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Synthesis, characterization and antimicrobial evaluation of triazole substituted pyrimidine derivatives

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The present investigation has been conducted with the purpose of creating a novel class of 1,2,3-triazole linked pyrimidine hybrids using Cu(I)-catalyzed azide-alkyne cycloaddition (NaAAC) with high yields (80-90%) by utilizing different poly-halogenated synthetic compounds. The structures of the newly synthesized compounds have been determined using ^{1}H and ^{13}C NMR, IR, and ESI-HRMS. Evidence of antimicrobial action by the synthetically produced pharmacologically active molecules have been provided. The efficacy of these synthetic compounds in eradicating bacteria and fungi has been examined and compared with that of established antibiotics such as ciprofloxacin and ketoconazol, respectively. Among all the compounds, **4a** and **4f** exhibit the highest levels of activity. The compounds have been tested for their properties against *Bacillus subtilis*, *Escherichia coli* and *Aspergillus flavus*, *Candida albicans*.

Keywords: Triazoles, Pyrimidine, Click reaction, Anti microbial activity

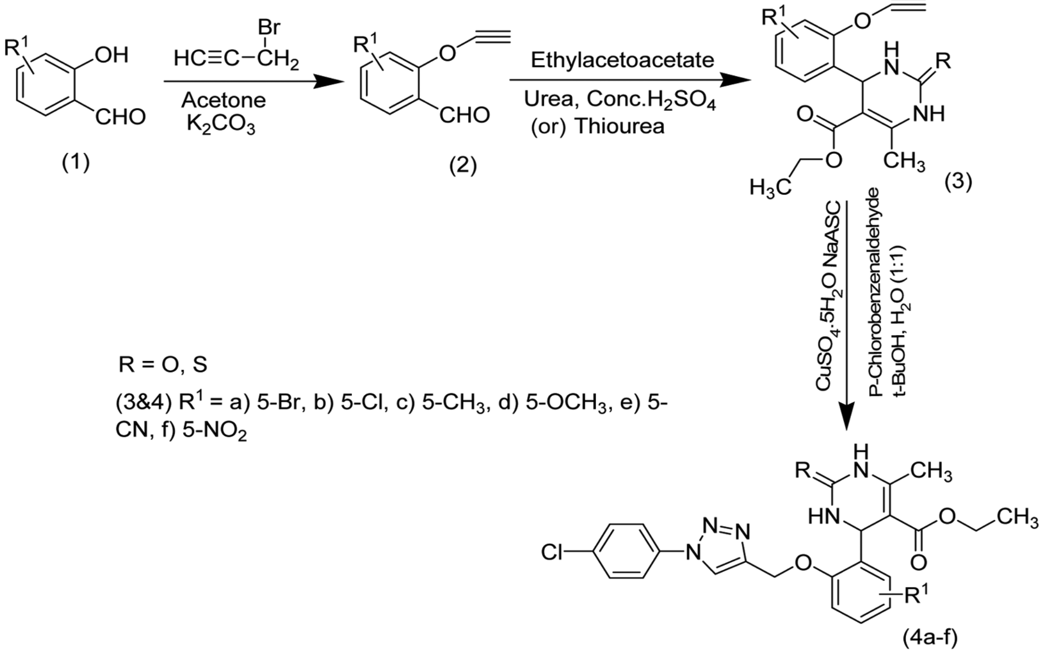
Pyridine nucleus-based medicines are fast becoming a significant class of therapeutic agents[1], with many present drugs expected to be replaced in the near future[2]. The pyridine nucleus, found in several physiologically active compounds, has various beneficial properties such as anti-tubercular, anti-viral, insecticidal, antibacterial, anti-neoplastic, and anti-tumor activity[3-10]. It has been noted that several Triazoles and other pyridine fused derivatives have antibacterial properties[11]. Whereas the 1,2,3-triazole scaffold primarily has unique structural features and shows promising physiologically[12]. There have been a number of recent publications discussing 1,2,3-triazole synthesis, anti-tubercular action, and potassium or sodium channel activating activities[13-15].

Developing upon our prior accomplishments in synthesizing potential molecules, we have developed a new set of compounds by combining pyridine, 1,2,3-triazole[16,17]. This has resulted in the creation of a hybrid molecule, and we have successfully produced a range of novel derivatives of ethyl 6-(2-((1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy) phenyl) -1,2,3,4-tetrahydro-4-methyl-2-oxopyrimidine-5-carboxylate[18]. Therefore, the current work produced a series of novel derivatives **4a-f** of hybrid compounds that included triazole systems, and then examined their antimicrobial ability[19,20].

Results and Discussion

5-Substituted 2-hydroxybenzaldehyde **1** (1.04 mL) with 3-bromoprop-1-yne (0.75 mL) in presence of potassium carbonate and acetone (50 mL) were taken round bottom flask and stirred for 6 h to get 2-(ethynyloxy)benzaldehyde **2**. In order to get **3**, 2-(ethynyloxy)benzaldehyde **2** is subjected to treatment with ethyl acetate and either urea or thiourea in the presence of conc. H_2SO_4 with different alkyl substituents. The click reaction is used to treat the propargylated benzaldehyde (146 mg, 0.001 mol) with aryl azides (26 mg, 0.002 mol). Furthermore, sodium ascorbate (38mg, 0.0002mol) and 48 mg of $CuSO_4.5H_2O$ and 15 mL of a 1:1 mixture of t-butanol and water were added to the reaction in an RB flask. The mixture was agitated overnight at RT to produce aryl triazole carboxylates **4a-f**. Following vigorous stirring of the reaction mixture, it was filtered and allowed to dry in a 1:1 ether:ethyl acetate combination. Analytical and spectral data (IR, ^{1}H and ^{13}C NMR and EI-MS) validated the structure of the novel compounds (Scheme 1).

Compound **4g** served as a sample example, and its ^{1}H NMR spectra showed a multiplet between δ 7.25 and 7.55, indicating the presence of aromatic protons and triazole protons, shows singlet at δ 8.30 and 8.71 attributed to $-NH_2$ protons. All of the molecular ion peaks of the named substances were visible in their



Scheme 1 — Synthetic pathway of compounds **4a-f**

Table 1 — Antimicrobial activities of compounds

Compd	Antimicrobial and antifungal activities of compounds – Zone of inhibition (mm)			
	Antibacterial activity		Antifungal activity	
	B. subtilis	*E. coli*	*A. flavus*	*C. albicans*
4a	36	34	32	34
4b	29	33	28	29
4c	25	26	25	24
4d	28	30	26	20
4e	21	23	22	21
4f	29	34	16	39
Ciprofloxacin	22	25	–	–
Ketoconazole	–	–	23	22

respective MS spectra. The structures of the stated compounds **4a-f** were also confirmed by IR, NMR, MS and elemental analysis.

Antimicrobial activity

Agar well diffusion was used to evaluate the antibacterial activity of the test compounds against *Bacillus subtilis* and *Escherichia coli* (Table 1). Mueller Hinton system agar was used for bacterial cultivation. The typical method for treating bacterial infections was followed when ciprofloxacin was provided. The samples were diluted in a 1 molar (M) dimethylsulfoxide (DMSO) solution until they reached a concentration of 1 mg/mL. The other compounds showed moderate to high levels of activity, whereas the compound 4a showed greater levels of activity.

There were 106 cells/mL of fungal spores suspended in sterile PBS. After being dipped into the fungal solution, a sterile swab was turned on top of the agar plate. In order to prepare the plates for the experiment, they were let to air dry at RT for 15 min. A solution containing the test chemical was then added to the well, with concentrations ranging from 20 g/mL to 80 g/mL. The plates were kept at 37°C during the incubation process. In order to test the antifungal activity, the inhibitory zones were measured in millimeters following a 48 h incubation period. Activity levels ranged from moderate to high for the other compounds, with the exception of **4f**, which showed high activity. The most effective antifungal drug is used as ketoconazole.

Experimental Section

We acquired all of our reagents from Sigma Aldrich. For this synthesis, we only employed solvents of the highest technical quality that had been recently distilled. Then we used an OrbitrapExactive LC-HRMS to conduct the ESI-HRMS. An ^{1}H NMR spectrum and ^{13}C (100 MHz) NMR spectra at 400 MHz was acquired using $CDCl_3$ as the solvent standard using a Bruker AM-400 spectrometer. The part per million (ppm) units are used for chemical shifts (δ), whereas the Hz units are used for coupling constants (*J*). In order to find the melting points, capillary tubes were used in a Buchi 510 apparatus.

General procedure for synthesis of compound 2

In a round-bottom flask, 1.04 mL of 5-substituted 2-hydroxybenzaldehyde dissolved in methanol and add 0.75 mL of 3-bromoprop-1-yne were combined with 50 mL of acetone and potassium carbonate. The mixture was agitated for 6-8hours to produce 2-(ethynyloxy) benzaldehyde (2). After the reaction mixture was vigorously stirred, it was filtered and allowed to dry. Yield 71%. m.p.221–223°C. IR (KBr): 2650 cm^{-1} (CHO); ^{1}H NMR ($CDCl_3$): δ 1.55 (s, 1H-CH), 6.99 (d, *J*=8.7Hz, 2H, Ar-H) 7.44 (m, 2H-ArH), 10.14 (s, 1H, CHO); ^{13}C NMR ($CDCl_3$): δ 55.40, 82.63, 120.25, 122.43, 131.35, 135.41, 161.03, 187.53; MS: *m/z* 146.04. Anal. Calcd for $C_9H_6O_2$: C, 73.97; H, 4.14; O, 21.90. Found: C, 73.99; H, 4.16; O, 21.95%.

General procedure for synthesis of compounds 3a and 3b

The reaction of 2-(ethynyloxy)benzaldehyde **2** with ethyl acetate and urea or thiourea in the presence of concentrated H_2SO_4 with different alkyl substituents gives **3**. It was filtered and left to dry after being recrystallized with chloroform and a vigorous stirring of the reaction mixture.

Ethyl 6-(2-bromo-6-(ethynyloxy)phenyl)-1,2,3,4-tetrahydro-4-methyl-2-oxopyrimidine-5-carboxylate, 3-R=O: Yield 69%. m.p.228–229°C. IR (KBr): 1610 (C=O), 3218 cm^{-1} (NH); ^{1}H NMR ($CDCl_3$): δ 1.28 (s, 1H-CH), 1.40 (s, 6H, CH_3) 2.40 (s, 2H, CH_2), 2.60 (s, 3H, Ar-H), 5.43 (s, 1H, NH), 7.04 (s, 2H, NH); ^{13}C NMR ($CDCl_3$): δ 14.70, 19.75, 49.32, 55.40, 61.50, 82.63, 103.03, 113.96, 121.91, 125.39, 126.83, 132.02, 141.01, 156.18, 162.22, 168.95; MS: *m/z* 371.21. Anal. Calcd for $C_{16}H_{15}BrN_2O_4$: C, 50.68; H, 3.97; N, 7.39. Found: C, 50.69; H, 3.99; N, 7.42%.

Ethyl 6-(2-bromo-6-(ethynyloxy)phenyl)-1,2,3,4-tetrahydro-4-methyl-2-thioxopyrimid ine-5-car boxylate, 3-R=S: Yield 72%. m.p.226–228°C. IR (KBr): 1615 (C=O), 3220 cm^{-1} (NH); ^{1}H NMR ($CDCl_3$): δ 1.35 (s, 1H-CH), 1.58 (s, 6H, CH_3) 4.36 (s, 2H, CH_2), 5.00 (s, 1H, CH), 6.77 (s, 2H, Ar-H), 7.04 (s, 2H, NH), 8.45 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 14.70, 19.75, 51.46, 55.40, 61.50, 82.63, 104.72, 113.96, 121.91, 1325.39, 126.83, 132.02, 142.89, 162.22, 168.95, 176.23; MS: *m/z* 395.27. Anal. Calcd for $C_{16}H_{15}BrN_2O_3S$: C, 48.62; H, 3.82; N, 7.09. Found: C, 48.65; H, 3.85; N, 7.13%.

General procedure for the synthesis of compounds 4a-f

The propargylated benzaldehyde (146 mg, 0.001 mol) is treated with aryl azides (26 mg, 0.002 mol) *via* the click reaction. In addition a 1:1 combination of *t*-butanol and water together with 48 mg of $CuSO_4.5H_2O$ were introduced to the reaction in an RB flask. Aryl triazole carboxylates **4a-f** were synthesized by stirring the mixture at RT during the night. The reaction mass was filtered and left to dry in a 1:1 ether:etyl acetate mixture when the reaction mixture was vigorously stirred subsequently.

Ethyl 6-(2-((1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-bromophenyl)-1,2,3,4-tetrahydro-4-methyl-2-oxopyrimidine-5-carboxylate, 4a: Yield 90%. m.p.225–227°C. IR (KBr): 1612 (C=O), 3225 cm^{-1} (NH); ^{1}H NMR ($CDCl_3$): δ 1.53 (s, 6H, CH_3) 4.35 (s, 2H, CH_2), 5.13 (s, 2H, NH), 5.20 (s, 2H, CH_2), 7.25 (m, 3H, Ar-H), 7.55 (s, 4H, Ar-H), 8.30 (s, 1H, Triazole-CH), 8.71 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 14.70, 19.75, 49.32, 59.85, 61.50, 100.83, 113.41, 119.40, 123.13, 123.99, 126.34, 129.21, 130.13, 132.65, 134.59, 136.01, 142.86, 147.86, 156.18, 157.78, 168.95; MS: *m/z* 545.05. Anal. Calcd for $C_{23}H_{21}BrClN_5O_4$: C, 50.52; H, 3.87; N, 12.81. Found: C, 50.55; H, 3.90; N, 12.84%.

Ethyl 6-(2-((1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-chlorophenyl)-1,2,3,4-tetrahydro-4-methyl-2-oxopyrimidine-5-carboxylate, 4b: Yield 73%. m.p.227–229°C. IR (KBr): 1620 (C=O), 3220 cm^{-1} (NH); ^{1}H NMR ($CDCl_3$): δ 1.61 (s, 6H,

CH_3) 4.43 (m, 4H, CH_2), 5.16 (s, 2H, NH), 5.20 (m, 3H, Ar-H), 7.48 (m, 3H, Ar-H), 8.29 (s, 1H, Triazole-CH), 8.66 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 14.70, 19.75, 49.32, 59.85, 61.50, 101.23, 114.67, 119.90, 123.12, 125.40, 129.20, 130.44, 131.89, 132.65, 134.94, 136.01, 143.92, 147.86, 156.18, 156.82, 168.95; MS: *m/z* 545.05. Anal. Calcd for $C_{23}H_{20}Cl_2N_5O_4$: C, 50.52; H, 3.47; N, 12.05. Found: C, 50.55; H, 3.49; N, 12.10%.

Ethyl 6-(2-((1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-methylphenyl)-1,2,3,4-tetrahydro-4-methyl-2-oxopyrimidine-5-carboxylate, 4c: Yield 73%. m.p.224–226°C. IR (KBr): 1622 (C=O), 3221 cm^{-1} (NH); ^{1}H NMR ($CDCl_3$): δ 1.39 (s, 6H, CH_3) 1.40 (s, 3H, CH_3), 4.42 (m, 4H, CH_2), 5.29 (s, 2H, NH), 7.34 (m, 5H, Ar-H), 7.98 (m, 2H, Ar-H), 8.08 (s, 1H, Triazole-CH), 8.80 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 14.70, 19.75, 21.21, 49.32, 57.96, 61.50, 100.70 115.23, 119.90, 123.12, 128.72, 129.20, 131.40, 132.22, 132.65, 136.01, 146.23, 147.86, 155.54, 156.18, 168.95; MS: *m/z* 481.15. Anal. Calcd for $C_{24}H_{24}ClN_5O_4$: C, 59.81; H, 5.02; N, 14.53. Found: C, 59.83; H, 5.05; N, 14.55%.

Ethyl 6-(2-((1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-methoxyphenyl)-1,2,3,4-tetrahydro-4-methyl-2-oxopyrimidine-5-carboxylate, 4d: Yield 73%. m.p.225–227°C. IR (KBr): 1615 (C=O), 3212 cm^{-1} (NH); ^{1}H NMR ($CDCl_3$): δ 1.57 (s, 6H, CH_3) 3.98 (s, 3H, OCH_3), 4.46 (m, 4H, CH_2), 5.04 (s, 2H, CH_2), 5.17 (s, 2H, NH), 7.53 (m, 3H, Ar-H), 7.72(m, 2H, Ar-H), 8.07 (s, 1H, Triazole-CH), 8.39 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 14.72, 19.75, 49.32, 56.79, 59.85, 61.50, 100.83, 116.06, 119.90, 123.12, 123.96, 125.50, 129.20, 132.65, 136.01, 142.86, 147.86, 148.52, 150.51, 150.51, 156.18, 168.95; MS: *m/z* 497.15. Anal. Calcd for $C_{24}H_{24}ClN_5O_4$: C, 57.89; H, 4.86; N, 14.06. Found: C, 57.92; H, 4.90; N, 14.10%.

Ethyl 6-(2-((1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-hydroxyphenyl)-1,2,3,4-tetrahydro-4-methyl-2-oxopyrimidine-5-carboxylate, 4e: Yield 70%. m.p.220–222°C. IR (KBr): 1622 (C=O), 3100 (OH), 3221 cm^{-1} (NH); ^{1}H NMR ($CDCl_3$): δ 1.54 (s, 6H, CH_3) 4.37 (m, 4H, CH_2), 5.06 (s, 2H, NH), 5.27 (m, 2H, CH), 7.72 (m, 5H, Ar-H), 8.08 (s, 1H, Triazole-CH), 8.63 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 14.85, 19.75, 49.32, 59.85, 61.50, 99.85, 1..83, 116.12, 119.90, 123.12, 125.98, 128.46, 129.20, 132.65, 133.00, 136.01, 142.86, 147.86, 156.18, 156.39, 168.95; MS: *m/z* 492.13. Anal. Calcd for $C_{24}H_{21}ClN_6O_4$: C, 58.48; H, 4.29; N, 14.53. Found: C, 58.49; H, 4.32; N, 14.57%.

Ethyl 6-(2-((1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-nitrophenyl)-1,2,3,4-tetrahydro-4-methyl-2-oxopyrimidine-5-carboxylate, 4f: Yield 71%. m.p.223–225°C; IR (KBr): 1625 (C=O), 1600 (NO_2), 3211 cm^{-1} (NH); ^{1}H NMR ($CDCl_3$): δ 1.50 (s, 6H, CH_3) 4.36 (m, 4H, CH_2), 5.02 (s, 2H, NH), 5.24 (m, 2H, CH_2), 7.52 (m, 4H, Ar-H), 8.08 (s, 1H, Triazole-CH), 8.37 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 14.75, 19.75, 49.32,57.96, 61.50, 100.70, 117.84, 119.90, 123.12, 126.38, 127.02, 129.20, 129.72, 132.65, 136.01, 142.92, 146.23, 147.86, 156.81, 162.40, 168.95; MS: *m/z* 512.12. Anal. Calcd for $C_{23}H_{21}ClN_6O_6$: C, 53.86; H, 4.13; N, 16.39. Found: C, 53.89; H, 4.16; N, 16.42%.

Conclusion

In conclusion this study aimed to develop a new class of 1,2,3-triazole linked Pyrimidines hybrids with excellent yields through the use of various synthesized poly-halogenated compounds in a Cu(I)-catalyzed azide-alkyne cycloaddition (NaAAC). ^{1}H and ^{13}C NMR, IR, and ESI-HRMS were used to identify the structures of the newly synthesized compounds. All substances that have been shown to have antimicrobial activity in pharmacological studies are included. Compared to ciprofloxacin and ketoconazol, the synthesized substances were tested for bacteria and fungal control. When compared to the other compounds, compounds **4a** and **4f** showed the most activity.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/5877.

Conflict of interest

The authors affirm no conflicts of interest.

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