Research Article

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Synthesis of novel triazole hybrids with pyrene, fluorene, and biphenyl groups and evaluation of their antimycobacterial activity

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Abstract: The synthesis of 1,2,3-triazoles, which are acting as promising pharmacophores with strong biological activity, is the focus of our work. Three obscure streaks of hybrid molecules with a 1,2,3-triazole nucleus have been synthesized by us. Compounds are synthesized in two steps: first, an azide is formed from its amine equivalent, next 1,2,3-triazole ring is formed utilizing azide-alkyne click chemistry. The obtained yields range from fair to good. After testing, it is discovered that the compounds 1c, 2a, 2c, 3a have good antibacterial properties and compounds 2b, 3a have promising antifungal properties. The compounds are found to be soluble in ethanol, ethyl acetate. The primary difficulty we encountered was with cyclopropane as the reaction needed to be carried out under reflux at 100°C, and its boiling point is 51°C.

Keywords: triazoles, antimycobacterial, hybrids, novel

1 Introduction

Since Sharpless and his colleagues early popularized the notion of “click chemistry,” the CuAAC has amassed extensive research attention published in article titled “Click Chemistry: Diverse Chemical Function from a few good reactions” [1,2]. Under mild circumstances, this reaction effectively harvests 1,4-disubstituted-1,2,3-triazoles at a remarkable pace [3,4]. This metal-catalysed process was disjointly unearthed by the faction of Sharpless and Meldal. It harvests a coalescence of 1,4- and 1,5-disubstituted triazoles, which is a considerable upgradation over the definitive Husigen thermal 1,3-dipolar cycloaddition [5,6]. “Click chemistry” exemplifies a contoured avenue for accruing new molecular scheme. The immense suitableness of CuAAC is evident from its use across manifold expanse of biological and material sciences [7]. Triazole-based hybrid molecules have been synthesized through uncomplicated and adept Cu(II) catalysed advent [8]. Enumerated modules on triazole chemistry have underscored its potential in biochemical probing, shifting from drug discovery and accession to designating biological stuff [9,10]. 1,2,3-Triazole and 1,2,4 triazole units are profoundly cherished as linkers due to their flexibleness contra to metabolic disintegration and their provision to form hydrogen bonds, which heightens both biomolecular target binding and solubility [11,12]. Furthermore, molecules featuring 1,2,3-triazoles manifest robust antibacterial effects against a broad range of clinically relevant bacteria, including drug-resistant strains [13]. Pyrene’s paramountcy as an environmental pollution indicator and its usefulness as a fluorescence analyst highlight its crucial position in applied scientific domains and analytical procedures. Pyrene affixed triazoles serve as exquisite chemo sensors [14,15]. Their fluorescence is dependent on circumferential polarity, viscosity, and alliance with secondary molecules [16]. The sequel compounds can be adopted as pH-sensitive researchers and environment-sensile agents by blending them alongside triazoles. Their supplementary work is to spot the potentiality of biomolecules [17]. The evident characteristics of fluorene can be varied by allocating a triazole share to it [18,19]. Through functionalization, they impart durable properties to advanced materials for conventional use and medical applications [20]. The biphenyl allied triazoles support in offering great antimicrobial activity [21].

2 Materials and methods

The materials utilized include t-BuOH, conc. HCl, distilled water, copper sulphate, sodium ascorbate, sodium nitrite,

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sodium azide, cyclopropylacetylene (A), cyclopentylacetylene (B), cyclohexylacetylene (C), biphenyl amine (1), pyrene-2-amine (2), and fluorene-2-amine (3). Two steps must be taken in order to synthesize the final product.

2.1 First step

Conc. HCl:H_2O (1:3) is used to treat the 1° amine 1 eq. (1) and a temperature range of 0–5°C should be maintained in ice bath to initiate protonation. After accruing pinch-wise $NaNO_2$ (2.5 eq.), whirl the mixture for 20 min relentlessly. Next, add pinch-wise NaN_3 (2.5 eq.), and whirl once more for 20 min. Please take token that the reaction should be conducted at a temperature between 0 and 5°C. Thin layer chromatography (TLC) analysis should be used to sustain the reaction's advancement. To filter the crude azide (1″), ice-cold water or broken ice is affixed into the mixture. The equivalent procedure is followed for amines 2 and 3. The analogous azides 2″ and 3″ are filtered as crude product (Scheme 1) [22].

2.2 Second step

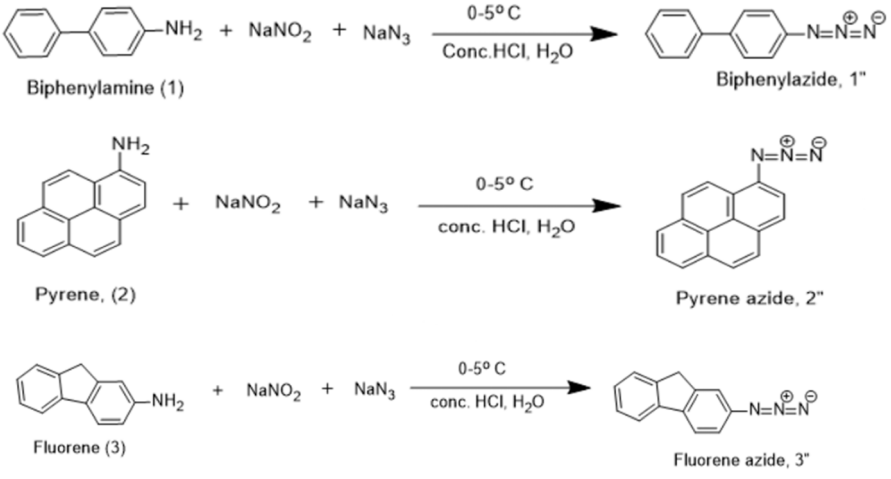
Alkyne (1 eq.) (A), $CuSO_4$ (0.8 eq.), sodium ascorbate (1.1 eq.), and azide (1 eq.) (1″) are mixed in a one-pot reaction employing t-BuOH:H_2O (1:1). After roughly an hour, the reaction mixture is agitated in an oil bath while under reflux. Using TLC, the reaction's progress is computed. The crude product 1a is filtered out of the reaction mixture by accruing crushed ice- or ice-cold water after the reaction is accomplished. The same azide 1″ is treated with other two alkynes B and C to form products 1b and 1c, respectively (Scheme 2) [23,24]. Ethanol is used in the recrystallization process to carve the crude product [25]. The same reaction is proceeded with azide 2″, 3″ and alkynes A, B, and C separately one by one to form products 2a, 2b, 2c and 3a, 3b, 3c (Schemes 3 and 4). Since the alkynes have low boiling temperatures, reflux was done scrupulously. ^{1}H-NMR, ^{13}C-NMR, and HRMS are used to characterize the synthesized molecules.

Using the usual disc-diffusion method [26], two Gram +ve and two Gram −ve microorganisms [27] were used to assess the made compounds' antibacterial activity. The minimum inhibitory concentration (MIC) values were computed and contrasted with the reference standards of ciprofloxacin and chloramphenicol (Table 1). *Aspergillus niger* and *Candida albicans* were used to investigate the antifungal activity. A comparison was made between the minimum fungicidal concentration and reference standards nystatin and griseofulvin [28] (Table 2).

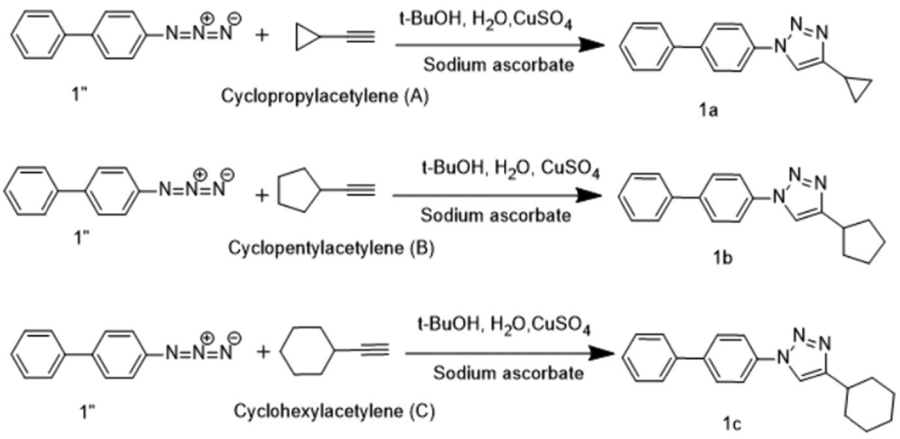
3 Results and discussion

Azides correspondent to Scheme 1 are pounded in marvellous yields (>80%). Exquisite yield of the synthesized compounds (Schemes 2–4) is earned (Table 3). Using spectrum techniques such as ^{1}H-NMR, ^{13}C-NMR, and HRMS, the synthesized compounds are verified. It is discovered that the spectral details agree with the suggested structures.

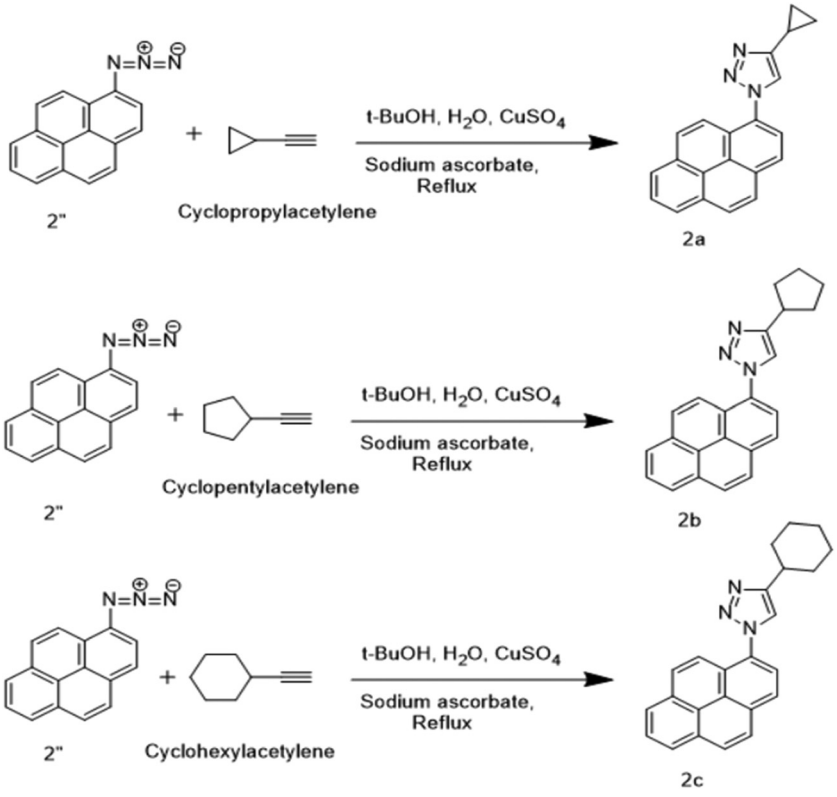
^{1}H and ^{13}C NMR: The structures of triazoles correspondent to Schemes 2–4 are corroborated by the presence of single signal at 8.08 ppm (1a, 1b, 1c), 8.31 ppm (for 2a, 2b, 2c), and 8.15 ppm (for 3a, 3b, 3c). Anisotropic effect downfields the shift as electronegative drift by N adhered to the ring



Scheme 1: Synthesis of azides of 1, 2, and 3 amino compounds [22].



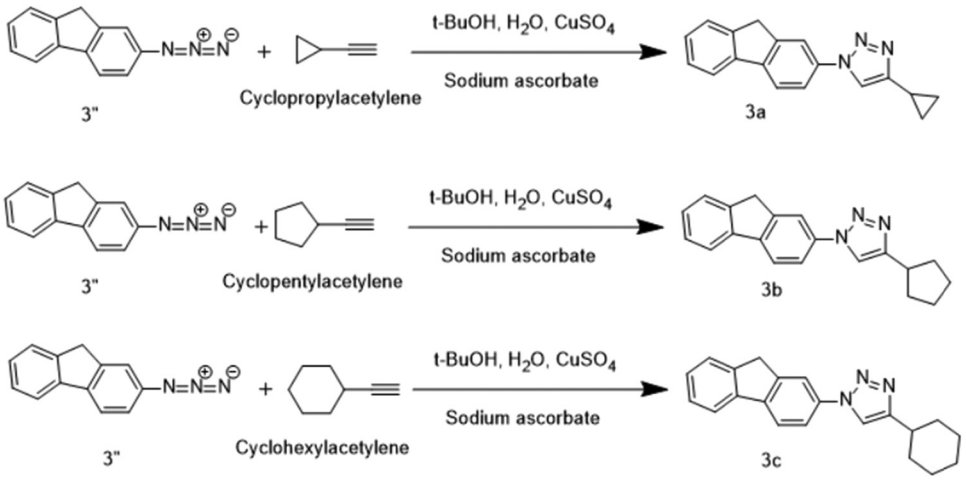
Scheme 2: Synthesis of substituted biphenyl triazoles [23,24].



Scheme 3: Synthesis of substituted pyrene triazoles [23,24].

decreased the e^- density encompassing proton. The potentiality of ^{1}H-NMR signals (multiplet) from δ 6.0 to 7.5 ppm portends the existence of aromatic hydrogens (sp^2) and signals from δ 1.5 to 4.0 ppm augurs the presence of sp^3 hybridized hydrogens. ^{13}C spectra manifest myriad peaks in the thick of 120–150 ppm which attested the ubiety of aromatic Cs.

HRMS: Molecular mass of molecular ion peak [M^+] as divulged in the HRMS spectra (determined using SYNAPT XS Mass Spectrometer), has been delineated to be commensurate to the compound's theoretically predicted molecular weight procured in HRMS spectra. **1a:** theoretical Mol. Wt. 261.311 g mol^{-1}, experimental Mol. Wt. 262.1339 $gmol^{-1}$; **1b:** theoretical Mol. Wt. 289.363 g mol^{-1}, experimental Mol. Wt.



Scheme 4: Synthesis of substituted fluorene triazoles [23,24].

Table 1: Antibacterial activity of targeted compounds [26,27]

Minimum inhibitory concentration (μg/mL)					
S. no.	**Compound**	***B. subtilis***	***S. aureus***	***E. coli***	***P. aeruginosa***
1	1a	250	100	250	250
2	1b	100	200	100	125
3	1c	125	125	**62.5**	100
4	2a	**62.5**	100	200	200
5	2b	100	100	100	125
6	2c	125	200	**62.5**	100
7	3a	100	**62.5**	200	250
8	3b	250	250	100	200
9	3c	200	125	200	100
Chloramphenicol		50	50	50	50
Ciprofloxacin		50	50	25	25

Table 2: Antifungal activity of the targeted compounds [28]

Minimum fungicidal concentration (μg/mL)			
S. no	**Compound**	***C. albicans***	***A. niger***
1	1a	1,000	500
2	1b	1,000	1,000
3	1c	500	500
4	2a	500	1,000
5	2b	**250**	500
6	2c	500	500
7	3a	**250**	1,000
8	3b	500	1,000
9	3c	1,000	500
Griseofulvin		500	100

Table 3: Yields and melting point of the targeted compounds

Compound	**Yield (%)**	**m.pt. (°C)**
1a	75	109
1b	84	120
1c	86	144
2a	76	135
2b	89	140
2c	91	158
3a	78	145
3b	88	155
3c	83	180

290.1654 g mol^{-1}; **1c:** theoretical Mol. Wt. 303.389 g mol^{-1}, experimental Mol. Wt. 304.1820 g mol^{-1}; **2a:** theoretical Mol. Wt. 309.351 g mol^{-1}, experimental Mol. Wt. 310.1393 g mol^{-1}; **2b:** theoretical Mol. Wt. 337.403 g mol^{-1} experimental Mol. Wt. 338.1702 g mol^{-1}; **2c:** theoretical Mol. Wt. 351.429 g mol^{-1}, experimental Mol. Wt. 352.1904 g mol^{-1}; **3a:** theoretical Mol.

Wt. 273.321 g mol^{-1}, experimental Mol. Wt. 274.1388 g mol^{-1}; **3b:** theoretical Mol. Wt. 301.373 g mol^{-1}, experimental Mol. Wt. 302.11706 g mol^{-1}; **3c:** theoretical Mol. Wt. 315.399 g mol^{-1}, experimental Mol. Wt. 316.1860 g mol^{-1}.

The MIC values for compounds 1c, 2c, and 3a against *B. subtilis* (Gram +ve), *E. coli* (Gram −ve), and *S. aureus* (Gram +ve), respectively, is 62.5 mg/mL. This is somewhat higher than the reference standards utilized. Therefore, more research on these substances as antibacterial agents is possible. In comparison to the reference standard griseofulvin, compounds 2b and 3a have superior antifungal efficacy (250 µg/mL) against *C. albicans* (500 µg/mL).

4 Conclusion

Innovative triazoles have been melded through a periodically used cycloaddition manner. Their germicidal property is tied to circumstantiated interpretation. Conspicuously, compounds 1c, 2c, and 3a egressed as persuasive antibacterial agents, while 2b and 3a evinced promising antifungal efficaciousness. In succinct, cyclopropyl ring is advantageous in professing agility counter to Gram +ve bacteria while cyclohexyl ring is advantageous counter to Gram −ve one. Further delving in these molecules for a voluminous leeway of biological activeness is in momentum.

The NMR spectra's details (determined using ^{1}H-$CDCl_3$ and ^{13}C-$CDCl_3$ Bruker Avance Neo 500 MHz NMR spectrometer) are listed below.

1a: ^{1}H-NMR analysis – **^{a*} δ 6.94 (m, 2H), 6.90 (m, 2H), 6.85 (m, 2H), 6.95 (m, 2H), 6.96 (m, 1H), 8.08 (s, 1H) ^{b*},** 1.42 (s, 1H), 0.93–1.00 (m, 4H).

^{13}C-NMR analysis – δ 161.46 (s), 140.41 (s), 136.44 (s), 134.35 (s), 129.02–128.81 (m), 128.33 (s), 127.40–127.18 (m), 126.70 (s), 124.86–124.68 (m), 124.68–124.48 (m), 8.69–8.37 (m), 7.55 (s).

1b: ^{1}H-NMR analysis – **(a*–b*)**, δ 2.79 (s, 1H), 2.05 (m, 4H), 1.67 (m, 4H).

^{13}C-NMR analysis – δ 154.42 (s), 140.41 (s), 136.44 (s), 134.35 (s), 129.41 (s), 129.02–128.81 (m), 128.33 (s), 127.40–127.18 (m), 124.86–124.68 (m), 124.68–124.48 (m), 39.24 (s), 34.36–33.96 (m), 26.23–26.01 (m).

1c: ^{1}H-NMR analysis –- **(a*–b*)**, δ 3.43 (s, 1H), 2.46 (m, 4H), 2.01–2.04 (m, 6H).

^{13}C-NMR analysis – δ 157.00 (s), 140.41 (s), 136.44 (s), 134.35 (s), 129.02–128.81 (m), 128.33 (s), 127.40–127.18 (m), 126.24 (s), 124.86–124.68 (m), 124.68–124.48 (m), 37.20 (s), 32.34–31.94 (m), 25.92 (s), 25.25–25.04 (m).

2a: ^{1}H-NMR analysis – **^{c*} δ 8.31 (s, 1H), 6.93 (d, 1H), 6.88 (d, 1H), 6.84 (d, 1H), 6.97 (d, 1H), 7.00 (d, 1H), 6.98 (d, 1H), 6.66 (d, 2H), 6.68 (m, 1H) ^{d*}**, 2.16 (m, 1H), 0.99 (m, 4H).

^{13}C-NMR analysis – δ 160.83 (s), 131.88–131.67 (m), 130.49 (s), 129.72 (s), 127.69 (s), 127.49–127.05 (m), 126.45 (s), 126.12 (s), 125.81 (s), 125.43 (s), 123.53 (s), 120.67 (s), 118.75 (s), 8.69–8.37 (m), 7.55 (s).

2b: ^{1}H-NMR analysis – **(c*–d*)**, δ 2.79 (m, 1H), 1.70 (m, 4H), 1.67 (m, 4H).

^{13}C-NMR analysis – δ 153.99 (s), 131.88–131.67 (m), 130.49 (s), 129.92 (s), 129.72 (s), 127.69 (s), 127.49–127.05 (m), 126.45 (s), 126.12 (s), 125.81 (s), 125.43 (s), 123.53 (s), 120.67 (s), 118.75 (s), 39.24 (s), 34.36–33.96 (m), 26.23–26.01 (m).

2c: ^{1}H-NMR analysis – **(c*–d*)**, δ 3.01 (m, 1H), 1.94 (m, 2H), 2.55 (m, 2H), 1.71–1.73 (m, 6H).

^{13}C-NMR analysis – δ 156.66 (s), 131.88–131.67 (m), 130.49 (s), 129.72 (s), 127.69 (s), 127.49–126.92 (m), 126.45 (s), 126.12 (s), 125.81 (s), 125.43 (s), 123.53 (s), 120.67 (s), 118.75 (s), 37.20 (s), 32.34–31.94 (m), 25.92 (s), 25.25–25.04 (m).

3a: ^{1}H-NMR analysis – **^{e*} δ 8.15 (s, 1H), 7.48 (s, 1H), 6.80 (d, 1H), 6.91 (d, 1H), 3.81 (m, 2H), 6.93 (d, 1H), 6.82 (d, 1H), 6.86 (m, 1H), 6.99 (m, 1H) ^{f*}**, 2.08 (m, 1H), 0.96 (m, 4H).

^{13}C-NMR analysis – δ 161.46 (s), 143.84 (s), 142.13 (s), 139.62 (s), 138.36 (s), 137.31 (s), 126.70 (s), 126.09 (s), 125.41 (d, J = 13.3 Hz), 122.09 (s), 121.47 (s), 121.03 (s), 120.63 (s), 35.36 (s), 8.69–8.37 (m), 7.55 (s).

3b: ^{1}H-NMR analysis – **(e*–f*)**, δ 2.79 (m, 1H), 1.83 (m, 4H), 1.67 (m, 4H).

^{13}C-NMR analysis – δ 154.42 (s), 143.84 (s), 142.13 (s), 139.62 (s), 138.36 (s), 137.31 (s), 129.41 (s), 126.09 (s), 125.41 (d, J = 13.3 Hz), 122.09 (s), 121.47 (s), 121.03 (s), 120.63 (s), 39.24 (s), 35.36 (s), 34.36–33.96 (m), 26.23–26.01 (m).

3c: ^{1}H-NMR analysis – **(e*–f*),** δ 3.42 (m, 1H), 1.75–1.79 (m, 8H), 2.44 (m, 2H).

^{13}C-NMR analysis – δ 156.99 (s), 143.84 (s), 142.13 (s), 139.62 (s), 138.36 (s), 137.31 (s), 126.17 (d, J = 18.2 Hz), 125.41 (d, J = 13.3 Hz), 122.09 (s), 121.47 (s), 121.03 (s), 120.63 (s), 37.20 (s), 35.36 (s), 32.34–31.94 (m), 25.92 (s), 25.25–25.04 (m).

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