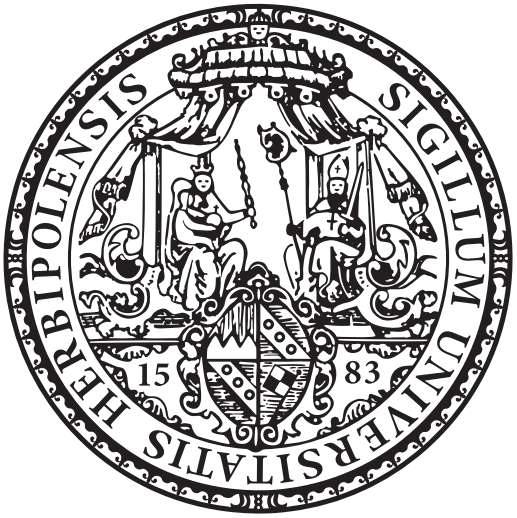
Development of Novel Quinolone Amides Against the African Sleeping Sickness - A Fluorine Walk

Dissertation zur Erlangung des
naturwissenschaftlichen Doktorgrades
der Julius-Maximilians-Universität Würzburg



vorgelegt von
Michael Berninger
aus Eschau

Würzburg 2018

Eingereicht bei der Fakultät für Chemie und Pharmazie am:

_____

Gutachter der schriftlichen Arbeit

1. Gutachter: _____

2. Gutachter: _____

Prüfer des öffentlichen Promotionskolloquiums

1. Prüfer: _____

2. Prüfer: _____

3. Prüfer: _____

Datum des öffentlichen Promotionskolloquiums:

_____

Doktorurkunde ausgehändigt am:

_____

Die vorliegende Arbeit wurde am Institut für Pharmazie und Lebensmittelchemie der Bayerischen Julius-Maximilians-Universität Würzburg auf Anregung und unter der Anleitung von

Frau Prof. Dr. Ulrike Holzgrabe

angefertigt.

Mein herzlicher Dank gilt ihr für die Aufnahme in die Arbeitsgruppe und das Anvertrauen des spannenden Projekts. Ich bedanke mich für das in mich gesetzte Vertrauen und die gewährten wissenschaftlichen Freiräume bei der Bearbeitung des Themas.

Des Weiteren bedanke ich mich beim Sonderforschungsbereich 630 für die finanzielle Unterstützung dieser Arbeit.

Weiterhin möchte ich folgenden Kooperationspartnern für die erfolgreiche Zusammenarbeit danken:

- Prof. Dr. Samuel Samnick, Dr. Ina Israel und Dr. Ehab Al-Momani, vom Universitätsklinikum Würzburg, Nuklearmedizin für die ^{18}F-Fluorierung und die anschließenden PET- bzw. autoradiographischen Untersuchungen.
- Antje Fuß und Dr. Joseph Skaf für die Substanztestung an *Trypanosoma brucei brucei*.
- Dr. Christine Erk für die Bestimmung der mikrosomalen Umsetzung und Identifizierung der Metabolite.
- PD Dr. Heike Bruhn und Elena Katzowitsch für die Zytotoxizitätsuntersuchungen.

Dem gesamten Arbeitskreis und allen Mitarbeitern bin ich für die angenehme Arbeitsatmosphäre und die schöne gemeinsame Zeit dankbar: Lina, Ines, Flo, Anna, Regina, David, Olli, Jogi, Jan, Miri, Nils, Raphael, Jens, Nina, Lu, Johannes, Christine, Christiane, Alex, Curd, Antonio, Anja, Paul, Niclas, Flo, Nicolas, Klaus, Patrick, Markus, Jonas, Joseph. Vielen Dank an alle Menschen, die mich während dieser Zeit ein Stück begleitet haben.

Teile der vorliegenden Arbeit sind Gegenstand folgender wissenschaftlichen Publikationen und Posterpräsentationen:

Publikationen:

Skaf, J.; Hamarsheh, O.; Berninger, M.; Balasubramanian, S.; Oelschlaeger, T. A.; Holzgrabe, U. Improving anti-trypanosomal activity of alkamides isolated from Achillea fragrantissima. *Fitoterapia* **2017,** 125, 191-198.

Pyrih, A.; Berninger, M.; Gzella, A.; Lesyk, R.; Holzgrabe, U. Synthesis and evaluation of antitrypanosomal activity of some thiosemicarbazide derivatives of 1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid. *Synth. Commun.* **2018,** 48, 1883-1891.

Berninger, M.; Erk, C.; Fuß, A.; Skaf, J.; Al-Momani, E.; Israel, I.; Raschig, M.; Güntzel, P.; Samnick, S.; Holzgrabe, U. Fluorine walk: The impact of fluorine in quinolone amides on their activity against African sleeping sickness. *Eur. J. Med. Chem.* **2018,** 152, 377-391.

Posterpräsentationen:

Berninger, M.; Hiltensperger, G.; Schleier, D.; Holzgrabe, U.: "Synthesis of novel Pyrazoloquinolinones against *Trypanosomoa brucei*", 8th Joint PhD Students Meeting of the SFB 630 and 766 and FOR 854, **2014**, Retzbach.

Berninger, M.; Güntzel, P.; Fuß, A.; Holzgrabe, U.: "4-Quinolone-3-carboxamides as active compounds against *Trypanosoma brucei*", Best of SFB 630 - and Future Perspectives, **2015**, Würzburg.

Berninger, M.; Fuß, A.; Al-Momani, E.; Isreal, I.; Raschig, M.; Güntzel, P.; Samnick, S.; Holzgrabe, U.: "Fluorine Walk: The role of fluorine in quinolone amides active against African sleeping sickness", XXIV EFMC International Symposium on Medicinal Chemistry, **2016**, Manchester (UK)

Meinen Eltern

Content

1 Introduction

Novel lead compounds in pre-clinical development against African sleeping sickness

M. Berninger, I. Schmidt, A. Ponte-Sucre and U. Holzgrabe, *Med. Chem. Commun.*, 2017, 8, 1872-1890. DOI**:** 10.1039/C7MD00280G - Reproduced by permission of The Royal Society of Chemistry.

Abstract

Human African trypanosomiasis (HAT), also known as African sleeping sickness, is caused by parasitic protozoa of the genus *Trypanosoma*. As the disease progresses, the parasites cross the blood brain barrier and are lethal for the patients if the disease is left untreated. Current therapies suffer from several drawbacks due to e.g. toxicity of the respective compounds or resistance to approved antitrypanosomal drugs. In this review, the different strategies of drug development against HAT are considered, namely the target-based approach, the phenotypic high throughput screening and the drug repurposing strategy. The most promising compounds emerging from these approaches entering an *in vivo* evaluation are mentioned herein. Of note, it may turn out to be difficult to confirm *in vitro* activity in an animal model of infection; however, possible reasons for the missing efficacy in unsuccessful *in vivo* studies are discussed.

1. Introduction

Human African trypanosomiasis (HAT) is also called sleeping sickness. The disease is mainly restricted to Africa, and depending on the affected region two types of human illness can be found: East and West African trypanosomiasis. Two species of trypanosomatides which are transmitted by the tsetse fly, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*, cause the ailment. The number of cases in Africa has dropped drastically, however, approximately 3000 new infections of both East and West African trypanosomiasis have been reported to the World Health

Organization in 2015.[1] However, underestimating the actual number of cases is a common problem due to weak health infrastructures and restricted access to medical facilities.[2]

HAT is mainly being spread through the bite of infected tsetse flies; infection seldomly occurs by blood transfusion or through placental transmission. Of note, the disease causes infertility and abortion in pregnant women or of childbearing age, respectively. This disease is invariably fatal if left untreated. In Africa, HAT-affected zones cover an area of about 8 million square kilometers between 14 degrees north latitude and 20 degrees south latitude.[3] In the 20th century three major HAT epidemics occurred, the first one between 1896 and 1906, the second in 1920, and the most recent one in 1970 lasting until the 1990s. Between these epidemics the number of cases constantly decreased to the currently known magnitiude.[4]

T. brucei rhodesiense causes the more virulent form of the disease which is called East African or Rhodesian African sleeping sickness. It is zoonotic, rare, and extremely virulent; patients usually die within a few months. *T. brucei gambiense* causes the West African or Gambian African sleeping sickness; the *gambiense* type shows both long latency and chronicity, whereas in the *gambiense* HAT, humans only constitute the main reservoir and transmission agent within the life cycle of the parasite.[1]

Two clinical stages define the status of the disease; i.e., a haemolymphatic initial systemic stage and the meningoencephalytic stage which is characterized by the invasion of the brain by parasites. The latter produces sensory, motor, and psychiatric disturbances, as well as typical and name-giving sleep alteration. The painful bite of an infected tsetse fly produces a distinctive local erythema, heat, edema, and tenderness leading to the appearance of the chancre, an ulcer that lasts for two or three weeks and appears where the parasites are present. The disease subsequently evolves into the two typical phases which have already been mentioned.[5,6]

The tsetse bite conduces the patient into the haemolymphatic stage I which is characterized by painful lymph nodes; eventually, intermittent fever episodes may occur as a result of the successive waves of trypanosome invasion within the blood stream. Adenopathy, splenomegaly, and liver disturbances are typical signals of the invasion of the reticuloendothelial system. Finally, skin rashes and pruritus with scratching skin lesions develop which become unbearable for the patient.[5–7]

Evolving into the meningoencephalitic stage II may take several months or even years. The parasites cross the blood–brain barrier (BBB), infect the central nervous system (CNS), and cause serious changes to the patient's sleep pattern which may be accompanied by confusion, motor, and mental coordination disorders. The cerebrospinal

fluid is invaded by enormous quantities of macrophages; non-specific perivascular inflammatory cell infiltrates occur in the leptomeninges and the white matter. Finally, a pronounced activation of microglia and astrocytes occurs.[5–7] The terminal phase of the disease is characterized by disturbances in consciousness, the development of dementia including incoherence, double incontinence, and seizures. The patient either dies from heart failure or – in the late stage – from encephalitis in a state of cachexia and physiological collapse.[5–7]

The Animal trypanosomiasis is known as Nagana. Two African trypanosome subgenera, Nannomona and Duttonella (*Trypanosoma congolense* and *Trypanosoma vivax*, respectively), as well as *Trypanosoma brucei brucei* cause the cattle sickness. From the veterinary point of view this is economically threatening as serious losses in pigs, camelids, goats, and sheep may vastly affect breeding within the affected rural areas.[8,9] *Trypanosoma evansi* (subgenus *Trypanozoon*) mostly infects mammals including horses, mules, camels, buffalo, cattle, and deer, however, cows, goats, sheep, dogs, and cats can also be affected. The parasite produces a disease called Surra, hip disease, Murrina, or Derrengadera (mal de cadeiras) of great economic impact for many geographic areas: morbidity and mortality rates are usually very high in tropical and semi-tropical regions such as North Africa, China, the Philippines, India, Indonesia, Malaysia, Russia, Central America, and South America.[10,11] In fact, in Africa, Asia and South America thousands of animals die from these diseases every year causing tremendous economic losses within these regions.[10,11] Besides the huge losses of working animals and deficiencies in milk production the reproductive deficiencies due to infertility also represent a major challenge. In addition, treatment costs are very high.[10,11] Of note, *Trypanosoma equiperdum* (subgenus *Trypanozoon*) infects equines under natural conditions; by venereal transmission it may also cause an equine disease called dourine.[12] Finally, when thinking of the optical identification of the parasites e.g. by microscopy it should be emphasized that *Trypanosoma evansi* (monomorphic) and *Trypanosoma equiperdum* (monomorphic but occasionally pleomorphic) are morphologically similar to the slender forms of *Trypanosoma brucei brucei*, as well as of *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*.[13,14] This makes a valid diagnostic very difficult.

2. Compounds in clinical stages

Only very few active compounds are available for the treatment of the disease, most of them only effective against one stage of the HAT or against one parasite species, i.e.

either *T. b. gambiense* or *T. b. rhodesiense*. The therapy is far away from being considered optimal and currently only two drugs, fexinidazole and the benzoxaborole SCYX-7158, have reached clinical trials. In addition, the development of resistance to clinically used drugs may aggravate the situation. No vaccines are available.[1] Taken together, new antitrypanosomal agents are urgently needed.[4] Hence, this review gives an insight in recent trypanocidal drug development. We applied strict selection criteria; in particular, at least *in vivo* studies and toxicological data should be available (Table 1). Especially in the last three years encouraging research has been performed in the field of antitrypanosomal agents, hence markedly plenty compounds with various scaffolds were tested for *in vivo* activity recently. Since the vast majority of compounds fails in subsequent clinical stages, a continuous refilling of the pipeline with innovative drug candidates is necessary.

Fexinidazole and SCYX-7158

Preclinical details of the current clinical candidates fexinidazole and SCYX-7158 (Fig. 1) have already been reviewed in the literature.[15–18] Fexinidazole initially was a designated antimicrobial agent against *T. brucei*, *Trichomonas*, *T. cruzi*, and *Entamoeba histolytica* by Sanofi-Aventis in 1978. Despite the promising activities of fexinidazole further progression was abandoned primarily due to the common prejudice against nitro-aromatics and their toxicological profile.[19] A screening approach by the drugs for neglected diseases initiative (DNDi) of 700 already known nitroheterocyclics – mainly nitro-imidazoles – against *T. brucei* rediscovered fexinidazole.[20] The convincing *in vivo* results particularly in the GVR35 mouse model (50 mg kg^{-1}, bid, ip, 4 days) demonstrated full cure (5/5).[21] In clinical phase I trials fexinidazole proved good tolerance in oral doses of 100 to 3600 mg.[22] Efficacy and safety of fexinidazole, currently being in clinical phase II/III, is tested in 394 acute stage patients in comparison to the nifurtimox-eflornithine combination therapy (NECT).[23] Moreover, the recruitment of patients for the phase III trial is ongoing, assessing the therapy success under real life conditions.[24] The DNDi expects a hopefully positive result from the regulatory authorities in late 2017, having “only” $45 million spent on the approval fexinidazole so far. This number reflects the money that was spent on drug research, early safety as well as proof-of-concept trials, and efficacy trials.[25]

The oxaborole SCYX-7158, also derived from phenotype screening, effectively cured (5/5) stage II trypanosomiasis in mice in tolerable doses (25 mg kg^{-1}, qd, orally, 7 days).[26] In the following Phase I trial, SCYX-7158 was administered to 128 healthy humans to find

the tolerated dose of 960 mg, proceeding with a phase II trial in the Democratic Republic of Congo (DRC) in 2016.[27] However, no data are reported yet.

Diamidines: from abandoned clinical trial to promising candidates again

In recent years several diamidines were evaluated as antitrypanosomal drug candidates due to their promising *in vitro* and *in vivo* activity against *Trypanosoma*. Especially the effective cure of stage I of HAT in mice was well established.[28,29] For example, pafuramidin (DB289, not shown) successfully completed phase III clinical trials. Disappointingly the progress to market authorization was terminated when unexpected severe nephrotoxic adverse effects emerged.[30,31] Although the side effects only appeared in a population in South Africa during the clinical trials, it was not possible to predicted them by preclinical safety models. Only a mouse diversity panel, i.e. well-characterized genetic variants of inbred mice, revealed the kidney safety liability of pafuramidin retrospectively.[32] However, the renal toxicity is not supposed to be a general adverse effect of the entire substance group, thus pursuing research on potent diamidines appears reasonable.[33] The final aim is to be able to cure both stages of HAT solely by oral administration. Therefore, recently discovered diamidines penetrating the CNS are prominent candidates for supplementing the drug pipeline.

At physiologic pH the diamidines are protonated twice. Since dicationic agents were thought to be unable to cross the BBB, it was even more unexpected that certain compounds like **1** (DB820) and **2** (DB829) (Fig. 2) were active in a stage II mouse model. In mice which had been infected with trypanosomes GVR35, compound **2** was superior to compound **1** (1/5 versus 5/5 cure rate) when 20 mg kg^{-1} were administered ip over 10 days. As a passive transport mechanism seems to be unlikely for ionized compounds, uptake transporters were suggested channeling these diamidines through the BBB.[34] Moreover, it was possible to make the compounds orally bioavailable by masking the amidine structure via a prodrug approach:[35] a methoxy group was attached to the amidine. This lowered the diamidines' pKa value in an extend that the molecules are largely unprotonated at physiological pH and warranted a rapid absorption in the gastrointestinal tract. In human liver microsomes these compounds were metabolized via *O*-demethylation and *N*-dehydroxylation into the respective active compounds.[36] Finally, the corresponding prodrugs **3** (DB844) and **4** (DB868) (Fig. 2) effectively cured all (5/5) mice in a CNS model, administering 100 mg kg^{-1}, po over 5 days.[29] In subsequent studies, vervet monkeys were infected with stage II developing strain *T. b. rhodesiense* KETRI 2537. After determining

the tolerable dose of 5 mg kg^{-1} per day, 8 monkeys were administered 5 mg kg^{-1}, po for 10 days and 6 mg kg^{-1}, po for 14 days, respectively, starting on day 28 pi. Following the monitoring time of 300 days, **3** partially cured 37.5% of the infected animals in lower doses and 42.5% in the elevated dosage regime, respectively.[37] Additionally, the pharmacokinetic and safety profiles of **4** were also tested in vervet monkeys for stage I of HAT. Herein, treating the *T. b. rhodesiense* KETRI 2537 infected animals started 7 days pi, and all animals were cured applying a dose of 3 mg kg^{-1} per day for 7 days. In this study, biomarkers like creatinine and urea concentration were monitored in order to exclude previously proclaimed nephrotoxicity and kidney liability.[33] Further pharmacokinetic investigation was arranged, particularly concerning the CNS exposure by different diamidines. For understanding the outstanding activity of **2** in stage II of HAT compared to other diamidines, Yang et al. proposed a higher unbound fraction of **2** both in plasma and brain (**1** $f_{u,plasma}$ = 21% / $f_{u,brain}$ = 0.2%; **2** $f_{u,plasma}$ = 40% / $f_{u,brain}$ = 1.5%). Thus, high AUC in plasma (7.6 µmol L^{-1} h^{-1}) and brain (508 µmol L^{-1} h^{-1} within 72 h) could be achieved. The CNS permeation was presumed to occur by preferential active transporter; likely the position of the nitrogen played a crucial role.[34,38] Finally, the mechanism of the traverse through the BBB should be fully elucidated before proceeding with clinical phases.

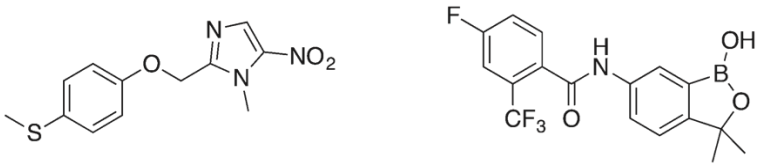


Fig. 1: Clinical candidates fexinidazole (left) and SCYX-7158.

Furthermore, Patrick *et al.* described diamidines derived from a m-terphenyl scaffold which cured 4/4 mice infected with *T. b. rhodesiense* STIB900 in doses of 5 mg kg^{-1}, ip, for 4 days.[39] The most promising candidates resulted by the replacement of the furan linker by a phenyl moiety. Hence, compound **5** (Fig. 2) – which is chemically closely related to **2** – was assessed in a CNS mouse model. Additionally, detailed pharmacokinetic parameters were examined. **5** (28DAP010) cured all infected mice (5/5) in this stage I model with 20 mg kg^{-1}, ip, for 10 days. The extent of its binding to mouse plasma proteins and brain tissue ($f_{u,plasma}$ = 26% / $f_{u,brain}$ = 0.69%) was slightly stronger when compared to **2**.[38] Nevertheless, similar AUC values were accomplished in brain tissue (469 µmol L^{-1} h^{-1} within 72 h), finally making it a backup compound in case of clinical failure of the diamidine **2**.

Besides the prodrug approach, Martinez *et al.* tried to enhance compound permeability through the BBB by reducing the ionization rate.[28] Compound **6** (Fig. 3) carrying N-hydroxy-derivatized imidazoline rings exhibited a pK_a of 7.43 and therefore, a less ionized ratio (51.8%) at physiological pH. In a stage I mouse model **6** was fully curative in doses of 20 mg kg^{-1}, ip, for 4 days, hence a model of stage II infection was designed afterwards. In this case, **6** was administered at a dose of 50 mg kg^{-1}, ip, bid, which – in comparison to the control – extended the mean days of relapse to 52. Furthermore, metabolic studies suggested that **6** possessed an intrinsic activity against the parasites without the need of previous bioactivation.[28]

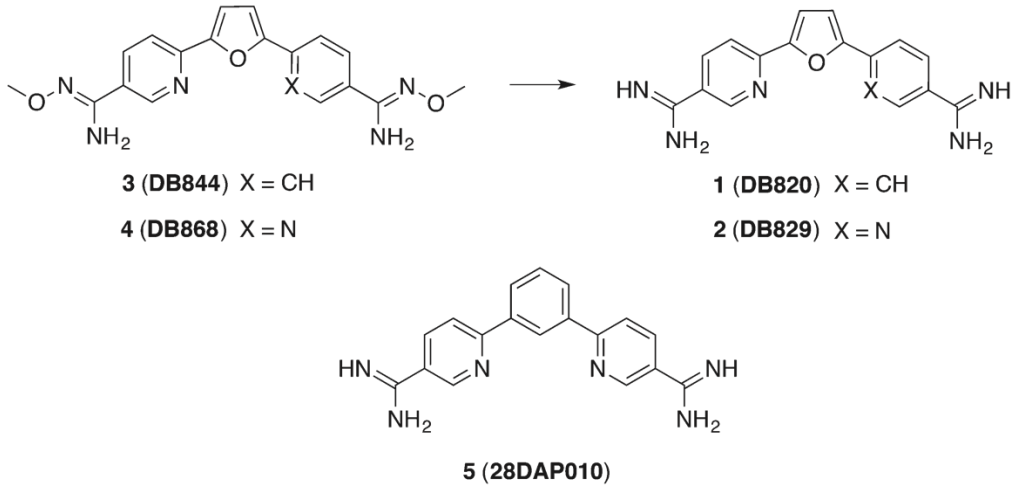


Fig. 2: Diamidine compounds being effective in CNS mouse model.

According to Vanden Eynde *et al.* the toxicity of pentamidine could be reduced by replacing the dioxypentyl linker by a diamidealkyl linker.[40] The most promising derivatives **7**, **8**, and **9** (Fig. 3) (IC_{50} = 2–9 nM, *T. b. brucei*; CC_{50} = 43.0 ≥ 100 µM, L6 cells) were tested in mice infected with drug-sensitive and drug-resistant strains of *T. b. brucei*. One daily dose was administered ip for 3 days, beginning 24 h pi. The three compounds cured all mice (3/3) infected with the drug-sensitive strains (Lab 110 EATRO and KETRI 2002) in a dose of 10 mg kg^{-1}. The most effective compound, **8**, cured even in doses lower than 1.0 and 2.5 mg kg^{-1}, respectively. For mice infected with the resistant strain KETRI2538 (refractory to eflornithine and arsenical drugs) a survival of over 30 days could be observed when treated with **7** and **8** (doses between 5 and 25 mg kg^{-1}), and all mice were considered as cured. However, all compounds tested including pentamidine were ineffective against strain Ketri 1992 (refractory to diamidines).[40]

Yang *et al.* focused on potent diamidines with **10** (Fig. 3) being the most selective scaffold. **10** exhibited an IC_{50} value of 7.7 nM against *T. b. brucei* and a CC_{50} value greater than 200 µM against both hepatocytes and kidney cells, resulting in a SI > 13 000. Hence, **10** proceeded to *in vivo* studies for the stage I of HAT. Since the administration of both 3 and 10 mg kg^{-1}, ip, for 4 days effectively cured all mice (5/5), reduced doses of 1 and 0.3 mg kg^{-1}, ip, were applied for 4 days. However, the lower doses were not effective anymore. In this case, A/T-rich DNA motifs (especially in the kinetoplast DNA) could be designated as drug target of the investigated diamidines. Moreover, two additional targets for **10** were identified, particularly the trypanosomal farnesyl diphosphate synthase (FPPS) (more selective to *Tb*FPPS then to human FPPS, representing an improvement towards **2**) and the uncoupling of the proton pump in the respiratory chain which is responsible for ATP synthesis.[41]

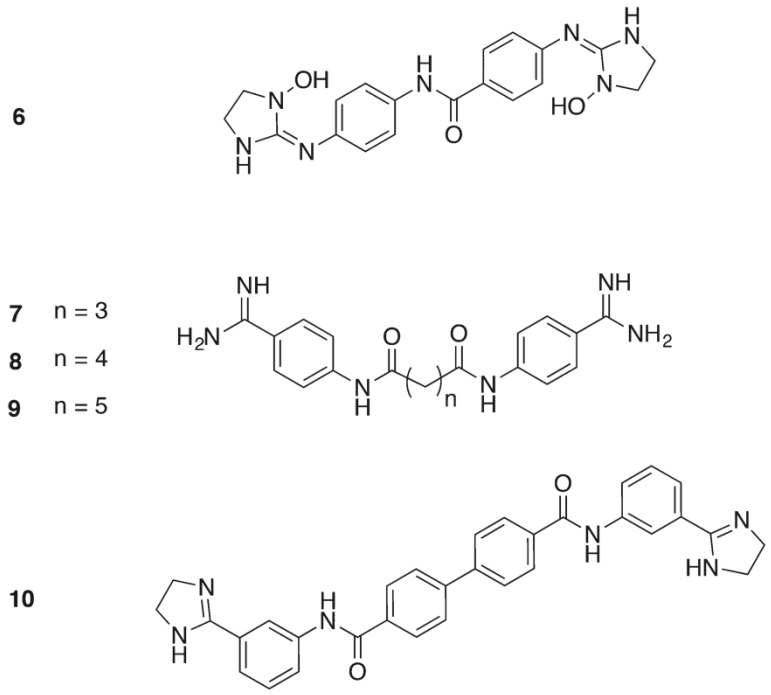


Fig. 3: Diamidines with varied linker.

3. Target-based approach

Drug discovery programs for neglected tropical diseases rely both on target-based approaches and phenotypic screening. In the target-based approaches compound libraries are screened against a certain molecular target, e.g. enzymes. In further steps, the initial hit is optimized regarding potency, selectivity, and pharmacokinetic profile. Thus,

a preliminary validation process is required to ensure that the compound actually binds to the supposed target.[42,43] Unfortunately, in the case of African sleeping sickness there are only few validated molecular targets. In the following sections the most distinguished target-based approach was taken into consideration.

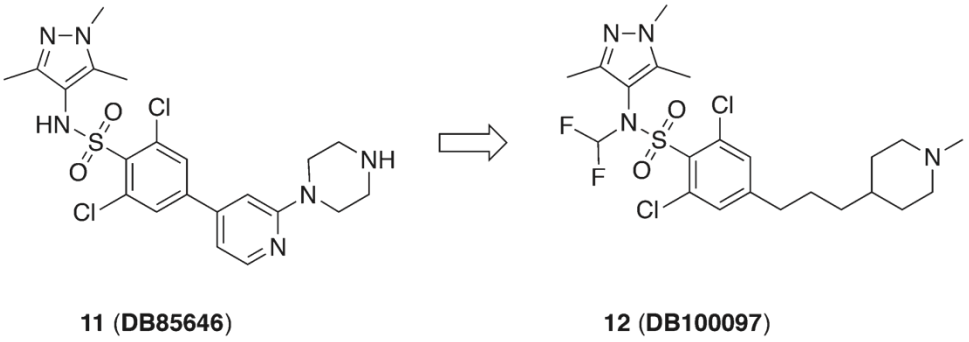
N-Myristoyltransferase inhibitors

RNA interference assays in *T. brucei* confirmed *N*-myristoyltransferase (NMT) as an essential protein in both life cycle stages of trypanosomes producing the diseases discussed herein, namely in the procyclic vector and the mammalian bloodstream form. Therefore, this enzyme was suggested to be an appropriate target for the development of anti-parasitic agents. The NMT catalyzes the cotranslational myristoylation of the *N*-terminal glycine of approximately 60 proteins which are predicted to be essential for the parasite growth. Which downstream peptides exactly inhibit the growth of parasites has not been elucidated yet, but pleiotropic effects are assumed.[44]

Brand *et al.* optimized the HTS hit which resulted in the very potent NMT-inhibitor **11** (DDD85646) (Fig. 4) (IC_{50} = 2 nM, *Tb*NMT).[45] For pharmacokinetic studies, female NMRI mice were treated with 3 mg kg^{-1}, iv and 10 mg kg^{-1}, po, respectively. It showed a low blood clearance (Cl_b = 6 mL min^{-1} kg^{-1}) and a small volume of distribution (V_d = 0.4 L kg^{-1}) with a moderate half-life value ($t_{1/2}$ = 1.2 h) and a favorable oral bioavailability (F = 19%).[46] For the *in vivo* efficacy studies, female NMRI mice were infected with *T. b. brucei* S427. The administration of **11** in a dose of 12.5 mg kg^{-1}, po, bid for 4 days could be considered an effective dose for this compound as no parasites were detectable after 30 days.[46] Notably, the minimal orally efficacious dose was 50 mg kg^{-1} for the more clinical relevant *T. b. rhodesiense* subspecies. A disadvantage was the low brain penetration of this compound (brain– blood-ratio <0.1). Due to lacking efficacy, a stage II HAT efficacy study at the maximum tolerated dose of 100 mg kg^{-1}, po, bid failed.[45] All in all, this study could prove NMT as a promising target against HAT, but the examined compound has some limitations, namely the lack of selectivity against the human isoform of NMT (IC_{50} = 3 nM, HsNMT; IC50 = 2 nM, *Tb*NMT) and its low CNS exposure.[45]

The Drug Discovery Unit in Dundee (DDU) consequentially guided compound **11** through lead optimization referring to the related crystal structures of NMT of *Aspergillus fumigator* and *Leishmania major*.[47] The replacement of the pyridine ring by a flexible alkyl linker achieved increased selectivity which was further refined addressing the CNS penetration. Therefore, the sulfonamide group which was suggested to be deprotonated at

physiological pH was capped. Since simple alkyl residues like methyl or ethyl groups were metabolized rapidly, a difluorinated methyl group was attached to increase both stability and CNS exposure. Finally, the improved compound **12** (DDD100097) was obtained. The selectivity against HsNMT was increased (IC_{50} = 12 nM, HsNMT versus 2 nM, *Tb*NMT) which was reflected in the advanced selectivity against MRC5 fibroblasts (CC_{50} = 0.3 µM versus 1 µM). Moreover, **12** exhibited an advanced permeability through the BBB (blood–brain-ratio = 1.6), contrary to **11**. After proof of full cure by using **12** in a stage I mouse model (*T. b. brucei* S427, 50 mg kg^{-1}, po, bid for 4 days) an evaluation of a stage II mouse model was performed. *T. b. brucei* GVR35 infected mice were treated with the maximum tolerated dose (100 mg kg^{-1}, bid, po) for 5 days but signs of toxicity occurred. Nevertheless, in order to achieve the proof-of-concept *in vivo* for stage II, other dosing schedules (e.g. extension of the treatment days or pulse dosing) were tested.



11 (**DB85646**) **12** (**DB100097**)

Fig. 4: TbNMT inhibitors identified through target-based approach.

Both a prolonged administration of 100 mg kg^{-1} bid for 8 days and three rounds of 3 days treatment with a two-day drug-free interval each partially cured mice (1/5). Unexpectedly, no absolute efficacy was obtained, though a dose of 20 mg kg−1, bid, po was predicted to be curative. The observed treatment failure might result from the discrepancy in the susceptibility of *T. b. brucei* strain GVR35 and *T. b. brucei* S427 towards **12**. Previous NMT inhibitors exhibited similar differences in activities (4–5-fold less active against *T. b. brucei* GVR35). Since the dosing regime was set on the basis of the efficacy against *T. b. brucei* S427, it was supposed that treatment failure originated from subtherapeutic doses. Unfortunately, the dose was at the limit of therapeutic tolerance, thus no further increase could be performed.[47]

4. Phenotypic HTS

The whole-cell screening of large compound libraries became feasible due to the application of the inexpensive, robust, and simple resazurin-based assay including 384- and 96-well formats. The phenotype screening hits provide a guaranteed impact on living organisms and hence possess better drug-like properties which is in contrast to compounds being obtained from the target-based approach. Consequently, a cytotoxicity assay against mammalian cells is performed to exclude general toxic molecules and to estimate selectivity.[42] Moreover, HTS of diverse libraries could identify novel and unknown drug targets, nevertheless it may be challenging to subsequently elucidate the accurate mode of action.[48–51] Of note, when looking at currently approved drugs against sleeping sickness only for eflornithine the mechanism of action could be elucidated, yet.

Oxazolopyridine derived lead compounds

Tatipaka *et al.* performed a phenotypic screening of 700000 compounds in cooperation with the Genomics Institution of the Novartis Research Foundation (GNF) to find new starting points for the lead optimization of compounds against HAT.[52] Scaffold **13** (Fig. 5), a substituted 2-(3-aminophenyl)- oxazolopyridine, was chosen for subsequent hit-to-lead optimization because it lacks stereogenic carbon atoms and possesses desirable properties such as low molecular weight and compliance to the Lipinski's rule of five. In the next step, 110 compounds were synthesized, revealing the most selective 2-(3-aminophenyl)imidazopyridine (**14**), exhibiting an IC_{50} of 2 nM in vitro against *T. brucei* S427 (Fig. 5). An enhanced potency arose from replacing chlorine with fluorine and substituting the furanoyl group by an urea group. In terms of potency, varying the oxazolopyridine core was well tolerated when replacing the oxygen with a nitrogen which resulted in an imidazopyridine scaffold. However, the leap in activity was achieved by attaching an aromatic moiety to C6. Finally, a moderate CC_{50} towards human lymphoblasts and hepatocytes (24 µM and 30 µM, respectively) suggested a SI value greater than 1000. The analysis of pharmacokinetic parameters indicated that **14** showed an oral bioavailability with sufficient plasma levels (C_{max} = 4.3 µM) for parasite inhibition.
Additionally, CNS exposure was firstly demonstrated by permeability studies across MDCK-MDR1 cells without the compound being substrate of the efflux pump MDR1. Secondly, the level of brain penetration was proven as brain-to-blood concentration ratio (0.5 and 1.1, determined by two independent experiments) after 60 min of ip dosing. The metabolism studies demonstrated a relatively short half-life of 31 min when incubated

utilizing human hepatic microsomes. The plasma clearance was determined to be 14.8 ml min^{-1} kg^{-1} and the half-life for plasma elimination was 204 min. For the *in vivo* studies mice were infected with *T. b. rhodesiense*. Compound **14** was administered orally 48 h pi. The lowest potent dose of 5 mg kg^{-1}, bid for 5 consecutive days, was able to cure all mice (5/5) after 60 days. Diminishing the dose to 2.5 mg kg^{-1} or reducing the administration to a qd regime cured 2 of 5 mice only. During a high dose study with 50 mg kg^{-1}, bid, po for **14** consecutive days neither a change in weight compared to mice treated with the vehicle, nor clinical toxicity could be observed. Altogether, these results indicated the high potential of **14** as a representative of a novel drug class against stage I of HAT. Further efforts to examine the potential for stage II are in progress.

Surprisingly, Sykes *et al.* also identified molecule **13** (Fig. 5) as a potential candidate for drug development in an independently performed whole-cell HTS of 87296 compounds.[48] Additionally, while screening 3 million compounds in a proliferation assay against kinetoplastid parasites *T. cruzi*, *T. brucei*, and *Leishmania donovani*, **13** stood out in being active against all three parasites (IC_{50} = 150 nM, *T. brucei*) and was advanced to lead compound **15** (GNF6702) (Fig. 5).

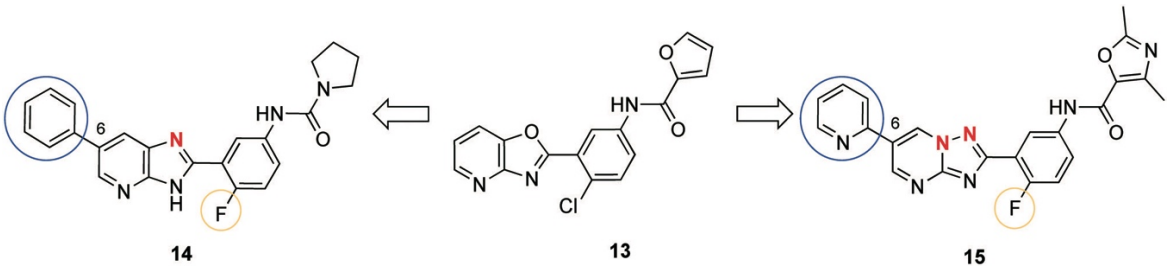


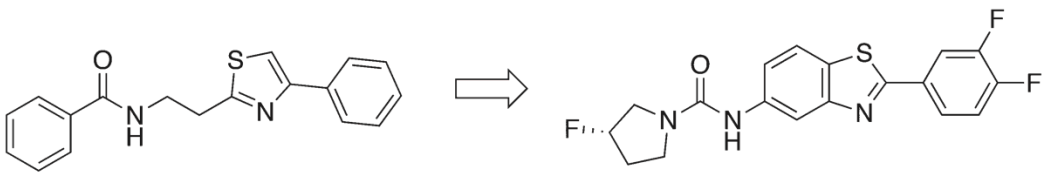
Fig. 5 Lead compounds derived from oxazolopyridine **13**.

The implemented modification comprised substituting chlorine with fluorine, as it was done likewise in the group of Tatipaka. The furanoyl residue was replaced by a dimethyl-oxazole ring due to the toxicity risks associated with furan. The core modification resulted in an imidazopyrimidine scaf- fold. Moreover, the C6-substitution initiated the substantial increase in activity against all kinetoplastids, whereas the pyrimidine moiety at C6 introduced the best selectivity towards macrophages and fibroblast cells. Conclusively, the lead compound was optimized regarding potency, oral bioavailability, and plasma clearance. Thus, compound **15** (IC_{50} = 70 nM) was set to a mouse model of stage II of HAT. However, from the chemical point of view, compounds **14** and **15** are quite similar.

The CNS infection was demonstrated in mice by luciferase expressing *T. brucei* parasites and detection of the bioluminescence. Treating the animals started 21 days pi with 100 mg kg^{-1}, po, qd, for 7 days of **15**; the compound was fully curative in all mice (6/6). Due to this outstanding result, efforts on target identification were made to start a target-based drug development. The first hint was gathered using cultivated, drug resistant strains of *Trypanosoma* which possessed mutations in the proteasome encoding genes. Further investigation of resistant parasites overexpressing PSMB4F24L (i.e., proteasome subunit beta type 4), revealed susceptibility towards **15**. Additionally, in contrast to the mammalian protease inhibitor bortezomib compound **15** did not affect the human proteasome but selectively blocked any chymotrypsin-like activity. In summary, the *T. brucei* proteasome was assessed as a validated drug target of **15** through various evidence such as the correlation between the inhibition of the parasite proteasome and the parasite growth, respectively. It is tempting to speculate that compound **14** may address the same target due to its chemical similarity. As a next step the preclinical toxicity of **15** should be evaluated and consideration about preclinical studies for stage II of HAT should be given.[53]

Thiazole-2-ethylamine derived lead compound

Patrick *et al.* subjected molecule **16**, one initial hit from the GNF library, to lead optimization (Fig. 6).[54] The structure modification focused on both ends of the molecule while retaining the thiazol-2-ethylamine core. Interestingly, Sykes *et al.* again independently confirmed structurally close related phenylthiazol-4-ylethylamides being promising hit scaffolds.[48] Thirty-three analogues of **16** having IC_{50} values below 0.2 µg mL^{-1} and SI values greater than 120 towards L6 cells were evaluated *in vivo* with a dose of 40 mg kg^{-1} administered ip.[54]



16 **17**

Fig. 6 Lead compound derived from thiazole-2-ethylamine.

Two mice infected with *T. b. rhodesiense* were treated for three consecutive days starting 24 h pi. Despite the good in vitro activity (e.g., $IC_{50} < 25$ nM) of certain molecules, none of the analogues was able to cure mice. Studies on the metabolic stability revealed half-lives ranging from 0.3 to 11 min, explaining the lack of *in vivo* efficacy. Since all the metabolically unstable compounds contained the thiazol-2-ethylamine core, this scaffold was considered as a major weakness.[54] Although details about the metabolic degradation remain unexplored, only replacing it by fused ring systems could enhance the stability of the compounds. Additionally, the metabolic stability of the molecule could be increased fourfold by fluorination of the pyrrolidine moiety. Finally, only the benzothiazole scaffold **17** exhibited prolonged half-lives ($t_{1/2} > 60$ min) with concurrently retained antitrypanosomal activity ($IC_{50} = 0.0348$ µM, *T. b. brucei*). Compound **17** was administered at 50 mg kg^{-1}, po, bid for 4 days, beginning two days pi and cured all mice (5/5) in a stage I mouse model. Moreover, **17** was given in doses of 50 mg kg^{-1}, po for 10 days, beginning 21 days pi in a stage II study with *T. b. brucei* TREU667 infected mice. Over a period of 180 days pi, all mice (5/5) were cured and free of parasites in tail blood samples. These results are outstanding for an oral treatment option for both stages and will be complemented by target identification in the near future.[55]

Indoline-2-carboxamides

Based on a protease inhibitor library containing 3400 compounds of the DDU in Dundee, hit compound **18** (Fig. 7) was identified ($IC_{50} = 30$ nM, *T. b. brucei*) with a SI greater than 1800 versus MRC-5 fibroblasts ($CC_{50} > 50$ µM). Extensive pharmacokinetic research demonstrated a microsomal metabolic instability of **18**. Therefore, the following *in vivo* experiments were conducted with hepatic CYP reductase null (HRN) mice having a suspended CYP450 activity. The HRN mice were infected with *T. b. brucei* S427 and the administration began three days pi with 3 mg kg^{-1}, ip, bid for 4 days. All mice (3/3) could be cured without a relapse up to 30 days pi. However, when 3 infected NMRI mice were treated with 10 mg kg^{-1}, bid for 4 days, only a partial cure was obtained (one mouse relapsed; two mice were free of parasites up to 30 days pi). Despite the compound blood concentration of **18** quickly decreased below the EC_{50} within 4 hours, it was at least partially effective. Hence, **18** is considered to selectively accumulate within the parasites or might have a rapid cidality. Thus, Cleghorn *et al.* made some efforts in order to prolong compound half-lives and substituted the potentially unstable ether group by a methylene linker.[56] Furthermore, regarding the antitrypanosomal activity the R configured enantiomer

emerged being superior to the racemate. Other variations addressing e.g. the indoline core or the amide was either not tolerated or only little beneficial. Lead compound **19** (Fig. 7) eventually possessed an improved pharmacokinetic profile regarding brain tissue binding, c_{max}, $t_{1/2}$, AUC, and brain-to-blood ratio, and was passed to a stage II mouse model. *T. b. brucei* (GVR35 strain) infected mice were treated with 12.5 mg kg^{-1}, ip, bid for 5 days beginning 21 days pi. One of five mice showed no parasitemia after 180 days pi what was specified as cured, while the remaining animals gradually relapsed within this period. The future challenge in optimizing the compound will include improving the microsomal stability.[56]

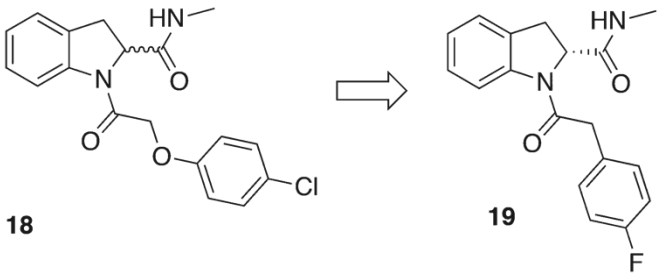
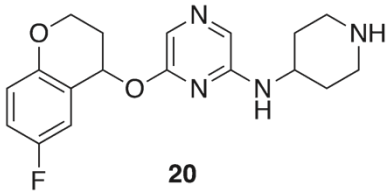


Fig. 7: HTS hit from protease inhibitor library and its lead compound.

2-Aminopyrazines / 2-aminopyridines

A screening of 5500 small molecules identified 2-aminopyrazines and 2-aminopyridines as hit structures.[57] Since the most effective compound **20** (Fig. 8) exhibited in vitro activity against *Trypanosoma* subsp. (IC_{50} = 0.12 µM) and a SI > 200 towards mammalian cell lines (MOLT4, L929), *in vivo* efficacy was studied in the stage I of HAT. A first treatment regime was designed to administer 20 mg kg^{-1}, ip, qd, for 5 days; it yielded a 50% curative outcome (2/4). Subsequently, treatment was changed to 25 mg kg^{-1}, bid for 10 days, as **20** was well tolerated previously; this higher dose effectively cured all mice (4/4) without any signs of toxicity. *In silico* and *in vitro* evaluation suggested appropriate pharmacokinetics with a high membrane permeability, low protein binding (f_u = 0.4), and high metabolic stability. However, more detailed *in vivo* investigation about bioavailability and other preclinical properties is in progress.[57]



20

Fig. 8: 2-Aminopyrazine from HTS.

5. Drug repurposing

In drug repurposing programs approved drugs are evaluated for novel fields of indication. Besides pharmacokinetics, toxicity, and metabolism it can be challenging to bring new chemical entities (NCEs) into clinical application.[50] These preclinical investigations are extremely time consuming and cost intensive factors of the drug development. However, in drug repurposing approaches the development can be accelerated since reliable safety and pharmacokinetic data are already available.[58] For pursuing this strategy, whole phenotypic screening as described before is applied.

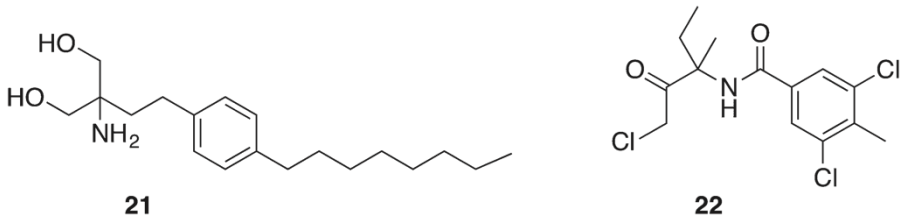
Fingolimod – screening hit derived from approved FDA drugs

According to the well-established HTS protocols against *T. brucei*, a library of 741 compounds – either in a late clinical stage or with FDA approval – was screened.[48] Compound **21** (fingolimod) (Fig. 9) is a registered drug representing an oral treatment option of multiple sclerosis and demonstrated a selective activity against several *T. brucei* subspecies (IC_{50} = 0.01–0.59 µM; SI = 16.8 towards HEK293).[59] In order to validate the target of the sphingolipid derivative **21**, other sphingosine analogues and sphingosine kinase inhibitors (SPHK) were tested for antitrypanosomal activity (IC_{50} = 0.02–10.79 µM). TbSPHK was validated as a drug target previously.[60] Unfortunately, no correlation between TbSPHK inhibition and activity against *T. b. brucei* was observed. Nevertheless, fingolimod was evaluated in an *in vivo* acute mouse model where mice were infected with *T. b. rhodesiense*. Disappointingly, **21** was not effective in curing mice due to unknown reasons.[59]

Zoxamide – screening hit derived from agrochemicals

Witschel *et al.* initially screened 600 agrochemicals supplied by BASF for their antiparasitic activity. Here, registration and admission requirements could be more stringent in certain

points than for pharmaceuticals because for such entities entering the food chain no tolerance towards side effects is allowed. Commercialized agrochemicals need to pass broad toxicological assessments including chronic and reprotoxicological studies in different mammalian species. Besides, beneficial features of these substances are a highly optimized temperature stability, their multi-ton scale production process, and low production costs. According to *in vitro* studies with *T. b. rhodesiense* STIB900, **22** (zoxamide) (Fig. 9) showed a similar activity (IC_{50} = 6 nM) as melarsoprol (IC_{50} = 6 nM). However, **22** was favored due to its oral LD_{50} of >5000 mg kg^{-1} in rats versus an LD_{50} iv of 44 mg kg^{-1} of melarsoprol in mice. For this reason, **22** was warranted for *in vivo* studies with female NMRI mice previously inoculated with *T. b. rhodesiense* STIB900. The treatment was initiated on day 3 pi and took 4 days with 200 mg kg^{-1}, ip, qd. No parasitemia was observed up to seven days pi. However, ten days pi all mice showed parasites in blood demonstrating that still efforts have to be done in optimizing these substances. Finally, agrochemicals could be considered being an interesting starting point with good prospects and a source of new lead structures against protozoal diseases which has been untapped so far.[61]



21 **22**

Fig. 9: Omnidirectional HTS hits of FDA approved drugs and agrochemicals, respectively.

Kinase-inhibitors

PI3K/mTOR inhibitors

The drug repurposing approach uncovered inhibitors of the phosphoinositide 3-kinase (PI3K) and the mammalian target of rapamycin (mTOR) kinase, both representing genetically validated potential drug targets in trypanosomes. Due to a remarkable structural homology between PI3K and mTOR in humans and trypanosomes, eight commercially available compounds were evaluated. **23** (NVP-BEZ235) (Fig. 10) inhibited both PI3Ks and mTOR in the sub-nanomolar concentration range. The in vitro assessment involving various *T. brucei* subsp. demonstrated an IC_{50} values from 0.18 to 16.3 nM. The consequent *in vivo* evaluation in *T. b. rhodesiense* EATRO3 infected mice which were

treated with 10 mg kg^{-1}, qd, ip for 4 days beginning 3 days pi, extended the mean survival to 13.4 days.[62,63]

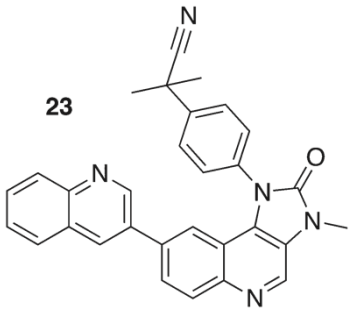
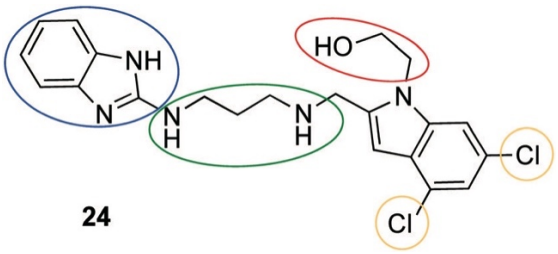


Fig. 10: Repurposed PI3K/mTOR inhibitor.

HTS of kinase inhibitors

Further drug repurposing approaches identified other human kinase inhibitors as potent antitrypanosomal agents.[60,63–65] A broadly designed kinase-targeted HTS of 42444 compounds including the published kinase inhibitor set by GlaxoSmithKline could confirm 797 selective hits. Regarding potency, predicted CNS permeability, physicochemical properties, and selectivity over human kinases, the three most attractive hits were assessed during pharmacokinetic studies. Subsequently, compound **24** (Neu-1053) (Fig. 11) was selected for being evaluated in an efficacy stage I mouse model due to appropriate blood exposure after ip administration, rapid onset of the antitrypanosomal effect *in vitro* (IC_{50} = 0.96 nM, *T. b. brucei*), and a high selectivity. The *T. b. rhodesiense* infected mice were successfully cured (4/4) after the administration of 10 mg kg^{-1}, ip, bid for 4 consecutive days, followed by a four-day treatment pause and a second round of 4 days of treatment. In a supplemental study with *T. b. brucei* infected mice compound **24**, which was given in the same regime, cured 3 of 4 mice for 90 days.[66] Subsequently, a structure–activity relationship (SAR) with 26 analogues of **24** was derived.[67] The hydroxyethyl moiety at the indole scaffold was suggested to be prone to metabolism, thus replacement with other residues (e.g. methyl-, amino- ethyl-, or methoxyethyl residues) was performed. Moreover, the linker between the two aromatic moieties was varied regarding length and rigidity. When replacing the benzimidazole by other scaffolds, the relevance of having a hydrogen bond donor in this region was unravelled. The dichloro substitution at the indole scaffold apparently affected the activity against *T. brucei*, as the omission of the lipophilic halogens resulted in a loss of potency. Finally, the structure variation of the primal hit compound **24** did not improve the potency of the compound. Therefore, a pharmacokinetic

study of **24** determining the brain-to-plasma ratio (0.39) was subsequently conducted. Since sufficient drug levels could be reached (31.6 ng g^{-1}) in murine brain, a murine CNS infection model was designed. Ten mice were infected with *T. b. brucei* (GVR 35 strain); five of these animals were administered 40 mg kg^{-1} diminazene aceturate on day 21 as a control. In comparison, **24** was given to the other half of the mice in doses of 20 mg kg^{-1} on day 21–25. Afterwards, the treatment was interrupted for 2 days and was continued with doses of 20 mg kg^{-1} on days 28–32. During the second period of treatment no parasitemia was detected in blood on day 35, however all mice relapsed on day 39.[67] The lack of stage II efficacy was suggested to be related to a high plasma protein binding (>99.9) and a rapid clearance (Cl_{int} = 24.9 µL min^{-1}/ 106 cells in rat hepatocyte). Target identification of **24** was carried out. Due to the structural similarity to previously reported *T. b.* methionyl-tRNA synthetase (MetRS) inhibitors, the inhibition of MetRS was confirmed by means of biochemical (*T. brucei* MetRS inhibition assay), cellular (comparing the reaction of wild-type *T. brucei* to *T. brucei* with upregulated MetRS mRNA), and crystallographic techniques. The binding mode of **24** to MetRs was quite similar to that of reported inhibitors. Furthermore, both the methionine substrate pocket and the auxiliary pocket were addressed by **24** as reported before.[68,69] Additionally, novel interactions within the TbruMetRS complex were shaped, especially the hydroxyethyl substituent made a hydrogen bond to an asparagine in distance of 2.6 Å.[67]



24

Fig. 11: Hit compound from kinase-targeted HTS and its SAR.

Tyrosine kinase inhibitors

The protein tyrosine kinase inhibitor (PTKI) tyrphostin A47 blocks the endocytosis of transferrin and inhibits trypanosome replication. Driven by the fact that the tyrosine kinase inhibitors (TKI) inhibited the growth of *T. b. brucei*, Katiyar *et al.* described the potency of **25** (lapatinib, a TKI applied for cancer) (Fig. 12) in trypanosomes. Since the *T. brucei* genome does not encode for EGFR or HER2, which are considered as actual targets of lapatinib in humans, the mode of action in *T. brucei* was investigated.[70] The addressed targets of lapatinib included three essential tyrosine kinases of *T. brucei* which prompted

to screen further PTKIs against *T. brucei*. Therefore, Behera *et al.* referred to other PTKI (e.g., erlotinib, imatinib, axitinib, and sunitinib) which at least were in an advanced phase of drug development and for which non-toxic profiles and a straightforward synthesis was known.[71] Three PTKI were selected for *in vivo* assessment of which compound **25** exclusively cured 1/4 of the trypanosome-infected mice when administering 100 mg kg^{-1}, po, bid, for 14 days.[71] A supplementary SAR study by Patel *et al.* explored analogues of **25** and could even reveal an increased selectivity due to the replacement of the moiety in position C6 with a 3-morpholinophenyl residue (Fig. 12). However, probably due to the high plasma protein binding survival of the animals could only be prolonged for a few days within *in vivo* experiments.[72] Hence, PTKIs can be considered as an important part in the portfolio of new chemical entities against trypanosomes.[73,74]

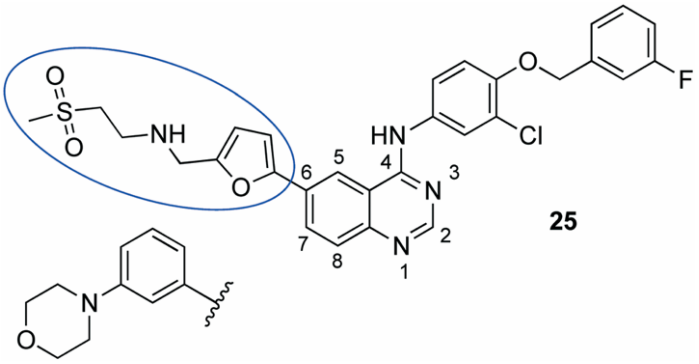
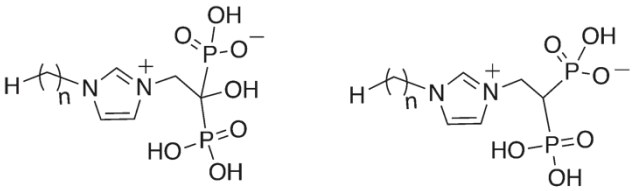


Fig. 12: The human tyrosine kinase inhibitor lapatinib (**25**) and its analogue.

Farnesyl diphosphate synthetase (FPPS) inhibitors

Initially, a library screen of 925 putative prenyl synthase inhibitors against the *T. b. brucei* farnesyl diphosphate synthase (TbFPPS) resulted in bisphosphonate molecules, particularly representing lipophilic analogues of the approved drug zoledronate. Since the FPPS gene was assumed to be essential for the trypanosomal sterol biosynthesis, target validation started to correlate TbFPPS inhibition with the antitrypanosomal activity. Interestingly, for none of the 56 hit compounds inhibition of TbFPPS correlated with the activity against *T. brucei*. However, different targets than the TbFPPS like the squalene synthase or the hexokinase might be addressed by the bisphosphonates which could be advantageous due to a hampered development of resistance. Nevertheless, compounds **26** and **27** (Fig. 13) with a C9 and C7 alkyl side chain, respectively, both exhibited an IC_{50} value of 1.0 µM against *T. brucei* and a CC_{50} values of 301 µM and 64 µM against

hepatocytes, respectively. These two most promising compounds were administered ip into *T. brucei* infected mice over a period of 4 days. A treatment with **26** at a dose of 3 mg kg^{-1}, bid led to a prolongation of life for a maximum of 16 days. Applying a treatment once a day survival was extended for 11 days, while the dose of 1 mg kg^{-1} prolonged life for 6 to 7 days. Compound **27** was not effective as a single dose of 1 mg kg^{-1}, but 3 mg kg^{-1} could extend the survival by a maximum of 8 days.[75]



26 n = 9 **27** n = 7

Fig. 13: Lipophilic bisphosphonates derived from FPPS targeted HTS.

Quinolones

Ciprofloxacin (**28**), an approved fluoroquinolone with broad-spectrum antibacterial activity, showed slight antitrypanosomal effects with an IC_{50} value of 52 µM against *T. b. brucei*.[76] The esterification of ciprofloxacin resulting in **29** (Fig. 14) exhibited a decreased IC_{50} value of 26 µM. However, *in vivo* experiments involving compound **30** (IC_{50} = 6.03 µM *T. b. gambiense*; CC_{50} > 30 µM, 3T6 fibroblast cells) with a single ip dose of 100 µmol kg^{-1} extended survival up to 8 days, but mice could not be fully cured.[77] Hiltensperger *et al.* synthesized novel quinolone amides and tested their activity against *T. brucei*.[78] The SAR studies led to the lead structure **31** which exhibited a promising in vitro activity against *T. b. brucei* (IC_{50} = 47 nM) and *T. b. rhodesiense* (IC_{50} = 9 nM) as well as a notably moderate cytotoxicity (CC_{50} = 57 µM, J774.1 macrophages), resulting in a SI of 6333. In order to overcome the poor water solubility of **31**, an amorphous formulation was developed by spraydrying the compound with Eudragit L100 which was dissolved in a delivery vehicle of glucose (5%), polysorbate 80 (1%), and PBS buffer (pH 7.4) prior to administration. A stringent mouse model was conducted initially starting on day 3 pi with a treatment of 3.5 mg kg^{-1}, bid, ip for 4 days. The resulting mean survival of mice was 17 days. Afterwards, 3/6 of the infected mice were cured by means of an early-treatment model, i.e. begin of treatment one day pi with the same dosage regime, indicating that **31** is a promising lead structure but demands for further improvement.[79]

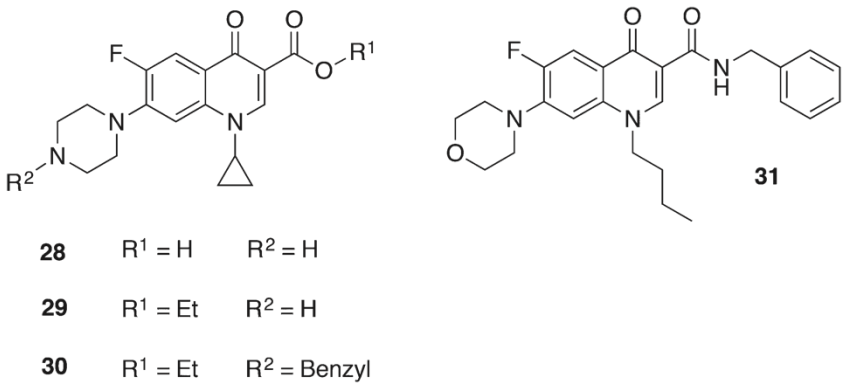


Fig. 14: Quinolone derived compounds.

Bisnaphthalimidopropyl

Bisnaphthalimidopropyl compounds have been evaluated as agents against cancer, bacteria, and Leishmania infections.[80–83] The application of **32** was extended to the kinetoplast-holding *T. b. brucei*, showing an IC_{50} value of 2.35 nM (Fig. 15). Further cytotoxic studies excluded any harmful effects on human cell lines (THP-1 macrophages, hepatocytes and neurons) within therapeutic doses, exhibiting an SI of 2514, 3916, and 878, respectively. Preliminary pharmacokinetic tests demonstrated 95–100% metabolic stability in mouse microsomes, and a 10 mg kg^{-1}, iv injection of **32** achieved plasma levels above the IC_{90} value for 24 h. The ensuing *in vivo* efficacy mice studies of stage I of HAT started 3 days pi by administering 10 or 20 mg kg^{-1}, iv, qd for 6 days. Treatment efficacy was monitored by bioluminescence in luciferase-positive *Trypanosoma* parasites; additionally, parasitemia was assessed. The elevated dose of 20 mg kg^{-1} demonstrated *in vivo* activity and could eliminate the parasite burden during treatment, though infection relapsed when the treatment was stopped at day 9 pi. Graca *et al*. searched for the reason of the lack of efficacy and proposed a potential invasion of parasites into the peritoneal cavity, i.e. into extravascular tissue next to the injection area. Optimization of **32** regarding improved pharmacokinetic and pharmacodynamic properties, especially with efficacy in a mouse model of stage II of HAT, is the future goal.[84] Quarternization of the nitrogen atoms in the middle chain revealed highly active bisnaphthalimido analogues with low cytotoxicities, however these compounds still are too toxic for animal studies.[85,86]

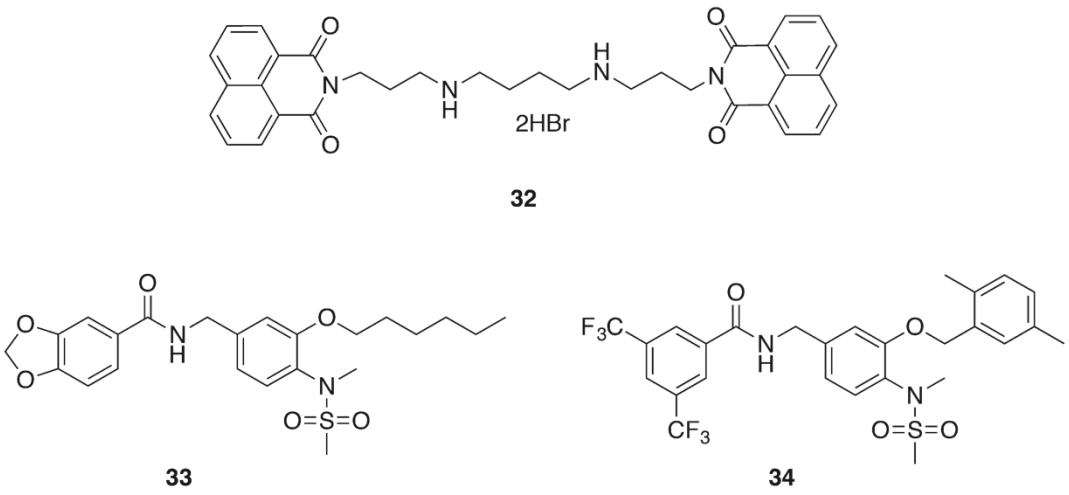


Fig. 15: Bisnaphthalimidopropyl and tubulin inhibitors.

Tubulin inhibitors

Tubulin inhibitors are well established compounds for the treatment of cancer. Tubulin is a crucial molecule in physiological processes involving locomotion and cell division in *T. b. brucei*. Starting with anti-cancer agents, Nanavaty *et al*. succeeded in selectively addressing trypanosomal tubulins.[87] Compounds **33** and **34** (Fig. 15) exhibited IC_{50} values of 0.82 µM and 0.68 µM, respectively, against *T. b. brucei*. The cytotoxic effects were evaluated against macrophages (RAW267.4) and kidney cells (HEK293) and resulted in a SI greater than 102. Therefore, each compound was administered qd to mice with a dose of 400 mg kg^{-1} and 200 mg kg^{-1}, respectively, using oral gavage. The level of parasitemia was determined by taking daily blood samples and counting the number of *T. brucei* cells. The mice relapsed on day 4 and day 3, respectively. Conclusively, the reasons for the inefficacy during the *in vivo* setting were suspected to be a low bioavailability of the compounds which possibly was due to a high first-pass effect or a high binding affinity to serum albumin. However, these highly selective inhibitors of tubulin could be the starting point for more potent inhibitors against *T. b. brucei*.[87]

HSP90 inhibitors

The heat shock protein 90 (HSP90) assists with proper protein folding and the stabilizing of client proteins regulating cell cycle, signal transduction, and receptor formation. For this reason, a couple of HSP90 inhibitors entered various stages of clinical trials against cancer.[88] Kinetoplastids express the HSP90 homologue HSP83 which is considered as an appropriate drug target.[89] Since **35** (17-DMAG) (Fig. 16) showed an IC_{50} value of 3.1 nM

against trypanosomes and a SI of 300 towards L1210 mammalian cells it was advanced into *in vivo* experiments. Administering **35** as a single dose of 150 mg kg^{-1}, ip, led to drop outs due to acute toxicity effects. This was the reason why the dose of 150 mg kg^{-1} was split over 5 days (i.e., 30 mg kg^{-1}, ip, qd). Hence, all mice were free of parasites from day 3 to 30; this implied a full cure (3/3). Additionally, administration by oral gavage for 5 days (50 mg kg^{-1}, qd) cured all but one mouse which died from toxicity and/or gavage-induced trauma. Finally, during the development of novel HSP90 inhibitors it might be valuable to add antitrypanosomal investigations.[88] Interestingly, the HSP90 inhibitor geldanamycin stood out from an independently applied screening by another research group.[59] These outcomes prompted Giannini *et al.* to investigate indicated HSP90 inhibitors for their antitrypanosomal activity (Fig. 14). In the beginning they pointed out that there is no correlation between HSP90 binding affinity (HSP90alpha enzyme assay) and in vitro activity against *T. b. rhodesiense*. An *in vitro* assay with *T. b. rhodesiense* (IC_{50} = 4–22 nM) and cytotoxicity tests with L6 cells (CC_{50} = 146–264 nM) revealed **36**, **37**, and **38** as promising test candidates with SI between 6 and 66. For **36** and **37** parasitemia could be decreased below the detection limit after day 4 by an ip treatment of 5 mg kg^{-1} over 4 days in *T. b. rhodesiense* infected mice. However, all mice relapsed on day 9 and 11, respectively. For compound **38** the effect of toxicity dominated and all mice died on day 2 after the 2nd injection which was not surprising due to the low SI.[90]

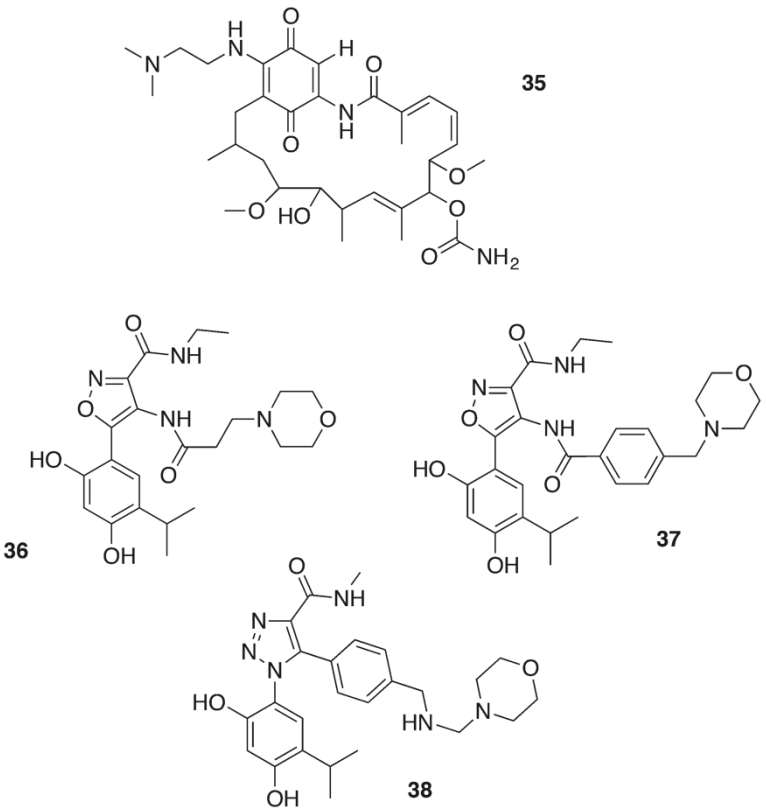
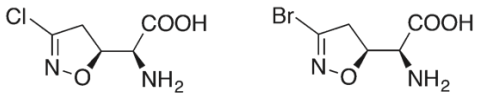


Fig. 16: HSP90 inhibitors.

CTP Synthetase inhibitor

It is known that *T. brucei* has low pools of cytidine triphosphate (CTP) in comparison to mammalian cells.[91] Nevertheless, CTP is essential for cell survival rendering the CTP synthetase (CTPS) an excellent target: the parasites are not able to compensate the inhibition of CTPS by the salvage of cytidine. Compound **39** (acivicin) (Fig. 17), primarily developed as an antitumor agent, showed good inhibitory activity on CTPS but failed in *in vivo* mouse models.[92] The mode of action involves a nucleophilic attack by a thiol group with the displacement of the chlorine atom. Therefore, the halogen atom plays an important role for the inhibitory activity. The exchange of the chlorine in **39** by a bromine atom in **40** (3-bromoacivicin) yielded a 12-fold higher *in vitro* activity against *T. brucei* (IC_{50} = 38 nM versus IC_{50} = 450 nM) while retaining a moderate cytotoxicity (CC_{50} = 12.91 µM versus 15.65 µM, HEK cells). For the *in vivo* studies, three female adult mice infected with *T. b. brucei* S427 were administered 50 mg kg^{-1} ip, for 4 consecutive days, beginning 2 days pi. Parasites could not be detected throughout the treatment days but re-emerged on day 8, hence the studies were discontinued on day 11. Conclusively, compound **40** failed to completely eradicate protozoa *in vivo* at its maximum tolerable dose.[93]



39 **40**

Fig. 17: CTP synthetase inhibitors acivicin and 3-bromoacivicin.

Carbazoles

Most recently, Thomas *et al.* made use of carbazole derived compounds, so-called curaxins, for their drug repositioning program[94] and warranted them to be tested against *T. brucei* for several reasons: most compounds previously showed oral bioavailability, a promising *in vivo* toxicity profile, and were accessible by straightforward synthesis. All advantageous aspects of drug repurposing that are mentioned herein could be utilized. Particularly compound **41** (Fig. 18) is trailed in clinic phase I against solid tumors which might speed up the development for an antiprotozoan drug. According to encouraging *in vitro* data (50% growth inhibitor concentration GI_{50} = 58 nM, *T. b. rhodesiense*; GI_{50} = 55 nM, *T. b. brucei*; CC_{50} = 2100 nM, HeLa cells) compound **41** was advanced in an acute mouse model. *T. b. brucei* infected mice were treated with 40 mg kg^{-1}, po, starting 1 day pi.

All mice (4/4) were cured since there was no detectable parasites in peripheral blood after 30 days. The fact that some curaxins intercalate into DNA prompted the authors to investigate their effect on DNA replication and mitosis. Interestingly, **41** blocked the execution of mitosis but did not affect DNA replication, hence other targets like proteins involved in DNA replication, e.g., CRK3, AUK1, and TLK1, were presumed.[94]

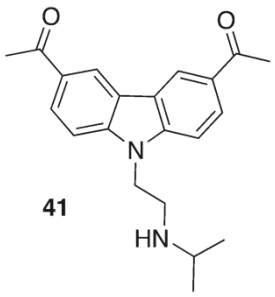


Fig. 18: Carbazole-derived structure.

6. Derived from plants

Medicinal plants are always a rich source for antiinfective drugs. e.g., The methanol extract of *Morinda lucida Benth*. possessed antitrypanosomal activity *in vivo*. Tetracyclic iridoids were identified as the causative agents by bioassay-guided fractionation.[95,96] The most potent compound, the iridoid **42**, (IC_{50} = 0.43 µM, *T. b. brucei*; CC_{50} = 4.7–18.14 µM, various fibroblast cells) was isolated from leaves of *M. lucida*. Administering 30 mg kg^{-1}, ip, for 5 days effectively cured all *T. b. brucei* (TC-221 strain) infected mice (5/5). Additionally, Kwofie *et al.* suggested the induction of apoptosis in trypanosomes to be the mode of action of **42** (Fig. 19) or, more specifically, the inhibition of the expression of paraflagellar rod subunit 2 (PFR-2). The pivotal role of the parasite flagellum both in cell cycle division and the migration is well known. The induced disorder of cell cycle and the flagellum having fragmented nuclei was revealed by means of Nexin assay, Western Blot, and fluorescence-activated cell sorting (FACS) analysis.[97]

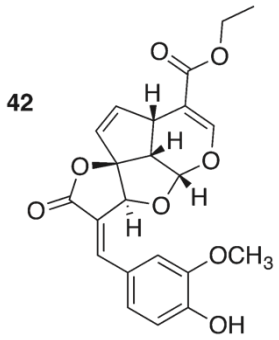


Fig. 19: Tetracyclic iridoid as active compound isolated from *Morinda lucida Benth*.

7. Adjunct: Nagana treatment options

The treatment of Nagana (Animal African Trypanosomiasis, cf. above) is restricted to three available drugs: diminazene diaceturate, ethidium bromide, and isometamidium chloride. Unfortunately, treatment options suffered a setback as drug-resistant strains evolved due to the application of these three drugs for 50 years.[98,99] For this reason, Gillingwater *et al.* recently tested the *in vivo* activity of the well-established diamidines against *T. congolense* and *T. vivax*. Among other diamidines particularly compounds **1** and **2** (Fig. 2) were both fully curative in a mouse model (4/4) when 2.5 mg kg^{-1} (ip, in a single bolus dose) and 1.25 mg kg^{-1}, respectively, were administered. However, a potential cross-resistance to the closely related compounds diminazene aceturate (diamidine) and isometamidium chloride (amidine) should be investigated for *T. congolense* and *T. vivax*.[100]

8. Conclusion

There are various starting points to generate hit compounds for the treatment of African sleeping sickness. Especially stage II of HAT which is very hard to treat poses a tough challenge for drug discovery programs as molecules inevitably need to cross the BBB. However, promising compounds (**2**, **15**, and **17**) are in the pipeline accomplishing these criteria for CNS mouse models, and in some cases even are orally bioavailable (**15** and **17**). Especially the large phenotypic screening campaigns performed by the GNF, GlaxoSmithKline, DDU, and Sykes *et al*. resulted in promising hits discussed herein. Nevertheless, it is not always easy to translate results from *in vitro* studies into *in vivo* efficacy like shown in several of the mentioned studies. The reasons for *in vivo* failures are multilayered and might originate from (I) extensive metabolism, (II) high plasma protein binding, (III) poor water solubility, (IV) efflux transporters, (V) different sensitivity for particular strains, (VI) reduced permeability, and (VII) growth inhibition rather than trypanocidal effects.

Table 1: *In vitro* and *in vivo* data from compounds mentioned herein.

compd	*T. brucei* IC_{50} [μM]	Cytotoxicity CC_{50} [μM]	SI	*in vivo* infection	treatment regime	curation	Lit.
6	0.89[a]	> 213[c]	> 240	*T. b. rhodesiense* STIB900	20 mg/kg, ip, for 4 days, begin 3 days pi	4/4	1
				T. b. brucei GVR35	50 mg/kg, ip, for 5 days, begin 17 days pi	1/5	
7	0.009[a] 0.096[b]	69[c]	7,667	*T. b. brucei* Lab 110 EATRO	10 mg/kg, ip, for 3 days,	3/3	2
				T. b. rhodesiense Ketri 2538	begin 1 day pi	3/3	
8	0.003[a] 0.002[b]	100[c]	33,333	*T. b. brucei* Lab 110 EATRO	5 mg/kg, ip, for 3 days, begin 1	3/3	2
				T. b. rhodesiense Ketri 2538	day pi	3/3	
9	0.002[a] 0.002[b]	43[c]	21,500	*T. b. brucei* Lab 110 EATRO	1 mg/kg, ip, for 3 days, begin 1	3/3	2
				T. b. rhodesiense Ketri 2002	day pi	3/3	
10	0.0077[a]	> 100[d] > 100[e]	> 13,000	*T. b. brucei* Lister 427	3 mg/kg, ip, qd, for 4 days,	5/5	3
11	0.002[a]	0.3[f]	150	*T. b. brucei* S427	12.5 mg/kg, po, bid, for 4 days, begin 3 days pi	5/5	4
				T. b. rhodesiense STIB900	50 mg/kg, po, bid, for 2 days, begin 3 pi	5/5	
				T. b. brucei GVR35	100 mg/kg, po, bid, for 5 days, begin 21 pi	0/5	5
12	0.001[a]	1[f]	1,000	*T. b. brucei* S427	50 mg/kg, po, bid, for 4 days, begin 3 days pi	5/5	6
				T. b. brucei GVR35	100 mg/kg, po, bid, for 5 days	0/5	
					50 mg/kg, po, bid, for 10 days	0/5	
					100 mg/kg, po, bid, for 8 days	1/5	
					100 mg/kg, po, bid, for 3 rounds of 3 day treatment with a two day hiatus between rounds	1/5	
14	0.002[a]	30[e] 24[g]	> 15,000	*T. b. rhodesiense* STIB9000	5 mg/kg, po, bid, for 5 days, begin 2 days pi	5/5	7
15	0.07[a]	> 20[h] > 50[i]	> 286 > 714	*T. b. brucei* GVR35-VSL2	100 mg/kg, qd, oral gavage, for 7 days, begin 21 days pi	6/6	8
16	0.191[a] 0.632[b]	103[c]	539 162	*T. b. rhodesiense* STIB900	50 mg/kg, ip, bid, for 3 days, begin 1 day pi	0	9
						5/5	10

compd	*T. brucei* IC_{50} [μM]	Cytotoxicity CC_{50} [μM]	SI	*in vivo* infection	treatment regime	curation	Lit.
17	0.0348[a]	> 50[g]	> 1,437	*T. b. rhodesiense* STIB900	50 mg/kg, po, bid, for 4 days, begin 2 days pi		
		> 50[e]	> 1,437	*T. b. brucei* TREU667	50 mg/kg, po, bid, for 10 days, begin 21 days pi	5/5	10
18	0.03[a]	> 50[f]	> 1,800	*T. b. brucei* S427	10 mg/kg, ip, bid, for 4 days	2/3	11
19	0.06[a]	> 50[f]	> 860	*T. b. brucei* GVR35	12.5 mg/kg, ip, bid, for 5 days, begin 21 days pi	1/5	
20	0.120[a]	25[j]	213	*T. b. brucei*	20 mg/kg, ip, qd, for 5 days, begin 1 day pi	2/4	12
		25[k]			25 mg/kg, ip, bid, for 10 days, begin 1 day pi	4/4	
21	0.590[a] 0.200[b] 0.01 (*Tbg)*		16.8		data not shown; according to Ref. 1	0/4	14
22	0.0059[a]			*T. b. rhodesiense* STIB900	200 mg/kg, ip, qd, for 4 days, begin 3 days pi	0/4	15
23	0.0163[a] 0.00073[b]			*T. b. rhodesiense* EATRO3	10 mg/kg, ip, qd, for 4 days begin 3 days pi	0/5	16
24	0.00096[a]	12[f]	12,500	*T. b. rhodesiense* TREU164	10 mg/kg, ip, bid, for 4 days - 4 days hiatus- 4 days; begin 3 days pi	4/4	17
				T. b. brucei Lister 427		3/4	
				T. b. brucei GVR35	20 mg/kg, ip, bid, for 4 days - 4 days hiatus- 4 days; begin 3 day pi	0/5	18
25	1.5[a]	> 20[l]	> 13	*T. b. brucei* CA427	100 mg/kg, po, bid, for 14 days, begin 1 day pi	1/4	19
26	1[a]	301[d]	293			11 days max survival	20
27	1[a]	65[d]	62	*T. b. brucei* s427	3 mg/kg, ip, qd, for 4 days	8 days max. survival	
30	6.03 (*Tbg)*	> 30[m]	5	*T. b. brucei* CMP	100 μmol/kg, ip, qd,	0/6	21
31	0.047[a] 0.001[b]	47.2[c] 57.0[i] 26.3[d]	1,004 1,212 560	*T. b. rhodesiense* STIB900	3.5 mg/kg, ip, bid, for 4 days, begin 3 days pi	mean survival 17 days	22
		47.0[e]	1,001		3.5 mg/kg, ip, bid, for 4 days, begin 1 day pi,	3/6	

compd	*T. brucei* IC_{50} [μM]	Cytotoxicity CC_{50} [μM]	SI	*in vivo* infection	treatment regime	curation	Lit.
32	0.0023[a]	9.19[e]	3,916	*T. b. brucei* Lister 427	20 mg/kg, iv, for 6 days, begin 3 days pi 30 mg/kg, ip, qd, begin 1 day pi,	0/4	23
33	0.82[a]	192[d]	234	*T. b. brucei* Lister 427	400 mg/kg, po, qd, 4 days, begin 1 day pi	0/4	24
34	0.68[a]	92.4[d]	136		200 mg/kg, oral gavage, qd, 4 days, begin 1 day pi	0/4	24
35	0.0031[a]	1[n]	300	*T. b. brucei* MiTat1.2 s427	30 mg/kg, ip, qd, begin 1 day pi, 50 mg/kg, po, qd, begin 1 day pi	3/3 2/3	25
36	0.022[b]	0.146[c]	6	*T. b. rhodesiense* STIB900	50 mg/kg, ip, qd, 4 days	relapse on day 11	26
37	0.0018[b]	0.201[c]	11			relapse on day 9	26
39	0.45[a]	15.7[d]	35	*T. b. brucei* TC221	5 mg/kg, ip, qd, for 30 days, begin 5 days pi		27
40	0.038[a]	12.9[d]	340	*T. b. brucei* S427	50 mg/kg, ip, qd, for 4 days, begin 2 days pi	0/3	28
41	0.055[a] 0.058[b]	2.1[e]	38	*T. b. brucei* RUMP528	40 mg/kg, po, qd, total 14 doses (4 days on/2 days off), begin 1day pi	4/4	29
42	0.430[a]	4.7[o] 10.9[p]	11 25	*T. b. brucei* TC-221	30 mg/kg, ip, qd, for 5 days, begin 6 h pi	5/5	30

[a]*T. b. brucei*, [b]*T. b. rhodesiense,* [c]L6 rat myoblasts, [d]HEK293T human embryonic kidney cells, [e]HepG2 human hepatocytes, [f]MRC5 human lung fibroblasts, [g]CRL-8155 human lymphoblasts, [h]3T3 fibroblasts, [i]macrophages, [j]MOLT4 human T lymphoblasts, [k]L929 fibroblasts, [l]HeLa cells, [m]3T6 fibroblasts, [n]L1210 lymphocytic B-cells, [o]skin fibroblasts, [p]lung fibroblasts

Abbreviations

AUC, Area under the curve; BBB, blood–brain barrier; bid, “bis in die”, twice a day; CC_{50}, Half maximal cytotoxic concentration; CNS, Central nervous system; CTP, Cytidine triphosphate; DDU, Drug Discovery Unit; DNDi, Drugs for neglected diseases initiative; EGFR, Epidermal growth factor receptor; GI_{50}, Half maximal growth inhibitor concentration; GNF, Genomics institute of the Novartis research foundation; HAT, Human African trypanosomaisis; HRN, Hepatic cytochrome P450 reductase null; HSP90, Heat shock protein 90; HTS, High throughput screening; IC_{50} Half maximal inhibitory concentration; ip, Intraperitoneal; iv, Intravenous; MDR1, Multidrug-resistance-protein 1; mTOR, Mammalian target of rapamycin; PI3K, Phosphoinositide 3-kinase; pi, Post infection; p.o., per os, orally; SI, Selectivity index, CC_{50}, (mammalian cell line)/IC50 (*T. brucei*); SAR, Structure–activity relationship; *T.b.b.*, *T. brucei brucei*; *T.b.r.*, *T. brucei rhodesiense*; *T.b.g.*, *T. brucei gambiense*; TbFPPS, T. b. brucei farnesyl diphosphate synthase; qd, “Quaque die”, once a day;

References

1 http://www.who.int/mediacentre/factsheets/fs259/en/, (accessed 27.01.17).

2 http://www.webmd.com/a-to-z-guides/trypanosomiasis?page=3, (accessed 27.01.17).

3 D. Steverding, Parasites Vectors, **2008**, 1, 3.

4 A. Stich, A. Ponte-Sucre and U. Holzgrabe, Lancet Infect. Dis., **2013**, 13, 733–734.

5 J. M. Sternberg, Parasite Immunol., **2004**, 26, 469–476.

6 P. G. E. Kennedy, Lancet Neurol., **2013**, 12, 186–194.

7 J. R. Franco, P. P. Simarro, A. Diarra and J. G. Jannin, Clin. Epidemiol., **2014**, 6, 257-275.

8 C. W. Duffy, L. J. Morrison, A. Black, G. L. Pinchbeck, R. M. Christley, A. Schoenefeld, A. Tait, C. M. Turner and A. MacLeod, Int. J. Parasitol., **2009**, 39, 1475–1483.

9 L. J. Morrison, Parasite Immunol., **2011**, 33, 448–455.

10 M. Desquesnes, A. Dargantes, D. H. Lai, Z. R. Lun, P. Holzmuller and S. Jittapalapong, BioMed Res. Int., **2013**, 321237.

11 M. Desquesnes, Livestock trypanosomoses and their vectors in Latin America. OIE (World Organisation for Animal Health), 2004.

12 R. Camargo, A. Izquier, G. L. Uzcanga, T. Perrone, A. Acosta-Serrano, L. Carrasquel, L. P. Arias, J. L. Escalona, V. Cardozo and J. Bubis, Vet. Parasitol., **2015**, 207, 17–33.

13 B. Blacklock and W. Yorke, Proc. R. Soc. London, Ser. B, **1913**, 87, 89–96.

14 R. Brun, H. Hecker and Z. R. Lun, Vet. Parasitol., **1998**, 79, 95–107.

15 R. Brun, R. Don, R. T. Jacobs, M. Z. Wang and M. P. Barrett, Future Microbiol., **2011**, 6, 677–691.

16 G. Eperon, M. Balasegaram, J. Potet, C. Mowbray, O. Valverde and F. Chappuis, Expert Rev. Anti-Infect. Ther., **2014**, 12, 1407–1417.

17 A. S. Nagle, S. Khare, A. B. Kumar, F. Supek, A. Buchynskyy, C. J. Mathison, N. K. Chennamaneni, N. Pendem, F. S. Buckner, M. H. Gelb and V. Molteni, Chem. Rev., **2014**, 114, 11305–11347.

18 R. T. Jacobs, J. J. Plattner, B. Nare, S. A. Wring, D. Chen, Y. Freund, E. G. Gaukel, M. D. Orr, J. B. Perales, M. Jenks, R. A. Noe, J. M. Sligar, Y. K. Zhang, C. J. Bacchi, N. Yarlett and R. Don, Future Med. Chem., **2011**, 3, 1259–1278.

19 S. Patterson and S. Wyllie, Trends Parasitol., **2014**, 30, 289–298.

20 E. Torreele, B. Bourdin Trunz, D. Tweats, M. Kaiser, R. Brun, G. Mazué, M. A. Bray and B. Pécoul, PLoS Neglected Trop. Dis., **2010**, 4, e923.

21 M. Kaiser, M. A. Bray, M. Cal, B. Bourdin Trunz, E. Torreele and R. Brun, Antimicrob. Agents Chemother., **2011**, 55, 5602–5608.

22 A. Tarral, S. Blesson, O. V. Mordt, E. Torreele, D. Sassella, M. A. Bray, L. Hovsepian, E. Evene, V. Gualano, M. Felices and N. Strub-Wourgaft, Clin. Pharmacokinet., **2014**, 53, 565–580.

23 http://www.clinicaltrials.gov/ Identifier: NCT01685827, (accessed 29.01.17).

24 http://www.clinicaltrials.gov/ Identifier: NCT03025789, (accessed 30.01.17).

25 A. Maxmen, Nature, **2016**, 536, 388–390.

26 R. T. Jacobs, B. Nare, S. A. Wring, M. D. Orr, D. Chen, J. M. Sligar, M. X. Jenks, R. A. Noe, T. S. Bowling, L. T. Mercer, C. Rewerts, E. Gaukel, J. Owens, R. Parham, R. Randolph, B. Beaudet, C. J. Bacchi, N. Yarlett, J. J. Plattner, Y. Freund, C. Ding, T. Akama, Y. K. Zhang, R. Brun, M. Kaiser, I. Scandale and R. Don, PLoS Neglected Trop. Dis., **2011**, 5, e1151.

27 http://www.dndi.org/2015/media-centre/press-releases/pr- scyx-7158/, (accessed 28.01.17).

28 C. H. Rios Martinez, F. Miller, K. Ganeshamoorthy, F. Glacial, M. Kaiser, H. P. de Koning, A. A. Eze, L. Lagartera, T. Herraiz and C. Dardonville, Antimicrob. Agents Chemother., **2015**, 59, 890–904.

29 T. Wenzler, D. W. Boykin, M. A. Ismail, J. E. Hall, R. R. Tidwell and R. Brun, Antimicrob. Agents Chemother., **2009**, 53, 4185–4192.

30 M. F. Paine, M. Z. Wang, C. N. Generaux, D. W. Boykin, W. D. Wilson, H. P. De Koning, C. A. Olson, G. Pohlig, C. Burri, R. Brun, G. A. Murilla, J. K. Thuita, M. P. Barrett and R. R. Tidwell, Curr. Opin. Invest. Drugs, **2010**, 11, 876–883.

31 G. Pohlig, S. C. Bernhard, J. Blum, C. Burri, A. Mpanya, J. P. Lubaki, A. M. Mpoto, B. F. Munungu, P. M. N'Tombe, G. K. Deo, P. N. Mutantu, F. M. Kuikumbi, A. F. Mintwo, A. K. Mununga, A. Dala, S. Macharia, C. M. Bilenge, V. K. Mesu, J. R. Franco, N. D. Dituvanga, R. R. Tidwell and C. A. Olson, PLoS Neglected Trop. Dis., **2016**, 10, e0004363.

32 A. H. Harrill, K. D. Desmet, K. K. Wolf, A. S. Bridges, J. S. Eaddy, C. L. Kurtz, J. E. Hall, M. F. Paine, R. R. Tidwell and P. B. Watkins, Toxicol. Sci., **2012**, 130, 416–426.

33 J. K. Thuita, K. K. Wolf, G. A. Murilla, Q. Liu, J. N. Mutuku, Y. Chen, A. S. Bridges, R. E. Mdachi, M. A. Ismail, S. Ching, D. W. Boykin, J. E. Hall, R. R. Tidwell, M. F. Paine, R. Brun and M. Z. Wang, PLoS Neglected Trop. Dis., **2013**, 7, e2230.

34 S. Yang, T. Wenzler, P. N. Miller, H. Wu, D. W. Boykin, R. Brun and M. Z. Wang, Antimicrob. Agents Chemother., **2014**, 58, 4064–4074.

35 W. D. Wilson, F. A. Tanious, A. Mathis, D. Tevis, J. E. Hall and D. W. Boykin, Biochimie, **2008**, 90, 999–1014.

36 J. H. Ansede, R. D. Voyksner, M. A. Ismail, D. W. Boykin, R. R. Tidwell and J. E. Hall, Xenobiotica, **2005**, 35, 211–226.

37 J. K. Thuita, M. Z. Wang, J. M. Kagira, C. L. Denton, M. F. Paine, R. E. Mdachi, G. A. Murilla, S. Ching, D. W. Boykin, R. R. Tidwell, J. E. Hall and R. Brun, PLoS Neglected Trop. Dis., **2012**, 6, e1734.

38 T. Wenzler, S. Yang, D. A. Patrick, O. Braissant, M. A. Ismail, R. R. Tidwell, D. W. Boykin, M. Z. Wang and R. Brun, Antimicrob. Agents Chemother., **2014**, 58, 4452–4463.

39 D. A. Patrick, M. A. Ismail, R. K. Arafa, T. Wenzler, X. Zhu, T. Pandharkar, S. K. Jones, K. A. Werbovetz, R. Brun, D. W. Boykin and R. R. Tidwell, J. Med. Chem., **2013**, 56, 5473–5494.

40 J. J. Vanden Eynde, A. Mayence, M. Mottamal, C. J. Bacchi, N. Yarlett, M. Kaiser, R. Brun and T. L. Huang, Pharmaceuticals, **2016**, 9, 20.

41 G. Yang, W. Zhu, Y. Wang, G. Huang, S. Y. Byun, G. Choi, K. Li, Z. Huang, R. Docampo, E. Oldfield and J. H. No, ACS Infect. Dis., **2015**, 1, 388–398.

42 I. H. Gilbert, J. Med. Chem., **2013**, 56, 7719–7726.

43 I. H. Gilbert, Parasitology, **2014**, 141, 28–36.

44 H. P. Price, M. R. Menon, C. Panethymitaki, D. Goulding, P. G. McKean and D. F. Smith, J. Biol. Chem., **2003**, 278, 7206–7214.

45 S. Brand, L. A. Cleghorn, S. P. McElroy, D. A. Robinson, V. C. Smith, I. Hallyburton, J. R. Harrison, N. R. Norcross, D. Spinks, T. Bayliss, S. Norval, L. Stojanovski, L. S. Torrie, J. A. Frearson, R. Brenk, A. H. Fairlamb, M. A. Ferguson, K. D. Read, P. G. Wyatt and I. H. Gilbert, J. Med. Chem., **2012**, 55, 140–152.

46 J. A. Frearson, S. Brand, S. P. McElroy, L. A. Cleghorn, O. Smid, L. Stojanovski, H. P. Price, M. L. Guther, L. S. Torrie, D. A. Robinson, I. Hallyburton, C. P. Mpamhanga, J. A. Brannigan, A. J. Wilkinson, M. Hodgkinson, R. Hui, W. Qiu, O. G. Raimi, D. M. van Aalten, R. Brenk, I. H. Gilbert, K. D. Read, A. H. Fairlamb, M. A. Ferguson, D. F. Smith and P. G. Wyatt, Nature, **2010**, 464, 728–732.

47 S. Brand, N. R. Norcross, S. Thompson, J. R. Harrison, V. C. Smith, D. A. Robinson, L. S. Torrie, S. P. McElroy, I. Hallyburton, S. Norval, P. Scullion, L. Stojanovski, F. R. Simeons, D. van Aalten, J. A. Frearson, R. Brenk, A. H. Fairlamb, M. A. Ferguson, P. G. Wyatt, I. H. Gilbert and K. D. Read, J. Med. Chem., **2014**, 57, 9855–9869.

48 M. L. Sykes, J. B. Baell, M. Kaiser, E. Chatelain, S. R. Moawad, D. Ganame, J. R. Ioset and V. M. Avery, PLoS Neglected Trop. Dis., **2012**, 6, e1896.

49 T. Bowling, L. Mercer, R. Don, R. Jacobs and B. Nare, Int. J. Parasitol.: Drugs Drug Resist., **2012**, 2, 262–270.

50 M. Njoroge, N. M. Njuguna, P. Mutai, D. S. Ongarora, P. W. Smith and K. Chibale, Chem. Rev., **2014**, 114, 11138–11163.

51 M. L. Sykes and V. M. Avery, J. Med. Chem., **2013**, 56, 7727–7740.

52 H. B. Tatipaka, J. R. Gillespie, A. K. Chatterjee, N. R. Norcross, M. A. Hulverson, R. M. Ranade, P. Nagendar, S. A. Creason, J. McQueen, N. A. Duster, A. Nagle, F. Supek, V. Molteni, T. Wenzler, R. Brun, R. Glynne, F. S. Buckner and M. H. Gelb, J. Med. Chem., **2014**, 57, 828–835.

53 S. Khare, A. S. Nagle, A. Biggart, Y. H. Lai, F. Liang, L. C. Davis, S. W. Barnes, C. J. N. Mathison, E. Myburgh, M.-Y. Gao, J. R. Gillespie, X. Liu, J. L. Tan, M. Stinson,

I. C. Rivera, J. Ballard, V. Yeh, T. Groessl, G. Federe, H. X. Y. Koh, J. D. Venable, B. Bursulaya, M. Shapiro, P. K. Mishra, G. Spraggon, A. Brock, J. C. Mottram, F. S. Buckner, S. P. S. Rao, B. G. Wen, J. R. Walker, T. Tuntland, V. Molteni, R. J. Glynne and F. Supek, Nature, **2016**, 537, 229–233.

54 D. A. Patrick, T. Wenzler, S. Yang, P. T. Weiser, M. Z. Wang, R. Brun and R. R. Tidwell, Bioorg. Med. Chem., **2016**, 24, 2451–2465.

55 D. A. Patrick, J. R. Gillespie, J. McQueen, M. A. Hulverson, R. M. Ranade, S. A. Creason, Z. M. Herbst, M. H. Gelb, F. S. Buckner and R. R. Tidwell, J. Med. Chem., **2017**, 60, 957–971.

56 L. A. Cleghorn, S. Albrecht, L. Stojanovski, F. R. Simeons, S. Norval, R. Kime, I. T. Collie, M. De Rycker, L. Campbell, I. Hallyburton, J. A. Frearson, P. G. Wyatt, K. D. Read and I. H. Gilbert, J. Med. Chem., **2015**, 58, 7695–7706.

57 S. K. Vodnala, T. Lundback, B. Sjoberg, R. Svensson, M. E. Rottenberg and L. G. Hammarstrom, Antimicrob. Agents Chemother., **2013**, 57, 1012–1018.

58 K. T. Andrews, G. Fisher and T. S. Skinner-Adams, Int. J. Parasitol.: Drugs Drug Resist., **2014**, 4, 95–111.

59 A. J. Jones, M. Kaiser and V. M. Avery, Antimicrob. Agents Chemother., **2015**, 60, 1859–1861.

60 D. A. Pasternack, A. I. Sharma, C. L. Olson, C. L. Epting and D. M. Engman, MBio, **2015**, 6, e01291-15.

61 M. Witschel, M. Rottmann, M. Kaiser and R. Brun, PLoS Neglected Trop. Dis., **2012**, 6, e1805.

62 R. Diaz-Gonzalez, F. M. Kuhlmann, C. Galan-Rodriguez, L. Madeira da Silva, M. Saldivia, C. E. Karver, A. Rodriguez, S. M. Beverley, M. Navarro and M. P. Pollastri, PLoS Neglected Trop. Dis., **2011**, 5, e1297.

63 J. D. Seixas, S. A. Luengo-Arratta, R. Diaz, M. Saldivia, D. I. Rojas-Barros, P. Manzano, S. Gonzalez, M. Berlanga, T. K. Smith, M. Navarro and M. P. Pollastri, J. Med. Chem., **2014**, 57, 4834–4848.

64 R. Urich, R. Grimaldi, T. Luksch, J. A. Frearson, R. Brenk and P. G. Wyatt, J. Med. Chem., **2014**, 57, 7536–7549.

65 R. O. Oduor, K. K. Ojo, G. P. Williams, F. Bertelli, J. Mills, L. Maes, D. C. Pryde, T. Parkinson, W. C. Van Voorhis and T. P. Holler, PLoS Neglected Trop. Dis., **2011**, 5, e1017.

66 R. Diaz, S. A. Luengo-Arratta, J. D. Seixas, E. Amata, W. Devine, C. Cordon-Obras, D. I. Rojas-Barros, E. Jimenez, F. Ortega, S. Crouch, G. Colmenarejo, J.

M. Fiandor, J. J. Martin, M. Berlanga, S. Gonzalez, P. Manzano, M. Navarro and M. P. Pollastri, PLoS Neglected Trop. Dis., **2014**, 8, e3253.

67 W. G. Devine, R. Diaz-Gonzalez, G. Ceballos-Perez, D. Rojas, T. Satoh, W. Tear, R. M. Ranade, X. Barros-Alvarez, W. G. Hol, F. S. Buckner, M. Navarro and M. P. Pollastri, ACS Infect. Dis., **2017**, 3, 225–236.

68 S. Shibata, J. R. Gillespie, A. M. Kelley, A. J. Napuli, Z. Zhang, K. V. Kovzun, R. M. Pefley, J. Lam, F. H. Zucker, W. C. Van Voorhis, E. A. Merritt, W. G. Hol, C. L. Verlinde, E. Fan and F. S. Buckner, Antimicrob. Agents Chemother., **2011**, 55, 1982–1989.

69 C. Y. Koh, J. E. Kim, A. B. Wetzel, W. J. de van der Schueren, S. Shibata, R. M. Ranade, J. Liu, Z. Zhang, J. R. Gillespie, F. S. Buckner, C. L. Verlinde, E. Fan and W. G. Hol, PLoS Neglected Trop. Dis., **2014**, 8, e2775.

70 S. Katiyar, I. Kufareva, R. Behera, S. M. Thomas, Y. Ogata, M. Pollastri, R. Abagyan and K. Mensa-Wilmot, PLoS One, 2013, 8, e56150.

71 R. Behera, S. M. Thomas and K. Mensa-Wilmot, Antimicrob. Agents Chemother., **2014**, 58, 2202–2210.

72 G. Patel, C. E. Karver, R. Behera, P. J. Guyett, C. Sullenberger, P. Edwards, N. E. Roncal, K. Mensa-Wilmot and M. P. Pollastri, J. Med. Chem., **2013**, 56, 3820–3832.

73 W. Devine, S. M. Thomas, J. Erath, K. A. Bachovchin, P. J. Lee, S. E. Leed, A. Rodriguez, R. J. Sciotti, K. Mensa-Wilmot and M. P. Pollastri, ACS Med. Chem. Lett., **2017**, 8, 350–354.

74 J. L. Woodring, G. Patel, J. Erath, R. Behera, P. J. Lee, S. E. Leed, A. Rodriguez, R. J. Sciotti, K. Mensa-Wilmot and M. P. Pollastri, MedChemComm, **2015**, 6, 339–346.

75 G. Yang, W. Zhu, K. Kim, S. Y. Byun, G. Choi, K. Wang, J. S. Cha, H. S. Cho, E. Oldfield and J. H. No, Antimicrob. Agents Chemother., 2015, 59, 7530–7539.

76 E. Nenortas, C. Burri and T. A. Shapiro, Antimicrob. Agents Chemother., **1999**, 43, 2066–2068.

77 S. Pomel, F. Dubar, D. Forge, P. M. Loiseau and C. Biot, Bioorg. Med. Chem., **2015**, 23, 5168–5174.

78 G. Hiltensperger, N. G. Jones, S. Niedermeier, A. Stich, M. Kaiser, J. Jung, S. Puhl, A. Damme, H. Braunschweig, L. Meinel, M. Engstler and U. Holzgrabe, J. Med. Chem., **2012**, 55, 2538–2548.

79 G. Hiltensperger, N. Hecht, M. Kaiser, J. C. Rybak, A. Hoerst, N. Dannenbauer, K. Muller-Buschbaum, H. Bruhn, H. Esch, L. Lehmann, L. Meinel and U. Holzgrabe, Antimicrob. Agents Chemother., **2016**, 60, 4442–4452.

80 M. Kopsida, G. A. Barron, G. Bermano, P. Kong Thoo Lin and M. Goua, Org. Biomol. Chem., **2016**, 14, 9780–9789.

81 T. M. Menzel, M. Tischer, P. Francois, J. Nickel, J. Schrenzel, H. Bruhn, A. Albrecht, L. Lehmann, U. Holzgrabe and K. Ohlsen, Antimicrob. Agents Chemother., **2011**, 55, 311–320.

82 J. Tavares, A. Ouaissi, P. Kong Thoo Lin, I. Loureiro, S. Kaur, N. Roy and A. Cordeiro-da-Silva, ChemMedChem, **2010**, 5, 140–147.

83 J. Oliveira, L. Ralton, J. Tavares, A. Codeiro-da-Silva, C. S. Bestwick, A. McPherson and P. K. Thoo Lin, Bioorg. Med. Chem., **2007**, 15, 541–545.

84 N. A. Graca, L. Gaspar, D. M. Costa, I. Loureiro, P. K. Thoo-Lin, I. Ramos, M. Roura, A. Pruvost, I. K. Pemberton, H. Loukil, J. MacDougall, J. Tavares and A. Cordeiro-da-Silva, Antimicrob. Agents Chemother., **2016**, 60, 2532–2536.

85 M. Muth, V. Hoerr, M. Glaser, A. Ponte-Sucre, H. Moll, A. Stich and U. Holzgrabe, Bioorg. Med. Chem. Lett., **2007**, 17, 1590–1593.

86 A. Ponte-Sucre, H. Bruhn, T. Schirmeister, A. Cecil, C. R. Albert, C. Buechold, M. Tischer, S. Schlesinger, T. Goebel, A. Fuss, D. Mathein, B. Merget, C. A. Sotriffer, A. Stich, G. Krohne, M. Engstler, G. Bringmann and U. Holzgrabe, Parasitol. Res., **2015**, 114, 501–512.

87 V. Nanavaty, R. Lama, R. Sandhu, B. Zhong, D. Kulman, V. Bobba, A. Zhao, B. Li and B. Su, PLoS One, **2016**, 11, e0146289.

88 K. J. Meyer and T. A. Shapiro, J. Infect. Dis., **2013**, 208, 489–499.

89 J. C. Pizarro, T. Hills, G. Senisterra, A. K. Wernimont, C. Mackenzie, N. R. Norcross, M. A. Ferguson, P. G. Wyatt, I. H. Gilbert and R. Hui, PLoS Neglected Trop. Dis., **2013**, 7, e2492.

90 G. Giannini and G. Battistuzzi, Bioorg. Med. Chem. Lett., 2015, 25, 462–465.

91 A. Hofer, D. Steverding, A. Chabes, R. Brun and L. Thelander, Proc. Natl. Acad. Sci. U. S. A., **2001**, 98, 6412–6416.

92 A. Fijolek, A. Hofer and L. Thelander, J. Biol. Chem., **2007**, 282, 11858–11865.

93 P. Conti, A. Pinto, P. E. Wong, L. L. Major, L. Tamborini, M. C. Iannuzzi, C. De Micheli, M. P. Barrett and T. K. Smith, ChemMedChem, **2011**, 6, 329–333.

94 S. M. Thomas, A. Purmal, M. Pollastri and K. Mensa- Wilmot, Sci. Rep., 2017, 7, 40565.

95 I. U. Asuzu and C. N. Chineme, J. Ethnopharmacol., **1990**, 30, 307–313.

96 M. Suzuki, N. H. Tung, K. D. Kwofie, R. Adegle, M. Amoa- Bosompem, M. Sakyiamah, F. Ayertey, K. B.-A. Owusu, I. Tuffour, P. Atchoglo, K. K. Frempong, W. K. Anyan, T. Uto, O. Morinaga, T. Yamashita, F. Aboagye, A. A. Appiah, R. Appiah-Opong, A. K. Nyarko, S. Yamaoka, Y. Yamaguchi, D. Edoh, K. Koram, N. Ohta, D. A. Boakye, I. Ayi and Y. Shoyama, Bioorg. Med. Chem. Lett., **2015**, 25, 3030–3033.

97 K. D. Kwofie, N. H. Tung, M. Suzuki-Ohashi, M. Amoa-Bosompem, R. Adegle, M. M. Sakyiamah, F. Ayertey, K. B.-A. Owusu, I. Tuffour, P. Atchoglo, K. K. Frempong, W. K. Anyan, T. Uto, O. Morinaga, T. Yamashita, F. Aboagye, A. A. Appiah, R. Appiah-Opong, A. K. Nyarko, Y. Yamaguchi, D. Edoh, K. A. Koram, S. Yamaoka, D. A. Boakye, N. Ohta, Y. Shoyama and I. Ayi, Antimicrob. Agents Chemother., **2016**, 60, 3283–3290.

98 E. Matovu, T. Seebeck, J. C. K. Enyaru and R. Kaminsky, Microbes Infect., **2001**, 3, 763–770.

99 M. Yaro, K. A. Munyard, M. J. Stear and D. M. Groth, Vet. Parasitol., **2016**, 225, 43–52.

100 K. Gillingwater, C. Kunz, B. C. Braghirolia, D. W. Boykin, R. R. Tidwell and R. Brun, Antimicrob. Agents Chemother., **2017**, 61, e02356-16.

2 Aims and Objectives

The Drug Repurposing strategy, such as testing approved fluoroquinolones against *T. b. brucei* was the starting point of the research efforts herein.[1] Certain quinolones exhibited moderate *in vitro* activity against *Trypanosoma* (e.g., IC_{50} = 70 μM for norfloxacin[1])[2,3], however, *in vivo* efficacy was not achieved.[2] Until this time, the structural variation mainly affected the nitrogen heterocycle in position 7 and the substituent of *N*-1. The carboxylic acid in position 3 was commonly retained since the beta-ketoacid is considered the pharmacophore of the commercial fluoroquinolones. They address the bacterial topoisomerase (DNA Gyrase or topoisomerase IV) and inhibit the DNA replication, and thus finally lead to bacteria cell death.[4]

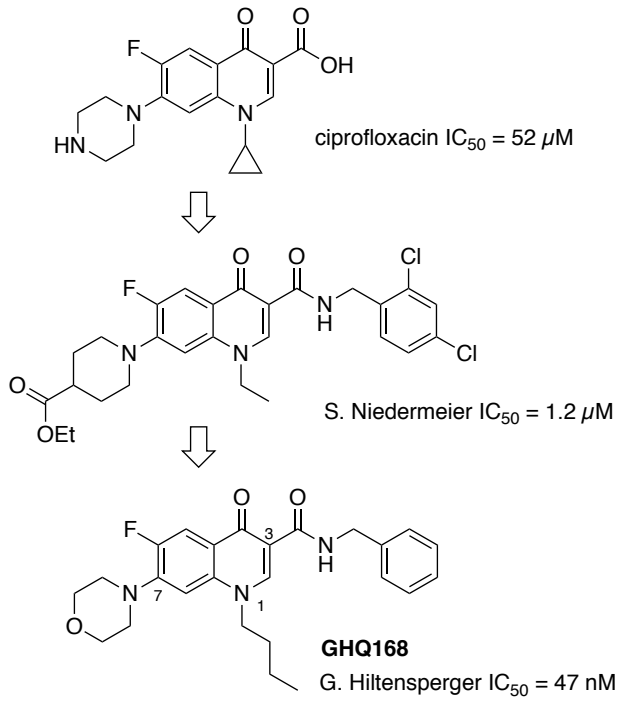


Figure 1: *SAR revealed lead compound* ***GHQ168*** *with the pivotal groups (benzylamide in position 3 and morpholine ring in position 7) for the highest activity against T. b. brucei.*

However, S. Niedermeier revealed a leap in the antitrypanosomal activity when the carboxylic acid is replaced by a benzylamide residue in position 3.[5] This result questioned the mechanism of action, and Hiltensperger *et al.*[6] could consequently exclude the trypanosomal mitochondrial topoisomerase II ($TbTopoII_{mt}$) as main target of the quinolone amides. Furthermore, G. Hiltensperger identified the morpholine ring in position 7 being the most potent substituent with superior activity against *T. brucei* (IC_{50} (*T. b. brucei*) = 47 nM, IC_{50} (*T. b. rhodesiense*) = 9 nM).[7] Providing the *in vivo* proof of concept of the lead compound **GHQ168** was challenging due to poor water solubility. Nevertheless, the administration of **GHQ168** could be achieved by a spray-dried formulation; 50% (3/6) of the mice which were infected with *T. b. rhodesinse* (STIB900) were cured.[8]

The aim of this thesis was to enlarge the substance library of the quinolone amides in order to deepen the insight into the structure-activity relationship. Here, the main focus

should be the modification of the quinolone core. In the course of changing the substitution patterns of **GHQ168**, a special attention was payed to the fluorine atom. The systematically scan of fluorine in different positions ('fluorine walk') should be applied. All synthesised compounds were characterised by their antitrypanosomal activity and cytotoxic potential within the SFB 630; promising candidates with beneficial/ comparable biological properties to **GHQ168** should be examined in physico-chemical experiments (logP, solubility, metabolism). Since poor water solubility still has remained an issue of the newly synthesised quinolone amides, an appropriate formulation for *in vivo* administration should be found. A suitable treatment of HAT stage II inevitably needs to pass the BBB. Therefore, the aim was to examine if the quinolone amides can cross the BBB, and thus could be a treatment option for the late-stage disease. A radiolabelled quinolone amide ought to be synthesised in cooperation with Prof. Dr. Samnick (Department of Nuclear Medicine, University Hospital of Würzburg) and administered to mice. The PET and *ex vivo* autoradiography experiments should demonstrate relevant concentrations of the [^{18}F]-labelled compound in the murine brain.

The second part of the work deals with a scaffold hopping approach: starting from the quinolone amides and concluding in the pyrazoloquinolin-3-ones. The intramolecular hydrogen bond should be replaced by a covalent bond in order to constrain the amide residue. The novel scaffold core should be compared to the parent quinolone skeleton in the scope of biological properties.

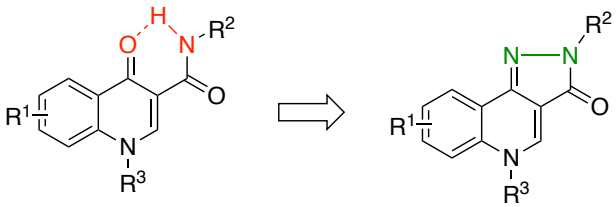


Figure 2: *Scaffold hopping: Conformal restriction of the benzylamide structure in the quinolone amides (left) leading to the pyrazoloquinolin-3-ones (right).*

3 Synthesis of the Fluoroquinolone Amides

3.1 Development of the 4-Quinolones as Antibiotics

In 1939 Gordon Gould and Walter Abraham Jacobs combined two previously developed procedures accomplishing their convenient quinolone synthesis. They applied the aniline condensation with diethyl ethoxymethylenemalonate (DEEMM), initially performed by Claisen[9], and subsequently realised the ring closure according to Camps[10]. However, the Gould-Jacobs reaction gained importance when the antibacterial activity of the quinolones was discovered in 1960.[11] A 7-chloro-quinolone was isolated as an active by-product of the synthesis of the antimalaria drug chloroquine.[11] Interestingly, George Lesher (acknowledged inventor of nalidixic acid, Figure 3) never published the rationale why the quinolone core of the hit compound was switched to the 1,8-naphthyridone scaffold.[12] An educated guess was provided by Bisacchi[12] suggesting that Lesher's research group has just circumvent the prior published patent GB830832[13]. This patent already claimed certain quinolones for their antibacterial activities, which was why Lesher needed to find options laying outside the scope of the GB830832 patent.[12] Thus, the naphthyridone nalidixic acid became a clinical candidate and finally was approved as NegGram®. Until today, the quinolones were modified many times with regard to their antibacterial potency. Just in order to give a little impression about the vast variety, certain representatives are depicted in Figure 3.

Since fluoroquinolones might be associated with serious and permanent side effects (e.g., tendonitis, tendon rupture, QT prolongation, phototoxicity), particular drugs were withdrawn from the market in the past. Most recently, the U.S. Food and Drug Administration reviewed the safety issue of fluoroquinolones due to severe events, and subsequently advised a more restricted use of these antibiotics.[14]

Some structural features that are associated with high antibacterial properties in these commercially available quinolones were adopted for the quinolone amides against *T. b. brucei*.

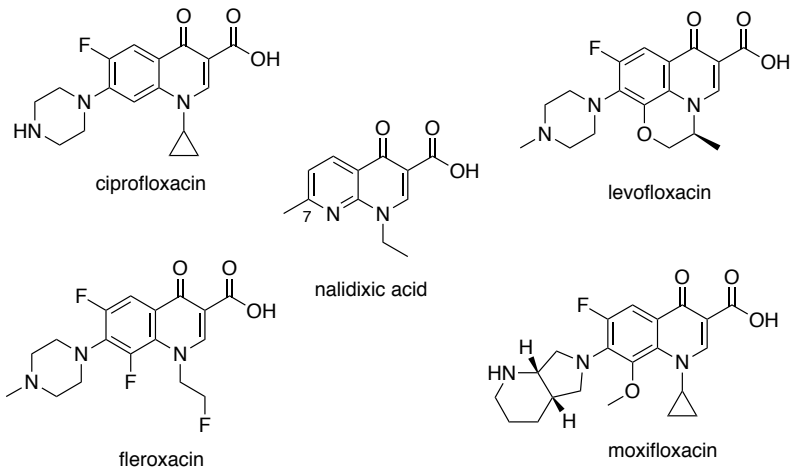


Figure 3: *Representatives of the fluoroquinolone compound class which gave rise to ideas for novel quinolone amides against T. b. brucei in this work. Nalidixic acid was the first one in class.*

3.2 Retrosynthesis of the Lead Compound

In the previous work of S. Niedermeier[5,15] and G. Hiltensperger[6,7], the reaction scheme of the quinolone amide was established. The lead compound **GHQ168** was synthesised according to the depicted retrosynthesis (Figure 4). The synthesis of the benzylamide in position 3 and the introduction of a nitrogen heterocycle in position 7 (e.g., morpholine, **4a**) were the last steps of the procedure. Accordingly, the compound library was conveniently extended by modifying these residues. The *N*-1 position was initially substituted with an *n*-butyl residue and the carboxylic ester was cleaved subsequently, as depicted in the retrosynthesis. The quinolone compound **2a** was obtained by an intramolecular cyclisation of compound **1a**. An aniline compound (e.g., 3-chloro-4-fluoraniline) was used as starting material. However, since the substitution pattern of the quinolone core in position 5, 6, 8 should be modified, different aniline molecules were applied to the reaction.

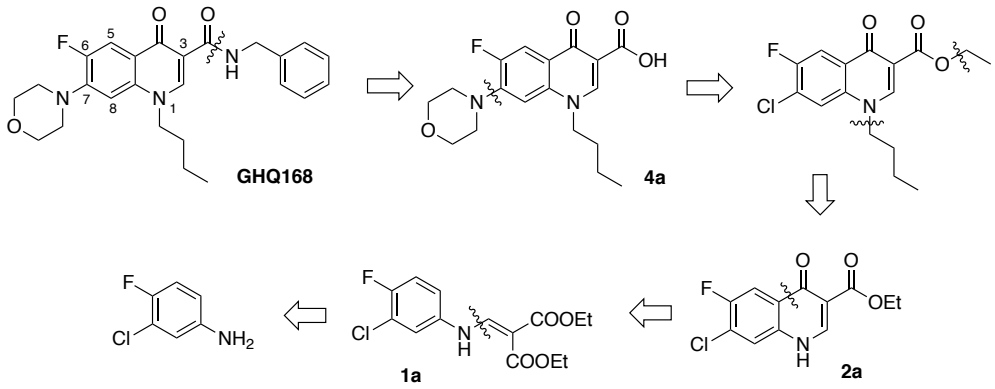


Figure 4: *Retrosynthesis of the quinolone amide **GHQ168** according to S. Niedermeier and G. Hiltensperger.*

3.3 Gould-Jacobs Protocol

The first step towards building up the desired quinolone scaffold is the reaction of aniline derivatives with diethyl ethoxymethylenemalonate (DEEMM) to give diethyl malonate molecules (Figure 5). Since there is a wide diversity of available aniline molecules, certain variation could easily be introduced to the quinolones. However, the particular modifications of the quinolone core are commonly accompanied with the new start of the Gould-Jacobs procedure. The aniline molecules were commercially purchased with consideration for the prospective substitution patterns of the quinolone core. For later modification reactions in the synthesis pathway, it was inevitably necessary to have a halogen atom as an appropriate leaving moiety in position 7 of the quinolone.

The isolated diethyl malonates were cyclised to the ethyl-4-hydroxyquinoline-3-carboxylate. As the ring cyclisation requires high temperature conditions, adequate high boiling solvents like diphenyl ether were used (Figure 5).

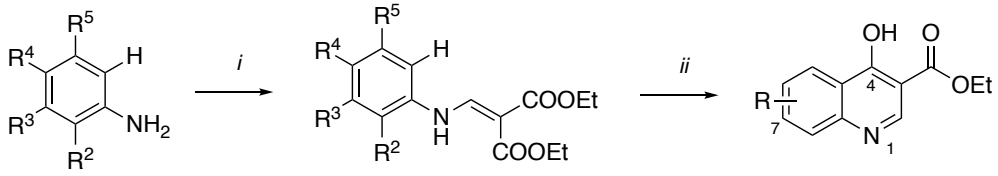


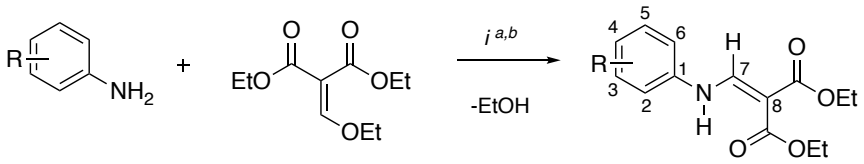
Figure 5: *Gould-Jacobs procedure; reagents and reaction conditions: i) DEEMM, toluene, 110 °C; ii) diphenyl ether, > 200°C;*

3.3.1 Synthesis of the Anilinomethylenmalonic Esters

As depicted in Figure 6, the aniline derivative reacted with the diethyl malonate (DEEMM), as originally reported by Claisen *et al.*[9] First, the aniline nitrogen reacts with the ethoxymethylene moiety, followed by an ethanol elimination, to give the anilinomethylenemalonic esters **1a-i**. According to Levya *et al.*[16], the reaction takes place in boiling toluene. Ethanol released during the reaction and high yields could be accomplished (60-98%).

Referring to Hiltensperger[7] reaction time (1-3 h) could be vastly shortened with consistent satisfying yields (82-94%) using microwave supported reaction for **1a**, **1b**, **1d**, **1e**, **1g**. Since apolar solvents (e.g., toluene) exhibit poor absorption capacity for microwave irradiation, *Weflon*® plates were added to the reaction solution. The ^{1}H NMR analysis of the enamines **1a-i** confirmed the intramolecular hydrogen bond between the carbonyl

oxygen and the *N*-H concurrent to literature.[17] The vicinal coupling constant of $^2J_{NH\text{-}H7}$ = 12 - 16 Hz for the =CH-NH group indicated a dihedral-angle of ca. 150-180 °C, which was in accordance with Karplus equation for antiperiplanar conformation.[18]



DEEMM **1a-i**

Compound / *Substituent R^x*	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**	**1i**
R^2	H	F	H	F	H	H	H	H	H
R^3	Cl	Cl	F	F	F	Br	F	Cl	H
R^4	F	H	H	F	F	H	CF_3	OCH_3	OCH_3
R^5	H	H	F	H	F	H	H	H	H
Yield [%]	94	94	98	86	82	94	86	75	60
Time [h]	2[a]	3[a]	20[b]	1[a]	3[a]	24[b]	2[a]	24[b]	20[b]

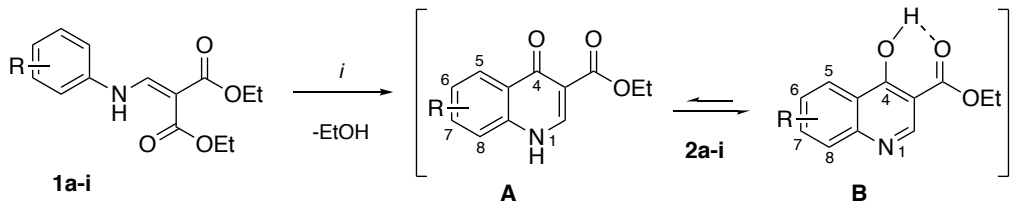
Figure 6: *Synthesis and yields of the anilinomethylenmalonic esters (**1a-i**). R^x with x is the atom number in **1a-i**. Reagents and reaction conditions: i) toluene, 110 °C; [a]microwave irradiation; [b]conventionally oil bath heating.*

3.3.2 Synthesis of the Ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylates

In a subsequent intramolecular electrophilic aromatic substitution (S_EAr), the C-6 carbon atom of the anilinomethylenemalonic ester (**1a-i**) attacked the carboxylic ester carbon to obtain the desired ring closure. High temperatures were essential to introduce this reaction, thus the cyclisation was performed in diphenyl ether.[16] Hiltensperger *et al.*[7] transferred the classical reaction approach to a microwave system, which was applied for the following reactions. Hence, the reaction was accomplished within maximum 90 min, and gave satisfying yields (35-91%). The resulting compounds ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (**A**) and its tautomer ethyl-4-hydroxyquinoline-3-carboxylate (**B**) are depicted in Figure 7.

According to literature[19], there is a higher prevalence of the enol tautomer (**B**) due to the intramolecular hydrogen bond stabilisation effects. However, in the ^{1}H NMR spectrum of compound **2d** for instance, the *N*-H signal emerged at δ = 12.77 ppm. Hence, the

corresponding 4-oxo tautomer (**A**) is obviously predominantly present in DMSO-d_6, since solvents of high polarity compete with the intramolecular formed hydrogen bond in the 4-hydroxy tautomer (**B**).



Compound / ***Substituent R^x***	**2a**[7]	**2b**[20]	**2c**[21]	**2d**[22]	**2e**[23]	**2f**[24]	**2g**[25]	**2h**[26]	**2i**[27]
R^5	H	H	F	H	F	H	H	H	H
R^6	F	H	H	F	F	H	CF_3	OCH_3	OCH_3
R^7	Cl	Cl	F	F	F	Br	F	Cl	H
R^8	H	F	H	F	H	H	H	H	H
Yield [%]	77	70	91	43	92	69	35	55	51
Time [min]	70	60	15	90	10	30	20	20	45

***Figure 7**: Yields and reaction times of the synthesised ethyl-4-oxo-1,4-dihydroquinoline-3-carboxyalte derivatives (**2a-i**). R^x with x is the atom number in **2a-i**. Reagents and reaction conditions: i) diphenyl ether, MW, 210 °C.*

Whenever a hydrogen atom is in position 2 of the anilinomethylenemalonic esters (**1a**, **1c**, **1e-i**) a bidirectional ring closure occurrs (Figure 8). Thus, Gould-Jacobs reaction suffers from regioselectivity. Nevertheless, two different regioisomers solely emerged with asymmetric anilinomethylenemalonic esters (**1a**, **1f-1h**). In these particular cases, the isomers of **2a**, **2f-2h** were not separated at the respective stage of the synthesis due to poor solubility. The desired isomers were isolated after alkylation of position *N*-1 via column chromatography (3.4.1). Remarkable short reaction times and high yields could be achieved with compounds **1c** and **1e** (91% in 10 min and 92% in 15 min, respectively), as position C-6 and C-2 are both involved in cyclisation reaction but afford the very same product (Figure 8).

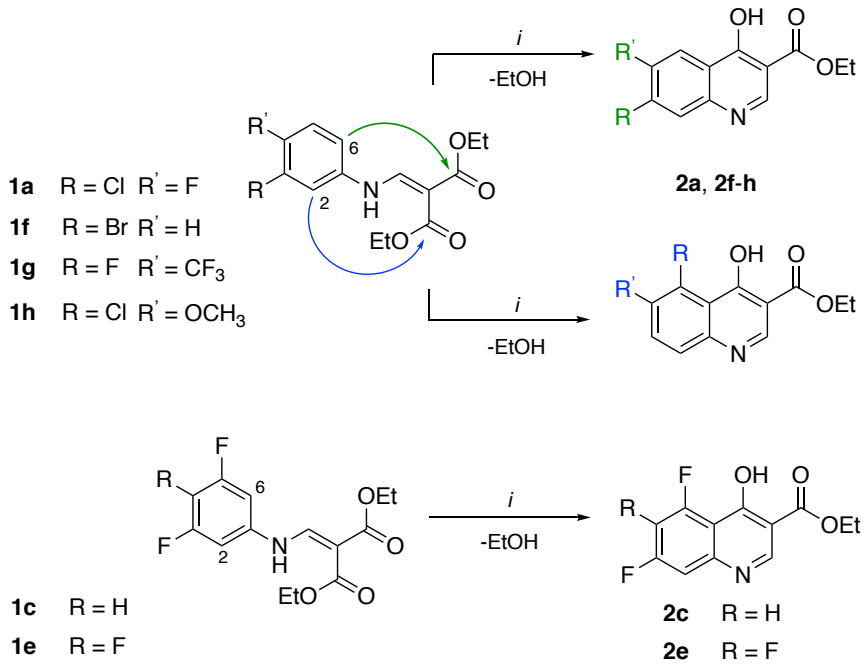


Figure 8: *Cyclisation with anilinomethylenmalonic esters having asymmetric (**1a**, **1f**-**1h**) and symmetric substitution patterns (**1c** and **1e**). In the first-mentioned case, two regioisomeres emerged. Reagents and reaction conditions: i) diphenyl ether, MW, 210°C.*

3.3.3 Synthesis Approach for the 1,8-Naphthyridine-3-carboxylic Acid

The first antibacterial quinolone nalidixic acid (Figure 3) was synthesised by Lesher *et al.*[11] using the Gould-Jacobs approach. The pyridine-2-amine (R = CH_3[11], H, Cl) was successfully reacted with DEEMM according to previous protocol (3.3.1).

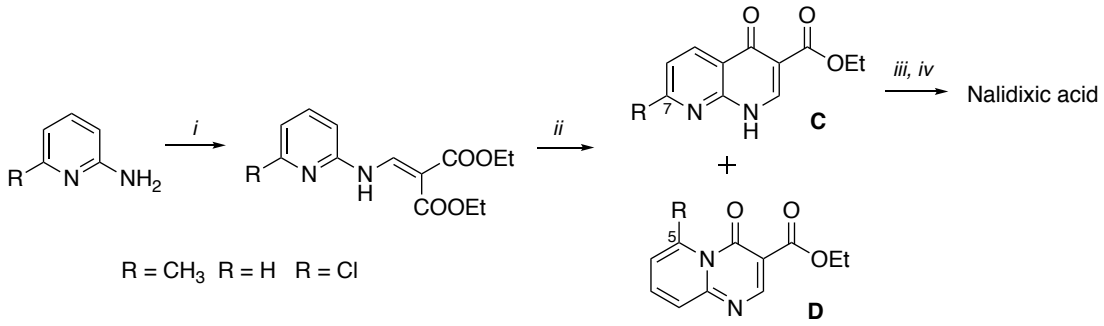


Figure 9: *Synthesis of the 1,8-naphthyridine-3-carboxylic acid via the Gould-Jacobs synthesis according to reference 21. Reagents and reaction conditions: i) DEEMM, toluene, 110 °C; ii) diphenyl ether, MW, 210 °C; iii) ethyl iodide, K_2CO_3, abs. DMF; iv) acidic/ basic hydrolysis.*

The ensuing ring closure with substituent bearing an alkyl residue (R = CH_3, CH_2CH_3) selectively gives **C** in a yield up to 90%.[28] However, applying the cyclisation protocol (3.3.2) to other reagents (e.g., R = H), solely isomer **D** could be isolated which was consistent with the literature (94% of **D**).[29] Steric hindrance could be considered a reason for the exclusive emergence of the regioisomer **C**. Afterwards, the 1,8-naphthyridone was alkylated with ethyl iodide and K_2CO_3. In a second step the carboxylic ester was saponified to the corresponding carboxylic acid affording the nalidixic acid.[11]

Since an appropriate leaving group (e.g., halogen) in position 7 was expedient, the 6-chloropyridine-2-amine (R = Cl) was utilized as starting material. The 'bulky' chlorine substituent should rather afford isomer **C**. Unfortunately, neither isomer **C** nor **D** could be isolated after cyclisation reaction in diphenyl ether. Presumably, the halogen (R = Cl) took part in some unwanted side reactions leading to decomposition during ring closure as it was proposed for R = Br.[30] Due to failure of the 1,8-naphthyridone synthesis via Gould-Jacobs, the Grohe-Heitzer procedure was applied for this purpose (3.8.1).

3.4 Alkylation and Hydrolysis to the 1-Butyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acids 3a-l

3.4.1 Synthesis of the 1-Butyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

According to Koga *et al.*[20], the ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylate was dissolved in abs. DMF and the vinylogous amide *N*-H was deprotonated by means of K_2CO_3 (4-5 eq). Hereafter, two functional nucleophiles were obtained due to mesomeric formulas presented in Figure 10, i.e. the *N*-1 atom and the enolate oxygen. Under basic conditions the intermediate **G** is most likely to occur because the negative charge is well stabilized through the beta-keto ester.[31] Referring to the HSAB concept[32], the 'soft' anion **G** selectively reacts with the 'soft' electrophile (e.g., alkyl bromides) in an orbital-controlled reaction to the *N*-alkylated product. In comparison, the 'hard' nucleophile **F** preferably reacts in a charge-controlled *O*-alkylation reaction.[31]

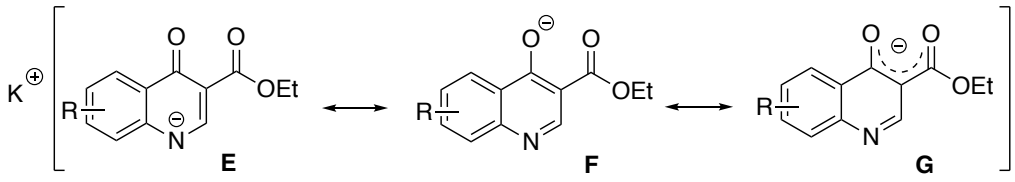
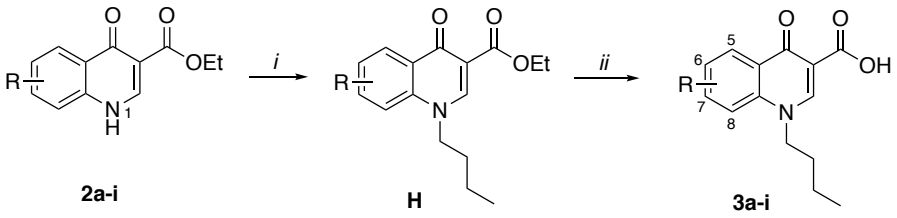


Figure 10: *Deprotonated 4-oxo-1,4-dihydoquinoline with mesomeric formulas **E**, **F**, and **G**.*

Hence, the *N*-1 position of compounds **2a-i** were selectively reacted with *n*-bromobutane and catalytic amount of KI. Finally, the *N*-alkylated 4-oxo-1,4-dihydroquinolin-3-carboxylic acid esters (**H**) were obtained after column chromatography. The two regioisomers emerged from prior cyclisation reaction (3.3.2, concerning **2a**, **g-h**) could be separated at this point of the synthesis. The intermediate ester **H** was subsequently hydrolysed to the corresponding carboxylic acid using HCl and KOH solution, respectively. The medium of choice for the hydrolysis was dependent on the susceptibility of the quinolone core for a nucleophilic attack. Preventing particular side reaction, the ester was hydrolysed in acidic conditions (**3b-e**, **3g**). On the contrary, compounds **3a**, **3f-i** were obtained by refluxing in basic conditions.



Compound / ***Substituent R^x***	**3a**[7]	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**
R^5	H	H	F	H	F	H	H	H	H
R^6	F	H	H	F	F	H	CF_3	OCH_3	OCH_3
R^7	Cl	Cl	F	F	F	Br	F	Cl	H
R^8	H	F	H	F	H	H	H	H	H
Yield [%]	76	55	88	62	28	68	68	55	73

Figure 11: *Overall yields of the synthesised 1-butyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid derivatives (**3a-i**). R^x with x is the atom number in **3a-i**. Reagents and reaction conditions: i) n-bromobutane, K_2CO_3, KI, abs. DMF, 70-90 °C; ii) acidic/basic hydrolysis.*

3.4.2 Side Reactions throughout the Alkylation and Hydrolysis Reactions

During the course of alkylation and hydrolysis (3.4.1), particular side reactions were observed. Applying the protocol of Koga *et al.*[20] led to unexpected products due to susceptibility for displacement reactions. Positions 5 and 7 are particularly prone to aromatic nucleophilic substitution (S_NAr) with *O*- and *N*-nucleophiles. The electron-withdrawing carbonyl moiety in position 4 induces certain resonance stabilization (Figure

17). However, particular quinolones favour the S_NAr more than others, apparently depending on their individual substitution patterns.

3.4.2.1 Synthesis of the 1-Butyl-7-ethoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid

In the hydrolysis step of compound **2a** for instance, no undesired nucleophilic displacement reactions neither in position 6 nor in position 7 have been observed yet. In contrast to that, under basic conditions the cleaved ethanolate of compound **I** attacked position 7 in a nucleophilic aromatic sequence, and the undesired side product **3j** emerged in a yield of 70% (Figure 12). A possible explanation for certain reactivity could be given by the 'element effect'.[33] The highly polarized C-F bond promotes the addition at the carbon atom, the rate determining step of the S_NAr reaction. Consequently, among the halogens, fluorine is displaced most rapidly in the S_NAr (reactivity order: F > Cl ≈ Br > I).[33,34] The ensuing elimination of the leaving group, however, does not significantly influence the reaction rate. Moreover, the two fluorine atoms in direct vicinity (position 6 and 8) bearing electron-withdrawing inductive effects, might rise the susceptibility for substitution reactions of **I**. Hence, potential nucleophiles (hydroxyl ions or emerging ethanolate ions through ester cleavage) should be generally avoided, in order to prevent the mentioned side reactions. This is the reason why acidic conditions were applied for certain compounds (**3b-e**, **3g**).

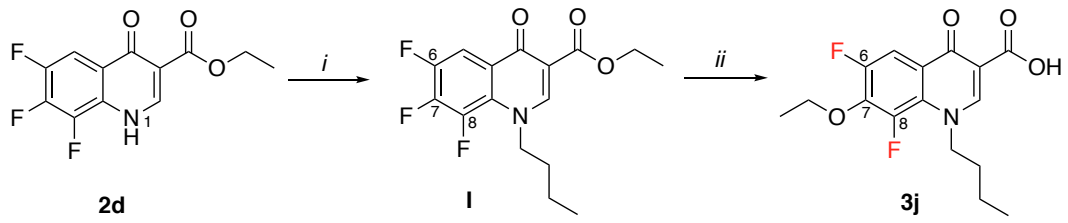


Figure 12: *Undesired displacement reaction in position 7 of the nascent ethanolate-ion. Reagents and reaction conditions: i) n-bromobutane, K_2CO_3, KI, DMF, 90 °C; ii) 3 M aqueous KOH, reflux.*

At the first glance, reaction control of the hydrolysis prompted to think just isolating the starting material (**2d**) again, as the signals of the ethyl ester were still apparent in the ^{1}H NMR spectrum. However, a closer look revealed that the signal splitting of H-5 did not comply with a 6,7,8-trifluoro substituted quinolone. The resulting doublet of a doublet rather reflects the coupling between H-5 and F-6 ($^3J_{H,F}$ = 11.2 Hz) and F-8 ($^5J_{H,F}$ = 2.4 Hz), respectively. For comparison, the 6,7,8-triffluoro quinolone **2d** generated the following signal splitting: ddd ($^3J_{H,F}$ = 10.7 Hz, $^4J_{H,F}$ = 8.2 Hz, $^5J_{H,F}$ = 2.4 Hz) for H-5. Additionally,

mass spectroscopy revealed a *m/z* = 326.3 $[M+H]^+$ which was consistent with the postulated compound **3j**. For final clarification, ^{19}F NMR (^1H decoupled) experiments of **3j** (red) were consulted in comparison to the desired compound **3d** (purple) (Figure 13). Both fluorine atoms F-6 (d, δ = -126.84 ppm) and F-8 (d, δ = -135.06 ppm) coupled to each other and a doublet ($^4J_{6,8}$ = 10.1 Hz) emerged. In turn, **3d** generated three signals: F-6 (δ = -134.44 ppm) and F-8 (δ = -140.68 ppm) made not much of a difference in the splitting pattern (doublet of a doublet), since there is a large ($^3J_{6,7}$ = 24.8 Hz; $^3J_{7,8}$ = 20.0 Hz) and a rather small coupling constant ($^4J_{6,8}$ = 6.9 Hz). The doublet of a doublet assigned to F-7 (δ = -149.05 ppm), nearly coincide to an apparent triplet, as the coupling constants are fairly similar.

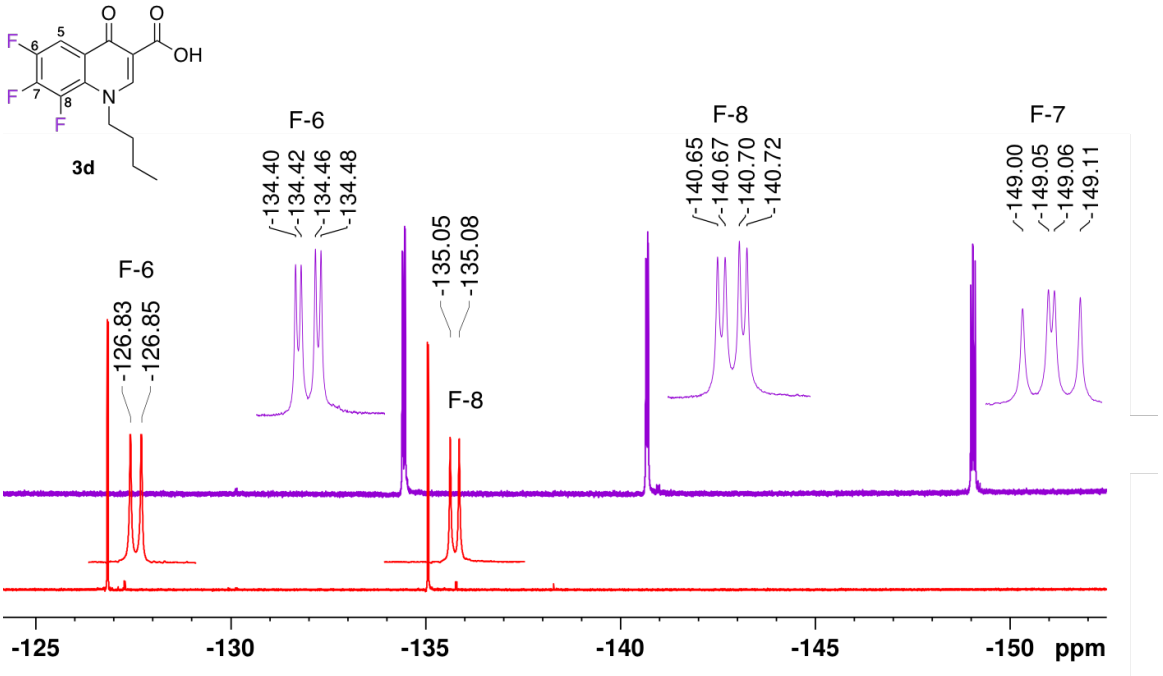
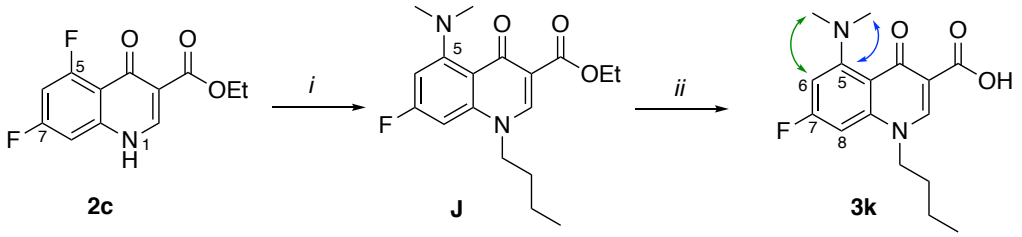


Figure 13: *^{19}F (^1H decoupled) NMR of the undesired compound **3j** (red) after basic hydrolysis and the requested compound **3d** (purple) after acidic hydrolysis of intermediate **I**.*

3.4.2.2 Synthesis of 1-Butyl-5-(dimethylamino)-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid

Additionally, particular attention should be paid to the purity and deterioration of DMF. When using non-freshly distilled DMF as solvent for **2c**, the degradation product *N,N*-dimethylamine underwent a displacement reaction at the quinolone core **J** (Figure 14). The mass spectroscopy confirmed a *m/z* = 307.3 $[M+H]^+$, but position 5 and position 7 are both potential reactive sites for S_NAr.[35] Here, once more the heteronuclear coupling

between fluorine and protons in the ^{1}H NMR spectrum facilitated the elucidation of the respective positional isomer.



2c **J** **3k**

Figure 14: *Undesired S_NAr reaction with the degradation product N,N-dimethylamine in position 5. Reagents and reactions conditions: i) n-bromobutane, K_2CO_3, KI, DMF, 90 °C; ii) 2 M HCl, reflux.*

The aromatic protons of **3k** gave a doublet of a doublet with a rather large and a small coupling constant (H-6: $^{3}J_{H,F}$ = 11.2 Hz, $^{4}J_{H,H}$ = 2.4 Hz; H-8: $^{3}J_{H,F}$ = 12.7, $^{4}J_{H,H}$ = 2.4 Hz), which suggested that there was no fluorine in position 5 anymore, but in position 7. Moreover, a subsequent two-dimensional heteronuclear multiple bond correlation (HMBC), displayed the proximity of the *N*-methyl groups to C-5 and C-6, respectively (Figure 15). The spectroscopic data were consistent with compound **3k**, which was afforded in a yield of 18%. This was a first hint, that C-5 might be privileged over C-7 for S_NAr. Hence, this should be considered in following displacement reactions affecting compounds **3c** and **3e** (3.5). Moreover, the S_NAr also succeeded with alcoholates as nucleophiles. Hence, in an intended S_NAr reaction of position 5, methanol was treated with sodium hydride and smoothly yielded compound **4l** (3.5.3).

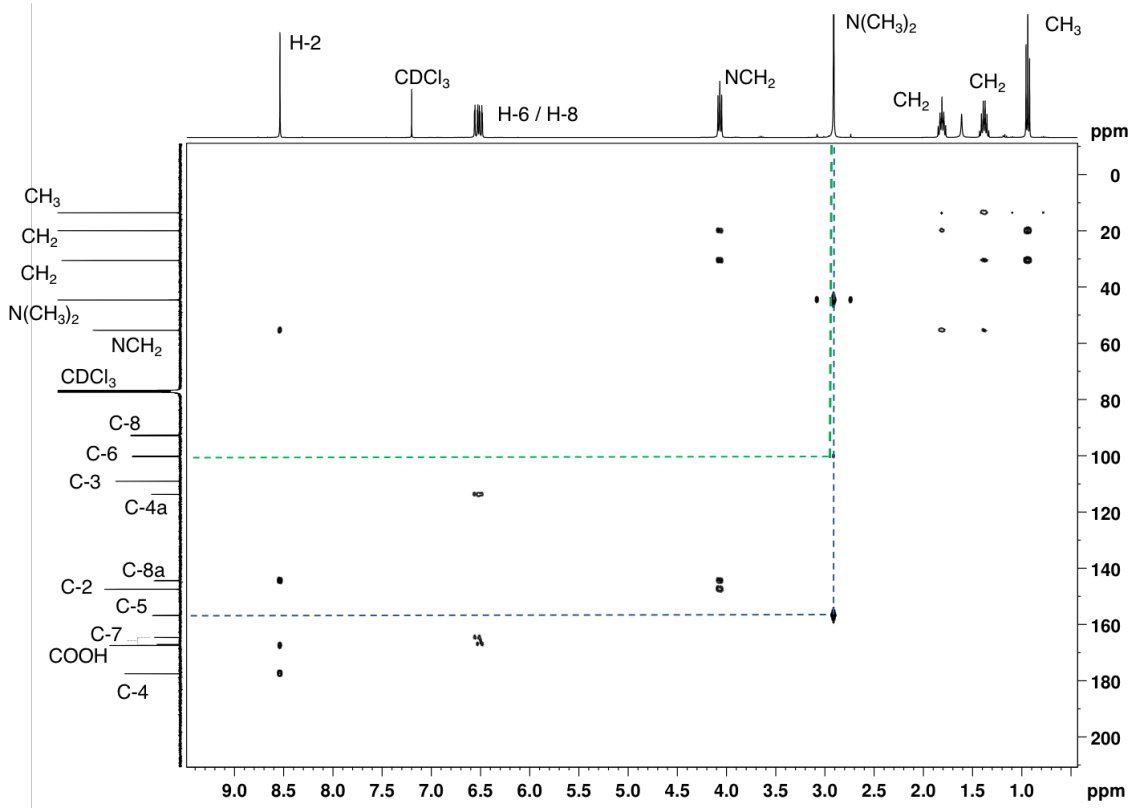
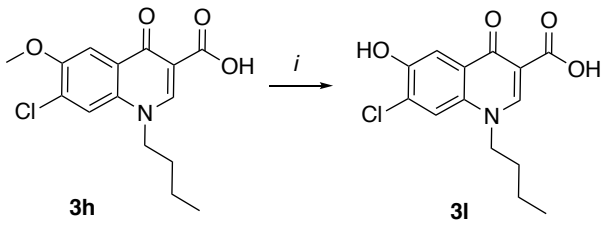


Figure 15: *Heteronuclear multiple bond correlation (HMBC) of* ***3k*** *illustrates the vicinity of N(C**H**3)2 to **C**-5 (blue) and **C**-6 (green), respectively.*

3.4.3 Derivatization of the 1-Butyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acids

After alkylation the *N*-1 position, the quinolone core was subsequently modified. First, the methoxy ether of **3h** was cleaved to the corresponding phenol. Since we have little information about the importance of the substituent in position 6 for the antitrypanosomal activity, it was tried to diversify this site. Therefore, the ether of compound **3h** was cleaved using boron tribromide (1 M solution in CH_2Cl_2). The broad signal in the ^1H NMR experiment of δ = 11.25 ppm was assigned to the phenolic alcohol group in **3l**.



3h **3l**

Figure 16: *Synthesis of derivative* ***3l*** *via an ether cleavage. Reagents and reaction conditions: i) BBr_3 (1 M in CH_2Cl_2), CH_2Cl_2, 0 °C-rt.*

Secondly, it was tried to introduce a nitro group to the quinolone skeleton, preferably in position 6. For this purpose, an electrophilic aromatic substitution (S_EAr) with **3f** was pursued. The protocol according Suzuki *et al.*[36] was applied, using a mixture of *conc.* sulfuric acid and *conc.* nitric acid (ratio 1:1). As depicted in Figure 17, the quinolone core **3f** is slightly deactivated for S_EAr because of the electron withdrawing carbonyl moiety in C-4, decreasing the electronic density in position 5 and 7 (*ortho* and *para*). Hence, C-6 and C-8 (*meta*) is worth to be considered for electrophilic substitution. The bromine atom in position 7 also weakly deactivates the S_EAr reaction by its –I effect. However, due to the predominant +M effect of bromine, positions 6 and 8 are still preferred for the reaction.

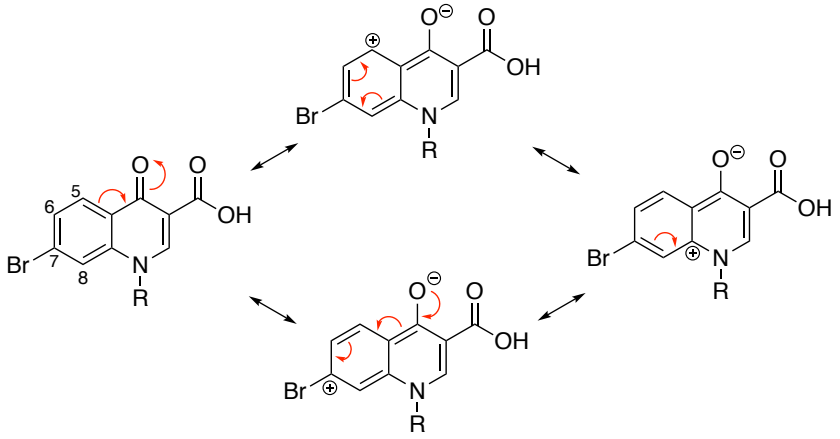


Figure 17: *Electronic densities of the quinolone **3f** emerging due to the electron withdrawing (-M effect) carbonyl moiety.*

To overcome the particular deactivating effects of the substituents, the reaction heated at 60 °C. Thus, Figure 18 illustrates that the *in situ* generated nitronium ion reacts at both sites, obtaining the nitrated quinolones **3m** (6-nitro quinolone) and **3m'** (8-nitro quinolone). The two isomers were separated by means of column chromatography, and the compounds **3m** (Yield 35%) and **3m'** (Yield 21%) could be isolated.

In contrast to the S_EAr, the quinolone core is generally activated for S_NAr in position 5 and 7, when bearing an appropriate leaving group in the particular sites.

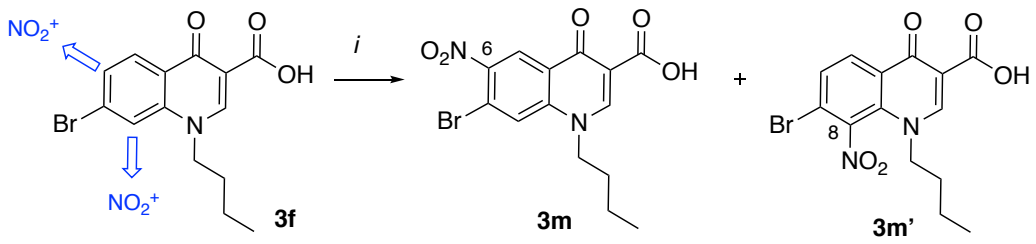


Figure 18: *The nitration of **3f** gave the 6-nitro quinolone **3m** and the 8-nitro quinolone **3m'**. Reagents and reaction conditions: i) conc. H_2SO_4, conc. HNO_3, 0 °C-60 °C.*

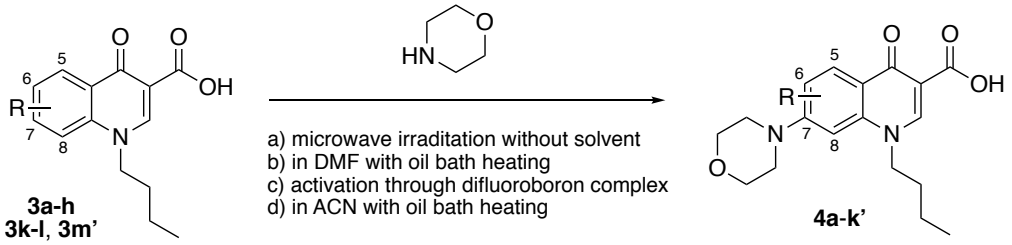
3.5 Nucleophilic Aromatic Substitution (S_NAr) of the 1-Alkyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acids

20 years after the initial discovery of nalidixic acid Koga *et al.*[20] achieved a significant breakthrough in antibiotic therapy introducing saturated nitrogen heterocycles in position 7. Since Hiltensperger *et al.*[7] ascertained the morpholine ring with prominent antitrypanosomal activity, the quinolones **3a**-**h**, **3k**-**l**, **3m'** were substituted with this particular residue, which is described herein. If C-7 carried halogen atoms (Cl, Br or F), the displacement reaction was conveniently accomplished to generate versatile analogues. For the reaction sequence, it is expedient to cleave the carboxylic ester in position 3 prior to the aromatic displacement reaction. Otherwise, the respective amide could be formed as side product.

3.5.1 Synthesis of the 1-Butyl-7-amino-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid

Since Hiltensperger[7] observed the time saving benefit of microwave assisted reactions (4-5 h), the synthesis of **4a**, **4f-h**, **4j-k'** was performed in the microwave with morpholine at 110 °C. Morpholine simultaneously acted as solvent and reactant, and afforded yields of 29-89%.

This straightforward microwave supported approach was initially applied for most of the quinolone derivatives, but gradually limitations regarding regioselectivity and feasibility occurred. For instance, performing the reaction with compound **3b** (7-chloro, 8-fluoro-substituation), no conversion was observed. Consequently, the more drastic procedure according to Grohe *et al.*[37] was applied (elevated temperature [145 °C] in combination with longer reaction time [48 h]): the carboxylic acid derivative **3b** was dissolved in abs. DMF, treated with morpholine (10 eq), and finally compound **4b** was obtained after 48 h. For the synthesis of compound **4d**, the suitable protocol according Sunduru *et al.*[38] was applied. The starting material **3d** dissolved in acetonitrile, reacted with morpholine (2 eq) and heated at 90 °C for 8 h. Compound **4d** was yielded in 36% after column chromatography. The particular activation forming the difluoroboron complex of **3c** and **3e** is discussed in the section hereinafter (3.5.4).



3a-h
3k-l, **3m'**

4a-k'

Compound	**4a**[7]	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**	**4k**	**4k'**
Substituent R^x												
R^5	H	H	F	H	F	H	H	H	$N(CH_3)_2$	H	H	H
R^6	F	H	H	F	F	H	CF_3	OCH_3	H	OH	NO_2	H
R^8	H	F	F	F	H	H	H	H	H	H	H	NO_2
Yield [%]	63	62	25	36	25	83	29	70	56	57	-	89
Time [h]	4[a]	48[b]	4[c]	8[d]	1[c]	4.5[a]	12[a]	6[a]	2[b]	10[a]	-	0.5[a]

Figure 19: *Synthesis procedure and yields of the 1-butyl-7-morpholino-4-oxo-1,4-dihdroquinoline-3-carboxylic acid derivatives (**4a-k'**). R^x with x is the atom number in **4a-k'**. [a]microwave irradiation without solvent; [b]dissolved in abs. DMF, 145 °C; [c]activated through difluoroboron complex (3.5.4); [d] dissolved in abs. ACN, 80 °C; "-" no product could be isolated.*

The nucleophilic displacement reaction of the halogen of the quinolone follows a two-step proceeding addition-elimination (S_NAr). The nucleophilic addition to the electron-deficient quinolone is activated by the electron-withdrawing 4-oxo group (Figure 17). On the one hand, this moiety reduces the electron density particularly in position 5 (*ortho*) and position 7 (*para*), and on the other hand, the negatively charged transition state (Meisenheimer complex) is stabilized. Forming the mesomeric stabilized Meisenheimer complex is the rate-determining step, while the release of the halide proceeds rather without hindrance. Special attention should be paid to compounds **3m** and **3m'** as the nitro group exerts supplementary activation to position 7 for S_NAr. For **4k'**, reaction was already completed after 30 min in a very good yield (89%). However, compound **4k** could not be isolated using these reaction conditions due to decomposition.

3.5.2 Synthesis of the 1-Butyl-5-amino-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid

The distinct reactivity of various fluorine substituted 4-oxo-1,4-dihydroquinoline-3-carboxylates for S_NAr was investigated in recent publications.[35,39,40] As mentioned before, positions 5 and 7 both should be considered as region for S_NAr, when bearing a halogen atom in particular positions (compounds **3c**, **3e**). Shibamori *et al.*[35] established a strategy to push the attacking nucleophile in a certain direction. It was supposed that displacement in C-7 is privileged when using polar solvents and bearing the carboxylic acid in C-3. If the C-5 substituted product is desired, the S_NAr should be performed in non-polar solvents (e.g., toluene) along with the corresponding carboxylic acid ester.[35,40]

The *N*-H of the attacking amine is assumed to form a hydrogen bond with the C-4 oxo moiety, drawing the nitrogen in a region, where C-5 gets easily attacked (Figure 20).[35] Consequently, non-polar solvents support this hydrogen bond-formation and promote 5-substituted products.

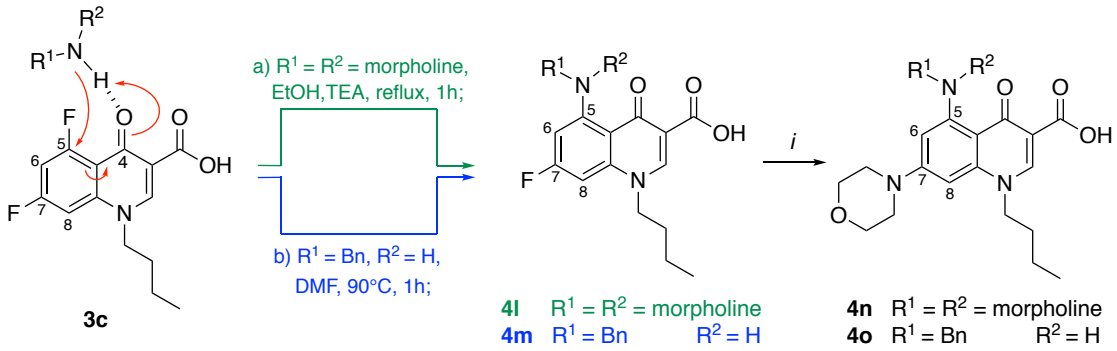


Figure 20: *The S_NAr of **3c** with morpholine and benzylamine, respectively, exclusively afforded the 5-substituted compounds **4l** and **4m**. The subsequent S_NAr reaction with morpholine yield compounds **4n** and **4o**. Reagents and reaction conditions: i) morpholine, MW, 110 °C.*

Considering the regioselectivity approach mentioned by Shibamori *et al.*[35], the carboxylic acid **3c** was dissolved in ethanol, and treated with equimolar amounts of morpholine and triethylamine (2 eq). Nevertheless, exclusively the undesired 5-substituted quinolone **4l** was generated in a yield of 56% (Figure 20). Moreover, benzylamine in abs. DMF preferentially nucleophilic attacked position 5 of the quinolone **3c**. Both examples with polar solvents demonstrated the challenge to control the regioselectivity by simply sticking to particular solvents. Since this approach failed, a strategy for selective activation was pursued (3.5.4).

However, in the certain case of benzylamine, the displacement of C-5 yielding compound **4m** was actually intended, as it can be utilized for the incorporation of a benzyl-protected amine.[41] Thus, an envisaged debenzylation could afford a sparfloxacin-related quinolone amide with a free amino moiety in position 5 (3.7.1).[41] Both 5-amino substituted molecules were reacted with morpholine, and compounds **4n** and **4o** were obtained in yields of 31% and 35%, respectively (Figure 20).

3.5.3 Synthesis of the 1-Butyl-5-alkyloxy-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid

For a better insight into SAR concerning position 5 and its contribution to the antitrypanosomal property, it is valuable having numerous structural variations here. Thus, the particular susceptibility of position 5 for S_NAr is exploited in this section. As Miyamoto *et al.*[41] introduced both amino and alkyloxy residues into the quinolones, it was attempted likewise.

Initially, a benzyloxy moiety was tried to be introduced in position 5 of **3c** (Figure 21). The benzyl residue in **K** was supposed to be the protecting group for an alcohol and should be removed at the very end of the quinolone amide synthesis. However, the reaction failed for unknown reasons, and a methoxy group was tried to be introduced alternatively. The methanolate ion, generated from the reaction of abs. methanol and sodium hydride (10 eq) attacked the C-5, and finally gave compound **4p** in a yield of 40%. The subsequent reaction with morpholine in the microwave supported synthesis gave compound **4q** (34%) within 5 h.

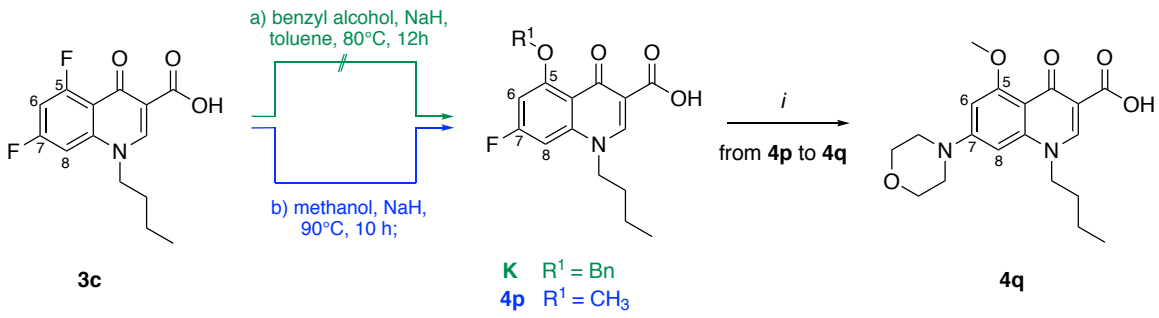


Figure 21: *Introducing of an alkoxy residue in position 5 and the subsequent substitution to compound* ***4q****. Reagents and reaction conditions: i) morpholine, MW, 110 °C.*

3.5.4 Synthesis of the 1-Butyl-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid via a Difluoroboron Complex

In order to activate the substitution of the fluorine atom in C-7 with a morpholine group, compounds **3c** and **3e** were activated by a difluoroboron complex **L** (Figure 22). According to Heravi *et al.*[42], the compounds were dissolved in $CHCl_3$ and reacted with boron trifluoride diethyl etherate and triethylamine. The intense yellow and fluorescing intermediate **L** was obtained.[35] The spectroscopic data of several difluoroboron complexes with fluoroquinolones have been well examined by Lin *et al.*[43] and comply with the obtained results. The most obvious change in the complex **L** regarding ^{1}H NMR was the disappearance of the signal at δ= 14.98 ppm (**3c**), which was assigned to the carboxylic acid protons. Additionally, the H-2 protons in the boron-chelated complex are shifted to lower field compared to H-2 in the quinolones. Moreover, the downfield shifts of protons R-6 and H-8 regarding intermediate **L** in comparison to **3c**, substantiated the electron-withdrawing effect.

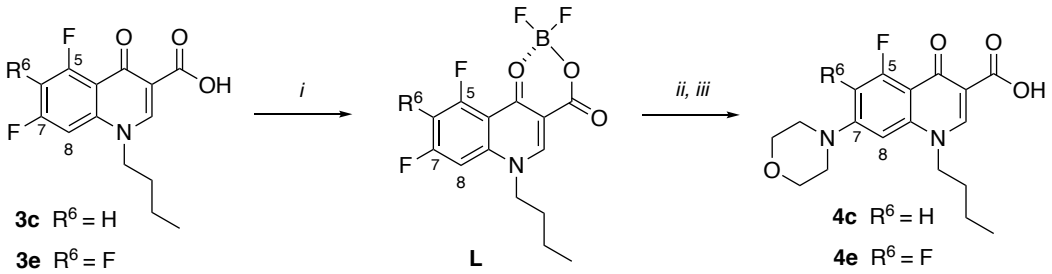


Figure 22: *The activation of compounds **3c** and **3e** for the S_NAr in position 7: Synthesis of the difluoroboron complex (**L**) and the regioselective substituted compounds **4c** and **4e**. Reagents and reaction conditions: i) BF_3•OEt_2 (1M solution in CH_2Cl_2), TEA, CH_2Cl_2, 45 °C; ii) morpholine, TEA, EtOH; iii) NaOH, reflux.*

Certain valence vibrations in the IR spectra of the difluoroboron complex differ from these in the corresponding quinolone. Since the proposed interaction between the boron atom and the quinolone is coordinated through the 4-oxo group and the carboxylate oxygen, these particular stretching vibrations were affected. The strong absorption bands of the quinolones in the region of $\tilde{\nu}$ = ~ 3400 cm^{-1} were assigned to the O-H stretching vibration of the carboxylic group. The related stretching vibration in the complex was not observed. In the difluoroboron complex, the C=O valence vibrations of C-4 ($\tilde{\nu}$ = 1625 cm^{-1}) and the carboxylic group ($\tilde{\nu}$ = 1715 cm^{-1}) were both high-frequency shifted with respect to those of the parent quinolones ($\tilde{\nu}$ = 1614 cm^{-1} and $\tilde{\nu}$ = 1708 cm^{-1}). Moreover, the B-F valence

vibrations ($\tilde{\nu}$ = 1137 cm^{-1}) and the B-O valence vibrations ($\tilde{\nu}$ = 1047 cm^{-1}) were solely present in the complex.

Next, the activated difluoroboron complex was dissolved in ethanol and reacted with morpholine and triethylamine under mild conditions (T= 60 °C for R^6 = H and rt was applied for R^6 = F, respectively). Finally, the complex was hydrolysed under basic conditions. After acidification the solution under ice cooling, compounds **4c** and **4e** were obtained. The circumvention was time-consuming, but successfully resulted in selectively 7-substituted quinolones in overall yields of 25%.

For the quinolones which were prone for side reaction in position 6 during S_NAr (e.g., **3a**, 6-fluoro, 7-chloro substituent), the difluoroboron complex was also utilized for privileging C-7 over C-6.[7,39] Hermecz *et al.*[39] proposed a stronger electron-withdrawing impact on C-7 ($\Delta\delta$= 3.7 ppm) than on C-6 ($\Delta\delta$= 1.4 ppm), since the particular signals in the ^{13}C NMR spectrum were shifted downfield to a greater extent.

In the case of **3c** and its activated boron complex, however, the particular enhanced activation of C-7 ($\Delta\delta$= 1.2 ppm) over C-5 ($\Delta\delta$= 5.0 ppm) did not correspond to the observed ^{13}C NMR data. Thus, the formation of the privileged 7-substituted product might have been favoured more likely due to sterical reason and to the unavailable hydrogen bonding with the 4-oxo group (Figure 20).

3.5.5 Bioisosterism of Morpholine

A saturated five- or six-membered nitrogen heterocycle in position 7 of the quinolones leads to beneficial pharmacokinetic and enhanced antibiotic activity.[44] Considering the antitrypanosomal activity of the quinolone amides, Hiltensperger *et al.*[7] revealed the exclusive superiority of the morpholine moiety in C-7 over other tested nitrogen heterocycles. However, bioisosteric related heterocycles to morpholine were evaluated for their antitrypanosomal activities in this work. The concept of isosterism is traced back to Langmuir[45] in the year 1919, as he defined groups of atoms possessing the same number of atoms and valence electrons. This initial definition was further developed and finally Friedman[46] introduced the term of *bioisosterism*. Chemical isosteric replacements do not necessarily imply an identical biological response. Thus, the bioisosteric moiety is rather less structural related than mimic the biological effect. However, this fact implicates that an effective applied bioisosteric replacement naturally cannot be transferred to another biological setting.[47] Nevertheless, the SwissBioisostere[48] database provides a source of similar structural elements regarding their biological efficacy in assays. For the replacement of the morpholine substituent in **GHQ168**, the particular database was consulted and promising proposed residues were applied in position 7.[49]

According to the procedure of Grohe *et al.*[37], the carboxylic acid **3a** was dissolved in abs. DMF and heated with the following amines (3-10 eq) at 110 °C (Figure 23): thiomorpholine (**4r**), 2,6-dimethylmorpholine (cis+trans) (**4s**), and 4-methoxypiperidine (**4t**). The crude products were recrystallised from ethyl acetate and mixture of ethyl acetate/ethanol (2:1) for **4s**. Particular substitution reactions with the depicted heterocycles were not successfully accomplished (**M-O**).

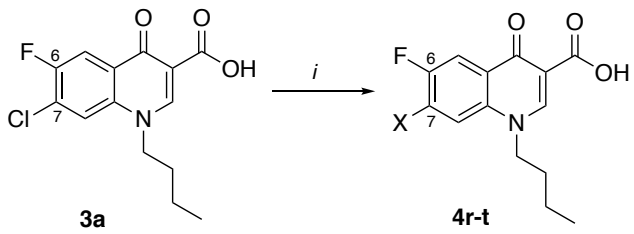
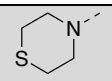
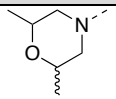
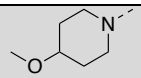
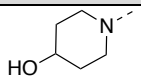
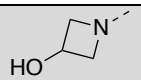
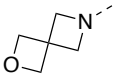


Figure 23: *Displacement reaction in position 7 with amino heterocycles. "-" no product could be isolated. Reagents and reaction conditions: i) H-X, abs. DMF, MW, 110°.*

compd	X =	Yield [%]
4r		33
4s		49
4t		67
M		-
N		-
O		-

3.6 Synthesis of the 1-Butyl-4-oxo-1,4-dihydroquinoline-3-carboxamides

Since Niedermeier[15] developed the convenient and efficient activation procedure, this established two-step method was retained for the synthesis of the respective amides. Besides other potential activation procedures ($SOCl_2$, PCl_5, DCC, HOBt), the mixed anhydride with *i*-butyl chloroformate (IBCF) was associated with less work up steps and higher yields.[5,6] Thus, the 1-alkyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acids **3i-k**, **4a-i, 4l**, **4n-o**, **4q-t** were dissolved in abs. DMF under argon atmosphere and deprotonated with *N*-methylmorpholine (*N*MM) under ice cooling. The *i*-butyl chloroformate (IBCF) was added and the mixed anhydride intermediate (**P**) reacted with the appropriate amine to achieve **GHQ168**, **5-29** (Figure 24). The respective amides were isolated in yields of 15-84% (Table 1, Table 2).

Attempts to convert compounds **4j** and **4k'** to the corresponding amides were not successful and the products could not be isolated purely. Indeed, the phenol moiety of **4j** apparently also reacted with IBCF to a mixed carbonate ester. Thus, additional potential nucleophilic sites in the quinolone should be avoided by using protecting groups. Unfortunately, the carbonate ester could not be removed selectively to obtain the parent phenol. The 8-nitro substituted compound **4k'** was reacted according to prior procedure but the resulted amide could not be separated from emerged side products.

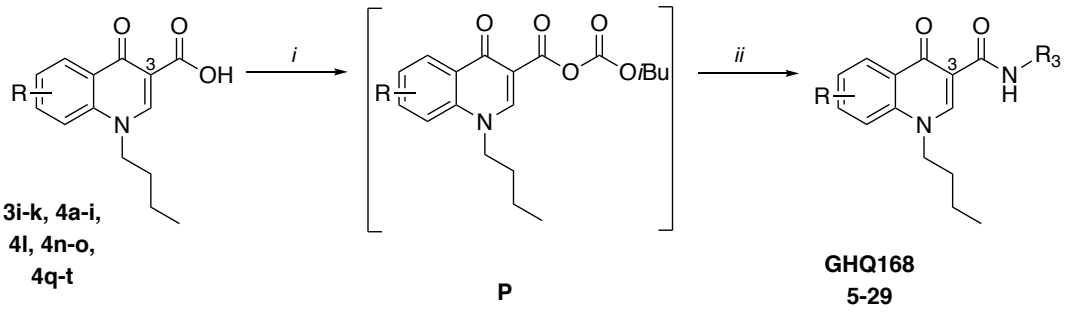
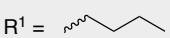
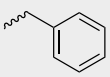
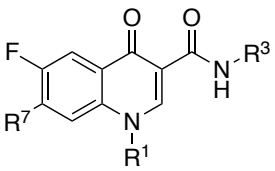


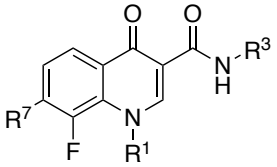
Figure 24: *Synthesis of the corresponding amide via a mixed anhydride. Reagents and reaction conditions: i) NMM, IBCF, abs. DMF, 0 °C; ii) NH-R_3, rt.*

***Table 1**: Overview of the synthesised quinolone amides (**GHQ168**, **5-12**, **18**). Yields are enclosed in brackets.*

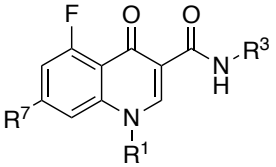
1-Butyl-7-morpholino-4-quinolone-3-carboxamides	R^1 = 	R^3 = 	R^7 = 



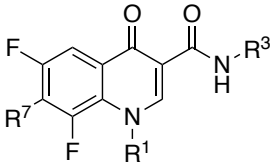
GHQ168 (39%)



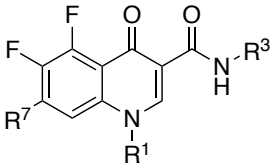
5 (62%)



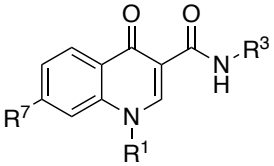
6 (40%)



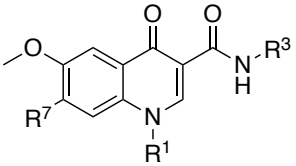
7 (25%)



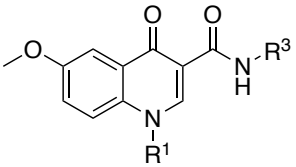
8 (44%)



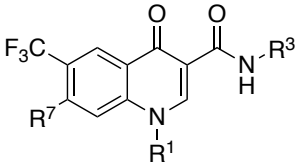
9 (17%)



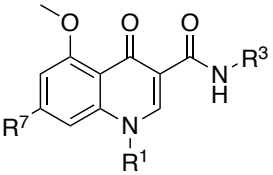
10 (34%)



11 (34%)



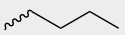
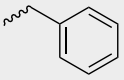
12 (15%)

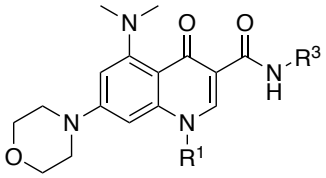


18 (21%)

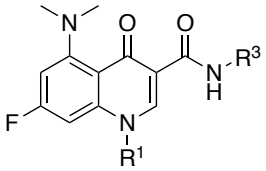
Table 2: *Overview of the synthesised quinolone amides (**13-17**, **19-29**). Yields are enclosed in brackets. Substitution pattern differs from the depicted quinolone scaffold: *compound **19** carries an additional fluorine in position 8; **compound **24** carries a fluorine in C-5 and no fluorine in C-6.*

1-Butyl-5-amino-4-quinolone-3-carboxamides

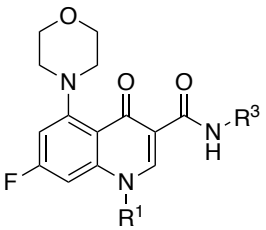
R^1 =  R^3 = 



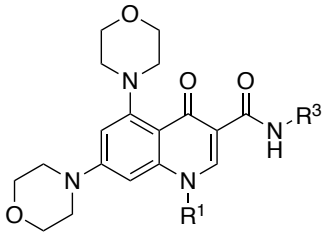
13 (42%)



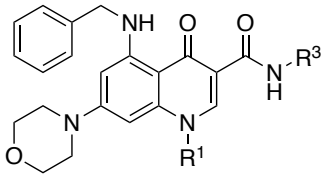
14 (24%)



15 (84%)

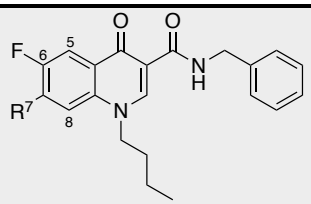


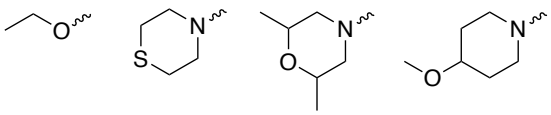
16 (51%)



17 (48%)

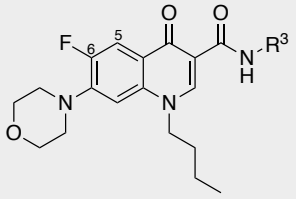
1-Butyl-6-fluoro-4-quinolone-3-carboxamides

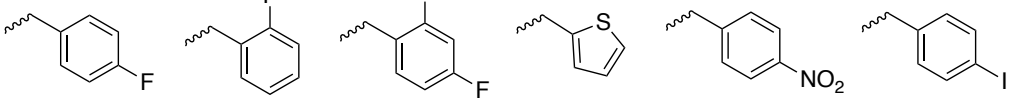


R^7 = 

19 (82%)* **20** (23%) **21** (26%) **22** (47%)

1-Butyl-6-fluoro-4-quinolone-3-carboxamides



R^3 = 

23 (58%) **24**** (31%) **25** (25%) **26** (30%) **27** (42%) **28** (47%) **29** (54%)

3.7 Derivatization of the Quinolone Amides

3.7.1 Derivatization of the N-Benzyl-5-(benzylamino)-1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide

The antibiotic sparfloxacin bears an amino group in position 5 and possesses an increased activity against gram-positive bacteria compared to the closely related ciprofloxacin. Hence, a 5-amino quinolone skeleton should be examined for its potential benefit against *T. b. brucei*. Miyamoto *et al.*[41] conveniently cleaved the benzyl protected amine in position 5 via a palladium-catalyzed hydrogenation in a very last step of the synthesis, finally leading to sparfloxacin. Applying these reaction conditions to the quinolone amide **18**, the desired compound **Q** could not be isolated (Figure 25). Neither the conventional approach in the autoclave (H_2 atmosphere, Pd/C, rt), nor the microwave assisted synthesis (pressurized H_2 atmosphere, 27 bar, Pd/C, 80 °C) was successful.

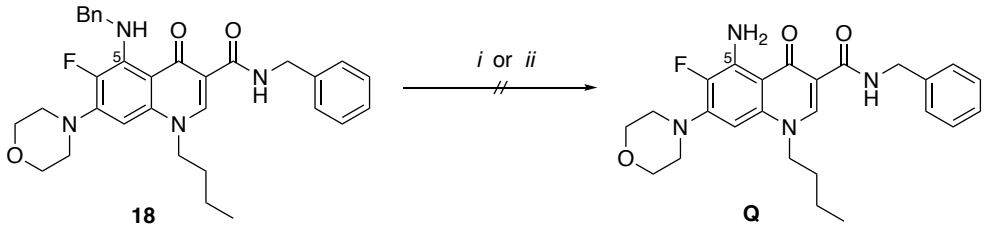


Figure 25: *Failed removing of the benzyl protecting group via hydrogenation uncover the free amino moiety in position 5. approach i) autoclave, Pd/C, $CHCl_3$, rt; approach ii) synthWave, Pd/C, $CHCl_3$, 80 °C.*

3.7.2 Derivatization of the N-Benzyl-1-butyl-7-ethoxy-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxamide

The initially unintended ethoxy substitution in position 7 (3.4.2.1, **3j**) was utilized in subsequent reactions (Figure 26). According to the SwissBioisostere Database[48,49] (3.5.5), even ethoxy and hydroxy moieties could possibly generate similar or advantageous biological response comparing with morpholine substituted molecules.

Compound **19** was reacted with BBr_3 and compound **30** was obtained in a yield of 79%. Voluminous benzyloxy substituents in positions 7 and 8 of the quinolones showed an increased activity against *Trypanosoma cruzi*.[3] This pathogen is closely related to the *Trypanosoma brucei* species, as it belongs to the same genus of *Trypanosoma*. Thus, this possibly advantageous residue was introduced reacting the phenol with K_2CO_3 and benzyl chloride, to achieve compound **31** in a yield of 56%.

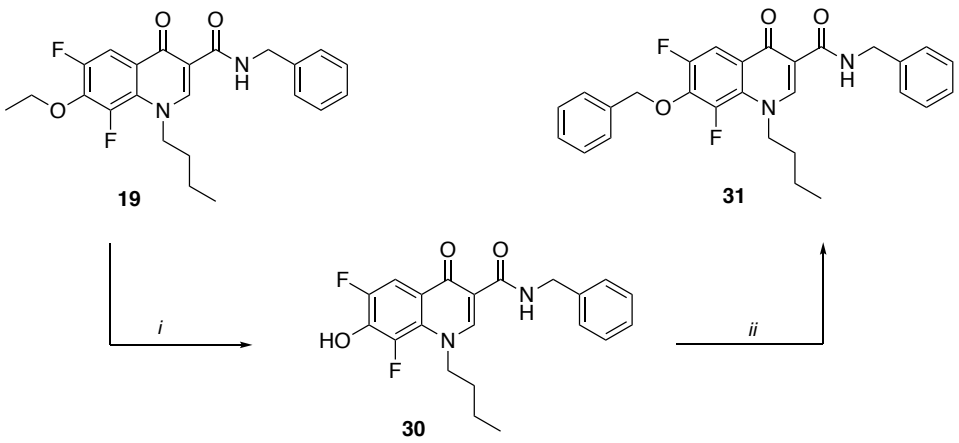


Figure 26: *The ether cleavage of compound **19** afforded the 7-hydroxy substituted compound **30**. Subsequent reaction with benzyl chloride yielded compound **31**. Reagents and reaction conditions: i) BBr_3 (1 M solution in CH_2Cl_2), CH_2Cl_2, 0 °C-rt; ii) Bn-Cl, K_2CO_3, abs. DMF, rt;*

3.7.3 Derivatization of N-Benzyl-1-butyl-6-fluoro-7-(4-methoxypiperidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxamide

The cleavage of the 4-methoxypiperidine ether in **22** was expediently performed after the amidation step (3.6). The ether was split using BBr_3 again.[50] However, the major products of this ether cleavage gave the undesired bromine containing quinolone **32** in a yield of 21% (Figure 27). At the first glance, the 4-hydroxypiperidine product was supposed to be obtained due to the missing ^1H NMR signal (δ = 3.29 ppm, OCH_3). The mass spectroscopy analysis, however, revealed a *m/z* = 514.9 / 517.9 $[M+H]^+$ which perfectly fitted to compound **32** and complied with the isotpe pattern of bromine. The mechanism is proposed to proceed through an adduct formation of the ethereal oxygen and the electrophilic boron (**R**). It is suggested, that a stabilized carbocationic transition state is initially established. Then, the nascent bromide ion nucleophilic attacks

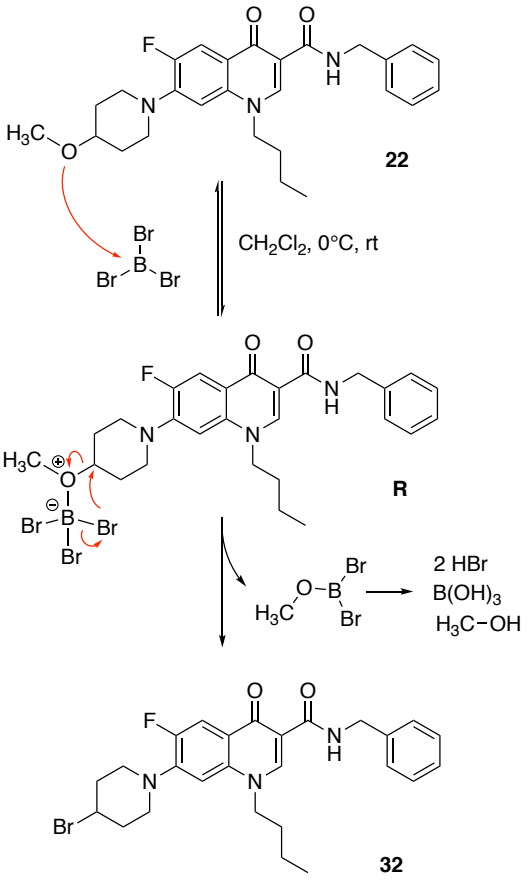


Figure 27: *Mechanism of the ether cleavage with BBr_3 applied for compound **22**.*

the secondary carbon atom of the piperidine. The assumed unimolecular process is favoured for ethers with branched alkyl substituents and implicates that forming of the adduct (**R**) is the rate determining step.[51] In contrast, mixed aryl alkyl ethers (**19**) undergo a rather different pathway with boron tribromide.[52] These kind of ethers selectively afford the alkyl bromide and the corresponding phenol derivative (Figure 26).

3.8 Grohe-Heitzer Protocol

The established Grohe and Heitzer cyclisation of enamines to the respective quinolone, opened the door for further substitution variations.[37] Thus, cyclopropyl (represented in ciprofloxacin, Figure 3) and aryl residues in the *N*-1 position were now accessible and even novel scaffold variations were accessible (e.g., enoxacin), including the synthesis of the 1,8-naphthyridone core which previously failed with the Gould-Jacobs approach (3.3.3).[53,54,55]

3.8.1 Synthesis of the 1,8-Naphthyridone Scaffold

The starting material ethyl 3-(2,6-dichloro-5-fluoropyridin-3-yl)-3-oxopropanoate was reacted with triethyl orthoformate and acetic anhydride for 4 h at 120 °C, achieving the acrylate (Figure 28). The volatile residues were evaporated and the oily intermediate was dissolved in CH_2Cl_2 without prior characterisation. The vinylogous ester was subsequently reacted with a slight excess of *n*-butylamine to give the respective E- und Z-isomers **33**. The oily product was crystallised obtaining a yellow solid in 36%.[54] According to Hiltensperger *et al.*[6] the Z-isomer is predominantly formed due to steric hindrance. The two isomers can be distinguished ^{1}H NMR spectroscopically (Figure 28). An intramolecular hydrogen bond is exclusively established between the *N*-H and the carbonyl carbon of the ester group of in the Z-isomer (indicated in blue). Accordingly, the electron density of the *N*-H is increased and the ^{1}H NMR signal is shifted downfield (δ = 11.01 ppm). For comparison, the respective signal of the E-isomer is located at δ = 9.67 ppm (indicated in green). Additionally, the H-3' signal of the E-isomer forms a hydrogen bond with the enclosed carboxylic ester, resulting in slight deshielding effects (δ = 8.23 ppm compared to δ = 8.15 ppm of the Z-isomer). The isomeric ratio was determined to be 1:5 (E/Z) by means of the integration of signal H-3'. The nucleophilic ring closure (S_NAr) was achieved at 100 °C, using K_2CO_3 and a polar aprotic solvent (e.g., acetonitrile). The ethyl-4-oxo-1,4-

dihydro-1,8-naphthyridin-3-carboxylate **34** was obtained in a yield of 64%. The feasibility of this cyclisation depends on the leaving group and the surrounding functionalities.

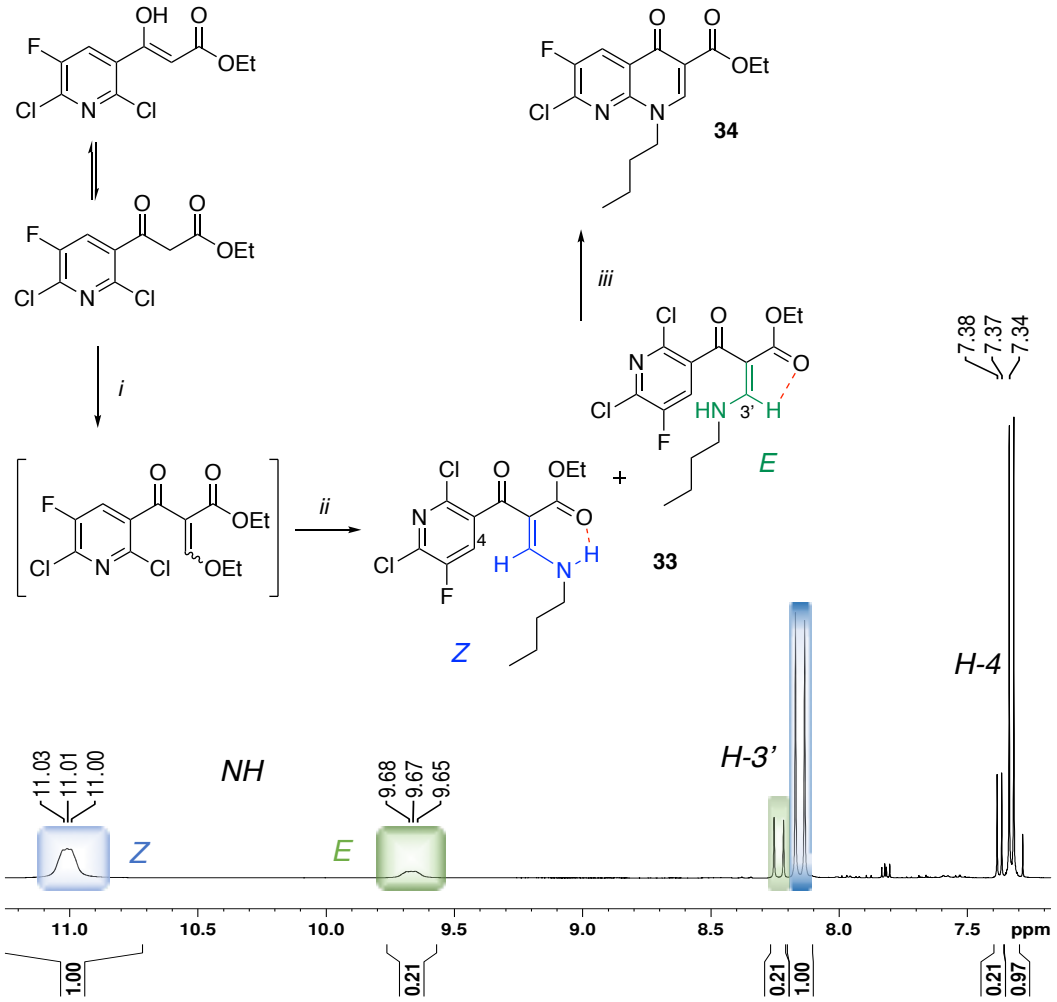


Figure 28: *Synthesis of ethyl 1,8-naphthyridine-3-carboxylate **34**. Reagents and reaction conditions: i) triethyl orthoformate, acetic anhydride, 120 °C; ii) n-butylamine, CH_2Cl_2, rt; iii) K_2CO_3, ACN, 100 °C. ^{1}H NMR spectrum of the isomeric mixture **33** recorded in $CDCl_3$. Signals of the Z-isomer are indicated with blue and the E-isomer in green.*

3.8.2 Synthesis of the N-Benzyl-1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide

Next, the morpholine ring should be 'added' to the 1,8-naphthyridone core. The substitution of chlorine in position 7 was difficult to achieve and solely the synthesis protocol according Tabarrini *et al.*[56] was successful. The carboxylic ester **35** was used as starting material, dissolved in DMF and reacted with DIPEA, and morpholine (Figure 29). Subsequently, the ester was cleaved to the corresponding carboxylic acid **36** (Yield 36%).

The final step forming the amide **37** could be accomplished according to the general procedure (3.6) in a yield of 90%.

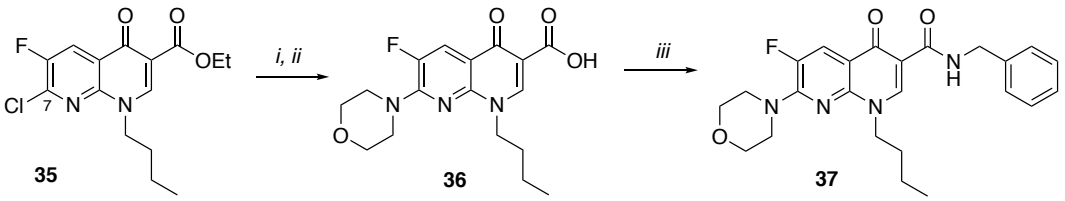


Figure 29: *Synthesis of the 1,8-naphthyridone amide **37**. Reagents and reaction conditions: i) morpholine, DIPEA, abs. DMF, 100 °C; ii) 2 M HCl, reflux; iii) NMM, IBCF, benzylamine, abs. DMF, 0 °C-rt.*

3.9 Synthesis of Quinolone Amides Derived from Commercially Available Quinolones

Since commercially available quinolone antibiotics of various generations were in stock, nalidixic acid was converted into the corresponding benzylamides (**38**) according to the established amidation method (3.6) in yields of 61% (Figure 30). Before the ciprofloxacin core was reacted likewise, the 7-chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid was substituted with morpholine in position 7 obtaining **40** in a yield of 93% (3.5.1). The enclosed amidation of **40** resulted in compound **41** (Yield 63%). Enoxacin was initially protected with Boc anhydride, then the amide conversion was applied (3.6) and the protecting group was removed. Finally, the free base **39** was obtained in yield of 27%.

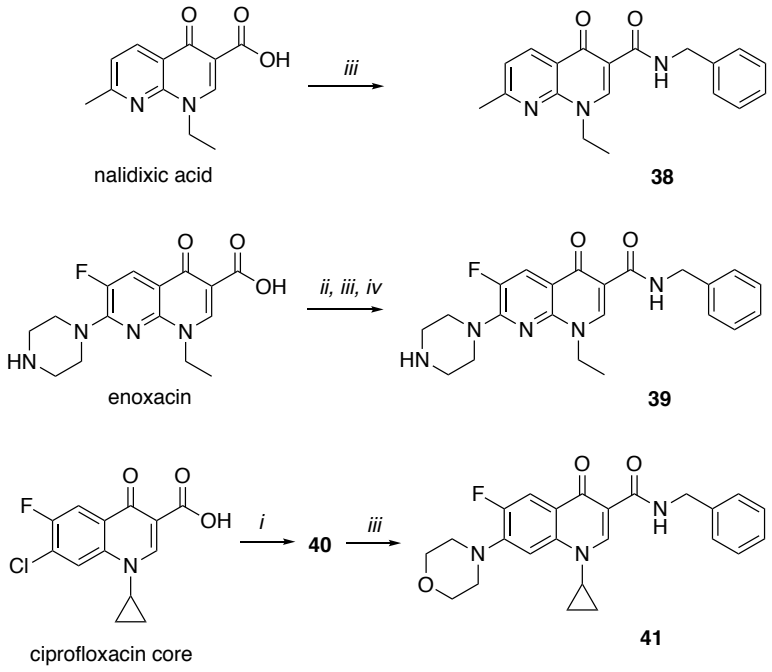


Figure 30: *Synthesis of compounds **38**-**41** which are associated with commercially quinolone antibiotics. Reagents and reaction conditions: i) morpholine, abs. DMF, 110 °C, MW; ii) Boc_2O, TEA, abs. DMF, 100 °C; iii) NMM, IBCF, benzylamine, abs. DMF, 0 °C-rt; iv) TFA, CH_2Cl_2, rt;*

4 Application of Fluorine

4.1 Role of Fluorine in Medicinal Chemistry

The history of fluorine in pharmaceutical products began with fludrocortisone in 1955, and a few years later 5-fluorouracil.[57] In 1970, there were only about 2% of fluorine-containing drugs approved. Since then, the proportion has been vastly increased to 20-30%.[58] Interestingly, fluorinated drugs are among the top selling pharmaceuticals, e.g., rosuvastatin, sitagliptin, efavirenz, and celecoxib. These few examples of frequently prescribed drugs also demonstrates broad applications of fluorine in all therapeutic areas. However, fluorine particularly plays a pivotal role in drugs targeting the CNS (e.g. paroxetine, fluoxetine, flunitrazepam, and haloperidol).[59] Since a fluorine atom at an aromatic ring commonly increases lipophilicity, the absorption across the blood-brain barrier (BBB) could be enhanced.

In general, fluorine can be applied for various strategical reasons in drug development e.g., influencing the physicochemical properties via its electron withdrawing effect (e.g., metabolism). Besides affecting the pK_a, the permeability and the potency of drug candidates, fluorine also emerges as prominent atom in nuclear medicine.[60]

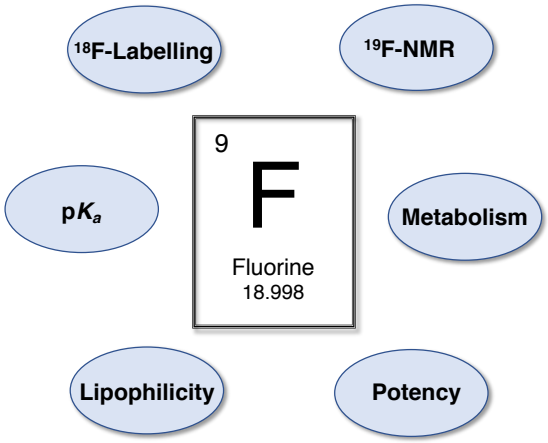


Figure 31: *Schematic overview of the applications and parameters which are associated with the fluorine atom.*

4.2 Fluorination Methods

Interestingly, there is only a handful organofluorine molecules which originate from nature. In contrast to the obviously limited biological fluorination reactions, fluorination reagents are in common use for chemical synthesis.[61] The development of several straightforward electrophilic and nucleophilic reagents for introducing fluorine contributed to its widespread application. Since fluoride is strongly solvated in protic solvents, aprotic solvents are in preferential use for the nucleophilic fluorination. Thus, tetraalkylammonium ions are applied as counterions (TBAF) in order to reduce certain ionic bond strength of fluoride in aprotic solvents.[62] Another convenient nucleophilic fluorination agent is (diethylamino)sulfurtrifluoride (DAST), in particular for converting alcohols into fluorides.
For a long period of time there were no electrophilic fluorination agents besides elemental fluorine. The inconvenience with the unselective and extremely reactive fluorine gas was overcome with the development of the stable and user-friendly *N*-F reagents (selectfluor and *N*-Fluorobenzenesulfonimide (NFSI)).[62]

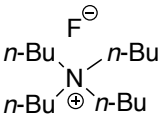
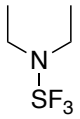
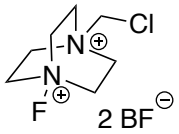
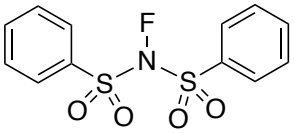
Nucleophilic fluorination reagent		**Electrophilic fluorination reagents**	
			
TBAF	DAST	Selectfluor	NFSI

Table 3: *Examples of commercially available nucleophilic and electrophilic fluorination reagents.*

4.3 Positron Emission Tomography

Positron emission tomography (PET) is a non-invasive nuclear imaging technique for assessing various biological processes. Once inside the body, the decay of the radioisotope emits a positron (beta decay) that travels a short distance until it collides with an electron present in the neighbouring tissue (Figure 32). The encounter generates two photons (annihiliation) with an energy of 511 keV that travel in approximately opposite directions.[63] The absorption of the coincidentally emitted gamma radiation by the surrounded photomultiplier tubes are the basis for detection. Finally, the registered events illustrate the spatial location of the radionuclide in a studied object over a time period.[64]
To date, it is widely applied in the field of oncology in combination with the radiotracer fluorodeoxyglucose ([^{18}F]-FDG), which illustrates the areas of glucose metabolism.[65] Since

most tumour cells exhibit high rates of glucose turnover, [^{18}F]-FDG uptake is extensively increased into the respective cells, where it undergoes phosphorylation by the hexokinase. Further glycolysis steps are halted, and the phosphorylated [^{18}F]-FDG is trapped in the malignant tumour cells, and the radiotracer accumulates within.[66] In contrast to the clinical diagnostic, PET imaging can provide eminent insights during drug discovery research (e.g., elucidation the mechanism of drug action, proof of concept), particularly in case of CNS drugs.[67]

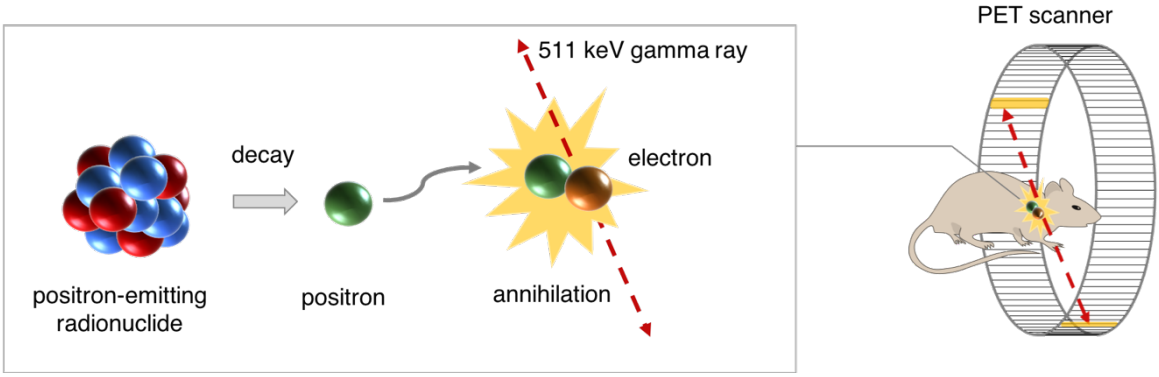


Figure 32: *A radionuclide emits a positron resulting in an annihiliation. The two emitting photons in opposed directions are simultaneously detected in a small-animal PET scanner.*

4.4 [^{18}F] Labelling

In the field of radiochemistry, the advantage of using [^{18}F]-fluorine relates to its most ideal half-life (110 min), ease of production, and its relatively low positron energy (0.64 MeV).[64] As low positron energy causes minor extension in tissue, particularly high resolution in positron emission tomography (PET) can be achieved. The respective half-life offers a sufficient time period for performing the radiolabelling, purification, and formulation for intravenous injection. However, the entire process should be accomplished within 3 hours.[60] Thus, radionuclide should be incorporated in a last stage of the synthetic pathway.

4.4.1 Nucleophilic [^{18}F]-Fluorination Strategies

[^{18}F]-Fluorine is usually generated in a cyclotron system by a proton bombardment of [^{18}O], a stabile naturally occurring isotope of oxygen. Depending on the target material, whether isotopically enriched [^{18}O]-H_2O or [^{18}O]-O_2 is used, either [^{18}F]-fluoride or [^{18}F]-fluorine is obtained (Figure 33).[68] The electrophilic [^{18}F]-F_2 gas is suitable for labelling electron rich aromatic rings and alkenes, but lacks certain regioselectivity which challenges purification efforts.[69]

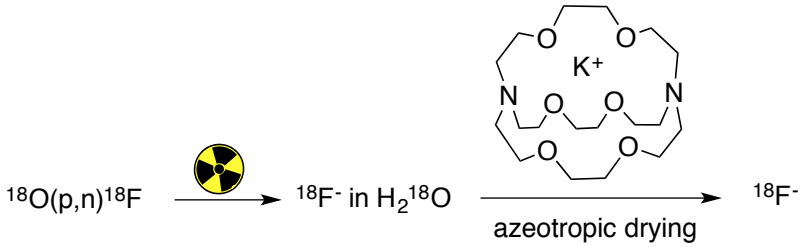


Figure 33: *General procedure of [^{18}F]-fluoride with the subsequent addition of Kryptofix 2.2.2 and azeotropic drying of residual water, inspired by ref. 69.*

Thus, radiofluorination approaches in the nuclear medicine are predominantly performed with the nucleophilic [^{18}F]-fluoride. However, the nucleophilicity of fluoride in aqueous solution is dramatically decreased due to formed hydrogen bonds. Interrupting these tight solvation shells can be achieved by the addition of cationic counter ion prior to the evaporation of water. For this reason an anion exchange resin cartridge is used and [^{18}F]-fluoride is eluted with either alkali carbonate solution (combined with Kryptofix 2.2.2) or tetrabutylammonium hydroxide (Figure 33). Consequently, the [^{18}F]-fluoride is only weakly bound to the Kryptofix 2.2.2 and the quaternary ammonium salt, respectively, increasing nucleophilic substitution reactions. The final step is the azeotropic drying process with ACN to clear the [^{18}F]-fluoride salt from any residual water.[60,65]

Besides the nucleophilicity, the solubility of the [^{18}F]-fluoride ion in organic solvents is also a major concern. To overcome this problem, phase transfer catalysts (the aminopolyether-complex Kryptofix 2.2.2, tetrabutylammonium cations) with dipolar aprotic solvents such as DMSO, DMF and ACN are commonly used.[65] The particular aprotic solvents possess advantageously rather high dielectric constants ε (ε>30) leading to dissociation of ions but without generating [^{18}F]-HF.

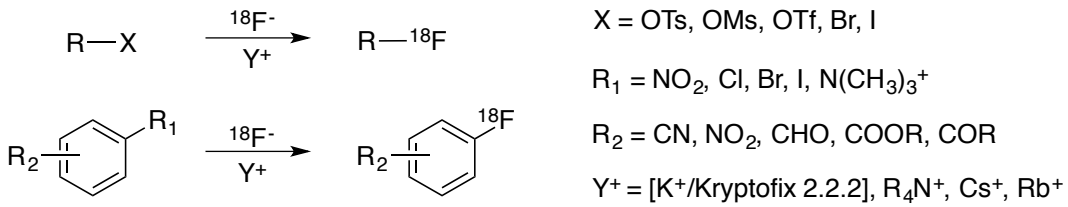


Figure 34: *General procedures for nucleophilic radiofluorination in alkenes and arenes.*

From a chemical point of view, the introduction of [^{18}F]-fluorine in molecules requires a carefully developed methodology as the carbon-fluorine bond is rather difficult to tie. The sulfonic esters (e.g. *p*-toluenesulfonyl ester, methanesulfonic ester, trifluoromethanesulfonic ester) are typically used as a prosthetic group for direct

nucleophilic radiolabelling, since they can be considered excellent leaving groups. However, due to their rather high reactivity, the particular precursors might be unstable and prone for side reactions. Thus, less reactive halogens as leaving groups (I > Br > Cl) could alternatively be beneficial (Figure 34). The reaction follows a S_N2 mechanism, implicating Walden inversion and causes the conversion of the configuration of a chiral carbon atom. The aromatic nucleophilic substitution is mostly limited to electron-deficient arenes combined with good leaving groups. Nitro and trimethylammonium groups are widely used in *ortho* or *para* position to the electron withdrawing groups (Figure 34).

4.4.2 Radiofluorination of the Quinolone Amide

Since adequate therapies of stage II trypanosomiasis must inevitably pass the blood-brain barrier (BBB) it is fairly interesting if the quinolone amides could successfully clear this hurdle.

In recent years, certain commercially available fluoroquinolones, such as lemofloxacin, trovafloxacin and fleroxacin were labelled with ^{18}F for pharmacokinetic studies in humans.[70-72] In some cases, an exchange of the native ^{19}F atoms by the ^{18}F isotopes could be exploited using the K[^{18}F]-fluoride.[70,71] However, this isotopic exchange reaction disadvantageously possesses only low specific activity. As the previously mentioned fluoroquinolones carry several fluorine atoms, the exact position of the nucleophilic isotope exchange reaction could not be determined. However, an isotope exchange of ciprofloxacin (only containing fluorine in C-6) failed.[73] The fluorine in position 6 might solely be replaced in intermediate **3a**, in an earlier stage of the synthesis.[73] But due to the half-life for ^{18}F of 110 min, the step back in the synthesis is limited. Contrary to ciprofloxacin the quinolone amides require two additional synthesis steps (S_NAr in position 7, amidation in position 3) after radiolabelling, what even questioned the successful outcome of this approach. Hence, there was the need for 'incorporating' an extra fluorine atom to **GHQ168**, since the native one is not accessible.

As structural deviation to **GHQ168** should be kept as slight as possible, it was reasonable to replace a hydrogen atom by a fluorine atom. Furthermore, the fluorine atom should be easily introduced into the scaffold regarding a 'cold' reference compound and a 'hot' labelled compound. For instance, the reference compound **23** (*p*-fluorine substituted benzylamide moiety) was conveniently afforded in the described straightforward synthesis (3.6). The incorporation of ^{18}F in the aromatic ring, however, calls for a time-consuming

detour. Since compound **29** (*p*-iodine substituted benzylamide moiety) is not activated for direct nucleophilic ^{18}F-radiolabelling, a 4-[^{18}F]-fluorobenzylamine derivative ought to be produced in a two-step synthesis.[74] To keep the radiofluorination more simple, a single-step labelling process in the very end was requested.

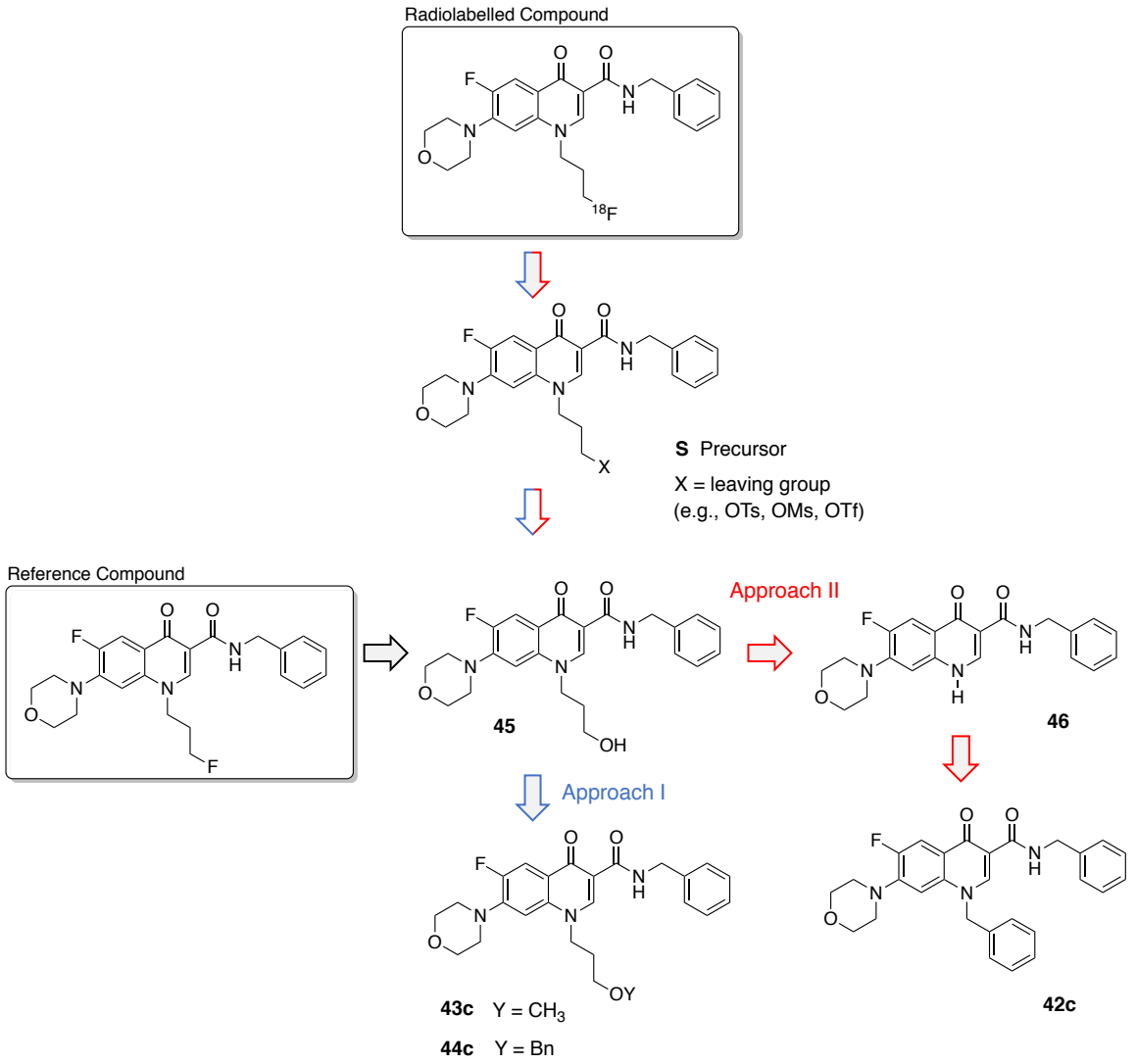


Figure 35: *Retrosynthesis of the radiolabelled quinolone amide and the reference compound. The sulfonic ester* ***S*** *(e.g., OTs, OMs, OTf) is a convenient precursor for nucleophilic aliphatic substitution. The hydroxypropyl residue in* ***45*** *can be obtained by either deprotecting the primary alcohol residue in compounds* ***43c*** *and* ***42c*** *(approach I), respectively, or by introducing the entire hydroxypropyl group into quinolone* ***46*** *(approach II).*

Hence, [^{18}F]-fluorine was intended to be introduced to the *N*-alkyl chain according to ^{18}F-labelled fleroxacin (Figure 35).[75] As depicted in the retrosynthesis, two approaches (blue and red) were pursued and an appropriate precursor was needed, bearing a good leaving group. In both approaches, the sulfonic esters **S** (X = OTs, OMs) were used as prosthetic groups. Compound **45** having a terminal hydroxyl group, is an essential intermediate of

these synthesis routes. Since the reactivity of an alcohol moiety could hamper the familiar synthesis of the quinolone amides (3.6), the hydroxyl group demanded for protection (Y = OCH_3, OBn, approach I). Alternatively, the hydroxypropyl moiety can be introduced into **46** (approach II). However, *N*-1 position must also be protected during quinolone amide synthesis due to nucleophilic property (e.g., benzyl protected in **42c**).

*4.4.2.1 Synthesis of the Protected Quinolone Amides **42a-44c***

Two synthetic routes (approach I and II) pursued the introduction of an alcohol (Figure 36). On the one hand, the terminal hydroxyl group of the alkyl chain was derived from an ether group (**43a** and **44a**). The ethers were intended to be the protecting groups during quinolone amide synthesis (approach Ia and Ib). On the other hand, the entire *N*-position was protected by means of a benzyl group in **42a**, which was considered to be stable towards many reaction conditions (approach II).

The starting material ethyl 1-benzyl-4-quinolone-3-carboxylate[†] was hydrolysed and the corresponding acid **42a** (55%) was obtained (Figure 36). The general procedure (3.4.1) for the alkylation of **2a** in the *N*-1 position was applied: the 1-chloro-3-methoxypropane and ((3-bromopropoxy)methyl)benzene, respectively, were added to a solution of **2a** in DMF. Compounds **43a** and **44a** were obtained in yields of 49% and 66%, respectively.
The subsequent S_NAr reaction (3.5.1) of compounds **42a-44a** gave compounds **42b-44b** (Yields 58-78%). According to the general procedures for the amidation (3.6), compounds **42c-44c** were achieved (Yields 52-76%) (Figure 36).

[†] This compound was thankfully provided by R. Messerer.

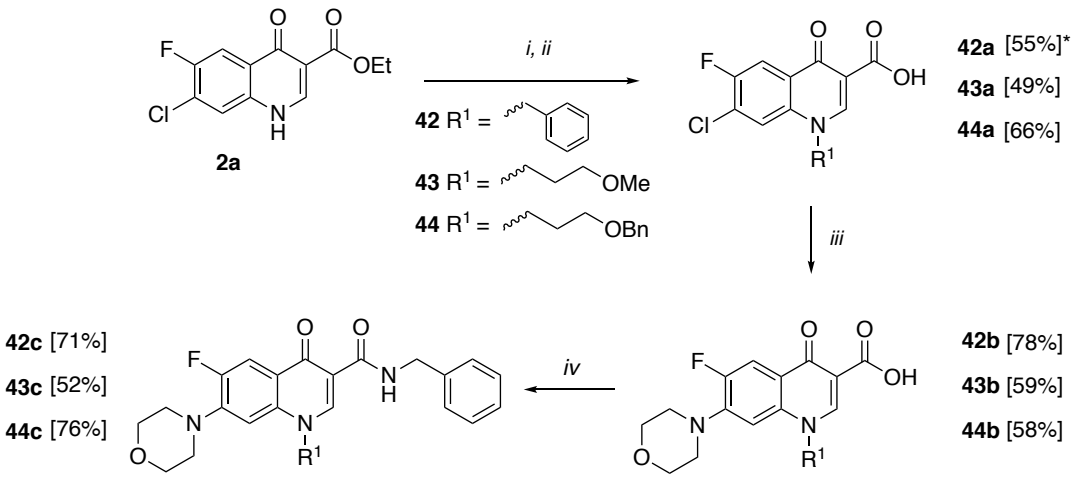


Figure 36: *Synthesis of the quinolone amides which were intended to act as precursor for the introduction of an alcohol at the N-1 position. Reagents and reaction conditions: i) R^1-Br, K_2CO_3, KI, abs. DMF, 80-85 °C; ii) KOH (3 M, aqueous solution), reflux; iii) morpholine, MW, 110 °C; iv) NMM, IBCF, benzylamine, abs. DMF, 0 °C-rt. *yield only refers to hydrolysis.*

4.4.2.2 Removing the Protecting Groups

a) Approach Ia

In order to obtain the primary alcohol in *N*-1 position, the methoxypropyl group of compound **43c** was attempted to cleave using BBr_3 (Figure 38). However, the isolated product did not contain the characteristic ^{1}H NMR signals of the morpholine ring in **43c** (δ = 3.92 – 3.89 ppm and 3.27 – 3.25 ppm) anymore. Hence, the morpholine was apparently susceptible for alkoxy cleavages, even applying these mild conditions. Due to failed removal of the methoxy group in **43c** another protecting group was required.

b) Approach Ib

Thus, the benzyloxy group (**44c**) was selected for protecting the primary alcohol. Compound **44c** was reacted with Pd/C under pressurised H_2 atmosphere, which did not affect other functionalities in the scaffold. The heterogeneous catalysed reaction starts with an oxidative addition of Pd(0) which implies the insertion of Pd(II) into the substrate (Figure 37). Next, the hydrogen adsorbs at the catalyst surface, subsequently gets activated and disassociates, respectively. One coordinated hydrogen atom is installed into the Pd(II) complex and another hydrogen forms the releasing alcohol. Eventually, a reductive elimination forms the starting material Pd(0) species and toluene. Here, a special value is assigned to the microwave which allows to highly pressurise the chamber with hydrogen (25 bar). Since the increased pressure elevates the boiling point of the solvent (CH_2Cl_2), an excessive temperature (100 °C) could be applied for the reaction.

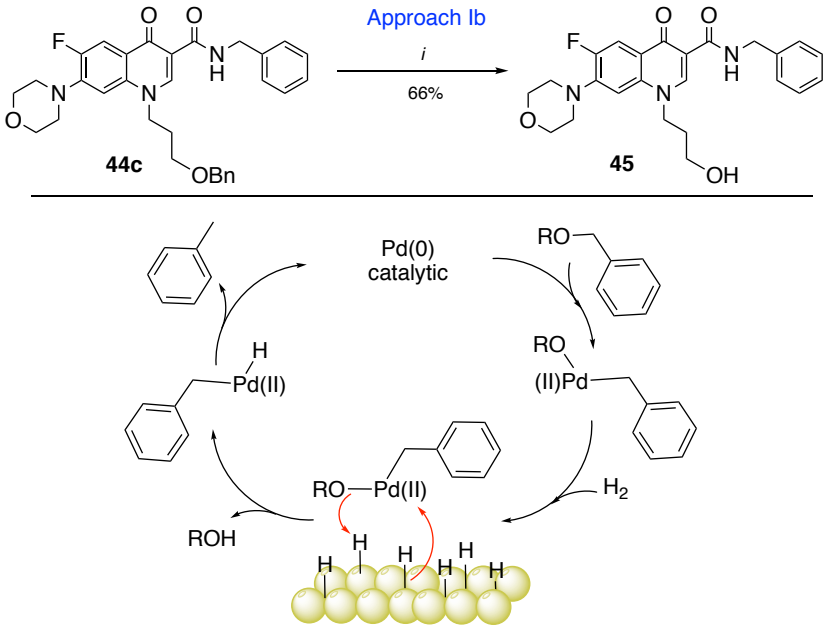


Figure 37: *Synthesis of compound **45** and the mechanistic consideration of the heterogeneous catalytic transfer hydrogenation. Reagents and reaction conditions: i) Pd/C, H_2 (25 bar), $CHCl_3$, MW, 100 °C. Adapted from ref. 76.*

c) Approach II

In another attempt the benzyl residue in the *N*-1 position of **42c** was removed by hydration with H_2 and catalytic amount of Pd/C. Compound **46** was obtained in a yield of 41% (Figure 38). However, the subsequent reaction of **46** with 3-brompropan-1-ol according to the protocol of Koga *et al.*[20] did not work, and merely the starting product was isolated.

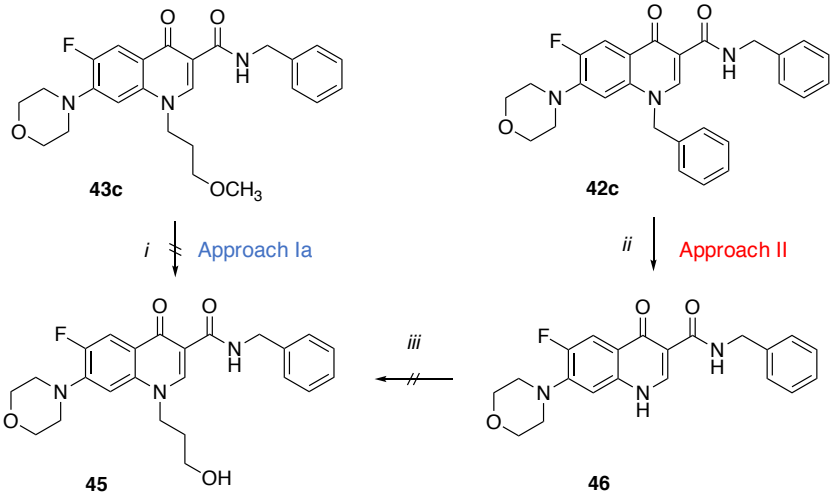


Figure 38: *Failed attempts to cleave the methoxypropyl ether in **43c** (approach Ia). Synthesis pathway to give **45**. The introduction of 3-hydroxypropyl moiety into compound **46** was not successful (approach II). Reagents and reaction conditions: i) BBr_3 (1 M solution in CH_2Cl_2), abs. CH_2Cl_2, 0 °C-rt; ii) Pd/C, H_2 (10 bar), $MeOH/CHCl_3$, rt; iii) 3-bromopropan-1-ol, K_2CO_3, abs. DMF, 90 °C.*

*4.4.3 Synthesis of the Precursor **S***

In the next step the primary alcohol of compound **45** was transformed to the reactive sulfonic esters (e.g., OTs and OMs) obtaining the precursor **S**. Adhering to approach I (Figure 35) the precursor **S** is a pivotal stage for radiolabelling.

According to the standard protocol[77] for the production of reactive sulfonates, *p*-toluenesulfonyl chloride was reacted in the presence of pyridine and an apolar solvent. However, since the reagent **45** was hardly soluble in certain solvents abs. DMF was used for the reaction approach (*a*, Figure 39). Unfortunately, the desired compound **U** was not isolated. Pyridine takes the hydrogen atom of the intermediate **T**, forming the pyridinium chloride and the desired tosylated compound **U**. Unintentionally, the reaction proceeded and the highly activated *p*-toluenesulfonic ester **U** was obviously attacked by the chloride ion, obtaining **47** in yields of 38%. The presumed mechanism is substantiated by the fact that polar aprotic solvents are supposed to accelerate such nucleophilic reactions. The cations (pyridinium ions) are well solvated by the aprotic solvents, while the chloride anions are exposed and thus perfectly act as nucleophiles. Recently, this undesired side reaction of tosyl chloride was exploited as mild approach for the transformation of alcohols into chlorides.[78] But even if the apolar dichloromethane was used in combination with trimethylamine, the chloride ions acted as nucleophile again (*b*, Figure 39). The undoubted characterisation of **47** was performed by LC-MS (calculated *m/z* 457.2 $[M + H]^+$, found *m/z* 458.7 $[M + H]^+$) in combination with NMR spectroscopy. The ^{13}C data confirmed the typical shift for **C**H_2-Cl (53.0 – 37.0 ppm) at δ = 40.1 ppm.

Since the continuing reaction of the tosylated compound could not be stopped at the definite stage (**U**), a high degree of reactivity of the leaving group could be claimed. Accordingly, a less reactive leaving group would be appropriate to circumvent undesired nucleophilic attacks. Hence, compound **46**, methanesulfonyl chloride, and triethylamine were dissolved in abs. CH_2Cl_2 to give the corresponding mesylate **48** in a yield of 52% (Figure 40).

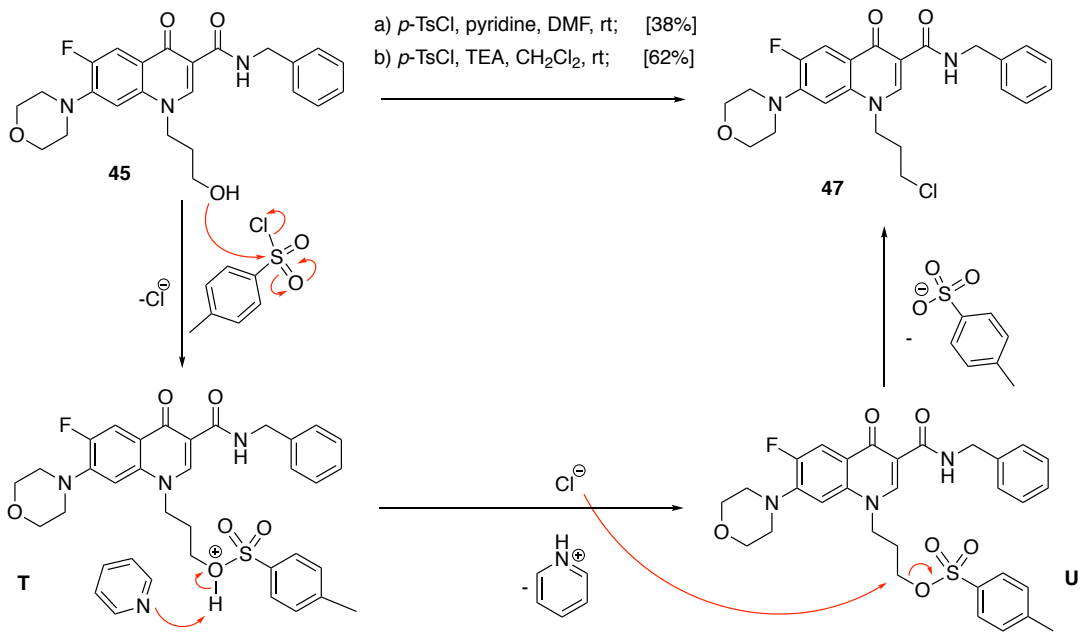


Figure 39: *Proposed mechanism of the unintentionally synthesized compound* ***47****.*

4.4.4 Synthesis of the Reference Compound ***49***

For verification purposes of the radiosynthesis, a non-radioactive 'cold' reference substance is requested. Consequently, the reference compound should possess a 3-fluoropropyl residue in position *N*-1, identically to the proposed radiolabelled compound. According to the protocol of Middleton[79], compound **46** was reacted with the nucleophilic fluorination agent diethylaminosulfor trifluoride (DAST, Table 3) to achieve the counterpart compound **49** (Figure 40). The primary alcohol nucleophilically attacks the sulfur atom and hydrogen fluoride is released. Hereafter, the formed alkoxyaminosulfur difluoride intermediate is subsequently nucleophilic attacked by fluoride. Compound **49** was obtained in a yield of 53%.

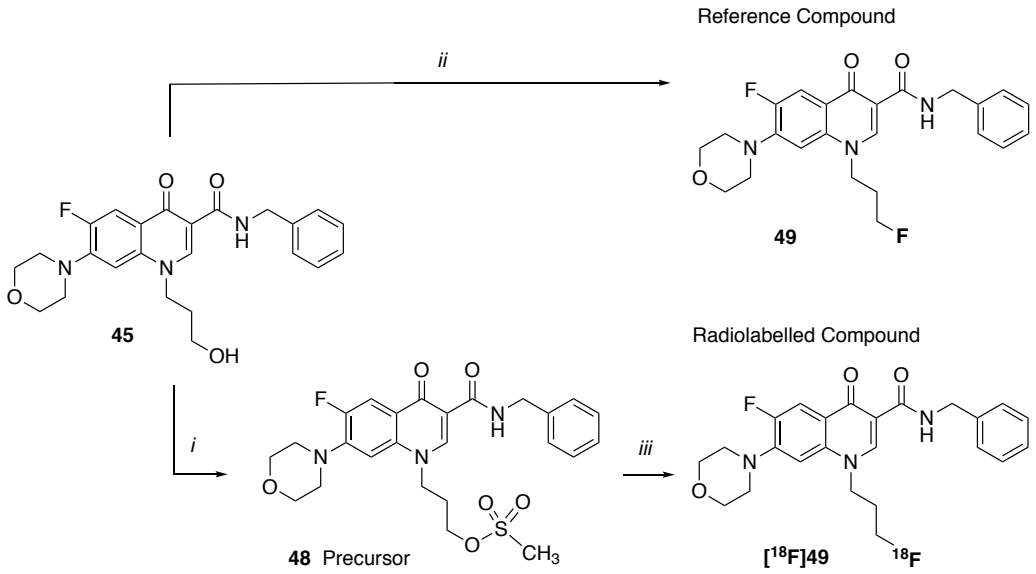


Figure 40: *Direct fluorination using the fluorinating reagent DAST, and the preparation of the precursor* ***48*** *with the subsequent radiofluorination. Reagents and reaction conditions: i) MsCl, TEA, abs. CH_2Cl_2, 0 °C-rt; ii) DAST, abs. CH_2Cl_2, 0 °C-rt; iii) $K^{18}F$, Kryptofix 2.2.2, ACN, 120 °C.*

4.4.5 Synthesis of the Radiolabelled Compound [^{18}F]49

The mesylated precursor **48** with its prosthetic group, was radiofluorinated according to a standard protocol using Kryptofix 2.2.2 and $K^{18}F$ (Figure 40). The purification was carried out by radio-HPLC and the radioactive fraction was collected (Figure 41 (A)). **[^{18}F]49** was afforded in a radiochemical yield of 60%. HPLC co-injection of the unlabelled compound **49** (t_R = 8.9 min) and the radiolabelled compound **[^{18}F]49** (t_R = 9.4 min), using UV trace in blue and radiotrace in red, confirmed the identity and radiochemical purity of the radiotracer (Figure 41 (B)).

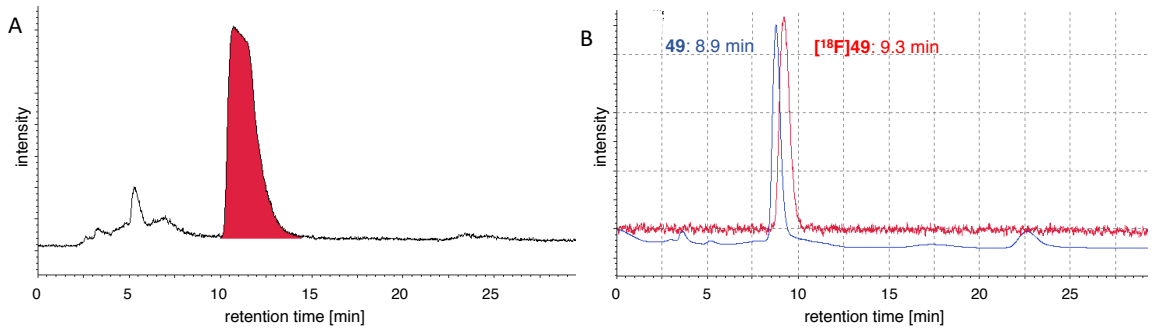


Figure 41: *A) Preparative radio-HPLC chromatogram of the reaction mixture; B) Co-injection of the unlabelled* ***49*** *(blue) compound with the purified radiofluorinated counterpart* ***[^{18}F]49*** *(red). Reproduced from ref. 80. Copyright © 2018 Elsevier Masson SAS. All rights reserved.*

4.5 Small-Animal PET

As mentioned in prior section adequate therapies of stage II trypanosomiasis must inevitably pass the BBB. For this purpose, the labelled quinolone amide **[^{18}F]49** (19.3±2.3 MBq) was administered into two B6/J mice via tail vein under 1.5% isoflurane anesthesia. Small-animal PET scans were performed after 10 min and 60 min post injection (pi). The labelled compound could not be traced in brain areas, neither after 10 min nor after 60 min pi. Instead, **[^{18}F]49** was predominantly detected rather quickly in the liver and the kidney, suggesting a high metabolic turnover (Figure 32). These pharmacokinetic results were rather unexpected since the quinolone amides were supposed to be metabolic stable ($t_{1/2}$ [GHQ168] = 5.8 h)[8].

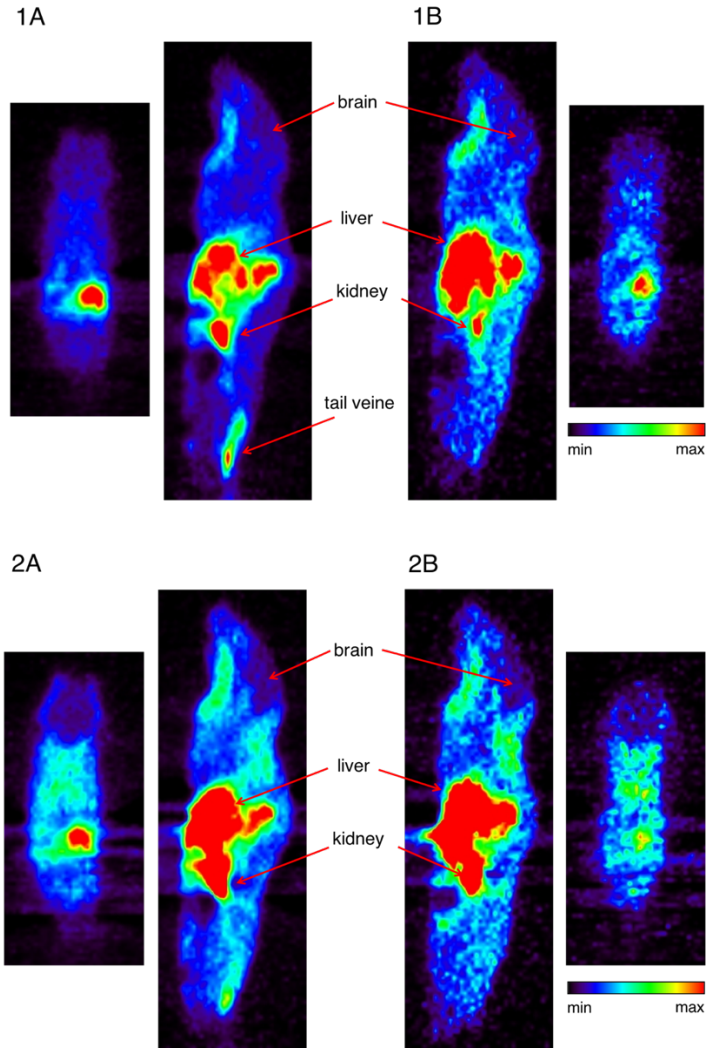


Figure 42: *PET scans of mouse I (1A) and mouse II (2A) after 10 min post injection. Maximal concentration of* ***[^{18}F]49*** *is detected in the liver and the kidney, but not in the area of the brain. Similarly, in the PET scans after 60 min post injection (1B and 2B), the radiolabelled compound could apparently not be traced in the brain.*

4.6 Ex vivo Autoradiography

After the PET experiments, both mice were sacrificed and the murine brain was dissected free. The brain was sectioned (3 mm) in sagittal direction (Figure 43) using a cryostat and was exposed on a phosphor image plate overnight. The image plate was read out on an image plate reader and data were analysed. The *ex vivo* autoradiography demonstrated the permeation of **[^{18}F]49** through the BBB in both mice (A, B, Figure 44). The labelled compound accumulated within the entire brain in medium concentrations (indicated by green). Additionally, the red colored regions in the inner brain sections (A: 2,3) designate even high concentrations of the quinolone amide. These images finally approve the transfer of quinolone amides over the healthy murine BBB.

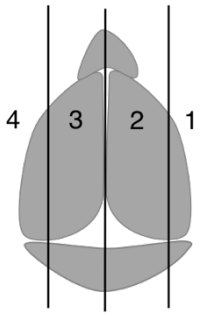


Figure 43: *Schematic murine brain in dorsal view with the particular sections (1-4).*

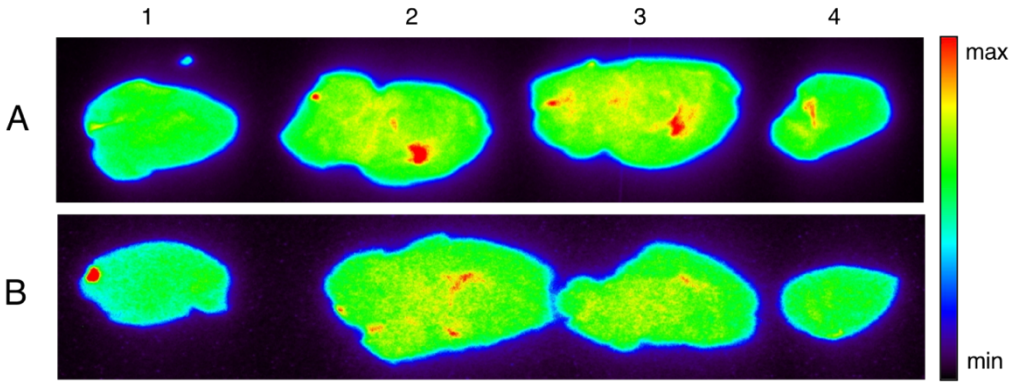


Figure 44: *The ex vivo autoradiography of the murine brain sections (1-4) in sagittal direction of two mice (A and B). Red areas indicate high concentration of the labelled quinolone amide* ***[^{18}F]49****. Reproduced from ref. 80. Copyright © 2018 Elsevier Masson SAS. All rights reserved.*

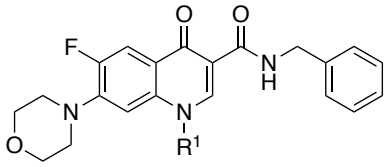
5 Structure-Activity Relationship of the Quinolone Amides

The synthesised compounds were examined for their biological properties within the SFB 630. A. Fuß (Medical Mission Institute, Würzburg) and J. Skaf (AK Holzgrabe) screened the compounds for their antitrypanosomal activity. The assay was conducted with the *T. brucei brucei* laboratory strain TC 221 indicating the viability of the trypanosomes by AlarmarBlue®. The respective IC_{50} values indicate the half maximal inhibitory growth concentration after 48 h. Additionally, cytotoxicity was examined against the macrophage cell line J774.1 with AlarmarBlue®. The cytotoxic effects are indicated in the CC_{50} value (i.e. concentration for half maximal growth inhibition).

We applied a drug repurposing strategy in combination with a phenotypic 'medium' throughput screening. Pursuing these respective approaches resulted in drug candidates which effectively take effect on living organism. However, the major drawback is the challenge in target elucidation after Hit-identification. Hence, the mode of action of the quinolone amides towards the trypanosomes is still unidentified. Nevertheless, the insight into SAR should be expanded by different substitution patterns herein. In particular the fluorine substitution pattern at the quinolone core was considered.

5.1 *N*-1 Position

Hiltensperger *et al.*[7] compared different alkyl chain lengths for their activity and claimed that the *n*-butyl residue is the most active option. Nevertheless, further options for the *N*-1 substitution were evaluated herein (Table 4). The cyclopropyl ring in **41** (derived from ciprofloxacin) could generally contribute to versatile features (e.g., enhancing potency).[81] In the quinolone amide **41**, however, these three coplanar carbon atoms did not contribute toward increased potency. The more 'bulky' benzyl residue in **42c** resulted in reduced activity against *Trypanosoma*. However, the omission of the entire substituent in the *N*-1, e.g. in the case of compound **46**, suffered the greatest loss of activity (IC_{50} = 1.90 μM). The hydroxypropyl residue led to a moderate trypanocidal activity of **45** (IC_{50} = 0.27 μM). As discussed in 5.4, the C-OH bond is considered to be bioisoster to the C-F bond.[82] Accordingly, compound **49** with the fluoropropyl residue exhibited similiar antitrypanosomal activity (IC_{50} = 0.12 μM).



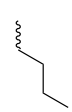
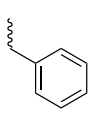
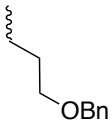
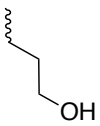
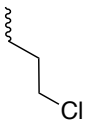
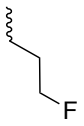
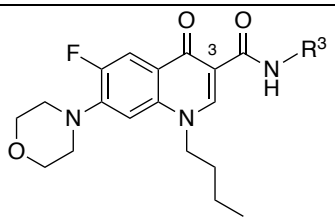
R^1 =								
	GHQ168	**41**	**42c**	**44c**	**45**	**46**	**47**	**49**
IC_{50} [μM] ***T.b.brucei***	0.047 ± 0.00	0.17 ± 0.06	1.09 ± 0.10	ND*	0.27 ± 0.07	1.90 ± 0.18	ND*	0.12 ± 0.06
CC_{50} [μM] **J774.1**	57	43	>100	>100	43	>100	51	42

Table 4: *Biological properties of quinolone amides with diverse substituents in the N-1 position. *not determined due to poor solubility in test medium;*

5.2 C-3 Position

In opposition to the carboxylic acid group, the benzylamide moiety in position 3 of the quinolones is pivotal for the antitrypanosomal activity (Table 5).[7] Investigating the impact of the fluorine atom revealed that *para*-fluorine (**23**) was well-tolerated regarding the biological property (IC_{50} = 0.05 μM). However, *ortho*-fluorine substituent (**25**) reduced the activity against *T. b. brucei* to an eighth of the **GHQ168**'s activity (IC_{50} = 0.41 μM). Interestingly, the nanomolar antitrypanosomal activity could be recovered and even slightly improved (IC_{50} = 0.03 μM) by the combined substitution pattern of both *ortho*- and *para*-fluorine (**26**). The thiophene ring, commonly considered bioisostere to the phenyl ring, could not be examined for its antitrypanosomal activity due to poor solubility in the test medium.



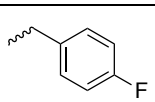
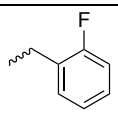
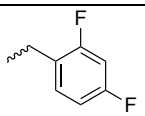
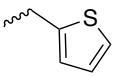
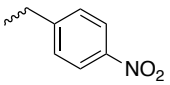
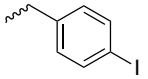
R^3 =						
	23	**25**	**26**	**27**	**28**	**29**
IC_{50} [μM] ***T.b.brucei***	0.05 ± 0.00	0.41 ± 0.07	0.03 ± 0.00	ND*	0.08 ± 0.04	ND*
CC_{50} [μM] **J774.1**	>20**	37	60	34	20	>100

Table 5: *Biological properties of quinolone amides with diverse substituents in position 3. *not determined due to poor solubility in test medium. **higher concentration could not be tested due to precipitation.*

5.3 C-5 Position

The chemical modifications of the fluoroquinolones against bacteria were predominantly focused on *N*-1 position and C-7 in the early days. Little attention has been paid to substituents in position 5 as a potential influencing factor until Miyamoto *et al.*[41] discovered sparfloxacin with its improved potency. Since the C-5 amino moiety was apparently beneficial for the antibacterial activity, this effect should be evaluated for the quinolone amides against *T. b. brucei* (Table 6).

Unfortunately, the synthesis of the corresponding compound **Q** (3.7.1) could not be accomplished. However, other substituents in C-5 were tested (**6**, **13-18**). The fluorine atom in position 5 (**6**) was well-tolerated regarding potency (IC_{50} = 0.05 μM) and even reduced cytotoxic effects (CC_{50} >100 μM). Hence, compound **6** showed a promising safety profile and high selectivity (expressed by selective index (SI) = CC_{50}/IC_{50} = >2000). In contrast to this, modestly sized residues like the dimethyl amino group (**13**) and the methoxy group (**18**) led to decreased activities (IC_{50} = 1.85 μM and IC_{50} = 0.76 μM, respectively). It was previously reported for quinolone carboxylic acids that methyl group of these groups are out of plane of the quinolone core.[83] Hence, it was supposed that the formation for **13** and **18** is likewise. Thus, the adverse effect on activity might be attributed to the steric bulkiness of these groups. The potency of **16** was consistent with this finding as the most sterically demanding residue (morpholine in C-5) was least active (IC_{50} = 5.68 μM).

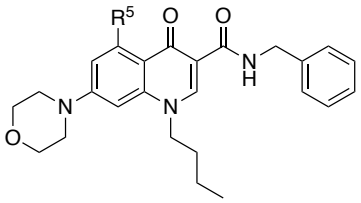
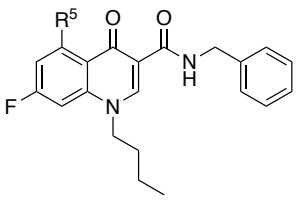
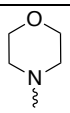
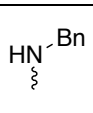
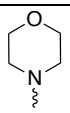
							
R^5 =	F	$N(CH_3)_2$			OCH_3	$N(CH_3)_2$	
	6	**13**	**16**	**17**	**18**	**14**	**15**
IC_{50} [μM] ***T.b.brucei***	0.05 ± 0.01	1.85 ± 0.26	5.68 ± 0.02	ND*	0.76 ± 0.01	16.3 ± 1.62	16.8 ± 0.09
CC_{50} [μM] **J774.1**	>100	44	ND*	ND*	52	>100	>100

Table 6: *Biological properties of quinolone amides with various substituents in position 5. *not determined due to poor solubility in test medium.*

Related compounds **14** and **15** had no morpholine in C-7 but a bulky residue in position 5 (dimethyl amino and morpholine, respectively). This series of compounds repeatedly confirmed the pivotal impact of morpholine in position C-7 for the potency towards *Trypanosoma*. Therefore, compounds **14** and **15** exhibited considerable lower activities (IC_{50} = 16.3 μM and IC_{50} = 16.8 μM).

5.4 C-6 Position and Fluorine Walk around the Quinolone Core

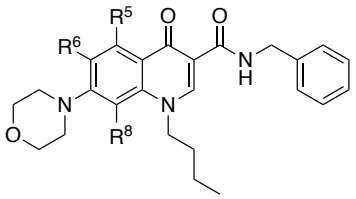
In terms of antibacterial activity, the fluorine atom in C-6 is associated with improved potency and was eponymous for the entire compound class (*fluoro*quinolones). Fluorine in position 6 of the quinolone amides also possessed appropriate properties against *Trypanosoma* as demonstrated for **GHQ168**.[7] Here, various fluorine substitution patterns were analysed (Table 7).

In medicinal chemistry hydrogen and fluorine atoms are commonly interchangeable and thus used as bioisosteres. However, in terms of size, bond length, and electronic effects the two atoms are fairly different. The van der Waals volume of the fluorine atom is about approx. twofold larger than the volume of the hydrogen atom (7.2 $Å^3$ vs. 13.3 $Å^3$).[82] Additionally, the length of C-F (1.35 Å) has closer similarity to the C-O bond (1.43 Å) than to the C-H bond (1.09 Å).[84] Moreover, the electronegativities of the two atoms vary in 1.78 causing the highly polarised C-F bond.[82] Because of these obvious differences, further substituents (e.g., OCH_3, CF_3) besides fluorine were tested in position 6.

First, the fluorine atom was left out of the molecule (**9**) denoted as 6-desfluoroquinolone amide. The strategy of nonfluoroquinolones was already successfully pursued for antibacterial applications, e.g., for nemonoxacin (recent approval in Taiwan and currently in clinical phase III in the U.S.).[85] However, compound **9** did not benefit from omitting fluorine and the activity was less comparing with **GHQ168** (IC_{50} = 0.23 μM). Consequently, the fluorine atom in C-6 apparently contributed to the trypanocidal activity of the quinolone amides.

The methoxy group in position 6 (**10**) is undoubtedly a more voluminous residue than fluorine, but the two atoms already demonstrated bioisosteric features in thrombin inhibitors[84,86] and in the approved drug ezetimibe[87,88]. Both groups (C-F and C-OCH_3) are considered to be electron acceptors, even though the methoxy moiety possesses a substantially higher proton affinity.[84] The biological evaluation of the quinolone amide **10** revealed a reduced antitrypanosomal activity (IC_{50} = 0.31 μM). Hence, a stronger H-bond acceptor did not lead to higher trypanocidal activities.

The trifluoromethyl residue in position 6 (**12**) poses an even more sterically demanding substituent (van der Waals volume: 39.8 $Å^3$ (CF_3), 13.3 $Å^3$ (F)), which is comparable to an ethyl group (van der Waals volume: 38.9 $Å^3$).[82] This bulky and lipophilic moiety disadvantageously influenced the biological activities since compound **12** merely exhibited an IC_{50} = 54.0 μM.



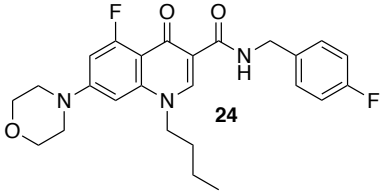
R^5 / R^6 / R^8 =	$R^5 = R^6 = H$ $R^8 = F$	$R^5 = H$ $R^6 = R^8 = F$	$R^5 = R^6 = F$ $R^8 = H$	$R^5 = R^6 = H$ $R^8 = H$	$R^6 = OCH_3$ $R^5 = R^8 = H$	$R^6 = CF_3$ $R^5 = R^8 = H$
	5	**7**	**8**	**9**	**10**	**12**
IC_{50} [μM] ***T.b.brucei***	0.79 ± 0.06	0.06 ± 0.00	0.04 ± 0.02	0.23 ± 0.02	0.31 ± 0.16	0.54 ± 0.01
CC_{50} [μM] **J774.1**	>25**	>20**	>25**	>100	42	79

Table 7: *Biological properties of quinolone amides with various substituents in position 6 and different fluorine susbstitution patterns. **higher concentration could not be tested due to precipitation.*

Next, the 'fluorine walk' was performed varying the fluorine substitution pattern of the quinolone core. The fluorine atom in C-6 was relocated to position C-8 (**5**) and resulted in decreased activity (IC_{50} = 0.79 μM). The loss in activity might be based on the missing substituent in position 6 as it was observed for **5**. Additionally, fluorine in C-8 seemed to negatively influence the biological activity. The 6,8-difluoro quinolone amide **7** showed restored antitrypanosomal activity (IC_{50} = 0.06 μM).

Compound **6** (discussed in the previous section) with fluorine in C-5 still exhibited the extraordinary activity against *T. b. brucei* (IC_{50} = 0.05 μM), even though fluorine in position 6 was absent. Hence, the fluorine atom in the quinolone amides was still pivotal, but either the C-5 or C-6 positions for fluorine substitution were selectable. Interestingly, this particular F-5 substitution pattern showed no cytotoxic effects (CC_{50} = >100 μM). Unfortunately, these results could not be substantiated in extended cytotoxic tests towards hepatocytes and nephrocytes, respectively, due to precipitation in test medium. The benefit from a double fluorination in 5,6-difluoro substituted quinolone amide **8** was only slightly beneficial (IC_{50} = 0.04 μM).

Finally, the most promising substitution patterns of compounds **6** (fluorine in position 5) and **23** (*p*-fluorobenzylamide) were combined in compound **24**. Thus, the antitrypanosomal activity was raised to an IC_{50} = 0.02 μM. No cytotoxic effects could be observed up to concentration of 25 μM (CC_{50} = >25 μM).



24

Figure 45: *Promising quinolone amide with improved biological activity.*

Furthermore, compound **24** exhibited improved metabolic stability (6.2) and promising physicochemical properties (6).

5.5 C-7 Position

The initial investigation of Hiltensperger *et al.*[7] concerning the influence of various substituents in position C-7 of the quinolone amides revealed the morpholine ring as pivotal element for their activities. To date, no other residues in position 7 could exceed this trypanocidal activity. The previously performed SAR analysis suggested that rather small moieties comprising a hydrogen bond acceptor would be favourable.[7] Hence, compound **20**-**22** were considered potentially appropriate to meet these demands (Table 8). However, both the thiomorpholine (**20**) and the 2,4-dimethyl morpholine (**21**) groups diminished the activity against *T. b. brucei* in comparison to **GHQ168** (IC_{50} = 0.44 μM for **20** and IC_{50} = 0.17 μM for **21**).

Aryloxy residues and hydroxyl in position C-7, respectively, were examined in compounds **19**, **30**, and **31**. Compound **19** could not be tested due to inadequate solubility and compound **30** showed no activity against the trypanosomes. A benzyloxy group in position 7 has already shown advantageous impact on the antitrypanosomal activity of quinolones.[3] Unfortunately, no statement about the biological properties of compound **31** could be made since the problem of low solubility occurred once more.

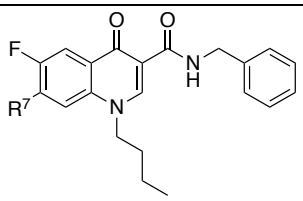
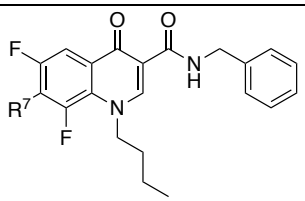
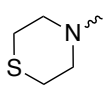
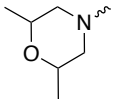
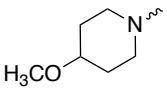
						
R^7 =				OEt	OH	OBn
	20	**21**	**22**	**19**	**30**	**31**
IC_{50} [μM] ***T.b.brucei***	0.44 ± 0.16	0.17 ± 0.03	0.24 ± 0.05	ND*	>40	ND*
CC_{50} [μM] **J774.1**	>20**	>20**	>20**	>100	>100	ND*

Table 8: *Biological properties of quinolone amides with various substituents in position 7. *not determined due to poor solubility in test medium. **higher concentration could not be tested due to precipitation.*

5.6 *N*-8 and Commercially Available Quinolones

The first compounds of the quinolone antibiotics (e.g., nalidixic acid) were, in fact, 1,8-naphthyridones. These first-in-class compounds lack potency against Gram-positive organisms, but the progressive fluoroquinolones (4-oxo-quinolone core) of advanced generations have been improved. Nevertheless, recently developed *quinolone* antibiotics returned back to the 1,8-naphthyridone core (e.g., zabofloxacin). According to this strategy, the respective 1,8-naphthyridones were transformed into their corresponding amide investigating their antitrypanosomal potency (Table 9).

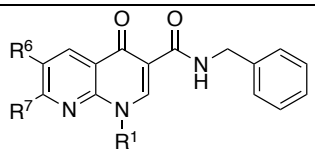
Core	**1,8-naphthyridone**		
			
R^1 / R^6 / R^7 =	R^1 = Et R^6 = H R^7 = Me	R^1 = Et R^6 = F R^7 = piperazinyl	R^1 = *n*-butyl R^6 = F R^7 = morpholinyl
	38	**39**	**37**
IC_{50} [μM] ***T.b.brucei***	ND*	3.51 ± 0.67	0.49 ± 0.11
CC_{50} [μM] **J774.1**	>100	40	>20**

Table 9: *Biological properties of 1,8-naphthyridone amides. *not determined due to poor solubility in test medium. **higher concentration could not be tested due to precipitation.*

Compound **38** derived from nalidixic acid was poorly soluble and activity evaluation was not possible. However, antitrypanosomal activity was assumed to presumably be rather low since a saturated heterocycle (e.g., morpholine) is missing in position 7. Compound **39** derived from enoxacin exhibited only moderate activity (IC_{50} = 3.51 μM). Comparing to **39**, compound **37** is structurally more similar to the lead **GHQ168** (morpholine in C-7 and *n*-butyl in *N*-1). Despite this similarity, **37** did not surpass the trypanocidal activity of the lead (IC_{50} = 0.49 μM). Thus, the nitrogen atom in position 8 did not advantageously impact the biological activity.

6 Physicochemical and Pharmacokinetic Properties

The physicochemical properties of a potential orally available drug candidate is generally expressed by the Lipinski's rule of 5[89] (MW < 500 g/mol, clogP < 5, HBA < 10, HBD < 5) and the criteria postulated by Veber *et al.*[90] (PSA < 140 Å^2 and RB < 10). These parameters strongly affect the ADME (**a**bsorption, **d**istribution, **m**etabolism and **e**xcretion) properties of a substance and are highly related to drug-likeness. Despite the quinolone amides met these features, the intrinsic solubility of this compound class was 'practically insoluble' according to Ph. Eur.[8,91]. This result was traced back to the compact arrangement within the crystalline structure and the strong intermolecular interaction. Hence, the resulting high crystal lattice energy negatively influences the dissolution rate.[8]

However, the lipophilicity and the intrinsic solubility of the novel quinolone amides are assessed herein. In particular the influence of the fluorine atom to the physicochemical properties was considered.

6.1 logP

The octanol-water partition coefficient logP describes the ratio of concentrations of a compound in two immiscible solvents (*n*-octanol and water; logD for pH 7.4). It is a tempting optimization strategy increasing the lipophilicity of the ligand, as it is often accompanied with improved *in vitro* potency. Furthermore, a higher lipophilicity is associated with a greater CNS exposure which is particularly interesting for addressing African Trypanosomiasis stage II.[92] However, an increased lipophilicity might include a greater likelihood for poor solubility, metabolic instability and lack of selectivity.[92,93] The logP values were measured experimentally using an HPLC method (10.11).

The influence of a fluorine substituent on the lipophilicity of the quinolone amides was evaluated. An additional fluorine atom at an aromatic ring generally leads to a more lipophilic compound.[87] The overlap between the fluorine orbitals with the corresponding orbital on carbons, presumably compensates the originally electron-withdrawing influence of the fluorine atom.[94] This effect holds true for compounds **7** and **23**, as lipophilicity was increased compared to **GHQ168** (ΔlogP = 0.47 and ΔlogP = 0.03).

However, there are exceptions wherein the fluorine is in close vicinity to an oxygen atom (distance F···O smaller than 3.1 Å) and the molecule consequently becomes more hydrophilic.[87] This observation was made for compound **6** and was likely attributable to two factors. The fluorine substituent in position 5 nearby the oxygen atom of the carbonyl group might increase the overall polarity of the quinolone molecule.[87] Additionally, the

electron-withdrawing fluorine might polarise the oxygen atom due to the particular proximity. Therefore, the established hydrogen bonds between the surrounding water molecules and the oxygen atom were strengthened.[87] Accordingly, the mono fluorinated **GHQ168** (logP = 4.10) possessed a higher logP value compared to the double fluorinated compounds **8** (ΔlogP = -0.74) and **24** (ΔlogP = -0.69), respectively.

As opposed to this, the introduction of a fluorine or a trifluoromethyl substituent into a saturated alkyl group usually decreased the compound's lipophilicity. The strongly polarised C-F and C-CF_3 bonds induce a dipole and consequently reduce lipophilicity.[95] This effect holds true for compound **49** since logP value was lowered comparing to **GHQ168** (ΔlogP = -0.76). In comparison, the 3-hydroxypropyl residue at the *N*-1 position lead to a considerable decrease in the lipophilicity (logP = 3.04).

6.2 Metabolism

Pharmacokinetics generally describe the various steps of chemical xenobiotics in the living organism. The relevant processes are described in the ADME scheme and thus, metabolic stability can determine the bioavailability of drugs.

After oral administration and resorption in the gastro-intestinal tract, the compound is transported via portal vein to the liver. The xenobiotics are metabolised in a Phase-I biotransformation, predominantly in the liver. Lipophilic drugs are transformed into more polar metabolites in the hepatic microsomes (containing cytochrome P450-dependent monooxygenases) and in the cytosol (containing hydrolases and aldehyde dehydrogenases). Particular enzymes modify the absorbed compounds by oxidation, reduction and hydrolysis and the metabolites are often conjugated subsequently with endogenous substrates like e.g., glucuronic acid and

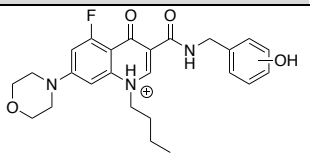
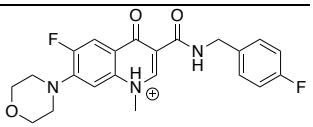
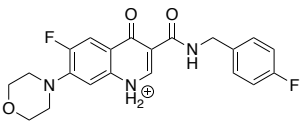
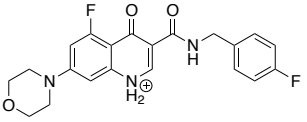
Parent Compound	Metabolite
6	 **M1**: m/z [M+H$^+$] 454.21
23	 **M2**: m/z [M+H$^+$] 414.16
	 **M3**: m/z [M+H$^+$] 400.15
24	 **M4**: m/z [M+H$^+$] 400.15

Table 10: *In vitro metabolism studies for compound **6**, **23**, **24** revealed structures **M1-4** that represent the major metabolites of the particular quinolones amides.[80]*

glutathione (phase II biotransformation). Phase-I metabolism of drug candidates can be simulated *in vivo* by liver microsomes (human and rat S9 liver enzymes) as they contain the particular CYP enzymes. Both the liver microsomes and the cytosolic metabolism were investigated and discussed herein.

The metabolites of the lead compound **GHQ168** and the respective half-life ($t_{1/2}$ = 5 h 46 min) were examined in previous studies.[8] Hydroxylated metabolites, presumably at the benzyl residue, were identified. To address this issue the well-established tool of fluorine substitution as direct replacement for a hydrogen atom was used. The substitution with fluorine at the metabolically labile benzyl site should prevent certain metabolism, based on the fact that the C-F bond is less prone to oxidative processes than the C-H bond. Moreover, the electron-density of an entire aromatic group can be reduced by fluorine and thus, being less susceptible towards oxidation.[60]

The following metabolism studies with compounds **6**, **23**, **24**, were carried out by C. Erk of our research group (AK Holzgrabe). The predominantly formed metabolites are depicted in Table 10. The investigation of compound **6** revealed eight metabolites, which mainly occurred due to aromatic and aliphatic hydroxylation (Figure 46). Additionally, the m/z values indicated the presence of an oxidative *N*-dealkylation in the *N*-1 position. The hydroxylated benzyl moiety **M1** emerged from compound **6** in highest proportion.[80]

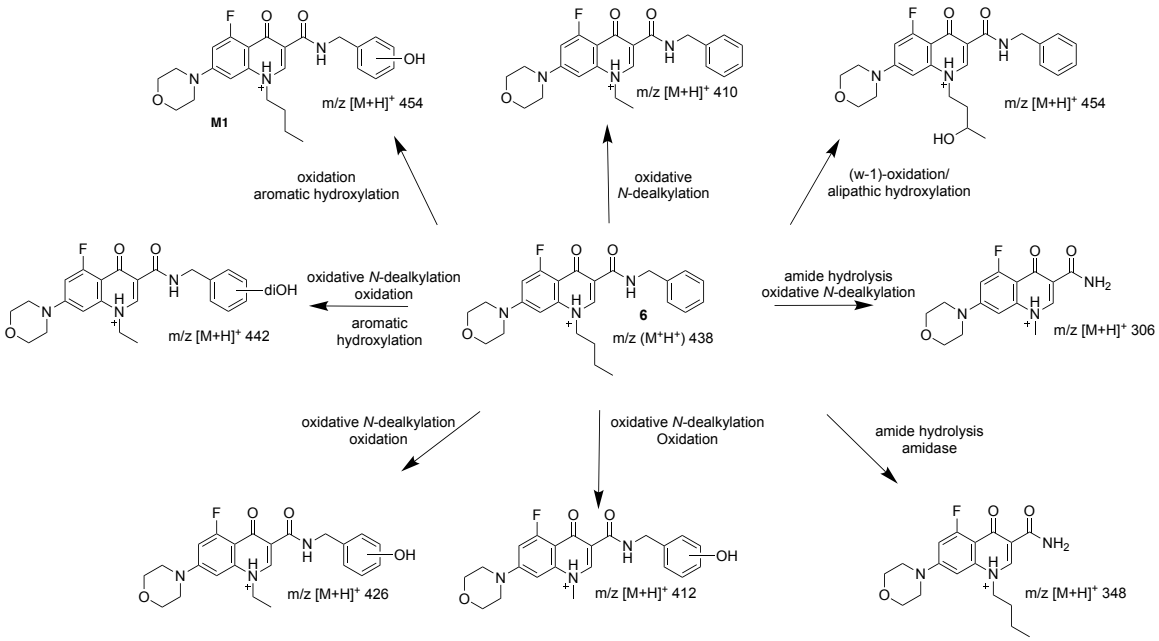


Figure 46: *Metabolic pathway of compound **6**. Reproduced from ref. 80. Copyright © 2018 Elsevier Masson SAS. All rights reserved.*

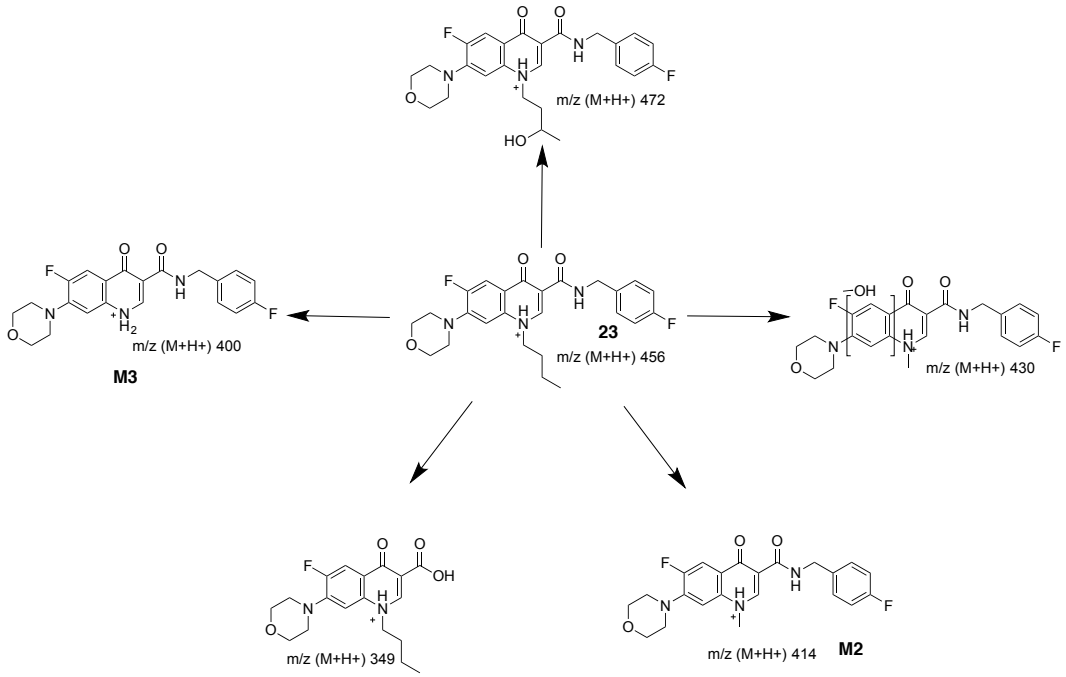


Figure 47: *Metabolic pathway of compound **23**. Reproduced from ref. 80. Copyright © 2018 Elsevier Masson SAS. All rights reserved.*

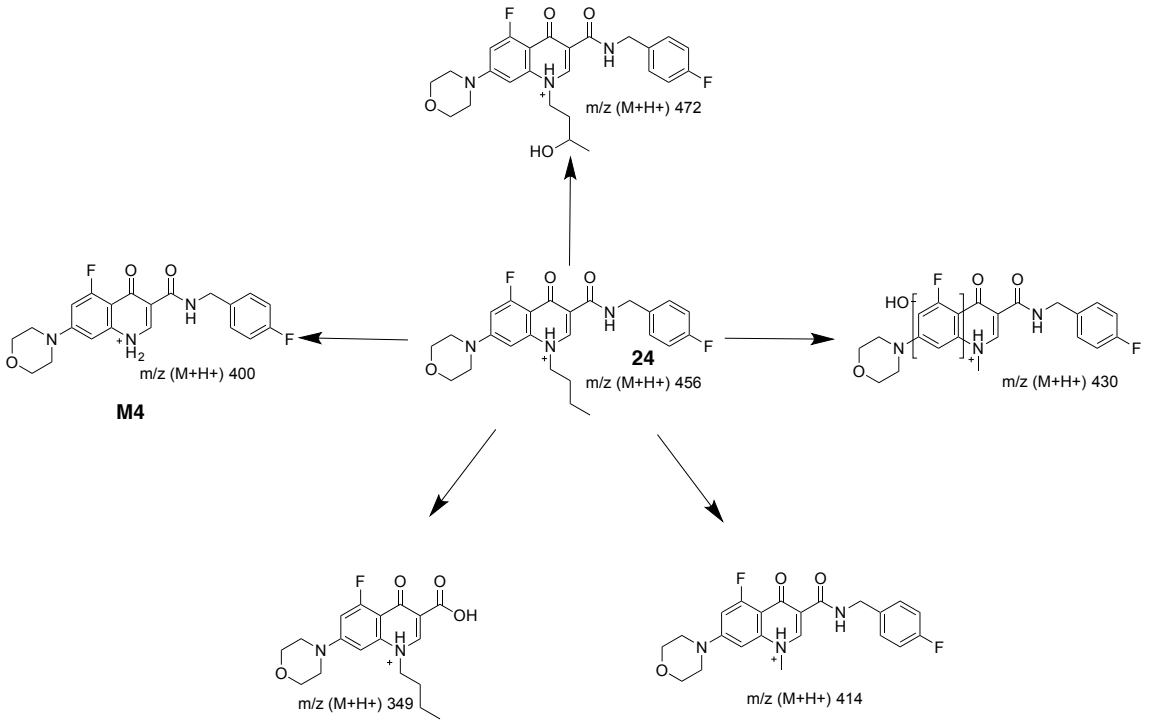


Figure 48: *Metabolic pathway of compound **24**. Reproduced from ref. 80. Copyright © 2018 Elsevier Masson SAS. All rights reserved.*

The aromatic ring in compound **23** and **24** is stabile towards metabolism due to *para* fluorine substitution. As expected, no hydroxylation at the benzyl moiety was noticed (Figure 47 and 48). Hence, the most dominating pathway for these compounds was the *N*-desalkylation resulting in metabolites **M2**, **M3** and **M4**.

Additionally, the microsomal turnover with male rat microsomes (M9566, Sigma Aldrich) was determined for compounds **6**, **23**, **24**. On the assumption of a hepatic elimination obeying a first-order kinetics, the elimination rate constant k_e can be calculated from the following equation:

$$\ln c_t = -k_e \times t + c_0$$

The elimination rate constant k_e and the resulting half-lifes $t_{1/2}$ are summarised in Table 11. In comparison to the lead compound **GHQ168**, the k_e values of the novel synthesised quinolone amides declined. Hence, the respective half-life could be prolonged for compounds **6**, **23**, and **24**. The additional fluorine at the benzyl residue in **23** expectedly enhanced its stability compared to **GHQ168** ($t_{1/2}$ = 5 h 46 min vs. 6 h 25 min). Interestingly, compound **6** was less prone to metabolism only because of the altered fluorine position (F-6 versus F-5). Thus, half-life was extended for 1 h 27 min in comparison to **GHQ168**. Consequently, combining together both fluorine substitution patterns (F-5 and *p*-fluorobenzyl) in **24**, the half-life was prolonged to 7 h 42 min. The hydrogen/fluorine exchanges increased the compound's metabolic stability, without compromising the biological activity (IC_{50} = 0.02 μM).

compound	**GHQ168**	**6**	**23**	**24**
k_e [min^{-1}]	0.0020[8]	0.0016	0.0018	0.0015
$t_{1/2}$	5 h 46 min[8]	7 h 13 min	6 h 25 min	7 h 42 min

Table 11: *The resulting elimination rate constant k_e and half-life $t_{1/2}$ due to the microsomal turnover of **GHQ168**, **6**, **23**, and **24**.*

6.3 Consideration for an *in vivo* Study

The preceding lead compound **GHQ168** was partially effective *in vivo* (3/6 mice were cured), but three mice died after dose administration. Hence, there was a need for a safer and more selective treatment option. Compound **6** was considered for an *in vivo* investigation due to a promising safety profile (CC_{50} = >100 μM, SI = >2000), very high

antitrypanosomal activity (IC_{50} = 0.05 μM), a desirable logP value, and an appropriate half-life ($t_{1/2}$ = 7 h 13 min, k_e = 0.0016 min^{-1}).

Subsequently, an *in silico* prediction of the pharmacokinetics using Simcyp® software was made. The simulation relies on the clearance (Cl) of the compound, which can be calculated from the volume of distribution V_d, the k_e, and the average weight of a mouse (m_{mouse} = 0.025 kg).

$$\mathrm{Cl} = k_e \times V_d \times m_{mouse}$$

According to the method of Berezhkovskiy[96], the software calculated the V_d based on the plasma protein binding and the logP value (logP = 3.36; V_d = 4.89 mL/kg). However, the affinity to plasma proteins could not be practically assessed for the newly developed quinolone amide **6**, as the solubility was too poor. Nonetheless, comparable protein bindings within the compound class of quinolone amides were assumed. This was why the particular protein binding of 80.5% for **GHQ168** was applied for compound **6**.

The clearance was 0.20 mL/min and the resulted simulation is depicted in Figure 49. In accordance to the *in vivo* experiments of **GHQ168** the study was designed for four days with i.p. administration twice a day.[8] The simulation predicted a maximal plasma level of 4.5 μM and a minimal concentration level of 0.5 μM. The latter value surpassed the IC_{50} against *T. b. brucei* by the factor 100. Additionally, drug concentrations of 4.5 μM were supposed not to cause toxic effects according CC_{50} value (CC_{50} = >100 μM). The simulated dose for administration was 3.5 mg/kg and thus a solubility of approx. 88 μg/mL was required (maximal volume of i.p. application was approx. 1 mL). This concentration exceeded the maximal water solubility of compound **6** (S_w = 0.12 μg/mL). Hence, **6** was formulated by analogy with **GHQ168** as spray dried amorphous nanoparticles.[8]

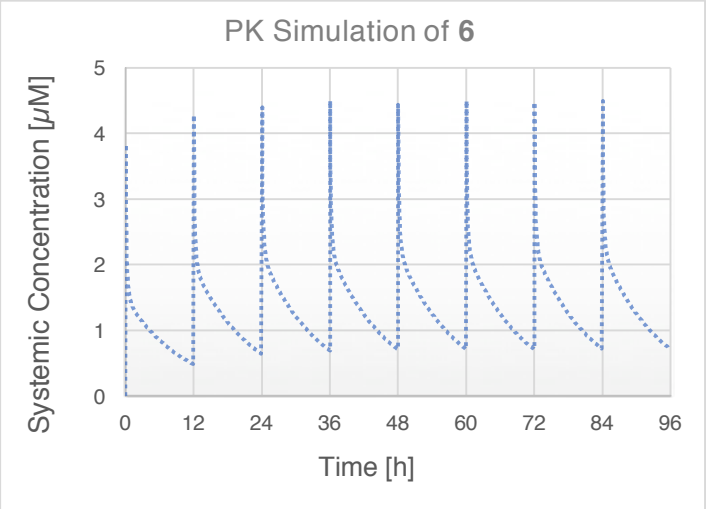


Figure 49: *Plasma concentration of compound **6** simulated by Simcyp® with administration of 3.5 mg/kg for 4 days twice a day.*

*6.3.1 Formulation for Compound **6***

The poorly water soluble quinolone amides challenged former *in vivo* studies, and thus, the lead compound **GHQ168** was previously formulated as an amorphous nanoparticle using the spray drying method.[8] The novel quinolone amide **6** was formulated in analogy with this procedure. Accordingly, compound **6** (30.0 mg) and Eudragit® L100 (270.0 mg) were dissolved in methanol and the solution was spray dried. The performance of this procedure was evaluated using x-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC). Crystalline substances produce characteristic, and sharply formed peaks in the diffractogram, as shown for the raw substance **6** (black, Figure 50). As opposed to this, amorphous materials show broad signals (blue, Spray drying #1). However, in the spray drying batch #2 sharp reflexes are still detectable to some extend (purple). This indicated that the spray drying process was not entirely completed and crystalline molecules were still present. The amorphous character of batch #1 was verified using DSC experiments. In the course of this, a glass transition could be monitored for the amorphous solid dispersion #1 and no sharp melting point was detected.

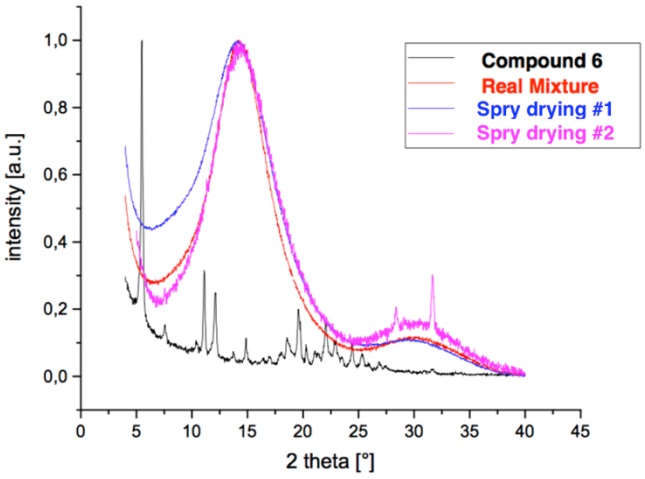


Figure 50: *Diffractogram of the raw compound **6**, the real mixture and spry drying batches #1 and #2.*

The dissolution of the spray drying formulation #1 was assessed using the quantification method described in 10.12. The maximum solubility initially reached 3.8 µg/mL (2 min) and rapidly decreased to 2.1 µg/mL afterwards, and remained at approx. 1.5 µg/mL for at least 360 min. Contrary to this, the raw substance **6** possessed a thermodynamic solubility of 0.12 µg/mL (assessed after 24 h). Hence, the supersaturated state exceeded the thermodynamic solubility of compound **6** by the factor 10. Nevertheless, this solubility enhancement was insufficient for i.p. administration since approx. 88 µg/mL were required. Eventually, no *in vivo* study could be performed for compound **6**.

7 Scaffold Hopping: Pyrazoloquinolin-3-ones

In general, scaffold hopping starts with an active lead compound and ends with a dissimilar core structure possessing similar biological activity to the parent scaffold.[97] The challenge of this approach is to retain enough similarity to remain active but at the same time modify the central template in order to positively influence the molecule's properties.[98] According to Sun *et al.*[97] the degree of changes associated with the 'hop' can be classified into distinct stages, e.g., a simple replacement of the heterocycle core (1° hop), and a topology/shape-based scaffold hopping approach (4° hop).

The change of the central core structure could aim several objectives:[99]

I. novel scaffolds are beneficial applying for a patent
II. metabolically instabilities could be overcome by certain variations improving pharmacokinetic properties
III. lipophilic compounds could be transformed in a more polar scaffold
IV. flexible residues are converted into a rather rigid scaffold and thus might superiorly interact with the target.

Starting point of the scaffold hopping approach was the latter fact (IV) combined with the previous SAR research of G. Hiltensperger. He revealed the importance of the intramolecular hydrogen bond between the *sec.* amide and the carbonyl oxygen in position 4 (Figure 51).[6] The antitrypanosomal activities of the corresponding benzyl ester and the *tert.* amide, respectively, dramatically decreased when the hydrogen bond could not be formed.[6] Accordingly, the hydrogen bond was replaced by a covalent bond which permanent locks the flexible benzylamide into its bioactive conformation. Hence, it does not need to adopt to its preferred conformation and an associated entropic loss might be evaded.[100] Referring to Sun *et al.*[97] this ring closure is considered as 2° hop. Therefore, the pyrazoloquinolin-3-ones were designed as conformationally constrained compounds of the established quinolone amides. This concept of accurately guiding the residue of the amide (R^1) was already successfully applied for quinolone amides targeting the CB_2 cannabinoid receptor.[101] Furthermore, Wentland *et al.*[102] rigidified the conformation of quinolones in position 3 to generate potent topoisomerase II inhibitors.

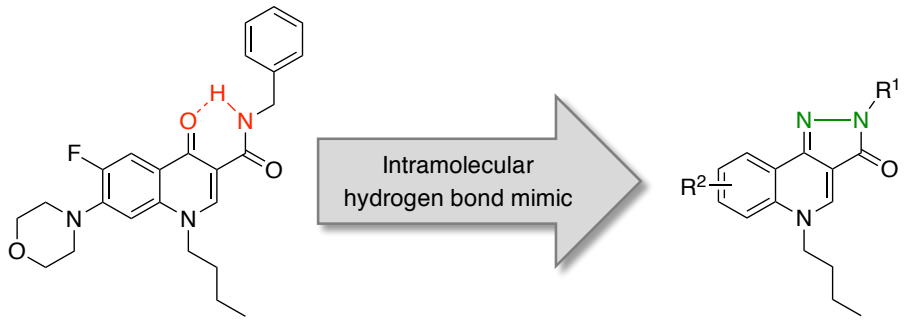


Figure 51: *Conformal-constrained design of the lead compound* ***GHQ168*** *resulting in a scaffold hopping approach leading to the pyrazoloquinolin-3-ones compound class; R_1= phenyl, benzyl; R_2= F-6/Cl-7 and H-6/H-7.*

7.1 The Pyrazoloquinolin-3-ones

In 1982, the 2-arylpyrazolo[4,3-*c*]quinolin-3-ones (**V**) were initially described by Yokoyama *et al.*[103] in the research about ligands of the benzodiazepine receptor and is still of high interest nowadays (Figure 52).[104] The early study revealed high receptor affinity of this compound class, combined with oral bioavailability and good blood-brain barrier penetration.[103] Since then, the pyrazoloquinolin-3-ones attracts several research groups utilizing the apparent multivalent scaffold for various biological applications, e.g, as allosteric modulators of M_1 muscarinic receptor[105], ligands for the benzodiazepine binding site of $GABA_A$ receptor[106], and agonists of the CB_2 cannabinoid receptor[101] just to mention a recent selection. Hence, the diverse biological activities and the chemistry of the pyrazolo[4,3-*c*]quinolones were already summarised in two reviews.[107,108]

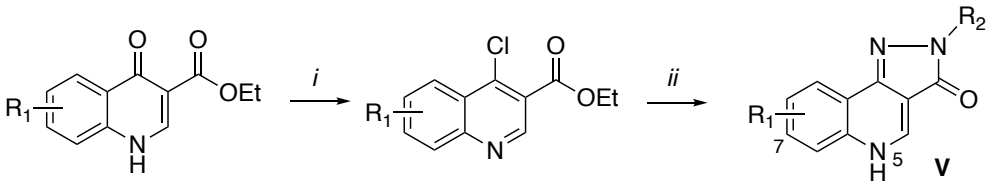


Figure 52: *Synthesis of the pyrazolo[4,3-c]quinolin-3-one scaffold* ***V*** *starting with the quinolone core. Reagents and reactions conditions: i) $POCl_3$, 100 °C; ii) H_2NHN-R_2, DMF, 120 °C.*

The quinolone core is inert to react directly with hydrazine derivatives to give the desired pyrazoloquinolones **V**. Thus, reactive 4-chloroquinoline intermediate is required (Figure 52): the 4-chloroquinoline is conveniently obtained using the chlorination agents, i.e. $POCl_3$[109,110] and $SOCl_2$[111,112]. Then, hydrazine compound nucleophilic attacks the activated intermediate, and the pyrazoloquinoline-3-ones **V** is obtained. In order to accomplish the highest possible likeness between **GHQ168** and the corresponding pyrazoloquinolone

compound, the morpholine in C-7 and the *n*-butyl chain in position *N*-5 is required. Hence, the reactions for introducing these substituents were performed afterwards.

7.2 Synthesis of the 4-Chloroquinoline

The ethyl 4-chloro-3-carboxylate **50a** was initially described by Kaslow *et al.*[109] in their study on antimalarials. The ethyl 4-hydroxy-1,4-dihydroquinoline-3-carboxylates were conveniently transformed into the corresponding 4-chloro-quinolines by reacting with $POCl_3$ (1.7 eq) for 45-60 min at 100 °C (Figure 53). The phenolic group of the 4-hydroxyquinoline (tautomer to the 4-quinolone) nucleophilic attacks the phosphorus atom while a chloride is leaving the molecule. In the following, the released chloride immediately reacts with the established intermediate. The oxophilicity of the phosphorus atom is considered to be the driving force of the reaction, forming the stable phosphenic chloride (PO_2Cl). Finally, **50a** and **50b** were obtained in adequate yields (73-77%).

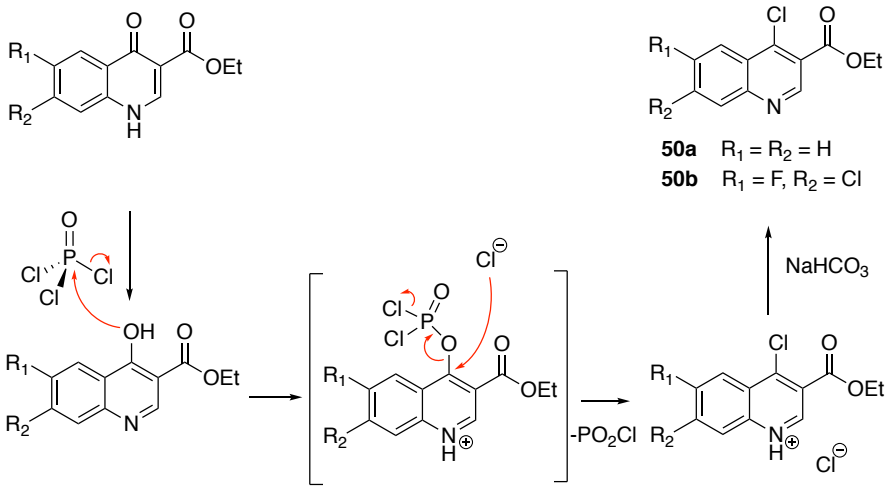


Figure 53: *Synthesis of compounds **50a** and **50b** and the corresponding mechanism.*

7.3 Synthesis of the 2-Aryl Pyrazolo[4,3-c]quinolin-3-one

According to the protocol of Lopez Rivilli *et al.*[111], **50a** and **50b** dissolved in abs. DMF were reacted with phenylhydrazine (Figure 54). After the reaction was completed the crude product was purified by column chromatography. Compounds **51a** and **51b** were isolated in yields of 50-65%. The reaction consists of an initial S_NAr of chlorine by the *prim.* amino group of phenylhydrazine, which is even accomplished at lower temperature.[103] The

electron density at the NH_2 group is higher in comparison to the adjacent *sec.* amine, whose nucleophilic ability is quite limited due to the conjugated phenyl moiety. Hence, this reaction obeys certain regioselectivity. Next, the nucleophilic attack of the *sec.* amine at the ester moiety completes the ring closure. Higher temperature and the immediate vicinity favour the intramolecular reaction over an attack of a further phenylhydrazine molecule.

The resulting pyrazoloquinolin-3-ones exist in three different tautomeric forms (**W**, **X**, **Y**) (Figure 54).[112] For this reason NMR spectroscopy is a very powerful technique for elucidating the tautomers and finding their forms at equilibrium. According to literature[103], the tautomeric form **W** is assigned to be the only prevalent structure in DMSO-d_6. It is also the thermodynamically favoured one and form **Y** is the least stable. The ^{1}H NMR signals of H-4 (s, δ = 8.77 / 8.73 ppm) and *N*-H in position 5 (br, δ = 12.90 / 12.80 ppm) are consistent with the pyrazoloquinoline-3-one structure **W**. However, the dihydropyrazolone substructure is known to underlay a lactam-lactim tautomerism (**X**, **Y**, Figure 54). Due to the highly conjugated electron system over the whole molecule, the compounds appear in an intense yellow-orange colour.

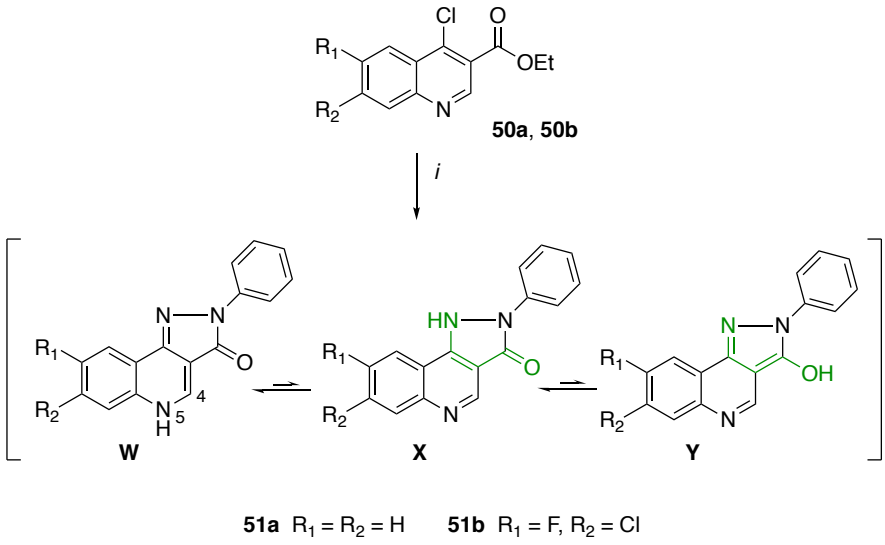


Figure 54: *Synthesis of the 2-aryl pyrazoloquinolin-3-ones* ***51a*** *and* ***51b****. The tautomeric equilibrium of the pyrazoloquinolones (****W, X, Y****). Reagents and reaction conditions: i)* H_2*NHN-Ph, DMF, 120 °C.*

7.4 Synthesis of the 2-Benzyl Pyrazolo[4,3-c]quinolin-3-one

The reactivity of benzyl hydrazine is quite different in comparison to the phenylhydrazine. Since the aryl substituent is not conjugated with the *sec.* amine and thus does not affect its electron density, the nucleophilicity of both nitrogen atoms is nearly the same. Hence, the 4-chloro position is presumable equally attacked by both nucleophilic positions. Compound **50b** was dissolved in DMF and was reacted with phenylhydrazine dihydrochloride and TEA (Figure 55). Applying these conditions, the regioisomers **52'** and **52** were obtained. After removing the solvent *in vacuo* the isomeric ratio was assessed to be 1:1 by ^{1}H NMR, with regard to the integrals of the H-4 signals (Figure 56). The resulting equal proportion of the isomers substantiates the identical nucleophilic potency of the nitrogens in phenylhydrazine.

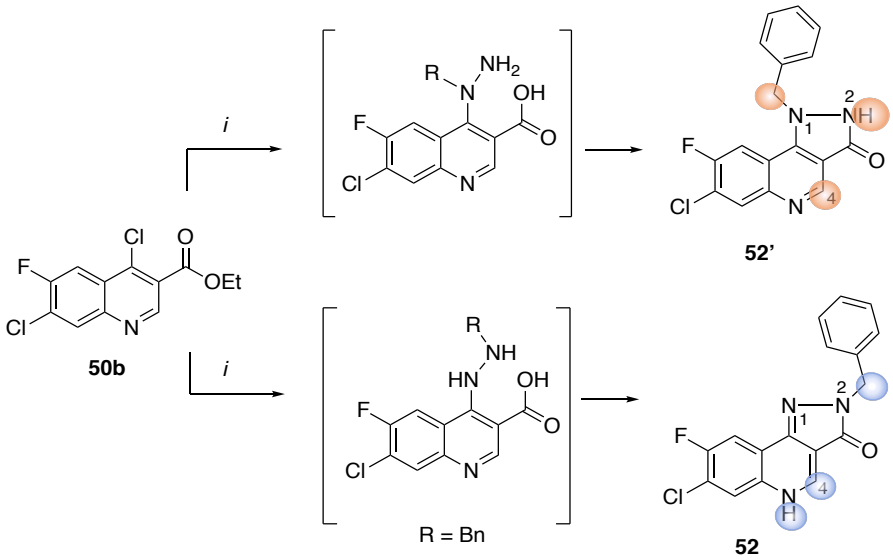


Figure 55: *Synthesis of the benzyl pyrazolo[4,3-c]quinoline-3-one with the emerging regioisomers* ***52'*** *and* ***52****. The tagged protons in orange and blue were consulted for distinction the two isomers. Reagents and reaction conditions: i) H_2NHN-Bn*2HCl, TEA, DMF, 120 °C;*

The goal to separate **52'** and **52** proved to be particularly demanding and some recent attempts to isolate certain benzyl pyrazoloquinoline-3-ones have already failed.[111] After a careful isolation of the apparent similar compounds (**52'**: R_f = 0.31; **52**: R_f = 0.24 (silica gel 0.020-0.060 mm; $CHCl_3$/EtOAc = 2:1)), an accurate characterisation could be achieved: Considering the ^{1}H NMR spectra of a mixture of **52/52'**, the broad signal at δ = 12.73 ppm is assigned to the *N*-H signal of position 5. This is in accordance with the typical shift for vinylic amidines (Figure 56). In comparison to this, the signal for *N*-H in position 2 is shifted more upfield (δ = 11.58 ppm), which is consistent with the literature.[113] Both *N*-H protons are exchangeable with D_2O. The H-4 protons can be assigned to the signals δ = 9.11 ppm

and δ = 8.73 ppm, respectively. Furthermore, the benzylic CH_2 group of the isomers can be distinguished since the *N*-2 substituted residue is notably more deshielded.

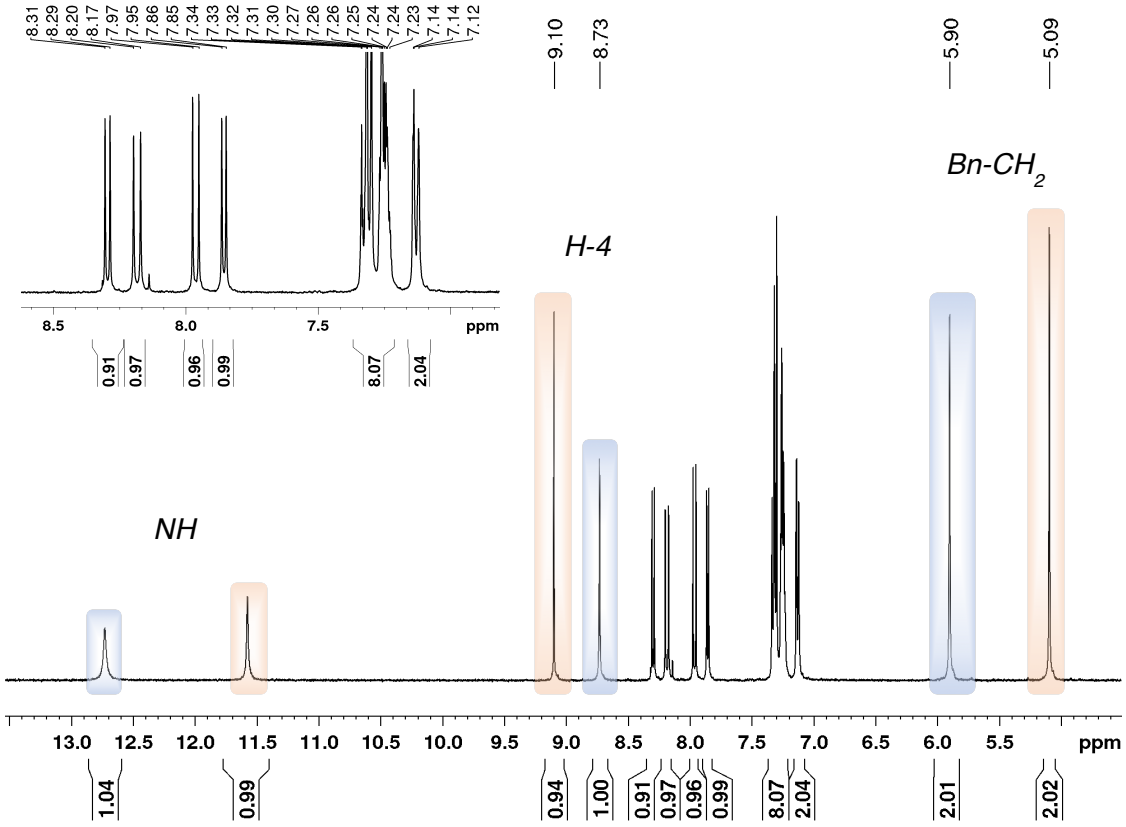


Figure 56: *^{1}H NMR of a mixture of the isomers **52'** (orange) and **52** (blue) in an equal proportion, recorded in DMSO-d_6.*

7.5 Synthesis of the Alkylated Pyrazoloquinolin-3-ones

The introduction of an *n*-butyl residue in position 5 of **51a**, **51b** and **52** permits to establish a link between the biological activity of the quinolone amides and the alkylated pyrazoloquinolin-3-ones. For this reason, **51a**, **51b** and **52**, respectively, were dissolved in abs. DMF. According to the procedure applied for the quinolones (3.4.1), reaction mixture was treated with K_2CO_3, *n*-bromobutane, and a catalytic amount of KI. Since the pK_a value of the pyrazolone has been ascertained to be 8.1[114], the basicity of K_2CO_3 (pK_a = 10.25) is just sufficient to deprotonate the enol group of the lactim-structure **Y** (Figure 54). The resulting mesomeric structures of the deprotonated pyrazoloquinolin-3-ones unveil three potentially nucleophilic sides.[115]

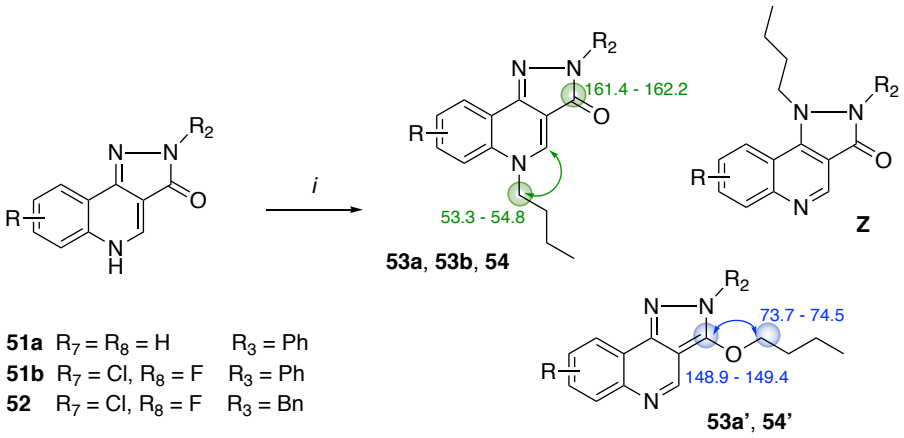


Figure 57: *Potentially products emerging from the alkylation reaction. Double arrows indicate long range ^{1}H-^{13}C coupling in the HMBC spectra. The chemical shifts in the ^{13}C spectra of the colored carbon atoms (green and blue) are depicted in ppm. Reagents and conditions: i) n-bromobutane, K_2CO_3, KI, DMF, 85-100 °C.*

In theory, all depicted products could be obtained from the alkylation reaction (Figure 57). However, under respective reaction conditions, only the *N*-5 alkylated products **53a**, **53b**, **54** and the *O*-alkylated molecules **53a'**, **54'** were isolated. After the reactions were completed the isolation of the desired isomers **53a**, **54** could be achieved using column chromatography. The undesired isomers **53a'**, **54'** eluted first on normal phase silica gel. The substances **53a**, **54** were achieved in yields of 20-29%, whereas the undesired molecules were obtained in 29-36%. Particular compound **53b** was selectively isolated by simple hot filtration with ethyl acetate of the crude product mixture (Yield 36%).

The ^{1}H NMR spectra of the *N*-alkylated and the *O*-alkylated isomers showed close similarity, but the ^{13}C NMR spectra did not. Hence, the ^{13}C NMR data were considered to confirm the respective regioisomers. The signal of the carbonyl group of position 3 appeared typically in the downfield area (δ = 161.4 – 162.2 ppm for **53a**, **53b**, **54**). Moreover, the signals obtained of δ = 53.3 – 54.8 ppm perfectly correlated to N**C**H_2 which coupled to the H-2 in the HMBC experiment (indicated by the green double arrow (Figure 57)).

On the contrary, the ^{13}C spectra of **53a'**, **54'** lacked the characteristic signal of a carbonyl group. The carbon atom of the respective enol was rather assigned to δ = 148.9 – 149.4 instead. The C-3 was in vicinity to the *n*-butyl moiety, since a coupling in the HMBC spectrum was apparent (blue double arrow). The significant shift of the methylene to the downfield (δ = 73.7 – 74.5 ppm) indicated that the alkyl residue was not connected to the nitrogen, but must be linked to the oxygen in position 3. Compound **Z** was not isolated.

7.6 Nucleophilic Aromatic Substitution of the Pyrazoloquinolin-3-ones

Since the morpholine residue leads to a significant increase of biological activity, it was pivotal introducing this moiety into the pyrazoloquinolones. Hence, the chlorine substituent of C-7 (**53b**) was tried to be substituted in a S_NAr reaction, by analogy with the quinolone amides (3.5). However, the position 7 was apparently not activated, since the nucleophilic attack failed (Figure 58). Moreover, changing the reaction sequence by trying to introduce the morpholine prior to the alkylation was not successful. Compound **51b** as a starting material for S_NAr led to product **55**, i.e. solely substitution of the fluorine atom in position 8 occurred. Referring to the ^1H NMR data (δ = 3.81 – 3.79 ppm, 4H; δ = 3.12 – 3.10 ppm, 4H), the morpholine was introduced into the pyrazoloquinolone scaffold. At a second glance, the characteristic couplings with the fluorine atoms were missing in the NMR experiments. Finally, the mass spectrum (*m/z* 381.9 $[M + H]^+$) confirmed the substitution of fluorine in C-8. According to literature[116], both positions are activated for S_NAr. The subsequent alkylation reaction with compound **55** leads to the *O*- and *N*-butyl isomers as described in the prior section.

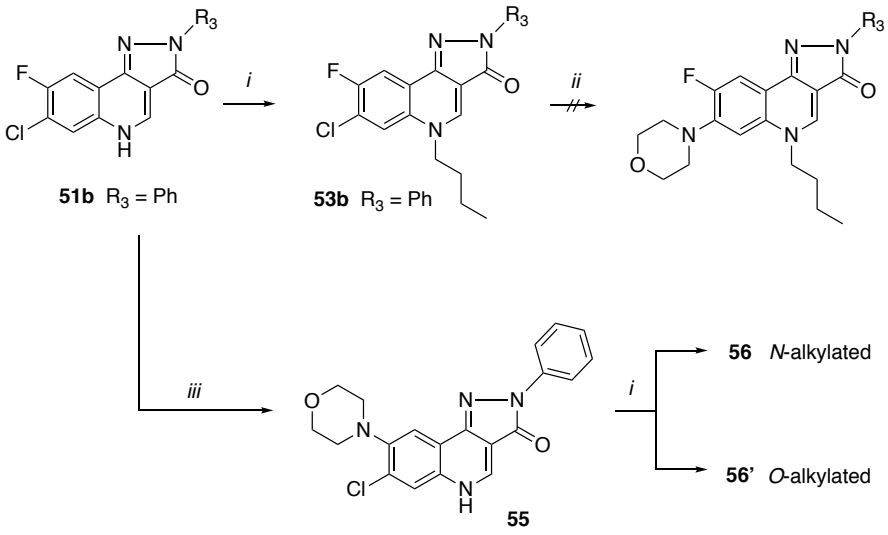


Figure 58: *S_NAr at the pyrazoloquinolin-3-one scaffold on the one hand utterly failed and on the other hand led to undesired substitution on position 8. In the subsequent alkylation reaction of **55**, the N-alkylated and the O-alkylated isomers were obtained in analogy to 7.5. Reagents and reaction conditions: i) n-bromobutane, K_2CO_3, KI, abs. DMF, 95 °C. ii) morpholine, 110 °C; iii) morpholine, 115 °C;*

7.7 Synthesis of the 4-Thioxo-quinoline-3-carboxylate

Since the formerly pursued synthesis route did not give a morpholine substituted pyrazoloquinolinone scaffold, the nitrogen heterocycle should be introduced in an earlier stage. For practical reasons, the well-established S_NAr procedure at the quinolone core was utilized (3.5). Once all substituents (*n*-butyl chain, morpholine) were attached to the molecule, the carboxylic acid (**4a**) was converted into the corresponding ethyl ester (**57**) using $SOCl_2$ in a mixture of ethanol/toluene. Afterwards the carbonyl group of C-4 was activated to the more reactive thiocarbonyl group (compound **58**) according to Wentland[102]. Herein, the easy to handle Lawesson's reagent was applied at 60 °C, obtaining the 4-thioxo moiety after 2.5 h in a good yield of 86%. Referring to the reaction procedure of El Bakali *et al.*[101], compound **58** was dissolved in ethanol, and refluxed with phenylhydrazine. The *prim*. amine initially attacks the 4-thioxo group. In a second step, the *sec.* amine of phenylhydrazine reacts with the carboxylic ester in an intramolecular cyclisation reaction, yielding 87% of compound **59**.

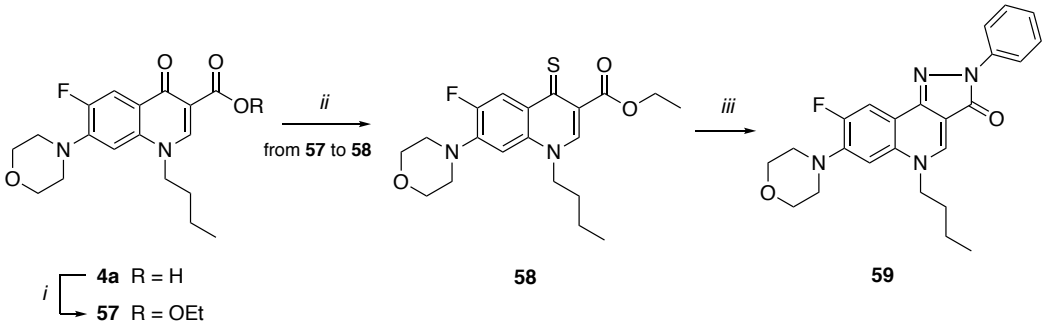


Figure 59: *Synthesis of the 4-thioxo-quinoline-3-carboxylate **58** to finally obtain compound **59**. Reagents and conditions: i) $SOCl_2$, ethanol/toluene; ii) Lawesson's reagent, toluene, 60 °C; iii) phenylhydrazine, abs. EtOH, reflux.*

7.8 Biological Evaluation of the Pyrazoloquinolin-3-ones

First, it was intended to show that the covalent bond of the pyrazoloquinolin-3-ones which replaced the hydrogen bond of the quinolone amides cause a similar biological effect. For this comparison, quinolone amides synthesised by E. Kugelmann (**EK01** and **EK02**)[117] were included (Figure 60). Compound **EK01** neither had an alkyl residue in *N*-1 nor a saturated heterocycle in C-7, consequently it was closely related to the pyrazoloquinolin-3-one **51a**. The activity against *T. b. brucei* was in the same range with an even slightly lower IC_{50} value for **51a** (IC_{50} = 23.6 μM).

The introduction of an alkyl moiety in the *N*-1 and *N*-5 position, respectively, resulted in a leap by the factor 4 for the IC_{50} values for both compounds **EK02** (IC_{50} = 7.9 μM) and **53a** (IC_{50} = 6.37 μM). The pyrazoloquinolin-3-one compound class obviously generated a comparable antitrypanosomal activity and thus, the covalent bond between the nitrogen atoms was considered bioisosteric to the hydrogen bond.

For the final evaluation, the lead compound **GHQ168** containing morpholine in C-7 should be consulted. Compound **59** also holds a morpholine ring in the relevant position, but its trypanocidal activity unfortunately could not be assessed due to poor solubility. Besides the missing information about its biological activity, **59** is apparently even less soluble than the quinolone amide. Conclusively, the novel pyrazoloquinolin-3-ones are presumably not superior to the quinolone amides.

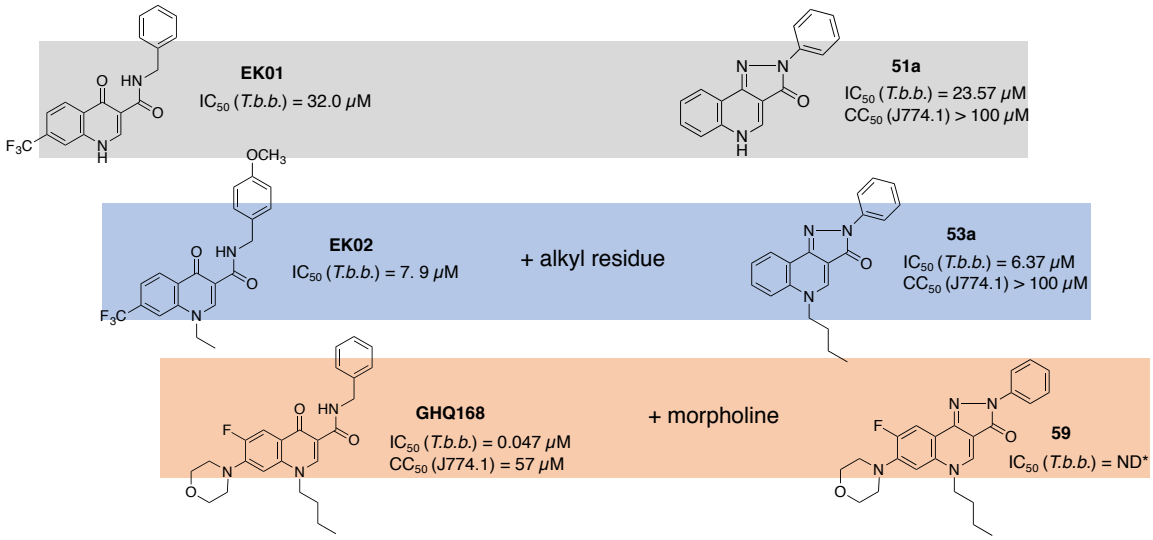


Figure 60: *The comparison of the antitrypanosomal activity of the quinolone amides versus the pyrazoloquinolin-3-ones. *not determined due to poor solubility in test medium.*

Taken together, the scaffold hopping approach demonstrates certain similarities between the quinolone amides and the pyrazoloquinolin-3-ones in general. However, no beneficial properties were gained due to the rigidification.

8 Summary

In recent years the transmission of the Human African Trypanosomiasis could be significantly reduced. The reported cases in 2016 reached a historic low level of 2184 cases and these achievements can be ascribed to intense control and surveillance programmes.[118] However, most of the reported cases (>1000 in 2015) occurred in the Democratic Republic of the Congo and thus, need to be treated adequately. In particular, when the parasites have traversed the blood-brain barrier (BBB), treatment proved to be even more difficult. In addition, the number of cases always came in waves due to many reasons, e.g., development of resistances. Thus, it can be expected from experiences of the past that the number of cases will increase again. Hence, novel chemical entities are desperately needed in order to overcome the drawbacks which are associated with the current treatment options.

Our drug discovery approach included an initial drug repurposing strategy combined with a phenotypic screening. S. Niedermeier found novel active compounds derived from commercial fluoroquinolones. The most promising hit compound was further developed by G. Hiltensperger resulting in the lead quinolone amide **GHQ168** (IC_{50} = 0.047 μM).

This doctoral thesis is about new insights into the SAR of the quinolone amides and the enhancement of the lead compound. Special consideration was given to the fluorine atom in the quinolone amides and how certain fluorine substitution patterns influence the antitrypanosomal activity, physicochemical properties and pharmacokinetics (i.e. 'fluorine walk'). Moreover, the ability of the compound class crossing the BBB should be investigated. This feature is inevitable necessary in order to potentially treat African sleeping sickness stage II.

The Gould-Jacobs protocol was predominantly used for the synthesis of the quinolone core. Since former SAR studies mainly concentrated on the variation in positions 1, 3 and 7, quinolone scaffolds (**2a-i**) with diverse substitution patterns regarding positions 5, 6, 7 and 8 were synthesised in this thesis. The resulting quinolone amides were evaluated for their antitrypanosomal activity.

Voluminous residues in position C-5 resulted in diminished activities (compounds **13**, **16** and **18**) and solely small-sized moieties were tolerated. In particular the fluorine atom in position 5 revealed beneficial trypanocidal effects as shown for compounds **6** (IC_{50} = 0.05 μM), **8** (IC_{50} = 0.04 μM), and **24** (IC_{50} = 0.02 μM). Furthermore, having fluorine only in position 5 of the quinolone core could considerably reduce the cytotoxic effects (CC_{50}

>100 μM, SI = >2000 for **6**). Hence, the 5-fluoro-substituted quinolone amides were considered superior to **GHQ168**.

Regarding the C-6 position all other moieties (e.g., H in **9**, OCH_3 in **10**, CF_3 in **12**) except of a fluorine atom decreased the activity against *Trypanosoma brucei brucei*. A double fluorination in C-6 and C-8 was not beneficial (IC_{50} = 0.06 μM for **7**) and a single fluorine atom in C-8 even showed a negative effect (IC_{50} = 0.79 μM for **5**).

The logP value is considered a surrogate parameter for lipophlicity and thus, affecting permeability and solubility processes. In particular the fluorine atom influences the lipophilicity due to versatile effects:[87] Lipophilicity is increased by additional fluorine atoms on aromatic rings (**7**, **23**) and reduced by fluorine atoms at an alkyl chain (**49**), respectively. Additionally, the 5-fluoro-substituted quinolone amides (**6**, **8**, and **24**) could prove the contrary effect of decreasing lipophilicity when the aromatic fluorine substituent is in vicinity to a carbonyl group.

For the most promising drug candidates **6**, **23**, and **24** the respective metabolites and the metabolic turnover were investigated by C. Erk. In comparison to **GHQ168** the hydroxylation of the benzylamide was prevented by the *para*-fluorine atom. Hence, half-life was extended for compound **23** ($t_{1/2}$ = 6.4 h) and *N*-desalkylation was the predominant pathway. Moreover, the respective fluorine substitution pattern of the quinolone core affected the metabolism of compound **6**. The 5-fluoro-substituted quinolone amide was less prone for biotransformation ($t_{1/2}$ = 7.2 h) and half-life could even be further prolonged for compound **24** ($t_{1/2}$ = 7.7 h).

Due to the most appropriate safety profile of compound **6**, this particular drug candidate was considered for *in vivo* study. Its poor solubility made a direct intraperitoneal administration unfeasible. Thus, an amorphous solid dispersion of **6** was generated using the spray-drying method according to the previous protocol.[8] Unfortunately, the required solubility for the predicted *in vivo* study was not achieved.

Furthermore, the compound class of the quinolone amide was evaluated for its ability for brain penetration. The methanesulfonyl precursor **48** was synthesised and subsequently radiofluorinated in the group of Prof. Dr. Samnick (Department of Nuclear Medicine, University Hospital of Würzburg). The labelled compound **[^{18}F]49** was administered to mice, and its distribution throughout the body was analysed using positron emission tomography and autoradiography, respectively. The autoradiography of the murine brains revealed medium to high concentrations of **[^{18}F]49**. Therefore, the quinolone amides are generally suitable for treating Human African Trypanosomiasis stage II.

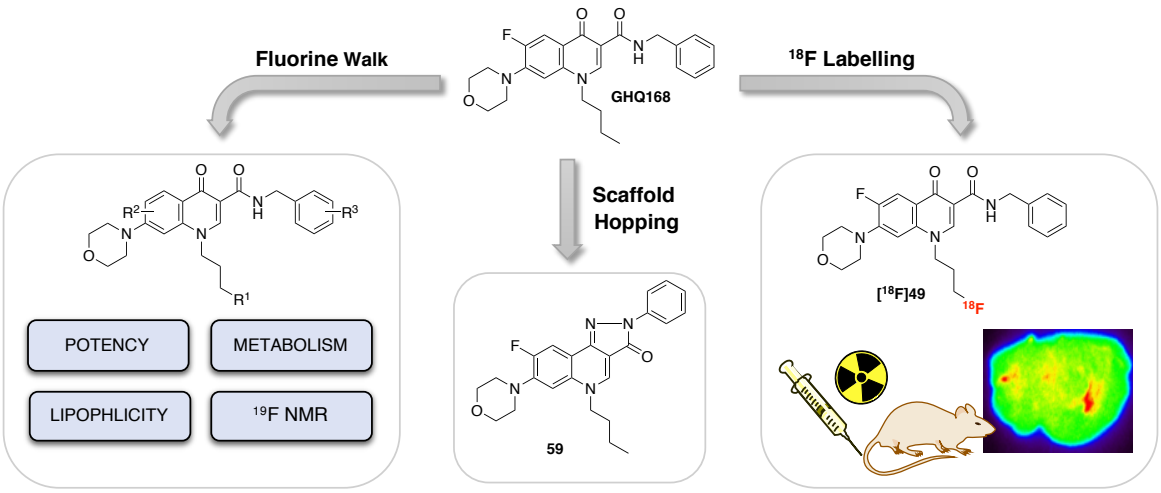


Figure 61: *Overview of the implemented projects in the scope of this thesis: the applications of fluorine regarding the quinolone amides and a scaffold hopping approach.*

A scaffold hopping approach was performed starting from the quinolone amides and concluding with the compound class of pyrazoloquinolin-3-ones. The intramolecular hydrogen bond between the *sec.* amide and the C-4 carbonyl moiety was replaced by a covalent bond. The two compound classes were comparable regarding the antitrypanosomal activity to some degree (IC_{50} = 7.9 μM (**EK02**) vs. 6.37 μM (**53a**)). However, a final evaluation of **59** was not possible due to poor solubility.

9 Zusammenfassung

Die Verbreitung der Afrikanischen Schlafkrankheit konnte in den vergangenen Jahren deutlich verringert werden. Die dokumentierten Fallzahlen aus 2016 erreichten ein historisch niedriges Niveau, was auf eine engmaschige Kontrolle und Überwachung zurückzuführen ist. Dennoch gibt es nach wie vor zahlreiche Krankheitsfälle (>1000 Fälle im Jahr 2015 für die Demokratische Republik Kongo), die entsprechend behandelt werden müssen. Die Therapie wird insbesondere dann erschwert, wenn die Parasiten die Blut-Hirn-Schranke überwunden haben. Außerdem treten die Krankheitsfälle aus mehreren Gründen, wie beispielsweise durch Resistenzentwicklung immer wieder schubweise auf. Die Erfahrungen aus der Vergangenheit zeigen, dass die Fallzahlen jederzeit wieder ansteigen können. Deshalb sind neue Arzneistoffe dringend notwendig, um die Nachteile der aktuellen Behandlungmöglichkeiten umgehen zu können. Unsere Suche nach neuen Wirkstoffen beinhaltete eine anfängliche Umwidmung eines zugelassenen Arzneistoffes in Verbindung mit einem Phänotyp-basierten Screening. S. Niedermeier entdeckte neue aktive Verbindungen, die sich von handelsüblichen Fluorchinolonen ableiteteten. Die vielversprechenste Substanz wurde von G. Hiltensperger zum Chinolonamid **GHQ168** (IC_{50} = 0.047 μM) weiter optimiert.

Diese Arbeit befasst sich mit neuen Erkenntnissen zur Struktur-Wirkungs Beziehung der Chinolonamiden und mit der Verbesserung der Leitsubstanz. Besondere Berücksichtigung fanden dabei die Fluor-Substitutionen an den Chinolonamiden. Es sollte untersucht werden, inwiefern gewisse Fluorsubstitutionsmuster die antitrypanosomale Wirkung, physiko-chemische Eigenschaften und Pharmakokinetik beeinflussen („Fluor Walk“). Außerdem sollte ermittelt werden, ob diese Substanzklasse die Blut-Hirn-Schranke überwinden kann. Dieses Merkmal muss unabdingbar vorhanden sein, um die Afrikanische Schlafkrankheit in Stufe II potentiell behandeln zu können.

Das Gould-Jacobs-Verfahren wurde hauptsächlich für die Synthese des Chinolongrundgerüstes angewandt. Da sich die vorausgegangene Analyse der Struktur-Wirkungs Beziehungen vornehmlich auf das Variieren der Substitutenten in den Positionen 1, 3 und 7 konzentrierte, wurden in dieser Arbeit Chinolone (**2a-i**) mit diversen Substitutionsmustern in den Positionen 5, 6, 7 und 8 synthetisiert. Die erhaltenen Chinolonamide wurden auf ihre antitrypanosomale Aktivität untersucht.

Voluminöse Reste in der Position C-5 verursachten verringerte Aktivitäten (Verbindungen **13**, **16** and **18**), d.h. nur kleine Reste waren hinnehmbar. Vor allem ein Fluor-Atom in

Position 5 wirkte sich günstig auf die antitrypanosomale Wirkung aus, was mit Verbindungen **6** (IC_{50} = 0.05 μM), **8** (IC_{50} = 0.04 μM), und **24** (IC_{50} = 0.02 μM) gezeigt werden konnte. Des Weiteren reduzierte sich die zytotoxische Wirkung (CC_{50} >100 μM, SI = >2000 für **6**), wenn sich das Fluor-Atom nur in Position 5 des Chinolongrundgerüsts befindet. Deshalb wurden die Chinolonamide mit Fluor in Position 5 gegenüber **GHQ168** als überlegen erachtet.

In Bezug auf Position 6 zeigen die Reste (z. B. H in **9**, OCH_3 in **10**, CF_3 in **12**), mit Ausnahme des Fluor-Atoms, eine verringerte Aktivität gegenüber *Trypanosoma brucei brucei*. Eine zweifache Fluorsubstitution in C-6 und C-8 war nicht vorteilhaft (IC_{50} = 0.06 μM für **7**) und ein einfaches Fluor-Atom in C-8 zeigte einen negativen Effekt (IC_{50} = 0.79 μM für **5**).

Der logP Wert wird als Surrogatparameter der Lipophilie betrachtet und wirkt sich somit auf Permeabilität- und Löslichkeitsprozesse aus. Insbesondere das Fluor-Atom beeinflusst die Lipophilie durch vielfältige Effekte:[87] die Lipophilie wird durch zusätzliche Fluor-Atome am Aromaten erhöht (**7**, **23**), beziehungsweise durch Fluor-Atome an einer Alkylkette verringert (**49**). Zusätzlich konnte für die 5-fluoro-substituierten Chinolonamide (**6**, **8**, **24**) der paradoxe Effekt gezeigt werden, dass die Lipophilie verringert wird, sobald ein aromatischer Fluorsubstituent in unmittelbarer Nähe zu einer Carbonylgruppe steht.

Für die vielversprechensten Wirkstoffkandidaten **6**, **23** und **24** wurden die jeweiligen Metabolite und der metabolische Umsatz von C. Erk untersucht. Im Vergleich zu **GHQ168** wurde einer Hydroxylierung des Benzylamid-Restes durch eine *para*-Fluor-Substitution vorgebeugt. Dadurch wurde die Halbwertszeit der Verbindung **23** verlängert ($t_{1/2}$ = 6.4 h) und eine *N*-Desalkylierung war der vorrangige Abbauweg. Außerdem wirkte sich das entsprechende Fluorsubstitutionsmuster auf den Metabolismus von Substanz **6** aus. Das 5-fluoro-substitutierte Chinolonamid war weniger anfällig für Biotransformationen ($t_{1/2}$ = 7.2 h) und die Halbwertszeit konnte für die Substanz **24** sogar noch weiter verlängert werden ($t_{1/2}$ = 7.7 h).

Aufgrund des geeigneten Sicherheitsprofils der Verbindung **6**, wurde für diesen Wirkstoffkandidaten eine *In-vivo*-Studie in Betracht gezogen. Die schlechte Wasserlöslichkeit machte jedoch eine direkte intraperitoneale Gabe unpraktikabel. Deshalb wurde durch Sprühtrocknung der Verbindung **6**, gemäß der früheren Vorgehensweise,[8] eine „amorphous solid dispersion“ erzeugt. Die benötigte Löslichkeit für die vorausberechnete *In-vivo*-Studie wurde dabei leider nicht erreicht.

Darüber hinaus wurde die Substanzklasse der Chinolonamide hinsichtlich ihrer Fähigkeit, ins Gehirn zu gelangen, untersucht. Dazu wurde die Methansulfonyl-Vorstufe **48** in der Gruppe von Prof. Dr. Samnick (Institut für Nuklearmedizin, Universitätsklink Würzburg) synthetisiert und mit [^{18}F]Fluor markiert. Die markierte Verbindung **[^{18}F]49** wurde anschließend in Mäuse injiziert und dessen Verteilung im Körper mittels Positronen-Emissions-Tomographie, beziehungsweise mittels Autoradiographie analysiert. Die Autoradiographie der Mäusegehirne zeigte mittlere bis hohe Konzentrationen von **[^{18}F]49**. Demnach sind die Chinolonamide generell dafür geeignet, die Afrikanische Schlafkrankheit in Stufe II zu behandeln.

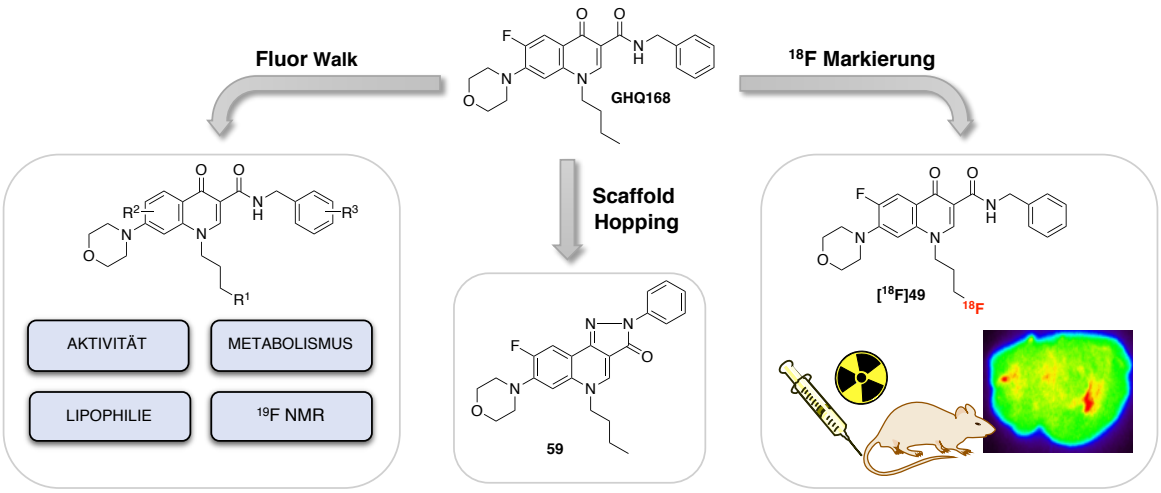


Figure 62: *Übersicht über die in dieser Arbeit umgesetzten Projekte: Die Anwendungsbereiche des Fluors bezogen auf die Chinolonamide und ein „Scaffold Hopping“-Ansatz.*

Ein “Scaffold Hopping”-Ansatz wurde für die Chinolonamide angestrebt und ergab schließlich die Substanzklasse der Pyrazolochinolin-3-one. Die intramolekulare Wasserstoffbrückenbindung zwischen dem *sek.* Amid und der Carbonylgruppe in C-4 wurde durch eine kovalente Bindung ersetzt. Die beiden Substanzklassen waren im Ansatz, bezogen auf ihre antitrypanosomale Wirkung, miteinander vergleichbar (IC_{50} = 7.9 μM (**EK02**) vs. 6.37 μM (**53a**)). Dennoch konnte eine abschließende Bewertung aufgrund mangelnder Löslichkeit nicht stattfinden.

10 Experimental Section

10.1 General Experimental Procedures and Equipment

Autoradiography

Brain tissues were exposed on a phosphor image plate (*Biostep*, Jahnsdorf, Germany) overnight. The image plate was read out on an image plate reader (*Dürr Medical*, Bietigheim-Bissingen, Germany) and data analysis was performed with the software AMIDE Medical Image Data Examiner (Version 1.0.4).

Chemicals

All chemicals were purchased from *Sigma-Aldrich* (Schnelldorf, Germany), *VWR* (Darmstadt, Germany), *Alfa-Aesar* (Karlsruhe, Germany) and *Merck* (Darmstadt, Germany), and were used without prior purification.

Chromatography

- Thin layer chromatography (TLC) was performed on pre-coated silica gel glass plates SIL 625 UV_{254} (*Macherey-Nagel*, Düren, Germany). The detection was made by quenching at 254 nm, intrinsic fluorescence at 366 nm or with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde (1g/100 mL) dissolved in a mixture of 25 mL conc. HCl and 75 mL methanol). The retardation factor R_f is defined by the ratio of the migration distance of the substance and the migration distance of the solvent front.
- For the general purification, a column chromatography driven by gravity with silica gel 60 (0.063-0.200 mm) and silica gel (0.040-0.063 mm) (*Merck*, Darmstadt, Germany) was used.
- HPLC (high performance liquid chromatography) method **I** for purity analysis:

  instrument — *Shimadzu* HPLC system (*Shimadzu Scientific Instruments*, Kyoto, Japan), equipped with a DGU-20A3R controller, LC20AB liquid chromatograph, and a SPD-20 UV/Vis detector.

  column — Synergi Fusion-RP (4 μm, 150 mm × 4.6 mm) (*Phenomenex*, Aschaffenburg, Germany)

eluent	water + 0.1% FA (A), MeOH + 0.1% FA (B)
gradient elution	0-8 min 5% → 90% (B)
	8-13 min 90% (B)
	13-15 min 90% → 5% (B)
detection	a) λ = 254 nm
	b) *Shimadzu* LCMS-2020 single quadrupole mass spectrometer; Ionization method: ESI
temperature	rt
injection volume	20 μL
flow	1 mL/min

- HPLC method **II** for logP determination and purity analysis:

instrument	*Agilent* HPLC system (1100 series) (*Agilent Technologies*, Böblingen, Germany) with an integrated degasser, binary pump, column thermostat, and a DAD detector.
column	Eurosphere II (5 μm, 150 mm × 4.6 mm) (*Knauer*, Berlin, Germany) and Nucleosil (5 μm, 150 mm × 4.6 mm) (*Macherey-Nagel*, Düren, Germany).
eluent	KH_2PO_4 buffer (10 mM, pH = 7.4)/ MeOH (30/70)
detection	λ = 254 nm
temperature	30 °C
injection volume	40 μL
flow	1 mL/min

- HPLC method **III** for solubility determination:

instrument	cf. HPLC method **II**
column	Eurosphere II (5 μm, 150 mm × 4.6 mm) (*Knauer*, Berlin, Germany)
eluent	ACN/water (72/28)
detection	λ = 280 nm
temperature	40 °C
injection volume	20 μL
flow	1 mL/min

- HPLC method **IV**:

instrument	cf. HPLC method **II**

column	Zorbax SB-C18 (3.5 μm, 100 mm × 3.0 mm) (*Agilent Technologies*, Böblingen, Germany)
eluent	water + 0.5% FA (A), ACN + 0.5% FA (B)
gradient elution	0-25 min 0% → 95% (B)
detection	a) λ = 254 nm b) *Agilent* 1100 LC/MSD Trap SL; Ionization method: ESI
temperature	25 °C
injection volume	5 μL
flow	0.5 mL/min

For the HPLC analysis exclusively deionised water, purified by a Milli-Q®-System of *Merck* (Darmstadt, Germany), was used. Additionally, Methanol (LiChrosolv®, HPLC gradient grade, *Merck*) and acetonitrile (LiChrosolv®, HPLC gradient grade, *Merck*) only in the appropriate purity grade was utilized.

Infrared Spectroscopy (IR)

IR spectra were recorded on JASCO FT-IR-6100 spectrometer (*Jasco*, Groß-Umstadt, Germany) in combination with a diamond ATR accessory. The wavenumber $\tilde{\nu}$ is specified as cm^{-1}.

Melting Point (mp)

The melting points were determined with a capillary melting point apparatus MPD350:BM 3.5 (*Sanyo*, Gallenkamp BV, Netherlands) and were not corrected.

Microwave Systems

For the microwave supported synthesis, the following systems (*Milestone*, Leutkirch, Germany) were applied:

- synthWAVE
- rotaPREP
- Ethos 1600

Since apolar solvents do not absorb microwave irradiation sufficiently, *Weflon*® plates (polytetrafluoroethylene with 10% graphit) were added to certain reaction mixture to increase the efficiency of absorption.

Nuclear Magnetic Resonance Spectroscopy (NMR Spectroscopy)

^{1}H (400.131 MHz) and ^{13}C (100.623 MHz) NMR spectra were recorded using a Bruker AV 400 NMR spectrometer (*Bruker Biospin*, Ettlingen, Germany) at 300 K. The signals of the deuterated solvents were used as internal standard (DMSO-d_6: ^{1}H 2.50 ppm, ^{13}C 39.43 ppm; $CDCl_3$: ^{1}H 7.26 ppm, ^{13}C 77.00 ppm). The measurements of ^{19}F were recorded on a Bruker Advance III HD 400 NMR spectrometer of *Bruker Biospin* (Ettlingen, Germany), with the external standard $CFCl_3$ (0 ppm). NMR data are presented with chemicals shift in ppm, the multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; sext, sextet; m, multiplet; dd, doublet of doublet, ddd, doublet of doublet; br, broad signal), coupling constants (*J* given in Hz). Topspin® (version 3.2-pl7) software (*Bruker Biospin,* Ettlingen, Germany) was used for processing and analysing the NMR spectra.

Radionuclide Production

[^{18}F] Fluoride was produced on the PETtrace® cyclotron (*GE Medical Systems*, Uppsala, Sweden) at the interdisciplinary PET centre of the University of Würzburg. [^{18}F] Fluoride was produced via a $^{18}O(p,n)^{18}F$ reaction by irradiating 3.0 mL of 95% enriched [^{18}O]water with 16.5 MeV protons.

Solvents

The used solvents were dried according to general procedures[119] and were then stored with molecular sieve (3.6 Å), and under argon atmosphere:

- Acetone was dried over $CaSO_4$ with an ensuing distillation.
- Acetonitrile was treated with NaH (1 g/L) and the dispersion was refluxed, following with a distillation shortly afterwards.
- Chloroform/dichloromethane was stored with $CaCl_2$ for at least 24 h and was subsequently distilled.
- Ethanol / methanol was dried with magnesium chips, refluxed and finally distilled.

- Dimethylformamide was treated with CaH_2 (5g/L) and the mixture stirred for 12 h, and then a distillation followed.

Spray Drying

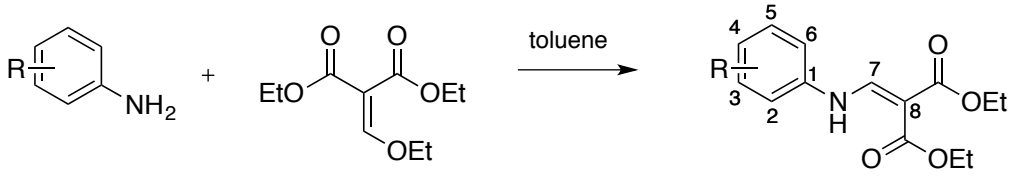
A solid dispersion was prepared using a Nano Spray Drayer B90 (*Büchi*, Flawil, Switzerland). The process was performed with an air flow of 150 L/min and inlet temperature of 60 °C through a 7 *µ*m mesh size (resulting pressure of 50 mbar).

Thermomixer

For the solubility determination, the samples were tempered (37 °C) and shaken (800 rpm) simultaneously in a thermomixer (*Eppendorf,* Hamburg, Germany).

10.2 Gould-Jacobs Approach[120]

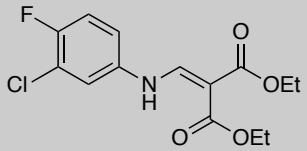
10.2.1 General Synthesis Procedure of the Anilinomethylenemalonic Esters 1a-i.

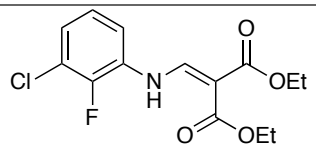
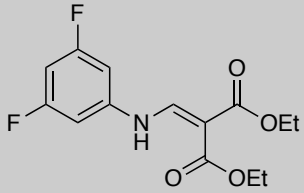
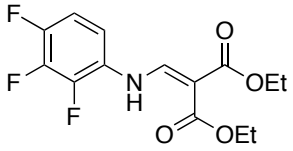
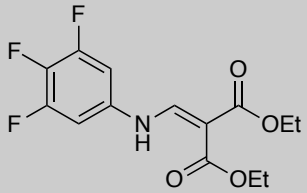
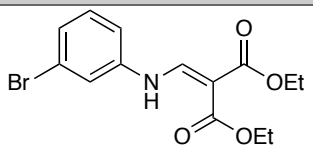
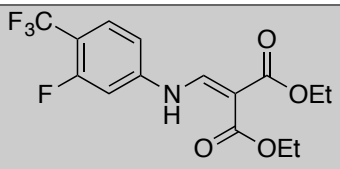
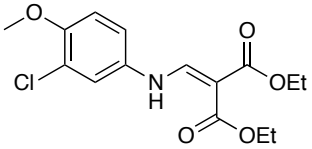
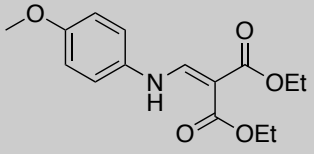


- Conventionally reaction procedure according to Leyva *et al.*[16]: A solution of the appropriate aniline (1 eq) in toluene (20-50 mL) was treated with diethyl methylenemalonate (DEEMM) (1.2 eq, M_r = 216.23 g/mol, ϱ = 1.07 g/mL) and was refluxed for 20-24 h in combination with a dean-shark apparatus to give compound **1c**, **1f**, **1h**, **1i**. The solvent was removed under reduced pressure and the crude product was crystallised from *n*-hexane at -20 °C.

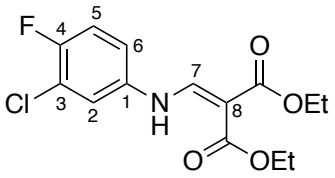
- Microwave supported reaction according to Hiltensperger *et al.*[7]: The aniline compound was dissolved in toluene (50 mL) in a three-way flask, and 5 *Weflon*® plates were added to the reaction. The solution heated under microwave irradiation (110 °C, 500 w, 1-3 h) in an *Ethos* microwave system. The solvent was removed *in vacuo* and the remaining oily residue was treated with *n*-hexane. The mixture was stored at -20 °C overnight and the precipitated solid was filtered off, washed with cold *n*-hexane and finally give **1a**, **1b**, **1d**, **1e**, **1g**.

Table 12: *Reagents and reaction conditions of the synthesis of the anilinomethylenemalonic esters.* [a]*microwave irradiation;* [b]*conventionally oil bath heating.*

Anilinomethylenemalonic Esters	Aniline Compounds	DEEMM	Reaction Time [h]
 **1a**	M_r = 145.56 g/mol n = 34.35 mmol m = 5.0 g	n = 41.22 mmol m = 8.91 g V = 8.33 mL	2[a]

Anilinomethylenemalonic Esters	Aniline Compounds	DEEMM	Reaction Time [h]
 **1b**	M_r = 145.56 g/mol n = 68.7 mmol m = 10.0 g	n = 82.44 mmol m = 17.83 g V = 16.66 mL	3[a]
 **1c**	M_r = 129.11 g/mol n = 77.45 mmol m = 10.0 g	n = 92.94 mmol m = 20.10 g V = 18.78 mL	20[b]
 **1d**	M_r = 147.10 g/mol n = 67.98 mmol m = 10.0 g	n = 81.42 mmol m = 17.61 g V = 16.46 mL	1[a]
 **1e**	M_r = 147.10 g/mol n = 40.79 mmol m = 6.0 g	n = 48.95 mmol m = 10.58 g V = 9.89 mL	3[a]
 **1f**	M_r = 172.02 g/mol n = 37.79 mmol m = 6.5 g	n = 45.34 mmol m = 9.80 g V = 9.16 mL	24[b]
 **1g**	M_r = 179.12 g/mol n = 30.70 mmol m = 5.5 g	n = 36.84 mmol m = 7.97 g V = 7.45 mL	2[a]
 **1h**	M_r = 157.03 g/mol n = 63.45 mmol m = 10.0 g	n = 76.14 mmol m = 16.34 g V = 15.27 mL	24[b]
 **1i**	M_r = 123.15 g/mol n = 24.36 mmol m = 3.0 g	n = 29.23 m = 6.32 g V = 5.91 mL	20[b]

Diethyl 2-(((3-chloro-4-fluorophenyl)amino)methylene)malonate, ***1a*** *(MB01)*



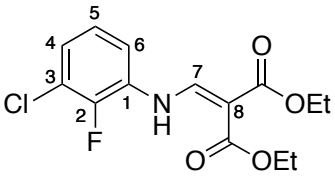
$C_{14}H_{15}ClFNO_4$

M_r = 315.73 g/mol

appearance:	white solid
yield:	10.2 g (32.3 mmol/ 94%, Lit[19]: 80%)
reaction control:	R_f = 0.34 (cyclohexane/EtOAc = 4:1)
melting point [°C]:	62-63 °C (Lit[19]: 58-60 °C)
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3260, 3075, 2979, 2904, 1683, 1629, 1506, 1423, 1236, 1020.

The obtained physical and spectroscopic data are consistent with that found in literature.[19]

Diethyl 2-(((3-chloro-2-fluorophenyl)amino)methylene)malonate, **1b** (MB378)



$C_{14}H_{15}ClFNO_4$

M_r = 315.73 g/mol

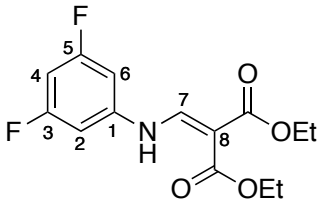
appearance:	white solid
yield:	20.5 g (64.9 mmol/ 94%)
reaction control:	R_f = 0.71 (cyclohexane/EtOAc = 1:1)
melting point [°C]:	102-103 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3092, 2991, 2900, 1677, 1644, 1600, 1573, 1468, 1414, 1238, 1220.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 10.85 (dd, 1H, ^3J = 13.2, N-**H**), 8.43 (d, 1H, ^3J = 13.2, **H**-7), 7.64 – 7.59 (m, 1H, **H**-6), 7.35 – 7.30 (m, 1H, **H**-5) 7.27 – 7.23 (m, 1H, **H**-4), 4.23 (q, 2H, ^3J = 7.2, OC**H$_2$**CH$_3$), 4.14 (q, 2H, ^3J = 7.2, OC**H$_2$**CH$_3$), 3.84 (s, 3H, OC**H$_3$**), 1.25 (t, 6H, ^3J = 7.2, (OCH$_2$C**H$_3$**)$_2$).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 167.4 (1C, **C**OOEt), 164.3 (1C, **C**OOEt), 150.7 (1C, **C**-7), 149.9 (d, 1C, $^3J_{C,F}$ = 244.0, **C**-2), 129.0 (d, 1C, $^2J_{C,F}$ = 9.0, **C**-1), 125.7 (d, 1C, $^3J_{C,F}$ = 4.8, **C**-4), 125.2 (1C, **C**-5), 120 (d, 1C, $^2J_{C,F}$ = 15.3, **C**-3), 116.4 (1C, **C**-6), 95.7 (1C, **C**-8), 59.9 (1C, O**C**H_2CH_3), 59.6 (1C, O**C**H_2CH_3), 14.0 (1C, OCH_2**C**H_3), 13.9 (1C, OCH_2**C**H_3).

Koga *et al.*[20] did not provide melting point, yield and NMR data.

Diethyl 2-(((3,5-difluorophenyl)amino)methylene)malonate, **1c** (MBMR01_1)



$C_{14}H_{15}F_2NO_4$

M_r = 299.27 g/mol

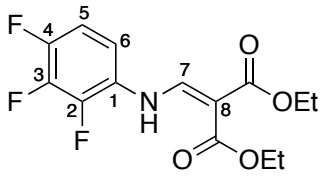
appearance:	white solid
yield:	22.7 g (75.9 mmol/ 98%)
reaction control:	R_f = 0.49 (PE/$CHCl_3$ = 1:1)
melting point [°C]:	94-95 °C (Lit[21]: 103-105 °C)
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3089, 2985, 2937, 2906, 1683, 1644, 1612, 1589, 1477, 1416, 1239, 1213, 797.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 10.98 (d, 1H, ^3J = 12.8, N-**H**), 8.37 (d, 1H, ^3J = 12.8, **H**-7), 6.70 – 6.64 (m, 2H, **H**-6/**H**-2), 6.61 – 6.56 (m, 1H, **H**-4) 4.31 (q, 2H, ^3J = 7.2, O**C**H_2CH_3), 4.25 (q, 2H, ^3J = 7.2, O**C**H_2CH_3), 1.38 (t, 3H, ^3J = 7.2, OCH_2**C**H_3), 1.33 (t, 3H, ^3J = 7.2, OCH_2**C**H_3).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 168.7 (1C, **C**OOEt), 165.2 (1C, **C**OOEt), 165.19 (1C, **C**-7), 163.4 (d, 2C, $^1J_{C,F}$ = 247.1, $^2J_{C,F}$ = 14.5, **C**-3/**C**-5), 141.7 (1C, **C**-1), 100.4 – 99.9 (m, 3C, **C**-2/**C**-4/**C**-6), 95.7 (1C, **C**-8) 60.8 (1C, O**C**H_2CH_3), 60.4 (1C, O**C**H_2CH_3), 14.4 (1C, OCH_2**C**H_3), 14.2 (1C, OCH_2**C**H_3).

Podányi *et al.*[21] did not provide yield and spectroscopic data.

Diethyl 2-(((2,3,4-trifluorophenyl)amino)methylene)malonate, ***1d*** *(MB283)*



$C_{14}H_{14}F_3NO_4$

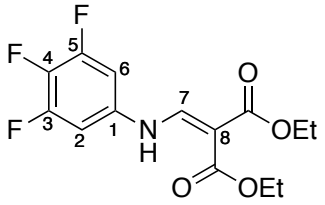
M_r = 317.26 g/mol

appearance:	white solid
yield:	18.5 g (58.4 mmol/ 86%; Lit[22]: 86%)
reaction control:	R_f = 0.79 (PE/EtOAc = 2:1)
melting point [°C]:	93-95 °C (Lit[22]: 94-95 °C)
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3075, 2985, 2941, 1689, 1649, 1620, 1606, 1507, 1425, 1347, 1203, 1023, 803.

^{19}F NMR (^{1}H decoupled, $CDCl_3$, δ [ppm], *J* [Hz]): 139.03 (d, $^2J_{3,4}$ = 22.1, $^4J_{2,4}$ = 3.8, **F**-4), 149.59 (d, $^2J_{2,3}$ = 20.8, $^4J_{2,4}$ = 3.8, **F**-2), 157.51 (dd, $^2J_{3,4}$ = 22.1, $^2J_{2,3}$ = 20.8, **F**-3).

The obtained physical and spectroscopic data are consistent with that found in literature.[38]

Diethyl 2-(((3,4,5-trifluorophenyl)amino)methylene)malonate, ***1e*** *(MB403)*



$C_{14}H_{14}F_3NO_4$

M_r = 317.26 g/mol

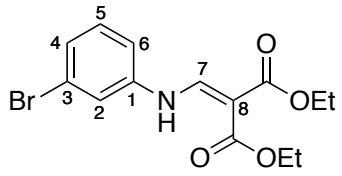
appearance:	white solid
yield:	10.6 g (33.4 mmol/ 82%; Lit[121]: 74%)
reaction control:	R_f = 0.77 (PE/EtOAc = 2:1)
melting point [°C]:	88-90 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3254, 2994, 1686, 1602, 1530 1471, 1431, 1377, 1227, 1044.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 10.93 (d, 1H, ^3J = 13.2, N-**H**), 8.28 (d, 1H, ^3J = 13.2, **H**-**7**), 6.77 – 6.74 (m, 2H, **H**-2/**H**-6), 4.32 (q, 2H, ^3J = 7.2, OC**H$_2$**CH$_3$), 4.24 (q, 2H, ^3J = 7.2, OC**H$_2$**CH$_3$), 1.41 – 1.22 (m, 6H, (OCH$_2$C**H$_3$**)$_2$).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 168.7 (1C, **C**OOEt), 165.2 (1C, **C**OOEt), 152.0 (ddd, 1C, $^{1}J_{C,F}$ = 249.0, $^{2}J_{C,F}$ = 10.7, $^{2}J_{C,F}$ = 5.4, **C**-4), 150.8 (1C, **C**-7), 136.5 (dt, 1C, $^{1}J_{C,F}$ = 248.1, $^{2}J_{C,F}$ = 15.2, **C**-5), 135.3 (dt, 1C, $^{3}J_{C,F}$ = 4.0, $^{3}J_{C,F}$ = 3.8, **C**-1), 125.24 (ddd, 1C, $^{1}J_{C,F}$ = 246.9, $^{2}J_{C,F}$ = 13.6, $^{2}J_{C,F}$ = 13.3, **C**-3), 101.7 – 101.6 (m, 2C, **C**-6/**C**-2), 95.7 (1C, **C**-8), 59.9 (1C, O**C**H$_2$CH$_3$), 59.6 (1C, O**C**H$_2$CH$_3$), 13.9 (1C, OCH$_2$**C**H$_3$), 13.8 (1C, OCH$_2$**C**H$_3$).

Cociorva *et al.*[121] do not provide melting point and NMR data.

Diethyl 2-(((3-bromophenyl)amino)methylene)malonate, ***1f*** *(MB10)*



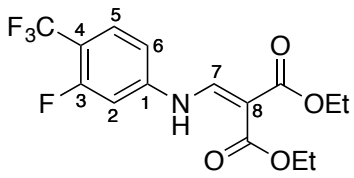
$C_{14}H_{16}BrNO_4$

M_r = 342.19 g/mol

appearance:	white solid
yield:	12.1 g (35.4 mmol/ 94%; Lit[24]: 40%)
reaction control:	R_f = 0.76 (EtOAc/PE = 2:1)
melting point [°C]:	76-77 °C (Lit[24]: 70-71 °C)
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3141, 2983, 2900, 1585, 1235, 1092, 768.

The obtained melting point and spectroscopic data are consistent with that found in literature.[24,122]

Diethyl 2-(((3-fluoro-4-(trifluoromethyl)phenyl)amino)methylene)malonate, ***1g*** *(MB396)*



$C_{15}H_{15}F_4NO_4$

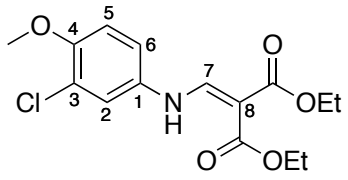
M_r = 349.28 g/mol

appearance:	white solid
yield:	9.4 g (26.7 mmol/ 86%; Lit[26]: 59%)
reaction control:	R_f = 0.79 (PE/EtOAc = 2:1)
melting point [°C]:	92-94 °C (Lit[26]: 88-89 °C; cyclohexane)

IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 2991, 2946, 2908, 1706, 1653, 1584, 1417, 1296, 1217, 1125.

The obtained physical and spectroscopic data are consistent with that found in literature.[26]

Diethyl 2-(((3-chloro-4-methoxyphenyl)amino)methylene)malonate, ***1h*** *(MB151)*



$C_{15}H_{18}ClNO_5$

M_r = 327.76 g/mol

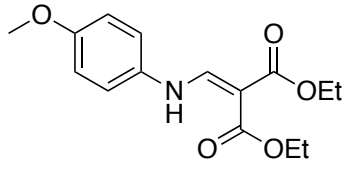
appearance:	white solid
yield:	15.4 g (47.0 mmol/ 75%)
reaction control:	R_f = 0.65 (cyclohexane/EtOAc = 1:1)
melting point [°C]:	105-106 °C (Lit[25]: 101-101.5 °C)
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3241, 2983, 2941, 2903, 1687, 1633, 1595, 1506, 1428, 1236, 1019.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 10.6 (d, 1H, ^3J = 16.0, N-**H**), 8.28 (d, 1H, ^3J = 14, **H**-7), 7.57 (d, 1H, ^4J = 2.8, **H**-2), 7.33 (dd, 1H, ^4J = 2.8, ^3J = 8.8, **H**-6), 7.15 (d, 1H, ^3J = 8.8, **H**-5), 4.20 (q, 2H, ^3J = 7.2, OC**H$_2$**CH$_3$), 4.11 (q, 2H, ^3J = 7.2, OC**H$_2$**CH$_3$), 3.84 (s, 3H, OC**H$_3$**), 1.27 – 1.21 (m, 6H, (OCH$_2$C**H$_3$**)$_2$).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 167.1 (1C, **C**OOEt), 164.9 (1C, **C**OOEt), 151.7 (1C, **C**-4), 151.5 (1C, **C**-7), 133.4 (1C, **C**-1), 121.6 (1C, **C**-3), 119.8 (1C, **C**-2), 117.0 (1C, **C**-6), 113.4 (1C, **C**-5), 92.8 (1C, **C**-8) 59.4 (1C, O**C**H$_2$CH$_3$), 59.3 (1C, O**C**H$_2$CH$_3$), 56.3 (1C, O**C**H$_3$), 14.2 (1C, OCH$_2$**C**H$_3$), 14.1 (1C, OCH$_2$**C**H$_3$).

Snyder *et al.*[25] do not provide NMR

Diethyl 2-(((4-methoxyphenyl)amino)methylene)malonate, ***1i*** *(MB26a)*



$C_{15}H_{19}NO_5$

M_r = 293.32 g/mol

appearance:	dark blue solid
yield:	4.3 g (14.5 mmol/ 60%; Lit[123]: 63%)
reaction control:	R_f = 0.84 (PE/EtOAc = 1:2)
melting point [°C]:	42-43 °C (Lit[124]: 38-40 °C)
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2945, 2910, 1690, 1633, 1620, 1595, 1508, 1425, 1209.

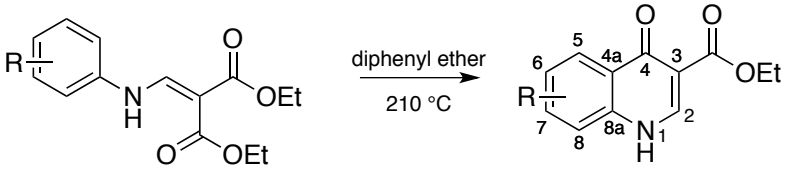
The obtained physical and spectroscopic data are consistent with that found in literature.[123]

10.2.2 General Synthesis of the Ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (2a-i).

The anilinomethylenemalonic ester **1a-i** as starting material was dissolved in 5-15 mL diphenyl ether and 5 *Weflon*® plates were added to the solution. The reaction solution heated 10-90 min at 210 °C in an open vessel apparatus in the rotaPREP microwave system. The solution was cooled down and then treated with diethyl ether to initiate precipitation of the product, which was filtrated and washed with cold diethyl ether (3x25 mL).

The detailed reaction conditions and compound properties are displayed Table 13. Spectroscopic data, particularly NMR data were limited accessible due to poor solubility in common deuterated solvents.

Table 13: *Reagent and reaction conditions of the synthesised ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylates (**2a-i**).*



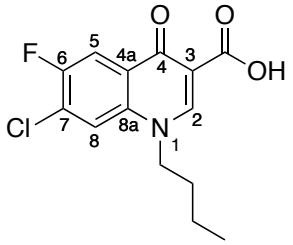
Compound		Formula M_r [g/mol]	Reagents and Conditions	Reaction Time [min]	Yield	Appearance	Melting Point [°C]	IR (ATR, $\tilde{\nu}$ [cm^{-1}])
2a	R^5=R^8=H; R^6=F; R^7=Cl	$C_{12}H_9ClFNO_3$ 269.66	**1a** 5.0 g, 15.8 mmol	70	3.3 g, 77% Lit[19]: 56%	white solid	309-311 Lit[19]: 290	3099, 2985, 1685, 1609, 1530, 1450, 1180, 1032.
2b	R^5=R^6=H; R^7=Cl; R^8=F	$C_{12}H_9ClFNO_3$ 269.66	**1b** 5.0 g, 15.8 mmol	60	3.0 g, 70% Lit[20]: 66%	beige brown	267-269 Lit[20]: 263-265	3049, 2982, 1709, 1616, 1571, 1531, 1446, 1284, 1201, 1030.
2c	R^5=R^7=F; R^6=R^8=H	$C_{12}H_9F_2NO_3$ 253.20	**1c** 5.0, 16.7 mmol	15	3.6 g, 91%	white solid	324 (dec.) Lit[21]: 313	3090, 2982, 1695, 1596, 1450,1363, 1290, 1117.

2d	R^5=H; R^6=R^7=R^8=F	$C_{12}H_8F_3NO_3$ 271.20	**1d** 3.0, 9.5 mmol	90	1.1 g, 43% Lit[22]: 83%	beige brown	275-277 Lit[22]: 279-280	3075, 2985, 2942, 1689, 1650, 1621, 1605, 1496, 1204, 1040.
2e	R^5=R^6=R^7=F; R^8=H	$C_{12}H_8F_3NO_3$ 271.20	**1e** 5.0 g, 15.8 mmol	10	3.9 g, 92% Lit[121]: 94%	grey solid	>325	3068, 2984, 1697, 1615, 1489, 1376, 1261, 1095, 1076.
2f	R^5=R^6=R^8=H; R^7=Br	$C_{12}H_{10}BrNO_3$ 296.12	**1f** 7.0 g, 20.5 mmol	30	4.1 g, 69% Lit[24]: 44%	pale grey solid	311-312 Lit[24]: 307-309	3144, 3084, 2976, 1695, 1607, 1580, 1548, 1523,1458, 1374, 1192.
2g	R^5=R^8=H; R^6=CF_3; R^7=F	$C_{13}H_9F_4NO_3$ 303.21	**1h** 5.0 g, 14.2 mmol	20	2.36 g, 55%	brown solid	325 (dec.)	3089, 1688, 1645, 1611, 1490, 1413, 1309, 1247, 1138, 1106.
2h	R^5=R^8=H; R^6=OCH_3; R^7=Cl	$C_{13}H_{12}ClNO_4$ 281.69	**1g** 4.0 g, 12.2 mmol	20	1.2 g, 35%	brownish solid	315-318 (dec.) Lit[25]: 299 (dec.)	3087, 2979,1701, 1612, 1578, 1521, 1476, 1376, 1196, 1171.
2i	R^5=R^7=R^8=H; R^6=OCH_3	$C_{13}H_{13}NO_4$ 247.25	**1i** 1.5 g, 5.2 mmol	45	0.66 g, 51%	grey solid	285-287 Lit[27]: 274-277	3066, 2954, 1698, 1613, 1559, 1543, 1314, 1201.

10.3 Alkylation of the *N*-H Position and Hydrolysis to the 1-Butyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acids

General procedure according to Koga *et al.*[20]: the corresponding ethyl-4-hydroxyquinoline-3-carboxylate **2a-i** (1 eq) and K_2CO_3 (4 eq) were suspended under Ar atmosphere in abs. DMF. The reaction mixture heated 30 min at 60 °C, following by adding catalytic amount of KI and *n*-bromobutane (5 eq). After 20-48 h of heating at 75-90 °C, the solvent was removed *in vacuo* and water added to the residue. The aqueous layer was extracted with EtOAc and the combined organic layers were dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* and the oily residue was subsequently purified by column chromatography on silica gel with the mobile phase specified in the respective procedures. Without further characterisation, the resulting products **2a-h** were hydrolysed using either 2 M HCl or 3 M KOH at 100 °C. If necessary, the solution was acidified to pH 2 and the precipitated product was collected. Afterwards, the solid was washed with cold water and dried *in vacuo* to give compound **3a-l**.

1-Butyl-7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***3a*** *(MB07)*



$C_{14}H_{13}ClFNO_3$

Mr = 297.71 g/mol

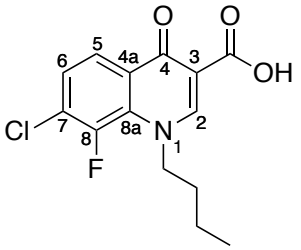
According to the general procedure 10.3, a solution of compound **2a** (500 mg, 1.86 mmol) and K_2CO_3 (1.0 g, 7.4 mmol) in abs. DMF (30 mL) was treated with *n*-bromobutane (996 µL, 9.30 mmol), and the reaction mixture heated at 80 °C for 48 h. The crude product was purified by column chromatography (silica gel; $CHCl_3$/*i*-Pr = 150:1) and then hydrolysed by refluxing under basic conditions (3 M KOH). After acidification with 2 M HCl under ice cooling, the precipitates were collected and dried *in vacuo* to yield **3a**.

appearance:	pale grey solid	
yield:	421 mg (1.41 mmol/ 76%; Lit[6]: 87%)	
reaction control:	alkylation	R_f = 0.48 ($CHCl_3$/*i*-Pr = 150:1)
	hydrolysis	R_f = 0.23 ($CHCl_3$/MeOH/FA = 100:1:1)

melting point [°C]:	227-228 °C (Lit[6]: 233-235 °C)
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3060, 3029, 2969, 2872, 1716, 1610, 1602, 1531, 1455.

The obtained physical and spectroscopic data are consistent with that found in literature.[6]

1-Butyl-7-chloro-8-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***3b*** *(MB380)*



$C_{14}H_{13}ClFNO_3$

M_r = 297.71 g/mol

According to the general procedure 10.3, a solution of compound **2b** (3.0 g, 11.1 mmol) and K_2CO_3 (6.2 g, 44.5 mmol) in abs. DMF (40 mL) was treated with *n*-bromobutane (6.0 mL, 55.6 mmol), and the reaction mixture heated at 80 °C for 48 h. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and then hydrolysed by refluxing under acidic conditions (2 M HCl). The precipitates were collected and dried *in vacuo* to yield **3b**.

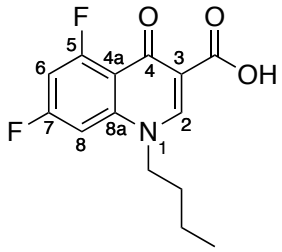
appearance:	yellowish solid	
yield:	1.8 g (6.1 mmol/ 55%)	
reaction control:	alkylation	R_f = 0.72 ($CHCl_3$/MeOH = 100:1)
	hydrolysis	R_f = 0.70 ($CHCl_3$/MeOH/FA = 100:1:1)
melting point [°C]:	211-213 °C	
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3091, 3048, 2964, 2930, 2858, 1713, 1620, 1602, 1541, 1440.	

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 14.85 (s, 1H, COO**H**), 9.01 (s, 1H, **H**-2), 8.20 (dd, 1H, ^3J = 7.2, ^5J = 1.6, **H**-5), 8.57 (dd, 1H, ^3J = 8.8, ^4J = 6.4, **H**-6), 4.60 – 4.56 (m, 2H, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 1.82 – 1.77 (m, 2H, NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.38 – 1.33 (m, 2H, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 0.94 (t, 3H, ^3J = 7.2, NCH$_2$CH$_2$CH$_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 177.7 (d, 1C, $^4J_{C,F}$ = 2.4, **C**-4), 165.1 (1C, **C**OOH), 152.1(1C, **C**-2), 147.2 (d, 1C, $^1J_{C,F}$ = 251.4, **C**-8), 129.7 (d, 1C, $^2J_{C,F}$ = 7.1, **C**-8a), 127.4 (1C, **C**-6), 126.8 (1C, **C**-4a), 126.4 (d, 1C, $^2J_{C,F}$ = 18.9, **C**-7), 122.5 (d, 1C, $^4J_{C,F}$ =

4.9, **C**-5), 108.1 (1C, **C**-3), 58.0 (d, 1C, $^{4}J_{C,F}$ = 14.4, N**C**$H_2CH_2CH_2CH_3$), 31.9 (d, 1C, $^{5}J_{C,F}$ = 4.1, NCH_2**C**$H_2CH_2CH_3$), 18.9 (1C, NCH_2CH_2**C**H_2CH_3), 13.3 (1C, $NCH_2CH_2CH_2$**C**H_3). **^{19}F NMR** (^{1}H decoupled, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): -121.47 (s, **F**-8).

1-Butyl-5,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***3c*** *(MBMR22)*



$C_{14}H_{13}F_2NO_3$

M_r = 281.26 g/mol

According to the general procedure 10.3, a solution of compound **2c** (10.0 g, 39.5 mmol) and K_2CO_3 (16.7 g, 158.0 mmol) in abs. DMF (100 mL) was treated with *n*-bromobutane (21.3 mL, 197.5 mmol), and the reaction mixture heated at 90 °C for 20 h. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and then hydrolysed by refluxing under acidic conditions (2 M HCl). The precipitates were collected and dried *in vacuo* to yield **3c**.

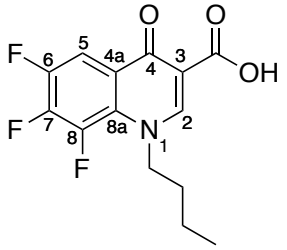
appearance:	white solid	
yield:	9.8 g (34.7 mmol/ 88%)	
reaction control:	alkylation	R_f = 0.86 ($CHCl_3$/MeOH = 100:1)
	hydrolysis	R_f = 0.53 ($CHCl_3$/MeOH/FA = 100:2:1)
melting point [°C]:	208-210 °C	
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3368, 3118, 2965, 2871, 1708, 1614, 1512, 1437, 1343, 1283, 1168, 1127, 1014.	

^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 14.98 (s, 1H, COO**H**), 8.98 (s, 1H, **H**-2), 7.78 – 7.75 (m, 1H, **H**-8), 7.51 – 7.46 (m, 1H, **H**-6), 4.47 (t, 2H, ^{3}J = 7.2, N**CH_2**$CH_2CH_2CH_3$), 1.76 – 1.68 (m, 2H, NCH_2**CH_2**CH_2CH_3), 1.34 (m, 2H, NCH_2CH_2**CH_2**CH_3), 0.89 (t, 3H, ^{3}J = 7.2, $NCH_2CH_2CH_2$**CH_3**).

^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 176.8 (d, 1C, $^{3}J_{C,F}$ = 1.6, **C**-4), 165.4 (**C**OOH), 164.6 (dd, 1C, $^{1}J_{C,F}$ = 250.4, $^{3}J_{C,F}$ = 15.2, **C**-5), 156.7 (dd, 1C, $^{1}J_{C,F}$ = 248.7, $^{3}J_{C,F}$ = 15.6, **C**-7), 150.1 (1C, **C**-2), 144.3 (dd, 1C, $^{3}J_{C,F}$ = 15.0, $^{3}J_{C,F}$ = 14.2, **C**-8a), 113.7 (dd, 1C, $^{2}J_{C,F}$ = 8.4, $^{4}J_{C,F}$ = 2.4, **C**-4a), 108.8 (1C, **C**-3), 100.1 (dd, 1C, $^{2}J_{C,F}$ = 25.1, $^{2}J_{C,F}$ = 25.1,

C-6), 92.6 (dd, 1C, $^{2}J_{C,F}$ = 26.7, $^{4}J_{C,F}$ = 4.5, **C**-8), 54.2 (1C, N**C**$H_2CH_2CH_2CH_3$), 30.3 (1C, NCH_2**C**$H_2CH_2CH_3$) 18.9 (1C, NCH_2CH_2**C**H_2CH_3), 13.5 (1C, $NCH_2CH_2CH_2$**C**H_3).

1-Butyl-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***3d*** *(MBPG12)*



$C_{14}H_{12}F_3NO_3$

M_r = 299.25 g/mol

According to the general procedure 10.3, a solution of compound **2d** (2.4 g, 8.9 mmol) and K_2CO_3 (4.9 g, 35.4 mmol) in abs. DMF (50 mL) was treated with *n*-bromobutane (5.75 mL, 44.3 mmol), and the reaction mixture heated at 90 °C for 48 h. The crude product was purified by column chromatography (silica gel; $CHCl_3$/*i*PrOH = 50:1) and then hydrolysed by refluxing under acidic conditions (2 M HCl). The precipitates were collected and dried *in vacuo* to yield **3d**.

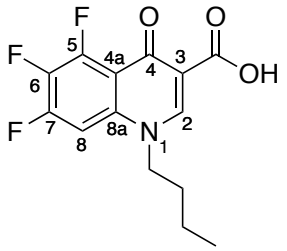
appearance:	white solid	
yield:	1.6 g (5.4 mmol/ 62%)	
reaction control:	alkylation	R_f = 0.49 ($CHCl_3$/*i*PrOH = 50:1)
	hydrolysis	R_f = 0.43 ($CHCl_3$/MeOH/FA = 100:2:1)
melting point [°C]:	216–218 °C	
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3056, 2963, 2934, 2875, 1713, 1614, 1560, 1519, 1482, 1455, 1412, 1390, 1284, 1110, 1056.	

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 14.48 (s, 1H, COO**H**), 9.04 (s, 1H, **H**-2), 8.24 – 8.19 (ddd, 1H, ^{3}J = 10.7, ^{4}J = 8.2, ^{5}J = 2.4, **H**-5), 4.62 – 4.57 (m, 2H, N**CH_2**$CH_2CH_2CH_3$), 1.84 – 1.80 (m, 2H, NCH_2**CH_2**CH_2CH_3), 1.38 – 1.33 (m, 2H, NCH_2CH_2**CH_2**CH_3), 0.93 (t, 3H, ^{3}J = 7.2, $NCH_2CH_2CH_2$**CH_3**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 175.4 (d, 1C, $^{4}J_{C,F}$ = 1.2, **C**-4), 164.6 (1C, **C**OOH), 152.6 (1C, **C**-2), 149.2 (m, 1C, **C**-6), 146.9 (m, 1C, **C**-8), 142.5 (m, 1C, **C**-7), 127.1 (1C, **C**-8a), 122.6 (d, 1C, $^{3}J_{C,F}$ = 6.8, **C**-4a), 108.2 (m, 1C, **C**-5), 107.4 (1C, **C**-3), 58.1 (d, $^{4}J_{C,F}$ = 13.5, N**C**$H_2CH_2CH_2CH_3$), 32.4 (d, $^{5}J_{C,F}$ = 3.9, NCH_2**C**$H_2CH_2CH_3$), 19.4 (1C, NCH_2CH_2**C**H_2CH_3), 13.9 (1C, $NCH_2CH_2CH_2$**C**H_3).

^{19}F NMR (^{1}H decoupled, DMSO-d_6, δ [ppm], J [Hz]): -134.44 (dd, $^{3}J_{6,7}$ = 24.8, $^{4}J_{6,8}$ = 6.9, **F**-6), -140.68 (dd, $^{3}J_{7,8}$ = 20.0, $^{4}J_{6,8}$ = 6.9, **F**-8), -149.05 (dd, $^{3}J_{6,7}$ = 24.8, $^{3}J_{7,8}$ = 20.0, **F**-7).

1-Butyl-5,6,7-trifluoro-4-oxo-1,4-diyhroquinoline-3-carboxylic acid, ***3e*** *(MB407)*



$C_{14}H_{12}F_3NO_3$

M_r = 299.25 g/mol

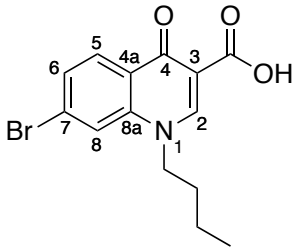
According to the general procedure 10.3, a solution of compound **2e** (9.12 g, 33.6 mmol) and K_2CO_3 (18.60 g, 134.4 mmol) in abs. DMF (75 mL) was treated with *n*-bromobutane (18.0 mL, 168.0 mmol), and the reaction mixture heated at 70 °C for 20 h. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and then hydrolysed by refluxing under acidic conditions (2 M HCl). The precipitates were collected, dried *in vacuo* and recrystallised from EtOAc to yield **3e**.

appearance:	white solid	
yield:	2.13 g (7.12 mmol/ 28%)	
reaction control:	alkylation	R_f = 0.81 ($CHCl_3$/MeOH = 100:1)
	hydrolysis	R_f = 0.51 ($CHCl_3$/MeOH/Fa = 100:2:1)
melting point [°C]:	233–235 °C	
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3099, 2964, 2879, 1715, 1617, 1455, 1346, 1297, 1110.	

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 9.00 (s, 1H, **H**-2), 8.12 – 8.07 (m, 1H, **H**-8), 4.49 (t, 2H, ^{3}J = 7.2, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 1.71 (quint, 2H, ^{3}J = 7.2, NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.34 (sext, 2H, ^{3}J = 7.2, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 0.89 (t, 3H, ^{3}J = 7.2, NCH$_2$CH$_2$CH$_2$C**H$_3$**).
^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 176.6 (1C, **C**-4), 165.3 (1C, **C**OOH), 153.2 (ddd, 1C, $^{1}J_{C,F}$ = 251.9, $^{2}J_{C,F}$ = 11.0, $^{3}J_{C,F}$ = 4.6, **C**-5), 150.0 (1C, **C**-2), 149.8 (ddd, 1C, $^{1}J_{C,F}$ = 264.2, $^{2}J_{C,F}$ = 10.8, $^{3}J_{C,F}$ = 5.0, **C**-7), 136.9 (dt, 1C, $^{1}J_{C,F}$ = 248.8, $^{2}J_{C,F}$ = 15.3, **C**-6), 136.5 (d, 1C, $^{3}J_{C,F}$ = 12.9, **C**-8a), 113.9 (d, 1C, $^{2}J_{C,F}$ = 5.3, **C**-4a), 108.4 (1C, **C**-3), 102.4 (dd, 1C, $^{2}J_{C,F}$ = 22.5, $^{3}J_{C,F}$ = 4.5, **C**-8), 54.1 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 20.3 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 18.9 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.4 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

^{19}F NMR (^{1}H coupled, DMSO-d_6, δ [ppm], J [Hz]): -123.76 (ddd, $^{3}J_{6,7}$ = 25.0, $^{4}J_{5,7}$ = 13.5, $^{3}J_{7,8}$ = 11.6, **F**-7), -133.87 (dd, $^{3}J_{5,6}$ = 19.4, $^{4}J_{5,7}$ = 13.7, **F**-5), -161.99 (ddd, $^{3}J_{6,7}$ = 24.9, $^{3}J_{5,6}$ = 21.2, $^{4}J_{6,8}$ = 6.8, **F**-6).

7-Bromo-1-butyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***3f*** *(MB12)*



$C_{14}H_{14}BrNO_3$

M_r = 324.17 g/mol

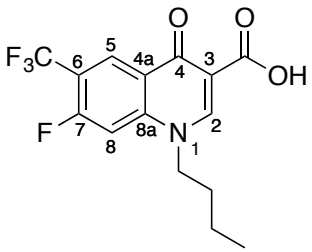
According to the general procedure 10.3, a solution of compound **2f** (3.2 g, 10.8 mmol) and K_2CO_3 (6.0 g, 43.2 mmol) in abs. DMF (30 mL) was treated with *n*-bromobutane (5.8 mL, 54.0 mmol), and the reaction mixture heated at 85 °C for 24 h. The crude product was purified by column chromatography (silica gel; $CHCl_3$/*i*PrOH = 75:1) and then hydrolysed by refluxing under basic conditions (3 M KOH). After acidification with 2 M HCl under ice cooling, the precipitates were collected and dried *in vacuo* yielding **3f**.

appearance:	pale brown solid	
yield:	2.4 g (7.3 mmol/ 68%)	
reaction control:	alkylation	R_f = 0.62 ($CHCl_3$/*i*PrOH = 75:1)
	hydrolysis	R_f = 0.29 ($CHCl_3$/*i*PrOH = 20:1)
melting point [°C]:	224-225 °C	
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3039, 2948, 2869, 1700, 1603, 1508, 1449, 1211, 949.	

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 14.94 (s, 1H, COO**H**), 9.02 (s, 1H, **H**-2), 8.29 (m, 2H, **H**-5/**H**-8), 8.57 (dd, 1H, ^{3}J = 8.8, ^{4}J = 1.2, **H**-6), 4.57 (t, 2H, ^{3}J = 7.2, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 1.74 (quint, 2H, ^{3}J = 7.2, NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.35 (sext, 2H, ^{3}J = 7.6, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 0.93 (t, 3H, ^{3}J = 7.2, NCH$_2$CH$_2$CH$_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 177.7 (1C, **C**-4), 165.6 (1C, **C**OOH), 150.0 (1C, **C**-2), 140.0 (1C, **C**-8a), 129.4 (1C, **C**-6), 128.3 (1C, **C**-4a), 127.8 (1C, **C**-5), 124.5 (1C, **C**-7), 120.5 (1C, **C**-8), 107.9(1C, **C**-3), 53.6 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 30.6 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 18.9 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.4 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

1-Butyl-7-fluoro-4-oxo-6-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylic acid, ***3g*** *(MB399)*



$C_{15}H_{13}F_4NO_3$

M_r = 331.27 g/mol

According to the general procedure 10.3, a solution of compound **2g** (5.2 g, 17.1 mmol) and K_2CO_3 (9.5 g, 68.4 mmol) in abs. DMF was treated with *n*-bromobutane (9.2 mL, 85.5 mmol), and the reaction mixture heated at 80 °C for 24 h. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and then hydrolysed by refluxing under acidic conditions (2 M HCl). The precipitates were collected and dried *in vacuo*, and recrystallised from ACN to yield **3g**.

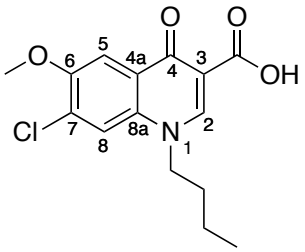
appearance:	yellowish solid	
yield:	3.1 g (9.4 mmol/ 55%)	
reaction control:	alkylation	R_f = 0.36 ($CHCl_3$/MeOH = 100:1)
	hydrolysis	R_f = 0.38 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	198-200 °C	
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3050, 2967, 2878, 1721, 1609, 1455, 1388 1304, 1257, 1139.	

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 14.45 (s, 1H, COO**H**), 9.11 (s, 1H, **H**-2), 8.57 (d, 1H, ^4J = 8.0, **H**-5), 8.30 (d, 1H, ^3J = 12.8, **H**-8), 4.54 (t, 2H, ^3J = 7.6, NC**H$_2$**$CH_2CH_2CH_3$), 1.76 (quint, 2H, ^3J = 7.6, NCH_2C**H$_2$**CH_2CH_3), 1.35 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.91 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2C$**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 177.4 (1C, **C**-4), 165.6 (1C, **C**OOH), 161.2 (d, 1C, $^1J_{C,F}$ = 257.0, **C**-7), 152.0 (1C, **C**-2), 144.0 (d, 1C, $^3J_{C,F}$ = 6.8, **C**-8a), 126.9 (m, 1C, **C**-5), 122.6 (d, 1C, $^4J_{C,F}$ = 1.8, **C**-4a), 122.3 (q, 1C, $^1J_{C,F}$ = 269.9, **C**F_3), 115.9–115.2 (m, 1C, **C**-6), 109.5 (1C, **C**-3), 107.6 (m, 1C, $^2J_{C,F}$ = 26.1, **C**-8), 54.3 (1C, N**C**$H_2CH_2CH_2CH_3$), 31.0 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.4 (1C, NCH_2CH_2**C**H_2CH_3), 13.9 (1C, $NCH_2CH_2CH_2$**C**H_3).

^{19}F NMR (^{1}H coupled, DMSO-d_6, δ [ppm], J [Hz]): -60.49 (d, ^4J = 12.2, C**F$_3$**), -107.92 (m, **F**-7).

1-Butyl-7-chloro-6-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***3h*** *(MB165)*



$C_{15}H_{16}ClNO_4$

M_r = 309.75 g/mol

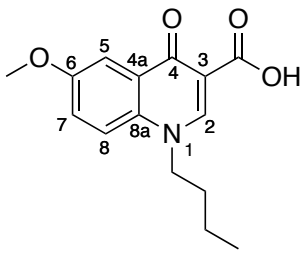
According to the general procedure 10.3, a solution of compound **2h** (5.5 g, 19.6 mmol) and K_2CO_3 (10.8 g, 78.4 mmol) in abs. DMF (50 mL) was treated with *n*-bromobutane (10.5 mL, 98.0 mmol), and the reaction mixture heated at 85 °C for 24 h. The crude product was purified by column chromatography (silica gel; $CHCl_3$/*i*PrOH = 150:1) and then hydrolysed by refluxing under basic conditions (3 M KOH). After acidification with 2 M HCl under ice cooling, the precipitates were collected and dried *in vacuo*. Finally, the solid was recrystallised from ACN to give **3h**.

appearance:	white solid	
yield:	4.1 g (13.3 mmol/ 68%)	
reaction control:	alkylation	R_f = 0.59 ($CHCl_3$/*i*PrOH = 150:1)
	hydrolysis	R_f = 0.74 ($CHCl_3$/MeOH/FA = 100:5:1)
melting point [°C]:	232-233 °C	
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3041, 2963, 2941, 2874, 1702, 1607, 1457, 1433, 1219, 1058.	

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 15.20 (s, 1H, COO**H**), 8.98 (s, 1H, **H**-2), 8.27 (s, 1H, **H**-8), 7.86 (s, 1H, **H**-5), 4.61 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 1.74 (quint, 2H, ^3J = 7.6, NCH_2C**H$_2$**CH_2CH_3), 1.32 (sext, 2H, ^3J = 7.6, NCH_2CH_2C**H$_2$**CH_3), 0.98 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2C$**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 176.3 (1C, **C**-4), 165.8 (1C, **C**OOH), 152.8 (1C, **C**-6), 148.3 (1C, **C**-2), 133.6 (1C, **C**-8a), 129.6 (1C, **C**-7), 125.8 (1C, **C**-4a), 119.9 (1C, **C**-8), 107.7 (1C, **C**-3), 106.6 (1C, **C**-5), 56.7 (1C, O**C**H_3), 53.5 (1C, N**C**$H_2CH_2CH_2CH_3$), 30.8 (1C, NCH_2**C**$H_2CH_2CH_3$), 18.9 (1C, NCH_2CH_2**C**H_2CH_3), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

1-Butyl-6-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***3i*** *(MB26c)*



$C_{15}H_{17}NO_4$

M_r = 275.30 g/mol

According to the general procedure 10.3, a solution of compound **2i** (0.6 g, 2.4 mmol) and K_2CO_3 (1.65 g, 9.6 mmol) in abs. DMF (20 mL) was treated with *n*-bromobutane (1.53 mL, 12.1 mmol), and the reaction mixture heated at 85 °C for 24 h. The crude product was purified by column chromatography (silica gel; $CHCl_3$/*i*PrOH= 75:1) and then hydrolysed by refluxing under basic conditions (3 M KOH). After acidification with 2 M HCl under ice cooling, the precipitates were collected and dried *in vacuo* to give **3i**.

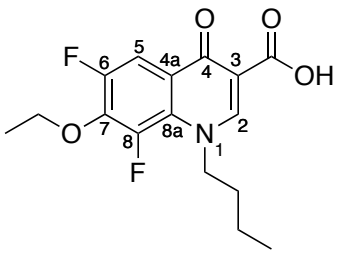
appearance:	white solid	
yield:	480 mg (1.74 mmol/ 73%; Lit[125]: 82%)	
reaction control:	alkylation	R_f = 0.37 ($CHCl_3$/*i*PrOH = 75:1)
	hydrolysis	R_f = 0.32 ($CHCl_3$/MeOH/Fa = 100:1:1)
melting point [°C]:	187-189 °C (Lit[125]: 219-221 °C, EtOH)	
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3033, 2970, 2940, 2866, 1719, 1604, 1457, 1219, 1128.	

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 15.12 (s, 1H, COO**H**), 8.74 (s, 1H, **H**-2), 7.88 (d, 1H, ^4J = 2.8, **H**-5), 7.56 (d, 1H, ^3J = 9.3, **H**-8), 7.45 (dd, 1H, ^3J = 9.2, ^4J = 2.8, **H**-7), 4.31 (t, 2H, ^3J = 7.6, NC**H$_2$**$CH_2CH_2CH_3$), 3.97 (s, 1H, OCH_3), 1.91 (quint, 2H, ^3J = 7.6, NCH_2C**H$_2$**CH_2CH_3), 1.44 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 1.00 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2C$**H$_3$**).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 172.3 (1C, **C**-4), 167.3 (1C, **C**OOH), 157.9 (1C, **C**-6), 146.7 (1C, **C**-2), 133.8 (1C, **C**-8a), 130.3 (1C, **C**-4a), 124.7 (1C, **C**-8), 118.0 (1C, **C**-7), 108.1 (1C, **C**-3), 106.3 (1C, **C**-5), 56.0 (1C, O**C**H_3), 54.9 (1C, N**C**$H_2CH_2CH_2CH_3$), 31.2 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.2 (1C, NCH_2CH_2**C**H_2CH_3), 13.5 (1C, $NCH_2CH_2CH_2$**C**H_3).

Baker *et al.*[125] do not provide spectroscopic data.

1-Butyl-7-ethoxy-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***3j*** *(MB290)*



$C_{16}H_{17}F_2NO_4$

M_r = 325.31 g/mol

The intermediate ethyl ester **I** (ethyl 1-butyl-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate, 590 mg, 1.80 mmol) was suspended in aqueous KOH (3 M) and refluxed for 24 h. Afterwards, aqueous HCl (2 M) was added to the reaction mixture under ice cooling. The resulting precipitate was filtrated and recrystallised from EtOH to give compound **3j**.

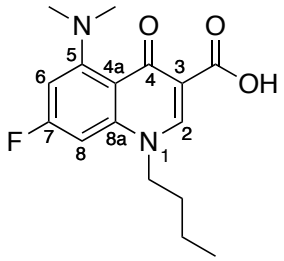
appearance:	white solid
yield:	410 mg (1.26 mmol/ 70%)
reaction control:	R_f = 0.52 ($CHCl_3$/MeOH/FA = 100:2:1)
melting point [°C]:	190-191 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3051, 2929, 1713, 1653, 1555, 1484, 1359, 1305, 1249, 1211, 1096.
mass:	*m/z* 326.3 $[M + H]^+$

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 14.71 (s, 1H, COO**H**), 8.98 (s, 1H, **H**-2), 8.02 (dd, 1H, ^3J = 11.2, ^5J = 2.4, **H**-5), 4.59 – 4.55 (m, 2H, NC**H$_2$**$CH_2CH_2CH_3$, 4.38 (q, 2H, ^3J = 6.8, OC**H$_2$**CH_3), 1.82 – 1.75 (m, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.39 – 1.32 (m, 2H, NCH_2CH_2C**H$_2$**CH_3/OCH_2C**H$_3$**), 0.92 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 175.6 (d, 1C, $^4J_{C,F}$ = 1.2, **C**-4), 165.3 (1C, **C**OOH), 154.3 (dd, 1C, $^1J_{C,F}$ = 249.2, $^3J_{C,F}$ = 6.2, **C**-6), 151.6 (1C, **C**-2), 146.3 (dd, 1C, $^1J_{C,F}$ = 256.2, $^3J_{C,F}$ = 6.2, **C**-8), 140.4 (d, 1C, $^2J_{C,F}$ = 15.7, **C**-7), 127.1 (m, 1C, **C**-8a), 121.5 (d, 1C, $^3J_{C,F}$ = 7.0, **C**-4a), 107.2 (dd, 1C, $^2J_{C,F}$ = 20.8, $^4J_{C,F}$ = 3.7, **C**-5), 107.0 (1C, **C**-3), 71.0 (1C, O**C**H_2H_3), 57.8 (d, 1C, $^4J_{C,F}$ = 14.5, N**C**$H_2CH_2CH_2CH_3$), 31.9 (d, 1C, $^5J_{C,F}$ = 4.2, NCH_2**C**$H_2CH_2CH_3$), 18.9 (1C, NCH_2CH_2**C**H_2CH_3), 15.2 (1C, O**C**H_2H_3), 13.3 (1C, $NCH_2CH_2CH_2$**C**H_3).

^{19}F NMR (^{1}H decoupled, DMSO, δ [ppm], *J* [Hz]): -126.83 (d, $J_{6,8}$ = 10.1, **F**-6), -135.06 (d, $J_{6,8}$ = 10.1, **F**-8).

1-Butyl-5-(dimethylamino)-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***3k*** *(MBMR01_2)*



$C_{16}H_{19}FN_2O_3$

M_r = 306.34 g/mol

A solution of ethyl 5,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **1c** (1 eq, 7.3 g, 29.3 mmol) in non-freshly distilled DMF (50 mL, containing the degradation product dimethyl amine) was treated with K_2CO_3 at 60 °C for 30 min. Afterwards, *n*-bromobutane (20.0 g, 146.3 mmol, 15.8 mL) and a catalytic amount of KI was added to the reaction solution and heated at 90 °C for 24 h. The solvent was removed under reduced pressure and the crude product was mixed with water (60 mL). The aqueous layer was extracted with EtOAc (3x50 mL), the organic layers were combined, and the solvent was evaporated. The intermediate carboxylic ethyl ester was hydrolysed in 2 M HCl by refluxing for 6 h. The mixture was consequently extracted with $CHCl_3$ (3x25 mL) and the combined organic layers were dried over anhydrous Na_2SO_4. After removing the solvent, the product was recrystallised from EtOH to give **3k**.

appearance:	yellow solid
yield:	1.6 g (5.2 mmol/ 18%)
reaction control:	R_f = 0.67 ($CHCl_3$/EtOAc = 20:1)
melting point [°C]:	205-206 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3057, 2952, 2867, 1716, 1628, 1563, 1513, 1436, 1276, 1231, 1187, 1167, 1124, 1029.
mass:	*m/z* 307.1 $[M + H]^+$

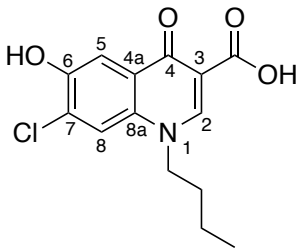
15.53 (s, 1H, COO**H**), 8.60 (s, 1H, **H**-2), 6.62 – 6.57 (m, 2H, **H**-6/**H**-8), 4.13 (t, 2H, ^3J = 7.2, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 2.97 (s, 6H, N(C**H**$_3$)$_2$), 1.91 – 1.83 (m, 2H, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.76 (sext, 2H, ^3J = 7.2, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 1.00 (t, 3H, ^3J = 7.2, NCH$_2$CH$_2$CH$_2$C**H**$_3$).

^{13}C NMR (100 MHz, $CDCl_3$ δ [ppm], *J* [Hz]): 177.1 (1C, **C**-4), 167.4 (1C, **C**OOH), 165.4 (d, 1C, $^1J_{C,F}$ = 249.0, **C**-7), 156.7 (d, 1C, $^3J_{C,F}$ = 13.0, **C**-5), 147.4 (1C, **C**-2), 144.3 (d, 1C, $^3J_{C,F}$ = 15.0, **C**-8a), 113.7 (d, 1C, $^4J_{C,F}$ = 1.2, **C**-4a), 109.0 (1C, **C**-3), 100.1 (d, 1C, $^2J_{C,F}$ = 25.0,

C-6), 92.6 (d, 1C, $^{2}J_{C,F}$ = 27.8, **C**-8), 55.3 (1C, N**C**$H_2CH_2CH_2CH_3$), 44.5 (2C, N(**C**$H_3)_2$), 30.4 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.9 (1C, NCH_2CH_2**C**H_2CH_3), 13.5 (1C, $NCH_2CH_2CH_2$**C**H_3).
^{19}F NMR (^{1}H decoupled, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): -102.56 (s, **F**-7).

10.3.1 Derivatization of the 1-Butyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acids

1-Butyl-7-chloro-6-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***3l*** *(MB173)*



$C_{14}H_{14}ClNO_4$
M_r = 295.72 g/mol

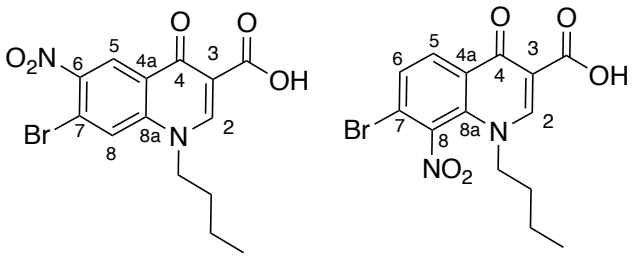
A solution of **3h** (1.38 mg, 4.1 mmol) in CH_2Cl_2 (15 mL) was cooled to 0 °C and BBr_3 (1 M in CH_2Cl_2) (2 eq, 8.2 mL, 12.3 mmol) was slowly added to the reaction. The reaction solution stirred for 12 h at rt and was subsequently quenched with EtOH (10 mL). The solvent was removed under reduced pressure and the product was purified by column chromatography (silica gel; $CHCl_3$/MeOH/FA = 100:5:1). Finally, the product was recrystallised from EtOH to give **3l**.

appearance:	white solid
yield:	450 mg (1.52 mmol/ 37%)
reaction control:	R_f = 0.73 ($CHCl_3$/MeOH/FA = 100:5:1)
melting point [°C]:	281-282 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3170, 2979, 2699, 1659, 1593, 1512, 1478, 1451, 1497, 1210.

^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 15.2 (s, 1H, COO**H**), 11.25 (s, 1H, O**H**), 8.91 (s, 1H, **H**-2), 8.17 (s, 1H, **H**-8), 7.85 (s, 1H, **H**-5), 4.54 (t, 2H, ^{3}J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 1.81 (quint, 2H, ^{3}J = 7.6, NCH_2C**H$_2$**CH_2CH_3), 1.33 (sext, 2H, ^{3}J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.90 (t, 3H, ^{3}J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).
^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 176.4 (1C, **C**-4), 166.0 (1C, **C**OOH), 151.8 (1C, **C**-2), 148.5 (1C, **C**-6), 132.6 (1C, **C**-8a), 128.7 (1C, **C**-7), 125.7 (1C, **C**-4a), 119.7

(1C, **C**-8), 109.7 (1C, **C**-5), 106.6 (1C, **C**-3), 53.4 (1C, N**C**$H_2CH_2CH_2CH_3$), 30.7 (1C, NCH_2**C**$H_2CH_2CH_3$), 18.9 (1C, NCH_2CH_2**C**H_2CH_3), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

7-Bromo-1-butyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***3m*** *and 7-Bromo-1-butyl-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,* ***3m'*** *(MB170)*



$C_{14}H_{13}BrN_2O_5$

M_r = 369.17 g/mol

According to the procedure of Suzuki *et al.*[36] compound **3f** (450 mg, 1.39 mmol) was dissolved in *conc*. H_2SO_4 (10 mL). The previously prepared mixture of *conc*. H_2SO_4 and *conc.* HNO_3 (1:1) was slowly added dropwise to the reaction solution. The reaction mixture was allowed to warm to rt, stirred for 6 h and poured onto ice. The products were extracted with $CHCl_3$ (3x40 mL) and the solvent was removed *in vacuo*. The crude products were purified and separated by column chromatography (silica gel; $CHCl_3$/MeOH = 20:1).

Compound **3m**:

appearance:	yellow solid
yield:	180 mg (0.49 mmol/ 35%)
reaction control:	R_f = 0.73 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	258-259 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3105, 3047, 2965, 2932, 1741, 1602, 1521, 1450, 1347, 1220, 1132.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 14.12 (s, 1H, COO**H**), 9.09 (s, 1H, **H**-2), 8.85 (s, 1H, **H**-5), 8.57 (s, 1H, **H**-8), 4.60 (t, 2H, ^3J= 7.6, N**CH_2**$CH_2CH_2CH_3$), 1.74 (quint, 2H, ^3J = 7.6, NCH_2**CH_2**CH_2CH_3), 1.34 (sext, 2H, ^3J= 7.6, NCH_2CH_2**CH_2**CH_3), 0.93 (t, 3H, ^3J= 7.6, $NCH_2CH_2CH_2$**CH_3**).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 177.7 (1C, **C**-4), 164.9 (1C, **C**OOH), 151.6 (1C, **C**-2), 146.3 (1C, **C**-6), 141.2 (1C, **C**-8a), 125.0 (1C, **C**-4a), 124.7 (1C, **C**-5), 123.4

(1C, **C**-8), 119.0 (1C, **C**-7), 109.1 (1C, **C**-3), 53.6 (1C, N**C**$H_2CH_2CH_2CH_3$), 30.6 (1C, NCH_2**C**$H_2CH_2CH_3$), 18.9 (1C, NCH_2CH_2**C**H_2CH_3), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

Compound **3m'**:

appearance:	yellow solid
yield:	110 mg (0.30 mmol/ 21%)
reaction control:	R_f = 0.45 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	195-196 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3055, 2852, 2870, 1707, 1656, 1597, 1573, 1534, 1481, 1402, 1384, 1350.

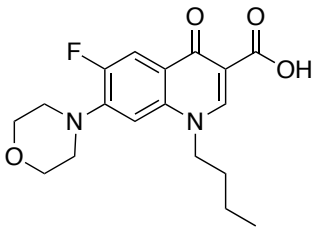
^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 13.00 (s, 1H, COO**H**), 8.71 (s, 1H, **H**-2), 8.46 (d, 1H, ^3J = 8.8, **H**-5), 7.82 (d, 1H, ^3J = 8.8, **H**-6), 4.10 (t, 2H, ^3J = 7.6, N**CH_2**$CH_2CH_2CH_3$), 1.81 (quint, 2H, ^3J = 7.6, NCH_2**CH_2**CH_2CH_3), 1.35 (sext, 2H, ^3J = 7.6, NCH_2CH_2**CH_2**CH_3), 0.96 (t, 3H, ^3J = 7.6, $NCH_2CH_2CH_2$**CH_3**).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 176.6 (1C, **C**-4), 168.5 (1C, **C**OOH), 151.8 (1C, **C**-2), 140.2 (1C, **C**-8), 135.1 (1C, **C**-8a), 130.6 (1C, **C**-6), 130.0 (1C, **C**-5), 127.8 (1C, **C**-4a), 122.9 (1C, **C**-7), 110.8 (1C, **C**-3), 56.7 (1C, N**C**$H_2CH_2CH_2CH_3$), 33.2 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.5 (1C, NCH_2CH_2**C**H_2CH_3), 13.3 (1C, $NCH_2CH_2CH_2$**C**H_3).

10.4 S_NAr of the 4-Oxo-1,4-dihydroquinoline-3-carboxylic acids.

General procedure according to Hiltensperger *et al.*[7]: The 1-alkyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acids **3a**, **3f-h**, **3k-l, 3m'** (1 eq), dissolved in 5-15 ml morpholine, heated for 4-10 h under microwave irradiation at 110 °C. The reaction solution was acidified with 2 M HCl at 0 °C (pH 2) and the precipitate was collected. The resulted yellow solid was subsequently dried *in vacuo* and recrystallised from EtOH/EtOAc.
If there is a deviation of the general procedure, the specific proceeding is described in the respective protocol hereinafter.

1-Butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***4a*** *(MB04)*



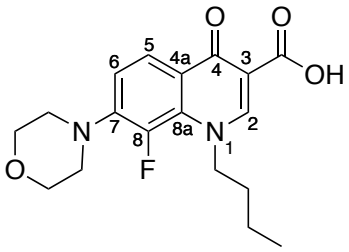
$C_{18}H_{21}FN_2O_4$
M_r = 348.37 g/mol

According to the general procedure 10.4, compound **3a** (400 mg, 1.34 mmol) was dissolved in morpholine (7.5 mL) and the reaction solution heated at 110 °C under microwave irradiation for 4 h. Subsequently, the reaction was acidified (pH 2) by means of aqueous HCl (2 M) under ice cooling. The resulting precipitates were collected, dried *in vacuo* and recrystallised from EtOH/EtOAc (1:1).

appearance:	pale yellow solid
yield:	295 mg (0.84 mmol/ 63%; Lit[7]: 68%)
reaction control:	R_f = 0.63 ($CHCl_3$/MeOH = 20:1)
melting point [°C]:	234-235 °C (Lit[7]: 242-243 °C)
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3060, 2965, 2873, 1715, 1650, 1467, 1458, 1257, 1114, 943.

The obtained physical and spectroscopic data are consistent with that found in literature.[7]

1-Butyl-8-fluoro-7-morpholino-1,4-dihydroquinoline-3-carboxylic acid, ***4b*** *(MB389)*



$C_{18}H_{21}FN_2O_4$

M_r = 348.37 g/mol

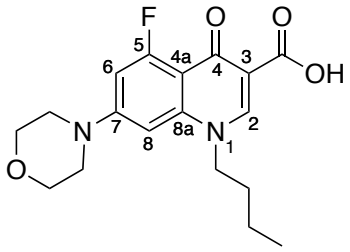
Compound **3b** (400 mg, 1.34 mmol) was dissolved in DMF (7.5 mL) and the reaction solution was treated with morpholine (10 eq, 1.17 mL, 13.44 mmol), and heated for 48 h at 145 °C. Afterwards, the solvent was removed under reduced pressure, the mixture was acidified to pH 2, and the yellow solid was collected. The crude product was recrystallised from EtOH to yield **4b**.

appearance:	greyish solid
yield:	290 mg (0.83 mmol/ 62%)
reaction control:	R_f = 0.39 ($CHCl_3$/MeOH/FA = 100:2:1)
melting point [°C]:	272-273 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3046, 2955, 2857, 1719, 1614, 1444, 1247, 1119, 926.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 15.1 (br, 1H, COO**H**), 8.58 (s, 1H, **H**-2), 8.26 (dd, 1H, ^3J = 8.8, ^5J = 1.6, **H**-5), 7.19 (m, 1H, **H**-6), 4.41 (m, 2H, NC**H$_2$**$CH_2CH_2CH_3$), 3.92 – 3.91 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.30 – 3.27 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.88 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.34 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.92 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 177.2 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 166.8 (1C, **C**OOH), 150.9 (1C, **C**-2), 144.7 (d, 1C, $^2J_{C,F}$ = 8.5, **C**-7), 143.2 (d, 1C, $^1J_{C,F}$ = 246.9, **C**-8), 129.9 (d, 1C $^2J_{C,F}$ = 6.3, **C**-8a), 123.4 (d, 1C, $^4J_{C,F}$ = 3.9, **C**-5), 122.1 (1C, **C**-4a), 117.3 (d, 1C, $^4J_{C,F}$ = 3.1, **C**-6), 108.1 (1C, **C**-3), 66.7 (2C, morpholino-**C**H_2-O-**C**H_2), 59.4 (d, 1C, $^4J_{C,F}$ = 16.0, N**C**$H_2CH_2CH_2CH_3$), 50.7 (d, 2C, $^4J_{C,F}$ = 4.2, morpholino-**C**H_2-N-**C**H_2), 32.8 (d, 1C, $^5J_{C,F}$ = 4.0, NCH_2**C**$H_2CH_2CH_3$), 19.7 (1C, NCH_2CH_2**C**H_2CH_3), 13.6 (1C, $NCH_2CH_2CH_2$**C**H_3).

1-Butyl-5-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***4c*** *(MBMR35)*



$C_{18}H_{21}FN_2O_4$

M_r 348.37 g/mol

According to Heravi *et al.*[42], compound **3c** (1.0 g, 3.8 mmol), TEA (750 µL, 5.4 mmol) and boron trifluoride diethyl etherate (675 µL, 5.36 mmol) were dissolved in CH_2Cl_2 and refluxed for 2 h. The solvent was removed under reduced pressure and the crude product was treated with water/MeOH (1:1). The resulting yellow precipitate was collected and dried *in vacuo*. Afterwards, the yellow borate complex (1 eq, 980 mg, 2.98 mmol) was dissolved in ethanol (25 mL) and the reaction mixture was treated with TEA (830 µL, 5.97 mmol) and morpholine (260 mL, 3.0 mmol), and heated for 4 h at 60 °C. The solvent was removed under reduced pressure and the intermediate was refluxed in 2 M NaOH for 2 h. Finally, compound **4c** precipitated after the addition of 2 M HCl.

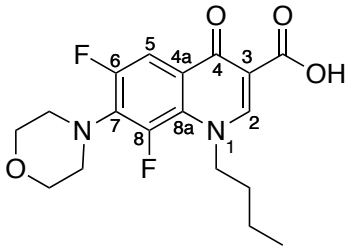
appearance:	yellow solid
yield:	260 mg (0.75 mmol/ 25%)
reaction control:	R_f = 0.45 ($CHCl_3$/MeOH = 100:3)
melting point [°C]:	257-260 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3389, 3049, 2975, 1701, 1630, 1539, 1519, 1448, 1365, 1265, 1216, 1160, 1118, 1051.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 15.0 (br, 1H, COO**H**), 8.54 (s, 1H, **H**-2), 6.71 (d, 1H, ^3J = 14.4, **H**-6), 6.45 (s, 1H, **H**-8), 4.18 (t, 2H, ^3J = 6.8, NC**H$_2$**$CH_2CH_2CH_3$), 3.92 – 3.90 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.40 – 3.38 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.90 – 1.87 (m, 2H, NCH_2C**H$_2$**CH_2CH_3), 1.42 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 1.09 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2C$**H$_3$**).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 177.1 (d, 1C, $^4J_{C,F}$ = 1.7, **C**-4), 167.2 (1C, **C**OOH), 163.4 (d, 1C, $^1J_{C,F}$ = 260.8, **C**-5), 154.5 (d, 1C, $^3J_{C,F}$ = 12.9, **C**-7), 148.3 (1C, **C**-2), 142.6 (d, 1C, $^3J_{C,F}$ = 5.7, **C**-8a), 108.6 (1C, **C**-3), 108.3 (d, 1C, $^2J_{C,F}$ = 9.9, **C**-4a), 100.4 (d, 1C, $^2J_{C,F}$ = 25.6, **C**-6), 94.0 (1C, **C**-8), 66.1 (2C, morpholino-**C**H_2-O-**C**H_2), 55.0 (1C,

N**C**$H_2CH_2CH_2CH_3$), 47.1 (2C, morpholino-**C**H_2-N-**C**H_2), 30.3 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.9 (1C, NCH_2CH_2**C**H_2CH_3), 13.6 (1C, $NCH_2CH_2CH_2$**C**H_3).

1-Butyl-6,8-difluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***4d*** *(MBPG18).*



$C_{18}H_{20}F_2N_2O_4$

M_r = 366.36 g/mol

According to to Sunduru *et al.*[38], compound **3d** (250 mg, 0.84 mmol) was dissolved in abs. ACN (20.0 mL) and the reaction solution treated with morpholine (0.15 mL, 1.7 mmol), and refluxed for 8 h. Afterwards, the solvent was removed under reduced pressure, the crude product was purified by means of column chromatography (silica gel; $CHCl_3$/MeOH/FA = 100:2:1) and was recrystallised from EtOH, yielding compound **4d**.

appearance:	white solid
yield:	240 mg (0.66 mmol/ 36%)
reaction control:	R_f = 0.31 ($CHCl_3$/MeOH/FA = 100:2:1)
melting point [°C]:	210-212 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3320, 3051, 2953, 2850, 1715, 1615, 1539, 1464, 1378, 1279, 1207, 1113, 1051, 1016.

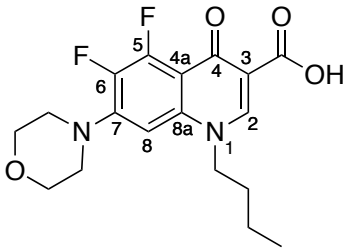
^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 14.73 (s, 1H, COO**H**), 8.91 (s, 1H, **H**-2), 7.86 (dd, 1H, ^3J = 11.2, ^5J = 2.4, **H**-5), 4.59 – 4.55 (m, 2H, N**CH_2**$CH_2CH_2CH_3$), 3.75 – 3.72 (m, 4H, morpholino-C**H_2**-O-C**H_2**), 3.34 (br, 4H, morpholino-C**H_2**-N-C**H_2**), 1.80 (quint, 2H, ^3J = 7.2, NCH_2C**H_2**CH_2CH_3), 1.30 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H_2**CH_3), 0.91 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H_3**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 175.6 (d, 1C, $^4J_{C,F}$ = 2.4, **C**-4), 165.4 (1C, **C**OOH), 154.3 (dd, 1C, $^1J_{C,F}$ = 248.4, $^3J_{C,F}$ = 6.2, **C**-6), 151.3 (1C, **C**-2), 146.3 (dd, 1C, $^1J_{C,F}$ = 249.7, $^3J_{C,F}$ = 6.6, **C**-8), 133.4 (m, 1C, **C**-7), 127.2 (dd, 1C, $^2J_{C,F}$ = 7.1, $^4J_{C,F}$ = 2.0, **C**-8a), 120.6 (m, 1C, **C**-4a), 107.2 (dd, 1C, $^2J_{C,F}$ = 22.8, $^4J_{C,F}$ = 2.7, **C**-5), 106.7 (1C, **C**-3), 66.6 (2C, morpholino-**C**H_2-O-**C**H_2), 57.9 (d, 1C, $^4J_{C,F}$ = 15.6, N**C**$H_2CH_2CH_2CH_3$), 47.1 (t, 2C,

$^4J_{C,F}$ = 3.8, morpholino-**C**H_2-N-**C**H_2), 31.9 (d, 1C, $^5J_{C,F}$ = 4.1, NCH_2**C**$H_2CH_2CH_3$), 18.9 (1C, NCH_2CH_2**C**H_2CH_3), 13.3 (1C, $NCH_2CH_2CH_2$**C**H_3).

^{19}F NMR (^{1}H decoupled, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): -119.37 (d, $^4J_{6,8}$ = 11.4), -129.13 (d, $^4J_{6,8}$ = 11.4).

1-Butyl-5,6-difluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***4e*** *(MB413, MB416, MB420).*



$C_{18}H_{20}F_2N_2O_4$

M_r = 366.36 g/mol

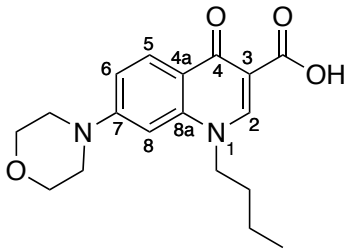
In accordance to the synthesis of compound **4c**, compound **3e** (650 mg, 2.17 mmol), TEA (453 µL, 3.26 mmol) and boron trifluoride diethyl etherate (465 µL, 3.26 mmol) were dissolved in CH_2Cl_2 and refluxed for 2 h. The solvent was removed under reduced pressure and a mixture of water/MeOH (1:1) was added to the residue. The resulting yellow precipitate was dried *in vacuo*. Afterwards, the yellow solid was dissolved in EtOH (25 mL) and was treated with TEA (453 µL, 3.26 mmol) and morpholine (208 µL, 2.39 mmol), and stirred for 1 h at rt. The solvent was removed under reduced pressure and the intermediate (310 mg) was refluxed in 2 M NaOH for 2 h. Finally, the compound **4e** precipitated after the addition of 2 M HCl.

appearance:	yellow solid
yield:	200 mg (0.55 mmol/ 25%)
reaction control:	R_f = 0.58 ($CHCl_3$/MeOH = 25:1)
melting point [°C]:	218-220 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3047, 2950, 2857, 1712, 1630, 1539, 1279, 1244, 1109.

^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 15.3 (br, 1H, COO**H**), 8.63 (s, 1H, **H**-2), 6.56 (d, 1H, ^4J = 5.6, **H**-8), 4.21 (t, 3H, ^3J = 7.2, N**C**$H_2CH_2CH_2CH_3$), 3.93 – 3.90 (m, 4H, morpholino-**C**H_2-O-**C**H_2), 3.34 – 3.32 (m, 4H, morpholino-**C**H_2-N-**C**H_2), 1.88 (quint, 2H, ^3J = 7.2, NCH_2**C**$H_2CH_2CH_3$), 1.45 (sext, 2H, ^3J = 7.2, NCH_2CH_2**C**H_2CH_3), 1.02 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$**C**H_3).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 175.0 (1C, **C**-4), 165.3 (1C, **C**OOH), 153.2 (dd, 1C, $^{1}J_{C,F}$ = 249.1, $^{2}J_{C,F}$ = 12.4, **C**-5), 149.8 (1C, **C**-2), 144.8 (m, 1C, **C**-7), 139.8 (d, 1C, $^{3}J_{C,F}$ = 10.4, **C**-8a), 138.6 (dd, 1C, $^{1}J_{C,F}$ = 250.0, $^{2}J_{C,F}$ = 14.5, **C**-6), 112.9 (d, 1C, $^{2}J_{C,F}$ = 6.8, **C**-4a), 110.1 (1C, **C**-3), 103.4 (d, 1C, $^{3}J_{C,F}$ = 4.5, **C**-8), 66.3 (2C, morpholino-**C**H_2-O-**C**H_2), 53.5 (1C, N**C**$H_2CH_2CH_2CH_3$), 48.9 (d, 2C, $^{4}J_{C,F}$ = 4.2, morpholino-**C**H_2-N-**C**H_2), 29.3 (1C, NCH_2**C**$H_2CH_2CH_3$), 18.9 (1C, NCH_2CH_2**C**H_2CH_3), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

1-Butyl-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***4f*** *(MB13)*



$C_{18}H_{22}N_2O_4$

M_r = 330.38 g/mol

According to the general procedure 10.4, compound **3f** (860 mg, 2.59 mmol) was dissolved in morpholine (10.0 mL) and the reaction solution heated for 4.5 h under microwave irradiation at 110 °C. After acidification (pH 2) the solution and drying the precipitate *in vacuo*, the solid was recrystallised from EtOH to yield **4f**.

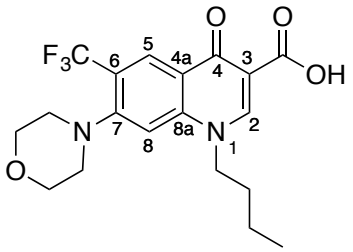
appearance:	yellow solid
yield:	710 mg (2.15 mmol/ 83%)
reaction control:	R_f = 0.68 ($CHCl_3$/MeOH = 10:1)
melting point [°C]:	229–230 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3066, 2956, 2862, 1714, 1615, 1519, 1444, 1243, 1103.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], J [Hz]): 15.33 (s, 1H, COO**H**), 8.62 (s, 1H, **H**-2), 8.34 (d, 1H, ^{3}J = 9.4, **H**-5), 7.13 (dd, 1H, ^{3}J = 9.2, ^{4}J = 2.4, **H**-6), 6.66 (d, 1H, ^{4}J = 2.4, **H**-8), 4.22 (t, 2H, ^{3}J = 7.6, N**C**$\mathbf{H_2}CH_2CH_2CH_3$), 3.92 – 3.90 (m, 4H, morpholino-**C**H_2-O-**C**H_2), 3.40 – 3.38 (m, 4H, morpholino-**C**H_2-N-**C**H_2), 1.91 (quint, 2H, ^{3}J = 7.6, NCH_2C$\mathbf{H_2}CH_2CH_3$), 1.47 (sext, 2H, ^{3}J = 7.6, NCH_2CH_2C$\mathbf{H_2}CH_3$), 1.02 (t, 3H, ^{3}J = 7.2, $NCH_2CH_2CH_2$C$\mathbf{H_3}$).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], J [Hz]): 177.48 (1C, **C**-4), 167.6 (1C, **C**OOH), 154.9 (1C, **C**-7), 148.0 (1C, **C**-2), 141.3 (1C, **C**-8a), 128.5 (1C, **C**-5), 118.4 (1C, **C**-4a), 114.5 (1C, **C**-6), 107.9 (1C, **C**-3), 97.8 (1C, **C**-8), 66.4 (2C, morpholino-**C**H_2-O-**C**H_2), 53.6 (1C,

N**C**$H_2CH_2CH_2CH_3$), 47.6 (2C, morpholino-**C**H_2-N-**C**H_2), 30.6 (1C, NCH_2**C**$H_2CH_2CH_3$), 18.9 (1C, NCH_2CH_2**C**H_2CH_3), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

1-Butyl-morpholino-4-oxo-6-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylic acid, ***4g*** *(MB402)*



$C_{19}H_{21}F_3N_2O_4$

M_r = 398.38 g/mol

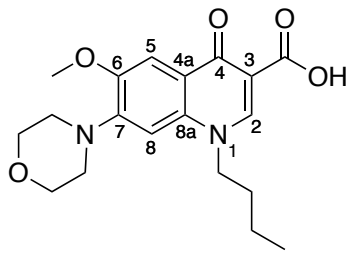
According to the general procedure 10.4, compound **3g** (600 mg, 1.81 mmol) was dissolved in morpholine (10.0 mL) and heated for 6 h under microwave irradiation at 110 °C. After acidification (pH 2) the solution and drying the precipitate *in vacuo*, the solid was recrystallised from EtOH to yield **4g**.

appearance:	yellow solid
yield:	510 mg (1.28 mmol/ 70%)
reaction control:	R_f = 0.38 ($CHCl_3$/MeOH/FA = 100:2:1)
melting point [°C]:	182-185 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3046, 2952, 2857, 1725, 1610, 1455, 1393, 1303, 1244, 1102.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 14.52 (s, 1H, COO**H**), 8.68 (s, 1H, **H**-2), 8.67 (s, 1H, **H**-5), 7.18 (s, 1H, **H**-8), 4.25 (t, 2H, ^3J = 7.6, NC**H$_2$**$CH_2CH_2CH_3$), 3.84 – 3.82 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.07 – 3.05 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.85 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.40 (sext, 2H, ^3J = 7.6, NCH_2CH_2C**H$_2$**CH_3), 0.91 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 177.4 (1C, **C**-4), 165.4 (1C, **C**OOH), 156.2 (1C, **C**-7), 149.4 (1C, **C**-2), 142.2 (1C, **C**-8a), 128.6 (q, 1C, $^3J_{C,F}$ = 5.5, **C**-5), 124.6 (q, 1C, $^2J_{C,F}$ = 30.6, **C**-6), 123.2 (q, 1C, $^1J_{C,F}$ = 278.5, **C**F_3), 121.7 (1C, **C**-4a), 109.4 (1C, **C**-8), 109.2 (1C, **C**-3), 66.8 (2C, morpholino-**C**H_2-O-**C**H_2), 54.3 (1C, N**C**$H_2CH_2CH_2CH_3$), 53.4 (2C, morpholino-**C**H_2-N-**C**H_2), 30.7 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.8 (1C, NCH_2CH_2**C**H_2CH_3), 13.5 (1C, $NCH_2CH_2CH_2$**C**H_3).

1-Butyl-6-methoxy-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***4h*** *(MB166)*



$C_{19}H_{24}N_2O_5$

M_r = 360.41 g/mol

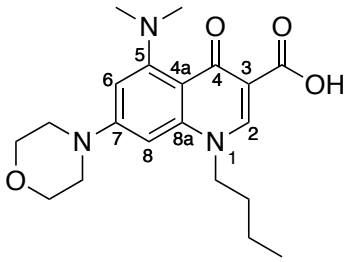
According to the general procedure 10.4, compound **3h** (330 mg, 1.07 mmol) was dissolved in morpholine (15.0 mL) and the reaction solution heated for 12 h under microwave irradiation at 110 °C. After acidification (pH 2) the solution and drying the precipitate *in vacuo*, the solid was recrystallised from EtOH to yield **4h**.

appearance:	grey solid
yield:	110 mg (0.31 mmol / 29%)
reaction control:	R_f = 0.58 ($CHCl_3$/MeOH/FA = 100:5:1)
melting point [°C]:	248-250 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3045, 2952, 1711, 1616, 1470, 1446, 1256, 1229, 1111, 1041, 1006.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 15.77 (s, 1H, COO**H**), 8.87 (s, 1H, **H**-2), 7.67 (s, 1H, **H**-5), 7.09 (s, 1H, **H**-8), 4.58 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.95 (s, 3H), 3.79 – 3.75 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.26 – 3.23 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.79 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.33 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.92 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 175.8 (1C, **C**-4), 166.5 (1C, **C**OOH), 150.7 (1C, **C**-7), 147.2 (1C, **C**-6), 147.1 (1C, **C**-2), 135.0 (1C, **C**-8a), 120.0 (1C, **C**-4a), 106.5 (1C, **C**-3), 104.9 (1C, **C**-5)**,** 104.8 (1C, **C**-8), 66.0 (2C, morpholino-**C**H_2-O-**C**H_2), 55.8 (1C, O**C**H_3), 53.2 (1C, N**C**$H_2CH_2CH_2CH_3$), 49.9 (2C, morpholino-**C**H_2-N-**C**H_2), 30.3 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.0 (1C, NCH_2CH_2**C**H_2CH_3), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

1-Butyl-5-(dimethylamino)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***4i*** *(MBMR10)*



$C_{20}H_{27}N_3O_4$

M_r = 373.45 g/mol

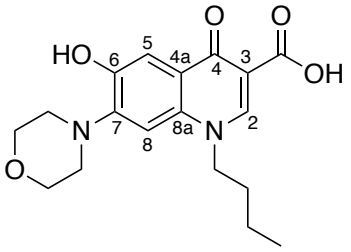
Compound **3k** (500 mg, 1.78 mmol) was dissolved in DMF (20 mL) and the reaction solution treated with morpholine (5 eq, 687 µL, 8.90 mmol), and heated for 2 h at 145 °C. Afterwards, the solvent was reduced under reduced pressure and the crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH/FA = 100:3:1). The crude product was recrystallised from EtOH to yield **4i**.

appearance:	greyish solid
yield:	160 mg (0.80 mmol/ 56%)
reaction control:	R_f = 0.61 ($CHCl_3$/MeOH/FA = 100:3:1)
melting point [°C]:	228-229 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2945, 2833, 1696, 1616, 1595, 1526, 1434, 1359, 1233, 1110.

^{1}H NMR (DMSO-d_6, δ [ppm], J [Hz]): 16.28 (s, 1H, COO**H**), 8.67 (s, 1H, **H**-2), 6.44 – 6.42 (m, 2H, **H**-6/**H**-8), 4.39 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.77 – 3.74 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.41 – 3.38 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 2.80 (s, 6H, N(C**H$_3$**)$_2$), 1.76 – 1.71 (m, 2H, NCH_2C**H$_2$**CH_2CH_3), 1.32 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.92 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (DMSO-d_6, δ [ppm], J [Hz]): 175.9 (1C, **C**-4), 167.0 (1C, **C**OOH), 154.7 (1C, **C**-7), 153.9 (1C, **C**-5), 147.6 (1C, **C**-2), 143.9 (1C, **C**-8a), 108.7 (1C, **C**-4a), 106.5 (1C, **C**-3), 99.7 (1C, **C**-6), 91.4 (1C, **C**-8), 65.8 (2C, morpholino-**C**H_2-O-**C**H_2), 53.5 (1C, N**C**$H_2CH_2CH_2CH_3$), 46.6 (2C, morpholino-**C**H_2-**C**H_2), 44.1 (2C, N(**C**H_3)$_2$), 29.7 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.0 (1C, NCH_2CH_2**C**H_2CH_3), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

1-Butyl-6-hydroxy-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***4j*** *(MB177)*



$C_{18}H_{22}N_2O_5$

M_r = 346.38 mg/mol

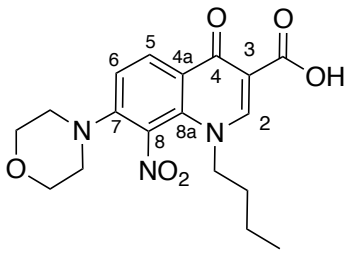
According to the general procedure 10.4, compound **3h** (300 mg, 1.01 mmol) was dissolved in morpholine (10.0 mL) and heated for 5 h under microwave irradiation at 110 °C. After acidification (pH 2) the solution and drying the precipitate *in vacuo*, the solid was recrystallised from EtOH to yield **4j**.

appearance:	brown solid
yield:	200 mg (0.87 mmol/ 57%)
reaction control:	R_f = 0.58 ($CHCl_3$/MeOH/FA = 100:5:1)
melting point [°C]:	268-269 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3269, 2934, 1726, 1621, 1527, 1506, 1419, 1302, 1257, 1222, 1122, 1041.

^{1}H NMR (DMSO-d_6, δ [ppm], J [Hz]): 15.9 (s, 1H, COO**H**), 8.82 (br, 1H, O**H**), 8.17 (s, 1H, **H**-2), 7.68 (s, 1H, **H**-5), 7.03 (s, 1H, **H**-8), 4.54 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.80 – 3.78 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.29 – 3.28 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.81 – 1.77 (m, 2H, NCH_2C**H$_2$**CH_2CH_3), 1.33 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.96 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (DMSO-d_6, δ [ppm], J [Hz]): 176.3 (1C, **C**-4), 167.2 (1C, **C**OOH), 149.5 (1C, **C**-7), 147.1 (2C, **C**-2/**C**-6), 134.6 (1C, **C**-8a), 120.7 (1C, **C**-4a), 109.5 (1C, **C**-5), 106.7 (1C, **C**-3), 105.2 (1C, **C**-8), 66.5 (2C, morpholino-**C**H_2-O-**C**H_2), 53.7 (1C, N**C**$H_2CH_2CH_2CH_3$), 49.9 (2C, morpholino-**C**H_2-**C**H_2), 30.9 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.6 (1C, NCH_2CH_2**C**H_2CH_3), 13.9 (1C, $NCH_2CH_2CH_2$**C**H_3).

1-Butyl-7-morpholino-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***4k'*** *(MB174)*



$C_{18}H_{21}N_3O_6$

M_r = 375.38 mg/mol

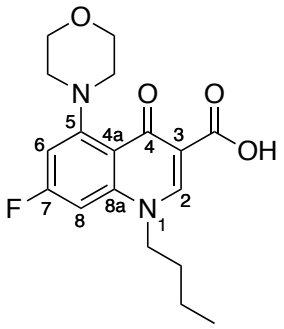
According to the general procedure 10.4, compound **3m'** (110 mg, 0.30 mmol) was dissolved in morpholine (7.5 mL) and heated for 1 h under microwave irradiation at 110 °C. After acidification (pH 2) the solution and drying the precipitate *in vacuo*, the solid was recrystallised from EtOH to yield **4k'**.

appearance:	yellow solid
yield:	100 mg (0.27 mmol/ 89%)
reaction control:	R_f = 0.73 ($CHCl_3$/MeOH/FA = 100:2:1)
melting point [°C]:	209-210 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3210, 2860, 2705, 1719, 1606, 1530, 1445, 1343, 1220.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 14.2 (s, 1H, COO**H**), 8.73 – 8.67 (m, 2H, **H**-2/ **H**-5), 7.45 (d, 1H, ^3J = 8.8, **H**-6), 4.06 (m, 2H, NC**H$_2$**$CH_2CH_2CH_3$), 3.83 – 3.81 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.12 – 3.09 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.76 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.32 (sext, 2H, ^3J = 7.2, $NCH_2CH_2CH_2CH_3$), 0.99 (t, 3H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 176.8 (1C, **C**-4), 165.7 (1C, **C**OOH), 151.2 (1C, **C**-7), 151.2 (1C, **C**-2), 140.4 (1C, **C**-8), 149.0 (1C, **C**-2), 132.6 (1C, **C**-8a), 130.6 (1C, **C**-5), 124.9 (1C, **C**-4a), 120.9 (1C, **C**-6), 110.2 (1C, **C**-3), 66.9 (2C, morpholino-**C**H_2-O-**C**H_2), 56.7 (1C, N**C**$H_2CH_2CH_2CH_3$), 53.6 (2C, morpholino-**C**H_2-N-**C**H_2), 33.0 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.5 (1C, NCH_2CH_2**C**H_2CH_3), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

1-Butyl-7-fluoro-5-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***4l*** *(MB333)*



$C_{18}H_{21}FN_2O_4$

M_r = 348.37 g/mol

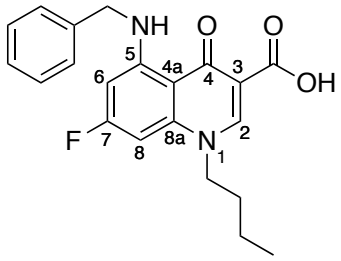
In accordance with the procedure mentioned by Shibamori *et al.*[35], compound **3c** (400 mg, 1.44 mmol) was dissolved in EtOH (15 mL) and treated with morpholine (1 eq, 114 µL, 1.44 mmol), and TEA (2 eq, 400 µL, 2.86 mmol). The reaction solution was refluxed for 1 h and the solvent removed under reduced pressure. The crude product was recrystallised from EtOAc/EtOH (5:1) to yield **4l**.

appearance:	yellowish solid
yield:	280 mg (0.80 mmol/ 56%)
reaction control:	R_f = 0.61 ($CHCl_3$/MeOH/TEA = 100:2:1)
melting point [°C]:	230-231 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3123, 3054, 2863, 2805, 1695, 1627, 1601, 1564, 1522, 1426, 1299, 1262, 1211, 1112, 1007.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 15.84 (s, 1H, COO**H**), 8.90 (s, 1H, **H**-2),), 7.31 (dd, 1H, ^3J = 10.8, ^4J = 2.0, **H**-8), 6.91 (dd, 1H, ^3J = 11.6, ^4J = 2.4, **H**-6), 4.44 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.82 – 3.79 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.08 (br, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.71 (m, 2H, NCH_2C**H$_2$**CH_2CH_3), 1.32 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.90 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 177.1 (1C, **C**-4), 166.2 (1C, **C**OOH), 166.5 (d, 1C, $^1J_{C,F}$ = 248.0, **C**-7), 156.7 (d, 1C, $^3J_{C,F}$ = 12.8, **C**-5), 149.0 (1C, **C**-2), 144.7 (d, 1C, $^3J_{C,F}$ = 15.0, **C**-8a), 115.4 (1C, **C**-4a), 108.8 (1C, **C**-3), 103.0 (d, 1C, $^2J_{C,F}$ = 24.8, **C**-6), 96.7 (d, 1C, $^2J_{C,F}$ = 27.4, **C**-8), 66.51 (2C, morpholino-**C**H_2-O-**C**H_2), 54.7 (1C, N**C**$H_2CH_2CH_2CH_3$), 53.4 (2C, morpholino-**C**H_2-N-**C**H_2), 30.7 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.5 (1C, NCH_2CH_2**C**H_2CH_3), 13.9 (1C, $NCH_2CH_2CH_2$**C**H_3).

5-(Benzylamino)-1-butyl-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***4m*** *(MB335)*



$C_{21}H_{21}FN_2O_3$

M_r = 368.41 g/mol

Compound **3c** (600 mg, 2.14 mmol) was dissolved in abs. DMF (10 mL) and treated with benzylamine (1.5 eq, 350 µL, 3.20 mmol). The reaction solution was heated at 90 °C for 1 h and the solvent subsequently removed under reduced pressure. After adding 5 mL HCl (2 M) to the reaction, the occurring precipitate was filtrated, and dried *in vacuo* to give compound **4m**.

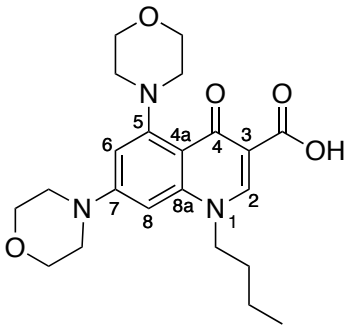
appearance:	yellow solid
yield:	530 mg (1.43 mmol / 67%)
reaction control:	R_f = 0.82 ($CHCl_3$/MeOH = 100:3)
melting point [°C]:	239-240 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3264, 3046, 2932, 2856, 1698, 1627, 1576, 1437, 1348, 1274, 1220, 1114, 1035, 934.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 14.87 (s, 1H, COO**H**), 10.12 – 10.09 (m, 1H, N-**H**), 8.85 (s, 1H, **H**-2), 7.42 – 7.35 (m, 4H, Bn-C**H**$_{aromat.}$), 7.30 – 7.27 (m, 1H, Bn-C**H**$_{aromat.}$), 6.78 (dd, 1H, ^3J = 11.6, ^4J = 2.4, **H**-6), 6.50 (dd, 1H, ^3J = 12.4, ^4J = 2.0, **H**-8), 4.52 (d, 2H, Bn-C**H$_2$**), 4.36 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 1.70 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.30 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.93 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 179.5 (1C, **C**-4), 165.6 (1C, **C**OOH), 165.2 (d, 1C, $^1J_{C,F}$ = 247.0, **C**-7), 153.6 (d, 1C, $^3J_{C,F}$ = 15.0, **C**-5), 140.1 (1C, **C**-2), 143.5 (1C, **C**-8a), 137.9 (1C, Bn-**C**$_{aromat.}$), 128.7 (2C, Bn-**C**H$_{aromat.}$), 127.2 (2C, Bn-**C**H$_{aromat.}$), 127.1 (1C, benzyl-**C**H$_{aromat.}$), 108.3 (d, 1C, **C**-4a), 107.7 (1C, **C**-3), 92.7 (d, 1C, $^2J_{C,F}$ = 26.9, **C**-6), 92.3 (d, 1C, $^2J_{C,F}$ = 28.5, **C**-8), 54.0 (1C, N**C**$H_2CH_2CH_2CH_3$), 46.1 (1C, Bn-**C**H_2), 29.9 (1C, NCH_2**C**$H_2CH_2CH_3$), 18.9 (1C, NCH_2CH_2**C**H_2CH_3), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

^{19}F NMR (^{1}H decoupled, DMSO-d_6, δ [ppm]): -100.38 (s, **F**-7).

1-Butyl-5,7-dimorpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***4n*** *(MBMS02)*



$C_{22}H_{29}N_3O_5$

M_r = 415.49 g/mol

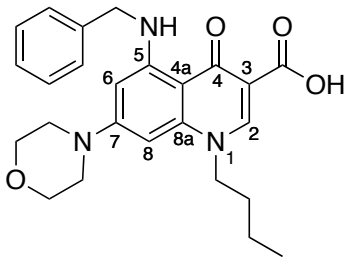
According to the general procedure 10.4, compound **4l** (400 mg, 1.15 mmol) was dissolved in morpholine (10 mL) and the reaction solution heated at 110 °C for 7 h under microwave irradiation. Afterwards, 10 mL aqueous HCl (2 M) was added to the mixture at 0 °C; the occurring precipitate was collected, and dried *in vacuo*. Finally, the residue was recrystallised from EtOAc to give **4n**.

appearance:	yellowish solid
yield:	150 mg (0.36 mmol/ 31%)
reaction control:	R_f = 0.62 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	223-226 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3400, 2960, 2862, 1687, 1595, 1527, 1433, 1362, 1298, 1249, 1213, 1187, 1104, 1052, 859.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 8.73 (s, 1H, **H**-2),), 6.54 – 6.53 (m, 2H, **H**-8/**H**-6), 4.43 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.80 – 3.75 (m, 8H, morpholino-C**H$_2$**-O-C**H$_2$**/morpholino-C**H$_2$**-O-C**H$_2$**), 3.43 – 3.41 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 3.08 (br, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.74 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.32 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.90 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 176.2 (1C, **C**-4), 166.9 (1C, **C**OOH), 154.5 (1C, **C**-7), 154.0 (1C, **C**-5), 147.6 (1C, **C**-2), 143.9 (1C, **C**-8a), 110.1 (1C, **C**-4a), 106.8 (1C, **C**-3), 101.5 (1C, **C**-6), 92.8 (1C, **C**-8), 66.5 (2C, morpholino-**C**H_2-O-**C**H_2), 65.8 (2C, morpholino-**C**H_2-O-**C**H_2), 54.7 (1C, N**C**$H_2CH_2CH_2CH_3$), 46.5 (2C, morpholino-**C**H_2-N-**C**H_2), 42.9 (2C, morpholino-**C**H_2-N-**C**H_2), 29.7 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.0 (1C, NCH_2CH_2**C**H_2CH_3), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

5-(Benzylamino)-1-butyl-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***4o*** *(MBMR34)*



$C_{25}H_{29}N_3O_4$

M_r = 435.52 g/mol

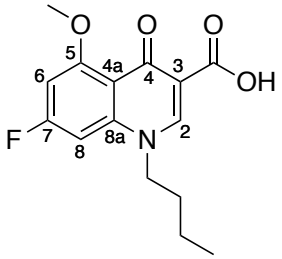
Compound **4m** (1.3 g, 3.6 mmol) was dissolved in morpholine (10 mL) and refluxed for 24 h. Afterwards, aqueous HCl (2 M) was added to acidify (pH 2) the solution under ice cooling, and the aqueous phase extracted with $CHCl_3$ (3x20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The oily residue was purified by means of column chromatography (silica gel; $CHCl_3$/MeOH = 20:1) and a brown oil was crystallised from EtOAc to give **4o**.

appearance:	yellow solid
yield:	560 mg (1.29 mmol/ 35%)
reaction control:	R_f = 0.48 ($CHCl_3$/MeOH = 20:1)
melting point [°C]:	207-210 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3271, 2963, 2919, 2875, 1722, 1627, 1593, 1553, 1516, 1433, 1355, 1275, 1175, 1119, 943.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 15.34 (s, 1H, COO**H**) 10.00 (t, 1H, ^3J = 5.2, N-**H**), 8.43 (s, 1H, **H**-2), 7.40 – 7.33 (m, 4H, Bn-C**H**$_{aromat.}$), 7.30 – 7.28 (m, 1H, Bn-C**H**$_{aromat.}$), 5.88 (d, 1H, ^4J = 2.0, **H**-6), 5.84 (d, 1H, ^4J = 2.0, **H**-8), 4.45 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 4.05 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.82 – 3.79 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.23 – 3.20 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.82 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.30 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.98 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 179.2 (d, 1C, $^3J_{C,F}$ = 1.7, **C**-4), 167.7 (1C, **C**ON), 155.5 (1C, **C**-7), 152.7 (1C, **C**-5), 147.3 (1C, **C**-2), 143.3 (1C, **C**-8a), 138.0 (1C, Bn-C$_{aromat.}$), 128.8 (2C, Bn-CH$_{aromat.}$), 127.3 (1C, Bn-CH$_{aromat.}$), 127.0 (2C, Bn-CH$_{aromat.}$), 107.2 (1C, **C**-3), 105.8 (d, 1C, $^3J_{C,F}$ = 8.5, **C**-4a), 91.8 (d, 1C, ^2J = 25.6, **C**-6), 87.0 (1C, **C**-8), 66.4 (2C, morpholino-**C**H_2-O-**C**H_2), 54.8 (1C, N**C**$H_2CH_2CH_2CH_3$), 47.4 (2C, morpholino-**C**H_2-N-**C**H_2), 47.3 (1C, Bn-CH_2), 30.0 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.9 (1C, NCH_2CH_2**C**H_2CH_3), 13.6 (1C, $NCH_2CH_2CH_2$**C**H_3).

1-Butyl-7-fluoro-5-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***4p*** *(MB446)*



$C_{15}H_{16}FNO_4$

M_r = 293.29 g/mol

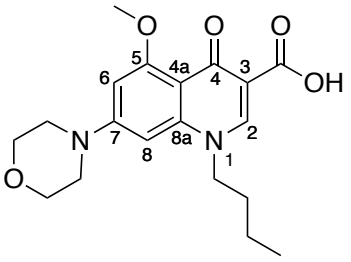
According to Shibamori *et al.*[41], a cold suspension of compound **3c** (1 eq, 1.3 g, 4.4 mmol) in abs. MeOH (30 mL) was treated with NaH (10 eq, 107 mg, 44.4 mmol) under Ar atmosphere and ice cooling. Afterwards, the reaction solution heated at 90 °C for 10 h and then was concentrated under reduced pressure. The reaction mixture was diluted with water and neutralised with aqueous HCl (3 M). The water layer was extracted with $CHCl_3$ (3x50 mL) and the combined organic layers were dried over Na_2SO_4 with subsequent removing of the solvent *in vacuo*.

appearance:	yellowish solid
yield:	523 mg (1.78 mmol / 40%)
reaction control:	R_f = 0.29 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	211-213 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3031, 2957, 2850, 1712, 1631, 1573, 1376, 1268, 1142, 1112.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 15.19 (s, 1H, COO**H**), 8.60 (s, 1H, **H**-2), 6.66 (dd, 1H, ^4J = 2.0, ^2J = 8.0, **H**-6), 6.64 (dd, 1H, ^4J = 2.0, ^2J = 10.8, **H**-8), 4.12 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.97 (s, 3H, OC**H$_3$**), 1.82 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.39 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.95 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, $CDCl_3$ δ [ppm], *J* [Hz]): 176.4 (1C, **C**-4), 166.1 (1C, **C**OOH), 165.8 (d, 1C, $^1J_{C,F}$ = 250.2, **C**-7), 159.7 (d, 1C, $^3J_{C,F}$ = 14.4, **C**-5), 148.2 (1C, **C**-2), 145.3 (d, 1C, $^3J_{C,F}$ = 14.6, **C**-8a), 113.3 (d, 1C, $^4J_{C,F}$ = 1.2, **C**-4a), 109.3 (1C, **C**-3), 96.3 (d, 1C, $^2J_{C,F}$ = 24.0, **C**-6), 92.4 (d, 1C, $^2J_{C,F}$ = 25.0, **C**-8), 55.9 (1C, O**C**H_3), 53.6 (1C, N**C**$H_2CH_2CH_2CH_3$), 30.3 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.3 (1C, NCH_2CH_2**C**H_2CH_3), 13.5 (1C, $NCH_2CH_2CH_2$**C**H_3).

1-Butyl-5-methoxy-7-morpholino-4-oxo-1,3-dihydroquinoline-3-carboxylic acid, ***4q*** *(MB447)*



$C_{19}H_{24}N_2O_5$

M_r = 360.41 g/mol

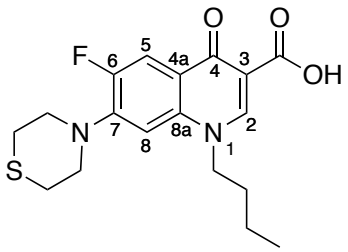
According to the general procedure 10.4, compound **4p** (520 mg, 1.77 mmol) was dissolved in morpholine (7.5 mL) and heated for 5 h under microwave irradiation at 110 °C. After acidification (pH2) the solution and drying the precipitate *in vacuo*, the solid was recrystallised from EtOAc to yield **4q**.

appearance:	yellow solid
yield:	220 mg (0.61 mmol/ 34%)
reaction control:	R_f = 0.31 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	258–259 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2961, 2863, 1704, 1624, 1600, 1545, 1421, 1375, 1263, 1115.
mass:	*m/z* 361.0 $[M + H]^+$

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 8.54 (s, 1H, **H**-2), 6.44 (d, 1H, ^4J = 1.6, **H**-6), 6.43 (d, 1H, ^4J = 1.6, **H**-8), 4.15 (t, 2H, ^3J = 7.6, NC**H$_2$**$CH_2CH_2CH_3$), 4.00 (s, 3H, OC**H$_3$**), 3.93 – 3.91 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.41 – 3.39 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.76 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.32 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.95 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 176.8 (1C, **C**-4), 165.8 (1C, **C**OOH), 151.4 (1C, **C**-2), 151.2 (1C, **C**-7), 139.8 (1C, **C**-8a), 130.6 (1C, **C**-5), 125.0 (1C, **C**-8), 124.9 (1C, **C**-4a), 120.9 (1C, **C**-6), 110.2 (1C, **C**-3), 66.9 (2C, morpholino-**C**H_2-O-**C**H_2), 56.3 (1C, N**C**$H_2CH_2CH_2CH_3$), 53.5 (2C, morpholino-**C**H_2-N-**C**H_2), 33.0 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.5 (1C, NCH_2CH_2**C**H_2CH_3), 13.3 (1C, $NCH_2CH_2CH_2$**C**H_3).

1-Butyl-6-fluoro-4-oxo-7-thiomorpholino-1,4-dihydroquinoline-3-carboxylic acid, ***4r*** *(MB306)*



$C_{18}H_{21}FN_2O_3S$

M_r = 364.44 g/mol

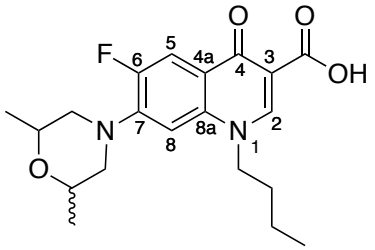
Compound **3a** (500 mg, 1.68 mmol) was dissolved in DMF (5 mL) and thiomorpholine (10 eq, 1.70 mL, 16.80 mmol) was added. The reaction solution heated at 110 °C under microwave irradiation for 4 h. Subsequently, the solvent was removed under reduced pressure and the residue was treated with aqueous HCl (2 M) under ice cooling. The resulting precipitates were collected, dried *in vacuo*, and recrystallised from EtOAc.

appearance:	brown solid
yield:	200 mg (0.55 mmol/ 33%)
reaction control:	R_f = 0.53 ($CHCl_3$/MeOH/FA = 100:2:1)
melting point [°C]:	178-180 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3051, 2962, 2873, 1717, 1625, 1450, 1420, 1303, 1248, 1207, 1103, 947.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 8.93 (s, 1H, **H**-2), 7.91 (d, 1H, ^3J = 13.2, **H**-5), 7.19 (d, 1H, ^4J = 7.6, **H**-8), 4.55 (t, 2H, ^3J = 7.6, NC**H$_2$**$CH_2CH_2CH_3$), 3.60 – 3.57 (m, 4H, thiomorpholino-C**H$_2$**-N-C**H$_2$**), 2.82 – 2.79 (m, 4H, thiomorpholino-C**H$_2$**-S-C**H$_2$**), 1.79 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.33 (sex, 2H, ^3J = 7.6, NCH_2CH_2C**H$_2$**CH_3), 0.92 (t, 3H, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 176.0 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 166.0 (1C, **C**OOH), 152.4 (d, 1C, $^1J_{C,F}$ = 247.8, **C**-6), 148.7 (1C, **C**-2), 145.7 (d, 1C, $^3J_{C,F}$ = 10.4, **C**-7), 137.3 (1C, **C**-8a), 119.3 (d, 1C, $^4J_{C,F}$ = 10.4, **C**-4a), 111.1 (d, 1C, $^2J_{C,F}$ = 23.2, **C**-5), 106.7 (**C**3), 106.5 (d, 1C, $^4J_{C,F}$ = 3.6, **C**-8), 53.3 (1C, N**C**$H_2CH_2CH_2CH_3$), 52.0 (d, 2C, $^4J_{C,F}$ = 4.8, thiomorpholino-**C**H_2-N-**C**H_2), 30.2 (1C, NCH_2**C**$H_2CH_2CH_3$), 26.5 (2C, thiomorpholino-**C**H_2-S-**C**H_2), 19.0 (1C, NCH_2CH_2**C**H_2CH_3), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

1-Butyl-7-(2,6-dimethylmorpholino)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***4s*** *(MB307)*



$C_{20}H_{25}FN_2O_4$

M_r = 376.43 g/mol

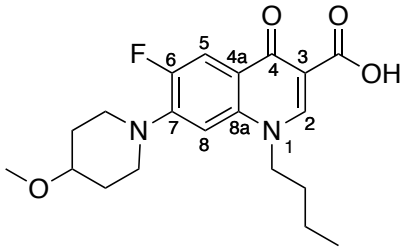
Compound **3a** (400 mg, 1.34 mmol) was dissolved in DMF (5 mL) and 2,6-dimethylmorpholine (cis + trans) (10 eq, 1.55 mL, 13.43 mmol) added. The reaction solution heated at 110 °C under microwave irradiation for 5 h. Subsequently, the solvent was removed under reduced pressure and the residue treated with aqueous HCl (2 M) under ice cooling. The resulting precipitates were collected, dried *in vacuo* and recrystallised from EtOAc/EtOH (2:1).

appearance:	yellow solid
yield:	250 mg (0.66 mmol/ 49%)
reaction control:	R_f = 0.54 ($CHCl_3$/MeOH/FA = 100:2:1)
melting point [°C]:	201-203 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3047, 2960, 2929, 2872, 1720, 1621, 1516, 1480, 1446, 1375, 1260, 1214, 1142, 1084, 948.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 14.9 (s, 1H, COO**H**), 8.65 (s, 1H, **H**-2), 8.04 (d, 1H, ^3J = 13.2, **H**-5), 6.90 (d, 1H, ^4J = 7.6, **H**-8), 4.41 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.95 – 3.90 (m, 2H, dimethylmorpholino-C**H**-O-C**H**), 3.52 – 3.48 (m, 2H, dimethylmorpholino-C**H$_2$**-N-C**H$_2$**), 2.67 – 2.61 (m, 2H, dimethylmorpholino-C**H$_2$**-N-C**H$_2$**), 1.91 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.40 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 1.27 (d, 6H, ^3J = 7.2, dimethylmorpholino-(C**H$_3$**)$_2$), 0.92 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 176.9 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 167.2 (1C, **C**OOH), 153.4 (d, 1C, $^1J_{C,F}$ = 247.8, **C**-6), 147.8 (1C, **C**-2), 145.7 (d, 1C, $^3J_{C,F}$ = 10.4, **C**-7), 137.3 (1C, **C**-8a), 119.3 (d, 1C, $^3J_{C,F}$ = 10.4, **C**-4a), 113.1 (d, 1C, $^2J_{C,F}$ = 23.2, **C**-5), 108.2 (**C**-3), 104.4 (d, 1C, $^4J_{C,F}$ = 3.6, **C**-8), 71.25 (2C, dimethylmorpholino-**C**H-O-**C**H), 55.4 (d, 2C, $^4J_{C,F}$ = 4.9, morpholino-**C**H_2-N-**C**H_2), 54.3 (1C, N**C**$H_2CH_2CH_2CH_3$), 30.2 (1C, NCH_2**C**$H_2CH_2CH_3$), 18.8 (1C, NCH_2CH_2**C**H_2CH_3), 18.4 (2C, dimethylmorpholino-(**C**H_3)$_2$), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

1-Butyl-6-fluoro-7-(4-methoxypiperidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***4t*** *(MB304)*



$C_{20}H_{25}FN_2O_4$

M_r = 376.43 g/mol

Compound **3a** (1.0 g, 2.9 mmol) was dissolved in DMF (5 mL) and 4-methoxypiperidine (3 eq, 1.0 mg, 8.6 mmol) added. The reaction solution was heated at 110 °C under microwave irradiation for 4.5 h. Subsequently, the solvent was removed under reduced pressure and the residue treated with aqueous HCl (2 M) under ice cooling. The resulting precipitates were collected, dried *in vacuo*, and recrystallised from EtOAc.

appearance:	white solid
yield:	720 mg (1.91 mmol/ 67%)
reaction control:	R_f = 0.31 ($CHCl_3$/MeOH/FA = 100:2:1)
melting point [°C]:	153-155 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3243, 3049, 2949, 2869, 1715, 1622, 1547, 1499, 1483, 1368, 1302, 1252, 1192, 1094, 1027, 937.

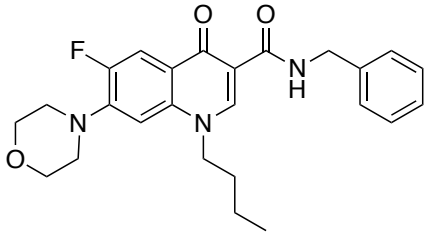
^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 15.38 (s, 1H, COO**H**), 8.92 (s, 1H, **H**-2), 7.90 (d, 1H, ^3J = 13.2, **H**-5), 7.15 (d, 1H, ^4J = 7.6, **H**-8), 4.56 (t, 2H, ^3J = 6.8, NC**H$_2$**$CH_2CH_2CH_3$), 3.52 (br, 2H, piperidinyl-C**H$_2$**), 3.49 – 3.42 (m, 1H, piperidinyl-C**H**), 3.30 (s, 3H, OC**H$_3$**), 3.16 – 3.10 (m, 2H, piperidinyl-C**H$_2$**), 2.00 (br, 2H, piperidinyl-C**H$_2$**), 1.78 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.67 – 162 (m, 2H, piperidinyl-C**H$_2$**), 1.32 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.92 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 176.5 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 166.6 (1C, **C**OOH), 153.4 (d, 1C, $^1J_{C,F}$ = 247.8, **C**-6), 149.2 (1C, **C**-2), 145.1 (d, 1C, $^3J_{C,F}$ = 10.4, **C**-7), 137.3 (1C, C-8a), 118.9 (d, 1C, $^4J_{C,F}$ = 10.4, **C**-4a), 111.1 (d, 1C, $^2J_{C,F}$ = 23.2, **C**-5), 106.7 (**C**-3), 105.7 (d, 1C, $^3J_{C,F}$ = 3.6, **C**-8), 74.3 (1C, piperidinyl-**C**H), 54.9 (1C, O**C**H_3), 53.3 (1C, N**C**$H_2CH_2CH_2CH_3$), 46.9 (2C, d, $^4J_{C,F}$ = 4.8, piperidinyl-**C**H_2), 30.2 (1C, NCH_2**C**$H_2CH_2CH_3$), 30.1 (1C, piperidinyl-**C**H_2), 19.0 (1C, NCH_2CH_2**C**H_2CH_3), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

10.5 *N*-Benzyl-4-oxo-1,4-dihydroquinoline-3-carboxamides

General procedure according Hiltensperger *et al.*[7]: The 1-alkyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acids **4a-q** (1 eq) and *N*-methylmorpholine (*N*MM, 5 eq) were dissolved in abs. DMF under Ar atmosphere. The reaction solution stirred for 1 h at 0 °C and then, *i*-butyl chloroformate (IBCF) (4 eq) was added and stirred for 1 h at 0 °C, until benzylamine derivative (4 eq) was added. After 45 min of stirring at rt, the solvent was removed *in vacuo* and the residue was purified by column chromatography on silica gel with a mobile phase which is specified in the respective proceeding hereinafter. Finally, the crude solid was recrystallised.

N-Benzyl-1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***GHQ168***



$C_{25}H_{28}FN_3O_3$

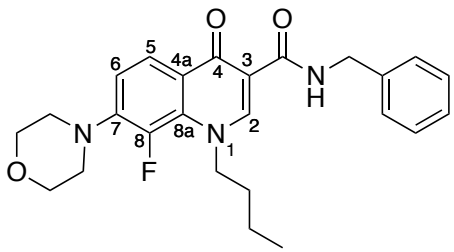
M_r = 437.52 g/mol

A solution of compound **4a** (660 mg, 1.89 mmol) in abs. DMF (20 mL) was treated with *N*MM (1.04 mL, 9.48 mmol), IBCF (0.99 mL, 7.58 mmol), and benzylamine (0.83 mL, 7.58 mmol) as specified in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and recrystallised from EtOAc to produce **GHQ168**.

appearance:	white solid
yield:	320 mg (0.73 mmol/ 39%; Lit[6]: 72%)
reaction control:	R_f = 0.71 ($CHCl_3$/MeOH = 100:1)
melting point [°C]:	162-163 °C (Lit[6]: 157-159 °C)
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3160, 2967, 2873, 2856, 1649, 1600, 1490, 1486, 1259, 1117, 940.

The obtained physical and spectroscopic data are consistent with that found in literature.[6]

N-Benzyl-1-butyl-8-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***5*** *(MB395, MB418)*



$C_{25}H_{28}FN_3O_3$

M_r = 437.52 g/mol

A solution of compound **4b** (230 mg, 0.66 mmol) in abs. DMF (15 mL) was treated with *N*MM (362 µL, 3.3 mmol), IBCF (343 µL, 2.64 mmol), and benzylamine (289 µL, 2.64 mmol) as depicted in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:3) and recrystallised from EtOH to give **5**.

appearance:	white solid
yield:	180 mg (0.41 mmol / 62%)
reaction control:	R_f = 0.28 ($CHCl_3$/MeOH = 100:3)
melting point [°C]:	170-171 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3110, 3039, 2951, 2854, 1654, 1590, 1555, 1549, 1447, 1243, 1121.
mass:	*m/z* 438.2 $[M + H]^+$
HPLC purity:	96% (HPLC method **I**)

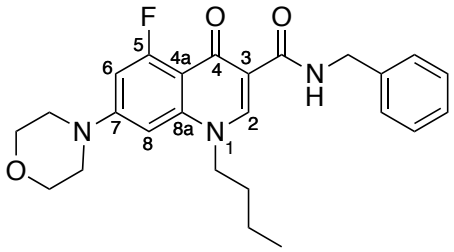
^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 10.25 (t, 1H, ^3J = 6.0, N-**H**), 8.72 (s, 1H, **H**-2), 8.08 (dd, 1H, ^3J = 8.8, ^5J = 0.8, **H**-5), 7.34 – 7.31 (m, 4H, Bn-C**H**$_{aromat.}$), 7.27 – 7.24 (m, 2H, Bn-C**H**$_{aromat.}$/**H**-6), 4.54 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 4.48 – 4.46 (m, 2H, NC**H$_2$**$CH_2CH_2CH_3$), 3.78 – 3.76 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.20 – 3.17 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.75 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.31 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.90 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 174.2 (d, 1C, $^4J_{C,F}$ = 1.7, **C**-4), 163.8 (1C, **C**ON), 150.5 (1C, **C**-2), 143.5 (d, 1C, $^2J_{C,F}$ = 8.4, **C**-7), 143.1 (d, 1C, $^1J_{C,F}$ = 246.1, **C**-8), 139.3 (1C, Bn-**C**$_{aromat.}$), 129.1 (d, 1C, $^2J_{C,F}$ = 5.9, **C**-8a), 128.3 (2C, Bn-**C**H$_{aromat.}$), 127.3 (2C, Bn-**C**H$_{aromat.}$), 126.8 (1C, Bn-**C**H$_{aromat.}$), 122.9 (1C, **C**-4a), 122.3 (d, 1C, $^4J_{C,F}$ = 3.6, **C**-5), 116.6 (d, 1C, $^3J_{C,F}$ = 2.4, **C**-6), 110.1 (1C, **C**-3), 66.0 (2C, morpholino-**C**H_2-O-**C**H_2), 57.5 (d, 1C, $^4J_{C,F}$ = 15.8, N**C**$H_2CH_2CH_2CH_3$), 50.3 (d, 2C, $^4J_{C,F}$ = 4.0, morpholino-**C**H_2-N-**C**H_2),

42.1 (1C, Bn-**C**H_2), 32.0 (d, 1C, $^5J_{C,F}$ = 3.8, NCH_2**C**$H_2CH_2CH_3$), 18.9 (1C, NCH_2CH_2**C**H_2CH_3), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

^{19}F NMR (^{1}H coupled, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): -134.80 (br, **F**-8).

N-Benzyl-1-butyl-5-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***6*** *(MBMR343)*



$C_{25}H_{28}FN_3O_3$

M_r = 437.52 g/mol

A solution of compound **4c** (260 mg, 0.75 mmol) in abs. DMF (15 mL) was treated with *N*MM (412 µL, 3.75 mmol), IBCF (390 µL, 3.00 mmol), and benzylamine (330 µL, 3.00 mmol) as depicted in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and recrystallised from EtOAc to give **6**.

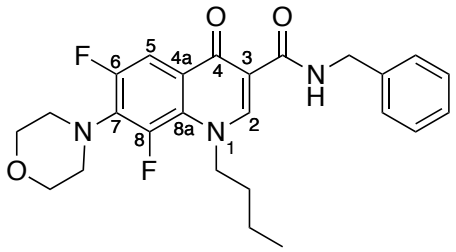
appearance:	white solid
yield:	130 mg (0.30 mmol/ 40%)
reaction control:	R_f = 0.28 ($CHCl_3$/MeOH = 100:1)
melting point [°C]:	189-191 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3100, 3030, 2964, 2945, 2983, 2856, 1662, 1624, 1573, 1530, 1497, 1467, 1264, 1215, 1117, 1003.
mass:	*m/z* 438.2 $[M + H]^+$
HPLC purity:	100% (HPLC method **I**)

^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 10.39 (t, 1H, ^3J = 6.0, N-**H**), 8.65 (s, 1H, **H**-2), 7.34 – 7.31 (m, 4H, Bn-C**H**$_{aromat.}$), 7.27 – 7.24 (m, 1H, Bn-C**H**$_{aromat.}$), 6.91 (dd, 1H, ^3J = 15.6, ^4J = 1.6, **C**-6), 6.65 (d, 1H, ^4J = 1.6, **C**-8), 4.52 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 4.37 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.76 – 3.73 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.39 – 3.37 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.72 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.31 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.90 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 174.3 (d, 1C, $^{3}J_{C,F}$ = 1.7, **C**-4), 164.1 (1C, **C**ON), 162.7 (d, 1C,$^{1}J_{C,F}$ = 256.1, **C**-5), 153.5 (d, 1C, $^{3}J_{C,F}$ = 13.1, **C**-7), 147.9 (1C, **C**-2), 141.9 (d, 1C, $^{3}J_{C,F}$ = 5.7, **C**-8a), 139.5 (1C, Bn-**C**$_{aromat.}$), 128.3 (2C, Bn-**C**H$_{aromat.}$), 127.3 (2C, Bn-**C**H$_{aromat.}$), 126.8 (1C, Bn-**C**H$_{aromat.}$), 110.7 (1C, **C**-3), 108.6 (d, 1C, $^{3}J_{C,F}$ = 8.5, **C**-4a), 99.2 (d, 1C, $^{2}J_{C,F}$ = 25.6, **C**-6), 94.3 (1C, **C**-8), 65.6 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 52.9 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 46.5 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 42.5 (1C, Bn-**C**H$_2$), 29.8 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.4 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

^{19}F NMR (^{1}H decoupled, DMSO-d_6, δ [ppm]): -109.86 (s, **F**-5).

N-Benzyl-1-butyl-6,8-difluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***7*** *(MBPG24)*



$C_{25}H_{27}F_2N_3O_3$

M_r = 455.51 g/mol

A solution of compound **4d** (190 mg, 0.52 mmol) in abs. DMF (15 mL) was treated with *N*MM (316 µL, 2.6 mmol), IBCF (270 µL, 2.1 mmol), and benzylamine (227 µL, 2.1 mmol) as depicted in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 50:1) and recrystallised from EtOAc to yield **7**.

appearance:	white solid
yield:	60 mg (0.13 mmol/ 25%)
reaction control:	R_f = 0.77 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	165–167 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3176, 3064, 3033, 2861, 1651, 1596, 1536, 1472, 1450, 1377, 1281, 1213, 1109, 1026.
mass:	*m/z* 456.2 $[M + H]^+$
HPLC purity:	99% (HPLC method **I**)

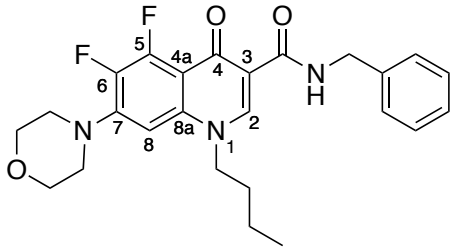
^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 10.16 (t, 1H, ^{3}J = 6.0, N-**H**), 8.74 (s, 1H, **H**-2), 7.80 (dd, 1H, ^{3}J = 10.8, ^{5}J = 1.6, **H**-5), 7.35 – 7.32 (m, 4H, Bn-C**H**$_{aromat.}$), 7.27 – 7.24 (m, 1H, Bn-C**H**$_{aromat.}$), 4.55 (d, 2H, ^{3}J = 6.0, Bn-**C**H$_2$), 4.48 – 4.44 (br, 2H,

NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.73 – 3.71 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.29 (br, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.80 – 1.73 (m, 2H, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.38 – 1.31 (m, 2H, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.91 (t, 3H, ^3J = 7.2, NCH$_2$CH$_2$CH$_2$C**H**$_3$).

^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 173.1 (d, 1C, $^4J_{C,F}$ = 1.4, **C**-4), 163.5 (1C, **C**ON), 153.6 (dd, 1C, $^1J_{C,F}$ = 246.0, $^3J_{C,F}$ = 6.0, **C**-6), 150.3 (1C, **C**-2), 146.3 (dd, 1C, $^1J_{C,F}$ = 249.0, $^3J_{C,F}$ = 7.0, **C**-8), 139.2 (1C, Bn-**C**$_{aromat.}$), 132.3 (t, 1C, $^2J_{C,F}$ = 14.0, **C**-7), 128.3 (2C, Bn-**C**H$_{aromat.}$), 127.3 (2C, Bn-**C**H$_{aromat.}$), 126.8 (1C, Bn-**C**H$_{aromat.}$), 126.6 (m, 1C, **C**-8a), 122.7 (d, 1C, $^3J_{C,F}$ = 8.0, **C**-4a), 109.7 (1C, **C**-3), 107.0 (dd, 1C, $^2J_{C,F}$ = 19.0, $^4J_{C,F}$ = 2.0, **C**-5), 66.6 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 57.7 (d, 1C, $^4J_{C,F}$ = 15.0, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 50.7 (t, 2C, $^4J_{C,F}$ = 6.0, morpholino-**C**H$_2$-N-**C**H$_2$), 42.1 (1C, Bn-**C**H$_2$), 32.0 (d, 1C, $^5J_{C,F}$ = 4.0, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 18.9 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.4 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

^{19}F NMR (^{1}H decoupled, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): -121.44 (d, $^4J_{6,8}$ = 9.6, **F**-6), -130.03 (d, $^4J_{6,8}$ = 9.6, **F**-8).

N-Benzyl-1-butyl-5,6-difluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***8*** *(MB421)*



$C_{25}H_{27}F_2N_3O_3$

M_r = 455.51 g/mol

A solution of compound **4e** (200 mg, 0.55 mmol) in abs. DMF (10 mL) was treated with *N*MM (300 µL, 2.75 mmol), IBCF (286 µL, 2.20 mmol), and benzylamine (240 µL, 2.20 mmol) as depicted in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and recrystallised from EtOAc to give **8**.

appearance:	white solid
yield:	110 mg (0.24 mmol/ 44%)
reaction control:	R_f = 0.33 ($CHCl_3$/MeOH = 100:1)
melting point [°C]:	179-180 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3130, 3035, 2958, 2867, 1655, 1629, 1602, 1484 1377, 1274, 1115, 1009.

mass: *m/z* 456.1 $[M + H]^+$

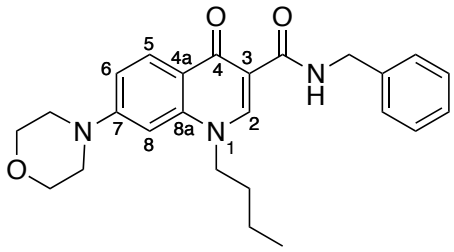
HPLC purity: 99% (HPLC method **I**)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 10.26 (t, 1H, ^3J = 6.0, N-**H**), 8.74 (s, 1H, **H**-2), 7.35 – 7.32 (m, 4H, Bn-C**H**$_{aromat.}$), 7.27 – 7.23 (m, 1H, Bn-C**H**$_{aromat.}$), 6.82 (d, 1H, ^4J = 6.4, **H**-8), 4.54 (d, 2H, ^3J = 6.0, Bn-**C**H$_2$), 4.43 (t, 2H, ^3J = 7.2, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 3.79 – 3.77 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.30 – 3.27 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.74 (quint, 2H, ^3J = 7.2, NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.31 (sext, 2H, ^3J = 7.2, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 0.91 (t, 3H, ^3J = 7.2, NCH$_2$CH$_2$CH$_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 174.2 (1C, **C**-4), 163.7 (1C, **C**ON), 149.4 (dd, 1C, $^1J_{C,F}$ = 257.7, $^2J_{C,F}$ = 13.2, **C**-5), 147.0 (1C, **C**-2), 144.1 (m, 1C, **C**-7), 140.6 (dd, 1C, $^1J_{C,F}$ = 245.6, $^2J_{C,F}$ = 13.7, **C**-6), 139.3 (1C, Bn-**C**$_{aromat.}$), 136.4 (d, 1C, $^3J_{C,F}$ = 3.9, **C**-8a), 128.3 (2C, Bn-**C**H$_{aromat.}$), 127.3 (2C, Bn-**C**H$_{aromat.}$), 126.7 (1C, Bn-**C**H$_{aromat.}$), 111.5 (d, 1C, $^2J_{C,F}$ = 5.4, **C**-4a), 110.9 (1C, **C**-3), 99.5 (1C, **C**-8), 65.7 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 53.0 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 49.3 (d, 2C, $^4J_{C,F}$ = 4.4, morpholino-**C**H$_2$-N-**C**H$_2$), 42.0 (1C, Bn-**C**H$_2$), 29.8 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.4 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

^{19}F NMR (^{1}H coupled, DMSO-d_6, δ [ppm], J [Hz]): -140.03 (d, $^3J_{5,6}$ = 18.0, **F**-5), -151.62 (dd, $^3J_{5,6}$ = 18.0, $^4J_{6,8}$ = 8.0, **F**-6).

N-Benzyl-1-butyl-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***9*** *(MB15)*



$C_{25}H_{29}N_3O_3$

M_r = 419.53 g/mol

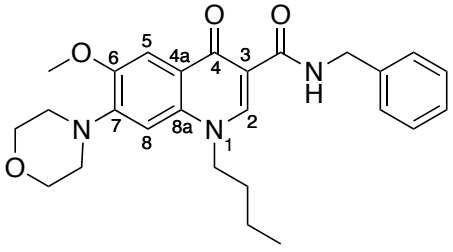
A solution of compound **4f** (460 mg, 1.39 mmol) in abs. DMF (20 mL) was treated with *N*MM (764 µL, 6.95 mmol), IBCF (723 µL, 5.56 mmol), and benzylamine (608 µL, 5.56 mmol) as depicted in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and recrystallised from EtOAc to give **9**.

appearance:	white solid
yield:	99 mg (0.24 mmol/ 17%)
reaction control:	R_f = 0.57 ($CHCl_3$/MeOH = 100:1)
melting point [°C]:	172 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3171, 3039, 2965, 2937, 2877, 2840, 1652, 1597, 1542, 1529, 1466, 1451, 1237, 1126.
mass:	*m/z* 420.3 $[M + H]^+$
HPLC purity:	97% (HPLC method **I**)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 10.5 (t**,** 1H, ^3J = 6.0, N-**H**), 8.71 (s, 1H, **H**-2), 8.12 (d, 1H, ^3J = 9.2, **H**-5), 7.35 – 7.33 (m, 5H, Bn-C**H**$_{aromat.}$), 7.20 (dd, 1H, ^3J = 9.2, ^4J = 0.8, **H**-6), 6.90 (d, 1H, ^4J = 0.8, **H**-8), 4.53 (d, 2H, ^3J = 5.6, Bn-**C**H_2), 4.42 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.77-3.79 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.34 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.75 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.32 (sext, 2H, ^3J = 7.6, NCH_2CH_2C**H$_2$**CH_3), 0.91 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 174.7 (1C, **C**-4), 164.4 (1C, **C**ON), 153.9 (1C, **C**-7), 147.8 (1C, **C**-2), 140.5 (1C, **C**-8a), 139.4 (1C, Bn-**C**$_{aromat.}$), 128.3 (2C, Bn-**C**H$_{aromat.}$), 127.3 (2C, Bn-**C**H$_{aromat.}$/**C**-5), 126.8 (1C, Bn-**C**H$_{aromat.}$), 119.0 (1C, **C**-4a), 113.4 (1C, **C**-6), 110.0 (1C, **C**-3), 98.4 (1C, **C**-8), 65.8 (2C, morpholino-**C**H_2-O-**C**H_2), 52.4 (1C, N**C**$H_2CH_2CH_2CH_3$), 47.0 (2C, morpholino-**C**H_2-N-**C**H_2), 42.0 (1C, Bn-**C**H_2), 30.2 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.1 (1C, NCH_2CH_2**C**H_2CH_3), 13.5 (1C, $NCH_2CH_2CH_2$**C**H_3).

N-Benzyl-1-butyl-6-methoxy-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***10*** *(MB168)*



$C_{26}H_{31}N_3O_4$

M_r = 449.55 g/mol

A solution of compound **4h** (230 mg, 0.63 mmol) in abs. DMF (10 mL) was treated with *N*MM (346 µL, 3.2 mmol), IBCF (327 µL, 2.52 mmol), and benzylamine (275 µL, 2.52 mmol) as depicted in the general procedure 10.5. The crude product was purified by

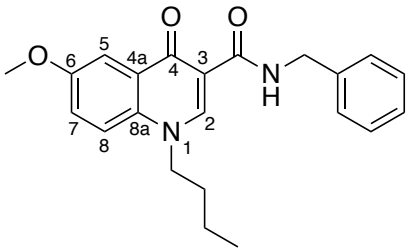
column chromatography (silica gel; $CHCl_3$/MeOH = 50:1) and recrystallised from ACN to give **10**.

appearance:	white solid
yield:	100 mg (0.22 mmol/ 34%)
reaction control:	R_f = 0.73 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	168 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3210, 3150, 3045, 2952, 1711, 1616, 1470, 1446, 1256, 1229, 1111, 1041, 1006.
mass:	*m/z* 450.3 $[M + H]^+$
HPLC purity:	96% (HPLC method **I**)

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 10.57 (t, 1H, ^3J = 5.2, N-**H**), 8.70 (s, 1H, **H**-2), 7.85 (s, 1H, **H**-5), 7.43 – 7.21 (m, 5H, Bn-C$\mathbf{H}_{aromat.}$), 6.82 (s, 1H, **H**-8), 4.71 (d, 2H, ^3J = 5.6, Bn-C$\mathbf{H_2}$), 4.22 (t, 2H, ^3J = 7.6, NC$\mathbf{H_2}CH_2CH_2CH_3$), 3.99 (s, 3H, OC$\mathbf{H_3}$), 3.94 – 3.92 (m, 4H, morpholino-C$\mathbf{H_2}$-O-C$\mathbf{H_2}$), 3.24 – 3.22 (m, 4H, morpholino-C$\mathbf{H_2}$-N-C$\mathbf{H_2}$), 1.90 (quint, 2H, ^3J = 7.2, $NCH_2C\mathbf{H_2}CH_2CH_3$), 1.4 (sext, 2H, ^3J = 7.2, $NCH_2CH_2C\mathbf{H_2}CH_3$), 1.0 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2C\mathbf{H_3}$).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 175.4 (1C, **C**-4), 165.4 (1C, **C**ON), 150.4 (1C, **C**-7), 146.6 (1C, **C**-2), 146.2 (1C, **C**-6), 138.9 (1C, Bn-$\mathbf{C}_{aromat.}$), 134.5 (1C, **C**-8a), 128.8 (2C Bn-**C**$H_{aromat.}$), 127.7 (2C, Bn-**C**$H_{aromat.}$), 127.0 (1C, Bn-**C**$H_{aromat.}$), 123.42 (1C, **C**-4a), 111.0 (1C, **C**-3), 106.6 (1C, **C**-5), 103.7 (1C, **C**-8), 66.8 (2C, morpholino-**C**H_2-O-**C**H_2), 55.5 (1C, O**C**H_3), 54.0 (1C, N**C**$H_2CH_2CH_2CH_3$), 50.7 (2C, morpholino-**C**H_2-N-**C**H_2), 43.3 (1C, Bn-**C**H_2), 31.0 (1C, $NCH_2\mathbf{C}H_2CH_2CH_3$), 20.0 (1C, $NCH_2CH_2\mathbf{C}H_2CH_3$), 13.6 (1C, $NCH_2CH_2CH_2\mathbf{C}H_3$).

N-Benzyl-1-butyl-6-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***11*** *(MB26d)*



$C_{22}H_{24}N_2O_3$

M_r = 364.45 g/mol

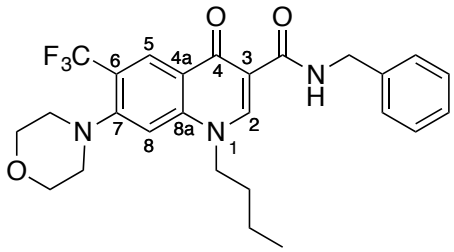
A solution of compound **3i** (450 mg, 1.63 mmol) in abs. DMF (10 mL) was treated with *N*MM (790 µL, 8.15 mmol), IBCF (849 µL, 6.52 mmol), and benzylamine (699 µL, 6.52 mmol) as depicted in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 50:1) and recrystallised from EtOAc to give **11**.

appearance:	white solid
yield:	150 mg (0.41 mmol/ 25%)
reaction control:	R_f = 0.60 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	114 - 115 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3179, 3032, 2928, 1649, 1548, 1490, 1453, 1220, 1027, 812.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 10.48 (t, 1H, ^3J = 4.8, N-**H**), 8.74 (s, 1H, **H**-2), 7.89 (d, 1H, ^3J = 2.8, **H**-5), 7.74 – 7.25 (m, 7H, **H**-8/ **H**-7/ Bn-C**H**$_{aromat.}$), 4.67 (d, 2H, Bn-C**H$_2$**), 4.24 (t, 2H, ^3J = 7.6, NC**H$_2$**$CH_2CH_2CH_3$), 3.91 (s, 1H, OC**H$_3$**), 1.87 (quint, 2H, ^3J = 7.6, NCH_2C**H$_2$**CH_2CH_3), 1.40 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.97 (t, 3H, ^3J = 7.6, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 175.9 (1C, **C**-4), 165.3 (1C, **C**ON), 157.2 (1C, **C**-6), 147.5 (1C, **C**-2), 138.9 (1C, Bn-**C**$_{aromat.}$), 133.5 (1C, **C**-8a), 129.5 (1C, **C**-4a), 128.7 (2C, Bn-**C**H$_{aromat.}$), 127.7 (2C, Bn-**C**H$_{aromat.}$), 127.0 (1C, Bn-**C**H$_{aromat.}$), 123.5 (1C, **C**-8), 117.5 (1C, **C**-7), 110.9 (1C, **C**-3), 106.5 (1C, **C**-5), 55.8 (1C, O**C**H_3), 54.2 (1C, N**C**$H_2CH_2CH_2CH_3$), 43.3 (1C, Bn-**C**H_2), 31.2 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.9 (1C, NCH_2CH_2**C**H_2CH_3), 13.6 (1C, $NCH_2CH_2CH_2$**C**H_3).

N-Benzyl-1-butyl-7-morpholino-4-oxo-6-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxamide, ***12*** *(MB412)*



$C_{26}H_{28}F_3N_3O_3$

M_r = 487.52 g/mol

A solution of compound **4g** (220 mg, 0.56 mmol) in abs. DMF (15 mL) was treated with *N*MM (307 µL, 2.80 mmol), IBCF (231 µL, 2.24 mmol), and benzylamine (214 µL, 2.56 mmol) as depicted in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and recrystallised from EtOAc to give **12**.

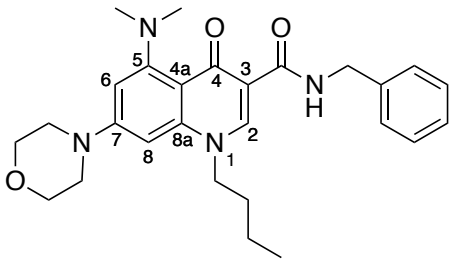
appearance:	white solid
yield:	40 mg (0.08 mmol/ 15%)
reaction control:	R_f = 0.67 ($CHCl_3$/MeOH = 100:1)
melting point [°C]:	168 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3232, 2958, 2935, 1659, 1626, 1598, 1486, 1452, 1267, 1240, 1108.
mass:	*m/z* 488.1 $[M + H]^+$
HPLC purity:	97% (HPLC method **I**)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 10.18 (t, 1H, ^3J = 7.6, N-**H**), 8.90 (s, 1H, **H**-2), 8.54 (s, 1H, **H**-5), 7.68 (s, 1H, **H**-8), 7.35 – 7.33 (m, 4H, Bn-C**H**$_{aromat.}$), 7.28 – 7.25 (m, 1H, Bn-C**H**$_{aromat.}$), 4.57 – 4.54 (m, 4H, NC**H$_2$**$CH_2CH_2CH_3$ / Bn-**C**H_2), 3.77 – 3.75 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.09 – 3.05 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.75 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.35 (sext, 2H, ^3J = 7.6, NCH_2CH_2C**H$_2$**CH_3), 0.92 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 174.6 (1C, **C**-4), 163.6 (1C, **C**ON), 154.9 (1C, **C**-7), 149.5 (1C, **C**-2), 142.0 (1C, **C**-8a), 139.1 (1C, Bn-**C**$_{aromat.}$), 128.4 (2C, Bn-**C**H$_{aromat.}$), 127.3 (2C, Bn-**C**H$_{aromat.}$), 126.6 (1C, Bn-**C**H$_{aromat.}$), 126.5 (q, 1C, $^3J_{C,F}$ = 5.3, **C**-5), 124.5 (q, 1C, $^1J_{C,F}$ = 278.5, **C**F_3), 122.7 (1C, **C**-4a), 121.8 (q, 1C, $^2J_{C,F}$ = 28.9, **C**-6), 112.0 (1C, **C**-8), 111.5 (1C, **C**-3), 66.3 (2C, morpholino-**C**H_2-O-**C**H_2), 52.9 (2C, morpholino-**C**H_2-

N-**C**H_2), 52.7 (1C, N**C**$H_2CH_2CH_2CH_3$), 42.1 (1C, Bn-**C**H_2), 30.4 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.0 (1C, NCH_2CH_2**C**H_2CH_3), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

N-Benzyl-1-butyl-5-(dimethylamino)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***13*** *(MB332)*



$C_{27}H_{34}N_4O_3$

M_r = 462.59 g/mol

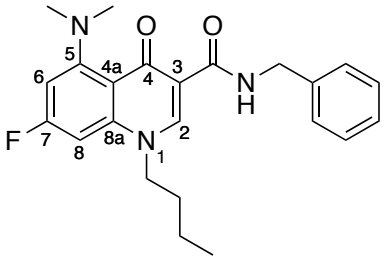
A solution of compound **4i** (350 mg, 0.94 mmol) in abs. DMF (15 mL) was treated with *N*MM (517 µL, 4.70 mmol), IBCF (490 µL, 3.78 mmol), and benzylamine (410 µL, 3.78 mmol) as depicted in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:3) and recrystallised from EtOH to give **12**.

appearance:	white solid
yield:	180 mg (0.40 mmol/ 42%)
reaction control:	R_f = 0.82 ($CHCl_3$/MeOH = 100:3)
melting point [°C]:	168-170 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3154, 3030, 2954, 2861, 2823, 1653, 1594, 1564, 1526, 1485, 1452, 1373, 1284, 1230, 1120, 1013, 741.
mass:	*m/z* 463.3 $[M + H]^+$
HPLC purity:	99% (HPLC method **I**)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 10.69 (t, 1H, ^3J = 6.0, N-**H**), 8.54 (s, 1H, **H**-2), 7.34 – 7.33 (m, 4H, Bn-C**H**$_{aromat.}$), 7.27 – 7.25 (m, 1H, Bn-C**H**$_{aromat.}$), 6.36 – 6.34 (m, 2H, **H**-6 / **H**-8), 4.52 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 4.29 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.76–3.74 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.34 – 3.31 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 2.75 (s, 6H), 1.74 – 1.70 (m, 2H, NCH_2C**H$_2$**CH_2CH_3), 1.33 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.91 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 174.8 (1C, **C**-4), 164.7 (1C, **C**ON), 154.5 (1C, **C**-5), 153.2 (1C, **C**-7), 146.3 (1C, **C**-2), 143.5 (1C, **C**-8a), 139.6 (1C, Bn-**C**$_{aromat.}$), 128.3 (2C, Bn-**C**H$_{aromat.}$), 127.2 (2C, Bn-**C**H$_{aromat.}$), 126.7 (1C, Bn-C**H**$_{aromat.}$), 111.1 (1C, **C**-4a), 110.5 (1C, **C**-3), 99.1 (1C, **C**-6), 91.2 (1C, **C**-8), 65.9 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 53.0 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 47.0 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 42.0 (2C, N(**C**H$_3$)$_2$), 40.1 (1C, Bn-**C**H$_2$), 29.9 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.5 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

N-Benzyl-1-butyl-5-(dimethylamino)-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***14*** *(MBMR13)*



$C_{23}H_{26}FN_3O_2$

M_r = 395.48 g/mol

According to the general procedure 10.5, a solution of **3k** (250 mg, 0.82 mmol) in abs. DMF (10 mL) was treated with *N*MM (449 µL, 4.08 mmol), IBCF (466 µL, 3.3 mmol), and benzylamine (356 µL, 3.3 mmol). The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH/TEA = 100:3:1) and recrystallised from EtOH to yield compound **14.**

appearance:	pale yellow solid
yield:	80 mg (0.20 mmol/ 24%)
reaction control:	R_f = 0.78 ($CHCl_3$/MeOH/TEA = 100:3:1)
melting point [°C]:	137–138 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3210, 3034, 2951, 2929, 2848, 2789, 1656, 1612, 1550, 1488, 1450, 1423, 1356, 1279, 1240, 1168, 1131, 1065, 1026, 944.
HPLC purity:	98% (HPLC method **II**)

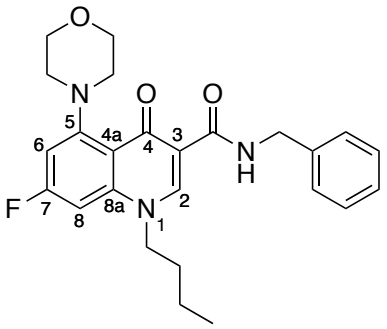
^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 10.56 (t, 1H, ^3J = 6.0, N-**H**), 8.60 (s, 1H, **H**-2), 7.39 – 7.31 (m, 4H, Bn-C**H**$_{aromat.}$), 7.25 – 7.21 (m, 1H, Bn-C**H**$_{aromat.}$), 6.53 (dd, 1H, ^3J = 13.6, ^4J = 2.0, **H**-6), 6.50 (dd, 1H, ^3J = 12.0, ^4J = 2.0, **H**-8), 4.66 (d, 2H, ^3J = 5.6, Bn-C**H$_2$**),

4.07 (t, 2H, ^3J = 7.2, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 2.93 (s, 6H, N(C**H**$_3$)$_2$), 1.87 – 1.83 (m, 2H, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.76 (sext, 2H, ^3J = 7.2, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 1.00 (t, 3H, ^3J = 7.2, NCH$_2$CH$_2$CH$_2$C**H**$_3$).

^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 176.1 (1C, **C**-4), 165.2 (1C, **C**ON), 164.8 (d, 1C, $^1J_{C,F}$ = 247.0, **C**-7), 156.7 (d, 1C, $^3J_{C,F}$ = 13.0, **C**-5), 146.4 (1C, **C**-2), 144.0 (d, 1C, $^3J_{C,F}$ = 14.0, **C**-8a), 139.0 (1C, Bn-**C**$_{aromat.}$), 128.5 (2C, Bn-**C**H$_{aromat.}$), 127.6 (2C, Bn-**C**H$_{aromat.}$), 126.9 (1C, Bn-**C**H$_{aromat.}$), 115.3 (1C, **C**-4a), 112.7 (1C, **C**-3), 99.3 (d, 1C, $^2J_{C,F}$ = 25.1, **C**-6), 92.3 (d, 1C, $^2J_{C,F}$ = 27.4, **C**-8), 54.8 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 44.5 (2C, N(**C**H$_3$)$_2$), 43.2 (1C, Bn-**C**H$_2$), 30.5 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.9 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.6 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

^{19}F NMR (^{1}H decoupled, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): -104.98 (s, **F**-7),

N-Benzyl-1-butyl-7-fluoro-5-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***15*** *(MBMS01, MB334)*



$C_{25}H_{28}FN_3O_3$

M_r = 437.52 g/mol

According to the general procedure 10.5, a solution of **4l** (200 mg, 0.57 mmol) in abs. DMF (10 mL) was treated with *N*MM (313 µL, 2.85 mmol), IBCF (296 µL, 2.28 mmol), and benzylamine (285 µL, 2.28 mmol). The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:3) and recrystallised from EtOAc to yield **15.**

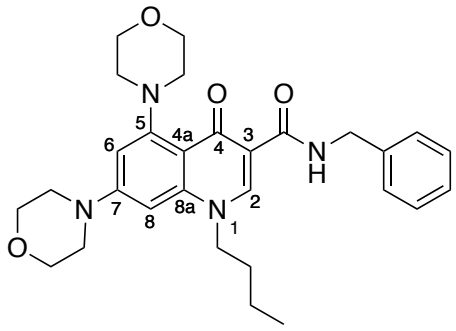
appearance:	white solid
yield:	210 mg (0.48 mmol / 84%)
reaction control:	R_f = 0.82 ($CHCl_3$/MeOH = 100:3)
melting point [°C]:	181–182 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3181, 3042, 2962, 2931, 2871, 2814, 1655, 1592, 1533, 1477, 1427, 1390, 1363, 1283, 1264, 1225, 1206, 1151.
HPLC purity:	99% (HPLC method **II**)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 10.48 (t, 1H, ^3J = 6.0, N-**H**), 8.72 (s, 1H, **H**-2), 7.34 – 7.32 (m, 4H, Bn-C**H**$_{aromat.}$), 7.27 – 7.23 (m, 1H, Bn-C**H**$_{aromat.}$), 7.16 (dd, 1H, ^3J = 10.8, ^4J = 2.0, **H**-8), 6.76 (dd, 1H, ^3J = 11.6, ^4J = 2.0, **H**-8), 4.55 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 4.33 (t, 2H, ^3J = 7.2, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 3.79 – 3.77 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.03 (br, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.70 (m, 2H, ^3J = 7.2, NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.33 (sext, 2H, ^3J = 7.2, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 0.89 (t, 3H, ^3J = 7.2, NCH$_2$CH$_2$CH$_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]):175.3 (1C, **C**-4), 164.2 (1C, **C**ON), 164.2 (d, 1C, $^1J_{C,F}$ = 245.0, **C**-7), 155.6 (d, 1C, $^3J_{C,F}$ = 12.4, **C**-7), 147.0 (1C, **C**-2), 143.6 (d, 1C, $^3J_{C,F}$ = 14.0 **C**-8a), 139.5 (1C, Bn-**C**$_{aromat.}$), 128.3 (2C, Bn-**C**H$_{aromat.}$), 127.1 (2C, Bn-**C**H$_{aromat.}$), 126.7 (1C, Bn-**C**H$_{aromat.}$), 116.1 (1C, **C**-4a), 112.0 (1C, **C**-3), 100.8 (d, 1C, ^2J = 24.4, **C**-6), 95.2 (d, 1C, $^2J_{C,F}$ = 26.9, **C**-8), 65.9 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 53.5 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 52.6 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 41.9 (1C, Bn-CH$_2$), 30.1 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.0 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.4 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

^{19}F NMR (^{1}H decoupled, DMSO-d_6, δ [ppm], J [Hz]): -104.09 (s, **F**-7).

N-Benzyl-1-butyl-5,7-dimorpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***16*** *(MB453)*



$C_{29}H_{36}N_4O_4$

M_r = 504.63 g/mol

According to the general procedure 10.5, a solution of **4n** (820 mg, 2.35 mmol) in abs. DMF (15 mL) was treated with *N*MM (1.30 mL, 11.75 mmol), IBCF (1.22 μL, 9.40 mmol), and benzylamine (1.22 μL, 9.49 mmol). The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and recrystallised from EtOH to yield compound **16.**

appearance:	white solid
yield:	610 mg (1.21 mmol/ 51%)
reaction control:	R_f = 0.62 ($CHCl_3$/MeOH = 100:1)

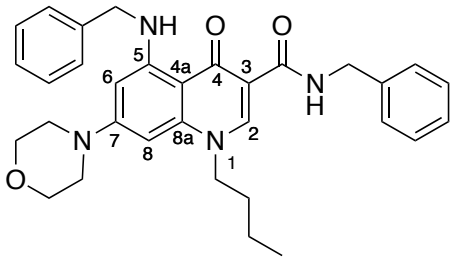
melting point [°C]: 186–187 °C

IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3130, 3046, 2951, 2850, 1654, 1659, 1530, 1435, 1110.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 10.72 (t, 1H, ^3J = 6.0, N-**H**), 8.59 (s, 1H, **H**-2), 7.36 – 7.31 (m, 4H, Bn-C**H**$_{aromat.}$), 7.27 – 7.3 (m, 1H, Bn-C**H**$_{aromat.}$), 6.45 – 6.44 (d, 1H, ^3J = 10.8, **H**-8/**H**-6), 4.53 (d, 2H, ^3J = 5.6, Bn-C**H$_2$**), 4.33 (t, 2H, ^3J = 7.2, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 3.78 – 3.75 (m, 8H, (morpholino-C**H$_2$**-O-C**H$_2$**)$_2$), 3.38 – 3.32 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 2.90 (br, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.73 (quint, 2H, ^3J = 7.2, NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.32 (sext, 2H, ^3J = 7.2, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 0.91 (t, 3H, ^3J = 7.2, NCH$_2$CH$_2$CH$_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 175.0 (1C, **C**-4), 164.8 (1C, **C**ON), 154.4 (1C, **C**-5), 153.2 (1C, **C**-7), 146.4 (1C, **C**-2), 143.5 (1C, **C**-8a), 139.6 (1C, Bn-**C**$_{aromat.}$), 128.3 (2C, Bn-**C**H$_{aromat.}$), 127.1 (2C, Bn-**C**H$_{aromat.}$), 126.7 (1C, Bn-**C**H$_{aromat.}$), 112.1 (1C, **C**-4a), 110.8 (1C, **C**-3), 100.7 (1C, **C**-6), 92.7 (1C, **C**-8), 66.1 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 65.8 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 53.2 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 52.8 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 46.8 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 41.8 (1C, Bn-**C**H$_2$), 29.8 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.4 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

N-Benzyl-5-(benzylamino)-1-butyl-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***17*** *(MB360)*



$C_{32}H_{36}N_4O_3$

M_r = 524.67 g/mol

According to the general procedure 10.5, a solution of **4o** (600 mg, 1.38 mmol) in abs. DMF (15 mL) was treated with *N*MM (759 µL, 6.90 mmol), IBCF (718 µL, 5.52 mmol), and benzylamine (642 µL, 5.52 mmol). The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and recrystallised from a mixture of EtOAc and MeOH (1:1) to yield compound **17.**

appearance: beige solid

yield: 350 mg (0.67 mmol/ 48%)

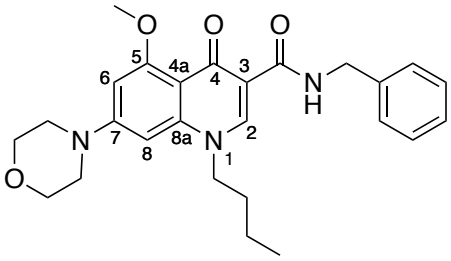
reaction control: R_f = 0.44 ($CHCl_3$/MeOH = 100:1)

melting point [°C]:	209–210 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3168, 3047, 2949, 2931, 2958, 1657, 1609, 1536, 1441, 1357, 1287, 1227, 1213, 1125, 1111, 1070, 812.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 10.45 (t, 1H, ^3J = 5.2, N-**H**), 10.38 (t, 1H, ^3J = 5.6, CON-**H**), 8.43 (s, 1H, **H**-2), 7.30 – 7.20 (m, 8H, Bn-C**H**$_{aromat.}$), 7.17 – 7.14 (m, 2H, Bn-CH$_{aromat.}$), 5.79 (d, 1H, ^4J = 2.0, **H**-6), 5.72 (d, 1H, ^4J = 2.0, **H**-8), 4.56 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 4.34 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 3.97 (t, 2H, ^3J = 7.2, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 3.72 – 3.69 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.10 – 3.07 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.75 (quint, 2H, ^3J = 7.2, NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.34 (sext, 2H, ^3J = 7.2, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 0.89 (t, 3H, ^3J = 7.2, NCH$_2$CH$_2$CH$_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 178.6 (1C, **C**-4), 165.2 (1C, **C**ON), 154.4 (1C, **C**-7), 152.6 (1C, **C**-5), 146.4 (1C, **C**-2), 142.6 (1C, **C**-8a), 138.8 (1C, Bn-**C**$_{aromat.}$), 138.3 (1C, Bn-**C**$_{aromat.}$), 128.3 (2C, Bn-**C**H$_{aromat.}$), 128.3 (2C, Bn-**C**H$_{aromat.}$), 127.0 (2C, Bn-**C**H$_{aromat.}$), 126.7 (3C, Bn-**C**H$_{aromat.}$/Bn-**C**H$_{aromat.}$), 126.6 (1C, Bn-**C**H$_{aromat.}$), 110.3 (1C, **C**-3), 106.9 (1C, **C**-4a), 91.1 (1C, **C**-6), 86.4 (1C, **C**-8), 66.1 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 54.1 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 47.3 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 46.8 (1C, Bn-**C**H$_2$), 42.6 (1C, Bn-**C**H$_2$), 29.7 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.6 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.3 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

N-Benzyl-1-butyl-5-methoxy-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***18*** *(MB452)*



$C_{26}H_{31}N_3O_4$

M_r = 449.55 g/mol

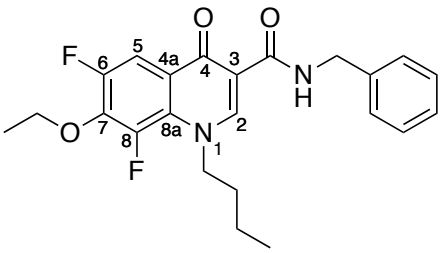
A solution of compound **4q** (220 mg, 0.61 mmol) in abs. DMF (10 mL) was treated with *N*MM (335 μL, 3.05 mmol), IBCF (317 μL, 2.44 mmol), and benzylamine (305 μL, 2.44 mmol) as depicted in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 50:1) and recrystallised from EtOH to give **18**.

appearance:	white solid
yield:	140 mg (0.31 mmol/ 51%)
reaction control:	R_f = 0.63 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	159-161 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3120, 2987, 2954, 2924, 2894, 1661, 1611, 1523, 1470, 1356, 1258, 1237.
mass:	*m/z* 450.1 $[M + H]^+$
HPLC purity:	98% (HPLC method **I**)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 10.67 (t, 1H, ^3J = 6.0, N-**H**), 8.57 (s, 1H, **H**-2), 7.35 – 7.32 (m, 4H, Bn-C**H**$_{aromat.}$), 7.28 – 7.25 (m, 1H, Bn-C**H**$_{aromat.}$), 6.53 (d, 1H, ^4J = 1.6, **H**-5), 6.43 (d, 1H, ^4J = 1.6, **H**-8), 4.50 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 4.32 (t, 2H, ^3J = 7.6, NC**H$_2$**$CH_2CH_2CH_3$), 3.82 (s, 3H, OC**H$_3$**), 3.78 – 3.76 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.39 – 3.37 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.72 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.31 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.91 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 175.25 (1C, **C**-4), 164.5 (1C, **C**ON), 161.8 (1C, **C**-5), 153.8 (1C, **C**-7), 146.8 (1C, **C**-2), 142.6 (1C, **C**-8a), 139.5 (1C, Bn-**C**$_{aromat.}$), 128.3 (2C, Bn-**C**H$_{aromat.}$), 127.3 (2C, Bn-**C**H$_{aromat.}$), 126.7 (1C, Bn-**C**H$_{aromat.}$), 110.9 (1C, **C**-4a), 110.2 (1C, **C**-3), 95.0 (1C, **C**-6), 91.3 (1C, **C**-8), 65.8 (2C, morpholino-**C**H_2-N-**C**H_2), 55.8 (1C, O**C**H_3), 53.0 (1C, N**C**$H_2CH_2CH_2CH_3$), 46.9 (2C, morpholino-**C**H_2-N-**C**H_2), 42.0 (1C, Bn-**C**H_2), 29.8 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.1 (1C, NCH_2CH_2**C**H_2CH_3), 13.5 (1C, $NCH_2CH_2CH_2$**C**H_3).

N-Benzyl-1-butyl-7-ethoxy-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***19*** *(MB319)*



$C_{23}H_{24}F_2N_2O_3$

M_r = 414.45 g/mol

A solution of compound **3j** (400 mg, 1.23 mmol) in abs. DMF (10 mL) was treated with *N*MM (676 µL, 6.15 mmol), IBCF (637 µL, 4.92 mmol), and benzylamine (573 µL, 4.92 mmol) as depicted in the general procedure 10.5. The crude product was purified by

column chromatography (silica gel; $CHCl_3$/PE = 10:1) and recrystallised from EtOAc to yield compound **19**.

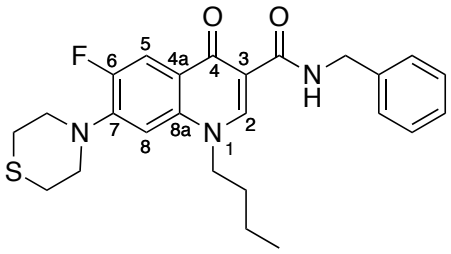
appearance:	white solid
yield:	420 mg (1.01 mmol/ 82%)
reaction control:	R_f = 0.55 ($CHCl_3$/PE = 10:1)
melting point [°C]:	147–148 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3184, 3051, 2961, 2865, 1654, 1598, 1574, 1531, 1466, 1336, 1317, 1247, 1213, 1133, 1025, 919, 804.
mass:	*m/z* 415.2 $[M + H]^+$
HPLC purity:	97% (HPLC method **I**)

^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 10.13 (t, 1H, ^3J = 6.0, N-**H**), 8.81 (s, 1H, **H**-2), 7.93 (dd, 1H, ^3J = 11.2, ^5J = 2.0, **H**-5), 7.35 – 7.32 (m, 4H, Bn-C**H**$_{aromat.}$), 7.29 – 7.24 (m, 1H, Bn-C**H**$_{aromat.}$), 4.55 (d, 2H, ^3J = 6.0, Bn-**C**H_2), 4.51 – 4.47 (br, 2H, NC**H$_2$**$CH_2CH_2CH_3$), 4.35 (q, 2H, ^3J = 6.8, OC**H$_2$**CH_3), 1.80 – 1.73 (m, 2H, NCH_2C**H$_2$**CH_2CH_3), 1.38 – 1.31 (m, 5H, NCH_2CH_2C**H$_2$**CH_3/OCH_2C**H$_3$**), 0.91 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 175.6 (d, 1C, $^4J_{C,F}$ = 1.2, **C**-4), 165.3 (1C, **C**ON), 152.4 (dd, 1C, $^1J_{C,F}$ = 247.0, $^3J_{C,F}$ = 4.7, **C**-6), 150.6 (1C, **C**-2), 146.9 (dd, 1C, $^1J_{C,F}$ = 256.2, $^3J_{C,F}$ = 4.0, **C**-8), 139.3 (1C, **C**-7), 139.2 (1C, Bn-**C**$_{aromat.}$), 128.3 (2C, Bn-**C**H$_{aromat.}$), 127.3 (2C, Bn-**C**H$_{aromat.}$), 126.8 (1C, Bn-**C**H$_{aromat.}$), 126.8 (m, 1C, **C**-8a), 123.3 (d, 1C, $^3J_{C,F}$ = 8.0, **C**-4a), 109.9 (1C, **C**-3), 107.2 (dd, 1C, $^2J_{C,F}$ = 19.0, $^4J_{C,F}$ = 4.0, **C**-5), 71.4 (m, 1C, O**C**H_2CH_3), 57.7 (1C, N**C**$H_2CH_2CH_2CH_3$), 42.1 (1C, Bn-**C**H_2), 32.6 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.5 (1C, NCH_2CH_2**C**H_2CH_3), 15.8 (1C, OCH_2**C**H_3), 13.9 (1C, $NCH_2CH_2CH_2$**C**H_3).

^{19}F NMR (^{1}H decoupled, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): -128.92 (d, $^4J_{6,8}$ = 8.6, **F**-6), -136.32 (d, $^4J_{6,8}$ = 8.6, **F**-8).

N-Benzyl-1-butyl-6-fluoro-4-oxo-7-thiomorpholino-1,4-dihydroquinoline-3-carboxamide, ***20*** *(MB310)*



$C_{25}H_{28}FN_3O_2S$

M_r = 453.58 g/mol

A solution of compound **4o** (170 mg, 0.47 mmol) in abs. DMF (10 mL) was treated with *N*MM (258 µL, 2.35 mmol), IBCF (245 µL, 1.88 mmol), and benzylamine (205 µL, 1.88 mmol) as specified in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and oily product was crystallised from ACN to give **20**.

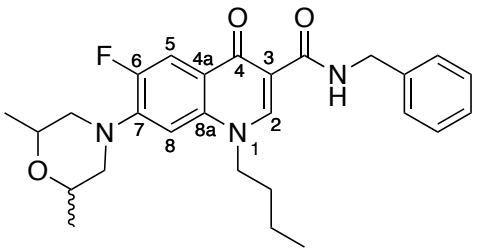
appearance:	pale yellow solid
yield:	50 mg (0.11 mmol/ 23%)
reaction control:	R_f = 0.56 ($CHCl_3$/MeOH = 100:1)
melting point [°C]:	155-156 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3145, 3031, 2923, 1650, 1600, 1486, 1486, 1451, 1381, 1288, 1245, 961, 907, 804.
HPLC purity:	96% (HPLC method **II**)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 10.36 (t, 1H, ^3J = 6.0, N-**H**), 8.78 (s, 1H, **H**-2), 7.78 (d, 1H, ^3J = 13.2, **H**-5), 7.34 – 7.30 (m, 4H, Bn-C**H**$_{aromat.}$), 7.27 – 7.25 (m, 1H, Bn-C**H**$_{aromat.}$), 7.12 (d, 1H, ^3J = 6.0, **H**-8), 4.54 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 4.46 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.53 – 3.51 (m, 4H, thiomorpholino-C**H$_2$**-N-C**H$_2$**), 2.81 – 2.79 (m, 4H, thiomorpholino-C**H$_2$**-S-C**H$_2$**), 1.77 (quint, 2H, ^3J = 7.6, NCH_2C**H$_2$**CH_2CH_3), 1.32 (sext, 2H, ^3J = 7.6, NCH_2CH_2C**H$_2$**CH_3), 0.92 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 174.0 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 164.0 (1C, **C**ON), 152.6 (d, 1C, $^1J_{C,F}$ = 247.8, **C**-6), 147.7 (1C, **C**-2), 144.9 (d, 1C, $^2J_{C,F}$ = 8.4, **C**-7), 139.3 (1C, Bn-**C**$_{aromat.}$), 136.5 (1C, **C**-8a), 128.3 (2C, Bn-**C**H$_{aromat.}$), 127.3 (2C, Bn-**C**H$_{aromat.}$), 126.7 (1C, Bn-**C**H$_{aromat.}$), 121.4 (d, 1C, $^3J_{C,F}$ = 2.8, **C**-4a), 111.4 (d, 1C, $^2J_{C,F}$ = 22.6, **C**-5), 109.9 (1C, **C**-3), 106.5 (d, 1C, $^3J_{C,F}$ = 2.8, **C**-8), 52.8 (1C, N**C**$H_2CH_2CH_2CH_3$), 52.7 (d, 2C, $^4J_{C,F}$ = 4.7, thiomorpholino-**C**H_2-N-**C**H_2), 42.1 (1C, Bn-**C**H_2), 30.2 (1C, NCH_2**C**$H_2CH_2CH_3$),

26.6 (2C, thiomorpholino-**C**H_2-S-**C**H_2), 19.0 (1C, NCH_2CH_2**C**H_2CH_3), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

N-Benzyl-1-butyl-7-(2,6-dimethylmorpholino)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***21*** *(MB312)*



$C_{27}H_{32}FN_3O_3$

M_r = 465.67 g/mol

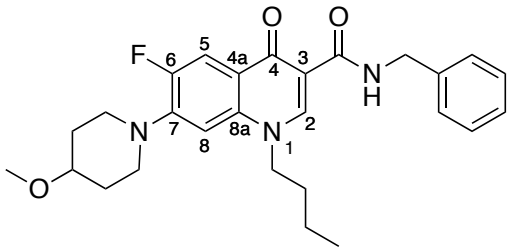
A solution of compound **4p** (250 mg, 0.66 mmol) in abs. DMF (15 mL) was treated with *N*MM (365 µL, 3.32 mmol), IBCF (343 µL, 2.66 mmol), and benzylamine (283 µL, 2.66 mmol) as specified in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and recrystallised from ACN to give **21**.

appearance:	white solid
yield:	80 mg (0.17 mmol/ 26%)
reaction control:	R_f = 0.76 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	165-166 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3121, 3060, 2971, 2933, 2863, 1654, 1624, 1602, 1540, 1487, 1375, 1257, 1212, 1143, 1080, 936, 806, 735.
HPLC purity:	95% (HPLC method **II**)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 10.36 (t, 1H, ^3J = 5.6, N-**H**), 8.78 (s, 1H, **H**-2), 7.85 (d, 1H, ^3J = 13.6, **H**-5), 7.35 – 7.33 (m, 4H, Bn-C**H**$_{aromat.}$), 7.28 – 7.24 (m, 1H, Bn-C**H**$_{aromat.}$), 7.08 (d, 1H, ^3J = 6.0, **H**-8), 4.55 (d, 2H, ^3J = 5.6, Bn-C**H$_2$**), 4.48 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.81 – 3.76 (m, 2H, dimethylmorpholino-C**H**-O-C**H**), 3.54 – 3.51 (m, 2H, dimethylmorpholino-C**H**-N-C**H**), 2.57 – 2.55 (m, 2H, dimethylmorpholino-C**H**-N-C**H**), 1.77 (quint, 2H, ^3J = 7.6, NCH_2C**H$_2$**CH_2CH_3), 1.33 (sext, 2H, ^3J = 7.6, NCH_2CH_2C**H$_2$**CH_3), 1.17 (s, 3H, dimethylmorpholino-C**H$_3$**), 1.15 (s, 3H, dimethylmorpholino-C**H$_3$**), 0.92 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 174.0 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 164.0 (1C, **C**ON), 152.6 (d, 1C, $^1J_{C,F}$ = 247.8, **C**-6), 147.7 (1C, **C**-2), 143.9 (d, 1C, $^2J_{C,F}$ = 11.8, **C**-7), 139.3 (1C, Bn-**C**$_{aromat.}$), 136.6 (1C, **C**-8a), 128.3 (2C, Bn-**C**H$_{aromat.}$), 127.3 (2C, Bn-**C**H$_{aromat.}$), 126.7 (1C, Bn-**C**H$_{aromat.}$), 121.3 (d, 1C, $^3J_{C,F}$ = 7.0, **C**-4a), 111.3 (d, 1C, $^2J_{C,F}$ = 22.6, **C**-5), 109.9 (1C, **C**-3), 105.6 (d, 1C, $^3J_{C,F}$ = 2.8, **C**-8), 70.7 (2C, dimethylmorpholino-**C**H$_2$-O-**C**H$_2$), 54.8 (d, 2C, $^4J_{C,F}$ = 4.0, dimethylmorpholino-**C**H$_2$-N-**C**H$_2$), 52.7 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 42.0 (1C, Bn-**C**H$_2$), 30.1 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.3 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

N-Benzyl-1-butyl-6-fluoro-7-(4-methoxypiperidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***22*** *(MB305)*



$C_{27}H_{32}FN_3O_3$

M_r = 465.67 g/mol

A solution of compound **4q** (500 mg, 1.33 mmol) in DMF (15 mL) was treated with *N*MM (730 μL, 6.65 mmol), IBCF (691 μL, 5.31 mmol), and benzylamine (581 μL, 5.31 mmol) as depicted in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 50:1) and recrystallised from ACN to give **22**.

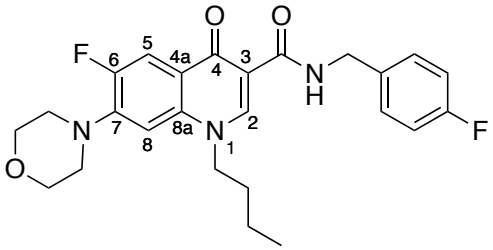
appearance:	white solid
yield:	290 mg (0.62 mmol/ 47%)
reaction control:	R_f = 0.36 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	134-135 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3177, 3051, 2930, 2825, 1654, 1625, 1600, 1553, 1536, 1484, 1359, 1242, 1095, 1027, 803.
HPLC purity:	96% (HPLC method **II**)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 10.37 (t, 1H, ^3J = 6.0, N-**H**), 8.78 (s, 1H, **H**-2), 7.85 (d, 1H, ^3J = 13.6, **H**-5), 7.34 – 7.33 (m, 4H, Bn-C**H**$_{aromat.}$), 7.28 – 7.24 (m, 1H, Bn-C**H**$_{aromat.}$), 7.07 (d, 1H, ^3J = 6.0, **H**-8), 4.54 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 4.46 (t, 2H, ^3J = 7.2, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 3.49 – 3.39 (m, 3H, piperidinyl-C**H$_2$**-N-C**H$_2$**/piperidinyl-C**H**), 3.29 (s,

3H, OC**H$_3$**), 3.09 – 3.03 (m, 2H, piperidinyl-C**H$_2$**), 2.01 – 1.97 (m, 2H, piperidinyl-C**H$_2$**), 1.77 (quint, 2H, ^3J = 7.6, NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.66 – 1.62 (m, 2H, piperidinyl-C**H$_2$**), 1.32 (sext, 2H, ^3J = 7.6, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 0.92 (t, 3H, ^3J = 7.2, NCH$_2$CH$_2$CH$_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 174.0 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 164.1 (1C, **C**ON), 152.5 (d, 1C, $^1J_{C,F}$ = 247.8, **C**-6), 147.6 (1C, **C**-2), 144.6 (d, 1C, $^2J_{C,F}$ = 10.4, **C**-7), 139.2 (1C, Bn-**C**$_{aromat.}$), 136.6 (1C, **C**-8a), 128.3 (2C, Bn-**C**H$_{aromat.}$), 127.2 (2C, Bn-**C**H$_{aromat.}$), 126.7 (1C, Bn-**C**H$_{aromat.}$), 121.1 (d, 1C, $^3J_{C,F}$ = 7.0, **C**-4a), 111.2 (d, 1C, $^2J_{C,F}$ = 22.9, **C**-5), 109.9 (1C, **C**-3), 105.6 (d, 1C, $^3J_{C,F}$ = 2.8, **C**-8), 74.4 (1C, piperidinyl-**C**H), 54.9 (1C, O**C**H$_3$), 52.7 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 47.1 (d, 2C, $^4J_{C,F}$ = 4.7, piperidinyl-**C**H$_2$-N-**C**H$_2$), 42.0 (1C, Bn-**C**H$_2$), 30.2 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 30.1 (2C, piperidinyl-**C**H$_2$-CH-**C**H$_2$), 19.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.3 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

1-Butyl-6-fluoro-N-(4-fluorobenzyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***23*** *(MB209)*



$C_{25}H_{27}F_2N_3O_3$

M_r = 455.51 g/mol

A solution of compound **4a** (90 mg, 0.26 mmol) in abs. DMF (7.5 mL) was treated with *N*MM (141 µL, 1.29 mmol), IBCF (134 µL, 1.02 mmol), and (4-fluorophenyl)methanamine (118 µL, 1.02 mmol) as depicted in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and recrystallised from EtOAc to give **23**.

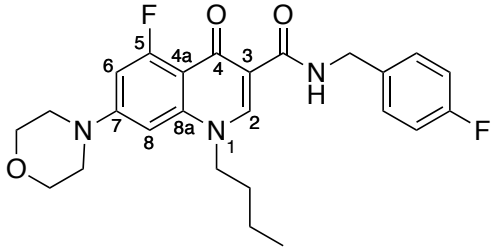
appearance:	white solid
yield:	70 mg (0.15 mmol / 58%)
reaction control:	R_f = 0.75 ($CHCl_3$/MeOH = 100:1)
melting point [°C]:	177-179 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3180, 3032, 2954, 2871, 1654, 1625, 1603, 1536, 1485, 1468, 1478, 1257, 1213, 1113
mass:	*m/z* 456.2 $[M + H]^+$
HPLC purity:	98% (HPLC method **I**)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 10.36 (t, 1H, ^3J = 6.0, N-**H**), 8.78 (s, 1H, **H**-2), 7.87 (d, 1H, ^3J = 13.6, **H**-5), 7.40 – 7.36 (m, 2H, Bn-C**H**$_{aromat.}$), 7.18 – 7.09 (m, 2H, Bn-C**H**$_{aromat.}$), 7.09 (d, 1H, ^4J = 7.6, **H**-8), 4.53 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 4.48 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.80 – 3.78 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.26 – 3.24 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.77 (quint, 2H, ^3J = 7.6, NCH_2C**H$_2$**CH_2CH_3), 1.32 (sext, 2H, ^3J = 7.6, NCH_2CH_2C**H$_2$**CH_3), 0.92 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 174.6 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 164.0 (1C, **C**ON), 160.2 (d, 1C, $^1J_{C,F}$ = 240.4, Bn-**C**H$_{aromat.}$), 152.4 (d, 1C, $^1J_{C,F}$ = 245.6, **C**-6), 147.7 (1C, **C**-2), 144.3 (d, 1C, $^2J_{C,F}$ = 10.3, **C**-6), 136.6 (1C, **C**-8a), 135.6 (d, 1C, $^4J_{C,F}$ = 3.0, Bn-**C**$_{aromat.}$), 129.3 (d, 2C, $^3J_{C,F}$ = 8.0, Bn-**C**H$_{aromat.,}$), 121.4 (d, 1C, $^3J_{C,F}$ = 6.9, **C**-4a), 115.1 (d, 2C, $^2J_{C,F}$ = 21.1, Bn-**C**H$_{aromat.}$), 111.0 (d, 1C, $^2J_{C,F}$ = 22.4, **C**-5), 109.9 (1C, **C**-3), 105.5 (d, 1C, $^3J_{C,F}$ = 3.3, **C**-8), 65.8 (2C, morpholino-**C**H_2-O-**C**H_2), 52.8 (1C, N**C**$H_2CH_2CH_2CH_3$), 49.8 (d, 2C, $^4J_{C,F}$ = 4.4, morpholino-**C**H_2-N-**C**H_2), 41.3 (1C, Bn-**C**H_2), 30.2 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.1 (1C, NCH_2CH_2**C**H_2CH_3), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

^{19}F NMR (^{1}H decoupled, DMSO-d_6, δ [ppm], J [Hz]): -116.04 (s, *p*-**F**), -123.79 (s, **F**-6).

1-Butyl-5-fluoro-N-(4-fluorobenzyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***24*** *(MB444)*



$C_{25}H_{27}F_2N_3O_3$

M_r = 455.51 g/mol

A solution of compound **4c** (100 mg, 0.29 mmol) in abs. DMF (7.5 mL) was treated with *N*MM (160 μL, 1.45 mmol), IBCF (150 μL, 1.16 mmol), and (4-fluorophenyl)methanamine (145 mg, 1.16 mmol) as depicted in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and recrystallised from EtOH to produce **24**.

appearance:	white solid
yield:	40 mg (0.09 mmol/ 31%)
reaction control:	R_f = 0.53 ($CHCl_3$/MeOH = 100:1)

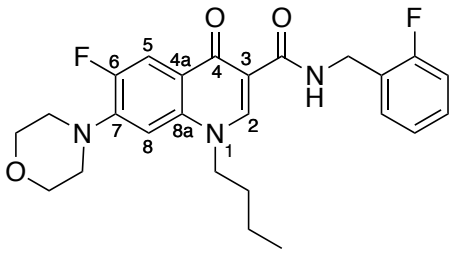
melting point [°C]:	191 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3112, 3063, 2958, 2860, 1662, 1629, 1603, 1583, 1550, 1534, 1508.
mass:	*m/z* 456.0 $[M + H]^+$
HPLC purity:	97% (HPLC method **I**)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 10.38 (t, 1H, ^3J = 6.0, N-**H**), 8.65 (s, 1H, **H**-2), 7.39 – 7.35 (m, 2H, Bn-C**H**$_{aromat.}$), 7.18 – 7.13 (m, 2H, Bn-C**H**$_{aromat.}$), 6.95 (dd, 1H, ^3J = 15.6, ^4J = 1.6, **H**-5), 6.65 (d, 1H, ^4J = 1.6, **H**-8), 4.50 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 4.37 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.76 – 3.73 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.39 – 3.37 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.72 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.31 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.90 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 174.2 (d, 1C, $^3J_{C,F}$ = 1.7, **C**-4), 164.1 (1C, **C**ON), 163.2 (d, 1C, $^1J_{C,F}$ = 256.1, **C**-5), 160.8 (d, 1C, $^1J_{C,F}$ = 240.8, Bn-**C**$_{aromat.}$), 153.5 (d, 1C, $^3J_{C,F}$ = 13.1, **C**-7), 147.9 (1C, **C**-2), 142.0 (d, 1C, $^3J_{C,F}$ = 5.7, **C**-8a), 135.7 (d, 1C, $^3J_{C,F}$ = 3.0, Bn-**C**$_{aromat.}$), 129.2 (d, 2C, $^3J_{C,F}$ = 8.1, Bn-**C**H$_{aromat.}$), 115.0 (d, 2C, $^2J_{C,F}$ = 21.1, Bn-**C**H$_{aromat.}$), 110.7 (1C, **C**-3), 108.7 (d, 1C, $^3J_{C,F}$ = 8.5, **C**-4a), 99.2 (d, 1C, ^2J = 25.6, **C**-6), 94.3 (1C, **C**-8), 65.6 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 52.9 (1C, N**C**H$_2$$CH_2CH_2CH_3$), 46.5 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 42.5 (1C, Bn-**C**H$_2$), 29.8 (1C, NCH_2**C**H$_2$$CH_2CH_3$), 19.1 (1C, NCH_2CH_2**C**H$_2$$CH_3$), 13.4 (1C, $NCH_2CH_2CH_2$**C**H$_3$).

^{19}F NMR (^{1}H coupled, DMSO-d_6, δ [ppm], *J* [Hz]): -109.89 (d, $^2J_{5,6}$ = 16.6, **F**-5), -116.08 (m, *p*-**F**).

1-Butyl-6-fluoro-N-(2-fluorobenzyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***25*** *(MB400)*



$C_{25}H_{27}F_2N_3O_3$

M_r = 455.51 g/mol

A solution of compound **4a** (210 mg, 0.60 mmol) in abs. DMF (10 mL) was treated with *N*MM (329 µL, 3.00 mmol), IBCF (328 µL, 2.40 mmol), and (2-fluorophenyl)methanamine (300 mg, 2.40 mmol) as depicted in the general procedure 10.5. The crude product was

purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and recrystallised from EtOAc to give **25**.

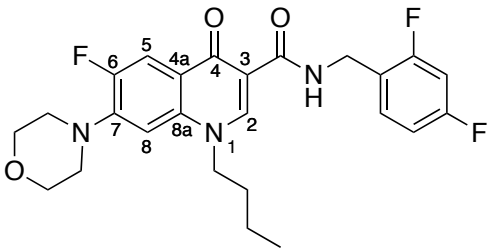
appearance:	white solid
yield:	70 mg (0.15 mmol/ 25%)
reaction control:	R_f = 0.34 ($CHCl_3$/MeOH = 100:1)
melting point [°C]:	154-156 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3136, 3053, 2963, 2848, 1651, 1604, 1482, 1452, 1305, 1267, 1247, 1122.
mass:	*m/z* 456.1 $[M + H]^+$
HPLC purity:	95% (HPLC method **I**)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 10.37 (t, 1H, ^3J = 6.0, N-**H**), 8.77 (s, 1H, **H**-2), 7.87 (d, 1H, ^3J = 13.6, **H**-8), 7.41 – 7.32 (m, 2H, Bn-C**H**$_{aromat.}$), 7.22 – 7.16 (m, 2H, Bn-C**H**$_{aromat.}$), 7.10 (d, 1H, ^4J = 7.2, **H**-8), 4.58 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 4.47 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.80 – 3.78 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.26 – 3.24 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.76 (quint, 2H, ^3J = 7.6, NCH_2C**H$_2$**CH_2CH_3), 1.31 (sext, 2H, ^3J = 7.6, NCH_2CH_2C**H$_2$**CH_3), 0.92 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 174.1 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 164.2 (1C, **C**ON), 160.1 (d, 1C, $^1J_{C,F}$ = 242.9, Bn-**C**$_{aromat.}$), 152.4 (d, 1C, $^1J_{C,F}$ = 246.1, **C**-6), 147.8 (1C, **C**-2), 144.3 (d, 1C, $^2J_{C,F}$ = 10.3, **C**-7), 136.6 (1C, **C**-8a), 129.7 (d, 1C, $^3J_{C,F}$ = 4.5, Bn-**C**H$_{aromat.}$), 129.0 (d, 1C, $^3J_{C,F}$ = 8.0, Bn-**C**H$_{aromat.}$), 125.9 (d, 1C, $^2J_{C,F}$ = 14.9, Bn-**C**$_{aromat.}$), 124.4 (d, $^4J_{C,F}$ = 3.4), 121.4 (d, 1C, $^3J_{C,F}$ = 6.9, **C**-4a), 115.1 (d, 1C, $^2J_{C,F}$ = 21.0, Bn-**C**H$_{aromat.}$), 111.4 (d, 1C, $^2J_{C,F}$ = 22.4, **C**-5), 109.9 (1C, **C**-3), 105.5 (d, 1C, $^3J_{C,F}$ = 3.3, **C**-8), 65.9 (2C, morpholino-**C**H_2-O-**C**H_2), 52.8 (1C, N**C**$H_2CH_2CH_2CH_3$), 49.8 (d, 2C, $^4J_{C,F}$ = 4.4, morpholino-**C**H_2-N-**C**H_2), 36.1 (d, 1C, $^3J_{C,F}$ = 4.2, Bn-**C**H_2), 30.2 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.1 (1C, NCH_2CH_2**C**H_2CH_3), 13.4 (1C, $NCH_2CH_2CH_2$**C**H_3).

^{19}F NMR (^{1}H coupled, DMSO-d_6, δ [ppm], *J* [Hz]): -118.89 (m, *o*-**F**), -123.70 (dd, $^3J_{5,6}$ = 14.4, $^4J_{6,8}$ = 7.7, **F**-6).

1-Butyl-N-(2,4-difluorobenzyl)-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***26*** *(MB401)*



$C_{25}H_{26}F_3N_3O_3$

M_r = 473.50 g/mol

A solution of compound **4a** (200 mg, 0.57 mmol) in abs. DMF (7.5 mL) was treated with *N*MM (313 µL, 3.00 mmol), IBCF (297 µL, 2.28 mmol), and (2-fluorophenyl)methanamine (326 mg, 2.28 mmol) as depicted in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and recrystallised from EtOH to give **26**.

appearance:	white solid
yield:	80 mg (0.17 mmol/ 30%)
reaction control:	R_f = 0.41 ($CHCl_3$/MeOH = 100:1)
melting point [°C]:	160-161 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3194, 3031, 2959, 2871, 1659, 1625, 1601, 1485, 1449, 1256, 1210, 1102.
mass:	*m/z* 474.1 $[M + H]^+$
HPLC purity:	98% (HPLC method **I**)

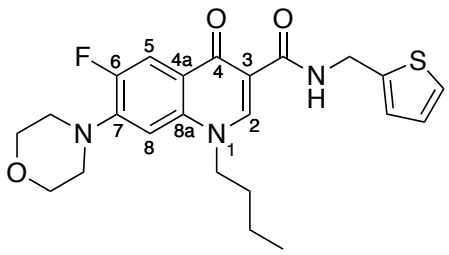
^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 10.36 (t, 1H, ^3J = 6.0, N-**H**), 8.76 (s, 1H, **H**-2), 7.88 (d, 1H, ^3J = 13.6, **H**-5), 7.46 – 7.40 (m, 1H, Bn-C**H**$_{aromat.}$), 7.26 – 7.21 (m, 1H, Bn-C**H**$_{aromat.}$), 7.10 (m, 2H, Bn-C**H**$_{aromat.}$/**H**-8), 4.56 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 4.46 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.80 – 3.78 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.26 – 3.24 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.76 (quint, 2H, ^3J = 7.6, NCH_2C**H$_2$**CH_2CH_3), 1.32 (sext, 2H, ^3J = 7.6, NCH_2CH_2C**H$_2$**CH_3), 0.91 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 174.0 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 164.2 (1C, **C**ON), 161.4 (dd, 1C, $^1J_{C,F}$ = 243.4, $^3J_{C,F}$ = 12.1, Bn-**C**$_{aromat.}$), 160.2 (dd, 1C, $^1J_{C,F}$ = 245.8, $^3J_{C,F}$ = 12.1, Bn-**C**$_{aromat.}$), 152.4 (d, 1C, $^1J_{C,F}$ = 246.1, **C**-6), 147.8 (1C, **C**-2), 144.3 (d, 1C, $^2J_{C,F}$ = 10.3, **C**-7), 136.6 (1C, **C**-8a), 130.8 (dd, 1C, $^3J_{C,F}$ = 9.8, $^3J_{C,F}$ = 6.8, Bn-**C**H$_{aromat.}$), 122.4 (dd, 1C, $^2J_{C,F}$ = 14.9, $^4J_{C,F}$ = 3.6, Bn-**C**H$_{aromat.}$), 124.4 (d, 1C, $^4J_{C,F}$ = 3.4, Bn-**C**H$_{aromat.}$), 121.4 (d, 1C, $^3J_{C,F}$ = 6.9, **C**-4a), 111.4 (d, 1C, $^2J_{C,F}$ = 22.4, **C**-5), 111.3 (dd, 1C, $^2J_{C,F}$ = 20.9,

$^4J_{C,F}$ = 3.5, Bn-**C**$_{aromat.}$), 109.9 (1C, **C**-3), 105.5 (d, 1C, $^3J_{C,F}$ = 3.3, **C**-8), 103.7 (t, 1C, $^2J_{C,F}$ = 25.6, Bn-**C**H$_{aromat.}$), 65.8 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 52.8 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 49.8 (d, 2C, $^4J_{C,F}$ = 4.4, morpholino-**C**H$_2$-N-**C**H$_2$), 36.1 (d, 1C, $^3J_{C,F}$ = 4.2, Bn-**C**H$_2$), 30.2 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.4 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

^{19}F NMR (^{1}H coupled, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): -111.89 (m, *p*-**F**), -114.45 (m, *o*-**F**), -123.72 (dd, $^3J_{5,6}$ = 14.4, $^4J_{6,8}$ = 7.7, **F**-6).

1-Butyl-6-fluoro-7-morpholino-4-oxo-N-(thiophen-2-ylmethyl)-1,4-dihydroquinoline-3-carboxamide, ***27*** *(MB271)*



$C_{23}H_{26}FN_3O_3S$

M_r = 443.54 g/mol

According to the general procedure 10.5, a solution of **4a** (240 mg, 0.69 mmol) in abs. DMF (10 mL) was treated with *N*MM (333 µL, 3.45 mmol), IBCF (359 µL, 2.76 mmol), and thiophen-2-ylmethanamine (284 µL, 2.76 mmol). The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH/FA = 100:5:1) and recrystallised from EtOAc to yield **27**.

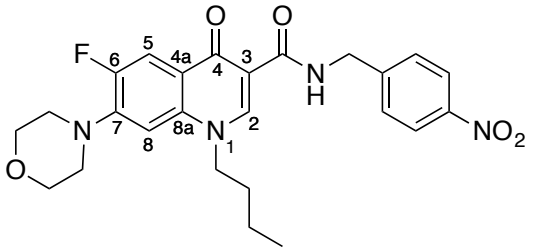
appearance:	white solid
yield:	130 mg (0.29 mmol/ 42%)
reaction control:	R_f = 0.63 ($CHCl_3$/MeOH = 100:5:1)
melting point [°C]:	175–176 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3128, 3049, 2962, 2934, 2864, 1655, 1624, 1501, 1442, 1305, 1250, 1208, 1110, 926
HPLC purity:	97% (HPLC method **II**)

^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 10.38 (t, 1H, ^3J = 5.6, N-**H**), 8.79 (s, 1H, **H**-2), 7.86 (d, 1H, ^3J = 13.2, **H**-5), 7.40 (dd, 1H, thiopheno-C**H**$_{aromat.}$), 7.10 (d, 1H, ^4J = 7.2, **H**-8), 7.05 – 7.00 (m, 1H, thiopheno-C**H**$_{aromat.}$), 6.97 – 6.92 (m, 1H, thiopheno-C**H**$_{aromat.}$), 4.70 (d, 2H, ^3J = 5.6, Bn-C**H$_2$**), 4.48 (t, 2H, ^3J = 7.2, N**CH$_2$**CH$_2$CH$_2$CH$_3$), 3.80 – 3.78 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.26 – 3.24 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.77 (quint, 2H, ^3J

= 7.6, $NCH_2C\mathbf{H}_2CH_2CH_3$), 1.32 (sext, 2H, ^3J = 7.2, $NCH_2CH_2C\mathbf{H}_2CH_3$), 0.92 (t, 3H, ^3J = 7.6, $NCH_2CH_2CH_2C\mathbf{H}_3$).

^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 173.9 (d, 1C, $^4J_{C,F}$ = 2.4, **C**-4), 163.9 (1C, **C**ON), 152.5 (d, 1C, $^1J_{C,F}$ = 245.4, **C**-6), 147.8 (1C, **C**-2), 144.3 (d, 1C, $^2J_{C,F}$ = 10.8, **C**-7), 142.2 (1C, thiopheno-$\mathbf{C}_{aromat.}$), 136.6 (1C, **C**-8a), 126.6 (1C, thiopheno-$\mathbf{C}H_{aromat.}$), 125.2 (1C, thiopheno-$\mathbf{C}H_{aromat.}$), 125.1 (1C, thiopheno-$\mathbf{C}H_{aromat.}$), 121.4 (d, 1C, $^3J_{C,F}$ = 7.0, **C**-4a), 111.3 (d, 1C, $^2J_{C,F}$ = 22.6, **C**-5), 109.7 (1C, **C**-3), 105.5 (d, 1C, $^4J_{C,F}$ = 3.0, **C**-8), 65.8 (2C, morpholino-$\mathbf{C}H_2$-O-$\mathbf{C}H_2$), 52.8 (1C, $N\mathbf{C}H_2CH_2CH_2CH_3$), 49.8 (d, 2C, $^4J_{C,F}$ = 4.4, morpholino-$\mathbf{C}H_2$-N-$\mathbf{C}H_2$), 36.9 (1C, thiopheno-$\mathbf{C}H_2$), 30.2 (1C, $NCH_2\mathbf{C}H_2CH_2CH_3$), 19.1 (1C, $NCH_2CH_2\mathbf{C}H_2CH_3$), 13.4 (1C, $NCH_2CH_2CH_2\mathbf{C}H_3$).

1-Butyl-6-fluoro-7-morpholino-N-(4-nitrobenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***28*** *(MB211)*



$C_{25}H_{27}FN_4O_5$

M_r = 482.51 g/mol

According to the general procedure 10.5, a solution of **4a** (200 mg, 0.57 mmol) in abs. DMF (7.5 mL) was treated with *N*MM (315 µL, 2.87 mmol), IBCF (297 µL, 2.30 mmol), and 4-nitrobenzylamine (349 mg, 2.30 mmol). The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 50:1) and recrystallised from EtOH to yield **28**.

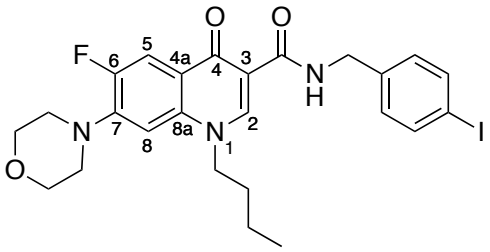
appearance:	yellowish solid
yield:	130 mg (0.47 mmol/ 47%)
reaction control:	R_f = 0.36 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	158–159 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3424, 3195, 3056, 2965, 2861, 1659, 1601, 1541, 1517, 1486, 1447, 1340, 1255, 1212, 1111, 930.

^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 10.50 (t, 1H, ^3J = 6.0, N-**H**), 8.78 (s, 1H, **H**-2), 8.20 (d, 2H, ^3J = 8.8, Bn-$\mathbf{C}H_{aromat.}$), 7.90 (d, 1H, ^3J = 13.6, **H**-5), 7.58 (d, 1H, ^3J = 6.0, Bn-$\mathbf{C}H_{aromat.}$), 4.69 (d, 2H, ^3J = 6.0, Bn-$\mathbf{C}H_2$), 4.48 (t, 2H, ^3J = 7.2, $N\mathbf{C}H_2CH_2CH_2CH_3$), 3.80 – 3.78 (m, 4H, morpholino-$\mathbf{C}H_2$-O-$\mathbf{C}H_2$), 3.27 – 3.25 (m, 4H, morpholino-$\mathbf{C}H_2$-N-$\mathbf{C}H_2$), 1.77

(quint, 2H, ^3J = 7.6, $NCH_2C\mathbf{H_2}CH_2CH_3$), 1.32 (sext, 2H, ^3J = 7.2, $NCH_2CH_2C\mathbf{H_2}CH_3$), 0.92 (t, 3H, ^3J = 7.6, $NCH_2CH_2CH_2C\mathbf{H_3}$).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 174.0 (d, 1C, $^4J_{C,F}$ = 2.4, **C**-4), 164.0 (1C, **C**ON), 152.5 (d, 1C, $^1J_{C,F}$ = 246.2, **C**-6), 147.8 (1C, Bn-**C**$_{aromat.}$), 147.7 (1C, **C**-2), 146.5 (1C, Bn-**C**$_{aromat.}$), 144.4 (d, 1C, $^2J_{C,F}$ = 10.3, **C**-7), 136.6 (1C, **C**-8a), 128.9 (2C, Bn-**C**H$_{aromat.}$), 123.5 (2C, Bn-**C**H$_{aromat.}$), 121.4 (d, 1C, $^3J_{C,F}$ = 6.8, **C**-4a), 111.3 (d, 1C, $^2J_{C,F}$ = 22.7, **C**-5), 109.8 (1C, **C**-3), 105.5 (d, 1C, $^4J_{C,F}$ = 3.2, **C**-8), 65.8 (2C, morpholino-**C**H_2-O-**C**H_2), 52.8 (1C, N**C**$H_2CH_2CH_2CH_3$), 49.8 (d, 2C, $^4J_{C,F}$ = 4.4, morpholino-**C**H_2-N-**C**H_2), 41.6 (1C, Bn-**C**H_2), 30.2 (1C, $NCH_2\mathbf{C}H_2CH_2CH_3$), 19.1 (1C, $NCH_2CH_2\mathbf{C}H_2CH_3$), 13.4 (1C, $NCH_2CH_2CH_2\mathbf{C}H_3$).

1-Butyl-6-fluoro-N-(4-iodobenzyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***29*** *(MB210)*



$C_{25}H_{27}FIN_3O_3$

M_r = 563.41 g/mol

According to general procedure 10.5, a solution of **4a** (310 mg, 0.89 mmol) in abs. DMF (15 mL) was treated with *N*MM (489 µL, 4.45 mmol), IBCF (460 µL, 3.56 mmol), and 4-iodobenzylamine (860 mg, 3.56 mmol). The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and recrystallised from EtOAc to yield **29**.

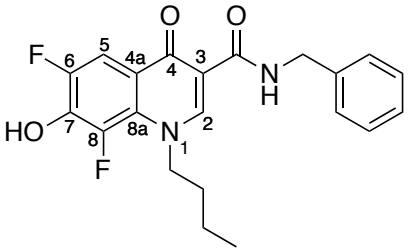
appearance:	white solid
yield:	270 mg (0.48 mmol/ 54%)
reaction control:	R_f = 0.39 ($CHCl_3$/MeOH = 100:1)
melting point [°C]:	185–186 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3038, 2951, 2854, 1652, 1600, 1535, 1483, 1440, 1301, 1253, 1001, 803.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 10.37 (t, 1H, ^3J = 6.0, N-**H**), 8.77 (s, 1H, **H**-2), 7.87 (d, 1H, ^3J = 13.2, **H**-5), 7.69 (d, 2H, ^3J = 8.0, Bn-C**H**$_{aromat.}$), 7.15 (d, 2H, ^3J = 8.0,

Bn-C**H**$_{aromat.}$), 7.09 (d, 1H, ^{4}J = 7.0, **H**-8), 4.50 – 4.46 (m, 4H, Bn-C**H$_2$**/NC**H$_2$**CH$_2$CH$_2$CH$_3$), 3.80 – 3.78 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.26 – 3.24 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.77 (quint, ^{3}J = 7.2, 2H, NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.32 (sext, 2H, ^{3}J = 7.2, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 0.92 (t, 3H, ^{3}J = 7.2, NCH$_2$CH$_2$CH$_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 174.0 (d, 1C, $^{4}J_{C,F}$ = 2.5, **C**-4), 164.0 (1C, **C**ON), 152.4 (d, 1C, $^{1}J_{C,F}$ = 247.0, **C**-6), 147.8 (1C, **C**-2), 144.4 (d, 1C, $^{2}J_{C,F}$ = 10.2, **C**-7), 139.4 (1C, Bn-**C**), 137.0 (2C, Bn-**C**H$_{aromat.}$), 136.6 (1C, **C**-8a), 129.7 (2C, Bn-**C**H$_{aromat.}$), 121.5 (d, 1C, $^{3}J_{C,F}$ = 6.9, **C**-4a), 111.5 (d, 1C, $^{2}J_{C,F}$ = 22.4, **C**-5), 109.9 (1C, **C**-3), 105.5 (d, 1C, $^{3}J_{C,F}$ = 3.3, **C**-8), 92.5 (1C, Bn-**C**$_{aromat.}$), 65.8 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 52.8 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 49.8 (d, 2C, $^{4}J_{C,F}$ = 4.4, morpholino-**C**H$_2$-N-**C**H$_2$), 41.5 (1C, Bn-**C**H$_2$), 30.2 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.4 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

N-Benzyl-1-butyl-6,8-difluoro-7-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***30*** *(MBPG25)*



$C_{21}H_{20}F_2N_2O_3$

M_r = 386.40 g/mol

Compound **19** (200 mg, 0.48 mmol) was dissolved in abs. CH_2Cl_2 (15 mL) under Ar atmosphere and the solution was cooled in an ice bath to 0 °C. A BBr_3 solution (1.0 M in CH_2Cl_2) (5 eq, 2.4 mL, 2.4 mmol) was added slowly to the solution. Afterwards, the reaction solution stirred for 6 d at rt. The reaction was quenched with 10 mL EtOH and the solvent was removed *in vacuo*. The crude product was purified by means of column chromatography (silica gel; $CHCl_3$/PE = 10:1).

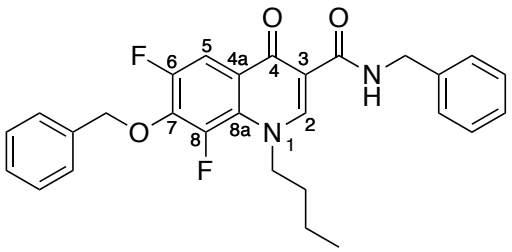
appearance:	white-grey solid
yield:	150 mg (0.38 mmol/ 79%)
reaction control:	R_f = 0.34 ($CHCl_3$/PE = 10:1)
melting point [°C]:	225–227 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3158, 2959, 2932, 2873, 2850, 1649, 1593, 1551, 1474, 1385, 1320, 1243, 1212, 883, 798.
HPLC purity:	96% (HPLC method **I**)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 11.60 (br, 1H, O-**H**), 10.21 (t, 1H, ^{3}J = 5.6, N-**H**), 8.74 (s, 1H, **H**-2), 7.84 (dd, 1H, ^{3}J = 10.8, ^{5}J = 1.6, **H**-5), 7.34 – 7.32 (m, 4H, Bn-C**H**$_{aromat.}$), 7.28 – 7.22 (m, 1H, Bn-C**H**$_{aromat.}$), 4.55 (d, 2H, ^{3}J = 5.6, Bn-**C**H$_2$), 4.48 – 4.44 (m, 2H, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 1.80 – 1.73 (m, 2H, NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.38 – 1.31 (m, 2H, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 0.91 (t, 3H, ^{3}J = 7.2, NCH$_2$CH$_2$CH$_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 173.9 (d, 1C, $^{4}J_{C,F}$ = 1.2, **C**-4), 164.2 (1C, **C**ON), 150.9 (dd, 1C, $^{1}J_{C,F}$ = 247.0, $^{3}J_{C,F}$ = 6.0, **C**-6), 150.5 (1C, **C**-2), 142.3 (dd, 1C, $^{1}J_{C,F}$ = 244.0, $^{3}J_{C,F}$ = 6.0, **C**-8), 139.8 (m, 1C, **C**-7), 139.8 (1C, Bn-**C**$_{aromat.}$), 128.9 (2C, Bn-**C**H$_{aromat.}$), 127.8 (2C, Bn-**C**H$_{aromat.}$), 127.0 (m, 1C, **C**-8a), 126.4 (1C, Bn-**C**H$_{aromat.}$), 120.0 (d, 1C, $^{3}J_{C,F}$ = 7.0, **C**-4a), 110.2 (1C, **C**-3), 107.4 (dd, 1C, $^{2}J_{C,F}$ = 19.0, $^{4}J_{C,F}$ = 2.0, **C**-5), 57.7 (d, 1C, $^{2}J_{C,F}$ = 14.0, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 42.7 (1C, Bn-**C**H$_2$), 32.6 (d, 1C, $^{5}J_{C,F}$ = 3.7, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.5 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.9 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

^{19}F NMR (^{1}H decoupled, DMSO-d_6, δ [ppm], J [Hz]): -132.87 (d, $^{4}J_{6,8}$ = 13.0, **F**-6), -142.49 (d, $^{4}J_{6,8}$ = 13.0, **F**-8).

N-Benzyl-7-(benzyloxy)-1-butyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***31*** *(MB349)*



$C_{28}H_{26}F_2N_2O_3$

M_r = 476.52 g/mol

Compound **30** (110 mg, 0.37 mmol) was dissolved in abs. DMF (20 mL) and was treated with K_2CO_3 (5 eq, 255 mg, 1.85 mmol), and benzyl chloride (5 eq, 213 μL, 1.85 mmol). The reaction solution stirred for 12 h at rt and the solvent was removed *in vacuo*. Water was added to the residue and the aqueous mixture was extracted with EtOAc (3x30 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo*. Finally, the product was recrystallised from EtOAc to yield **31**.

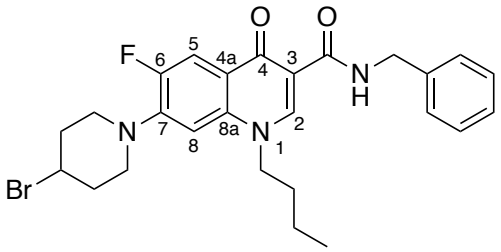
appearance:	white-grey solid
yield:	98 mg (0.21 mmol/ 56%)
reaction control:	R_f = 0.79 ($CHCl_3$/MeOH = 100:1)

melting point [°C]:	183–184 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3173, 3064, 3030, 2956, 2928, 2871, 1651, 1597, 1546, 1512, 1470, 1378, 1341, 1250, 1219, 1079.
mass:	*m/z* 477.1 $[M + H]^+$
HPLC purity:	99% (HPLC method **I**)

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 10.18 (t, 1H, ^3J = 5.6, N-**H**), 8.65 (s, 1H, **H**-2), 8.02 (dd, 1H, ^3J = 10.2, ^5J = 1.6, **H**-5), 7.44 – 7.32 (m, 8H, Bn-C**H**$_{aromat.}$), 7.30 – 7.23 (m, 2H, Bn-C**H**$_{aromat.}$), 5.32 (s, 2H, Bn-**C**H_2), 4.55 (d, 2H, ^3J = 5.6, Bn-**C**H_2), 4.33 – 4.29 (m, 2H, NC**H$_2$**$CH_2CH_2CH_3$), 1.76 – 1.73 (m, 2H, NCH_2C**H$_2$**CH_2CH_3), 1.40 – 1.34 (m, 2H, NCH_2CH_2C**H$_2$**CH_3), 0.96 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2C$**H$_3$**).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 174.3 (t, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 164.2 (1C, **C**ON), 153.4 (dd, 1C, $^1J_{C,F}$ = 247.0, $^3J_{C,F}$ = 3.6, **C**-6), 150.0 (1C, **C**-2), 146.2 (dd, 1C, $^1J_{C,F}$ = 250.3, $^3J_{C,F}$ = 5.1, **C**-8), 139.5 (t, 1C, $^3J_{C,F}$ = 15.0, **C**-7), 138.7 (1C, Bn-**C**$_{aromat.}$), 135.5 (1C, Bn-**C**$_{aromat.}$), 128.9 (2C, Bn-**C**H$_{aromat.}$), 128.6 (2C, Bn-**C**H$_{aromat.}$), 128.6 (2C, Bn-**C**H$_{aromat.}$, 128.5 (1C, Bn-**C**H$_{aromat.}$), 127.6 (2C, Bn-**C**H$_{aromat.}$), 127.1 (1C, Bn-**C**H$_{aromat.}$), 126.3 (dd, 1C, $^2J_{C,F}$ = 5.8, $^4J_{C,F}$ = 2.2, **C**-8a), 124.3 (d, 1C, $^3J_{C,F}$ = 7.0, **C**-4a), 111.0 (1C, **C**-3), 108.5 (dd, 1C, $^2J_{C,F}$ = 21.0, $^4J_{C,F}$ = 3.4, **C**-5), 76.5 (m, 1C, Bn-**C**H_2), 58.2 (d, 1C, $^2J_{C,F}$ = 14.0, N**C**$H_2CH_2CH_2CH_3$), 42.7 (1C, Bn-**C**H_2), 32.6 (d, 1C, $^5J_{C,F}$ = 3.7, NCH_2**C**$H_2CH_2CH_3$), 19.6 (1C, NCH_2CH_2**C**H_2CH_3), 13.5 (1C, $NCH_2CH_2CH_2$**C**H_3).

N-Benzyl-7-(4-bromopiperidin-1-yl)-1-butyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***32*** *(MB311)*



$C_{26}H_{29}BrFN_3O_2$

M_r = 514.44 g/mol

A solution of **22** (150 mg, 0.32 mmol) in CH_2Cl_2 (10 mL) was cooled to 0 °C and BBr_3 (1 M in CH_2Cl_2) (5 eq, 1.61 mL, 1.61 mmol) was slowly added to the reaction. The reaction solution stirred for 12 h at rt and was subsequently quenched with EtOH (5 mL). The solvent was removed under reduced pressure and the product was purified by column

chromatography (silica gel; $CHCl_3$/MeOH = 50:1). Finally, the product was recrystallised from EtOH to give **32**.

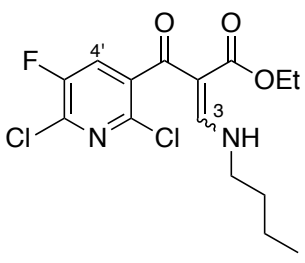
appearance:	white solid
yield:	30 mg (0.07 mmol/ 21%)
reaction control:	R_f = 0.73 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	171-172 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3171, 3053, 2956, 2931, 2872, 1654, 1625, 1600, 1536, 1483, 1451, 1257, 1209, 1180, 999, 803.
mass:	*m/z* 514.9 $[M + H]^+$

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 10.37 (t, 1H, ^3J = 5.6, N-**H**), 8.64 (s, 1H, **H**-2), 7.86 (d, 1H, ^3J = 13.2, **H**-5), 7.34 – 7.21 (m, 4H, Bn-C**H**$_{aromat.}$), 7.20 – 7.16 (m, 1H, Bn-C**H**$_{aromat.}$), 6.74 (d, 1H, ^3J = 7.2, **H**-8), 4.61 (d, 2H, ^3J = 5.6, Bn-C**H$_2$**), 3.46 – 3.41 (m, 2H, piperidinyl-C**H**), 4.12 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.46 – 3.41 (m, 2H, piperidinyl-C**H$_2$**-N-C**H$_2$**), 3.17 – 3.12 (m, 2H, piperidinyl-C**H$_2$**), 2.31 – 2.26 (m, 2H, piperidinyl-C**H$_2$**), 2.19 – 2.13 (m, 2H, piperidinyl-C**H$_2$**), 1.81 (quint, 2H, ^3J = 7.6, NCH_2C**H$_2$**CH_2CH_3), 1.32 (sext, 2H, ^3J = 7.6, NCH_2CH_2C**H$_2$**CH_3), 0.94 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 175.2 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 165.0 (1C, **C**ON), 152.4 (d, 1C, $^1J_{C,F}$ = 247.8, **C**-6), 147.1 (1C, **C**-2), 144.6 (d, 1C, $^2J_{C,F}$ = 11.8, **C**-7), 138.8 (1C, Bn-**C**$_{aromat.}$), 136.6 (1C, **C**-8a), 128.5 (2C, Bn-**C**H$_{aromat.}$), 127.6 (2C, Bn-**C**H$_{aromat.}$), 127.0 (1C, Bn-**C**H$_{aromat.}$), 122.6 (d, 1C, $^3J_{C,F}$ = 7.0, **C**-4a), 112.8 (d, 1C, $^2J_{C,F}$ = 22.6, **C**-5), 111.1 (1C, **C**-3), 104.1 (1C, **C**-8), 54.0 (1C, O**C**H_3), 52.7 (1C, N**C**$H_2CH_2CH_2CH_3$), 48.7 (1C, piperidinyl-**C**H), 48.2 (d, 2C, $^4J_{C,F}$ = 4.7, piperidinyl-**C**H_2-N-**C**H_2), 43.2 (1C, Bn-**C**H_2), 30.2 (1C, NCH_2**C**$H_2CH_2CH_3$), 30.7 (2C, piperidinyl-**C**H_2-CH-**C**H_2), 19.9 (1C, NCH_2CH_2**C**H_2CH_3), 13.6 (1C, $NCH_2CH_2CH_2$**C**H_3).

10.6 Grohe-Heitzer approach[37]

Ethyl 3-(butylamino)-2-(2,6-dichloro-5-fluoronicotinoyl)acrylate, ***33*** *(MB280)*



$C_{15}H_{17}Cl_2FN_2O_3$

M_r = 363.21 g/mol

Ethyl 3-(2,6-dichloro-5-fluoropyridin-3-yl)-3-oxopropanoate (3.0 g, 10.6 mmol) was dissolved in triethyl orthoacetate (2.6 mL, 15.9 mmol) and acetic anhydride (2.5 mL, 26.5 mmol), and heated at 120 °C for 4 h. The volatile residues were removed *in vacuo* and the oily intermediate ethyl 2-(2,6-dichloro-5-fluoronicotinoyl)-3-ethoxyacrylate was dissolved in CH_2Cl_2 (30 mL), and treated with *n*-butylamine (1.54 mL, 15.9 mmol). The solution was stirred for 3 d at rt and the solvent was subsequently removed under reduced pressure. The oily product was crystallised from EtOH/PE (5:1) at -20 °C to yield in compound **33**.

appearance:	yellow solid	
yield:	1.4 g (3.9 mmol/ 36%)	
reaction control:	intermediate	R_f = 0.67 (EtOAc/PE = 1:2)
	product	R_f = 0.72 (EtOAc/PE = 1:2)
melting point [°C]:	86-88 °C	
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3210, 3041, 2960, 2930, 2875, 2360, 1673, 1622, 1583, 1553, 1436, 1392, 1318, 1269, 1269, 1137, 1220, 1047.	
isomeric ratio:	1:5 (E/Z)	

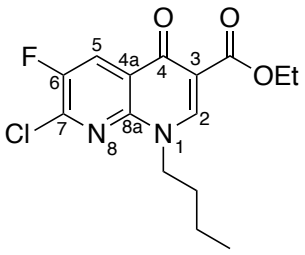
Z-Isomer

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 11.00 (br, 1H, N-**H**), 8.16 (d, 1H, ^3J= 14.4, **H**-3), 7.34 (d, 1H, ^3J = 7.2, **H**-4), 4.02 (q, 2H, ^3J = 7.2, O**CH_2**CH_3), 3.47 (m, 2H, N**CH_2**$CH_2CH_2CH_3$), 1.72 – 1.66 (m, 2H, NCH_2**CH_2**CH_2CH_3), 1.47 – 1.41 (m, 2H, NCH_2CH_2**CH_2**CH_3), 1.06 – 1.00 (m, 6H, OCH_2**CH_3**/$NCH_2CH_2CH_2$**CH_3**).

E-Isomer

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 9.69 (br, 1H, N-**H**), 8.25 (d, 1H, ^3J= 14.8, **H**-3), 7.38 (d, 1H, ^3J = 7.2, H-4), 3.98 (q, 2H, ^3J = 7.2, O**CH_2**CH_3), 2.82 (t, 2H, ^3J =7.2, N**CH_2**$CH_2CH_2CH_3$), 1.63 – 1.50 (m, 2H, NCH_2**CH_2**CH_2CH_3), 1.39 – 1.37 (m, 2H, NCH_2CH_2**CH_2**CH_3), 0.95 – 0.89 (m, 6H, OCH_2**CH_3**/$NCH_2CH_2CH_2$**CH_3**).

Ethyl 1-butyl-7-chloro-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate, ***34*** *(MB289)*



$C_{15}H_{16}ClFN_2O_3$

M_r = 326.75 g/mol

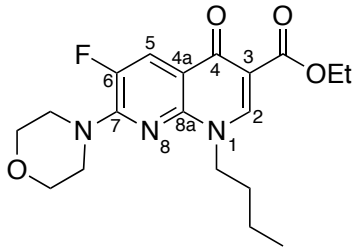
Compound **33** (5.0 g, 14.9 mmol) was dissolved in ACN (50 mL) and was treated with K_2CO_3 (2.5 g, 17.9 mmol). The suspension heated at 100 °C for 3 h and finally the solvent was removed under reduced pressure. The residue was treated with water (30 mL) and the aqueous layer was extracted with EtOAc (3x30 mL). The combined organic layers were dried over Na_2SO_4; the solvent was removed *in vacuo* and gave compound **34**.

appearance:	yellow solid
yield:	3.1 g (9.6 mmol/ 64%)
reaction control:	R_f = 0.66 ($CHCl_3$/MeOH = 20:1)
melting point [°C]:	129-130 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3073, 3038, 2964, 1689, 1627, 1594, 1488, 1255, 1218, 1179, 1131, 1043, 943.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 8.60 (s, 1H, **H**-2), 8.48 (d, 1H, ^3J = 13.2, **H**-5), 4.46 – 4.37 (m, 4H, NC**H$_2$**$CH_2CH_2CH_3$/OC**H$_2$**CH_3), 1.89 (quint, 2H, ^3J = 7.6, NCH_2C**H$_2$**CH_2CH_3), 1.46 – 1.40 (m, 5H, NCH_2CH_2C**H$_2$**CH_3/OCH_2C**H$_3$**), 1.02 (t, 3H, ^3J = 7.6, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100, $CDCl_3$, δ [ppm], *J* [Hz]): 172.3 (d, 1C, $^4J_{C,F}$ = 1.0, **C**-4), 164.9 (1C, **C**OOEt), 152.4 (d, 1C, $^1J_{C,F}$ = 259.2, **C**-6), 149.2 (1C, **C**-2), 144.4 (d, 1C, $^4J_{C,F}$ = 2.2, **C**-8a), 142.4 (d, 1C, $^2J_{C,F}$ = 22.4, **C**-7), 124.2 (d, 1C, $^3J_{C,F}$ = 2.7, **C**-4a), 123.3 (d, 1C, $^2J_{C,F}$ = 20.3, **C**-5), 112.3 (1C, **C**-3), 61.3 (1C, O**C**H_2CH_3), 51.9 (1C, N**C**$H_2CH_2CH_2CH_3$), 31.5 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.8 (1C, NCH_2**C**$H_2CH_2CH_3$), 14.7 (1C, OCH_2**C**H_3), 13.5 (1C, $NCH_2CH_2CH_2$**C**H_3).

Ethyl 1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate, ***35*** *(MBPG11)*



$C_{19}H_{24}FN_3O_4$

M_r = 377.42 g/mol

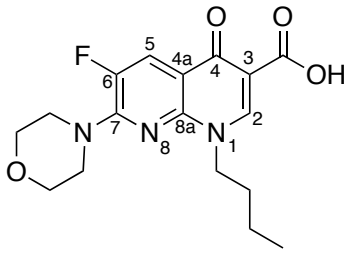
Compound **34** (600 mg, 1.84 mmol) was dissolved in abs. DMF (20 mL) and was treated with morpholine (800 µL, 9.18 mmol) and DIPEA (1.6 mL, 9.18 mmol). The solution heated at 100 °C for 24 h. Afterwards the solvent was removed *in vacuo* and the oily residue was suspended in $CHCl_3$. The organic layer was extracted with water (3x50 mL), dried over Na_2SO_4, and finally removed under reduced pressure. The subsequent column chromatography (silica gel; $CHCl_3$/MeOH/FA = 100:5:1) yielded in the oily product, which was crystallised overnight at rt.

appearance:	yellow solid
yield:	650 mg (1.72 mmol/ 93%)
reaction control:	R_f = 0.60 ($CHCl_3$/MeOH/FA = 100:5:1)
melting point [°C]:	89-91 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3039, 2968, 2937, 2862, 1671, 1620, 1459, 1437, 1307, 1259, 1232, 1213, 1102, 914, 804.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 8.42 (s, 1H, **H**-2), 8.13 (d, 1H, ^3J = 13.6, **H**-5), 4.36 (q, 4H, ^3J = 7.2, OC**H$_2$**CH_3), 4.24 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.85 – 3.82 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.75 – 3.73 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.81 (quint, 2H, ^3J = 7.6, NCH_2C**H$_2$**CH_2CH_3), 1.41 – 1.38 (m, 5H, NCH_2CH_2C**H$_2$**CH_3/OCH_2C**H$_3$**), 0.96 (t, 3H, ^3J = 7.6, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100, $CDCl_3$, δ [ppm], *J* [Hz]): 173.5 (d, 1C, $^4J_{C,F}$ = 2.6, **C**-4), 165.7 (1C, **C**OOEt), 149.8 (d, 1C, $^2J_{C,F}$ = 9.8, **C**-7), 147.8 (1C, **C**-2), 146.3 (d, 1C, $^1J_{C,F}$ = 252.5, **C**-6), 144.4 (d, 1C, $^4J_{C,F}$ = 0.6, **C**-8a), 119.4 (d, 1C, $^2J_{C,F}$ = 21.4, **C**-5), 117.0 (d, 1C, $^3J_{C,F}$ = 3.3, **C**-4a), 111.6 (1C, **C**-3), 66.6 (2C, morpholino-C**H$_2$**-O-C**H$_2$**), 60.9 (1C, O**C**H_2CH_3), 51.6 (1C, N**C**$H_2CH_2CH_2CH_3$), 47.4 (d, 2C, $^4J_{C,F}$ = 7.5, morpholino-C**H$_2$**-N-C**H$_2$**), 31.5 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.9 (1C, NCH_2**C**$H_2CH_2CH_3$), 14.4 (1C, OCH_2**C**H_3), 13.6 (1C, $NCH_2CH_2CH_2$**C**H_3).

1-Butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid, ***36*** *(MBPG17)*



$C_{17}H_{20}FN_3O_4$

M_r = 349.36 g/mol

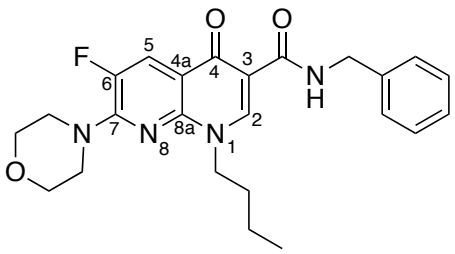
Compound **35** (650 mg, 1.72 mmol) was dissolved in HCl (3 M, 100 mL) and refluxed for 6 h. Afterwards the solution was treated with cold water (20 ml) and stored at 0 °C for 1 h. The white precipitate was collected, washed with water, and subsequently dried *in vacuo* over phosphorus pentoxide.

appearance:	white solid
yield:	380 mg (1.09 mmol/ 63%)
reaction control:	R_f = 0.25 ($CHCl_3$/MeOH/FA = 50:1:1)
melting point [°C]:	215 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3046, 2959, 2846, 2675, 1718, 1626, 1545, 1515, 1470, 1442, 1119, 949.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 15.3 (br, 1H, COO**H**), 8.42 (s, 1H, **H**-2), 8.13 (d, 1H, ^3J = 13.6, **H**-5), 4.36 (q, 4H, ^3J = 7.2, OC**H$_2$**CH_3), 4.24 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.85 – 3.82 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.75 – 3.73 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.81 (quint, 2H, ^3J = 7.6, NCH_2C**H$_2$**CH_2CH_3), 1.41 – 1.38 (m, 5H, NCH_2CH_2C**H$_2$**CH_3/OCH_2C**H$_3$**), 0.96 (t, 3H, ^3J = 7.6, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100, DMSO-d_6, δ [ppm], J [Hz]): 173.5 (d, 1C, $^4J_{C,F}$ = 2.6, **C**-4), 165.7 (1C, **C**OOEt), 149.8 (d, 1C, $^2J_{C,F}$ = 9.8, **C**-7), 147.8 (1C, **C**-2), 146.3 (d, 1C, $^1J_{C,F}$ = 252.5, **C**-6), 144.4 (d, 1C, $^4J_{C,F}$ = 0.6, **C**-8a), 119.4 (d, 1C, $^2J_{C,F}$ = 21.4, **C**-5), 117.0 (d, 1C, $^3J_{C,F}$ = 3.3, **C**-4a), 111.6 (1C, **C**-3), 66.6 (2C, morpholino-C**H$_2$**-O-C**H$_2$**), 60.9 (1C, O**C**H_2CH_3), 51.6 (1C, N**C**$H_2CH_2CH_2CH_3$), 47.4 (d, 2C, $^4J_{C,F}$ = 7.5, morpholino-C**H$_2$**-N-C**H$_2$**), 31.5 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.9 (1C, NCH_2**C**$H_2CH_2CH_3$), 14.4 (1C, OCH_2**C**H_3), 13.6 (1C, $NCH_2CH_2CH_2$**C**H_3).

N-Benzyl-1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide, ***37*** *(MBPG20)*



$C_{24}H_{27}FN_4O_3$

M_r = 438.50 g/mol

A solution of compound **36** (380 mg, 1.09 mmol) in abs. DMF (15 mL) was treated with *N*MM (598 µL, 5.45 mmol), IBCF (567 µL, 4.36 mmol), and benzylamine (476 µL, 4.36 mmol) as depicted in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1) and recrystallised from EtOAc to give **37**.

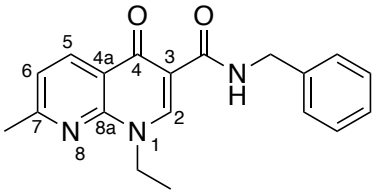
appearance:	white solid
yield:	430 mg (0.98 mmol/ 90%)
reaction control:	R_f = 0.21 ($CHCl_3$/MeOH = 100:1)
melting point [°C]:	178-179 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3157, 3033, 2957, 2927, 2870, 1655, 1618, 1598, 1537, 1461, 1433, 1356, 1300, 1253, 1121, 1109, 932, 804.
HPLC purity:	99% (HPLC method **II**)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 10.31 (t, 1H, ^3J = 5.6, N-**H**), 8.79 (s, 1H, **H**-2), 8.00 (d, 1H, ^3J = 13.6, **H**-5), 7.34 – 7.33 (m, 4H, Bn-C**H**$_{aromat.}$), 7.28 – 7.24 (m, 1H, Bn-C**H**$_{aromat.}$), 4.54 (d, 2H, ^3J = 5.6, Bn-C**H$_2$**), 4.39 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.74 (br, 8H, morpholino-C**H$_2$**-O-C**H$_2$**/morpholino-C**H$_2$**-N-C**H$_2$**), 1.74 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.32 (sext, 2H, ^3J = 7.2, NCH_2CH_2C**H$_2$**CH_3), 0.90 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 175.0 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 164.3 (1C, **C**ON), 150.0 (d, 1C, $^2J_{C,F}$ = 8.4, **C**-7), 147.3 (1C, **C**-2), 146.1 (d, 1C, $^1J_{C,F}$ = 257.0, **C**-6), 144.8 (1C, **C**-8a), 139.8 (1C, Bn-**C**$_{aromat.}$), 128.9 (2C, Bn-**C**H$_{aromat.}$), 127.8 (2C, Bn-**C**H$_{aromat.}$), 127.4 (1C, Bn-**C**H$_{aromat.}$), 120.5 (d, 1C, $^2J_{C,F}$ = 21.4, **C**-5), 114.9 (d, 1C, $^3J_{C,F}$ = 2.8, **C**-4a), 111.8 (1C, **C**-3), 66.4 (2C, morpholino-**C**H_2-O-**C**H_2), 52.8 (1C, N**C**$H_2CH_2CH_2CH_3$), 47.5 (d, 2C, $^4J_{C,F}$ = 7.5, morpholino-**C**H_2-N-**C**H_2), 42.6 (1C, Bn-**C**H_2), 31.4 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.8 (1C, NCH_2CH_2**C**H_2CH_3), 14.0 (1C, $NCH_2CH_2CH_2$**C**H_3).

10.7 Synthesis of Quinolone Amides Derived from Commercially Available Quinolones

N-Benzyl-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide, ***38*** *(MB263)*



$C_{19}H_{19}N_3O_2$

M_r = 321.38 g/mol

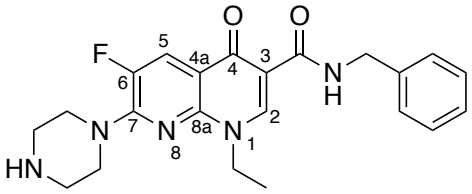
A solution of nalidixic acid (250 mg, 1.1 mmol) in abs. DMF (10 mL) was treated with *N*MM (592 µL, 5.38 mmol), IBCF (560 µL, 4.30 mmol), and benzylamine (470 µL, 4.30 mmol) as described in the general procedure 10.5. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 20:1) and recrystallised from EtOAc to produce **38**.

appearance:	white solid
yield:	210 mg (0.65 mmol/ 61%)
reaction control:	R_f = 0.62 ($CHCl_3$/MeOH = 20:1)
melting point [°C]:	205-206 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3120, 3031, 2929, 1655, 1603, 1532, 1492, 1431, 1349.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 10.40 (t, 1H, ^3J = 6.0, N-**H**), 8.94 (s, 1H, **H**-2), 8.53 (d, 1H, ^3J = 8.0, **H**-5), 7.37 (d, 1H, ^3J = 8.4, **H**-6), 7.30 – 7.29 (m, 4H, Bn-C**H**$_{aromat.}$), 7.23 – 7.21 (m, H, Bn-C**H**$_{aromat.}$), 4.59 – 4.53 (m, 4H, NC**H$_2$**CH_3/ Bn-C**H$_2$**), 2.64 (s, 3H, C**H$_3$**), 1.41 (t, 3H, ^3J = 7.2, NCH_2C**H$_3$**).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 176.5 (1C, **C**-4), 164.3 (1C, **C**ON), 163.5 (1C, **C**-7), 148.6 (1C, **C**-8a), 148.1 (1C, **C**-2), 139.5 (1C, Bn-**C**$_{aromat.}$), 139.1 (1C, **C**-5), 128.7 (2C, Bn-**C**H$_{aromat.}$), 127.6 (2C, Bn-**C**H$_{aromat.}$), 127.2 (1C, Bn-**C**H$_{aromat.}$), 121.5 (1C, **C**-6), 120.1 (1C, **C**-4a), 112.4 (1C, **C**-3), 52.9 (1C, N**C**$H_2CH_2CH_2CH_3$), 46.5 (1C, **C**H_3), 42.5 (1C, Bn-**C**H_2), 25.0 (1C, N**C**H_2CH_3), 15.1 (1C, NCH_2**C**H_3).

N-Benzyl-1-ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydro-1,8-naphthyridine-3-carboxamide, ***39*** *(MB297)*



$C_{22}H_{24}FN_5O_2$

M_r = 409.47 g/mol

Enoxacin (0.5 g, 1.56 mmol) was dissolved in abs. DMF (10 mL) and was treated with TEA (3 eq, 648 µL, 4.7 mmol), and Boc_2O (2 eq, 0.7 g, 3.1 mmol). The reaction mixture was heated at 100 °C for 4 h. The solvent was evaporated and water (20 mL) was added to the residue. The aqueous layer was extracted with CH_2Cl_2 (3x30 mL), the combined organic layers were dried over Na_2SO_4, and the solvent was removed *in vacuo.* The resulted white solid (640 mg, 1.52 mmol) was dissolved in abs. DMF (7.5 mL) treated with *N*MM (840 µL, 7.60 mmol), IBCF (794 µL, 6.08 mmol), and benzylamine (664 µL, 6.08 mmol) as depicted in the general procedure 10.5. After column chromatography (silica gel; $CHCl_3$/EtOAc = 20:1), the white solid was dissolved in CH_2Cl_2 and TFA (4 mL) added to the reaction mixture. The solution was stirred for 12 h at rt and the reaction quenched by a saturated Na_2CO_3 solution (20 mL). The water layer was separated and extracted with CH_2Cl_2 (3x30 mL). The organic layers were dried over Na_2SO_4 and the solvent removed *in vacuo*. The crude product was recrystallised from EtOAc to give **39**.

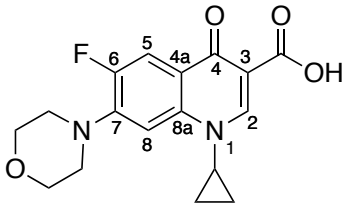
appearance:	white solid
yield:	170 mg (0.42 mmol/ 27%)
reaction control:	R_f = 0.55 ($CHCl_3$/EtOAc = 20:1)
melting point [°C]:	185-186 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3117, 3048, 2928, 1649, 1621, 1599, 1549, 1351, 1126.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 10.34 (t, 1H, ^3J = 6.0, N-**H**), 8.81 (s, 1H, **H**-2), 7.96 (d, 1H, ^3J = 14.0, **H**-5), 7.34 – 7.32 (m, 4H, Bn-C**H**$_{aromat.}$), 7.27 – 7.24 (m, H, Bn-C**H**$_{aromat.}$), 4.54 (d, 2H, ^3J = 7.2, Bn-C**H$_2$**), 4.41 (q, 2H, ^3J = 7.2, NC**H$_2$**CH_3), 3.70 – 3.67 (m, 4H, piperazino-C**H$_2$**-N-C**H$_2$**), 2.85 – 2.83 (m, 4H, piperazino-C**H$_2$**-NH-C**H$_2$**), 1.36 (t, 3H, ^3J = 7.6, NCH_2C**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 174.4 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 163.8 (1C, **C**ON), 149.5 (d, 1C, $^2J_{C,F}$ = 8.8, **C**-7), 146.3 (1C, **C**-2), 146.2 (d, 1C, $^1J_{C,F}$ = 255.4, **C**-6), 144.2 (1C, **C**-8a), 139.3 (1C, Bn-**C**$_{aromat.}$), 128.3 (2C, Bn-**C**H$_{aromat.}$), 127.2 (2C, Bn-**C**H$_{aromat.}$),

126.8 (C, Bn-**C**H$_{aromat.}$), 119.7 (d, 1C, $^{2}J_{C,F}$ = 21.4, **C**-5), 113.9 (d, 1C, $^{3}J_{C,F}$ = 3.1, **C**-4a), 111.3 (1C, **C**-3), 48.0 (2C, $^{3}J_{C,F}$ = 7.4, piperazino-**C**H$_2$-N-**C**H$_2$), 46.4 (1C, N**C**H$_2$CH$_3$), 45.4 (d, 2C, piperazino-**C**H$_2$-NH-**C**H$_2$), 41.9 (1C, Bn-**C**H$_2$), 14.6 (1C, NCH$_2$**C**H$_3$).

1-Cyclopropyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***40*** *(MB284)*



$C_{17}H_{17}FN_2O_4$

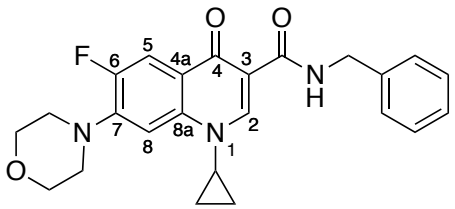
M_r = 332.33 g/mol

According to the general procedure 10.4, 7-chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2.0 g, 7.1 mmol) was dissolved in morpholine (10 mL) and heated for 6 h under microwave irradiation at 110 °C. After acidification (pH 2) the solution and drying the precipitate *in vacuo*, the solid was recrystallised from EtOH to yield **40**.

appearance:	yellow solid
yield:	2.21 g (6.65 mmol/ 94%; Lit[126]: 87%)
reaction control:	R_f = 0.40 ($CHCl_3$/MeOH/FA = 100:5:1)
melting point [°C]:	292-293 °C [126]: 293-295 °C]
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2985, 1689, 1650, 1621, 1607, 1507, 1473, 1425, 1347, 1241, 1168, 1924.

The obtained physical and spectroscopic data are consistent with that found in literature.[126]

N-Benzyl-1-cyclopropyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***41*** *(MB288)*



$C_{24}H_{24}FN_3O_3$

M_r = 421.47 g/mol

According to the general procedure 10.5, a solution of **40** (500 mg, 1.50 mmol) in abs. DMF (10 mL) was treated with *N*MM (835 µL, 7.52 mmol), IBCF (783 µL, 6.02 mmol), and benzylamine (659 µL, 6.02 mmol). The crude product was purified by column chromatography (silica gel; $CHCl_3$/*i*PrOH = 50:1) and recrystallised from toluene/EtOAc (2:1) to yield **41**.

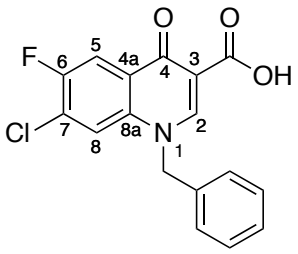
appearance:	white solid
yield:	270 mg (0.48 mmol/ 54%)
reaction control:	R_f = 0.42 ($CHCl_3$/*i*PrOH = 50:1)
melting point [°C]:	161–162 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3260, 3072, 2965, 2833, 1660, 1625, 1608, 1536, 1478, 1448, 1428, 1340, 1168.
HPLC purity:	97% (HPLC method II)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 10.30 (t, 1H, ^3J = 6.0, N-**H**), 8.66 (s, 1H, **H**-2), 7.85 (d, 1H, ^3J = 13.6, **H**-5), 7.49 (d, 1H, ^4J = 7.6, **H**-8), 7.51 – 7.31 (m, 4H, Bn-C$\mathbf{H}_{aromat.}$), 7.27 – 7.24 (m, 1H, Bn-C$\mathbf{H}_{aromat.}$), 4.54 (d, 2H, ^3J = 6.0, Bn-C$\mathbf{H_2}$), 3.82 – 3.80 (m, 4H, morpholino-C$\mathbf{H_2}$-O-C$\mathbf{H_2}$), 3.75 – 3.73 (m, 1H, cyclopropylo-**H**), 3.33 – 3.25 (m, 4H, morpholino-C$\mathbf{H_2}$-N-C$\mathbf{H_2}$), 1.31 – 1.29 (m, 2H, cyclopropylo-**H**), 1.18 – 1.10 (m, 2H, cyclopropylo-**H**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 174.2 (d, 1C, $^4J_{C,F}$ = 2.2, **C**-4), 163.9 (1C, **C**ON), 154.1 (d, 1C, $^1J_{C,F}$ = 249.0, **C**-6), 146.8 (1C, **C**-2), 144.1 (d, 1C, $^2J_{C,F}$ = 10.6, **C**-7), 139.3 (1C, Bn-**C**), 138.2 (1C, **C**-8a), 128.0 (2C, Bn-**C**H$_{aromat.}$), 127.2 (2C, Bn-**C**H$_{aromat.}$), 120.7 (d, 1C, $^3J_{C,F}$ = 6.9, **C**-4a), 111.1 (d, 1C, $^2J_{C,F}$ = 22.5, **C**-5), 109.9 (1C, **C**-3), 105.8 (d, 1C, $^3J_{C,F}$ = 2.4, **C**-8), 65.7 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 49.7 (d, 2C, $^4J_{C,F}$ = 4.4, morpholino-**C**H$_2$-N-**C**H$_2$), 42.0 (1C, Bn-**C**H$_2$), 34.9 (1C, cyclopropylo-**C**), 7.4 (2C, cyclopropylo-**C**).

10.8 Synthesis Approach for the ^{18}F Labelling

1-Benzyl-7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***42a*** *(MB222)*



$C_{17}H_{11}ClFNO_3$

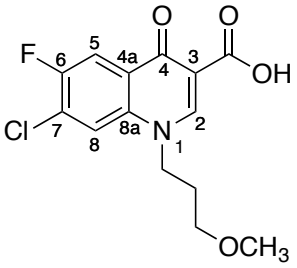
M_r = 331.73 g/mol

The starting compound ethyl 1-benzyl-7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate‡ (0.50 g, 1.39 mmol) was hydrolysed by refluxing the reaction solution in basic conditions (3 M KOH). After acidification with 2 M HCl under ice cooling, the precipitates were collected and dried *in vacuo*.

appearance:	yellowish solid
yield:	3.1 g (9.4 mmol/ 55%)
reaction control:	R_f = 0.47 ($CHCl_3$/MeOH/FA= 100:5:1)
melting point [°C]:	242-244 °C [127]: 252-253 °C, DMF]
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3045, 1707, 1614, 1567, 1544, 1465, 1376, 1266, 1215, 1026, 763.

The obtained physical and spectroscopic data are consistent with that found in literature.[127]

7-Chloro-6-fluoro-1-(3-methoxypropy)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***43a*** *(MB122)*



$C_{14}H_{13}ClFNO_4$

M_r = 313.71 g/mol

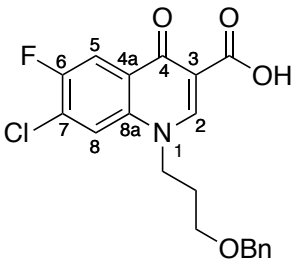
‡ This compound was synthesized and kindly provided by R. Messerer

A solution of compound **1a** (1 eq, 1.4 g, 5.2 mmol) and K_2CO_3 (4 eq, 2.9 g, 20.8 mmol) in abs. DMF (20 mL) was treated with 1-chloro-3-methoxypropane (5 eq, 2.8 mL, 25.9 mmol), and the reaction solution heated at 80 °C for 48 h. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 50:1) and then hydrolysed by refluxing under basic conditions (3 M KOH). After acidification with 2 M HCl under ice cooling, the precipitates were collected and dried *in vacuo* to yield **43a**.

appearance:	beige solid
yield:	800 mg (2.55 mmol/ 49%)
reaction control:	R_f = 0.31 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	226-227 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3040, 2936, 1718, 1609, 1479, 1384, 1151, 896.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 14.73 (s, 1H, COO**H**), 8.98 (s, 1H, **H**-2), 8.39 (d, 1H, ^4J = 6.4, **H**-8), 8.20 (d, 1H, ^3J = 8.8, **H**-5), 4.62 (t, 2H, ^3J = 6.8, NC**H$_2$**$CH_2CH_2OCH_3$), 3.34 (t, 2H, ^3J = 5.6, NCH_2CH_2C**H$_2$**OCH_3), 3.19 (s, 3H, $NCH_2CH_2CH_2OC$**H$_3$**), 2.04 (quint, 2H, ^3J = 6.0, NCH_2C**H$_2$**CH_2OCH_3).
^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 176.7 (d, 1C, $^4J_{C,F}$ = 3.1, **C**-4), 165.6 (1C, **C**OOH), 154.4 (d, $^1J_{C,F}$ = 247.8, **C**-6), 150.3 (1C, **C**-2), 136.4 (d, 1C, $^4J_{C,F}$ = 4.1, **C**-8a), 127.2 (d, 1C, $^2J_{C,F}$ = 21.4, **C**-7), 125.9 (d, 1C, $^3J_{C,F}$ = 6.4, **C**-4a), 121.0 (1C, **C**-8), 111.8 (d, 1C, $^2J_{C,F}$ = 23.2, **C**-5), 107.5 (1C, **C**-3) 68.2 (1C, NCH_2CH_2**C**H_2OCH_3), 57.8 (1C, $NCH_2CH_2CH_2O$**C**H_3), 51.4 (1C, N**C**$H_2CH_2CH_2OCH_3$), 28.2 (1C, NCH_2**C**$H_2CH_2OCH_3$).

1-(3-(Benzyloxy)propyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***44a*** *(MB298)*



$C_{20}H_{17}ClFNO_4$
M_r = 389.81 g/mol

According to the general procedure 10.3, a solution of compound **1a** (1 eq, 1.0 g, 4.1 mmol) and K_2CO_3 (4 eq, 2.3 g, 16.6 mmol) in abs. DMF (20 mL) was treated with ((3-

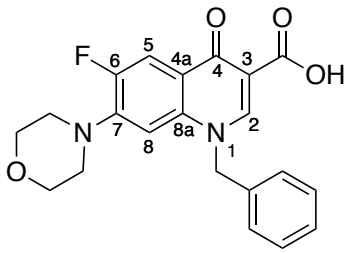
bromopropoxy)methyl)benzene (1.5 eq, 1.1 mL, 6.2 mmol), and the reaction solution heated at 85 °C for 48 h. The crude product was purified by column chromatography (silica gel; $CHCl_3$/EtOAc = 20:1) and then hydrolysed by refluxing under basic conditions (3 M KOH). After acidification with 2 M HCl under ice cooling, the precipitates were collected and dried *in vacuo* to yield **44a**.

appearance:	beige solid
yield:	1.1 g (2.7 mmol/ 66%)
reaction control:	R_f = 0.40 ($CHCl_3$/EtOAc = 20:1)
melting point [°C]:	179-180 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3046, 2864, 1715, 1613, 1557, 1508, 894.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 9.01 (s, 1H, **H**-2), 8.40 (d, 2H, ^4J = 6.0, **H**-8), 8.17 (d, 1H, ^3J = 9.2, **H**-5), 7.31–7.23 (m, 5H, C**H**$_{aromat.}$), 4.66 (t, 2H, ^3J = 6.0, NC**H$_2$**CH_2CH_2OBn), 4.39 (s, 2H, Bn-C**H$_2$**), 3.49 (t, 2H, ^3J = 6.0, NCH_2CH_2C**H$_2$**OBn), 2.08 (quint, 2H,^3J = 6.0, NCH_2C**H$_2$**CH_2OBn).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 176.4 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 165.4 (1C, **C**OOH), 155.6 (d, 1C, $^1J_{C,F}$ = 247.8, **C**-6), 150.3 (1C, **C**-2), 137.9 (1C, Bn-**C**$_{quart.}$), 136.4 (d, 1C, $^4J_{C,F}$ = 1.7, **C**-8a), 128.1 (2C, Bn-**C**$_{aromat.}$), 127.4 (2C, Bn-**C**$_{aromat.}$), 127.3 (1C, Bn-**C**$_{aromat.}$) 127.1 (1C, **C**-4a), 125.9 (d, 1C, $^2J_{C,F}$ = 6.5, **C**-7), 121.0 (1C, **C**-8), 111.8 (d, 1C, $^2J_{C,F}$ = 22.7, **C**-5), 107.6 (1C, **C**-3), 72.0 (1C, Bn-**C**H_2), 66.2 (1C, NCH_2CH_2**C**H_2OBn), 51.6 (1C, N**C**$H_2CH_2CH_2$OBn), 28.3 (1C, NCH_2**C**H_2CH_2OBn).

1-Benzyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***42b*** *(MB225)*



$C_{21}H_{19}FN_2O_4$

M_r = 382.39 g/mol

According to the general procedure 10.4, compound **42a** (300 mg, 0.90 mmol) was dissolved in 10.0 mL morpholine and heated for 6 h under microwave irradiation at 110 °C.

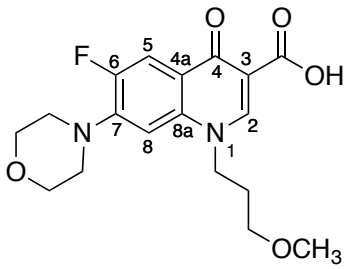
After acidification and drying the precipitate *in vacuo*, the yellow solid was recrystallised from EtOAc to yield **42b**.

appearance:	yellow solid
yield:	270 mg (0.71 mmol/ 78%)
reaction control:	R_f = 0.43 ($CHCl_3$/MeOH/FA = 100:5:1)
melting point [°C]:	304-306 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3045, 1707, 1614, 1567, 1544, 1465, 1376, 1266, 1215, 1026, 763.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 14.26 (s, 1H, COO**H**), 9.21 (s, 1H, **H**-2), 7.92 (d, 1H, ^3J = 13.4, **H**-5), 7.40 – 7.30 (m, 5H, C**H**$_{aromat.}$), 7.10 (d, 1H, ^4J = 7.2, **H**-8), 5.85 (s, 2H, Bn-C**H$_2$**), 3.72 – 3.70 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.12 – 3.09 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 176.4 (1C, **C**-4), 165.9 (1C, **C**OOH), 152.2 (d, 1C, $^1J_{C,F}$ = 249.0, **C**-6), 149.5 (1C, **C**-2), 144.7 (d, 1C, $^2J_{C,F}$ = 10.6, **C**-7), 137.4 (1C, **C**-8a), 135.2 (1C, Bn-**C**$_{aromat.}$), 128.9 (2C, Bn-**C**H$_{aromat.}$), 128.1 (2C, Bn-**C**H$_{aromat.}$), 126.9 (1C, Bn-**C**H$_{aromat.}$), 119.8 (d, 1C, $^3J_{C,F}$ = 7.6, **C**-4a), 111.1 (d, 1C, $^2J_{C,F}$ = 22.5, **C**-5), 107.1 (1C, **C**-3), 106.6 (d, 1C, $^3J_{C,F}$ = 2.4, **C**-8), 65.6 (1C, NCH$_2$CH$_2$**C**H$_2$OBn), 65.7 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 56.7 (1C, Bn-**C**H$_2$), 49.6 (d, 2C, $^4J_{C,F}$ = 4.7, morpholino-**C**H$_2$-N-**C**H$_2$).

*6-Fluoro-1-(3-methoxypropyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, **43b** (MB129)*



$C_{18}H_{21}FN_2O_5$

M_r = 364.37 g/mol

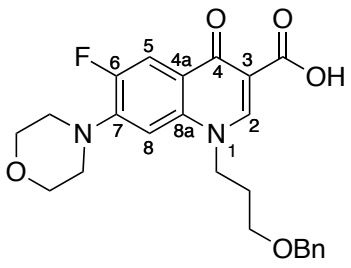
According to the general procedure 10.4, compound **43b** (580 mg, 1.85 mmol) was dissolved in 15.0 mL morpholine and heated for 5 h under microwave irradiation at 110 °C. After acidification and drying the precipitate *in vacuo*, the yellow solid was recrystallised from MeOH to yield in compound **43b**.

appearance:	yellow solid
yield:	400 mg (1.10 mmol/ 59%)
reaction control:	R_f = 0.28 ($CHCl_3$/MeOH/FA = 50:1:1)
melting point [°C]:	226-227 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2864, 1709, 1650, 1453, 1407, 1301, 1287, 1100.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 15.02 (s, 1H, COO**H**), 8.63 (s, 1H, **H**-2), 8.08 (d, 1H, ^3J = 13.6, **H**-5), 7.04 (d, 1H, ^4J = 7.2, **H**-8), 4.39 (t, 2H, ^3J = 7.0, NC**H$_2$**$CH_2CH_2OCH_3$), 3.92 – 3.90 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.42 (t, 2H, ^3J = 5.6, NCH_2CH_2C**H$_2$**OCH_3), 3.39 (s, 3H, $NCH_2CH_2CH_2OC$**H$_3$**), 3.33 – 3.30 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 2.18 – 2.11 (m, 2H, NCH_2C**H$_2$**CH_2OCH_3).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 176.5 (d, 1C, $^4J_{C,F}$ = 1.7, **C**-4), 166.3 (1C, **C**OOH), 152.3 (d, 1C, $^1J_{C,F}$ = 248.0, **C**-6), 147.7 (1C, **C**-2), 144.0 (d, 1C, $^2J_{C,F}$ = 10.8, **C**-7), 137.4 (1C, **C**-8a), 119.8 (d, 1C, $^3J_{C,F}$ = 10.2, **C**-4a), 112.5 (d, 1C, $^2J_{C,F}$ = 22.4, **C**-5), 107.0 (1C, **C**-3), 104.1 (d, 1C, $^3J_{C,F}$ = 4.8, **C**-8), 68.1 (1C, $NCH_2CH_2CH_2O$**C**H_3), 66.6 (2C, morpholino-**C**H_2-O-**C**H_2), 59.1 (1C, NCH_2CH_2**C**H_2OCH_3), 51.4 (1C, N**C**$H_2CH_2CH_2OCH_3$), 49.9 (d, 2C, $^4J_{C,F}$ = 4.7, morpholino-**C**H_2-N-**C**H_2), 29.0 (1C, NCH_2**C**$H_2CH_2OCH_3$).

1-(3-(Benzyloxy)propyl)-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, ***44b*** *(MB300)*



$C_{24}H_{25}FN_2O_5$

M_r = 440.47 g/mol

According to the general procedure 10.4, compound **44a** (4.0 g, 10.3 mmol) was dissolved in morpholine (10.0 mL) and heated for 6 h under microwave irradiation at 110 °C. After acidification and drying the precipitate *in vacuo*, the yellow solid was recrystallised from EtOAc/$CHCl_3$ (10:1) to yield in compound **44b**.

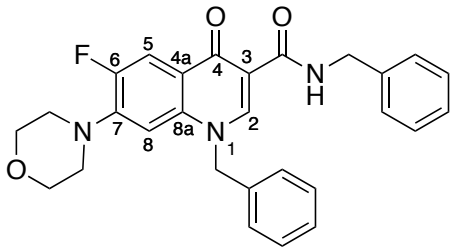
appearance:	yellow solid
yield:	2.6 g (5.9 mmol/ 58%)
reaction control:	R_f = 0.27 ($CHCl_3$/MeOH/FA = 100:2:1)

melting point [°C]:	191-192 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2858, 1713, 1626, 1508, 1453, 1406, 1354, 1302, 1265, 1206, 1101, 1025.

^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 14.7 (s, 1H, COO**H**), 8.91 (s, 1H, **H**-2), 7.91 (d, 1H, ^3J = 13.6, **H**-5), 7.33 – 7.28 (m, 5H, C**H**$_{aromat.}$), 7.19 (d, 1H, ^4J = 7.2, **H**-8), 4.64 (t, 2H, ^3J = 6.8, NC**H$_2$**CH$_2$CH$_2$OBn), 4.44 (s, 2H, Bn-C**H$_2$**), 3.72 – 3.70 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.49 (t, 2H, ^3J = 5.6, NCH$_2$CH$_2$C**H$_2$**OBn), 3.23 – 3.22 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 2.11 (quint, 2H, ^3J = 6.0, NCH$_2$C**H$_2$**CH$_2$OBn).

^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 176.1 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 166.0 (1C, **C**OOH), 152.4 (d, 1C, $^1J_{C,F}$ = 247.8, **C**-6), 149.1 (1C, **C**-2), 145.1 (d, 1C, $^2J_{C,F}$ = 10.4, **C**-7), 138.1 (1C, Bn-**C**$_{aromat.}$), 137.3 (1C, **C**-8a), 128.2 (2C, Bn-**C**H$_{aromat.}$), 128.1 (2C, Bn-**C**H$_{aromat.}$), 127.5 (1C, Bn-**C**H$_{aromat.}$), 119.3 (d, 1C, $^3J_{C,F}$ = 10.4, **C**-4a), 111.1 (d, 1C, $^2J_{C,F}$ = 23.2, **C**-5), 106.8 (1C, **C**-3), 105.7 (d, 1C, $^3J_{C,F}$ = 4.8, **C**-8), 72.1 (1C, Bn-**C**H$_2$), 66.4 (1C, NCH$_2$CH$_2$**C**H$_2$OBn), 65.7 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 51.6 (1C, N**C**H$_2$CH$_2$CH$_2$OBn), 49.6 (d, 2C, $^4J_{C,F}$ = 4.7, morpholino-**C**H$_2$-N-**C**H$_2$), 28.1 (1C, NCH$_2$**C**H$_2$CH$_2$OBn).

N,1-Dibenzyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***42c*** *(MB227)*



$C_{28}H_{26}FN_3O_3$

M_r = 471.53 g/mol

A solution of compound **42b** (230 mg, 0.60 mmol) in abs. DMF was treated with *N*MM (330 µL, 3.00 mmol), IBCF (313 µL, 2.40 mmol), and benzylamine (263 µL, 2.40 mmol) as depicted in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 20:1) and recrystallised from EtOAc to give **42c**.

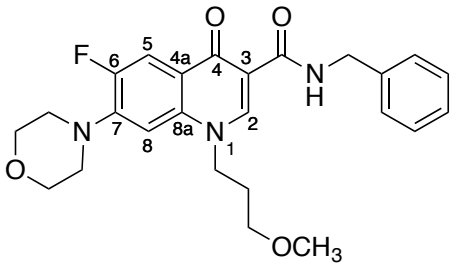
appearance:	white solid
yield:	200 mg (0.42 mmol/ 71%)
reaction control:	R_f = 0.82 ($CHCl_3$/MeOH = 20:1)

melting point [°C]:	232-233 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3049, 2959, 2852, 1648, 1549, 1486, 1443, 1117.
HPLC purity:	99% (HPLC method **II**)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 10.36 (t, 1H, ^3J = 6.5, N-**H**), 9.05 (s, 1H, **H**-2), 7.85 (d, 1H, ^3J = 13.6, **H**-5), 7.40 – 7.25 (m, 10H, Bn-C**H**$_{aromat.}$), 7.04 (d, 1H, ^4J = 7.2, **H**-8), 5.77 (s, 2H, Bn-**C**H$_2$), 4.57 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 3.75 – 3.70 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.49 (t, 2H, ^3J = 5.6, NCH$_2$CH$_2$C**H$_2$**OBn), 3.10 – 3.07 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 176.4 (d, 1C, $^4J_{C,F}$ = 2.3, **C**-4), 164.6 (**C**ON), 152.2 (d, 1C, $^1J_{C,F}$ = 242.2, **C**-6), 148.4 (1C, **C**-2), 143.9 (d, 1C, $^3J_{C,F}$ = 10.3, **C**-7), 139.9 (1C, Bn-**C**$_{aromat.}$), 136.8 (1C, **C**-8a), 135.7 (1C, Bn-**C**$_{aromat.}$), 128.9 (2C, Bn-**C**H$_{aromat.}$), 128.3 (2C, Bn-**C**H$_{aromat.}$), 127.9 (1C, Bn-**C**H$_{aromat.}$), 127.3 (2C, Bn-**C**H$_{aromat.}$), 126.8 (1C, Bn-**C**H$_{aromat.}$), 126.8 (2C, Bn-**C**H$_{aromat.}$), 121.5 (d, 1C, $^3J_{C,F}$ = 6.9, **C**-4a), 111.3 (d, 1C, $^2J_{C,F}$ = 22.5, **C**-5), 110.3 (1C, **C**-3), 106.3 (d, 1C, $^3J_{C,F}$ = 3.0, **C**-8), 65.6 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 56.7 (1C, Bn-**C**H$_2$), 50.3 (d, 2C, $^4J_{C,F}$ = 5.0, morpholino-**C**H$_2$-N-**C**H$_2$), 42.6 (1C, Bn-**C**H$_2$).

N-Benzyl-6-fluoro-1-(3-methoxypropyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***43c*** *(MB133)*



$C_{25}H_{28}FN_3O_4$

M_r = 453.51 g/mol

A solution of compound **43b** (200 mg, 0.55 mmol) in abs. DMF was treated with *N*MM (288 µL, 2.75 mmol), IBCF (295 µL, 2.20 mmol), and benzylamine (249 µL, 2.20 mmol) as depicted in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 20:1) and recrystallised from toluene to give **43c**.

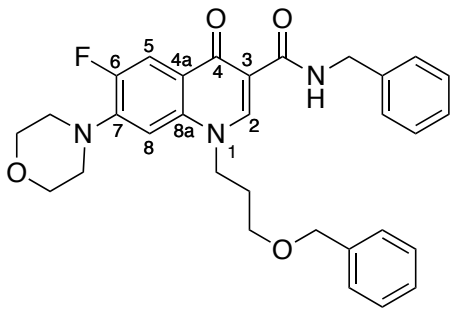
appearance:	white solid
yield:	130 mg (0.29 mmol/ 52%)

reaction control:	R_f = 0.60 ($CHCl_3$/MeOH = 20:1)
melting point [°C]:	200-201 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3184, 3033, 2930, 2876, 1654, 1537, 1586, 1490, 1446, 1360, 1253, 1118.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 10.42 (t, 1H, ^3J = 5.6, N-**H**), 8.73 (s, 1H, **H**-2), 8.06 (d, 1H, ^3J = 13.2, **H**-5), 7.39 – 7.16 (m, 5H, Bn-C**H**$_{aromat.}$), 6.88 (d, 1H, ^4J = 6.8, **H**-8), 4.67 (d, 2H, ^3J = 5.6, Bn-C**H$_2$**), 4.34 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2OCH_3$) 3.92 – 3.89 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.41 (t, 2H, ^3J = 5.2, NCH_2CH_2C**H$_2$**OCH_3), 3.39 (s, 3H, $NCH_2CH_2CH_2OC$**H$_3$**), 3.27 – 3.25 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 2.15 – 2.09 (m, 2H, NCH_2C**H$_2$**CH_2OCH_3).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 175.4 (d, 1C, $^4J_{C,F}$ = 2.4), 165.0 (1C, **C**ON), 152.4 (d, 1C, $^1J_{C,F}$ = 247.0, **C**-6), 147.4 (1C, **C**-2), 144.8 (d, 1C, $^2J_{C,F}$ = 10.2, **C**-7), 138.8 (1C, Bn-**C**H$_{aromat.}$), 136.6 (1C, **C**-8a), 128.5 (2C, Bn-**C**H$_{aromat.}$), 127.6 (2C, Bn-**C**H$_{aromat.}$), 127.0 (1C, Bn-**C**$_{aromat.}$), 122.7 (d, 1C, $^3J_{C,F}$ = 7.0, **C**-4a), 112.9 (d, 1C, $^2J_{C,F}$ = 23.0, **C**-5), 111.4 (1C, **C**-3), 104.1 (d, 1C, $^3J_{C,F}$ = 4.4, **C**-8), 68.3 (1C, $NCH_2CH_2CH_2O$**C**H_3), 66.7 (2C, morpholino-**C**H_2-O-**C**H_2), 59.0 (1C, NCH_2CH_2**C**H_2OCH_3), 50.8 (1C, N**C**$H_2CH_2CH_2OCH_3$), 50.2 (d, 2C, $^4J_{C,F}$ = 4.7, morpholino-**C**H_2-N-**C**H_2) 43.2 (1C, Bn-**C**H_2), 29.1 (1C, NCH_2**C**$H_2CH_2OCH_3$).

N-Benzyl-1-(3-(benzyloxy)propyl)-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***44c*** *(MB301)*



$C_{31}H_{32}FN_3O_4$

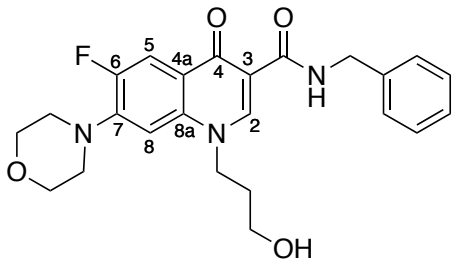
M_r = 529.61 g/mol

A solution of compound **44b** (400 mg, 0.90 mmol) in abs. DMF (15 mL) was treated with *N*MM (463 µL, 4.50 mmol), IBCF (468 µL, 3.60 mmol), and benzylamine (393 µL, 3.60 mmol) as depicted in the general procedure 10.5. The crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:3) and recrystallised from EtOAc to give **44c**.

appearance:	white solid
yield:	360 mg (0.68 mmol/ 76%)
reaction control:	R_f = 0.56 ($CHCl_3$/MeOH = 100:3)
melting point [°C]:	174-175 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3181, 3038, 2939, 2856, 1652, 1536, 1586, 1358, 1266.
mass:	*m/z* 530.1 $[M + H]^+$
HPLC purity:	100% (HPLC method **I**)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 10.37 (t, 1H, ^3J = 6.8, N-**H**), 8.81 (s, 1H, **H**-2), 7.87 (d, 1H, ^3J = 13.6, **H**-5), 7.36 – 7.25 (m, 10H, Bn-C**H**$_{aromat.}$), 7.13 (d, 1H, ^4J = 7.2, **H**-8), 4.58 – 4.55 (m, 4H, Bn-C**H$_2$**/NC**H$_2$**CH_2CH_2OBn), 4.44 (s, 2H, Bn-C**H$_2$**), 3.75 – 3.70 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.49 (t, 2H, ^3J = 5.6, NCH_2CH_2C**H$_2$**OBn), 3.20 – 3.18 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 2.10 (quint, 2H, ^3J = 6.0, NCH_2C**H$_2$**CH_2OBn).
^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 174.7 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 164.6 (1C, **C**ON), 153.2 (d, 1C, $^1J_{C,F}$ = 247.8, **C**-6), 148.6 (1C, **C**-2), 144.8 (d, 1C, $^2J_{C,F}$ = 10.4, **C**-7), 139.9 (1C, Bn-**C**$_{aromat.}$), 138.1 (1C, Bn-**C**$_{aromat.}$), 128.9 (2C, Bn-**C**H$_{aromat.}$), 128.7 (2C, Bn-**C**H$_{aromat.}$), 128.7 (1C, Bn-**C**H$_{aromat.}$), 128.1 (2C, Bn-**C**H$_{aromat.}$), 127.8 (1C, Bn-**C**H$_{aromat.}$), 127.9 (2C, **C**H$_{aromat.}$), 122.0 (d, 1C, $^4J_{C,F}$ = 7.0, **C**-4a), 112.1 (d, 1C, $^2J_{C,F}$ = 22.9, **C**-5), 110.6 (1C, **C**-3), 106.0 (d, 1C, $^3J_{C,F}$ = 4.8, **C**-8), 72.1 (1C, Bn-**C**H$_2$), 66.8 (1C, NCH_2CH_2**C**H_2OBn), 66.7 (2C,), 51.6 (1C, N**C**$H_2CH_2CH_2$OBn), 50.3 (d, 2C, $^4J_{C,F}$ = 4.7, morpholino-**C**H_2-O-**C**H_2), 42.6 (1C, Bn-**C**H_2), 28.8 (1C, NCH_2**C**H_2CH_2OBn).

N-Benzyl-6-fluoro-1-(3-hydroxypropyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***45*** *(MB303)*



$C_{24}H_{26}FN_3O_4$
M_r = 439.49 g/mol

Compound **44c** (0.20 g, 0.38 mmol) and a catalytical amount of Pd/C were suspended in $CHCl_3$ (15 mL). The mixture was pressurized with H_2 (25 bar) and heated in a *synthWAVE* microwave system (100 °C/500 W) for 12 h. The solvent was removed *in vacuo* and the

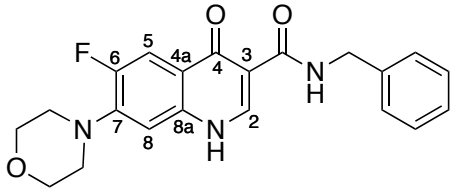
residue was purified via column chromatography (silica gel; $CHCl_3$/MeOH = 20:1). The white residue was recrystallised from EtOAc to give **45**.

appearance:	pale white solid
yield:	110 mg (0.25 mmol/ 66%)
reaction control:	R_f = 0.22 ($CHCl_3$/MeOH = 20:1)
melting point [°C]:	182 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3384, 3070, 2858, 1651, 1628, 1600, 1536, 1488, 1448, 1364, 1259, 1170, 1036.
mass:	*m/z* 440.1 $[M + H]^+$
HPLC purity:	99% (HPLC method **I**)

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 10.36 (t, 1H, ^3J = 6.8, N-**H**), 8.78 (s, 1H, **H**-2), 7.87 (d, 1H, ^3J = 13.6, **H**-5), 7.34 – 7.33 (m, 4H, Bn-C**H**$_{aromat.}$), 7.28 – 7.24 (m, 1H, Bn-C**H**$_{aromat.}$), 7.20 (d, 1H, ^4J = 7.2, **H**-8), 4.79 (t, 1H, ^3J = 4.8, $NCH_2CH_2CH_2O$**H**), 4.44 – 4.49 (m, 4H NC**H$_2$**CH_2CH_2OH / Bn-C**H$_2$**), 3.79 – 3.77 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.49 – 3.42 (m, 2H, NCH_2CH_2C**H$_2$**OH), 3.26 – 3.23 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 1.94 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2OH).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 174.0 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 164.0 (1C, **C**ON), 153.3 (d, 1C, $^1J_{C,F}$ = 247.8, **C**-6), 147.9 (1C, **C**-2), 144.1 (d, 1C, $^2J_{C,F}$ = 10.4, **C**-7), 139.2 (1C, Bn-**C**H$_{aromat.}$), 136.6 (1C, **C**-8a), 128.3 (2C, Bn-**C**H$_{aromat.}$), 127.1 (2C, Bn-**C**H$_{aromat.}$), 126.7 (1C, Bn-**C**$_{aromat.}$), 121.3 (d, 1C, $^3J_{C,F}$ = 7.0, **C**-4a), 111.2 (d, 1C, $^2J_{C,F}$ = 22.9, **C**-5), 109.9 (1C, **C**-3), 105.5 (d, 1C, $^3J_{C,F}$ = 4.8, **C**-8), 65.7 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 57.0 (1C, NCH_2CH_2**C**H_2OH), 50.2 (1C, N**C**$H_2CH_2CH_2OH$), 49.7 (d, 2C, $^4J_{C,F}$ = 4.7, morpholino-**C**H$_2$-N-**C**H$_2$) 41.9 (1C, Bn-**C**H$_2$), 31.1 (1C, NCH_2**C**H_2CH_2OH).

N-Benzyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***46*** *(MB231, MB259)*



$C_{21}H_{20}FN_3O_3$

M_r = 381.41 g/mol

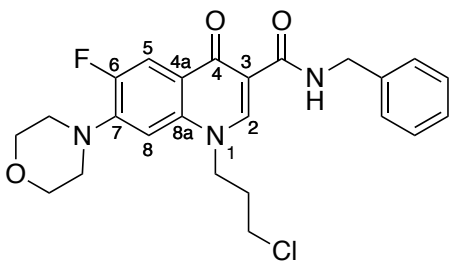
Compound **42c** (60 mg, 0.13 mmol) was dissolved in a mixture of MeOH and $CHCl_3$ (10 mL, 2:1) and a catalytic amount of Pd/C was added. The reaction solution stirred for 24 h in a hydration autoclave apparatus with an atmosphere of hydrogen of 10 bar. The reaction suspension was filtered through *Celite* and the filtrate was concentrated under reduced pressure. The subsequent column chromatography (silica gel; $CHCl_3$/MeOH = 20:1) resulted in an oily residue which was crystallised from EtOAc to give **46**.

appearance:	white solid
yield:	20 mg (0.05 mmol/ 41%)
reaction control:	R_f = 0.64 ($CHCl_3$/MeOH = 20:1)
melting point [°C]:	289-291 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3949, 3122, 3064, 2843, 1743, 1636, 1525, 1486, 1245, 1200, 1117.
HPLC purity:	99% (HPLC method **II**).

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 12.53 (br, 1H, N-**H**), 10.40 (t, 1H, ^3J = 6.0, N-**H**), 8.70 (s, 1H, **H**-2), 7.78 (d, 1H, ^3J = 13.6, **H**-5), 7.34 – 7.13 (m, 5H, C**H**$_{aromat.}$), 7.04 (d, 1H, ^4J = 7.2, **H**-8), 4.57 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 3.79 – 3.76 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.16 – 3.14 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 176.7 (d, 1C, $^4J_{C,F}$ = 2.4, **C**-4), 164.3 (1C, **C**ON), 152.5 (d, 1C, $^1J_{C,F}$ = 245.4, **C**-6), 144.3 (d, 1C, $^3J_{C,F}$ = 10.8, **C**-7), 143.2 (1C, **C**-2), 139.4 (1C, Bn-**C**$_{aromat.}$), 136.8 (1C, **C**-8a), 128.3 (2C, Bn-**C**H$_{aromatisch.}$), 127.2 (2C, Bn-**C**H$_{aromat.}$), 126.7 (1C, Bn-**C**H$_{aromat.}$), 120.3 (d, 1C, $^3J_{C,F}$ = 7.0, **C**-4a), 110.4 (d, 1C, $^2J_{C,F}$ = 22.6, **C**-5), 109.9 (1C, **C**-3), 106.7 (d, 1C, $^4J_{C,F}$ = 3.0, **C**-8), 65.8 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 49.7 (d, 2C, $^4J_{C,F}$ = 4.5, morpholino-**C**H$_2$-N-**C**H$_2$), 42.0 (1C, Bn-**C**H$_2$).

N-Benzyl-1-(3-chloropropyl)-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***47*** *(MB313)*



$C_{24}H_{25}ClFN_3O_3$

M_r = 457.93 g/mol

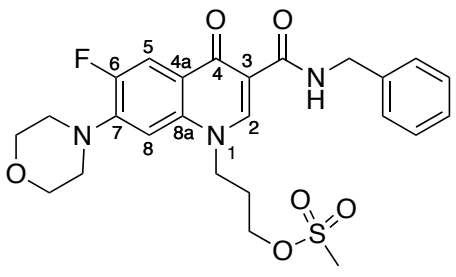
Compound **45** (90 mg, 0.20 mmol) was dissolved in abs. DMF (10 mL) and the solution was treated with pyridine (10 eq, 161 μL) under ice cooling. Subsequently, *p*-toluenesulfonyl chloride (3 eq, 110 mg, 0.58 mmol) was added to the reaction mixture and stirred for 5 h at rt. The crude product was purified by column chromatography (silica gel; $CHCl_3$/PE = 10:1) to give **47**.

appearance:	white solid
yield:	35 mg (0.08 mmol/ 38%)
reaction control:	R_f = 0.67 ($CHCl_3$/PE = 10:1)
melting point [°C]:	194-195 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3134, 3048, 2959, 2913, 2862, 1652, 1626, 1601, 1535, 1486, 1309, 1251, 1111.
mass:	*m/z* 458.7 $[M + H]^+$

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 10.34 (t, 1H, ^3J = 7.2, N-**H**), 8.79 (s, 1H, **H**-2), 7.86 (d, 1H, ^3J = 13.6, **H**-5), 7.35 – 7.44 (m, 4H, Bn-C**H**$_{aromat.}$), 7.28 – 7.25 (m, 1H, Bn-C**H**$_{aromat.}$), 7.10 (d, 1H, ^4J = 7.2, **H**-8), 4.58 – 4.54 (m, 5H, Bn-C**H$_2$**/NC**H$_2$**CH_2CH_2Cl), 3.80 – 3.77 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.73 – 3.70 (m, 2H, NC**H$_2$**CH_2CH_2Cl), 3.26 – 3.24 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 2.29 – 2.27 (m, 2H, NCH_2C**H$_2$**CH_2Cl).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 174.2 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 164.0 (1C, **C**ON), 152.7 (d, 1C, $^1J_{C,F}$ = 247.8, **C**-6), 148.0 (1C, **C**-2), 144.4 (d, 1C, $^2J_{C,F}$ = 10.4, **C**-7), 139.3 (1C, Bn-**C**$_{aromat.}$), 136.6 (1C, **C**-8a), 128.4 (2C, Bn-**C**H$_{aromat.}$), 127.3 (2C, Bn-**C**H$_{aromat.}$), 126.8 (1C, Bn-**C**H$_{aromat.}$), 121.4 (d, 1C, $^3J_{C,F}$ = 7.0, **C**-4a), 111.4 (d, 1C, $^2J_{C,F}$ = 22.9, **C**-5), 110.2 (1C, **C**-3), 105.4 (d, 1C, $^3J_{C,F}$ = 4.8, **C**-8), 65.8 (2C, morpholino-**C**H_2-O-**C**H_2), 50.6 (1C, N**C**$H_2CH_2CH_2Cl$), 49.7 (d, 2C, $^4J_{C,F}$ = 4.2, morpholino-**C**H_2-N-**C**H_2), 40.2 (1C, Bn-**C**H_2), 40.1 (1C, NCH_2CH_2**C**H_2Cl), 30.9 (1C, NCH_2**C**H_2CH_2Cl).

N-Benzyl-1-(3-((methylsulfonyl)oxy)propyl)-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***48*** *(MB347, MB351)*



$C_{25}H_{28}FN_3O_6S$

M_r = 517.57 g/mol

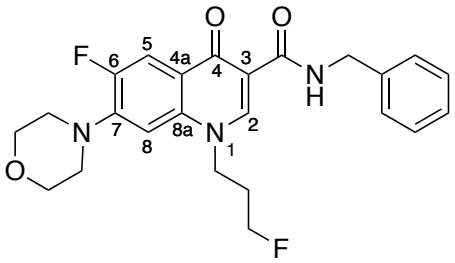
Compound **45** (0.23 mg, 0.52 mmol), TEA (507 μL, 3.66 mmol) and methanesulfonyl chlorid (80 μL, 1.05 mmol) were dissolved in abs. CH_2Cl_2 under Ar atmosphere at 0 °C. After 12 h of stirring at room temperature, the solvent was removed under reduced pressure to give the crude product. The purification was carried out by subsequent column chromatography (silica gel; $CHCl_3$/MeOH = 100:3), and yielded in **48**.

appearance:	white solid
yield:	141 mg (0.27 mmol/ 52%)
reaction control:	R_f = 0.49 ($CHCl_3$/MeOH = 100:3)
melting point [°C]:	232–234 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3168, 3045, 2935, 2830, 1654, 1625, 1601, 1541, 1487, 1352, 1263, 1250, 1169.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 10.37 (t, 1H, ^3J = 6.0, N-**H**), 8.72 (s, 1H, **H**-2), 8.06 (d, 1H, ^2J = 13.2, **H**-5), 7.38 – 7.32 (m, 4H, Bn-C**H**$_{aromat.}$), 7.28 – 7.22 (m, 1H, Bn-C**H**$_{aromat.}$), 6.82 (d, 1H, ^4J = 7.2, **H**-8), 4.66 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 4.40 (t, 2H, ^3J = 7.2, NC**H$_2$**CH_2CH_2OMs), 4.33 (t, 2H, ^3J = 5.2, NC**H$_2$**CH_2CH_2OMs), 3.91 – 3.89 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.29 – 3.27 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 3.08 (s, 3H, $NCH_2CH_2CH_2O$**Ms**), 2.38–2.32 (m, 2H, NCH_2C**H$_2$**CH_2OMs).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 175.3 (d, 1C, $^4J_{C,F}$ = 2.2, **C**-4), 164.8 (1C, **C**ON), 153.1 (d, 1C, $^1J_{C,F}$ = 248.1, **C**-6), 147.1 (1C, **C**-2), 145.2 (d, 1C, $^3J_{C,F}$ = 10.5, **C**-7), 138.7 (1C, Bn-**C**$_{aromat.}$), 136.5 (1C, **C**-8a), 128.6 (2C, Bn-**C**H$_{aromat.}$), 127.6 (2C, Bn-**C**H$_{aromat.}$), 127.1 (1C, Bn-**C**H$_{aromat.}$), 122.4 (d, 1C, $^3J_{C,F}$ = 7.4, **C**-4a), 113.2 (d, 1C, $^2J_{C,F}$ = 22.9, **C**-5), 111.7 (1C, **C**-3), 103.2 (d, 1C, $^4J_{C,F}$ = 2.9, **C**-8), 66.6 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 66.3 (1C, NCH_2CH_2**C**H_2OMs), 50.3 (1C, N**C**$H_2CH_2CH_2OMs$), 50.2 (d, 2C, $^4J_{C,F}$ = 4.2, morpholino-**C**H$_2$-N-**C**H$_2$), 43.3 (1C, Bn-**C**H$_2$), 37.6 (1C, $NCH_2CH_2CH_2O$**Ms**), 28.8 (1C, NCH_2**C**H_2CH_2OMs).

N-Benzyl-6-fluoro-1-(3-fluoropropyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide, ***49*** *(MB352)*



$C_{24}H_{25}F_2N_3O_3$

M_r = 441.48 g/mol

A solution of *N,N*-diethylaminosulfur trifluoride (123 µl, 0.93 mmol) in CH_2Cl_2 (3 mL) was transferred to a solution of **45** (0.21 mg, 0.47 mmol) in abs. CH_2Cl_2 (15 mL) at 0 °C. The reaction solution stirred for 20 h at rt and reaction was quenched by means of 5% $NaHCO_3$ (5 mL). The aqueous layer was extracted with CH_2Cl_2 (4x30 mL), dried over Na_2SO_4, and the solvent was removed under reduced pressure. The solid residue was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:3) to give **49**.

appearance:	white solid
yield:	110 mg (0.25 mmol/ 53%)
reaction control:	R_f = 0.59 ($CHCl_3$/MeOH = 100:3)
melting point [°C]:	152 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3196, 3059, 2965, 2906, 2855, 1654, 1627, 1604, 1537, 1485, 1449, 1377, 1359, 1303, 1254, 1206, 1172.
mass:	*m/z* 442.2 $[M + H]^+$
HPLC purity:	97% (HPLC method **I**)

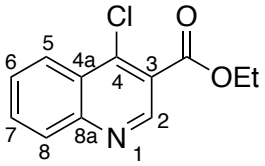
^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 10.32 (t, 1H, ^3J = 6.0, N-**H**), 8.67 (s, 1H, **H**-2), 8.01 (d, 1H, ^3J = 13.2, **H**-5), 7.32 – 7.24 (m, 4H, Bn-C**H**$_{aromat.}$), 7.17 – 7.15 (m, 1H, Bn-C**H**$_{aromat.}$), 6.81 (d, 1H, ^4J = 7.2, **H**-8), 4.60 (d, 2H, ^3J = 6.0, Bn-C**H$_2$**), 4.49 (dt, 2H, ^2J = 46.8, ^3J = 5.2, $NCH_2CH_2C\mathbf{H_2}F$), 4.32 (t, 2H, ^3J = 5.2, $NC\mathbf{H_2}CH_2CH_2F$), 3.85 – 3.82 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.21 – 3.18 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 2.28 – 2.16 (m, 2H, $NCH_2C\mathbf{H_2}CH_2F$).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 175.3 (d, 1C, $^4J_{C,F}$ = 2.2, **C**-4), 164.8 (1C, **C**ON), 153.3 (d, 1C, $^1J_{C,F}$ = 248.1, **C**-6), 147.1 (1C, **C**-2), 145.1 (d, 1C, $^2J_{C,F}$ = 10.6, **C**-7), 138.8 (1C, Bn-**C**$_{aromat.}$), 136.7 (1C, **C**-8a), 128.6 (2C, Bn-**C**H$_{aromat.}$), 127.6 (2C, Bn-**C**H$_{aromat.}$), 127.1 (1C, Bn-**C**H$_{aromat.}$), 122.5 (d, 1C, $^3J_{C,F}$ = 7.3, **C**-4a), 113.1 (d, 1C, $^2J_{C,F}$ = 22.9, **C**-5), 111.8 (1C, **C**-3), 103.2 (m, **C**-8), 80.2.3 (d, 1C, $^1J_{C,F}$ = 165.4, $NCH_2CH_2\mathbf{C}H_2F$), 66.6 (2C,

morpholino-**C**H_2-O-**C**H_2), 50.2 (d, 2C, $^4J_{C,F}$ = 4.2, morpholino-**C**H_2-N-**C**H_2), 49.8 (d, 1C, $^3J_{C,F}$ = 3.1, N**C**$H_2CH_2CH_2F$), 43.3 (1C, Bn-**C**H_2), 37.6 (d, 1C, $^2J_{C,F}$ = 19.9, NCH_2**C**H_2CH_2F). **^{19}F NMR** (^{1}H decoupled, DMSO-*d_6*, δ [ppm], *J* [Hz]): -123.80 (s, **F**-6), -219.87 (s, $NCH_2CH_2CH_2$**F**).

10.9 Scaffold Hopping Approach: Pyrazolo[4,3-c]quinolin-3-one

Ethyl 4-chloroquinoline-3-carboxylate, ***50a*** *(MBDS04)*



$C_{12}H_{10}ClNO_2$

M_r = 235.67 g/mol

According to Kaslow *et al.*[109] the reagent ethyl 4-hydroxy-1,4-dihydroquinoline-3-carboxylate (1.00 g, 4.60 mmol) dissolved in $POCl_3$ (0.73 mL, 7.83 mmol) heated at 100 °C for 45 min. The reaction mixture was poured into ice water and neutralized with saturated $NaHCO_3$ solution. Afterwards, the water phase was extracted with CH_2Cl_2 (3x30 mL) and the combined organic layers were dried over anhydrous Na_2SO_4. Removing the solvent *in vacuo* afforded a brown crude solid, which was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 100:1).

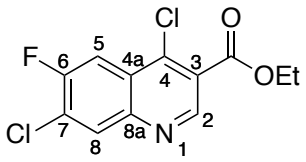
appearance:	white solid
yield:	0.79 g (3.36 mmol/ 73%, Lit[109]: 83%)
reaction control:	R_f = 0.86 ($CHCl_3$/MeOH = 20:1)
melting point [°C]:	53-54 °C [109]: 46-47 °C]
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2980, 1716, 1612, 1578, 1555, 1483, 1229, 1208, 1168, 1004, 760.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 9.20 (s, 1H, **H**-2), 8.41 (ddd, 1H, ^3J= 8.4, ^4J= 1.2, ^5J= 0.4, **H**-5), 8.14 (d, 1H, ^3J= 8.4, ^4J= 0.4, **H**-8), 7.84 (ddd, 1H, ^3J= 8.4, ^3J= 6.8, ^4J= 1.6, **H**-7), 7.70 (ddd, 1H, ^3J= 8.0, ^3J= 6.8, ^4J= 1.2, **H**-6), 4.50 (q, 2H, ^3J = 7.2, C**H_2**CH_3), 1.46 (t, 3H, ^3J = 7.2, CH_2C**H_3**).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 164.7 (1C, **C**OOEt), 150.2 (1C, **C**-2), 149.7 (1C, **C**-4), 143.6 (1C, **C**-8a), 132.0 (1C, **C**-7), 130.0 (1C, **C**-8), 128.5 (1C, **C**-6), 126.3 (1C, **C**-4a), 125.6 (1C, **C**-5), 123.2 (1C, **C**-3), 61.5 (1C, **C**H_2CH_3), 13.6 (1C, CH_2**C**H_3).

Kaslow *et al.*[109] did not provide IR and NMR data.

Ethyl 4,7-dichloro-6-fluoroquinoline-3-carboxylate, ***50b*** *(MB201)*



$C_{12}H_8Cl_2FNO_2$

M_r = 288.10 g/mol

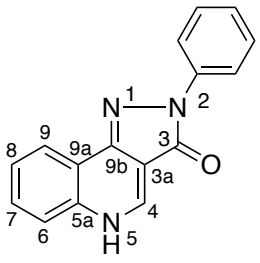
According to Kaslow *et al.*[109] compound **2a** (1.0 g, 3.5 mmol) was dissolved in $POCl_3$ (0.55 mL, 5.9 mmol) and the mixture was stirred at 100 °C for 1 h. Subsequently, the mixture was poured on ice water and neutralised with saturated $NaHCO_3$ solution. The water phase was extracted with CH_2Cl_2 (3x30 mL) and the combined organic layers were dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and finally purified with column chromatography (silica gel; PE/EtOAc = 5:1).

appearance:	grey solid
yield:	0.77 g (2.67 mmol/ 77%)
reaction control:	R_f = 0.53 (PE/EtOAc = 5:1)
melting point [°C]:	109-111 °C [128]: 112-114 °C]
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3068, 2981, 1725, 1614, 1579, 1549, 1447, 1284, 1191, 1031.

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 163.4 (1C, **C**OOEt), 156.2 (d, 1C, ^1J = 249.0, **C**-6), 150.2 (d, 1C, ^3J = 3.0, **C**-2), 145.7 (1C, **C**-4), 140.8 (d, 1C, ^3J = 5.9, **C**-8a), 131.4 (1C, **C**-8), 126.4 (d, 1C, ^2J = 21.1, **C**-7), 125.3 (d, 1C, ^2J = 9.4, **C**-4a), 123.9 (1C, **C**-3), 110.6 (d, 1C, ^3J = 24.0, **C**-6), 62.1 (1C, **C**H_2CH_3), 13.9 (1C, CH_2**C**H_3).

Cai *et al.*[128] did not give IR and ^{13}C NMR data.

2-Phenyl-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one, ***51a*** *(MBDS199)*



$C_{16}H_{11}N_3O$

M_r = 261.28 g/mol

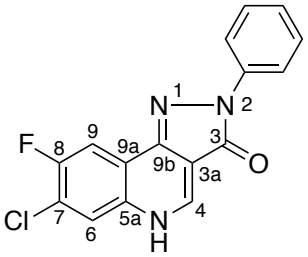
According to López Rivilli *et al.*[111], compound **50a** (0.50 g, 2.12 mmol) was dissolved in DMF and the solution was treated with phenylhydrazine (250 µL, 2.54 mmol). The reaction solution heated at 120 °C for 4 h, the solvent was removed under reduced pressure, and the crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 20:1) resulting in the orange product **51a**.

appearance:	orange solid
yield:	0.36 g (1.38 mmol / 65%)
reaction control:	R_f = 0.51 ($CHCl_3$/MeOH = 20:1)
melting point [°C]:	> 315 °C [103]: 328-331°C]
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2680, 1627, 1577, 1477, 1449, 1366, 1334, 883, 780.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 12.80 (br, 1H, N-**H**), 8.73 (s, 1H, **H**-4), 8.24 – 8.21 (m, 3H, Ph-C**H**$_{aromat.}$/**H**-8), 7.73 – 7.66 (m, 2H, **H**-7/**H**-6), 7.58 – 7.54 (m, 1H, **H**-9), 7.47 – 7.43 (m, 2H, Ph-C**H**$_{aromat.}$), 7.19 – 7.15 (m, 1H, Ph-C**H**$_{aromat.}$).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 161.5 (1C, **C**-3), 142.9 (1C, **C**-9b), 140.0 (1C, Ph-**C**$_{aromat.}$), 139.3 (1C, **C**-4), 135.4 (1C, **C**-5a), 130.0 (1C, **C**-7), 128.5 (2C, Ph-**C**H$_{aromat.}$), 126.9 (1C, **C**-9), 123.9 (1C, Ph-**C**H$_{aromat.}$), 122.0 (1C, **C**-8), 119.4 (1C, **C**-6), 118.6 (2C, Ph-**C**H$_{aromat.}$), 118.5 (1C, **C**-9a), 106.1 (1C, **C**-3a).

7-Chloro-8-fluoro-2-phenyl-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one, ***51b*** *(MBDS202)*



$C_{16}H_9ClFN_3O$

M_r = 313.72 g/mol

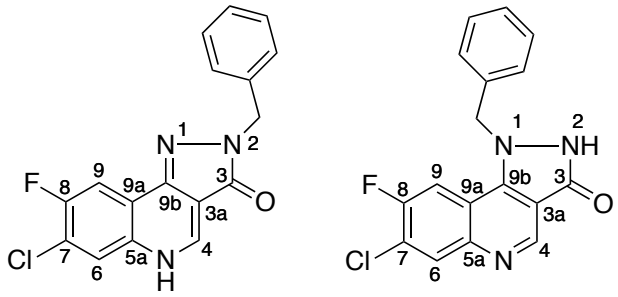
According to López Rivilli *et al.*[111], compound **50b** (0.51 g, 1.77 mmol) was dissolved in DMF and was treated with phenylhydrazine (193 μL, 2.12 mmol). The solution heated at 120 °C for 15 h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 20:1) to give compound **51b**.

appearance:	yellow solid
yield:	0.33 g (1.05 mmol/ 50%)
reaction control:	R_f = 0.31 ($CHCl_3$/MeOH = 20:1)
melting point [°C]:	> 315 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2833, 1609, 1585, 1475, 1453, 1346, 1305, 1254, 1143, 957.

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 161.3 (1C, **C**-3), 154.8 (1C, $^1J_{C,F}$ = 241.5, **C**-8), 141.9 (d, 1C, $^4J_{C,F}$ = 2.7, **C**-9b), 140.0 (2C, **C**-4/Ph-**C**$_{aromat.}$), 132.6 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-5a), 128.6 (2C, Ph-**C**H$_{aromat.}$), 124.2 (1C, Ph-**C**H), 121.9 (1C, $^2J_{C,F}$ = 20.2, **C**-7), 121.4 (1C, **C**-6), 119.0 (d, 1C, $^3J_{C,F}$ = 10.2, **C**-9a), 118.7 (2C, Ph-**C**H$_{aromat.}$), 108.9 (d, 1C, $^2J_{C,F}$ = 22.4, **C**-9), 105.8 (1C, **C**-3a).

The spectroscopic data are in accordance with Kaplan *et al.*[116]

2-Benzyl-7-chloro-8-fluoro-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one, ***52*** *and 1-Benzyl-7-chloro-8-fluoro-1,2-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one,* ***52'*** *(MBDS215)*



$C_{17}H_{11}ClFN_3O$

M_r = 327.74 g/mol

According to López Rivilli *et al.*[111], compound **50b** (0.60 g, 2.08 mmol) was dissolved in DMF and was treated with TEA (581 µL, 4.16 mmol), and benzylhydrazine dihydrochloride (0.49 g, 2.50 mmol). The reaction solution heated at 120 °C for 15 h. The solvent was removed under reduced pressure and the regioisomers **52** and **52'** could be separated by column chromatography (silica gel [0.020-0.060 mm]; $CHCl_3$/EtOAc = 2:1).

Compound **52**:

appearance:	yellow solid
yield:	120 mg (0.37 mmol/ 18%)
reaction control:	R_f = 0.31 ($CHCl_3$/EtOAc = 2:1)
melting point [°C]:	277-278 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2920, 2853, 1592, 1479, 1449, 1344, 1251, 880.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 12.72 (br, 1H, N-**H**), 8.71 (s, 1H, **H**-4), 7.94 (d, 1H, ^3J= 9.6, **H**-9), 7.85 (d, 1H, ^4J= 6.4, **H**-6), 7.33 – 7.23 (m, 5H, Bn-**C**$H_{aromat.}$), 5.09 (s, 2H, Bn-C**H_2**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 161.5 (1C, **C**-3), 154.7 (1C, $^1J_{C,F}$ = 245.8, **C**-8), 140.6 (d, 1C, $^4J_{C,F}$ = 2.9, **C**-9b), 139.5 (1C, **C**-4) 138.4 (1C, Bn-**C**$_{aromat.}$), 132.4 (d, 1C, $^4J_{C,F}$ = 1.7, **C**-5a), 128.2 (2C, Bn-**C**$H_{aromat.}$), 127.3 (2C, Bn-**C**$H_{aromat.}$), 126.9 (1C, Bn-**C**$H_{aromat.}$), 121.5 (1C, **C**-6), 121.0 (d, 1C, $^2J_{C,F}$ = 20.1 **C**-7), 119.3 (d, 1C, $^3J_{C,F}$ = 8.0, **C**-9a), 108.4 (d, 1C, $^2J_{C,F}$ = 22.7, **C**-9), 104.8 (1C, **C**-3a), 47.5 (1C, Bn-**C**H_2).

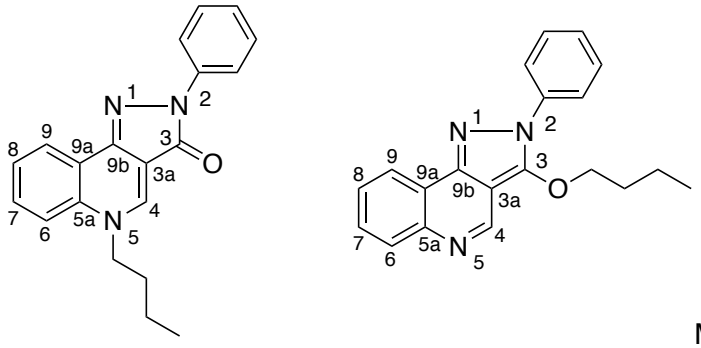
Compound **52'**:

appearance:	yellow solid
yield:	80 mg (0.21 mmol/ 10%)
reaction control:	R_f = 0.24 ($CHCl_3$/EtOAc = 2:1)
melting point [°C]:	299-301 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2960, 1587, 1520, 1468, 1446, 1241, 1386, 1172, 1111.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 11.58 (br, 1H, N-**H**), 9.10 (s, 1H, **H**-4), 8.28 (d, 1H, ^3J = 10.4, **H**-9), 8.28 (d, 1H, ^4J = 7.6, **H**-6), 7.32 – 7.11 (m, 5H, Bn-**C**H$_{aromat.}$), 5.89 (s, 2H, Bn-C**H$_2$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 162.9 (1C, **C**-3), 154.6 (1C, $^1J_{C,F}$ = 245.8, **C**-8), 145.6 (1C, **C**-4), 142.8 (d, 1C, $^4J_{C,F}$ = 1.7, **C**-9b), 138.0 (1C, **C**-5a), 136.9 (1C, Bn-**C**$_{aromat.}$), 131.2 (1C, **C**-6), 128.7 (2C, Bn-**C**H$_{aromat.}$), 127.5 (1C, Bn-**C**H$_{aromat.}$), 126.2 (2C, Bn-**C**H$_{aromat.}$), 121.0 (d, 1C, $^2J_{C,F}$ = 20.1 **C**-7), 114.8 (d, 1C, $^3J_{C,F}$ = 8.0, **C**-9a), 108.8 (d, 1C, $^2J_{C,F}$ = 23.4, **C**-9), 106.6 (1C, **C**-3a), 53.8 (1C, Bn-**C**H$_2$).

5-Butyl-2-phenyl-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one, ***53a*** *and 3-butoxy-2-phenyl-2H-pyrazolo[4,3-c]quinolone,* ***53a'*** *(MBDS200)*



$C_{20}H_{19}N_3O$

M_r = 317.39 g/mol

Compound **51a** (0.30 g, 0.96 mmol) was dissolved in DMF and was treated with K_2CO_3 (0.52 g, 3.78 mmol). Catalytic amount of KI and *n*-bromobutane (0.40 mL, 3.78 mmol) was added and the reaction mixture heated for 20 h at 100 °C. The solvent was removed *in vacuo*, and the crude product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 20:1) isolating the two products.

Compound **53a**:

appearance:	yellow solid
yield:	60 mg (0.19 mmol/ 20%)
reaction control:	R_f = 0.67 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	200-201 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3028, 2867, 1656, 1629, 1528, 1457, 1395, 1122, 976, 896.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 9.29 (s, 1H, **H**-4), 8.35 (dd, 1H, ^3J = 8.8, ^4J = 1.6, **H**-9), 8.26 – 8.23 (m, 2H, Ph-C**H**$_{aromat.}$), 7.97 (d, 1H, ^3J = 8.8, **H**-6), 7.80 (ddd, 1H, ^3J = 8.8, ^3J = 7.2, ^4J = 1.2, **H**-7), 7.66 (ddd, 1H, ^3J = 8.0, ^4J = 0.8, **H**-8), 7.51 – 7.46 (m, 2H, Ph-C**H**$_{aromat.}$), 7.22 (m, 1H, Ph-C**H**$_{aromat.}$), 4.51 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 1.83 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.40 (sext, 2H, ^3J = 7.6, NCH_2CH_2C**H$_2$**CH_3), 0.96 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2C$**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 161.4 (1C, **C**-3), 143.7 (1C, **C**-4), 142.6 (1C, **C**-9b), 140.7 (1C, Ph-**C**$_{aromat.}$), 135.4 (1C, **C**-5a), 130.5 (1C, **C**-7), 128.7 (2C, Ph-**C**H$_{aromat.}$), 126.6 (1C, **C**-8), 124.0 (1C, Ph-C**H**$_{aromat.}$), 122.7 (1C, **C**-9), 119.9 (1C, **C**-9a), 118.6 (2C, Ph-**C**H$_{aromat.}$), 118.2 (1C, **C**-6), 106.1 (1C, **C**-3a), 53.3 (1C, N**C**$H_2CH_2CH_2CH_3$), 30.6 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.2 (1C, NCH_2CH_2**C**H_2CH_3), 13.5 (1C, $NCH_2CH_2CH_2$**C**H_3).

Compound **53a'**:

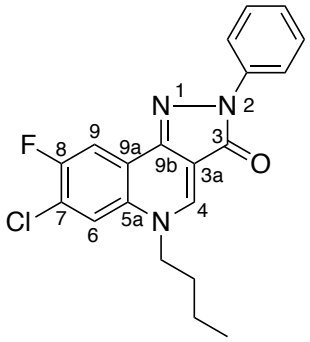
appearance:	yellow solid
yield:	110 mg (0.35 mmol / 36%)
reaction control:	R_f = 0.74 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	165-167 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2965, 2873, 1595, 1521, 1456, 1374, 1307, 1135, 756

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 9.29 (s, 1H, **H**-4), 8.35 (dd, 1H, ^3J = 8.0, ^4J = 1.2, **H**-9), 7.96 (dd, 1H, ^3J = 8.0, ^4J = 1.2, **H**-8), 7.86 – 7.83 (m, 2H, Ph-C**H**$_{aromat.}$), 7.71 – 7.61 (m, 1H, **H**-7), 7.63 – 7.58 (m, 3H, Ph-C**H**$_{aromat.}$/**H**-6), 7.50 – 7.46 (m, 1H, Ph-C**H**$_{aromat.}$), 4.84 (t, 2H, ^3J = 7.2, OC**H$_2$**$CH_2CH_2CH_3$), 1.83 (m, 2H, OCH_2C**H$_2$**CH_2CH_3), 1.48 (sext, 2H, ^3J = 7.6, OCH_2CH_2C**H$_2$**CH_3), 0.94 (t, 3H, ^3J = 7.2, $OCH_2CH_2CH_2C$**H$_3$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 149.4 (1C, **C**-3), 147.4 (1C, **C**-4), 145.6 (1C, **C**-9b), 144.7 (1C, **C**-5a), 138.7 (1C, Ph-**C**$_{aromat.}$), 129.7 (1C, **C**-8), 129.7 (2C, Ph-**C**H$_{aromat.}$), 129.4 (1C, **C**-7), 128.5 (1C, Ph-C**H**$_{aromat.}$), 127.3 (1C, **C**-6), 124.2 (2C, Ph-**C**H$_{aromat.}$), 121.8 (1C, **C**-9) 120.1 (1C, **C**-9a), 102.4 (1C, **C**-3a), 74.5 (1C,

O**C**$H_2CH_2CH_2CH_3$), 31.5 (1C, OCH_2**C**$H_2CH_2CH_3$), 18.9 (1C, OCH_2CH_2**C**H_2CH_3), 14.0 (1C, $OCH_2CH_2CH_2$**C**H_3).

5-Butyl-7-chloro-8-fluoro-2-phenyl-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one, ***53b*** *(MBDS212)*



$C_{20}H_{17}ClFN_3O$

M_r = 369.82 g/mol

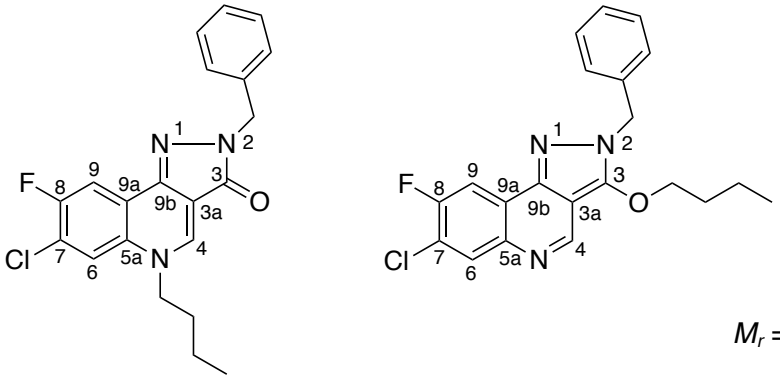
Compound **50b** (0.65 g, 2.07 mmol) was dissolved in abs. DMF (15 mL) and was treated with K_2CO_3 (1.15 g, 8.03 mmol). *N*-Bromobutane (0.9 mL, 8.34 mmol) was added and the reaction mixture heated at 95 °C for 15 h. The solvent was removed *in vacuo* and **53b** was selectively isolated by hot filtration with EtOAc.

appearance:	yellow solid
yield:	0.26 g (0.70 mmol / 34%)
reaction control:	R_f = 0.48 ($CHCl_3$/MeOH = 10:1)
melting point [°C]:	296 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2954, 1651, 1634, 1592, 1530, 1488, 1464, 1308.
mass:	*m/z* 370.8 $[M + H]^+$

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 8.24 (s, 1H, **H**-4), 8.19 – 8.16 (m, 3H, **H**-9/Ph-C**H**$_{aromat.}$), 7.47 – 7.43 (m, 2H, Ph-C**H**$_{aromat.}$), 7.21 – 7.20 (m, 1H, Ph-C**H**$_{aromat.}$), 4.48 (t, 2H, ^3J = 6.8, NC**H$_2$**$CH_2CH_2CH_3$), 1.76 (quint, 2H, ^3J = 7.6, NCH_2C**H$_2$**CH_2CH_3), 1.47 (sext, 2H, ^3J = 7.6, NCH_2CH_2C**H$_2$**CH_3), 1.02 (t, 3H, ^3J = 7.6, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR data were not accessible due to poor solubility in common deuterated solvents. However, signal for N**C**$H_2CH_2CH_2CH_3$ at 53.2 ppm is identifiable with ^{1}H-^{13}C correlation (HMBC) between **H**-2, ensuring the depicted *N*5-alkylated isomer was isolated.

2-Benzyl-5-butyl-7-chloro-8-fluoro-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one, ***54*** *and 2-benzyl-3-butoxy-7-chloro-8-fluoro-2H-pyrazolo[4,3-c]quinolone,* ***54'*** *(MB241)*



$C_{21}H_{19}ClFN_3O$

M_r = 383.85 g/mol

Compound **52** (60 mg, 0.18 mmol) was dissolved in DMF (10 mL) and was treated with K_2CO_3 (125 mg, 0.90 mmol). *N*-Bromobutane (80 μL, 0.72 mmol) was added and the reaction mixture heated at 85 °C for 12 h. The solvent was removed *in vacuo* and the regioisomers **54** and **54'** could be separated by column chromatography (silica gel; $CHCl_3$/MeOH = 50:1).

Compound **54**:

appearance:	yellow solid
yield:	20 mg (0.05 mmol/ 29%)
reaction control:	R_f = 0.21 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	256-257 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2970, 2930, 2858, 1626, 1512, 1562, 1402, 1366, 1258, 1217.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 8.22 (s, 1H, **H**-4), 8.05 (d, 1H, ^3J = 8.8, **H**-9), 7.55 (d, 1H, ^4J = 6.0, **H**-6), 7.38 – 7.24 (m, 5H, Bn-C**H**$_{aromat.}$), 5.21 (s, 2H, Bn-C**H$_2$**), 4.19 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 1.88 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.44 (sext, 2H, ^3J = 7.6, NCH_2CH_2C**H$_2$**CH_3), 1.00 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 162.2 (1C, **C**-3), 155.7 (1C, $^1J_{C,F}$ = 250.3, **C**-8), 141.3 (1C, **C**-4), 140.9 (d, 1C, $^4J_{C,F}$ = 1.7, **C**-9b), 137.7 (1C, Bn-**C**$_{aromat.}$), 131.9 (1C, $^4J_{C,F}$ = 2.5, **C**-5a), 128.4 (2C, Bn-**C**H$_{aromat.}$), 128.0 (1C, Bn-**C**H$_{aromat.}$), 127.3 (2C, Bn-**C**H$_{aromat.}$), 123.3 (d, 1C, $^2J_{C,F}$ = 20.1 **C**-7), 121.3 (d, 1C, $^3J_{C,F}$ = 7.9, **C**-9a), 118.8 (1C, **C**-6), 110.3 (d,

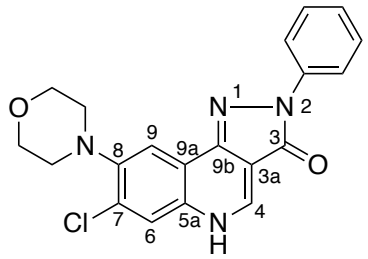
1C, $^{2}J_{C,F}$ = 22.4, **C**-9), 106.9 (1C, **C**-3a), 54.8 (1C, N**C**$H_2CH_2CH_2CH_3$), 48.6 (1C, Bn-**C**H_2), 30.8 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.9 (1C, NCH_2CH_2**C**H_2CH_3), 13.5 (1C, $NCH_2CH_2CH_2$**C**H_3).

Compound **54'**:

appearance:	white solid
yield:	20 mg (0.05 mmol/ 29%)
reaction control:	R_f = 0.80 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	185-186 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2960, 2874, 1587, 1520, 1468, 1368, 1241, 1111, 1059.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 8.98 (s, 1H, **H**-4), 8.06 (d, 1H, ^{3}J = 8.8, **H**-9), 8.00 (d, 1H, ^{4}J = 6.0, **H**-6), 7.26 – 7.19 (m, 5H, Bn-C**H**$_{aromat.}$), 5.36 (s, 2H, Bn-C**H$_2$**), 4.57 (t, 2H, ^{3}J = 7.2, OC**H$_2$**$CH_2CH_2CH_3$), 1.83 (quint, 2H, ^{3}J = 7.2, OCH_2C**H$_2$**CH_2CH_3), 1.43 (sext, 2H, ^{3}J = 7.6, OCH_2CH_2C**H$_2$**CH_3), 0.95 (t, 3H, ^{3}J = 7.2, $OCH_2CH_2CH_2$C**H$_3$**).
^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 156.7 (1C, $^{1}J_{C,F}$ = 250.3, **C**-8), 148.9 (1C, **C**-3), 146.1 (1C, **C**-4) 144.8 (d, 1C, $^{4}J_{C,F}$ = 1.7, **C**-9b), 141.4 (1C, $^{4}J_{C,F}$ = 2.5, **C**-5a), 135.6 (1C, Bn-**C**$_{aromat.}$), 131.2 (1C, **C**-6), 128.8 (2C, Bn-**C**H$_{aromat.}$), 128.1 (1C, Bn-**C**H$_{aromat.}$), 127.7 (2C, Bn-**C**H$_{aromat.}$), 122.0 (d, 1C, $^{2}J_{C,F}$ = 19.9, **C**-7), 120.1 (d, 1C, $^{3}J_{C,F}$ = 7.9, **C**-9a), 107.9 (d, 1C, $^{2}J_{C,F}$ = 22.9, **C**-9), 101.4 (1C, **C**-3a), 73.7 (1C, O**C**$H_2CH_2CH_2CH_3$), 52.3 (1C, Bn-**C**H_2), 31.4 (OCH_2**C**$H_2CH_2CH_3$), 18.8 (1C, OCH_2CH_2**C**H_2CH_3), 13.6 (1C, $OCH_2CH_2CH_2$**C**H_3)

7-Chloro-8-morpholino-2-phenyl-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one ***55*** *(MBDS204)*



$C_{20}H_{17}ClN_4O_2$
M_r = 380.83 g/mol

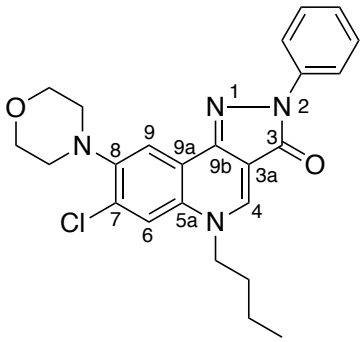
Compound **51b** (0.15 g, 0.48 mmol) dissolved in morpholine (7.5 mL) heated at 115 °C for 72 h. Afterwards the reaction mixture was neutralized with 2 M HCl and was extracted with $CHCl_3$ (3x20 mL). The combined organic layers were dried with anhydrous Na_2SO_4, the solvent was removed *in vacuo*, and the crude product was recrystallised from EtOH affording compound **55**.

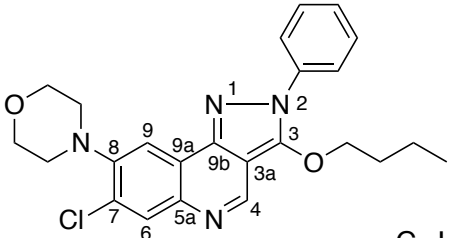
appearance:	yellow solid
yield:	50 mg (0.13 mmol/ 27%)
reaction control:	R_f = 0.61 ($CHCl_3$/MeOH = 20:1)
melting point [°C]:	> 315 °C
mass:	*m/z* 381.9 $[M + H]^+$
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3064, 2848, 1600, 1572, 1443, 1357, 1227, 1112, 960.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 12.30 (b, 1H, N-**H**), 8.70 (s, 1H, **H**-4), 8.23 – 8.21 (m, 2H, Ph-C**H**$_{aromat.}$), 7.78 (d, 2H, **H**-6/**H**-9), 7.46 – 7.42 (m, 2H, Ph-C**H**$_{aromat.}$), 7.19 - 7.15 (m, 1H, Ph-C**H**$_{aromat.}$), 3.81 – 3.79 (m, 4H, morpholine-C**H$_2$**-O-C**H$_2$**), 3.12 – 3.10 (m, 4H, morpholine-C**H$_2$**-N-C**H$_2$**).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], *J* [Hz]): 161.5 (1C, **C**-3), 147.0 (1C, **C**-8), 142.3 (1C, **C**-9b), 140.0 (1C, Ph-**C**$_{aromat.}$), 139.0 (1C, **C**-4), 131.9 (1C, **C**-5a), 129.8 (1C, **C**-7), 128.6 (2C, Ph-**C**H$_{aromat.}$), 123.9 (1C, Ph-**C**H$_{aromat.}$), 121.3 (1C, **C**-6), 118.6 (2C, Ph-**C**H$_{aromat.}$), 118.4 (1C, **C**-9a), 112.1 (1C, **C**-6), 105.8 (1C, **C**-3a), 66.1 (2C, morpholine-**C**H$_2$-O-**C**H$_2$), 51.4 (2C, morpholine-**C**H$_2$-N-**C**H$_2$).

5-Butyl-7-chloro-8-morpholino-2-phenyl-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one, ***56*** *and 5-butyl-7-chloro-8-morpholino-2-phenyl-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one,* ***56'*** *(MBDS245)*





$C_{24}H_{25}ClN_4O_2$

M_r = 436.94 g/mol

Compound **55** (250 mg, 0.66 mmol) was dissolved in DMF (7.5 mL) and was treated with K_2CO_3 (362 mg, 2.6 mmol). *N*-Bromobutane (280 µL, 2.6 mmol) was added and the reaction mixture heated at 80 °C for 4 h. The solvent was removed *in vacuo* and the regioisomers **56** and **56'** could be separated by column chromatography (silica gel; $CHCl_3$/PE = 50:1).

Compound **56**:

appearance:	yellow solid
yield:	180 mg (0.41 mmol/ 62%)
reaction control:	R_f = 0.65 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	267-268 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2954, 2848, 1625, 1592, 1467, 1446, 1307, 1112.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 8.29 (s, 1H, **H**-4), 8.21 – 8.19 (m, 2H, Ph-C**H**$_{aromat.}$), 7.99 (d, 1H, **H**-9), 7.56 (s, 1H, **H**-6), 7.47 – 7.43 (m, 2H, Ph-C**H**$_{aromat.}$), 4.20 (t, 2H, ^3J = 7.2, NC**H$_2$**$CH_2CH_2CH_3$), 3.94 – 3.92 (4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.22 – 3.19 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.89 (quint, 2H, ^3J = 7.6, NCH_2C**H$_2$**CH_2CH_3), 1.47 (sext, 2H, ^3J = 7.6, NCH_2CH_2C**H$_2$**CH_3), 1.00 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 161.7 (1C, **C**-3), 147.8 (1C, **C**-8), 142.3 (1C, **C**-9b), 141.0 (1C, **C**-4), 139.7 (1C, Ph-**C**$_{aromat.}$), 131.5 (1C, **C**-5a), 131.4 (1C, **C**-7), 128.4 (2C, Ph-**C**H$_{aromat.}$), 124.9 (1C, Ph-**C**H$_{aromat.}$), 120.4 (1C, **C**-9a), 120.1 (2C, Ph-**C**H$_{aromat.}$), 119.0 (1C, **C**-9), 113.8 (1C, **C**-6), 107.6 (1C, **C**-3a), 67.1 (2C, morpholino-**C**H_2-O-**C**H_2), 54.8 (1C, N**C**$H_2CH_2CH_2CH_3$), 51.8 (2C, morpholino-**C**H_2-N-**C**H_2), 30.9 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.2 (1C, NCH_2CH_2**C**H_2CH_3), 13.5 (1C, $NCH_2CH_2CH_2$**C**H_3).

Compound **56'**:

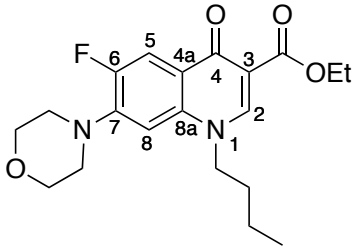
appearance:	brown solid
yield:	90 mg (0.21 mmol/ 31%)
reaction control:	R_f = 0.80 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	148-149 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2963, 2952, 1594, 1585, 1516, 1443, 1374, 1205, 1111.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 9.08 (s, 1H, **H**-4), 8.09 (s, 1H, **H**-9), 8.02 (s, 1H, **H**-6), 7.87 – 7.84 (m, 2H, Ph-C**H**$_{aromat.}$), 7.56 – 7.53 (m, 2H, Ph-C**H**$_{aromat.}$), 7.46 – 7.42 (m, 1H, Ph-C**H**$_{aromat.}$), 4.70 (t, 2H, ^3J = 6.4, OC**H$_2$**$CH_2CH_2CH_3$), 3.95 – 3.92 (4H, morpholine-C**H$_2$**-O-C**H$_2$**), 3.23 – 3.20 (m, 4H, morpholine-C**H$_2$**-N-C**H$_2$**), 1.94 – 1.87 (m, 2H, OCH_2C**H$_2$**CH_2CH_3), 1.50 (sext, 2H, ^3J = 7.6, OCH_2CH_2C**H$_2$**CH_3), 0.99 (t, 3H, ^3J = 7.2, $OCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 149.1 (1C, **C**-9b), 148.1 (1C, **C**-8), 145.3 (1C, **C**-3), 145.0 (1C, **C**-4), 140.8 (1C, **C**-5a), 138.1 (1C, Ph-**C**$_{aromat.}$), 131.0 (1C, **C**-6), 130.7

(1C, **C**-7), 129.1 (1C, Ph-**C**$H_{aromat.}$), 128.2 (2C, Ph-**C**$H_{aromat.}$), 124.0 (2C, Ph-**C**$H_{aromat.}$), 119.6 (1C, **C**-9a), 111.7 (1C, **C**-9), 102.4 (1C, **C**-3a), 74.2 (1C, O**C**$H_2CH_2CH_2CH_3$), 67.1 (2C, morpholino-**C**H_2-O-**C**H_2), 52.1 (2C, morpholino-**C**H_2-N-**C**H_2), 31.4 (1C, OCH_2**C**$H_2CH_2CH_3$), 19.0 (1C, OCH_2CH_2**C**H_2CH_3), 13.6 (1C, $OCH_2CH_2CH_2$**C**H_3).

Ethyl 1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylate, ***57*** *(MB364)*



$C_{20}H_{25}FN_2O_4$

M_r = 376.43 g/mol

Compound **4a** (530 mg, 1.52 mmol) was suspended in a mixture of toluene/ethanol (10 mL/5 mL) and thionyl chloride (2 mL) was added to the mixture. The reaction solution heated at 70 °C for 4 h. Subsequently, the solution was poured on ice and was neutralized with saturated $NaHCO_3$ solution. The aqueous layer was extracted with $CHCl_3$ (3x30 mL) and the organic layers were dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* and the oily product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 50:1). The oily brown product crystallised at rt overnight to compound **57**.

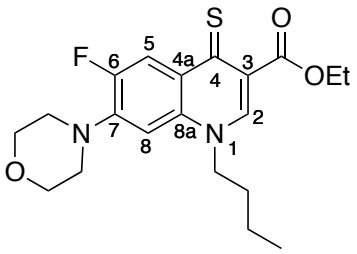
appearance:	brown solid
yield:	380 mg (1.01 mmol/ 66%)
reaction control:	R_f = 0.35 ($CHCl_3$/MeOH = 50:1))
melting point [°C]:	148-150 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2956, 2931, 2906, 2856, 1722, 1617, 1586, 1504, 1481, 1374, 1302, 1251, 1213.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 8.35 (s, 1H, **H**-2), 8.04 (d, 1H, ^3J = 13.2, **H**-5), 6.69 (d, 1H, ^4J = 6.8, **H**-8), 4.36 (q, 2H, ^3J = 7.6, O**C**H_2CH_3), 4.11 (t, 2H, ^3J = 7.6, N**C**$H_2CH_2CH_2CH_3$), 3.90 – 3.89 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.23 – 3.20 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.85 (quint, 2H, ^3J = 7.2, NCH_2**C**$H_2CH_2CH_3$), 1.46 – 1.37 (m, 5H, NCH_2CH_2**C**H_2CH_3/OCH_2**C**H_3), 0.99 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$**C**H_3).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 172.0 (d, 1C, $^4J_{C,F}$ = 2.5, **C**-4), 165.9 (1C, **C**OOEt), 152.6 (d, 1C, $^1J_{C,F}$ = 247.8, **C**-6), 148.5 (1C, **C**-2), 144.4 (d, 1C, $^3J_{C,F}$ = 10.4, **C**-

7), 136.2 (1C, **C**-8a), 124.0 (d, 1C, $^{4}J_{C,F}$ = 10.4, **C**-4a), 113.7 (d, 1C, $^{2}J_{C,F}$ = 22.9, **C**-5), 110.2 (**C**-3), 103.7 (d, 1C, $^{4}J_{C,F}$ = 3.6, **C**-8), 66.5 (2C, morpholino-**C**H_2-O-**C**H_2), 60.8 (1C, O**C**H_2CH_3), 53.3 (1C, N**C**$H_2CH_2CH_2CH_3$), 50.2 (d, 2C, $^{4}J_{C,F}$ = 4.8, morpholino-**C**H_2-N-**C**H_2), 30.6 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.8 (1C, NCH_2CH_2**C**H_2CH_3), 14.4 (1C, OCH_2**C**H_3), 13.6 (1C, $NCH_2CH_2CH_2$**C**H_3).

Ethyl 1-butyl-6-fluoro-7-morpholino-4-thioxo-1,4-dihydroquinoline-3-carboxylate, ***58*** *(MB366)*



$C_{20}H_{25}FN_2O_3S$

M_r =392.49 g/mol

According to Wentland *et al.*[102], compound **57** (210 mg, 0.56 mmol) was dissolved in abs. toluene (15 mL) and Lawesson's reagent (271 mg, 0.67 mmol) was added to the reaction. The reaction mixture heated at 60 °C for 2.5 h and the solvent was removed *in vacuo*. The crude red product was purified by column chromatography (silica gel; $CHCl_3$/MeOH = 50:1) to give **58**.

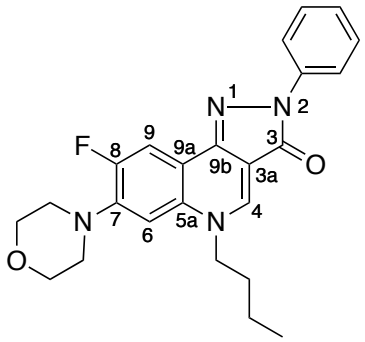
appearance:	yellow solid
yield:	190 mg (0.48 mmol/ 86%)
reaction control:	R_f = 0.33 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	160-161 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	2963, 2935, 2860, 2820, 1679, 1621, 1602, 1497, 1484, 1450, 1389, 1322, 1254, 1244.

^{1}H NMR (400 MHz, DMSO-d_6, δ [ppm], J [Hz]): 8.60 (d, 1H, ^{3}J = 15.2, **H**-5), 7.79 (s, 1H, **H**-2), 6.65 (d, 1H, ^{4}J = 7.2, **H**-8), 4.40 (q, 2H, ^{3}J = 7.2, O**C**H_2CH_3), 4.17 (t, 2H, ^{3}J = 7.2, N**C**$H_2CH_2CH_2CH_3$), 3.93 – 3.91 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.28 – 3.26 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.81 (quint, 2H, ^{3}J = 7.2, NCH_2C**H**$_2CH_2CH_3$), 1.45 – 1.38 (m, 5H, NCH_2CH_2C**H**$_2CH_3$/OCH_2C**H**$_3$), 0.98 (t, 3H, ^{3}J = 7.2, $NCH_2CH_2CH_2$C**H**$_3$).

^{13}C NMR (100 MHz, DMSO-d_6, δ [ppm], J [Hz]): 194.3 (d, 1C, $^{4}J_{C,F}$ = 2.5, **C**-4), 166.4 (1C, **C**OOEt), 152.6 (d, 1C, $^{1}J_{C,F}$ = 248.0, **C**-6), 144.8 (d, 1C, $^{3}J_{C,F}$ = 11.2, **C**-7), 139.2 (1C, **C**-

2), 132.0 (1C, **C**-8a), 131.1 (d, 1C, $^4J_{C,F}$ = 9.8, **C**-4a), 125.6 (**C**-3), 117.0 (d, 1C, $^2J_{C,F}$ = 24.8, **C**-5), 103.1 (d, 1C, $^4J_{C,F}$ = 3.6, **C**-8), 66.5 (2C, morpholino-**C**H_2-O-**C**H_2), 61.3 (1C, O**C**H_2CH_3), 54.8 (1C, N**C**$H_2CH_2CH_2CH_3$), 50.0 (d, 2C, $^4J_{C,F}$ = 4.8, morpholino-**C**H_2-N-**C**H_2), 30.6 (1C, NCH_2**C**$H_2CH_2CH_3$), 19.9 (1C, NCH_2CH_2**C**H_2CH_3), 14.2 (1C, OCH_2**C**H_3), 13.6 (1C, $NCH_2CH_2CH_2$**C**H_3).

5-Butyl-8-fluoro-7-morpholino-2-phenyl-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one, ***59*** *(MB363)*



$C_{24}H_{25}FN_4O_2$

M_r = 420.49 g/mol

According to El Bakali *et al.*[101], compound **58** (70 mg, 0.19 mmol) was dissolved in abs. EtOH and phenylhydrazine (25 µL, 0.23 mmol) added to the solution. The reaction solution refluxed for 15 h and after cooling down, the resulting precipitate was collected; the yellow solid was recrystallised from MeOH to give **59**.

appearance:	yellow solid
yield:	70 mg (0.17 mmol/ 87%)
reaction control:	R_f = 0.40 ($CHCl_3$/MeOH = 50:1)
melting point [°C]:	260-262 °C
IR (ATR, $\tilde{\nu}$ [cm^{-1}]):	3208, 2959, 2870, 2805, 1654, 1628, 1592, 1524, 1492, 1446, 1395, 1305, 1256, 1117.

^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 8.49 (s, 1H, **H**-4), 8.19 – 8.04 (m, 2H, Ph-C$H_{aromat.}$) 8.05 (d, 1H, ^3J = 12.4, **H**-9), 7.47 – 7.36 (m, 2H, Ph-C$H_{aromat.}$), 7.26 – 7.20 (m, 1H, Ph-C**H**), 6.92 (d, 1H, ^4J = 7.2, **H**-6), 4.25 (t, 2H, ^3J = 7.2, N**C**$H_2CH_2CH_2CH_3$), 3.94 – 3.92 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.23 – 3.21 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.91 (quint, 2H, ^3J = 7.2, NCH_2C**H$_2$**CH_2CH_3), 1.45 (sext, 2H, NCH_2CH_2C**H$_2$**CH_3), 1.02 (t, 3H, ^3J = 7.2, $NCH_2CH_2CH_2$C**H$_3$**).

^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm], *J* [Hz]): 161.1 (1C, **C**-3), 15247 (1C, $^1J_{C,F}$ = 249.7, **C**-8), 142.6 (d, 1C, $^4J_{C,F}$ = 1.7, **C**-9b), 142.4 (d, 1C, $^2J_{C,F}$ = 10.2, **C**-7), 141.9 (1C, **C**-4), 139.6 (1C, Ph-**C**$_{aromat.}$), 131.9 (1C, $^4J_{C,F}$ = 2.5, **C**-5a), 128.4 (2C, Ph-**C**H$_{aromat.}$), 125.1 (1C, Ph-**C**H$_{aromat.}$), 120.2 (2C, Ph-**C**H$_{aromat.}$), 115.3 (d, 1C, $^3J_{C,F}$ = 7.9, **C**-9a), 110.2 (d, 1C, $^2J_{C,F}$ = 22.4, **C**-9), 107.1 (1C, **C**-3a), 105.6 (d, 1C, $^3J_{C,F}$ = 2.7, **C**-6), 66.7 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 55.0 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 50.5 (d, 2C, $^4J_{C,F}$ = 4.2, morpholino-**C**H$_2$-N-**C**H$_2$), 30.8 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.9 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.6 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

10.10 Cell Viability Assay

All cell culture experiments were performed under sterile conditions using a class II laminar airflow safety cabinet. The macrophage cell line J774.1 was maintained in RPMI-1640 medium, supplemented with 10% FCS, 10 U/mL penicillin G, 10 μg/mL streptomycin, and 50 μM 2-mercaptoethanol in a humidified (95%) atmosphere at 37 °C and 5% CO_2. Cell density was daily monitored and medium was renewed every 2-3 days. The macrophages were seeded at a density of 1-3 x 10^5 cells per mL; 70-80% confluency (5-7 x 10^5 cells per mL) was reached after 2-3 days.

For the experimental procedure, previously reported protocol was applied.[7,8] The cells were detached from the flasks with a cell scraper, and cell density was adjusted (final cell density in the assay: 2 x 10^5 / mL). J774.1 macrophages were seeded into the chambers of the 96-well plates and were incubated overnight to allow attachment and recovery. Various concentrations of the compounds were added to the cells; positive (macrophages in culture medium) and negative controls (culture medium without cells) were run with each plate. Incubation time period was 24 h at 37 °C, 5% CO_2, 95% humidity. Following the addition of AlamarBlue ® (20 µL), the plates were further incubated at similar conditions. The absorbance was read at a wavelength of 550 nm (reference wavelength 630 nm) after 24 and 48 h. Absorbance in the absence of compounds was set as 100% of growth control. The CC_{50} values are presented as mean values of two independent experiments.

10.11 LogP Determination

The logarithmized capacity factor of the calibration substances was correlated with the experimental octanol/water logP values, and the resulted linear equation was applied to calculate logP value of the tested compounds.[6] The test substances were dissolved in concentration of 4 mg/mL in DMSO and subsequent diluted to 4 µg/mL with mobile phase. The samples were measured with HPLC method **II** and the capacity factor *k'* was determined as

$$k' = \frac{(t_R - t_0)}{t_0}$$

t_R = retention time of the analyte
t_0 = dead time of the column

A linear regression was performed for the log k'/logP data of the reference compounds. The following substances with the corresponding logP values according literature, were used for calibration. The regression equation was used to calculate the logP of the analytes.

reference substances	dead time t_0 [41]	retention time t_R [41]	capacity factor *k'*	log*k'*	logP value
acetanilide	1.60	2.62	0.64	-0.19	1.10
2-phenylethanol	1.60	2.89	0.80	-0.10	1.36
benzene	1.60	5.22	2.26	0.36	2.13
toluene	1.60	7.77	3.86	0.59	2.74
biphenyl	1.60	17.50	9.94	1.00	4.01
anthracene	1.60	27.90	3.44	1.22	4.45

Table 14: *Data for the logP calibration curve*

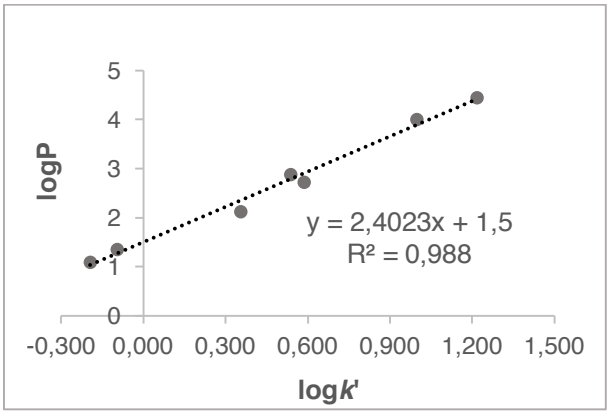


Figure 63: *Calibration curve for determination of logP values*

10.12 Solubility

For the assessment of the thermodynamic solubility, the continuous shake flask protocol according to reference[8] was applied. The compound was dosed in excess into Eppendorf vials and dissolved in PBS buffer (pH 7.4) Throughout a period of 24 h of continuous shaking (800 rpm) and constant warming (37 °C), samples (approx. 100 µL) were taken. After centrifugation (13.000 rpm, 1 min), the supernatant (80 µL) was analysed with HPLC method **III**. Data are representative of three independent experiments and values are expressed in mean (SD).

For the calibration equation, approx. 2 mg of the analysed compound was dissolved in MeOH and diluted to concentrations of 100 µg/mL, 50 µg/mL, 10 µg/mL, 1 µg/mL, 0.5 µg/mL, and 0.1 µg/mL. The peak areas and the concentrations were plotted against each other to give the calibration curve.

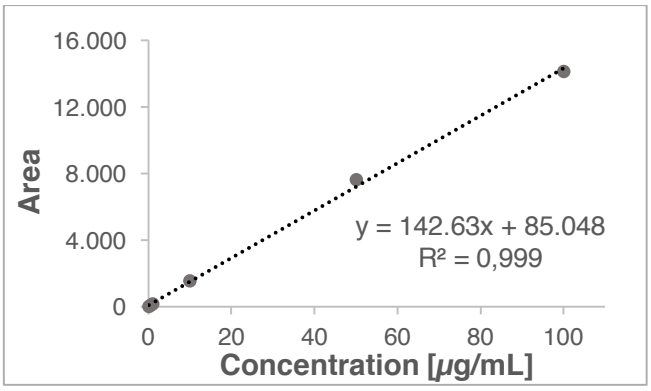


Figure 64: *Calibration curve (0.1 µg/mL-100 µL) of compound* ***6****.*

11 Appendix

11.1 Supplemental HMBC spectra

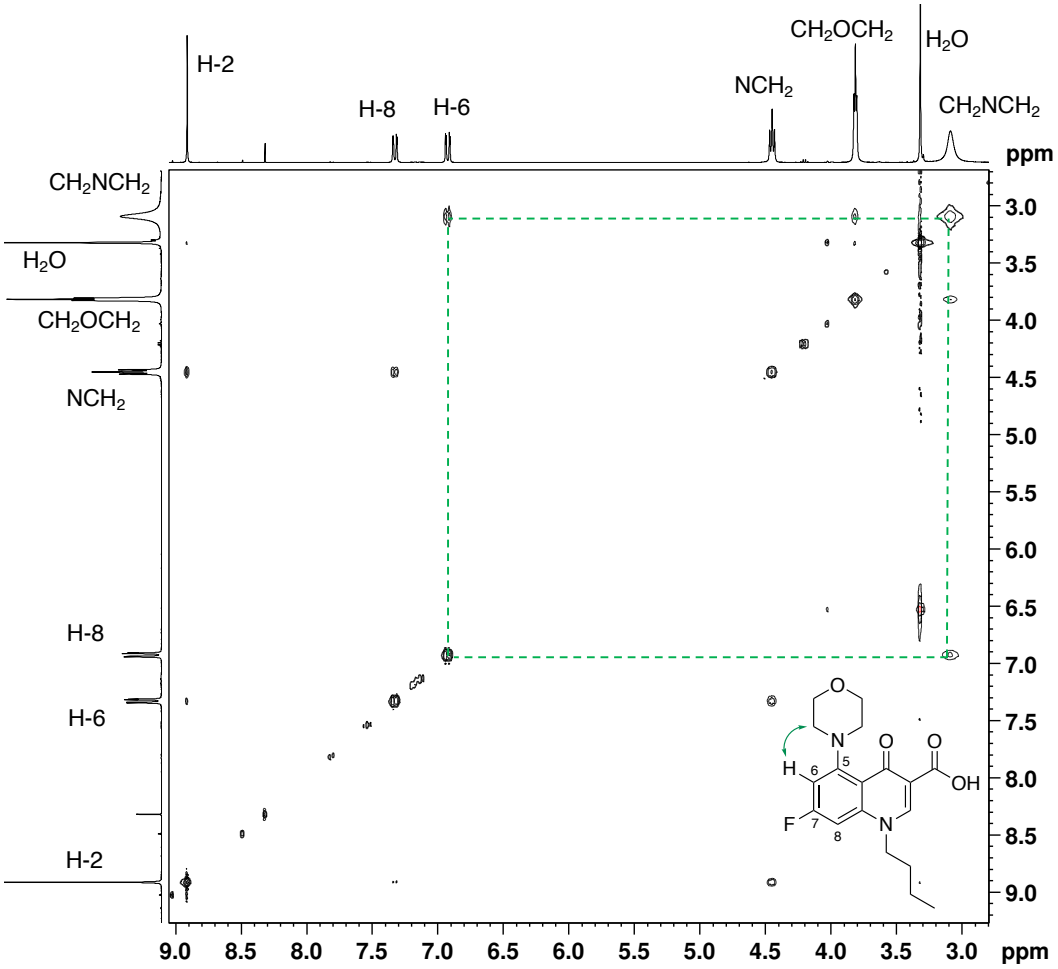


Figure 65: *HMBC analysis of compound **4l** illustrates the vicinity of **C**H_2-N-**C**H_2 of the morpholine ring and the **H**-6 (green) of the quinolone core.*

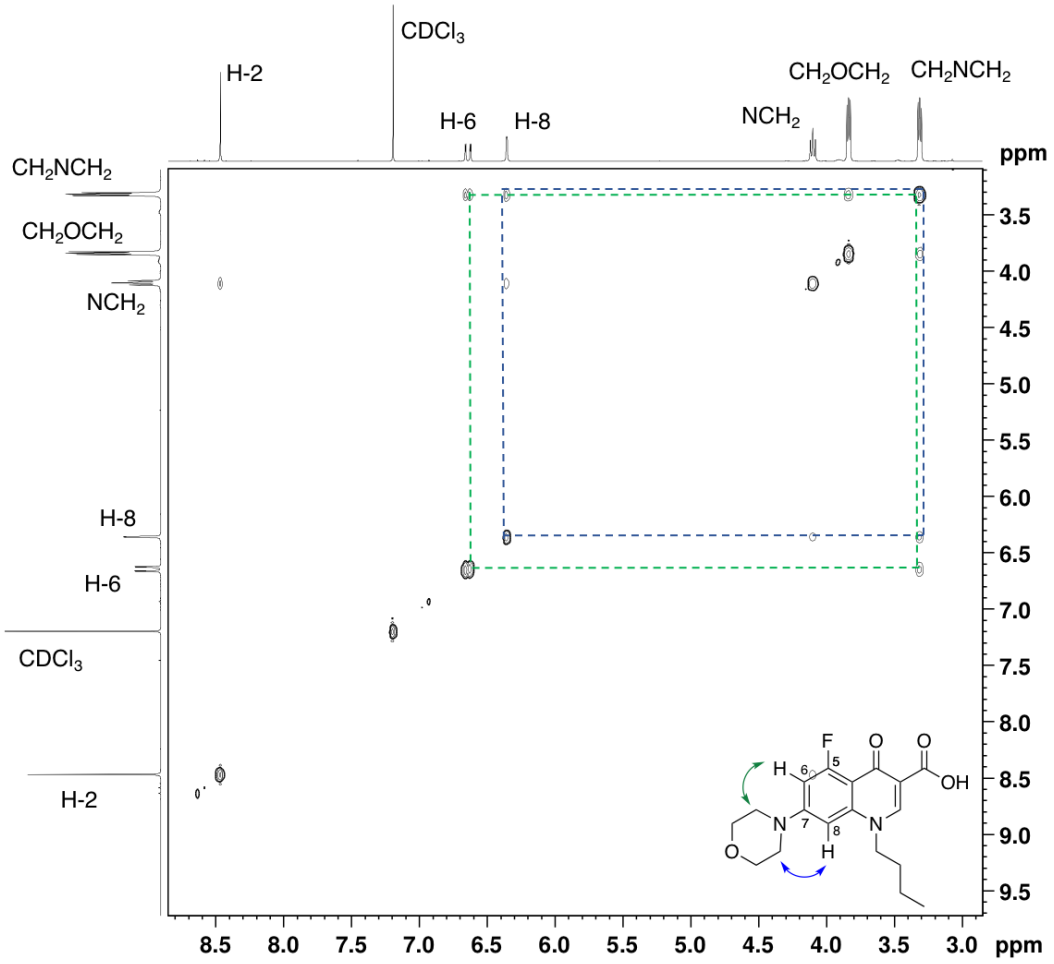
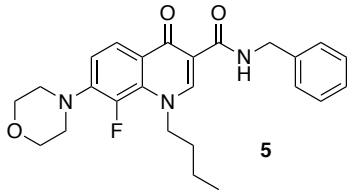
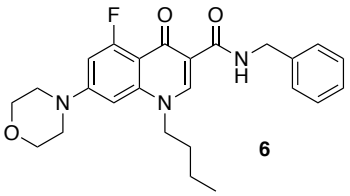
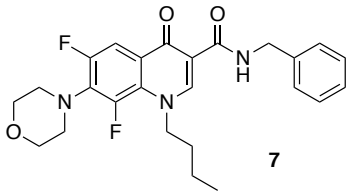


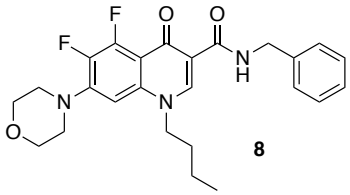
Figure 66: *HMBC analysis of compound **4e** illustrates the vicinity of **C**H_2-N-**C**H_2 and both the **H**-6 (green) and the **H**-8 (blue) of the quinolone core.*

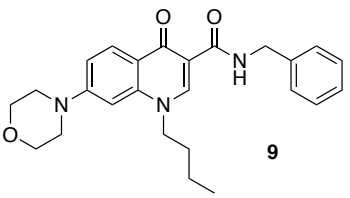
11.2 Overview of the synthesised Quinolone Amides

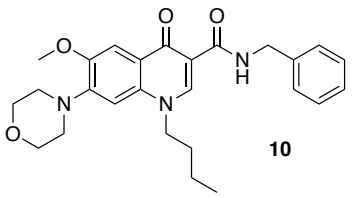

5

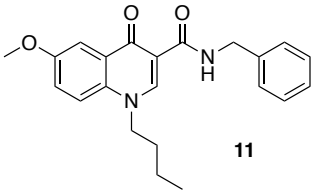

6

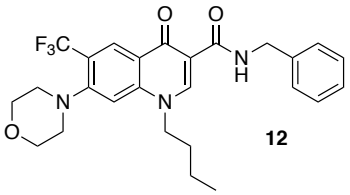

7

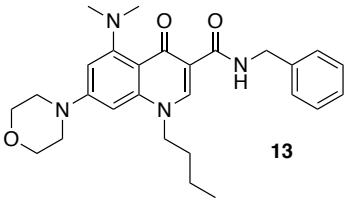

8

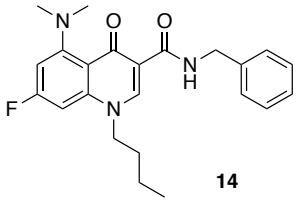

9

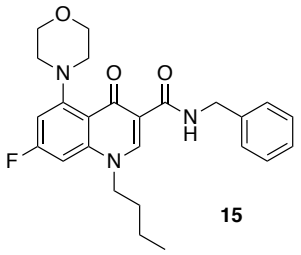

10

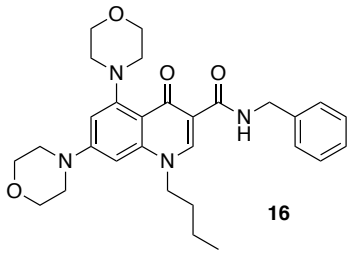

11

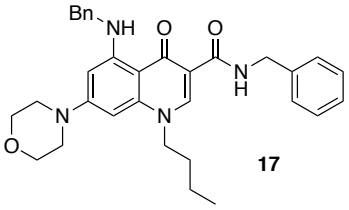

12

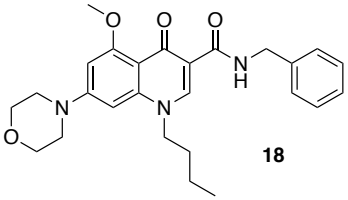

13

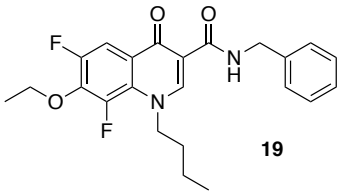

14

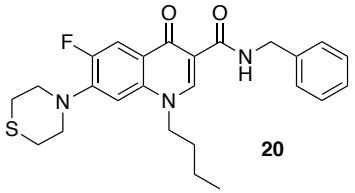

15

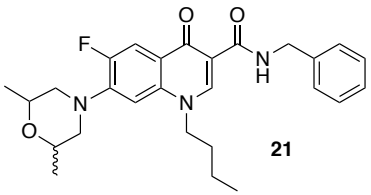

16

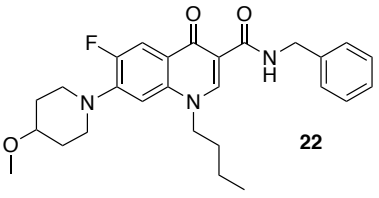

17

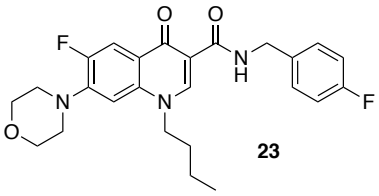

18

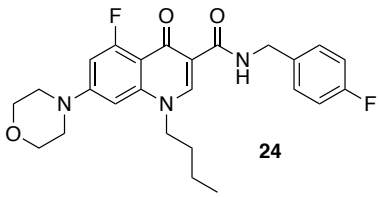

19

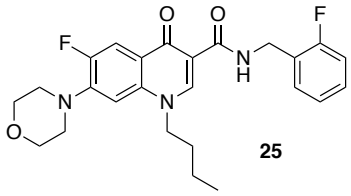

20

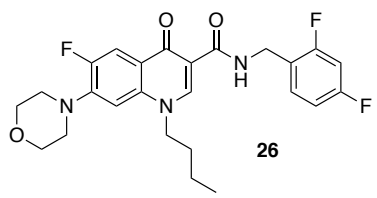

21


22

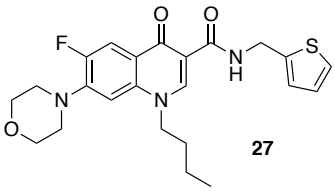

23


24

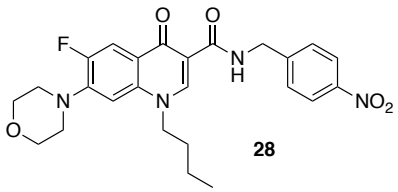

25



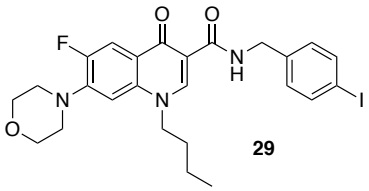
26



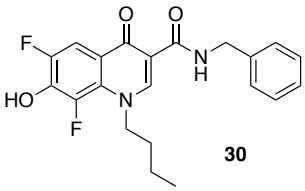
27



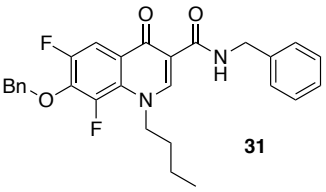
28



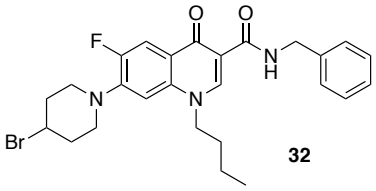
29



30

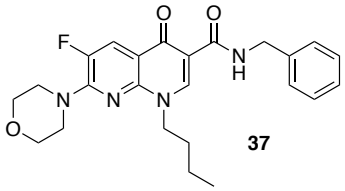


31

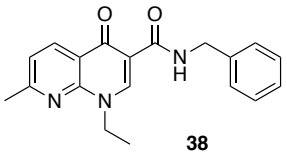


32

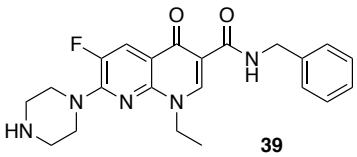
11.3 Overview of the synthesised Quinolone Amides derived from commercially Fluoroquinolones and 1,8-Naphthyridones



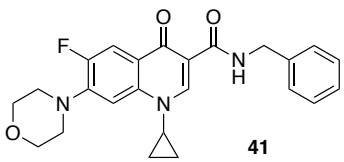
37



38

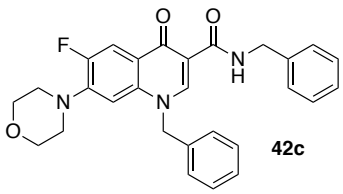


39

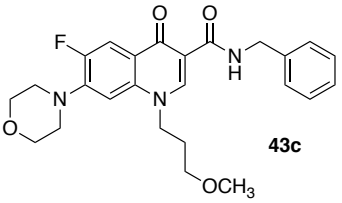


41

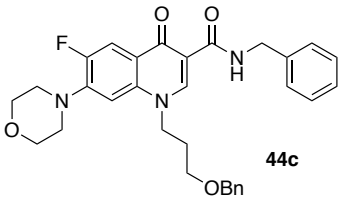
11.4 Overview of the synthesised Quinolone Amides for the ^{18}F Labelling approach



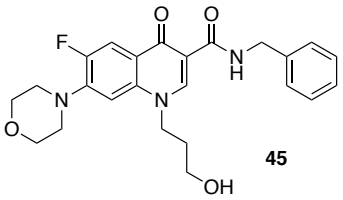
42c



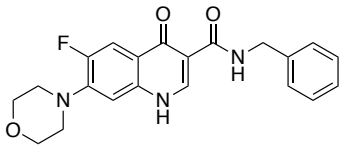
43c



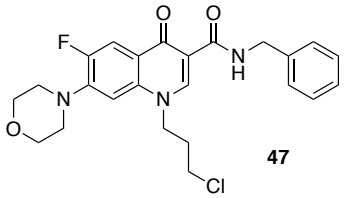
44c



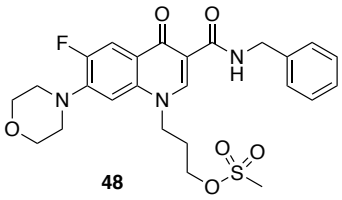
45



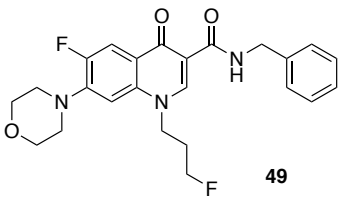
46



47

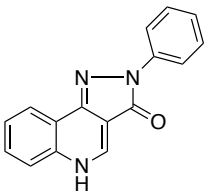


48

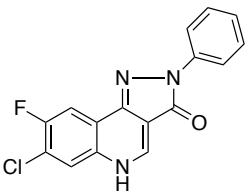


49

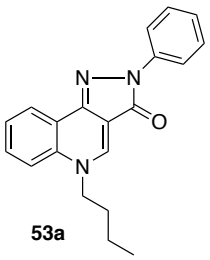
11.5 Overview of the synthesised Pyrazolo[4,3-c]quinolin-3-ones



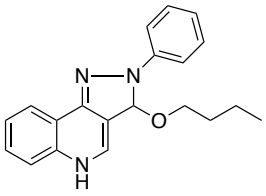
51a



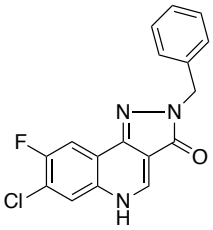
51b



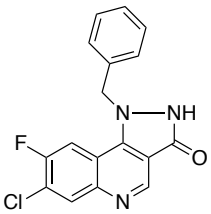
53a



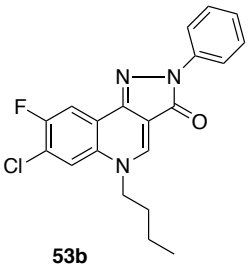
53a'



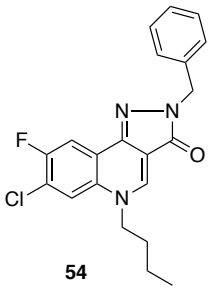
52



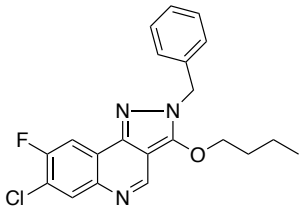
52'



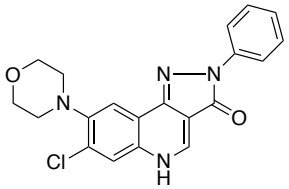
53b



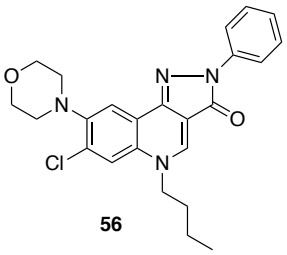
54



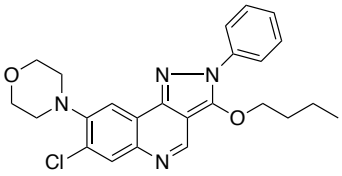
54'



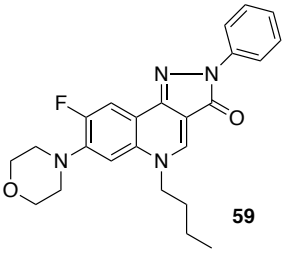
55



56



56'



59

11.6 Documentation of Authorship

This section contains a list of the individual contribution for each author of the publication reprinted in this thesis:

Novel lead compounds in pre-clinical development against African sleeping sickness, M. Berninger, I. Schmidt, A. Ponte-Sucre and U. Holzgrabe, *Med. Chem. Commun.*, 2017, 8, 1872-1890.

Author	**1**	**2**	**3**	**4**
Study design / concept development	x	x		x
Literature analysis and interpretation	x	x	x	
Manuscript planning	x	x		x
Manuscript writing	x		x	
Correction of manuscript	x		x	x
Supervision of Michael Berninger				x

12 References

1 Nenortas, E.; Burri, C.; Shapiro, T. A. Antitrypanosomal Activity of Fluoroquinolones. *Antimicrob. Agents Chemother.* **1999,** 43, 2066-2068.

2 Keiser, J.; Burri, C. Evaluation of quinolone derivatives for antitrypanosomal activity. *Trop. Med. Int. Health* **2001,** 6, 369-389.

3 Ma, X.; Zhou, W.; Brun, R. Synthesis, in vitro antitrypanosomal and antibacterial activity of phenoxy, phenylthio or benzyloxy substituted quinolones. *Bioorg. Med. Chem. Lett.* **2009,** 19, 986-989.

4 Shen, L. L.; Mitscher, L. A.; Sharma, P. N.; O'Donnell, T. J.; Chu, D. W. T.; Cooper, C. S.; Rosen, T.; Pernet, A. G. Mechanism of inhibition of DNA gyrase by quinolone antibacterials: a cooperative drug-DNA binding model. *Biochemistry* **1989,** 28, 3886-3894.

5 Niedermeier, S. *4-Chinolone als Ausgangspunkt für antiparasitäre und antivirale Wirkstoffe*. Dissertation, Julius-Maximilians-Universität Würzburg, 2010.

6 Hiltensperger, G. *4-Chinolon-3-carboxamide Entwicklung neuer Wirkstoffe zur Behandlung der afrikanischen Schlafkrankheit*. Dissertation, Julius-Maximilians-Universität Würzburg, 2013.

7 Hiltensperger, G.; Jones, N. G.; Niedermeier, S.; Stich, A.; Kaiser, M.; Jung, J.; Puhl, S.; Damme, A.; Braunschweig, H.; Meinel, L.; Engstler, M.; Holzgrabe, U. Synthesis and structure-activity relationships of new quinolone-type molecules against Trypanosoma brucei. *J. Med. Chem.* **2012,** 55, 2538-48.

8 Hiltensperger, G.; Hecht, N.; Kaiser, M.; Rybak, J.-C.; Hoerst, A.; Dannenbauer, N.; Müller-Buschbaum, K.; Bruhn, H.; Esch, H.; Lehmann, L.; Meinel, L.; Holzgrabe, U. Quinolone Amides as Antitrypanosomal Lead Compounds with In Vivo Activity. *Antimicrob. Agents Chemother.* **2016,** 60, 4442-4452.

9 Claisen, L. Untersuchungen über die Oxymethylenverbindungen. *Justus Liebigs Ann. Chem.* **1894,** 281, 306-313.

10 Camps, R. Von der Amidophenylpropiolsäure zur Kynurensäure und deren Verwandten. *Berichte der deutschen chemischen Gesellschaft* **1901,** 34, 2703-2718.

11 Lesher, G. Y.; Froelich, E. J.; Gruett, M. D.; Bailey, J. H.; Brundage, R. P. 1,8-Naphthyridine Derivatives. A New Class of Chemotherapeutic Agents. *J. Med. Pharm. Chem.* **1962,** 5, 1063-1065.

12 Bisacchi, G. S. Origins of the Quinolone Class of Antibacterials: An Expanded “Discovery Story”. *J. Med. Chem.* **2015,** 58, 4874-4882.

13 Barton, N.; Crowther, A. F.; Hepworth, W.; Richardson, N. D.; Driver, G. W. New Quinolones and Therapeutic Compositions Containing Them. GB830832, **1960**.

14 https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm513183.htm (13.03.18)

15 Niedermeier, S.; Singethan, K.; Rohrer, S. G.; Matz, M.; Kossner, M.; Diederich, S.; Maisner, A.; Schmitz, J.; Hiltensperger, G.; Baumann, K.; Holzgrabe, U.; Schneider-Schaulies, J. A Small-Molecule Inhibitor of Nipah Virus Envelope Protein-Mediated Membrane Fusion. *J. Med. Chem.* **2009,** 52, 4257-4265.

16 Leyva, E.; Monreal, E.; Hernández, A. Synthesis of fluoro-4-hydroxyquinoline-3-carboxylic acids by the Gould–Jacobs reaction. *J. Fluorine Chem.* **1999,** 94, 7-10.

17 Bünz, C.; Heber, D.; Holzgrabe, U.; Sürig, U. E–Z isomerism of anilinomethylenemalonic acid derivatives. *Magn. Reson. Chem.* **1993,** 31, 371-374.

18 Karplus, M. Vicinal Proton Coupling in Nuclear Magnetic Resonance. *J. Am. Chem. Soc.* **1963,** 85, 2870-2871.

19 de la Cruz, A.; Elguero, J.; Goya, P.; Martínez, A.; Pfleiderer, W. Tautomerism and acidity in 4-quinolone-3-carboxylic acid derivatives. *Tetrahedron* **1992,** 48, 6135-6150.

20 Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. Structure-activity relationships of antibacterial 6,7- and 7,8-disubstituted 1-alkyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acids. *J. Med. Chem.* **1980,** 23, 1358-63.

21 Podányi, B.; Keresztúri, G.; Vasvári-Debreczy, L.; Hermecz, I.; Tóth, G. An NMR Study of Halogenated 1,4-Dihydro-1-ethyl-4-oxoquinoline-3-carboxylates. *Magn. Reson. Chem.* **1996,** 34, 972-978.

22 Leyva, S.; Leyva, E. Thermochemical reaction of 7-azido-1-ethyl-6,8-difluoroquinolone-3-carboxylate with heterocyclic amines. An expeditious synthesis of novel fluoroquinolone derivatives. *Tetrahedron* **2007,** 63, 2093-2097.

23 Watanuki, S.; Koga, Y.; Moritomo, H.; Tsukamoto, I. Quinolone derivative or salt thereof. *PCT Int. Appl.* WO 2005009971 A1 20050203, **2005**.

24 Conroy, A. E.; Mosher, H. S.; Whitmore, F. C. Heterocyclic Basic Compounds. XII. 7-Bromo-and 7-Iodo-quinolines. *J. Am. Chem. Soc.* **1949,** 71, 3236-3237.

25 Snyder, H. R.; Freier, H. E.; Kovacic, P.; Heyningen, E. M. v. Synthesis of 4-Hydroxyquinolines. VIII. Some Halogen Containing 4-Aminoquinoline Derivatives. *J. Am. Chem. Soc.* **1947,** 69, 371-374.

26 Massari, S.; Daelemans, D.; Manfroni, G.; Sabatini, S.; Tabarrini, O.; Pannecouque, C.; Cecchetti, V. Studies on anti-HIV quinolones: new insights on the C-6 position. *Biorg. Med. Chem.* **2009,** 17, 667-74.

27 Price, C. C.; Roberts, R. M. The Synthesis of 4-Hydroxyquinolines. I. Through Ethoxymethylenemalonic Ester. *J. Am. Chem. Soc.* **1946,** 68, 1204-1208.

28 Hermecz, I.; Meszaros, Z.; Vasvari-Debreczy, L.; Horvath, A.; Horvath, G.; Pongor-Csakvari, M. Nitrogen bridgehead compounds. Part 4. 1-3 N-C-acyl migration. Part 2. *J. Chem. Soc., Perkin Trans. 1* **1977**, 789-795.

29 Adams, R.; Pachter, I. J. Ultraviolet Spectra and Structures of the Pyrido [1,2-a]pyrimidones. *J. Am. Chem. Soc.* **1952,** 74, 5491-5497.

30 Lappin, G. R. Cyclization of 2-Aminopyridine Derivatives. I. Substituted Ethyl 2-Pyridylaminomethylenemalonates1,2. *J. Am. Chem. Soc.* **1948,** 70, 3348-3350.

31 Makara, G.; Keser, G. M.; Kovacs, A. On the mechanism of the alkylation of quinoline and naphthyridine derivatives. *Journal of the Chemical Society, Perkin Transactions 2* **1994**, 591-594.

32 Pearson, R. G. Hard and Soft Acids and Bases. *J. Am. Chem. Soc.* **1963,** 85, 3533-3539.

33 Bunnett, J. F.; Garbisch, E. W.; Pruitt, K. M. The “Element Effect” as a Criterion of Mechanism in Activated Aromatic Nucleophilic Substitution Reactions1,2. *J. Am. Chem. Soc.* **1957,** 79, 385-391.

34 Senger, N. A.; Bo, B.; Cheng, Q.; Keeffe, J. R.; Gronert, S.; Wu, W. The Element Effect Revisited: Factors Determining Leaving Group Ability in Activated Nucleophilic Aromatic Substitution Reactions. *J. Org. Chem.* **2012,** 77, 9535-9540.

35 Shibamori, K.-i.; Egawa, H.; Miyamoto, T.; Nishimura, Y.; Itokawa, A.; Nakano, J.; Matsumoto, J.-i. Regioselective Displacement Reactions of 1-Cyclopropyl-5, 6, 7, 8-tetrafluoro-4(1H)-oxoquinoline-3-carboxylic Acid with Amine Nucleophiles. *Chem. Pharm. Bull. (Tokyo)* **1990,** 38, 2390-2396.

36 Suzuki, N.; Tanaka, Y.; Dohmori, R. Synthesis of Antimicrobial Agents. I. Synthesis and Antimicrobial Activities of Thiazoloquinoline Derivatives. *Chem. Pharm. Bull. (Tokyo)* **1979,** 27, 1-11.

37 Grohe, K.; Heitzer, H. Cycloaracylierung von Enaminen, I. Synthese von 4-Chinolon-3-carbonsäuren. *Liebigs Ann. Chem.* **1987,** 1987, 29-37.

38 Sunduru, N.; Gupta, L.; Chauhan, K.; Mishra, N. N.; Shukla, P. K.; Chauhan, P. M. Synthesis and antibacterial evaluation of novel 8-fluoro Norfloxacin derivatives as potential probes for methicillin and vancomycin-resistant Staphylococcus aureus. *Eur. J. Med. Chem.* **2011,** 46, 1232-44.

39 Hermecz, I.; Vasvári-Debreczy, L.; Podányi, B.; Kereszturi, G.; Balogh, M.; Horváth, Á.; Várkonyi, P. Regioselective nucleophilic substitution of halogen derivatives of 1-substituted 4-oxo-1,4-dihydroquinoline-3-carboxylic acids. *Heterocycles* **1998,** 48, 1111-1116.

40 Moran, D. B.; Ziegler, C. B.; Dunne, T. S.; Kuck, N. A.; Lin, Y. I. Synthesis of novel 5-fluoro analogs of norfloxacin and ciprofloxacin. *J. Med. Chem.* **1989,** 32, 1313-1318.

41 Miyamoto, T.; Matsumoto, J.; Chiba, K.; Egawa, H.; Shibamori, K.; Minamida, A.; Nishimura, Y.; Okada, H.; Kataoka, M.; Fujita, M.; Hirose, T.; Nakano, J. Synthesis and structure-activity relationships of 5-substituted 6,8-difluoroquinolones, including sparfloxacin, a new quinolone antibacterial agent with improved potency. *J. Med. Chem.* **1990,** 33, 1645-56.

42 Heravi, M. M.; Jaddi, Z. S.; Oskooie, H. A.; Khaleghi, S.; Ghassemzadeh, M. Regioselective synthesis of quinolone antibacterials via borate complex of quinolone carboxylic acid. *J. Chem. Res.* **2005,** 2005, 578-579.

43 Lin, L.; Zhai, Y.; Wang, D.; Yin, G.; Fan, L.; Hu, Y. Preparation, characterization and spectroscopic properties of difluoroboron complexes with some fluoroquinolones. *J. Fluorine Chem.* **2016,** 182, 7-11.

44 Mitscher, L. A. Bacterial Topoisomerase Inhibitors: Quinolone and Pyridone Antibacterial Agents. *Chem. Rev.* **2005,** 105, 559-592.

45 Langmuir, I. The Structure of Atoms and the Octet Theory of Valence. *Proc. Natl. Acad. Sci. U. S. A.* **1919,** 5, 252-259.

46 Friedman, H. *Influence of isosteric replacements upon biological activity*. NAS-NRS Publication No. 206: 1951.

47 Meanwell, N. A. Synopsis of Some Recent Tactical Application of Bioisosteres in Drug Design. *J. Med. Chem.* **2011,** 54, 2529-2591.

48 Wirth, M.; Zoete, V.; Michielin, O.; Sauer, W. H. B. SwissBioisostere: a database of molecular replacements for ligand design. *Nucleic Acids Res.* **2013,** 41, D1137-D1143.

49 http://www.swissbioisostere.ch/ (06.12.17).

50 Benton, F. L.; Dillon, T. E. The Cleavage of Ethers with Boron Bromide. I. Some Common Ethers. *J. Am. Chem. Soc.* **1942,** 64, 1128-1129.

51 Sousa, C.; Silva, P. J. BBr3-Assisted Cleavage of Most Ethers Does Not Follow the Commonly Assumed Mechanism. *Eur. J. Org. Chem.* **2013,** 2013, 5195-5199.

52 Kosak, T. M.; Conrad, H. A.; Korich, A. L.; Lord, R. L. Ether Cleavage Re-Investigated: Elucidating the Mechanism of BBr3-Facilitated Demethylation of Aryl Methyl Ethers. *Eur. J. Org. Chem.* **2015,** 2015, 7460-7467.

53 Grohe, K. Zur Bedeutung des Cycloaracylierungsverfahrens für die Synthese moderner Fluorchinolone. *Journal für Praktische Chemie/Chemiker-Zeitung* **1993,** 335, 397-409.

54 Bouzard, D.; Di Cesare, P.; Essiz, M.; Jacquet, J. P.; Remuzon, P.; Weber, A.; Oki, T.; Masuyoshi, M. Fluoronaphthyridines and quinolones as antibacterial agents. 1. Synthesis and structure-activity relationship of new 1-substituted derivatives. *J. Med. Chem.* **1989,** 32, 537-542.

55 Egawa, H.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Okada, H.; Uno, H.; Matsumoto, J. Pyridonecarboxylic acids as antibacterial agents. Synthesis and antibacterial activity of 7-(3-amino-1-pyrrolidinyl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid and its analogs. *J. Med. Chem.* **1984,** 27, 1543-1548.

56 Tabarrini, O.; Massari, S.; Sancineto, L.; Daelemans, D.; Sabatini, S.; Manfroni, G.; Cecchetti, V.; Pannecouque, C. Structural Investigation of the Naphthyridone Scaffold: Identification of a 1,6-Naphthyridone Derivative with Potent and Selective Anti-HIV Activity. *ChemMedChem* **2011,** 6, 1249-1257.

57 Wang, J.; Sánchez-Roselló, M.; Aceña, J. L.; del Pozo, C.; Sorochinsky, A. E.; Fustero, S.; Soloshonok, V. A.; Liu, H. Fluorine in Pharmaceutical Industry:

Fluorine-Containing Drugs Introduced to the Market in the Last Decade (2001–2011). *Chem. Rev.* **2014,** 114, 2432-2506.

58 Zhou, Y.; Wang, J.; Gu, Z.; Wang, S.; Zhu, W.; Aceña, J. L.; Soloshonok, V. A.; Izawa, K.; Liu, H. Next Generation of Fluorine-Containing Pharmaceuticals, Compounds Currently in Phase II–III Clinical Trials of Major Pharmaceutical Companies: New Structural Trends and Therapeutic Areas. *Chem. Rev.* **2016,** 116, 422-518.

59 Shengguo, S.; Adeboye, A. Fluorinated Molecules as Drugs and Imaging Agents in the CNS. *Curr. Top. Med. Chem.* **2006,** 6, 1457-1464.

60 Gillis, E. P.; Eastman, K. J.; Hill, M. D.; Donnelly, D. J.; Meanwell, N. A. Applications of Fluorine in Medicinal Chemistry. *J. Med. Chem.* **2015,** 58, 8315-8359.

61 Liang, T.; Neumann, C. N.; Ritter, T. Introduction of Fluorine and Fluorine-Containing Functional Groups. *Angew. Chem. Int. Ed.* **2013,** 52, 8214-8264.

62 Kirk, K. L. Fluorination in Medicinal Chemistry: Methods, Strategies, and Recent Developments. *Organic Process Research & Development* **2008,** 12, 305-321.

63 Shukla, A. K.; Kumar, U. Positron emission tomography: An overview. *Journal of Medical Physics / Association of Medical Physicists of India* **2006,** 31, 13-21.

64 Li, Z.; Conti, P. S. Radiopharmaceutical chemistry for positron emission tomography. *Advanced Drug Delivery Reviews* **2010,** 62, 1031-1051.

65 Jacobson, O.; Chen, X. PET Designated Flouride-18 Production and Chemistry. *Curr. Top. Med. Chem.* **2010,** 10, 1048-1059.

66 Saleem, A.; Charnley, N.; Price, P. Clinical molecular imaging with positron emission tomography. *Eur. J. Cancer* **2006,** 42, 1720-1727.

67 Piel, M.; Vernaleken, I.; Rösch, F. Positron Emission Tomography in CNS Drug Discovery and Drug Monitoring. *J. Med. Chem.* **2014,** 57, 9232-9258.

68 Kilbourn, M. R.; Huizenga, J. R. *Fluorine-18 Labeling of Radiopharmaceuticals*. National Academy Press: Washington, DC, 1990.

69 Jacobson, O.; Kiesewetter, D. O.; Chen, X. Fluorine-18 Radiochemistry, Labeling Strategies and Synthetic Routes. *Bioconjugate Chem.* **2015,** 26, 1-18.

70 Babich, J. W.; Rubin, R. H.; Graham, W. A.; Wilkinson, R. A.; Vincent, J.; Fischman, A. J. 18F-labeling and biodistribution of the novel fluoro-quinolone antimicrobial agent, trovafloxacin (CP 99,219). *Nucl. Med. Biol.* **1996,** 23, 995-998.

71 Tewson, T. J.; Yang, D.; Wong, G.; Macy, D.; DeJesus, O. J.; Nickles, R. J.; Perlman, S. B.; Taylor, M.; Frank, P. The synthesis of fluorine-18 lomefloxacin and its preliminary use in human studies. *Nucl. Med. Biol.* **1996,** 23, 767-772.

72 Fischman, A. J.; Livni, E.; Babich, J.; Alpert, N. M.; Liu, Y. Y.; Thom, E.; Cleeland, R.; Prosser, B. L.; Correia, J. A.; Strauss, H. W. Pharmacokinetics of [18F]fleroxacin in healthy human subjects studied by using positron emission tomography. *Antimicrob. Agents Chemother.* **1993,** 37, 2144-2152.

73 Langer, O.; Mitterhauser, M.; Brunner, M.; Zeitlinger, M.; Wadsak, W.; Mayer, B. X.; Kletter, K.; Müller, M. Synthesis of fluorine-18-labeled ciprofloxacin for PET studies in humans. *Nucl. Med. Biol.* **2003,** 30, 285-291.

74 Turkman, N.; Shavrin, A.; Paolillo, V.; Yeh, H. H.; Flores, L.; Soghomonian, S.; Rabinovich, B.; Volgin, A.; Gelovani, J.; Alauddin, M. Synthesis and preliminary evaluation of [18F]-labeled 2-oxoquinoline derivatives for PET imaging of cannabinoid CB2 receptor. *Nucl. Med. Biol.* **2012,** 39, 593-600.

75 Livni, E.; Babich, J.; Alpert, N. M.; Liu, Y.-Y.; Thom, E.; Cleeland, R.; Prosser, B. L.; Correia, J. A.; Strauss, H. W.; Rubin, R. H.; Fischman, A. J. Synthesis and biodistribution of 18F-labeled Fleroxacin. *Nucl. Med. Biol.* **1993,** 20, 81-87.

76 Soom, N. A.; Thiemann, T. Hydrogenation of Alkenes with NaBH4,CH3CO2H, Pd/C in the Presence of O- and N-Benzyl Functions. *International Journal of Organic Chemistry* **2016,** 06, 1-11.

77 Kabalka, G. W.; Varma, M.; Varma, R. S.; Srivastava, P. C.; Knapp, F. F. The tosylation of alcohols. *J. Org. Chem.* **1986,** 51, 2386-2388.

78 Ding, R.; He, Y.; Wang, X.; Xu, J.; Chen, Y.; Feng, M.; Qi, C. Treatment of Alcohols with Tosyl Chloride Does Not always Lead to the Formation of Tosylates. *Molecules* **2011,** 16, 5665.

79 Middleton, W. J. New fluorinating reagents. Dialkylaminosulfur fluorides. *J. Org. Chem.* **1975,** 40, 574-578.

80 Berninger, M.; Erk, C.; Fuß, A.; Skaf, J.; Al-Momani, E.; Israel, I.; Raschig, M.; Güntzel, P.; Samnick, S.; Holzgrabe, U. Fluorine walk: The impact of fluorine in quinolone amides on their activity against African sleeping sickness. *Eur. J. Med. Chem.* **2018,** 152, 377-391.

81 Talele, T. T. The “Cyclopropyl Fragment” is a Versatile Player that Frequently Appears in Preclinical/Clinical Drug Molecules. *J. Med. Chem.* **2016,** 59, 8712-8756.

82 Meanwell, N. A. Fluorine and Fluorinated Motifs in the Design and Application of Bioisosteres for Drug Design. *J. Med. Chem.* **2018**.

83 Yoshida, T.; Yamamoto, Y.; Orita, H.; Kakiuchi, M.; Takahashi, Y.; Itakura, M.; Kado, N.; Mitani, K.; Yasuda, S.; Kato, H.; Itoh, Y. Studies on Quinolone Antibacterials. IV. Structure-Activity Relationship of Antibacterial Activity and Side Effects for 5- and 8-Substituted and 5,8-Disubstituted-7-(3-amino-1-pyrrolidinyl)-1-cyclopropyl-1,4-dihydro-4-oxoquinoline-3-carboxylic Acids. *Chem. Pharm. Bull.* **1996,** 44, 1074-1085.

84 Müller, K.; Faeh, C.; Diederich, F. Fluorine in Pharmaceuticals: Looking Beyond Intuition. *Science* **2007,** 317, 1881.

85 Poole, R. M. Nemonoxacin: First Global Approval. *Drugs* **2014,** 74, 1445-1453.

86 Eliane, S.; Anja, H. R.; Kaspar, S.; A., O. J.; Christoph, F.; Paul, S.; Ulrike, O. S.; Björn, W.; Manfred, K.; François, D. A Fluorine Scan at the Catalytic Center of Thrombin: C-F, C-OH, and C-OMe Bioisosterism and Fluorine Effects on pKa and log D Values. *ChemMedChem* **2006,** 1, 611-621.

87 Böhm, H. J.; Banner, D.; Bendels, S.; Kansy, M.; Kuhn, B.; Müller, K.; Obst-Sander, U.; Stahl, M. Fluorine in Medicinal Chemistry. *Chembiochem* **2004,** 5, 637-643.

88 Clader, J. W. The Discovery of Ezetimibe: A View from Outside the Receptor. *J. Med. Chem.* **2004,** 47, 1-9.

89 Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews* **2001,** 46, 3-26.

90 Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. Molecular Properties That Influence the Oral Bioavailability of Drug Candidates. *J. Med. Chem.* **2002,** 45, 2615-2623.

91 *European Pharmacopoeia 9th Edition (2017), EDQM Council of Europe, Strasbourg*.

92 Rankovic, Z. CNS Drug Design: Balancing Physicochemical Properties for Optimal Brain Exposure. *J. Med. Chem.* **2015,** 58, 2584-2608.

93 Leeson, P. D.; Springthorpe, B. The influence of drug-like concepts on decision-making in medicinal chemistry. *Nature Reviews Drug Discovery* **2007,** 6, 881.

94 Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. Fluorine in medicinal chemistry. *Chem. Soc. Rev.* **2008,** 37, 320-330.

95 Smart, B. E. Fluorine substituent effects (on bioactivity). *J. Fluorine Chem.* **2001,** 109, 3-11.

96 Berezhkovskiy, L. M. Volume of Distribution at Steady State for a Linear Pharmacokinetic System with Peripheral Elimination. *J. Pharm. Sci.* **2004,** 93, 1628-1640.

97 Sun, H.; Tawa, G.; Wallqvist, A. Classification of scaffold-hopping approaches. *Drug Discovery Today* **2012,** 17, 310-324.

98 Langdon, S. R.; Ertl, P.; Brown, N. Bioisosteric Replacement and Scaffold Hopping in Lead Generation and Optimization. *Mol. Inform.* **2010,** 29, 366-385.

99 Böhm, H.-J.; Flohr, A.; Stahl, M. Scaffold hopping. *Drug Discovery Today: Technologies* **2004,** 1, 217-224.

100 Fang, Z.; Song, Y. n.; Zhan, P.; Zhang, Q.; Liu, X. Conformational restriction: an effective tactic in 'follow-on'-based drug discovery. *Future Med. Chem.* **2014,** 6, 885-901.

101 El Bakali, J.; Muccioli, G. G.; Body-Malapel, M.; Djouina, M.; Klupsch, F.; Ghinet, A.; Barczyk, A.; Renault, N.; Chavatte, P.; Desreumaux, P.; Lambert, D. M.; Millet, R. Conformational Restriction Leading to a Selective CB2 Cannabinoid Receptor Agonist Orally Active Against Colitis. *ACS Med. Chem. Lett.* **2015,** 6, 198-203.

102 Wentland, M. P.; Aldous, S. C.; Gruett, M. D.; Perni, R. B.; Powles, R. G.; Danz, D. W.; Klingbeil, K. M.; Peverly, A. D.; Robinson, R. G.; Corbett, T. H.; Rake, J. B.; Coughlin, S. A. The antitumor activity of novel pyrazoloquinoline derivatives. *Bioorg. Med. Chem. Lett.* **1995,** 5, 405-410.

103 Yokoyama, N.; Ritter, B.; Neubert, A. D. 2-Arylpyrazolo[4,3-c]quinolin-3-ones: a novel agonist, a partial agonist and an antagonist of benzodiazepines. *J. Med. Chem.* **1982,** 25, 337-339.

104 Knutson, D. E.; Kodali, R.; Divović, B.; Treven, M.; Stephen, M. R.; Zahn, N. M.; Dobričić, V.; Huber, A. T.; Meirelles, M. A.; Verma, R. S.; Wimmer, L.; Witzigmann, C.; Arnold, L. A.; Chiou, L.-C.; Ernst, M.; Mihovilovic, M. D.; Savić, M. M.; Sieghart, W.; Cook, J. M. Design and Synthesis of Novel Deuterated Ligands Functionally Selective for the γ-Aminobutyric Acid Type A Receptor (GABAAR) α6 Subtype with Improved Metabolic Stability and Enhanced Bioavailability. *J. Med. Chem.* **2018**, 61, 2422–2446.

105 Han, C.; Chatterjee, A.; Noetzel, M. J.; Panarese, J. D.; Niswender, C.; Conn, P. J.; Lindsley, C. W.; Stauffer, S. R. Discovery and SAR of muscarinic receptor subtype 1 (M1) allosteric activators from a molecular libraries high throughput screen. Part I: 2,5-dibenzyl-2H-pyrazolo[4,3-c]quinolin-3(5H)-ones as positive allosteric modulators. *Bioorg. Med. Chem. Lett.* **2015,** 25, 384-388.

106 Hintermann, S.; Hurth, K.; Nozulak, J.; Tintelnot-Blomley, M.; Aichholz, R.; Blanz, J.; Kaupmann, K.; Mosbacher, J. Exploring subtype selectivity and metabolic stability of a novel series of ligands for the benzodiazepine binding site of the GABAA receptor. *Bioorg. Med. Chem. Lett.* **2011,** 21, 1523-1526.

107 Gaurav, A.; Gautam, V.; Singh, R. An Overview on Synthetic Methodologies and Biological Activities of Pyrazoloquinolines. *Mini-Reviews in Medicinal Chemistry* **2010,** 10, 1194-1210.

108 Mekheimer, R. A.; Ahmed, E. A.; Sadek, K. U. Recent developments in the chemistry of pyrazolo[4,3-c]quinolines. *Tetrahedron* **2012,** 68, 1637-1667.

109 Kaslow, C. E.; Clark, W. R. Quinolinemethanols. *J. Org. Chem.* **1953,** 18, 55-58.

110 Savini, L.; Massarelli, P.; Nencini, C.; Pellerano, C.; Biggio, G.; Maciocco, A.; Tuligi, G.; Carrieri, A.; Cinone, N.; Carotti, A. High affinity central benzodiazepine receptor ligands: synthesis and structure–activity relationship studies of a new series of pyrazolo[4,3-c]quinolin-3-ones. *Biorg. Med. Chem.* **1998,** 6, 389-399.

111 López Rivilli, M. J.; Moyano, E. L.; Yranzo, G. I. An alternative approach toward 2-aryl-2H-pyrazolo[4,3-c]-quinolin-3-ones by a multistep synthesis. *Tetrahedron Lett.* **2010,** 51, 478-481.

112 Ferreira, V. F.; Leal, K. Z.; Lindgren, E. B.; de Oliveira, M. R. P.; de Souza, M. C. B. V.; Vasconcelos, T. R. A.; Wardell, J. L.; Wardell, S. M. S. V.; Yoneda, J. D. Structural evaluation of three 2-phenylpyrazolo[4,3-c]quinolin-3-one monohydrates. *J. Mol. Struct.* **2013,** 1051, 299-309.

113 Pal, S.; Mareddy, J.; Devi, N. S. High speed synthesis of pyrazolones using microwave-assisted neat reaction technology. *J. Braz. Chem. Soc.* **2008,** 19, 1207-1214.

114 Masoud, M.; Abd El Zaher Mostafa, M.; Ahmed, R.; Abd El Moneim, N. Structures and Chemical Equilibria of Some N-Heterocycles Containing Amide Linkages. *Molecules* **2003,** 8, 430.

115 Beshore, D. C.; DiPardo, R. M.; Kuduk, S. D. Preparation of 2,4,5-trisubstituted pyrazolo[4,3-c]quinolin-3-ones. *Tetrahedron Lett.* **2010,** 51, 970-973.

116 Kaplan, A. P.; Gupta, V.; Wasley, J. W. F. Therapeutic Pyrazoloquinoline Derivatives. *US Patent Application Publication* US 20080306049 A1, **2008**.

117 Kugelmann, E. *Random Chemistry-Leitstruktursuche mittels Fentons Reagenz*. Dissertation, Julius-Maximilians-Universität Würzburg, 2011.

118 http://www.who.int/trypanosomiasis_african/country/risk_AFRO/en/ (25.06.18)

119 Hünig, S.; Kreitmeier, P.; Märkl, G.; Sauer, J. *Arbeitsmethoden in der organischen Chemie*. Lehmanns Verlag: Berlin, 2006.

120 Gould, R. G.; Jacobs, W. A. The Synthesis of Certain Substituted Quinolines and 5,6-Benzoquinolines. *J. Am. Chem. Soc.* **1939,** 61, 2890-2895.

121 Cociorva, O.; Li, B.; Szardenings, K.; Fukuda, Y.; Nomura, M.; Seto, S.; Yumoto, K.; Okada, K.; Nakamura, A. Aminoquinolones as GSK-3 Inhibitors. *PCT Int. Appl.* WO 2007106537 A2 20070920, **2007**.

122 Chen, Y.-L.; Zacharias, J.; Vince, R.; Geraghty, R. J.; Wang, Z. C-6 aryl substituted 4-quinolone-3-carboxylic acids as inhibitors of hepatitis C virus. *Biorg. Med. Chem.* **2012,** 20, 4790-4800.

123 Muñoz, H.; Tamariz, J.; Zamora, H. S.; Lázaro, M.; Labarrios, F. Preparation of Propanedioic Acid, (Anilinomethylene) Alkyl Esters by Direct Condensation. *Synth. Commun.* **1987,** 17, 549-554.

124 Kermack, W. O.; Storey, N. E. 115. Attempts to find new antimalarials. Part XXIX. The synthesis of various derivatives of 2:3-benz-[γ]-carboline. *J. Chem. Soc.* **1950**, 607-612.

125 Baker, B. R.; Bramhall, R. R. Irreversible enzyme inhibitors. 190. Inhibition of some dehydrogenases by 1-substituted-1,4-dihydro-4-quinolone-3-carboxylic acids. *J. Med. Chem.* **1972,** 15, 233-235.

126 Lippur, K.; Tiirik, T.; Kudrjashova, M.; Järving, I.; Lopp, M.; Kanger, T. Amination of quinolones with morpholine derivatives. *Tetrahedron* **2012,** 68, 9550-9555.

127 Wallace, M. D.; Waraich, N. F.; Debowski, A. W.; Corral, M. G.; Maxwell, A.; Mylne, J. S.; Stubbs, K. A. Developing ciprofloxacin analogues against plant DNA gyrase: a novel herbicide mode of action. *Chem. Commun.* **2018,** 54, 1869-1872.

128 Cai, Z.; Zhou, W.; Sun, L. Synthesis and HMG CoA reductase inhibition of 4-thiophenyl quinolines as potential hypocholesterolemic agents. *Biorg. Med. Chem.* **2007,** 15, 7809-7829.