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Synthesis and Antimicrobial Evaluation of Novel Triazole Based Pyrazole Derivatives

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Abstract: *Pyrazole is well-known intermediate in the synthesis of a wide range of biologically active heterocyclic compounds, including imidazoles, pyrazoles, and pyrimidines. Additionally, they play a vital role in the construction of various bioactive scaffolds such as flavones, flavanols, anthocyanins, pyrazolines, benzal coumarones, deoxybenzoins, and hydantoins. In the present work, novel triazole-based pyrazole derivatives were synthesized through the reaction of N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)acetamide with a series of aromatic aldehydes, resulting in the formation of chalcone intermediates. These intermediates were subsequently reacted with 4H-1,2,4-triazole-3-amine to obtain a new series of triazole-linked pyrazoles. The structures of all synthesized compounds were confirmed through various spectroscopic methods and evaluated for their antimicrobial activity against both gram-positive and gram-negative bacterial strains.*
Keywords: *Antimicrobial Activity, Pyrazole, Aldehyde, 4H-1,2,4-triazole-3-amine and Spectroscopy.*

1. INTRODUCTION

Chalcones, chemically known as 1,3-diaryl-2-propen-1-ones, are a class of open-chain flavonoids characterized by the presence of an α,β-unsaturated carbonyl system connecting two aromatic rings. They are widely regarded as valuable synthetic intermediates and core scaffolds in medicinal chemistry due to their structural simplicity, ease of synthesis, and broad spectrum of biological activities [1,2].

Naturally occurring chalcones are found in various plant species, especially in fruits, vegetables, spices, and teas, where they contribute to pigmentation and exhibit antioxidant properties. Over the past decades, both natural and synthetic chalcones have shown a wide array of pharmacological properties, including antimicrobial, anti-inflammatory, anticancer, antioxidant, antimalarial, and antiviral activities [3–5].

The α,β-unsaturated carbonyl group in chalcones acts as a Michael acceptor, enabling interactions with nucleophilic sites in biological systems. This reactivity is crucial for many of their observed biological effects, particularly their ability to inhibit enzymes or modulate cellular signaling pathways [6].

Moreover, chalcones serve as important precursors in the synthesis of various heterocyclic compounds such as flavones, flavanones, aurones, pyrazolines, imidazoles, and triazoles, many of which exhibit enhanced biological activity [7,8]. This synthetic flexibility makes chalcones a key starting point in the development of novel therapeutic agents.

Pyrazoles are a significant class of five-membered heterocyclic compounds containing two adjacent nitrogen atoms within the ring structure. Since their discovery in the late 19th century, pyrazoles have attracted considerable attention due to their wide range of pharmacological and biological activities. These compounds have demonstrated diverse therapeutic properties, including antimicrobial, anti-inflammatory, analgesic, anticancer, antiviral, and antidiabetic activities [9–11].

The structural versatility of pyrazoles allows for extensive chemical modifications, making them ideal scaffolds in the design and development of novel bioactive molecules. Substituted pyrazoles are frequently found in numerous drugs and agrochemicals, such as celecoxib (a selective COX-2 inhibitor) and rimonabant (an anti-obesity agent), which further highlights their medicinal relevance [12,13].

Moreover, the pyrazole ring is often incorporated into more complex molecular frameworks to enhance biological activity or target specificity. Its ability to engage in hydrogen bonding and π-π interactions also contributes to its favorable interaction with biological targets [14].

Given their broad spectrum of activity and structural tunability, pyrazoles continue to be of great interest in medicinal chemistry and drug discovery. In the presence study, we have synthesized triazole based pyrazole derivatives from novel chalcones on condensation reaction with 4H-1,2,4-triazole-3-amine and characterized all compounds using spectroscopic techniques. All prepared compounds were also tested for their antimicrobial activity against gram-positive and gram-negative bacterial.

2. METHODS AND MATERIALS

2.1 Chemicals and Reagents

All chemicals such as aromatic aldehydes, 4H-1,2,4-triazole-3-amine, Sodium hydroxide (NaOH), N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl) acetamide and ethanol were obtained directly from Merck (Mumbai, India) and used as received without additional purification.

2.2 Experimental

The analytical instrumentation employed in this study includes:

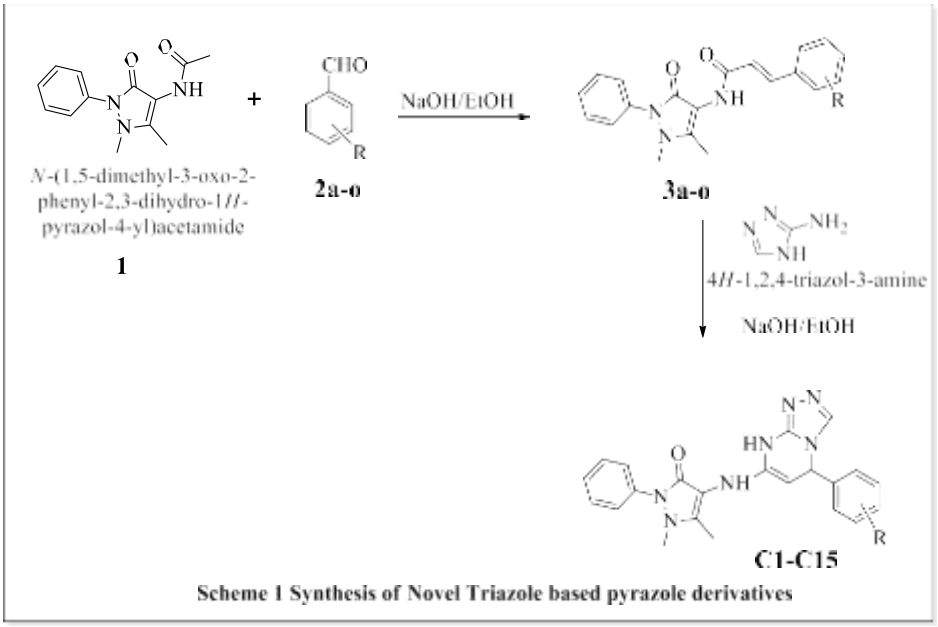
- A Bruker Avance-400 MHz spectrometer for ^{1}H NMR measurements and a 100 MHz instrument for ^{13}C NMR, with chemical shifts reported in parts per million (ppm).
- An ABB Bomem FT-IR 3000 spectrophotometer to record infrared spectra, with data expressed in wavenumbers (cm^{-1}).
- A Shimadzu LCMS-2010 system was used to obtain mass spectral data.
- A PerkinElmer 2400 Series II CHNS/O elemental analyzer measured the carbon, hydrogen, nitrogen, sulfur, and oxygen content of the compounds.

All spectral and compositional data were analyzed following standard procedures.

2.3 Method of Synthesis

2.3.1 Synthesis of Various Triazole Based Pyrazoles C1-C15

In a 250 mL round-bottom flask, combine 0.01 mol of N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)acetamide with 0.01 mol of the chosen aromatic aldehyde in 50 mL of ethanol. Add 10 mL of 10% NaOH and swirl the mixture for 2–3 hours to synthesize chalcones (3a–3o). Next, add 0.01 mol of 4H-1,2,4-triazole-3-amine to the chalcone reaction mixture and reflux in ethanol for 1.5–3 hours, again using 10 mL of 10% NaOH, to form pyrazole derivatives (**C1-C15**). Completion of reaction was checked by Thin Layer Chromatography (TLC). Reaction **scheme 1** shows the synthesis of novel pyrazoles.



Scheme 1 Synthesis of Novel Triazole based pyrazole derivatives

3. RESULT AND DISCUSSION

Table 1 Data showing synthesis of triazole based pyrazoles C1-C15

Sr. No.	Compounds Code	R	Reaction Time (hr)	% Yield[b]	Melting Point (°C)
1	**C1**	-H	3.5	82	235
2	**C2**	4-OH	4.5	78	260
3	**C3**	3-OH	4.5	78	240
4	**C4**	2-OH	4.5	76	255
5	**C5**	2- OCH_3	5	73	238
6	**C6**	4-OCH_3	5	74	257

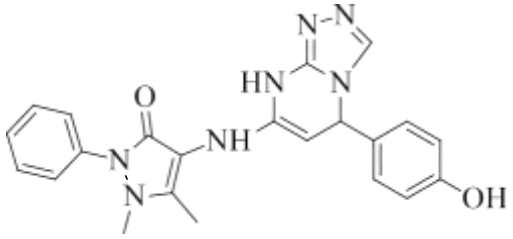
7	**C7**	2-Cl	4.5	81	247
8	**C8**	4-Cl	4.5	82	230
9	**C9**	3-Cl	4.5	82	232
10	**C10**	2-NO_2	4	83	262
11	**C11**	4-NO_2	4	84	267
12	**C12**	3-NO_2	4	81	256
13	**C13**	3-Br	4.5	80	228
14	**C14**	2- Br	4.5	81	221
15	**C15**	4- Br	4.5	82	234

[a]Reaction is monitored by TLC, [b]Isolated yield

Table 1 summarizes the synthesis of various pyrazoles obtained through the reaction of N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)acetamide with different aromatic aldehydes in ethanol using NaOH as a base. The resulting chalcones were further reacted with 4H-1,2,4-triazole-3-amine to yield the final products. From the data in Table 1, it's evident that substrates bearing electron-withdrawing groups underwent the reaction more rapidly than those with electron-donating substituents. For example, compounds C10-C12 (with electron-withdrawing groups) were formed in just 4hr, while compounds C5 and C6 (featuring electron-donating groups) required approximately 5hr to complete the synthesis.

Characterization

Compound C2 was selected as a representative example. Its ^{1}H NMR spectrum displays distinctive peaks corresponding to various types of protons and functional groups, which are assigned based on expected shielding and deshielding patterns. Aromatic protons are found in the downfield region, typically between 6.5 and 8.5 ppm. The detailed spectroscopic data (^{1}H NMR, ^{13}C NMR, IR, and MS) for compound C2 are presented below.

Compound code: C2	
$C_{22}H_{21}N_6O_2$	
M. P. (°C): 255	4-((5-(4-hydroxyphenyl)-5,8-dihydro-[1,2,4]triazolo[4,3-*a*]pyrimidin-7-yl)amino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:	2.2 (s, 1H, -NH), 2.5 (s, 3H, -CH_3), 3.2 (s, 3H, -CH_3-N), 3.6 (d, 1H), 4.2 (s, 1H, -NH ring), 4.4 (d, 1H, -vinylic H), 5.5 (s, 1H, -OH), 6.5-8.5 (10H, Ar-H, complex).
^{13}C NMR (100 MHz, $CDCl_3$) δ ppm:	32.1, 42.2, 58.2, 61.2, 130.4, 132.4, 135.8, 140.1, 142.5, 143.7, 148.5, 149.1, 160.8.
IR cm^{-1} (KBr):	3411, 3350, 3345, 3110, 3042, 2953, 2852, 1685, 1592, 1570, 753.
Mass (M+1):	415.5
Elemental analysis:	**Calculated (%) :** C: 63.60; H: 5.09; N: 23.60. **Found (%) :** C: 63.64; H: 5.07; N: 23.62

Antimicrobial Activity

Preparation of Media

Nutrient agar was prepared using the following formulation per liter of distilled water:

- 15 g agar
- 5 g peptone
- 5 g sodium chloride
- (Optional): 3 g beef extract (or 1–1.5 g beef extract + 1.5–2 g yeast extract)

To prepare the medium, the solids were dissolved in 1 L of distilled water by heating and boiling with stirring. The pH was adjusted to around 6.8–7.4. Then the mixture was sterilized by autoclaving at 121 °C and ~15 psi for 15 minutes. Once cooled to 45–50 °C, approximately 20 mL of agar was poured into each sterile Petri dish and left to solidify overnight.
For liquid cultures, nutrient broth was made with:

- 10 g peptone
- 10 g beef extract
- 5 g NaCl per liter

This mixture was dissolved in distilled water, sterilized at 121 °C for 15 minutes, and then cooled to room temperature before inoculation.

5.2Experimental Data of Antimicrobial Study.

Table 2 Antibacterial Activities of Compounds C1-C15.

Samples	S.aureus (+Ve)	B.megaterium (+Ve)	E.coli (-Ve)	P.vulgaris (-Ve)
C1	10	10	8	9
C2	8	9	9	8
C3	10	8	4	9
C4	11	8	12	5
C5	10	8	8	9
C6	8	5	7	8
C7	5	6	8	9
C8	7	11	9	11
C9	9	10	10	10
C10	10	7	8	9
C11	8	4	12	11
C12	10	9	6	10
C13	4	11	10	9
C14	11	8	9	10
C15	10	6	7	7
Ampicillin	15	14	17	16
Gentamycin	16	15	14	16

Figure-1 shows the antimicrobial activity of synthesized compounds C1-C15 with its highest and lowest value of zone of inhibition as follow.

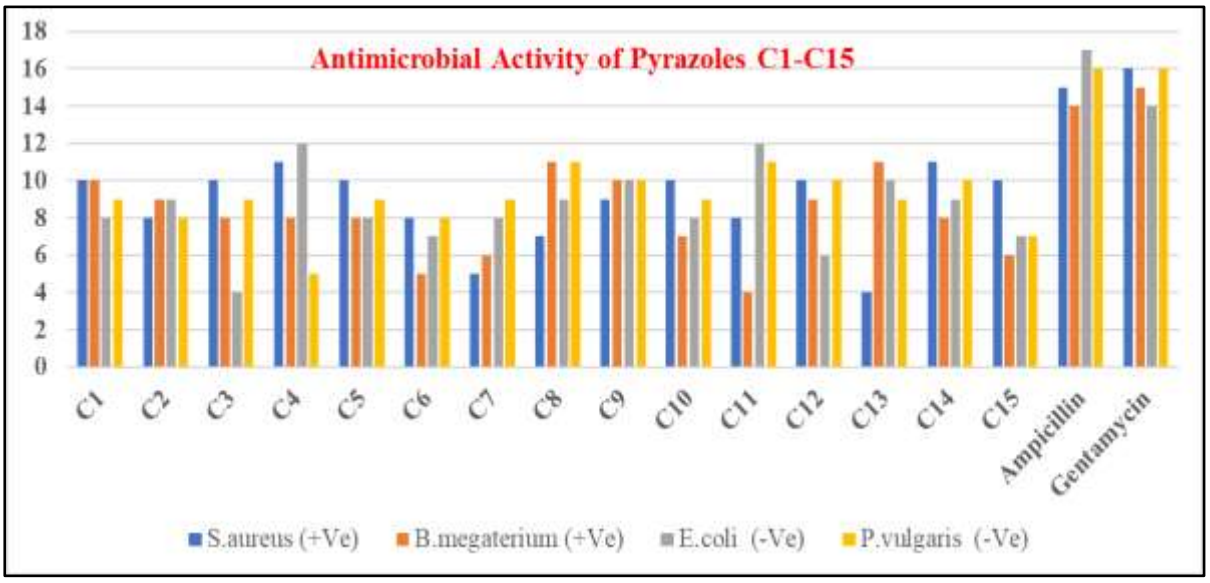


Figure-1 Antimicrobial Activity of pyrazoles C1-C15.

I. Staphylococcus Aureus

- **Highest activity:** Compounds C-4 and C-14; zone of inhibition is 11.0 mm.
- **Lowest activity:** Compound C-13; zone of inhibition is 5.0 mm

Ii. Bacillus Megaterium

- **Highest activity:** Compounds C-8 and C-13; zone of inhibition is 11.0 mm.
- **Lowest activity:** Compound C-4; zone of inhibition is 4.0 mm.

III. Escherichia Coli

- **Highest activity:** Compounds C-4 and C-11; zone of inhibition is 12.0 mm.
- **Lowest activity:** Compound C-3; zone of inhibition is 4.0 mm.

IV. Proteus Vulgaris

- **Highest activity:** Compounds C-8 and C-11; zone of inhibition is 11.0 mm.
- **Lowest activity:** Compound C-4; zone of inhibition is 5.0 mm.

4. CONCLUSION

In conclusion, a diverse series of highly functionalized pyrazole derivatives was synthesized efficiently from readily available starting materials. This compound library features reactive pyrazole scaffolds designed for potential biological applications. Spectroscopic characterization confirmed the structures of all synthesized compounds, and antimicrobial screening revealed notable activity in several members—particularly C-4, C-8, C-11, C-13 and C-14.

These findings align with broader research indicating the efficacy of substituted pyrazoles as antimicrobial agents. For example, many 4-functionalized pyrazole derivatives have shown promising activity against both Gram-positive and Gram-negative bacteria.

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Conflict of Interest

Author does not have conflict of Interest for this work.

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