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Synthesis and in vitro antimycobacterial activity of N^1-nicotinoyl-3-(4′-hydroxy-3′-methyl phenyl)-5-[(sub)phenyl]-2-pyrazolines

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Abstract—A series of N^1-nicotinoyl-3- (4′-hydroxy-3′-methyl phenyl)-5-(substituted phenyl)-2-pyrazolines were synthesized by the reaction between isoniazid (INH) and chalcones and were tested for their antimycobacterial activity in vitro against *Mycobacterium tuberculosis* H37Rv (MTB) and INH-resistant *M. tuberculosis* (INHR-MTB) using the agar dilution method. Among the synthesized compounds, compound **(i)** N^1-nicotinyl-3-(4′-hydroxy-3′-methyl phenyl)-5-(1″-chlorophenyl)-2-pyrazoline was found to be the most active agent against MTB and INHR-MTB, with minimum inhibitory concentration of 0.26 μm. When compared to INH-compound **i** was found to be 2.8- and 43.7-fold more active against MTB and INHR-MTB, respectively.

Tuberculosis (TB) is considered by the WHO to be the most important chronic communicable disease in the world.[1] The emergence of AIDS, decline of socioeconomic standards and a reduced emphasis on tuberculosis control programs contribute to the disease's resurgence in industrialized countries.[2] In most parts of the world, we are limited to combinations of five drugs to treat TB effectively, namely rifampicin, isoniazid (INH), ethambutol, streptomycin and pyrazinamide. Problems in the chemotherapy of tuberculosis arise when patients develop bacterial resistance to any of these first-line drugs and because second-line drugs, such as ethionamide, aminosalicylic acid, cycloserine, amikacin, kanamycin and capreomycin are too toxic and cannot be employed simultaneously.[3] Resistance of *Mycobacterium tuberculosis* (MTB) strains to antimycobacterial agents is an increasing problem worldwide.[4–6] The key tasks for an antituberculosis regimens are (a) the development of long-acting drugs with large dosage intervals in order to facilitate Directly Observed Treatment Short Course (DOTS) and enhance patient compliance; (b) the prevention of multidrug-resistant MTB (MDR-TB) strains using drugs that exhibit potent early microbicidal activity; and (c) the eradication of slowly metabolizing and, if possible, dormant populations of MTB organisms that cause relapse, using new classes of antituberculous drugs. Despite the current use of rifampicin and pyrazinamide, therapy for TB requires at least 6 months. To decrease the risk of bacteriological reactivation of TB, long-term therapies for more than 6 months are generally needed. This frequently leads to patient non-adherence, thereby causing the emergence/increase of MDR-MTB strains. Notably, the cost of treating MDR-TB patients is much greater than that for patients carrying drug-susceptible MTB strains. In addition, there are thought to be two billion people latently infected with TB, more are exposed. Because dormant types of MTB organisms may survive in vivo for as long as several decades, new drugs effective against dormant MTB organisms would be highly useful in preventing bacteriological reactivation of MTB in such populations. Thus, the development of new anti-TB drugs that are effective against a persistent MTB infection is urgently desired. Among the standard antimycobacterial agents, in spite of toxicity on repeated dosing isoniazid (INH) is still considered to be a first-line drug for chemotherapy of tuberculosis.[7] On the other hand, pyrazoline derivatives were active against many mycobacterias.[8,9] The current work describes the incorporation of INH in a pyrazoline

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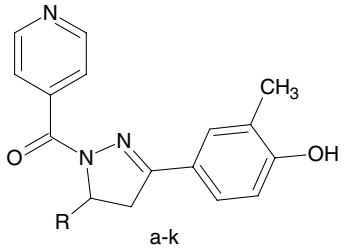
moiety and screen its antimycobacterial activity against *M. tuberculosis* $H_{37}Rv$ and INH-resistant *M. tuberculosis* (INHR-MTB).

N^1-Nicotinoyl-3-(4′-hydroxy-3′-methyl phenyl)-5-[(sub-) phenyl]-2-pyrazolines **a**–**k** described in this study are shown in Table 1, and a reaction sequence for the preparation is outlined in Scheme 1. The required chalcones were prepared by reacting 3-methyl-4-hydroxy acetophenone with appropriate aldehyde in presence of base by conventional Claisen-Schmidt condensation. Reaction between chalcone with isonicotinyl hydrazide in ethanolic solution in the presence of glacial acetic acid (reaction time varies from 8 to 14 h) afforded titled thiazolines **a**–**k** in 65–94% yield after recrystallization with methanol. The purity of the compounds was checked by TLC and elemental analyses. Both analytical and spectral data (^{1}H NMR, IR) of all the synthesized compounds were in full agreement with the proposed structures. In general, Infrared spectra (IR) revealed OH, C=O, C=N and C—N peak at 3307, 1640, 1590 and 1320 cm^{-1}, respectively. In the nuclear magnetic resonance spectra (^{1}H NMR) the signals of the respective protons of the prepared titled compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed a singlet at δ 2.2 ppm corresponding to methyl group; singlet at δ 2.57 ppm corresponding to C4-methylene group; singlet at δ 5.78 ppm corresponding to C5 proton; and multiplet at δ 8.22–8.5 ppm for pyridyl proton. The elemental

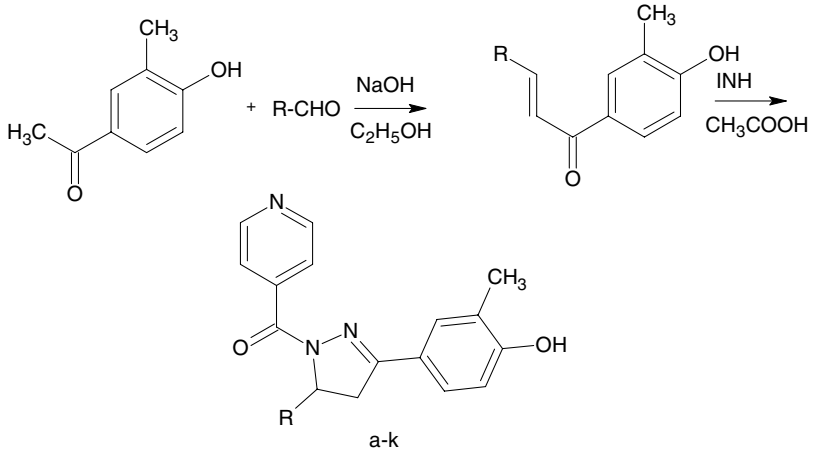
Table 1. Physical constants and antimycobacterial activity of the synthesized compounds



a-k

Compound	R	Yield (%)	MP (°C)	Molecular formula	Molecular weight	MIC (μM)	
						MTB[a]	MTB[b]
a	4-Methoxy phenyl-	72	119	$C_{23}H_{21}N_3O_3$	387.4	16.13	8.05
b	4-Chloro phenyl-	78	166	$C_{22}H_{18}ClN_3O_2$	391.8	1.99	3.98
c	4-Dimethylamino phenyl-	66	110	$C_{24}H_{24}N_4O_2$	400.47	1.95	1.95
d	Phenyl-	80	140	$C_{22}H_{19}N_3O_2$	357.4	8.73	4.36
e	3,4-Dimethoxy phenyl-	82	138	$C_{24}H_{23}N_3O_4$	417.4	1.87	0.93
f	2,3,4-Trimethoxy phenyl-	86	186	$C_{25}H_{25}N_3O_5$	447.4	3.93	0.87
g	Furyl-	94	152	$C_{20}H_{17}N_3O_3$	347.3	0.58	0.58
h	4-Fluoro phenyl-	86	146	$C_{22}H_{18}FN_3O_2$	375.3	0.27	0.54
i	2-Chloro phenyl-	80	194	$C_{22}H_{18}ClN_3O_2$	391.8	0.26	0.26
j	2,6-Dichloro phenyl-	78	212	$C_{22}H_{17}Cl_2N_3O_2$	426.2	0.47	0.23
k	3-Nitro Phenyl-	65	112	$C_{22}H_{18}N_4O_4$	402.4	15.53	7.75
INH	—	—	—	—	—	0.73	11.37

[a] *Mycobacterium tuberculosis* $H_{37}R_v$.
[b] INH-resistant *Mycobacterium tuberculosis*.



a-k

Scheme 1. Protocol for the synthesis of N^1-nicotinoyl-3-(4′-hydroxy-3′-methyl phenyl)-5-[(sub)phenyl]-2-pyrazolines.

analysis results were within ±0.4% of the theoretical values.

The synthesized compounds **a**–**k** were tested for their antimycobacterial activity in vitro against MTB and INHR-MTB by agar dilution method in Middlebrook 7H11 supplemented with OADC media (Hi-Media) using double dilution technique (drug concentrations of 12.5, 6.25, 3.125, etc.) similar to that recommended by the National Committee for Clinical Laboratory Standards[10] for the determination of minimum inhibitory concentration (MIC). The INHR-MTB clinical isolate was obtained from Tuberculosis Research Center, Chennai, India. The MIC was defined as the minimum concentration of compound required to inhibit 99% of bacterial growth and MIC's of the compounds are reported in Table 1 with standard drug INH for comparison.

Among the 11 compounds synthesized four compounds were found to be most active compounds with minimum inhibitory concentration of less than 1 μM and were found to be more active than INH against MTB. Compounds with halogen substituted phenyl group showed more activity. Among them compounds with 2-chlorophenyl (**i**) and 4-fluorophenyl (**h**) substituents were found to be most active and were >2-fold more active than INH against *M. tuberculosis* with MIC of ~0.26 μM. Against INHR-MTB, all the synthesized compounds were more active than INH with MIC of less than 8 μM. Among them compounds **i** and 2,6-dichlorophenyl substituent **j** were found to be promising and were ~50-fold more potent than INH.

Three compounds (**h**–**j**) were further examined for toxicity (IC_{50}) in a mammalian Vero cell line at concentrations of 62.5 μg/mL. After 72 h exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay.[11] These compounds were found to be non-toxic till 62.5 μg/mL.

Among the newer derivatives, compound **i** showed a promising activity in vitro. It is conceivable that these derivatives showing antimycobacterial activity can be further modified to exhibit better potency than the standard drugs. Further studies to acquire more information about structure–activity relationships are in progress in our laboratory.

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