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Synthesis and Screening of Biological Activities of Some Important Pyrazoline Derivatives

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ABSTRACT: An innovative progression of synthetic pyrazolines derivatives (4a-f) have been synthesized via usual process producing high-quality yield in ethanol through cyclization reaction of chalcones (3a-f), by means of hydrazine hydrate and few drops of glacial acetic acid. Those prepared compounds had been screened for their antimicrobial activities which give an idea about reasonable to good activity against a variety of strains of bacteria and fungi employed. These prepared compounds had been established with IR, ^{1}HNMR and mass spectral statistics.

KEYWORDS: Chalcones, pyrazolines, antibacterial, antifungal agents.

I. INTRODUCTION

Fluorine atom acting a significant role in the field of chemical and biochemical sciences. The literature review exposed that after nitrogen; fluorine occupies the position of the second most hetero element in the field of life science oriented research. More than 10% of recently registered pharmaceutical drugs and close to about 40% of newly registered agrochemicals include one or more atom [1]. Fluorinated heterocycles have been coupled with anti-inflammatory agents and psychopharmaceuticals [2], antimicrobial [3], antilung cancer [4] and act as selective inhibitors of biosynthesis of amineric neurotransmitters [5].

Chalcones are natural compounds found in various plants or they are synthetically synthesized. These substances are of a high attention due to their uses as preliminary compounds in the synthesis of a number of heterocyclic compounds [6-7]. Chalcones are unsaturated compounds that are major intermediates in the synthesis of natural products [8].They are known to have various biological activities resembling fungicidal [9] properties.

A variety of substituted pyrazolines [10] and their derivatives are vital biological agents and significant amounts of research action have been directed in the direction of this class of compounds. Nitrogen containing five membered heterocyclic compounds, natural in addition to synthetic, have been received significant attention due to the broad range of pharmacological activities. Pyrazoline shows one of the energetic classes of compounds associating a broad spectrum of biological activities. Pyrazoline has been reported to acquire anti-diabetic [11], anti-diuretic [12], anti-analgesic [13], anti-helimentic [14], anti-hypolipaemic [15], anti-malarial [16], and anti-depressant [17] activities.

In view of these inspections and in persistence of the research work on bioactive heterocycles [18-19]. It was anticipated to plan and viewing them for antimicrobial activities.

II. MATERIALS AND METHODS

In this investigation chemicals were purchased from local dealer with s.d fine make was used. Chemicals were 99.99% pure, purity has been checked by thin layer chromatography and melting point and mixed melting point. Conventional method has been used for synthesis of ortho-hydroxyacetophenone as a precursor. Stirring and reflux method were used for synthesis of chalcones (3a-f) and pyrazolines (4a-f) respectively.

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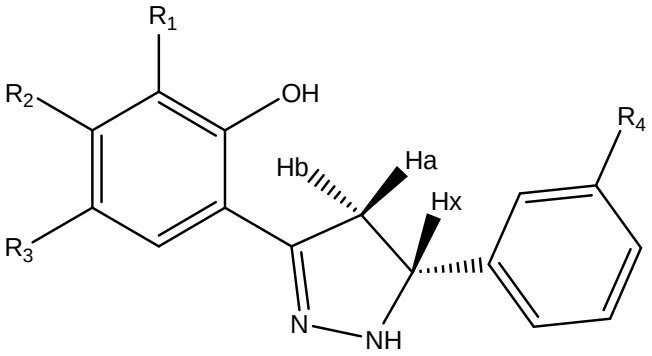
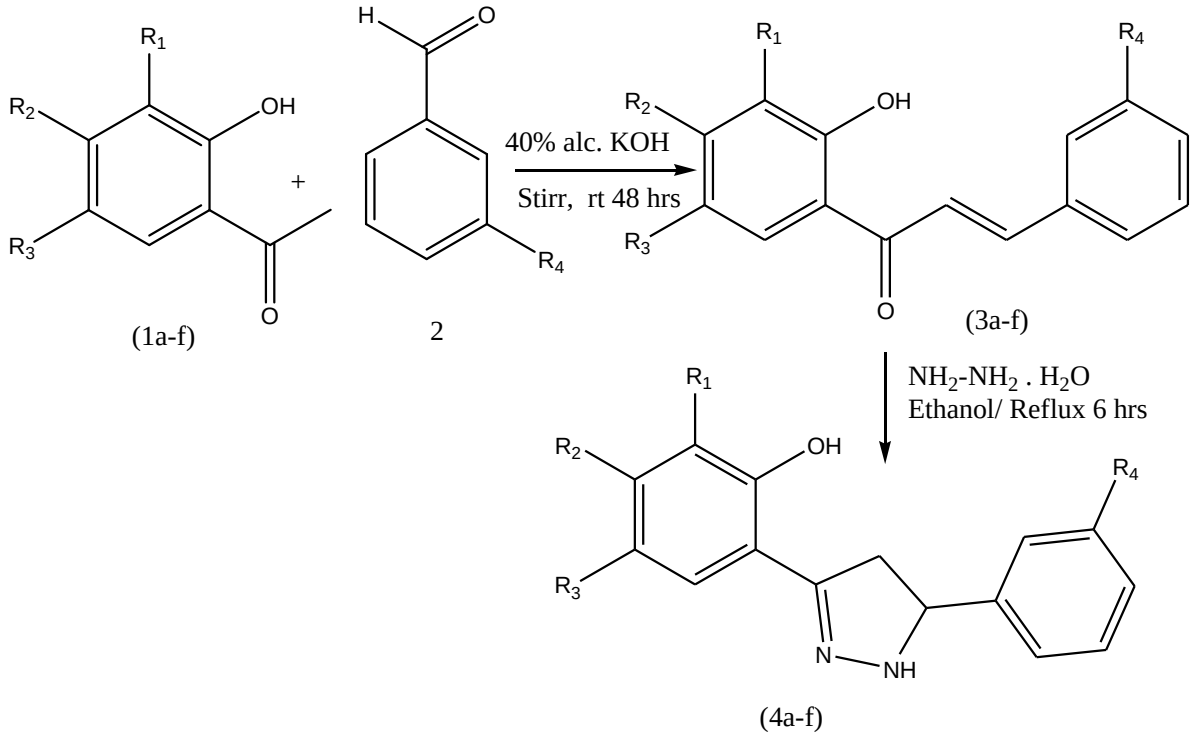
III. RESULTS AND DISCUSSION

Synthesis of 2-(*R*)-4-(3-fluorophenyl)cyclopent-1-ethyl-4-methylphenol is summarized in scheme-I. The starting compound (*E*)-3-(3-fluorophenyl)-1-(2-hydroxy-5-methylphenyl)prop-2-en-1-one was prepared by the claisen-schmidt condensation of a variety of substituted acetophenones with aromatic aldehydes in presence of ethanol/KOH.

The chalcones (3a-f) were characterized by IR, ^{1}HNMR, (LCMS) Mass Spectrometry. The ^{1}HNMR spectra of pyrazolines 4b exhibit characteristic feature signals due to the ABX system. The Ha proton which is eclipsed by Hx proton indicates an apparent doublet of doublet at 3.61 with JHaHx ≈ 5.72Hz and JHaHb ≈ 10.88 Hz. The Hb proton shows a doublet of a doublet with JHbHx ≈ 7.48Hz and 9.12Hz. The Hx proton appeared downfield at δ 5.0 as a doublet of a doublet with JHaHx ≈ 10.40 Hz and JHbHx ≈ 9.60 Hz.



IV. SCHEME-1



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V. EXPERIMENTAL SECTION

All the recorded melting points were determined in open capillaries into liquid paraffin bath and are uncorrected. The development of reaction was monitored with thin layer chromatography by means of silica gel-G(Merck). IR spectra have been recorded on a Perkin Elmer Spectrum Version 10.4.2 FTIR Spectrophotometer.^{1}HNMR spectra have been recorded on Bruker Avance-II 400 MHz NMR spectrophotometer by means of $CDCl_3$ as a solvent and tetra methyl silane as internal standard. Peak values have been indicated in δ (ppm). The Mass spectra were scaned on a Waters, Q-TOF Micromass (LC-MS) mass spectrometer. The antimicrobial activity of the synthesized compounds has been tested by disc diffusion method.

Synthesis of (*E*)-3-(3-fluorophenyl)-1-(2-hydroxy-5-methylphenyl)prop-2-en-1-one (3a)
3-fluorocarbaldehyde **1** (0.02 mol) and substituted 2- hydroxy acetophenone **2** (0.02 mol) were dissolved in 30 ml of alcohol. To this reaction mixture 40% KOH (10 ml) was added. The reaction mixture was stirred at room temperature for 48 hours. The content was poured over crushed ice and neutralized by means of concentrated hydrochloric acid. Yellow products thus obtained was filtered, dried and crystallized from suitable solvent to give pure compound **3a.** The compounds synthesized by above procedure are shown in **TABLE- 1**.
3a IR (cm^{-1}): 3354 (O-H), 1692 (Conj. C=O), 1617, (C=C), 1224 (C-F)
^{1}HNMR (δ): 11.15 (s, O-H), 7.17 to 8.20 (7 H Ar-H), 6.97 (dd, 1H, 16.12Hz), 5. 4 (dd, 1H, 15.2Hz), 2.36 (s, Ar-CH_3).
MS (m/z): 257 (M^{+1})
3b IR (cm^{-1}): 3073 (O-H), 1647 (Conj. C=O), 1580 (C=C), 1219 (C-F), 785(C-Cl)
^{1}H NMR (δ): 13.25 (s, O-H), 7.17 to 7.96 (8H Ar-H and –CH=CH-).
MS (m/z): 312 (M^{+1})
3c IR (cm^{-1}): 3072 (O-H), 1644 (Conj. C=O), 1572, (C=C), 1165 (C-F), 741 (C-Br)
^{1}H NMR (δ): 12.65 (s, O-H), 6.96 to 8.01 (8H Ar-H and –CH=CH-)
MS (m/z): 320 (M^{+1}), 322 (M^{+2})

Synthesis of 2-(*R*)-4-(3-fluorophenyl)cyclopent-1-ethyl-4-methylphenol (4a-f):
Compound 3a (0.001 mol), and Hydrazine hydrate (0.001 mol) were taken in 15 ml ethanol to this reaction mixture 2-3 drops of glacial acetic acid were added. The reaction mixture was heated at reflux for 6 hours. The progress of reaction was monitored by TLC. After completion of reaction the reaction mixture was poured over crushed ice. The products were separated by filtration, washed with water, dried and purified by recrystalization from ethanol to obtain the pyrazoline derivatives 4a. Compounds 4(b-f) were also synthesized in a similar way.
4a IR (cm^{-1}): 3361 (O-H), 3072 (N-H), 1485 (C=N), 1220 (-C-F).
^{1}HNMR (400 MHz, $CDCl_3$, δ): 2.35 (s, Ar-CH_3), 11.55 (s, -OH), 3.14 (dd, Hb, J = 9.12 Hz & 7.48 Hz), 3.61 (dd, Ha, J = 10.88 Hz & 5.72 Hz), 5.00 (dd, Hx, J = 10.4Hz & 9.60 Hz), 7.01-7.39 (m, 7H aromatic), 6.17 (s, 1H, pyrazoline).
MS (m/z): 271 (M^{+1})
4b IR (cm^{-1}): 3347 (O-H), 3072 (N-H), 1452 (C=N), 1263 (-C-F), 715 (C-Cl)
^{1}HNMR (400 MHz, $CDCl_3$, δ): 11.55 (s, -OH), 3.14 δ (dd, Hb, J = 9.12 Hz & 7.48 Hz), 3.61 (dd, Ha, J = 10.88 Hz & 5.72 Hz), 5.00 δ (dd, Hx, J = 10.4Hz & 9.60 Hz), 7.01-7.39 (m, 6H aromatic), 6.17 (s, 1H, pyrazoline).
MS (m/z):325 (M^{+1}), 327 (M^{+2}).
4c IR (cm^{-1}): 3459 (O-H), 3377 (N-H), 1582 (C=N), 1271 (-C-F), 780 (C-Br)
^{1}HNMR (400 MHz, $CDCl_3$, δ): 12.65 (s, -OH), 3.30 δ (dd, Hb, J = 8.96 Hz & 7.56 Hz), 3.60 (dd, Ha, J = 10.76 Hz & 5.76 Hz), 5.50 δ (dd, Hx, J = 9.92Hz & 9.72 Hz), 7.14-8.02 (m, 7H aromatic), 4.90.17 (s, 1H, pyrazoline).
MS m/z) : 335(M^{+1}), 337(M^{+2}).

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VI. **TABLE-1**

Characterization data of synthesized compounds (3a-f) and (4a-f)

Compound	R_1	R_2	R_3	R_4	M.P. (^{0}C)	Yield (%)
3a	H	H	CH_3	F	141	78
3b	Cl	H	Cl	F	182	79
3c	H	H	Br	F	178	62
3d	H	H	Cl	F	184	71
3e	H	CH_3	Cl	F	240	76
3f	H	H	H	F	80	75
4a	H	H	CH_3	F	140	72
4b	Cl	H	Cl	F	138	68
4c	H	H	Br	F	160	76
4d	H	H	Cl	F	162	74
4e	H	CH_3	Cl	F	165	69
4f	H	H	H	F	152	70

VII. ANTIMICROBIAL ACTIVITY

Synthesized compounds had been screened for its in vitro antibacterial activity against gram negative organism E. Coli and gram positive organism pseudomonas aeruginosa and staphylococcus aureus using Gentamycin as a reference standard by paper disc diffusion method. Antifungal activity has been evaluated with Candida sp against Nystatin as a reference standard. The tested compounds have been evaluated at 100 µg/ml concentration. The Muller Hinton agar used as a culture media. The zone of inhibition was measured in mm after 24 hrs of incubation at 37^{0}C. Microbial data of synthesized compounds are summarized in TABLE-2.

Compounds 3f, 4a, 4b, 4c, 4f were found to be excellent antifungal activity for candida sp. (15-16mm).An observation of data showed that 4c and 4d have shown moderate activity for gram positive bacteria Staphylococus aureus ATCC 25923 (11-12mm).

VIII. **TABLE-2**

Antibacterial and Antifungal activities of some representative compounds.

Compounds	*E-Coli* ATCC 25922	*Pseudomones arruginosa* ATCC27853	*Staphylococcus aureus* ATCC 25923	*Candida sp.*
3a	--	--	--	--
3b	--	--	--	--
3c	--	--	--	--
3d	--	--	--	--
3e	--	--	--	--
3f	--	--	--	**13mm**
4a	--	--	--	**13mm**
4b	--	--	--	**14mm**
4c	--	--	**11mm**	**13mm**
4d	--	--	**12mm**	**16mm**
4e	--	--	--	--
4f	--	--	--	**15mm**
Gentamicin	**22mm**	**38mm**	**29mm**	--
Nystatin	--	--	--	**21mm**

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IX. CONCLUSION

The newly synthesized pyrazoline derivatives exhibited moderate to promising antimicrobial activity against standard strains. This class of compounds certainly holds great promise to discover novel classes of antimicrobial agents. All these reactions are very easy to carry out giving high yield. These results make interesting lead molecule for further synthetic and biological evaluation.

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REFERENCES

[1]Cottet, f., Marrul, M., Lefebvre, O. and Schlosser M, " Recommendable routes to trifluoromethyl substituted pyridine and quinoline carboxylic acid" Eur J Org Chem, Vol.2003, issue 8, pp.1559-1568, 2003.
[2]Ghotekar, D. S., Joshi, R. S., Mandhane, P. G., Bhagat, S. S., & Gill, C. H., "Synthesis of some biologically important fluorinated 3-chlorochromones and 1,5-benzothiazepines as antimicrobial and antifungal agents", Ind J of Chem, Vol-49B, pp. 1267-1270, 2010.
[3]Chavhan, N.M., Badadhe, P.V., Joshi R.S., Mandhane, P.G., and Gill, C.H., "Synthesis and antibacterial screening of some fluoroflavones'', Indian J Het Chem, Vol. 19, pp-163-166, 2009.
[4]Bhat, B. A., Dhar, K. L., Puri, S. C., Saxsena, A. K., Shanmugavel, M., and Qazi, G.N.," Synthesis and biological evaluation of chalcones and their derived pyrazoles as potential cytotoxic agents", Bioorg Med Chem Lett, Vol.15, pp. 3177, 2005.
[5]Kollonitsch, J., Patchett, A. A., Marberg, S., Maycock, A. L., Perkins, L. M., Doldouras, G. A., Duggan, D. E., and Aster, A. D., "Selective inhibitors of biosynthesis of aminergic neurotransmitters", Nature, Vol. 274, pp. 906, 1978.
[6]Singh, O. V., & Muthukrishnan, M., "Synthesis of isoflavones containing naturally occurring substitution pattern by oxidative rearrangement of respective flavones using thallium(III) p-tosylate", Ind J of Chem, Vol-44B, pp. 2575-2581, 2005.
[7]Saundane, A. R., & Prabhakar, W., "Synthesis, antimicrobial & antioxidant activities of some indole analogues containing naphyridine and pyrimidonaphthyridine systems", Ind J of Chem, Vol-51B, pp. 1593-1606, 2012.
[8]Singh, J. P., Dulwat, M., Jaitawat, N., Chundawat, S. S., Devpura, A., & Dulwat, S. S., " Microwave enhanced Claisen-Schmidt condensation: A green route to chalcones", Ind J Chem, Vol- 51B, pp. 1623-1627, 2012.
[9]Shelke, S. N., Mhaske, G. R., Bonifacio, V. D. B., Gawande, M. B., " Green Synthesis & anti-infectives of fluorinated pyrazoline derivatives', Bioorg Med Chem Lett, Vol-22, pp. 5727-5730 2012.
[10]Badadhe, P. V., Chavan, N. M., Mandhane, P. G., Joshi, R. S., Nagargoje, D. R., and Gill C. H., "Synthesis and characterization of some novel isoxazolines and pyrazolines as potent antimicrobial agents" Indian J Chem, Vol. 50B, pp. 879, 2011.
[11]Bhaskar, M. & Reddy, M. K., " Synthesis and antibacterial activity of prenyloxy chalcones and prenyloxy aurones", Ind J Het Chem, Vol. 21, pp. 49-52, 2011.
[12]Patil, S. Y., Oswal, R. J., Sayare, A. S., Landge, S. L., & Antre, R. V., "Synthesis, characterization & Antibacterial evaluation of novel 2-pyrazoline derivatives", Der Pharma Chemica Vol- 4(1) pp. 33-38, 2012.
[13]Sawant, A. B., Gill, C. H., and Nirwan, R. S., " Synthesis and biological activities of some flavones", Ind J Het Chem, Vol. 21, pp. 297-300, 2012.
[14]Kotla, V. V., Dalavai, V. K., & Chundari, V. R., " Synthesis & biological activity studies of some novel pyrazoline derivatives", Der Pharma Chemica, Vol. 4(5), pp. 2003-2008, 2012.
[15]Patel, M. R., Dodiya, B. L., Chetiya, R. M., Joshi, K. A., Vekaria, P. B., Bapodara, A. H., & Joshi, H. S., "Synthesis & Antimicrobial evaluation of pyrazoline derivatives", Int J Chem Tech Research, Vol-3(2), pp . 967-974, 2011.
[16]Acharya, B. N., Saraswat, D., Tiwari, M., Shrivastva, A. K., Ghorpade, R., Bapna, S., & Kaushik, M. P., " Synthesis & antimicrobial evaluation of 1,2,5-trisubstituted pyrazolines", Eur J Med Chem, Vol-45, pp. 430-438, 2010.
[17]Chate, A. V., Joshi, R. S., Mandhane, P. G., & Gill, C. H., " Synthesis, characterization & antimicrobial screening of some novel chalcones & their derivatives", Ind J of Chem, Vol-51B, pp. 1642-1648, 2012.
[18]Diwakar, S. D., Bhagat, S. S., Shingare, M. S., and Gill, C. H., " Synthesis and Antimicrobial screening of some novel 2-(5-(4-(1H-Benzo[d][1,2,3]triazol-1-yl)phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenols incorporated by triazole moiety" Bioorg Med Chem Lett, Vol. 18, pp. 4678, 2008.
[19]Bhagat, S. S., Ghotekar, D. S., Badadhe, P. V., Chavan, N. M., Dixit, P. P., and Gill, C. H., "Synthesis and antimicrobial screening of pyrazolines and benzothiazepines incorporated with thiophene", Indian J Het Chem, Vol. 20, pp. 355-358, 2011.