Chemical Modifications of Quinolone Amides Against African Trypanosomiasis:

Balancing Solubility, Bioactivity, and Cytotoxicity

Dissertation zur Erlangung des naturwissenschaftlichen

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Table of abbreviations

9-BBN-H   9-borabicyclo[3.3.1]nonan

acac   acetylacetone

ADME   absorption, distribution, metabolism, and excretion

AlOx   aluminium oxide

atm   standard atmosphere

ATR   attenuated total reflectance

BBB   blood-brain barrier

Boc   *tert*-butyloxycarbonyl

CAN   ceric ammonium nitrate

CATT   card agglutination test for trypanosomiasis

CC_{50} concentration that reduced cell proliferation by 50 %

cf.   confer

CNS   central nervous system

CSF   cerebrospinal fluid

dba   dibenzylideneacetone

DBPO   dibenzoylperoxid

DCC   *N,N'*-dicyclohexylcarbodiimide

DIBAL   diisobutylaluminium hydride

DIPEA   *N,N'*-diisopropylethylamine

DMEM   Dulbecco's Modified Eagle's Medium

DMF   *N,N'*-dimethylformamide

DMSO   dimethyl sulfoxide

DNA   deoxyribonucleic acid

DND*i*   Drugs for Neglected Diseases *initiative*

EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EMA	European Medicines Agency
eq	equivalent
ESI	electrospray ionization
et al.	*et alii*
HAT	human African trypanosomiasis
HBA	hydrogen bond acceptor
HBD	hydrogen bond donor
HBTU	hexafluorophosphate benzotriazole tetramethyl uronium
HMI-9	Hirumi's modified Iscove's medium 9
HMPA	hexamethylphosphoramide
HOBt	hydroxybenzotriazole
HPLC	high-performance liquid chromatography
i.p.	intraperitoneal
IBCF	isobutyl chloroformate
IC_{50}	half maximal inhibitory concentration
IR	infrared
kDNA	kinetoplast
LC-MS	liquid chromatography-mass spectrometry
LDA	lithium diisopropylamide
LHMDS	lithium bis(trimethylsilyl)amide
Lit.	literature
logP	logarithm of the partition coefficient
MBDA	magnesium bis(diisopropylamide)
mCPBA	*meta*-chloroperoxybenzoic acid
MEHQ	monomethyl ether hydroquinone
mp	melting point

MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NAD	nicotinamide adenine dinucleotide
NBS	*N*-bromosuccinimide
NECT	nifurtimox-eflornithine combination therapy
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy
NTD	neglected tropical disease
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PE	petroleum ether
Ph. Eur.	European Pharmacopoeia
ppm	parts per million
p-TsOH	*para*-toluenesulfonic acid
PyBOP	benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate
r.t.	room temperature
RNA	ribonucleic acid
RP	reversed phase
rpm	revolutions per minute
RPMI-1640	Roswell Park Memorial Institute 1640 medium
RuPhos	2-dicyclohexylphosphino-2′,6′-diisopropoxybiphenyl
SI	selectivity index
$S_N Ar$	nucleophilic aromatic substitution
S_w	aqueous solubility
TFA	trifluoroacetic acid
TfOH	trifluoromethanesulfonic acid
THF	tetrahydrofuran

TLC	thin-layer chromatography
TLF	trypanosome lytic factor
TPAP	tetrapropylammonium perruthenate
UV	ultraviolet
WHO	World Health Organization
wt%	mass percentage (mass fraction multiplied by 100)

1 <u>Introduction</u>

1.1 Human African trypanosomiasis – a neglected disease

The human African trypanosomiasis, also known as sleeping sickness, is a parasitic infection and belongs to the so-called neglected tropical diseases (NTDs).[1] These chronic and debilitating diseases occur among the extreme poor in developing countries in Africa, Asia, and South America.[2] They arise in areas with limited access to adequate sanitation, clean water, and healthcare, and people living in proximity to animals and infective vectors.[3] All NTDs combined were affecting close to 2 billion people at the turn of the millennium, resulting in approximately 534,000 deaths annually.[4] Furthermore, these diseases have not only an impact on health aspects, but also indirect, more subtle, consequences, as they are keeping children out of school, adults out of work, and charge households with considerable costs, resulting in vicious cycles of poverty.[3, 5] Although causing a comparable burden as malaria, they have remained mainly neglected in the global health agenda.[3] For a long period, pharmaceutical companies regarded the medical treatment of NTDs as commercially unattractive, due to the required high research and development investment compared to the possible income. There is a 13-fold greater chance of a drug being brought to market for central-nervous-system disorders or cancer than for a neglected disease.[6]

In the year 2005, the world health organization (WHO) drew up an innovative strategy to control, eliminate, and eradicate all NTDs, consisting of rapid impact medical solutions and lasting transmission preventions.[7] Although the implementation of these strategies progressed slow, the WHO presented an NTD roadmap in 2012 to further enhance and accelerate the countermeasures.[8] These inspiring aims resulted in the London Declaration on Neglected Tropical Diseases, which was signed by officials from the WHO, the World Bank, philanthropic foundations, the world's leading pharmaceutical companies, and endemic countries.[9] The ambitious coordinated effort set the eradication of Guinea worm disease and the elimination of four further NTDs, including the human African trypanosomiasis, as its main objective. Both, the WHO roadmap and the London Declaration, have been a game-changer in the NTD treatment, scaling up sharply in the last decade and reaching 1.12 billion people in the year 2018, which represented 65 % of those at risk. Even though there has been a huge progress in the combat of NTDs, international donors tend to focus on other projects today, so the risk arises that the diseases slide back into neglect once again.[3]

One of Africa's most deadly diseases, the human African trypanosomiasis (HAT), has seen an impressive decline since the engagement of the WHO. In the year 1998, a report suggested about 300,000 cases of HAT every year, whereas merely 977 cases were reported in 2018.[10, 11] These numbers demonstrate clearly that the goal of the WHO, the elimination of sleeping

sickness as a public health problem, is within grasp. Nevertheless, there have been several major epidemics before and a re-emergence is possible at any time due to the unstable social circumstances.

1.2   Epidemiology of the HAT

The human African trypanosomiasis is caused by protozoan parasites of the genus *Trypanosoma* and transmitted by the bite of the blood-sucking tsetse fly of the genus *Glossina*.[12] The disease occurs in the sub-Saharan Africa, where 70 million people are at risk, and is restricted by the suitable habitats of its vector, yet the fly infestation covers about 10 million km^2 of the continent.[10] It appears mainly in remote rural regions, though cases in urban areas have also been reported.[13] The HAT can develop in variable sized areas, from single villages to entire regions. Even within an infected area, the intensity can vary from one village to another.[14] The local population has known the existence of the severe disease occurring in cattle and also in humans for centuries, calling it nagana. In 1899, the British microbiologist David Bruce could solve the cause of the mysterious cattle epidemic, identified the causative protozoan, and proved that the tsetse fly transmitted it from wild to domestic animals. In honour of his discoveries, the microorganisms were named *Trypanosoma brucei*.[12] This species of protozoan contains three subspecies. The kinetoplastids of *T. brucei brucei* are responsible for the animal trypanosomiasis. Although they are non-infective to humans due to lysis by immune molecules called trypanosome lytic factors (TLFs), the infection of domestic animals is a burden to the economic development of affected rural areas.[14, 15] The human African trypanosomiasis originates from the two other subspecies and the course of disease takes two different forms, depending on the parasite involved. *Trypanosoma brucei gambiense* is responsible for the main proportion of reported cases, nearly 98 %, and is found in 24 countries in west and central Africa.[14] An infection with this subspecies causes a chronic form of the sleeping sickness, which lasts for months or even years without recognizable symptoms. Once signs of the disease emerge, the patient is already in an advanced stage, in which the central nervous system is affected. The less common *Trypanosoma brucei rhodesiense* domiciles 13 countries in eastern and southern Africa and accounts for only 2 % of cases. However, this form causes an acute and severe illness, producing observable symptoms after a few weeks or months. This latter variant constitutes for most HAT cases in non-endemic countries, imported by American or European tourists, which visited African safari parks.[10, 16]

These two subspecies were strictly geographically separated for centuries. However, the steadily spread of *Trypanosoma brucei rhodesiense* towards the northwest led to an overlap of both forms of the disease in Uganda.[1] This coexistence complicates the patient treatment, which is different for each species, as microscopy alone is not sufficient to distinguish between the two variants. Furthermore, there is now the possibility of a coinfection in some patients.[12]

1.3 Transmission

The vectors of the human African trypanosomiasis are the blood-feeding tsetse flies. About 30 species and subspecies of these animals can transmit the parasites. Of these species, the ability of transmission as well as the preferred habitats differ slightly.[1] The parasitic cycle, which is depicted in Figure 1, starts with the feeding of the tsetse fly on an infected mammalian host. Humans are the main reservoir for *T. b. gambiense*, while animals, especially domestic cattle, are the main reservoir for *T. b. rhodesiense*.

After the bite, the trypanosomes enter the digestive tract of the fly. In the next 3-5 weeks, the parasites undergo a complex series of differentiation steps, including anatomical and biochemical changes, resulting in the infective forms of the trypanosomes in the salivary glands of the fly.[12] This process, which can be seen in Figure 1, depends on a variety of factors, therefore, the completion of the cycle in the fly is rare. Only about 0.1 % of the flies host a mature infection, which can be transmitted.[1] Furthermore, the parasites cannot be passed on from the mother fly to newly-hatched ones. However, the tsetse fly remains infectious for life.
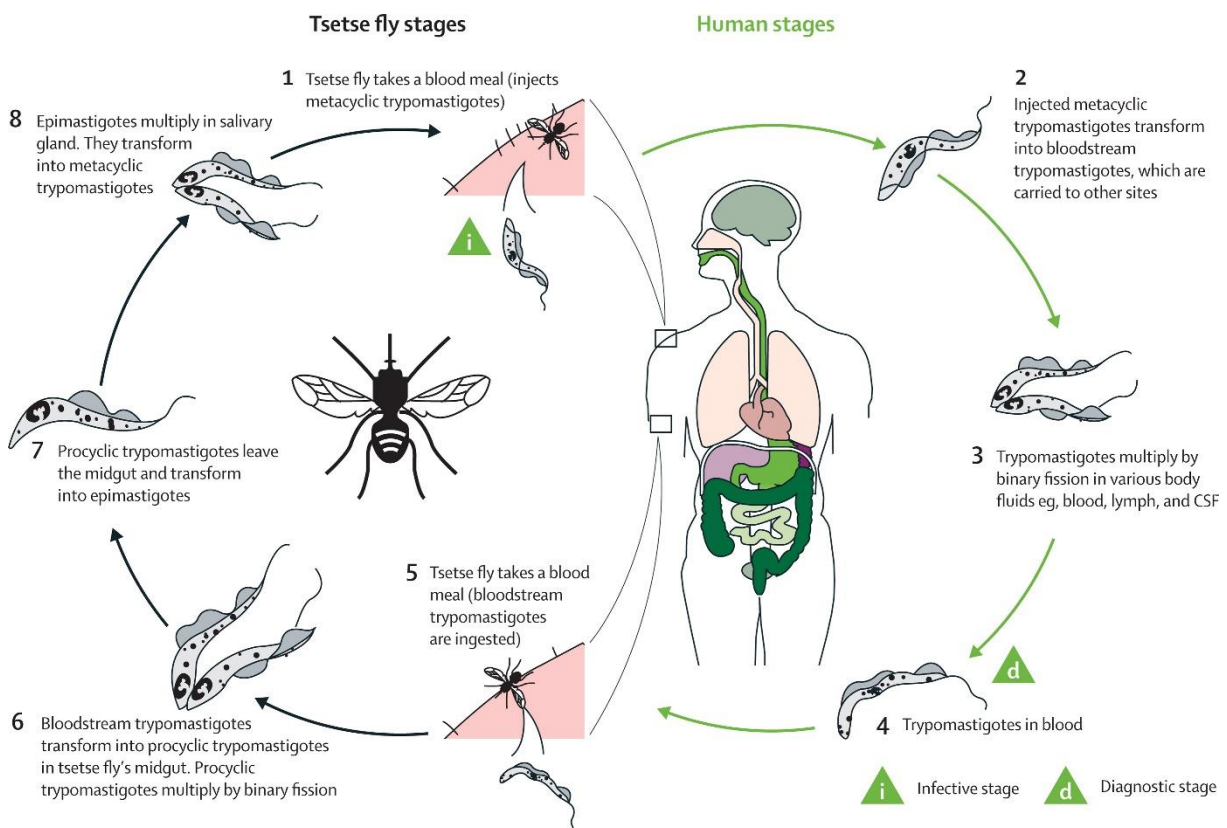
Figure 1. The life cycle of the African trypanosome in vector and human.[12]

By biting the human host, the tsetse fly injects the metacyclic trypomastigotes, the flagellated stage of trypanosomes, which enter the bloodstream. Here, they transform to trypomastigotes and spread in the bloodstream, lymph nodes, and organs, including spleen, heart, and liver. Even the eyes and the endocrine organs are affected.[12] This is known as stage 1 or the

haemolymphatic stage, in which the trypomastigotes multiply by binary fission. When a tsetse fly afterwards takes a blood meal, the bloodstream trypomastigotes are ingested and the cycle restarts. In the infected human host, the parasites cross the blood-brain barrier and the central nervous system after a period, typically a few weeks in the case of *T. b. rhodesiense* and several months in the case of *T. b. gambiense*.[12]

Next to the transmission through the bite of the tsetse fly, there are more possibilities of infection for humans, like a mother-to-child infection. Trypanosomes are known to cross the placenta and infect the fetus. Furthermore, the illness can also be spread through sexual contact of the patients.[14]

An untreated HAT infection usually leads to death, although, in the last years, some cases of tolerant or resistant individuals were reported. The tolerent humans show no symptoms of the disease, analogous to certain trypanotolerant cattle breeds.[10, 17] Some patients maintain an asymptomatic state after the infection and are even able to be aparasitaemic and seronegative.[18, 19] Rare examples of individuals declining a treatment and self-curing also exist, with these being regarded as resistant.[18] A stunning case is the presentation of HAT in a male after at least 29 years after infection.[20] Until now, it is unclear how common these tolerances are and whether the affected seropositive patients should be regarded as a risk of infection for others.[10] A recent study revealed that the human skin is also an anatomical reservoir for African trypanosomes. Capewell *et al.* reported a substantial quantity of trypanosomes in the skin of undiagnosed individuals, which can be transmitted to the tsetse vectors, even when the parasites could not be detected in the blood.[21] These findings and the fact that cattle serves as a suitable reservoir question the long-term success of eliminating the human African trypanosomiasis.

1.4 Course of the human African trypanosomiasis

The first recognizable symptom after the infection with HAT is the trypanosomal chancre, an itchy, inflammatory reaction at the location of the bite. However, it appears only in 19 % of patients with *T. b. rhodesiense* and is rarely seen with *T. b. gambiense*.[1] The illness can be separated in two phases, the first haemolymphatic and the second meningo-encephalitic one. Early symptoms, after one to three weeks after the bite, include headache, malaise, arthralgia, weight loss, fatigue, and fever with rigors. These are quite non-specific and can be easily confused with malaria.[12] As the disease progresses, the patients develop more severe features, as myocarditis, endocrine dysfunction, and fertility problems, including sterility, abortion, and stillbirths. A typical symptom of *T. b. gambiense* is the so-called Winterbottom's sign, a posterior cervical lymphadenophaty.[22]

In the second phase of the illness, when the trypomastigotes have crossed the blood-brain barrier and have infected the central nervous system (CNS), almost all regions of the nervous system are involved and a wide range of symptoms occurs. This includes mental, sensory, and motor system disturbances, as well as abnormal reflexes.[12] The typical sleep disturbances are the most prominent and name-giving feature, appearing in 74 % of patients. These sleep irregularities include a reversal of the normal sleep/wake cycle, uncontrollable episodes of sleep, and alterations of the sleep structure itself, with an early onset of the REM-phase.[12, 23] The development of the disease and the duration of the stages differentiate in the two forms of HAT. An infection with *T. b. rhodesiense* results in a more acute illness lasting several weeks if untreated, whereas a *T. b. gambiense* case lasts for months or even years.[24]

Symptoms of the human African trypanosomiasis in travellers from non-endemic countries are quite atypical and similar for both variants. They present more frequently a chancre at the biting site and a trypanosomal rash. Additionally, the patients suffer from severe haematological disorders, impaired kidney function, electrolyte disturbances, and high concentrations of liver enzymes.[1, 25, 26] The typical lymphadenopathy and sleep abnormalities are only rarely found, but the development to the late-stage illness is rapid.[1, 27]

1.5 Diagnosis

A three-step approach is used for the diagnosis and staging of the human African trypanosomiasis. At first, there is the screening for a potential infection, which involves checking for symptoms, like the Winterbottom's sign, and serological testing for antibodies.[14] This card agglutination test for trypanosomiasis (CATT) is fast, practical, and efficient, allowing mass population screening in endemic areas.[28] Unfortunately, it is only available for *T. b. gambiense*.[1] Additionally, the CATT results might be misleading in the absence of specific antigens, showing a negative result for an infected person.[29] After the screening, there has to be a diagnostic confirmation whether the trypanosomes are present in body fluids. The norm is a microscopic examination of lymph node aspirate or blood. Different concentration methods should be used because of the low sensitivity.[1] A more accurate alternative is the detection of parasite nucleic acids by PCR, which is commonly done in travellers from non-endemic countries. However, advanced testing facilities do not exist in field conditions in the affected rural areas.[24] Finally, the state of disease progression has to be determined, as the treatment differs significantly between the two stages. The common method for late-stage diagnosis of both HAT forms is the examination of the cerebrospinal fluid (CSF). A lumbar puncture is performed directly after the diagnosis and the number of white blood cells in the CSF and the presence of trypanosomes is measured.[30]

The diagnosis should be done as early as possible in order to avoid the progressing to the neurological state. However, this turns out to be difficult due to the long asymptomatic first

stage. New diagnostic tests with suitable protocols for the endemic regions are still needed for an effective control and surveillance of HAT. Furthermore, an active screening of the population of risk is recommended by the WHO, to identify patients at the beginning of the illness and prevent transmission.[14, 30]

1.6 Treatment

After diagnosis of a HAT case, an immediate therapy is indispensable, as an untreated infection almost always leads to death. To this point, only five drugs are registered for the treatment of human African trypanosomiasis and these have to be differentiated between first-stage and second-stage treatment, and the two subspecies. Pentamidine (**1**), first used in 1940, is the first-line treatment for *T. b. gambiense* and is administered intramuscularly as an isethionate salt (cf. Figure 2).[31] Its mechanism of action is still unknown, but an inhibition of the production of DNA, RNA, and proteins is suspected to be involved.[32] Although it is generally well tolerated, there are potential side effects, like hyperglycaemia or hypoglycaemia, prolongation of the QT interval, hypotension, and gastrointestinal complications.[1, 33] Theoretically, suramin (**2**) can also be used for this subspecies. Unfortunately, these trypanosomes share their habitats in western and central Africa with the parasitic roundworms *Onchocerca*. The intravenous administered suramin (**2**) has a high activity against these parasites and can evoke severe allergic reactions in patients.[1] Therefore, it is mainly used for the treatment of *T. b. rhodesiense*. An inhibition of the energy metabolism by blocking glycolytic enzymes is assumed as its mechanism of action.[34] Possible complications are renal failure, skin lesions, bone marrow toxicity, and peripheral neuropathy.[35] Both of these drugs, pentamidine (**1**) and suramin (**2**), are fully ionised at physiological pH value and cannot cross the blood-brain barrier. As a result, they are only effective in the haemolymphatic phase.[36, 37]
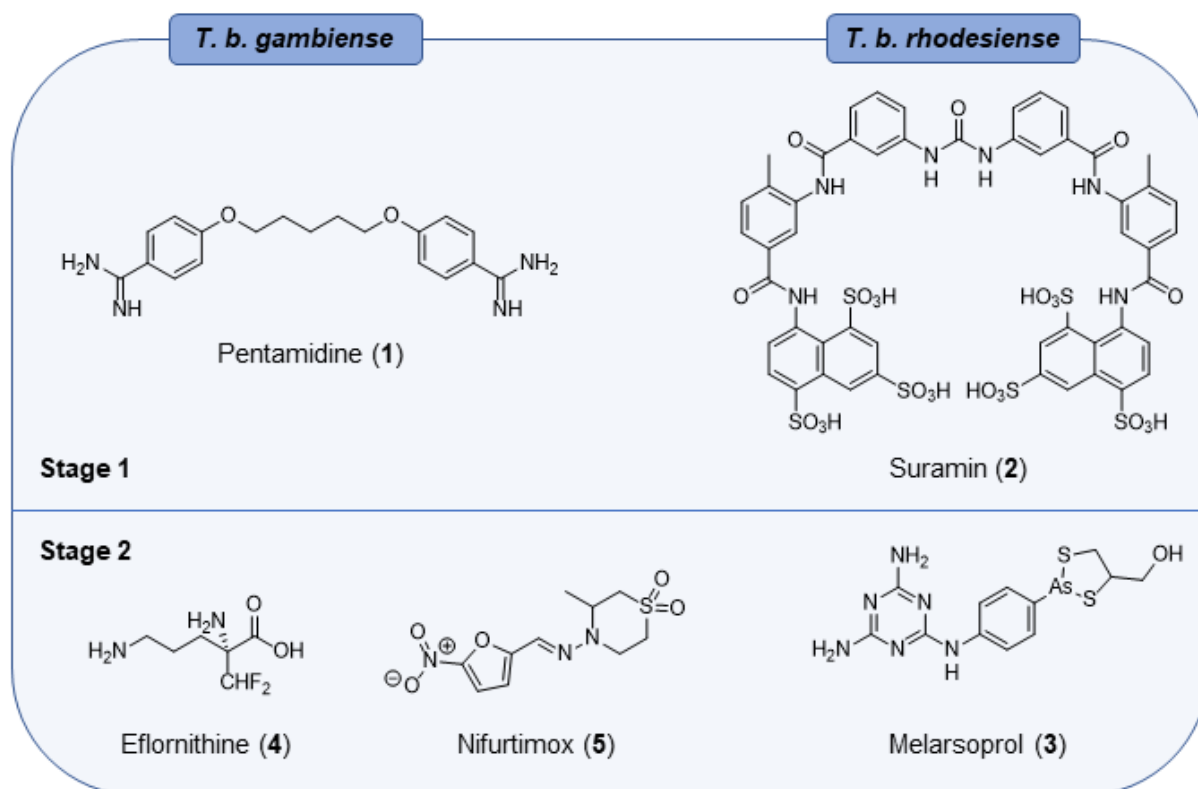
Figure 2. Currently used antitrypanosomal drugs, classified according to subspecies and disease phase.

The treatment of the second stage, after the invasion of the CNS, is challenging, as the drugs used are more toxic. The arsenic containing melarsoprol (**3**) is effective in the second stage of both HAT forms. Its primary target is a vital enzyme, the so-called trypanothione.[38] Melarsoprol (**3**) has to be given intravenously in propylene glycol due to its very poor solubility in water.[39] Unfortunately, the administration is accompanied with frequent and severe adverse effects. Within nearly 10 % of patients, a reactive encephalopathy occurs, leading to an overall mortality rate of about 5 %.[1] Therefore, it is mainly used for the treatment of *T. b. rhodesiense*, for which no other drug is registered, and in very resource-poor countries also for *T. b. gambiense*.

The first-in-line medical treatment for the subspecies *T.b. gambiense* is the less toxic eflornithine (**4**), which is an ornithine decarboxylase inhibitor. Its negative aspect is the difficult administration: a successful treatment requires a slow intravenous infusion every 6 hours for 14 days.[36, 40] An important development was the nifurtimox-eflornithine combination therapy (NECT), whichs adds the oral administered drug nifurtimox (**5**), originally registered for the Chagas disease. This combination shortens the time and cost of the treatment. Sadly, this was the only advance in the treatment of human African trypanosomiasis in the last decades.

All available drugs have significant drawbacks, like severe adverse effects and an inappropriate administration. Furthermore, the therapy has to start in an early stage of the infection to be effective, relying on a quick detection. The lack of new drugs led to a growing

resistance in trypanosomes, resulting in an alarming melarsoprol failure rate of 20 to 30 %.[41, 42] A vaccination against HAT is not possible at the present time due to the rapid variation of expressed surface glycoproteins.[12] Therefore, there is an urgent need for the development of novel drugs for the treatment of human African trypanosomiasis.[43]

1.7 New drugs emerging the pipeline

The Drugs for Neglected Diseases *initiative* (DND*i*) rediscovered fexinidazole (**6**) in their search for new antiparasitic compounds in 2005; the molecular structure is depicted in Figure 3.[44] The drug was initially developed as a broad-spectrum antimicrobial agent by Hoechst AG (now Sanofi) in the 1970s. Already in 1983, the *in vivo* activity of fexinidazole (**6**) against African trypanosomes was reported.[45] However, the development was not further pursued. Fexinidazole (**6**) is a nitroimidazole, a known class of trypanocidal compounds, containing the extremely effective megazol, whose development was stopped due to its mutagenicity.[46] They are also widely used as antibiotics, displaying acceptable activity/toxicity profiles.[47]
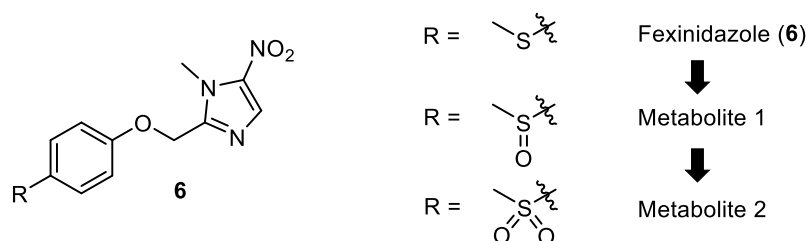

Figure 3. Molecular structure of fexinidazole and its metabolites.

The advantage of fexinidazole (**6**) is the oral administration, replacing the labour-intensive infusions and reducing the cost of treatment. After the absorption in the digestive tract, the 5-nitroimidazole is bioactivated by parasitic nitroreductase enzymes, generating reactive amines, which inhibit the DNA synthesis of trypanosomes.[48] Fexinidazole (**6**) exhibits an activity against *T. b. gambiense*, *T. b. rhodesiense*, and *T. b. brucei* in murine models. The compound is metabolised to the sulfoxide and the sulfone, both having comparable effectivity as their parent compound.[49] All three molecules are able to cross the blood-brain barrier, therefore, it can be used in the stage 2 of HAT. With an oral administration for 10 days, fexinidazole (**6**) presents an equivalent efficacy to pentamidine (**1**) in the first-stage and to NECT in the second-stage. Only in severe second-stage cases NECT shows a superior efficacy.[48]

The European Medicines Agency (EMA) recommended fexinidazole (**6**) for the use outside of the European Union in November 2018. One year later, the Democratic Republic of Congo approved the new drug for the treatment of first-stage and second-stage *T. b. gambiense*.[44] In 2021, fexinidazole (**6**) was authorized in Uganda and in other endemic countries a registration is underway.[44] Sanofi committed to donate fexinidazole (**6**) to the WHO, which will distribute it to respective states.[44]

The only other current candidate in the pipeline is the oxaborole acoziborole (**7**), formerly known as SCYX-7158 (cf. Figure 4). The advantage over fexinidazole (**6**) is the potential single oral dose due to its long half-life.[36] It finished the phase I clinical trials in 2015 and proceeds now with phase II/III.[50]
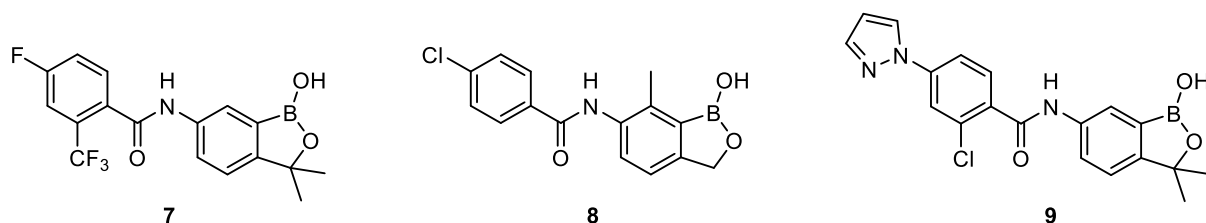

Figure 4. The molecular structures of oxaborole HAT candidates.

There are two similar compounds, the molecules SCYX-1330682 (**8**) and SCYX-1608210 (**9**), which both demonstrated to cure stage 2 HAT in murine models.[51] However, due to the success of acoziborole (**7**), the development of these two oxaboroles was put on hold in 2013 and will only recommence if problems occur in the clinical trials of acoziborole.[52]

1.8   Fluorquinolones

Quinolones are widely used as antibiotics and offer nearly ideal attributes, like a broad spectrum of activity, good bioavailability, and a low incidence of side effects.[53] In 1962, Lesher *et al.* discovered an impurity with an antibacterial activity during their synthesis of chloroquine. A subsequent structural optimization led to the nalidixic acid (**10**), which was commercialised for the treatment of urinary tract infections.[54] Although the 1,8-naphthyridone derivative **10** is officially not a quinolone, it is regarded as the first member of this drug family. Compared to currently known antibiotics, it has a poor potency and a small activity spectrum. A broader antibacterial spectrum was achieved by the addition of a piperazine ring in position 7, resulting in zwitterionic substances like pipemidic acid.[55] The introduction of a fluorine in position 6 resulted in a 100-fold decrease in the minimum inhibitory concentration and heralded the era of the second generation of quinolones.[53] All members of this generation have an enhanced activity due to the fluorine-substitution, however, mainly against Gram-negative pathogens.[56] The most known member of this group is ciprofloxacin (**11**), which enabled a treatment of *Pseudomonas aeruginosa* for the first time. Unfortunately, the widespread use of this fluoroquinolone, even for minor or viral infections, led to a bacterial resistance. Previous drawbacks were overcome by the introduction of the third generation. These compounds, like levofloxacin (**12**), possess activity against Gram-positive bacteria, as well as an activity against ciprofloxacin-resistant pneumococci.[56]
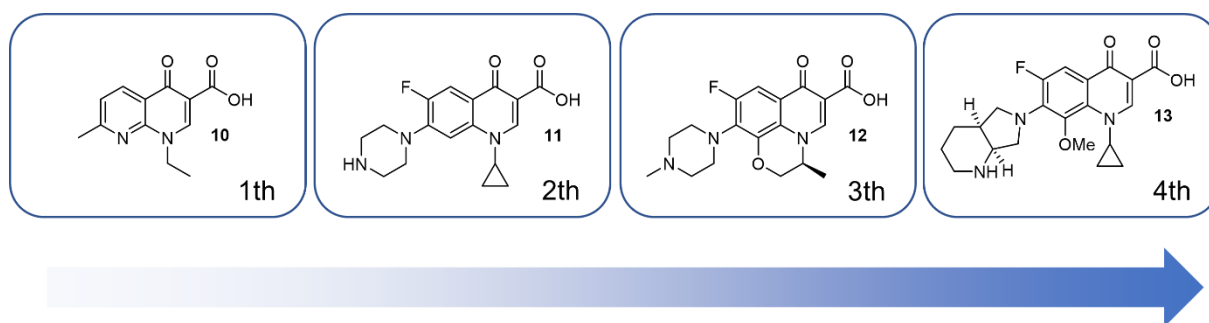
Figure 5. Examples for every generation of antimicrobial fluoroquinolones.

All antibacterial quinolones act by interfering with the DNA synthesis and thereby the replication pathway. Their targets are DNA topoisomerases, which are essential for the bacterial DNA replication, while missing in human cells. These enzymes are responsible for the unwinding process of the double-stranded DNA into two single-stranded templates.[57, 58] The newest generation of antibacterial quinolones, with members like moxifloxacin (**13**), has an equal affinity for topoisomerase II (DNA gyrase) and topoisomerase IV, which leads to potency against a wide range of pathogens and decreases the probability of resistance when there are mutations on a single enzyme.[59] During the last 50 years, an enormous number of quinolone candidates are or were in development, resulting in over 10,000 patented molecules and an considerable market share of antibiotics, which is even expected to grow in the next years.[53, 60]

1.9 Antitrypanosomal fluoroquinolones

Trypanosomes have an abundancy of topoisomerases II in the nucleus and mitochondrion.[61] Furthermore, they possess a unique type of mitochondrial DNA, the kinetoplast DNA (kDNA), which is a complex network of thousands of interlocked circular DNAs. Topoisomerases are mandatory for its replication and, therefore, trypanosomes produce unusual types that are only dedicated to the kDNA metabolism. The human body has no ortholog for this enzyme, making it an ideal drug target.[62] With this background knowledge, Shapiro *et al.* tested fluoroquinolones against *T. b. brucei* cells and reported a clear activity of bicyclic and tricyclic compounds.[63] The clinical used drugs, norfloxacin (**14**) and ciprofloxacin (**11**), had an EC_{50} in the micromolar range in *in vitro* assays. Unfortunately, both compounds were cytotoxic, resulting in low selectivity indices.[64] Furthermore, Shapiro *et al.* demonstrated the correlation of the antitrypanosomal activity with the ability to inhibit the nucleic acid biosynthesis and stabilisation of cleavable protein-DNA complexes. The research group of Burri continued this research, finding active quinolone compounds with IC_{50} values between 100 to 900 ng/mL. Pyrrolidine substituents in position 7 generally led to an increase in activity. Additionally, they tested all drugs with an IC_{50} below 1 μg/mL and a selectivity index of more than 10 in *in vivo* experiments. For unknown reasons, the *in vitro* results could not be reproduced in mice - no

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parasitological cure was achieved.[65] Until that point, most structure-activity relationship optimizations focused on a derivatisation at position 7 and all tested candidates had a carboxylic acid in position 3, which derived from the antimicrobial origin of the fluoroquinolones. Brun *et al.* showed that this functional group is not mandatory, and small esters and primary amides still possess an activity against trypanosomes.[66]

Based on the discussed findings, Niedermeier discovered promising antitrypanosomal fluorquinolones by an amidation of the carboxylic acid in position 3, which is shown in Figure 6.[67] Her research started with the relative simple quinolone derivate **15**, which possessed no activity. An amidation with benzylic substituents resulted in a significant increase in activity. It is important to mention that aniline substituents completely averted this improvement. A substitution of the trifluoromethyl group in position 7 with an amine function enhanced the antiparasitic effect even further, leading to the depicted compound **16**, which served as a starting point for further structure-activity relationship optimizations.
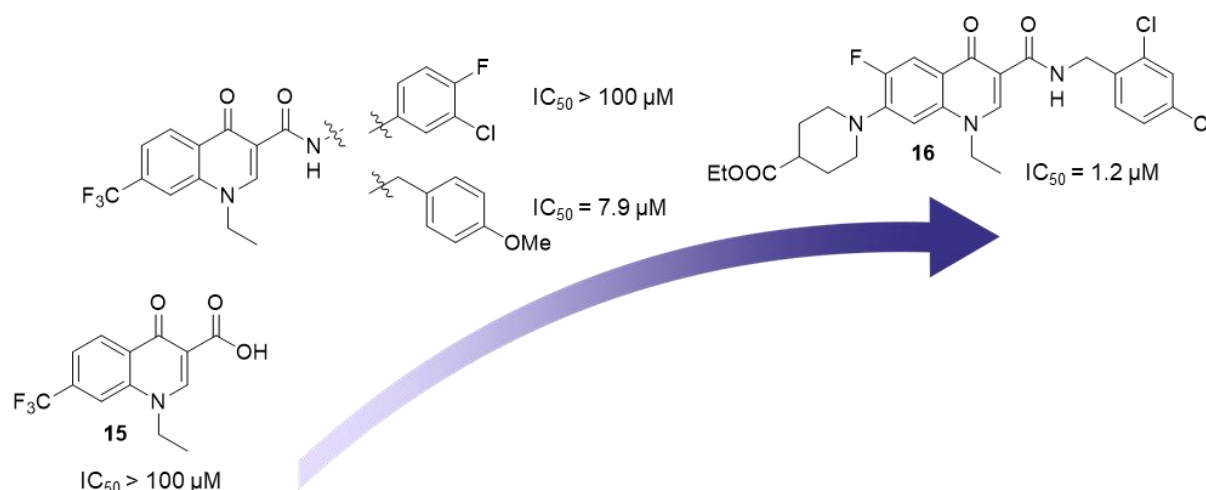

*Figure 6. Observed increase in antitrypanosomal activity by an amidation with benzylic residues and discovery of the lead compound **16** by Niedermeier.[67]*

Hiltensperger synthesized an array of fluoroquinolones with a SAR optimization in position 1, 3, and 7 to draw the following conclusions. An elongation of the aliphatic chain to a *n*-butyl increased the antitrypanosomal activity, succeeding aromatic residues. Benzylamides with hydrogen bond donors (HBD) decreased the potency, whereas unsubstituted and benzylamides with hydrogen bond acceptors (HBA) increased it. Secondary amines in position 7 were superior to aliphatic primary ones, whereby a morpholine substituent was ideal. This analysis resulted in the lead structure **GHQ168** (cf. Figure 7) with an outstanding antitrypanosomal activity (IC$_{50}$ (*T. b. brucei*) = 47 nM and IC$_{50}$ (*T. b. rhodesiense*) = 9 nM) and excellent selectivity (SI = 1140).[68, 69] A fluorescence microscopy based screening was performed with this promising compound to seek out the target of these quinolone amides and a change in the mitochondrial morphology was observed.[68] An additional cell cycle analysis

of cells treated with **GHQ168** showed an increased percentage of segregated kinetoplasts, indicating an interference in the correct segregation of the kinetoplast, which was not happening in ciprofloxacin (**11**) treated cells. However, experiments of *T. brucei* with knocked-down mitochondrial topoisomerases II without quinolone amide treatment did not lead to a segregation defect, excluding the possibility that compound **GHQ168** targets the topoisomerase II in trypanosomes alone and rather other proteins involved in the kinetoplast segregation.[68] These observations are consistent with the requirement that only *β*-keto-carboxylates are able to bind to the topoisomerase II.[70] Therefore, the target of antitrypanosomal quinolone amides is still unknown.
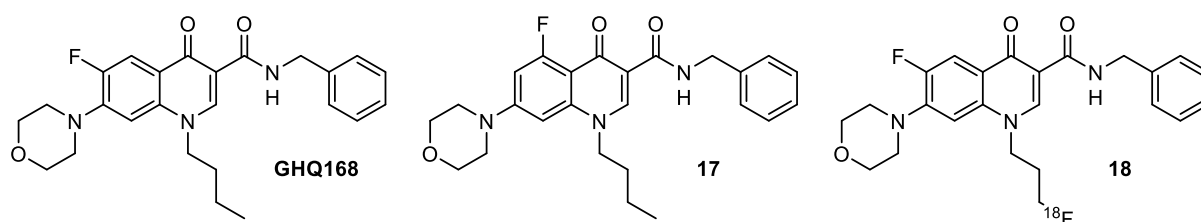

*Figure 7. Molecular structures of the most promising compounds, **GHQ168** and **17**, and the ^{18}F labelled quinolone.[71]*

Further investigations by Berninger revealed that a shift of the fluorine from position 6 to position 5, as seen in structure **17** in Figure 7, reduced the cytotoxicity ($CC_{50} > 100$ µM) while maintaining a comparable antitrypanosomal activity ($IC_{50} = 0.05$ µM, SI > 2000).[51, 71, 72]

A permeation of drugs through the blood-brain barrier is essential for the treatment of second-stage of human African trypanosomiasis. Therefore, autoradiography experiments with [^{18}F]-labelled fluoroquinolones were conducted. The direct nucleophilic exchange of ^{19}F to ^{18}F in position 6, which is reported for other commercially available fluoroquinolones, was not successful.[73, 74] Hence, the radiolabelling was performed at the aliphatic chain in position 1 by substitution of a leaving group, as can be seen in structure **18** in Figure 7. This marked compound was administered to mice and the following *ex vivo* autoradiography demonstrated an accumulation of the fluoroquinolone in the entire brain in medium concentrations, proving the permeation of the fluoroquinolone through the blood-brain barrier (BBB) and, therefore, theoretically enabling a treatment of both stages of HAT.[71, 72] Furthermore, both lead structures, quinolone **GHQ168** and **17**, fulfil the Lipinski's rule of five, indicating suitable molecular properties for the required pharmacokinetics for an orally active drug, including absorption, distribution, metabolism, and excretion (ADME).[75]

Based on these promising results, first *in vivo* experiments in mice with compound **GHQ168** were planned. Unfortunately, usual aqueous DMSO mixtures could not be used due to the very poor solubility of the fluoroquinolone. Therefore, a preliminary oral formulation was developed and orally administered to *T. b. rhodesiense* infected mice. However, no *in vivo* efficacy was

observed.[68] To exclude the influence of the oral formulation, the quinolone was administered intraperitoneally (i.p.) as a lipid formulation, which had to be aborted due to the low tolerance to this by the test animals. Finally, a spray-dried formulation of **GHQ168** proved the *in vivo* efficacy, curing 50 % (3 out of 6) of mice infected with *T. b. rhodesiense*.[76]

2   <u>Aims of the thesis</u>

Earlier in our research group synthesized quinolone amides possessed an excellent activity against trypanosomes, while showing a good selectivity. Although the lead compounds, **GHQ168** and **17**, have favourable structural properties, fulfilling the Lipinski's rule of 5, their aqueous solubility was extremely poor, according to the classification by Ph. Eur. 'practically insoluble'.[69, 72] This characteristic hampered *in vitro* bioactivity measurements and *in vivo* experiments were only executable with a spray-dried formulation of **GHQ168**.[76] Furthermore, an identification of the biological target of the quinolone amides was not accomplished. The crystal structure of **GHQ168** revealed a strong π-π stacking, which is probably responsible for the low solubility.[68, 69]

The aim of this thesis was a chemical modification of the quinolone amide structure to impede the stacking in the crystal structure and, therefore, increase the aqueous solubility, while maintaining an acceptable antitrypanosomal activity. This should be accomplished by the insertion of rigid steric-demanding residues in position 1 (yellow) and in the benzylic position (orange), which is depicted in Figure 8.
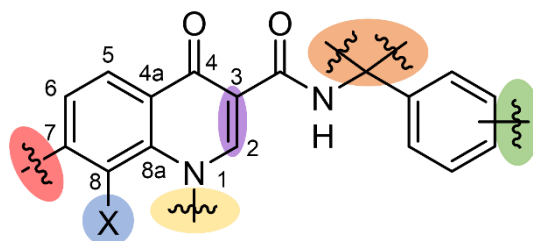

Figure 8. Sites of chemical modifications of the quinolone amide structure.

Another objective was the saturation of the aromatic system in position 2 and 3 (purple). The following disruption of planarity of the molecular structure should lead to an improved solubility. Furthermore, an incorporation of steric-demanding substituents in position 8 (blue), should result in a distortion in the otherwise planar quinolone structure and weaken the intermolecular interactions. The substitution of the morpholine ring in position 7 (red) with oligo(ethylene glycol)methyl ether chains should increase the hydrophilicity, as wells as reduce aggregation. Finally, an introduction of primary amines in the benzylic residue (green) was planned. The main reason for this modification was not a hinderance of the π-π stacking, rather than an increase in solubility due to the basic properties of the amine group.

After their synthesis, the physicochemical properties (logP, solubility) of all compounds should be examined. Furthermore, the quinolone amides are tested for their antitrypanosomal activity, as well as the cytotoxicity. The expansion of the substance library in this thesis is intended to

improve our understanding of the structure-activity relationship of quinolone amides against trypanosomes.

3   Synthesis of the quinolone amides

## 3.1   General synthetic approach for the quinolone amides

A general synthetic route for quinolone amides has been established in prior works, which is shown as a retrosynthesis for compound **19** in Scheme 1.[67, 69] The majority of the tested compounds in this thesis was synthesized analogously to this scheme. The synthetic procedure starts with the respective aniline derivative **20**, depending on the desired fluorine substitution pattern of the 5-, 6-, or 8-position in the final compound. This aniline derivative **20** reacts with diethyl malonate to the anilinomethylenemalonic ester **21**, which yields after an intramolecular cyclization the quinoline structure **22**. Subsequently, the *N*-1 position is substituted with an alkyl group and the carboxylic ester in position 3 is cleaved. Afterwards, the halogen in position 7 is substituted by a morpholine ring and, in the last step, the carboxylic acid is coupled with a benzylic amine.
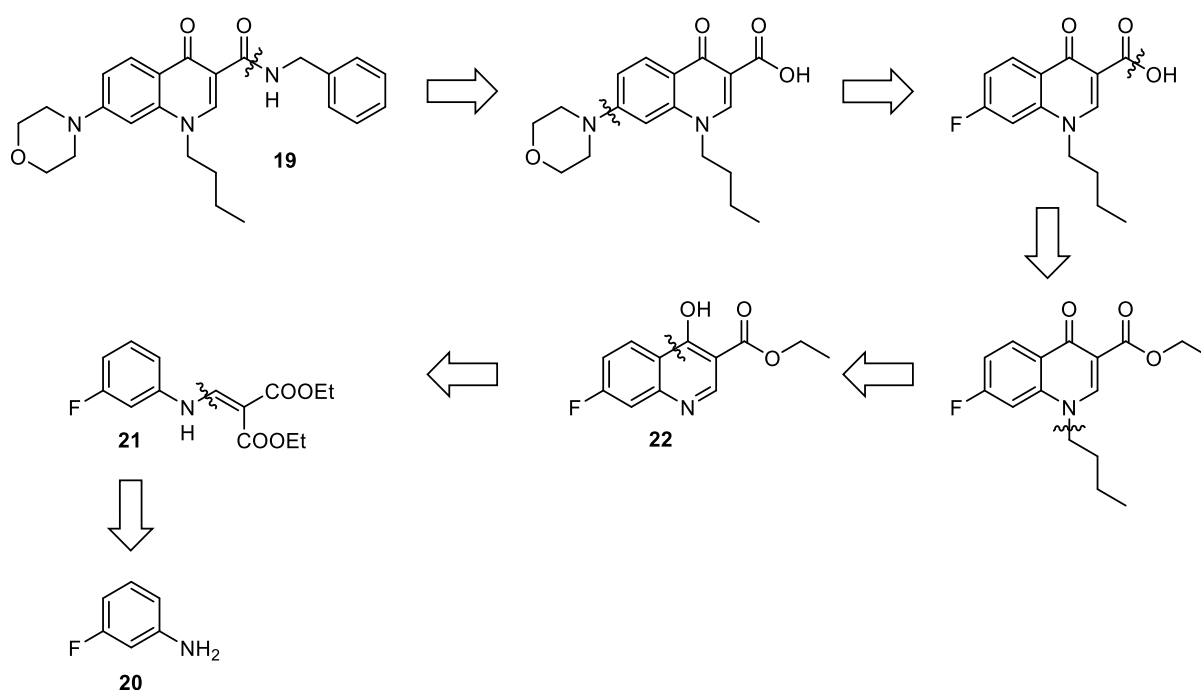
*Scheme 1. Retrosynthesis for the established synthetic route for quinolone amide **19**.*

3.2   Gould-Jacobs procedure

The Gould-Jacobs reaction is a thermal cyclization for the synthesis of the 4-quinoline scaffold, an indispensable heterocycle in drug discovery.[77] It consists of two consecutive steps, which are shown in Scheme 2, and begins with a condensation of an aniline derivative and diethyl ethoxymethylenemalonate. The resulting anilinomethylenemalonic ester **21** undergoes a cyclization at high temperatures to yield the respective quinoline **22**.[78]
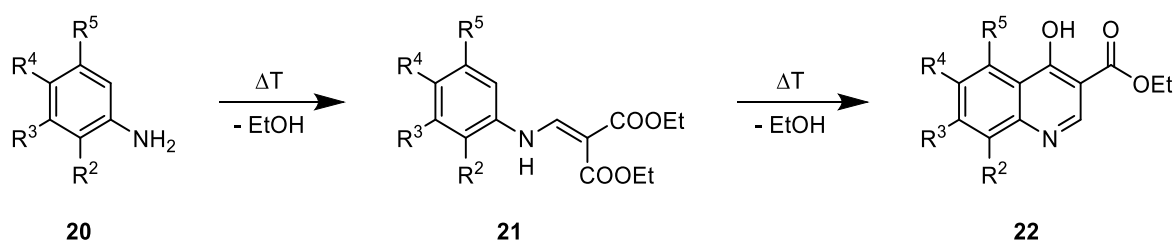
Scheme 2. General reaction scheme of the Gould-Jacobs procedure.

The first step can be carried out in a classical approach or a microwave-assisted synthesis, while the latter shortens the required reaction times quite drastically (1-3 h).[69] In this work, the condensation was done under classical heating in a bomb tube for one day due to the easy reaction setup and comparable yields, which are listed in Table 1. All products were crystallized in cold pentane and unused liquid starting materials were separated by washing with pentane.

The halogen substitution pattern in the aniline derivatives was chosen in respect to the desired substitution in the final compounds. A fluorine or chlorine substituent in position 3 was always required due to the morpholine substitution in a later step.
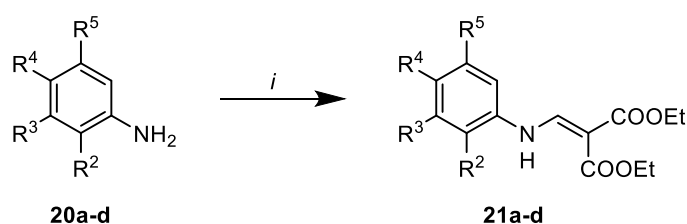

Table 1. Synthesis of anilinomethylenemalonic esters **21a-d** and their respective yields. Reagents and reaction conditions: i) diethyl methylenemalonate, toluene, 110 °C.

Compound	R^2	R^3	R^4	R^5	Yield [%]
21a	H	Cl	F	H	86
21b	H	F	H	H	57
21c	H	F	H	F	90
21d	F	F	F	H	94

The cyclization in the second step of the Gould-Jacobs procedure requires high temperatures (>200 °C). Like the step before, this reaction can be done under classical heating or microwave irradiation. The advantages of the microwave-assisted synthesis are short reaction times (25 min) and high yields.[69, 72] Therefore, this method was used here. Diphenylether was chosen as a suitable solvent for these high temperatures. Unfortunately, it has a poor microwave absorption due to its low dielectric constant.[79] To overcome this problem, *Weflon*® plates were added to the reaction solution, enabling a heating up to 210 °C.
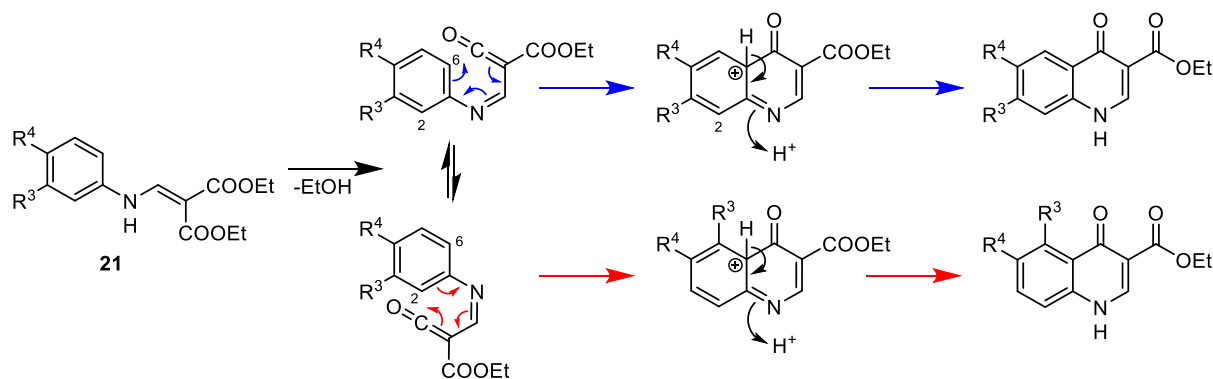
Scheme 3. The reaction mechanism of the electrocyclization in the Gould-Jacobs protocol.

When there are hydrogen substituents in both *ortho*-positions 2 and 6, the Gould-Jacobs reaction lacks regioselectivity, which is illustrated by the reaction mechanism in Scheme 3. At high temperatures, the anilinomethylenemalonic ester **21** forms a ketene by an elimination of ethanol.[80] Afterwards, a 6π-electronic cyclization occurs in position 2 (red pathway) or 6 (blue pathway) of the aniline moiety, followed by a rearomatization resulting in the respective quinoline. Once an unsymmetrical aniline derivative is used, two possible isomers are formed. When the starting materials **21a,b** were cyclized, these isomers were not separated by chromatography due to their poor solubility rather than used directly in the alkylation of the *N*-1 position, after which a purification was easily possible. Therefore, Table 2 contains the combined yields for both isomers.

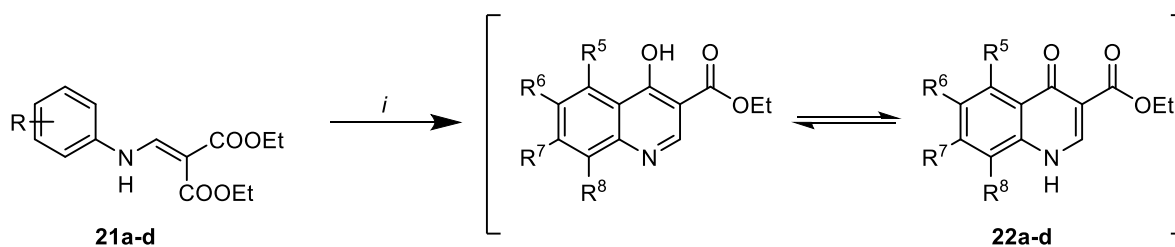*Table 2. Synthesis of 4-quinoline derivatives **22a-d** and their respective yields. Reagents and reaction conditions: i) diphenyl ether, 210 °C.*

Compound	R^5	R^6	R^7	R^8	Yield [%]
22a	H	F	Cl	H	50
22b	H	H	F	H	56
22c	F	H	F	H	68
22d	H	F	F	F	44

The products **22a-d** exist as two tautomers, being in an equilibrium of the 4-hydroxy form and the 4-oxo form. Semi-empirical quantum chemistry based investigations predicted that the hydroxy form is more stable.[81, 82] However, NMR observations in DMSO-d_6 and UV spectra in water showed that the 4-oxo form is predominant in polar solvents.[72, 81]

3.3 Modifications in position 1

3.3.1 Cycloalkylmethyl substituents

In former studies, the *N*-1 position possessed small aliphatic (e.g., ethyl, *n*-propyl, *n*-butyl, cyclopropyl) or aromatic substituents (2-fluorophenyl).[67, 69, 72] In this thesis, more bulky aliphatic groups were integrated to the quinolone amide structure in order to prevent the molecular π-π-stacking of the solid phase. These substituents were introduced under the same conditions as in prior publications, which are shown in Table 3.[69] The 4-oxo-1,4-dihydroquinoline-3-carboxylic ester **22b** was dissolved in abs. DMF, an excess of K_2CO_3 was added, and the suspension was stirred at 60 °C for 30 min to deprotonate the amine. Afterwards, the respective cycloalkylmethylbromide (R^1Br) and a catalytic amount of KI were added, and the mixture was stirred at 100 °C for three days.
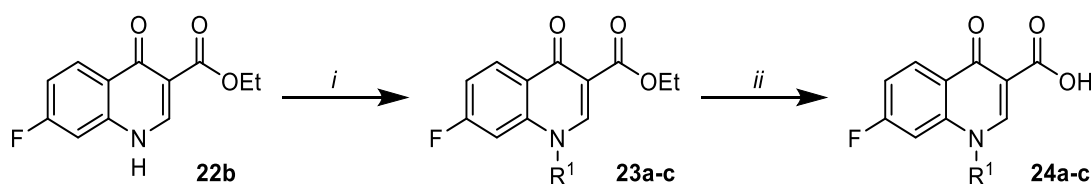

Table 3. Synthesis of 1-(cylcoalkylmethyl)-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acids. Reagents and reaction conditions: i) K_2CO_3, DMF, 60 °C for 30 min, then cat. KI, R^1Br, 100 °C; ii) 2 M HCl, EtOH, reflux.

Compound	R1	Yield [%]
24a		43
24b		54
24c		54

A purification by column chromatography was possible after this step due to the higher solubility compared to the starting material **22b**, which enabled a separation from the 5-fluoro isomer originating from the step before and additional minor side products, which derived from an *O*-alkylation or dimethylamine substitution in position 7 due to a decomposition of DMF. The alkylated products **23a-c** were used directly without further characterization in the next step, the cleavage of the 3-carboxylic ester, which is possible under basic or acidic conditions.

However, a substitution with a hydroxy group occurred in position 7 as a side reaction under basic conditions. Therefore, the esters **23a-c** were dissolved in ethanol and heated with a 2 M HCl solution, which had the additional advantage that the forming carboxylic acids **24a-c** precipitated, allowing for a convenient work-up.

In the next step, the morpholino substituent was introduced in position 7 by a nucleophilic aromatic substitution, which involves an addition-elimination mechanism. In the first and rate-limiting step, the morpholine attacks the aromatic system, forming the resonance-stabilized Meisenheimer complex **25**, which is shown in Scheme 4.[83] The electron-withdrawing 4-oxo group helps to stabilize this σ-complex, which lowers the activation energy of the nucleophilic attack. The following elimination of the fluoride leads to rearomatization of the ring.[84]
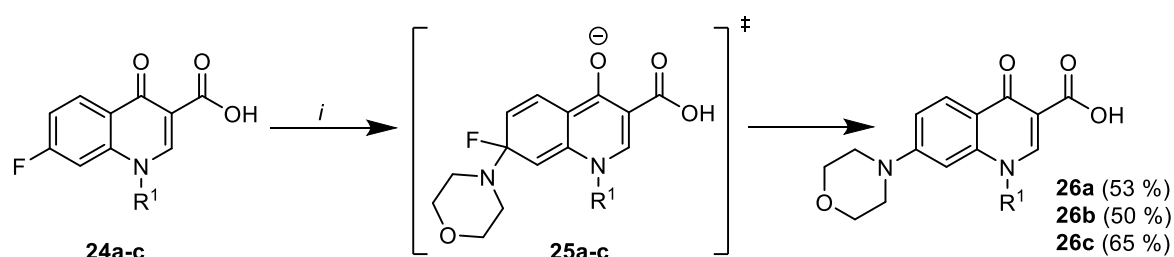
*Scheme 4. Synthesis of 7-morpholine quinolones **26a-c** by a nucleophilic aromatic substitution. Reagents and reaction conditions: i) morpholine, 110 °C.*

Since the quinolonic acids **24a-c** had only one potential leaving group and no further substitutions were possible, the reaction was run neat in morpholine without additional solvent. The starting materials were dissolved in morpholine and rotated under microwave irradiation, followed by an acidification that led to precipitation of the morpholine substituted products **26a-c**.

In the last step, the carboxylic acids **26a-c** were coupled with benzylamine to yield the final quinolone amides **27a-c**, which can be seen in Scheme 5. These reactions were accomplished under standard peptide coupling conditions, utilizing the reagent benzotriazole-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) under basic conditions. The carboxylic acids form the respective HOBt ester with PyBOP, which is more reactive due to the good leaving group, enabling the acylation of the amine. PyBOP is the further developed BOP coupling reagent and does not form the carcinogenic hexamethylphosphoramide (HMPA) during the reaction.[85] It is also superior to carbodiimides reagents, like DCC or EDC, which have the disadvantage of a slow formation of unreactive *N*-acylurea derivatives,[86] and HBTU, which releases the volatile cytotoxic tetramethylurea as a sideproduct.[87]
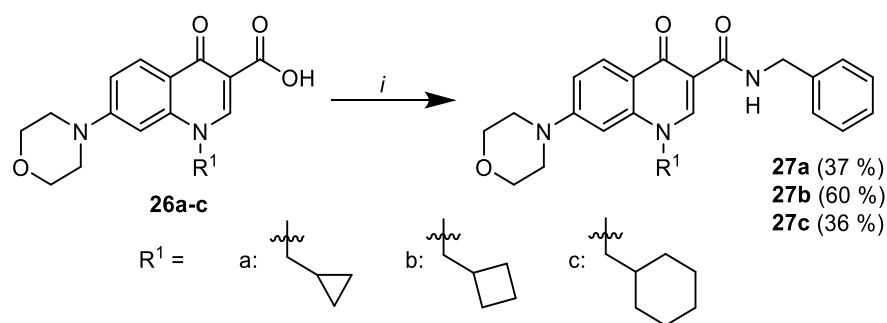
*Scheme 5. Amide coupling of the compounds **26a-c** with benzylamine. Reagents and reaction conditions: i) PyBOP, DIPEA, BnNH$_2$, DMF.*

The three synthesized quinolone amides **27a-c** were purified by column chromatography and subsequent recrystallizations from mixtures of CHCl$_3$ and EtOH.

3.3.2 Bicyclic substituents

In recent years, bicyclic molecule residues have been frequently used as a benzene bioisostere in medicinal chemical research, in attempts to improve potency, physicochemical properties, and metabolic stability.[88-91] In this work, the flexible butyl chain in position 1 was replaced by the simplest member of the bicyclic bridged compounds, the bicyclo[1.1.1]pentane, analogous to the preceding cycloalkylmethyl substituents. The newly introduced bulky three-dimensional residue, which can be seen in Figure 9, should hinder the intermolecular interactions in the crystal lattice and, therefore, enhance the aqueous solubility.
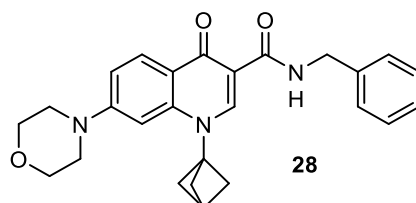

*Figure 9. Molecular structure of the target compound **28**, which contains a bicyclic bridged moiety in position 1.*

The 1-bicyclo[1.1.1]pentan-1-yl quinolone amide **28** could not be synthesized using the general route depicted in Chapter 3.1 due to the limited availability of 1-halogene bicyclo[1.1.1]pentane derivatives, which are expensive and can only be synthesized by extensive procedures.[92, 93] Since bicyclo[1.1.1]pentane amine was commercially available, this synthetic building block was used in the quinolone 3-carboxylic acid synthetic route established by Grohe and Heitzer, which was already used by Hiltensperger for similar derivatives in our research group.[69, 94] The first step of this procedure is the treatment of 2,4-dichlorobenzoic acid (**29**) with thionyl chloride to yield the respective acyl chloride **30**, as seen in Scheme 6. The excess of thionyl chloride was removed by distillation and the residue was dissolved in toluene, which was subsequently removed *in vacuo* to remove slight amounts of remaining thionyl chloride. This procedure was repeated several times. Magnesium ethoxide was freshly prepared by

suspending magnesium turnings in ethanol and adding a few drops of carbon tetrachloride until a modest boiling was achieved. Diethyl malonate was added to this solution and the mixture was transferred to the prior prepared acyl chloride **30** to yield compound **31**, which was singly decarboxylated by heating with *para*-toluenesulfonic acid in water without any prior purifications. The resulting substance **32** was purified by column chromatography and isolated in a yield of 59 % over these three steps.
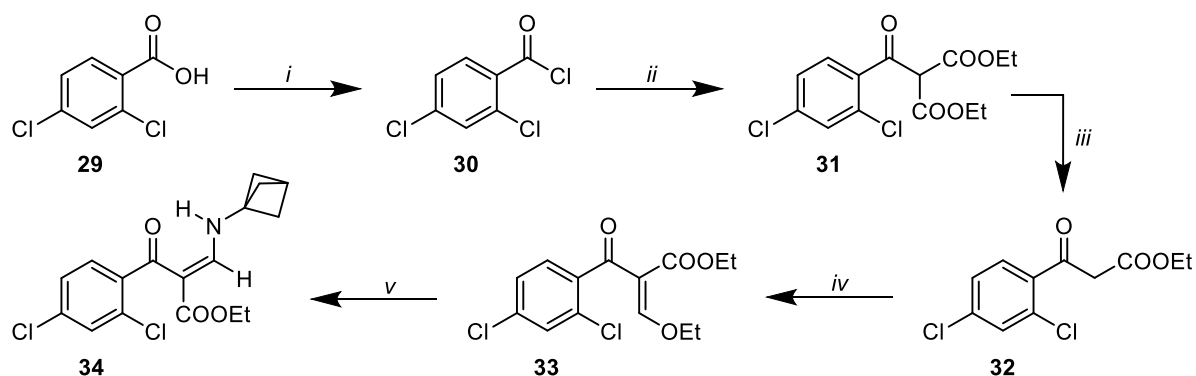
*Scheme 6. Synthesis of compound **34**. Reagents and reaction conditions: i) SOCl₂, reflux; ii) Mg, CH₂(COOEt)₂, CCl₄, EtOH; iii) p-TsOH, H₂O, reflux; iv) triethyl orthoformate, acetic anhydride, 115 °C; v) 1-bicyclo[1.1.1]pentylamine hydrochloride, K₂CO₃, EtOH, reflux.*

Compound **32** existed as two tautomers, the keto and the enol form, which was observable by NMR spectroscopy. Both, the ^{1}H NMR and the ^{13}C NMR spectrum, contained a double set of signals. The tautomeric ratio was determined by integration of the ^{1}H NMR spectrum, for which an excerpt is shown in Figure 10. The keto form had a CH_2 signal at a chemical shift of 4.00 ppm, whereas the enol form had a CH signal at 5.55 ppm and an OH signal at 12.48 ppm. The integrals indicated a ratio of about 1:1, which was identical to the derivative synthesized by Hiltensperger and the literature.[69, 95]
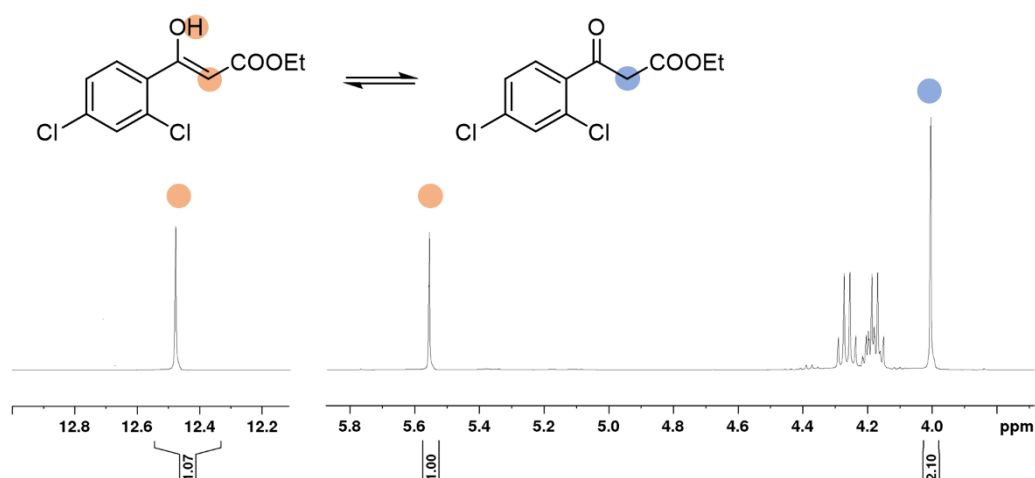

*Figure 10. An excerpt from the ^{1}H NMR spectrum (400 MHz, CDCl₃) of compound **32** that illustrates the tautomeric equilibrium.*

Compound **32** was dissolved in acetic anhydride and rotated with triethyl orthoformate under microwave irradiation to yield product **33**. In this reaction, the enol form attacks the triethyl orthoformate, resulting in a substitution of an ethanolat, which gets intercepted by the acetic anhydride. A subsequent elimination of ethanol generates the conjugated π-electron system (Scheme 6). After the reaction, all volatile substances were removed *in vacuo* and compound **33** was directly used in the substitution of the vinylogous ester with the bicyclo[1.1.1]pentane amine. After a purification by column chromatography, the desired product **34** was isolated by precipitation from petroleum ether at – 20 °C overnight. As Jürgens reported in a publication of the synthesis of *N*-aryl substituted quinolone antibacterials,[96] this intermediate was present as an *E*- and a *Z*-isomer, indicated by the two sets of signals in the ^{1}H NMR spectrum. The isomeric ratio of three to one, with the *E*-isomer being the major product, was determined by the integration of the respective *β*-protons at 8.03 and 8.11 ppm, which is shown in Figure 11. Compared to previously published *N*-aryl substituted derivatives, the surplus of the *E* isomer is significantly lower (7:1).[69, 96]
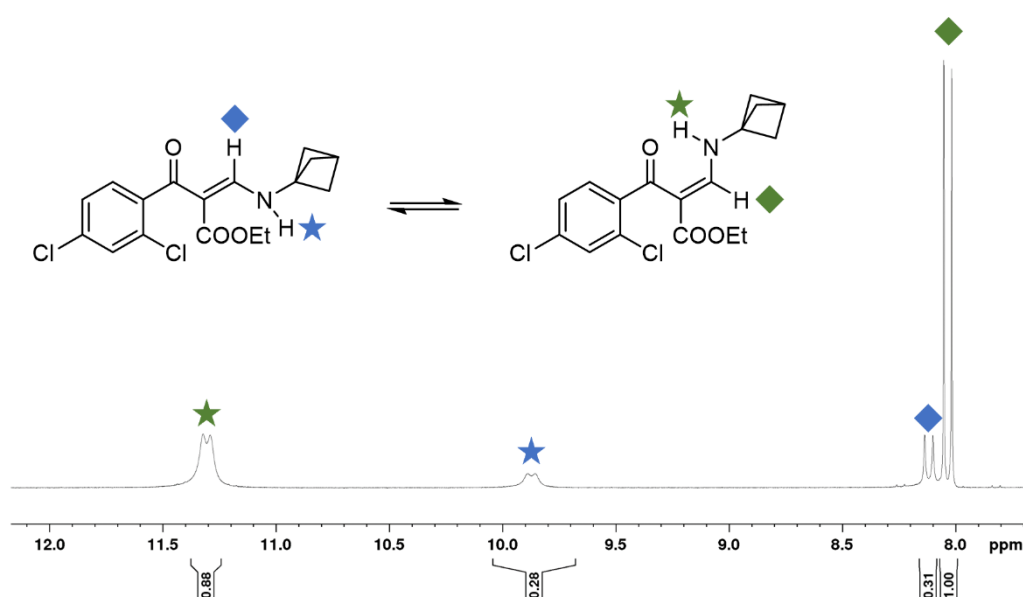
*Figure 11. An excerpt from the ^{1}H NMR spectrum (400 MHz, CDCl$_3$) of compound **34**.*

The ring closure was performed by an intramolecular nucleophilic aromatic substitution, in which the vinylogous amide attacked the aromatic *ortho*-position, replacing the chloride substituent. To facilitate this reaction, the mixture was stirred with K$_2$CO$_3$ at 110 °C for 2 h. The crude product **35** was directly used in the ester cleavage without further purification by heating with a 2 M HCl solution to yield the quinolonic acid **36**, which is shown in Scheme 7.
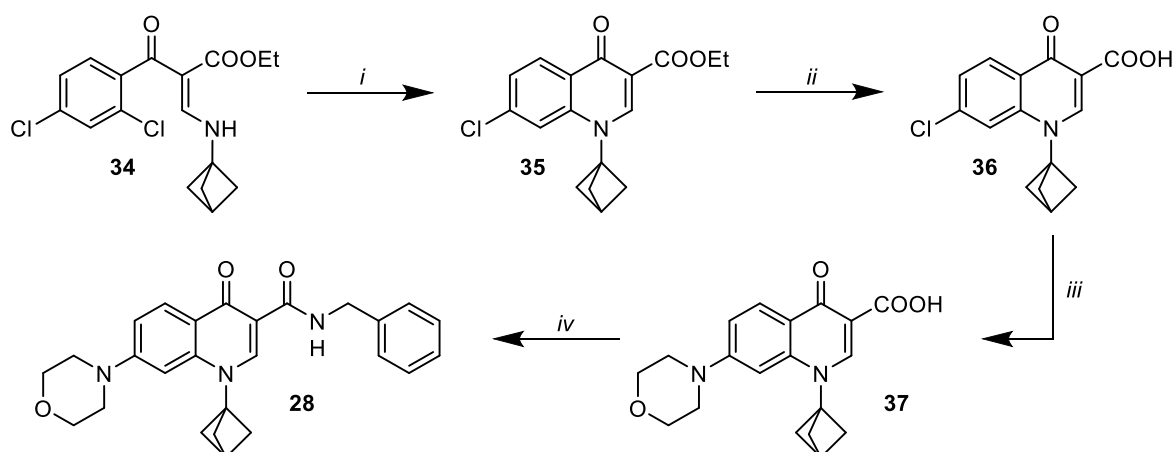
*Scheme 7. Synthesis of the quinolone amide **28**. Reagents and reaction conditions: i) K$_2$CO$_3$, DMF, 110 °C; ii) 2 M HCl, EtOH, 100 °C; iii) morpholine, 110 °C; iv) PyBOP, DIPEA, BnNH$_2$, DMF, r.t.*

Analogous to the already described general procedure in Chapter 3.1, compound **36** was substituted in position 7 with a morpholine ring in a microwave-assisted reaction and the resulting carboxylic acid **37** was coupled with benzyl amine to yield the desired quinolone amide **28**.

3.4 Modifications in position 3

3.4.1 Introduction of aliphatic cyclic benzyl amines

The crystal structure of **GHQ168** has shown that the flexible benzyl moiety in position 3 arranged itself in the remaining free space of the crystal lattice and hereby enables the π-π-stacking.[69] An insertion of a sterically demanding substituent in the benzylic position to limit the free rotatability of the residue should prevent this intermolecular interaction. Therefore, cycloalkyl substituents were introduced in the benzylic position of the benzylamine moiety. The amines **41a,b** were prepared through a three-step synthetic sequence, which is shown in Scheme 8.[97]
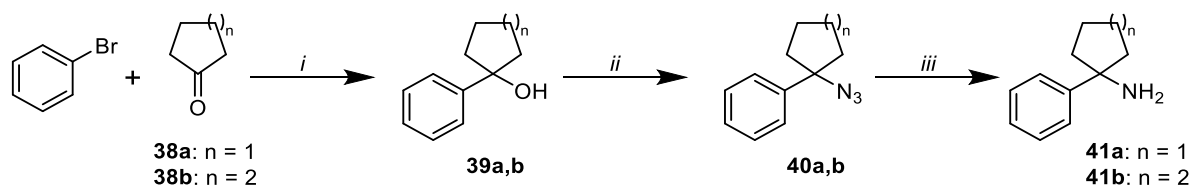
*Scheme 8. Synthesis of the cycloalkylamines **41a** and **41b** through a three-step synthetic route. Reagents and reaction conditions: i) Mg, abs. THF, 0 °C → r.t.; ii) NaN$_3$, TFA, CH$_2$Cl$_2$, 0 °C → r.t.; LiAlH$_4$, THF, 0 °C → r.t.*

The first step was a nucleophilic addition of freshly prepared phenylmagnesium bromide to the commercially available cycloalkyl ketones **38**. The Grignard reagent was made by treating bromobenzene with magnesium turnings, which were activated by a small amount of iodine to initiate the reaction, and added to the cooled ketone solutions through a filter cannula. The resulting tertiary alcohol derivatives **39** were directly used in the following substitution reaction

with sodium azide in the presence of trifluoroacetic acid. Afterwards, the azides **40** were reduced by lithium aluminium hydride and purified by extraction to the water phase after protonation, followed by a basification and an extraction to the organic phase to yield the desired cycloalkylamines **41a** and **41b**.

The cyclopropylamine **41c** could not be synthesized by the above depicted synthetic route due to the highly reactive nature of cyclopropanone, deriving from the high ring strain.[98] However, Bertus *et al.* developed a direct synthesis that originates from benzonitrile, which is shown in Scheme 9.[99]
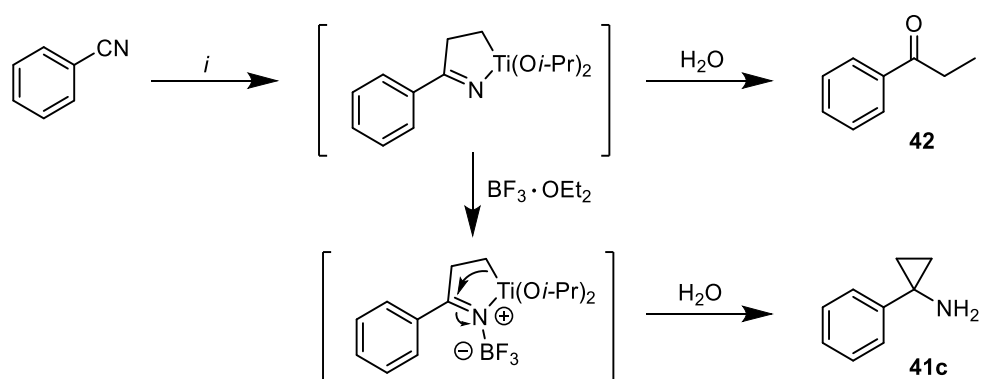
Scheme 9. Preparation of cyclopropylamine 41c by Bertus et al.[99] Reagents and reaction conditions: i) C_2H_5MgBr, Ti(Oi-Pr)$_4$, abs. THF, -78 °C → r.t., then BF$_3$·OEt$_2$.

When benzonitrile is nucleophilic attacked by ethylmagnesium bromide in the presence of titanium isopropoxide, a five-membered ring is formed as an intermediate. If the reaction is directly worked up by the addition of water, the main product is propiophenone (**42**) and the desired cyclopropylamine **41c** can only be isolated in low yields. However, the addition of boron trifluoride before hydrolysis decreases the electronic density at the imine carbon and promotes the ring closure. Therefore, the Lewis acid shifts the ratio of these two products in favor of the amine. Bertus *et al.* noted that, in contrast to aliphatic nitrile starting materials, the addition of the Grignard reagent to benzonitrile must be done at −78 °C, otherwise the yield drops significantly.[99]

The compound **41c** was synthesized according to the literature[99] and purified by extraction, analogous to the other cyclic benzyl amine derivatives **41a,b**. Remaining impurities were removed by column chromatography with ammonia-deactivated silica gel and the cyclopropylamine **41c** was isolated in a yield of 37 %.

Besides the already shown cycloalkyl substituents **41a-c**, a tetrahydro-2*H*-pyran and the respective thiopyran were introduced in the benzylic position of the quinolone amide structure. With an additional hydrogen bond acceptor, the number of potential hydrogen bonds increases and, therefore, the water solubility of the corresponding quinolone amide should also increase. The necessary pyran amines **47a,b** were synthesized by a three-step reaction sequence,

which was developed by Nitta *et al.*[100] The first two steps, a lithiation and a Ritter reaction, are shown in Scheme 10.

43a: X = O
43b: X = S

44a,b

45a: X = O
45b: X = S
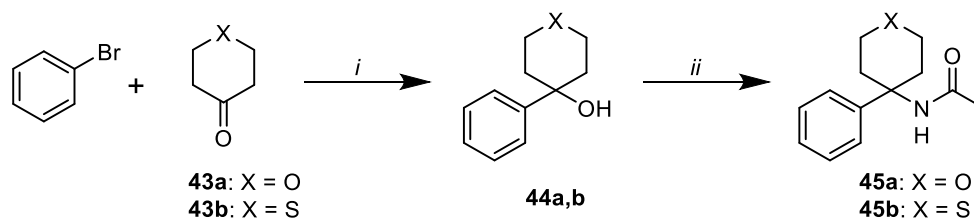
*Scheme 10. Synthetic sequence to the tetrahydropyranacetamides **45**. Reagents and reaction conditions: i) nBuLi in hexane (2.5 M), abs. THF, -78 °C → r.t.; ii) conc. H_2SO_4, MeCN, 0 °C.*

Analogous to the synthesis of cycloalkyl derivatives **41a,b**, the synthetic route started with bromobenzene, which was lithiated with *n*-butyllithium at – 78 °C. This reactive species was added to the commercially available tetrahydro-4*H*-pyran-4-one (**43a**) and thiopyranone **43b**, respectively, to yield the tertiary alcohols **44a** and **44b** (42–43 %). Subsequently, a Ritter reaction was carried out with concentrated sulfuric acid and acetonitrile, which simultaneously acted as solvent and reagent. The mechanism of this step is shown in Scheme 11. The strong acid protonates the respective tertiary alcohol, leading to an elimination of water. The resulting carbenium ion, which is stabilized by the neighbouring aromatic system, is attacked by the nitrogen of the acetonitrile. Afterwards, there is a nucleophilic addition of water to the nitrilium, followed by a hydrogen shift and a deprotonation to yield the desired acetamides **45**.
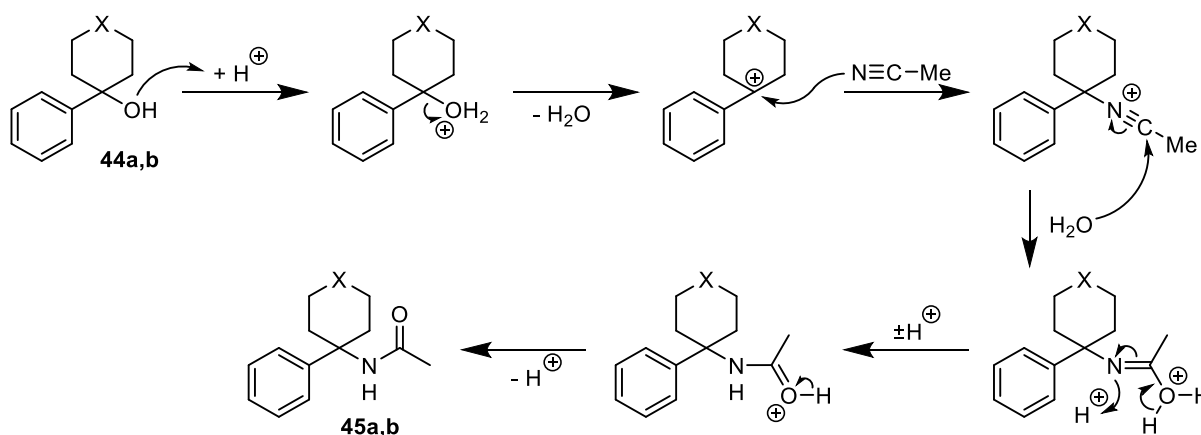
*Scheme 11.Reaction mechanism of the Ritter reaction of the tertiary alcohol **44** to acetamide **45**.*

The Ritter reaction was performed at 0 °C and the mixture was stirred for 2 h. The acetamides, **45a** and **45b**, were extracted and could only be purified by reversed phase flash chromatography. Due to the harsh reaction conditions, some side products were formed, decreasing the yields (a: 32 %, b: 57 %) of the isolated acetamides.

The last step was the deacetylation of the acetamides **45a** and **45b**. Nitta *et al.* used two different ways for this deprotection: a relative harsh method by stirring the acetamide in a 6 M

HCl solution under reflux and a mild one utilizing titanium isopropoxide and diphenylsilane.[100] For the products **45a,b**, the strong acidic conditions led to an elimination of acetamide and the conjugated product, the respective 4-phenyl-3,6-dihydropyran (**46a** or **46b**) was formed (Scheme 12, A). Therefore, the milder method was chosen, which was originally developed by Lee *et al.* based on the research of Buchwald *et al.* (Scheme 12, B).[101, 102] The acetamides, **45a** and **45b**, titanium isopropoxide, and diphenylsilane were dissolved in abs. THF and these solutions were heated until an effervescence started. The mixtures were periodically heated to maintain a steady effervescence. After two hours, no further gas evolution was observed and the reaction was quenched by the addition of 2 M HCl solution. Afterwards, the crude products were purified by extraction to the water phase, followed by basification and further extraction to the organic phase to yield the desired amines **47a** (54 %) and **47b** (64 %).
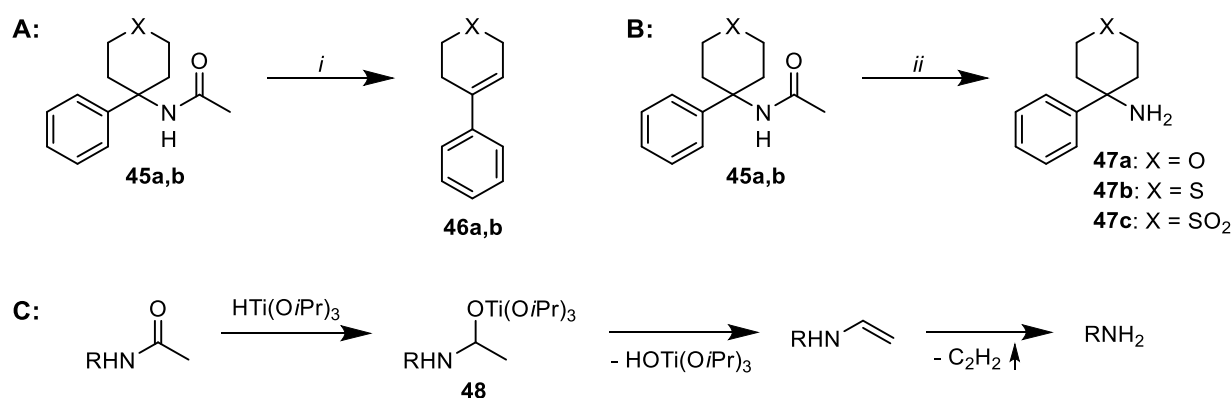
*Scheme 12. Deacetylation of the acetamides **45a** and **45b**. A: Elimination of acetamide under strong acidic conditions leading to the undesired product **46**. B: Deprotection of the amines under milder conditions. C. Proposed stepwise mechanism of the deacetylation utilizing titanium isopropoxide and diphenylsilane.[102] Reagents and reaction conditions: i) 6 M HCl, reflux; ii) Ti(OiPr)$_4$, Ph$_2$SiH$_2$, abs. THF, periodical heating to maintain effervescence.*

Although the exact mechanism of this reaction is still unclear, Buchwald *et al.* proposed that a titanium hydride-like complex (HTi(OiPr)$_3$) is formed by a σ-bond metathesis reaction between titanium isopropoxide and diphenylsilane.[102] This reactive reductant can lead to the hemiaminal **48** through oxidative addition to the titanium while the carbonyl is reduced, followed by elimination of the oxidized titanium complex and acetylene, which explains the observable gas evolution.

However, the NMR characterization of the tetrahydrothiopyran derivative **47b** revealed that not the desired product was isolated, but rather an oxidation of the sulfide to the respective sulfone **47c** had occurred. A comparison with the acetylated amine **45b** showed a significant low-field shift of the neighbouring protons. The signals appeared at 2.94 and 2.67 ppm in the acetylated molecule, in comparison with the respective signals at 3.63 and 2.85 ppm in the free amine **47c**. A similar low-field shift was observable in the ^{13}C NMR spectra, from 36.1 to 48.0 ppm. These observations indicated an oxidation to a sulfone with a higher electronegativity, which led to a lower electron density at the neighbouring atoms and, therefore, a low-field shift. NMR

measurements of similar compounds in the literature support these findings.[103, 104] Furthermore, the LC/MS measurement of the afterwards coupled quinolone amide showed the mass/charge ratio of the sulfone-containing product.

This oxidation was in contrast to the literature by Nitta *et al.*, in which the authors only isolated the corresponding sulfide after the deacetylation reaction.[100] In a later step, they intentionally oxidized it to the sulfone, but had to use strong oxidants, like TPAP or mCPBA. However, the sulfone group also represented an interesting structural motive, increasing the polarity of its final compound when incorporated in the benzylic position and, therefore, should improve the water solubility.

Subsequently, all the above mentioned cyclic benzylic amines, **41a-c** and **47a,c**, were linked to a quinoline moiety through peptide coupling, whereby the three different quinoline structures **26d-f** were used that are shown in Scheme 13.

26d: R^5, R^6 = H
26e: R^5 = H, R^6 = F
26f: R^5 = F, R^6 = H
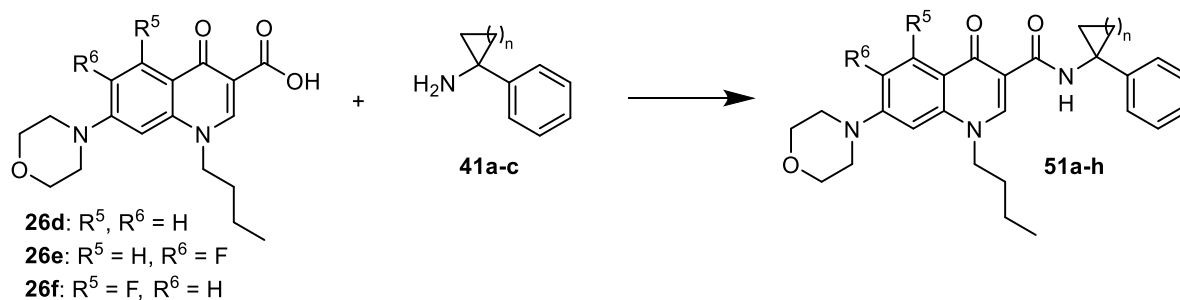
*Scheme 13. Amide coupling of the quinolonic acids **26d-f** with the cyclic benzylic amines **41a-c**.*

The necessary 7-morpholino and the 6-fluor-7-morpholino quinolinic acids, **26d** and **26e**, were synthesized analogously to the already seen *N*-cycloalkylquinolines **26a-c** (cf. Chapter 3.3.1), only shifting to 3-chloro-4-fluoroaniline as the starting material for **26e** and using 1-bromobutane in the *N*-1 alkylation step. A slightly different synthetic approach had to be chosen for the 5,7-difluor substituted acid **24f** due to the missing regioselectivity during the introduction of the morpholino ring, which additionally leads to the 5-substituted and the twofold substituted product. Shibamori *et al.* proposed a selective substitution strategy for this synthetic problem by selecting a suitable solvent.[105] They suggested that the attacking amine should be precoordinated by a hydrogen bonding to the carbonyl group in position 4 during a substitution in position 5 of the quinolone moiety. Therefore, this precoordination should favour the 5-substituted product in a non-polar solvent. In a polar solvent, this effect should be minimized and the C-7 substitution is preferred.[105] Nevertheless, experiments by Berninger have disproved this hypothesis; a regioselectivity by the choice of solvent was not detected.[72]

Using another approach, our research group established a procedure for the regioselective substitution, based on a publication by Heravi *et al.*.[69, 72, 106] The researchers utilized a

complexation of the carboxylic acid and the ketone in position 4 with boron trifluoride for a selective substitution in their synthesis of ciprofloxacin (**11**), which is shown in Scheme 14.
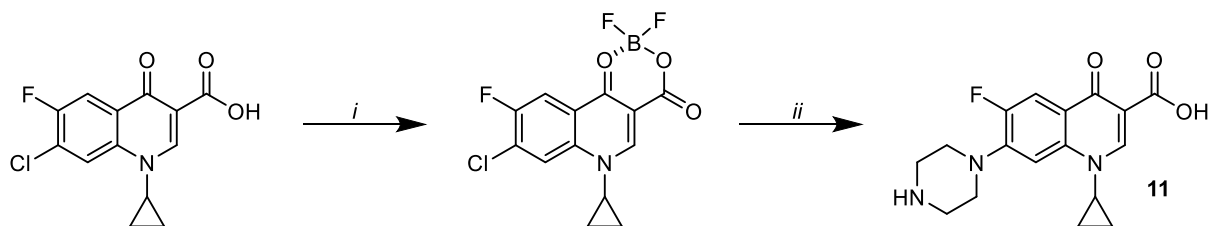
Scheme 14. Chelation with boron trifluoride for a selective halogen substitution by Heravi et al.[106] Reagents and reaction conditions: i) BF₃·OEt₂, NEt₃, CH₂Cl₂, reflux; ii) piperazine, NEt₃, abs. DMSO, then NaOH, reflux.

Although a lack of regioselectivity was not observable in the synthesis of the similar 6-fluoro-7-chloroquinolinic acid **26e** in this work, whereby morpholine even acted as the solvent of the reaction, the method of chelation has been adopted for the substitution of the 5,7-difluoroquinolinic acid **24f**, which is shown in Scheme 15. Therefore, the acid **24f** was heated with boron trifluoride etherate to form the desired chelate **49**, which was afterwards substituted in position 7 by morpholine. The cleavage of the boronic ester was performed under basic conditions, yielding the desired mono-substituted product **26f**.
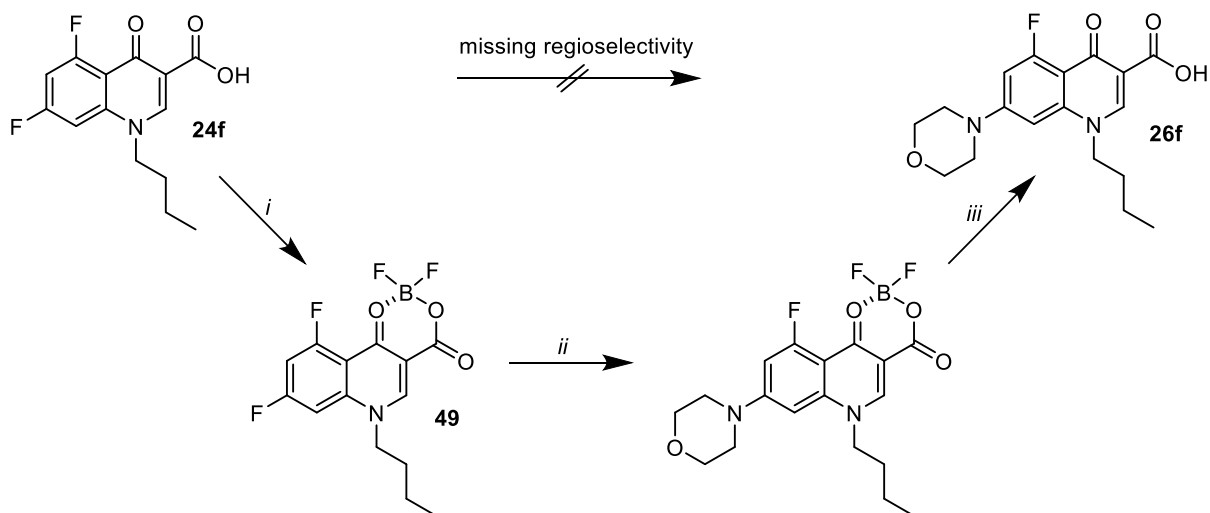
*Scheme 15. Regioselective nucleophilic aromatic substitution of compound **24f** in position 7. Reagents and reaction conditions: i) BF₃·OEt₂, NEt₃, CH₂Cl₂, reflux; ii) morpholine, NEt₃, EtOH, reflux; iii) 2 M NaOH, reflux.[72]*

In the case of Heravi's 6,7-dihalogenic substituted quinoline, the boronic ester acted as a Lewis acid and lowered the electronic density in the *para*-position. Therefore, the chlorine in position 7 was favoured in the piperazine substitution. In our synthesis, both halogen-containing positions, in *ortho*- and *para*-position to the *β*-carbonyl function, are activated by the electronic withdrawing effect. A stronger activation of position 7 compared to position 5 could not be detected in the ¹³C NMR spectrum of the boron chelate **49** by Berninger.[72] The preferred substitution in position 7 may occur due to a missing precoordination of the morpholine to the

carbonyl function in position 4, based on hydrogen bonding, as a result of the steric hinderance to the boronic ester.

The amide coupling products **50a-h** of the three quinolonic acids **26d-f** with the synthesized cycloalkyl benzylic amines **41a-c** are summarized in the following Table 4.

26d: R^5, R^6 = H
26e: R^5 = H, R^6 = F
26f: R^5 = F, R^6 = H
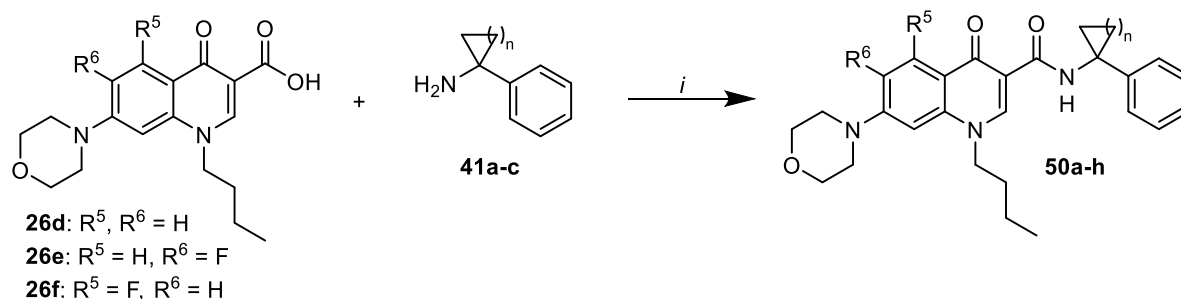
Table 4. Synthesis of cycloalkyl containing quinolone amides and their respective yields. Reagents and reaction conditions: i) i-Butyl-chloroformate, N-methylmorpholine, abs. DMF, 0 °C to r.t.

Compound	R^5	R^6	n	Yield [%]
50a	H	H	1	73
50b	H	F	1	84
50c	F	H	1	44
50d	H	H	3	95
50e	H	F	3	69
50f	H	H	4	63
50g	H	F	4	61
50h	F	H	4	82

These amide couplings were achieved by activation of the acids **26d-f** by a mixed anhydride (Scheme 16). Prior investigations by Niedermeier showed higher yields for this synthetic method, compared to a PyBOP-catalyzed coupling.[67] Therefore, the compounds **26d-f** were deprotonated by *N*-methylmorpholine and *i*-butyl chloroformate was added at 0 °C, forming the anhydride **51**. Afterwards, the cycloalkyl benzylic amines **41a-c** could attack this active intermediate to obtain the desired amides. However, the respective urea derivates **52** were formed as side products in this synthetic approach, which is shown in Scheme 16. These impurities were not detectable in the LC/MS chromatogram at 254 nm, only in the NMR spectra. They were removed by intensive washing with a saturated ammonium chloride solution. In further coupling reactions using the mixed anhydride approach, the crude products were already treated multiple times with a saturated ammonium chloride solution before purification by column chromatography.
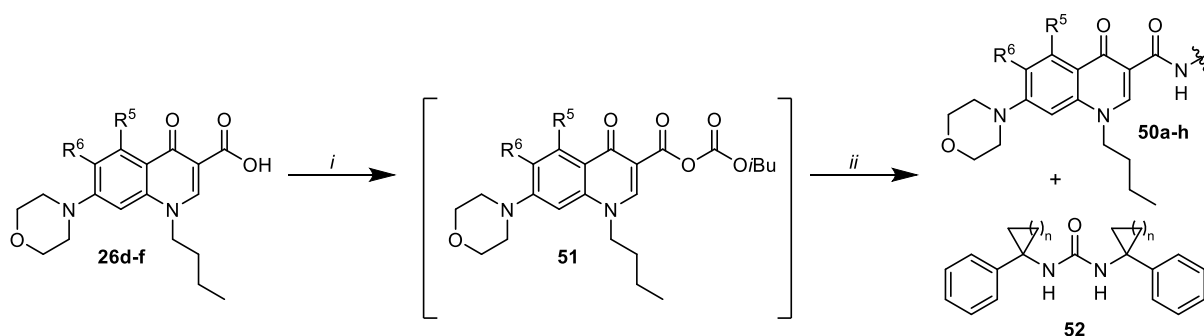
*Scheme 16. Amide coupling of the quinolonic acids **26d-f** with the synthesized cycloalkyl benzylic amines **41a-c** by an activated mixed anhydride **51** as an intermediate. Reagents and reaction conditions: i) i-Butyl chloroformate, N-methylmorpholine, abs. DMF, 0 °C; ii) cycloalkyl benzyl amines **41a-c**, abs. DMF, r.t.*

The heteroatom-containing cycloalkyl amines **47a,c** were also coupled with the quinolonic acid **26d**. Therefore, PyBOP was chosen as a suitable reagent, because under these conditions no side products were formed. The products were purified by column chromatography and recrystallized from EtOH to yield the desired amides **53a** and **53c**, which are shown in Scheme 17.
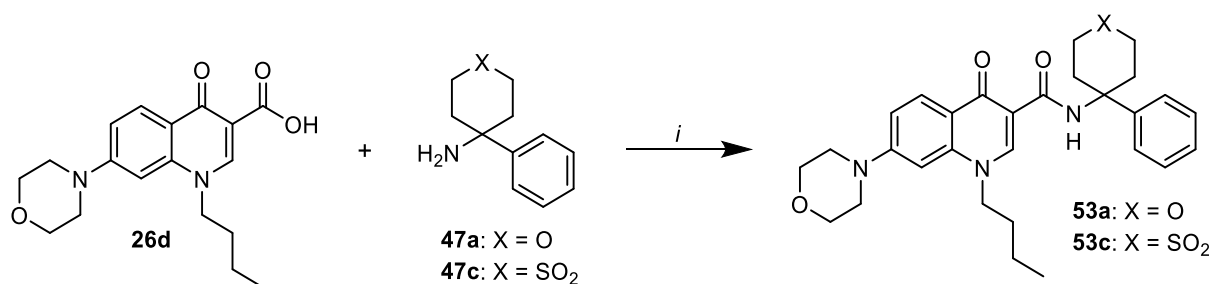
*Scheme 17. Amide coupling of the quinolonic acid **26a** with the heteroatom-containing cycloalkyl amines **47a,c**. Reagents and reaction conditions: i) PyBOP, DIPEA, BnNH$_2$, DMF, r.t.*

3.4.2 Introduction of a propellane-like aliphatic amide in position 3

In earlier projects, the benzylic moiety in position 3 was exchanged with similar aromatic derivatives.[69, 72] In this work, the influence of a bulky aliphatic residue was investigated. Therefore, the already used bicyclo[1.1.1]pentanyl amine was linked to the quinolonic acid **26d** under standard peptide coupling conditions using PyBOP.
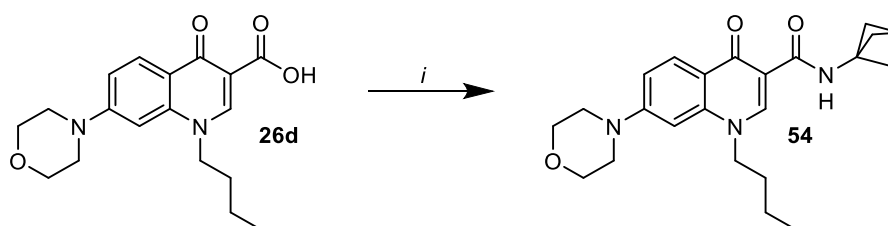
Scheme 18. Introduction of a bulky aliphatic residue in position 3. Reagents and reaction conditions: i) 1-bicyclo[1.1.1]pentylamine hydrochloride, PyBOP, DIPEA, abs. DMF, r.t.

After the successful reaction, the desired product **54** was purified by column chromatography and recrystallized from EtOH.

3.4.3 Introduction of benzylic primary amines in position 3

As another approach for the structural modifications in this position, the introduction of a primary amine was considered. The main reason for this step was not a hinderance of the mentioned π-π-stacking but rather an increase of solubility due to the basic properties of the free amine. Furthermore, the research group of Hergenrother has discovered in their studies that the insertion of a primary amine in selected antibiotic compounds generates an additional bioactivity against Gram-negative bacteria. They argue that the protonated amine enables better penetration of the substances through the porins.[107] Here a similar strategy was chosen to reproduce a corresponding preservation of bioactivity against trypanosomes by the introduction of a primary amines in the quinolone amides with a simultaneous increase in aqueous solubility. The benzylic moiety was selected for this modification because of the relatively easy synthetic accessibility, and *meta*- as well as *para*- substituted derivatives were synthesized.

Two synthetic approaches were possible for these primary amine derivatives, which are shown, exemplarily for the *meta*-substituted compounds, in Scheme 19. They can either be synthesized by an amide coupling with a single Boc-protected diamine and a subsequent deprotection (method **A**), or by the coupling with aminomethylbenzonitrile, followed by a reduction of the cyano group (method **B**).
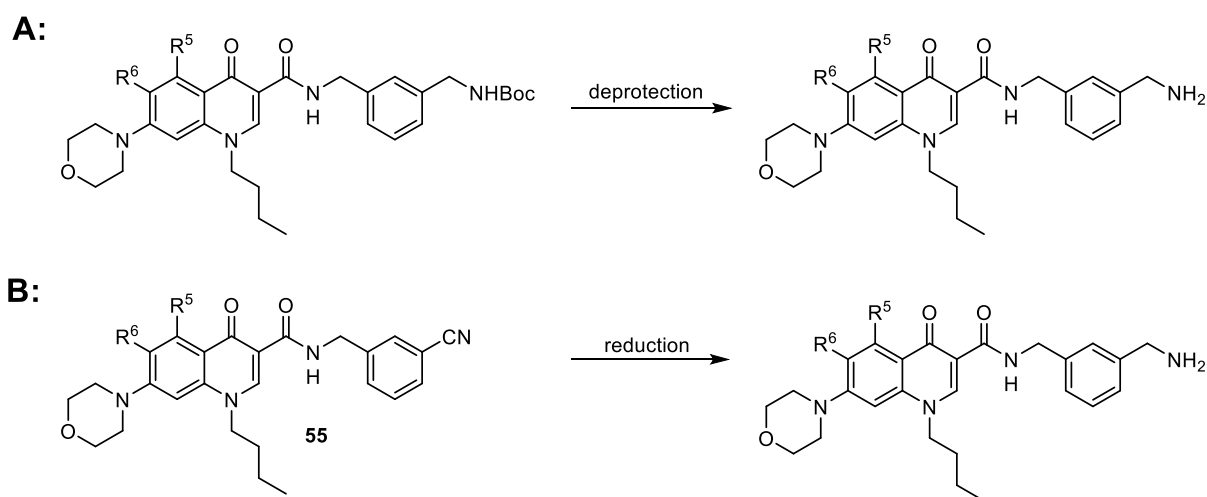
Scheme 19. Synthetic approaches for the introduction of primary amines in the benzylic moiety. Approach A: Deprotection of a Boc-protected amine. Approach B: Reduction of a nitrile group.

Test reactions revealed that minor unknown impurities were produced in the synthetic route **A** which could only be removed by several purifications using flash chromatography, whereas in the second approach the nitrile derivative **55** could be easily purified by recrystallization.

Therefore, the procedure **B** utilizing the reduction was chosen for the synthesis of the desired compounds. The benzonitrile derivatives, **56a** and **56b**, required for this purpose were prepared by a three-step synthesis starting from the respective methylbenzonitrile **57**, which is shown in Scheme 20. In the first step, a bromine substituent was introduced to the methyl group in a Wohl-Ziegler-bromination using dibenzoyl peroxide (DBPO) as a radical starter, following Kompella *et al.*[108, 109] The reaction was performed in chloroform, as the forming *N*-succinimide was insoluble in this solvent, enabling a convenient separation.
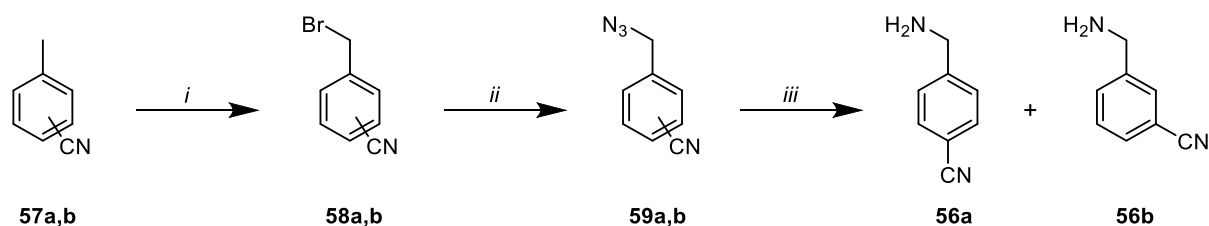
*Scheme 20. Synthesis of the benzonitrile derivatives **56a** and **56b**. Reagents and reaction conditions: i) NBS, DBPO, CHCl$_3$, reflux; ii) NaN$_3$, DMF, r.t.; iii) PPh$_3$, THF, H$_2$O, r.t.*

The single brominated product **58** was directly used in the subsequent substitution with sodium azide. In the last step, the azide group was reduced in a Staudinger reaction, based on the protocol of Deb *et al.*[110] Therefore, compound **59** was dissolved in a THF/water-mixture with PPh$_3$ as a reducing agent, which selectively reduced only the azide function. The present water in the reaction mixture hydrolysed the forming phosphazene, leading to the desired amines **56a** and **56b**, which were purified by protonation and extraction to the water phase, followed by basification and further extraction to the organic phase. It was discovered that these amines are not stable over a longer period and should be directly used in the following synthetic step.

The three already shown quinolonic acids **26d-f** were coupled with these aminomethylbenzonitriles **56a,b** by an activation via a mixed anhydride under the same conditions as already discussed in Chapter 3.4.1. The products **55a-f** are summarized in the following Table 5.
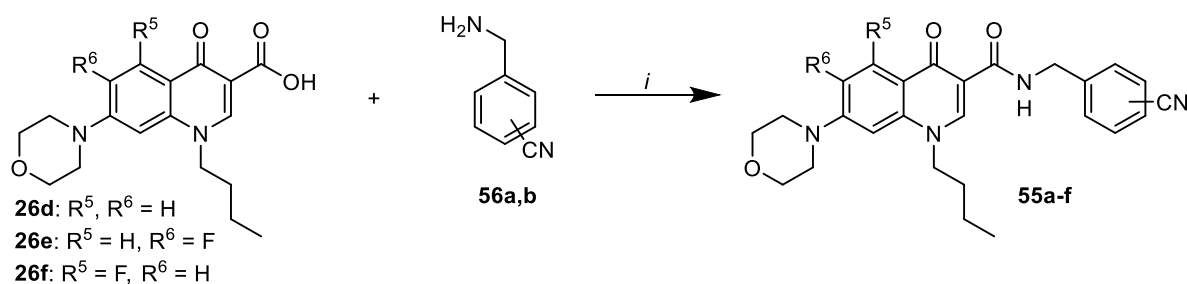
*Table 5. Synthesis of quinolone amides with a nitrile function **55a-f** and their respective yields. Reagents and reaction conditions: i) i-Butyl-chloroformate, N-methylmorpholine, abs. DMF, 0 °C.*

Compound	R^5	R^6	Position of nitrile function	Yield [%]
55a	H	H	*para*	51
55b	H	F	*para*	75
55c	F	H	*para*	81
55d	H	H	*meta*	88
55e	H	F	*meta*	76
55f	F	H	*meta*	73

In the next step, suitable conditions for a reduction of the nitrile function had to be found. Therefore, small test reactions were performed with nitrile **55a** as an exemplary compound. Several common reducing agents, which are listed in Table 6, were tested and the respective outcome was analyzed by LC/MS. A reduction with LiAlH$_4$ led to a decomposition of the starting material **55a** and several unwanted products, whereas in the experiment using borane as a reducing agent, even at elevated temperatures, no conversion of the starting material **55a** was detected.[111]

Sodium borohydride is a mild reducing agent with a broader functional group compatibility, compared to LiAlH$_4$. Generally, it is not strong enough to reduce a cyano group.[112] However, its reactivity can be enhanced by the addition of transition metal salts, the most reliable of such are nickel and cobalt salts.[112, 113] Therefore, a test reaction was performed with NaBH$_4$ and catalytic amounts of NiCl$_2$ in methanol. A first reaction control showed a slow formation of the desired primary amine, next to an unknown product with a lower mass-to-charge ratio and mainly the starting material **55a**. The addition of one additional equivalent of sodium borohydride and stirring overnight led to a complete conversion of the starting material **55a** to the unknown product. To avoid this formation, a similar approach was tested under the same conditions with the addition of Boc$_2$O, in order to trap the formed amine in the Boc-protected form, which was established by Caddick *et al.*[114] The desired protected amine was formed; in addition to an array of side products, which would have made the purification more difficult.
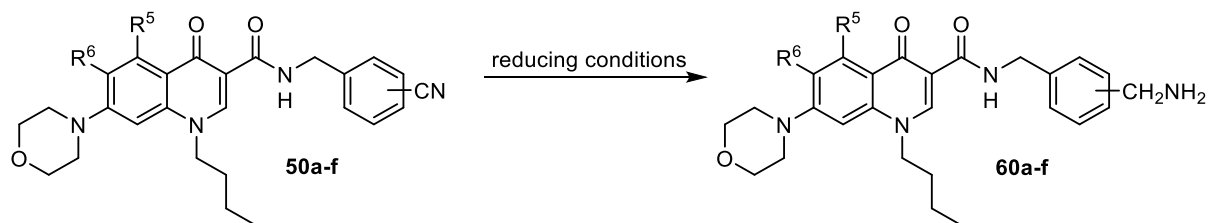
Table 6. Carried out test reactions for finding suitable reduction conditions and their outcome, which was observed by LC/MS analysis.

Reducing conditions	Observable reaction outcome
LiAlH$_4$, THF, r.t.	Decomposition of starting material
BH$_3$, THF, r.t.	No conversion
BH$_3$, THF, reflux	No conversion
NaBH$_4$, NiCl$_2$, MeOH, r.t.	Conversion to an unknown product
NaBH$_4$, NiCl$_2$, Boc$_2$O, MeOH, r.t.	Formation of some side products
Pd/C, 1 atm. H$_2$, MeOH	No conversion
Pd/C, 10 atm. H$_2$, MeOH	No conversion
Raney-Ni, 10 atm. H$_2$, MeOH/CH$_2$Cl$_2$	Mainly product formation

In a next attempt, the cyano group should be reduced with palladium in a hydrogen atmosphere.[115, 116] The test reaction under one standard atmosphere of hydrogen showed no conversion at all, only the starting material was detected in the LC/MS analysis. Even an increase of the hydrogen pressure to 10 atm did not lead to any conversion of the starting material. The shift to Raney-Nickel as the catalyst under 10 atm of hydrogen enabled a reduction of the nitrile function and resulted in the desired product with nearly no side products.[117] Therefore, this approach was chosen for the reduction of the quinolone amides with a nitrile function **55a-f** and the isolated amines **60a-f** were purified by flash chromatography.

3.5 Modifications in position 7

3.5.1 Triethylene glycol derivatives

The incorporation of oligo(ethylene glycol)methyl ether chains into small lipophilic compounds is a common procedure to increase the solubility in polar solvents, with applications in all fields of chemistry.[118-121] Next to its increase of hydrophilicity, the longer side chains can also provide an introduction of steric hinderance and reduce aggregation through π-π stacking interactions.[118] In this work, tri(ethylene glycol)methyl derivatives were introduced in position 7 of the initial quinolone amide structure. Three different substituents were planned, each with a methoxy group at the end of the linear chain, which are depicted in Figure 12. The first ethylene glycol chain was directly linked with the free hydroxy group, whereas the second had a primary amine in this position. The last derivative was coupled via the secondary amine of a piperidine moiety.
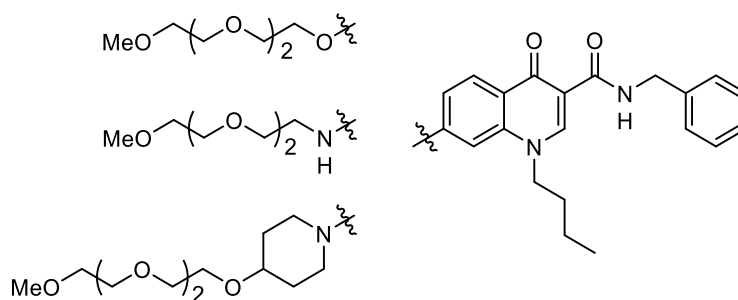
Figure 12. Overview of the planned triethylene glycol derivatives.

The synthesis of all three derivatives originated from triethylene glycol, which was singly methylated with dimethyl sulfate in the first step.[122] The product **61** was used as the first triethylene glycol substituent and was taken as a starting point for the other derivatives. Therefore, the remaining alcohol group was activated with tosyl chloride and, for the second derivative, substituted with sodium azide.[123, 124] The resulting azide **62**, which is shown in Scheme 21, was reduced in the following step by LiAlH$_4$, which led to the desired primary amine **63**.[125]
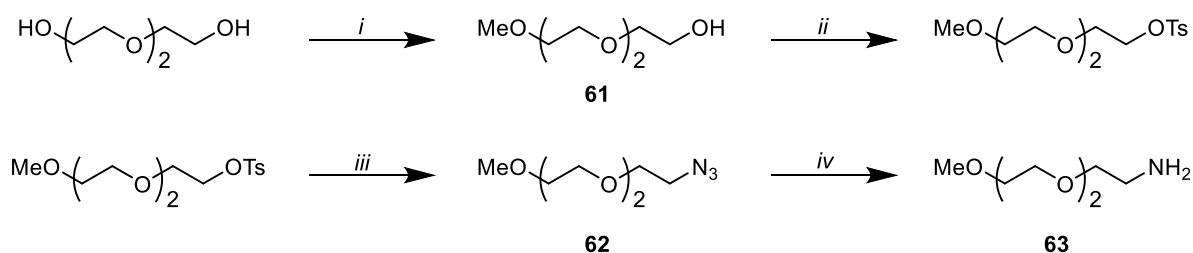
*Scheme 21. Synthesis of the triethylene glycol chains **61** and **63**. Reagents and reaction conditions: i) Me$_2$SO$_4$, NaOH, 110 °C; ii) TsCl, NEt$_3$, CH$_2$Cl$_2$, 0 °C to r.t.; iii) NaN$_3$, DMF, 80 °C; iv) LiAlH$_4$, THF, 0 °C to r.t.*

The last ethylene glycol chain was synthesized by the substitution of the tosyl-activated compound with prior *N*-Boc-protected 4-hydroxypiperidine under basic conditions, followed by a deprotection with TFA to yield the desired product **64**, which can be seen in Scheme 22. Both derivatives with an amine function, **63** and **64**, were purified by a protonation and extraction to the water phase, followed by a basification and a further extraction to the organic phase.
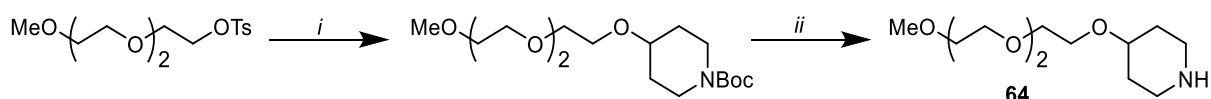
*Scheme 22. Synthesis of the triethylene glycol derivative **64**. Reagents and reaction conditions: i) N-Boc-4-hydroxypiperidine, NaH, THF, r.t.; ii) TFA, THF, r.t.*

Afterwards, the three triethylene glycol derivatives **61**, **63**, and **64** were linked to the quinolonic acid **24d** by a nucleophilic aromatic substitution in position 7, which is shown in Scheme 23. Two different approaches were used for this step: For compound **61**, the hydroxy group was deprotonated using sodium hydride and the substitution was performed at room temperature, whereas the amines **63** and **64** required elevated temperatures and DIPEA as a suitable base. Nevertheless, all three reactions needed several days for completion.
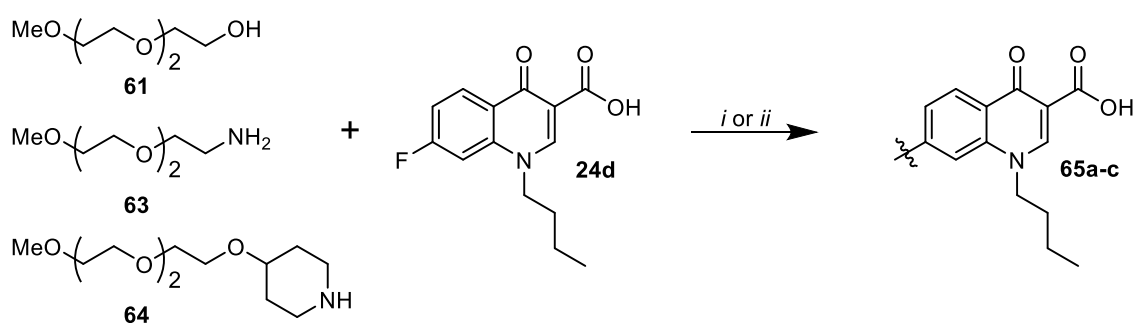
Scheme 23. Introduction of the triethylene glycol side chains to the quinolonic moiety. Reagents and reaction conditions: i) NaH, THF, r.t.; ii) DIPEA, DMF, 110 °C.

The resulting quinolonic acids **65** were purified by column chromatography with one percent of formic acid as an additive. Due to their hydrophilic properties, the compounds interacted strongly with the silica gel and could only be detached from the column using a high polar eluent mixture. This led to remaining impurities in compounds **65a** and **65b**, which were used directly in the next step without further characterization.

In the last step, the quinolonic acids **65a-c** were coupled with benzyl amine using the two already mentioned methods. The acids **65a** and **65b** were activated with isobutyl chloroformate, whereas the peptide coupling reagent PyBOP was used for compound **65c**. Both amide coupling procedures were successful and yielded the triethylene glycol modified quinolone amides **66a-c**, which are shown in Scheme 24.
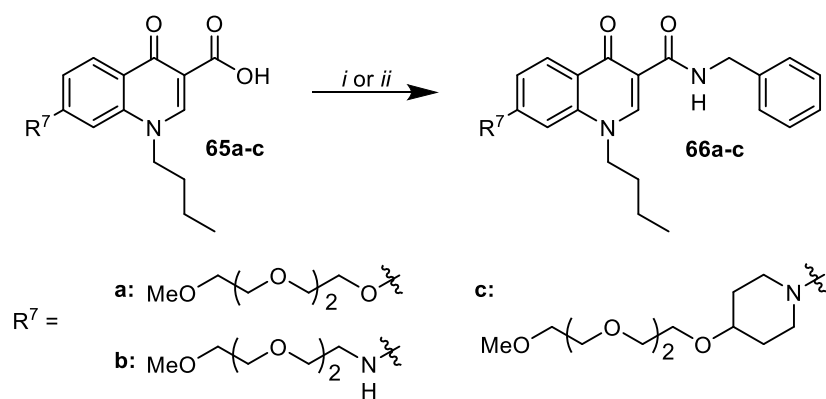
*Scheme 24. Amide coupling of quinolonic acids **65a-c** with benzyl amine. Reagents and reaction conditions: i) i-Butyl-chloroformate, N-methylmorpholine, benzylamine, abs. DMF, 0 °C to r.t.; ii) PyBOP, DIPEA, BnNH₂, DMF, r.t.*

3.5.2 1,4-Diazepanyl derivatives

Formerly published quinolone amides by our research group had mostly six-membered heterocycles in position 7, such as morpholine, thiomorpholine, piperazine-, or piperidine-derivatives.[68, 69, 72] In this work, the influence of a seven-membered heterocycle was investigated. Therefore, a 4-methyl-1,4-diazepanyl moiety was introduced in position 7 of the quinolone amide structure (Scheme 25).
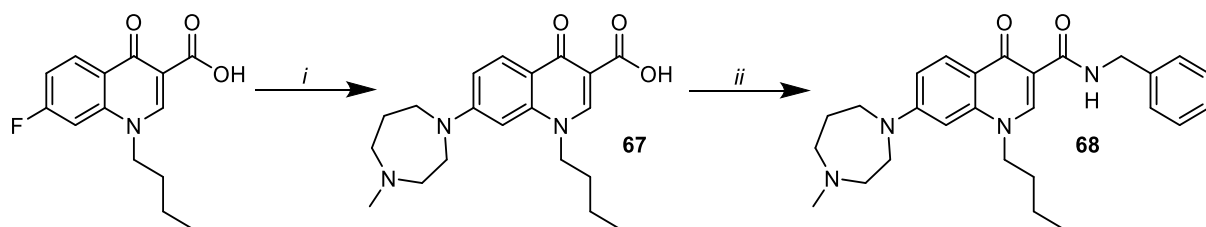
*Scheme 25. Synthesis of the 4-methyl-1,4-diazepanyl-containing quinolone amide **68**. Reagents and reaction conditions: i) 4-methyl-1,4-diazepane, DMF, 67 °C; ii) PyBOP, DIPEA, BnNH₂, DMF, r.t.*

The synthetic route started with the quinolonic acid **24d**, which was substituted with 4-methyl-1,4-diazepane under mild conditions in the microwave. The resulting compound **67** was purified by reversed phase chromatography. However, due to the formation of a zwitterion, the purification was challenging and the acid **67** contained slight impurities. In the next step, compound **67** was coupled with benzylamine under standard conditions, yielding the desired quinolone amide **68**.

3.6 Modifications in position 8

In their synthesis of a novel antibacterial quinolone, Kuramoto *et al.* showed by X-ray structures that a chlorine substituent in the position 8, neighboured by substituents in position 1 and 7, induced a slight distortion in the otherwise planar quinoline structure.[126] This deviation of the molecular structure even enhanced the biological activity compared to similar planar derivatives. It was assumed that in this work an analogous distortion could impair the π-π stacking, weaken the intermolecular interactions and, therefore, increase the water solubility of quinolone amides. For this reason, a chlorine as well as bigger non-polar substituents should be introduced in position 8 of our quinolone structure.

The original synthetic plan, which is shown in Scheme 26, was to start with 2,3-dichloroaniline (**69**) and proceed after the Gould-Jacobs procedure, using the same protocol as already reported in Chapter 3.2. However, the introduction of the butyl chain in position 1 was not possible under normal reaction conditions and only the starting material **70** was isolated. This was probably due to the steric hindrance to the chlorine substituent in position 8.
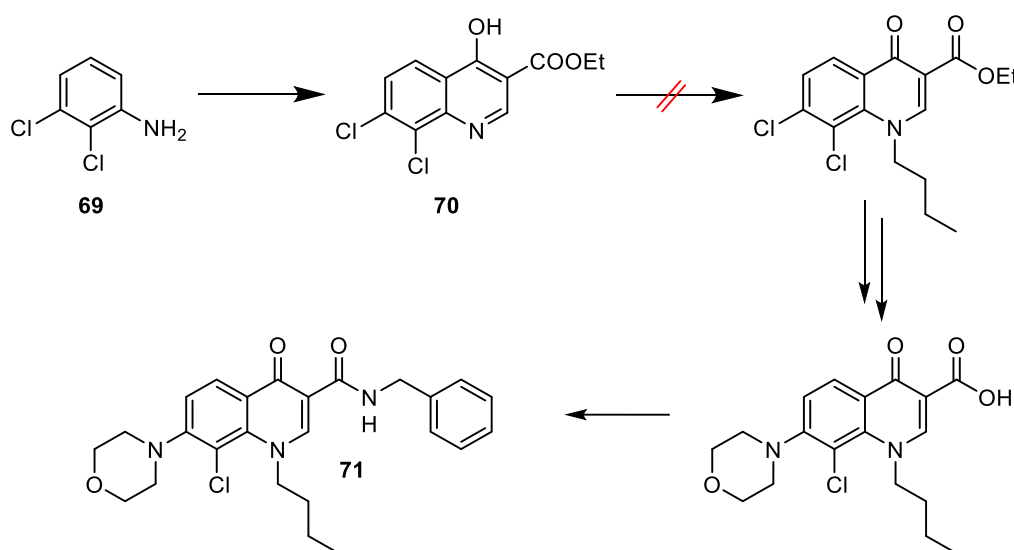
*Scheme 26. Original synthetic route for the 8-chlorine-substituted derivative **71**.*

After this unsuccessful attempt, the chlorine was implemented by an electrophilic aromatic substitution. The already synthesized 6-fluorine-substituted quinolonic acid **26e** was chosen as a suitable starting point for this synthetic route. This derivative was used to guarantee a mono chlorination, as with the 1-butyl-7-morpholino-1,4-dihydro-quinolonic acid **26d** a chlorination was possible in position 6 and 8. The reaction was performed with sulfuryl chloride under mild conditions and the resulting acid **72** was coupled with benzyl amine, which is shown in the following Scheme 27.[127]
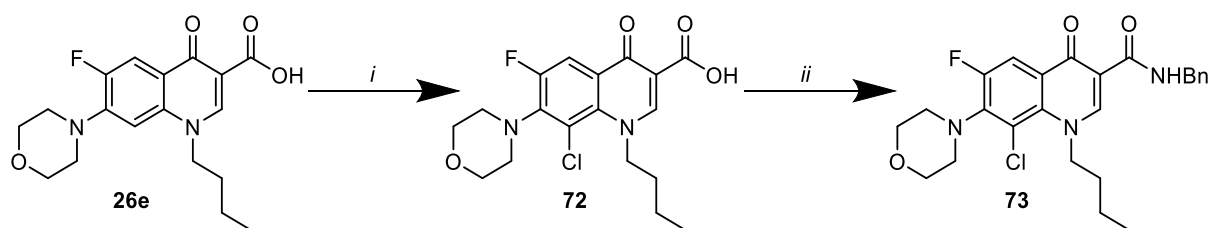
*Scheme 27. Chlorination of the quinolonic acid **26e** in position 8 and the respective coupled quinolone amide **73**. Reagents and reaction conditions: i) SO₂Cl₂, abs. CH₂Cl₂, 0 °C; i) i-Butyl chloroformate, N-methylmorpholine, abs. DMF, 0 °C to r.t.*

Further, it was planned to introduce larger substituents in position 8 by a nucleophilic aromatic substitution. As the rate-determining step for an S$_N$Ar reaction is the attack of the nucleophile, which is promoted by the electronegativity of the halogen leaving group, the available 6,8-difluoro quinolone amide **74** was chosen as the starting material for substitution test reactions, which is depicted in Scheme 28. The first attempt was a substitution with sodium methoxide in abs. DMF. In order to prevent a mixture of different singly substituted products, 2.5 equivalents NaOMe were used. Despite the high reactivity of the reagent, relatively drastic conditions were necessary to observe any substitution at position 8.
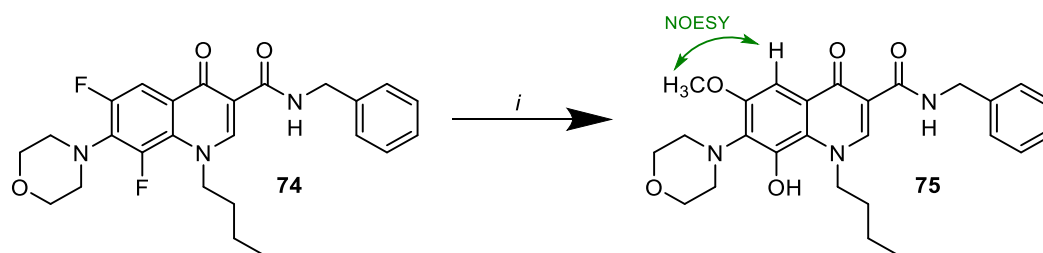
*Scheme 28. Substitution of the 6,8-difluoro quinolone amide **74** with sodium methoxide. Reagents and reaction conditions: i) NaOMe (25 wt% in MeOH), abs. DMF, 120 °C.*

However, the solvent contained small amounts of remaining water and the resulting hydroxide attacked at position 8 during the reaction. Consequently, the undesired compound **75** was isolated as the main product. The constitution of the product was determined by the NOESY NMR spectrum with an observable interaction between the methoxy group and the hydrogen in position 5, which is shown in Scheme 28. Although this compound was not planned originally, its bioactivity against trypanosomes was tested (see Chapter 8.3).

The following substitution test reaction aimed to introduce the next bigger moiety, an ethoxy group. For that reason, the starting material **74** and sodium ethoxide were stirred in freshly distilled DMF at 120 °C. The reaction was significantly slower than the methoxide substitution, which was visible by reaction control with LC/MS. No starting material was observed after six days and the desired product **76** was isolated.
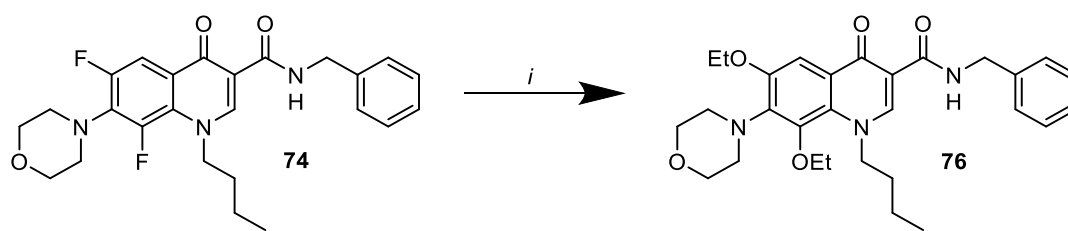
*Scheme 29. Substitution of the 6,8-difluoro quinolone amide **74** with sodium ethoxide. Reagents and reaction conditions: i) NaOEt (21 wt% in EtOH), abs. DMF, 120 °C.*

An analogous reaction with sodium isopropoxide was unsuccessful. Even after two weeks reaction time at 120 °C, no substitution at position 8 was detected. An increase of the reaction temperature led to a variety of unknown products, indicating decomposition of the starting material **74**. Apparently, a substitution at the desired position 8 is not possible for sterically demanding moieties under these conditions. Therefore, the strategy for introducing more bulky substituents shifted from a nucleophilic aromatic substitution to metal-catalyzed coupling reactions.

The Kumada-Corriu cross coupling was chosen to introduce aliphatic substituents in position 8. This coupling method uses a transition metal catalyst to link an aryl halide with a Grignard reagent.[128, 129] An array of catalysts was investigated, which are listed in Table 7. Palladium complexes are normally known only for cross coupling reactions of aryl bromides or iodides due to their lower bond dissociation energies. However, there are some examples for reactions with electronic rich aromatic systems.[130] Therefore, a test reaction with $Pd(PPh_3)_4$ and isopropylmagnesium bromide was performed, but only the starting material **72** was isolated.
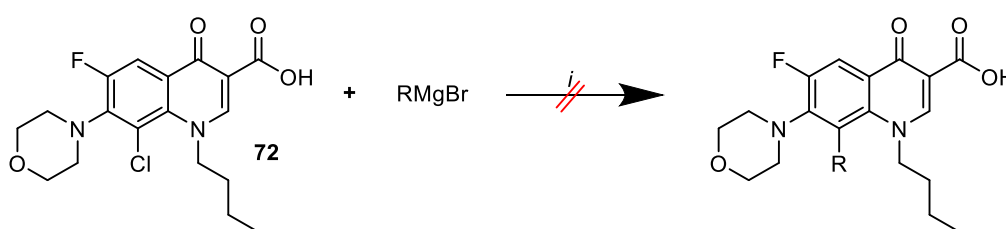
*Table 7. Attempted Kumada-Corriu cross coupling reactions of compound **72** with different catalysts. Reagents and reaction conditions: i) RMgBr, catalyst, THF, 0 °C to r.t.*

Grignard reagent RMgBr	Catalyst
$(CH_3)_2CHMgBr$	$Pd(PPh_3)_4$
$(CH_3)_2CHMgBr$	$Ni(acac)_2$
$(CH_3)_2CHMgBr$	$NiCl_2$
$(CH_3)_2CHMgBr$	$Fe(acac)_3$
$C_6H_{13}MgBr$	$Fe(acac)_3$
$C_6H_{11}MgBr$	$Fe(acac)_3$

Nickel catalysts are cheaper and undergo more willingly an oxidative addition into the aryl halide bond, which is often the rate-determining step of the coupling reaction.[131] Therefore, two Ni(II) catalysts were tested for the reaction with the isopropyl Grignard reagent, but no conversion of the starting material **72** was observed. Fürstner *et al.* demonstrated that iron catalysts are well suited for the insertion into the aryl chloride bond and perform cross coupling reactions even at low temperatures.[132] Therefore, the reaction with the isopropyl Grignard reagent was performed with their proposed catalyst Fe(acac)$_3$, but was not successful. As Fürstner *et al.* used long *n*-alkylmagnesium bromides in their coupling reactions, an attempt with the *n*-hexyl Grignard reagent was conducted.[132] Furthermore, a test reaction with cyclohexylmagnesium bromide was done.

To exclude an interference of the carboxylic acid function with the metal catalyst, also the already amide coupled compound **73**, which is shown in Scheme 30, was used in two test reactions. These carbon-carbon coupling attempts were done with the most promising Fe(acac)$_3$ catalyst, and isopropylmagnesium bromide and *n*-hexylmagnesium bromide as substrates. Despite the absence of the carboxylic acid function, no conversion was detected. Therefore, the next strategy for a successful coupling was the introduction of a bromine substituent in position 8.
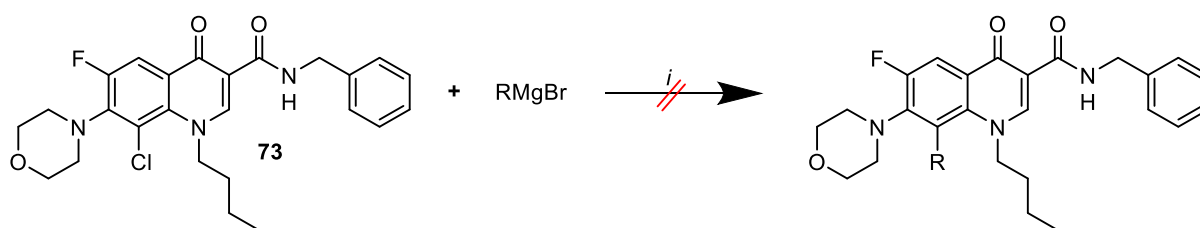
*Scheme 30. Attempted Kumada-Corriu cross coupling with quinolone amide **73**. Reagents and reaction conditions: i) (CH$_3$)$_2$CHMgBr or C$_6$H$_{13}$MgBr, catalyst, THF, 0 °C to r.t.*

Since already a chlorine substituent caused problems during the synthesis of the quinolone backbone (Scheme 26) and the electrophilic aromatic substitution occurred under mild conditions, the same procedure was chosen for the introduction of a bromine substituent. Starting with the quinolonic acid **26e**, a variety of bromination reagents were tested, which are listed in Table 8.
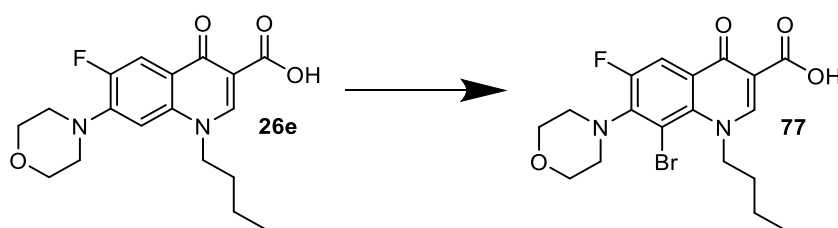
Table 8. Electrophilic aromatic bromination attempts of the quinolonic acid **26e**.

Reagents	Solvent	Reaction conditions
HBr	DMSO	r.t.
HBr, DMSO	AcOH	r.t.
HBr, DMSO	EtOAC	65 °C
Br$_2$	CH$_2$Cl$_2$	r.t.
Br$_2$, AlCl$_3$	CH$_2$Cl$_2$	r.t.
Br$_2$, FeCl$_3$	CH$_2$Cl$_2$	r.t.
NBS	DMF	r.t.
NBS	ACN	r.t.
NBS	CCl$_4$	60 °C, bomb tube
NBS, H$_2$SO4	Acetone	r.t.
NBS, TFA	Acetone	r.t.
NBS	TFA	r.t.

The first attempts for the bromination of compound **26e** were done with bromodimethylsulfonium bromide, which is prepared *in situ* by the combination of HBr and DMSO.[133] Following the general procedures established by Majetich *et al.*, DMSO was used once as the solvent and in another test reaction as a reagent with acetic acid as the solvent.[133] Both attempts resulted only in the starting material **26e**, which was monitored by TLC. The group of Jiao showed in many examples that the same reagent is suitable for a late-stage functionalization, using ethyl acetate as a solvent and elevated temperature.[134] However, these conditions did not result in the desired product.

The next attempt was a bromination with molecular bromine, as well as with the addition of the two Lewis acids, FeCl$_3$ and AlCl$_3$. The resulting increase in polarity of the bromine bond promotes an electrophilic halogenation, but the reaction control by TLC showed only the spot of the starting material **26e**.[135, 136]

The next tested bromination reagent was *N*-bromosuccinimide, which normally reacts under mild conditions. Two polar solvents were used, DMF and acetonitrile, which favour the electrophilic halogenation, but no reaction was observed at room temperature.[137-140] Pelleter *et al.* reported that aromatic compounds can be easily brominated by heating with NBS in CCl$_4$, even under continuous flow conditions.[141] To mimic their closed flow reactor and not

evaporate the by heating produced bromine, the test reaction was carried out in a closed bomb tube, but without success. Another strategy to increase the reactivity of *N*-bromsuccinimide is the activation with strong acids.[140] A common method is the addition of conc. sulfuric acid.[142, 143] The group of Esteves showed that TFA can be used for an activation of tribromoisocyanuric acid, a similar compound to NBS.[144] Hence, TFA was added to the compound **26e** and NBS in acetone. Both acid activations led to no conversion of the starting material. Inspired by the work of Esteves, the final successful attempt was the usage of the strong acid TFA not as a reagent but as a solvent.[144] This resulted in short reaction times with no starting material being detected after two hours.

Once compound **26e** was brominated in position 8, two Kumada-Corriu cross couplings with the resulting quinolonic acid **77** were tested, analogous to the conditions used in the reactions of the 8-chloro derivative **72** with the model substrate *n*-hexylmagnesium bromide and the two catalysts Ni(acac)$_2$ and Pd(PPh$_3$)$_4$. Since no carbon-carbon coupling was observed, the quinolonic acid **77** was coupled with benzyl amine under standard conditions to yield the quinolone amide **78**. Afterwards, several attempts for a carbon-carbon cross coupling in position 8 were carried out, which are listed in Table 9.
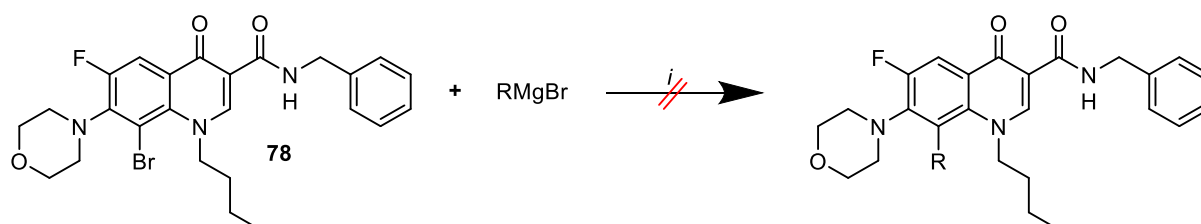
Table 9. Attempted coupling reactions of quinolone amide **78** with different coupling reagents and catalysts in THF.

Coupling reagent	Catalyst	Reaction conditions
C$_6$H$_{13}$MgBr	Pd(PPh$_3$)$_4$	0 °C to r.t.
C$_6$H$_{13}$MgBr	Ni(acac)$_2$	0 °C to r.t.
C$_6$H$_{13}$MgBr	Ni(dppe)Cl$_2$	0 °C to r.t.
Octen, 9-BBN-H	Pd(PPh$_3$)$_4$	0 °C to r.t. to reflux
Octen, 9-BBN-H	Pd(dppf)Cl$_2$	0 °C to r.t. to reflux

Since the Kumada-Corriu cross coupling was not successful, two Suzuki-Miyaura reactions were tested. Therefore, 9-BBN-H and oct-1-en were stirred at room temperature to form the required organoborane compound. Afterwards, the quinolone amide **78** and the respective catalyst were added and the solution was stirred at reflux. Surprisingly, a reductive elimination occurred in the reaction mixture with Pd(PPh$_3$)$_4$ and the quinolone amide **GHQ168** with a hydrogen in position 8 was formed. This observation suggests that an oxidative addition of the quinolone to the metal complex occurred. However, a reductive elimination was preferred over

the desired carbon-carbon coupling. Apparently, an introduction of a substituent in position 8 with two large neighbouring residues is not possible via coupling reactions under standard conditions. Therefore, further experiments were omitted, before testing the bioactivity of the already prepared 8-halogen derivatives **71** and **78**.

3.7 Synthesis of 2,3-saturated quinolone amide derivatives

The planarity and symmetry of a molecular structure have a significant impact on the crystal packing in the solid state. Therefore, a disruption of this planarity and a rise in the three-dimensionality of a compound are expected to decrease the efficiency of crystal packing, according to Ishikawa *et al.*[145] In this work, the planarity of the quinolonic structure was abrogated by a saturation of the carbon atoms C-2 and C-3. The respective retrosynthetic pathway for the synthesis of this 2,3-saturated quinolone amide **79** is depicted in the following Scheme 31.
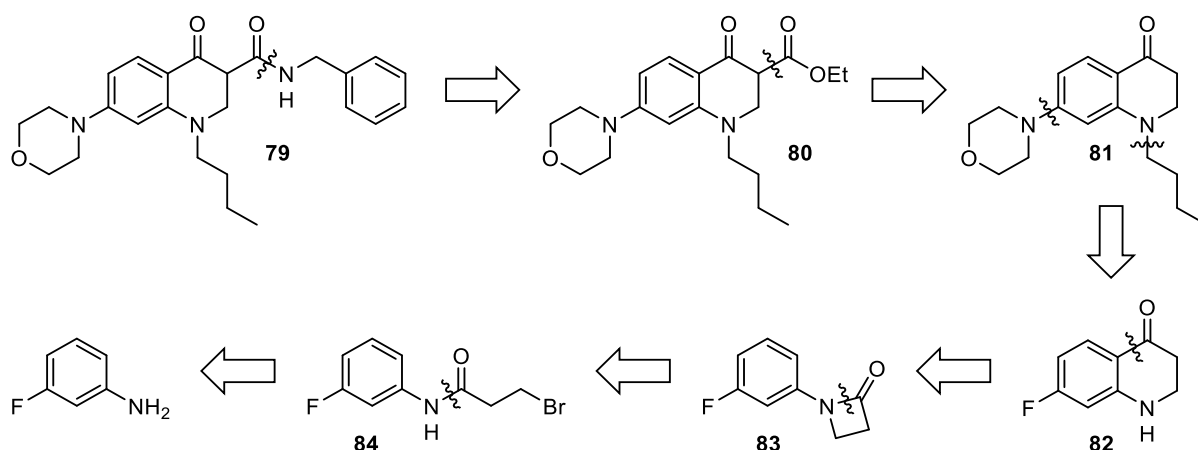
*Scheme 31. Planned retrosynthetic pathway for the synthesis of the 2,3-saturated quinolone amide **79**.*

Like in earlier synthesis pathways, the amide bond should be formed in the last step. However, the amidation must be done directly with the carboxylic ester **80**. A prior ester cleavage to the respective carboxylic acid is not possible, as this structure will most likely be oxidized under basic conditions, releasing CO_2 as a gas. The required ester **80** was planned to be synthesized by a Claisen condensation between the dihydroquinoline ketone **81** and ethyl chloroformate. In the prior step, the morpholino moiety is introduced in position 7 by a nucleophilic aromatic substitution and the butyl chain gets linked to the nitrogen in position 1. The dihydroquinoline ring system **82** should be synthesized by a Fries rearrangement of a 2-azetidinone structural unit under acidic conditions. This β-lactam **83** should be formed in an intramolecular substitution of amide **84**, which originates from 3-fluoroaniline.

In the first step, the amide coupling of 3-fluoroaniline was performed with 3-bromopropanoyl chloride, which resulted in a quick reaction. After the workup, the crude product was washed with a saturated $NaHCO_3$ solution to remove minor amounts of 3-bromopropanoic acid, which was produced during the quenching. Consequently, the amide **84** was deprotonated with sodium hydride, which led to an intramolecular substitution of the bromine and the desired β-lactam **83** was formed, which is shown in Scheme 32.
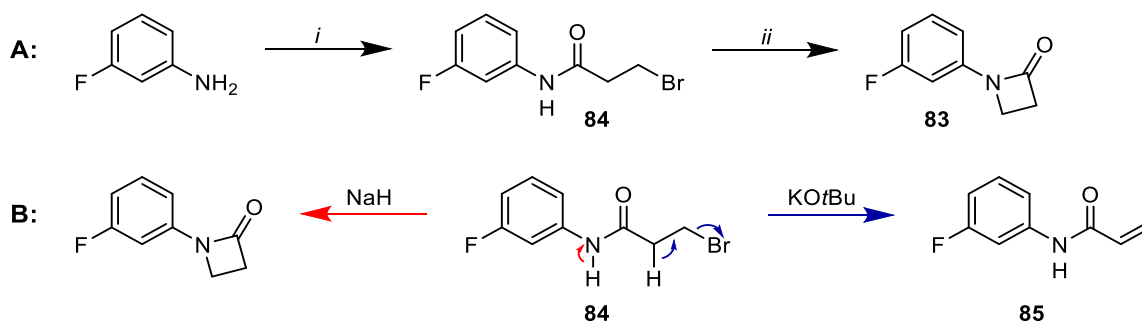
*Scheme 32. A: Synthesis of the 2-azetidinone **83**. Reagents and reaction conditions: i) K_2CO_3, 3-bromopropanoyl chloride, abs. CH_2Cl_2, 0 °C to r.t.; ii) NaH, abs. DMF, r.t.; B: Two possible deprotonations with different bases and their resulting products.*

Theoretically, there are two possible positions for a deprotonation in compound **84**, the amide function and the hydrogen in α-position relatively to the carbonyl group. Since the literature used potassium *tert*-butoxide, this base was chosen for the first test reactions.[146] However, not only the desired product **83** was formed, but also an elimination occurred (blue pathway in Scheme 32), which resulted in *N*-(3-fluorophenyl)acrylamide (**85**) as the major product. When sodium hydride was used as a base, only the amide function was deprotonated and the 2-azetidinone structural motif was formed (red pathway in Scheme 32).

The following Fries rearrangement of the 2-azetidinone **83** is an intramolecular Friedel-Crafts acylation. The mechanism is shown in Scheme 33. The driving force of this reaction is the decrease of ring tension of the β-lactam. Therefore, compound **83** was treated with triflic acid (TfOH), which led to a cleavage of the amide bond. Afterwards, the aniline ring attacks the acyl cation in an electrophilic aromatic substitution. However, this reaction can happen at both *ortho*-positions, leading to the isomers **82a** and **82b**. The ^{1}H NMR characterization of the isolated isomeric mixture revealed a product ratio of 1.5/1 (**82a/82b**).
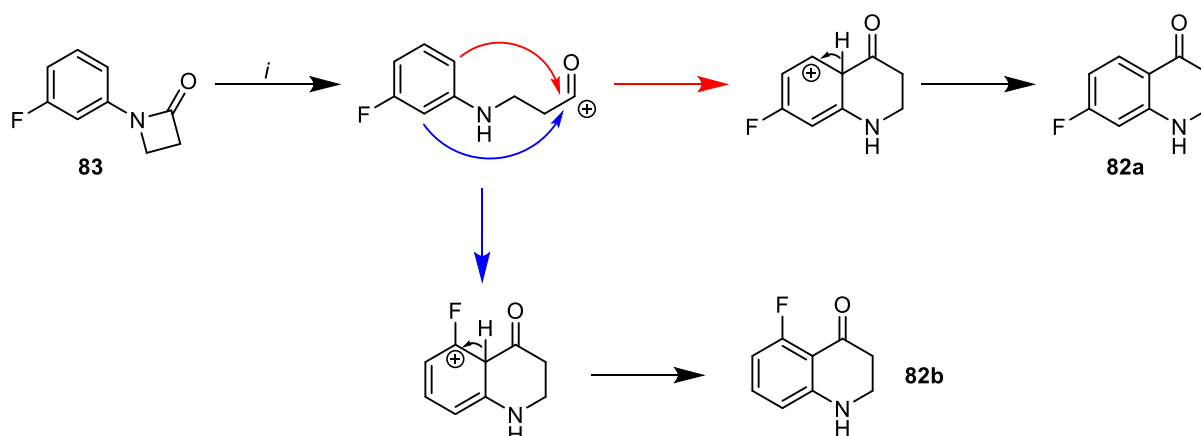
*Scheme 33. Fries rearrangement of compound **83** under acidic conditions. Reagents and reaction conditions: i) TfOH, abs. CH_2Cl_2, r.t.*

The two isomers **82a** and **82b** showed a different chromatographic behavior, which enabled a separation by column chromatography. Afterwards, the butyl group was introduced by

nucleophilic substitution. Test reactions, analogous to the unsaturated derivatives **24a-c** (cf. Chapter 3.3.1), with K_2CO_3 and catalytic amounts of KI resulted in an isolation of the starting material **82a**. Also, no conversion was observed with the organic base DIPEA. However, the use of the strong base NaH led to an immediate colour change at room temperature and no starting material was detected after two hours. The resulting product **86** was substituted in position 7 with morpholine. Therefore, it was dissolved in morpholine and heated to 110 °C under microwave irradiation, which led to compound **81** (Scheme 34).
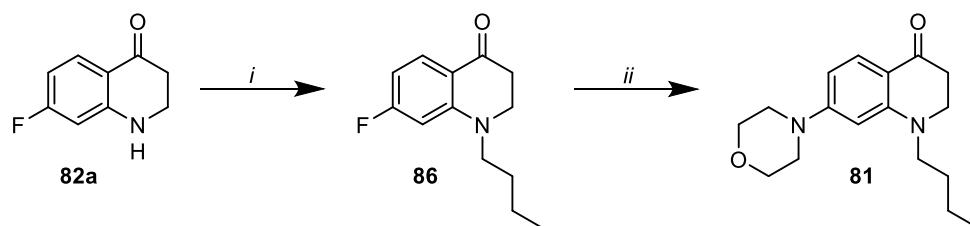
Scheme 34. Introduction of the n-*butyl chain in position 1 and the morpholino substituent in position 7. Reagents and reaction conditions: i) NaH,* n-*butylbromide, abs. DMF, r.t.; ii) morpholine, 110 °C.*

The original synthetic plan was to introduce the ester function in position 3 via a Claisen condensation. Therefore, the dihydroquinoline ketone **81** was deprotonated by a strong base, forming the required enolate, which should afterwards attack a reactive ester species. Several test reactions were done for this synthetic step, which are summarized in Table 10.
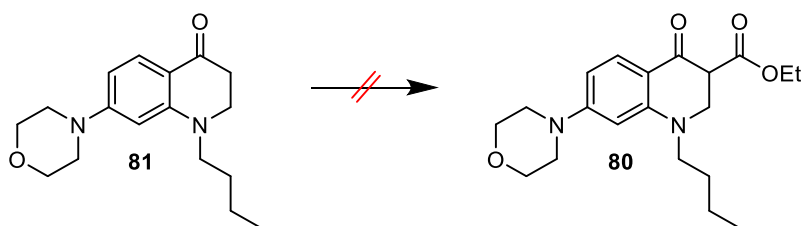
Table 10. Reaction conditions for the Claisen condensation of compound **81 with a reactive ester**.

Base	Reactive ester	Solvent	Temperature
LDA	Ethyl chloroformate	THF	-78 °C → r.t.
LDA	Diethyl carbonate	THF	-78 °C → r.t.
LHMDS	Ethyl chloroformate	THF	-78 °C → r.t.
LHMDS	Diethyl carbonate	THF	-78 °C → r.t.
NaOEt	Ethyl chloroformate	EtOH	r.t.
NaOEt	Diethyl carbonate	EtOH	r.t.
NaH	Ethyl chloroformate	THF	0 °C → r.t.
NaH	Ethyl chloroformate	DMF	0 °C → r.t.
NaH	Ethyl chloroformate	THF	60 °C
NaH	Ethyl chloroformate	DMF	120 °C
NaH	Diethyl carbonate	DMF	120 °C
NaOMe	Ethyl chloroformate	THF	r.t.
NaOMe	Diethyl carbonate	THF	r.t.

Despite numerous experiments with different combinations of base, solvent, reactive ester, and reaction temperature, it was not possible to synthesize the desired product **80**. In most cases, merely the starting material **81** was isolated. Only with the use of LDA or NaOMe as a base and at low temperatures, minor amounts of the product **80** were detected by a reaction control with LC/MS. However, no product signals were observed after the following work-up, mainly the starting material **81** was isolated. Additionally, small amounts of the 2,3-unsaturated derivative **87** were formed.
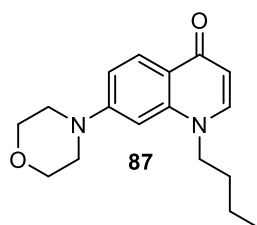
Figure 13. The molecular structure of the isolated 2,3-unsaturated byproduct **87**.

In a publication of Kobayashi *et al.* about the syntheses of similar 2,3-saturated quinolone compounds, the authors were also facing problems with the synthesis of derivatives with a proton in position 3. They claimed an instability against air without explaining this behaviour.[147]

However, the authors were able to synthesize the respective derivatives with an additional methyl substituent in position 3. Inspired by their work, a synthetic route for the saturated 3-methyl derivative **88** was developed, which is shown in Scheme 35.
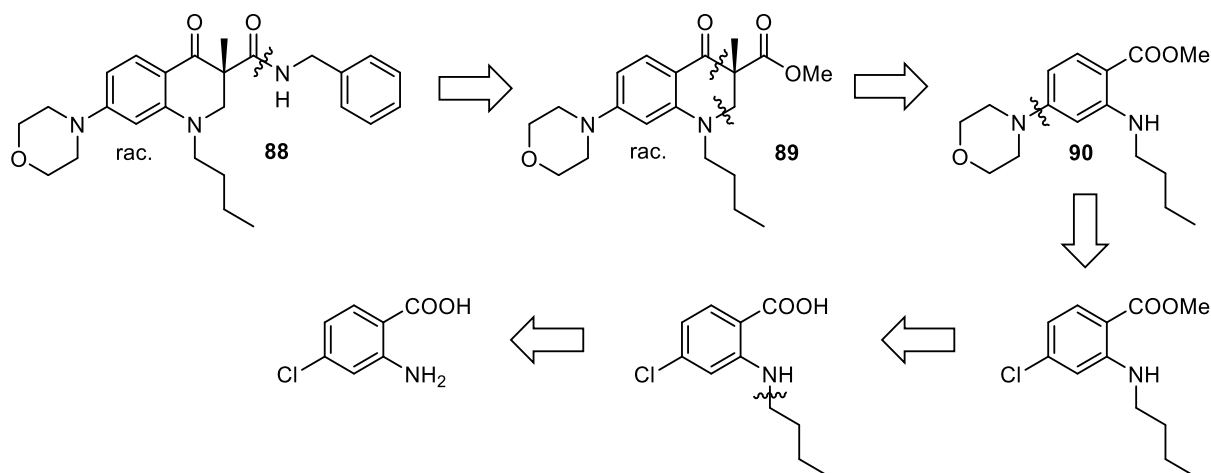
*Scheme 35. Retrosynthesis of the saturated 3-methyl quinolone derivative **88**.*

Like in the planned synthesis of compound **79**, the amidation in the last step must be done directly with the quinolonic ester **89** without a prior ester cleavage. The formation of the 2,3-saturated quinolonic unit should be accomplished in a cascade reaction of the secondary amine **90** with methyl methacrylate in the presence of a magnesium amide, based on the work of Kobayashi *et al.*[147] The earlier introduction of the morpholino ring and the butyl chain should be done via standard substitution reactions, starting from 2-amino 4-chlorobenzoic acid. The 4-fluoro derivative would have been an even better starting point, enabling an easier nucleophilic aromatic substitution of the morpholine; however, the compound was not commercially available at the time of this thesis.

In the first step, the butyl chain was linked to the amino function of 2-amino-4-chlorobenzoic acid by a S$_N$2 reaction with 1-bromobutane, resulting in the secondary amine **91**, which is shown in Scheme 36.[148] Afterwards, an esterification of the carboxylic acid group with thionyl chloride and methanol was performed.[149]
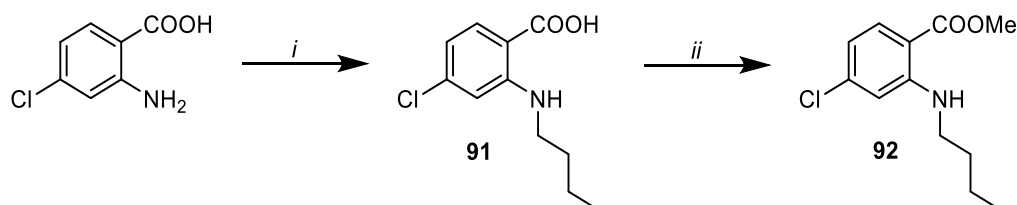
Scheme 36. Introduction of the butyl chain by an S$_N$2 reaction and the following esterification. Reagents and reaction conditions: i) n-butylbromide, K$_2$CO$_3$, DMF, 100 °C; ii) SOCl$_2$, MeOH, abs. toluene, 80 °C.

It was planned to introduce the morpholino moiety in the following step by a nucleophilic aromatic substitution. Unfortunately, not even heating the reaction to reflux in morpholine over

days led to any conversion of the starting material **92**. Substitution attempts before the esterification were also not successful.

A strategy to circumvent this substitution problem, was the activation of the benzoic ester **93** with trifluoroacetic anhydride, which is shown in Scheme 37. Due to the electronegativity of the CF$_3$-group, the electronic density is withdrawn from the aromatic ring, promoting a nucleophilic substitution. Consequently, the trifluoroacetamide can be easily cleaved under basic aqueous conditions.[150]
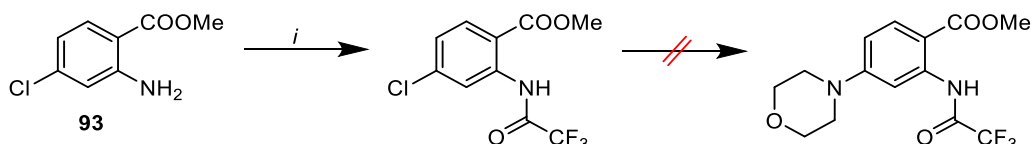
*Scheme 37. Amidation of the benzoic ester **93** with trifluoracetic anhydride and the attempted following morpholine substitution. Reagents and reaction conditions: i) trifluoroacetic anhydride, abs. CH$_2$Cl$_2$, 0 °C; ii) morpholine, 110 °C.*

Even with this activation, no substitution reaction occurred. Therefore, the synthetic strategy was shifted to an introduction of the morpholino moiety by a Buchwald-Hartwig amination.[151] This synthetic route, which is depicted in Scheme 38, started from the 4-chloro 2-nitrobenzoic acid, since an amino function in the starting material could have interfered in the coupling reaction. After an esterification under analogous conditions as already mentioned, the C-N coupling was done with Pd$_2$(dba)$_3$ and RuPhos, which are the standard reagents for the coupling of a secondary amine.[152]
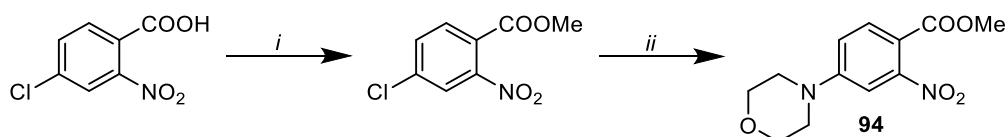
Scheme 38. Esterification of 4-chloro 2-nitrobenzoic acid and the following Buchwald-Hartwig coupling. Reagents and reaction conditions: i) SOCl$_2$, MeOH, reflux ii) Pd$_2$(dba)$_3$, RuPhos, Cs$_2$CO$_3$, morpholine, abs. 1,4-dioxane, 100 °C.

In contrast to the prior substitution attempts, the coupling reaction yielded the desired product **94**. In the following step, the nitro function was reduced with zinc dust in a mixture of acetic acid and water.[153] The reduction occured quite fast, which is shown in Scheme 39, since no starting material was observed by a reaction control via TLC after 15 minutes. Earlier test reactions with longer reaction times provided lower yields, indicating a decomposition of the aniline derivative **95**. Therefore, continuous reaction controls and a fast work-up were necessary for this step.
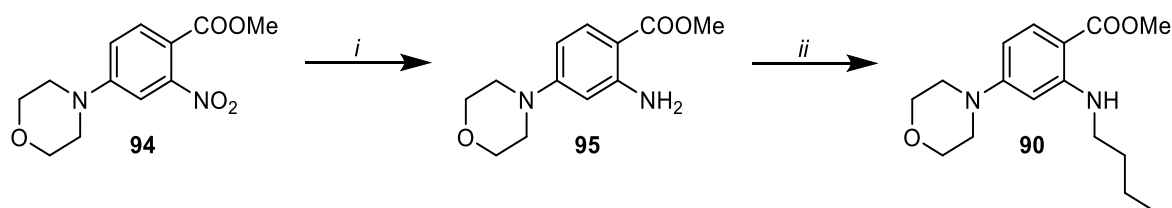
*Scheme 39. Reduction of the nitro function of compound **94** and the introduction of the butyl chain via a reductive alkylation. Reagents and reaction conditions: i) Zn, AcOH, H$_2$O, r.t.; ii) butyraldehyde, AcOH, NaBH(OAc)$_3$, abs. CH$_3$CN, r.t.*

The resulting amine **95** should be linked to an *n*-butyl chain in the next step. Conversions using conditions analogous to the alkylation of quinoline-4(1*H*)-one derivatives **24a-c** (cf. Chapter 3.3.1) with 1-bromobutane, potassium carbonate, and catalytic amounts of KI were unsuccessful, even with the use of five equivalents of the alkylbromide. For this reason, a reductive amination strategy was chosen for this alkylation step.

Byun *et al.* reported a one-pot reductive mono-alkylation of aniline derivatives under Pd/C catalysis with the respective aldehydes.[154] The required hydrogen was developed *in situ* by ammonium formate. As the published reductions proceeded smoothly with good yields at room temperature, the conditions were applied for the synthesis of the secondary amine **90**. However, no reaction occurred, only the starting material was isolated. In contrast, the reductive amination with the application of NaBH(OAc)$_3$ as a reducing agent under acidic conditions was successful, although quite slow and the addition of more equivalents of the boron hydride was necessary.[155, 156]

Kobayashi *et al.* developed a convenient synthetic method for the preparation of 4-oxo-tetrahydroquinoline-3-carboxylates via a conjugate addition/Claisen-type condensation sequence between 2-aminobenzoates and acrylates.[147] This cascade reactions happened under the treatment with magnesium bis(diisopropylamide) (further abbreviated as MBDA), which was generated *in situ* from the combination of ethylmagnesium bromide and diisopropylamine. This procedure was tested for the synthesis of the desired 2,3-saturated quinolone structure **89**, using the secondary amine **90** and methyl methacrylate, which is shown in Scheme 40.[147]
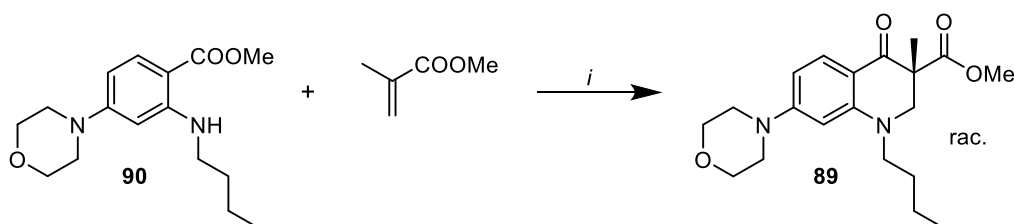
*Scheme 40. Synthesis of the 2,3-saturated quinolonic ester **89** via a conjugate addition/Claisen-type condensation sequence. Reagents and reaction conditions: i) EtMgBr, C$_6$H$_{15}$N, abs. 1,4-dioxane, 0 °C to r.t.*

Although compound **90** was very similar to the starting material in the publication of Kobayashi *et al.*, only about five percent product formation was observed by LC/MS after a reaction time of 16 hours. Longer reaction times did not result in a higher conversion rate. Next to freshly distilled dry diethyl ether, which was used in the literature as the solvent of choice, THF and 1,4-dioxane were tested, but did not lead to any improvement. The same holds true for the filtration of methyl methacrylate over Al_2O_3 before the addition to remove the polymerisation inhibitor MEHQ, which is added to commercially available acrylates in low concentrations (ppm scale).

Since all these attempts did not lead to acceptable yields, new methods for this reaction were tested. The use of other strong bases (NaH, KOtBu, LiHMDS, LDA) resulted in no conversion, always the starting material **90** was isolated after the work-up. Yamazaki *et al.* reported similar reactions under catalytic conditions with different Lewis acids.[157, 158] Therefore, the cascade reaction was tested by application of $Zn(OTf)_2$ and $TiCl_4$. However, also in these cases, no conversion was observed. Since only with the use of MBDA product formation was detected, it was decided to return to these conditions and optimize them. The freshly prepared MBDA was placed in abs. 1,4-dioxane and this solution was cooled as much as possible without freezing. The starting material **90** was added and, after a complete mixing, the suspension was completely frozen. Consequently, the methyl methacrylate was added and the ice-bath was removed, which led to a slow melting of the reaction mixture. With this approach, the yield could be raised up to 65 percent.

In the last step, the carboxylic ester group should be directly converted into the desired benzyl amine without a prior cleavage of the ester function, due to the possibility of an oxidation reaction under the release of CO_2. At first, a direct amination in benzylamine at high temperatures was tested. The oldest method for this kind of reactions requires very harsh conditions.[159, 160] When the starting material **89** was heated in benzylamine under 120 °C, no reaction occurred. Higher temperatures resulted in the undesired ester cleavage and following oxidation, leading to the 2,3-unsaturated 3-methyl quinolone **96** (Scheme 41, B).

Jeon *et al.* reported a direct conversion of esters to amides under mild conditions using DIBAL and forming reactive diisobutyl(amino)aluminium derivatives, but in the case of the 2,3-saturated quinolone **89**, no reaction was observed.[161] Therefore, the highly reactive $AlMe_3$ was used, which reacts with amines readily under the evolution of methane to yield dimethylaluminium amides.[162] The application of this reagent resulted in the formation of the desired product **88** in a yield of 32 percent (Scheme 41, A). Since incomplete reaction and recovery of starting material was observed, more than one equivalent of $AlMe_3$ was added in another reaction attempt. Surprisingly, this addition led not to a higher conversion of the starting material **89** to the final compound **88**, but to an unknown product **97**. The isolation of

this substance and the following characterization by means of NMR and LC/MS revealed its molecular structure, which is depicted in Scheme 41. The compound was isolated as a mixture of the *E*- and *Z*-imine isomer, in a ratio of 10 to 1. Only the major *E*-isomer is shown in Scheme 41.
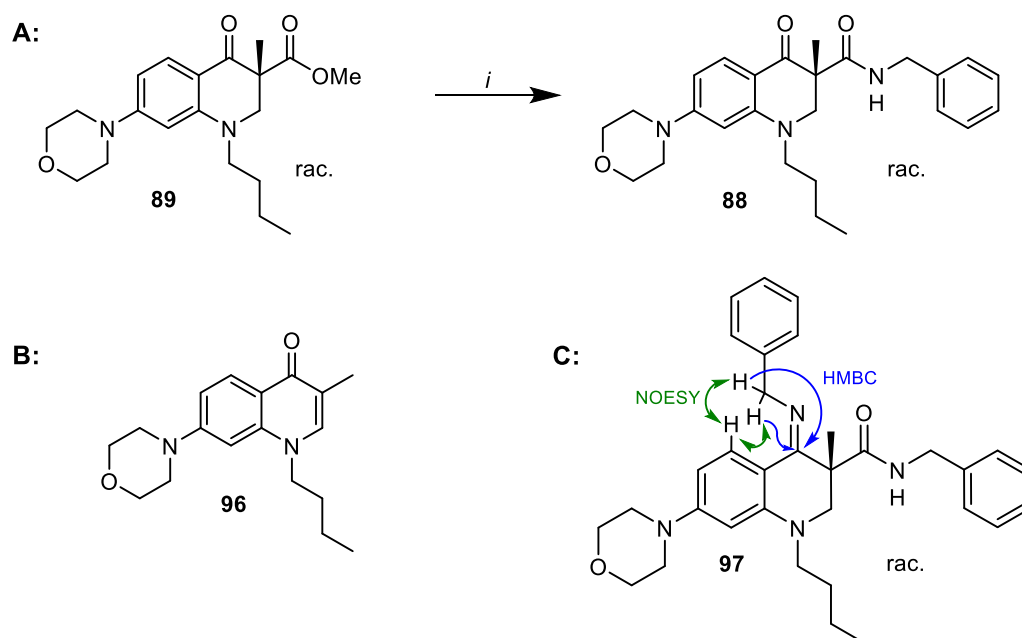
Scheme 41. A: Direct amination of the 2,3-saturated quinolonic ester 89 with benzylamine. Reagents and reaction conditions: i) benzylamine, AlMe₃, abs. toluene, abs. CH₂Cl₂, r.t. B: The molecular structure of the oxidation product 96, which was formed at high temperatures in benzylamine; C: The undesired product 97, formed in the presence of more equivalents AlMe₃.

The constitution of compound **97** was elucidated by 2D NMR techniques. A NOESY interaction between the CH_2 group neighbouring the ketimine and the hydogen in position 5 was observed, as well as a HMBC signal between the CH_2 group and the carbon C-4 (Scheme 41, C). To avoid the formation of product **97**, only one equivalent of $AlMe_3$ was used, even though this resulted in a low yield. The final 2,3-saturated quinolone amide **88** was synthesized successfully and purified by normal phase and subsequent reversed phase chromatography. The resulting racemic mixture was not separated at this point and used directly in the bioactivity studies. Only in the case of promising results, an enantiomer separation would be carried out.

As a second 2,3-saturated compound, the 2,3-dimethyl derivative was planned, due to its relatively easy synthetic accessibility. Kobayashi *et al.* reported in their publication on the synthesis of tetrahydroquinoline-3-carboxylates also the formation of this structural motif.[147] Starting from the already synthesized secondary amine **90**, the cascade ring closure reaction was performed with methyl (*E*)-2-methylbut-2-enoate, which is shown in Scheme 42.
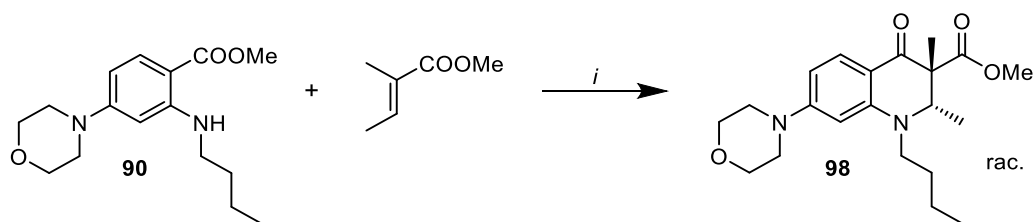
*Scheme 42. Synthesis of the 2,3-dimethyl saturated quinolonic ester **98**. Reagents and reaction conditions: i) EtMgBr, $C_6H_{15}N$, abs. 1,4-dioxane, 0 °C to r.t.*

The conjugate addition/Claisen-type condensation sequence with the dimethyl acrylate resulted in even lower yields than the reaction with the methyl acrylate, leading to the desired saturated quinolonic ester **98** in a yield of 17 %. Kobayashi *et al.* claimed that in this step only the trans diastereomer was formed. They reported an interaction of the 3-CH$_3$ group and the hydrogen in position 2 in NOE experiments.[147] This observation could not be reproduced in this work. Despite only one set of signals was visible in the NMR spectra, which indicated the presence of only one diastereomer, NOESY measurements showed an interaction between the 3-CH$_3$ group and the hydrogen in position 2, as well as a signal to the 2-CH$_3$ group. It could be possible that the NOE signal to both substituents in position 2 was caused by the conformational isomerism of the partially saturated ring system and that Kobayashi *et al.* could not see both signals due to the field strength of their NMR spectrometer.[147]
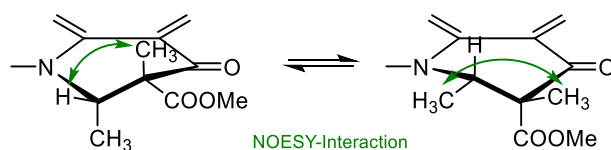
*Scheme 43. Both conformers of the 2,3-dimethyl saturated quinolonic ester **98** and the possible NOESY interactions. Only the affected ring is shown, the rest was omitted for clarity.*

Analogous to compound **88**, the 2,3-dimethyl saturated quinolonic ester **98** should be converted to the respective amide **99**, which is shown in Scheme 44. However, several test reactions with AlMe$_3$ and benzylamine led to the formation of an unknown product with a lower mass-to-charge ratio than the starting material **98**. Since at this point of this work, the monomethyl 2,3-saturated compound **88** already demonstrated a significantly lower bioactivity compared to the aromatic derivative, an isolation and a structural elucidation of the unknown substance, as well as further amide formation attempts, were neglected.
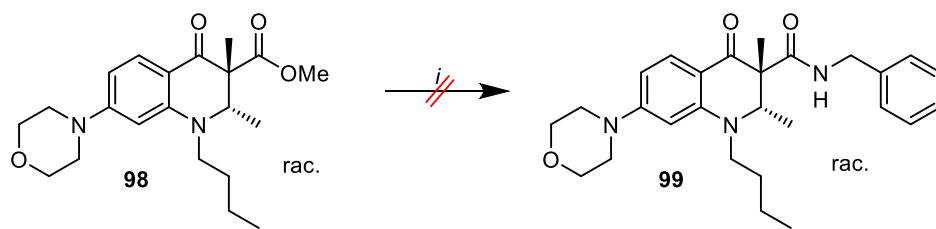
*Scheme 44. Unsuccessful attempted synthesis of the 2,3-dimethyl saturated quinolone amide **99**. Reagents and reaction conditions: i) benzylamine, AlMe₃, abs. toluene, abs. CH₂Cl₂, r.t.*

4 Physicochemical properties of the quinolone amides

Solubility is one of the most important parameters to achieve the necessary concentration of a drug in the systemic circulation that is required for a pharmacological response.[163] However, it is still a major challenge in drug discovery; since over 40 % of new chemical entities developed in the pharmaceutical industry are practically insoluble in water.[164] A low aqueous solubility is adverse to the bioavailability of a substance and can completely exclude an oral administration.[163] However, this type of administration is desired for antitrypanosomal drugs due to the difficulty of medical care in the areas affected by this disease.

The likelihood that a given small molecule will be orally available is described by Lipinski's rule of five.[165] It predicts that good absorption or permeation is more likely when there are less than 5 hydrogen bond donors, 10 hydrogen bond acceptors, the molecular weight is less than 500, and the calculated logP value does not exceed 5.[75, 166] The previous lead compounds, **GHQ168** and **17**, possess these favorable structural properties, which are predicting an oral bioavailability. However, most quinolone amides developed so far showed an extremely poor aqueous solubility. According to the classification by Ph. Eur. the substances are 'practically insoluble'.[69, 72, 167] In some cases, this property even impeded *in vitro* bioactivity measurements. Furthermore, an *in vivo* experiment was only possible with a spray-dried formulation of lead compound **GHQ168**.[76] Up to this date, the actual target of the quinolone amides could not be identified, partly due to the poor solubility. This adverse physicochemical property is probably based on the π-π stacking of the molecules in the crystal structure, resulting in a high crystal lattice energy, which must be raised for a dissolution.[69] The chemical modified quinolone amides of this work should intervene this intermolecular interaction and, therefore, increase the aqueous solubility. All following solubility values represent the thermodynamic solubility in a PBS buffer (pH = 7.4) at 37 °C, analogous to the conditions in the bioactivity assay.[68]

The partition coefficient logP is defined as the ratio of the concentrations of a compound in organic and aqueous phase at equilibrium. In the absence of ionization or dissociation, the partition coefficient can be viewed as an indicator of lipophilicity.[168] Therefore, it strongly affects the ADME (absorption, distribution, metabolism, and excretion) properties of a substance. A low lipophilicity prevents a permeation through the blood-brain barrier, which is essential for the treatment of stage II HAT.[169, 170] However, a high lipophilicity can result in a poor bioavailability and an accumulation in fatty tissue, which impedes the excretion.[171] Therefore, the partition coefficient is a decisive parameter in the development of new drug candidates.

In this chapter, only the modified quinolone amides without a fluorine substituent in position 5 or 6 are compared with compound **19** for reasons of clarity. The quinolone amide **19**, which is shown in Figure 14, possessed an aqueous solubility of 1.36 µg/mL and a logP value of 3.51.[71]
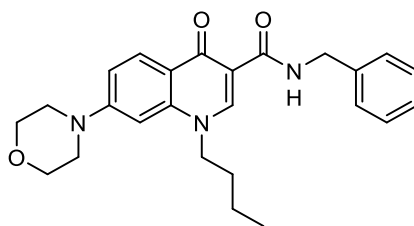
*Figure 14. Molecular structure of reference compound **19**.*

4.1 Modifications in position 1

An exchange of the *n*-butyl chain in position 1 with small cycloalkylmethyl substituents led to an increase in aqueous solubility, which is shown in Table 11. A clear trend was observable: The cyclopropylmethyl group resulted in the greatest improvement and the solubility decreased with an increasing ring size. The cyclobutyl group still had a small positive effect, whereas the solubility of the cyclohexylmethyl quinolone amide **27c** was extremely low. It could not be determined by HPLC measurement, as the corresponding peak was below the limit of quantification. As expected, the logP values of the quinolone amides **27a-c** correlated with the respective ring size. An enlargement of the aliphatic ring resulted in an increase of the logP value.

Table 11. Modifications in position 1 and the respective physicochemical properties.
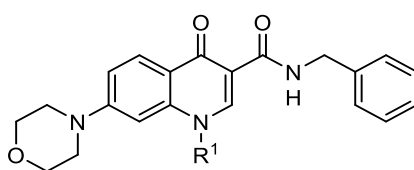
Compound	R^1	Solubility [µg/mL]	LogP
27a		5.81 (± 1.54)	3.24
27b		1.66 (± 0.08)	3.68
27c		< 0.1	4.59
28		4.06 (± 0.99)	4.57

The introduction of the 1-bicyclo[1.1.1]pentan-1-yl substituent in position 1 resulted also in a slightly improved aqueous solubility, whereas the logP value was comparable with compound **27c**. Although the improvements in solubility were small, these examples show that the solubilities of the substances do not directly correlate with the lipophilicity, but rather that the crystal packing has an influence as well.
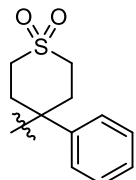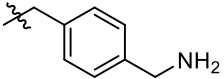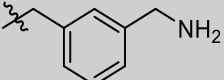
4.2 Modifications in position 3

The introduction of a cycloalkyl moiety in the benzylic position was done to hinder the free orientation of the aromatic system in position 3 in the crystal lattice. However, this approach did not lead to an improvement in aqueous solubility, as shown by compounds **50a,d,f** in Table 12. Contrary, it was significantly decreased for all three derivatives, lying below the limit of quantification by HPLC. The incorporation of a heteroatom into the cyclohexyl substituent in compounds **53a** and **53c** was able to counteract this effect, which resulted in a solubility comparable to the initial compound **19** for the oxygen-containing derivative **53a**. In the case of sulfone **53c**, the lipophilicity declined and the solubility was slightly increased (S_w = 3.35 µg/mL).

Table 12. Modifications in position 3 and the respective physicochemical properties.
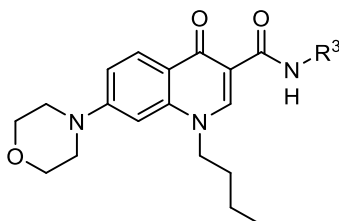
Compound	R³	Solubility [µg/mL]	LogP
50a		< 0.1	3.76
50d		< 0.1	4.57
50f		< 0.1	4.94
53a		1.03 (± 0.24)	3.60

53c		3.35 (± 0.01)	2.83
54		3.02 (± 0.17)	3.74
60a		> 1000	3.24
60b		> 1000	3.31
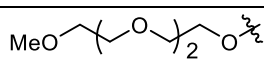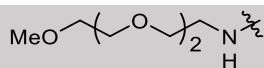
The complete exchange of the aromatic benzylic system with the bulky aliphatic 1-bicyclo[1.1.1]pentan-1-yl substituent in compound **54** enhanced the aqueous solubility, although the lipophilicity increased. As expected, the introduction of a primary amine raised the solubility significantly, up to more than 1 mg/mL for all derivatives **60a-f**, regardless of a fluorine substitution.
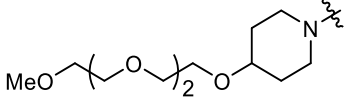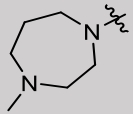
4.3   Modifications in position 7

The replacement of the morpholine ring in position 7 with triethylene glycol substituents resulted in a strong improvement in solubility, which is shown in Table 13. The effect was in the same order of magnitude for all three derivatives **66a-c**, whereas the linkage by a secondary amine in compound **66b** showed the lowest rise. A 20-fold increase in aqueous solubility was observed for the synthesized 1-methyl-1,4-diazepane containing quinolone amide **68**. The logP values rose for the structures **66c** and **68**.

Table 13. Quinolone amides with different substituents in position 7, their solubility, and their partition coefficients.
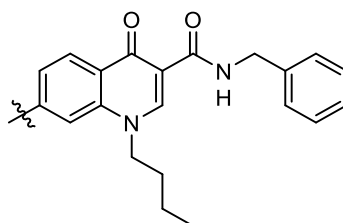
Compound	R⁷	Solubility [µg/mL]	LogP
66a		93.0 (± 9.73)	3.57
66b		41.0 (± 14.3)	3.38

66c		86.9 (± 6.90)	4.05
68		20.9 (± 1.98)	4.51

4.4 Modifications in position 8

The insertion of a halogen substituent in position 8 in compounds **73** and **78** led to a significant increase in lipophilicity and a drop in aqueous solubility. The same results were observed for the substitution with hydroxy- or alkoxy-groups, which is shown in Table 14. In all cases the solubility was below the limit of quantification by means of HPLC (< 0.1 µg/mL).

Table 14. Modifications in position 3 and the respective physicochemical properties.
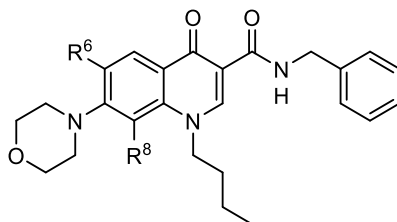
Compound	R^6	R^8	Solubility [µg/mL]	LogP
73	F	Cl	< 0.1	5.01
78	F	Br	< 0.1	5.05
75	OMe	OH	< 0.1	4.51
76	OEt	OEt	< 0.1	5.51

The modifications in position 8 were performed to achieve an impeded arrangement of the molecular structures in the crystal lattice and, therefore, a rise in solubility. However, this effect was not observed or outweighed by the resulting high lipophilicity.

The 2,3-saturated quinolone amide **88** possessed a slightly lower lipophilicity (logP = 3.04) compared to the unsaturated compound **19**, whereas the solubility (S_w = 10.4 µg/mL) was enhanced.

5 Structure-activity relationship of the quinolone amides

5.1 The AlamarBlue® assay

The antitrypanosomal activity and the cytotoxicity of the synthesized quinolone amides were determined using an AlamarBlue® assay, which utilizes resazurin as the active ingredient. The compound is water-soluble, stable in culture medium, nontoxic, and cell-permeable.[172] When dissolved, it possesses a deep blue/purple colour. In a reductive environment, resazurin is reduced to resorufin, which results in a colour change to pink. Therefore, it can be used as an indicator of cell viability, as the aerobic respiration of metabolically active cells is able to reduce resazurin.[172] This change of oxidation state can be quantified by colorimetric or fluorometric measurements. Already in 1929, Pesch and Simmert used this technique to quantify bacteria in milk.[173, 174]
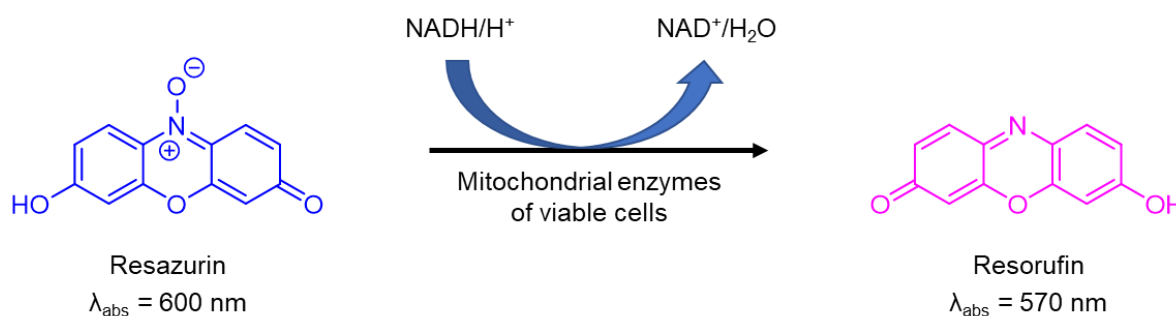
Scheme 45. Reduction of resazurin to resorufin in the presence of metabolically active cells.

The AlamarBlue® assay shows comparable results to formazan- (MTT) or [³H]-thymidine-based cell proliferation assays.[172] Räz *et al.* showed the potential of AlamarBlue® for the determination of drug sensitivities for African trypanosomes *in vitro*.[175] Based on their findings, our research group, in collaboration with other participants of the former SFB630, deployed the assay to measure bioactivities of a variety of quinolone amides in the past.[71] Therefore, the determination of trypanocidal potential of the synthesized compounds was planned to be performed using the same procedure. However, a few adjustments were necessary.

In earlier studies, the absorbance measurements were carried out after 48 h and 72 h. In many cases, the colorimetric determination after 72 h resulted in higher IC_{50} values than in the 48 h measurement, representing a lower bioactivity.[68, 71] The reason of this drop in activity is the emergence of the positive control (only trypanosomes in cell medium) from the exponential growth phase while the wells with a lower cell concentration, resulting from the presence of test substances, were still in this growth phase. As a result, falsely lower bioactivities were measured. Therefore, the absorbance measurement was only performed after 48 h. Another minor difference was the use of HMI-9 medium that contained phenol red, in contrast to

previous assays without phenol red. However, this addition does not affect the outcome of the assay, it only shifts all absorption values 0.03 units higher.[172]

Apart from these small adjustments, the protocol was exactly followed. However, the trypanosomes did not multiply like intended after cell seeding, but rather were dying. The addition of AlamarBlue® did not lead to a colour change visible to the eye and the absorbance measurement showed no conversion of resazurin. To identify the unknown cause, various adaptions were tested: higher seeding concentrations of cells, use of a laboratory heating mat during the single steps, lower DMSO concentrations, well plates of different manufacturers. However, none of these adjustments resulted in the survival of the trypanosomes. The solution to the problem was a preincubation of the well plates after the addition of the compounds to the presented medium in an atmosphere of 37 °C, 5 % CO_2, and 95 % humidity for 6 h. A cell seeding after this preincubation led to the desired cell growth and a determination of antitrypanosomal activity of the test substances was possible. The measurements were monitored using pentamidine (IC_{50} = 5.3 nM) as a reference substance.

The cytotoxicity of the synthesized quinolone amides was examined against the murine macrophage cell line J774A.1. A similar protocol as in prior publications was used.[68, 71, 72] However, the cells were cultured in a DMEM medium instead of an RPMI-1640 medium.[72] The poor solubility of the compounds was a limiting factor, as relatively high DMSO concentrations were necessary to prevent a precipitation during the assay procedure. Therefore, the effect of DMSO against the macrophage cells was tested alone. No cytotoxic effect was observed up to a DMSO concentration of 1.25 % in cell medium. Higher concentrations led to a sharp decrease in cell viability. With this amount of DMSO, a maximum concentration of test substances of 50 µM was achieved. Higher concentrations of quinolone amides led to a precipitation during the cell assay.

The structure-activity relationships of the synthesized compounds are discussed below. The three starting substances of this work, which are shown in the following Figure 15, serve as references.

IC_{50} = 0.23 µM
CC_{50} > 100 µM

IC_{50} = 0.047 µM
CC_{50} = 57 µM

IC_{50} = 0.05 µM
CC_{50} > 100 µM
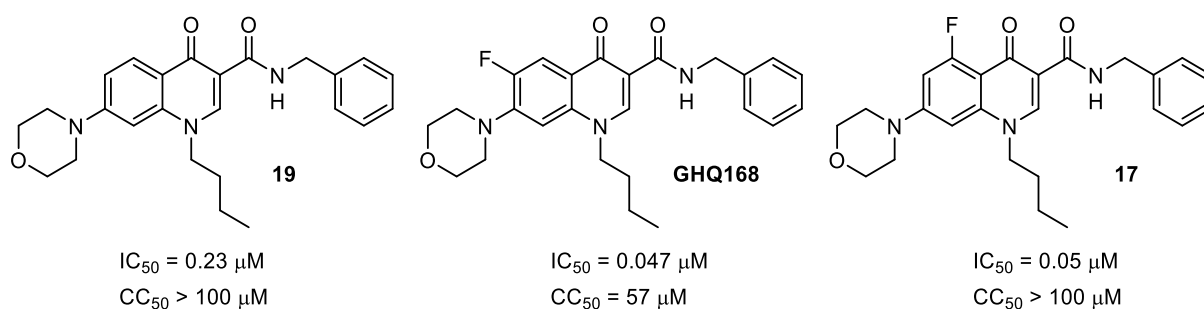
Figure 15. Prior quinolone amides of our research group and their biological activities.[69, 72]

5.2 Modifications in position 1

The exchange of the butyl chain in position 1 with cycloalkylmethyl substituents resulted in a small, but significant decrease in antitrypanosomal activity in comparison with compound **19** (IC_{50} = 0.23 µM). A clear trend was visible: The activity tend to decline with an increasing ring size (Table 15). No cytotoxicity was observed for the propyl and the hexyl residue. Only in the case of the butyl substituent, a low cytotoxic effect (CC_{50} = 29.89 µM) was measured.

Table 15. Biological data of synthesized quinolone amides with different N-1 substituents.
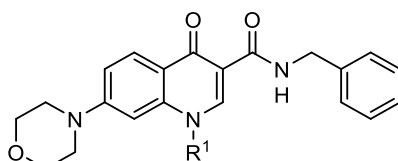
Compound	Substituent R^1	*T. b. brucei* IC_{50} [µM]	J774A.1 CC_{50} [µM]
27a		0.55 (± 0.14)	> 50
27b		0.80 (± 0.04)	29.89
27c		0.86 (± 0.04)	> 50
28		0.13 (± 0.02)	48.44

The introduction of the 1-bicyclo[1.1.1]pentan-1-yl substituent led to higher trypanocidal activity of compound **28** (IC_{50} = 0.13 µM). However, a slight bioactivity against macrophage cells was detected (CC_{50} = 48.44 µM).

5.3 Modifications in position 3

Prior quinolone amides had a benzyl substituent in position 3 (Figure 15).[69, 72] Here, aliphatic ring systems were introduced in the benzylic position. Simultaneously, the impact of the fluorine substitution pattern was investigated, analogous to prior projects.[71] The introduction of a cyclopropyl unit resulted in a decline of the antitrypanosomal activity for all three derivatives (**50a-c**). Earlier observations that a fluorine substituent increases the activity were confirmed, whereas no clear differences were measured between a fluorine in position 5 or 6 (Table 16). All three cyclopropyl derivatives possessed no cytotoxic effect below 50 µM.

Table 16. Biological data of synthesized quinolone amides with modifications in position 3.
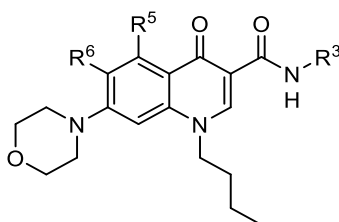
Compound	R^3	R^5	R^6	*T. b. brucei* IC$_{50}$ [µM]	J774A.1 CC$_{50}$ [µM]
50a		H	H	0.72 (± 0.13)	> 50
50b		H	F	0.15 (± 0.03)	> 50
50c		F	H	0.18 (± 0.05)	> 50
50d		H	H	4.40 (± 0.07)	34.11
50e		H	F	1.83 (± 0.12)	47.39
50f		H	H	4.18 (± 0.59)	36.99
50g		H	F	4.72 (± 0.50)	30.97
50h		F	H	4.22 (± 0.34)	> 50
53a		H	H	> 20	> 50
53c		H	H	> 20	> 50
54		H	H	3.72 (± 0.23)	> 50

The introduction of a cyclopentyl ring led to a sharp drop in trypanocidal activity for compound **50d** (IC$_{50}$ = 4.40 µM), while a fluorine substituent in position 6 in compound **50e** still had a positive effect on the activity (IC$_{50}$ = 1.83 µM). Furthermore, the bigger ring size resulted in a measurable cytotoxicity for both derivatives (Table 16). The further increase of the ring size resulted in comparable low potencies for the cyclohexyl derivatives **50f-h**. A fluorine substitution had no positive effect on the antitrypanosomal activity. Additionally, a slight cytotoxicity was measured for derivatives **50f** and **50g** (Table 16). Compound **50h** with a fluorine in position 5 showed no cytotoxic effect, which is consistent with the previous observations of Berninger.[71, 72]

Additional hetero-atoms in the six-membered ring substituents in compounds **53a** and **53c** resulted in a complete decline of bioactivity (IC$_{50}$ > 20 µM). Apparently, the introduction of bigger ring systems in the benzylic position of quinolone amides has an adverse effect on their activity against trypanosomes. In the case of cyclohexyl residues not even a fluorine substitution in position 5 or 6 can compensate this effect.

The exchange of the benzyl substituent in position 3 with the bulky aliphatic 1-bicyclo[1.1.1]pentan-1-yl residue in compound **54** led to a drop in potency (IC$_{50}$ = 3.72 µM). This observation demonstrated the necessity of a benzylic system in this position.

The following quinolone amides **60a-f** with an additional primary amine function showed severe stability problems. A decomposition occurred for all derivatives within weeks. Small amounts of the desired amines could be extracted from the decomposition mixtures and were purified by reversed phase chromatography. Using this procedure, the quinolone amides **60a-e** were reisolated in a purity of > 90 percent and tested against T. b. brucei. The amine **60f** could not be recovered due to a complete decomposition.

The introduction of a primary amine function in the benzylic residue resulted in a slight decrease of the antitrypanosomal activity (Table 17). A *meta*- or *para*-substitution did not lead to any significant differences. All compounds showed a low cytotoxicity (Table 17). Therefore, the fluorine-containing substances **60b**,**c**,**d** still possess a good selectivity. Considering their excellent aqueous solubility, these observations make them attractive substances for further investigations.

*Table 17. Biological data of synthesized quinolone amides with primary benzylamines in position 3 and the intermediates 55a,c,f. *not determined due to decomposition.*
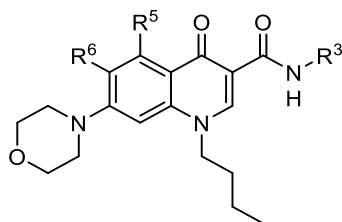
Compound	R³	R⁵	R⁶	*T. b. brucei* IC$_{50}$ [µM]	J774A.1 CC$_{50}$ [µM]
60a		H	H	1.55 (± 0.05)	> 50
60b		H	F	0.30 (± 0.00)	49.21
60c		F	H	0.25 (± 0.07)	> 50
60d		H	H	1.08 (± 0.03)	> 50
60e		H	F	0.52 (± 0.02)	41.78
60f		F	H	ND*	ND*
55a		H	H	0.35 (± 0.04)	> 50
55c		F	H	0.10 (± 0.01)	> 50
55f		F	H	0.022 (±0.005)	> 50

The measurement of the synthetic precursors **55a** and **55c** with a cyano group instead of the primary amine in *para*-position revealed a slight decline in trypanocidal activity while maintaining no observable cytotoxicity (Table 17). Compound **55f** with a nitrile function in *meta*-position showed a higher bioactivity (IC$_{50}$ = 22 nM) than the previous lead compounds **GHQ168** (IC$_{50}$ = 47 nM) and **17** (IC$_{50}$ = 50 nM). Furthermore, no cytotoxic effect was measured for compound **55f**, resulting in an excellent selectivity. However, quinolone amide **55f** had a comparable solubility (0.24 µg/mL) to substance **17.**

5.4 Modifications in position 7

In former publications, a morpholino substituent in position 7 of quinolone amides excelled through a good potency against trypanosomes and low cytotoxicity in addition to a positive effect on the aqueous solubility.[68, 72] The exchange of this morpholino residue by triethylene glycol chains led to a loss in bioactivity, with all three derivatives **66a-c** possessing an IC_{50} value above 1 µM (Table 18). The different linkages of the triethylene glycol side chain had no impact on the antitrypanosomal activity. Apparently, a long flexible chain in position 7 has a strong adverse effect on the bioactivity. This decrease in trypanocidal activity is partially compensated by the significantly better solubility of substances **66a-c**. The cytotoxicity remained low for all three derivatives (Table 18).

Table 18. Biological data of synthesized quinolone amides with different substituents in position 7.
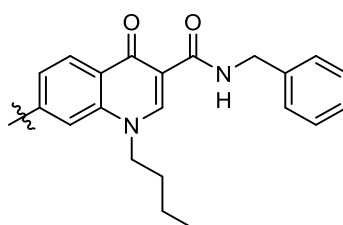
Compound	R³	*T. b. brucei* IC_{50} [µM]	J774A.1 CC_{50} [µM]
66a	MeO~(~O~)₂~O	1.12 (± 0.44)	> 50
66b	MeO~(~O~)₂~NH	1.15 (± 0.01)	> 50
66c	MeO~(~O~)₂~O-piperidine-N	1.01 (± 0.19)	48.51
68	N-methyl-1,4-diazepane	2.97 (± 0.18)	14.20

The introduction of a 1-methyl-1,4-diazepane substituent in position 7 led to a significant drop in antitrypanosomal activity (IC_{50} = 2.97 µM). Furthermore, a cytotoxicity (CC_{50} = 14.20 µM) was measured in the macrophage assay. Hiltensperger has shown in his PhD thesis that substitution with different piperazine derivatives caused increased cytotoxicity.[69] Apparently, a basic amine function (secondary or tertiary) in this position produces a toxic effect.

5.5 Modifications in position 8

The introduction of non-polar sterically demanding substituents in position 8 led to a slight decline in antitrypanosomal activity for the halogenated compounds **73** and **78** (Table 19) compared to substance **GHQ168** (IC_{50} = 0.047 µM). Both compounds showed no cytotoxicity in the macrophage cell assay.

Table 19. Biological data of synthesized quinolone amides with different substituents in position 8.
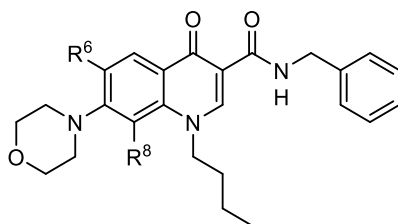
Compound	R^6	R^8	*T. b. brucei* IC_{50} [µM]	J774A.1 CC_{50} [µM]
73	F	Cl	0.18 (± 0.05)	> 50
78	F	Br	0.47 (± 0.08)	> 50
75	OMe	OH	2.25 (± 0.45)	37.06
76	OEt	OEt	1.93 (± 0.15)	33.16

A sharp loss of bioactivity was measured for the alkoxide-substituted quinolone amides **75** and **76** with IC_{50} values in the micromolar range. Furthermore, slight cytotoxicity was observed for both compounds (Table 19).

The saturation in position 2 and 3 and the introduction of a methyl-substituent in position 3 resulted in a drop in trypanocial activity (IC_{50} = 3.74 µM). No cytotoxicity was measured for compound **88** (Figure 16). The loss in activity could be caused by the loss of planarity, the necessity of π-electrons in this position, or an additional steric hinderance by the added methyl group. However, the introduction of the methyl group was necessary to ensure stability of the saturated compound (cf. Chapter 3.7).

IC_{50} = 3.74 µM
CC_{50} > 50 µM
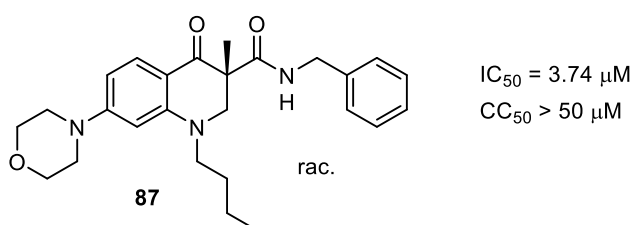
*Figure 16. The 2,3-saturated compound **88** and its measured biological data.*

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6 <u>Summary</u>

The human African trypanosomiasis is a devastating parasitic infection and occurs in developing countries in sub-Saharan Africa.[1] This neglected tropical disease is caused by the protozoan *T. brucei* and transmitted by the bite of the tsetse fly.[12] After an infection, the patients show non-specific symptoms in the first weeks, which often leads to a false diagnosis. However, an immediate therapy is indispensable, as an untreated infection usually results in death.[12]

Although the number of cases of HAT is declining in the last years, a re-emergence of the disease is possible at any time due to the unstable social circumstances in the affected areas.[3, 11] Asymptomatic courses of the disease, cattle as a suitable reservoir, and recent reports about substantial quantities of trypanosomes in the skin of undiagnosed humans question the WHO's long-term goal of eradicating the HAT by 2030.[14, 19, 21]

There are only a few drugs available for treatment and these further divide in the two infection stages. Furthermore, the available drugs are only effective for the chronic or acute form of the disease. All authorized compounds have significant drawbacks and, except for fexinidazole, have been in use for decades. The alarming rise of resistances, a near impossible vaccination, and the lack of new drug candidates in the pipeline require the search for novel chemical entities with antitrypanosomal activity.[12, 41, 43]

Over the last years, our research group has synthesized a variety of quinolone amides with good antitrypanosomal activity.[67, 69, 72] Through structural optimization, Berninger discovered lead compound **17** with an excellent selectivity (IC_{50} = 0.05 µM, CC_{50} > 100 µM).[71] Although cell lysis experiments were performed, the actual target site of the quinolone amides is still unknown up to this date.[68] Further target elucidations and *in vivo* studies were impeded by the poor aqueous solubility of the compounds.[76]

In this thesis, new quinolone amides were synthesized to deepen our understanding of their structure-activity relationship. The quinolinic core structure was constructed by the Gould-Jacob and Grohe-Heitzer protocol.[78, 94] Voluminous substituents were introduced in position 1, 3, 7, and 8 (cf. Figure 17) in order to prevent a π- π stacking in the crystal structure, which presumably causes the poor solubility.[69] Furthermore, primary amines were incorporated in the benzylic residue in position 3 to enhance the aqueous solubility. In another attempt, a non-planar, 2,3-saturated quinolone amide **88** was synthesized. The physicochemical properties, trypanocidal activity, and cytotoxicity of all compounds were determined.
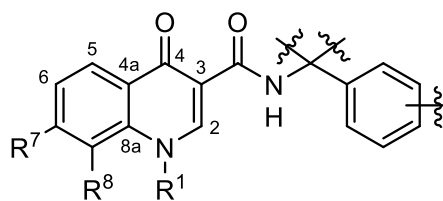
Figure 17. General quinolone amide structure and sites of chemical modifications.

The exchange of the butyl chain in position 1 with cycloalkylmethyl substituents resulted in a lower bioactivity compared to the non-fluorinated lead compound **19** (IC_{50} = 0.23 µM), whereby a clear trend was observed: the bigger the ring size, the lower the antitrypanosomal activity. Compound **28** with a 1-bicyclo[1.1.1]pentan-1-yl substituent in position 1 showed a higher bioactivity (IC_{50} = 0.13 µM), while possessing a higher aqueous solubility. Only low cyto-toxicities were observed for all modifications in position 1.

All compounds with a cycloalkyl ring in benzylic position had a sharp loss in solubility (< 0.1 µg/mL). The trypanocidal activity of the cyclopropyl derivatives **50a-c** was slightly lower compared to the respective parent compound, whereas a significant drop was observed for the higher ring sizes. In the case of cyclohexyl substituents, even the introduction of a fluorine in position 5 or 6 could not enhance the bioactivity. No cytotoxicity was evaluated for the cyclopropyl structures **50a-c** and the pentyl- and hexyl derivatives, **50d-e** and **50f-h**, showed a low toxic effect. Although the insertion of heteroatoms into the cyclohexyl ring in compounds **53a,c** restored the aqueous solubility, the antitrypanosomal activity was diminished (IC_{50} > 20 µM). The complete exchange of the benzyl unit in position 3 with a 1-bicyclo[1.1.1]pentan-1-yl residue led to a higher solubility (3.02 µg/mL) but the bioactivity was lowered (IC_{50} = 3.72 µM).

The introduction of primary amines in the benzylic residue resulted in a significant rise in solubility, which exceeded 1 mg/mL for all compounds **60a-e**. However, these substances were not stable. The bioactivities of compounds **60a-e** were slightly lower compared to the respective reference compound, while showing no or only a small cytotoxicity. Considering the excellent solubility, these observations make the amines **60a-e** attractive structures for further investigations. Furthermore, the nitrile derivatives **55a,c,f**, intermediates in the synthesis of the respective primary amines, were tested against *T. b. brucei*. The *para*-derivatives **55a** and **55c** showed a slightly lower bioactivity, whereas compound **55f** with a nitrile function in *meta*-position had a high antitrypanosomal activity (IC_{50} = 22 nM), surpassing the lead compound **17**. No cytotoxicity was measured for all nitrile derivatives, leading to an excellent selectivity. The solubility of compound **55f** (0.24 µg/mL) was comparable to prior lead compound **17**.

The introduction of triethylene glycol chains in position 7 in quinolone amides **66a-c** led to a sharp increase in aqueous solubility, up to 93 µg/mL for compound **66a**. All three derivatives

had an IC_{50} value of about 1 µM and showed nearly no cytotoxicity. A low trypanocidal activity was measured for the quinolone amide **68** with a 1-methyl-1,4-diazepane substituent in position 7. Furthermore, compound **68** had the highest cytotoxic effect (CC_{50} = 14.20 µM) of tested molecules in this thesis.

All modification in position 8 resulted in a decrease in aqueous solubility (< 0.1 µg/mL). The introduction of a chlorine or bromine substituent led to a moderate loss of bioactivity, while no cytotoxicity was observed. The methoxy and ethoxy derivatives **75** and **76** had a low antitrypanosomal activity and a slight cytotoxic effect was detected.

The 2,3-saturated quinolone amide **88** possessed an enhanced solubility (10.4 µg/mL) without the introduction of an additional polar functional group. However, the bioactivity of compound **88** dropped into the micromolar range, whereas no cytotoxicity was measured.

In summary, a variety of quinolone amides with antitrypanosomal activity were synthesized, providing new knowledge about the structure-activity relationship. Furthermore, compounds with increased solubility and the promising substance **55f** (cf. Figure 18) with excellent selectivity were discovered.

Compound	IC_{50} [µM]	CC_{50} [µM]	Solubility [µg/mL]
17	0.05	> 100	0.12
55f	0.022	> 50	0.24

17: R = H
55f: R = CN
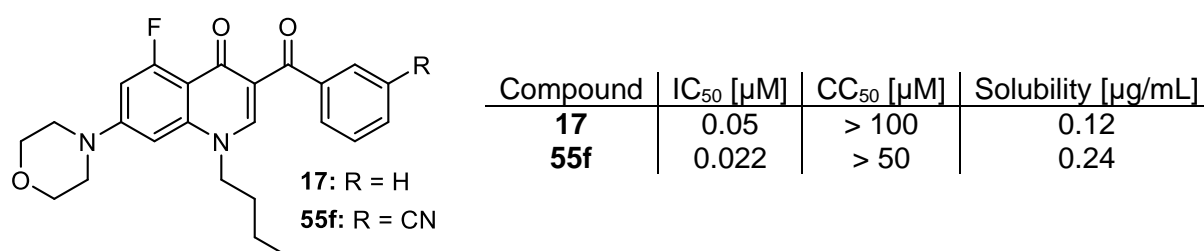
*Figure 18. Quinolone amides **17** and **55f** with their biological activities and solubility.*

7 <u>Zusammenfassung</u>

Die Humane Afrikanische Trypanosomiasis, auch als Schlafkrankheit bezeichnet, ist eine verheerende parasitäre Infektion, welche in Entwicklungsländern in Afrika südlich der Sahara vorkommt.[1] Diese vernachlässigte Tropenkrankheit wird durch die Protozoen *T. brucei* verursacht und durch den Stich der Tsetsefliege übertragen.[12] In den ersten Wochen nach einer Infektion zeigen die Patienten meist nur unspezifische Symptome, was häufig zu einer Fehldiagnose führt. Allerdings ist ein sofortiger Therapiebeginn unerlässlich, da eine unbehandelte Infektion in der Regel zum Tod führt.[12]

Obwohl die Anzahl an HAT-Fälle in den letzten Jahren stark rückläufig ist, kann eine neue Krankheitswelle aufgrund der instabilen sozialen Strukturen in den betroffenen Gebieten jederzeit wieder auftreten.[3, 11] Asymptomatische Krankheitsverläufe, Rinder als geeignetes Reservoir und jüngste Berichte über erhebliche Mengen an Trypanosomen in der Haut nicht-diagnostizierter Menschen stellen das langfristige Ziel der WHO in Frage, die HAT bis 2030 auszurotten.[14, 19, 21]

Nur wenige Medikamente stehen für eine Behandlung der HAT zur Verfügung und diese können wiederum nur in einem der beiden Infektionsstadien eingesetzt werden. Desweitern sind die vorhandenen Medikamente entweder nur in der chronischen oder in der akuten Form wirksam. Alle zugelassenen Präparate haben erhebliche Nachteile und werden, mit Ausnahme von Fexinidazol, seit Jahrzehnten eingesetzt. Die daraus folgende alarmierende Zunahme von Resistenzen, eine nahezu unmögliche Impfung und der Mangel an neuen Medikamenten in der „Pipeline" erfordern die Suche nach neuen antitrypanosomalen Wirkstoffen.[12, 41, 43]

Unsere Arbeitsgruppe hat in den letzten Jahren eine Reihe von 4-Chinolon-3-carboxamiden mit antitrypanosomaler Aktivität synthetisiert.[67, 69, 72] Durch Strukturoptimierung entdeckte Berninger die Leitverbindung **17**, welche eine hervorragende Selektivität (IC_{50} = 0,05 µM, CC_{50} > 100 µM) besitzt.[71] Obwohl bereits Versuche mittels Zelllyse behandelter Trypanosomen durchgeführt worden sind, ist der Angriffspunkt der Chinoloncarboxamide bis heute noch nicht entdeckt worden.[68] Weitere Untersuchungen für eine Identifizierung des „Targets" und größer angelegte *In-vivo*-Studien wurden durch die schlechte Wasserlöslichkeit der Verbindungen verhindert.[76]

In dieser Arbeit wurden neue 4-Chinolon-3-carboxamide synthetisiert, um unser Verständnis der Struktur-Wirkungs-Beziehung zu vertiefen. Die Chinolin-Kernstruktur wurde nach dem Gould-Jacob- und Grohe-Heitzer-Protokoll aufgebaut. In den Positionen 1, 3, 7 und 8 wurden sterisch anspruchsvolle Substituenten eingeführt, um π-π-Wechselwirkungen in der Kristallstruktur zu verhindern, die vermutlich die Ursache für die schlechte Wasserlöslichkeit sind.[69,

[78, 94] Außerdem sollten primäre Amine in der Benzylgruppe in Position 3 die Löslichkeit verbessern. In einem weiteren Ansatz wurde ein nicht-planares, 2,3-gesättigtes Chinoloncarboxamid **88** synthetisiert. Die physicochemischen Eigenschaften, die trypanocide Aktivität sowie die Zytotoxizität aller hergestellten Verbindungen wurden bestimmt.
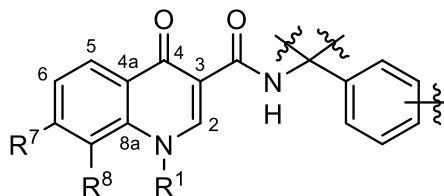
Figure 19. Eine allgemeine Struktur eines 4-Chinolon-3-carboxamids und gekennzeichnete chemische Modifikationen.

Der Austausch der Butylgruppe in Position 1 durch Cycloalkylmethyl-Substituenten führte zu einer geringeren Bioaktivität im Vergleich zu der nicht-fluorierten Leitstruktur **19** (IC_{50} = 0,23 µM). Je größer hierbei der Alkylring war, desto geringer war die antitrypanosomale Aktivität. Die Verbindung **28**, welche einen 1-Bicyclo[1.1.1]pentan-1-yl-Substituenten in Position 1 hatte, zeigte eine höhere Bioaktivität (IC_{50} = 0,13 µM). Gleichzeitig besaß sie eine größere Wasserlöslichkeit. Nur eine geringe Zytotoxizität wurde für alle in Position 1 modifizierten Substanzen gemessen.

Das Einführen eines Cycloalkylrings in der benzylischen Stellung in Position 3 führte in allen Verbindungen zu einer Abnahme der Löslichkeit (< 0,1 µg/mL). Die antitrypanosomale Aktivität der Cyclopropyl-Derivate **50a-c** war im Vergleich zu der jeweiligen Ausgangssubstanz nur geringfügig niedriger. Allerdings wurde ein deutlicher Rückgang bei den höheren Ringgrößen beobachtet, der auch nicht durch das Einfügen eines Fluor-Substituenten in Position 5 oder 6 ausgeglichen wurde. Für die Verbindungen **50a-c** mit einem Propylring wurde keine Zytotoxizität festgestellt. Die Pentyl- und Hexyl-Derivate, **50d-e** und **50f-h**, zeigten nur eine geringe toxische Wirkung. Zusätzliche Heteroatome in den Cyclohexyl-Substituenten in den Verbindungen **53a,c** erhöhten zwar die Wasserlöslichkeit, allerdings wurde die anti-trypanosomale Aktivität hiermit deutlich verringert (> 20 µM). Der vollständige Austausch des Benzylrestes in Position 3 durch einen 1-Bicyclo[1.1.1]pentan-1-yl-Substituenten führte zu einer höheren Löslichkeit (3,02 µg/mL), aber die Bioaktivität (IC_{50} = 3,72 µM) nahm ab.

Primäre Amine in der Benzyleinheit in Position 3 führten zu einem signifikanten Anstieg der Wasserlöslichkeit. Diese lag bei allen Verbindungen **60a-e** über 1 mg/mL. Allerdings hatten diese Verbindungen deutliche Stabilitätsprobleme. Die Bioaktivität der Verbindungen **60a-e** war im Vergleich zu den Referenzstrukturen etwas geringer, wobei sie keine oder nur eine geringe Zytotoxizität aufwiesen. In Anbetracht ihrer hervorragenden Löslichkeit macht dies die Amine zu interessanten Strukturen für weitere Untersuchungen. Darüber hinaus wurden die

Nitril-Derivate **55a,c,f**, welche Vorstufen in der Synthese der primären Amine waren, gegen *T. b. brucei* getestet. Die Chinoloncarboxamide **55a** und **55c** mit einer Nitril-Funktion in *para*-Stellung zeigten eine leicht geringere Bioaktivität im Vergleich zu den bisherigen Leitstrukturen. Verbindung **55f** mit einer Nitril-Gruppe in *meta*-Stellung hatte eine sehr hohe antitrypanosomale Aktivität (IC_{50} = 22 nM) und übertraf damit die bisherige Leitverbindung **17**. Es wurde keine Zytotoxizität für alle Nitril-Derivate festgestellt, woraus eine ausgezeichnete Selektivität für Verbindung **55f** folgt. Die Wasserlöslichkeit von Verbindung **55f** (0,24 µg/mL) war vergleichbar mit der Ausgangssubstanz **17**.

Die Implementierung von Triethylenglykol-Ketten in Position 7 in den Chinoloncarboxamiden **66a-c** führte zu einem starken Anstieg der Wasserlöslichkeit. Für alle drei Derivate wurde eine geringe Bioaktivität von etwa 1 µM und nahezu keine Zytotoxizität gemessen. Das Chinolonamid **68** mit einem 1-Methyl-1,4-diazepan-Substituenten in Position 7 zeigte ebenfalls eine schwächere antitrypanosomale Wirksamkeit. Darüber hinaus hatte die Verbindung **68** die höchste zytotoxische Wirkung (CC_{50} = 14,20 µM) der in dieser Arbeit getesteten Moleküle.

Alle Modifikationen in Position 8 führten zu einer Verringerung der Löslichkeit (< 0,1 µg/mL). Die Einführung eines Chlor- oder Brom-Substituenten bewirkte einen moderaten Verlust an Bioaktivität. Desweiteren wurde für diese Derivate **73** und **78** keine Zytotoxizität beobachtet. Die Methoxy- und Ethoxy-Derivate, **75** and **76**, hatten eine geringe antitrypanosomale Aktivität und es wurde eine leichte zytotoxische Wirkung festgestellt.

Das 2,3-gesättigte Chinoloncarboxamid **88** besaß ohne eine zusätzliche polare funktionelle Gruppe eine verbesserte Wasserlöslichkeit (10,4 µg/mL). Allerdings fiel die Bioaktivität von Verbindung **88** jedoch in den mikromolaren Bereich. Es wurde keine Zytotoxizität für diese Substanz beobachtet.

Insgesamt wurde eine Vielzahl von neuen 4-Chinolon-3-carboxamiden mit antitrypanosomaler Aktivität synthetisiert, welche neue Erkenntnisse über die Struktur-Wirkungsbeziehungen lieferten. Des Weiteren wurden einige Verbindungen mit erhöhter Löslichkeit und die vielversprechende Substanz **55f** mit ausgezeichneter Selektivität entdeckt.

17: R = H
55f: R = CN
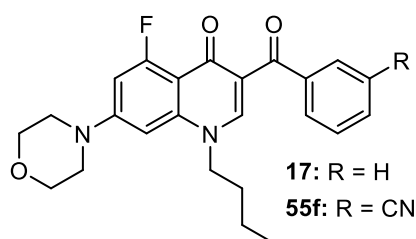
Substanz	IC_{50} [µM]	CC_{50} [µM]	Löslichkeit [µg/mL]
17	0,05	> 100	0,12
55f	0,022	> 50	0,24

*Figure 20. Die Verbindungen **17** und **55f** mit ihren biologischen Aktivitäten und Löslichkeiten.*

8 <u>Experimental Part</u>

8.1 General methods and used equipment

Infrared spectroscopy (IR)

All IR spectra were recorded on a JASCO FT-IR-6100 spectrometer (*Jasco*, Groß-Umstadt, Germany) equipped with an ATR unit. All spectra were measured at room temperature. The intensities of the absorption bands are illustrated by the following abbreviations: s = strong, m = medium, w = weak.

Melting points (m.p.)

All melting points were measured with a capillary melting point apparatus MPD350:BM 3.5 (*Sanyo*, Gallenkamp BV, Netherlands) and a MP70 melting point system (*Mettler Toledo*, Greifensee, Switzerland).

Nuclear magnetic resonance spectroscopy (NMR)

All ^{1}H (400.13 MHz) and ^{13}C NMR (100.61 MHz) spectra were recorded using a Bruker AV 400 NMR spectrometer (*Bruker Biospin*, Ettlingen, Germany) at 300 K. The processing of the spectra was done with the software 'Topspin' (*Bruker Biospin*, Ettlingen, Germany). Chemical shifts are given in units of the δ scale and are calibrated on the trace proton signals of the used deuterated solvents for ^{1}H NMR spectra [δ(CDCl$_3$) = 7.26 ppm, δ(MeOD) = 3.31 ppm, δ(DMSO) = 2.50 ppm] and ^{13}C signals of the solvents for ^{13}C spectra [δ(CDCl$_3$) = 77.16 ppm, δ(MeOD) = 49.00 ppm, δ(DMSO) = 39.52 ppm].[176] Coupling constants are given in Hertz (Hz). Spin multiplicities are given by the following abbreviations: s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublets of doublets, t = triplet, q = quartet, quint = quintet, sext = sextet, m = multiplet, br = broad.

Thermomixer

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

Microwave system

The microwave-supported syntheses were carried out in a synthWAVE microwave (*Milestone*, Leutkirch, Germany). When an apolar solvent was required for the synthesis, *Weflon*® plates (polytetrafluroethylene with 10 % graphit) were added to the reaction mixture in order to increase the microwave irradiation absorption efficiency.

Thin-layer chromatography

The thin-layer chromatography was performed on silica gel 60 F_{254} plates from *Macherey-Nagel* (Düren, Germany). The detection was carried out by irradiation and subsequent fluorescence quenching at 254 nm or excitation at 366 nm. Depending on the monitored molecules, the silica gel plates were stained with iodine vapor, ninhydrin, potassium permanganate, or bromocresol green. Azides were reduced to amines on the TLC plate with a 10 % PPh_3 solution in CH_2Cl_2 and stained with ninhydrin.[177]

Column chromatography

For column chromatography, silica gel (0.063 - 0.2 nm) from *Merck* (Darmstadt, Germany) was used. When amines were purified, the silica gel was deactivated by addition of 7.5 wt% ammonia. The columns were prepared by the wet method and the composition of the eluent is given in volume percent.

Flash chromatography

The purifications by flash chromatography were done on a puriFlash® system (*Interchim*, Montluçon, France) with pre-packed columns (silica 50 µm F0040).

High performance liquid chromatography (HPLC)

Method **I** for purity analysis

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

column	Synergi Fusion-RP (4 µm, 150 mm x 4.6 mm), (*Phenomenex*, Aschaffenburg, Germany)
eluent	A = water + 0.1 % formic acid
	B = methanol + 0.1 % formic acid

gradient elution	0 – 8 min	5 % B → 100 % B
	8 – 12 min	100 % B
	12 – 16 min	100 % B → 5 % B
	16 – 18 min	5 % B

detection	λ = 254 nm
	Shimadzu LCMS-2020 single quadrupole mass spectrometer, ionization method: ESI
temperature	r.t.
flow	1 mL/min

Method **II** for logP determination

instrument	*Agilent* HPLC system 1200 series
column	Synergi Max-RP (4 µm, 150 mm x 4.6 mm), (*Phenomenex*, Aschaffenburg, Germany)
eluent	10 mM KH_2PO_4 buffer (pH = 7.4)/ MeOH (30/70)
detection	λ = 254 nm
temperature	30 °C
flow	1 mL/min

Method **III** for solubility determination

instrument	*Agilent* HPLC system 1200 series
column	Eurosphere II (5 µm, 150 mm x 4.6 mm) (*Knauer*, Berlin, Germany)
eluent	water/ACN (30/70)

detection	a) λ = 254 nm
	b) λ = 280 nm
	c) λ = 220 nm
temperature	30 °C
flow	1 mL/min

Method **IV** for solubility determination of primary amines **60a-f**

instrument	*Agilent* HPLC system 1200 series
column	Eurosphere II (5 µm, 150 mm x 4.6 mm) (*Knauer*, Berlin, Germany)
eluent	water + 0.1 % TFA / ACN + 0.1 % TFA (30/70)
detection	λ = 254 nm
temperature	30 °C
flow	1 mL/min

Water, used in HPLC analysis, was purified by a Milli-Q® system (*Merck*, Darmstadt, Germany). HPLC gradient grade acetonitrile and HPLC gradient grade methanol were purchased from *Sigma Aldrich* (Schnelldorf, Germany).

Mass spectrometry

Mass spectrometry (MS) was performed using an LCMS-2020 single quadrupole mass spectrometer running in positive ionization mode.

High resolution mass spectrometry (HRMS) was performed using a Sciex X500R QTOF mass spectrometer (Concord, Ontario, Canada) equipped with a Turbo Vᴛᴍ Ion Source (ESI). Automatic calibration of the mass spectrometer was performed using the provided tuning solution for ESI (*Sciex*, Concord, Ontario, Canada).

Used chemicals

All chemicals were purchased from *Sigma Aldrich* (Schnelldorf, Germany), *VWR* (Darmstadt, Germany), and *TCI* (Eschborn, Germany), and were used without further purification.

The solvents were dried according to general procedure and stored over molecular sieve (3.6 Å) under argon atmosphere.[178]

- Acetonitrile was stirred with CaH_2 overnight and subsequently distilled.
- Dichloromethane/Chloroform was stored over $CaCl_2$ for at least 24 h and subsequently distilled.
- Methanol was dried with magnesium shavings, refluxed, and distilled.
- Dimethylformamide was stirred with CaH_2 overnight and subsequently distilled.
- Tetrahydrofuran/diethyl ether were pre-dried over CaH_2 and decanted. Sodium and benzophenone were added and the mixture was heated at reflux for several hours until the solvent turned deep blue. Afterwards, the solvent was distilled.

Dry 1,4-dioxane was bought at *Sigma Aldrich* (Schnelldorf, Germany) and was used without prior purification.

8.2 Syntheses

8.2.1 General synthetic procedure (A) for anilinomethylenemalonic ester derivatives

The appropriate aniline derivative (1 eq) and diethyl methylenemalonate (1.2 eq) were dissolved in toluene and stirred under reflux for 16-24 h. The solvent was removed *in vacuo* and the residue was crystallized from pentane at -20 °C to yield the desired compounds.

Diethyl 2-(((3-chloro-4-fluorophenyl)amino)methylene)malonate (**21a**)
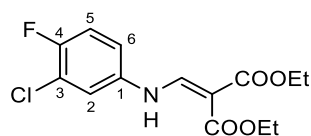
According to the general procedure (**A**), 3-chloro-4-fluoroaniline (5.00 g, 34.4 mmol) was dissolved in toluene (30 mL) and treated with diethyl methylenemalonate (7.64 mL, 37.8 mmol) for 16 h.

Chemical formula: $C_{14}H_{15}ClFNO_4$

Molar mass: 315.7 g/mol

Appearance: colourless solid

Yield: 9.33 g (29.6 mmol, 86 %, Lit: 94 %)[72]

Melting point: 72 °C (Lit.: 62-63 °C)[72]

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3193 (w), 3078 (w), 2978 (w), 2909 (w), 1717 (m), 1655 (m), 1618 (m), 1505 (m), 1216 (s), 1069 (s), 794 (s).

^{1}H NMR (400 MHz, CDCl$_3$): δ = 11.0 (d, 1H, ^3J = 13.4 Hz, N**H**), 8.37 (d, 1H, ^3J = 13.4 Hz, NH**CH**), 7.20 (dd, 1H, $^4J_{H,F}$ = 6.0 Hz, $^4J_{H,H}$ = 2.8 Hz, 2-**H**), 7.15 (m, 1H, 5-**H**), 6.99 (m, 1H, 6-**H**), 4.31 (q, 2H, ^3J = 7.2 Hz, OC**H$_2$**CH$_3$), 4.25 (q, 2H, ^3J = 7.2 Hz, OC**H$_2$**CH$_3$), 1.38 (t, 3H, ^3J = 7.2 Hz, OCH$_2$C**H$_3$**), 1.33 (t, 3H, ^3J = 7.2 Hz, OCH$_2$C**H$_3$**) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 169.0 (1C, **C**OOEt), 165.6 (1C, **C**OOEt), 155.4 (d, 1C, $^1J_{C,F}$ = 247.2 Hz, **C**-4), 151.8 (1C, NH**C**H), 136.4 (d, 1C, $^4J_{C,F}$ = 3.3 Hz, **C**-1), 122.5 (d, 1C, $^2J_{C,F}$ = 19.1 Hz, **C**-3), 119.3 (1C, **C**-2), 117.7 (d, 1C, $^2J_{C,F}$ = 22.6 Hz, **C**-5), 117.0 (d, 1C, $^3J_{C,F}$ = 7.0 Hz, **C**-6), 94.8 (1C, **C**(COOEt)$_2$), 60.7 (1C, O**C**H$_2$CH$_3$), 60.4 (1C, O**C**H$_2$CH$_3$), 14.5 (1C, OCH$_2$**C**H$_3$), 14.4 (1C, OCH$_2$**C**H$_3$) ppm.

The spectroscopic data are in accordance with literature.[72]

Diethyl 2-(((3-fluorophenyl)amino)methylene)malonate (**21b**)
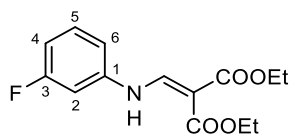
According to the general procedure (**A**), 3-fluoroaniline (4.31 mL, 45.0 mmol) was dissolved in toluene (30 mL) and treated with diethyl methylenemalonate (10.9 mL, 54.0 mmol) for 24 h.

Chemical formula: $C_{14}H_{16}FNO_4$

Molar mass: 281.3 g/mol

Appearance: colourless solid

Yield: 7.26 g (25.8 mmol, 57 %, Lit: 93 %)[179]

Melting point: 49 °C (Lit: 48 °C)[180]

IR (ATR, $\tilde{v}$ [cm⁻¹]): 3179 (w), 3051 (w), 2983 (w), 2902 (w), 1684 (m), 1637 (m), 1606 (m), 1426 (m), 1248 (s), 1144 (m), 1099 (m), 798 (s).

¹H NMR (400 MHz, DMSO-d_6): δ = 11.0 (d, 1H, ^3J = 13.4 Hz, N**H**), 8.44 (d, 1H, ^3J = 13.4 Hz, NHC**H**), 7.31 (m, 1H, 2-**H**), 6.79-6.91 (m, 3H, 4-**H** + 5-**H** + 6-**H**), 4.30 (q, 2H, ^3J = 7.2 Hz, OC**H₂**CH₃), 4.24 (q, 2H, ^3J = 7.2 Hz, OC**H₂**CH₃), 1.37 (t, 3H, ^3J = 7.2 Hz, OCH₂C**H₃**), 1.32 (t, 3H, ^3J = 7.2 Hz, OCH₂C**H₃**) ppm.

¹³C NMR (100 MHz, DMSO-d_6): δ = 168.8 (1C, **C**OOEt), 165.4 (1C, **C**OOEt), 163.6 (d, 1C, $^1J_{C,F}$ = 247.1 Hz, **C**-3), 151.3 (1C, CH=**C**), 140.9 (1C, $^3J_{C,F}$ = 10.1 Hz, **C**-1), 131.1 (d, 1C, $^3J_{C,F}$ = 9.4 Hz, **C**-5), 112.8 (d, 1C, $^4J_{C,F}$ = 2.9 Hz, **C**-6), 111.5 (d, 1C, $^2J_{C,F}$ = 21.3 Hz, **C**-4), 104.3 (d, 1C, $^2J_{C,F}$ = 25.5 Hz, **C**-2), 94.5 (1C, NHC**H**=C), 60.5 (1C, O**C**H₂CH₃), 60.2 (1C, O**C**H₂CH₃), 14.3 (1C, OCH₂**C**H₃), 14.2 (1C, OCH₂**C**H₃) ppm.

The spectroscopic data are in accordance with literature.[181]

Diethyl 2-(((3,5-difluorophenyl)amino)methylene)malonate (**21c**)
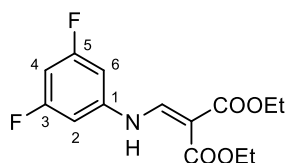
According to the general procedure (**A**), 3,5-difluoroaniline (4.50 mL, 45.0 mmol) was dissolved in toluene (30 mL) and treated with diethyl methylenemalonate (10.9 mL, 54.0 mmol) for 18 h.

Chemical formula: $C_{14}H_{15}F_2NO_4$

Molar mass: 299.3 g/mol

Appearance: colourless solid

Yield: 12.1 g (40.4 mmol, 90 %, Lit: 98 %)[72]

Melting point: 102 °C (Lit: 103-105 °C)[182]

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3168 (w), 3089 (w), 2986 (w), 2906 (w), 1684 (m), 1645 (m), 1589 (s), 1416 (m), 1241 (s), 981 (m), 798 (s).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.6 (d, 1H, ^3J = 13.6 Hz, N**H**), 8.31 (d, 1H, ^3J = 13.6 Hz, NHC**H**), 6.55-6.63 (m, 2H, 2-**H** + 6-**H**), 6.48-6.55 (m, 1H, 4-**H**), 4.21 (q, 2H, ^3J = 7.1 Hz, OC**H$_2$**CH$_3$), 4.13 (q, 2H, ^3J = 7.1 Hz, OC**H$_2$**CH$_3$), 1.25 (t, 3H, ^3J = 7.1 Hz, OCH$_2$C**H$_3$**), 1.25 (t, 3H, ^3J = 7.1 Hz, OCH$_2$C**H$_3$**) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 168.7 (1C, **C**OOEt), 165.2 (1C, **C**OOEt), 164.0 (d, 1C, $^1J_{C,F}$ = 248.8 Hz, **C**-F), 163.9 (d, 1C, $^1J_{C,F}$ = 248.8 Hz, **C**-F), 150.6 (1C, CH=**C**), 141.7 (t, 1C, $^3J_{C,F}$ = 12.6 Hz, **C**-1), 100.3 (d, 2C, $^2J_{C,F}$ = 29.2 Hz, **C**-2, **C**-6), 100.2 (t, 1C, $^2J_{C,F}$ = 13.5 Hz, **C**-4), 95.6 (1C, NH**C**H=C), 59.9 (1C, O**C**H$_2$CH$_3$), 59.7 (1C, O**C**H$_2$CH$_3$), 14.2 (1C, OCH$_2$**C**H$_3$), 14.1 (1C, OCH$_2$**C**H$_3$) ppm.

The spectroscopic data are in accordance with literature.[72]

Diethyl-2-(((2,3,4-trifluorophenyl)amino)methylene)malonate (**21d**)
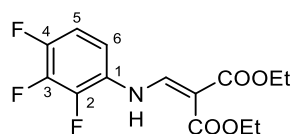
According to the general procedure (**A**), 3,4,5-trifluoroaniline (4.00 mL, 37.8 mmol) was dissolved in toluene (40 mL) and treated with diethyl methylenemalonate (9.17 mL, 45.4 mmol) for 24 h.

Chemical formula: $C_{14}H_{14}F_3NO_4$

Molar mass: 317.3 g/mol

Appearance: colourless solid

Yield: 11.3 g (35.6 mmol, 94 %, Lit: 86 %)[72]

Melting point: 90 °C (Lit: 93-95 °C)[72]

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3146 (w), 3077 (w), 2984 (w), 1690 (m), 1650 (m), 1621 (m), 1507 (m), 1426 (m), 1244 (s), 1040 (m), 795 (s).

^{1}H NMR (400 MHz, CDCl$_3$): δ = 11.0 (d, 1H, ^3J = 13.1 Hz, N**H**), 8.35 (d, 1H, ^3J = 13.1 Hz, NHC**H**), 6.99 (m, 2H, 5-**H** + 6-**H**), 4.29 (q, 2H, ^3J = 7.1 Hz, OC**H$_2$**CH$_3$), 4.22 (q, 2H, ^3J = 7.1 Hz, OC**H$_2$**CH$_3$), 1.34 (t, 3H, ^3J = 7.1 Hz, OCH$_2$C**H$_3$**), 1.30 (t, 3H, ^3J = 7.1 Hz, OCH$_2$C**H$_3$**) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 168.6 (1C, **C**OOEt), 165.4 (1C, **C**OOEt), 151.0 (1C, CH=**C**), 147.9 (ddd, 1C, $^1J_{C,F}$ = 248.2 Hz, $^2J_{C,F}$ = 10.2 Hz, $^3J_{C,F}$ = 2.4 Hz, **C**-2), 142.6 (ddd, 1C, $^1J_{C,F}$ = 250.6 Hz, $^2J_{C,F}$ = 12.4 Hz, $^3J_{C,F}$ = 3.3 Hz, **C**-4), 140.6 (ddd, 1C, $^1J_{C,F}$ = 252.7 Hz, $^2J_{C,F}$ = 16.2 Hz, $^2J_{C,F}$ = 13.3 Hz, **C**-3), 125.9 (dd, 1C, $^2J_{C,F}$ = 8.4 Hz, $^3J_{C,F}$ = 3.5 Hz, **C**-1), 112.4 (dd, 1C, $^2J_{C,F}$ = 18.6 Hz, $^3J_{C,F}$ = 4.0 Hz, **C**-5), 110.3 (m, 1C, **C**-6), 96.3 (1C, NH**C**H=C), 60.8 (1C, O**C**H$_2$CH$_3$), 60.5 (1C, O**C**H$_2$CH$_3$), 14.4 (1C, OCH$_2$**C**H$_3$), 14.3 (1C, OCH$_2$**C**H$_3$) ppm.

The spectroscopic data are in accordance with literature.[72]

8.2.2 General synthesis procedure (B) for ethyl 4-oxo-1,4-dihydroquinoline-3-carboxylate derivatives

The respective anilinomethylenemalonic ester derivative **21** was dissolved in diphenyl ether (10-25 mL) and 8-12 *Weflon*® discs were added. The solution was heated at 210 °C for 25 min in an open vessel apparatus in a rotaPREP microwave system. The solution was cooled to r.t. and diethyl ether (50-100 mL) was added. The resulting precipitate was filtered and washed with diethyl ether (3×25 mL). Since the resulting products were hardly soluble in any solvent, they were used directly in the next step without characterization and further purification.

Ethyl 6-fluoro-7-chloro-4-hydroxyquinoline-3-carboxylate (**22a**)
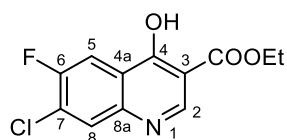
Chemical formula: $C_{12}H_9ClFNO_3$

Molar mass: 269.7 g/mol

Appearance: colourless solid

Amount of starting material: 9.13 g

Yield: 3.92 g (14.5 mmol, 50 %, Lit: 77 %)[72]

Ethyl 7-fluoro-4-hydroxyquinoline-3-carboxylate (**22b**)
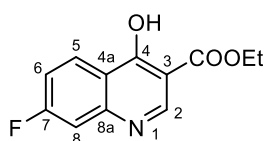
Chemical formula: $C_{12}H_{10}FNO_3$

Molar mass: 235.2 g/mol

Appearance: colourless solid

Amount of starting material: 7.26 g

Yield: 3.42 g (14.5 mmol, 56 %, Lit: 51 %)[183]

Ethyl 5,7-difluoro-4-hydroxyquinoline-3-carboxylate (**22c**)
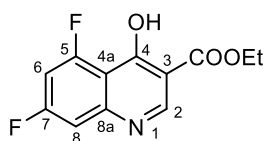
Chemical formula: $C_{12}H_9F_2NO_3$

Molar mass: 253.2 g/mol

Appearance: colourless solid

Amount of starting material: 10.0 g

Yield: 5.75 g (22.7 mmol, 68 %, Lit: 91 %)[72]

Ethyl 6,7,8-trifluoro-4-hydroxyquinoline-3-carboxylate (**22d**)
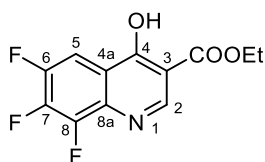
Chemical formula: $C_{12}H_8F_3NO_3$

Molar mass: 271.2 g/mol

Appearance: colourless solid

Amount of starting material: 10.8 g

Yield: 4.07 g (15.0 mmol, 44 %, Lit: 43 %)[72]

8.2.3 General synthetic procedure (C) for 1-alkyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acids

The corresponding ethyl-4-hydroxyquinoline-3-carboxylate **22** (1 eq) and K_2CO_3 (4 eq) were dissolved in abs. DMF and stirred at 60 °C for 30 min. A catalytic amount of KI and the respective alkyl bromide (4 eq) were added and the reaction mixture was stirred at 80-100 °C for 20-72 h. The solvent was removed *in vacuo* and distilled water and CH_2Cl_2 were added to the resulting residue. After shaking, the phases were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH (100/1)). The resulting oil was dissolved in EtOH (10 mL) and 2 M HCl (50 mL) and stirred at reflux for 12 h. The precipitated product was filtered, washed with water, and dried over P_2O_5.

1-(Cyclopropylmethyl)-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**24a**)
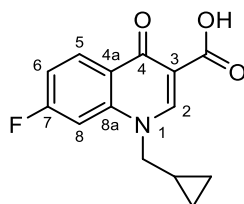
According to the general procedure (**C**), compound **22b** (536 mg, 2.28 mmol) and K_2CO_3 (630 mg, 4.56 mmol) were suspended in abs. DMF (50 mL) and stirred with (bromomethyl)cyclopropane (400 mg, 2.96 mmol) at 100 °C for 72 h. The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH (100/1)) and hydrolysed with a 2 M HCl solution. The precipitated product was filtered, washed with water, and dried over P_2O_5. The crude product was purified by recrystallization from $CHCl_3$/EtOH to yield the desired product as a colourless solid.

Chemical formula: $C_{14}H_{12}FNO_3$

Molar mass: 261.3 g/mol

Appearance: colourless solid

Yield: 258 mg (988 µmol, 43 %)

Melting point: 187 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3050 (w), 2978 (w), 2922 (w), 1701 (m), 1604 (m), 1458 (m), 1246 (s), 958 (m), 794 (s).

¹H NMR (400 MHz, CDCl₃): δ = 14.77 (s, 1H, CO**OH**), 8.86 (s, 1H, 2-**H**), 8.58 (m, 1H, 5-**H**), 7.38 (dd, 1H, $^3J_{H,F}$ = 10.2 Hz, ^4J = 2.0 Hz, 8-**H**), 7.32 (m, 1H, 6-**H**), 4.12 (d, 2H, ^3J = 7.0 Hz, NC**H₂**), 1.39 (m, 1H, NCH₂C**H**), 0.83 (m, 2H, C**H₂**), 0.53 (m, 2H, C**H₂**) ppm.

¹³C NMR (100 MHz, CDCl₃): δ = 178.0 (1C, **C**-4), 166.9 (1C, **C**OOH), 166.1 (d, 1C, $^1J_{C,F}$ = 255.8 Hz, **C**-7), 148.6 (1C, **C**-2), 141.5 (d, 1C, $^3J_{C,F}$ = 11.4 Hz, **C**-8a), 130.6 (d, 1C, $^3J_{C,F}$ = 10.8 Hz, **C**-5), 123.4 (d, 1C, $^4J_{C,F}$ = 1.7 Hz, **C**-4a), 115.3 (d, 1C, $^2J_{C,F}$ = 23.0 Hz, **C**-6), 109.3 (1C, **C**-3), 103.1 (d, 1C, $^2J_{C,F}$ = 26.9 Hz, **C**-8), 59.1 (1C, N**C**H₂), 10.0 (1C, NCH₂**C**H), 5.0 (2C, 2 x **C**H₂) ppm.

1-(Cyclobutylmethyl)-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**24b**)
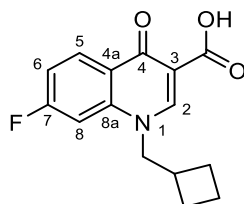
According to the general procedure (**C**), compound **22b** (520 mg, 2.21 mmol) and K₂CO₃ (917 mg, 6.63 mmol) were suspended in abs. DMF (50 mL) and stirred with (bromomethyl)cyclobutane (659 mg, 4.42 mmol) at 100 °C for 72 h. The crude product was purified by column chromatography (silica gel, CH₂Cl₂/MeOH (100/1)) and hydrolysed with a 2 M HCl solution. The precipitated product was filtered, washed with water, and dried over P₂O₅. The crude product was purified by recrystallization from CHCl₃/EtOH to yield the desired product as a colourless solid.

Chemical formula: C₁₅H₁₄FNO₃

Molar mass: 275.3 g/mol

Appearance: colourless solid

Yield: 327 mg (1.19 mmol, 54 %)

Melting point: 232 °C

IR (ATR, $\tilde{v}$ [cm⁻¹]): 3046 (w), 2969 (w), 2862 (w), 1698 (m), 1607 (m), 1455 (m), 1243 (m), 959 (s), 792 (s).

Mass: *m/z* 275.95 [M + H]⁺

¹H NMR (400 MHz, DMSO-d_6): δ = 15.02 (s, 1H, COOH), 9.05 (s, 1H, 2-**H**), 8.42 (dd, 1H, ^3J = 8.9 Hz, $^4J_{H,F}$ = 6.5 Hz, 5-**H**), 7.98 (dd, 1H, $^3J_{H,F}$ = 11.2 Hz, ^4J = 2.0 Hz, 8-**H**), 7.54 (m, 1H, 6-**H**), 4.58 (d, 2H, ^3J = 7.5 Hz, NC**H₂**), 2.81 (m, 1H, NCH₂C**H**), 1.76–1.97 (br, 6H, 3 x C**H₂**) ppm.

¹³C NMR (100 MHz, DMSO-d_6): δ = 177.5 (1C, **C**-4), 166.3 (1C, **C**OOH), 165.8 (d, 1C, $^1J_{C,F}$ = 251.6 Hz, **C**-7), 150.4 (1C, **C**-2), 141.8 (d, 1C, $^3J_{C,F}$ = 12.4 Hz, **C**-8a), 129.7 (d, 1C, $^3J_{C,F}$ = 11.1 Hz, **C**-5), 123.0 (d, 1C, $^4J_{C,F}$ = 1.3 Hz, **C**-4a), 115.7 (d, 1C, $^2J_{C,F}$ = 23.6 Hz, **C**-6), 108.2 (1C, **C**-3), 105.3 (d, 1C, $^2J_{C,F}$ = 27.3 Hz, **C**-8), 58.0 (1C, N**C**H₂), 34.3 (1C, NCH₂**C**H), 25.5 (2C, 2 x **C**H₂), 18.1 (1C, **C**H₂) ppm.

1-(Cyclohexylmethyl)-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**24c**)
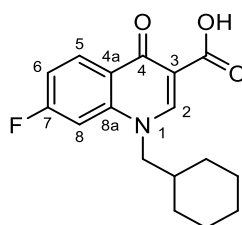
According to the general procedure (**C**), compound **22b** (630 mg, 2.68 mmol) and K₂CO₃ (1.11 g, 8.04 mmol) were suspended in abs. DMF (100 mL) and stirred with (bromomethyl)cyclohexane (1.12 mL, 8.04 mmol) at 100 °C for 72 h. The crude product was purified by column chromatography (silica gel, CH₂Cl₂/MeOH (100/1)) and hydrolysed with a 2 M HCl solution. The precipitated product was filtered, washed with water, and dried over P₂O₅. The crude product was purified by recrystallization from CHCl₃/EtOH to yield the desired product as a colourless solid.

Chemical formula: C₁₇H₁₈FNO₃

Molar mass: 303.3 g/mol

Appearance: colourless solid

Yield: 440 mg (1.45 mmol, 54 %)

Melting point: 211 °C

IR (ATR, $\tilde{v}$ [cm⁻¹]): 3055 (w), 2928 (w), 2847 (w), 1711 (s), 1614 (s), 1464 (s), 1256 (m), 939 (m), 793 (s).

Mass: *m/z* 303.90 [M + H]⁺

¹H NMR (400 MHz, DMSO-d_6): δ = 15.04 (s, 1H, COOH), 8.98 (s, 1H, 2-**H**), 8.45 (dd, 1H, ^3J = 9.0 Hz, $^4J_{H,F}$ = 6.4 Hz, 5-**H**), 7.98 (dd, 1H, $^3J_{H,F}$ = 11.2 Hz, ^4J = 2.2 Hz, 8-**H**), 7.55 (m, 1H,

6-**H**), 4.41 (d, 2H, ^3J = 7.5 Hz, NC**H₂**), 1.84 (m, 1H, NCH₂C**H**), 1.60 (m, 5H, C**H₂**), 1.09 (m, 5H, C**H₂**) ppm.

¹³C NMR (100 MHz, DMSO-d_6): δ = 177.6 (1C, **C**-4), 166.3 (1C, **C**OOH), 165.8 (d, 1C, $^1J_{C,F}$ = 251.7 Hz, **C**-7), 150.9 (1C, **C**-2), 141.9 (d, 1C, $^3J_{C,F}$ = 12.4 Hz, **C**-8a), 129.7 (d, 1C, $^3J_{C,F}$ = 11.1 Hz, **C**-5), 123.0 (d, 1C, $^4J_{C,F}$ = 1.6 Hz, **C**-4a), 115.7 (d, 1C, $^2J_{C,F}$ = 23.6 Hz, **C**-6), 108.0 (1C, **C**-3), 105.3 (d, 1C, $^2J_{C,F}$ = 27.3 Hz, **C**-8), 59.2 (1C, N**C**H₂), 36.7 (1C, NCH₂**C**H), 29.7 (2C, 2 x **C**H₂), 26.2 (2C, 2 x **C**H₂), 18.1 (1C, **C**H₂) ppm.

1-Butyl-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**24d**)
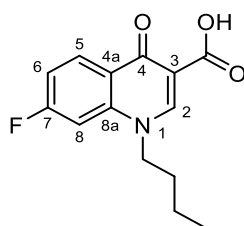
According to the general procedure (**C**), compound **22b** (2.47 g, 10.5 mmol) and K₂CO₃ (5.81 g, 42.0 mmol) were suspended in abs. DMF (200 mL) and stirred with 1-bromobutane (4.53 mL, 5.76 g, 42.0 mmol) at 80 °C for 24 h. The crude product was purified by column chromatography (silica gel, CH₂Cl₂/MeOH (100/1)) and hydrolysed with a 2 M HCl solution. The precipitated product was filtered, washed with water, and dried over P₂O₅. The crude product was purified by recrystallization from CHCl₃/EtOH to yield the desired product as a colourless solid.

Chemical formula: C₁₄H₁₄FNO₃

Molar mass: 263.3 g/mol

Appearance: colourless solid

Yield: 1.88 g (7.14 mmol, 68 %)

Melting point: 196 °C

IR (ATR, $\tilde{v}$ [cm⁻¹]): 3051 (w), 2963 (w), 2865 (w), 1704 (w), 1609 (m), 1456 (m), 1248 (m), 957 (m), 862 (m), 793 (s).

¹H NMR (400 MHz, DMSO-d_6): δ = 15.03 (s, 1H, COO**H**), 9.04 (s, 1H, 2-**H**), 8.43 (dd, 1H, ^3J = 9.0 Hz, $^4J_{H,F}$ = 6.5 Hz, 5-**H**), 7.96 (dd, 1H, $^3J_{H,F}$ = 11.2 Hz, ^4J = 2.1 Hz, 8-**H**), 7.52 (m, 1H, 6-**H**), 4.53 (t, 2H, ^3J = 7.4 Hz, NC**H₂**CH₂CH₂CH₃), 1.75 (quint, 2H, ^3J = 7.4 Hz,

NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.35 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.91 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 177.1 (1C, **C**-4), 165.8 (1C, **C**OOH), 165.3 (d, 1C, $^1J_{C,F}$ = 251.6 Hz, **C**-7), 150.2 (1C, **C**-2), 141.1 (d, 1C, $^3J_{C,F}$ = 12.3 Hz, **C**-8a), 129.3 (d, 1C, $^3J_{C,F}$ = 11.1 Hz, **C**-5), 122.5 (d, 1C, $^4J_{C,F}$ = 1.5 Hz, **C**-4a), 115.2 (d, 1C, $^2J_{C,F}$ = 23.7 Hz, **C**-6), 107.8 (1C, **C**-3), 104.6 (d, 1C, $^2J_{C,F}$ = 27.2 Hz, **C**-8), 53.6 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 30.6 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.0 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.5 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

1-Butyl-7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**24e**)
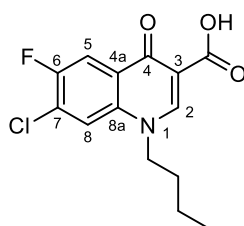
According to the general procedure (**C**), compound **22a** (1.80 g, 6.68 mmol) and K$_2$CO$_3$ (3.69 g, 26.7 mmol) were suspended in abs. DMF (150 mL) and stirred with 1-bromobutane (2.88 mL, 3.66 g, 26.7 mmol) at 80 °C for 48 h. The crude product was purified by column chromatography (silica gel, CH$_2$Cl$_2$/MeOH (100/1)) and hydrolysed with a 2 M HCl solution. The precipitated product was filtered, washed with water, and dried over P$_2$O$_5$. The crude product was purified by recrystallization from CHCl$_3$/EtOH to yield the desired product as a colourless solid.

Chemical formula: C$_{14}$H$_{13}$ClFNO$_3$

Molar mass: 297.7 g/mol

Appearance: colourless solid

Yield: 1.62 g (5.44 mmol, 82 %, Lit: 76 %)[72]

^{1}H NMR (400 MHz, DMSO-d_6): δ = 14.77 (s, 1H, COO**H**), 9.06 (s, 1H, 2-**H**), 8.43 (d, 1H, $^4J_{H,F}$ = 6.1 Hz, 8-**H**), 8.21 (d, 1H, $^3J_{H,F}$ = 9.1 Hz, 5-**H**), 4.58 (t, 2H, ^3J = 7.4 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 1.78 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.34 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.91 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 176.5 (d, 1C, $^4J_{C,F}$ = 2.7 Hz, **C**-4), 165.5 (1C, **C**OOH), 154.9 (1C, $^1J_{C,F}$ = 249.6 Hz, **C**-6), 150.0 (1C, **C**-2), 136.4 (1C, $^4J_{C,F}$ = 1.9 Hz, **C**-8a), 127.4 (d, 1C, $^2J_{C,F}$ = 20.1 Hz, **C**-7), 126.1 (d, 1C, $^3J_{C,F}$ = 6.7 Hz, **C**-4a), 121.1 (1C, **C**-8), 112.0 (d, 1C, $^2J_{C,F}$ =

22.8 Hz, **C**-5), 107.7 (1C, **C**-3), 53.7 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 30.8 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.0 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.5 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

The spectroscopic data are in accordance with literature.[72]

1-Butyl-5,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**24f**)
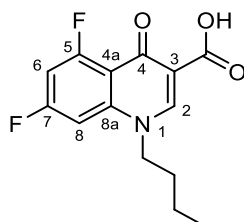
According to the general procedure (**C**), compound **22c** (2.82 g, 11.1 mmol) and K$_2$CO$_3$ (4.62 g, 33.4 mmol) were suspended in abs. DMF (150 mL) and stirred with 1-bromobutane (3.58 mL, 4.58 g, 33.4 mmol) at 80 °C for 48 h. The crude product was purified by column chromatography (silica gel, CH$_2$Cl$_2$/MeOH (100/1)) and hydrolysed with a 2 M HCl solution. The precipitated product was filtered, washed with water, and dried over P$_2$O$_5$. The crude product was purified by recrystallization from CHCl$_3$/EtOH to yield the desired product as a colourless solid.

Chemical formula: C$_{14}$H$_{13}$F$_2$NO$_3$

Molar mass: 281.3 g/mol

Appearance: colourless solid

Yield: 597 mg (2.12 mmol, 19 %, Lit: 88 %)[72]

^{1}H NMR (400 MHz, DMSO-d_6): δ = 14.98 (s, 1H, COO**H**), 9.01 (s, 1H, 2-**H**), 7.79 (m, 1H, 8-**H**), 7.48-7.56 (m, 1H, 6-**H**), 4.49 (t, 2H, ^3J = 7.4 Hz, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 1.73 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 1.34 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 0.90 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$**CH$_3$**) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 176.8 (d, 1C, $^3J_{C,F}$ = 1.7 Hz, **C**-4), 165.5 (1C, **C**OOH), 164.7 (dd, 1C, $^1J_{C,F}$ = 253.9 Hz, $^3J_{C,F}$ = 16.7 Hz, **C**-7), 162.4 (dd, 1C, $^1J_{C,F}$ = 262.2 Hz, $^3J_{C,F}$ = 16.8 Hz, **C**-5), 150.2 (1C, **C**-2), 142.3 (dd, 1C, $^3J_{C,F}$ = 14.4 Hz, $^3J_{C,F}$ = 5.5 Hz, **C**-8a), 113.1 (dd, 1C, $^2J_{C,F}$ = 8.6 Hz, $^4J_{C,F}$ = 2.8 Hz, **C**-4a), 108.8 (1C, **C**-3), 102.8 (dd, 1C, $^2J_{C,F}$ = 27.0 Hz, $^2J_{C,F}$ = 25.5 Hz, **C**-6), 101.0 (dd, 1C, $^2J_{C,F}$ = 24.8 Hz, $^4J_{C,F}$ = 2.4 Hz, **C**-8), 54.1 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 30.3 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.0 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.5 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

The spectroscopic data are in accordance with literature.[72]

1-Butyl-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**24g**)
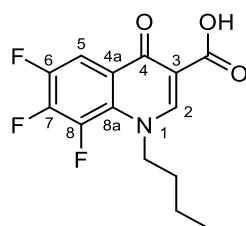
According to the general procedure (**C**), compound **22d** (4.10 g, 15.1 mmol) and K_2CO_3 (8.36 g, 60.5 mmol) were suspended in abs. DMF (250 mL) and stirred with 1-bromobutane (4.89 mL, 6.21 g, 45.4 mmol) at 80 °C for 48 h. The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH (100/1)) and hydrolysed with a 2 M HCl solution. The precipitated product was filtered, washed with water, and dried over P_2O_5. The crude product was purified by recrystallization from $CHCl_3$/EtOH to yield the desired product as a colourless solid.

Chemical formula: $C_{14}H_{12}F_3NO_3$

Molar mass: 299.3 g/mol

Appearance: colourless solid

Yield: 2.65 mg (8.86 mmol, 59 %, Lit: 62 %)[72]

Melting point: 197 °C (Lit: 216-218 °C)[72]

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3057 (w), 2962 (w), 2874 (w), 1714 (s), 1617 (m), 1561 (s), 1461 (s), 1286 (m), 1058 (s), 919 (m), 807 (s).

Mass: *m/z* 299.90 [M + H]$^+$

^{1}H NMR (400 MHz, DMSO-d_6): δ = 14.48 (s, 1H, COO**H**), 9.04 (s, 1H, 2-**H**), 8.20 (ddd, 1H, $^3J_{H,F}$ = 9.9 Hz, $^4J_{H,F}$ = 8.4 Hz, $^5J_{H,F}$ = 1.8 Hz, 5-**H**), 4.59 (m, 2H, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 1.81 (m, 2H, NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.35 (m, 2H, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 0.91 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$-CH$_2$C**H$_3$**) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 175.4 (1C, **C**-4), 165.1 (1C, **C**OOH), 152.1 (1C, **C**-2), 149.4 (m, 1C, **C**-6), 147.0 (m, 1C, **C**-8), 142.2 (m, 1C, **C**-7), 133.4 (1C, **C**-8a), 127.2 (1C, **C**-8a), 122.7 (d, 1C, $^3J_{C,F}$ = 5.2 Hz, **C**-4a), 108.2 (dd, 1C, $^2J_{C,F}$ = 18.5 Hz, $^3J_{C,F}$ = 3.4 Hz, **C**-5), 107.6 (1C, **C**-3), 57.7 (d, 1C, $^4J_{C,F}$ = 13.8 Hz, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 31.9 (d, 1C, $^5J_{C,F}$ = 3.9 Hz, 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$_3$) ppm.

The spectroscopic data are in accordance with literature.[72]

8.2.4 S$_N$Ar at position 7 of the 1-alkyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acids

General procedure (**D**) for 1-alkyl-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acids:

The corresponding 1-alkyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **24** was dissolved in morpholine and the solution was heated at 110 °C under microwave irradiation for 4-6 h. The reaction solution was acidified with a 2 M HCl solution until the product precipitated. The solid was filtered, washed with water, and dried over P_2O_5. The crude product was purified by recrystallization from CHCl$_3$/EtOH.

1-(Cyclopropylmethyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**26a**)
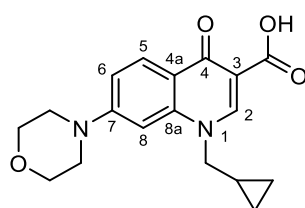
According to the general procedure (**D**), compound **24a** (220 mg, 842 µmol) was dissolved in morpholine (20 mL) and heated at 110 °C under microwave irradiation for 4 h.

Chemical formula: $C_{18}H_{20}N_2O_4$

Molar mass: 328.4 g/mol

Appearance: yellow solid

Yield: 146 mg (445 µmol, 53 %)

Melting point: 243 °C

IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3060 (w), 2979 (w), 2845 (w), 1714 (w), 1615 (m), 1454 (m), 1240 (m), 1107 (m), 966 (m), 790 (m).

^{1}H NMR (400 MHz, CDCl$_3$): δ = 15.28 (s, 1H, COO**H**), 8.74 (s, 1H, 2-**H**), 8.39 (d, 1H, ^3J = 9.0 Hz, 5-**H**), 7.35 (d, 1H, ^3J = 9.0 Hz, 6-**H**), 6.99 (s, 1H, 8-**H**), 4.08 (d, 2H, ^3J = 6.8 Hz, NC**H$_2$**), 3.96 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.44 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.40 (m, 1H, NCH$_2$C**H**), 0.81 (m, 2H, C**H$_2$**), 0.52 (m, 2H, C**H$_2$**) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 177.5 (1C, **C**-4), 167.6 (1C, **C**OOH), 154.1 (1C, **C**-7), 147.7 (1C, **C**-2), 141.8 (1C, **C**-8a), 128.7 (1C, **C**-5), 119.3 (1C, **C**-4a), 114.9 (1C, **C**-6), 108.5 (1C, **C**-3), 99.6 (1C, **C**-8), 66.3 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 58.8 (1C, N**C**H$_2$), 48.5 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 10.0 (1C, NCH$_2$**C**H), 5.0 (2C, 2 x **C**H$_2$) ppm.

1-(Cyclobutylmethyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**26b**)
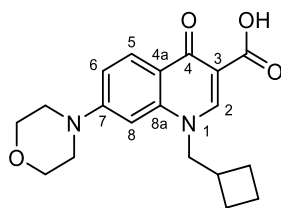
According to the general procedure (**D**), compound **24b** (283 mg, 1.03 mmol) was dissolved in morpholine (20 mL) and heated at 110 °C under microwave irradiation for 4 h.

Chemical formula: $C_{19}H_{22}N_2O_4$

Molar mass: 342.4 g/mol

Appearance: yellow solid

Yield: 176 mg (514 µmol, 50 %)

Melting point: 216 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3087 (w), 2933 (w), 2858 (w), 1707 (m), 1618 (s), 1445 (s), 1244 (s), 1119 (s), 970 (s), 809 (s).

Mass: *m/z* 343.00 [M + H]$^+$

^{1}H NMR (400 MHz, DMSO-d_6): δ = 15.70 (s, 1H, COO**H**), 8.87 (s, 1H, 2-**H**), 8.16 (d, 1H, ^3J = 9.2 Hz, 5-**H**), 7.35 (dd, 1H, ^3J = 9.2 Hz, ^4J = 2.0 Hz, 6-**H**), 7.54 (d, 1H, ^4J = 2.0 Hz, 8-**H**), 4.56 (d, 2H, ^3J = 7.4 Hz, NC**H**$_2$), 3.79 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.46 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 2.85 (m, 1H, NCH$_2$C**H**), 1.76–1.97 (br, 6H, 3 x C**H**$_2$) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 176.0 (1C, **C**-4), 167.0 (1C, **C**OOH), 155.1 (1C, **C**-7), 149.1 (1C, **C**-2), 142.0 (1C, **C**-8a), 127.5 (1C, **C**-5), 117.1 (1C, **C**-4a), 114.9 (1C, **C**-6), 106.9 (1C, **C**-3), 99.1 (1C, **C**-8), 66.3 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 57.6 (1C, N**C**H$_2$), 47.3 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 34.3 (1C, N**C**H$_2$**C**H), 25.8 (2C, 2 x **C**H$_2$), 18.2 (1C, **C**H$_2$) ppm.

1-(Cyclohexylmethyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**26c**)
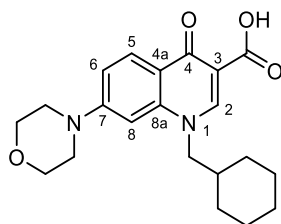
According to the general procedure (**D**), compound **24c** (400 mg, 1.32 mmol) was dissolved in morpholine (20 mL) and heated at 110 °C under microwave irradiation for 4 h.

Chemical formula: $C_{21}H_{26}N_2O_4$

Molar mass: 370.5 g/mol

Appearance: colourless solid

Yield: 316 mg (853 µmol, 65 %)

Melting point: 232 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3044 (w), 2921 (w), 2844 (w), 1699 (m), 1612 (s), 1446 (s), 1245 (s), 956 (s), 790 (s).

Mass: *m*/*z* 371.20 [M + H]$^+$

^{1}H NMR (400 MHz, CDCl$_3$): δ = 14.65 (s, 1H, COO**H**), 8.57 (s, 1H, 2-**H**), 8.40 (d, 1H, ^3J = 9.1 Hz, 5-**H**), 7.18 (dd, 1H, ^3J = 9.1 Hz, ^4J = 1.8 Hz, 6-**H**), 6.89 (br, 1H, 8-**H**), 4.04 (d, 2H, ^3J = 7.2 Hz, NC**H**$_2$), 3.97 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.41 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.94 (m, 1H, NCH$_2$C**H**), 1.65-1.83 (m, 5H, C**H**$_2$), 1.03-1.28 (m, 5H, C**H**$_2$) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 177.6 (1C, **C**-4), 167.6 (1C, **C**OOH), 165.8 (1C, **C**-7), 148.8 (1C, **C**-2), 141.7 (1C, **C**-8a), 128.8 (1C, **C**-5), 119.7 (1C, **C**-4a), 114.9 (1C, **C**-6), 108.2 (1C, **C**-3), 100.0 (1C, **C**-8), 66.3 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 61.0 (1C, N**C**H$_2$), 48.5 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 37.1 (1C, N**C**H$_2$**C**H), 31.0 (2C, 2 x **C**H$_2$), 26.1 (1C, **C**H$_2$), 25.6 (2C, 2 x **C**H$_2$) ppm.

1-Butyl-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**26d**)
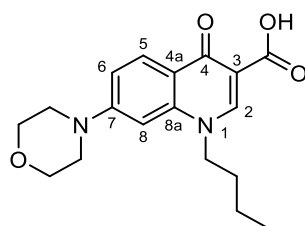
According to the general procedure (**D**), compound **24d** (51.6 mg, 196 µmol) was dissolved in morpholine (20 mL) and heated at 110 °C under microwave irradiation for 4 h.

Chemical formula: $C_{18}H_{22}N_2O_4$

Molar mass: 330.4 g/mol

Appearance: colourless solid

Yield: 53.2 mg (161 µmol, 82 %)

Melting point: 228 °C (Lit: 229-230 °C)[72]

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3061 (w), 2956 (w), 2858 (w), 1713 (m), 1610 (m), 1442 (m), 1233 (m), 1101 (m), 970 (m), 792 (s).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 15.69 (s, 1H, COO**H**), 8.85 (s, 1H, 2-**H**), 8.15 (d, 1H, ^3J = 9.2 Hz, 5-**H**), 7.33 (dd, 1H, ^3J = 9.2 Hz, ^4J = 1.6 Hz, 6-**H**), 7.17 (d, 1H, ^4J = 1.6 Hz, 8-**H**), 4.51 (t, 2H, ^3J = 7.4 Hz, N**CH$_2$**CH$_2$CH$_2$CH$_3$), 3.78 (m, 4H, morpholino-**CH$_2$**-O-**CH$_2$**), 3.45 (m, 4H, morpholino-**CH$_2$**-N-**CH$_2$**), 1.76 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$**CH$_2$**CH$_2$CH$_3$), 1.32 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$**CH$_2$**CH$_3$), 0.91 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$**CH$_3$**) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 176.4 (1C, **C**-4), 166.5 (1C, **C**OOH), 154.6 (1C, **C**-7), 148.9 (1C, **C**-2), 141.2 (1C, **C**-8a), 127.0 (1C, **C**-5), 116.7 (1C, **C**-4a), 114.4 (1C, **C**-6), 106.6 (1C, **C**-3), 98.4 (1C, **C**-8), 65.8 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 52.9 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 46.8 (2C, morpholino-**C**H$_2$-N-**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$_3$) ppm.

The spectroscopic data are in accordance with literature.[72]

1-Butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**26e**)
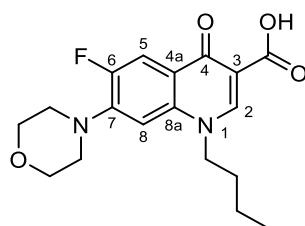
According to the general procedure (**D**), compound **24e** (3.45 g, 11.6 mmol) was dissolved in morpholine (20 mL) and heated at 110 °C under microwave irradiation for 6 h.

Chemical formula: $C_{18}H_{21}FN_2O_4$

Molar mass: 348.4 g/mol

Appearance: yellow solid

Yield: 2.62 g (7.52 mmol, 65 %, Lit: 63)[72]

Melting point: 238 °C (Lit: 234-235 °C)[72]

IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3055 (w), 2957 (w), 2871 (w), 1719 (m), 1626 (m), 1460 (s), 1257 (s), 1116 (s), 942 (m), 804 (m).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 15.31 (s, 1H, COO**H**), 8.94 (s, 1H, 2-**H**), 7.93 (d, 1H, $^3J_{H,F}$ = 13.4 Hz, 5-**H**), 7.17 (d, 1H, $^4J_{H,F}$ = 7.3 Hz, 8-**H**), 4.57 (t, 2H, ^3J = 7.4 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.78-3.82 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.29-3.33 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.79 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.33 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.92 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 176.2 (1C, **C**-4), 166.1 (1C, **C**OOH), 152.9 (1C, $^1J_{C,F}$ = 218.0 Hz, **C**-6), 148.9 (1C, **C**-2), 145.3 (1C, $^2J_{C,F}$ = 10.8 Hz, **C**-7), 137.3 (1C, **C**-8a), 119.3 (1C, **C**-4a), 111.2 (d, 1C, $^2J_{C,F}$ = 23.3 Hz, **C**-5), 106.9 (1C, **C**-3), 105.9 (1C, **C**-8), 65.8 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 52.9 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 49.7 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 30.3 (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$_3$) ppm.

The spectroscopic data are in accordance with literature.[72]

1-Butyl-5-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**26f**)
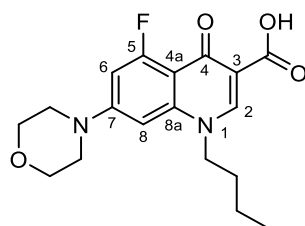
Compound **24f** (900 mg, 3.20 mmol), triethylamine (665 µL, 4.80 mmol), and boron trifluoride (2.41 mL, 19.2 mmol) were dissolved in abs. CH_2Cl_2 (90 mL) and stirred under reflux for 5 h. The reaction was quenched with water (3 mL) and the solvent was removed *in vacuo*. The solid residue was washed with MeOH and dried over P_2O_5. The intermediate, morpholine (276 µL, 3.20 mmol), and triethylamine (665 µL, 4.80 mmol) were dissolved in EtOH (80 mL) and stirred under reflux for 4 h. The solvent was removed *in vacuo*, the residue was dissolved in a 2 M NaOH solution (80 mL) and stirred at reflux for 2 h. After cooling to r.t., the aqueous solution was acidified with a 2 M HCl solution until the product precipitated. The solid was filtered, washed with water, and dried over P_2O_5. The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH/FA (100/1/1)) to yield the desired product as a yellow solid.

Chemical formula: $C_{18}H_{21}FN_2O_4$

Molar mass: 348.4 g/mol

Appearance: yellow solid

Yield: 270 mg (775 µmol, 24 %, Lit: 25 %)[72]

Melting point: 272 °C (Lit: 257-260 °C)[72]

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3044 (w), 2970 (w), 2874 (w), 1699 (m), 1627 (m), 1447 (m), 1265 (m), 1119 (m), 992 (m), 811 (s).

¹H NMR (400 MHz, DMSO-d_6): δ = 15.67 (br, 1H, COO**H**), 8.82 (s, 1H, 2-**H**), 7.10 (dd, 1H, $^3J_{H,F}$ = 15.6 Hz, ^4J = 2.0 Hz, 6-**H**), 6.74 (d, 1H, ^4J = 2.0 Hz, 8-**H**), 4.47 (t, 2H, ^3J = 7.4 Hz, NC**H₂**CH₂CH₂CH₃), 3.73-3.77 (m, 4H, morpholino-**CH₂**-O-**CH₂**), 3.44-3.48 (m, 4H, morpholino-**CH₂**-N-**CH₂**), 1.74 (quint, 2H, ^3J = 7.4 Hz, NCH₂**CH₂**CH₂CH₃), 1.32 (sext, 2H, ^3J = 7.4 Hz, NCH₂CH₂**CH₂**CH₃), 0.91 (t, 3H, ^3J = 7.4 Hz, NCH₂CH₂CH₂**CH₃**) ppm.

¹³C NMR (100 MHz, DMSO-d_6): δ = 176.1 (1C, **C**-4), 166.3 (1C, **C**OOH), 162.1 (1C, ^1J = 255.3 Hz, **C**-5), 149.3 (1C, **C**-2), 142.4 (1C, **C**-8a), 107.2 (1C, **C**-4a), 100.6 (1C, **C**-6), 94.7 (1C, **C**-8), 65.7 (2C, morpholino-**CH₂**-O-**CH₂**), 53.5 (1C, N**CH₂**CH₂CH₂CH₃), 46.5 (2C, mor-

pholino-**CH**₂-N-**CH**₂), 29.9 (1C, NCH₂**C**H₂CH₂CH₃), 19.1 (1C, NCH₂CH₂**C**H₂CH₃), 13.5 (1C, NCH₂CH₂CH₂**C**H₃) ppm.

The ^{13}C signal of C-3 was not detectable due to low intensity caused by the low solubility of the substance. The spectroscopic data are in accordance with literature.[72]

1-Butyl-6,8-difluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**26g**)
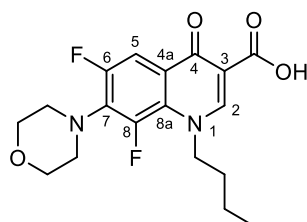
Compound **24g** (2.61 g, 8.72 mmol) and morpholine (1.50 mL, 17.4 mmol) were dissolved in DMF (20 mL) and the solution was heated at 110 °C under microwave irradiation for 4 h. The solvent was removed *in vacuo*. CH₂Cl₂ (100 mL) and a saturated ammonium chloride solution (100 mL) were added. After shaking, the phases were separated and the water phase was extracted with CH₂Cl₂. The combined organic phases were dried over Na₂SO₄, filtered, and the solvent was removed *in vacuo*. The crude product was purified by recrystallization from EtOH.

Chemical formula: $C_{18}H_{20}F_2N_2O_4$

Molar mass: 366.4 g/mol

Appearance: yellow solid

Yield: 1.97 g (5.38 mmol, 62 %, Lit: 36 %)[72]

Melting point: 198 °C (Lit: 210 °C)[72]

IR (ATR, $\tilde{\nu}$ [cm⁻¹]): 3051 (w), 2952 (w), 2849 (w), 1714 (m), 1617 (m), 1510 (w), 1467 (s), 1279 (m), 1206 (m), 1115 (s), 1051 (s), 1016 (m), 918 (s), 806 (s).

Mass: *m/z* 366.95 [M + H]⁺

¹H NMR (400 MHz, DMSO-d_6): δ = 14.87 (s, 1H, COO**H**), 8.92 (s, 1H, 2-**H**), 7.88 (d, 1H, $^3J_{H,F}$ = 12.0 Hz, 5-**H**), 4.56 (m, 2H, N**CH**₂CH₂CH₂CH₃), 3.73 (m, 4H, morpholino-**CH**₂-O-**CH**₂), 3.34 (m, 4H, morpholino-**CH**₂-N-**CH**₂), 1.79 (quint, 2H, ^3J = 7.4 Hz, NCH₂**CH**₂CH₂CH₃), 1.32 (sext, 2H, ^3J = 7.4 Hz, NCH₂CH₂**CH**₂CH₃), 0.91 (t, 3H, ^3J = 7.4 Hz, NCH₂CH₂CH₂**CH**₃) ppm.

¹³C NMR (100 MHz, DMSO-d_6): δ = 175.5 (1C, **C**-4), 165.5 (1C, **C**OOH), 154.7 (dd, 1C, $^1J_{C,F}$ = 249.6 Hz, $^3J_{C,F}$ = 6.1 Hz, **C**-6), 151.4 (1C, **C**-2), 145.5 (dd, 1C, $^1J_{C,F}$ = 250.7 Hz, $^3J_{C,F}$ = 6.6 Hz, **C**-8), 127.3 (m, 1C, **C**-8a), 127.0 (d, 1C, ^3J = 8.7 Hz **C**-4a), 107.3 (dd, 1C, $^2J_{C,F}$ = 22.6 Hz, $^4J_{C,F}$ = 2.3 Hz, **C**-5), 106.8 (1C, **C**-3), 66.6 (2C, morpholino-**C**H₂-O-**C**H₂), 58.0 (d, 1C, $^4J_{C,F}$ = 15.2 Hz, N**C**H₂CH₂CH₂CH₃), 50.8 (2C, morpholino-**C**H₂-N-**C**H₂), 32.0 (d, 1C, $^5J_{C,F}$ = 4.2 Hz, NCH₂-**C**H₂CH₂CH₃), 19.0 (1C, NCH₂CH₂**C**H₂CH₃), 13.4 (1C, NCH₂CH₂CH₂**C**H₃) ppm.

The spectroscopic data are in accordance with literature.[72]

8.2.5 Synthesis of 1-cycloalkylmethyl quinolone amides

N-Benzyl-1-(cyclopropylmethyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**27a**)
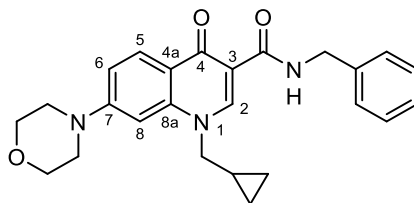
The carboxylic acid **26a** (75 mg, 228 µmol), benzylamine (50.0 µL, 457 µmol), and PyBOP (113 mg, 297 µmol) were dissolved in abs. DMF (80 mL). DIPEA (79.8 µL, 457 mmol) was added and the solution was stirred at r.t. overnight. A saturated ammonium chloride solution (100 mL) was added and the mixture was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH (100/0.5)) and recrystallized from $CHCl_3$/EtOH to yield the desired product.

Chemical formula: $C_{25}H_{27}N_3O_3$

Molar mass: 417.5 g/mol

Appearance: colourless crystals

Yield: 35.7 mg (85.5 µmol, 37 %)

Melting point: 199 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3149 (w), 3037 (w), 2959 (w), 2826 (w), 1652 (m), 1600 (m), 1529 (m), 1445 (m), 1244 (s), 1117 (m), 967 (m), 819 (m).

HRMS: [M+H]$^+$ 418.21252 m/z, found 418.21218 m/z.

Purity (HPLC method I): 99.7 %

logP (HPLC method II): 3.24

^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.51 (t, 1H, ^3J = 5.9 Hz, CON**H**), 8.79 (s, 1H, 2-**H**), 8.14 (d, 1H, ^3J = 9.1 Hz, 5-**H**), 7.34 (m, 4H, Bn-C**H**$_{arom.}$), 7.25 (m, 2H, Bn-C**H**$_{arom.}$ + 6-**H**), 7.02 (d, 1H, ^4J = 2.0 Hz, 8-**H**), 4.54 (d, 2H, ^3J = 5.9 Hz, NHC**H**$_2$), 4.30 (d, 2H, ^3J = 7.2 Hz, NC**H**$_2$), 3.78 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.40 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.37 (m, 1H, C**H**), 0.58 (m, 2H, C**H**$_2$), 0.46 (m, 2H, C**H**$_2$) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 174.8 (1C, **C**-4), 164.5 (1C, **C**ONH), 154.0 (1C, **C**-7), 147.3 (1C, **C**-2), 141.0 (1C, **C**-8a), 139.5 (1C, Bn-**C**$_{arom.}$), 128.4 (2C, 2 x Bn-**C**$_{arom.}$), 127.3 (2C,

Bn-$C_{arom.}$), 127.3 (1C, **C**-5), 126.8 (1C, Bn-$C_{arom.}$), 119.0 (1C, **C**-4a), 113.5 (1C, **C**-6), 110.0 (1C, **C**-3), 98.7 (1C, **C**-8), 65.9 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 56.3 (1C, N**C**H$_2$), 47.2 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 42.1 (1C, NH**C**H$_2$), 10.2 (1C, **C**H), 3.99 (2C, 2 x **C**H$_2$) ppm.

N-Benzyl-1-(cyclobutylmethyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**27b**)
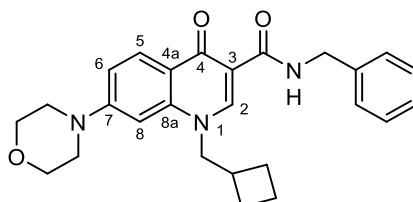
The carboxylic acid **26b** (176 mg, 514 µmol), benzylamine (68.0 µL, 617 µmol), and PyBOP (321 mg, 617 µmol) were dissolved in abs. DMF (80 mL). DIPEA (224 µL, 1.29 mmol) was added and the solution was stirred at r.t. overnight. A saturated ammonium chloride solution (100 mL) was added and the mixture was extracted with CH$_2$Cl$_2$. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH$_2$Cl$_2$/MeOH (100/0.5)) and recrystallized from CHCl$_3$/EtOH to yield the desired product.

Chemical formula: C$_{26}$H$_{29}$N$_3$O$_3$

Molar mass: 431.5 g/mol

Appearance: colourless crystals

Yield: 134 mg (311 µmol, 60 %)

Melting point: 181 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3167 (w), 3054 (w), 2967 (w), 2850 (w), 1655 (m), 1613 (m), 1600 (m), 1464 (m), 1244 (m), 1123 (m), 965 (m), 837 (m).

HRMS: [M+H]$^+$ 432.22817 m/z, found 432.22798 m/z.

Purity (HPLC method I): 99.8 %

logP (HPLC method II): 3.68

¹H NMR (400 MHz, CDCl$_3$): δ = 10.52 (br, 1H, CON**H**), 8.69 (s, 1H, 2-**H**), 8.35 (d, 1H, ^3J = 9.0 Hz, 5-**H**), 7.39 (d, 1H, ^3J = 7.3 Hz, Bn-C**H**$_{arom.}$), 7.32 (t, 1H, ^3J = 7.3 Hz, Bn-C**H**$_{arom.}$), 7.23 (t, 1H, ^3J = 7.3 Hz, Bn-C**H**), 7.06 (d, 1H, ^3J = 9.0 Hz, 6-**H**), 6.62 (br, 1H, 8-**H**), 4.67 (d, 2H, ^3J = 5.7 Hz, NHC**H**$_2$), 4.18 (d, 2H, ^3J = 6.8 Hz, NC**H**$_2$), 3.90 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$),

3.34 (m, 4H, morpholino-CH_2-N-CH_2), 2.92 (sept, 1H,^3J = 7.6 Hz, NHCH$_2$C**H**), 2.11 (m, 2H, C**H$_2$**), 1.92 (m, 4H, 2 x C**H$_2$**) ppm.

^{13}**C NMR** (100 MHz, CDCl$_3$): δ = 176.1 (1C, **C**-4), 165.5 (1C, **C**ONH), 154.3 (1C, **C**-7), 147.5 (1C, **C**-2), 141.2 (1C, **C**-8a), 139.1 (1C, Bn-**C**$_{arom.}$), 128.65 (2C, 2 x Bn-**C**$_{arom.}$), 128.60 (1C, **C**-5), 127.8 (2C, Bn-**C**$_{arom.}$), 127.1 (1C, Bn-**C**$_{arom.}$), 120.6 (1C, **C**-4a), 113.9 (1C, **C**-6), 111.4 (1C, **C**-3), 98.4 (1C, **C**-8), 66.6 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 58.6 (1C, N**C**H$_2$), 48.1 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 43.4 (1C, NH**C**H$_2$), 34.3 (1C, N**C**H$_2$**C**H), 26.5 (2C, 2 x **C**H$_2$), 18.3 (1C, **C**H$_2$) ppm.

N-Benzyl-1-(cyclohexylmethyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**27c**)
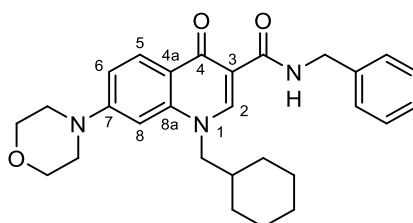
The carboxylic acid **26c** (140 mg, 378 µmol), benzylamine (62.0 µL, 567 µmol), and PyBOP (275 mg, 529 µmol) were dissolved in abs. DMF (80 mL). DIPEA (145 µL, 831 µmol) was added and the solution was stirred at r.t. overnight. A saturated ammonium chloride solution (100 mL) was added and the mixture was extracted with CH$_2$Cl$_2$. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH$_2$Cl$_2$/MeOH (100/0.5)) and recrystallized from CHCl$_3$/EtOH to yield the desired product.

Chemical formula: C$_{28}$H$_{33}$N$_3$O$_3$

Molar mass: 459.6 g/mol

Appearance: colourless needles

Yield: 62.0 mg (135 µmol, 36 %)

Melting point: 216 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3173 (w), 3026 (w), 2965 (w), 2849 (w), 1652 (m), 1614 (m), 1598 (m), 1553 (s), 1450 (m), 1242 (m), 1122 (m), 794 (m).

HRMS: [M+H]$^+$ 460.25947 m/z, found 460.25897 m/z.

Purity (HPLC method I): 99.3 %

logP (HPLC method II): 4.59

¹H NMR (400 MHz, CDCl$_3$): δ = 10.52 (t, 1H, ^3J = 5.7 Hz, CON**H**), 8.61 (s, 1H, 2-**H**), 8.34 (d, 1H, ^3J = 9.2 Hz, 5-**H**), 7.38 (m, 2H, Bn-C**H**$_{arom.}$), 7.30 (m, 2H, Bn-C**H**$_{arom.}$), 7.22 (m, 1H, Bn-C**H**$_{arom.}$), 7.05 (d, 1H, ^3J = 9.2 Hz, ^4J = 2.2 Hz, 6-**H**), 6.60 (d, 1H, ^4J = 2.2 Hz, 8-**H**), 4.66 (d, 2H, ^3J = 5.7 Hz, NHC**H**$_2$), 3.97 (d, 2H, ^3J = 7.1 Hz, NC**H**$_2$), 3.90 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.29 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.91 (m, 1H, C**H**), 1.64-1.79 (m, 5H, C**H**$_2$), 1.02-1.25 (m, 5H, C**H**$_2$) ppm.

¹³C NMR (100 MHz, CDCl$_3$): δ = 176.1 (1C, **C**-4), 165.5 (1C, **C**ONH), 154.3 (1C, **C**-7), 148.1 (1C, **C**-2), 141.3 (1C, **C**-8a), 139.1 (1C, Bn-**C**$_{arom.}$), 128.70 (1C, **C**-5), 128.68 (2C, 2 x Bn-**C**$_{arom.}$), 127.8 (2C, Bn-**C**$_{arom.}$), 127.1 (1C, Bn-**C**$_{arom.}$), 120.7 (1C, **C**-4a), 113.9 (1C, **C**-6), 111.2 (1C, **C**-3), 98.5 (1C, **C**-8), 66.7 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 60.3 (1C, N**C**H$_2$), 48.0 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 43.4 (1C, NH**C**H$_2$), 37.1 (1C, N**C**H$_2$**C**H), 31.1 (2C, 2 x **C**H$_2$), 26.2 (1C, **C**H$_2$), 25.7 (2C, 2 x **C**H$_2$) ppm.

8.2.6 Synthesis of 1-bicyclo[1.1.1]pentan-1-yl quinolone amides

Ethyl 3-(2,4-dichlorophenyl)-3-oxopropanoate (**32**)
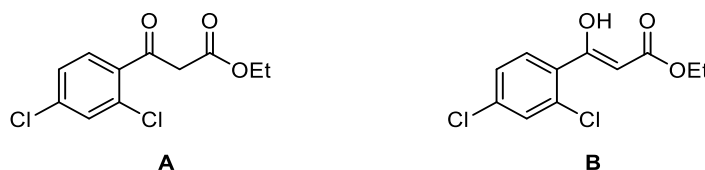
2,4-Dichlorobenzoic acid (2.00 g, 10.5 mmol) was suspended in thionyl chloride (10 mL) and stirred under reflux for 2 h. All volatile substances were removed *in vacuo*. The residue was twice dissolved in toluene (10 mL) and the solvent was removed *in vacuo*.

In another flask, magnesium turnings (382 mg, 15.7 mmol) were suspended in abs. EtOH (20 mL) and a few drops of CCl_4 were added until a slight boiling was achieved. A solution of diethyl malonate (2.40 mL, 15.7 mmol) in EtOH (4 mL) and toluene (15 mL) was added dropwise over 15 min. The reaction mixture was stirred at r.t. for 30 min and, afterwards, a solution of the prior synthesized 2,4-dichlorobenzoyl chloride in toluene (5 mL) was added. The solution was stirred at r.t. for 16 h and the solvent was removed *in vacuo*. The residue was dissolved in 10 % sulfuric acid (100 mL) and EtOAc (100 mL), the phases were separated, and the water phase was extracted with EtOAc. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*.

The residue was suspended in water (10 mL) and *para*-toluenesulfonic acid (1.80 g, 10.5 mmol) was added. The suspension was stirred under reflux for 4 h and extracted with EtOAc. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, PE/EtOAc (20/1)) to yield the desired product as a yellow oil.

Chemical formula: $C_{11}H_{10}Cl_2O_3$

Molar mass: 261.1 g/mol

Appearance: yellow oil

Yield: 1.60 g (6.13 mmol, 59 %)

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3093 (w), 2984 (w), 1737 (m), 1700 (m), 1625 (m), 1583 (m), 1270 (m), 1238 (s), 1192 (s), 1105 (m), 1024 (m), 805 (s).

Isomer A:

¹H NMR (400 MHz, CDCl₃): δ = 7.58 (d, 1H, ^3J = 8.4 Hz, 6-**H**), 7.44 (m, 1H, 3-**H**), 7.30 (m, 1H, 5-**H**), 4.18 (m, 2H, OC**H₂**CH₃), 4.00 (s, 2H, **CH₂**), 1.24 (m, 3H, OCH₂C**H₃**) ppm.

¹³C NMR (100 MHz, CDCl₃): δ = 193.5 (1C, **C**OCH₂), 166.9 (1C, **C**OOEt), 138.5 (1C, **C**-2), 135.9 (**C**-1), 132.8 (1C, **C**-4), 131.1 (1C, **C**-6), 130.6/130.7 (1C, **C**-3), 127.3/127.6 (1C, **C**-5), 61.7 (1C, O**C**H₂CH₃), 49.2 (1C, **C**H₂), 14.1 (1C, OCH₂**C**H₃) ppm.

Isomer B:

¹H NMR (400 MHz, CDCl₃): δ = 12.48 (s, 1H, O**H**), 7.52 (d, 1H, ^3J = 8.4 Hz, 6-**H**), 7.44 (m, 1H, 3-**H**), 7.30 (m, 1H, 5-**H**), 5.55 (s, 1H, **CH**), 4.26 (m, 2H, OC**H₂**CH₃), 4.00 (s, 2H, **CH₂**), 1.32 (t, 3H, ^3J = 7.1 Hz, OCH₂C**H₃**) ppm.

¹³C NMR (100 MHz, CDCl₃): δ = 172.6 (1C, **C**OOEt), 169.2 (1C, **C**OHCH), 136.6 (1C, **C**-2), 133.1 (1C, **C**-4), 132.1 (**C**-1), 131.4 (1C, **C**-6), 130.6/130.7 (1C, **C**-3), 127.3/127.6 (1C, **C**-5), 93.7 (1C, COH**C**H), 60.8 (1C, O**C**H₂CH₃), 14.3 (1C, OCH₂**C**H₃) ppm.

The spectroscopic data are in accordance with literature.[184]

Ethyl 3-(bicyclo[1.1.1]pentan-1-ylamino)-2-(2,4-dichlorobenzoyl)acrylate (**34**)
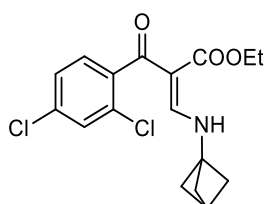
Ethyl 3-(2,4-dichlorophenyl)-3-oxopropanoate (**32**) (872 mg, 3.34 mmol) and triethyl orthoformate (2.75 mL, 16.7 mmol) were dissolved in acetic anhydride (20 mL) and rotated under microwave irradiation (600 W, 115 °C) for 45 min. The volatile substances were removed *in vacuo* and the residue was dissolved in EtOH (50 mL). 1-Bicyclo[1.1.1]pentylamine hydrochloride (399 mg, 3.34 mmol) and K₂CO₃ (462 mg, 3.34 mmol) were added. The reaction mixture was stirred under reflux for 4 h. The solvent was removed *in vacuo* and the residue was purified by column chromatography (silica gel, CH₂Cl₂/PE (4/1)). The resulting oil was dissolved in PE (20 mL) and stored at -20 °C overnight to yield the desired product as a colourless solid.

Chemical formula: C₁₇H₁₇Cl₂NO₃

Molar mass: 354.2 g/mol

Appearance: colourless solid

Yield: 435 mg (1.23 mmol, 37 %)

Melting point: 105 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3137 (br), 3016 (w), 2987 (w), 2874 (w), 1681 (m), 1602 (m), 1543 (m), 1415 (m), 1239 (s), 1123 (m), 1019 (m), 808 (m).

The product was present as an *E*- and a *Z*-isomer, indicated by the two sets of signals in the ^{1}H NMR spectrum. The isomeric ratio of three to one, with the *E*-isomer being the major product, was determined by the integration of the respective *β*-protons at 8.03 and 8.11 ppm. Only the main isomer (*E*-isomer) was evaluated in the NMR characterization.

^{1}H NMR (400 MHz, CDCl$_3$): δ = 11.31 (d, 1H, ^3J = 13.8 Hz, CHN**H**), 8.03 (d, 1H, ^3J = 13.8 Hz, C**H**NH), 7.35 (d, 1H, ^4J = 1.8 Hz, 3-**H**), 7.24 (m, 1H, 5-**H**), 7.12 (d, 1H, ^3J = 8.2 Hz, 6-**H**), 3.98 (q, 2H, ^3J = 7.1 Hz, OC**H$_2$**CH$_3$), 2.58 (s, 1H, C**H**), 2.13 (s, 6H, 3 x C**H$_2$**), 0.96 (t, 3H, ^3J = 7.1 Hz, OCH$_2$C**H$_3$**) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 192.8 (1C, **C**O), 166.8 (1C, **C**OOEt), 157.8 (1C, **C**HNH), 141.4 (1C, **C**-1), 134.5 (1C, **C**-2), 130.9 (1C, **C**-4), 129.0 (1C, **C**-3), 128.2 (1C, **C**-6), 126.9 (1C, **C**-5), 101.4 (1C, CO**C**CH), 60.0 (1C, O**C**H$_2$CH$_3$), 53.0 (1C, **C**(CH$_2$)$_3$), 52.4 (3C, 3 x **C**H$_2$), 22.8 (1C, **C**H), 13.9 (1C, OCH$_2$**C**H$_3$) ppm.

1-(Bicyclo[1.1.1]pentan-1-yl)-7-chloro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**36**)
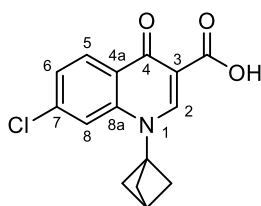
The acrylate **34** (358 mg, 1.01 mmol) and K$_2$CO$_3$ (210 mg, 1.52 mmol) were dissolved in abs. DMF and stirred at 110 °C for 2 h. The solvent was removed *in vacuo*. Water (50 mL) and EtOAc (50 mL) were added. After shaking, the phases were separated and the water phase was extracted with EtOAc. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed *in vacuo*. The residue was dissolved in EtOH (15 mL) and a 2 M HCl solution (30 mL) was added. The mixture was stirred at 100 °C for 16 h, the precipitate was filtered, and washed with EtOH. The solid was recrystallized from CHCl$_3$/EtOH to yield the desired product as grey crystals.

Chemical formula: $C_{15}H_{12}ClNO_3$

Molar mass: 289.7 g/mol

Appearance: grey crystals

Yield: 242 mg (835 mmol, 83 %)

Melting point: 251 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3599 (w), 3079 (w), 2984 (w), 2886 (w), 1714 (s), 1601 (s), 1449 (s), 1304 (m), 1219 (m), 1085 (m), 917 (s), 801 (s).

^{1}H NMR (400 MHz, CDCl$_3$): δ = 14.71 (s, 1H, COO**H**), 8.57 (s, 1H, 2-**H**), 8.40 (d, 1H, ^3J = 8.7 Hz, 5-**H**), 8.16 (d, 1H, ^4J = 1.8 Hz, 8-**H**), 7.75 (dd, 1H, ^3J = 8.7 Hz, ^4J = 1.8 Hz, 6-**H**), 2.87 (s, 1H, C**H**), 2.62 (s, 6H, 3 x C**H$_2$**) ppm.

The ^{13}C NMR spectrum could not be evaluated due to low intensities caused by the low solubility of the substance.

1-(Bicyclo[1.1.1]pentan-1-yl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**37**)
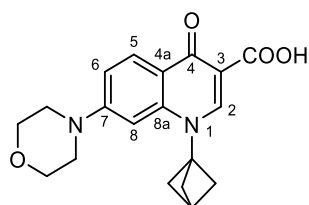
The acid **36** (227 mg, 784 µmol) was dissolved in morpholine (20 mL) and the solution was heated at 110 °C under microwave irradiation for 6 h. The reaction solution was acidified with a 2 M HCl solution until the product precipitated. The solid was filtered, washed with water, and dried over P$_2$O$_5$. The crude product was purified by recrystallization from EtOH.

Chemical formula: $C_{19}H_{20}N_2O_4$

Molar mass: 340.4 g/mol

Appearance: colourless solid

Yield: 190 mg (558 µmol, 71 %)

Melting point: 175 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3072 (w), 2969 (w), 2876 (w), 1704 (m), 1613 (m), 1511 (m), 1448 (m), 1245 (m), 965 (m), 792 (s).

¹H NMR (400 MHz, CDCl₃): δ = 15.47 (s, 1H, COO**H**), 8.45 (s, 1H, 2-**H**), 8.17 (d, 1H, ^3J = 9.3 Hz, 5-**H**), 7.39 (dd, 1H, ^3J = 9.3 Hz, ^4J = 1.9 Hz , 6-**H**), 7.16 (d, 1H, ^4J = 1.9 Hz, 8-**H**), 3.79 (m, 4H, morpholino-C**H₂**-O-C**H₂**), 3.42 (m, 4H, morpholino-C**H₂**-N-C**H₂**), 2.86 (s, 1H, C**H**), 2.60 (s, 6H, 3 x C**H₂**) ppm.

¹³C NMR (100 MHz, CDCl₃): δ = 176.7 (1C, **C**-4), 166.2 (1C, **C**OOH), 154.4 (1C, **C**-7), 145.9 (1C, **C**-2), 141.5 (1C, **C**-8a), 127.1 (1C, **C**-5), 116.2 (1C, **C**-4a), 114.6 (1C, **C**-6), 106.8 (1C, **C**-3), 99.1 (1C, **C**-8), 65.8 (2C, morpholino-**C**H₂-O-**C**H₂), 57.6 (1C, **C**(CH₂)₃), 52.6 (3C, 3 x **C**H₂), 46.7 (2C, morpholino-**C**H₂-N-**C**H₂), 22.6 (1C, **C**H) ppm.

N-Benzyl-1-(bicyclo[1.1.1]pentan-1-yl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**28**)
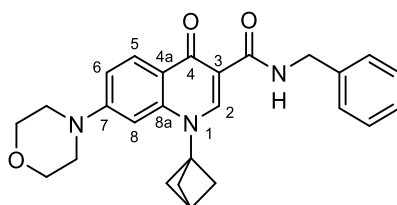
The carboxylic acid **37** (85.0 mg, 250 µmol), benzylamine (41.0 µL, 375 µmol), and PyBOP (182 mg, 350 µmol) were dissolved in abs. DMF (100 mL). DIPEA (131 µL, 749 µmol) was added and the solution was stirred at r.t. overnight. A saturated ammonium chloride solution (100 mL) was added and the mixture was extracted with CH₂Cl₂. The combined organic phases were dried over Na₂SO₄, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH₂Cl₂/MeOH (100/0.5)) and recrystallized from EtOH to yield the desired product.

Chemical formula: C₂₆H₂₇N₃O₃

Molar mass: 429.5 g/mol

Appearance: colourless crystals

Yield: 83.0 mg (193 µmol, 77 %)

Melting point: 300 °C

IR (ATR, $\tilde{\nu}$ [cm⁻¹]): 3197 (w), 3027 (w), 2955 (w), 2818 (w), 1666 (m), 1617 (w), 1599 (m), 1536 (m), 1240 (s), 1121 (m), 963 (m), 730 (m).

HRMS: [M+H]⁺ 430.21252 m/z, found 430.21275 m/z.

Purity (HPLC method I): 98.9 %

logP (HPLC method II): 4.57

¹H NMR (400 MHz, DMSO-d_6): δ = 10.39 (t, 1H, ^3J = 6.0 Hz, CON**H**), 8.51 (s, 1H, 2-**H**), 8.13 (d, 1H, ^3J = 9.2 Hz, 5-**H**), 7.33 (m, 4H, 4 x Bn-C**H**arom.), 7.26 (m, 2H, 6-**H** + Bn-C**H**arom.), 7.10 (d, 1H, ^4J = 2.0 Hz, 8-**H**), 4.54 (d, 2H, ^3J = 6.0 Hz, NHC**H₂**), 3.79 (m, 4H, morpholino-C**H₂**-O-C**H₂**), 3.36 (m, 4H, morpholino-C**H₂**-N-C**H₂**), 2.83 (s, 1H, C**H**), 2.56 (s, 6H, 3 x C**H₂**) ppm.

¹³C NMR (100 MHz, DMSO-d_6): δ = 174.9 (1C, **C**-4), 164.2 (1C, **C**ON), 153.8 (1C, **C**-7), 144.7 (1C, **C**-2), 140.8 (1C, Bn-**C**arom.), 139.5 (1C, **C**-8a), 128.4 (2C, Bn-**C**arom.), 127.4 (1C, **C**-5.), 127.3 (2C, Bn-**C**arom.), 126.8 (1C, Bn-**C**arom.), 118.4 (1C, **C**-4a), 113.7 (1C, **C**-6), 110.3 (1C, **C**-3), 99.2 (1C, **C**-8), 65.9 (2C, morpholino-**C**H₂-O-**C**H₂), 57.3 (1C, **C**(CH₂)₃), 52.5 (3C, 3 x **C**H₂), 47.0 (2C, morpholino-**C**H₂-N-**C**H₂), 42.1 (NH**C**H₂), 22.7 (1C, **C**H) ppm.

8.2.7 *N*-Bicyclo[1.1.1]pentanyl quinolone amides

N-(Bicyclo[1.1.1]pentan-1-yl)-1-butyl-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**54**)
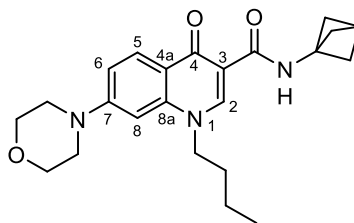
The carboxylic acid **26d** (138 mg, 418 µmol), 1-bicyclo[1.1.1]pentylamine hydrochloride (50.0 mg, 418 µmol), and PyBOP (261 mg, 502 µmol) were dissolved in abs. DMF (100 mL). DIPEA (219 µL, 1.25 mmol) was added and the solution was stirred at r.t. overnight. A saturated ammonium chloride solution (100 mL) was added and the mixture was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH (100/1)) and recrystallized from EtOH to yield the desired product.

Chemical formula: $C_{23}H_{29}N_3O_3$

Molar mass: 395.5 g/mol

Appearance: colourless crystals

Yield: 91.5 mg (231 µmol, 55 %)

Melting point: 225 °C

IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3201 (w), 3059 (w), 2958 (w), 2869 (w), 1651 (m), 1619 (m), 1524 (m), 1468 (m), 1247 (m), 1126 (m), 970 (m), 715 (s).

HRMS: [M+H]$^+$ 396.22817 m/z, found 396.22809 m/z.

Purity (HPLC method I): 99.6 %

logP (HPLC method II): 3.74

^{1}H NMR (400 MHz, CDCl$_3$): δ = 10.48 (s, 1H, CON**H**), 8.63 (s, 1H, 2-**H**), 8.12 (d, 1H, ^3J = 9.2 Hz, 5-**H**), 7.22 (d, 1H, ^3J = 9.2 Hz, ^4J = 1.9 Hz, 6-**H**), 6.89 (d, 1H, ^3J = 1.9 Hz, 8-**H**), 4.40 (t, 2H, ^3J = 7.3 Hz, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 3.77 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.38 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 2.46 (s, 1H, C**H**), 2.08 (s, 6H, 3 x C**H$_2$**), 1.74 (quint, 2H, ^3J = 7.3 Hz, NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.30 (sext, 2H, ^3J = 7.3 Hz, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 0.90 (t, 3H, ^3J = 7.3 Hz, NCH$_2$CH$_2$CH$_2$C**H$_3$**) ppm.

13C NMR (100 MHz, DMSO-d_6): δ = 174.6 (1C, **C**-4), 164.3 (1C, **C**ONH), 154.0 (1C, **C**-7), 147.6 (1C, **C**-2), 140.6 (1C, **C**-8a), 127.3 (1C, **C**-5), 119.0 (1C, **C**-4a), 113.5 (1C, **C**-6), 109.9 (1C, **C**-3), 98.5 (1C, **C**-8), 65.8 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 52.5 (3C, 3 x **C**H$_2$), 52.4 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 48.3 (1C, **C**(CH$_2$)$_3$), 47.6 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 30.2 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 24.7 (1C, **C**H), 19.2 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.5 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

8.2.8 Synthesis of *N*-1-phenylcycloalkyl quinolone amides

1-Phenylcyclopropylamine (**41c**)
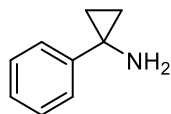
Under argon atmosphere, magnesium shavings (2.68 g, 110 mmol) were covered with abs. THF (20 mL). A solution of ethyl bromide (1.63 mL, 22.0 mmol) in abs. THF (50 mL) was added dropwise, at a rate that maintained a steady reflux. After complete addition, the suspension was stirred under reflux for 1 h. The reaction solution was transferred through a filter canula to a solution of benzonitrile (990 µL, 9.70 mmol) and Ti(O*i*-Pr)$_4$ (3.24 mL, 10.7 mmol) in abs. THF (100 mL) at -78 °C. The mixture was stirred at -78 °C for 15 min. After the solution was warmed to r.t. over the duration of 1 h, boron trifluoride etherate (2.46 mL, 19.4 mmol) was added and the reaction mixture was stirred for 1 h. A 1 M HCl solution (10 mL) and Et$_2$O (30 mL) were added to the stirring mixture. The water phase was basified by adding a 2 M NaOH solution, the phases were separated, and the water phase was extracted with Et$_2$O. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (deactivated silica gel, CH$_2$Cl$_2$/MeOH (100/3)) to yield the product as a yellow oil.

Chemical formula: C$_9$H$_{11}$N

Molar mass: 133.2 g/mol

Appearance: yellow oil

Yield: 483 mg (3.63 µmol, 37 %, Lit: 65 %)[99]

¹H NMR (400 MHz, DMSO-*d₆*): δ = 7.30-7.35 (m, 4H, C**H**$_{arom.}$), 7.20 (m, 1H, C**H**$_{arom.}$), 2.08 (br, 2H, N**H**$_2$), 1.08 (m, 2H, C**H**$_2$CH$_2$), 0.99 (m, 2H, C**H**$_2$CH$_2$) ppm.

1-Phenylcyclopentylamine (**41a**)
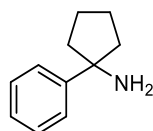
Under argon atmosphere, magnesium shavings (2.96 g, 122 mmol) were covered with abs. THF (20 mL). A solution of phenyl bromide (2.55 mL, 24.3 mmol) in abs. THF (50 mL) was added dropwise, at a rate which maintained a steady reflux. After complete addition, the suspension was stirred under reflux for 1 h. The reaction solution was transferred through a

filter canula to a solution of cyclopentanone (1.96 mL, 22.1 mmol) in abs. THF (50 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and at r.t. for 1 h. The reaction was quenched by addition of a saturated ammonium chloride solution (5 mL) and water (50 mL) to the stirring mixture. The phases were separated and the water phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (50 mL) and sodium azide (3.16 g, 48.7 mmol) was added. The suspension was cooled to -5 °C and a solution of TFA (14.2 mL, 186 mmol) in CH_2Cl_2 (15 mL) was added dropwise over 30 min. The mixture was stirred at 0 °C for 1 h and at r.t. for 1 h. Water (50 mL) was added to the stirring mixture. The phases were separated, and the water phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, n-hexane) to yield 1-(1-azidocyclopentyl)benzene with slight impurities. The crude intermediate (350 mg, 1.87 mmol) was dissolved in abs. THF (20 mL) and cooled to 0 °C. $LiAlH_4$ (70.9 mg, 1.87 mmol) was added and the suspension was stirred at r.t. for 1 h. A 2 M HCl solution (10 mL) was added to the stirring mixture, the phases were separated, and the water phase was washed with CH_2Cl_2. Afterwards, the aqueous solution was basified using a 6 M NaOH solution and extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo* to yield the desired product as a yellow oil.

Chemical formula: $C_{11}H_{15}N$

Molar mass: 161.3 g/mol

Appearance: yellow oil

Yield: 198 mg (1.23 µmol, 6 %, Lit: 16 %)[97]

¹H NMR (400 MHz, DMSO-d_6): δ = 7.47 (m, 2H, **CH**arom.), 7.28 (m, 2H, **CH**arom.), 7.15 (m, 1H, **CH**arom.), 1.63-1.91 (br, 10H, 4x**CH**$_2$ + N**H**$_2$) ppm.

¹³C NMR (100 MHz, DMSO-d_6): δ = 150.5 (1C, **C**arom.), 127.7 (2C, **C**arom.), 125.5 (1C, **C**arom.), 125.4 (2C, **C**arom.), 63.4 (1C, **C**CH$_2$), 41.5 (2C, C**C**H$_2$CH$_2$), 23.4 (2C, CCH$_2$**C**H$_2$) ppm.

1-Phenylcyclohexylamine (**41b**)
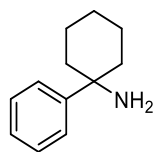
Under argon atmosphere, magnesium shavings (2.45 g, 101 mmol) were covered with abs. THF (20 mL). A solution of phenyl bromide (3.52 mL, 33.6 mmol) in abs. THF (50 mL) was added dropwise, at a rate which maintained a steady reflux. After complete addition, the suspension was stirred under reflux for 1 h. The reaction solution was transferred through a filter canula to a solution of cyclohexanone (3.16 mL, 30.6 mmol) in abs. THF (50 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and at r.t. for 1 h. The reaction was quenched by addition of a saturated ammonium chloride solution (5 mL) and water (50 mL) to the stirring mixture. The phases were separated and the water phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (50 mL) and sodium azide (3.97 g, 61.1 mmol) was added. The suspension was cooled to -5 °C and a solution of TFA (19.7 mL, 257 mmol) in CH_2Cl_2 (20 mL) was added dropwise over 30 min. The mixture was stirred at 0 °C for 1 h and at r.t. for 1 h. Water (50 mL) was added to the stirring mixture, the phases were separated, and the water phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, n-hexane) to yield 1-(1-azidocyclohexyl)benzene with slight impurities. The crude intermediate was dissolved in abs. THF (20 mL) and cooled to 0 °C. $LiAlH_4$ (1.16 mg, 30.6 mmol) was added and the reaction was stirred at r.t. for 1 h. A 2 M HCl solution (20 mL) was added to the stirring mixture, the phases were separated, and the water phase was washed with CH_2Cl_2. Afterwards, the aqueous solution was basified using a 6 M NaOH solution and extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo* to yield the desired product as a yellow oil.

Chemical formula: $C_{12}H_{17}N$

Molar mass: 175.3 g/mol

Appearance: yellow oil

Yield: 3.25 mg (18.5 mmol, 61 %, Lit: 60 %)[185]

1H NMR (400 MHz, DMSO-d_6): δ = 7.53 (m, 2H, **CH**arom.), 7.29 (m, 2H, **CH**arom.), 7.16 (m, 1H, **CH**arom.), 1.19-1.82 (m, 12H, 5 x **CH₂** + **NH₂**) ppm.

13C NMR (100 MHz, DMSO-d_6): δ = 151.5 (1C, **C**arom.), 127.8 (2C, **C**arom.), 125.6 (1C, **C**arom.), 125.1 (2C, **C**arom.), 53.4 (1C, **C**q), 38.9 (2C, **C**H$_2$), 25.5 (1C, **C**H$_2$), 21.9 (2C, **C**H$_2$) ppm.

General synthetic procedure (**E**) for *N*-Benzyl-4-oxo-1,4-dihydroquinoline-3-carboxamides:

The 1-alkyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acids **26d-f** (1 eq) and *N*-methylmorpholine (4-5 eq) were dissolved in abs. DMF and cooled to 0 °C. *i*-Butyl chloroformate (2-4 eq) was added and the solution was stirred at 0 °C for 1 h. The benzylamine derivative (2-4 eq) was added and the reaction solution was stirred at r.t. for 1 h. The solvent was removed *in vacuo*, a saturated ammonium chloride solution was added to the residue, and the solution was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography to yield the desired product.

1-Butyl-7-morpholino-4-oxo-*N*-(1-phenylcyclopropyl)-1,4-dihydroquinoline-3-carboxamide (**50a**)
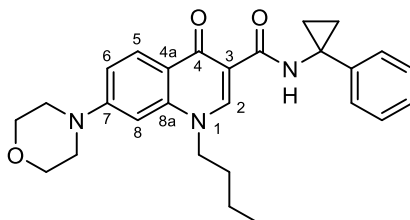
According to the general procedure (**E**), compound **26d** (91.7 mg, 278 μmol) was dissolved in abs. DMF (10 mL) and treated with NMM (153 μL, 1.39 mmol), IBCF (108 μL, 833 μmol), and 1-phenylcyclopropylamine (**41c**) (111 mg, 833 μmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (deactivated silica gel, $CHCl_3$/MeOH (100/0.5)) and afterwards recrystallized from $CHCl_3$/MeOH.

Chemical formula: $C_{27}H_{31}N_3O_3$

Molar mass: 445.6 g/mol

Appearance: colourless needles

Yield: 90.0 mg (202 μmol, 73 %)

Melting point: 249 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3153 (w), 3048 (w), 2969 (w), 2858 (w), 1660 (m), 1617 (w), 1602 (m), 1526 (m), 1446 (m), 1242 (s), 1126 (m), 964 (m), 753 (s).

HRMS: [M+H]$^+$ 446.24382 m/z, found 446.24475 m/z.

Purity (HPLC method I): 99.5 %

logP (HPLC method II): 3.76

¹H NMR (400 MHz, CDCl₃): δ = 10.73 (s, 1H, CON**H**), 8.65 (s, 1H, 2-**H**), 8.37 (d, 1H, ^3J = 9.2 Hz, 5-**H**), 7.34 (m, 2H, Bn-C**H**$_{arom.}$), 7.26 (m, 2H, Bn-C**H**$_{arom.}$), 7.14 (m, 1H, Bn-C**H**$_{arom.}$), 7.08 (dd, 1H, ^3J = 9.2 Hz, ^4J = 2.2 Hz, 6-**H**), 6.63 (d, 1H, ^4J = 2.2 Hz, 8-**H**), 4.14 (t, 2H, ^3J = 7.4 Hz, NC**H**₂CH₂CH₂CH₃), 3.90 (m, 4H, morpholino-C**H**₂-O-C**H**₂), 3.35 (m, 4H, morpholino-C**H**₂-N-C**H**₂), 1.87 (m, 2H, NCH₂C**H**₂CH₂CH₃), 1.41 (m, 4H, NCH₂CH₂C**H**₂CH₃ + C**H**₂CH₂), 1.32 (m, 4H, C**H**₂C**H**₂), 0.99 (t, 3H, ^3J = 7.4 Hz, NCH₂CH₂CH₂C**H**₃) ppm.

¹³C NMR (100 MHz, CDCl₃): δ = 176.2 (1C, **C**-4), 166.1 (1C, **C**ON), 154.4 (1C, **C**-7), 147.7 (1C, **C**-2), 143.3 (1C, Bn-**C**$_{arom.}$), 141.0 (1C, **C**-8a), 128.7 (1C, **C**-5), 128.3 (2C, Bn-**C**$_{arom.}$), 126.1 (1C, Bn-**C**$_{arom.}$), 125.8 (2C, Bn-**C**$_{arom.}$), 120.7 (1C, **C**-4a), 113.9 (1C, **C**-6), 111.7 (1C, **C**-3), 98.2 (1C, **C**-8), 66.7 (2C, morpholino-**C**H₂-O-**C**H₂), 53.9 (1C, N**C**H₂CH₂CH₂CH₃), 48.0 (2C, morpholino-**C**H₂-N-**C**H₂), 34.8 (1C, NH**C**), 30.8 (1C, NCH₂**C**H₂CH₂CH₃), 20.1 (1C, NCH₂CH₂**C**H₂CH₃), 17.8 (2C, **C**H₂-**C**H₂), 13.8 (1C, NCH₂CH₂CH₂**C**H₃) ppm.

1-Butyl-6-fluoro-7-morpholino-4-oxo-*N*-(1-phenylcyclopropyl)-1,4-dihydroquinoline-3-carboxamide (**50b**)
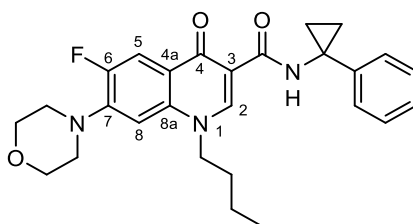
According to the general procedure (**E**), compound **26e** (200 mg, 574 µmol) was dissolved in abs. DMF (30 mL) and treated with NMM (252 µL, 2.30 mmol), IBCF (149 µL, 1.15 mmol), and 1-phenylcyclopropylamine (**41c**) (153 mg, 1.15 mmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (silica gel, CHCl₃/MeOH (100/1)) and afterwards recrystallized from CHCl₃/MeOH.

Chemical formula: C₂₇H₃₀FN₃O₃

Molar mass: 463.6 g/mol

Appearance: colourless needles

Yield: 224 mg (483 µmol, 84 %)

Melting point: 230 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3184(w), 3037 (w), 2950 (w), 2855 (w), 1660 (s), 1623 (w), 1604 (m), 1529 (s), 1485 (s), 1254 (s), 1117 (m), 896 (m), 751 (s).

HRMS: [M+H]$^+$ 464.23440 m/z, found 464.23502 m/z.

Purity (HPLC method I): 99.7 %

logP (HPLC method II): 4.46

^{1}H NMR (400 MHz, CDCl$_3$): δ = 10.49 (s, 1H, CON**H**), 8.54 (s, 1H, 2-**H**), 7.96 (d, 1H, $^3J_{H,F}$ = 13.3 Hz, 5-**H**), 7.21 (m, 2H, Bn-C**H**$_{arom.}$), 7.14 (m, 2H, Bn-C**H**$_{arom.}$), 7.03 (m, 1H, Bn-C**H**$_{arom.}$), 6.65 (d, 1H, $^4J_{H,F}$ = 6.9 Hz, 8-**H**), 4.04 (t, 2H, ^3J = 7.4 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.80 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.14 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.77 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.29 (m, 4H, NCH$_2$CH$_2$C**H**$_2$CH$_3$ + NHCC**H**$_2$), 1.21 (m, 2H, NHCC**H**$_2$), 0.87 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 175.5 (d, 1C, $^4J_{C,F}$ = 2.4 Hz, **C**-4), 165.7 (1C, **C**ON), 153.3 (d, 1C, $^1J_{C,F}$ = 249.4 Hz, **C**-6), 147.4 (1C, **C**-2), 145.0 (d, 1C, $^2J_{C,F}$ = 10.7 Hz, **C**-7), 143.1 (1C, Bn-**C**$_{arom.}$), 136.8 (1C, **C**-8a), 128.3 (2C, Bn-**C**$_{arom.}$), 126.1 (1C, Bn-**C**$_{arom.}$), 125.8 (2C, Bn-**C**$_{arom.}$), 122.9 (d, 1C, $^3J_{C,F}$ = 7.2 Hz, **C**-4a), 113.2 (d, 1C, $^2J_{C,F}$ = 23.0 Hz, **C**-5), 111.6 (1C, **C**-3), 103.8 (d, 1C, $^3J_{C,F}$ = 3.1 Hz, **C**-8), 66.8 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 54.2 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 50.4 (d, 2C, $^4J_{C,F}$ = 4.6 Hz, morpholino-**C**H$_2$-N-**C**H$_2$), 34.9 (2C, NH**C**), 30.9 (1C, NCH$_2$**C**H$_2$-CH$_2$CH$_3$), 20.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 17.8 (2C, 2 x NHCC**H**$_2$), 13.7 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

1-Butyl-5-fluoro-7-morpholino-4-oxo-*N*-(1-phenylcyclopropyl)-1,4-dihydroquinoline-3-carboxamide (**50c**)
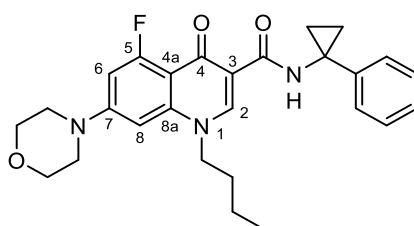
According to the general procedure (**E**), compound **26f** (100 mg, 287 µmol) was dissolved in abs. DMF (20 mL) and treated with NMM (126 µL, 1.15 mmol), IBCF (149 µL, 1.15 mmol), and 1-phenylcyclopropylamine (**41c**) (153 mg, 1.15 mmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (silica gel, CHCl$_3$/MeOH (100/1)) and afterwards recrystallized from CHCl$_3$/MeOH. One last remaining impurity was removed by

washing with saturated ammonium chloride solution and repeated recrystallization from $CHCl_3$/MeOH.

Chemical formula: $C_{27}H_{30}FN_3O_3$

Molar mass: 463.6 g/mol

Appearance: colourless needles

Yield: 58.0 mg (125 µmol, 44 %)

Melting point: 230 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3160 (w), 3044 (w), 2959 (w), 2858 (w), 1663 (m), 1624 (m), 1600 (m), 1444 (m), 1262 (s), 1006 (s), 796 (s).

HRMS: [M+H]$^+$ 464.23440 m/z, found 464.23428 m/z.

Purity (HPLC method I): 99.5 %

logP (HPLC method II): 3.54

^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.67 (s, 1H, CON**H**), 8.59 (s, 1H, 2-**H**), 7.34 (m, 2H, Bn-C**H**$_{arom.}$), 7.26 (m, 2H, Bn-C**H**$_{arom.}$), 7.14 (m, 1H, Bn-C**H**$_{arom.}$), 6.66 (d, 1H, $^3J_{H,F}$ = 15.0 Hz, ^4J = 2.1 Hz, 6-**H**), 6.39 (d, 1H, ^4J = 2.1 Hz, 8-**H**), 4.09 (t, 2H, ^3J = 7.4 Hz, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 3.89 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.33 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.85 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.43 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 1.31 (m, 4H, 2 x C**H$_2$**), 0.99 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H$_3$**) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.9 (1C, **C**-4), 165.7 (1C, **C**ON), 164.4 (d, 1C, $^1J_{C,F}$ = 196.7 Hz, **C**-5), 154.2 (1C, **C**-7), 147.8 (1C, **C**-2), 143.1 (1C, Bn-**C**$_{arom.}$), 142.7 (1C, **C**-8a), 128.3 (2C, Bn-**C**$_{arom.}$), 126.1 (1C, Bn-**C**$_{arom.}$), 126.0 (2C, Bn-**C**$_{arom.}$), 110.8 (1C, **C**-4a), 99.9 (1C, **C**-6), 94.0 (1C, **C**-8), 66.5 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 54.5 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 47.5 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 34.9 (1C, NH**C**), 30.5 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 20.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 17.6 (2C, C-**C**H$_2$-**C**H$_2$), 13.8 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

The assignment of the quaternary aromatic carbon atoms was done using the 2D spectra due to low concentration due to low solubility. The signal of C-3 was not found due to no interactions in the HMBC spectrum.

1-Butyl-7-morpholino-4-oxo-*N*-(1-phenylcyclopentyl)-1,4-dihydroquinoline-3-carboxamide (**50d**)
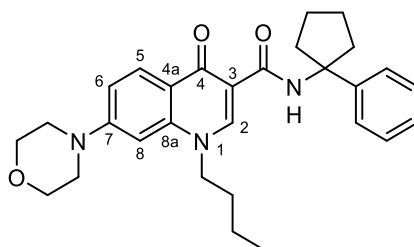
According to the general procedure (**E**), compound **26d** (81.1 mg, 245 µmol) was dissolved in abs. DMF (20 mL) and treated with NMM (134 µL, 982 mmol), IBCF (63.9 µL, 491 µmol), and 1-phenylcyclopentylamine (**41a**) (79.2 mg, 245 µmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (silica gel, CHCl$_3$/MeOH (100/1)) and afterwards recrystallized from CHCl$_3$/MeOH.

Chemical formula: C$_{29}$H$_{35}$N$_3$O$_3$

Molar mass: 473.6 g/mol

Appearance: colourless needles

Yield: 111 mg (234 µmol, 95 %)

Melting point: 209 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3181 (w), 3051 (w), 2953 (w), 2860 (w), 1663 (m), 1601 (m), 1542 (s), 1451 (s), 1237 (s), 1117 (s), 963 (m), 754 (s), 699 (s).

HRMS: [M+H]$^+$ 474.27512 m/z, found 474.27642 m/z.

Purity (HPLC method I): 99.2 %

logP (HPLC method II): 4.57

^{1}H NMR (400 MHz, CDCl$_3$): δ = 10.55 (s, 1H, CON**H**), 8.51 (s, 1H, 2-**H**), 8.34 (d, 1H, ^3J = 9.2 Hz, 5-**H**), 7.45 (m, 2H, Bn-C**H**$_{arom.}$), 7.23 (m, 2H, Bn-C**H**$_{arom.}$), 7.11 (m, 1H, Bn-C**H**$_{arom.}$), 7.03 (dd, 1H, ^3J = 9.2 Hz, ^4J = 2.2 Hz, 6-**H**), 6.57 (d, 1H, ^4J = 2.2 Hz, 8-**H**), 4.04 (t, 2H, ^3J = 7.4 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.85 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.29 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 2.50 (m, 2H, NHCC**H**$_2$), 2.09 (m, 2H, NHCC**H**$_2$), 1.89 (m, 2H, NHCCH$_2$C**H**$_2$), 1.79 (m, 4H, NHCCH$_2$C**H**$_2$ + NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.35 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.91 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 176.3 (1C, **C**-4), 164.4 (1C, **C**ON), 154.3 (1C, **C**-7), 147.5 (1C, **C**-2), 146.0 (1C, Bn-**C**$_{arom.}$), 141.0 (1C, **C**-8a), 128.7 (1C, **C**-5), 128.1 (2C, Bn-**C**$_{arom.}$), 126.3 (1C, Bn-**C**$_{arom.}$), 126.1 (2C, Bn-**C**$_{arom.}$), 120.7 (1C, **C**-4a), 113.7 (1C, **C**-6), 112.3 (1C, **C**-3), 98.1 (1C, **C**-8), 66.7 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 66.3 (1C, NH**C**), 53.8 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 48.0 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 39.5 (2C, NHC**C**H$_2$), 30.8 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 23.5 (2C, NHCCH$_2$**C**H$_2$), 20.2 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.8 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

1-Butyl-6-fluoro-7-morpholino-4-oxo-*N*-(1-phenylcyclopentyl)-1,4-dihydroquinoline-3-carboxamide (**50e**)
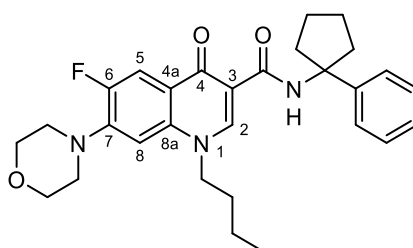
According to the general procedure (**E**), compound **26e** (82.3 mg, 236 µmol) was dissolved in abs. DMF (20 mL) and treated with NMM (104 µL, 945 mmol), IBCF (61.5 µL, 472 µmol), and 1-phenylcyclopentylamine (**41a**) (76.2 mg, 472 µmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (silica gel, CHCl$_3$/MeOH (100/1)) and afterwards recrystallized from CHCl$_3$/MeOH.

Chemical formula: C$_{29}$H$_{34}$FN$_3$O$_3$

Molar mass: 491.6 g/mol

Appearance: colourless needles

Yield: 79.6 mg (162 µmol, 69 %)

Melting point: 205 °C

IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3188 (w), 3059 (w), 2953 (w), 2862 (w), 1662 (m), 1623 (w), 1599 (w), 1549 (m), 1454 (m), 1257 (s), 1110 (m), 754 (s), 697 (s).

HRMS: [M+H]$^+$ 492.26570 m/z, found 492.26678 m/z.

Purity (HPLC method I): 99.6 %

logP (HPLC method II): 5.24

^{1}H NMR (400 MHz, CDCl$_3$): δ = 10.43 (s, 1H, CON**H**), 8.52 (s, 1H, 2-**H**), 8.05 (d, 1H, $^3J_{H,F}$ = 13.4 Hz, 5-**H**), 7.44 (m, 2H, Bn-C**H**$_{arom.}$), 7.23 (m, 2H, Bn-C**H**$_{arom.}$), 7.11 (m, 1H, Bn-C**H**$_{arom.}$),

6.70 (d, 1H, $^4J_{H,F}$ = 6.9 Hz, 8-**H**), 4.06 (t, 2H, ^3J = 7.4 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.86 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.19 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 2.48 (m, 2H, NHCC**H**$_2$), 2.08 (m, 2H, NHCC**H**$_2$), 1.87 (m, 2H, NHCCH$_2$C**H**$_2$), 1.78 (m, 4H, NHCCH$_2$C**H**$_2$ + NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.35 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.91 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 175.6 (d, 1C, $^4J_{C,F}$ = 2.4 Hz, **C**-4), 164.1 (1C, **C**ON), 153.3 (d, 1C, $^1J_{C,F}$ = 249.2 Hz, **C**-6), 147.2 (1C, **C**-2), 145.9 (1C, Bn-**C**$_{arom.}$), 145.0 (d, 1C, $^2J_{C,F}$ = 10.8 Hz, **C**-7), 136.8 (1C, **C**-8a), 128.1 (2C, Bn-**C**$_{arom.}$), 126.3 (1C, Bn-**C**$_{arom.}$), 126.1 (2C, Bn-**C**$_{arom.}$), 122.9 (d, 1C, $^3J_{C,F}$ = 7.1 Hz, **C**-4a), 113.2 (d, 1C, $^2J_{C,F}$ = 23.1 Hz, **C**-5), 112.2 (1C, **C**-3), 103.7 (1C, **C**-8), 66.8 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 66.4 (1C, NH**C**), 54.2 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 50.4 (d, 2C, $^4J_{C,F}$ = 4.5 Hz, morpholino-**C**H$_2$-N-**C**H$_2$), 39.4 (2C, NHC**C**H$_2$), 30.9 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 23.4 (2C, NHCCH$_2$**C**H$_2$), 20.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.7 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

1-Butyl-7-morpholino-4-oxo-*N*-(1-phenylcyclohexyl)-1,4-dihydroquinoline-3-carboxamide (**50f**)
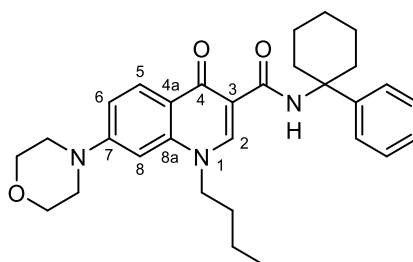
According to the general procedure (**E**), compound **26d** (200 mg, 605 µmol) was dissolved in abs. DMF (50 mL) and treated with NMM (266 µL, 2.42 mmol), IBCF (236 µL, 1.82 mmol), and 1-phenylcyclohexylamine (**41b**) (318 mg, 1.82 mmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (silica gel, CHCl$_3$/MeOH (100/1)) and afterwards recrystallized from CHCl$_3$/MeOH.

Chemical formula: C$_{30}$H$_{37}$N$_3$O$_3$

Molar mass: 487.6 g/mol

Appearance: colourless needles

Yield: 187 mg (383 µmol, 63 %)

Melting point: 213 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3188 (w), 3055 (w), 2952 (w), 2858 (w), 1664 (m), 1601 (m), 1543 (s), 1459 (s), 1236 (s), 1118 (m), 963 (m), 698 (s).

HRMS: [M+H]$^+$ 488.29077 m/z, found 488.29249 m/z.

Purity (HPLC method I): 99.5 %

logP (HPLC method II): 4.94

^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.65 (s, 1H, CON**H**), 8.56 (s, 1H, 2-**H**), 8.20 (d, 1H, ^3J = 9.1 Hz, 5-**H**), 7.38 (m, 2H, Bn-C**H**$_{arom.}$), 7.26 (m, 3H, 6-**H** + 2 x Bn-C**H**$_{arom.}$), 7.16 (m, 1H, Bn-C**H**$_{arom.}$), 6.89 (d, 1H, ^4J = 1.5 Hz, 8-**H**), 4.36 (t, 2H, ^3J = 7.1 Hz, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 3.78 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.40 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 2.37 (d, 2H, ^3J = 12.5 Hz, 2 x C**H**), 1.56-1.80 (m, 9H, 7 x C**H** + NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.30 (m, 3H, C**H** + NCH$_2$CH$_2$C**H$_2$**CH$_3$), 0.90 (t, 3H, ^3J = 7.3 Hz, NCH$_2$CH$_2$CH$_2$C**H$_3$**) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 175.1 (1C, **C**-4), 163.1 (1C, **C**ON), 153.9 (1C, **C**-7), 148.1 (1C, Bn-**C**$_{arom.}$), 147.5 (1C, **C**-2), 140.7 (1C, **C**-8a), 127.9 (2C, Bn-**C**$_{arom.}$), 127.4 (1C, **C**-5), 125.8 (1C, Bn-**C**$_{arom.}$), 125.1 (2C, Bn-**C**$_{arom.}$), 119.0 (1C, **C**-4a), 113.4 (1C, **C**-6), 110.8 (1C, **C**-3), 98.3 (1C, **C**-8), 65.9 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 57.4 (1C, NH**C**(CH$_2$)$_2$), 52.4 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 47.0 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 36.0 (2C, NHC(**C**H$_2$)$_2$), 30.3 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 24.9 (1C, **C**H$_2$), 21.9 (2C, 2 x **C**H$_2$), 19.2 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.5 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

1-Butyl-6-fluoro-7-morpholino-4-oxo-*N*-(1-phenylcyclohexyl)-1,4-dihydroquinoline-3-carboxamide (**50g**)
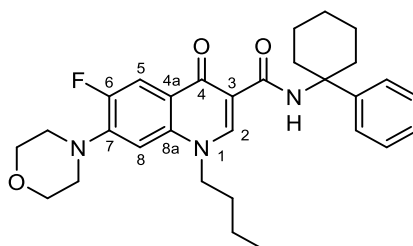
According to the general procedure (**E**), compound **26e** (200 mg, 574 µmol) was dissolved in abs. DMF (50 mL) and treated with NMM (252 µL, 2.30 mmol), IBCF (224 µL, 1.72 mmol), and 1-phenylcyclohexylamine (**41b**) (302 mg, 1.72 mmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (silica gel, CHCl$_3$/MeOH (100/1)) and afterwards recrystallized from CHCl$_3$/MeOH.

Chemical formula: C$_{30}$H$_{36}$FN$_3$O$_3$

Molar mass: 505.6 g/mol

Appearance: colourless needles

Yield: 239 mg (472 µmol, 82 %)

Melting point: 207 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3195 (w), 3059 (w), 2934 (w), 2850 (w), 1661 (s), 1627 (m), 1553 (m), 1487 (s), 1262 (s), 1122 (m), 948 (m), 760 (m), 698 (s).

HRMS: [M+H]$^+$ 506.28134 m/z, found 506.28240 m/z.

Purity (HPLC method I): 99.8 %

logP (HPLC method II): 5.64

^{1}H NMR (400 MHz, CDCl$_3$): δ = 10.52 (s, 1H, CON**H**), 8.54 (s, 1H, 2-**H**), 8.11 (d, 1H, $^3J_{H,F}$ = 13.4 Hz, 5-**H**), 7.42 (m, 2H, Bn-C**H**$_{arom.}$), 7.23 (m, 2H, Bn-C**H**$_{arom.}$), 7.12 (m, 1H, Bn-C**H**$_{arom.}$), 6.72 (d, 1H, $^4J_{H,F}$ = 6.9 Hz, 8-**H**), 4.07 (t, 2H, ^3J = 7.4 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.87 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.21 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 2.50 (m, 2H, 2 x C**H**), 1.76 (m, 10H, 8 x C**H** + NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.36 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.92 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 175.8 (d, 1C, $^4J_{C,F}$ = 2.5 Hz, **C**-4), 163.8 (1C, **C**ON), 153.3 (d, 1C, $^1J_{C,F}$ = 249.2 Hz, **C**-6), 148.1 (1C, **C**-2), 147.3 (1C, Bn-**C**$_{arom.}$), 145.0 (d, 1C, $^2J_{C,F}$ = 10.7 Hz, **C**-7), 136.9 (1C, **C**-8a), 128.3 (2C, Bn-**C**$_{arom.}$), 126.3 (1C, Bn-**C**$_{arom.}$), 125.3 (2C, Bn-**C**$_{arom.}$), 122.9 (d, 1C, $^3J_{C,F}$ = 7.1 Hz, **C**-4a), 113.3 (d, 1C, $^2J_{C,F}$ = 23.0 Hz, **C**-5), 112.3 (1C, **C**-3), 103.7 (d, 1C, $^3J_{C,F}$ = 3.1 Hz, **C**-8), 66.8 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 58.2 (2C, NH**C**), 54.2 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 50.4 (d, 2C, $^4J_{C,F}$ = 4.5 Hz, morpholino-**C**H$_2$-N-**C**H$_2$), 36.7 (2C, 2 x NHC**C**H$_2$), 30.9 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 25.8 (1C, **C**H$_2$), 22.5 (2C, 2 x **C**H$_2$), 20.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.7 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

1-Butyl-5-fluoro-7-morpholino-4-oxo-*N*-(1-phenylcyclohexyl)-1,4-dihydroquinoline-3-carboxamide (**50h**)
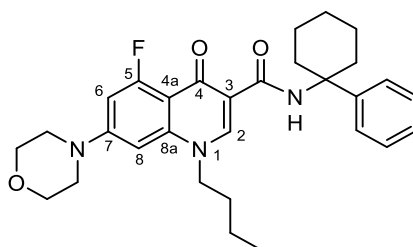
According to the general procedure (**E**), compound **26f** (160 mg, 459 µmol) was dissolved in abs. DMF (50 mL) and treated with NMM (202 µL, 1.84 mmol), IBCF (179 µL, 1.38 mmol), and 1-phenylcyclohexylamine (**41b**) (242 mg, 1. mmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (silica gel, CHCl$_3$/MeOH (100/1)) and afterwards recrystallized from CHCl$_3$/MeOH.

Chemical formula: C$_{30}$H$_{36}$FN$_3$O$_3$

Molar mass: 505.6 g/mol

Appearance: colourless crystals

Yield: 142 mg (281 µmol, 61 %)

Melting point: 219 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3198 (w), 3052 (w), 2945 (w), 2856 (w), 1662 (m), 1624 (m), 1529 (m), 1446 (m), 1267 (m), 1124 (m), 1006 (m), 823 (m), 698 (s).

HRMS: [M+H]$^+$ 506.28135 m/z, found 506.28169 m/z.

Purity (HPLC method I): 99.0 %

logP (HPLC method II): 4.97

¹H NMR (400 MHz, CDCl$_3$): δ = 10.57 (s, 1H, CON**H**), 8.52 (s, 1H, 2-**H**), 7.47 (m, 2H, Bn-C**H**$_{arom.}$), 7.29 (m, 3H, 6-**H** + 2 x Bn-C**H**$_{arom.}$), 7.16 (m, 1H, Bn-C**H**$_{arom.}$), 6.68 (dd, 1H, $^3J_{H,F}$ = 15.1 Hz, ^4J = 2.1 Hz, 6-**H**), 6.39 (d, 1H, ^4J = 2.1 Hz, 8-**H**), 4.04 (t, 2H, ^3J = 7.4 Hz, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 3.89 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.33 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 2.55 (d, 2H, ^3J = 7.4 Hz, 2 x C**H**), 1.66-1.87 (m, 9H, 7 x C**H** + NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.41 (sext, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 1.30 (m, 1H, C**H**), 0.97 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H$_3$**) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 176.2 (1C, **C**-4), 164.1 (d, 1C, $^1J_{C,F}$ = 260.3 Hz, **C**-5), 163.7 (1C, **C**ON), 154.0 (d, 1C, $^3J_{C,F}$ = 13.1 Hz, **C**-7), 148.2 (1C, Bn-**C**$_{arom.}$), 147.7 (1C, **C**-2), 142.7 (d, 1C, $^3J_{C,F}$ = 5.7 Hz, **C**-8a), 128.2 (2C, Bn-**C**$_{arom.}$), 126.3 (1C, Bn-**C**$_{arom.}$), 125.3 (2C, Bn-**C**$_{arom.}$), 113.3 (1C, **C**-3), 110.7 (d, 1C, $^2J_{C,F}$ = 8.4 Hz, **C**-4a), 99.9 (d, 1C, $^2J_{C,F}$ = 26.6 Hz, **C**-6), 94.0 (d, 1C, ^4J = 2.8 Hz, **C**-8), 66.5 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 58.2 (1C, NH**C**(CH$_2$)$_2$), 54.5 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 47.5 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 36.7 (2C, NHC(**C**H$_2$)$_2$), 30.5 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 25.8 (1C, **C**H$_2$), 22.6 (2C, 2 x **C**H$_2$), 20.2 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.8 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

8.2.9 Synthesis of heteroatom-containing *N*-1-phenylcycloalkyl quinolone amides

4-Phenyltetrahydro-2*H*-pyran-4-ol (**44a**)
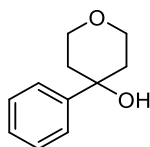
Bromobenzene (502 µL, 4.79 mmol) was dissolved in abs. THF (50 mL) and cooled to –78 °C. A 2.5 M *n*-butyllithium solution in hexane (2.33 mL, 5.59 mmol) was added dropwise and the solution was stirred at –78 °C for 20 min. Tetrahydro-4*H*-pyrane-4-one (400 mg, 3.99 mmol) was added and the mixture was stirred at –78 °C for 5 min. Afterwards, the reaction was allowed to adopt to r.t. and quenched with water. The phases were separated and the water phase was extracted with diethyl ether and EtOAc. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silical gel, hexane/EtO_2 (9/1)) to yield the desired product.

Chemical formula: $C_{11}H_{14}O_2$

Molar mass: 178.2 g/mol

Appearance: Colourless solid

Yield: 349 mg (1.96 mmol, 41 %)

Melting point: 98 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3372 (m), 3084 (w), 2954 (m), 2922 (w), 2887 (w), 1490 (w), 1445 (w), 1407 (m), 1221 (m), 1015 (s), 964 (s).

¹H NMR (400 MHz, CDCl₃): δ = 7.50 (d, 2H, ^3J = 7.7 Hz, **CH**arom.), 7.38 (t, 2H, ^3J = 7.7 Hz, **CH**arom.), 7.29 (m, 1H, **CH**arom.), 3.95 (m, 2H, **CH**₂OC**H**₂), 3.88 (m, 2H, **CH**₂OC**H**₂), 2.19 (m, 2H, **CH**₂CC**H**₂), 1.69 (m, 3H, **CH**₂CC**H**₂ + O**H**) ppm.

¹³C NMR (100 MHz, CDCl₃): δ = 148.2 (1C, **C**arom.), 128.7 (2C, **CH**arom.), 127.5 (1C, **CH**arom.), 124.6 (2C, **CH**arom.), 70.9 (1C, **C**OH), 64.1 (2C, **CH**₂OC**H**₂), 39.0 (2C, **CH**₂C**CH**₂) ppm.

The spectroscopic data are in accordance with literature.[186]

4-Phenyltetrahydro-2*H*-thiopyran-4-ol (**44b**)
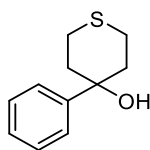
Bromobenzene (440 µL, 4.12 mmol) was dissolved in abs. THF (50 mL) and cooled to –78 °C. A 2.5 M *n*-butyllithium solution in hexane (2.33 mL, 5.59 mmol) was added dropwise and the solution was stirred at –78 °C for 20 min. Tetrahydro-4*H*-thiopyran-4-one (400 mg, 3.44 mmol) was added and the mixture was stirred at –78 °C for 5 min. Afterwards, the reaction was allowed to adopt to r.t. and quenched with water. The phases were separated and the water phase was extracted with diethyl ether and EtOAc. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silical gel, hexane/EtO$_2$ (9/1)). The product was isolated as a 3:1 mixture of the desired product and the starting material, tetrahydro-4*H*-thiopyran-4-one.

Chemical formula: $C_{11}H_{14}OS$

Molar mass: 194.3 g/mol

Appearance: Colourless solid

Yield: 337 mg (1.73 mmol, 42 %)

Melting point: 54 °C (Lit.: 78 °C)[187]

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3409 (m), 3057 (w), 2915 (w), 1493 (m), 1423 (m), 1274 (m), 1227 (m), 1063 (s), 927 (s), 759 (s).

¹H NMR (400 MHz, CDCl$_3$): δ = 7.49 (m, 2H, **CH**$_{arom.}$), 7.37 (t, 2H, ^3J = 7.7 Hz, **CH**$_{arom.}$), 7.27 (m, 1H, **CH**$_{arom.}$), 3.23 (m, 2H, **CH**$_2$S**CH**$_2$), 2.48 (m, 2H, **CH**$_2$S**CH**$_2$), 2.19 (m, 3H, **CH**$_2$C**CH**$_2$), 2.03 (m, 2H, **CH**$_2$C**CH**$_2$), 1.51 (br, 1H, CO**H**) ppm.

¹³C NMR (100 MHz, CDCl$_3$): δ = 149.2 (1C, **C**$_{arom.}$), 128.7 (2C, **CH**$_{arom.}$), 127.3 (1C, **CH**$_{arom.}$), 124.4 (2C, **CH**$_{arom.}$), 72.1 (1C, **C**OH), 39.8 (2C, **CH**$_2$S**CH**$_2$), 24.4 (2C, **CH**$_2$C**CH**$_2$) ppm.

The spectroscopic data are accordance with literature.[187]

N-(4-Phenyltetrahydro-2*H*-pyran-4-yl)acetamide (**45a**)
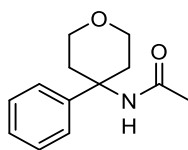
4-Phenyltetrahydro-2*H*-pyran-4-ol (**44a**) (670 mg, 3.76 mmol) was dissolved in acetonitrile (30 mL) and cooled to 0 °C. The cooled solution was added to a mixture of conc. sulfuric acid (5 mL) and acetonitrile (5 mL) at 0 °C. This mixture was stirred at 0 °C for 2 h. Water (50 mL), a 2 M NaOH solution (5 mL), and EtOAC (50 mL) were added to the stirring mixture. The phases were separated and the water phase was extracted with EtOAc. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (RP-18, H_2O/MeOH (70/30)) to yield the desired product.

Chemical formula: $C_{13}H_{17}NO_2$

Molar mass: 219.3 g/mol

Appearance: Colourless solid

Yield: 261 mg (1.19 mmol, 32 %)

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3280 (w), 3060 (w), 2953 (w), 2858 (w), 1651 (s), 1543 (s), 1299 (m), 1105 (s), 976 (w), 697 (s).

¹H NMR (400 MHz, CDCl₃): δ = 7.36 (m, 4H, **CH**$_{arom.}$), 7.24 (m, 1H, **CH**$_{arom.}$), 5.62 (br, 1H, **NH**), 3.86 (m, 2H, **CH₂**OC**H₂**), 3.74 (m, 2H, **CH₂**OC**H₂**), 2.36 (m, 2H, **CH₂**CC**H₂**), 2.18 (m, 2H, **CH₂**CC**H₂**), 2.02 (s, 3H, **CH₃**) ppm.

¹³C NMR (100 MHz, CDCl₃): δ = 169.5 (1C, NH**C**O), 145.3 (1C, **C**$_{arom.}$), 128.6 (2C, **CH**$_{arom.}$), 127.2 (1C, **CH**$_{arom.}$), 125.2 (2C, **CH**$_{arom.}$), 64.0 (2C, **CH₂**OC**H₂**), 56.1 (1C, **C**NH), 36.3 (2C, **CH₂**CC**H₂**), 24.8 (1C, **CH₃**) ppm.

N-(4-Phenyltetrahydro-2*H*-thiopyran-4-yl)acetamide (**45b**)
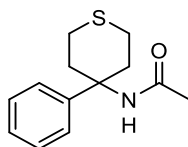
4-Phenyltetrahydro-2*H*-thiopyran-4-ol (**44b**) (300 mg, 1.54 mmol) was dissolved in acetonitrile (20 mL) and cooled to 0 °C. The cooled solution was added to a mixture of conc. sulfuric acid

(5 mL) and acetonitrile (5 mL) at 0 °C. This mixture was stirred at 0 °C for 2 h. Water (50 mL), a 2 M NaOH solution (5 mL), and EtOAC (50 mL) were added to the stirring mixture. The phases were separated and the water phase was extracted with EtOAc. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (RP-18, H_2O/MeOH (70/30)) to yield the desired product.

Chemical formula: $C_{13}H_{17}NOS$

Molar mass: 235.4 g/mol

Appearance: Colourless solid

Yield: 206 mg (875 µmol, 57 %)

IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3284 (w), 3057 (w), 2919 (w), 2849 (w), 1656 (s), 1545 (s), 1296 (m), 1029 (s), 796 (m), 699 (s).

^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.35 (m, 4H, C**H**$_{arom.}$), 7.24 (m, 1H, C**H**$_{arom.}$), 5.48 (br, 1H, N**H**), 2.94 (m, 2H, C**H**$_2$SCH$_2$), 2.67 (m, 2H, CH$_2$SC**H**$_2$), 2.56 (m, 2H, C**H**$_2$CCH$_2$), 2.20 (m, 2H, CH$_2$CC**H**$_2$), 2.07 (s, 3H, C**H**$_3$) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 168.2 (1C, NH**C**O), 144.8 (1C, **C**$_{arom.}$), 127.6 (2C, **C**H$_{arom.}$), 126.0 (1C, **C**H$_{arom.}$), 123.8 (2C, **C**H$_{arom.}$), 56.7 (1C, **C**NH), 36.1 (2C, **C**H$_2$S**C**H$_2$), 24.8 (1C, **C**H$_3$), 23.1 (2C, **C**H$_2$C**C**H$_2$) ppm.

4-Phenyltetrahydro-2*H*-pyran-4-amine (**47a**)
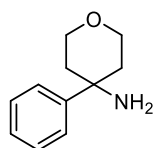
N-(4-Phenyltetrahydro-2*H*-pyran-4-yl)acetamide (**45a**) (261 mg, 1.19 mmol) was dissolved in abs. THF (150 mL). Titanium isopropoxide (720 µL, 2.38 mmol) and diphenylsilane (665 µL, 3.57 mmol) were added. The solution was heated under stirring until an effervescence started. The reaction was stirred for 2 h and was periodically heated to maintain effervescence. A 2 M HCl solution (100 mL) was added to the stirring solution and the phases were separated. The water phase was washed with diethyl ether. Afterwards, the aqueous solution was basified using a 6 M NaOH solution and extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo* to yield the desired product as a yellow oil.

Chemical formula: $C_{11}H_{15}NO$

Molar mass: 177.3 g/mol

Appearance: Yellow oil

Yield: 113 mg (638 µmol, 54 %)

¹H NMR (400 MHz, C_6D_6): δ = 7.31 (m, 4H, **CH**arom.), 7.19 (m, 1H, **CH**arom.), 3.92 (m, 2H, **CH₂**OC**H₂**), 3.80 (m, 2H, **CH₂**OC**H₂**), 2.05 (m, 2H, **CH₂**CC**H₂**), 1.31 (m, 2H, **CH₂**CC**H₂**), 0.83 (s, 2H, N**H₂**) ppm.

¹³C NMR (100 MHz, CDCl₃): δ = 150.4 (1C, **C**arom.), 128.2 (2C, **CH**arom.), 126.2 (1C, **CH**arom.), 124.7 (2C, **CH**arom.), 63.6 (2C, **CH₂**OC**H₂**), 51.3 (1C, **C**NH), 39.0 (2C, **CH₂**CC**H₂**) ppm.

1,1-Dioxido-4-phenyltetrahydro-2*H*-thiopyran-4-amine (**47c**)
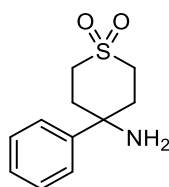
N-(-4-Phenyltetrahydro-2*H*-thiopyran-4-yl)acetamide (**45b**) (34.0 mg, 144 µmol) was dissolved in abs. THF (20 mL). Titanium isopropoxide (87.4 µL, 289 mmol) and diphenylsilane (80.7 µL, 433 mmol) were added. The solution was heated under stirring until an effervescence started. The reaction was stirred for 2 h and was periodically heated to maintain the effervescence. A 2 M HCl solution (20 mL) was added to the stirring solution and the phases were separated. The water phase was washed with diethyl ether. Afterwards, the aqueous solution was basified using a 6 M NaOH solution and extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo* to yield the desired product as a yellow oil.

Chemical formula: $C_{11}H_{15}NO_2S$

Molar mass: 225.3 g/mol

Appearance: Yellow oil

Yield: 18.0 mg (93.1 µmol, 64 %)

¹H NMR (400 MHz, CD_2Cl_2): δ = 7.48 (m, 2H, **CH**arom.), 7.39 (m, 2H, **CH**arom.), 7.28 (m, 1H, **CH**arom.), 3.63 (m, 2H, **CH₂**SC**H₂**), 2.85 (m, 2H, **CH₂**SC**H₂**), 2.63 (m, 2H, **CH₂**CC**H₂**), 1.98 (m, 2H, **CH₂**CC**H₂**), 1.66 (br, 2H, N**H₂**) ppm.

13C NMR (100 MHz, CD$_2$Cl$_2$): δ = 149.0 (1C, **C**$_{arom.}$), 129.0 (2C, **C**H$_{arom.}$), 127.5 (1C, **C**H$_{arom.}$), 124.7 (2C, **C**H$_{arom.}$), 52.9 (1C, **C**NH), 48.0 (2C, **C**H$_2$S**C**H$_2$), 37.1 (2C, **C**H$_2$C**C**H$_2$) ppm.

1-Butyl-7-morpholino-4-oxo-*N*-(4-phenyltetrahydro-2*H*-pyran-4-yl)-1,4-dihydroquinoline-3-carboxamide (**53a**)
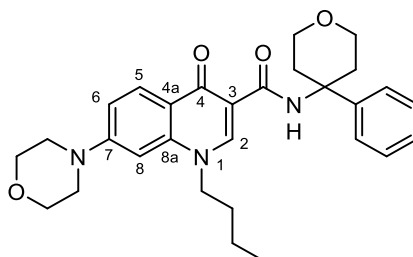
The carboxylic acid **26d** (205 mg, 621 µmol), amine **47a** (110 mg, 621 µmol), and PyBOP (388 mg, 745 µmol) were dissolved in abs. DMF (80 mL). DIPEA (271 µL, 1.55 mmol) was added and the solution was stirred at r.t. overnight. A saturated ammonium chloride solution (100 mL) was added to the stirring solution and the mixture was extracted with CH$_2$Cl$_2$. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH$_2$Cl$_2$/MeOH (100/0.5 → 100/1)) and recrystallized from EtOH several times to yield the desired product.

Chemical formula: C$_{29}$H$_{35}$N$_3$O$_4$

Molar mass: 489.6 g/mol

Appearance: Colourless crystals

Yield: 132 mg (270 µmol, 43 %)

Melting point: 214 °C

IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3181 (w), 3052 (w), 2956 (w), 2858 (w), 1664 (m), 1613 (m), 1604 (m), 1525 (s), 1241 (s), 1173 (m), 966 (m), 753 (s).

HRMS: [M+H]$^+$ 490.27003 m/z, found 490.27043 m/z.

Purity (HPLC method I): 95.6 %

logP (HPLC method II): 3.60

1H NMR (400 MHz, CDCl$_3$): δ = 10.91 (s, 1H, N**H**), 8.55 (s, 1H, 2-**H**), 8.41 (d, 1H, ^3J = 9.2 Hz, 5-**H**), 7.48 (d, 2H, ^3J = 7.8 Hz, Bn-C**H**$_{arom.}$), 7.32 (t, 2H, ^3J = 7.8 Hz, Bn-C**H**$_{arom.}$), 7.20 (m, 1H, Bn-C**H**$_{arom.}$), 7.10 (dd, 1H, ^3J = 9.2 Hz, ^4J = 1.5 Hz, 6-**H**), 6.64 (d, 1H, ^4J = 1.5 Hz, 8-**H**), 4.11 (t, 2H, ^3J = 7.4 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.92 (m, 8H, morpholino-C**H**$_2$-O-C**H**$_2$ + C**H**$_2$OC**H**$_2$), 3.36

(m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 2.51 (m, 2H, **CH**$_2$CC**H**$_2$), 2.21 (m, 2H, **CH**$_2$CC**H**$_2$), 1.85 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$**CH**$_2$CH$_2$CH$_3$), 1.42 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$**CH**$_2$CH$_3$), 0.97 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$**CH**$_3$) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 176.5 (1C, **C**-4), 164.6 (1C, **C**ON), 154.5 (1C, **C**-7), 147.5 (1C, **C**-2), 146.7 (1C, Bn-**C**$_{arom.}$), 141.1 (1C, **C**-8a), 128.7 (1C, **C**-5), 128.5 (2C, Bn-**C**$_{arom.}$), 126.7 (1C, Bn-**C**$_{arom.}$), 125.3 (2C, Bn-**C**$_{arom.}$), 120.6 (1C, **C**-4a), 114.0 (1C, **C**-6), 112.0 (1C, **C**-3), 98.1 (1C, **C**-8), 66.6 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 64.2 (2C, **CH**$_2$O**CH**$_2$), 55.8 (1C, CH$_2$**C**CH$_2$), 53.9 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 48.0 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 36.6 (2C, **C**H$_2$C**C**H$_2$), 30.8 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 20.2 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.8 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

1-Butyl-7-morpholino-4-oxo-*N*-(1,1-dioxido-4-phenyltetrahydro-2*H*-thiopyran-4-yl)-1,4-dihydroquinoline-3-carboxamide (**53b**)
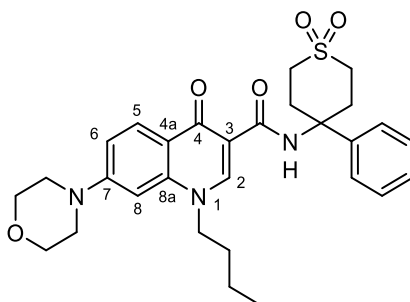
The carboxylic acid **26d** (29.1 mg, 87.9 μmol), amine **47c** (17.0 mg, 87.9 μmol), and PyBOP (45.8 mg, 87.9 μmol) were dissolved in abs. DMF (20 mL). DIPEA (38.4 μL, 220 μmol) was added and the solution was stirred at r.t. overnight. A saturated ammonium chloride solution (10 mL) was added to the stirring solution and the mixture was extracted with CH$_2$Cl$_2$. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH$_2$Cl$_2$/MeOH (100/0.5 → 100/1)) to yield the desired product.

Chemical formula: C$_{29}$H$_{35}$N$_3$O$_5$S

Molar mass: 537.7 g/mol

Appearance: Yellow solid

Yield: 16.1 mg (30.7 μmol, 35 %)

Melting point: > 230 °C decomposition

IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3181 (w), 3052 (w), 2956 (w), 2858 (w), 1664 (m), 1613 (m), 1604 (m), 1525 (m), 1242 (m), 1119 (s), 966 (m), 753 (m).

HRMS: [M+H]$^+$ 538.23702 m/z, found 538.23800 m/z.

Purity (HPLC method I): 95.5 %

logP (HPLC method II): 2.83

^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.23 (s, 1H, N**H**), 8.56 (s, 1H, 2-**H**), 8.38 (d, 1H, ^3J = 9.2 Hz, 5-**H**), 7.45 (d, 2H, ^3J = 7.7 Hz, Bn-C**H**$_{arom.}$), 7.33 (t, 2H, ^3J = 7.7 Hz, Bn-C**H**$_{arom.}$), 7.23 (m, 1H, Bn-C**H**$_{arom.}$), 7.13 (dd, 1H, ^3J = 9.2 Hz, ^4J = 1.3 Hz, 6-**H**), 6.67 (d, 1H, ^4J = 1.3 Hz, 8-**H**), 4.16 (t, 2H, ^3J = 7.4 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.92 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.51 (m, 2H, C**H**$_2$SCH$_2$), 3.39 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 3.03 (m, 4H, C**H**$_2$SCH$_2$ + C**H**$_2$CCH$_2$), 2.74 (m, 2H, C**H**$_2$CCH$_2$), 1.89 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.44 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.99 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 176.4 (1C, **C**-4), 165.6 (1C, **C**ON), 154.6 (1C, **C**-7), 147.5 (1C, **C**-2), 144.2 (1C, Bn-**C**$_{arom.}$), 141.1 (1C, **C**-8a), 128.8 (2C, Bn-**C**$_{arom.}$), 128.7 (1C, **C**-5), 127.5 (1C, Bn-**C**$_{arom.}$), 125.1 (2C, Bn-**C**$_{arom.}$), 120.3 (1C, **C**-4a), 114.3 (1C, **C**-6), 111.2 (1C, **C**-3), 98.1 (1C, **C**-8), 66.6 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 56.8 (1C, CH$_2$**C**CH$_2$), 54.2 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 48.1 (2C, **C**H$_2$SCH$_2$), 48.0 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 35.1 (2C, **C**H$_2$CCH$_2$), 30.8 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 20.2 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.8 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

8.2.10 Synthesis of quinolone amides with a primary amine function

General synthetic procedure (**F**) for 3- and 4-(aminomethyl)benzonitriles:

3-Methylbenzonitrile (**57a**) or 4-methylbenzonitrile (**57b**), NBS, and DBPO were dissolved in CHCl$_3$ (150 mL) and stirred under reflux for 3 h. A saturated sodium thiosulfate solution (3 mL) and a saturated ammonium chloride solution (50 mL) were added to the stirring solution. The phases were separated and the water phase was extracted with CH$_2$Cl$_2$. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed *in vacuo*.[109]

The residue was dissolved in abs. DMF (150 mL) and sodium azide was added. After stirring at r.t. for 12 h, the solvent was removed *in vacuo*. Water (30 mL) and CH$_2$Cl$_2$ (30 mL) were added. After shaking, the phases were separated and the water phase was extracted with CH$_2$Cl$_2$. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed *in vacuo*.

The obtained residue was dissolved in a THF/water mixture (150 mL, 9/1) and cooled to 0 °C. Triphenylphosphane was added portionwise and the reaction solution was stirred at 0 °C for 30 min. After removing the ice-bath, the solution of **56a** was stirred at r.t. for 12 h, the solution of **56b** for 7 d. The solvent was removed *in vacuo* and the residue was dissolved in water (30 mL) and CH$_2$Cl$_2$ (30 mL). The water phase was acidified with a 4 M HCl solution, the phases were separated, and the water phase was washed with CH$_2$Cl$_2$. Afterwards, the water phase was basified with a 2 M NaOH solution and extracted with CH$_2$Cl$_2$. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed *in vacuo* to yield the desired products.[110]

*Table 20. Used amounts in the synthesis of **56a** and **56b**.*

Product		Sample weight [g]	Substance amount [mmol]
	57a	5.00	42.7
	NBS	7.60	42.7
56a	DBPO	0.27	1.14
	NaN3	2.78	42.7
	PPh3	11.2	42.7
	57b	5.00	42.7
	NBS	7.60	42.7
56b	DBPO	0.27	1.14
	NaN3	4.16	42.7
	PPh3	11.2	42.7

4-(Aminomethyl)benzonitrile (**56a**)
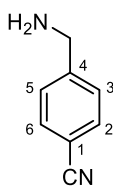
Chemical formula: $C_8H_8N_2$

Molar mass: 132.2 g/mol

Appearance: yellow oil

Yield: 3.28 g (24.8 mmol, 58 %)

¹H NMR (400 MHz, DMSO-d_6): δ = 7.95 (s, 2H, N**H**$_2$), 7.75 (d, 2H, ^3J = 8.1 Hz, 2-**H** + 6-**H**), 7.53 (d, 2H, ^3J = 8.1 Hz, 3-**H** + 5-**H**), 3.79 (s, 2H, C**H**$_2$) ppm.

The spectroscopic data are in accordance with literature.[188]

3-(Aminomethyl)benzonitrile (**56b**)
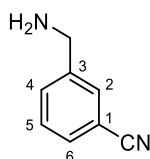
Chemical formula: $C_8H_8N_2$

Molar mass: 132.2 g/mol

Appearance: yellow oil

Yield: 2.82 g (21.3 mmol, 50 %)

¹H NMR (400 MHz, DMSO-d_6): δ = 7.95 (s, 2H, N**H**$_2$), 7.78 (s, 1H, 6-**H**), 7.66-7.67 (m, 1H, 5-**H**), 7.64-7.65 (m, 1H, 2-**H**), 7.50 (t, 1H, ^3J = 7.7 Hz, 4-**H**), 3.75 (s, 2H, C**H**$_2$) ppm.

The spectroscopic data are in accordance with literature.[189]

1-Butyl-*N*-(4-cyanobenzyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**55a**)
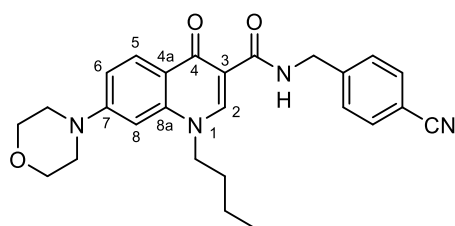
According to the general procedure (**E**), carboxylic acid **26d** (224 mg, 678 µmol) was dissolved in abs. DMF (50 mL) and treated with NMM (298 µL, 2.71 mmol), IBCF (265 µL, 2.03 mmol), and 4-(aminomethyl)benzonitrile (269 mg, 2.03 mmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (silica gel, $CHCl_3$/MeOH (100/1 → 100/3)) and recrystallized from $CHCl_3$/EtOH to yield the desired product as colourless needles.

Chemical formula: $C_{26}H_{28}N_4O_3$

Molar mass: 444.2 g/mol

Appearance: colourless needles

Yield: 153 mg (344 µmol, 51 %)

Melting point: 191 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3201 (w), 3056 (w), 2959 (w), 2858 (w), 2225 (w), 1661 (s), 1614 (s), 1532 (s), 1467 (s), 1243 (s), 1119 (s), 820 (s).

Mass: *m/z* 445.15 [M + H]$^+$

^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.60 (t, 1H, ^3J = 6.0 Hz, CON**H**), 8.71 (s, 1H, 2-**H**), 8.15 (d, 1H, ^3J = 9.1 Hz, 5-**H**), 7.80 (d, 2H, ^3J = 8.1 Hz, Bn-C**H**$_{arom.}$), 7.51 (d, 2H, ^3J = 8.1 Hz, Bn-C**H**$_{arom.}$), 7.23 (dd, 1H, ^3J = 9.1 Hz, ^4J = 1.2 Hz, 6-**H**), 7.09 (d, 1H, ^4J = 1.2 Hz, 8-**H**), 4.63 (d, 2H, ^3J = 6.0 Hz, NHC**H**$_2$), 4.42 (t, 2H, ^3J = 7.3 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.78 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.39 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 2.38 (d, 2H, ^3J = 12.6 Hz, 2 x C**H**), 1.75 (quint, 2H, ^3J = 7.3 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.32 (sext, 2H, ^3J = 7.3 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.91 (t, 3H, ^3J = 7.3 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 174.7 (1C, **C**-4), 164.8 (1C, **C**ON), 154.0 (1C, **C**-7), 147.8 (1C, **C**-2), 145.7 (1C, Bn-**C**$_{arom.}$), 140.7 (1C, **C**-8a), 132.4 (2C, Bn-**C**$_{arom.}$), 128.5 (2C, Bn-**C**$_{arom.}$), 127.4 (1C, **C**-5), 119.3 (1C, **C**-4a), 118.9 (1C, Bn-**C**$_{arom.}$), 113.5 (1C, **C**-6), 109.9 (1C, Bn-**C**N), 109.6 (1C, **C**-3), 98.6 (1C, **C**-8), 66.0 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 52.6 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 47.2 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 41.8 (1C, NH**C**H$_2$), 30.5 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.3 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.4 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

1-Butyl-*N*-(4-cyanobenzyl)-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**55b**)
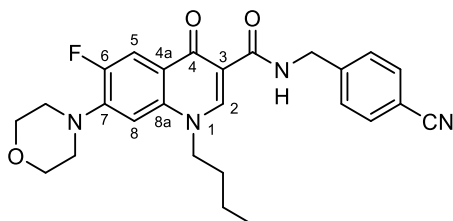
According to the general procedure (**E**), carboxylic acid **26e** (231 mg, 663 µmol) was dissolved in abs. DMF (45 mL) and treated with NMM (365 µL, 3.32 mmol), IBCF (259 µL, 1.99 mmol), and 4-(aminomethyl)benzonitrile (263 mg, 1.99 mmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (silica gel, CHCl$_3$/MeOH (100/1 → 100/3)) and afterwards recrystallized from CHCl$_3$/MeOH.

Chemical formula: C$_{26}$H$_{27}$FN$_4$O$_3$

Molar mass: 462.5 g/mol

Appearance: colourless needles

Yield: 230 mg (497 µmol, 75 %)

Melting point: 204 °C

IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3201 (w), 3052 (w), 2958 (w), 2858 (w), 2224 (w), 1660 (s), 1624 (w), 1605 (w), 1541 (m), 1489 (s), 1256 (s), 1114 (s), 803 (m).

^{1}H NMR (400 MHz, DMSO-*d$_6$*): δ = 10.45 (t, 1H, ^3J = 6.1 Hz, CON**H**), 8.78 (s, 1H, 2-**H**), 7.90 (d, 1H, $^3J_{H,F}$ = 13.4 Hz, 5-**H**), 7.80 (d, 2H, ^3J = 8.4 Hz, Bn-C**H**$_{arom.}$), 7.51 (d, 2H, ^3J = 8.4 Hz, Bn-C**H**$_{arom.}$),7.10 (d, 1H, $^4J_{H,F}$ = 7.3 Hz, 8-**H**), 4.63 (d, 2H, ^3J = 6.1 Hz, CONHC**H**$_2$), 4.48 (t, 2H, ^3J = 7.4 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.82-3.77 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.24-3.28 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.77 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.32 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.92 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, DMSO-*d$_6$*): δ = 174.1 (1C, **C**-4), 164.5 (1C, **C**ONH), 152.5 (d, 1C, $^1J_{C,F}$ = 247.6 Hz, **C**-6), 147.9 (1C, **C**-2), 145.6 (1C, NHCH$_2$**C**), 144.4 (d, 1C, $^2J_{C,F}$ = 10.4 Hz, **C**-7), 136.7 (1C, **C**-8a), 132.3 (2C, Bn-**C**$_{arom.}$), 128.1 (2C, Bn-**C**$_{arom.}$), 121.5 (d, 1C, $^3J_{C,F}$ = 7.1 Hz, **C**-4a), 118.9 (1C, **C**N), 111.5 (d, 1C, $^2J_{C,F}$ = 22.8 Hz, **C**-5), 109.9 (1C, **C**-3), 109.5 (1C, **C**CN), 105.6 (d, 1C, $^3J_{C,F}$ = 2.7 Hz, **C**-8), 65.9 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 52.9 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 49.8 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 41.9 (1C, NH-**C**H$_2$), 30.3 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.2 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.5 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

1-Butyl-*N*-(4-cyanobenzyl)-5-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**55c**)
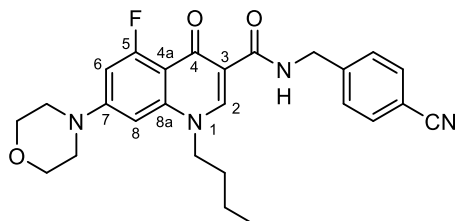
The carboxylic acid **26f** (63 mg, 181 µmol), 4-(aminomethyl)benzonitrile (47.8 mg, 362 µmol), and PyBOP (113 mg, 217 µmol) were dissolved in abs. DMF (15 mL). DIPEA (63.2 µL, 362 µmol) was added and the solution was stirred at r.t. overnight. A saturated ammonium chloride solution (40 mL) was added to the stirring solution and the mixture was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The crude product was purified by column chromatography (silica gel, $CHCl_3$/MeOH (100/1 → 100/3)) to yield the desired product as a colourless solid.

Chemical formula: $C_{26}H_{27}FN_4O_3$

Molar mass: 462.5 g/mol

Appearance: colourless solid

Yield: 61.2 mg (132 µmol, 73 %)

Melting point: 206 °C

IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3177 (w), 3064 (w), 2967 (w), 2871 (w), 2227 (w), 1662 (s), 1631 (s), 1605 (m), 1533 (m), 1472 (m), 1261 (m), 1121 (m), 811 (m).

HRMS: [M+H]$^+$ 463.21399 m/z, found 463.21405 m/z.

Purity (HPLC method I): 99.5 %

logP (HPLC method II): 2.90

^{1}H NMR (400 MHz, CDCl$_3$): δ = 10.64 (t, 1H, ^3J = 5.9 Hz, CONH), 8.59 (s, 1H, 2-**H**), 7.59 (d, 2H, ^3J = 8.1 Hz, Bn-C**H**$_{arom.}$), 7.46 (d, 2H, ^3J = 8.1 Hz, Bn-C**H**$_{arom.}$), 6.58 (dd, 1H, $^3J_{H,F}$ = 14.9 Hz, ^4J = 1.4 Hz, 6-**H**), 6.40 (br, 1H, 8-**H**), 4.67 (d, 2H, ^3J = 5.9 Hz, CONHC**H**$_2$), 4.13 (t, 2H, ^3J = 7.3 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.88 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.33 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.86 (quint, 2H, ^3J = 7.3 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.44 (sext, 2H, ^3J = 7.3 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.99 (t, 3H, ^3J = 7.3 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 175.8 (1C, **C**-4), 165.5 (1C, **C**ONH), 164.1 (d, 1C, $^1J_{C,F}$ = 261.0 Hz, **C**-5), 154.1 (d, 1C, $^3J_{C,F}$ = 13.1 Hz, **C**-7), 147.8 (1C, **C**-2), 144.7 (1, NHCH$_2$**C**), 142.5

(d, 1C, $^3J_{C,F}$ = 5.6 Hz, **C**-8a), 132.5 (2C, Bn-**C**$_{arom.}$), 128.3 (2C, Bn-**C**$_{arom.}$),119.1 (1C, **C**N), 112.0 (1C, **C**-CN), 110.9 (1C, **C**-3), 110.5 (d, 1C, $^2J_{C,F}$ = 8.2 Hz, **C**-4a), 100.0 (d, 1C, $^2J_{C,F}$ = 26.2 Hz, **C**-6), 94.0 (d, 1C, $^4J_{C,F}$ = 2.5 Hz, **C**-8), 66.4 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 54.6 (1C, N**C**H$_2$-CH$_2$CH$_2$CH$_3$), 47.4 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 42.9 (1C, NH-**C**H$_2$), 30.4 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 20.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.8 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

1-Butyl-*N*-(3-cyanobenzyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**55d**)
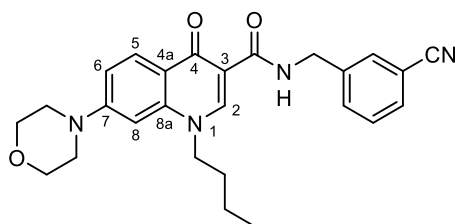
According to the general procedure (**E**), carboxylic acid **26d** (210 mg, 636 µmol) was dissolved in abs. DMF (50 mL) and treated with NMM (280 µL, 2.54 mmol), IBCF (331 µL, 2.54 mmol), and 3-(aminomethyl)benzonitrile (336 mg, 2.54 mmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (silica gel, CHCl$_3$/MeOH (100/1 → 100/3)) and recrystallized from CHCl$_3$/EtOH to yield the desired product as colourless needles.

Chemical formula: C$_{26}$H$_{28}$N$_4$O$_3$

Molar mass: 444.2 g/mol

Appearance: colourless needles

Yield: 250 mg (562 µmol, 88 %)

Melting point: 186 °C

IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3158 (w), 3083 (w), 2955 (w), 2872 (w), 2228 (w), 1650 (m), 1619 (m), 1599 (m), 1532 (s), 1473 (s), 1243 (s), 1116 (m), 785 (s).

Mass: *m*/*z* 445.15 [M + H]$^+$

^{1}H NMR (400 MHz, DMSO-*d$_6$*): δ = 10.57 (t, 1H, ^3J = 6.0 Hz, CON**H**), 8.71 (s, 1H, 2-**H**), 8.14 (d, 1H, ^3J = 9.2 Hz, 5-**H**), 7.50-7.80 (m, 4H, 4 x Bn-C**H**$_{arom.}$), 7.23 (dd, 1H, ^3J = 9.2 Hz, ^4J = 1.6 Hz, 6-**H**), 6.90 (d, 1H, ^4J = 1.6 Hz, 8-**H**), 4.59 (d, 2H, ^3J = 6.0 Hz, NHC**H**$_2$), 4.42 (t, 2H, ^3J = 7.3 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.78 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.39 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.75 (quint, 2H, ^3J = 7.3 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.32 (sext, 2H, ^3J = 7.3 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.91 (t, 3H, ^3J = 7.3 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

¹³C NMR (100 MHz, DMSO-*d₆*): δ = 174.6 (1C, **C**-4), 164.8 (1C, **C**ON), 154.1 (1C, **C**-7), 148.0 (1C, **C**-2), 141.8 (1C, Bn-**C**arom.), 140.9 (1C, **C**-8a), 132.2 (1C, Bn-**C**arom.), 130.9 (1C, Bn-**C**arom.), 130.6 (1C, Bn-**C**arom.), 129.7 (1C, Bn-**C**arom.), 127.4 (1C, **C**-5), 119.3 (1C, **C**-4a), 118.9 (1C, **C**CN), 113.5 (1C, **C**-6), 111.3 (1C, C**C**N), 109.9 (1C, **C**-3), 98.6 (1C, **C**-8), 65.9 (2C, morpholino-**C**H₂-O-**C**H₂), 52.5 (1C, N**C**H₂CH₂CH₂CH₃), 47.1 (2C, morpholino-**C**H₂-N-**C**H₂), 41.4 (1C, NH**C**H₂), 30.5 (1C, NCH₂**C**H₂CH₂CH₃), 19.2 (1C, NCH₂CH₂**C**H₂CH₃), 13.5 (1C, NCH₂CH₂CH₂**C**H₃) ppm.

1-Butyl-*N*-(3-cyanobenzyl)-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**55e**)
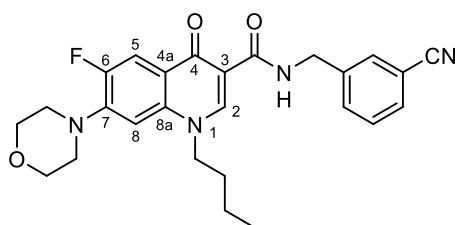
According to the general procedure (**E**), carboxylic acid **26e** (325 mg, 933 μmol) was dissolved in abs. DMF (45 mL) and treated with NMM (514 μL, 4.66 mmol), IBCF (365 μL, 2.80 mmol), and 3-(aminomethyl)benzonitrile (370 mg, 2.80 mmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (silica gel, CHCl₃/MeOH (100/1 → 100/3)) to yield the desired product as a colourless solid.

Chemical formula: $C_{26}H_{27}FN_4O_3$

Molar mass: 462.5 g/mol

Appearance: colourless solid

Yield: 328 mg (709 μmol, 76 %)

Melting point: 178 °C

IR (ATR, $\tilde{\nu}$ [cm⁻¹]): 3167 (w), 3056 (w), 2958 (w), 2859 (w), 2227 (w), 1654 (s), 1627 (m), 1604 (m), 1538 (s), 1486 (s), 1254 (s), 1118 (m), 803 (m).

¹H NMR (400 MHz, DMSO-*d₆*): δ = 10.43 (t, 1H, ³*J* = 6.0 Hz, CON**H**), 8.78 (s, 1H, 2-**H**), 7.89 (d, 1H, ³*J*ₕ,ₑ = 13.6 Hz, 5-**H**), 7.77 (s, 1H, Bn-C**H**arom.), 7.72 (d, 1H, ³*J* = 7.8 Hz, Bn-C**H**arom.), 7.68 (d, 1H, ³*J* = 7.8 Hz, Bn-C**H**arom.), 7.55 (t, 1H, ³*J* = 7.8 Hz, Bn-C**H**arom.), 7.10 (d, 1H, ⁴*J*ₕ,ₑ = 7.3 Hz, 8-**H**), 4.59 (d, 2H, ³*J* = 6.0 Hz, NHC**H₂**), 4.48 (t, 2H, ³*J* = 7.3 Hz, NC**H₂**CH₂CH₂CH₃), 3.82-3.77 (m, 4H, morpholino-C**H₂**-O-C**H₂**), 3.28-3.24 (m, 4H, morpholino-

CH$_2$-N-CH$_2$), 1.77 (quint, 2H, ^3J = 7.3 Hz, NCH$_2$CH$_2$CH$_2$CH$_3$), 1.33 (sext, 2H, ^3J = 7.3 Hz, NCH$_2$CH$_2$CH$_2$CH$_3$), 0.92 (t, 3H, ^3J = 7.3 Hz, NCH$_2$CH$_2$CH$_2$CH$_3$) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 174.1 (1C, **C**-4), 164.1 (1C, **C**ONH), 151.0 (d, 1C, $^1J_{C,F}$ = 231.8 Hz, **C**-6), 147.9 (1C, **C**-2), 144.3 (1C, **C**-7), 141.5 (1C, NHCH$_2$**C**), 136.7 (1C, **C**-8a), 132.5 (1C, Bn-**C**$_{arom.}$), 130.9 (1C, Bn-**C**$_{arom.}$), 130.6 (1C, Bn-**C**$_{arom.}$), 129.6 (1C, Bn-**C**$_{arom.}$), 121.5 (1C, **C**-4a), 118.8 (1C, **C**N), 111.6 (1C, **C**CN), 111.3 (1C, **C**-5), 109.9 (1C, **C**-3), 105.6 (1C, **C**-8), 65.9 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 52.9 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 49.9 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 41.5 (1C, NH-**C**H$_2$), 30.3 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.2 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.5 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

The assignment of the quaternary aromatic carbon atoms was done using the 2D spectra due to low concentration due to low solubility.

1-Butyl-*N*-(3-cyanobenzyl)-5-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**55f**)
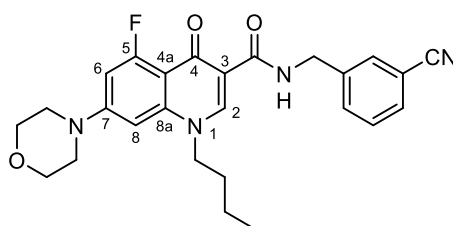
According to the general procedure (**E**), carboxylic acid **26f** (187 mg, 534 µmol) was dissolved in abs. DMF (45 mL) and treated with NMM (236 µL, 2.15 mmol), IBCF (209 µL, 1.61 mmol), and 3-(aminomethyl)benzonitrile (213 mg, 1.61 mmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (silica gel, CHCl$_3$/MeOH (100/1 → 100/3)) to yield the desired product as a colourless solid.

Chemical formula: C$_{26}$H$_{27}$FN$_4$O$_3$

Molar mass: 462.5 g/mol

Appearance: colourless solid

Yield: 202 mg (81 %)

Melting point: 208 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3213 (w), 3061 (w), 2967 (w), 2874 (w), 2226 (w), 1655 (m), 1625 (m), 1522 (s), 1457 (s), 1261 (s), 1112 (s), 810 (s).

HRMS: [M+H]$^+$ 463.21399 m/z, found 463.21565 m/z.

Purity (HPLC method I): 98.8 %

logP (HPLC method II): 2.98

¹H NMR (400 MHz, CDCl$_3$): δ = 10.65 (t, 1H, ^3J = 5.9 Hz, CON**H**), 8.63 (s, 1H, 2-**H**), 7.67 (m, 1H, Bn-C**H**$_{arom.}$), 7.62 (m, 1H, Bn-C**H**$_{arom.}$), 7.54 (m, 1H, Bn-C**H**$_{arom.}$), 7.43 (t, 1H, ^3J = 7.7 Hz, Bn-C**H**$_{arom.}$), 6.68 (dd, 1H, $^3J_{H,F}$ = 14.9 Hz, ^4J = 2.1 Hz, 6-**H**), 6.43 (d, 1H, ^4J = 2.1 Hz, 8-**H**), 4.67 (d, 2H, ^3J = 5.9 Hz, NHC**H**$_2$), 4.16 (t, 2H, ^3J = 7.4 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.91 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.35 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.90 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.47 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 1.03 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

¹³C NMR (100 MHz, CDCl$_3$): δ = 175.8 (1C, **C**-4), 165.5 (1C, **C**ONH), 164.3 (d, 1C, $^1J_{C,F}$ = 247.9 Hz, **C**-5), 154.1 (d, 1C, $^3J_{C,F}$ = 12.5 Hz, **C**-7), 147.8 (1C, **C**-2), 142.6 (d, 1C, $^3J_{C,F}$ = 4.2 Hz, **C**-8a), 140.8 (1C, NHCH$_2$**C**), 132.2 (1C, Bn-**C**$_{arom.}$), 131.3 (1C, Bn-**C**$_{arom.}$), 130.9 (1C, Bn-**C**$_{arom.}$), 129.4 (1C, Bn-**C**$_{arom.}$), 118.8 (1C, **C**N), 112.7 (1C, **C**CN), 112.1 (1C, **C**-3), 110.6 (1C, **C**-4a), 100.1 (d, 1C, $^2J_{C,F}$ = 25.6 Hz, **C**-6), 94.0 (1C, **C**-8), 66.4 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 54.6 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 47.4 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 42.6 (1C, NH-**C**H$_2$), 30.5 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 20.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.8 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

General synthetic procedure (**G**) for *N*-((aminomethyl)benzyl))-1,4-dihydroquinoline-3-carbox-amides:

The compounds **55a-f** were dissolved in a mixture of MeOH and CH_2Cl_2 and transferred to a sealed pressure tube. A Raney nickel suspension (2 mL) was added and the reaction was stirred at r.t. under a 10 bar H_2 atmosphere for 12 h. The suspension was filtered through celite and the filter cake was washed with MeOH. The solvent of the filtrate was removed *in vacuo* and water was added to the residue. The aqueous solution was acidified with a 2 M HCl solution and washed with CH_2Cl_2. Afterwards, the aqueous solution was basified with a 2 M NaOH solution and extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (RP-18, H_2O + 0.1 % FA/MeOH + 0.1 % FA). The products were dried by repeatedly suspending in pentane and removing the solvent under vacuum.

*Table 21. Used amounts in the synthesis of the primary amines **60a-f**.*

Nitrile	Product	Sample weight [mg]	Amount of substance [µmol]
55a	60a	59.3	135
55b	60b	160	360
55c	60c	223	482
55d	60d	252	545
55e	60e	328	709
55f	60f	220	476

N-(4-(Aminomethyl)benzyl)-1-butyl-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**60a**)
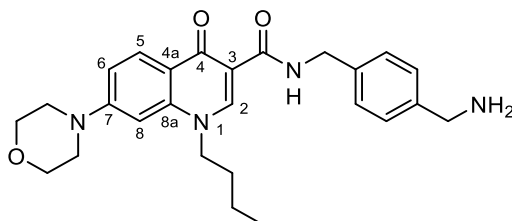
Chemical formula: $C_{26}H_{32}N_4O_3$

Molar mass: 448.6 g/mol

Appearance: colourless solid

Yield: 23 mg (51.3 µmol, 38 %)

Melting point: 169 °C

Mass: *m/z* 449.20 [M + H]$^+$

logP (HPLC method II): 3.24

¹H NMR (400 MHz, DMSO-*d$_6$*): δ = 10.45 (t, 1H, ^3J = 5.6 Hz, CON**H**), 8,71 (s, 1H, 2-**H**), 8.12 (d, 1H, ^3J = 9.1 Hz, 5-**H**), 7.19-7.31 (m, 5H, 4 x Bn-C**H**$_{arom.}$ + 6-**H**), 6.89 (d, 1H, ^4J = 1.7 Hz, 8-**H**), 4.50 (d, 2H, ^3J = 5.6 Hz, NHC**H₂**), 4.43 (t, 2H, ^3J = 7.3 Hz, NC**H₂**CH₂CH₂CH₃), 3.77 (m, 4H, morpholino-C**H₂**-O-C**H₂**), 3.69 (s, 2H, C**H₂**NH₂), 3.38 (m, 4H, morpholino-C**H₂**-N-C**H₂**), 1.74 (quint, 2H, ^3J = 7.3 Hz, NCH₂C**H₂**CH₂CH₃), 1.32 (sext, 2H, ^3J = 7.3 Hz, NCH₂CH₂C**H₂**CH₃), 0.91 (t, 3H, ^3J = 7.3 Hz, NCH₂CH₂CH₂C**H₃**) ppm.

¹³C NMR (100 MHz, DMSO-*d$_6$*): δ = 175.2 (1C, **C**-4), 164.9 (1C, **C**ONH), 154.4 (1C, **C**-7), 148.3 (1C, **C**-2), 143.3 (1C, **C**CH₂NH₂), 141.5 (1C, **C**-8a), 138.0 (1C, NHCH₂**C**), 126.9-128.4 (5C, 4 x Bn-**C**$_{arom.}$ + **C**-5), 119.4 (1C, **C**-4a), 114.1 (1C, **C**-6), 109.9 (1C, **C**-3), 99.1 (1C, **C**-8), 66.4 (2C, morpholino-**C**H₂-O-**C**H₂), 53.0 (1C, N**C**H₂CH₂CH₂CH₃), 47.6 (2C, morpholino-**C**H₂-N-**C**H₂), 45.7 (1C, **C**H₂NH₂), 42.5 (1C, NH**C**H₂), 30.8 (1C, NCH₂**C**H₂CH₂CH₃), 19.6 (1C, NCH₂CH₂**C**H₂CH₃), 14.2 (1C, NCH₂CH₂CH₂**C**H₃) ppm.

Only small amounts of quinolone amides **60a-f** with a primary amine function were reisolated after their decomposition. Therefore, no IR spectrum was measured. The assignment of the quaternary aromatic carbon atoms was done using the 2D spectra due to low concentration.

N-(4-(Aminomethyl)benzyl)-1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**60b**)
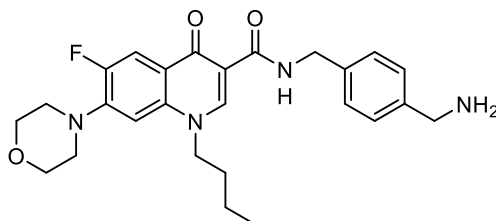
Chemical formula: $C_{26}H_{31}FN_4O_3$

Molar mass: 466.6 g/mol

Appearance: colourless solid

Yield: 120 mg (257 µmol, 53 %)

Melting point: 173 °C

HRMS: [M+H]$^+$ 467.24529 m/z, found 467.24515 m/z.

logP (HPLC method II): 3.80

The NMR characterisation was done with the formate salt of compound **60b**.

^{1}H NMR (400 MHz, DMSO-*d$_6$*): δ = 10.35 (t, 1H, ^3J = 5.8 Hz, CON**H**), 8,78 (s, 1H, 2-**H**), 7.87 (d, 1H, $^3J_{H,F}$ = 13.6 Hz, 5-**H**), 7.35 (m, 4H, 4 x Bn-C**H**$_{arom.}$), 7.10 (d, 1H, $^4J_{H,F}$ = 7.4 Hz, 8-**H**), 4.53 (d, 2H, ^3J = 5.8 Hz, NHC**H$_2$**), 4.48 (t, 2H, ^3J = 7.3 Hz, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 3.87 (s, 2H, C**H$_2$**NH$_2$), 3.78-3.81 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.23-3.27 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.77 (quint, 2H, ^3J = 7.3 Hz, NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.32 (sext, 2H, ^3J = 7.3 Hz, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 0.92 (t, 3H, ^3J = 7.3 Hz, NCH$_2$CH$_2$CH$_2$C**H$_3$**), ppm.

^{13}C NMR (100 MHz, DMSO-*d$_6$*): δ = 174.0 (1C, **C**-4), 164.1 (1C, **C**ONH), 152.3 (1C, $^1J_{C,F}$ = 195.5 Hz, **C**-6), 147.8 (1C, **C**-2), 144.3 (1C, **C**-7), 138.5 (1C, NHCH$_2$**C**), 137.7 (1C, **C**CH$_2$NH$_2$), 137.0 (1C, **C**-8a), 128.2 (2C, Bn-**C**$_{arom.}$), 127.4 (2C, Bn-**C**$_{arom.}$), 121.4 (1C, **C**-4a), 110.0 (1C, **C**-5), 105.6 (1C, **C**-8), 65.9 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 54.9 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 49.9 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 43.2 (1C, **C**H$_2$NH$_2$), 41.9 (1C, NH**C**H$_2$), 30.3 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.2 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.5 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

Only small amounts of quinolone amides **60a-f** with a primary amine function were reisolated after their decomposition. Therefore, no IR spectrum was measured. The assignment of the quaternary aromatic carbon atoms was done using the 2D spectra due to low concentration. The signal of C-3 was not found due to no interactions in the HMBC spectrum.

N-(4-(Aminomethyl)benzyl)-1-butyl-5-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**60c**)
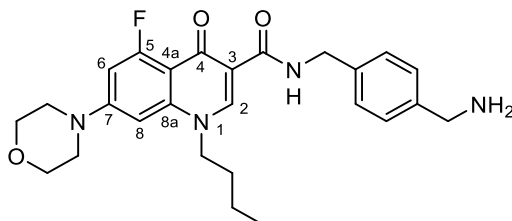
Chemical formula: $C_{26}H_{31}FN_4O_3$

Molar mass: 466.6 g/mol

Appearance: colourless solid

Yield: 100 mg (214 μmol, 39 %)

Melting point: 172 °C

HRMS: [M+H]$^+$ 467.24529 m/z, found 467.24563 m/z.

logP (HPLC method II): 2.72

^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.33 (t, 1H, ^3J = 5.7 Hz, CON**H**), 8.65 (s, 1H, 2-**H**), 7.24-7.31 (m, 4H, 4 x Bn-C**H**$_{arom.}$), 6.92 (dd, 1H, $^3J_{H,F}$ = 15.8 Hz, ^4J = 1.7 Hz, 6-**H**), 6.66 (d, 1H, ^4J = 1.7 Hz, 8-**H**), 4.57 (d, 2H, ^3J = 5.7 Hz, NHC**H**$_2$), 4.38 (t, 2H, ^3J = 7.3 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.70 (s, 2H, C**H**$_2$NH$_2$), 3.73-3.77 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.36-3.41 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.72 (quint, 2H, ^3J = 7.3 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.31 (sext, 2H, ^3J = 7.3 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.91 (t, 3H, ^3J = 7.3 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 174.3 (1C, **C**-4), 164.0 (1C, **C**ONH), 163.4 (d, 1C, $^1J_{C,F}$ = 297.2 Hz, **C**-5), 154.1 (1C, **C**-7), 147.9 (1C, **C**-2), 143.0 (1C, **C**CH$_2$NH$_2$), 142.1 (1C, **C**-8a), 137.0 (1C, NHCH$_2$**C**), 128.1 (4C, 4 x Bn-**C**$_{arom.}$), 108.7 (1C, **C**-4a), 99.4 (1C, **C**-6), 94.5 (1C, **C**-8), 65.7 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 53.0 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 46.6 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 45.2 (1C, **C**H$_2$NH$_2$), 41.9 (1C, NH**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$_3$) ppm.

Only small amounts of quinolone amides **60a-f** with a primary amine function were reisolated after their decomposition. Therefore, no IR spectrum was measured. The assignment of the quaternary aromatic carbon atoms was done using the 2D spectra due to low concentration. The signal of C-3 was not found due to no interactions in the HMBC spectrum.

N-(3-(Aminomethyl)benzyl)-1-butyl-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**60d**)

Chemical formula: $C_{26}H_{32}N_4O_3$

Molar mass: 448.6 g/mol

Appearance: colourless solid

Yield: 110 mg (245 µmol, 68 %)

Melting point: 161 °C

HRMS: [M+H]$^+$ 449.25472 m/z, found 449.25649 m/z.

logP (HPLC method II): 3.31

¹H NMR (400 MHz, DMSO-*d₆*): δ = 10.47 (t, 1H, ^3J = 5.8 Hz, CON**H**), 8,72 (s, 1H, 2-**H**), 8.13 (d, 1H, ^3J = 9.1 Hz, 5-**H**), 7.15-7.32 (m, 5H, 4 x Bn-C**H**$_{arom.}$ + 6-**H**), 6.90 (d, 1H, ^4J = 1.7 Hz, 8-**H**), 4.52 (d, 2H, ^3J = 5.8 Hz, NHC**H₂**), 4.44 (t, 2H, ^3J = 7.3 Hz, NC**H₂**CH₂CH₂CH₃), 3.78 (m, 4H, morpholino-C**H₂**-O-C**H₂**), 3.71 (s, 2H, C**H₂**NH₂), 3.39 (m, 4H, morpholino-C**H₂**-N-C**H₂**), 1.76 (quint, 2H, ^3J = 7.3 Hz, NCH₂C**H₂**CH₂CH₃), 1.33 (sext, 2H, ^3J = 7.3 Hz, NCH₂CH₂C**H₂**CH₃), 0.92 (t, 3H, ^3J = 7.3 Hz, NCH₂CH₂CH₂C**H₃**) ppm.

¹³C NMR (100 MHz, DMSO-*d₆*): δ = 175.2 (1C, **C**-4), 165.0 (1C, **C**ONH), 154.4 (1C, **C**-7), 148.3 (1C, **C**-2), 144.8 (1C, **C**CH₂NH₂), 141.5 (1C, **C**-8a), 139.7 (1C, NHCH₂**C**), 128.7 (1C, Bn-**C**$_{arom.}$), 127.8 (1C, C-5), 126.7 (1C, Bn-**C**$_{arom.}$), 126.2 (1C, Bn-**C**$_{arom.}$), 125.8 (1C, Bn-**C**$_{arom.}$), 119.6 (1C, **C**-4a), 114.0 (1C, **C**-6), 110.6 (1C, **C**-3), 99.0 (1C, **C**-8), 66.3 (2C, morpholino-**C**H₂-O-**C**H₂), 52.9 (1C, N**C**H₂CH₂CH₂CH₃), 47.6 (2C, morpholino-**C**H₂-N-**C**H₂), 46.1 (1C, **C**H₂NH₂), 42.7 (1C, NH**C**H₂), 30.7 (1C, NCH₂**C**H₂CH₂CH₃), 19.7 (1C, NCH₂CH₂**C**H₂CH₃), 14.0 (1C, NCH₂CH₂CH₂**C**H₃) ppm.

Only small amounts of quinolone amides **60a-f** with a primary amine function were reisolated after their decomposition. Therefore, no IR spectrum was measured.

N-(3-(Aminomethyl)benzyl)-1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**60e**)

Chemical formula: $C_{26}H_{31}FN_4O_3$

Molar mass: 466.6 g/mol

Appearance: colourless solid

Yield: 98 mg (210 µmol, 30 %)

Melting point: 176 °C
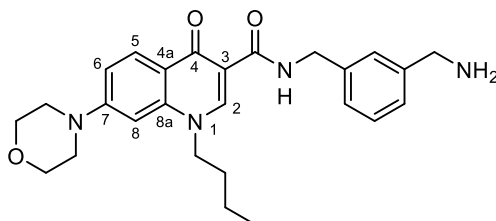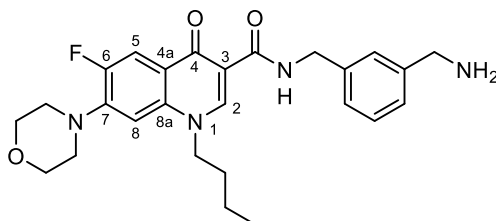
HRMS: $[M+H]^+$ 467.24529 m/z, found 467.24656 m/z.

logP (HPLC method II): 3.83

The NMR characterisation was performed with the formate salt of compound **60e**.

^{1}H NMR (400 MHz, DMSO-*d$_6$*): δ = 10.35 (t, 1H, ^3J = 5.8 Hz, CON**H**), 8.79 (s, 1H, 2-**H**), 7.88 (d, 1H, $^3J_{H,F}$ = 13.6 Hz, 5-**H**), 7.35 (s, 1H, Bn-C**H**$_{arom.}$), 7.24-7.34 (m, 3H, Bn-3 x C**H**$_{arom.}$), 7.10 (d, 1H, $^4J_{H,F}$ = 7.2 Hz, 8-**H**), 4.54 (d, 2H, ^3J = 5.8 Hz, NHC**H**$_2$), 4.49 (t, 2H, ^3J = 7.3 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.85 (s, 2H, C**H**$_2$NH$_2$), 3.77-3.82 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.23-3.28 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.77 (quint, 2H, ^3J = 7.3 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.33 (sext, 2H, ^3J = 7.3 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.92 (t, 3H, ^3J = 7.3 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, DMSO-*d$_6$*): δ = 175.3 (1C, **C**-4), 164.1 (1C, **C**ONH), 151.8 (1C, $^1J_{C,F}$ = 231.8 Hz, **C**-6), 147.8 (1C, **C**-2), 144.2 (1C, **C**-7), 140.5 (1C, **C**CH$_2$NH$_2$), 139.4 (1C, NHCH$_2$**C**), 136.6 (1C, **C**-8a), 126.2-128.5 (4C, 4 x Bn-**C**$_{arom.}$), 121.5 (1C, **C**-4a), 110.0 (1C, **C**-5), 105.6 (1C, **C**-8), 66.4 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 53.3 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 50.3 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 44.4 (1C, **C**H$_2$NH$_2$), 42.6 (1C, NH**C**H$_2$), 30.3 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.2 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.5 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

Only small amounts of quinolone amides **60a-f** with a primary amine function were reisolated after their decomposition. Therefore, no IR spectrum was measured. The assignment of the quaternary aromatic carbon atoms was done using the 2D spectra due to low concentration. The signal of C-3 was not found due to no interactions in the HMBC spectrum.

N-(3-(Aminomethyl)benzyl)-1-butyl-5-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**60f**)

Chemical formula: $C_{26}H_{31}FN_4O_3$

Molar mass: 466.6 g/mol

Appearance: colourless solid

Yield: 123 mg (264 μmol, 55 %)

Melting point: 159 °C

Mass: *m/z* 467.20 [M + H]$^+$

¹H NMR (400 MHz, DMSO-*d₆*): δ = 10.35 (t, 1H, ^3J = 5.8 Hz, CON**H**), 8.66 (s, 1H, 2-**H**), 7.52-7.65 (m, 1H, Bn-C**H**$_{arom.}$), 7.17-7.36 (m, 3H, Bn-C**H**$_{arom.}$), 6.93 (d, 1H, $^3J_{H,F}$ = 15.7 Hz, 6-**H**), 6.66 (s, 1H, 8-**H**), 4.52 (d, 2H, ^3J = 5.8 Hz, NHC**H₂**), 4.38 (t, 2H, ^3J = 7.3 Hz, NC**H₂**CH₂CH₂CH₃), 3.83 (s, 2H, C**H₂**NH₂), 3.72-3.77 (m, 4H, morpholino-C**H₂**-O-C**H₂**), 3.37-3.42 (m, 4H, morpholino-C**H₂**-N-C**H₂**), 1.72 (quint, 2H, ^3J = 7.3 Hz, NCH₂C**H₂**CH₂CH₃), 1.32 (sext, 2H, ^3J = 7.3 Hz, NCH₂CH₂C**H₂**CH₃), 0.91 (t, 3H, ^3J = 7.3 Hz, NCH₂CH₂CH₂C**H₃**) ppm.

¹³C NMR (100 MHz, DMSO-*d₆*): δ = 174.7 (1C, **C**-4), 164.5 (1C, **C**ONH), 163.4 (d, 1C, $^1J_{C,F}$ = 279.0 Hz, **C**-5), 154.2 (1C, **C**-7), 148.5 (1C, **C**-2), 144.9 (1C, **C**CH₂NH₂), 142.5 (1C, **C**-8a), 139.7 (1C, NHCH₂**C**), 128.7 (1C, Bn-**C**$_{arom.}$), 126.7 (1C, Bn-**C**$_{arom.}$), 126.1 (1C, Bn-**C**$_{arom.}$), 125.8 (1C, Bn-**C**$_{arom.}$), 109.1 (1C, **C**-4a), 100.0 (1C, **C**-6), 95.0 (1C, **C**-8), 65.9 (2C, morpholino-**C**H₂-O-**C**H₂), 53.1 (1C, N**C**H₂CH₂CH₂CH₃), 46.6 (2C, morpholino-**C**H₂-N-**C**H₂), 45.7 (1C, **C**H₂NH₂), 42.2 (1C, NH**C**H₂), 29.9 (1C, NCH₂**C**H₂CH₂CH₃), 19.1 (1C, NCH₂CH₂**C**H₂CH₃), 13.5 (1C, NCH₂CH₂CH₂**C**H₃) ppm.

Only small amounts of quinolone amides **60a-f** with a primary amine function were reisolated after their decomposition. Therefore, no IR spectrum was measured. The assignment of the quaternary aromatic carbon atoms was done using the 2D spectra due to low concentration. The signal of C-3 was not found due to no interactions in the HMBC spectrum.

8.2.11 Synthesis of quinolone amides with a triethylene glycol residue in position 7

2-(2-(2-Methoxyethoxy)ethoxy)ethan-1-ol (**61**)
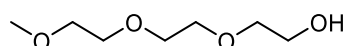
Triethylenglycol (5.00 mL, 36.6 mmol) and NaOH (732 mg, 18.3 mmol) were suspended and stirred at 70 °C for 30 min. Dimethyl sulfate (868 µL, 9.16 mmol) was added dropwise and the mixture was stirred at 110 °C for 1 h. Water (20 mL) and CH_2Cl_2 (20 mL) were added to the stirring mixture, the phases were separated, and the water phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH (100/4)) to yield the desired product.

Chemical formula: $C_7H_{16}O_4$

Molar mass: 164.2 g/mol

Appearance: colourless oil

Yield: 733 mg (4.46 mmol, 12 %, Lit: 33 %)[122]

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3356 (br), 2920 (w), 1647 (m), 1457 (w), 1352 (w), 1090 (m), 625 (s).

^{1}H NMR (400 MHz, CDCl$_3$): δ = 3.72 (m, 2H, C**H**$_2$), 3.67 (m, 6 H, 3 x C**H**$_2$), 3.61 (m, 2H, C**H**$_2$), 3.56 (m, 2H, C**H**$_2$), 3.38 (s, 3H, OC**H**$_3$), 2.51 (t, 1H, ^3J = 6.0 Hz, O**H**) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 72.6 (1C, **C**H$_2$), 72.1 (1C, **C**H$_2$), 70.8 (1C, **C**H$_2$), 70.7 (1C, **C**H$_2$), 70.5 (1C, **C**H$_2$), 61.9 (1C, **C**H$_2$), 59.2 (1C, O**C**H$_3$) ppm.

The spectroscopic data are in accordance with literature.[122]

2-(2-(2-Methoxyethoxy)ethoxy)ethyl-4-methylbenzenesulfonate
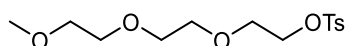
Compound **61** (725 mg, 4.42 mmol) and triethylamine (1.22 mL, 8.83 mmol) were dissolved in abs. CH_2Cl_2 (20 mL) and cooled to 0 °C. Tosyl chloride (842 mg, 4.42 mmol) was added portionwise and the mixture was stirred at r.t. overnight. Water (2 mL) was added and the solvent was removed *in vacuo*. The residue was dissolved in a mixture of a saturated NaHCO$_3$ solution (50 mL) and CH_2Cl_2 (50 mL). After shaking, the phases were separated and the water phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4,

filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH (100/1)).

Chemical formula: $C_{14}H_{22}O_6S$

Molar mass: 318.4 g/mol

Appearance: colourless oil

Yield: 362 mg (1.14 mmol, 26 %, Lit.: 79 %)[123]

IR (ATR, $\tilde{v}$ [cm^{-1}]): 2954 (m), 2922 (s), 2852 (m), 1736 (w), 1458 (m), 1376 (w), 1259 (m), 1097 (m), 1020 (m), 800 (m).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.80 (d, 2H, ^3J = 8.4 Hz, C**H**$_{arom.}$), 7.34 (d, 2H, ^3J = 8.4 Hz, C**H**$_{arom.}$), 4.16 (m, 2H, C**H**$_2$), 3.68 (m, 2H, C**H**$_2$), 3.57-3.62 (m, 6H, 3 x C**H**$_2$), 3.53 (m, 2H, C**H**$_2$), 3.37 (s, 3H, OC**H**$_3$), 2.44 (s, 3H, C**H**$_3$) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 144.8 (1C, **C**CH$_3$), 133.1 (1C, SO$_2$**C**), 129.8 (2C, 2 x **C**H$_{arom.}$), 128.0 (2C, 2 x **C**H$_{arom.}$), 71.9 (1C, **C**H$_2$), 70.8 (1C, **C**H$_2$), 70.6 (1C, **C**H$_2$), 70.6 (1C, **C**H$_2$), 69.2 (1C, **C**H$_2$), 68.7 (1C, **C**H$_2$), 59.0 (1C, O**C**H$_3$), 21.6 (1C, **C**H$_3$) ppm.

The spectroscopic data are in accordance with literature.[123]

1-Azido-2-(2-(2-methoxyethoxy)ethoxy)ethane (**62**)
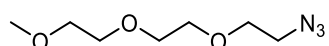
2-(2-(2-Methoxyethoxy)ethoxy)ethyl-4-methylbenzenesulfonate (240 mg, 754 µmol) and sodium azide (98.0 mg, 1.51 mmol) were suspended in abs. DMF (50 mL). The suspension was stirred at 80 °C for 12 h. The solvent was removed *in vacuo* and the residue was dissolved in a mixture of water (50 mL) and CH_2Cl_2 (50 mL). After shaking, the phases were separated and the water phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2) to yield the desired product.

Chemical formula: $C_7H_{15}N_3O_3$

Molar mass: 189.2 g/mol

Appearance: yellow oil

Yield: 121 mg (639 µmol, 85 %, Lit: 100 %)[124]

¹H NMR (400 MHz, CDCl₃): δ = 3.72 (m, 2H, C**H**₂), 3.67 (m, 6H, C**H**₂), 3.61 (m, 2H, C**H**₂), 3.56 (m, 2H, C**H**₂), 3.38 (s, 3H, OC**H**₃) ppm.

¹³C NMR (100 MHz, CDCl₃): δ = 71.9 (1C, **C**H₂), 70.7 (1C, **C**H₂), 70.7 (1C, **C**H₂), 70.6 (1C, **C**H₂), 70.0 (1C, **C**H₂), 59.0 (1C, O**C**H₃), 50.7 (1C, **C**H₂) ppm.

The spectroscopic data are in accordance with literature.[123]

1-Amino-2-(2-(2-methoxyethoxy)ethoxy)ethane (**63**)
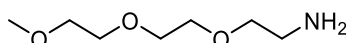
Compound **62** (45.0 mg, 238 µmol) was dissolved in abs. THF and cooled to 0 °C. LiAlH₄ (18.0 mg, 476 µmol) was added, the ice-bath was removed, and the reaction was stirred at r.t. for 1 h. The reaction was quenched by the addition of water (10 mL) and a 1 M HCl solution (2 mL) was added. The solution was washed with EtOAc and basified with a 1 M NaOH solution. The aqueous solution was extracted with EtOAc. The combined organic phases were dried over Na₂SO₄, filtered, and the solvent was removed *in vacuo* to yield the desired product.

Molar mass: 163.2 g/mol

Appearance: yellow oil

Yield: 38.1 mg (233 mmol, 98 %, Lit.: 99 %)[125]

IR (ATR, $\tilde{v}$ [cm⁻¹]): 3439 (br), 3396 (w), 2920 (m), 2871 (m), 1455 (w), 1350 (w), 1247 (w), 1199 (w), 1098 (s), 848 (m).

¹H NMR (400 MHz, CDCl₃): δ = 3.71 (m, 2H, C**H**₂), 3.65 (m, 6H, C**H**₂), 3.59 (m, 2H, C**H**₂), 3.54 (m, 2H, C**H**₂), 3.37 (s, 3H, OC**H**₃), 2.63 (br, 2H, N**H**₂) ppm.

¹³C NMR (100 MHz, CDCl₃): δ = 72.6 (1C, **C**H₂), 72.0 (1C, **C**H₂), 70.7 (1C, **C**H₂), 70.6 (1C, **C**H₂), 70.4 (1C, **C**H₂), 61.8 (1C, **C**H₂), 59.1 (1C, O**C**H₃) ppm.

The spectroscopic data are in accordance with literature.[190]

N-Boc-4-hydroxypiperidine
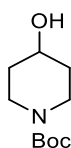
4-Hydroxpiperidine (800 mg, 7.91 mmol) and triethylamine (1.10 mL, 7.91 mmol) were dissolved in abs. THF (60 mL). A solution of di-*tert*-butyl dicarbonate in THF (2 M, 3.95 mL, 7.91 mmol) was added and the mixture was stirred at r.t. for 12 h. Water (40 mL) and diethyl ether (30 mL) were added to the stirring mixture. The phases were separated and the water phase was extracted with diethyl ether. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The crude product was used without further purifications.

Chemical formula: $C_{10}H_{19}NO_3$

Molar mass: 201.3 g/mol

Appearance: yellow oil

Yield: 1.38 g (6.86 mmol, 87 %)

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3458 (m), 2951 (w), 2858 (w), 1657 (s), 1423 (s), 1168 (s), 1067 (s), 770 (m).

¹H NMR (400 MHz, CDCl$_3$): δ = 3.84 (m, 3H, **CH₂**NC**H₂** + C**H**), 3.02 (m, 2H, C**H₂**NC**H₂**), 1.85 (m, 2H, C**H₂**CC**H₂**), 1.59 (s, 1H, O**H**), 1.41-1.50 (m, 11H, C(C**H₃**)₃ + C**H₂**CC**H₂**) ppm.

¹³C NMR (100 MHz, CDCl$_3$): δ = 154.8 (1C, **C**ON), 79.5 (1C, **C**OCON), 67.8 (1C, **C**OH), 41.3 (2C, **C**H₂), 34.2 (2C, **C**H₂), 28.4 (3C, **C**H₃) ppm.

The spectroscopic data are in accordance with literature.[191]

4-(2-(2-(2-Methoxyethoxy)ethoxy)ethoxy)piperidine (**64**)
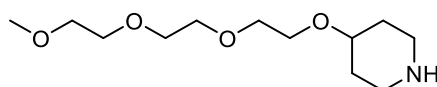
2-(2-(2-Methoxyethoxy)ethoxy)ethyl-4-methylbenzenesulfonate (583 mg, 2.90 mmol) and *N*-Boc-4-hydroxypiperidine (923 mg, 2.90 mmol) were dissolved in abs. THF (200 mL). A sodium hydride suspension (60 wt%, 116 mg, 2.90 mmol) was added and the solution was stirred at r.t. for 12 h. Water (50 mL) and diethyl ether (50 mL) were added to the stirring solution. The phases were separated and the water phase was extracted with diethyl ether. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The

residue was dissolved in THF (50 mL) and TFA (666 µL, 8.70 mmol) was added. The mixture was stirred at r.t. for 3 h. Water (30 mL) and diethyl ether (30 mL) were added to the stirring mixture. The phases were separated and the water phase was washed with diethyl ether. Afterwards, the water phase was basified with a 2 M NaOH solution and extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*.

Chemical formula: $C_{12}H_{25}NO_4$

Molar mass: 247.3 g/mol

Appearance: yellow oil

Yield: 517 mg (2.09 mmol, 72 %)

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3386 (br), 2919 (w), 1647 (m), 1456 (m), 1276 (m), 1254 (m), 1083 (s), 1035 (w), 631 (s).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.60-3.69 (m, 10H, 5 x OC**H**$_2$), 3.55 (m, 2H, OC**H**$_2$), 3.41 (m, 1H, C**H**), 3.38 (s, 3H, OC**H**$_3$), 3.10 (m, 2H, C**H**$_2$NC**H**$_2$), 2.66 (m, 2H, C**H**$_2$NC**H**$_2$), 1.94 (m, 4H, C**H**$_2$CC**H**$_2$) ppm.

1-Butyl-7-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**65a**)
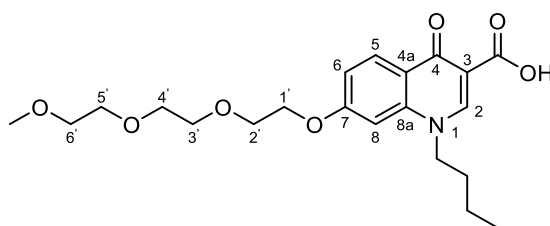
Carboxylic acid **24d** (111 mg, 423 µmol) and compound **61** (69.0 mg, 423 µmol) were dissolved in abs. DMF (50 mL). A sodium hydride suspension (60 wt%, 37.2 mg, 930 µmol) was added and the mixture was stirred at r.t for 3 d. The solvent was removed *in vacuo*. Water (50 mL) and CH_2Cl_2 (50 mL) were added. After shaking, the phases were separated and the water phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH/FA (100/1/1 → 100/10/1) to yield a mixture of the desired product **65a** and the used carboxylic acid **24d** (1/0.6 ratio). Various attempts to separate the mixture by column chromatography were not successful.

Chemical formula: $C_{21}H_{29}NO_7$

Molar mass: 407.5 g/mol

Appearance: yellow solid

Yield: 74.0 mg of the mixture (131 µmol of **65a**, 31 %)

¹H NMR (400 MHz, DMSO-d_6): δ = 15.12 (s, 1H, COO**H**), 8.67 (s, 1H, 2-**H**), 8.46 (d, 1H, ^3J = 9.0 Hz, 5-**H**), 7.17 (dd, 1H, ^3J = 9.0 Hz, ^4J = 1.7 Hz, 6-**H**), 6.99 (d, 1H, ^4J = 1.7 Hz, 8-**H**), 4.30 (m, 2H, 1'-**H**₂), 4.24 (m, 2H, NC**H₂**CH₂CH₂CH₃), 3.94 (m, 2H, 2'-**H**₂), 3.76 (m, 2H, 3'-**H**₂), 3.70 (m, 2H, 4'-**H**₂), 3.65 (m, 2H, 5'-**H**₂), 3.54 (m, 2H, 6'-**H**₂), 3.37 (s, 3H, OC**H₃**), 1.90 (m, 2H, NCH₂C**H₂**CH₂CH₃), 1.46 (m, 2H, NCH₂CH₂C**H₂**CH₃), 1.01 (m, 3H, NCH₂CH₂CH₂C**H₃**) ppm.

N-Benzyl-1-butyl-7-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**66a**)
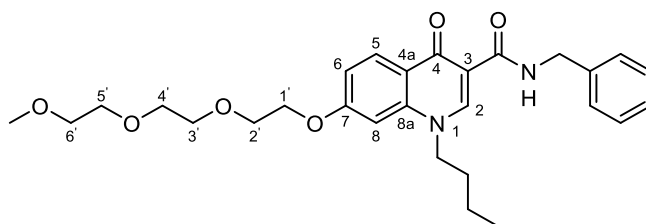
According to the general procedure (**E**), the mixture of carboxylic acid **65a** (74.0 mg, 131 µmol of **65a**) was dissolved in abs. DMF (20 mL) and treated with NMM (79.9 µL, 726 µmol), IBCF (94.5 µL, 726 µmol), and benzylamine (79.4 µL, 726 µmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (silica gel, CH₂Cl₂/MeOH (100/1 → 100/10)) to yield the desired product.

Chemical formula: C₂₈H₃₆N₂O₆

Molar mass: 496.6 g/mol

Appearance: yellow solid

Yield: 45.3 mg (91.2 mmol, 50 %)

Melting point: 68 °C

IR (ATR, $\tilde{v}$ [cm⁻¹]): 3179 (w), 3031 (w), 2954 (w), 2871 (w), 1649 (m), 1619 (w), 1599 (m), 1542 (m), 1468 (m), 1257 (m), 1103 (s), 841 (m), 735 (s).

HRMS: [M+H]⁺ 497.26461 m/z, found 497.26430 m/z.

Purity (HPLC method I): 99.4 %

logP (HPLC method II): 3.57

¹H NMR (400 MHz, CDCl$_3$): δ = 10.47 (t, 1H, ^3J = 5.7 Hz, N**H**), 8.73 (s, 1H, 2-**H**), 8.43 (d, 1H, ^3J = 9.0 Hz, 5-**H**), 7.38 (m, 2H, Bn-C**H**$_{arom.}$), 7.32 (m, 2H, Bn-C**H**$_{arom.}$), 7.23 (m, 1H, Bn-C**H**$_{arom.}$), 7.07 (dd, 1H, ^3J = 9.0 Hz, ^4J = 2.0 Hz, 6-**H**), 6.91 (d, 1H, ^4J = 2.0 Hz, 8-**H**), 4.67 (d, 2H, ^3J = 5.7 Hz, Bn-C**H**$_2$), 4.27 (t, 2H, ^3J = 4.8 Hz, 1'-**H**$_2$), 4.18 (t, 2H, ^3J = 7.4 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.92 (t, 2H, ^3J = 4.8 Hz, 2'-**H**$_2$), 3.76 (m, 2H, 3'-**H**$_2$), 3.69 (m, 2H, 4'-**H**$_2$), 3.65 (m, 2H, 5'-**H**$_2$), 3.54 (m, 2H, 6'-**H**$_2$), 3.37 (s, 3H, OC**H**$_3$), 1.87 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.44 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.99 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

¹³C NMR (100 MHz, CDCl$_3$): δ = 176.3 (1C, **C**-4), 165.3 (1C, **C**ON), 162.7 (1C, **C**-7), 148.0 (1C, **C**-2), 140.9 (1C, **C**-8a), 139.0 (1C, Bn-**C**$_{arom.}$), 129.5 (1C, **C**-5), 128.7 (2C, Bn-**C**$_{arom.}$), 127.8 (2C, Bn-**C**$_{arom.}$), 127.1 (1C, Bn-**C**$_{arom.}$), 122.4 (1C, **C**-4a), 113.5 (1C, **C**-6), 111.7 (1C, **C**-3), 100.3 (1C, **C**-8), 72.1 (1C, **C**-6'), 71.0 (1C, **C**-3'), 70.8 (1C, **C**-4'), 70.8 (1C, **C**-5'), 69.7 (1C, **C**-2'), 68.1 (1C, **C**-1'), 59.2 (1C, O**C**H$_3$), 54.1 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 43.4 (1C, Bn-**C**H$_2$), 30.9 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 20.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.8 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

1-Butyl-7-((2-(2-(2-methoxyethoxy)ethoxy)ethyl)amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**65b**)
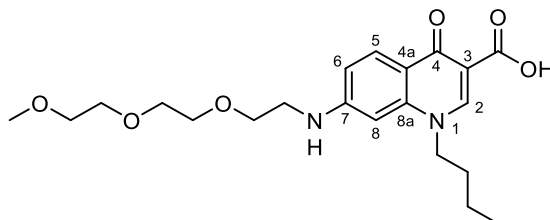
Carboxylic acid **24d** (395 mg, 1.50 mmol), amine **63** (245 mg, 1.50 mmol), and triethylamine (520 μL, 3.75 mmol) were dissolved in abs. DMF (50 mL) and stirred at 110 °C for 7 d. The solvent was removed *in vacuo*. Water (50 mL) and CH$_2$Cl$_2$ (50 mL) were added. After shaking, the phases were separated and the water phase was extracted with CH$_2$Cl$_2$. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH$_2$Cl$_2$/MeOH/FA (100/1/1 → 100/10/1)) to yield the desired product, which still contained some minor impurities. It was directly used in the next step without further purifications.

Chemical formula: C$_{21}$H$_{30}$N$_2$O$_6$

Molar mass: 406.5 g/mol

Appearance: yellow solid

Yield: 183 mg of impure product

N-Benzyl-1-butyl-7-((2-(2-(2-methoxyethoxy)ethoxy)ethyl)amino)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**66b**)
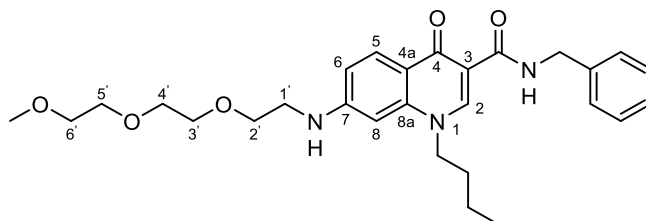
According to the general procedure (**E**), carboxylic acid **65b** (183 mg, 450 µmol) was dissolved in abs. DMF (20 mL) and treated with NMM (198 µL, 1.80 mmol), IBCF (234 µL, 1.80 µmol), and benzylamine (197 µL, 1.80 µmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH (100/1 → 100/10)).

Chemical formula: $C_{28}H_{37}N_3O_5$

Molar mass: 495.6 g/mol

Appearance: colourless solid

Yield: 73.0 mg (147 mmol, 33 %)

Melting point: 88 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3313 (w), 3054 (w), 2955 (w), 2868 (w), 1649 (m), 1623 (w), 1594 (m), 1555 (m), 1505 (m), 1108 (m), 795 (s).

HRMS: [M+H]$^+$ 496.28060 m/z, found 496.28053 m/z.

Purity (HPLC method I): 96.2 %

logP (HPLC method II): 3.38

¹H NMR (400 MHz, CDCl$_3$): δ = 10.56 (t, 1H, ^3J = 5.8 Hz, CON**H**), 8.57 (s, 1H, 2-**H**), 8.19 (d, 1H, ^3J = 8.9 Hz, 5-**H**), 7.32 (m, 2H, Bn-C**H**$_{arom.}$), 7.24 (m, 2H, Bn-C**H**$_{arom.}$), 7.16 (m, 1H, Bn-C**H**$_{arom.}$), 6.68 (dd, 1H, ^3J = 8.9 Hz, ^4J = 2.0 Hz, 6-**H**), 6.31 (d, 1H, ^4J = 2.0 Hz, 8-**H**), 4.89 (t, 1H, ^3J = 4.8 Hz, N**H**), 4.60 (d, 2H, ^3J = 5.8 Hz, Bn-C**H**$_2$), 4.07 (t, 2H, ^3J = 7.4 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.71 (t, 2H, ^3J = 5.1 Hz, 2'-**H**$_2$), 3.61 (m, 6H, 3'-**H**$_2$ + 4'-**H**$_2$ + 5'-**H**$_2$), 3.50 (m, 2H, 6'-**H**$_2$), 3.32 (m, 5H, 1'-**H**$_2$ + OC**H**$_3$), 1.80 (m, 2H, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.37 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$-C**H**$_2$CH$_3$), 0.92 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

¹³C NMR (100 MHz, CDCl₃): δ = 176.1 (1C, **C**-4), 165.7 (1C, **C**ON), 152.2 (1C, **C**-7), 147.2 (1C, **C**-2), 141.5 (1C, **C**-8a), 139.2 (1C, Bn-**C**arom.), 128.8 (1C, **C**-5), 128.6 (2C, Bn-**C**arom.), 127.8 (2C, Bn-**C**arom.), 127.0 (1C, Bn-**C**arom.), 119.4 (1C, **C**-4a), 113.4 (1C, **C**-6), 111.2 (1C, **C**-3), 94.7 (1C, **C**-8), 72.1 (1C, **C**-6'), 70.8 (1C, **C**-5'), 70.7 (1C, **C**-4'), 70.5 (1C, **C**-3'), 69.3 (1C, **C**-2'), 59.2 (1C, O**C**H₃), 53.9 (1C, N**C**H₂CH₂CH₂CH₃), 43.3 (1C, Bn-**C**H₂), 43.2 (1C, **C**-1'), 30.8 (1C, NCH₂**C**H₂CH₂CH₃), 20.1 (1C, NCH₂CH₂**C**H₂CH₃), 13.8 (1C, NCH₂CH₂CH₂**C**H₃) ppm.

1-Butyl-7-(4-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)piperidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**65c**)
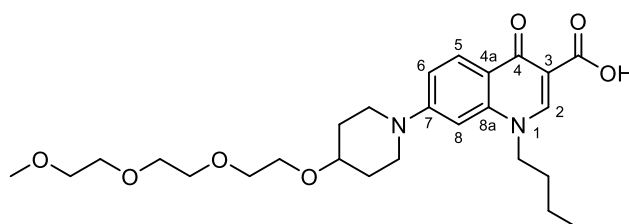
Carboxylic acid **24d** (400 mg, 1.52 mmol), amine **64** (752 mg, 3.04 mmol), and DIPEA (796 µL, 4.56 mmol) were dissolved in abs. DMF (20 mL) and stirred at 110 °C for 2 d. The solvent was removed *in vacuo*. Water (50 mL) and CH₂Cl₂ (50 mL) were added. After shaking, the phases were separated and the water phase was extracted with CH₂Cl₂. The combined organic phases were dried over Na₂SO₄, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH₂Cl₂/MeOH/FA (100/4/1)) to yield the still impure product **65c**. The oil was dissolved in CH₂Cl₂ and extracted with a 1 M NaOH solution. The phases were separated, the water phase was acidified with a 2 M HCl solution, and extracted with CH₂Cl₂. The combined organic phases were dried over Na₂SO₄, filtered, and the solvent was removed *in vacuo* to yield the desired product as a yellow oil.

Chemical formula: C₂₆H₃₈N₂O₇

Molar mass: 490.6 g/mol

Appearance: yellow oil

Yield: 456 mg (929 µmol, 61 %)

IR (ATR, ṽ [cm⁻¹]): 3476 (br), 3055 (w), 2922 (w), 2871 (w), 1712 (m), 1615 (s), 1519 (m), 1461 (s), 1233 (m), 1099 (m), 957 (w), 796 (w).

¹H NMR (400 MHz, CDCl₃): δ = 15.51 (s, 1H, COO**H**), 8.57 (s, 1H, 2-**H**), 8.30 (d, 1H, ³*J* = 9.2 Hz, 5-**H**), 7.12 (dd, 1H, ³*J* = 9.2 Hz, ⁴*J* = 2.1 Hz, 6-**H**), 6.63 (d, 1H, ⁴*J* = 2.1 Hz, 8-**H**), 4.19 (t, 2H, ³*J* = 7.4 Hz, NC**H₂**CH₂CH₂CH₃), 3.62–4.19 (m, 13H, 5 x OC**H₂** + C**H₂**NC**H₂** +

CH$_2$CHCH$_2$), 3.54 (m, 2H, H$_3$COC**H**$_2$), 3.37 (s, 3H, OC**H**$_3$), 3.29 (m, 2H, C**H**$_2$NC**H**$_2$), 2.01 (m, 2H, CH$_2$CHC**H**$_2$), 1.89 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.78 (m, 2H C**H**$_2$CHC**H**$_2$), 1.45 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 1.01 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 177.4 (1C, **C**-4), 167.9 (1C, **C**ON), 154.6 (1C, **C**-7), 147.9 (1C, **C**-2), 141.7 (1C, **C**-8a), 128.5 (1C, **C**-5), 117.6 (1C, **C**-4a), 115.0 (1C, **C**-6), 108.1 (1C, **C**-3), 97.6 (1C, **C**-8), 74.1 (1C, CH$_2$**C**HCH$_2$), 72.1 (1C; H$_3$CO**C**H$_2$), 71.0 (1C, O**C**H$_2$), 70.8 (1C, O**C**H$_2$), 70.8 (1C, O**C**H$_2$), 70.7 (1C, O**C**H$_2$), 67.7 (1C, O**C**H$_2$), 59.2 (1C, O**C**H$_3$), 54.3 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 45.2 (2C, **C**H$_2$N**C**H$_2$), 30.7 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 30.5 (2C, **C**H$_2$CH**C**H$_2$), 20.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.7 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

N-Benzyl-1-butyl-7-(4-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)piperidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**66c**)
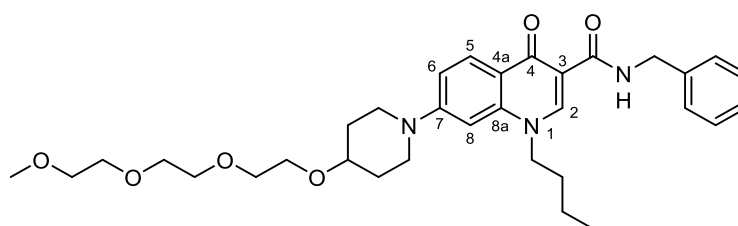
The carboxylic acid **65c** (228 mg, 465 µmol), benzylamine (254 µL, 2.32 mmol), and PyBOP (339 mg, 651 µmol) were dissolved in abs. DMF (15 mL). DIPEA (244 µL, 1.39 mmol) was added and the solution was stirred at r.t. overnight. A saturated ammonium chloride solution (40 mL) was added and the mixture was extracted with CH$_2$Cl$_2$. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed *in vacuo*. The crude product was purified by column chromatography (silica gel, CH$_2$Cl$_2$/MeOH (100/4)) and flash chromatography (RP-18, H$_2$O/MeOH) to yield the desired product as a colourless oil.

Chemical formula: C$_{33}$H$_{45}$N$_3$O$_6$

Molar mass: 579.7 g/mol

Appearance: colourless oil

Yield: 129 mg (223 µmol, 48 %)

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3203 (w), 3051 (w), 2919 (w), 2852 (w), 1655 (m), 1613 (m), 1529 (s), 1461 (s), 1230 (m), 1096 (s), 699 (m).

HRMS: [M+H]$^+$ 580.33811 m/z, found 580.33856 m/z.

Purity (HPLC method I): 98.3 %

logP (HPLC method II): 4.05

^{1}H NMR (400 MHz, CDCl$_3$): δ = 10.58 (t, 1H, ^3J = 5.6 Hz, CON**H**), 8.66 (s, 1H, 2-**H**), 8.31 (d, 1H, ^3J = 9.2 Hz, 5-**H**), 7.39 (d, 2H, ^3J = 7.4 Hz, Bn-C**H**$_{arom.}$), 7.31 (t, 2H, ^3J = 7.4 Hz, Bn-C**H**$_{arom.}$), 7.23 (t, 1H, ^3J = 7.4 Hz, Bn-C**H**$_{arom.}$), 7.07 (dd, 1H, ^3J = 9.2 Hz, ^4J = 1.6 Hz, 6-**H**), 6.63 (br, 1H, 8-**H**), 4.67 (d, 2H, ^3J = 5.6 Hz, Bn-C**H**$_2$), 4.16 (t, 2H, ^3J = 7.4 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.59–3.73 (m, 13H, 5 x OC**H**$_2$ + C**H**$_2$NC**H**$_2$ + CH$_2$CHC**H**$_2$), 3.54 (m, 2H, H$_3$COC**H**$_2$), 3.37 (s, 3H, OC**H**$_3$), 3.31 (m, 2H, C**H**$_2$NC**H**$_2$), 2.00 (m, 2H, C**H**$_2$CHC**H**$_2$), 1.88 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.77 (m, 2H, C**H**$_2$CHC**H**$_2$), 1.45 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 1.00 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 176.1 (1C, **C**-4), 165.6 (1C, **C**ON), 154.1 (1C, **C**-7), 147.5 (1C, **C**-2), 141.1 (1C, **C**-8a), 139.2 (1C, Bn-**C**$_{arom.}$), 128.7 (2C, Bn-**C**$_{arom.}$), 128.6 (1C, **C**-5), 127.8 (2C, Bn-**C**$_{arom.}$), 127.1 (1C, Bn-**C**$_{arom.}$), 120.0 (1C, **C**-4a), 114.4 (1C, **C**-6), 111.4 (1C, **C**-3), 98.1 (1C, **C**-8), 74.4 (1C, CH$_2$**C**HCH$_2$), 72.1 (1C, H$_3$CO**C**H$_2$), 71.0 (1C, O**C**H$_2$), 70.8 (1C, O**C**H$_2$), 70.8 (1C, O**C**H$_2$), 70.7 (1C, O**C**H$_2$), 67.6 (1C, O**C**H$_2$), 59.2 (1C, O**C**H$_3$), 53.8 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 45.6 (2C, **C**H$_2$N**C**H$_2$), 43.4 (1C, Bn-**C**H$_2$), 30.8 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 30.6 (2C, **C**H$_2$CH**C**H$_2$), 20.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.8 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

8.2.12 Synthesis of 7-(4-methyl-1,4-diazepan-1-yl) quinolone amides

1-Butyl-7-(4-methyl-1,4-diazepanyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**67**)
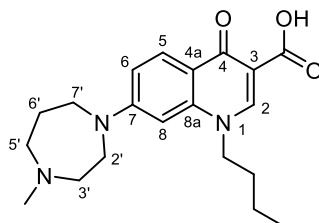
Compound **24d** (1.03 g, 3.92 mmol) and 4-methyl-1,4-diazepane (1.46 mL, 11.8 mmol) were dissolved in DMF (100 mL) and heated under microwave irradiation at 67 °C for 7 h. The solvent was removed *in vacuo* and the residue was purified by flash chromatography (RP-18, H_2O/MeOH + 0.1 % FA).

Chemical formula: $C_{20}H_{27}N_3O_3$

Molar mass: 357.5 g/mol

Appearance: yellow solid

Yield: 1.08 g (2.68 mmol, 68 %)

Melting point: 208 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3075 (w), 2936 (w), 2855 (w), 1705 (m), 1623 (s), 1480 (s), 1224 (m), 1009 (m), 805 (s).

Mass: *m/z* 358.35 [M + H]$^+$

¹H NMR (400 MHz, DMSO-*d₆*): δ = 15.52 (br, 1H, COO**H**), 8.37 (s, 1H, 2-**H**), 8.19 (d, 1H, ^3J = 9.3 Hz, 5-**H**), 6.87 (dd, 1H, ^3J = 9.3 Hz, ^4J = 1.9 Hz, 6-**H**), 6.34 (d, 1H, ^4J = 1.9 Hz, 8-**H**), 4.10 (t, 1H, ^3J = 7.4 Hz, NC**H₂**CH₂CH₂CH₃), 3.74 (m, 2H, 2'-**H₂**), 3.56 (t, 2H, ^3J = 6.2 Hz, 7'-**H₂**), 2.97 (m, 2H, 3'-**H₂**), 2.87 (m, 2H, 5'-**H₂**), 2.50 (s, 3H, C**H₃**), 2.18 (m, 2H, 6'-**H₂**), 1.82 (quint, 2H, ^3J = 7.4 Hz, NCH₂C**H₂**CH₂CH₃), 1.38 (sext, 2H, ^3J = 7.4 Hz, NCH₂CH₂C**H₂**CH₃), 0.93 (t, 3H, ^3J = 7.4 Hz, NCH₂CH₂CH₂C**H₃**) ppm.

The ¹³C NMR spectrum could not be evaluated due to low concentration due to low solubility.

N-Benzyl-1-butyl-7-(4-methyl-1,4-diazepanyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (**68**)
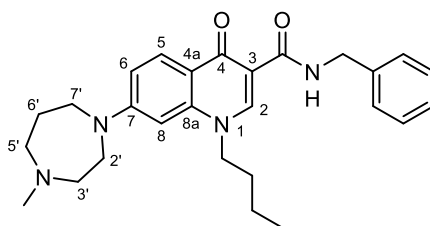
The carboxylic acid **67** (100 mg, 280 µmol), benzylamine (45.9 µL, 420 µmol), and PyBOP (204 mg, 392 µmol) were dissolved in abs. DMF (20 mL). DIPEA (147 µL, 839 mmol) was added and the solution was stirred at r.t. overnight. The solvent was removed *in vacuo* and the residue was purified by flash chromatography (RP-18, H_2O/MeOH) and column chromatography (silica gel, CH_2Cl_2/MeOH (100/2 → 100/10) to yield the desired product.

Chemical formula: $C_{27}H_{34}N_4O_2$

Molar mass: 446.6 g/mol

Appearance: colourless solid

Yield: 64.0 mg (144 µmol, 51 %)

Melting point: 174 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3160 (w), 3037 (w), 2925 (w), 2791 (w), 1649 (m), 1611 (m), 1596 (m), 1542 (m), 1461 (m), 1123 (m), 921 (w), 808 (s).

Mass: [M+H]$^+$ 447.27545 m/z, found 447.27530 m/z.

Purity (HPLC method I): 98.0 %

logP (HPLC method II): 3.77

^{1}H NMR (400 MHz, CDCl$_3$): δ = 10.62 (t, 1H, ^3J = 5.7 Hz, CON**H**), 8.63 (s, 1H, 2-**H**), 8.29 (d, 1H, ^3J = 9.2 Hz, 5-**H**), 7.39 (m, 2H, Bn-C**H**$_{arom.}$), 7.31 (m, 2H, Bn-C**H**$_{arom.}$), 7.22 (m, 1H, Bn-C**H**$_{arom.}$), 6.88 (dd, 1H, ^3J = 9.2 Hz, ^4J = 2.2 Hz, 6-**H**), 6.38 (d, 1H, ^4J = 2.2 Hz, 8-**H**), 4.67 (d, 2H, ^3J = 5.7 Hz, NHC**H**$_2$), 4.14 (t, 2H, ^3J = 7.4 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.70 (m, 2H, 2'-**H**$_2$), 3.60 (t, 2H, ^3J = 6.3 Hz, 7'-**H**$_2$), 2.79 (m, 2H, 3'-**H**$_2$), 2.63 (m, 2H, 5'-**H**$_2$), 2.42 (s, 3H, C**H**$_3$), 2.09 (m, 2H, 6'-**H**$_2$), 1.88 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.44 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.99 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 176.1 (1C, **C**-4), 165.7 (1C, **C**ON), 152.5 (1C, **C**-7), 147.4 (1C, **C**-2), 141.4 (1C, **C**-8a), 139.2 (1C, Bn-**C**$_{arom.}$), 128.8 (1C, **C**-5), 128.6 (2C, Bn-**C**$_{arom.}$), 127.8 (2C, Bn-**C**$_{arom.}$), 127.1 (1C, Bn-**C**$_{arom.}$), 118.4 (1C, **C**-4a), 111.4 (1C, **C**-6), 111.1 (1C, **C**-3), 94.5 (1C, **C**-8), 57.7 (1C, **C**-3'), 57.1 (1C, **C**-5'), 53.8 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 48.8 (1C, **C**-2'), 48.5

(1C, **C**-7'), 46.8 (1C, **C**H$_3$), 43.3 (NH**C**H$_2$), 30.7 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 27.5 (1C, **C**-6'), 20.1 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.8 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

8.2.13 Modifications of position 8

1-Butyl-6,8-difluoro-7-morpholino-4-oxo-N-(1-phenylcyclopropyl)-1,4-dihydroquinoline-3-carboxamide (**74**)
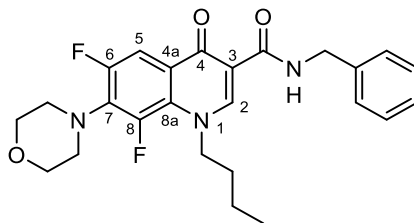
According to the general procedure (**E**), compound **26g** (590 mg, 1.61 mmol) was dissolved in abs. DMF (100 mL) and treated with NMM (708 µL, 6.44 mmol), IBCF (838 µL, 6.44 mmol), and benzylamine (704 µL, 6.44 mmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (silica gel, $CHCl_3$/MeOH (100/1)) and afterwards recrystallized from $CHCl_3$/MeOH.

Chemical formula: $C_{25}H_{25}F_3N_3O_3$

Molar mass: 455.5 g/mol

Appearance: colourless needles

Yield: 478 mg (1.05 mmol, 65 %, Lit.: 25 %)[72]

Melting point: 164 °C (Lit.: 165-167 °C)[72]

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3173 (w), 3063 (w), 3033 (w), 2956 (w), 2871 (w), 1653 (m), 1536 (s), 1472 (s), 1241 (s), 1111 (s), 803 (s), 695 (s).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.17 (t, 1H, ^3J = 5.8 Hz, CON**H**), 8.75 (s, 1H, 2-**H**), 7.81 (d, 1H, $^3J_{H,F}$ = 12.3 Hz, 5-**H**), 7.21-7.37 (m, 5H, Bn-C**H**$_{arom.}$), 4.55 (d, 2H, 4.36 ^3J = 5.8 Hz, NHC**H**$_2$), 4.47 (m, 2H, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.72 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.27 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.76 (quint, 2H, ^3J = 7.3 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.31 (sext, 2H, ^3J = 7.3 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.91 (t, 3H, ^3J = 7.3 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 173.2 (1C, **C**-4), 163.9 (1C, **C**ONH), 154.1 (d, 1C, $^1J_{C,F}$ = 247.3 Hz, **C**-6), 150.5 (1C, **C**-2), 146.2 (d, 1C, $^1J_{C,F}$ = 254.3 Hz, **C**-8), 139.3 (1C, Bn-**C**$_q$), 132.5 (m, 1C, **C**-7), 128.4 (2C, Bn-**C**H$_{arom.}$), 127.4 (2C, Bn-**C**H$_{arom.}$), 127.0 (1C, Bn-**C**H$_{arom.}$), 126.7 (m, 1C, **C**-8a), 122.9 (d, 1C, $^3J_{C,F}$ = 7.9 Hz, **C**-4a), 109.8 (1C, **C**-3), 107.2 (d, 1C, $^2J_{C,F}$ = 22.6 Hz, **C**-5), 66.7 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 57.4 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 50.9 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 32.0 (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$_3$) ppm.

The spectroscopic data are in accordance with literature.[72]

N-Benzyl-1-butyl-8-hydroxy-6-methoxy-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**75**)
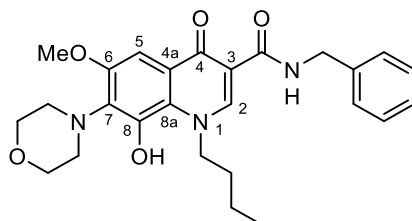
Compound **74** (120 mg, 263 µmol) was dissolved in abs. DMF (15 mL). A sodium methoxide solution (25 wt% in MeOH, 150 µL, 659 µmol) was added and the solution was stirred at 120 °C for 12 h. The solvent was removed *in vacuo* and water (20 mL) and a saturated ammonium chloride solution (2 mL) were added. The mixture was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH (100/0.5 → 100/1)) and recrystallized from EtOH to yield the desired product.

Chemical formula: $C_{26}H_{31}N_3O_5$

Molar mass: 465.6 g/mol

Appearance: yellow crystals

Yield: 55.0 mg (118 µmol, 45 %)

Melting point: 173 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3181 (w), 3085 (br), 2959 (w), 2859 (w), 1653 (m), 1601 (m), 1523 (m), 1459 (m), 1111 (s), 742(m).

HRMS: [M+H]$^+$ 466.23365 m/z, found 466.23444 m/z.

Purity (HPLC method I): 97.0 %

logP (HPLC method II): 4.41

¹H NMR (400 MHz, DMSO-d_6): δ = 10.44 (t, 1H, ^3J = 5.8 Hz, CON**H**), 9.55 (s, 1H, O**H**), 8.67 (s, 1H, 2-**H**), 7.36 (m, 5H, 5-**H** + 4 x Bn-C**H**$_{arom.}$), 7.27 (m, 1H, Bn-C**H**$_{arom.}$), 4.71 (t, 2H, ^3J = 7.4 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 4.55 (d, 2H, ^3J = 5.8 Hz, NHC**H**$_2$), 3.90 (s, 3H, OC**H**$_3$), 3.81 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 1.77 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.30 (sext 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.90 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

The missing ^{1}H NMR signal (morpholino-C**H**$_2$-N-C**H**$_2$) resonates beneath the water signal. It was detectable in the HMQC NMR spectrum.

^{13}**C NMR** (100 MHz, DMSO-d_6): δ = 174.0 (1C, **C**-4), 164.3 (1C, **C**ONH), 155.9 (1C, **C**-7), 148.6 (1C, **C**-2), 145.7 (1C, **C**-8a), 139.3 (1C, NHCH$_2$**C**), 129.2 (1C, **C**-6/8), 128.4 (2C, Bn-**C**H$_{arom.}$), 127.9 (1C, **C**-6/8), 127.3 (2C, Bn-**C**H$_{arom.}$), 126.9 (1C, Bn-**C**H$_{arom.}$), 123.0 (1C, **C**-4), 108.9 (1C, **C**-3), 97.0 (1C, **C**-5), 66.3 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 57.3 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 55.5 (1C, O**C**H$_3$), 49.6 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 42.2 (1C, NH**C**H$_2$), 33.3 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.0 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.6 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

N-Benzyl-1-butyl-6,8-diethoxy-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**76**)
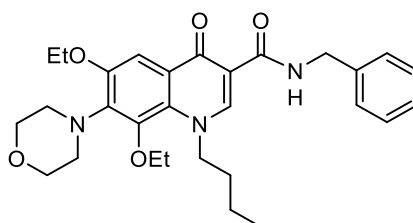
Compound **74** (110 mg, 242 µmol) was dissolved in abs. DMF (10 mL). A sodium ethoxide solution (21 wt% in EtOH, 289 µL, 776 µmol) was added and the solution was stirred at 120 °C for 6 d. The solvent was removed *in vacuo* and water (20 mL) and a saturated ammonium chloride solution (2 mL) were added. The mixture was extracted with CH$_2$Cl$_2$. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH$_2$Cl$_2$/MeOH (100/0.5 → 100/1)) and recrystallized from EtOH to yield the desired product.

Chemical formula: C$_{29}$H$_{37}$N$_3$O$_5$

Molar mass: 507.6 g/mol

Appearance: red crystals

Yield: 63.0 mg (124 µmol, 51 %)

Melting point: 161 °C

IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3177 (w), 3025 (w), 2972 (w), 2847 (w), 1650 (m), 1594 (m), 1542 (m), 1457 (s), 1108 (s), 951 (m), 753 (m).

HRMS: [M+H]$^+$ 508.28060 m/z, found 508.28201 m/z.

Purity (HPLC method I): 96.4 %

logP (HPLC method II): 5.51

¹H NMR (400 MHz, DMSO-d_6): δ = 10.43 (t, 1H, ^3J = 5.8 Hz, CON**H**), 8.66 (s, 1H, 2-**H**), 7.54 (s, 1H, 5-**H**), 7.34 (m, 4H, 4 x Bn-C**H**$_{arom.}$), 7.27 (m, 1H, Bn-C**H**$_{arom.}$), 4.60 (t, 2H, ^3J = 7.3 Hz, NC**H₂**CH₂CH₂CH₃), 4.55 (d, 2H, ^3J = 5.8 Hz, NHC**H₂**), 4.11 (q, 2H, ^3J = 6.9 Hz, OC**H₂**CH₃), 3.86 (q, 2H, ^3J = 7.0 Hz, OC**H₂**CH₃), 3.71 (m, 4H, morpholino-C**H₂**-O-C**H₂**), 3.25 (m, 4H, morpholino-C**H₂**-O-C**H₂**), 1.58 (quint, 2H, ^3J = 7.3 Hz, NCH₂C**H₂**CH₂CH₃), 1.41 (t, 3H, ^3J = 6.9 Hz, OCH₂C**H₃**), 1.36 (t, 3H, ^3J = 7.0 Hz, OCH₂C**H₃**), 1.13 (sext, 2H, ^3J = 7.3 Hz, NCH₂CH₂C**H₂**CH₃), 0.82 (t, 3H, ^3J = 7.3 Hz, NCH₂CH₂CH₂C**H₃**) ppm.

¹³C NMR (100 MHz, DMSO-d_6): δ = 174.0 (1C, **C**-4), 164.3 (1C, **C**ONH), 152.8 (1C, **C**-7), 149.1 (1C, **C**-2), 143.4 (1C, **C**-8a), 139.4 (1C, NHCH₂**C**), 139.1 (1C, **C**-6/8), 128.9 (1C, **C**-4), 128.4 (2C, Bn-**C**H$_{arom.}$), 127.3 (2C, Bn-**C**H$_{arom.}$), 126.9 (1C, Bn-**C**H$_{arom.}$), 124.3 (1C, **C**-6/8), 109.3 (1C, **C**-3), 102.4 (1C, **C**-5), 70.8 (1C, O**C**H₂CH₃), 67.0 (2C, morpholino-**C**H₂-O-**C**H₂), 63.8 (1C, O**C**H₂CH₃), 57.0 (1C, N**C**H₂CH₂CH₂CH₃), 50.6 (2C, morpholino-**C**H₂-N-**C**H₂), 42.1 (1C, NH**C**H₂), 31.6 (1C, NCH₂**C**H₂CH₂CH₃), 18.9 (1C, NCH₂CH₂**C**H₂CH₃), 14.6 (1C, OCH₂**C**H₃), 14.5 (1C, OCH₂**C**H₃), 13.5 (1C, NCH₂CH₂CH₂**C**H₃) ppm.

1-Butyl-8-chloro-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**72**)
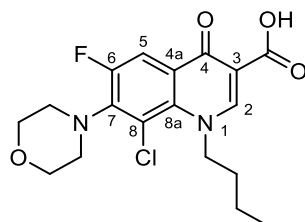
The carboxylic acid **26e** (142 mg, 408 µmol) was dissolved in abs. CH₂Cl₂ (15 mL) and cooled to 0 °C. Sulfuryl chloride (66.0 µL, 817 mmol) was added and the solution was stirred at 0 °C for 40 min. Water (10 mL) and a saturated NaHCO₃ solution (3 mL) were added to the stirring solution. The phases were separated and the water phase was extracted with CH₂Cl₂. The combined organic phases were dried over Na₂SO₄, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CHCl₃/MeOH/FA (100/1/1)) to yield the desired product as a yellow solid.

Chemical formula: C₁₈H₂₀ClFN₂O₄

Molar mass: 382.8 g/mol

Appearance: yellow solid

Yield: 109 mg (285 µmol, 70 %)

¹H NMR (400 MHz, DMSO-d_6): δ = 14.63 (br, 1H, COO**H**), 8.96 (s, 1H, 2-**H**), 8.05 (d, 1H, $^3J_{H,F}$ = 11.9 Hz, 5-**H**), 4.84 (t, 2H, ^3J = 7.5 Hz, NC**H₂**CH₂CH₂CH₃), 3.76 (m, 4H, morpholino-C**H₂**-O-C**H₂**), 3.32 (m, 4H, morpholino-C**H₂**-N-C**H₂**), 1.72 (quint, 2H, ^3J = 7.5 Hz, NCH₂C**H₂**CH₂CH₃), 1.22 (sext, 2H, ^3J = 7.5 Hz, NCH₂CH₂C**H₂**CH₃), 0.87 (t, 3H, ^3J = 7.5 Hz, NCH₂CH₂CH₂C**H₃**) ppm.

The missing ¹H NMR signal of the morpholino ring resonates underneath the water signal. It was detectable in the HMQC NMR spectrum.

¹³C NMR (100 MHz, DMSO-d_6): δ = 176.0 (1C, **C**-4), 165.3 (1C, **C**ON), 155.8 (d, 1C, $^1J_{C,F}$ = 239.0 Hz, **C**-6), 153.1 (1C, **C**-2), 143.3 (d, 1C, $^2J_{C,F}$ = 14.3 Hz, **C**-7), 136.2 (1C, **C**-8a), 123.7 (1C, **C**-8), 120.6 (1C, **C**-4a), 111.2 (d, 1C, $^2J_{C,F}$ = 23.9 Hz, **C**-5), 107.6 (1C, **C**-3), 66.8 (2C, morpholino-**C**H₂-O-**C**H₂), 57.6 (1C, N**C**H₂CH₂CH₂CH₃), 51.3 (2C, morpholino-**C**H₂-N-**C**H₂), 32.0 (1C, NCH₂**C**H₂CH₂CH₃), 19.0 (1C, NCH₂CH₂**C**H₂CH₃), 13.5 (1C, NCH₂CH₂CH₂**C**H₃) ppm.

1-Butyl-8-chloro-6-fluoro-7-morpholino-4-oxo-*N*-benzyl-1,4-dihydroquinoline-3-carboxamide (**71**)
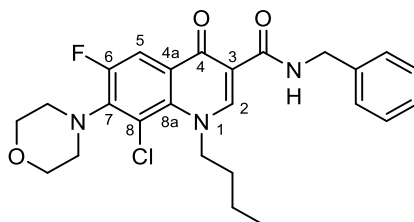
According to the general procedure (**E**), compound **72** (246 mg, 642 µmol) was dissolved in abs. DMF (50 mL) and treated with DIPEA (561 µL, 3.21 mmol), IBCF (334 µL, 2.57 mmol), and benzylamine (281 µL, 2.57 mmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (silica gel, CH₂Cl₂/MeOH (100/1)) to yield the desired product.

Chemical formula: C₂₅H₂₇ClFN₃O₃

Molar mass: 472.0 g/mol

Appearance: orange solid

Yield: 80.9 mg (171 µmol, 27 %)

Melting point: 142 °C

IR (ATR, $\tilde{v}$ [cm⁻¹]): 3253 (w), 3048 (w), 2966 (w), 2849 (w), 1657 (s), 1593 (m), 1549 (m), 1442 (s), 1254 (m), 1115 (s), 945 (s), 802 (s).

HRMS: [M+H]$^+$ 472.17977 m/z, found 472.18084 m/z.

Purity (HPLC method I): 97.4 %

logP (HPLC method II): 5.01

^{1}**H NMR** (400 MHz, CDCl$_3$): δ = 10.08 (t, 1H, ^3J = 5.3 Hz, CON**H**), 8.66 (s, 1H, 2-**H**), 8.06 (d, 1H, $^3J_{H,F}$ = 12.0 Hz, 5-**H**), 7.29 (m, 4H, Bn-C**H**$_{arom.}$), 7.19 (m, 1H, Bn-C**H**$_{arom.}$), 4.59 (m, 4H, Bn-C**H**$_2$ + NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.81 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.26 (br, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.74 (m, 2H, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.26 (m, 2H, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.88 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}**C NMR** (100 MHz, CDCl$_3$): δ = 174.9 (d, 1C, $^4J_{C,F}$ = 2.5 Hz, **C**-4), 164.5 (1C, **C**ON), 156.7 (d, 1C, $^1J_{C,F}$ = 252.0 Hz, **C**-6), 151.7 (1C, **C**-2), 142.8 (d, 1C, $^2J_{C,F}$ = 14.7 Hz, **C**-7), 138.8 (1C, Bn-**C**$_{arom.}$), 136.0 (1C, **C**-8a), 128.7 (2C, Bn-**C**$_{arom.}$), 127.8 (2C, Bn-**C**$_{arom.}$), 127.3 (1C, Bn-**C**$_{arom.}$), 127.0 (d, 1C, $^3J_{C,F}$ = 7.4 Hz, **C**-8), 120.1 (d, 1C, $^3J_{C,F}$ = 5.1 Hz, **C**-4a), 112.7 (d, 1C, $^2J_{C,F}$ = 23.2 Hz, **C**-5), 111.7 (1C, **C**-3), 67.6 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 58.1 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 51.5 (d, 1C, $^4J_{C,F}$ = 4.9 Hz, morpholino-**C**H$_2$-N-**C**H$_2$), 43.5 (1C, NH**C**), 32.8 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.8 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.8 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

1-Butyl-8-bromo-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**77**)
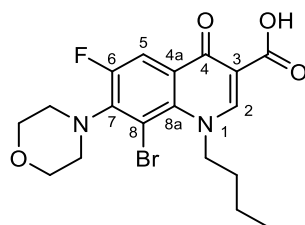
The carboxylic acid **26e** (664 mg, 1.91 mmol) and NBS (848 mg, 4.76 mmol) were dissolved in TFA (10 mL). The solution was stirred at r.t. for 2 h. A saturated sodium sulfite solution (1 mL) was added to quench traces of bromine. Water (20 mL) was added, the solution was basified with a saturated NaHCO$_3$ solution, and extracted with CH$_2$Cl$_2$. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CHCl$_3$/MeOH/FA (100/1/1)) to yield the desired product as an orange solid.

Chemical formula: C$_{18}$H$_{20}$BrFN$_2$O$_4$

Molar mass: 427.3 g/mol

Appearance: orange solid

Yield: 407 mg (953 µmol, 50 %)

Melting point: 164 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3041 (w), 2954 (w), 2850 (w), 1711 (m), 1610 (m), 1441 (s), 1249 (m), 1113 (m), 948 (m), 804 (m).

Mass: m/z 428.85 [M + H]$^+$

^{1}H NMR (400 MHz, DMSO-d_6): δ = 14.56 (s, 1H, COO**H**), 8.98 (s, 1H, 2-**H**), 8.08 (d, 1H, $^3J_{H,F}$ = 11.8 Hz, 5-**H**), 4.88 (t, 2H, ^3J = 7.4 Hz, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 3.78 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.32 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 1.66 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.16 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 0.84 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H$_3$**) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 176.0 (d, 1C, $^4J_{C,F}$ = 2.5 Hz, **C**-4), 165.2 (1C, **C**ON), 156.0 (d, 1C, $^1J_{C,F}$ = 252.9 Hz, **C**-6), 153.0 (1C, **C**-2), 144.8 (d, 1C, $^2J_{C,F}$ = 13.9 Hz, **C**-7), 138.1 (d, 1C, $^4J_{C,F}$ = 1.3 Hz, **C**-8a), 124.6 (d, 1C, $^3J_{C,F}$ = 7.8 Hz, **C**-8), 111.9 (d, 1C, $^2J_{C,F}$ = 23.4 Hz, **C**-5), 110.6 (d, 1C, $^3J_{C,F}$ = 4.4 Hz, **C**-4a), 107.9 (1C, **C**-3), 66.6 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 57.0 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 51.3 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 31.6 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 18.8 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.4 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

1-Butyl-8-bromo-6-fluoro-7-morpholino-4-oxo-*N*-benzyl-1,4-dihydroquinoline-3-carboxamide (**78**)
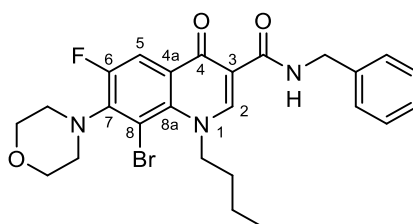
According to the general procedure (**E**), compound **77** (380 mg, 888 µmol) was dissolved in abs. DMF (50 mL) and treated with NMM (390 µL, 3.55 mmol), IBCF (347 µL, 2.66 mmol), and benzylamine (291 µL, 2.66 mmol). The solution was stirred at r.t. for 1 h. The crude product was purified by column chromatography (silica gel, CH$_2$Cl$_2$/MeOH (100/1)) to yield the desired product.

Chemical formula: C$_{25}$H$_{27}$BrFN$_3$O$_3$

Molar mass: 516.4 g/mol

Appearance: colourless solid

Yield: 351 mg (680 µmol, 77 %)

Melting point: 121 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3197 (w), 3078 (w), 2955 (w), 2847 (w), 1666 (m), 1581 (m), 1550 (m), 1430 (m), 1111 (m), 943 (m), 722 (s).

HRMS: [M+H]$^+$ 516.12926 m/z, found 516.13129 m/z.

Purity (HPLC method I): 99.0 %

logP (HPLC method II): 5.05

^{1}H NMR (400 MHz, CDCl$_3$): δ = 10.05 (t, 1H, ^3J = 5.5 Hz, CON**H**), 8.70 (s, 1H, 2-**H**), 8.10 (d, 1H, $^3J_{H,F}$ = 11.9 Hz, 5-**H**), 7.30 (m, 4H, Bn-C**H**$_{arom.}$), 7.20 (m, 1H, Bn-C**H**$_{arom.}$), 4.62 (m, 4H, Bn-C**H**$_2$ + NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.83 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.27 (br, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.70 (m, 2H, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.21 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.86 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 175.0 (d, 1C, $^4J_{C,F}$ = 2.4 Hz, **C**-4), 164.4 (1C, **C**ON), 156.9 (d, 1C, $^1J_{C,F}$ = 253.3 Hz, **C**-6), 151.6 (1C, **C**-2), 144.2 (d, 1C, $^2J_{C,F}$ = 14.6 Hz, **C**-7), 138.8 (1C, Bn-**C**$_{arom.}$), 137.9 (d, 1C, $^4J_{C,F}$ = 1.4 Hz, **C**-8a), 128.7 (2C, Bn-**C**$_{arom.}$), 127.9 (d, 1C, $^3J_{C,F}$ = 7.0 Hz, **C**-8), 127.8 (2C, Bn-**C**$_{arom.}$), 127.3 (1C, Bn-**C**$_{arom.}$), 113.6 (d, 1C, $^2J_{C,F}$ = 23.5 Hz, **C**-5), 112.1 (1C, **C**-3), 111.2 (d, 1C, $^3J_{C,F}$ = 4.3 Hz, **C**-4a), 67.5 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 57.5 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 51.7 (d, 2C, $^4J_{C,F}$ = 5.1 Hz, morpholino-**C**H$_2$-N-**C**H$_2$), 43.5 (1C, NH**C**), 32.5 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.7 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.7 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

8.2.14 Synthesis of 2,3-saturated quinolone amides

3-Bromo-*N*-(3-fluorophenyl)propenamide (**84**)
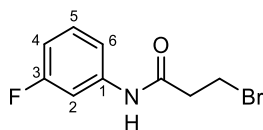
3-Fluoroaniline (1.16 g, 10.4 mmol) and potassium carbonate (1.60 g, 11.6 mmol) were dissolved in abs. CH_2Cl_2 (20 mL) and cooled to 0 °C. 3-Bromopropanoyl chloride (1.30 mL, 12.9 mmol) was added and the solution was stirred at 0 °C for 15 min. The ice bath was removed and the reaction mixture was stirred at r.t. for 2 h. Afterwards, the reaction was quenched with water and the phases were separated. The organic phase was washed with a saturated $NaHCO_3$ solution and the combined water phases were extracted with CH_2Cl_2. The combined organic phases were washed with a 2 M HCl solution, dried over Na_2SO_4, and filtered. The solvent was removed *in vacuo* to yield the desired product as a colourless solid.

Chemical formula: C_9H_9BrFNO

Molar mass: 246.1 g/mol

Appearance: colourless solid

Yield: 2.48 g (10.1 mmol, 97 %, Lit: 99 %)[146]

Melting point: 107 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3272 (w), 3154 (w), 3104 (w), 1662 (m), 1604 (s), 1489 (s), 1420 (s), 1261 (m), 1142 (s), 860 (s), 680 (s).

^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.50 (d, 1H, $^3J_{H,F}$ = 10.8 Hz, 2-**H**), 7.28 (m, 1H, 5-**H**), 7.15 (d, 1H, ^3J = 8.0 Hz, 6-**H**), 6.82 (m, 1H, 4-**H**), 3.71 (t, 2H, ^3J = 6.4 Hz, C**H**$_2$Br), 2.95 (t, 2H, ^3J = 6.4 Hz, C**H**$_2$) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 167.9 (1C, **C**O), 163.2 (d, 1C, $^1J_{C,F}$ = 255.5 Hz, **C**-3), 138.8 (1C, **C**-1), 130.3 (d, 1C, $^3J_{C,F}$ = 9.2 Hz, **C**-5), 115.2 (1C, **C**-6), 111.6 (d, 1C, $^2J_{C,F}$ = 21.2 Hz, **C**-4), 107.7 (d, 1C, $^2J_{C,F}$ = 26.9 Hz, **C**-2), 40.9 (1C, **C**H$_2$), 26.9 (1C, **C**H$_2$) ppm.

1-(3-Fluorophenyl)azetidine-2-one (**83**)

The amide **84** (2.00 g, 8.13 mmol) was dissolved in abs. DMF (40 mL) and sodium hydride (390 mg, 60 wt%, 9.75 mmol) was added. The mixture was stirred at r.t. overnight. The reaction was quenched by the addition of water and the reaction solution was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo* to yield the desired product.

Chemical formula: C_9H_8FNO

Molar mass: 165.2 g/mol

Appearance: colourless solid.

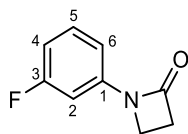Yield: 934 mg (5.65 mmol, 69 %)

Melting point: 70 °C

IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3081 (w), 2960 (w), 2913 (w), 1730 (s), 1612 (m), 1581 (m), 1382 (s), 1195 (s), 1146 (s), 850 (s), 774 (s).

^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.29 (m, 1H, 5-**H**), 7.12 (m, 2H, 2-**H** + 6-**H**), 6.77 (td, 1H, $^3J_{H,F}$ = 12.6 Hz, ^3J = 2.0 Hz, 4-**H**), 3.68 (t, 2H, ^3J = 4.2 Hz, **CH$_2$**), 3.13 (t, 2H, ^3J = 4.2 Hz, **CH$_2$**) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 165.4 (1C, **C**O), 163.3 (d, 1C, $^1J_{C,F}$ = 246.0 Hz, **C**-3), 139.9 (d, 1C, $^3J_{C,F}$ = 10.5 Hz, **C**-1), 130.6 (d, 1C, $^3J_{C,F}$ = 9.2 Hz, **C**-5), 111.8 (d, 1C, $^4J_{C,F}$ = 3.0 Hz, **C**-6), 110.8 (d, 1C, $^2J_{C,F}$ = 21.4 Hz, **C**-4), 104.1 (d, 1C, $^2J_{C,F}$ = 25.8 Hz, **C**-2), 38.5 (1C, **CH$_2$**), 36.6 (1C, **CH$_2$**) ppm.

7-Fluoro-2,3-dihydroquinolin-4(1*H*)-one (**82**)
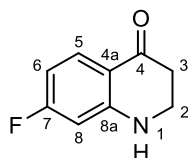
Compound **83** (1.02 g, 6.18 mmol) was dissolved in abs. CH_2Cl_2 (20 mL) and trifluoromethanesulfonic acid (1.08 mL, 12.4 mmol) was added dropwise. The solution was stirred at r.t. for 2 h. Water (5 mL) was added to the stirring solution and the mixture was

neutralized using a 4 M NaOH solution. The phases were separated and the water phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2) to yield the desired product.

Chemical formula: C_9H_8FNO

Molar mass: 165.2 g/mol

Appearance: yellow solid

Yield: 348 mg (2.11 mmol, 34 %, Lit: 99 %)[146]

Melting point: 101 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3341 (w), 3298 (w), 2960 (w), 2854 (w), 1613 (s), 1578 (m), 1245 (s), 1188 (s), 845 (s), 795 (s).

¹H NMR (400 MHz, CDCl$_3$): δ = 7.86 (dd, 1H, ^3J = 8.7 Hz, $^4J_{H,F}$ = 6.6 Hz, 5-**H**), 6.43 (m, 1H, 6-**H**), 6.33 (dd, 1H, $^3J_{H,F}$ = 10.4 Hz, ^3J = 2.1 Hz, 8-**H**), 3.59 (t, 2H, ^3J = 6.9 Hz, C**H**$_2$), 2.69 (t, 2H, ^3J = 6.9 Hz, C**H**$_2$) ppm.

¹³C NMR (100 MHz, CDCl$_3$): δ = 192.8 (1C, **C**O), 167.4 (d, 1C, $^1J_{C,F}$ = 231.7 Hz, **C**-7), 153.7 (1C, **C**-8a), 131.0 (d, 1C, $^3J_{C,F}$ = 12.8 Hz, **C**-5), 116.6 (1C, **C**-4a), 106.6 (d, 1C, $^2J_{C,F}$ = 22.0 Hz, **C**-6), 101.5 (d, 1C, $^2J_{C,F}$ = 23.4 Hz, **C**-8), 42.4 (1C, **C**H$_2$), 37.9 (1C, **C**H$_2$) ppm.

7-Fluoro-2,3-dihydroquinolin-4(1*H*)-one (**86**)
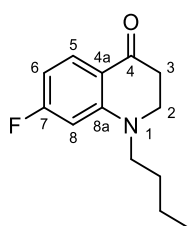
Compound **82** (186 mg, 1.13 mmol) was dissolved in abs. DMF (20 mL) and sodium hydride (90.2 mg, 60 wt%, 2.26 mmol) was added. After stirring at r.t. for 15 min, 1-bromobutane (120 µL, 155 mg) was added and the mixture was stirred at r.t. for 2 h. The reaction was quenched by the addition of water and the phases were separated. The water phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH (100/0 → 100/1)) to yield the desired product.

Chemical formula: $C_{13}H_{16}FNO$

Molar mass: 221.3 g/mol

Appearance: yellow solid

Yield: 96.1 mg (434 µmol, 39 %)

Melting point: 51 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3048 (w), 2955 (m), 2870 (w), 1656 (m), 1616 (s), 1240 (s), 1157 (s), 934 (m), 830 (s).

¹H NMR (400 MHz, CDCl$_3$): δ = 7.89 (dd, 1H, ^3J = 8.7 Hz, $^4J_{H,F}$ = 7.1 Hz, 5-**H**), 6.36 (m, 1H, 6-**H**), (dd, 1H, $^3J_{H,F}$ = 12.0 Hz, ^4J = 2.2 Hz, 8-**H**), 3.53 (t, 2H, ^3J = 7.1 Hz, NC**H$_2$**), 3.30 (t, 2H, ^3J = 7.4 Hz, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 2.66 (t, 2H, ^3J = 7.1 Hz, COC**H$_2$**), 1.61 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.41 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 0.98 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H$_3$**) ppm.

¹³C NMR (100 MHz, CDCl$_3$): δ = 192.3 (1C, **C**O), 168.1 (d, 1C, $^1J_{C,F}$ = 252.2 Hz, **C**-7), 153.3 (d, 1C, $^3J_{C,F}$ = 12.3 Hz, **C**-8a), 131.5 (d, 1C, $^3J_{C,F}$ = 12.0 Hz, **C**-5), 116.6 (1C, **C**-4a), 104.6 (d, 1C, $^2J_{C,F}$ = 23.2 Hz, **C**-6), 99.1 (d, 1C, $^2J_{C,F}$ = 23.4 Hz, **C**-8), 53.4 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 51.7 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 49.3 (1C, N**C**H$_2$), 37.7 (1C, CO**C**H$_2$), 28.5 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 20.5 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 14.0 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

1-Butyl-7-morpholino-2,3-dihydroquinolin-4(1*H*)-one (**81**)
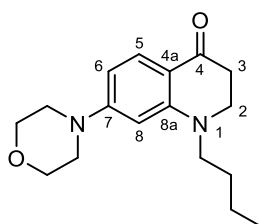
The compound **86** (176 mg, 795 µmol) was dissolved in morpholine (20 mL) and heated at 110 °C under microwave irradiation for 8 h. The solution was acidified (pH ≈ 5) with a 6 M HCl solution and extracted with CH$_2$Cl$_2$. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH$_2$Cl$_2$/MeOH (100/0 → 100/1)) to yield the product as a yellow solid.

Chemical formula: C$_{17}$H$_{24}$N$_2$O$_2$

Molar mass: 288.4 g/mol

Appearance: yellow solid

Yield: 156 mg (541 µmol, 68 %)

Melting point: 68 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3044 (w), 2952 (w), 2865 (w), 1646 (m), 1593 (s), 1449 (m), 1287 (m), 1210 (s), 1118 (s), 946 (m), 803 (s).

^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.84 (d, 1H, ^3J = 8.9 Hz, 5-**H**), 6.33 (td, 1H, ^3J = 8.9 Hz, ^4J = 2.1 Hz, 6-**H**), 6.19 (br, 1H, 8-**H**), 3.90 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.50 (t, 2H, ^3J = 6.9 Hz, NC**H**$_2$), 3.34 (t, 2H, ^3J = 7.4 Hz, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.31 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 2.63 (t, 2H, ^3J = 6.9 Hz, COC**H**$_2$), 1.64 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.43 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.90 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 191.8 (1C, **C**O), 156.0 (1C, **C**-7), 152.9 (1C, **C**-8a), 130.5 (1C, **C**-5), 113.0 (1C, **C**-4a), 104.9 (1C, **C**-6), 96.0 (1C, **C**-8), 66.7 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 51.4 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 49.2 (1C, N**C**H$_2$), 48.0 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 37.8 (1C, CO**C**H$_2$), 28.7 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 20.6 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 14.1 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

2-(Butylamino)-4-chlorobenzoic acid (**91**)
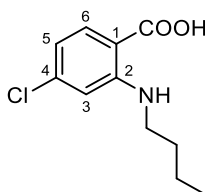
2-Amino-4-chlorobenzoic acid (466 mg, 2.72 mmol), 1-bromobutane (879 µL, 8.15 mmol), and potassium carbonate (1.50 g, 10.9 mmol) were suspended in DMF (100 mL) and stirred at 100 °C for 5 d. The reaction mixture was filtered and the solvent was removed *in vacuo*. The residue was dissolved in EtOH (1 mL) and a 0.5 M HCl solution (30 mL) was added. The forming precipitate was filtered and washed with water. The solid was recrystallized from EtOH to yield the desired product as a colourless solid.

Chemical formula: C$_{11}$H$_{14}$ClNO$_2$

Molar mass: 227.7 g/mol

Appearance: colourless solid

Yield: 471 mg (2.07 µmol, 76 %, Lit.: 50 %)[148]

¹H NMR (400 MHz, DMSO-d_6): δ = 12.78 (br, 1H, COO**H**), 7.76 (d, 1H, ^3J = 8.5 Hz, 6-**H**), 6.73 (d, 1H, ^4J = 2.0 Hz, 3-**H**), 6.55 (dd, 1H, ^3J = 8.5 Hz, ^4J = 2.0 Hz, 5-**H**), 3.16 (t, 2H, ^3J = 7.3 Hz, NC**H₂**CH₂CH₂CH₃), 1.56 (quint, 2H, ^3J = 7.3 Hz, NCH₂C**H₂**CH₂CH₃), 1.38 (sext, 2H, ^3J = 7.3 Hz, NCH₂CH₂C**H₂**CH₃), 0.92 (t, 3H, ^3J = 7.3 Hz, NCH₂CH₂CH₂C**H₃**) ppm.

¹³C NMR (100 MHz, DMSO-d_6): δ = 169.4 (1C, **C**OOH), 151.7 (1C, **C**-2), 139.4 (1C, **C**-4), 133.4 (1C, **C**-6), 113.9 (1C, **C**-5), 110.4 (1C, **C**-3), 108.7 (1C, **C**-1), 41.7 (1C, N**C**H₂CH₂CH₂CH₃), 30.5 (1C, NCH₂**C**H₂CH₂CH₃), 19.7 (1C, NCH₂CH₂**C**H₂CH₃), 13.6 (1C, NCH₂CH₂CH₂**C**H₃) ppm.

Methyl 2-(butylamino)-4-chlorobenzoate (**92**)
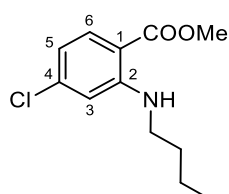
The acid **91** (64.0 mg, 281 µmol) was dissolved in abs. toluene (5 mL) and thionylchloride (39.0 µL, 556 µmol) was added. The mixture was stirred at 80 °C for 1 h and methanol (10 mL) was added. The solution was stirred at r.t. until the yellow colour disappeared and a saturated NaHCO₃ solution (20 mL) was added to the stirring solution. The phases were separated and the water phase was extracted with EtOAc. The combined organic phases were dried over Na₂SO₄, filtered, and the solvent was removed *in vacuo* to yield the desired product as a yellow oil.

Chemical formula: $C_{12}H_{16}ClNO_2$

Molar mass: 241.7 g/mol

Appearance: yellow oil

Yield: 65.0 mg (269 µmol, 96 %, Lit.: 74 %)[149]

¹H NMR (400 MHz, CDCl₃): δ = 7.81 (d, 1H, ^3J = 8.6 Hz, 6-**H**), 6.68 (d, 1H, ^4J = 1.5 Hz, 3-**H**), 6.54 (dd, 1H, ^3J = 8.6 Hz, ^4J = 1.5 Hz, 5-**H**), 3.85 (s, 3H, COOC**H₃**), 3.16 (t, 2H, ^3J = 7.3 Hz, NC**H₂**CH₂CH₂CH₃), 1.68 (quint, 2H, ^3J = 7.3 Hz, NCH₂C**H₂**CH₂CH₃), 1.47 (sext, 2H, ^3J = 7.3 Hz, NCH₂CH₂C**H₂**CH₃), 0.97 (t, 3H, ^3J = 7.3 Hz, NCH₂CH₂CH₂C**H₃**) ppm.

Methyl 4-chloro-2-(2,2,2-trifluoroacetamido)benzoate
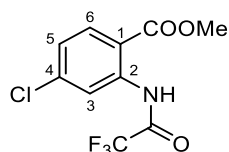
Methyl 4-chlorobenzoate (880 mg, 4.74 mmol) was dissolved in abs. CH_2Cl_2 (20 mL) and the solution was cooled to 0 °C. Trifluoroacetic anhydride (989 μL, 7.11 mmol) was added dropwise and the reaction mixture was stirred at 0 °C for 30 min. The reaction was quenched by the addition of MeOH. Water (30 mL) was added to the stirring mixture, the phases were separated, and the water phase was extracted with CH_2Cl_2. The combined organic phases were washed with a saturated $NaHCO_3$ solution (100 mL), dried over Na_2SO_4, and filtered. The solvent was removed *in vacuo* and the residue was purified by column chromatography (silica gel, CH_2Cl_2) to yield the desired product.

Chemical formula: $C_{10}H_7ClF_3NO_3$

Molar mass: 281.6 g/mol

Appearance: yellow crystals

Yield: 973 mg (3.46 mmol, 73 %)

¹H NMR (400 MHz, DMSO-d_6): δ = 12.32 (s, 1H, N**H**CO), 8.73 (d, 1H, ^4J = 2.0 Hz, 3-**H**), 8.03 (d, 1H, ^3J = 8.6 Hz, 6-**H**), 7.22 (dd, 1H, ^3J = 8.6 Hz, ^4J = 2.0 Hz, 5-**H**), 3.98 (s, 3H, COOC**H₃**) ppm.

Methyl 4-chloro-2-nitrobenzoate
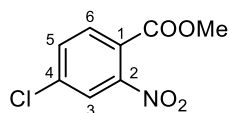
2-Nitro-4-chlorobenzoic acid (2.00 g, 9.92 mmol) was suspended in abs. MeOH (35 mL) and cooled to 0 °C. Thionylchloride (4.32 mL, 59.5 mmol) was added dropwise and the solution was stirred at 0 °C for 10 min. The ice-bath was removed and the reaction mixture was stirred at r.t. for 20 min. Afterwards, the solution was stirred under reflux for 5 h and quenched by addition of water. A saturated $NaHCO_3$ solution (100 mL) and CH_2Cl_2 (100 mL) were added to the stirring mixture. The phases were separated and the water phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo* to yield the desired product as a yellow oil.

Chemical formula: $C_8H_6ClNO_4$

Molar mass: 215.6 g/mol

Appearance: yellow oil

Yield: 1.76 g (8.16 mmol, 82 %, Lit.: 67 %)[192]

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3101 (w), 2958 (w), 1734 (s), 1603 (w), 1540 (s), 1350 (m), 1278 (s), 1102 (s).

^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.86 (d, 1H, ^4J = 2.0 Hz, 3-**H**), 7.74 (d, 1H, ^3J = 8.3 Hz, 6-**H**), 7.64 (dd, 1H, ^3J = 8.3 Hz, ^4J = 2.0 Hz, 5-**H**), 3.92 (s, 3H, COOC**H**$_3$) ppm.

The spectroscopic data are in accordance with literature.[192]

Methyl 4-morpholino-2-ntirobenzoate (**94**)
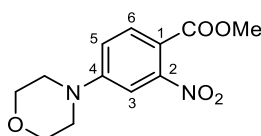
Methyl 4-chloro-2-nitrobenzoate (959 mg, 4.45 mmol), Pd$_2$(dba)$_3$ (204 mg, 222 µmol), caesium carbonate (4.35 g, 13.3 mmol), and RuPhos (208 mg, 445 µmol) were dissolved in abs. 1,4-dioxane (50 mL). Freshly through AlOx filtered morpholine (460 µL, 5.34 mmol) was added and the reaction mixture was stirred at 100 °C for 5 d. Diethylether (100 mL) and water (100 mL) were added to the stirring mixture. The phases were separated and the water phase was extracted with diethylether. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH$_2$Cl$_2$) to yield the desired product as a yellow oil.

Chemical formula: $C_{12}H_{14}N_2O_5$

Molar mass: 266.3 g/mol

Appearance: yellow oil

Yield: 969 mg (3.64 mmol, 82 %)

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3089 (w), 2954 (w), 2858 (w), 1720 (m), 1613 (s), 1537 (s), 1297 (m), 1123 (m), 965 (m), 769 (w).

¹H NMR (400 MHz, CDCl₃): δ = 7.77 (d, 1H, ³J = 8.8 Hz, 6-**H**), 7.05 (d, 1H, ⁴J = 2.5 Hz, 3-**H**), 6.96 (dd, 1H, ³J = 8.8 Hz, ⁴J = 2.5 Hz, 5-**H**), 3.86 (m, 7H, COOC**H**₃ + morpholino-C**H**₂-O-C**H**₂), 3.31 (m, 4H, morpholino-C**H**₂-N-C**H**₂) ppm.

¹³C NMR (100 MHz, CDCl₃): δ = 164.9 (1C, **C**OOCH₃), 153.6 (1C, **C**-4), 151.8 (1C, **C**-2), 132.3 (1C, **C**-6), 115.6 (1C, **C**-5), 114.2 (1C, **C**-1), 108.4 (1C, **C**-3), 66.4 (1C, morpholino-**C**H₂-O-**C**H₂), 52.8 (1C, COO**C**H₃), 47.4 (1C, morpholino-**C**H₂-N-**C**H₂) ppm.

Methyl 2-amino-4-morpholinobenzoate (**95**)
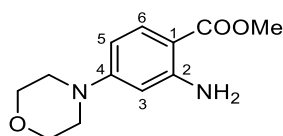
Methyl 4-morpholino-2-ntirobenzoate (953 mg, 3.58 mmol) was dissolved in acetic acid (25 mL) and water (2.5 mL). Zinc powder (2.34 g, 35.8 mmol) was added and the solution was stirred at r.t. for 15 min. The reaction mixture was filtered through cellite to remove the zinc powder. Water was added and the solution was extracted with CH₂Cl₂. The combined organic phases were dried over Na₂SO₄, filtered, and the solvent was removed *in vacuo*. Remaining acetic acid was removed by washing the crude product with a saturated NaHCO₃ solution to yield the product as the free amine.

Chemical formula: C₁₂H₁₆N₂O₃

Molar mass: 236.3 g/mol

Appearance: orange solid

Yield: 771 mg (3.26 mmol, 91 %, Lit.: 94 %)[193]

Melting point: 204 °C

IR (ATR, $\tilde{v}$ [cm⁻¹]): 3442 (w), 3341 (w), 3024 (w), 2946 (w), 2835 (w), 1685 (m), 1611 (m), 1433 (m), 1240 (m), 1087 (s), 957 (m), 772 (m).

¹H NMR (400 MHz, CDCl₃): δ = 7.74 (d, 1H, ³J = 9.0 Hz, 6-**H**), 6.96 (dd, 1H, ³J = 9.0 Hz, ⁴J = 2.3 Hz, 5-**H**), 6.00 (d, 1H, ⁴J = 2.3 Hz, 3-**H**), 5.70 (br, 2H, N**H**₂), 3.82 (m, 7H, COOC**H**₃ + morpholino-C**H**₂-O-C**H**₂), 3.22 (m, 4H, morpholino-C**H**₂-N-C**H**₂) ppm.

¹³C NMR (100 MHz, CDCl₃): δ = 168.4 (1C, **C**OOCH₃), 155.3 (1C, **C**-4), 152.1 (1C, **C**-2), 132.7 (1C, **C**-6), 104.6 (1C, **C**-5), 103.0 (1C, **C**-1), 99.9 (1C, **C**-3), 66.8 (1C, morpholino-**C**H₂-O-**C**H₂), 51.3 (1C, COO**C**H₃), 47.8 (1C, morpholino-**C**H₂-N-**C**H₂) ppm.

The spectroscopic data are in accordance with literature.[193]

Methyl 2-(butylamino)-4-morpholinobenzoate (**90**)
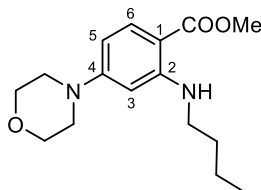
Methyl 2-amino-4-morpholinobenzoate (454 mg, 1.92 mmol), butyraldehyde (242 μL, 3.46 mmol), acetic acid (198 μL, 3.46 μmol), and sodium triacetoxyborohydride (814 mg, 3.84 mmol) were dissolved in abs. MeCN (15 mL) and stirred at r.t. overnight. A reaction control by TLC indicated an incomplete reaction. Therefore, additional sodium triacetoxyborohydride (814 mg, 3.84 mmol) was added and the mixture was stirred at r.t. overnight. The reaction was quenched by the addition of water (50 mL) and CH_2Cl_2 (50 mL) was added to the stirring mixture. The phases were separated and the water phase was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH_2Cl_2) to yield the desired product.

Chemical formula: $C_{16}H_{24}N_2O_3$

Molar mass: 292.4 g/mol

Appearance: colourless solid

Yield: 507 mg (1.73 mmol, 90 %)

Melting point: 61 °C

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3347 (w), 3027 (w), 2955 (w), 2854 (w), 1665 (m), 1609 (m), 1572 (m), 1434 (m), 1212 (s), 1097 (s), 993 (m), 758 (m).

¹H NMR (400 MHz, CDCl₃): δ = 7.78 (d, 1H, ^3J = 9.0 Hz, 6-**H**), 7.74 (br, 1H, N**H**), 6.14 (dd, 1H, ^3J = 9.0 Hz, ^4J = 2.4 Hz, 5-**H**), 5.97 (d, 1H, ^4J = 2.4 Hz, 3-**H**), 3.83 (m, 4H, morpholino-C**H₂**-O-C**H₂**), 3.80 (s, 3H, COOC**H₃**), 3.26 (m, 4H, morpholino-C**H₂**-N-C**H₂**), 3.16 (m, 2H, NC**H₂**CH₂CH₂CH₃), 1.68 (quint, 2H, ^3J = 7.3 Hz, NCH₂C**H₂**CH₂CH₃), 1.47 (sext, 2H, ^3J = 7.3 Hz, NCH₂CH₂C**H₂**CH₃), 0.97 (t, 3H, ^3J = 7.3 Hz, NCH₂CH₂CH₂C**H₃**) ppm.

¹³C NMR (100 MHz, CDCl₃): δ = 168.9 (1C, **C**OOCH₃), 155.8 (1C, **C**-2), 152.9 (1C, **C**-4), 133.1 (1C, **C**-6), 102.3 (1C, **C**-5), 101.8 (1C, **C**-1), 94.8 (1C, **C**-3), 66.8 (2C, morpholino-**C**H₂-O-**C**H₂),

51.1 (1C, COOC**H**$_3$), 47.9 (2C, morpholino-**C**H$_2$-N-**C**H$_2$), 42.6 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 31.3 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 20.5 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 14.0 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

Methyl 1-butyl-3-methyl-7-morpholino-4-oxo-1,2,3,4-tetrahydroquinoline-3-carboxylate (**89**)
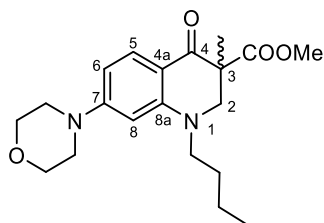
Diisopropylamine (216 µL, 1.54 mmol) was dissolved in abs. 1,4-dioxane (3 mL), cooled to 0 °C, and a 3 M ethylmagnesium bromide solution (205 mg, 1.54 mmol) was added dropwise. The mixture was allowed to adopt to room temperature and stirred at 30 °C for 1 h. Afterwards, the turbid solution was cooled to 0 °C, methyl 2-(butylamino)-4-morpholinobenzoate (225 mg, 770 µmol) and methyl methacrylate (492 µL, 4.62 mmol) were added, and the reaction mixture was stirred at r.t. overnight. A saturated aqueous NH$_4$Cl solution (4 mL) and EtOAc (4 mL) were added to the stirring mixture, the phases were separated, and the water phase was extracted with EtOAc. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH$_2$Cl$_2$/MeOH (100/1)) and additionally by flash chromatography (RP-18, H$_2$O/MeOH) to yield the desired product as a yellow oil.

Chemical formula: C$_{20}$H$_{28}$N$_2$O$_4$

Molar mass: 360.5 g/mol

Appearance: yellow oil

Yield: 180 mg (499 µmol, 65 %)

IR (ATR, $\tilde{v}$ [cm^{-1}]): 3027 (w), 2955 (w), 2856 (w), 1732 (m), 1655 (m), 1594 (s), 1550 (m), 1230 (s), 1121 (s), 959 (m), 811 (m).

Mass *m/z* 361.35 [M + H]$^+$

^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.85 (d, 1H, ^3J = 9.0 Hz, 5-**H**), 6.32 (dd, 1H, ^3J = 9.0 Hz, ^4J = 2.3 Hz, 6-**H**), 5.93 (d, 1H, ^4J = 2.3 Hz, 8-**H**), 3.84 (m, 5H, C**H**$_2$ + morpholino-C**H**$_2$-O-C**H**$_2$), 3.66 (s, 3H, COOC**H**$_3$), 3.23–3.40 (m, 7H, C**H**$_2$ + morpholino-C**H**$_2$-N-C**H**$_2$ + NC**H**$_2$CH$_2$CH$_2$CH$_3$), 1.61 (quint, 2H, ^3J = 7.4 Hz, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.41 (s, 3H, C**H**$_3$), 1.39 (sext, 2H, ^3J = 7.4 Hz, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.98 (t, 3H, ^3J = 7.4 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

¹³C NMR (100 MHz, CDCl₃): δ = 189.8 (1C, **C**-4), 173.1 (1C, **C**OOCH₃), 156.4 (1C, **C**-8a), 152.2 (1C, **C**-7), 131.0 (1C, **C**-5), 111.3 (1C, **C**-4a), 105.3 (1C, **C**-6), 95.3 (1C, **C**-8), 66.8 (2C, morpholino-**C**H₂-O-**C**H₂), 58.5 (1C, **C**H₂), 53.0 (1C, **C**CH₃), 52.7 (1, COO**C**H₃), 51.2 (1C, N**C**H₂CH₂CH₂CH₃), 47.7 (2C, morpholino-**C**H₂-N-**C**H₂), 28.8 (1C, NCH₂**C**H₂CH₂CH₃), 20.5 (1C, NCH₂CH₂**C**H₂CH₃), 18.1 (1C, **C**H₃), 14.1 (1C, NCH₂CH₂CH₂**C**H₃) ppm.

N-Benzyl-1-butyl-3-methyl-7-morpholino-4-oxo-1,2,3,4-tetrahydroquinoline-3-carboxamide (**88**)
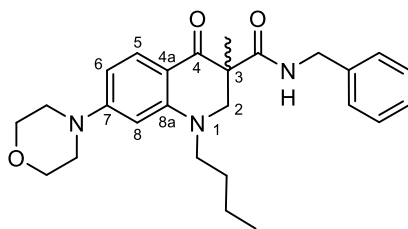
Benzylamine (28.1 µL, 262 µmol) was dissolved in abs. CH₂Cl₂ (2 mL) and a 2 M tri-methylaluminium solution in toluene (131 µL, 262 µmol) was added. The mixture was stirred at r.t. for 30 min. Compound **89** (86.0 mg, 239 µmol) was added and the solution was stirred at r.t. for 48 h. A saturated aqueous NH₄Cl solution (4 mL) and CH₂Cl₂ (4 mL) were added to the stirring mixture, the phases separated, and the water phase was extracted with CH₂Cl₂. The combined organic phases were dried over Na₂SO₄, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH₂Cl₂/MeOH (100/1)) and flash chromatography (RP-18, H₂O/MeOH) to yield the desired product as a yellow oil.

Chemical formula: C₂₆H₃₃N₃O₃

Molar mass: 435.6 g/mol

Appearance: yellow oil

Yield: 33.0 mg (75.8 µmol, 32 %)

IR (ATR, $\tilde{v}$ [cm⁻¹]): 3305 (w), 3032 (w), 2958 (w), 2857 (w), 1661 (m), 1632 (m), 1590 (s), 1229 (s), 1103 (m), 957 (m), 808 (m).

HRMS: [M+H]⁺ 436.25947 m/z, found 436.25945 m/z.

Purity (HPLC method I): 99.8 %

logP (HPLC method II): 3.04

¹H NMR (400 MHz, CDCl₃): δ = 7.81 (d, 1H, ³*J* = 9.1 Hz, 5-**H**), 7.81 (br, 1H, N**H**), 7.23 (m, 5H, Bn-C**H**ₐᵣₒₘ.), 6.29 (dd, 1H, ³*J* = 9.1 Hz, ⁴*J* = 2.2 Hz, 6-**H**), 5.90 (d, 1H, ⁴*J* = 2.2 Hz, 8-**H**), 4.49 (dd, 1H, ²*J* = 15.1 Hz, ³*J* = 5.7 Hz, Bn-C**H₂**), 4.33 (dd, 1H, ²*J* = 15.1 Hz, ³*J* = 5.7 Hz, Bn-C**H₂**), 3.83 (m, 5H, C**H₂** + morpholino-C**H₂**-O-C**H₂**), 3.50 (d, 1H, ²*J* = 12.7 Hz, C**H₂**), 3.39 (m, 2H, NC**H₂**CH₂CH₂CH₃), 3.30 (m, 4H, morpholino-C**H₂**-N-C**H₂**), 1.65 (quint, 2H, ³*J* = 7.4 Hz, NCH₂C**H₂**CH₂CH₃), 1.42 (m, 5H, C**H₃** + NCH₂CH₂C**H₂**CH₃), 0.98 (t, 3H, ³*J* = 7.4 Hz, NCH₂CH₂CH₂C**H₃**) ppm.

¹³C NMR (100 MHz, CDCl₃): δ = 193.7 (1C, **C**ONH), 171.4 (1C, **C**-4), 156.8 (1C, **C**-8a), 152.9 (1C, **C**-7), 138.5 (1C, Bn-**C**q), 131.2 (1C, **C**-5), 128.7 (2C, Bn-**C**Hₐᵣₒₘ.), 127.4 (2C, Bn-**C**Hₐᵣₒₘ.), 127.3 (1C, Bn-**C**Hₐᵣₒₘ.), 110.6 (1C, **C**-4a), 105.3 (1C, **C**-6), 94.7 (1C, **C**-8), 66.7 (2C, morpholino-**C**H₂-O-**C**H₂), 56.7 (1C, **C**H₂), 51.3 (1C, **C**CH₃), 51.2 (1C, N**C**H₂CH₂CH₂CH₃), 47.5 (2C, morpholino-**C**H₂-N-**C**H₂), 43.5 (Bn-**C**H₂), 28.6 (1C, NCH₂**C**H₂CH₂CH₃), 21.5 (1C, **C**H₃), 20.5 (1C, NCH₂CH₂**C**H₂CH₃), 14.1 (1C, NCH₂CH₂CH₂**C**H₃) ppm.

(*E*)-*N*-Benzyl-4-(benzylimino)-1-butyl-3-methyl-7-morpholino-1,2,3,4-tetrahydroquinoline-3-carboxamide (**97**)
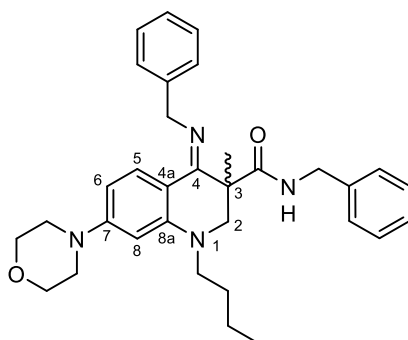
Chemical formula: C₃₃H₄₀N₄O₂

Molar mass: 524.7 g/mol

Appearance: yellow oil

Yield: 46.6 mg (75.8 µmol, 32 %)

Mass *m*/*z* 525.20 [M + H]⁺

NMR spectra only stated for the major isomer (*E*).

¹H NMR (400 MHz, CDCl₃): δ = 8.17 (m, 1H, N**H**), 7.15-7.31 (m, 9H, 5-**H**, 8 x Bn-C**H**ₐᵣₒₘ.), 6.97 (m, 2H, 2 x Bn-C**H**ₐᵣₒₘ.), 6.29 (dd, 1H, ³*J* = 8.8 Hz, ⁴*J* = 2.3 Hz, 6-**H**), 6.05 (d, 1H, ⁴*J* = 2.3 Hz, 8-**H**), 4.98 (br, 2H, Bn-C**H₂**), 4.42 (dd, 1H, ²*J* = 15.1 Hz, ³*J* = 5.6 Hz, Bn-C**H₂**), 4.26 (dd, 1H, ²*J*

= 15.1 Hz, ^3J = 5.6 Hz, Bn-C**H**$_2$), 3.85 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.80 (d, 1H, ^2J = 12.6 Hz, C**H**$_2$), 3.45 (d, 1H, ^2J = 12.6 Hz, C**H**$_2$), 3.34 (m, 2H, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.23 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.63 (m, 2H, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.48 (s, 3H, C**H**$_3$), 1.38 (m, 2H, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.96 (t, 3H, ^3J = 7.3 Hz, NCH$_2$CH$_2$CH$_2$C**H**$_3$) ppm.

**^{13}C NMR** (100 MHz, CDCl$_3$): δ = 173.7 (1C, **C**ONH), 165.5 (1C, **C**-4), 153.9 (1C, **C**-8a), 149.1 (1C, **C**-7), 141.7 (1C, Bn-**C**$_q$), 138.7 (1C, Bn-**C**$_q$), 131.3 (1C, **C**-5), 128.53 (2C, Bn-**C**H$_{arom.}$), 128.55 (2C, Bn-**C**H$_{arom.}$), 127.41 (2C, Bn-**C**H$_{arom.}$), 127.40 (2C, Bn-**C**H$_{arom.}$), 127.0 (1C, Bn-**C**H$_{arom.}$), 126.6 (1C, Bn-**C**H$_{arom.}$), 107.3 (1C, **C**-4a), 102.5 (1C, **C**-6), 96.6 (1C, **C**-8), 66.9 (1C, morpholino-**C**H$_2$-O-**C**H$_2$), 57.7 (1C, **C**H$_2$), 57.0 (1C, Bn-**C**H$_2$), 51.2 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 49.4 (1C, **C**CH$_3$), 48.3 (1C, morpholino-**C**H$_2$-N-**C**H$_2$), 43.3 (Bn-**C**H$_2$), 28.6 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 22.3 (1C, **C**H$_3$), 20.6 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 14.1 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$) ppm.

Methyl 1-butyl-2,3-*trans*-dimethyl-7-morpholino-4-oxo-1,2,3,4-tetrahydroquinoline-3-carboxylate (**98**)
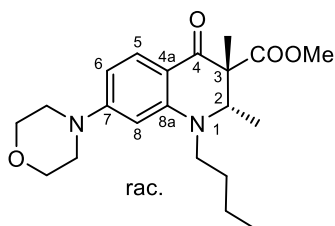
Diisopropylamine (385 μL, 2.74 mmol) was dissolved in abs. 1,4-dioxane (3 mL), cooled to 0 °C, and a 3 M ethylmagnesium bromide solution (912 mg, 2.74 mmol) was added dropwise. The mixture was allowed to adopt to room temperature and stirred at 30 °C for 1 h. Afterwards, the turbid solution was cooled to 0 °C, methyl 2-(butylamino)-4-morpholinobenzoate (400 mg, 1.37 mmol) and methyl (*E*)-2methylbut-2-enoate (997 μL, 8.21 mmol) were added, and the reaction mixture was stirred at r.t. overnight. A saturated aqueous NH$_4$Cl solution (4 mL) and EtOAc (4 mL) were added to the stirring mixture, the phases were separated, and the water phase was extracted with EtOAc. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, CH$_2$Cl$_2$/MeOH (100/1)) and flash chromatography (RP-18, H$_2$O/MeOH) to yield the desired product as a yellow oil.

Chemical formula: C$_{21}$H$_{30}$N$_2$O$_4$

Molar mass: 374.5 g/mol

Appearance: yellow oil

Yield: 86 mg (230 µmol, 17 %)

IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3048 (w), 2955 (w), 2921 (w), 2851 (w), 1731 (m), 1661 (m), 1594 (s), 1230 (s), 1122 (m), 810 (w).

Mass m/z 375.40 [M + H]$^+$

^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.81 (d, 1H, ^3J = 8.9 Hz, 5-**H**), 6.26 (dd, 1H, ^3J = 8.9 Hz, ^4J = 2.2 Hz, 6-**H**), 5.93 (d, 1H, ^4J = 2.2 Hz, 8-**H**), 3.91 (q, 1H, ^3J = 6.8 Hz, C**H**), 3.81 (m, 4H, morpholino-C**H$_2$**-O-C**H$_2$**), 3.58 (s, 3H, COOC**H$_3$**), 3.39 (m, 1H, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 3.26 (m, 4H, morpholino-C**H$_2$**-N-C**H$_2$**), 2.98 (m, 1H, NC**H$_2$**CH$_2$CH$_2$CH$_3$), 1.61 (m, 2H, NCH$_2$C**H$_2$**CH$_2$CH$_3$), 1.38 (m, 2H, NCH$_2$CH$_2$C**H$_2$**CH$_3$), 1.33 (s, 3H, C**H$_3$**), 1.06 (d, 3H, ^3J = 6.8 Hz, C**H$_3$**), 0.98 (t, 3H, ^3J = 7.3 Hz, NCH$_2$CH$_2$CH$_2$C**H$_3$**) ppm.

^{13}C NMR (100 MHz, CDCl$_3$): δ = 189.7 (1C, **C**-4), 174.0 (1C, **C**OOCH$_3$), 156.5 (1C, **C**-8a), 150.1 (1C, **C**-7), 130.1 (1C, **C**-5), 111.7 (1C, **C**-4a), 104.4 (1C, **C**-6), 95.6 (1C, **C**-8), 66.7 (1C, morpholino-**C**H$_2$-O-**C**H$_2$), 62.6 (1C, **C**-3), 56.8 (1C, **C**-2), 52.6 (1, COO**C**H$_3$), 49.9 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 47.6 (1C, morpholino-**C**H$_2$-N-**C**H$_2$), 30.0 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 20.4 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 17.3 (1C, 3-**C**H$_3$), 14.1 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$), 10.7 (1C, 2-**C**H$_3$) ppm.

8.3 Bioactivity assay

Antitrypanosomal Assay. Trypomastigote forms of *T. brucei brucei* laboratory strain M1.2wt Lister 427 were cultured in HMI-9 medium, supplemented with 10 % (v/v) FBS.[194] The AlamarBlue® assay was performed according to previously reported procedure.[68, 71, 76, 175] A defined number of parasites (10^4 trypanosomes per mL) was exposed in preincubated 96 well plates to various concentration levels of the test substances in a final volume of 200 µL. Positive (trypanosomes in culture medium) and negative controls (test substance without trypanosomes) were run with each plate. The plates were then incubated at 37 °C in an atmosphere of 5 % CO$_2$ for a total time of 48 h. The effect of test substances was quantified in IC$_{50}$ values by linear interpolation of two different measurements. The activity of the test substances was measured by light absorption in a Tecan M200 Infinite plate reader at a wavelength of 570 nm with a reference wavelength of 600 nm, using AlamarBlue®. The tests were performed in triplicate and IC$_{50}$ values are presented as mean values of three independent experiments.

8.4 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 cultured in DMEM medium (high glucose), supplemented with 10% FCS, 10 U/mL penicillin G, and 10 µg/mL streptomycin in an atmosphere of 37 °C, 5% CO_2, and 95% humidity. For the experiments, a 0.1 mL cell suspension (cell density: 1 x 10^4/well) was transferred to 96-well plates and incubated overnight. Compound stock solutions were prepared in DMSO and diluted with culture medium. A diluted substance solution (0.1 mL) was added to the cells and incubated for 24 h. Final DMSO concentrations did not exceed 1.25%. Positive (macrophages in culture medium) and negative controls (culture medium without cells) were run with each plate. Following the addition of AlamarBlue® (20 µL), the plates were further incubated for 24 h. The absorbance was read at a wavelength of 570 nm (reference wavelength 600 nm) indicating the viability. The CC_{50} values are presented as mean values of three independent experiments against the macrophages.

8.5 LogP determination

The experimental determination of the logP values of the synthesized compounds was carried out analogously to prior works of our research group.[69, 72, 195] By means of HPLC, the retention time t_R of references and the synthesised substances were measured, from which the capacity factor k' correlating with the logP value was calculated according to the following equation

$$k' = \frac{(t_R - t_0)}{t_0}$$

where t_0 corresponds to the dead time of the column.

All substances were dissolved in MeOH, diluted with MeOH to a concentration of 10 µg/mL, and measured with HPLC method **II**. In the following table, the reference substances and their corresponding logP values according to literature are stated.[196]

Table 22. Reference substances and their logP values.

Reference substance	Capacity factor k'	logk'	logP value
2-Butanone	0.28	-0.55	0.3
Acetanilide	0.36	-0.45	1.0
2-Phenylethanol	0.58	-0.23	1.4
Benzene	1.46	0.17	2.1
Toluene	2.46	0.39	2.7
Chlorobenzene	2.31	0.36	2.8
Ethylbenzene	3.81	0.58	3.2
Thymol	2.54	0.40	3.3
Diphenylamine	2.63	0.42	3.4
Biphenyl	5.63	0.75	4.0
Diphenyl ether	5.84	0.77	4.2
Phenanthrene	8.82	0.95	4.5
Triphenylamine	26.60	1.42	5.7

Using the determined k' and the logP values, a calibration line was calculated by linear regression. The regression equation was used to calculate the logP values of the test compounds.
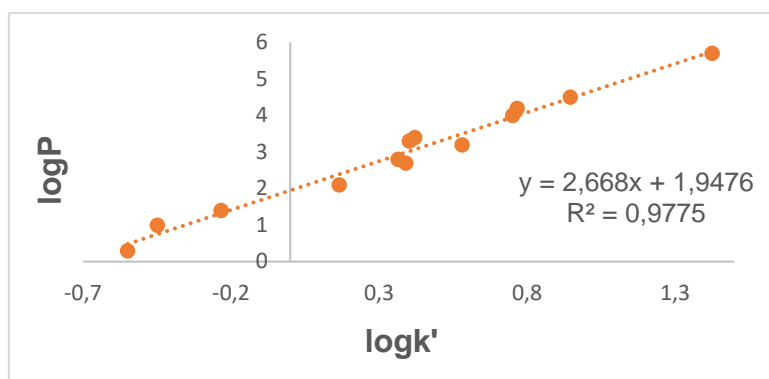
Figure 21. Calibration line of the logP value determination.

8.6 Solubility

The thermodynamic solubility was determined by continuous shake flask experiments, analogous to earlier work of our research group.[69, 72] The substance was suspended in excess in a PBS buffer (pH 7.4) and shook continuously (800 rpm) at constant warming (37 °C). After 24 h, the non-dissolved remains were separated by centrifugation (13.000 rpm, 1 min), samples were taken of the supernatant, and analysed by HPLC (method **III**). Three independent experiments were performed and the solubilities are represented as mean values.

For the calibration equation, the analysed compound was dissolved in MeOH and diluted to various concentrations, dependent on the solubility of the respective substance. The measured peak areas were plotted against the concentrations to yield the calibration line.
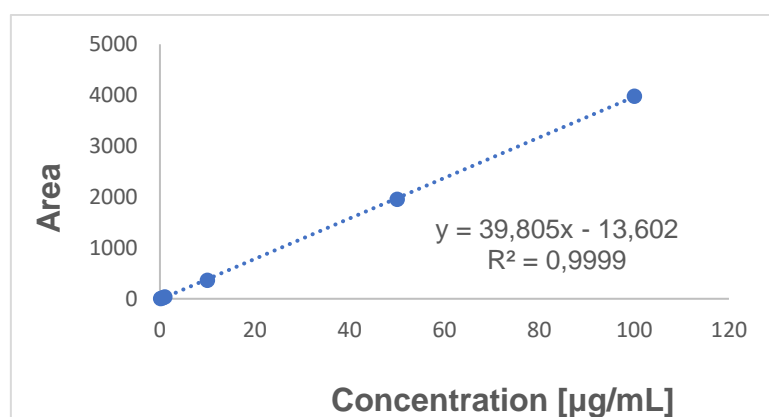
*Figure 22. Calibration line of compound **54**.*

9 <u>**References**</u>

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10 <u>Appendix</u>

10.1 Overview of synthesized quinolone amides

27a

27b

27c

28

54

50a

50b

50c

50d

50e

50f

50g

50h

53a

53b

75

76

68

55c

55f

60a

60d

60b

60e

60c

60f

71

78

66a

66b

66c

rac. **88**