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Original article

Synthesis and antituberculosis activity of new thiazolylhydrazone derivatives

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Abstract

The increasing clinical importance of drug-resistant mycobacterial pathogens has lent additional urgency to microbiological research and new antimycobacterial compound development. For this purpose, new thiazolylhydrazone derivatives were synthesized and evaluated for antituberculosis activity. The reaction of thiosemicarbazide with acetophenone derivatives gave 1-(1-arylethylidene)thiosemicarbazide (**1**). The *N*-(1-arylethylidene)-*N*′-[4-(indan-5-yl)thiazol-2-yl]hydrazone (**3**) derivatives were synthesized by reacting 1-(1-arylethylidene)thiosemicarbazide with 1-(5-indanyl)-2-bromoethanone (**2**). The chemical structure of the compounds was elucidated by elemental analyses, IR, ^{1}H NMR, MS-FAB$^+$ spectral data. Antituberculosis activities of the synthesized compounds were determined by broth microdilution assay, the Microplate Alamar Blue Assay, in BACTEC12B medium and the results were screened in vitro, using BACTEC 460 Radiometric System against *Mycobacterium tuberculosis* H_{37}Rv (ATCC 27294) at 6.25 μg/ml and some of the tested compounds showed important inhibition ranging from 92% to 96%. The compounds were also investigated for their cytotoxic properties on normal mouse fibroblast (NIH/3T3) cell line and the results obtained here showed that all the compounds used have no significant cytotoxicity at the concentrations under 50 μg/ml.

Keywords: Thiazolylhydrazone; Antituberculosis activity; Toxicity

1. Introduction

According to alarming data from the World Health Organisation, tuberculosis (TB) has spread to every corner of the globe [1]. As much as one-third of the world's population is currently infected and more than 5000 people die from TB every day. A large number of the infected people are carriers of the latent form, which creates a potentially dangerous source of the illness for the future. The HIV pandemic has led to the rapid growth of the TB epidemic and increased the likelihood of people dying from TB. Another factor contributing to the rise in TB infections, and consequently to the increased number of deaths, is the emergence of multiple drug resistance (MDR) [2–4].

There is an urgent need to develop new TB drugs [5]. However, no new TB drugs have been developed in about 40 years. Although TB can be cured with the current therapy, the six months needed to treat the disease is too long, and the treatment often has significant toxicity. These factors make patient compliance to therapy very difficult and this noncompliance frequently selects for drug-resistant TB bacteria. The current TB problem clearly demonstrates the need for a reevaluation of our knowledge of the current TB drugs and chemotherapy and the need for new and better drugs that are not only active against drug-resistant TB but also, more importantly, shorten the requirement for six months of therapy [6].

To pursue this goal, our research efforts are directed to find new chemical classes of antimycobacterially active agents. The methods of investigation of structure–activity relationships

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(SARs) enabled us to find some new pharmacophores of the above-mentioned activity. Many studies were carried out on heterocyclic systems bearing a hydrazone structure as a pharmacophore [7–13]. Among them especially thiazole residue [14–17] had taken our interest.

Keeping these observations in mind, we decided to undertake the synthesis of *N*-(1-arylethylidene)-*N*′-[4-(indan-5-yl) thiazol-2-yl]hydrazones carrying a thiazolylhydrazone moiety and to study their antituberculosis activity and toxicity.

2. Chemistry

The synthetic route of compounds is outlined in Scheme 1. For the synthesis of the title compounds, 1-(1-arylethylidene)-thiosemicarbazide (**1**) required as starting material was prepared by the reaction of acetophenone derivatives with thiosemicarbazide [18]. The reaction of equimolar quantities of thiosemicarbazide (**1**) with 1-(5-indanyl)-2-bromoethanone (**2**) in the presence of isopropyl alcohol resulted in the formation of the title compounds (**3a–f**) (Table 1).

3. Biology

3.1. Antituberculosis activity

All of the compounds were evaluated for in vitro antituberculosis activity against *Mycobacterium tuberculosis*, as a part of the TAACF TB screening program under direction of the US National Institute of Health, the NIAID division. Rifampicin was used as a reference drug. Primary screening was conducted at a single concentration, 6.25 μg/ml, against *M. tuberculosis* $H_{37}Rv$ (ATTCC 27294), in BACTEC 12B medium, using the Microplate Alamar Blue Assay (MABA) [19]. Compounds effecting <90% inhibition in the primary screening (MIC > 6.25 μg/ml) were not generally evaluated further. Some of the compounds showed significant antituberculosis activity as can be inferred from Table 2.

3.2. Toxicity

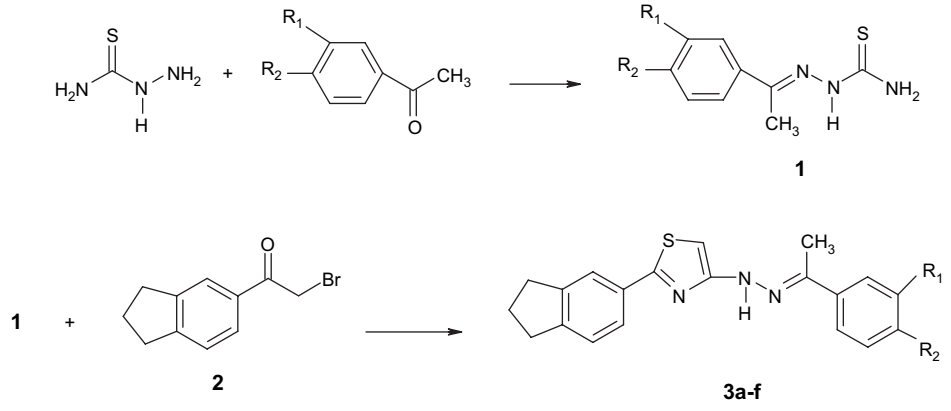
The level of cellular MTT (Sigma) reduction was quantified as previously described in the literature with small modifications [20–22].

4. Results, discussion and conclusion

In the present work, six new compounds were synthesized. The formulas of compounds (**3a–f**) were found by elemental analyses and their structures were determined by IR, ^{1}H NMR and MS-FAB$^+$ spectral data. The IR data were very informative and provided evidence for the formation of the expected structures. In the IR spectra, some significant stretching bands due N–H, C=N and C=C were at 3309–3224 cm^{-1}, 1612–1484 cm^{-1}, respectively. The ^{1}H NMR spectra data were also consistent with the assigned structures. In the 250 MHz ^{1}H NMR spectrum of compounds, protons C_2 of indane resonated as a pentaplet at 1.95–2.10 ppm, protons C_1 and C_3 of indane resonated as a quartet at 2.70–3.00 ppm, NH proton was observed as a broad at 5.95–6.90 ppm, and all the other aromatic and aliphatic protons were observed at expected regions. The mass spectra (MS(FAB)) of compounds showed [M + 1] peaks, in agreement with their molecular formula. All compounds gave satisfactory elemental analysis.

In conclusion, a series of novel *N*-(1-arylethylidene)-*N*′-[4-(indan-5-yl)thiazol-2-yl]hydrazone (**3**) derivatives (**3a–f**) were synthesized and their antituberculosis activities and toxicity have been evaluated. According to the primary screening, the compounds **3b**, **3c**, **3e** and **3f** showed the highest inhibitions with 96%, 96%, 94%, 92%, respectively. SAR observation showed that a thiazolylhydrazone structure and a substitution on phenyl affect the activity.

The IC_{50} values obtained for the six compounds are shown in Table 3. As can be seen for the six compounds, there were no significant cytotoxicity at the concentrations under 49 μg/ml after 24 h incubation. Among the compounds used, compound **3b** showed greater toxicity at 49–200 μg/ml range. On the other hand, **3a** and **3c** showed no significant toxicity at the concentrations used. Also compounds **3d**, **3e** and **3f**



Scheme 1. Synthetic route of the title compounds.

Table 1
Some characteristics of the compounds

Comp.	R_1	R_2	M.p. (°C)	Yield (%)	Mol. for.	MW
3a	H	H	258–260 dec.	70	$C_{20}H_{19}N_3S$	333
3b	H	CH_3	248–250 dec.	64	$C_{21}H_{21}N_3S$	347
3c	H	OCH_3	253–255	61	$C_{21}H_{21}N_3OS$	363
3d	H	NO_2	212–214	78	$C_{20}H_{18}N_4O_2S$	378
3e	H	Cl	243–244 dec.	63	$C_{20}H_{18}ClN_3S$	367
3f	Cl	Cl	237–239	59	$C_{20}H_{17}Cl_2N_3S$	401

showed moderate cytotoxic effects on NIH/3T3 cell line, demonstrating that **3d**, **3e** and **3f** were the cause of 90, 120 and 110 μg/ml IC_{50} values, respectively.

In comparison of the results of toxicity and antimicrobial activity tests, it is seen that the antimicrobial activity of the compounds is not due to their general toxicity effect, however, their antimicrobial activity can be possibly because of their selective antimicrobial effect.

We concluded from our investigations that **3b**, **3c**, **3e** and **3f** may be considered promising for the development of new antituberculosis agents with their antituberculosis activity and toxicity screening.

5. Experimental

5.1. 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 250 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.

5.1.1. Preparation of 1-(1-arylethylidene)thiosemicarbazide (*1*)

In a flask equipped with a reflux condenser, a mixture of thiosemicarbazide (40 mmol) and the appropriate acetophenone derivatives (40 mmol) are reacted in 80 ml isopropyl alcohol in the presence of a catalytic amount of acetic acid. The mixture is then refluxed for 1 h and the obtained solid is filtered and used without further purification.

5.1.2. Preparation of N-(1-arylethylidene)-N′-[4-(indan-5-yl) thiazol-2-yl]hydrazones (*3a–f*)

1-(1-Arylethylidene)thiosemicarbazide (**1**) (20 mmol) and 1-(5-indanyl)-2-bromoethanone (**2**) (20 mmol) are stirred in refluxing isopropyl alcohol (80 ml) to complete dissolution, until a white foaming product is formed. The mixture is then allowed to cool and the solid is filtered, washed with saturated $NaHCO_3$ water solution and then with cool water, dried, and crystallised from ethanol.

Table 2
Antituberculosis activity of the compounds

Comp.	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	Rifampicin
MIC (μg/ml)	>6.25	<6.25	<6.25	>6.25	<6.25	<6.25	0.25
% Inhibition	89	96	96	54	94	92	98

Table 3
Cytotoxicity of compounds to mouse fibroblast (NIH/3T3) cell line

Comp.	Cytotoxicity IC_{50} (μg/ml)[a]
3a	200 ± 9.1
3b	49 ± 7.6
3c	180 ± 8.5
3d	90 ± 3.3
3e	120 ± 15
3f	110 ± 5.0

[a] Incubation for 24 h. IC_{50} is the drug concentration required to inhibit 50% of the cell growth. The values represent mean ± standard deviation of triplicate determinations.

Some characteristics of the synthesized compounds are shown in Table 1. Analytical and spectral data (IR, ^{1}H NMR, MS-FAB$^+$) confirmed the structures of the new compounds.

Compounds **3a–f**: IR (KBr) ν_{max} (cm^{-1}): 3309–3224 (NH), 1612–1484 (C=C and C=N).

*5.1.2.1. N-(1-Phenylethylidene)-N′-[4-(indan-5-yl)thiazol-2-yl] hydrazone (**3a**).* ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.95–2.15 (2H, pentaplet, protons C_2 of indane), 2.85 (3H, s, CH_3), 2.80–3.00 (4H, q, protons C_1 and C_3 of indane), 5.95 (1H, br, NH), 7.20–7.85 (9H, m, aromatic protons). For $C_{20}H_{19}N_3S$ calculated: 72.04% C, 5.74% H, 12.60% N; found: 72.16% C, 5.82% H, 12.64% N. MS-FAB$^+$: *m/z*: 334 [M + 1].

*5.1.2.2. N-(1-(4-Methylphenyl)ethylidene)-N′-[4-(indan-5-yl) thiazol-2-yl]hydrazone (**3b**).* ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.95–2.10 (2H, pentaplet, protons C_2 of indane), 2.40–2.45 (6H, two s, CH_3), 2.75–2.95 (4H, q, protons C_1 and C_3 of indane), 6.90 (1H, br, NH), 7.10–7.70 (8H, m, aromatic protons). For $C_{21}H_{21}N_3S$ calculated: 72.59% C, 6.09% H, 12.09% N; found: 72.56% C, 6.04% H, 12.12% N. MS-FAB$^+$: *m/z*: 347 [M], 348 [M + 1].

*5.1.2.3. N-(1-(4-Methoxyphenyl)ethylidene)-N′-[4-(indan-5-yl) thiazol-2-yl]hydrazone (**3c**).* ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.90–2.10 (2H, pentaplet, protons C_2 of indane), 2.30 (3H, s, CH_3), 2.70–2.95 (4H, q, protons C_1 and C_3 of indane), 3.80 (3H, s, OCH_3), 5.95 (1H, br, NH), 7.00 (2H, d, $J = 8.89$ Hz, phenyl C_2 and C_6 protons), 7.20 (1H, s, indane C_4 proton), 7.25 (1H, d, $J = 7.80$ Hz, indane C_7 proton), 7.60 (1H, d, $J = 7.94$ Hz, indane C_6 proton), 7.70 (1H, s, thiazole C_5 proton), 7.75 (2H, d, $J = 8.88$ Hz, phenyl C_3 and C_5 protons). For $C_{21}H_{21}N_3OS$ calculated: 69.39% C, 5.82% H, 11.56% N; found: 69.38% C, 5.82% H, 11.54% N. MS-FAB$^+$: *m/z*: 364 [M + 1].

*5.1.2.4. N-(1-(4-Nitrophenyl)ethylidene)-N′-[4-(indan-5-yl)thiazol-2-yl]hydrazone (**3d**).* ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.90–2.00 (2H, br, protons C_2 of indane), 2.35

(3H, s, CH_3), 2.70–2.90 (4H, br, protons C_1 and C_3 of indane), 6.50 (1H, br, NH), 7.40–8.30 (8H, m, aromatic protons). For $C_{20}H_{18}N_4O_2S$ calculated: 63.47% C, 4.79% H, 14.80% N; found: 63.55% C, 4.84% H, 14.91% N. MS-FAB$^+$: *m/z*: 379 [M + 1].

*5.1.2.5. N-(1-(4-Chlorophenyl)ethylidene)-N′-[4-(indan-5-yl) thiazol-2-yl]hydrazone (**3e**).* ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.90–2.10 (2H, pentaplet, protons C_2 of indane), 2.80 (3H, s, CH_3), 2.80–2.95 (4H, q, protons C_1 and C_3 of indane), 6.85 (1H, br, NH), 7.25 (1H, s, indane C_4 proton), 7.30 (1H, d, $J = 7.37$ Hz, indane C_7 proton), 7.50 (2H, d, $J = 8.63$ Hz, phenyl C_2 and C_6 protons), 7.65 (1H, d, $J = 7.49$ Hz, indane C_6 proton), 7.75 (1H, s, thiazole C_5 proton), 7.80 (2H, d, $J = 8.64$ Hz, phenyl C_3 and C_5 protons). For $C_{20}H_{18}ClN_3S$ calculated: 65.30% C, 4.93% H, 11.42% N; found: 65.29% C, 4.99% H, 11.54% N. MS-FAB$^+$: *m/z*: 367 [M], 368 [M + 1], 369 [M + 2].

*5.1.2.6. N-(1-(3,4-Dichlorophenyl)ethylidene)-N′-[4-(indan-5-yl)thiazol-2-yl]hydrazone (**3f**).* ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 1.95–2.10 (2H, pentaplet, protons C_2 of indane), 2.35 (3H, s, CH_3), 2.70–2.95 (4H, q, protons C_1 and C_3 of indane), 6.40 (1H, br, NH), 7.20–8.00 (7H, m, aromatic protons). For $C_{20}H_{17}Cl_2N_3S$ calculated: 59.71% C, 4.26% H, 10.44% N; found: 59.89% C, 4.43% H, 10.59% N. MS-FAB$^+$: *m/z*: 401 [M], 402 [M + 1], 403[M + 2].

5.2. Biology

5.2.1. 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 more, or suspension of organism isolated earlier on conventional medium. The culture was mixed well 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 McFarland 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 compared with that in the drug vial. The following formula was used to interpret results:

$$\Delta\text{GI control} > \Delta\text{GI drug} = \text{Susceptible}$$
$$\Delta\text{GI control} < \Delta\text{GI drug} = \text{Resistant}$$

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 1 or 2 additional days to establish a definite pattern of ΔGI differences.

5.3. Toxicity

5.3.1. Cell culture and drug treatment

NIH/3T3 cells were obtained from the American Type Culture Collection (ATCC, USA). The cells were incubated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Life Technologies, UK), 100 IU/ml penicillin (Gibco, Paisley, Scotland) and 100 μg/ml streptomycin (Gibco) at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Exponentially growing cells were plated at 5×10^4 cells/ml into 96-well microtiter tissue culture plates (Nunc, Denmark) and incubated for 24 h before the addition of the drugs (the optimum cell number for cytotoxicity assays was determined in preliminary experiments). Stock solutions of compounds were prepared in dimethyl sulphoxide (DMSO; Sigma–Aldrich, Poole, UK) and further dilutions were made with fresh culture medium (the concentration of DMSO in the final culture medium was <0.6% which had no effect on the cell viability).

5.3.2. Cell culture and drug treatment

It is widely used as a measure of cytotoxicity. After 24 h of preincubation, the tested compounds were added to give final concentration in the range of 1.56–200 μg/ml and the cells were incubated for 24 h. At the end of this period, MTT was added to a final concentration of 0.5 mg/ml and the cells were incubated for 4 h at 37 °C. After the medium was removed, the formazan crystals formed by MTT metabolism were solubilized by addition of 200 μl DMSO to each well and absorbance was read at 540 nm with a microtitre plate spectrophotometer (Elx808-IU Bio-Tek plate reader). Every concentration was repeated in three wells and IC_{50} values were defined as the drug concentrations that reduced absorbance to 50% of control values.

Data presentation and statistics. Statistical analyses were carried out by the one-way analyses of variance (ANOVA) test and criterion of the differences between means (±SEM) was $P < 0.05$.

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