Bioorganic & Medicinal Chemistry Letters 22 (2012) 4353–4357

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

# Regioselective synthesis and antimicrobial studies of ester linked 1,4-disubstituted 1,2,3-bistriazoles

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ARTICLE INFO

*Article history:*
Received 17 March 2012
Revised 21 April 2012
Accepted 2 May 2012
Available online 12 May 2012

*Keywords:*
Click chemistry
1,4-Disubstituted 1,2,3-triazoles
Antibacterial activity
Antifungal activity
Docking studies

ABSTRACT

A series of 1,4-disubstituted 1,2,3-bistriazoles was synthesized via click chemistry by cycloaddition of various bisalkynes with benzyl/2-phenylethyl azide. Synthesized triazoles were characterized by IR, $^1$H NMR, $^{13}$C NMR and mass spectral techniques. All the compounds were evaluated for antibacterial/antifungal activities and found to possess moderate to good antimicrobial activities. Further the docking study for the most active compound against DNA Gyrase was also carried out.



The 1,2,3-triazole heterocycles have gained significant interest of synthetic organic chemists for the development of new biologically active molecules. The basic triazole moiety does not occur in nature, although it has gained considerable attention as this ring is a potential pharmacophore. The 1,2,3-triazoles and its derivatives are reported as anti-HIV,[1] antimicrobial,[2] antiallergic,[3] antifungal,[4] antitumor,[5] and selective $\beta_3$ adrenergic receptor agonist.[6] Some substituted 1,2,3-triazoles are also found to be antitubercular agents[7] and cannabinoid CB1 receptor antagonists.[8] 1,3-dipolar cycloaddition of azides and terminal alkynes is the most widely used method for the synthesis of 1,2,3-triazoles. The classical Huisgens 1,3-dipolar thermal cycloaddition is relatively slow, occurs at high temperature resulting into a mixture of 1,4- and 1,5-disubstituted 1,2,3-triazoles.[9] Recently, Meldal and Sharpless independently discovered the Cu(I) catalyzed Huisgen 1,3-dipolar cycloaddition, which has emerged as a novel alternative for the selective synthesis of 1,4-disubstituted 1,2,3-triazoles with excellent yield.[10] Even though this reaction requires only benign reaction conditions, simple workup and purification procedures, still it can create molecular diversity by joining molecular building blocks. The better regioselectivity, broad scope and the bio-compatibility of the compounds have made it one of the most powerful click reaction in material, biological and pharmaceutical sciences.[11] In addition, the use of click reaction has also been reported for the synthesis of ionic receptors,[12] triazolophanes,[13] dendrimers,[14] cyclic peptides,[15] peptide nanotubes,[16] peptidomimetics[17] etc.

Herein, we report the synthesis of a series of ester linked 1,4-disubstituted 1,2,3-bistriazoles (Table 1) from various bisalkynes and their antibacterial/antifungal activities. To the best of our knowledge all the prepared compounds are new except **3a**.[18] The bisalkynes were prepared by treating acid dichlorides with propargyl alcohol in presence of 4-(Dimethylamino)pyridine (DMAP) in dichloromethane according to the literature procedure.[5] The click reaction between bisalkynes (except **1e**) and the azides (**2a**, **2b**) was carried out in acetonitrile with Cu(I) as the catalyst and diisopropylethylamine (DIPEA) as the base. It afforded the desired products (**3a**–**3h**, **3k**–**3l**) in good yield, i.e., 65–85%. However, these conditions could not be applied for the preparation of compounds containing pyridine moiety (**3i**, **3j**). These compounds were synthesized by reacting bisalkyne **1e** with azides **2a**/**2b** in 1:1 water–THF mixture containing copper sulfate and sodium ascorbate (Scheme 1).

All the synthesized bistriazoles were well characterized by IR, $^1$H NMR, $^{13}$C NMR spectroscopy and mass spectrometry. The formation of triazoles was apparent from the absorption band in the region 3120–3140 cm$^{-1}$ due to =C–H (stretching) of triazole ring in the IR spectra. The appearance of characteristic singlet in $^1$H NMR due to triazolyl protons in the region of $\delta$ 7.52–7.68 and $\delta$ 123.60–124.77 in $^{13}$C NMR due to C-5 of the triazole ring showed the formation of triazole ring. To further confirm the structure of bistriazoles, an X-ray crystallographic study of compound **3j** was also carried out (Fig. 1).[19]

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http://dx.doi.org/10.1016/j.bmcl.2012.05.008

**Table 1**
Synthesis of 1,4 disubstituted 1,2,3-bistriazoles **3a–l**

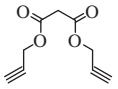
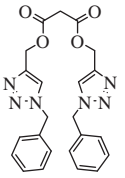
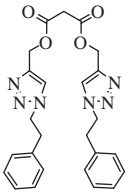
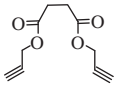
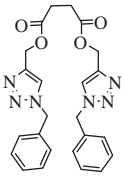
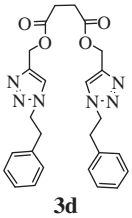
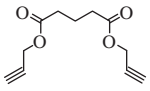
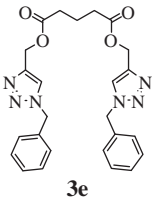
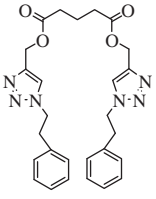
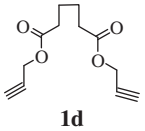
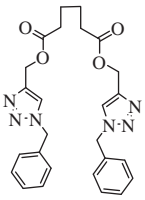
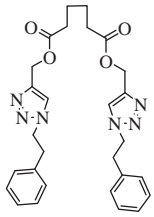
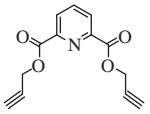
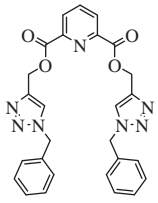
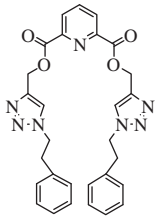
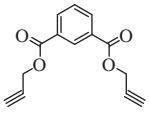
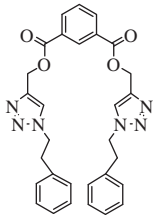
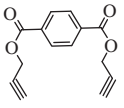
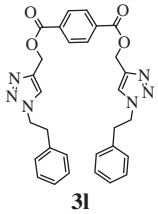
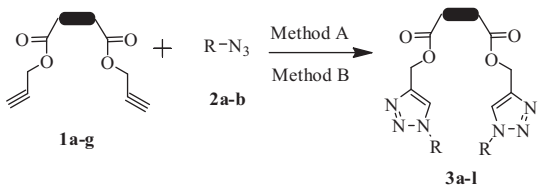
| Entry | Alkyne | R | Compound |
|---|---|---|---|
| 1 |  **1a** |  **2a** |  **3a** |
| 2 | **1a** |  **2b** |  **3b** |
| 3 |  **1b** | **2a** |  **3c** |
| 4 | **1b** | **2b** |  **3d** |
| 5 |  **1c** | **2a** |  **3e** |
| 6 | **1c** | **2b** |  **3f** |
| 7 |  **1d** | **2a** |  **3g** |

**Table 1** (*continued*)

| Entry | Alkyne | R | Compound |
|---|---|---|---|
| 8 | **1d** | **2b** |  **3h** |
| 9 |  **1e** | **2a** |  **3i** |
| 10 | **1e** | **2b** |  **3j** |
| 11 |  **1f** | **2b** |  **3k** |
| 12 |  **1g** | **2b** |  **3l** |




**Scheme 1.** Synthesis of 1,4 disubstituted 1,2,3-bistriazoles **3a–3l**.[22] Reagents and conditions: Method A: Cu(I), DIPEA, MeCN, rt. Method B: $CuSO_4 \cdot 5H_2O$, sodium ascorbate, $THF/H_2O$ (1:1), rt.

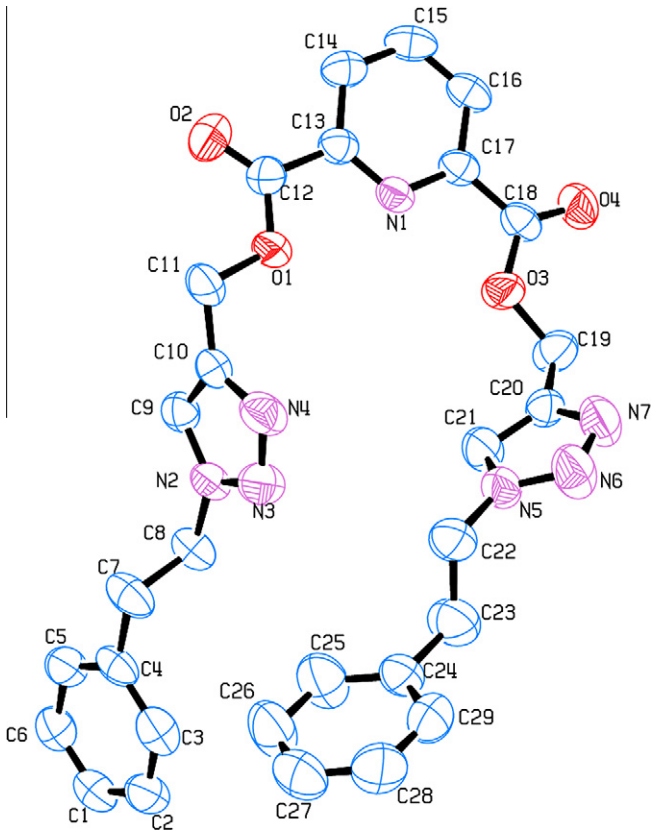



**Figure 1.** X-ray structure of **3j**.

The synthesized triazoles were screened for their in vitro antibacterial and antifungal activities. The in vitro antibacterial activities were tested against Gram-positive bacteria *Bacillus subtilis* (MTCC 441) and Gram-negative bacteria *Escherichia coli* (MTCC 7443) by standard serial dilution method[20] using a stock solution of 100 μg/ml concentration. Double strength nutrient broth was used as culture media and dimethylsulphoxide was used as solvent control. The stock solutions of the test compounds were serially diluted in test tubes containing 1 ml of sterile medium to get the concentration of 50–3.12 μg/ml and then inoculated with 100 μL of suspension of respective microorganism in sterile saline. Norfloxacin was used as standard drug. The inoculated test tubes were incubated at 37 ± 1 °C for 24 h. Perusal of the activity data shows that compound **3i** is most potent against *B. subtilis* and *E. coli*. Further the triazoles derived from aromatic alkynes were found to be more active against both the bacterial strains as compared to that derived from aliphatic one. The higher activity of compounds **3i**–**3l** can be due to more rigidity resulting from presence of pyridine and benzene nucleus. In case of triazoles derived from aliphatic alkynes compound **3d** and **3g** are comparatively more active against both strains. For *B. subtilis* the activity of compound **3i** is comparable to the Norfloxacin (standard), while in case of *E. coli* the activities of compounds **3i**, **3d** and **3g** are nearer to the reference.

**Table 2**
In vitro antibacterial and antifungal screening studies of the title compounds (MIC, μmol/mL)

| Entry | Compound | *B. subtillis* | *E. coli* | *A. niger* | *C. albicans* |
|---|---|---|---|---|---|
| 1 | **3a** | 0.0280 | 0.0280 | 0.0140 | 0.0140 |
| 2 | **3b** | 0.0263 | 0.0263 | 0.0263 | 0.0263 |
| 3 | **3c** | 0.0271 | 0.0271 | 0.0271 | 0.0135 |
| 4 | **3d** | 0.0256 | 0.0128 | 0.0256 | 0.0128 |
| 5 | **3e** | 0.0263 | 0.0263 | 0.0263 | 0.0135 |
| 6 | **3f** | 0.0249 | 0.0249 | 0.0249 | 0.0249 |
| 7 | **3g** | 0.0256 | 0.0128 | 0.0128 | 0.0128 |
| 8 | **3h** | 0.0242 | 0.0242 | 0.0242 | 0.0121 |
| 9 | **3i** | 0.0123 | 0.0123 | 0.0246 | 0.0123 |
| 10 | **3j** | 0.0233 | 0.0233 | 0.0233 | 0.0233 |
| 11 | **3k** | 0.0233 | 0.0233 | 0.0233 | 0.0233 |
| 12 | **3l** | 0.0233 | 0.0233 | 0.0233 | 0.0233 |
| 13 | Norfloxacin | 0.0098 | 0.0098 | — | — |
| 14 | Fluconazole | — | — | 0.0102 | 0.0102 |

The in vitro antifungal activities were evaluated against *Candida albicans* (MTCC 183) and *Aspergillus niger* (MTCC 282) by serial dilution method using a stock solution of 100 μg/ml concentration. Sabouraud dextrose broth was employed as culture media and dimethylsulphoxide as solvent control. The stock solutions of the test compounds were serially diluted in test tubes containing 1 ml of sterile medium to get the concentration of 50–3.12 μg/ml and then inoculated with 100 μL of suspension of respective microorganism in sterile saline. Fluconazole was used as standard drug. The inoculated test tubes were incubated at 25 ± 1 °C for 48 h in case of *C. albicans* and at 25 ± 1 °C for 120 h in case of *A. niger*. Antifungal activity was determined by measuring minimum inhibitory

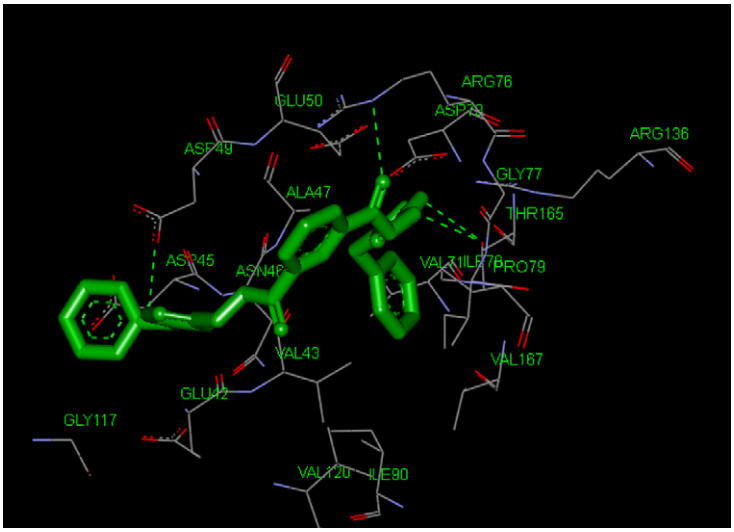



**Figure 2.** Binding mode of ligand **3i** to 1KZN by docking simulation.

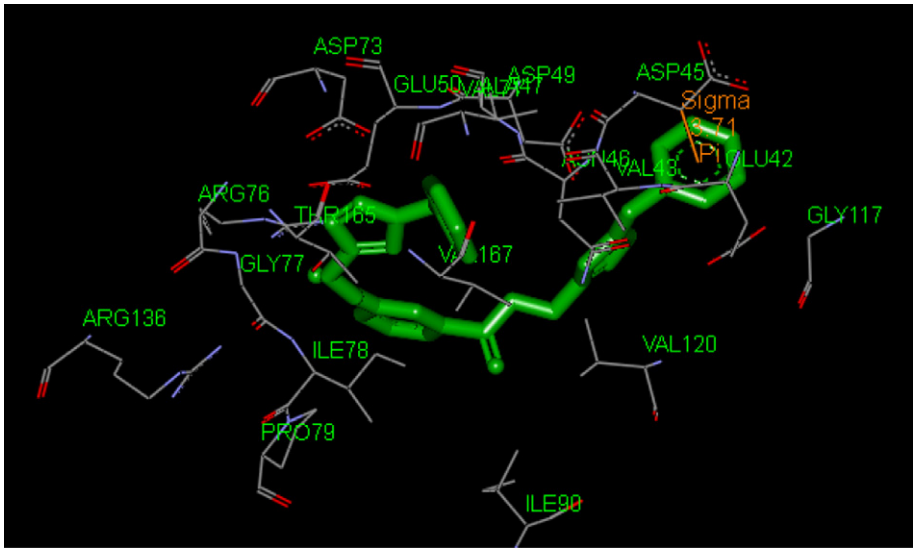



**Figure 3.** Sigma–pi interaction of benzene ring of ligand **3i** to Asp-45.

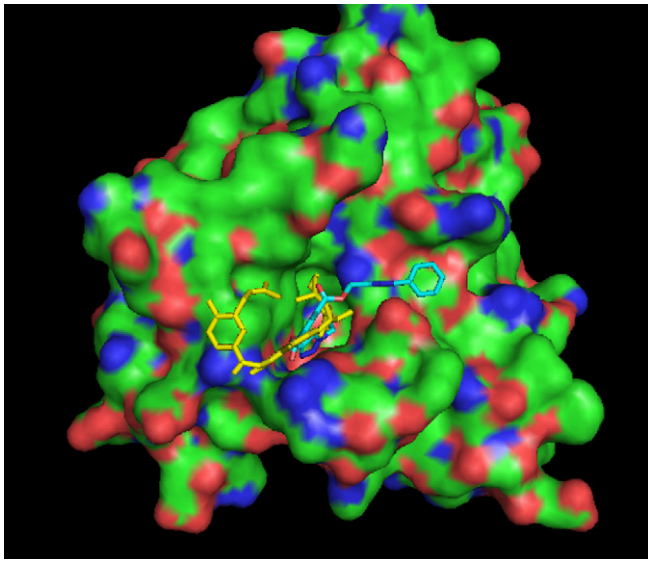


**Figure 4.** Surface diagram of the docked molecule (blue color) along with co-crystallized ligand (yellow color).

concentration. Compounds **3h**, **3i**, **3d**, **3c** and **3a** are more active than others against *C. albicans*, whereas **3g** and **3a** have more efficacy than other compounds in case of *A. niger*. Further, compounds **3a** and **3g** exhibited good activity against both the fungal strains. The antifungal activity of compounds **3a**, **3g** and **3a**, **3c**, **3d**, **3e**, **3g**, **3h**, **3i** are comparable to standard against *A. niger* and *C. albicans*, respectively. No specific activity trend was observed for antifungal studies. The in vitro antibacterial and antifungal activities data of the tested compounds is depicted in Table 2 as MIC values (μmol/mL).

*Docking studies:* In order to investigate a plausible mechanism of action of the most potent compound **3i** against bacteria *E. coli*, docking studies[21] into the crystal structure of topoisomerase II DNA Gyrase B complexed with the natural inhibitor clorobiocin (1 kzn) was performed. The binding mode of most active compound **3i** against *E. coli* was studied.

Clorobiocin, the co-crystallized ligand forms hydrogen bonds with Asp-73 and Thr-165. For the synthesized molecule **3i** (Fig. 2), the oxygen atom on carbonyl of ester linkage forms hydrogen bond with Arg-76. Both triazole moieties generated hydrogen bonds with Thr-165 and Asp-79. There is one sigma–pi interaction between benzene ring of the molecule and carbon chain of Asp-45 as shown in Figure 3. The surface diagram of the docked molecule along with co-crystallized ligand is depicted in Figure 4. Thus these hydrogen bonds, sigma–pi and other vander waal's interactions confers good inhibitory activity to the compound under study.

In conclusion, synthesis of some ester linked 1,4-disubstituted 1,2,3-bistriazoles have been reported by Cu(I) catalyzed azide-alkyne cycloaddition using different aliphatic/aromatic moieties. The antimicrobial activity studies revealed that all the compounds screened showed moderate to good activities. Fitness of most active compound **3i** against *E. coli* in Topoisomerase II was studied using *in silico* tools.

## Acknowledgments

Authors wish to acknowledge SAIF, Panjab University, Chandigarh for providing NMR/Mass spectra, Professor T. N. Guru Row, Indian Institute of Science, Bangalore for providing the single crystal X-ray diffraction facility and University Grants Commission, New Delhi for financial support to carry out above work through Minor and Major research projects (K.L., C.P.K.).

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.008.

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22. *General procedure for the synthesis of 1,4-disubstituted 1,2,3 bistriazoles:* Method A:[13a] The bisalkyne (1 mmol), diisopropylethylamine (2.2 mmol) and azide (2.2 mmol) were dissolved in dry acetonitrile under Nitrogen atmosphere. To this solution was added Cu(I) (0.22 mmol) and the reaction mixture was stirred overnight. The acetonitrile was evaporated, dissolved the residues in ethyl acetate, filtered, washed the filtrate with 2N $H_2SO_4$, water and finally with saturated aqueous $NaHCO_3$ solution. The organic layer was dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuum to yield 1, 4-disubstituted 1,2,3-triazoles. *Bis((1-benzyl-1H-1,2,3-triazol-4-yl)methyl) adipate* ***[3g]***: White solid, mp: 121–124 °C, IR(KBr): 3122, 3059, 2943, 1728, 1458, 1448, 1250 $cm^{-1}$. $^1H$ NMR (500 MHz, $CDCl_3$): 1.60(br s, 4H), 2.30(br s, 4H), 5.18(s, 4H), 5.52(s, 6H), 7.27–7.79(m, 4H), 7.34–7.40(m, 6H), 7.52 (s, 2H). $^{13}C$ NMR (100 MHz, $CDCl_3$): 24.1, 33.6, 54.2, 57.6, 123.6, 128.2, 128.9, 129.2, 134.4, 143.3, 173.1. MS *m*/*z*: 489.5 $[M+H]^+$, 511.5 $[M+Na]^+$. *Method B*: Bisalkyne(1 mmol) and azide (2.2 mmol) were suspended in a 1:1 mixture of water and THF(3 ml each). To this reaction mixture was added sodium ascorbate (0.2 mmol) and $CuSO_4{\cdot}5H_2O$ (0.1 mmol). The reaction mixture was stirred overnight. Upon completion of reaction the reaction mixture was diluted with cold water (20 ml) and extracted with ethyl acetate (10 × 2 ml ml). The combined organic layer was washed with aqueous solution of $NH_4Cl$:$NH_3$ (9:1) and dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuum to yield 1, 4-disubstituted 1,2,3-triazoles. *Bis((1-benzyl-1H-1,2,3-triazol-4-yl)methyl) pyridine-2,6-dicarboxylate* ***[3i]*****:** White solid, mp: 87–89 °C, IR(KBr): 3136, 3092, 1734, 1452, 1252 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): 5.52(s, 8H), 7.27–7.38(m, 4H), 7.35–7.38 (m, 6H), 7.68 (s, 2H), 7.97(t, 1H, *J* = 7.8), 8.24(d, 2H, *J* = 7.7Hz). $^{13}C$ NMR (100 MHz, $CDCl_3$): 54.3, 59.1, 124.4, 128.2, 128.3, 128.9, 129.2, 134.3, 138.4, 142.6, 148.0, 1164.1. MS *m*/*z*: 510.2 $[M+H]^+$.