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Microwave assisted one-pot synthesis of highly potent novel isoniazid analogues

Thimmappa H. Manjashetty, Perumal Yogeeswari, Dharmarajan Sriram *

Medicinal Chemistry and Antimycobacterial Research Laboratory, Department of Pharmacy, Birla Institute of Technology and Science, Pilani, Hyderabad Campus, Jawahar Nagar, Hyderabad 500 078, India

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ABSTRACT

A series of novel isoniazid (INH) analogues were synthesized by microwave assisted one pot reaction of INH, various benzaldehydes and dimedone in water with catalytic amount of DBSA. The synthesized compounds were evaluated for their anti-TB activity against *Mycobacterium tuberculosis* H37Rv (MTB) and multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB). Among the 29 compounds, compound *N*-[9-[2-(benzyloxy)phenyl]-3,3,6,6-tetramethyl-1,8-dioxo-2,3,4,5,6,7,8,9-octahydro-10(1*H*)-acridinyl] isonicotinamide (**12**) inhibited MTB with MIC of <0.17 μM and MDR-TB with MIC of 0.69 μM.

Tuberculosis (TB) is the leading cause of mortality among all infectious diseases worldwide and is responsible for over two million deaths annually.[1] The major concerns for current TB treatment are its latency, co-infection with HIV, poor patient compliance, and drug resistance issues caused by the emergence of MDR-TB and the recent advent of extensively drug resistant tuberculosis (XDR-TB).[2] It is estimated that between 2002 and 2020, approximately 1 billion people will be newly infected, more than 150 million people will get sick, and 36 million will die of TB if new disease prevention and treatment measures are not developed.[3] Hence, there is an urgent need to develop novel, highly effective, and fast acting anti-TB drugs with low toxicity profiles and performing activity against both drug-sensitive and drug resistant MTB.

Isoniazid (INH) has become the single most researched antitubercular agent which is not effective against MDR-TB. Several recent experiments indicate that the incorporation of hydrophobic moieties into the framework of INH can enhance penetration of the drug into the tissues of the mammalian host and into the waxy cell wall of the bacterium. Previous work on several members of this class, particularly the (hetero)aromatic derivatives, had been carried out and had demonstrated good activities by the methods then available (Fig. 1).[4–8] The important relationship between serum levels of INH and the emergence of INH-resistant cultures in patients with pulmonary tuberculosis became evident in careful studies done some time ago.[9] These studies stressed the importance of early intensive chemotherapy, with an emphasis on initial peak serum concentrations of INH, rather than on concentrations measured three or more hours after the dose. Further, the improved therapeutic outcome of certain treatment regimens was attributed to the higher early peak serum concentrations of INH that they permitted, rather than to the period that minimum inhibitory concentrations (MIC) of INH were maintained in the serum.[10] Serum concentrations are influenced by a number of factors, but among the most important of these is the enzymatic acetylation of INH by *N*-acetyl transferase (NAT). This represents a major metabolic pathway for INH in human beings. Acetylation greatly reduces the therapeutic activity of the drug. NAT is also present in mycobacterial pathogens. Resistance to INH in mycobacteria can thus be related to increased expression of NAT.[11]

Chemical modification of the hydrazine unit of INH with a functional group that blocks acetylation, while maintaining strong anti-TB action, has the potential to improve clinical outcomes and reduce the emergence in patients of acquired INH resistance. The goal of our study was to investigate such chemical modification. In the present study we incorporated the INH moiety in the decahydroacridine scaffold and evaluated for in vitro MTB and MDR-TB activities.

The synthetic procedure (Scheme 1) consists of the microwave assisted reaction of 5,5-dimethylcyclohexane-1,3-dione (dimedone) with the various benzaldehydes and the INH in water with catalytic amount *p*-dodecylbenezenesulfonic acid (DBSA)[12] (Table 1). The selection of reaction conditions (in particularly the solvent and the catalyst) appears to be the most critical. Although today's environmental consciousness imposes the use of water as a

* Corresponding author. Tel.: +91 4066303506.
E-mail addresses: dsriram@bits-hyderabad.ac.in, drdsriram@yahoo.com (D. Sriram).

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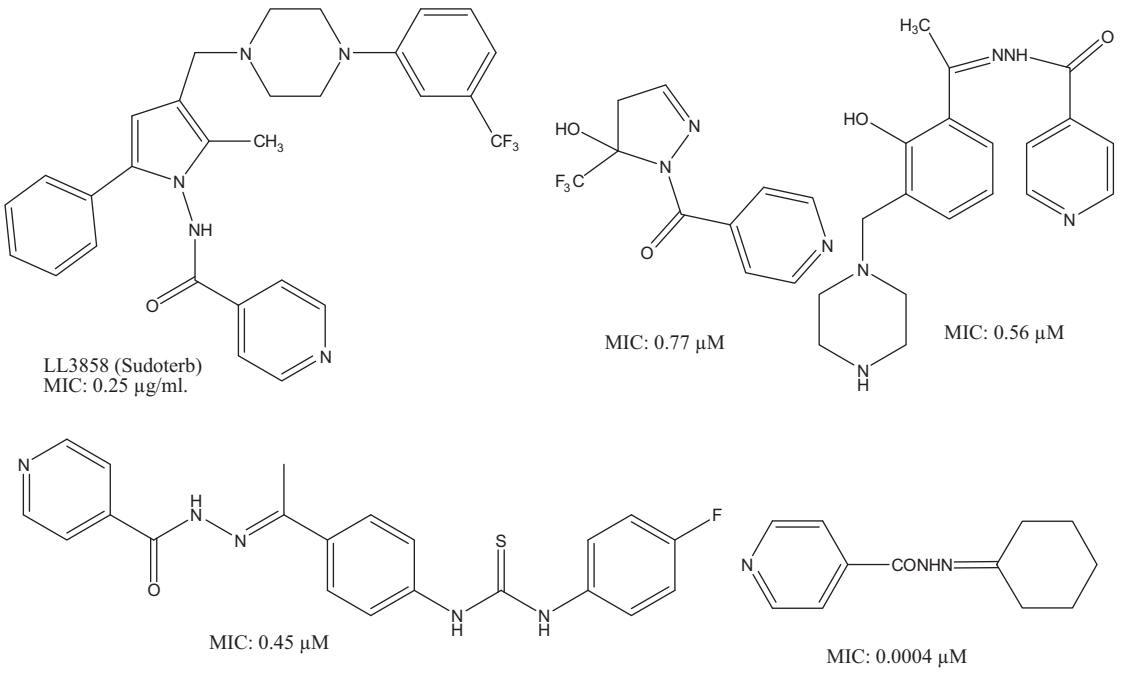
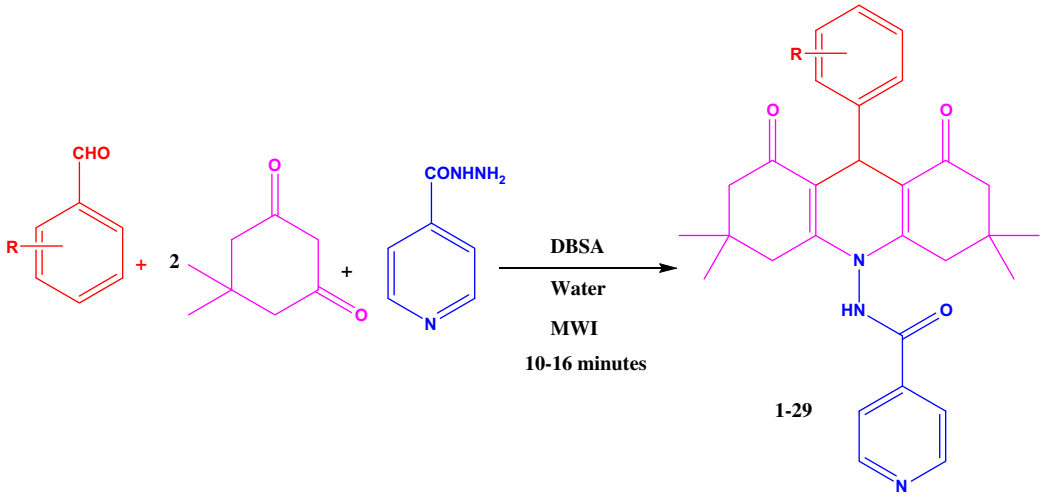


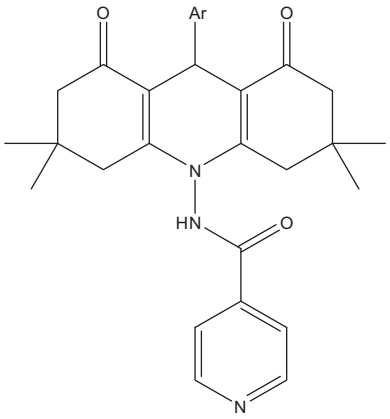
Figure 1. Potential reported isoniazid analogues.



Scheme 1. Synthetic protocol of the compounds.

solvent on both industrial and academic chemists, organic solvents are still used instead of water for that most organic substances are insoluble in water and many reactive substrates, reagents and catalysts are decomposed or deactivated by water. DBSA is a Brønsted acid-surfactant-combined catalyst, and the surfactant used in water can make organic materials soluble or form colloidal dispersions, and the Brønsted acid is stable in water, so it can solve the two drawbacks of the reactions in water. DBSA has been used in a number of organic reactions as a good catalyst. The effect of electron and the nature of substituents on the aromatic ring did not show expected strong effects in terms of yields under these reaction conditions. Benzaldehyde containing electron-withdrawing groups (such as nitro, halide groups) or electron-donating groups (such as hydroxy, alkoxyl groups) were employed and they were found to react well to give the corresponding *N*-[9-(aryl)-3,3,6,6-tetramethyl-1,8-dioxo-2,3,4,5,6,7,8,9-octahydro-10(1*H*)-acridinyl]isonicotinamides in good to excellent yields (65–96%). Use of 10 mol % DBSA in refluxing water is sufficient to push the reaction forward. The yields are, in general, very high regardless of the structural variations in benzaldehyde. In this synthetic protocol we have utilized microwave irradiation, which when compared to conventional reflux (6 h), took just 10–16 min. The purity of compounds **1–29** was checked by TLC and elemental analyses. Both analytical and spectral data (^{1}H NMR, 13C NMR, and mass spectra) of all the synthesized compounds were in full agreement with the proposed structures.[13] The ^{1}H NMR spectra of compounds 1–29 show singlets at 0.80–0.84 and 0.90–0.95 ppm which are assigned to the protons of the four methyl groups in positions 3 and 6, quadrates for the CH_2 group protons of the cyclohexane rings in the range 1.80–2.21 ppm, a singlet for the methine proton at 5.24–5.40 ppm, and signals for the aromatic protons in the range 6.80–8.72 ppm. Lipophilicity of the synthesized derivatives and that of the parent compound, INH, are expressed in terms of their log *P* values (Table 1). These values were computed with a routine

Table 1
Physical constants, in vitro anti-tubercular and cytotoxicity activities



No.	Ar	Yield (%)	Mp (°C)	*C* Log *P*[a]	CC_{50} in μM[b]	MIC in μM[c]	
						MTB	MDR-TB
1	Phenyl	91	146–148	4.32	—	6.66	—
2	2-Methylphenyl	82	142–144	4.77	—	3.22	—
3	3-Methylphenyl	70	151–154	4.82	—	6.44	—
4	4-Methylphenyl	94	82–84	4.82	—	6.44	—
5	2-Hydroxyphenyl	77	182–184	3.45	—	>12.87	—
6	3-Hydroxyphenyl	71	218–221	3.65	—	>12.87	—
7	4-Hydroxyphenyl	70	252–254	3.65	—	12.87	—
8	2-Methoxyphenyl	68	132–136	3.84	—	12.51	—
9	4-Methoxyphenyl	71	98–100	4.24	—	12.51	—
10	4-Dimethylaminophenyl	89	148–150	4.49	—	6.10	—
11	4-Isopropylphenyl	96	178–180	5.75	—	1.52	—
12	2-Benzyloxyphenyl	65	96–98	5.61	>108.56	<0.17	0.69
13	3-Benzyloxyphenyl	75	146–148	6.01	>108.56	0.17	1.35
14	4-Benzyloxyphenyl	78	134–136	6.01	>108.56	0.17	0.69
15	4-Hydroxy-3-methoxyphenyl	67	182–184	3.50	—	3.02	—
16	2,3,4-Trimethoxyphenyl	76	152–154	3.62	—	11.16	—
17	2-Trifluoromethylphenyl	89	126–128	5.20	>116.26	0.36	1.45
18	3-Trifluoromethylphenyl	68	84–86	5.20	>116.26	0.18	1.45
19	4-Trifluoromethylphenyl	68	110–112	5.20	>116.26	0.36	0.74
20	4-Chlorophenyl	86	94–96	5.03	>124.00	0.79	1.54
21	2-Bromophenyl	67	172–174	5.18	>113.95	0.18	2.84
22	3-Bromophenyl	79	174–178	5.18	<113.95	0.73	2.84
23	4-Bromophenyl	67	120–122	5.18	<113.95	0.73	2.84
24	2-Fluorophenyl	66	102–104	4.46	>128.18	0.41	1.59
25	3-Fluorophenyl	65	90–92	4.46	>128.18	0.41	0.82
26	4-Fluorophenyl	79	106–108	4.46	>128.18	0.20	0.20
27	2-Nitrophenyl	74	234–236	3.98	<121.46	0.19	1.51
28	3-Nitrophenyl	67	120–122	4.06	<121.46	0.77	3.03
29	4-Nitrophenyl	75	90–92	4.06	<121.46	0.77	3.03
Isoniazid				−0.66	>455.73	0.73	45.57

[a] Calculated with ChemBioDraw Ultra 11.0.
[b] Cytotocity in VERO cell line.
[c] Minimum inhibitory concentration.

method called calculated log *P* (*C* log *P*) using ChemBioDraw Ultra 11.0 software.

The compounds **1–29** were screened for their in vitro antimycobacterial activity against log-phase cultures of MTB and MDR-TB in Middlebrook 7H11 agar medium supplemented with OADC by agar dilution method similar to that recommended by the National Committee for Clinical Laboratory Standards for the determination of MIC in triplicate.[14] The MIC is defined as the minimum concentration of a compound required to completely inhibit the bacterial growth. The MIC's of the synthesized compounds along with the standard drug INH for comparison are reported in Table 1. In the first phase of screening, all the compounds showed in vitro activity against MTB with MIC ranging from <0.17 to >12.87 μM. Sixteen compounds (**12–14, 17–29**) inhibited MTB with less than 1 μM. When compared to standard first line drug INH (MIC of 0.73 μM); 10 compounds (**12–14, 17–19, 21, 24–27**) were more potent with MIC less than 0.73 μM and two compounds (**22–23**) were equally active. Compound *N*-[9-[2-(benzyloxy)phenyl]-3,3,6,6-tetramethyl-1,8-dioxo-2,3,4,5,6,7,8,9-octahydro-10(1*H*)-acridinyl]isonicotinamide (**12**) was found to be the most promising compound with MIC of <0.17 μM. Compound **12** was found to be more than four times active as parent drug INH. The enhanced activity might be due to its hydrophobicity, *C* log *P* of this compound **12** was 5.61 whereas parent compound INH is −0.66. With respect to structure anti-TB activity, the substituent in the 9th position dictates the activity. Electron donating group like hydroxyl, methyl and methoxyl groups are detrimental; among alkyl substituents isopropyl (**11**) showed better activity than methyl (**2–4**) group. Among alkoxy/aryloxy substituents benzyloxy group showed maximum activity and 2-benzyloxy group was found to

be more potent than 3 and 4-benzyloxy group. Electron withdrawing groups like halogen and nitro group showed better activity. The compounds which showed activity against MTB with less than 1 μM were further screened against MDR-TB. The MDR-TB clinical isolate was obtained from Tuberculosis Research Center, Chennai, India, and was resistant to INH, rifampicin, ethambutol and ofloxacin. The screened compounds inhibited MDR-TB with MIC ranging from 0.20 to 2.84 μM and all the 16 screened compounds were more potent than INH (MIC of 45.57 μM). Compounds with 4-fluorophenyl substituent showed very good activity with MIC of 0.20 μM and was found to be 227 times more potent than INH against MDR-TB.

Some of the compounds were further examined for toxicity (CC_{50}) in a mammalian Vero cell line at concentrations of 62.5 μg/ml.[15] After 72 h of exposure of tested compounds, 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 and reported in Table 1. Compounds with bromo and nitro substituted phenyl at 9th position showed toxicity at 62.5 mg/ml (56–72%). Compound **18** showed maximum selectivity index (CC_{50}/MIC) of >645. These results are important as nitrophenyl substituted analog **27** was more active and at the same time it was cytotoxic. Certainly, the development of any new compounds for TB would depend on an excellent safety profile since it would need to be used for several months in combination with other antitubercular agents.

Screening of the antimycobacterial activity of these novel series identified novel INH analogue with high activity toward MDR-TB, with MIC values between 0.2 and 2.84 μM. In conclusion, it has been shown that the potency, selectivity, and low cytotoxicity of these compounds make them valid leads for synthesizing new compounds that possess better activity. Further structure-activity and mechanistic studies should prove fruitful.

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