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Environmentally Benign Ultrasound Promoted Synthesis of Some Important Pyrazoline Derivatives as Antibacterial and Antifungal Agents

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ABSTRACT:A new series of pyrazoline derivatives (4a-f) has been synthesized by ultrasonication methods and evaluated for its antibacterial and antifungal activity. The chalcones (3a-f) were transformed into respective pyrazoline derivatives by using hydrazine hydrate and few drops of glacial acetic acid in ethanol. All the synthesized compounds were confirmed by FTIR, ^{1}HNMR and mass spectral data.

KEYWORDS: Chalcones, o-hydroxyacetophenone, pyrazolines, antibacterial, antifungal agents.

I. INTRODUCTION

Ultrasound has increasingly been used in organic synthesis[1,2]. This method is more convenient as it requires shorter time for completion of reaction and higher yields are obtained as compare to conventional methods.

Pyrazolines and their derivatives have been found to possess a broad spectrum of biological activities such as anti-bacterial[3-5], anti-depressant[6], anti-convulsant[7-9], anti-hypertensive[10], anti-oxidant[11], anti-tumor[12] and anti-cancer activities[13,14]. Recently these classes of compounds are reported to possess potential anti-viral activity against flavivirus[15], HIV[16] and anti-malarial activity[17].

Pyrazolines are well known and nitrogen-containing five membered heterocyclic compounds. Several pyrazoline derivatives have been found to possess wide range of biological activities[18]. Benzofuran bearing 1,3,5-trisubstituted pyrazoline exhibited antitubercular, antimicrobial and anti-inflammatory activities[19]. Pyrazoline derivatives[20] have been associated with various bioactivities hence various methods have been worked out for its synthesis. The discovery of the analgesic and antipyretic properties of antipyrine, phenylbutanolinone, the considerable biological activity of fused pyrazoline and the excellent dying properties of Pyrazolyl azo derivatives (eg restazine) have undoubtedly promted interest in development of new derivatives.

Literature survey indicated that the only few pyrazoline are naturally occurring. This is due to the difficulty of living organisms to build an N-N bond pyrazoline itself and many N unsubstituted derivatives are inhibitors and deactivators of liver alcohol dehydrogenage. The use of pyrazoline derivatives in medicine are undoubtedly the principle application. Some of the most important aromatic pyrazolines with biological properties like amebicide,vrichomonacidal, hypoglycaemic, antipsychotic, sedative, hypnotic were reported. Pyrazolines of sulphonamides possess wide range of bacteriostatic and fugicidal actions. For examples bacteriostastic action in vivo for a prolonged time was noted in orisul. Certain alkyl pyrazolines were also found to have the above properties. A sharply pronounced sedative action on the central nervous system was shown by alkyl aryl pyrazolines. Stanazolol is used as an analbolic steroid with no adverse side effects.

Keeping in view of these observations and in continuation of our work on chalcones and pyrazoline derivatives herein we wish to report synthesis of these heterocycles (scheme-I) by using ultrasound method containing benzofuran moiety.

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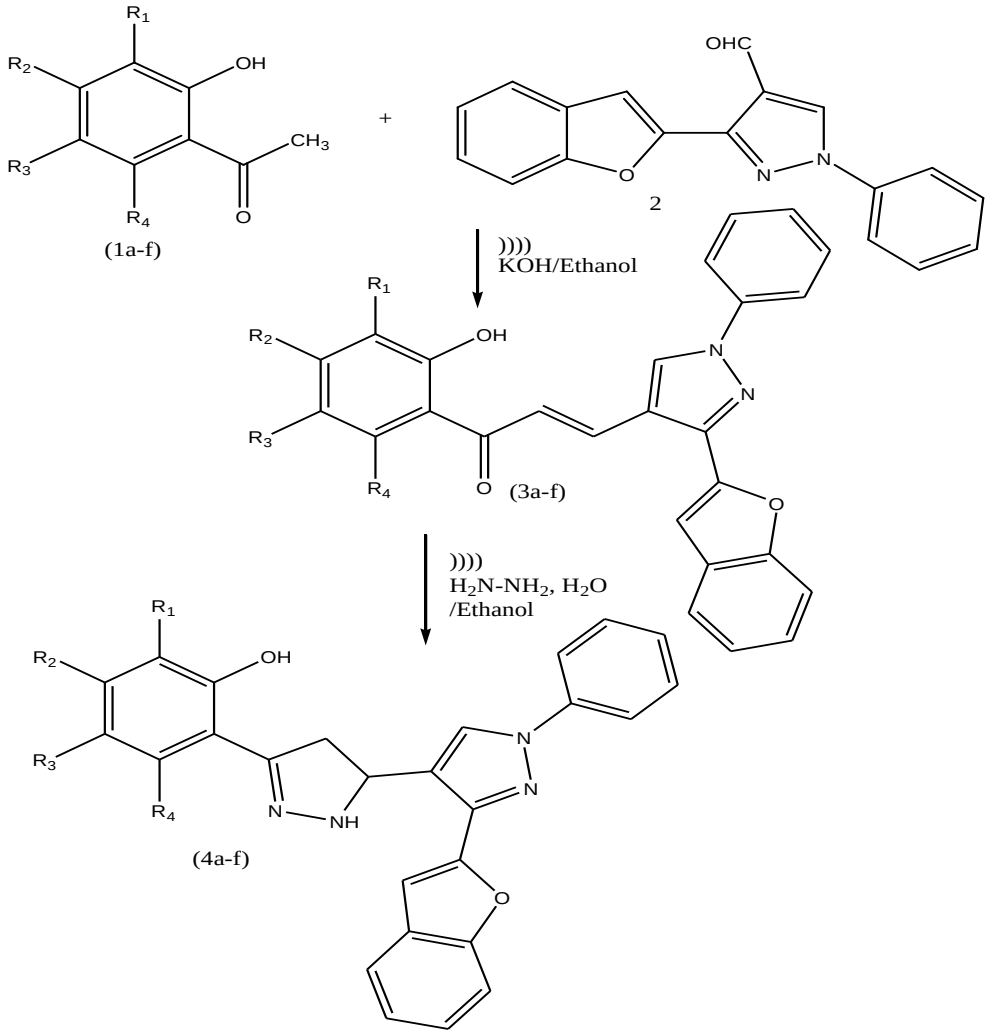
II. MATERIALS AND DISCUSSION

For this invention chemicals with s.d fine and Aldrich make were used from local dealer. 99.99 % pure chemicals, thin layer chromatography and melting point, mixed melting point had been used to check the purity of the chemicals. O- hydroxy acetophenones as a precursor synthesized by conventional method. Chalcones (3a-f) and pyrazolines (4a-f) were synthesized by non conventional method.

III. RESULTS AND DISCUSSION

All experiments under ultrasonication were carried out in bath type ultrasonicator model EN-20U-S manufactured by Enertech Electronica Pvt. Ltd. Mumbai, India having maximum power out put of 100w and 33KHz frequency. All the newly synthesized compounds were screened for its in vitro antibacterial activity against Pseudomonus aerugninasa, Straphylococcus aureus and E. coli using Gentamicin and tetracycline as a reference standard by paper disc diffusion method. Antifungal activity was evaluated against Candida sp. Using Ketoconazole as a standard drug. All the tested compounds were evaluated at 100 ug/ml conc. Some of the compound has shown moderate antimicrobial activities.

IV. SCHEME-I



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V. EXPERIMENTAL SECTION

All the recorded melting points were determined in open capillaries in liquid paraffin bath and are uncorrected. The progress of reaction was monitored with thin layer chromatography using 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 using $CDCl_3$ as a solvent and tetra methyl silane as internal standard. Signal values have been shown in δ (ppm). The Mass spectra have been recorded on a Waters, Q-TOF Micromass (LC-MS) mass spectrometer. The antimicrobial activity of the synthesized compounds have been tested by disc diffusion method.

Synthesis of 3-(3-benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one (3a):

Equimolar amount of pyrazole aldehyde **1** (0.02 mol) and substituted 2- hydroxy acetophenones **2** (0.02 mol) were dissolved in 30 ml of absolute ethanol. To this content 40% potassium hydroxide (10 ml) were added. The content was stirred at room temperature for 36 hours. Then the reaction mixture was poured into crushed ice and neutralized with concentrated hydrochloric acid. Yellow solid thus obtained was filtered, dried and crystallized from suitable solvent to get pure compound **3a.**The compounds synthesized by above procedure are shown in table **1**.

3a: IR (cm^{-1}): 3150 (O-H), 1649 (Conj. C=O), 1430, (C=N), 1120 (C-O).
^{1}H NMR (δ): 12.93 (1H, s, O-H), 7.07 (1H, d, HC=CH) 7.93 (1H, d, HC=CH) 8.39 (1H, s, pyrazole) 9.96 to 8.20 (14H, m, Ar-H).
MS :(m/z): 407 (M^{+1}).
3b: IR (cm^{-1}): 3218 (O-H), 1651(Conj. C=O), 1410 (C=N), 1130 (C-O).
^{1}H NMR (δ): 12.50 (1H, s, O-H), 6.90 (1H, d, HC=CH), 7.02 (1H, d, HC=CH), 7.20 to 8.18 (12H, m, Ar-H), 8.40 (1H, s, pyrazole), 2.36 (3H, s, $-CH_3$).
MS :(m/z): 455.5 (M^{+1}).
3c: IR (cm^{-1}): 3130 (O-H), 1660 (Conj. C=O), 1418 (C=N), 1122 (C-O).
^{1}H NMR (δ): 12.35 (1H, s, O-H), 6.80 (1H, d, HC=CH), 7.00 (1H, d, HC=CH), 7.12 to 8.10 (13H, m, Ar-H), 8.50 (1H, s, pyrazole), 2.33 (3H, s, $-CH_3$).
MS :(m/z): 421(M^{+1}).

Synthesis of 2-(5-(3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-yl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenol:

Compound 3a (0.001 mol), and Hydrazine hydrate (0.001 mol) was 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 8 hours. The progress of reaction was monitored by thin layer chromatography. After completion of reaction the content was poured over crushed ice. The products were separated by filtration, washed with water, dried and purified by recrystalization from ethanol to get the pyrazoline derivative 4a. Compounds 4(b-f) were also synthesized in a similar manner.

4a : IR (cm^{-1}): 3340 (N-H), 3147 (O-H), 1402 (C=N), 1118 (-C-O).
^{1}HNMR (400 MHz, $CDCl_3$, δ): 11.07 1H, s, -OH), 8.11 (1H, s, pyrazole), 5.49 (1H, s, -NH), 3.22 (1H, m, pyrazoline), 3.74 (2H, m, pyrazoline), 6.77 to 7.91 (15H, m, Ar-H).
MS: (M^{+1}) : m/z: 422.
4b : IR (cm^{-1}): 3230 (N-H), 3110 (O-H), 1430 (C=N), 1123 (-C-O).
^{1}HNMR (400 MHz, $CDCl_3$, δ): 11.20 (1H, s, -OH), 8.20 (1H,s, pyrazole), 5.20 (1H, s, NH), 3.25 (1H, m, pyrazoline), 3.65 (2H, m, pyrazoline), 2.32 (3H, s, CH_3), 7.10 to 7.85 (12H, m, Ar-H).
MS: (M^{+1}) 468.5.
4c : IR (cm^{-1}): 3250 (N-H), 3150 (O-H), 1440 (C=N), 1126 (-C-O).
^{1}HNMR (400 MHz, $CDCl_3$, δ): 11.25 (1H, s, -OH), 8.15 (1H, s, pyrazole), 5.35 (1H, s, NH), 3.10 (1H, m, pyrazoline), 3.68 (2H, m, pyrazoline), 2.38 (3H, s, $-CH_3$), 7.22 to 7.95 (13H, m, Ar-H).
MS: (M^{+1}) 435.

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VI. **TABLE-1**

Physical 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	H	H	225	92
3b	H	CH_3	Cl	H	199	80
3c	H	H	CH_3	H	147	73
3d	Cl	H	Cl	H	278	74
3e	H	H	Cl	H	222	72
3f	CH_3	H	CH_3	H	192	76
4a	H	H	H	H	221	72
4b	H	CH_3	Cl	H	173	76
4c	H	H	CH_3	H	172	75
4d	Cl	H	Cl	H	111	67
4e	H	H	Cl	H	225	89
4f	CH_3	H	CH_3	H	110	72

VII. ANTIMICROBIAL ACTIVITY

These synthesized compounds were screened for its in vitro antimicrobial activity against gram positive organism pseudomonas aeruginosa and staphylococcus aureus and gram negative organism E. Coli using Gentamycin and Cefixine as a reference standard by paper disc diffusion method. Antifungal activity was evaluated with Ketaconazole against Candida as a standard. All these tested compounds were evaluated at 100 µg/ml concentration. Muller Hinton agar was used culture media. The zone of inhibition was measured in mm after 24 hr of incubation at 37^{0}C. Microbial data for corresponding compounds are summarized in Table-2.

An examination of data revealed that 3c and 4b have shown moderate activity for gram positive bacteria Staphylococus aureus ATCC 25923 (10-13mm). Compound 4b was found to be more potent with respect to Cefixime. Similarly, these compounds have shown moderate antifungal activity for Candida sp. (13-16 mm).

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	--	--	**10mm**	**13mm**
3d	--	--	--	--
3e	--	--	--	--
3f	--	--	--	--
4a	--	--	**13mm**	**16mm**
4b	--	--	--	--
4c	--	--	--	--

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4d	--	--	--	--
4e	--	--	--	--
4f	--	--	--	--
Gentamicin	**20mm**	**20mm**	**25mm**	--
Cefixime	**28mm**	--	**15mm**	--
Ketoconazole	--	--	--	**23mm**

IX. CONCLUSION

The newly synthesized pyrazoline derivatives are very easy to carry out giving high yield, shorter time, environmentally benign reactions. These results make interesting lead molecule for further synthetic and biological evaluation.

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