Full Paper

Synthesis and Antituberculosis Activity of New Hydrazide Derivatives

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The increasing clinical importance of drug-resistant mycobacterial pathogens, especially *Mycobacterium tuberculosis*, has lent additional urgency to microbiological research and new antimycobacterial compound development. For this purpose, new hydrazide derivatives of imidazo[1,2-a]pyridine were synthesized and evaluated for antituberculosis activity. The reaction of 2-[(2-carboxyimidazo[1,2-*a*]pyridine-3-yl)sulfanyl]acetic acid hydrazide with various benzaldehydes gave *N*-(arylidene)-2-[(2-carboxyimidazo[1,2-*a*]pyridine-3-yl)sulfanyl]acetic acid hydrazide derivatives. The chemical structures of the compounds were elucidated by IR, ^{1}H-NMR, FAB-MS spectral data and elemental analysis. Antituberculosis activities of the synthesized compounds were determined by broth microdilution assay, the Microplate Alamar Blue Assay in BACTEC 12B medium. The results were screened *in vitro,* using the BACTEC 460 Radiometric System against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) at 6.25 μg/mL; the tested compounds showed significant inhibition.

Keywords: Antituberculosis activity / Hydrazide / Imidazo[1,2-*a*]pyridine

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Introduction

Tuberculosis (TB) has re-emerged both in industrial and developing countries [1 – 3]. Further contributing to the increased morbidity is the emergence of new strains of *Mycobacterium tuberculosis* resistant to some or all currently used antitubercular drugs [4, 5]. Particularly multidrug-resistant TB (MDR-TB) is alarming. The standard TB therapy is non-effective in controlling MDR-TB in high MDR-TB incidence areas [6]. There is great fear that the TB situation may get even worse with the spread of HIV worldwide [1] and this is one among other reasons for an urgent need to develop new TB drugs. The Alliance aims to get improved TB drugs to those who need them, drugs, which shorten or simplify treatment of TB or provide a more effective treatment of multidrug-resistant TB; or improve the treatment of latent TB infection or some combination of these [7]. As medicinal chemists, we may handle the problem of obtaining new TB drugs for a fast and better treatment, in two approaches: (i) synthesis of analogues, obtained by modifying or derivating existing chemical structures; (ii) and in case of the multidrug-resistant TB treatment, building the anti-TB strategy on the novelty of the chemical structure for the benefical advantage that the TB organism has not the chance to develop resistant [7, 8].

The heterocyclic hydrazones constitute an important class of biologically active drug molecules, which have attractive attention of medicinal chemists due to their antituberculosis activities [9 – 12]. On the other hand, a lot of studies were carried out on heterocyclic systems bearing an alkylsulfanyl group as a pharmacophore for antituberculosis activity. QSAR calculations carried out on various types of heterocycles proved that the activity is enhanced with electron-withdrawing substituents. An alkylsulfanyl group bound to an electron-deficient car-

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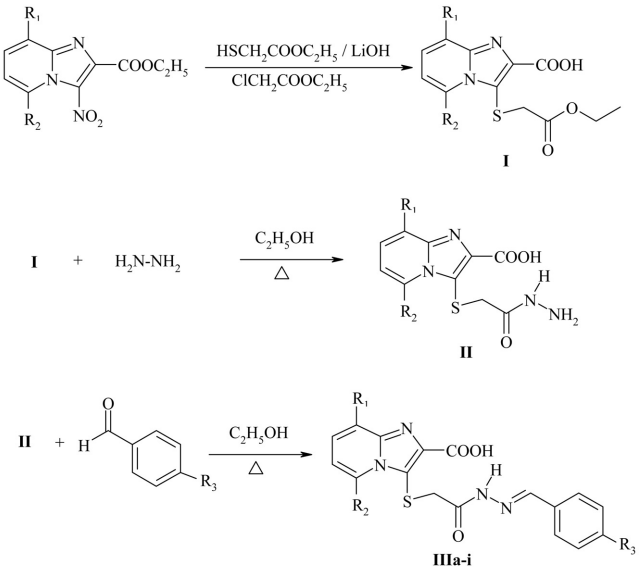
Abbreviations: growth index (GI); multidrug-resistant TB (MDR-TB); (quantitative) structure-activity relationship ((Q)SAR)

Table 1. Some characteristics of the compounds.

Compound	R_1	R_2	R_3	Yield (%)	Mp. (°C)	M.W.	Formula
IIIa	H	H	Cl	75	192–193	388	$C_{17}H_{13}ClN_4O_3S$
IIIb	H	H	CH_3	72	138–140	368	$C_{18}H_{16}N_4O_3S$
IIIc	H	H	OCH_3	68	160–162	384	$C_{18}H_{16}N_4O_4S$
IIId	H	H	NO_2	75	186–188	399	$C_{17}H_{13}N_5O_5S$
IIIe	CH_3	H	Cl	68	210–212	402	$C_{18}H_{15}ClN_4O_3S$
IIIf	CH_3	H	CH_3	73	190–191	382	$C_{19}H_{18}N_4O_3S$
IIIg	CH_3	H	NO_2	70	226–227	413	$C_{18}H_{15}N_5O_5S$
IIIh	H	CH_3	Cl	74	148–150	402	$C_{18}H_{15}ClN_4O_3S$
IIIi	H	CH_3	CH_3	69	136–137	382	$C_{19}H_{18}N_4O_3S$

bon atom in various heterocycles is responsible for antimycobacterial activity [13–16].

In view of these data, we aimed at the synthesis and antituberculosis evaluations of new *N*-(benzylidene)-2-[(2-carboxyimidazo[1,2-*a*]pyridine-3-yl)sulfanyl]acetic acid hydrazide derivatives. We have chosen imidazo[1,2-*a*]pyridines, which have emerged as potentially interesting drugs, particularly with regard to their antituberculosis activity [17] among the various heterocycles that have attracted the attention as potential antitubercular agents as the basic heterocyclic moiety.



Scheme 1. Synthesis of the *N*-(arylidene)-2-[(2-carboxyimidazo[1,2-*a*]pyridine-3-yl)sulfanyl]acetic acid hydrazide derivatives **IIIa–i**.

Results and discussion

In this present work, a series of nine new compounds were synthesized. Scheme 1 illustrates the way used for the preparation of target compounds. As starting materials, ethyl 3-nitroimidazo[1,2-*a*]pyridine-2-yl carboxylates were used to produce ethyl 2-[(2-carboxyimidazo[1,2-*a*]pyridine-3-yl)sulfanyl]acetates. The 2-[(2-carboxyimidazo[1,2-*a*]pyridine-3-yl)sulfanyl]acetic acid hydrazides **II** were prepared by reacting ethyl 2-[(2-carboxyimidazo[1,2-*a*]pyridine-3-yl)sulfanyl]acetates **I** with hydrazine hydrate. The condensation of the acid hydrazides with appropriate benzaldehydes resulted in the formation of *N*-(arylidene)-2-[(2-carboxyimidazo[1,2-*a*]pyridine-3-yl)sulfanyl]acetic acid hydrazide derivatives **IIIa–i**. Some characteristics of the synthesized compounds are shown in Table 1.

The structures of the obtained compounds were elucidated by spectral data. In the IR spectra, some significant stretching bands due the N-H, C=O, C=N and C-O-C were at about 3220–3195 cm^{-1}, 1670–1645 cm^{-1}, 1605–1545 cm^{-1} and 1250–1210 cm^{-1}, respectively.

In the ^{1}H-NMR spectra, the signal due to S-CH_2 protons and N=CH proton present in all compounds, appeared at 3.75–3.90 ppm and 8.40–8.60 ppm as singlet, respectively. The NH proton was observed at 12.00–12.40 ppm as a doublet. All the other aromatic and aliphatic protons were observed at the expected regions. All compounds gave satisfactory elemental analyses. Mass spectra (MS (FAB)) of the compounds showed a [M+1] peaks in agreement with their molecular weight.

The antituberculosis activities of the synthesized compounds were screened *in vitro* using a BACTEC 460 radiometric system against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) at 6.25 μg/mL. Rifampicin was used as the test standard. All of the tested compounds showed significant antituberculosis activity as can be inferred from Table 2. The compounds **IIId** and **IIIg** which the 4-nitrobenzylidene derivatives showed the highest inhibitions with 68%. Other compounds showed varying inhibition values between 45–53%.

SAR observation showed that 4-nitro substitution on benzylidene affects the activity.

Table 2. Antituberculosis activity of the compounds.

Compound	**IIIa**	**IIIb**	**IIIc**	**IIId**	**IIIe**	**IIIf**	**IIIg**	**IIIh**	**IIIi**	Rifampicin
MIC (μg/mL)	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	0.25
Inhibition (%)	45	50	48	68	52	47	68	53	49	98

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The authors have declared no conflict of interest.

Experimental

Chemistry

All reagents were purchased from commercial suppliers and were used without further purification. Melting points were determined by using an Electrothermal 9100 digital melting point apparatus (Barnstead International, Dubuque, IA, USA) and were uncorrected. The compounds were checked for purity by TLC on silica gel 60 F_{254} (Merck, Darmstadt, Germany). Spectroscopic data were recorded on the following instruments: Elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyser (Perkin Elmer, Wellesley, MA, USA), IR (ν, cm^{-1}), Shimadzu 435 IR spectrophotometer (Shimadzu, Tokyo, Japan); ^{1}H-NMR spectra (δ, ppm, Hz) were recorded on a Bruker 250 MHz spectrometer (Bruker, Billerica, MA, USA) in DMSO-d_6 with TMS as an internal standard. MS-FAB$^+$ was recorded on VG Quattro mass spectrometer (Agilent Technologies, Santa Clara, CA, USA).

General procedure for synthesis of the compounds Ethyl 2-[(2-carboxyimidazo[1,2-a]pyridine-3-yl)sulfanyl]acetates I

These compounds were prepared as starting materials in accordance with the method described in the literature [18].

2-[(2-Carboxyimidazo[1,2-a]pyridine-3-yl)sulfanyl]acetic acid hydrazides II

These compounds were prepared according to the literature, by reacting ethyl 2-[(2-carboxyimidazo[1,2-*a*]pyridine-3-yl)sulfanyl]acetates **I** with hydrazine hydrate [19].

N-(arylidene)-2-[(2-carboxyimidazo[1,2-a]pyridine-3-yl)sulfanyl]acetic acid hydrazides IIIa – i

Equimolar quantities of acid hydrazides **II** (30 mmol) and appropriate benzaldehydes in 25 mL of absolute ethanol were refluxed for 3-5 h. The resulting solid was filtered and recrystallized from ethanol. **IIIa – i**: IR (KBr, cm^{-1}): 3195 – 3220 (NH), 1645 – 1670 (CO), 1605 – 1545 (C=N) and (C=C), 1250 – 1210 (C-O-C).

N-(4-chlorobenzylidene)-2-[(2-carboxyimidazo[1,2-a]pyridine-3-yl)sulfanyl]acetic acid hydrazide IIIa

^{1}H-NMR (250 MHz, DMSO-d_6, δ ppm): 3.85 (s, 2H, S-CH_2), 7.15 – 7.80 (m, 7H, aromatic protons), 8.60 (s, 1H, N=CH), 8.70 – 8.85 (m, 1H, aromatic proton), 12.10 (s, 1H, NH). MS (FAB) [M+1]: *m*/*z* 389. Anal. Calc. for $C_{17}H_{13}ClN_4O_3S$:C, 52.51; H, 3.37; N, 14.41. Found: C, 52.53; H, 3.39; N, 14.43.

N-(4-methylbenzylidene)-2-[(2-carboxyimidazo[1,2-a]pyridine-3-yl)sulfanyl]acetic acid hydrazide IIIb

^{1}H-NMR (250 MHz, DMSO-d_6, δ ppm): 2.35 (s, 3H, CH_3), 3.80 (s, 2H, S-CH_2), 7.10 – 7.85 (m, 7H, aromatic protons), 8.55 (s, 1H, N=CH), 8.60 – 8.80 (m, 1H, aromatic proton), 12.00 (s, 1H, NH). MS (FAB) [M+1]: *m*/*z* 369. Anal. Calc. for $C_{18}H_{16}N_4O_3S$:C, 58.68; H, 4.38; N, 15.21. Found: C, 58.73; H, 4.39; N, 15.20.

N-(4-methoxybenzylidene)-2-[(2-carboxyimidazo[1,2-a]pyridine-3-yl)sulfanyl]acetic acid hydrazide IIIc

^{1}H-NMR (250 MHz, DMSO-d_6, δ ppm): 3.80 (s, 2H, S-CH_2), 3.90 (s, 3H, OCH_3), 7.05 – 7.75 (m, 7H, aromatic protons), 8.45 (s, 1H, N=CH), 8.60 – 8.70 (m, 1H, aromatic proton), 12.15 (s, 1H, NH). MS (FAB) [M+1]: *m*/*z* 385. Anal. Calc. for $C_{18}H_{16}N_4O_4S$:C, 56.24; H, 4.20; N, 14.57. Found: C, 56.21; H, 4.25; N, 14.60.

N-(4-nitrobenzylidene)-2-[(2-carboxyimidazo[1,2-a]pyridine-3-yl)sulfanyl]acetic acid hydrazide IIId

^{1}H-NMR (250 MHz, DMSO-d_6, δ ppm): 3.85 (s, 2H, S-CH_2), 7.10 – 7.80 (m, 3H, imidazopyridine protons), 8.00 (d, *J* = 8.76 Hz, 2H, aromatic protons), 8.35 (d, *J* = 8.78 Hz, 2H, aromatic protons), 8.70 – 8.85 (m, 2H, N=CH and imidazopyridine proton), 12.40 (s, 1H, NH). MS (FAB) [M+1]: *m*/*z* 400. Anal. Calc. for $C_{17}H_{13}N_5O_5S$:C, 51.13; H, 3.28; N, 17.54. Found: C, 51.15; H, 3.34; N, 17.51.

N-(4-chlorobenzylidene)-2-[(2-carboxy-8-methylimidazo[1,2-a]pyridine-3-yl)sulfanyl]acetic acid hydrazide IIIe

^{1}H-NMR (250 MHz, DMSO-d_6, δ ppm): 2.65 (s, 3H, CH_3), 3.95 (s, 2H, S-CH_2), 7.05 – 7.65 (m, 6H, aromatic protons), 8.50 (s, 1H, N=CH), 8.60 – 8.75 (m, 1H, aromatic proton), 12.00 (s, 1H, NH). MS (FAB) [M+1]: *m*/*z* 403. Anal. Calc. for $C_{18}H_{15}ClN_4O_3S$:C, 53.73; H, 3.73; N, 13.93. Found: C, 53.74; H, 3.72; N, 13.95.

N-(4-methylbenzylidene)-2-[(2-carboxy-8-methylimidazo[1,2-a]pyridine-3-yl)sulfanyl]acetic acid hydrazide IIIf

^{1}H-NMR (250 MHz, DMSO-d_6, δ ppm): 2.35 (s, 3H, phenyl-CH_3), 2.60 (s, 3H, CH_3), 3.75 (s, 2H, S-CH_2), 7.10 – 7.65 (m, 6H, aromatic protons), 8.45 (s, 1H, N=CH), 8.50 – 8.60 (m, 1H, aromatic proton), 12.05 (s, 1H, NH). MS (FAB) [M+1]: *m*/*z* 383. Anal. Calc. for $C_{19}H_{18}N_4O_3S$:C, 59.67; H, 4.74; N, 14.65. Found: C, 59.70; H, 4.72; N, 14.65.

N-(4-nitrobenzylidene)-2-[(2-carboxy-8-methylimidazo[1,2-a]pyridine-3-yl)sulfanyl]acetic acid hydrazide IIIg

^{1}H-NMR (250 MHz, DMSO-d_6, δ ppm): 2.55 (s, 3H, CH_3), 3.75 (s, 2H, S-CH_2), 7.00 – 8.20 (m, 6H, aromatic protons), 8.50 – 8.65 (m, 2H, N=CH and imidazopyridine proton), 12.10 (s, 1H, NH). MS (FAB) [M+1]: *m/z* 414. Anal. Calc. for $C_{18}H_{15}N_5O_5S$:C, 52.30; H, 3.66; N, 16.94. Found: C, 52.34; H, 3.69; N, 16.91.

N-(4-chlorobenzylidene)-2-[(2-carboxy-5-methylimidazo[1,2-a]pyridine-3-yl)sulfanyl]acetic acid hydrazide IIIh

^{1}H-NMR (250 MHz, DMSO-d_6, δ ppm): 2.75 (s, 3H, CH_3), 3.90 (s, 2H, S-CH_2), 7.00 – 7.70 (m, 6H, aromatic protons), 8.45 (s, 1H, N=CH), 8.50 – 8.70 (m, 1H, aromatic proton), 12.10 (s, 1H, NH). MS (FAB) [M+1]: *m/z* 403. Anal. Calc. for $C_{18}H_{15}ClN_4O_3S$:C, 53.73; H, 3.73; N, 13.93. Found: C, 53.65; H, 3.69; N, 13.94.

N-(4-methylbenzylidene)-2-[(2-carboxy-5-methylimidazo[1,2-a]pyridine-3-yl)sulfanyl]acetic acid hydrazide IIIi

^{1}H-NMR (250 MHz, DMSO-d_6, δ ppm): 2.30 (s, 3H, phenyl-CH_3), 2.75 (s, 3H, CH_3), 3.85 (s, 2H, S-CH_2), 7.15 – 7.65 (m, 6H, aromatic protons), 8.40 (s, 1H, N=CH), 8.45 – 8.65 (m, 1H, aromatic proton), 12.15 (s, 1H, NH). MS (FAB) [M+1]: *m/z* 383. Anal. Calc. for $C_{19}H_{18}N_4O_3S$:C, 59.67; H, 4.74; N, 14.65. Found: C, 59.65; H, 4.70; N, 14.60.

Microbiology

In-vitro evaluation of antimycobacterial activity against Mycobacterium tuberculosis $H_{37}Rv$

Antituberculotic activities of the compounds were tested at the center of Tuberculosis Antimicrobial Acquisiton & Coordinating Facility (TAACF). Compounds were tested for *in-vitro* antituberculosis activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) at 6.25 μg/mL, in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA). Compounds exhibiting fluorescence are tested in the BACTEC 460 Radiometric System [20].

BACTEC radiometric method of susceptibility testing

Inocula for susceptibility testing were either from a positive BACTEC isolation vial with a growth index (GI) of 500 and more, or suspension of organism isolated earlier on conventional medium. The culture was well mixed with a syringe and 0.1 mL of a positive BACTEC culture was added to each of the vials containing the test drugs. The drug vials contained rifampicin (0.25 μg/mL). A control vial was inoculated with a 1 : 100 microdilution of the culture. A suspension equivalent to a Mc-Farland No.1 standard was prepared in the same manner as a BACTEC positive vial, when growth from a solid medium was used. Each vial was tested immediately on a BACTEC instrument to provide CO_2 in the headspace. The vials were incubated at 37°C and tested daily with a BACTEC instrument. When the GI in the control read at least 30, the increase in GI (ΔGI) from the previous day in the control was compare with that in the drug vial. The following formula was used to interpret results:

ΔGI control > ΔGI drug = Susceptible

ΔGI control < ΔGI drug = Resistant

If a clear susceptibility pattern (the difference of ΔGI of control and the drug bottle) was not seen at the time, the control ΔGI is 30, the vials were read for one or two additional days to establish a definite pattern of ΔGI differences.

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