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Research Article

ANTIMICROBIAL STUDIES OF SOME SUBSTITUTED PYRAZOLINE DERIVATIVES DERIVED FROM ACETYL HYDRAZINES

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ABSTRACT

Objectives: Several pyrazoline derivatives have been developed as chemotherapeutic agents and have found wide clinical applications such as anticancer (4), antibacterial (4), antifungal (4), and antitubercular (4) agents. Chalcones with an enone system between two aromatic rings exhibit interesting pharmacological activities such as antiinflammatory, antileishmanial, antibacterial, antifungal, antitumor, antimalarial, and antitubercular activity. To synthesize the series of pyrazolines from chalcones and to evaluate the antimicrobial activities of the synthesized compounds.

Materials and Methods: Chalcones were synthesized from various substituted aldehydes condensing with various substituted acetophenones and cyclized into pyrazolines using aryloxy acetyl hydrazines. Antimicrobial and antitubercular activity studies were carried out.

Results: Antimicrobial studies for the synthesized pyrazolines revealed that some compounds have showed promising activity.

Conclusions: The above results proved that pyrazolines are found to be interesting lead molecules for further synthesis as antimicrobial and antitubercular agents.

Keywords: Chalcones, Pyrazolines, Antimicrobial activity, Antitubercular activity.

INTRODUCTION

Nitrogen heterocyclic compounds like pyrazolines have received considerable attention in the recent years due to their diverse pharmacological and biological activities such as antitubercular [1], antidepressant [2], anticonvulsant [2], antitumor [3], antiinflammatory [4], analgesic [4], antibacterial [5], antifungal [5], and anticancer [6] activities.

The intermediate used are substituted chalcones derived from various aldehydes and ketones which are known for their antimalarial [7], anticancer [8], antioxidant [8], analgesic [9], and antiinflammatory [9] activities. Based on the above observations, it was contemplated to synthesize a novel series of 1,3,5-trisubstituted pyrazolines derivatives (1-9) derived from aryloxy acetyl hydrazines. The new compounds have been screened for their *in vitro* antibacterial, antifungal, and antitubercular activities.

MATERIALS AND METHODS

Melting points were determined by capillary method and were uncorrected. The infrared (IR) spectra arerecorded by using Shimadzu Perkin Ekmer 8201 Pc IR spectrometer using a thin film on potassium bromide pellets techniques and frequencies are expressed in cm^{-1}. The PMR spectra were recorded on Bruker Avance II 400 nuclear magnetic resonance (NMR) spectrometer. All spectra were obtained in $CDCl_3$ and DMSO. Chemical shift values are reported as values in ppm relative to tetramethylsilane (δ=0) as an internal standard. The fast atom bombardment (FAB) mass spectra were recorded on JEOL SX-102/DA-6000 Mass spectrometer using Argon/Xenon (6 Kv, 10 Ma) as the FAB gas. The elemental analyses have been obtained using Vairo Elementar Model, CHN analyzer and the results were found to be within ±0.4%. The homogeneity of the compound was checked on silica gel-coated plates by using acetone:benzene (1:1) as a solvent and observed in UV light.

General methods of synthesis of aryloxy acetyl hydrazine

A mixture of ethyl aryloxy acetate (0.5 ml) and hydrazine hydrate (99%, 0.075 mol) in ethanol was refluxed for 6 hr. The product obtained on cooling was re-crystallized from ethanol.

Synthesis of substituted 1,3-diphenyl propen-1-one from substituted aromatic aldehydes and substituted aromatic ketones [10]

A mixture of substituted aromatic aldehyde (0.01 mol) and substituted aromatic ketone (0.01 mol) in absolute ethanol (20 ml) was stirred together for 24 hr in the presence of 20% NaOH (3-4 ml). The reaction mixture was poured into crushed ice and acidified with HCl. The separated solid was filtered, washed with water, and recrystallized from ethanol.

Synthesis of substituted 3,5 substituted phenyl 2-chlorophenoxy acetyl, 4,5-dihydro-1h-pyrazol-5yl)phenol(1-9) [11]

A solution of chalcone (0.01 mol) and aryloxy acetyl hydrazine (0.02 mol) in 20 ml glacial acetic acid was refluxed for 10 hr, product was cooled and filtered and recrystallized from ethanol.

Spectral data

Compound 2: 4-{(1-(2-chlorophenoxy)acetyl)-3-3-nitrophenyl)4,5-dihydro-1H-pyrazol-5yl)} phenol.

IR (cm^{-1}): 3386 (O-H str), 1651 (C=O str), 3092 (N-N Str), 1598 (C=N str), 745 (C-Cl), 1524 and 1347 (Ar N=O str of NO_2), 1560 (Ar C=C str), 2980 (aliphatic C-H str), 3085 (Ar C-H str).

^{1}H NMR (δ ppm): 9.6 (s, 1H, OH), 6.8-7.2 (m, 12H, Ar-H), 4.72 (s, 2H, $-CH_2$), 3.7 (dd, Ha, 1H, pyrazoline), 3.2 (dd, Hb, 1H, pyrazoline), 5.5 (dd, Hc, 1H, pyrazoline).

Mass (m/z) (M^+) 451, (M^+ 1) 452.

Anal. Calcd for C, 61.19; H, 3.99, Found: C, 61.16; H, 3.98.

Compound 4: 2-{(3-(4-chlorophenyl)acetyl)-1-(2-nitrophenoxy) acetyl)-4,5-dihydro-1H-pyrazol-5yl)} phenol.

IR (cm^{-1}): 3137 (O-H str), 1645 (C=O str), 3058 (N-N Str), 1498 (C=N str), 764 (C-Cl), 1420 and 1340 (Ar N=O str of NO_2), 1555 (Ar C=C str), 2972 (aliphatic C-H str), 3076 (Ar C-H str).

^{1}H NMR (δ ppm): 9.5 (s, 1H, OH), 6.8-7.4 (m, 12H, Ar-H), 4.7 (s, 2H, -CH_2), 3.6dd, Ha, 1H, pyrazoline), 3.1 (dd, Hb, 1H, pyrazoline), 5.5 (dd, Hc, 1H, pyrazoline).

Mass (m/z) (M^+) 451, (M^+ 1) 452.

Anal. Calcd for C, 61.19; H, 3.99, Found: C, 61.18; H, 4.01.

Compound 6: 2-{(1-(3-aminophenoxy)acetyl)-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5yl)} phenol.

IR (cm^{-1}): 3676 (O-H str), 1650 (C=O str), 3131 (N-N Str), 1492 (C=N str), 764 (C-Cl), 3058 (NH str).

^{1}H NMR (δ ppm): 10.16 (s, 1H, OH), 7.58-6.80 (m, 12H, Ar-H), 5.50 (s, 2H, -CH_2), 3.94 (dd, Ha,1H, pyrazoline), 3.4 (dd, Hb,1H, pyrazoline), 5.1 (dd, Hc,1H, pyrazoline).

Mass (m/z) (M^+) 421, (M^+ 1) 422.

Anal. Calcd for C, 66.44; H, 6.86, Found: C, 63.18; H, 6.80 (Table 1).

Antimicrobial activity [12,13]

The antimicrobial activity of synthesized compounds was determined by cup plate method.

The *in vitro* antimicrobial activity was carried out against 24 hr old cultures of three bacteria and two fungi. The bacteria used were *Bacillus subtilis, Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* and fungi used were *Candida albicans and Aspergillus niger*. The zone of inhibition was determined for all the tested compounds. The fungicidal and antibacterial activity is proportional to the diameter (mm) of the zone of inhibition. The plates were incubated at 37°C for 24 hr and 25°C for 48 hr in the case of antibacterial and antifungal activity studies. Most of the tested compounds showed significant antibacterial activity against the standard drug ampicillin, and majority of compound showed good antifungal activity against the standard drug griseofulvin (Table 2).

Antitubercular activity using Microplate Alamar Blue Assay (MABA) [14]

The antimycobacterial activity of compounds was assessed against *Mycobacterium tuberculosis* using MABA. The 96-well plates received 100 µl of the middlebrook 7 H 9 broth containing *M. tuberculosis* and serial dilution of compounds were made directly on a plate. The final drug concentrations tested were 0.2-100.0 µg/ml and standards used are INH. Plates were covered and sealed with parafilm and incubated at 37°C for 7 days. After this time, 25 µl of freshly prepared 1:1 mixture of alamar blue reagent and 10% tween 80 was added to the plate and incubated for 24 hr. The blue color in the well was interpreted as of no bacterial growth, and pink color was scored as growth. The minimum inhibitory concentrations (MIC) was defined as lowest drug concentration which prevented the color change from blue to pink. The MIC data are given in Table 3. Compound 1, 2, 3, 4, and 8 have shown significant antitubercular activity with MIC ranging from 6.25 to 25 µg.

RESULTS AND DISCUSSION

Various 1, 3, 5 substituted pyrazoline derivatives were synthesized by the reactions of chalcones with aryloxy acetyl hydrazines in the presence of glacial acetic acid afforded title compounds with good yield (70-80%). Synthesized compounds were screened for antibacterial, antifungal, and antitubercular activity studies revealed

Table 1: Physical data of synthesized 1, 3, 5 substituted pyrazoline derivatives

Compound	Physical state	Molecular formula	Molecular weight	M.P (°C)	% yield
1	White crystals	$C_{23}H_{18}O_3N_2Cl_2$	442	239-241	70
2	Yellow crystals	$C_{23}H_{18}O_5N_3Cl$	451	198-200	73
3	Yellow amorphous	$C_{23}H_{18}O_3N_2Cl_2$	442	188-190	70
4	Brown crystals	$C_{23}H_{18}O_5N_3Cl$	451	138-140	71
5	White crystals	$C_{24}H_{21}O_6N_3$	447	157-160	72
6	Violet crystals	$C_{23}H_{20}O_3N_3Cl$	421	117-119	71
7	Brown crystals	$C_{23}H_{18}O_5N_3Cl$	451	110-111	78
8	White crystals	$C_{23}H_{18}O_3N_2Cl$	406	170-172	71
9	White crystals	$C_{23}H_{18}O_5N_3$	417	127-129	73

Table 2: Antimicrobial activity of the synthesized compounds

Compound no	Diameter of the inhibition zone (mm)					
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. niger*	*C. albicans*
1	16	17	18	13	13	12
2	16	17	18	12	14	11
3	15	16	17	13	13	12
4	15	16	17	13	13	11
5	14	15	16	12	14	12
6	16	16	15	12	12	11
7	15	15	16	11	13	10
8	14	13	14	10	12	10
9	12	14	14	09	09	09
Ampicillin	18	20	21	15	-	-
Griseofulvin	-	-	-		16	13

B. subtilis: Bacillus subtilis, S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, A. niger: Aspergillus niger, C. albicans: Candida albicans

Table 3: MIC results of substituted compounds for anti-tubercular activity

Compound	MIC in µg/ml
1	6.25
2	25
3	25
4	25
5	50
6	50
7	50
8	25
9	50
INH	3.125

MIC: Minimum inhibitory concentrations

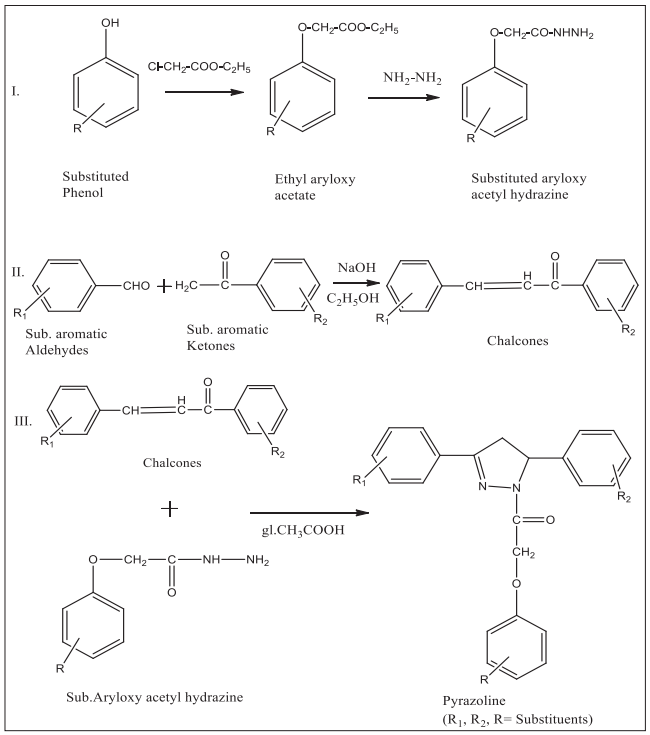

Fig. 1: Reaction scheme

that compounds showed significant activity against ampicillin and compounds showed moderate to significant antifungal activity against griseofulvin. Antibacterial activity studies showed that compounds exhibited good antitubercular activities against INH as standard drug.

CONCLUSIONS

The synthesis of 1, 3, 5 substituted pyrazolines by the above method resulted in very good yields and confirmed by IR, NMR, MASS, and elemental spectral analysis. Biological activity studies showed that compounds exhibited significant antibacterial, antifungal, and moderate antitubercular activities.

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