Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 15 (2005) 4502–4505

Synthesis and in vitro and in vivo antimycobacterial activity of isonicotinoyl hydrazones

Dharmarajan Sriram,* Perumal Yogeeswari and Kasinathan Madhu

Medicinal Chemistry Research Laboratory, Pharmacy Group, Birla Institute of Technology and Science, Pilani 33031, India

Received 16 February 2005; revised 5 July 2005; accepted 7 July 2005
Available online 22 August 2005

Abstract—The purpose of this study was to prepare various isoniazid derivatives by introducing the isoniazid pharmacophore into several molecules and screening for antimycobacterial activity. Ortho-hydroxy acetophenone reacts with isoniazid to form acid hydrazones. The C-Mannich bases of the above acid hydrazones were prepared by reacting them with formaldehyde and various secondary amines. The synthesized compounds were screened against *M. tuberculosis* $H_{37}R_v$ using the alamar blue susceptibility test. The synthesized compounds inhibit *Mycobacterium tuberculosis* strain $H_{37}R_v$ with minimum inhibitory concentrations ranging from 0.56 to 4.61 μM. Compound *N*′-{1-[2-hydroxy-3-(piperazin-1-ylmethyl)phenyl]ethylidene}isonicotinohydrazide **8** was found to be the most active compound with an MIC of 0.56 μM, and was more potent than isoniazid (MIC of 2.04 μM). After 10 days of treatment, compound **8** decreased the bacterial load in murine lung tissue by 3.7-log 10 as compared to controls, which was equipotent to isoniazid. The results demonstrate the potential and importance of developing new isoniazid derivatives against mycobacterial infections.

1. Introduction

Tuberculosis (TB) is one of the most common infectious diseases known to man. About 32% of the world's population (1.9 billion people) is infected with TB. Every year, approximately 8 million of these infected people develop active TB, and almost 2 million of them die from the disease,[1] a life lost to TB every 15 s. The incidence of TB infection has steadily risen in the last decade and this increase can be attributed to a similar increase in human immunodeficiency virus (HIV) infection.[2] The association of TB and HIV infections is so dramatic that, in some cases, nearly two-thirds of the patients diagnosed with TB are also HIV-1 seropositive.[3] Furthermore, numerous studies have shown that TB is a cofactor in the progression of HIV infection.[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 (INH) 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 secondly to chromosomal mutations in inhA and kasA.[7] Antibacterial resistance to a drug can be counteracted by designed new derivatives.[8] Further, pharmacokinetic properties and cellular permeability of a drug can be modulated by derivatization to bioreversible forms of this drug, namely hydrazones.[9] Preparation of Mannich bases of isonicotinoyl hydrazone have improved the lipid solubility.[10] Here, we report the synthesis and preliminary antimycobacterial activity data of newer isonocotinoyl hydrazones.

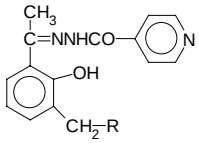
2. Chemistry

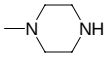
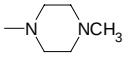
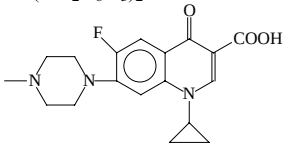
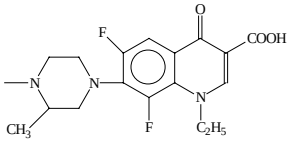
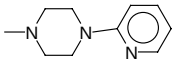
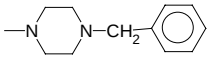
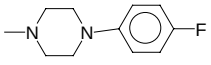
Isonicotinylhydrazones **1**–**15** described in this study is shown in Table 1, and a reaction sequence for the preparation is outlined in Figure 1. 2-Hydroxy acetophenone reacts with isoniazid in the presence of glacial acetic acid in ethanolic medium refluxed for 9 h to form acid hydrazones (yield: 72.6%, mp: 242 °C),[14] which in turn, reacts with formaldehyde and appropriate secondary amines in a microwave oven at an intensity of 80% with 30 s/cycle. The number of cycles in turn depended on the completion of the reaction, which was checked by TLC. The reaction timing varied from 1.5–3 min to form targeted compounds (55–64% yields). The purity of the

Keywords: Isoniazide derivatives; Antimycobacterial.
* Corresponding author. Tel.: +91 1596 244684; fax: +91 1596 244183; e-mail: dsriram@bits-pilani.ac.in

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.07.011

Table 1. Physical data and antimycobacterial activity of synthesized compounds



Compound	R	Molecular formula	Yield (%)	Mp (°C)	log P[a]	MIC[b] (μM)	IC_{50}[c] (μM)
1	$-N(CH_3)_2$	$C_{17}H_{20}N_4O_2$	56	234	2.32	2.49	>20.00
2	$-N(C_2H_5)_2$	$C_{19}H_{24}N_4O_2$	62	213	2.75	2.29	>18.35
3	$-N(C_4H_9)_2$	$C_{23}H_{32}N_4O_2$	52	208	5.29	3.93	>15.76
4		$C_{20}H_{24}N_4O_2$	62	186	3.83	4.43	>17.73
5		$C_{19}H_{22}N_4O_2$	58	194	3.36	4.61	>18.47
6		$C_{19}H_{22}N_4O_3$	55	219	2.05	2.20	>17.63
7	$-N(C_6H_5)_2$	$C_{27}H_{24}N_4O_2$	61	208	6.16	3.57	>14.32
8		$C_{19}H_{23}N_5O_2$	50	183	2.61	0.56	>17.68
9		$C_{20}H_{21}N_5O_2$	54	194	2.02	8.61	>17.20
10	$-N(CH_2C_6H_5)_2$	$C_{29}H_{28}N_4O_2$	46	190	6.07	3.36	>13.46
11		$C_{32}H_{31}N_6O_5F$	64	237	1.18	1.30	>10.44
12		$C_{32}H_{32}N_6O_5F_2$	63	230	1.42	1.26	>10.10
13		$C_{24}H_{26}N_6O_2$	61	51	4.87	0.90	>14.52
14		$C_{26}H_{29}N_5O_2$	57	62	6.01	0.88	>14.09
15		$C_{25}H_{26}N_5O_2F$	55	57	3.12	0.87	>13.97
Isoniazid	—	—	—	—	−0.58	2.04	>45.57

[a] log P was calculated using online www.logp.com site.
[b] MIC = minimum inhibitory concentration.
[c] IC_{50} = Cytotoxicity represented as inhibitory concentration in 50% of cell line.

compounds was checked by TLC and elemental analyses, and the compounds of this study were identified by spectral data. In general, IR spectra[15] showed C=N (azomethine) peak at 1640 cm^{-1} and CH_2 (Mannich methylene) peak at 2860 and 2840 cm^{-1}. In the ^1H NMR spectra, the signals of the respective protons of the prepared derivatives were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The spectra of all the compounds showed a singlet at δ 4.8–5.1 ppm corresponding to $-NCH_2N-$ group. The elemental analysis results were within ±0.4% of the theoretical values. Lipophilicity of the synthesized derivatives **1**–**15** and the parent compound, INH, is 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.

3. Antimycobacterial activity

The synthesized compounds **1**–**15** were tested for their antimycobacterial activity in vitro against *Mycobacterium tuberculosis* $H_{37}R_v$ using the microplate alamar blue assay method[11] in duplicate and MICs of the compounds were reported in Table 1. MIC is defined as the minimum concentration of compound required to give 90% inhibition of bacterial growth. Results show that compounds **1**–**15** exhibited excellent antimycobacterial

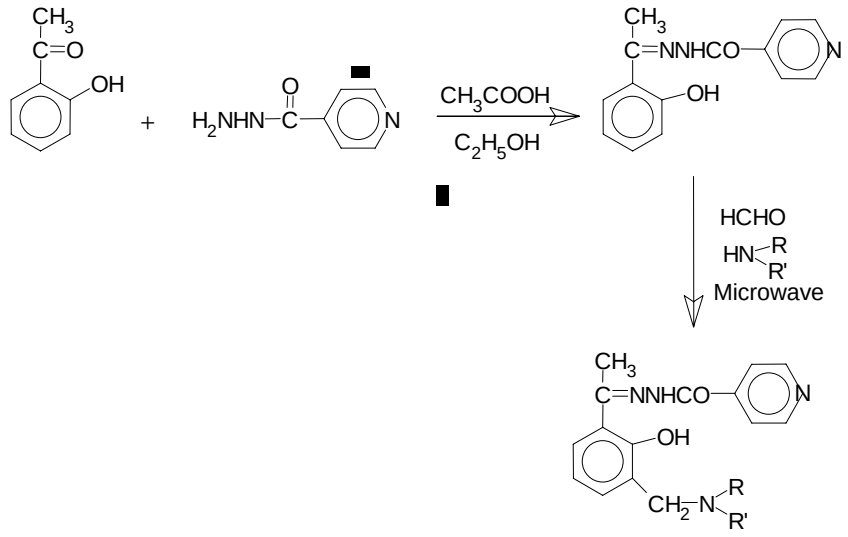


Figure 1. Synthetic protocol of the compounds.

Table 2. In vivo activity data of 8 and isoniazid against *Mycobacterium tuberculosis* in mice

Compound	Lungs (log CFU ± SEM)[a]
Control	9.78 ± 0.12
8 (25 mg/kg)	6.08 ± 0.19
Isoniazid (25 mg/kg)	6.38 ± 0.18

[a] CFU = colony forming units; SEM = standard error in mean.

activity. The lipophilicity of the synthesized compounds increased remarkably compared with the parent drug, INH. This may render them more capable of penetrating various biomembranes[12], consequently improving their permeation properties through mycobacterial cell membranes. All the synthesized compounds inhibit *M. tuberculosis* with MIC ranging from 0.56 to 4.61 μM. Six compounds (**8**, **11**–**15**) were more potent than INH and compound **8** (MIC 0.56 μM) emerged as the most potent derivative, being three times more effective than INH (MIC 2.04 μM) in vitro.

All the compounds were further examined for toxicity (IC_{50}) in a mammalian cell line, VERO cells by the TAACF. The compounds were non-toxic as represented in Table 1 and the selectivity index (IC_{50}/MIC) for the most active compound **8** was more than 30.

Subsequently, compound **8** was tested for efficacy against MTB at a dose of 25 mg/kg in mouse model.[13] Briefly, 30 inbred female AKR mice (as this strain is used in the previous report as mentioned in Ref. 13), weighing 18–20 g were infected iv via the *lateral tail vein* with 10^7 CFU of *M. tuberculosis* $H_{37}R_v$. They were divided into three groups of 10 mice each after 2 days. One group received daily for 10 days the aqueous suspension of the test compound **8** by intraperitoneal route at a dose of 25 mg/kg body weight. The second group received INH at 25 mg/kg body weights for 10 days by ip route, whereas the third group served as the control receiving no drug. The control groups did not receive sham injections. Bacterial counts were measured by plating lung homogenates on day 28 (Table 2). Compound **8** decreased the bacterial load by 3.7-log 10 while INH decreased counts by 3.4-log 10. Statistical analysis (Student's *t*-test) suggested that compound **8** and INH were equipotent.

4. Conclusion

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.

Acknowledgment

The authors are thankful to Dr. S. Ananthan from the Southern Research Institute, Birmingham, AL, USA, for the in vitro evaluation of antimycobacterial activity.

References and notes

1. Frieden, T. R.; Sterling, T. R.; Munsiff, S. S.; Watt, C. J.; Dye, C. *Lancet* **2003**, *362*, 887.
2. Currie, C. S.; Williams, B. G.; Cheng, R. C.; Dye, C. *AIDS* **2003**, *17*, 2508.
3. Williams, B. G.; Dye, C. *Science* **2003**, *301*, 1537.
4. Toossi, Z.; Mayanja-Kizza, H.; Hirsch, C. S.; Edmonds, K. L.; Spahlinger, T.; Hom, D. L.; Aung, H.; Mugyenyi, P.; Ellner, J. J.; Whalen, C. W. *Clin. Exp. Immunol.* **2001**, *123*, 8.
5. Sbarbaro, J. A. *Chest* **1997**, *111*, 1151.
6. Blair, I. A.; Timoco, R. M.; Brodie, M. J.; Clarc, R. A.; Dollery, T.; Timbrell, J. A.; Beever, I. A. *Hum. Toxicol.* **1985**, *4*, 202.
7. Mondell, G. L.; Sande, M. A. In *The Pharmacological Basis of Therapeutics*; Gilman, A. G., Rull, T. W., Nies, A. S., Taylor, P., Eds., 8th ed.; Pergamon Press: New York, 1991; p 1147.

8. Wechter, W. J.; Johnson, M. A.; Hall, C. M.; Warner, D. T.; Berger, A. E.; Wenzel, A. H.; Gish, D. T.; Neil, G. L. *J. Med. Chem.* **1975**, *18*, 344.
9. Maccari, R.; Ottana, R.; Monforte, F.; Vigorita, M. G. *Antimicrob. Agent Chemother.* **2002**, *46*, 294.
10. Joshi, C.; Khosla, N.; Tiwari, P. *Bioorg. Med. Chem.* **2004**, *14*, 571.
11. Collins, L.; Frazblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.
12. Moss, J.; Bundgaard, H. *Pharm. Res.* **1990**, *7*, 892.
13. Katiyar, D.; Tiwari, V. K.; Tripathi, R. P.; Srivastava, A.; Chaturvedi, V.; Srivastava, R.; Srivastava, B. S. *Bioorg. Med. Chem.* **2003**, *11*, 4369.
14. Characterization data for *N′*-[1-(2-hydroxyphenyl)ethylidene]pyridohydrazide: IR (KBr): 3210, 2820, 1640, 1628 cm^{-1}; ^1H NMR, δ (ppm): 2.18 (s, 3H, CH_3), 6.9–8.77 (m, 8H, Ar-H), 9.9 (s, 1H, OH, D_2O exchangeable), 10.85 (s, 1H, CONH, D_2O exchangeable); calcd for $C_{14}H_{13}N_3O_2$: C, 65.87; H, 5.13; N, 16.46. Found: C, 65.66; H, 5.19, N, 16.42.
15. Characterization data for compound **8** IR (KBr): 3210, 3010, 2858, 2840, 2820, 1680, 1636, 1506, 1236, 1125 cm^{-1}; ^1H NMR, δ (ppm): 2.26 (s, 3H, CH_3), 2.5–2.65 (m, 8H, $-N(CH_2CH_2)_2N-$), 6.7–8.6 (m, 8H, Ar-H), 9.96 (s, 1H, OH, D_2O exchangeable), 10.88 (s, 1H, CONH, D_2O exchangeable); calcd for $C_{19}H_{23}N_5O_2$: C, 64.57; H, 6.56; N, 19.82. Found: C, 64.66; H, 6.49, N, 18.72.