INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

ANTIMICROBIAL STUDIES OF SOME NEW NOVEL PYRAZOLINE DERIVATIVES

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ABSTRACT

10 compounds of 3, 5-diphenyl substituted Δ^2-pyrazoline derivatives were synthesized. The structures of the synthesized compounds were confirmed by FTIR, ^{1}H NMR, and FAB MASS spectral data. Synthesized compounds were screened for their antimicrobial activity. Their antimicrobial activities against *Staphylococcus aureus* (NCIM-2901), *Bacillus subtilis* (MTCC-441), *Escherichia coli* (NCIM-2810), and *Pseudomonas aeruginosa* (NCIM-2036) were investigated. A significant level of activity was observed.

Keywords: Δ^2-pyrazolines, 2-hydroxyacetophenone, chalcones, Antimicrobial activity.

INTRODUCTION

Combat against bacterial infections has resulted in the development of a wide variety of antibiotics. After years of misuse and overuse of antibiotics, bacteria are becoming resistant to antibiotic resulting in a potential global health crisis leading to increase in mortality. Frequently, it is recommended to use new antibacterial agents with enhanced broad-spectrum potency. Therefore, recent efforts have been directed toward exploring novel antibacterial agents[1].

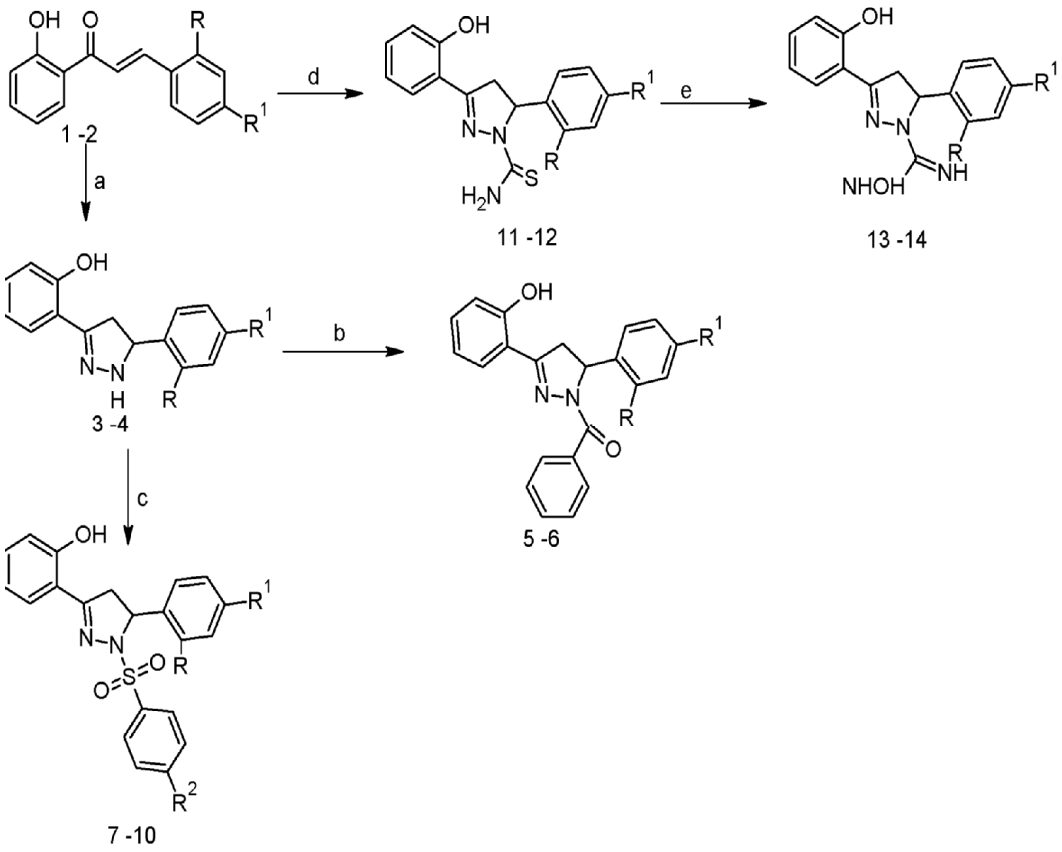
In order to overcome this rapid development of drug resistance, new agents should preferably consist of chemical characteristics that clearly differ from those of existing agents. In drug designing programs an essential component of the search for new leads is the synthesis of molecules, which are novel yet resemble known biologically active molecules by virtue of the presence of critical structural features. Certain small heterocyclic molecules act as highly functionalized scaffolds and are known pharmacophores of a number of biologically active and medicinally useful molecules[2, 3].

Electron-rich nitrogen heterocyclic plays an important role in diverse biological activities. A second nitrogen in the five-membered ring also influences the activity or pharmacokinetic profile of molecule[4, 5]. Δ^2-Pyrazolinederivatives have also been reported in the literature to exhibit various biological activities such as antimicrobial[6-11] analgesic and anti-inflammatory[12-16], antihypertensive[17], and antidepressant etc[18-21].

CHEMISTRY

The compounds were synthesized as reported earlier[22-24]. The synthetic route has been outlined in Scheme 1. Hydroxy chalcones 1 and 2 were prepared through Claisen–Schmidt condensation of 2-hydroxy acetophenone with either 2-hydroxy benzaldehyde or 4-hydroxy benzaldehyde. Compounds 3 and 4 were

synthesized by the reaction of hydrazine hydrate with 1 and 2, respectively in ethanol. Reaction of benzoyl chloride with compounds 3 and 4 in pyridine provided compounds 5 and 6, respectively. Reaction of benzene sulfonyl chloride and p-toluene sulphonyl chloride with compound 3 and 4 in tetrahydrofuran provided compounds 7–10. Reaction of thiosemicarbazide with 1 and 2 in ethanol and 2 M equivalent of sodium hydroxide provided compounds 11 and 12 respectively. Compounds 11 and 12 upon treatment with methyl iodide and then with hydroxylamine in methanol provided compounds 13 and 14, respectively. Structures, physico-chemical and spectral characterization of the synthesized compounds are given in the data.



Scheme 1: Synthesis of compounds 3–14. Reagents and conditions: (a) $NH_2NH_2.H_2O$ (80%) excess, C_2H_5OH, reflux 3–6 h; (b) C_6H_5COCl, pyridine, reflux 3 h; (c) R^2-$C_6H_4SO_2Cl$, THF, stirring, 0.5–1 h; (d) thiosemicarbazide, C_2H_5OH, reflux 8–10 h; (e) CH_3I / NH_2OH, stirring 6–12 h.

Code	R	R^1	R^2
3	-OH	-H	-
4	-H	-OH	-
5	-OH	-H	-
6	-H	-OH	-
7	-OH	-H	-
8	-H	-OH	-
9	-OH	-H	-
10	-H	-OH	$-CH_3$
13	-OH	-H	$-CH_3$
14	-H	-OH	-

EXPERIMENTAL

All the chemicals and solvents were purchased from CDH, New Delhi. Chemicals and solvents were of reagent grade and solvents were purified before use by standard procedures. Melting points were determined by using Thermonik Melting Point Apparatus (Campbell electronics, India) by open capillary method and are uncorrected. The IR Spectra of compounds synthesized were taken on SHIMADZU-FTIR 8400S, from 4000-400 cm^{-1} using KBr discs. The FAB mass spectra of synthesized compounds were recorded on a Jeol SX 102/Da-600 mass spectrometer/Data System using Argon/Xenon as the FAB gas. The accelerating voltage was 10kV and spectra were recorded at room temperature. M-Nitro benzyl alcohol (NBA) was used as the matrix. ^{1}HNMR spectra were recorded at 300MHz in $CDCl_3$ using Bruker Avance 400 instrument. Progress and completion of the reaction were checked by TLC in a solvent vapour saturated chamber on glass plates coated with silica gel-G / Silica Gel GF254, followed by detection using iodine chamber / UV light (254nm) in LAMAG TLC visualizing chamber. Purification of intermediates and final compounds were done by recrystallization using appropriate solvents. Pure intermediates, monitored on TLC, were analyzed using FTIR and final compounds were subjected to ^{1}H NMR and FAB-Mass Spectroscopy.

General procedure for the synthesis of the compounds

Synthesis of chalcones

2-hydroxy acetophenone (0.01M) and suitable substituted aldehydes (0.01M) were dissolved in ethanol (10ml). Then the solution of 60% potassium hydroxide was added to the resulting solution with continuous stirring at 0 ^{0C}. The reaction mixture was allowed to kept at room temperature for about 48 hours with occasional shaking. After 48 hours it was poured into ice-cold water, and then neutralized to pH-2 using 6N hydrochloric acid. The yellow precipitate obtained was filtered, washed, dried and recrystallised from suitable solvent. The intermediate "1" and "2" were obtained.

2-5[-(2-hydroxyphenyl)-4, 5-dihydro-1H pyrazol-3-yl] phenol (Compound-3)

The intermediate "1" (2gm) was treated with hydrazine hydrate (10ml) in ethanol (50ml) and refluxed for 3-6 hours. Then the hot reaction mixture was poured into ice-cold water. The solid separated out was filtered, washed with water, dried and recrystallised from ethanol to afford Compound 3.

2-[5-(4-hydroxyphenyl)-4, 5-dihydro-1H pyrazol-3-yl] phenol (Compound4)

The intermediate "2" (2gm) was treated with hydrazine hydrate (10ml) in ethanol (50ml) and refluxed for 3-6 hours. Then the hot reaction mixture was poured into ice-cold water. The solid separated out was filtered, washed with water, dried and recrystallised from ethanol to afford Compound 4.

2-[1-benzoyl-5-(2-hydroxy phenyl)-4, 5-dihydro-1H-pyrazol-3-yl] phenol (Compound5)

To compound 3 (0.001M) in pyridine (10ml), benzoyl chloride (0.002M) was added. The reaction mixture was heated on water bath for 3hours and poured over crushed ice mixed with dilute hydrochloric acid. The solid separated out was filtered, washed with water, dried and recrystallised from ethanol to afford Compound 5[25].

2-[1-benzoyl-5-(4-hydroxy phenyl)-4, 5-dihydro-1H-pyrazol-3-yl] phenol (Compound6)

To compound 4 (0.001M) in pyridine (10ml), benzoyl chloride (0.002M) was added. The reaction mixture was heated on water bath for 3hours and poured over crushed ice mixed with dilute hydrochloric acid. The solid separated out was filtered, washed with water, dried and recrystallised from ethanol to afford Compound 6.

2-[5-(2-hydroxyphenyl)-1(phenylsulfonyl)-4,5-dihydro-1H-pyrazol-3-yl]phenol (Compound7)

Benzene sulfonyl chloride (0.0025) was dissolved in tetrahydrofuran (2ml) with stirring. The stirred mixture was cooled in an ice bath to 10-15^{0C}; followed by gradual addition of a solution of compound 3 in tetrahydrofuran so that the temperature was maintained between 10-20^{0C}. Stirring was continued for 15 minutes after the addition was complete. The solid separated out was filtered, dried and recrystallised from methanol to afford Compound 7.

2-[5-(4-hydroxy phenyl)-1(phenyl sulfonyl)-4, 5-dihydro-1H-pyrazol-3-yl] phenol (Compound8)

Benzene sulfonyl chloride (0.0025) was dissolved in tetrahydrofuran (2ml) with stirring. The stirred mixture was cooled in an ice bath to 10-15^{0C}; followed by gradual addition of a solution of compound 4 in tetrahydrofuran so that the temperature was maintained between 10-20^{0C}.Stirring was continued for 15 minutes after the addition was complete. The solid separated out was filtered, dried and recrystallised from methanol to afford Compound 8.

2-[5-(2-hydroxy phenyl)-1-tosyl-4, 5-dihydro-1H-pyrazol-3-yl] phenol (Compound9)

P-Toluene sulfonyl chloride (0.0025) was dissolved in tetrahydrofuran (2ml) with stirring. The stirred mixture was cooled in an ice bath to 10-15^{0C}; followed by gradual addition of a solution of compound 3 in tetrahydrofuran so that the temperature was maintained between 10-20^{0C}. Stirring was continued for 15 minutes after the addition was complete. The solid separated out was filtered, dried and recrystallised from methanol to afford Compound 9[26].

2-[5-(4-hydroxy phenyl)-1tosyl-4, 5-dihydro-1H-pyrazol-3-yl] phenol (Compound10)

P-Toluene sulfonyl chloride (0.0025) was dissolved in tetrahydrofuran (2ml) with stirring. The stirred mixture was cooled in an ice bath to 10-15^{0C}; followed by gradual addition of a solution of compound 4 in tetrahydrofuran so that the temperature was maintained between 10-20^{0C}. Stirring was continued for 15 minutes after the addition was complete. The solid separated out was filtered, dried and recrystallised from methanol to afford Compound 10.

(N-hydroxy-3,5-bis(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboximidamide (Compound13)

The intermediate "1" (0.01M) was treated with thiosemicarbazide (0.0125M), sodium hydroxide (0.025M) and ethanol (20ml) and refluxed for 8 hours. The reaction mixture while hot was poured in ice-cold water and acidified with dilute hydrochloric acid. The precipitate (11) obtained was filtered, dried and recrystallised from methanol. The intermediate "11" (500mg) was treated with methyl iodide and hydroxyl amine in methanol and continuously stirred until there is no change in color of lead acetate paper. The precipitate obtained was filtered and washed with cold methanol and dried to afford Compound 13.

(N-hydroxy-3-(2-hydroxy phenyl)-5-(4-hydroxy phenyl)-4, 5-dihydro-1H-pyrazole-1-carboximidamide (Compound14)

The intermediate "2" (0.01M) was treated with thiosemicarbazide (0.0125M), sodium hydroxide (0.025M) and ethanol (20ml) and refluxed for 8 hours. The reaction mixture while hot was poured in ice-cold water and acidified with dilute hydrochloric acid. The precipitate (12) obtained was filtered, dried and recrystallised from methanol. The intermediate "12" (500mg) was treated with methyl iodide and hydroxyl amine in methanol and continuously stirred until there is no change in color of lead acetate paper. The precipitate obtained was filtered and washed with cold methanol and dried to afford Compound 14.

Antimicrobial activity[27]

Antibacterial activities of compounds were tested using cup-plate method in nutrient agar medium against *Staphylococcus aureus* (NCIM-2901), *Bacillus subtilis* (MTCC-441), *Escherichia coli* (NCIM-2810) and *Pseudomonas aeroginosa* (NCIM-2036). Nutrient Broth (single strength) medium was used for revival, culturing and sub culturing of the bacteria. Inoculated a previously liquefied nutrient agar medium (for 100ml) appropriate to the assay with the requisite quantity (0.5ml) of 24hrs suspension of the required micro organism, at a temperature between 40^{0C} and 50^{0C} was used to prepare Petri dishes of 3 to 4 mm depth. Test compounds and standard drug (Ciprofloxacin) were dissolved in Dimethyl Sulfoxide (DMSO) to give a concentration of 2000 µg/mL. Stock solutions were diluted with the sterile DMSO to prepare concentration of 100, 50, 25, 12.5, 6.25 µg/ml of the compounds. Cylinders/cups were prepared by using sterile cork borer of 5mm internal diameter. 0.1ml of the different concentrations of the synthesized compounds was added to the cups uniformly. The Petri plates were incubated for about 24 hrs at the temperature of 37^{0C}. Accurately measured the diameters of the circular inhibition zones and the MIC values were calculated. The observed data on the antibacterial activity of the

compounds and control drugs are given in Table-2.

RESULTS, DISCUSSION AND CONCLUSION

Compounds were synthesized as per scheme-1. FTIR, FAB-MASS and ^{1}H NMR spectra of the compounds confirm the formation of the desired compounds. The percentage zone of inhibition of the test compounds were compared with that of Ciprofloxacin. The test compounds 3, 8 & 9 were found to have moderate activity against *Staphylococcus aureus* (NCIM-2901) and *Escherichia coli* (NCIM-2810).The test compound 3 had moderate activity against *Bacillus subtilis* (MTCC-441), where as Compounds 7 & 9 were found to have moderate activity against *Pseudomonas aeruginosa* (NCIM-2036), when compared to ciprofloxacin. All other compounds showed low activity against all bacterial strains, used in this experiment.In summery, out of 10 Compounds synthesized, 4 Compounds (3, 7, 8 and 9) were found to possess antibacterial activity. Interestingly one of the two N-Unsubstituted and 3 out of 4 Aryl sulphonyl substituted derivatives were found to have antibacterial activity.

ACKNOWLEDGEMENT

We are grateful to the Head, SAIF, CDRI, Lucknow for providing ^{1}H NMR and FAB-MS Spectral data.

Table 1: Structure and physico-chemical characterization of compounds (3-14)

Compound	M. F	M.W	M.P(°C)	Yield (%)
3	$C_{15}H_{16}N_4O_3$	254	120-122	71
4	$C_{15}H_{14}N_2O_3$	254	100-102	71
5	$C_{22}H_{18}N_2O_3$	358	105-107	97
6	$C_{22}H_{18}N_2O_3$	358	185-188	83
7	$C_{21}H_{18}N_2O_4S$	394	140-144	51
8	$C_{21}H_{18}N_2O_4S$	394	190-193	51
9	$C_{22}H_{20}N_2O_4S$	408	78-80	54
10	$C_{22}H_{20}N_2O_4S$	408	200-202	35
13	$C_{16}H_{16}N_4O_3$	312	190-192	30
14	$C_{16}H_{16}N_4O_3$	312	150-152	60

Table 2: Antibacterial Profile of Δ^2-pyrazolines (3-14)

Compound	*Staphylococcus aureus* NCIM-2901		*Bacillus subtilis* MTCC-441		*Escherichia coli* NCIM-2810		*Pseudomonas aeruginosa* NCIM-2036	
	ZOI	MIC	ZOI	MIC	ZOI	MIC	ZOI	MIC
3	10	50	10	100	10	100	_	_
4	_	_	_	_	_	_	_	_
5	_	_	_	_	_	_	_	_
6	_	_	_	_	_	_	_	_
7	_	_	_	_	_	_	10	50
8	9	100	_	_	14	100	_	_
9	9	100	_	_	12	50	12	100
10	_	_	_	_	_	_	_	_
13	_	_	_	_	_	_	_	_
14	_	_	_	_	_	_	_	_
Ciprofloxacin	14	0.79	12	0.2	12	0.79	10	0.2

''-'' Indicates bacteria are resistant to the compounds at concentration 100 μg/ml; MIC values are given in (μg/ml) minimum inhibitory concentration, i.e., lowest concentration to completely inhibit bacterial growth; zone of inhibition is expressed in mm.

Table 3: Spectral data of hydroxyl Δ^2 pyrazolines (3-14)

Compound	FTIR (KBr) ν/cm^{-1}	FAB Mass (m/z)	^{1}H NMR ($CDCl_3$) δ
3	3267(OH), 3049(Ar-H), 1593 (C=N), 1492 (C=C).	254(M^+), 255(M+1, base peak)	10.7 (1H, s, Ar-NH), 9.06 (1H, s, Ar-OH), 6.8-7.3 (8H, m, Ar-H), 3.21 (H_A, dd, J_{AM}=15Hz, J_{AX}=14.4 Hz), 3.55 (H_M, dd, J_{MA}=15.9 Hz, J_{MX}=10.5 Hz), 4.9 (H_X, dd, J_{MX}=11.5 Hz, J_{AX}=12 Hz)
4	3259(OH), 3010(Ar-H), 1598(C=N), 1496(C=C).	254(M^+), 255(M+1, base peak)	11.04 (1H, s, Ar-NH), 6.8-7.2 (8H, m, Ar-H), 3.12 (H_A, dd, J_{AM}=16.2 Hz, J_{AX}=8.1 Hz), 3.53 (H_M, dd, J_{MA}=16.2 Hz, J_{MX}=10.5 Hz), 4.83 (H_X, dd, J_{MX}=9.75 Hz, J_{AX}=8.4 Hz)
5	3200(OH), 3064(Ar-H), 1573(C=N), 1699(C=O), 1498(C=C).	358(M^+), 359(M+1)	8.81 (1H, s, Ar-OH), 6.7-7.6 (13H, m, Ar-H), 6.05-3.4 (d, pyrazoline H)
6	3203(OH), 3063(Ar-H), 1572(C=N), 1626(C=O), 1498(C=C).	358(M^+), 359(M+1)	10 (1H, s, Ar-OH), 6.6-7.8 (13H, m, Ar-H), 5.7-3.3 (d, pyrazoline H)
7	3443(OH), 3064(Ar-H), 1597(C=N),1500(C=C), 1176(O=S=O).	395(M+1),396(M+2)	10 (1H, s, Ar-OH), 6.8-7.3 (13H, m, Ar-H), 3.26 (H_A, dd, J_{AM}=17.25 Hz, J_{AX}=9.3 Hz), 3.62 (H_M, dd, J_{MA}=11.7 Hz, J_{MX}=16.8 Hz), 5.20 (H_X, dd, J_{MX}=9.9 Hz, J_{AX}=9.9 Hz)
8	3535(OH), 3070(Ar-H), 1614(C=N), 1512(C=C), 1176(O=S=O).	395(M+1), 396(M+2)	10.29 (1H, s, Ar-OH), 6.8-7.8 (13H, m, Ar-H), 3.2 (H_A, dd, J_{AM}=17.8 Hz, J_{AX}=7.8 Hz), 3.62 (H_M, dd,J_{MA}=14.5 Hz, J_{MX}=12 Hz), 4.94 (H_X, dd, J_{MX}=9.6 Hz, J_{AX}=9.3 Hz)
9	3205(OH), 3057(Ar-H), 1600(C=N), 1498(C=C), 1172(O=S=O).	408(M^+), 409(M+1, base peak)	10.35 (1H, s, Ar-OH), 6.8-7.3 (13H, m, Ar-H), 2.406 (3H, s, Ar-CH_3) 3.25 (H_A, dd, J_{AM}=17.25 Hz, J_{AX}=9.6 Hz), 3.61 (H_M, dd, J_{MA}=16.9 Hz, J_{MX}=11.4 Hz), 5.15 (H_X, dd, J_{MX}=10.2 Hz, J_{AX}=10.2 Hz)
10	3225(OH), 3074(Ar-H), 1616(C=N), 1516(C=C), 1168(O=S=O).	408(M^+), 409(M+1, base peak)	10.34 (1H, s, Ar-OH), 6.7-7.7 (13H, m, Ar-H), 2.405 (3H, s, Ar-CH_3) 3.18 (H_A, dd, J_{AM}=16.8 Hz, J_{AX}=9 Hz), 3.6 (H_M, dd, J_{MA}=16.95 Hz, J_{MX}=11.7 Hz), 4.89 (H_X, dd, J_{MX}=9.9 Hz, J_{AX}=9.9 Hz)
13	3201(OH), 1660(C=N), 1489(C=C),1350(C-	312(M^+), 313(M+1)	
14	3280(OH), 1641(C=N), 1490(C=C),1346(C-N).	312(M^+), 313(M+1)	

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