

Synthesis and antitubercular evaluation of 2-iminothiazolidine-4-ones

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ABSTRACT

In the present manuscript, we report synthesis of new 3 and 5 substituted 2-imino thiazolidine-4-ones by three step synthetic protocols from 3-trifluoromethyl aniline or 2-amino heterocycle. The compounds were evaluated for *in vitro* activities against *Mycobacterium tuberculosis* (MTB) in presence and absence of efflux pump inhibitor, cytotoxicity against RAW 264.7 cells. Among the thirty six compounds, 2-imino-3-(5-nitrothiazol-2-yl)-5-(3,4,5-trimethoxybenzylidene)thiazolidin-4-one (**5g**) was found to be the most active compound *in vitro* with MICs of 3.31 μM against log-phase culture of MTB and also non-toxic up to 100 μM . Compound **5g** showed minimum inhibitory concentration (MIC) of 0.82 μM against MTB in presence of efflux pump inhibitor verapamil.

1. Introduction

Despite the existence of treatments for tuberculosis (TB), nine million people are currently infected and one and a half million die, each year [1]. The disease also represents an escalating threat for global health, with the increasing prevalence of multi drug resistant (MDR) and extensively drug resistant (XDR) TB strains [2]. Despite this, the development of new drugs with novel modes of action for the treatment of TB would likely still be the most cost-effective way of tackling the pandemic. Specifically, any new drug should be able to shorten the duration of treatment, avoid significant drug-drug interaction with current regimens, to treat MDR as well as XDR-TB patients [3,4].

Target-based approaches are widely used in drug discovery; questions have been raised about the efficiency of this approach given the very high attrition rates that these projects have historically shown in the anti-infective field [5,6]. Furthermore, the state of affairs is a symptom of wider inconsistencies between results obtained with animal models of infection and their translation to clinical therapeutic value in humans. Compounds identified in whole-cell screens fulfil a double function: (i) they provide lead structures for further optimization within the drug development progression sequence, and (ii) they can be exploited as tools to identify new

targets. Notably, cell-based hits already fulfil some important criteria, including permeability issues and, given the progression criteria in the high-throughput screen, higher activity against mycobacteria than mammalian cells. Thus, they provide suitable chemical and biological starting points.

The Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) was established by the National Institute of Allergy and Infectious Diseases (NIAID) in 1994 to allow researchers access to high quality screening services in order to encourage antituberculosis drug discovery research. Recently TAACF reported their anti-TB high-throughput results of large libraries of drug like small molecules [7-9], among them one of the molecule SID: 24823007 5-(furan-2-yl methylene)-2-imino-3-(thiazol-2-yl)thiazolidin-4-one showed good activity against *Mycobacterium tuberculosis* H37Rv with minimum inhibitory concentration of < 0.2 μM and selectivity index of > 115 (Figure 1).

We have taken SID: 24823007 as the starting point to design more analogues by keeping 2-imino thiazolidine-4-one nucleus intact and modify 3rd and 5th position with various aryl and heteroaryl moiety to study the structure activity relationship (SAR) of the lead compound.

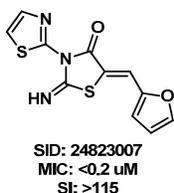


Figure 1. Antitubercular lead compound.

2. Experimental

2.1. Instrumentation

All the reagents obtained from commercial sources were used without further purification. All reactions were run under inert atmosphere of nitrogen or argon. All the reactions were monitored by thin layer chromatography (TLC) on silica gel 40 F₂₅₄ (Merck, Darmstadt, Germany) coated on aluminium plates. ¹H and ¹³C NMR spectra were recorded on a Bruker AM-300 or 400 NMR spectrometer, Bruker BioSpin Corp., Germany. Chemical shifts are in parts per million (ppm). In the Nuclear Magnetic resonance spectra (¹H NMR and ¹³C NMR), the signals of the respective protons of the prepared 2-iminothiazolidin-4-one derivatives were verified on the basis of their chemical shifts, multiplicities, and coupling constants. Temperatures are reported in degrees Celsius and are uncorrected. Compounds were analysed for C, H, N and analytical results obtained were within $\pm 0.4\%$ of the calculated values for the formula shown. Molecular weights of the synthesised compounds were checked by (Shimadzu, LC/MS-2020) ESI-MS method.

2.2. General procedure for synthesis of N-substituted chloroacetamides (2a-c)

Compound **1** (1.0 equiv.) and chloroacetyl chloride (1.0 equiv.) were taken in benzene and the reaction mixture was refluxed for 6 h. after completion of the reaction monitored by TLC, the reaction mixture was diluted with EtOAc and washed with sat NaHCO₃, H₂O and Brine. The combined organic layer was dried over anhydrous Na₂SO₄, evaporated to get compound **2**.

2-Chloro-N-(3-(trifluoromethyl)phenyl)acetamide (2a): 3-(Trifluoromethyl)aniline (3.0 g, 18.63 mmol) and chloroacetyl chloride (1.48 mL, 18.63 mmol) were taken in benzene (25 mL) and the reaction mixture was refluxed for 6 h. The reaction mixture was diluted with EtOAc and washed with sat. NaHCO₃ (3 \times 30 mL), H₂O (2 \times 30 mL) and Brine (2 \times 20 mL). The combined organic layer was dried over anhydrous Na₂SO₄, evaporated under vacuum to get solid product, the solids were washed with hexanes to get 2-chloro-N-(3-(trifluoromethyl)phenyl)acetamide. Color: Off-white solid. Yield: 3.60 g, 81%. ¹H NMR (400 MHz, CDCl₃, δ , ppm): 8.21 (s, 1H, Ar-H), 7.77 (s, 1H, NH), 7.54 (d, J = 8.4 Hz, 1H, Ar-H), 7.49 (d, J = 7.6 Hz, 1H, Ar-H), 7.36 (t, J = 7.6 Hz, 1H, Ar-H), 4.23 (s, 2H, CH₂). MS (EI, m/z (%)): 238 [M+H]⁺.

2-Chloro-N-(5-nitrothiazol-2-yl)acetamide (2b): 5-Nitrothiazol-2-amine (3.0 g, 20.80 mmol) and chloroacetyl chloride (1.64 mL, 20.80 mmol) were taken in benzene (25 mL) and the reaction mixture was refluxed for 6 h. The reaction mixture was diluted with EtOAc and washed with sat. NaHCO₃ (3 \times 30 mL), H₂O (2 \times 30 mL) and Brine (2 \times 20 mL). The combined organic layer was dried over anhydrous Na₂SO₄, evaporated under vacuum to get solid product, the solids were washed with hexanes to get 2-chloro-N-(5-nitrothiazol-2-yl)acetamide. Color: Off-white solid. Yield: 3.40 g, 74%. ¹H NMR (400 MHz, DMSO-*d*₆, δ , ppm): 9.43 (s, 1H, NH), 8.64 (s, 1H, Ar-H), 4.24 (s, 2H, CH₂). MS (EI, m/z (%)): 222 [M+H]⁺.

2-Chloro-N-(6-nitrobenzo[d]thiazol-2-yl)acetamide (2c): 6-Nitrobenzo[d]thiazol-2-amine (3.0 g, 15.38 mmol) and chloroacetyl chloride (1.21 mL, 15.38 mmol) were taken in

benzene (25 mL) and the reaction mixture was refluxed for 6 h. The reaction mixture was diluted with EtOAc and washed with sat. NaHCO₃ (3 \times 30 mL), H₂O (2 \times 30 mL) and Brine (2 \times 20 mL). The combined organic layer was dried over anhydrous Na₂SO₄, evaporated under vacuum to get solid product, the solids were washed with hexanes to get 2-chloro-N-(6-nitrobenzo[d]thiazol-2-yl)acetamide. Color: Off-white solid. Yield: 2.97 g, 71%. ¹H NMR (400 MHz, DMSO-*d*₆, δ , ppm): 10.03 (s, 1H, NH), 8.49 (s, 1H, Ar-H), 8.32 (d, J = 8.4 Hz, 1H, Ar-H), 8.22 (d, J = 8.0 Hz, 1H, Ar-H), 4.26 (s, 2H, CH₂). MS (EI, m/z (%)): 272 [M+H]⁺.

2.3. General procedure for synthesis of 3-substituted-2-iminothiazolidin-4-one (3a-c)

Compound **2a-c** (1.0 equiv) and KSCN (1.6 equiv) were taken in dry acetone and refluxed for 2 h. The reaction mixture was concentrated and obtained solid was washed with H₂O and dried in vacuum oven to get compound **3a-c**.

2-Imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (3a): 2-Chloro-N-(3-(trifluoromethyl)phenyl)acetamide (3.60 g, 15.18 mmoles) and KSCN (2.36 g, 24.30 mmoles) were taken in dry acetone and refluxed for 2 h. The reaction mixture was concentrated and obtained solid was washed with H₂O and dried in vacuum oven to get compound 2-imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one. Yield: 3.30 g, 83%. Color: Off-white solid. ¹H NMR (400 MHz, CDCl₃, δ , ppm): 9.12 (s, 1H, NH), 7.78 (s, 1H, Ar-H), 7.64 (d, J = 8.0 Hz, 1H, Ar-H), 7.54 (d, J = 7.6 Hz, 1H, Ar-H), 7.45 (t, J = 7.6 Hz, 1H, Ar-H), 4.32 (s, 2H, CH₂). MS (EI, m/z (%)): 261 [M+H]⁺.

2-Imino-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (3b): 2-Chloro-N-(5-nitrothiazol-2-yl)acetamide (3.40 g, 15.38 mmoles) and KSCN (2.40 g, 24.61 mmoles) were taken in dry acetone and refluxed for 2 h. The reaction mixture was concentrated and obtained solid was washed with H₂O and dried in vacuum oven to get compound 2-imino-3-(5-nitrothiazol-2-yl)thiazolidin-4-one. Yield: 3.15 g, 84%. Color: Off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆, δ , ppm): 9.97 (s, 1H, NH), 8.81 (s, 1H, Ar-H), 4.27 (s, 2H, CH₂). MS (EI, m/z (%)): 245 [M+H]⁺.

2-Imino-3-(6-nitrobenzo[d]thiazol-2-yl)thiazolidin-4-one (3c): 2-Chloro-N-(6-nitrobenzo[d]thiazol-2-yl)acetamide (2.97 g, 10.95 mmoles) and KSCN (1.71 g, 17.53 mmoles) were taken in dry acetone and refluxed for 2 h. The reaction mixture was concentrated and obtained solid was washed with H₂O and dried in vacuum oven to get compound 2-imino-3-(6-nitrobenzo[d]thiazol-2-yl)thiazolidin-4-one. Yield: 2.34 g, 72%. Color: Off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆, δ , ppm): 9.72 (s, 1H, NH), 8.53 (s, 1H, Ar-H), 8.34 (d, J = 8.4 Hz, 1H, Ar-H), 8.17 (d, J = 8.0 Hz, 1H, Ar-H), 4.20 (s, 2H, CH₂). MS (EI, m/z (%)): 295 [M+H]⁺.

2.4. General procedure for synthesis of compounds 4a-l, 5a-l and 6a-l

2-Imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (**3a**) (0.25 g, 0.96 mmol), NaOAc (0.15 g, 1.92 mmoles) and 2-hydroxybenzaldehyde (0.14 g, 1.15 mmol) were taken in acetic acid (2.0 mL) and heated at 100 °C for 3 hours, the solids formed in the reaction mixture were filtered and washed with water, little amount of ethanol and hexanes to afford title compound (0.38 g, 90%) as an off-white solid (**4a**).

5-(2-Hydroxybenzylidene)-2-imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (4a): Color: Brown. Yield: 90%. ¹H NMR (400 MHz, DMSO-*d*₆, δ , ppm): 11.51 (s, 1H, OH), 10.02 (s, 1H, NH), 8.30 (s, 1H, Ar-H), 7.81 (d, J = 8.0 Hz, 1H, Ar-H), 7.78-7.60 (m, 4H, Ar-H + CH), 7.54-7.36 (m, 3H, Ar-H). ¹³C NMR (100 MHz, DMSO-*d*₆, δ , ppm): 169.0, 153.4, 152.3, 144.9, 136.5, 133.2, 131.5, 128.4, 127.3, 127.0, 126.4, 125.7, 124.4, 123.6, 122.4, 121.3, 119.4. MS (EI, m/z (%)): 365 [M+H]⁺. Anal. calcd. for

$C_{17}H_{11}F_3N_2O_2S$: C, 56.04; H, 3.04; N, 7.69. Found: C, 56.12; H, 3.10; N, 7.72%.

5-(4-Hydroxybenzylidene)-2-imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (4b): Color: White. Yield: 88%. 1H NMR (400 MHz, DMSO- d_6 , δ , ppm): 12.91 (s, 1H, OH), 9.42 (s, 1H, NH), 8.34 (s, 1H, Ar-H), 7.99 (d, $J = 7.6$ Hz, 2H, Ar-H), 7.72-7.63 (m, 4H, Ar-H + CH), 7.27 (d, $J = 8.0$ Hz, 2H, Ar-H). ^{13}C NMR (100 MHz, DMSO- d_6 , δ , ppm): 166.8, 160.6, 152.4, 149.4, 134.5, 133.4, 130.5 (2C), 129.8, 129.1, 127.4, 126.3, 125.4, 124.7, 123.4 (2C), 119.7. MS (EI, m/z (%)): 365 [M+H] $^+$. Anal. calcd. for $C_{17}H_{11}F_3N_2O_2S$: C, 56.04; H, 3.04; N, 7.69. Found: C, 56.14; H, 3.10; N, 7.77%.

2-Imino-5-(4-methoxybenzylidene)-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (4c): Color: Brown. Yield: 79%. 1H NMR (300 MHz, δ , ppm): 12.40 (s, 1H, NH), 8.78-7.20 (m, 3H, Ar-H), 7.92-7.77 (m, 4H, Ar-H + CH), 7.69-7.54 (m, 2H, Ar-H), 4.05 (s, 3H, CH $_3$). ^{13}C NMR (75 MHz, DMSO- d_6 , δ , ppm): 163.5, 156.4, 149.4, 144.5, 136.4, 135.3, 133.4 (2C), 132.6, 130.5, 129.5, 127.6, 126.0, 125.4, 124.6, 123.3 (2C), 120.6. MS (EI, m/z (%)): 379 [M+H] $^+$. Anal. calcd. for $C_{18}H_{13}F_3N_2O_2S$: C, 57.14; H, 3.46; N, 7.40. Found: C, 57.21; H, 3.48; N, 7.43%.

5-(4-(Benzoyloxy)benzylidene)-2-imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (4d): Color: Brown. Yield: 93%. 1H NMR (300 MHz, CDCl $_3$, δ , ppm): 12.45 (s, 1H, NH), 8.56 (s, 1H, Ar-H), 8.12 (d, $J = 7.6$ Hz, 2H, Ar-H), 7.99-7.72 (m, 6H, Ar-H + CH), 7.63-7.51 (m, 5H, Ar-H), 4.99 (s, 2H, CH $_2$). ^{13}C NMR (75 MHz, CDCl $_3$, δ , ppm): 167.3, 161.1, 154.3, 148.7, 139.5, 138.4, 137.4, 136.3 (2C), 134.5, 133.2 (2C), 131.4, 130.4 (2C), 129.6, 128.4, 127.4, 126.3 (2C), 125.2, 123.4, 120.5, 69.3. MS (EI, m/z (%)): 455 [M+H] $^+$. Anal. calcd. for $C_{24}H_{17}F_3N_2O_2S$: C, 63.43; H, 3.77; N, 6.16. Found: C, 63.52; H, 3.87; N, 6.25%.

2-Imino-5-(4-methylbenzylidene)-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (4e): Color: White. Yield: 81%. 1H NMR (300 MHz, DMSO- d_6 , δ , ppm): 12.24 (s, 1H, NH), 8.34 (s, 1H, Ar-H), 7.89 (d, $J = 7.6$ Hz, 2H, Ar-H), 7.81-7.72 (m, 6H, Ar-H + CH), 2.52 (s, 3H, CH $_3$). ^{13}C NMR (75 MHz, DMSO- d_6 , δ , ppm): 164.3, 159.4, 155.3, 152.4, 141.3, 139.5, 138.4, 136.4, 135.4 (2C), 133.2, 130.4 (2C), 127.4, 126.4, 125.3, 122.4, 22.5. MS (EI, m/z (%)): 363 [M+H] $^+$. Anal. calcd. for $C_{18}H_{13}F_3N_2O_2S$: C, 59.66; H, 3.62; N, 7.73. Found: C, 59.73; H, 3.67; N, 7.87%.

5-(4-(Dimethylamino)benzylidene)-2-imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (4f): Color: Brown. Yield: 84%. 1H NMR (400 MHz, CDCl $_3$, δ , ppm): 9.99 (s, 1H, NH), 8.28 (s, 1H, Ar-H), 7.97 (d, $J = 7.6$ Hz, 2H, Ar-H), 7.82-7.74 (m, 2H, Ar-H + CH), 7.63-7.45 (m, 4H, Ar-H), 3.15 (s, 6H, 2CH $_3$). ^{13}C NMR (100 MHz, CDCl $_3$, δ , ppm): 164.4, 154.4, 152.6, 143.4, 136.4, 134.5, 133.6 (2C), 132.9, 132.1, 131.3 (2C), 127.5, 126.4, 124.6, 123.4, 120.4, 43.2 (2C). MS (EI, m/z (%)): 392 [M+H] $^+$. Anal. calcd. for $C_{19}H_{16}F_3N_3O_2S$: C, 58.30; H, 4.12; N, 10.74. Found: C, 58.41; H, 4.18; N, 10.83%.

2-Imino-3-(3-(trifluoromethyl)phenyl)-5-(3,4,5-trimethoxybenzylidene)thiazolidin-4-one (4g): Color: White. Yield: 89%. 1H NMR (400 MHz, CDCl $_3$, δ , ppm): 12.60 (s, 1H, NH), 8.49 (s, 1H, Ar-H), 8.10-8.01 (m, 2H, Ar-H), 7.99 (s, 1H, CH), 7.63 (d, $J = 7.2$ Hz, 1H, Ar-H), 7.49 (s, 2H, Ar-H), 4.12 (s, 9H, 3CH $_3$). ^{13}C NMR (100 MHz, CDCl $_3$, δ , ppm): 168.3, 162.4 (2C), 161.1, 152.4, 149.6, 142.3, 138.5, 137.4, 135.9, 133.0, 131.4, 128.4, 126.2, 124.2, 123.4 (2C), 63.4, 61.2 (2C). MS (EI, m/z (%)): 439 [M+H] $^+$. Anal. calcd. for $C_{20}H_{17}F_3N_2O_4S$: C, 54.79; H, 3.91; N, 6.39. Found: C, 54.82; H, 3.98; N, 6.36%.

5-(2-Fluorobenzylidene)-2-imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (4h): Color: Light Yellow. Yield: 80%. 1H NMR (400 MHz, DMSO- d_6 , δ , ppm): 12.42 (s, 1H, NH), 7.99 (s, 1H, Ar-H), 7.92 (s, 1H, CH), 7.81-7.74 (m, 2H, Ar-H), 7.63 (d, $J = 6.8$ Hz, 1H, Ar-H), 7.58-7.49 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, DMSO- d_6 , δ , ppm): 167.2, 162.2, 154.3, 149.6, 135.3, 134.6, 133.2, 129.2, 126.3, 126.0, 125.2, 123.7, 123.2, 122.6, 121.4, 120.3, 119.9. MS (EI, m/z (%)): 367 [M+H] $^+$. Anal. calcd. for $C_{17}H_{10}F_4N_2O_2S$: C, 55.74; H, 2.75; N, 7.65. Found: C, 55.81; H, 2.81; N, 7.74%.

5-(4-Chlorobenzylidene)-2-imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (4i): Color: Light Yellow. Yield: 79%. 1H NMR (400 MHz, DMSO- d_6 , δ , ppm): 12.88 (s, 1H, NH), 8.21 (s, 1H, Ar-H), 7.81 (d, $J = 7.2$ Hz, 2H, Ar-H), 7.77 (d, $J = 6.8$ Hz, 1H, Ar-H), 7.72-7.68 (m, 3H, Ar-H + CH), 7.35 (d, $J = 8.0$ Hz, 2H, Ar-H). ^{13}C NMR (100 MHz, DMSO- d_6 , δ , ppm): 167.2, 163.3, 155.2, 148.9, 136.8, 136.2, 135.3 (2C), 133.8, 130.4, 129.4, 127.2, 126.4, 124.7 (2C), 123.1, 120.6. MS (EI, m/z (%)): 383 [M+H] $^+$. Anal. calcd. for $C_{17}H_{10}ClF_3N_2O_2S$: C, 53.34; H, 2.63; N, 7.32. Found: C, 53.37; H, 2.72; N, 7.41%.

2-Imino-5-(3-nitrobenzylidene)-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (4j): Color: Yellow. Yield: 76%. 1H NMR (400 MHz, DMSO- d_6): 12.94 (s, 1H, NH), 8.34 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 7.98 (d, $J = 7.6$ Hz, 1H, Ar-H), 7.81-7.74 (m, 3H, Ar-H + CH), 7.63-7.54 (m, 3H, Ar-H). ^{13}C NMR (100 MHz, DMSO- d_6 , δ , ppm): 164.6, 162.2, 151.2, 146.4, 137.8, 136.3, 134.2, 133.8, 132.6, 131.6, 130.4, 128.2, 126.7, 125.1, 124.2, 123.2, 121.5. MS (EI, m/z (%)): 394 [M+H] $^+$. Anal. calcd. for $C_{17}H_{10}F_3N_3O_3S$: C, 51.91; H, 2.56; N, 10.68. Found: C, 51.94; H, 2.62; N, 10.73%.

2-Imino-5-((5-nitrofuranyl)methylene)-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (4k): Color: Yellow. Yield: 92%. 1H NMR (400 MHz, CDCl $_3$, δ , ppm): 12.63 (s, 1H, NH), 8.31 (d, $J = 7.2$ Hz, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 7.92 (d, $J = 7.2$ Hz, 1H, Ar-H), 7.81-7.72 (m, 2H, Ar-H + CH), 7.58 (d, $J = 7.2$ Hz, 1H, Ar-H), 7.54 (t, $J = 7.2$ Hz, 1H, Ar-H). ^{13}C NMR (100 MHz, CDCl $_3$, δ , ppm): 168.1, 160.2, 153.2, 152.1, 149.3, 136.3, 135.3, 134.9, 133.9, 131.4, 127.2, 126.4, 124.2, 122.1, 120.5. MS (EI, m/z (%)): 384 [M+H] $^+$. Anal. calcd. for $C_{15}H_8F_3N_3O_4S$: C, 47.00; H, 2.10; N, 10.96. Found: C, 47.11; H, 2.13; N, 10.98%.

2-Imino-5-((5-nitrothiophen-2-yl)methylene)-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (4l): Color: Light Yellow. Yield: 72%. 1H NMR (400 MHz, CDCl $_3$, δ , ppm): 12.51 (s, 1H, NH), 8.29 (d, $J = 7.2$ Hz, 1H, Ar-H), 8.19 (s, 1H, Ar-H), 7.89 (d, $J = 7.2$ Hz, 1H, Ar-H), 7.79-7.69 (m, 2H, Ar-H + CH), 7.63 (d, $J = 7.2$ Hz, 1H, Ar-H), 7.58 (t, $J = 7.2$ Hz, 1H, Ar-H). ^{13}C NMR (100 MHz, CDCl $_3$, δ , ppm): 166.9, 161.1, 152.3, 150.3, 148.2, 135.5, 134.1, 133.4, 132.9, 130.2, 128.4, 126.2, 122.3, 120.3, 118.8. MS (EI, m/z (%)): 400 [M+H] $^+$. Anal. calcd. for $C_{15}H_8F_3N_3O_3S_2$: C, 45.11; H, 2.02; N, 10.52. Found: C, 45.19; H, 2.10; N, 10.59%.

5-(2-Hydroxybenzylidene)-2-imino-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (5a): Color: White. Yield: 88%. 1H NMR (400 MHz, DMSO- d_6 , δ , ppm): 12.42 (s, 1H, OH), 9.70 (s, 1H, NH), 8.31 (s, 1H, Ar-H), 7.92 (d, $J = 7.2$ Hz, 1H, Ar-H), 7.81 (s, 1H, CH), 7.72-7.69 (m, 2H, Ar-H), 7.63 (t, $J = 7.6$ Hz, 1H, Ar-H). ^{13}C NMR (100 MHz, DMSO- d_6 , δ , ppm): 172.6, 163.4, 156.4, 149.4, 137.4, 131.4, 128.5, 126.4, 125.2, 123.8, 121.4, 120.4, 119.3. MS (EI, m/z (%)): 349 [M+H] $^+$. Anal. calcd. for $C_{13}H_8N_4O_4S_2$: C, 44.82; H, 2.31; N, 16.08. Found: C, 44.92; H, 2.41; N, 16.12%.

5-(4-Hydroxybenzylidene)-2-imino-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (5b): Color: White. Yield: 79%. 1H NMR (400 MHz, DMSO- d_6 , δ , ppm): 12.78 (s, 1H, OH), 9.21 (s, 1H, NH), 8.31 (s, 1H, Ar-H), 7.92 (s, 1H, CH), 7.63 (d, $J = 7.6$ Hz, 2H, Ar-H), 7.32 (d, $J = 8.0$ Hz, 2H, Ar-H). ^{13}C NMR (100 MHz, DMSO- d_6 , δ , ppm): 169.9, 163.6, 154.9, 148.5, 134.5, 132.4, 129.5 (2C), 127.8, 124.9, 123.1 (2C), 121.5. MS (EI, m/z (%)): 349 [M+H] $^+$. Anal. calcd. for $C_{13}H_8N_4O_4S_2$: C, 44.82; H, 2.31; N, 16.08. Found: C, 44.94; H, 2.34; N, 16.18%.

2-Imino-5-(4-methoxybenzylidene)-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (5c): Color: Light Yellow. Yield: 94%. 1H NMR (300 MHz, DMSO- d_6 , δ , ppm): 13.12 (s, 1H, NH), 8.31 (s, 1H, Ar-H), 7.69 (s, 1H, CH), 7.65 (d, $J = 7.5$ Hz, 2H, Ar-H), 7.29 (d, $J = 7.2$ Hz, 2H, Ar-H), 3.99 (s, 3H, CH $_3$). ^{13}C NMR (75 MHz, DMSO- d_6 , δ , ppm): 171.9, 166.6, 156.2, 150.5, 141.5, 136.2, 132.5 (2C), 128.3, 126.3, 122.4 (2C), 120.6, 60.3. MS (EI, m/z (%)): 363 [M+H] $^+$. Anal. calcd. for $C_{14}H_{10}N_4O_4S_2$: C, 46.40; H, 2.78; N, 15.46. Found: C, 46.51; H, 2.81; N, 15.52%.

5-(4-(Benzoyloxy)benzylidene)-2-imino-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (5d): Color: Brown. Yield: 93%. 1H NMR (300 MHz, CDCl $_3$, δ , ppm): 13.12 (s, 1H, NH), 8.53 (s, 1H, Ar-H), 7.81 (s, 1H, CH), 7.72 (d, $J = 7.2$ Hz, 2H, Ar-H), 7.67-7.54 (m, 7H, Ar-

H), 5.22 (s, 2H, CH₂). ¹³C NMR (75 MHz, CDCl₃, δ, ppm): 169.6, 162.6, 154.7, 144.9, 139.6, 136.6, 134.2, 133.2 (2C), 132.5, 132.0 (2C), 130.5, 128.2 (2C), 126.2, 124.8 (2C), 120.6, 70.2. MS (EI, *m/z* (%)): 439 [M+H]⁺. Anal. calcd. for C₂₀H₁₄N₄O₄S₂: C, 54.78; H, 3.22; N, 12.78. Found: C, 54.85; H, 3.26; N, 12.81%.

2-Imino-5-(4-methylbenzylidene)-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (5e): Color: Light Yellow. Yield: 89%. ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): 12.54 (s, 1H, NH), 8.41 (s, 1H, Ar-H), 7.71 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.67 (s, 1H, CH), 7.58 (d, *J* = 7.2 Hz, 2H, Ar-H), 2.49 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃, δ, ppm): 172.1, 166.4, 152.5, 149.4, 138.4, 136.3, 135.2 (2C), 134.2, 131.4 (2C), 126.2, 122.1, 22.3. MS (EI, *m/z* (%)): 347 [M+H]⁺. Anal. calcd. for C₁₄H₁₀N₄O₃S₂: C, 48.54; H, 2.91; N, 16.17. Found: C, 48.61; H, 2.93; N, 16.21%.

5-(4-(Dimethylamino)benzylidene)-2-imino-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (5f): Color: Yellow. Yield: 76%. ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): 12.87 (s, 1H, NH), 8.35 (s, 1H, Ar-H), 7.72 (s, 1H, CH), 7.63 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.54 (d, *J* = 8.0 Hz, 2H, Ar-H), 3.15 (s, 6H, 2CH₃). ¹³C NMR (100 MHz, DMSO-*d*₆, δ, ppm): 169.9, 164.1, 153.3, 149.4, 141.4, 136.4, 133.2 (2C), 130.9, 128.8, 126.3 (2C), 125.5, 44.4 (2C). MS (EI, *m/z* (%)): 376 [M+H]⁺. Anal. calcd. for C₁₅H₁₃N₅O₃S₂: C, 47.99; H, 3.49; N, 18.65. Found: C, 47.97; H, 3.58; N, 18.71%.

2-Imino-3-(5-nitrothiazol-2-yl)-5-(3,4,5-trimethoxybenzylidene)thiazolidin-4-one (5g): Color: Yellow. Yield: 88%. ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): 13.23 (s, 1H, NH), 8.45 (s, 1H, Ar-H), 7.72 (s, 1H, CH), 7.11 (s, 2H, Ar-H), 3.90 (s, 9H, 3CH₃). ¹³C NMR (100 MHz, DMSO-*d*₆, δ, ppm): 171.7, 166.2, 152.1 (2C), 148.2, 146.3, 142.3, 137.2, 135.9, 132.6, 130.3, 126.4 (2C), 61.3, 60.6 (2C). MS (EI, *m/z* (%)): 423 [M+H]⁺. Anal. calcd. for C₁₆H₁₄N₄O₆S₂: C, 45.49; H, 3.34; N, 13.26. Found: C, 45.52; H, 3.41; N, 13.32%.

5-(2-Fluorobenzylidene)-2-imino-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (5h): Color: Light Yellow. Yield: 78%. ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): 11.91 (s, 1H, NH), 8.31 (s, 1H, Ar-H), 8.18 (s, 1H, CH), 7.94 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.81-7.63 (m, 3H, Ar-H). ¹³C NMR (100 MHz, DMSO-*d*₆, δ, ppm): 168.3, 163.5, 156.1, 146.2, 134.8, 134.2, 133.5, 132.9, 128.4, 126.9, 124.4, 120.3, 119.4. MS (EI, *m/z* (%)): 350 [M+H]⁺. Anal. calcd. for C₁₃H₇FN₄O₃S₂: C, 44.57; H, 2.01; N, 15.99. Found: C, 44.61; H, 1.98; N, 16.01%.

5-(4-Chlorobenzylidene)-2-imino-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (5i): Color: Brown. Yield: 81%. ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): 13.02 (s, 1H, NH), 8.41 (s, 1H, Ar-H), 7.81 (s, 1H, CH), 7.72 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.56 (d, *J* = 8.0 Hz, 2H, Ar-H). ¹³C NMR (100 MHz, DMSO-*d*₆, δ, ppm): 166.9, 160.2, 152.7, 146.8, 137.4, 134.8, 133.3(2C), 132.3, 128.4(2C), 126.2, 121.5. MS (EI, *m/z* (%)): 367 [M+H]⁺. Anal. calcd. for C₁₃H₇ClN₄O₃S₂: C, 42.57; H, 1.92; N, 15.27. Found: C, 42.63; H, 1.99; N, 15.32%.

2-Imino-5-(3-nitrobenzylidene)-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (5j): Color: Yellow. Yield: 88%. ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): δ 13.11 (s, 1H, NH), 8.32 (s, 1H, Ar-H), 8.27 (s, 1H, CH), 7.94-7.76 (m, 3H, Ar-H), 7.62 (t, *J* = 7.6 Hz, 1H, Ar-H). ¹³C NMR (100 MHz, DMSO-*d*₆, δ, ppm): 170.1, 163.2, 152.2, 147.3, 138.1, 136.2, 135.0, 133.3, 132.4, 127.3, 125.9, 123.3, 121.6. MS (EI, *m/z* (%)): 378 [M+H]⁺. Anal. calcd. for C₁₃H₇N₅O₅S₂: C, 41.38; H, 1.87; N, 18.56. Found: C, 41.41; H, 1.96; N, 18.61%.

2-Imino-5-((5-nitrofuran-2-yl)methylene)-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (5k): Color: Brown. Yield: 69%. ¹H NMR (400 MHz, CDCl₃, δ, ppm): 13.21 (s, 1H, NH), 8.49 (s, 1H, Ar-H), 8.39 (d, *J* = 6.8 Hz, 1H, Ar-H), 8.21 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.99 (s, 1H, CH). ¹³C NMR (100 MHz, CDCl₃, δ, ppm): 172.1, 163.3, 152.8, 150.8, 143.2, 142.4, 136.3, 133.9, 126.8, 124.2, 123.6. MS (EI, *m/z* (%)): 368 [M+H]⁺. Anal. calcd. for C₁₁H₅N₅O₆S₂: C, 35.97; H, 1.37; N, 19.07. Found: C, 36.02; H, 1.39; N, 19.19%.

2-Imino-3-(5-nitrothiazol-2-yl)-5-((5-nitrothiophen-2-yl)methylene)thiazolidin-4-one (5l): Color: Brown. Yield: 76%. ¹H NMR (400 MHz, CDCl₃, δ, ppm): 13.14 (s, 1H, NH), 8.53 (s, 1H, Ar-H), 8.34 (d, *J* = 6.8 Hz, 1H, Ar-H), 8.17 (d, *J* = 7.2 Hz, 1H, Ar-

H), 7.82 (s, 1H, CH). ¹³C NMR (100 MHz, CDCl₃, δ, ppm): 169.4, 161.3, 151.4, 150.3, 142.8, 141.3, 135.2, 132.7, 125.9, 123.9, 122.4. MS (EI, *m/z* (%)): 384 [M+H]⁺. Anal. calcd. for C₁₁H₅N₅O₅S₃: C, 34.46; H, 1.31; N, 18.27. Found: C, 34.52; H, 1.32; N, 18.29%.

5-(2-Hydroxybenzylidene)-2-imino-3-(6-nitrobenzo[d]thiazol-2-yl)thiazolidin-4-one (6a): Color: Light Yellow. Yield: 83%. ¹H NMR (400 MHz, CDCl₃, δ, ppm): 12.60 (s, 1H, OH), 9.49 (s, 1H, NH), 8.31 (d, *J* = 7.2 Hz, 1H, Ar-H), 8.12 (s, 1H, CH), 8.01 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.81-7.74 (m, 4H, Ar-H), 7.63 (d, *J* = 8.0 Hz, 1H, Ar-H). ¹³C NMR (100 MHz, DMSO-*d*₆, δ, ppm): 169.6, 161.1, 155.3, 148.8, 138.0, 136.1, 132.2, 130.5, 128.1, 127.4, 126.3, 125.8, 125.0, 124.0, 122.1, 120.6, 120.0. MS (EI, *m/z* (%)): 399 [M+H]⁺. Anal. calcd. for C₁₇H₁₀N₄O₄S₂: C, 51.25; H, 2.53; N, 14.06. Found: C, 51.30; H, 2.65; N, 14.16%.

5-(4-Hydroxybenzylidene)-2-imino-3-(6-nitrobenzo[d]thiazol-2-yl)thiazolidin-4-one (6b): Color: Light Yellow. Yield: 81%. ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): 13.05 (s, 1H, OH), 9.36 (s, 1H, NH), 8.21 (d, *J* = 7.2 Hz, 1H, Ar-H), 8.18 (s, 1H, Ar-H), 8.01 (d, *J* = 6.8 Hz, 1H, Ar-H), 7.92 (s, 1H, CH), 7.77 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.54 (d, *J* = 8.0 Hz, 2H, Ar-H). ¹³C NMR (100 MHz, DMSO-*d*₆, δ, ppm): 166.4, 162.6, 157.6, 153.7, 148.4, 143.4, 137.5, 133.4 (2C), 130.6, 129.3, 127.2, 125.6, 124.4 (2C), 122.4, 120.6. MS (EI, *m/z* (%)): 399 [M+H]⁺. Anal. calcd. for C₁₇H₁₀N₄O₄S₂: C, 51.25; H, 2.53; N, 14.06. Found: C, 51.29; H, 2.62; N, 14.12%.

2-Imino-5-(4-methoxybenzylidene)-3-(6-nitrobenzo[d]thiazol-2-yl)thiazolidin-4-one (6c): Color: Brown. Yield: 84%. ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): 12.98 (s, 1H, NH), 8.47 (s, 1H, Ar-H), 8.39 (d, *J* = 6.8 Hz, 1H, Ar-H), 8.29 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.87 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.83 (s, 1H, CH), 7.72 (d, *J* = 7.2 Hz, 2H, Ar-H), 3.94 (s, 3H, CH₃). ¹³C NMR (100 MHz, DMSO-*d*₆, δ, ppm): 165.3, 160.6, 156.5, 152.4, 144.3, 142.4, 139.3, 132.9 (2C), 130.4, 128.6, 126.9, 126.0, 123.9 (2C), 122.4, 120.3, 60.1. MS (EI, *m/z* (%)): 413 [M+H]⁺. Anal. calcd. for C₁₈H₁₂N₄O₄S₂: C, 52.42; H, 2.93; N, 13.58. Found: C, 52.49; H, 3.00; N, 13.67%.

5-(4-(Benzyloxy)benzylidene)-2-imino-3-(6-nitrobenzo[d]thiazol-2-yl)thiazolidin-4-one (6d): Color: Brown. Yield: 91%. ¹H NMR (400 MHz, CDCl₃, δ, ppm): 12.42 (s, 1H, NH), 8.61 (s, 1H, Ar-H), 8.32 (d, *J* = 7.2 Hz, 1H, Ar-H), 8.10 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.99 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.87-7.69 (m, 6H, Ar-H + CH), 7.63-7.58 (m, 2H, Ar-H), 5.15 (s, 2H, CH₂). ¹³C NMR (75 MHz, CDCl₃, δ, ppm): 168.7, 163.0, 155.4, 145.4, 141.6, 139.2, 138.3, 136.4, 135.3, 134.2, 133.8(2C), 132.6, 131.8 (2C), 131.2, 130.7, 127.4 (2C), 125.9, 125.2 (2C), 121.5, 71.1. MS (EI, *m/z* (%)): 489 [M+H]⁺. Anal. calcd. for C₂₄H₁₆N₄O₄S₂: C, 59.00; H, 3.30; N, 11.47. Found: C, 59.12; H, 3.34; N, 11.52%.

2-Imino-5-(4-methylbenzylidene)-3-(6-nitrobenzo[d]thiazol-2-yl)thiazolidin-4-one (6e): Color: Yellow. Yield: 88%. ¹H NMR (400 MHz, CDCl₃, δ, ppm): 12.33 (s, 1H, NH), 8.32 (s, 1H, Ar-H), 8.21 (d, *J* = 7.2 Hz, 1H, Ar-H), 8.01 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.72 (s, 1H, CH), 7.63 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.56 (d, *J* = 7.2 Hz, 2H, Ar-H), 2.43 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃, δ, ppm): 166.3, 161.8, 153.3, 149.6, 141.3, 139.2, 135.6(2C), 134.1 (2C), 133.2, 131.5, 128.6, 127.2, 126.2, 124.6, 123.4, 21.9. MS (EI, *m/z* (%)): 397 [M+H]⁺. Anal. calcd. for C₁₈H₁₂N₄O₃S₂: C, 54.53; H, 3.05; N, 14.13. Found: C, 54.61; H, 3.09; N, 14.21%.

5-(4-(Dimethylamino)benzylidene)-2-imino-3-(6-nitrobenzo[d]thiazol-2-yl)thiazolidin-4-one (6f): Color: Yellow. Yield: 72%. ¹H NMR (400 MHz, CDCl₃, δ, ppm): 12.40 (s, 1H, NH), 8.34 (s, 1H, Ar-H), 8.19 (d, *J* = 7.2 Hz, 1H, Ar-H), 8.01 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.72 (s, 1H, CH), 7.69-7.63 (m, 2H, Ar-H), 7.54 (d, *J* = 8.0 Hz, 2H, Ar-H), 3.12 (s, 6H, 2CH₃). ¹³C NMR (100 MHz, DMSO-*d*₆, δ, ppm): 168.7, 163.2, 156.4, 149.4, 144.5, 143.4, 138.6, 136.2, 134.0 (2C), 133.4, 130.4, 127.9, 126.8 (2C), 125.1, 123.4, 44.1 (2C). MS (EI, *m/z* (%)): 426 [M+H]⁺. Anal. calcd. for C₁₉H₁₅N₅O₃S₂: C, 53.63; H, 3.55; N, 16.46. Found: C, 53.69; H, 3.62; N, 16.51%.

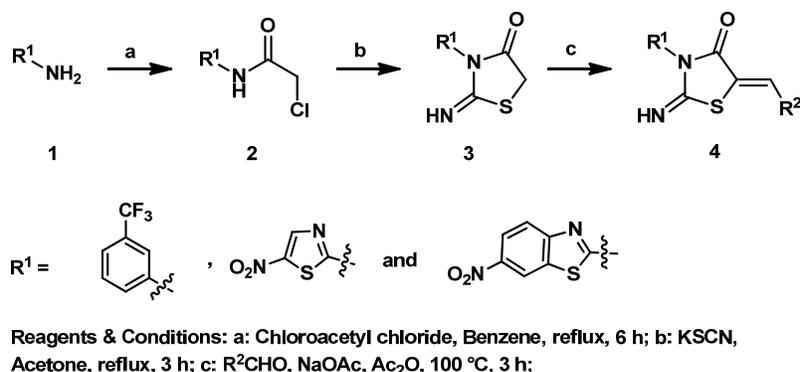


Figure 2. General synthetic procedure.

2-Imino-3-(6-nitrobenzo[d]thiazol-2-yl)-5-(3,4,5-trimethoxybenzylidene)thiazolidin-4-one (6g): Color: Light Yellow. Yield: 83%. ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): 13.03 (s, 1H, NH), 8.55 (s, 1H, Ar-H), 8.44 (d, *J* = 7.2 Hz, 1H, Ar-H), 8.26 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.72 (s, 1H, CH), 7.54 (s, 2H, Ar-H), 3.96 (s, 9H, 3CH₃). ¹³C NMR (100 MHz, DMSO-*d*₆, δ, ppm): 168.4, 162.3, 154.8, 154.2 (2C), 146.2, 145.6, 138.7, 136.9, 135.6, 134.5, 133.2, 132.6, 131.3, 130.6, 126.2 (2C), 61.6, 61.2 (2C). MS (EI, *m/z* (%)): 473 [M+H]⁺. Anal. calcd. for C₂₀H₁₆N₄O₆S₂: C, 50.84; H, 3.41; N, 11.86. Found: C, 50.92; H, 3.45; N, 11.92%.

5-(2-Fluorobenzylidene)-2-imino-3-(6-nitrobenzo[d]thiazol-2-yl)thiazolidin-4-one (6h): Color: Brown. Yield: 79%. ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): 10.43 (s, 1H, NH), 8.44 (s, 1H, Ar-H), 8.31 (d, *J* = 7.2 Hz, 1H, Ar-H), 8.22 (d, *J* = 6.8 Hz, 1H, Ar-H), 7.89 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.81-7.69 (m, 3H, Ar-H), 7.65 (d, *J* = 7.6 Hz, 1H, Ar-H). ¹³C NMR (100 MHz, DMSO-*d*₆, δ, ppm): 166.5, 160.6, 153.3, 147.4, 137.4, 136.4, 134.9, 132.5, 131.3, 129.6, 127.4, 126.7, 124.6, 123.3, 122.4, 121.3, 120.6. MS (EI, *m/z* (%)): 401 [M+H]⁺. Anal. calcd. for C₁₇H₉FN₄O₃S₂: C, 50.99; H, 2.27; N, 13.99. Found: C, 51.10; H, 2.29; N, 14.06%.

5-(4-Chlorobenzylidene)-2-imino-3-(6-nitrobenzo[d]thiazol-2-yl)thiazolidin-4-one (6i): Color: Light Yellow. Yield: 72%. ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): 12.79 (s, 1H, NH), 8.24 (d, *J* = 7.2 Hz, 1H, Ar-H), 8.12 (d, *J* = 6.8 Hz, 1H, Ar-H), 7.99 (s, 1H, Ar-H), 7.81 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.79 (s, 1H, CH), 7.77 (d, *J* = 8.0 Hz, 2H, Ar-H). ¹³C NMR (100 MHz, DMSO-*d*₆, δ, ppm): 165.3, 158.8, 154.4, 146.2, 139.3, 136.9, 135.1, 133.4, 132.8 (2C), 129.1 (2C), 127.4, 125.1, 123.7, 121.4, 120.0. MS (EI, *m/z* (%)): 417 [M+H]⁺. Anal. calcd. for C₁₇H₉ClN₄O₃S₂: C, 48.98; H, 2.18; N, 13.44. Found: C, 49.03; H, 2.23; N, 13.53%.

2-Imino-3-(6-nitrobenzo[d]thiazol-2-yl)-5-(3-nitrobenzylidene)thiazolidin-4-one (6j): Color: Yellow. Yield: 84%. ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): 12.75 (s, 1H, NH), 8.31 (s, 1H, Ar-H), 8.16 (d, *J* = 7.2 Hz, 1H, Ar-H), 8.11 (s, 1H, Ar-H), 7.92 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.82 (s, 1H, CH), 7.72-7.62 (m, 3H, Ar-H). ¹³C NMR (100 MHz, DMSO-*d*₆, δ, ppm): 167.1, 161.4, 156.2, 147.2, 142.4, 139.4, 138.1, 136.2, 134.5, 133.0, 132.4, 128.0, 126.0, 124.1, 123.6, 121.6, 120.4. MS (EI, *m/z* (%)): 428 [M+H]⁺. Anal. calcd. for C₁₇H₉N₅O₅S₂: C, 47.77; H, 2.12; N, 16.39. Found: C, 47.81; H, 2.16; N, 16.44%.

2-Imino-3-(6-nitrobenzo[d]thiazol-2-yl)-5-((5-nitrofuran-2-yl)methylene)thiazolidin-4-one (6k): Color: Brown. Yield: 70%. ¹H NMR (400 MHz, CDCl₃, δ, ppm): 12.33 (s, 1H, NH), 8.31 (d, *J* = 7.2 Hz, 1H, Ar-H), 8.21 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.08 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.99 (s, 1H, CH), 7.91 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.80 (s, 1H, Ar-H). ¹³C NMR (100 MHz, CDCl₃, δ, ppm): 170.2, 161.1, 156.2, 152.4, 144.6, 140.4, 138.3, 136.5, 134.8, 133.3, 129.4, 126.4, 124.6, 123.6, 121.5. MS (EI, *m/z* (%)): 418 [M+H]⁺. Anal. calcd. for C₁₅H₇N₅O₆S₂: C, 43.17; H, 1.69; N, 16.78. Found: C, 43.22; H, 1.72; N, 16.82%.

2-Imino-3-(6-nitrobenzo[d]thiazol-2-yl)-5-((5-nitrothiophen-2-yl)methylene)thiazolidin-4-one (6l): Color: Yellow. Yield: 72%. ¹H NMR (400 MHz, CDCl₃, δ, ppm): 12.44 (s, 1H, NH), 8.29 (d, *J* = 7.2 Hz, 1H, Ar-H), 8.17 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.01 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.99 (s, 1H, CH), 7.89 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.72 (s, 1H, Ar-H). ¹³C NMR (100 MHz, CDCl₃, δ, ppm): 171.3, 160.4, 156.6, 153.0, 145.3, 142.0, 139.1, 137.2, 134.7, 133.3, 128.9, 127.1, 125.2, 124.2, 121.9. MS (EI, *m/z* (%)): 434 [M+H]⁺. Anal. calcd. for C₁₅H₇N₅O₅S₃: C, 41.57; H, 1.63; N, 16.16. Found: C, 41.62; H, 1.69; N, 16.24%.

2.5. In vitro MTB screening

Two-fold serial dilutions of each test compound/drug were prepared and incorporated into Middle-Brook 7H11 agar medium with oleic acid, albumin, dextrose, and catalase (OADC) growth supplement to get final concentrations of 50, 25, 12.5, 6.25, 3.13, 1.56, and 0.78 μg/mL. Inoculum of *M. tuberculosis* H37Rv ATCC 27294 was prepared from fresh Middlebrook 7H11 agar slants with OADC (Difco) growth supplement adjusted to 1 mg/mL (wet weight) in Tween 80 (0.05%) saline diluted to 10⁻² to give a concentration of ~10⁷ cfu/mL. Five microliters of this bacterial suspension was spotted onto 7H11 agar tubes containing different concentrations of the drug as discussed above [10]. The tubes were incubated at 37 °C, and final readings (as MIC in μg/mL) were determined after 28 days. The MIC is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth. This method is similar to that recommended by the National Committee for Clinical Laboratory Standards for the determination of MIC in triplicate.

Susceptibility testing in the presence of the efflux inhibitors verapamil was carried out by adding the respective inhibitor at sub inhibitory concentrations (0.25 × MIC) to the MTB cultures in the assay. The MICs of verapamil > 200 μg (taken as 200 μg).

2.6. In vitro cytotoxicity screening

Some compounds were further examined for toxicity in a RAW 264.7 cell line at the concentration of 50 μM. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay.

3. Results and discussion

3.1. Chemistry

The target molecules were synthesised by following the three step synthetic protocol (Figure 2).

Table 1. Biological activities of synthesized compounds.

Compound	R ²	Yield (%)	M.p. (°C)	C log P	MTB MIC (μM)	MTB MIC (μM) (in presence of verapamil)	Cytotoxicity at 100 μM (RAW 264.7 cells) % Inhibition
4a	2-Hydroxyphenyl	90	208-209	4.64	65.24	ND*	ND
4b	4-Hydroxyphenyl	88	221-222	4.64	16.31	ND	22.60
4c	4-Methoxyphenyl	79	189-190	5.22	4.12	2.06	17.82
4d	4-Benzyloxyphenyl	93	218-220	6.99	6.88	6.88	19.04
4e	4-Methylphenyl	81	205-207	5.80	138.12	ND	ND
4f	4-(Dimethylamino)phenyl	84	234-235	5.47	110.13	ND	ND
4g	3,4,5-Trimethoxyphenyl	89	208-209	4.61	7.11	1.77	21.64
4h	2-Fluorophenyl	80	201-203	5.45	4.25	1.06	22.80
4i	4-Chlorophenyl	79	209-211	6.02	130.54	ND	ND
4j	3-Nitrophenyl	76	180-181	5.05	63.45	ND	ND
4k	5-Nitrofuran-2-yl	92	231-232	4.22	4.07	4.07	26.72
4l	5-Nitrothiophen-2-yl	72	241-243	4.78	12.53	ND	24.62
5a	2-Hydroxyphenyl	88	218-220	1.99	117.3	ND	12.86
5b	4-Hydroxyphenyl	79	213-214	1.99	39.1	ND	18.98
5c	4-Methoxyphenyl	84	204-205	2.58	3.78	1.89	14.56
5d	4-Benzyloxyphenyl	93	241-243	4.35	6.40	1.60	16.40
5e	4-Methylphenyl	89	260-262	3.16	63.12	ND	ND
5f	4-(Dimethylamino)phenyl	76	290-291	2.82	118.48	ND	ND
5g	3,4,5-Trimethoxyphenyl	88	207-208	1.96	3.31	0.82	12.40
5h	2-Fluorophenyl	78	236-238	2.80	3.90	3.90	16.86
5i	4-Chlorophenyl	81	222-223	3.37	59.95	ND	ND
5j	3-Nitrophenyl	88	196-198	2.41	14.63	ND	20.62
5k	5-Nitrofuran-2-yl	69	232-233	1.58	7.49	3.74	24.62
5l	5-Nitrothiophen-2-yl	76	241-242	2.14	7.21	3.60	18.72
6a	2-Hydroxyphenyl	83	218-220	3.54	44.85	ND	28.72
6b	4-Hydroxyphenyl	81	227-228	3.54	22.42	ND	16.32
6c	4-Methoxyphenyl	84	232-233	4.13	17.22	ND	22.30
6d	4-Benzyloxyphenyl	91	208-210	5.89	15.26	ND	20.63
6e	4-Methylphenyl	88	249-250	4.71	54.18	ND	ND
6f	4-(Dimethylamino)phenyl	72	281-283	4.37	132.62	ND	ND
6g	3,4,5-Trimethoxyphenyl	83	210-213	3.51	7.40	3.7	14.80
6h	2-Fluorophenyl	79	223-225	4.35	4.26	2.13	20.62
6i	4-Chlorophenyl	72	243-244	4.92	85.30	ND	ND
6j	3-Nitrophenyl	84	204-206	3.95	66.31	ND	ND
6k	5-Nitrofuran-2-yl	70	254-255	3.13	6.81	1.70	18.92
6l	5-Nitrothiophen-2-yl	72	241-243	3.69	6.52	1.63	23.26
Isoniazid					0.72	0.72	ND
Rifampicin					0.24	0.24	ND
Ethambutol					7.64	3.82	ND
Pyrazinamide					50.77	ND	ND
Ciprofloxacin					4.71	4.71	ND

* ND indicates not determined.

In the first step we treated the amines (**1**) like 3-trifluoromethylaniline, 2-amino-5-nitrothiazole and 2-amino-6-nitrobenzothiazole with chloro acetylchloride without using any base under thermal conditions to get corresponding chloroacetamide derivatives (**2**).

Refluxing of chloroacetamide derivatives (**2**) and potassium thiocyanate in acetone for 3 hours yield the corresponding cyclised 3-substituted-2-iminothiazolidine-4-ones (**3**). In final step we used Knoevenagel condensation [11] of the compound **3** with various substituted aldehydes using NaOAc/acetic acid at 100 °C, to produce title compounds (**4a-l**, **5a-l** and **6a-l**). These reactions were also successfully carried out using piperidine/ethanol at 90 °C, but former reaction conditions favoured the easy purification, due to the lower solubility of formed products in acetic acid. Direct filtration of reaction mixture and washing of residue with excess water, cold ethanol, ethers and hexanes produced the final compounds with good purity and in excellent yields (Table 1).

3.2. Anti-TB activity and structure-activity relationship

The compounds were screened for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv by

microplate alamar blue assay method [12] for the determination of MIC in duplicate. The minimum inhibitory concentration is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth. MICs of the synthesized compounds along with the standard drugs for comparison are reported (Table 1). The compounds showed MIC's ranging from 3.31-138.12 μM; and fifteen compounds showed promising activity with MIC of less than 10 μM. When compared to original lead compound SID: 24823007; all the compounds were found to be less active. When compared to standard first line antitubercular drug ethambutol (MIC of 7.64 μM), fifteen compounds were found to be more active and when compared to pyrazinamide (MIC of 50.77 μM), twenty-five compounds were more active. All the molecules were found to be less active than isoniazid (MIC of 0.72 μM) and rifampicin (MIC of 0.24 μM) but seven compounds were more active than DNA gyrase inhibitor ciprofloxacin (MIC of 4.71 μM). Among the compounds, 2-imino-3-(5-nitrothiazol-2-yl)-5-(3, 4, 5-trimethoxybenzylidene)thiazolidin-4-one (**5g**) was found to be the most active compound *in vitro* with MICs of 3.31 μM. To study the SAR we prepared the compounds with variations in N-3 and C-5 positions. In N-3 position we have tried with 3-trifluoromethylphenyl (**4a-l**), 5-nitrothiazol-2-yl

(5a-l) and 6-nitrobenzothiazol-2-yl (6a-l) groups. The order of activity with respect to N-3 position were; 6-nitrobenzothiazol-2-yl > 3-trifluoromethylphenyl > 5-nitrothiazol-2-yl group. In the C-5 position we have prepared molecules with phenyl ring with both electron donating and electron withdrawing groups and also with few heterocycles. In the case of phenyl ring at position C-5; 4-hydroxyl substituent (4b, 5b, and 6b) were found to be two to four times more potent than 2-hydroxyl substituent (4a, 5a, and 6a). Replacement of 4-hydroxyl group with 4-methoxyl (4c, 5c, and 6c) and 4-benzyloxy group (4d, 5d, and 6d) increases the activity; whereas methyl group (4e, 5e and 6e) and dimethylamino group (4f, 5f, and 6f) decreases the activity drastically. Tri substitution with 3,4,5-trimethoxyl group (4g, 5g, and 6g) showed good potency with MIC of <8 μM . In the case of electron withdrawing groups; substitution with 2-fluoro group (4h, 5h, and 6h) showed good activity with MIC of <5 μM ; whereas substituents like 4-chloro group (4i, 5i and 6i) and 3-nitro groups (4j, 5j and 6j) were detrimental for activity. With respect to heterocyclic substituents; we prepared with 5-nitrofuran-2-yl and 5-nitrothiophen-2-yl groups and both of them provides good activity [13]. We have also calculated ClogP with Chemdraw 8.0 for all the compounds to see the relationship between lipophilicity and antitubercular activity; but it was not correlating (Table 1).

When compared to lead compound SID: 24823007, all the synthesized compounds showed less activity. It could be due to involvement of wide array of efflux mechanisms mediated by several ABC (ATP-binding cassette) transporters and major facilitator superfamily (MFS) proteins, or antibiotic-modifying and -degrading enzymes, to name a few possibilities [14]. Multiple drugs like verapamil, reserpine, phenothiazines such as thioridazine, and piperine have been shown to inhibit bacterial efflux pumps in vitro [15]. In general, the mechanisms by which these agents act are poorly understood. Several models have been proposed [16], such as: (1) direct binding and inhibition of pump assembly or function; (2) disruption of the transmembrane gradients utilized by secondary transporters; (3) inhibitor binding to the antimicrobial compound; (4) competition for efflux. We have tested some selected compounds [MIC of < 10 μM], in presence of reported efflux pump inhibitor verapamil; and most of the cases MIC decreased 2 to 4 fold when compared to absence of efflux pump. Most active compound 5g showed MIC of 0.82 μM

3.3. Cytotoxicity study

Compounds which showed MIC of less than 50 μM were further examined for cytotoxicity in a RAW 264.7 cell line (mouse leukaemic monocyte macrophage) at single concentration of 100 μM . We have selected this macrophage cell line to test the toxicity because generally MTB used to reside inside the macrophages and the new molecules should not possess any toxicity against macrophages. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay. Most of the tested compounds were not cytotoxic to RAW 264.7 cells and their percentage growth inhibitions were reported in Table 1. The most active anti-TB compound 5g has shown toxicity of 12.4% at 100 μM with selectivity index of > 30 for MTB.

4. Conclusion

In this study we have designed, synthesized and study the SAR of various inhibitors of MTB based on the lead compound SID: 24823007 reported by TAACF. Among the compounds, 2-imino-3-(5-nitrothiazol-2-yl)-5-(3,4,5-trimethoxybenzylidene) thiazolidin-4-one (19) was found to be the most active compound *in vitro* with MICs of 3.31 μM against log-phase culture of MTB and also non-toxic up to 100 μM , but compound 5g is less potent than lead compound SID: 24823007.

Compound 5g showed MIC of 0.82 μM in presence of efflux pump inhibitor. Further structural optimization required to get the compounds with better potency than the lead compound and also to explore the possible mechanism of action against various MTB essential enzymes. In addition, efflux could be the rate-limiting step in the discovery of novel anti-TB compounds, as has already been recognized in the discovery of drugs for Gram-negative bacterial infections. The discovery of new pumps with multiple specificities in MTB and the impact these pumps have on antitubercular therapy by conferring resistance to many of the new molecules discovered necessitate the study of efflux mechanisms as an important therapeutic target.

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