Bioorganic & Medicinal Chemistry Letters 20 (2010) 1881–1884

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis, antibacterial and antifungal activities of some carbazole derivatives

Fei-Fei Zhang [a], Lin-Ling Gan [b], Cheng-He Zhou [a,*]

[a] School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China
[b] School of Pharmaceutical Sciences, Southwest University, Chongqing 400715, China

ARTICLE INFO

Article history:
Received 30 July 2009
Revised 22 December 2009
Accepted 29 January 2010
Available online 4 February 2010

Keywords:
Carbazole
Imidazole
1,2,4-Triazole
Triazolium
Antibacterial
Antifungal

ABSTRACT

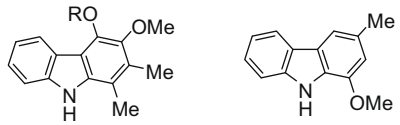
A series of N-substituted carbazole derivatives were synthesized and evaluated for antibacterial and antifungal activities against *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus proteus*, *Candida albicans* and *Aspergillus fumigatus* by two fold serial dilution technique. Some of the synthesized compounds displayed comparable or even better antibacterial and antifungal activities than reference drugs fluconazole, chloramphenicol and norfloxacin against tested strains.

Disease-causing microbes that have become resistant to drug therapy are an increasing public health problem particularly during the last decade. The hospital-acquired infections are resistant to the most powerful antibiotics available methicillin and vancomycin. These drugs are reserved to treat only the most intractable infections in order to retard the development of resistance to them.[1] Therefore it is imperative to design and develop new antimicrobial agents with novel chemical structures possibly having modes of action rather than analogues of the existing ones.

Carbazole and its derivatives are an important type of nitrogen-containing aromatic heterocyclic compounds, possess desirable electronic and charge-transport properties, as well as large π-conjugated system, and the various functional groups are easily introduced into the structurally rigid carbazolyl ring. These characteristics result in the extensive potential applications of carbazole-based derivatives in the field of chemistry (photoelectrical materials,[2] dyes,[3] supramolecular recognition[4] etc.) and medicinal chemistry[5] (antitumor, antimicrobial, antihistaminic, antioxidative, anti-inflammatory, psychotropic agents etc.). Carbazole rings are present in a variety of naturally occurring medicinally active substances.[5] For example, the carbazomycins are an unprecedented class of antibiotics with a carbazole framework.[6] Carbazomycins A and B (Fig. 1) inhibit the growth of phytopathogenic fungi and have antibacterial and anti-yeast activities. However, Murrayafoline A exhibited strong fungicidal activity against *Cladosporium cucumerinum* at the dose of 12.5 μg, which was isolated from *Murraya euchrestifolia* Hayata collected in Taiwan.[7]

Numerous researches have focused on the isolation, purification and biological activity of natural carbazole compounds as well as the total synthesis of biologically active carbazole alkaloids and their analogues. Recently, the novel artificial synthetic carbazole derivatives as antibiotics have attracted special attention, when it was found that carbazole-based macrocyclic carbazolophanes exhibited significant antibacterial and antifungal activities.[8] Some carbazole derivatives combined via spacers gave good antibacterial activities against tested human pathogens compared to commercial antibiotics (benzalkonium chloride, cetylpyridinium chloride and tetracycline).[9] However, to the best of our knowledge, imidazole or 1,2,4-triazole-based N-substituted carbazole derivatives have not been reported. Herein, we wish to report the synthesis, antibacterial and antifungal activities of a series of N-substituted carbazole derivatives including carbazole-based halides **1a–c**, imidazoles **2a–c**, triazoles **3a–c**, triazolium **4** and bis-carbazoles **5a–c**.

It is well-known that azole moieties such as imidazole and triazole nucleus as important pharmacophore appear extensively in



Carbazomycin A, R = Me
Carbazomycin B, R = H

Murrayafoline A

Figure 1. Structures of some biologically active carbazole compounds.

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

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.01.159

various types of pharmaceutical agents, widely implicate in biochemical processes and display diversity of pharmacological activities.[10] A large number of azole compounds are used as antimicrobial drugs in clinic, for example, miconazole, clotrimazole and econazole are administered topically, while ketoconazole, itraconazole and fluconazole are useful in the treatment of systemic infections. Furthermore, it has been found that some azoles such as miconazole gave remarkable antibacterial activity against MRSA.[11] The widespread use of azole antimicrobial drugs led numerous efforts to develop some azole derivatives as new antimicrobial agents.

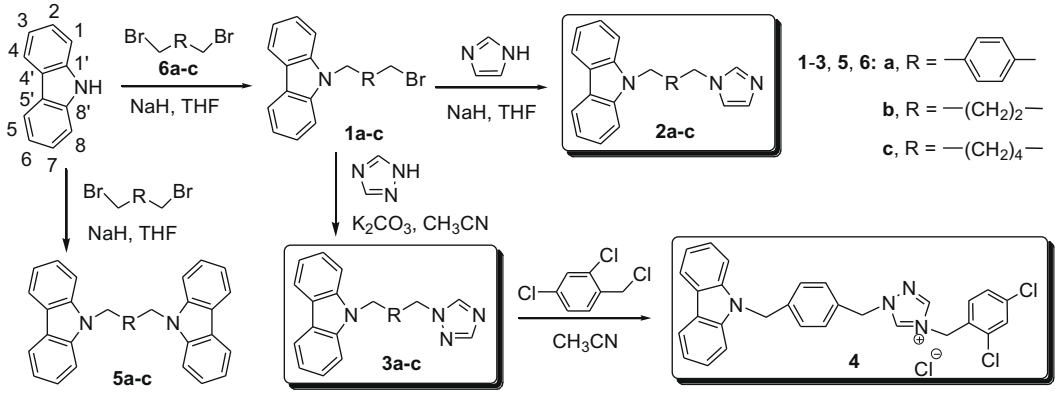
In this connection, a series of carbazole-based azole derivatives such as imidazole, 1,2,4-triazole ones and so on were designed and synthesized for the first time, and their antibacterial and antifungal activities were evaluated against *Staphylococcus aureus*, MRSA, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus proteus*, *Candida albicans* and *Aspergillus fumigatus*. Recent studies found that the linkers have an important effect on the antimicrobial activities, particularly the six-carbon chain spacer gave better biological activities.[12] Therefore various functional groups including aryl and alkyl bridged types as well as different lengths of alkyl chains were introduced into the target compounds in order to investigate their structure–activity relationships. Some works have shown that introduction of electropositive groups in triazole derivatives is useful to increase the water solubility and membrane permeability, and thereby improve their biological activities.[12] In view of this, 1,2,4-triazolium derivative **4** was designed and synthesized by the use of 2,4-dichlorobenzyl chloride, because halobenzyl moiety was found to be biologically important and could improve the antimicrobial activities. In addition, bis-carbazole compounds **5a–c** were prepared by the replacement of triazolyl group with carbazolyl ring in target molecules **3a–c** in order to further investigate the effect of carbazolyl group on the antimicrobial activities, as a comparison with triazole nucleus.

The synthetic route of N-substituted carbazole derivatives is shown in Scheme 1. The target carbazole-based azole derivatives were synthesized by using commercially available carbazole as starting materials. The reaction of carbazole with alkyl or aryl dibromides at room temperature produced the halides **1a–c** with the yields of 41–77.5% in the presence of sodium hydride under a stream of nitrogen. Carbazole halides **1a–c** reacted with imidazole using NaH as the base to afford the imidazole derivatives **2a–c** with yields ranging from 83.5% to 89.2%. Triazole compounds **3a–c** were successfully synthesized in good yields (76.4–91.1%) by mixing intermediates **1a–c**, 1,2,4-triazole and potassium carbonate in acetonitrile at 45 °C. The quaternization of compound **3a** with commercially available 2,4-dichlorobenzyl chloride gave desired triazolium derivative **4** in good yield after purification via washing with petroleum ether (30–60 °C). Bis-carbazoles **5a–c** were conveniently prepared by the reaction of carbazole with corresponding alkyl or aryl dibromides. Some synthetic data were given in Table 1. These new compounds were confirmed by MS, HRMS, IR and ^{1}H, ^{13}C, ^{1}H–^{1}H COSY, ^{1}H–^{13}C HETCOR, APT and DEPT NMR spectra.[13]

All of the synthesized compounds were screened in vitro for antibacterial activities against Gram-positive *S. aureus*, MRSA and *B. subtilis*, Gram-negative *E. coli*, *P. aeruginosa* and *B. proteus* as well as antifungal activities against *C. albicans* and *A. fumigatus* by two-fold serial dilution technique.[14] All compounds were evaluated 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 microorganisms compared with that of the current antimicrobial drugs fluconazole, chloramphenicol and norfloxacin in clinic. The data of antibacterial and antifungal tests are depicted in Table 1.

The obtained results showed that the synthesized compounds **1–5** exhibited poor to good activities against all tested strains. As noted in Table 1, all carbazole derivatives, except for bis-carbazole **5b** bridged by $(CH_2)_4$ linker, could effectively inhibit the growth of *S. aureus*. Imidazole-derived carbazole compounds **2a–c** showed good antibacterial activities against bacteria with MIC values of 1–8 μg/mL and moderate antifungal activity (MIC = 16 μg/mL) against *C. albicans*, and exhibited better antibacterial activity than chloramphenicol or almost equipotent to norfloxacin. In addition, compounds **2a–c** showed potent activity against MRSA with MIC values of 4–8 μg/mL. Triazole compounds **3a–c** gave comparable activities against *C. albicans* (MIC = 2–4 μg/mL) with corresponding reference drug fluconazole (MIC = 0.5 μg/mL). The triazole derivative **3c** containing a $(CH_2)_6$ spacer also showed moderate potency against MRSA, *E. coli* and *P. aeruginosa*.

Compared to imidazoles **2a–c** and triazoles **3a–c**, carbazole N-substituted bromides **1a–c** and bis-carbazole compounds **5a–c** showed poor antimicrobial activities. Unexpectedly, the introduction of 1,2,4-triazole moiety in carbazole compounds **3a–c** instead of imidazole ring decreased their antibacterial activities, and increased their antifungal activities against *C. albicans*, in comparison with imidazoles **2a–c** and triazoles **3a–c**. These facts suggested that the azole (imidazole or 1,2,4-triazole) residue should be specially helpful for biological activity, imidazole moiety seems to be favourable for antibacterial efficacy against tested bacteria, while 1,2,4-triazole group is conducive to antifungal activities such as *C. albicans*. Moreover, the structural factors of linker including the type of the bridged groups such as aryl and alkyl ones, as well as the length of the bridged alkyl chain could efficiently affect their antimicrobial activities. Here an effect of the bridged linkers on the antibacterial and antifungal activities was not obvious, and further



Scheme 1. Synthetic route of N-substituted carbazole derivatives.

Table 1
Some characteristic, in vitro antibacterial and antifungal activities as MIC_{50} (μg/mL) for synthetic N-substituted carbazole derivatives **1–5**

Compd	Mp (°C)	Yield (%)	*S. aureus*	MRSA	*B. subtilis*	*E. coli*	*P. aeruginosa*	*B. proteus*	*C. albicans*	*A. fumigatus*
1a	143–144	41.0	128	>512	256	256	256	512	>512	>512
1b	123–124	77.5	256	>512	>512	>512	128	>512	512	>512
1c	58–59	60.0	128	>512	>512	>512	>512	>512	>512	512
2a	97–99	89.2	1	8	4	8	1	4	16	>512
2b	73–74	84.9	2	4	4	8	1	1	16	64
2c	Oil	83.5	8	8	8	4	1	1	16	128
3a	121–123	91.1	256	>512	>512	32	>512	>512	4	>512
3b	79–80	86.6	256	>512	>512	32	128	>512	2	>512
3c	78–79	76.4	32	64	>512	16	32	>512	2	>512
4	229–230	82.4	2	8	2	2	4	8	32	64
5a	246–248	78.2	256	>512	>512	>512	>512	>512	>512	>512
5b	228–229	86.4	>512	>512	>512	>512	>512	64	>512	>512
5c	118–119	84.7	128	>512	>512	>512	>512	>512	>512	256
Fluconazole	—	—	—	—	—	—	—	—	0.5	512
Chloramphenicol	—	—	64	8	>512	64	>512	16	—	—
Norfloxacin	—	—	8	1	1	0.5	2	1	—	—

study is necessary in order to deduce the structure–activity relationships.

The carbazole triazolium compound **4**, a quaternization product of triazole **3a**, displayed comparable or even better efficacy than the standard drugs chloramphenicol or norfloxacin against all tested strains with MIC values ranging from 1 to 64 μg/mL. In comparison with triazole **3a**, its corresponding triazolium **4** gave excellent antibacterial and antifungal activities except for *C. albicans* (see Table 1). It was also noteworthy that anti-MRSA activity of compound **4** was comparable to that of chloramphenicol. This means the halobenzyl triazolium electropositive moiety plays an important role in antimicrobial activity, which could not only broaden the antimicrobial spectrum of activity, but also greatly increase potency. On the other hand, amphiphilic quaternary ammonium compounds especially imidazoliums are generally known to be utilized for the recognition to anions such as halides, dihydrogen phosphate and dicarboxylates by the strong $(C–H)^{+}\cdots X^{-}$ hydrogen binding between imidazolium moieties and these anions.[15] However, the studies about anion receptors dealing with the triazolium which is the analogue of imidazolium are seldom observed. Carbazole-based derivatives, having desirable electronic and charge-transport properties as well as large π-conjugated system, as good fluorophoric receptors were found to be able to be used as phosphate and fluoride ion sensors and as fluorescence markers for cancer cells.[16] Our preliminary researches have revealed that compound **4** containing triazolium group and carbazole moiety showed high binding affinity with anions by virtue of forming strong $[C–H]^{+}\cdots$anion hydrogen bonds. Further study on anion recognition towards various biologically important anions and the effects of anions on antimicrobial activities is in progress.

In conclusion, a series of carbazole-based azole derivatives were designed and synthesized for the first time via an easy, convenient and efficient synthetic route. The antimicrobial results showed that carbazoles in combination with imidazole derivatives **2a–c** were the most active compounds against *S. aureus*, MRSA, *B. subtilis*, *E. coli*, *P. aeruginosa* and *B. proteus* (MIC values of 1–8 μg/mL), and carbazole-triazole compounds **3a–c** possessed significant antifungal activities against *C. albicans*. Meanwhile, carbazole triazolium compound **4** exhibited good activity against both bacteria and fungi. Introduction of azole moiety (imidazole or 1,2,4-triazole) and electropositive group in carbazole derivatives could improve the antimicrobial activities. The minimum inhibition concentrations of several compounds were $\leqslant$4 μg/mL, which should be a good starting point for us to optimize the structure and obtain more potent antibacterial and antifungal agents with these scaffolds. Moreover, compounds **2a–c** and **4** should also be worthy to further investigate as potential lead compounds for new antibacterial agents against MRSA strain. Further in vivo and cytotoxicity studies of these potential compounds are necessary. Compound **4** as artificial anion receptor is expected to play important roles in supramolecular recognition to various anions via non-covalent interactions by the use of the desirable electronic and charge-transport properties, as well as large π-conjugated system, electropositive triazolium moiety in carbazole triazolium **4**. Synthesis of other similar carbazole derivatives and their antibacterial and antifungal activities including the effects of anions on antimicrobial efficacy as well as the mode of action of the prepared compounds and anion recognition towards various biologically important anions are under active investigation, which will be discussed in detail in the future paper.

Acknowledgments

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

References and notes

1. Moellering, R. C.; Graybill, J. R.; McGowan, J. E.; Corey, L. *Am. J. Med.* **2007**, *120*, 4.
2. Morin, J. F.; Leclerc, M.; Adès, D.; Siove, A. *Macromol. Rapid. Commun.* **2005**, *26*, 761.
3. Kim, D.; Lee, J. K.; Kang, S. O.; Ko, J. *Tetrahedron* **2007**, *63*, 1913.
4. (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* **2009**, *52*, 415; (b) Zhou, C. H.; Zhang, F. F.; Gan, L. L.; Zhang, Y. Y.; Geng, R. X. *Sci. China, Ser. B* **2009**, *39*, 208. in Chinese.
5. Knölker, H. J.; Reddy, K. R. *Chem. Rev.* **2002**, *102*, 4303.
6. (a) Knölker, H. J.; Fröhner, W.; Reddy, K. R. *Eur. J. Org. Chem.* **2003**, *4*, 740; (b) Hagiwara, H.; Choshi, T.; Fujimoto, H.; Sugino, E.; Hibino, S. *Tetrahedron* **2000**, *56*, 5807.
7. Cuong, N. M.; Wilhelm, H.; Porzel, A.; Arnold, N.; Wessjohann, L. *Nat. Prod. Res.* **2008**, *22*, 1428.
8. Rajakumar, P.; Sekar, K.; Shanmugaiah, V.; Mathivanan, N. *Eur. J. Med. Chem.* **2009**, *44*, 3040.
9. Rajakumar, P.; Sekar, K.; Shanmugaiah, V.; Mathivanan, N. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4416.
10. (a) Mi, J. L.; Zhou, C. H.; Bai, X. *Chin. J. Antibiot.* **2007**, *32*, 587. in Chinese; (b) Cai, J. L.; Li, S.; Zhou, C. H.; Gan, L. L.; Wu, J. *Chin. J. New Drugs* **2009**, *18*, 598. in Chinese; (c) Bai, X.; Zhou, C. H.; Mi, J. L. *Chem. Res. Appl.* **2007**, *19*, 721. in Chinese.
11. Güven, Ö. Ö.; Erdoğan, T.; Göker, H.; Yıldız, S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2233.
12. (a) 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; (b) Muroi, H.; Nihei, K. I.; Tsujimoto, K.; Kubo, I. *Bioorg. Med. Chem.* **2004**, *12*, 583.
13. *Experimental*: Melting points are uncorrected and were recorded on X-6 melting point apparatus. TLC analyses were done using pre-coated silica gel plates and visualization was done using UV lamp at 254 nm. IR spectra were recorded on a Bio-Rad FTS-185. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV 300 or Varian 400 spectrometer using TMS as an internal standard. Chemical shifts were given in δ ppm and signals are described as singlet (s),

doublet (d), triplet (t), quartet (q), broad (br) and multiplet (m). All the solvents used were analytical grade only. The mass spectra were recorded on FINIGAN TRACE GC/MS and HRMS.

Synthesis of 9-(4-(-imidazol-1-yl)butyl)-9H-carbazole (**2b**): To a 100 ml round flask, 9-(4-bromobutyl)-9*H*-carbazole (1.76 g, 6 mmol), imidazole (0.49 g, 7 mmol) NaH (0.2 g, 8 mmol) and THF (10 mL) solution were added. The mixture was stirred at room temperature for 48 h under a stream of nitrogen. After the reaction came to the end (monitored by TLC, eluent, chloroform/methanol, 10/1, V/V), THF was removed by a rotary evaporator and then the mixture was cooled. Water (30 mL) was added. The resulting solution was extracted with CH_2Cl_2 (3×30 mL). All the combined CH_2Cl_2 solutions were dried with anhydrous Na_2SO_4 and then evaporated under reduced pressure. The residue was purified via silica gel column chromatography (chloroform/methanol, 10/1, V/V) to give compound **2b** (1.43 g) as white solid. Yield 84.9%; mp 73–74 °C; IR (KBr) *v*: 3050 (Ar-H), 2931, 2863 (CH_2), 1594, 1484, 1452 (aromatic frame), 1325, 1230, 1152, 1076, 815, 751, 724, 664 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.76–1.84 (m, 2H, imidazole-CH_2CH_2), 1.88–1.92 (m, 2H, carbazole-CH_2CH_2), 3.79–3.82 (t, 2H, *J* = 6.8 Hz, imidazole-CH_2), 4.32–4.36 (t, 2H, *J* = 6.4 Hz, carbazole-CH_2), 6.76 (s, 1H, imidazole 4-H), 7.01 (s, 1H, imidazole 5-H), 7.23–7.27 (m, 2H, carbazole 3,6-H), 7.35 (m, 2H, carbazole 2,7-H), 7.37 (s, 1H, imidazole 2-H), 7.45–7.49 (m, 2H, carbazole 1,8-H), 8.09–8.12 (m, 2H, carbazole 4,5-H) ppm; MS (*m*/*z*): 312 $[M+Na]^+$, 290 $[M]^+$, 222 [M–imidazole]$^+$; HRMS (TOF) calcd for $C_{19}H_{20}N_3$ $[M+H]^+$, 290.1657; found, 290.1652.

Synthesis of 9-(6-(1H-1,2,4-triazol-1-yl)hexyl)-9H-carbazole (**3c**): A mixture of 1*H*-1,2,4-triazole (0.50 g, 7 mmol), 9-(6-bromohexyl)-9*H*-carbazole (1.44 g, 5 mmol), potassium carbonate (1.43 g, 10 mmol) and TBAB (tetrabutyl ammonium bromide, 5 mg) in acetonitrile (30 mL) was stirred at 45 °C. After the reaction came to the end (monitored by TLC, eluent, chloroform/methanol, 8/1, V/V), the solvent was evaporated and then water (30 mL) was added. The resulting mixture was extracted with CH_2Cl_2 (3×30 mL), the combined organic phase was dried over anhydrous Na_2SO_4 and then evaporated under reduced pressure. The resulting residue was purified via silica gel column chromatography (chloroform/methanol, 8/1, V/V) to give compound **3c** (1.06 g) as white solid. Yield 76.4%; mp 79–80 °C; IR (KBr) *v*: 3100, 3052 (Ar-H), 2928, 2857 (CH_2), 1595, 1511, 1485, 1454 (aromatic frame), 1274, 1235 (triazole frame), 1136, 1016, 885, 754, 725, 683 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.26–1.35 (m, 2H, carbazole-$CH_2CH_2CH_2$), 1.36–1.42 (m, 2H, triazole-$CH_2CH_2CH_2$), 1.78–1.84 (m, 2H, carbazole-CH_2CH_2), 1.85–1.91 (m, 2H, triazole-CH_2CH_2), 4.05–4.08 (t, 2H, *J* = 7 Hz, carbazole-CH_2), 4.28–4.31 (t, 2H, *J* = 7 Hz, triazole-CH_2), 7.21–7.23 (d, 1H, *J* = 6.8 Hz, carbazole 3-H), 7.24–7.25 (d, 1H, *J* = 4 Hz, carbazole 6-H), 7.36 (s, 1H, carbazole 2-H), 7.38 (s, 1H, carbazole 7-H), 7.44–7.48 (m, 2H, carbazole 1,8-H), 7.91 (s, 1H, carbazole 4-H), 7.95 (s, 1H, triazole 5-H), 8.09 (s, 1H, carbazole 5-H), 8.11 (s, 1H, triazole 3-H) ppm; MS (*m*/*z*): 341 $[M+Na]^+$, 319 $[M]^+$; HRMS (TOF) calcd for $C_{20}H_{23}N_4$ $[M+H]^+$, 319.1923; found, 319.1928.

Synthesis of 1-(4-((9H-carbazol-9-yl) methyl) benzyl-4- (2,4-dichlorobenzyl)-1H-1,2,4-triazolium chloride (**4**): A mixture of 9-(4-((1*H*-1,2,4-triazol-1-yl)methyl)benzyl)-9*H*-carbazole (0.74 g, 2.2 mmol) and 2,4-dichlorobenzyl chloride (0.49 g, 2.5 mmol) in acetonitrile (15 mL) was stirred at 80 °C. After the reaction came to the end, the solvent was evaporated under reduced pressure. The residue was washed three times with petroleum ether (30–60 °C) and dried to give compound **4** (0.97 g) as white solid. Yield 82.4%; mp 229–230 °C; IR (KBr) *v*: 3093, 3009 (Ar-H), 2940, 2817 (CH_2), 1630, 1594, 1573, 1528, 1483, 1459 (aromatic frame), 1347, 1329, 1183, 928, 775, 740, 559 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 5.52 (s, 2H, carbazole-CH_2), 5.55 (s, 2H, PhCH_2-triazole), 5.67 (s, 2H, triazole-CH_2-(2,4-dichlorobenzyl)), 7.18–7.19 (d, 2H, *J* = 3.2 Hz, Ph-H), 7.20–7.21 (d, 2H, *J* = 3 Hz, 2,4-dichlorobenzyl 5,6-H), 7.23 (s, 1H, 2,4-dichlorobenzyl 3-H), 7.30–7.32 (d, 2H, *J* = 8 Hz, Ph-H), 7.40–7.44 (m, 2H, carbazole 3,6-H), 7.51–7.54 (m, 1H, carbazole 2-H), 7.58 (s, 1H, carbazole 7-H), 7.59–7.60 (d, 1H, *J* = 3.6 Hz, carbazole 8-H), 7.61 (s, 1H, triazole 5-H), 7.74–7.75 (d, 1H, *J* = 2.4 Hz, carbazole 1-H), 8.16 (s, 1H, carbazole 4-H), 8.18 (s, 1H, carbazole 5-H), 9.23 (s, 1H, triazole 3-H) ppm; MS(*m*/*z*): 497 $[M-Cl]^+$; HRMS (TOF) calcd for $C_{29}H_{24}Cl_3N_4$ $[M+H]^+$, 533.1067; found, 533.1068.

Synthesis of 1,4-bis((9H-carbazol-9-yl)methyl) benzene (**5a**): To a 100 ml round flask, carbazole (2.17 g, 8 mmol), 1,4-bis(bromomethyl)benzene (1.08 g, 4 mmol), NaH (0.24 g, 10 mmol) and THF (10 mL) solution were added. The mixture was stirred at room temperature for 48 h under a stream of nitrogen. After the reaction came to the end (monitored by TLC, eluent, petroleum ether/chloroform, 2/1, V/V), THF was removed by a rotary evaporator. The residue was cooled and water (30 mL) was added. The resulting solution was extracted with CH_2Cl_2 (3×30 mL). All the combined CH_2Cl_2 solutions were dried with anhydrous Na_2SO_4 and then evaporated under reduced pressure. The resulting residue was purified via silica gel column chromatography (petroleum ether/chloroform, 2/1, V/V) to give compound **5a** (1.36 g) as white solid. Yield 78.2%; mp 246–248 °C; IR (KBr) *v*: 3046, 3026 (Ar-H), 2913, 2853 (CH_2), 1628, 1595, 1511, 1484, 1459, 1436 (aromatic frame), 1327, 1209, 1152, 1116, 848, 748, 716 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 5.45 (s, 4H, N–CH_2), 7.02 (s, 4H, Ph-H), 7.21–7.26 (m, 4H, carbazole 3,6-H), 7.30–7.32 (m, 4H, carbazole 2,7-H), 7.38–7.40 (d, 2H, *J* = 4 Hz, carbazole 1-H), 7.40–7.41 (d, 2H, *J* = 4 Hz, carbazole 8-H), 8.10–8.12 (m, 4H, carbazole 4,5-H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$) δ: 136.6 (carbazole 1′,8′-C), 126.8 (Ph 1-C), 125.8 (Ph 2-C), 122.5 (carbazole 4′,5′-C), 120.4 (carbazole 2,7-C), 119.2 (carbazole 4,5-C), 116.3 (carbazole 3,6-C), 108.8 (carbazole 1,8-C), 46.2 (N-CH_2) ppm; MS (*m*/*z*): 459 $[M+Na]^+$, 436 $[M]^+$; HRMS (TOF) calcd for $C_{32}H_{25}N_2$ $[M+H]^+$, 437.2018; found, 437.2015.

14. (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. Experimental determination of antibacterial and antifungal activities. The minimal inhibitory concentrations (MICs) of the title compounds were determined in vitro by the modified microbroth dilution method according to the methods defined by the National Committee for Clinical Laboratory Standards. The test strains were provided by the School of Pharmaceutical Sciences, Southwest University. Fluconazole and chloramphenicol obtained from their respective manufacturers served as controls. The prepared compounds **1**–**5** were evaluated for their antibacterial activity against *S. aureus* ATCC 29213, MRSA N 315 and *B. subtilis* ATCC 21216 as Gram-positive, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *B. proteus* ATCC 49027 as Gram-negative bacteria. The bacterial suspension was adjusted with sterile saline to a concentration of 1×10^5 CFU. The test compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare the stock solutions. The test compounds and reference drugs were prepared in Mueller–Hinton broth (Guangdong huaikai microbial sci. & tech co., Ltd, Guangzhou, Guangdong, China) by twofold serial dilution to obtain the required concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5 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 new compounds were evaluated for their antifungal activity against *C. albicans* ATCC 76615 and *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 $1–5 \times 10^3$ 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 (0.5–512 μg/mL) of each tested compound. These dilutions were inoculated and incubated at 35 °C for 24 h. The drug MIC_{50} was defined as the first well with an approximate 50% reduction in growth compared to the growth of the drug-free well. The minimum inhibitory concentration (MIC_{50}) values (in μg/mL) were summarized in Table 1.
15. Yun, S.; Ihm, H.; Kim, H. G.; Kim, J. K.; Kim, K. S. *J. Org. Chem.* **2003**, *68*, 2467.
16. Chang, C. C.; Kuo, I. C.; Ling, I. F.; Chen, C. T.; Chen, H. C.; Lou, P. J.; Lin, J. J.; Chang, T. C. *Anal. Chem.* **2004**, *76*, 4490.