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Research Article

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Regioselective synthesis and antimicrobial activity of 1,2,3-triazolophanes

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ABSTRACT

*A series of 1,2,3-triazolphanes has been synthesized from dialkynes and diazides through copper (I)-catalyzed cycloaddition reaction. The structures of all the newly synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, MS spectral techniques. All the compounds were screened for in vitro antibacterial activity against Bacillus subtilis, Staphylococcus aureus, Escherichia coli and antifungal activity against Candida albicans and Aspergillus niger. The antimicrobial activity indicated that compounds exhibited good to high activity. Further, compound **3a** and **5b** displayed promising activity against B. subtilis and E. coli respectively.*

Keywords: 1,2,3-triazolphanes, click chemistry, antimicrobial activity

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INTRODUCTION

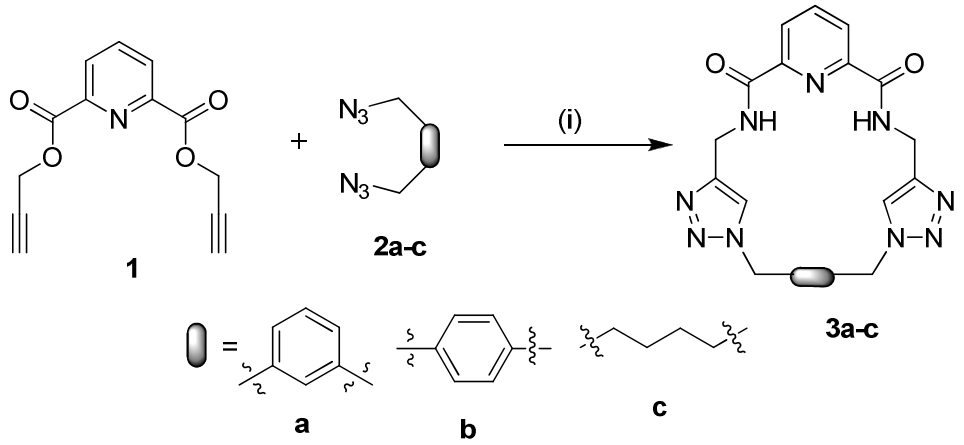
1,4-Disubstituted 1,2,3-Triazolophanes have attracted considerable attention since the advent of copper (I)-catalyzed azide alkyne cycloaddition [1-2]. Triazolophanes are the macrocyclic compounds containing one or more 1,2,3-triazole moiety. This class of compounds find applications in sensors [3], ionic receptors [4], peptide nanotubes [5], β-turn peptidomimetics [6], cyclic peptides [7] and molecular hosts [8]. Moreover, compounds containing 1,2,3-triazole ring are reported to possess important biological activities such as antimicrobial [9], antiprotozoal [10], anti-HIV [11], anticancer [12] and antitubercular [13]. Due to their easy access and high stability, 1,2,3-triazole systems are frequently used as building blocks in drug design. Keeping in view of these findings we report herein, the synthesis and antimicrobial activity of 1,4-disubstituted 1,2,3-triazolophanes.

EXPERIMENTAL SECTION

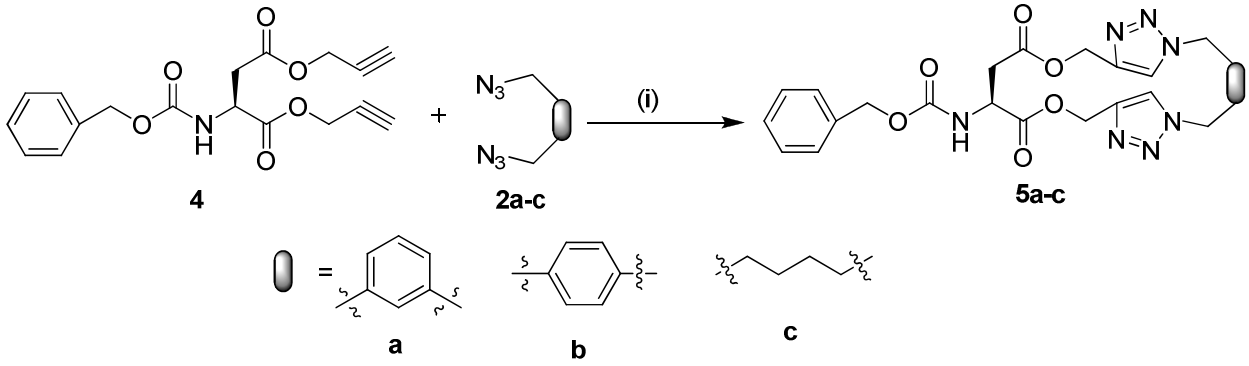
Melting points (ºC) were determined in open capillaries and are uncorrected. The FTIR spectra were obtained on SHIMAZDU IR AFFINITY-I FTIR spectrophotometer using potassium bromide (KBr). The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance III 400 nano bay spectrophotometer operating at 400 and 100 MHz respectively, in the indicated solvents with tetramethysilane (TMS) as the internal standard (chemical shift in δ, ppm). Coupling constant (J) values are in Hertz (Hz). Mass spectra were recorded with LCMS-QTOF Module No. G6540 A (UHD) instrument. The completion of reactions and the purity of the compounds was analyzed by thin layer chromatography (TLC) using readymade silica gel plates (SIL G/UV254, ALUGRAM) and visualized under Ultraviolet lamp. Chemicals employed were procured from commercial suppliers and were used as such.

General method for the preparation the synthesis of 1,2,3-triazoles (3a-c and **5a-c):** To a stirred solution of copper sulphate (5 mol %) and sodium ascorbate (10 mol %) in DMF/water (9:1) was added a solution of substituted benzyl bromide (1 mmol), sodium azide (1 mmol), alkynes (0.5 mmol) drop wise in 2-3 hours. The reaction mixture

was stirred overnight at room temperature and the progress was monitored by TLC. Upon completion of the reaction ice cold water (25 ml) was added to the reaction mixture, precipitates were collected by filtration and washed with aqueous ammonia solution followed by water. To remove traces of reactant, precipitates were further washed with ethyl acetate and dried under vacuum to afford pure product in moderate to good yields (**3a-b**). In case of **3c** and **5a-c**, the reaction mixture was extracted with ethyl acetate (3x15mL). Combined organic layer was washed with aqueous ammonia solution followed by water and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum to get crude products which were purified by column chromatography (hexane/ethyl acetate gradient).



SCHEME: 1. Synthesis of 1,2,3-triazolophanes. Reagents and conditions (i) NaN_3, sodium ascorbate, $CuSO_4.5H_2O$, DMF/ H_2O, RT.



SCHEME: 2. Synthesis of Aspartic acid based 1,2,3-triazolophanes. Reagents and conditions (i) NaN_3, sodium ascorbate, $CuSO_4.5H_2O$, DMF/ H_2O, RT.

Antimicrobial Activity

All the newly synthesized 1,2,3-triazolophanes were tested *in vitro* for antimicrobial activity against two Gram-positive bacteria *S. aureus* (MTCC 3160); *B. subtilis* (MTCC 121), one Gram-negative bacteria *E. coli* (MTCC 40) and two fungal strains viz. *A. niger* (MTCC 281); *C. albicans* (MTCC 183) by standard serial dilution method[14] using a stock solution of 50 µg/mL concentration. Ciprofloxacin and Fluconazole were used as reference while carrying out antibacterial and antifungal activity respectively. The inoculated test tubes were incubated at 37±1°C for 24 h for all the three bacterial strains, while for antifungal activity the inoculated test tubes were incubated at 37±1°C for 48 h in case of *C. albicans* and at 25 ± 1^0C for 120 h in case of *A. niger*. Minimum inhibitory concentrations (MIC) were determined in terms of µmol/mL and are presented in Table 1.

Table 1: *In vitro* antibacterial and antifungal activities of the 1,2,3-triazolophanes (3a-c and 5a-c).

Compounds	Minimum inhibitory concentration (MIC) in µmol/mL				
	BACTERIA			**FUNGI**	
	S. aureus	*B. subtilis*	*E. coli*	*A. niger*	*C. albicans*
3a	0.0145	0.0036	0.0073	0.0073	0.0145
3b	0.0145	0.0073	0.0073	0.0073	0.0073
3c	0.0152	0.0076	0.0152	0.0152	0.0152
5a	0.0059	0.0059	0.0118	0.0118	0.0118
5b	0.0059	0.0118	0.0029	0.0059	0.0118
5c	0.0061	0.0122	0.0122	0.0122	0.0122
Ciprofloxacin	0.0047	0.0047	0.0047	----	----
Fluconazole	----	----	----	0.0051	0.0051

*Triazolophane [**3a**]:* Yellowish solid, Yield: 55%; m.p. = >250 ºC, IR (KBr): 3139, 3045, 2958, 1734, 1601, 1456 cm^{-1}. ^{1}HNMR (400 MHz, DMSO-d_6): 5.44 (s, 4H), 5.60 (s, 4H), 7.27 (brs, 2H), 7.35 (s, 2H), 8.16 (d, 1H, J = 6.4 Hz), 8.23 (d, 2H, J = 7.2 Hz), 8.32 (s, 2H). ^{13}CNMR (100 MHz, DMSO-d_6): 53.1, 59.0, 125.87, 128.2, 128.4, 128.8, 129.8, 136.9, 139.8, 142.2, 148.0, 164.2. MS: m/z 432.4 $(M+H)^+$.

*Triazolophane [**3b**]:* Yellowish solid, Yield: 65%, m.p. = >250 ºC, IR (KBr): 3136, 3040, 2960, 1731, 1600, 1452 cm^{-1}. ^{1}HNMR (400 MHz, DMSO-d_6): 5.42 (s, 4H), 5.60 (s, 4H), 7.33 (s, 4H), 8.23 (brs, 1H), 8.26 (brs, 2H), 8.31 (s, 2H). ^{13}CNMR (100 MHz, DMSO-d_6): 52.7, 59.0, 125.8, 128.8, 128.9, 129.0, 136.3, 139.8, 142.2, 148.0, 164.2. MS: m/z 432.4 $(M+H)^+$, 454.3 $(M+Na)^+$.

*Triazolophane [**3c**]:* Whitish semisolid, Yield: 40%; IR (KBr): 3134, 3043, 2954, 1735, 1603, 1454 cm^{-1}. ^{1}HNMR (400 MHz, $CDCl_3$): 1.38-1.44 (m, 4H), 1.93.1.99 (m, 4H), 4.10 (t, 4H, J = 6.9 Hz), 5.70 (s, 4H), 8.22 (brs, 1H), 8.29 (brs, 2H), 8.35 (s, 2H). ^{13}CNMR (100 MHz, $CDCl_3$): 26.6, 27.4, 56.7, 60.0, 125.2, 128.4, 139.5, 142.6, 148.4, 164.9. MS: m/z 412.2 $(M+H)^+$.

*Triazolophane [**5a**]:* Whitish semisolid, Yield: 60%; IR (KBr): 3370, 3132, 3035, 2958, 1740, 1598, 1450 cm^{-1}. ^{1}HNMR (400 MHz, $CDCl_3$): 2.67-2.94 (m, 2H), 4.57-4.63 (m, 1 H), 5.04 (s, 4H), 5.14 (s, 2H), 5.59 (s, 4H), 6.14 (brs, 1H), 7.01-7.37 (m, 9H), 7.59 (s, 1H), 7.62 (s, 1H). ^{13}CNMR (100 MHz, $CDCl_3$): 36.3, 50.4, 53.9, 58.1, 58.7, 67.1, 124.2, 124.5, 127.0, 127.6, 127.8, 128.1, 128.2, 128.3, 128.6, 129.5, 129.7, 129.9, 130.2, 135.7, 136.1, 142.5, 156.0, 170.4, 170.6. MS: m/z 532.5 $(M+H)^+$, 554.4 $(M+Na)^+$.

*Triazolophane [**5b**]:* Whitish semisolid, Yield: 62%; IR (KBr): 3374, 3135, 3033, 2955, 1738, 1595, 1453 cm^{-1}. ^{1}HNMR (400 MHz, $CDCl_3$): 2.59-2.90 (m, 2H), 4.56-4.60 (m, 1 H), 5.03 (s, 4H), 5.13 (s, 2H), 5.43 (s, 4H), 6.05 (brs, 1H), 7.07-7.29 (m, 9H), 7.55 (s, 1H), 7.60 (s, 1H). ^{13}CNMR (100 MHz, $CDCl_3$): 36.5, 50.4, 51.6, 53.8, 58.0, 58.7, 67.1, 124.1, 124.4, 127.9, 128.1, 128.3, 128.6, 128.7, 131.4, 135.3, 136.1, 142.4, 142.6, 155.9, 170.4, 170.5. MS: m/z 532.3 $(M+H)^+$, 554.3 $(M+Na)^+$.

*Triazolophane [**5c**]:* Off white semisolid, Yield: 45%; IR (KBr): 3369, 3129, 3036, 2950, 1742, 1603, 1452 cm^{-1}. ^{1}HNMR (400 MHz, $CDCl_3$): 1.37-1.42 (m, 4H), 1.91-2.02 (m, 4H), 2.85-3.12 (m, 2H), 4.33 (t, 4H, J = 7.1 Hz), 5.26 (s, 2H), 5.59 (s, 4H), 5.88 (brs, 1H), 7.33 (s, 5H), 7.59 (s, 1H), 7.63 (s, 1H). ^{13}CNMR (100 MHz, $CDCl_3$): 26.0, 26.1, 28.5, 28.6, 29.9, 30.0, 36.6, 50.3, 50.4, 51.1, 51.2, 58.2, 58.9, 67.2, 124.0, 124.3, 128.1, 128.3, 128.6, 136.1, 142.0, 142.2, 155.9, 170.5. MS: m/z 512.4 $(M+H)^+$.

RESULTS AND DISCUSSION

The title compounds (3a-c and 5a-c) were synthesized from dialkynes **1** and **4**. The starting material **1** was prepared from 2,6-pyridine dicarbonylchloride and propargyl alcohol, according to literature procedure [15]. Likewise, **4** was prepared from N-Cbz-(S)-aspartic acid and propargyl bromide as per reported procedure [16]. The diazides **2a-c** were synthesized according to literature method [15]. The dialkynes **1** and **4** were reacted with equivalent of diazides **2a-c** in presence of copper sulfate and sodium ascorbate in water/DMF mixture. The newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS spectral techniques. The IR spectrum of all the triazolophanes showed bands in the region 3139-3129 and 1742-1731 cm^{-1} which were attributed to the stretching vibrations of =C-H of triazole ring and C=O of carbonyl group respectively.The ^{1}H NMR spectra of all the compounds supported their structures. The ^{1}H NMR spectra of **3a** exhibited two singlets at δ 5.44 and 5.60 each integrating for four protons which can be assigned to pair of methylene and a characteristic singlet at δ 8.32 due to two triazolyl protons. In the ^{1}H NMR spectra of **5a** two singlet were observed at δ 7.59 and 7.62 indicating the presence of two triazolyl protons. The ^{13}C NMR spectra of **3a** exhibited signal at δ 53.1 and 58.7 corresponding to four methylene carbons. Two characteristic signals at δ 125.9 and 164.2 were assigned to C-5 of triazoles ring and carbonyl carbon respectively. In the ^{13}C NMR spectra of **5a** two signals at δ 124.2 and 124.5 were observed due to C-5 of two triazoles rings. The signal at δ 156.0, 170.4 and 170.6 indicates the presence of three carbonyl carbons.

The perusal of antimicrobial screening of newly synthesized compounds indicated that all the compounds exhibited good to high activity. In case of *S. aureus*, compounds derived from aspartic acid (**5a-c**) showed better activity compared to that containing pyridine moiety (**3a-c**) but this trend reversed for *B. subtilis*. Compounds **3a** and **5b** were found to be more potent among all the tested compounds as well as reference drug, against *B. subtilis* and *E. coli*. Compound **5b** displayed comparable activity to fluconazole and found to be most active than others against *A.niger*.

CONCLUSION

A small series of 1,2,3-triazolophanes has been synthesized by copper (I)-catalyzed click reaction and investigated for their antimicrobial activity. The antimicrobial activity data indicated that all the newly synthesized compounds exhibited good activity. Compounds **3a** and **5b** showed promising antibacterial activity against *B. subtilis* and *E. coli* respectively. Likewise 5b showed good and comparable activity to fluconazole against *A.niger*.

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