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Benzothiazinone-piperazine derivatives as efficient *Mycobacterium tuberculosis* DNA gyrase inhibitors

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ARTICLE INFO

Article history:
Received 20 January 2015
Received in revised form
18 February 2015
Accepted 24 February 2015
Available online 26 March 2015

Keywords:
Tuberculosis
DNA gyrase
Supercoiling
Minimum inhibitory concentration

ABSTRACT

Background and objectives: Bacterial DNA topoisomerases are unique in maintaining the DNA topology for cell viability. *Mycobacterium tuberculosis* (MTB) DNA gyrase, a sole type II topoisomerase has a larger scope as a target for developing novel therapeutics. In this study, an effort was made towards the design and synthesis of benzothiazinone-piperazine hybrid analogues to obtain the possibility of it to lead development through the molecular hybridization technique.

Methods: A five-step scheme was followed to obtain a series of 36 benzothiazinone-piperazine derivatives and to evaluate them for MTB DNA gyrase inhibition, antimycobacterial and cytotoxicity studies.

Results: Compound N-(4-chlorophenyl)-4-(6-nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperazine-1-carbothioamide (**18**) showed greater inhibitory potential with an IC_{50} of 0.51 ± 0.16 μM in the DNA supercoiling assay of MTB with a moderate anti-tubercular activity of 4.41 μM. The compound even passed the safety profile of eukaryotic cell cytotoxicity with a 1.81% inhibition in the RAW 264.7 cell line at 100 μM concentration.

Conclusions: This study describes the discovery of benzothiazinone as gyrase inhibitors with potent MTB MIC and inhibitory profiles of the gyrase enzyme with less cytotoxic effect. Furthermore, it is believed that this class of compounds has the potential to be further developed as an anti-TB drug candidate.

Introduction

Tuberculosis (TB) is a major threat to mankind and claims approximately 1.5 million lives each year according to the World Health Organization (WHO) [1]. Statistics report that one-third of the global population is infected by the latent bacilli *Mycobacterium tuberculosis* (MTB). Moreover, in 2012 globally an estimated half a million people fell ill with multidrug-resistant tuberculosis (MDR-TB) [2]. Furthermore, the first-line TB drugs currently in use are at least 40 years old with an increase in drug-resistant strains of MTB. Similarly, the second-line drugs used mainly for the treatment of multidrug-resistant and extensively drug-resistant (XDR) TB induce serious side effects according to WHO [3]; there is an

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[1] Equal contribution.
Peer review under responsibility of Asian African Society for Mycobacteriology.
http://dx.doi.org/10.1016/j.ijmyco.2015.02.002

urgent need to discover novel antitubercular agents with newer combination regimens for treating MDR and XDR-TB. Presently, bedaquiline [4] is the only approved drug by the FDA, whereas delamanid [5], SQ109 [6], oxazolidinones [7] and fluoroquinolones [8] are still in the clinical development stage. Hence, there is an urgent need for discovery of novel antimycobacterial agents to replenish the TB pipeline drugs.

DNA topoisomerases maintain the DNA topology during replication, transcription and recombination. Among many topoisomerases, DNA gyrase is the sole topoisomerase II enzyme present in MTB [9]. It mainly consists of GyrA and GyrB domains, in the holoenzyme complex as A2B2. While the GyrA subunit mainly interacts with the DNA and possesses the active-site tyrosine responsible for DNA cleavage and re-ligation, the GyrB subunit helps in the ATP hydrolysis, thus acting as a catalytic site for the enzyme [10,11]. This enzyme is absent in the eukaryotic organisms, though a less homologous enzyme does exist; thus, it seems to be an attractive target for developing novel drugs against TB. Though fluoroquinolones have been efficient bactericidal antimicrobials, they are losing their potency due to the resistance being developed rapidly. Hence, in the present study, a drug discovery program was initiated through molecular hybridization targeting DNA gyrase of MTB; herein this study describes the design and development of benzothiazinone hybrids as novel compounds using the chemical structure of the previously reported antitubercular benzothiazinone bearing (left hand side) derivative PBTZ169 [12,13] and the MTB DNA gyrase inhibitor bearing aryl (thio) urea right hand side chain [14,15].

Materials and methods

General

All commercially available chemicals and solvents were used without further purification. TLC experiments were performed on alumina-backed silica gel 40 F_{254} plates (Merck, Darmstadt, Germany). Homogeneity of the compounds was monitored by thin layer chromatography (TLC) on silica gel 40 F_{254} coated on aluminium plates, visualized by UV light and $KMnO_4$ treatment. All ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-300 (300.12 MHz, 75.12 MHz) NMR spectrometer, BrukerBioSpin Corp, Germany. Chemical shifts were reported in ppm (δ) with reference to the internal standard TMS. The signals were designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Molecular weights of the synthesized compounds were checked by LCMS 6100B series Agilent Technology. Elemental analyses were carried out on an automatic Flash EA 1112 Series, CHN Analyzer (Thermo).

General procedure for the synthesis of 5-substituted-2-chlorbenzamides (2a–c)

To a stirred solution of the corresponding acid (**1a–c**) (1 mmol) in dichloromethane (15 ml) at −10 °C was added oxalyl chloride (2.5 mmol). The solution was refluxed for about 6 h (monitored by TLC and LCMS for completion), and solvent evaporated under reduced pressure. The residue was further diluted with acetonitrile (30 ml), cooled to −20 °C and added ammonium hydroxide solution drop wise and allowed to stir for 30 min. The resulting solid was filtered out to afford the corresponding amide (**2a–c**) in good yield.

2-Chloro-5-nitrobenzamide (2a)

The compound was synthesized according to the general procedure using 2-chloro-5-nitrobenzoic acid (**1a**) (5.0 g, 0.02 mmol), oxalyl chloride (5.3 ml, 0.05 mmol) and aqueous ammonium hydroxide (50 ml) to afford **2a** (3.9 g, 78.4%) as yellow solid. M.p: 185–187 °C. ^1H NMR (DMSO-d_6): δ_H 8.28–7.83 (m, 3H), 7.52 (b, 1H). ^{13}C NMR (DMSO-d_6): δ_C 168.2, 146.2, 140.3, 130.7, 128.5, 122.1. ESI-MS *m/z* 201 $(M+H)^+$. Anal. calcd. for $C_7H_5ClN_2O_3$: C, 41.92; H, 2.51; N, 13.97; Found: C, 41.89; H, 2.54; N, 13.99.

2-Chloro-5-(trifluoromethyl)benzamide (2b)

The compound was synthesized according to the general procedure using 2-chloro-5-(trifluoromethyl)benzoic acid (**1b**) (4.0 g, 0.01 mmol), oxalyl chloride (3.8 ml, 0.04 mmol) and aqueous ammonium hydroxide (40 ml) to afford **2b** (1.84 g, 46.2%) as white solid. M.p: 176–178 °C. ^1H NMR (DMSO-d_6): δ_H 8.02–7.64 (m, 3H), 7.54 (b, 1H). ^{13}C NMR (DMSO-d_6): δ_C 168.4, 137.7, 132.3, 129.7, 129.5, 128.1, 125.3. 123.5. ESI-MS *m/z* 224 $(M+H)^+$. Anal. calcd. for $C_8H_5ClF_3NO$: C, 42.98; H, 2.25; N, 6.26; Found: C, 41.89; H, 2.54; N, 13.99.

2,5-Dichlorobenzamide (2c)

The compound was synthesized according to the general procedure using 2,5-dichlorobenzoic acid (**1c**) (4.0 g, 0.02 mmol), oxalyl chloride (4.5 ml, 0.05 mmol) and aqueous ammonium hydroxide (40 ml) to afford **2c** (2.12 g, 53.4%) as white solid. M.p: 181–183 °C. ^1H NMR (DMSO-d_6): δ_H 7.89–7.63 (m, 3H), 7.52 (b, 1H). ^{13}C NMR (DMSO-d_6): δ_C 168.6, 133.5, 132.3, 132.1, 129.4, 128.6. ESI-MS *m/z* 192 $(M+H)^+$. Anal. calcd. for $C_7H_5Cl_2NO$: C, 44.24; H, 2.65; N, 7.37; Found: C, 44.26; H, 2.64; N, 7.39.

General procedure for the synthesis of 5-substituted-2-(methylthio)-4H-benzo[e][1,3]thiazin-4-one (3a–c)

To a stirred solution of the corresponding benzamide (**2a–c**) (1 mmol) in DMSO (15 ml) at 10 °C was added carbon disulphide (3 mmol), sodium hydroxide (2 mmol), and the mixture was allowed to stand for 15 min. Subsequently, methyl iodide (1.2 mmol) was added. The reaction mixture was allowed to stand for another 30 min, and 50 ml of water was added. The resulting white solid separated by filtration to afford the corresponding benzothiazinone (**3a–c**) in good yield.

2-(Methylthio)-6-nitro-4H-benzo[e][1,3]thiazin-4-one (3a)

The compound was synthesized according to the general procedure using 2-chloro-5-nitrobenzamide (**2a**) (2.0 g, 9.97 mmol), carbon disulphide (1.8 ml, 29.9 mmol), sodium hydroxide (0.79 g, 19.9 mmol) and methyl iodide (0.73 ml, 11.9 mmol) to afford **3a** (1.82 g, 71.9%) as yellow solid. M.p:

211–213 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.36–7.82 (m, 3H), 2.72 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 162.4, 145.3, 143.6, 138.4, 130.5, 128.4, 123.6, 14.2. ESI-MS *m*/z 255 $(M+H)^+$. Anal. calcd. for $C_9H_6N_2O_3S_2$: C, 42.51; H, 2.38; N, 11.02; Found: C, 42.54; H, 2.36; N, 11.05.

2-(Methylthio)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (3b)

The compound was synthesized according to the general procedure using 2-chloro-5-(trifluoromethyl)benzamide (**2b**) (1.5 g, 6.71 mmol), carbon disulphide (1.2 ml, 20.1 mmol), sodium hydroxide (0.53 g, 13.4 mmol) and methyl iodide (0.49 ml, 8.1 mmol) to afford **3b** (1.63 g, 88.1%) as white solid. M.p: 208–210 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.35–7.78 (m, 3H), 2.72 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 162.5, 140.3, 137.6, 131.4, 130.6, 128.4, 126.6, 123.5, 14.3. ESI-MS *m*/z 278 $(M+H)^+$. Anal. calcd. for $C_{10}H_6F_3NOS_2$: C, 43.32; H, 2.18; N, 5.05; Found: C, 43.33; H, 2.16; N, 5.03.

6-Chloro-2-(methylthio)-4H-benzo[e][1,3]thiazin-4-one (3c)

The compound was synthesized according to the general procedure using 2,5-dichlorobenzamide (**2c**) (2.0 g, 10.52 mmol), carbon disulphide (1.9 ml, 31. mmol), sodium hydroxide (0.84 g, 21.1 mmol) and methyl iodide (0.78 ml, 12.6 mmol) to afford **3c** (1.57 g, 61.3%) as white solid. M.p: 219–221 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.36–7.74 (m, 3H), 2.72 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.8, 162.5, 138.3, 135.6, 134.4, 131.6, 131.4, 130.6, 14.3. ESI-MS *m*/z 244 $(M+H)^+$. Anal. calcd. for $C_9H_6ClNOS_2$: C, 44.35; H, 2.48; N, 5.75; Found: C, 44.33; H, 2.46; N, 5.73.

General procedure for the synthesis of 6-substitutedo-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-ones (4a–c)

To a stirred solution of the corresponding benzothiazinone (**3a**) (1 mmol) in ethanol (15 ml) at room temperature was added 1-Boc-piperazine (1 mmol). The solution was refluxed for about 12 h (monitored by TLC and LCMS for completion), and solvent evaporated under reduced pressure. The residue was further diluted with water (30 ml) and ethyl acetate (50 ml) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding *N*-boc protected piperazine benzothiazinones in good yield, which was taken in dichloromethane (60 ml) and was cooled to 0 °C and was added trifluoroacetic acid (8 ml) and stirred the reaction at room temperature for 1 h. After completion of the reaction by TLC, the reaction mixture was cooled to 0 °C and basified to pH ~8.0 using saturated aqueous $NaHCO_3$ solution. The organic layer was separated, washed with water (2 × 20 ml) and brine (1 × 20 ml) and dried over anhydrous sodium sulphate. The organic layer was concentrated under vacuum afforded the free amine as pale brown oil. The crude material was used for final reactions without purification.

6-Nitro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (4a)

The compound was synthesized according to the general procedure using 2-(methylthio)-6-nitro-4H-benzo[e][1,3]thiazin-4-one (**3a**) (1.8 g, 7.08 mmol), 1-Boc-piperazine (1.31 g, 7.08 mmol) and trifluoroacetic acid (2 ml) to afford **4a** (1.33 g, 64.5%) as yellow solid. M.p: 208–210 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.33–7.24 (m, 3H), 5.25 (b, 2H), 3.12–1.88 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.3, 145.6, 143.6, 138.4, 130.3, 128.4, 123.6, 46.7, 41.5 (2C), 32.2 (2C). ESI-MS *m*/z 307 $(M+H)^+$. Anal. calcd. for $C_{13}H_{14}N_4O_3S$: C, 50.97; H, 4.61; N, 18.29; Found: C, 50.96; H, 4.65; N, 18.31.

2-(Piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (4b)

The compound was synthesized according to the general procedure using 2-(methylthio)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**3b**) (1.6 g, 5.77 mmol), 1-Boc-piperazine (1.07 g, 5.77 mmol) and trifluoroacetic acid (2 ml) to afford **4b** (1.28 g, 70.7%) as white solid. M.p: 221–223 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.32–7.27 (m, 3H), 5.26 (b, 2H), 3.14–1.85 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.2, 159.4, 140.7, 137.6, 131.4, 130.3, 128.5, 126.8, 123.3, 46.7, 41.4 (2C), 32.5 (2C). ESI-MS *m*/z 330 $(M+H)^+$. Anal. calcd. for $C_{14}H_{14}F_3N_3OS$: C, 51.06; H, 4.28; N, 12.76; Found: C, 51.08; H, 4.27; N, 12.73.

6-Chloro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (4c)

The compound was synthesized according to the general procedure using 6-chloro-2-(methylthio)-4H-benzo[e][1,3]thiazin-4-one (**3c**) (1.5 g, 6.15 mmol), 1-Boc-piperazine (1.14 g, 6.15 mmol) and trifluoroacetic acid (2 ml) to afford **4c** (1.44 g, 83.2%) as white solid. M.p: 217–219 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.33–7.28 (m, 3H), 5.24 (b, 2H), 3.12–1.88 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.5, 138.6, 135.1, 134.4, 131.3, 131.1, 130.2, 46.8, 41.4 (2C), 32.2 (2C). ESI-MS *m*/z 296 $(M+H)^+$. Anal. calcd. for $C_{13}H_{14}ClN_3OS$: C, 52.79; H, 4.77; N, 14.21; Found: C, 52.82; H, 4.76; N, 14.25.

General procedure for the synthesis of 4-(6-substituted-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-arylpiperazine-1-carboxamide derivatives (5–16)

To a cooled solution of 6-substituted-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (1 mmol) in anhydrous DCM (2 ml) was added corresponding isocyanate (1 mmol), triethylamine (1 mmol) and stirred the reaction mixture at room temperature for 12 h (monitored by TLC and LCMS for completion), and solvent evaporated under reduced pressure. The residue was further diluted with water (30 ml) and ethyl acetate (50 ml) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding urea derivative (**5–16**) in good yield.

4-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-phenylpiperazine-1-carboxamide (**5**)

The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4a**) (0.1 g, 0.34 mmol) and Phenyl isocyanate (0.04 g, 0.34 mmol) to afford **5** (0.06 g, 46.18%) as yellow solid. M.p: 211–213 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.59 (b, 1H), 8.56–7.48 (m, 8H), 3.12–1.88 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.2, 155.7, 145.3, 143.6, 139.1, 138.2, 130.2, 128.7 (3C), 128.1, 123.5, 121.5 (2C), 51.6 (2C), 48.5 (2C). ESI-MS *m*/*z* 412 (M+H)$^+$. Anal. calcd. for $C_{19}H_{17}N_5O_4S$: C, 55.47; H, 4.16; N, 17.02; Found: C, 55.44; H, 4.52.15; N, 17.06.

N-(4-Chlorophenyl)-4-(6-nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperazine-1-carboxamide (**6**)

The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4a**) (0.1 g, 0.34 mmol) and 4-chlorophenyl isocyanate (0.05 g, 0.34 mmol) to afford **6** (0.08 g, 53.10%) as yellow solid. M.p: 203–205 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.58 (b, 1H), 8.54–7.43 (m, 7H), 3.12–1.88 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.3, 155.5, 145.7, 143.4, 138.2, 137.2, 133.7, 130.1, 129.5 (2C), 128.5, 123.7, 120.5 (2C), 51.6 (2C), 48.4 (2C). ESI-MS *m*/*z* 446 (M+H)$^+$. Anal. calcd. for $C_{19}H_{16}ClN_5O_4S$: C, 51.18; H, 3.62; N, 15.71; Found: C, 51.21; H, 3.64; N, 15.69.

4-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(p-tolyl)piperazine-1-carboxamide (**7**)

The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4a**) (0.1 g, 0.34 mmol) and *p*-tolyl isocyanate (0.04 g, 0.34 mmol) to afford **7** (0.05 g, 35.73%) as yellow solid. M.p: 208–210 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.56 (b, 1H), 8.57–7.46 (m, 7H), 3.11–1.87 (m, 8H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.2, 155.7, 145.3, 143.2, 138.6, 136.8, 136.3, 130.5, 129.5 (2C), 128.8, 123.6, 121.3 (2C), 51.3 (2C), 48.4 (2C), 21.2. ESI-MS *m*/*z* 426 (M+H)$^+$. Anal. calcd. for $C_{20}H_{19}N_5O_4S$: C, 56.46; H, 4.50; N, 16.46; Found: C, 56.43; H, 4.54; N, 16.43.

4-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(4-nitrophenyl)piperazine-1-carboxamide (**8**)

The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4a**) (0.1 g, 0.34 mmol) and 4-nitrophenyl isocyanate (0.05 g, 0.34 mmol) to afford **8** (0.02 g, 14.73%) as yellow solid. M.p: 226–228 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.56 (b, 1H), 8.58–7.48 (m, 7H), 3.14–1.89 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 159.4, 154.1, 145.7, 145.2, 143.6, 143.4, 138.5, 130.9, 128.7, 124.2 (2C), 123.6, 119.5 (2C), 51.5, 48.6 (2C). ESI-MS *m*/*z* 457 (M+H)$^+$. Anal. calcd. for $C_{19}H_{16}N_6O_6S$: C, 50.00; H, 3.53; N, 18.41; Found: C, 50.02; H, 3.51; N, 18.38.

4-(4-Oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)-N-phenylpiperazine-carboxamide (**9**)

The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**4b**) (0.1 g, 0.31 mmol) and phenyl isocyanate (0.03 g, 0.31 mmol) to afford **9** (0.08 g, 62.39%) as white solid. M.p: 213–215 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.52 (b, 1H), 8.51–7.40 (m, 8H), 3.12–1.84 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.7, 159.4, 155.6, 140.7, 139.4, 137.6, 131.3, 130.6, 128.7 (2C), 128.2, 126.1, 123.4, 121.5 (2C), 51.2 (2C), 48.4 (2C). ESI-MS *m*/*z* 435 (M+H)$^+$. Anal. calcd. for $C_{20}H_{17}F_3N_4O_2S$: C, 55.29; H, 3.94; N, 12.90; Found: C, 55.26; H, 3.95; N, 12.88.

N-(4-Chlorophenyl)-4-(4-oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)piperazine-1-carboxamide (**10**)

The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**4b**) (0.1 g, 0.31 mmol) and 4-chlorophenyl isocyanate (0.04 g, 0.31 mmol) to afford **10** (0.05 g, 34.95%) as white solid. M.p: 211–213 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.51 (b, 1H), 8.50–7.38 (m, 7H), 3.14–1.82 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.6, 155.7, 140.2, 137.7, 137.3, 133.2, 131.2, 130.4, 129.1 (2C), 128.5, 126.6, 123.6, 120.7 (2C), 51.5 (2C), 48.4 (2C). ESI-MS *m*/*z* 469 (M+H)$^+$. Anal. calcd. for $C_{20}H_{16}ClF_3N_4O_2S$: C, 51.23; H, 3.44; N, 11.95; Found: C, 51.25; H, 3.45; N, 11.96.

4-(4-Oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)-N-(p-tolyl)piperazine-1-carboxamide (**11**)

The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**4b**) (0.1 g, 0.31 mmol) and *p*-tolylisocyanate (0.04 g, 0.31 mmol) to afford **11** (0.04 g, 32.33%) as white solid. M.p: 216–218 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.54 (b, 1H), 8.51–7.41 (m, 7H), 3.13–1.83 (m, 8H), 2.38 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 159.5, 155.6, 140.7, 137.2, 136.6, 131.2, 130.2, 129.3 (2C), 128.3, 126.6, 123.6, 121.4 (2C), 51.2 (2C), 48.1 (2C), 21.3. ESI-MS *m*/*z* 449 (M+H)$^+$. Anal. calcd. for $C_{21}H_{19}F_3N_4O_2S$: C, 56.24; H, 4.27; N, 12.49; Found: C, 56.28; H, 4.24; N, 12.47.

N-(4-Nitrophenyl)-4-(4-oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)piperazine-1-carboxamide (**12**)

The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**4b**) (0.1 g, 0.31 mmol) and 4-nitrophenyl isocyanate (0.05 g, 0.31 mmol) to afford **12** (0.03 g, 25.64%) as white solid. M.p: 201–203 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.55 (b, 1H), 8.55–7.46 (m, 7H), 3.15–1.85 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.7, 159.2, 155.1, 145.8, 143.2, 140.6, 137.2, 131.3, 130.2, 128.4, 126.3, 124.4 (2C), 123.6, 119.6 (2C), 51.5 (2C), 48.8 (2C). ESI-MS *m*/*z* 480 (M+H)$^+$. Anal. calcd. for $C_{20}H_{16}F_3N_5O_4S$: C, 50.10; H, 3.36; N, 14.61; Found: C, 50.13; H, 3.34; N, 14.58.

4-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-phenylpiperazine-1-carboxamide (**13**)

The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4c**) (0.1 g, 0.35 mmol) and phenyl isocyanate (0.04 g,

0.35 mmol) to afford **13** (0.04 g, 34.44%) as white solid. M.p: 217–219 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.50 (b, 1H), 8.54–7.39 (m, 8H), 3.16–1.89 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.5, 155.2, 139.8, 138.2, 135.6, 134.2, 131.3, 131.2, 130.4, 128.7 (2C), 128.4, 121.6 (2C), 51.6 (2C), 48.4 (2C). ESI-MS *m*/*z* 401 $(M+H)^+$. Anal. calcd. for $C_{19}H_{17}ClN_4O_2S$: C, 56.93; H, 4.27; N, 13.98; Found: C, 56.90; H, 4.29; N, 13.95.

4-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(4-chlorophenyl)piperazine-1-carboxamide (14)

The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4c**) (0.1 g, 0.35 mmol) and 4-chlorophenyl isocyanate (0.05 g, 0.35 mmol) to afford **14** (0.05 g, 36.89%) as white solid. M.p: 222–224 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.51 (b, 1H), 8.56–7.42 (m, 7H), 3.14–1.86 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 159.2, 155.4, 138.8, 137.2, 135.5, 134.2, 133.5, 131.4, 131.1, 130.5, 129.7 (2C), 120.6 (2C), 51.4 (2C), 48.2 (2C). ESI-MS *m*/*z* 436 $(M+H)^+$. Anal. calcd. for $C_{19}H_{16}Cl_2N_4O_2S$: C, 52.42; H, 3.70; N, 12.87; Found: C, 52.43; H, 3.68; N, 12.89.

4-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(p-tolyl)piperazine-1-carboxamide (15)

The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4c**) (0.1 g, 0.35 mmol) and *p*-tolylisocyanate (0.04 g, 0.35 mmol) to afford **15** (0.06 g, 44.82%) as white solid. M.p: 197–199 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.49 (b, 1H), 8.51–7.42 (m, 7H), 3.14–1.84 (m, 8H), 2.34 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.7, 155.1, 138.7, 136.2, 135.5, 134.3, 131.5, 131.3, 130.1, 129.5 (2C), 121.7 (2C), 51.5 (2C), 48.4 (2C), 21.4. ESI-MS *m*/*z* 415 $(M+H)^+$. Anal. calcd. for $C_{20}H_{19}ClN_4O_2S$: C, 57.90; H, 4.62; N, 13.50; Found: C, 57.89; H, 4.59; N, 13.52.

4-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(4-nitrophenyl)piperazine-1-carboxamide (16)

The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4c**) (0.1 g, 0.35 mmol) and 4-nitrophenyl isocyanate (0.05 g, 0.35 mmol) to afford **16** (0.08 g, 51.82%) as white solid. M.p: 215–217 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.52 (b, 1H), 8.53–7.47 (m, 7H), 3.15–1.81 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.7, 159.3, 155.2, 145.7, 143.2, 138.5, 135.3, 134.5, 131.3, 131.1, 130.5, 124.3 (2C), 119.8 (2C), 51.5 (2C), 48.6 (2C). ESI-MS *m*/*z* 446 $(M+H)^+$. Anal. calcd. for $C_{19}H_{16}ClN_5O_4S$: C, 51.18; H, 3.62; N, 15.71; Found: C, 51.19; H, 3.65; N, 15.74.

General procedure for the synthesis of 4-(6-substituted-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-arylpiperazine-1-carbothioamide derivatives (17–28)

To a cooled solution of 6-substituted-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (1 mmol) in anhydrous DCM (2 ml) was added corresponding isothiocyanate (1 mmol), triethylamine (1 mmol) and stirred the reaction mixture at room temperature for 12 h (monitored by TLC and LCMS for completion), and solvent evaporated under reduced pressure. The residue was further diluted with water (30 ml) and ethyl acetate (50 ml) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding thiourea derivative (**17–28**) in good yield.

4-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-phenylpiperazine-1-carbothioamide (17)

The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4a**) (0.1 g, 0.34 mmol) and phenyl isothiocyanate (0.04 g, 0.34 mmol) to afford **17** (0.06 g, 46.50%) as yellow solid. M.p: 221–223 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.97 (b, 1H), 8.52–7.71 (m, 8H), 3.06–1.84 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 181.6, 167.5, 159.2, 145.2, 143.7, 138.2, 138.1, 130.3, 129.3 (2C), 128.4, 128.1 (2C), 126.5 (2C), 123.3, 56.7 (2C), 48.5 (2C). ESI-MS *m*/*z* 428 $(M+H)^+$. Anal. calcd. for $C_{19}H_{17}N_5O_3S_2$: C, 53.38; H, 4.01; N, 16.38; Found: C, 53.40; H, 4.02; N, 16.37.

N-(4-Chlorophenyl)-4-(6-nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperazine-1-carbothioamide (18)

The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one **4a**) (0.1 g, 0.34 mmol) and 4-Chlorophenyl isothiocyanate (0.05 g, 0.34 mmol) to afford **18** (0.07 g, 45.56%) as yellow solid. M.p: 189–191 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.95 (b, 1H), 8.55–7.73 (m, 7H), 3.04–1.86 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 181.4, 167.6, 159.4, 145.7, 143.2, 138.4, 136.1, 133.3, 131.3 (2C), 130.4, 129.1 (2C), 128.5, 123.4, 56.6 (2C), 48.8 (2C). ESI-MS *m*/*z* 462 $(M+H)^+$. Anal. calcd. for $C_{19}H_{16}ClN_5O_3S_2$: C, 49.40; H, 3.49; N, 15.16; Found: C, 49.43; H, 3.51; N, 15.19.

4-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(p-tolyl)piperazine-1-carbothioamide (19)

The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4a**) (0.1 g, 0.34 mmol) and *p*-tolyl isothiocyanate (0.05 g, 0.34 mmol) to afford **19** (0.07 g, 48.33%) as yellow solid. M.p: 186–188 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.93 (b, 1H), 8.58–7.81 (m, 7H), 3.07–1.88 (m, 8H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 181.3, 167.5, 159.8, 145.6, 143.1, 138.4, 137.1, 135.3, 130.3, 129.4 (2C), 128.1, 126.5 (2C), 123.5, 56.8 (2C), 48.3 (2C), 21.4. ESI-MS *m*/*z* 442 $(M+H)^+$. Anal. calcd. for $C_{20}H_{19}N_5O_3S_2$: C, 54.41; H, 4.34; N, 15.86; Found: C, 54.39; H, 4.35; N, 15.89.

4-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(4-nitrophenyl)piperazine-1 carbothioamide (20)

The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4a**) (0.1 g, 0.34 mmol) and 4-nitrophenyl isothiocyanate (0.06 g, 0.34 mmol) to afford **20** (0.04 g, 26.60%) as yellow solid. M.p: 192–194 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.95 (b, 1H), 8.61–7.42 (m, 7H), 3.08–1.81 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 181.5, 167.3, 159.5, 145.8, 144.6, 143.4, 143.1,

138.3, 130.2, 128.4, 124.5 (2C), 124.2 (2C), 123.7, 56.6 (2C), 48.5 (2C). ESI-MS *m/z* 473 $(M+H)^+$. Anal. calcd. for $C_{19}H_{16}N_6O_5S_2$: C, 48.30; H, 3.41; N, 17.79; Found: C, 48.33; H, 3.45; N, 17.80.

4-(4-Oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)-N-phenylpiperazine-1-carbothioamide (21)

The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**4b**) (0.1 g, 0.31 mmol) and phenyl isothiocyanate (0.04 g, 0.31 mmol) to afford **21** (0.05 g, 40.58%) as white solid. M.p: 179–181 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.97 (b, 1H), 8.62–7.45 (m, 8H), 3.10–1.85 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 181.1, 167.9, 159.6, 140.8, 138.6, 137.4, 131.6, 130.3, 129.2 (2C), 128.4, 128.2, 126.2 (3C), 123.5, 56.7 (2C), 48.2 (2C). ESI-MS *m/z* 451 $(M+H)^+$. Anal. calcd. for $C_{20}H_{17}F_3N_4OS_2$: C, 53.32; H, 3.80; N, 12.44; Found: C, 55.29; H, 3.91; N, 12.42.

N-(4-Chlorophenyl)-4-(4-oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)piperazine-1-carbothioamide (22)

The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**4b**) (0.1 g, 0.31 mmol) and 4-chlorophenyl isothiocyanate (0.05 g, 0.31 mmol) to afford **22** (0.06 g, 40.29%) as white solid. M.p: 193–195 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.94 (b, 1H), 8.63–7.41 (m, 7H), 3.12–1.88 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 181.8, 167.7, 159.4, 140.5, 137.6, 136.4, 133.6, 131.3, 131.2 (2C), 130.4, 129.2 (2C), 128.2, 126.5, 123.6, 56.8 (2C), 48.6 (2C). ESI-MS *m/z* 485 $(M+H)^+$. Anal. calcd. for $C_{20}H_{16}ClF_3N_4OS_2$: C, 49.53; H, 3.33; N, 11.55; Found: C, 49.51; H, 3.35; N, 11.54.

4-(4-Oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)-N-(p-tolyl)piperazine-1-carbothioamide (23)

The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**4b**) (0.1 g, 0.31 mmol) and *p*-tolyl isothiocyanate (0.04 g, 0.31 mmol) to afford **23** (0.06 g, 45.46%) as white solid. M.p: 183–185 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.91 (b, 1H), 8.59–7.63 (m, 7H), 3.15–1.85 (m, 8H), 2.35 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 181.5, 167.1, 159.9, 140.2, 137.4, 137.2, 135.4, 131.6, 130.3, 129.2 (2C), 128.4, 126.5, 126.2 (2C), 123.5, 56.4 (2C), 48.2 (2C), 21.4. ESI-MS *m/z* 465 $(M+H)^+$. Anal. calcd. for $C_{21}H_{19}F_3N_4OS_2$: C, 54.30; H, 4.12; N, 12.06; Found: C, 54.32; H, 4.16; N, 12.07.

N-(4-Nitrophenyl)-4-(4-oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)piperazine-1-carbothioamide (24)

The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**4b**) (0.1 g, 0.31 mmol) and 4-nitrophenyl isothiocyanate (0.05 g, 0.31 mmol) to afford **24** (0.08 g, 52.16%) as white solid. M.p: 192–194 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.98 (b, 1H), 8.62–7.61 (m, 7H), 3.11–1.82 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 181.1, 167.3, 159.4, 144.2, 143.4, 140.2, 137.4, 131.6, 130.3, 128.2, 126.4, 124.5 (2C), 124.2 (2C), 123.7, 56.3 (2C), 48.7 (2C). ESI-MS *m/z* 496 $(M+H)^+$. Anal. calcd. for $C_{20}H_{16}F_3N_5O_3S_2$: C, 48.48; H, 3.25; N, 14.13; Found: C, 48.49; H, 3.22; N, 14.17.

4-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-phenylpiperazine-1-carbothioamide (25)

The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4c**) (0.1 g, 0.35 mmol) and phenyl isothiocyanate (0.04 g, 0.35 mmol) to afford **25** (0.05 g, 34.47%) as white solid. M.p: 199–201 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.92 (b, 1H), 8.58–7.66 (m, 8H), 3.14–1.85 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 181.5, 167.5, 159.4, 138.6, 138.4, 135.2, 134.4, 131.6, 131.2, 130.2, 129.4 (2C), 128.5, 126.6 (2C), 56.3 (2C), 48.6 (2C). ESI-MS *m/z* 417 $(M+H)^+$. Anal. calcd. for $C_{19}H_{17}ClN_4OS_2$: C, 54.73; H, 4.11; N, 13.44; Found: C, 54.75; H, 4.14; N, 13.42.

4-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(4-chlorophenyl)piperazine-1-carbothioamide (26)

The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4c**) (0.1 g, 0.35 mmol) and 4-chlorophenyl isothiocyanate (0.05 g, 0.35 mmol) to afford **26** (0.03 g, 24.34%) as white solid. M.p: 206–208 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.89 (b, 1H), 8.53–7.74 (m, 7H), 3.09–1.89 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 181.1, 167.4, 159.8, 138.9, 136.4, 135.5, 134.7, 133.6, 131.4, 131.2, 131.1 (2C), 130.4, 129.5 (2C), 56.5 (2C), 48.5 (2C). ESI-MS *m/z* 452 $(M+H)^+$. Anal. calcd. for $C_{19}H_{16}Cl_2N_4OS_2$: C, 50.56; H, 3.57; N, 12.41; Found: C, 50.57; H, 3.59; N, 12.39.

4-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(p-tolyl)piperazine-1-carbothioamide (27)

The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4c**) (0.1 g, 0.35 mmol) and *p*-tolyl isothiocyanate (0.05 g, 0.35 mmol) to afford **27** (0.07 g, 47.73%) as white solid. M.p: 221–223 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.91 (b, 1H), 8.54–7.71 (m, 7H), 3.05–1.92 (m, 8H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 181.7, 167.5, 159.1, 138.2, 137.4, 135.5, 135.3, 134.6, 131.4, 131.2, 130.3, 129.4 (2C), 126.5 (2C), 56.3 (2C), 48.9 (2C), 21.4. ESI-MS *m/z* 431 $(M+H)^+$. Anal. calcd. for $C_{20}H_{19}ClN_4OS_2$: C, 55.74; H, 4.44; N, 13.00; Found: C, 55.73; H, 4.45; N, 13.03.

4-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(4-nitrophenyl)piperazine-1-carbothioamide (28)

The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4c**) (0.1 g, 0.35 mmol) and 4-nitrophenyl isothiocyanate (0.06 g, 0.35 mmol) to afford **28** (0.05 g, 31.72%) as white solid. M.p: 227–229 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.91 (b, 1H), 8.61–7.74 (m, 7H), 3.08–1.91 (m, 8H), 2.36. ^{13}C NMR (DMSO-d_6): δ_C 181.8, 167.1, 159.2, 144.2, 143.4, 138.5, 135.7, 134.4, 131.4, 131.3, 130.5, 124.4 (2C), 124.1 (2C), 56.5 (2C), 48.7 (2C). ESI-MS *m/z* 462 $(M+H)^+$. Anal. calcd. for $C_{19}H_{16}ClN_5O_3S_2$: C, 49.40; H, 3.49; N, 15.16; Found: C, 49.44; H, 3.52; N, 15.13.

General procedure for the synthesis of 2-(4-benzylpiperazin-1-yl)-6-substituted-4H-benzo[e][1,3]thiazin-4-one derivatives (29–40)

To a cooled solution of 6-substituted-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (1 mmol) in methanol (2 ml) was added aldehyde (1 mmol) and sodium cyanoborohydride (1 mmol) and stirred the reaction mixture at room temperature for 12 h (monitored by TLC and LCMS for completion), and solvent evaporated under reduced pressure. The residue was further diluted with water (30 ml) and ethyl acetate (50 ml) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane: ethyl acetate as eluent to give the corresponding final derivative (**29–40**) in good yield.

2-(4-Benzylpiperazin-1-yl)-6-nitro-4H-benzo[e][1,3]thiazin-4-one (29)

The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4a**) (0.1 g, 0.34 mmol), benzaldehyde (0.03 g, 0.34 mmol) and sodium cyanoborohydride (0.02 g, 0.34 mmol) to afford **29** (0.04 g, 35.16%) as yellow solid. M.p: 189–191 °C. ^{1}H NMR (DMSO-d_6):): δ_H 8.52–7.49 (m, 8H), 3.51 (s, 2H), 3.12–1.78 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.4, 145.2, 143.8, 138.5, 138.3, 130.4, 128.6, 128.4 (2C), 128.2 (2C), 127.5, 123.1, 64.5, 54.3 (2C), 49.6 (2C). ESI-MS *m*/*z* 383 $(M+H)^+$. Anal. calcd. for $C_{19}H_{18}N_4O_3S$: C, 59.67; H, 4.74; N, 14.65; Found: C, 59.66; H, 4.71; N, 14.67.

2-(4-(4-Chlorobenzyl)piperazin-1-yl)-6-nitro-4H-benzo[e][1,3]thiazin-4-one (30)

The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4a**) (0.1 g, 0.34 mmol) 4-chlorobenzaldehyde (0.04 g, 0.34 mmol) and sodium cyanoborohydride (0.02 g, 0.34 mmol) to afford **30** (0.06 g, 46.28%) as yellow solid. M.p: 204–206 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.53–7.47 (m, 7H), 3.54 (s, 2H), 3.14–1.80 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.6, 145.4, 143.7, 138.5, 136.7, 132.6, 131.4 (2C), 130.6, 128.4, 128.2 (2C), 123.7, 64.5, 54.4 (2C), 49.5 (2C). ESI-MS *m*/*z* 417 $(M+H)^+$. Anal. calcd. for $C_{19}H_{17}ClN_4O_3S$: C, 54.74; H, 4.11; N, 13.44; Found: C, 54.72; H, 4.15; N, 13.47.

2-(4-(4-Methylbenzyl)piperazin-1-yl)-6-nitro-4H-benzo[e][1,3]thiazin-4-oneone (31)

The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4a**) (0.1 g, 0.34 mmol), 4-tolualdehyde (0.04 g, 0.34 mmol) and sodium cyanoborohydride (0.02 g, 0.34 mmol) to afford **31** (0.05 g, 39.08%) as yellow solid. M.p: 217–219 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.55–7.44 (m, 7H), 3.54 (s, 2H), 3.12–1.78 (m, 8H), 2.98 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.6, 145.4, 143.7, 138.5, 136.7, 135.6, 130.6, 130.4 (2C), 128.6, 128.5 (2C), 123.2, 64.5, 54.4 (2C), 49.5 (2C), 21.4. ESI-MS *m*/*z* 397 $(M+H)^+$. Anal. calcd. for $C_{20}H_{20}N_4O_3S$: C, 60.59; H, 5.08; N, 14.13; Found: C, 60.62; H, 5.10; N, 14.11.

6-Nitro-2-(4-(4-nitrobenzyl)piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (32)

The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4a**) (0.1 g, 0.34 mmol), 4-Nitro benzaldehyde (0.05 g, 0.34 mmol) and sodium cyanoborohydride (0.02 g, 0.34 mmol) to afford **32** (0.05 g, 39.98%) as yellow solid. M.p: 201–203 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.58–7.47 (m, 7H), 3.56 (s, 2H), 3.11–1.79 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.3, 146.5, 145.5, 144.7, 143.6, 138.4, 130.6, 129.4 (2C), 128.4, 123.5, 123.2 (2C), 64.6, 54.2 (2C), 49.6 (2C). ESI-MS *m*/*z* 428 $(M+H)^+$. Anal. calcd. for $C_{19}H_{17}N_5O_5S$: C, 53.39; H, 4.01; N, 16.38; Found: C, 53.37; H, 4.02; N, 16.36.

2-(4-Benzylpiperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (33)

The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**4b**) (0.1 g, 0.31 mmol), benzaldehyde (0.03 g, 0.31 mmol) and sodium cyanoborohydride (0.02 g, 0.31 mmol) to afford **33** (0.08 g, 64.52%) as white solid. M.p: 186–188 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.60–7.52 (m, 8H), 3.54 (s, 2H), 3.14–1.82 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.2, 159.5, 140.5, 138.4, 137.5, 131.7, 130.6, 128.7 (2C), 128.4 (2C), 128.2, 127.4, 126.2, 123.2, 64.4, 54.8 (2C), 49.7 (2C). ESI-MS *m*/*z* 406 $(M+H)^+$. Anal. calcd. for $C_{20}H_{18}F_3N_3OS$: C, 59.25; H, 4.47; N, 10.36; Found: C, 59.24; H, 4.49; N, 10.35.

2-(4-(4-Chlorobenzyl)piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (34)

The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**4b**) (0.1 g, 0.31 mmol), 4-chloro benzaldehyde (0.04 g, 0.31 mmol) and sodium cyanoborohydride (0.02 g, 0.31 mmol) to afford **34** (0.08 g, 63.05%) as white solid. M.p: 182–184 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.59–7.51 (m, 7H), 3.55 (s, 2H), 3.16–1.80 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.5, 140.5, 137.4, 136.5, 132.7, 131.6 (3C), 130.4, 128.5 (2C), 128.2, 126.2, 123.2, 64.5, 54.7 (2C), 49.6 (2C). ESI-MS *m*/*z* 440 $(M+H)^+$. Anal. calcd. for $C_{20}H_{17}ClF_3N_3OS$: C, 54.61; H, 3.90; N, 9.55; Found: C, 54.60; H, 3.88; N, 9.56.

2-(4-(4-Methylbenzyl)piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (35)

The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**4b**) (0.1 g, 0.31 mmol), 4-tolualdehyde (0.03 g, 0.31 mmol) and sodium cyanoborohydride (0.02 g, 0.31 mmol) to afford **35** (0.06 g, 48.09%) as white solid. M.p: 193–195 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.61–7.54 (m, 7H), 3.52 (s, 2H), 3.14–1.83 (m, 8H), 2.98 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.3, 140.4, 137.4, 136.1, 135.7, 131.5, 130.4, 130.1 (2C),

128.7 (2C), 128.2, 126.4, 123.7, 64.6, 54.3 (2C), 49.6 (2C), 21.1. ESI-MS *m/z* 420 $(M+H)^+$. Anal. calcd. for $C_{21}H_{20}F_3N_3OS$: C, 60.13; H, 4.81; N, 10.02; Found: C, 60.15; H, 4.79; N, 10.01.

2-(4-(4-Nitrobenzyl)piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (36)

The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (**4b**) (0.1 g, 0.31 mmol), 4-nitrobenzaldehyde (0.04 g, 0.31 mmol) and sodium cyanoborohydride (0.02 g, 0.31 mmol) to afford **36** (0.08 g, 58.77%) as white solid. M.p: 183–185 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.58–7.52 (m, 7H), 3.54 (s, 2H), 3.14–1.83 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.7, 159.3, 146.4, 144.2, 140.1, 137.7, 131.5, 130.2, 129.7 (2C), 128.4, 126.2, 123.4, 123.2 (2C), 64.5, 54.4 (2C), 49.8 (2C). ESI-MS *m/z* 451 $(M+H)^+$. Anal. calcd. for $C_{20}H_{17}F_3N_4O_3S$: C, 53.33; H, 3.80; N, 12.44; Found: C, 53.29; H, 3.81; N, 12.43.

2-(4-Benzylpiperazin-1-yl)-6-chloro-4H-benzo[e][1,3]thiazin-4-one (37)

The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4c**) (0.1 g, 0.35 mmol), benzaldehyde (0.03 g, 0.35 mmol) and sodium cyanoborohydride (0.02 g, 0.35 mmol) to afford **37** (0.07 g, 54.55%) as white solid. M.p: 197–199 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.63–7.55 (m, 8H), 3.56 (s, 2H), 3.12–1.81 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 138.4, 138.2, 135.1, 134.7, 131.5, 131.2, 130.7, 128.4 (2C), 128.2 (2C), 127.4, 64.4, 54.6 (2C), 49.7 (2C). ESI-MS *m/z* 372 $(M+H)^+$. Anal. calcd. for $C_{19}H_{18}ClN_3OS$: C, 61.36; H, 4.88; N, 11.30; Found: C, 61.33; H, 4.89; N, 11.31.

6-Chloro-2-(4-(4-chlorobenzyl)piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-oneone (38)

The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4c**) (0.1 g, 0.35 mmol), 4-chlorobenzaldehyde (0.05 g, 0.35 mmol) and sodium cyanoborohydride (0.02 g, 0.35 mmol) to afford **38** (0.07 g, 50.62%) as white solid. M.p: 205–207 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.60–7.54 (m, 7H), 3.55 (s, 2H), 3.11–1.82 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 159.4, 138.4, 136.2, 135.4, 134.6, 132.5, 131.6, 131.3, 131.1 (2C), 130.4, 128.4 (2C), 64.5, 54.3 (2C), 49.7 (2C). ESI-MS *m/z* 407 $(M+H)^+$. Anal. calcd. for $C_{19}H_{17}Cl_2N_3OS$: C, 56.16; H, 4.22; N, 10.34; Found: C, 56.17; H, 4.25; N, 10.33.

6-Chloro-2-(4-(4-methylbenzyl)piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (39)

The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4c**) (0.1 g, 0.35 mmol), 4-tolualdehyde (0.04 g, 0.35 mmol) and sodium cyanoborohydride (0.02 g, 0.35 mmol) to afford **39** (0.06 g, 50.38%) as white solid. M.p: 211–213 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.62–7.52 (m, 7H), 3.52 (s, 2H), 3.12–1.81 (m, 8H), 2.98 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 138.5, 136.2, 135.4, 135.2, 134.5, 131.2, 131.1, 130.4, 130.1 (2C), 128.6 (2C), 64.4, 54.6 (2C), 49.6 (2C), 21.1 (2C). ESI-MS *m/z* 386 $(M+H)^+$. Anal. calcd. for $C_{20}H_{20}ClN_3OS$: C, 62.25; H, 5.22; N, 10.89; Found: C, 62.25; H, 5.24; N, 10.88.

6-Chloro-2-(4-(4-nitrobenzyl)piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (40)

The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (**4c**) (0.1 g, 0.35 mmol), 4-nitrobenzaldehyde (0.05 g, 0.35 mmol) and sodium cyanoborohydride (0.02 g, 0.35 mmol) to afford **40** (0.07 g, 52.72%) as white solid. M.p: 215–217 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.64–7.56 (m, 7H), 3.56 (s, 2H), 3.12–1.81 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.3, 146.5, 144.2, 138.4, 135.4, 134.9, 131.6, 131.1, 130.4, 129.8 (2C), 123.7 (2C), 64.4, 54.2 (2C), 49.4 (2C). ESI-MS *m/z* 417 $(M+H)^+$. Anal. calcd. for $C_{19}H_{17}ClN_4O_3S$: C, 54.74; H, 4.11; N, 13.44; Found: C, 54.76; H, 4.13; N, 13.42.

Enzymology

In vitro MTB DNA supercoiling assay

Supercoiling assay involves the GyrA and GyrB subunits. The holoenzyme performs the function of supercoiling the DNA. The assay was performed using MTB DNA supercoiling assay kits (Inspiralis Ltd., Norwich, UK). Briefly, the assay was performed in a 30μL reaction volume for 30 min at 37 °C in an assay buffer containing 50 mM HEPES–KOH (pH 7.9), 6 mM magnesium acetate, 4 mM dithiothreitol (DTT), 1 mM ATP, 100 mM potassium glutamate, 2 mM spermidine and 0.05 mg/mL albumin. During the assay, 1U of DNA gyrase was incubated with 0.5 μg of relaxed pBR322 in the assay buffer for 30 min. Various concentrations of the compounds were diluted and incubated along with the reactants. The incubation time was optimized based on the interaction and activity of the protein with the double stranded DNA. Subsequently, the reaction was quenched by addition of an equal volume of 30 μL of chloroform:isoamyl alcohol (24:1) and STEB buffer [sucrose–Tris–HCl–ethylene diamine tetra-acetic acid (EDTA)–bromophenol blue], with a brief vortex followed by centrifugation [14]. Products were analyzed by electrophoresis on 1% agarose gels after staining with ethidium bromide. Using Image Lab TM software (Bio-Rad), the intensity of bands was measured and analyzed to determine enzyme inhibition by relative band intensity comparing with the control. In this assay too, novobiocin was set as a standard.

In vitro MTB MABA assay

Briefly, the MTB inoculum was prepared from fresh LJ medium re-suspended in 7H9-S medium (7H9 broth, 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase [OADC]), adjusted to a McFarland tube No. 1, and diluted 1:20; 100 μL was used as inoculum [15]. Each drug stock solution was thawed and diluted in 7H9-S at four-fold the final highest concentration tested. Serial twofold dilutions of each drug were prepared directly in a sterile 96-well microtiter plate using 100 μL 7H9-S. A growth control containing no antibiotic and a sterile control were also

prepared on each plate. Sterile water was added to all perimeter wells to avoid evaporation during the incubation. The plate was covered, sealed in plastic bags and incubated at 37 °C in normal atmosphere. After 7 days incubation, 30 μL of alamar blue solution was added to each well, and the plate was re-incubated overnight. A change in colour from blue (oxidized state) to pink (reduced) indicated the growth of bacteria, and the MIC was defined as the lowest concentration of drug that prevented this change in colour.

In vitro cytotoxicity studies

Eukaryotic RAW 264.7 mouse macrophage cells were used to test the cytotoxic activity of all the compounds [16]. The toxicity was measured by incubating the test compounds in 96 flat-bottomed well plates containing a cell count of 5×10^5 at different concentrations, with 5% CO_2 and 95% O_2 atmosphere for 48 h at 37 °C. About 4 h, before the end of incubation period 10 μL of MTT reagent (10 mg mL^{-1}) was added, the plate was centrifuged at 1200 rcf for about 3 min to obtain a clear supernatant, the supernatant was removed, and subsequently to each well 200 μL of DMSO was added to dissolve the formed formazan crystals [6]. The absorbance was measured at a wavelength of 560 nm on Perkin Elmer Victor X3 microplate reader against the blank after a span of 10 min. The assay was performed in triplicates for each concentration of drug to minimize the error rate. The cytotoxicity of each compound was expressed as% inhibition at that particular concentration.

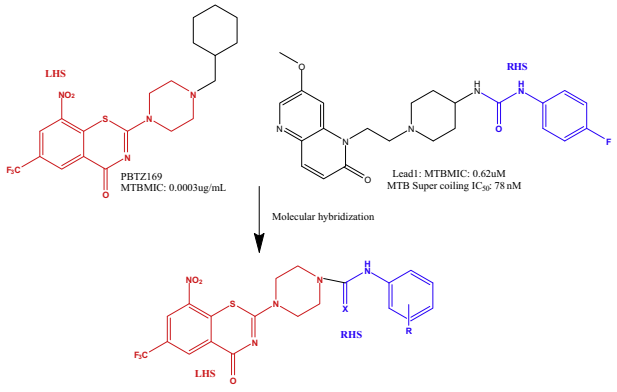
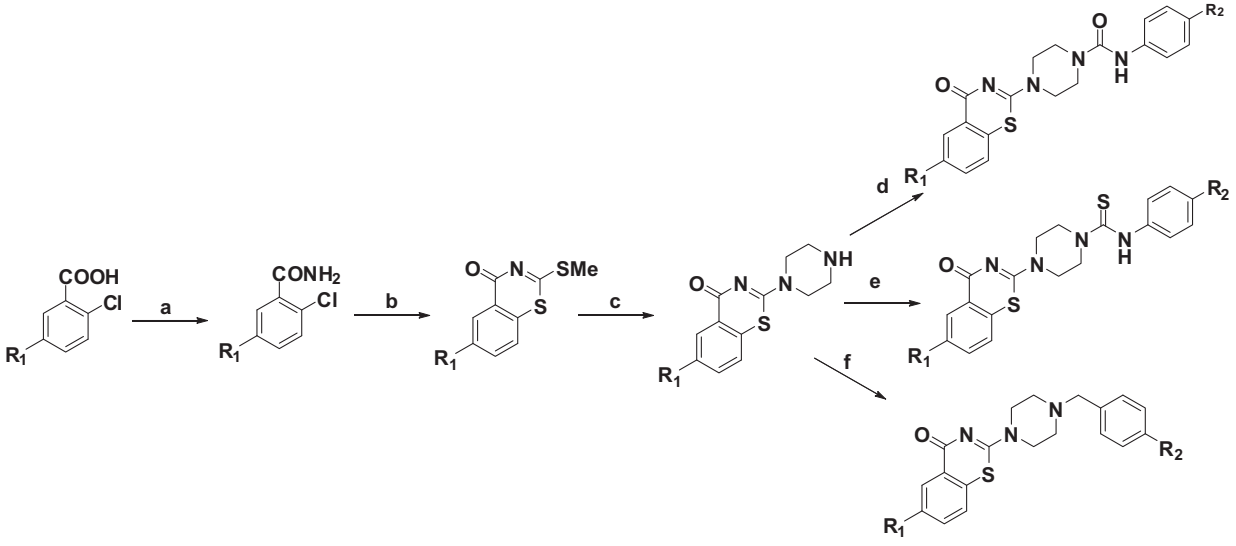


Figure 1 – Strategy employed for designing the lead. Chemical structure of previously reported antitubercular benzothiazinone bearing (left-hand side) derivative PBTZ169 and MTB DNA gyrase inhibitor bearing aryl (thio) urea right hand side chain and the inhibitor designed through molecular hybridization.

Results and discussion

Design and synthesis

In this paper, novel MTB DNA gyrase inhibitors were designed by using a molecular hybridization technique. This novel terminology in the field of drug design and development where fusion/hybridization of two or more pharmacophoric subunits occurs from the molecular structure of ligands/prototypes was previously reported to have an inhibitory effect against the targeted disease. This newly designed hybrid can lead to compounds with improved affinity and efficacy with lower side effects than the parent template compounds, while retaining the desired characteristics of the original template. Earlier, various literatures have explored this methodology in designing newer analogues as potential candidate drugs for biological evaluation. Encouraged by the previous successful research efforts in this regard [17], it was decided to further extend the above methodology to identify novel starting points to design inhibitors for ATPase domain of mycobacterial gyrase (see Fig. 1).

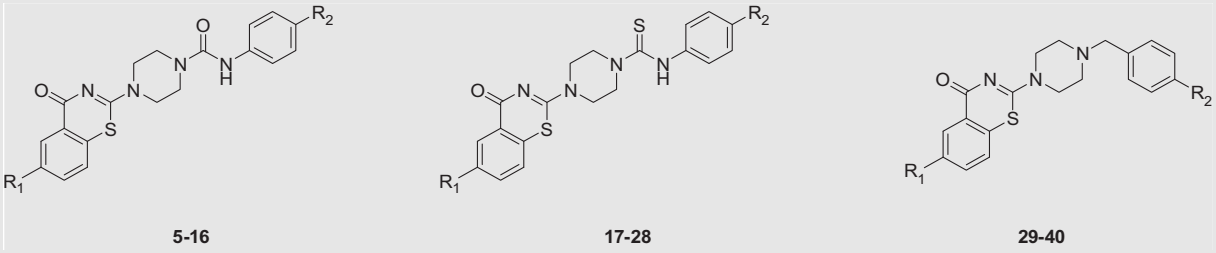


Reagents & conditions : (a) (i) Oxalyl chloride, DCM (ii) NH_4OH, ACN, 6h (b) CS_2, MeI, DMSO, 1h (c) 1-Boc-piperazine , TFA, DCM, 6h (d) R_2NCO, TEA, DCM, 12h (e) R_2NCS, TEA, DCM, 12h (f) R_2CHO, $NaBH_3CN$, CH_3OH, 12h.

Scheme 1 – Synthetic protocol adopted for the lead derivatization.

The synthetic pathway used to achieve the target compounds has been delineated in Scheme 1. Synthesis of the compounds started with conversion of commercially available substituted 2-chlorobenzoic acids into corresponding 2-chlorobenzoyl chlorides by DMF-catalyzed treatment in the presence of oxalyl chloride in dichloromethane. The obtained 2-chlorobenzoyl chlorides were converted into corresponding amide intermediate by drop wise addition of 25% aqueous ammonia at −20 °C. The amide intermediates were further treated with carbon disulphide, methyl iodide and sodium hydroxide in DMSO to afford the thioalkylated products, which upon treatment with 1-Boc-piperazine in ethanol followed by deprotection using trifluoroacetic acid gave the scaffolds in good yield. The final library was then assembled by treating the obtained scaffolds with the desired isocyanates/isothiocyanates and aldehydes to afford compound **5–40** in excellent yields. A series of 36 derivatives were prepared using the above method for the present study; both analytical and spectral data (^{1}H NMR, ^{13}C NMR, and mass spectra) of all the synthesized compounds were in full agreement with the proposed structures.

Table 1 – Enzyme inhibitory effect of synthesized compounds and their antitubercular activity.



Comp	R_1	R_2	Supercoiling assay (IC_{50}) (μM)	MIC (μM)	Cytotoxicity in RAW 264.7 cells
5	NO_2	H	16.84 ± 0.27	30.38	ND
6	NO_2	Cl	23.71 ± 0.21	28.03	ND
7	NO_2	CH_3	9.62 ± 0.17	14.68	16.24
8	NO_2	NO_2	9.11 ± 0.34	13.67	15.23
9	CF_3	H	10.95 ± 0.22	19.18	ND
10	CF_3	Cl	10.52 ± 0.26	13.32	10.24
11	CF_3	CH_3	5.26 ± 0.35	11.54	7.56
12	CF_3	NO_2	26.45 ± 0.31	52.14	ND
13	Cl	H	17.51 ± 0.28	15.59	16.52
14	Cl	Cl	10.46 ± 0.24	17.91	2.56
15	Cl	CH_3	2.62 ± 0.13	6.02	5.14
16	Cl	NO_2	4.61 ± 0.18	5.24	3.56
17	NO_2	H	0.77 ± 0.06	1.82	3.25
18	NO_2	Cl	0.51 ± 0.16	4.41	1.81
19	NO_2	CH_3	0.82 ± 0.03	2.83	4.22
20	NO_2	NO_2	2.59 ± 0.14	5.29	2.21
21	CF_3	H	0.83 ± 0.18	4.62	6.48
22	CF_3	Cl	4.23 ± 0.33	8.59	3.15
23	CF_3	CH_3	4.51 ± 0.26	9.54	16.45
24	CF_3	NO_2	2.16 ± 0.11	5.04	5.48
25	Cl	H	10.93 ± 0.34	11.22	2.35
26	Cl	Cl	6.86 ± 0.24	6.92	25.64
27	Cl	CH_3	11.56 ± 0.18	14.5	2.48
28	Cl	NO_2	6.23 ± 0.15	13.52	1.45
29	NO_2	H	3.21 ± 0.32	4.08	5.41
30	NO_2	Cl	3.68 ± 0.19	5.99	1.56
31	NO_2	CH_3	8.93 ± 0.24	15.76	26.54
32	NO_2	NO_2	4.21 ± 0.33	7.31	21.35
33	CF_3	H	11.52 ± 0.17	15.41	ND
34	CF_3	Cl	3.86 ± 0.13	6.31	8.45
35	CF_3	CH_3	11.63 ± 0.98	16.73	18.56
36	CF_3	NO_2	4.55 ± 0.31	5.19	14.56
37	Cl	H	17.64 ± 0.66	33.61	ND
38	Cl	Cl	12.11 ± 0.42	15.38	5.36
39	Cl	CH_3	21.54 ± 0.59	24.25	2.36
40	Cl	NO_2	7.33 ± 0.36	14.99	5.48
Novobiocin			0.068 ± 0.07	>200	7.56

Biological evaluation

The supercoiling of the DNA is required to maintain the bacteria in a viable state. All the 36 inhibitors synthesized were screened for their effectivity to inhibit the supercoiling activity of the DNA gyrase. Among the tested compounds, (**18**) emerged at sub micro molar inhibitory profile. The nitro group at the R_1 position and the chloro group at the R_2 positions were favourable for the effective inhibition of the benzothiazinone derivative with an IC_{50} of 0.51 ± 0.16 μM. Compounds synthesized had various strong electron withdrawing substitutions like nitro, trifluoro and weak chloro groups, at the substituted R_1 position while the R_2 was substituted with hydrogen, chloro, methyl and nitro groups. Among the twelve R_1 substituted nitro groups, ten molecules showed an IC_{50} of less than 10 μM, only compound (**5**) and (**6**) had lower inhibition rates highlighting the importance of the nitro group substitution at this position. All the enzyme studies were carried out in the presence of standard drug novobiocin with an IC_{50} was 68 nM. Among the 36 compounds subjected to inhibitory assays, compound (**18**) N-(4-chlorophenyl)-4-(6-nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperazine-1-carbothioamide was the most potent analogue. Four compounds (**17**, **18**, **19** and **21**) showed nano molar range inhibitions in the supercoiling assay as shown in Table 1. Furthermore, all the compounds were then subjected to a number of tertiary screens in order to assess their *in vitro* anti-tubercular potency and cell cytotoxicity insights. Moreover, one of the major hurdles in target-based drug discovery is the lack of translation of the drug potency and selectivity observed at the enzyme level to that of mycobacterial cidality. In order to re-ascertain the compounds efficiencies, molecules were first subjected to whole cell screening against MTB H37Rv strain using the broth dilution method [15] with compound concentrations ranging from 50 to 1.56 μg/mL in triplicates. Fifteen compounds showed an MIC of less than 10 μM concentration highlighting the importance of this series in the bactericidal activity. Compounds (**17**), (**19**) and (**21**) had MIC less than 5 μM. The most active compound (**18**) in the DNA gyrase enzyme assay showed 4.41 μM MIC probably due to the cell efflux pumps, while the compound (**17**) had a better MIC than (**18**) owing to its neutral R_2 positions H group. The standard compound novobiocin which showed excellent activity in MTB DNA gyrase was completely inactive in the log phase culture of MTB. None of the compounds were more potent than earlier reported benzothiazinone derivatives [12,13]. Further, all the synthesized compounds were further screened for their *in vitro* cytotoxicity's in the RAW 264.7 mouse macrophage cell lines [16], since the predominant host cells for MTB are the lung macrophages. The concentration was set at 100 μM and the assay was done by the (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Compounds with an MIC of less than 15 μM alone were tested for the cytotoxicity studies. All those compounds tested showed below 25% cell toxicities. The most active compound (**18**) had 1.81% inhibition comparatively much less than the standard novobiocin. All the results are represented in Table 1 [18,19].

Conclusion

In the continuous efforts to discover novel antimicrobial compounds with anti-gyrase activity, this study has described the discovery of benzothiazinone as a gyrase inhibitor with potent MTB MIC and inhibitory profiles of the gyrase enzyme with a well correlated structural activity relationship and less cytotoxic effect. Furthermore, it is believed that this class of compounds has further potential to be developed as an anti-TB drug candidate.

Conflict of interest

No conflict of interest is perceived and none declared.

Acknowledgement

DS acknowledges University Grants Commission, New Delhi, India for UGC Research Award.

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