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Preliminary communication

Synthesis and antituberculosis activity of some *N*-pyridyl-*N*′-thiazolylhydrazine derivatives

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ABSTRACT

In this study, new *N*-(1-arylethylidene)-*N*′-(4-arylthiazol-2-yl)hydrazine derivatives were synthesized and evaluated for their antituberculosis activity. The chemical structures of the compounds were elucidated by IR, NMR and FAB$^+$-MS spectral data and Elemental Analyses. The initial screen was conducted against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA). The VERO cell cytotoxicity assay was done in parallel with the TB Dose Response assay. Viability was assessed using Promega's Cell Titer-Glo Luminescent Cell Viability Assay. Cytotoxicity was determined from the dose–response curve as the CC50 using a curve-fitting program. One of the compounds showed high activity with low toxicity.

1. Introduction

Tuberculosis (TB) is the leading infectious disease among adults and youth, with one-third of the world's population infected with *Mycobacterium tuberculosis*. According to the World Health Organization (WHO), in 2006 there were 9.2 million new cases and 1.7 million deaths from TB around the world. The incidence of TB infection has steadily risen in the last decade [1]. The reemergence of TB infection has been further complicated. Two developments make the resurgence in TB especially alarming. The first is pathogenic synergy with HIV. The overall incidence of TB in HIV-positive patients is 50 times that of the rate for HIV-negative individuals [2]. The second is the emergence of drug-resistant and multi-drug-resistant TB (MDR-TB). In the WHO report, Anti-Tuberculosis Drug Resistance in the World, is based on information collected between 2002 and 2006 on 90 000 TB patients in 81 countries. It is also found that extensively drug-resistant tuberculosis (XDR-TB), a virtually untreatable form of the respiratory disease, has been recorded in 45 countries [1,3].

The standard "short" course treatment for tuberculosis (TB), is isoniazid (INH), rifampicin, pyrazinamide, and ethambutol for two months, then INH and rifampicin alone for a further four months. The patient is considered cured at six months. For latent tuberculosis, the standard treatment is six to nine months of INH alone. Non-compliance has contributed to the appearance of XDR-TB strains. MDR-TB is resistant to, at least, INH and rifampicin, often taking a further two years to treat with second-line drugs. XDR-TB also exhibits resistance to second-line drugs including fluoroquinolones and one of capreomycin, kanamycin or amikacin [4,5].

So there are three reasons usually given for needing new tuberculosis drugs: (i) to improve current treatment by shortening the total duration of treatment and/or by providing for more widely spaced intermittent treatment, (ii) to improve the treatment of MDR-TB, and (3) to provide for more effective treatment of latent tuberculosis infection (LTBI) [6].

There are two basic approaches to develop a new drug for TB: (i) synthesis of analogues, modifications or derivatives of existing compounds for shortening and improving TB treatment and, (ii) searching novel structures which the TB organism has never encountered with before, for the treatment MDR-TB [7].

To pursue this goal, our research efforts are directed to the modification of INH, which is a well known antituberculosis agent bearing pyridine and hydrazine moieties. Modifying either of these molecules has been a challenge taken up by several research groups [8–12].

The literature survey showed an important relationship between serum levels of INH and the emergence of INH-resistant cultures in patients with tuberculosis [13]. Serum concentrations are influenced by a number of factors, but among them the most important factor is the enzymic acetylation of INH by *N*-acetyltransferase

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(NAT). This represents a major metabolic pathway for INH in human beings. NAT is also present in mycobacteria. It was found that recombinant NAT from *M. tuberculosis* acetylates INH *in vitro* and it was proposed that NAT in mycobacteria could have a role in acetylating and hence inactivating INH [14]. It is assumed that resistance to INH in mycobacteria can thus be related to increased expression of NAT and it is proposed that the chemical modification of the hydrazine unit of INH with a functional group blocks acetylation, maintaining strong activity and improving clinical outcomes [11,12]. This hypothesis was verified by many studies with their significant results [8–12,15–17].

In this study, we planned to synthesize new molecules bearing modified hydrazine moieties on different positions of pyridine ring. We chose the thiazole ring for modification because of the importance of thiazole derivatives as antituberculosis agents [18–21].

2. Chemistry

The synthetic route of the compounds is outlined in Scheme 1. For the synthesis of the title compounds, 1-(1-arylethylidene)-thiosemicarbazide (**1a–c**) required as starting material was prepared by the reaction of acetylpyridines with thiosemicarbazide [22]. The reaction of equimolar quantities of thiosemicarbazide (**1a–c**) with 1-(2-thiophenyl)-2-bromoethanone or 1-(2-hydroxy-5-methoxyphenyl)-2-bromoethanone in the presence of isopropyl alcohol resulted in the formation of the title compounds (**2a–f**) (Table 1).

3. Pharmacology

3.1. Antituberculosis activity and cytotoxicity

The initial screen is conducted against *M. tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA) [23]. One of the compounds showed significant antituberculosis activity as can be inferred from Table 2.

The VERO cell cytotoxicity assay [24] is done in parallel with the TB Dose Response assay. Viability is assessed using Promega's Cell Titer-Glo Luminescent Cell Viability Assay [25].

4. Results and discussion

The structures of compounds **2a–f** were confirmed by elemental analyses, MS-FAB, IR and ^{1}H NMR spectral data. All compounds gave satisfactory elemental analysis. The mass spectra (MS (FAB)) of the compounds showed M + 1 peaks, in agreement with their molecular formula. The IR spectra of the compounds showed NH bands at 3335–3199 cm^{-1} and C=C, C=N bands at 1600–1435 cm^{-1} regions respectively.

In the 400 MHz ^{1}H NMR spectra of the compounds, the CH_3 protons were observed as singlet at 2.30–2.45 ppm. The NH proton appeared at 10.50–10.90 ppm. The OCH_3 and OH protons of **2d–f** were observed at 3.70–3.80 ppm and 11.50–11.90 ppm respectively. All the other aromatic protons were observed at expected regions.

The results of antituberculosis and cytotoxicity screening of newly prepared compounds **2a–f** are expressed in Table 2. The very important result was observed at antituberculosis activity screening for one of the compounds. The compound **2d** showed high antituberculosis activity (IC_{50}: 6.22 μg/mL and IC_{90}: 6.78 μg/mL) and low cytotoxicity (CC_{50}: >40 μg/mL). Because of SI value of the compound **2d** ≥10, further tests are in progress.

5. Conclusion

A series of novel *N*-(1-arylethylidene)-*N*′-(4-arylthiazol-2-yl)hydrazine derivatives **2** were synthesized and their antituberculosis activities have been evaluated. Among these series, compound **2d** including 2-pyridyl and 2-hydroxy-5-methoxyphenyl showed significant antituberculosis activity. On the other hand compound **2a**, which also includes 2-pyridyl moiety, showed notable antituberculosis activity. As a result, we can say that the 2-pyridyl derivatives are more active than other pyridyl derivatives when their antituberculosis activity is compared.

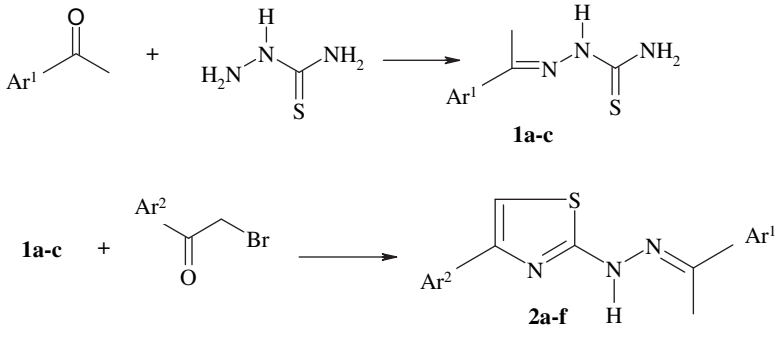
6. Experimental

6.1. Chemistry

All melting points (m.p.) were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck). Spectroscopic data were recorded on the following instruments: IR, Shimadzu 435 IR spectrophotometer; ^{1}H NMR, Bruker 400 MHz NMR spectrometer in DMSO-d_6 using TMS as an internal standard; elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyser; MS-FAB$^+$, VG Quattro mass spectrometer.

6.1.1. Preparation of 1-(1-arylethylidene)thiosemicarbazide (*1a–c*)

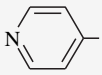
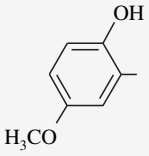
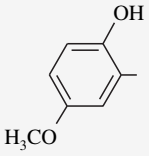
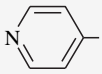
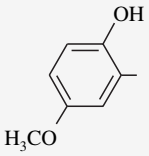
In a flask equipped with a reflux condenser, a mixture of thiosemicarbazide (50 mmol) and the appropriate acetylpyridine (50 mmol) is reacted in *n*-butanol (100 ml) in the presence of



Ar^1: 2-pyridyl, 3-pyridyl, 4-pyridyl Ar^2: 2-thiophenyl, 2-hydroxy-5-metoxyphenyl

Scheme 1. Synthetic protocol of the title compounds.

Table 1
Some characteristics of the compounds.

Comp.	Ar^1	Ar^2	m.p. (°C)	Yield (%)	Mol. formula	M.W.
2a			244–246	75	$C_{14}H_{12}N_4S_2$	300
2b			247–249	72	$C_{14}H_{12}N_4S_2$	300
2c			227–228	78	$C_{14}H_{12}N_4S_2$	300
2d			249–251	85	$C_{17}H_{16}N_4O_2S$	340
2e			219–221	88	$C_{17}H_{16}N_4O_2S$	340
2f			275 dec.	82	$C_{17}H_{16}N_4O_2S$	340

a catalytic amount of acetic acid. The mixture is then refluxed for 3 h and the obtained solid is filtered and used without further purification [22].

*6.1.2. Preparation of N-(1-arylethylidene)-N′-(4-arylthiazol-2-yl)-hydrazines (**2a–f**)*

1-(1-Arylethylidene)thiosemicarbazide (**1a–c**) (20 mmol) and 1-(2-thiophenyl)-2-bromoethanone or 1-(2-hydroxy-5-methoxyphenyl)-2-bromoethanone (20 mmol) are stirred in refluxing isopropyl alcohol (50 ml) for 3 h. The mixture is then allowed to cool, basified with saturated $NaHCO_3$ water solution, filter and then wash with cool water, dried, and crystallized from ethanol. Some characteristics of the synthesized compounds are shown in Table 1.

Table 2
Antituberculosis activity and cytotoxicity of the compounds

Comp.	MABA: $H_{37}Rv$ data		Cell Titer-Glo: Vero Cell CC_{50} (μg/mL)	SI (CC_{50}/IC_{90})
	IC_{50} (μg/mL)	IC_{90} (μg/mL)		
2a	48.39	55.23	–	
2b	92.16	>100	–	–
2c	>100	>100	–	–
2d	6.22	6.78	>40	>5.89
2e	>100	>100	–	–
2f	>100	>100	–	–

Compounds **2a–f**: IR (KBr) υ_{max} (cm^{-1}): 3335–3199 (NH), 1600–1435 (C=C and C=N).

*6.1.2.1. N-[1-(2-Pyridyl)ethylidene]-N′-[4-(2-thiophenyl)thiazol-2-yl]-hydrazine (**2a**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.40 (3H, s, CH_3), 7.90–9.40 (8H, m, aromatic protons), 10.90 (1H, s, NH). For $C_{14}H_{12}N_4S_2$ calculated: 55.98% C, 4.03% H, 18.65% N; found: 55.99% C, 4.07% H, 18.69% N. MS-FAB$^+$: *m/z*: 301(100%) [M + 1].

*6.1.2.2. N-[1-(3-Pyridyl)ethylidene]-N′-[4-(2-thiophenyl)thiazol-2-yl]-hydrazine (**2b**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.40 (3H, s, CH_3), 7.70–9.30 (8H, m, aromatic protons), 10.50 (1H, s, NH). For $C_{14}H_{12}N_4S_2$ calculated: 55.98% C, 4.03% H, 18.65% N; found: 55.95% C, 4.00% H, 18.66% N. MS-FAB$^+$: *m/z*: 301(100%) [M + 1].

*6.1.2.3. N-[1-(4-Pyridyl)ethylidene]-N′-[4-(2-thiophenyl)thiazol-2-yl]-hydrazine (**2c**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.30 (3H, s, CH_3), 7.10–8.90 (8H, m, aromatic protons), 10.60 (1H, s, NH). For $C_{14}H_{12}N_4S_2$ calculated: 55.98% C, 4.03% H, 18.65% N; found: 56.01% C, 4.01% H, 18.61% N. MS-FAB$^+$: *m/z*: 301(100%) [M + 1].

*6.1.2.4. N-[1-(2-Pyridyl)ethylidene]-N′-[4-(2-hydroxy-5-methoxyphenyl)thiazol-2-yl]hydrazine (**2d**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.35 (3H, s, CH_3), 3.80 (3H, s, OCH_3), 6.70–8.60 (8H, m, aromatic protons), 10.80 (1H, s, NH), 11.60 (1H, s, OH). For

$C_{17}H_{16}N_4O_2S$ calculated: 59.98% C, 4.74% H, 16.46% N; found: 59.95% C, 4.77% H, 16.48% N. MS-FAB$^+$: m/z: 341(100%) [M + 1].

*6.1.2.5. N-[1-(3-Pyridyl)ethylidene]-N′-[4-(2-hydroxy-5-methoxyphenyl)thiazol-2-yl]hydrazine (**2e**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.40 (3H, s, CH_3), 3.75 (3H, s, OCH_3), 6.80–9.00 (8H, m, aromatic protons), 10.75 (1H, s, NH), 11.50 (1H, s, OH). For $C_{17}H_{16}N_4O_2S$ calculated: 59.98% C, 4.74% H, 16.46% N; found: 59.97% C, 4.78% H, 16.50% N. MS-FAB$^+$: m/z: 341(100%) [M + 1].

*6.1.2.6. N-[1-(4-Pyridyl)ethylidene]-N′-[4-(2-hydroxy-5-methoxyphenyl)thiazol-2-yl]hydrazine (**2f**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.35 (3H, s, CH_3), 3.70 (3H, s, OCH_3), 6.70–8.80 (8H, m, aromatic protons), 10.60 (1H, s, NH), 11.90 (1H, s, OH). For $C_{17}H_{16}N_4O_2S$ calculated: 59.98% C, 4.74% H, 16.46% N; found: 60.01% C, 4.74% H, 16.42% N. MS-FAB$^+$: m/z: 341(100%) [M + 1].

6.2. Pharmacological evaluation

6.2.1. Primary screen (dose response)

6.2.1.1. Determination of a 90% inhibitory concentration (IC_{90}). The initial screen is conducted against *M. tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA) [23]. Compounds are tested in ten 2-fold dilutions, typically from 100 μg/mL to 0.19 μg/mL. The IC_{90} is defined as the concentration effecting a reduction in fluorescence of 90% relative to controls. This value is determined from the dose–response curve using a curve-fitting program. Any IC_{90} value of $\leq$10 μg/mL is considered "Active" for antitubercular activity. The "Active" compounds are considered for "Secondary Screening".

6.2.2. Secondary screen

6.2.2.1. Determination of mammalian cell cytotoxicity (CC_{50}). The VERO cell cytotoxicity assay [24] is done in parallel with the TB Dose Response assay. After 72 h exposure, viability is assessed using Promega's Cell Titer-Glo Luminescent Cell Viability Assay [25], a homogeneous method of determining the number of viable cells in culture based on quantitation of the ATP present. Cytotoxicity is determined from the dose–response curve as the CC_{50} using a curve-fitting program. Ultimately, the CC_{50} is divided by the IC_{90} to calculate an SI (Selectivity Index) value. SI values of $\geq$10 are considered for further testing.

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