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Graphical Abstract

4-Amino Quinoline Derivatives as Novel *Mycobacterium tuberculosis* GyrB Inhibitors: Structural Optimization, Synthesis and Biological Evaluation

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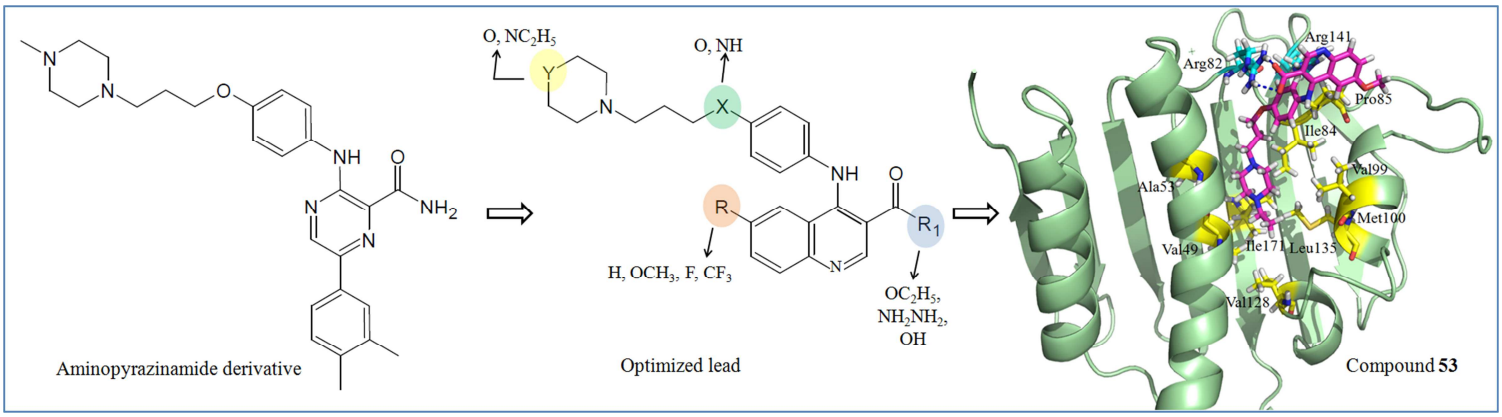

Compound **53** was identified to be potent GyrB inhibitor with 0.86 µM and an MIC of 3.3 µM.

SHORT COMMUNICATION

4-Aminoquinoline Derivatives as Novel *Mycobacterium tuberculosis* GyrB Inhibitors: Structural Optimization, Synthesis and Biological Evaluation

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Abstract

Mycobacterial DNA gyrase B subunit has been identified to be one of the potentially underexploited drug targets in the field of antitubercular drug discovery. In the present study, we employed structural optimization of the reported GyrB inhibitor resulting in synthesis of a series of 46 novel quinoline derivatives. The compounds were evaluated for their *in vitro Mycobacterium smegmatis* GyrB inhibitory ability and *M. tuberculosis* DNA supercoiling inhibitory activity. The antitubercular activity of these compounds was tested over Mtb H37Rv strain and their safety profile was checked against mouse macrophage RAW 264.7 cell line. Among all, three compounds (**23**, **28**, and **53**) emerged to be active displaying IC_{50} values below 1 µM against Msm GyrB and were found to be non-cytotoxic at 50 µM concentration. Compound **53** was identified to be potent GyrB inhibitor with 0.86±0.16 µM and an MIC (minimum inhibitory concentration) of 3.3 µM. The binding affinity of this compound towards GyrB protein was analyzed by differential scanning fluorimetry which resulted in a positive shift of 3.3 ^{o}C in melting temperature (T_m) when compared to the native protein thereby reacertaining the stabilization effect of the compound over protein.

Keywords: *Mycobacterium tuberculosis*, DNA gyrase B, DNA supercoil assay, cytotoxicity, differential scanning fluorimetry.

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1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, continues to be one of the deadliest infectious diseases with mortality rate of around 1.5 million lives in 2013 as reported by World Health Organization (WHO) [1]. Though several effective anti-tubercular drugs such as isoniazid, rifampicin, ethambutol, fluoroquinolones, exist in the market, emergence of new Mtb strains as like multi drug resistant (MDR), extensively drug resistant (XDR) and the latest total drug resistant (TDR) TB indicate the urgency in the development of newer anti-tubercular agents with newer combination therapy so as to be effective against these strains [2,3,4]. Unlike the conventional anti-tubercular drugs in the market, there is a need to discover newer anti-tubercular agents which can target a novel pathway thereby destroying the viability of the pathogen.

DNA gyrase (topoisomerase type II) of Mtb can be an attractive target in this prospect due to the uniqueness of the Mtb genome which codes for only two types of topoisomerases (type I and II) unlike other pathogens. DNA gyrase, a crucial enzyme, causes negative supercoiling of DNA which relieves strain during the DNA unwinding [5]. Functional DNA gyrase usually exists as a heterotetramer (A_2B_2) with two A subunits and two B subunits [6]. Fluoroquinolones which target gyrase A subunit have been facing a major hurdle of their resistance developed by Mtb which makes gyrase B subunit a druggable target for discovery of potent anti-tubercular agents. DNA gyrase B subunit is involved in the process of ATP hydrolysis which in turn provides energy to gyrase A subunit for maintaining the DNA topological state [7]. Novobiocin and coumermycin are the reported Mtb GyrB inhibitors [7].

Several compounds have been reported as DNA GyrB inhibitors which include benzimidazole ureas [8], pyrazinamides [9] and triazolopyridine ureas [10]. In the present study, structural optimization of the reported lead, aminopyrazinamide derivative from AstraZeneca, was carried out substituting at suitable positions and were synthetically derivatized to a series of 46 compounds. These compounds were subjected to *in vitro M. smegmatis* GyrB inhibition and Mtb DNA supercoiling assay so as to study their inhibitory profile. Structure activity relationship of the compounds was build up relating their GyrB activity and *in silico* docking pattern. Further, the compounds were tested over *in vitro* drug sensitive Mtb cultures using microplate alamar blue assay (MABA) and their cytotoxicity studies were carried out using macrophage cell line from mouse.

2. Results and Discussion

In the present study, the reported aminopyrazinamide derivative from AstraZeneca [9] was considered for further molecular optimization process which can be targeted against Mtb GyrB enzyme. The interaction profile of the compound at the protein active site was analyzed using molecular docking studies. The compound, 6-(3,4-dimethylphenyl)-3-[[4-[3-(4-methylpiperazin-1-yl)propoxy]phenyl]amino] pyrazine-2-carboxamide, was subjected to molecular docking at the ATPase site of *M. smegmatis* GyrB using Glide *v*5.8 [11]. The interaction pattern of the compound, shown in **figure 1** [12], revealed the presence of two hydrogen bonds, one between the free amino group of compound and Asp79 and the second one between the nitrogen atom of piperazine and Arg82. The orientation of the compound at the active site revealed the presence of a highly hydrophobic pocket where the 3,4-dimethyl phenyl moiety was found to be stabilized by non-polar interactions with residues Val99, Met100, Val128, Leu135 and Leu171. Also residues such as Ile84 and Pro85 were found to be involved in hydrophobic interactions with the compound.

This compound was further considered for molecular reengineering by retaining the crucial groups for GyrB activity and substituting particular sites with various suitable moieties. **Figure 2** demonstrates the substitutions made over the aminopyrazinamide compound ending up with a molecular lead. The 6-(3,4-dimethyl phenyl) pyrazine group which imparts for the hydrophobic interactions of the compound was replaced by quinoline moiety so as to observe the effect of fused ring system over the activity. The carboxamide group over pyrazine in the aminopyrazinamide compound, which was reported to be crucial for activity, was also modified by substituting amine group with ethoxy, hydrazine and hydroxyl moieties. The secondary amine linking the pyrazine and phenyl moiety was retained. The oxygen connecting the butyl chain with phenyl ring was retained and also substituted by amine group. The final modification was done at the N-methyl group of piperazine by substituting with oxygen (O) and N-ethyl group.

The titled compounds were synthesized by following multi-step synthesis protocol. Initially, we synthesized the amine derivatives (**4a-b** and **7a-b**) starting with simple 1,4-phenylene diamine and 4-aminophenol which were selectively alkylated with 1,3-dibromo propane using K_2CO_3 in DMF heating at 110 ^oC for 3 h. This was followed by reacting them with morpholine and N-ethyl piperazine under similar conditions, subsequent de-protection of BOC to get the amine derivatives (**4a-b** and **7a-b**). The reaction sequences were showed in **Scheme 1**. These amine

derivatives were alkylated with different substituted 4-chloro quinoline analogues (**11a-d**) using *para*-tolulene sulfonic acid (PTSA) as catalyst in methanol in a Biotage microwave vial by irradiating at 120 ^{o}C for 45 min to get substituted ethyl phenyl aminoquinoline-3-carboxylates (**12-27**)[13]. These were further converted to their corresponding hydrazide (**28-43**) and acid derivatives (**44-57**). The substituted 4-chloro quinoline analogues (**11a-d**) were prepared by a 3 step synthesis process according to the reported procedure[14,15] starting with **8a-d** were converted to diethyl phenyl amino methylidene propanedioate derivatives (**9a-d)** by condensing with diethyl ethoxy methylene malonate. These (**9a-d**) were cyclized intramolecularly to 4-hydroxy quinoline 3-carboxylate derivatives (**10a-d**) by heating them at 75 ^{o}C for 12 h in polyphosphoric acid and phosphorous oxy chloride (catalytic). In this method, the yield (89-92%) was improved much better than the reported procedure using Dowtherm and subsequent refluxing in phosphorous oxy chloride yielded the 4-chloroquinoline 3-carboxylate derivatives (**11a-d**). The reaction sequences are shown in **Scheme 2**.

Mycobacterium smegmatis (Msm) DNA GyrB was used for performing the gyrase ATPase assay. The surrogate Msm GyrB protein was used as Mtb was found to be slow growing organism[9]. Moreover, the sequence similarity between the GyrB protein domains of both the organisms was found to be 87%, with most of the catalytic site being conserved which proves a higher degree of homology in their ATP binding pocket[16]. The Msm GyrB gene was cloned into a prokaryotic expression vector pQE2 and was expressed in *BL21 (DE3) pLys*S competent cells. The expressed protein was further induced with IPTG (Isopropyl-β-D-thiogalactopyranoside) for increased production of the desired protein and the desired GyrB protein was subsequently purified by Ni-NTA column. The purified protein was confirmed by running 10% SDS- PAGE (sodium dodecyl sulphate – polyacrylamide gel electrophoresis). Msm DNA GyrB enzyme follows greater than first-order kinetics [17]. However, at a constant enzyme concentration of 15 μM a hyperbolic dependence of rate on substrate (ATP) concentration was observed as the GyrB ATPase activity of Msm does not follow Michaelis–Menten kinetics. The apparent K_m^{app} and V_{max}^{app} determined experimentally were 300 μM and 2.1 μmol/s respectively as illustrated in **figure 3.**

Ideally, novobiocin was used as a standard inhibitor in these assays. Furthermore, according to scientists from AstraZeneca, a tight correlation of <3–5 fold variation in IC_{50} values were acceptable between the ATPase activity of Msm and the supercoiling activity of Mtb [9]; this

had given us enough confidence to use Msm as a surrogate enzyme for DNA GyrB ATPase activity. Moreover, to eliminate the possibilities of aggregation of the protein and also auto-fluorescence artifacts, non-specific inhibition detergents were added in all our biological experiments [18]. The series of 46 compounds synthesized were biologically evaluated for their *in vitro* Msm GyrB ATPase assay using the protocol described in the experimental section. The compounds showed an IC_{50} values ranging between 0.86 to 72.48 µM and are given in **table 1**. Compound **53** was found to be most activewith an IC_{50} of 0.86 ± 0.42 µM against Msm GyrB and its dose response curve was plotted using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA) by taking log (inhibitor concentration) on the X-axis and its response (%inhibition) on the Y-axis as shown in **figure 4**, while the standard novobiocin showed an IC_{50} of 180 ± 3.9 nM. Further, compound **53** selectively inhibited mycobacterial DNA GyrB and was found inactive against other bacterial GyrB from *Staphylococcus aureus* and *Escherichia coli* (Inspiralis) even at 100 µM concentrations confirming its specificity towards mycobacterial species.

Subsequently, the compounds inhibiting the ATPase activity of GyrB protein should also inhibit the supercoiling activity of the gyrase holoenzyme as the GyrA and GyrB domains constitute the DNA gyrase. The supercoiling assay was performed in our laboratory using the DNA supercoiling assay kit (Inspiralis Pvt. limited, Norwich) as per the protocol described in the experimental section given in supporting information. All the reactions were carried on Mtb DNA gyrase enzyme using compounds dose dependently starting from 100 µM, 50 µM, 25 µM, 12.5 µM, 6.25 µM, 3.125 µM, 1.56 µM and 0.75 µM. The most active compound **53** showed an IC_{50} of 0.63±0.19 µM as illustrated in **table 1** and the agarose gel picture depicting the inhibitory activity of compound **53** over DNA at various concentrations is shown in **figure 5.** Compounds **23**, **28, 42** and **57** also exhibited sub-micromolar GyrB inhibitory concentrations highlighting this series of 46 compounds as efficient inhibitors of DNA GyrB protein. To keep a check on the assay conditions, novobiocin was considered as a standard compound in this assay too. IC_{50} for all the compounds was calculated based on relative quantification using Image lab software, Bio-Rad, compared to the control in the assay. These compounds were also screened for *in vitro* antimycobacterial activity against Mtb H37Rv strain using microplate alamar blue assay (MABA) [19]. The MICs for the compounds are given in **table 1**.

The synthesized 46 compounds were subjected to molecular docking studies in order to analyze their binding interactions and active site orientation so as to build a structure activity relationship (SAR) for the series with respect to their GyrB activity. Among the series, compound **53** was found to be exhibiting the most promising GyrB inhibitory activity with an IC_{50} of 0.86 µM. This compound also correlated well in terms of its supercoiling activity with 0.63 µM IC_{50} and Mtb MIC of 3.3 µM. The compound's activity was well supported by its binding mode at the ATPase site of Msm GyrB where it was found to be involved in two hydrogen bond interactions with Arg82 and a cation-π interaction with Arg141 as shown in **figure 6**. The compound was oriented such that the quinoline moiety was directed towards the solvent accessible surface area with the carboxylic group over it involving in the hydrogen bonding. The N-ethyl piperazine was set in to the GyrB hydrophobic pocket where it was found to be stabilized by hydrophobic interactions with residues Val49, Ile84, Pro85, Val99, Met100, Val128, Leu135 and Ile171.

The compounds in the present series can be divided into three groups for convenience according to their R_1 substitutions – (i) compounds **12-27**, (ii) compounds **28-43** and (iii) compounds **44-57**. Of the compounds **12-27** with ethoxy substitution at R_1 position, compound **23** was found to be the top active one with GyrB inhibitory activity of 0.97 µM and Mtb MIC of 2.94 µM. The interaction profile of the compound revealed the presence of polar contact between Arg82 and nitrogen of piperazine ring as shown in **figure 7(a)**. Unlike compound **53**, compound **23** was oriented such that the quinoline group was embedded into the hydrophobic pocket and involved in non-polar interactions with Ile84, Pro85, Val99, Met100, Val128, and Leu135. The trifluoro methyl group over quinoline added up the hydrophobicity of the moiety making it highly stable at the pocket. Compounds **12-19** with morpholine ring in place of piperazine were found to be inactive with an exception of compound **19** which was found with IC_{50} of 6.62 µM. This loss in activity might be attributed for the absence of N-ethyl substitution at Y-position for hydrogen bonding. The importance of $-NC_2H_5$ can be understood from the interaction profiles of compounds **23** and **15** given in **figure S1** in supporting informtion. The compounds **20**, **21** and **22** were found to be lesser active when compared to compound **23** which may be explained by the presence of trifluoro methyl group at R substitution. It was observed that with an increase in hydrophobic component at R position, as seen in compounds **20**, **21**, **22** and **23**, the activity was found to be improved starting from >50 µM to 0.97 µM. Replacement of oxygen at X-position in compounds **20**, **21** and **22** by nitrogen resulted in compounds **24**, **25** and **26** with improved GyrB

activity which can be explained by the participation of nitrogen in hydrogen bonding with residues Glu48 or Glu56 which enhanced the inhibitory activity by ~2 folds.

Considering compounds **28-43** with hydrazine at R_1 position, compound **28** was found to be the best active one with GyrB IC_{50} of 0.97 µM and corresponding Mtb MIC of 2.4 µM. The compound was found interacting by hydrogen bonding with residues Asn52, Val77 and Asp79 as shown in **figure 7(b)**. Interesting point is that the hydrazine group was found to be involved in two hydrogen bonds of three orienting the compound in such a way that quinoline bound in to the hydrophobic pocket. The compound was also involved in non-polar interactions with Ile84, Pro85, Val99, Met100 and Val128. The difference in GyrB activity of compounds **12** and **28** by almost 23 folds can be described by the presence of hydrazine in compound **28**, as shown in **figure S2** in supporting information. On substitution of methoxy (compound **29**), fluorine (compound **30**) and trifluoro methyl (compound **31**) at R position of compound **28**, activity was found to be continuously decreased. This may be attributed to the steric bulk of the substitutions which made the compound orient such that hydrazine was moved away from the interacting residues thereby making them inactive. The substitution of nitrogen in place of oxygen at X-position resulted in complete loss of GyrB inhibitory activity as observed in compounds **32-35**. The GyrB activity was found to be improved up on the presence of N-ethyl substitution at Y-position making the compounds active by several folds when compared to their morpholine counterparts. This might be reasoned for the addition of N-ethyl moiety over the compounds in which it was found participating in strong hydrophobic interactions. The hydrazine group facing the solvent was found to be involved in polar contacts with residues such as Asp55 (as in compounds **36**, **37**, **38, 41**), Asn52 (compounds **38**, **40**, **41** and **42**).

The final set of compounds includes **44-57** with hydroxyl group with an IC_{50} of 7.89 µM. The GyrB inhibitory activity and DNA supercoiling activity was found to be improved among the compounds **52-57** with N-ethyl substitution. The difference in the activity of the compounds set **44-51** and **52-57** can be probably explained in terms of their hydroxyl and N-ethyl substitution. In this set of **44-57** compounds, the presence of hydroxyl group at the R_1 position reversed the pose of the compounds making them orient such that the hydroxyl group facing the solvent due to its high polar nature. In compounds **44-51**, the morpholine group was oriented in the pocket which was found with less non-polar interactions. Conversely, in compounds **52-57** the presence of N-ethyl piperazine ring in the active site pocket was found to be highly stabilized and

interacting with residues Val49, Ile84, Pro85, Val99, Met100, Val128 and Leu135. On the whole, quinoline-3-carboxylic acid (-OH at R_1) and quinoline-3-carbohydrazide (-$NHNH_2$ at R_1) derivatives with ethyl piperazine (-NC_2H_5 at Y) moiety were found to be desirable substitutions resulting in compounds with efficient GyrB inhibitory potential.

Additionally, the eukaryotic cell safety profile of the synthesized compounds was analyzed by testing against mouse leukemic monocyte macrophage cell line RAW 264.7 cells at 50 µM concentration using MTT assay[20]. All the tested compounds demonstrated a good safety profile with very low inhibitory potential against the eukaryotic cell line. The assays were performed in triplicates, twice to record the precise inhibitions [21].The inhibitions were within a range of 0.07 to 44.56% at 50 µM inhibitor concentration. Further, the most active compound **53** showed 0.66% inhibition at 50 µM concentration illustrating its safety profile in the eukaryotes. The cytotoxicity profiles of all the synthesized compounds are given in **table 1**.

Furthermore, to re-ascertain the binding affinity of the DNA GyrB protein with the ligand and to estimate the thermal stabilityof the protein-ligand complex, differential scanning fluorimetry experiments were performed. The DNA GyrB DSF experiments were performed as described in the protocol in experimental section. Initially, purified concentrated protein of 2 mg/mL concentration was subjected to sequential (0.6 ^{o}C/min) increasing temperatures starting from an optimized 25 ^{o}C.

Subsequently, the native protein and the protein-ligand complex were heated from 25 ^{o}C to 95 ^{o}C in steps of 0.1 ^{o}C increment in the presence of 50 X SYPRO orange dye. As the dye has more affinity towards the hydrophobic residues, the fluorescence increases with the increase in the exposure and binding of the dye to the exposed hydrophobic residues of the protein[22]. Thus, it can be concluded that the affinity of the protein-ligand complex is proportional to temperature shift between the melting temperature (T_m) of the native protein and the protein-ligand complex. In short, greater the shift in the temperature towards right, higher is the stability of the complex. Major shift was observed by taking the GyrB protein in complex with compound **53** as the shift in T_m was found to be 3.3 ^{o}C. The native protein T_m was observed at 45 ^{o}C (green) whereas the protein-ligand complex (red) showed T_m of 48.3 ^{o}C as depicted in **figure 8.**

3. Conclusions

Concluding the study, in the present work we report a new class of N-phenyl quinoline amines as mycobacterial GyrB inhibitors with three compounds exhibiting activity below 1 µM. The *in vitro* GyrB activity of the compounds was well correlated to their structures with respect to their *in silico* binding pattern thereby establishing a valid structure activity relationship (SAR) for the compounds. In the present scenario where there is an immediate necessity of newer potential antitubercular drugs, these inhibitors, offering synthetic feasibility and least cytotoxicity, can be handful for futher optimization to a drug candidate.

4. Experimental Section

4.1. General: All commercially available chemicals and solvents were used without further purification. Melting points of the synthesized compounds were determined by Buchi B-540 open capillary instrument and wereuncorrected. The homogeneity of the compounds was monitored by TLC (Thin layer chromatography) on silica gel 40 F254 coated on aluminium plates, visualized by UV or iodine chamber and $KMnO_4$ treatment. All ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-300 (300.12 MHz, 75.12 MHz) NMR spectrometer and BrukerBioSpin Corp, Germany respectively. Molecular weights of the synthesized compounds were checked by SHIMADZU LCMS-2020 series in ESI mode. Chemical shifts are 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. Elemental analyses were carried out on ElementarVario MICRO CUBE CHN Analyser.

4.1.1. Synthesis of tert-butyl (4-aminophenyl)carbamate **(1):** To a stirred solution of *p*-phenylenediamine (10.8 g, 100 mmol) and triethylamine (Et_3N) (21.8 g, 216 mmol) in *N*,*N*-dimethyl formamide (DMF) (150 mL) Boc-anhydride (28.3 g, 130 mmol) was added slowly at 0 ºC allowed to stir for 3 h at room temperature (rt). The reaction was monitored by TLC, after completion DMF was removed by high vacuum pump and diluted with ethylacetate (300 mL) and the organic layer was washed with brine (150 mL). The organic layer was evaporated under reduce pressure to get crude brown solid which was further purified by flash column chromatography by 230-400 mesh silica gel using ethylacetate:hexane as eluent to afford brown solid (15 g, 75%); mp: 112-114°C. ESI-MS was found at m/z 209.43 $[M+H]^+$.^{1}H NMR (300 MHz, $CDCl_3$, TMS):δ = 9.68 (s, 1H), 7.56 (d, *J* = 7.6 Hz, 2H), 6.79 (d, *J* = 7.8 Hz, 2H),5.89 (s, 2H) 1.36 (s, 9H).

***4.1.2. General synthetic procedure for the preparation of intermediates 2 and 6*; *General procedure* A:** To a stirred solution of compound **1** or **5** (65.0 mmol**)**, poatassium carbonate (K_2CO_3) (97.5 mmol) and 1,3-dibromopropane (78.0 mmol) in DMF (100 ml) was heated to 110 ºC for 3h. After completion of the reaction (monitored by TLC), DMF was removed by under vacuo. To the crude reaction mixture crushed ice was added and diluted with ethylacetate (150 mL). The organic layer was washed with brine (100 mL), separated and dried over anhydrous sodium sulphate (anhy. Na_2SO_4) and evaporated under reduced pressure to get crude product which was further purified by flash column chromatography by 230-400 silica gel using ethylacetate:hexane as eluent to afford corresponding bromo derivative as a solid.

***4.1.2.1. Tert-butyl (4-((3-bromopropyl) amino) phenyl)carbamate* (2):** The compound was prepared according to the general procedure A using compound **1** (13.53g, 65mmol), 1,3-dibromopropane (15.74 g, 78 mmol) as an off-white solid (12.8g, 60%);m.p: 113-115°C; ESI-MS was found at m/z 330.43 $[M+H]^+$.^{1}H NMR (300 MHz, $CDCl_3$, TMS):δ = 9.98 (s, 1H), 7.53 (d, *J* = 7.4 Hz, 2H), 6.72 (d, *J* = 8.1Hz, 2H), 5.68 (s, 1H), 3.56 (t, *J* = 7.0 Hz, 2H), 3.31 (t, *J* = 6.9 Hz, 2H), 2.01 (m, 2H), 1.39 (s, 9H).

***4.1.2.2. Tert-butyl (4-(3-bromopropoxy)phenyl)carbamate*(6)**: The compound was prepared according to the general procedure A using compound **5** (13.58 g, 65 mmol), 1,3-dibromopropane (15.76 g, 78 mmol) as pale brown solid (10.4 g, 70%);m.p: 100-102°C; ESI-MS was found at m/z 331.36 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$, TMS):δ = 9.76 (s, 1H), 6.78(d, *J* = 7.8 Hz, 2H), 6.68 (d, *J* = 7.6 Hz, 2H),4.08 (t, *J* = 6.8 Hz, 2H), 3.58 (t, *J* = 7.0 Hz, 2H), 1.98 (m, 2H), 1.29 (s, 9H).

***4.1.3. General synthetic procedure for the preparation of intermediate 3 and 7*; *General procedure* B:** To a stirred solution of intermediate **3** (20 mmol) or **6** (20 mmol,**)**, potassium carbonate (30 mmol) (K_2CO_3) and 1.1 equivalent of morpholine/1-ethylpiperazine in DMF was heated to 110 ºC for 3 h. TLC analysis indicated that the reaction was complete. DMF was removed by high vacuum pump. To the crude reaction mixture crushed ice was added and diluted with ethylacetate (150 mL). The organic layer was washed with brine (100 mL), separated, dried over anhydrous Na_2SO_4 and evaporated to get crude product which was further purified by flash column chromatography by 230-400 silica gel using ethyl acetate:hexane as eluent to afford desired compounds (**3a-b**, **7a-b**), followed by Boc de-protection of **3a-3b** &intermediateof **step f_1**with trifluoro acetic acid (TFA) 10 equiv (~10 mL) in 15 mL of DCM at

0 ºC to room temperature for 4 h gives the corresponding amine (**4a-b**& **7a-7b**) derivatives in good yields.

4.1.3.1. Tert-butyl (4-((3-morpholinopropyl)amino)phenyl)carbamate **(3a):** The compound was prepared according to the general procedure Busing compound **2** (6.58 g, 20 mmol), morpholine (1.9 g, 22 mmol) as an off-white solid (4.75 g, 71%);m.p: 66-68 °C; ESI-MS was found at m/z 336.48 $[M+H]^+$.^{1}H NMR (300 MHz, $CDCl_3$, TMS):δ = 9.98 (s, 1H), 7.57 (d, *J* = 7.1 Hz, 2H), 6.73 (d, *J* = 7.6 Hz, 2H), 5.49 (s, 1H), 3.40 (t,*J* = 6.8 Hz,4H), 2.41 (m, 2H),2.36 (t, *J* = 6.4 Hz, 4H), 2.34 (s, 6H), 1.67 (m, 2H), 1.32 (s, 9H).

4.1.3.2. Tert-butyl (4-((3-(4-ethylpiperazin-1-yl)propyl)amino)phenyl) carbamate **(3b):** The compound was prepared according to the general procedure B using compound **2** (6.58 g, 20 mmol), 1-ethylpiperazine (2.5 g, 22 mmol) as a pale brown solid (5g, 70%);mp: 97-98°C; ESI-MS was found at m/z 363.56 $[M+H]^+$.^{1}H NMR (300 MHz, $CDCl_3$, TMS):δ = 10.02 (s, 1H), 7.60 (d, *J* = 7.3 Hz, 2H), 6.68 (d, *J* = 7.7 Hz, 2H), 5.38 (s, 1H), 3.38 (t, *J* = 7.0 Hz, 2H), 2.40 (m, 2H), 2.37 (t , *J* = 6.8 Hz, 4H), 2.31 (s, 6H), 1.69 (m, 2H), 1.21 (s, 9H), 1.02 (t, *J* = 6.8 Hz, 3H).

4.1.3.3. N1-(3-Morpholinopropyl)benzene-1,4-diamine **(4a):** The compound was prepared according to the general procedure B using compound **3a** (4.0 g, 12 mmol), TFA (13.64 g, 119 mmol) as pale brown liquid (2.52 g, 90%); ESI-MS was found at m/z 236.50 $[M+H]^+$.^{1}H NMR (300 MHz, $CDCl_3$, TMS):δ = 6.74 (d, *J* = 7.6 Hz, 2H), 6.56 (d, *J* = 7.2 Hz, 2H), 6.02-5.49 (bs, 3H), 3.58 (t, *J* = 7.0 Hz, 4H), 3.31 (t, *J* = 6.8 Hz, 2H), 2.29 (s, 6H), 1.62 (m, 2H).

4.1.3.4. N1-(3-(4-Ethylpiperazin-1-yl)propyl)benzene-1,4-diamine **(4b):** The compound was prepared according to the general procedure B using compound **3b** (4.35 g, 12 mmol), TFA (13.68 g, 119 mmol) as pale brown liquid; Yield ; ESI-MS was found at m/z 263.55 $[M+H]^+$.^{1}H NMR (300 MHz, $CDCl_3$, TMS):δ = 6.57 (d, *J* = 7.7 Hz, 2H), 6.36 (d, *J* = 7.4 Hz, 2H), 5.56 (bs, 3H), 3.35 (t, *J* = 7.0 Hz, 2H), 2.42 (m, 2H), 2.39 (t, *J* = 6.8 Hz, 4H), 2.28 (s, 6H), 1.26 (m, 2H), 1.04 (t, *J* = 7.0 Hz, 3H).

4.1.3.5. 4-(3-Morpholinopropoxy)aniline **(7a):** The compound was prepared according to the general procedure B using compound **6** (3.22 g, 14 mmol), morpholine (1.34 g, 15.4 mmol) aspale brown liquid (2.8 g, 85%); ESI-MS was found at m/z 237.20 $[M+H]^+$.^{1}H NMR (300 MHz, $CDCl_3$, TMS):δ = 6.76 (d, *J* = 7.1 Hz, 2H), 6.62 (d, *J* = 7.5 Hz, 2H), 6.02 (bs, 2H), 4.03 (t, *J*= 6.8 Hz, 2H), 3.64 (t, *J* = 7.0 Hz, 4H), 2.30 (s, 6H), 1.71 (m, 2H).

***4.1.3.6. 4-(3-(4-Ethylpiperazin-1-yl)propoxy)aniline* (7b):** The compound was prepared according to the general procedure B using compound **6** (3.22 g, 14 mmol), 1-ethylpiperazine (1.75 g, 15.4 mmol) as pale brown liquid (3.2g, 87%); ESI-MS was found at m/z 264.73 $[M+H]^+$.^{1}H NMR (300 MHz, $CDCl_3$, TMS):δ = 6.74 (d, *J* = 7.6 Hz, 2H), 6.65 (d, *J* = 7.4 Hz, 2H), 6.03 (bs, 2H), 4.03 (t, *J* = 7.0 Hz, 2H), 2.41 (q,*J* = 7.2 Hz, 2H), 2.34 (t, *J* = 6.8 Hz, 4H), 2.29 (s, 6H), 1.77 (m, 2H), 1.05 (t,*J* = 7.0 Hz,3H).

***4.1.4. General procedure for synthesis of diethyl 2-((phenyl/4-methoxy/4-fluoro/4-trifluoromethylphenylamino)methylene)malonate*; *General procedure* C (9a-d):** To a stirred solution of substituted aniline **8** (50 mmol), diethyl 2-(ethoxymethylene)malonate(55 mmol) in ethanol (EtOH) (250 mL) was refluxed for 3 h. TLC analysis indicated that the reaction was complete. After cooling the reaction mixture solid was separated. The separated solid was filtered and washed with 3% ethyl acetate:hexane for further purification to afford desired compound as an off-white solid in good yield (89-92%).

***4.1.4.1. Diethyl 2-((phenylamino)methylene)malonate* (9a):** The compound was prepared according to the general procedure C using compound **8a** (4.65 g, 50 mmol), diethyl 2-(ethoxymethylene)malonate (11.87 g, 55 mmol) as an off-white solid (11.83 g, 90%);m.p: 94-96 °C; ESI-MS was found at m/z 264.23 $[M+H]^+$.^{1}H NMR (300 MHz, $CDCl_3$, TMS):δ = 8.46 (s, 1H), 7.32-6.84 (m, 5H), 6.62 (s, 1H), 4.35 (q,*J* = 7.0 Hz, 4H), 1.32 (t,*J* = 6.8 Hz, 6H).

***4.1.4.2. Diethyl 2-(((4-methoxyphenyl)amino)methylene)malonate* (9b):** The compound was prepared according to the general procedure C using compound **8b** (6.15 g, 50 mmol), diethyl 2-(ethoxymethylene)malonate (11.86 g, 55 mmol) as an off-white solid (13.1 g, 89%);m.p: 40-42° C; ESI-MS was found at m/z 294.42 $[M+H]^+$.^{1}H NMR (300 MHz, $CDCl_3$, TMS):δ = 8.43 (s, 1H), 7.46 (d, *J* = 6.3Hz, 2H), 6.81 (d,*J*= 6.5Hz, 2H), 6.68 (s, 1H),4.37 (q,*J* = 7.0 Hz, 4H), 3.81 (s, 3H), 1.36 (t,*J* = 6.8 Hz, 6H).

***4.1.4.3. Diethyl 2-(((4-fluorophenyl)amino)methylene)malonate* (9c):** The compound was prepared according to the general procedure C using compound **8c** (5.55 g, 50 mmol), diethyl 2-(ethoxymethylene)malonate (11.87 g, 55 mmol) as an off white solid (12.68 g, 91%) ;m.p: 74-76°C; ESI-MS was found at m/z 282.31 $[M+H]^+$.^{1}H NMR (300 MHz, $CDCl_3$, TMS):δ = 8.47 (s, 1H), 7.11 (d, *J* = 7.2 Hz, 2H), 6.43 (d, *J* = 7.1Hz, 2H), 6.72 (s, 1H), 4.36 (q,*J* = 7.0 Hz,4H), 1.35 (t,*J* = 7.0 Hz, 6H).

4.1.4.4. Diethyl 2-(((4-(trifluoromethyl)phenyl)amino)methylene)malonate **(9d):** The compound was prepared according to the general procedure C using compound **8d** (8.0 g, 50 mmol), diethyl 2-(ethoxymethylene)malonate (11.87 g, 55 mmol) as an off white solid (14.8 g, 91%);m.p: 95-97°C; ESI-MS was found at m/z 332.32 $[M+H]^+$.^{1}H NMR (300 MHz, $CDCl_3$, TMS):δ = 8.45 (s, 1H), 7.46 (d, *J* = 6.8Hz, 2H), 6.46 (d, *J* = 6.7Hz, 2H), 6.70 (s, 1H), 4.37 (q,*J* = 7.0 Hz, 4H), 1.33 (t,*J* = 6.8 Hz, 6H).

4.1.5. General procedure for synthesis of ethyl 6-unsubtituted/methoxy/fluoro/trifluoromethyl-4-hydroxyquinoline-3-carboxylate **(10a-d):** Polyphosphoric acid (PPA) (2 equiv by weight) was added to corresponding intermediate **1** (40 mmol) to thisphosphorousoxychloride ($POCl_3$) (10mmol) was added. The reaction mixture was heated to 75 ºC for 8 h, monitored by TLC, after completion of the reaction. The reaction mixture was quenched slowly with 10% sodium hydroxide solution by keeping it in an ice bath and the P^H was adjusted to a pH~7, the solid separated was filtered, dried and washed with diethyl ether (3*20 mL) to afford desired compound in good yield.

4.1.5.1. Ethyl 4-hydroxyquinoline-3-carboxylate **(10a):** Pale yellow solid (5.64 g, 65%);m.p: 276-278 °C; ESI-MS was found at m/z 218.32 $[M+H]^+$.^{1}H NMR (300 MHz, DMSO-d6, TMS):δ = 10.72 (brs, 1H), 8.91 (s, 1H), 8.46 -7.78 (m, 4H), 4.29 (q,*J* = 7.1 Hz, 2H), 1.29 (t, *J* = 7.1Hz, 3H).

4.1.5.2. Ethyl 4-hydroxy-6-methoxyquinoline-3-carboxylate **(10b):** yellow solid (6.51 g, 66%);m.p: 274-276°C; ESI-MS was found at m/z 248.32 $[M+H]^+$.^{1}H NMR (300 MHz, DMSO-d6, TMS):δ = 10.67 (brs, 1H), 8.82 (s, 1H), 8.14 (d, *J* = 9.0Hz, 1H), 7.73 (d, *J* = 7.2 Hz, 1H), 7.14 (s, 1H), 4.27 (q, *J* = 7.2 Hz, 2H), 1.29 (t,*J* = 7.0 Hz, 3H).

4.1.5.3. Ethyl 6-fluoro-4-hydroxyquinoline-3-carboxylate **(10c):** Pale yellow solid (6.58 g, 70%);m.p: 288-290°C; ESI-MS was found at m/z 236.22 $[M+H]^+$.^{1}H NMR (300 MHz, DMSO-d6, TMS):δ = 10.45 (brs, 1H), 8.86 (s, 1H), 8.16 (d, *J* = 7.7 Hz, 1H), 7.81 (s, 1H), 7.34 (d, *J* = 7.4 Hz, 1H), 4.23 (q, *J* = 6.8 Hz, 2H), 1.29 (t, *J* = 6.8 Hz, 3H).

4.1.5.4. Ethyl 4-hydroxy-6-(trifluoromethyl)quinoline-3-carboxylate **(10d):** Pale brown solid (7.75 g, 68%); m.p: 290-292° C; ESI-MS was found at m/z 286.34 $[M+H]^+$.^{1}H NMR (300 MHz, DMSO-d6, TMS):δ = 10.52 (brs, 1H), 8.91 (s, 1H), 8.61 (d, *J* = 7.8 Hz, 1H), 8.37 (s, 1H), 8.11 (d, *J* = 7.6 Hz, 1H), 4.27 (q, *J* = 7.0 Hz, 2H), 1.28 (t, *J* = 6.8 Hz, 3H).

***4.1.6. General procedure for synthesis of ethyl 6-unsubtituted/methoxy/fluoro/trifluoromethyl-4-chloroquinoline-3-carboxylate* (11a-d):** To the corresponding intermediate **10** (25 mmol), $POCl_3$ (125 mmol) was added slowly and refluxed for 3 h at 105 ºC. TLC analysis indicated that the reaction was completed. Excess $POCl_3$ was removed under reduced pressure and the crude reaction mass was quenched with crushed ice then neutralized with saturated sodiumbicarbonatesolution (100 mL) and extracted with ethylacetate (3*100 mL). The organic layer was dried over anhy.Na_2SO_4, filtered and evaporated under reduced pressure to get corresponding desired chloro intermediate.

***4.1.6.1. Ethyl 4-chloroquinoline-3-carboxylate* (11a):** Yellow solid (4.74 g, 81%);m.p: 43-45 °C; ESI-MS was found at m/z 236.67 $[M+H]^+$.^{1}H NMR (300 MHz, $CDCl_3$, TMS):δ = 8.92 (s, 1H), 8.46-7.78 (m, 4H), 4.28 (q, *J* = 6.8 Hz, 2H), 1.27 (t, *J* = 7.3Hz, 3H).

***4.1.6.2. Ethyl 4-chloro-6-methoxyquinoline-3-carboxylate* (11b):** Pale yellow solid (5.44 g, 82%); m.p: 79-81° C; ESI-MS was found at m/z 266.68 $[M+H]^+$.^{1}H NMR (300 MHz, $CDCl_3$, TMS):δ = 8.84 (s, 1H), 8.13 (d, *J* = 9.1Hz, 1H), 7.72 (d, *J* = 7.2 Hz, 1H), 7.15 (s, 1H), 4.26 (q, *J* = 7.0 Hz, 2H), 1.28 (t,*J* = 6.8 Hz, 3H).

***4.1.6.3. Ethyl 4-chloro-6-fluoroquinoline-3-carboxylate* (11c):** Yellow solid (5.64 g, 89%);m.p: 62-64°C; ESI-MS was found at m/z 254.60 $[M+H]^+$.^{1}H NMR (300 MHz, $CDCl_3$, TMS):δ = 8.87 (s, 1H), 8.17 (d, *J* = 7.5 Hz, 1H), 7.83 (s, 1H), 7.36 (d, *J* = 7.6 Hz, 1H), 4.22 (q, *J* = 7.0 Hz, 2H), 1.29 (t, *J* = 7.0 Hz, 3H).

***4.1.6.4. Ethyl 4-chloro-6-(trifluoromethyl)quinoline-3-carboxylate* (11d):** Pale yellow solid (6.5 g, 86%);m.p: 90-92 °C; ESI-MS was found at m/z 304.64 $[M+H]^+$.^{1}H NMR (300 MHz, $CDCl_3$, TMS):δ = 8.95 (s, 1H), 8.62 (d, *J* = 7.7 Hz, 1H), 8.38 (s, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 4.28 (q, *J* = 7.1 Hz, 2H), 1.29 (t, *J* = 7.0 Hz, 3H).

***4.1.7. General procedure for the preparation of titled phenyl-amino-quinoline ethyl ester derivatives* (12-27):** The alkylation of amine intermediates (**4a-b** &**7a-b**) was carried in a Biotage microwave vial. Compound **11** (4.5 mmol) (1 equiv), **4** or **7** (1.2 equiv) and *para*-tolulenesufonic acid (PTSA) 5-6 mg catalytic amount in 15 ml methanol and then subjected to microwave irradiation at 120 ºC for 45 min. The reaction mixture was concentrated under vacuo. The residue was dissolved in water to this saturated $NaHCO_3$ solution was added to make alkaline, and extracted thrice with 5% MeOH:DCM solution. Combined organic layers were dried over anhydrous Na_2SO_4 and evaporated under vacuo, purification of the resultant product

in silica gel by flash column chromatography using 5-10 % MeOH:DCM as eluent to get final ethyl ester derivatives.

4.1.7.1. Ethyl 4-((4-(3-morpholinopropoxy)phenyl)amino) quinoline-3-carboxylate **(12):** Pale yellow gammy (1.10 g, 60%);^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 10.21 (s, 1H), 9.31 (s,1H), 8.36 (d, *J* = 8.8 Hz, 1H), 7.99 (m, 3H),7.52 (d, *J* = 8.2 Hz, 2H), 6.97 (d,*J* = 8.4 Hz, 2H), 4.32 (q, *J* = 7.0 Hz, 2H), 4.02 (t, *J* = 6.8 Hz, 2H), 3.67 (t, *J* = 7.0 Hz, 4H), 2.37 (s, 6H), 1.79 (m, 2H), 1.28 (t, *J* = 7.0 Hz, 3H).^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 168.5, 153.0, 152.2, 149.9, 141.3, 132.8(2C), 129.7, 127.4, 126.0, 121.7(2C), 120.9, 115.8(2C), 113.6, 73.4, 67.1(2C), 63.4(2C), 61.6, 59.1, 28.3, 14.6. ESI-MS m/z (Calcd. for $C_{25}H_{29}N_3O_4$: 435.22); Found: 436.29 $(M+H)^+$. Anal calcd.for $C_{25}H_{29}N_3O_4$: C, 68.95; H, 6.71; N, 9.65; Found: C, 68.91; H, 6.67; N, 9.62.

4.1.7.2. Ethyl 6-methoxy-4-((4-(3-morpholinopropoxy)phenyl)amino) quinoline-3-carboxylate **(13):** Brown solid (1.2 g, 58%); m.p: 170-172°C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ = 10.01 (s, 1H), 8.91 (s,1H), 8.31 (d, *J* = 8.6 Hz, 1H), 8.01-7.53 (m, 4H), 6.98 (d, *J* = 8.2 Hz, 2H), 4.33 (q, *J* = 7.0 Hz, 2H), 4.03 (t, *J* = 6.8 Hz, 2H), 3.81 (s, 3H), 3.65 (t, *J* = 7.1 Hz, 4H), 2.41 (s, 6H), 1.80 (m, 2H), 1.29 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 168.5, 157.3, 152.0, 150.2(2C), 136.9, 132.7, 131.4, 124.8, 122.3(3C), 115.9(2C), 114.2, 103.7, 73.5, 67.2(2C), 63.6(2C), 61.3, 59.0, 56.4, 28.5, 14.8. ESI-MS m/z (Calcd. for $C_{26}H_{31}N_3O_5$: 465.23); Found: 464.05 $(M-H)^-$. Anal calcd.for $C_{26}H_{31}N_3O_5$: C, 67.08; H, 6.71; N, 9.03; Found: C, 67.11; H, 6.74; N, 9.01.

4.1.7.3. Ethyl 6-fluoro-4-((4-(3-morpholinopropoxy)phenyl)amino) quinoline-3-carboxylate **(14):** Off-white solid (1.16 g, 57%);m.p: 93-95°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.89 (s, 1H), 9.01 (s,1H), 8.32 (d, *J* = 8.8 Hz, 1H), 7.96-7.49 (m, 4H), 6.98 (d, *J* = 8.1 Hz, 2H), 4.33 (q, *J* = 7.1 Hz, 2H), 4.03 (t, *J* = 6.8 Hz, 2H), 3.59 (t, *J* = 6.9 Hz, 4H), 2.36 (s, 6H), 1.80 (m, 2H), 1.26 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 168.3, 160.8, 153.0, 151.9, 149.7, 138.3, 133.0, 130.5, 124.2, 121.8(3C), 116.0(2C), 114.7, 104.5, 73.8, 67.5(2C), 63.2(2C), 61.4, 59.0, 28.6, 14.9. ESI-MS m/z (Calcd. for $C_{25}H_{28}FN_3O_4$: 453.21); Found: 452.21 $(M-H)^-$. Anal calcd.for $C_{25}H_{28}FN_3O_4$: C, 66.21; H, 6.22; N, 9.27; Found: C, 66.19; H, 6.25; N, 9.24.

4.1.7.4. Ethyl 4-((4-(3-morpholinopropoxy)phenyl)amino)-6-(trifluoromethyl) quinoline-3-carboxylate **(15):** Pale yellow solid (1.3 g, 58%);m.p: 98-100 °C;^{1}H NMR (300 MHz, DMSO-d_6,

TMS):δ = 10.20 (s, 1H), 8.87 (s,1H), 8.37 (d, *J* = 8.1 Hz, 1H), 7.96 (m, 2H), 7.51 (d, *J* = 8.4 Hz, 2H), 6.98 (d, *J* = 8.2 Hz, 2H), 4.30 (q, *J* = 7.0 Hz, 2H), 4.04 (t, *J* = 6.8 Hz, 2H), 3.68 (t, *J* = 6.8 Hz, 4H), 2.37 (s, 6H), 1.80 (m, 2H), 1.29 (t, *J* = 6.4 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 168.5, 155.0, 154.3, 149.9, 142.5, 132.8, 131.6, 129.1, 127.9, 125.0, 121.8(2C), 120.5(2C), 116.2(2C), 115.4, 73.7, 67.3(2C), 63.5(2C), 61.8, 59.2, 28.5, 14.7. ESI-MS m/z (Calcd. for $C_{26}H_{28}F_3N_3O_4$: 503.20); Found: 502.02 $(M-H)^-$. Anal calcd.for $C_{26}H_{28}F_3N_3O_4$: C, 62.02; H, 5.61; N, 8.35; Found: C, 62.04; H, 5.58; N, 8.32.

***4.1.7.5. Ethyl 4-((4-((3-morpholinopropyl)amino)phenyl)amino)quinoline-3-carboxylate* (16):** Pale yellow solid (1.15 g, 59%);m.p: 240-242°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.99 (s, 1H), 8.89 (s,1H), 7.99-7.48 (m, 4H), 6.85 (d, *J* = 8.1 Hz, 2H), 6.73 (d, *J* = 8.2 Hz, 2H), 5.51 (brs, 1H), 4.30 (q, *J* = 7.4 Hz, 2H), 3.68 (t, *J* = 7.0 Hz, 4H),3.35 (t, *J* = 6.8 Hz, 2H), 2.39 (s, 6H), 1.69 (m, 2H), 1.26 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 168.3, 153.1, 152.4, 140.9, 138.2, 134.9, 132.7, 129.5, 127.0, 126.2, 121.5, 119.0(2C), 118.8(2C), 113.6, 67.3(2C), 63.5(2C), 61.2, 52.7, 41.5, 27.8, 14.6. ESI-MS m/z (Calcd. for $C_{25}H_{30}N_4O_3$: 434.23); Found: 435.25 $(M+H)^+$. Anal calcd.for $C_{25}H_{30}N_4O_3$: C, 69.10; H, 6.96; N, 12.89; Found: C, 69.13; H, 6.94; N, 12.87.

***4.1.7.6. Ethyl 6-methoxy-4-((4-((3-morpholinopropyl)amino)phenyl)amino) quinoline-3-carboxylate* (17):** Brown solid (1.2 g, 60%);m.p: 138-140°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 10.01 (s, 1H), 8.89 (s,1H), 8.30 (d, *J* = 8.8 Hz, 1H), 8.04-7.55 (m, 2H), 6.61 (d, *J* = 9.1 Hz, 2H), 6.59 (d, *J* = 8.4 Hz, 2H), 5.53 (brs, 1H), 4.31 (q, *J* = 7.2 Hz, 2H), 3.66 (t, *J* = 7.0 Hz, 4H),3.82 (s, 3H),3.43 (t, *J* = 6.8 Hz, 2H), 2.41 (s, 6H), 1.81 (m, 2H), 1.26 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 168.5, 157.3, 152.0, 150.2, 138.0, 136.9, 135.1, 131.3, 125.0, 122.2, 119.4(2C), 119.0(2C), 114.3, 103.9, 67.4(2C), 63.6(2C), 61.3, 56.0, 52.8, 41.6, 27.9, 14.5. ESI-MS m/z (Calcd. for $C_{26}H_{32}N_4O_4$: 464.24); Found: 465.31 $(M+H)^+$. Anal calcd.for $C_{26}H_{32}N_4O_4$: C, 67.22; H, 6.94; N, 12.06; Found: C, 67.24; H, 6.91; N, 12.03.

***4.1.7.7. Ethyl 6-fluoro-4-((4-((3-morpholinopropyl)amino)phenyl)amino) quinoline-3-carboxylate* (18):** Pale green solid (1.2 g, 59%);m.p: 116-118°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.96 (s, 1H), 8.82 (s,1H), 7.76-6.98 (m, 3H), 6.63 (d, *J* = 8.4 Hz, 2H), 6.59 (d, *J* = 8.2 Hz, 2H), 5.52 (brs, 1H), 4.35 (q, *J* = 7.1 Hz, 2H), 3.57 (t, *J* = 6.8 Hz, 4H),3.42 (t, *J* = 7.1 Hz, 2H), 2.35 (s, 6H), 1.81 (m, 2H), 1.23 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 168.3, 160.9, 152.7, 151.6, 138.3(2C), 134.9, 130.4, 124.2, 121.8, 119.3(2C), 119.0(2C),

114.9, 104.7, 67.4(2C), 63.5(2C), 61.7, 52.8, 41.6, 27.4, 14.7. ESI-MS m/z (Calcd. for $C_{25}H_{29}FN_4O_3$: 452.22); Found: 451.09 $(M-H)^-$. Anal calcd.for $C_{25}H_{29}FN_4O_3$: C, 66.35; H, 6.46; N, 12.38; Found: C, 66.33; H, 6.48; N, 12.41.

***4.1.7.8. Ethyl 4-((4-((3-morpholinopropyl)amino)phenyl)amino)-6-(trifluoro methyl)quinoline-3-carboxylate* (19):** Brown solid (1.25 g, 56%);m.p: 101-103°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 10.01 (s, 1H), 8.83 (s,1H), 7.96-7.01 (m, 3H), 6.54 (d, *J* = 8.0 Hz, 2H), 6.49 (d, *J* = 8.1 Hz, 2H), 5.51 (brs, 1H), 4.32 (q, *J* = 7.0 Hz, 2H), 3.64 (t, *J* = 7.2 Hz, 4H),3.52 (t, *J* = 6.7 Hz, 2H), 2.40 (s, 6H), 1.81 (m, 2H), 1.27 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 168.5, 154.9, 154.3, 142.6, 138.1, 134.8, 131.6, 129.0, 127.8, 125.1, 120.5(2C), 119.4(2C), 118.9(2C), 115.2, 67.2(2C), 63.4(2C), 61.7, 52.6, 41.8, 27.5, 14.7. ESI-MS m/z (Calcd. for $C_{26}H_{29}F_3N_4O_3$: 502.22); Found: 503.21 $(M+H)^+$. Anal calcd.for $C_{26}H_{29}F_3N_4O_3$: C, 62.14; H, 5.82; N, 11.15; Found: C, 62.08; H, 5.85; N, 11.12.

***4.1.7.9. Ethyl 4-((4-(3-(4-ethylpiperazin-1-yl)propoxy)phenyl)amino)quinoline-3-carboxylate* (20):** Brown solid(1.2 g, 59%);m.p: 110-112°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.82 (s, 1H), 8.87 (s, 1H), 8.43-7.73 (m, 4H), 7.48 (d, *J* = 8.2 Hz, 2H), 6.58 (d, *J* = 8.2 Hz, 2H), 4.23 (q, *J* = 7.0 Hz, 2H), 4.02 (m, 2H), 2.47-2.32 (m, 12H), 1.83 (m, 2H), 1.29 (t, *J* = 7.0 Hz, 3H), 1.04 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 167.3, 152.5, 151.7, 150.1,139.2, 132.3(2C), 129.1, 126.5, 125.2, 122.3 (3C), 115.2(2C), 113.3, 73.5, 61.1, 58.1(3C), 57.5(2C), 49.8, 27.8, 14.3, 13.4. ESI-MS m/z (Calcd. for $C_{27}H_{34}N_4O_3$: 462.58); Found: 463.34 $(M+H)^+$. Anal calcd.for $C_{27}H_{34}N_4O_3$: C, 70.10; H, 7.41; N, 12.11; Found: C, 70.09; H, 7.43; N, 12.24.

***4.1.7.10. Ethyl4-((4-(3-(4-ethylpiperazin-1-yl)propoxy)phenyl)amino)-6-methoxyquinoline-3-carboxylate* (21):** Brown gammy (1.32 g, 60%); ^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.92 (s, 1H), 8.86 (s, 1H), 7.81-7.45 (m, 3H), 7.43 (d, *J* = 8.4 Hz, 2H), 6.57 (d, *J* = 8.1 Hz, 2H), 4.19 (q, *J* = 7.0 Hz, 2H), 4.01 (t, *J* = 6.8 Hz, 2H), 3.54 (s, 3H), 2.48-2.31 (m, 12H), 1.82 (m, 2H), 1.31 (q, *J* = 7.1 Hz, 3H), 1.03 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 168.3, 157.5, 156.2, 150.4(2C), 136.9, 133.0, 131.3, 125.1, 122.4(3C), 115.9(2C), 114.3, 103.7, 73.8, 61.4, 59.1(3C), 58.5(2C), 56.3, 50.1, 28.4, 14.7, 13.9. ESI-MS m/z (Calcd. for $C_{28}H_{36}N_4O_4$: 492.27); Found: 493.52 $(M+H)^+$. Anal calcd.for $C_{28}H_{36}N_4O_4$: C, 68.27; H, 7.37; N, 11.37; Found: C, 68.25; H, 7.33; N, 11.35.

***4.1.7.11. Ethyl 4-((4-(3-(4-ethylpiperazin-1-yl)propoxy)phenyl)amino)-6-fluoroquinoline-3-carboxylate* (22):** Pale yellow solid (1.27 g, 59%);m.p: 99-101°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.94 (s, 1H), 8.87 (s, 1H), 8.01 (d, *J* = 8.8 Hz, 1H), 7.73 (d, *J* = 8.1 Hz, 1H), 7.63 (dd, *J* = 8.1 Hz,2.7 Hz, 1H), 6.92 (d, *J* = 8.6 Hz, 2H), 6.58 (d, *J* = 8.0 Hz, 2H), 4.28 (q, *J* = 6.8 Hz, 2H), 3.98 (t, *J* = 7.0 Hz, 2H), 2.49-2.32 (m, 12H), 1.83 (m, 2H), 1.32 (t, *J* = 6.8 Hz, 3H), 1.03 (t, *J* = 7.2 Hz, 3H).^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 168.6, 160.5, 152.7, 151.9, 150.2, 138.0, 132.8, 130.5, 124.2, 121.9(3C), 115.7(2C), 114.8, 104.5, 73.7, 61.4, 59.4(3C), 58.6(2C), 50.2, 28.2, 14.5, 13.8. ESI-MS m/z (Calcd. for $C_{27}H_{33}FN_4O_3$: 480.25); Found: 479.16 $(M-H)^-$. Anal calcd.for $C_{27}H_{33}FN_4O_3$: C, 67.48; H, 6.92; N, 11.66; Found: C, 67.51; H, 6.64; N, 11.67.

***4.1.7.12. Ethyl4-((4-(3-(4-ethylpiperazin-1-yl)propoxy)phenyl)amino)-6-(trifluoromethyl)quinoline-3-carboxylate* (23):** Pale yellow gammy (1.3 g, 54%);m.p: 240°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 10.01 (s, 1H), 8.89 (s, 1H), 8.47 (d, *J* = 2.6 Hz, 1H), 8.29 (dd, *J* = 8.4 Hz, 2.4 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 7.43 (d, *J* = 8.6 Hz, 2H), 6.59 (d, *J* = 8.2 Hz, 2H), 4.16 (q, *J* = 7.0 Hz, 2H), 3.99 (t, *J* = 7.1 Hz, 2H), 2.47-2.35 (m, 12H), 1.82 (m, 2H), 1.31 (t, *J* = 6.8 Hz, 3H), 1.03 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 168.5, 154.8(2C), 150.1, 142.6, 132.9, 131.6, 129.1, 127.8, 125.0, 121.8(2C), 120.9(2C), 115.7(3C), 73.5, 70.3, 59.0(3C), 58.5(2C), 50.2, 28.4, 14.6, 13.7. ESI-MS m/z (Calcd. for $C_{28}H_{33}F_3N_4O_3$: 530.25); Found: 529.11 $(M-H)^-$. Anal calcd.for $C_{28}H_{33}F_3N_4O_3$: C, 63.38; H, 6.27; N, 10.56; Found: C, 63.35; H, 6.24; N, 10.58.

***4.1.7.13. Ethyl 4-((4-((3-(4-ethylpiperazin-1-yl)propyl)amino)phenyl)amino) quinoline-3-carboxylate* (24):** Pale yellow solid (1.2 g, 57%);m.p: 90-92°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.97 (s, 1H), 8.81 (s, 1H), 8.39-7.71 (m, 4H), 6.57 (d, *J* = 8.0 Hz, 2H), 6.45 (d, *J* = 8.2 Hz, 2H), 5.54 (brs, 1H), 4.19 (q, *J* = 7.0 Hz, 2H), 3.41 (t, *J* = 6.8 Hz, 2H), 2.48-2.32 (m, 12H), 1.82 (m, 2H), 1.36 (t, *J* = 7.0 Hz, 3H), 1.02 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 168.3, 153.5, 152.4, 141.0, 138.2, 134.9, 132.6, 129.7, 127.3, 126.1, 121.4, 119.3(2C), 119.0(2C), 113.8, 61.5, 58.9(2C), 38.1(2C), 52.4, 50.2, 41.7, 27.5, 14.8, 13.9. ESI-MS m/z (Calcd. for $C_{27}H_{35}N_5O_2$: 461.28); Found: 462.41 $(M+H)^+$. Anal calcd.for $C_{27}H_{35}N_5O_2$: C, 70.25; H, 7.64; N, 15.17; Found: C, 70.27; H, 7.61; N, 15.19.

***4.1.7.14. Ethyl4-((4-((3-(4-ethylpiperazin-1-yl)propyl)amino)phenyl)amino)-6-methoxyquinoline-3-carboxylate* (25):** Yellow gummy (1.2 g, 55%); ^{1}H NMR (300 MHz,

DMSO-d_6, TMS):δ = 9.97 (s, 1H), 8.87 (s, 1H), 7.78 (d, *J* = 8.6 Hz, 1H), 7.65 (d, *J* = 2.2 Hz, 1H), 7.39 (dd, *J* = 8.2 Hz, 2.4 Hz, 1H), 6.60 (d, *J* = 8.2 Hz, 2H), 6.54 (d, *J* = 8.1 Hz, 2H), 5.52 (brs, 1H), 4.21 (q, *J* = 7.0 Hz, 2H), 3.58 (s, 3H), 3.42 (t, *J* = 6.8 Hz, 2H), 2.47-2.30 (m, 12H), 1.83 (m, 2H), 1.35 (t, *J* = 7.0 Hz, 3H), 1.02 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 168.5, 157.3, 152.0, 150.2, 138.4, 136.9, 135.1, 131.2, 124.9, 122.2, 119.3(2C), 118.9(2C), 114.4, 103.7, 61.5, 59.1(2C), 58.4(2C), 56.3, 52.5, 50.2, 41.7, 27.9, 14.5, 13.8. ESI-MS m/z (Calcd. for $C_{28}H_{37}N_5O_3$: 491.29); Found: 490.18 $(M-H)^-$. Anal calcd.for $C_{28}H_{37}N_5O_3$: C, 68.41; H, 7.59; N, 14.25; Found: C, 68.43; H, 7.61; N, 14.27.

***4.1.7.15. Ethyl 4-((4-((3-(4-ethylpiperazin-1-yl)propyl)amino)phenyl)amino)-6-fluoroquinoline-3-carboxylate* (26):** Pale yellow solid (1.27 g, 59%);m.p: 106-108 °C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.97 (s, 1H), 8.88 (s, 1H), 7.78 (d, *J* = 8.2 Hz, 1H), 7.60 (d, *J* = 2.2 Hz, 1H), 7.49 (dd, *J* = 8.6 Hz, 2.4 Hz, 1H), 6.91 (d, *J* = 8.1 Hz, 2H), 6.59 (d, *J* = 8.2 Hz, 2H), 5.51(brs, 1H), 4.20 (q, *J* = 7.0 Hz, 2H), 3.54 (t, *J* = 6.7 Hz, 2H), 2.47-2.31 (m, 12H), 1.84 (m, 2H), 1.33 (t, *J* = 7.1 Hz, 3H), 1.01 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 168.3, 160.7, 152.5, 151.8, 138.3(2C), 134.9, 130.4, 124.2, 121.9, 119.1(2C), 118.8(2C), 115.0, 104.7, 61.4, 59.2(2C), 58.6(2C), 52.4, 50.2, 41.7, 27.9, 14.5, 13.7. ESI-MS m/z (Calcd. for $C_{27}H_{34}FN_5O_2$: 479.27); Found: 480.11 $(M+H)^+$. Anal calcd.for $C_{27}H_{34}FN_5O_2$: C, 67.62; H, 7.15; N, 14.60; Found: C, 67.65; H, 7.13; N, 14.57.

***4.1.7.16. Ethyl 4-((4-((3-(4-ethylpiperazin-1-yl)propyl)amino)phenyl)amino)-6-(trifluoro methyl)quinoline-3-carboxylate* (27):** Yellow solid (1.28 g, 54%);m.p: 98-100°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.99 (s, 1H), 8.87 (s, 1H), 8.51 (d, *J* = 2.2 Hz, 1H), 8.29 (dd, *J* = 8.6 Hz, 2.4 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 6.56 (d, *J* = 8.4 Hz, 2H), 6.48 (d, *J* = 8.1 Hz, 2H), 5.51 (brs, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.51 (t, *J* = 6.7 Hz, 2H), 2.48-2.34 (m, 12H), 1.83 (m, 2H), 1.36 (t, *J* = 7.0 Hz, 3H), 1.03 (t, *J* = 6.9 Hz, 3H).^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 168.6, 155.0, 154.5, 142.7, 138.2, 134.9, 131.8, 129.0, 127.9, 125.2, 120.6, 119.8, 118.9(2C), 118.7(2C), 115.4, 61.6, 59.1(2C), 58.5(2C), 52.7, 50.3, 41.7, 27.5, 14.9, 13.8. ESI-MS m/z (Calcd. for $C_{28}H_{34}F_3N_5O_2$: 529.27); Found: 530.29 $(M+H)^+$. Anal calcd.for $C_{28}H_{34}F_3N_5O_2$: C, 63.50; H, 6.47; N, 13.22; Found: C, 63.48; H, 6.44; N, 13.26.

***4.1.8. General procedure for the preparation of titled phenyl-amino-quinolineacidhydrazide derivatives* (28-43):** The corresponding ethyl ester derivatives (0.5 mmol) (**12-27**) were converted to acid hydrazides by refluxing them with hydrazine hydrate (1.5 mmol) in ethanol for

6h to get corresponding acid hydrazide derivative which were further purified by recrystallization from ethanol.

4.1.8.1. 4-((4-(3-Morpholinopropoxy)phenyl)amino)quinoline-3-carbohydrazide **(28):** Yellow solid (0.126 g, 60%);m.p: 289-291°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 10.03 (s, 1H), 9.29 (s, 1H), 8.82 (s, 1H), 8.30 (d, *J* = 8.8 Hz, 1H), 8.01 (m, 3H), 7.52 (d,*J* = 8.2 Hz, 2H), 6.95 (d, *J* = 8.5 Hz, 2H), 4.01 (t, *J* = 7.1 Hz, 2H), 3.68 (t, *J* = 6.8 Hz, 4H), 2.46 (s, 6H), 2.21 (brs, 2H),1.98 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 165.5, 152.2, 150.0, 146.9, 138.1, 133.0, 132.6, 129.4, 127.1, 126.3, 123.9, 121.7(2C), 115.9(2C), 113.4, 73.6, 67.4(2C), 63.7(2C), 59.1, 28.4. ESI-MS m/z (Calcd. for $C_{23}H_{27}N_5O_3$: 421.21); Found: 422.19 $(M+H)^+$. Anal calcd.for $C_{23}H_{27}N_5O_3$: C, 65.54; H, 6.46; N, 16.62; Found: C, 65.51; H, 6.48; N, 16.64.

4.1.8.2. 6-Methoxy-4-((4-(3-morpholinopropoxy)phenyl)amino)quinoline-3-carbohydrazide **(29):** Yellow solid (0.13 g, 58%);m.p: 298-300°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 10.02 (s, 1H), 8.93 (s,1H), 8.84 (s, 1H), 8.12 (d, *J* = 8.6 Hz, 1H), 7.61 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.39 (d, *J* = 2.3 Hz, 1H), 7.28 (dd, *J* = 8.6 Hz, 2.4 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 2H), 4.04 (t, *J* = 7.1 Hz, 2H), 3.82 (s, 3H), 3.64 (t, *J* = 6.8 Hz, 4H), 2.31 (s, 6H), 2.19 (brs, 2H), 1.81 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 163.3, 157.5, 151.2, 150.0, 144.5, 133.7, 132.9, 131.1, 124.8(2C), 122.0(2C), 115.7(2C), 113.9, 103.6, 73.8, 67.3(2C), 63.5(2C), 59.1, 56.3, 28.5. ESI-MS m/z (Calcd. for $C_{24}H_{29}N_5O_4$: 451.22); Found: 452.45 $(M+H)^+$. Anal calcd.for $C_{24}H_{29}N_5O_4$: C, 63.84; H, 6.47; N, 15.51; Found: C, 63.87; H, 6.45; N, 15.56.

4.1.8.3. 6-Fluoro-4-((4-(3-morpholinopropoxy)phenyl)amino)quinoline-3-carbohydrazide **(30):** Brown solid (0.124 g, 57%);m.p: 290-292°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.90 (s, 1H), 9.02 (s, 1H), 8.87(s, 1H), 7.99 (d, *J* = 8.8 Hz, 1H), 7.57 (d, *J* = 8.2 Hz, 2H),7.46 (d, *J* = 2.4 Hz, 1H), 7.28 (dd, *J* = 8.6 Hz, 2.5 Hz, 1H), 6.86 (d, *J* = 8.3 Hz, 2H), 4.02 (t, *J* = 7.0 Hz, 2H), 3.57 (t, *J* = 7.1 Hz, 4H), 2.34 (s, 6H), 2.20 (brs, 2H), 1.84 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 165.4, 160.6, 151.9, 150.0, 145.8, 135.2, 132.7, 130.1, 124.6, 123.9, 122.2(2C), 115.8(2C), 114.4, 104.5, 73.8, 67.5(2C), 63.7(2C), 59.1, 28.3. ESI-MS m/z (Calcd. for $C_{23}H_{26}FN_5O_3$: 439.20); Found: 440.51 $(M+H)^+$. Anal calcd.for $C_{23}H_{26}FN_5O_3$: C, 62.86; H, 5.96; N, 15.94; Found: C, 68.84; H, 5.93; N, 15.89.

4.1.8.4. 4-((4-(3-Morpholinopropoxy)phenyl)amino)-6-(trifluoromethyl) quinoline-3-carbohydrazide **(31):** Yellow solid (0.143 g, 59%);m.p: 281-283°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 10.09 (s, 1H), 8.99 (s,1H), 8.87 (s, 1H), 8.51 (d, *J* = 2.1 Hz, 1H), 8.19 (dd,*J* = 8.6

Hz, 2.3 Hz, 1H), 8.14 (d, *J* = 8.2 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 8.5 Hz, 2H), 4.03 (t, *J* = 7.0 Hz, 2H), 3.69 (t, *J* = 6.7 Hz, 4H), 2.33 (s, 6H), 2.03 (brs, 2H), 1.82 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 165.3, 153.1, 149.9, 148.8, 139.2, 132.7, 131.4, 128.8, 127.6, 125.2, 122.9(3C), 120.7, 116.1(2C), 114.6, 73.8, 67.3(2C), 63.5(2C), 59.1, 28.4. ESI-MS m/z (Calcd. for $C_{24}H_{26}F_3N_5O_3$: 489.20); Found: 490.25 $(M+H)^+$. Anal calcd.for $C_{24}H_{26}F_3N_5O_3$: C, 58.89; H, 5.35; N, 14.31; Found: C, 58.91; H, 5.33; N, 14.28.

***4.1.8.5. 4-((4-((3-Morpholinopropyl)amino)phenyl)amino)quinoline-3-carbohydrazide* (32):** Pale green solid (0.128 g, 61%);m.p: 310-312°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 10.01 (s, 1H), 8.90 (s, 1H), 8.88 (s, 1H), 8.20-7.46 (m, 4H), 6.86 (d, *J* = 8.4 Hz, 2H), 6.41 (d, *J* = 8.6 Hz, 2H), 5.52 (brs, 1H), 3.65 (t, *J* = 6.8 Hz, 4H),3.36 (t, *J* = 7.0 Hz, 2H), 2.35 (s, 6H), 2.02 (s, 2H), 1.78 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 165.3, 152.0, 146.7, 138.2(2C), 134.9, 132.5, 129.3, 127.0, 126.2, 123.9, 119.1(2C), 118.7(2C), 113.2, 67.5(2C), 63.3(2C), 52.9, 41.6, 27.8. ESI-MS m/z (Calcd. for $C_{23}H_{28}N_6O_2$: 420.23); Found: 419.05 $(M-H)^-$. Anal calcd.for $C_{23}H_{28}N_6O_2$: C, 65.69; H, 6.71; N, 19.99; Found: C, 65.64; H, 6.73; N, 19.96.

***4.1.8.6. 6-Methoxy-4-((4-((3-morpholinopropyl)amino)phenyl)amino)quinoline-3-carbohydrazide*(33):** Yellow solid (0.126 g, 56%);m.p: 291-293°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.98 (s, 1H), 8.90 (s, 1H), 8.79 (s, 1H), 8.05 (d, *J* = 8.6 Hz, 1H), 7.52 (d, *J* = 2.4 Hz, 1H), 7.31 (dd, *J* = 8.2 Hz, 2.3 Hz, 1H), 6.61 (d, *J* = 7.8 Hz, 2H), 6.42 (d, *J* = 8.4 Hz, 2H), 5.52 (brs, 1H), 3.83 (s, 3H), 3.65 (m, 4H), 3.44 (t, *J* = 7.0 Hz, 2H),2.36 (s, 6H), 2.04 (brs, 2H), 1.85 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 165.4, 157.1, 150.9, 144.3, 138.5, 134.8, 133.6, 130.9, 125.0(2C), 119.2(2C), 118.9(2C), 113.7, 103.5, 67.5(2C), 63.7(2C), 56.3, 52.8, 41.9, 27.6. ESI-MS m/z (Calcd. for $C_{24}H_{30}N_6O_3$: 450.24); Found: 451.26 $(M+H)^+$. Anal calcd.for $C_{24}H_{30}N_6O_3$: C, 63.98; H, 6.71; N, 18.65; Found: C, 63.95; H, 6.73; N, 18.67.

***4.1.8.7. 6-Fluoro-4-((4-((3-morpholinopropyl)amino)phenyl)amino)quinoline-3-carbohydrazide* (34):** Brown solid (0.127 g, 58%);m.p: 289-291°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.99 (s, 1H), 9.21 (s, 1H), 8.85 (s,1H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.41 (d, *J* = 2.4 Hz, 1H), 7.29 (dd, *J* = 8.6 Hz, 2.5 Hz, 1H), 6.58 (d, *J* = 8.2 Hz, 2H), 6.40 (d, *J* = 8.4 Hz, 2H), 5.51 (brs, 1H), 3.58 (t, *J* = 7.0 Hz, 4H),3.44 (t, *J* = 6.8 Hz, 2H), 2.33 (s, 6H), 2.03 (s, 2H), 1.81 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 165.3, 160.5, 151.8, 146.0, 138.2, 135.1(2C), 130.3, 124.9(2C), 119.1(2C), 118.8(2C), 114.2, 104.7, 67.3(2C), 63.6(2C), 52.9, 41.6,

27.8. ESI-MS m/z (Calcd. for $C_{23}H_{27}FN_6O_2$: 438.22); Found: 437.11 $(M-H)^-$. Anal calcd.for $C_{23}H_{27}FN_6O_2$: C, 63.00; H, 6.21; N, 19.17; Found: C, 63.04; H, 6.22; N, 19.15.

***4.1.8.8. 4-((4-((3-Morpholinopropyl)amino)phenyl)amino)-6-(trifluoromethyl)quinoline-3-carbohydrazide* (35):** Brown solid (0.14 g, 57%);m.p: 302-304°C; ^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.98 (s, 1H), 9.38 (s, 1H), 8.81 (s,1H), 8.28 (d, *J* = 2.4 Hz, 1H), 8.18 (dd, *J* = 8.8 Hz, 2.2 Hz, 1H), 8.14 (d, *J* = 8.1 Hz, 1H), 6.55 (d, *J* = 8.4 Hz, 2H), 6.38 (d, *J* = 8.2 Hz, 2H), 5.52 (brs, 1H), 3.66 (t, *J* = 7.0 Hz, 4H),3.54 (t, *J* = 6.7 Hz, 2H), 2.31 (s, 6H), 2.06 (s, 2H), 1.86 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 165.3, 153.1, 148.9, 139.2, 138.4, 134.8, 131.5, 128.9, 127.7, 125.0, 122.8, 120.6, 119.2(2C), 118.8(2C), 114.7, 67.3(2C), 63.5(2C), 52.7, 41.4, 27.6. ESI-MS m/z (Calcd. for $C_{24}H_{27}F_3N_6O_2$: 488.21); Found: 487.05 $(M-H)^-$. Anal calcd.for $C_{24}H_{27}F_3N_6O_2$: C, 59.01; H, 5.57; N, 17.20; Found: C, 59.05; H, 5.61; N, 17.15.

***4.1.8.9. 4-((4-(3-(4-Ethylpiperazin-1-yl)propoxy)phenyl)amino)quinoline-3-carbohydrazide*(36):** Brown solid (0.134 g, 60%);m.p: 320-322°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.86 (s, 1H), 8.90 (s, 1H), 8.86 (s, 1H), 8.34-7.59 (m, 4H), 7.56 (d, *J* = 8.6 Hz, 2H), 6.59 (d, *J* = 8.4 Hz, 2H), 4.03 (t, *J* = 7.0 Hz, 2H), 2.49-2.31 (m, 12H), 2.11 (s, 2H), 1.86 (m, 2H), 1.05 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 165.2, 152.0, 149.9, 146.6, 137.8, 132.7(2C), 129.3, 127.1, 126.3, 123.8, 122.0(2C), 115.9(2C), 113.3, 73.7, 59.1(3C), 58.3(2C), 50.4, 28.3, 14.0. ESI-MS m/z (Calcd. for $C_{25}H_{32}N_6O_2$: 448.26); Found: 449.46 $(M+H)^+$. Anal calcd.for $C_{25}H_{32}N_6O_2$: C, 66.94; H, 7.19; N, 18.74; Found: C, 66.97; H, 7.21; N, 18.69.

***4.1.8.10. 4-((4-(3-(4-Ethylpiperazin-1-yl)propoxy)phenyl)amino)-6-methoxy quinoline-3-carbohydrazide* (37):** Yellow solid (0.14 g, 59%);m.p: 302-304°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.97 (s, 1H), 8.97 (s, 1H), 8.74 (s, 1H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.60 (d, *J* = 8.8 Hz, 2H), 7.43 (d, *J* = 2.4 Hz, 1H), 7.28 (dd, *J* = 8.4 Hz, 2.5 Hz, 1H), 6.78 (d, *J* = 8.2 Hz, 2H), 4.04 (t, *J* = 7.0 Hz, 2H), 3.55 (s, 3H), 2.49-2.30 (m, 12H), 2.02 (s, 2H), 1.79 (m, 2H), 1.04 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 165.3, 157.1, 150.9, 149.7, 144.5, 133.8(2C), 130.8, 125.1(2C), 121.9(2C), 115.7(2C), 113.4, 103.6, 73.4, 59.2(3C), 58.5(2C), 56.1, 50.3, 28.1, 13.9. ESI-MS m/z (Calcd. for $C_{26}H_{34}N_6O_3$: 478.27); Found: 477.04 $(M-H)^-$. Anal calcd.for $C_{26}H_{34}N_6O_3$: C, 65.25; H, 7.16; N, 17.56; Found: C, 66.28; H, 7.11; N, 17.52.

***4.1.8.11. 4-((4-(3-(4-Ethylpiperazin-1-yl)propoxy)phenyl)amino)-6-fluoro quinoline-3-carbohydrazide* (38):** Brown solid (0.128 g, 55%);m.p: 320-322°C;^{1}H NMR (300 MHz, DMSO-

d_6, TMS):δ = 9.96 (s, 1H), 9.34 (s, 1H), 8.89 (s, 1H), 7.88 (d, *J* = 8.6 Hz, 1H), 7.64 (d, *J*= 8.8 Hz, 2H), 7.39 (d, *J* = 2.5 Hz, 1H), 7.31 (dd, *J* = 8.4 Hz, 2.2 Hz, 1H),6.57 (d, *J* = 8.2 Hz, 2H), 3.99 (t, *J* = 7.1 Hz, 2H), 2.48-2.30 (m, 12H), 2.05 (s, 2H), 1.86 (m, 2H), 1.03 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 165.4, 160.6, 151.9, 150.1, 145.8, 135.0, 132.7, 130.2, 124.5(2C), 121.9(2C), 115.7(2C), 114.3, 104.5, 73.8, 59.5(3C), 58.3(2C), 50.2, 28.4, 13.9. ESI-MS m/z (Calcd. for $C_{25}H_{31}FN_6O_2$: 466.25); Found: 467.48 $(M+H)^+$. Anal calcd.for $C_{25}H_{31}FN_6O_2$: C, 64.36; H, 6.70; N, 18.01; Found: C, 64.39; H, 6.72; N, 17.98.

4.1.8.12. 4-((4-(3-(4-Ethylpiperazin-1-yl)propoxy)phenyl)amino)-6-(trifluoromethyl)quinoline-3-carbohydrazide **(39):** Yellow solid (0.154 g, 60%);m.p: 300-302°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.99 (s, 1H), 9.48 (s, 1H), 8.90 (s, 1H), 8.38 (d, *J* = 2.5 Hz, 1H), 8.20 (dd, *J* = 8.6 Hz, 2.4 HZ, 1H), 8.14 (d, *J* = 8.8 Hz, 1H), 7.64 (d, *J* = 8.4 Hz, 2H), 6.61 (d, *J* = 8.2 Hz, 2H), 4.01 (t, *J* = 6.8 Hz, 2H), 2.49-2.33 (m, 12H), 2.10 (brs, 2H), 1.86 (m, 2H), 1.02 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 165.4, 153.1, 150.0, 149.2, 139.4, 132.9, 131.5, 128.7, 127.6, 125.1, 122.8, 121.6(2C), 120.4, 115.8(2C), 114.6, 73.4, 59.0(3C), 58.5(2C), 50.2, 28.6, 14.0. ESI-MS m/z (Calcd. for $C_{26}H_{31}F_3N_6O_2$: 516.25); Found: 517.29 $(M+H)^+$. Anal calcd.for $C_{26}H_{31}F_3N_6O_2$: C, 60.45; H, 6.05; N, 16.27; Found: C, 60.47; H, 6.01; N, 16.29.

4.1.8.13. 4-((4-((3-(4-Ethylpiperazin-1yl)propyl)amino)phenyl)amino)quinoline-3-carbohydrazide **(40):** Yellow solid (0.127 g, 57%);m.p: 310-312°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.98 (s, 1H), 9.39 (s, 1H), 8.86 (s, 1H), 7.54 (m, 4H), 6.73 (d, *J* = 8.4 Hz, 2H), 6.58 (d, *J* = 8.2 Hz, 2H), 5.52 (brs, 1H), 3.42 (t, *J* = 7.0 Hz, 2H), 2.50-2.33 (m, 12H), 2.06 (brs, 2H), 1.71 (m, 2H), 1.01 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 165.4, 152.1, 146.6, 138.3, 137.9, 134.7, 132.5, 129.3, 126.8, 125.9, 123.7, 119.1(2C), 118.8(2C), 113.4, 59.0(2C), 58.6(2C), 52.4, 50.2, 41.8, 27.5, 14.0. ESI-MS m/z (Calcd. for $C_{25}H_{33}N_7O$: 447.27); Found: 448.15 $(M+H)^+$. Anal Calcd.for $C_{25}H_{33}N_7O$: C, 67.09; H, 7.43; N, 21.91; Found: C, 67.12; H, 7.39; N, 21.93.

4.1.8.14. 4-((4-((3-(4-Ethylpiperazin-1-yl)propyl)amino)phenyl)amino)-6-methoxyquinoline-3-carbohydrazide **(41):** Yellow solid (0.13 g, 55%);m.p: 306-308°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 10.01 (s, 1H), 9.16 (s, 1H), 8.89 (s, 1H), 7.81 (d, *J* = 8.8 Hz, 1H), 7.46 (d, *J* = 2.4 Hz, 1H), 7.29 (dd, *J* = 8.4 Hz, 2.5 Hz, 1H), 6.56 (d, *J* = 8.2 Hz, 2H), 6.51 (d, *J* = 8.4 Hz, 2H), 5.52 (brs, 1H), 3.58 (s, 3H), 3.46 (t, *J* = 6.8 Hz, 2H), 2.49-2.29 (m, 12H), 2.08 (s, 2H), 1.70 9m, 2H), 1.01 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 165.4, 157.2, 150.9,

144.5, 138.3, 135.0, 133.6, 130.9, 125.1(2C), 119.4(2C), 119.0(2C), 113.7, 103.5, 59.0(2C), 58.4(2C), 56.2, 52.5, 50.1, 41.8, 27.5, 13.9. ESI-MS m/z (Calcd. for $C_{26}H_{35}N_7O_2$: 477.29); Found: 478.36 $(M+H)^+$. Anal calcd.for $C_{26}H_{35}N_7O_2$: C, 65.38; H, 7.39; N, 20.53; Found: C, 65.41; H, 7.37; N, 20.55.

***4.1.8.15. 4-((4-((3-(4-Ethylpiperazin-1-yl)propyl)amino)phenyl)amino)-6-fluoroquinoline-3-carbohydrazide* (42):** Yellow solid (0.13 g, 56%);m.p: 298-300°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 10.02 (s, 1H), 9.28 (s, 1H), 8.67 (s, 1H), 7.91 (d, *J* = 8.6 Hz, 1H), 7.42 (d, *J* = 2.5 Hz, 1H), 7.31(dd, *J* = 8.8 Hz, 2.4 Hz, 1H), 6.61 (d, *J* = 8.2 Hz, 2H), 6.39 (d, *J* = 8.4 Hz, 2H), 5.52(brs, 1H), 3.55 (t, *J* = 6.8 Hz, 2H), 2.50-2.30 (m, 12H), 2.09 (s, 2H), 1.71 (m, 2H), 1.02 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 165.5, 160.6, 151.8, 146.0, 138.2, 135.1(2C), 130.3, 124.9(2C), 119.3(2C), 119.0(2C), 114.3, 104.9, 59.0(2C), 58.7(2C), 52.4, 50.2, 41.5, 27.8, 14.2.ESI-MS m/z (Calcd. for $C_{25}H_{32}FN_7O$: 465.27); Found: 466.42 $(M+H)^+$. Anal calcd.for $C_{25}H_{32}FN_7O$: C, 64.50; H, 6.93; N, 21.06; Found: C, 64.53; H, 6.95; N, 21.01.

***4.1.8.16. 4-((4-((3-(4-Ethylpiperazin-1-yl)propyl)amino)phenyl)amino)-6-(trifluoromethyl)quinoline-3-carbohydrazide* (43):** Yellow solid (0.138 g, 54%);m.p: 268-270°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 9.98 (s, 1H), 9.45 (s, 1H), 8.43 (s, 1H), 8.40 (d, *J* = 2.5 Hz, 1H), 8.21 (dd, *J* = 8.6 Hz, 2.5 Hz, 1H), 6.59 (d, *J* = 8.4 Hz, 2H), 6.44 (d, *J* = 8.2 Hz, 2H), 5.50 (brs, 1H), 3.52 (t, *J* = 6.8 Hz, 2H), 2.49-2.33 (m, 12H), 2.11 (s, 2H), 1.73 (m, 2H), 1.01 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 165.4, 153.2, 149.0, 139.5, 138.3, 134.9, 131.4, 128.7, 127.8, 125.3, 122.6, 120.8, 119.4(2C), 118.9(2C), 114.5, 59.1(2C), 58.4(2C), 52.6, 50.3, 41.6, 27.8, 14.0. ESI-MS m/z (Calcd. for $C_{26}H_{32}F_3N_7O$: 515.26); Found: 516.31 $(M+H)^+$. Anal calcd.for $C_{26}H_{32}F_3N_7O$: C, 60.57; H, 6.26; N, 19.02; Found: C, 60.54; H, 6.29; N, 19.04.

***4.1.9. General procedure for the preparation of titled phenyl-amino-quinoline acid derivatives* (44-57):** The corresponding ethyl ester derivatives (0.5 mmol) (**12-27**) were hydrolyzed by lithium hydroxide monohydrate (1.25 mmol) in 6mL of 1:1:1 ratio of methanol; tetrahydrofuran; distilled water as a solvent and acidified with 2N HCl by maintaingpH 5-6. The solid precipitated was filtered, dried and diethyl ether, ethyl acetate washings were given for further purification to get the corresponding acid derivatives in moderate yield.

***4.1.9.1. 4-((4-(3-Morpholinopropoxy)phenyl)amino)quinoline-3-carboxylic acid* (44):** Yellow gammy (0.11 g, 55%); ^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 11.51 (s, 1H), 9.86 (s, 1H),

9.34 (s,1H), 8.18 (dd, *J* = 8.6 Hz, *J* = 2.4 Hz, 1H), 7.83 (dd,*J* = 8.8 Hz, *J* = 2.5 Hz, 1H), 7.76-7.60 (m, 2H), 7.49 (d, *J* = 8.4 Hz, 2H), 6.98 (d, *J* = 8.2 Hz, 2H), 4.03 (t, *J* = 7.0 Hz, 2H), 3.66 (t,*J* = 6.8 Hz, 4H), 2.34 (s, 6H), 1.79 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 170.0, 153.2, 152.4, 149.8, 140.7, 132.5(2C), 129.3, 127.0, 126.2, 121.9(3C), 115.8(2C), 114.3, 73.6, 67.4(2C), 63.6(2C), 59.2, 28.5. ESI-MS m/z (Calcd. for $C_{23}H_{25}N_3O_4$: 407.18); Found: 408.25 $(M+H)^+$. Anal calcd.for $C_{23}H_{25}N_3O_4$: C, 67.80; H, 6.18; N, 10.31; Found: C, 67.76; H, 6.21; N, 10.33.

***4.1.9.2. 6-Methoxy-4-((4-(3-morpholinopropoxy)phenyl)amino)quinoline-3-carboxylic acid* (45):** Pale yellow solid (0.11 g, 53%);m.p: 239-241°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 11.65 (brs, 1H), 9.92 (s, 1H), 8.94 (s,1H), 7.84 (d, *J* = 8.6 Hz, 1H), 7.63 (d, *J* = 8.2 Hz, 2H), 7.43 (d, *J* = 2.5 Hz, 1H), 7.28 (dd, *J* = 8.4Hz, 2.5 Hz, 1H), 6.86 (d, *J* = 8.0 Hz, 2H), 4.01 (t, *J* = 7.1 Hz, 2H), 3.82 (s, 3H), 3.66 (t, *J* = 6.8 Hz, 4H), 2.36 (s, 6H), 1.80 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 169.9, 157.3, 151.8, 150.2(2C), 136.4, 132.7, 130.9, 125.0, 121.8(3C), 116.1(2C), 114.9, 103.5, 73.7, 67.4(2C), 63.5(2C), 59.2, 56.4, 28.6. ESI-MS m/z (Calcd. for $C_{24}H_{27}N_3O_5$: 437.20); Found: 436.11 $(M-H)^-$. Anal calcd.for $C_{24}H_{27}N_3O_5$: C, 65.89; H, 6.22; N, 9.60; Found: C, 65.87; H, 6.25; N, 9.61.

***4.1.9.3. 6-Fluoro-4-((4-(3-morpholinopropoxy)phenyl)amino)quinoline-3-carboxylic acid* (46):** Off-white solid (0.11 g, 56%);m.p: 199-201°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 11.69 (brs, 1H), 9.93 (s, 1H), 8.92 (s,1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.45 (d, *J* = 2.4 Hz, 1H), 7.36 (dd, *J* = 8.6 Hz, 2.5 Hz, 1H), 6.98 (d, *J* = 8.1 Hz, 2H), 4.02 (t, *J* = 6.9 Hz, 2H), 3.61 (t, *J* = 7.0 Hz, 4H), 2.35 (s, 6H), 1.81 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 170.1, 160.8, 152.4, 151.6, 149.9, 137.7, 133.0, 130.4, 124.1, 121.9(3C), 116.0(2C), 115.3, 104.5, 73.7, 67.3(2C), 63.6(2C), 59.1, 28.4. ESI-MS m/z (Calcd. for $C_{23}H_{24}FN_3O_4$: 425.18); Found: 426.35 $(M+H)^+$. Anal calcd.for $C_{23}H_{24}FN_3O_4$: C, 64.93; H, 5.69; N, 9.88; Found: C, 64.95; H, 5.66; N, 9.91.

***4.1.9.4. 4-((4-(3-Morpholinopropoxy)phenyl)amino)-6-(trifluoromethyl) quinoline-3-carboxylic acid* (47):** Pale green solid (0.144 g, 61%);m.p: 248-250°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 11.72 (brs, 1H), 9.91 (s, 1H), 8.87 (s,1H), 8.31 (d, *J* = 2.5 Hz, 1H), 8.24 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H), 8.18 (d, *J* = 8.6 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 6.96 (d, *J* = 8.0 Hz, 2H), 3.99 (t, *J* = 7.0 Hz, 2H), 3.60 (t, *J* = 6.8 Hz, 4H), 2.31 (s, 6H), 1.82 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 169.9, 154.6(2C), 149.8, 142.2, 132.7, 131.5, 129.0, 127.8,

125.3, 122.1(2C), 119.9(2C), 115.7(3C), 73.5, 67.2(2C), 63.6(2C), 59.1, 28.3. ESI-MS m/z (Calcd. for $C_{24}H_{24}F_3N_3O_4$: 475.17); Found: 476.38 $(M+H)^+$. Anal calcd.for $C_{24}H_{24}F_3N_3O_4$: C, 60.63; H, 5.09; N, 8.84; Found: C, 60.68; H, 5.06; N, 8.81.

***4.1.9.5. 4-((4-((3-Morpholinopropyl)amino)phenyl)amino)quinoline-3-carboxylic acid* (48):** Brown solid (0.118 g, 58%);m.p: 187-189°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 11.67 (brs, 1H), 9.94 (s, 1H), 8.87 (s, 1H), 8.21 (d, *J* = 2.5 Hz, 1H), 7.92 (d, *J* = d, *J* = 8.4 Hz, 1H), 7.81-7.61 (m, 2H), 6.71 (d, *J* = 8.2 Hz, 2H), 6.40 (d, *J* = 8.2 Hz, 2H), 5.51 (brs, 1H), 3.64 (t, *J* = 7.0 Hz, 4H), 3.34 (t, *J* = 6.8 Hz, 2H), 2.30 (s, 6H), 1.69 (m, 2H).^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 170.1, 153.3, 152.0, 140.9, 138.2, 134.8, 132.6, 129.3, 127.0, 125.9, 120.7, 119.2(2C), 118.6(2C), 114.2, 67.1(2C), 63.6(2C), 52.9, 41.6, 27.5. ESI-MS m/z (Calcd. for $C_{23}H_{26}N_4O_3$: 406.20); Found: 407.19 $(M+H)^+$. Anal calcd.for $C_{23}H_{26}N_4O_3$: C, 67.96; H, 6.45; N, 13.78; Found: C, 67.94; H, 6.47; N, 13.81.

***4.1.9.6. 6-Methoxy-4-((4-((3-morpholinopropyl)amino)phenyl)amino) quinoline-3-carboxylic acid* (49):** Brown solid (0.128 g, 59%);m.p: 199-201°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 11.52 (brs, 1H), 9.93 (s, 1H), 8.88 (s, 1H), 8.34 (m, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.54 (d, *J* = 2.5 Hz, 1H), 7.31 (d, *J* = 8.2 Hz, 2.4 Hz, 1H), 6.54 (d, *J* = 8.2 Hz, 2H), 6.40 (d, *J* = 8.0 Hz, 2H), 5.51 (brs, 1H), 3.84 (s, 3H), 3.64 (t, *J* = 6.8 Hz, 4H),3.45 (t, *J* = 7.0 Hz, 2H), 2.36 (s, 6H), 1.82 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 170.2, 157.5, 151.8, 150.2, 138.3, 136.7, 134.9, 131.0, 125.2, 121.8, 119.0(2C), 118.7(2C), 114.9, 103.6, 67.3(2C), 63.4(2C), 56.0, 52.9, 41.7, 27.5. ESI-MS m/z (Calcd. for $C_{24}H_{28}N_4O_4$: 436.21); Found: 437.17 $(M+H)^+$. Anal calcd.for $C_{24}H_{28}N_4O_4$: C, 66.04; H, 6.47; N, 12.84; Found: C, 66.07; H, 6.51; N, 12.81.

***4.1.9.7. 6-Fluoro-4-((4-((3-morpholinopropyl)amino)phenyl)amino)quinoline-3-carboxylic acid* (50):** Red solid (0.118 g, 56%);m.p: 216-218°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 11.71 (brs, 1H), 9.97 (s, 1H), 8.82 (s, 1H), 7.98 (d, *J* = 8.0 Hz, 1H), 7.52 (d, *J* = 2.5 Hz, 1H), 7.34 (dd, *J* = 8.2 Hz, 2.5 Hz, 1H), 6.56 (d, *J* = 8.0 Hz, 2H), 6.40 (d, *J* = 8.2 Hz, 2H), 5.52 (brs, 1H), 3.63 (t, *J* = 7.0 Hz, 4H), 3.45 (t, *J* = 6.8 Hz, 2H), 2.34 (s, 6H), 1.81 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 169.9, 160.7, 152.5(2C), 138.3(2C), 134.9, 130.1, 124.3, 121.8, 119.5(2C), 119.1(2C), 115.4, 104.2, 67.5(2C), 63.4(2C), 52.9, 41.5, 27.7. ESI-MS m/z (Calcd. for $C_{23}H_{25}FN_4O_3$: 424.19); Found: 425.15 $(M+H)^+$. Anal calcd.for $C_{23}H_{25}FN_4O_3$: C, 65.08; H, 5.94; N, 13.20; Found: C, 65.09; H, 5.91; N, 13.22.

***4.1.9.8. 4-((4-((3-Morpholinopropyl)amino)phenyl)amino)-6-(trifluoromethyl) quinoline-3-carboxylic acid* (51):** Brown solid (0.141 g, 60%);m.p: 223-224°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 11.89 (brs, 1H), 9.98 (s, 1H), 8.80 (s, 1H), 8.29 (d, *J* = 2.5 Hz, 1H), 8.20-8.16 (m, 2H), 6.57 (d, *J* = 8.2 Hz, 2H), 6.38 (d, *J* = 8.0 Hz, 2H), 5.53 (brs, 1H), 3.64 (t, *J* = 7.0 Hz, 4H), 3.44 (t, *J* = 6.7 Hz, 2H), 2.33 (s, 6H), 1.82 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 169.8, 155.2, 154.0, 142.5, 138.3, 134.9, 131.6, 128.8, 127.9, 125.1, 120.3(2C), 119.0(2C), 118.7(2C), 115.8, 67.5(2C), 63.6(2C), 52.7, 41.4, 27.6. ESI-MS m/z (Calcd. for $C_{24}H_{25}F_3N_4O_3$: 474.19); Found: 475.13$(M+H)^+$. Anal calcd.for $C_{24}H_{25}F_3N_4O_3$: C, 60.75; H, 5.31; N, 11.81; Found: C, 60.71; H, 5.28; N, 11.76.

***4.1.9.9. 4-((4-(3-(4-Ethylpiperazin-1-yl)propoxy)phenyl)amino)quinoline-3-carboxylic acid* (52):** Brown gammy (0.127 g, 59%); ^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 11.79 (brs, 1H), 9.86 (s, 1H), 8.85 (s, 1H), 8.20 (d, *J* = 7.8 Hz, 1H), 7.98 (d, *J* = 8.0 Hz, 1H), 7.80-7.63 (m, 2H), 7.59 (d, *J* = 8.0 Hz, 2H), 6.92 (d, *J* = 8.2 Hz, 2H), 4.01 (t, *J* = 7.0 Hz, 2H), 2.48-2.30 (m, 12H), 1.83 (m, 2H), 1.05 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 170.0, 152.9, 152.3, 150.1, 140.8, 132.9(2C), 128.9, 127.4, 126.2, 121.7(2C), 120.6, 115.8(2C), 114.3, 73.6, 59.2(3C), 58.6(2C), 50.2, 28.3, 14.1. ESI-MS m/z (Calcd. for $C_{25}H_{30}N_4O_3$: 434.23); Found: 433.16 $(M-H)^-$. Anal calcd.for $C_{25}H_{30}N_4O_3$: C, 69.10; H, 6.96; N, 12.89; Found: C, 69.14; H, 6.92; N, 12.91.

***4.1.9.10. 4-((4-(3-(4-Ethylpiperazin-1-yl)propoxy)phenyl)amino)-6-methoxyquinoline-3-carboxylic acid* (53):** Pale green solid (0.131 g, 57%);m.p: 282-284°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 11.72 (brs, 1H), 9.96 (s, 1H), 8.87 (s, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.52 (d, *J* = 2.5 Hz, 1H), 7.31 (dd, *J* = 8.1 Hz, 2.5 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 2H), 4.03 (t, *J* = 7.0 Hz, 2H), 3.55 (s, 3H), 2.47-2.30 (m, 12H), 1.82 (m, 2H), 1.04 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 169.9, 157.3, 151.7, 150.2(2C), 136.4, 132.8, 131.1, 125.0, 121.9(3C), 115.7(2C), 114.9, 103.5, 73.7, 59.3(3C), 58.3(2C), 56.5, 50.1, 28.3, 14.1. ESI-MS m/z (Calcd. for $C_{26}H_{32}N_4O_4$: 464.24); Found: 465.28 $(M+H)^+$. Anal calcd.for C, 67.22; H, 6.94; N, 12.06; Found: C, 67.25; H, 6.91; N, 12.09.

***4.1.9.11. 4-((4-(3-(4-Ethylpiperazin-1-yl)propoxy)phenyl)amino)-6-fluoroquinoline-3-carboxylic acid* (54):** Yellow gammy (0.137 g, 61%); ^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 11.54 (brs, 1H), 9.95 (s, 1H), 8.86 (s, 1H), 7.94 (d, *J* = 8.1 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.46 (s, 1H), 7.31 (dd, *J* = 8.2 Hz, 2.4 Hz, 1H), 6.68 (d, *J* = 8.2 Hz, 2H), 3.99 (t, *J* = 7.0 Hz, 2H),

2.47-2.30 (m, 12H), 1.82 (m, 2H), 1.02 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 170.1, 160.7, 152.4, 151.5, 149.9, 137.6, 132.8, 130.3, 124.1, 122.0(3C), 115.9(3C), 104.5, 73.7, 59.2(3C), 58.4(2C), 50.1, 28.5, 14.0. ESI-MS m/z (Calcd. for $C_{25}H_{29}FN_4O_3$: 452.22); Found: 453.38 $(M+H)^+$. Anal calcd.for $C_{25}H_{29}FN_4O_3$: C, 66.35; H, 6.46; N, 12.38; Found: C, 66.33; H, 6.42; N, 12.41.

***4.1.9.12. 4-((4-(3-(4-Ethylpiperazin-1-yl)propoxy)phenyl)amino)-6-(trifluoromethyl)quinoline-3-carboxylic acid* (55):** Yellow solid (0.14 g, 56%);m.p: 153-155°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 11.56 (brs, 1H), 9.97 (s, 1H), 8.88 (s, 1H), 8.36 (s, 1H), 8.21 (dd, *J* = 8.0 Hz, 2.5 Hz, 1H), 8.13 (d, *J* = 8.2 Hz, 1H), 7.82 (d, *J* = 8.6 Hz, 2H), 6.95 (d, *J* = 8.4 Hz, 2H), 3.98 (t, *J* = 7.0 Hz, 2H), 2.46-2.34 (m, 12H), 1.82 (m, 2H), 1.03 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 169.9, 154.7(2C), 150.1, 142.3, 132.9, 131.6, 129.2, 128.0, 125.2, 121.8(2C), 120.5(2C), 115.9(3C), 73.6, 59.1(3C), 58.3(2C), 50.0, 28.4, 14.1. ESI-MS m/z (Calcd. for $C_{26}H_{29}F_3N_4O_3$: 502.22); Found: 503.36 $(M+H)^+$. Anal calcd.for $C_{26}H_{29}F_3N_4O_3$: C, 62.14; H, 5.82; N, 11.15; Found: C, 62.11; H, 5.85; N, 11.12.

***4.1.9.13. 4-((4-((3-(4-Ethylpiperazin-1-yl)propyl)amino)phenyl)amino) quinoline-3-carboxylic acid* (56):** Brown solid (0.127 g, 59%);m.p: 188-190°C;^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 11.72 (brs, 1H), 9.96 (s, 1H), 8.86 (s, 1H), 8.13-7.61 (m, 4H), 6.58 (d, *J* = 8.2 Hz, 2H), 6.34 (d, *J* = 8.0 Hz, 2H), 5.52 (brs, 1H), 3.51 (t, *J* = 7.0 Hz, 2H), 2.46-2.31 (m, 12H), 1.83 (m, 2H), 1.04 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 169.8, 153.0, 152.5, 140.9, 138.2, 134.8, 132.6, 129.3, 127.1, 126.2, 120.9, 119.4(2C), 118.9(2C), 114.3, 59.0(2C), 58.5(2C), 52.7, 50.2, 41.8, 27.6, 13.9. ESI-MS m/z (Calcd. for $C_{25}H_{31}N_5O_2$: 433.25); Found: 432.09 $(M-H)^-$. Anal calcd.for $C_{25}H_{31}N_5O_2$: C, 69.26; H, 7.21; N, 16.15; Found: C, 69.21; H, 7.23; N, 16.18.

***4.1.9.14. 4-((4-((3-(4-Ethylpiperazin-1-yl)propyl)amino)phenyl)amino)-6-methoxyquinoline-3-carboxylic acid* (57):** Yellow gummy (0.138 g, 60%); ^{1}H NMR (300 MHz, DMSO-d_6, TMS):δ = 11.58 (brs, 1H), 9.98 (s, 1H), 8.89 (s, 1H), 7.79 (d, *J* = 8.4 Hz, 1H), 7.51 (s, 1H), 7.34 (dd, *J* = 7.8 Hz, 2.4 Hz, 1H), 6.57 (d, *J* = 8.2 Hz, 2H),6.46 (d, *J* = 8.0 Hz, 2H), 5.51 (s, 1H), 3.86 (s, 3H), 3.36 (t, *J* = 7.0 Hz, 2H), 2.48-2.29 (m, 12H), 1.83 (m, 2H), 1.04 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ = 170.0, 157.3, 151.9, 150.2, 138.4, 136.7, 134.8, 131.0, 124.9, 122.1, 119.2(2C), 118.7(2C), 115.2, 103.4, 59.2(2C), 58.5(2C), 56.3, 52.4, 50.1, 41.9, 27.7, 14.1. ESI-MS m/z (Calcd. For $C_{26}H_{33}N_5O_3$: 463.26); Found: 464.35 $(M+H)^+$. Anal calcd.for $C_{26}H_{33}N_5O_3$: C, 67.36; H, 7.18; N, 15.11; Found: C, 67.32; H, 7.21; N, 15.07.

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FIGURES LEGENDS

Figure 1: Interaction profile of aminopyrazinamide compound at the Msm GyrB ATPase domain (PDB ID – 4B6C).

Figure 2: Process of lead optimization with substituents at the respective positions

Figure 3: ATPase activity of *Mycobacterium smegmatis* DNA GyrB protein as a function of substrate (ATP) concentration at a constant enzyme concentration.

Figure 4: Percentage inhibition of Msm GyrB by compound **53** at various concentrations

Figure 5: DNA supercoiling assay picture of compound **53** at five different concentrations of 6 µM, 3 µM, 1.5 µM, 0.8 µM and 0.4 µM where B – relaxed DNA substrate + DMSO, C – relaxed DNA substrate with DNA gyrase enzyme + DMSO.

Figure 6: Interaction profile of **(a)** aminopyrazinamide derivative (PDB – 4B6C) (purple sticks) at the ATPase domain of *M. smegmatis* GyrB with residues involved in hydrogen bond and hydrophobic interactions in green and yellow respectively **(b)** compound **53** (pink sticks) at the ATPase domain of Msm GyrB with residues involved in hydrogen bonds and hydrophobic interactions in cyan and yellow respectively. Blue dashed lines indicate hydrogen bonds.

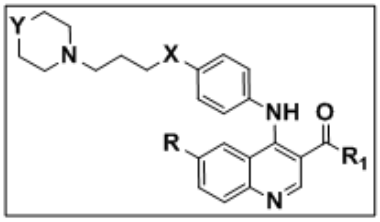
Figure 7: Interaction pattern of **(a)** compound **23** and **(b)** compound **28** at Msm GyrB ATPase domain.

Figure 8: Differential scanning fluorimetry curve for GyrB protein complexed with compound **53** (red) and native GyrB protein (green). A positive shift in T_m of about 3.3 ^oC was observed in protein-ligand complex when compared to native protein.

Scheme 1. Synthetic protocol for the amine intermediate compounds **4a-b** and **7a-b**.

Scheme 2. Synthetic protocol for the title compounds.

Table 1.*In vitro* biological activity results for the synthesized compounds **12-57**.



Compd.	R	R_1	X	Y	MsmGyr B assay[a] (IC_{50} in µM)	MTB Supercoiling assay[b](IC_{50} µM)	Mtb MIC[c] (µM)	Cytotoxicity at 50 µM[d] (% inhibition)
12	H	OC_2H_5	O	O	22.83±0.38	7.53±0.16	28.70	17.67
13	OCH_3	OC_2H_5	O	O	14.92±0.46	13.32±0.11	13.35	21.23
14	F	OC_2H_5	O	O	16.87±0.31	7.63±0.18	21.72	44.06
15	CF_3	OC_2H_5	O	O	38.84±0.66	16.42±0.21	43.10	20.21
16	H	OC_2H_5	NH	O	17.92±0.29	8.73±0.13	14.38	1.82
17	OCH_3	OC_2H_5	NH	O	22.31±0.31	9.32±0.15	23.36	19.33
18	F	OC_2H_5	NH	O	11.34±0.51	4.61±0.19	13.45	19.61
19	CF_3	OC_2H_5	NH	O	6.62±0.22	3.12±0.21	49.75	20.22
20	H	OC_2H_5	O	NC_2H_5	>50	23.92±0.31	13.51	21.41
21	OCH_3	OC_2H_5	O	NC_2H_5	15.92±0.61	6.85±0.31	11.58	21.55
22	F	OC_2H_5	O	NC_2H_5	23.61±0.38	11.83±0.51	3.25	21.61
23	CF_3	OC_2H_5	O	NC_2H_5	0.97±0.18	0.69±0.15	2.94	27.30
24	H	OC_2H_5	NH	NC_2H_5	11.72±0.62	3.25±0.16	6.77	20.4
25	OCH_3	OC_2H_5	NH	NC_2H_5	9.55±0.44	8.52±0.18	25.43	21.41
26	F	OC_2H_5	NH	NC_2H_5	16.92±0.39	7.99±0.16	13.03	20.22
27	CF_3	OC_2H_5	NH	NC_2H_5	8.82±0.64	3.77±0.11	1.47	20.15
28	H	$NHNH_2$	O	O	0.97±0.25	0.72±0.15	2.4	44.56
29	OCH_3	$NHNH_2$	O	O	10.58±0.34	10.41±0.42	55.37	6.26
30	F	$NHNH_2$	O	O	20.88±0.65	21.42±0.31	28.44	13.72
31	CF_3	$NHNH_2$	O	O	38.66±0.60	19.93±0.29	36.38	0.07
32	H	$NHNH_2$	NH	O	>50	22.87±0.51	44.86	6.48
33	OCH_3	$NHNH_2$	NH	O	44.93±0.34	15.32±0.22	27.75	9.59
34	F	$NHNH_2$	NH	O	18.67±0.64	10.55±0.21	23.56	29.08
35	CF_3	$NHNH_2$	NH	O	47.25±0.26	19.88±0.35	25.59	7.94
36	H	$NHNH_2$	O	NC_2H_5	28.44±0.61	16.66±0.38	13.93	18.24
37	OCH_3	$NHNH_2$	O	NC_2H_5	12.63±0.31	5.89±0.25	11.63	2.72
38	F	$NHNH_2$	O	NC_2H_5	2.92±0.16	2.96±0.21	3.34	11.95
39	CF_3	$NHNH_2$	O	NC_2H_5	11.88±0.52	9.33±0.16	11.51	20.14
40	H	$NHNH_2$	NH	NC_2H_5	10.83±0.23	9.42±0.18	13.49	3.36
41	OCH_3	$NHNH_2$	NH	NC_2H_5	3.26±0.54	2.55±0.19	13.09	2.56
42	F	$NHNH_2$	NH	NC_2H_5	1.15±0.16	0.82±0.15	3.3	6.11
43	CF_3	$NHNH_2$	NH	NC_2H_5	26.34±0.63	10.95±0.22	30.03	1.59
44	H	OH	O	O	>50	21.82±0.29	30.68	11.28
45	OCH_3	OH	O	O	20.56±0.54	12.9±0.31	57.14	11.42
46	F	OH	O	O	31.28±0.48	16.93±0.54	14.69	10.85
47	CF_3	OH	O	O	27.83±0.41	14.33±0.31	13.15	22.57
48	H	OH	NH	O	11.33±0.61	5.56±0.61	7.69	0.55
49	OCH_3	OH	NH	O	38.92±0.71	12.55±0.55	28.64	16.07
50	F	OH	NH	O	14.98±0.29	4.44±0.23	29.46	13.12

51	CF_3	OH	NH	O	7.89±0.31	3.77±0.15	6.59	19.06
52	H	OH	O	NC_2H_5	21.66±0.51	10.22±0.18	27.19	13.37
53	OCH_3	OH	O	NC_2H_5	0.86±0.16	0.63±0.15	3.3	0.66
54	F	OH	O	NC_2H_5	6.82±0.32	5.96±0.22	6.91	24.16
55	CF_3	OH	O	NC_2H_5	7.91±0.31	3.54±0.26	19.75	13.37
56	H	OH	NH	NC_2H_5	11.32±0.52	5.88±0.24	11.80	32.28
57	OCH_3	OH	NH	NC_2H_5	1.32±0.21	0.93±0.21	3.48	20.10
Novobiocin					180±3.9 nM	46±10 nM	>200	nd
Moxifloxacin					>50	11.2±0.36	1.26	nd
Isoniazid					nd	nd	0.66	nd
Rifampicin					nd	nd	0.23	nd

IC_{50} – 50% inhibitory concentration; MIC – minimum inhibitory concentration; Mtb – *Mycobacterium tuberculosis*; nd – not determined.

[a]MsmgyrB ATPase activity.

[b]Mtb DNA gyrase supercoiling activity.

[c] MIC of the compounds on Mtb H37Rv.

[d] Cytotoxicity of compounds at 50 µM against RAW 264.7 cell line.

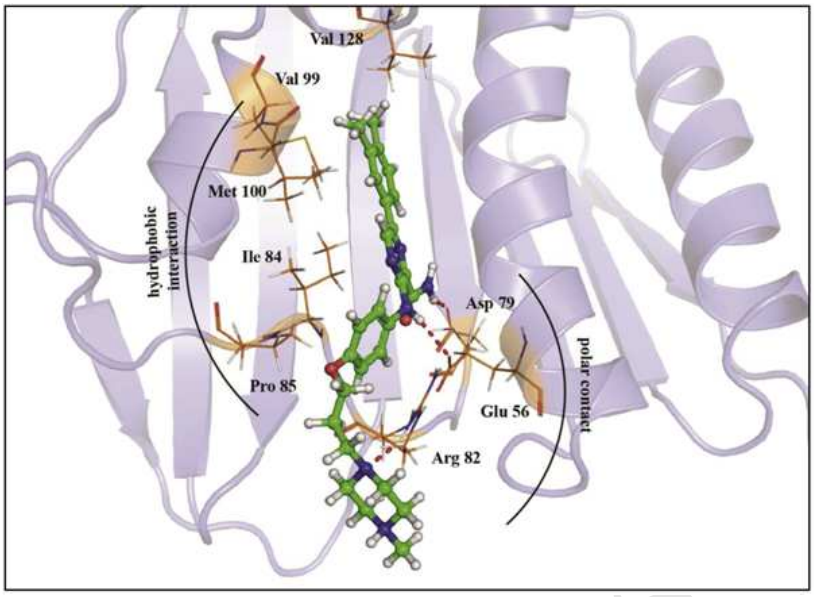


Figure 1: Interaction profile of aminopyrazinamide compound at the MsmGyrB ATPase domain (PDB ID – 4B6C).

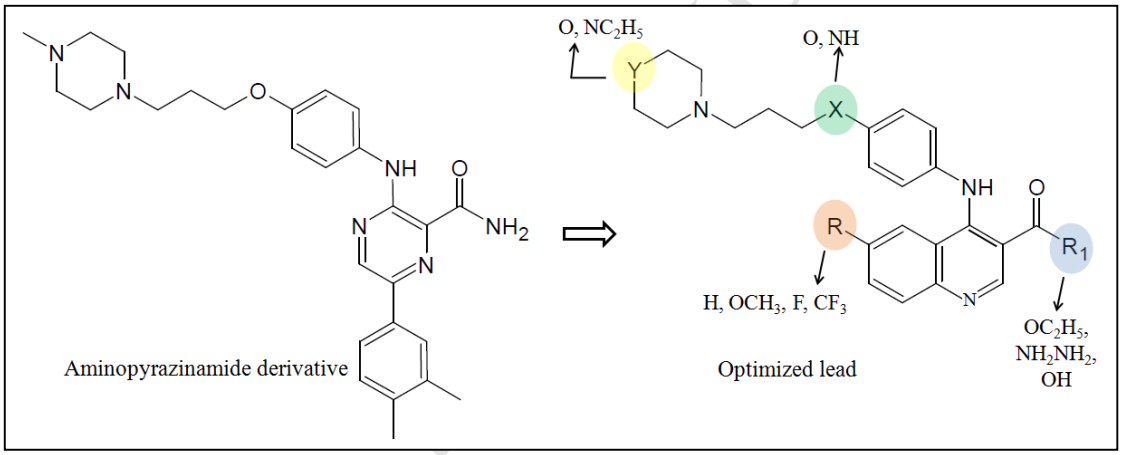


Figure 2: Process of lead optimization with substituents at the respective positions

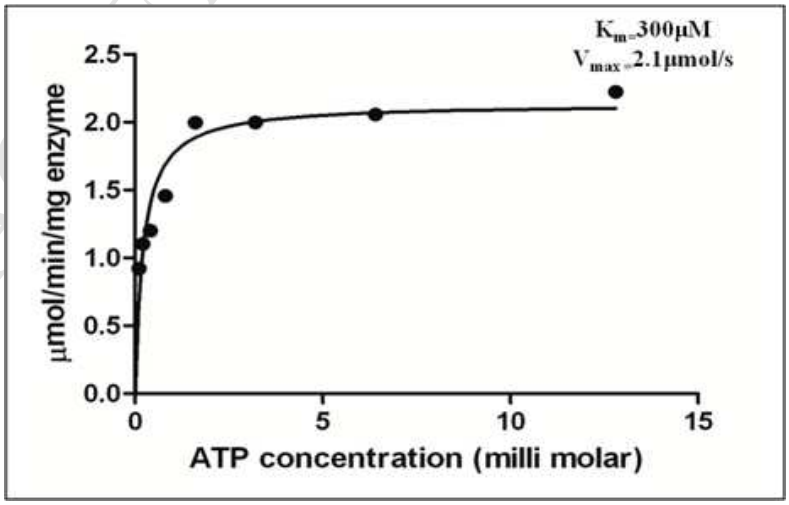


Figure 3: ATPase activity of *Mycobacterium smegmatis* DNA GyrB protein as a function of substrate (ATP) concentration at a constant enzyme concentration.

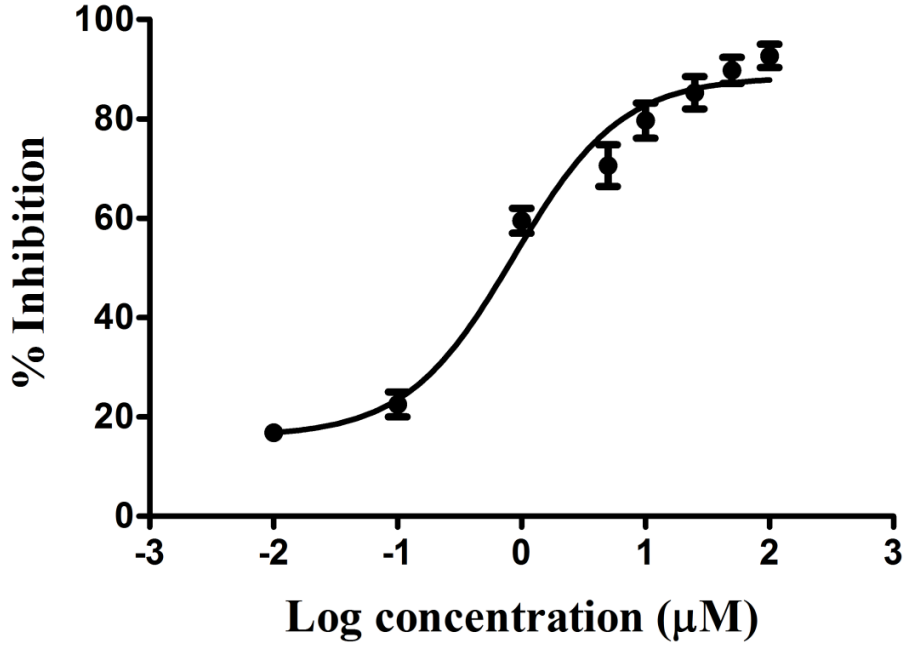


Figure 4: Percentage inhibition of Msm GyrB by compound **53** at various concentrations

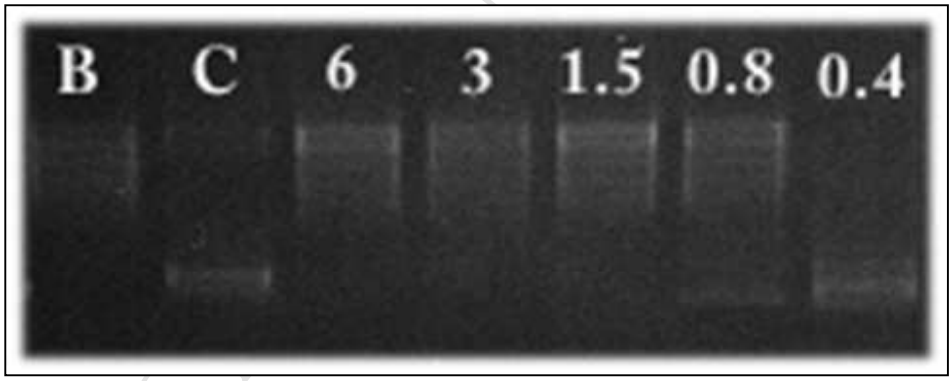


Figure 5: DNA supercoiling assay picture of compound **53** at five different concentrations of 6 µM, 3 µM, 15 µM, 0.8 µM and 0.4 µM where B – relaxed DNA substrate + DMSO, C-relaxed DNA substrate with DNA gyrase enzyme + DMSO.

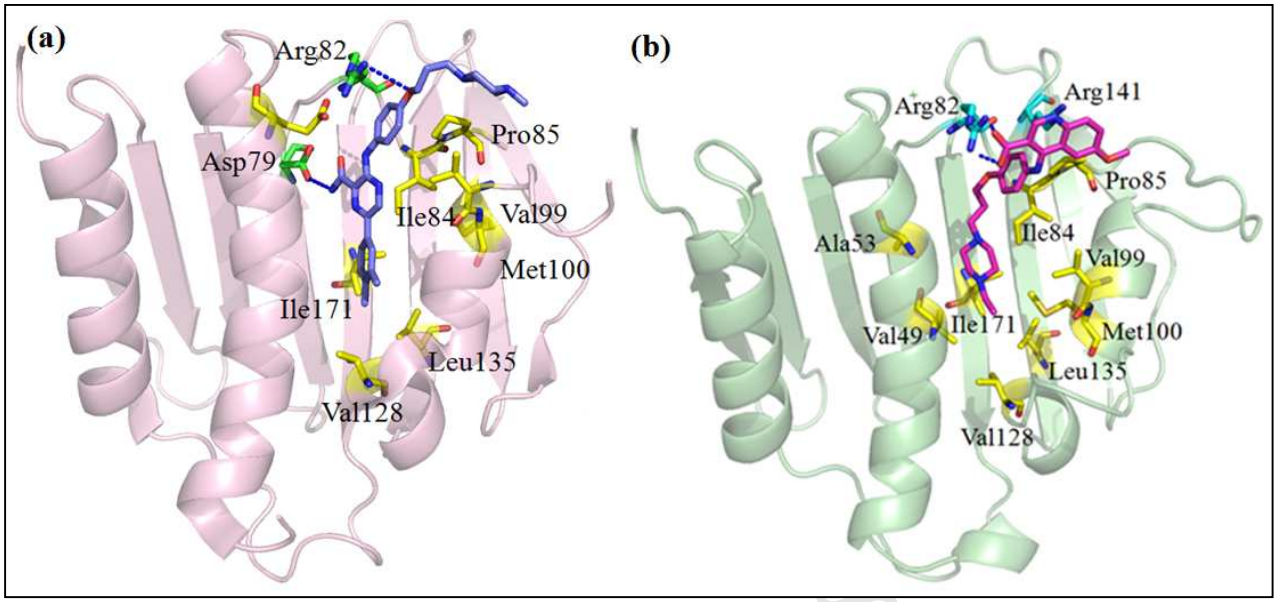


Figure 6: Interaction profile of **(a)** aminopyrazinamide derivative (PDB – 4B6C) (purple sticks) at the ATPase domain of *M. smegmatis* GyrB with residues involved in hydrogen bond and hydrophobic interactions in green and yellow respectively. **(b)** compound **53** (pink sticks) at the ATPase domain of Msm GyrB with residues involved in hydrogen bonds and hydrophobic interactions in cyan and yellow respectively. Blue dashed lines indicate hydrogen bonds.

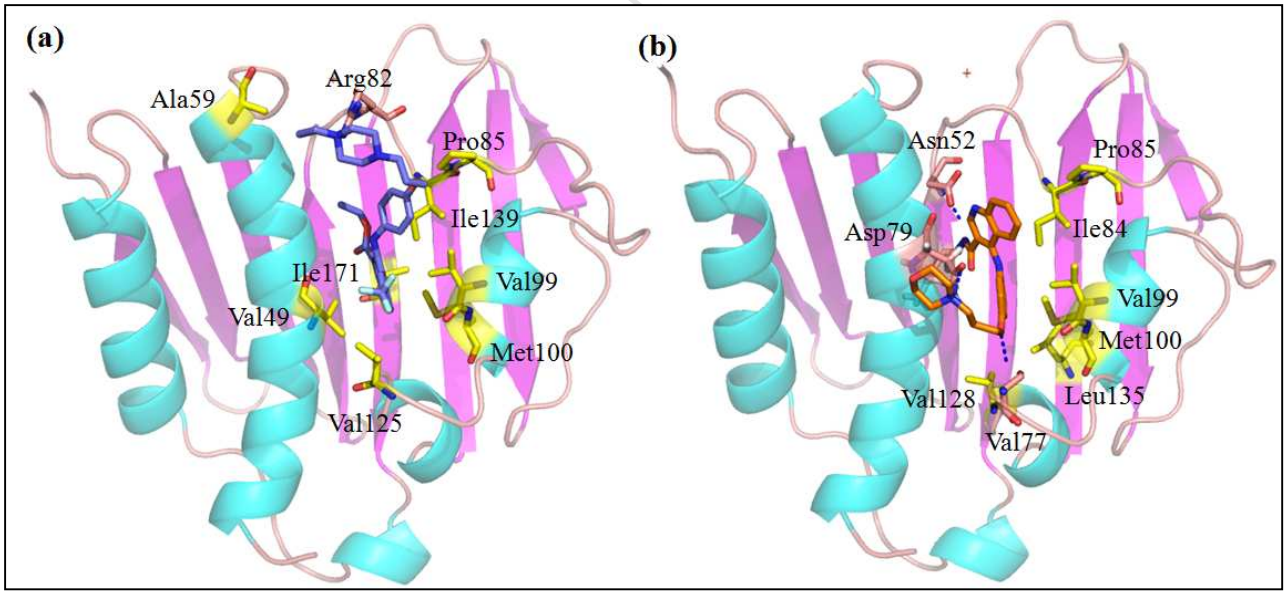


Figure 7: Interaction pattern of **(a)** compound **23** and **(b)** compound **28** at Msm GyrB ATPase domain.

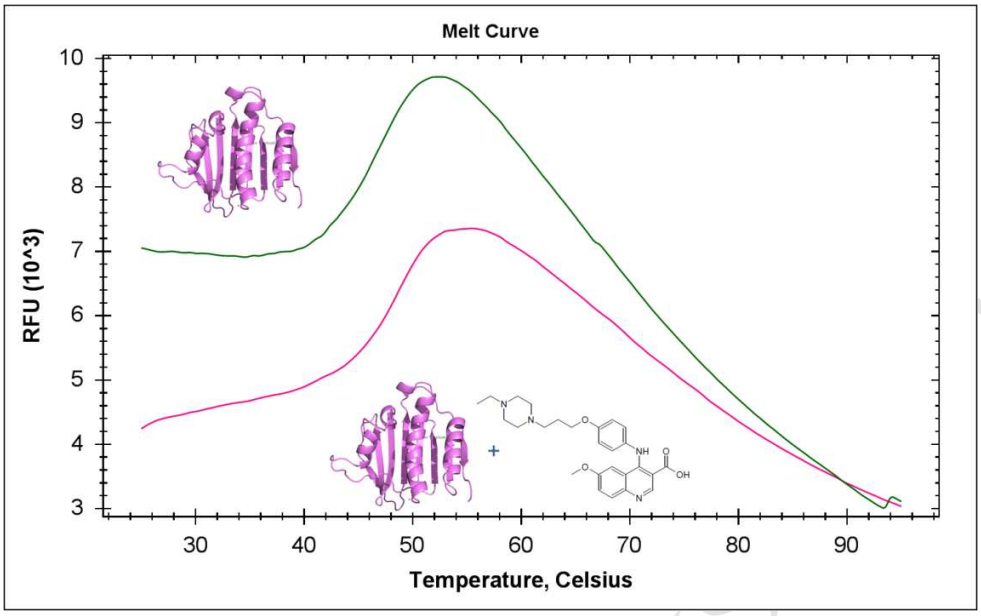
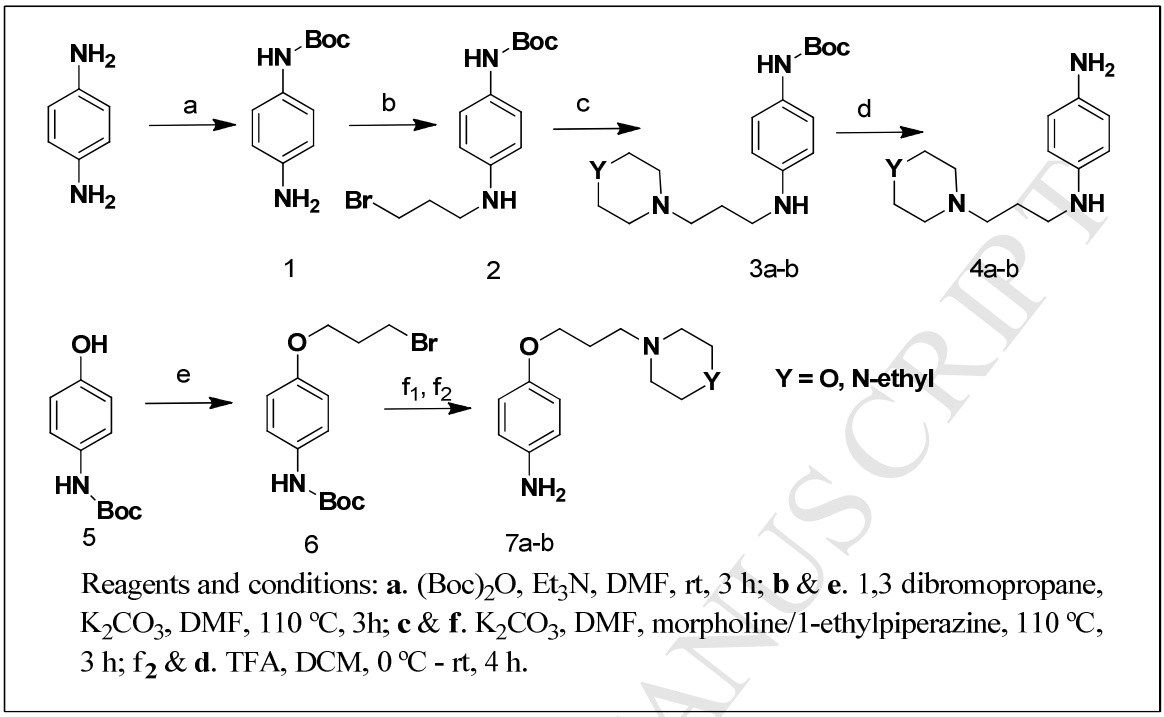
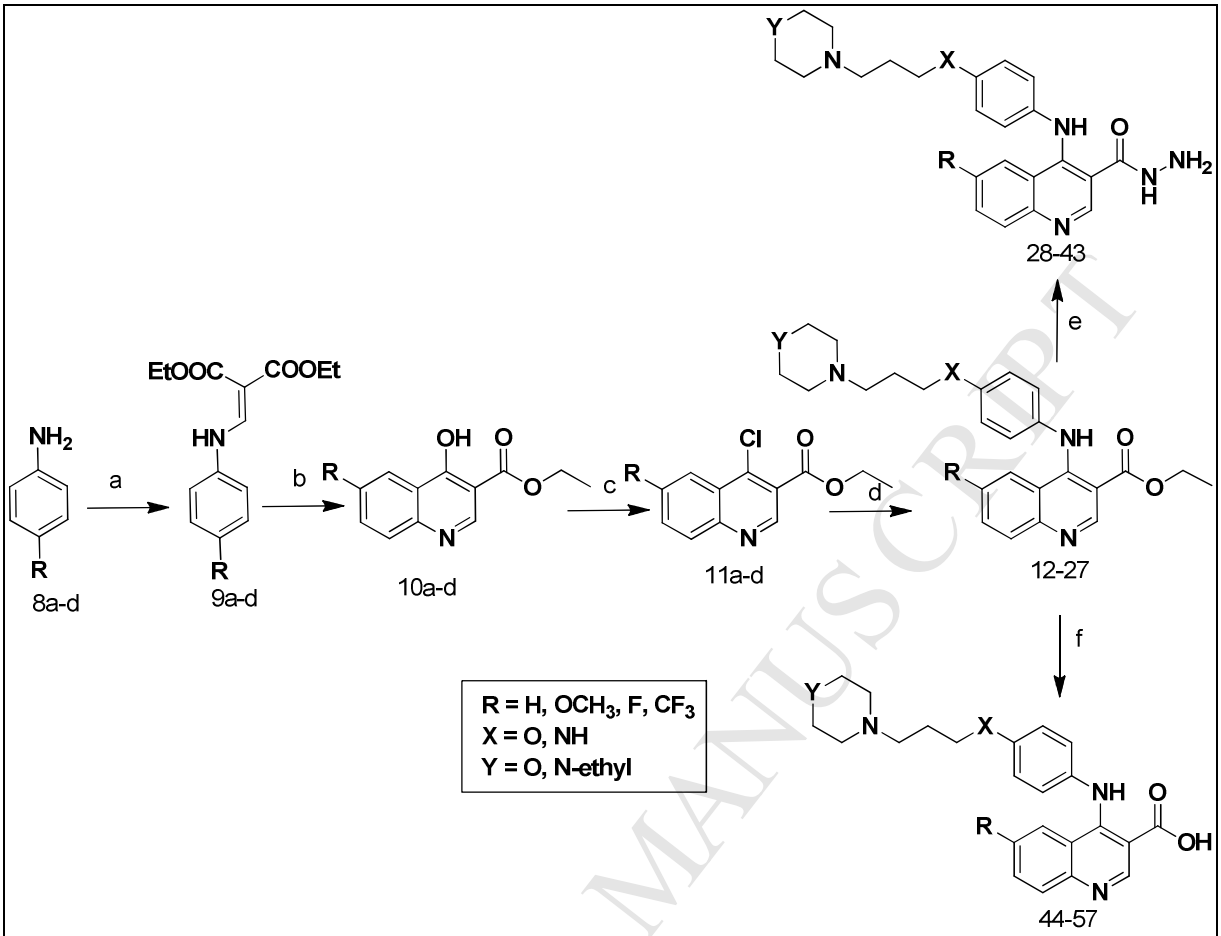


Figure 8: Differential scanning fluorimetry curve for GyrB protein complexed with compound **53** (red) and native GyrB protein (green). A positive shift in T_m of about 3.3 ^oC was observed in protein-ligand complex when compared to native protein.



Scheme 1. Synthetic protocol for the amine intermediate compounds **4a-b** and **7a-b**.



Reagents and conditions: **a**. Substituted aniline, diethyl 2-(ethoxymethylene)malonate, EtOH, 110 ° C, 4h; **b**. PPA, $POCl_3$, 75 ºC, 12 h; **c**. $POCl_3$, 90 ºC, 3 h; **d**. PTSA (catalytic), MeOH, MW at 120 ºC, 1 h; **e**. NH_2-NH_2.H_2O, EtOH, 85 ºC, 4 h; **f**. LiOH.H_2O, 1:1 ratio of MeOH and water, rt, 3 h.

Scheme 2. Synthetic protocol for the title compounds

Highlights

- We designed novel leads for MTB DNA gyrase inhibition
- We synthesized forty six novel quinolone derivatives
- The compounds were screened for GyrB, DNA gyrase supercoiling, MTB MIC
- One compound (**53**) showed GyrB IC_{50} of 0.86±0.16 µM.

Supporting Information

4-Aminoquinoline Derivatives as Novel *Mycobacterium tuberculosis* GyrB Inhibitors: Structural Optimization, Synthesis and Biological Evaluation

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Figures

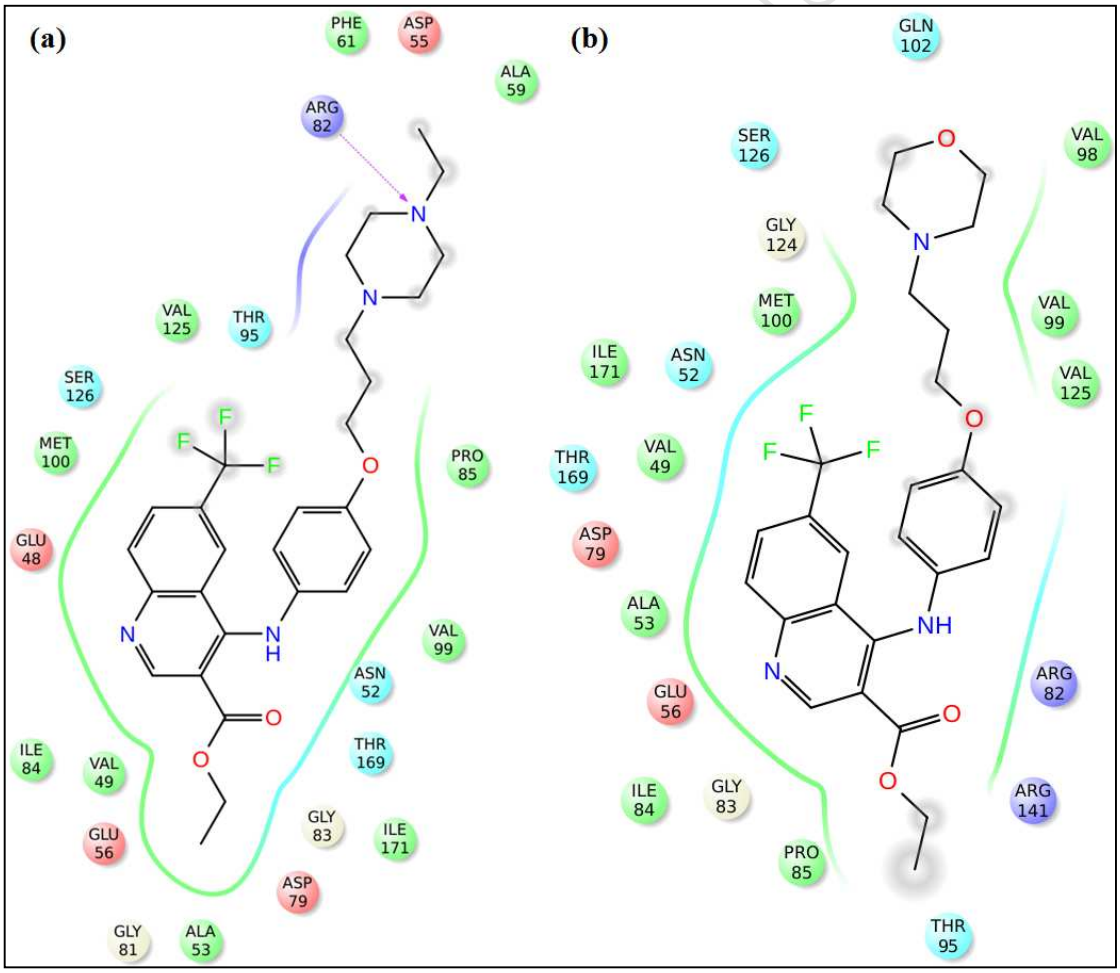

Figure S1: Interaction profile of (a) compound **23** and (b) compound **15** at *M. smegmatis* GyrB ATPase domain active site.

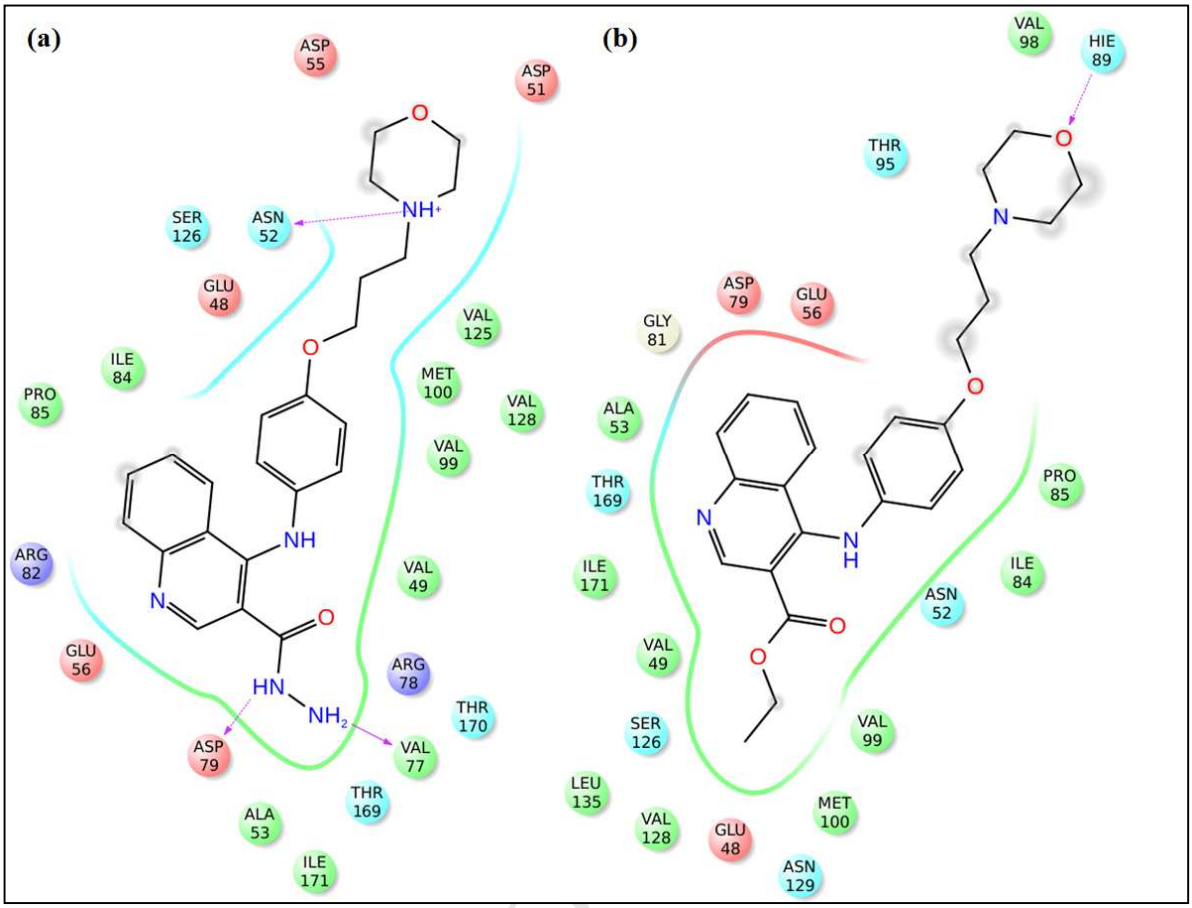

Figure S2: Interaction profile of (a) compound **28** and (b) compound **12** at *M. smegmatis* GyrB ATPase domain active site.

1. Experimental section

1.1. Cloning and purification of Msm GyrB

Cloning of *Mycobacterium smegmatis* GyrB was done by amplifying the gene from mc^2155 strains genomic DNA using the specific forward and reverse primers 5' CACCCATATGGTGGCTGCCCAGAAGAACAA 3' (NdeI), and 5' AGCTAAGCTTTTAAACATCCAGGAAGCGAA 3' (Hind III) respectively. The final PCR amplicons were cloned in expression vector pQE2 (Qiagen) with a 6-His-tag and were then transformed into *BL21 (DE3) pLysS* cells, as the compatibility of the host system for GyrB

protein expression and protein folding was high. The vector transformed cells were later grown at 37 °C in LB broth containing 100 µg/mL ampicillin to an optical density (OD) of 0.5 absorbance read at 595 nm (A_{595}). They were induced with isopropyl-β-D-thiogalactopyranoside (IPTG) at a final concentration of 0.1 mM while the cells were in exponential growth phase and the cell growth was further continued for another 12h at 18 ^{o}C as low temperatures promote the slow release of the protein. The bacterial cells were then centrifuged at 10,000 rpm (rotations per minute), 4 °C for 15 min. The cell pellets were re-suspended in PBSG buffer (PBS containing 5% glycerol), further lysed using sonicator (20 sec pulse and 45 sec halt) and centrifuged the crude lysate at 8000 rpm, 4 °C for 10 min. Subsequently, centrifugation was repeated for the supernatant of previous step at 10,000 rpm at 4 °C for 35 min for a clear supernatant. The cell extract was later transferred to a pre-equilibrated Ni-NTA column (Bio-Rad) and was washed with wash buffer (5% glycerol in PBS and 500 mM NaCl).The protein was eluted using different concentrations of imidazole ranging from 10 mM to 200 mM using elution buffer (5% Glycerol, 140 mM NaCl in 25 mM Tris-Cl (pH 8.0)). Fractions containing desired GyrB subunit were identified by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), subsequently pooled the 100 mM and 200 mM fractions and dialyzed against the dialyses buffer (15% Glycerol, 140 mMNaCl in 25 mMTris-HCl (pH 7.4)),aliquoted and stored at -80°C.

1.2. *In vitro* Msm ATPase assay

As the GyrB protein is the catalytic domain, it is involved in the hydrolysis of ATP resulting in energy generation. This ATPase assay was performed similar to the previously reported protocol[1]. The assay was carried out in 30 µL reaction volume for 100 min at 25 °C in reaction buffer containing 60 mM HEPES-KOH (pH 7.7), 250 mM potassium glutamate, 200 mMKCl, 2 mM $MgCl_2$, 1 mM DTT, 2% Glycerol, 4% DMSO, 0.001% BriJ-35, 0.65 mM ATP, 40 nM

GyrB. The assay was performed in 96 well (Polystyrene untreated) flat-bottomed plates. Drug concentrations of the compounds were placed in the assay well, followed by 6 µL of 5X assay buffer mixed with substrate along with 1 µL of enzyme later were added and incubated. The sequential addition of the above was of importance for the binding and interaction. Consequently, the enzyme reaction was initiated by adding 14 µL of $MgCl_2$ solution. The reaction was allowed to proceed for 100 min at room temperature. Further, the reaction was quenched by adding 20 µL malachite green reagent (Bioassay systems). Inorganic phosphates (Pi) released during the reaction were measured at 620 nm after 20 min incubation. Novobiocin was used as a standard compound.

1.3. *In vitro*Mtb DNA supercoiling assay

Supercoiling assay involves bothGyrA and GyrB subunits. The holoenzyme performs the function of supercoiling the DNA. The assay was performed using Mtb DNA supercoiling assay kits (Inspiralis Ltd., Norwich, UK). Briefly, the assay was performed in a 30 µL reaction volume for 30 min at 37 ^{0}C in an assay buffer containing 50 mM HEPES–KOH (pH 7.9), 6 mM magnesium acetate, 4 mMdithiothreitol (DTT), 1 mM ATP, 100 mM potassium glutamate, 2 mMspermidine and 0.05 mg/mL albumin. During the assay, 1U of MtbDNAgyrase was incubated with 0.5 µg of relaxed pBR322 in the assay buffer for 30 min. various concentrations of the compounds were diluted and incubated along with the reactants. The incubation time was optimized based on the interaction and activity of the protein with the double stranded DNA. Later, the reaction was quenched by addition of an equal volume of 30 µL of chloroform:isoamyl alcohol (24:1) and STEB buffer [sucrose–Tris–HCl–ethylene diamine tetra-acetic acid (EDTA)–bromophenol blue] with a brief vortex followed by centrifugation. Products were analyzed by electrophoresis on 1% agarose gels after staining with ethidium bromide. The intensity of bands

was measured and analyzed to determine enzyme inhibition by relative band intensity comparing with the control using Image Lab TM software (Bio-Rad). In this assay too,novobiocin was set as a standard.

1.4. *In vitro*Mtbmicroplatealamarblue assay (MABA)

The synthesized compounds were further tested for their *in vitro*antimycobacterial activity against drug sensitive Mtb H37Rv strain using microplatealamar blue assay (MABA)[2]. Briefly explaining the protocol, the Mtb inoculum was prepared from fresh LJ medium, resuspended in 7H9 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 to 1:20; from which 100 mL was used as inoculum. Each compound stock solutionwas thawed and diluted using 7H9-S at four-fold the final highest concentration tested. Two-fold serial dilutions of each compound were directly prepared in sterile 96-well microtiter plate using 100 mL 7H9-S. Two controls, one as growth control with no antibiotic and another a sterile control, were prepared in each plate. In order to avoid evaporation during incubation, sterile water was added to all perimeter wells of the plate. The plates were covered and sealed using plastic bags and incubated at 37 ^{o}C in normal atmosphere. After a period of 7 days incubation, to each well 30 mL of alamar blue solution was added and the plate was incubated overnight. A change in colour from blue (oxidized) to pink (reduced) indicating the growth of bacteria. The MIC (minimum inhibitory concentration) which is defined as lowest inhibitory concentration of the compound was determined based on the change in colour.

1.5. *In vitro* cytotoxicity screening

Eukaryotic RAW 264.7 mouse macrophages cells were used to test the cytotoxic activity of all the compounds[3]. The toxicity was measured by incubating the test compounds in 96 flat-

bottomed well plate containing a cell count of 5×10^5 at different concentrations, with 5% CO_2 and 95% O_2 atmosphere for 48 h at 37 °C. About 4 h, before the end of incubation period, 10 µL of MTT reagent (10 mg/mL) was added, the plate was centrifuged at 1200 rcf for about 3 min to obtain a clear supernatant, the supernatant was removed, and subsequently to each well 200 µL of DMSO was added to dissolve the formed formazan crystals[4]. The absorbance was measured at a wavelength of 560 nm on Perkin Elmer Victor X3 microplate reader against the blank after a span of 10 min. The assay was performed in triplicates for each concentration of drug to minimize the error rate. The cytotoxicity of each compound was expressed as percentage inhibition at that particular concentration.

1.6. Biophysical characterization using differential scanning fluorimetry (DSF)

The most active compound was further investigated using a biophysical technique. The ability of the compound to stabilize the *M. Smegmatis* GyrB protein was assessed using this technique by measuring fluorescence of the native protein and the protein-ligand complexes in the presence of a fluorescent dye SYPRO-orange whose fluorescence increases when exposed to non-polar residues of the protein and reaches a maximum when the protein denatures, this happens as it binds with maximum number of residues in uncoiled state [5]. Complex with the compound was heated stepwise from 25 to 95 ^oC in steps of 0.1 ^oC in the presence of the fluorescent dye, whose fluorescence increases as it interacts with protein. A right side positive shift of T_m in comparison to native protein means higher stabilization of the protein-ligand complex, which is a consequence of the thermally stable inhibitor binding profile[6].

1.7. Molecular modeling studies

The synthesized set of molecules which were assayed for their GyrB inhibitory activity and supercoiling activity where further docked to the active site of GyrB ATPase domain of *M.*

smegmatis which was retrieved from Protein Data Bank with accession ID 4B6C[1]. The retrieved protein was initially subjected to addition of hydrogen atoms, bond order correction, and removal of water molecules using Protein Preparation Wizard of Schrödinger Suite 2012[7]. The hydrogen-bonding network of the protein was optimized and was finally subjected to energy minimization using OPLS2005 force field[8]. The molecules to be docked were sketched in the Maestro panel of Schrödinger and were subjected to conformation generation employing LigPrep of Schrödinger 2012[9]. The docking of these molecules at the active pocket of the protein was carried out using Glide *v*5.8[10].

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