European Journal of Medicinal Chemistry 45 (2010) 4388–4398

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis and biological activities of novel amine-derived bis-azoles as potential antibacterial and antifungal agents

Bo Fang [a], Cheng-He Zhou [a,*], Xian-Cai Rao [b]

[a] Laboratory of Bioorganic & Medicinal Chemistry, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, People's Republic of China
[b] Department of Microbiology, College of Medicine, Third Military Medical University, Chongqing 400038, People's Republic of China

ARTICLE INFO

Article history:
Received 31 March 2010
Received in revised form
4 June 2010
Accepted 5 June 2010
Available online 12 June 2010

Keywords:
Antibacterial
Antifungal
Triazole
Imidazole
Benzimidazole

ABSTRACT

A series of novel amine-derived bis-azole compounds were designed by the systematical structural modification of Fluconazole and synthesized by a convenient and efficient method, and the antimicrobial activities for all prepared compounds were evaluated *in vitro* against six representative bacterial strains and two fungal strains. Bioactive results indicated that some synthesized compounds exhibited moderate or even better activities in comparison with the reference drugs. Especially, bis-imidazole **5b** and its salts gave significant antibacterial efficacy against all tested bacteria strains including MRSA, while bis-triazoles **4b–c** and their corresponding salts exhibited better activities against *Candida albicans*, *Bacillus proteus* than standard drugs Fluconazole and Norfloxacin respectively. Unexpectedly, bis-bromides **3a–f** presented excellent activities against all tested microbial strains.

1. Introduction

Infectious diseases have been serious and growing threatens to human health during the past few decades [1,2]. The decrease of sensibility to antimicrobial agents in current use has also been increasing for a great variety of pathogens and the resistance to multiple drugs is more and more prevalent for several microorganisms, especially for Gram-positive bacteria [3] and some intractable fungi [4]. Furthermore, infections by methicillin-resistant *Staphylococcus aureus* (MRSA) and *Aspergillus* presented difficult problems for medicine [5,6]. Therefore the urgent need for discovery or optimization of antimicrobial agents active against these resistant strains is of paramount importance [7,8].

Azole derivatives including triazole, imidazole and benzimidazole ones etc. are an important class of aromatic heterocyclic compounds with broad spectrum of biological activities such as antimicrobial [9–11], anticancer [12], anti-inflammatory [13], antivirus [14], anticonvulsant [15]. Particularly, the investigations of azole-based antimicrobial agents have attracted considerable attention in recent years. Numerous efforts have been directed toward the development of novel azole antimicrobial agents [16–19], and some excellent drugs such as Fluconazole and Miconazole have been successfully developed and widely used in clinic. Their inhibitory properties as regard representative fungi have been extensively exploited [20–22]. Especially, it is worthy to note that Fluconazole, the first-line triazole-antifungal drug recommended by World Health Organization (WHO), has established an exceptional therapeutic record for *Candida* infections, and become the first choice in the treatment of infections by *Candida albicans* and *Cryptococcus neoformans* due to its potent activity, excellent safety profile, and favorable pharmacokinetic characteristics [23]. However, Fluconazole is not effective against invasive aspergillosis and is not fungicidal. In addition, extensive clinical use of Fluconazole has resulted in the increasing Fluconazole-resistant *C. albicans* isolates [24]. Thus, many researches have been devoted to the further development of Fluconazole in order to broaden its antimicrobial spectrum and increase its therapeutic indexes. These works mainly included three aspects: (a) To optimize and modify the chemical structure of Fluconazole by introducing functional groups into hydroxyl or triazolyl moiety [25]. Fosfluconazole, an ester prodrug which was obtained by the esterification of hydroxyl group of Fluconazole with phosphoric acid, was one of the successful examples that has been marketed in 2003 [26]; (b) To expand the new potential applications of Fluconazole in supramolecular chemistry and develop the Fluconazole-based supramolecular drugs [27,28]; (c) To develop various analogues of Fluconazole [29,30]. A large number of researches have been focused on the analogues of Fluconazole in recent years, and some

* Corresponding author. Tel./fax: +86 23 68254967, +86 23 68254165.
E-mail address: zhouch@swu.edu.cn (C.-H. Zhou).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.06.012

outstanding achievements have been obtained in this aspect. Many azole derivatives, for instance, Voriconazole, Posaconazole and Ravuconazole were marketed or in the late stages of clinical trials. Moreover, it was also found that some azoles were effective inhibitors for enoyl acyl carrier protein reductase (FabI), a novel antibacterial target [31], while some azole compounds showed remarkable antibacterial efficacy against MRSA [32].

Prompted by these observations and in continuation of our researches for bioactive molecules [33,34], we modified the structure of Fluconazole from the following respects (Fig. 1):

(1) Tertiary amino moiety as bioisoster was selected to replace the tertiary alcohol of Fluconazole. Compared with tertiary alcohol, the tertiary amino group could not only be liable to form hydrogen bonds, but also accept protons or form quaternary salts which result in the increase of water solubility [35], or coordinate with metal ions [36], which probably lead to enhance affinity, selectivity and potency in biological properties.
(2) On the basis of above changed structural modification, various halobenzyl moieties were introduced into target compounds to substitute 2,4-diflurophenyl group of Fluconazole. Many studies showed that incorporation of halobenzyl moiety into organic molecules could greatly improve the pharmacological properties [37,38], which could enhance the rate of absorption and transport of drugs *in vivo* with increased lipid solubility [39]. Moreover, benzyl moiety with better flexibility compared to phenyl group, could possibly improve molecular biological properties.
(3) Various azolyl rings like triazolyl and its bioisosters imidazolyl as well as benzimidazolyl groups were introduced into the above modified structures to investigate the effect of azolyl rings on biological activities, since it was found that some bis-imidazole derivatives [40] and structurally simple alkyl azoles gave good antimicrobial activities [41].
(4) The methylene bridge between tertiary alcohol group and triazolyl moiety in Fluconazole was replaced by ethylene chain to increase molecular flexibility, which might be helpful to improve molecular binding ability to the microbial target [42].

Based on the above considerations, a series of novel halobenzyl amine-derived bis-azoles and their hydrochlorides and nitrates including bis-triazoles, bis-imidazoles and bis-benzimidazoles as the analogues of Fluconazole were designed and synthesized by a convenient and efficient method. Their antibacterial and antifungal activities were evaluated, and the structure–activity relationships were also investigated.

2. Chemistry

The synthetic route of target amine bis-azoles **4**–**6** and corresponding salts **7**–**12** was shown in Scheme 1. The intermediates **2a**–**f** could be efficiently prepared with high yields (95.8%–98.5%) by the N-alkylation of diethanolamine in acetonitrile with halobenzyl halides **1a**–**f**, and then diols **2a**–**f** were brominated in chloroform using phosphorus tribromide to afford the bromides **3a**–**f** in good yields at 20–60 °C. Compounds **3a**–**f** reacted with 1*H*-1,2,4-triazole or 2-methyl-5-nitro-1*H*-imidazole in the presence of weak base such as potassium carbonate in acetonitrile at 40–70 °C to give bis-triazoles (**4a**–**f**) and bis-nitroimidazole compound **5g**. However, under the same condition above, it was difficult to produce the corresponding bis-imidazoles (**5a**–**f**, **5h**, **5i**) and bis-benzimidazoles (**6a**–**c**), whereas the presence of strong base NaH in anhydrous THF could effectively result in the formation of target bis-azoles **5a**–**f**, **5h**, **5i** and **6a**–**c** in moderate to high yields at 40–70 °C. The hydrochlorides **7**–**9** or nitrates **10**–**12** were easily prepared by the reaction of compounds **4**–**6** with hydrochloric acid or nitric acid respectively.

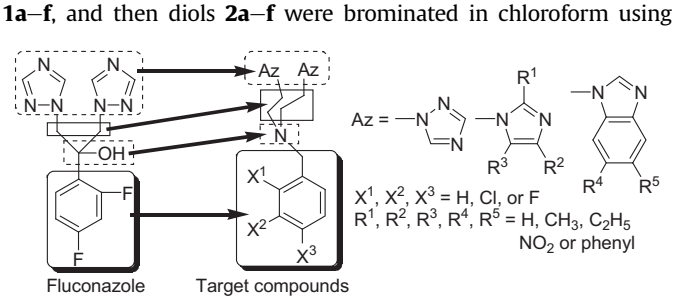


Fig. 1. Structures of Fluconazole and amine-derived bis-azoles.

3. Pharmacology

The *in vitro* antimicrobial screening for all the prepared compounds were evaluated against three Gram-positive organisms viz. *S. aureus*, MRSA, *Bacillus subtilis* and three Gram-negative organisms viz. *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus proteus* as well as fungi viz. *C. albicans*, *Aspergillus fumigatus* by the micro-broth dilution method in 96-well microtest plates according to the methods recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [43]. The minimum inhibitory concentrations (MIC) were determined and compared to Chloramphenicol, Norfloxacin and Fluconazole as standard drugs. All these antimicrobial data are given in Table 4 and 5.

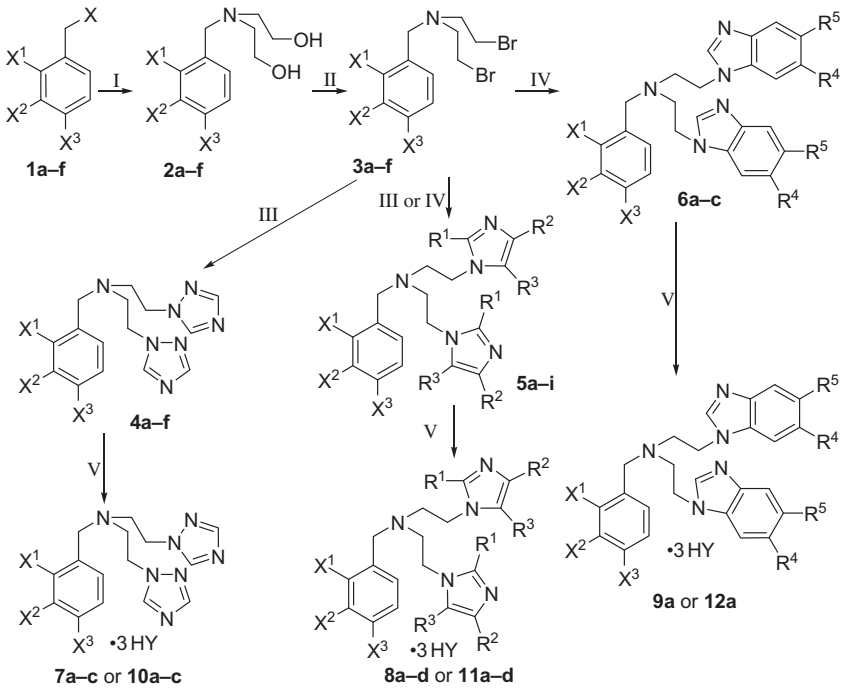
4. Results and discussion

4.1. Chemistry

The structures of the synthesized compounds (Table 1) were characterized by MS, IR and ^{1}H NMR spectra as well as elemental analyses. The analytical data were consistent with the assigned structures and listed in the experimental section. The MS spectra of all the compounds showed a $[M + H]^+$ or $[M + H - 3HY]^+$ peak, in agreement with their molecular formula.

In IR spectra, the intermediates **2a**–**f** gave broad absorption bands in 3382–3358 cm^{-1} region which indicated the presence of the OH group, while the disappearance of the characteristic hydroxy group stretching frequencies in bromides **3a**–**f** suggested that the hydroxy group underwent bromination. In addition, the aryl characteristic C–H bands of compounds **2a**–**f** and **3a**–**f** appeared in the region between 3078 and 3027 cm^{-1}. Target bis-azoles **4**–**12** can be recognized by the presence of specific absorption bands of azolyl moiety in 3126–3082 cm^{-1} region. All the other absorption bands were also observed at expected regions.

In ^{1}H NMR spectra, intermediates **2a**–**f** showed a singlet at δ 3.02–3.66 ppm due to the presence of OH protons, protons H^c and H^d gave signal as triplet at δ 2.54–2.68 ppm, 3.48–3.59 ppm respectively. Compared with the diols **2a**–**f**, bromides **3a**–**f** displayed upfield shifts for H^d (0.15–0.35 ppm), and downfield shifts for H^c (0.19–0.32 ppm) signal because OH moieties were substituted by Br groups (some ^{1}H NMR data were listed in Table 2). When the Br atoms of the compounds (**3a**–**f**) were replaced by azolyl groups, the resulting bis-azoles **4**–**6** showed significant downfield shifts for H^d and upfield shifts for H^a. The proton chemical shifts of imiazolyl groups in compounds **5a**–**f** were in the order H-2 > H-4 > H-5, and bis-imidazoles **5a**–**f** displayed higher field chemical shifts for H^d, H^e and H^f as compared to corresponding triazoles **4a**–**f**. Moreover, the presence of nitro group in compound **5g** resulted in large downfield chemical shifts of H^d and H^e, when compared with other bis-imidazoles. No large differences were found in H^a chemical shifts for the protons of phenyl ring in bis-azoles **4**–**6**.



Scheme 1. Synthetic route of intermediates **2**, **3** and amine-derived bis-azoles **4**–**12**. Reagents and reaction conditions: (I) $NH(CH_2CH_2OH)_2$, CH_3CN, 50 °C; (II) PBr_3, $CHCl_3$, 60 °C; (III) 1*H*-1,2,4-triazole or 2-methyl-5-nitro-1*H*-imidazole, K_2CO_3, CH_3CN, 60 °C; (IV) 1*H*-imidazole or substituted 1*H*-imidazole or 1*H*-benzimidazole or substituted 1*H*-benzimidazole, NaH, THF, 60 °C; (V) $Et_2O/CHCl_3$, 4 mol/L HCl or HNO_3.

In comparison with free bis-azoles **4**–**6**, their corresponding hydrochlorides **7**–**9** and nitrates **10**–**12** showed large downfield shifts for some protons (some ^{1}H NMR data were listed in Table 3). Especially, compounds **7**–**12** displayed remarkable downfield shifts for protons H^d (0.68–1.23 ppm) and H^e, H^f (0.48–2.56 ppm). These facts demonstrated that the positive charge in nitrogen atoms could obviously affect the chemical shifts of bis-azoles.

4.2. Antibacterial activity

The obtained results (Table 4) showed that most of dihalobenzyl amine bis-azoles and all bromides **3a**–**f** exhibited moderate to excellent efficacy against tested bacterial strains, especially all salts **7**–**12** gave better activities than their corresponding precursors **4**–**6**.

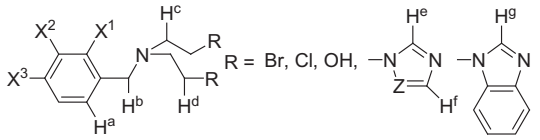
For the tested bis-triazoles **4a**–**f**, some compounds showed poor to moderate antibacterial activities, and dihalobenzyl bis-triazoles **4a**–**c** displayed better activities against the tested bacterial strains than the corresponding monochlorobenzyl compounds **4d**–**e**. Particularly, 3,4-dichlorobenzyl compound **4c** exhibited excellent activity against *B. proteus* (MIC = 0.5 μg/mL), which was 2-fold and 16-fold more potent than Norfloxacin and Chloramphenicol respectively. These results suggested that the presence of dichlorobenzyl moiety in bis-triazole compounds could improve effectively the antibacterial activity to some extent.

Compared to bis-triazoles **4a**–**f**, bis-imidazoles **5a**–**f** exhibited stronger efficacy against *S. aureus*, MRSA, *B. subtilis* and *E. coli*, and

Table 1
Structures of intermediates **1**–**3** and amine-derived bis-azoles **4**–**12**.

Compd.	X^1	X^2	X^3	X	R^1	R^2	R^3	Compd.	X^1	X^2	X^3	R^1	R^2	R^3	R^4	R^5	Y
1a–3a	F	H	F	Br	–	–	–	**5i**	Cl	H	Cl	C_6H_5	H	H	–	–	–
1b–3b	Cl	H	Cl	Cl	–	–	–	**6a**	F	H	F	–	–	–	H	H	–
1c–3c	H	Cl	Cl	Cl	–	–	–	**6b**	Cl	H	Cl	–	–	–	H	H	–
1d–3d	Cl	H	H	Cl	–	–	–	**6c**	Cl	H	Cl	–	–	–	CH_3	CH_3	–
1e–3e	H	Cl	H	Cl	–	–	–	**7a**	F	H	F	–	–	–	–	–	Cl
1f–3f	H	H	Cl	Cl	–	–	–	**7b**	Cl	H	Cl	–	–	–	–	–	Cl
4a	F	H	F	–	–	–	–	**7c**	H	Cl	Cl	–	–	–	–	–	Cl
4b	Cl	H	Cl	–	–	–	–	**8a**	F	H	F	H	H	H	–	–	Cl
4c	H	Cl	Cl	–	–	–	–	**8b**	Cl	H	Cl	H	H	H	–	–	Cl
4d	Cl	H	H	–	–	–	–	**8c**	H	Cl	Cl	H	H	H	–	–	Cl
4e	H	Cl	H	–	–	–	–	**8d**	Cl	H	Cl	C_6H_5	H	H	–	–	Cl
4f	H	H	Cl	–	–	–	–	**9a**	F	H	F	H	H	–	H	H	Cl
5a	F	H	F	–	H	H	H	**10a**	F	H	F	–	–	–	–	–	NO_3
5b	Cl	H	Cl	–	H	H	H	**10b**	Cl	H	Cl	–	–	–	–	–	NO_3
5c	H	Cl	Cl	–	H	H	H	**10c**	H	Cl	Cl	–	–	–	–	–	NO_3
5d	Cl	H	H	–	H	H	H	**11a**	F	H	F	H	H	H	–	–	NO_3
5e	H	Cl	H	–	H	H	H	**11b**	Cl	H	Cl	H	H	H	–	–	NO_3
5f	H	H	Cl	–	H	H	H	**11c**	H	Cl	Cl	H	H	H	–	–	NO_3
5g	Cl	H	Cl	–	CH_3	NO_2	H	**11d**	Cl	H	Cl	C_6H_5	H	H	–	–	NO_3
5h	Cl	H	Cl	–	C_2H_5	CH_3	H	**12a**	F	H	F	H	H	–	H	H	NO_3

Table 2
Some ^{1}H NMR data (δ ppm) of intermediates **2d**–**f**, **3d**–**f** and corresponding amine-derived bis-azoles **4**–**6**.



Compd.	2d	2e	2f	3d	3e	3f	4d	4e	4f	5d	5e	5f	5g	6b	6c
H^a	7.15	7.20	7.31	7.19	7.14	7.17	6.89	6.83	6.89	7.09	6.96	7.06	6.95	6.70	6.72
H^b	3.71	3.61	3.79	3.79	3.60	3.59	3.66	3.56	3.54	3.77	3.58	3.61	3.71	3.67	3.65
H^c	2.63	2.62	2.66	2.95	2.86	2.85	2.92	2.94	2.93	2.80	2.76	2.78	2.83	2.83	2.82
H^d	3.52	3.56	3.58	3.30	3.25	3.24	3.96	4.03	4.03	3.82	3.82	3.80	4.20	3.90	3.87
H^e	–	–	–	–	–	–	7.75	7.82	7.81	7.34	7.34	7.38	–	–	–
H^f	–	–	–	–	–	–	7.79	7.89	7.88	7.01	7.02	7.01	7.86	–	–
H^g	–	–	–	–	–	–	–	–	–	–	–	–	–	7.73	7.45

especially dihalobenzyl compounds **5a**–**c** presented remarkable antibacterial activities against all the tested strains. It seemed that imidazolyl ring be favorable for antibacterial efficacy in comparison with triazolyl moiety. Among compounds **5a**–**c**, 2,4-dichlorobenzyl bis-imidazole **5b** was the most active one which showed noticeable and broad spectrum antibacterial activities with MIC values in the range of 4–32 μg/mL. Compound **5b** exhibited excellent activity against *P. aeruginosa* with the MIC value of 4 μg/mL, which was 16-fold more potent than the reference drug Chloramphenicol. It also revealed equivalent activities against *S. aureus* and MRSA with the MIC values of 8 and 16 μg/mL, respectively. Interestingly, the three substituted-imidazoles **5g**–**5i**, whose activities were inferior to bis-imidazole **5b**, showed that the introduction of electron-donor or electron-withdrawing substituent into imidazolyl ring seemed to be unfavorable for their antibacterial efficacy. However, bis-2-phenylimidazoles **5i** displayed better activities against all tested strains than compounds **5g** and **5h** with 2-methyl-4-nitro-imidazolyl and 2-ethyl-4-methylimidazolyl group respectively, this might be attributed to the large lipophilic phenyl groups at 2-position of imidazolyl ring.

Bis-benzimidazoles **6a**–**c** showed comparable antibacterial efficiency with triazole compounds **4a**–**f**, but weaker activities than imidazole derivatives **5a**–**i**. Particularly, compound **6b** gave relatively good activities against all tested bacterial strains with the MIC values ranging from 16 to 128 μg/mL.

In addition, some azole compounds with good activities were chosen to transform into water-soluble hydrochlorides **7**–**9** and nitrates **10**–**12**. Such modification resulted in remarkable improvement in their antibacterial efficacy. Among these salts, compound **8d** showed best antibacterial activities (MIC values ranging from 2 to 8 μg/mL) against all tested bacteria strains except *B. proteus*, which displayed much better efficacy than standard drug Chloramphenicol. While the strain *B. proteus* were much sensitive to hydrochloride **7c** and nitrate **10c** with the same MIC value of 0.25 μg/mL, which were 2-fold more potent than that of their precursor **4c** and even 4-fold and 32-fold more potent than standard drugs Norfloxacin and Chloramphenicol respectively. Moreover, hydrochlorides **8b**–**d** and nitrate **11d** exhibited stronger activities against MRSA (MIC = 8 μg/mL) than Chloramphenicol. The enhanced activities for all these bis-azole hydrochlorides and nitrates salts **7**–**12** might be attributed to the improvement of the water solubility in comparison with their corresponding precursors.

In order to further investigate different groups in titled tertiary amines on the antimicrobial activities, intermediate diols **2a**–**f** and dibromides **3a**–**f**, which may be considered as the bioisosteres of chlorine in nitrogen mustards, were also evaluated for their antimicrobial activities, since much works have been found that nitrogen mustards as clinical anticancer drugs [44] in extensive use have significant antimicrobial efficacy [45,46]. To our surprise, amine-derived dibromides **3a**–**f** exhibited moderate to excellent antibacterial activities against all tested strains. Especially, compound **3b** with 2,4-dichlorobenzyl group displayed prominent activities towards the tested bacterial strains with MIC values between 0.25 μg/mL and 2 μg/mL, which was equivalent or even much better in comparison with the standard drug Norfloxacin. However, the bis-hydroxy compounds **2a**–**f** as the precursors of bromides **3a**–**f**, displayed poor or no obvious activities against the tested strains.

In general, the target compounds **4c**, **5a**–**c**, hydrochlorides **7c**, **8a**–**d**, nitrates **10c**, **11a**–**d** as well as all bromides **3a**–**f** showed moderate or even excellent antibacterial activities in comparison with the reference drugs (Norfloxacin and Chloramphenicol) against tested bacteria strains including MRSA. Meanwhile, the above discussion demonstrated that the antibacterial efficacy should be related to azolyl ring, halobenzyl group as well as water solubility of prepared compounds to some extent. For these serial compounds, the dihalobenzyl and imidazolyl moieties in compounds **2**–**6** seemed to be two favorable factors for antibacterial activities, and the salts **7**–**12** gave better inhibitory activities

Table 3
Some ^{1}H NMR data (δ ppm) of amine-derived bis-azoles **4**–**6** and corresponding salts **7**–**12**.

Compd.	4a	4b	5a	5b	6a	7a	7b	8a	8b	9a	10a	10b	11a	11b	12a
H^a	6.91	6.90	6.94	6.85	6.52	7.14	7.37	7.00	7.11	6.57	7.17	7.14	7.05	7.09	6.93
H^b	3.58	3.61	3.60	3.65	3.32	4.31	4.09	3.92	3.92	3.85	4.39	3.91	3.95	3.90	3.74
H^c	2.92	2.80	2.71	2.74	2.52	3.46	3.29	3.10	3.12	3.17	3.42	3.18	3.14	3.09	3.10
H^d	4.02	3.84	3.79	3.80	3.59	4.75	4.63	4.51	4.54	4.82	4.92	4.52	4.54	4.52	4.66
H^e	7.78	8.10	7.26	7.29	–	8.47	8.58	9.30	9.36		8.54	8.78	9.31	9.25	–
H^f	7.84	8.10	6.94	6.96	–	9.26	9.45	7.76	7.73	–	9.37	9.59	7.78	7.71	–
H^g	–	–	–	–	7.47	–	–	–	–	10.03	–	–	–	–	9.56

Table 4
Antibacterial data as MIC (μg/mL) for intermediates **2**, **3** and azole derivatives **4**–**12**[a,b]

Compd.	*S. aureus*	MRSA	*B. subtilis*	*E. coli*	*P. aeruginosa*	*B. proteus*
2a	256	>256	256	128	256	256
2b	128	256	256	256	128	128
2c	256	>256	128	128	>256	128
2d	>256	>256	>256	>256	>256	256
2e	>256	>256	>256	>256	>256	>256
2f	>256	>256	>256	256	256	256
3a	1	2	0.5	1	4	4
3b	2	4	0.25	2	0.25	0.5
3c	0.5	4	4	2	1	2
3d	8	32	64	32	16	16
3e	16	32	128	64	64	32
3f	32	32	32	16	16	8
4a	128	>256	64	64	8	>256
4b	64	256	>256	64	32	16
4c	64	256	32	32	64	0.5
4d	>256	>256	>256	256	128	>256
4e	256	256	>256	>256	16	32
4f	>256	>256	>256	256	16	128
5a	32	32	32	64	128	64
5b	8	16	32	16	4	32
5c	16	32	16	16	64	64
5d	128	256	>256	16	256	>256
5e	64	>256	128	32	32	64
5f	64	64	64	32	64	128
5g	256	>256	32	16	32	32
5h	16	128	64	256	8	16
5i	16	16	32	64	64	16
6a	64	>256	64	128	>256	64
6b	32	64	32	128	32	32
6c	128	>256	64	16	128	32
7a	64	>256	32	64	8	256
7b	16	128	128	32	16	4
7c	32	32	16	32	32	0.25
8a	16	16	8	16	64	32
8b	4	8	8	16	4	32
8c	4	8	4	4	32	16
8d	2	8	4	2	2	4
9a	32	128	>256	>256	>256	64
10a	128	>256	32	128	16	>256
10b	32	256	128	64	16	8
10c	16	64	16	32	64	0.25
11a	32	32	16	32	64	64
11b	16	16	16	32	4	64
11c	16	16	16	4	64	32
11d	4	8	8	4	4	16
12a	128	256	>256	>256	>256	128
Norfloxacin	0.25	0.25	1	0.5	2	1
Chloramphenicol	8	16	8	8	64	8

[a] Minimum inhibitory concentrations were determined by micro-broth dilution method.
[b] *S. aureus*, *Staphylococcus aureus* ATCC 29213; MRSA, *Staphylococcus aureus* N 315; *B. subtilis*, *Bacillus subtilis* ATCC 21216; *E. coli*, *Escherichia coli* ATCC 25922; *P. aeruginosa*, *Pseudomonas aeruginosa* ATCC 27853; *B. Proteus*, *Bacillus proteus* ATCC 13315.

than their precursors **4**–**6**. Further study is necessary to elucidate their mechanism and structure–activity relationships.

4.3. Antifungal activity

The antifungal evaluation showed that the activities for most bis-azoles **4**–**12** were relatively weak compared to their antibacterial activities (Table 5). However, some dihalobenzyl compounds, their corresponding hydrochlorides and nitrates as well as bromides **3a**–**f** exhibited significant antifungal activities against tested strains.

Some triazole compounds and their salts exhibited better activities against *C. albicans* than corresponding bis-imidazole and bis-benzimidazole derivatives, especially 2,4-dicholobenzyl bis-triazole **4b**, its hydrochloride **7b** and nitrate **10b** gave the best

Table 5
Antifungal data as MIC (μg/mL) for intermediates **2**, **3** and azole derivatives **4**–**12**.[a]

Compd.	*C. albicans*	*A. fumigatus*
2a	256	>256
2b	128	256
2c	>256	>256
2d	>256	>256
2e	>256	>256
2f	>256	>256
3a	0.5	64
3b	0.25	64
3c	2	128
3d	32	128
3e	8	64
3f	16	128
4a	>256	>256
4b	0.25	>256
4c	>256	>256
4d	>256	>256
4e	>256	>256
4f	>256	>256
5a	>256	>256
5b	128	>256
5c	>256	64
5d	>256	>256
5e	>256	>256
5f	>256	>256
5g	>256	>256
5h	32	>256
5i	>256	>256
6a	>256	>256
6b	>256	>256
6c	>256	>256
7a	32	256
7b	0.25	128
7c	128	256
8a	128	>256
8b	64	>256
8c	>256	32
8d	64	128
9a	>256	>256
10a	64	>256
10b	0.25	128
10c	256	>256
11a	128	>256
11b	128	>256
11c	>256	64
11d	64	256
12a	>256	>256
Fluconazole	0.5	256

[a] *C. albicans*, *Candida albicans* ATCC 76615; *A. fumigatus*, *Aspergillus fumigatus* ATCC 96918.

antifungal activities (MIC = 0.25 μg/mL), which were two-fold more potent than clinical antifungal drug Fluconazole (MIC = 0.5 μg/mL).

Bis-imidazole compounds **5h**, **8b**, **8d** and **11d** presented better antifungal activities against *C. albicans* than other imidazoles and benzimidazoles with the MIC values ranging from 32 to 64 μg/mL. Unfortunately, most compounds showed poor or no obvious activity (MIC ≥ 256 μg/mL) against *A. fumigatus*, with the exception of 3,4-dicholobenzyl compounds **5c**, **8c**, **11c** and 2,4-dicholobenzyl salts **7b**, **8d** and **10b** (MIC = 32–128 μg/mL), which were found to exhibit better activity than Fluconazole (MIC = 256 μg/mL). In addition, dihalobenzyl compounds **4a**–**c** and **5a**–**c** have stronger antifungal efficiency than monohalobenzyl ones **4d**–**f** and **5d**–**f** respectively, similar to the antibacterial activities. These results indicated that triazolyl moiety seemed to be more helpful than imidazolyl or benzimidazolyl group for enhancing the antifungal activities against *C. albicans*, and their corresponding salts were favorable for improving their antifungal efficacy.

The antifungal activities showed that the intermediates bromides **3a**–**f** exhibited excellent antifungal activities against *C. albicans* and *A. fumigatus*, especially dihalobenzyl compounds **3a**

and **3b** gave the MIC value of 0.5, 0.25 μg/mL against *C. albicans* and 64, 64 μg/mL against *A. fumigatus* which were comparable or even superior to Fluconazole.

5. Conclusion

In conclusion, a series of novel halobenzyl amine-derived bis-azoles were designed by the systematical structural modification of Fluconazole and successfully synthesized by a convenient and efficient method. All new compounds were characterized by MS, IR, ^{1}H NMR and elemental analyses. The *in vitro* antimicrobial activities of these azole derivatives and their intermediates were determined against six bacterial strains and two fungal strains. The results showed that some azole derivatives exhibited significant antibacterial and antifungal activities, even comparable or superior to reference drugs (Fluconazole, Norfloxacin and Chloramphenicol). Imidazoles **5b**, **8a**–**d** and **11a**–**d** bearing dihalobenzyl groups presented better antibacterial activities than other compounds against all tested bacteria strains. However, bis-triazoles **4c**, **7c** and **10c** gave remarkable efficacy (MIC values of 0.25–0.5 μg/mL) against *B. proteus*. Moreover, bis-triazoles **4b**, **7b** and **10b** exhibited more potent antifungal activity than the clinically prevalent antifungal drug Fluconazole against *C. albicans* with MIC value of 0.25 μg/mL. Surprisingly, bromides **3a**–**f** also showed excellent antibacterial and antifungal activities. These antimicrobial results indicated that structural factors such as the type of azolyl rings, halobenzyl moieties and the water solubility of target compounds could significantly affect their antimicrobial activities. In these serial compounds, it was showed that dihalobenzyl groups are more helpful for increasing antibacterial and antifungal efficacy in comparison with the monohalobenzyl ones. For another, imidazolyl moiety seems to be favorable for antibacterial efficacy against tested bacteria, while 1,2,4-triazolyl group is conducive to antifungal activities. In addition, the water-soluble salts could further enhance the inhibitory activities of these compounds.

6. Experimental protocol

6.1. General methods

Melting points were determined on X-6 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Bio-Rad FTS-185 spectrometer by using KBr disks. ^{1}H NMR spectra were recorded using TMS as an internal reference standard either on a Bruker AV 300 or Varian-Mercury 400 spectrometer. Chemical shifts are reported in parts per million (δ). Coupling constants (J) are reported in hertz (Hz). The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. The following abbreviations were used to designate azolyl groups: Tri, triazolyl; Im, imidazolyl; Benim, benzoimidazolyl. The mass spectra were recorded on FINNIGAN TRACE GC–MS 2000 mass spectrometer. Elemental analyses were carried out on a Carlo Erba model EA 1106 elemental analyzer. TLC analyses were done using pre-coated silica gel plates. Column chromatographies were performed on silica gel (Merck; 70–230 mesh) column. Developed plates were visualized by UV light. All solvents were reagent grade and, if necessary, were purified and dried by standard methods.

6.1.1. General procedure for the preparation of compounds ***2a–f***

A mixture of diethanolamine (6.31 g, 0.06 mol) and compound **1** (**1a**–**f**) (0.05 mol) in acetonitrile was stirred and heated at 50 °C. After the reaction was completed (monitored by TLC, dichloromethane/light petroleum (1/100–1/20, V/V)), the mixture was cooled to room temperature and the solvent was removed under reduced pressure, and then the residue was basified with saturated aqueous sodium bicarbonate and extracted with chloroform. Subsequently, the organic extracts were collected, washed with brine, dried over anhydrous sodium sulfate and filtered. The filtrate was evaporated to afford the crude product, which was used in the next step without further purification.

*6.1.1.1. 2,2′-(2,4-Difluorobenzylazanediyl)diethanol (**2a**).* Compound **2a** was obtained as yellow oil (11.12 g). Yield: 96.3%; IR (KBr, cm^{-1}) ν: 3382, 2953, 2884, 1619, 1504, 1456, 1276, 1137, 961, 851; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.25–7.15 (m, 1H, Ph 6-*H*), 6.73–6.61 (m, 2H, Ph 3, 5-*H*), 3.59 (s, 2H, PhCH_2), 3.48 (t, J = 5.1 Hz, 4H, NCH$_2$CH_2), 3.21 (s, 2H, O*H*), 2.54 (t, J = 5.1 Hz, 4H, NCH_2); MS m/z: 232 [M + H]$^+$.

*6.1.1.2. 2,2′-(2,4-Dichlorobenzylazanediyl)diethanol (**2b**).* Compound **2b** was obtained as yellow oil (12.89 g). Yield: 97.6%; IR (KBr, cm^{-1}) ν: 3374, 2950, 1588, 1561, 1470, 1374, 1244, 1081, 867, 824; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.46–7.19 (m, 3H, Ar-*H*), 3.75 (s, 2H, PhCH_2), 3.59 (t, J = 5.1 Hz, 4H, NCH$_2$CH_2), 3.32 (s, 2H, O*H*), 2.68 (t, J = 5.1 Hz, 4H, NCH_2); MS m/z: 264 [M + H]$^+$.

*6.1.1.3. 2,2′-(3,4-Dichlorobenzylazanediyl)diethanol (**2c**).* Compound **2c** was obtained as yellow oil (12.65 g). Yield: 96.2%; IR (KBr, cm^{-1}) ν: 3358, 2927, 1593, 1562, 1471, 1288, 1204, 1130, 1031, 880, 822, 770; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.42 (s, 1H, Ph 2-*H*), 7.35 (d, J = 8.2 Hz, 1H, Ph 5-*H*), 7.16 (d, J = 8.2 Hz, 1H, Ph 6-*H*), 3.62 (s, 2H, PhCH_2), 3.59 (t, J = 5.0 Hz, 4H, NCH$_2$CH_2), 3.40 (s, 2H, O*H*), 2.65 (t, J = 5.0 Hz, 4H, NCH_2); MS m/z: 264 [M + H]$^+$.

*6.1.1.4. 2,2′-(2-chlorobenzylazanediyl)diethanol (**2d**).* Compound **2d** was obtained as yellow oil (11.11 g). Yield: 97.0%; IR (KBr, cm^{-1}) ν: 3382, 3066, 2927, 1594, 1572, 1472, 1275, 1203, 1144, 1035, 876, 753; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.37–7.34 (m, 1H, Ph 3-*H*), 7.28–7.25 (m, 1H, Ph 5-*H*), 7.20–7.09 (m, 2H, Ph 4,6-*H*), 3.71 (s, 2H, PhCH_2), 3.52 (t, J = 5.2 Hz, 4H, NCH$_2$CH_2), 3.02 (s, 2H, O*H*), 2.63 (t, J = 5.2 Hz, 4H, NCH_2); MS m/z: 230 [M + H]$^+$.

*6.1.1.5. 2,2′-(3-Chlorobenzylazanediyl)diethanol (**2e**).* Compound **2e** was obtained as yellow oil (11.28 g). Yield: 98.5%; IR (KBr, cm^{-1}) ν: 3368, 3072, 2927, 1596, 1568, 1285, 1203, 1135, 1032, 878, 762; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.48 (d, J = 7.9 Hz, 1H, Ph 4-*H*), 7.38–7.32 (m, 1H, Ph 5-*H*), 7.23–7.16 (m, 2H, Ph 2, 6-*H*), 3.61 (s, 2H, PhCH_2), 3.56 (t, J = 5.2 Hz, 4H, NCH$_2$CH_2), 3.18 (s, 2H, O*H*), 2.62 (t, J = 5.2 Hz, 4H, NCH_2); MS m/z: 230 [M + H]$^+$.

*6.1.1.6. 2,2′-(4-Chlorobenzylazanediyl)diethanol (**2f**).* Compound **2f** was obtained as yellow oil (10.97 g). Yield: 95.8%; IR (KBr, cm^{-1}) ν: 3359, 2949, 2882, 2827, 1615, 1597, 1490, 1275, 1143, 1087, 901, 876, 841, 808; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.36–7.26 (m, 4H, Ph 2,3,5, 6-*H*), 3.79 (s, 2H, PhCH_2), 3.66 (s, 2H, O*H*), 3.58 (t, J = 4.9 Hz, 4H, NCH$_2$CH_2), 2.66 (t, J = 4.9 Hz, 4H, NCH_2); MS m/z: 230 [M + H]$^+$.

6.1.2. General procedure for the preparation of compounds ***3a–f***

To a stirred solution of compound **2** (**2a**–**f**) (0.04 mol) in chloroform was added tribromophosphine (13.54 g, 0.05 mol) dropwise. The resulting mixture was stirred at room temperature for 2 h, and then heated at 60 °C. After the reaction was completed (monitored by TLC, methanol/chloroform (1/20–1/8, V/V)), the reaction was cooled to room temperature and treated with ice water. After basified with saturated aqueous sodium bicarbonate, the mixture was extracted with chloroform, and the combined organic extracts were washed with brine, dried over sodium sulfate and filtered. The filtrate was concentrated to give crude product, and the crude product was purified by column chromatography on silica gel eluting with dichloromethane/light petroleum (1/20–1/6,

V/V) to afford the pure target **3a**–**f**. Characterization data of **3a**–**f** are given below.

*6.1.2.1. 2-Bromo-N-(2-bromoethyl)-N-(2,4-difluorobenzyl)ethanamine (**3a**).* Compound **3a** was obtained as yellow oil (12.25 g). Yield: 85.8%; IR (KBr, cm^{-1}) ν: 3078, 2965, 2846, 1618, 1502, 1457, 1278, 1136,1094, 970, 851; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.32 (d, J = 7.1 Hz, 1H, Ph 6-*H*), 6.76–6.71 (m, 2H, Ph 3, 5-*H*), 3.64 (s, 2H, PhCH_2), 3.25 (t, J = 7.2 Hz, 4H, NCH$_2$CH_2), 2.85 (t, J = 7.2 Hz, 4H, NCH_2); MS m/z: 358 $[M + H]^+$.

*6.1.2.2. 2-Bromo-N-(2-bromoethyl)-N-(2,4-dichlorobenzyl)ethanamine (**3b**).* Compound **3b** was obtained as yellow oil (14.88 g). Yield: 95.6%; IR (KBr, cm^{-1}) ν: 2960, 2923, 2850, 1588, 1561, 1469, 1379, 1282, 1206, 1096, 1048, 865, 822; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.26–7.54 (m, 3H, Ar-*H*), 3.82 (s, 2H, PhCH_2), 3.44 (t, J = 7.1 Hz, 4H, NCH$_2$CH_2), 3.00 (t, J = 7.1 Hz, 4H, NCH_2); MS m/z: 390 $[M + H]^+$.

*6.1.2.3. 2-Bromo-N-(2-bromoethyl)-N-(3,4-dichlorobenzyl)ethanamine (**3c**).* Compound **3c** was obtained as yellow oil (14.36 g). Yield: 92.3%; IR (KBr, cm^{-1}) ν: 3058, 3012, 2963, 2926, 2830, 1593, 1562, 1469, 1282, 1205, 1093, 1030, 819; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.36 (s, 1H, Ph 2-*H*), 7.28 (d, J = 8.2 Hz, 1H, Ph 5-*H*), 7.10 (d, J = 8.2 Hz, 1H, Ph 6-*H*), 3.57 (s, 2H, PhCH_2), 3.24 (t, J = 7.1 Hz, 4H, NCH$_2$CH_2), 2.84 (t, J = 7.1 Hz, 4H, NCH_2); MS m/z: 390 $[M + H]^+$.

*6.1.2.4. 2-Bromo-N-(2-bromoethyl)-N-(2-chlorobenzyl)ethanamine (**3d**).* Compound **3d** was obtained as yellow oil (11.97 g). Yield: 84.3%; IR (KBr, cm^{-1}) ν: 3064, 3015, 2966, 2837, 1593, 1572, 1471, 1441, 1282, 1206,1092, 1037, 754; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.49 (d, J = 7.0 Hz, 1H, Ph 3-*H*), 7.42–7.34 (m, 1H, Ph 5-*H*), 7.22–7.17 (m, 2H, Ph 4,6-*H*), 3.79 (s, 2H, PhCH_2), 3.30 (t, J = 7.2 Hz, 4H, NCH$_2$CH_2), 2.95 (t, J = 7.2 Hz, 4H, NCH_2); MS m/z: 356 $[M + H]^+$.

*6.1.2.5. 2-Bromo-N-(2-bromoethyl)-N-(3-chlorobenzyl)ethanamine (**3e**).* Compound **3e** was obtained as yellow oil (10.31 g). Yield: 72.6%; IR (KBr, cm^{-1}) ν: 3060, 3014, 2964, 2830, 1597, 1575, 1474, 1430, 1282, 1204,1099, 1077, 971, 889, 780; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.17–7.11 (m, 4H, Ph 2,4,5, 6-*H*), 3.60 (s, 2H, PhCH_2), 3.25 (t, J = 7.2 Hz, 4H, NCH$_2$CH_2), 2.86 (t, J = 7.2 Hz, 4H, NCH_2); MS m/z: 356 $[M + H]^+$.

*6.1.2.6. 2-Bromo-N-(2-bromoethyl)-N-(4-chlorobenzyl)ethanamine (**3f**).* Compound **3f** was obtained as yellow oil (12.31 g). Yield: 86.7%; IR (KBr, cm^{-1}) ν: 3027, 2925, 2852, 1596, 1541, 1490, 1281, 1225,1091, 878, 833, 806; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.21–7.13 (m, 4H, Ph 2,3,5, 6-*H*), 3.59 (s, 2H, PhCH_2), 3.24 (t, J = 7.2 Hz, 4H, NCH$_2$CH_2), 2.85 (t, J = 7.2 Hz, 4H, NCH_2); MS m/z: 356 $[M + H]^+$.

*6.1.3. General procedure for the preparation of compounds **4a**–**f** and **5g***

To a stirred suspension of potassium carbonate (1.04 g, 7.5 mmol) in 10 mL of acetonitrile was added 1*H*-1,2,4-triazole (0.48 g, 6.9 mmol) or 2-methyl-5-nitro-1*H*-imidazole (0.88 g, 6.9 mmol). The mixture was stirred at 60 °C for 1 h. After cooled to room temperature, the compound **3** (**3a**–**f**) (3.0 mmol) was added and the mixture was stirred at 60 °C. The absence of compound **3** monitored by TLC (dichloromethane/light petroleum) (1/20–1/2, V/V) indicated that the reaction was completed. Subsequently, the mixture was cooled to room temperature, the solvent was removed under reduced pressure, and the residue was partitioned between chloroform and saturated aqueous sodium bicarbonate. The organic extracts were collected, washed with brine, dried over sodium sulfate and filtered. After the filtrate was concentrated, the crude product was purified by column chromatography on silica gel eluting with acetone/ethylacetate (1/8–1/1, V/V). Characterization data of **4a**–**f** and **5g** are given below.

*6.1.3.1. N-(2-(1H-1,2,4-Triazol-1-yl)ethyl)-N-(2,4-difluorobenzyl)-2-(1H-1,2,4-triazol-1-yl)ethanamine (**4a**).* Compound **4a** was obtained as a yellow solid (0.84 g). Yield: 83.8%, mp: 90–93 °C; IR (KBr, cm^{-1}) ν: 3118, 2955, 2841, 1619, 1506, 1431, 1275, 1140, 962, 855; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.84 (d, J = 3.6 Hz, 2H, Tri 3-*H*), 7.78 (d, J = 4.0 Hz, 2H, Tri 5-*H*), 6.93–6.88 (m, 1H, Ph 6-*H*), 6.77–6.72 (m, 2H, Ph 3, 5-*H*), 4.03 (t, J = 6.4 Hz, 4H, Tri-CH_2), 3.58 (s, 2H, PhCH_2), 2.93 (t, J = 6.4 Hz, 4H, Tri-CH$_2$CH_2); MS m/z: 334 $[M + H]^+$; Anal. Calcd. for $C_{15}H_{17}F_2N_7$: C, 54.05; H, 5.14; N, 29.41. Found: C, 54.17; H, 5.12; N, 29.68.

*6.1.3.2. N-(2-(1H-1,2,4-Triazol-1-yl)ethyl)-N-(2,4-dichlorobenzyl)-2-(1H-1,2,4-triazol-1-yl)ethanamine (**4b**).* Compound **4b** was obtained as colorless oil (0.90 g). Yield: 82.0%; IR (KBr, cm^{-1}) ν: 3123, 3106, 3067, 3056, 2968, 2829, 1508, 1473, 1450, 1383, 1201, 1141, 1046, 1007, 876, 816; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 8.10 (m, 4H, Tri 3-*H*, 5-*H*), 7.10–6.69 (m, 3H, Ar-*H*), 3.84 (t, J = 5.6 Hz, 4H, Tri-CH_2), 3.61 (s, 2H, PhCH_2), 2.80 (t, J = 5.6 Hz, 4H, Tri-CH$_2$CH_2); MS m/z: 366 $[M + H]^+$; Anal. Calcd. for $C_{15}H_{17}Cl_2N_7$: C, 49.19; H, 4.68; N, 26.77. Found: C, 49.32; H, 4.63; N, 26.59.

*6.1.3.3. N-(2-(1H-1,2,4-Triazol-1-yl)ethyl)-N-(3,4-dichlorobenzyl)-2-(1H-1,2,4-triazol-1-yl)ethanamine (**4c**).* Compound **4c** was obtained as colorless oil (0.83 g). Yield: 75.6%; IR (KBr, cm^{-1}) ν: 3118, 2953, 2832, 1508, 1470, 1275, 1139, 960, 875; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.86 (s, 2H, Tri 3-*H*), 7.80 (s, 2H, Tri 5-*H*), 7.23 (t, J = 4.8 Hz, 1H, Ph 5-*H*), 7.00 (d, J = 1.6 Hz, 1H, Ph 2-*H*), 6.72–6.70 (m, 1H, Ph 6-*H*), 4.03 (t, J = 6.0 Hz, 4H, Tri-CH_2), 3.48 (s, 2H, PhCH_2), 2.90 (t, J = 6.0 Hz, 4H, Tri-CH$_2$CH_2); MS m/z: 366 $[M + H]^+$; Anal. Calcd. for $C_{15}H_{17}Cl_2N_7$: C, 49.19; H, 4.68; N, 26.77. Found: C, 49.28; H, 4.60; N, 26.82.

*6.1.3.4. N-(2-(1H-1,2,4-Triazol-1-yl)ethyl)-N-(2-chlorobenzyl)-2-(1H-1,2,4-triazol-1-yl)ethanamine (**4d**).* Compound **4d** was obtained as a white solid (0.66 g). Yield: 66.3%, mp: 91–93 °C; IR (KBr, cm^{-1}) ν: 3121, 3110, 3058, 2951, 2881, 2826, 1594, 1507, 1465, 1136, 1045, 857, 779; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.79 (s, 2H, Tri 3-*H*), 7.75 (s, 2H, Tri 5-*H*), 7.26–7.23 (m, 1H, Ph 3-*H*), 7.13–7.04 (m, 2H, Ph 4, 5-*H*), 6.91–6.87 (m, 1H, Ph 6-*H*), 3.96 (t, J = 6.0 Hz, 4H, Tri-CH_2), 3.66 (s, 2H, PhCH_2), 2.92 (t, J = 6.0 Hz, 4H, Tri-CH$_2$CH_2); MS m/z: 332 $[M + H]^+$; Anal. Calcd. for $C_{15}H_{18}ClN_7$: C, 54.30; H, 5.47; N, 29.55. Found: C, 54.18; H, 5.41; N, 29.76.

*6.1.3.5. N-(2-(1H-1,2,4-Triazol-1-yl)ethyl)-N-(3-chlorobenzyl)-2-(1H-1,2,4-triazol-1-yl)ethanamine (**4e**).* Compound **4e** was obtained as a white solid (0.50 g). Yield: 50.2%, mp: 93–94 °C; IR (KBr, cm^{-1}) ν: 3117, 3059, 2957, 2882, 2816, 1598, 1575, 1509, 1466, 1382, 1207, 1141, 880, 768; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.89 (s, 2H, Tri 3-*H*), 7.82 (s, 2H, Tri 5-*H*), 7.20–7.13 (m, 2H, Ph 4, 5-*H*), 6.98 (d, J = 1.6 Hz, 1H, Ph 2-*H*), 6.84–6.82 (m, 1H, Ph 6-*H*), 4.03 (t, J = 6.0 Hz, 4H, Tri-CH_2), 3.56 (s, 2H, PhCH_2), 2.94 (t, J = 6.0 Hz, 4H, Tri-CH$_2$CH_2); MS m/z: 332 $[M + H]^+$; Anal. Calcd. for $C_{15}H_{18}ClN_7$: C, 54.30; H, 5.47; N, 29.55. Found: C, 54.15; H, 5.58; N, 29.84.

*6.1.3.6. N-(2-(1H-1,2,4-Triazol-1-yl)ethyl)-N-(4-chlorobenzyl)-2-(1H-1,2,4-triazol-1-yl)ethanamine (**4f**).* Compound **4f** was obtained as colorless oil (0.57 g). Yield: 57.2%; IR (KBr, cm^{-1}) ν: 3114, 2953, 2894, 2831, 1670, 1597, 1577, 1507, 1275, 1207, 1141, 846, 752; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.88 (s, 2H, Tri 3-*H*), 7.81 (s, 2H, Tri 5-*H*), 7.18 (d, J = 6.4 Hz, 2H, Ph 3, 5-*H*), 6.89 (d, J = 8.4 Hz, 2H, Ph 2,

6-*H*), 4.03 (t, J = 6.0 Hz, 4H, Tri-CH_2), 3.54 (s, 2H, PhCH_2), 2.93 (t, J = 6.0 Hz, 4H, Tri-CH$_2$CH_2); MS m/z: 332 $[M + H]^+$; Anal. Calcd. for $C_{15}H_{18}ClN_7$: C, 54.30; H, 5.47; N, 29.55. Found: C, 54.52; H, 5.38; N, 29.79.

*6.1.3.7. N-(2,4-Dichlorobenzyl)-2-(2-methyl-4-nitro-1H-imidazol-1-yl)-N-(2-(2-methyl-4-nitro-1H-imidazol-1-yl)ethyl)ethanamine (**5g**).* Compound **5g** was obtained as a white solid (1.22 g). Yield: 84.4%, mp: 189–191 °C; IR(KBr, cm^{-1}): 3126, 2815, 1587, 1536, 1496, 1386, 1183, 1112, 826, 750; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.86 (s, 2H, Im 4-*H*), 7.62–6.95 (m, 3H, Ph 3, 5, 6-*H*), 4.20 (t, J = 5.8 Hz, 4H, Im-CH_2), 3.71 (s, 2H, PhCH_2), 2.83 (t, J = 5.8 Hz, 4H, Im-CH$_2$CH_2), 2.31 (s, 6H, Im-CH_3); MS m/z: 482 $[M + H]^+$; Anal. Calcd. for $C_{19}H_{21}Cl_2N_7O_4$: C, 47.31; H, 4.39; N, 20.33. Found: C, 47.09; H, 4.46; N, 20.12.

*6.1.4. General procedure for the preparation of compounds **5**–**6***

To a stirred suspension of sodium hydride (0.18 g, 7.5 mmol) in 10 mL of tetrahydrofuran was added 1*H*-imidazole (0.47 g, 6.9 mmol) or 4-ethyl-2-methyl-1*H*-imidazole (0.76 g, 6.9 mmol) or 2-phenyl-1*H*-imidazole (0.99 g, 6.9 mmol) or 1*H*-benzoimidazole (0.82 g, 6.9 mmol) or 5,6-dimethyl-1*H*-benzoimidazole (1.00 g, 6.9 mmol), the mixture was heated at 60 °C for 1 h. Then the mixture was cooled to room temperature, the compound **3** (**3a**–**f**) (3.0 mmol) was added and the mixture was stirred at 60 °C. The reaction was monitored by TLC (dichloromethane/light petroleum (1/20–1/2, V/V)) when the absence of compound **3** indicated that the reaction was completed. Then the reaction was cooled to room temperature and quenched with ice water, the mixture was partitioned between chloroform and saturated aqueous sodium bicarbonate, the combined organic extracts were washed with brine, dried over sodium sulfate and filtered, the filtrate was concentrated. The crude product was purified by column chromatography on silica gel eluting with acetone/ethylacetate (1/8–1/2, V/V). Characterization data of **5**–**6** are given below.

*6.1.4.1. N-(2-(1H-Imidazol-1-yl)ethyl)-N-(2,4-difluorobenzyl)-2-(1H-imidazol-1-yl)ethanamine (**5a**).* Compound **5a** was obtained as colorless oil (0.86 g). Yield: 86.3%; IR (KBr, cm^{-1}) ν: 3112, 2947, 2834, 1618, 1505, 1452, 1280, 1137, 961, 850; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.26 (s, 2H, Im 2-*H*), 6.94 (d, J = 3.0 Hz, 3H, Im 4-*H*, Ph 6-*H*), 6.76–6.71 (m, 4H, Im 5-*H*, Ph 3, 5-*H*), 3.79 (t, J = 4.5 Hz, 4H, Im-CH_2), 3.60 (s, 2H, PhCH_2), 2.71 (t, J = 4.5 Hz, 4H, Im-CH$_2$CH_2); MS m/z: 332 $[M + H]^+$; Anal. Calcd. for $C_{17}H_{19}F_2N_5$: C, 61.62; H, 5.78; N, 21.13. Found: C, 61.81; H, 5.72; N, 21.34.

*6.1.4.2. N-(2-(1H-Imidazol-1-yl)ethyl)-N-(2,4-dichlorobenzyl)-2-(1H-imidazol-1-yl)ethanamine (**5b**).* Compound **5b** was obtained as colorless oil (0.93 g). Yield: 85.2%; IR (KBr, cm^{-1}) ν: 2924, 2853, 1644, 1590, 1562, 1505, 1453, 1287, 1079, 821, 736; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.29 (s, 2H, Im 2-*H*), 7.23 (s, 1H, Ph 3-*H*), 7.05 (d, J = 8.3 Hz, 1H, Ph 5-*H*), 6.96 (bs, 2H, Im 4-*H*), 6.85 (d, J = 8.3 Hz, 1H, Ph 6-*H*), 6.69 (bs, 2H, Im 5-*H*), 3.80 (t, J = 6.0 Hz, 4H, Im-CH_2), 3.65 (s, 2H, PhCH_2), 2.74 (t, J = 6.0 Hz, 4H, Im-CH$_2$CH_2); MS m/z: 364 $[M + H]^+$; Anal. Calcd. for $C_{17}H_{19}Cl_2N_5$: C, 56.05; H, 5.26; N, 19.23. Found: C, 56.23; H, 5.35; N, 19.37.

*6.1.4.3. N-(2-(1H-Imidazol-1-yl)ethyl)-N-(3,4-dichlorobenzyl)-2-(1H-imidazol-1-yl)ethanamine (**5c**).* Compound **5c** was obtained as a white solid (0.79 g). Yield: 72.3%, mp: 91–93 °C; IR (KBr, cm^{-1}) ν: 3093, 3050, 2965, 2930, 2859, 1594, 1505, 1486, 1265, 1212, 1139, 847, 752,720; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.25 (s, 2H, Im 2-*H*), 7.24 (d, J = 2.4 Hz, 1H, Ph 5-*H*), 7.10 (s, 1H, Ph 2-*H*), 6.96 (bs, 2H, Im 4-*H*), 6.80–6.78 (m, 1H, Ph 6-*H*), 6.68 (bs, 2H, Im 5-*H*), 3.80 (t, J = 6.0 Hz, 4H, Im-CH_2), 3.49 (s, 2H, PhCH_2), 2.70 (t, J = 6.0 Hz, 4H, Im-CH$_2$CH_2); MS m/z: 364 $[M + H]^+$; Anal. Calcd. for $C_{17}H_{19}Cl_2N_5$: C, 56.05; H, 5.26; N, 19.23. Found: C, 56.32; H, 5.19; N, 19.04.

*6.1.4.4. N-(2-(1H-Imidazol-1-yl)ethyl)-N-(2-chlorobenzyl)-2-(1H-imidazol-1-yl)ethanamine (**5d**).* Compound **5d** was obtained as colorless oil (0.62 g). Yield: 62.6%; IR (KBr, cm^{-1}) ν: 3112, 2986, 2951, 2899, 2825, 1594, 1571, 1550, 1506, 1377, 1229, 1079, 1035, 888, 820, 755; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.35–7.33 (m, 3H, Im 2-*H*, Ph 3-*H*), 7.34 (s, 2H, Im 2-*H*), 7.20–7.17 (m, 2H, Ph 4, 5-*H*), 7.10–7.07 (m, 1H, Ph 6-*H*), 7.01 (t, J = 1.2 Hz, 2H, Im 4-*H*), 6.76 (t, J = 1.2 Hz, 2H, Im 5-*H*), 3.82 (t, J = 6.0 Hz, 4H, Im-CH_2), 3.77 (s, 2H, PhCH_2), 2.80 (t, J = 6.0 Hz, 4H, Im-CH$_2$CH_2); MS m/z: 330 $[M + H]^+$; Anal. Calcd. for $C_{17}H_{20}ClN_5$: C, 61.91; H, 6.11; N, 21.23. Found: C, 61.78; H, 6.20; N, 21.08.

*6.1.4.5. N-(2-(1H-Imidazol-1-yl)ethyl)-N-(3-chlorobenzyl)-2-(1H-imidazol-1-yl)ethanamine (**5e**).* Compound **5e** was obtained as colorless oil (0.58 g). Yield: 58.2%; IR (KBr, cm^{-1}) ν: 3109, 2946, 2829, 1634, 1596 1509, 1473, 1374, 1231, 747; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.34 (s, 2H, Im 2-*H*), 7.20 (d, J = 2.4 Hz, 1H, Ph 4-*H*), 7.18 (d, J = 8.0 Hz, 1H, Ph 5-*H*), 7.11 (s, 1H, Ph 2-*H*), 7.02 (s, 2H, Im 4-*H*), 6.97–6.94 (m, 1H, Ph 6-*H*), 6.74 (t, J = 1.2 Hz, 2H, Im 5-*H*), 3.82 (t, J = 6.0 Hz, 4H, Im-CH_2), 3.58 (s, 2H, PhCH_2), 2.76 (t, J = 6.0 Hz, 4H, Im-CH$_2$CH_2); MS m/z: 330 $[M + H]^+$; Anal. Calcd. for $C_{17}H_{20}ClN_5$: C, 61.91; H, 6.11; N, 21.23. Found: C, 61.69; H, 6.06; N, 21.41.

*6.1.4.6. N-(2-(1H-Imidazol-1-yl)ethyl)-N-(4-chlorobenzyl)-2-(1H-imidazol-1-yl)ethanamine (**5f**).* Compound **5f** was obtained as colorless oil (0.62 g). Yield: 62.2%; IR (KBr, cm^{-1}) ν: 3113, 2937, 1597, 1508, 1493, 1285, 1233, 1108, 1081, 915, 808, 748; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.38 (s, 2H, Im 2-*H*), 7.25 (d, J = 2.4 Hz, 2H, Ph 3, 5-*H*), 7.06 (d, J = 2.4 Hz, 2H, Ph 2, 6-*H*), 7.01 (s, 2H, Im 4-*H*), 6.81 (t, J = 1.2 Hz, 2H, Im 5-*H*), 3.80 (t, J = 6.0 Hz, 4H, Im-CH_2), 3.61 (s, 2H, PhCH_2), 2.78 (t, J = 6.0 Hz, 4H, Im-CH$_2$CH_2); MS m/z: 330 $[M + H]^+$; Anal. Calcd. for $C_{17}H_{20}ClN_5$: C, 61.91; H, 6.11; N, 21.23. Found: C, 61.75; H, 6.17; N, 21.09.

*6.1.4.7. N-(2,4-Dichlorobenzyl)-2-(2-ethyl-4-methyl-1H-imidazol-1-yl)-N-(2-(2-ethyl-4-methyl-1H-imidazol-1-yl)ethyl)ethanamine (**5h**).* Compound **5h** was obtained as a white solid (1.14 g). Yield: 84.8%, mp: 142–144 °C; IR (KBr, cm^{-1}): 3132, 2975, 2510, 1644, 1588, 1503, 1382, 1101, 1047, 863, 823, 750; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.86–7.32 (m, 3H, Ph 3, 5, 6-*H*), 6.97 (s, 2H, Im 5-*H*), 3.83 (t, J = 6.0 Hz, 4H, Im-CH_2), 3.62 (s, 2H, PhCH_2), 2.84 (t, J = 6.0 Hz, 4H, Im-CH$_2$CH_2), 2.59 (q, J = 5.4 Hz, 4H, Im-CH_2CH$_3$), 2.27 (s, 6H, Im-CH_3), 1.53 (t, J = 5.4 Hz, 6H, Im-CH$_2$CH_3); MS m/z: 448 $[M + H]^+$; Anal. Calcd. for $C_{23}H_{31}Cl_2N_5$: C, 61.60; H, 6.97; N, 15.62. Found: C, 61.81; H, 6.88; N, 15.46.

*6.1.4.8. N-(2,4-Dichlorobenzyl)-2-(2-phenyl-1H-imidazol-1-yl)-N-(2-(2-phenyl-1H-imidazol-1-yl)ethyl)ethanamine (**5i**).* Compound **5i** was obtained as a white solid (1.35 g). Yield: 87.2%, mp: 146–145 °C; IR (KBr, cm^{-1}): 3106, 2958, 2804, 1587, 1363, 1136, 1115, 836, 776; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.37 (m, 10H, Ar-*H*), 7.22–6.64 (m, 7H, Ar-*H*), 3.83 (t, J = 5.6 Hz, 4H, Im-CH_2), 3.36 (s, 2H, PhCH_2), 2.52 (t, J = 5.6 Hz, 4H, Im-CH$_2$CH_2); MS m/z: 516 $[M + H]^+$; Anal. Calcd. for $C_{29}H_{27}Cl_2N_5$: C, 67.44; H, 5.27; N, 13.56. Found: C, 67.66; H, 5.35; N, 13.42.

*6.1.4.9. N-(2-(1H-Benzoimidazol-1-yl)ethyl)-2-(1H-benzoimidazol-1-yl)-N-(2,4-difluorobenzyl)ethanemine (**6a**).* Compound **6a** was obtained as a white solid (1.02 g). Yield: 78.9%, mp: 114–115 °C; IR (KBr, cm^{-1}) ν: 3100, 2941, 2854, 1619, 1501, 1459, 1285, 1139, 957, 846, 742; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.47 (d, J = 8.0 Hz, 2H, Benim 2-*H*), 7.26 (bs, 2H, Benim 4-*H*) 6.96–6.88 (m, 4H, Benim 6,7-*H*),

6.78–6.76 (m, 2H, Benim 5-*H*, Ph 3, 5-*H*), 6.54–6.49 (m, 1H, Ph 6-*H*), 6.40–6.34 (m, 1H, Ph 5-*H*), 6.26–6.22 (m, 1H, Ph 3-*H*), 3.59 (t, $J = 6.4$ Hz, 4H, Benim-CH_2), 3.32 (s, 2H, PhCH_2), 2.52 (t, $J = 6.0$ Hz, 4H, Benim-CH$_2$CH_2); MS *m*/*z*: 432 $[M + H]^+$; Anal. Calcd. for $C_{25}H_{23}F_2N_5$: C, 69.59; H, 5.37; N, 16.23. Found: C, 69.82; H, 5.45; N, 16.41.

*6.1.4.10. N-(2-(1H-Benzoimidazol-1-yl)ethyl)-2-(1H-benzoimidazol-1-yl)-N-(2,4-dichlorobenzyl)ethanemine (**6b**).* Compound **6b** was obtained as a white solid (1.25 g). Yield: 89.8%, mp: 144–146 °C; IR (KBr, cm^{-1}): 3082, 2939 2823, 1587, 1496, 1455, 1365, 1286, 1202, 864, 742; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.73 (s, 2H, Benim 2-*H*), 7.50–6.70 (m, 11H, Ar-*H*), 3.90 (t, $J = 7.2$ Hz, 4H, Benim-CH_2), 3.67 (s, 2H, PhCH_2), 2.83 (t, $J = 7.2$ Hz, 4H, Benim-CH$_2$CH_2); MS *m*/*z*: 464 $[M + H]^+$; Anal. Calcd. for $C_{25}H_{23}Cl_2N_5$: C, 64.66; H, 4.99; N, 15.08. Found: C, 64.91; H, 5.06; N, 15.29.

*6.1.4.11. N-(2,4-Dichlorobenzyl)-2-(5,6-dimethyl-1H-benzoimidazol-1-yl)-N-(2-(5,6-dimethyl-1H-benzoimidazol-1-yl)ethyl)ethanamine (**6c**).* Compound **6c** was obtained as a white solid (1.36 g). Yield: 87.2%, mp: 190–192 °C; IR (KBr, cm^{-1}): 3085, 2936, 2830, 1585, 1499, 1466, 1356, 1221, 1139, 845; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.45 (s, 2H, benzimi 2-*H*), 7.19–6.72 (m, 7H, Ar-*H*), 3.87 (t, $J = 6.8$ Hz, 4H, Benim-CH_2), 3.65 (s, 2H, PhCH_2), 2.82 (t, $J = 6.8$ Hz, 4H, Benim-CH$_2$CH_2), 2.28 (s, 12H, Ar-CH_3); MS *m*/*z*: 520 $[M + H]^+$; Anal. Calcd. for $C_{29}H_{31}Cl_2N_5$: C, 66.92; H, 6.00; N, 13.46. Found: C, 66.64; H, 6.08; N, 13.57.

*6.1.5. General procedure for the preparation of hydrochlorides **7**–**9***

Hydrochloric acid (4 mol/L) was added dropwise into a solution of compound **4a** (167 mg, 0.5 mmol), or **4b** (183 mg, 0.5 mmol), or **4c** (183 mg, 0.5 mmol), or **5a** (166 mg, 0.5 mmol), or **5b** (182 mg, 0.5 mmol), or **5c** (182 mg, 0.5 mmol), or **5i** (258 mg, 0.5 mmol), or **6a** (216 mg, 0.5 mmol) in ethyl ether/chloroform (4/1, V/V), stopped the addition when no more precipitate formed. After the addition, the formed precipitate was filtered, and washed with chloroform and then with light petroleum ether and dried by freeze drying oven to afford hydrochlorides **7**–**9**.

*6.1.5.1. N-(2-(1H-1,2,4-Triazol-1-yl)ethyl)-N-(2,4-difluorobenzyl)-2-(1H-1,2,4-triazol-1-yl)ethanamine hydrochloride (**7a**).* Compound **7a** was obtained as a white solid (0.16 g). Yield: 72.2%, mp: 126–128 °C; IR (KBr, cm^{-1}) ν: 3068, 3021, 2941, 1637, 1572, 1520, 1377, 1111, 886, 825, 721; ^{1}H NMR (400 MHz, DMSO, ppm): δ 9.26 (s, 2H, Tri 3-*H*), 8.47 (s, 2H, Tri 5-*H*), 7.67 (d, $J = 6.0$ Hz, 1H, Ph 3-*H*), 7.34 (t, $J = 8.4$ Hz, 1H, Ph 5-*H*), 7.14 (t, $J = 8.4$ Hz, 1H, Ph 6-*H*), 4.75 (s, 4H, Tri-CH_2), 4.31 (s, 2H, PhCH_2), 3.46 (s, 4H, Tri-CH$_2$CH_2); MS *m*/*z*: 334 $[M + H - 3HCl]^+$; Anal. Calcd. for $C_{15}H_{20}Cl_3F_2N_7$: C, 40.69; H, 4.55; N, 22.15. Found: C, 40.83; H, 4.61; N, 22.32.

*6.1.5.2. N-(2-(1H-1,2,4-Triazol-1-yl)ethyl)-N-(2,4-dichlorobenzyl)-2-(1H-1,2,4-triazol-1-yl)ethanamine hydrochloride (**7b**).* Compound **7b** was obtained as a white solid (0.18 g). Yield: 75.6%, mp: 110–112 °C; IR (KBr, cm^{-1}) ν: 3093, 3046, 3021, 2938, 1630, 1582, 1528, 1380, 1101, 904, 850, 823; ^{1}H NMR (400 MHz, DMSO, ppm): δ 9.45 (s, 2H, Tri 3-*H*), 8.58 (s, 2H, Tri 5-*H*), 7.61 (s, 1H, Ph 3-*H*), 7.37 (s, 2H, Ph 5, 6-*H*), 4.63 (s, 4H, Tri-CH_2), 4.09 (s, 2H, PhCH_2), 3.29 (s, 4H, Tri-CH$_2$CH_2); MS *m*/*z*: 366 $[M + H - 3HCl]^+$; Anal. Calcd. for $C_{15}H_{20}Cl_5N_7$: C, 37.88; H, 4.24; N, 20.61. Found: C, 37.69; H, 4.19; N, 20.72.

*6.1.5.3. N-(2-(1H-1,2,4-Triazol-1-yl)ethyl)-N-(3,4-dichlorobenzyl)-2-(1H-1,2,4-triazol-1-yl)ethanamine hydrochloride (**7c**).* Compound **7c** was obtained as a white solid (0.16 g). Yield: 67.2%, mp: 124–126 °C; IR (KBr, cm^{-1}) ν: 3116, 2952, 2838, 1506, 1476, 1278, 1140, 958, 876; ^{1}H NMR (400 MHz, DMSO, ppm): δ 9.19 (s, 2H, Tri 3-*H*), 8.42 (s, 2H, Tri 5-*H*), 7.80 (s, 1H, Ph 5-*H*), 7.67 (d, $J = 8.4$ Hz, 1H, Ph 2-*H*), 7.51 (s, 1H, Ph 6-*H*), 4.74 (s, 4H, Tri-CH_2), 4.33 (s, 2H, PhCH_2), 3.47 (s, 4H, Tri-CH$_2$CH_2); MS *m*/*z*: 366 $[M + H - 3HCl]^+$; Anal. Calcd. for $C_{15}H_{20}Cl_5N_7$: C, 37.88; H, 4.24; N, 20.61. Found: C, 37.72; H, 4.20; N, 20.69.

*6.1.5.4. N-(2-(1H-Imidazol-1-yl)ethyl)-N-(2,4-difluorobenzyl)-2-(1H-imidazol-1-yl)ethanamine hydrochloride (**8a**).* Compound **8a** was obtained as a white solid (0.15 g). Yield: 68.0%, mp: 107–109 °C; IR (KBr, cm^{-1}) ν: 3089, 2954, 2857, 1620, 1501, 1457, 1276, 1141, 960, 856; ^{1}H NMR (400 MHz, DMSO, ppm): δ 9.30 (s, 2H, Im 2-*H*), 7.76 (s, 2H, Im 4-*H*), 7.63 (s, 2H, Im 5-*H*), 7.22–7.18 (m, 2H, Ph 2, 5-*H*), 7.00 (t, $J = 7.6$ Hz, 1H, Ph 6-*H*), 4.51 (s, 4H, Im-CH_2), 3.92 (s, 2H, PhCH_2), 3.10 (s, 4H, Im-CH$_2$CH_2); MS *m*/*z*: 332 $[M + H - 3HCl]^+$; Anal. Calcd. for $C_{17}H_{22}Cl_3F_2N_5$: C, 46.33; H, 5.03; N, 15.89. Found: C, 46.49; H, 4.97; N, 15.70.

*6.1.5.5. N-(2-(1H-Imidazol-1-yl)ethyl)-N-(2,4-dichlorobenzyl)-2-(1H-imidazol-1-yl)ethanamine hydrochloride (**8b**).* Compound **8b** was obtained as a white solid (0.16 g). Yield: 67.5%, mp: 127–128 °C; IR (KBr, cm^{-1}) ν: 3121, 3038, 2961, 2931, 2846, 1601, 1504, 1455, 1268, 1200, 1138, 958, 853; ^{1}H NMR (400 MHz, DMSO, ppm): δ 9.36 (s, 2H, Im 2-*H*), 7.73 (s, 2H, Im 4-*H*), 7.68 (s, 2H, Im 5-*H*), 7.55 (s, 1H, Ph 3-*H*), 7.30 (s, 1H, Ph 5-*H*), 7.11 (s, 1H, Ph 6-*H*), 4.54 (s, 4H, Im-CH_2), 3.92 (s, 2H, PhCH_2), 3.12 (s, 4H, Im-CH$_2$CH_2); MS *m*/*z*: 364 $[M + H - 3HCl]^+$; Anal. Calcd. for $C_{17}H_{22}Cl_5N_5$: C, 43.11; H, 4.68; N, 14.79. Found: C, 43.32; H, 4.62; N, 14.92.

*6.1.5.6. N-(2-(1H-Imidazol-1-yl)ethyl)-N-(3,4-dichlorobenzyl)-2-(1H-imidazol-1-yl)ethanamine hydrochloride (**8c**).* Compound **8c** was obtained as a white solid (0.18 g). Yield: 75.9%, mp: 129–131 °C; IR (KBr, cm^{-1}) ν: 3109, 3046, 2958, 2928, 2856, 1602, 1509, 1452, 1261, 1201, 1140, 949, 850, 750; ^{1}H NMR (400 MHz, DMSO, ppm): δ 9.34 (s, 2H, Im 2-*H*), 7.79 (s, 2H, Im 4-*H*), 7.66 (s, 2H, Im 5-*H*), 7.52 (d, $J = 8.0$ Hz, 1H, Ph 2-*H*), 7.26 (s, 1H, Ph 5-*H*), 7.18 (s, 1H, Ph 6-*H*), 4.50 (s, 4H, Im-CH_2), 3.84 (s, 2H, PhCH_2), 3.08 (s, 4H, Im-CH$_2$CH_2); MS *m*/*z*: 364 $[M + H - 3HCl]^+$; Anal. Calcd. for $C_{17}H_{22}Cl_5N_5$: C, 43.11; H, 4.68; N, 14.79. Found: C, 43.33; H, 4.73; N, 14.61.

*6.1.5.7. N-(2,4-Dichlorobenzyl)-2-(2-phenyl-1H-imidazol-1-yl)-N-(2-(2-phenyl-1H-imidazol-1-yl)ethyl)ethanamine hydrochloride (**8d**).* Compound **8d** was obtained as a white solid (0.23 g). Yield: 73.5%, mp: 137–139 °C; IR (KBr, cm^{-1}): 3101, 3038, 2952, 2921, 2843, 1598, 1505, 1458, 1402, 1271, 1109, 831, 772; ^{1}H NMR (400 MHz, DMSO, ppm): δ 7.72 (s, 2H, Im 4-*H*), 7.63 (s, 2H, Im 5-*H*), 7.43–7.35 (m, 4H, Ar-*H*), 6.95–6.82 (m, 9H, Ar-*H*), 4.52 (s, 4H, Im-CH_2), 3.91 (s, 2H, PhCH_2), 3.13 (s, 4H, Im-CH$_2$CH_2); MS *m*/*z*: 516 $[M + H - 3HCl]^+$; Anal. Calcd. for $C_{29}H_{30}Cl_5N_5$: C, 55.65; H, 4.83; N, 11.19. Found: C, 55.43; H, 4.77; N, 11.32.

*6.1.5.8. N-(2-(1H-Benzoimidazol-1-yl)ethyl)-2-(1H-benzoimidazol-1-yl)-N-(2,4-difluorobenzyl)ethanamine hydrochloride (**9a**).* Compound **9a** was obtained as a white solid (0.20 g). Yield: 73.9%, mp: 119–121 °C; IR (KBr, cm^{-1}) ν: 3117, 3025, 2921, 2841, 1619, 1552, 1503, 1381, 1280, 1101, 820, 732; ^{1}H NMR (400 MHz, DMSO, ppm): δ 10.03 (s, 2H, Benim 2-*H*), 7.98 (d, $J = 8.0$ Hz, 2H, Benim 4-*H*), 7.84 (d, $J = 8.0$ Hz, 2H, Benim 7-*H*), 7.60–7.52 (m, 4H, Benim 5, 6-*H*), 7.02 (s, 1H, Ph 3-*H*), 6.80 (s, 1H, Ph 5-*H*), 6.57 (s, 1H, Ph 6-*H*), 4.82 (s, 4H, Benim-CH_2), 3.85 (s, 2H, PhCH_2), 3.17 (s, 4H, Benim-CH$_2$CH_2); MS *m*/*z*: 432 $[M + H - 3HCl]^+$; Anal. Calcd. for $C_{25}H_{26}Cl_3F_2N_5$: C, 55.52; H, 4.85; N, 12.95. Found: C, 55.39; H, 4.90; N, 12.86.

*6.1.6. General procedure for the preparation of nitrates **10**–**12***

The nitric acid (4 mol/L) was added dropwise into a solution of compound **4a** (0.17 g, 0.5 mmol), or **4b** (0.18 g, 0.5 mmol), or **4c**

(0.18 g, 0.5 mmol), or **5a** (0.17 g, 0.5 mmol), or **5b** (0.18 g, 0.5 mmol), or **5c** (0.18 g, 0.5 mmol), or **5i** (0.26 g, 0.5 mmol), or **6a** (0.22 g, 0.5 mmol) in ethyl ether/chloroform (4/1, V/V), stopped the addition when no more precipitate formed. After the addition, the formed precipitate was filtered, and the precipitate was washed with chloroform and light petroleum ether by turns, and then dried by freeze drying oven to afford compounds **10**–**12**.

*6.1.7. N-(2-(1H-1,2,4-Triazol-1-yl)ethyl)-N-(2,4-difluorobenzyl)-2-(1H-1,2,4-triazol-1-yl)ethanamine nitrate (**10a**)*

Compound **10a** was obtained as a white solid (0.17 g). Yield: 65.1%, mp: 147–149 °C; IR (KBr, cm^{-1}) ν: 3123, 3056, 3034, 2957, 1621, 1563, 1509, 1341, 1123, 837, 821, 720; ^{1}H NMR (400 MHz, DMSO, ppm): δ 9.37 (s, 2H, Tri 3-*H*), 8.54 (s, 2H, Tri 5-*H*), 7.74 (d, J = 6.0 Hz, 1H, Ph 3-*H*), 7.38 (t, J = 8.0 Hz, 1H, Ph 5-*H*), 7.17 (t, J = 8.0 Hz, 1H, Ph 6-*H*), 4.92 (s, 4H, Tri-CH_2), 4.39 (s, 2H, PhCH_2), 3.42 (s, 4H, Tri-CH$_2$CH_2); MS *m*/*z*: 334 [M + H − 3HNO$_3$]$^+$; Anal. Calcd. for $C_{15}H_{20}F_2N_{10}O_9$: C, 34.49; H, 3.86; N, 26.81. Found: C, 34.66; H, 3.81; N, 26.74.

*6.1.8. N-(2-(1H-1,2,4-Triazol-1-yl)ethyl)-N-(2,4-dichlorobenzyl)-2-(1H-1,2,4-triazol-1-yl)ethanamine nitrate (**10b**)*

Compound **10b** was obtained as a white solid (0.16 g). Yield: 57.7%, mp: 133–135 °C; IR (KBr, cm^{-1}) ν: 3098, 3050, 3019, 2946, 1632, 1588, 1560, 1384, 1108, 851, 827, 724; ^{1}H NMR (400 MHz, DMSO, ppm): δ 9.59 (s, 2H, Tri 3-*H*), 8.78 (s, 2H, Tri 5-*H*), 7.53 (d, J = 2.0 Hz, 1H, Ph 3-*H*), 7.32–7.29 (m, 1H, Ph 5-*H*), 7.14 (d, J = 8.0 Hz, 1H, Ph 6-*H*), 4.52 (t, J = 6.0 Hz, 4H, Tri-CH_2), 3.91 (s, 2H, PhCH_2), 3.18 (t, J = 6.0 Hz, 4H, Tri-CH$_2$CH_2); MS *m*/*z*: 366 [M + H − 3HNO$_3$]$^+$; Anal. Calcd. for $C_{15}H_{20}Cl_2N_{10}O_9$: C, 32.44; H, 3.63; N, 25.22. Found: C, 32.69; H, 3.56; N, 25.41.

*6.1.9. N-(2-(1H-1,2,4-Triazol-1-yl)ethyl)-N-(3,4-dichlorobenzyl)-2-(1H-1,2,4-triazol-1-yl)ethanamine nitrate (**10c**)*

Compound **10c** was obtained as a white solid (0.17 g). Yield: 61.3%, mp: 129–131 °C; IR (KBr, cm^{-1}) ν: 3121, 2946, 2828, 1502, 1470, 1288, 1142, 960, 871; ^{1}H NMR (400 MHz, DMSO, ppm): δ 9.21 (s, 2H, Tri 3-*H*), 8.47 (s, 2H, Tri 5-*H*), 7.86 (s, 1H, Ph 5-*H*), 7.72 (d, J = 8.4 Hz, 1H, Ph 2-*H*), 7.54 (s, 1H, Ph 6-*H*), 4.78 (s, 4H, Tri-CH_2), 4.35 (s, 2H, PhCH_2), 3.50 (s, 4H, Tri-CH$_2$CH_2); MS *m*/*z*: 366 [M + H − 3HNO$_3$]$^+$; Anal. Calcd. for $C_{15}H_{20}Cl_2N_{10}O_9$: C, 32.44; H, 3.63; N, 25.22. Found: C, 32.62; H, 3.68; N, 25.05.

*6.1.10. N-(2-(1H-Imidazol-1-yl)ethyl)-N-(2,4-difluorobenzyl)-2-(1H-imidazol-1-yl)ethanamine nitrate (**11a**)*

Compound **11a** was obtained as a white solid (0.14 g). Yield: 53.8%, mp: 126–128 °C; IR (KBr, cm^{-1}) ν: 3063, 2949, 2853, 1616, 1503, 1452, 1266, 1137, 957, 854; ^{1}H NMR (400 MHz, DMSO, ppm): δ 9.31 (s, 2H, Im 2-*H*), 7.78 (s, 2H, Im 4-*H*), 7.65 (s, 2H, Im 5-*H*), 7.23–7.20 (m, 2H, Ph 2, 5-*H*), 7.05 (t, J = 7.2 Hz, 1H, Ph 6-*H*), 4.54 (s, 4H, Im-CH_2), 3.95 (s, 2H, PhCH_2), 3.14 (s, 4H, Im-CH$_2$CH_2); MS *m*/*z*: 332 [M + H − 3HNO$_3$]$^+$; Anal. Calcd. for $C_{17}H_{22}F_2N_8O_9$: C, 39.24; H, 4.26; N, 21.53. Found: C, 39.45; H, 4.31; N, 21.70.

*6.1.11. N-(2-(1H-Imidazol-1-yl)ethyl)-N-(2,4-dichlorobenzyl)-2-(1H-imidazol-1-yl)ethanamine nitrate (**11b**)*

Compound **11b** was obtained as a white solid (0.15 g). Yield: 54.2%, mp: 138–140 °C; IR (KBr, cm^{-1}) ν: 3116, 3054, 2956, 2928, 2856, 1608, 1501, 1457, 1209, 1141, 961, 850; ^{1}H NMR (400 MHz, DMSO, ppm): δ 9.25 (s, 2H, Im 2-*H*), 7.71 (s, 2H, Im 4-*H*), 7.58 (s, 2H, Im 5-*H*), 7.51 (s, 1H, Ph 3-*H*), 7.28 (s, 1H, Ph 5-*H*), 7.09 (s, 1H, Ph 6-*H*), 4.52 (s, 4H, Im-CH_2), 3.90 (s, 2H, PhCH_2), 3.09 (s, 4H, Im-CH$_2$CH_2); MS *m*/*z*: 364 [M + H − 3HNO$_3$]$^+$; Anal. Calcd. for $C_{17}H_{22}Cl_2N_8O_9$: C, 36.90; H, 4.01; N, 20.25. Found: C, 36.68; H, 4.06; N, 20.14.

*6.1.12. N-(2-(1H-Imidazol-1-yl)ethyl)-N-(3,4-dichlorobenzyl)-2-(1H-imidazol-1-yl)ethanamine nitrate (**11c**)*

Compound **11c** was obtained as a white solid (0.17 g). Yield: 61.5%, mp: 142–144 °C; IR (KBr, cm^{-1}) ν: 3118, 3038, 2964, 2921, 2853, 1609, 1504, 1456, 1251, 1138, 857, 746, 720; ^{1}H NMR (400 MHz, DMSO, ppm): δ 9.32 (s, 2H, Im 2-*H*), 7.78 (s, 2H, Im 4-*H*), 7.63 (s, 2H, Im 5-*H*), 7.62 (d, J = 8.0 Hz, 1H, Ph 2-*H*), 7.23 (s, 1H, Ph 5-*H*), 7.18 (s, 1H, Ph 6-*H*), 4.48 (s, 4H, Im-CH_2), 3.81 (s, 2H, PhCH_2), 3.05 (s, 4H, Im-CH$_2$CH_2); MS *m*/*z*: 364 [M + H − 3HNO$_3$]$^+$; Anal. Calcd. for $C_{17}H_{22}Cl_2N_8O_9$: C, 36.90; H, 4.01; N, 20.25. Found: C, 36.72; H, 3.96; N, 20.37.

*6.1.13. N-(2,4-Dichlorobenzyl)-2-(2-phenyl-1H-imidazol-1-yl)-N-(2-(2-phenyl-1H-imidazol-1-yl)ethyl)ethanamine nitrate (**11d**)*

Compound **11d** was obtained as a white solid (0.23 g). Yield: 65.2%, mp: 143–145 °C; IR (KBr, cm^{-1}): 3112, 3054, 2961, 2928, 2852, 1599, 1503, 1456, 1259, 1106, 829, 770; ^{1}H NMR (400 MHz, DMSO, ppm): δ 7.83 (s, 2H, Im 4-*H*), 7.74 (s, 2H, Im 5-*H*), 7.54–7.46 (m, 4H, Ar-*H*), 6.93–6.76 (m, 9H, Ar-*H*), 4.54 (s, 4H, Im-CH_2), 3.93 (s, 2H, PhCH_2), 3.12 (s, 4H, Im-CH$_2$CH_2); MS *m*/*z*: 516 [M + H − 3HNO$_3$]$^+$; Anal. Calcd. for $C_{29}H_{30}Cl_2N_8O_9$: C, 49.37; H, 4.29; N, 15.88. Found: C, 49.56; H, 4.34; N, 15.72.

*6.1.14. N-(2-(1H-Benzoimidazol-1-yl)ethyl)-2-(1H-benzoimidazol-1-yl)-N-(2,4-difluorobenzyl)ethanamine nitrate (**12a**)*

Compound **12a** was obtained as a white solid (0.17 g). Yield: 54.8%, mp: 124–126 °C; IR (KBr, cm^{-1}) ν: 3114, 3016, 2907, 2850, 1620, 1548, 1510, 1384, 1279, 1106, 824, 730; ^{1}H NMR (400 MHz, DMSO, ppm): δ 9.56 (s, 2H, Benim 2-*H*), 7.93 (m, 2H, Benim 4-*H*), 7.82 (m, 2H, Benim 7-*H*), 7.62–7.53 (m, 4H, Benim 5, 6-*H*), 6.96–6.90 (m, 1H, Ph 6-*H*), 6.78–6.73 (m, 1H, Ph 5-*H*), 6.57–6.53 (m, 1H, Ph 3-*H*), 4.66 (t, J = 6.0 Hz, 4H, Benim-CH_2), 3.74 (s, 2H, PhCH_2), 3.10 (t, J = 6.0 Hz, 4H, Benim-CH$_2$CH_2); MS *m*/*z*: 432 [M + H − 3HNO$_3$]$^+$; Anal. Calcd. for $C_{25}H_{26}F_2N_8O_9$: C, 48.39; H, 4.22; N, 18.06. Found: C, 48.04; H, 4.36; N, 18.19.

6.2. Biological assays

The *in vitro* minimal inhibitory concentrations (MICs) of the target compounds and their precursors were determined by the micro-broth dilution method in 96-well microtest plates according to the methods recommended by the National Committee for Clinical Laboratory Standards (NCCLS). The tested strains were provided by Department of Microbiology, College of Medicine, Third Military Medical University, China. Fluconazole and Chloramphenicol obtained from their respective manufacturers served as the controls.

6.2.1. Antibacterial assay

The prepared compounds **2**–**12** were evaluated for their antibacterial activities against *S. aureus* ATCC 29213, *S. aureus* N 315 (MRSA), *B. subtilis* ATCC 21216 as Gram-positive, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *B. proteus* ATCC 13315 as Gram-negative bacteria. The bacterial suspension was adjusted with sterile saline to a concentration of 1×10^4–1×10^5 CFU. The tested compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare the stock solutions. The tested compounds and reference drugs were prepared in Mueller-Hinton Broth (Guangdong huaikai microbial sci.& tech co., Ltd, Guangzhou, Guangdong, China) by two-fold serial dilution to obtain the required concentrations of 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, and 0.25 μg/mL. These dilutions were inoculated and incubated at 37 °C for 24 h. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment. The growth was monitored visually and

spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). The minimum inhibitory concentrations were summarized in Table 4.

6.2.2. Antifungal assay

The new compounds were evaluated for their antifungal activities against *C. albicans* ATCC 76615, *A. fumigatus* ATCC 96918. A spore suspension in sterile distilled water was prepared from 1-day-old culture of the fungi growing on Sabouraud agar (SA) media. The final spore concentration was $1–5\times10^3$ spore mL^{-1}. From the stock solutions of the tested compounds and reference antifungal Fluconazole, dilutions in sterile RPMI 1640 medium (Neuronbc Laboraton Technology CO., Ltd, Beijing, China) were made resulting in eleven wanted concentrations (0.25–256 μg/mL) of each tested compounds. These dilutions were inoculated and incubated at 35 °C for 24 h. The growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of fungi was regarded as minimum inhibitory concentration (MIC). The minimum inhibitory concentrations were summarized in Table 5.

Acknowledgments

This work was partially supported by the Natural Science Foundation of Chongqing (CSCT: 2007BB5369, 2009BB5296) and Talents Introduction Foundation of Southwest University (SWUB2006018, XSGX0602).

References

[1] G.H. Talbot, J. Bradley, J.E. Edwards, D. Gilbert, M. Scheld, J.G. Bartlett, Clin. Infect. Dis. 42 (2006) 657–668.
[2] P.L. Shao, L.M. Huang, P.R. Hsueh, Int. J. Antimicrob. Agents 30 (2007) 487–495.
[3] A.J. O'Neill, Expert. Opin. Investig. Drugs 17 (2008) 297–302.
[4] B.S. Dawane, S.G. Konda, G.G. Mandawad, B.M. Shaikh, Eur. J. Med. Chem. 45 (2010) 387–392.
[5] T. Lorand, B. Kocsis, Mini. Rev. Med. Chem. 7 (2007) 900–911.
[6] H. Bayrak, A. Demirbas, N. Demirbas, S.A. Karaoglu, Eur. J. Med. Chem. 44 (2009) 4362–4366.
[7] D.J. Payne, M.N. Gwynn, D.J. Holmes, D.L. Pompliano, Nat. Rev. Drug Discov. 6 (2007) 29–40.
[8] H. Bayrak, A. Demirbas, S.A. Karaoglu, N. Demirbas, Eur. J. Med. Chem. 44 (2009) 1057–1066.
[9] A. Khalafi-Nezhad, M.N.S. Rad, H. Mohabatkar, Z. Asrari, B. Hemmateenejada, Bioorg. Med. Chem. 13 (2005) 1931–1938.
[10] J.L. Cai, S. Li, C.H. Zhou, L.L. Gan, J. Wu, Chin. J. New Drugs 18 (2009) 598–608 (in Chinese).
[11] Z. Rezaei, S. Khabnadideh, K. Pakshir, Z. Hossaini, F. Amiri, E. Assadpour, Eur. J. Med. Chem. 44 (2009) 3064–3067.
[12] J. Mann, A. Baron, Y. Opoku-Boahen, E. Johansson, G. Parkinson, L.R. Kelland, S. Neidle, J. Med. Chem. 44 (2001) 138–144.
[13] J.L. Adams, J.C. Bocham, T.F. Gallagher, S. Kassis, E.F. Webb, R. Hall, M. Sorenson, R. Garigipati, D.E. Grisvold, J.C. Lee, Bioorg. Med. Chem. Lett. 11 (2001) 2867–2870.
[14] R.K. Ujjinamatada, A. Baier, P. Borowski, R.S. Hosmane, Bioorg. Med. Chem. Lett. 17 (2007) 2285–2288.
[15] S.A.F. Rostom, H.M.A. Ashour, H.A.A.E. Razik, A.E.F.H.A.E. Fattah, N.N. El-Din, Bioorg. Med. Chem. 17 (2009) 2410–2422.
[16] R. Silvestri, M. Artico, G.L. Regina, A.D. Pasquali, G.D. Martino, F.D. D'Auria, L. Nencioni, A.T. Palamara, J. Med. Chem. 47 (2004) 3924–3926.
[17] F. Bellina, S. Cauteruccio, R. Rossi, Tetrahedron 63 (2007) 4571–4624.
[18] J.L. Mi, C.H. Zhou, X. Bai, Chin. J. Antibiot. 32 (2007) 587–593 (in Chinese).
[19] X. Bai, C.H. Zhou, J.L. Mi, Chem. Res. Appl. 19 (2007) 721–729 (in Chinese).
[20] G.L. Regina, F.D. D'Auria, A. Tafi, F. Piscitelli, S. Olla, F. Caporuscio, L. Nencioni, R. Cirilli, F.L. Torre, N.R.D. Melo, S.L. Kelly, D.C. Lamb, M. Artico, M. Botta, A.T. Palamara, R. Silvestri, J. Med. Chem. 51 (2008) 3841–3855.
[21] E. Menegola, M.L. Broccia, F.D. Renzo, E. Giavini, Reprod. Toxicol. 22 (2006) 186–195.
[22] P.T.M. Nguyen, J.D. Baldeck, J. Olsson, R.E. Marquis, Oral Microbiol. Immunol. 20 (2005) 93–100.
[23] X.Y. Che, C.Q. Sheng, W.Y. Wang, Y.B. Cao, Y.L. Xu, H.T. Ji, G.Q. Dong, Z.Y. Miao, J.Z. Yao, W.N. Zhang, Eur. J. Med. Chem. 44 (2009) 4218–4226.
[24] L. Jeu, F.J. Piacenti, A.G. Lyakhovetskiy, H.B. Fung, Clin. Ther. 25 (2003) 1321–1381.
[25] H.W. Boucher, A.H. Groll, C.C. Chiou, T.J. Walsh, Drugs 64 (2004) 1997–2020.
[26] A. Bentley, M. Butters, S.P. Green, W.J. Learmonth, J.A. MacRae, M.C. Morland, G. O'Connor, J. Skuse, Organ. Prog. Res. Develop. 6 (2002) 109–112.
[27] K. Wan, L.L. Gan, C.H. Zhou, Y.Y. Zhang, Chin. Pharm. J, article in press.
[28] C.H. Zhou, L.L. Gan, Y.Y. Zhang, F.F. Zhang, G.Z. Wang, L. Jin, R.X. Geng, Sci. China Ser. B-Chem. 52 (2009) 415–458.
[29] J. Bartroli, E. Turmo, M. Algueró, E. Boncompte, M.L. Vericat, J. García-Rafanell, J. Forn, J. Med. Chem. 38 (1995) 3918–3932.
[30] Q.Y. Sun, W.N. Zhang, J.M. Xu, Y.B. Cao, Q.Y. Wu, D.Z. Zhang, C.M. Liu, S.C. Yu, Y.Y. Jiang, Eur. J. Med. Chem. 42 (2007) 1151–1157.
[31] D.A. Heerding, G. Chan, W.E. DeWolf, A.P. Fosberry, C.A. Janson, D.D. Jaworski, E. McManus, W.H. Miller, T.D. Moore, D.J. Payne, X.Y. Qiu, S.F. Rittenhouse, C. Slater-Radosti, W. Smith, D.T. Takata, K.S. Vaidya, C.C.K. Yuan, W.F. Huffman, Bioorg. Med. Chem. Lett. 11 (2001) 2061–2065.
[32] S.H. Lee, C.J. Kim, J. Microbiol. Biotechnol. 9 (1999) 572–575.
[33] Y. Luo, Y.H. Lu, L.L. Gan, C.H. Zhou, J. Wu, R.X. Geng, Y.Y. Zhang, Arch. Pharm. Chem. Life Sci. 342 (2009) 386–393.
[34] F.F. Zhang, L.L. Gan, C.H. Zhou, Bioorg. Med. Chem. Lett. 20 (2010) 1881–1884.
[35] Z. Nowakowska, B. Kędzia, G. Schroeder, Eur. J. Med. Chem. 43 (2008) 707–713.
[36] M.H. Klingele, S. Brooker, Coord. Chem. Rev. 241 (2003) 119–132.
[37] Ö.Ö. Güven, T. Erdoğan, H. Göker, S. Yıldız, Bioorg. Med. Chem. Lett. 17 (2007) 2233–2236.
[38] P. Karegoudar, D.J. Prasad, M. Ashok, M. Mahalinga, B. Poojary, B.S. Holla, Eur. J. Med. Chem. 43 (2008) 808–815.
[39] R.H. Lin, P.J. Connolly, S.L. Huang, S.K. Wetter, Y.H. Lu, W.V. Murray, S.L. Emanuel, R.H. Gruninger, A.R. Fuentes-Pesquera, C.A. Rugg, S.A. Middleton, L.K. Jolliffe, J. Med. Chem. 48 (2005) 4208–4211.
[40] D. Zampieri, M.G. Mamolo, L. Vio, E. Banfi, G. Scialino, M. Fermeglia, M. Ferronec, S. Pricl, Bioorg. Med. Chem. 15 (2007) 7444–7458.
[41] S. Khabnadideh, Z. Rezaei, A. Khalafi-Nezhad, R. Bahrinajafi, R. Mohamadi, A.A. Farrokhroz, Bioorg. Med. Chem. Lett. 13 (2003) 2863–2865.
[42] H. Muroi, K.I. Nihei, K. Tsujimoto, I. Kubo, Bioorg. Med. Chem. 12 (2004) 583–587.
[43] National Committee for Clinical Laboratory Standards Approved standard Document, M27–A2, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. National Committee for Clinical Laboratory Standards, Wayne, PA, 2002.
[44] Y.Y. Zhuang, C.H. Zhou, Y.F. Wang, D.H. Li, Chin. Pharm. J. 43 (2008) 1281–1287 (in Chinese).
[45] Z.Y. Wang, J.H. Shi, H.X. Shi, Chin. J. Syn. Chem. 3 (1995) 226–230 (in Chinese).
[46] L. Han, Z.M. Li, Y. Zhang, W.M. Guo, Chin. J. Org. Chem. 26 (2006) 242–246 (in Chinese).