Synthesis and Antitubercular Activity Studies of Substituted 2-Pyrazolines

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Certain substituted 2-pyrazolines were synthesized by reaction between isoniazid and chalcones of type $A_{(1-9)}$. All compounds were tested for antitubercular activity against *M. tuberculosis* ($H_{37}Rv$) strain by using alamar blue assay. Of all the pyrazoline compounds tested, compounds P_1, P_4, P_5, P_7 and P_9 showed good antimycobacterial activity comparable to standard drug Isoniazid.

Tuberculosis continues to be a major concern, all over the world, despite all the advances in medical sciences, as tuberculosis kills more number of people than AIDS, Malaria and other infectious diseases combined together. The present scenario is further worsened by the advent of MDR–TB which arises from resistance to isoniazid and rifampicin the two most powerful antituberculosis drugs. So MDR–TB[1-4] needs to have newer antitubercular agents with high efficacy and safety profile and therefore one of the approaches to go for new synthetic analogs is by targeted structure modification of presently available antitubercular drugs. Literature survey shows that pyrazolines exhibit antitubercular activity[5]. Isoniazid is a well established drug used in the treatment of tuberculosis. So it was thought whether the pyrazolines of isoniazid could be explored for its antitubercular activity.

With the aim mentioned above, different substituted 2-pyrazolines of isonicotinic acid hydrazide were prepared $P_{(1-9)}$. All compounds were tested against *M. tuberculosis* H_{37} *Rv* strain. Isoniazid (INH) was used as standard drug. Primary screening was conducted at 0.5, 1.0 and 2 μg/ml against *M. tuberculosis* ($H_{37}Rv$) strain by alamar blue assay method.

Melting points were determined in open capillary tubes and uncorrected. Purity of the compounds were checked by precoated TLC plates. IR spectra were recorded using KBr pellets on a Jasco IR spectrometer and ^{1}H NMR spectra were recorded on a Bruker AC 300 MHz FTNMR using TMS as internal standard and chemical shifts were expressed in δ ppm. Elemental analysis was carried out using a Perkin Elmer –2400 CHN analyzer. The compounds $A_{(1-9)}$ were prepared according to the standard procedure[6].

A mixture of chalcone (A) (0.01mol) in ethanol (25 ml), isoniazid (0.01 mol) and piperidine (1 ml) was refluxed in a boiling water bath for 3 h.(Fig. 1) The resultant solution was concentrated, cooled and poured into ice cold water and recrystallised from ethanol[7]. Nine such compounds $P_{(1-9)}$ were synthesized and characterized (Table 1).

Following the above procedure, 1-(pyridine-4-carbonyl)-3-(4'-methoxyphenyl)-5-(4"- chlorophenyl) pyrazoline (P_5) was obtained as a crystalline product. IR (KBr) spectrum of compound P_5 exhibited bands at 1602.56 (C = O), 1511.92 (C=N), 1493.60 (C=C), 1254.47 (C-N of N-N-C), 1089.58 (C-Cl), ^{1}H NMR ($CDCl_3$) spectrum of compound P_5 displayed signals at δ 3.3 (OCH_3), 3.8(CH_2), 5.9 (CH) and 7.0 – 8.4 (aromatic and pyridinyl protons).

Mycobacterium tuberculosis ($H_{37}Rv$) strain maintained on Lowenstein–Jensen Medium at Rajiv Gandhi center for Biotechnology, Trivandrum was used as the test organism for antimycobacterial screening studies[8].

Stock solutions of newly synthesized compounds were prepared in dimethyl sulphoxide (DMSO) or dimethyl formamide (DMF), filter sterilized and were added to 450 μl of TB broth in 1.5 ml sterile disposable microcentrifuge

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TABLE 1: PHYSICAL, ANALYTICAL AND ANTITUBERCULAR ACTIVITY DATA OF COMPOUNDS $P_{(1-9)}$

Compd	R_1	R_2	R_3	mp	Yield %	Mol. Formula (mol. wt.)	% of C,H,N calculated (found)			Antitubercular activity		
							C	H	N	0.5 µg/ml	1 µg/ml	2 µg/ml
P_1	H	OH	OCH_3	205	60	$C_{22}H_{19}N_3O_3$ (373)	70.84 (70.95)	5.13 (5.21)	11.27 (11.21)	+	+	+
P_2	OCH_3	OH	OCH_3	122	80	$C_{23}H_{21}N_3O_4$ (403)	68.55 (68.67)	5.25 (5.31)	10.43 (10.35)	–	–	+
P_3	NO_2	OH	OCH_3	210	55	$C_{22}H_{18}N_4O_5$ (418)	63.22 (63.13)	4.34 (4.25)	13.40 (13.48)	–	–	–
P_4	H	Cl	H	85	65	$C_{21}H_{16}N_3OCl$ (361)	69.87 (69.75)	4.47 (4.36)	11.64 (11.75)	+	+	+
P_5	OCH_3	Cl	H	90	70	$C_{22}H_{18}N_3O_2Cl$ (391)	67.58 (67.63)	4.64 (4.78)	10.74 (10.80)	+	+	+
P_6	NO_2	Cl	H	87	55	$C_{21}H_{15}N_4O_3Cl$ (406)	62.13 (62.21)	3.72 (3.84)	13.80 (13.89)	–	–	–
P_7	H	OCH_3	OCH_3	100	80	$C_{23}H_{21}N_3O_3$ (387)	71.38 (71.49)	5.47 (5.35)	10.86 (10.75)	+	+	+
P_8	OCH_3	OCH_3	OCH_3	105	70	$C_{24}H_{23}N_3O_4$ (417)	69.13 (69.25)	5.56 (5.62)	10.08 (10.15)	–	–	–
P_9	NO_2	OCH_3	OCH_3	204	65	$C_{23}H_{20}O_5N_4$ (432)	63.95 (63.82)	4.67 (4.54)	12.97 (13.01)	+	+	+

Solvent system used for TLC is n-hexane:ethylacetate:glacial acetic acid (5:5:0.1). +=Blue colour (sensitive), –=Pink colour (Resistant). Positive control (INH at 0.1, 0.2 and 0.4 µg/ml) in the assay showed Blue colour. Negative control (DMSO and DMF) in the assay showed Pink colour.

tubes to achieve final concentrations of 0.5, 1.0 and 2.0 µg/ml. INH (at 0.1,0.2 and 0.4 µg/ml) was set up simultaneously as the positive control and three tubes that did not have any test compound, but only solvents (DMSO or DMF) were used as negative control. Colonies from 4-week-old subcultures were transferred to tubes containing 0.85% saline, thoroughly vortex mixed and the suspensions were allowed to stand for five min. Fifty microlitres of the supernatant were inoculated into all the tubes containing different concentrations of newly synthesized compounds and INH. The solvents of the compounds were also incorporated in the control tubes. The tubes were mixed well and incubated at 37° without shaking.

On the 7th day, 25 ml of alamar blue solution was added to the first negative control tube. The colour changed from blue to pink which indicates that the solvents (DMSO or DMF) did not affect the growth of *Mycobacterium tuberculosis*. So the dye was added to all tubes and observed for 6 h. Blue colour in the tube indicated sensitivity of *M. tuberculosis* to the newly synthesized compounds and pink colour indicated resistance of *M. tuberculosis* to them.

The results of antitubercular screening are shown in Table 1. All the newly synthesized compounds were screened for antitubercular activity against *M. tuberculosis*. The critical concentration of INH used in studies with clinical isolates is 0.2 µg/ml. As the potency of the newly synthesized compounds were unknown, various concentrations (0.5, 1.0 and 2.0 µg/ml) of compounds were used in this study. Of the nine compounds in pyrazoline series, compound P_2 (methoxy phenyl derivative of

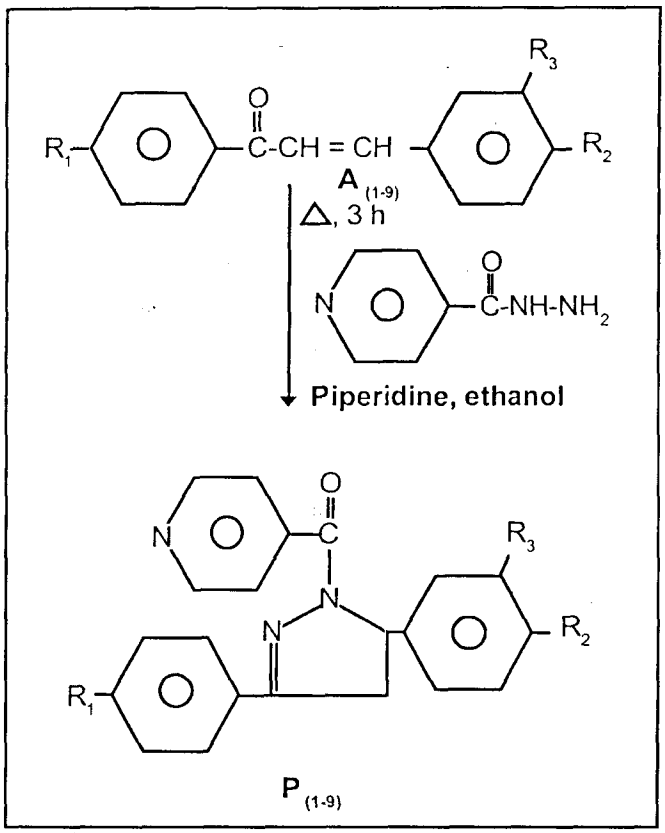

Fig. 1: Reaction scheme

pyrazoline) showed inhibitory activity only at the highest concentration of 2 µg/ml and compounds P_1 (Phenyl derivative of pyrazoline), P_4 (chlorophenyl derivative of pyrazoline), P_5 (methoxy phenyl derivative of pyrazoline), P_7 (dimethoxy phenyl derivative of pyrazoline) and P_9 (nitro phenyl derivative of pyrazoline) showed higher degree of antitubercular activity against *M. tuberculosis* at all the three concentrations used, and the above compounds showed activity comparable with that of the antitubercular activity of standard drug INH used at 0.4 µg/ml concentration and these compounds can serve as potent lead moieties in order to obtain ideal antitubercular agent.

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Estimation of Valdecoxib in Tablets by RP-HPLC Method

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A simple, efficient and reproducible reverse phase HPLC method has been developed for the determination of valdecoxib in tablets. The analyte was resolved by using a mobile phase

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