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Design, Synthesis, and in vitro Antifungal Activity of 1-[(4-Substituted-benzyl)methylamino]-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ols

Rémi Guillon,[a] Fabrice Pagniez,[b] Francis Giraud,[a] Damien Crépin,[a] Carine Picot,[b] Marc Le Borgne,[a] Florent Morio,[b] Muriel Duflos,[a] Cédric Logé,*[a] and Patrice Le Pape[b]

As part of our studies focused on the design of 1-[((hetero)aryl- and piperidinylmethyl)amino]-2-phenyl-3-(1*H*-1,2,4-triazol-1-yl)-propan-2-ols as antifungal agents, we report the development of new extended benzylamine derivatives substituted at the *para* position by sulfonamide or retrosulfonamide groups linked to alkyl or aryl chains. These molecules have broad-spectrum antifungal activities not only against *Candida* spp., including fluconazole-resistant strains, but also against a filamentous species (*A. fumigatus*). Concerning fluconazole resistance, selected compounds exhibit the capacity to overcome *CDR* and *ERG11* gene upregulation and to maintain antifungal activity despite a recognized critical CYP51 substitution in *C. albicans* isolates. Synthesis, investigation of the mechanism of action by sterol analysis in a *C. albicans* strain, and structure–activity relationships (SARs) are reported.

Introduction

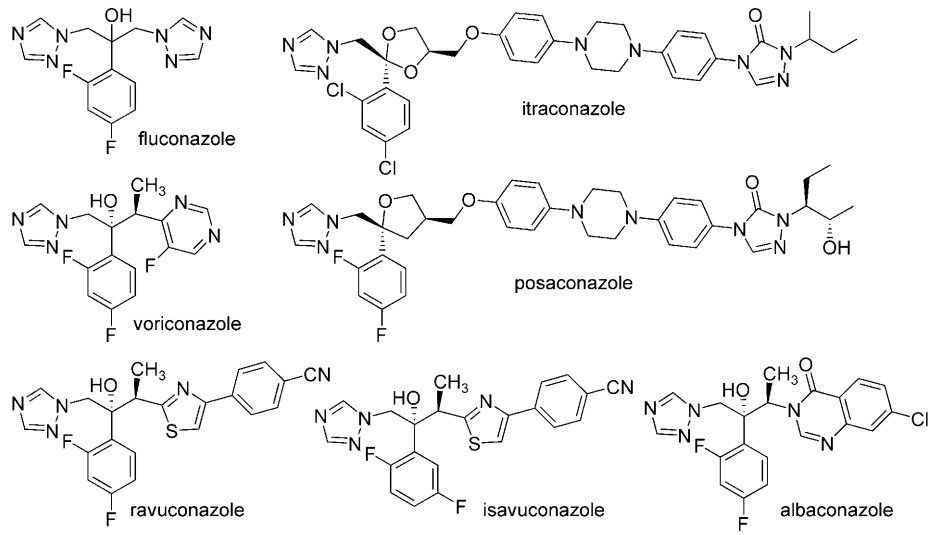
During the last few decades, fungi have become a major threat to many hospitalized patients particularly for those who are severely immunocompromised and are therefore highly susceptible to systemic fungal infections, most often caused by *Candida* and *Aspergillus* species.[1] Currently, four main chemical families (having four global cellular targets in fungal cells) are used for the treatment of invasive fungal infections: polyenes, pyrimidine analogues, azoles, and echinocandins. Among them, azoles are the largest class of antifungal agents in clinical use. They mainly target P450-dependent sterol 14α-demethylase (CYP51, Erg11), encoded by the *ERG11* gene, a key enzyme in fungal ergosterol biosynthesis.[2] This inhibition leads to depletion of ergosterol in the cell membrane and accumulation of toxic intermediate sterols, causing increased membrane permeability and inhibition of fungal growth. The 'first-generation' triazoles (fluconazole and itraconazole) considerably improved the treatment of some serious fungal infections, such as candidiasis. However, these earlier agents have limitations related to their narrow-spectrum antifungal activity.[3] The lack of activity against filamentous fungi and emergence of acquired resistance of some *Candida* isolates represent well-known limits of fluconazole, whereas itraconazole has an unpredictable absorption in oral formulations and considerable metabolic variability.[4] Currently, among the 'second-generation' triazoles, including voriconazole,[5] posaconazole,[6] ravuconazole,[7] isavuconazole,[8] and albaconazole,[9] only voriconazole and posaconazole are available for clinical management of invasive fungal infections. Both compounds have a very broad-spectrum of antifungal activities that includes *Candida* species, filamentous, and dimorphic fungi. Ravuconazole, isavuconazole, and albaconazole are currently in various stages of development and have shown very potent in vitro activity against species of *Candida*, *Cryptococcus*, and *Aspergillus*.[10] However, despite the arrival of these new effective drugs, some therapeutic problems remain, in particular emerging fungal species, variable drug bioavailability, some toxicity, lack of either oral or intravenous preparations, significant drug interactions for some agents, and development of resistance.[11]

Antifungal resistance is a serious concern due to the limited number of available agents. In *Candida* species, four major mechanisms of resistance to azoles have been described: 1) decreased intracellular accumulation of azoles due to the overexpression of genes encoding efflux transporters belonging to the ATP-binding cassette (ABC) superfamily (*CDR1* and *CDR2*) or major facilitator superfamily (*MDR1*);[12,13] 2) genetic alterations in the *ERG11* gene leading to amino acid substitutions in the target enzyme CYP51 that decrease drug binding;[14–16] 3) alterations in the sterol biosynthesis pathway that bypass the accumulation of toxic sterols;[17,18] and 4) overexpression of the *ERG11* gene.[19] Therefore, the antifungal agent is overwhelmed, and routine therapeutic concentrations can no longer effectively inhibit ergosterol synthesis.[20]

To date, more than 140 different amino acid substitutions have been detected in CYP51 of clinical isolates of *C. albi-*

[a] Dr. R. Guillon, Dr. F. Giraud, D. Crépin, Prof. M. Le Borgne, Prof. M. Duflos, Dr. C. Logé
Université de Nantes, Nantes Atlantique Universités, Département de Pharmacochimie, Cibles et Médicaments des Infections, de l'Immunité et du Cancer, IICIMED-EA 1155, UFR Sciences Pharmaceutiques, 1 rue Gaston Veil, 44035 Nantes Cedex 1 (France)
Fax: (+33) 240-412-876
E-mail: cedric.loge@univ-nantes.fr

[b] Dr. F. Pagniez, C. Picot, F. Morio, Prof. P. Le Pape
Université de Nantes, Nantes Atlantique Universités, Département de Parasitologie et Mycologie Médicale, Cibles et Médicaments des Infections, de l'Immunité et du Cancer, IICIMED-EA 1155, UFR Sciences Pharmaceutiques, 1 rue Gaston Veil, 44035 Nantes Cedex 1 (France)

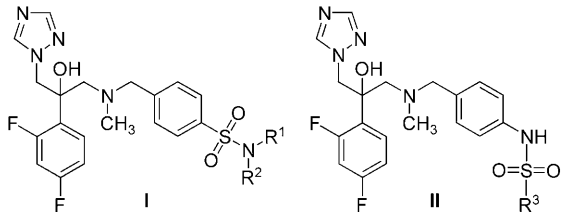


cans.[21] Most of these substitutions, instead of being randomly dispersed, are clustered into three hot spot regions ranging from amino acids 105 to 165, 266 to 287, and 405 to 488.[22] The contribution of each individual mutation to azole resistance is, however, difficult to estimate because *ERG11* mutations often occur in combination (between two and four combined mutations) in the same allele and because resistance mechanisms are often combined in azole-resistant *C. albicans* isolates.[18,22,23]

The long-awaited structural information of CYP51 from pathogenic fungi could certainly accelerate the discovery of novel antifungal agents as well as provide insight into a better understanding of the resistance mechanisms. In the absence of such crystal structures, several three-dimensional models of CYP51 from *C. albicans*, *A. fumigatus*, or *Cryptococcus neoformans* have been reported[24–27] on the basis of a prokaryotic CYP51 from *M. tuberculosis*[28] and interactions with azoles have been explored by flexible molecular docking. In particular, the receptor-based pharmacophore model published by Sheng et al. was used to guide the rational optimization of the azole antifungal agents and highlighted the importance of Tyr118 and Ser378 residues of *C. albicans* sterol 14α-demethylase P450 (CACYP51) in the stabilization of inhibitors.[29] Tyr118, a highly conserved residue in the CYP51 family, may be a potential site for the π–π interaction with the inhibitor, whereas Ser378, a conserved amino acid across the fungal CYP51 enzymes, could form a specific hydrogen bonding interaction. The role of the Tyr118 residue in determining azole susceptibility was also validated by site-directed mutagenesis.[30,31] Moreover, to combat infection more efficiently, one strategy is to increase the size of the inhibitor to cover a larger area within the active site of fungal CYP51.[32,33] In particular, Xiao et al. provided a model for the binding of azoles with extended side chains in CYP51 from *C. albicans* and *A. fumigatus*. Specifically, itraconazole and posaconazole appear to be less affected by mutations near the heme site than either fluconazole or voriconazole and this could be explained by tighter affinity and/or compensatory adjustments within the active site.[24]

As part of our research project focused on the optimization of azole antifungal agents based on the modification of the side chain attached to the pharmacophore, we recently reported on the design and synthesis of 1-[((hetero)aryl- or piperidinylmethyl)amino]-2-phenyl-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ols showing various degrees of antifungal activity against *Candida albicans* and *Aspergillus fumigatus* strains.[34–37] The benzylamine series bearing H-bond acceptor entities in the *para* position of the benzyl group and a *N*-methyl group in the linker gave the most active compounds both in *C. albicans* and in *A. fumigatus* strains, in accordance with the receptor-based pharmacophore model published by Sheng et al.[29]

Herein, we report the development of new benzylamine derivatives substituted in the *para* position by sulfonamide (**I**) or retrosulfonamide groups (**II**) linked to alkyl or aryl chains.

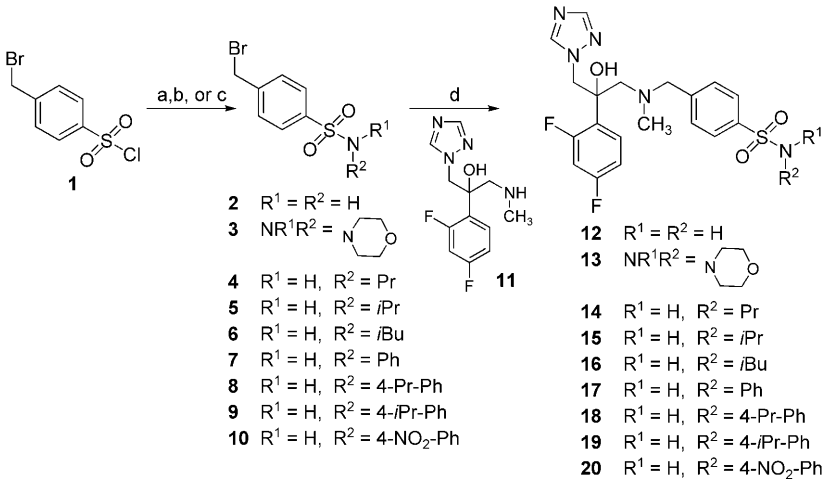


These structures are easily prepared, retained all the main characteristics mentioned above, and may be of sufficient size to avoid mutations near the heme site. The synthesis and biological in vitro evaluation against various *Candida* spp. and *A. fumigatus* strains are described.

Results and Discussion

Chemistry

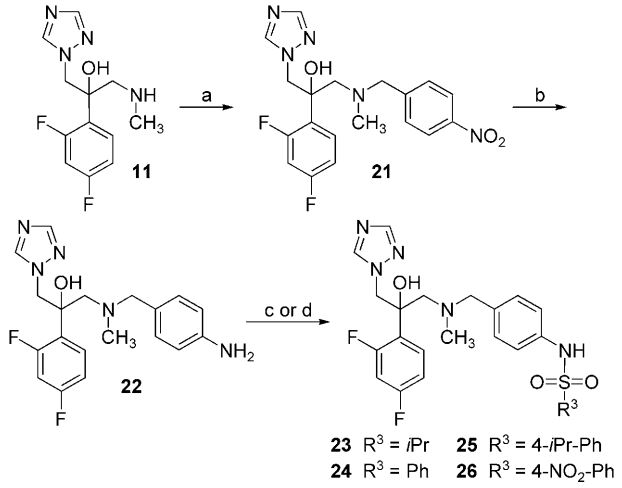
The synthetic route of target compounds **12**–**20** is outlined in Scheme 1. These molecules were obtained by nucleophilic substitution of previously described[36] key intermediate **11** with appropriate substituted 4-(bromomethyl)benzenesulfonamides prepared by the reaction of commercially available 4-(bromomethyl)benzenesulfonylchloride **1** with amines. Compound **2** was obtained following a synthetic procedure reported in the literature by the action of aqueous ammonia in THF.[38] Sulfonamides **3**–**6** and **7**–**10** were respectively synthesized in good yields by reaction of **1** with alkylamines in the presence of Hünig's base in THF or with anilines in dichloromethane and pyridine. The nucleophilic substitution reaction by the amino



Scheme 1. Reagents and conditions: a) NH_4OH, THF, RT, 1 h, 98%; b) alkylamines, *N*,*N*-diisopropylethylamine, THF, RT, 1 h, 70–97%; c) anilines, pyridine, CH_2Cl_2, 0 °C, 2 h, 81–90%; d) **2**–**10**, *N*,*N*-diisopropylethylamine, CH_3CN, RT, 24 h, 61–85%.

alcohol **11** on derivatives **2**–**10** in the presence of Hünig's base in acetonitrile afforded nine original compounds (**12**–**20**).

Scheme 2 outlines the synthesis of compounds **23**–**26** starting from the key intermediate **11**. Reaction with 4-nitrobenzylbromide using Hünig's base in acetonitrile gave compound **21**



Scheme 2. Reagents and conditions: a) 4-nitrobenzylbromide, *N*,*N*-diisopropylethylamine, CH_3CN, RT, 24 h, 77%; b) 5% Pd/C, H_2, EtOH, RT, 5 bar, 2 h, 72%; c) isopropylsulfonyl chloride, pyridine, RT, 2 h, 47%; d) benzenesulfonyl chlorides, In, CH_3CN, RT, 15 h, 29–68%.

in 77% yield. Catalytic hydrogenation of the nitro aromatic group using 5% Pd/C in ethanol afforded the corresponding amine **22**, which was condensed with isopropylsulfonyl chloride in pyridine to give molecule **23**.[39] Targeted aromatic compounds **24**–**26** were prepared by indium catalysis in acetonitrile with benzenesulfonyl chlorides.[40]

Antifungal activity

The in vitro antifungal activities of all compounds were evaluated against human pathogenic fungi (*C. albicans*, *C. krusei*, *C. glabrata*, *C. parapsilosis*, and *A. fumigatus*) and are summarized in Table 1. The MIC values (in $\mu g\,mL^{-1}$) of fluconazole, voriconazole, and itraconazole are also shown as reference.

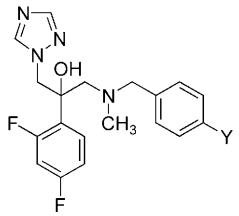
With the *C. albicans* CA98001 strain, all compounds displayed a high level of activity, with MIC values ranging from 1.0 to 69.0 $ng\,mL^{-1}$, similar to that of voriconazole or fluconazole. For the *A. fumigatus* AF98003 strain, biological results are promising with MIC values mostly ranging from 767.0 to 3570.0 $ng\,mL^{-1}$, only two- to eightfold greater than that of itraconazole. On both of these strains, compounds bearing the most extreme side chain from a steric point of view (**12** compared with **19** or **20**) share the same antifungal values, and comparison of sulfonamides **15**, **17**, **19**, and **20** with their retrosulfonamide analogues **23**, **24**, **25**, and **26**, did not reveal significant differences in activity, except for compound **23** (the sole representative in the alkyl series) on the *A. fumigatus* strain.

A significant broad-spectrum antifungal activity was also observed for some molecules (**15**–**20** and **25**) against *C. krusei*, *C. glabrata*, and *C. parapsilosis* strains confirming their potential in targeting fluconazole low-susceptible strains and fluconazole-intrinsically resistant *Candida* species.

Sterol analysis

The mechanism of action of this series of azoles was investigated by studying inhibition of *C. albicans* CA98001 ergosterol biosynthesis after treatment by **17**, one of the most active compounds. As shown in Table 2, this molecule strongly inhibited ergosterol biosynthesis while lanosterol accumulated. About 60% of ergosterol biosynthesis inhibition was obtained with a concentration of 0.020 $\mu g\,mL^{-1}$, near the MIC value (4.0 $ng\,mL^{-1}$). At a higher concentration (0.1 $\mu g\,mL^{-1}$), this effect was maximal showing that inhibition of ergosterol biosynthesis by this compound is dose-dependent. Also, methylated sterols at the C14 position also accumulated (eburicol, 14-methylfecosterol and 14-methylepisterol) in a dose-dependent manner. Accumulation of lanosterol and production of 14-methylated sterols are typically a result of a 14α-demethylase blockage by triazole derivatives. However, the low proportion of 14-methyl-3,6-diol could also be explained by further inhibition of Δ5,6-desaturase encoded by the *ERG3* gene.[41]

Table 1. In vitro antifungal activities of compounds **12**–**20** and **23**–**26** against *Candida* and *A. fumigatus* strains.



Compd	Y	MIC [μg mL^{-1}][a]						
		CA98001	CK506	CK8	CG468	CAPA1	CAPA2	AF98003
12	-SO_2NH_2	0.013 ± 0.001	13.0 ± 0.4	13.2 ± 1.1	3.4 ± 1.6	0.91 ± 0.12	0.75 ± 0.16	2.45 ± 0.04
13	-SO_2-morpholine	0.018 ± 0.001	13.4 ± 0.7	2.7 ± 1.0	0.021 ± 0.002	0.88 ± 0.17	1.1 ± 0.3	3.25 ± 0.10
14	-SO_2-NH-Pr	< 0.001	12.6 ± 0.4	1.8 ± 0.1	1.57 ± 0.03	0.91 ± 0.07	1.5 ± 0.5	0.767 ± 0.24
15	-SO_2-NH-*i*Pr	0.002 ± 0.001	5.7 ± 0.1	1.8 ± 0.36	0.014 ± 0.001	0.58 ± 0.01	0.76 ± 0.01	2.35 ± 0.05
16	-SO_2-NH-*i*Bu	< 0.001	2.2 ± 0.7	1.0 ± 0.3	< 0.001	0.038 ± 0.030	0.84 ± 0.2	1.88 ± 0.20
17	-SO_2-NH-Ph	0.004 ± 0.001	1.6 ± 0.1	1.8 ± 0.1	2.0 ± 1.0	0.10 ± 0.02	0.12 ± 0.06	3.08 ± 0.10
18	-SO_2-NH-Ph-(*p*)Pr	0.069 ± 0.051	1.2 ± 0.1	1.8 ± 0.1	1.6 ± 0.03	0.12 ± 0.02	0.23 ± 0.05	1.56 ± 0.44
19	-SO_2-NH-Ph-(*p*)*i*Pr	0.030 ± 0.003	1.8 ± 0.1	0.85 ± 0.22	< 0.001	0.10 ± 0.01	0.62 ± 0.06	3.33 ± 0.06
20	-SO_2-NH-Ph-(*p*)NO_2	0.018 ± 0.002	3.5 ± 0.7	1.9 ± 0.2	< 0.001	0.017 ± 0.005	0.73 ± 0.16	2.57 ± 0.11
23	-NH-SO_2-*i*Pr	0.026 ± 0.008	17.5 ± 0.2	15.1 ± 0.6	14.6 ± 0.3	4.5 ± 1.4	3.1 ± 0.9	29.0 ± 0.50
24	-NH-SO_2-Ph	0.015 ± 0.004	10.6 ± 1.9	1.7 ± 0.2	1.40 ± 0.03	1.2 ± 0.2	0.58 ± 0.10	3.29 ± 0.05
25	-NH-SO_2-Ph-(*p*)*i*Pr	0.023 ± 0.001	2.0 ± 0.1	1.5 ± 0.1	1.9 ± 0.1	0.15 ± 0.03	0.18 ± 0.01	2.89 ± 0.06
26	-NH-SO_2-Ph-(*p*)NO_2	0.030 ± 0.008	15.3 ± 1.6	18.3 ± 2.0	18.3 ± 0.2	1.91 ± 0.36	1.7 ± 0.2	3.57 ± 0.06
fluconazole	–	0.036 ± 0.021	> 30	12.9 ± 0.9	7.7 ± 0.1	> 30	> 30	–
voriconazole	–	0.005 ± 0.001	1.3 ± 0.3	0.24 ± 0.07	0.45 ± 0.04	0.95 ± 0.13	0.36 ± 0.01	0.15 ± 0.01
itraconazole	–	–	–	–	–	–	–	0.42 ± 0.04

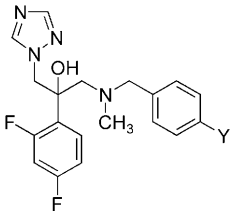
[a] Values represent the mean ± SD of experiments performed in triplicate; *C. albicans* (CA98001), *C. krusei* (CK506, CK8), *C. glabrata* (CG468), *C. parapsilosis* (CAPA1, CAPA2), and *A. fumigatus* (AF98003).

Table 2. Effect of compound **17** on the sterol composition of *C. albicans* CA98001.

Sterols[a]	Control	**17** [μg mL^{-1}]	
		0.020	0.1
lanosterol	0.5	22.5	38.3
eburicol	0.6	31.0	44.2
zymosterol	0.3	0.9	–
ergosta-5,8-dienol	1.3	2.6	–
episterol	0.3	0.7	–
14-methylfecosterol	–	2.1	5.7
14-methylepisterol	–	3.2	9.4
14-methyl-3,6-diol	–	0.4	2.4
ergosterol	97.0	36.7	0.0

[a] Sterols of interest were identified by their mass spectra; the area under the curve (AUC) of each peak was used to calculate the ratio of (sterol AUC)/(sum of sterols AUC).

Table 3. In vitro antifungal activities of compounds **17**–**19** and **25** against *C. albicans* strains with reduced fluconazole susceptibility.



Compd	Y	MIC [μg mL^{-1}][a]	
		DSY735	CAAL-74
17	-SO_2-NH-Ph	1.03 ± 0.26	0.06 ± 0.01
18	-SO_2-NH-Ph-(*p*)Pr	0.29 ± 0.01	0.011 ± 0.001
19	-SO_2-NH-Ph-(*p*)*i*Pr	1.05 ± 0.31	0.17 ± 0.01
25	-NH-SO_2-Ph-(*p*)*i*Pr	0.24 ± 0.01	0.12 ± 0.03
fluconazole	–	12.55	> 30

[a] Values represent the mean ± SD of experiments performed in triplicate.

Biological evaluation against *C. albicans* isolates with decreased fluconazole susceptibility

To evaluate more precisely the biological interest of these compounds, we investigated their potency against two *C. albicans* strains having identified mechanisms of resistance. These include *TAC1* (transcriptional activator of *CDR* genes) mutation and *TAC1* and *ERG11* upregulation after formation of isochromosome 5L (DSY735) and amino acid substitutions Tyr132Phe/Gly448Val (CAAL-74). Compounds **17**–**19** and **25** were selected because of their broad-spectrum antifungal activity and relatively long side chain positioned in the benzylamine linker. The MIC values (in μg mL^{-1}) are presented in Table 3.

Interestingly, compared to fluconazole, all these extended compounds exhibited high antifungal activities against the selected strains, showing that overexpression of efflux pumps and *ERG11* or specific point substitutions in the CYP51 enzyme, which result in fluconazole resistance, could be rationally overcome.

Molecular modeling

Despite a mechanism of action that could imply both inhibition of 14α-demethylase (CYP51) and Δ5,6-desaturase, we performed the docking of compound **17** in our model of *C. albicans* CYP51.[37,42] According to the receptor-based pharmacophore model,[29] the putative binding solution suggests key hydrogen bonding interactions between the sulfonyl group and both Ser378 and His377 residues (Figure 1). On the *C. albicans*

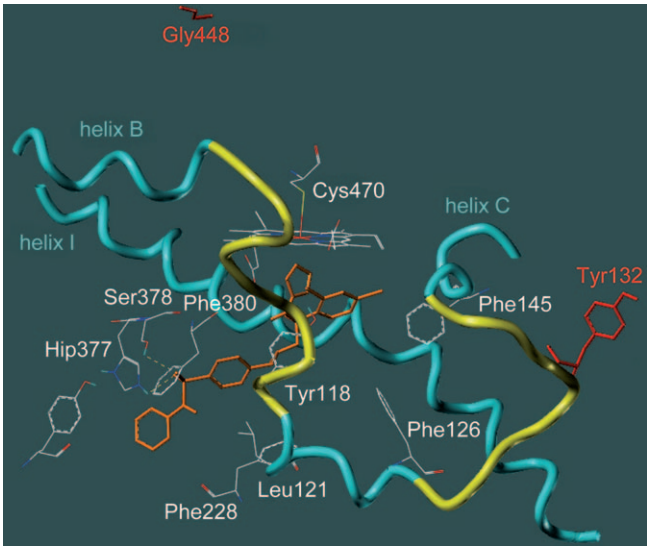


Figure 1. Proposed binding solution of compound (*S*)-**17** in the putative active site pocket (channel 2) of *C. albicans* CYP51. Hip377 is the protonated form of the histidine residue. The backbone is shown in tube representation (Sybyl v. 8.0, Tripos Associates Inc., St. Louis, MO, USA).

CAAL-74 strain, substitutions of Tyr132Phe is known to confer azole resistance.[43] Tyr132 is located on the enzyme's flexible BC loop, which adopts the open conformation observed in the crystal structure of CYP51 from *M. tuberculosis* which is used as a template. This conformation positions this residue away from the active site. However, we can assume that, given the recent crystal structures of CYP51 from human and *Trypanosomatidae* in complex with azole antifungal inhibitors,[44,45] this residue could come into close proximity with the active site as the BC loop adapts a closed conformation. Thus, the activity observed for the larger compound **17** relative to fluconazole could be explained by tighter affinity and/or compensatory adjustments within the active site. The involvement of the Gly448Val substitution is more difficult to explain because this amino acid is only described in combination with Tyr132 in clinical isolates,[21,43b] and is part of a large specific loop insertion, located far from the active site, not modeled in detail.

Conclusions

In summary, we report on the synthesis and biological evaluation of novel triazole derivatives. These extended molecules have broad-spectrum antifungal activities not only against *Candida* spp. including fluconazole-resistant strains but also against a filamentous species (*A. fumigatus*), which are the most prevalent fungal pathogens. Concerning fluconazole resistance, selected compounds demonstrate a capacity to overcome *CDR* and *ERG11* gene upregulation and to maintain antifungal activity despite a recognized critical CYP51 substitution in *C. albicans* isolates. Additional investigations including emerging molds (*Scedosporium* spp., *Fusarium* spp., and *Zygomycetes*), a large number of azole-resistant clinical isolates, in vitro evaluation on recombinant CYP51 from *C. albicans* and toxicity studies could be undertaken to check the usefulness of these compounds for further optimization.

Experimental Section

Chemistry

General methods: Melting points were determined using an Electrothermal IA9300 digital melting point apparatus and are reported uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC250 (250 MHz) or on Bruker Avance 400 spectrometer (400 MHz). Chemical shifts are expressed as δ values (ppm) relative to tetramethylsilane as internal standard (in NMR description, s = singlet, d = doublet, t = triplet, q = quadruplet, sext = sextuplet, m = multiplet, and b = broad). Coupling constants *J* are given in Hertz. IR spectra were obtained in KBr pellets using a PerkinElmer Paragon FTIR 1000 PC spectrometer. Only the most significant absorption bands have been reported. Electrospray mass spectrometric analysis was performed on a Waters Acquity UPLC System ZQ 2000 single quadrupole. All tested compounds displayed more than 95% purity. All reactions were monitored by thin-layer chromatography (TLC) using 0.2 mm-thick silica gel plates 60F-254 (5735 Merck). Column chromatography was carried out using silica gel 60 (70–230 Mesh, ASTM, Merck). Chemicals and solvents used are commercially available.

4-(Bromomethyl)benzenesulfonamide (2): NH_4OH (28% in H_2O) (0.93 mL, 13.83 mmol) was added to a stirred solution of 4-bromomethylbenzenesulfonyl chloride **1** (1.0 g, 3.71 mmol) in THF (12 mL) at 0 °C. The solution was stirred at RT for 1 h. the mixture was diluted with H_2O and the product was extracted with EtOAc. Organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo to give compound **2** as a white powder (910 mg, 98%): R_f = 0.40 (CH_2Cl_2/EtOH 19:1); mp: 176–177 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 4.80 (s, 2H), 7.43 (s, 2H), 7.67 (d, 2H, *J* = 8.2 Hz), 7.84 ppm (d, 2H, *J* = 8.2 Hz); IR (KBr): $\tilde{\nu}$ = 3361, 2957, 2929, 1569, 1507, 1463, 1329, 1153, 673 cm^{-1}; MS (ESI) *m/z* (%): 78.9 (71), 80.9 (100), 248.0 (58), 250.0 (71) [*M*−H]$^+$.

4-{[4-(Bromomethyl)phenyl]sulfonyl}morpholine (3): *N*,*N*-diisopropylethylamine (154 μL, 0.89 mmol) was added to a stirred solution of morpholine (71 μL, 0.82 mmol) in THF (3 mL) at 0 °C followed by dropwise addition of 4-bromomethylbenzenesulfonyl chloride **1** (200 mg, 0.74 mmol) in THF (4 mL). The solution was stirred at RT for 1 h. The mixture was diluted with H_2O and the product was extracted with CH_2Cl_2. Organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified with silica gel column chromatography (CH_2Cl_2) to yield compound **3** as a white powder (166 mg, 70%): R_f = 0.40 (CH_2Cl_2); mp: 147–148 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 2.90 (t, 4H, *J* = 4.6 Hz), 3.66 (t, 4H, *J* = 4.6 Hz), 4.84 (s, 2H), 7.77 ppm (s, 4H); IR (KBr): $\tilde{\nu}$ = 1339, 1164, 1099, 940, 729 cm^{-1}; MS (ESI) *m/z* (%): 82.8 (100), 319.8 (6), 321.9 (6) [*M*+H]$^+$.

4-(Bromomethyl)-*N*-(propyl)benzenesulfonamide (4): Using the synthetic procedure used for compound **3** starting from 4-bromomethylbenzenesulfonyl chloride **1** (300 mg, 1.11 mmol) and *n*-propylamine (183 μL, 2.22 mmol) to yield compound **4** as a light brown powder (265 mg, 82%): R_f = 0.35 (CH_2Cl_2); mp: 71–72 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 0.82 (t, 3H, J = 7.4 Hz), 1.40 (sext, 2H, J = 7.4 Hz), 2.71 (m, 2H), 4.80 (s, 2H), 7.65 (t, 1H, J = 6.0 Hz), 7.69 (d, 2H, J = 8.4 Hz), 7.80 ppm (d, 2H, J = 8.4 Hz); IR (KBr): $\tilde{\nu}$ = 3266, 2965, 2878, 1323, 1153, 669 cm^{-1}; MS (ESI) *m/z* (%): 82.9 (100), 291.9 (8), 293.9 (8) [*M*+H]$^+$.

4-(Bromomethyl)-*N*-(isopropyl)benzenesulfonamide (5): Using the synthetic procedure used for compound **3** starting from 4-bromomethylbenzenesulfonyl chloride **1** (200 mg, 0.74 mmol) and isopropylamine (127 μL, 1.48 mmol) to yield compound **5** as a white powder (210 mg, 97%): R_f = 0.40 (CH_2Cl_2); mp: 95–96 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 0.97 (d, 6H, J = 6.7 Hz), 3.29 (sep, 1H, J = 6.7 Hz), 4.80 (s, 2H), 7.67 (m, 3H), 7.82 ppm (dd, 2H, J = 6.4 Hz, J = 1.8 Hz); IR (KBr): $\tilde{\nu}$ = 3299, 1420, 1312, 1253, 676 cm^{-1}; MS (ESI) *m/z* (%): 82.9 (100), 291.9 (2), 293.9 (2) [*M*+H]$^+$.

4-(Bromomethyl)-*N*-(isobutyl)benzenesulfonamide (6): Using the synthetic procedure used for compound **3** starting from 4-bromomethylbenzenesulfonyl chloride **1** (300 mg, 1.11 mmol) and isobutylamine (221 μL, 2.22 mmol) to yield compound **6** as a white powder (300 mg, 88%): R_f = 0.50 (CH_2Cl_2); mp: 98–99 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 0.84 (d, 6H, J = 6.7 Hz), 1.65 (m, 1H, J = 6.7 Hz), 2.59 (d, 2H, J = 6.7 Hz), 4.80 (s, 2H), 7.68 (m, 3H), 7.81 ppm (d, 2H, J = 6.4 Hz); IR (KBr): $\tilde{\nu}$ = 3265, 2959, 1416, 1320, 1149, 674 cm^{-1}; MS (ESI) *m/z* (%): 82.9 (100), 305.9 (10), 308.0 (12) [*M*+H]$^+$.

4-(Bromomethyl)-*N*-(phenyl)benzenesulfonamide (7): Pyridine (60 μL, 0.74 mmol) was added to a stirred solution of aniline (62 μL, 0.67 mmol) in CH_2Cl_2 (5 mL) at 0 °C followed by the dropwise addition of 4-bromomethylbenzenesulfonyl chloride **1** (200 mg, 0.74 mmol) in CH_2Cl_2 (3 mL). The solution was stirred at 0 °C for 2 h. The mixture was diluted with CH_2Cl_2 and washed with 5 M HCl. Organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified on silica gel column chromatography (CH_2Cl_2) to yield compound **7** as a white powder (185 mg, 84%): R_f = 0.35 (CH_2Cl_2); mp: 124–125 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 4.74 (s, 2H), 7.06 (t, 1H, J = 7.2 Hz), 7.12 (d, 2H, J = 7.2 Hz), 7.26 (t, 2H, J = 7.2 Hz), 7.63 (d, 2H, J = 8.4 Hz), 7.77 (d, 2H, J = 8.4 Hz), 10.36 ppm (s, 1H); IR (KBr): $\tilde{\nu}$ = 3232, 1594, 1484, 1338, 1163, 698 cm^{-1}; MS (ESI) *m/z* (%): 78.9 (26), 80.8 (25), 324.0 (94), 326.0 (100) [*M*−H]$^+$.

4-(Bromomethyl)-*N*-(4-propylphenyl)benzenesulfonamide (8): Using the synthetic procedure used for compound **7** starting from 4-bromomethylbenzenesulfonyl chloride **1** (200 mg, 0.74 mmol) and 4-propylaniline (100 μL, 0.67 mmol) to yield compound **8** as a light red powder (225 mg, 90%): R_f = 0.60 (CH_2Cl_2); mp: 85–86 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 0.86 (t, 3H, J = 7.3 Hz), 1.52 (sext, 2H, J = 7.3 Hz), 2.46 (t, 2H, J = 7.3 Hz), 4.74 (s, 2H), 7.01 (d, 2H, J = 8.4 Hz), 7.07 (d, 2H, J = 8.4 Hz), 7.62 (d, 2H, J = 8.3 Hz), 7.74 (d, 2H, J = 8.3 Hz), 10.21 ppm (s, 1H); IR (KBr): $\tilde{\nu}$ = 3274, 2928, 2860, 1508, 1387, 1328, 1159, 686 cm^{-1}; MS (ESI) *m/z* (%): 78.8 (28), 80.9 (26), 366.1 (100), 368.1 (87) [*M*−H]$^+$.

4-(Bromomethyl)-*N*-(4-isopropylphenyl)benzenesulfonamide (9): Using the synthetic procedure used for compound **7** starting from 4-bromomethylbenzenesulfonyl chloride **1** (200 mg, 0.74 mmol) and 4-isopropylaniline (92 μL, 0.67 mmol) to yield compound **9** as a light red powder (205 mg, 81%): R_f = 0.45 (CH_2Cl_2); mp: 100–101 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 1.14 (d, 6H, J = 6.4 Hz), 2.81 (m, 1H), 4.75 (s, 2H), 7.03 (d, 2H, J = 7.5 Hz), 7.13 (d, 2H, J = 7.5 Hz), 7.63 (d, 2H, J = 7.5 Hz), 7.77 (d, 2H, J = 7.5 Hz), 10.24 ppm (s, 1H); IR (KBr): $\tilde{\nu}$ = 3268, 2963, 1510, 1446, 1326, 1161, 683 cm^{-1}; MS (ESI) *m/z* (%): 82.8 (100), 367.9 (4), 369.9 (4) [*M*+H]$^+$.

4-(Bromomethyl)-*N*-(4-nitrophenyl)benzenesulfonamide (10): Using the synthetic procedure used for compound **7** starting from 4-bromomethylbenzenesulfonyl chloride **1** (200 mg, 0.74 mmol) and 4-nitroaniline (93 mg, 0.67 mmol) to yield crude product **10** as a light brown powder (200 mg); MS (ESI) *m/z* (%): 369.0 (96), 371.0 (100) [*M*−H]$^+$.

4-({[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]methylamino}methyl)benzenesulfonamide (12): *N*,*N*-diisopropylethylamine (114 μL, 0.66 mmol) was added to a stirred solution of **11** (161 mg, 0.60 mmol) in CH_3CN (5 mL) followed by the addition of compound **2** (150 mg, 0.60 mmol). The solution was stirred at RT for 24 h. The solvent was removed under reduced pressure and the residue was partitioned between CH_2Cl_2 and H_2O. Organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified using silica gel column chromatography (CH_2Cl_2 and CH_2Cl_2/EtOH 99:1) to yield compound **12** as a white powder (215 mg, 82%): R_f = 0.75 (CH_2Cl_2/EtOH 95:5); mp: 151–152 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 2.09 (s, 3H), 2.79 (d, 1H, J = 14.6 Hz), 3.10 (d, 1H, J = 14.6 Hz), 3.47 (d, 1H, J = 14.0 Hz), 3.70 (d, 1H, J = 14.0 Hz), 4.57 (s, 2H), 5.83 (s, 1H), 7.01 (ddd, 1H, J_{H-F} = J_{H-H} = 8.4 Hz, J_{H-H} = 2.4 Hz), 7.21 (ddd, 1H, J_{H-F} = J'_{H-F} = 9.2 Hz, J_{H-H} = 2.4 Hz), 7.24 (d, 2H, J = 8.3 Hz), 7.32 (s, 2H), 7.46 (ddd, 1H, J_{H-H} = 8.4 Hz, J_{H-F} = J'_{H-F} = 6.8 Hz), 7.71 (d, 2H, J = 8.3 Hz), 7.80 (s, 1H), 8.33 ppm (s, 1H); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 43.7, 56.1, 62.0, 63.3, 75.5, 103.9, 110.8, 125.6 (2C), 126.2, 128.0 (2C), 130.0, 142.8, 143.5, 145.1, 150.7 ppm, (CF not visible); IR (KBr): $\tilde{\nu}$ = 3312, 1612, 1499, 1417, 1336, 1155 cm^{-1}; MS (ESI) *m/z* (%): 438.0 (100) [*M*+H]$^+$; UPLC purity 96%.

2-(2,4-Difluorophenyl)-1-{methyl[4-(morpholinosulfonylphenyl)-methyl]amino}-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (13): Using the synthetic procedure used for compound **12** starting from **11** (100 mg, 0.37 mmol) and **3** (119 mg, 0.37 mmol) to yield compound **13** as a white powder (160 mg, 85%): R_f = 0.15 (CH_2Cl_2/EtOH 98:2); mp: 57–58 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 2.15 (s, 3H), 2.79 (d, 1H, J = 13.7 Hz), 2.86 (m, 4H), 3.06 (d, 1H, J = 13.7 Hz), 3.55 (d, 1H, J = 14.0 Hz), 3.66 (m, 4H), 3.73 (d, 1H, J = 14.0 Hz), 4.52 (d, 1H, J = 14.3 Hz), 4.61 (d, 1H, J = 14.3 Hz), 5.84 (s, 1H), 7.00 (ddd, 1H, J_{H-F} = J_{H-H} = 8.4 Hz, J_{H-H} = 2.4 Hz), 7.17 (ddd, 1H, J_{H-F} = J'_{H-F} = 9.2 Hz, J_{H-H} = 2.4 Hz), 7.44 (m, 3H), 7.64 (d, 2H, J = 8.3 Hz), 7.78 (s, 1H), 8.32 ppm (s, 1H); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 44.1, 46.1 (2C), 56.1, 62.2, 63.3, 65.5 (2C), 75.5, 103.9, 110.9, 126.3, 127.7 (2C), 129.5 (2C), 130.1, 132.9, 145.1, 145.5, 150.7 ppm, (CF not visible); IR (KBr): $\tilde{\nu}$ = 3423, 1615, 1503, 1348, 1270, 1167, 1102 cm^{-1}; MS (ESI) *m/z* (%): 508.0 (100) [*M*+H]$^+$; UPLC purity 97%.

4-({[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]methylamino}methyl)-*N*-(propyl)benzenesulfonamide (14): Using the synthetic procedure used for compound **12** starting from **11** (138 mg, 0.51 mmol) and **4** (150 mg, 0.51 mmol) to yield compound **14** as a light brown powder (150 mg, 61%): R_f = 0.40 (CH_2Cl_2/EtOH 95:5); mp: 52–53 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 0.81 (t, 3H, J = 7.2 Hz), 1.38 (m, 2H), 2.13 (s, 3H), 2.69 (q, 2H, J = 7.2 Hz), 2.80 (d, 1H, J = 13.6 Hz), 3.07 (d, 1H, J = 13.6 Hz), 3.50 (d, 1H, J = 14.0 Hz), 3.69 (d, 1H, J = 14.0 Hz), 4.53 (d, 1H, J = 14.4 Hz), 4.59 (d, 1H, J = 14.4 Hz), 5.83 (s, 1H), 7.00 (ddd, 1H, J_{H-F} = J_{H-H} = 8.4 Hz, J_{H-H} = 2.4 Hz), 7.18 (ddd, 1H, J_{H-F} = J'_{H-F} = 9.2 Hz, J_{H-H} = 2.4 Hz), 7.29 (d, 2H, J = 7.6 Hz), 7.45 (ddd, 1H, J_{H-H} = 8.4 Hz, J_{H-F} = J'_{H-F} = 6.8 Hz), 7.54 (m, 1H), 7.68 (d, 2H, J = 7.6 Hz), 7.79 (s, 1H),

8.33 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 11.3, 22.5, 43.9, 44.5, 56.0, 62.1, 63.2, 75.5, 103.9, 110.8, 126.2, 126.4 (2C), 129.1 (2C), 130.0, 139.2, 144.1, 145.1, 150.7, 159.2, 161.9 ppm; IR (KBr): $\tilde{\nu}$ = 3452, 3285, 2965, 2873, 1617, 1502, 1323, 1273, 1160, 1092 cm^{-1}; MS (ESI) *m/z* 480.2 (100) [*M*+H]$^+$; UPLC purity 100%.

4-({[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]methylamino}methyl)-*N*-(isopropyl)benzenesulfonamide (15): Using the synthetic procedure used for compound **12** starting from **11** (130 mg, 0.49 mmol) and **5** (142 mg, 0.49 mmol) to yield compound **15** as a white powder (mg, 80%): R_f = 0.20 (CH_2Cl_2/EtOH 98:2); mp: 135–136 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 0.95 (d, 6H, *J* = 6.4 Hz), 2.13 (s, 3H), 2.79 (d, 1H, *J* = 13.7 Hz), 3.06 (d, 1H, *J* = 13.7 Hz), 3.25 (m, 1H), 3.50 (d, 1H, *J* = 13.4 Hz), 3.69 (d, 1H, *J* = 13.4 Hz), 4.51 (d, 1H, *J* = 14.3 Hz), 4.60 (d, 1H, *J* = 14.3 Hz), 5.82 (s, 1H), 7.00 (ddd, 1H, $J_{H-F} = J_{H-H}$ = 8.4 Hz, J_{H-H} = 2.4 Hz), 7.18 (ddd, 1H, $J_{H-F} = J'_{H-F}$ = 9.2 Hz, J_{H-H} = 2.4 Hz), 7.29 (d, 2H, *J* = 8.0 Hz), 7.45 (ddd, 1H, J_{H-H} = 8.4 Hz, $J_{H-F} = J'_{H-F}$ = 6.8 Hz), 7.54 (d, 1H, *J* = 7.3 Hz), 7.70 (d, 2H, *J* = 8.0 Hz), 7.78 (s, 1H), 8.32 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 23.3 (2C), 44.0, 45.4, 56.0, 62.1, 63.2, 75.5, 103.9, 110.8, 126.1, 126.4 (2C), 129.2 (2C), 130.1 (CH), 140.5, 144.0, 145.1, 150.7, 159.2, 161.9 ppm; IR (KBr): $\tilde{\nu}$ = 3440, 3281, 2970, 1616, 1504, 1429, 1316, 1148 cm^{-1}; MS (ESI) *m/z* (%): 480.0 (100) [*M*+H]$^+$; UPLC purity 95%.

4-({[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]methylamino}methyl)-*N*-(isobutyl)benzenesulfonamide (16): Using the synthetic procedure used for compound **12** starting from **11** (131 mg, 0.49 mmol) and **6** (150 mg, 0.49 mmol) to yield compound **16** as a colorless oil (195 mg, 81%): R_f = 0.25 (CH_2Cl_2/EtOH 98:2); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 0.82 (d, 6H, *J* = 6.7 Hz), 1.63 (m, 1H), 2.12 (s, 3H), 2.78 (d, 1H, *J* = 13.7 Hz), 3.07 (d, 1H, *J* = 13.7 Hz), 3.35 (m, 2H), 3.49 (d, 1H, *J* = 13.7 Hz), 3.70 (d, 1H, *J* = 13.7 Hz), 4.52 (d, 1H, *J* = 14.3 Hz), 4.60 (d, 1H, *J* = 14.3 Hz), 5.82 (s, 1H), 7.00 (ddd, 1H, $J_{H-F} = J_{H-H}$ = 8.4 Hz, J_{H-H} = 2.4 Hz), 7.18 (ddd, 1H, $J_{H-F} = J'_{H-F}$ = 9.2 Hz, J_{H-H} = 2.4 Hz), 7.28 (d, 2H, *J* = 8.5 Hz), 7.45 (ddd, 1H, J_{H-H} = 8.4 Hz, $J_{H-F} = J'_{H-F}$ = 6.8 Hz), 7.56 (d, 1H, *J* = 6.1 Hz), 7.68 (d, 2H, *J* = 8.5 Hz), 7.79 (s, 1H), 8.32 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 20.1 (2C), 28.2, 44.0, 50.2, 56.0, 62.1, 63.1, 75.5, 103.9, 110.8, 126.2, 126.4 (2C), 129.2 (2C), 130.2, 139.3, 144.1, 145.1, 150.7, 159.2, 161.9 ppm; IR (NaCl): $\tilde{\nu}$ = 3267, 2937, 1600, 1472, 1313, 1138 cm^{-1}; MS (ESI) *m/z* (%): 494.0 (100) [*M*+H]$^+$; UPLC purity 97%.

4-({[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]methylamino}methyl)-*N*-(phenyl)benzenesulfonamide (17): Using the synthetic procedure used for compound **12** starting from **11** (82 mg, 0.31 mmol) and **7** (100 mg, 0.31 mmol) to yield compound **17** as a light brown powder (130 mg, 82%): R_f = 0.20 (CH_2Cl_2/EtOH 98:2); mp: 128–129 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 2.08 (s, 3H), 2.76 (d, 1H, *J* = 13.6 Hz), 3.03 (d, 1H, *J* = 13.6 Hz), 3.45 (d, 1H, *J* = 14.4 Hz), 3.64 (d, 1H, *J* = 14.4 Hz), 4.51 (d, 1H, *J* = 14.0 Hz), 4.57 (d, 1H, *J* = 14.0 Hz), 5.79 (s, 1H), 6.97 (ddd, 1H, $J_{H-F} = J_{H-H}$ = 8.4 Hz, J_{H-H} = 2.4 Hz), 7.04 (t, 1H, *J* = 7.2 Hz), 7.09–7.16 (m, 3H), 7.23–7.26 (m, 4H), 7.42 (ddd, 1H, J_{H-H} = 8.4 Hz, $J_{H-F} = J'_{H-F}$ = 6.8 Hz), 7.64 (d, 2H, *J* = 8.0 Hz), 7.78 (s, 1H), 8.31 (s, 1H), 10.24 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 43.9, 56.0, 62.1, 63.3, 75.5, 103.8, 110.7, 120.2 (2C), 124.2, 126.2, 126.6 (2C), 129.1 (2C), 129.3 (2C), 130.1, 137.9, 138.1, 144.8, 145.1, 150.6 ppm, (CF not visible); IR (KBr): $\tilde{\nu}$ = 3465, 1613, 1498, 1339, 1271, 1159, 1092 cm^{-1}; MS (ESI) *m/z* (%): 514.1 (100) [*M*+H]$^+$; UPLC purity 95%.

4-({[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]methylamino}methyl)-*N*-(4-propylphenyl)benzenesulfonamide (18): Using the synthetic procedure used for compound **12** starting from **11** (87 mg, 0.33 mmol) and **8** (120 mg, 0.33 mmol) to yield compound **18** as a white powder (130 mg, 72%): R_f = 0.25 (CH_2Cl_2/EtOH 98:2); mp: 77–78 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 0.84 (t, 3H, *J* = 7.2 Hz), 1.51 (sext, 2H, *J* = 7.2 Hz), 2.08 (s, 3H), 2.45 (t, 2H, *J* = 7.2 Hz), 2.76 (d, 1H, *J* = 14.0 Hz), 3.02 (d, 1H, *J* = 14.0 Hz), 3.45 (d, 1H, *J* = 14.0 Hz), 3.64 (d, 1H, *J* = 14.0 Hz), 4.51 (d, 1H, *J* = 13.2 Hz), 4.57 (d, 1H, *J* = 13.2 Hz), 5.79 (s, 1H), 6.95–7.06 (m, 5H), 7.12 (ddd, 1H, $J_{H-F} = J'_{H-F}$ = 9.2 Hz, J_{H-H} = 2.4 Hz), 7.22 (d, 2H, *J* = 7.6 Hz), 7.42 (ddd, 1H, J_{H-H} = 8.4 Hz, $J_{H-F} = J'_{H-F}$ = 6.8 Hz), 7.61 (d, 2H, *J* = 7.6 Hz), 7.78 (s, 1H), 8.31 (s, 1H), 10.09 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 13.7, 24.1, 36.6, 43.9, 56.0, 62.1, 63.2, 75.5, 103.9, 110.8, 120.7 (2C), 126.1, 126.6 (2C), 129.1 (4C), 130.1, 135.4, 138.2, 138.3, 144.7, 145.1, 150.1 ppm, (CF not visible); IR (KBr): $\tilde{\nu}$ = 3455, 2929, 2860, 1615, 1507, 1460, 1335, 1277, 1159, 1093 cm^{-1}; MS (ESI) *m/z* (%): 556.1 (100) [*M*+H]$^+$; UPLC purity 99%.

4-({[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]methylamino}methyl)-*N*-(4-isopropylphenyl)benzenesulfonamide (19): Using the synthetic procedure used for compound **12** starting from **11** (87 mg, 0.33 mmol) and **9** (120 mg, 0.33 mmol) to yield compound **19** as a white powder (135 mg, 75%): R_f = 0.20 (CH_2Cl_2/EtOH 98:2); mp: 67–68 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.13 (d, 6H, *J* = 6.8 Hz), 2.08 (s, 3H), 2.74–2.82 (m, 2H), 3.03 (d, 1H, *J* = 13.6 Hz), 3.45 (d, 1H, *J* = 14.4 Hz), 3.65 (d, 1H, *J* = 14.4 Hz), 4,51 (d, 1H, *J* = 14.0 Hz), 4.57 (d, 1H, *J* = 14.0 Hz), 5.79 (s, 1H), 6.97 (m, 1H), 7.00 (d, 2H, *J* = 8.0 Hz), 7.10 (m, 3H), 7.23 (d, 2H, *J* = 7.6 Hz), 7.42 (ddd, 1H, J_{H-H} = 8.4 Hz, $J_{H-F} = J'_{H-F}$ = 6.8 Hz), 7.63 (d, 2H, *J* = 7.6 Hz), 7.77 (s, 1H), 8.31 (s, 1H), 10.12 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 24.9 (2C), 32.8, 44.0, 56.0, 62.1, 63.2, 75.5, 103.9, 110.8, 120.7 (2C), 126.2, 126.6 (2C), 127.0 (2C), 129.1 (2C), 130.0, 135.5, 138.4, 144.4, 144.7, 145.1, 150.1, 159.1, 161.8 ppm; IR (KBr): $\tilde{\nu}$ = 3448, 2960, 2864, 1616, 1508, 1461, 1327, 1275, 1160, 1092 cm^{-1}; MS (ESI) *m/z* (%): 556.1 (100) [*M*+H]$^+$; UPLC purity 97%.

4-({[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]methylamino}methyl)-*N*-(4-nitrophenyl)benzenesulfonamide (20): Using the synthetic procedure used for compound **12** starting from **11** (145 mg, 0.54 mmol) and **10** (200 mg, 0.54 mmol) to yield compound **20** as a yellow powder (125 mg, 33% in two steps): R_f = 0.25 (CH_2Cl_2/EtOH 98:2); mp: 96–97 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 2.09 (s, 3H), 2.75 (d, 1H, *J* = 13.6 Hz), 3.02 (d, 1H, *J* = 13.6 Hz), 3.47 (d, 1H, *J* = 14.4 Hz), 3.65 (d, 1H, *J* = 14.4 Hz), 4.48 (d, 1H, *J* = 14.0 Hz), 4.56 (d, 1H, *J* = 14.0 Hz), 5.79 (s, 1H), 6.96 (ddd, 1H, $J_{H-F} = J_{H-H}$ = 8.4 Hz, J_{H-H} = 2.4 Hz), 7.11 (ddd, 1H, $J_{H-F} = J'_{H-F}$ = 9.2 Hz, J_{H-H} = 2.4 Hz), 7.29–7.33 (m, 4H), 7.41 (ddd, 1H, J_{H-H} = 8.4 Hz, $J_{H-F} = J'_{H-F}$ = 6.8 Hz), 7.76–7.78 (m, 3H), 8.16 (d, 2H, *J* = 8.8 Hz), 8.29 (s, 1H), 11.26 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 44.0, 56.0, 62.0, 63.2, 75.5, 103.7, 111.0, 118.1 (2C), 125.6 (2C), 126.1, 126.7 (2C$_t$), 129.5 (2C), 130.1, 137.6, 142.7, 144.5, 145.1, 145.6, 150.6 ppm, (CF not visible); IR (KBr): $\tilde{\nu}$ = 3444, 1599, 1500, 1342, 1276, 1161, 1090 cm^{-1}; MS (ESI) *m/z* (%): 559.0 (100) [*M*+H]$^+$; UPLC purity 99%.

2-(2,4-Difluorophenyl)-1-[methyl(4-nitrobenzyl)amino]-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (21): Using the synthetic procedure used for compound **12** starting from **11** (1.24 g, 4.63 mmol) and 4-nitrobenzylbromide (1.00 g, 4.63 mmol) to yield compound **21** as an orange oil (1.44 g, 77%): R_f = 0.25 (CH_2Cl_2/EtOH 98:2); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 2.13 (s, 3H), 2.79 (d, 1H, *J* = 13.7 Hz), 3.10 (d, 1H, *J* = 13.7 Hz), 3.56 (d, 1H, *J* = 14.3 Hz), 3.77 (d, 1H, *J* = 14.3 Hz), 4.53 (d, 1H, *J* = 14.0 Hz), 4.61 (d, 1H, *J* = 14.0 Hz), 5.85 (s, 1H), 7.00 (ddd, 1H, $J_{H-F} = J_{H-H}$ = 8.4 Hz, J_{H-H} = 2.4 Hz), 7.19 (ddd, 1H,

$J_{H-F}=J'_{H-F}=9.2$ Hz, $J_{H-H}=2.4$ Hz), 7.38 (d, 2H, $J=8.5$ Hz), 7.46 (ddd, 1H, $J_{H-H}=8.4$ Hz, $J_{H-F}=J'_{H-F}=6.8$ Hz), 7.79 (s, 1H), 8.13 (d, 2H, $J=8.5$ Hz), 8.32 ppm (s, 1H); IR (NaCl): $\tilde{\nu}=3381$, 2948, 1609, 1514, 1421, 1347, 1272, 1102 cm^{-1}. MS (ESI) *m/z* (%): 404.0 (100) $[M+H]^+$; UPLC purity 100%.

2-(2,4-Difluorophenyl)-1-[methyl(4-aminobenzyl)amino]-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (22): Active charcoal-supported Pd (5%, 140 mg) was added to a stirred solution of **21** (1.4 g, 3.47 mmol) in EtOH (100 mL). The solution was stirred at RT for 2 h under H_2 atmosphere (5 bar). The mixture was filtered through a pad of Celite, and the filtrate was concentrated. The residue was purified using silica gel column chromatography (CH_2Cl_2 and CH_2Cl_2/EtOH 98:2) to give compound **22** as a light brown powder (940 mg, 72%): $R_f=0.40$ (CH_2Cl_2/EtOH 95:5); mp: 86–87 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=2.07$ (s, 3H), 2.78 (d, 1H, $J=14.0$ Hz), 2.93 (d, 1H, $J=14.0$ Hz), 3.26 (d, 1H, $J=14.0$ Hz), 3.34 (d, 1H, $J=14.0$ Hz), 4.48 (d, 1H, $J=14.3$ Hz), 4.56 (d, 1H, $J=14.3$ Hz), 4.95 (s, 2H), 5.70 (s, 1H), 6.47 (d, 2H, $J=8.2$ Hz), 6.77 (d, 2H, $J=8.2$ Hz), 6.98 (ddd, 1H, $J_{H-F}=J_{H-H}=8.4$ Hz, $J_{H-H}=2.4$ Hz), 7.17 (ddd, 1H, $J_{H-F}=J'_{H-F}=9.2$ Hz, $J_{H-H}=2.4$ Hz), 7.43 (ddd, 1H, $J_{H-H}=8.4$ Hz, $J_{H-F}=J'_{H-F}=6.8$ Hz), 7.75 (s, 1H), 8.31 ppm (s, 1H); IR (KBr): $\tilde{\nu}=3308$, 3211, 1617, 1507, 1280, 1129 cm^{-1}; MS (ESI) *m/z* (%): 105.7 (100), 374.1 (12) $[M+H]^+$.

***N*-[4-({[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)-propyl]methylamino}methyl)phenyl]propane-2-sulfonamide (23)**: Isopropylsulfonyl chloride (49 μL, 0.44 mmol) was added to a stirred solution of **22** (150 mg, 0.40 mmol) in pyridine (2 mL). The solution was stirred at RT for 2 h. The solvent was removed under reduced pressure and the residue was partitioned between CH_2Cl_2 and H_2O. Organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified using silica gel column chromatography (CH_2Cl_2 and CH_2Cl_2/EtOH 99:1) to give compound **23** as a white powder (90 mg, 47%): $R_f=0.10$ (CH_2Cl_2/EtOH 98:2); mp: 60–61 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=1.24$ (d, 6H, $J=6.8$ Hz), 2.10 (s, 3H), 2.77 (d, 1H, $J=13.6$ Hz), 2.98 (d, 1H, $J=13.6$ Hz), 3.20 (m, 1H), 3.40 (d, 1H, $J=13.2$ Hz), 3.50 (d, 1H, $J=13.2$ Hz), 4.47 (d, 1H, $J=14.4$ Hz), 4.56 (d, 1H, $J=14.4$ Hz), 5.74 (s, 1H), 7,00 (ddd, 1H, $J_{H-F}=J_{H-H}=8.4$ Hz, $J_{H-H}=2.4$ Hz), 7.04 (d, 2H, $J=8.8$ Hz), 7.14 (d, 2H, $J=8.8$ Hz), 7.17 (ddd, 1H, $J_{H-F}=J'_{H-F}=9.2$ Hz, $J_{H-H}=2.4$ Hz), 7.44 (ddd, 1H, $J_{H-H}=8.4$ Hz, $J_{H-F}=J'_{H-F}=6.8$ Hz), 7.77 (s, 1H), 8.31 (s, 1H), 9.70 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=16.3$ (2C), 43.9, 51.3, 56.0, 62.2, 63.0, 75.1, 103.7, 110.9, 119.4 (2C), 126.4, 129.8 (2C), 130.1, 134.4, 137.4, 145.0, 150.6 ppm, (CF not visible); IR (KBr): $\tilde{\nu}=3445$, 1620, 1508, 1326, 1140 cm^{-1}; MS (ESI) *m/z* (%): 480.0 (100) $[M+H]^+$; UPLC purity 95%.

***N*-[4-({[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)-propyl]methylamino}methyl)phenyl]benzenesulfonamide (24)**: Indium powder (5 mg, 0.04 mmol) was added to a stirred solution of **22** (150 mg, 0.40 mmol) in CH_3CN (3 mL) under argon followed by the addition of benzenesulfonyle chloride (52 μL, 0.40 mmol). The solution was stirred at RT overnight. The solvent was removed under reduced pressure and the residue was partitioned between CH_2Cl_2 and H_2O. Organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified using silica gel column chromatography (CH_2Cl_2 and CH_2Cl_2/EtOH 99:1) to yield compound **24** as a white powder (140 mg, 68%): $R_f=0.15$ (CH_2Cl_2/EtOH 98:2); mp: 125–126 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=2.04$ (s, 3H), 2.73 (d, 1H, $J=13.6$ Hz), 2.95 (d, 1H, $J=13.6$ Hz), 3.31 (d, 1H, $J=13.2$ Hz), 3.44 (d, 1H, $J=13.2$ Hz), 4.47 (d, 1H, $J=14.4$ Hz), 4.54 (d, 1H, $J=14.4$ Hz), 5.72 (s, 1H), 6.94–7.00 (m, 5H), 7.13 (ddd, 1H, $J_{H-F}=J'_{H-F}=9.2$ Hz, $J_{H-H}=2.4$ Hz), 7.41 (ddd, 1H, $J_{H-H}=8.4$ Hz, $J_{H-F}=J'_{H-F}=6.8$ Hz), 7.56 (d, 2H, $J=7.6$ Hz), 7.63 (s, 1H), 7.75–7.77 (m, 3H), 8.30 (s, 1H), 10.19 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=43.8$, 56.0, 62.2, 63.0, 75.2, 103.8, 110.7, 120.2 (2C), 126.3, 126.8 (2C), 129.4 (2C), 129.5 (2C), 130.0, 133.0, 135.0, 136.5, 139.7, 145.0, 150.6 ppm, (CF not visible); IR (KBr): $\tilde{\nu}=3450$, 1619, 1506, 1331, 1158 cm^{-1}; MS (ESI) *m/z* (%): 514.0 (100) $[M+H]^+$; UPLC purity 95%.

***N*-[4-({[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)-propyl]methylamino}methyl)phenyl]-4-isopropylbenzenesulfonamide (25)**: Using the synthetic procedure used for compound **24** starting from with **22** (100 mg, 0.27 mmol) and 4-isopropylbenzenesulfonyle chloride (48 μL, 0.27 mmol) to yield compound **25** as a light brown powder (100 mg, 67%): $R_f=0.50$ (CH_2Cl_2/EtOH 95:5); mp: 92–93 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=1.19$ (d, 6H, $J=6.8$ Hz), 2.04 (s, 3H), 2.73 (d, 1H, $J=13.6$ Hz), 2.93–2.98 (m, 2H), 3.31 (d, 1H, $J=13.2$ Hz), 3.45 (d, 1H, $J=13.2$ Hz), 4.48 (d, 1H, $J=14.4$ Hz), 4.54 (d, 1H, $J=14.4$ Hz), 5.72 (s, 1H), 6.94–7.01 (m, 5H), 7.11 (ddd, 1H, $J_{H-F}=J'_{H-F}=9.2$ Hz, $J_{H-H}=2.4$ Hz), 7.38–7.44 (m, 3H), 7.69 (d, 2H, $J=8.4$ Hz), 7.76 (s, 1H), 8.30 (s, 1H), 10.19 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=23.5$ (2C), 33.5, 43.8, 56.0, 62.2, 63.2, 75.2, 103.8, 110.7, 119.8 (2C), 126.3, 126.9 (2C), 127.3 (2C), 129.6 (2C), 130.0, 134.8, 136.7, 137.4, 145.0, 150.6, 153.7 ppm, (CF not visible); IR (KBr): $\tilde{\nu}=3450$, 2958, 1618, 1508, 1328, 1162, 1087 cm^{-1}; MS (ESI) *m/z* (%): 556.2 (100) $[M+H]^+$; UPLC purity 98%.

***N*-[4-({[2-(2,4-Difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)-propyl]methylamino}methyl)phenyl]-4-nitrobenzenesulfonamide (26)**: Using the synthetic procedure used for compound **24** starting from **22** (150 mg, 0.40 mmol) and 4-nitrobenzenesulfonyle chloride (89 mg, 0.40 mmol) to yield compound **26** as a light yellow powder (65 mg, 29%): $R_f=0.10$ (CH_2Cl_2/EtOH 98:2); mp: 159–160 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=2.07$ (s, 3H), 2.72 (d, 1H, $J=14.0$ Hz), 2.93 (d, 1H, $J=14.0$ Hz), 3.33 (d, 1H, $J=13.2$ Hz), 3.45 (d, 1H, $J=13.2$ Hz), 4.42 (d, 1H, $J=14.0$ Hz), 4.54 (d, 1H, $J=14.0$ Hz), 5.73 (s, 1H), 6.94–7.00 (m, 5H), 7.12 (ddd, 1H, $J_{H-F}=J'_{H-F}=9.2$ Hz, $J_{H-H}=2.4$ Hz), 7.40 (ddd, 1H, $J_{H-H}=8.4$ Hz, $J_{H-F}=J'_{H-F}=6.8$ Hz), 7.75 (s, 1H), 7.99 (d, 2H, $J=8.8$ Hz), 8.30 (s, 1H), 8.39 (d, 2H, $J=8.8$ Hz), 10.54 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=43.9$, 55.6, 61.8, 62.8, 75.3, 103.5, 110.5, 120.8 (2C), 124.8 (2C), 126.4, 128.4 (2C), 129.8 (2C), 130.1, 135.7, 135.8, 145.0, 145.1, 150.6, 150.1 ppm, (CF not visible); IR (KBr): $\tilde{\nu}=3450$, 1623, 1528, 1350, 1166 cm^{-1}; MS (ESI) *m/z* (%): 559.2 (100) $[M+H]^+$; UPLC purity 96%.

Biological assays

Antifungal activity: All these compounds were screened for their antifungal activity against *C. albicans* (CA98001, DSY735, CAAL-74), *C. krusei* (CK506, CK8), *C. glabrata* (CG468), *C. parapsilosis* (CAPA1, CAPA2), and *A. fumigatus* (AF98003) strains. DSY735 was a gift from Pr. D. Sanglard (Institute of Microbiology, University of Lausanne, Switzerland). All other strains were issued from our collection. Inhibition growth was measured as previously described.[46] Fluconazole, voriconazole, and itraconazole were used as positive controls. MICs were defined as the concentrations that inhibit growth of *Candida* spp. or *A. fumigatus* by 50% or 80% respectively as recommended by the Clinical and Laboratory Standards Institute document M27A3 and M38A2 guidelines.[47] MIC values are expressed in $\mu g\,mL^{-1}$.

Sterol extraction and analysis: To study sterol synthesis, *C. albicans* CA98001 cells were incubated in 50 mL Sabouraud broth medium (Sigma–Aldrich) for 18 h at 35 °C with stirring. Compound **17** was introduced into the culture medium before incubation. Cells were

collected by centrifugation at 1500 g. The pellet was suspended in 3 mL of saponification medium (25 g of KOH, 36 mL of distilled H_2O and brought to 100 mL with 100% EtOH). Then suspension was vortexed for 1 min and incubated at 80 °C for 60 min. Sterols were then extracted by addition of a mixture of 1 mL of distilled H_2O and 4 mL of *n*-hexane (Merck, Darmstadt, Germany). Hexane extract was then evaporated. Samples were derivatized with 100 μL of silylating mixture (Fluka, Saint Quentin Fallavier, France) at RT for 30 min, evaporated, and diluted in 500 μL of *n*-hexane. Two microliters were injected into a gas chromatograph (model 6890N, Agilent Technologies, Palo Alto, CA, USA) coupled with a quadrupole mass spectrometer (5973i, Agilent Technologies, Palo Alto, CA, USA). Analyses were carried out in a splitless mode with helium as carrier gas (constant rate of 1.2 mL min^{-1}) and the injector temperature was 250 °C. The transfer line between the gas chromatograph and the mass spectrometer was operated at 290 °C and the EI source was held at 280 °C. The GC capillary column was an HP-5MS (30 m × 0.25 mm ID, 0.25 μm film thickness, Agilent Technologies). The GC oven was programmed as follows: initial temperature 150 °C, hold for 0.5 min, ramp to 280 °C at 40 °C min^{-1}, and ramp to 300 °C at 5 °C min^{-1}, hold for 6 min. Sterols of interest were identified by their mass spectrum. With a view to investigating the influence of treatment on sterol abundance, the area under the curve (AUC) of each peak was used to calculate the ratio of (sterol AUC)/(sum of sterols AUC).

Molecular modeling

Molecular modeling studies were performed using Sybyl software version 8.0[48] running on a DELL precision T3400 workstation. The structure of CYP51 from *M. tuberculosis* complexed with fluconazole (PDB code, 1A1)[28] was used as the template for the homology model of *C. albicans* CYP51. The protocol is described in detail elsewhere.[37,42] Flexible docking of azoles into the enzyme active site was performed using GOLD software.[49] A distance constraint was applied from N-4 of the triazole ring to the heme iron ($2.0 < d < 2.4$ Å). For each compound, the most stable docking model was selected according to the best scored conformation as predicted by the GoldScore scoring function.

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Keywords: antifungal agents · *Candida albicans* · CYP51 inhibitors · structure–activity relationships · triazoles

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