Bioorganic & Medicinal Chemistry Letters 19 (2009) 1811–1814

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis, and biological evaluation of novel 1-(1*H*-1,2,4-triazole-1-yl)-2-(2,4-difluorophenyl)-3-substituted benzylamino-2-propanols

Xiaoyun Chai, Jun Zhang, Shichong Yu, Honggang Hu, Yan Zou, Qingjie Zhao, Zhigang Dan, Dazhi Zhang, Qiuye Wu *

Department of Organic Chemistry, College of Pharmacy, Second Military Medical University, Guohe Road 325, Shanghai 200433, People's Republic of China

ARTICLE INFO

Article history:
Received 24 September 2008
Revised 19 December 2008
Accepted 9 January 2009
Available online 21 January 2009

Keywords:
Triazole
Synthesis
Antifungal activity
CYP51
Molecular docking

ABSTRACT

Based on the results of computational docking to the active site of the cytochrome P450 14α-demethylase (CYP51), a series of 1-(1*H*-1,2,4-triazole-1-yl)-2-(2,4-difluorophenyl)-3-substituted benzylamino-2-propanols as analogs of fluconazole were designed, synthesized, and evaluated as antifungal agents. Results of preliminary antifungal tests against eight human pathogenic fungi in vitro showed that all the title compounds exhibited excellent activities with broad spectrum.

During the past several decades, systemic fungal infection had become a momentous complication and a major cause of morbidity and mortality in immunocompromised individuals.[1,2] Currently, several clinical drugs such as azoles, amphotericin B, 5-fluorocytosine, and caspofungin have been developed to reduce the impact of fungal diseases infected by candidosis, aspergillosis, and cryptococcosis.[3,4] Among these antifungal agents, azoles were used widely and efficiently, especially triazole, such as fluconazole, voriconazole, and itraconazole, now play a leading role in the treatment of invasive fungal infections. These antifungal drugs act by inhibiting CYP51 in the process of biosynthesis of ergosterol through a mechanism in which the heterocyclic nitrogen atom (N-4 of triazole) binds to the heme iron atom.[5] However, the increasing administration of such antifungal agents has led to the development of fungal resistance. Survey reveals genetic mutations that result in resistance to clinically used drugs, especially fluconazole, may also result in resistance to new structurally related azoles such as voriconazole and ravuconazole.[6–8] The emergence of resistance shows the need of the discovery of new antifungal compounds. And so, there is a must need for broad spectrum and low-toxicity antifungal agents genuinely, although it is developed rapidly recently.

The crystal structure of CYP51 of fungi has not been obtained. Ji et al. constructed a 3D model of CYP51 from *Candida albicans*.[9] In general, the active site of CYP51 for ligand binding can be divided into four subsites: a coordination bond with iron of the heme group, the hydrophilic H-bonding region, the hydrophobic region, and the narrow hydrophobic cleft formed by the residues in the helix B′-meander 1 loop and N-terminus of helix I.[10] Based on the results of Ji et al., we constructed a 3D model of CYP51 from *C. albicans* and analyzed the binding between fluconazole and CYP51. As shown in Figure 2, fluconazole binds to the active site of CYP51 via coordination of the N atom of the triazole nucleus with iron of the heme group. The diflurophenyl group is located in the hydrophobic binding cleft lined with Ala114, Phe126, Leu139, Met140, Phe145, Ile304, Met306, and Gly307. Several residues lined with Leu121, Thr122, Phe228, Thr311, Pro375, Leu376, His377, Ser378, Met508, Val509, and Val510 were observed to form indirect nonbonding interactions with another triazole ring.[11]

Researches indicated that the triazole ring, the diflurophenyl group and the hydroxyl group were the pharmacophores of antifungal agents. But the side chains which located in the narrow

Figure 1. Chemical structure of fluconazole and modified positions of the compounds.

* Corresponding author. Tel./fax: +86 21 25070381.
E-mail address: wuqy6439@sohu.com (Q. Wu).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.01.048

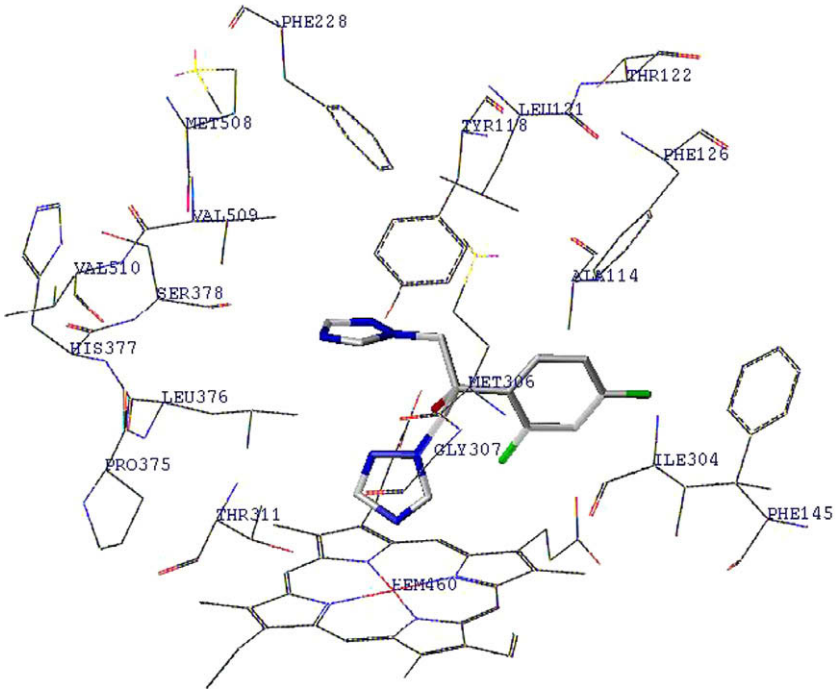


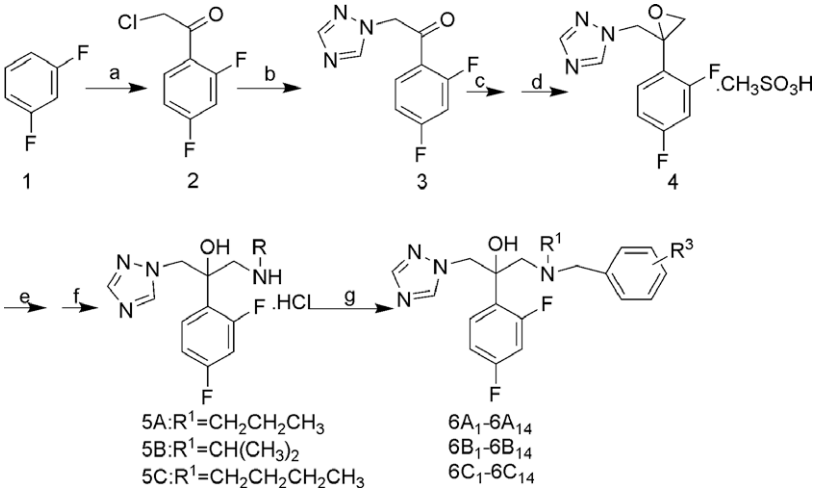
Figure 2. Computed binding geometry of the clinical used inhibitor fluconazole in the active site of CYP51.

hydrophobic cleft were also important. And the optimization of the side chain attached to the pharmacophore remains attractive to the current researches. We intended to alter the side chains to find potent systemic antifungal agents with a broad antifungal spectrum and less potential to develop resistance.

In our compounds design, we systematically altered the structure of fluconazole (Fig. 1) based on the results of computational docking to the active site of the CYP51, and the modification was focused on one triazole moiety of fluconazole.

Synthesis of the target compounds **$6A_n$–$6C_n$** was accomplished using chemistry illustrated in Scheme 1. The intermediate oxirane **4** was synthesized with known procedures.[12] Oxirane **4** was allowed to react with *n*-propylamine in the presence of triethylamine in ethanol, and then added HCl gas to formed compounds **$5A_n$**. To a stirred mixture of **$5A_n$** and substituted benzyl bromide in the presence of KI, K_2CO_3 in acetonitrile, we afforded compounds **$6A_n$**. The target compounds **$6B_n$–$6C_n$** were synthesized in the same way as **$6A_n$**. All the new compounds (**$6A_n$–$6C_n$**) described above were characterized by IR, LC–MS, and NMR spectroscopic analysis.[13]

The in vitro minimal inhibitory concentrations (MICs) of the compounds were determined by the micro-broth dilution method in 96-well microtest plates according to the methods defined by the National Committee for Clinical Laboratory Standards (NCCLS).[14] *C. albicans* and *Cryptococcus neoformans* are ATCC standard strains, and others are clinic isolates. Fluconazole, itraconazole, ketoconazole, voriconazole, amphotericin B, and terbinafine were obtained from their respective manufacturers served as the positive control. The minimum 80% inhibitory concentration (MIC_{80}) values are summarized in Table 1.



Scheme 1. Synthetic route to the title compounds. Reagents and conditions: (a) $ClCH_2COCl$, $AlCl_3$, 50 °C, 5 h, in 87% yield; (b) $C_6H_5CH_3$, $NaHCO_3$, 1*H*-1,2,4-triazole, reflux, 5 h, in 87% yield; (c) $C_6H_5CH_3$, $(CH_3)_3SOI$, NaOH, centylmethylammonium bromide, 60 °C, 3 h, in 86% yield; (d) CH_3SO_3H, 0 °C, 1 h, in 89%yield; (e) CH_3CH_2OH, Et_3N, primary amine, reflux, 6 h; (f) $HCl_{(g)}$, in 80–90% yield; (g) CH_3CN, KI, K_2CO_3, substituted benzyl bromide, rt, 5–6 h, in 50–70% yield.

Table 1
Antifungal activities of the title compounds in vitro (MIC_{80}, μg/mL)

Compound	R^1	R^3	*C. alb.*	*C. neo.*	*C. par.*	*C. tro.*	*T. rub.*	*C. kru.*	*M. gyp.*	*A. fum.*
6A$_1$	$-(CH_2)_2CH_3$	H	0.0156	0.0625	0.0625	0.0625	0.0625	0.0625	0.0156	>64
6A$_2$	$-(CH_2)_2CH_3$	2-F	0.0156	0.25	0.0625	0.0625	1	0.0625	0.0156	>64
6A$_3$	$-(CH_2)_2CH_3$	3-F	0.0156	0.0625	0.0625	0.25	1	0.0625	0.0625	>64
6A$_4$	$-(CH_2)_2CH_3$	4-F	0.0625	0.0625	0.0625	0.25	1	0.0625	0.0039	>64
6A$_5$	$-(CH_2)_2CH_3$	2-Cl	0.25	0.0625	0.25	0.25	4	0.25	0.0156	>64
6A$_6$	$-(CH_2)_2CH_3$	3-Cl	0.0625	0.25	0.25	0.25	4	0.25	0.0625	>64
6A$_7$	$-(CH_2)_2CH_3$	4-Cl	0.0625	0.0625	0.25	0.25	1	0.0625	0.0625	64
6A$_8$	$-(CH_2)_2CH_3$	2-Br	0.0625	0.25	0.0039	0.25	1	0.25	0.0625	>64
6A$_9$	$-(CH_2)_2CH_3$	4-Br	0.0625	0.25	0.0156	0.0625	0.25	0.25	0.0625	>64
6A$_{10}$	$-(CH_2)_2CH_3$	2-CH_3	0.0625	0.25	0.0625	0.0625	4	0.0625	0.0039	>64
6A$_{11}$	$-(CH_2)_2CH_3$	4-CH_3	0.0625	0.25	0.0625	0.0625	0.25	0.0625	0.0156	>64
6A$_{12}$	$-(CH_2)_2CH_3$	4-NO_2	0.0156	0.25	0.0625	0.25	0.25	0.0625	0.0156	64
6A$_{13}$	$-(CH_2)_2CH_3$	4-CH_2CH_3	0.0156	1	0.25	0.25	1	1	0.25	>64
6A$_{14}$	$-(CH_2)_2CH_3$	2,4-Cl,Cl	0.25	1	1	4	1	1	0.0156	>64
6B$_1$	$-CH(CH_3)_2$	H	0.0156	0.25	0.0625	0.25	0.0625	0.25	0.0156	64
6B$_2$	$-CH(CH_3)_2$	2-F	0.0625	0.25	0.0625	0.25	1	0.25	0.0625	>64
6B$_3$	$-CH(CH_3)_2$	3-F	0.0625	0.0625	0.0625	0.0625	0.25	0.0625	0.0625	64
6B$_4$	$-CH(CH_3)_2$	4-F	0.0625	0.0625	0.0625	0.25	0.25	0.0625	0.0625	>64
6B$_5$	$-CH(CH_3)_2$	2-Cl	0.0625	0.25	0.25	0.25	0.25	0.0625	0.0625	>64
6B$_6$	$-CH(CH_3)_2$	3-Cl	0.0156	1	0.25	1	0.25	0.25	1	>64
6B$_7$	$-CH(CH_3)_2$	4-Cl	0.0625	0.25	0.0625	0.25	0.25	0.0625	0.0625	1
6B$_8$	$-CH(CH_3)_2$	2-Br	0.25	0.25	0.0625	0.0625	0.0625	0.25	0.0156	>64
6B$_9$	$-CH(CH_3)_2$	4-Br	0.0039	0.25	0.0625	0.0625	0.0625	0.25	0.0156	4
6B$_{10}$	$-CH(CH_3)_2$	2-CH_3	0.0156	0.25	0.0625	0.25	0.25	0.25	0.0625	>64
6B$_{11}$	$-CH(CH_3)_2$	4-CH_3	1	0.25	0.25	1	0.0625	0.25	0.0625	64
6B$_{12}$	$-CH(CH_3)_2$	4-NO_2	0.0156	0.25	0.0625	0.25	0.25	0.0625	0.0156	64
6B$_{13}$	$-CH(CH_3)_2$	4-CH_2CH_3	0.0625	1	1	1	1	1	0.25	>64
6B$_{14}$	$-CH(CH_3)_2$	2,4-Cl,Cl	0.25	16	1	1	0.25	1	1	>64
6C$_1$	$-(CH_2)_3CH_3$	H	0.0625	0.0625	0.0625	0.0625	0.25	0.25	0.0156	>64
6C$_2$	$-(CH_2)_3CH_3$	2-F	0.0625	0.25	0.25	0.25	1	0.25	0.25	16
6C$_3$	$-(CH_2)_3CH_3$	3-F	0.0156	0.25	0.25	0.25	1	1	0.0625	>64
6C$_4$	$-(CH_2)_3CH_3$	4-F	0.25	0.25	0.0625	0.25	1	1	0.0156	>64
6C$_5$	$-(CH_2)_3CH_3$	2-Cl	1	0.25	0.25	0.25	1	1	0.25	>64
6C$_6$	$-(CH_2)_3CH_3$	3-Cl	1	0.25	0.25	1	1	1	0.25	>64
6C$_7$	$-(CH_2)_3CH_3$	4-Cl	1	1	1	1	1	1	1	>64
6C$_8$	$-(CH_2)_3CH_3$	2-Br	1	0.25	0.25	0.25	1	1	0.25	>64
6C$_9$	$-(CH_2)_3CH_3$	4-Br	4	0.25	0.25	1	0.25	1	1	>64
6C$_{10}$	$-(CH_2)_3CH_3$	2-CH_3	1	0.25	0.25	0.25	1	1	0.0156	>64
6C$_{11}$	$-(CH_2)_3CH_3$	4-CH_3	4	0.25	1	1	1	1	0.25	>64
6C$_{12}$	$-(CH_2)_3CH_3$	4-NO_2	0.0156	0.0625	0.25	0.0625	0.25	1	0.0625	>64
6C$_{13}$	$-(CH_2)_3CH_3$	4-CH_2CH_3	0.0039	0.25	4	1	4	1	1	>64
6C$_{14}$	$-(CH_2)_3CH_3$	2,4-Cl,Cl	0.0039	1	4	1	16	16	0.25	>64
ICZ	—	—	0.0625	0.125	0.125	0.125	0.125	0.5	0.0625	0.5
KCZ	—	—	0.0156	0.0625	0.0625	0.0625	0.0625	0.25	0.0156	1
FCZ	—	—	0.5	1	1	1	4	4	0.25	>64
VCZ	—	—	0.0156	0.0156	0.0039	0.0039	0.0625	0.0625	0.0039	0.25
AMB	—	—	2	2	2	2	2	2	2	64
TRB	—	—	4	1	0.25	0.0625	0.0625	16	4	0.0625

Abbreviations: C .alb, *Candida albicans*; *C. neo*, *Cryptococcus neoformans*; *C. par*, *Candida parapsilosis*; *C. tro*, *Candida tropicalis*; *T. rub*, *Trichophyton rubrum*; *C. kri*, *Candida Krusei*; *M .gyp*, *Microsporum gypseum*; *A. fum*, *Aspergillus fumigatus*.
ICZ, itraconazole; KCZ, ketoconazole; FCZ, fluconazole; VCZ, voriconazole; AMB, amphotericin B; TRB, terbinafine.

Compared to the most prescribed antifungals in clinic voriconazole, these compounds also exhibited excellent activities and broad spectrum. The MIC_{80} values indicate that the series of **6A$_n$** and **6B$_n$** showed more excellent antifungal activities against the fungi than that of the **6C$_n$** series. Noticeably, the **6A$_n$** and **6B$_n$** series showed higher activity against nearly all the fungi tested except against *Aspergillus fumigatus*. Noticeably, the MIC value of compound **6A$_4$** and **6A$_{10}$** is 64 times lower than that of fluconazole against *Microsporum gypseum* in vitro. And compound **6B$_9$**, **6C$_{13}$**, and **6C$_{14}$** showed 128 times higher activity (with the MIC_{80} value of 0.0039 mg/mL) than that of fluconazole against *C. albicans* and also showed higher activities than that of the other positive controls. These results clearly indicated that the substituted benzyl side chain linked to the triazole pharmacophore greatly enhanced the antifungal activity of these analogs against *Candida* species, and that the amine side chain showed higher activity as it is shortened. In addition, despite the positive control drug itraconazole and voriconazole showing cross-resistance to fluconazole, almost all of our target compounds displayed certain extent of activities against fluconazole-resistant strains of *C. albicans*. The *Trichophyton* species *Trichophyton rubrum* was found to be much less sensitive to these derivatives, as indicated by their MIC_{80} values showing moderate activity. As far as we all know *A. fumigatus* possesses an intrinsic mechanism resistant to triazole antifungals.[15]

We proposed a likely binding mode for **6B$_9$** to the active site of CYP51 based on computational docking results (Fig. 3). In our previous study, both *R* and *S* isomers of the compounds interacted with CYP51 through a similar binding mode. However, *R* isomers showed lower interaction energy with CYP51 than the *S* isomers, which indicated that the *R* isomers might have better antifungal activity than the *S* isomers.[11] In the following discussion, all the docked conformations refer to the *R* configuration of the compounds. As usual, the triazole interacts with iron of the heme group, while the diflurophenyl group in the designed compound could be placed into the hydrophobic pocket formed by Ala114, Phe126, Leu139, Met140, Phe145, Ile304, Met306, and Gly307. And the 4-bromobenzyl group would generate π–π stacking interactions with the Tyr-118. The N-substituted group of the linker

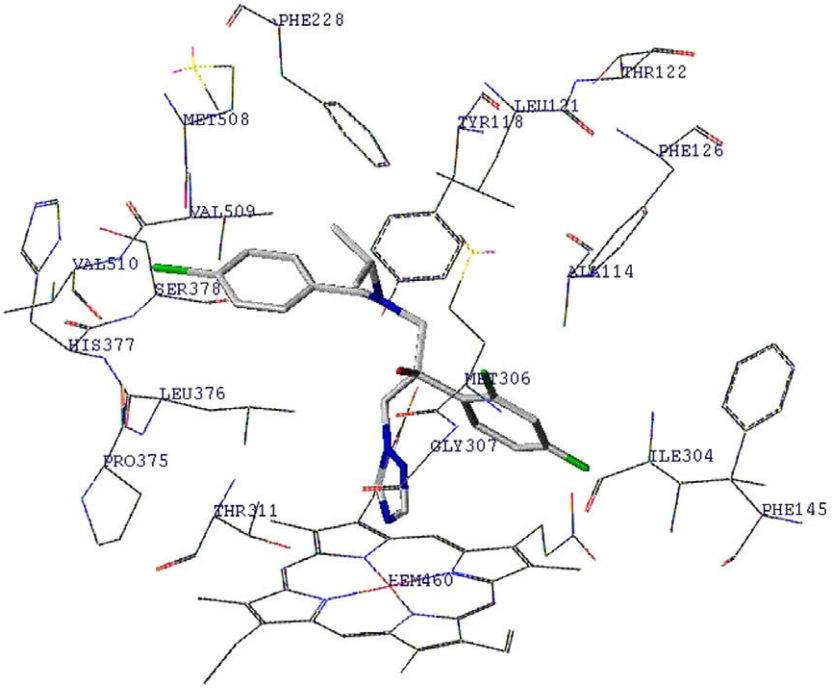


Figure 3. Computed binding geometry of the new inhibitor **6B$_9$** in the active site of CYP51.

would be oriented to interact with a hydrophobic pocket formed by Leu121, Thr122, Phe228, Thr311, Pro375, Leu376, His377, Ser378, Met508, Val509, and Val510.

In addition, all of the side chains were of the pharmacophores, and the spatial orientations of the pharmacophores were just oriented in the hydrophobic pocket. The side chains of inhibitors were not the determinants for activity, but were very important. They played a role in adjusting the physico-chemical properties of the whole molecule to avoid some dissatisfying side effects and improve their pharmacokinetic and pharmacodynamic behaviors.

In conclusion, an efficient method which depended on computational docking experiments has been developed for the synthesis of diversified novel triazole derivatives. Results of preliminary antifungal tests against eight human pathogenic fungi in vitro showed that these analogs exhibited excellent activities with broad spectrum. The research has led to the discovery of a series of compounds for further optimization.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 20772153) and by Shanghai Leading Academic Discipline Project No. B906. We thank Dr. Wannian Zhang for his advice and discussions.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.01.048.

References and notes

1. Fridkin, S. K.; Jarvis, W. R. *Clin. Microbiol. Rev.* **1996**, *9*, 499.
2. Wingard, J. R.; Leather, H. *Biol. Blood Marrow Transplant.* **2004**, *10*, 73.
3. Latge, J. P. *Clin. Microbiol. Rev.* **1999**, *12*, 310.
4. Steenbergen, J. N.; Casadevall, A. J. *J. Clin. Microbiol.* **2000**, *38*, 1974.
5. Georgopapadakou, N. H.; Walsh, T. J. *Antimicrob. Agents Chemother.* **1996**, *40*, 279.
6. Pfaller, M. A.; Messer, S. A.; Hollis, R. J.; Jones, R. N. *Antimicrob. Agents Chemother.* **2001**, *45*, 2862.
7. Jeu, L.; Piacenti, F. J.; Lyakhovetskiy, A. G.; Fung, H. B. *Clin. Pharmaocol. Ther.* **2003**, *25*, 1321.
8. Lepesheva, G. I.; Zaitseva, N. G.; Nes, W. D.; Zhou, W.; Arase, M.; Liu, J.; Hill, G. C.; Waterman, M. R. *J. Biol. Chem.* **2006**, *281*, 3577.
9. Ji, H.; Zhang, W.; Zhou, Y.; Zhang, M.; Zhu, J.; Song, Y.; Lü, J.; Zhu, J. *J. Med. Chem.* **2000**, *43*, 2493.
10. Sheng, C.; Zhang, W.; Ji, H. *Chin. Chem. Lett.* **2004**, *15*, 404.
11. Sheng, C.; Zhang, W.; Ji, H.; Zhang, M.; Song, Y.; Xu, H.; Zhu, J.; Miao, Z.; Jiang, Q.; Yao, J.; Zhou, Y.; Zhu, J.; Lü, J. *J. Med. Chem.* **2006**, *49*, 2512.
12. Richardson, K. US Patent 4404216, 1983.
13. *Representative analytical data for compound* ***6A$_1$***. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.05 (1H, s, triazole-H), 7.73 (1H, s, triazole-H), 6.74–7.61 (8H, m, Ar-H), 5.37 (1H, br, OH), 4.36–4.47 (2H, dd, J = 14.4 Hz, triazole-CH_2–), 3.30–3.51 (2H, dd, J = 14.0 Hz, Ar-CH_2–), 2.83–3.17 (2H, dd, J = 14.0 Hz, CCH_2N), 2.23–2.27 (2H, t, $NCH_2CH_2CH_3$), 1.26–1.33 (2H, m, $NCH_2CH_2CH_3$), 0.67–0.71 (3H, t, CH_3); IR (KBr): 3200, 3173, 3066, 2952, 2869, 1614, 1535, 1477, 1251, 1106, 750, 695; LC–MS, *m/z:* 387.2 $(M+H)^+$.
Compound ***6B$_9$***. ^{1}H NMR (400 MHz,$CDCl_3$) δ: 8.07 (1H, s, triazole-H), 7.77 (1H, s, triazole-H), 6.73–7.53 (7H, m, Ar-H), 5.34 (1H, br, OH), 4.35–4.51 (2H, dd, J = 14.0 Hz, triazole-CH_2–), 3.21–3.37 (2H, dd, J = 14.0 Hz, Ar-CH_2–), 2.83–3.06 (2H, dd, J = 14.0 Hz, CH_2), 2.59–2.62 (1H, m, CH), 0.81–0.88 (6H, dd, J = 6.8 Hz, 2$\times$ CH_3); IR (KBr): 3197, 3108, 3002, 2985, 2822, 1650, 1545, 1407, 1384, 1035, 957, 780; LC–MS, *m/z:* 456.1 $(M+H)^+$.
Compound ***6C$_{12}$***. ^{1}H NMR (400 MHz,$CDCl_3$) δ: 8.04 (1H, s, triazole-H), 7.76 (1H, s, triazole-H), 6.75–8.14 (7H, m, Ar-H), 5.35 (1H, br, OH), 4.42–4.57 (2H, dd, J = 14.4 Hz, triazole-CH_2–), 3.49–3.61 (2H, dd, J = 14.4 Hz, Ar-CH_2–), 2.85–3.19 (2H, dd, J = 14.0 Hz, CCH_2N), 2.27–2.31 (2H, t, $NCH_2CH_2CH_2CH_3$), 1.22–1.34 (2H, m, $NCH_2CH_2CH_2CH_3$), 1.06–1.14 (2H, m, $NCH_2CH_2CH_2CH_3$), 0.75–0.79 (3H, t, CH_3); IR (KBr): 3105, 2984, 2833, 1615, 1518, 1497, 1260, 1109, 960, 845; LC–MS, *m/z:* 446.2 $(M+H)^+$.
14. National Committee for Clinical Laboratory Standards, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts Approved standard. Document M27-A2, National Committee for Clinical Laboratory Standards, Wayne, PA, 2002.
15. Mellado, E.; Garcia-Effron, G.; Alcázar-Fuoli, L.; Melchers, W. J. G.; Verweij, P. E.; Cuenca-Estrella, M.; Rodriguez-Tudela, J. L. *Antimicrob. Agents Chemother.* **2007**, *51*, 1897.