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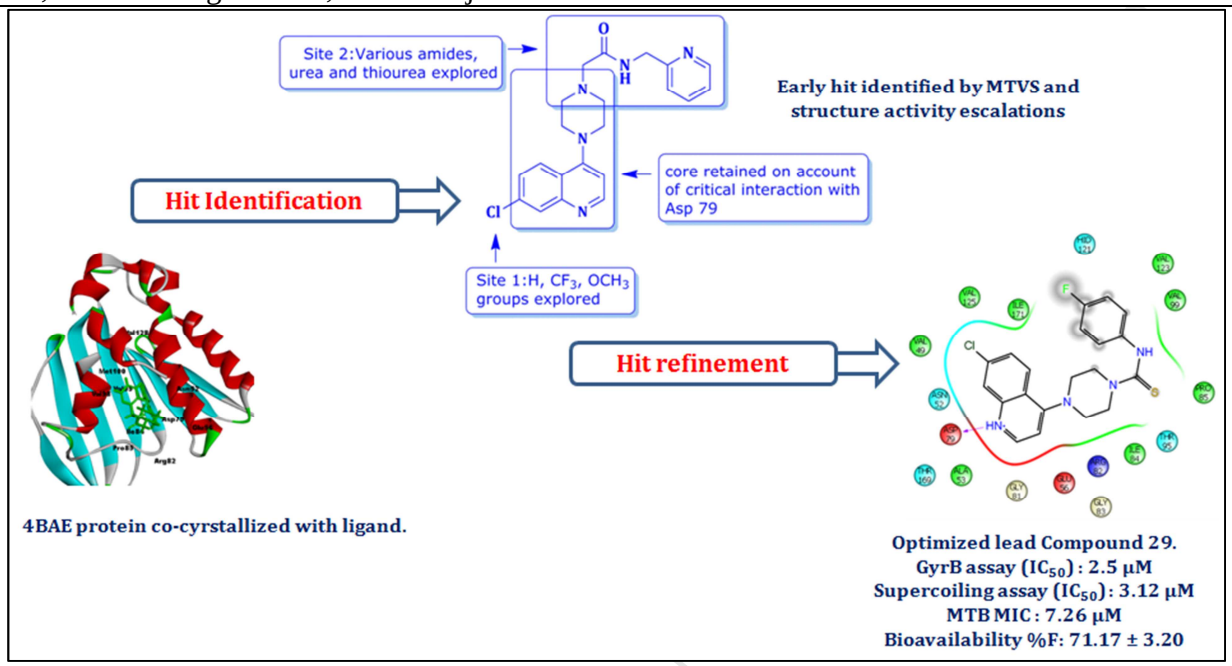

A structure based medium throughput virtual screening of in house chemical library to identify novel binders of *Mycobacterium tuberculosis* gyrase ATPase domain led to the discovery of a quinoline scaffold. Initial hit is further optimized to study SAR and biological evaluation.

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Abstract:

A structure based medium throughput virtual screening campaign of BITS-Pilani in house chemical library to identify novel binders of *Mycobacterium tuberculosis* gyrase ATPase domain led to the discovery of a quinoline scaffold. Further medicinal chemistry explorations on the right hand core of the early hit, engendered a potent lead demonstrating superior efficacy both in the enzyme and whole cell screening assay. The binding affinity shown at the enzyme level was further corroborated by biophysical characterization techniques. Early pharmacokinetic evaluation of the optimized analogue was encouraging and provides interesting potential for further optimization.

Keywords: Medium throughput virtual screening, *Mycobacterium tuberculosis*, DNA Gyrase, differential scanning fluorimetry.

1. Introduction:

Poor patient compliance and irrational prescribing practices have ruined the effectiveness of the currently available drug regime for tuberculosis. The emergence of the drug resistant strains and TB co-infection in HIV/AIDS patient are other factors that have made the treatment for TB more challenging [1-3]. To combat these issues and to meet future therapeutic needs, structurally unique chemical entities that target new targets/pathways are needed.

The druggability of DNA Gyrase has been well established clinically and its inhibition has shown catastrophic effect on bacterial cell growth and survival. DNA gyrase is an essential enzyme that introduces negative supercoils into DNA and regulates the superhelical state of the bacterial chromosomes. The functional DNA gyrase enzyme exists as a heterotetramer with two A subunits and two B subunits (A_2B_2). The A subunit (90 to 100 kDa, depending on the bacterial species) carries the breakage-reunion active site, whereas the B subunit (70 to 90 kDa) promotes ATP hydrolysis, providing sufficient amount of energy for the DNA super coiling.

Unlike other bacterial genome DNA-gyrase is the sole Type II topoisomerase in *Mycobacterium tuberculosis*, making it more attractive from a drug discovery perspective as it makes the enzyme more vulnerable to inhibition and hence a novel DNA gyrase lead can be effectively nurtured into an anti-tubercular drug [4-7].

Flouroquinolones (FQs), that acts through the inhibition of gyrA domain of DNA gyrase are the most researched anti-tubercular DNA gyrase inhibitors, with two candidates (Moxifloxacin [MXF] and Gatifloxacin [GAT] being trailed at Phase III of current anti-TB clinical portfolio [8-10]. MXF has demonstrated promising activity against both drug sensitive and drug resistant strains of MTB and had shown indication of its usefulness in reducing the length of TB treatment

regimens in their early *in vitro* and murine studies [11-12]. However a recent meta-analysis for clinical trials of MXF or GAT containing regimen to evaluate their treatment efficacy and safety as part of first line therapy of drug sensitive tuberculosis have indicated that MXF of GAT might not have the ability to shorten treatment duration in the initial therapy for tuberculosis [13]. These coupled with prevalence of pre-existing resistance to FQs suggest that, although flouroquinolones could probably replace isoniazid in the first line therapy of tuberculosis because of their superior bactericidal activity and may also help in treating drug-resistant tuberculosis but they are not going to revolutionize the treatment of tuberculosis. [14].

The ATPase domain; that makes the other half of functional gyrase heterotetramer (A_2B_2) complex promotes ATP hydrolysis, providing sufficient amount of energy for the DNA super coiling activity. In the absence of the ATP, DNA gyrase catalyzes only the relaxation of supercoiled DNA but not the introduction of negative supercoils. Depriving the enzyme the source of energy via inhibition of GyrB domain should still exert the same phenotypic effect on the bacterial viability to the one exhibited by fluoroquinolones, that inhibits the gyrA domain. Moreover mutations in gyrase that confer resistance to fluoroquinolones are outside the 43 kDa N-terminal domain that is required for ATPase activity. However, there has not been any effective therapeutics developed against this target for TB. Thus inhibitors that target ATPase domain, the focus of the present study may prove beneficial to replace the fluoroquinolones when resistance for that class of drug becomes regnant [4-7, 15-20].

2. Results and Discussion:

Combination of ligand based modeling and virtual screening is an emerging concept in drug design for identification of newer chemotypes as attractive starting points for medicinal chemistry SAR exploration. In the present work we report the development of a novel class of

anti-tubercular agent acting through the inhibition of the mycobacterial Gyrase ATPase domain identified through structure based screening of diverse compound collection of BITS Pilani in house database. Recent efforts from Astra Zeneca reports the identification of novel class of pyrrolamides as potential Gyrase ATPase inhibitors with promising antimicrobial potency against *Mycobacterium tuberculosis* H37Rv strain as well as 99 drug resistant, clinical isolates of *Mycobacterium tuberculosis* [16]. Reported crystal structure of the *Mycobacterium smegmatis* GyrB protein co-crystallized with most potent ligand from this study [Gyrase inhibitory IC_{50} < 5 nM, PDB entry 4BAE] [16] retrieved from the Protein Data Bank (www.rcsb.org) was utilized as a structural framework in our docking explorations to identify newer chemotypes as putative binders of Gyrase ATPase. Glide XP (extra precision) module of Schrödinger 9.2 (Glide, version 5.7, Schrödinger, LLC, New York, NY, 81 2011) [21] was utilized for docking. An initial validation of the active site pocket was performed by redocking the crystal ligand 2-[4-(3-bromo-4-chloro-5-methyl-1H-pyrrole-2-amido)-3-methoxypiperidin-1-yl]-4-(1-methyl-1H-1,2,4-triazol-5-yl)-1,3-thiazole-5-carboxylic acid with the active site residues of the *Mycobacterium smegmatis* GyrB protein. Redocking results showed that the compound exhibited similar interactions [**Figure S1** in supplementary information] as that of the original crystal structure which was further confirmed with RMSD of 1.45 Å. Later, BITS Pilani chemical library consisting of little more than 3000 diverse compounds were first subjected to a Glide based Medium Throughput Virtual Screening (MTVS), a method specifically proven to discard noticeable nonbinders with minimal computational time. The molecules that came out of this exercise were then subjected to a second round of filtering for medchem tractability based on physicochemical parameters as predicted using QikProp module of Schrödinger, LLC, and undesirable chemical features. The basic goal of this strategy was to decrease the enormous

virtual chemical space of small organic molecules to a manageable number of compounds that could inhibit the protein with the highest chance to lead to a drug candidate. Four hundred eighty ligands that satisfied the above criteria and thus were drug like were further flexibly re-docked together with the crystal ligand using the more computationally expensive Glide standard precision (SP) scoring; this led to the selection of 120 compounds. To evaluate precisely the binding interactions that these ligands maintained in the active site cavity, the hits obtained from Glide SP docking were further evaluated with Glide XP. The Glide XP combines accurate, physics-based scoring terms and thorough sampling, and the results gave scores ranging from −5.01 to −7.10 kcal mol^{-1}. Final short listing of possible hit compounds was based on visual inspection of the important amino acid residues in the active site cleft involved in binding that included hydrogen bonds to Asp79, Arg141 and Arg82 and hydrophobic interactions with Ala53, Ile171, Val49, Val77, Val50, Val125, Met100, Val99, Ile84 and Val123, analogues to the one observed with the reported pyrrolamide ligand used as template in this study [PDB entry 4BAE, **Figure S1** in supplementary information].

The selected hits retrieved from the BITS Pilani database were then experimentally evaluated for their *in vitro Mycobacterium smegmatis* ATPase inhibitory potency at a single concentration of 50 µM in triplicate by using malachite green based assay adapted to a 96-well plate format and finally for a dose–response estimation in more detail as described previously[19,22-24]. The ATPase assay was performed on *Mycobacterium smegmatis* DNA gyraseB protein due to the low specific activity of *Mycobacterium tuberculosis* ATPase. The use of the *Mycobacterium smegmatis* GyrB protein as a surrogate for the GyrB protein from *Mycobacterium tuberculosis* has been well demonstrated in the literature [22] Novobiocin, which has been previously demonstrated to be a potent inhibitor of DNA GyrB,[18] was used as a positive control in this

study. Negative controls (0% inhibition) did not contain any inhibitory compounds. Compounds were also tested in the presence of Brij-35, a nonanionic detergent, to ascertain whether these inhibitions were an artifact due to sequestration of the enzyme by drug aggregates. Other artifacts, like auto absorbance of the drug, were also ruled out by nullifying its absorbance during the reaction. The working outline utilized for identifying the inhibitors has been depicted in **Figure 1** and the lig. plot representation of the best six ligands (hits) together with their docking scores, fitness and hydrogen bonding has been depicted in **Figure 2**.

Among the small number of hits identified Compound **1**, [**2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)-N-(pyridin-2-ylmethyl)acetamide** with gyr B inhibitory IC_{50} = 12.2 ± 0.09 µM emerged as the most promising candidate. To have an structural insight into the orientation and the possible binding interactions that the hit molecule maintained in the active site of the protein that could possibly be exploited in the subsequent hit expansion step to deliver more potent and selective inhibitor, the ligand was analyzed in more detail. In the docking explorations, Compound **1** exhibited a docking score of -6.26 $kcal/mol^{-1}$ and was found to be in the vicinity of the amino acid Ala53, Asp79, Val125, Val49, Ile171, Val77, Val98,Val99, Pro85, Asn52, Glu56 and Gly83 amino acid residues (which is also characterized to be the active site pocket). A closer look at the interaction profile diagram of the molecule [**Figure 2**, ligand 2 and **Figure S2** in supplementary information] showed the quinoline nitrogen (N-4) to be involved in a prominent hydrogen bonding interaction with Asp 79, analogues to the one observed in the crystal ligand [PDB entry 4BAE, **Figure S1** in supplementary information]. This interaction is believed to be critical in retaining the activity. An additional hydrogen bonding interaction was observed between the pyridyl nitrogen on the right hand core and NH_2 of His121.Furthermore the compound was also found to be stabilized by the hydrophobic interaction with Val125,

Val49, Ile171, Val77, Ala53, Ile84, Val98, Val99, and Val123 amino acid residues. However the molecule failed to retain hydrogen bonding interaction with Arg141 and the pi-stacking interaction with Arg82 as observed in the reference crystal structure; probably critical interactions that might have reduced the potency of the molecules in the present study compared to the reference pyrrolamide ligand that exhibited *in vitro* GyrB IC_{50} in the nanomolar range.

Based on the findings from the protein-ligand interaction of compound **1** in the active site of protein, the following modification (and combinations thereof) were explored in the first hit expansion step (i) replacing the 7-chloro substituted quinoline nucleus with a 7–CF_3 7-OCH_3 substituted and a un- substituted quinoline core. (ii) extending/reducing the chain length of the amino-pyridyl nucleus on the right hand side (iii) introducing various aryl/heteroaryl nucleus of varied chain length on the right hand core as a possible replacement for the 2-aminomethyl pyridine nucleus.

Thus a series of 13 molecules were synthesized using the synthetic protocol described in **Scheme 1** in the first stage of hit expansion as steps towards the derivation of structure-activity relationships and hit optimization.

The synthesized derivatives (cmpd. **1**, **2 – 14**) were then evaluated for the GyrB inhibitory potency using the previously described malachite green assay. Among the 13 derivatives evaluated for their GyrB inhibitory potency, eight compounds showed an inhibitory IC_{50} of less than 25 µM; out of which two compounds exhibited strong inhibition of GyrB activity with IC_{50} less than 10 µM [**Table 1**]. Compound **6** and **13** emerged as the most promising leads with an inhibitory IC_{50} of 9.1 ± 0.5 µM and 7.6 ± 0.7 µM respectively.

With respect to the structure-activity relationship study, the various substitutions attempted at the C-7 position of the quinolone nucleus (cmpd. **2–4**) was detrimental to bioactivity as none of the

modified molecules showed any significant effect on the GyrB activity. Docking experiments revealed that unlike the chloro substituted analogue that oriented nicely into the hydrophobic pocket, the various substitutions attempted at the C-7 position changed the orientation of these molecules (cmpd. **3–4**) completely, taking it out of the active site pocket thereby loosing the important hydrophobic interactions, a fatal determinant of inhibitory potency. A similar trend was also observed for un-substituted analogue (cmpd. **1**) as well. These findings emphasized the importance of chloro group at this position; primarily due to the hydrophobic interaction that this group maintained in the active site pocket.

With respect to the modifications attempted to understand the effect of chain length on the aminopyridyl ring in activity determination (cmpd. **5** – **6**). It was found that an increase in chain length by one carbon atom as in the case of compound **5** was seen to significantly hamper the activity, this as understood from the docking studies was due to the loss of two important hydrogen bonding that the hit molecule (cmpd. **1**) maintained with Asp79 and His 121 [**Figure S3** in supplementary information]. However chopping the chain length of the aminopyridyl ring by one carbon atom (as in compound **6**) did not have notable reduction in potency. The molecule could still hold on to the important interaction with Asp 79 observed in the active site pocket and the additional hydrogen bond with His 141 outside the active site cavity, analogues to the one observed in the hit molecule compound **1**; accounting for its good GyrB inhibitory IC_{50} of 9.1 ± 0.5 µM.

Among the various aryl/heteroaryl substituted amides attempted in synthesis (cmpd. **7** - **14**); compound **13** emerged as the most promising lead with a GyrB inhibitory IC_{50} of 7.6 ± 0.7 µM. The binding analysis of this compound [**Figure S4** in supplementary information] showed the molecule oriented nicely into the active site cavity in a pattern similar to the crystal ligand and

was found to be involved in three hydrogen bonding interactions with Asp79, Arg141 and Arg82 amino acid residues, in addition to the hydrophobic interactions with the active site residues.

Keeping these findings in mind, a second subset was synthesized by introducing various substituents on the right hand core of the 7-chloro-4-(piperazin-1-yl)quinoline nucleus that mimicked the pyridyl nucleus (cmpd. **15 – 27**) in the hit molecule compound **1**. A further *in vitro* GyrB evaluation of the synthesized molecules showed that 9 out of 13 molecules tested under these conditions showed IC_{50} less than 15 µM [**Table 1**] with thiocarbamide (cmpd. **24**) and carbamide (cmpd**. 25**) derivatives emerging as most potent leads with an IC_{50} of 5.5 ± 0.1 µM and 6.1 ± 1.3 µM respectively. A closer look at the interaction profile diagram of these molecules [Figure **S5** in supplementary information] showed that molecule oriented deeply into the hydrophobic cavity in the active site pocket; exhibiting good fitness score and retaining the critical hydrogen bonding interaction with Asp79 as well as the non-polar interaction in the hydrophobic pocket.

Based on these observations, another 18 derivatives exploring various aliphatic and aromatic carbamide and thiocarbamide derivatives as the right hand core of the 7-chloro-4-(piperazin-1-yl)quinoline nucleus (cmpd. **28 – 45**) were synthesized and evaluated for their GyrB inhibitory potency. *In vitro* characterization of these analogues revealed the molecules to exhibit good to very promising GyrB inhibitory potency in the lower micromolar range. Compound **29** and **36**, the 4-fluorophenyl substituted thiocarbamide and the carbamide derivatives emerged as the most promising optimized derivatives with a GyrB inhibitory IC_{50} of 2.5 ± 0.1 µM and 3.1 ± 0.2 µM respectively. The docking studies [Figure **S6 & S7** in supplementary information] showed the molecules to orient in a manner analogues to that virtual screening hit and also to that of the second generation thiocarbamide/carbamide analogues (cmpd. **24** and **25**); retaining the critical

interaction with Asp 79. The molecules nicely oriented into the hydrophobic cavity and were found to be stabilized there by hydrophobic interactions with Val49, Val, Ala53, Ile171, Val125, and Val99 amino acid residues. A closer look at the interaction pattern of the less active analogue (cmpd. **34**) [Figure **S8** in supplementary information] in this class showed that the introduction of the bulkier acetyl group at the para position of the right hand phenyl core changed the orientation completely, thus the molecule though retained the hydrophobic interaction but failed to maintain the hydrogen bonding interaction with Asp79 thus accounting for the loss in activity.

The aliphatic thiocarbamide/carbamide derivatives (cmpd. **42** - **45**) attempted in synthesis also turned out to be completely inactive. An in-silico investigation into the same [Figure **S9** in supplementary information] revealed that the molecules oriented in a completely opposite manner to the active analogues, throwing the quinoline nucleus out of the pocket. Although, the molecules still retained the interaction with Asp 79 but this was rather observed with the right hand carbamide and thiocarbamide NH and not with the quinoiline nitrogen (N-4) as in the case of hit compound **1**. Also these molecules failed in maintaining hydrophobic interaction with amino acid residues present in the active site of protein highlighting the presence of hydrophobic ring on the right hand core as an important determinant of inhibitory potency.

Thus the hydrogen bonding interaction with Asp 79 in the active site pocket and the non polar interactions retained in the hydrophobic cavity of the active site could be considered as the critical factors that drove the bioactivity.

Furthermore, the binding affinity of the most potent analogue was evaluated by measuring the thermal stability of the protein–ligand complex using biophysical differential scanning fluorimetry experiments (**Figure 3**) using a previously demonstrated protocol [23-25].

Compound **29** displayed a T_M shift of 4.1°C (T_M = 48.1°C) compared with the native protein (T_M = 44°C), a repercussion of strong binding of the ligand to the protein and highly correlating with its GyrB IC_{50} of 2.5 ± 0.1 µM.

All the synthesized molecules were also subjected to a number of secondary screenings that included the *Mycobacterium tuberculosis* DNA supercoiling assay; followed by *in vitro* evaluation of their antimycobacterial potency and safety profile.

The DNA supercoiling assay would be an indirect measurement of their GyrB inhibitory potency as any inhibition of ATPase activity conferred by the DNA GyrB subunit should also inhibit the supercoiling activity performed by gyrA domain. Thus all the compounds were further evaluated for their supercoiling inhibition studies using *Mycobacterium tuberculosis* DNA gyrase supercoiling kit from Inspiralis (Inspiralis, Norwich) [26]. In general, a good correlation was observed between the *in vitro* GyrB potency and *in vitro* supercoiling activity [**Table 1**]. The hit molecule compound **1** exhibited a supercoiling inhibitory IC_{50} of 6.25±0.8 µM. Out of 44 molecule studied, 35 molecules exhibited an inhibitory IC_{50} of < 25 µM, out of which 23 molecules exhibited an inhibitory IC_{50} of < 10 µM and 12 molecules exhibited an inhibitory IC_{50} of < 5 µM [**Table 1**]. The optimized analogues Compound **29** and **36** showed an IC_{50} of 2.7 ± 0.14 µM and 3.125 ± 0.9 µM, respectively well correlating with and GyrB inhibitory IC_{50} of 2.5 ± 0.1 µM and 3.1 ± 0.2 µM, respectively.

The antimycobacterial potency of these molecules were evaluated by *in vitro* MABA assay [27]. Out of the 44 molecules tested 33 molecules showed MIC less than 50 µM; out of which 16 molecules showed MIC less than 25 µM and 2 molecules exhibited MIC less than 10 µM [**Table 1**]. The optimized analogues, compounds **29** and **36** showed an *in vitro Mycobacterium tuberculosis* MIC of 7.8 µM and 15.3 µM respectively synchronizing well with their GyrB

inhibitory IC_{50} of 2.5 ± 0.1and 3.1 ± 0.2 µM and supercoiling IC_{50} of 2.7 ± 0.14 and 3.125 ± 0.9 µM.

Finally the toxicity profile of all the compounds were also tested against the mouse macrophage RAW 264.7 cell lines at 100 µM concentration using (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [28]. All the compounds displayed relatively good safety profile except for the few nitro substituted analogues that exhibited slight inhibition at this tested concentration, with Compound **38** exhibiting maximum inhibition of 42%, but can be considered relatively insignificant at this stage of the study as their gyrase inhibitory IC_{50} were less than 20 µM.

that showed slight toxicity at the concentration tested [**Table 1**].

Pharmacokinetic profile of potent analogue compound **29** was evaluated in male Wistar rats following intravenous (1 mg/kg) and oral (20 mg/kg) administration using a protocol as detailed in the experimental section of supplementary information. Post to intravenous administration, compound **29** exhibited favorable pharmacokinetic properties with moderate distribution outside of vascular system (0.99 L/kg) and low systemic clearance (**Figure 5** and **Table 2**). Following oral administration (**Figure 5**), compound **29** was rapidly absorbed (T_{max}~ 2h) and showed high plasma exposure resulting fairly good oral bioavailability (68-75 %). From the oral profile, it is evident that concentration levels of compound **29** were well above the IC_{50} (~1.0 µg/ml) for more than 6h. Such a high exposure could ensure therapeutic effectiveness of the compound against TB infections for extended duration. It is well known that the free drug concentration in the blood influences pharmacokinetic and pharmacodynamic properties of a molecule [29]. Therefore, we investigated protein binding ability of compound **29** in human and rat plasma matrices. Compound **29** showed extensive protein binding (> 90 %) across the species and the

297 binding rate of compound **29** with plasma protein was concentration-dependent (**Table 2**). In
298 order to predict metabolic clearance in humans, we investigated *in vitro* metabolic stability
299 profile of compound **29** in human liver microsomes and extrapolated for human clearance
300 (CL_{blood}). Microsomal stability study predicts slow hepatic clearance (10.01 mL/min/kg) of
301 compound **29** in humans. In conclusion, our studies demonstrate favorable pharmacokinetic
302 properties of compound **29**, encouraging further *in vivo* studies and consideration of compound
303 **29** as a suitable candidate to be worked out from a pharmaceutical point of view as potential anti-
304 tubercular lead.

305 **3. Conclusion**:

306 The present study describes the identification of novel structural motif from a medium
307 throughput virtual screening campaign of an in-house 3000-member compound library for
308 Mycobacterium tuberculosis DNA gyraseB inhibitors. Synthesis and structure activity
309 relationship (SAR) studies around initial hit led to several analogues, the most potent of which
310 displayed an *in vitro* gyrB inhibitory IC_{50} value of 2.5 ± 0.1 µM. A correlation between *in vitro*
311 gyrB inhibitory activity and *in vitro* antimycobacterial activity against the lab sensitive H37Rv
312 strain of Mycobacterium tuberculosis has been demonstrated. A preliminary evaluation of
313 ADME parameters; demonstrated favorable pharmacokinetic properties of compound **29** and
314 provides an important advance within the context of new antituberculosis chemotypes.

315 **4. Experimental section:**

316 **4.1. Chemistry:**

317 **4.1.1. General:**

318 All commercially available chemicals and solvents were used without further purification. TLC
319 experiments were performed on alumina-backed silica gel 40 F254 plates (Merck, Darmstadt,

Germany). Homogeneity of the compounds was monitored by thin layer chromatography (TLC) on silica gel 40 F254 coated on aluminum plates, visualized by UV light and $KMnO_4$ treatment. Flash chromatography was performed on a Biotage Isolera with prepackaged disposable normal phase silica columns. All ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-300 (300.12 MHz, 75.12 MHz) NMR spectrometer, Bruker BioSpin 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; dd, doublet of doublets; 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). The purity of the final compounds was examined by HPLC (Shimadzu, Japan, (on Phenomenex C8 (150 * 4.6 mm, 5µm, 100 Å) double end-capped RP-HPLC column)) and was greater than 95%. 4,7-dichloroquinoline, the precursor for preparing compounds **1**, **5-45** was procured from Sigma-Aldrich (Cas no: 86-98-6). The precursor 4-chloroquinoline, 4-chloro-7-(trifluoromethyl)quinoline, 4-chloro-7-methoxyquinoline utilized for generating compounds **2**, **3** and **4** were synthesized utilizing the literature protocol [30-32] respectively.

4.1.2. 7-Chloro-4-(piperazin-1-yl)quinoline: To a suspension of 4,7-dichloroquinoline (2.5g, 12.6 mmol) and potassium carbonate (2.1, 15.1 mmol) in acetonitrile (20 mL) was added piperazine (1.1g, 12.6 mmol) at 30^{o}C. The reaction mixture was then heated to 80^{o}C for 1-2 h (monitored by TLC and LCMS for completion), cooled to 30^{o}C. The mixture was then filtered through celite bed, and acetonitrile was evaporated in vacuo. The resultant residue was diluted with water (10 mL) and dichloromethane (20 mL) and the layers separated. The aqueous layer was re-extracted with dichloromethane (2 x 25 mL). The combined organic extract was washed with brine, dried over sodium sulphate, and evaporated in vacuo. The resultant residue was the

purified by column chromatography on neutral alumina using hexane:ethylacetate as eluent to give **7-chloro-4-(piperazin-1-yl)quinoline** (1.9g, 61.3%) as an off-white solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.92 - 3.03 (m, 4H), 3.05 - 3.13, (m, 4H), 6.95 (d, *J* = 5.1Hz, 1H), 7.52 (dd, *J* = 8.7 Hz, *J* = 1.8 Hz, 1H), 7.92 - 8.04 (m, 2H), 8.67 (d, *J* = 4.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δc. 156.7, 152.1, 149.6, 133.4, 128, 125.9, 125.5, 121.3, 109.2, 52.8, 45.3. ESI-MS *m*/*z* 248.1 $(M+1)^+$. Anal Calcd for $C_{13}H_{14}ClN_3$; C, 63.03; H, 5.70; N, 16.96; Found: 62.99; H, 5.67; N, 16.93

4.1.3. Methyl 2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)acetate: To a suspension of 7-chloro-4-(piperazin-1-yl)quinoline (2.5g, 10 mmol) and potassium carbonate (1.66 g, 12 mmol) in acetonitrile (20 mL) was added methylbromoacetate (1.53 g, 10 mmol) at 30^{o}C. The reaction mixture was then heated to 60^{o}C for 1h (monitored by TLC and LCMS for completion), cooled to 30^{o}C. The mixture was then filtered through celite bed, and acetonitrile was evaporated in vacuo. The resultant residue was diluted with water and dichloromethane, and the layers separated. The aqueous layer was re-extracted with dichloromethane (2 x 40 mL). The combined organic extract was washed with brine, dried over sodium sulphate, and evaporated in vacuo The resultant residue was the purified by column chromatography on neutral alumina using hexane: ethylacetate as eluent to give **methyl 2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)acetate** (2.23g, 67.6%) as an off-white solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.73– 2.82 (m, 4H), 3.01 – 3.12 (m, 4H), 3.36 (s, 2H), 3.68 (s, 3H), 6.98 (d, *J* = 5.1Hz, 1H), 6.93 – 8.61 (m, 4H). ^{13}C NMR (DMSO-d_6): δc. 170.3, 156.5, 151.9, 149.7, 133.3, 128.1, 125.7, 125.3, 121.5, 108.9, 57.1, 55.3, 53.9, 51.2. ESI-MS *m*/*z* 320.1 $(M+H)^+$. Anal Calcd for $C_{16}H_{18}ClN_3O_2$; C, 60.09; H, 5.67; N, 13.14; Found: C, 60.15; H, 5.64; N, 13.11

4.1.4. 2-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)acetic acid: To a solution of methyl 2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)acetate (1.5g, 4.7 mmol) in THF:H_2O:CH_3OH system (1:1:1) was added lithium hydroxide (0.3 g, 7.1 mmol) at 0^oC. The reaction mixture was slowly warmed to 30^oC then stirred at 30^oC for 3-4h (monitored by TLC and LCMS for completion). The reaction mixture was then cooled to 0^oC and acidified to a pH of 3-4 with 1N HCl. and extracted with dichloromethane (3 x 50mL). The combined organic extract was successively washed with water and brine, dried over sodium sulphate, and evaporated in vacuo to give **2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)acetic acid** (0.7g, 49%) as white solid. ^{1}H NMR (DMSO-d_6): δ_H 2.63 – 2.69 (m, 4H), 2.98 – 3.09 (m, 4H), 3.24 (s, 2H), 6.97 – 8.63 (m, 5H) 12.1 (s, 1H). ^{13}C NMR (DMSO-d_6): δc. 175.8, 156.9, 151.8, 149.3, 133.6, 127.8, 126, 125.6, 121.1, 109, 60.6, 55.6, 54.1. ESI-MS *m/z* 304.1 $(M-H)^+$. Anal Calcd for $C_{15}H_{16}ClN_3O_2$; C, 58.92; H, 5.27; N, 13.74; Found: C, 58.87; H, 5.24; N, 13.78.

4.1.5. General procedure for the synthesis of amide derivatives (1 –14): To a solution of 2-(4-(7-sub:quinolin-4-yl)piperazin-1-yl)acetic acid (1 mmol) in dry dichloromethane (3 mL) was added triethyl amine (1.5 mmol) and corresponding amine (1 mmol) at 0^oC. Propylphosphonic anhydride (2 mmol) was then added drop wise to the reaction mixture and the reaction mixture was stirred at 30^oC for 6h, (monitored by TLC & LCMS for completion). The reaction mixture was washed with water (2 mL), brine (2 mL), dried over anhydrous sodium sulphate and evaporated in vacuo to give the desired product as mentioned below

4.1.5.1. 2-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-N-(pyridin-2-ylmethyl)acetamide (**1**): The compound was synthesized according to the above general procedure using 2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)acetic acid (0.25g, 0.82mmol), pyridin-2-ylmethanamine (0.089g mmol, 0.82mmol), triethylamine (0.125g, 1.23 mmol), propylphosphonic anhydride

(0.51g, 1.6mmol) to afford **1** (0.25g, 75%) as off white solid. M.p: 155-157 ^{o}C. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.56 - 2.69 (m, 4H), 3.07 – 3.16 (m, 4H), 3.36 (s, 2H), 4.51 (s, 2H), 6.93 (d, *J* = 5.2Hz, 1H), 7.24 – 8.64 (m, 9H). ^{13}C NMR (DMSO-d_6): δc. 171.4, 156.9, 155.8, 152.7, 150.2, 149.3, 140, 136.9, 132.6, 128.6, 125.8, 124.3, 123.1, 121.2, 109.6, 60.3, 52.3, 47.6, 45.9 ESI-MS *m*/*z* 396.1 $(M+H)^{+}$. Anal Calcd for $C_{21}H_{22}ClN_5O$; C, 63.71; H, 5.60; N, 17.69; Found: C, 63.66; H, 5.63; N, 17.74.

4.1.5.2. 2-(4-(Quinolin-4-yl)piperazin-1-yl)-N-(pyridin-2-ylmethyl)acetamide (**2**): The compound was synthesized according to the above general procedure using 2-(4-(quinolin-4-yl)piperazin-1-yl)acetic acid (0.25g, 0.92mmol), pyridin-2-ylmethanamine (0.099g mmol, 0.92mmol), triethylamine (0.139g, 1.38 mmol), propylphosphonic anhydride (0.41g, 1.8mmol) to afford **2** (0.23g, 69.7%) as off white solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.49 - 2.57 (m, 4H), 3.01 – 3.11 (m, 4H), 3.32 (s, 2H), 4.47 (s, 2H), 6.98 (d, *J* = 5.1 Hz, 1H), 7.24 – 8.64 (m, 10H). ^{13}C NMR (DMSO-d_6): δc. 171.1, 157.3, 156, 151.6, 148.9, 140.4, 139.9, 130.6, 130, 128.7, 128.2, 127, 124.3, 121.1, 114.9, 60.1, 52.6, 47.8, 46.1. ESI-MS *m*/*z* 362.1 $(M+H)^{+}$. Anal Calcd for $C_{21}H_{23}N_5O$; C, 69.78; H, 6.41; N, 19.38; Found: 69.84; H, 6.36; N, 19.34

4.1.5.3. 2-(4-(7-Trifluoromethyl quinolin-4-yl)piperazin-1-yl)-N-(pyridin-2-ylmethyl)acetamide (**3**): The compound was synthesized according to the above general procedure using 2-(4-(7-trifluoromethyl quinolin-4-yl)piperazin-1-yl)acetic acid (0.25g, 0.74mmol), pyridin-2-ylmethanamine (0.08g mmol, 0.74mmol), triethylamine (0.11g, 1.11 mmol), propylphosphonic anhydride (0.32g, 1.4mmol) to afford **3** (0.18g, 58%) as off white solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.52 - 2.63 (m, 4H), 3.04 – 3.17 (m, 4H), 3.29 (s, 2H), 4.51 (s, 2H), 7.08 (d, *J* = 5.2 Hz, 1H), 7.32 – 8.78 (m, 9H). ^{13}C NMR (DMSO-d_6): δc. 171.3, 157.7, 156.2, 152.9, 148.8, 147.9, 139.8, 132.7, 129.1, 128.8, 126.5, 124.3, 124.1, 121.8, 121, 120.4,

59.9, 52.4, 47.9, 46. I-MS *m/z* 430.2 $(M+H)^+$. Anal Calcd for $C_{22}H_{22}F_3N_5O$; C, 61.53; H, 5.16; N, 16.31; Found C, 61.48; H, 5.17; N, 16.27.

4.1.5.4. 2-(4-(7-Methoxy quinolin-4-yl)piperazin-1-yl)-N-(pyridin-2-ylmethyl)acetamide (**4**): The compound was synthesized according to the above general procedure using 2-(4-(7-methoxy quinolin-4-yl)piperazin-1-yl)acetic acid (0.25g, 0.83mmol), pyridin-2-ylmethanamine (0.09g mmol, 0.83mmol), triethylamine (0.13g, 1.3 mmol), propylphosphonic anhydride (0.37g, 1.6mmol) to afford **4** (0.24g, 75%) as off white solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.46 - 2.55 (m, 4H), 2.98 – 3.09 (m, 4H), 3.34 (s, 2H), 3.86 (s, 3H), 4.49 (s, 2H), 6.73 (d, *J* = 5.1 Hz, 1H), 7.27 – 8.78 (m, 9H). ^{13}C NMR (DMSO-d_6): δc. 170.9, 156.9, 156, 152.1, 150.9, 148.9, 147.6, 139.9, 126.4, 123.8, 123.3, 121.3, 117.6, 116.2, 107.9, 60, 56.2, 52.3, 47.6, 46.1. ESI-MS *m/z* 392.1 $(M+H)^+$. Anal Calcd for $C_{22}H_{25}N_5O_2$; C, 67.5; H, 6.44; N, 17.89; Found C, 67.45; H, 6.46; N, 17.93.

4.1.5.5. 2-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-N-(2-(pyridin-2-yl)ethyl)acetamide (**5**): The compound was synthesized according to the above general procedure using 2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)acetic acid (0.25g, 0.82mmol), 2-(pyridin-2-yl)ethanamine (0.1g mmol, 0.82mmol), triethylamine (0.125g, 1.23 mmol), propylphosphonic anhydride (0.51g, 1.6mmol) to afford **5** (0.25g, 73.5%) as off white solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.54 – 2.62 (m, 4H), 3.06 – 3.19 (m, 6H), 3.27 (s, 2H), 3.63 (t, *J* = 7.1 Hz, 2H), 6.93 (d, *J* = 8.1Hz, 1H), 7.24 – 8.67 (m, 9H). ^{13}C NMR (DMSO-d_6): δc. 170.6, 158.1, 157.6, 152.3, 149.8, 147.9, 136.6, 133.7, 129.6, 129, 125.9, 123, 122.6, 121.2, 109.3, 60.2, 52.6, 47.9, 40.9, 35.8. ESI-MS *m/z* 410.1 $(M+H)^+$. Anal Calcd for $C_{22}H_{24}ClN_5O$; C, 64.46; H, 5.90; N, 17.09; Found: C, 64.41; H, 5.94; N, 17.13.

4.1.5.6. 2-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-N-(pyridin-2-yl)acetamide (**6**): The compound was synthesized according to the above general procedure using 2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)acetic acid (0.25g, 0.82mmol), pyridin-2-amine (0.77g, 0.82mmol), triethylamine (0.125g, 1.23 mmol), propylphosphonic anhydride (0.51g, 1.6mmol) to afford **6** (0.2g, 63%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.59 – 2.71 (m, 4H), 3.08 – 3.16 (m, 4H), 3.31 (s, 2H), 7.01 (d, *J* = 5.2 Hz, 1H), 7.28 – 8.71 (m, 9H). ^{13}C NMR (DMSO-d_6): δc. 168.7, 157.8, 152.6, 151.5, 149.9, 146.4, 139.2, 133.9, 129.8, 129, 125.9, 124.7, 122.7, 115.4, 109.4, 62.9, 52.8, 47.6. SI-MS *m*/*z* 382.1 $(M+H)^+$. Anal Calcd for $C_{20}H_{20}ClN_5O$; C, 62.91; H, 5.28; N, 18.34; Found: C, 62.86; H, 5.32; N, 18.28.

4.1.5.7. *N*-Benzyl-2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)acetamide (7): The compound was synthesized according to the above general procedure using 2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)aceticacid (0.25g, 0.82mmol), phenylmethanamine(0.088g mmol, 0.82mmol), triethylamine (0.125g, 1.23 mmol), propylphosphonic anhydride (0.51g, 1.6mmol) to afford **7** (0.27g, 84%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.49 – 2.56 (m, 4H), 2.96 – 3.08 (m, 4H), 3.23 (s, 2H), 4.21 (s, 2H), 6.93 (d, *J* = 5.1 Hz, 1H), 7.16 – 8.59 (m, 10H). ^{13}C NMR (DMSO-d_6): δc. 171.3, 157.2, 152.4, 149.8, 138.2, 133.1, 129.8, 128.6, 128.8, 127.1, 126.8, 125.8, 122.3, 109.2, 59.2, 52.4, 47.4, 43.2. ESI-MS *m*/*z* 395.1 $(M+H)^+$. Anal Calcd for $C_{22}H_{23}ClN_4O$; C, 66.91; H, 5.87; N, 14.19; Found: C, 66.95; H, 5.91; N, 14.23.

4.1.5.8. 2-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-N-phenylacetamide (**8**) The compound was synthesized according to the above general procedure using 2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)acetic acid (0.25g, 0.82mmol), aniline (0.076g mmol, 0.82mmol), triethylamine (0.125g, 1.23 mmol), propylphosphonic anhydride (0.51g, 1.6mmol) to afford **8** (0.14g, 45%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.53 – 2.61 (m, 4H), 3.07 – 3.16 (m, 4H), 3.37 (s, 2H), 6.89 (d, *J*

= 5.1 Hz, 1H), 7.13 – 8.62 (m, 10H). ^{3}C NMR (DMSO-d_6): δc. 168.3, 157.6, 152.7, 149.6, 138.3, 133.3, 129.8, 129.2, 128.8, 127.9, 126.2, 122.3, 121.6, 109.6, 63.4, 52.6, 47.8. ESI-MS *m*/*z* 381.1 $(M+H)^+$. Anal Calcd for $C_{21}H_{21}ClN_4O$; C, 66.22; H, 5.56; N, 14.71; Found: C, 66.27; H, 5.59; N, 14.75.

4.1.5.9. 2-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-N-(thiophen-2-ylmethyl)acetamide (**9**): The compound was synthesized according to the above general procedure using 2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)acetic acid (0.25g, 0.82mmol), thiophen-2-ylmethanamine (0.093g mmol, 0.82mmol), triethylamine (0.125g, 1.23 mmol), propylphosphonic anhydride (0.51g, 1.6mmol) to afford **9** (0.21g, 64%) as pale brown solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.49 – 2.56 (m, 4H), 2.91 – 3.03 (m, 4H), 3.23 (s, 2H), 5.02 (s, 2H), 6.83 – 8.49 (m, 9H). ^{13}C NMR (DMSO-d_6): δc. 171.1, 157.4, 152.8, 149.7, 141.1, 133.2, 129.8, 129, 127.2, 126.6, 125.8, 125.3, 122.9, 109.8, 59.8, 52.7, 47.6, 42.8. ESI-MS *m*/*z* 401.3 $(M+H)^+$. Anal Calcd for $C_{20}H_{21}ClN_4OS$; C, 59.91; H, 5.28; N, 13.97; Found: C, 59.86; H, 5.25; N, 13.91.

4.1.5.10. 2-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-N-(furan-2-ylmethyl)acetamide (**10**) The compound was synthesized according to the above general procedure using 2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)aceticacid(0.25g, 0.82mmol), furan-2-ylmethanamine (0.079g mmol, 0.82mmol), triethylamine (0.125g, 1.23 mmol), propylphosphonic anhydride (0.51g, 1.6mmol) to afford **10** (0.19g, 59%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.57 – 2.64 (m, 4H), 2.97 – 3.07 (m, 4H), 3.27 (s, 2H), 5.23 (s, 2H), 6.33 – 6.46 (m, 2H), 6.89 (d, *J* = 5.2 Hz, 1H), 7.39 – 8.59 (m, 6H). ^{13}C NMR (DMSO-d_6): δc. 170.8, 157.2, 152.7, 149.9, 146.3, 141.8, 133.4, 129.8, 129, 125.8, 122.6, 110.3, 110.1, 109.7, 59, 52.5, 47.2, 36.8. ESI-MS *m*/*z* 385.2 $(M+H)^+$. Anal Calcd for $C_{20}H_{21}ClN_4O_2$; C, 62.42; H, 5.50; N, 14.56; Found: C, 62.37; H, 5.55; N, 14.52.

4.1.5.11. 2-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-N-(5-nitrothiazol-2-yl)acetamide (**11**) The compound was synthesized according to the above general procedure using 2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)acetic acid (0.25g, 0.82mmol), 2-amino-5-nitrothiazole (0.093g mmol, 0.82mmol), triethylamine (0.125g, 1.23 mmol), propylphosphonic anhydride (0.51g, 1.6mmol) to afford **11** (0.24g, 68.6%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.61 – 2.73 (m, 4H), 3.06 – 3.16 (m, 4H), 3.31 (s, 2H), 6.89 (d, *J* = 5.1Hz, 1H), 7.32 – 8.61 (m, 6H). ^{13}C NMR (DMSO-d_6): δc. 168.7, 162.8, 157.6, 152.8, 150.1, 147.6, 136.2, 133.3, 130.2, 129.3, 126.1, 122.8, 109.6, 64.1, 52.9, 47.8. ESI-MS *m*/*z* 433.2 $(M+H)^+$. Anal Calcd for $C_{18}H_{17}ClN_6O_3S$; C, 49.94; H, 3.96; N, 19.41; Found: C, 49.88; H, 3.91; N, 19.37.

4.1.5.12. 2-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-N-(6-nitrobenzo[d]thiazol-2-yl)acetamide (**12**) The compound was synthesized according to the above general procedure using 2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)acetic acid (0.25g, 0.82mmol),6-nitro benzo[*d*]thiazol-2-amine (0.16g mmol, 0.82mmol), triethylamine (0.125g, 1.23 mmol), propylphosphonic anhydride (0.51g, 1.6mmol) to afford **12** (0.32g, 81%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.56 – 2.64 (m, 4H), 3.01 – 3.09 (m, 4H), 3.27 (s, 2H), 6.96 (d, J = 5.1 Hz, 1H), 7.26 – 8.82 (m, 8H). ^{13}C NMR (DMSO-d_6): δc. 175.4, 168.8, 158.3, 157.8, 152.8, 150, 143.8, 133.2, 131.6, 130.1, 129.1, 125.8, 122.3, 121.1, 118.6, 117.6, 109.8, 63.8, 52.7, 47.6. ESI-MS *m*/*z* 483.2 $(M+H)^+$. Anal Calcd for $C_{22}H_{19}ClN_6O_3S$; C, 54.71; H, 3.97; N, 17.40; Found: C, 59.77; H,4.02; N, 17.45.

4.1.5.13. *N*-(4-Acetylphenyl)-2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)acetamide (**13**) The compound was synthesized according to the above general procedure using 2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)acetic acid (0.25g, 0.82mmol), 1-(4-aminophenyl)ethanone (0.11g mmol, 0.82mmol), triethylamine (0.125g, 1.23 mmol), propylphosphonic anhydride

(0.51g, 1.6mmol) to afford **13** (0.28 g, 80%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.46 – 2.54 (m, 4H), 2.58 (s, 3H), 2.96 – 3.05 (m, 4H), 3.37 (s, 2H), 6.93 (d, J = 5.1 Hz, 1H), 7.29 – 8.63 (m, 8H). ^{13}C NMR (DMSO-d_6): δc. 189.3, 168.7, 157.6, 152.7, 149.8, 141.8, 136.9, 133.3, 129.8, 128.9, 128.7, 125.8, 122.8, 121.3, 109.7, 63.9, 52.8, 47.3, 26.1. SI-MS *m*/*z* 423.3 $(M+H)^+$. Anal Calcd for $C_{23}H_{23}ClN_4O_2$; C, 65.32; H, 5.48; N, 13.25; Found: C, 65.39; H, 5.51; N, 13.28

4.1.5.14. *N*-(2-Chloro-5-(trifluoromethyl)phenyl)-2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)acetamide (**14**) The compound was synthesized according to the above general procedure using 2-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)acetic acid (0.25g, 0.82mmol), 2-chloro-5-(trifluoromethyl) benzenamine (0.16g mmol, 0.82mmol), triethylamine (0.125g, 1.23 mmol), propylphosphonic anhydride (0.51g, 1.6mmol) to afford **14** (0.25g, 64%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.51 – 2.59 (m, 4H), 2.99 – 3.07 (m, 4H), 3.37 (s, 2H), 6.99 (d, J = 5.2 Hz, 1H), 7.27 – 8.61 (m, 8H). ^{13}C NMR (DMSO-d_6): δc. 169.1, 157.9, 152.6, 150, 138.1, 133.5, 129.8, 129.6, 129.4, 129.1, 126.1, 125.9, 124.3, 122.8, 121.9, 117.9, 109.7, 64.2, 52.9, 47.8. ESI-MS *m*/*z* 484.2 $(M+H)^+$. Anal Calcd for $C_{22}H_{19}Cl_2F_3N_4O$; C, 54.67; H, 3.96; N, 11.59; Found: C, 54.71; H, 4.01; N, 11.63.

4.1.6. General procedure for the synthesis of amide derivatives (15 – 20): To a solution of 7-chloro-4-(piperazin-1-yl)quinoline (1 mmol) in dry dichloromethane (3 mL) was added triethyl amine (1.5 mmol) and corresponding acid (1 mmol) at 0^{o}C. propylphosphonic anhydride (2 mmol) was then added drop wise to the reaction mixture and the reaction mixture was stirred at 30^{o} for 6h (monitored by TLC & LCMS for completion). The reaction mixture was washed with water (2 mL), brine (2 mL), dried over anhydrous sodium sulphate and evaporated in vacuo to give the desired product as mentioned below.

4.1.6.1. (4-(7-Chloroquinolin-4-yl)piperazin-1-yl)(1H-indol-2-yl)methanone (**15**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), indole-2-carboxylic acid (0.16g 1 mmol), triethylamine (0.15 g, 1.5 mmol), propylphosphonic anhydride (0.64g, 2 mmol) to afford **15** (0.33g, 84.6%) as off white solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.29 – 3.33 (m, 4H), 3.96 – 4.09 (m, 4H), 6.91 – 8.72 (m, 10H), 10.91 (bs, 1H). ^{13}C NMR (DMSO-d_6): δc. 162.3, 155.8, 152.6, 149.8, 139.6, 133.7, 133.3, 131.6, 129.8, 128.9, 125.8, 122.7, 121.5, 120.9, 119.4, 114.2, 110.8, 109.9, 51.6, 43.2. ESI-MS *m*/*z* 391.2 $(M+H)^+$. Anal Calcd for $C_{22}H_{19}ClN_4O$; C, 67.60; H, 4.90; N, 14.33; Found C, 67.55; H, 4.95; N, 14.37.

4.1.6.2. (4-(7-Chloroquinolin-4-yl)piperazin-1-yl)(5-fluoro-1*H*-indol-2-yl)methanone (**16**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), 5-fluoro-1*H*-indole-2-carboxylic acid (0.18g 1 mmol), triethylamine (0.15 g, 1.5 mmol), propylphosphonic anhydride (0.64g, 2 mmol) to afford **16** (0.26g, 78.8%) as off white solid. M.p: 229-231^{o}C. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.23 – 3.29 (m, 4H), 3.98 – 4.12 (m, 4H), 6.86 (s, 1H), 7.03 – 7.12 (m, 2H), 7.34 – 7.48 (m, 2H), 7.55 – 7.62 (m, 2H), 7.98 – 8.03 (m, 1H), 8.12 – 8.18 (m, 1H), 8.70 – 8.76 (m, 1H). ^{13}C NMR (DMSO-d_6): δc. 161.9, 156, 152.1, 149.6, 133.7, 132.7, 131.5, 128, 126.9, 126.8, 126, 121.3, 113.3, 112.1, 111.8, 109.6, 105.6, 104.2, 51.8, 42.9. ESI-MS *m*/*z* 409.2 $(M+H)^+$. Anal Calcd for $C_{22}H_{18}ClFN_4O$; C, 64.63; H, 4.44; N, 13.70, Found, C, 64.69; H, 4.41; N, 13.75.

4.1.6.3. (4-(7-Chloroquinolin-4-yl)piperazin-1-yl)(5-chloro-1*H*-indol-2-yl)methanone (**17**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol),5-chloro-1*H*-indole-2-carboxylic acid (0.2g 1 mmol), triethylamine (0.15 g, 1.5 mmol), propylphosphonic anhydride (0.64g, 2 mmol) to afford **17**

(0.32g, 74.4%) as off white solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.27 – 3.32 (m, 4H), 3.93 – 4.03 (m, 4H), 6.89 – 8.68 (m, 9H), 10.71 (bs, 1H). ^{13}C NMR (DMSO-d_6): δc. 162.3, 156.4, 152.3, 149.8, 136.4, 133.6, 133.3, 132.5, 129.6, 128.7, 125.8, 124.9, 122.7, 122.4, 121.9, 114.3, 113.8, 109.6, 51.9, 43.1. ESI-MS *m*/*z* 426.1 $(M+H)^+$. Anal Calcd for $C_{22}H_{18}Cl_2N_4O$; C, 62.13; H, 4.27; N, 13.17; Found C, 62.17; H, 4.31; N, 13.22.

4.1.6.4. (4-(7-Chloroquinolin-4-yl)piperazin-1-yl)(5-methoxy-1*H*-indol-2-yl)methanone (**18**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol),5-methoxy-1*H*-indole-2-carboxylic acid (0.19g 1 mmol), triethylamine (0.15 g, 1.5 mmol), propylphosphonic anhydride (0.64g, 2 mmol) to afford **18** (0.29g, 69%) as off white solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.26 – 3.31 (m, 4H), 3.86 (s, 3H), 3.99 – 4.09 (m, 4H), 6.83 – 8.56 (m, 9H), 10.89 (bs, 1H). ^{13}C NMR (DMSO-d_6): δc. 162.1, 156.4, 152.4, 149.8, 136.4, 133.6, 133.1, 132.6, 129.6, 128.7, 125.8, 124.9, 122.7, 122.4, 121.9, 114.3, 113.8, 109.8, 51.9, 43.1. ESI-MS *m*/*z* 421.11 $(M+H)^+$. Anal Calcd for $C_{23}H_{21}ClN_4O_2$; C, 65.63; H, 5.03; N, 13.31; Found C, 65.56; H, 5.07; N, 13.26.

4.1.6.5. (4-(7-Chloroquinolin-4-yl)piperazin-1-yl)(1*H*-pyrrol-2-yl)methanone (**19**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), 1H-pyrrol-2-carboxylic acid (0.11g 1 mmol), triethylamine (0.15 g, 1.5 mmol), propylphosphonic anhydride (0.64g, 2 mmol) to afford **19** (0.26g, 76.5%) as off white solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.31 – 3.37 (m, 4H), 3.96 – 4.03 (m, 4H), 6.79 – 8.71 (m, 9H). ^{13}C NMR (DMSO-d_6): δc. 161.8, 156.6, 152.3, 149.7, 133.5, 129.8, 128.6, 126.5, 125.7, 122.3, 120.7, 110.3, 109.8, 109.3, 52, 42.8. ESI-MS *m*/*z* 341.1 $(M+H)^+$. Anal Calcd for $C_{18}H_{17}ClN_4O$; C, 63.44; H, 5.03; N, 16.44; Found C, 63.49; H, 4.98; N, 16.47.

4.1.6.6. (4-(7-Chloroquinolin-4-yl)piperazin-1-yl)(pyridin-2-yl)methanone (**20**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), picolinic acid (0.12g 1 mmol), triethylamine (0.15 g, 1.5 mmol), propylphosphonic anhydride (0.64g, 2 mmol) to afford **20** (0.27g, 71.4%) as off white solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.26 – 3.32 (m, 4H), 3.99 – 4.10 (m, 4H), 7.06 (d, *J* = 5.2Hz, 1H), 7.39 – 8.89 (m, 8H). ^{13}C NMR (DMSO-d_6): δc. 161.9, 156.8, 156.1, 152.1, 149.8, 147.6, 137.1, 133.4, 129.8, 128.7, 126.4, 125.7, 122.3, 121.8, 109.3, 51.7, 42.9. ESI-MS *m*/*z* 353.1 $(M+H)^+$. Anal Calcd for $C_{19}H_{17}ClN_4O$; C, 64.68; H, 4.86; N, 15.88; Found C, 64.77; H, 4.89; N, 15.82.

4.1.7. General procedure for the synthesis of N-alkyl derivatives (22 & 23):

To a solution of 7-chloro-4-(piperazin-1-yl)quinoline (1 mmol) in dry dichloroethane (6 mL) was added the corresponding aldehyde (1.1 mmol), freshly activated 3 Å molecular sieves (0.25 g) and catalytic amount of acetic acid. The reaction mixture was stirred at 30^{o}C for 6 h and filtered through celite bed (under N_2 atmosphere). The solvent was removed under reduced pressure and residue further diluted with dry methanol (6 mL) and cooled to 0^{o}C. Sodium triacetoxy borohydride (1.5 mmol) was added (portion-wise) and the reaction was stirred at 30^{o}C for 6 h (monitored by TLC & LCMS for completion for completion). The solvent was then removed under reduced pressure and the residue diluted with water (5 mL) and extracted with dichloromethane (10 mL). The aqueous phase was back-extracted with dichloromethane (2 x 10 mL) and dried over sodium sulphate. The combined organic phases were concentrated under reduced and residue purified by column chromatography using hexane: ethylacetate as eluent to give the desired product in good yield.

4.1.7.1. 4-(4-((1*H*-Indol-3-yl)methyl)piperazin-1-yl)-7-chloroquinoline (**22**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), indole-2-carboxaldehyde (0.16g, 1.1mmol), to afford **22** (0.23g, 60.5%) as buff coloured solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.59 – 2.64 (m, 2H), 3.12 – 3.19 (m, 4H), 3.56 (s, 2H), 7.06 – 8.63 (m, 10H), 9.78 (bs, 1H). ^{13}C NMR (DMSO-d_6): δc. 155.9, 152.5, 149.6, 136.9, 133.4, 129.9, 128.6, 127.5, 125.7, 122.8, 122.3, 121.4, 119.3, 118.2, 111.9, 110.7, 109.8, 55.6, 51.8, 43.2. ESI-MS *m*/*z* 377.2 $(M+H)^+$. Anal Calcd for $C_{22}H_{21}ClN_4$; C, 70.11; H, 5.62; N, 14.87; Found C, 70.19; H, 5.67; N, 14.91.

4.1.7.2. 4-(4-((1*H*-Pyrrol-2-yl)methyl)piperazin-1-yl)-7-chloroquinoline (**23**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), 1H-pyrrole-2-carboxaldhyde(0.1g, 1.1mmol), to afford **23** (0.25g, 75.7%) as buff coloured solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.61 – 2.67 (m, 4H), 3.13 – 3.19 (m, 4H), 3.72 (s, 2H), 5.76 – 5.86 (m, 2H), 6.79 – 8.63 (m, 7H). ^{13}C NMR (DMSO-d_6): δc. 156.4, 152.5, 150, 133.6, 130.2, 129.8, 128.6, 126.1, 122.7, 117.3, 109.6, 108.3, 107.2, 56.9, 51.9, 42.7. ESI-MS *m*/*z* 327.1 $(M+H)^+$. Anal Calcd for $C_{18}H_{19}ClN_4$; C, 66.15; H, 5.86; N, 17.14; Found C, 66.19; H, 5.83; N, 17.21.

4.1.8. General procedure for the synthesis of *N*-alkyl derivatives (21, 26 & 27): To a suspension of 7-chloro-4-(piperazin-1-yl)quinoline (1mmol) and potassium carbonate (1.5mmol) in acetonitrile was added the corresponding alkyl halide (1mmol) at 30^{o}C. The reaction mixture was then heated to 80^{o}C for 1h (monitored by TLC and LCMS for completion) and cooled to 30^{o}C. The mixture was then filtered through celite bed, and acetonitrile was evaporated in vacuo. The resultant residue was diluted with water and dichloromethane, and the layers separated. The

aqueous layer was re-extracted with dichloromethane (2 x 5 mL). The combined organic extract was washed with brine, dried over sodium sulphate and evaporated in vacuo.

4.1.8.1. 4-(4-((1*H*-Benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-chloroquinoline (**21**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), potassium carbonate (0.21g, 1.5mmol) and 2-(chloromethyl)-1*H*-benzo[*d*]imidazole (0.17g, 1 mmol) to afford **21** (0.21g, 55.2%) as solid. M.p: 230-231 ^{o}C. ^{1}H NMR ($CDCl_3$): $\delta_{H.}$ 2.72 – 2.81 (m, 4H), 3.07 – 3.18 (m, 4H), 4.47 (s, 2H), 6.97 (d, J = 5.2 Hz, 1H), 7.23 – 8.47 (m, 8H). ^{13}C NMR ($CDCl_3$): δc.157.6, 152.6, 149.8, 141.2, 139.3, 133.4, 129.8, 129.1, 125.7, 123.1, 122.4, 115.4, 109.9, 63.3, 54.2, 49.3. ESI-MS *m*/*z* 378.1 $(M+H)^+$. Anal Calcd for $C_{21}H_{20}ClN_5$ C, 66.75; H, 5.33; N, 18.53; Found C, 66.83; H, 5.39; N, 18.49.

4.1.8.2. 7-Chloro-4-(-4-(5-nitrothiazol-2-yl)piperazin-1-yl)quinoline (**26**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), potassium carbonate (0.21g, 1.5mmol) and 2-chloro-5-nitrothiazole(0.17g, 1 mmol) to afford **26** (0.29g, 76.3%) as orange solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.21 – 3.28 (m, 8H), 6.97 (d, J = 5.1 Hz, 1H), 7.53 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H), 7.86 (s, 1H), 8.11 – 8.67 (m, 3H). ^{13}C NMR (DMSO-d_6): δc. 157.6, 154.3, 152.7, 150.1, 147.3, 136.4, 133.6, 130, 129, 126, 122.3, 109.6, 47.3, 43.6. ESI-MS *m*/*z* 376.1$(M+H)^+$. Anal Calcd for $C_{16}H_{14}ClN_5O_2S$ C, 51.13; H, 3.75; N, 18.63; Found C, 51.06; H, 3.69; N, 18.68.

4.1.8.3. 4-(4-(7-Chloroquinolin-4-yl)piperazin-1-yl)-7-nitrobenzo[c][1,2,5]oxadiazole (**27**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), potassium carbonate (0.21g, 1.5mmol) and 4-chloro-7-nitrobenzo[c][1,2,5]oxadiazole (0.2g mmol, 1 mmol) to afford **27** (0.27g, 65.8%) as dark red

solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.23 – 3.29 (m, 4H), 3.56 – 3.63 (m, 4H), 6.97 (d, J = 5.1 Hz, 1H), 7.26 – 7.41 (m, 2H), 7.83 (s, 1H), 8.06 – 8.71 (m, 3H). ^{13}C NMR (DMSO-d_6): δc. 157.8, 152.6, 149.8, 143.7, 140.6, 137.9, 133.8, 133.4, 129.9, 129, 126.3, 123.3, 122.8, 109.6, 98.4, 45.4, 44.8. ESI-MS *m/z* 411.1 $(M+H)^+$. Anal Calcd for $C_{19}H_{15}ClN_6O_3$ C, 55.55; H, 3.68; N, 20.46; Found C, 55.61; H, 3.63; N, 20.49.

4.1.9. General procedure for the synthesis of urea/thiourea derivatives (24, 25, 28-45): To a solution of 7-chloro-4-(piperazin-1-yl)quinoline (1 mmol) in dry dichloromethane (3 mL) was added triethyl amine (1.5 mmol) and the corresponding isocyanate/isothiocyanate (1 mmol) at 0^{o}C and the reaction mixture was slowly warmed to 30^{o}C and stirred at 30^{o}C for 6-8 h (monitored by TLC & LCMS for completion). The reaction mixture was washed with water (2 mL), brine (2 mL), dried over anhydrous sodium sulphate and evaporated in vacuo to give the desired product as mentioned below

4.1.9.1. *N*-Benzyl-4-(7-chloroquinolin-4-yl)piperazine-1-carbothioamide (**24**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), benzylisothiocyanate (0.15g 1 mmol) and triethylamine (0.15 g, 1.5 mmol) to afford **25** (0.33g, 82.5%) as off white solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.21 – 3.32 (m, 4H), 4.03 – 4.11 (m, 4H), 4.73 (s, 2H), 7.03 (d, *J* = 5.1 Hz, 1H), 7.21 – 8.69 (m, 10H). ^{13}C NMR (DMSO-d_6): δc. 181.2, 157.8, 152.3, 149.7, 140.3, 133.7, 129.6, 128.5, 128.2, 127.1, 126.6, 125.8, 121.9, 109.8, 51.5, 50.6, 47.6. ESI-MS *m/z* 397.2 $(M+H)^+$. Anal Calcd for $C_{21}H_{21}ClN_4S$; C, 63.54; H, 5.33; N, 14.11; Found C, 63.47; H, 5.37; N, 14.15.

4.1.9.2. *N*-Benzyl-4-(7-chloroquinolin-4-yl)piperazine-1-carboxamide (**25**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), benzylisocyanate (0.15g, 1 mmol) and triethylamine (0.15 g, 1.5

mmol) to afford **24** (0.25g, 65.8%) as off white solid. M.p: 143-145 ^{o}C. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.06 – 3.19 (m, 4H), 3.58 – 3.67 (m, 4H), 4.29 (d, *J* = 5.7 Hz, 2H), 7.04 (d, *J* = 5.1Hz, 1H), 7.15 – 7.35 (m, 6H), 7.56 (dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 1H), 7.99 (d, *J* = 2.1 Hz, 1H), 8.09 (d, *J* = 9 Hz, 1H), 8.72 (d, *J* = 4.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δc.157.5, 156.2, 152.2, 149.6, 140.9, 133.6, 128.1, 128, 127, 126.4, 126, 125.8, 121.4, 114.5, 109.6, 51.7, 50.3, 43.5. ESI-MS *m*/*z* 381.1 $(M+H)^{+}$. Anal Calcd for $C_{21}H_{21}ClN_4O$; C, 66.22; H, 5.56; N, 14.71; Found C, 66.29; H, 5.61; N, 14.75.

4.1.9.3. 4-(7-Chloroquinolin-4-yl)-N-phenylpiperazine-1-carbothioamide (**28**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), phenylisothiocyanate (0.14g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) to afford **28** (0.27g, 71%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.27 – 3.36 (m, 4H), 4.13 – 4.23 (m, 4H), 6.87 – 8.72 (m, 11H). ^{13}C NMR (DMSO-d_6): δc. 181.6, 157.7, 152.3, 149.6, 138.6, 133.5, 129.4, 129, 128.6, 128.1, 126.3, 125.8, 121.8, 109.3, 51.3, 47.6. ESI-MS *m*/*z* 383.1 $(M+H)^{+}$. Anal Calcd for $C_{20}H_{19}ClN_4S$: C, 62.73; H, 5.00; N, 14.63; Found C, 62.66; H, 4.96; N, 14.58.

4.1.9.4. 4-(7-Chloroquinolin-4-yl)-N-(4-fluorophenyl)piperazine-1-carbothioamide (**29**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), 4-fluorophenylisothiocyante (0.15g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) to afford **29** (0.31g, 77.5%) as solid. M.p: 203-205 ^{o}C. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.25 – 3.34 (m, 4H), 4.16 – 4.24 (m, 4H), 7.06 (d, *J* = 5.1 Hz, 1H), 7.10 – 7.19 (m, 2H), 7.29 – 7.36 (m, 2H), 7.58 (dd, *J* = 9 Hz, *J* = 2.1 Hz, 1H), 8.01 (d, *J* = 2.1 Hz, 1H), 8.14 (d, *J* = 9 Hz,1H), 8.73 (d, *J* = 5.1 Hz, 1H), 9.47 (s, 1H). ^{13}C NMR (DMSO-d_6): δc. 181.9, 157.6, 155.7, 152.2, 149.6, 137.2, 133.6, 128.1, 127.7, 127.6, 126.2, 125.9, 121.3, 114.8, 114.5,

109.6, 51.2, 47.8. ESI-MS *m/z* 401.3 $(M+H)^+$. Anal Calcd for $C_{20}H_{18}ClFN_4S$: C, 59.92; H, 4.53; N, 13.98; Found C, 59.85; H, 4.47; N, 14.04.

4.1.9.5. 4-(7-Chloroquinolin-4-yl)-N-(4-chlorophenyl)piperazine-1-carbothioamide (**30**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), 4-chlorophenylisothiocyanate (0.17g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) to afford **30** (0.31g, 73.8%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.29 – 3.37 (m, 4H), 4.13 – 4.24 (m, 4H), 6.67 – 8.73 (m, 10H). ^{13}C NMR (DMSO-d_6): δc. 181.7, 157.8, 152.5, 149.4, 136.2, 133.4, 132.6, 130.5, 129.4, 128.8, 128.4, 125.8, 122.1, 109.8, 51.1, 47.5. ESI-MS *m/z* 418.1 $(M+1)^+$. Anal Calcd for $C_{20}H_{18}Cl_2N_4S$: C, 57.56; H, 4.35; N, 13.42; Found C, 57.62; H, 4.38; N, 13.36.

4.1.9.6. 4-(7-Chloroquinolin-4-yl)-N-(4-nitrophenyl)piperazine-1-carbothioamide (**31**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), 4-nitrophenylisothiocyanate (0.18g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) to afford **31** (0.34g, 79%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.32 – 3.38 (m, 4H), 4.17 – 4.25 (m, 4H), 6.79 – 8.69 (m, 10H). ^{13}C NMR (DMSO-d_6): δc. 182, 157.8, 152.4, 149.7, 144.3, 143.2, 133.6, 129.6, 128.6, 126, 124.6, 123.9, 122.3, 109.6, 51.2, 47.9. ESI-MS *m/z* 428.3 $(M+H)^+$. Anal Calcd for $C_{20}H_{18}ClN_5O_2S$: C, 56.14; H, 4.24; N, 16.37; Found C, 56.11; H, 4.19; N, 16.31.

4.1.9.7. 4-(7-Chloroquinolin-4-yl)-N-(4-methoxyphenyl)piperazine-1-carbothioamide (**32**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), 4-methoxyphenylisothiocyanate (0.17g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) to afford **32** (0.32g, 76.2%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$

3.19 – 3.27 (m, 4H), 3.86 (s, 3H), 4.09 – 4.18 (m, 4H), 6.46 – 6.99 (m, 5H), 7.27 – 8.76 (m, 5H). ^{13}C NMR (DMSO-d_6): δc. 181.8, 158.8, 157.6, 152.3, 149.8, 133.7, 129.6, 128.7, 127.2, 125.8, 122, 113.6, 112.4, 109.8, 56.3, 51.3, 47.8. ESI-MS *m*/*z* 413.2 $(M+H)^+$. Anal Calcd for $C_{21}H_{21}ClN_4OS$: C, 61.08; H, 5.13; N, 13.57; Found C, 61.01; H, 5.18; N, 13.62.

4.1.9.8. 4-(7-Chloroquinolin-4-yl)-N-p-tolylpiperazine-1-carbothioamide (**33**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), tolylisothiocyanate (0.15g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) to afford **33** (0.33g, 82.5%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.29 (s, 3H), 3.23 – 3.29 (m, 4H), 4.13 -4.21 (m, 4H), 6.46 -7.08 (m, 5H), 7.29 -8.83 (m, 5H). ^{13}C NMR (DMSO-d_6): δc. 181.5, 157.3, 152.8, 149.4, 136.9, 135.7, 133.5, 129.6, 128.9, 128.6, 126.1, 125.7, 121.8, 109.8, 50.9, 47.5, 21.6. ESI-MS *m*/*z* 397.1 $(M+H)^+$. Anal Calcd for $C_{21}H_{21}ClN_4S$: C, 63.54; H, 5.33; N, 14.11; Found C, 63.6; H, 5.28; N, 14.07.

4.1.9.9. *N*-(4-Acetylphenyl)-4-(7-chloroquinolin-4-yl)piperazine-1-carboxthioamide (**34**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), 4-acetylphenylisothiocyanate (0.18g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) to afford **34** (0.36g, 83.7%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.64 (s, 3H), 3.24 – 3.31 (m, 4H), 4.13 - 4.18 (m, 4H), 6.72 – 6.97 (m, 3H), 7.29 – 8.91 (m, 7H). ^{13}C NMR (DMSO-d_6): δc. 191.2, 181.7, 157.4, 152.7, 150, 141.7, 136.8, 133.4, 130, 128.9, 128.6, 125.8, 125.6, 121.6, 109.7, 51.2, 47.8, 26.1. ESI-MS *m*/*z* 425.3 $(M+H)^+$. Anal Calcd for $C_{22}H_{21}ClN_4OS$: C, 62.18; H, 4.98; N, 13.18; Found C, 62.25; H, 4.94; N, 13.13.

4.1.9.10. 4-(7-Chloroquinolin-4-yl)-N-phenylpiperazine-1-carboamide (**35**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-

yl)quinoline (0.25g, 1 mmol), phenylisocyanate (0.12g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) to afford **35** (0.27g, 72.9%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.19 – 3.26 (m, 4H), 3.61 – 3.69 (m, 4H), 7.07 (d, J = 5.1Hz, 1H), 7.17 – 8.67 (m, 10H). ^{13}C NMR (DMSO-d_6): δc. 157.6, 155.3, 152.7, 149.2, 138.7, 133.2, 128.8, 128.6, 128.1, 127.6, 126.1, 121.9, 121.3, 109.7, 50.7, 43.1. ESI-MS m/z 367.2 $(M+H)^+$. Anal Calcd for $C_{20}H_{19}ClN_4O$: C, 65.48; H, 5.22; N, 15.27; Found C, 65.57; H, 5.18; N, 15.31.

4.1.9.11. 4-(7-Chloroquinolin-4-yl)-N-(4-fluorophenyl)piperazine-1-carboxamide (**36**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), 4-fluorophenylisocyanate (0.14g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) to afford **36** (0.31g, 79.5%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.17 – 3.23 (m, 4H), 3.61 – 3.67 (m, 4H), 7.01 (d, J = 5.1Hz, 1H), 7.21 – 8.72 (m, 9H). ^{13}C NMR (DMSO-d_6): δc. 158.1, 157.6, 155.1, 152.4, 149.6, 134.3, 133.7, 129.4, 128.7, 125.8, 121.7, 119.6, 114.8, 109.9, 50.6, 43.4. ESI-MS m/z 385.3 $(M+H)^+$. Anal Calcd for $C_{20}H_{18}ClFN_4O$: C, 62.42; H, 4.71; N, 14.56; Found C, 62.37; H, 4.67; N, 14.64.

4.1.9.12. 4-(7-Chloroquinolin-4-yl)-N-(4-chlorophenyl)piperazine-1-carboxamide (**37**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), 4-chlorophenylisocyanate (0.15g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) to afford **37** (0.29g, 72.5%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.21 – 3.29 (m, 4H), 3.67 – 3.74 (m, 4H), 6.99 (d, J = 5.2Hz, 1H), 7.34 – 8.81 (m, 9H). ^{13}C NMR (DMSO-d_6): δc. 157.9, 155.1, 152.8, 149.4, 136.8, 133.7, 132.8, 129.6, 129, 128.7, 126, 122.2, 119.9, 109.6, 50.4, 43.3. ESI-MS m/z 402.1 $(M+H)^+$. Anal Calcd for $C_{20}H_{18}Cl_2N_4O$: C, 59.86; H, 4.52; N, 13.96; Found C, 59.79; H, 4.54; N, 14.02.

4.1.9.13. 4-(7-Chloroquinolin-4-yl)-N-(4-nitrophenyl)piperazine-1-carboxamide (**38**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), 4-nitrophenylisocyanate (0.16g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) to afford **38** (0.3g, 73.1%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.29 – 3.37 (m, 4H), 3.69 – 3.77 (m, 4H), 7.34 – 8.89 (m, 9H). ^{13}C NMR (DMSO-d_6): δc. 157.8, 155.3, 152.9, 149.8, 145.3, 142.9, 133.8, 130, 128.9, 126.2, 123.7, 122.8, 120.3, 109.8, 50.9, 43.6. ESI-MS *m*/*z* 412.1 $(M+H)^+$. Anal Calcd for $C_{20}H_{18}ClN_5O_3$: C, 58.33; H, 4.41; N, 17.00; Found C, 58.41; H, 4.37; N, 16.95.

4.1.9.14. 4-(7-Chloroquinolin-4-yl)-N-(4-methoxyphenyl)piperazine-1-carboxamide (**39**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), 4-methoxyphenylisocyanate (0.15g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) to afford **39** (0.27g, 67.5%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 3.13 – 3.19 (m, 4H), 3.59 – 3.67 (m, 4H), 3.86 (s, 3H), 6.93 – 8.79 (m, 10H). ^{13}C NMR (DMSO-d_6): δc. 158.1, 157.7, 155.1, 152.6, 149.4, 133.6, 131.4, 129.9, 128.7, 125.7, 122.8, 119.7, 113.8, 109.6, 55.3, 50.3, 43.1. ESI-MS *m*/*z* 397.3 $(M+H)^+$. Anal Calcd for $C_{21}H_{21}ClN_4O_2$: C, 63.55; H, 5.33; N, 14.12; Found C, 63.48; H, 5.36; N, 14.17.

4.1.9.15. 4-(7-Chloroquinolin-4-yl)-N-p-tolylpiperazine-1-carboxamide (**40**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), tolylisocyanate (0.13g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) to afford **40** (0.29g, 76.3%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.37 (s, 3H), 3.18 – 3.26 (m, 4H), 3.59 – 3.64 (m, 4H), 6.97 (d, *J* = 5.1Hz, 1H), 7.24 – 8.67 (m, 9H). ^{13}C NMR (DMSO-d_6): δc. 157.6, 154.9, 152.3, 149.1, 136.7, 135.9, 133.7, 130, 128.9, 128.4, 126, 122.1, 120.8,

109.8, 50.4, 43.3, 21.3. ESI-MS *m/z* 381.2 $(M+H)^+$. Anal Calcd for $C_{21}H_{21}ClN_4O$: C, 66.22; H, 5.56; N, 14.71; Found C, 66.32; H, 5.51; N, 14.65.

4.1.9.16. ***N*-(4-Acetylphenyl)-4-(7-chloroquinolin-4-yl)piperazine-1-carboxamide** (**41**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), 4-acetylphenylisocyanate (0.16g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) to afford **41** (0.3g, 73.2%) as solid. ^{1}H NMR (DMSO-d_6): $\delta_{H.}$ 2.59 (s, 3H), 3.24 – 3.31 (m, 4H), 3.64 – 3.72 (m, 4H), 7.06 (d, *J* = 5.1Hz, 1H), 7.36 – 8.82 (m, 9H). ^{13}C NMR (DMSO-d_6): δc. 193.2, 157.3, 155.3, 152.7, 149.8, 142.9, 136.2, 133.4, 130.2, 128.7, 128.5, 126, 122.3, 120.8, 109.7, 50.6, 43.2, 26.4. ESI-MS *m/z* 409.2 $(M+H)^+$. Anal Calcd for $C_{22}H_{21}ClN_4O_2$: C, 64.62; H, 5.18; N, 13.70; Found C, 64.53; H, 5.15; N, 13.63.

4.1.9.17. **4-(7-Chloroquinolin-4-yl)-N-ethylpiperazine-1-carbothioamide** (**42**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), ethylisothiocyante (0.087g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) toto afford **42** (0.2g, 58.8%) as pale yellow solid. ^{1}H NMR ($CDCl_3$): $\delta_{H.}$ 1.32 (t, *J* = 7.2Hz, 3H), 3.31 – 3.38 (m, 4H), 4.13 – 4.27 (m, 6H), 6.96 (d, *J* = 5.1Hz, 1H), 7.31 – 8.73 (m, 4H). ^{13}C NMR ($CDCl_3$): δc. 181.1, 157.6, 152.7, 149.6, 133.7, 129.8, 128.7, 126.1, 122.4, 109.7, 51.4, 47.6, 40.3, 15.6. ESI-MS *m/z* 335.1 $(M+H)^+$. Anal Calcd for $C_{16}H_{19}ClN_4S$ C, 57.39; H, 5.72; N, 16.73; Found C, 57.47; H, 5.69; N, 16.68.

4.1.9.18. **4-(7-Chloroquinolin-4-yl)-N-isopropylpiperazine-1-carbothioamide** (**43**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), propylisothiocyante (0.1g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) toto afford **43** (0.23g, 65.7%) as pale yellow solid. ^{1}H NMR

(CDCl$_3$): $\delta_{H.}$ 1.11 (d, *J* = 6.9Hz, 6H), 3.34 – 3.41 (m, 4H), 4.18 – 4.26 (m, 4H), 4.49 (m, 1H), 7.02 (d, *J* = 5.1 Hz, 1H), 7.34 – 8.73 (m, 4H). ^{13}C NMR (CDCl$_3$): δc. 181, 157.8, 153, 150, 133.6, 129.8, 128.9, 125.8, 122.3, 109.8, 51.9, 51.6, 47.4, 22.9. ESI-MS *m*/*z* 349.1 $(M+H)^+$. Anal Calcd for $C_{17}H_{21}ClN_4S$: C, 58.52; H, 6.07; N, 16.06; Found C, 57.45; H, 6.01; N, 15.99.

4.1.9.19. 4-(7-Chloroquinolin-4-yl)-N-ethylpiperazine-1-carboxamide (**44**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol), ethylisocyante (0.02g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) to afford **44** (0.19g, 59.4%) as solid. ^{1}H NMR (CDCl$_3$): $\delta_{H.}$ 1.17 (t, J = 7.1 Hz, 3H), 3.19 – 3.29 (m, 6H), 3.61 – 3.67 (m, 4H), 6.97 (d, *J* = 5.1Hz, 1H), 7.34 – 8.73 (m, 4H). ^{13}C NMR (CDCl$_3$): δc. 158.1, 157.6, 152.6, 149.7, 133.7, 129.8, 128.7, 125.6, 122.3, 109.7, 50.6, 43.4, 33.7, 13.3. ESI-MS *m*/*z* 319.1 $(M+H)^+$. Anal Calcd for $C_{16}H_{19}ClN_4O$: C, 60.28; H, 6.01; N, 17.57; Found C, 60.39; H, 5.96; N, 17.61.

4.1.9.20. 4-(7-Chloroquinolin-4-yl)-N-isopropylpiperazine-1-carboxamide (**45**): The compound was synthesized according to the above general procedure using 7-chloro-4-(piperazin-1-yl)quinoline (0.25g, 1 mmol),isopropylisocyante (0.085g, 1 mmol) and triethylamine (0.15 g, 1.5 mmol) toto afford **45** (0.16g, 48.5%) as pale yellow solid. ^{1}H NMR (CDCl$_3$): $\delta_{H.}$ 1.37 (d, J = 6.7 Hz, 6H), 3.16 – 3.22 (m, 4H), 3.58 – 3.66 (m, 4H), 4.19 (m, 1H), 6.93 (d, J = 5.1Hz, 1H), 7.36 – 8.69 (m, 4H). ^{13}C NMR (CDCl$_3$): δc. 157.6, 157.3, 152.6, 149.7, 133.6, 129.8, 128.6, 125.8, 122.7, 109.6, 50.4, 43.2, 44.8, 21.7, ESI-MS *m*/*z* 333.1 $(M+H)^+$. Anal Calcd for $C_{17}H_{21}ClN_4O$: C, 61.35; H, 6.36; N, 16.83; Found C, 61.27; H, 6.4; N, 16.89.

4.2. Biological evaluation

4.2.1. *Mycobacterium smegmatis* gyrase ATPase assay:

Being the gyrase enzyme catalytic site the gyraseB domain performs the ATPase assay with the sole GyrB subunit. The assay was performed in 30 µL reaction volume for 120 min at 25°C in reaction buffer containing 60 mM HEPES-KOH pH 7.7, 250 mM potassium glutamate, 200 mM KCl, 2 mM magnesium chloride, 1 mM DTT, 2% Glycerol, 4% DMSO, 0.001% BriJ, 0.65 mM ATP, 40 nM GyrB as previously published method [17, 20-21]. All the test compounds were diluted in 4% DMSO to about eight concentrations for the determination of IC_{50}. ATPase assay was performed in V-shaped 96-well plates (Polystyrene untreated). Initially 15 µL of 2x assay buffer containing purified GyrB enzyme and substrate mix were placed in the assay well followed by 1 µL of test compound, subsequently the enzyme reaction was initiated by adding 14 µL of $MgCl_2$ solution, as metal ion triggers the enzyme. The reaction was allowed to proceed for 120 min at room temperature. At the end, 20µL malachite green reagent (Bioassay systems) was added to quench the reaction and incubated for 20 min to determine the inorganic phosphates (Pi) released when measured at 635 nm wavelength against the blank absorbance. In this assay, novobiocin was considered as positive control and moxifloxacin as the negative control.

4.2.2. *In vitro Mycobacterium tuberculosis* MABA assay:

The compounds were further screened for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv by microplate Alamar blue assay method [23]. Briefly, the 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. Each drug stock solution was thawed and diluted in 7H9-S at four-fold the final highest concentration tested. Serial two-

836 fold dilutions of each drug were prepared directly in a sterile 96-well microtiter plate using 100
837 µl 7H9-S. A growth control containing no antibiotic and a sterile control were also prepared on
838 each plate. Sterile water was added to all perimetre wells to avoid evaporation during the
839 incubation. The plate was covered, sealed in plastic bags and incubated at 37^{o}C in normal
840 atmosphere. After 7 days incubation, 30 ml of alamar blue solution was added to each well, and
841 the plate was re-incubated overnight. A change in color from blue (oxidised state) to pink
842 (reduced) indicated the growth of bacteria, and the MIC was defined as the lowest concentration
843 of drug that prevented this change in color.

844 **Supporting information**: Supporting information contains the details regarding the protocol
845 utilised for cloning and purification of protien, supercoiling assay, docking and interaction
846 profile of compounds, toxicity evaluation, DSF experiments and pharmacokinetics.

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849 **Appendix A. Supplementary data**

850 Supplementary data related to this article can be found at

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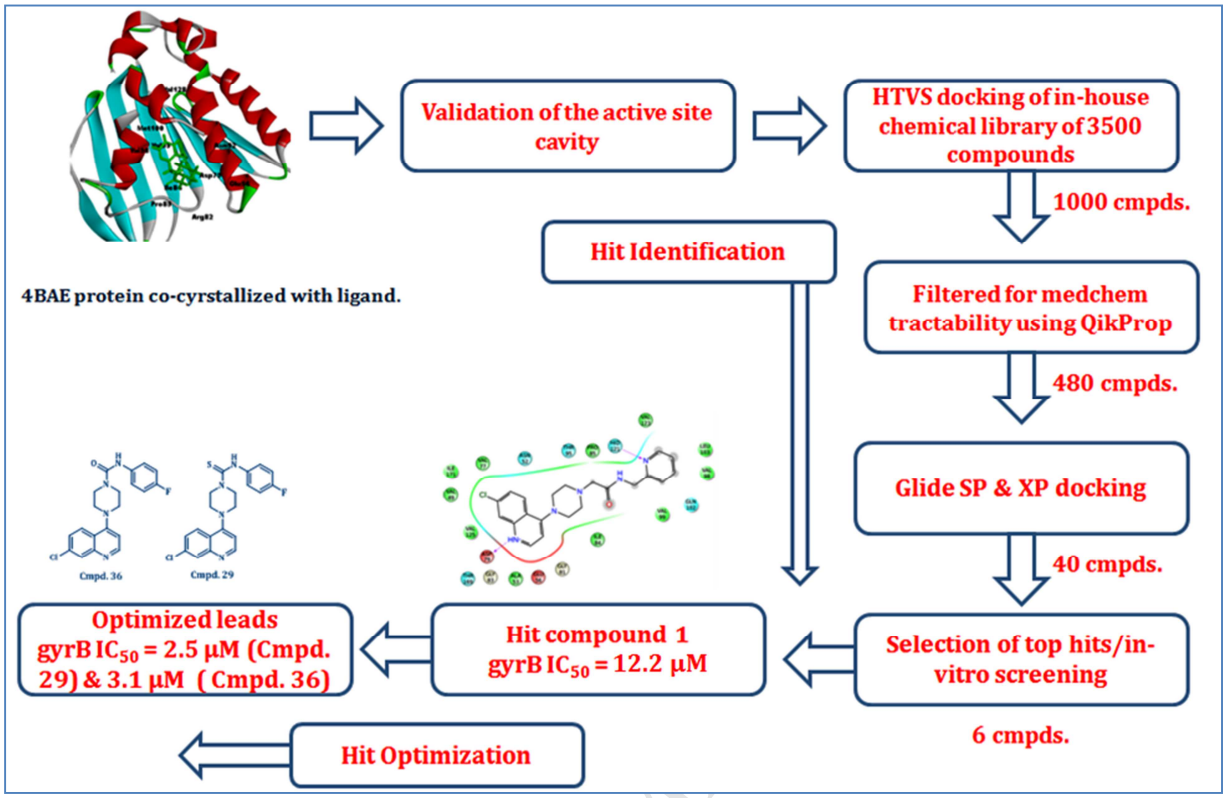

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947 **Figure 1**: Strategy employed for hit identification and optimization.

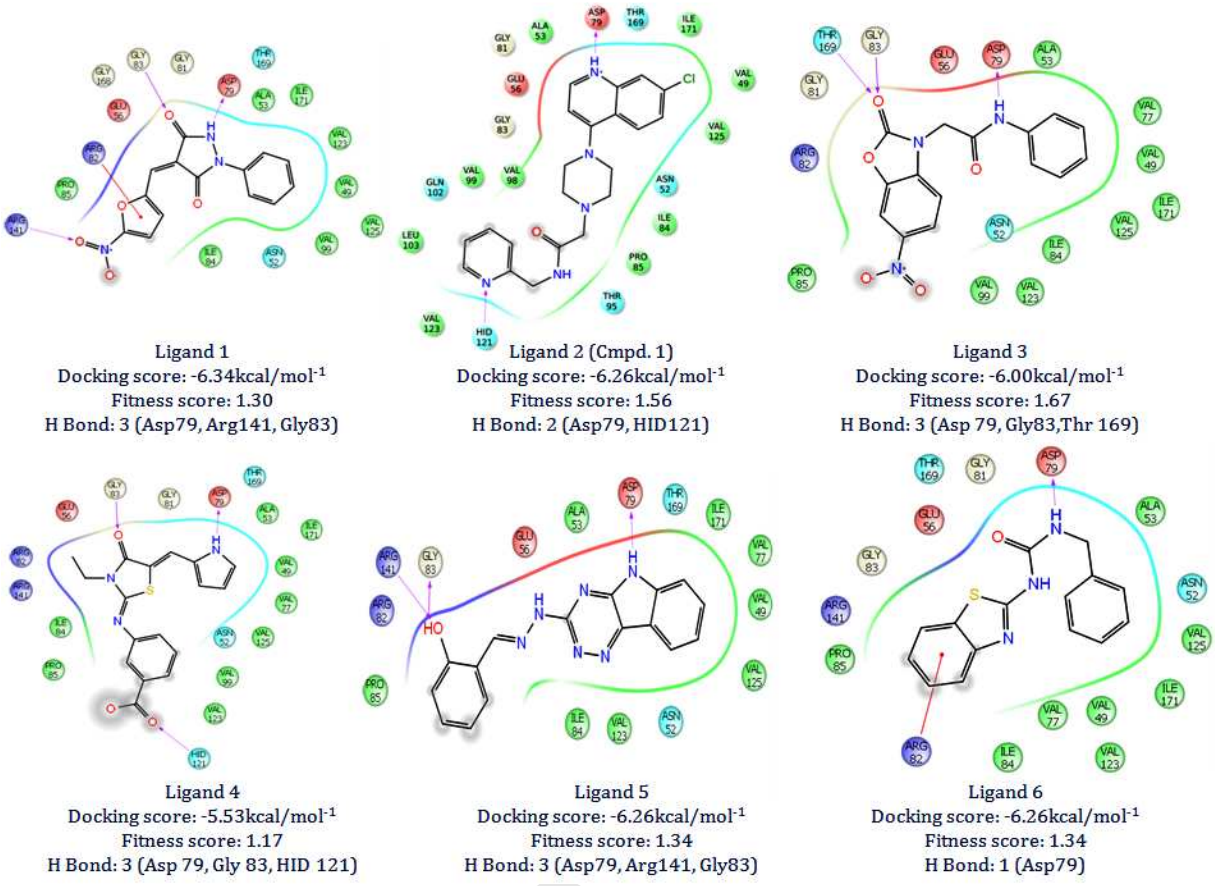
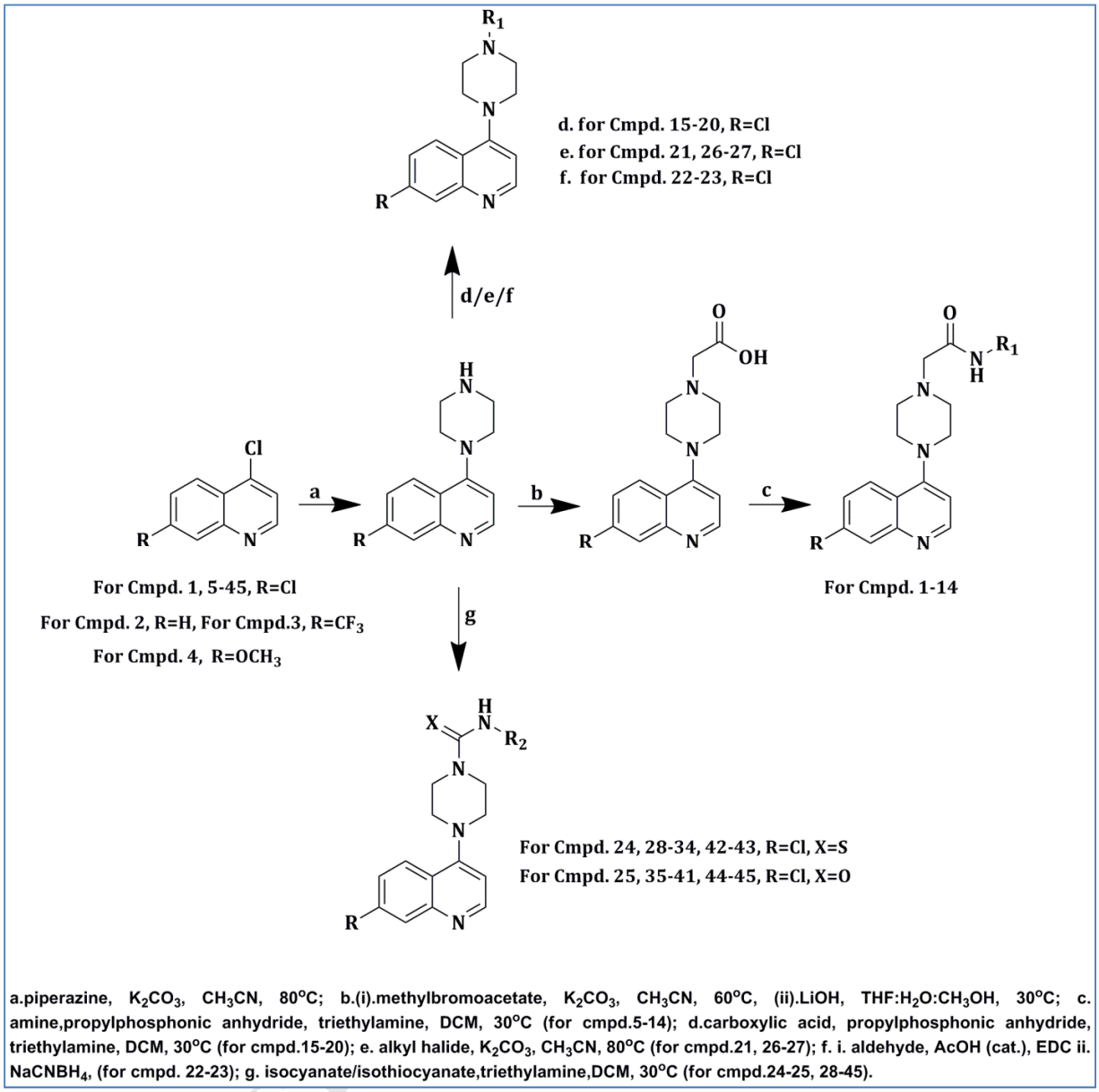


Figure 2: Lig plot diagram of the best six ligands together with their docking scores, fitness and hydrogen bonding interactions.



Scheme 1: Synthetic protocol utilized for developing the designed ligands.

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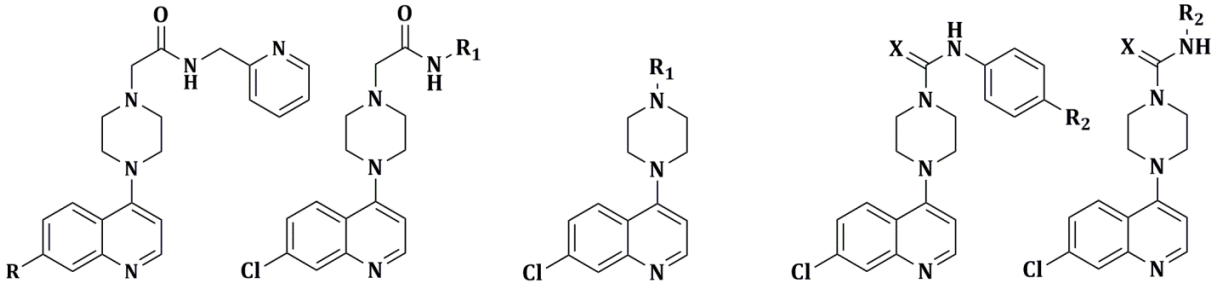
968 **Table 1**:

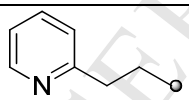
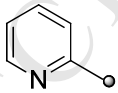
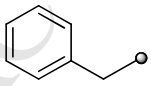
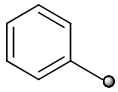
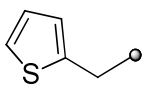
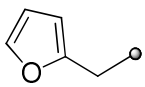


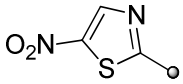
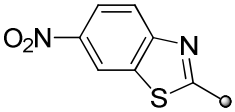
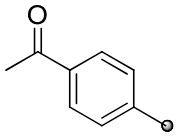
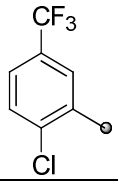
Cmpd. 1 - 4 Cmpd. 5 - 14 Cmpd. 15 - 27 Cmpd. 28 - 41 Cmpd. 42 - 45

969 Subset 1 Subset 2 Subset 3

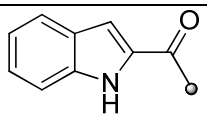
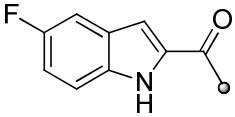
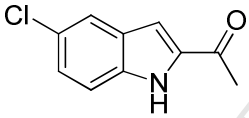
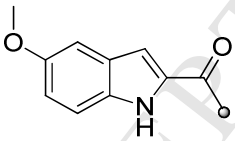
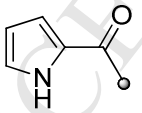
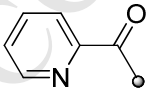
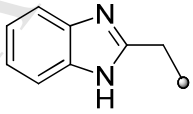
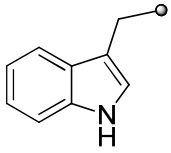
Subset 1

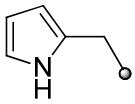
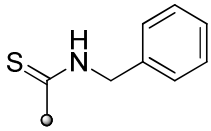
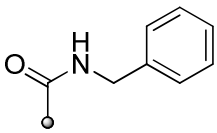
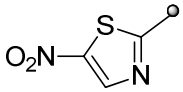
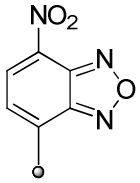
Cmpd.	R	GyrB assay (IC_{50}) (µM)[a]	Supercoiling assay (IC_{50}) (µM)[b]	MIC (µM)[c]	Cytotoxicity (% inhib: at 100 µM)[d]
1	Cl	12.2±0.09	6.25±0.8	31.57	36.42
2	H	>75	>75	138.33	23.53
3	CF_3	>75	>75	116.43	51.24
4	OCH_3	51.6±3.1	63.2±4.3	63.86	33.53

Cmpd.	R_1	GyrB assay (IC_{50}) (µM)[a]	Supercoiling assay (IC_{50}) (µM)[b]	MIC µM[c]	Cytotoxicity (% inhib: at 100 µM)[d]
5		30.4±3.2	12.5±1.7	61	26.32
6		9.1±0.5	6.125±1.2	17.71	25.42
7		23.8±2.5	12.5±1.1	63.3	15.34
8		33.1±4.1	28.3±2.7	35.52	26.24
9		14.8±1.3	6.125±0.6	31.2	33.42
10		16.2±1.29	5.33±0.7	32.5	21.14

11		14.6±2.1	6.125±2.1	31.2	11.34
12		16.1±2.1	6.125±1.3	25.8	23.65
13		7.6±0.7	3.125±0.3	14.5	29.53
14		31.3±4.1	12.5±2.4	24.2	24.66

Subset 2

Cmpd.	R_1	GyrB assay (IC_{50}) (µM)[a]	Supercoiling assay (IC_{50}) (µM)[b]	MIC µM[c]	Cytotoxicity (% inhib: at 100 µM)[d]
15		8.53±1.2	3.125±0.8	15.99	34.65
16		13.6±1.6	6.125±2.1	15.29	23.26
17		10.3±1.6	12.1±3.1	14.7	29.77
18		24.3±4.3	26.1±3.7	29.7	16.35
19		14.2±1.6	6.125±0.8	18.33	34.23
20		11±0.7	3.125±0.4	35.42	23.65
21		32.6±1.4	25±2.7	27.3	34.76
22		8.3±2.6	1.82±0.5	16.25	22.23

23		16.9±2.3	4.15±1.6	76.55	12.56
24		5.5±0.1	2.7±0.8	15.75	19.76
25		6.1±1.3	3.125±0.5	17.66	25.21
26		9.6±2.1	6.125±1.7	33.2	35.11
27		10.3±2.6	3.125±0.3	7.26	37.23

Subset 3

Cmpd.	X	R_2	GyrB assay (IC_{50}) (µM)[a]	Supercoiling assay (IC_{50}) (µM)[b]	MIC (µM)[c]	Cytotoxicity(% inhib:at 100 µM)[d]
28	S	H	4.12±0.3	3.125±0.8	16.32	16.54
29	S	F	2.5±0.1	2.7±0.4	7.8	23.87
30	S	Cl	10.7±1.3	8.5±1.1	30	34.23
31	S	NO_2	6.8±0.9	3.125±1.1	14.32	39.87
32	S	OCH_3	11±2.2	19.3±3.4	30.27	23.12
33	S	CH_3	13.8±2.4	25±4.8	62.98	15.76
34	S	$COCH_3$	27±3.2	>25	58.8	27.23
35	O	H	7.6±0.8	12.5±1.2	17.03	30.90
36	O	F	3.1±0.2	3.125±0.9	15.27	17.56
37	O	Cl	9.9±3.2	16.3±4.2	31.5	26.23
38	O	NO_2	9.2±1.3	8.5±2.1	30.35	41.76

39	O	OCH_3	21.1±3.1	12.5±0.3	31.5	25.34
40	O	CH_3	16±3.5	12.5±2.7	32.8	33.87
41	O	$COCH_3$	18.8±3.3	14.9±2.4	30.6	26.23
42	S	C_2H_5	40.6±4.2	>25	74.6	21.87
43	S	$CH(CH_3)_2$	40.4±3.5	>25	71.6	36.33
44	O	C_2H_5	45.3±5.1	>25	156.8	26.98
45	O	$CH(CH_3)_2$	43.9±4.7	>25	154.9	34.43
	Novobiocin		0.046±10	0.180±3.9	nd	nd
	Isoniazid		nd	nd	0.66	nd
	Rifampicin		nd	nd	0.23	nd
	Ofloxacin		nd	nd	2.16	nd

[a]*Mycobacterium smegmatis* GyrB ATPase activity; [b]*Mycobacterium tuberculosis* DNA Gyrase super coiling activity, [c]*in vitro Mycobacterium tuberculosis* activity, [d]At 100 µM against RAW 264.7 cells, nd: indicates not determined.

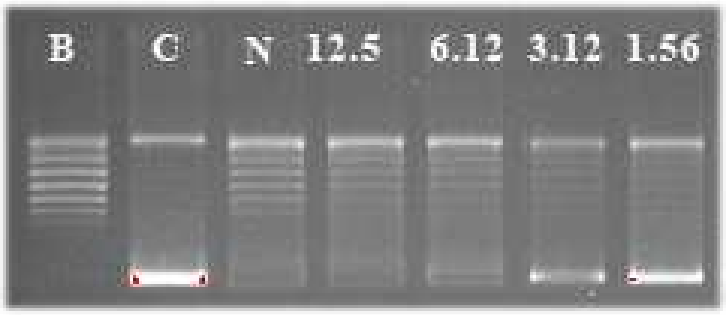


Figure 3: Inhibitory profile of *Mycobacterium tuberculosis* DNA Gyrase supercoiling activity by compound **29**.

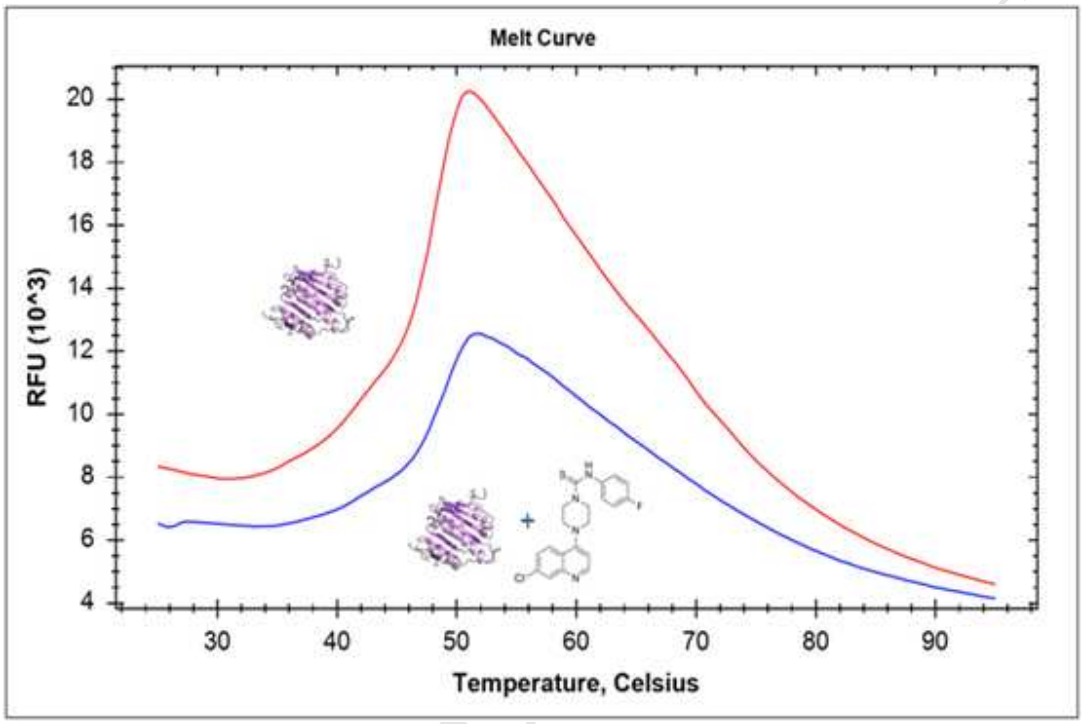
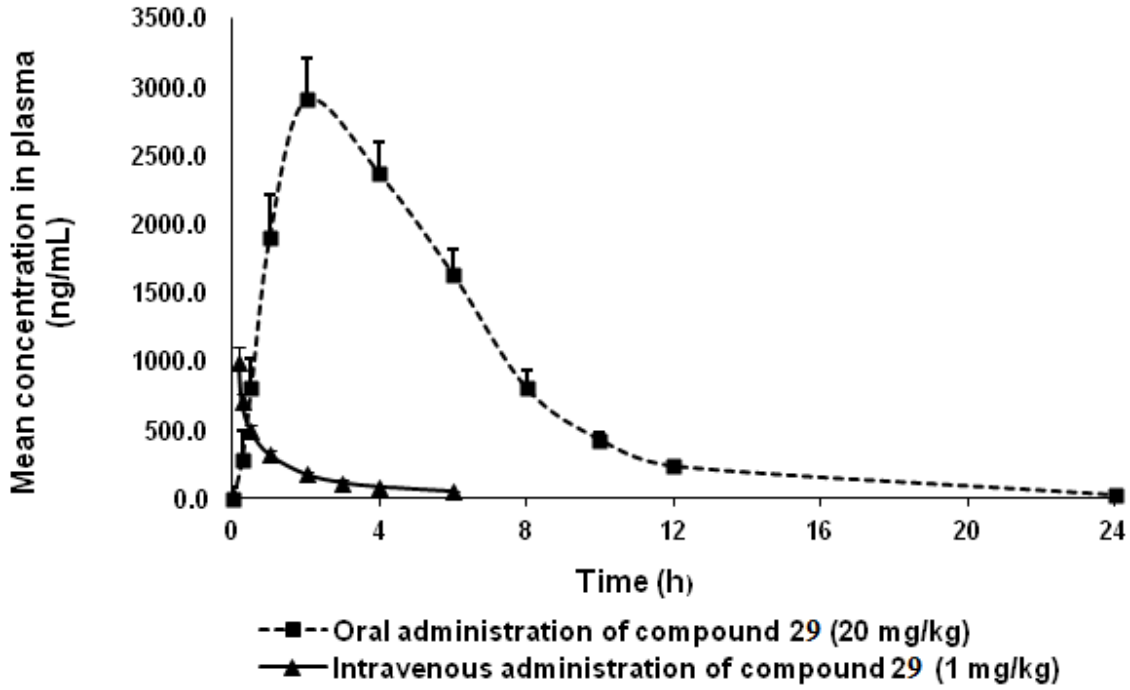


Figure 4: DSF curves for compound **29** (protein-ligand complex, blue curve) showing an increase in the thermal shift of 4.1°C when compared to the native GyrB protein (red curve).



995

996 **Figure 5:** Mean plasma concentration-time profile of compound **29** following intravenous and
997 oral administration to male Wistar rats. Each point represents mean ± SD (n = 5).

998 **Table 2**. Pharmacokinetic parameters of compound **29** following intravenous (1 mg/kg) and oral
999 administration (20 mg/kg) to rats.

Pharmacokinetic parameters	**Unit**	**Intravenous**	**Oral**
AUC_{0-t}	h*ng/mL	1240.66 ± 90.45	18759.93 ± 1256.39
$AUC_{0-\infty}$	h*ng/mL	1354.69 ± 93.45	19283.49 ± 1498.56
C_{max}	ng/mL		2906.67± 308.24
T_{max}	h		2.20 (1-4)*
$t_{1/2}$	h	2.04 ± 0.09	-
Cl_{Total}	L/h/kg	0.80 ± 0.05	-
Vd	L/kg	0.99 ± 0.08	-
Bioavailability (% F)		-	71.17 ± 3.20

1000 Values represent mean ± SD (n = 5). * Values are given in range.

1001 **Table 3.** Protein binding ability of compound **29** in rat and human plasma matrices.

Plasma protein binding		
Concentration (ng/ml)	**Rat**	**Human**
100	93.5 ± 1.8	92.2 ± 1.5
2500	94.8 ± 2.1	93.6 ± 1.8
5000	95.4 ± 1.9	94.3 ± 2.2

1002 Plasma protein binding was determined by ultra-filtration
1003 method. Values represent mean ± SD (n = 3).
1004

Highlights:

- We report a new class of small molecule inhibitor of *M. tuberculosis* gyrase ATPase domain
- A structure based medium throughput virtual screening identified an initial hit compound **1**.
- Hit expansion, leads compound **29** as potent GyrB inhibitory IC_{50} of 2.5 ± 0.1 µM.
- The molecules exhibited promising in-vitro MTB potency.
- The binding affinity of the inhibitor towards the GyrB domain was re-ascertained by DSF.