Scientia Pharmaceutica (Sci. Pharm.) **72**, 35–41 (2004)

Synthesis of Tetrahydro-2H-[1, 3, 5]thiadiazine-5-(4-pyridylcarboxamido)-2-thione with antitubercular activity

D. Sriram*, K. Jyothi Mallika, P. Yogeeswari

Medicinal Chemistry Research Laboratory, Pharmacy Group, Birla Institute of Technology and Science, Pilani 333031, India

Abstract

3-Substituted-5-(4-pyridylcarboxamide)tetrahydro-2H-[1,3,5]thiadizine-2-thione derivatives **(1-9)** were synthesized as derivatives of isoniazid (INH) to overcome the resistance developed with its therapeutic use. The structures were confirmed by their spectral and elemental analyses data. These derivatives revealed higher lipophilicity compared with INH. The antimycobacterial activity of the synthesized compounds and INH was evaluated *in vitro* against *Mycobacterium tuberculosis* $H_{37}R_v$ at 6.25μg/ml in BACTEC 12B medium using the BACTEC 460 radiometric system. The derivatives exhibited antitubercular activity.

Keywords: Isoniazid, Antitubercular.

Introduction

Tuberculosis 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]. Resistance of *Mycobacterium tuberculosis* strains to antimycobacterial agents is an increasing problem worldwide [3-5]. 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 a failure of the drug to penetrate or to be taken up by the microorganisms [7]. Fortunately, pharmacokinetic properties and cellular permeability of a drug can be modulated by derivatization to bioreversible forms of this

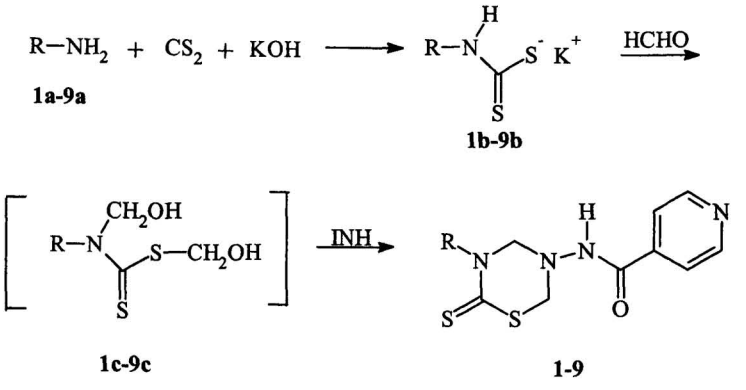
drug, namely prodrugs [8]. Moreover, resistance development to a drug can be also obviated through prodrug approach [9, 10].

Tetrahydro-2H-[1,3,5]thiadiazine-2-thione derivatives were found to insinuate the criterion of the prodrug approach that impart the desirable physicochemical properties to attached drugs, and liberate the parent drugs through chemical or enzymatic degradation [11-13]. Accordingly the current work describes the incorporation of INH in a tetrahydro-2H-[1, 3, 5]thiadiazine-2-thione moiety (THTT).

Investigation and Results

The prodrugs **1-9** were synthesized in a two-step reaction (Scheme). In the first step of the reaction dithiocarbamic acid salts **1b-9b** were formed by reaction of primary amines **1a-9a** with carbon disulphide and potassium hydroxide. However the second step of the reaction involved addition of formaline to the previously formed **1b-9b** to afford compounds 1c-9c (*in situ*). This was followed by progressive addition of INH solution in ethanolic phosphate buffer (pH 7.8) to provide the designed derivatives **1-9**. Structures of these synthesized compounds were verified on the basis of spectral and elemental methods of analyses.

The most differentiating stretching bands in IR spectra of the prodrugs **1-9** are: stretching absorption for the amidic NH at the range of 3340- 3400 cm^{-1}, aliphatic C-H stretching around 2855- 3011 cm^{-1}, aromatic C-H stretching around 3060- 3120 cm^{-1}, amidic C=O stretching at 1660-1676 cm^{-1}, pyridine C=N stretching at 1485-1496 cm^{-1} and C-S stretching around 1163- 1249 cm^{-1}. With the exception of the N^3- substituents of the THTT moiety, the ^1H NMR resonance of the remaining sites of the protons of the synthesized derivatives is almost superimposable. The ^1H NMR spectrum of the prodrug **1** revealed the presence of signals at 4.77 (bs, 4H, 4-CH_2, 6-CH_2), 7.7-8.9 (m, 8H, Ar C-H) and 10.5 (s, 1H, CONH, D_2O exchangeable) and the prodrug **3** showed signals at 3.25 (s, 3H, CH_3), 4.75 (bs, 4H, 4-CH_2, 6-CH_2), 7.75-8.82 (m, 7H, Ar C-H) and 10.75 (s, 1H, CONH, D_2O exchangeable). Lipophilicity of the synthesized derivatives **1-9** 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.



	R
1	2-pyridyl
2	3-methyl-2-pyridyl
3	4-methyl-2-pyridyl
4	5-methyl-2-pyridyl
5	3-hydroxy-2-pyridyl
6	3-carboxy-2-pyridyl
7	Pyridyl-4-carboxamido
8	Pyrimidin-2-yl
9	1,2,3,4-tetrazol-5-yl

Scheme. Synthetic protocol of the compounds

The synthesized prodrugs **1-9** were tested for their antimycobacterial activity *in vitro* against *Mycobacterium tuberculosis* $H_{37}R_v$ using the BACTEC 460 radiometric system. The results are summarized in Table 1.

Discussion

Rapid glance to the obtained results revealed that the prodrugs **1-9** exhibited antimycobacterial activity. The lipophilicity of the synthesized prodrugs increased remarkably compared with the parent drug, INH. This may render them more capable of

penetrating various biomembranes [14], consequently improving their permeation properties through mycobacterial cell membranes. The results showed that there was no improvement in antitubercular activity compared to the parent drug. Among the newer derivatives, compounds **7** and **8** showed a percentage activity of 85. 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.

Experimental

Synthesis of compounds

Melting points were determined on an electro thermal melting point apparatus (Buchi BM530) in open capillary tubes and are uncorrected. IR spectra (KBr disc) were recorded on Jasco IR Report 100 spectrometer. ^{1}H NMR spectra were scanned on a JEOL FX 90Q (Fourier Transform) NMR spectrometer using DMSO-d_6 as solvent. Chemical shifts are expressed in δ (ppm) relative to TMS as an internal standard. Elemental analyses (C, H, N and S) were performed on Perkin-Elmer model 240c analyzer.

Synthesis of 3-substituted-5-(4-pyridylcarboxamido) tetrahydro-2H-[1, 3, 5] thiadiazine -2- thione derivatives 1-9: General method

Carbon disulphide (60mmol) was added portion wise to a stirred mixture of the appropriate pyridyl or pyrimidinyl or tetrazolylamine **1a-9a** (10mmol) and potassium hydroxide (20%, 10mmol) in alcohol: water (2:1) water, stirring was continued at ambient temperature for 6h. Formaldehyde solution (35%, 22mmol) was added for the mixture and stirring was continued for further 2h. To the resulting clear solution, a solution of INH (10mmol) in a mixture of phosphate buffer (pH 7.8, 5ml) and ethanol (10ml) was added portion wise during 15min. After stirring for 6h at ambient temperature, dilute hydrochloric acid (5%, 5ml) was added and stirring was continued for further 1hr. The formed precipitate was collected by filtration, washed with methanol and dried. The crude product was crystallized from chloroform/methanol (1:1) to afford compounds **1-9**. Yields, m.p.s and physical data are given in Table 1.

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

Compound	M.P. (°C)	Yield (%)	Molecular Formula[a]	Scilog P[b]	Percentage inhibition[c] against *Mycobacterium tuberculosis* H37 Rv
1	154	62	$C_{14}H_{13}N_5OS_2$	1.694	82
2	164	42	$C_{15}H_{15}N_5OS_2$	1.708	86
3	140	66	$C_{15}H_{15}N_5OS_2$	1.780	83
4	145	67	$C_{15}H_{15}N_5OS_2$	1.619	83
5	147	65	$C_{14}H_{13}N_5O_2S_2$	1.003	84
6	170	45	$C_{15}H_{13}N_5O_3S_2$	0.513	82
7	150	63	$C_{15}H_{14}N_6O_2S_2$	1.150	85
8	151	49	$C_{13}H_{12}N_6OS_2$	1.507	85
9	155	47	$C_{10}H_{10}N_8OS_2$	2.076	79
INH	-	-	-	0.702	95
Rifampin	-	-	-	-	98

[a] Elemental analyses for C, H, N, S are within ± 0.5% of the theoretical values
[b] SciLog P was calculated using Alchemy 2000 software.
[c] Compounds were tested at a single concentration of 6.25 µg/ml

In vitro antimycobacterial screening

The synthesized prodrugs **1-9** were tested for their antimycobacterial activity *in vitro* against *Mycobacterium tuberculosis* $H_{37}R_v$ using the BACTEC 460 radiometric system [15, 16]. Stock solutions of test compounds were prepared in DMSO at 1mg/ml and sterilized by passage through 0.22mm PFTE filters (Millex- FG, Millipore, Bedford, MA). Controls received 50 ml DMSO. Rifampin (Sigma Chemical Co, St.Louis, MO) was included as a positive drug control. *Mycobacterium tuberculosis* $H_{37}R_v$ (ATCC 27294; American Type Culture Collection, Rockville, MD) was cultured at 37°c on a rotary shaker in Middle brook 7H9 broth (Difco Laboratories, Detroit MI) supplemented with 0.2%v/v glycerol and 0.5%v/v Tween 80 until the culture turbidity achieved an optical density 0.45- 0.55 at 550 nm. Assays were usually completed in 5-8 days and percent inhibition was measured as (1- GI of test sample/ GI of control) x 100 where GI represents growth index.

Acknowledgements

The authors are thankful to Dr. S. Ananthan from the Southern Research Institute, Birmingham, Alabama, USA, for the *in vitro* evaluation of antimycobacterial activity and the University Grants commission for funding the project.

Reference

1 Bloom B R, Murray C J L,
Tuberculosis: commentary on a reemergent killer.
Science 1992; 257, 1055-64.

2 Barnes P F, Blotch A B, Davidson P T, Snider D E, Jr.,
Tuberculosis in patients with human immunodeficiency virus infection.
N. Engl. J. Med. 1991; 324, 1644-50.

3 Sbarbaro J A,
'Multidrug'-resistant tuberculosis. It is time to focus on the private sector of medicine.
Chest 1997; 111, 1149-51.

4 Fujiwara P I, Cook S V, Rutherford C M, Crawford J T, Glickman S E, Kreiswirth B N, Sachdev P S, Osahan S S, Ebrahimzadeh A, Frieden T R,
A continuing survey of drug-resistant tuberculosis, New York City, April 1994.
Arch. Intern. Med. 1997; 157, 531-6.

5 Schaberg T, Gloger G, Reichert B, Mauch H, Lode H,
Resistant lung tuberculosis in Berlin 1987-1993.
Pneumologie 1996; 50, 21-7.

6 Blair I A, Timoco R M, Brodie M J, Clarc R A, Dollery T, Timbrell J A, Beever I A,
Plasma hydrazine concentrations in man after isoniazid and hydralazine administration.
Hum. Toxicol. 1985; 4, 195-202.

7 Mondell G L Sande M A,
In: The Pharmacological Basis of Therapeutics. 8th ed. Gilman A G, Rull T W, Nies A S, Taylor P.
New York: Pergamon Press, 1991: 1147.

8 Conradi R A, Burton P S, Borchardt R T,
In: Lipohilicity in Drug Action and Toxicology
Monographs of Methods and Principles in Medicinal Chemistry. 1st ed. Volume 4, Pliska V, Testa B, Waterbeemd H.
New York, Basel, Cambridge, Tokyo: VCH, Weinheim, 1996: 233.

9 Wechter W J, Johnson M A, Hall C M, Warner D T, Berger A E, Wenzel A H, Gish D T, Neil G L,
ara-Cytidine acylates. Use of drug design predictors in structure-activity relationship correlation.

J. Med. Chem. 1975: 18, 339-44.

10 Ryu E K, Ross R J, Matsushita T, MacCoss M, Hong C I, West C R,
Phospholipid-nucleoside conjugates. 3. Syntheses and preliminary biological evaluation of 1-beta-D-arabinofuranosylcytosine 5'-monophosphate-L-1,2-dipalmitin and selected 1-beta-D-arabinofuranosylcytosine 5-diphosphate-L-1,2-diacylglycerols.
J. Med. Chem. 1982; 25, 1322-9.

11 Ertan M, Tayhan A B, Yulung N,
Synthesis and antimicrobial activities of some new tetrahydro-2H-1,3,5-thiadiazine-2-thione derivatives of ampicillin.
Arch. Pharm. (Weinheim) 1990; 323, 605-9.

12 Aboul-Fadl T, El-Shorbagi A,
New carriers for representative peptides and peptide drugs.
Arch. Pharm. Pharm. Med. Chem. 1997: 330, 327-32.

13 Aboul-Fadl T, Hassanin K,
Tetrahydro-2H-1,3,5-thiadiazine-5-(4-pyridylcarboxamide)-2-thione derivatives as prodrugs for isoniazid; synthesis, investigations and in vitro antituberculous activity.
Pharmazie 1999: 54, 244-7.

14 Moss J, Bundgaard H,
Prodrugs of peptides. 6. Bioreversible derivatives of thyrotropin-releasing hormone (TRH) with increased lipophilicity and resistance to cleavage by the TRH-specific serum enzyme.
Pharm. Res. 1990: 7, 885-92.

15 Inderleid C B, Salfinger M,
In: Manual of Clinical Microbiology, 6th ed. Murray P R, Baron E J, Pfaller M A, Tenover F C, Yoken R H.
Washington/Main: ASM Press,1995: 1385.

16 Inderleid C B, Nash K A,
In: Antibiotics in Laboratory Medicine, 4th ed. Lorian V.
Baltimore/Main: Williams & Wilkins, 1996: 127.

Received April 8th, 2003
Accepted November 11th, 2003