

# Design, Synthesis and Relational Biological Evaluation of Novel Diphenylpiperazine 1,2,3-triazole Derivatives

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## Gan *et al.*: Synthesis and Biological Evaluation of Novel Diphenylpiperazine 1,2,3-triazole Derivatives

A series of novel diphenylpiperazine 1,2,3-triazole derivatives were designed, synthesized and characterized by proton nuclear magnetic resonance, carbon-13 nuclear magnetic resonance, infrared and mass spectroscopy. The prepared compounds were investigated *in vitro* for antibacterial, antifungal and cytotoxic activities. Preliminary results showed that nitroimidazole piperazine 14b was active against bacteria *Bacillus subtilis*, *Micrococcus luteus*, *Bacillus proteus*, *Escherichia coli* and *Bacillus typhi* with MIC values of 25 µg/ml, but showed moderate activity against *Candida albicans* and *Saccharomyces cerevisiae* (MIC- 50 µg/ml). Moreover, the 1,2,3-triazole-linked nitroimidazole piperazine compound 15b and benzimidazole piperazine 15e were found to be effective *in vitro* against the PC-3 cell line and the cell survival rates were only 67 and 60 %, respectively at a concentration of 100 µM in a dose-dependent manner. Further, molecular docking experiments suggested that the nitro and hydroxyl groups of compound 14b could insert into base-pairs of DNA hexamer duplex by the formation of hydrogen bonds with guanine of DNA.

**Key words:** Diphenylpiperazine, 1,2,3-triazole, antibacterial, antifungal, cytotoxicity

Increasing emergence of bacterial and fungal infections and new cancer cell lines has become growing threats to human health in the past few decades. More and more diseases and infections caused by some resistant microorganisms or cancers have failed to respond to conventional treatment, and in some cases, even last resort defenses have become ineffective. This situation has stimulated an urgent need to develop novel compounds with intriguing activities, broad biological spectra, fewer adverse effects, lower toxicity, especially with mechanisms of action distinct from the well-known clinical agents<sup>[1]</sup>.

It is well known that piperazine nucleus is an important fragment for bioactive compounds. This skeleton is able to form hydrogen bonds, coordinate with metal ions, accept protons and perform quaternization, and could not only regulate physicochemical properties of desired molecules but also interact with various enzymes and receptors in biological system which exerts broad bioactive spectrum<sup>[2,3]</sup>. Therefore, piperazine fragment has been widely presented in clinical drugs especially in antimicrobial field. The introduction of diphenyl moiety into molecules made them easily penetrate

the cell membrane with improved liposolubility and enhanced biological activities<sup>[4,5]</sup>. Recent research has revealed that diphenylpiperazine fragment possessed multiple pharmacological activities and compounds with this fragment included antihistamines, cetirizine and buclizine, antihypertensives, cinnarizine and dipfluzine, antimigraine agent lomerizine, anticancer agents and so on. Notably, this structure could directly react with the multidrug resistance-associated protein and inhibit its transportation to exhibit strong bioactivities<sup>[6]</sup>. Therefore, several investigators explored this scaffold to synthesize novel molecules with distinct mechanisms of action. Previous studies from our laboratory reported that diphenylpiperazine GLLa bearing imidazole ring exhibited equivalent bioactivity against *Staphylococcus aureus* similar to the standard chloromycin with a minimum inhibitory

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concentration (MIC) value of 3.1  $\mu\text{g/ml}$ , and also showed desirable antifungal activities against *Candida albicans* and *Saccharomyces cerevisiae* with MIC values between 3.1 and 12.5  $\mu\text{g/ml}$ , which were nearly close to fluconazole. The substitution of imidazole ring with benzimidazole moiety yielded compound GLLb, which resulted in good activity against the human prostatic carcinoma (PC-3) cell line<sup>[7]</sup>. This compelled the current investigation to prepare diphenylpiperazine-derived compounds with intriguing bioactivities. 1,2,3-Triazole, an unique electron-rich ring, endows its derivatives with extensive potential in medicinal chemistry<sup>[8]</sup>. This investigation dealt with the development of diphenylpiperazine 1,2,3-triazole derivatives as newer agents, which expected to play a large role in the treatment of fungal and bacterial infections, and cancer (fig. 1).

With the introduction of click chemistry, 1,2,3-triazole derivatives have opened up a new opportunity toazole compounds with interesting activity. 1,4-Disubstituted 1,2,3-triazoles could be easily employed as hydrogen bond acceptor due to the large dipole moment, which are favourable for binding to biological target sites and improving solubility<sup>[9]</sup>. Furthermore, 1,2,3-triazole could also act as an attractive bridge group to connect two pharmacophores and biologically beneficial fragments into one molecule to generate innovative multifunctional compounds<sup>[10]</sup>. Noticeably, the bioisosteric replacement between 1,2,3-triazole moiety and its bioisoster 1,2,4-triazole has received special attention in medical chemistry, which represented an efficient concept for the discovery and development of novel triazole drugs, significantly extending the chemical space of triazole scaffolds possessing potential activities or enhancing biological activities<sup>[11]</sup>.

In view of such exciting properties and as an extension of earlier studies on the development of diphenylpiperazine azoles, 1,2,3-triazole was

introduced into the diphenylpiperazine scaffold with the aim to investigate the effect on antimicrobial and anticancer activities. Various azolyl rings like 1,2,4-triazolyl<sup>[12]</sup> and its bioisosters imidazolyl<sup>[13]</sup> and benzimidazolyl<sup>[14-18]</sup> groups were introduced into the above modified structures to investigate the effect of azolyl rings on biological activities<sup>[19]</sup>. Moreover, aromatic 2,4-dichlorophenyl and naphthalenyl groups were incorporated to improve the pharmacological properties. Additionally, bisphenol A and enoxacin as important pharmacophores were present in the target molecules to study their effects on the bioactivity. The newly synthesized diphenylpiperazine 1,2,3-triazole derivatives were screened for antibacterial and antifungal activities as well as antitumor activity against PC-3 *in vitro*. Moreover, molecular docking studies were employed to study the interaction behaviours between the prepared compound and DNA hexamer duplex<sup>[20]</sup>.

## MATERIALS AND METHODS

Melting points (MPs) were recorded on X-6 melting point apparatus and uncorrected. Thin-layer chromatography (TLC) was performed on pre-coated silica gel plates. Fourier-transform infrared spectra were carried out on Bruker RFS100/S spectrophotometer (Bio-Rad, Cambridge, MA, USA) using KBr pellets in the 400-4000  $\text{cm}^{-1}$  range. Proton nuclear magnetic resonance (<sup>1</sup>H NMR) and <sup>13</sup>C NMR spectra were recorded on a Bruker AV 300 spectrometer using tetramethylsilane (TMS) as an internal standard. The following abbreviations were used to designate aryl groups, Pip for piperazine. Mass spectra were recorded on Finigan Trace GC/MS. All chemicals and solvents were commercially available and were used without further purification.

### Synthesis:

1-(Prop-2-ynyl)-1*H*-1,2,4-triazole 2a was prepared according to the literature, starting from 1*H*-1,2,4-triazole (2.00 g, 29.0 mmol) and 3-bromo-1-propyne 1 (2.10 g, 17.6 mmol). Yield: 71 %; MP: 56-57<sup>o</sup><sup>[21]</sup>. 4-Nitro-1-(prop-2-ynyl)-1*H*-imidazole 2b was prepared starting from 4-nitro-1*H*-imidazole (2.00 g, 17.6 mmol) and 3-bromo-1-propyne 1 (2.10 g, 17.6 mmol). Yield: 71 %; MP: 126-128<sup>o</sup><sup>[22]</sup>. 2-Methyl-5-nitro-1-(prop-2-ynyl)-1*H*-imidazole 2c was prepared starting from 2-methyl-5-nitro-1*H*-imidazole (2.00 g, 15.7 mmol) and 3-bromo-1-propyne 1 (1.85 g, 15.7 mmol). Yield: 80 %; MP: 130-132<sup>o</sup><sup>[22]</sup>.

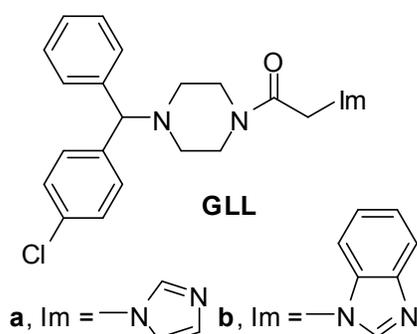


Fig. 1: Structures of 1-((4-chlorophenyl)(phenyl)methyl)piperazine derivatives

### Synthesis of 2-phenyl-1-(prop-2-ynyl)-1H-imidazole 2d:

To a solution of 2-phenyl-1H-imidazole (1.00 g, 6.9 mmol) and sodium hydride (0.334 g, 13.8 mmol) in dry tetrahydrofuran 10 ml was added 3-bromo-1-propyne 1 (1.20 g, 10.2 mmol) at 40°. After 5 h, the reaction system was added 1 N hydrochloric acid to pH=7 at 0°. The solvent was removed and the residue was extracted with chloroform (3×20 ml), dried over anhydrous sodium sulphate, and then the solvent chloroform was removed *in vacuo* to give compound 2d as oil. Yield: 70 %; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ: 7.50-7.48 (m, 5H, Ar-H), 7.02 (s, 1H, imidazole-H), 6.92 (s, 1H, imidazole-H), 3.11 (s, 2H, CH<sub>2</sub>), 1.78 (s, 1H, CH) ppm; MS (m/z): 182 [M]<sup>+</sup>. Compound 1-(prop-2-ynyl)-1H-benzo[d]imidazole 2e was prepared according to the procedure described for compound 2d, starting from benzimidazole (1.20 g, 10.2 mmol) and 3-bromo-1-propyne (1.20 g, 10.2 mmol). The pure product 2e was obtained as yellow oil. Yield: 19 %<sup>[23]</sup>.

### Synthesis of 2,4-dichloro-1-(prop-2-ynyloxy)benzene 3a:

A suspension of 2,4-dichlorophenol (1.00 g, 6.1 mmol), potassium carbonate (1.64 g, 11.9 mmol) and tetrabutylammonium iodide (trace) was stirred in acetone/water (1/1, 10 ml). After 2 h, 3-bromo-1-propyne (2.10 g, 17.6 mmol) was added and the reaction system was stirred at room temperature continuously. After the reaction was completed (monitored by TLC, eluent, petroleum ether, R<sub>f</sub>=0.5), the solvent was removed and the residue was extracted with ethyl acetate (3×20 ml), dried over anhydrous sodium sulphate and purified by silica gel column chromatography (eluent, petroleum ether) to afford compound 3a as white solid. Yield: 60 %; MP: 56-58°<sup>[24]</sup>. Compound 2-(prop-2-ynyloxy)naphthalene 3b was prepared according to the procedure described for compound 3a, starting from naphthalen-2-ol (1.5 g, 10.4 mmol) and 3-bromo-1-propyne (2.1 g, 17.6 mmol). The pure product 3b was obtained as yellow solid. Yield: 65 %; MP: 69-71°<sup>[25]</sup>. Compound 4,4'-(propane-2,2-diyl)bis((prop-2-ynyloxy)benzene) 4 was prepared according to the procedure described for compound 3a, starting from bisphenol A (1.35 g, 5.9 mmol) and 3-bromo-1-propyne (2.10 g, 17.6 mmol). The pure product 4 was obtained as yellow solid. Yield: 54 %; MP: 70-71°<sup>[25]</sup>.

### Synthesis of 1-ethyl-6-fluoro-4-oxo-7-(4-(prop-2-ynyl)piperazin-1-yl)-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid 5:

A suspension of enoxacin (0.20 g, 0.625 mmol) and potassium carbonate (0.087 g, 0.625 mmol) was stirred in acetonitrile (20 ml). 3-Bromo-1-propyne (0.12 g, 1.00 mmol) was added at 0°, and after 1 h, the reaction system was stirred at room temperature continuously. After the reaction was completed (monitored by TLC, eluent, chloroform/methanol, 15/1, V/V, R<sub>f</sub>=0.5), the reaction system was filtered, and the residue was extracted with acetonitrile (3×20 ml), dried over anhydrous sodium sulphate and purified by silica gel column chromatography (eluent, chloroform/methanol, 30/1, V/V) to afford compound 5 as white solid. Yield: 50 %; MP: 260°; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ: 15.06 (s, 1H, COOH), 8.70 (s, 1H, enoxacin-2-H), 8.12 (d, J=12.0 Hz, 2H), 4.45-4.27 (q, J=12.0 Hz, 2H, CH<sub>2</sub>CH<sub>3</sub>), 3.94 (s, 4H, Pip), 3.41 (s, 2H, CH<sub>2</sub>), 2.75 (s, 4H, Pip), 2.30 (s, 1H, CH), 1.51 (t, J=6.0 Hz, 3H, CH<sub>2</sub>CH<sub>3</sub>) ppm; MS (m/z): 359 [M]<sup>+</sup>.

Compound (4-chlorophenyl)(phenyl)methanol 7 was prepared according to the procedure described in the literature, starting from (4-chlorophenyl)(phenyl)methanone (10.02 g, 46.4 mmol) and sodium borohydride (2.01 g, 53 mmol). The pure product 7 was obtained as white solid. Yield: 95 %; MP: 65-67°<sup>[7]</sup>. Compound 1-chloro-4-(chloro(phenyl)methyl)benzene 8 was prepared according to the procedure described in the literature, starting from (4-chlorophenyl)(phenyl)methanol (9.03 g, 41.1 mmol) and thionyl chloride (6.16 g, 52 mmol). The pure product 8 was obtained as yellow oil. Yield: 95 %<sup>[7]</sup>. Compound 1-((4-chlorophenyl)(phenyl)methyl)piperazine 9 was prepared according to the procedure described in the literature, starting from 1-chloro-4-(chloro(phenyl)methyl)benzene (6.02 g, 25.3 mmol) and piperazine (10.00 g, 116 mmol). The pure product 9 was obtained as white solid. Yield: 62 %; MP: 66-68°<sup>[7]</sup>.

Compound 1-chloro-3-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)propan-2-ol 10 was prepared according to the procedure described in the literature, starting from 1-[(4-chlorophenyl)(phenyl)methyl]piperazine (5.02 g, 17.4 mmol) and 2-(chloromethyl)oxirane (4.81 g, 52.2 mmol). The pure product 10 was obtained as white solid. Yield: 40 %; MP: 70-71°<sup>[7]</sup>. Compound 2-chloro-1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)ethanone 11 was prepared according to the procedure described in the literature, starting from 1-[(4-chlorophenyl)(phenyl)methyl]piperazine (7.02 g, 24.4 mmol) and 2-chloroacetyl chloride (6.03 g, 53.0 mmol). The pure product 11 was obtained as yellow oil. Yield: 45 %<sup>[7]</sup>.

### Synthesis of 1-azido-3-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)propan-2-ol 12:

A suspension of compound 10 (2.35 g, 6.2 mmol) and sodium azide (0.61 g, 9.3 mmol) was stirred in acetone/water (1/1, V/V, 30 ml) at 40° overnight. After the reaction was completed, the solvent was removed *in vacuo* and the residue was extracted with ethyl acetate (3×20 ml), dried over anhydrous sodium sulphate, and then the solvent ethyl acetate was removed *in vacuo* to give compound 12 as yellow oil. Yield: 95 %; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ 7.45-7.35 (m, 9H, Ar-H), 4.23 (s, 1H, Ar<sub>2</sub>CH), 3.65 (m, 2H), 2.91 (m, 2H), 2.74-2.62 (m, 8H, Pip) ppm.

Compound 2-azido-1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)ethanone 13 was prepared according to the procedure described for compound 12, starting from compound 11 (2.01 g, 5.5 mmol) and sodium azide (1.07 g, 16.5 mmol). The pure product 13 was obtained as oil. Yield: 87 %; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ 7.43-7.22 (m, 9H, Ar-H), 4.31 (s, 1H, Ar<sub>2</sub>CH), 4.02 (s, 2H, COCH<sub>2</sub>), 3.70 (bs, 2H, Pip), 3.52 (bs, 2H, Pip), 2.50-2.41 (bs, 4H, Pip) ppm.

### Synthesis of 1-(4-((1H-1,2,4-triazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)-3-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)propan-2-ol 14a:

To a solution of compound 12 (0.10 g, 0.66 mmol) in a *t*-BuOH/acetone/H<sub>2</sub>O (10 ml, 1/1/1, v/v/v) mixture were added sodium ascorbate (0.4 eq.) and copper (II) sulphate pentahydrate (0.2 eq.) successively. Here after compound 2a (0.245 g, 0.66 mmol) was added, and the mixture was stirred at 60°. After the reaction came to the end (monitored by TLC, eluent, chloroform/methanol, 50/1, V/V, R<sub>f</sub>=0.3), the solvent was removed and the residue was extracted with chloroform (3×20 ml), dried over anhydrous sodium sulphate and purified by silica gel column chromatography (eluent, chloroform/methanol, 100/1, V/V) to afford compound 14a as white solid. Yield: 60 %; MP: 127-128°; IR (KBr): ν 3059, 3027, 2967, 2921, 2860, 1658 (C=O), 1495, 1457, 1423, 1238, 1137, 1049, 850, 744, 702 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ: 8.20 (s, 1H, 1,2,4-triazole 3-H), 7.94 (s, 1H, 1,2,4-triazole 5-H), 7.81 (s, 1H, 1,2,3-triazole 5-H), 7.34-7.26 (m, 9H, Ar-H), 5.50 (s, 2H, CH<sub>2</sub>-1,2,4-triazole), 4.52 (d, 1H, *J*=12.0 Hz, 1,2,3-triazole-CH<sub>2</sub>), 4.32-4.25 (m, 1H, CH(OH)), 4.20 (s, 1H, Ar<sub>2</sub>CH), 4.13-4.08 (m, 1H, 1,2,3-triazole-CH<sub>2</sub>), 2.69 (bs, 2H, NCH<sub>2</sub>CH(OH)), 2.46-2.18 (m, 8H, Pip) ppm; MS (m/z): 515 [M+Na]<sup>+</sup>.

Compound 1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)-3-(4-((4-nitro-1H-imidazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propan-2-ol 14b was prepared according to the procedure described for compound 14a, starting from compound 12 (0.50 g, 1.29 mmol) and compound 2b (0.20 g, 1.29 mmol). The pure product 14b was obtained as white solid. Yield: 72 %; MP: 117-118°; IR (KBr): ν 3143, 3027, 2952, 2882, 1545, 1490, 1451, 1337, 1135, 1052, 823, 757, 702 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ: 7.87 (s, 1H, 1,2,3-triazole 5-H), 7.85 (s, 1H, nitroimidazole-5H), 7.58 (s, 1H, nitroimidazole-2H), 7.35-7.21 (m, 9H, Ar-H), 5.30 (s, 2H, CH<sub>2</sub>-nitroimidazole), 4.57 (d, 1H, *J*=13.7 Hz, 1,2,3-triazole-CH<sub>2</sub>), 4.34-4.27 (m, 1H, CH(OH)), 4.23 (s, 1H, Ar<sub>2</sub>CH), 4.13 (d, 1H, 1,2,3-triazole-CH<sub>2</sub>), 2.77 (bs, 2H, NCH<sub>2</sub>CH(OH)), 2.56-2.33 (m, 8H, Pip) ppm; MS (m/z): 559 [M+Na]<sup>+</sup>, 537 [M]<sup>+</sup>.

Compound 2-(4-((1H-1,2,4-triazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)-1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)ethanone 15a was prepared according to the procedure described for compound 14a, starting from compound 13 (0.35 g, 0.95 mmol) and compound 2a (0.10 g, 0.95 mmol). The pure product 15a was obtained as white solid. Yield: 66 %; MP: 107-108°; IR (KBr): ν 3122, 3027, 2961, 2861, 1661 (C=O), 1561, 1506, 1487, 1451, 1419, 1138, 1051, 851, 703 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ: 8.20 (s, 1H, 1,2,4-triazole 3-H), 7.94 (s, 1H, 1,2,4-triazole 5-H), 7.78 (s, 1H, 1,2,3-triazole 5-H), 7.34-7.26 (m, 9H, Ar-H), 5.50 (s, 2H, CH<sub>2</sub>-1,2,3-triazole), 5.19 (s, 2H, COCH<sub>2</sub>), 4.24 (s, 1H, Ar<sub>2</sub>CH), 3.63-3.54 (d, 4H, Pip), 2.41 (bs, 4H, Pip) ppm; MS (m/z): 509 [M+Na]<sup>+</sup>.

Compound 1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)-2-(4-((4-nitro-1H-imidazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)ethanone 15b was prepared according to the procedure described for compound 14a, starting from compound 13 (0.24 g, 0.66 mmol) and compound 2b (0.10 g, 0.66 mmol). The pure product 15b was obtained as white solid. Yield: 60 %; MP: 127-128°; IR (KBr): ν 3059, 3027, 2967, 2921, 2860, 1658 (C=O), 1545, 1495, 1457, 1423, 1337, 1238, 1138, 1049, 850, 744, 702 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ: 7.87 (s, 1H, 1,2,3-triazole 5-H), 7.82 (s, 1H, nitroimidazole 5-H), 7.57 (s, 1H, nitroimidazole 2-H), 7.35-7.27 (m, 9H, Ar-H), 5.32 (s, 2H, CH<sub>2</sub>-nitroimidazole), 5.24 (s, 2H, COCH<sub>2</sub>), 4.27 (s, 1H, Ar<sub>2</sub>CH), 3.65-3.57 (bs, 4H, Pip), 2.43 (bs, 4H, Pip) ppm; MS (m/z): 543 [M+Na]<sup>+</sup>.

Compound 1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)-2-(4-((2-methyl-5-nitro-1H-imidazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)ethanone 15c was prepared according to the procedure described for compound 14a, starting from compound 13 (0.44 g, 1.21 mmol) and compound 2c (0.20 g, 1.21 mmol). The pure product 15c was obtained as white solid. Yield: 62 %; MP: 173-175 °; IR (KBr):  $\nu$  3059, 3027, 2967, 2921, 2860, 1658 (C=O), 1545, 1495, 1457, 1423, 1337, 1238, 1138, 1049, 850, 744, 702  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ : 7.74 (s, 2H, 1,2,3-triazole 5-H, imidazole 4-H), 7.35-7.26 (m, 9H, Ar-H), 5.22 (s, 4H, imidazole- $\text{CH}_2$ ,  $\text{COCH}_2$ ), 4.25 (s, 1H,  $\text{Ar}_2\text{CH}$ ), 3.63 (s, 2H, Pip), 3.55 (s, 2H, Pip), 2.50 (s, 3H,  $\text{CH}_3$ ), 2.40 (bs, 4H, Pip) ppm; MS (m/z): 535  $[\text{M}]^+$ .

Compound 1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)-2-(4-((2-phenyl-1H-imidazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)ethanone 15d was prepared according to the procedure described for compound 14a, starting from compound 13 (0.38 g, 1.02 mmol) and compound 2d (0.19 g, 1.02 mmol). The pure product 15d was obtained as white solid. Yield: 62 %; MP: 96-98 °;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ : 7.63-7.10 (m, 17H, Ar-H, 1,2,3-triazole 5-H, imidazole-H), 5.39 (s, 2H, imidazole- $\text{CH}_2$ ), 5.32 (s, 2H,  $\text{COCH}_2$ ), 4.23 (s, 1H,  $\text{Ar}_2\text{CH}$ ), 3.70 (bs, 2H, Pip), 3.50 (bs, 2H, Pip), 2.39 (bs, 4H, Pip) ppm; MS (m/z): 552  $[\text{M}]^+$ .

Compound 2-(4-((1H-benzo[d]imidazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)-1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)ethanone 15e was prepared according to the procedure described for compound 14a, starting from compound 13 (0.35 g, 0.96 mmol) and compound 2e (0.15 g, 0.96 mmol). The pure product 15e was obtained as white solid. Yield: 60 %; MP: 93-95 °;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ : 7.99 (s, 1H, benzimidazole-H), 7.79 (s, 1H, benzimidazole-H), 7.51 (s, 1H, 1,2,3-triazole 5-H), 7.44 (s, 1H, benzimidazole-H), 7.32-7.21 (m, 10H, Ar-H, benzimidazole-H), 5.47 (s, 2H, benzimidazole- $\text{CH}_2$ ), 5.01 (s, 2H,  $\text{COCH}_2$ ), 4.20 (s, 1H,  $\text{Ar}_2\text{CH}$ ), 3.58 (s, 2H, Pip), 3.47 (s, 2H, Pip), 2.35 (bs, 4H, Pip) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$ : 162.9, 143.9, 142.8, 141.1, 140.3, 133.5, 133.0, 129.0, 128.9, 128.8, 127.7, 127.6, 123.9, 123.2, 122.4, 120.4, 109.8, 75.0, 51.5, 51.1, 50.8, 45.4, 42.5, 40.5 ppm; MS (m/z): 526  $[\text{M}]^+$ .

Compound 1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)-3-(4-((2,4-dichlorophenoxy)methyl)-1H-1,2,3-triazol-1-yl)propan-2-ol 16a was prepared according to the procedure described for compound

14a, starting from compound 12 (0.39 g, 1.0 mmol) and compound 3a (0.20 g, 1.0 mmol). The pure product 16a was obtained as white solid. Yield: 70 %; MP: 82-83 °; IR (KBr):  $\nu$  3145, 3027, 2967, 2921, 2860, 1545, 1518, 1490, 1451, 1238, 1138, 1049, 823, 757, 702  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ : 7.86 (s, 1H, 1,2,3-triazole 5-H), 7.35-7.16 (m, 11H, Ar-H), 7.06-7.03 (d, 1H,  $J=8.8$  Hz, Ar-H), 5.3 (s, 2H,  $\text{OCH}_2$ ), 4.53 (d, 1H,  $J=14.0$  Hz, 1,2,3-triazole- $\text{CH}_2$ ), 4.35-4.29 (m, 1H,  $\text{CH}(\text{OH})$ ), 4.20 (s, 1H,  $\text{Ar}_2\text{CH}$ ), 4.16-4.09 (m, 1H, 1,2,3-triazole- $\text{CH}_2$ ), 2.68 (bs, 2H,  $\text{NCH}_2\text{CH}(\text{OH})$ ), 2.43-2.18 (m, 8H, pip) ppm; MS (m/z): 588  $[\text{M}]^+$ .

Compound 1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)-3-(4-((naphthalen-2-yloxy)methyl)-1H-1,2,3-triazol-1-yl)propan-2-ol 16b was prepared according to the procedure described for compound 14a, starting from compound 12 (0.65 g, 1.7 mmol) and compound 3b (0.31 g, 1.7 mmol). The pure product 16b was obtained as white solid. Yield: 85 %; MP: >260 °; IR (KBr):  $\nu$  3145, 3027, 2967, 2921, 2860, 1599, 1545, 1518, 1451, 1238, 1138, 1049, 823, 757, 702  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ : 7.85 (s, 1H, 1,2,3-triazole 5-H), 7.76-6.82 (m, 16H, Ar-H), 5.3 (s, 2H,  $\text{OCH}_2$ ), 4.53 (d, 1H,  $J=14.0$  Hz, 1,2,3-triazole- $\text{CH}_2$ ), 4.35-4.29 (m, 1H,  $\text{CH}(\text{OH})$ ), 4.20 (s, 1H,  $\text{Ar}_2\text{CH}$ ), 4.16-4.09 (m, 1H, 1,2,3-triazole- $\text{CH}_2$ ), 2.68 (bs, 2H,  $\text{NCH}_2\text{CH}(\text{OH})$ ), 2.43-2.18 (m, 8H, Pip) ppm; MS (m/z): 569  $[\text{M}]^+$ .

Compound 1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)-2-(4-((2,4-dichlorophenoxy)methyl)-1H-1,2,3-triazol-1-yl)ethanone 17a was prepared according to the procedure described for compound 14a, starting from compound 13 (0.37 g, 1.0 mmol) and compound 3a (0.20 g, 1.0 mmol). The pure product 17a was obtained as white solid. Yield: 70 %; MP: 112-114 °; IR (KBr):  $\nu$  3145, 3027, 2967, 2923, 1664 (C=O), 1483, 1416, 1238, 1136, 1056, 804, 758  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ : 7.83 (s, 1H, 1,2,3-triazole 5-H), 7.36-7.16 (m, 11H, Ar-H), 7.05-7.03 (d, 1H,  $J=8.8$  Hz, Ar-H), 5.26 (s, 2H,  $\text{OCH}_2$ ), 5.19 (s, 2H,  $\text{COCH}_2$ ), 4.23 (s, 2H,  $\text{Ar}_2\text{CH}$ ), 3.63-3.53 (d, 4H, Pip), 2.39 (s, 4H, Pip) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$ : 163.02 (C=O), 152.57, 143.60, 141.10, 140.36, 132.99, 130.02, 129.05, 128.88, 128.81, 127.70, 127.64, 127.56, 126.45, 124.76, 123.99, 115.10, 75.00, 63.41, 51.55, 51.12, 50.88, 45.41, 42.43 ppm; MS (m/z): 595  $[\text{M}+\text{Na}]^+$ .

Compound 1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)-2-(4-((naphthalen-2-yloxy)methyl)-1H-1,2,3-triazol-1-yl)ethanone 17b was prepared

according to the procedure described for compound 14a, starting from compound 13 (0.63 g, 1.7 mmol) and compound 3b (0.31 g, 1.7 mmol). The pure product 17b was obtained as white solid. Yield: 85 %; MP: >260°; IR (KBr):  $\nu$  3145, 3059, 3027, 2960, 2922, 2862, 1665 (C=O), 1599, 1510, 1451, 1238, 1141, 1049, 838, 757  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ : 7.83 (s, 1H, 1,2,3-triazole 5-H), 7.78-6.85 (m, 16H, Ar-H), 5.34 (s, 2H,  $\text{OCH}_2$ ), 5.20 (s, 2H,  $\text{COCH}_2$ ), 4.21 (s, 1H,  $\text{Ar}_2\text{CH}$ ), 3.63-3.54 (d, 4H, Pip), 2.39 (s, 4H, Pip) ppm;  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 75 MHz)  $\delta$ : 163.15(C=O), 156.18, 144.45, 141.19, 140.41, 134.45, 133.67, 129.54, 129.22, 129.09, 128.91, 128.84, 127.76, 127.63, 126.92, 126.44, 124.41, 123.88, 118.85, 107.35, 75.03, 62.06, 51.65, 51.19, 50.91, 45.58, 42.52 ppm; MS (m/z): 575  $[\text{M}+\text{Na}]^+$ .

Compound 2,2'-(4,4'-(4,4'-(propane-2,2-diyl)bis(4,1-phenylene))bis(oxy)bis(methylene)bis(1H-1,2,3-triazole-4,1-diyl))bis(1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)ethanone) 18 was prepared according to the procedure described for compound 14a, starting from compound 13 (0.98 g, 2.64 mmol) and compound 4 (0.4 g, 1.32 mmol). The pure product 18 was obtained as white solid. Yield: 69 %; MP: >260°; IR (KBr):  $\nu$  3144, 3060, 3028, 2965, 2868, 1664 (C=O), 1607, 1509, 1451, 1237, 1138, 1048, 830, 758  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ : 7.78 (s, 2H, 1,2,3-triazole 5-H), 7.35-6.85 (m, 28H, Ar-H), 5.20 (s, 8H,  $\text{OCH}_2$ ,  $\text{COCH}_2$ ), 4.23 (s, 2H,  $\text{Ar}_2\text{CH}$ ), 3.62-3.53 (d, 8H,  $\text{N}(\text{CH}_2\text{CH}_2)_2\text{NCO}$ ), 2.39 (s, 8H,  $\text{N}(\text{CH}_2\text{CH}_2)_2\text{NCO}$ ) ppm;  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 75 MHz)  $\delta$ : 163.40(C=O), 156.38, 144.94, 143.87, 141.42, 140.63, 133.26, 129.31, 129.12, 129.05, 128.01, 127.98, 127.78, 124.55, 114.40, 75.26, 62.29, 51.85, 51.40, 51.10, 45.80, 42.73, 41.95, 31.22 ppm; MS (m/z): 382  $[\text{M}]^+$ .

#### Synthesis of 7-(4-(2-(4-((4-chlorophenyl)(phenyl)methyl)piperazine-1-yl)-2-oxoethyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid 19:

To a solution of enoxacin (0.20 g, 0.625 mmol) and potassium carbonate (0.087 g, 0.625 mmol) in acetonitrile 20 ml of compound 11 (0.23 g, 0.625 mmol) was added at 0°. After 1 h, the mixture was stirred at room temperature for 12 h, the solvent was removed and the residue was extracted with chloroform (320 ml), dried over anhydrous sodium sulphate and purified by silica gel column chromatography (eluent, ethyl acetate/petroleum ether, 2/1, V/V) to afford compound 19

as white solid. Yield: 49 %; MP: 165-167°;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ : 15.04 (s, 1H, COOH), 8.56 (s, 1H, enoxacin-2-H), 8.10 (d,  $J=12.0$  Hz, 2H), 7.35-7.22 (m, 9H, Ar-H), 4.43-4.36 (q,  $J=6.0$  Hz, 2H,  $\text{CH}_2\text{CH}_3$ ), 4.23 (s, 1H,  $\text{Ar}_2\text{CH}$ ), 3.88 (s, 4H, Pip), 3.60 (s, 4H, Pip), 3.25 (s, 2H,  $\text{COCH}_2$ ), 2.69 (s, 4H, Pip), 2.38 (s, 4H, Pip), 1.49 (t,  $J=6.0$  Hz, 3H,  $\text{CH}_2\text{CH}_3$ ) ppm; MS (m/z): 647  $[\text{M}]^+$ .

#### Antibacterial and antifungal assays:

MIC ( $\mu\text{g/ml}$ ) is defined as the lowest concentration of target compounds that completely inhibit the growth of bacteria, by means of standard two-fold serial dilution method in 96-well microtiter plates according to the National Committee for Clinical Laboratory Standards (NCCLS). The tested microorganism strains were provided by the School of Pharmaceutical Sciences, Southwest University and the College of Pharmacy, Third Military Medical University. Chloramphenicol and fluconazole, were used as control drugs. Dimethylsulfoxide (DMSO) with bacterial culture and without test compounds was used as positive control to ensure that the solvent had no effect on bacteria growth. All the bacterial and fungal growth was monitored visually and spectrophotometrically, and the experiments were performed in triplicate.

#### Antibacterial assays:

The prepared compounds 14-19 were evaluated for antibacterial activities against Gram-positive bacteria (*S. aureus* ATCC 6538, methicillin-resistant *S. aureus* N315 (MRSA), *Micrococcus luteus* and *Bacillus subtilis* ATCC 21216), Gram-negative bacteria (*Escherichia coli* ATCC 8099, *Pseudomonas aeruginosa* ATCC 27853, *B. typhi* and *B. proteus* ATCC 13315). The bacterial suspension was adjusted with sterile saline to a concentration of  $1 \times 10^5$  CFU. Initially the compounds were dissolved in DMSO to prepare the stock solutions, then the tested compounds and reference drugs were prepared in Mueller-Hinton broth (Guangdong Huaikai Microbial Science & Tech Co., Ltd., Guangzhou, Guangdong, China) to obtain the required concentrations of 400, 200, 100, 50, 25, 12.5, 6.25, 3.1, 1.6, 0.8 and 0.4  $\mu\text{g/ml}$ . These dilutions were inoculated and incubated at 37° for 24 h.

#### Antifungal assays:

The newly synthesized compounds 14-19 were evaluated for antifungal activities against *C. albicans* ATCC 76615, *Aspergillus fumigatus* ATCC 96918, *Calathea utilis*, *S. cerevisiae* and *A. flavus*. A spore

suspension in sterile distilled water was prepared from one day old culture of the fungi growing on Sabouraud agar (SA) media. The final spore concentration was  $1-5 \times 10^3$  spore/ml. From the stock solutions of the tested compounds and reference antifungal drug fluconazole, dilutions in sterile RPMI 1640 medium (Neuronbc Laboraton Technology CO., Ltd, Beijing, China) were made resulting in eleven wanted concentrations (0.4 to 400  $\mu\text{g/ml}$ ) of each tested compound. These dilutions were inoculated and incubated at  $35^\circ$  for 24 h.

### 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay for cytotoxic activity:

PC-3 cell line was purchased from the Shanghai Institutes for Biological Sciences, China. Ham's F12 nutrient mixture, enriched with 10 % heat inactivated foetal bovine serum and 1 % of penicillin-streptomycin was used for cell culture tests. The cytotoxic activity was investigated using MTT assay<sup>[26]</sup>. Stock solutions (100 mM) of test compounds 14a–b and 15a–e were prepared in DMSO and stored at  $-20^\circ$  prior to make dilutions for the biological assay. Cell suspensions were diluted to  $10^5$  cells/ml, suitably prepared and distributed in plates of culture with 96 wells (225  $\mu\text{l}$  in each well), then incubated at  $37^\circ$  in a humid atmosphere with 5 % of  $\text{CO}_2$ . After 24 h, 25  $\mu\text{l}$  of the test compounds were added to each well. The plates were incubated again at  $37^\circ$  for 24 h. Then, 25  $\mu\text{l}$  of MTT solution (5 mg/ml) was added to each well, and the mixture was incubated at  $37^\circ$  for 2 h. At the end of this period, the culture medium with the MTT excess was aspirated and after that, 100  $\mu\text{l}$  of DMSO was added to each well to dissolve the formazan crystals. The optical density of the wells was measured at 490 nm. Results were evaluated by comparing the absorbance of the wells containing compound-treated cells with the absorbance of wells containing 0.1 % DMSO alone (solvent control). Conventionally, cell viability was estimated to be 100 % in the solvent control. All assays were performed in triplicate and mean $\pm$ SD values were used to estimate cell growth inhibition rate. The cell survival rates were shown in fig. 2.

### Molecular docking:

All the docking studies were carried out using Surflex-Docking 2.0 on a window 7 workstation. The crystal structure of DNA (PDB entry 3FT6) was downloaded from the protein data bank and used for docking studies. Water molecules were removed from protein PDB files, and hydrogen atoms were added. The 3D structures of

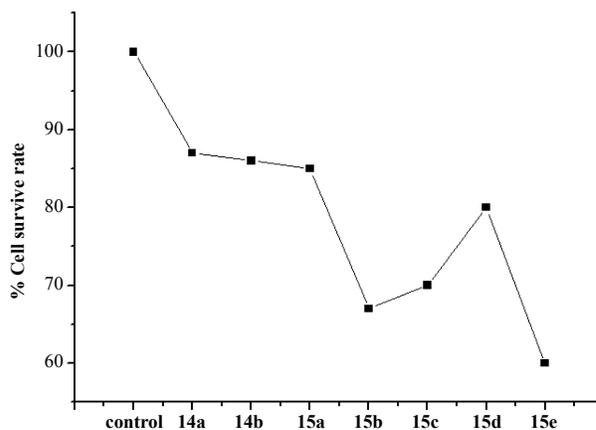


Fig. 2: Tumor cell survival rates after exposure to test compounds

PC-3 cell survival rates after exposure to 100  $\mu\text{M}$  each of 14a–b and 15a–e. ■—■ PC-3

compound 14b were first built using Surflex-Docking 2.0 sketch followed by energy minimization. At last we used the Surflex-Docking program to automatically dock the drugs into the binding pockets of DNA. In the docking process, 20 conformations were obtained, and among them the lowest free energy solution (or the highest total score) was chosen for our compounds modelling.

## RESULTS AND DISCUSSION

The target diphenylpiperazine 1,2,3-triazole derivatives were prepared via multi-step reactions and the synthetic process was outlined in figs. 3 and 4. Commercially available 3-bromoprop-1-yne was reacted with azoles in acetonitrile to produce compounds 1a–e, and while it was treated with phenol or bisphenol A in acetone/water (1/1) in the presence of potassium carbonate, propargyl ether compounds 2a–b or 3 were obtained in yields of 54-65 %. Furthermore, 3-bromoprop-1-yne could react with enoxacin and sulfonamide via N-alkylation reaction to give the corresponding alkynes 4 and 5 (fig. 3).

The (4-chlorophenyl)(phenyl)methanone 6 was reduced by sodium borohydride to produce alcohol 7, and then further treated by thionyl chloride to give chloride 8. The latter was subsequently reacted with piperazine to afford 1-((4-chlorophenyl)(phenyl)methyl) piperazine 9. The N-alkylation of piperazine ring of compound 9 with 2-(chloromethyl)oxirane or chloroacetyl chloride afforded the compounds 10 and 11 in 40 and 45 % yields, respectively. Further substitution of compounds 10 and 11 with sodium azide was performed to provide the important intermediates 12 and 13. The 1,3-dipolar

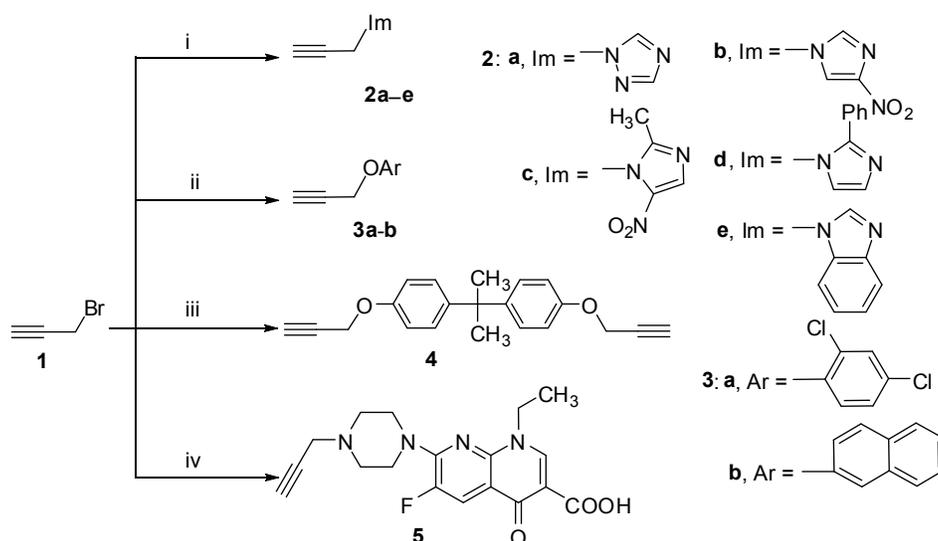


Fig. 3: Synthesis of intermediates 1–5

Reagents and conditions: (i) azole, potassium carbonate or sodium hydride, acetonitrile, 50°; (ii) phenol, potassium carbonate, acetone/water (1/1, V/V), rt; (iii) bisphenol A, potassium carbonate, acetone/water (1/1, V/V), rt; (iv) enoxacin, potassium carbonate, acetonitrile, 0°

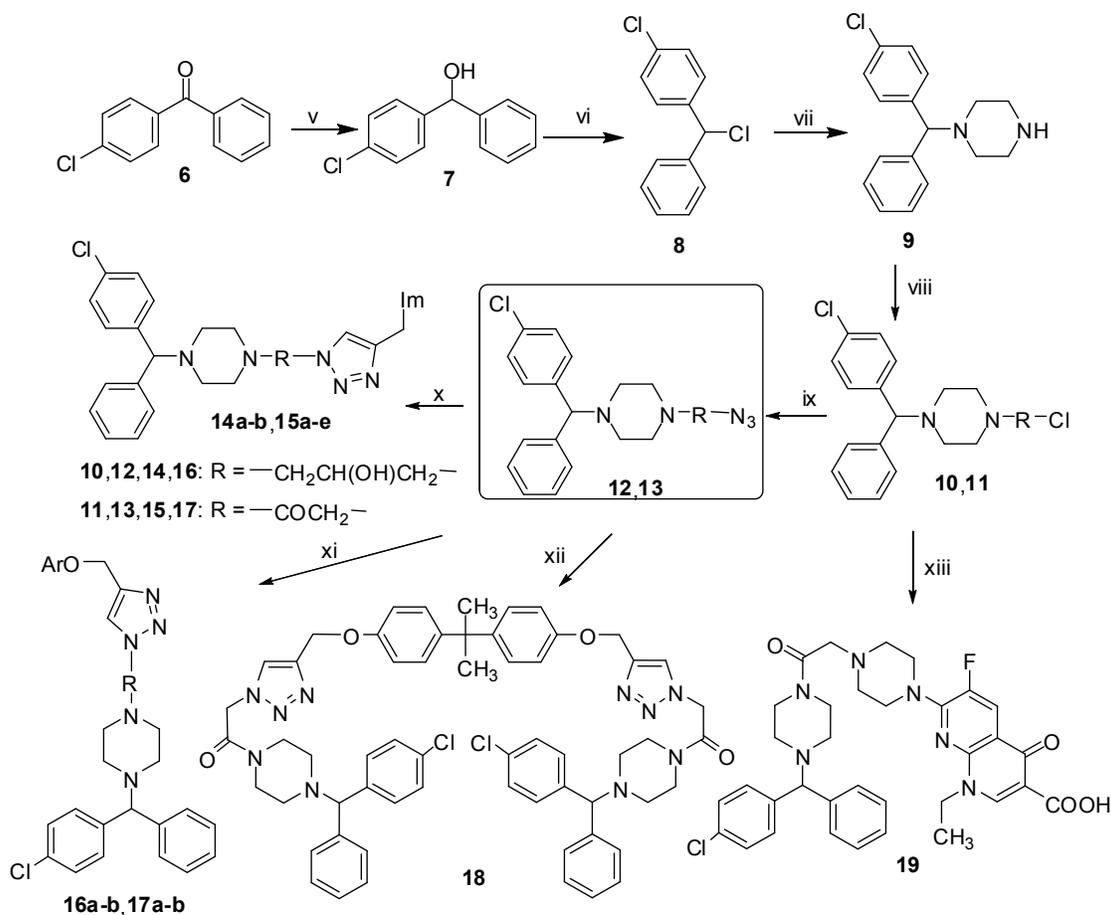


Fig. 4: Synthesis of diphenylpiperazine derivatives 14–19

Reagents and conditions: (v) sodium borohydride, methanol, r.t.; (vi) thionyl chloride, chloroform, 0–70°; (vii) piperazine, potassium carbonate, N,N-dimethylformamide, 80°; (viii) 2-(chloromethyl)oxirane, ethanol, r.t.; or chloroacetyl chloride, dichloromethane, triethylamine, 0–5°; (ix) sodium azide, acetone/water (1/1, V/V), 40°; (x) compounds 2a-e, sodium ascorbate (0.4 eq.),  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (0.2 eq.), *t*-BuOH/acetone/ $\text{H}_2\text{O}$  (1/1/1, V/V/V), 60°; (xi) compounds 3a,b, sodium ascorbate (0.4 eq.),  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (0.2 eq.), *t*-BuOH/acetone/ $\text{H}_2\text{O}$  (1/1/1, V/V/V), 60°; (xii) compound 4, sodium ascorbate (0.4 eq.),  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (0.2 eq.), *t*-BuOH/acetone/ $\text{H}_2\text{O}$  (1/1/1, V/V/V), 60°; (xiii) enoxacin, potassium carbonate, acetonitrile, 0°

cycloaddition of azides with propargyl azoles, propargyl ethers or propargyl sulfonamide using catalytic amount of copper sulphate and sodium ascorbate in *t*-BuOH/acetone/H<sub>2</sub>O (1/1/1, V/V/V) mixture at 60° afforded diphenylpiperazine 1,2,3-triazole derivatives 14a–b, 15a–e, 16a–b, 17a–b and 18 in moderate to good yields ranging from 60 to 85 %. However, the enoxacin contained diphenyl 1,2,3-triazole compound was not synthesized unfortunately. In order to exploration the important role of 1,2,3-triazole fragment to the bioactivity, the hydrophobic group ((4-chlorophenyl)(phenyl)methyl)piperazine was introduced into the C-7 position of enoxacin piperazine ring (19) to improve antimicrobial activity and broaden spectrum (fig. 4).

1,3-Dipolar cycloadditions of azide with alkyne to form five-membered 1,2,3-triazole ring are classic click reaction, and have been extensively researched. To further manifest this reaction, the reaction of compound 12 with compound 2a was employed as a model reaction, and some influential factors such as solvent and catalyst were optimized to improve the transformation yields for different substrates. As shown in Table 1, nearly no reaction occurred when CuSO<sub>4</sub>·5H<sub>2</sub>O (0.1 eq.) and sodium ascorbate (0.2 eq.) were employed as catalysts (entries 1 and 2). The highest yield (60 %) and shortest reaction time (1 h) were obtained when the ratio of catalyst was improved to CuSO<sub>4</sub>·5H<sub>2</sub>O (0.2 eq.) and sodium ascorbate (0.4 eq., entry 4). Therefore *t*-BuOH/acetone/H<sub>2</sub>O (1/1/1, V/V/V) were chosen as solvent medium, and CuSO<sub>4</sub>·5H<sub>2</sub>O (0.2 eq.) and sodium ascorbate (0.4 eq.) were employed as catalysts to perform this transformation.

All the new compounds were characterized by IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and MS spectra. Their spectral analyses were consistent with the assigned structures and listed in the experimental section. The mass spectra for compounds gave a major fragment of [M+H]<sup>+</sup> or [M+Na]<sup>+</sup> according to their molecular formula.

In IR spectra, all the synthesized compounds gave absorptions in 3026–3100 cm<sup>-1</sup>, which was attributed to the stretching vibration of C–H bonds of the aromatic

ring. Compounds 15a–e, 17a–b and 18 exhibited strong absorption bands at 1658–1665 cm<sup>-1</sup> region, which indicated the presence of carbonyl groups, whereas two absorption between 1545 and 1337 cm<sup>-1</sup> were attributed to the NO<sub>2</sub> group in compounds 14b and 15b. Moreover, the IR spectra of target compounds 16a–b and 17a–b also exhibited characteristic strong absorption at 1237–1238 cm<sup>-1</sup> attributable to the stretching vibration of C–O bonds. Notably, the characteristic C–N bands of piperazine ring in all piperazine derivatives appeared in the region between 1135–1141 cm<sup>-1</sup> and 1048–1056 cm<sup>-1</sup>. All the other absorption bands were also observed at the expected regions.

In <sup>1</sup>H NMR spectra, compounds 14–18 gave singlets at 5.22–5.50 ppm assigned to the CH<sub>2</sub> protons linked to C-4 of the 1,2,3-triazole ring. The singlets at 4.57–4.09 ppm of compounds 14 and 16 were assigned to the CH<sub>2</sub> protons linked with N-1 of the 1,2,3-triazole ring, while the N-CH<sub>2</sub> protons in compounds 15, 17 and 18 gave higher shift signals at 5.19–5.24 ppm due to the presence of the strong electron-withdrawing carbonyl group. Moreover, for compounds 15 and 17 the CH<sub>2</sub> protons of piperazine ring were observed at 3.63–3.53 ppm and 2.39–2.43 ppm, but only one signal at 2.56–2.18 ppm was found for compound 4. All the other aromatic and aliphatic protons appeared at the appropriate chemical shifts and integral values.

The *in vitro* antimicrobial screening of all the synthesized compounds was performed on four Gram-positive bacteria (*S. aureus* ATCC 6538, MRSA, *M. luteus* ATCC 4698 and *B. subtilis* ATCC 21216), four Gram-negative bacteria (*E. coli* ATCC 8099, *P. aeruginosa* ATCC 27853, *B. typhi* and *B. proteus* ATCC 13315) and three fungi (*C. albicans* ATCC 76615, *S. cerevisiae* and *A. flavus*) using two fold serial dilution technique recommended by the NCCLS with clinically used antimicrobial drugs chloromycin, enoxacin and fluconazole serving as positive controls.

The synthesized compounds were also evaluated for cytotoxic activity on a human tumor cell line, PC-3 using the MTT assay. Results of each tested compound

**TABLE 1: SCREENING OF THE OPTIMAL REACTION CONDITIONS FOR COMPOUND 14A**

Entry	Solvent(V/V)	Catalyst	Time (h)	Yield <sup>a</sup> (%)
1	Acetone/water (1/1)	CuSO <sub>4</sub> ·5H <sub>2</sub> O (0.1 eq.), sodium ascorbate (0.2 eq.)	10	Trace
2	<i>t</i> -BuOH/H <sub>2</sub> O (1/1)	CuSO <sub>4</sub> ·5H <sub>2</sub> O (0.1 eq.), sodium ascorbate (0.2 eq.)	10	Trace
3	<i>t</i> -BuOH/H <sub>2</sub> O (1/1),	CuSO <sub>4</sub> ·5H <sub>2</sub> O (0.15 eq.), sodium ascorbate (0.3 eq.)	10	20
4	<i>t</i> -BuOH/acetone/H <sub>2</sub> O (1/1/1)	CuSO <sub>4</sub> ·5H <sub>2</sub> O (0.2 eq.), sodium ascorbate (0.4 eq.)	1	60

General conditions: To a solution of compound 12 (0.10 g, 0.66 mmol) in solvent were added sodium ascorbate and copper (II) sulphate pentahydrate successively. Here after compound 2a (0.245 g, 0.66 mmol) was added, and the mixture was stirred at 60°. <sup>a</sup> Yield of the isolated product 14a after silica gel chromatography

were reported as the percent survived cells out of the treated cells compared to the untreated control cells<sup>[7]</sup>. The cell survival rates were summarized in fig. 2.

The *in vitro* antifungal evaluation displayed that only some compounds exhibited inhibitory activity against *C. albicans* and *S. cerevisiae*. Diphenylpiperazine 1,2,3-triazole derivatives 14a, 14b and 15b exhibited moderate activity with MIC values of 50-100 µg/ml against *C. albicans* and *S. cerevisiae*. Compound 19 bearing enoxacin fragment displayed relatively better antifungal activity on *C. albicans*, *S. cerevisiae* and *A. flavus*. It was noteworthy that the 4-nitro imidazole contained compound 14b and 15b exhibited 2-fold more potent activity against *A. flavus* in

**TABLE 2: ANTIFUNGAL ACTIVITY OF COMPOUNDS 14–19**

Compounds	<i>C. albicans</i>	<i>S. cerevisiae</i>	<i>A. flavus</i>
14a	100	100	200
14b	50	50	100
15a	400	200	400
15b	100	100	100
15c	400	400	400
15d	400	400	400
15e	400	400	400
16a	400	400	400
16b	>400	>400	>400
17a	400	400	400
17b	>400	>400	>400
18	>400	>400	>400
19	200	200	200
Fluconazole	3.1	6.2	200

Minimal inhibitory concentrations (MIC, µg/ml) were determined by micro broth dilution method for microdilution plates

comparison to fluconazole (MIC=200 µg/ml; Table 2). The *in vitro* antibacterial screening demonstrated that some of the newly synthesized compounds could effectively inhibit the growth of all the tested microorganisms. Specially, nitroimidazole piperazine 14b gave good activity against *B. subtilis*, *M. luteus*, *B. proteus*, *E. coli* and *B. typhi* with MIC values of 25 µg/ml and it exhibited moderate inhibition of the growth of *S. aureus* and MRSA with MIC value of 50 µg/ml. The substitution of 4-nitro imidazole by triazole ring, which yielded compound 14a, resulted in decreased antibacterial activity. Furthermore, the replacement of hydroxyl group of compound 14 by carbonyl moiety, which gave compounds 15a–e, also led to weak activity against the tested bacterial strains (Table 3).

The results of preliminary cytotoxic activity showed that the tested compounds exhibited weak growth inhibition against PC-3 cell line at the concentration of 100 µM (fig. 2). Among the tested piperazine derivatives, compounds 15b and 15e showed inhibitory activity against PC-3 cell line at the concentration of 100 µM with the cell survive rate only of 67 and 60 %, respectively. It was thought that the presence of nitroimidazole and benzimidazole ring would afford beneficial effect to antiproliferative properties. Meanwhile, the antiproliferative results indicated that all the compounds showed dose-dependent antiproliferative activity in the tested cell lines. Further studies are in progress to define the important mechanisms of action of the above mentioned compounds.

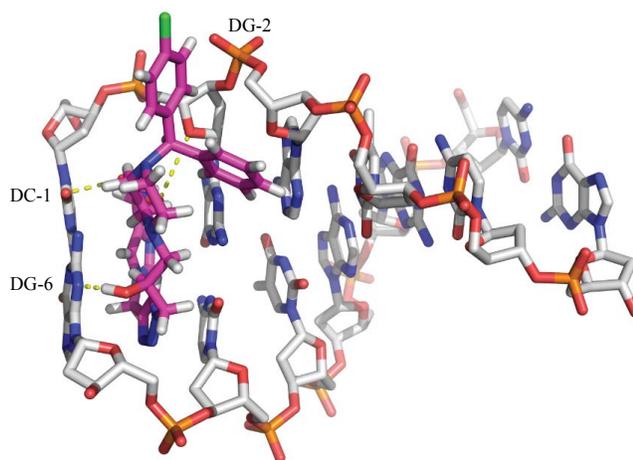
**TABLE 3: ANTIBACTERIAL ACTIVITY OF COMPOUNDS 14–19**

Compounds	Gram-positive bacteria				Gram-negative bacteria			
	<i>S. aureus</i>	MRSA	<i>B. subtilis</i>	<i>M. luteus</i>	<i>B. proteus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. typhi</i>
14a	100	100	200	200	200	100	200	100
14b	50	50	25	100	25	25	25	25
15a	400	200	400	400	200	200	200	200
15b	200	200	100	100	400	200	200	100
15c	200	200	200	400	100	400	400	400
15d	400	400	400	400	400	400	400	400
15e	200	200	100	200	400	400	400	400
16a	400	400	400	400	>00	400	400	400
16b	>400	>400	>400	>400	>400	>400	>400	>400
17a	400	400	400	400	400	400	400	400
17b	>400	>400	>400	>400	>400	>400	>400	>400
18	>400	>400	>400	>400	>400	>400	>400	>400
19	200	200	200	200	50	100	100	100
Chloromycin	3.1	6.2	3.1	1.6	6.2	3.1	3.1	1.6
Enoxacin	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4

Minimal inhibitory concentrations (MIC, µg/ml) were determined by micro broth dilution method for microdilution plates. MRSA, methicillin-resistant *Staphylococcus aureus* (N315)

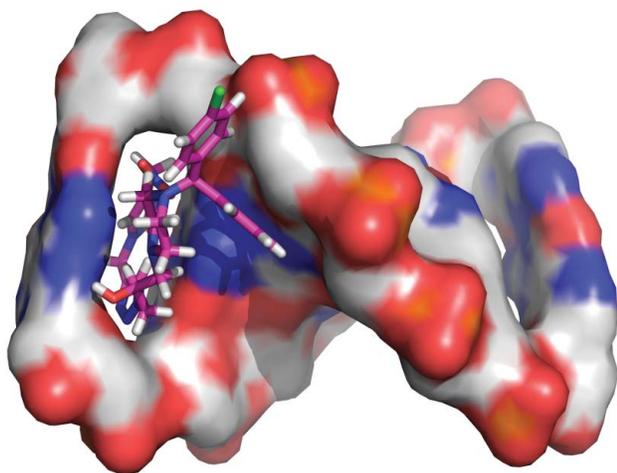
Molecular docking study as a useful strategy is extensively employed to research the binding modes of small molecules to DNA<sup>[20]</sup>. In our research, molecular docking study was performed between compound 14b and DNA hexamer duplex (PDB code: 3FT6) to understand the binding model. The docking mode with lowest binding free energy ( $-27.8 \text{ kJ}\cdot\text{mol}^{-1}$ ) is shown in figs. 5 and 6. The results showed that the nitro and hydroxyl groups of compound 14b could form hydrogen bonds with the guanine of DNA, and thus prevent the formation of hydrogen bond between cytosine in DNA. This kind of interaction resulted in decreased stability of DNA, thus inhibiting its physiological function.

In conclusion, a novel series of diphenylpiperazine 1,2,3-triazole derivatives have been successfully prepared starting from commercially available



**Fig. 5: Molecular modeling of compound 14b and DNA hexamer duplex (PDB: 3FT6)**

The dashed lines represent the hydrogen bonding interactions between compound 14b and DNA hexamer duplex (total binding score is 5.04)



**Fig. 6: Stereo view of the compound 14b–DNA complex**  
Stereo view of the conformation of compound 14b intercalated to DNA hexamer duplex to form compound 14b–DNA complex

4-chlorophenyl)(phenyl)methanone. The new compounds were confirmed by <sup>1</sup>H NMR, <sup>13</sup>C NMR, IR and MS spectra. The assessment of *in vitro* antibacterial and antifungal activities against eight bacterial strains and three fungal strains showed that some compounds exhibited moderate to good antibacterial and antifungal activities, especially nitroimidazole piperazine 14b gave good activity against bacteria *B. subtilis*, *M. luteus*, *B. proteus*, *E. coli* and *B. typhi* with MIC values of 25  $\mu\text{g}/\text{ml}$ , respectively, and it showed moderate activity against *C. albicans* and *S. cerevisiae* (MIC=50  $\mu\text{g}/\text{ml}$ ). Moreover, the 1,2,3-triazole-linked nitroimidazole diphenylpiperazine compound 15b and benzimidazole contained one 15e were found to be effective *in vitro* against the PC-3 cell line, and the cell survive rates only of 67 and 60 %, respectively at concentration of 100  $\mu\text{M}$  in a dose-dependent manner. Furthermore, molecular docking experiments suggested that the nitro and hydroxyl groups of compound 14b could insert into base-pairs of DNA hexamer duplex by the formation of hydrogen bonds with guanine of DNA. All these results opened up a promising starting point to optimize the structures of diphenylpiperazine 1,2,3-triazole derivatives as potent biological agents.

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### Conflict of interest:

There are no conflicts of interest.

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