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Synthesis and in vitro antitubercular activity of some 1-[(4-sub)phenyl]-3-(4-{1-[(pyridine-4-carbonyl) hydrazono]ethyl}phenyl)thiourea

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Abstract—Various isonicotinyl hydrazones were prepared by reacting isonicotinyl hydrazide [INH] with 1-(4-acetylphenyl)-3-[(4-sub)phenyl]thiourea and were tested for their antimycobacterial activity in vitro against *Mycobacterium tuberculosis* $H_{37}R_v$ and INH-resistant *M. tuberculosis* using the BACTEC 460 radiometric system. Among the synthesized compounds, 1-(4-fluorophenyl)-3-(4-{1-[(pyridine-4-carbonyl)-hydrazono]ethyl}phenyl)thiourea (**4d**) was found to be the most potent compound with a minimum inhibitory concentration of 0.49 μM against *M. tuberculosis* $H_{37}R_v$ and INH-resistant *M. tuberculosis*. When compared to INH, **4d** was found to be 3 and 185 times more active against *M. tuberculosis* $H_{37}R_v$ and INH-resistant *M. tuberculosis*, respectively, with a selectivity index of >300.

Tuberculosis (TB) is the most prevalent infectious disease worldwide and a leading killer caused by a single infectious agent, that is, *Mycobacterium tuberculosis*.[1,2] According to a World Health Organization (WHO) report [3], *M. tuberculosis* currently infects over 2 billion people worldwide, with 30 million new cases reported each year. This intracellular infection accounts for at least 3 million deaths annually. 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.[4] The reemergence of TB infection is further complicated by an increase in cases, which are resistant to conventional antitubercular drug therapy.[5] On the other hand, in spite of toxicity on repeated dosing isoniazid is still considered to be a first-line drug for chemotherapy of tuberculosis.[6] Recently, it was suggested that the mechanism of resistance to INH is related to katG mutations and deletions, and second to chromosomal mutations in inhA and kasA.[4] Antibacterial resistance to a drug can be counteracted by designed new derivatives.[7] Further, pharmacokinetic properties and cellular permeability of a drug can be modulated by derivatization to bioreversible forms of this drug, namely hydrazones.[8,9] On the other hand, thiourea derivatives were active against many Mycobacteria.[10] The current work describes the incorporation of INH in a *N*,*N*′-diaryl thiourea moiety and screening for activity against *M. tuberculosis* $H_{37}R_v$ and an INH-resistant clinical isolate.

Isonicotinyl hydrazones **4a**–**4f** described in this study are shown in Table 1, and a reaction sequence for the preparation is outlined in Scheme 1. The starting compounds (sub)-phenyl isothiocyanates **1a**–**1f** were prepared according to known procedures[11] from appropriate anilines. Phenyl isothiocyanates were converted to thiourea **3a**–**3f** by refluxing with 4-amino acetophenone **2** for 17 h. The reaction between compounds **3a**–**3f** with isonicotinyl hydrazide in ethanolic solution took place in the presence of glacial acetic acid. On cooling, the precipitate was collected, washed with cold ethanol, and recrystallized from a mixture of DMF and water which afforded hydrazones **4a**–**4 f** with 50–64% yield. The purity of compounds was checked by TLC and elemental analyses. Both analytical and spectral data (^{1}H NMR,

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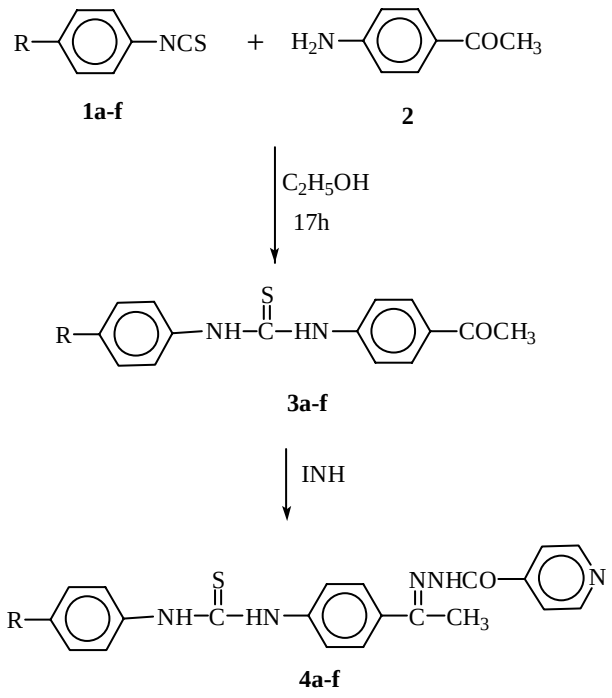
Table 1. Physical constants and antimycobacterial activity of the synthesized compounds

Compound	R	Molecular formula	Yield (%)	Mp (°C)	log P[a]	IC_{50} (μM)	MIC[b] (μM)	
							M. tuberculosis $H_{37}R_v$	INH-resistant *M. tuberculosis*
4a	H	$C_{21}H_{19}N_5SO$	50	144	3.24	160.47	4.00	4.00
4b	4-CH_3	$C_{22}H_{21}N_5SO$	55	234	3.18	154.89	1.93	3.86
4c	4-Br	$C_{21}H_{18}N_5SOBr$	51	218	3.05	133.44	1.66	1.66
4d	4-F	$C_{21}H_{18}N_5SOF$	59	241	3.19	>153.4	0.49	0.49
4e	4-OCH_3	$C_{22}H_{21}N_5SO_2$	64	223	3.27	>149	0.47	1.85
4f	4-OC_2H_5	$C_{23}H_{23}N_5SO_2$	50	214	3.45	144.16	0.89	1.79
INH	—	—	—	—	0.58	>454.7	1.46	90.94

[a] log P was calculated using online www.logp.com site.
[b] MIC = minimum inhibitory concentration.



Scheme 1. Synthetic protocol of isonicotinoyl hydrazones.

^{13}C NMR, and mass spectra) of all the synthesized compounds were in full agreement with the proposed structures.[12] Lipophilicity of the synthesized derivatives **4a**–**4f** and that of the parent compound, INH, are expressed in terms of their log P values. These values were computed with a routine method called calculated log P (C log P) using Alchemy software.

The synthesized compounds **4a**–**4f** were tested for their antimycobacterial activity in vitro against *M. tuberculosis* $H_{37}R_v$ and clinical isolate of INH-resistant *M. tuberculosis* [obtained from Tuberculosis Research Center, Chennai, India] using the BACTEC 460 radiometric system. All the compounds were further examined for toxicity (IC_{50}) in a mammalian cell line, VERO cells. The results are summarized in Table 1. Rapid glance at the obtained results revealed that the compounds **4a**–**4f** exhibited very good antimycobacterial activity. Among the synthesized compounds, compounds **4d** and **4e** were found to be the most potent compounds [MIC: 0.49 and 0.47 μM, respectively] and were more active than INH [MIC: 1.46 μM] against *M. tuberculosis* $H_{37}R_v$. All the compounds were more potent than INH [MIC: 90.94 μM] against INH-resistant *M. tuberculosis* with MICs ranging from 0.49 to 4.0 μM. Compound **4d** was found to be 185 times more active than INH against this isolate. The order of activity with respect to the substituents in the 4th position is >F > OCH_3 > OC_2H_5 > Br > CH_3 >H.

All the compounds were further examined for toxicity (IC_{50}) in a mammalian VERO cell line at concentrations of 6.25 and 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.[13] The compounds were non-toxic as represented in Table 1 and the selectivity index (IC_{50}/MIC) for the most active compound **4d** was more than 312.

The lipophilicity of the synthesized compounds increased remarkably compared with that of the parent drug, INH. This may render them more capable of penetrating various biomembranes,[14] consequently improving their permeation properties through mycobacterial cell membranes.

Among the newer derivatives, compound **4d** 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. These results need to be refined in terms of degradation kinetic measurements and stability studies of the synthesized derivatives.

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