RASĀYAN *J. Chem.*
Vol. 19 | No. 4 | 2508-2516 | October-December | 2026
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

DEVELOPMENT OF NOVEL 6-FLUOROCHROMANE-LINKED PHENYL PYRAZOLINE HYBRIDS AS PROMISING ANTIMICROBIAL AGENTS: SYNTHESIS AND SAR STUDIES

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ABSTRACT

The emergence of resistant microbial pathogens continues to challenge the effectiveness of existing antimicrobial therapies, creating a demand for chemically diverse molecules with improved biological potential. In this study, a series of novel 6-fluorochromane-linked phenyl pyrazoline derivatives was designed and synthesized through a convenient multistep synthetic strategy. Structural confirmation of the synthesized molecules was achieved using infrared spectroscopy, proton nuclear magnetic resonance spectroscopy, carbon-13 nuclear magnetic resonance spectroscopy and mass spectrometry. The antimicrobial potential of the synthesized derivatives was assessed against representative Gram-positive and Gram-negative bacteria together with selected pathogenic fungi. Several molecules exhibited encouraging antimicrobial activity, demonstrating that appropriate structural modification of the aromatic ring markedly influences biological performance. Structure–activity relationship evaluation indicated that the combined presence of the fluorochromane framework and phenyl pyrazoline nucleus, together with suitable electronic substitution on the aromatic ring, contributes to enhanced antimicrobial properties. These findings offer valuable guidance for the future design and optimization of fluorinated heterocyclic molecules and support Sustainable Development Goal 3 (Good Health and Well-being) by contributing to ongoing efforts aimed at combating infectious diseases.

Keywords: Heterocyclic Chemistry, 6-Fluorochromane, Phenyl Pyrazoline, Molecular Hybridization, Antimicrobial Agents, Structure–Activity Relationship.

RASĀYAN *J. Chem.*, Vol. 19, No.4, 2026

INTRODUCTION

Heterocyclic compounds constitute an important class of organic molecules widely recognized for their significant pharmacological significance in medicinal chemistry.[1] Among these, nitrogen-containing heterocyclic moieties have attracted considerable scientific attention owing to their remarkable therapeutic potential.[2] Pyrazoline derivatives, in particular, have gained significant attention for exhibiting diverse biological activities like antimicrobial, antioxidant and anticancer effects.[3-7] The structural versatility of the pyrazoline ring and the ease of structural modification associated with the pyrazoline ring make it an important pharmacophore for the development of new bioactive molecules.[8-10] Chromane-based derivatives represent another important class of heterocyclic compounds widely reported for their biological significance.[11] Chalcones are known for their presence in natural products as well as synthetic drugs.[12] Furthermore, incorporation of fluorine into medicinally important molecules is an accepted approach for enhancing biological performance.[13] Fluorine substitution enhances lipophilicity, chemical stability and bioavailability, thereby increasing the interaction of molecules with biological targets. Consequently, fluorochromane-based derivatives represent an attractive area for further investigation; they have also gained increasing interest in recent years.[14] Chalcones are well-known intermediates in organic synthesis and are easily synthesized through reactions between aromatic aldehydes and suitable ketones. Due to their α, β-unsaturated carbonyl system, chalcones exhibit significant reactivity and biological activity.[15,16] They are widely utilized as valuable intermediates for constructing diverse heterocyclic frameworks.[17] The most important transformations in chalcones are their cyclisation with phenylhydrazine, leading to the formation of phenyl pyrazoline derivatives with enhanced biological

http://doi.org/10.31788/RJC.2026.1949982
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properties.[18] The emergence of antimicrobial resistance has become a serious global health challenge, substantially reducing the effectiveness of currently available antimicrobial drugs and creating an urgent demand for structurally novel therapeutic agents with improved efficacy.[19,20] Although pyrazoline, chromane and carboxamide derivatives have individually demonstrated promising antimicrobial potential, reports describing the integration of these three pharmacologically important motifs into a single fluorinated molecular framework remain comparatively limited. Furthermore, the influence of electronic and positional substitution on the antimicrobial behaviour of 6-fluorochromane-carboxamide-linked phenyl pyrazoline derivatives has not been comprehensively investigated, representing an important research gap in this field. To address this challenge, the present study was undertaken to design and synthesise a series of novel 6-fluorochromane-carboxamide-based phenyl pyrazoline derivatives. The findings of this investigation are expected to contribute to the rational development of fluorinated heterocyclic scaffolds as potential antimicrobial lead molecules for future medicinal chemistry research. The present investigation is aligned with United Nations Sustainable Development Goal 3 (Good Health and Well-being) by supporting the discovery of innovative antimicrobial candidates that may contribute to combating antimicrobial resistance and improving global healthcare outcomes.

EXPERIMENTAL

Materials and Methods

All chemical reagents were procured from reputable pharmaceutical companies located in Ankleshwar, India. Solvents used in this investigation were of analytical grade and were obtained from commercial suppliers. 6-fluoro-3,4-dihydro-chromene-2-carboxylic acid, substituted aromatic benzaldehydes, phenyl hydrazine hydrochloride, sodium hydroxide, potassium hydroxide, and ethanol was used as received from Sigma-Aldrich and others without further purification.

Characterization Techniques

The progress of each reaction was monitored by thin-layer chromatography (TLC) using silica gel 60 F-254 pre-coated aluminium plates, and the chromatograms were visualised under ultraviolet light. All reactions were performed on a magnetic stirrer equipped with temperature control. FT-IR spectra were recorded on a Perkin-Elmer 100 spectrometer in KBr discs. ^{1}H-NMR spectra were taken at SICART lab on a 400 MHz Bruker Avance 3 Bruker using DMSO as solvent. ^{13}C-NMR spectra were taken on a Bruker Avance 400 NMR Spectrometer at SICART, Vallabh Vidyanagar, Anand, India. LC-MS analysis of all newly synthesised compounds was performed on an LCQ Fleet and TSQ Quantum Access with a thermos-specific system at SICART, Anand, India.

General Synthetic Procedure

Synthesis of N-(4-acetyl phenyl)-6-fluoro-3,4-dihydro-2H-chromene-2-carboxamide[3]

Thionyl chloride (99.6 g, 0.835 mol) was charged into a clean RBF. 6-fluoro-3,4-dihydro-chromene-2-carboxylic acid (25.0 g, 0.083 mol) was added to the RBF by maintaining the temperature < 25°C. The reaction was maintained at 75-80°C with continuous stirring for 3 h. The ongoing reaction was monitored by TLC in chloroform: methanol (8:2 system. Excess T.C. was distilled off under reduced pressure. A fresh ethylene dichloride (200 mL) was added in RM dropwise with 4-amino acetophenone 12.5 g, 0.060 mol. Triethylamine (8.45 g, 0.083 mol) was also included in the reaction as a base at 15°C. The reaction mixture was refluxed for 3 h. After that, water was added to the mixture to separate the layers. organic layer was washed with 5% HCl, with 10% sodium bicarbonate solution and with a final water wash. Precipitates were recrystallised from chloroform.

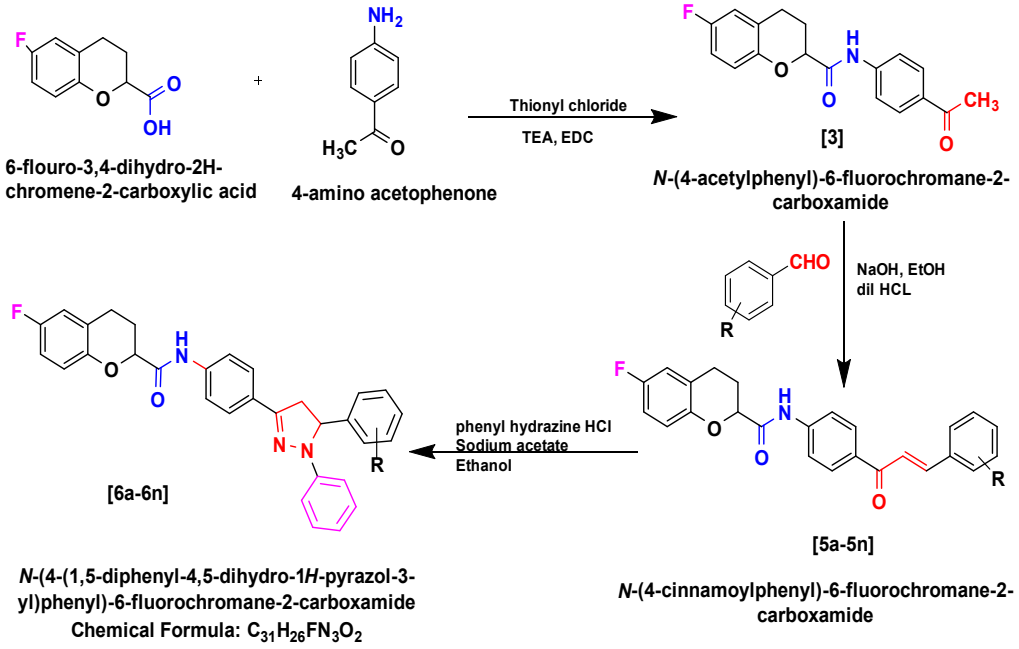
Synthesis of (E)-6-fluoro-N-[4-(3-(phenyl)-2-propenoyl) phenyl]chromane-2-carboxamide [5]

Take a 10 g of N-(4-acetyl phenyl)-6-fluoro-3,4-dihydro-2H-chromene-2-carboxamide diluted in 10 millimeters of ethanol. Then, NaOH (1.02 g, 0.039 mol) was diluted in 10 mL of water. It was slowly added to the reaction mixture, and then dropwise benzaldehyde (2.6 g, 0.036 mol) was also added, below 25°C. It was stirred for 5-6 hours. The flask was cooled, and the reaction mixture was poured into a glass beaker, acidified with dilute hydrochloric acid, and filtered; it was also dried. Finally, chalcone was

recrystallised from alcohol. Similarly, carboxamide [3] reacted with various aromatic aldehydes [4a-4n] to give related chalcones [5a-5n].

Synthesis of N-(4-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-6-fluorochromane-2-carboxamide [6]

(E)-6-fluoro-N-[4-(3-(phenyl)-2-propenoyl)phenyl]chromane-2-carboxamide (5g, 0.012 mol) was dissolved in 40 ml of ethanol. 1.92 g of potassium hydroxide was added as a catalyst, then phenylhydrazine hydrochloride (2 g, 0.028 mol) was slowly added to the reaction mixture. The reaction mixture became yellow in colour. The temperature rose to 82°C with refluxed for eight hours. TLC system toluene: ethyl acetate – 8:2 was used to check completion of the reaction. RM was poured into beaker had ice cold water with continue stirring. The solid was collected by filtration and recrystallized from alcohol. The final product appeared as a light-yellow colored powder. The same procedure was used for the synthesis of all new phenyl pyrazoline derivatives [6a-n].



Where R:- 6a= -H, 6b= 4-OCH_3, 6c= 2-Cl, 6d= 4-CH_3, 6e= 3,4-CH_3, 6f= 3-Br, 6g= 3-OH, 6h= 4-OH, 6i= 4-OH,3-OCH_3, 6j= 3-OH,4-OCH_3, 6k= 3,4-OCH_3, 6l= 3-NO_2, 6m= 2-OH, 6n= 4-Br

Scheme-1: Synthesis of Phenyl Pyrazoline derivatives [6a-6n]

RESULTS AND DISCUSSION

Chemistry

The formation of the target phenyl pyrazoline derivatives (6a-6n) proceeds through a well-defined sequence of bond-forming transformations. Initially, the chalcone intermediates are generated via base-catalysed Claisen–Schmidt condensation, involving nucleophilic addition of the enolate ion to the carbonyl carbon of the substituted aromatic benzaldehydes, followed by dehydration to afford the α,β-unsaturated carbonyl system. Subsequently, the chalcone undergoes reaction with phenylhydrazine hydrochloride. where the nucleophilic $–NH_2$ group attacks the β-carbon of the chalcone, leading to the formation of a hydrazone intermediate. This intermediate further undergoes intramolecular cyclisation, resulting in the formation of the five-membered pyrazoline ring through carbon-nitrogen bond formation. The cyclisation step is facilitated by the nucleophilic attack of the second nitrogen atom on the carbonyl carbon, followed by proton transfer and stabilisation of the heterocyclic framework. The overall transformation involves sequential nucleophilic addition, condensation, cyclisation, and ring closure, leading to the formation of 4,5-dihydro-1H-pyrazoline derivatives. Furthermore, the presence of the 6-

fluorochromane carboxamide moiety remains intact throughout the reaction sequence, contributing to structural rigidity and electronic modulation of the final compounds. The substituents on the aromatic ring influence the electron density on the chalcone system, thereby affecting the rate of cyclisation and overall yield of the final products.

Spectral Characterisation

The synthesised fluorinated phenyl pyrazoline derivatives containing a chromane scaffold were successfully established through detailed spectral investigations.

FT-IR Spectra

The characteristic absorption bands observed in the region of 3300-3325 cm^{-1} confirmed the presence of amide N–H stretching vibrations, while intense bands around 1650-1665 cm^{-1} indicated the carbonyl functionality of the carboxamide moiety. The appearance of absorption bands near 1530-1545 cm^{-1}further supported the formation of the pyrazoline ring through C=N stretching vibrations. In addition, the fluorinated chromane framework was evidenced by characteristic C–F stretching absorptions in the 1050-1100 cm^{-1} spectral region. Figure 1 shows the FT-IR spectra of compound 6a [FT-IR cm^{-1}**:** 3324(N-H str.), 3003(Ar-H), 1676 (C=O str. of amide bond), 1523 (C=N str. of pyrazoline), 1198 (C-N of pyrazoline), 1268(C-O of chromane), 1082 (C-F str.), 827(C-H str.)]

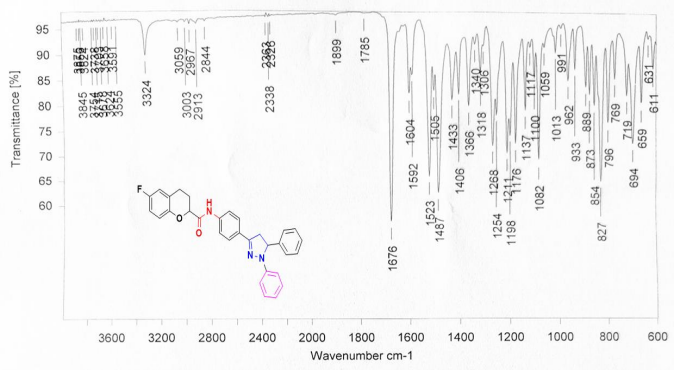


Fig.-1: FT-IR Spectra of Phenyl Pyrazoline Derivative (6a)

^{1}H NMR Spectra

It was displayed characteristic resonance patterns associated with the pyrazoline nucleus and chromane ring system. The methylene protons of the pyrazoline moiety resonated within δ 3.10–3.90 ppm, whereas the methine proton appeared around δ 4.40–5.40 ppm, confirming successful cyclisation of chalcone intermediates into the corresponding pyrazoline derivatives. Aromatic protons associated with both the substituted phenyl ring and fluorinated chromane scaffold appeared as multiple signals in the region of δ 6.70–8.10 ppm. The amide N–H proton consistently appeared as a downfield singlet due to hydrogen-bonding interactions around 10-11 ppm. Figure-2 showed ^{1}H-NMR spectra of compound 6a.[δ: 2.25 (2H, d, aliphatic methyl), 2.8(2H, dt, chroman), 4.73(1H, s, C-O-C), 4.83(1H, s, Pyrazoline), 6.9-7.8(18H, m, Ar-H of benzene), 10.06(1H, s, NH of pyrazoline), 10.16(1H, s, CONH)].

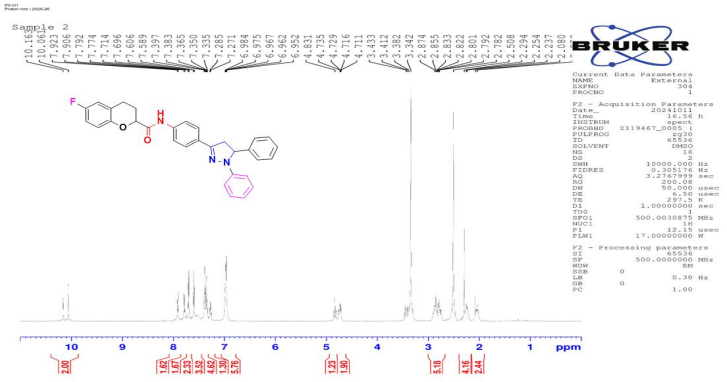


Fig.-2: ^{1}H-NMR Spectra of Phenyl Pyrazoline Derivative(6a)

^{13}C NMR Spectra

The aliphatic carbons generally resonated in the region of δ 15-25 ppm, while the methine carbon attached to the pyrazoline ring appeared around δ 60-65 ppm. Signals corresponding to methoxy carbons in substituted derivatives were observed near δ 55-57 ppm. Aromatic carbons of both the phenyl and chromane systems appeared within δ 110-145 ppm. The carbon directly attached to fluorine in the chromane scaffold showed a characteristic deshielded resonance around δ 158-162 ppm due to fluorine coupling effects. Furthermore, the amide carbonyl carbon consistently appeared in the downfield region near δ 165-169 ppm, thereby supporting the successful incorporation of the carboxamide functionality into the synthesised framework.^{13}C-NMR spectra of compound 6a are shown in Fig.-3. [δ: 14.96 (1C, s, aliphatic), 23.73, 24.44 (2C, s, aliphatic), 64.12 (1C, s, Pyrazoline), 75.49 (1C, s, C-O-C), 114.18-155.48 (23C, m, aromatic benzene) , 157.87 (pyrazoline C), 168.99 (1C, s, C-F), 169.18 (1C, s, C=O of Amide)]

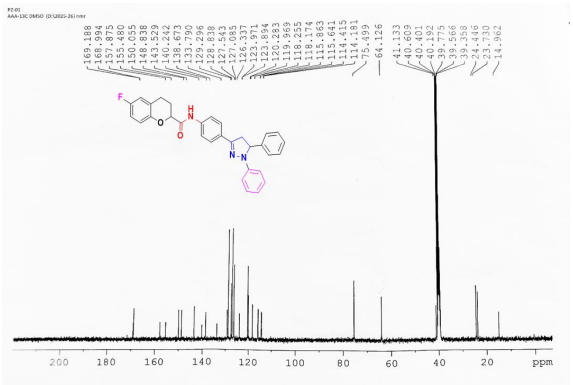


Fig.-3: ^{13}C-NMR Spectra of Phenyl Pyrazoline Derivative(6a)

Mass Spectra

This analysis revealed molecular ion peaks consistent with the calculated molecular masses of the synthesised phenyl pyrazoline derivatives, thereby confirming the successful formation of the targeted 6-fluorochromane-linked phenyl pyrazoline analogues. Figure-4 confirmed the mass of compound 6a. Molecular Formula: $C_{31}H_{26}FN_3O_2$, Formula Weight: 491.5 gm/mol. Found: M+1=492.4.

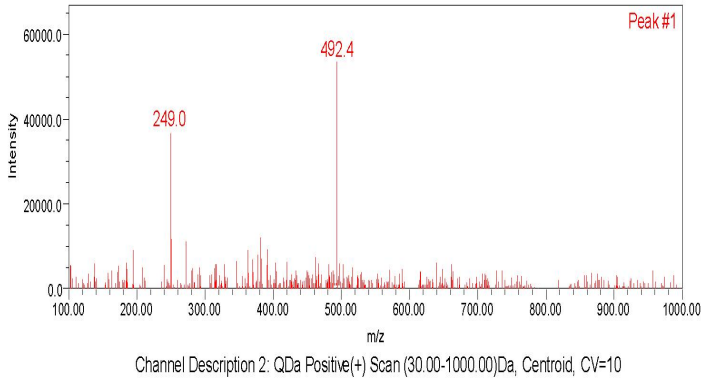
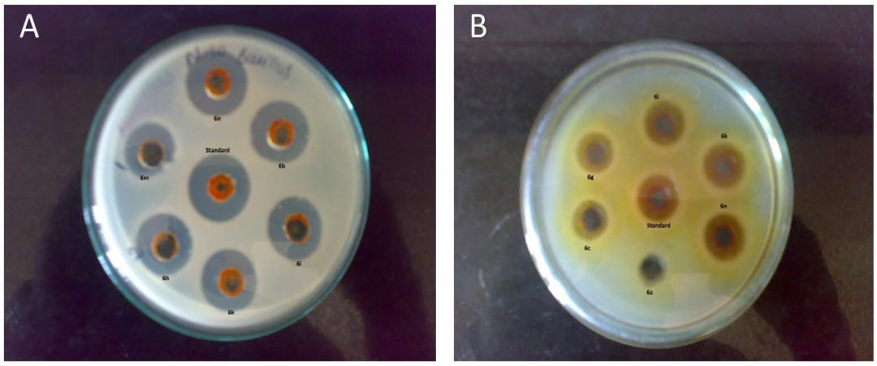


Fig.-4: Mass Spectra of Phenyl Pyrazoline Derivative(6a)

Antimicrobial Activity

The antimicrobial activity of 6-fluorochromane-based phenyl pyrazoline derivatives (6a-6n) was evaluated against selected Gram-positive bacteria (*Streptococcus pyogenes, Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*), as well as fungal strains (*Aspergillus niger, Candida albicans*) using the agar diffusion method. All results are presented in Tables-1 and 2. The obtained results indicate that the synthesised compounds exhibit a broad spectrum of antimicrobial activity with varying degrees of inhibition against the tested microorganisms. These findings demonstrate that incorporation of fluorochromane and pyrazoline pharmacophores within a

single molecular framework is an effective strategy for generating compounds with promising antimicrobial potential. Among the series, compounds 6l (3-NO_2), 6f (3-Br) and 6n (4-Br) showed significant activity against both bacterial and fungal strains. The enhanced activity of phenyl pyrazolines may be attributed to the presence of electron-withdrawing substituents, which increase lipophilicity and facilitate improved penetration through microbial cell membranes. Compounds 6d (4-CH_3), 6i (4-OH,3-OCH_3) and 6j (3-OH,4-OCH_3) exhibited moderate antimicrobial activity, while compounds 6a (H), 6g (3-OH) and 6m (2-OH) showed relatively lower activity. The remaining compounds demonstrated moderate inhibition depending on the type of microorganism. These variations indicate that both the electronic characteristics and positional arrangement of substituents on the phenyl ring play a significant role in regulating antimicrobial efficacy. In general, derivatives containing electron-withdrawing groups exhibited superior activity compared to those bearing electron-donating substituents. Overall, the study highlights that the combination of the fluoro chromane nucleus and pyrazoline moiety provides an effective pharmacophoric framework for antimicrobial activity, and appropriate structural modification can lead to compounds with enhanced biological potential. The above discussion is illustrated in Fig.-5.



A: Antibacterial activity of the synthesized phenyl pyrazoline derivative 6b,6h,6n,6l,6k,6m Against *Escherichia coli* .

B: Antifungal activity of the synthesized phenyl pyrazoline derivative 6l,6k,6n,6a,6c,6g Against *Aspergillus niger*.

Fig.-5: Representative Antimicrobial Screening of Synthesized 6-fluorochromane-Phenyl Pyrazoline Derivatives by Agar Disc Diffusion Method

Structure-Activity Relationship (SAR) Studies

The biological evaluation of the synthesised fluoro-chromane-incorporated phenyl pyrazoline derivatives (6a–6n) revealed a distinct correlation between the nature of aromatic substitution and antimicrobial potency. As shown in Fig.-6, the biological response was governed by the electronic nature and positional orientation of the substituents of 6-fluoro-chromane phenyl pyrazoline derivatives. The fluorine contributes to enhanced lipophilicity and physicochemical properties; the chromane scaffold provides a conformationally rigid framework that supports effective molecular recognition. The carboxamide linkage which may strengthen hydrogen bonding interactions with microbial targets. Together, these structural components establish a favourable molecular framework for antimicrobial activity. Among the synthesised derivatives, compounds 6k, 6m, and 6n exhibited the highest activity and the most promising antimicrobial profile against both bacterial strains as well as fungal species. In particular, derivatives containing multiple substituents on the phenyl ring showed superior activity, indicating that increased substitution enhances lipophilicity and facilitates better penetration across microbial membranes, as shown in Fig.-7.

Conversely, unsubstituted and weakly substituted derivatives displayed only moderate activity. Overall, SAR findings suggest that careful structural modification provides a strategy for developing new antimicrobial agents, and these findings support United Nations Sustainable Development Goal 3 (good health and well-being). The presence of the pyrazoline pharmacophore along with the fluoro chromane carboxamide scaffold plays a crucial role in enhancing the biological efficacy of these compounds, suggesting that further structural optimisation may lead to potent antimicrobial candidates.

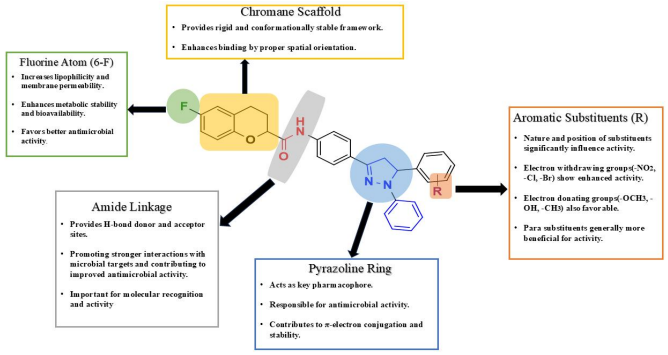


Fig.-6: Structure-activity Relationship (SAR) of 6-Fluorochromane Phenyl Pyrazoline Derivative

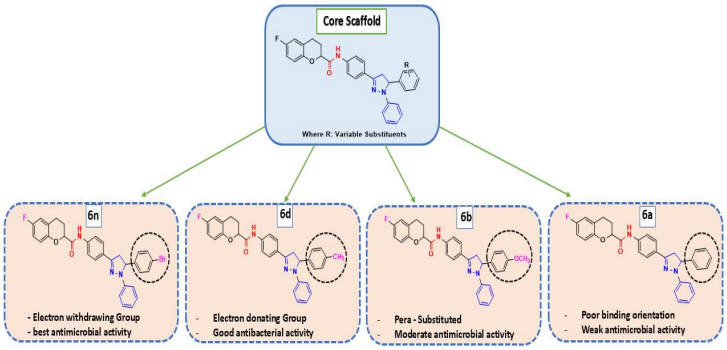


Fig.-7: Effect of Structural Substituents(R) on the Antimicrobial Activity of 6-Fluorochromane-Phenyl Pyrazoline Derivatives

Table-1: Antibacterial Activity of Phenyl Pyrazoline Derivatives

S. No.	Compounds	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus pyrogens*
1	6a	10	8	12	11
2	6b	15	14	14	13
3	6c	17	16	19	18
4	6d	14	12	16	15
5	6e	15	13	17	16
6	6f	11	9	10	12
7	6g	12	10	13	14
8	6h	13	11	15	13
9	6i	14	12	16	15
10	6j	16	14	18	17
11	6k	18	16	20	19
12	6l	15	13	17	16
13	6m	18	15	18	19
14	6n	19	12	20	20
Standard 1	Ampicillin	25	20	26	28
Standard 2	Ciprofloxacin	30	28	28	26
Control	DMSO	-	-	-	-

Note: Zone of inhibition expressed in mm; concentration of test compounds = 100 µg/ml. Ampicillin and ciprofloxacin were used as reference drugs.

Table-2: Antifungal Activity of Phenyl Pyrazoline Derivatives

S. No.	Compounds	*Candida Albicans*	*Aspergillus Niger*
1	6a	10	9
2	6b	12	10
3	6c	15	12

4	6d	13	10
5	6e	14	11
6	6f	10	8
7	6g	12	9
8	6h	13	11
9	6i	12	10
10	6j	14	9
11	6k	16	14
12	6l	12	11
13	6m	17	15
14	6n	16	18
Standard	Nystatin (mm)	25	20
Control	DMSO	-	-

Note: Antifungal activity expressed as zone of inhibition(mm). Nystatin was used as reference drug.

CONCLUSION

The present study successfully established a series of 6-fluorochromane carboxamide-based phenyl pyrazoline derivatives through an efficient synthetic approach and demonstrated their promising antimicrobial potential. The structure–activity relationship investigation revealed that the fluorine substituent, chromane scaffold, carboxamide linkage, pyrazoline pharmacophore, and aromatic substitution pattern collectively influenced the biological performance of the synthesised molecules. These findings provide valuable insight into the rational design of fluorinated heterocyclic compounds and identify the fluoro-chromane–carboxamide–pyrazoline framework as a promising platform for the development of new antimicrobial lead molecules. Future investigations involving molecular docking, toxicity evaluation, pharmacokinetic assessment and in vivo studies are warranted to explore their therapeutic potential. The outcomes of this work support United Nations Sustainable Development Goal 3 (Good Health and Well-Being) by contributing to the discovery of structurally innovative antimicrobial candidates for addressing infectious diseases and antimicrobial resistance.

ACKNOWLEDGMENTS

The authors are grateful to Kenny Pharma Pvt. Ltd. in G.I.D.C. Ankleshwar for providing the research facility for this research work.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-Being.* All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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Cite this article as: V. V. Desai *et al.*, *Rasayan J. Chem.*, 19(4), 2508-2516(2026), https://doi.org/10.31788/RJC.2026.1949982

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