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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ANALYSIS OF VARIOUS SUBSTITUTED 2-(5-(3-(5-BROMOTHIOPHEN-2-YL)-1-(4-FLUOROPHENYL)-1*H*-PYRAZOL-4-YL)-4,5-DIHYDRO-1*H*-PYRAZOL-3-YL)PHENOL

Shirsat A. J., Rupnar B. D., Bhagat S. S. and Kakade G. K.[1]
Department of Chemistry, R. B. Attal College, Georai, Dist. Beed (M. S.)
[1]Department of Chemistry, Arts, Commerce & Science College, Kille, Dharur, Dist. Beed (M. S.)
shirsatamol222@gmail.com

ABSTRACT
A series of substituted pyrazolines synthesis, used procedure was a simple, efficient and green. The reaction between acrolein, and α, β- enone in presence of phenylhydrazine yield simple pyrazoline via. Cyclization. The easy work-up of product under mild condition with fast reaction this significant feature of synthesis of pyrazolines. Further the structures of pyrazoline derivatives were elucidated by IR, ^{1}H NMR and mass spectral analysis. The compounds were evaluated for their antibacterial activity using Gram + positive and Gram - negative bacteria.

Keywords: *Pyrazolines, chalcones, synthesis, spectral data, antibacterial activity.*

INTRODUCTION
Many heterocyclic compounds due to their definite activity are employed in the treatment of several infectious diseases. Their use in the treatment is attributed to their intrinsic toxicity to different pathogens. Among a broad range of heterocyclic compounds that have been explored for the development of pharmaceutically significant molecules, pyrazolines constitute an interesting class of heterocycles due to their synthetic versatility and large variety of biological activities like acyl-CoA inhibitory [1], antioxidant[2], anticancer[3], antifungal[4], antibacterial[5], antidepressant[6-8], anticonvulsant[9], antiinflammatory[10], antitumor[11], analgesic[12], neuroprotective [13] properties.

EXPERIMENTAL
For the synthesis of the compounds, all required chemicals were obtained from SD Fine chemicals and Sigma Aldrich. Melting points are uncorrected and were recorded in open capillaries. By using Bruker Avance II 400 MHz NMR Spectrophotometer, solvent is DMSO-d6 and TMS as an internal standard, 1H NMR spectra were recorded. On FT-IR Spectrophotometer Model RZX (Perkin Elmer) on potassium bromide disk, the infra-red spectra were recorded. By using electro-spray method (ES), Mass spectra were recorded on Macromass mass spectrophotometer (Waters). Synthesized compounds purity was checked on TLC plate which is coated by silica gel as stationary phase which is obtained from Merck. In this, mobile phase is solvent mixture of hexane / ethyl acetate (80:20).

GENERAL PROCEDURE
Compound **1c** Chalcone (0.01mol) was dissolved in 20ml ethanol. To this reacting mixture, 0.02 mol of hydrazine hydrate was slowly added. These contents were heated for 4 hr. under mild reflux and then in to the reaction mixture, glacial acetic acid (4-5 drops) was added and heating was continued to 3hr and then cooled up to room temperature. Cold water (60ml) was slowly added to the flask and product was separated. This product was filtered, washed with cold water for many times and recrystallized in ethanol. The compounds **2(a-g)** were prepared by following above general procedure. Physical data of synthesized compounds are recorded in **Table 1**. Confirmed synthesized compounds structures by ^{1}HNMR, Mass and IR spectra.

IR (2c) (cm^{-1}):965(C-Cl), 1070(Ar-Br), 1555(C=C), 1598(C=N), 3121(O-H), 3310(N-H).

^{1}H NMR (2c) (DMSO-d_6)δ ppm: 3.1124-3.1665(dd, 1H, -CH_a-, *J*=12.08 Hz & *J*=9.56Hz),

3.5231-3.5713(dd,1H,-CH_b-, *J*= 12.12Hz & *J*=7.16Hz), 4.7452-4.7901(ddd,1H, -CH_c-, *J*=6.24Hz, *J*=7.12Hz & *J*=5.36Hz), 6.9190-6.9341(d, 1H, -NH-, *J*=6.04Hz), 7.3218-7.4123(m, 2H, Ar-H), 7.4352-7.5424(m, 1H, Ar-H), 7.6389-7.7093(m, 2H, Ar-H), 7.8326(s, 1H, Ar-H), 7.8587-7.8998(m, 1H, Ar-H), 7.9362(s,1H, Ar-H), 7.9842(s, 1H, Ar-H), 8.5863(s,1H, pyrazole-H), 11.3586(s, 1H, Ar–OH).

ES-MS (2c) (m/z):517.2(M+1), 518.2(M+2), 519.2(M+3), 521.2(M+5).

IR (2f) (cm^{-1}): 955(C-Cl), 1065(Ar-Br), 1575(C=C), 1600(C=N), 3231(O-H), 3342(N-H).

^{1}H NMR (2f) (DMSO-d_6)δ ppm: 2.4123(s, 3H, -CH_3), 3.1293-3.1785(dd, 1H, -CH_a-, *J*=10.58 Hz & *J*=9.10Hz), 3.4819-3.5211(dd,1H,-CH_b-, *J*= 8.50Hz & *J*=7.18Hz), 4.7251-4.7552(ddd,1H, -CH_c-, *J*=4.12Hz, *J*=3.92Hz & *J*=4.00Hz), 6.8970-6.9202(d, 1H, -NH-, *J*=9.2Hz), 7.1987-7.2835(m, 2H, Ar-H), 7.4567-7.5364(m, 1H, Ar-H),

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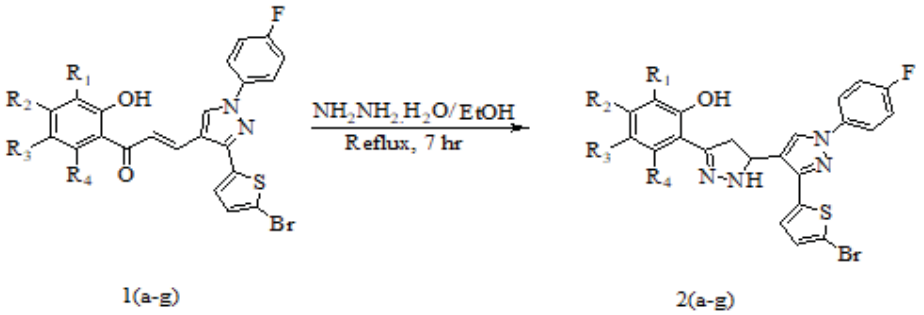
7.5825-7.5998(d, 1H, *J*=6.92 Hz), 7.8254(s, 1H, Ar-H), 7.8325-7.8496(d, 1H, Ar-H, *J*=6.84Hz), 7.8751(s,1H, Ar-H), 7.9053(s, 1H, Ar-H), 8.6587(s,1H, pyrazole-H), 11.8521(s, 1H, Ar–OH).

ES-MS (2f) (m/z): 531.1(M+1), 532.1(M+2), 533.1M+3), 535.1(M+5).



Scheme-1: Synthesis of various 2-(5-(3-(5-bromothiophen-2-yl)-1-(4-fluorophenyl)-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenol

Table-1: Physical data of compounds (2a-g)

Comp.	R_1	R_2	R_3	M.P. (^oC)	Yield (%)
2a	H	H	H	162-164	69
2b	H	H	CH_3	182-184	72
2c	H	H	Cl	190-192	74
2d	Cl	H	Cl	180-182	77
2e	H	H	F	212-214	69
2f	H	CH_3	Cl	168-170	81
2g	H	H	Br	206-208	78

RESULT AND DISCUSSION

The synthetic work was carried out beginning from chalcones with hydrazine hydrate in ethanol by cyclization pyrazolines are formed successfully in moderate to good yields. All newly synthesized compounds were identified on the basis of ^{1}H NMR, melting point range, Mass spectral analysis & IR. Using disc diffusion method, newly synthesized derivatives were evaluated for antimicrobial activity.

Antimicrobial activity: Compounds 2(a-g) were analyzed for their in vitro antimicrobial activity against *Escherichia coli (ATCC 25922)*, *Staphylococcus aureus (ATCC 25923)*, *Pseudomonas aeruginosa (ATCC 27853)* by paper disc diffusion method and reference standard drug is Gentamycin. Antifungal activity was analyzed against Candida sp. using Nystatin as standard drug. At 100 µg/ml concentration, all the tests were evaluated. Muller Hinton agar was the culture media. The region of inhibition was measured in mm after 24 hr of incubation at 37oC. Microbial data for compounds 2(a-g) are summarized below in Table 2.

Table-2: Antimicrobial Analysis Data

Sr. No.	Comp. No.	*Escherichia coli (ATCC 25922)*	*Staphylococcus aureus (ATCC 25923)*	*Pseudomonas aeruginosa (ATCC 27853)*	*Candida sp.*
1	2a	No Zone	No Zone	No Zone	No Zone
2	2b	No Zone	No Zone	No Zone	No Zone
3	2c	No Zone	No Zone	No Zone	No Zone
4	2d	No Zone	No Zone	No Zone	No Zone
5	2e	No Zone	No Zone	No Zone	No Zone
6	2f	No Zone	No Zone	No Zone	No Zone
7	2g	No Zone	No Zone	No Zone	No Zone
8	Gentamycin	28 mm	23 mm	32 mm	--
9	Nystatin	--	--	--	23 mm

CONCLUSION

Starting from Chalcones 1(a-g), different new cyclized derivatives of pyrazoline have been synthesized to good yield and characterized by IR, ^{1}HNMR and Mass spectral data. The newly synthesized derivatives of pyrazolines were evaluated against Candida sp. and Gram positive as well as Gram negative bacterial strains.

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