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Synthesis of Novel Halobenzyloxy and Alkoxy 1,2,4-Triazoles and Evaluation for Their Antifungal and Antibacterial Activities

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A new class of halobenzyloxy or alkoxy 1,2,4-triazoles and their hydrochlorides were synthesized through cyclization starting from commercially available phenylhydrazine. The structures were characterized by MS, IR and ^{1}H NMR spectra as well as elemental analyses. All the synthesized compounds were screened for their antibacterial activities *in vitro* against *Staphylococcus aureus* (ATCC29213), methicillin-resistant *Staphylococcus aureus* (N315), *Bacillus subtilis*, *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Eberthella typhosa*, and antifungal activities against *Candida albicans* (ATCC76615), *Aspergillus fumigatus* by broth microdilution assay method. The results of preliminary bioassay indicated that 3-(2,4-difluorobenzyloxy)-1-phenyl-1*H*-1,2,4-triazole hydrochloride exhibited the best inhibitory activity with an MIC value of 56.25 μM against *P. aeruginosa* superior to Chloramphenicol, and showed comparable activity with Chloramphenicol against *E. coli* (ATCC25922).

Key Words: 1,2,4-Triazole, Cyclization, Phenylhydrazine, Antifungal, Antibacterial

Introduction

The incidence of systemic fungal infections such as candidosis, cryptococcosis, and aspergillosis has been rapidly increasing mainly due to the growing number of immunocompromised hosts in the past few decades. Similarly, the dramatically rising prevalence of multi-drug resistant microbial infections has recently become a serious health care problem. Particularly, the emergence of multi-drug-resistant Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) has been a problem of ever-increasing significance in both community and hospital acquired infections.[1] Thus, there is a real perceived need for the discovery of new compounds with high efficiency, broad spectrum and low toxicity, which are structurally distinct from those of well known classes of antimicrobial agents to which many clinically relevant pathogens are now resistant. Consequently, the search for new molecular scaffolds will always remain an important and challenging task for medicinal chemists.

Azole compounds especially 1,2,4-triazole derivatives are quite important types of nitrogen-containing aromatic heterocyclic compounds endowed with excellent safety profile, favorable pharmacokinetic characteristics and wide biological activities.[2-9] Noticeably, 1,2,4-triazoles as antimicrobial agents have aroused special attention in medicinal chemistry in recent years due to their prominent activity, low toxicity as well as broad spectrum.[10] So far a large number of 1,2,4-triazole antimicrobial compounds have been extensively used in clinic.[11] For example, Fluconazole shows good antifungal activity with relatively low toxicity and has been preferred as first-line antifungal therapy.[12] However, Fluconazole is not effective against invasive aspergillosis and has suffered severe drug resistance. Itraconazole is an improvement of Fluconazole in terms of having a broad antifungal spectrum and better toleration, whereas its use is hampered by variable oral absorption and low bioavailability. This situation has led to an ongoing search for new azoles. Very recently, a substantial effort has been devoted to the modification and optimization of the existing triazole antimicriobial agents which relied on the empirical development of a structure-activity relationship (SAR).[13] On the other hand, it is also an increasing prevalence of one such strategy that has been pursued in recent years to develop new antimicrobial agents with novel chemical structures, which could have modes of action rather than analogs of the existing ones.

In our ongoing interest in the development of new antimicrobial agents,[14-18] our attention has been focusing on the discovery of novel *N*-substituted 1,2,4-triazole compounds with structurally difference from the well known ones *via N*-alkylation of 1,2,4-triazole ring.[14,15] Except that, there are still so many approaches that are available for the insertion of specific functionalities into the 1,2,4-triazole nucleus to build up diversified substituted 1,2,4-triazoles.[19-25] The alterations in chemical structure of substituted 1,2,4-triazoles could affect their interactions with cells and tissues, thereby exhibiting different biological effects. Furthermore, much research manifested that incorporation of fluorine or chlorine moiety into an organic molecule could efficiently improve the pharmacological properties,[26-29] which resulted in increasing lipid solubility, accordingly enhancing the rate of absorption and transport of drugs *in vivo*. The replacement of hydrogen or hydroxyl group by fluorine or chlorine represents a valuable strategy and was extensively used in drug development to alter biological function.[30,31] Meanwhile, the introduction of alkyl moiety into various heterocyclic systems as the large non-polar portion could play an important role in modulating the physicochemical properties of the whole molecule that possibly avoid some side effects and improve their pharmacokinetic and pharmacodynamic behaviors. This is also helpful to increase their biological activities.[32-34]

On the basis of all above observations and as an extension of our studies on biologically active *N*-substituted 1,2,4-triazol-

es derivatives, herein a series of novel halobenzyloxy and alkoxy 1,2,4-triazole compounds have been synthesized and evaluated for their antibacterial and antifungal activities *in vitro*. Additionally, their corresponding hydrochlorides were also prepared in order to investigate the effect of water solubility of target triazoles on the antimicrobial activities, and their structure-activity relationships were also discussed.

Results and Discussion

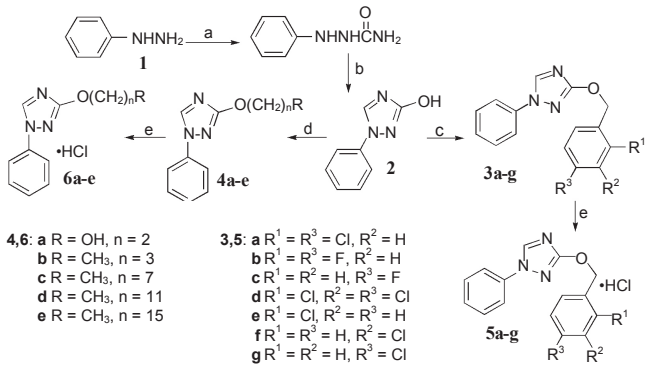
Chemistry. The synthetic route of halobenzyloxy or alkoxy 1,2,4-triazole derivatives **3-4** and their hydrochlorides **5-6** was outlined in Scheme 1. Phenylhydrazine **1** was first reacted with urea to give the intermediate phenylhydrazinecarboxamide, and then the later was cyclized with formic acid to afford the corresponding hydroxytriazole **2**.[35] The etherification of 1-phenyl-1,2,4-triazole-3-ol (**2**) using halogen-substituted benzyl or alkyl halides in the presence of anhydrous potassium carbonate and TBAI afforded halobenzyloxy or alkoxy 1,2,4-triazoles (**3a-g**, **4a-e**) in moderate to excellent yields (from 56% to 89%), and then treated in ethyl ether with HCl (4 mol/L) to give the corresponding hydrochlorides (**5a-g**, **6a-e**) in high yields ranging from 81% to 88%.

Experimental results revealed that the ratio of reactants, solvent, reaction time, amount of catalyst as well as the addition rate of concentration sulfuric acid exerted important influences on the yield of hydroxytriazole (**2**). Therefore, we optimized the cyclization conditions, and exploited an one-pot, three-component synthesis strategy to construct the 1,2,4-triazole ring, which represented an easy, convenient and efficient synthetic route.

The intermediate hydroxytriazole **2** involves two tautomeric forms. When it reacted with halogen-substituted benzyl or alkyl halides, it may be in the wake of obtaining the *N*-alkylation products in the *O*-alkylation reaction. The *O*-alkylation reaction was found to be highly sensitive to reaction temperature, solvent and pH. When the temperature was above 80 ºC, the reaction became complex, and thus led to poor yields. However, too low temperature (below 55 ºC) would not only greatly decrease the reactivity but also make the reaction sluggish enough to lower the yields. The optimal reaction temperature was at 70 - 75 ºC in our study, which gave the highest yields. Moreover, the reaction failed as the pH was higher than 10 or less than 6. The results confirmed that the presence of weak base (K_2CO_3) in acetonitrile at 70 - 75 ºC with pH 7.5 - 8.5 was favorable to this *O*-alkylation reaction.

It was also noticed that the presence of the triazole hydrochloride in compounds **5-6** resulted in a better water solubility in comparison with the corresponding precursors **3-4**, while alkoxy triazole hydrochlorides possessed better solubility than halobenzyloxy ones in other solvents such as chloroform, tetrahydrofuran and acetone.

Analysis of spectra. All the synthesized compounds were confirmed by MS, IR and ^{1}H NMR spectra as well as elemental analyses. The spectral analyses were in accordance with the assigned structures, and all the characterization data were given in the experimental section. The mass spectra for the target compounds showed a major fragment of $[M]^+$, $[M+H]^+$ or $[M-HCl]^+$, in agreement with their molecular formula.



4,6: **a** R = OH, n = 2
b R = CH_3, n = 3
c R = CH_3, n = 7
d R = CH_3, n = 11
e R = CH_3, n = 15

3,5: **a** $R^1 = R^3$ = Cl, R^2 = H
b $R^1 = R^3$ = F, R^2 = H
c $R^1 = R^2$ = H, R^3 = F
d R^1 = Cl, $R^2 = R^3$ = Cl
e R^1 = Cl, $R^2 = R^3$ = H
f $R^1 = R^3$ = H, R^2 = Cl
g $R^1 = R^2$ = H, R^3 = Cl

Scheme 1. Synthetic route of halobenzyloxy and alkoxy triazole derivatives **3-6**. Reagents and conditions: (a) $NH_2CONH_2/H_2SO_4/H_2O$, 100 ºC; (b) HCOOH / H_2SO_4, 125 ºC; (c) halobenzyl bromide or chloride, TBAI, K_2CO_3/CH_3CN, 70 ºC; (d) $R(CH_2)_nBr$, TBAI, K_2CO_3/CH_3CN, 75 ºC; (e) 4 mol/L HCl/ethyl ether, rt.

IR spectra: The IR spectra of compounds **3-6** exhibited that one moderate and two characteristic strong absorption bands at 3200 - 3000 cm^{-1}, 1290 - 1200 cm^{-1} and 1110 - 1000 cm^{-1} were respectively attributable to the stretching vibration of Ar-H, the anti-symmetric stretching vibration and the symmetric stretching vibration of C-O-C bonds. Moreover, it was found that all the vibration frequency in halobenzyloxy or alkoxy 1,2,4-triazoles was obviously shifted to higher wave numbers in contrast to their corresponding hydrochlorides, which was mainly responsible for the inductive effects of the positive charges in triazole moiety. For another, the H_2C-O absorption bands in halobenzyloxy 1,2,4-triazoles were observed at higher frequency in comparison with that in alkoxy ones owing to the electrophilic inductive effects fluorine or chlorine moiety and conjugation effects of benzene ring in compounds **3a-g** and **5a-g**.

^{1}H NMR spectra: The ^{1}H NMR spectra showed that two singlets at the region of 5.62 - 4.34 ppm and 8.95 - 7.84 ppm were assigned to the methylene protons of alkoxy or halobenzyloxy group and the N = C*H* protons of triazole ring in all title compounds respectively. Furthermore, the downfield shift of the methylene protons of the halobenzyl compounds was observed, in comparison with that in alkyl substituted 1,2,4-triazoles. In the ^{1}H NMR spectra, it was also found that all the triazole N = C*H* protons and the methylene protons in the hydrochlorides showed large downfield shifts compared to the corresponding precursors, probably ascribed to the presence of positive charges in the hydrochlorides. All the other aromatic and aliphatic protons appeared at the appropriate chemical shifts and integral values.

Biological activity. These halobenzyloxy and alkoxy 1,2,4-triazoles **3-4** and their hydrochlorides **5-6** were evaluated for their antimicrobial activities against *MRSA* (N315), *S. aureus* (ATCC29213) and *B. subtilis* as Gram-positive, *E. coli* (ATCC-25922), *P. aeruginosa*, *S. dysenteriae* and *E. typhosa* as Gram-negative bacteria, as well as *C. albicans* (ATCC76615) and *A. fumigatus* as fungi by broth microdilution assay method. Clinical antimicrobial drugs Chloramphenicol and Fluconazole were served as the positive control. The minimum inhibitory concentration (MIC, μM) values were compiled in Table 1.

Table 1. *In vitro* antibacterial and antifungal activities of the title compounds **3-6**[a,b,c,d]

	MIC μM[d]								
	Fungal strains		Baterial strains						
Compd.	*C. albicans*	*A. fumigatus*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*E. coli*	*S. dysenteriae*	*MRSA*	*E. typhosa*
3a	400	800	800	>1600	800	1600	800	1600	800
3b	900	900	450	225	112.5	112.5	450	1800	450
3c	950	>1900	950	1900	950	950	>1900	>1900	1900
3d	>1600	>1600	>1600	1600	1600	1600	>1600	>1600	>1600
3e	1800	1800	>1800	1800	1800	1800	1800	>1800	900
3f	>1800	1800	1800	1800	1800	1800	900	>1800	1800
3g	1800	1800	>1800	900	900	900	1800	>1800	1800
4a	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500
4b	>2360	>2360	>2360	>2360	>2360	>2360	>2360	>2360	>2360
4c	1880	1880	1880	940	1880	940	>1880	>1880	470
4d	780	>1560	780	780	1560	390	1560	>1560	1560
4e	>1330	>1330	>1330	>1330	>1330	>1330	>1330	>1330	>1330
5a	200	800	400	>1600	400	800	400	800	400
5b	450	450	450	112.5	56.25	56.25	225	450	450
5c	950	>1900	950	950	475	475	>1900	>1900	950
5d	>1600	>1600	>1600	800	800	800	>1600	>1600	>1600
5e	900	900	>1800	900	>1800	450	900	>1800	450
5f	900	900	450	450	900	900	450	>1800	900
5g	900	900	>1800	900	450	450	900	>1800	900
6a	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500	>2500
6b	>2360	>2360	>2360	2360	2360	>2360	>2360	>2360	2360
6c	940	940	940	235	470	470	>1880	>1880	235
6d	390	1560	195	390	780	195	780	1560	780
6e	>1330	>1330	>1330	1330	>1330	1330	>1330	>1330	665
A	-	-	198.1	>1584	>1584	49.52	49.52	49.52	49.52
B	52.24	>1671	-	-	-	-	-	-	-

[a]Minimum inhibitory concentrations were determined by micro broth dilution method for microdilution plates. [b]**A** = Chloramphenicol, **B** = Fluconazole. [c]*C. albicans*, Candida albicans ATCC76615; *A. fumigatu*s, Aspergillus fumigatus; *S. aureus*, Staphylococcus aureus ATCC29213, *B. subtilis*, Bacillus subtilis; *E. coli*, Escherichia coli ATCC25922; *P. aeruginosa*, Pseudomonas aeruginosa; *S. dysenteriae,* Shigella dysenteriae; *MRSA*, Methicillin-Resistant *Staphylococcus aureus* N315; *E. typhosa*, Eberthella typhosa. [d]MIC values was in μM.

Antibacterial activity: The investigation of antibacterial screening data revealed that some target compounds exhibited significant inhibitory activities against some tested strains such as *B. subtilis* and *P. aeruginosa* at 56.25 - 475 μM *in vitro*. Especially, it was worthy to note that the compounds **3a** and **3b** as well as their corresponding hydrochlorides **5a** and **5b** bearing 2,4-difluorobenzyloxy and 2,4-dichlorobenzyloxy groups exhibited the most prominent activity against all bacterial strains tested at the concentration of 56.25 - 450 μM, in comparison with other halobenzyloxy 1,2,4-triazoles, which was possibly attributed to the presence of pharmacologically active dihalophenyl moiety at position 3 of the triazole ring. That suggested that 2,4-dichlorobenzyl or 2,4-difluorobenzyl substitution in the triazole ring was suitable for antibacterial activity.

On basis of the bioactive data, it was observed that the antibacterial activities for alkoxy derivatives **4a-e** and **6a-e** were weaker in comparison to halobenzyloxy compounds **3a-g** and **5a-g**. However, compounds **4c**, **4d** and their hydrochlorides **6c**, **6d** containing long alkyl chains with lengths in the range of C8-C12 gave significant activities against some tested strains with MIC values ranging from 195 μM to 470 μM. Conversely, the antibacterial results showed no obvious inhibition for compounds **4a**, **4b** including their hydrochlorides **6a**, **6b** with short C2-C4 alkyl chain. Therefore, the introduction of linear alkyl with lengths in the range of C8-C12 into 1,2,4-triazole ring could enhance antibacterial activities.

The 4-nitrogen of triaozle ring is liable to quaternary, and formed into quaternary ammonium salt, which is favourable to improve water-solubility and enhance activities. *In vitro* studies demonstrated that all the halobenzyloxy and alkoxy 1,2,4-triazole hydrochlorides **5-6** exhibited better activities compared to the corresponding precursors **3**-**4**, in particular, compound **5b** possessed dramatic biological activities against nearly all tested strains with MIC values ranging from 56.25 μM to 450 μM, shown in Table 1. Noticeably, compound **5b** gave 15 - 30 times higher potency than Chloramphenicol against *B. subtilis* and *P. aeruginosa*, with MIC values of 112.5 μM and 56.25 μM respectively, and exerted almost comparable inhibitory activity

with respect to the standard drug Chloramphenicol against *E. coli* (ATCC25922). Surprisingly, compound **5b** was observed to show potent activity against methicillin-resistant *S. aureus* (N315) with an MIC value of 450 μM.

Generally, the halobenzyloxy triazole derivatives **3a**, **3b**, **3c** and **4d** along with their hydrochlorides **5a**, **5b**, **5c** and **6d** containing dichlorobenzyloxy, difluorobenzyloxy, 4-fluorobenzyloxy and dodecyloxy moieties respectively, showed more potent antibacterial activity than other new compounds against some tested bacteria species, while the hydrochlorides with better water solubility exhibited enhanced activity in contrast to their corresponding compounds. All the results suggested the significant effect of the water-solubility of the compounds, the substitution of benzyloxy group as well as the length of linear alkyl chain in the 1,2,4-triazole ring on antibacterial activity.

Antifungal activity: The antifungal evaluation revealed that for almost all the synthesized halobenzyloxy and alkoxy 1,2,4-triazole derivatives, the activities were relatively weak compared to their antibacterial efficacy. However, it's worth mentioning that the 1,2,4-triazole hydrochlorides **5a** and **5b** displayed higher inhibitory activities against *A. fumigatus* than the reference drug Fluconazole with the low MIC values of 800 μM and 450 μM, respectively. All the newly synthesized compounds almost had no obvious inhibitory activity against *C. albicans* except for compounds **5a** with 2,4-difluorobenzyloxy group and **6d** consisting of the linear alkyl chain with length in C12, which showed remarkable antifungal activity toward *C. albicans* with MIC values of 200 μM and 390 μM, respectively. The other compounds exhibited poor or weak antifungal activity against all fungi tested.

Conclusion

In summary, a new type of halobenzyloxy 1,2,4-triazoles (**3a-g**) and alkoxy 1,2,4-triazoles (**4a-e**) as well as their corresponding hydrochlorides (**5a-g**, **6a-e**) were synthesized successfully *via* cyclization using phenylhydrazine as starting material, and their antimicrobial activities were also evaluated. All these new compounds were confirmed by IR, MS, ^{1}H NMR spectra and elemental analyses. All results obtained from antibacterial and antifungal tests revealed that some of the present series carrying dihalobenzyloxy, octyloxy or dodecyloxy groups exhibited moderate antimicrobial activities *in vitro*. Noticeably, compound **3b** containing difluorobenzyloxy and its hydrochloride **5b** showed the most potent activity against *P. aeruginosa* and *E. coli* with the MIC values of 112.5 μM and 56.25 μM respectively. The study of preliminary structure-activity relationships delineated that the type of substituent and the length of alkyl chain in the new compounds are responsible for the variation of the antimicrobial activities. Moreover, the water-solubility of the tested compounds has important effect on their biological activities. Further investigations are in progress for this class of heterocyclic compounds.

Experimental

Chemistry. Melting points are uncorrected and were recorded on X-6 melting point apparatus. Thin layer chromatography (TLC) was performed using pre-coated silica gel plates and visualization was obtained by exposure to iodine vapors and / or under UV light (254 nm). FT-IR spectra were recorded on Bruker RFS100/S spectrophotometer (USA) using KBr pellets in the 400 - 4000 cm^{-1} range. ^{1}H-NMR spectra were determined with a Bruker AV 300 MHz spectrometer and were reported in δ units (ppm) relative to tetramethylsilane (TMS) as an internal standard. The chemical shifts are expressed in parts per million (d) and following abbreviations were used: s = singlet; bs = broad singlet; d = doublet; t = triplet; m = multiplet. The mass spectra were recorded on FINNIGAN TRACE GC-MS 2000 mass spectrometer (Thermo Electron Corporation, Bremen, Germany). Elemental analyses were carried out on a ERBA1106 (Carlo Erba, Milan, Italy). All chemicals and solvents were commercially available, reagent grade, and were used without further purification.

Synthesis of 1-phenyl-1*H*-1,2,4-triazol-3-ol (2). The mixture of phenylhydrazine (2.8 g, 26 mmol) and urea (4.9 g, 82 mmol) was dissolved in 6 mL H_2O with stirring at 90 ºC. Subsequently, concentrated sulfuric acid (4.2 g, 42 mmol) was added by a dropping funnel with stirring. After that, the mixture was refluxed for 5 - 9 h at 100 ºC until the starting material was almost consumed (monitored by TLC, eluent, chloroform/acetone, 10/1, v/v), and then set down the temperature to 90 ºC, added HCOOH (3.7 g, 82 mmol) to the flask and stirred for 16 - 20 h under 125 ºC until the reaction was over (monitored by TLC, eluent, chloroform/acetone, 3/1, v/v). The reacting mixture was cooled to room temperature, and then the resultant yellow precipitate was filtered and washed with water until pH 6.5. The crude product was purified by recrystallization from acetic acid to afford white solid in 88% yield, mp 281 - 282 ºC, which is according with the reference.[35]

Synthesis of halobenzyloxy 1,2,4-triazoles (3a-g). To a flask containing hydroxyltriazole **2** (200 mg) in 10 mL CH_3CN was added 200 mg (14.5 mmol) of anhydrous potassium carbonate and stirred for 30 min at 65 ºC. Then 5 mg of tetrabutyl ammonium iodide (TBAI) and equimolar halobenzyl bromide or chloride were added and the mixture was stirred at 70 - 75 ºC for 5 - 7 h (monitored by TLC, eluent, chloroform). After cooling, the reaction mixture was evaporated under reduced pressure, treated with water (10 mL), and then extracted with chloroform (3 × 10 mL). The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified *via* silica gel column chromatography (eluent, chloroform) and recrystallized from the mixture solvent of chloroform and petroleum ether (30 - 60 ºC) to yield the compounds **3a-g**.

3-(2,4-Dichlorobenzyloxy)-1-phenyl-1*H*-1,2,4-triazole (3a): This compound **3a** (342 mg) was obtained as colourless needles in 86% yield, mp 138 - 139 ºC; IR (KBr) ν 3122, 3072 (Ar-H), 2921, 2868 (CH_2), 1600, 1553, 1448, 1410, 1361 (aromatic frame), 1250, 1094 (C-O-C), 869, 859, 813, 736, 637 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, *J* in Hz) δ 8.28 (s, 1H, triazole 5-H), 7.64-7.58 (m, 3H, Ar H), 7.49 (t, 2H, ^3J = 7.60, Ar H), 7.43-7.37 (m, 2H, Ar H), 7.30-7.29 (m, 1H, Ar H), 5.48 (s, 2H, OCH_2) ppm; MS *m*/*z* 320 [M]$^+$; Anal. Calcd. for $C_{15}H_{11}Cl_2N_3O$: C, 56.32; H, 3.50; N, 13.16. Found: C, 56.27; H, 3.46; N, 13.12.

3-(2,4-Difluorobenzyloxy)-1-phenyl-1*H*-1,2,4-triazole

(3b): This compound **3b** (316 mg) was obtained as white solid in 88% yield, mp 100 - 101 ºC. IR (KBr) ν 3131, 3084 (Ar-H), 2968, 2968 (CH_2), 1622, 1546, 1473, 1445, 1333 (aromatic frame), 1280, 1080 (C-O-C), 853, 830, 787, 739, 672 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, *J* in Hz) δ 8.28 (s, 1H, triazole 5-H), 7.64-7.58 (m, 2H, Ar H), 7.56-7.53 (m, 1H, Ar H), 7.51-7.48 (m, 2H, Ar H), 7.46-7.36 (m, 1H, Ar H), 6.89-6.82 (m, 2H, Ar H), 5.47 (s, 2H, OCH_2) ppm; MS *m*/*z* 288 $[M+H]^+$; Anal. Calcd. for $C_{15}H_{11}F_2N_3O$: C, 62.76; H, 3.90; N, 14.64. Found: C, 62.72; H, 3.86; N, 14.63.

3-(4-Fluorobenzyloxy)-1-phenyl-1*H*-1,2,4-triazole (3c): This compound **3c** (297 mg) was obtained as white solid in 89% yield, mp 102 - 103 ºC; IR (KBr) ν 3122, 3065 (Ar-H), 2972, 2853 (CH_2), 1599, 1555, 1480, 1452, 1332 (aromatic frame), 1222, 1055 (C-O-C), 859, 823, 805, 757, 680 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, *J* in Hz) δ 8.26 (s, 1H, triazole 5-H), 7.62 (d, 2H, ^3J = 7.83, Ar H), 7.51-7.45 (m, 4H, Ar H), 7.38-7.33 (t, 1H, ^3J = 7.32, Ar H), 7.10-7.04 (t, 2H, ^3J = 8.64, Ar H), 5.37 (s, 2H, OCH_2) ppm; MS *m*/*z* 270 $[M+H]^+$; Anal. Calcd. for $C_{15}H_{12}FN_3O$: C, 66.94; H, 4.53; N, 15.64. Found: C, 66.91; H, 4.49; N, 15.60.

3-(3,4-Dichlorobenzyloxy)-1-phenyl-1*H*-1,2,4-triazole (3d): This compound **3d** (337 mg) was obtained as white solid in 85% yield, mp 98 - 100 ºC; IR (KBr) ν 3122, 3089 (Ar-H), 2973, 2915 (CH_2), 1597, 1568, 1479, 1448, 1329 (aromatic frame), 1234, 1056 (C-O-C), 860, 824, 799, 758, 689 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, *J* in Hz) δ 8.27 (s, 1H, triazole 5-H), 7.62 (d, 3H, ^3J = 8.22, Ar H), 7.51-7.44 (m, 3H, Ar H), 7.39-7.32 (m, 2H, Ar H), 5.36 (s, 2H, OCH_2) ppm; MS *m*/*z* 320 $[M]^+$; Anal. Calcd. for Anal. Calcd. for $C_{15}H_{11}Cl_2N_3O$: C, 56.30; H, 3.50; N, 13.17. Found: C, 56.27; H, 3.46; N, 13.12.

3-(2-Chlorobenzyloxy)-1-phenyl-1*H*-1,2,4-triazole (3e): This compound **3e** (315 mg) was obtained as white solid in 89% yield, mp 123 - 125 ºC; IR (KBr) ν 3121, 3092 (Ar-H), 2973, 2869 (CH_2), 1599, 1565, 1458, 1435, 1332 (aromatic frame), 1234, 1047 (C-O-C), 870, 846, 774, 746, 678 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, *J* in Hz) δ 7.84 (s, 1H, triazole 5-H), 7.49-7.45 (m, 4H, Ar H), 7.32-7.29 (m, 5H, Ar H), 5.42 (s, 2H, OCH_2) ppm; MS *m*/*z* 285 $[M]^+$; Anal. Calcd. for $C_{15}H_{12}ClN_3O$: C, 63.09; H, 4.29; N, 14.75. Found: C, 63.05; H, 4.23; N, 14.71.

3-(4-Chlorobenzyloxy)-1-phenyl-1*H*-1,2,4-triazole (3f): This compound **3f** (304 mg) was obtained as white solid in 86% yield, mp 130 - 131 ºC; IR (KBr) ν 3120, 3056 (Ar-H), 2970, 2852 (CH_2), 1597, 1568, 1479, 1448, 1334 (aromatic frame), 1234, 1056 (C-O-C), 863, 846, 811, 758, 688 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, *J* in Hz) δ 8.27 (s, 1H, triazole 5-H), 7.62 (d, 3H, ^3J = 7.83, Ar H), 7.51-7.44 (m, 4H, Ar H), 7.38-7.34 (m, 2H, Ar H), 5.38 (s, 2H, OCH_2) ppm; MS *m*/*z* 285 $[M]^+$; Anal. Calcd. for $C_{15}H_{12}ClN_3O$: C, 63.07; H, 4.25; N, 14.75. Found: C, 63.05; H, 4.23; N, 14.71.

3-(3-Chlorobenzyloxy)-1-phenyl-1*H*-1,2,4-triazole (3g): This compound **3g** (308 mg) was obtained as white solid in 87% yield, mp 104 - 106 ºC; IR (KBr) ν 3121, 3070 (Ar-H), 2973, 2852 (CH_2), 1598, 1551, 1478, 1448, 1337 (aromatic frame), 1235, 1054 (C-O-C), 860, 836, 784, 756, 688 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, *J* in Hz) δ 8.27 (s, 1H, triazole 5-H), 7.62 (d, 2H, ^3J = 7.86, Ar H), 7.52-7.45 (m, 3H, Ar H), 7.38-7.36 (m, 2H, Ar H), 7.32-7.30 (m, 2H, Ar H), 5.39 (s, 2H, OCH_2) ppm; MS *m*/*z* 285 $[M]^+$; Anal. Calcd. for $C_{15}H_{12}ClN_3O$: C, 63.06; H, 4.30; N, 14.75. Found: C, 63.05; H, 4.28; N, 14.71.

Synthesis of alkoxy 1,2,4-triazoles (4a-e). A suspension of hydroxytriazole **2** (200 mg) and anhydrous potassium carbonate (200 mg, 14.5 mmol, 1.2 equiv) in CH_3CN (10 mL) was stirred for 30 min at 65 ºC. Then tetrabutyl ammonium iodide (TBAI, 5 mg) and equimolar alkyl bromide were added to the suspension and the mixture was stirred at 70 - 75 ºC for 5 - 7 h (monitored by TLC, eluent, chloroform). After cooling, the solvent was evaporated under reduced pressure. Then, the residue was poured into water (10 mL) and extracted with chloroform (3 × 10 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give the crude product, which was purified by silica gel column chromatography (eluent, chloroform) to give the desired compounds **4a-e**.

2-(1-Phenyl-1*H*-1,2,4-triazol-3-yloxy)ethanol (4a): This compound **4a** (143 mg) was obtained as white solid in 56% yield, mp 132 - 133 ºC; IR (KBr) ν 3512 (O-H), 3153, 3099 (Ar-H), 2973, 2894 (CH_2), 1607, 1596, 1498, 1464, 1321 (aromatic frame), 1241, 1092 (C-O-C), 1015 (C-O), 894, 841, 752, 664 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, *J* in Hz) δ 8.28 (s, 1H, triazole 5-H), 7.62 (d, 2H, ^3J = 7.86, Ph 2,6-H), 7.50 (t, 2H, ^3J = 7.41, Ph 3,5-H), 7.37 (t, 1H, ^3J = 7.26, Ph 4-H), 4.51 (t, 2H, ^3J = 3.81, OCH_2CH$_2$OH), 4.00 (t, 2H, ^3J = 4.02, OCH$_2$CH_2OH) ppm; MS *m*/*z* 206 $[M]^+$; Anal. Calcd. for $C_{10}H_{11}N_3O_2$: C, 58.56; H, 5.43; N, 20.51. Found: C, 58.53; H, 5.40; N, 20.48.

3-Butoxy-1-phenyl-1*H*-1,2,4-triazole (4b): This compound **4b** (135 mg) was obtained as colorless liquid in 50% yield; ^{1}H-NMR (300 MHz, $CDCl_3$, *J* in Hz) δ 8.28 (s, 1H, triazole 5-H), 7.62 (d, 2H, ^3J = 7.55, Ph 2,6-H), 7.37 (t, 2H, ^3J = 7.42, Ph 3,5-H), 7.36 (t, 1H, ^3J = 7.08, Ph 4-H), 4.34 (t, 2H, ^3J = 6.45, OCH_2), 1.64-1.61 (m, 2H, OCH$_2$CH_2), 1.43-1.40 (m, 2H, CH_2CH$_3$), 0.96 (t, 3H, CH$_3$) ppm; MS *m*/*z* 217 $[M]^+$; Anal. Calcd. for $C_{12}H_{15}N_3O$: C, 66.37; H, 7.01; N, 19.38. Found: C, 66.34; H, 6.96; N, 19.34.

3-(Octyloxy)-1-phenyl-1*H*-1,2,4-triazole (4c): This compound **4c** (221 mg) was obtained as white solid in 66% yield, mp 57 - 58 ºC; IR (KBr) ν 3126, 3088 (Ar-H), 2983, 2896 (CH_2), 1607, 1597, 1478, 1437, 1337 (aromatic frame), 1237, 1033 (C-O-C), 883, 842, 785, 756, 681 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, *J* in Hz) δ 8.25 (s, 1H, triazole 5-H), 7.89 (d, 2H, ^3J = 6.75, Ph 2,6-H), 7.47 (t, 2H, ^3J = 7.50, Ph 3,5-H), 7.34 (t, 1H, ^3J = 7.18, Ph 4-H), 4.35 (t, 2H, ^3J = 6.54, OCH_2), 1.75-1.69 (m, 2H, OCH$_2$-CH_2), 1.46-1.43 (m, 10H, (CH_2)$_5$CH$_3$), 0.87 (t, 3H, CH$_2$CH_3) ppm; MS *m*/*z* 270 $[M]^+$; Anal. Calcd. for $C_{16}H_{23}N_3O$: C, 70.34; H, 8.51; N, 15.40. Found: C, 70.30; H, 8.48; N, 15.37.

3-(Dodecyloxy)-1-phenyl-1*H*-1,2,4-triazole (4d): This compound **4d** (221 mg) was obtained as white solid in 54% yield, mp 81 - 82 ºC; IR (KBr) ν 3127, 3062 (Ar-H), 2977, 2889 (CH_2), 1611, 1596, 1464, 1435, 1331 (aromatic frame), 1235, 1026 (C-O-C), 887, 846, 784, 757, 669 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$, *J* in Hz) δ 8.25 (s, 1H, triazole 5-H), 7.62 (d, 2H, ^3J = 7.86, Ph 2,6-H), 7.47 (t, 2H, ^3J = 7.70, Ph 3,5-H), 7.35 (t, 1H, ^3J = 7.35, Ph 4-H), 4.35 (t, 2H, ^3J = 6.57, OCH_2), 1.81-1.78 (m, 2H, OCH$_2$-CH_2), 1.49-1.43 (m, 2H, OCH$_2$CH$_2$CH_2), 1.28-1.25 (m, 16H, (CH_2)$_8$CH$_3$), 0.87 (t, 3H, CH$_2$CH_3) ppm; MS *m*/*z* 330 $[M]^+$; Anal. Calcd. for $C_{20}H_{31}N_3O$: C, 73.96; H, 9.52; N, 12.79. Found: C, 72.91; H, 9.48; N, 12.75.

3-(Hexadecyloxy)-1-phenyl-1*H*-1,2,4-triazole (4e): This

compound **4e** (320 mg) was obtained as white solid in 67% yield, mp 111 - 112 ºC; IR (KBr) ν 3132, 3089 (Ar-H), 2973, 2882 (CH_2), 1602, 1596, 1478, 1448, 1321 (aromatic frame), 1243, 1044 (C-O-C), 895, 838, 795, 766, 677 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, *J* in Hz) δ 8.24 (s, 1H, triazole 5-H), 7.62 (d, 2H, ^3J = 8.0, Ph 2,6-H), 7.47 (t, 2H, ^3J = 7.85, Ph 3,5-H), 7.34 (t, 1H, ^3J = 7.5, Ph 4-H), 4.34 (t, 2H, ^3J = 6.60, OCH_2), 1.86-1.78 (m, 2H, OCH_2CH_2), 1.48-1.43 (m, 2H, $OCH_2CH_2CH_2$), 1.25 (m, 24H, $(CH_2)_{12}CH_3$), 0.87 (t, 3H, CH_2CH_3) ppm; MS *m*/*z* 385 $[M]^+$; Anal. Calcd. for $C_{24}H_{39}N_3O$: C, 74.78; H, 10.20; N, 10.94. Found: C, 74.76; H, 10.19; N, 10.90.

Synthesis of halobenzyloxy 1,2,4-triazole hydrochlorides (5a-g). To a solution of halobenzyloxy 1,2,4-triazoles **3a-g** (100 mg) in ethyl ether (10 mL) was added hydrochloride acid (10 mL, 4 mol/L). The mixture was stirred at room temperature for 5 - 6 h and the solvent was evaporated to afford the corresponding hydrochlorides **5a-g**. Some salts were highly hydroscopic and no sharp melting points could be observed.

3-(2,4-Dichlorobenzyloxy)-1-phenyl-1*H*-1,2,4-triazole hydrochloride (5a): This hydrochloride **5a** (98 mg) was obtained as white solid in 88% yield, mp 238 - 239 ºC; IR (KBr) ν 3132, 3085 (Ar- H), 2945, 2874 (CH_2), 1635, 1573, 1469, 1446, 1341 (aromatic frame), 1265, 1079 (C-O-C), 887, 863, 825, 748, 645 cm^{-1}; ^{1}H- NMR (300 MHz, DMSO-d_6) δ 8.77 (s, 1H, triazole 5-H), 7.85- 7.80 (m, 3H, Ar H), 7.59-7.54 (m, 2H, Ar H), 7.41-7.37 (m, 2H, Ar H), 7.36-7.30 (m, 1H, Ar H), 5.57 (s, 2H, OCH_2) ppm; MS *m*/*z* 320 $[M\text{-}HCl]^+$.

3-(2,4-Difluorobenzyloxy)-1-phenyl-1*H*-1,2,4-triazole hydrochloride (5b): This hydrochloride **5b** (96 mg) was obtained as white solid in 88% yield; No sharp melting points; IR (KBr) ν 3143, 3092 (Ar-H), 2974, 2970 (CH_2), 1641, 1562, 1494, 1467, 1369 (aromatic frame), 1295, 1107 (C-O-C), 874, 842, 790, 742, 673 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.68 (s, 1H, triazole 5-H), 7.84-7.80 (m, 2H, Ar H), 7.63-7.60 (m, 1H, Ar H), 7.57-7.53 (m, 2H, Ar H), 7.41-7.38 (m, 1H, Ar H), 7.04-7.01 (m, 2H, Ar H), 5.51 (s, 2H, OCH_2) ppm; MS *m*/*z* 288 $[M\text{-}Cl]^+$.

3-(4-Fluorobenzyloxy)-1-phenyl-1*H*-1,2,4-triazole hydrochloride (5c): This hydrochloride **5c** (98 mg) was obtained as white solid in 86% yield; 215 - 217 ºC; IR (KBr) ν 3140, 3076 (Ar-H), 2981, 2869 (CH_2), 1613, 1563, 1485, 1459, 1342 (aromatic frame), 1254, 1095 (C-O-C), 863, 829, 805, 764, 686 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.61 (s, 1H, triazole 5-H), 7.88-7.84 (m, 4H, Ar H), 7.70-7.67 (m, 2H, Ar H), 7.49-7.46 (m, 2H, Ar H), 7.34-7.29 (m, H, Ar H), 5.41 (s, 2H, OCH_2) ppm; MS *m*/*z* 270 $[M\text{-}Cl]^+$.

3-(3,4-Dichlorobenzyloxy)-1-phenyl-1*H*-1,2,4-triazole hydrochloride (5d): This hydrochloride **5d** (96 mg) was obtained as white solid in 86% yield, mp 176 - 178 ºC; IR (KBr) ν 3135, 3076 (Ar-H), 2985, 2937 (CH_2), 1623, 1575, 1487, 1450, 1339 (aromatic frame), 1254, 1091 (C-O-C), 867, 834, 782, 755, 689 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.84 (s, 1H, triazole 5-H), 7.85-7.87 (m, 3H, Ar H), 7.69-7.64 (m, 3H, Ar H), 7.45-7.40 (m, 2H, Ar H), 5.43 (s, 2H, OCH_2) ppm; MS *m*/*z* 320 $[M\text{-}HCl]^+$.

3-(2-Chlorobenzyloxy)-1-phenyl-1*H*-1,2,4-triazole hydrochloride (5e): This hydrochloride **5e** (91 mg) was obtained as white solid in 81% yield, mp 191 - 193 ºC; IR (KBr) ν 3137, 3090 (Ar-H), 2981, 2884 (CH_2), 1619, 1570, 1454, 1432, 1339 (aromatic frame), 1260, 1084 (C-O-C), 879, 849, 784, 745, 678 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.64 (s, 1H, triazole 5-H), 7.79-7.72 (m, 4H, Ar H), 7.47-7.42 (m, 5H, Ar H), 5.49 (s, 2H, OCH_2) ppm; MS *m*/*z* 285 $[M\text{-}HCl]^+$.

3-(4-Chlorobenzyloxy)-1-phenyl-1*H*-1,2,4-triazole hydrochloride (5f): This hydrochloride **5f** (94 mg) was obtained as white solid in 83% yield, mp 209 - 211 ºC; IR (KBr) ν 3143, 3079 (Ar-H), 2991, 2847 (CH_2), 1623, 1575, 1477, 1454, 1341 (aromatic frame), 1254, 1091 (C-O-C), 876, 854, 819, 760, 688 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.81 (s, 1H, triazole 5-H), 7.78-7.72 (m, 3H, Ar H), 7.62-7.58 (m, 4H, Ar H), 7.47-7.42 (m, 2H, Ar H), 5.46 (s, 2H, OCH_2) ppm; MS *m*/*z* 285 $[M\text{-}HCl]^+$.

3-(3-Chlorobenzyloxy)-1-phenyl-1*H*-1,2,4-triazole hydrochloride (5g): This hydrochloride **5g** (98 mg) was obtained as white solid in 87% yield, mp 172 - 175 ºC; IR (KBr) ν 3136, 3067 (Ar-H), 2982, 2846 (CH_2), 1620, 1564, 1482, 1451, 1342 (aromatic frame), 1248, 1094 (C-O-C), 869, 841, 782, 758, 695 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.79 (s, 1H, triazole 5-H), 7.78-7.74 (m, 2H, Ar H), 7.64-7.60 (m, 3H, Ar H), 7.49-7.45 (m, 2H, Ar H), 7.39-7.36 (m, 2H, Ar H), 5.47 (s, 2H, OCH_2) ppm; MS *m*/*z* 285 $[M\text{-}HCl]^+$.

Synthesis of alkoxy 1,2,4-triazole hydrochlorides (6a-e). To a well-stirred suspension of alkoxy 1,2,4-triazoles **4a-e** (100 mg) in ethyl ether (10 mL) was added hydrochloride acid (10 mL, 4 mol/L). After the addition, the resulting mixture was stirred at room temperature for 5 - 6 h, and then the solvent was removed to afford the hydrochlorides **6a-e**.

2-(1-Phenyl-1*H*-1,2,4-triazol-3-yloxy)ethanol hydrochloride (6a): This hydrochloride **6a** (96 mg) was obtained as white solid in 81% yield, mp 198 - 201 ºC; IR (KBr) ν 3542 (O-H), 3169, 3098 (Ar-H), 2984, 2897 (CH_2), 1626, 1604, 1512, 1479, 1346 (aromatic frame), 1274, 1108 (C-O-C), 1045 (C-O), 899, 854, 752, 671 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.97 (s, 1H, triazole 5-H), 7.74-7.70 (m, 2H, Ph H), 7.59-7.55 (m, 2H, Ph H), 7.41-7.38 (m, 1H, Ph H), 4.63-4.60 (m, 2H, OCH_2CH_2OH), 4.13-4.09 (m, 2H, OCH_2CH_2OH) ppm; MS *m*/*z* 206 $[M\text{-}HCl]^+$.

3-Butoxy-1-phenyl-1*H*-1,2,4-triazole hydrochloride (6b): This hydrochloride **6b** (97 mg) was obtained as colorless liquid in 83% yield; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.94 (s, 1H, triazole 5-H), 7.73 (m, 2H, Ph-H), 7.49-7.45 (m, 2H, Ph H), 7.40-7.36 (m, 1H, Ph H), 4.45-4.42 (m, 2H, OCH_2), 1.64-1.61 (m, 2H, OCH_2CH_2), 1.47-1.45 (m, 2H, CH_2CH_3), 1.01 (t, 3H, CH_3) ppm; MS *m*/*z* 217 $[M\text{-}HCl]^+$.

3-(Octyloxy)-1-phenyl-1*H*-1,2,4-triazole hydrochloride (6c): This hydrochloride **6c** (97 mg) was obtained as white solid in 85% yield; mp 101 - 103 ºC; IR (KBr) ν 3137, 3089 (Ar-H), 2991, 2895 (CH_2), 1623, 1594, 1484, 1436, 1346 (aromatic frame), 1250, 1047 (C-O-C), 889, 842, 784, 751, 682 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.91 (s, 1H, triazole 5-H), 7.92-7.88 (m, 2H, Ph H), 7.54-7.50 (m, 2H, Ph H), 7.42-7.39 (m, 1H, Ph H), 4.43-4.40 (m, 2H, OCH_2), 1.82-1.79 (m, 2H, OCH_2CH_2), 1.48-1.45 (m, 10H, $(CH_2)_5CH_3$), 0.86 (t, 3H, CH_3) ppm; MS *m*/*z* 270 $[M\text{-}HCl]^+$.

3-(Dodecyloxy)-1-phenyl-1*H*-1,2,4-triazole hydrochloride (6d): This hydrochloride **6d** (97 mg) was obtained as white solid in 87% yield; mp 135 - 137 ºC; IR (KBr) ν 3135, 3071 (Ar-H), 2984, 2895 (CH_2), 1625, 1598, 1476, 1449, 1346 (aromatic frame), 1251, 1085 (C-O-C), 893, 849, 782, 757, 671 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.95 (s, 1H, triazole 5-H), 7.71-7.68

(m, 2H, Ph H), 7.51-7.48 (m, 2H, Ph H), 7.42-7.39 (m, 1H, Ph H), 4.41-4.38 (m, 2H, OCH_2), 1.85-1.81 (m, 2H, OCH_2CH_2), 1.46-1.42 (m, 2H, $OCH_2CH_2CH_2$), 1.26-1.23 (m, 16H, $(CH_2)_8$-CH_3), 0.88 (t, 3H, CH_3) ppm; MS *m*/*z* 330 $[M\text{-}HCl]^+$.

3-(Hexadecyloxy)-1-phenyl-1*H*-1,2,4-triazole hydrochloride (6e): This hydrochloride **6e** (95 mg) was obtained as white solid in 87% yield, mp 181 - 183 ºC; IR (KBr) ν 3142, 3095 (Ar-H), 2979, 2887 (CH_2), 1632, 1611, 1495, 1451, 1336 (aromatic frame), 1248, 1078 (C-O-C), 897, 845, 799, 771, 686 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.89 (s, 1H, triazole 5-H), 7.73-7.69 (m, 2H, Ph H), 7.52-7.49 (m, 2H, Ph H), 7.40-7.37 (m, 1H, Ph H), 4.42-4.45 (m, 2H, OCH_2), 1.87-1.85 (m, 2H, OCH_2CH_2), 1.47-1.42 (m, 2H, $OCH_2CH_2CH_2$), 1.27-1.23 (m, 24H, $(CH_2)_{12}$-CH_3), 0.87 (t, 3H, CH_3) ppm; MS *m*/*z* 385 $[M\text{-}HCl]^+$.

Antibacterial and antifungal assays. The *in vitro* minimal inhibitory concentrations (MICs) of the target compounds were determined by broth microdilution assay method in 96-well microtest plates.[36] The tested microorganism strains were provided by the School of Pharmaceutical Sciences, Southwest University and the College of Pharmacy, Third Military Medical University. Fluconazole and Chloramphenicol obtained from their respective manufacturers served as controls.

All compounds were evaluated for their antibacterial activities against *S. aureus* (ATCC29213) and *B. subtilis* as Gram-positive, *E. coli* (ATCC25922), *P. aeruginosa*, *S. dysenteriae* and *E. typhosa* as Gram-negative bacteria, as well as their antifungal activity against *C. albicans* (ATCC76615) and *A. fumigatu*s, at the concentrations of the antimicrobial agents ranging from 0.5 μg/mL to 512 μg/mL and scored for MIC_{50} as the level of growth inhibition of the tested microorganisms. The minimum inhibitory concentration (MIC) values (in μM) were summarized in Table 1.

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References

1. (a) Dismukes, W. E. *Clin. Infect. Dis.* **2006**, *42*, 1289. (b) DeMarco, C. E.; Cushing, L. A.; Frempont-Manso, E. *Antimicrob. Agents Chemother.* **2007**, *51*, 3235. (c) Lorand, T.; Kocsis, B. *Mini. Rev. Med. Chem.* **2007**, *7*, 900.
2. Yu, G.-P.; Xu, L.-Z.; Yi, X.; Bi, W.-Z.; Zhu, Q.; Zhai, Z.-W. *J. Agric. Food Chem.* **2009**, *57*, 4854.
3. Ming, Z.-H.; Xu, S.-Z.; Zhou, L.; Ding, M.-W.; Yang, J.-Y.; Yang, S.; Xiao, W.-J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3938.
4. Chen, J.; Sun, X.-Y.; Chai, K.-Y.; Lee, J.-S.; Song, M.-S.; Quana, Z.-S. *Bioorg. Med. Chem.* **2007**, *15*, 6775.
5. Küçükgüzel, İ.; Tatar, E.; Küçükgüzel, Ş. G.; Rollas, S.; De Clercq, E. *Eur. J. Med. Chem.* **2008**, *43*, 381.
6. Micheli, F.; Bonanomi, G.; Blaney, F. E.; Braggio, S.; Capelli, A. M.; Checchia, A.; Curcuruto, O.; Damiani, F.; Fabio, R. D.; Donati, D.; Gentile, G.; Gribble, A.; Hamprecht, D.; Ted esco, G.; Terreni, S.; Tarsi, L.; Lightfoot, A.; Stemp, G.; MacDonald, G.; Smith, A.; Pecoraro, M.; Petrone, M.; Perini, O.; Jacqui, P.; Rossi, T.; Worby, A.; Pilla, M.; Valerio, E.; Griffante, C.; Mugnaini, M.; Wood, M.; Scott, C.; Andreoli, M.; Lacroix, L.; Schwarz, A.; Gozzi, A.; Bifone, A.; Ashby, C. R., Jr.; Hagan, J. J.; Heidbreder, C. *J. Med. Chem.* **2007**, *50*, 5076.
7. Xiang, J.; Wan, Z.-K.; Li, H.-Q.; Ipek, M.; Binnun, E.; Nunez, J.; Chen, L.; McKew, J. C.; Mansour, T. S.; Xu, X.; Suri, V.; Tam, M.; Xing, Y.; Li, X.-P.; Hahm, S.; Tobin, Js.; Saiah, E. *J. Med. Chem.* **2008**, *51*, 4068.
8. Ahmad, S.; Madsen, C. S.; Stein, P. D.; Janovitz, E.; Huang, C.; Ngu, K.; Bisaha, S.; Kennedy, L. J.; Chen, B.-C.; Zhao, R.-L.; Sitkoff, D.; Monshizadegan, H. *J. Med. Chem.* **2008**, *51*, 2722.
9. Garfunkle, J.; Ezzili, C.; Rayl, T. J.; Hochstatter, D.G.; Hwang, I.; Boger, D. L. *J. Med. Chem.* **2008**, *51*, 937.
10. De Pauw, B. E. *Int. J. Antimicrob. Agents* **2000**, *16*, 147.
11. Johnsonl, L. B.; Kauffman, C. A. *Clin. Infect. Dis.* **2003**, *36*, 630.
12. (a) Wan, K.; Zhou, C.-H. *Chin. Pharm. J.* **2010**, (in press). (b) Cha, R.; Sobel, J. D. *Expert. Rev. Anti-Infect. Ther.* **2004**, *2*, 357.
13. (a) Liu, P.; Zhu, S.-L.; Li, P.; Xie, W.-J.; Jin, Y.-S.; Sun, Q.-Y.; Wu, Q.-Y.; Sun, P.; Zhang, Y.-J.; Yang, X.-H.; Jiang, Y.-Y.; Zhang, D.-Z. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3261. (b) Meerpoel, L.; Backx, L. J. J.; Van der Veken, L. J. E. ; Heeres, J. ; Corens, D.; De Groot, A.; Odds, F. C.; Van Gerven, F.; Woestenborghs, F. A. A.; Van Breda, A.; Oris, M. ; van Dorsselaer, P.; Willemsens, G. H. M.; Vermuyten, K. J. P.; Marichal, P. J. M. G.; Vanden Bossche, H. F.; Ausma, J.; Borgers, M. *J. Med. Chem.* **2005**, *48*, 2184. (c) La Regina, G.; D'Auria, F. D.; Tafi, A.; Piscitelli, F.; Olla, S.; Caporuscio, F.; Nencioni, L.; Cirilli, R.; La Torre, F.; De Melo, N. R.; Kelly, S. L.; Lamb, D. C.; Artico, M.; Botta, M.; Palamara, A. T.; Silvestri, R. *J. Med. Chem.* **2008**, *51*, 3841.
14. (a) Zhou, C.-H.; Gan, L.-L.; Zhang, Y.-Y.; Zhang, F.-F.; Wang, G.-Z.; Jin, L.; Geng, R.-X. *Sci. China. Ser. B-Chem.* **2009**, *52*, 415. (b) Zhou, C.-H.; Zhang, F.-F.; Gan, L.-L.; Zhang, Y.-Y.; Geng, R.-X. *Sci. China Ser. B-Chem.* **2009**, *39*, 208 (in Chinese).
15. Luo, Y.; Lu, Y.-H.; Gan, L.-L.; Zhou, C.-H.; Wu, J.; Geng, R.-X.; Zhang, Y.-Y. *Arch. Pharm. Chem. Life Sci.* **2009**, *342*, 386.
16. Zhang, F.-F.; Gan, L.-L.; Zhou, C.-H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1881.
17. Mi, J.-L.; Zhou, C.-H.; Bai, X. *Chin. J. Antibiot.* **2007**, *32*, 587.
18. Mi, J.-L.; Wu, J.; Zhou, C.-H. *West China J. Parm. Sci.* **2008**, *23*, 84.
19. Mavrova, A. T.; Wesselinova, D.; Tsenov, Y. A.; Denkova, P. *Eur. J. Med. Chem.* **2009**, *44*, 63.
20. Salgın-Gökşen, U.; Gökhan-Kelekçi, N.; Özgür, G.; Köysal, Y.; Kılıç, E.; Işık, Ş.; MeralÖzalp, G. A. *Bioorg. Med. Chem.* **2007**, *15*, 5738.
21. Bayrak, H.; Demirbas, A.; Karaoglu, S. A.; Demirbas, N. *Eur. J. Med. Chem.* **2009**, *44*, 1057.
22. Sato, T.; Ashizawa, N.; Iwanaga, T.; Nakamura, H.; Matsumoto, K.; Inoue, T.; Nagata, O. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 184.
23. Navidpour, L.; Shafaroodi, H.; Abdi, K.; Amini, M.; Ghahremani, M. H.; Dehpour, A. R.; Shafiee, A. *Bioorg. Med. Chem.* **2006**, *14*, 2507.
24. Kuş, C.; Ayhan-Kılcıgil, G.; Özbey, S.; Kaynak, F. B.; Kaya, M.; Can-Eke, T. Ć. B. *Bioorg. Med. Chem.* **2008**, *16*, 4294.
25. Turan-Zitouni, G.; Kaplancıklı, Z.; TahaYıldız, M.; Chevallet, P.; Kaya, D. *Eur. J. Med. Chem.* **2005**, *40*, 607.
26. Khanmohammadi, H.; Abnosi, M. H.; Hosseinzadeha, A.; Erfantalab, M. *Spectrochimica Acta Part A* **2008**, *71*, 1474.
27. Lin, R.-H.; Connolly, P. J.; Huang, S.; Wetter, S. K.; Lu,Y.-H.; Murray, W. V.; Emanuel, S. L.; Gruninger, R. H.; Fuentes-Pesquera, A. R.; Rugg, C. A.; Middleton, S. A.; Jolliffe, L. K. *J. Med. Chem.* **2005**, *48*, 4208.
28. Karegoudar, P.; Prasad, D. J.; Ashok, M.; Mahalinga, M.; Poojary, B.; Holla, B. S. *Eur. J. Med. Chem.* **2008**, *43*, 808.
29. Karthikeyan, M. S.; Holla, B. S.; Kumari, N. S. *Eur. J. Med. Chem.* **2008**, *43*, 309.
30. Kagoshima, Y.; Konosu, T. *J. Fluorine Chem.* **2006**, *127*, 643.
31. (a) Suzuki, T.; Moriya, M.; Sakamoto, T.; Suga, T.; Kishino, H.; Takahashi, H.; Ishikawa, M.; Nagai, K.; Imai, Y.; Sekino, E.; Ito, M.; Iwaas, H.; Ishihara, A.; Tokita, S.; Kanatani, A.; Sato, N.; Fukami, T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3072. (b) Jadhav, G. R.; Shaikh, M. U.; Kale, R. P.; Shiradkar, M. R.; Gill, C. H. *Eur. J. Med. Chem.* **2009**, *44*, 2930.

32. Chai, X.-Y.; Zhang, J.; Song, Y.-L.; Hu, H.-G.; Zou, Y.; Zhao, Q. -J.; Dan, Z.-G.; Zhang, D.-Z.; Wu, Q.-Y. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1811.
33. Sheng, C.-Q.; Zhang, W.-N.; Ji, H.-T.; Zhang, M.; Song, Y.-L.; Xu, H.; Zhu, J.; Miao, Z.-Y.; Jiang, Q.-F.; Yao, J.-Z.; Zhou, Y.-J.; Zhu, J.; Lü, J.-G. *J. Med. Chem.* **2006**, *49*, 2512.
34. Rezaei, Z.; Khabnadideh, S.; Pakshir, K.; Hossaini, Z.; Amiri, F.; Assadpour, E. *Eur. J. Med. Chem.* **2009**, *44*, 3064.
35. Koeh, M.; Pstein, E. P.; Stahler, G.; Main, F. US Patent 4467098, 1984.
36. (a) Kadi, A. A.; El-Brollosy, N. R.; Al-Deeb, O. A.; Habib, E. E.; Ibrahim, T. M.; El-Emam, A. A. *Eur. J. Med. Chem.* **2007**, *42*, 235. (b) Özbek, N.; Katırcıoğlu, H.; Karacan, N.; Baykal, T. *Bioorg. Med. Chem*. **2007**, *15*, 5105.