IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Synthesis, Characterization, and antimicrobial Evaluation of Novel Nitro Pyrazole Derivatives

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Abstract

The global rise of multidrug-resistant bacterial and fungal pathogens poses a critical challenge to clinical medicine, driving the demand for novel pharmacophores. Pyrazole heterocycles represent a privileged scaffold capable of bypassing established resistance mechanisms. Here, we report the design, synthesis, structural characterization, and *in vitro* antimicrobial evaluation of a novel clubbed bis-pyrazole entity: 2'-(4-chlorophenyl)-1-(2,4-dinitrophenyl)-5'-methyl-5-phenyl-2',4,4',5-tetrahydro-1H,3'H-[3,4'-bipyrazol]-3'-one. Synthesized via a multi-step condensation approach, the target compound was structurally elucidated using FTIR, 1H NMR, 13C NMR and Mass Spectral elemental analysis. *In vitro* screening against standard bacterial (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*) and fungal (*Candida albicans*, *Aspergillus niger*) strains was performed using agar-well diffusion and microdilution assays. The compound demonstrated potent antimicrobial efficacy, characterized by significant zones of inhibition and low minimum inhibitory concentration (MIC) values. These results highlight the synergistic potential of integrating a halogenated phenyl moiety with a formylated bipyrazole core to develop potent, broad-spectrum antimicrobial agents.

Key words: Pyrazole, oxobutyrates, Antimicrobial activity, MIC

Introduction

Based on the chemical name provided, the compound 2'-(4-chlorophenyl)-1-(2,4-dinitrophenyl)-5'-methyl-5-phenyl-2',4,4',5-tetrahydro-1H,3'H-[3,4'-bipyrazol]-3'-one appears to be a highly functionalized, nitrogen-containing heterocycle. It belongs to the bipyrazolone family, which is known for significant biological activities and applications in materials science. Cancer is the name given to a large group of related diseases, which can affect almost any part of the body. It occurs when damaged cells, failed to undergo self-destruction and instead they grow, proliferate and spread abnormally[I]. Disruption of the normal regulation of cell cycle progression and proliferation are the major events leading to cancer. The excess cells so formed may continue to divide indefinitely and form growths called tumour which tend to metastasize in some cases [II]. The tumour micro-environment and the stress signals, such as those caused by damaged DNA, are the regulating factors that determine whether cancer cells proliferate or die [III].

Heterocyclic chemistry is an ever-evolving field. The quest for new anticancer drugs is underway all over the world and the heterocyclic compounds serve as key structural components for this process [IV].Pyrazole ring has attracted much attention due to its presence in Antipyrine. In recent years, pyrazole systems, have attracted more attention as biomolecules due to their interesting biological activities [V]. A number of drugs containing pyrazole as main nucleus available in the market, which possess good pharmacological as well as physiological properties. The presently available pyrazole molecules includes Celecoxib as an anti-inflammatory agent, CDPPB as an antipsychotic agent, Difenamizole as an analgesic agent, Betazole as a H_2receptor agonist, Fezolamine as an antidepressant, Lozanolac as an anti-

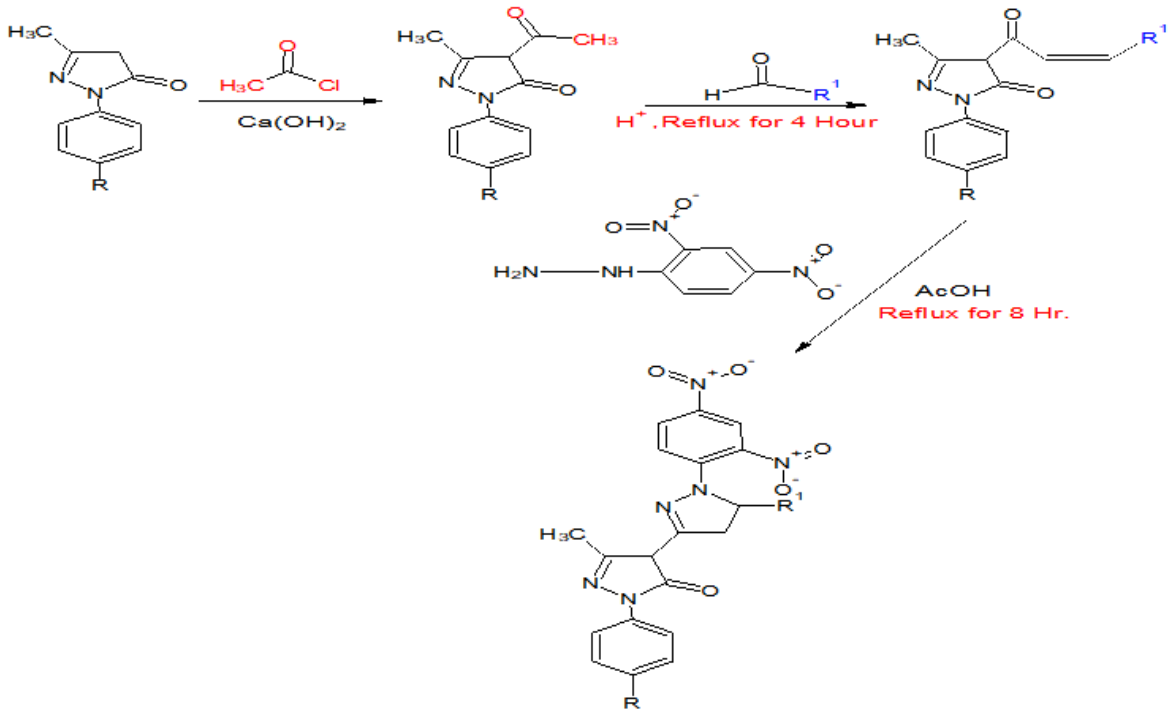
inflammatory agent, Rimonabant as an antiobesity agent, Mepirizole as an anti-inflammatory agent[VI].
The literature of Pyrazoles is enriched with diversified activities. They are reported to possess various biological activities like antibacterial[VII],anti-inflammatory[VIII], antioxidant[IX], anti-tumor[X], analgesic[XI], anti-tubercular[XII], etc., At present Ruxolitinib & Crizotinibare the currently available drugs in the market as antitumor agents containing pyrazole moiety and plays a vital role in influencing the activity[XIII]. One of the compound CAN508, is currently emerged as a new anti-proliferative agent with azo and pyrazole group having some sort of selectivity for cancer cells [XIII- XV].
Similarly compounds having hydrazano or azo groups are able to display a wide range of biological activities. The literature shows the importance of substitution of azo compounds on the various heterocyclic compounds, in increasing the biological potency The incorporation of azo group directly enhances the activity and they are reported with various activities like anti-HIV [XVI] and antimicrobial [XVII] properties.
Keeping in view, of the above facts and the biological importance of aryl hydrazone group, and in continuation of work on 3,5-dimetyl arylazo pyrazoles [XVIII-XX], in the present work we report the synthesis and cytotoxicity evaluation of a new series of 3,5-dimetyl arylazo pyrazoles.

EXPERIMENTAL

Melting points were determined using open capillary tube method and are uncorrected. Alpha bruker FT-IR- Spectrometer was used for recording IR spectra (cm^{-1}). Bruker Advance-II NMR spectrometer (USA) was employed to record ^{1}H-NMR spectra operating at 400 MHz with DMSO/$CDCl_3$ as a solvent. TMS was served as an internal standard. The recording of the mass spectra was carried out by Perkin -Elmer GC-MS.TLC plates were used to monitor the progress of the chemical reaction.

General procedure for Synthesis of 2'-(4-chlorophenyl)-1-(2,4-dinitrophenyl)-5'-methyl-5-phenyl-2',4,4',5-tetrahydro-1H,3'H-[3,4'-bipyrazol]-3'-one

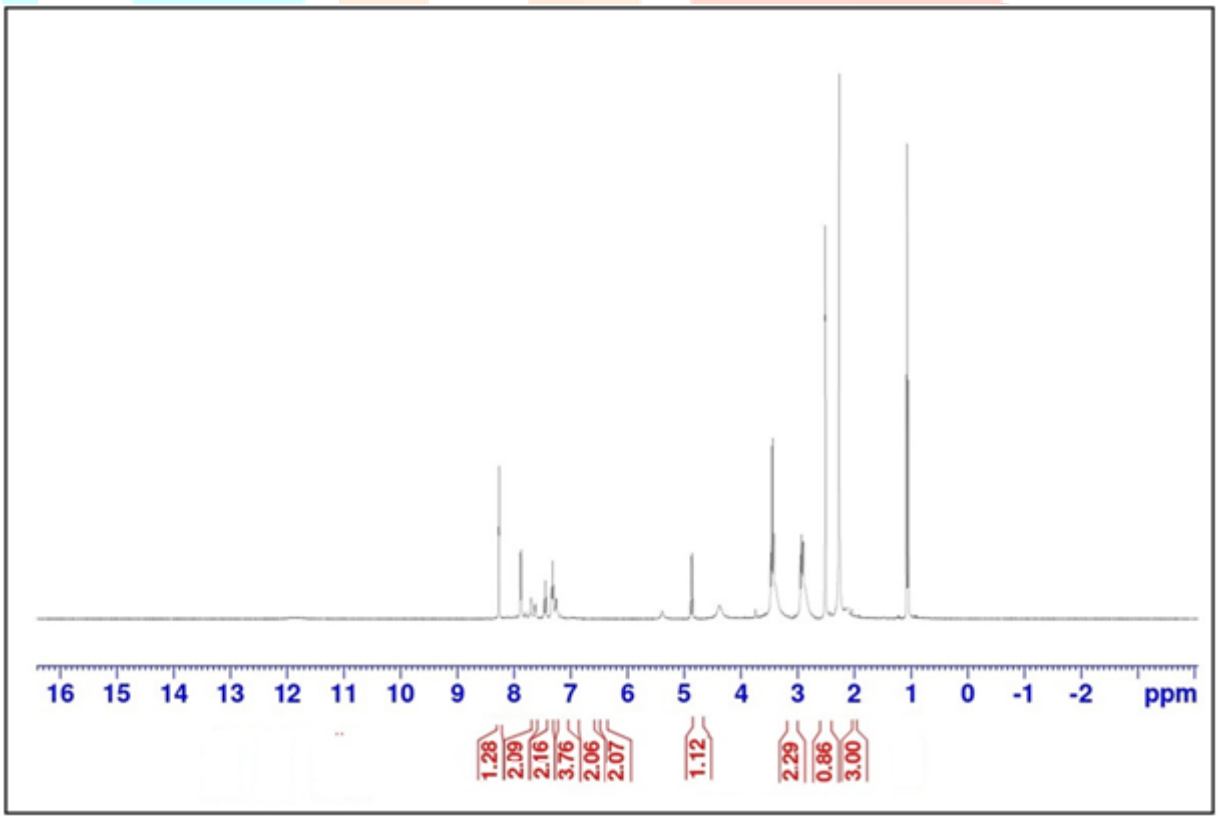
Procedure for synthesis of pyrazoline (D1):- A mixture of Chalcone (3, 10.0mmol), hydrazine hydrate (4, 50.0mmol) and Acetic acid (40ml) were refluxed for 8 hours continuously. Reaction was monitored by TLC continuously using solvent system Petroleum ether: Ethylacetate, resulting solution was poured into ice cold water and allowed to stand overnight. Precipitate formed were filtered and washed with cold water. The product was recrystallized with ethanol. Similar procedure was adopted for synthesis of other derivatives(D1-D5). $C_{25}H_{19}ClN_6O_5$: Yield, 72%; m.p. 185-190 °C. ESI-MS (m/z): 320.34[M]+, 318.04[M+2]+. Found (%): C, 64.69, H, 4.77, N, 14.81. calculated: C, 64.87, H, 4.99, N, 14.91; ^{1}H NMR (DMSO-d6 400 MHz): 6.55-7.98 (12H, m, Ar-H), 08.10 (1H, -carbonyl proton); ^{13}C NMR (DMSO-d6 100 MHz): δ:25.23, 38.85, 42.85, 62.08, 128.25-138.25(12C, Ar-C), 140.12, 145.32, 145.46, 160.17, 162.24, 175.17,; FTIR (KBr. cm-1): 1660-1680 Amide Carbonyl (C=O), 1590-1610 (C=O, Pyrazolone Carbonyl), 1510 (C=N stretch), 1450 (C=C aromatic), 3105 (C-H aromatic), 1060 (C-Cl aromatic). The synthetic route of targeted pyrazole derivatives is shown in Scheme 1.



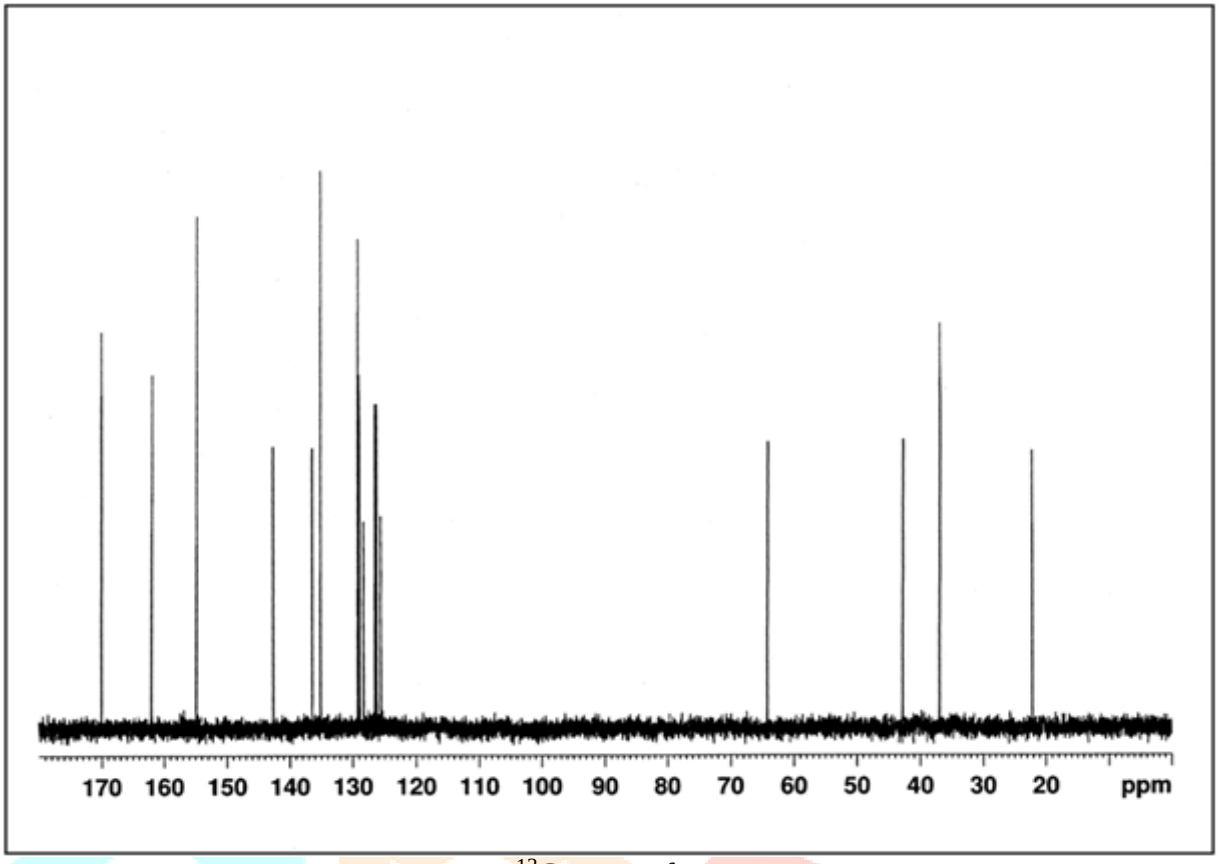
Where R = Cl

R^1 =Phenyl (D1), 2-methyl phenyl(D2), 2-hydroxy phenyl(D3), 4-hydroxy phenyl(D4), 4-chloro phenyl(D5)

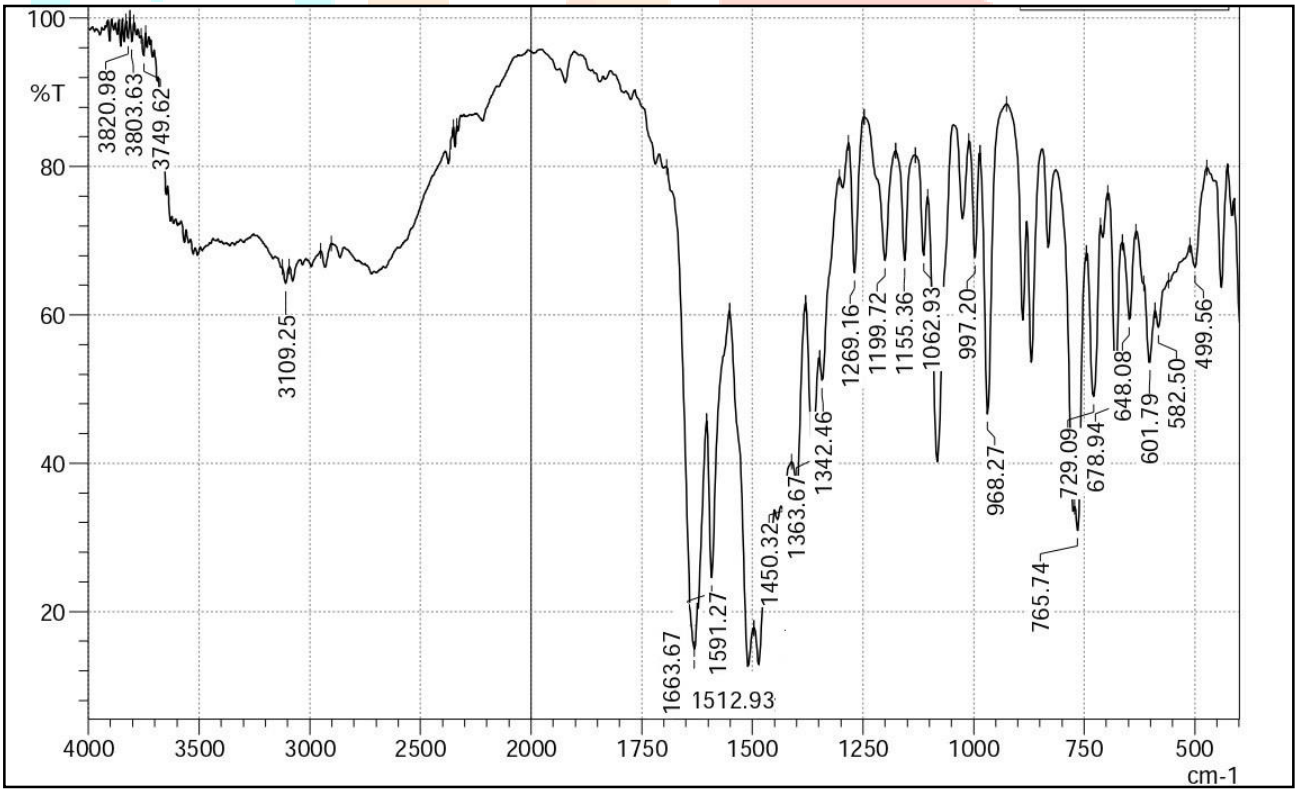
Scheme 1. Synthesis of Pyrazole derivative (D)



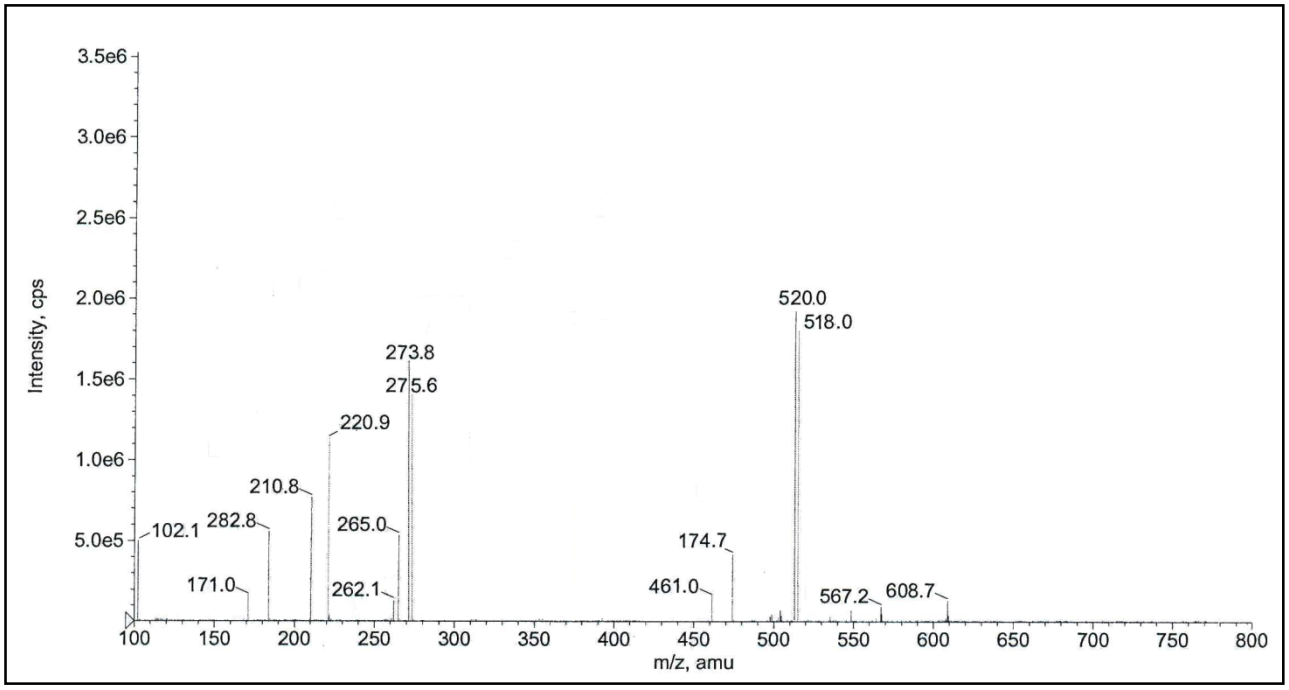
^{1}H NMR of D1



^{13}C NMR of D1



IR Spectrum of D1



Mass Spectra D1

RESULTS AND DISCUSSIONS

Melting points

All melting points were determined in open capillaries in a liquid paraffin bath and are uncorrected. The IR spectra were recorded with KBr pellets on Perkin - Elmer - 783 spectrophotometer and 1H NMR spectra were recorded on a Varian Geminy 200 MHz spectrophotometer with $CDCl_3$ / $DMSOd_6$ as a solvent using tetramethylsilane (T.M.S.) as an internal standard; the chemical shift values are in d ppm. The purity of the compounds was checked by thin layer chromatography (T.L.C.) on silica gel coated glass plates.

Table 1

Analytical and physiochemical parameters of synthesized compound

No.	Code No.	Molecular Formula	Molecular Weight (g/m)	Yield (%)	M.P. °C	C %	H %	N %
						Found		
1	D1	$C_{25}H_{19}ClN_6O_5$	518.91	73	156	72.13	5.10	17.74
2	D2	$C_{26}H_{21}ClN_6O_5$	532.94	72	108	69.35	5.29	16.17
3	D3	$C_{25}H_{19}ClN_6O_6$	534.11	72	108	69.35	5.29	16.17
4	D4	$C_{25}H_{19}ClN_6O_6$	534.11	72	164	68.66	4.80	16.85
5	D5	$C_{25}H_{19}ClN_6O_5$	518.91	75	152	65.02	4.31	15..92

Biological Evaluation (Antimicrobial Assay)

Antibacterial and Antifungal Screenings

The in vitro antibacterial efficacy was tested against standard pathogenic bacteria: Gram-positive organisms (*Staphylococcus aureus* MTCC 96, *S.pyogenus* MTCC 442) and Gram-negative organisms (*Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 1688).[XXI] Antifungal activity was profiled using Candida albicans and Aspergillus niger. The tested bacterial and fungal strains were prepared in the luria broth and incubated at 37 °C and 200 rpm in an orbital incubator for overnight. Sample solutions were prepared in DMSO for concentration 200, 150, 100, 50, 25, 12 and 6µg/mL. The standard drug solution of Chloramphenicol (antibacterial drug) and Nystatin (antifungal drug) were prepared in DMSO. Serial broth micro dilution was adopted as a reference method. 10 µl solution of test compound was inoculated in 5 mL luria broth for each

concentration respectively and additionally one test tubes was kept as control. Each of the test tubes was inoculated with a suspension of standard microorganism to be tested and incubated at 35 °C for 24 h. At the end of the incubation period, the tubes were examined for the turbidity. Turbidity in the test tubes indicated that microorganism growth has not inhibited by the antibiotic contained in the medium at the test concentration.[XXII] The antimicrobial activity tests were run in triplicate.

Results and Discussion

Antimicrobial Profile

The test candidate exhibited promising bio-efficacy values across multiple pathogen models:

Table-2: Antimicrobial activity of D1-D5

Compound	Zone of Inhibition (in mm) Antibacterial Activity				Antifungal Activity	
	Gram positive		Gram negative		C. Albicance	A. Niger
	S.Pyogenus	S.Aureus	E.Coli	Ps.Aeruginosa		
D1	72	46	53	56	150	200
D2	75	42	56	52	200	150
D3	82	45	58	56	150	150
D4	75	47	54	54	100	100
D5	77	49	53	58	150	150

The standard drugs minimal inhibition concentration

Drug	E.Coli	P.Aeruginosa	S.Aureus	S.Pyogenus
	MTCC 443	MTCC 1688	MTCC 96	MTCC 442
Chloramphenicol	50	50	50	50

Minimal Fungicidal Concentration

Drug	C.Albicans	A.Niger
	MTCC 227	MTCC 282
Nystatin	100	100

CONCLUSION

A novel 2'-(4-chlorophenyl)-1-(2,4-dinitrophenyl)-5'-methyl-5-phenyl-2',4,4',5-tetrahydro-1H,3'H-[3,4'-bipyrazol]-3'-one, was successfully designed, synthesized, and structurally confirmed. The in vitro antimicrobial profile highlighted significant inhibitory potential against Gram-positive bacteria (*S. aureus* and *B. pyogenus*) along with moderate antifungal efficacy against *C. albicans*.. The combination of a highly functionalized formylated bipyrazole core and a chlorinated aromatic ring provides a promising template for developing potent new antibacterial agents. Future work will focus on molecular docking studies to clarify the exact mechanism of action within bacterial transpeptidase or DNA gyrase enzymes, alongside optimization of the phenyl ring substituents to maximize broader biological potency.

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