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Research Article

PYRAZOLINES AS POTENT ANTITUBERCULAR AND CYTOTOXIC AGENTS

ISHWAR BHAT K, ABHISHEK KUMAR*

Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences, Nitte University, Paneer, Deralakatte, Mangalore, Karnataka, India. Email: abhi12bunty@gmail.com

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ABSTRACT

Objective: Pyrazolines are known to exhibit different biological and pharmacological properties such as anticancer, antibacterial, antifungal and antitubercular activities. Chalcones with an enone group between two aromatic rings exhibit remarkable pharmacological activities such as anti-inflammatory, antibacterial, antitumor, antifungal, and antimalarial activity. A series of pyrazolines from chalcones have been synthesized and evaluated for antitubercular and cytotoxic activity studies.

Methods: Chalcones [3-substituted phenyl-1-(p-tolyl)prop-2-en-1-one] were synthesized from various substituted aldehydes and 4-methyl acetophenone and cyclized into pyrazolines [5-substituted phenyl-3-(p-tolyl)-4,5-dihydro-1H-pyrazole] using hydrazine hydrate. Antitubercular and cytotoxic activity studies were carried out.

Results: Antitubercular and cytotoxic activity studies of synthesized pyrazoline revealed that some compounds have showed promising activity.

Conclusion: The observed results proved that pyrazolines are found to be interesting lead molecules for further synthesis as antitubercular and cytotoxic agents.

Keywords: Chalcones, Pyrazoline, Antitubercular activity, Cytotoxic activity.

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INTRODUCTION

Nitrogen-containing heterocyclic compounds such as pyrazolines have received remarkable attention in the recent years due to their diverse pharmacological and biological activities such as antitubercular [1], antidepressant [2], anticonvulsant [3], antitumor [4], anti-inflammatory [5], analgesic [6], antibacterial [7], and anticancer [8]. The intermediate used are substituted chalcones derived from various substituted aldehydes and ketones which are known for their anticancer [9], antioxidant [10], analgesic [11], anti-inflammatory [12], and antimalarial [13] activities. Based on the observations, it was contemplated to synthesize a novel series pyrazoline derivatives derived from substituted chalcones. All the synthesized compounds have been screened for their *in vitro* antitubercular and cytotoxic activity studies.

METHODS

All the chemicals used such as 4-methyl acetophenone, substituted benzaldehydes, hydrazine hydrate, sodium hydroxide, ethanol, and glacial acetic acid used were of analytical grade. Melting points were determined by the capillary method and were uncorrected. The infrared (IR) spectra were recorded by using Shimadzu Perkin Ekuner-8201 Pc IR spectrometer using a thin film on potassium bromide pellets techniques, and absorption frequencies are expressed in cm^{-1}. The ^1H Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance 11400 NMR spectrometer using deuterochloroform and DMSO as solvent. Chemical shift values were reported as values in ppm relative to tetramethylsilane (δ=0) as an internal standard. Mass spectra were recorded on JEOL SX-102/DA-6000 mass spectrometer using Argon/Xenon (6 kV, 10 Ma) as the FAB gas. The purity of the compounds was checked on silica gel coated plates by using ethyl acetate: chloroform (1:9) as a solvent and observed in ultraviolet light.

General procedure

Synthesis of 3-substituted phenyl-1-(p-tolyl)prop-2-en-1-one [14]

A mixture of 4-methyl acetophenone (0.01 mol) and substituted benzaldehydes (0.01 mol) in ethanol (20 ml) was stirred for 24 hrs in the presence of 20% NaOH (4-5 ml). The mixture was poured into crushed ice and acidified with 5% HCl. The product obtained was filtered, washed with water, and recrystallized from ethanol.

Synthesis of 5-substituted phenyl-3-(p-tolyl)-4,5-dihydro-1H-pyrazole [15]

A mixture of substituted chalcones (0.01 mol) in 20 ml of glacial acetic acid and hydrazine hydrate (0.01 mol) were added and refluxed for 16-20 hrs. After the completion of the reaction, the reaction mixture was poured into 250 ml of ice cold water. The solid separated was filtered, washed with cold water, dried, and recrystallized by using ethanol/chloroform. The purity of the compound was checked by using ethyl acetate: chloroform (1:9) as a solvent for TLC.

Spectral data

5-(4-chlorophenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazole (PZ_1)

IR (KBr) cm^{-1}: 1641(C=N str), 2921(C-H aliphatic), 3018(C-H aromatic), 730(C-Cl str), 1550(C=C str), 1324(C-N str), 3258(N-H str); ^1H NMR (δ ppm): 2.3 (s, 3H of CH_3), 7.2-7.5 (m, 9H, Ar-H), 3.3-3.6 (dd, IH of H_a), 3.6-3.9 (dd, IH of H_b), 5.3-5.8 (dd, IH of H_c), 7.7 (s, 1H of NH); MS: m/z 271 (M+1).

3,5-di-p-tolyl-4,5-dihydro-1H-pyrazole (PZ_2)

IR (KBr) cm^{-1}: 1639(C=N str), 2918(C-H aliphatic), 3015(C-H aromatic), 1545 (C=C str), 1318(C-N str); ^1H NMR (δ ppm): 2.2 (s, 3H of CH_3), 7.1-7.5 (m, 9H, Ar-H), 3.4-3.7(dd, IH of H_a), 3.7-4.0 (dd, IH of H_b), 5.4-5.8 (dd, IH of H_c), 7.6 (s, 1H of NH); MS: m/z 250 (M^+).

5-(4-nitrophenyl)- 3-(p-tolyl)- 4,5-dihydro-1H-pyrazole (PZ_3)

IR (KBr) cm^{-1}: 1648(C=N str), 2914(C-H aliphatic), 3024(C-H aromatic str), 1540(C=C str), 1314(C-N str), 1428(Ar-NO_2 str); ^1H NMR (δ ppm): 2.1 (s, 3H of CH_3), 7.2-7.6 (m, 9H, Ar-H), 3.5-3.7 (dd, IH of H_a),

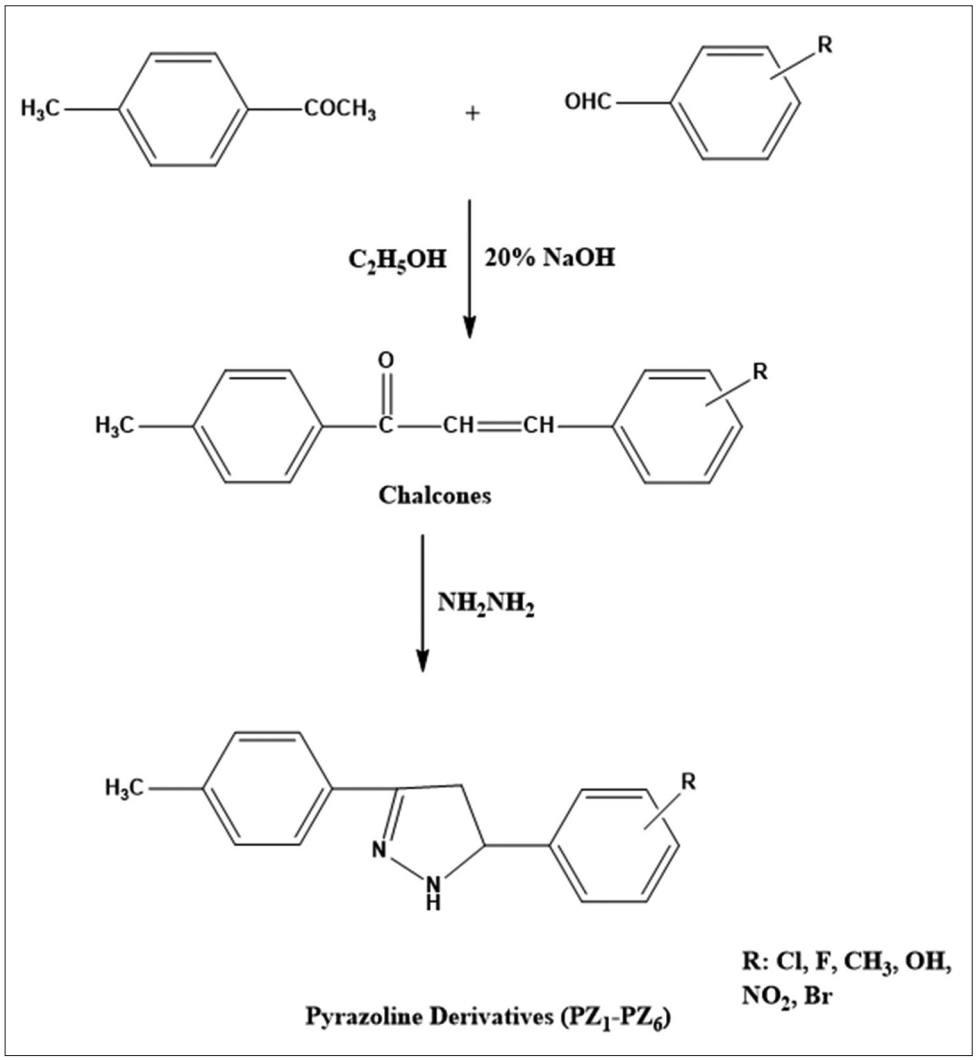

Fig. 1: Scheme for pyrazoline derivatives

Table 1: Physical data of the synthesized compounds

Compound code	R	Molecule weight	M.P°C	Physical state	% yield
PZ_1	4-Cl	270	136-138	White crystals	72
PZ_2	4-CH_3	250	92-94	White crystals	71
PZ_3	4-NO_2	281	168-170	Yellow crystals	69
PZ_4	4-OH	252	116-118	Brown crystals	65
PZ_5	4-Br	301	176-178	Orange crystals	74
PZ_6	4-F	254	152-154	White crystals	73

3.8-4.1 (dd, IH of H_b), 5.4-5.9 (dd, IH of H_c), 7.8 (s, 1H of NH); MS: m/z 281 (M^+).

Antitubercular activity using microplate alamar blue assay (MABA) [16]

The antimycobacterial activity of synthesized compounds was assessed against *Mycobacterium tuberculosis* using MABA. The 96 well plate received 10 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on the plate. The final drug concentrations of the tested compounds were 0.2-100 µl/ml and standards used are INH. Plates were covered and sealed with parafilm and incubated at 37°C for 7 days. After this, 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 hrs. The presence of blue color in the well indicated no bacterial growth and appearance of pink color indicated the growth. The minimum inhibitory concentration (MIC) was defined as lowest drug concentration which prevented the color change from blue to pink. The MIC data are given in Table 2. Compounds PZ_1, PZ_3, PZ_4, PZ_5, and PZ_6 have shown significant antitubercular activity with MIC ranging from 7.5 to 18 µg/ml.

Cytotoxic activity

All the test compounds were screened for cytotoxic activity against Ehrlich Ascites Carcinoma (EAC) cells. The tumor cells aspirated from the peritoneal cavity of tumor-bearing mice was washed thrice with normal saline and checked for viability using Trypan blue exclusion method [17]. The cell suspension (1 million cells in 0.1 ml) was added to tubes containing various concentrations of the test compounds and volume was made up to 1 ml using phosphate buffered saline. Control tubes contained only cell suspension. The assay mixtures were incubated for 3 hrs, at 37°C, and then, percent of dead cells were evaluated by trypan blue exclusion method. Compounds PZ_1, PZ_3, and PZ_5 induced

Table 2: Antitubercular activity of compounds (PZ_1-PZ_6) by microplate alamar blue assay

Compounds	MIC in µg/ml
PZ_1	7.5
PZ_2	40
PZ_3	10
PZ_4	18
PZ_5	17
PZ_6	15
INH	3.125

MIC: Minimum inhibitory concentration

Table 3: Cytotoxic activity of compounds (PZ_1-PZ_6) by Trypan blue exclusion method

Compounds	Number of dead cells (%) at different concentrations (µg/ml)				
	10	20	50	100	200
Control	-	-	-	-	-
PZ_1	11	21	36	53	65
PZ_2	07	16	20	35	35
PZ_3	10	23	37	54	70
PZ_4	05	19	29	37	42
PZ_5	05	20	30	31	50
PZ_6	12	32	46	56	75
5-fluorouracil	20	35	50	85	95

the greatest effect on EAC cells with an activity more than 60% at a concentration of 200 µg/ml. The results are summarized in Table 3.

RESULTS AND DISCUSSION

Antitubercular activity

The test compounds were evaluated for their antitubercular activity against *M. tuberculosis* using MABA. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. This indicates that the test compound has potent antitubercular activity under *in vitro* condition. Compounds PZ_1, PZ_3, PZ_4, PZ_5, and PZ_6 have shown significant antitubercular activity with MIC ranging from 7.5 to 18 µg/ml compared to the standard drug isoniazid. The presence of pyrazoline moiety with substitution and groups such as chloro, nitro, hydroxy, bromo, and fluoro resulted in significant antitubercular activity.

Cytotoxic activity

The test compounds were screened for their cytotoxic activity against EAC cells using trypan blue exclusion method. Compounds PZ_1, PZ_3, and PZ_5 induced significant effect on EAC cells with an activity more than 60% at a concentration of 200 µg/ml. The presence of pyrazoline moiety with electron withdrawing groups such as chloro, nitro, and bromo has accounted for their remarkable cytotoxic activity.

CONCLUSION

The study reports the successful synthesis of pyrazoline derivatives with moderate yields, and most of the synthesized compounds showed potent antitubercular and cytotoxic activities.

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