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SHORT COMMUNICATION

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Azobenzene derivatives with activity against drug-resistant *Candida albicans* and *Candida auris*

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Abstract

Increasing resistance against antimycotic drugs challenges anti-infective therapies today and contributes to the mortality of infections by drug-resistant *Candida* species and strains. Therefore, novel antifungal agents are needed. A promising approach in developing new drugs is using naturally occurring molecules as lead structures. In this work, 4,4'-dihydroxyazobenzene, a compound structurally related to antifungal stilbene derivatives and present in *Agaricus xanthodermus* (yellow stainer), served as a starting point for the synthesis of five azobenzene derivatives. These compounds prevented the growth of both fluconazole-susceptible and fluconazole-resistant *Candida albicans* and *Candida auris* strains. Further in vivo studies are required to confirm the potential therapeutic value of these compounds.

KEYWORDS
antifungal drug, azobenzenes, *Candida albicans*, *Candida auris*

1 | INTRODUCTION

To date, more than 200 *Candida* species are known.[1] The most prominent among these is *Candida albicans*, present in the commensal flora of about 75% of all healthy persons.[2] While *Candida* spp. are usually harmless, immunocompromised patients may suffer from deep-seated candidiasis, bloodstream infections, or superficial infections.[3–6]

In recent years, the number of infections by *Candida* spp. increased, at least in part driven by the widespread use of broad-spectrum antimicrobials and the rising rate of surgical procedures.[4] Currently, most nosocomial infections are caused by *Candida albicans* and the frequency of other species such as *Candida glabrata* (mainly in northern Europe, United States, and Canada) and *Candida parapsilosis* (mainly in southern Europe, Asia, and South Africa) is increasing.[4,7–9] In addition, *Candida auris* recently emerged as a multidrug-resistant species and described for the first time in Japan in 2009[10] with global reports since then.[4] Infections by *C. albicans* are commonly treated with fluconazole, which inhibits ergosterol biosynthesis. However, *C. albicans* can develop fluconazole resistance by various mechanisms, including mutations in the target enzyme, upregulation of ergosterol biosynthesis genes, or constitutive over-expression of genes encoding multidrug efflux pumps.[11] On the other hand, most *C. auris* isolates are already fluconazole-resistant, and many of them are also resistant to other antifungals, including echinocandins and amphotericin B.[4] Therefore, the development of novel antimycotic drugs is needed.[4] Along these lines, several studies have shown that stilbene derivatives were active against *C. albicans*,[12–14] although this was not confirmed for the stilbene resveratrol.[15] As some stilbene derivatives were cytotoxic and mutagenic[16] structurally similar azobenzene derivatives were tested for their activity against *C. albicans*.[17,18] These azobenzenes had promising minimum inhibitory concentration (MIC) values exceeding

the MIC of fluconazole. In addition, an antimicrobial potential of cationic amphiphilic azobenzene derivatives has already been demonstrated on azobenzene-imidazolium conjugates and metallo azobenzene isothiouronium salts.[19,20]

This contribution is in light of these serious threats. Therefore, five cationic amphiphilic azobenzene bromide salts were synthesized according to syntheses known from the literature[21,22] and their activity was tested against *C. albicans* and *C. auris*, including fluconazole-resistant strains. Furthermore, their cytotoxicity and MIC values were determined, respectively.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

4,4'-Dihydroxyazobenzene, a naturally azo compound, is one constituent of *Agaricus xanthodermus* (Figure 1a).[23] This basic structure can be found in various compounds with antimicrobial potential.[24] Among others, antimicrobial substances were synthesized in which this azobenzene core was modified only by alkyl chains (Figure 1b) as well as amphiphilic cationic azobenzene derivatives (Figure 1c).[17-20]

Three cationic amphiphilic azobenzene bromide salts (**5a–c**), which have already been investigated as photoliquefiable ionic crystals,[21,22] and two structurally similar compounds (**5d,e**) were synthesized and tested for their activity against different *C. albicans* and *C. auris* strains (Scheme 1). A phenyl ring was modified with a hydrophobic alkyloxy chain before the basic azobenzene structure was synthesized. Since the hydrophobic part of the molecule was structurally identical in all the compounds, compound **2** was synthesized in a nucleophilic reaction from 4-acetamidophenol and 1-bromohexane in a 74% yield. Starting from this, the modified azobenzene core **3** was synthesized in an azo coupling with a 53% yield. Subsequently, the second phenyl ring of the azobenzene core structure was modified with two different alkyl chains, to determine the influence of the distance between the azobenzene core and the permanent positive charge. For this, compound **3** was treated with 1,4-dibromobutane or 1,2-dibromoethane to give **4a** and **4b** in 83% and 46% yield, respectively. To obtain amphiphilic compounds, different trialkylamines or trialkylphosphine were coupled to compounds **4a** and **4b**, to obtain permanently positively charged quaternary ammonium or phosphonium salts. Due to the significantly longer alkyl chains on the amine, the synthesis of compounds **5a** (13%) and **5b** (36%) had lower yields as compounds **5d** (88%) and **5e** (71%). The synthesis of compound **5c** (71%) provided similar yields compared to the synthesis with trimethylamine, so there is no difference between ammonium and phosphonium salts in terms of synthesis.[21] NMR spectra are provided in the Supporting Information: Figures S1–S5.

2.2 | Pharmacology/Biology

2.2.1 | MIC

The compounds were tested for their activity against four *C. albicans* and two *C. auris* strains (Table 1). SC5314 is a fluconazole-sensitive

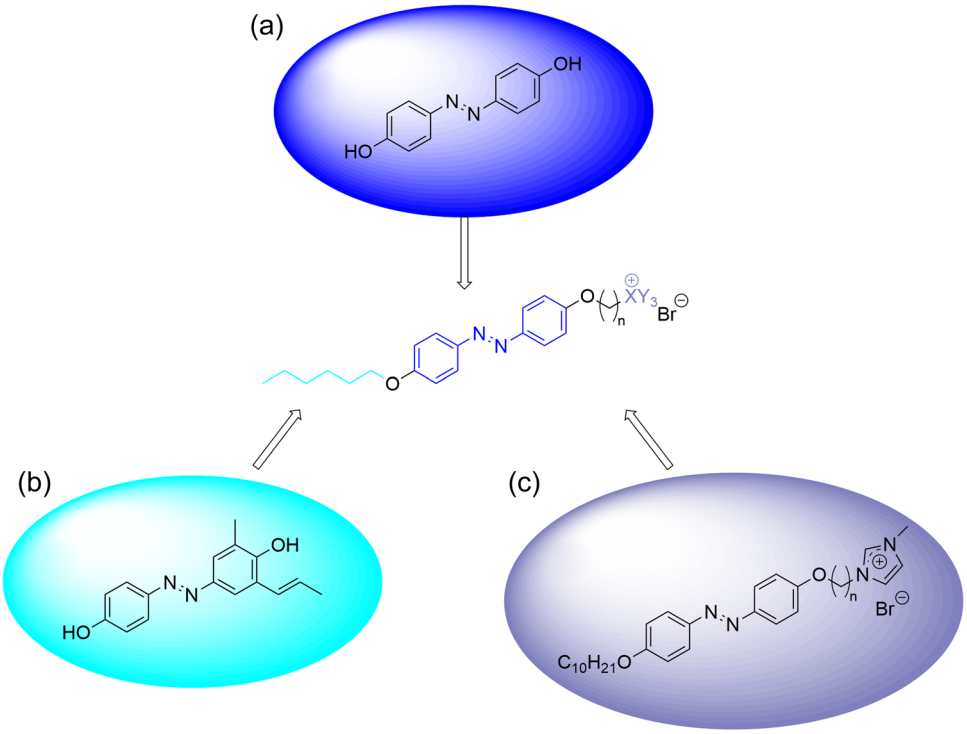
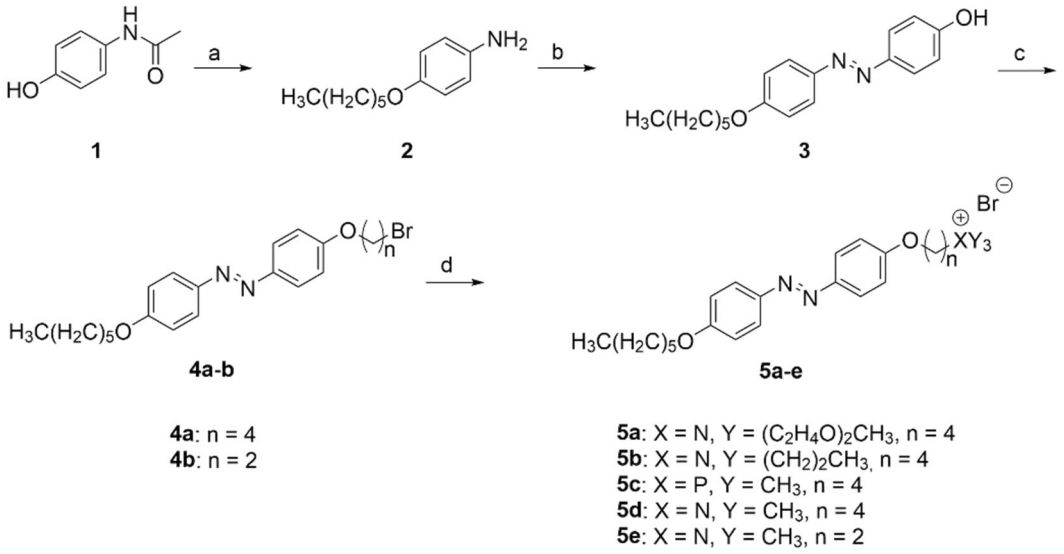


FIGURE 1 Antimicrobial substances that inspired the design of tested compounds. (a) azobenzene core structure 4,4'-dihydroxyazobenzene, (b) alkyl chain substituated azobenzene derivatives and (c) amphiphilic cationic azobenzene derivatives. (X) Nitrogen and Phosphorus replacement and (Y) alkyl/alkyloxy substitutes.



SCHEME 1 Synthesis of compounds **5a–e**. Reagents and conditions: (a) 1. 1-bromohexane, K_2CO_3, acetone, reflux, 2. HCl, methanol; (b) 1. $NaNO_2$, HCl, H_2O, acetone, 2. phenol, NaOH; (c) dibromoalkane, K_2CO_3, acetone, reflux; (d) trialkylamine/trialkylphosphine, acetonitrile, reflux/ 40°C/ 60-70°C.

TABLE 1 Minimal inhibitory concentration (MIC) values of compounds **5a–e**

Strain	Fluconazole MIC (µg/ml)	MIC (µg/ml)				
		5a	5b	5c	5d	5e
SC5314 (*Candida albicans*)[25]	0.5	1.89	1.44	2.55	2.46	4.64
SCMEUTR34 (*C. albicans*)[26]	>256	3.78	2.88	2.55	2.46	4.64
TW1 (*C. albicans*)[27]	1	1.89	1.44	2.55	2.46	2.32
TW17 (*C. albicans*)[27]	>256	3.78	2.88	2.55	2.46	4.64
DSMZ 21092 (*Candida auris*)	8	3.78	2.88	5.09	4.93	9.29
NR2-2015-214 (*C. auris*)	>128	7.57	2.88	5.09	4.93	9.29

Note: Average values from two biological replicates.

C. albicans reference strain.[25] SCMEUTR34 is a genetically engineered derivative of strain SC5314 exhibiting several fluconazole resistance mechanisms. It contains a resistance mutation in the drug target enzyme, a gain-of-function mutation in the transcription factor Upc2 that causes overexpression of ergosterol biosynthesis genes, and gain-of-function mutations in the transcription factors Mrr1 and Tac1, resulting in constitutive overexpression of genes encoding the multidrug efflux pumps Mdr1 and Cdr1/Cdr2, respectively.[26] The clinical isolates TW1 and TW17 are the first and last of a series of *C. albicans* isolates obtained from different episodes of oropharyngeal candidiasis in an HIV patient.[27] DSMZ 21092 is the *C. auris* reference strain, and NR2-2015-214 a clinical *C. auris* isolate.

The MIC values for both fluconazole-susceptible *C. albicans* wild-type reference strain SC5314 and clinical isolate TW1 were between 1.4 and 4.6 µg/ml. Four of the azobenzenes studied here, except for compound **5e**, prevented the growth of *C. albicans* SC5314 at lower concentrations compared to a previously reported antifungal uncharged azobenzene.[18] However, the charged azobenzene derivatives reported here were less active against the fluconazole-susceptible *C. albicans* strains than fluconazole. In contrast to fluconazole, all herein-tested azo compounds showed antifungal activity against fluconazole-resistant *C. albicans* strains and *C. auris* strains, respectively. The MIC values for fluconazole-resistant *C. albicans* strains SCMEUTR34 and TW17 were 2.5 to 4.6 µg/ml and, therefore, in the range of the values for fluconazole-susceptible *C. albicans* strains. These data sets suggested that the resistance mechanism against fluconazole did not affect the activity of the tested compounds here, and we conclude that the fluconazole-resistance mechanisms did not impair azobenzene activity.

The MIC values for *C. auris* strains DSMZ 21092 and NR2-2015-214 were between 2.9 and 9.3 µg/ml and, therefore, significantly higher as compared to *C. albicans*. All tested azobenzenes stopped the growth of the tested *C. auris* strains.

One aspect driving efficacy within the relatively small structural design space reported here was the distance between the azobenzene

scaffold and the positive charge (*n*). For example, compound **5e** with an ethyl group between the azobenzene scaffold and the positive charge had the lowest antifungal activity for *C. albicans* and *C. auris* strains, respectively. Furthermore, the activity was improved by shielding the positive charge with long alkyl chains (Y). Thus, compound **5b** —its charge is shielded by a propyl (Y = $(CH_2)_2CH_3$) chain —displayed the best results against both *C. albicans* and *C. auris* strains, respectively. At this stage, this structure–activity relationship (SAR) correlation is preliminary, and further studies are needed. For example, the narrow data set did not allow a distinction of the effects of ammonium versus phosphonium groups.

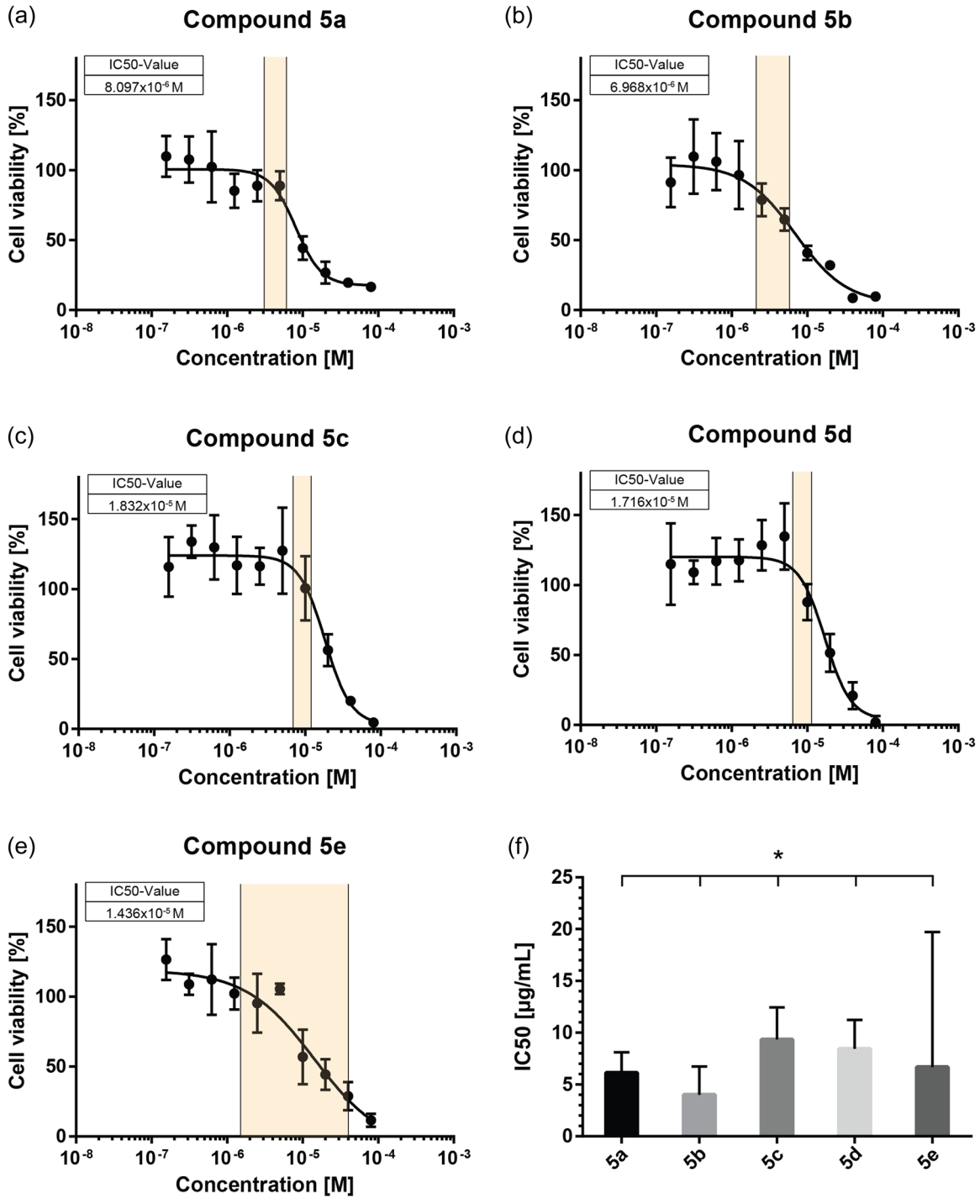


FIGURE 2 (a–e) Cell viability of HaCat cells determined with WST-1 ($n = 4$, outliers were determined with Dixon-Q-test, IC_{50} values were determined using a Sigmoidal 4PL plot. Yellow areas are the 95% confidence interval for the IC_{50} values). (f) IC_{50} values (means ± 95% confidence interval, significance represented by analysis of variance and Tukey multiple-comparison test with $^*p \leq 0.05$)

2.2.2 | Cell cytotoxicity

HaCat cells were exposed to serial dilutions of each compound and the cell viability was determined with a colorimetric WST-1 assay (Figure 2). IC_{50} values ranged from 4.02 to 9.33 µg/ml for all compounds. Therefore, the tested compounds were active against all *C. albicans* strains in a noncytotoxic range. However, if one considers the MIC values for *C. auris*, **5e** was only effective at a level that was also cytotoxic, and **5a** inhibited the growth of NR2-2015-214 (*C. auris*) only in concentrations exceeding its IC_{50} value. These findings suggested a narrow therapeutic window for both compounds **5e** and **5a**, respectively (Figure 2). This finding constitutes the preliminary character of this study, aiming at expanding the structural class of azobenzenes as antifungals. To which extent the cytotoxic effects seen in cell culture are relevant, for example, for topical use on (compromised) skin as well as the therapeutic value of the azobenzenes remains to be shown in future studies. These applications would not be adequately reflected by cytotoxicity experiments as carried out here but should include dermal safety studies in the future.

3 | CONCLUSION

We expanded the class of azobenzene derivatives as potential antifungals. All tested azobenzene derivatives had promising results both in terms of their antifungal activity as well as their toxicological profiles. Since the values for *C. albicans* strains are near the cytotoxic range and for multiresistant *C. auris* strains are in the cytotoxic range, application may be focused on external, topical use on the skin.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Commonly available chemicals and solvents used for syntheses and purification were purchased from Sigma-Aldrich, Fluka, Merck, and Fisher Scientific and used without further purification. Acetone and acetonitrile were dried using a molecular sieve (3 Å) and stored under argon atmosphere. An MLS-Ethos 1600 system from MLS-GmbH was used. All experiments were performed on an Avance 400 spectrometer (^{1}H spectra: 400.132 MHz) from Bruker BioSpin MRI GmbH. For the calibration of the spectra, the residual protons of the corresponding deuterated solvent (δ ($CDCl_3$) = 7.26 ppm, δ (DMSO) = 2.50 ppm, δ (MeOD) = 3.31 ppm) were used. Coupling constants ^{n}J were given in Hz and the chemical shifts in ppm. All samples were prepared in brown glass NMR tubes.

The InChI codes of the investigated compounds, together with some biological activity data, are provided as Supporting Information.

4.1.2 | General procedure for the synthesis of azobenzene derivatives 5a–e

The azobenzene derivatives **5a–e** were synthesized in a four-step synthesis. In a nucleophilic reaction with subsequent deprotection, 4-(hexyloxy)aniline **2** was synthesized from 4-acetamidophenol **1**. In an azo coupling, 4-(hexyloxy)aniline **2** was converted into 4-hexyloxy-4′-hydroxyazobenzene **3**. In a nucleophilic reaction with 1,4-dibromobutane or 1,2-dibromoethane, 4-hexyloxy-4′-(4-bromobutyloxy)-azobenzene **4a** and 4-hexyloxy-4′-(2-bromoethyloxy)-azobenzene **4b** were synthesized, respectively. To synthesize the ammonium-based salts **5a**, **5b**, **5d**, **5e**, **4a**, and **4b** were refluxed or stirred at 40°C with trialkylamines, respectively. To obtain the phosphonium-based salt **5d, 4a** was stirred at 40°C with trimethylphosphine.

All compounds were synthesized as previously reported and further extended with modifications (vide infra).[21,22] For this reason, the reactions are only briefly summarized below.

*4-(Hexyloxy)anilin (**2**)*

A solution of 4-acetamidophenol (20.0 g, 132.3 mmol), 1-bromohexane (28.4 g, 24.1 ml, 172.0 mmol), and potassium carbonate (47.5 g, 344.0 mmol) in anhydrous acetone (200 ml) was stirred for 4 days before water was added to obtain a colorless solid. After purification the solid was dissolved in a mixture of methanol and 35% HCl (200 ml, 6:4), refluxed for 2 days and purified to obtain **2** as a brown solid (19.0 g, 98.3 mmol, 74%). ^{1}H-NMR ($CDCl_3$, δ [ppm], *J* [Hz]) 6.78–6.72 (m, 2H), 6.70–6.63 (m, 2H), 3.88 (t, *J* = 6.6 Hz, 2H), 1.78–1.69 (m, 2H), 1.49–1.40 (m, 2H), 1.38–1.29 (m, 4H), 0.93–0.87 (m, 3H).

*4-Hexyloxy-4′-hydroxyazobenzene (**3**)*

A solution of sodium nitrite (2.4 g, 34.4 mmol) in water (15 ml) was added to a solution of 4-hexyloxyanilin (**2**) (5.0 g, 25.9 mmol) and 35% HCl (6.9 ml, 77.6 mmol) in a mixture of water and acetone (230 ml, 1:1). After adding a solution of phenol (3.3 g, 34.9 mmol) and sodium hydroxide (3.1 g, 77.6 mmol) in water (50 ml) the mixture was stirred for 4 h at 0°C. The solution was stirred overnight at room temperature and purified to obtain **3** as a red solid (4.1 g, 13.6 mmol, 53%). ^{1}H-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 7.95–7.78 (m, 4H), 7.05–6.89 (m, 4H), 4.02 (t, *J* = 6.6 Hz, 2H), 1.87–1.76 (m, 2H), 1.55–1.43 (m, 2H), 1.39–1.31 (m, 4H), 0.95–0.89 (m, 3H).

*4-Hexyloxy-4′-(4-bromobutyloxy)-azobenzene (**4a**)*

4-Hexyloxy-4′-hydroxyazobenzene (**3**) (9.49 g; 31.8 mmol), 1,4-dibromobutane (35.5 g, 160 mmol) and potassium carbonate (13.8 g, 99.9 mmol) were suspended in acetone (300 ml) and refluxed for 12 h. After purification **4a** was obtained as a yellow solid (11.5 g, 83%). ^{1}H-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 7.77–7.94 (m, 4H), 6.93–7.04 (m, 4H), 4.08 (t, *J* = 6.0, 2H), 4.03 (t, *J* = 6.6, 2H), 3.51 (t, *J* = 6.4, 2H), 2.04–2.16 (m, 2H), 1.93–2.04 (m, 2H), 1.82 (quin, *J* = 6.5, 2H), 1.42–1.56 (m, 2H), 1.28–1.42 (m, 4H), 0.92 (t, *J* = 7.1, 3H).

*4-Hexyloxy-4′-(2-bromoethyloxy)-azobenzene (**4b**)*

A suspension of 4-hexyloxy-4′-hydroxyazobenzene (**3**) (10.4 g, 34.9 mmol), 1,2-dibromoethane (32.8 g, 15.1 ml, 174.4 mmol), and potassium carbonate (14.5 g, 104.7 mmol) in acetone was refluxed for 21 h. After purification **4b** was obtained as yellow solid (6.5 g, 16.1 mmol, 46%). ^{1}H-NMR ($CDCl_3$, δ [ppm], J [Hz]): 7.94–7.82 (m, 4H), 7.06–6.94 (m, 4H), 4.36 (t, J = 6.3 Hz, 2H), 4.03 (t, J = 6.6 Hz, 2H), 3.67 (t, J = 6.3 Hz, 2H), 1.88–1.76 (m, 2H), 1.56–1.44 (m, 2H), 1.41–1.29 (m, 4H), 0.97–0.87 (m, 3H).

*Compound **5a***

4-Hexyloxy-4′-(4-bromobutyloxy)-azobenzene (**4a**) (5.8 g, 13.3 mmol) and tris[2-(2-methoxyethoxy)ethyl]amine (13.0 g, 40.1 mmol) were suspended in acetonitrile (20 ml) and refluxed for 96 h. After purification **5a** was obtained as yellow solid (1.3 g, 1.76 mmol, 13%). ^{1}H-NMR ($CDCl_3$, δ [ppm], J [Hz]): 8.03–7.68 (m, 4H), 7.07–6.92 (m, 4H), 4.10 (t, J = 5.9 Hz, 2H), 4.07–4.03 (m, 2H), 4.03–3.97 (m, 6H), 3.97–3.89 (m, 6H), 3.80–3.71 (m, 2H), 3.71–3.63 (m, 6H), 3.54–3.45 (m, 6H), 3.34 (s, 9H), 2.15–1.98 (m, 2H), 1.95–1.86 (m, 2H), 1.86–1.74 (m, 2H), 1.55–1.42 (m, 2H), 1.42–1.30 (m, 4H), 0.97–0.85 (m, 3H).

*Compound **5b***

4-Hexyloxy-4′-(4-bromobutyloxy)-azobenzene (**4a**) (3.0 g, 7.0 mmol) and tripropylamine (3.0 g, 20.0 mmol) were refluxed in acetonitrile (20 ml) for 96 h. After purification **5b** was obtained as yellow solid (1.4 g, 2.5 mmol, 36%). ^{1}H-NMR (MeOD, δ [ppm], J [Hz]): 8.00–7.68 (m, 4H), 7.19–6.93 (m, 4H), 4.18 (t, J = 5.4 Hz, 2H), 4.07 (t, J = 6.4 Hz, 2H), 3.39–3.34 (m, 2H), 3.27–3.20 (m, 6H), 2.02–1.87 (m, 4H), 1.87–1.79 (m, 2H), 1.78–1.69 (m, 6H), 1.57–1.46 (m, 2H), 1.42–1.33 (m, 4H), 1.01 (t, J = 7.3 Hz, 9H), 0.97–0.91 (m, 3H).

*Compound **5c***

4-Hexyloxy-4′-(4-bromobutyloxy)-azobenzene (**4a**) (1.7 g, 3.9 mmol) and trimethylphosphine (0.45 g, 5.9 mmol) were stirred in acetonitrile (20 ml) at 40°C for 120 h. After purification **5c** was obtained as yellow solid (1.4 g, 2.8 mmol, 71%). ^{1}H-NMR (DMSO, δ [ppm], J [Hz]): 7.90–7.76 (m, 4H), 7.18–7.04 (m, 4H), 4.13 (t, J = 6.1 Hz, 2H), 4.06 (t, J = 6.5 Hz, 2H), 2.35–2.23 (m, 2H), 1.92–1.80 (m, 11H), 1.79–1.63 (m, 4H), 1.49–1.38 (m, 2H), 1.38–1.25 (m, 4H), 0.93–0.84 (m, 3H).

*Compound **5d***

4-Hexyloxy-4′-(4-bromobutyloxy)-azobenzene (**4a**) (1.4 g, 3.3 mmol) and trimethylamine (0.26 g, 4.4 mmol) were stirred in acetonitrile (20 ml) at 40°C for 120 h. After purification **5d** was obtained as a yellow solid (1.4 g, 2.9 mmol, 88%). ^{1}H-NMR (DMSO, δ [ppm], J [Hz]): 7.97–7.73 (m, 4H), 7.27–6.99 (m, 4H), 4.14 (t, J = 6.0 Hz, 2H), 4.06 (t, J = 6.5 Hz, 2H), 3.46–3.36 (m, 2H), 3.08 (s, 9H), 1.96–1.83 (m, 2H), 1.83–1.67 (m, 4H), 1.50–1.38 (m, 2H), 1.38–1.24 (m, 4H), 0.95–0.82 (m, 3H).

*Compound **5e***

A solution of 4-hexyloxy-4′-(2-bromoethyloxy)-azobenzene (**4b**) (3.0 g, 7.4 mmol) and trimethylamine (1.3 g, 22.2 mmol) in acetonitrile (10 ml) is heated to 60°C - 70°C by means of microwave radiation and stirred for 11 h. After purification, **5e** was obtained as a yellow solid (2.5 g, 5.3 mmol, 71%). ^{1}H-NMR (DMSO, δ [ppm], J [Hz]): 7.94–7.78 (m, 4H), 7.25–7.05 (m, 4H), 4.57 (s, 2H), 4.06 (t, J = 6.5 Hz, 2H), 3.88–3.79 (m, 2H), 3.21 (s, 9H), 1.80–1.69 (m, 2H), 1.48–1.37 (m, 2H), 1.37–1.26 (m, 4H), 0.92–0.84 (m, 3H).

4.2 | Pharmacological/biological assays

4.2.1 | Strains and growth conditions

C. albicans and *C. auris* strains were stored as glycerol stocks (17.2% glycerol) at -80°C. To grow subcultures, the strains were streaked out on YPD agar plates and incubated for 2 days at 30°C.

4.2.2 | Azobenzene derivatives MIC assay

The MIC was determined according to a previously described method,[28,29] with slight modifications. First, stock solutions of the compounds were prepared in water with 1% DMSO. In 96-well plates, dilution series of each azobenzene compound were prepared in water in a concentration range from 80 to 0.04 µM. For each *Candida* species, two colonies from a YPD agar plate were suspended in 2 ml of a 0.9% NaCl solution. After mixing 4 µl of each solution with 2 ml 2× SD-CSM medium (13.4 g yeast nitrogen base without amino acids [YNB; BIO 101, Vista, Calif.], 40 g glucose, and 1.54 g complete supplement medium [CSM, BIO101]), 100 µl of the cell suspension was mixed with 100 µl of each azobenzene derivative dilution, resulting in a concentration range from 40 to 0.02 µM. The 96-well plates were incubated for 48 h at 37°C. The MIC of the substances is determined as the concentration that abolished or drastically reduced visible growth compared to a drug-free control.

4.2.3 | Cell culture

HaCat cells were cultured in growth medium (DMEM containing 4500 mg/ml glucose, 2 mM glutamine, 10% fetal calf serum, 100 U/ml penicillin, 100 g/ml streptomycin) in a T75 flask at 37°C and 5% CO_2.

4.2.4 | Cell proliferation assay (WST-1)

For cytotoxicity assay, 5000 HaCat cells/well were seeded in 96-well plates and incubated overnight at 37°C, 5% CO_2. The prepared stock solutions were serially diluted with GM (DMEM containing 4500 mg/ml glucose, 2 mM glutamine, 10% Fetal calf serum, 100 U/ml penicillin, 100 g/ml streptomycin) in a concentration range from 0.16 to 80 µM, resulting in a maximal DMSO concentration of 1%. The dilution series were added to the cells and incubated for 24 h at

37°C and 5% CO_2. After GM media was aspirated and cells were washed with PBS, diluted WST-1 was added to the cells and incubated for 4 h at 37°C and 5% CO_2. The absorption of formazan formed because an enzymatic reaction of living cells was detected at 450 nm using a SPECTRAmax250 automated microtiter plate reader. The positive (no HaCat cells + WST-1) and negative (HaCat cells + WST-1) control was performed in the same way. The cell viability was calculated with the negative control normalized to 100% and the positive control normalized to 0%.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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