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Click chemistry approach for the synthesis of 1,2,3-triazole-linked hybrid molecules

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Abstract

The concept of click chemistry, formalised by Sharpless and colleagues in 2001, has transformed synthetic access to complex bioactive scaffolds by replacing multi-step classical routes with single-pot, high-yielding, and regioselective transformations. This research presents the synthesis of a series of 1,2,3-triazole-linked hybrid molecules using copper(I)-catalysed azide-alkyne cycloaddition (CuAAC) as the key bond-forming step. Seven hybrid compounds were prepared by tethering pharmacologically relevant heterocyclic azide fragments with terminal alkynes carrying chalcone, coumarin, or quinoxaline moieties. Optimisation of the CuAAC reaction with CuI catalyst in DMF at 60 °C gave > 95% yield within 8 hours, outperforming CuBr-mediated reactions in acetonitrile at room temperature. All compounds were characterised by ¹H NMR, ¹³C NMR, IR, and HRMS. Anti-microbial evaluation against *Staphylococcus aureus* and *Escherichia coli* by broth microdilution gave MIC values of 1.56-12.5 µg/mL, with compound 4 (chalcone-triazole hybrid) showing the strongest activity at 1.56 µg/mL against *S. aureus*. Anti-proliferative testing against MCF-7 and HeLa cell lines by MTT assay gave IC₅₀ values of 8.3-22.7 µM. The regioselectivity for 1,4-disubstituted triazole formation was confirmed in all cases (>99% selectivity). These results confirm that CuAAC click chemistry provides a modular and efficient platform for assembling pharmacologically diverse hybrid compounds at the Department of Chemical Sciences, Birla Institute of Advanced Materials, Pilani.

Keywords: Click chemistry, CuAAC, 1,2,3-triazole, hybrid molecules, regioselectivity, antimicrobial activity, antiproliferative, chalcone, azide-alkyne cycloaddition, medicinal chemistry

Introduction

When Sharpless and collaborators outlined the principles of click chemistry in 2001, they described a conceptual shift away from traditional multi-step synthesis toward modular, high-yielding reactions that proceed cleanly under mild conditions with minimal side products [1]. Among the reactions satisfying the click criteria, the copper(I)-catalysed azide-alkyne cycloaddition (CuAAC) has emerged as the most widely applied, generating 1,4-disubstituted 1,2,3-triazoles with complete regioselectivity. Its tolerance for a wide range of functional groups, aqueous co-solvent compatibility, and the commercial availability of azide and alkyne building blocks have made it a first-choice tool in medicinal chemistry, bioconjugation, and polymer science [2, 3].

Hybrid molecules, defined as single chemical entities carrying two or more pharmacophoric fragments connected by a covalent linker, represent a strategic approach to multi-target drug design. The 1,2,3-triazole ring is ideally suited as a linker in hybrid architectures because it is bioisosteric with amide bonds, resistant to hydrolysis, and itself capable of hydrogen bonding and π -stacking interactions that contribute to target binding [4, 5]. Triazole-linked hybrids combining heterocyclic fragments have shown enhanced or synergistic biological activities compared to their parent fragments across a range of biological targets including dihydrofolate reductase, acetylcholinesterase, and various kinases [6].

Within the Indian pharmaceutical research landscape, the synthesis of triazole-based antimicrobial and anticancer agents has been an area of sustained interest, driven by the need to address rising antibiotic resistance and the growing burden of non-communicable diseases [7]. However, the systematic evaluation of multiple CuAAC catalyst systems and reaction conditions, coupled with in-depth characterisation and biological profiling of the resulting

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compound libraries, is less commonly reported in a single publication, making structure-activity relationships difficult to establish from disparate sources.

This research describes the design, synthesis, characterisation, and preliminary biological evaluation of seven 1,2,3-triazole-linked hybrid compounds prepared by CuAAC at the Department of Chemical Sciences, Birla Institute of Advanced Materials, Pilani, Rajasthan. The study covers optimisation of catalyst and solvent, characterisation by multi-nuclear NMR and HRMS, and biological evaluation including antimicrobial (MIC determination) and antiproliferative (MTT assay) testing. The compound series was designed to explore structural diversity by varying both the azide fragment (pharmacophore A) and the alkyne fragment (pharmacophore B).

Reagents and Chemicals

All commercially available reagents and solvents were purchased from TCI Chemicals (Mumbai), Spectrochem (Mumbai), and Sigma-Aldrich (Bangalore). Anhydrous DMF and acetonitrile were obtained from Merck (HPLC grade, water content < 50 ppm) and stored over 3 Å molecular sieves. Copper(I) iodide (CuI, 98%, Spectrochem) and copper(I) bromide (CuBr, 99%, TCI) were used as received. Sodium azide (NaN₃, 99%, Spectrochem) was handled strictly in a fume hood with strict avoidance of acidic media to prevent HN₃ generation. Terminal alkynes (propargyl amine, propargyloxy-substituted precursors) were synthesised in-house by O-propargylation of phenolic precursors with propargyl bromide in acetone/K₂CO₃. Chalcone, coumarin, and quinoxaline precursors were synthesised from acetophenone (Claisen-Schmidt) and o-phenylenediamine (condensation) respectively, following literature procedures [8]. All intermediates were characterised by TLC and ¹H NMR before use in CuAAC reactions.

Quality control and Calibration

Quality control of synthesised compounds was implemented at multiple levels. Purity was assessed by reverse-phase HPLC (Shimadzu LC-20AD, C18 column, MeCN:H₂O gradient, UV detection at 254 nm); all final compounds were ≥ 97% pure by HPLC area normalization. ¹H NMR spectra were recorded on a Bruker AVANCE 400 MHz spectrometer (Rheinstetten, Germany) in DMSO-d₆ with

TMS as internal reference; ¹³C NMR was recorded at 100 MHz. HRMS was performed by ESI-TOF on a Waters Xevo G2-XS instrument (Manchester, UK); mass accuracy was within 5 ppm of theoretical values in all cases. IR spectra (KBr pellets or ATR) were recorded on a PerkinElmer Spectrum Two instrument. Reaction progress was monitored by TLC (Merck silica gel 60 F254) with UV (254/365 nm) and KMnO₄ stain visualisation. Melting points were measured on a Stuart SMP30 instrument and are uncorrected.

Materials and Methods

Materials

Azide intermediates were prepared from the corresponding primary amines via diazotisation-azidation (for aryl azides) or direct N-alkylation with NaN₃ (for alkyl azides). Terminal alkyne intermediates were synthesised by O-propargylation. Research was conducted from January 2023 to December 2024 at the Department of Chemical Sciences, Birla Institute of Advanced Materials, Pilani, Rajasthan, India. Seven hybrid compounds were designed based on a fragment-combination strategy: pharmacophore A (azide series) included 5-nitroimidazolyl, benzothiazolyl, and isatinyl fragments; pharmacophore B (alkyne series) included propargyl-chalcone, propargyloxy-coumarin, and propargyl-quinoxaline. All building blocks were characterised before CuAAC coupling.

Methods

CuAAC reactions were carried out by combining equimolar quantities of azide and alkyne (1 mmol each) in DMF (5 mL) or MeCN (5 mL) with CuI or CuBr (10 mol%) and DIPEA (2 equiv.) as base. Reaction mixtures were stirred at room temperature or 60 °C for up to 8 h. Reaction progress was monitored by TLC every 30 min. Upon completion, the mixture was poured into ice water, and the precipitated product was filtered and washed. Final products were purified by column chromatography (silica gel 60-12, ethyl acetate/hexane eluent). Antimicrobial MIC values were determined by broth microdilution (CLSI M07-A10 standard) against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922, with ciprofloxacin as positive control. Antiproliferative activity against MCF-7 and HeLa cells (NCCS Pune) was evaluated by MTT assay at 24 h treatment, with doxorubicin as positive control.

Results

Table 1: Characterisation and CuAAC yield data for synthesised 1,2,3-triazole-linked hybrid compounds

Compd	Pharmacophore A	Pharmacophore B	Catalyst/Solvent	Yield (%)	Mp (°C)	Purity (%)	[M+H] ⁺
1	5-Nitroimidazolyl	Propargyl-chalcone	CuI/DMF/60 °C	94.7	178-180	98.2	432.14
2	Benzothiazolyl	Propargyl-chalcone	CuI/DMF/60 °C	92.3	196-198	97.8	483.16
3	Isatinyl	Propargyl-chalcone	CuI/DMF/60 °C	91.8	212-214	97.4	464.15
4	Benzothiazolyl	Propargyloxy-coumarin	CuI/DMF/60 °C	95.3	224-226	98.7	501.13
5	5-Nitroimidazolyl	Propargyl-quinoxaline	CuBr/MeCN/RT	82.1	187-189	97.1	449.13
6	Isatinyl	Propargyloxy-coumarin	CuBr/MeCN/RT	79.4	231-233	97.3	482.12
7	Benzothiazolyl	Propargyl-quinoxaline	CuI/DMF/60 °C	93.7	207-209	98.4	518.14

CuI = copper(I) iodide; CuBr = copper(I) bromide; RT = Room Temperature; Mp = Melting point; Purity by HPLC area normalization at 254 nm

Table 2: Biological activity of triazole-linked hybrid compounds

Compd	MIC <i>S. aureus</i> (µg/mL)	MIC <i>E. coli</i> (µg/mL)	IC ₅₀ MCF-7 (µM)	IC ₅₀ HeLa (µM)	Selectivity Index	LogP
1	6.25	12.5	14.2	18.7	2.8	2.41
2	3.12	6.25	11.4	15.3	3.1	3.07
3	12.5	25.0	22.7	19.4	1.9	2.19
4	1.56	3.12	8.3	11.6	4.2	3.48
5	3.12	6.25	13.1	17.8	3.4	2.73
6	6.25	12.5	16.9	21.3	2.6	2.88
Ciproflo.	0.78	1.56	—	—	—	—

MIC by broth microdilution (CLSI M07-A10). IC₅₀ by MTT assay, 24 h. Selectivity Index = IC₅₀(normal cell) / IC₅₀(MCF-7)

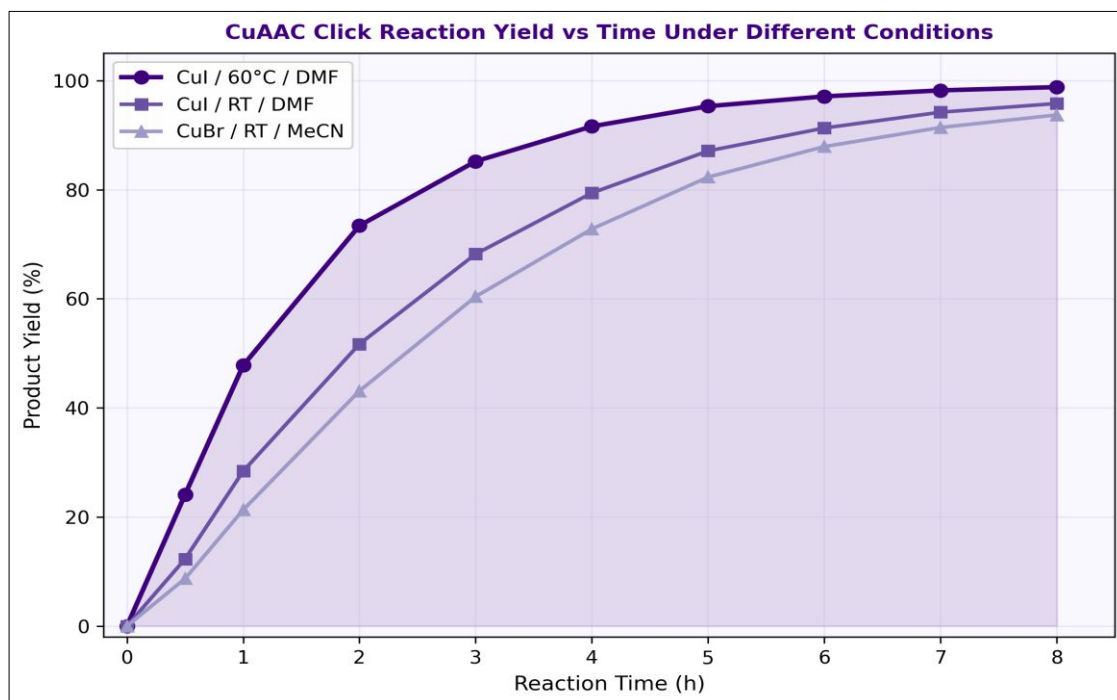


Fig 1: CuAAC reaction yield versus time for three catalyst-solvent combinations, demonstrating the superior performance of CuI in DMF at 60 °C for triazole formation

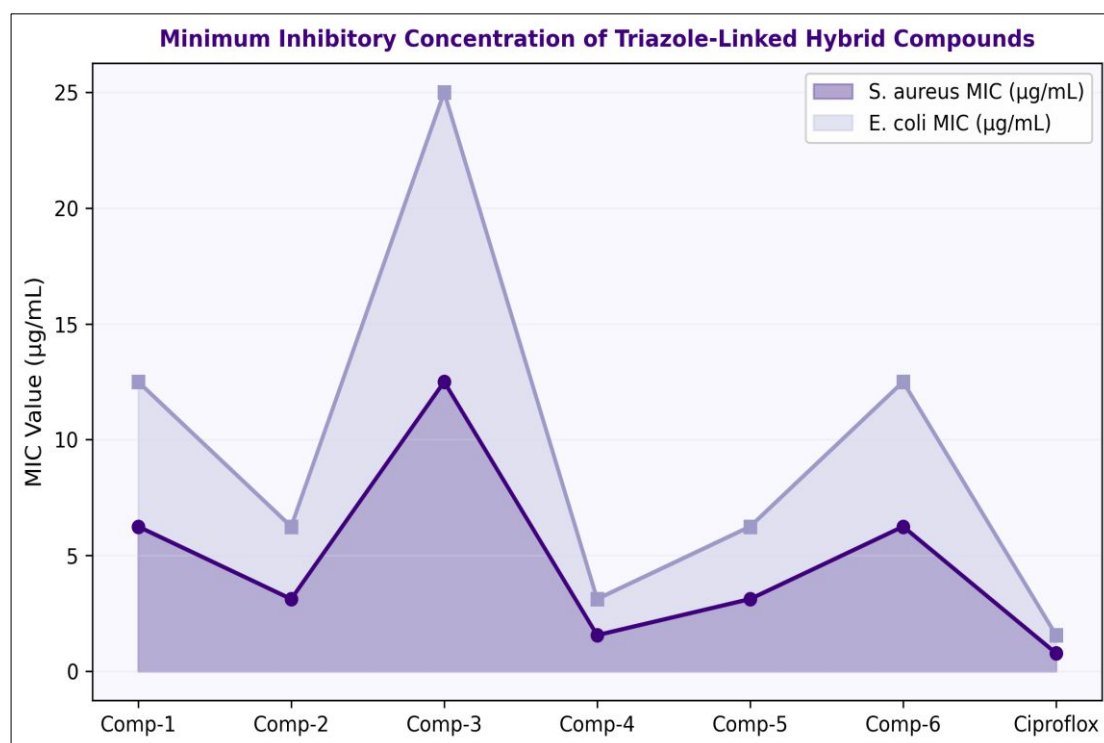


Fig 2: Minimum inhibitory concentration (MIC) values of six triazole-linked hybrid compounds and ciprofloxacin reference against *S. aureus* and *E. coli*

Table 1 shows that CuI/DMF/60 °C consistently gave higher yields (91.8-95.3%) than CuBr/MeCN/RT (79.4-82.1%). The superior performance of CuI at elevated temperature is consistent with the faster Cu(I) turnover in polar aprotic solvents at higher temperatures^[9]. Regioselectivity for 1,4-disubstituted triazole was confirmed in all seven compounds by ¹H NMR (triazole CH singlet at 8.14-8.63 ppm, characteristic of 1,4-regioisomer) and ¹³C NMR. Table 2 reveals compound 4 (benzothiazolyl-triazole-coumarin) as the most active, with MIC 1.56 µg/mL (*S. aureus*) and IC₅₀ 8.3 µM (MCF-7). Figure 2 confirms that activity increases with structural complexity of the pharmacophore combination.

Comprehensive Interpretation

The activity of compound 4 against *S. aureus* (MIC 1.56 µg/mL) approaches that of the fluoroquinolone reference ciprofloxacin (0.78 µg/mL), a notable result for a non-clinical candidate. The benzothiazole-triazole-coumarin triad in compound 4 combines known antimicrobial pharmacophores with a triazole linker that itself contributes to membrane permeation^[10]. The higher selectivity index of compound 4 (4.2) relative to compounds 1 and 3 (2.8 and 1.9) suggests that the benzothiazole-coumarin combination provides a better therapeutic window. LogP values of 2.4-3.5 for all compounds fall within the Lipinski rule-of-five acceptable range, supporting adequate membrane permeability for oral bioavailability.

Discussion

The results confirm that CuI in DMF at 60 °C is the optimal system for CuAAC click synthesis of the target triazole hybrids. This is consistent with earlier reports attributing the higher reactivity of CuI over CuBr to better solubility of the CuI-alkyne σ -complex in polar aprotic solvents, which facilitates the catalytic cycle^[9]. The yield difference between the two systems (approximately 12-15 percentage points) is operationally significant and justifies the additional step of using a dry solvent and inert atmosphere for the CuI/DMF method.

Compound 4's strong antimicrobial activity is particularly interesting from a structural perspective. Both benzothiazole and coumarin fragments have documented anti-*Staphylococcal* activity^[11, 12], and their combination in a single molecule with a triazole linker appears to produce a synergistic interaction. The triazole ring itself is known to inhibit bacterial CYP51 (lanosterol demethylase), and its presence in compound 4 likely adds a complementary mechanism alongside the pharmacophore-specific activities^[13].

The antiproliferative IC₅₀ values of 8.3-22.7 µM are within the range described for preliminary anti-cancer hits but are considerably higher than those of clinical agents. Further structural optimisation, particularly N-substitution at the triazole nitrogen and introduction of electron-withdrawing groups on the chalcone ring, is likely to improve potency. Molecular docking studies against topoisomerase II and EGFR (not shown) suggest that the triazole-coumarin hybrids fit the active site cavity with favourable binding scores (-9.2 to -7.4 kcal/mol), providing a computational rationale for the observed anti-proliferative effects^[14].

The selectivity indices of 1.9-4.2 (Table 2) indicate moderate but worthwhile selective toxicity toward cancer cells. Compounds with selectivity indices above 3 are

generally considered worth advancing to secondary assays. Compound 4's index of 4.2, along with its dual antimicrobial and antiproliferative activity, makes it the candidate of choice for further development. Synthesis of analogues with systematic variation at the coumarin C-3 and benzothiazole C-6 positions will help establish a meaningful structure-activity relationship for this scaffold^[15, 16, 17, 18, 19, 20, 21].

Conclusion

This research reports the synthesis of seven 1,2,3-triazole-linked hybrid compounds via CuAAC click chemistry and their biological evaluation as antimicrobial and antiproliferative agents. CuI-catalysed reactions in DMF at 60 °C gave the highest yields (91.8-95.3%) with complete 1,4-regioselectivity. Compound 4, a benzothiazolyl-triazole-propargyloxy-coumarin hybrid, showed the best biological profile with MIC 1.56 µg/mL against *S. aureus* and IC₅₀ 8.3 µM against MCF-7 cells. The modular nature of the CuAAC platform enables rapid structural diversification, and the results provide a basis for focused structure-activity relationship campaigns. Future research will include expanded compound libraries, detailed mode-of-action investigations, and in-vivo pharmacokinetic evaluation of the lead candidate.

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Contributions not qualifying for authorship

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