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RESEARCH ARTICLE

Novel 1-(4-substituted benzylidene)-4-(1-(substituted methyl)-2,3-dioxoindolin-5-yl)semicarbazide derivatives for use against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) and MDR-TB strain

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Abstract A series of eighteen new 1-(4-substituted benzylidene)-4-(1-(substituted methyl)-2,3-dioxoindolin-5-yl)semicarbazide derivatives were designed, synthesized and characterized by spectral and elemental analyses. The derivatives were screened in vitro for antimicrobial activity against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) and MDR-TB strains. The activity was expressed as the minimum inhibitory concentration in μg/mL. Among the tested compounds **7j**, **7m**, **7o** and **7q** possesses equipotent activity as standard drug Isoniazid against MTB while **7m** and **7q** exhibited higher activity against MDR-TB strain when compared with both the reference drugs isoniazid and rifampicin. Basic structure activity relationships are presented.

Keywords Isatin · Antitubercular agents · *Mycobacterium tuberculosis*

Introduction

In recent time's tuberculosis, TB is a foremost public health problem and over 35 % of the world population was infected with this disease caused by *Mycobacterium tuberculosis* (Pablos-Mendez et al. 1998). The successful treatment of TB becomes more complex due to the resistance developed by bacteria, *M. tuberculosis*. It is accountable for more than 1.8 million people died and 9.5 million new cases worldwide in 2010 are reported as per World Health Organization (WHO) survey. It has been estimated that up to 50 million citizens are infected with drug-resistant forms of TB. There are estimated 1.4 million extensively/multi drug resistant TB (XDR/MDR-TB) cases will need to be treated between 2010 and 2015 (WHO 2010). The treatment of TB with existing drugs are associated with severe side effects including itching, thrombocytopenia, hepatotoxicity, rashes, fever, drug induced hepatitis, etc. (Yee et al. 2003). Despite the efforts of academic institutions and the pharmaceutical companies engaged in the design, synthesis, and development of new antitubercular regimens, the current TB therapeutic arsenal is poor. Only a few derivatives were found endowed with some antimycobacterial activity, including diarylquinoline (TMC207) (Andries et al. 2005), nitroimidazoles (OPC67683 and PA824) (Matsumoto et al. 2005), fluoroquinolones (Gatifloxacin and Moxifloxacin) (Alvirez-Freites et al. 2002), and 1,2-diamine (SQ109) (Lee et al. 2003).

A wide-ranging literature survey reveals that heterocyclic compounds, viz. derivatives of benzimidazole (Gill et al. 2008), pyrrole (Mariangela et al. 2009), dihydrobenzofuran (Tripathi et al. 2010), indole (Karthikeyan et al. 2009), furan (Tangallapally et al. 2004), imidazole (Pandey et al. 2009) and benzotriazole (Dixit et al. 2006) showed excellent antimycobacterial properties. In addition, among the heterocyclic compounds, Isatin a privileged lead molecule for scheming potential bioactive agents, and were found to have various biological activities. They have been shown to possess anti-viral (Kang et al. 2011), anti-tumor (Gudipati et al. 2011), anti-mycobacterial (Sriram et al.

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2006), anti-fungal (Singh et al. 2010), anti-malarial (Hans et al. 2011), anti-oxidant (Andreani et al. 2010), anti-convulsant (Praveen et al. 2011), anti-HIV (Banerjee et al. 2011) anti-glycation, (Khan et al. 2009) and potent inhibitors against caspase-3 (Jiang et al. 2011). These exciting properties encouraged many efforts toward the synthesis and pharmacological screening of many isatin derivatives. Hence it is worth to synthesize such compounds.

Many of the drug candidates are currently undergoing clinical trials against different strains of *M. tuberculosis.* Some of the successful clinical trial candidates (Nitazoxanide, SCV-07 & LL-3858) and its common similarity in its structure are represented in Fig. 1. Motivated by the aforementioned findings and with the aim of obtaining novel antimicrobial compounds we hereby report the synthesis and antimycobacterial studies of 1-(4-substituted benzylidene)-4-(1-(substituted methyl)-2,3-dioxoindolin-5-yl)semicarbazide derivatives.

Materials and methods

Chemistry

The chemicals and reagents used were obtained from various chemical units Merck India Ltd., Qualigens, E. CDH, and SD Fine Chem. All the solvents used were of LR grade and purified before their use. All the reaction steps were monitored until completion using thin layer chromatography (TLC). Solvent systems were used ethyl acetate: *n*-hexane (1:1), and detection was made using UV light at 254 nm and iodine spotting. All the melting points were taken in open glass capillary and are uncorrected. ^{1}H-NMR spectra were taken on a Bruker ultra shield (300 MHz) NMR spectrometer in $CDCl_3$ and dimethyl sulphoxide (DMSO)-d_6 using tetramethylsilane [$(CH_3)_4Si$] as internal standard. Chemical shift (δ) are expressed in ppm. The multiplicities of the signals in the ^{1}H-NMR are abbreviated by s (singlet), t (triplet), br (broad) and m (multiplet). The *J* constant was given in (Hz). Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. All the IR spectra were recorded in KBr pellets on a Jasco FT-IR 410 spectrometer. Elemental analyses were performed on a Perkine Elmer model 240C analyzer and were within ±0.4 % of the theoretical values.

General procedure for the synthesis of title compounds

*Preparation of 5-nitroisatin (**2**)*

The 5-nitroisatin (**2**) was prepared according to the reported literature (Siddiqui et al. 2011). Briefly, in a mixture of 50 g (35 mL, 0.50 mol) of conc. nitric acid and 74 g (40 mL, 0.75 mol) conc. sulfuric acid, isatin (48.50 g, 0.33 mol) was added slowly with frequent shaking in 500 mL round bottomed flask. Then the mixture was cooled by immersing the flask in crushed ice cold water. After adding all isatin, flask was fitted with reflux condenser and the mixture was heated on water bath maintaining the temperature at 60 °C for 1 h to obtain the desired compound 5-nitroisatin (**2**). Then the entire content was then transferred into a beaker containing 500 mL cold water, stirred in order to wash out as much acid from the desired product. When compound **2** settled completely to the bottom, the upper acid layer was removed from the mixture. Then the bottom layer was transferred to the separating funnel and shaked vigorously with about 50 mL of water. Then the residual layer was collected, dried with anhydrous calcium chloride and finally filtered to obtain the pure compound (**2**). Yield 65 %, Mp 230 °C; IR (KBr) cm^{-1}: 3342 (NH_{str}), 2996 (Ar–CH_{str}), 1732 (C=O, Isatin), 1570 (NO_{str}-asym.), 1348 (NO_{str}-sym.); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.02–7.94 (m, 3H, Ar–H), 8.92 (s, 1H, NH-Isatin); MS (EI) *m/z* 192 [M^+]; Anal. Calcd for

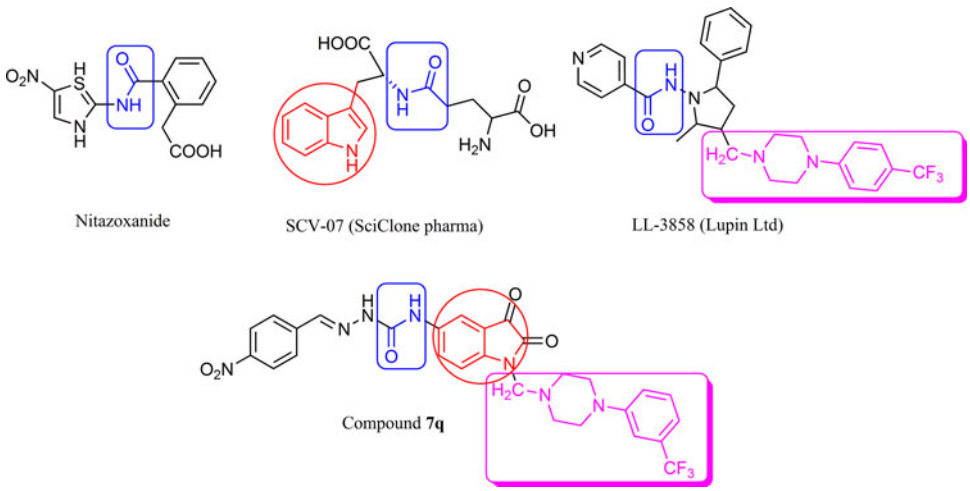


Fig. 1 Compounds currently undergoing clinical evaluation against TB and active compound **7q**

$C_8H_4N_2O_4$: C, 50.01; H, 2.10; N, 14.58. Found: C, 49.91; H, 2.12; N, 14.62.

*5-Nitro-1-substituted indolin-2,3-dione (**3a–3f**)*

To an ethanolic solution of 5-nitro isatin (0.04 mol) was added an equimolar amount of various secondary amines, dissolved in 95 % absolute ethanol or dimethyl sulfoxide. To this mixture, an equimolar amount of 37 % formaldehyde solution was then added and this mixture was then stirred at room temperature for 3 h, and kept aside for 2 days in refrigerator to form crystals. Finally the products in the form of crystals were separated by filtration, washed with hexane, and vacuum dried. Desired compounds were finally recrystallized with ethanol to obtain pure product.

*1-((Dimethylamino)methyl)-5-nitro indolin-2,3-dione (**3a**)* Yield 74 %, Mp 274–276 °C; IR (KBr) cm^{-1}: 2984 (Ar–CH_{str}); 1738 (C=O, Isatin); 1542 (NO_{str}-asym.); 1358 (NO_{str}-sym.); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.34 (s, 6H, CH_3); 4.44 (s, 2H, –CH_2); 7.32–7.80 (m, 3H, Ar–H); MS (EI) *m/z* 249 [M^+]; Anal. Calcd for $C_{11}H_{11}N_3O_4$: C, 53.01; H, 4.45; N, 16.86. Found: C, 53.19; H, 4.47; N, 16.90.

*5-Nitro-1-(pyrrolidin-1-ylmethyl)indolin-2,3-dione (**3b**)* Yield 69 %, Mp 210–212 °C; IR (KBr) cm^{-1}: 3010 (Ar–CH_{str}), 1720 (C=O, Isatin), 1532 (NO_{str}-asym.), 1326 (NO_{str}-sym.); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.48–1.66 (m, 4H, pyrrolidine); 2.32–2.54 (*t*, 4H, $J = 5.6$ Hz, pyrrolidine); 4.52 (s, 2H, –CH_2); 7.10–7.72 (m, 3H, Ar–H); MS (EI) *m/z* 275 [M^+]; Anal. Calcd for $C_{13}H_{13}N_3O_4$: C, 56.72; H, 4.76; N, 15.27. Found: C, 56.86; H, 4.78; N, 15.32.

*5-Nitro-1-(piperazin-1-ylmethyl)indolin-2,3-dione (**3c**)* Yield 80 %, Mp 226–228 °C; FT-IR (KBr): cm^{-1} 3062 (Ar C–H_{Str}); 1742 (C=O, isatin); 1544 (NO_{str}-asym.), 1352 (NO_{str}-sym.); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.60–2.94 (m, 8H, piperazine); 3.65 (br, 1H, NH); 4.50 (s, 2H, –CH_2); 6.90–7.54 (m, 3H, Ar–CH); MS (EI) *m/z*: 290 [M^+]; Anal. Calcd for $C_{13}H_{14}N_4O_4$: C, 53.79; H, 4.86; N, 19.30. Found: C, 53.92; H, 4.88; N, 19.37.

*1-((4-Methyl piperazin-1-yl)methyl)-5-nitro indolin-2,3-dione (**3d**)* Yield 78 %, Mp 242–244 °C; FT-IR (KBr): cm^{-1} 3032 (Ar C–H_{Str}); 1746 (C=O, isatin); 1538 (NO_{str}-asym.), 1366 (NO_{str}-sym.); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.32 (s, 3H, CH_3); 2.72–2.98 (m, 8H, piperazine); 4.62 (s, 2H, –CH_2); 7.08-7.64 (m, 3H, Ar–CH); MS (EI) *m/z*: 304 [M^+]; Anal. Calcd for $C_{14}H_{16}N_4O_4$: C, 55.26; H, 5.30; N, 18.41. Found: C, 55.44; H, 5.28; N, 18.47.

*5-Nitro-1-((4-phenyl piperazin-1-yl)methyl)indolin-2,3-dione (**3e**)* Yield 66 %, Mp 250–252 °C; FT-IR (KBr): cm^{-1} 3040 (Ar C–H_{Str}); 1730 (C=O, isatin); 1546 (NO_{str}-asym.), 1372 (NO_{str}-sym.); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.64–2.92 (m, 8H, piperazine); 4.45 (s, 2H, –CH_2); 7.12–7.82 (m, 8H, Ar–CH); MS (EI) *m/z*: 366 [M^+]; Anal. Calcd for $C_{19}H_{18}N_4O_4$: C, 62.29; H, 4.95; N, 15.29. Found: C, 62.08; H, 4.97; N, 15.34.

*5-Nitro-1-((4-(3-trifluoromethyl phenyl)piperazin-1-yl)methyl)indolin-2,3-dione (**3f**)* Yield 84 %, Mp 268–270 °C; FT-IR (KBr): cm^{-1} 3042 (Ar C–H_{Str}); 1722 (C=O, isatin); 1538 (NO_{str}-asym.), 1348 (NO_{str}-sym.); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.54–2.96 (m, 8H, piperazine); 4.24 (s, 2H, –CH_2); 7.02-7.78 (m, 7H, Ar–CH); MS (EI) *m/z*: 434 [M^+]; Anal. Calcd for $C_{20}H_{17}F_3N_4O_4$: C, 55.30; H, 3.94; N, 12.90. Found: C, 55.48; H, 3.96; N, 12.94.

*5-Amino-1-substituted indolin-2,3-dione (**4a–4f**)*

To a solution of 5-nitro-1-substituted indolin-2,3-dione (**3a–3f**) (0.01 mol) in absolute ethanol (100 mL) was added iron powder (0.01 mol) in a round bottomed flask, the mixture was heated on a oil bath until the temperature reaches to 80–85 °C. After that, in the reaction flask 4 mL of hydrochloric acid (1.2 M) was added and the content was stirred in the same temperature for 4 h.

Finally the slurry was filtered and pH of the filtrate was adjusted to 7–8 with sodium bicarbonate to get the precipitate of compound **4a–4f**. Recrystallization from ethanol gave desired products in pure form.

*1-((Dimethylamino)methyl)-5-amino indolin-2,3-dione (**4a**)* Yield 65 %, Mp 284–286 °C; IR (KBr) cm^{-1}: 3384 (NH_{str}); 2988 (Ar–CH_{str}); 1732 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.38 (s, 6H, CH_3); 4.26 (s, 2H, –CH_2); 5.49 (s, 2H, –NH_2); 7.12–7.78 (m, 3H, Ar–H); MS (EI) *m/z* 219 [M^+]; Anal. Calcd for $C_{11}H_{13}N_3O_2$: C, 60.26; H, 5.98; N, 19.17. Found: C, 60.44; H, 5.96; N, 19.22.

*5-Amino-1-(pyrrolidin-1-ylmethyl)indolin-2,3-dione (**4b**)* Yield 68 %, Mp 270–272 °C; IR (KBr) cm^{-1}: 3392 (NH_{str}); 2966 (Ar–CH_{str}); 1744 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.52–1.78 (m, 4H, pyrrolidine); 2.24–2.62 (*t*, 4H, $J = 5.6$ Hz, pyrrolidine); 4.32 (s, 2H, –CH_2); 5.80 (s, 2H, –NH_2); 7.02–7.64 (m, 3H, Ar–H); MS (EI) *m/z* 245 [M^+]; Anal. Calcd for $C_{13}H_{15}N_3O_2$: C, 63.66; H, 6.16; N, 17.13. Found: C, 63.46; H, 6.18; N, 17.19.

5-Amino-1-(piperazin-1-ylmethyl)indolin-2,3-dione ***(4c)*** Yield 74 %, Mp 252–254 °C; IR (KBr) cm^{-1}: 3354 (NH_{str}); 3014 (Ar–CH_{str}); 1724 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.72–2.98 (m, 8H, piperazine); 3.54 (br, 1H, NH piperazine); 4.38 (s, 2H, –CH_2); 5.68 (s, 2H, –NH_2); 7.12–7.84 (m, 3H, Ar–H); MS (EI) *m/z* 260 [M^+]; Anal. Calcd for $C_{13}H_{16}N_4O_2$: C, 59.99; H, 6.20; N, 21.52. Found: C, 60.18; H, 6.22; N, 21.59.

1-((4-Methyl piperazin-1-yl) methyl)-5-amino indolin-2,3-dione ***(4d)*** Yield 65 %, Mp 272–274 °C; IR (KBr) cm^{-1}: 3372 (NH_{str}); 3046 (Ar–CH_{str}); 1728 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.40 (s, 3H, CH_3); 2.84–3.10 (m, 8H, piperazine); 4.44 (s, 2H, –CH_2); 5.74 (s, 2H, –NH_2); 7.10–7.70 (m, 3H, Ar–H); MS (EI) *m/z* 274 [M^+]; Anal. Calcd for $C_{14}H_{18}N_4O_2$: C, 61.30; H, 6.61; N, 20.42. Found: C, 61.46; H, 6.63; N, 20.48.

5-Amino-1-((4-phenyl piperazin-1-yl)methyl)indolin-2,3-dione ***(4e)*** Yield 75 %, Mp 294–296 °C; IR (KBr) cm^{-1}: 3358 (NH_{str}); 3024 (Ar–CH_{str}); 1736 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.74–3.12 (m, 8H, piperazine); 4.48 (s, 2H, –CH_2); 5.52 (s, 2H, –NH_2); 7.04–7.78 (m, 8H, Ar–H); MS (EI) *m/z* 336 [M^+]; Anal. Calcd for $C_{19}H_{20}N_4O_2$: C, 67.84; H, 5.99; N, 16.66. Found: C, 68.06; H, 6.01; N, 16.70.

5-Amino-1-((4-(3-(trifluoromethyl)phenyl)piperazin-1-yl) methyl)indolin-2,3-dione ***(4f)*** Yield 64 %, Mp 288–290 °C; IR (KBr) cm^{-1}: 3386 (NH_{str}); 2994 (Ar–CH_{str}); 1728 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.66–2.90 (m, 8H, piperazine); 4.56 (s, 2H, –CH_2); 5.48 (s, 2H, –NH_2); 6.88–7.70 (m, 7H, Ar–H); MS (EI) *m/z* 404 [M^+]; Anal. Calcd for $C_{20}H_{19}F_3N_4O_2$: C, 59.40; H, 4.74; N, 13.85. Found: C, 59.62; H, 4.76; N, 13.91.

*1-(1-Substituted 2,3-dioxoindolin-5-yl)urea (**5a**–**5f**)*

Compound **4a**–**4f** (0.01 mol) was dissolved separately in 10 mL glacial acetic acid and the volume was diluted to 100 mL with the same in a conical flask. NaOCN (sodium cyanate) (0.01 mol) and 50 mL of warm water was then added slowly with continuous stirring. Then the reaction mixture was allowed to stand for 1 h, and then cooled in crushed ice, allowed to stand for further 30 min. The product thus obtained was filtered and washed with cold water, finally dried at 100 °C. The product was recrystallized at least once from ethanol to give the pure form.

1-(1-((Dimethylamino)methyl)-2,3-dioxoindolin-5-yl)urea ***(5a)*** Yield 65 %, Mp 248–250 °C; IR (KBr) cm^{-1}: 3358 (NH_{str}); 3024 (Ar–CH_{str}); 1748 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.48 (s, 6H, CH_3); 4.44 (s, 2H, –CH_2); 5.88 (s, 2H, –NH_2); 6.86–7.84 (m, 3H, Ar–H); 8.28 (s, 1H, Ar–NH); MS (EI) *m/z* 262 [M^+]; Anal. Calcd for $C_{12}H_{14}N_4O_3$: C, 54.96; H, 5.38; N, 21.36. Found: C, 55.12; H, 5.40; N, 21.42.

1-(2,3-Dioxo-1-(pyrrolidin-1-ylmethyl)indolin-5-yl)urea ***(5b)*** Yield 78 %, Mp 192–196 °C; IR (KBr) cm^{-1}: 3356 (NH_{str}); 3030 (Ar–CH_{str}); 1742 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.56-1.82 (m, 4H, pyrrolidine); 2.30–2.64 (*t*, 4H, $J = 5.6$ Hz, pyrrolidine); 4.24 (s, 2H, –CH_2); 5.92 (s, 2H, –NH_2); 6.92-7.84 (m, 3H, Ar–H); 8.32 (s, 1H, Ar–NH); MS (EI) *m/z* 288 [M^+]; Anal. Calcd for $C_{14}H_{16}N_4O_3$: C, 58.32; H, 5.59; N, 19.43. Found: C, 58.46; H, 5.61; N, 19.48.

1-(2,3-Dioxo-1-(piperazin-1-ylmethyl)indolin-5-yl)urea ***(5c)*** Yield 72 %, Mp 258–260 °C; IR (KBr) cm^{-1}: 3372 (NH_{str}); 3028 (Ar–CH_{str}); 1740 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.68–2.94 (m, 8H, piperazine); 3.50 (br, 1H, NH piperazine); 4.60 (s, 2H, –CH_2); 5.70 (s, 2H, –NH_2); 7.10–7.92 (m, 3H, Ar–H); 8.34 (s, 1H, Ar–NH); MS (EI) *m/z* 303 [M^+]; Anal. Calcd for $C_{14}H_{17}N_5O_3$: C, 55.44; H, 5.65; N, 23.09. Found: C, 55.58; H, 5.63; N, 23.14.

1-(1-((4-Methyl piperazin-1-yl)methyl)-2,3-dioxoindolin-5-yl)urea ***(5d)*** Yield 69 %, Mp 280–282 °C; IR (KBr) cm^{-1}: 3410 (NH_{str}); 3084 (Ar–CH_{str}); 1718 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.24 (s, 3H, CH_3); 2.68–2.96 (m, 8H, piperazine); 4.58 (s, 2H, –CH_2); 5.90 (s, 2H, –NH_2); 7.12–7.84 (m, 3H, Ar–H); 8.30 (s, 1H, Ar–NH); MS (EI) *m/z* 317 [M^+]; Anal. Calcd for $C_{15}H_{19}N_5O_3$: C, 56.77; H, 6.03; N, 22.07. Found: C, 56.60; H, 6.05; N, 22.12.

1-(2,3-Dioxo-1-((4-phenyl piperazin-1-yl)methyl)indolin-5-yl)urea ***(5e)*** Yield 81 %, Mp 238-240 °C; IR (KBr) cm^{-1}: 3392 (NH_{str}); 3068 (Ar–CH_{str}); 1722 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.82–3.24 (m, 8H, piperazine); 4.62 (s, 2H, –CH_2); 5.86 (s, 2H, –NH_2); 6.96–7.84 (m, 8H, Ar–H); 8.28 (s, 1H, Ar–NH); MS (EI) *m/z* 379 [M^+]; Anal. Calcd for $C_{20}H_{21}N_5O_3$: C, 63.31; H, 5.58; N, 18.46. Found: C, 63.45; H, 5.60; N, 18.52.

1-(2,3-Dioxo-1-((4-(3-(trifluoromethyl) phenyl)piperazin-1-yl) methyl)indolin-5-yl)urea ***(5f)*** Yield 70 %, Mp 222–224 °C; IR (KBr) cm^{-1}: 3358 (NH_{str}); 3046 (Ar–CH_{str}); 1748 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.70–2.98 (m, 8H, piperazine); 4.44 (s, 2H, –CH_2); 5.96 (s, 2H, –NH_2); 6.80–7.78 (m, 7H, Ar–H); 8.34 (s, 1H, Ar–NH); MS (EI) *m/z* 447 [M^+]; Anal. Calcd for $C_{21}H_{20}F_3N_5O_3$:

C, 56.37; H, 4.51; N, 15.65. Found: C, 56.52; H, 4.53; N, 15.70.

*4-((1-Substituted)-2,3-dioxoindolin-5-yl)semicarbazide (**6a–6f**)*

The compounds **5a–5j** (0.01 mol) was dissolved in 30 mL of absolute ethanol. To this was added 2.5 mL of hydrazine hydrate and entire mixture was refluxed for 12–15 h with stirring. The contents were concentrated to half of its volume and poured onto crushed ice. The resultant precipitate was filtered, thoroughly washed with water, dried and recrystallized from ethanol.

*4-(1-((Dimethylamino)methyl)-2,3-dioxoindolin-5-yl)semicarbazide (**6a**)* Yield 68 %, Mp 248–250 °C; IR (KBr) cm^{-1}: 3384 (NH_{str}); 2958 ($Ar–CH_{str}$); 1714 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.20 (s, 6H, CH_3); 4.32 (s, 2H, $–CH_2$); 5.60 (s, 2H, $–NH_2$); 6.80–7.80 (m, 3H, Ar–H); 8.24 (s, 1H, Ar–NH); 9.94 (1H, CONH); MS (EI) *m/z* 277 [M^+]; Anal. Calcd for $C_{12}H_{15}N_5O_3$: C, 51.98; H, 5.45; N, 25.26. Found: C, 52.15; H, 5.47; N, 25.35.

*4-(2,3-Dioxo-1-(pyrrolidin-1-ylmethyl)indolin-5-yl)semicarbazide (**6b**)* Yield 74 %, Mp 241–243 °C; IR (KBr) cm^{-1}: 3342 (NH_{str}); 3054 ($Ar–CH_{str}$); 1722 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.30–1.88 (m, 4H, pyrrolidine); 2.34–2.70 (*t*, 4H, $J = 5.6$ Hz, pyrrolidine); 4.38 (s, 2H, $–CH_2$); 5.72 (s, 2H, $–NH_2$); 6.90–7.74 (m, 3H, Ar–H); 8.28 (s, 1H, Ar–NH); 9.90 (1H, CONH); MS (EI) *m/z* 303 [M^+]; Anal. Calcd for $C_{14}H_{17}N_5O_3$: C, 55.44; H, 5.65; N, 23.09. Found: C, 55.59; H, 5.67; N, 23.30.

*4-(2,3-Dioxo-1-(piperazin-1-ylmethyl)indolin-5-yl)semicarbazide (**6c**)* Yield 86 %, Mp 265–267 °C; IR (KBr) cm^{-1}: 3392 (NH_{str}); 2958 ($Ar–CH_{str}$); 1730 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.72–2.90 (m, 8H, piperazine); 3.46 (br, 1H, NH); 4.45 (s, 2H, $–CH_2$); 5.75 (s, 2H, $–NH_2$); 7.05–7.84 (m, 3H, Ar–H); 8.24 (s, 1H, Ar–NH); 9.92 (1H, CONH); MS (EI) *m/z* 318 [M^+]; Anal. Calcd for $C_{14}H_{18}N_6O_3$: C, 52.82; H, 5.70; N, 26.40. Found: 52.99; H, 5.68; N, 26.49.

*4-(1-((4-Methylpiperazin-1-yl)methyl)-2,3-dioxoindolin-5-yl)semicarbazide (**6d**)* Yield 78 %, Mp 261–263 °C; IR (KBr) cm^{-1}: 3380 (NH_{str}); 3068 ($Ar–CH_{str}$); 1722 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.28 (s, 3H, CH_3); 2.72-2.94 (m, 8H, piperazine); 4.30 (s, 2H, $–CH_2$); 5.74 (s, 2H, $–NH_2$); 6.94–7.74 (m, 3H, Ar–H); 8.28 (s, 1H, Ar–NH); 9.88 (1H, CONH); MS (EI) *m/z* 332 [M^+]; Anal. Calcd for $C_{15}H_{20}N_6O_3$: C, 54.21; H, 6.07; N, 25.29. Found: C, 54.40; H, 6.09; N, 25.35.

*4-(2,3-Dioxo-1-((4-phenylpiperazin-1-yl)methyl)indolin-5-yl)semicarbazide (**6e**)* Yield 66 %, Mp 277–279 °C; IR (KBr) cm^{-1}: 3385 (NH_{str}); 3054 ($Ar–CH_{str}$); 1744 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.72–3.22 (m, 8H, piperazine); 4.56 (s, 2H, $–CH_2$); 5.80 (s, 2H, $–NH_2$); 6.90–7.82 (m, 8H, Ar–H); 8.22 (s, 1H, Ar–NH); 9.92 (1H, CONH); MS (EI) *m/z* 394 [M^+]; Anal. Calcd for $C_{20}H_{22}N_6O_3$: C, 60.90; H, 5.62; N, 21.31. Found: C, 61.12; H, 5.64; N, 21.39.

*4-(2,3-Dioxo-1-((4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)methyl)indolin-5-yl)semicarbazide (**6f**)* Yield 71 %, Mp 243–245 °C; IR (KBr) cm^{-1}: 3368 (NH_{str}); 2984 ($Ar–CH_{str}$); 1726 (C=O, Isatin); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.65-2.94 (m, 8H, piperazine); 4.32 (s, 2H, $–CH_2$); 5.76 (s, 2H, $–NH_2$); 6.88–7.69 (m, 7H, Ar–H); 8.24 (s, 1H, Ar–NH); 9.96 (1H, CONH); MS (EI) *m/z* 462 [M^+]; Anal. Calcd for $C_{21}H_{21}F_3N_6O_3$: C, 54.54; H, 4.58; N, 18.17. Found: C, 54.68; H, 4.60; N, 18.23.

*Synthesis of title compounds (**7a–7r**)*

Title compounds **7a–7r** was synthesized by adding different aromatic aldehydes (0.01 mol) in fraction with the well stirred mixture of **6a–6f** (0.01 mol) in 50 mL ethanol and 2–3 drops of glacial acetic acid. The pH was maintained to 5–6. The reaction mixture was refluxed for 3–6 h and kept aside for 4 h. The products were separated by filtration, washed and vacuum dried. Finally the products were recrystallized using ethanol to get pure form. The method used for the preparation and isolation of the compounds gave materials of good purity, as evidenced by their spectral analyses and by TLC. The title compounds are found to be soluble in chloroform, dimethyl sulfoxide, and dimethylformamide.

*1-(4-Chlorobenzylidene)-4-(1-((dimethylamino)methyl)-2,3-dioxoindolin-5-yl)semicarbazide (**7a**)* FT-IR (KBr): cm^{-1} 2915 (Ar C–H_{Str}); 1710 (C=O, isatin); 1534 (CH=N); 748 (C–Cl); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.20 (s, 6H, $N(CH_3)_2$); 4.10 (s, 2H, $–CH_2$); 7.12–7.86 (m, 7H, Ar–CH); 8.24 (s, 1H, Ar–NH); 8.60 (s, 1H, CH=N); 9.84 (s, 1H, CONH); MS (EI) *m/z*: 401 [M+2]; Anal. Calcd for $C_{19}H_{18}ClN_5O_3$: C, 57.07; H, 4.54; N, 17.52. Found: C, 57.24; H, 4.56; N, 17.59.

*1-(4-Nitrobenzylidene)-4-(1-((dimethylamino)methyl)-2,3-dioxoindolin-5-yl)semicarbazide (**7b**)* FT-IR (KBr): cm^{-1} 2958 (Ar C–H_{Str}); 1722 (C=O, isatin); 1535 (CH=N); 1574 & 1326 (C-NO_2); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.32 (s, 6H, $N(CH_3)_2$); 4.35 (s, 2H, $–CH_2$); 6.98–7.96 (m, 7H, Ar–CH); 8.24 (s, 1H, Ar–NH); 8.52 (s, 1H, CH=N); 9.88 (s, 1H, CONH); MS (EI) *m/z*: 410 [M^+]; Anal. Calcd

for $C_{19}H_{18}N_6O_5$: C, 55.61; H, 4.42; N, 20.48. Found: C, 55.80; H, 4.44; N, 20.56.

*1-(4-Methylbenzylidene)-4-(1-((dimethylamino)methyl)-2,3-dioxoindolin-5-yl)semicarbazide (**7c**)* FT-IR (KBr): cm^{-1} 2900 (Ar C–H_{Str}); 1740 (C=O, isatin); 1532 (CH=N); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.21 (s, 6H, $N(CH_3)_2$); 2.45 (s, 3H, CH_3); 4.30 (s, 2H, –CH_2); 6.86–7.84 (m, 7H, Ar–CH); 8.20 (s, 1H, Ar–NH); 8.44 (s, 1H, CH=N); 9.84 (s, 1H, CONH); MS (EI) *m/z*: 379 [M^+]; Anal. Calcd for $C_{20}H_{21}N_5O_3$: C, 63.31; H, 5.58; N, 18.46. Found: C, 63.52; H, 5.60; N, 18.53.

*1-(4-Chlorobenzylidene)-4-(2,3-dioxo-1-(pyrrolidin-1-yl methyl) indolin-5-yl)semicarbazide (**7d**)* FT-IR (KBr): cm^{-1} 2920 (Ar C–H_{Str}); 1730 (C=O, isatin); 1554 (CH=N); 732 (C–Cl); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.52-1.71 (m, 4H, CH_2-pyrrolidine); 2.60 (*t*, $J = 5.8$, 4H, CH_2-pyrrolidine); 4.45 (s, 2H, –CH_2); 7.00–7.92 (m, 7H, Ar–CH); 8.20 (s, 1H, Ar–NH); 8.43 (s, 1H, CH=N); 9.90 (s, 1H, CONH); MS (EI) *m/z*: 428 [M+2]; Anal. Calcd for $C_{21}H_{20}ClN_5O_3$: C, 59.23; H, 4.73; N, 16.44. Found: C, 59.42; H, 4.71; N, 16.50.

*1-(4-Nitrobenzylidene)-4-(2,3-dioxo-1-(pyrrolidin-1-yl methyl) indolin-5-yl)semicarbazide (**7e**)* FT-IR (KBr): cm^{-1} 3010 (Ar C–H_{Str}); 1744 (C=O, isatin); 1562 (CH=N); 1572 & 1346 (C-NO_2); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.42–1.60 (m, 4H, CH_2-pyrrolidine); 2.53 (*t*, $J = 5.8$, 4H, CH_2-pyrrolidine); 4.35 (s, 2H, –CH_2); 7.12–7.84 (m, 7H, Ar–CH); 8.12 (s, 1H, Ar–NH); 8.32 (s, 1H, CH=N); 9.88 (s, 1H, CONH); MS (EI) *m/z*: 436 [M^+]; Anal. Calcd for $C_{21}H_{20}N_6O_5$: C, 57.79; H, 4.62; N, 19.26. Found: C, 57.96; H, 4.64; N, 19.34.

*1-(4-Methylbenzylidene)-4-(2,3-dioxo-1-(pyrrolidin-1-yl methyl) indolin-5-yl)semicarbazide (**7f**)* FT-IR (KBr): cm^{-1} 3024 (Ar C–H_{Str}); 1740 (C=O, isatin); 1546 (CH=N); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.56–1.68 (m, 4H, CH_2-pyrrolidine); 2.32 (s, 3H, CH_3); 2.58 (*t*, $J = 5.6$, 4H, CH_2-pyrrolidine); 4.52 (s, 2H, –CH_2); 6.98–7.80 (m, 7H, Ar–CH); 8.22 (s, 1H, Ar–NH); 8.38 (s, 1H, CH=N); 9.92 (s, 1H, CONH); MS (EI) *m/z*: 405 [M^+]; Anal. Calcd for $C_{22}H_{23}N_5O_3$: C, 65.17; H, 5.72; N, 17.27. Found: C, 65.38; H, 5.74; N, 17.34.

*1-(4-Chlorobenzylidene)-4-(2,3-dioxo-1-(piperazin-1-ylmethyl) indolin-5-yl)semicarbazide (**7g**)* FT-IR (KBr): cm^{-1} 3058 (Ar C–H_{Str}); 1752 (C=O, isatin); 1538 (CH=N); 744 (C–Cl); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.70–2.92 (m, 8H, piperazine); 3.66 (br, 1H, NH); 4.62 (s, 2H, –CH_2); 6.85–7.76 (m, 7H, Ar–CH); 8.28 (s, 1H, Ar–NH); 8.48 (s, 1H, CH=N); 9.90 (s, 1H, CONH); MS (EI) *m/z*: 441 [M+2]; Anal. Calcd for $C_{21}H_{21}ClN_6O_3$: C, 57.21; H, 4.80; N, 19.06. Found: C, 57.38; H, 4.82; N, 19.13.

*1-(4-Nitrobenzylidene)-4-(2,3-dioxo-1-(piperazin-1-yl methyl) indolin-5-yl)semicarbazide (**7h**)* FT-IR (KBr): cm^{-1} 2952 (Ar C–H_{Str}); 1732 (C=O, isatin); 1546 (CH=N); 1545 & 1364 (C-NO_2); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.64–2.90 (m, 8H, piperazine); 3.46 (br, 1H, NH); 4.45 (s, 2H, –CH_2); 6.94-7.88 (m, 7H, Ar–CH); 8.32 (s, 1H, Ar–NH); 8.56 (s, 1H, CH=N); 9.82 (s, 1H, CONH); MS (EI) *m/z*: 451 [M^+]; Anal. Calcd for $C_{21}H_{21}N_7O_5$: C, 55.87; H, 4.69; N, 21.72. Found: C, 56.04; H, 4.71; N, 21.80.

*1-(4-Methylbenzylidene)-4-(2,3-dioxo-1-(piperazin-1-ylmethyl) indolin-5-yl)semicarbazide (**7i**)* FT-IR (KBr): cm^{-1} 3010 (Ar C–H_{Str}); 1722 (C=O, isatin); 1532 (CH=N); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.34 (s, 3H, CH_3); 2.68–2.94 (m, 8H, piperazine); 3.58 (br, 1H, NH); 4.64 (s, 2H, –CH_2); 7.08-7.92 (m, 7H, Ar–CH); 8.38 (s, 1H, Ar–NH); 8.62 (s, 1H, CH=N); 9.88 (s, 1H, CONH); MS (EI) *m/z*: 420 [M^+]; Anal. Calcd for $C_{22}H_{24}N_6O_3$: C, 62.84; H, 5.75; N, 19.99. Found: C, 62.98; H, 5.77; N, 20.07.

*1-(4-Chlorobenzylidene)-4-(1-((4-methyl piperazine-1-yl) methyl)-2,3-dioxoindolin-5-yl)semicarbazide (**7j**)* FT-IR (KBr): cm^{-1} 3068 (Ar C–H_{Str}); 1724 (C=O, isatin); 1562 (CH=N); 768 (C–Cl); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.38 (s, 3H, CH_3); 2.74–3.12 (m, 8H, piperazine); 4.54 (s, 2H, –CH_2); 6.82–7.90 (m, 7H, Ar–CH); 8.30 (s, 1H, Ar–NH); 8.54 (s, 1H, CH=N); 9.84 (s, 1H, CONH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 46.2 (CH_3); 72.1 (2C), 64.5 (2C) (Piperazinyl); 75.2 (CH_2); 110.3, 126.6, 127.2 (CH, Indolyl); 128.5 (2C), 129.8 (2C) (CH_{arom}); 131.2, 132.3, 137.5 (C, Indolyl); 134.7, 138.6 (C_{arom}); 145.1 (C=N); 162.5 (C=O, Urea); 176.2, 182.5 (C=O, Indolyl). MS (EI) *m/z*: 455 [M+2]; Anal. Calcd for $C_{22}H_{23}ClN_6O_3$: C, 58.09; H, 5.10; N, 18.47. Found: C, 58.28; H, 5.08; N, 18.54.

*1-(4-Nitrobenzylidene)-4-(1-((4-methyl piperazine-1-yl) methyl)-2,3-dioxoindolin-5-yl) semicarbazide (**7k**)* FT-IR (KBr): cm^{-1} 3024 (Ar C–H_{Str}); 1718 (C=O, isatin); 1498 (CH=N); 1556 & 1388 (C-NO_2); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.40 (s, 3H, CH_3); 2.64-2.96 (m, 8H, piperazine); 4.34 (s, 2H, –CH_2); 6.96–7.80 (m, 7H, Ar–CH); 8.22 (s, 1H, Ar–NH); 8.60 (s, 1H, CH=N); 9.82 (s, 1H, CONH); MS (EI) *m/z*: 465 [M^+]; Anal. Calcd for $C_{22}H_{23}N_7O_5$: C, 56.77; H, 4.98; N, 21.06. Found: C, 56.92; H, 5.00; N, 21.14.

*1-(4-Methylbenzylidene)-4-(1-((4-methyl piperazine-1-yl) methyl)-2,3-dioxoindolin-5-yl)semicarbazide (**7l**)* FT-IR (KBr): cm^{-1} 3054 (Ar C–H_{Str}); 1728 (C=O, isatin); 1522 (CH=N); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.20 (s, 3H, CH_3); 2.42 (s, 3H, N–CH_3); 2.5–2.80 (m, 8H, piperazine); 4.55 (s, 2H, –CH_2); 7.02-7.88 (m, 7H, Ar–CH); 8.28 (s, 1H, Ar–NH); 8.54 (s, 1H, CH=N); 9.90 (s, 1H, CONH); MS (EI) *m/z*: 434 [M^+]; Anal. Calcd for $C_{23}H_{26}N_6O_3$: C, 63.58; H, 6.03; N, 19.34. Found: C, 63.72; H, 6.05; N, 19.41.

*1-(4-Chlorobenzylidene)-4-(2,3-dioxo-1-((4-phenyl piperazin-1-yl)methyl)indolin-5-yl)semicarbazide (**7m**)* FT-IR (KBr): cm^{-1} 2984 (Ar C–H_{Str}); 1732 (C=O, isatin); 1560 (CH=N); 774 (C–Cl); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.58–2.94 (m, 8H, piperazine); 4.62 (s, 2H, –CH_2); 6.78–7.98 (m, 12H, Ar–CH); 8.12 (s, 1H, Ar–NH); 8.46 (s, 1H, CH=N); 9.94 (s, 1H, CONH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 72.6 (2C), 58.6 (2C) (Piperazinyl); 74.4 (CH_2); 112.6, 122.9, 130.4 (CH, Indolyl); 116.3 (2C), 120.4, 125.8 (2C), 127.4 (2C), 128.2 (2C) (CH_{arom}); 131.4, 133.6, 136.2 (C, Indolyl); 132.5, 139.1, 142.6 (C_{arom}); 144.3 (C=N); 162.9 (C=O, Urea); 176.8, 181.8 (C=O, Indolyl). MS (EI) *m/z*: 519 [M+2]; Anal. Calcd for $C_{27}H_{25}ClN_6O_3$: C, 62.73; H, 4.87; N, 16.26. Found: C, 62.92; H, 4.89; N, 16.32.

*1-(4-Nitrobenzylidene)-4-(2,3-dioxo-1-((4-phenyl piperazin-1-yl)methyl)indolin-5-yl)semicarbazide (**7n**)* FT-IR (KBr): cm^{-1} 3030 (Ar C–H_{Str}); 1738 (C=O, isatin); 1492 (CH=N); 1538 & 1374 (C-NO_2); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.58–2.84 (m, 8H, piperazine); 4.50 (s, 2H, –CH_2); 6.66–7.88 (m, 12H, Ar–CH); 8.26 (s, 1H, Ar–NH); 8.62 (s, 1H, CH=N); 9.92 (s, 1H, CONH); MS (EI) *m/z*: 527 [M^+]; Anal. Calcd for $C_{27}H_{25}N_7O_5$: C, 61.47; H, 4.78; N, 18.59. Found: C, 61.68; H, 4.80; N, 18.66.

*1-(4-Methylbenzylidene)-4-(2,3-dioxo-1-((4-phenyl piperazin-1-yl)methyl)indolin-5-yl)semicarbazide (**7o**)* FT-IR (KBr): cm^{-1} 3042 (Ar C–H_{Str}); 1740 (C=O, isatin); 1510 (CH=N); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.32 (s, 3H, CH_3); 2.62–2.90 (m, 8H, piperazine); 4.64 (s, 2H, –CH_2); 6.70–7.92 (m, 12H, Ar–CH); 8.30 (s, 1H, Ar–NH); 8.50 (s, 1H, CH=N); 9.87 (s, 1H, CONH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 38.4 (CH_3); 72.4 (2C), 59.8 (2C) (Piperazinyl); 73.8 (CH_2); 112.8, 126.4, 130.8 (CH, Indolyl); 114.4 (2C), 118.5, 124.9 (2C), 128.3 (2C), 129.3 (2C) (CH_{arom}); 131.9, 132.8, 136.6 (C, Indolyl); 134.5, 137.5, 141.2 (C_{arom}); 142.5 (C=N); 163.6 (C=O, Urea); 177.6, 180.6 (C=O, Indolyl). MS (EI) *m/z*: 496 [M^+]; Anal. Calcd for $C_{28}H_{28}N_6O_3$: C, 67.73; H, 5.68; N, 16.92. Found: C, 67.94; H, 5.70; N, 16.98.

*1-(4-Chlorobenzylidene)-4-(2,3-dioxo-1-((4-(3-(trifluoromethyl) phenyl) piperazin-1-yl) methyl)indolin-5-yl)semicarbazide (**7p**)* FT-IR (KBr): cm^{-1} 2986 (Ar C–H_{Str}); 1744 (C=O, isatin); 1572 (CH=N); 745 (C–Cl); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.52–2.78 (m, 8H, piperazine); 4.42 (s, 2H, –CH_2); 6.66–7.84 (m, 11H, Ar–CH); 8.24 (s, 1H, Ar–NH); 8.52 (s, 1H, CH=N); 9.92 (s, 1H, CONH); MS (EI) *m/z*: 587 [M+2]; Anal. Calcd for $C_{28}H_{24}ClF_3N_6O_3$: C, 57.49; H, 4.14; N, 14.37. Found: C, 57.70; H, 4.16; N, 14.42.

*1-(4-Nitrobenzylidene)-4-(2,3-dioxo-1-((4-(3-(trifluoromethyl phenyl)piperazin-1-yl) methyl)indolin-5-yl)semicarbazide (**7q**)* FT-IR (KBr): cm^{-1} 3036 (Ar C–H_{Str}); 1750 (C=O, isatin); 1534 (CH=N); 1562 & 1344 (C-NO_2); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.84–3.06 (m, 8H, piperazine); 4.32 (s, 2H, –CH_2); 6.72–7.92 (m, 11H, Ar–CH); 8.36 (s, 1H, Ar–NH); 8.58 (s, 1H, CH=N); 9.91 (s, 1H, CONH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 70.2 (2C), 62.4 (2C) (Piperazinyl); 72.4 (CH_2); 113.6, 123.8, 129.6 (CH, Indolyl); 120.3 (CF_3); 116.2, 118.9, 122.6, 125.6, 126.8 (2C), 127.9 (2C), (CH_{arom}); 130.2, 133.4, 136.2 (C, Indolyl); 131.8, 138.6, 140.8, 144.6 (C_{arom}); 143.9 (C=N); 162.4 (C=O, Urea); 174.2, 182.3 (C=O, Indolyl). MS (EI) *m/z*: 595 [M^+]; Anal. Calcd for $C_{28}H_{24}F_3N_7O_5$: C, 56.47; H, 4.06; N, 16.46. Found: C, 56.66; H, 4.07; N, 16.50.

*1-(4-Methylbenzylidene)-4-(2,3-dioxo-1-((4-(3-(trifluoromethyl) phenyl)piperazin-1-yl) methyl)indolin-5-yl)semicarbazide (**7r**)* FT-IR (KBr): cm^{-1} 2998 (Ar C–H_{Str}); 1734 (C=O, isatin); 1530 (CH=N); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.34 (s, 3H, CH_3); 2.72–2.98 (m, 8H, piperazine); 4.36 (s, 2H, –CH_2); 6.70-7.84 (m, 11H, Ar–CH); 8.22 (s, 1H, Ar–NH); 8.48 (s, 1H, CH=N); 9.88 (s, 1H, CONH); MS (EI) *m/z*: 564 [M^+]; Anal. Calcd for $C_{29}H_{27}F_3N_6O_3$: C, 61.70; H, 4.82; N, 14.89. Found: C, 61.92; H, 4.81; N, 14.95.

Evaluation of antimycobacterial activity

The target compounds were screened for their in vitro antimycobacterial activity against *M. tuberculosis* H37Rv (ATCC 27294) and MDR-TB by resazurin assay method (Taneja et al. 2007) and their MIC values were determined. Resazurin reduction assays were developed to screen for drugs and compounds active against growing and dormant mycobacteria. The MDR-TB strain was obtained from Tuberculosis Research Center, Chennai, India, and was resistant to rifampicin, isoniazid, ethambutol and ofloxacin. *M. tuberculosis* strains were developed in Middlebrook 7H9 broth supplemented with 10 % albumin dextrose complex, 0.05 % tween 80 and 0.05 % glycerol. The culture was diluted to McFarland 2 standard with the similar medium. From this, 50 µL of this bacterial culture was

added to 150 μL of fresh medium in black, clear-bottomed, 96-well microplates (The use of black microtitre plates permitted measurement of fluorescence with a minimum background and subsequent MIC determination post resazurin reduction). The stock solutions (1 mg/mL) of the test compounds were prepared in DMSO. The compounds were tested to begin with 1, 10 and 100 μg/mL concentrations. Further the subsequent level testing was carried out at concentrations of 0.3125, 0.625, 1.25, 2.5, and 5 μg/mL. Control experiments were done using a growth media free from drugs or the tested compounds. Rifampicin and isoniazid was used as the standard compounds. The plates were incubated at 37 °C for 7 days. Thereafter, 20 μL of 0.01 % resazurin (Sigma, St. Louis, USA) and 12.5 μL of 20 % tween 80 were added to each well. The wells were observed after 24 and 48 h for a colour change from blue to pink. Resazurin, a redox dye, was blue in colour in its oxidized state and pink colour when it gets reduced by the growth of viable cells. The control tubes showed a colour change from blue to pink after 1 h at 37 °C. Compounds which prevented the change of colour of the resazurin dye were considered to be inhibitory to *M. tuberculosis* strains. The MIC was defined as the lowest concentration of a test compound at which the visible inhibition of the growth of the bacteria occurred.

Results and discussion

Synthesis

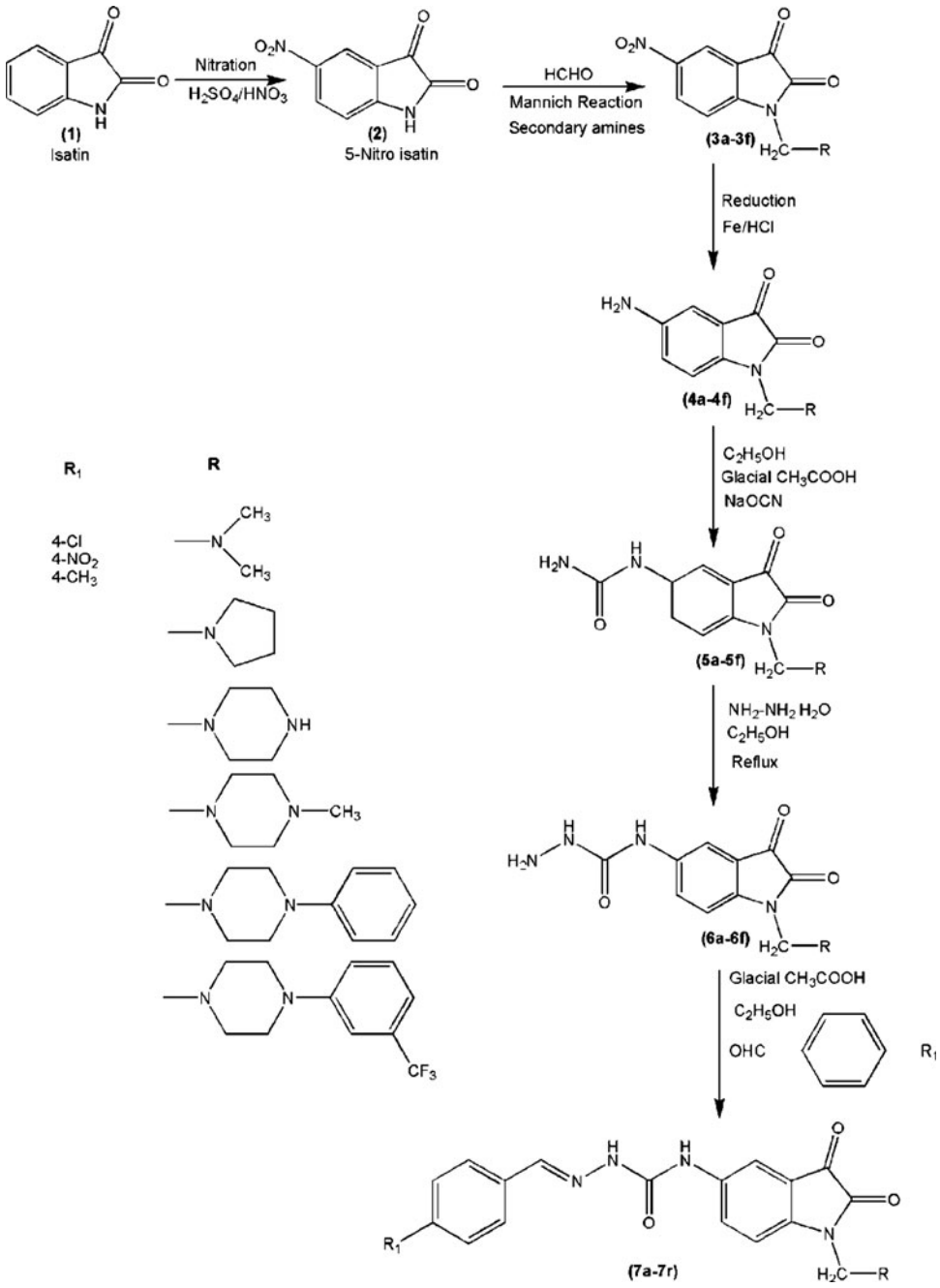
All the intermediate and title compounds are synthesized according to Scheme 1. Many compounds are obtained in moderate to good yield. The structures of the synthesized 1-(4-substituted benzylidene)-4-((1-substituted)-2,3-dioxo indolin-5-yl)semicarbazide derivatives (**7a**–**7r**) were established by their consistent spectral (IR, ^{1}H-NMR & Mass) and elemental analyses data. For example, the presence of nitro group in compound **2** was characterized by the presence of two strong bands in its IR spectrum at 1,348 and 1,570 cm^{-1} which arises from the symmetrical and asymmetrical stretching modes. The formation of compounds **3a**–**3f** were confirmed by the appearance of singlet at δ 4.24–4.62 ppm for two protons in its ^{1}H-NMR spectra which might be assigned to –NCH$_2$N-group connecting the isatin nucleus with secondary amines through Mannich reaction. The conversion of amine derivatives **4a**–**4f** from nitro **3a**–**3f** was confirmed by presence of strong absorption peak at 3,354–3,392 cm^{-1} in IR due to N–H stretching and absence of symmetrical and asymmetrical NO$_2$ stretching peaks in the same spectrum.

The appearance of peak at 2,900–3,100 cm^{-1} indicates presence of aromatic portions in all the synthesized compounds. The presence of aromatic protons was also confirmed by the multiplet signal in the range of δ 6.50–8.00 ppm. The appearance of singlet signal at δ 8.32–8.62 ppm revealed the formation of azomethine linkage (CH=N) bond in all the final compounds (**7a**–**7r**). The presence of the C=O functional group was indicated by the appearance of a strong stretching vibration band around 1,700–1,750 cm^{-1}, which is the characteristic of an keto group of isatin. The classic C–Cl stretching bands were observed at 748, 732, 744, 768, 774 and 745 cm^{-1} in final compound **7a**, **7d**, **7g**, **7j**, **7m** and **7p** respectively. Further, the aromatic nitro stretching around 1,350 cm^{-1} (symmetric NO$_2$ stretching) depicted the presence of nitro functional group in synthesized compounds **7c**, **7e**, **7h**, **7k**, **7n** and **7q**.

A typical spectral detail of compound **7q** has been discussed in the following section. The FTIR spectrum of title compound **7q** over the 3,036 cm^{-1} showed multiple weak absorption peaks corresponding to Ar–H stretching vibration. The strong absorption at 1,750 cm^{-1} is due to the C=O stretching vibration and the moderate intensity absorption at 1,534 cm^{-1} corresponds to a CH=N stretching vibration. The strong symmetrical stretching vibration at 1,344 cm^{-1} arises due to presence of C–NO$_2$ bond. It's ^{1}H-NMR spectrum showed a singlet at δ 8.58 ppm due to the proton attached to the imine carbon. A group of signals appeared between δ 6.72 and 7.92 ppm corresponds to Ar–H protons. The presence of CH=N stretching vibration at 1,534 cm^{-1} in IR spectrum and a singlet for a proton attached to the imine carbon at δ 8.58 ppm in ^{1}H-NMR confirms the formation of **7q**. Further mass spectrum confirmed their purity and molecular weight.

Lipophilicity

It is well documented that the lipophilicity of a molecule plays a vital role in determining its suitability as a drug candidate. The lipophilic properties of molecules influence essentially blood brain barrier distribution, cell membrane penetration, drug–receptor interaction, drug absorption processes in biological systems etc. (Kulig et al. 2003). It has been shown that the improved lipophilicity can be usually associated with the increased biological activity. Thus, hydrophobic parameter of a drug candidate seems to be the most important physicochemical parameter in the structure activity relationship studies. Lipophilicity of the final synthesized derivatives was expressed in the term of their clog *P* values which were calculated with the aid of Chem office 2009 software. As shown in Table 1, majority of the compounds except **7b**, **7e**, **7h** and **7k** have moderate to excellent lipophilicity character as evidenced by its clog *P* values (0.64–5.17). This may be rendering them more capable of penetrating various biomembranes, as a result



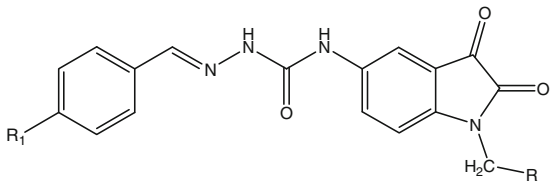
Scheme 1 Synthetic protocol of intermediates and target compounds

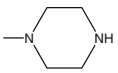
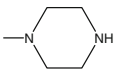
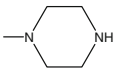
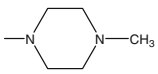
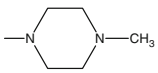
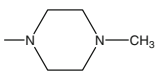
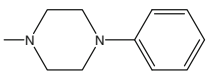
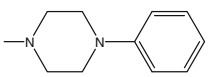
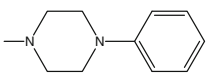
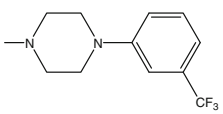
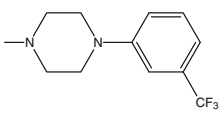
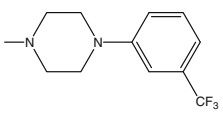
improving their penetrability towards mycobacterial cell membrane. In other words, the remarkable lipophilic character of the target compounds probably enhances their antimycobacterial activity.

Antimycobacterial evaluation

The in vitro antimycobacterial activity was evaluated against *M. tuberculosis* H37Rv (ATCC 27294), and MDR-TB strain by resazurin assay method. The MIC of the test compounds along with the standard drugs for comparison was reported in Table 2. Majority of the synthesized compounds exhibited an interesting activity profile against the tested mycobacterial strain. The initial antimycobacterial screening of the title compounds was carried out at different concentrations of 1, 10 and 100 μg/mL. From the result, it was observed that the compounds **7a**, **7c**, **7d**, **7f**, **7g**, **7i**, **7j** and **7l–7r** were active either at 1 μg/mL or ≤10 μg/mL concentrations against *M. tuberculosis* H37Rv strain. The active compounds from the initial exploration were further subjected to subsequent level of testing. The compounds which were active at ≥100 μg/mL concentration in the preliminary screening were omitted for further studies. The subsequent level testing was carried out at

Table 1 Physicochemical characterization of synthesized compounds **7a–7r**



Compd	R	R_1	Mol. formula	% Yield	Mp (°C)	R_f^a	C log P^b
7a	$-N(CH_3)_2$	Cl	$C_{19}H_{18}ClN_5O_3$	72	262–264	0.84	2.41
7b	$-N(CH_3)_2$	NO_2	$C_{19}H_{18}N_6O_5$	62	228–231	0.65	–
7c	$-N(CH_3)_2$	CH_3	$C_{20}H_{21}N_5O_3$	87	170–174	0.78	2.34
7d		Cl	$C_{21}H_{20}ClN_5O_3$	76	272–274	0.96	2.73
7e		NO_2	$C_{21}H_{20}N_6O_5$	78	218–220	0.85	–
7f		CH_3	$C_{22}H_{23}N_5O_3$	82	168–170	0.66	2.66
7g		Cl	$C_{21}H_{21}ClN_6O_3$	76	248–250	0.74	1.79
7h		NO_2	$C_{21}H_{21}N_7O_5$	81	276–278	0.80	–
7i		CH_3	$C_{22}H_{24}N_6O_3$	75	266–268	0.92	1.72
7j		Cl	$C_{22}H_{23}ClN_6O_3$	74	252–254	0.82	2.17
7k		NO_2	$C_{22}H_{23}N_7O_5$	72	290–292	0.52	–
7l		CH_3	$C_{23}H_{26}N_6O_3$	86	278–280	0.68	2.10
7m		Cl	$C_{27}H_{25}ClN_6O_3$	80	310–312	0.60	4.24
7n		NO_2	$C_{27}H_{25}N_7O_5$	84	282–284	0.76	0.64
7o		CH_3	$C_{28}H_{28}N_6O_3$	78	228–230	0.86	4.17
7p		Cl	$C_{28}H_{24}ClF_3N_6O_3$	83	244–246	0.62	5.17
7q		NO_2	$C_{28}H_{24}F_3N_7O_5$	81	232–234	0.71	1.62
7r		CH_3	$C_{29}H_{27}F_3N_6O_3$	88	294–296	0.85	5.09

[a] Solvent system used was ethyl acetate/*n*-hexane (1:1)

[b] Log *P* was calculated with Chem office 2009 software

Table 2 In vitro antimycobacterial screening data (MIC) of the target compounds **7a–7r** and reference drugs

Compound	Initial in vitro screening results: MIC (μg/mL)	Subsequent level screening results: MIC (μg/mL)	
	MTB[a]	MTB[a]	MDR-TB[b]
7a	10	2.5	>50
7b	>100	–	–
7c	10	5	>50
7d	10	2.5	>50
7e	>100	–	–
7f	10	5	>50
7g	1	1.25	25
7h	>100	–	–
7i	10	2.5	>50
7j	1	0.625	12.5
7k	>100	–	–
7l	10	5	>50
7m	1	0.625	6.25
7n	10	1.25	12.5
7o	1	0.625	12.5
7p	1	1.25	25
7q	1	0.625	6.25
7r	1	1.25	25
INH[c]	1	0.625	12.5
RIF[d]	1	0.312	25

[a] *Mycobacterium tuberculosis*

[b] *Mycobacterium tuberculosis* resistant to at least three drugs viz., isoniazid, rifampicin, ethambutol

[c] Isoniazid

[d] Rifampicin

concentrations of 0.3125, 0.625, 1.25, 2.5, and 5 μg/mL. Amongst the tested compounds, **7j**, **7m**, **7o** and **7q** (MIC—0.625 μg/mL) showed equipotent activity when compared with first line drug such as Isoniazid (MIC—0.625 μg/mL) against *M. tuberculosis* H37Rv strain and compounds **7 g**, **7n**, **7p** and **7r** (MIC—1.25 μg/mL) are also showed significant activity. It is interesting to note that the compounds showed better activity against *M. tuberculosis* H37Rv strain (with the MIC ranging from 0.625 to 1.25 μg/mL) are contain either chloro or nitro group on the distal phenyl ring except **7r** and **7o** (which had methyl group). Moreover, N1 position of isatin substituted by *N*-methyl piperazine or *N*-phenyl piperazine (**7j**, **7m**, **7o** and **7q**) exhibited remarkable activity (MIC—0.625 μg/mL). However, when N1 was substituted by dimethylamino or five membered pyrrolidine ring (compounds **7a–7f**), it resulted in compounds with decreased antimycobacterial activity. Further, the compounds **7j**, **7n** and **7o** displayed substantial activity against the MDR-TB strain (MIC—12.5 μg/mL) whereas, **7m** and **7q** exhibited promising activity against MDR-TB strain (MIC—6.25 μg/mL). All the compounds showed less active than rifampicin (MIC—0.312 μg/mL) against *M. tuberculosis* H37Rv strain.

Conclusion

In conclusion, a novel series of 1-(4-substituted benzylidene)-4-(1-(substituted methyl)-2,3-dioxoindolin-5-yl)semicarbazide derivatives (**7a–7r**) were designed, synthesized and screened for their in vitro antimycobacterial activity. Results of bioassay indicated that the compounds **7j**, **7m**, **7o** and **7q** possesses equipotent activity as standard drug Isoniazid against MTB while **7m** and **7q** exhibited higher activity against MDR-TB strain when compared with both the reference drugs. The remaining compounds showed good-to-moderate activity. Compounds 1-(4-chlorobenzylidene)-4-(2,3-dioxo-1-((4-phenyl piperazin-1-yl)methyl)indolin-5-yl)semicarbazide (**7m**) and 1-(4-nitrobenzylidene)-4-(2,3-dioxo-1-((4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)methyl)indolin-5-yl)semicarbazide (**7q**) may be further evaluated in other mycobacterium strains and in vivo animal models in the line of further development, and can serve up as a prototype molecule of new class of antimycobacterial agents.

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References

Alvirez-Freites, E.J., J.L. Carter, and M.H. Cynamon. 2002. *In vitro* and in vivo activities of gatifloxacin against *Mycobacterium tuberculosis*. *Antimicrobial Agents and Chemotherapy* 46: 1022–1025.

Andreani, A., S. Burnelli, M. Granaiola, A. Leoni, A. Locatelli, R. Morigi, M. Rambaldi, L. Varoli, M.A. Cremonini, G. Placucci, R. Cervellati, and E. Greco. 2010. New isatin derivatives with antioxidant activity. *European Journal of Medicinal Chemistry* 45: 1374–1378.

Andries, K., P. Verhasselt, J. Guillemont, H.W.H. Gohlmann, J.M. Neefs, H. Winkler, J.V. Gestel, P. Timmerman, M. Zhu, E. Lee, P. Williams, D. Chaffoy, E. Huitric, S. Hoffner, E. Cambau, C. Truffot-Pernot, N. Lounis, and V. Jarlier. 2005. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science* 307: 223–227.

Banerjee, D., P. Yogeeswari, P. Bhat, A. Thomas, M. Srividya, and D. Sriram. 2011. Novel isatinyl thiosemicarbazones derivatives as potential molecule to combat HIV-TB co-infection. *European Journal of Medicinal Chemistry* 46: 106–121.

Dixit, P.P., V.J. Patil, P.S. Nair, S. Jain, N. Sinha, and S.K. Arora. 2006. Synthesis of 1-[3-(4-benzotriazol-1/2-yl-3-fluorophenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-substituted-thiourea derivatives as antituberculosis agents. *European Journal of Medicinal Chemistry* 41: 423–428.

Gill, C., G. Jadhav, M. Shaikh, R. Kale, A. Ghawalkar, D. Nagargoje, and M. Shiradkar. 2008. Clubbed [1,2,3]triazoles by fluorine benzimidazole: A novel approach to H37Rv inhibitors as a potential treatment for tuberculosis. *Bioorganic & Medicinal Chemistry Letters* 18: 6244–6247.

Gudipati, R., R.N.R. Anreddy, and M. Sarangapani. 2011. Synthesis, characterization and anticancer activity of certain 3-{4-(5-mercapto-1,3,4-oxadiazole-2-yl)phenylimino}indolin-2-one derivatives. *Saudi Pharmaceutical Journal* 19: 153–158.

Hans, R.H., I.J.F. Wiid, P.D. Van Helden, B. Wan, S.G. Franzblau, J. Gut, P.J. Rosenthal, and K. Chibale. 2011. Novel thiolactone-isatin hybrids as potential antimalarial and antitubercular agents. *Bioorganic & Medicinal Chemistry Letters* 21: 2055–2058.

Jiang, Y., and T.V. Hansen. 2011. Isatin 1,2,3-triazoles as potent inhibitors against caspase-3. *Bioorganic & Medicinal Chemistry Letters* 21: 1626–1629.

Kang, I.J., L.W. Wang, T.A. Hsu, A. Yueh, C.C. Lee, Y.C. Lee, C.Y. Lee, Y.S. Chao, S.R. Shih, and J.H. Chern. 2011. Isatin-β-thiosemicarbazones as potent herpes simplex virus inhibitors. *Bioorganic & Medicinal Chemistry Letters* 21: 1948–1952.

Karthikeyan, S.V., S. Perumal, A.S. Krithika, P. Yogeeswari, D. Sriram, and K.K. Balasubramanian. 2009. A microwave assisted facile regioselective Fischer Indole synthesis and antitubercular evaluation of novel 2-aryl-3,4-dihydro-2H-thieno[3,2-b]indoles. *Bioorganic & Medicinal Chemistry Letters* 19: 3006–3009.

Khan, K.M., M. Khan, M. Ali, M. Taha, S. Rasheed, S. Perveen, and M.I. Choudhary. 2009. Synthesis of bis-Schiff bases of isatins and their antiglycation activity. *Bioorganic & Medicinal Chemistry* 17: 7795–7801.

Kulig, K., and B. Malawska. 2003. Estimation of the lipophilicity of antiarrhythmic and antihypertensive active 1-substituted pyrrolidin-2-one and pyrrolidine derivatives. *Biomedical Chromatography* 17: 318–324.

Lee, R.E., M. Protopopova, E. Crooks, R.A. Slayden, M. Terrot, and C.E. Barry. 2003. Combinatorial lead optimization of [1,2]-diamines based on ethambutol as potential antituberculosis preclinical candidates. *Journal of Combinatorial Chemistry* 5: 172–187.

Mariangela, B., C.P. Giulio, P. Giovanna, L. De Alessandro, M. Rita, R. De Edda, M. Fabrizio, and B. Maurizio. 2009. 1,5-Diaryl-2-ethyl pyrrole derivatives as antimycobacterial agents: Design, synthesis, and microbiological evaluation. *European Journal of Medicinal Chemistry* 44: 4734–4738.

Matsumoto, M., H. Hshizume, T. Tomishige, and M. Kawasaki. 2005. In vitro and in vivo efficacy of novel antituberculous candidate OPC-67683. *American Society for Microbiology* 34: 204.

Pablos-Mendez, A., M.C. Raviglione, A. Laszlo, N. Binkin, H.L. Reider, F. Bustreo, D.L. Cohn, C.S. Lambregts-van Weezenbeek, S.J. Kim, P. Chaulet, and P.N. Nunn. 1998. Global surveillance for antituberculosis-drug resistance, 1994–1997. *New England Journal of Medicine* 338: 1641–1649.

Pandey, J., V.K. Tiwari, S.S. Verma, V. Chaturvedi, S. Bhatnagar, S. Sinha, A.N. Gaikwad, and R.P. Tripathi. 2009. Synthesis and antitubercular screening of imidazole derivatives. *European Journal of Medicinal Chemistry* 44: 3350–3355.

Praveen, C., A. Ayyanar, and P.T. Perumal. 2011. Practical synthesis, anticonvulsant, and antimicrobial activity of N-allyl and N-propargyl di(indolyl)indolin-2-ones. *Bioorganic & Medicinal Chemistry Letters* 21: 4072–4077.

Siddiqui, N., M.S. Alam, and J.P. Stables. 2011. Synthesis and anticonvulsant properties of 1-(amino-N-arylmethanethio)-3-(1-substituted benzyl-2, 3-dioxoindolin-5-yl) urea derivatives. *European Journal of Medicinal Chemistry* 46: 2236–2242.

Singh, D.P., K. Kumar, and C. Sharma. 2010. New 14-membered octaazamacrocyclic complexes: Synthesis, spectral, antibacterial and antifungal studies. *European Journal of Medicinal Chemistry* 45: 1230–1236.

Sriram, D., A. Aubry, P. Yogeeswari, and L.M. Fisher. 2006. Gatifloxacin derivatives: Synthesis, antimycobacterial activities, and inhibition of *Mycobacterium tuberculosis* DNA gyrase. *Bioorganic & Medicinal Chemistry Letters* 16: 2982–2985.

Taneja, N.K., and J.S. Tyagi. 2007. Resazurin reduction assays for screening of anti-tubercular compounds against dormant and actively growing *Mycobacterium tuberculosis*, Mycobacterium bovis BCG and *Mycobacterium smegmatis*. *Journal of Antimicrobial Chemotherapy* 60: 288–293.

Tangallapally, R.P., R. Yendapally, R.E. Lee, K. Hevener, V.C. Jones, A.J. Lenaerts, M.R. McNeil, Y. Wang, S. Franzblau, and R.E. Lee. 2004. Synthesis and evaluation of nitrofuranylamides as novel antituberculosis agents. *Journal of Medicinal Chemistry* 47: 5276–5283.

Tripathi, R.P., A.K. Yadav, A. Ajay, S.S. Bisht, V. Chaturvedi, and S.K. Sinha. 2010. Synthesis of 1-(2,3-dihydrobenzofuran-2-yl-methyl[1,2,3]-triazoles and their antitubercular evaluations. *European Journal of Medicinal Chemistry* 45: 142–148.

WHO report, Global Tuberculosis Control Surveillance, Financing and Planning 2010.

Yee, D., C. Valiquette, M. Pelletier, I. Parison, I. Rocher, and D. Menzies. 2003. Incidence of serious side effects from first-line antituberculosis drugs among patients treated for active tuberculosis. *American Journal of Respiratory and Critical Care Medicine* 167(11): 1472–1477.