

## Synthesis and antitubercular activity of a series of hydrazone and nitrovinyl analogs derived from heterocyclic aldehydes

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### Abstract

A series of hydrazone and 3-nitrovinyl analogs of indole-3-carboxaldehydes and related compounds were synthesized and screened for antitubercular activity against *Mycobacterium tuberculosis* H37R<sub>V</sub> in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA). Several compounds showed inhibitory activity against *M. tuberculosis* in primary screening assays at a concentration of 6.25 µg/mL; subsequent dose-response studies indicated that the most active compounds, **3d**, **3e** & **8b**, had IC<sub>50</sub> values of 5.96, 5.4 & 1.6 µg/mL, respectively. These compounds represent potential leads for the further development of novel antitubercular agents.

**Keywords:** *Indole-3-hydrazones, benzo[b]thiophene-3-hydrazones, thiophene-3-hydrazones, 3-nitrovinylindoles, antitubercular activity*

### Introduction

Tuberculosis is a contagious disease caused by *Mycobacterium tuberculosis*. Despite the ready availability of effective treatments, tuberculosis today still represents one of the major worldwide public health problems. After a long period in which this disease seemed to be declining, in the last two decades an unexpected return has been observed [1]. The World Health Organization has recently estimated that every year about eight million new cases of tuberculosis occur, and up to three million individuals die from the disease [2]. Isoniazid, pyrazinamide, ethambutol, rifampicin and streptomycin, drugs generally used in combination, are still the current drugs of choice in the therapy of tuberculosis [3]. However, the protracted use of these molecules overtime represents the main cause in the outbreak of new resistant bacterial strains. Therefore, there is now an urgent need for the development of new antitubercular drugs.

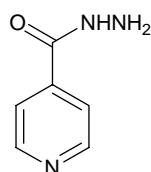
Numerous recent reports in the literature provide evidence of a renewed interest in the search for new antitubercular agents. The discovery that the

thiosemicarbazone analog of indole-3-carboxaldehyde is active against *M. tuberculosis* in mice [4,5] provided the stimulus for us to prepare a variety of structurally related compounds. A wide range of biological activities has been claimed for derivatives of indole-3-carboxaldehydes [6–11]. Importantly, the isonicotinylhydrazone of 1-benzylindole-3-carboxaldehyde is claimed to have antitubercular activity [12].

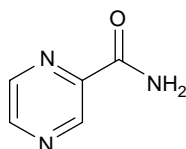
A variety of nitrovinylindoles derived from indole-3-carboxaldehydes have also been shown to possess antifungal [13] and amoebicidal activity [14]. However, there are no reports on the antitubercular activity of nitrovinylindoles. Thus, several nitrovinylindoles have also been investigated for antitubercular activity.

To study the structure-activity relationships of these compounds, we have synthesized a series of hydrazone and nitrovinyl analogs (figure 1) derived from a variety of substituted indole-3-carboxaldehydes, benzo[b]thiophen-3-carboxaldehyde and thiophene-3-carboxaldehyde, which were tested against *M. tuberculosis*, in order to uncover new leads in the development of novel antitubercular agents.

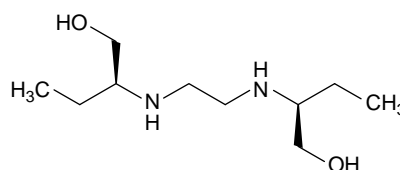
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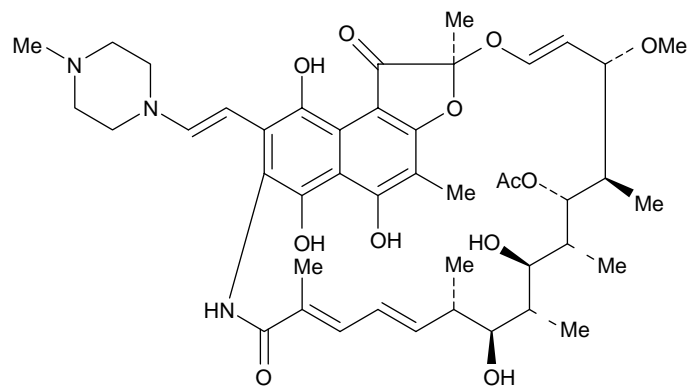
Isoniazid



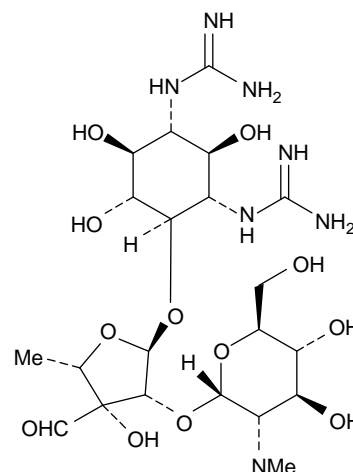
Pyraziniamide



Ethambutol



Rifampicin



Streptomycin

## Materials and methods

All reagents utilized were of general purpose or analytical grade and were purchased from Aldrich Chemical Company (St. Louis, MO).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Varian Mercury-300 spectrometer, operating at 300 MHz and 75 MHz respectively, using an appropriate deuterated solvent ( $\text{CDCl}_3$  or  $\text{DMSO-d}_6$ ). Chemical shifts are given in parts per million (ppm) relative to the internal standard tetramethylsilane. GC-MS was recorded on an Agilent 6890 Series GC System interfaced to a 5973 Mass Selective Detector and utilizing a Fused Silica Capillary Column ( $30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$  film thickness). Mass spectra were acquired by the University of Kentucky Mass Spectrometry Facility.

MALDI mass spectra were obtained on a Kratos Compact SEQ time-of-flight mass spectrometer (Manchester, UK), using alpha-cyano-4-hydroxycinnamic acid as the matrix.

GC-Mass spectra were recorded on an Agilent 6890 GC incorporating an Agilent 7683 autosampler and an Agilent 5973 MSD (Agilent Technologies, Wilmington, DE) interfaced to a Hewlett-Packard KAYAK XM600 computer (Hewlett-Packard Co., Palo Alto, CA) with MSD Productivity ChemStation Software (Rev. C.00.01) (Agilent Technologies, Palo Alto, CA) for analysis and data processing. An HP-5MS GC column ( $30\text{ m} \times 0.25\text{ mm I.D.} \times 0.25\text{ }\mu\text{m}$  film thickness) (Agilent Co., Wilmington, DE) was utilized for analyses,

with ultrapure helium as carrier gas at a flow rate of  $1.0\text{ mL/min}$  and an average velocity of  $38\text{ cm/sec}$  in constant flow mode. The temperature at the injection port, MS interface, electron impact source, and mass filter was set constant at  $280^\circ\text{C}$ ,  $280^\circ\text{C}$ ,  $230^\circ\text{C}$ , and  $150^\circ\text{C}$ , respectively. The oven temperature was initially set at  $120^\circ\text{C}$  and increased to  $160^\circ\text{C}$  at the rate of  $10^\circ\text{C/min}$ ; after holding at  $160^\circ\text{C}$  for 1 min, the oven temperature was increased to  $275^\circ\text{C}$  at the rate of  $60^\circ\text{C/min}$  and held at this temperature for 10 min, to elute possible high boiling point interfering impurities. The MS analyzer was set in the scan mode.

High resolution electron impact ionization mass spectra were recorded at  $70\text{ eV}$  on a JEOL JMS-700T MStation (magnetic sector instrument) at a resolution of greater than 10,000. Samples were introduced via a heatable direct probe inlet. Perfluorokerosene (pfk) was used to produce reference masses.

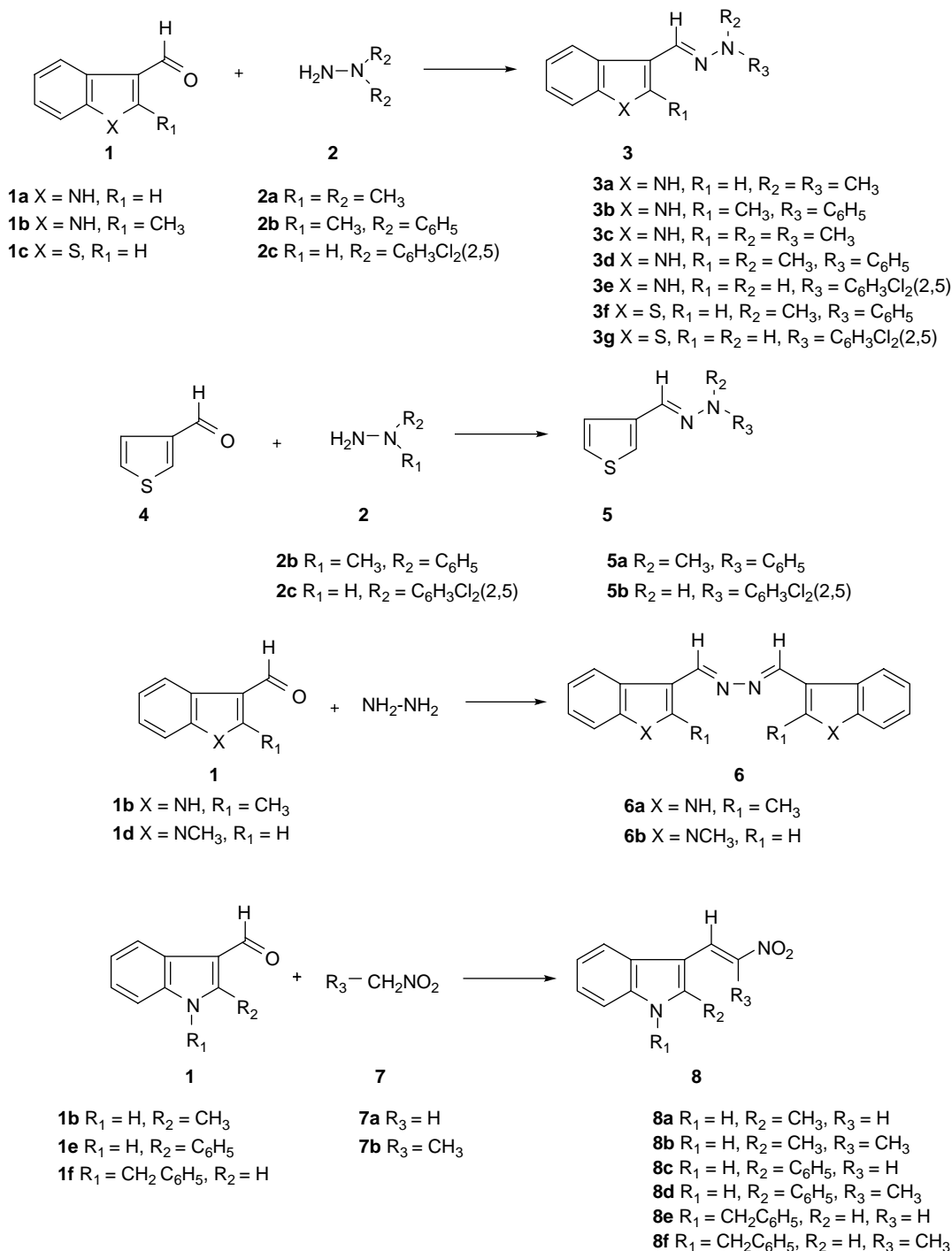
Melting points were determined on a Fisher-Johns Scientific melting point apparatus and are uncorrected.

## Chemistry

The general method for the preparation of hydrazones **3**, **5** & **6** and nitrovinylindoles **8** is summarized in Scheme 1. Indole-3-carboxaldehyde (**1a**) was generally reacted under reflux with *N,N*-dimethylhydrazine (**2a**), *N*-methyl-*N*-phenylhydrazine (**2b**) and

2,5-dichlorophenylhydrazine (**2c**) to afford *N'*-(1*H*-indol-3-yl-methylene)-*N,N*-dimethylhydrazine (**3a**), (*E*)-*N'*-(1*H*-indol-3-ylmethylene)-*N*-methyl-*N*-phenylhydrazine (**3b**), and (*E*)-*N*-(2,5-dichlorophenyl)-*N'*-(1*H*-indol-3-ylmethylene)hydrazine (**3e**), respectively. Also, 2-methylindole-3-carboxaldehyde was reacted with **2a** and **2b** under identical conditions to yield *N,N*-dimethyl-*N'*-(2-methyl-1*H*-indol-3-ylmethylene)hydrazine (**3c**), (*E*)-*N'*-(2-methyl-1*H*-indol-3-ylmethylene)-*N*-phenylhydrazine (**3d**), respectively.

In a similar manner the sulfur isostere of indole-3-carboxaldehyde, benzo[*b*]thiophene-3-carboxaldehyde (**1c**), was reacted with **2b** and **2c** to afford (*E*)-*N'*-benzo[*b*]thiophen-3-yl-methylene-*N*-methyl-*N*-phenylhydrazine (**3f**) and (*E*)-*N*-benzo[*b*]thiophen-3-ylmethylene-*N'*-(2,5-dichlorophenyl)hydrazine (**3g**), respectively. Additionally, thiophene analogs **5a** and **5b** were prepared by the reaction of thiophene-3-carboxaldehyde (**4**) with **2b** and **2c** to yield (*E*)-*N*-methyl-*N*-phenyl-*N'*-thiophen-3-ylmethylenehydra-



Scheme 1. Synthesis of the titled compounds.

zine (**5a**), and (*E*)-*N*-(2,5-dichlorophenyl)-*N'*-thiophen-3-ylmethylene-hydrazine (**5b**), respectively. Unexpectedly, the reaction of **1b** and **1d** with hydrazine under similar conditions to those described above resulted in the formation of the dimeric compounds: *N,N'*-bis-(2-methyl-1*H*-indol-3-yl-methylene)hydrazine (**6a**) and *N,N'*-bis(1-methyl-1*H*-indol-3-ylmethylene)hydrazine (**6b**), respectively.

The nitrovinylindole analogs **8a-f** were prepared by the reaction of indole-3-carboxaldehydes (**1b**, **1e** & **1f**) with nitromethane (**7a**) or nitroethane (**7b**) in the presence of ammonium acetate as the catalyst, to afford the desired nitrovinylindole, (*E*)-2-methyl-3-(2-nitrovinyl)-1*H*-indole (**8a**), (*E*)-2-methyl-3-(2-methyl-2-nitrovinyl)-1*H*-indole (**8b**), (*E*)-2-phenyl-3-(2-nitrovinyl)-1*H*-indole (**8c**), (*E*)-2-phenyl-3-(2-methyl-2-nitrovinyl)-1*H*-indole (**8d**), (*E*)-1-benzyl-3-(2-nitrovinyl)-1*H*-indole (**8e**), and (*E*)-1-benzyl-3-(2-methyl-2-nitrovinyl)-1*H*-indole (**8f**), in moderate yield (49–65%).

The structure and purity of the above indole derivatives were verified by <sup>1</sup>H & <sup>13</sup>C NMR spectroscopy, GC-MS and MALDI-TOF mass spectrometry. X-ray crystallographic analysis of four representative compounds has been previously reported [15–17], in every case the C=N and C=C bonds had the *E*-geometry.

*N'*-(1*H*-Indol-3-yl-methylene)-*N,N*-dimethyl-hydrazine **3a**. A mixture of indole-3-carboxaldehyde (1.45 g, 0.01 mol) and *N,N*-dimethylhydrazine (0.6 g, 0.01 mol) dissolved in ethanol (25 mL) was heated under reflux for 2 h. The reaction mixture was cooled to room temperature and the crystals that separated were collected by filtration. Recrystallisation from ethanol afforded *N'*-(1*H*-indol-3-yl-methylene)-*N,N*-dimethylhydrazine (72%) as pale yellow crystals: mp 101–103°C. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 2.93 (6H, s), 7.16–7.23 (2H, m), 7.29–7.36 (2H, m), 7.70 (1H, s), 8.19 (1H, sb), 8.29 (1H, d), 8.32 (1H, t) ppm; <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 43.8, 111.1, 115.0, 120.6, 122.1, 123.0, 124.3, 125.0, 132.1, 136.8 ppm. GC: t<sub>R</sub> = 9.88 min, purity >99% (single peak); MS (m/z) 187 (M<sup>+</sup>).

(*E*)-*N'*-(1*H*-Indol-3-ylmethylene)-*N*-methyl-*N*-phenyl-hydrazine **3b**. A mixture of indole-3-carboxaldehyde (1.45 g, 0.01 mol) and *N*-methyl-*N*-phenylhydrazine (1.22 g, 0.01 mol) dissolved in ethanol (25 mL) was heated under reflux for 2 h. The reaction mixture was cooled to room temperature and the crystals that separated were collected by filtration. Recrystallisation from ethanol afforded *N'*-(1*H*-indol-3-ylmethylene)-*N*-methyl-*N*-phenylhydrazine (64%) as pale yellow crystals: mp 104–106°C. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 3.38 (3H, s), 6.8 (1H, t), 7.18

(2H, m), 7.27 (4H, m), 7.63 (1H, d), 8.0 (1H, s), 8.25 (1H, m), 11.25 (1H, s) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>): δ 32.27, 111.59, 113.46, 113.65, 118.59, 119.82, 121.21, 122.0, 123.91, 127.38, 128.85, 131.20, 136.79, 147.88 ppm. GC: t<sub>R</sub> = 15.77 min, purity >99% (single peak); MS (m/z) 249 (M<sup>+</sup>).

*N,N*-Dimethyl-*N'*-(2-methyl-1*H*-indol-3-ylmethylene)hydrazine **3c**. A mixture of 2-methylindole-3-carboxaldehyde (1.59 g, 0.01 mol) and *N,N*-dimethylhydrazine (0.6 g, 0.01 mol) dissolved in ethanol (25 mL) was heated under reflux for 2 h. The reaction mixture was cooled to room temperature and the crystals that separated were collected by filtration. Recrystallisation from ethanol afforded *N,N*-dimethyl-*N'*-(2-methyl-1*H*-indol-3-ylmethylene)hydrazine (68%) as pale yellow crystals: mp 73–75°C. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 2.54 (3H, s), 2.93 (6H, s), 7.16–7.22 (2H, m), 7.25–7.30 (1H, m), 7.75 (1H, s), 7.98 (1H, sb), 8.26–8.36 (1H, m) ppm; <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 12.3, 44.0, 110.0, 110.2, 120.5, 121.3, 121.9, 126.3, 133.0, 134.8, 135.5 ppm. GC: t<sub>R</sub> = 10.58 min, purity >99% (single peak); MS (m/z) 201 (M<sup>+</sup>).

(*E*)-*N*-Methyl-*N'*-(2-methyl-1*H*-indol-3-ylmethylene)-*N*-phenylhydrazine **3d**. A mixture of 2-methylindole-3-carboxaldehyde (1.59 g, 0.01 mol) and *N*-methyl-*N*-phenylhydrazine (1.22 g, 0.01 mol) dissolved in ethanol (25 mL) was heated under reflux for 2 h. The reaction mixture was cooled to room temperature and crystals that separated were collected by filtration. Recrystallisation from ethanol afforded (*E*)-*N*-methyl-*N'*-(2-methyl-1*H*-indol-3-ylmethylene)-*N*-phenylhydrazine (59%) as pale yellow crystals: mp 123–125°C. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 2.57 (3H, s), 3.42 (3H, s), 6.81 (1H, t), 7.10 (2H, h), 7.37 (5H, m), 7.97 (1H, s), 8.20 (1H, m), 11.22 (1H, s) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>): δ 11.84, 32.30, 108.86, 110.54, 113.53, 118.42, 119.40, 120.20, 121.05, 125.22, 128.83, 130.68, 135.40, 136.53, 148.02 ppm. GC: t<sub>R</sub> = 16.34 min, purity >99% (single peak); MS (m/z) 263 (M<sup>+</sup>).

(*E*)-*N*-(2,5-Dichlorophenyl)-*N'*-(1*H*-indol-3-ylmethylene)hydrazine **3e**. A mixture of indole-3-carboxaldehyde (1.45 g, 0.01 mol) and 2,5-dichlorophenylhydrazine (1.77 g, 0.01 mol) dissolved in ethanol (25 mL) was heated under reflux for 2 h. The reaction mixture was cooled to room temperature and the crystals that separated were collected by filtration. Recrystallisation from ethanol afforded *N*-(2,5-dichlorophenyl)-*N'*-(1*H*-indol-3-ylmethylene)hydrazine (70%) as pale brown crystals: mp 163–165°C. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 6.75 (1H, dd), 7.20 (2H, m), 7.34 (1H, d), 7.45 (2H, q),

7.73 (1H, d), 8.16 (1H, t), 8.54 (1H, s), 9.68 (1H, s), 11.5 (1H, s) ppm;  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  112.53, 114.01, 114.22, 115.75, 118.88, 121.49, 122.85, 123.77, 125.84, 128.91, 131.14, 134.75, 138.82, 140.51, 144.38 ppm. GC:  $t_{\text{R}} = 12.09$  min, purity >99% (single peak); MS (m/z) 303 ( $\text{M}^+$ ).

*(E)-N'*-Benzo[b]thiophen-3-ylmethylene-*N*-methyl-*N*-phenylhydrazine **3f**. A mixture of benzo[b]thiophene-3-carboxaldehyde (0.487 g, 3 mmol) and *N*-methyl-*N*-phenylhydrazine (0.366 g, 3 mmol) dissolved in methanol (15 mL) was heated under reflux for 2 h. The reaction mixture was cooled to room temperature and the crystals that separated were collected by filtration. Recrystallisation from methanol afforded *(E)-N'*-benzo[b]thiophen-3-ylmethylene-*N*-methyl-*N*-phenylhydrazine (80%) as pale yellow crystals: mp 74–76°C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.47 (3H, s), 6.97 (1H, tt), 7.35–7.52 (6H, m), 7.54 (1H, s), 7.87 (1H, s), 7.89 (1H, t), 8.79 (1H, dq) ppm;  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  33.4, 115.6, 120.8, 122.7, 124.86, 124.94, 125.0, 125.9, 129.2, 129.3, 133.4, 136.4, 140.9, 148.1 ppm. GC:  $t_{\text{R}} = 13.29$  min, purity >99% (single peak); MS (m/z) 266 ( $\text{M}^+$ ).

*(E)-N*-Benzo[b]thiophen-3-ylmethylene-*N'*-(2,5-dichlorophenyl)hydrazine **3g**. A mixture of benzo[b]thiophene-3-carboxaldehyde (0.487 g, 3 mmol) and 2,5-dichlorophenylhydrazine (0.531 g, 3 mmol) dissolved in methanol (15 mL) was heated under reflux for 2 h. The reaction mixture was cooled to room temperature and the crystals that separated were collected by filtration. Recrystallisation from methanol afforded *(E)-N*-benzo[b]thiophen-3-ylmethylene-*N'*-(2,5-dichlorophenyl)hydrazine (78%) as brown crystals: mp 148–150°C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  6.78 (1H, dd), 7.42 (1H, d), 7.44 (1H, t), 7.55 (1H, t), 7.59 (1H, d), 7.61 (1H, s), 7.88 (1H, d), 8.00 (1H, s), 8.69 (1H, d) ppm;  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  114.0, 114.01, 115.2, 119.9, 122.8, 124.9, 125.3, 125.4, 128.8, 130.1, 131.4, 134.1, 136.0, 137.1, 140.8, 141.4 ppm. GC:  $t_{\text{R}} = 16.39$  min, purity >99% (single peak); MS (m/z) 320 ( $\text{M}^+$ ).

*(E)-N*-Methyl-*N*-phenyl-*N'*-thiophen-3-ylmethylene hydrazine **5a**. A mixture of thiophene-3-carboxaldehyde (0.336 g, 3 mmol) and *N*-methyl-*N*-phenylhydrazine (0.366 g, 3 mmol) dissolved in methanol (15 mL) was heated under reflux for 2 h. The reaction mixture was cooled to room temperature and the crystals that separated were collected by filtration. Recrystallisation from methanol afforded *(E)-N*-methyl-*N*-phenyl-*N'*-thiophen-3-ylmethylene hydrazine (82%) as pale yellow crystals: mp 80–82°C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.39 (3H, s), 6.91–6.96 (1H, m), 7.29–7.38 (6H, m), 7.58 (1H, dd),

7.60 (1H, s) ppm;  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  33.4, 115.3, 120.6, 122.3, 125.3, 126.1, 128.2, 129.1, 140.0, 147.9 ppm. GC:  $t_{\text{R}} = 10.38$  min, purity >99% (single peak); MS (m/z) 216 ( $\text{M}^+$ ).

*(E)-N*-(2,5-Dichlorophenyl)-*N'*-thiophen-3-ylmethylene hydrazine **5b**. A mixture of thiophene-3-carboxaldehyde (0.336 g, 3 mmol) and 2,5-dichlorophenylhydrazine (0.531 g, 3 mmol) dissolved in methanol (15 mL) was heated under reflux for 2 h. The reaction mixture was cooled to room temperature and the crystals that separated were collected by filtration. Recrystallisation from methanol afforded *(E)-N*-(2,5-dichlorophenyl)-*N'*-thiophen-3-ylmethylenehydrazine (79%) as brown crystals: mp 94–96°C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  6.75 (1H, dd), 7.17 (1H, d), 7.35 (1H, q), 7.43 (1H, dd), 7.55 (1H, t), 7.57 (1H, d), 7.89 (2H, s) ppm;  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  114.1, 115.1, 119.8, 124.9, 125.2, 126.7, 130.0, 134.0, 136.3, 137.7, 141.4 ppm. GC:  $t_{\text{R}} = 11.43$  min, purity >99% (single peak); MS (m/z) 270 ( $\text{M}^+$ ).

*N,N'*-bis-(2-Methyl-1*H*-indol-3-yl-methylene)hydrazine **6a**. A mixture of 2-methylindole-3-carboxaldehyde (1.59 g, 0.01 mol) and hydrazine (0.64 g, 0.02 mol) dissolved in ethanol (25 mL) was heated under reflux for 2 h. The reaction mixture was cooled to room temperature and the crystals that separated were collected by filtration. Recrystallisation from methanol afforded *N,N'*-bis-(2-methyl-1*H*-indol-3-yl-methylene)hydrazine (65%) as yellow crystals: mp > 300°C.  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  3.25 (6H, s), 7.00–7.03 (4H, m), 7.22–7.25 (2H, m), 8.21 (2H, d), 8.83 (2H, s), 11.44 (2H, s) ppm;  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  11.7, 107.8, 110.7, 120.2, 121.4, 121.6, 125.8, 135.6, 141.3, 153.7 ppm. MALDI-MS (m/z) 315 ( $\text{MH}^+$ ).

*N,N'*-bis-(1-Methyl-1*H*-indol-3-yl-methylene)hydrazine **6b**. A mixture of 1-methylindole-3-carboxaldehyde (1.59 g, 0.01 mol) and hydrazine (0.64 g, 0.02 mol) dissolved in ethanol (25 mL) was heated under reflux for 2 h. The reaction mixture was cooled to room temperature and the crystals that separated were collected by filtration. Recrystallisation from methanol afforded *N,N'*-bis-(2-methyl-1*H*-indol-3-yl-methylene)hydrazine (62%) as yellow crystals: mp 224–226°C.  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  3.84 (6H, s), 7.18–7.30 (4H, m), 7.51 (2H, d), 7.88 (2H, s), 8.34 (2H, dd), 8.86 (2H, s) ppm;  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  32.9, 110.2, 111.0, 120.7, 122.1, 122.6, 125.0, 135.2, 137.6, 154.5 ppm. MALDI-MS (m/z) 315 ( $\text{MH}^+$ ).

*General method for the preparation of 3-(2-nitrovinyl)indoles 8a-f.* Solid ammonium acetate (0.3 g, 3.8 mmol) was added to a suspension of the appropriately substituted indole-3-carboxaldehyde (6.8 mmol) in nitromethane or nitroethane (3 mL). The mixture was vigorously stirred and heated at 120–130°C for 2 h. The mixture was then cooled in an ice bath and the resulting solid which appeared was filtered and recrystallised from methanol.

*(E)-2-Methyl-3-(2-nitrovinyl)-1H-indole 8a.* Yellow crystals (62%): mp 190–192°C. <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): δ 2.60 (3H, s), 7.15–7.22 (2H, p), 7.38–7.42 (1H, m), 7.80–7.84 (1H, m), 7.88 (1H, d), 8.30 (1H, d), 12.20 (1H, b) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>): δ 11.93, 105.08, 111.79, 120.01, 121.80, 122.72, 125.10, 129.50, 133.19, 136.24, 147.55 ppm. GC: t<sub>R</sub> = 12.51 min, purity >99% (single peak); MS (m/z) 202 (M<sup>+</sup>).

*(E)-2-Methyl-3-(2-methyl-2-nitrovinyl)-1H-indole 8b.* Brown crystals (56%): mp 154–156°C. <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): δ 2.25 (3H, s), 2.42 (3H, s), 7.08–7.13 (2H, pd), 7.37 (1H, dd), 7.47 (1H, dd), 8.28 (1H, s), 11.92 (1H, s) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>): δ 12.58, 15.82, 105.29, 111.45, 119.39, 120.30, 121.65, 125.57, 129.12, 135.86, 141.46, 141.63 ppm. GC: t<sub>R</sub> = 11.63 min, purity >99% (single peak); MS (m/z) 216 (M<sup>+</sup>).

*(E)-2-Phenyl-3-(2-nitrovinyl)-1H-indole 8c.* Yellow crystals (58%): mp 220–222°C. <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): δ 7.24–7.35 (2H, m), 7.53 (1H, d), 7.57–7.68 (5H, m), 8.0 (1H, d), 8.06 (1H, dd), 8.15 (1H, dd), 12.60 (1H, b) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>): δ 104.93, 112.47, 120.94, 122.23, 123.78, 125.07, 129.03, 129.61, 129.74, 129.88, 131.52, 133.90, 136.89, 147.70 ppm. GC: t<sub>R</sub> = 17.41 min, purity >99% (single peak); MS (m/z) 264 (M<sup>+</sup>).

*(E)-2-Phenyl-3-(2-methyl-2-nitrovinyl)-1H-indole 8d.* Orange crystals (49%): mp 228–230°C. <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): δ 2.20 (3H, s), 7.14–7.27 (2H, m), 7.46–7.64 (7H, m), 8.19 (1H, s), 12.28 (1H, b) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>): δ 15.65, 105.03, 112.12, 119.98, 120.67, 122.75, 125.89, 128.67, 128.86, 129.17, 131.00, 136.47, 141.17, 144.03 ppm. GC: t<sub>R</sub> = 15.71 min, purity >99% (single peak); MS (m/z) 278 (M<sup>+</sup>).

*(E)-1-Benzyl-3-(2-nitrovinyl)-1H-indole 8e.* Yellow crystals (65%): mp 128–130°C. <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): δ 5.52 (2H, s), 7.25–7.34 (7H, m), 7.63 (1H, dd), 7.99–8.06 (2H, m), 8.40 (2H, dd) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>): δ 49.73, 107.78, 111.58, 120.72, 122.13, 123.40, 125.16, 127.13, 127.65, 128.59, 131.41, 133.87, 136.56, 137.33, 138.55 ppm. GC: t<sub>R</sub> = 17.15 min, purity >99% (single peak); MS (m/z) 278 (M<sup>+</sup>).

*(E)-1-Benzyl-3-(2-methyl-2-nitrovinyl)-1H-indole 8f.* Yellow crystals (63%): mp 114–116°C. <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): δ 2.48 (3H, s), 5.56 (2H, s), 7.19–7.31 (7H, m), 7.55 (1H, dd), 7.86 (1H, dd), 8.31 (1H, s), 8.44 (1H, dd) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>): δ 14.83, 49.84, 107.70, 111.22, 118.51, 121.30, 123.05, 125.86, 127.09, 127.54, 128.12, 128.53, 133.04, 135.78, 136.93, 141.18 ppm. GC: t<sub>R</sub> = 16.69 min, purity >99% (single peak); MS (m/z) 292 (M<sup>+</sup>).

### Microbiology

All compounds were evaluated for antimycobacterial activity against *Mycobacterium tuberculosis H37Rv*. Antitubercular activities of the compounds were performed by the Center of Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) at Southern Research Institute. Compounds were tested for in vitro antituberculosis activity against *Mycobacterium tuberculosis H37Rv* (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 were tested in the BACTEC 460 Radiometric System [18–19]. Compounds effecting <90% inhibition in the primary screen (i.e., MIC >6.25 µg/mL) were generally not evaluated further. Compounds exhibiting growth inhibition of >90% in the primary screen at 6.25 µg/mL were retested at lower concentration against *M. tuberculosis H37Rv* to determine the actual Minimum Inhibitory Concentration (MIC) in the broth microdilution MABA assay. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. Also the cytotoxicity (IC<sub>50</sub>) of compounds against cultured VERO cells, as well as their selectivity index (SI), defined as IC<sub>50</sub>/MIC, were determined. Rifampicin was utilized as the standard compound in these assays.

### Results and discussion

As shown in Table I, the compounds **3b**, **3d**, **3e**, **3g** and **8b** exhibited growth inhibition >90% in the primary screen at 6.25 µg/mL, and were therefore retested at lower concentration against *M. tuberculosis H37Rv* to determine the actual Minimum Inhibitory Concentration (MIC) in the MABA assay.

Also, the cytotoxicity (IC<sub>50</sub>) of compounds **3b**, **3d**, **3e** & **8b** against VERO cells as well as their selectivity index (SI), were determined. Unfortunately the solubility of compound **3g** in the tissue culture medium was too low for an IC<sub>50</sub> to be determined.

Based on the data from the antitubercular MABA assays for compounds **3a-g**, **5a**, **5b**, **6a**, **6b**, and **8a-f**, using *M. tuberculosis H37Rv* the following observations can be made. In the indole series, hydrazones **3a** and **3c** derived from *N,N*-dimethylhydrazine did not

Table I. Primary antitubercular activity screening results of **3a-g**, **5a-b**, **6a-b** & **8a-f** and IC<sub>50</sub> values for **3b**, **3d**, **3e** & **8b**.

Compound No.	TAACF No.	Inhibition %	MIC $\mu\text{g/mL}$	IC <sub>50</sub> $\mu\text{g/mL}$
<b>3a</b>	152888	9	> 6.25	
<b>3b</b>	152889	91	< 6.25	> 10
<b>3c</b>	154668	0	> 6.25	
<b>3d</b>	154667	95	< 6.25	5.96
<b>3e</b>	154670	97	< 6.25	5.40
<b>3f</b>	160822	34	> 6.25	
<b>3g</b>	160823	96	< 6.25	
<b>5a</b>	160821	50	> 6.25	
<b>5b</b>	160824	70	> 6.25	
<b>6a</b>	152887	10	> 6.25	
<b>6b</b>	152886	2	> 6.25	
<b>8a</b>	152877	17	> 6.25	
<b>8b</b>	152878	91	< 6.25	1.6
<b>8c</b>	152881	38	> 6.25	
<b>8d</b>	152882	39	> 6.25	
<b>8e</b>	152883	46	> 6.25	
<b>8f</b>	152884	30	> 6.25	

MIC<sup>a</sup> Minimum Inhibitory Concentration

Selectivity Index (SI): **3b** = > 1.6, **3d** = 0.95, **3e** = 0.86 and **8b** = 0.26

Rifampicin (IC<sub>50</sub> = 0.125  $\mu\text{g/mL}$ )<sup>20</sup>

exhibit any significant growth inhibition (i.e. 9% and 0% inhibition, respectively). However, the compounds derived from *N*-methyl-*N*-phenylhydrazine in the indole series (i.e. **3b** and **3d**) were active, exhibiting 91% & 95% growth inhibition of *M. tuberculosis H37Rv*, respectively. Thus, it appears that the presence of an *N,N*-dimethyl moiety in the molecule affords poor inhibitory activity; however, when one of the *N*-methyl groups is replaced with a phenyl or substituted phenyl group, inhibitory activity is greatly improved. It is also apparent that the presence or absence of an indolic 2-methyl substituent has no effect on inhibitory potency. Interestingly, compound **3e**, which is a mono *N*-2,5-dichlorophenyl substituted hydrazone, was the most potent compound (IC<sub>50</sub> = 5.4  $\mu\text{g/mL}$ ) in the series, suggesting that *N*-aryl mono substitution may be optimal for antitubercular activity in this series of indole hydrazones.

The benzo[b]thiophene analog **3g**, which is isosteric with **3e** (i.e. the only difference is the replacement of the indole NH moiety with an S atom), exhibited potent inhibitory activity against *M. tuberculosis H37Rv*, further establishing that a mono substituted *N*-2,5-dichlorophenyl substituent is a requirement for potent inhibitory activity in both the indole and benzo[b]thiophene series of analogs. However, when the indole moiety in the *N*-methyl-*N*-phenyl analog **3b** is replaced with a benzo[b]thiophene moiety (**3f**), there is considerable loss in inhibitory activity (i.e. 91% for **3b**, decreasing to 34% for **3f**). We also observed that replacing the benzo[b]thiophene moiety in **3g** with a thiophene moiety (**5b**) still maintained moderate but reduced inhibitory activity (i.e. 96% versus 70% inhibition, respectively). However, replacement of the indole

moiety in **3b** with a thiophene moiety to afford **5a**, resulted in a significant loss of inhibitory activity. Interestingly, none of the *bis* analogs **6a** and **6b** exhibited any significant inhibitory activity in the MABA assay.

These results suggest that hydrazones derived from aromatic aldehydes possessing a 6 + 4 $\pi$  electron system, and having one *N*-phenyl or substituted *N*-phenyl group on the terminal nitrogen affords compounds with maximum inhibitory activity as antitubercular agents. The most active compounds contained an *N*-2,5-dichlorophenyl moiety.

The nitrovinyl analogs **8a-8f** also were evaluated in the MABA assay for antitubercular activity. The results indicate that the 2-methylindole analog **8b** was the most potent compound exhibiting 91% inhibition in the primary screen. Replacing the 2-methyl group in compound **8b** with a 2-phenyl group resulted in compound **8d**, which had reduced inhibitory activity, suggesting that introduction of a bulky 2-substituent into the indole nucleus leads to a significant reduction in inhibitory activity. Additionally, introduction of an *N*-benzyl moiety into the **8b** molecule to afford **8f** resulted in a significant loss of inhibitory activity, suggesting that steric bulk at the indolic N-atom also results in loss of inhibitory potency.

Concurrent with the determination of MIC values, compounds which were active (i.e. MIC's > 90%) were also tested for cytotoxicity (IC<sub>50</sub>) in VERO cells at concentrations less than or equal to 62.5  $\mu\text{g/mL}$ , or 10 times the MIC for *M. tuberculosis H37Rv*. After 72 hours exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 non-radioactive cell proliferation assay. Compounds **3b**, **3d**, **3e**, & **8b** afforded IC<sub>50</sub> values of > 10, 5.96, 5.40,

and 1.6  $\mu\text{g/mL}$ , respectively, in the cytotoxicity assay. Compound **3g** was not evaluated due to solubility problems.

These preliminary studies have identified three lead molecules **3d**, **3e**, & **8b** from both the hydrazone and nitrovinyl series of indole derivatives investigated. The nitrovinyl analog **8b** was the most potent compound examined, and was approximately one-tenth as active as rifampin in the VERO cell assay for cytotoxicity. Compounds **3d**, **3e**, & **8b** represent potential lead molecules for the further development of novel antitubercular agents.

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