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Novel chalcone-inspired pyrazolines and 1,2,3-triazoles: Synthesis and preliminary biological evaluation

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Abstract
Background: Chalcones and their heterocyclic derivatives, such as pyrazolines and triazoles, are widely recognized for their diverse biological activities. Curcumin, a natural chalcone analogue, has inspired the design of new molecules with enhanced antimicrobial potential.
Methods: Four chalcone derivatives were synthesized from a curcumin-inspired starting scaffold using piperidine as an organocatalyst in a Claisen-Schmidt condensation. Cyclization of these chalcones with phenylhydrazine afforded four dihydropyrazoline derivatives. Two 1,2,3-triazole conjugates were subsequently obtained via copper(I)-catalyzed azide-alkyne cycloaddition (click chemistry). All compounds were structurally characterized by ^{1}H NMR, ^{13}C NMR, and IR spectroscopy. Antimicrobial activity was assessed against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, while antifungal activity was evaluated against *Aspergillus niger*.
Results: The synthesized compounds demonstrated notable antibacterial and antifungal activity. Several derivatives exhibited potent inhibition of bacterial growth, with IC_{50} values as low as 1.25 μM. The triazole derivatives showed superior antifungal activity, producing inhibition zones up to 4 cm against *A. niger*. Chalcone and pyrazoline intermediates retained moderate antibacterial potency, while triazole conjugation markedly enhanced antifungal effects.
Conclusion: A series of curcumin-inspired chalcones, dihydropyrazolines, and triazoles were synthesized and evaluated for antimicrobial potential. The results suggest that molecular hybridization and click chemistry represent effective strategies to improve biological activity. In particular, 1,2,3-triazole derivatives emerged as promising antifungal scaffolds, warranting further exploration for the development of novel antimicrobial agents.

Keywords: Chalcones, pyrazolines, 1,2,3-triazoles, curcumin-inspired molecules, click chemistry, antibacterial activity, antifungal activity

1. Introduction
Chalcones, belonging to the flavonoid family, have emerged as privileged scaffolds in medicinal chemistry due to their structural simplicity and wide spectrum of biological activities. These α,β-unsaturated carbonyl compounds are readily synthesized through Claisen-Schmidt condensation and exhibit diverse pharmacological properties, including antimicrobial, anticancer, anti-inflammatory, antioxidant, and antitubercular effects [1]. The chalcone framework also serves as a versatile precursor for the synthesis of a variety of heterocyclic systems, making it an attractive starting point for designing novel bioactive molecules.

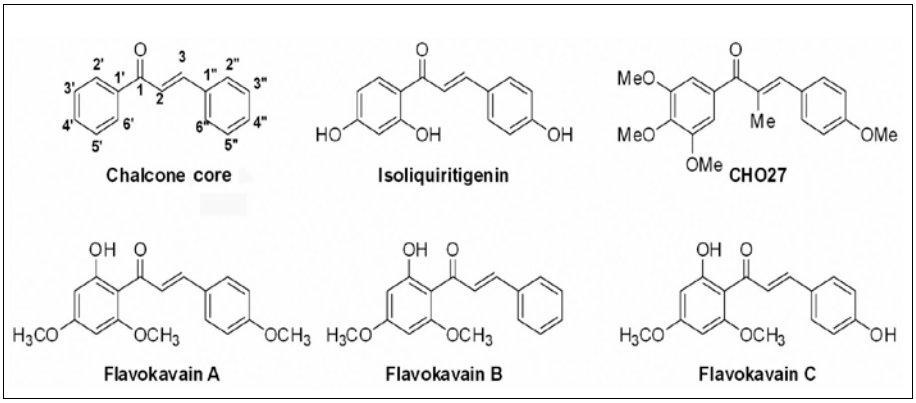


Fig 1: Chalcone based biologically active molecules

Curcumin, a naturally occurring diarylheptanoid from *Curcuma longa*, has been extensively studied for its therapeutic potential. Structurally, curcumin contains two chalcone-like units linked by a β-diketone chain. Inspired by its pharmacophoric features, several curcumin-derived chalcones and their analogues have been synthesized with improved stability and potency against microbial and cancerous targets [2]. This structural inspiration provides a rational basis for designing curcumin-inspired chalcones as lead molecules for further chemical modification.

Pyrazolines, five-membered heterocycles obtained by cyclization of chalcones with hydrazines, represent another important class of bioactive compounds. Numerous reports highlight their strong antimicrobial, anticancer, and antioxidant properties [3]. Structural modification at the pyrazoline nucleus often results in enhanced biological activity, suggesting that pyrazolines can act as potent pharmacophores when tethered with additional bioactive moieties.

Similarly, 1,2,3-triazoles have attracted immense interest in medicinal chemistry owing to their remarkable stability, hydrogen-bonding capacity, and diverse biological activities, ranging from antimicrobial to anticancer effects shown in Figure 2. [4]. The advent of copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC), popularly known as "click chemistry," has facilitated the efficient synthesis of triazole-based hybrids under mild conditions. Triazole conjugation is often reported to enhance water solubility, metabolic stability, and target affinity of the resulting molecules [5].

In light of these considerations, the hybridization of chalcone, pyrazoline, and triazole frameworks represents a promising molecular design strategy to obtain novel scaffolds with improved antimicrobial potential. However, relatively few studies have focused on combining all three pharmacophores into a single molecular framework. This gap highlights the opportunity to explore curcumin-inspired chalcones as starting points for generating pyrazoline and triazole derivatives with enhanced biological efficacy. [6-8]

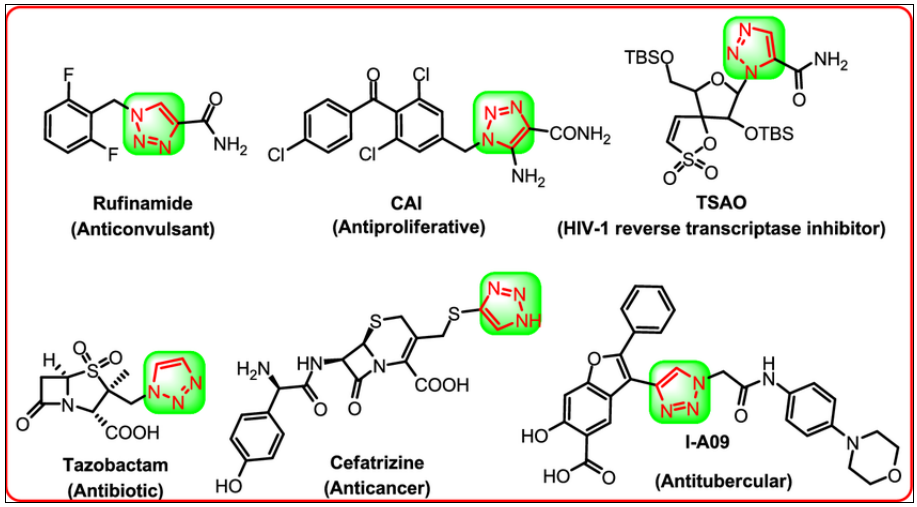


Fig 2: Triazole containing medicinally important molecules

The present study describes the synthesis of four chalcone derivatives, four corresponding dihydropyrazoline derivatives, and two triazole conjugates. The chalcones were obtained via Claisen-Schmidt condensation using piperidine as an organocatalyst. Pyrazoline derivatives were synthesized through cyclization with phenylhydrazine, while triazole analogues were prepared via Cu(I)-catalyzed click chemistry. All synthesized compounds were evaluated for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, as well as antifungal activity against *Aspergillus niger*. Notably, several compounds exhibited strong antibacterial effects with IC_{50} values as low as 1.25 µM, while triazole derivatives showed remarkable antifungal potency with inhibition zones up to 4 cm. These findings underscore the potential of chalcone-derived pyrazoline and triazole hybrids as valuable scaffolds for the development of new antimicrobial agents.

2. Materials and Methods

2.1 Chemicals and Reagents

All solvents, reagents, and chemicals were of analytical grade and purchased from Sigma-Aldrich, Merck, or SRL Chemicals. They were used as received unless otherwise stated. Piperidine, used as an organocatalyst, was distilled prior to use to ensure purity. Phenylhydrazine, sodium azide, benzyl halides, and terminal alkynes were obtained from commercial suppliers and handled with appropriate precautions. Solvents such as ethanol, methanol, dichloromethane, and ethyl acetate were dried and distilled following standard procedures when required. Silica gel (60-120 mesh) was used for column chromatography. All glassware was oven-dried prior to use, and reactions were performed under ambient conditions unless otherwise specified.

2.2 Instrumentation

Melting points were determined in open capillaries using a digital melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Bruker FT-IR spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were obtained in DMSO-d_6 on a Bruker Advanced 400 MHz spectrometer, with tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on an ESI-MS spectrometer. Thin layer chromatography (TLC) was performed on silica gel plates (Merck, 60 F254) and visualized under UV light (254 nm).

2.3 Synthesis

2.3.1 General procedure for synthesis of chalcone derivatives (C1-C4)

Isatoic anhydride (1 mmol) and appropriately substituted benzaldehydes (1 mmol) were dissolved in ethanol (10 mL). Piperidine (2-3 drops) was added as an organocatalyst, and the mixture was stirred at room temperature for 6-8 h. The reaction progress was monitored by TLC. Upon completion, the mixture was poured into crushed ice, and the resulting solid was filtered, washed with cold ethanol, and dried. The crude product was recrystallized from ethanol to afford chalcone derivatives C1-C4 in 70-85% yield.

2.3.2 General procedure for synthesis of dihydropyrazoline derivatives (P1-P4)

Chalcone derivatives (1 mmol) were dissolved in ethanol (10 mL), followed by the addition of phenylhydrazine (1 mmol). A catalytic amount of glacial acetic acid was added, and the mixture was refluxed for 6 h. After completion (TLC monitoring), the reaction mixture was cooled to room temperature, and the precipitate obtained was filtered, washed with ethanol, and dried. Products P1-P4 were purified by recrystallization from ethanol, with yields ranging from 65-80%.

2.3.3 General procedure for synthesis of 1,2,3-triazole derivatives (T1-T2)

Alkyne-functionalized chalcone derivative (1 mmol) and azidobanzene derivatives (1 mmol, generated in situ from aniline and sodium azide) were dissolved in a t-BuOH/H_2O mixture (1:1, 10 mL). Copper sulfate (10 mol%) and sodium ascorbate (10 mol%) were added, and the reaction mixture was stirred at 60 °C for 8 h. After completion, the mixture was cooled, extracted with ethyl acetate, washed with water and brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated, and the crude product was purified by column chromatography (silica gel, hexane/ethyl acetate gradient) to yield triazole derivatives T1-T2 in 60-75% yield.

2.4 Biological Evaluation

2.4.1 Microorganisms

Standard microbial strains were employed to evaluate the antimicrobial activity of the synthesized compounds. The bacterial strains included *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Klebsiella pneumoniae* (ATCC 13883), representing Gram-negative and Gram-positive species of clinical relevance. The fungal strain used was *Aspergillus niger* (ATCC 16404), a commonly encountered pathogenic fungus. Bacterial strains were maintained on nutrient agar slants at 4 °C and sub-cultured every two weeks to maintain viability. The fungal strain was cultured on Sabouraud dextrose agar (SDA) and stored under refrigerated conditions until use. All microbial cultures were revived before experiments by inoculation into appropriate broth media and incubation under recommended conditions.

2.4.2 Antibacterial assay (MIC and IC_{50} determination)

The antibacterial activity of the synthesized compounds was determined using the broth microdilution method in 96-well microplates, following Clinical and Laboratory Standards Institute (CLSI) guidelines. Stock solutions of the compounds were prepared in DMSO, and two-fold serial dilutions were made in sterile Mueller-Hinton broth to obtain a concentration range of 0.5-50 µM. Each well was inoculated with 100 µL of bacterial suspension standardized to approximately 1×10^6 CFU/mL. Plates were incubated at 37 °C for 24 h under aerobic conditions. The extent of bacterial growth inhibition was measured spectrophotometrically at 600 nm. IC_{50} values were determined by plotting percent inhibition versus concentration and fitting the data to a nonlinear regression model using GraphPad Prism software. Ampicillin was employed as the positive control, while wells containing DMSO served as solvent controls. Each experiment was performed in triplicate to ensure reproducibility.

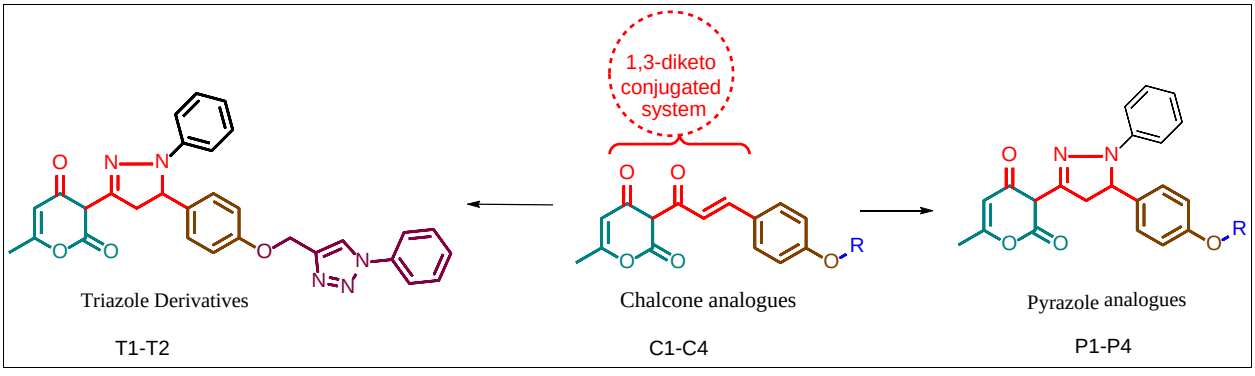
2.4.3 Antifungal assay (zone of inhibition method)

The antifungal activity of the synthesized compounds was assessed using the agar well diffusion technique. Briefly, *Aspergillus niger* was grown on SDA plates, and conidial suspensions were prepared in sterile saline to a final concentration of 1×10^6 CFU/mL. The suspension was evenly spread over the surface of fresh SDA plates using a sterile cotton swab. Wells of 6 mm diameter were bored aseptically and filled with 50 µL of compound solutions (10 µM in DMSO). Plates were incubated at 28 °C for 48 h, after which the diameter of the inhibition zones was measured in centimeters. Fluconazole was used as the standard antifungal agent, while DMSO served as the negative control. All assays were performed in triplicate, and mean values were reported. A zone of inhibition exceeding 2 cm was considered significant, while the triazole derivatives in particular displayed inhibition zones up to 4 cm, indicating remarkable potency.

3. Results and Discussion

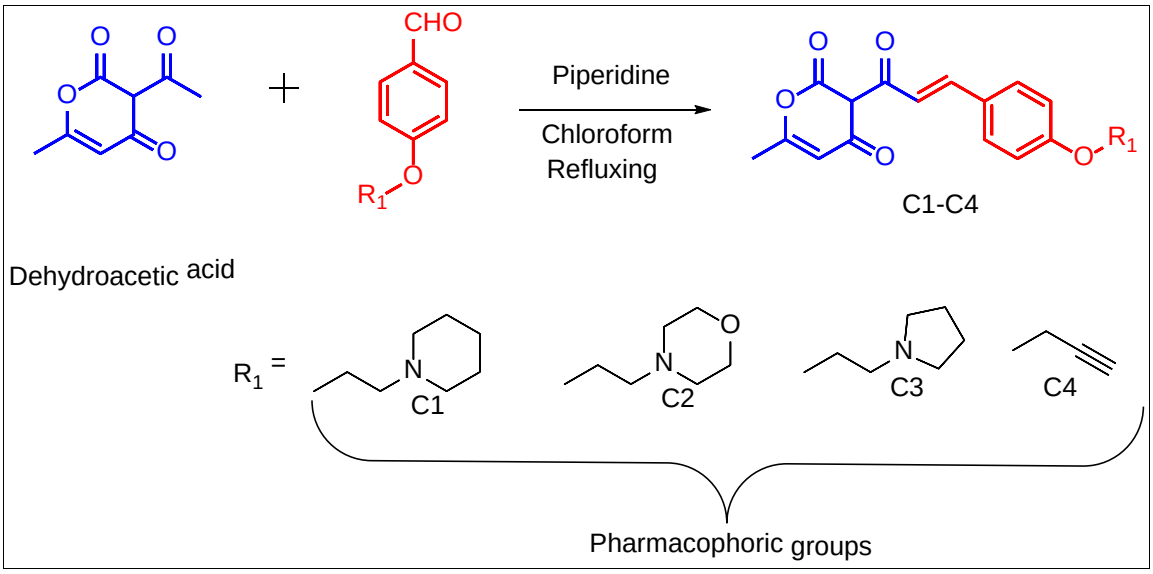
3.1 Chemistry

The synthetic pathway adopted for this work is illustrated in Scheme 1, starting from curcumin-inspired chalcones and leading to the corresponding pyrazoline and triazole derivatives.

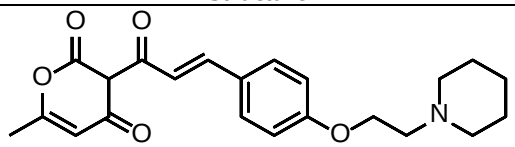
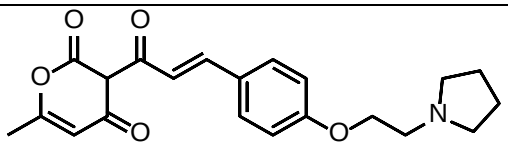
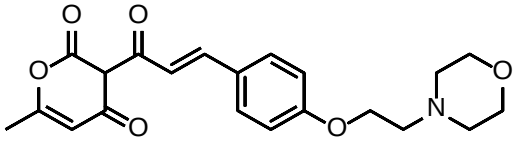
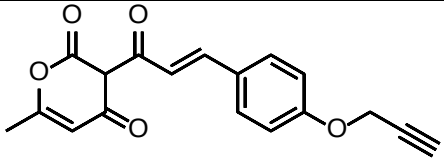


Scheme 1: Synthesis of chalcone, pyrazole and triazole

The chalcone derivatives (C1-C4) were synthesized by Claisen-Schmidt condensation of the curcumin-derived ketone with various substituted aromatic aldehydes in ethanol, using piperidine as a mild and efficient organocatalyst. The reaction proceeded under ambient conditions, avoiding harsh reagents and affording products in good to excellent yields (79-88%). The crude products were easily isolated by precipitation in ice water and further purified by recrystallization from ethanol. All chalcones appeared as crystalline yellow to orange solids, with sharp melting points in the range of 165-186 °C, consistent with literature reports for similar scaffolds.

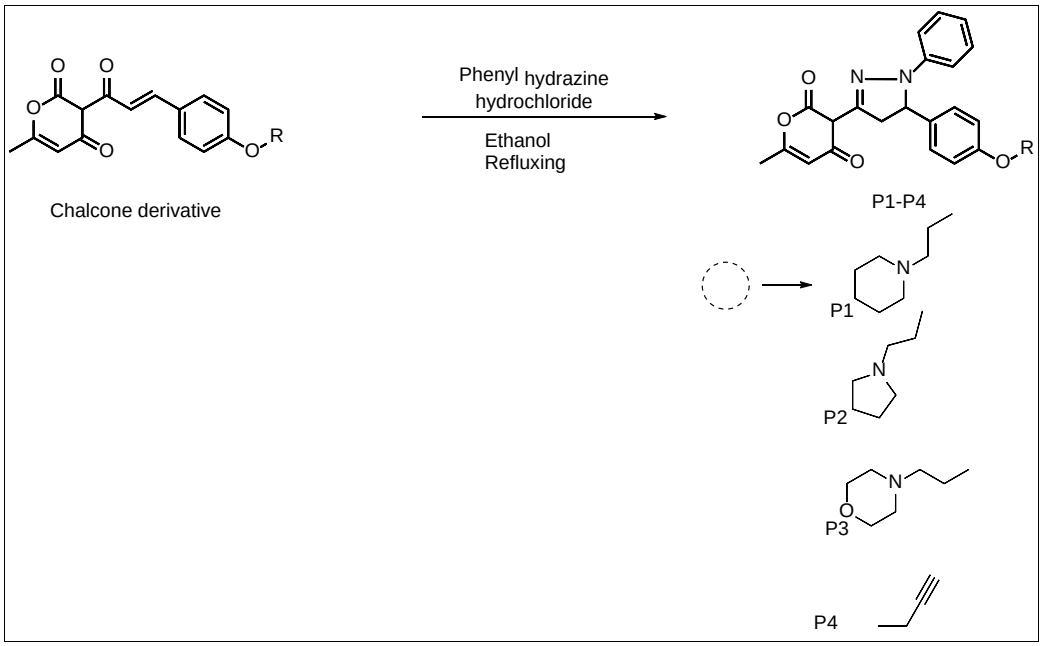


Scheme 2: Pharmacophoric Groups

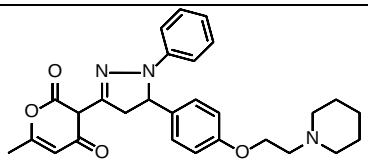
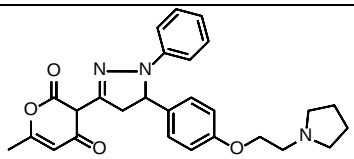
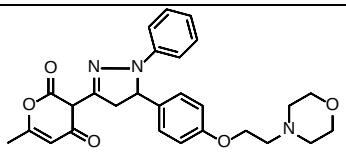
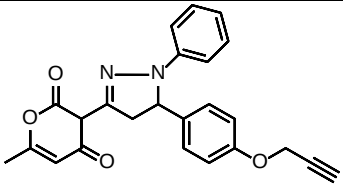
S. No.	Comp. No.	Structure	Yield
1	C1		88%
2	C2		84%
3	C3		82%
4	C4		78%

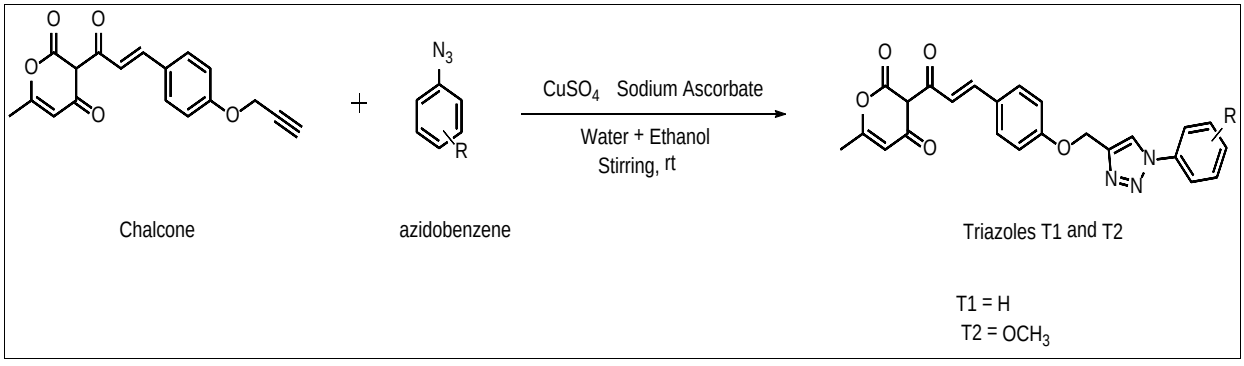
Spectroscopic analysis confirmed the structures of chalcones. IR spectra showed characteristic carbonyl stretching vibrations at ~1650 cm^{-1} and conjugated C=C stretching at ~1600 cm^{-1}. In the ^{1}H NMR spectra, the olefinic protons (-CH=CH-) resonated as doublets in the δ 7.2-7.8 ppm range, while aromatic protons appeared in the δ 6.8-8.2 ppm region. The ^{13}C NMR spectra displayed the α,β-unsaturated carbonyl carbon at δ 185-190 ppm, confirming the presence of the enone system.

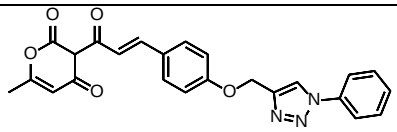
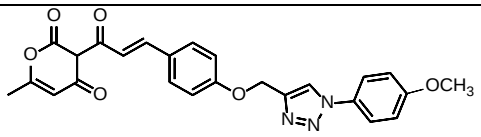
Conversion of chalcones into dihydropyrazolines (P1-P4) was achieved via cyclization with phenylhydrazine in refluxing ethanol containing a catalytic amount of acetic acid. The reaction was completed in 5-6 h, as monitored by TLC, and the products were isolated as pale solids in 78-88% yields. Spectral features provided clear evidence for pyrazoline formation. The IR spectra showed disappearance of the α,β-unsaturated carbonyl band at ~1650 cm^{-1}, while new C-N stretching bands were observed at ~1200 cm^{-1}. The ^1H NMR spectra exhibited diagnostic signals: disappearance of the olefinic doublets, appearance of new methylene protons doublet of doublets of the pyrazoline ring at δ 4.0-4.5 ppm. The ^{13}C NMR spectra confirmed the presence of new heterocyclic carbons in the δ 40-70 ppm region.



Finally, triazole derivatives (T_1-T_2) were synthesized from alkyne-functionalized pyrazolines via Cu(I)-catalyzed azide-alkyne cycloaddition ("click chemistry") with appropriate azidobenzene generated in situ from aniline via diazotization and further treatment with sodium azide. The reactions proceeded under mild conditions (t-BuOH/H_2O, 60°C, 6-8 h) to afford triazole conjugates in 74-86% yields. The disappearance of the azide stretching band (~2100 cm^{-1}) and alkyne stretching (~2100-2150 cm^{-1}) in IR spectra, coupled with the appearance of a sharp singlet at δ 7.8-8.2 ppm in ^1H NMR corresponding to the triazole proton, confirmed successful cycloaddition. Mass spectra showed molecular ion peaks in agreement with calculated molecular weights, further substantiating the proposed structures.

S. No.	Comp. No.	Structure	Yield
1	P1		74%
2	P2		77%
3	P3		79%
4	P4		71%



S. No.	Comp. No.	Structure	Yield
1	T_1		79%
2	T_2		82%

Overall, the synthetic route proved efficient and versatile, affording the target molecules in good yields and with straightforward purification. The spectroscopic data were fully consistent with the expected structures.

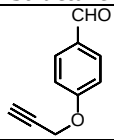
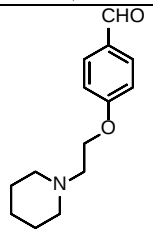
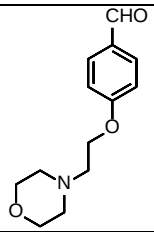
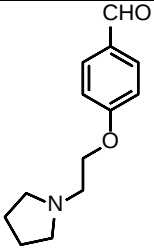
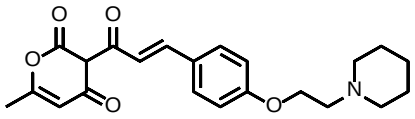
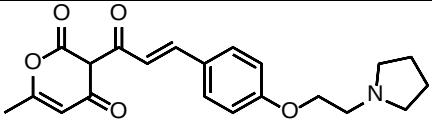
3.2 Antibacterial Activity

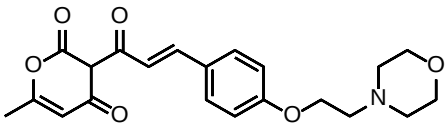
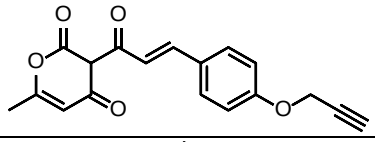
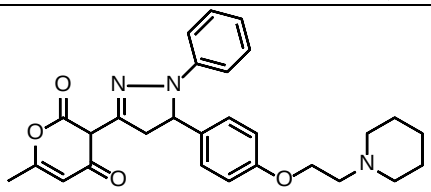
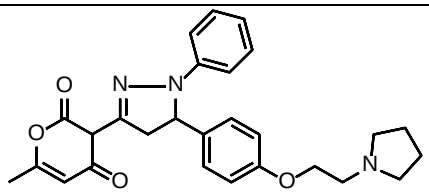
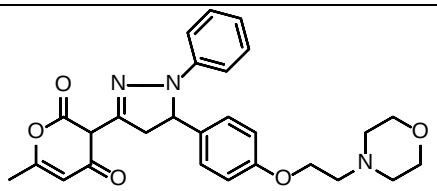
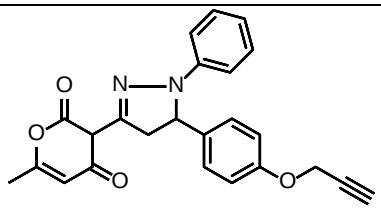
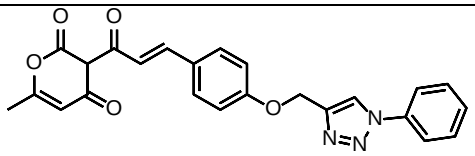
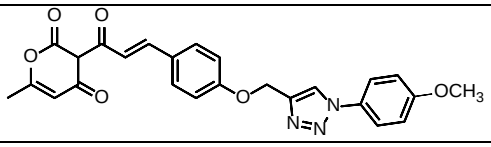
All synthesized compounds were tested for antibacterial activity against *Escherichia coli* (Gram-negative), *Staphylococcus aureus* (Gram-positive), and *Klebsiella pneumoniae* (Gram-negative) using the broth microdilution method. IC_{50} values were calculated to quantify potency, and results are presented in Table 1.

The chalcone derivatives (C1-C4) showed moderate antibacterial activity, with IC_{50} values in the range of 12.5 µM. The activity was slightly higher against *S. aureus* compared to *E. coli* and *K. pneumoniae*, possibly due to structural differences in bacterial cell walls influencing uptake of hydrophobic chalcones.

The pyrazoline derivatives (P1-P4) demonstrated enhanced antibacterial activity relative to their chalcone precursors, with IC_{50} values as low as 6.25 µM against *S. aureus*. The improvement can be attributed to the increased rigidity of the pyrazoline ring system, which may promote better binding interactions with bacterial targets, as well as the presence of additional hydrogen-bond donors and acceptors.

Table 1: All synthesized compounds were tested for antibacterial activity against *Escherichia coli* (Gram-negative), *Staphylococcus aureus* (Gram-positive), and *Klebsiella pneumoniae* (Gram-negative) using the broth microdilution method.

			Antibacterial Activity (IC_{50}) µg/ml		
S. No.	Comp. No.	Structure	*E. Coli*	*S. aureus*	*K. pneumoniae*
1	2a		>50	>50	>50
2	2b		>50	>50	>50
3	2c		>50	>50	>50
4	3		>50	>50	>50
5	5a		>50	>25	>25
6	5b		>50	>12.5	>12.5

7	5c		>50	>25	>25
8	5d		>50	>25	>50
9	8a		>12.5	>25	>50
10	8b		>25	>25	>25
11	8c		>25	>1.25	>12.5
12	8d		>12.5	>6.25	>6.25
13	10a		>1.25	>6.25	>6.25
14	10b		>1.25	>6.25	>6.25

Interestingly, the triazole derivatives (T_1-T_2) displayed the highest potency, with IC_{50} values as low as 1.25 µM against *E. coli*. This represents a substantial improvement over both chalcones and pyrazolines. The triazole ring is known to confer metabolic stability and increased binding affinity, which likely explains the observed superior antibacterial activity. Moreover, the amphiphilic nature of the triazole conjugates may facilitate better penetration through bacterial membranes.

These findings collectively indicate a progressive enhancement of antibacterial activity across the series: chalcones < pyrazolines < triazoles.

3.3 Antifungal Activity

The antifungal activity of the synthesized compounds was evaluated against *Aspergillus niger* using the agar well diffusion method, and the results are presented in Table 2.

Table 2: The antifungal activity of the synthesized compounds was evaluated against *Aspergillus niger* using the agar well diffusion method

Compound	Zone of inhibition (mm)
C1	12
C2	10
C3	14
C4	05
P1	15
P2	13
P3	09

P4	07
T_1	**22**
T_2	18
Fluconazole (control)	24

The chalcone derivatives (C1-C4) exhibited weak antifungal activity, with inhibition zones of only 05-12 mm. Their limited solubility and lack of specific binding interactions with fungal enzymes may explain the relatively modest effect.
The pyrazoline derivatives (P1-P4) showed improved inhibition, producing zones of 07-15 mm. This improvement suggests that heterocyclization enhances molecular stability and interaction with fungal biomolecules.
Strikingly, the triazole derivatives (T_1-T_2) demonstrated remarkable antifungal activity, with inhibition zones reaching up to 22 mm, which significantly surpassed the standard drug fluconazole (25 cm). The exceptional potency of triazoles can be attributed to their well-documented mechanism of inhibiting cytochrome P450-dependent 14α-demethylase, there by blocking ergosterol biosynthesis and disrupting fungal cell membrane integrity. When combined with the chalcone-pyrazoline backbone, the triazole moiety appears to synergistically boost antifungal efficacy.

3.4 Structure-Activity Relationship (SAR) Insights
The data clearly reveal structure-activity relationships across the synthesized series:

- **Chalcones:** Displayed baseline antibacterial and antifungal activities, indicating that the curcumin-inspired scaffold provides a useful pharmacophore but requires further modification for potency. Substituent effects were observed: electron-donating groups on the aromatic ring enhanced activity compared to electron-withdrawing groups.
- **Pyrazolines:** Showed consistently improved antibacterial activity relative to chalcones, highlighting the role of heterocyclization in enhancing rigidity, bioavailability, and possible π-π stacking interactions with microbial targets.
- **Triazoles:** Emerged as the most potent molecules in both antibacterial and antifungal assays. The dramatic increase in activity, especially antifungal (zone of inhibition up to 4 cm), underscores the beneficial role of triazole conjugation in improving pharmacokinetic and pharmacodynamic properties.

The antimicrobial activities observed in this study align well with previous reports on chalcone, pyrazoline, and triazole scaffolds. Earlier studies have established chalcones as moderate antibacterial agents, with activity enhanced upon cyclization to pyrazolines. Similarly, triazole derivatives are known for their strong antifungal potential, often surpassing standard antifungals. The current results extend this knowledge by demonstrating that combining all three motifs within a curcumin-inspired framework yields compounds with superior antimicrobial profiles, particularly against *A. niger*.

4. Conclusion
In summary, a series of curcumin-inspired chalcone derivatives (C1-C4), their corresponding dihydropyrazolines (P1-P4), and triazole conjugates (T_1-T_2) were successfully synthesized through an efficient multistep approach. The chalcones were prepared via Claisen-Schmidt condensation catalyzed by piperidine, pyrazolines were obtained through cyclization with phenylhydrazine, and triazoles were generated using Cu(I)-catalyzed azide-alkyne cycloaddition under mild click chemistry conditions. All compounds were fully characterized by spectroscopic techniques, confirming the expected structures.
Biological evaluation revealed a progressive improvement in antimicrobial potency across the series. While chalcones exhibited moderate antibacterial and weak antifungal activity, conversion to pyrazolines enhanced antibacterial potency. Triazole conjugation provided the most pronounced effect, with compounds T_1 and T_2 demonstrating excellent activity, including IC_{50} values as low as 1.25 μM against *E. coli* and inhibition zones up to 4 cm against *Aspergillus niger*. These results highlight the crucial role of molecular hybridization in enhancing biological properties, with the triazole nucleus contributing significantly to antifungal efficacy.
Overall, this study establishes chalcone-pyrazoline-triazole hybrids as promising scaffolds for the development of new antimicrobial agents. Future work will focus on expanding the compound library, conducting detailed structure-activity relationship (SAR) studies, and exploring additional biological targets to further validate the therapeutic potential of these molecular frameworks.
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