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Synthesis and structure–activity relationships of 2-phenyl-1-[(pyridinyl- and piperidinylmethyl)amino]-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ols as antifungal agents

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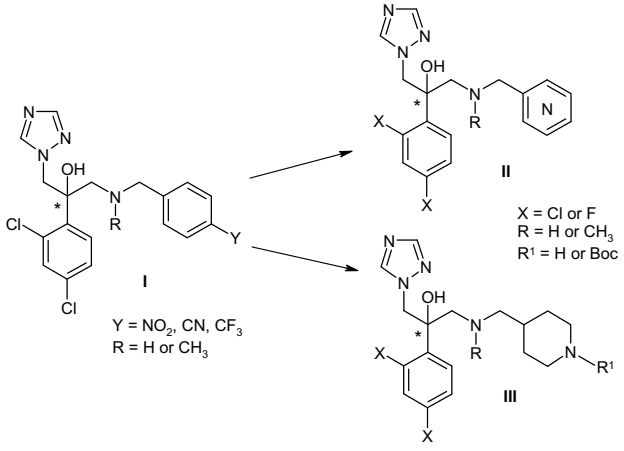
ABSTRACT

Continuous efforts on the synthesis and structure–activity relationships (SARs) studies of modified 1-benzylamino-2-phenyl-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ols as antifungal agents, allowed identification of new 1-[(pyridinyl- and piperidinylmethyl)amino] derivatives with MIC_{80} values ranging from 1410.0 to 23.0 ng mL^{-1} on *Candida albicans*. These results confirmed both the importance of π–π stacking and hydrogen bonding interactions in the active site of CYP51-*C. albicans*.

During the past two decades, the frequency of invasive and systemic fungal infections has increased dramatically due mainly to *Candida* species among which *Candida albicans*, at the origin of the greatest number of pathologies. Because of their safety profile and high therapeutic index, azoles are the most widely used and studied class of antifungal agents. They target the biosynthesis of ergosterol, a major component of fungal membranes (thereby preventing fungal growth), by inhibiting the cytochrome P450-dependent lanosterol 14α-demethylase (CYP51), encoded by the *ERG11* gene. Unfortunately, azoles are fungistatic against yeasts and the broad usage of these compounds led to development of resistance showing the urgent need for new and effective antifungal agents.

Recent studies showed that typical azole inhibitors were able to fit the putative active site of CYP51 by a combination of heme coordination, hydrogen bonding, π–π stacking and hydrophobic interactions.[1–3] In particular, the receptor-based pharmacophore model published by Sheng et al.[3] highlighted the importance of Tyr118 and Ser378 residues in the stabilization of the inhibitors within the channel 2 which is oriented to the FG loop.

In an attempt to find potent azole antifungal agents focused on 1-benzylamino-2-phenyl-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ols, we previously studied compounds bearing H-bond acceptors entities



Scheme 1. General structures of synthesized compounds.

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in *para* position of the benzyl group (**I**, Scheme 1). These compounds showed MIC_{80} values ranging from 0.37 to 30.0 ng mL^{-1}.[4]

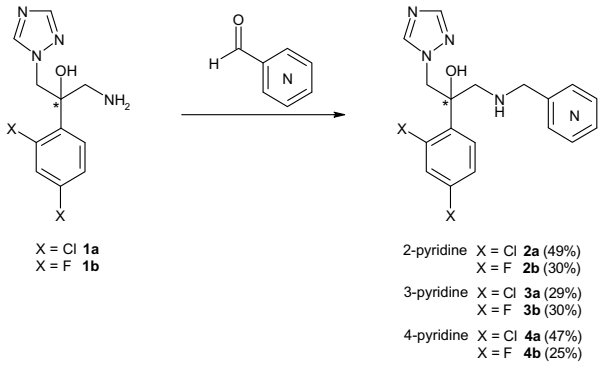
In this letter, we describe our continued efforts in the structure–activity relationships (SARs) studies toward the identification of new 1-[(pyridinyl- and piperidinylmethyl)amino] derivatives (**II** and **III**, Scheme 1).

1-[(pyridinylmethyl)amino] derivatives **2a**, **2b**, **3a**, **3b**, **4a**, and **4b** were synthesized from previously described key intermediates **1a** or **1b**[4] by reductive amination with the corresponding pyridinecarboxaldehydes in moderate yields (Scheme 2).[5]

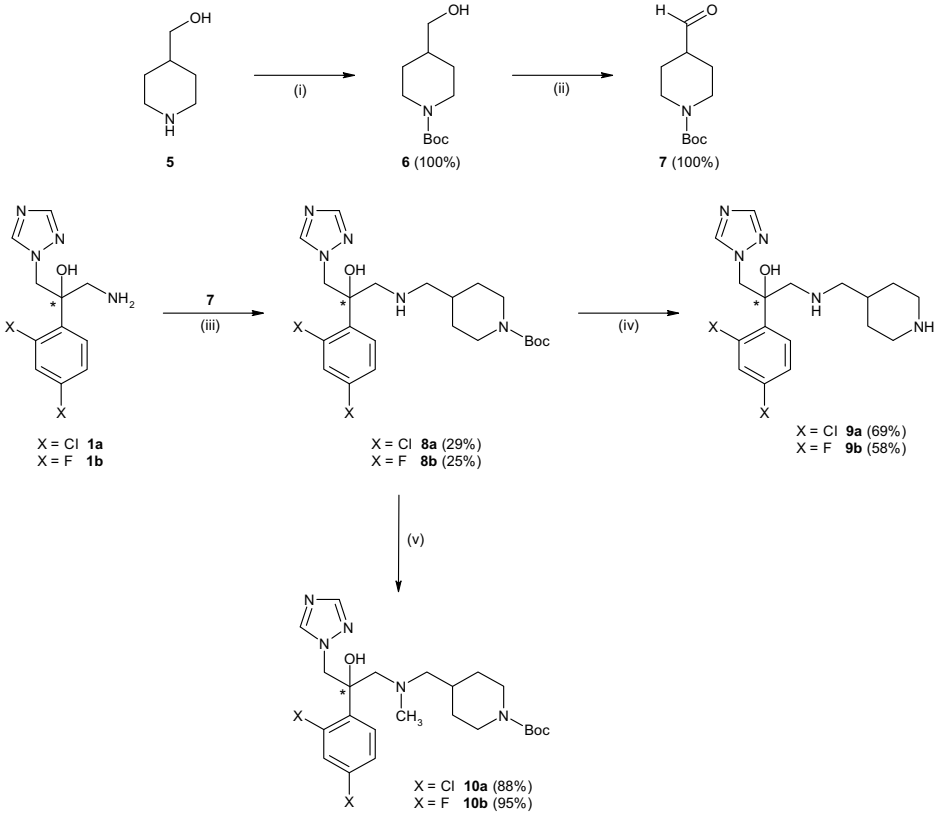
Scheme 3 outlines the general synthesis of 1-[(piperidinylmethyl)amino] derivatives **8a**, **8b**, **9a**, **9b**, **10a**, and **10b**. Treatment of the commercially available 4-piperidinemethanol **5** with di-*tert*-butyl dicarbonate afforded **6** in quantitative yield.[6] The primary alcohol was then converted into aldehyde **7** via a Swern oxidation using dimethyl sulfoxide, oxalyl chloride and triethylamine.[7] Next, compounds **8a** and **8b** were prepared from the appropriate amino-alcohol **1a** or **1b** according to the procedure described in Scheme 2.[8] Removal of the Boc protective group with trifluoroacetic acid led to amines **9a** and **9b**.[9] Finally, N-methylation was achieved by reductive amination of **8a** and **8b** with formaldehyde to afford two additional products **10a and 10b**.[10]

All these compounds were screened for their antifungal activity against *Candida albicans* CA98001 and *Aspergillus fumigatus* AF98003 strains. Inhibition growth was measured as previously described.[11] Fluconazole and itraconazole were used as positive controls. The minimum inhibitory concentration (MIC_{80}) values (in ng mL^{-1}) are presented in Table 1.

On *C. albicans*, the biological results are relatively heterogeneous ($23.0 < MIC_{80}$ (ng mL^{-1}) < 1410.0). Chlorinated compounds bearing a pyridine moiety (**2a**, **3a**, and **4a**) exhibited significant level of activity with MIC values 5- to 8-fold lower than that of fluconazole. Fluorinated analogues (**2b**, **3b**, and **4b**) are less active (MIC_{80} values of 220.0, 130.0, and 83.0 ng mL^{-1}, respectively). Interestingly, replacement of the pyridine ring (**4a**, MIC_{80} = 36.0 ng mL^{-1} and **4b**, MIC_{80} = 83.0 ng mL^{-1}) by a piperidine ring (**9a**, MIC_{80} = 120.0 ng mL^{-1} and **9b**, MIC_{80} = 1410.0 ng mL^{-1}) led to weaker inhibitors whatever the dihalogenophenyl group. In contrast, the corresponding *N*-Boc compounds **8a** and **8b** considerably improve the efficacy of this series to reach high inhibitory activities (MIC_{80} values of 26.0 and 27.0 ng mL^{-1}, respectively). Taken together, these results seem to imply the role of both aromatic and hydrogen bond interactions within the active site.

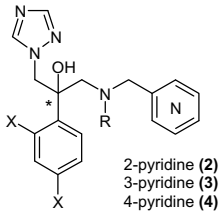


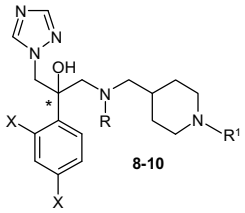
Scheme 2. Preparation of targeted compounds **2a**, **2b**, **3a**, **3b**, **4a**, and **4b**. Reagents and conditions: 2-pyridinecarboxaldehyde, 3-pyridinecarboxaldehyde (0.50 equiv) or 4-pyridinecarboxaldehyde (1.00 equiv), $NaBH_3CN$, AcOH/MeOH 2% v/v, CH_2Cl_2, rt, 16 h.



Scheme 3. Preparation of targeted compounds **8a**, **8b**, **9a**, **9b**, **10a**, and **10b**. Reagents and conditions: (i) Boc_2O, CH_2Cl_2, rt, 1 h 30 min; (ii) oxalyl chloride, DMSO, Et_3N, CH_2Cl_2, −70 °C to rt, 3 h; (iii) **7**, $NaBH_3CN$, AcOH/MeOH 2% v/v, rt, 16 h; (iv) TFA, CH_2Cl_2, rt, 16 h; (v) HCHO, $NaBH_3CN$, AcOH/MeOH 2% v/v, CH_2Cl_2, rt, 24 h.

Table 1
In vitro antifungal activity of pyridinyl- and piperidinylmethylamino derivatives





Compound	R	X	R^1	MIC_{80} values[a] (ng mL^{-1})	
				Candida albicans CA98001	*Aspergillus fumigatus* AF98003
2a	H	Cl	–	23.0 (±4.00)	na
2b	H	F	–	220.0 (±24.0)	na
3a	H	Cl	–	37.0 (±0.40)	na
3b	H	F	–	130.0 (±35.0)	na
4a	H	Cl	–	36.0 (±0.40)	na
4b	H	F	–	83.0 (±17.0)	na
8a	H	Cl	Boc	26.0 (±1.00)	na
8b	H	F	Boc	27.0 (±5.00)	na
9a	H	Cl	H	120.0 (±40.0)	na
9b	H	F	H	1410.0 (±350.0)	na
10a	CH_3	Cl	Boc	39.0 (±2.00)	na
10b	CH_3	F	Boc	153.0 (±83.0)	na
Fluconazole				190.0 (±6.0)	–
Itraconazole				–	420.0 (±40.0)

[a] Values are means of triplicate, standard deviation is given in parentheses (na, not active; MIC_{80} >30,000.0 ng mL^{-1}).

To investigate the structure-activity relationships, we performed the docking of one of the most active compounds, 2-(2,4-dichlorophenyl)-1-[(pyridin-4-ylmethyl)amino]-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol **4a**, in our model of CYP51-*C. albicans* (Fig. 1).[12] We realized the docking with (*S*)-configuration since in a precedent series, these isomers were much more active than (*R*)-enantiomers.[13] The pyridine ring should exploit an aromatic stacking interaction with residue Tyr118 but the distances between the nitrogen atom and key amino acids His377 and Ser378 are too large (higher than 6.00 Å) to allow the formation of an appropriate hydrogen bond. The reported moderate activities compared to those previously observed for our benzylamine series (**I**, Scheme 1),[4] are consistent with that observation.

Replacement of the pyridine moiety by non-substituted piperidine derivatives **9a** and **9b** seems to be detrimental for high potency (Table 1) and this could be explained by the additional loss of the π–π interaction with the phenol group of Tyr118.

Of particular interest are piperidines bearing *N*-Boc groups (compounds **8a and 8b**; Table 1) compared to compounds lacking such a substituent. Modeling of **8a** suggests a hydrogen bonding interaction between the carbonyl group and the imidazole side chain of His377 that could be important for the antifungal activity (Fig. 2). Even if interaction with Ser378 does not seem to occur, this result is consistent with the recent predicted pharmacophore model.[3]

Finally, all these compounds were inactive on the *A. fumigatus* strain whereas an emergence of activity was observed in our previous benzylamine series (**I**, Scheme 1), especially for those bearing a *N*-methyl group in the linker with MIC_{80} values ranging from 1960.0 to 2410.0 ng mL^{-1} for the best compounds (Y = NO_2 and

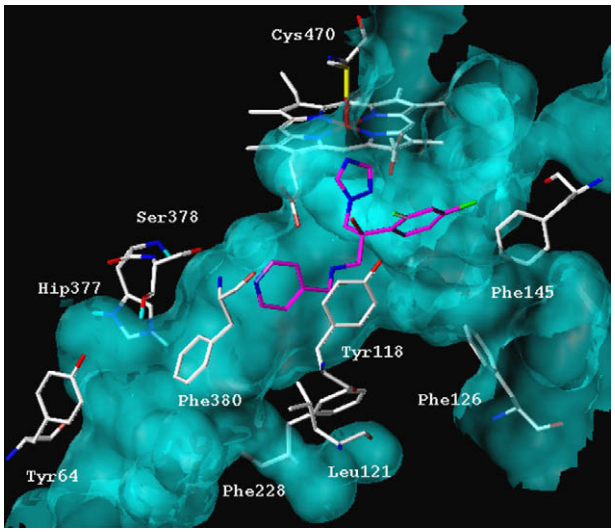


Figure 1. Docking solution of compound (*S*)-**4a** in the active site of CYP51-*Candida albicans*. Hip377 is the protonated form of histidine residue.

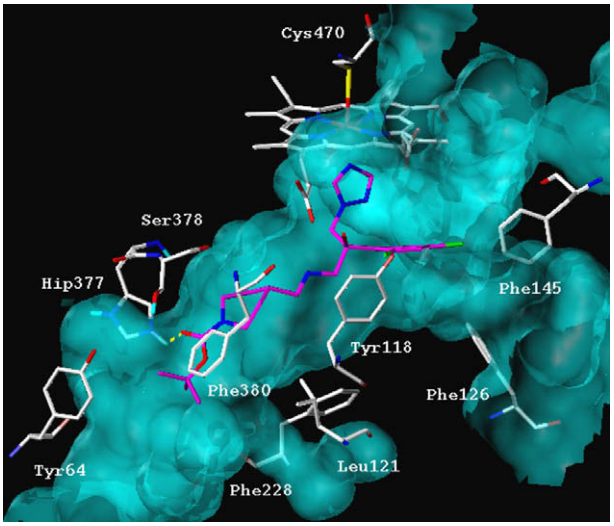


Figure 2. Docking solution of compound (*S*)-**8a** in the active site of CYP51-*Candida albicans*.

CN).[4] Since in this study, only piperidine derivatives **10a** and **10b** contain such a substituent, we can speculate that the loss of the aromatic ring and/or the *N*-methyl group are sufficient to induce negative effects within the active site of CYP51-*A. fumigatus*.

In conclusion, most of these compounds are more active than fluconazole on the *C. albicans* strain but are less active than our previously synthesized 1-benzylamino derivatives bearing H-bond acceptors entities in *para* position of the benzyl group,[4] thus confirming both the importance of π–π stacking and hydrogen bonding interactions in the active site of CYP51-*C. albicans*. However, even if azoles are known to inhibit mainly CYP51 enzymes, we can not exclude other mechanisms and only results on CYP51 isolated could confirm our hypotheses. Optimization of those series is ongoing and will be reported subsequently.

References and notes

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5. *Synthesis of 2-(2,4-dichlorophenyl)-1-[(pyridin-4-ylmethyl)amino]-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**4a**):* To a solution of **1a** (486 mg, 1.69 mmol) in 10 mL of methanol and 0.2 mL of acetic acid was added 4-pyridinecarboxaldehyde (0.16 mL, 1.69 mmol) under argon at room temperature. Then sodium cyanoborohydride (128 mg, 2.03 mmol) was added and the solution was stirred for 16 h. Mixture was diluted with water and product was extracted with dichloromethane. Organic layers were combined, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified on silica gel column chromatography (dichloromethane/ethanol, 10:1) and compound **4a** was obtained in a 47% yield as a yellow oil. ^{1}H NMR (DMSO-d_6): δ 3.00 (d, 1H, ^2J = 12.2 Hz), 3.28 (d, 1H, ^2J = 12.2 Hz), 3.70 (s, 2H), 4.69 (d, 1H, ^2J = 14.7 Hz), 4.92 (d, 1H, ^2J = 14.7 Hz), 5.94 (s, 1H, OH), 7.28 (d, 2H, ^3J = 5.8 Hz), 7.34 (dd, 1H, ^3J = 8.6 Hz, ^4J = 2.1 Hz), 7.54 (d, 1H, ^4J = 2.1 Hz), 7.57 (d, 1H, ^3J = 8.6 Hz), 7.75 (s, 1H), 8.32 (s, 1H), 8.49 (d, 2H, ^3J = 5.8 Hz). IR (NaCl cm^{-1}): 806 (υ C—Cl), 1271 (υ C—N), 1460, 1506, 1603 (υ C=C and υ C=N), 2930, 2955 (υ $CH_{aliph.}$), 3340 (υ O—H). MS *m/z* 378.0 (M^+).
6. *Synthesis of N-tert-butoxycarbonyl-4-hydroxymethyl piperidine (**6**).* To a solution of 4-piperidinemethanol **5** (1 g, 8.68 mmol) in 10 mL of dichloromethane was added di-*tert*-butyl dicarbonate (2.08 g, 9.55 mmol). The solution was stirred for 1.5 h at room temperature. Mixture was diluted with water and product was extracted with dichloromethane. Organic layers were combined, dried over anhydrous Na_2SO_4 and evaporated to get the right product **6** (quantitative yield, white powder) which was used without further purification. Mp 74–75 °C; ^{1}H NMR (DMSO-d_6): δ 0.91–1.07 (m, 2H), 1.42 (s, 9H), 1.62–1.65 (m, 2H), 2.59–2.84 (m, 2H), 3.27 (t, 2H, ^3J = 5.2 Hz), 3.94–3.99 (m, 2H), 4.50 (t, 1H, ^3J = 5.2 Hz, OH). IR (KBr cm^{-1}): 1256 (υ C—N), 1671 (υ C=O), 2940, 2961 (υ $CH_{aliph.}$), 3472 (υ O—H).
7. *Synthesis of N-tert-butoxycarbonyl-4-formyl piperidine (**7**). To a solution of dimethyl sulfoxyde (1.42 mL, Synthesis of N-tert-butoxycarbonyl-4-formyl piperidine (**7**).* To a solution of dimethyl sulfoxyde (1.42 mL20.05 mmol) in 10 mL of dichloromethane at −70 °C was added dropwise successively a solution of oxalyl chloride (0.96 mL, 11.03 mmol) in 30 mL of dichloromethane, and a solution of **6** (2.16 g, 10.02 mmol) in 10 mL of dichloromethane. The mixture was stirred for 15 min at −70 °C, and then triethylamine (7.27 mL, 52.12 mmol) was added. After being stirred for 1 h at −70°C, the resulting solution was allowed to reach to room temperature. Mixture was diluted with water and product was extracted with dichloromethane. Organic layers were combined, washed with saturated sodium bicarbonate, dried over anhydrous Na_2SO_4 and evaporated to get the right product **7** (quantitative yield, colorless oil) which was used without further purification. ^{1}H NMR (DMSO-d_6): δ 0.99–1.07 (m, 2H), 1.42 (s, 9H), 1.81–1.88 (m, 2H), 2.87–2.97 (m, 2H), 3.79–3.86 (m, 2H), 9.62 (s, 1H). IR (NaCl cm^{-1}): 1281 (υ C—N), 1689 (υ C=O), 2931 (υ $CH_{aliph.}$).
8. *Synthesis of 2-(2,4-dichlorophenyl)-1-{[(1-tert-butoxycarbonylpiperidin-4-yl)methyl]amino}-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**8a**).* Compound **8a** was prepared from **1a** according to the same protocol as described for compound **4a**. The right product was obtained in a 26% yield as a brown oil. ^{1}H NMR (DMSO-d_6): δ 0.84–0.94 (m, 2H), 1.41 (s, 9H), 1.46–1.48 (m, 1H), 1.51–1.62 (m, 2H), 2.28–2.40 (m, 2H), 2.59–2.71 (m, 2H), 3.06 (d, 1H, ^2J = 12.4 Hz), 3.28 (d, 1H, ^2J = 12.4 Hz), 3.86–3.94 (m, 2H), 4.66 (d, 1H, ^2J = 14.4 Hz), 4.92 (d, 1H, ^2J = 14.4 Hz), 5.96 (s, 1H, OH), 7.33 (dd, 1H, ^3J = 8.4 Hz, ^4J = 2.0 Hz), 7.55 (d, 1H, ^3J = 8.4 Hz), 7.56 (d, 1H, ^4J = 2.0 Hz), 7.76 (s, 1H), 8.33 (s, 1H). IR (NaCl cm^{-1}): 738 (υ C—Cl), 1274 (υ C—N), 1470, 1511, 1600 (υ C=C and υ C=N), 1681 (υ C=O), 2929, 2960 (υ $CH_{aliph.}$), 3422 (υ O—H and υ N—H). MS *m/z* 484.0 (M+H), 384.2 (M−Boc).
9. *Synthesis of 2-(2,4-dichlorophenyl)-1-[(piperidin-4-ylmethyl)amino]-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**9a**).* To a solution of **8a** (240 mg, 0.50 mmol) in 0.3 mL of dichloromethane was added 0.5 mL of trifluoroacetic acid. The solution was stirred for 16 h at room temperature. The mixture was basified with NaOH 1 M and extracted with dichloromethane. The organic layers were combined, washed with HCl 1 M, dried over anhydrous Na_2SO_4 and evaporated to get the right product **9a** (69% yield, brown oil). ^{1}H NMR (DMSO-d_6): δ 0.85–1.03 (m, 2H), 1.33–1.48 (m, 1H), 1.51–1.65 (m, 2H), 2.23–2.45 (m, 4H), 2.84–2.96 (m, 2H), 3.05 (d, 1H, ^2J = 12.5 Hz), 3.27 (d, 1H, ^2J = 12.5 Hz), 4.65 (d, 1H, ^2J = 13.4 Hz), 4.90 (d, 1H, ^2J = 13.4 Hz), 5.95 (s, 1H, OH), 7.33 (dd, 1H, ^3J = 8.4 Hz, ^4J = 2.0 Hz), 7.55 (d, 1H, ^3J = 8.4 Hz), 7.56 (d, 1H, ^4J = 2.0 Hz), 7.76 (s, 1H), 8.33 (s, 1H). IR (NaCl cm^{-1}): 735 (υ C—Cl), 1273 (υ C—N), 1463, 1507, 1589 (υ C=C and υ C=N), 2929, 2957 (υ $CH_{aliph.}$), 3422 (υ O—H and υ N—H). MS *m/z* 384.4 (M+H).
10. *Synthesis of 2-(2,4-dichlorophenyl)-1-{methyl[(1-tert-butoxycarbonylpiperidin-4-yl)methyl]amino}-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**10a**).* To a solution of **8a** (835 mg, 1.72 mmol) in 12 mL of methanol and 0.24 mL of acetic acid was added formaldehyde (30% weight solution, 0.159 mL, 1.72 mmol) under argon at room temperature. Then sodium cyanoborohydride (130 mg, 2.06 mmol) was added and the solution was stirred for 24 h. Mixture was diluted with water and the product was extracted with dichloromethane. Organic layers were combined, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified on silica gel column chromatography (dichloromethane/ethanol, 10:1) and compound **10a** was obtained in a 88% yield as a yellow powder. Mp 53–54 °C; ^{1}H NMR (DMSO-d_6): δ 0.68–0.78 (m, 2H), 1.40–1.50 (m, 12H), 2.09 (s, 3H), 2.18–2.26 (m, 2H), 2.55–2.65 (m, 2H), 2.72 (d, 1H, ^2J = 13.7 Hz), 3.33 (d, 1H, ^2J = 13.7 Hz), 3.80–3.90 (m, 2H), 4.64 (d, 1H, ^2J = 14.3 Hz), 4.86 (d, 1H, ^2J = 14.3 Hz), 5.80 (s, 1H, OH), 7.35 (dd, 1H, ^3J = 8.5 Hz, ^4J = 2.1 Hz), 7.57 (d, 1H, ^4J = 2.1 Hz), 7.62 (d, 1H, ^3J = 8.5 Hz), 7.79 (s, 1H), 8.33 (s, 1H). IR (KBr cm^{-1}): 803 (υ C—Cl), 1277 (υ C—N), 1455 (υ C=C), 1688 (υ C=O), 2931 (υ $CH_{aliph.}$), 3429 (υ O—H). MS *m/z* 498.0 (M^+).
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12. Giraud, F. Ph.D. Thesis, Université de Nantes, Nantes Atlantique Universités, October 2007. Briefly, the structure of CYP51 from *Mycobacterium tuberculosis* complexed with fluconazole (PDB code, 1EA1) was used as the template for the homology model of CYP51-*C. albicans*. Multiple alignment of the CYP51-*Mycobacterium tuberculosis* sequence with those of CYP51-*C. albicans* (PIR code, P10613) and CYP51-*A. fumigatus* (PIR code, Q9P8R0) was performed using ClustalW. This alignment was further checked by comparing a secondary structure elements prediction for CYP51-*C. albicans*, obtained through the PSIPRED protein structure prediction server (UCL, Department of Computer Science, Bioinformatics Group, London), with the experimental secondary structure assignments for CYP51-*M. tuberculosis* deduced from the PDB file. The 3D model of CYP51-*C. albicans* was then constructed by the Nest program from the protein structure modeling package JACKAL (Honig Lab, Columbia University, New York). The resulting model was further subjected to an energy minimization using Powell's method available in Maximin2 procedure with the MMFF94 force field and a dielectric constant of 4.0 until the gradient value reached 0.1 kcal/mol Å. During the optimization procedure, the structure was checked periodically by Verify-3D and Ramachandran plots. If present, improper geometries were manually corrected and the structure minimized again with the above procedure.
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