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Graphical Abstract

Benzo[*d*]thiazol-2-yl(piperazin-1-yl)methanones as New Anti-mycobacterial Chemotypes: Design, Synthesis, Biological Evaluation and 3D-QSAR Studies

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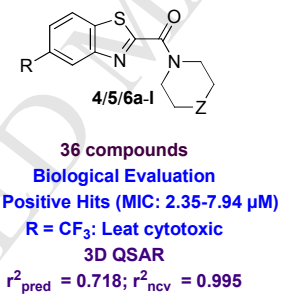

The benzo[*d*]thiazol-2-yl(piperazin-1-yl)methanones scaffold has been identified as new anti-mycobacterial chemotypes. The quantitative structure activity relationship has been established adopting a statistically reliable CoMFA model showing high prediction (r^2_{pred} = 0.718, r^2_{ncv} = 0.995).

Benzo[*d*]thiazol-2-yl(piperazin-1-yl)methanones as New Anti-mycobacterial Chemotypes: Design, Synthesis, Biological Evaluation and 3D-QSAR Studies

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Abstract: The benzo[*d*]thiazol-2-yl(piperazin-1-yl)methanones scaffold has been identified as new anti-mycobacterial chemotypes. Thirty-six structurally diverse benzo[*d*]thiazole-2-carboxamides have been prepared and subjected to assessment of their potential anti-tubercular agents through in vitro testing against *M. tuberculosis* $H_{37}Rv$ strain and evaluation of cytotoxicity against RAW 264.7 cell lines. Seventeen compounds showed anti-mycobacterial potential having MICs in the low (1-10) µM range. The 5-trifluoromethyl benzo[*d*]thiazol-2-yl(piperazin-1-yl)methanones emerged to be the most promising resulting in six positive hits (2.35-7.94 µM) and showed low-cytotoxicity (<50% inhibition at 50 µg/mL). The therapeutic index of these hits is 8–64. The quantitative structure activity relationship has been established adopting a statistically reliable CoMFA model showing high prediction (r^2_{pred} = 0.718, r^2_{ncv} = 0.995).

Keywords: Tuberculosis, benzo[*d*]thiazole-2-carboxamides, new antibacterial chemotypes, Anti-mycobacterial activity, COMFA, and 3D-QSAR.

1. Introduction

Tuberculosis (TB), caused by the infectious mycobacteria *Mycobacterium tuberculosis* (Mtb),[1] is responsible for the highest death globally. The disease affected about 9.0 million people and caused 1.5 million deaths [includes 360,000 patients co-infected with HIV (Human Immunodeficiency Virus] in 2013. Being next to Acquired Immuno Deficiency Syndrome (AIDS), TB is the second cause of death amongst the infectious diseases in the global scenario.[2]

The growth of TB is a threat to public health majorly due to the development of multi (MDR-TB; tolerant to isoniazid and rifampicin), extensively (XDR-TB; tolerant to all the first and a few second line drugs),[3] and the lately covered totally drug resistant Mtb (TDR-TB)[4] strains. The spread of MDR-TB could increase the cost of the available treatment between 100 and 1400 times apart from the threat to make TB incurable. At present, 5.3% of TB incidences are due to MDR-TB. The World Health Organization (WHO) reports yearly 490,000 new MDR-TB infections causing > 110,000 deaths and has announced TB a worldwide health crisis.[3] Most of first line TB drugs had been discovered in 1950s and 1960s. For nearly half a century, TB treatment lacked the availability of new drug until bedaquiline was launched at the end of 2012 to treat MDR-TB.[5] All the above facts necessitate the re-engineering and repositioning of synthetic bioactive for the development of new anti-TB drugs. The present work relates to the findings of benzo[*d*]thiazol-2-yl(piperazin-1-yl)methanones as new chemotypes with anti-TB activity.

Insert Figure 1 here.

1.1. Design

Encouraged by our recent findings on the anti-TB activity of benzo[*d*]thiazole-2-carboxamides,[6] we planned to search for more effective anti-TB new molecular entities and adopted scaffold hopping strategy[7] to construct new anti-bacterial chemotypes. In search for different antibacterial scaffolds we noticed that diverse new chemical entities bearing functionalized piperazine motif exhibited anti-TB potential (motifs A-H, Figure 1)[8-15].

In the present study, we designed the benzo[*d*]thiazol-2-yl(piperazin-1-yl)methanone scaffold (**I**) as new anti-TB chemotype through hybridization by covalent attachment of the piperazine moiety with the benzo[*d*]thiazole-2-carboxamide framework (Figure 2). The current pharmaceutical applications of benzothiazoles for treatment of AIDS[16] suggest anti-TB potency of the benzo[*d*]thiazol-2-yl(piperazin-1-yl)methanones through synergistic intervention of the TB-AIDS co-infection and provide support to the design of scaffold **I**.

Insert Figure 2 here.

2. Results and Discussion

2.1. Chemistry

The target benzo[*d*]thiazol-2-carboxamides can be prepared through two step reactions: aquatic cyclocondensation of aldehydes with 2-aminothiophenol **1a** at 110 ˚C[17] or at room temperature under the catalytic presence of SDOSS (sodium dioctyl sulfosuccinate) [18] followed by NH_4Cl catalyzed amide coupling[6] with diverse alicyclic amines **3a-l** (Scheme 1). The SDOSS-catalysed reaction (5 h) of **1a** with ethyl glyoxalate in water formed **2a** (83%). Following similar procedures two more derivatives ethyl 5-chloro benzo[*d*]thiazole-2-carboxylate **2b** and ethyl 5-trifluoromethyl benzo[*d*]thiazole-2-carboxylate **2c** were synthesized from the corresponding 4-chloro (**1b)** and 4-trifluoromethyl (**1c)** substituted 2-aminothiophenols.

Insert Scheme 1 here.

The desired amides **4**/**5**/**6a-l** were formed from ethyl benzo[*d*]thiazole-2-carboxylate derivatives **2a**/**2b**/**2c** and diverse piperazine derivatives **3a-l** under neat condition using NH_4Cl as catalyst (20 mol%) at 100 °C in moderate to good yields (Table 1). Dilution of the reaction mixture with cold water, filtration of the precipitated solid afforded the desired products (total thirty-six compounds) without any further requirement of purification.

Insert Table 1 here.

2.2. Biological Evaluation

The anti-tuberculosis activity of the synthesized thirty-six benzo[*d*]thiazole-2-carboxamide conjugates were evaluated against *M. tuberculosis* $H_{37}Rv$ (ATCC27294) strain.[19] The minimum inhibitory concentration (MIC; µg/mL) values of all the

synthesized compound along with the standard drugs Isoniazid (INH), Rifampin (R), Ethambutol (E), Pyrazinamide (Z) and Ciprofloxacin (Cfx) were determined thrice at pH 7.4 (Table 1). All the synthesized compounds exhibited MICs in the micromolar range, varying from 0.78-25 µg/mL. Out of thirty six compounds, eighteen compounds (**4a**, **4d**, **4e**, **4f**, **4g**, **4i**, **4j**, **4l**, **5g**, **5h**, **5i**, **5l**, **6a**, **6b**, **6e**, **6h**, **6j** and **6k**) exhibited MICs values of 0.78 - 3.125 µg/mL. In comparison with standard drugs E (1.56 µg/mL), Cfx (1.56 µg/mL), and Z (6.25 µg/mL) the synthesized compounds **4l**, **5l**, **6a** and **6b** (MIC of 0.78 µg/mL) were found to be more potent.

2.3. Cytotoxicity Evaluation

The cytotoxicity (determined at 50 µg/mL) of the compounds with anti-mycobacterial potency ≤ 6.25 µg/mL was evaluated against RAW 264.7 (mouse leukemic monocyte macrophage) cell lines by adopting (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) MTT assay (Table 1). The graphical representation of the anti-TB activity (Figure 3) and cytotoxicity of the synthesized compounds (activity ≤ 6.25 µg/mL; Figure 4) indicate that all CF_3 derivatives are less-cytotoxic compared to H and Cl derivatives. Out of the most active compounds (**4l**, **5l**, **6a** and **6b**), the compound **6a** and **6b** found to be more potent (MIC of 2.47 and 2.35 µM) and less cytotoxic (24.56% and 18.12% inhibition) and therapeutic index >60.

Insert Figure 3 here.

Insert Figure 4 here.

2.4. Evaluation of drug-like properties of the active compounds

The intermediate **2a** was thirty-two fold less active than **4l**. By attaching the 1-(diphenylmethyl)piperazine group, the lipophilicity of **2a** (cLogP, calculated using ChemDraw Ultra version 12.0.) increased from 2.3 to 4.72. The lipophilicity is an important attribute for the permeation of the compounds across the high lipid containing mycobacterium cell wall.[20] The most promising compound **5l** (MIC of 1.74 µM) has been found with higher cLogP value (5.44) and comparison of the cLogP data of compounds with those of the standard drugs reveal that the synthetic analogues are more lipophilic in nature compared to the first line drugs i.e., INH (-0.668), E (0.118), and Z (-0.676). The compounds with MIC ≤ 6.25 µg/mL were further subjected to analysis

for drug likeness such as the "Lipinski rule of 5".[21] The Lipinski properties (molecular weight, no. of hydrogen bond donors, no. of hydrogen bond acceptors and no. of rotational bonds) were computed using Accelrys Discovery Studio 2.5 and all the twenty-five hits qualify drug likeness property (Table 2).

Insert Table 2 here.

A complete structure activity relationship (SAR) can be drawn by correlating the anti-mycobacterial activity (μM) of the tested compounds (Table 1). Among the initially tested compounds **4a-c**, **5a-c** and **6a-c** by exploring combination of morpholine, thiamorpholine and piperidine ring with their corresponding 5-H, 5-Cl and 5-CF_3 derivatives, trifluoromethyl group gave superior activity among their other counterpart (Figure 5). Due to the limitation of further diversity generation in the scaffold with morpholine, thiamorpholine and piperidine fragment, piperazine derivatives were explored. Among twenty-seven piperazine derivatives synthesized, biological activity of fourteen compounds (**4e**, **4f**, **4g**, **4i**, **4j**, **4l**, **5g**, **5h**, **5i**, **5l**, **6e**, **6h**, **6j** and **6k**) were found to be between 1-10 μM.

Insert Figure 5 here.

To establish quantitative correlation among various piperazine derivatives the 3D-QSAR technique comparative molecular filed analysis (CoMFA)[7a,22] was applied.

2.5. 3D-QSAR

Using SYBYL 7.1 (Tripos Inc., USA) molecular modeling package,[23] CoMFA model was developed to recognize the morphological characteristics requisite for the anti-TB potential. Alignment of compounds in their lowest energy state was considered using benzothiazole as common fragment (Figure 6). The statistically competent CoMFA model was developed and corroborated by the partial least squares (PLS) method. The regression analysis of CoMFA field energies has been performed using PLS algorithm with the leave-one-out (LOO) method acquired for cross validation. The statistical parameters of the model derived from the benzo[*d*]thiazole-2-carboxamides series are summarized in Table 2.

The CoMFA gave the prediction of the activity of the test set compounds within statistically permissible range. Plot of experimental pMIC versus the predicted pMIC value of the test set and training set (Figure 8) demonstrated the reliability of the CoMFA model

to serve as useful tool for prediction of the anti-mycobacterial potential of newly designed benzo[*d*]thiazole-2-carboxamides (Table 3).

Insert Figure 6 here.

Insert Table 3 here.

2.5.1. Data Set and Biological Data

For 3D-QSAR analyses, 23 compounds with defined MIC values were employed. Total compounds were divided into a training set of 18 compounds, and a test set of 5 compounds. The most important step in the QSAR is the selection of a suitable training set with wide activity range, responsible for determining the quality of the generated QSAR model. The calculated MIC values (pMIC = -log MIC) of dataset spanned across a small range from 4.99 to 7.91. These activity values were rescaled to the range of four log units to develop statistically reliable model. To rescale the activity data following formula was employed. The test compounds were selected manually considering the structural diversity and wide range of activity in the data set.

Rescaling data set:

Existing series = a-b, Rescale series = x-y, Rescale value = x + (n-a)*[(y-x)/(b-a)]

=3.92+(n-4.04)*[4/1.84]

Where n is query. In our study, x= 3.92, y=7.92, a= 3.92, b= 5.76

2.5.2. Molecular modeling

All the molecular modeling calculations were performed using SYBYL 7.1 (Tripos Inc., USA) molecular modeling package installed on a Silicon Graphics Fuel Workstation running IRIX 6.5. Structures of all monophosphonate derivatives were generated using sketch molecule module. Compound **5l** being the most potent was selected as a template molecule (Figure 6). Geometry-optimized was carried out by applying Tripos molecular mechanics force field with conjugate gradient method. No constraints were applied on the internal geometries of the molecules. The minimization was terminated when the energy gradient convergence criterion of 0.001 kcal/mol was reached or when the 10,000 steps

minimization cycle was exceeded. Gasteiger-Hückel charges were applied to all the molecules of dataset, used for 3D-QSAR studies.

Insert Figure 7 here.

Molecular alignment is considered one of the most sensitive parameter in CoMFA analysis. Each molecule was aligned to the lowest energy conformation of the most active compound **5l**, (Figure 7) by performing an rms fitting of each conformer to those of the template using the alignment function of the Sybyl 7.1. The aligned molecules obtained through pairwise super positioning using the maximum common subgroup method, placed all molecules in the same reference frame as the reference compound shown in Figure 6.

2.5.3. Calculation of CoMFA descriptors

For generating CoMFA contour maps, a 3D cubic lattice with grid spacing of 2.0 Å was created around aligned molecule. For CoMFA, the steric (The Lennard-Jones potential) and electrostatic (Coulombic potential) field at each lattice point was calculated using the default probe, a sp^3 carbon atom with a charge of +1 and a van der Waals radius of 1.52 Å. The steric and electrostatic fields at these lattice points were calculated using Tripos force field. Energy values for these fields were truncated at 30 kcal/mol. The minimum column filtering was set to 2.0 kcal/mol to improve the signal-to noise ratio by retaining best fit model while omitting those lattice points whose energy variation was below this threshold.

2.5.4. Calculation of CoMFA descriptors

The optimal number of components in the final model was determined by using leave-one-out (LOO), a cross-validation method. The non-cross-validated conventional analysis was produced with the optimal number of components equal to that yielding the highest r^2_{cv}. Final model was evaluated based on the standard error of estimation values (SEE), non-cross validated r^2_{ncv} value and also by the F test value.

2.5.5. Predictive correlation coefficient

The predictive abilities were determined from a test set of 5 compounds. MIC values for test molecules were predicted by using developed CoMFA. The predictive correlation coefficient (r^2_{pred}), based on the molecules of test set, was calculated according to the equation shown below;

$$r^2_{pred} = \frac{(SD - PRESS)}{(SD)}$$

Where SD is the sum of squared deviations between the inhibitory activities of the test set and mean activities of the training molecules and PRESS is the sum of squared deviations between predicted and actual activity values for each molecule in the test set.

All test set compounds were predicted within statistically acceptable range using the CoMFA model. The scattered plots of experimental pMIC against the predicted pMIC value of the training set and test set is shown in Figure 8. These results suggest that the model is reliable and it could serve as a useful tool for predicting the MIC values of newly designed benzo[*d*]thiazole-2-carboxamides (Table 4).

Insert Figure 8 here.

Insert Table 4 here.

2.5.6. CoMFA Contour Maps and Discussion

Insert Figure 9 here.

The steric and electrostatic contributions in the contour maps for the statistically reliable CoMFA model were observed to be 47.6% and 52.4%, respectively. Green and yellow contours represent the steric interactions whilst red and blue contours represent the electrostatic interactions as shown in Figure 9. Green contours represent the place where bulkier groups are expected to favour the anti-TB activity while the yellow contours coded for less favored bulky groups for biological activity. In a similar way, the red contours display the region bearing electronegative group that is anticipated to enhance the anti-mycobacterial potential and the blue plots predict the region having positive charge that is anticipated to contribute to increase the anti-TB activity.

Figure 9a shows the distribution of steric fields generated around the compound **4i**. A large green contour present at the center of the phenyl ring shows favorable activity for the presence of a bulky group such as aromatic substituent's **4f** and **5f** compared to their aliphatic counterpart **4d** and **5d** respectively. However, in the case of CF_3 substituent **6f** there is reverse trend. For CF_3 substituents, hydrophobic group are unfavorable (**6f** and **6l**) whereas aliphatic (**3d**, **3e**) and heterocyclic rings (**6a**, **6b**, **6j**, **6k**) show positive correlation

with their biological activity. This is the reason why in case of CF_3 derivative, hydrophobic substituent (**6f-l**) gave unexpected low activity compared to their H (**4f-l**) and Cl (**5f-l**) counterparts. Similar reverse trend is observed in case of small green contour present at *ortho* methoxy substituent, where H and Cl derivative (**4g**, **5g**) shows favorable activity, on the other hand, CF_3 derivative **6g** show lower activity. Moreover, presence of yellow contour at the *para* position suggests avoiding the hydrophobic groups in future design.

In Figure 9b, two red contours were sighted in close proximity to the *ortho* and *para* position of the phenyl ring. The red contour indicates that electronegative group at the *ortho* and *para* position augments the anti-tuberculosis activity. As seen in SAR, the presence of acetyl or methoxy group at the para position of the phenyl ring gave positive impact in all three derivatives **4i**, **5h**, **5i**, **6h**, **6i** (except **4h**) compared to unsubstituted phenyl ring **4f**, **5f** and **6f** respectively. Presence of small blue contour at the *meta* position of the phenyl ring suggests to incorporate electropositive group in future design of the molecules.

Apart from this, in case of morpholine, thiamorpholine and piperidine derivatives, future direction include, there is either scope to incorporate more variation in the benzenoid nucleus of the benzo[*d*]thiazole ring or keeping CF_3 position constant or even bioisosteric replacement of benzo[*d*]thiazole ring with benzoxazole and benzimidazole ring might enhance anti-TB activity. Overall SAR of this series suggests that introduction of various aryl substituent in H and Cl derivative improves the anti-TB activity whereas heteroaryl and alicyclic amine will enhance the anti-TB activity of the CF_3 derivatives. As per the 3D-QSAR's steric and electrostatic contour there is scope to incorporate more variation by incorporating electron donating and withdrawing functional group in the diphenyl substituent of the **4l** and **5l**.

3. Conclusions

In conclusion, in this work a new series of benzo[*d*]thiazol-2-yl(piperazin-1-yl)methanones have been designed through molecular hybridization of *N*-benzyl benzo[*d*]thiazole-2-carboxamides and alicyclic piperazines. Thirty-six new analogues were prepared by amide coupling of ethyl benzo[*d*]thiazole-2-carboxyalte with various cyclic amines **3a-l** using ammonium chloride as catalyst under solvent free condition in moderate to good yields. Adopting the MABA assay, all these thirty six derivatives have been evaluated i*n vitro* for

anti-TB activity against *M. tuberculosis* $H_{37}Rv$ (Mtb) strain. Out of thirty six compounds synthesized, twenty-seven compounds have shown biological activity comparable to clinically used standard drug pyrazinamide (6.25 µg/mL) and nineteen compounds displayed anti-TB activity ranging from 0.78 to 3.125 µg/mL. The cytotoxicity of all the potent compounds was performed against RAW 264.7 cell lines and found among three series, CF_3 derivatives (**6a-l**) are specifically having low cytotoxicity. CF_3 derivative stood different from H and Cl derivative by being potent and non-cytotoxic, providing future direction for design. The results described here demonstrate the potential utility of benzo[*d*]thiazol-2-yl(piperazin-1-yl)methanones as anti-tubercular agents. The favourable drug like properties in conformity with the Lipinski rule and devoid of cytotoxicity of the most active compounds would provide further impetus to derive new chemical entities with optimized structure as potent anti-mycobacterial drug candidate.

4. Experimental Section

Chemicals and all solvents were commercially available (Aldrich Chemical, Merck AG, Fluka, Alfa Aesar and S-D Fine Chemicals) and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Advance DX spectrometer at 400 and 100 MHz, respectively, with TMS as an internal standard and using $CDCl_3$/MeOD/DMSO as solvent. Coupling constants were reported in hertz (Hz). ^{13}C NMR spectra were fully decoupled. For analysis of the results of NMR, Topspin software was used. The abbreviations used to characterize the signals are as follows: s = singlet, m = multiplet, d = doublet, dd = doublet of doublet, dt = doublet of triplet, t = triplet, q = quartet, br s = broad singlet. Mass spectra were measured in the APCI mode at an ionization potential of 70 eV with LCMS MSD (Hewlett Packard) and on a GCMS-QP 5000 (Shimadzu) (for EI) mass spectrometers; Infra-red spectra were recorded on Perkin Elmer FT-IR spectrometer in the range of 4000-600 cm^{-1} either as neat samples or using KBr for preparing pellets for solid samples or in solvent. Compounds were routinely checked for their purity on the silica gel GF-254 and visualized under UV at wavelength 254 nm. Melting points were measured with Gupta Scientific melting point apparatus. Evaporation of solvent was performed at reduced pressure, using a Buchi rotary evaporator. HPLC (model SCL-10AVP, Shimadzu, Japan) of all the target compounds has been performed using Qualisil Gold C18 column (4.6 × 250 mm, 5 micron, LCGC Chromatography Solution Pvt. Ltd) and acetonitrile :

water (80:20) as the mobile phase at 30 ºC utilizing 20 µL of the sample with flow rate of 1 mL min^{-1} using binary pump. Photo diode array detector (model no. SPD-M20A) was used for variable wavelength ranging from 200-800 nM. For interpretation of the results of HPLC, Class VP software was used. Elemental analyses were performed on organic element analyzer (Thermo SCIENTIFIC FLASH 2000) and, indicated by the symbols of the elements or functions were within ± 0.4 % of the theoretical values. For interpretation of the results of elemental analysis, Eager Xperience software was used.

The following compounds [**4a-d**, **4i**, **4l**, **5d**, **5f** and **4f**] are commercially available and intermediates (**2a-c**)[6] are thus reported.

4.1 Experimental procedure for the synthesis

Synthesis of ethyl 5-substituted benzo[*d*]thiazole-2-carboxlate (2a/2b/2c)

To a magnetically stirred miceller solution of SDOSS (44 mg, 10 mol %) in demineralised water (2 mL), was added 2-aminothiophenol **1a** (0.12 mL, 1 mmol, 1 equiv) and ethyl glyoxalate (0.118 mL, 1 mmol, 1.2 equiv) and the mixture was stirred at room temperature. After completion of the reaction (5 h, TLC), the reaction mixture was extracted with EtOAc (4 × 15 mL). The combined EtOAc extracts were washed with saturated brine (15 mL), dried (anhydrous Na_2SO_4), filtered and concentrated under vacuum rotary evaporation. The crude product was purified by passing through a column of silica gel (60-120 mesh) and eluted with hexane-EtOAc to afford the pure **2a**. Following this general procedure **2b** and **2c** were prepared by the cyclocondensation of ethyl glyoxalate with 2-amino-4-chlorothiophenol **1b** and 2-amino-4-(trifluoromethyl)thiophenol **1c**, respectively.

4.1.1 Ethyl benzo[*d*]thiazole-2-carboxylate (2a): Yield: 172 mg, 83% (yellow solid), mp 68-72°C. IR (KBr) ν: 1750 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm): 8.25 (d, J = 8.16 Hz, 1H), 7.97 (d, J = 7.92 Hz, 1H), 7.60-7.52 (m, 2H), 4.56 (q, J = 7.12 Hz, 2H), 1.49 (t, J = 7.12, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ(ppm): 160.68, 158.57, 153.22, 136.78, 127.54, 127.09, 125.52, 122.08, 63.13, 14.29. MS (APCI) *m/z* 208.21 $(M + H)^+$.

4.1.2 Ethyl 5-chlorobenzo[*d*]thiazole-2-carboxylate (2b): Yield: 176 mg, 73% (yellowish solid), mp 91-93°C. IR (KBr) ν: 1743 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.24 (d, J = 1.88 Hz, 1H), 7.91 (d, J = 8.68 Hz, 1H), 7.53 (dd, J = 8.64, 1.96 Hz), 4.57 (q, J = 7.16

Hz, 2H), 1.50 (t, *J* = 7.12 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 160.35, 160.31, 153.95, 134.98, 133.28, 128.26, 125.05, 122.89, 63.37, 14.27. MS (ESI) (*m/z*) 242.23 $(M)^+$.

4.1.3 Ethyl 5-(trifluoromethyl)benzo[*d*]thiazole-2-carboxylate (**2c**): Yield: 193 mg, 70% (white solid), mp 73-75 °C. IR (KBr) ν: 1736 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.53 (s, 1H), 8.13 (d, *J* = 8.52 Hz, 1H), 7.79 (dd, *J* = 8.52, 1.32 Hz, 1H), 4.60 (q, *J* = 7.12 Hz, 2H), 1.52 (t, *J* = 7.12 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 160.80, 160.01, 152.87, 139.93, 130.22, 129.89, 123.62, 123.59, 122.77, 122.49, 63.22, 14.05. MS (APCI) (*m/z*) 275.97 $(M+H)^+$.

General procedure for the synthesis of library of compounds

Method: The 5-substituted benzo[*d*]thiazole-2-carboxylate **2a**/**2b**/**2c** (1 mmol) was taken in a round bottom flask (10 mL). To it the amine (1 equiv) was added along with catalytic amount (20 mol%) of NH_4Cl and reaction mixture was heated at 100 ºC for 1-2 h under magnetic stirring. After completion of reaction (TLC), ice-cold water (2 mL) was added to the reaction mixture and the mixture was allowed to stir for 10 min. The solid precipitate was filtered and air dried to furnish the final product that did not require any further purification.

4.1.4 Benzo[*d*]thiazol-2-yl(morpholino)methanone (**4a**): Yield: 219 mg, 88% (white solid), IR ($CHCl_3$) ν: 1621 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.01 (d, *J* = 6.56 Hz, 1H), 7.99 (d, *J* = =7.6 Hz, 1H), 7.59-7.49 (m, 2H), 4.54 (t, *J* = 4.64 Hz, 2H), 3.88-3.83 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 164.50, 159.72, 153.07, 136.19, 126.79, 126.62, 124.64, 121.87, 67.24, 66.90, 47.18, 43.91. MS (EI) (*m/z*) 249.10 $(M+H)^+$. HPLC analysis: retention time = 4.384 min; peak area, 100%.

4.1.5 Benzo[*d*]thiazol-2-yl(thiomorpholino)methanone (**4b**): Yield: 233 mg, 88% (yellowish solid), mp 112-115 ºC. IR ($CHCl_3$) ν: 1618, 1498 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.11 (d, *J* = 7.92 Hz, 1H), 7.98 (d, *J* = 8.04 Hz, 1H), 7.59-7.49 (m, 2H), 4.66 (t, *J* = 4.56 Hz, 2H), 4.12 (t, *J* = 4.76 Hz, 2H), 4.82 (q, *J* = 5.96 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 164.35, 160.08, 152.99, 136.15, 126.78, 126.61, 124.66, 121.86, 49.44, 46.35, 28.53, 27.55. MS (APCI) (*m/z*) 265.06 $(M+H)^+$. HPLC analysis: retention time = 4.405 min; peak area, 100%.

4.1.6 Benzo[*d*]thiazol-2-yl(piperidin-1-yl)methanone (**4c**): Yield: 165 mg, 67% (brownish yellow solid), mp 80-83 ºC. IR (KBr) ν: 1615, 1505 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.10 (d, *J* = 8.2 Hz, 1H), 7.97 (d, *J* = 7.68 Hz, 1H), 7.56-7.46 (m, 2H), 4.26 (t, *J* = 9.96 Hz, 2H), 3.79 (t, *J* = 5.4 Hz, 2H), 1.74 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 164.91 (C=O), 159.90, 153.04, 136.08, 126.47, 126.42, 124.51, 121.79, 47.69, 44.74, 26.75, 25.80, 24.57. MS (APCI) (*m/z*) 247.13 $(M+H)^+$. HPLC analysis: retention time = 4.821 min; peak area, 98.79%.

4.1.7 Benzo[*d*]thiazol-2-yl(4-methylpiperazin-1-yl)methanone (**4d**): Yield: 97 mg, 37% (white solid). IR ($CHCl_3$) ν: 3432, 1630 cm^{-1}. ^{1}H NMR (400 MHz, $[D_4]$MeOD) δ (ppm): 8.12-8.07 (m, 2H), 7.63-7.54 (m, 2H), 4.42 (t, *J* = 4.72 Hz, 2H), 3.86 (t, *J* = 4.2 Hz, 2H), 2.60 (t, *J* = 4.52 Hz, 4H), 2.37 (s, 3H, $-OCH_3$). ^{13}C NMR (100 MHz, $[D_4]$MeOD) δ (ppm): 163.82 (C=O), 159.99, 152.79, 135.77, 126.77, 126.82, 126.66, 124.11, 121.73, 54.79, 54.14, 45.95, 44.54, 42.82 ($-OCH_3$). MS (APCI) (*m/z*) 262.11 $(M)^+$. HPLC analysis: retention time = 4.363 min; peak area, 95.65%.

4.1.8 1-(4-(benzo[*d*]thiazole-2-carbonyl)piperazin-1-yl)ethanone (**4e**): Yield: 159 mg, 55% (yellowish solid), mp 79-91 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.14-8.08 (m, 1H), 7.99 (d, *J* = 7.8 Hz, 1H), 7.59-7.50 (m, 2H), 4.58-4.48 (m, 2H), 3.90-3.85 (m, 2H), 3.81-3.78 (m, 2H), 3.67-3.63 (m, 2H), 2.19 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 169.29 (C=O), 164.03, 160.10, 153.03, 136.19, 126.93, 126.72, 124.80, 124.62, 121.93, 121.87, 46.68, 46.35, 45.94, 43.59, 43.46, 41.87, 41.13, 21.42. MS (APCI) (*m/z*) 290.93 $(M)^+$. Anal. Calcd for $C_{14}H_{15}N_3O_2S$: C, 58.11; H, 5.23; N, 14.52; S, 11.08. Found: C, 58.40; H, 5.49; N, 14.47; S, 11.35. HPLC analysis: retention time = 4.413 min; peak area, 98.99%.

4.1.9 Benzo[*d*]thiazol-2-yl(4-phenylpiperazin-1 -yl)methanone (**4f**): Yield: 246 mg, 76% (pale yellow solid), mp 95-98 ºC. IR (neat) ν: 1622, 1597 cm^{-1}. ^{1}H NMR (400 MHz, $[D_6]$DMSO) δ (ppm): 8.22 (d, *J* = 7.6 Hz, 1H), 8.17 (d, *J* = 7.91 Hz, 1H), 7.62 (quin, *J* = 6.92 Hz, 2H), 7.25 (t, *J* = 7.8 Hz, 2H), 7.00 (d, *J* = 8.12 Hz, 2H), 6.84 (t, *J* = 7.2 Hz, 1H), 4.47 (s, 2H), 3.87 (s, 2H), 3.29 (s, 4H).^{13}C NMR (100 MHz, $[D_6]$DMSO) δ (ppm): 164.65 (C=O), 158.83, 152.47, 150.59, 135.34, 128.99, 126.97, 124.29, 122.47, 119.38, 115.85, 48.77, 48.25, 45.74, 42.90. Anal. Calcd for $C_{18}H_{17}N_3OS$: C, 66.85; H, 5.30; N, 12.99; S, 9.91. Found: C, 66.54; H, 5.26; N, 13.24; S, 9.99. HPLC analysis: retention time = 5.397 min; peak area, 98.04%.

4.1.10 1-(Benzo[*d*]thiazol-2-yl)-2-(4-(2-methoxyphenyl)piperazin-1-yl)ethanone (**4g**): Yield: 194 mg, 55% (white solid). IR ($CHCl_3$) ν: 3410, 1626 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.10 (d, J = 8 Hz, 1H), 7.96 (d, J = 7.72 Hz, 1H), 7.56-7.47 (m, 2H), 7.07-7.03 (m, 1H), 6.95-6.89 (m, 3H), 4.65 (s, 2H), 4.05 (s, 2H), 3.90 (s, 3H), 3.2 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 164.82 (C=O), 159.74, 153.12, 152.30, 140.62, 136.22, 126.68, 126.55, 124.62, 123.61, 121.86, 121.10, 118.52, 111.33, 55.46 (-OCH_3), 51.34, 50.72, 46.84, 43.87. MS (APCI) (*m/z*) 354.24 $(M+H)^+$. Anal. Calcd for $C_{19}H_{19}N_3O_2S$: C, 64.57; H, 5.42; N, 11.89; S, 9.07. Found: C, 64.27; H, 5.24; N, 11.81; S, 8.99. HPLC analysis: retention time = 3.445 min; peak area, 98.56%.

4.1.11 1-(Benzo[*d*]thiazol-2-yl)-2-(4-(4-methoxyphenyl)piperazin-1-yl)ethanone (**4h**): Yield: 187 mg, 53% (yellowish solid). IR ($CHCl_3$) ν: 1623 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.13 (d, J = 7.88 Hz, 1H), 8.00 (d, J = 7.64 Hz, 1H), 7.59-7.50 (m, 2H), 6.98-6.88 (m, 4H), 4.65 (s, 2H), 4.04 (s, 2H), 3.81 (s, 3H, -OCH_3), 3.23 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 164.68 (C=O), 159.73, 154.44, 153.10, 145.23, 136.22, 126.74, 126.59, 124.65, 118.95, 114.57, 55.57 (-OCH_3), 51.59, 51.05, 46.60, 43.71. MS (EI) (*m/z*) 353 $(M)^+$. Anal. Calcd for $C_{19}H_{19}N_3O_2S$: C, 64.57; H, 5.42; N, 11.89; S, 9.07. Found: C, 64.25; H, 5.21; N, 11.80; S, 8.95. HPLC analysis: retention time = 4.928 min; peak area, 95.66%.

4.1.12 1-(4-(4-(Benzo[*d*]thiazole-2-carbonyl)piperazin-1-yl)phenyl)ethanone (**4i**): Yield: 183 mg, 50% (dark yellow solid), mp 150-154 ºC. IR (neat) ν: 1712 (w; *ν*(O=CMe)), 1670 (s; *ν*(O=C-N)) cm^{-1}. ^1H NMR (400 MHz, [D_6]DMSO) δ (ppm): 8.25-8.16 (m, 2H), 7.85 (d, J = 8.72 Hz, 2H), 7.66-7.58 (m, 2H), 7.02 (d, J = 8.72 Hz, 2H), 4.49 (s, 2H), 3.87 (s, 2H), 3.54 (s, 4H), 2.47 (s, 3H). ^{13}C NMR (100 MHz, [D_6]DMSO) δ (ppm): 196.16 (-C=OMe), 165.15 (C=O), 159.42, 153.82, 152.99, 135.86, 130.59, 127.49, 127.38, 124.79, 122.99, 113.70, 47.35, 46.64, 45.83, 43.16, 26.60 (-CH_3). MS (EI) (*m/z*) 364.99 $(M)^+$. HPLC analysis: retention time = 4.256 min; peak area, 95.62%.

4.1.13 Benzo[*d*]thiazol-2-yl(4-(pyridin-4-yl)piperazin-1-yl)methanone (**4j**): Yield: 175 mg, 54% (white solid), mp 149-153 ºC. IR (neat) ν: 1622 cm^{-1}. ^1H NMR (400 MHz, [D_4]MeOD) δ (ppm): 8.18-8.08 (m, 4H), 7.64-7.55 (m, 2H), 7.25 (d, J = 6.32 Hz, 2H), 4.59 (d, J = 4.44 Hz, 2H), 3.97 (t, J = 4.48 Hz, 2H), 3.63-3.59 (m, 4H). ^{13}C NMR (100 MHz, [D_4]MeOD) δ (ppm): 163.91 (C=O), 160.05, 155.21, 152.89, 148.74, 135.87, 126.87,

126.67, 124.17, 121.75, 108.23, 45.73, 45.57, 44.87, 42.79. Anal. Calcd for $C_{17}H_{16}N_4OS$: C, 62.94; H, 4.97; N, 17.27; S, 9.88. Found: C, 62.72; H, 4.96; N, 17.13; S, 9.96. MS (APCI) (*m/z*) 324.96 $(M+H)^+$. HPLC analysis: retention time = 3.307 min; peak area, 100%.

4.1.14 Benzo[*d*]thiazol-2-yl(4-(pyrazin-2-yl)piperazin-1-yl)methanone (**4k**): Yield: 120 mg, 37% (yellow solid), mp 80-83 ºC. IR (KBr) ν: 1620, 1574 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.13-8.08 (m, 34), 7.64-7.55 (m, 2H), 6.09 (t, *J* = 4.44 Hz, 2H), 3.97 (t, *J* = 4.48, 2H), 3.62-3.59 (m, 4H), 6.90 (*J* = 6.32 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 163.91 (C=O), 160.05, 155.21, 152.89, 148.74, 135.87, 132.11, 126.87, 126.67, 124.17, 121.75, 108.23, 45.73, 45.57, 44.87, 42.79. MS (APCI) (*m/z*) 325.13 $(M+H)^+$. HPLC analysis: retention time = 4.085 min; peak area, 95.25%.

4.1.15 (4-Benzhydrylpiperazin-1-yl)(benzo[*d*]thiazol-2-yl)methanone (**4l**): Yield: 261 mg, 63% (pale yellow solid), mp 133-136 ºC. IR ($CHCl_3$) ν: 3434, 1622 cm^{-1}. ^1H NMR (400 MHz, $[D_6]$DMSO) δ (ppm): 8.19 (d, *J* = 7.32 Hz, 1H), 8.08 (d, *J* = 7.6 Hz, 1H), 7.61-7.54 (m, 2H), 7.46 (d, *J* = 7.48 Hz, 4H), 7.32 (t, *J* = 7.44 Hz, 4H), 7.21 (t, *J* = 7.24 Hz, 2H), 4.40 (s, 1H), 4.31 (s, 2H), 3.75 (s, 2H), 2.44 (s, 4H). ^{13}C NMR (100 MHz, $[D_6]$DMSO) δ (ppm): 164.47 (C=O), 158.70, 152.39, 142.30, 135.23, 128.58, 127.60, 126.98, 126.90, 124.23, 122.47,74.51, 51.84, 51.19, 46.00, 43.05. Anal. Calcd for $C_{25}H_{23}N_3OS$: C, 72.61; H, 5.61; N, 10.16; S, 7.75. Found: C, 72.41; H, 5.67; N, 9.82; S, 7.54. MS (EI) (*m/z*) 414.05 $(M+H)^+$. HPLC analysis: retention time = 9.109 min; peak area, 100%.

4.1.16 (5-Chlorobenzo[*d*]thiazol-2-yl)(morpholino)methanone (**5a**): Yield: 217 mg, 77% (white solid), IR (KBr) ν: 1618 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.11 (s, 1H), 7.91 (d, *J* = 8.6 Hz, 1H), 7.50 (d, *J* = 8 Hz, 1H), 4.52 (t, *J* = 4.68, 2H), 3.87-3.83 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 166.50, 159.25, 153.85, 134.48, 132.70, 127.44, 124.32, 122.66, 67.20, 66.88, 47.15, 44.00. MS (APCI) (*m/z*) 283 $(M+H)^+$. Anal. Calcd for $C_{12}H_{11}ClN_2O_2S$: C, 50.97; H, 3.92; N, 9.91; S, 11.34. Found: C, 50.91; H, 3.89; N, 9.55; S, 11.22. HPLC analysis: retention time = 4.395 min; peak area, 97.73%.

4.1.17 (5-Chlorobenzo[*d*]thiazol-2-yl)(thiomorpholino)methanone (**5b**): Yield: 200 mg, 67% (brown solid), mp 109-111 ºC. IR (DMSO) ν: 3432, 1651 cm^{-1}. ^1H NMR (400 MHz, ^1H NMR $[D_6]$DMSO) δ (ppm): 8.23 (s, 2H), 7.64 (s, 1H), 4.43 (s, 2H), 3.96 (s, 2H), 2.77 (s,

4H). ^{13}C NMR (100 MHz, [D_6]DMSO) δ (ppm): 166.56 (C=O), 158.88, 153.08, 133.99, 131.58, 127.03, 124.00, 123.49, 48.75, 45.62, 27.44, 26.57. HRMS (ESI): *m/z* for $C_{12}H_{11}ClN_2NaOS_2$ [M + Na^+] calc.: 320.9899; observed: 320.9899. HPLC analysis: retention time = 5.376 min; peak area, 97.73%.

4.1.18 (5-Chlorobenzo[*d*]thiazol-2-yl)(piperidin-1-yl)methanone (**5c**): Yield: 216 mg, 77% (pale yellow solid), mp 50-52 ºC. IR (DMSO) ν: 3432, 1652 cm^{-1}. ^{1}H NMR (400 MHz, [D_6]DMSO) δ (ppm): 8.28 – 8.22 (m, 2H), 7.64-7.62 (m, 1H), 4.12 (t, *J* = 5.2 Hz, 2H), 3.68 (t, *J* = 5.24 Hz, 2H), 1.68-1.62 (m, 6H). ^{13}C NMR (100 MHz, [D_6]DMSO) δ (ppm): 166.92 (C=O), 158.49, 153.16, 133.92, 131.52, 126.90, 123.95, 123.43, 46.85, 43.91, 26.22, 25.34, 23.78. HRMS (ESI): *m/z* for $C_{13}H_{13}ClN_2NaOS$ [M + $Na]^+$, calc.: 303.0335; observed: 303.0335. HPLC analysis: retention time = 6.251 min; peak area, 91.06%.

4.1.19 (5-Chlorobenzo[*d*]thiazol-2-yl)(4-methylpiperazin-1-yl)methanone (**5d**): Yield: 172 mg, 58% (white solid), ^{1}H NMR (400 MHz, [D_6]DMSO) δ (ppm): 8.09 (d, *J* = 1.88 Hz, 1H), 7.88 (d, *J* = 8.44 Hz, 1H), 7.47 (dd, *J* = 9, 2.04 Hz, 1H), 4.44 (t, *J* = 4.28 Hz, 2H), 3.87 (t, *J* = 4.4 Hz, 2H), 2.54 (q, *J* = 4.64 Hz, 4H), 2.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 166.51 (C=O), 159.30, 153.58, 134.66, 132.26, 129.08, 127.84, 124.14, 52.84, 52.40, 43.55, 42.72. HPLC analysis: retention time = 3.147 min; peak area, 94.28%.

4.1.20 1-(4-(5-Chlorobenzo[*d*]thiazole-2-carbonyl)piperazin-1-yl)ethanone (**5e**): Yield: 266 mg, 82% (white solid), mp 103-106 ºC. IR (MeOH) ν: 3409, 1621 cm^{-1}. ^{1}H NMR (400 MHz, [D_4]MeOD) δ (ppm): 8.14-8.06 (m, 2H), 7.56 (dd, *J* = 8.64, 1.68 Hz, 1H), 4.50-4.40 (m, 2H), 3.91-3.82 (m, 2H), 3.77-3.72 (m, 4H), 2.19 (d, *J* = 4.2 Hz, 3H, -CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 170.78 (-COMe), 166.07 (C=O), 159.71, 153.59, 134.38, 132.57, 127.24, 123.56, 122.99, 45.92, 43.31, 43.02, 19.85 (-CH_3). Anal. Calcd for $C_{14}H_{14}ClN_3O_2S$: C, 51.93; H, 4.36; N, 12.98; S, 9.90. Found: C, 51.55; H, 4.35; N, 12.89; S, 9.57. MS (ESI) (*m/z*) 323.87 $(M)^+$. HPLC analysis: retention time = 3.765 min; peak area, 94.02%.

4.1.21 (5-Chlorobenzo[*d*]thiazol-2-yl)(4-phenylpiperazin-1-yl)methanone (**5f**): Yield: 279 mg, 78% (buff white solid), mp 108-111 ºC. IR (neat) ν: 3454, 1622, 1598 cm^{-1}. ^{1}H NMR (400 MHz, [D_6]DMSO) δ (ppm): 8.25 (s, 2H), 7.64 (d, *J* = 8.12 Hz, 1H), 7.25 (t, *J* =

7.08 Hz, 2H), 7.00 (d, *J* = 7.32 Hz, 2H), 6.83 (t, *J* = 6.4 Hz, 1H), 4.43 (s, 2H), 3.87 (s, 2H), 3.28 (s, 4H). ^{13}C NMR (100 MHz, [D$_6$]DMSO) δ (ppm): 167.38 (C=O), 159.07, 153.79, 151.07, 134.63, 132.17, 129.50, 127.65, 124.61, 124.10, 119.90, 116.37, 49.26, 48.72, 46.26, 43.43. MS (EI) (*m/z*) 358.06 $(M+H)^+$. HPLC analysis: retention time = 11.040 min; peak area, 96.77%.

4.1.22 1-(5-Chlorobenzo[*d*]thiazol-2-yl)-2-(4-(2-methoxyphenyl)piperazin-1-yl)ethanone (**5g**): Yield: 221 mg, 57% (brick red solid), mp 116-118 ºC. IR (KBr) ν: 3453, 1637, 1589 cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO) δ (ppm): 8.28-8.25 (m, 2H), 7.66-7.63 (m, 1H), 7.02-6.89 (m, 4H), 4.41 (t, *J* = 4.24 Hz, 2H), 3.86 (t, *J* = 4.28 Hz, 2H), 3.81 (s, 3H), 3.08 (q, *J* = 4.76 Hz, 4H). ^{13}C NMR (100 MHz, [D$_6$]DMSO) δ (ppm): 167.36 (C=O), 159.05, 153.76, 152.48, 140.95, 134.59, 132.13, 127.61, 125.09, 124.57, 124.09, 123.49, 121.28, 112.30, 55.81, 50.99, 50.52, 46.78, 43.76. HRMS (ESI): *m/z* for $C_{19}H_{19}ClN_3O_2S$ $[M + H]^+$, calc.: 388.0887; observed: 388.0886. HPLC analysis: retention time = 3.179 min; peak area, 95.54%.

4.1.23 1-(5-Chlorobenzo[*d*]thiazol-2-yl)-2-(4-(4-methoxyphenyl)piperazin-1-yl)ethanone (**5h**): Yield: 209 mg, 54% (brick red solid), mp 125-127 ºC. IR (KBr) ν: 3456, 1615, 1506 cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO) δ (ppm): 8.28-8.25 (m, 2H), 7.65 (dd, *J* = 8.64, 2 Hz, 1H), 6.96 (d, *J* = 9.04 Hz, 2H), 6.85 (d, *J* = 9.04 Hz, 2H), 4.40 (t, *J* = 4.48 Hz, 2H), 3.86 (t, *J* = 4.32 Hz, 2H), 3.69 (s, 3H), 3.14 (q, *J* = 5.84 Hz, 4H). ^{13}C NMR (100 MHz, [D$_6$]DMSO) δ (ppm): 167.35 (C=O), 159.03, 153.85, 153.75, 145.38, 134.60, 132.15, 127.62, 127.52, 125.08, 124.57, 124.08, 123.69, 118.56, 114.76, 55.63 (-OCH$_3$), 50.76, 50.29, 46.44, 43.54. Anal. Calcd for $C_{19}H_{18}ClN_3O_2S$ C, 58.83; H, 4.68; N, 10.83; S, 8.27. Found: C, 58.43; H, 4.51; N, 10.82; S, 8.57. HRMS (ESI): *m/z* for $C_{19}H_{19}ClN_3O_2S$ $[M + H]^+$, calc.: 388.0887; observed: 388.0869. HPLC analysis: retention time = 6.304 min; peak area, 96.75%.

4.1.24 1-(4-(4-(5-Chlorobenzo[*d*]thiazole-2-carbonyl)piperazin-1-yl)phenyl)ethanone (**5i**): Yield: 216 mg, 54% (orange solid), mp 150-153 ºC. IR (KBr) ν: 1660, 1598 cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO) δ (ppm): 8.26-8.25 (m, 1H), 7.84 (d, *J* = 8.8 Hz, 2H), 7.65 (dd, *J* = 8.6, 1.92 Hz, 1H), 7.02 (d, *J* = 8.92 Hz, 3H), 4.44 (t, *J* = 5.08 Hz, 2H), 3.86 (t, *J* = 4.6 Hz, 2H), 3.56-3.52 (m, 4H), 2.47 (s, 3H). ^{13}C NMR (100 MHz, [D$_6$]DMSO) δ (ppm): 195.54 (-COMe), 166.80 (C=O), 158.60, 153.22, 134.08, 131.60, 130.01, 127.10, 126.83,

124.04, 123.93, 123.52, 113.14, 46.74, 46.04, 45.27, 42.61, 26.04. Anal. Calcd for $C_{20}H_{18}ClN_3O_2S$: C, 60.67; H, 4.54; N, 10.51; S, 8.02. Found: C, 61.05; H, 4.90; N, 10.61; S, 7.99. HPLC analysis: retention time = 5.163 min; peak area, 96.27%.

4.1.25 (5-Chlorobenzo[*d*]thiazol-2-yl)(4-(pyridin-4-yl)piperazin-1-yl)methanone (**5j**): Yield: 209 mg, 58% (pale yellow solid), mp 143-147 ºC. IR (MeOH) ν: 1626, 1595 cm^{-1}. ^1H NMR (400 MHz, [D_6]DMSO) δ (ppm): 8.28-8.20 (m, 4H), 7.65 (d, J = 8.28 Hz, 1H), 6.88 (d, J = 5.2 Hz, 2H), 4.43 (s, 2H), 3.84 (s, 2H), 3.55 (s, 4H). ^{13}C NMR (100 MHz, [D_6]DMSO) δ (ppm): 166.79 (C=O), 158.64, 154.11, 153.21, 149.20, 134.07, 131.67, 127.12, 124.06, 123.52, 108.23, 45.45, 45.08, 44.71, 42.46. HRMS (ESI): *m/z* for $C_{17}H_{16}ClN_4OS$ $[M + H]^+$, calc.: 359.0733; observed: 359.0748. HPLC analysis: retention time = 3.595 min; peak area, 96.86%.

4.1.26 (5-Chlorobenzo[*d*]thiazol-2-yl)(4-(pyrazin-2-yl)piperazin-1-yl)methanone (**5k**): Yield: 169 mg, 47% (white solid), mp 90-95 ºC. ^1H NMR (400 MHz, [D_6]DMSO) δ (ppm): 8. 22 (s, 1H), 8.13 (s, 2H), 7.95 (s, 1H), 7.92 (d, J = 8.56 Hz, 1H), 7.51 (dd, J = 8.52, 1.32 Hz, 1H), 4.64 (t, J = 4.84 Hz, 2H), 4.02 (t, J = 4.88 Hz, 2H), 3.82-3.79 (m, 4H). ^{13}C NMR (100 MHz, [D_6]DMSO) δ (ppm): 165.41 (C=O), 158.43, 153.62, 152.85, 140.82, 133.50, 132.80, 131.74, 130.02, 126.49, 123.36, 121.67, 44.89, 44.01, 43.22, 42.26. HRMS (ESI): *m/z* for $C_{16}H_{15}ClN_5NaOS$ $[M + Na]^+$, calc.: 360.0686; observed: 360.0689. HPLC analysis: retention time = 4.843 min; peak area, 99.84%.

4.1.27 (4-Benzhydrylpiperazin-1-yl)(5-chlorobenzo[*d*]thiazol-2-yl)methanone (**5l**): Yield: 345 mg, 77% (pale yellow solid), mp 149-153 ºC. IR ($CHCl_3$) ν: 3398, 1634 cm^{-1}. ^1H NMR (400 MHz, [D_6]DMSO) δ (ppm): 8.18 (t, J = 8.56 Hz, 1H), 8.12 (br s, 1H), 7.44 (d, J = 7.28 Hz, 4H), 7.30 (t, J = 7.2 Hz, 4H), 7.21 (t, J = 7.04 Hz, 2H), 4.43 (s, 1H), 4.22 (s, 2H), 3.75 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 166.89 (C=O), 159.09, 153.86, 142.09, 134.45, 132.53, 128.68, 128.47, 127.89, 127.24, 124.21, 122.60, 75.99, 52.41, 51.70, 46.67, 43.88. Anal. Calcd for $C_{25}H_{22}ClN_3OS$: C, 67.03; H, 4.95; N, 9.38; S, 7.16. Found: C, 67.66; H, 4.89; N, 9.27; S, 7.04. HPLC analysis: retention time = 13.195 min; peak area, 100%.

4.1.28 Morpholino(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)methanone (**6a**): Yield: 278 mg, 88% (yellow solid), mp 119-121 ºC. IR ($CHCl_3$) ν: 1627 cm^{-1}. ^1H NMR (400 MHz,

$CDCl_3$) δ (ppm): 8.38 (s, 1H), 8.09 (d, J = 8.48 Hz, 1H), 7.73 (dd, J = 1.36, 8.44 Hz, 1H), 4.53 (t, J = 4.72, 2H), 3.88-3.82 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 166.95, 159.02, 152.60, 139.50, 129.54, 129.21, 123.05, 122.67, 122.03, 67.18, 66.86, 47.13, 44.0. HRMS (ESI): *m/z* for $C_{13}H_{12}F_3N_2O_2S$ [M + H^+] calc.: 317.0572; observed: 317.0572. HPLC analysis: retention time = 4.384 min; peak area, 98.02%.

4.1.29 Thiomorpholino(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)methanone (**6b**): Yield: 306 mg, 92% (yellow solid), mp 82-85 ºC. IR ($CHCl_3$) ν: 1626 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.23 (s, 1H), 7.64 (d, J = 8.44 Hz, 1H), 7.73 (dd, J = 1.28, 8.52 Hz, 2H), 4.65 (t, J = 4.92 Hz, 2H), 4.11 (t, J = 5.04 Hz, 2H), 2.84-2.79 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 166.83 (C=O), 159.33, 152.52, 139.46, 129.53, 125.34, 123.05, 122.66, 122.02, 49.41, 46.53, 28.54, 27.58. HRMS (ESI): *m/z* for $C_{13}H_{11}F_3N_2NaOS_2$ [M + Na^+] calc.: 355.0163; observed: 355.0160. HPLC analysis: retention time = 5.280 min; peak area, 100%.

4.1.30 Piperidin-1-yl(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)methanone (**6c**): Yield: 233 mg, 74% (orange yellow solid), mp 85-87 ºC. IR ($CHCl_3$) ν: 1625 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.38 (s, 1H), 8.07 (d, J = 8.4 Hz, 1H), 7.71 (dd, J = 1.4, 8.48 Hz), 4.28 (t, J = 5.8 Hz, 2H), 3.79 (t, J = 5.48 Hz, 2H), 1.74 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 167.42 (C=O), 159.11, 152.61, 139.48, 129.31, 125.41, 122.74, 122.57, 121.91, 47.64, 44.96, 26.75, 25.82, 24.51. HRMS (ESI): *m/z* for $C_{14}H_{13}F_3N_2NaOS$ [M + $Na]^+$, calc.: 337.0598; observed: 337.0594. HPLC analysis: retention time = 6.144 min; peak area, 98.78%.

4.1.31 (4-Methylpiperazin-1-yl)(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)methanone (**6d**): Yield: 254 mg, 77% (yellowish brown solid), mp 44-46 ºC. IR ($CHCl_3$) ν: 1627 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.38 (s, 1H), 8.08 (d, J = 8.48 Hz, 1H), 7.72 (d, J = 8.48 Hz, 1H), 4.48 (t, J = 4.64 Hz, 2H), 3.89 (t, J = 4.92 Hz, 2H), 2.56 (q, J = 4.96 Hz, 4H), 2.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 167.14 (C=O), 159.01, 152.60, 139.51, 129.44, 129.11, 122.93, 122.64, 121.95, 55.40, 54.68, 46.32, 45.91, 43.61 (-OCH_3). HRMS (ESI): *m/z* for $C_{14}H_{15}F_3N_3OS$ [M + $H]^+$, calc.: 330.0888; observed: 330.0888. HPLC analysis: retention time = 6.709 min; peak area, 100%.

4.1.32 1-(4-(5-(Trifluoromethyl)benzo[*d*]thiazole-2-carbonyl)piperazin-1-yl)ethanone (**6e**): Yield: 314 mg, 88% (pale yellow solid), mp 167-169 ºC. IR ($CHCl_3$) ν: 1630 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.41 (d, J = 16.84 Hz, 1H), 8.12 (d, J = 8.36 Hz, 1H), 7.76 (dd, J = 1.12, 8.48 Hz, 1H), 4.59-4.49 (m, 2H), 3.92-3.80 (m, 4H), 3.69-3.65 (m, 4H), 2.19 (s, 3H, $-CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 169.25 (-COMe), 164.20 (C=O), 159.07, 152.57, 141.32, 125.30, 123.18, 122.77, 122.68, 122.16, 46.61, 46.31, 41.80, 41.09, 21.41 ($-CH_3$). HRMS (ESI): *m/z* for $C_{14}H_{15}F_3N_3OS$ $[M + H]^+$, calc.: 330.0837; observed: 330.0835. HPLC analysis: retention time = 3.733 min; peak area, 98.79%.

4.1.33 (4-Phenylpiperazin-1-yl)(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)methanone (**6f**): Yield: 282 mg, 72% (yellowish white solid), mp 108-111 ºC. IR ($CHCl_3$) ν: 1627 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.43 (s, 1H), 8.12 (d, J = 8.48 Hz, 1H), 7.76 (dd, J = 1.24, 8.48 Hz, 1H), 7.36-7.32 (m, 2H), 7.01-6.94 (m, 3H), 4.68 (t, J = 5 Hz, 2H), 4.05 (t, J = 4.96 Hz, 2H), 3.38-3.35 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 167.11 (C=O), 159.02, 152.64, 150.82, 139.55, 129.51, 129.33, 122.01, 120.70, 116.92, 116.70, 50.08, 49.52, 46.37, 43.74. HRMS (ESI): *m/z* for $C_{19}H_{17}F_3N_3OS$ $[M + H]^+$, calc.: 392.1044; observed: 392.1046. HPLC analysis: retention time = 6.891 min; peak area, 96.42%.

4.1.34 (4-(2-Methoxyphenyl)piperazin-1-yl)(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)methanone (**6g**): Yield: 325 mg, 77% (pale yellow solid), mp 124-126 ºC. IR ($CHCl_3$) ν: 1627 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.40 (s, 1H), 8.09 (d, J = 8.48 Hz, 1H), 7.73 (d, J = 8.48 Hz, 1H), 7.08-7.04 (m, 1H), 6.96-6.90 (m, 3H), 4.66 (t, J = 4.64 Hz, 2H), 4.06 (t, J = 4.76 Hz, 2H), 3.91 (s, 3H), 3.21 (t, J = 5 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 167.27 (C=O), 160.92, 159.01, 152.65, 152.29, 140.48, 139.55, 129.12, 123.69, 122.94, 122.65, 121.97, 121.10, 118.51, 111.34, 55.46, 51.29, 50.69, 46.80, 44.03. HRMS (ESI): *m/z* for $C_{20}H_{19}F_3N_3O_2S$ $[M + H]^+$, calc.: 422.1150; observed: 422.1151. HPLC analysis: retention time =6.688 min; peak area, 98.32%.

4.1.35 2-(4-(4-Methoxyphenyl)piperazin-1-yl)-1-(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)ethanone (**6h**): Yield: 371 mg, 88% (brick red solid), mp 167-170 ºC. IR ($CHCl_3$) ν: 1628 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.40 (s, 1H), 8.09 (d, J = 8.52 Hz, 1H), 7.73 (d, J = 8.44 Hz, 1H), 6.96-6.92 (m, 2H), 6.90-6.83 (m, 2H), 4.63 (t, J = 4.84 Hz, 2H), 4.02 (t, J = 4.84 Hz, 2H), 3.78 (s, 3H), 3.22-3.20 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 167.14 (C=O), 159.01, 154.51, 152.63, 145.12, 139.54, 129.34, 124.71, 123.03,

122.67, 121.99, 118.99, 118.68, 114.59, 114.49, 55.57 (-OCH_3), 51.57, 51.06, 46.56, 43.88. HRMS (ESI): *m/z* for $C_{20}H_{19}F_3N_3O_2S$ $[M + H]^+$, calc.: 422.1150; observed: 422.1151. HPLC analysis: retention time = 6.197 min; peak area, 96.75%.

4.1.36 1-(4-(4-(5-(Trifluoromethyl)benzo[*d*]thiazole-2-carbonyl)piperazin-1-yl)phenyl)ethanone (**6i**): Yield: 334 mg, 77% (orange solid), mp 104-107 ºC. IR ($CHCl_3$) ν: 1667, 1627, 1598 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.40 (s, 1H), 8.11 (d, *J* = 8.4 Hz, 2H), 7.92 (d, *J* = 8.92 Hz, 2H), 7.75 (d, *J* = 8.48 Hz, 1H), 6.92 (d, *J* = 8.06 Hz, 2H), 4.69 (t, *J* = 4.96 Hz, 2H), 4.03 (t, *J* = 4.92 Hz, 2H), 3.55-3.53 (m, 4H), 2.55 (s, 3H). ^{13}C NMR (100 MHz, $[D_6]$DMSO) δ (ppm): 196.90 (-COMe), 166.87 (C=O), 159.11, 153.61, 152.62, 139.55, 130.47, 129.59, 129.26, 128.48, 125.33, 123.14, 122.74, 122.64, 122.03, 113.87, 48.02, 47.31, 45.89, 43.37, 26.20. HRMS (ESI): *m/z* for $C_{21}H_{19}F_3N_3O_2S$ $[M + H]^+$, calc.: 434.1150; observed: 434.1139. HPLC analysis: retention time = 6.165 min; peak area, 98.39%.

4.1.37 (4-(Pyridin-4-yl)piperazin-1-yl)(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)methanone (**6j**): Yield: 290 mg, 74% (pale yellow solid), mp 130-132 ºC. IR ($CHCl_3$) ν: 1627 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.40 (s, 1H), 8.35 (d, *J* = 5.96 Hz, 2H), 8.11 (d, *J* = 8.48 Hz, 1H), 7.75 (d, *J* = 8.48 Hz, 1H), 6.73 (d, *J* = 6.44 Hz, 2H), 4.69 (t, *J* = 5.08 Hz, 2H), 4.02 (t, *J* = 5.08 Hz, 2H), 3.55 (t, *J* = 5.4 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 166.77 (C=O), 159.14, 154.65, 152.61, 150.03, 139.54, 129.62, 129.29, 125.31, 123.22, 122.22, 122.60, 122.04, 108.51, 46.44, 45.68, 45.62, 43.14. HRMS (ESI): *m/z* for $C_{18}H_{16}F_3N_4OS$ $[M + H]^+$, calc.: 393.0997; observed: 393.0992. HPLC analysis: retention time = 1.685 min; peak area, 99.85%.

4.1.38 (4-(Pyrazin-2-yl)piperazin-1-yl)(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)methanone (**6k**): Yield: 295 mg, 75% (pale yellow solid), mp 97-100 ºC. IR ($CHCl_3$) ν: 1627 cm^{-1}.^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.41 (s, 1H), 8.21 (s, 1H), 8.12-8.09 (m, 2H) 7.94 (d, *J* = 2.48 Hz, 1H), 7.75 (d, *J* = 8.48 Hz, 1H), 4.65 (t, *J* = 4.92 Hz, 2H), 4.01 (t, *J* = 4.76 Hz, 2H), 3.81-3.78 (m, 4H). ^{13}C NMR (100 MHz, $[D_6]$DMSO) δ (ppm): 166.88 (C=O), 159.23, 154.62, 152.62, 141.85, 139.55, 133.88, 131.04, 129.52, 129.27, 123.16, 122.72, 122.10, 45.91, 45.03, 44.25, 43.34. HRMS (ESI): *m/z* for $C_{17}H_{15}F_3N_5OS$ $[M + Na]^+$, calc.: 394.0949; observed: 394.0949. HPLC analysis: retention time = 4.821 min; peak area, 98.41%.

4.1.39 (4-Benzhydrylpiperazin-1-yl)(5-(trifluoromethyl)benzo[d]thiazol-2-yl)methanone (**6l**): Yield: 356 mg, 74% (pale yellow solid), mp 136-139 ºC. IR ($CHCl_3$) ν: 1629 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.30 (s, 1H), 8.05 (d, J = 8.44 Hz, 1H), 7.69 (d, J = 8.48 Hz, 1H), 7.45-7.38 (m, 5H), 7.31-7.18 (m, 5H), 4.47 (t, J = 4.64 Hz, 2H), 4.30 (s, 1H), 3.86 (t, J = 4.8 Hz, 2H), 2.55 (q, J = 5.12, 4H). ^{13}C NMR (100 MHz, $CHCl_3$) δ (ppm): 167.30 (C=O), 158.87, 152.60, 142.04, 139.49, 129.39, 128.67, 127.89, 127.81, 127.25, 122.90, 122.61, 121.90, 75.97, 52.39, 51.70, 46.68, 43.96. HRMS (ESI): *m/z* for $C_{26}H_{23}F_3N_3OS$ $[M + H]^+$, calc.: 482.1514; observed: 482.1507. HPLC analysis: retention time = 27.659 min; peak area, 96.94%.

4.2. Biological Evaluation

4.2.1 Determination of the minimal inhibitory concentration (MIC) of 2 and 4, 5, 6 benzo[*d*]thiazole-2-carboxamides:

The drug susceptibility of *Mycobacterium tuberculosis* strain $H_{37}Rv$ was determined using the method recommended by the National Committee for Clinical Laboratory Standards for the determination of MIC in triplicate. Two-fold serial dilutions (50.0, 25.0, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.4 µg/mL) of each test compounds and drugs were prepared and incorporated into Middlebrook 7H11 agar medium with OADC Growth Supplement. Inoculum of *M. tuberculosis* H37Rv ATCC 27294 was prepared from fresh Middlebrook 7H11 agar slants with OADC (oleic acid, albumin, dextrose and catalase; 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 ~ 10^7 cfu/mL. A 5 µL amount of bacterial suspension was spotted into 7H11 agar tubes containing 10-fold serial dilutions of drugs per mL. The tubes were incubated at 37 ºC, and final readings were recorded after 28 days.[7] Isoniazid (INH), Rifampin (R), Ethambutol (E) and Pyrazinamide (Z) were purchased from Lupin Pharmaceuticals, Inc. and Ciprofloxacin (Cfx) was procured from Matrix laboratories Limited.

4.2.2 Cytotoxicity Evaluation

Anti-TB active compounds with MIC ≤ 12.5μg/mL were further examined for toxicity evaluated against RAW 264.7 (mouse leukemic monocyte macrophage) cell lines by using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) MTT assay at the concentration of 50 μg/mL. 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.

5. References

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FIGURE CAPTIONS

Figure 1. Reported bioactive compounds with piperazine scaffold having anti-TB activity.[8-15]

Figure 2. Design of benzo[*d*]thiazol-2-yl(piperazin-1-yl)methanone **I** as new anti-TB scaffold.

Figure 3. Graphical representation of the anti-TB activity of the synthesized compounds with MIC ≤ 1.56 µg/mL in comparison with standard drugs.

Figure 4. Graphical representation of the in-vitro cell viability profile of the synthesized compounds with MIC ≤ 6.25 µg/mL.

Figure 5. Structure activity correlation chart of the compounds **4**/**5**/**6a-c**.

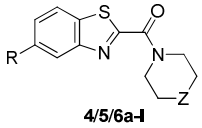
Figure 6. Confirmation of the template molecule compound 5l.

Figure 7. Alignment of all molecules used for CoMFA molecular field generation.

Figure 8. Correlation plot of the predicted pMIC with the experimental pMIC values generated through CoMFA.

Figure 9. CoMFA contours for benzo[*d*]thiazole-2-carboxamides: (a) steric, and (b) electrostatic interaction regions of **4i**.

Table 1. Yields of the 5-substituted benzo[*d*]thiazole-2-carboxylates **(2a/b/c)** and carboxamides (**4**/**5**/**6a-l**) and their biological activities[a]



4/5/6a-l

Entry	R	Z	R^1	Compd no.	Yield[b] (%)	MIC[c] (μg/mL)	MIC (μM)	Cytotoxicity[d] (% Inhibition)
1	-	-	-	**2a**	83	25	120.63	-
2	-	-	-	**2b**	73	3.125	12.93	37.40
3	-	-	-	**2c**	70	6.25	22.71	29.12
4	H	O	-	**4a**	88	1.56	6.28	36.16
5	H	S	-	**4b**	83	25	94.56	-
6	H	CH_2	-	**4c**	67	25	101.49	-
7	H	NR^1	Me	**4d**	56	3.125	11.96	52.10
8	H	NR^1	COMe	**4e**	55	1.56	5.39	48.82
9	H	NR^1	C_6H_5	**4f**	76	1.56	4.82	67.23
10	H	NR^1	2-OMe-C_6H_4	**4g**	55	3.125	8.84	53.36
11	H	NR^1	4-OMe-C_6H_4	**4h**	53	12.5	35.37	-
12	H	NR^1	4-COMe-C_6H_4	**4i**	51	1.56	4.27	62.34
13	H	NR^1	4-Pyridyl	**4j**	54	3.125	9.63	61.95
14	H	NR^1	2-Pyrazinyl	**4k**	77	6.25	19.21	65.34
15	H	NR^1	$CH(C_6H_5)_2$	**4l**	63	0.78	1.89	60.66
16	Cl	O	-	**5a**	77	6.25	22.10	26.12
17	Cl	S	-	**5b**	67	6.25	20.92	62.54
18	Cl	CH_2	-	**5c**	77	12.5	44.52	-
19	Cl	NR^1	Me	**5d**	60	25	84.52	-
20	Cl	NR^1	COMe	**5e**	82	12.5	38.60	-
21	Cl	NR^1	C_6H_5	**5f**	78	6.25	17.46	41.21
22	Cl	NR^1	2-OMe-C_6H_4	**5g**	57	3.125	8.06	63.36
23	Cl	NR^1	4-OMe-C_6H_4	**5h**	54	3.125	8.06	42.81

24	Cl	NR[1]	4-COMe-C_6H_4	**5i**	54	1.56	3.90	30.76
25	Cl	NR[1]	4-Pyridyl	**5j**	58	12.5	34.83	-
26	Cl	NR[1]	2-Pyrazinyl	**5k**	47	6.25	17.37	54.89
27	Cl	NR[1]	$CH(C_6H_4)_2$	**5l**	77	0.78	1.74	52.86
28	CF_3	O	-	**6a**	88	0.78	2.47	24.56
29	CF_3	S	-	**6b**	92	0.78	2.35	18.12
30	CF_3	CH_2	-	**6c**	74	12.5	39.77	-
31	CF_3	NR[1]	Me	**6d**	77	6.25	18.98	12.58
32	CF_3	NR[1]	COMe	**6e**	88	1.56	4.37	20.16
33	CF_3	NR[1]	C_6H_4	**6f**	72	12.5	31.94	-
34	CF_3	NR[1]	2-OMe-C_6H_4	**6g**	77	25	59.32	-
35	CF_3	NR[1]	4-OMe-C_6H_4	**6h**	88	3.125	7.42	19.41
36	CF_3	NR[1]	4-COMe-C_6H_4	**6i**	77	6.25	14.42	30.56
37	CF_3	NR[1]	4-Pyridyl	**6j**	74	3.125	7.96	17.52
38	CF_3	NR[1]	2-Pyrazinyl	**6k**	75	3.125	7.94	28.12
39	CF_3	NR[1]	$CH(C_6H_4)_2$	**6l**	74	25	51.92	-
40	-	-	-	INH	-	0.098	0.72	-
41	-	-	-	R	-	0.197	0.24	-
42	-	-	-	E	-	1.56	7.64	-
43	-	-	-	Z	-	6.25	50.77	-
44	-	-	-	Cfx	-	1.56	4.71	-

[a] The mixture of **2a**/**2b**/**2c,** the cyclic amine (1.0 equiv), and NH_4Cl (20 mol%) was heated for 1 h at 100 °C under solvent free condition. [b] Yield of the product after isolation. [c] MIC: Complete inhibition of growth of *M. tuberculosis* $H_{37}Rv$ (ATCC 27294). [d] % inhibitory concentration determined at 50 µg/mL of respective compound determined against RAW 264.7 cell lines. INH: Isoniazid, R: Rifampin, E: Ethambutol, Z: pyrazinamide and Cfx: ciprofloxacin.

Table 2. Drug-likeness and molecular property prediction of the target compounds with MIC ≤ 6.25 µg/mL.

Entry	Compd. No.	MIC[a] (µg/mL)	MIC[a] (µM)	ClogP[b]	MW[b]	No. of HBA[c]	No. of HBD[c]	No. of RB[c]
1	**4a**	1.56	6.28	1.09	248.30	0	1	2

2	**4d**	3.125	11.96	1.65	261.34	0	1	2
3	**4e**	1.56	5.39	0.67	289.35	0	1	3
4	**4f**	1.56	4.82	3.09	323.41	0	1	3
5	**4g**	3.125	8.84	3.11	353.44	0	1	4
6	**4i**	1.56	4.27	2.84	365.45	0	1	4
7	**4j**	3.125	9.63	2.14	324.40	1	1	3
8	**4k**	6.25	19.21	1.37	325.39	2	1	3
9	**4l**	0.78	1.89	4.72	413.53	0	1	5
10	**5a**	6.25	22.10	1.81	282.75	0	1	2
11	**5b**	6.25	20.92	2.54	298.81	0	1	2
12	**5f**	6.25	17.46	3.80	357.86	0	1	3
13	**5g**	3.125	8.06	3.82	387.88	0	1	4
14	**5h**	3.125	8.06	3.82	387.88	0	1	4
15	**5i**	1.56	3.90	3.55	399.89	0	1	4
16	**5k**	6.25	17.37	2.09	359.83	2	1	3
17	**5l**	0.78	1.74	5.44	447.98	0	1	5
18	**6a**	0.78	2.47	2.01	316.30	0	1	3
19	**6b**	0.78	2.35	2.74	332.36	0	1	3
20	**6d**	6.25	18.98	2.56	329.34	0	1	3
21	**6e**	1.56	4.37	1.58	357.35	0	1	4
22	**6h**	3.125	7.42	4.02	421.44	0	1	5
23	**6i**	6.25	14.42	3.74	433.45	0	1	5
24	**6j**	3.125	7.96	3.04	392.40	1	1	4
25	**6k**	3.125	7.94	2.28	393.39	2	1	4

[a]Molecular weight and ClogP (hydrophobicity) calculated using the Chem Draw Ultra, version 12.0. [b]No. of hydrogen bond acceptor and no. of hydrogen bond donors calculated using Accelrys Discovery Studio 2.5.

Table 3. The various parameters derived from CoMFA.

r^2_{cv} [a]	r^2_{ncv} [b]	SEE[c]	ONC[d]	F[e]	r^2_{pred} [f]	Field contribution	
						Steric	Electro-static
0.758	0.995	0.075	6	381.89	0.718	47.6	52.4

[a]Leave one out (LOO) cross-validated correlation coefficient, [b]no validation correlation coefficient, [c]Standard Error of Estimate, [d]Optimal number of components, [e]F test value, [f]Predictive correlation coefficient.

Table 4. Experimental and CoMFA-predicted MIC values of molecules in both training set and test set.

Compd No.	pMIC			Δ pMIC[a]
	Experimental	Rescaled	Predicted	
4d	4.92	6.10	6.11	-0.01
4e	5.27	6.85	6.84	0.01
4f*	5.32	6.95	6.47	0.48
4g	5.05	6.38	6.35	0.03
4h	4.45	5.07	6.11	-1.04
4i	5.37	7.07	7.11	-0.04
4j	5.02	6.30	6.29	0.01
4k	4.72	5.65	5.72	-0.07
4l	5.72	7.84	7.89	-0.05
5e	4.41	4.99	4.97	0.02
5f*	4.76	5.74	6.01	-0.27
5g	5.09	6.47	6.46	0.01
5h	5.09	6.47	6.44	0.03
5i	5.41	7.15	7.10	0.05
5j	4.46	5.09	5.09	0.00
5k*	4.76	5.74	5.71	0.03
5l	5.76	7.91	7.88	0.03
6d	4.72	5.66	6.26	-0.60
6e	5.36	7.04	7.06	-0.02
6h*	5.13	6.55	6.58	-0.03
6i	4.84	5.92	5.92	0.00
6j	5.10	6.48	6.42	0.06
6k*	5.10	6.48	6.46	0.02

* indicates molecules of test set.

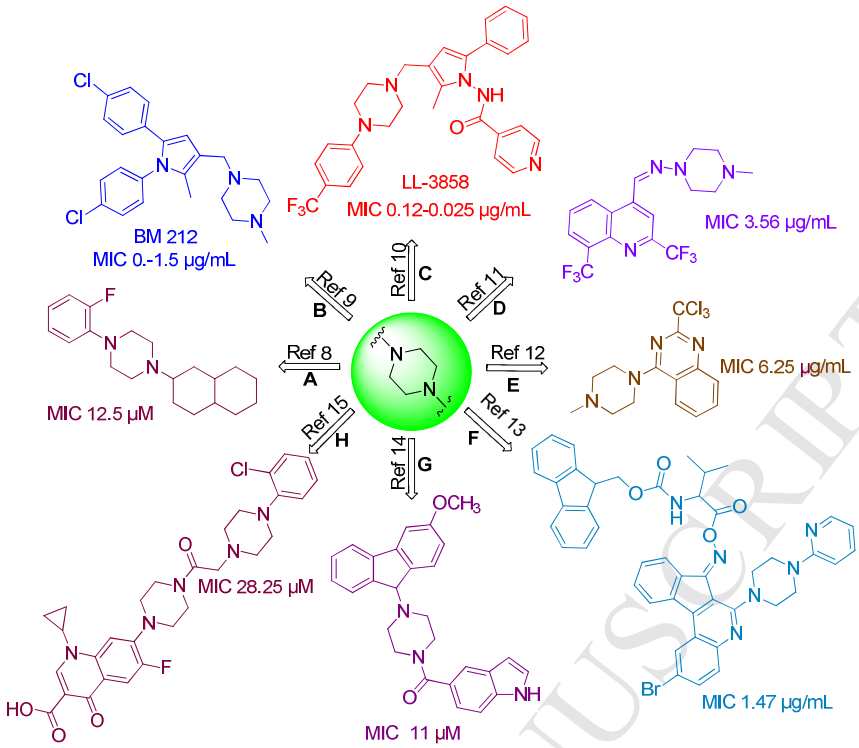
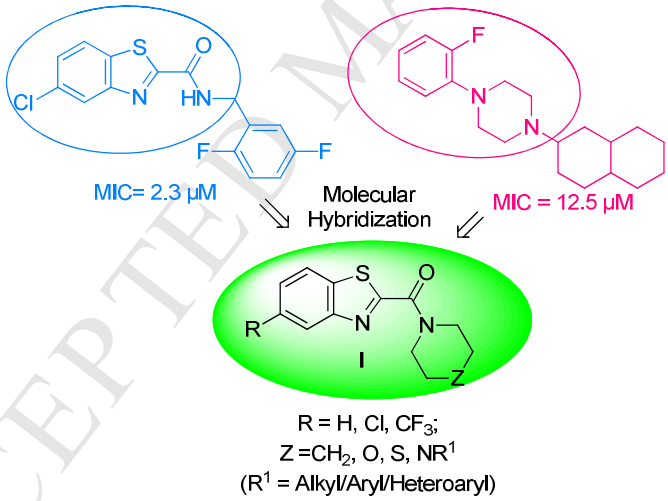


Figure 1.



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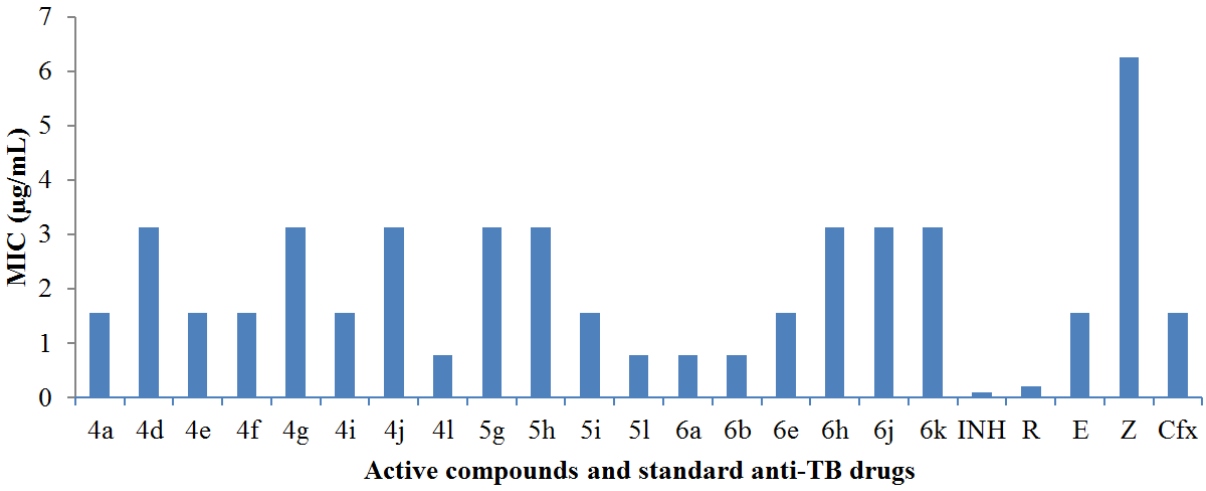

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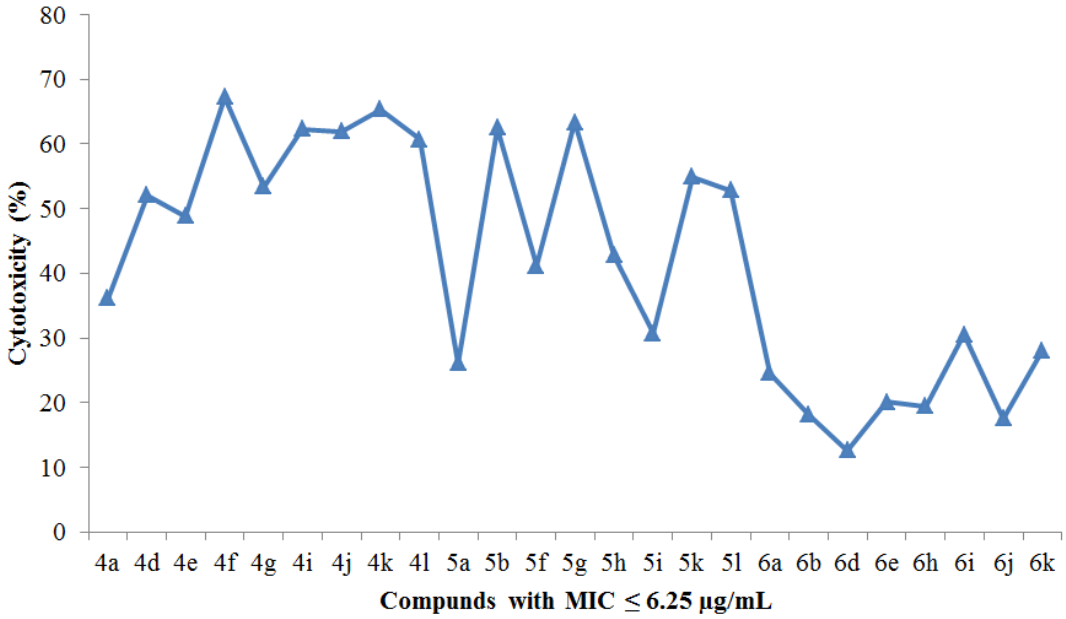

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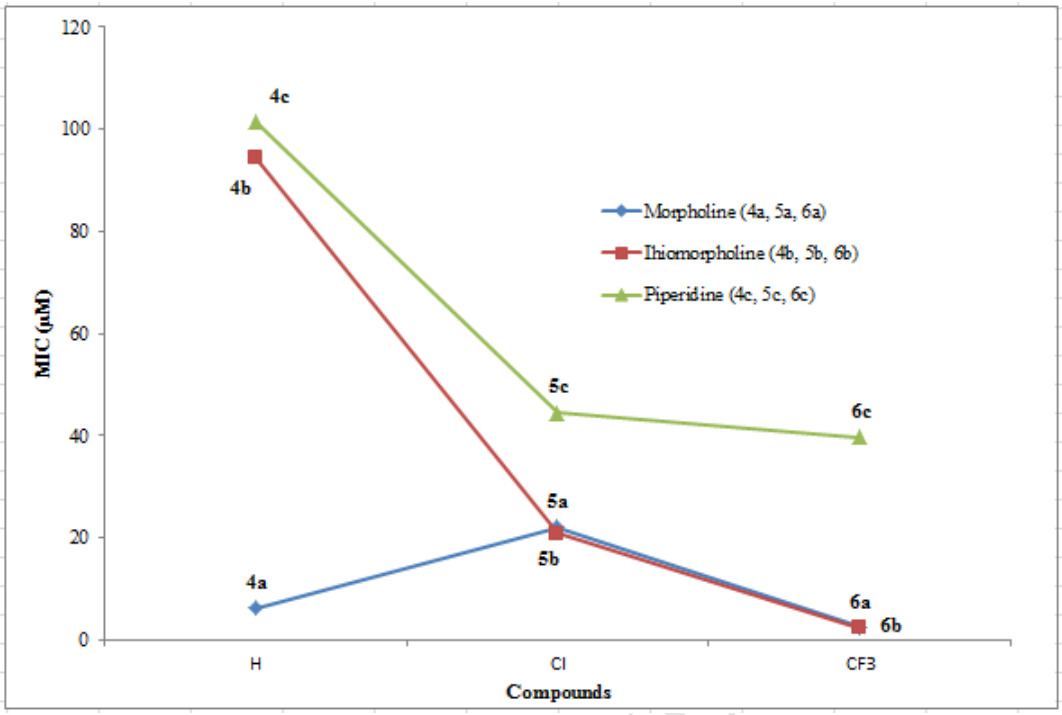

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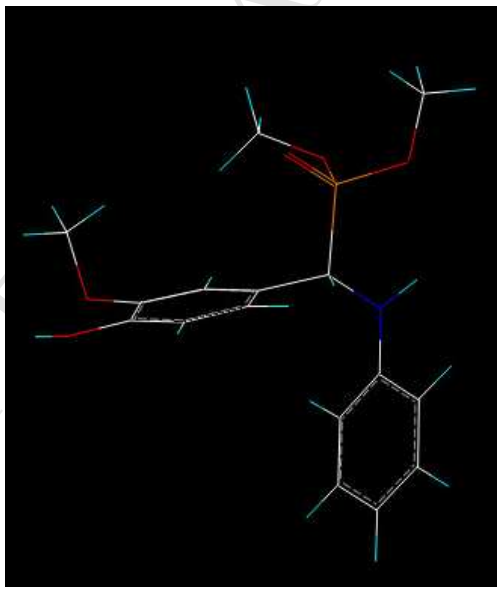

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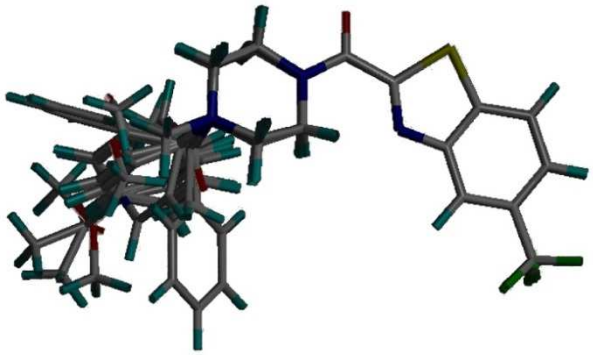

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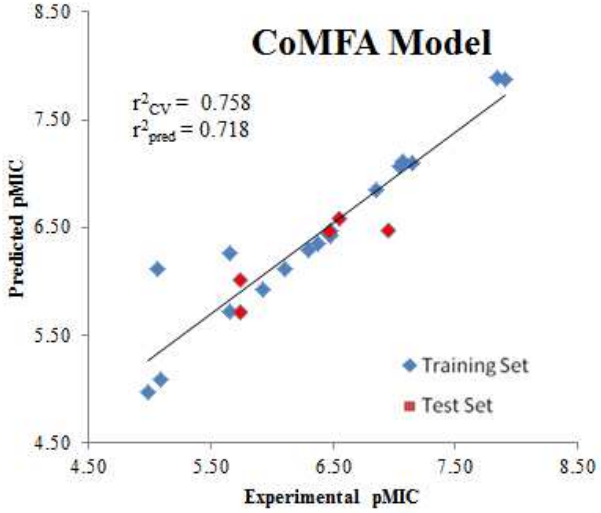

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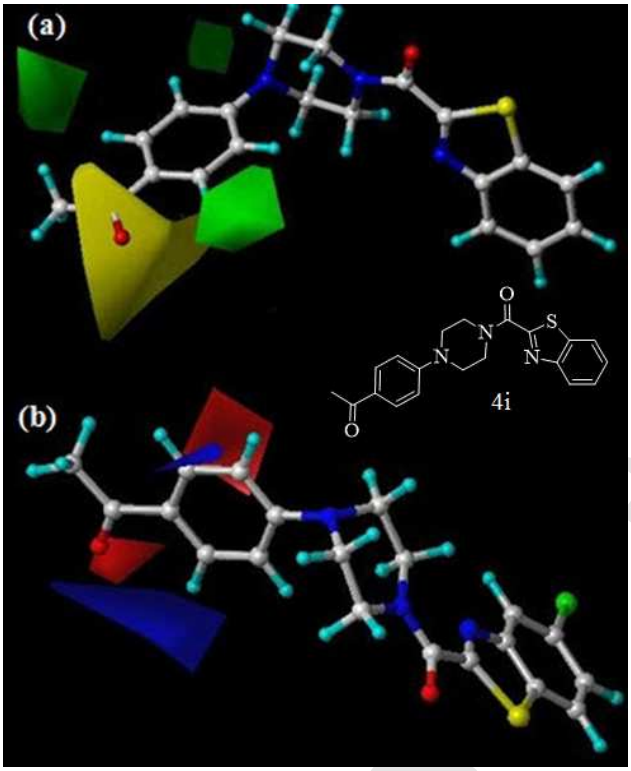

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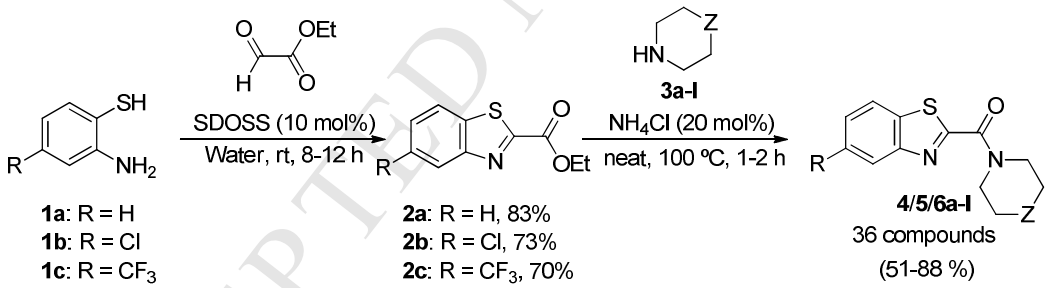

Scheme 1. Synthesis of benzo[*d*]thiazole-2-carboxamide **4**/**5**/**6a-l** via ammonium chloride catalyzed coupling of **2a**/**2b**/**2c** with the alicyclic amine **3a-l**.

Benzo[*d*]thiazol-2-yl(piperazin-1-yl)methanones as New Anti-mycobacterial Chemotypes: Design, Synthesis, Biological Evaluation and 3D-QSAR Studies

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36 compounds were synthesized using green synthetic protocol.

Twenty-one compounds displayed good *in vitro* anti-mycobacterial activity.

The most potent 3 compounds exhibited MIC of 0.78 μg/mL with therapeutic index > 60.

The 3D-QSAR for has been established with significant CoMFA model.