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Design, synthesis, and evaluation of 1-(*N*-benzylamino)-2-phenyl-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ols as antifungal agents

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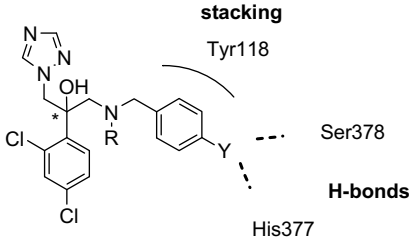
Abstract—A series of 1-(*N*-benzylamino)-2-phenyl-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ols **6a–c**, **7a–c**, **8a**, and **9a** were prepared in five steps and evaluated for their antifungal activity. The most active compound **7b** was docked into a home-made 3D model of the targeted enzyme confirming the importance of Tyr118, His377, and Ser378 residues in its binding mode.

Invasive fungal infections are frequently observed in immune-compromised patients suffering from AIDS or subjected to invasive surgery, anti-cancer therapy or graft receivers. Several treatments have been developed to reduce the impact of fungal diseases such as azoles (fluconazole, itraconazole, voriconazole, posaconazole), amphotericin B, 5-fluorocytosine, and caspofungin. Each molecule is targeting diverse biological pathways which are essential for the fungi. Unfortunately, massive use of those compounds as a curative or prophylactic approach has favored the emergence of resistance showing the need of the discovery of new antifungal compounds.

Our group is involved in the design and synthesis of azole antifungals for several years now.[1,2] We decided to use molecular modeling tools with the aim to rationalize our previous results and to suggest original structures to the synthesis. A homology model would help us to identify potential inhibitors of the lanosterol 14α-demethylase (CYP51) of *Candida albicans* and *Aspergillus fumigatus*.

Recent works were published by the group of Sheng et al.[3] who mentioned a promising approach for the design CYP51 inhibitors from *C. albicans*. They studied a large library of derivatives from the azole family and suggested a pharmacophoric model. They noticed the importance of Tyr118 and Ser378 key residues in the stabilization of the inhibitors within the channel 2 which is oriented to the FG loop.

Thus to verify their hypotheses and to confirm our own observations, we decided to build chlorinated analogues



Scheme 1. General structure of compounds **6a–c**, **7a–c**, **8a** and **9a** and targeted interactions.

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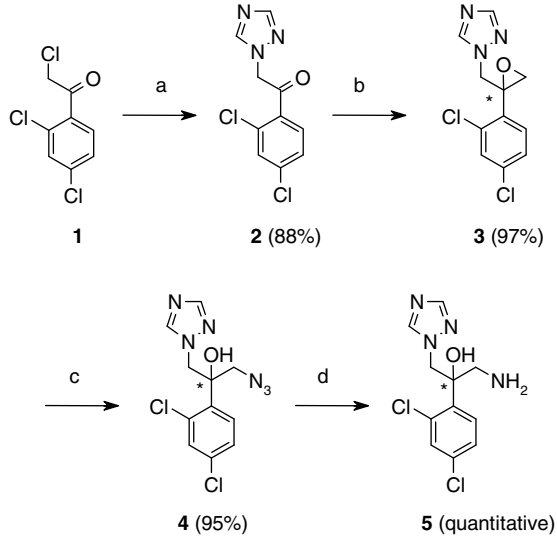
of their benzylamine series (Scheme 1). A new synthetic strategy was performed for the design of our 1-(*N*-benzylamino)-2-phenyl-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ols. Introduction of adequate substituents in position Y such as nitro, nitrile or trifluoromethyl groups would create potential *H*-bond interactions with key amino acids. On the other hand, the aromatic ring would generate the π–π stacking with the Tyr118. We chose to compare the biological activity of the nitro compounds with their analogues bearing an amino group in Y.

We herein describe the synthesis of benzylamine derivatives and their antifungal activities on *C. albicans* and *A. fumigatus* strains. Additionally SAR studies will be discussed with the help of molecular modeling.

Our laboratory already synthesized several families of azole compounds and the use of microwave irradiation provided us with an efficient procedure to access to the key intermediate **3** (Scheme 2).[4,5] So in the first step, 1*H*-1,2,4-triazole was alkylated with 2,2′,4′-trichloroacetophenone (**1**) in the presence of potassium carbonate as a base, affording the 2-(1*H*-1,2,4-triazol-1-yl)-2′,4′-dichloroacetophenone (**2**) in a 88% yield. Then, Corey–Chaykovsky reaction was performed using the reaction couple sodium hydroxide/trimethylsulfoxonium iodide (TMSOI) and led to the desired oxirane **3** in excellent yield.

Whereas the group of Sheng used the nucleophilicity of *N*-methylbenzylamines to open the oxirane ring,[3] we developed an original synthetic route to prepare our compounds (Schemes 2 and 3).

In the third step, the ring of epoxide **3** is opened by the use of sodium azide in the presence of ammonium chloride in methanol and after one night under reflux, intermediate **4** was isolated in a 95% yield.[6] Reduction of the azide moiety to amine is described in several papers. Fringuelli et al.[7] developed a copper-catalyzed preparation of amino-alcohols, Kempf et al.[6] and Shiozaki et al.[8] showed that the use of ammonium chloride could reduce azides into amines. Other systems like the couple zinc powder/ammonium formiate in methanol can induce in situ generation of hydrogen to reduce the azide moiety.[9] Moreover the use of triphenylphosphine according to Staudinger conditions could lead to the formation of the amino-alcohol.[10] Usually most of these techniques need an aqueous work-up. We noticed that amino-alcohol **3** was partially soluble in water, thus we used an easier procedure based on a hydrogenated reduction and we were able to isolate 1-amino-2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (**5**) using a simple filtration of the reaction medium in quantitative yield.[11]

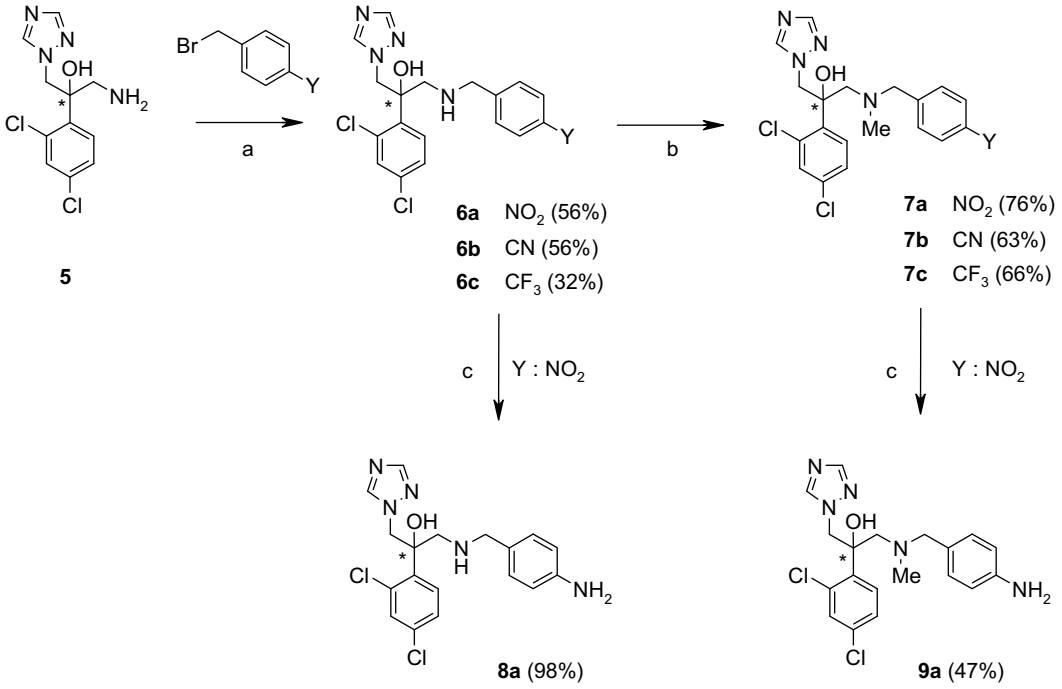


Scheme 2. General synthetic route for the preparation of intermediate **5**. Reagents and conditions: (a) 1*H*-1,2,4-triazole, K_2CO_3, CH_3CN, MW, 50W, 85 °C, 50 min; (b) $NaOH_{aq}$, TMSOI, toluene, MW, 10W, 80 °C, 50 min; (c) NaN_3, NH_4Cl, MeOH, reflux, 15 h; (d) H_2 (5 bars), Pd/C 5% (10% weight), EtOH, rt, 16 h.

The following steps of the synthesis are described in Scheme 3. The main reaction is a nucleophilic substitution of amino-alcohol **5** with appropriate substituted benzyl bromides in the presence of the Hünig base. Experimental protocol was optimized to afford the mono-*N*-substituted derivatives. Two equivalents of amino-alcohol **5** was placed in the presence of the base in a diluted medium and a solution of one equivalent of bromide was slowly added via a syringe. Depending on the volume added, several hours are needed for the complete addition.[12] We isolated three original derivatives **6a–c** in moderate to good yields. Furthermore, a methyl group was introduced on the amine spacer using formaldehyde and reductive amination conditions.[13] This led to the preparation of three additional derivatives **7a–c** in satisfactory yields.[14] Amino compounds **8a** and **9a** were obtained from their nitro analogues **6a** and **7a** using reducing conditions.[15]

Compounds **6a–c**, **7a–c**, **8a**, and **9a** were screened for their antifungal activity on *C. albicans* CA98001 and *A. fumigatus* AF98003 strains. Inhibition growth was measured according to the protocol described in a previous journal.[16] Fluconazole and itraconazole were used as positive controls. The minimum inhibitory concentration (MIC_{80}) values (in ng mL^{-1}) are summarized in Table 1.

On the *C. albicans* strain, our compounds showed a high level of activity with MIC values 6- to 500-fold lower than that of fluconazole. The 4-{*N*-[2-(2,4-dichlorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-*N*-methyl}aminomethylbenzonitrile (**7b**) showed a MIC_{80} value of 0.37 ng mL^{-1} and was the most active compound of the series whereas compound **8a** (Y = NH_2, linker N–H, MIC_{80} = 18,830.0 ng mL^{-1}) was inactive. A simple comparison of the two subseries (linker N–CH_3 or N–H) showed that, excepted for compounds **6c** and **7c**, the methyl group would be favorable for the activity. For example, compound **9a** bearing an amino group in position Y and a N–CH_3 linker showed a MIC_{80} value of 29.0 ng mL^{-1}. It was almost 1000-fold more active than its analogue **8a**. Comparison of **7b** (MIC_{80} = 0.37 ng mL^{-1}) which



Scheme 3. Final steps for the preparation of targeted compounds **6a–c**, **7a–c**, **8a**, and **9a**. Reagents and conditions: (a) benzyl bromide (0.50 equiv), $(i\text{-Pr})_2$NEt (0.55 equiv), CH_3CN, rt, 16–48 h; (b) HCHO, $NaBH_3CN$, AcOH/MeOH 2% v/v, rt, 16 h; (c) H_2 (5 bars), Pd/C 5% (10% weight), EtOH, rt, 16 h.

Table 1. In vitro antifungal activity of benzylamine derivatives **6a–c**, **7a–c**, **8a**, and **9a**

Compound	R	Y	MIC_{80} values (ng mL^{-1})	
			Candida albicans CA98001	*Aspergillus fumigatus* AF98003
6a	H	NO_2	6.0 ± 1.3	27,020.0 ± 840.00
6b	H	CN	2.8 ± 0.4	4020.0 ± 800.00
6c	H	CF_3	24.0 ± 2.0	24,040.0 ± 2230.00
8a	H	NH_2	18,830.0 ± 2750.00	3060.0 ± 120.00
7a	CH_3	NO_2	0.6 ± 1.3	1960.0 ± 170.00
7b	CH_3	CN	0.37 ± 0.16	2410.0 ± 40.00
7c	CH_3	CF_3	30.0 ± 3.0	21,130.0 ± 1380.00
9a	CH_3	NH_2	29.0 ± 2.0	2320.0 ± 120.00
Fluconazole			190.0 ± 6.0	—
Itraconazole			—	420.0 ± 40.0

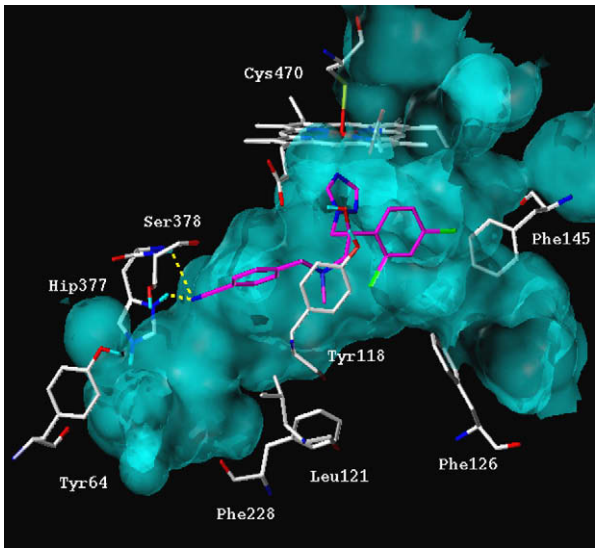
was 10-fold more active than its analogue **6b** which has a nitrile group and a non-substituted spacer (MIC_{80} = 2.8 ng mL^{-1}) led to the same conclusion. The presence of a methyl group would play a major role in the orientation of the inhibitors within the active site.

In addition the most suitable groups in position Y are *H*-bond acceptor entities. Indeed compounds **6a** (Y = NO_2, MIC_{80} = 6.0 ng mL^{-1}), **6b** (Y = CN, MIC_{80} = 2.8 ng mL^{-1}), and **6c** (Y = CF_3, MIC_{80} = 24.0 ng mL^{-1}) are almost 1000-fold more active than their amine analogue **8a**.

In our precedent series, (*S*)-isomers were the most active compounds.[5,17] Based on this observation, we realized the docking of the most active compound **7b** under this configuration in our model of CYP51-*C. albicans* (Scheme 4).[18] These studies helped us to understand the structure–activity relationship of this compound within the active site. The nitrile group should share two *H*-bonds with the key amino acids His377 and Ser378 whereas the *N*-methyl group of the linker would be oriented to a hydrophobic pocket (Tyr118, Leu121, Phe126, and Phe228).

These results are in accordance with the conclusions of the group of Sheng and additionally we identified a new residue which seems to be essential for the binding of inhibitors: His377.

Furthermore, biological results showed the emergence of activity of our compounds on the *Aspergillus* strain with MIC_{80} values ranging from 1960.0 to 2410.0 ng mL^{-1}. The *N*-methyl group seems to be also important here. Indeed, 2-(2,4-dichlorophenyl)-1-[*N*-methyl-*N*-(4-nitro-



Scheme 4. Docking of compound (*S*)-**7b** in the active site of CYP51-*Candida albicans*. Hip377 is the protonated form of histidine residue.

benzyl)amino]-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (**7a**) was the most active compound on the *A. fumigatus* strain and showed the lowest MIC_{80} value of 1960.0 ng mL^{-1}.

All these compounds could bind to the active site by *H*-bond interactions.

To verify our conclusions, an HPLC separation of compounds **6a–c**, **7a–c**, **8a**, and **9a** should be undertaken to check if the most active isomers are in the (*S*)-configuration as previously observed. Moreover, a homology model of the CYP51-*A. fumigatus* enzyme should be build according to the same procedure. More insightful observations of the active site would give us some key informations for further design of broad-spectrum antifungal agents.

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12. Synthesis of 2-(2,4-dichlorophenyl)-1-(4-nitrobenzylamino)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (**6a**). To a solution of 1-amino-2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (**5**) (613 mg, 2.13 mmol) in 24 mL of acetonitrile was added N,N′-diisopropylethylamine (0.17 mL, 1.17 mmol) under argon at room temperature. A solution of 4-nitrobenzyl bromide (230 mg, 1.06 mmol) in 10 mL of acetonitrile was slowly added to the mixture in 40 min and the solution was stirred for 24 h at room temperature. Solvent was removed under reduced pressure and residue was partitioned between dichloromethane and water. Product was extracted with dichloromethane and organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified on silica gel column chromatography (ethanol/dichloromethane 1:10) and compound **6a** was obtained in a 56% yield as a yellow oil. ^{1}H NMR (DMSO-d_6): δ 3.02 (d, 1H, ^2J = 12.5 Hz), 3.29 (d, 1H, ^2J = 12.5 Hz), 3.82 (s, 2H), 4.68 (d, 1H, ^2J = 14.1 Hz), 4.90 (d, 1H, ^2J = 14.1 Hz), 5.95 (s, 1H, OH), 7.34 (dd, 1H, ^3J = 8.8 Hz, ^4J = 2.4 Hz), 7.53–7.59 (m, 4H), 7.75 (s, 1H), 8.19 (d, 2H, ^3J = 8.8 Hz), 8.32 (s, 1H). IR (NaCl, cm^{-1}): 805 (*v* C–Cl), 1266 (*v* C–N), 1348 (*v* NO_2 sym), 1512 (*v* NO_2 asym), 1589 (*v* C=C and *v* C=N), 2927 (*v* C–$H_{aliph.}$), 3318 (*v* O–H). MS *m*/*z* 423.1 (M+H).

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14. Synthesis of 2-(2,4-dichlorophenyl)-1-[*N*-methyl-*N*-(4-nitrobenzyl)amino]-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (**7a**). To a solution of 2-(2,4-dichlorophenyl)-1-(4-nitrobenzylamino)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (**6a**) (116 mg, 0.27 mmol) in 2 mL of methanol and 2% of acetic acid was added formaldehyde (30% weight solution, 22.5 μL, 0.27 mmol) under argon at room temperature. Then sodium cyanoborohydride (21 mg, 0.39 mmol) was added and the mixture stirred for one night at room temperature. It was then diluted with water and the product was extracted with dichloromethane. Organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified on silica gel column chromatography (ethanol/dichloromethane 2:98) and compound **7a** was obtained in a 43% yield as a yellow oil. ^{1}H NMR (DMSO-d_6): δ 2.10 (s, 3H, CH_3), 2.85 (d, 1H, ^2J = 13.7 Hz), 3.47 (d, 1H, ^2J = 13.7 Hz), 3.54 (d, 1H, ^2J = 14.0 Hz), 3.76 (d, 1H, ^2J = 14.0 Hz), 4.69 (d, 1H, ^2J = 14.1 Hz), 4.86 (d, 1H, ^2J = 14.1 Hz), 5.95 (s, 1H, OH), 7.31 (d, 2H, ^3J = 8.8 Hz), 7.34 (dd, 1H, ^3J = 8.2 Hz, ^4J = 2.1 Hz), 7.58 (d, 1H, ^4J = 2.1 Hz), 7.60 (d, 1H, ^3J = 8.2 Hz), 7.78 (s, 1H), 8.10 (d, 2H, ^3J = 8.8 Hz), 8.33 (s, 1H). IR (NaCl, cm^{-1}): 1274 (ν C–N), 1347 (ν NO_2 sym), 1519 (ν NO_2 asym), 1464, 1602 (ν C=C and ν C=N), 2927 (ν C–$H_{aliph.}$), 3370 (ν O–H). MS *m*/*z* 436.2 (M^+).
15. Compounds **8a** and **9a** were prepared from **6a** and **7a** according to the same protocol as described for compound **5** but the reaction time was reduced to 1.5 h. Products were purified on silica gel column chromatography (ethanol/dichloromethane 1:10).
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