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RESEARCH ARTICLE

Indole-based hydrazide-hydrazones and 4-thiazolidinones: synthesis and evaluation as antitubercular and anticancer agents

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Abstract

A new series of indolylhydrazones (**6**) and indole-based 4-thiazolidinones (**7**, **8**) have been designed, synthesized and screened for *in vitro* antitubercular activity against *Mycobacterium tuberculosis* H37Rv. 4-Thiazolidinone derivatives **7g**–**7j**, **8g**, **8h** and **8j** displayed notable antituberculosis (anti-TB) activity showing 99% inhibition at MIC values ranging from 6.25 to 25.0 μg/ml. Compounds **7g**, **7h**, **7i**, **8h** and **8j** demonstrated anti-TB activity at concentrations 10-fold lower than those cytotoxic for the mammalian cell lines. The indolylhydrazone derivative **6b** has also been evaluated for antiproliferative activity against human cancer cell lines at the National Cancer Institute (USA). Compound **6b** showed an interesting anticancer profile against different human tumor-derived cell lines at sub-micromolar concentrations with obvious selectivity toward colon cancer cell line COLO 205.

Keywords

Anticancer activity, antitubercular activity, hydrazide-hydrazone, indole, 4-thiazolidinone

History

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Introduction

Tuberculosis (TB) represents an enduring deadly disease and appears as the second leading cause of infectious disease mortality worldwide, after the acquired immunodeficiency syndrome, caused by the human immunodeficiency virus (HIV). In 2011, WHO reported about 8.7 million new cases of TB (13% co-infected with HIV) with 1.4 million deaths including almost 1 million HIV-negative and 430 000 HIV-positive individuals[1]. The rise in the incidence is partly due to the poverty and inequity and partly to coinfection with HIV, which greatly increases the risk of progressing new or latent TB infections to active diseases. Incomplete drug treatments in third-world countries fuel the appearance of multi-drug-resistant (MDR) and extensively-drug-resistant (XDR) strains of the pathogen[2]. In addition, patient non-compliance resulting from the long-term therapy of the infection lasting between 6 and 9 months has contributed to the emergence of MDR-TB[3]. Therefore, there is an urgent need to develop new antitubercular drugs to combat the spread of TB, particularly its hard-to-kill MDR and latent forms.

The synthetically accessible and pharmacologically active 4-thiazolidinone scaffold has provided the impetus for the discovery of a number of novel antitubercular agents in recent years. An early representative actithiazic acid, (−)-2-(5-carboxypentyl)thiazolidin-4-one (Figure 1), isolated from the culture broth of a strain of streptomyces shows highly specific *in vitro* activity against MTB[4,5]. 2-Arylhydrazono-4-oxo-3-phenyl-5-thiazolidinone acetic acids[6], piperidone based 1,3-thiazolidin-4-ones[7], isonicotinyl hydrazide derivatives containing the 4-thiazolidinone nucleus[8] and numerous different 2,3-disubstituted or 2,3,5-trisubstituted 4-thiazolidinones[9–11] have been reported to possess appreciable *in vitro* antitubercular activity. In a recent report by Pathak et al., 3-(4-chlorophenyl)-*N*-[4-oxo-2-(substitutedphenyl)-1,3-thiazolidin-3-yl]-1*H*-pyrazole-5-carboxamides have been described as promising antitubercular agents with MIC values in the range of 0.4–4 μg/ml[12]. Likewise, spirothiazolidinone featured molecules, *N*-alkyl substituted spirothiazolidinones[13], spirothiazolidinones bearing the imidazo[2,1-*b*]thiazole residue[14] and indole-2-carboxamides with a spirothiazolidinone moiety[15], have been found to be effective as growth inhibitors of MTB. Besides, several small synthetic molecules with an indole nucleus[16–18] or acyl/aroyl hydrazone moieties[19,20] have been reported to possess antitubercular potential. We have recently reported on the synthesis of novel spirothiazolidinone derivatives of the 5-fluoro-3-phenyl-1*H*-indole scaffold with promising *in vitro* antitubercular properties against the *Mycobacterium tuberculosis* H_{37}Rv strain[21]. Studies on the identification of the molecular target for indole-2-carboxamides suggest that they may inhibit *M. tuberculosis* growth by acting on the MmpL3 protein, which belongs to the family of membrane transporters[22].

Several studies have been devoted to the antiproliferative activity of hydrazide–hydrazone (–CO–NH–N=CH–) based compounds that have structural similarity to our target indolylhydrazones (**6**). In an early report, Germain et al. identified a series of acyl hydrazones to be selectively lethal to breast cancer stem cell enriched populations[23]. Many acyl/aroyl hydrazones with a variety of heterocyclic spacers were reported for their antiproliferative properties[24–27]. More relevant to the present study was the

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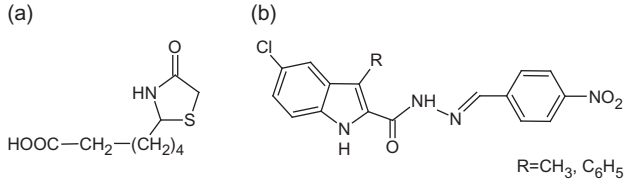


Figure 1. The structure of actithiazic acid (a) and apoptosis inducers (b).

identification of 5-chloro-3-methyl/phenyl-indole-2-carboxylic acid benzylidenehydrazides as anticancer agents by Zhang et al. (Figure 1). These compounds were found to be potent inducers of apoptosis and inhibited tubulin polimerization in G_2/M phase in breast cancer cell line T47D[28]. Moreover, some of the indolyhydrazones synthesized as precursors of 4-thiazolidinone derivatives in the present study (**6a**, **6d–h**) were previously patented by Bamaung et al. as angiogenesis inhibitors[29].

Encouraged by the above data and in continuation of an ongoing program aiming at the discovery of new leads with antitubercular activity, here we report the synthesis, structural identification and *in vitro* antitubercular activity of a new series of 5-fluoro-*N*-[5-(non)substituted-2-(non)substitutedphenyl-4-oxo-1,3-thiazolidin-3-yl]-3-phenyl-1*H*-indole-2-carboxamides (**7a–7l**, **8a–8l**) and their precursors 5-fluoro-N^2-[(non)substitutedbenzylidene]-3-phenyl-1*H*-indole-2-carbohydrazides (**6a–6l**) against *M. tuberculosis* H_{37}Rv. In view of the utility of hydrazide–hydrazone-based compounds for the discovery of novel antiproliferative agents, we also report on the preliminary antitumor screening results of 5-fluoro-N^2-(4-methylbenzylidene)-3-phenyl-1*H*-indole-2-carbohydrazide (**6b**), selected by the National Cancer Institute (NCI) as a prototype.

Experimental

Chemistry

Melting points were determined in open capillary tubes with a Buchi B-540 melting point apparatus (Büchi Labortechnik AG, Flawil, Switzerland) and are uncorrected. Microanalyses were performed on a Thermo Finnigan Flash EA 1112 elemental analyzer (Thermo Scientific, Waltham, MA). IR spectra were recorded in KBr discs (ν_{max} in cm^{-1}) on a Perkin-Elmer 1600 FTIR and Shimadzu IRAffinity-1 FTIR (Perkin-Elmer, Waltham, MA) spectrophotometer (Shimadzu Corporation, Kyoto, Japan). ^{1}H NMR (DMSO-d_6),^{13}C NMR (proton decoupled, DEPT-135) (DMSO-d_6) and heteronuclearcorrelation ^{1}H–^{13}C (HSQC, HMBC) (DMSO-d_6) spectra were run on Bruker AC 200 (200 MHz; Bruker BioSpin GmbH, Rheinstetten/Karlsruhe, Germany) and Varian^{UNITY}INOVA (500 MHz) instruments. Chemical shifts are reported as δ (ppm) relative to TMS as internal standard and coupling constants (J) are given in hertz (Hz). MS (ESI-) were determined on a Finnigan LCQ Advantage Max mass spectrometer (Thermo Scientific, Waltham, MA). Analytical HPLC was performed on a Shimadzu LC-10AD *VP* Liquid Chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with Shimadzu SCL-10A *VP* System Controller, Shimadzu DGU-14A Degasser, Shimadzu CTO-10AS *VP* Column Oven, Shimadzu SIL-10AD *VP* Auto Injector and Shimadzu SPD-M10A *VP* Diode Array Detector set at 304 nm. The data were collected and analyzed by using LC Workstation (Class *VP* System, Shimadzu). Chromatographic resolutions were carried out at room temperature on a Kromasil 100-5SIL microporous silica column (25 cm × 4.6 mm). Hexane–ethyl acetate (80:20) was used as the mobile phase at a flow rate of 1.2 mL/min. All solvents were HPLC grade (br.: broad, bnz.: benzylidene, ind.: indole, thz.: thiazolidinone).

Ethyl 2-benzyl-2-(4-fluorophenylhydrazono)acetate (3)

To a solution of **1** (0.02 mol) in ethanol (10 mL), water (10 mL) and conc. HCl (6 mL), 7% aqueous $NaNO_2$ solution (10 mL) was added dropwise at 0 °C with stirring. The resulting solution of diazonium salt (**2**) was poured into a cooled (0 °C) mixture of ethyl 2-benzyl-3-oxo-butanoate (0.02 mol), ethanol (10 mL), water (10 mL) and KOH (5.4 g) while stirring. The resulting mixture was refrigerated overnight. The red oily residue thus obtained was separated, washed with water and used without further purification.

Ethyl 5-fluoro-3-phenyl-1H-indole-2-carboxylate (4)

A solution of **3** (0.02 mol) in conc. HCl (20 mL) was heated under reflux for 4 h. The crude product was filtered off, washed with water until tested neutral to litmus and used without further purification.

5-Fluoro-3-phenyl-1H-indole-2-carbohydrazide (5)

A mixture of **4** (0.02 mol), ethanol (20 mL) and $H_2NNH_2{\cdot}H_2O$ (98%, 8 mL) was heated under reflux for 6 h. The resulting brown crystals were filtered off and recrystallized from ethanol–chloroform. m.p. 222–225 °C; IR(KBr): ν_{max} 3279 (N–H), 1624 (C=O); ^{1}H NMR (DMSO-d_6/200 MHz): δ 4.48 (s, 2H, NH_2), 7.10 (td, 1H, J = 9.1, 2.2, H6-ind.), 7.21 (dd, 1H, J = 9.8, 2.0, H4-ind.), 7.34–7.38 (m, 1H, H7-ind.), 7.42–7.61 (m, 5H, 3-C_6H_5-ind.), 8.86 (s, 1H, CONH), 11.82 (s, 1H, NH); ^{13}C NMR (proton decoupled, DMSO-d_6/125 MHz): δ 104.65 (d, J = 23.7, C4-ind.), 112.78 (d, J = 26.1, C6-ind.), 114.24 (d, J = 10.0, C7-ind.), 117.50 (d, J = 4.3, C3-ind.), 127.36 (d, J = 8.7, C3a-ind.), 129.21 (3-C_6H_5(C4)-ind.), 129.94 (3-C_6H_5(C3,C5)-ind.), 130.26 (C2-ind.), 130.41 (3-C_6H_5(C2,C6)-ind.), 132.82 (C7a-ind.), 134.27 (3-C_6H_5(C1)-ind.), 158.28 (d, J = 232.2, C5-ind.), 162.23 (C=O).

*General procedure for the synthesis of 5-fluoro-N^2-[(non) substitutedbenzylidene]-3-phenyl-1H-indole-2-carbohydrazides (**6a–6l**)*

A mixture of **5** (0.005 mol) and an appropriate benzaldehyde (0.006 mol) was refluxed in 15 mL abs. ethanol for 3 h. The precipitate obtained was purified either by recrystallization from ethanol–chloroform or by washing with hot ethanol.

5-Fluoro-N^2-(4-methylbenzylidene)-3-phenyl-1H-indole-2-carbohydrazide (**6b**). Brown crystals (91%); m.p. 250–252 °C; IR(KBr): ν_{max} 3314, 3280 (N–H), 1651 (C=O); ^{1}H NMR (DMSO-d_6/500 MHz): δ 2.32 (s, 3H, 4-CH_3), 7.14 (br. t, 1H, J = 8.3 Hz, H6-ind.), 7.24 (br. d, 2H, J = 4.4 Hz, H3,H5-bnz.), 7.32 (br. d, 2H, J = 9.3 Hz, H4,3-C_6H_5(H4)-ind.), 7.46–7.56 (m, 7H, H2,H6-bnz. and H7,3-C_6H_5(H2,H3,H5,H6)-ind.), 8.04 (s, 1H, N=CH), 11.33 (s, 1H, CONH), 12.04 (s, 1H, NH); ^{13}C NMR (HSQC, HMBC) (DMSO-d_6/125 MHz): δ 21.70 (4-CH_3), 104.85 (d, J = 23.5 Hz, C4-ind.), 113.28 (d, J = 25.1 Hz, C6-ind.), 114.38 (d, J = 10.0 Hz, C7-ind.), 118.67 (C3-ind.), 127.08 (d, C3a-ind.), 127.42 (3-C_6H_5(C4)-ind.), 127.77 (C2, 6-bnz.), 129.15 (3-C_6H_5(C3,5)-ind.), 129.95 (C3,5-bnz. and C2-ind.), 130.13 (3-C_6H_5(C2,6)-ind.), 131.98 (C1-bnz.), 133.01 (C7a-ind.), 134.01 (3-C_6H_5(C1)-ind.), 140.72 (C4-bnz.), 148.13 (C=N), 158.39 (d, J = 233.9 Hz, C5-ind.), 158.89 (CONH); MS (ESI-) m/z (%): 370 ($M-H^-$, 100). Anal calcd for $C_{23}H_{18}FN_3O$ (371.41): C, 74.38; H, 4.88; N, 11.31. Found: C, 74.39; H, 5.09; N, 11.15.

*(E)-5-Fluoro-N^2-(4-methoxybenzylidene)-3-phenyl-1H-indole-2-carbohydrazide (**6c**)*. See Ref[30].

*5-Fluoro-3-phenyl-N^2-[4-(trifluoromethyl)benzylidene]-1H-indole-2-carbohydrazide(**6i**)*. Yellow needles (92%); m.p. 254–255 °C; IR(KBr): ν_{max} 3270 (N–H), 1638 (C=O); ^{1}H NMR

(DMSO-d_6/500 MHz): δ 7.15 (td, 1H, $J = 8.8$; 2.0 Hz, H6-ind.), 7.32–7.53 (m, 7H, H4, H7,3-C_6H_5-ind.), 7.78 (br. s, 2H, H2,H6-bnz.), 7.90 (br. s, 2H, H3,H5-bnz.), 8.16 (s, 1H, N=CH), 11.61 (s, 1H, CONH), 12.05 (s, 1H, NH). Anal calcd for $C_{23}H_{15}F_4N_3O$ (425.38): C, 64.74; H, 3.55; N, 9.88. Found: C, 64.44; H, 3.47; N, 9.63.

*5-Fluoro-N^2-[4-(methoxycarbonyl)benzylidene]-3-phenyl-1H-indole-2-carbohydrazide (**6j**)*. Yellow crystals (94%); m.p. 256–258.5 °C; IR(KBr): ν_{max} 3263 (N–H), 1703 (C=O), 1649 (C=O); ^{1}H NMR (DMSO-d_6/500 MHz): δ 3.85 (s, 3H, 4-$COOCH_3$), 7.15 (td, 1H, $J = 9.0$; 2.0 Hz, H6-ind.), 7.33–7.53 (m, 7H, H4,H7,3-C_6H_5-ind.), 7.82 (br. s, 2H, H2,H6-bnz.), 8.00 (br. s, 2H, H3,H5-bnz.), 8.14 (s, 1H, N=CH), 11.58 (s, 1H, CONH), 12.07 (s, 1H, NH); ^{13}C NMR (HSQC) (DMSO-d_6/125 MHz): δ 52.91 (4-$COOCH_3$), 104.91 (d, $J = 23.5$ Hz, C4-ind.), 113.31 (C6-ind.), 114.44 (d, $J = 8.6$ Hz, C7-ind.), 119.13 (C3-ind.), 126.95 (C3a-ind.), 127.48 (3-C_6H_5(C4)-ind.), 127.90 (C2,6-bnz.), 129.15 (3-C_6H_5(C3,5)-ind.), 129.57 (C4-bnz. and C2-ind.), 130.26 (C3,5-bnz. and 3-C_6H_5(C2,6)-ind.), 131.18 (C7a-ind.), 133.18 (3-C_6H_5(C1)-ind.), 139.10 (C1-bnz.), 146.58 (C=N), 154.96 (CONH), 158.41 (d, $J = 233.9$ Hz, C5-ind.), 166.50 (4-$COOCH_3$); MS (ESI-) *m/z* (%): 414 ($M-H^-$, 100). Anal calcd for $C_{24}H_{18}FN_3O_3$ (415.42): C, 69.39; H, 4.37; N, 10.12. Found: C, 69.34; H, 4.63; N, 9.76.

*N^2-(2,6-dichlorobenzylidene)-5-fluoro-3-phenyl-1H-indole-2-carbohydrazide (**6k**)*. Yellow needles (89%); m.p. 205–206 °C; IR(KBr): ν_{max} 3309 (N–H), 1673 (C=O); ^{1}H NMR (DMSO-d_6/500 MHz): δ 7.15 (br. t, 1H, H6-ind.), 7.30–7.51 (m, 10H, H3-5-bnz. and H4,H7,3-C_6H_5-ind.), 8.27 (s, 1H, N=CH), 11.71 (s, 1H, CONH), 12.07 (s, 1H, NH). Anal calcd for $C_{22}H_{14}Cl_2FN_3O$ (426.27): C, 61.99; H, 3.81; N, 9.86. Found: C, 62.12; H, 4.03; N, 9.89.

*N^2-(2-chloro-6-fluorobenzylidene)-5-fluoro-3-phenyl-1H-indole-2-carbohydrazide (**6l**)*. Yellow needles (87%); m.p. 223–225 °C; IR(KBr): ν_{max} 3324, 3169 (N–H), 1654 (C=O); ^{1}H NMR (DMSO-d_6/500 MHz): δ 7.15 (br. t, 1H, $J = 8.8$ Hz, H6-ind.), 7.30–7.52 (m, 10H, H3-5-bnz. and H4,H7,3-C_6H_5-ind.), 8.32 (s, 1H, N=CH), 11.67 (s, 1H, CONH), 12.05 (s, 1H, NH); MS (ESI-) *m/z* (%): 410/408 ($M-H^-$, 28/100). Anal calcd for $C_{22}H_{14}ClF_2N_3O$ (409.82): C, 64.48; H, 3.84; N, 10.25. Found: C, 64.43; H, 4.10; N, 10.16.

*General procedure for the synthesis of 5-fluoro-N-[5-(non) substituted-2-(non)substitutedphenyl-4-oxo-1,3-thiazolidin-3-yl]-3-phenyl-1H-indole-2-carboxamides (**7a–7l, 8a–8l**)*

A mixture of **6a–6l** (0.0025 mol) and mercaptoacetic acid or 2-mercaptopropionic acid (0.01 mol) was refluxed in 30 mL dry benzene for 5–6 h using a Dean-Stark water separator. Excess benzene was evaporated *in vacuo*. The resulting residue was triturated with saturated $NaHCO_3$ solution until CO_2 evolution ceased and was allowed to stand overnight or in some cases refregerated until solidification. The solid thus obtained was washed with water, dried and recrystallized from ethanol or ethanol–chloroform.

*5-Fluoro-N-(4-oxo-2-phenyl-1,3-thiazolidin-3-yl)-3-phenyl-1H-indole-2-carboxamide (**7a**)*. *Wh*ite powder (62%); m.p. 219–220.5 °C; IR(KBr): ν_{max} 3364 (N–H), 1708, 1678 (C=O); ^{1}H NMR (DMSO-d_6/500 MHz): δ 3.75 (d, 1H, $J = 15.6$ Hz, H5-thz.), 3.89 (dd, 1H, $J = 15.9$; 1.5 Hz, H5-thz.), 5.84 (s, 1H, H2-thz.), 7.12 (td, 1H, $J = 9.3$; 2.4 Hz, H6-ind.), 7.18 (dd, 1H, $J = 9.8$; 2.4 Hz, H4-ind.), 7.25 (br. s, 5H, 3-C_6H_5-ind.), 7.37–7.39 (m, 3H, 2-C_6H_5(H3-5)-thz.), 7.44–7.48 (m, 3H, 2-C_6H_5(H2,H6)-thz. and H7-ind.), 9.96 (s, 1H, CONH), 11.84 (s, 1H, NH). Anal calcd for $C_{24}H_{18}FN_3O_2S$ (431.48): C, 66.81; H, 4.20; N, 9.74. Found: C, 67.15; H, 3.90; N, 9.69.

*5-Fluoro-N-[2-(4-methylphenyl)-4-oxo-1,3-thiazolidin-3-yl]-3-phenyl-1H-indole-2-carboxamide (**7b**)*. White needles (61%); m.p. 221–222 °C; IR(KBr): ν_{max} 3357, 3286 (N–H), 1704, 1676 (C=O); ^{1}H NMR (DMSO-d_6/500 MHz): δ 2.32 (s, 3H, 4-CH_3), 3.74 (d, 1H, $J = 16.1$ Hz, H5-thz.), 3.87 (dd, 1H, $J = 16.1$; 1.5 Hz, H5-thz.), 5.80 (s, 1H, H2-thz.), 7.12 (td, 1H, $J = 9.3$; 2.4 Hz, H6-ind.), 7.16–7.19 (m, 3H, 2-C_6H_5(H3,H5)-thz. and H4-ind.), 7.26 (br. s, 5H, 3-C_6H_5-ind.), 7.32 (d, 2H, $J = 8.3$ Hz, 2-C_6H_5(H2,H6)-thz.), 7.47 (dd, 1H, $J = 8.8$; 4.4 Hz, H7-ind.), 9.92 (s, 1H, CONH), 11.83 (s, 1H, NH); ^{13}C NMR (HSQC, HMBC) (DMSO-d_6/125 MHz): δ 21.49 (4-CH_3), 30.02 (C5-thz.), 62.44 (C2-thz.), 104.92 (d, $J = 24.0$ Hz, C4-ind.), 113.81 (d, $J = 26.8$ Hz, C6-ind.), 114.62 (d, $J = 9.1$ Hz, C7-ind.), 119.79 (d, $J = 4.8$ Hz, C3-ind.), 127.08 (d, $J = 10.1$ Hz, C3a-ind.), 127.26 (C2/3-C_6H_5(C4)-ind.), 127.38 (C2/3-C_6H_5(C4)-ind.), 128.32 (2-C_6H_5(C2,6)-thz.), 129.03 (3-C_6H_5(C3,5)-ind.), 129.83 (2-C_6H_5(C3,5)-thz.), 130.20 (3-C_6H_5(C2,6)-ind.), 133.19 (C7a-ind.), 133.37 (3-C_6H_5(C1)-ind.), 135.83 (2-C_6H_5(C1)-thz.), 139.11 (2-C_6H_5(C4)-thz.), 158.36 (d, $J = 233.9$ Hz, C5-ind.), 160.90 (CONH), 169.65 (CO-thz.); MS (ESI-) *m/z* (%): 444 ($M-H^-$, 100). Anal calcd for $C_{25}H_{20}FN_3O_2S$ (445.51): C, 67.40; H, 4.22; N, 9.43. Found: C, 67.24; H, 3.92; N, 9.66.

*5-Fluoro-N-[2-(4-methoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-3-phenyl-1H-indole-2-carboxamide (**7c**)*. White powder (65%); m.p. 184–185.5 °C; IR(KBr): ν_{max} 3352, 3292 (N–H), 1697, 1670 (C=O); ^{1}H NMR (DMSO-d_6/500 MHz): δ 3.74 (d, 1H, $J = 16.1$ Hz, H5-thz.), 3.77 (s, 3H, 4-OCH_3), 3.86 (dd, 1H, $J = 16.1$; 1.5 Hz, H5-thz.), 5.80 (s, 1H, H2-thz.), 6.92 (d, 2H, $J = 8.8$ Hz, 2-C_6H_5(H3,H5)-thz.), 7.12 (td, 1H, $J = 9.3$; 2.4 Hz, H6-ind.), 7.18 (dd, 1H, $J = 9.8$; 2.4 Hz, H4-ind.), 7.26–7.30 (m, 5H, 3-C_6H_5-ind.), 7.37 (d, 2H, $J = 8.8$ Hz, 2-C_6H_5(H2,H6)-thz.), 7.48 (dd, 1H, $J = 9.0$; 4.4 Hz, H7-ind.), 9.88 (s, 1H, CONH), 11.83 (s, 1H, NH). Anal calcd for $C_{25}H_{20}FN_3O_3S$ (461.51): C, 65.06; H, 4.37; N, 9.10. Found: C, 65.18; H, 4.32; N, 9.13.

*N-[2-(4-chlorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-5-fluoro-3-phenyl-1H-indole-2-carboxamide (**7d**)*. Beige crystals (89%); m.p. 234–236 °C; IR(KBr): ν_{max} 3291 (N–H), 1713, 1658 (C=O); ^{1}H NMR (DMSO-d_6/500 MHz): δ 3.75 (d, 1H, $J = 15.6$ Hz, H5-thz.), 3.91 (dd, 1H, $J = 16.0$; 1.8 Hz, H5-thz.), 5.85 (s, 1H, H2-thz.), 7.12 (td, 1H, $J = 9.0$; 2.3 Hz, H6-ind.), 7.18 (dd, 1H, $J = 9.8$; 2.5 Hz, H4-ind.), 7.24–7.27 (m, 5H, 3-C_6H_5-ind.), 7.43 (d, 2H, $J = 8.2$ Hz, 2-C_6H_5(H3,H5)-thz.), 7.46–7.49 (m, 3H, 2-C_6H_5(H2,H6)-thz. and H7-ind.), 10.01 (s, 1H, CONH), 11.85 (s, 1H, NH); ^{13}C NMR (HSQC) (DMSO-d_6/125 MHz): δ 29.94 (C5-thz.), 61.77 (C2-thz.), 104.93 (d, $J = 24.0$ Hz, C4-ind.), 113.80 (d, $J = 26.4$ Hz, C6-ind.), 114.61 (d, $J = 9.6$ Hz, C7-ind.), 119.72 (d, $J = 5.3$ Hz, C3-ind.), 127.06 (d, $J = 10.1$ Hz, C3a-ind.), 127.33 (C2/3-C_6H_5(C4)-ind.), 127.35 (C2/3-C_6H_5(C4)-ind.),128.98 (3-C_6H_5(C3,5)-ind.), 129.29 (2-C_6H_5(C3,5)-thz.), 130.18 (3-C_6H_5(C2,6)-ind.), 130.24 (2-C_6H_5(C2,6)-thz.), 133.22 (C7a-ind.), 133.37 (3-C_6H_5(C1)-ind.), 134.20 (2-C_6H_5(C1/C4)-thz.), 138.20 (2-C_6H_5(C1/C4)-thz.), 158.37 (d, $J = 233.9$ Hz, C5-ind.), 161.01 (CONH), 169.51 (CO-thz.). Anal calcd for $C_{24}H_{17}ClFN_3O_2S$ (465.93): C, 61.87; H, 3.88; N, 9.02. Found: C, 62.21; H, 4.21; N, 8.92.

*N-[2-(4-bromophenyl)-4-oxo-1,3-thiazolidin-3-yl]-5-fluoro-3-phenyl-1H-indole-2-carboxamide (**7e**)*. Beige powder (84%); m.p. 228–229 °C; IR(KBr): ν_{max} 3295 (N–H), 1713, 1656 (C=O); ^{1}H NMR (DMSO-d_6/500 MHz): δ 3.75 (d, 1H, $J = 16.0$ Hz, H5-thz.), 3.91 (dd, 1H, $J = 16.0$; 1.4 Hz, H5-thz.), 5.84 (s, 1H, H2-thz.), 7.12 (td, 1H, $J = 9.2$; 2.3 Hz, H6-ind.), 7.18 (dd, 1H, $J = 9.6$; 2.3 Hz, H4-ind.), 7.26 (br. s, 5H, 3-C_6H_5-ind.), 7.41 (d, 2H, $J = 8.7$ Hz, 2-C_6H_5(H2,H6)-thz.), 7.47 (dd, 1H, $J = 9.2$; 4.6 Hz, H7-ind.), 7.57 (d, 2H, $J = 8.7$ Hz, 2-C_6H_5(H3,H5)-thz.), 10.01 (s, 1H, CONH), 11.85 (s, 1H, NH); MS (ESI-) *m/z* (%): 510/508

(M-H$^-$, 100/87). Anal calcd for $C_{24}H_{17}BrFN_3O_2S$ (510.38): C, 56.58; H, 3.56; N, 8.23. Found: C, 56.94; H, 3.79; N, 8.12.

*5-Fluoro-N-[2-(4-fluorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-3-phenyl-1H-indole-2-carboxamide (**7f**)*. Beige crystals (89%); m.p. 217–219 °C; IR(KBr): ν_{max} 3284 (N–H), 1704, 1657 (C=O); ^{1}H NMR (DMSO-d$_6$/500 MHz): δ 3.75 (d, 1H, J = 16.0 Hz, H5-thz.), 3.90 (dd, 1H, J = 16.0; 1.8 Hz, H5-thz.), 5.85 (s, 1H, H2-thz.), 7.12 (td, 1H, J = 9.2; 2.7 Hz, H6-ind.), 7.17–7.20 (m, 3H, 2-C$_6$H$_5$(H3,H5)-thz. and H4-ind.), 7.27 (br. s, 5H, 3-C$_6$H$_5$-ind.), 7.46–7.52 (m, 3H, 2-C$_6$H$_5$ (H2,H6)-thz. and H7-ind.), 9.98 (s, 1H, CONH), 11.84 (s, 1H, NH). Anal calcd for $C_{24}H_{17}F_2N_3O_2S$ (449.47): C, 64.13; H, 3.91; N, 9.35. Found: C, 64.48; H, 4.17; N, 9.18.

*N-[2-(4-cyanophenyl)-4-oxo-1,3-thiazolidin-3-yl]-5-fluoro-3-phenyl-1H-indole-2-carboxamide (**7g**)*. White crystals (80%); m.p. 253–255 °C; IR(KBr): ν_{max} 3299 (N–H), 2231 (C≡N), 1713, 1655 (C=O); ^{1}H NMR (DMSO-d$_6$/500 MHz): δ 3.77 (d, 1H, J = 15.6 Hz, H5-thz.), 3.95 (dd, 1H, J = 16.0; 1.5 Hz, H5-thz.), 5.93 (s, 1H, H2-thz.), 7.12 (td, 1H, J = 9.3; 2.4 Hz, H6-ind.), 7.19 (dd, 1H, J = 9.8; 2.4 Hz, H4-ind.), 7.22–7.28 (m, 5H, 3-C$_6$H$_5$-ind.), 7.47 (dd, 1H, J = 9.0; 4.4 Hz, H7-ind.), 7.64 (d, 2H, J = 8.3 Hz, 2-C$_6$H$_5$(H2,H6)-thz.), 7.84 (d, 2H, J = 8.3 Hz, 2-C$_6$H$_5$ (H3,H5)-thz.), 10.11 (s, 1H, CONH), 11.87 (s, 1H, NH); MS (ESI-) *m/z* (%): 455 (M−H$^-$, 100). Anal calcd for $C_{25}H_{17}FN_4O_2S$ (456.49): C, 65.78; H, 3.75; N, 12.27. Found: C, 65.83; H, 3.70; N, 12.07.

*5-Fluoro-N-[2-(4-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]-3-phenyl-1H-indole-2-carboxamide (**7h**)*. Yellow crystals (78%); m.p. 258–260 °C; IR(KBr): ν_{max} 3300 (N–H), 1716, 1654 (C=O); ^{1}H NMR (DMSO-d$_6$/500 MHz): δ 3.79 (d, 1H, J = 16.1 Hz, H5-thz.), 3.97 (dd, 1H, J = 16.0; 1.5 Hz, H5-thz.), 6.00 (d, 1H, J = 1.0 Hz, H2-thz.), 7.12 (td, 1H, J = 9.3; 2.4 Hz, H6-ind.), 7.17–7.23 (m, 4H, H4-ind. and 3-C$_6$H$_5$(H3-5)-ind.), 7.27 (dd, 2H, J = 8.1; 1.5 Hz, 3-C$_6$H$_5$(H2,H6)-ind.), 7.47 (dd, 1H, J = 8.8; 4.4 Hz, H7-ind.), 7.72 (d, 2H, J = 8.8 Hz, 2-C$_6$H$_5$(H2,H6)-thz.), 8.21 (d, 2H, J = 8.8 Hz, 2-C$_6$H$_5$(H3,H5)-thz.), 10.16 (s, 1H, CONH), 11.88 (s, 1H, NH). Anal calcd for $C_{24}H_{17}FN_4O_4S$ (476.48): C, 60.50; H, 3.60; N, 11.76. Found: C, 60.58; H, 3.62; N, 11.77.

*5-Fluoro-N-{4-oxo-2-[4-(trifluoromethyl)phenyl]-1,3-thiazolidin-3-yl}-3-phenyl-1H-indole-2-carboxamide (**7i**)*. Beige crystals (73%); m.p. 211–212.5 °C; IR(KBr): ν_{max} 3332, 3176 (N–H), 1726, 1647 (C=O); ^{1}H NMR (DMSO-d$_6$/500 MHz): δ 3.78 (d, 1H, J = 16.0 Hz, H5-thz.), 3.94 (dd, 1H, J = 16.0; 1.8 Hz, H5-thz.), 5.95 (s, 1H, H2-thz.), 7.12 (td, 1H, J = 9.1; 2.3 Hz, H6-ind.), 7.17–7.20 (m, 4H, H4,3-C$_6$H$_5$(H3-5)-ind.), 7.24–7.27 (m, 2H, 3-C$_6$H$_5$(H2,H6)-ind.), 7.47 (dd, 1H, J = 9.2; 4.6 Hz, H7-ind.), 7.66 (d, 2H, J = 8.5 Hz, 2-C$_6$H$_5$(H2,H6)-thz.), 7.73 (d, 2H, J = 8.7 Hz, 2-C$_6$H$_5$(H3,H5)-thz.), 10.01 (s, 1H, CONH), 11.88 (s, 1H, NH); ^{13}C NMR (HSQC) (DMSO-d$_6$/125 MHz): δ 29.89 (C5-thz.), 61.65 (C2-thz.), 104.92 (d, J = 24.0 Hz, C4-ind.), 113.79 (d, J = 26.4 Hz, C6-ind.), 114.60 (d, J = 9.6 Hz, C7-ind.), 119.68 (d, J = 5.2 Hz, C3-ind.), 124.83 (d, J = 272.2 Hz, 4-CF$_3$), 126.23 (d, J = 3.8 Hz, 2-C$_6$H$_5$(C3,5)-thz.), 127.02 (d, J = 9.6 Hz, C3a-ind.), 127.23 (C2/3-C$_6$H$_5$(C4)-ind.), 127.41 (C2/3-C$_6$H$_5$(C4)-ind.), 128.88 (2-C$_6$H$_5$ (C2,6)-thz./3-C$_6$H$_5$(C3,5)-ind.), 128.93 (2-C$_6$H$_5$(C2,6)-thz./3-C$_6$H$_5$(C3,5)-ind.), 129.92 (d, J = 31.6 Hz, 2-C$_6$H$_5$(C4)-thz.), 130.13 (3-C$_6$H$_5$(C2,6)-ind.), 133.24 (C7a-ind.), 133.33 (3-C$_6$H$_5$(C1)-ind.), 144,20 (2-C$_6$H$_5$(C1)-thz.), 158.37 (d, J = 233.9 Hz, C5-ind.), 161.25 (CONH), 169.66 (CO-thz.). Anal calcd for $C_{25}H_{17}F_4N_3O_2S$ (499.48): C, 60.12; H, 3.43; N, 8.41. Found: C, 60.34; H, 3.76; N, 8.40.

*5-Fluoro-N-{2-[4-(methoxycarbonyl)phenyl]-4-oxo-1,3-thiazolidin-3-yl}-3-phenyl-1H-indole-2-carboxamide (**7j**)*. White powder (65%); m.p. 141–143 °C; IR(KBr): ν_{max} 3244 (N–H), 1718, 1695, 1653 (C=O); ^{1}H NMR (DMSO-d$_6$/500 MHz): δ 3.78 (d, 1H, J = 16.0 Hz, H5-thz.), 3.87 (s, 3H, 4-COOCH$_3$), 3.93 (dd, 1H, J = 15.8; 1.8 Hz, H5-thz.), 5.92 (s, 1H, H2-thz.), 7.12 (td, 1H, J = 9.2; 2.8 Hz, H6-ind.), 7.18 (dd, 1H, J = 9.8; 2.3 Hz, H4-ind.), 7.19–7.27 (m, 5H, 3-C$_6$H$_5$-ind.), 7.46 (dd, 1H, J = 9.2; 4.6 Hz, H7-ind.), 7.59 (d, 2H, J = 8.7 Hz, 2-C$_6$H$_5$(H2,H6)-thz.), 7.94 (d, 2H, J = 8.2 Hz, 2-C$_6$H$_5$(H3,H5)-thz.), 10.08 (s, 1H, CONH), 11.85 (s, 1H, NH); ^{13}C NMR (HSQC) (DMSO-d$_6$/125 MHz): δ 29.91 (C5-thz.), 52.95 (4-COOCH$_3$), 61.87 (C2-thz.), 104.91 (d, J = 23.5 Hz, C4-ind.), 113.79 (d, J = 26.8 Hz, C6-ind.), 114.60 (d, J = 9.6 Hz, C7-ind.), 119.74 (d, J = 4.8 Hz, C3-ind.), 127.03 (d, J = 9.6 Hz, C3a-ind.), 127.31 (C2/3-C$_6$H$_5$(C4)-ind.), 127.34 (C2/3-C$_6$H$_5$(C4)-ind.), 128.51 (2-C$_6$H$_5$(C2,6)-thz.), 128.94 (3-C$_6$H$_5$(C2,6)-ind.), 130.14 (2-C$_6$H$_5$(C3,5)-thz./3-C$_6$H$_5$(C3,5)-ind.), 130.16 (2-C$_6$H$_5$(C3,5)-thz./3-C$_6$H$_5$(C3,5)-ind.), 130.70 (2-C$_6$H$_5$(C4)-thz.), 133.20 (C7a-ind.), 133.34 (3-C$_6$H$_5$(C1)-ind.), 144.59 (2-C$_6$H$_5$(C1)-thz.), 158.36 (d, J = 233.9 Hz, C5-ind.), 161.09 (CONH), 166.60 (4-COOCH$_3$), 169.60 (CO-thz.); MS (ESI-) *m/z* (%): 488 (M-H$^-$, 100). Anal calcd for $C_{26}H_{20}FN_3O_4S.1/2H_2O$ (498.52): C, 62.64; H, 4.25; N, 8.43. Found: C, 62.88; H, 4.48; N, 8.26.

*N-[2-(2,6-dichlorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-5-fluoro-3-phenyl-1H-indole-2-carboxamide (**7k**)*. White needles (67%); m.p. 215–217 °C; IR(KBr): ν_{max} 3298, 3219 (N–H), 1720, 1638 (C=O); ^{1}H NMR (DMSO-d$_6$/500 MHz): δ 3.87 (d, 1H, J = 16.1 Hz, H5-thz.), 3.91 (dd, 1H, J = 15.9; 2.0 Hz, H5-thz.), 6.73 (d, 1H, J = 1.5 Hz, H2-thz.), 7.12–7.17 (m, 2H, H4,H6-ind.), 7.26–7.36 (m, 5H, 3-C$_6$H$_5$-ind.), 7.42 (t, 1H, J = 8.3 Hz, 2-C$_6$H$_5$(H4)-thz.), 7.47–7.53 (m, 3H, 2-C$_6$H$_5$(H3,H5)-thz. and H7-ind.), 9.97 (s, 1H, CONH), 11.86 (s, 1H, NH); ^{13}C NMR (HSQC) (DMSO-d$_6$/125 MHz): δ 30.91 (C5-thz.), 57.01 (C2-thz.), 104.95 (d, J = 24.0 Hz, C4-ind.), 114.08 (d, J = 26.8 Hz, C6-ind.), 114.72 (d, J = 9.6 Hz, C7-ind.), 120.46 (d, J = 5.3 Hz, C3-ind.), 126.79 (C2-ind.), 127.13 (d, J = 10.7 Hz, C3a-ind.), 127.59 (3-C$_6$H$_5$(C4)-ind.), 129.08 (3-C$_6$H$_5$(C3,5)-ind.), 129.62 (2-C$_6$H$_5$(C3/5)-thz.), 130.21 (3-C$_6$H$_5$(C2,6)-ind.), 131.88 (2-C$_6$H$_5$(C3/5)-thz.), 131.92 (2-C$_6$H$_5$(C4)-thz.), 133.22 (C7a-ind.), 133.48 (3-C$_6$H$_5$(C1)-ind.), 135.71 (2-C$_6$H$_5$(C2,6/C1)-thz.), 135.99 (2-C$_6$H$_5$(C2,6/C1)-thz.), 158.38 (d, J = 233.9 Hz, C5-ind.), 161.41 (CONH), 169.12 (CO-thz.). Anal calcd for $C_{24}H_{16}Cl_2FN_3O_2S$ (500.37): C, 57.61; H, 3.22; N, 8.40. Found: C, 57.53; H, 3.51; N, 8.24.

*N-[2-(2-chloro-6-fluorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-5-fluoro-3-phenyl-1H-indole-2-carboxamide (**7l**)*. White powder (67%); m.p. 174–175.5 °C; IR(KBr): ν_{max} 3290, 3209 (N–H), 1722, 1641 (C=O); ^{1}H NMR (DMSO-d$_6$/500 MHz): δ 3.81 (d, 1H, J = 15.6 Hz, H5-thz.), 3.87 (br. d, 1H, J = 15.1 Hz; H5-thz.), 6.40 (s, 1H, H2-thz.), 7.13 (td, 1H, J = 9.3; 2.4 Hz, H6-ind.), 7.18 (br. d, 1H, J = 9.8 Hz, H4-ind.), 7.23–7.38 (m, 7H, 2-C$_6$H$_5$(H3,H5)-thz. and 3-C$_6$H$_5$-ind.), 7.44–7.50 (m, 2H, 2-C$_6$H$_5$(H4)-thz. and H7-ind.), 10.21 (s, 1H, CONH), 11.86 (s, 1H, NH); ^{13}C NMR (HSQC, HMBC) (DMSO-d$_6$/125 MHz): δ 30.05 (C5-thz.), 55.75 (C2-thz.), 104.94 (d, J = 23.5 Hz, C4-ind.), 113.96 (d, J = 26.4 Hz, C6-ind.), 114.69 (d, J = 9.6 Hz, C7-ind.), 116.89 (d, J = 23.0 Hz, 2-C$_6$H$_5$(C5)-thz.), 120.17 (br. d, C3-ind.), 124.78 (d, J = 11.5 Hz, 2-C$_6$H$_5$(C1)-thz.), 126.37 (2-C$_6$H$_5$(C3)-thz.), 127.03 (d, J = 7.2 Hz, C3a-ind.), 127.14 (C2-ind.), 127.49 (3-C$_6$H$_5$(C4)-ind.), 129.04 (3-C$_6$H$_5$(C3,5)-ind.), 130.18 (3-C$_6$H$_5$(C2,6)-ind.), 132.28 (d, J = 10.5 Hz, 2-C$_6$H$_5$(C4)-thz.), 133.23 (C7a-ind.), 133.47 (3-C$_6$H$_5$(C1)-ind.), 134.15 (br. d, 2-C$_6$H$_5$(C2)-thz.), 158.38 (d, J = 233.9 Hz, C5-ind.), 162.43 (d, J = 256.4 Hz, 2-C$_6$H$_5$(C6)-thz.), 168.77 (CO-thz.); MS (ESI-) m/z (%): 484.5/482.5 (M-H$^-$, 40/100). Anal calcd for $C_{24}H_{16}ClF_2N_3O_2S.H_2O$ (501.93): C, 57.23; H, 3.22; N, 8.37. Found: C, 56.90; H, 3.40; N, 8.19.

*5-Fluoro-N-(5-methyl-4-oxo-2-phenyl-1,3-thiazolidin-3-yl)-3-phenyl-1H-indole-2-carboxamide (**8a**)*. White powder (68%); m.p. 233–236 °C; IR(KBr): ν_{max} 3368 (N–H), 1708, 1677

(C=O); ^{1}H NMR (DMSO-d$_6$/500 MHz): δ 1.51 (d, 3H, J = 6.7 Hz, 5-CH$_3$-thz.), 4.05 and 4.14 (q and br. q, 1H, J = 6.8 and J = 6.8 Hz, H5-thz.), 5.82 and 5.85 (2s, 1H, H2-thz.), 7.10–7.19 (m, 2H, H4,H6-ind.); 7.24 (br. s, 5H, 3-C$_6$H$_5$-ind.), 7.39–7.47 (m, 6H, 2-C$_6$H$_5$-thz. and H7-ind.), 10.01 (br. s, 1H, CONH), 11.84 (br. s, 1H, NH). Anal calcd for $C_{25}H_{20}FN_3O_2S$ (445.51): C, 67.40; H, 4.92; N, 9.43. Found: C, 67.63; H, 5.31; N, 9.51.

*5-Fluoro-N-[5-methyl-2-(4-methylphenyl)-4-oxo-1,3-thiazolidin-3-yl]-3-phenyl-1H-indole-2-carboxamide (**8b**)*. Beige needles (60%); m.p. 206–210 °C; IR(KBr): ν_{max} 3234 (N–H), 1700, 1654 (C=O); ^{1}H NMR (DMSO-d$_6$/500 MHz): δ 1.50–1.53 (m, 3H, 5-CH$_3$-thz.), 2.32 (s, 3H, 4-CH$_3$), 4.04 and 4.12 (q and qd, 1H, J = 6.8 and J = 6.8; 1.5 Hz, H5-thz.), 5.79 and 5.81 (s and br. s, 1H, H2-thz.), 7.12 (td, 1H, J = 9.3; 2.4 Hz, H6-ind.), 7.15–7.19 (m, 3H, 2-C$_6$H$_5$(H3,H5)-thz. and H4-ind.), 7.24–7.27 (m, 5H, 3-C$_6$H$_5$-ind.), 7.32 (d, 2H, J = 8.0 Hz, 2-C$_6$H$_5$(H2,H6)-thz.), 7.45–7.49 (m, 1H, H7-ind.), 9.94 and 9.98 (s, 1H, CONH), 11.81 and 11.88 (s, 1H, NH); MS (ESI-) *m/z* (%): 458 (M−H$^-$, 100). Anal calcd for $C_{26}H_{22}FN_3O_2S$ (459.54): C, 67.96; H, 4.83; N, 9.14. Found: C, 67.89; H, 4.94; N, 8.97.

*5-Fluoro-N-[2-(4-methoxyphenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-3-phenyl-1H-indole-2-carboxamide (**8c**)*. White powder (70%); m.p. 188–189 °C; IR(KBr): ν_{max} 3293 (N–H), 1714, 1639 (C=O); ^{1}H NMR (DMSO-d$_6$/500 MHz): δ 1.50–1.53 (m, 3H, 5-CH$_3$-thz.), 3.78 (s, 3H, 4-OCH$_3$), 4.03 and 4.11 (q and qd, 1H, J = 6.8 and J = 7.1; 1.5 Hz, H5-thz.), 5.78 and 5.81 (2s, 1H, H2-thz.), 6.91–6.94 (m, 2H, 2-C$_6$H$_5$(H3,H5)-thz.), 7.12 (td, 1H, J = 9.0; 2.4 Hz, H6-ind.), 7.15–7.19 (m, 1H, H4-ind.), 7.23–7.29 (m, 5H, 3-C$_6$H$_5$-ind.), 7.36 (d, 2H, J = 8.8 Hz, 2-C$_6$H$_5$(H2,H6)-thz.), 7.45–7.49 (m, 1H, H7-ind.), 9.90 and 9.94 (2s, 1H, CONH), 11.81 and 11.88 (2s, 1H, NH); ^{13}C NMR (HSQC, HMBC) (DMSO-d$_6$/125 MHz): δ 19.97, 20.71 (5-CH$_3$-thz.), 38.93, 39.25 (C5-thz.), 55.94 (4-OCH$_3$), 60.95, 61.21 (C2-thz.), 104.92 (d, J = 24.0 Hz, C4-ind.), 113.77, 113.84 (2d, J = 26.8, J = 26.4 Hz, C6-ind.), 114.55, 114.69 (2-C$_6$H$_5$(C3,5)-thz.), 114.62 (d, J = 8.1 Hz, C7-ind.), 119.58, 119.82 (2d, C3-ind.), 127.05 (d, C3a-ind.), 127.09, 127.19 (3-C$_6$H$_5$(C4)-ind.), 127.38, 127.41 (C2-ind.), 129.05 (3-C$_6$H$_5$(C3,5)-ind.), 129.66, 129.84 (2-C$_6$H$_5$(C2,6)-thz.), 130.19, 130.22 (3-C$_6$H$_5$(C2,6)-ind.), 130.47 (2-C$_6$H$_5$(C1)-thz.), 133.17 (C7a-ind.), 133.36 (3-C$_6$H$_5$(C1)-ind.), 158.35 (d, J = 233.9 Hz, C5-ind.), 160.46, 160.59 (2-C$_6$H$_5$(C4)-thz.), 160.88, 160.94 (CONH), 172.39, 172.43 (CO-thz.). Anal calcd for $C_{26}H_{22}FN_3O_3S$ (475.54): C, 65.57; H, 4.66; N, 8.54. Found: C, 65.23; H, 4.83; N, 8.21.

*N-[2-(4-chlorophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-5-fluoro-3-phenyl-1H-indole-2-carboxamide (**8d**)*. White needles (71%); m.p. 211–214 °C; IR(KBr): ν_{max} 3245 (N–H), 1700, 1655 (C=O); ^{1}H NMR (DMSO-d$_6$/500 MHz): δ 1.51 and 1.52 (2d, 3H, J = 6.8 Hz, 5-CH$_3$-thz.), 4.06 and 4.16 (q and qd, 1H, J = 6.8 and J = 7.1; 1.5 Hz, H5-thz.), 5.83 and 5.86 (s and d, 1H, J = 1.5 Hz, H2-thz.), 7.12 (td, 1H, J = 9.0; 2.4 Hz, H6-ind.), 7.16–7.20 (m, 1H, H4-ind.), 7.22–7.26 (m, 5H, 3-C$_6$H$_5$-ind.), 7.41–7.49 (m, 5H, 2-C$_6$H$_5$-thz. and H7-ind.), 10.03 (br. s, 1H, CONH), 11.84 and 11.91 (2s, 1H, NH). Anal calcd for $C_{25}H_{19}ClFN_3O_2S$ (479.95): C, 62.56; H, 3.99; N, 8.76. Found: C, 62.75; H, 4.21; N, 8.85.

*N-[2-(4-bromophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-5-fluoro-3-phenyl-1H-indole-2-carboxamide (**8e**)*. Beige powder (57%); m.p. 198–200 °C; IR(KBr): ν_{max} 3244 (N–H), 1700, 1654 (C=O); ^{1}H NMR (DMSO-d$_6$/500 MHz): δ 1.51 and 1.52 (2d, 3H, J = 6.8 Hz, 5-CH$_3$-thz.), 4.06 and 4.15 (q and qd, 1H, J = 6.8 and J = 6.8; 1.5 Hz, H5-thz.), 5.82 and 5.84 (s and d, 1H, J = 1.5 Hz, H2-thz.), 7.12 (td, 1H, J = 9.3; 2.4 Hz, H6-ind.), 7.15–7.20 (m, 1H, H4-ind.), 7.23–7.26 (m, 5H, 3-C$_6$H$_5$-ind.), 7.39 (d, 2H, J = 8.8 Hz, 2-C$_6$H$_5$(H2,H6)-thz.), 7.45–7.48 (m, 1H, H7-ind.), 7.54–7.58 (m, 2H, 2-C$_6$H$_5$(H3,H5)-thz.), 10.06 (br. s, 1H, CONH), 11.86 (br. s, 1H, NH); ^{13}C NMR (HSQC) (DMSO-d$_6$/125 MHz): δ 20.20, 20.53 (5-CH$_3$-thz.), 38.81, 39.33 (C5-thz.), 60.49, 60.82 (C2-thz.), 104.91 (d, J = 23.5 Hz, C4-ind.), 113.81, 113.85 (2d, J = 26.4 Hz, C6-ind.), 114.62 (d, J = 9.6 Hz, C7-ind.), 119.76 (d, J = 4.8 Hz, C3-ind.), 122.70, 122.93 (2-C$_6$H$_5$(C4)-thz.), 127.11 (d, J = 9.6 Hz, C3a-ind.), 127.25, 127.29 (3-C$_6$H$_5$(C4)-ind.), 127.31, 127.49 (C2-ind.), 128.97 (3-C$_6$H$_5$(C3,5)-ind.), 130.16, 130.18 (3-C$_6$H$_5$(C2,6)-ind.), 130.44, 130.77 (2-C$_6$H$_5$(C2,6)-thz.), 132.20, 132.27 (2-C$_6$H$_5$(C3,5)-thz.), 133.22 (C7a-ind.), 133.34 (3-C$_6$H$_5$(C1)-ind.), 137.93, 138.70 (2-C$_6$H$_5$(C1)-thz.), 158.35 (d, J = 233.9 Hz, C5-ind.), 161.10, 161.14 (CONH), 172.34, 172.46 (CO-thz.); MS (ESI-) *m/z* (%): 524/522 (M−H$^-$, 100/92). Anal calcd for $C_{25}H_{19}BrFN_3O_2S$ (524.41): C, 57.26; H, 3.95; N, 8.01. Found: C, 57.22; H, 4.22; N, 8.30.

*5-Fluoro-N-[2-(4-fluorophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-3-phenyl-1H-indole-2-carboxamide (**8f**)*. Beige powder (67%); m.p. 219–220 °C; IR(KBr): ν_{max} 3242 (N–H), 1700, 1661 (C=O); ^{1}H NMR (DMSO-d$_6$/500 MHz): δ 1.51 and 1.52 (2d, 3H, J = 6.8 Hz, 5-CH$_3$-thz.), 4.05 and 4.15 (q and qd, 1H, J = 6.8 and J = 7.3; 1.5 Hz, H5-thz.), 5.83 and 5.85 (s and d, 1H, J = 1.5 Hz, H2-thz.), 7.12 (td, 1H, J = 9.3; 2.4 Hz, H6-ind.), 7.16–7.18 (m, 1H, H4-ind.), 7.20 (d, 2H, J = 8.8 Hz, 2-C$_6$H$_5$(H3,H5)-thz.), 7.23–7.26 (m, 5H, 3-C$_6$H$_5$-ind.), 7.48–7.52 (m, 3H, 2-C$_6$H$_5$(H2,H6)-thz. and H7-ind.), 9.99 and 10.03 (2s, 1H, CONH), 11.83 and 11.90 (2s, 1H, NH). Anal calcd for $C_{25}H_{19}F_2N_3O_2S$ (463.50): C, 64.88; H, 4.13; N, 9.07. Found: C, 65.05; H, 4.47; N, 8.97.

*N-[2-(4-cyanophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-5-fluoro-3-phenyl-1H-indole-2-carboxamide (**8g**)*. White powder (73%); m.p. 199–200 °C; IR(KBr): ν_{max} 3314 (N–H), 2228 (C≡N), 1716, 1636 (C=O); ^{1}H NMR (DMSO-d$_6$/500 MHz): δ 1.51 and 1.52 (2d, 3H, J = 6.8 Hz, 5-CH$_3$-thz.), 4.09 and 4.20 (q and qd, 1H, J = 6.8 and J = 7.1; 1.5 Hz, H5-thz.), 5.92 and 5.93 (s and d, 1H, J = 1.5 Hz, H2-thz.), 7.12 (td, 1H, J = 9.3; 2.4 Hz, H6-ind.), 7.16–7.27 (m, 6H, 3-C$_6$H$_5$,H4-ind.), 7.44–7.48 (m, 1H, H7-ind.), 7.62 (d, 2H, J = 8.3 Hz, 2-C$_6$H$_5$(H2,H6)-thz.), 7.82–7.85 (m, 2H, 2-C$_6$H$_5$(H3,H5)-thz.), 10.13 and 10.17 (2s, 1H, CONH), 11.86 and 11.93 (2s, 1H, NH); ^{13}C NMR (HSQC,DEPT) (DMSO-d$_6$/125 MHz): δ 20.34, 20.45 (5-CH$_3$-thz.), 38.69, 39.44 (C5-thz.), 60.30, 60.68 (C2-thz.), 104.93 (d, J = 24.0 Hz, C4-ind.), 112.04, 112.25 (2-C$_6$H$_5$(C4)-thz.), 113.76 (d, J = 26.8 Hz, C6-ind.), 114.56 (d, J = 10.1 Hz, C7-ind.), 119.23 (4-CN), 119.58, 119.75 (2d, J = 5.0 Hz, C3-ind.), 127.04 (d, C3a-ind.), 127.24, 127.30 (3-C$_6$H$_5$(C4)-ind.), 127.50 (C2-ind.), 128.91, 128.98 (3-C$_6$H$_5$(C3,5)-ind.), 129.27 (2-C$_6$H$_5$(C2,6)-thz.), 130.13 (3-C$_6$H$_5$(C2,6)-ind.), 133.24 (C7a-ind./3-C$_6$H$_5$(C1)-ind.), 133.27, 133.33 (2-C$_6$H$_5$(C3,5)-thz.), 144.41, 145.12 (2-C$_6$H$_5$(C1)-thz.), 158.36 (d, J = 234.4 Hz, C5-ind.), 161.26 (CONH), 172.37, 172.56 (CO-thz.); MS (ESI-) m/z (%): 469 (M−H$^-$, 100). Anal calcd for $C_{26}H_{19}FN_4O_2S$ (470.52): C, 66.37; H, 4.07; N, 11.81. Found: C, 66.35; H, 4.37; N, 11.53.

*5-Fluoro-N-[5-methyl-2-(4-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]-3-phenyl-1H-indole-2-carboxamide (**8h**)*. Yellow crystals (66%); m.p. 193–195 °C; IR(KBr): ν_{max} 3285 (N–H), 1716, 1638 (C=O); ^{1}H NMR (DMSO-d$_6$/500 MHz): δ 1.52 and 1.53 (2d, 3H, J = 6.8 Hz, 5-CH$_3$-thz.), 4.11 and 4.21 (q and qd, 1H, J = 6.8 and J = 7.1; 1.5 Hz, H5-thz.), 5.98 and 5.99 (s and d, 1H, J = 1.0 Hz, H2-thz.), 7.12 (td, 1H, J = 9.3; 2.4 Hz, H6-ind.), 7.15–7.27 (m, 6H, 3-C$_6$H$_5$,H4-ind.), 7.44–7.48 (m, 1H, H7-ind.), 7.71 (d, 2H, J = 8.3 Hz, 2-C$_6$H$_5$(H2,H6)-thz.), 8.18–8.22 (m, 2H, 2-C$_6$H$_5$(H3,H5)-thz.), 10.16 and 10.20 (2s, 1H, CONH), 11.85 and 11.93 (2s, 1H, NH); ^{13}C NMR (HSQC) (DMSO-d$_6$/125 MHz): δ 20.32, 20.44 (5-CH$_3$-thz.), 38.72, 39.48 (C5-thz.), 60.00, 60.38 (C2-thz.), 104.92 (d, J = 23.5 Hz, C4-ind.), 113.76, 113.83 (2d, J = 26.8, J = 26.4 Hz, C6-ind.), 114.55, 114.60 (2d, J = 9.6 Hz, C7-ind.), 119.61, 119.74 (2d, J = 4.8 Hz, C3-ind.),

124.42, 124.50 (2-C_6H_5(C3,5)-thz.), 126.98, 127.08 (2d, J = 9.6 Hz, C3a-ind.), 127.19, 127.24 (3-C_6H_5(C4)-ind.), 127.31, 127.50 (C2-ind.), 128.88, 128.89 (3-C_6H_5(C3,5)-ind.), 129.37, 129.66 (2-C_6H_5(C2,6)-thz.), 130.15, 130.18 (3-C_6H_5(C2,6)-ind.), 133.23, 133.24 (C7a-ind.), 133.30, 133.33 (3-C_6H_5(C1)-ind.), 146.36, 147.09 (2-C_6H_5(C1)-thz.), 148,30, 148.43 (2-C_6H_5(C4)-thz.), 158.36 (d, J = 233.9 Hz, C5-ind.), 161.27 (CONH), 172.32, 172.53 (CO-thz.). Anal calcd for $C_{25}H_{19}FN_4O_4S.1/2H_2O$ (499.51): C, 60.11; H, 4.08; N, 11.22. Found: C, 60.29; H, 4.25; N, 11.25.

5-Fluoro-N-{5-methyl-4-oxo-2-[4-(trifluoromethyl)phenyl]-1,3-thiazolidin-3-yl}-3-phenyl-1H-indole-2-carboxamide ***(8i)***. White needles (53%); m.p. 217–219 °C; IR(KBr): ν_{max} 3303 (N–H), 1716, 1636 (C=O); ^{1}H NMR (DMSO-d_6/500 MHz): δ 1.51–1.54 (m, 3H, 5-CH_3-thz.), 4.09 and 4.18 (q and qd, 1H, J = 6.8 and J = 6.8; 1.5 Hz, H5-thz.), 5.94 and 5.96 (s and d, 1H, J = 1.5 Hz, H2-thz.), 7.12 (td, 1H, J = 9.3; 2.4 Hz, H6-ind.), 7.14–7.20 (m, 4H, 3-C_6H_5(H3-5),H4-ind.), 7.22–7.25 (m, 2H, 3-C_6H_5(H2,H6)-ind.), 7.44–7.48 (m, 1H, H7-ind.), 7.65 (d, 2H, J = 7.8 Hz, 2-C_6H_5(H2,H6)-thz.), 7.71–7.74 (m, 2H, 2-C_6H_5(H3,H5)-thz.), 10.15 (br. s, 1H, CONH), 11.93 (br. s, 1H, NH). Anal calcd for $C_{26}H_{19}F_4N_3O_2S$ (513.51): C, 60.81; H, 3.93; N, 8.18. Found: C, 60.70; H, 4.25; N, 8.22.

5-Fluoro-N-{2-[4-(methoxycarbonyl)phenyl]-5-methyl-4-oxo-1,3-thiazolidin-3-yl}-3-phenyl-1H-indole-2-carboxamide ***(8j)***. White needles (72%); m.p. 129–131 °C; IR(KBr): ν_{max} 3300 (N–H), 1713, 1672, 1638 (C=O); ^{1}H NMR (DMSO-d_6/500 MHz): δ 1.51 and 1.52 (2d, 3H, J = 6.8 Hz, 5-CH_3-thz.), 3.87 (s, 3H, 4-$COOCH_3$), 4.08 and 4.18 (q and qd, 1H, J = 6.8 and J = 6.8; 1.5 Hz, H5-thz.), 5.91 and 5.92 (s and br. s, 1H, H2-thz.), 7.12 (td, 1H, J = 9.3; 2.4 Hz, H6-ind.), 7.15–7.26 (m, 6H, 3-C_6H_5,H4-ind.), 7.44–7.48 (m, 1H, H7-ind.), 7.58 (d, 2H, J = 8.3 Hz, 2-C_6H_5(H2,H6)-thz.), 7.92–7.96 (m, 2H, 2-C_6H_5(H3,H5)-thz.), 10.11 (br. s, 1H, CONH), 11.83 and 11.90 (2s, 1H, NH); MS (ESI-) m/z (%): 502 ($M-H^-$, 100). Anal calcd for $C_{27}H_{22}FN_3O_4S.1/2H_2O$ (512.55): C, 63.27; H, 4.54; N, 8.20. Found: C, 63.15; H, 4.73; N, 8.08.

N-[2-(2,6-dichlorophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-5-fluoro-3-phenyl-1H-indole-2-carboxamide ***(8k)***. White powder (78%); m.p. 205.5–207 °C; IR(KBr): ν_{max} 3295, 3212 (N–H), 1715, 1639 (C=O); ^{1}H NMR (DMSO-d_6/500 MHz): δ 1.50 (d, 3H, J = 7.3 Hz, 5-CH_3-thz.), 4.17 (qd, 1H, J = 7.1; 2.0 Hz, H5-thz.), 6.69 (d, 1H, J = 2.0 Hz, H2-thz.), 7.14 (td, 1H, J = 9.3; 2.4 Hz, H6-ind.), 7.17 (dd, 1H, J = 9.2; 2.4 Hz, H4-ind.), 7.23–7.31 (m, 3H, 3-C_6H_5(H3-5)-ind.), 7.35 (dd, 2H, J = 8.1; 2.0 Hz, 3-C_6H_5(H2,H6)-ind.), 7.42 (t, 1H, J = 8.3 Hz, 2-C_6H_5(H4)-thz.), 7.46–7.50 (m, 2H, 2-C_6H_5(H3/H5)-thz. and H7-ind.), 7.53 (dd, 1H, J = 8.1; 1.5 Hz; 2-C_6H_5(H3/H5)-thz.), 10.11 (s, 1H, CONH), 11.91 (s, 1H, NH); ^{13}C NMR (HSQC) (DMSO-d_6/125 MHz): δ 20.70 (5-CH_3-thz.), 39.76 (C5-thz.), 55.79 (C2-thz.), 104.96 (d, J = 24.0 Hz, C4-ind.), 114.02 (d, J = 26.8 Hz, C6-ind.), 114.67 (d, J = 9.6 Hz, C7-ind.), 120.36 (d, J = 5.8 Hz, C3-ind.), 126.90 (C2-ind.), 127.34 (d, J = 9.6 Hz, C3a-ind.), 127.52 (3-C_6H_5(C4)ind.), 129.02 (3-C_6H_5(C3,5)-ind.), 129.64 (2-C_6H_5(C3/5)-thz.), 130.20 (3-C_6H_5(C2,6)-ind.), 131.88 (2-C_6H_5(C3/5)-thz.), 131.93 (2-C_6H_5(C4)-thz.), 133.23 (C7a/3-C_6H_5(C1)-ind.), 133.50 (C7a/3-C_6H_5(C1)-ind.), 135.50 (2-C_6H_5(C2,6/C1)-thz.), 136.06 (2-C_6H_5(C2,6/C1)-thz.), 158.37 (d, J = 233.9 Hz, C5-ind.), 161.50 (CONH), 171.96 (CO-thz.). Anal calcd for $C_{25}H_{18}Cl_2FN_3O_2S$ (514.40): C, 58.37; H, 3.53; N, 8.17. Found: C, 58.38; H, 3.43; N, 8.01.

N-[2-(2-chloro-6-fluorophenyl)-5-methyl-4-oxo-1,3-thiazolidin-3-yl]-5-fluoro-3-phenyl-1H-indole-2-carboxamide ***(8l)***. White powder (74%); m.p. 234–236 °C; IR(KBr): ν_{max} 3287, 3214 (N–H), 1716, 1642 (C=O); ^{1}H NMR (DMSO-d_6/500 MHz): δ 1.51–1.53 (m, 3H, 5-CH_3-thz.), 4.14–4.17 (m, 1H, H5-thz.), 6.37 and 6.41 (s and br. s, 1H, H2-thz.), 7.13 (td, 1H, J = 9.3; 2.4 Hz, H6-ind.), 7.18 (br. d, 1H, J = 9.8 Hz, H4-ind.), 7.24–7.38 (m, 7H, 2-C_6H_5(H3,H5)-thz. and 3-C_6H_5-ind.), 7.44–7.49 (m, 2H, 2-C_6H_5(H4)-thz. ve H7-ind.), 10.23 and 10.29 (2s, 1H, CONH), 11.87 and 11.90 (2s, 1H, NH); MS (ESI-) m/z (%): 498.5/496.5 ($M-H^-$, 45/100). Anal calcd for $C_{25}H_{18}ClF_2N_3O_2S$ (497.95): C, 60.30; H, 3.84; N, 8.44. Found: C, 60.14; H, 4.08; N, 8.50.

Biological assays

BACTEC 460TB assay

The new compounds were evaluated for *in vitro* antitubercular activity against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294) by the BACTEC 460TB (Becton Dickinson, Sparks, MD) system. This rapid and reliable radiometric method is based on the fact that mycobacteria metabolize fatty acids to CO_2. If these fatty acids are radioactively labeled with ^{14}C, the $^{14}CO_2$ end product can be quantitatively determined by the Bactec instrument.

All test compounds were dissolved in dimethyl sulfoxide (DMSO) at a stock concentration of 4000 μg/ml and kept at −20 °C until used. The final concentrations were prepared with 7H9 broth medium for mycobacteria. The final DMSO concentration was adjusted to 1% and the final concentration of the tested compounds was adjusted to 25 μg/ml in the BACTEC 12B medium. Inoculum for susceptibility testing was used from a positive BACTEC isolation vial with a growth index (GI) of 500 or more. The BACTEC culture was well mixed and 0.1 ml of culture was added to each of the vials containing the test compounds (25 μg/ml). A control vial was inoculated with a 1:100 dilution of the culture. The standard vials contained rifampicin (0.25 μg/ml). Vials were incubated at 37 °C and each vial was checked daily with a BACTEC 460 instrument. When the control GI reading was at least 30, the results were interpreted by calculating the increase in GI from the previous day. If the daily increase of GI of the control was greater than the daily GI increase of the test compound vial, the mycobacteria were reported as susceptible. If the daily increase of GI of the control was equal to or less than that of the test compound vial, the organisms were reported as resistant. The compounds demonstrating antitubercular activity at 25 μg/ml were further tested at twofold dilutions to determine the actual MIC[31–33].

MTT proliferation assay

The cytotoxicity of the active compounds was determined by the MTT assay using the rat kidney epithelial cell line (NRK-52E)[34,35]. The cell line was obtained from American Type Culture Collection (ATCC, USA). Cells were incubated in Dulbecco's modified Eagle's medium with 10% fetal bovine serum and antibiotics (1% penicillin, 1% streptomycin) in 95% O_2/5% CO_2 at 37 °C. Culture medium was changed every 2 days. The monolayer cells grown to 75–85% confluency were detached with trypsin-ethylenediaminetetraacetic acid to make single cell suspensions. Viable cells were determined using the trypan blue exclusion test and diluted with medium to give a final density of 10^5 cells/ml. The passage number range for the cell line was maintained between 20 and 25. One hundred microliters of cell suspension was seeded into 96-well plate at a plating density of 10^4 cells/well and incubated to allow for cell attachment at 37 °C in a humidified 5% CO_2 atmosphere for 24 h. After 24 h, the cells were treated with serial concentrations of the test compounds. Test compounds were initially dissolved in DMSO) and further diluted in distilled water to produce the end concentrations. Ten microliters of each concentration was added to plates to obtain final concentrations of 8–250 μg/ml. The final volume in each well was 100 μl and the plates were incubated at 37 °C in a humidified 5% CO_2

atmosphere for 24 h. Each concentration was tested in triplicate and each test was repeated. The medium containing no sample (growth control) and 0.5% DMSO (solvent control) served as the controls. There was no difference in the cell count between the solvent and growth controls.

The growth inhibitory activities of the tested compounds were determined using the standard colorometric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Twenty microliters of MTT solution (5 mg/mL in phosphate buffered saline) was added to each well and incubated at 37 °C for 4 h. The medium with MTT was removed, the formed formazan crystals were solubilized in 100 µl of DMSO and the absorbance was measured at 570 nm Epoch microplate spectrophotometer system (BioTek Instruments, Winooski, VT). The percentage cell viability was calculated with respect to the solvent control as follows:

$$\%\,\text{Cell viability} = \frac{\text{Abs}_{\text{test}}}{\text{Abs}_{\text{solvent control}}} \times 100.$$

Anticancer screening

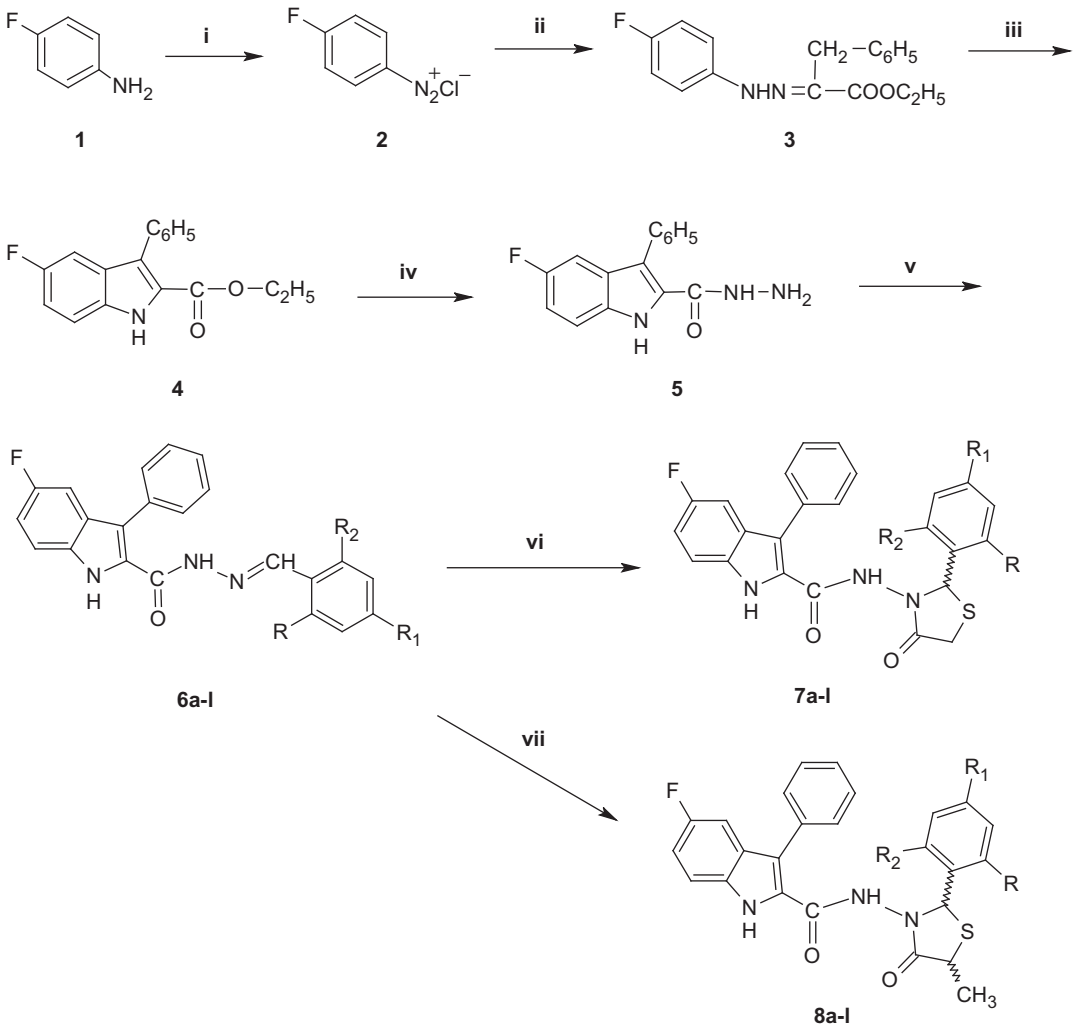
Primary anticancer assay was performed in accordance with the protocol of the Drug Evaluation Branch of the National Cancer Institute (Bethesda, MD). The procedure utilized basicly measured the cellular protein content of cultures stained with the protein dye sulforhodamine B spectrophotometrically[36].

Results and discussion

Chemistry

The synthetic approach utilized for the target compounds is outlined in Scheme 1. The key intermediate **5** and indolylhydrazones **6a** and **6d–6h** were previously synthesized and patented by Bamaung et al. as angiogenesis inhibitors[29].

The structures of the new compounds were characterized by microanalysis, IR, ^{1}H NMR, ^{13}C NMR (proton decoupled and DEPT-135), 2D NMR (HSQC and HMBC), electrospray



6-8a: R,R_1,R_2=H
6-8b: R,R_2=H; R_1=CH_3
6-8c: R,R_2=H; R_1=OCH_3
6-8d: R,R_2=H; R_1=Cl
6-8e: R,R_2=H; R_1=Br
6-8f: R,R_2=H; R_1=F
6-8g: R,R_2=H; R_1=CN
6-8h: R,R_2=H; R_1=NO_2
6-8i: R,R_2=H; R_1=CF_3
6-8j: R,R_2=H; R_1=$COOCH_3$
6-8k: R,R_2=Cl; R_1=H
6-8l: R=Cl; R_1=H; R2=F

* Compounds **6a** and **6d-h** were previously reported by Ba-Maung et al. (29)

Scheme 1. Synthesis of **6–8**. Reagents and conditions: (i) 7% $NaNO_2$, EtOH, conc. HCl, 0 °C; (ii) ethyl 2-benzyl-3-oxo-butanoate, KOH, EtOH, 0 °C; (iii) conc. HCl, reflux, 4 h; (iv) $H_2NNH_2{\cdot}H_2O$, EtOH, reflux, 6 h; (v) (non)substituted benzaldehyde, abs. EtOH, reflux, 5–6 h; (vi) mercaptoacetic acid, dry benzene, reflux, 5–6 h and (vii) 2-mercaptopropionic acid, dry benzene, reflux, 5–6 h.

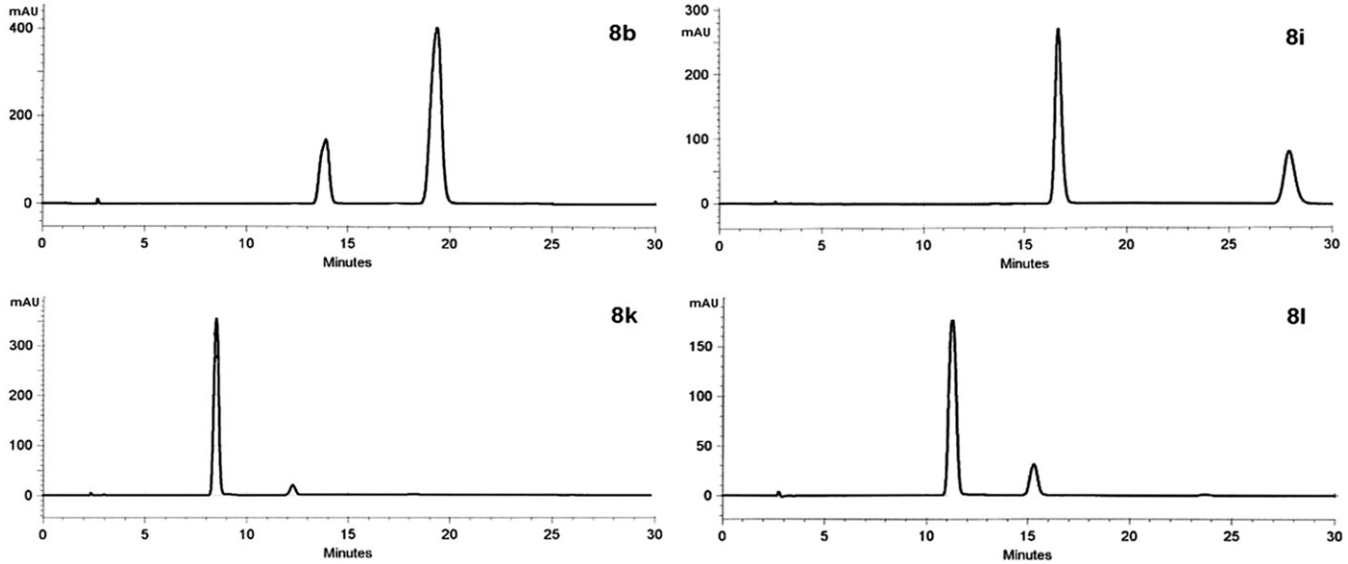


Figure 2. Normal-phase HPLC diastereomeric resolution of compounds **8b**, **8i**, **8k** and **8l**. Column: Kromasil 100-5SIL microporous silica column (25 cm × 4.6 mm); eluent: hexane–ethyl acetate (80:20); flow rate:1.2 mL/min; detection: 304 nm.

ionization mass spectrometry (ESI-MS) and HPLC studies. The absolute stereochemistry of **6c** was determined by an X-ray diffraction study[30]. The absence of the N–H_2 resonance of the intermediate hydrazide (**5**) at δ 4.48 ppm together with the new signals of the N=CH protons at δ 8.00–8.32 ppm in the ^{1}H NMR spectra of **6** supported the structure of the new adducts. Observation of new lactam C=O bands (1697–1726 cm^{-1}) besides C=O amide bands (1636–1678 cm^{-1}) in the IR spectra of **7** and **8** proved the aimed cyclization. The 4-thiazolidinone C2-H of **7** was observed at about δ 5.80–6.73 ppm as a singlet or a doublet (J = 1.0–1.5 Hz) and the C5-H_2 resonated at about δ 3.74–3.97 ppm as a double doublet with coupling constants in the range of 15.6–16.1 and 1.4–2.0 Hz. Large couplings about 15 Hz characteristic of geminal interactions supported the chiral nature of the 4-thiazolidinone C2 carbon and small splittings indicated a long-range interaction between the *cis* C2-H and C5-H protons of the rigid 4-thiazolidinone system. These four-bond couplings (W-coupling) across the C2-H and C5-H protons were also observed in the ^{1}H NMR spectra of **8** with coupling constants of about 1.0–1.5 Hz. Peaks associated with the indole subunit were observed in the expected regions and were assigned on the basis of ^{1}H–^{1}H and ^{1}H–^{19}F couplings.

^{13}C NMR experiments (proton decoupled and DEPT-135) run on **5**, **8g** and 2D NMR experiments (HSQC and HMBC) run on **6b**, **6j**, **7b**, **7d**, **7i–7l**, **8c**, **8e**, **8g**, **8h**, **8k** allowed unambiguous assingment of the proton and carbon chemical shifts. The carbocyclic indole carbons which explicitly showed the ^{13}C–^{19}F couplings of the 5-fluoro indole core were observed as separate doublets with characteristic coupling constants related to the ipso, ortho, meta and para positions and allowed definite positional assignment of the C3, C3a, C4–7 and C7a carbons of **6–8**. The transformation of **6** to **7** or **8** proceeds via the nucleophilic addition of the SH function to the C=N double bond. Upfield shifts observed in the ^{13}C NMR resonances of the C=N carbons of **6b** and **6g** (δ 148.13 and 146.58 ppm) supported the expected addition as the resulting typical sp^3 hybridized 4-thiazolidinone C5 absorbed at δ 29.89–39.76 ppm. Resonances assigned to the 4-thiazolidinone C2 (δ 55.75–62.44 ppm) and endocyclic C=O (δ 168.77–172.56 ppm) further confirmed the aimed conversion. Cross peaks observed between the 4-thiazolidinone 5-CH_3, C5-H protons and the lactam C=O in the HMBC spectrum of **7a**, **7l** and **8c** enabled definite assignment of the lactam C=O (δ 168.77–172.56 ppm) and the CONH (δ 160.88–161.50 ppm) carbons.

The ^{1}H NMR spectra of **8** displayed two sets of signals for most of the protons, except for the 2,6-dichloro substituted derivative, **8k**. The 4-thiazolidinone C2-H protons resonated as a singlet and a broad singlet/doublet and C5-H protons resonated as a quartet and a doublet of quartets. Indole N–H and CONH protons were observed as two separate singlets or one broad singlet. Most of the proton resonances appeared as duplicate signals or distorted multiplets (see Experimental section for details). The novel 2,3,5-trisubstituted-4-thiazolidinones (**8a–8l**) bear chiral C-2 and C-5 atoms and were possibly obtained as mixtures of cis and trans diastereomers. The stereoisomeric content and the purity of selected entries **8b**, **8i**, **8k** and **8e** were confirmed by analytical HPLC studies. A simple and rapid isocratic HPLC method was developed for the determination of the diastereomers of compounds **8b**, **8i**, **8k** and **8l** chosen as examples. The resolution of the diastereomers was achieved by normal-phase HPLC on a conventional silica column (Kromasil 100-5SIL microporous silica column) with hexane–ethyl acetate (80:20) as the mobil phase (Figure 2). The retention times and peak-area percentages are presented in Table 1. Compound **8k**, which did not display duplicate signals in ^{1}H NMR and ^{13}C NMR spectra, displayed two peaks on the chromatogram in an approximate ratio of 95:5 with a large excess of one of the diastereomers. The 5-unsubstituted derivatives **7b**, **7i**, **7k** and **7l** having a single chiral center at C-2 were also analyzed for comparison. As expected, the chromatograms showed only single peaks (for representative NMR spectra, see Supplementary materials).

ESI-MS was used to verify the molecular weights of compounds **6b**, **6j**, **6l**, **7–8b**, **e**, **g**, **j** and **l**. All compounds were analyzed under negative-ion ESI conditions since none of the compounds were responsive to the positive-ion mode. Deprotonated [M−H]− ions observed in the ESI-MS, confirmed the calculated molecular weight of compounds **6b**, **6j**, **6l**, **7–8b**, **8c**, **8g** and **8l**.

Biological activity

The novel indolylhydrazones (**6**) and indole-based 4-thiazolidinones (**7**, **8**) were tested for *in vitro* antitubercular activity against

Table 1. Retention times and peak area percentages of compounds **7–8b**, **8i**, **8k**, **8l** in NP-HPLC.[a]

	7b	**7i**	**7k**	**7l**	**8b**	**8i**	**8k**	**8l**
Retention time (min) (Peak-area percentage)	29.51 (100%)	23.75 (100%)	21.75 (100%)	23.69 (100%)	13.88 (23.49%) 19.30 (76.51%)	16.63 (66.05%) 27.92 (33.95%)	8.47 (94.27%) 12.27 (5.73%)	11.25 (84.82%) 15.28 (15.18%)

[a]Column: Kromasil 100-5SIL microporous silica column (25 cm × 4.6 mm); eluent: hexane–ethyl acetate (80:20); flow rate: 1.2 mL/min; detection: 304 nm.

Table 2. *In vitro* antitubercular activity and cytotoxicity screening results of compounds **6–8**.

Compound	R	R_1	R_2	R_3	MIC (μg/ml)[a]	IC_{50} (μg/ml)[b]
6b	H	CH_3	H	–	>25	NT[c]
6c	H	OCH_3	H	–	>25	NT
6i	H	CF_3	H	–	>25	NT
6j	H	$COOCH_3$	H	–	>25	NT
6k	Cl	H	Cl	–	>25	NT
6l	Cl	H	F	–	>25	NT
7a	H	H	H	H	>25	NT
7b	H	CH_3	H	H	>25	NT
7c	H	OCH_3	H	H	>25	NT
7d	H	Cl	H	H	>25	NT
7e	H	Br	H	H	>25	NT
7f	H	F	H	H	>25	NT
7g	H	CN	H	H	**6.25**	**113.4**
7h	H	NO_2	H	H	**6.25**	**>125**
7i	H	CF_3	H	H	**12.5**	**>250**
7j	H	$COOCH_3$	H	H	**25.0**	**>125**
7k	Cl	H	Cl	H	>25	NT
7l	Cl	H	F	H	>25	NT
8a	H	H	H	CH_3	>25	NT
8b	H	CH_3	H	CH_3	>25	NT
8c	H	OCH_3	H	CH_3	>25	NT
8d	H	Cl	H	CH_3	>25	NT
8e	H	Br	H	CH_3	>25	NT
8f	H	F	H	CH_3	>25	NT
8g	H	CN	H	CH_3	**12.5**	**60.0**
8h	H	NO_2	H	CH_3	**12.5**	**125.0**
8i	H	CF_3	H	CH_3	>25	NT
8j	H	$COOCH_3$	H	CH_3	**25.0**	**>250**
8k	Cl	H	Cl	CH_3	>25	NT
8l	Cl	H	F	CH_3	>25	NT
Rifampicin	–	–	–	–	0.25	**>250**

[a]MIC, minimum inhibitory concentration required to inhibit the growth of 99% of *M. tuberculosis* H37Rv (ATCC 27294).
[b]IC_{50}, 50% cytotoxic concentration against NRK-52E cells *in vitro*.
[c]NT, not tested.
Values in bold indicate the MIC and IC_{50} values of the most active compounds **7g–j**, **8g**, **8h** and **8j**.

M. tuberculosis H37Rv using the BACTEC 460TB system[31–33]. The antituberculosis (anti-TB) drug, rifampicin, was used as the positive control. The minimum concentration of compound required to hinder 99% of bacterial growth in the culture was referred as the MIC. Compounds **7g–7j**, **8g**, **8h** and **8j** were assayed using twofold dilutions starting at 25.0 μg/ml. As reported in Table 2, compounds **7g–7j**, **8g**, **8h** and **8j** exhibited significant anti-TB activity showing 99% inhibition at MIC values ranging from 25.0 to 6.25 μg/ml. The most potent entries were the CN an NO_2 substituted derivatives (**7g** and **7h**) with MIC values of 6.25 μg/ml.

The screening results showed that the 4-thiazolidinone scaffold had an essential role in anti-TB activity since precursors (**6**) were inactive at the tested concentrations. When the structures of the active molecules were examined, it seemed that substitutions on the benzene ring had a profound effect on the activity. Introduction of electron-withdrawing substituents CN, NO_2, CF_3 and $COOCH_3$ at the para-position of the phenyl ring, as in **7g–7j**, **8g**, **8h** and **8j**, enhanced the biological activity in the majority of the compounds. The screening results also showed that, substitution at the 5-position of the 4-thiazolidinone system had a negative effect. The 5-nonsubstituted-4-thiazolidinone derivatives (**7g–7i**) were found to be more active in comparison with their 5-methyl substituted congeners (**8g–8i**), except for compounds **7j** and **8j** which displayed similar MIC values (Table 2).

The active compounds, **7g–7j**, **8g**, **8h** and **8j**, were further examined for cytotoxicity using the rat kidney epithelial cell line (NRK-52E) up to 125.0 or 250.0 μg/ml. Higher concentrations could not tested due to solubility problems. The cytotoxicity results presented in Table 2 are expressed as the concentrations inhibiting 50% of the cell growth (IC_{50}). The tested compounds were found not to be cytotoxic at concentrations up to 125.0 or 250.0 μg/ml, except for 4-CN substituted derivatives **7g** and **8g** which exhibited low cytotoxicity at 113.4 and 60.0 μg/ml, respectively. All the tested compounds showed a differential between active and cytotoxic doses. Compounds **7g**, **7h**, **7i**, **8h** and **8j** may be considered for further development as potential anti-TB agents, as they demonstrated anti-TB activity at concentrations 10-fold lower than those cytotoxic for the mammalian cell lines[37].

5-Fluoro-N^2-(4-methylbenzylidene)-3-phenyl-1*H*-indole-2-carbohydrazide (**6b**, NSC 752358) was selected by the NCI (USA) as a prototype for evaluation in the *in vitro* preclinical antitumor screening program against 60 human tumor cell lines derived from nine different types of cancer, namely, leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate and kidney[36]. This *in vitro* screen was subdivided into a pretest and a main test. Within the one-dose pretest consisting of approximately 60 tumor cell lines, compound **6b** was added at a single dose of 10 μM to each cell line. The results were reported as the percentage of growth of the treated cells in comparison with that of the untreated control cells. Compound **6b** which satisfied predetermined threshold inhibition criteria was passed on for evaluation in the main test, consisting of 60 cell lines over a five-dose range (0.01–100 μM).

Table 3 shows the anticancer data for compound **6b**. The NCI standard anticancer agent 5-fluorouracil (NSC 19893) was used as the reference compound[38]. As can be seen from Table 3, **6b** exhibited significant anticancer activity at sub-micromolar concentrations against most of the tested cell lines. The GI_{50} values were between 0.01– and 0.1 μM with a full panel mean value of 0.039 μM, except for SF-268 (CNS Cancer), SK-MEL-28 (melanoma) and T-47D (breast cancer) cell lines. Compound **6b** demostrated higher 50% growth inhibition activity than the standard 5-fluorouracil (NSC 19893) did against all of the cell lines tested except for SR (leukemia), MALME 3M (melanoma), OVCAR-3 (ovarian cancer) and T-47D (breast cancer). It is especially noteable that **6b** had an obvious selectivity toward colon cancer COLO 205 cell line at both the GI_{50} (0.018 μM),

Table 3. In vitro anticancer activity of compound **6b** against 60 human tumor cell lines at five-dose levels in comparison with data of NCI's standard 5-fluorouracil.[a]

	NSC 752358 (**6b**)			NSC 19893 (5-fluorouracil)		
Panel/cell line	GI_{50}[b]	TGI[c]	LC_{50}[d]	GI_{50}	TGI	LC_{50}
Leukemia						
CCRF-CEM	0.033	>100	>100	10.0	100.0	100.0
HL-60(TB)	0.026	0.072	>100	2.51	100.0	100.0
K-562	0.038	15.5	>100	4.00	100.0	100.0
MOLT-4	0.038	15.5	70.8	0.32	50.1	100.0
RPMI-8226	0.033	13.8	>100	0.050	50.1	100.0
SR	0.028	40.7	>100	0.025	10.0	100.0
Non-small cell lung cancer						
A549/ATCC	0.034	>100	>100	0.20	63.1	100.0
EKVX	0.074	>100	>100	63.1	100.0	100.0
HOP-62	0.033	0.81	>100	0.40	100.0	100.0
HOP-92	0.036	30.2	>100	79.4	100.0	100.0
NCI-H23	0.031	19.1	>100	0.32	39.8	100.0
NCI-H322M	0.039	26.9	>100	0.20	7.94	100.0
NCI-H460	0.036	14.5	>100	0.063	50.1	100.0
NCI-H522	0.013	0.040	>100	7.94	63.1	100.0
Colon cancer						
COLO 205	0.018	0.034	0.063	0.16	63.1	100.0
HCC-2998	0.030	21.4	>100	0.050	39.8	100.0
HCT-116	0.023	0.069	>100	0.25	3.98	25.1
HCT-15	0.025	0.083	>100	0.10	50.1	100.0
HT29	0.025	NT[e]	>100	0.16	63.1	100.0
KM12	0.025	0.066	17.4	0.20	39.8	100.0
SW-620	0.039	>100	>100	1.00	100.0	100.0
CNS cancer						
SF-268	1.32	34.7	>100	1.58	100.0	100.0
SF-295	0.019	0.060	50.1	0.25	3.98	100.0
SF-539	0.025	0.070	38.0	0.063	79.4	100.0
SNB-19	0.050	>100	>100	4.00	79.4	100.0
SNB-75	0.032	11.7	83.2	79.4	100.0	100.0
U251	0.036	34.7	>100	1.00	79.4	100.0
Melanoma						
LOX IMVI	0.028	>100	>100	0.25	50.1	79.4
MALME-3M	0.067	66.1	>100	0.050	2.51	100.0
M14	0.022	0.061	>100	1.00	100.0	100.0
MDA-MB-435	0.021	0.048	2.75	0.079	79.4	100.0
SK-MEL-2	0.031	NT	>100	63.1	100.0	100.0
SK-MEL-28	0.25	>100	>100	1.00	63.1	100.0
SK-MEL-5	0.020	0.045	0.29	0.50	39.8	79.4
UACC-257	0.061	>100	>100	4.00	79.4	100.0
UACC-62	0.042	34.7	>100	0.50	39.8	100.0
Ovarian cancer						
IGROV1	0.032	1.82	>100	1.26	31.6	100.0
OVCAR-3	0.025	0.048	NT	0.015	0.32	50.1
OVCAR-4	0.046	>100	>100	4.00	79.4	100.0
OVCAR-5	0.050	>100	>100	10.0	50.1	100.0
OVCAR-8	0.037	>100	>100	1.58	31.6	100.0
NCI/ADR-RES	0.022	0.053	>100	0.32	12.6	100.0
SK-OV-3	0.027	0.077	>100	19.9	63.1	100.0
Renal cancer						
786-0	0.031	12.3	>100	0.79	50.1	100.0
A498	0.038	24.5	>100	0.40	100.0	100.0
ACHN	0.048	>100	>100	0.32	31.6	100.0
CAKI-1	0.033	3.63	>100	0.079	2.00	100.0
RXF 393	0.025	0.085	63.1	2.51	31.6	100.0
SN12C	0.045	>100	>100	0.50	25.1	100.0
TK-10	0.044	>100	>100	1.26	79.4	100.0
UO-31	0.038	21.4	>100	1.58	50.1	100.0
Prostate cancer						
PC-3	0.034	20.4	>100	2.51	100.0	100.0
DU-145	0.023	0.052	0.29	0.40	100.0	100.0
Breast cancer						
MCF7	0.029	>100	>100	0.079	50.1	100.0
MDA-MB-231/ATCC	0.033	0.23	>100	6.31	39.8	100.0
HS 578T	0.027	1.78	>100	10.0	100.0	100.0
BT-549	0.032	2.34	>100	10.0	100.0	100.0
T-47D	24.0	>100	>100	7.94	50.1	100.0
MDA-MB-468	0.023	0.067	>100	NT	NT	NT
MG_MID[f]	0.039	4.37	63.1	0.85	42.7	95.5

[a]Data obtained from the NCI's in vitro disease-oriented human tumor cell screen. All values were given in μM. Highest concentration was 100 μM unless otherwise reported.
[b]Molar concentration that inhibited 50% net cell growth.
[c]Molar concentration leading to total growth inhibition.
[d]Molar concentration leading to 50% cell death.
[e]Not tested.
[f]Average activity parameter over all cell lines.

Molar concentration leading to total growth inhibition (TGI) (0.034 μM) and LC_{50} (0.063 μM) levels. Compound **6b** (NSC 752358) was retested using the five-dose screen and the reproducibility of its cellular actions was confirmed.

Conclusion

In the search for effective and selective antitubercular agents, we achieved the synthesis of novel hydrazone (**6**) and 4-thiazolidinone (**7**, **8**) derivatives of the 5-fluoro-3-phenyl-1*H*-indole scaffold. All the newly synthesized compounds (**6–8**) were evaluated for *in vitro* anti-TB activity against *M. tuberculosis* H37Rv. The 4-thiazolidinone derivatives **7g–7j**, **8g**, **8h** and **8j** displayed appreciable anti-TB activity showing 99% inhibition at MIC values ranging from 25.0 to 6.25 μg/ml along with rather low cytotoxicity against mammalian cell lines. Compounds **7g**, **7h**, **7i**, **8h** and **8j** demonstrated anti-TB activity at concentrations 10-fold lower than those cytotoxic for the mammalian cell lines. The screening results warrant further investigation to evaluate *in vivo* anti-TB activity of promising analogs and elucidate the mechanism of the anti-TB potential of this class of compounds.

The antitumor potential of compound **6b**, which was selected by the NCI as a prototype, has been also investigated. Although the biochemical targets of the molecule have not been identified yet, it showed an interesting anticancer profile against different human tumor-derived cell lines at sub-micromolar concentrations with GI_{50} values between 0.01 and 0.1 μM with a full panel mean value of 0.039 μM, except for SF-268 (CNS cancer), SK-MEL-28 (melanoma) and T-47D (breast cancer) cell lines. Compound **6b** demonstrated an obvious selectivity toward colon cancer COLO 205 cell line at both the GI_{50} (0.018 μM), TGI (0.034 μM) and LC_{50} (0.063 μM) levels. These encouraging preliminary results make relevant structures an interesting avenue toward the discovery of a new class of anticancer agents worthy of further examination and scientific scrutiny.

Investigations directed toward the modification of the indole substituents as well as the substitution on the indole-2-carboxamide/carbohydrazide moiety are underway for enhanced antitubercular and anticancer activity.

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Declaration of interest

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