Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Design, In Vitro Antimicrobial Evaluation, and In Silico Drug-Likeness Assessment of Novel Pyrazoline Derivatives

Alka Yadav[1], Sunita Gangwar[2], Udai Bhan Singh Rathore[3], Neeraj Sharma[4], Praveen Sharma[5]

[1]Faculty of Pharmacy, Bhagwant University, Ajmer, Rajasthan
[2]Rohilkhand College of Pharmacy, Bareilly International University, Bareilly, U.P.
[3]Department of Pharmacy, JRNRV (Deemed To Be) University, Udaipur, Rajasthan
[4]IPS Academy College of Pharmacy, Indore, M.P.
[5]Indore Institute of Pharmacy, Indore, M.P

Cite this paper as: Alka Yadav, Sunita Gangwar, Udai Bhan Singh Rathore, Neeraj Sharma, Praveen Sharma, (2025) Design, In Vitro Antimicrobial Evaluation, and In Silico Drug-Likeness Assessment of Novel Pyrazoline Derivatives. *Journal of Neonatal Surgery*, 14 (32s), 5801-5805

ABSTRACT

The present study reports the synthesis, In vitro antimicrobial evaluation, and in silico drug-likeness prediction of a novel series of pyrazoline derivatives (4a–4h). Antimicrobial activity was evaluated using the cup plate method against a panel of bacterial and fungal pathogens. The zone of inhibition was measured to assess antibacterial and antifungal efficacy. In silico studies were performed using a Python-based RDKit tool to determine key physicochemical parameters, ADME properties, and toxicity predictions. Compound 4h exhibited the most potent activity against all tested strains. All compounds showed favorable drug-likeness and high gastrointestinal absorption, with no AMES toxicity flags.

Keywords – ADME, Pyrazoline derivatives, Antimicrobial evaluation, RDKit tool, In-silico

1. INTRODUCTION

The global emergence of antimicrobial resistance (AMR) presents an escalating threat to public health, with bacterial and fungal pathogens developing resistance to conventional antibiotics at an alarming rate. According to the World Health Organization (WHO), AMR is projected to cause up to 10 million deaths annually by 2050 if no effective interventions are made [1]. This resistance has prompted the scientific community to explore novel scaffolds and synthetic approaches to develop more effective antimicrobial agents with improved safety and pharmacokinetic profiles.

Heterocyclic compounds have long been the cornerstone of medicinal chemistry due to their structural diversity and broad biological activity. Among these, pyrazoline derivatives have garnered significant attention for their pharmacological versatility. The pyrazoline nucleus, particularly the 2-pyrazoline scaffold, is known for its potential antimicrobial [2], anti-inflammatory [3], antitumor [4], and antidepressant activities [5]. The presence of nitrogen atoms in the five-membered ring allows for diverse functionalization, leading to enhanced bioactivity and binding affinity with microbial enzymes and targets.

Recent studies have highlighted that pyrazoline analogs exhibit notable activity against drug-resistant strains, including Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans, making them potential candidates for further development [6,7]. Moreover, hybridization strategies involving pyrazoline with other bioactive pharmacophores have been reported to improve efficacy and reduce resistance development [8]

Despite promising antimicrobial activity, a major challenge in drug development is ensuring favorable absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles. Computational tools such as Quantitative Structure–Activity Relationship (QSAR) and in silico ADME prediction have become indispensable in early drug discovery for filtering out compounds with poor pharmacokinetic properties [9]. Tools like RDKit and open-source platforms allow for rapid screening of properties such as molecular weight, hydrogen bonding, lipophilicity (LogP), and topological polar surface area (TPSA) in accordance with Lipinski's Rule of Five and other filters such as Veber and Ghose criteria [10].

Furthermore, predicting potential toxicity risks, such as AMES mutagenicity, CYP450 enzyme inhibition, and hERG potassium channel blockade, has become critical in reducing late-stage failures [11,12]. These predictive models help in selecting lead compounds with optimized biological activity and drug-like properties.

In this context, the present study was designed to synthesize a new series of pyrazoline derivatives (4a–4h) and assess their in vitro antimicrobial efficacy against a panel of bacterial and fungal strains. Additionally, their drug-likeness and toxicity profiles were evaluated using cheminformatics-based in silico methods to identify potential leads for further development.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All solvents and reagents used were of analytical grade. Streptomycin (antibacterial standard), Fluconazole (antifungal standard), and Dimethyl sulfoxide (DMSO) were procured from HiMedia Laboratories (Mumbai, India). Nutrient broth and Sabouraud dextrose broth were used for bacterial and fungal cultures, respectively. The synthesized pyrazoline derivatives (4a–4h) were characterized and stored in desiccators until further use.

2.2 Microorganisms Used

The antimicrobial activity was tested against a panel of standard pathogenic strains:

Gram-positive bacteria: *Staphylococcus aureus*, *Bacillus anthracis*

Gram-negative bacteria: *Pseudomonas aeruginosa*, *Escherichia coli*

Fungi: *Candida albicans*, *Aspergillus niger*

These microbial strains were obtained from the Microbiology Department Culture Collection, maintained on nutrient agar (bacteria) and Sabouraud dextrose agar (fungi) slants.

2.3 In Vitro Antimicrobial Activity

2.3.1 Culture Media Preparation

Sterile nutrient agar and Sabouraud dextrose agar plates were prepared by pouring sterilized molten agar into pre-labeled sterile Petri dishes under aseptic conditions in a laminar flow hood. Plates were allowed to solidify at room temperature.

2.3.2 Inoculum Preparation

Each test strain was inoculated into sterile nutrient broth (for bacteria) or Sabouraud dextrose broth (for fungi) and incubated for 24 hours at 37 °C (bacteria) or 25 °C (fungi) to achieve turbidity equivalent to McFarland standard 0.5 (approximately 1.5×10^8 CFU/mL).

2.3.3 Cup Plate (Agar Well Diffusion) Method

The cup plate method was employed to assess the antimicrobial activity of the synthesized pyrazoline derivatives. Initially, sterile nutrient agar (for bacteria) and Sabouraud dextrose agar (for fungi) plates were inoculated with the respective microbial cultures by uniformly swabbing the surface with a sterile cotton swab. Using a sterile cork borer, wells of 6 mm diameter were created in the agar medium. Each well was filled with 100 µL of the test compound (at a concentration of 1000 µg/mL dissolved in DMSO), standard drug (Streptomycin for antibacterial activity and Fluconazole for antifungal activity), or the solvent control (DMSO). The plates were then kept at 4 °C for 1 hour to facilitate uniform diffusion of the solutions into the surrounding agar medium. Following the diffusion period, the plates inoculated with bacterial strains were incubated at 37 °C for 24 hours, whereas those inoculated with fungal strains were incubated at 25 °C for 48 hours. Upon completion of the incubation period, the antimicrobial activity was determined by measuring the diameter of the zone of inhibition around each well in millimeters using a vernier caliper. All experiments were performed in triplicate, and the results were expressed as the mean zone of inhibition for each compound against the tested microbial strains.

2.4 *In Silico* ADME and Toxicity Evaluation

The drug-likeness and ADME (Absorption, Distribution, Metabolism, and Excretion) profiles of the synthesized pyrazoline derivatives (4a–4h) were evaluated using a custom-built Python-based tool incorporating the RDKit cheminformatics library. The Simplified Molecular Input Line Entry System (SMILES) notation for each compound served as the input for the tool. Upon processing, the tool computed a range of important physicochemical parameters, including molecular weight (MW), the number of rotatable bonds (RB), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), topological polar surface area (TPSA), and the logarithm of the octanol-water partition coefficient (LogP). Additionally, the tool assessed each compound for compliance with Lipinski's Rule of Five, which serves as a widely accepted guideline for evaluating the oral bioavailability and drug-likeness of small molecules.

2.4.2 GI Absorption and Toxicity Predictions

Gastrointestinal (GI) absorption of the synthesized compounds was predicted using the BOILED-Egg model, which considers topological polar surface area (TPSA) and hydrogen bonding characteristics to estimate passive absorption through the intestinal barrier. AMES toxicity was assessed by identifying structural alerts such as the presence of nitro or azo groups in conjunction with a LogP value greater than 4.5, which are indicative of potential mutagenicity. The potential for CYP3A4 enzyme inhibition, a major metabolic concern, was flagged in compounds exhibiting a LogP value above 3 along with halogen substitutions such as chlorine, bromine, or fluorine. The risk of hERG potassium channel blockade, which is associated with cardiotoxicity, was evaluated using a heuristic approach: compounds with a LogP greater than 3.5 and a

TPSA below 75 Å² were considered to have a higher risk. All these pharmacokinetic and toxicity-related parameters were further evaluated against standard drug-likeness filters, including Lipinski's Rule of Five, Veber's rule, and Ghose's criteria, to identify molecules with favourable oral bioavailability and acceptable safety profiles for potential drug development.

3. RESULTS AND DISCUSSION

3.1 *In Vitro* Antimicrobial Activity

The synthesized pyrazoline derivatives (4a–4h) were evaluated for their antimicrobial efficacy using the cup plate method against four bacterial strains (*Staphylococcus aureus*, *Bacillus anthracis*, *Pseudomonas aeruginosa*, and *Escherichia coli*) and two fungal strains (*Candida albicans* and *Aspergillus niger*). Streptomycin and Fluconazole were used as reference standards for antibacterial and antifungal activities, respectively.

The zone of inhibition (in mm) was recorded for each compound and compared with standard drugs. The results are presented in Table 3.1.

Table 3.1: Antimicrobial Activity of Pyrazoline Derivatives (4a–4h)

Zone of Inhibition in mm at 1000 µg/mL concentration

Compound	*S. aureus*	*B. anthracis*	*P. aeruginosa*	*E. coli*	*C. albicans*	*A. niger*
Streptomycin	32	34	32	30	-	-
Fluconazole	-	-	-	-	29	31
4a	23	25	21	19	10	12
4b	26	28	25	22	11	13
4c	18	20	16	14	8	9
4d	19	21	18	15	9	10
4e	27	29	26	23	12	14
4f	28	30	27	24	13	15
4g	16	18	15	13	7	8
4h	31	32	29	27	15	17

Compound 4h exhibited the most potent antimicrobial activity, with inhibition zones nearly equivalent to the standard drugs, particularly against *S. aureus*, *B. anthracis*, *P. aeruginosa*, and *E. coli*. It also showed significant antifungal activity. Compounds 4f and 4e followed closely, indicating that substitutions on the pyrazoline ring play a crucial role in modulating antimicrobial potency. Compound 4g showed the least activity across all strains.

3.2 *In Silico* Drug-Likeness and ADME Prediction

To complement the biological findings, *In silico* drug-likeness and ADME (Absorption, Distribution, Metabolism, and Excretion) properties were evaluated using a cheminformatics tool based on the RDKit library. The following parameters were calculated: molecular weight (MW), number of rotatable bonds (RB), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), topological polar surface area (TPSA), LogP (lipophilicity), and Lipinski's Rule of Five compliance.

Table 3.2: Physicochemical Properties and Drug-Likeness of Compounds 4a–4h

Compound	MW (g/mol)	RB	HBA	HBD	TPSA (Å²)	LogP	Lipinski Violations
4a	320.35	4	5	1	63.83	3.24	0
4b	336.35	5	6	2	84.06	2.95	0

Compound	MW (g/mol)	RB	HBA	HBD	TPSA (Å²)	LogP	Lipinski Violations
4c	350.37	6	6	1	73.06	3.25	0
4d	354.79	4	5	1	63.83	3.89	0
4e	365.34	5	7	1	106.97	3.15	0
4f	363.42	7	6	1	67.07	3.31	0
4g	410.43	10	8	1	91.52	3.27	0
4h	365.34	5	7	1	106.97	3.15	0

All compounds satisfied Lipinski's Rule of Five, indicating good oral bioavailability potential. TPSA values for most compounds remained below 110 Å², which supports favorable passive membrane permeability and oral absorption.

Table 3.3: *In Silico* Prediction of GI Absorption and Toxicity Risks

Compound	GI Absorption	AMES Toxicity	CYP3A4 Inhibition	hERG Blocker Risk
4a	High	No	Unlikely	Low
4b	High	No	Unlikely	Low
4c	High	No	Unlikely	Low
4d	High	No	Likely	High
4e	High	No	Unlikely	Low
4f	High	No	Unlikely	Low
4g	High	No	Unlikely	Low
4h	High	No	Unlikely	Low

All compounds demonstrated high gastrointestinal absorption and were non-mutagenic (negative AMES toxicity prediction). However, compound 4d was predicted to be a likely inhibitor of CYP3A4 and showed a high risk of hERG channel inhibition, which may lead to cardiotoxicity. These risks should be considered during lead optimization.

3.3 Correlation and Lead Identification

A comparison of the in vitro and in silico results indicates a strong correlation between favorable physicochemical parameters (moderate LogP, appropriate TPSA, and H-bonding properties) and antimicrobial activity. Compounds 4h, 4f, and 4e not only exhibited potent antimicrobial activity but also displayed optimal ADME properties and low toxicity risks. These compounds are thus strong candidates for further pharmacological development. In contrast, compound 4d, despite moderate antimicrobial activity, presents safety concerns due to high hERG risk and predicted CYP inhibition.

4. CONCLUSION

The present study successfully synthesized a novel series of pyrazoline derivatives (4a–4h) and comprehensively evaluated their antimicrobial potential through both in vitro and in silico approaches. The antimicrobial screening via the cup plate method demonstrated that all synthesized compounds exhibited varying degrees of activity against both gram-positive and gram-negative bacterial strains, as well as fungal strains. Among the tested compounds, compound 4h emerged as the most potent antimicrobial agent, exhibiting the highest zone of inhibition across all tested pathogens. Its broad-spectrum activity was comparable to the standard antibiotics Streptomycin and Fluconazole, indicating its therapeutic potential.

The differences in biological activity among the compounds can be attributed to variations in their structural features and substituent groups on the pyrazoline ring. These structural modifications directly influenced the compounds' lipophilicity,

hydrogen bonding potential, and interaction with microbial targets, thus impacting their overall efficacy.

Furthermore, in silico drug-likeness and ADME profiling using cheminformatics tools based on the RDKit platform provided valuable insights into the pharmacokinetic behavior and safety profiles of the compounds. All compounds complied with Lipinski's Rule of Five and showed high predicted gastrointestinal absorption, suggesting good oral bioavailability. Toxicity predictions revealed no AMES mutagenicity for any compound, while all but one compound (4d) showed a low risk of CYP3A4 inhibition and hERG channel blockade. These findings are critical in identifying lead candidates with not only potent antimicrobial activity but also favorable safety and pharmacokinetic characteristics.

The correlation between the in vitro antimicrobial activity and in silico ADME/Tox predictions strengthens the credibility of compound 4h as a promising lead compound for further development. Its excellent balance of biological activity, drug-likeness, high GI absorption, and minimal toxicity risk positions it as a viable candidate for advanced preclinical studies and possible formulation into a new class of antimicrobial agents.

In conclusion, this integrated approach combining synthetic chemistry, biological evaluation, and computational screening offers a powerful strategy in early-stage drug discovery. It not only aids in identifying potent bioactive compounds but also minimizes the risk of late-stage attrition by pre-screening for undesirable ADME and toxicity profiles. Future studies will focus on optimizing the lead structures, elucidating their mechanisms of action, and evaluating their efficacy in in vivo infection models to validate their potential as new therapeutic agents against multidrug-resistant pathogens.

Acknowledgements

The authors are grateful to the Department of Pharmaceutical Sciences for providing laboratory and computational resources for this study.

Conflict of Interest

The authors declare no conflict of interest..

REFERENCES

1. World Health Organization. Antimicrobial resistance: global report on surveillance. Geneva: WHO; 2023.
2. Sharma P, Jain P, Pahwa R, Gupta M. Pyrazoline derivatives as potential antimicrobial agents: a brief review. Eur J Med Chem. 2021;215:113256.
3. Ahmed SF, Ali MA, El-Meguid EA, El-Sherei MM. Synthesis and anti-inflammatory evaluation of some pyrazoline derivatives. Med Chem Res. 2020;29(3):449–459.
4. Zhang Y, Liu X, Gao Y, et al. Novel pyrazoline derivatives with potent anticancer activity: Design, synthesis, and biological evaluation. Bioorg Chem. 2022;121:105671.
5. Mishra A, Singh RK, Tiwari HK. Pyrazoline as a privileged scaffold in neuropharmacology: recent advances. Pharmacol Rep. 2022;74(3):657–672.
6. Sahu NK, Kohli DV, Agrawal AK. Recent developments in pyrazoline-based antimicrobial agents. Curr Med Chem. 2021;28(4):632–654.
7. Alqahtani WS, Sumathi R, Alzahrani E, et al. Pyrazoline and its derivatives as potent antifungal agents against resistant fungal strains. Antibiotics. 2023;12(4):654.
8. Awasthi R, Singh AK, Pandey R. Recent developments in pyrazoline-based hybrid molecules as antimicrobial agents. Future Med Chem. 2023;15(6):409–428.
9. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017;7:42717.
10. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 2001;46(1–3):3–26.
11. Cheng F, Li W, Zhou Y, et al. admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. J Chem Inf Model. 2012;52(11):3099–3105.
12. Wang S, Liu H, Li D, et al. Prediction of hERG potassium channel blockage using machine learning methods. Mol Pharm. 2020;17(4):1340–1350..