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Synthesis and Biological Screening of Fluorinated Analogues of 4-aminoquinoline Derivatives as Antitubercular Agents

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ABSTRACT

A series of structurally diverse 4-aminoquinoline derivatives were synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral study. All the newly synthesized compounds were inspected for their *in vitro* antitubercular activity against *Mycobacterium tuberculosis* (Mtb) H37Ra and BCG using an established XTT Reduction Menadione (XRMA) and nitrate reductase assay, respectively. The newly synthesized compounds exhibited minimum inhibitory concentration (IC_{50}) ranging from 1.881 to >30 (µg/mL) against MtbH37Ra as Compounds **9p** (IC_{50}: 8.971 µg/mL), **9r** (IC_{50}: 1.881 µg/mL), **9t** (IC_{50}: 2.192 µg/mL) and **9u** (IC_{50}: 2.505 µg/mL). All the compounds further evaluated for their cytotoxic activity against HeLa, MCF-7 and THP-1 cell lines. The antibacterial screening study of these compounds was conducted against four different bacteria to asses there selectivity towards *M. tuberculosis*. Furthermore, molecular docking studies revealed the binding modes of the compounds in the binding site of the ATP-synthase of *M. tuberculosis*. These findings open the possibility for potential lead for antituberculosis chemotherapy.

KEYWORDS

Quinoline analogues, Antimycobacterium, Cytotoxicity, Molecular docking, ATP synthase.

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INTRODUCTION

There is extreme rise in the applications of enormous therapeutic agents as driving force for curing infectious diseases. Tuberculosis is one of the leading infectious diseases caused by *Mycobacterium tuberculosis*. Nowadays, the problem of multidrug resistance (MDR) diseases becoming a serious problem leading a great damage to society as well as economical slow down of the countries [1,2]. The nosocomial infections are caused by the resistance of *Acinetobacter baumannii* to most of the drugs such as aminoglycoside, cefetime, fluoroquinolone and most of the antibiotics [3,4]. Moreover, tuberculosis infections continues to be a measure cause of morbidity and mortality all over the world [5]. There is development of clinical pulmonary tuberculosis among the tuberculosis infected people leading to approximately three million annual death [6,7]. The first line drugs such as isoniazid, streptomycin,

ethambutol, rifampicin, pyrazilnamid, *etc*. have become insensitive against *Mycobacterium tuberculosis* strain [8-10]. Approximately one third of world population is currently at risk of tuberculosis infection and even further all the HIV infected patients are at highest risk of tuberculosis co-infection [11]. Hence, there is an urgent need to search more potent drug candidate against *Mycobacterium tuberculosis*.

Quinoline was found to be versatile building block for the synthesis of pharmacologically active compounds displaying a broad range of bioactivities such as antiviral [11,12], anticancer [13], antibacterial [14], antifungal [15], antiobesity [16], anti-inflammatory [17] and anti-TB [18] reported so far. During the World War-II, quinoline analogues such as chloroquinine and 4-aminoquinone derivatives are progressed globally for the effective treatment of malaria [19-21]. Several quinoline and 4-aminoquinoline analogues have been reported for the treatment of *Mycobacterium tuberculosis* infection [5,22-24]. Majority of 4-aminoquinoline derivatives are known for their antimalarial potential and displaying moderate anti-TB activity (Fig. 1).

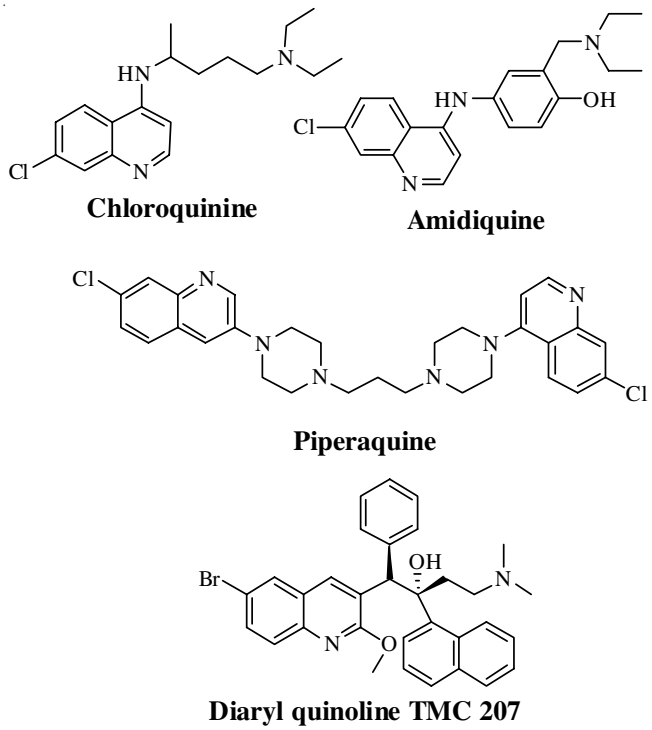


Fig. 1. Potentially active 4-aminoquinoline derivatives

As per our proposed strategy, quinoline derivatives studied so far lack C4-NH_2 side chain modification as amide. Moreover, introduction of conformational flexibility in quinoline C4-NH_2 side chain showed poor growth inhibitory activity against *Mycobacterium tuberculosis* cytochrome bc1 [25]. Hence, the designing of quinoline derivatives is attempted to incorporate chlorinated and fluorinated aryl amide side chain (Fig. 2).

The quinoline derivatives such as diaryl quinoline (TMC207) developed by Johnson and Johnson Pharmaceutical Research and Development showed remarkable anti-TB activity owing to its adenonosine triphosphate (ATP) synthase enzyme inhibitory activity [26]. ATP synthase is a key enzyme indispensible for virtually all living cells and act as the energy source for the bacterium. The mechanistic studies of how TMC207 binds at the binding site of ATP synthase has been well documented in several reports [27-32]. Recently, near full-length X-ray crystal structures of hexameric *M. tuberculosis* proteasomal ATPase Mpa (ATP synthase) has been reported (PDB: 5KWA and 5KZF) [33]. *M. tuberculosis* ATP syntase composed of membrane-embedded F0 core structure made up of subunits a (1), b (2) and c (6) and a hydrophilic F1 part, consisting of subunits α (3), β (3), γ, δ and ε. The numbers in bracket indicates number of units. The subunits c has the binding site for ADP. The residues Tyr452, Gly255, Thr300, Lys299 and Gly296 at the binding site are important in making the hydrogen bond interaction and formation of ATP. In order to gain deeper insights of the possible modes of binding of synthesized compounds at the binding site, we also undertook the docking studies.

EXPERIMENTAL

All the reagents used during the study were purchased from Aldrich and Spectro-Chem and used as such. Solvents were dried and redistilled before use. Melting points were recorded on digital electro-thermal melting point apparatus (VEEGO, VMP-DS) and are uncorrected. Reaction monitoring was done using thin layer chromatography (TLC) using precoated silica gel 60 F_{254} plates with layer thickness 0.25 nm purchased from Merck Ltd. TLC plates were visualized under ultraviolet light at 254 nm wavelength. IR spectra were recorded on KBr discs on Shimazdzu 470 IR spectrophotometer. ^{1}H NMR was recorded on Varian-NMR mercury 300 MHz spectrometer in $CDCl_3$ or DMSO-d_6 using TMS as an internal standard. Chemical shifts values (δ) are expressed parts per million (ppm). Mass spectra were recorded on Varian MAT311A at 70 ev.

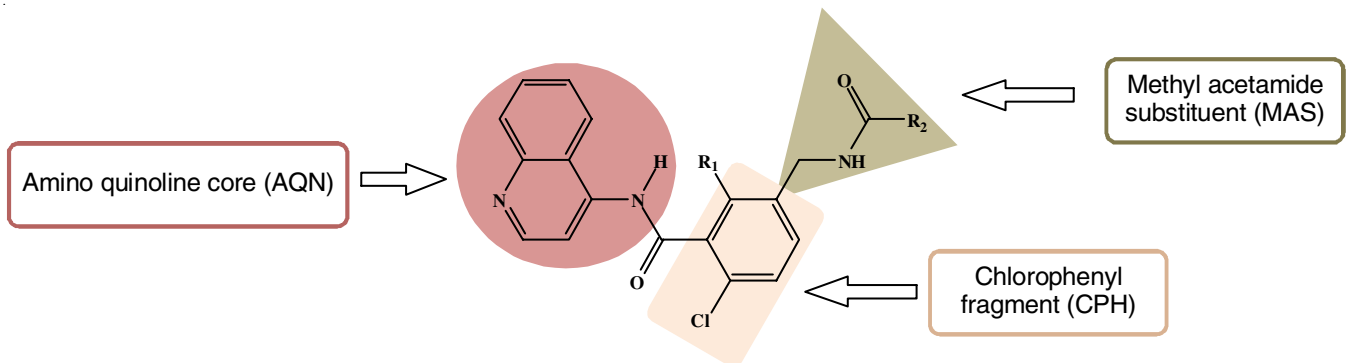


Fig. 2. Design of side chain modified (C_4-NH_2) 4-aminoquinoline derivatives

For biological investigations, all the related chemicals such as sodium salt XTT and MTT, DMSO, ampicillin and rifampicin were purchased from Sigma-Aldrich, USA. Dubos medium was purchased from DIFCO, USA. Synthesized compounds were dissolved in DMSO and used as stock solution (10 mg/mL) for further biological testing.

Synthesis of quinoline N-oxide (2): To a mixture of quinoline (20 g, 0.15 mol) in acetic acid (60 mL), hydrogen peroxide (35 % in water, 0.23 mol, 22 mL) was added. The reaction mixture was stirred at 60-65 ºC for 8 h. After completion of reaction (as indicated by TLC), reaction mixture was cooled and pH of the solution was adjusted to 8 using aq. 1 N NaOH solution. The reaction mixture was then extracted with dichloromethane (3 × 50 mL). The organic layer was dried over anhydrous sodium sulphate and solvent was removed under reduced pressure. The product was crystallized from ethyl acetate to afford compound **2** (17.20 g, 76.51 %) as yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.15 (dd, *J* = 2.5 Hz, 8.4 Hz, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.75 (d, *J* = 8.4 Hz, 1H), 8.43 (d, *J* = 6.3 Hz, 1H), 8.63 (d, *J* = 8.7 Hz, 1H); m.f. C_9H_7ON (m.w. 145.16), mass: *m/z* 145.59(M^+).

Synthesis of 4-nitroquinoline *N*-oxide (3): A mixture of quinoline N-oxide (17.10 g, 0.117 mol) and conc. H_2SO_4 (50 mL) heated to 65 ºC. To this hot mixture nitric acid (65 %, 1.1 eq, 0.129 mol, 12.6 mL) was slowly added. The reaction mixture was further stirred at 65 ºC for 2 h. After completion of reaction (as indicated by TLC), reaction mixture was cooled and poured on ice. The product separated as yellow solid, which was then filtered, washed with 10 %t sodium bicarbonate solution (20 mL), water (100 mL), dried and recrystallized by ethanol to afford compound **3** (20.65 g, 92.18 %) as yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.85-7.94 (m, 2H), 8.21 (d, *J* = 6.6 Hz, 1H), 8.53 (d, *J* = 7.3 Hz, 1H), 8.73-8.86 (m, 1H); m.f. C_9H_7ON (m.w. 190.16), mass: *m/z* 191.45 $(M+H)^+$.

Synthesis of quinolin-4-amine (4): 4-nitroquinoline N-oxide (20.50 g, 0.107 mol) was dissolved in acetic acid (100 mL) followed by iron powder (12.94 g, 0.539 mol, 5 eq.). The reaction mixture was refluxed for 3 h. After completion of reaction (as indicated by TLC), reaction mixture was cooled to room temperature and basified by saturated sodium carbonate solution (pH 8). The solution was filtered through celite bed and filtrate was diluted with water and extracted with ethyl acetate, separate layer, organic phase was dried over anhydrous sodium sulphate and solvent was removed under reduced pressure and the residue was crystallized from diethyl ether to gave 4-amino quinoline as a greenish-black solid (13.98 g, 89.41 %). ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.53 (d, *J* = 5.4 Hz, 1H), 6.75 (s, -NH_2, 2H, exchangeable with D_2O), 7.39 (dd, *J* = 2.5 Hz, 8.4 Hz, 1H), 7.60 (dd, *J* = 2.5 Hz, 8.4 Hz, 1H), 7.75 (d, *J* = 8.4 Hz, 1Hz), 8.14 (d, *J* = 8.4 Hz, 1H), 8.30 (d, *J* = 5.4 Hz, 1H); m.f. $C_9N_2H_8$ (m.w. 144.17), mass: *m/z* 145.57$(M+H)^+$.

General procedure for the synthesis of 6a and 6b: To a mixture of compound **5a** or **5b** (25 g, 0.159 mol) in concentrated sulfuric acid (200 mL) was added 2,2,2-trifluoro-N-(hydroxymethyl)acetamide (22.84 g, 0.159 mol). The reaction mixture was stirred at 25-30 ºC for 12 h. After completion of reaction (as indicated by TLC), reaction mixture was cooled and poured in ice water and stirred for 2 h. The product precipitated as a white solid, which was filtered off and recrystallized from toluene:butan-2-one (7:1). Purity of compound was monitored by HPLC (98.50 %) to give compound **6a** white solid (22.80 g) Yield: 50.71 %); ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.42 (d, Ar-CH_2, *J* = 6 Hz, 2H), 7.43 (d, *J* = 9.9 Hz, 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.71 (s, 1H), 10.06 (s, -$NHCOCF_3$, 1H, exchangeable with D_2O), 13.47 (s, -COOH, 1H, exchangeable with D_2O). m.f. $C_{10}H7NO_3F_3Cl$ (m.w. 281.62), mass: *m/z* 282.5 $(M+H)^+$; **6b**) ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.56 (d, Ar-CH_2, *J* = 6 Hz, 2H), 7.35 (d, *J* = 9.9 Hz, 1H), 7.84 (d, *J* = 8.4 Hz, 1H), 8.22 (s, -$NHCOCF_3$, 1H, exchangeable with D_2O), 9.83 (s, -COOH, 1H, exchangeable with D_2O); m.f. $C_{10}H_6NF_4Cl$ (m.w. 299.62), mass: *m/z* 300.55$(M+H)^+$.

Synthesis of 4-((2,2,2-trifluoroacetamido)methyl)-2-chloro-N-(quinolin-4-yl)benzamide (7a)/3-[(2,2,2-trifluoroacetamido)methyl]-6-chloro-2-fluoro-N-(quinolin-4-yl)-benzamide (7b): To a solution of 5-[(2,2,2-trifluoroacetamido)-methyl]-2-chlorobenzoic acid (**6a**)/3-[(2,2,2-trifluoroacetamido)-methyl]-6-chloro-2-fluorobenzoic acid (**6b**) (9.22 g, 0.034 mol) in dry DCM (96 mL, 8 vol) was added oxalyl chloride (3.6 mL, 0.051 mol) at room temperature. 1-2 drops of DMF was added in reaction mixture. Reaction mixture was stirred at 25-30 ºC for 3 h. Reaction was monitored by TLC. After completion of reaction, the solvent was evaporated under vacuum at room temperature to dryness. In another round bottom flask taken 4-amino quinoline (5 g, 0.034 mol) in dry THF, cool to 0 ºC was added triethylamine (7.1 mL, 0.051mol). Stirred at 0 ºC for 20 min, then slowly added above acid chloride solution in the reaction mixture. The reaction was stirred at 50 ºC for 4 h. Reaction was monitored by TLC. After completion of reaction, the reaction mixture was quenched in ice cold water, extracted by EtOAC, separate layer. EtOAc layer washed by sodium bicarbonate solution, the organic phase was dried over anhydrous sodium sulphate then evaporated under reduced pressure and the residue was crystallized from diethyl ether to give **7a** & **7b**, respectively as a faint yellow solid (12.2 g) Yield= 87.14 %; **7a**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.48 (d, Ar-CH_2, *J* = 6 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.63 (m, 3H), 7.79 (d, *J* = 8.4 Hz, 1H), 8.10 (dd, *J* = 2.6 Hz, 8.4 Hz, 2H), 8.36 (d, *J* = 8.7 Hz, 1H), 8.89 (d, *J* = 4.8 Hz, 1H), 10.12 (s, 1H, Ar-amide, exchangeable with D_2O), 10.98 (s, -$NHCOCF_3$, exchangeable with D_2O, 1H); m.f. $C_{19}H_{13}N_3O_2ClF_3$ (407.77), mass: *m/z* 408.45 $(M+H)^+$. **7b**) ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.62 (d, Ar-CH_2, *J* = 6 Hz, 2H), 7.47 (d, *J* = 8.7 Hz, 1H), 7.65 (d, *J* = 7.2 Hz, 1H), 7.79 (d, *J* = 8.7 Hz, 2H), 8.10 (dd, *J* = 2.6 Hz, 8.4 Hz, 2H), 8.34 (d, *J* = 8.4 Hz, 1H), 8.89 (d, *J* = 4.8 Hz, 1H), 9.95 (s, 1H, Ar-amide, exchangeable with D_2O), 10.96 (s, -NH-$COCF_3$, exchangeable with D_2O, 1H); m.f. $C_{19}H_{12}N_3O_2F_4Cl$ (m.w. 425.76), mass: *m/z* 424.28 $(M-H)^+$.

Synthesis of 4-(aminomethyl)-2-chloro-N-(quinolin-4-yl-benzamide) (8a)/3-(aminomethyl)-6-chloro-2-fluoro-N-(quinolin-4-yl)benzamide (8b): To a mixture of 4-((2,2,2-trifluoroacetamido)methyl)-2-chloro-N-(quinolin-4-yl)-benzamide (**7a**)/3-[(2,2,2-trifluoroacetamido)methyl]-6-chloro-2-fluoro-*N*-(quinolin-4-yl)benzamide (**7b**), (10 g, 0.024 mol) in THF:H_2O (50:10), NaOH (1.47 g, 0.036 mol) was added at 25 ºC. Reaction mixture was stirred at 25 ºC for 1 h. After completion of reaction, THF was removed under reduced

pressure, solid precipitated out filtered and collected to afford **8a** & **8b**, respectively as a yellow solid (6.89 g) Yield = 90.65 %; **8a**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.82 (s, Ar-CH_2, 2H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.62 (d, *J* = 4.8 Hz, 1H), 7.69 (s, 1H), 7.78 (d, *J* = 7.8 Hz, 1H), 8.06 (dd, *J* = 2.6 Hz, 8.4 Hz, 2H), 8.36 (d, *J* = 7.8 Hz, 1H), 8.87 (d, *J* = 4.8 Hz, 1H); m.f. $C_{17}H_{14}N_3OCl$ (m.w. 311.50), mass: *m/z* 312.69 $(M+H)^+$; **8b**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.85 (s, Ar-CH_2, 2H), 7.33 (dd, *J* = 2.5 Hz, 8.4 Hz, 1H), 7.68 (d, *J* = 7.6 Hz, 2H), 7.80 (dd, *J* = 2.5 Hz, 8.4 Hz, 1H), 8.04 (d, *J* = 8.4 Hz, 1H), 8.10 (d, *J* = 8.4 Hz, 1H), 8.34 (d, *J* = 7.6 Hz, 1H), 8.88 (d, *J* = 4.8 Hz, 1H), 10.91 (s, Ar-amide, exchangeable with D_2O, 1H); m.f. $C_{17}H_{13}N_3OFCl$ (m.w. 329.76), mass: *m/z* 330.18 $(M+H)^+$.

General procedure for the synthesis of 9a-x: To a solution of 4-(aminomethyl)-2-chloro-*N*-(quinolin-4-ylbenzamide) (200 mg, 0.642 mmol) in dry DCM, added triethyl amine (0.25 mL, 1.92 mmol), stirred at 0 ºC for 10 min. Then slowly added acid chloride (0.77mmol, 1.2 eq) was added slowly at 0 ºC. The reaction mixture was stirred at 25 ºC for 3-4 h. After completion of reaction (as indicated by TLC), reaction mixture was quenched in water and extracted in DCM. DCM layer was washed by sodium bicarbonate solution; the organic phase was dried over anhydrous sodium sulphate. Solvent was removed under reduced pressure and collected crude products were purified by column chromatography (2 % methanol:dichloromethane).

4-((2,2,2-Trifluoroacetamido)methyl)-2-chloro-N(quinolin-4-yl)benzamide (9a): Faint yellow solid (yield = 95 %), m.p. 162-163 ºC; IR (KBr, ν_{max}, cm^{-1}): 3285 (NH amide), 3035 (C-H alkane), 1728, 1651 (C=O amide), 1531 (aromatic carbon), 1219 (C-N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.48 (d, Ar-CH_2, *J* = 6 Hz, 2H), 7.47 (d, J = 8.4 Hz, 1H), 7.63 (m, 3H), 7.79 (d, *J* = 8.4 Hz, 1H), 8.10 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.36 (d, *J* = 8.7 Hz, 1H), 8.89 (d, *J* = 4.8 Hz), 10.12 (s, -NHCOCF_3, 1H, exchangeable with D_2O), 10.98 (s, Ar-amide, exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6): δ 42.2, 113.8, 121.9, 123.2, 126.5, 128.7, 129.3, 129.8, 130.0, 130.3, 130.7, 136.9, 137.4, 141.5, 149.2, 151.2, 166.5; m.f. $C_{19}H_{13}N_3O_2ClF_3$ (m.w. 407.77); mass: *m/z* (%) 408.45 (M+H).

2-Chloro-5-((pivalamidomethyl)-*N*-(quinolin-4-yl) benzamide (9b): White solid (yield = 97 %), m.p. 170-171 º C, IR (KBr, ν_{max}, cm^{-1}): 3361 (NH amide), 2966 (C-H stretching of alkane), 1642 (C=O amide), 1527 (aromatic carbon), 1313 (C-N), 1169, 1118 (C-O), 1428 (C-F); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.14 (s, -COCMe_3, 9H), 4.33 (d, Ar-CH_2, *J* = 5.7 Hz, 2H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.62-7.53 (m, 3H), 7.79 (d, *J* = 8.4 Hz, 1H), 8.05 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.20 (s, -NHCOCMe_3, exchangeable with D_2O ,1H), 8.35 (d, *J* = 8.4 Hz, 1H), 8.89 (d, *J* = 4.8 Hz, 1H), 10.97 (s, Ar-amide, exchangeable with D_2O, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 27.8, 38.5, 41.8, 113.9, 121.9, 123.2, 126.5, 128.0, 128.4, 129.8, 129.9, 130.0, 130.1, 136.5, 140.2, 141.6, 149.2, 151.2, 166.7, 178.0; m.f. $C_{22}H_{22}N_3O_2Cl$ (m.w. 395.88), Mass: *m/z* (%) 397.16 $(M+H)^+$.

2-Chloro-5-((isobutyramido)methyl)-N-(quinolin-4-yl) benzamide (9c): White solid; (yield = 94 %), m.p. 176-177 ºC. IR (KBr, ν_{max}, cm^{-1}): 3372 (NH amide), 2969 (C-H stretching of alkane), 1665, 1642 (C=O amide), 1526, 1504 (aromatic carbon), 1315 (C-N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.05 (d, -CHMe_2, *J* = 6.9 Hz, 6H), 2.44 (m, -CHMe_2, 1H), 4.33 (d, Ar-CH_2, *J* = 5.7 Hz, 2H), 7.41 (d, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 2H), 7.64 (d, *J* = 8.4 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 8.07 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.35 (d, *J* = 7.8 Hz, 1H), 8.39 (s, -NHCOCHMe_2, exchangeable with D_2O, 1H), 8.89 (d, *J* = 4.8 Hz, 1H), 10.96 (s, Ar-amide, exchangeable with D_2O, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.5, 34.0, 40.9, 113.4, 121.4, 122.7, 126.0, 127.6, 128.0, 129.3, 129.5, 129.5, 129.8, 136.1, 139.4, 141.1, 148.7, 150.7, 166.2, 176.2; m.f. $C_{21}H_{20}N_3O_2Cl$ (m.w. 381.86). Mass *m/z* (%) 383.36$(M+H)^+$.

2-Chloro-5-((cyclobutanecarboxamido)methyl)-N-(quinolin-4-yl)benzamide (9d): White solid; (yield = 92 %) m.p. 180-181 ºC. IR (KBr, ν_{max}, cm^{-1}): 3263 (NH amide), 2937 (C-H stretching of alkane), 1662, 1634 (C=O amide), 1526, 1504 (aromatic carbon), 1317 (C-N). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.77-2.18 (m, cyclobutane CH_2, 6H), 3.10 (m, cyclobutane -CH-, 1H), 4.33 (d, Ar-CH_2, *J* = 6 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.64 (d, *J* = 8.4 Hz, 1H), 7.81 (d, *J* = 7.8 Hz, 1H), 8.09 (dd, *J* = 2.5 Hz, 8.4 Hz, 2H), 8.30 (d, *J* = 7.8 Hz, 1H), 8.35 (d, *J* = 8.4 Hz, 1H), 8.69 (s, cyclobutane amide, exchangeable with D_2O,1H), 8.89 (d, *J* = 4.8 Hz, 1H), 10.96 (s, Ar-amide, exchangeable with D_2O, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.7, 24.6, 38.6, 41.0, 113.3, 121.4, 122.7, 126.0, 127.7, 128.1, 129.3, 129.5, 129.5, 129.9, 136.1, 139.3, 141.0, 148.7, 150.7, 166.2, 174.0; m.f. $C_{22}H_{20}N_3O_2Cl$ (m.w. 393.87). Mass *m/z* (%) 394.51 $(M+H)^+$.

2-Chloro-5-((cyclopropanecarboxamido)methyl)-*N*-(quinolin-4yl) benzamide (9e): Off white solid; (yield 89 %), m.p. 184-185 ºC. IR (KBr, ν_{max}, cm^{-1}): 3272 (NH amide), 1662, 1634 (C=O amide), 1525, 1504 (aromatic carbon), 1315 (C-N). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.67 (m, cyclopropyl CH_2, 4H), 1.62 (m, cyclopropyl CH, 1H), 4.36 (d, Ar-CH_2, *J* = 5.7 Hz, 2H), 7.43 (d, *J* = 7.8 Hz, 1H), 7.58 (d, *J* = 7.8 Hz, 2H), 7.65 (d, *J* = 7.8 Hz, 1H), 7.81 (d, *J* = 7.8 Hz, 1H), 8.10 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.36 (d, *J* = 7.8 Hz, 1H), 8.69 (s, cyclopropylamide, exchangeable with D_2O, 1H), 8.89 (d, *J* = 5.1 Hz, 1H), 10.96 (s, Ar-amide, exchangeable with D_2O, 1H); m.f. $C_{21}H_{18}N_3O_2Cl$ (m.w. 379.11). Mass: *m/z* (%) 380.68 $(M+H)^+$.

N-((3-(quinolin-4-ylcarbamoyl)-4-chlorophenyl)-methyl)furan-2-carboxamide (9f): White solid; (Yield 84 %). m.p. 197-198 ºC. IR (KBr, ν_{max}, cm^{-1}): 3414 (NH amide), 1691 (C=O amide), 1594 (aromatic carbon); ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.39 (d, Ar-CH_2, *J* = 5.7 Hz, 2H), 6.52 (dd, furan ring -CH, *J* = 3.6 Hz, 1H), 7.05 (d, C3-furan ring -CH, *J* = 3.6 Hz, 1H), 7.11 (d, C4-furan ring -CH, *J* = 3.6 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.65-7.85 (m, 6H), 7.98 (d, *J* = 8.4 Hz, 1H), 8.13 (d, *J* = 8.4 Hz, 1H), 8.93 (d, *J* = 4.8 Hz, 1H), 8.95 (s, furan amide, exchangeable with D_2O, 1H), 10.95 (s, Ar-amide, exchangeable with D_2O, 1H); m.f. $C_{22}H_{16}N_3O_3Cl$ (m.w. 405.83). Mass: *m/z* (%) 406.43 $(M+H)^+$.

2-Chloro-5-((cyclohexanecarboxamido)methyl)-*N*-(quinolin-4yl) benzamide (9g): Yellow solid; (yield 93 %), m.p. 168-169 ºC. IR (KBr, ν_{max}, cm^{-1}): 3294 (NH amide), 2928 (C-H stretching of alkane), 1665, 1641 (C=O amide), 1526, 1504 (aromatic carbon), 1315 (C-N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.13-1.12 (m, cyclohexane CH_2, 6H), 1.70-1.61 (m, cyclohexane CH_2, 4H), 2.17 (m, cyclohexane CH, 1H), 4.31 (d, Ar-CH_2, *J* = 5.4 Hz, 2H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.63-7.52 (m,

3H), 7.80 (d, *J* = 7.8 Hz, 1H), 8.04 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.31 (d, *J* = 8.1 Hz, 1H), 8.34 (s, cyclohexane amide, exchangeable with D_2O, 1H), 8.88 (d, *J* = 4.8 Hz, 1H), 10.94 (s, Ar-amide, exchangeable with D_2O, 1H); m.f. $C_{24}H_{24}N_3O_2Cl$ (m.w. 421.92). Mass: *m/z* (%) 423.12 $(M+H)^+$.

2,4-Dichloro-N-((4-chloro-3-(quinolin-4-ylcarbamoyl)-benzyl benzamide (9h): White solid; (yield 89 %). m.p. 202-203 ºC. IR (KBr, ν_{max}, cm^{-1}): 3265 (NH amide), 1665, 1639 (C=O amide), 1528 (aromatic carbon), 1314 (C-N); ^1H NMR (300 MHz, DMSO-d_6): δ 4.53 (d, Ar-CH_2, *J* = 5.7 Hz, 2H), 7.70-7.51 (m, 7H), 7.78 (d, *J* = 8.7 Hz, 1H), 8.04 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.35 (d, *J* = 8.7 Hz, 1H), 8.88 (d, *J* = 4.8 Hz, 1H), 9.16 (s, -CH_2 amide, exchangeable with D_2O, 1H), 10.97 (s, Ar-amide, exchangeable with D_2O, 1H); m.f. $C_{24}H_{16}N_3O_2Cl_3$ (m.w. 484.76). Mass *m/z* (%) 486.96 $(M+H)^+$.

2-Chloro-5-((3-chlorobenzamido)methyl)-*N*-(quinolin-4-yl)benzamide (9i): White solid; (yield = 91 %), m.p. 181-182 ºC. IR (KBr, ν_{max}, cm^{-1}): 3304 (NH amide), 1665, 1639 (C=O amide), 1528 (aromatic carbon), 1314 (C-N); ^1H NMR (300 MHz, DMSO-d_6): δ 4.56 (d, Ar-CH_2, *J* = 5.4 Hz, 2H), 7.67-7.51 (m, 6H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 7.95 (s, 1H), 8.08 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.35 (d, *J* = 7.8 Hz, 1H), 8.88 (d, *J* = 5.1 Hz, 1H), 9.29 (s, -CH_2 amide, exchangeable with D_2O, 1H), 10.97 (s, Ar-amide, exchangeable with D_2O, 1H); m.f. $C_{24}H_{17}N_3O_2Cl_2$ (m.w. 450.32). Mass *m/z* (%) 450.40 $(M+)^+$.

2-Chloro-5-((fluorobenzamido)methyl)-N-(quinolin-4-yl)benzamide (9j): Off white solid; (yield = 95 %), m.p. = 185-186 ºC. IR (KBr, ν_{max}, cm^{-1}): 3283 (NH amide), 1702, 1631 (C=O amide), 1526 (aromatic carbon), 1310 (C-N); ^1H NMR (300 MHz, DMSO-d_6): δ 4.56 (d, Ar-CH_2, *J* = 6.3 Hz, 2H), 7.47-7.41 (m, 8H), 7.78 (d, *J* = 7.8 Hz, 2H), 8.09 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.35 (d, *J* = 8.4 Hz, 1H), 8.88 (d, *J* = 4.8 Hz, 1H), 9.26 (s, -CH_2 amide, exchangeable with D_2O, 1H), 10.97 (s, Ar-amide, exchangeable with D_2O, 1H); m.f. $C_{24}H_{17}N_3O_2ClF$ (m.w. 433.86). Mass *m/z* (%) 434.77 $(M+H)^+$.

***N*-((3-(Quinolin-4-ylcarbamoyl)-4-chlorophenyl)-methyl)piperidine-1-carboxamide (9k):** Off white solid; (yield = 75 %), m.p. 167-168 ºC. IR (KBr, ν_{max}, cm^{-1}): 3352 (NH amide), 2934 (C-H stretching of alkane), 1677, 1622 (C=O amide), 1528 (aromatic carbon), 1312 (C-N); ^1H NMR (300 MHz, DMSO-d_6): δ 1.41 (m, piperidine CH_2, 4H), 1.51 (m, piperidine CH_2, 2H), 3.28 (d, piperidine -NCH_2, 4H), 4.29 (d, Ar-CH_2, *J* = 5.4 Hz, 2H), 7.12 (s, -piperidine-CH_2 amide, exchangeable with D_2O, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.64 (d, *J* = 8.4 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 8.09 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.36 (d, *J* = 8.4 Hz, 1H), 8.88 (d, *J* = 4.8 Hz, 1H), 10.97 (s, Ar-amide, exchangeable with D_2O, 1H); m.f. $C_{23}H_{23}N_4O_2Cl$ (m.w. 422.91). Mass *m/z* (%) 423.44 $(M+H)^+$.

***N*-((3-(Quinolin-4-ylcarbamoyl)-4-chlorophenyl)methyl)-2,6-dichloropyridine-3-carboxamide (9l):** Off white solid; (yield = 82 %), m.p. 198-199 ºC. IR (KBr, ν_{max}, cm^{-1}): 3222 (NH amide), 1657, 1610 (C=O amide), 1509 (aromatic carbon), 1341 (C-N); ^1H NMR (300 MHz, DMSO-d_6): δ 4.56 (d, Ar-CH_2, *J* = 6.3 Hz, 2H), 7.56 (d, pyridine CH, *J* = 9 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.70 (d, pyridine CH, *J* = 9 Hz, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 8.02 (d, *J* = 7.8 Hz, 1H), 8.08 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.36 (d, *J* = 8.4 Hz, 1H), 8.89 (s, 1H), 9.30 (s, pyridine-CH_2 amide, exchangeable with D_2O, 1H), 10.99 (s, Ar-amide, exchangeable with D_2O, 1H); m.f. $C_{23}H_{15}N_4O_2Cl_3$ (m.w. 485.75). Mass *m/z* (%) 485.39 $(M+)^+$.

2-Chloro-5-((3-methylbenzamido)methyl)-*N*-(quinolin-4-yl)benzamide (9m): Off white solid; (yield = 87 %), m.p. 178-179 ºC. IR (KBr, ν_{max}, cm^{-1}): 3405 (NH amide and OH alcohol), 2971 CH), 2226 (C≡C), 1660 (C=O amide), 1502 (aromatic carbon), 1325 (C=N), 1165, 1125 (C-O), 1440 (C-F); ^1H NMR (300 MHz, DMSO-d_6): δ 2.36 (s, Ar-CH_3, 3H), 4.55 (d, Ar-CH_2, *J* = 6.3 Hz, 2H), 7.35 (d, 2H), 7.72-7.51 (m, 7H), 8.09-8.02 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.35 (d, *J* = 8.4 Hz, 2H), 8.88 (s, 1H), 9.12 (s, CH_2-amide, exchangeable with D_2O, 1H), 10.98 (s, Ar-amide, exchangeable with D_2O, 1H); m.f. $C_{25}H_{20}N_3O_2Cl$ (m.w. 429.9). Mass *m/z* (%) 430.69 $(M+H)^+$.

3-((2,2,2-Trifluoroacetamido)methyl)-6-chloro-2-fluoro-*N*-(quinolin-4-yl)benzamide (9n): Faint yellow solid (yield = 92 %), m.p. 168-169 ºC; IR (KBr, ν_{max}, cm^{-1}): 3321 (NH amide), 2965 (C-H alkane), 1721, 1662 (C=O amide), 1532 (aromatic carbon), 1325 (C-N); ^1H NMR (300 MHz, DMSO-d_6): δ 4.62 (d, Ar-CH_2, *J* = 6 Hz, 2H), 7.47 (d, *J* = 8.7 Hz, 1H), 7.65 (d, *J* = 7.2 Hz, 1H), 7.79 (d, *J* = 8.7 Hz, 2H), 8.10 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.34 (d, *J* = 8.4 Hz, 1H), 8.89 (d, *J* = 4.8 Hz, 1H), 9.95 (1H, CH_2-amide, exchangeable with D_2O), 10.96 (s, 1H, Ar-amide, exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6): δ 34.2, 113.4, 114.4, 114.6, 121.4, 122.6, 122.7, 126.1, 129.3, 129.6, 129.9, 130.0, 133.6, 141.1, 148.7, 150.7, 160.3, 162.8, 165.1; m.f. $C_{19}H_{12}N_3O_2ClF_4$ (m.w. 425.76). Mass *m/z* (%): 424.28 $(M-H)^+$.

6-Chloro-2-fluoro-3-((pivalamido)methyl)-*N*-(quinolin-4-yl)benzamide (9o): White solid (yield = 96 %), m.p. 174-175 ºC; IR (KBr, ν_{max}, cm^{-1}): 3401 (NH amide), 2965 (C-H stretching of alkane), 1645 (C=O amide), 1527 (aromatic carbon), 1313 (C-N); ^1H NMR (300 MHz, DMSO-d_6): δ 1.08 (s, -$CHMe_3$, 9H), 4.45 (d, Ar-CH_2, *J* = 5.7 Hz, 2H), 7.40 (d, *J* = 8.7 Hz, 1H), 7.62 (d, *J* = 8.7 Hz, 1H), 7.71-7.87 (m, 4H), 8.05 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.10 (s, CH_2-amide, exchangeable with D_2O, 1H), 8.35 (d, *J* = 8.7 Hz, 1H), 8.89 (d, *J* = 5.1 Hz, 1H), 10.94 (s, Ar-amide, exchangeable with D_2O, 1H); m.f. $C_{22}H_{21}N_3O_2ClF$ (m.w. 413.87). Mass *m/z* (%): 414.37$(M+H)^+$.

6-Chloro-2-fluoro-3-((isobutyramido)methyl)-N-(quinolin-4-yl)benzamide (9p): White solid; (yield = 90 %), m.p. 179-180 ºC. IR (KBr, ν_{max}, cm^{-1}): 3389 (NH amide), 2970 (C-H stretching of alkane), 1646 (C=O amide), 1526 (aromatic carbon), 1313 (C-N); ^1H NMR (300 MHz, DMSO-d_6): δ 1.00 (d, -$CHMe_2$, *J* = 6.9 Hz, 6H), 2.39 (m, -$CHMe_2$, 1H), 4.47 (d, Ar-CH_2, *J* = 5.7 Hz, 2H), 7.43 (d, *J* = 8.7 Hz, 1H), 7.63 (d, *J* = 8.7 Hz, 1H), 7.79 (dd, *J* = 2.4 Hz, 8.4 Hz, 1H), 8.05 (d, *J* = 8.7 Hz, 2H), 8.14 (s, CH_2-amide, exchangeable with D_2O, 1H), 8.35 (d, *J* = 8.7 Hz, 1H), 8.89 (d, *J* = 4.8 Hz, 1H), 10.95(s, Ar-amide, exchangeable with D_2O, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 19.6, 33.9, 34.5, 113.7, 144.5, 121.6, 122.9, 124.8, 125.0, 126.4, 129.5, 129.9, 132.1, 133.7, 141.3, 148.7, 150.9, 160.4, 166.0, 176.3; m.f. $C_{21}H_{19}N_3O_2ClF$ (m.w. 399.85). Mass *m/z* (%): 400.47 $(M+H)^+$.

6-Chloro-3-((cyclobutanecarboxamido)methyl)-2-fluoro-*N*-(quinolin-4-yl)benzamide (9q): White solid; (yield = 92 %), m.p. 182-183°C. IR (KBr, ν_{max}, cm^{-1}): 3289 (NH amide), 2983, 2938 (C-H stretching of alkane), 1663, 1640 (C=O amide), 1523, 1501 (aromatic carbon), 1313 (C-N); ^1H NMR (300 MHz, DMSO-

d_6): δ 1.75-2.15 (m, cyclobutane -CH_2, 6H), 3.05 (m, 1H), 4.46 (d, Ar-CH_2, *J* = 6 Hz, 2H), 7.42 (d, *J* = 8.7 Hz, 1H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.81 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.05 (d, *J* = 8.1 Hz, 2H), 8.09 (s, CH_2-amide, exchangeable with D_2O, 1H), 8.34 (d, *J* = 8.4 Hz, 1H), 8.89 (d, *J* = 4.8 Hz, 1H), 10.92 (s, Ar-amide, exchangeable with D_2O, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 16.4, 23.2, 33.0, 37.0, 112.1, 112.9, 120.0, 121.4, 123.4, 123.5, 124.7, 127.9, 130.6, 130.7, 132.3, 139.7, 147.4, 149.4, 159.2, 164.4, 172.3; m.f. $C_{22}H_{19}N_3O_2ClF$ (m.w. 411.86). Mass m/z (%): 412.64 $(M+H)^+$.

6-Chloro-3-((cyclopropanecarboxamido)methyl)-2-fluoro-*N*-(quinolin-4yl)benzamide (9r): Off white solid; (yield = 85 %), m.p. 180-181 ºC. IR (KBr, ν_{max}, cm^{-1}): 3272 (NH amide), 1662, 1634 (C=O amide), 1525, 1504 (aromatic carbon), 1315 (C-N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.66 (m, cyclopropyl-CH_2, 4H), 1.61 (m, cyclopropyl-CH, 1H), 4.50 (d, Ar-CH_2, *J* = 5.7 Hz, 2H), 7.43 (d, *J* = 8.7 Hz, 1H), 7.65 (d, *J* = 8.7 Hz, 1H), 7.79 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.05 (d, *J* = 7.8 Hz, 1H), 8.11 (d, *J* = 7.8Hz, 1H), 8.35 (d, *J* = 8.7 Hz, 1H), 8.47 (s, CH_2-amide, exchangeable with D_2O, 1H), 8.90 (d, *J* = 5.1 Hz, 1H), 10.95 (s, Ar-amide, exchangeable with D_2O, 1H); m.f. $C_{21}H_{17}N_3O_2ClF$ (397.83) Mass *m/z* (%): 398.35 $(M+H)^+$.

N-((3-(Quinolin-4-ylcarbamoyl)-4-chloro-2-fluorophenyl)methyl)furan-2-carboxamide (9s): White solid; (yield = 87 %), m.p. 199-200 ºC. IR (KBr, ν_{max}, cm^{-1}): 3217 (NH amide), 2993, 2960 (C-H stretching of alkane), 1701, 1671 (C=O amide), 1591, 1557 (aromatic carbon), 1309 (C-N), 1181 (C-O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.54 (d, *J* = 5.7 Hz, 2H), 6.61 (dd, furan ring -CH, *J* = 3.6 Hz, 1H), 7.11 (d, C_3-furan ring-CH, *J* = 3.6 Hz, 1H), 7.12 (d, C_4-furan ring-CH, *J* = 3.6 Hz, 1H), 7.34 (d, *J* = 8.7 Hz, 1H), 7.73 (d, *J* = 8.1 Hz, 1H), 7.82 (m, 3H), 8.04 (d, *J* = 8.4 Hz, 1H), 8.13 (d, *J* = 8.4 Hz, 1H), 8.88 (s, exchangeable with D_2O, 1H), 8.99 (d, *J* = 4.8 Hz, 1H), 10.95 (s, exchangeable with D_2O, 1H); m.f. $C_{22}H_{15}N_3O_3ClF$ (m.w. 423.82). Mass *m/z* (%): 425.24 $(M+H)^+$.

6-Chloro-3-((cyclohexanecarboxamido)methyl)-2-fluoro-N-(quinolin-4yl)benzamide (9t): Yellow solid; (yield = 90 %). m.p. 172-173 ºC. IR (KBr, ν_{max}, cm^{-1}): 3280 (NH amide), 2928, 2853 (C-H stretching of alkane), 1661, 1639 (C=O amide), 1525, 1504 (aromatic carbon), 1313 (C-N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.16-1.67 (m, cyclohexane-CH_2, 10H), 2.13 (m, cyclohexane-CH, 1H), 4.45 (d, Ar-CH_2, *J* = 5.4 Hz, 2H), 7.42 (d, *J* = 8.7 Hz, 1H), 7.62 (d, *J* = 8.7 Hz, 1H), 7.81 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.05 (d, *J* = 8.4 Hz, 2H), 8.08 (s, CH_2-amide, exchangeable with D_2O, 1H), 8.34 (d, *J* = 8.34 Hz, 1H), 8.89 (d, *J* = 5.1 Hz, 1H), 10.93 (s, exchangeable with D_2O, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.5, 25.4, 29.1, 34.2, 43.6, 113.4, 114.4, 121.3, 122.7, 124.9, 126.0, 129.2, 129.3, 129.5, 131.9, 133.5, 141.0, 148.7, 150.7, 160.5, 165.7, 174.9; m.f. $C_{24}H_{23}N_3O_2ClF$ (m.w. 439.91). Mass *m/z* (%): 440.45 $(M+H)^+$.

6-Chloro-3-((2,4-dichlorobenzamido)methyl-2-fluoro-*N*-(quinolin-4-yl) benzamide (9u): White solid; (yield = 85 %), m.p. 204-205 ºC. IR (KBr, ν_{max}, cm^{-1}): 3254 (NH amide), 2955, 2925 (C-H stretching of alkane), 1652 (C=O amide), 1587, 1524 (aromatic carbon), 1312, 1285 (C-N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.65 (d, Ar-CH_2, *J* = 5.7 Hz, 2H), 7.41 (d, *J* = 8.4Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.83 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.05 (d, *J* = 8.4Hz, 1H), 8.11 (s, 1H), 8.3 (d, *J* = 8.1 Hz, 1H), 8.89 (d, *J* = 4.8Hz,1H), 8.98 (s, CH_2-amide, exchan-geable with D_2O, 1H), 10.96 (s, Ar-amide, exchangeable with D_2O, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 34.8, 113.4, 114.4, 121.3, 122.7, 126.0, 127.1, 127.4, 129.0, 129.3, 129.5, 130.1, 130.2, 131.2, 132.3, 132.9, 134.5,135.3, 136.4, 148.7, 150.7, 165.1, 165.7, 165.8; m.f. $C_{24}H_{15}N_3O_2Cl_3F$ (m.w. 501.02). Mass *m/z* (%): 502.5 $(M+H)^+$.

6-Chloro-3-((3-chlorobenzamido)methyl-2-fluoro-N-(quinolin-4-yl)benzamide (9v): White solid; (yield = 86 %), m.p. 183-184 ºC. IR (KBr, ν_{max}, cm^{-1}): 3232 (NH amide), 2975, 2938 (C-H stretching of alkane), 1667, 1651 (C=O amide), 1530, 1503 (aromatic carbon), 1312 (C-N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.67 (d, Ar-CH_2, *J* = 5.4 Hz, 2H), 7.39-7.91 (m, 8H), 8.10 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.34 (d, *J* = 8.4Hz, 1H), 8.89 (d, 1H), 9.04 (s, CH_2-amide, exchangeable with D_2O, 1H), 10.96 (s, Ar-amide, exchangeable with D_2O, 1H); m.w. $C_{24}H_{16}N_3O_2Cl_2F$ (m.w. 468.31). Mass *m/z* (%): 468.51 $(M+)^+$.

6-Chloro-2-fluoro-3-((3-fluorobenzamido)methyl-N-(quinolin-4-yl)benzamide (9w): Off white solid; (Yield = 89 %). m.p. 187-188 ºC. IR (KBr, ν_{max}, cm^{-1}): 3283 (NH amide), 1702, 1631 (C=O amide), 1526 (aromatic carbon), 1310 (C-N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.68 (d, Ar-CH_2, *J* = 6.3 Hz, 2H), 7.41 (d, *J* = 8.7 Hz, 2H), 7.50-7.74 (m, 6H), 8.09 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 8.35 (d, *J* = 7.8 Hz, 1H), 8.89 (d, *J* = 8.4 Hz, 1H), 9.01 (s, CH_2-amide, exchangeable with D_2O, 1H), 10.95 (s, Ar-amide, exchangeable with D_2O, 1H); m.f. $C_{24}H_{16}N_3O_2ClF_2$ (m.w. 451.85). Mass *m/z* (%): 452.64 $(M+H)^+$.

N-((3-(Quinolin-4-ylcarbamoyl)-4-chloro-2-fluorophenyl)methyl)-2,6-dichloropyridine-3-carboxamide (9x): Off white solid; (yield = 82 %), m.p. 170-171 ºC. IR (KBr, ν_{max}, cm^{-1}): 3222 (NH amide), 1657, 1610 (C=O amide), 1509 (aromatic carbon), 1341 (C-N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.68 (d, Ar-CH_2, *J* = 6.3 Hz, 2H), 7.47 (d, pyridine-CH, *J* = 8.7 Hz, 1H), 7.65 (d, *J* = 7.8 Hz, 2H), 7.79 (dd, *J* = 2.4 Hz, 8.4 Hz, 2H), 7.96 (d, *J* = 7.8 Hz, 1H), 8.05 (d, pyridine-CH, *J* = 8.7 Hz, 1H), 8.11 (d, *J* = 7.8 Hz, 1H), 8.90 (d, *J* = 5.1 Hz, 1H), 9.11 (s, CH_2-amide, exchangeable with D_2O, 1H), 10.95 (s, Ar-amide, exchangeable with D_2O, 1H); m.f. $C_{23}H_{14}N_4O_2Cl_3F$ (m.w. 503.74). Mass *m/z* (%): 504.52 $(M+H)^+$.

Cultivation of mycobacterium: Microbial strains such as *Mycobacterium tuberculosis* H37Ra (ATCC 25177) was obtained from Astra-Zeneca, India. The stock culture was maintained at -80 ºC and subcultured once in a liquid medium before inoculation into an experimental culture. Cultures were grown in Dubos media (enrichment media). For antimycobacterial assay, *M. pheli* medium (minimal essential medium) was used. It contains 0.5 g KH_2PO_4, 0.25 g trisodium citrate, 60 mg $MgSO_4$, 0.5 g aspargine and 2 mL glycerol in distilled water (100 mL) followed by pH adjustment to 6.6. All bacterial stock cultures were first grown in Dubos media at 37 ºC at 150 rpm. It takes at least 8-10 days for optical density 1 at 620 nm. The antimycobacterial assay was performed in 96-well plates for active as well as dormant stages.

Antitubercular activity: The antitubercular activity for each synthesized compound was determined by measuring inhibition of growth against a virulent strain of *M. tuberculosis* H37Ra (MTB, Dormant stage) (ATCC 25177) in liquid medium

using an established XTT Reduction Menadione assay (XRMA) method. The optical density was read on a micro plate reader (Spectramax plus384 plate reader, Molecular Devices Inc) at 470 nm filter against a blank prepared from cell-free wells. Absorbance given by cells treated with the vehicle alone was taken as 100 % cell growth. Initially primary screening was done at 30, 10 and 3 µg/mL. Compounds showing 90 % inhibition of bacilli at 30 µg/mL which were selected for further dose response curve. MIC and IC_{50} values of selected compound were calculated from their dose response curves (30 to 0.23 µg/mL) by using Origin 6 software. Percent inhibition was calculated by using the following formula:

$$\text{Inhibition } (\%) = \frac{(A_{control} - A_{test})}{(A_{control} - A_{blank})} \times 100$$

where, control is the medium with bacilli along with vehicle and blank is cell free medium. The drug in clinical use, rifampicin was used as reference.

Antibacterial activity: Bacterial strains *E. coli* (NCIM 2688), *P. aeruginosa* (NCIM 2036) as Gram-negative and *B. subtilus* (NCIM 2079), *S. aureus* (NCIM 2010) as Gram-positive were obtained from NCIM (NCL, Pune) and were grown in Luria Burtony medium from Himedia, India. Once the culture reaches 0.1 $O.D_{620}$, it was used for anti-bacterial assay. Briefly, 0.1 OD_{620} bacterial culture was treated with synthesized compound at 30, 10 and 3 µg/mL concentration and incubated for 8 h at 37 ºC. Ampicillin served as positive control for antibacterial activity.

Cytotoxicity assay: Three human cancer cell lines, HeLa (human cervical cancer), THP-1 (human monocytic leukemia) cell line were used to check the cytotoxicity of compounds. The cell lines were obtained from the National Center for Cell Science (NCCS), Pune and maintained in T25 flasks with 10 % (v/v) fetal bovine serum (FBS) containing Dulbecco's Modified Eagle Medium (DMEM). Cell line containing T25 flasks were maintained at 37 ºC under 5 % CO_2 and 95 % air in a humidified atmosphere. Medium were replaced twice a week. All the compounds were tested for their cytotoxicity against the mentioned cell line by using modified MTT assay. Briefly, HeLa and MCF-7 cells were seeded at the density of 1×10^4 cells/mL in a 96 well plate. The plates were incubated overnight into CO_2 incubator (37 ºC under 5 % CO_2 and 95 % air in a humidified atmosphere) to adhere the cells. Next day, cells were treated with different concentration of test compounds (30 µg/mL) and incubated for additional 48 h. Post incubation, cell medium was replaced with MTT (0.5 mg/mL) PBS medium and incubated for 2-4 h to form the reduced MTT or formazan crystals. The reduced MTT or formazan crystals were solubilized by addition of acidified isopropanol. The optical density was read on a micro-plate reader (Spectramax plus384 plate reader, Molecular Devices Inc) at 570 nm filter against a blank prepared from cell-free wells. Absorbance given by cells treated with the vehicle alone was taken as 100 % cell growth. IC_{50} and MIC values were calculated by plotting the graphs, by using Origin Pro software. The viability and growth in the presence of test material is calculated by using the following formula:

$$\text{Cytotoxicity } (\%) = \frac{(A_{control} - A_{test})}{(A_{control} - A_{blank})} \times 100$$

where, control is the culture medium with cells and DMSO and blank is the culture medium without cells.

Molecular docking: Crystal structure of *M. tuberculosis* ATP synthase (PDB: 5KWA) downloaded from www.rcsb.org was used for docking studies. Docking simulation was carried out using Autodock Vina [34]. The 2D structures of all the compounds were drawn with MarvinSketch 5.6.0.0 (2011) and subsequently converted into 3D geometry. The geometry of 3D molecules was optimized using UCSF Chimera 1.8 [35]. Gasteiger charges were added during geometry optimization and energy minimization was carried out with the combination of steepest descent and conjugate gradient geometry search criteria until gradient converses to 0.05 and 0.001, respectively. The crystal structure of ATP synthase was refined by removing water and other non-standard residues. The crystal structure was further curetted by energy minimization in UCSF Chimera with Amber ff12SB force field. During docking simulation polar hydrogen were added to protein structure. Grid box of size 14 × 14 × 14 with 1 Å spacing was chosen to cover the binding site of the protein. The grid box was centered on the postion of co-crystallized adenosine diphosphate (ADP). Docking protocol was validated for its use in calculating the binding free energy values for test compounds. Autodock Vina docking program is known for its speed and accuracy but the prepration of protein structure, optimization of the ligand structures and defining the appropriate grid box so as to encompass the entire binding site of protein is also crucial docking. The validation of docking procedure involves testing the docking programs ability to predict the crystallographic or experimental pose of ligand. The spatial similarity between predicted pose and crystallographic pose is generally measured by RMSD value. The docking protocol is considered to be validated if the RMSD between experimantal and predicted pose is below 2 Å [36]. The RMSD between crystallographic and predicted pose of ADP was found to be 0.929 Å (Fig. 3). The test compounds were docked using similar validated docking protocol at the binding site of ATP synthase. LigPlot+ program was utilized to conduct interaction analysis and PyMol was employed to render the 3D molecular graphics illustration [37,38]. Docking simulation results were analyzed by comparing the binding free energy in Kcal/mol and interactions of ligands with residues at the active site.

RESULTS AND DISCUSSION

In continuation to our ongoing research on the synthesis of biologically active compounds [39-44] in the present work, we have reported the design, synthesis and anti-*Mycobacterium tuberculosis*, cytotoxicity and molecular docking study of series of novel structurally diverse side chain modified 4-aminoquinoline derivatives (**Schemes I-III**).

The detailed synthetic pathway for quinoline derivatives is depicted in **Scheme-III**. The synthesis of quinoline *N*-oxide (**2**) was conducted from commercially available quinoline using H_2O_2, in acetic acid at 60-65 ºC. The quinoline-*N*-oxide (**2**) on nitration at 65 ºC afforded 4-nitroquinoline *N*-oxide (**3**) which on reaction with Fe/AcOH afforded 4-aminoquinoline (**4**) in 89.41% yield (**Scheme-I**). The compound **6** was obtained by the straight forward reaction of compound **5** with *N*-hydroxy

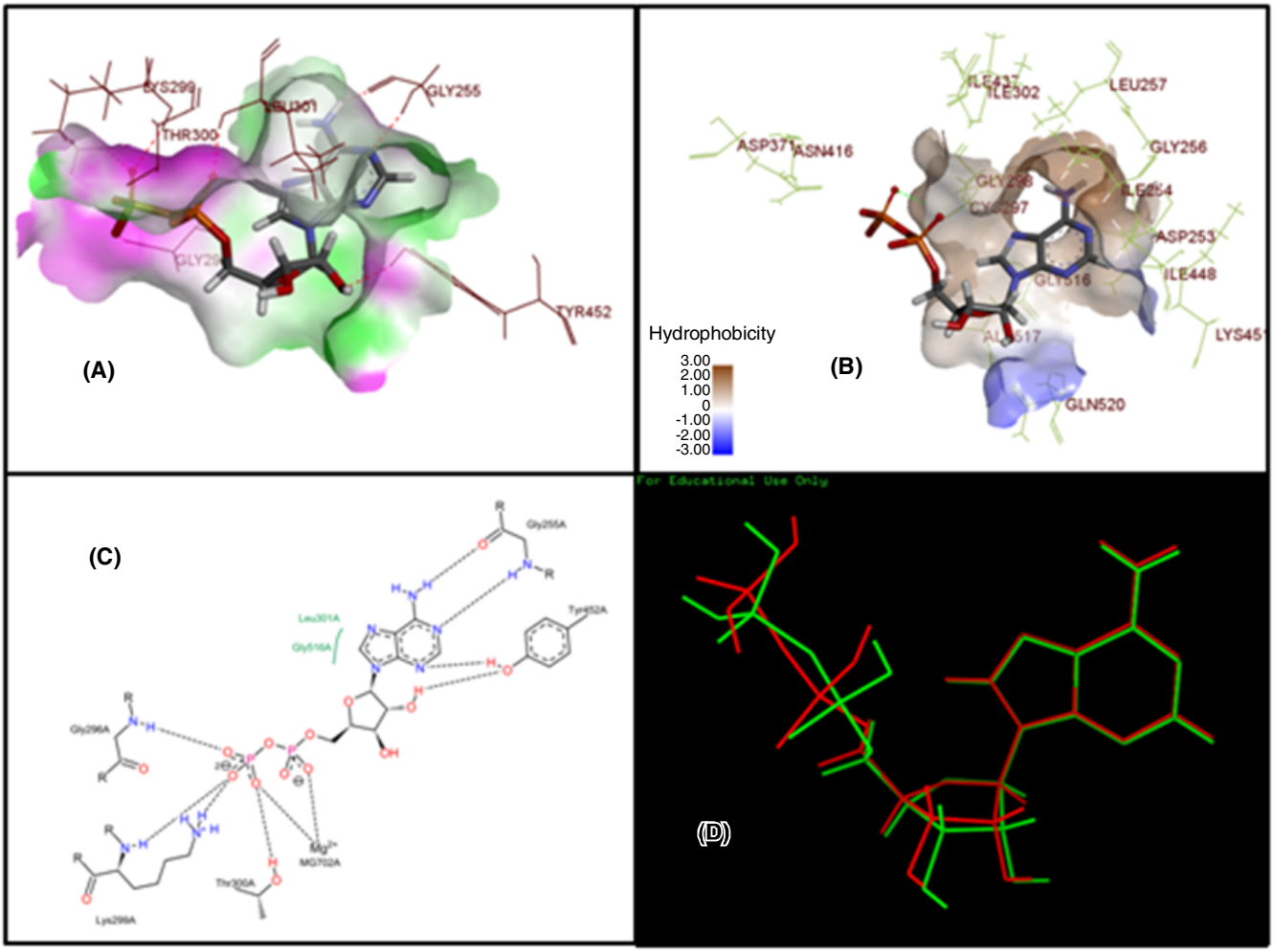
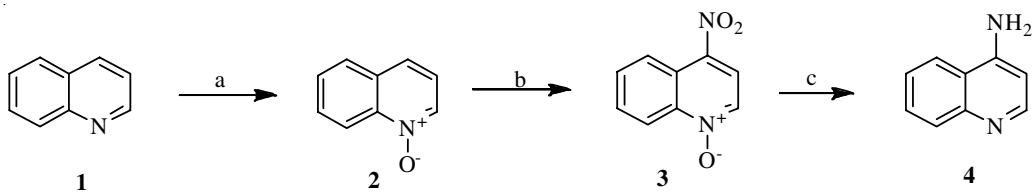
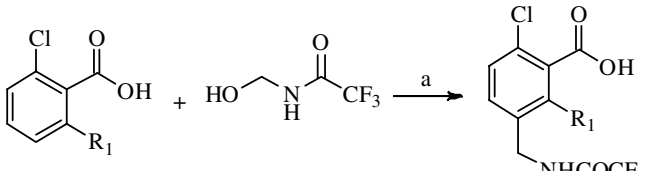


Fig. 3. Binding mode of ADP at the binding site of ATP synthase. A) ADP at the binding site surrounded by residues forming hydrogen bonds; B) ADP at the binding site surrounded by residues forming hydrophobic interactions; C) Poseview image of ADP interactions; D) validation of docking protocol: crystalographic pose of ADP in green line, docked pose of ADP in red line



Reagents and conditions: a) H_2O_2, (35 % in water), AcOH, 60-65 °C, 8 h; b) Conc. H_2SO_4, Conc. HNO_3, 65 °C, 2 h; c) Fe/AcOH, reflux, 2 h

Scheme-I: Synthesis of 4-aminoquinoline



5a:. R1 = H;
5b: R1 = F;

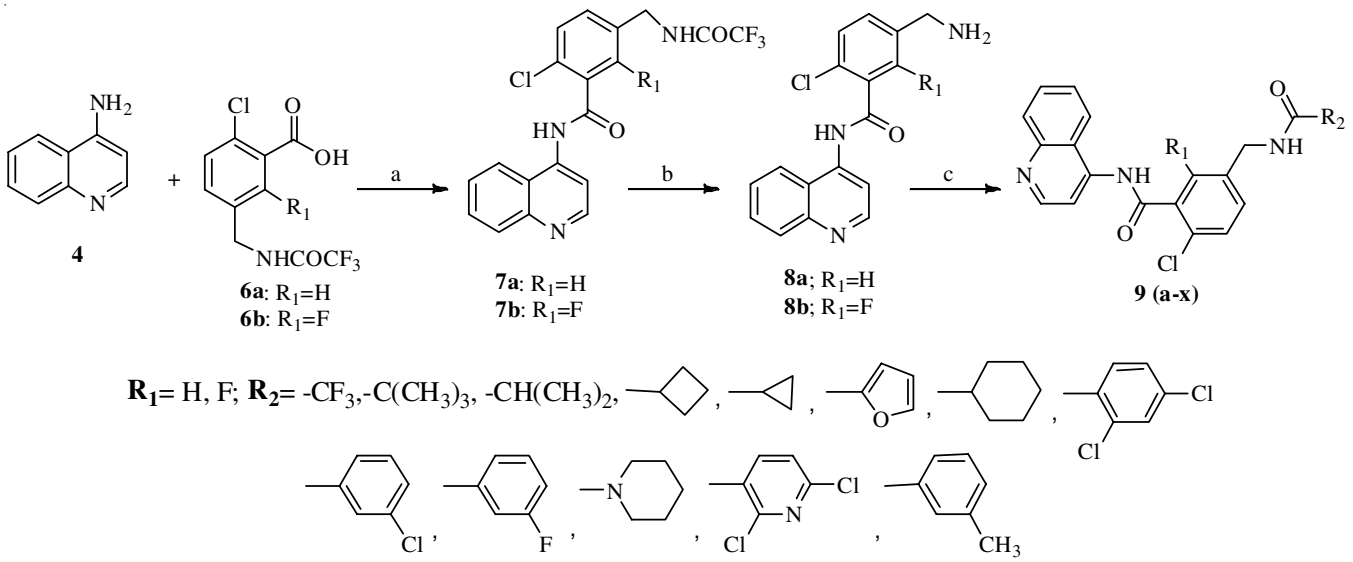
6a:. R1 = H;
6b: R1 = F;

Reagents and conditions: a) Conc. H_2SO_4, 2,2,2-trifluoro-*N*-(hydroxymethyl)acetamide, 25 °C, 12 h

Scheme-II: Synthesis of 5-[(2,2,2-trifluoroacetamido)methyl]-2-chlorobenzoic acid derivatives

methyl trifluoroacetamide in conc. H_2SO_4 at ambient temperature. Further, compound **6** on reaction with oxalyl chloride in dichloromethane gives acid chloride followed by the reaction with 4-aminoquinoline in alkaline medium gave compound **7**. The deprotection of compound **7** in alkaline media resulted in corresponding amides (**8**). The compound **8** on reaction with acids gave compound **9(a-x)** in good yields (Table-1). The purification of all the synthesized compounds was carried out by column chromatography using methanol:dichloromethane (2 %) as eluent system.

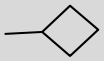
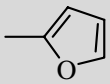
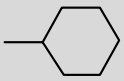
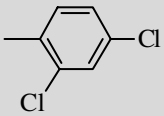
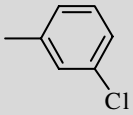
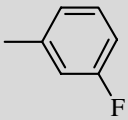
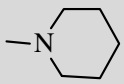
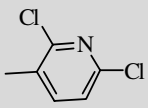
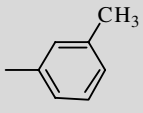
All the synthesized compounds were characterized by IR, ^{1}H NMR and mass spectral analysis. IR spectrum of compound

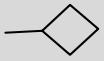
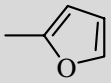
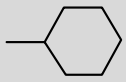
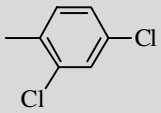
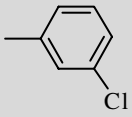
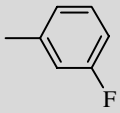
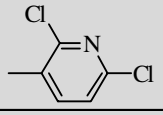


Reagents and conditions: a) i) Oxalyl chloride, CH_2Cl_2, 25 °C, 3 h; ii) TEA, acid chloride, THF, 50 °C, 4 h; b) NaOH, THF, 25 °C, 1 h; c) TEA, R_2COCl, 0-25 °C, DCM, 4 h

Scheme-III: Synthesis of 2-chloro-5-[(aryl carboxamido)methyl]-*N*-(quinolin-4yl)benzamide derivatives

TABLE-1
SYNTHESIS OF SOME NOVEL SIDE CHAIN MODIFIED 4-AMINOQUINOLINE DERIVATIVES (**9a-x**)

Entry	R_1	R_2	Time (h)	Yield (%)[a,b,c]	m.p. (°C)
9a	H	$-CF_3$	3.0	95	162-163
9b	H	$-C(CH_3)_3$	2.5	97	170-171
9c	H	$-CH(CH_3)_2$	2.6	94	176-177
9d	H		2.6	92	180-181
9e	H		2.4	89	184-185
9f	H		3.2	84	197-198
9g	H		3.0	93	168-169
9h	H		4.0	89	202-203
9i	H		4.0	91	181-182
9j	H		4.1	95	185-186
9k	H		4.5	75	167-168
9l	H		5.0	82	198-199
9m	H		4.5	87	178-179
9n	F	$-CF_3$	3.0	92	168-169
9o	F	$-C(CH_3)_3$	2.5	96	174-175

9p	F	-CH(CH$_3$)$_2$	2.6	90	179-180
9q	F		2.6	92	182-183
9r	F		2.4	85	180-181
9s	F		3.2	87	199-200
9t	F		3.0	90	172-173
9u	F		4.0	85	204-205
9v	F		4.0	86	183-184
9w	F		4.1	89	187-188
9x	F		4.5	82	170-171

[a]Isolated yields of the products. [b]All the products were characterized by IR, ^{1}H NMR, ^{13}C NMR and Mass.

9a showed absorption at 3285 cm^{-1} due to amide (-NH strechhing), 3035 cm^{-1} due to C-H stretching of alkane, band at1728 & 1651 cm^{-1} due to C=O stretching of amide group, 1531 cm^{-1} indicates presence of aromatic C-C stretching, IR band at 1219 cm^{-1} due to C-N stretching frequency.

All the newly synthesized compounds (**9a-x**) were tested against a virulent strain MTB H37Ra (ATCC 25177) using XTT reduction menadione assay (XRMA) and against *M. bovis* BCG (ATCC 35734) using nitrate reductase assay in liquid *M. pheli* medium, both of them were developed earlier in our lab. Clinically used drug, rifampicin was used as reference standards. The primary screening results showed that four compounds **9p**, **9r**, **9t** and **9u** in particular exhibit > 80 % inhibitory activity against MTB H37Ra and two compounds **9e** and **9t** exhibit > 80 % against *M. bovis* BCG strain. Compounds **9e**, **9p**, **9r**, **9t** and **9u** have been selected for further evaluation to dose response and the IC_{50} and MIC_{90} (minimum concentration bringing 90 % inhibition) values are presented in Table-2. 4-Aminoquinoline with fluorine (R_1) and cyclopropyl (R_2) substitution (**9r**) appear to provide strong activity against dormant MTB H37Ra (IC_{50} = 1.881 μg/mL). Compounds **9t** having R_1 = -F and R_2 = cyclohexyl substituent showed good activity against MTB H37Ra as well as BCG with IC_{50} value of 2.192 μg/mL and 9 μg/mL, respectively. Compounds **9u** (R_1 = F, R_2 = 3-fluorophenyl) and **9p** (R_1 = F, R_2 = isopropyl) showed moderate activity with IC_{50} of 2.505 and 8.971 μg/mL, respectively. Importantly, compound **9e** (R_1 = F, R_2 = cyclopropyl) showed activity only against BCG but not against MTB H37Ra.

All the compounds (**9a-x**) were further screened against Gram +ve (*B. subtilis* and *S. aureus*) and Gram -ve (*E. coli* and *P. aeroginosa*) bacteria to asses there selectivity towards MTB. None of the compound showed significant activity towards any of the screened bacterial strain.

TABLE-2
IC_{50} AND MIC VALUES OF THE ACTIVE COMPOUNDS FROM PRIMARY SCREENING AGAINST MTBH_{37}Ra AND Bovis BCG

Entry	MTB H37Ra		BCG	
	MIC	IC_{50}	MIC	IC_{50}
9e	> 30	> 30	29.57	15.58
9p	> 30	8.97	> 30	27.38
9r	> 30	1.88	> 30	25.99
9t	> 30	2.19	29.69	9
9u	> 30	2.51	> 30	> 30

The cytotoxicity of most potent antitubercular agents from *in vitro* MTBH37Ra and BCG screening (**9e**, **9p**, **9r**, **9t** and **9u**) was further assessed against three human cancer cell lines HeLa (human epithelial cervical cancer), MCF-7 (human mammary epithelial cells) and THP-1 (acute monocytic leukemia) using modified MTT cell viability assay. Notably, none of the tested compound show cytotoxicity (GI_{90} > 30 μg/mL) against these cell lines (Table-3). These results suggest the selectivity of the compounds towards MTB.

Due to restricted resources available in our laboratory for carrying out enzyme-based experimental studies and our curiosity to find out the best possible mode of action of synthesized 4-aminoquinoline analogues, we performed docking studies against *M. tuberculosis* ATP synthase enzyme. The binding pocket of ATP synthase consists of a various key residues which make the hydrogen bond and the hydrophobic π-π stacking interactions with ADP hydrogen bond acceptor/donor atoms and purine ring, respectively. As shown in Fig. 3, the residues Tyr452, Gly255, Thr300, Lys299, Gly296, and Leu301 make

TABLE-3
GROWTH INHIBITION GI_{50} AND GI_{90} IN PANEL OF CELL LINES BY COMPOUNDS

Entry	HeLa		MCF-7		THP-1	
	GI_{50}	GI_{90}	GI_{50}	GI_{90}	GI_{50}	GI_{90}
9e	> 30	> 30	> 30	> 30	> 30	> 30
9k	> 30	> 30	> 30	> 30	> 30	> 30
9p	> 30	> 30	> 30	> 30	> 30	> 30
9r	> 30	> 30	> 30	> 30	> 30	> 30
9t	> 30	> 30	> 30	> 30	> 30	> 30
9u	> 30	> 30	> 30	> 30	> 30	> 30
9x	> 30	> 30	> 30	> 30	> 30	> 30

hydrogen bond interactions. The binding pocket where the purine ring of ADP binds is surrounded by residues Tyr452, Gly255 and Leu301. This pocket is reasonably hydrophobic due to the presence of residues involved in hydrophobic interactions like Leu257, Gly256, Ile254, Ile448, Lys451, Gly298 and Cys297 which are buried in this pocket. The residues Thr300, Lys299 and Gly296 form a hydrophilic pocket where the ribose and diphosphate group of ADP bind. The nucleophilic residues Asp371 and Asn416 are involved in slow hydrolysis of ATP reside near to this hydrophilic pocket. The optimized structure of ADP could be docked with our chosen docking protocol, in the binding site of ATP synthase, which generated almost similar pose as that of crystallographic pose of ADP with RMSD below 1 Å (Fig. 3). When the synthesized compounds were docked into this binding site, the most active compounds **9p**, **9r**, **9t** and **9u** were found making almost similar type of interactions (Fig. 4) as that of ADP. Careful observation of interaction data from Table-4 clearly indicates that the most active compound against *M. tuberculosis*, compound **9r** from present series forms hydrogen bonding interaction with Tyr452, Gly296, Gly298, Leu301, Thr300 residues. The AQN core part of this compound binds in the hydrophilic binding pocket and the quinoline ring forms the π-π stacking interaction with Lys299. The central CPH fragment forms π-π stacking interaction with Leu301. Interestingly, methyl acetamide substituent (MAS) bearing hydrophobic cyclopropyl ring binds in the pocket where the purine ring binds and forms the hydrophopic interactions with the residues Ile448, Ile254, Leu257. The other active compounds **9t**, **9u** and **9P** also form similar hydrogen bond interactions and hydrophobic interactions at the binding site. In compound **9t**, cyclohexyl ring on MAS part could not produce the latter hydrophobic interactions and may be thus less active than the compound **9r**. In compound **9u**, 2,4-dichlorophenyl substituent forms hydrophobic interactions with the residues Leu301, Leu257 and Ile448. The presence of chloro groups on phenyl ring orients the compound in less favourable pose at the binding site. Similarly, in compound **9x**, 2,4-dichloropyridyl substituent may be reasoned for its less activity. The compound **9p** with isopropyl substituent on the MAS part was also found not forming any hydrophobic interactions in this pocket. The compound **9k** with 1-piperidinyl substituent forms hydrophobic interactions with Ile254,

TABLE-4
DOCKING STUDY PARAMETERS FOR THE SYNTHESIZED COMPOUNDS

Compd.	Binding free energy (kcal/mol)	Hydrogen bond	Residues in hydrophobic interaction or Van der Waals contact with the core parts of molecules		
			AQN	CPH	MAS
9a	-7.9	–	Lys299, Glu372	Leu301	ASP253, Gln520
9b	-7.2	Thr300	Ala517, Leu301	Gly296	–
9c	-7.3	Asn416, Gly298, Tyr452	Lys299, Gly296	Ala517, Leu301	–
9d	-7.7	Gly296, Tyr452	Asp371, Lys299	Leu301, Ala517	Ile448, Ile254, Leu257
9e	-8.1	Glu520, Tyr452, Asp253	Val249, Lys451	Leu301	Ile448, Ile254
9f	-8	Glu520, Gly255, Asp253	Val249, Lys451	Leu301	Ile448
9g	-8	Glu520, Asp253	Lys451	Leu301	Ile448, Ile254
9h	-7.1	–	–	–	Ala517, Gly516, Leu301, Tyr452, Ile448
9i	-7.9	–	Ala517	Cys297, Ile448, Leu301	Val249
9j	-7	–	Lys299	Gly296, Ala517	Val249
9k	-7.9	Gln520, Tyr452, Asp371, Thr300	Lys299	Leu301	Ile448, Ile254
9l	-7.8	Asp371	Glu372, Lys299	Ala517,	Tyr452, Val249
9m	-8	Asp253	Val249	Leu301	Ala517, Gly516, Ile448, Lys451
9n	-8	Thr300, Asn416	Gln520	Leu301	–
9o	-6.9	–	–	Leu301, Lys304, Val249	–
9p	-8.2	Tyr452, Gly296, Thr300, Leu301, Gly298, Asp371	Lys299	–	–
9q	-7.7	Gln520	–	Ala517, Gly296, Leu301	Ile254, Leu257, Ile448
9r	-8.4	Tyr452, Gly296, Gly298, Leu301, Thr300	Lys299	–	Ile448, Ile254, Leu257
9s	-7.1	Gly298, Lys299, Thr300	Leu301	Ala517	Lys299
9t	-8.3	Gln520, Tyr452, Thr300, Asp371	Lys299	Leu301	Val249
9u	-8.3	Tyr452	Glu372, Lys299	Ala517, Gln520	Ile448, Leu257, Leu301
9v	-7	Asn416	Lys299, Gly296	Ala517	Leu301, Val249
9w	-7	Glu372	Leu301	Ala517	Lys299, Asn416, Asp371
9x	-8	Gly296	Val249, Leu301	Ala517	Ala414, Lys299

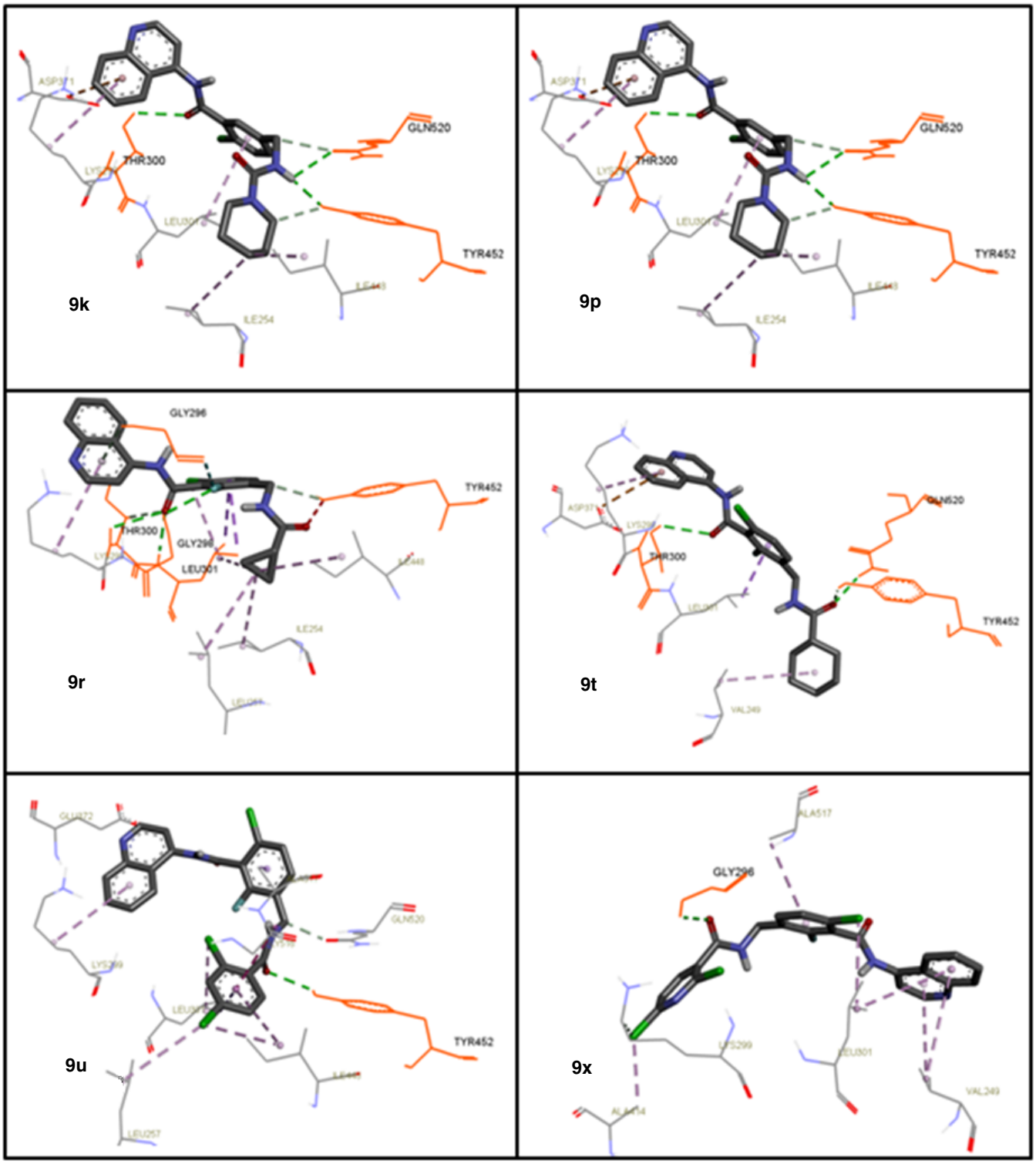


Fig. 4. Illustration showing docked complexes of promising 4-aminoquinoline compounds (residues involved in hydrogen bonds are depicted in orange shade)

Ile448 and Leu301, but it lacks fluoro atom which is essential in some key interactions at the binding site. As shown in docking data in Table-4, all other compounds either lacks hydrogen bond interactions with the key residues or lacks the hydrophobic interactions of the central CPH fragment and MAS substituent with the hydrophobic residues at the binding site. These observations demonstrate the trivial role of central CPH fragment and MAS substituent and thus might be replaced with more suitable functional groups in further optimization of series. However, AQN core part appears to play an important role in antitubercular effect of newly synthesized 4-amino-quinoline compounds and thus must be retained during advance activity optimization procedure. It is also to be noted that promising anti-tubercular compound **9r** (IC_{50} value 1.88) also demonstrates best free energy value (-8.4 Kcal/mol) amongst the tested compounds, followed by comparable results for

compound **9e**, **9k**, **9p**, **9r** and **9x**. The binding free energy values are in good agreement with the experimental antitubercular activity of compounds. Other compounds shows moderate to weak activity in antitubercular screening and as evident from the docking studies these compounds could not form the key hydrogen bonding or hydrophobic interactions at the binding site of ATP synthase.

Conclusion

In summary, we have synthesized a series of structurally diverse 4-aminoquinoline derivatives and evaluated their antitubercular activity. The synthesized compound demonstrated significant antimycobacterial activity in both MtbH37Ra (**9p**, **9r**, **9t** and **9u**) and BCG (**9e**, **9k**, **9p**, **9r**, **9t** and **9x**) with negligible cytotoxicity. The docking studies against ATP synthase revealed that these most active compounds showed hydrogen bonding and hydrophobic interactions with the key residues at the binding site of ATP synthase. It suggests that mechanism of action for the antitubercular activity of these compounds might be through inhibition of *M. tuberculosis* ATP synthase. So, these compounds can be further optimized for drug development which can give promising chemical leads for treatment of tuberculosis.

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REFERENCES

1. L.E. Cowen, The Evolution of Fungal Drug Resistance: Modulating the Trajectory from Genotype to Phenotype, *Nat. Rev. Microbiol.*, **6**, 187 (2008); https://doi.org/10.1038/nrmicro1835.
2. X.-D. Wang, W. Wei, P.-F. Wang, Y.-T. Tang, R.-C. Deng, B. Li, S.-S. Zhou, J.-W. Zhang, L. Zhang, Z.-P. Xiao, H. Ouyang and H.-L. Zhu, Novel 3-Arylfuran-2(5*H*)-one-fluoroquinolone Hybrid: Design, Synthesis and Evaluation as Antibacterial Agent, *Bioorg. Med. Chem.*, **22**, 3620 (2014); https://doi.org/10.1016/j.bmc.2014.05.018.
3. M.L. Cristina, A.M. Spagnolo, N. Cenderello, P. Fabbri, M. Sartini, G. Ottria and P. Orlando, Multidrug-Resistant *Acinetobacter baumannii* Outbreak: An Investigation of the Possible Routes of Transmission, *Public Health*, **127**, 386 (2013); https://doi.org/10.1016/j.puhe.2013.01.025.
4. R. Zarrilli, S. Pournaras, M. Giannouli and A. Tsakris, Global Evolution of Multidrug-Resistant *Acinetobacter baumannii* Clonal Lineages, *Int. J. Antimicrob. Agents*, **41**, 11 (2013); https://doi.org/10.1016/j.ijantimicag.2012.09.008.
5. A. Mital, V.S. Negi and U. Ramachandran, Synthesis and Antimycobacterial Activities of Certain Trifluoromethylaminoquinoline Derivatives, *ARKIVOC*, **10**, 220 (2006); https://doi.org/10.3998/ark.5550190.0007.a25.
6. E.L. Corbett, C.T.J. Watt, N. Walker, D. Maher, B.G. Williams, M.C. Raviglione and C. Dye, The Growing Burden of Tuberculosis Global Trends and Interactions With the HIV Epidemic, *Arch. Intern. Med.*, **163**, 1009 (2003); https://doi.org/10.1001/archinte.163.9.1009.
7. World Health Organization, 'The WHO Global Tuberculosis Program', (2003); http://www.who.int/gtb/.
8. S. Houston and A. Fanning, Current and Potential Treatment of Tuberculosis, *Drugs*, **48**, 689 (1994); https://doi.org/10.2165/00003495-199448050-00004.
9. D. Alland, G.E. Kalkut, A.R. Moss, R.A. Mc Adam, J.A. Hahn, W. Bosworth, E. Drucker and B.R. Bloom, Transmission of Tuberculosis in New York City-An Analysis by DNA Fingerprinting and Conventional Epidemiologic Methods, *Engl. J. Med. Chem.*, **330**, 1710 (1994); https://doi.org/10.1056/NEJM199406163302403.
10. A.C. Weltman and D.N. Rose, Tuberculosis Susceptibility Patterns, Predictors of Multidrug Resistance and Implications for Initial Therapeutic Regimens at a New York City Hospital, *Arch. Intern. Med.*, **154**, 2161 (1994); https://doi.org/10.1001/archinte.1994.00420190058007.
11. A. Mahapatra, S.P.N. Mativandlela, B. Binneman, P.B. Fourie, C.J. Hamilton, J.J.M. Meyer, F. van der Kooy, P. Houghton and N. Lall, Activity of 7-Methyljuglone Derivatives Against *Mycobacterium tuberculosis* and as Subversive Substrates for Mycothiol Disulfide Reductase, *Bioorg. Med. Chem.*, **15**, 7638 (2007); https://doi.org/10.1016/j.bmc.2007.08.064.
12. M. Font, A. Monge, T. Ruiz and B. Heras, Structure-Activity Relationships in Quinoline Reissert Derivatives with HIV-1 Reverse Transcriptase Inhibitory Activity, *Drug Des. Discov.*, **14**, 259 (1997).
13. T. Nakamura, M. Oka, K. Aizawa, H. Soda, M. Fukuda, K. Terashi, K. Ikeda, Y. Mizuta, Y. Noguchi, Y. Kimura, T. Tsuruo and S. Kohno, Direct Interaction between a Quinoline Derivative, MS-209, and Multidrug Resistance Protein (MRP) in Human Gastric Cancer Cells, *Biochem. Biophys. Res. Commun.*, **255**, 618 (1999); https://doi.org/10.1006/bbrc.1999.0245.
14. D. Kaminsky and R.I. Meltzer, Quinoline Antibacterial Agents. Oxolinic Acid and Related Compounds, *J. Med. Chem.*, **11**, 160 (1968); https://doi.org/10.1021/jm00307a041.
15. R. Musiol, J. Jampilek, V. Buchta, L. Silva, H. Niedbala, B. Podeszwa, A. Palka, K. Majerz-Maniecka, B. Oleksyn and J. Polanski, Antifungal Properties of New Series of Quinoline Derivatives, *Bioorg. Med. Chem.*, **14**, 3592 (2006); https://doi.org/10.1016/j.bmc.2006.01.016.
16. N.C. Warshakoon, J. Sheville, R.T. Bhatt, W. Ji, J.L. Mendez-Andino, K.M. Meyers, N. Kim, J.A. Wos, C. Mitchell, J.L. Paris, B.B. Pinney, O. Reizes and X.E. Hu, Design and Synthesis of Substituted Quinolines as Novel and Selective Melanin Concentrating Hormone Antagonists as Anti-Obesity Agents, *Bioorg. Med. Chem. Lett.*, **16**, 5207 (2006); https://doi.org/10.1016/j.bmcl.2006.07.006.
17. A.E. Slobda, D. Powell, J.F. Poletto, W.C. Pickett, J.J. Gibbons Jr., D.H. Bell, A.L. Oronsky and S.S. Kerwar, Antiinflammatory and Antiarthritic Properties of a Substituted Quinoline Carboxylic Acid, *J. Rheumatol.*, **18**, 855 (1991).
18. M.V.N. de Souza, K.C. Pais, C.R. Kaiser, M.A. Peralta, M. de L. Ferreira and M.C.S. Lourenço, Synthesis and *in vitro* Antitubercular Activity of a Series of Quinoline Derivatives, *Bioorg. Med. Chem.*, **17**, 1474 (2009); https://doi.org/10.1016/j.bmc.2009.01.013.
19. M. Foley and L. Tilley, Quinoline Antimalarials: Mechanisms of Action and Resistance and Prospects for New Agents, *Pharmacol. Ther.*, **79**, 55 (1998); https://doi.org/10.1016/S0163-7258(98)00012-6.
20. K. Kaur, M. Jain, R.P. Reddy and R. Jain, Quinolines and Structurally Related Heterocycles as Antimalarials, *Eur. J. Med. Chem.*, **45**, 3245 (2010); https://doi.org/10.1016/j.ejmech.2010.04.011.
21. A.P. Gorka, A. de Dios and P.D. Roepe, Quinoline Drug-Heme Interactions and Implications for Antimalarial Cytostatic *versus* Cytocidal Activities, *J. Med. Chem.*, **56**, 5231 (2013); https://doi.org/10.1021/jm400282d.
22. E. Berning, The Role of Fluoroquinolones in Tuberculosis Today, *Drugs*, **61**, 9 (2001); https://doi.org/10.2165/00003495-200161010-00002.
23. T.E. Renau, J.P. Sanchez, M.A. Shapiro, J.A. Dever, S.J. Gracheck and J.M. Domagala, Effect of Lipophilicity at N-1 on Activity of Fluoroquinolones against Mycobacteria, *J. Med. Chem.*, **38**, 2974 (1995); https://doi.org/10.1021/jm00015a021.
24. T.E. Renau, J.P. Sanchez, J.W. Gage, J.A. Dever, M.A. Shapiro, S.J. Gracheck and J.M. Domagala, Structure-Activity Relationships of the Quinolone Antibacterials against Mycobacteria:? Effect of Structural Changes at N-1 and C-7, *J. Med. Chem.*, **39**, 729 (1996); https://doi.org/10.1021/jm9507082.

25. R. Rustomjee, A.H. Diacon, J. Allen, A. Venter, C. Reddy, R.F. Patientia, T.C.P. Mthiyane, T. De Marez, R. van Heeswijk, R. Kerstens, A. Koul, K. De Beule, P.R. Donald and D.F. McNeeley, Early Bactericidal Activity and Pharmacokinetics of the Diarylquinoline TMC207 in Treatment of Pulmonary Tuberculosis, *Antimicrob. Agents Chemother.*, **52**, 2831 (2008); https://doi.org/10.1128/AAC.01204-07.
26. N. Phummarin, H.I. Boshoff, P.S. Tsang, J. Dalton, S. Wiles, C.E. Barry 3rd and B.R. Copp, SAR and Identification of 2-(Quinolin-4-yloxy)-acetamides as *Mycobacterium tuberculosis* Cytochrome bc_1 Inhibitors, *MedChemComm*, **7**, 2122 (2016); https://doi.org/10.1039/C6MD00236F.
27. P. Lu, H. Lill and D. Bald, ATP Synthase in Mycobacteria: Special Features and Implications for a Function as Drug Target, *Biochim. Biophys. Acta*, **1837**, 1208 (2014); https://doi.org/10.1016/j.bbabio.2014.01.022.
28. E. Segala, W. Sougakoff, A. Nevejans-Chauffour, V. Jarlier and S. Petrella, New Mutations in the Mycobacterial ATP Synthase: New Insights into the Binding of the Diarylquinoline TMC207 to the ATP Synthase C-Ring Structure, *Antimicrob. Agents Chemother.*, **56**, 2326 (2012); https://doi.org/10.1128/AAC.06154-11.
29. A. Koul, N. Dendouga, K. Vergauwen, B. Molenberghs, L. Vranckx, R. Willebrords, Z. Ristic, H. Lill, I. Dorange, J. Guillemont, D. Bald and K. Andries, Diarylquinolines Target Subunit c of Mycobacterial ATP Synthase, *Nat. Chem. Biol.*, **3**, 323 (2007); https://doi.org/10.1038/nchembio884.
30. R.S. Upadhayaya, J.K. Vandavasi, N.R. Vasireddy, V. Sharma, S.S. Dixit and J. Chattopadhyaya, Design, Synthesis, Biological Evaluation and Molecular Modelling Studies of Novel Quinoline Derivatives Against *Mycobacterium tuberculosis*, *Bioorg. Med. Chem.*, **17**, 2830 (2009); https://doi.org/10.1016/j.bmc.2009.02.026.
31. S. Singh, K.K. Roy, S.R. Khan, V.K. Kashyap, A. Sharma, S. Jaiswal, S.K. Sharma, M.Y. Krishnan, V. Chaturvedi, J. Lal, S. Sinha, A. Dasgupta, R. Srivastava and A.K. Saxena, Novel, Potent, Orally Bioavailable and Selective Mycobacterial ATP Synthase Inhibitors that Demonstrated Activity Against Both Replicating and Non-Replicating, *Bioorg. Med. Chem.*, **23**, 742 (2015); https://doi.org/10.1016/j.bmc.2014.12.060.
32. R.S. Upadhayaya, G.M. Kulkarni, N.R. Vasireddy, J.K. Vandavasi, S.S. Dixit, V. Sharma and J. Chattopadhyaya, *Bioorg. Med. Chem.*, **17**, 4681 (2009); https://doi.org/10.1016/j.bmc.2009.04.069.
33. Y. Wu, K. Hu, D. Li, L. Bai, S. Yang, J.B. Jastrab, S. Xiao, Y. Hu, S. Zhang, K.H. Darwin, T. Wang and H. Li, Design, Synthesis and Biological Evaluation of Novel Triazole, Urea and Thiourea Derivatives of Quinoline against *Mycobacterium tuberculosis*, *Mol. Microbiol.*, **105**, 227 (2017); https://doi.org/10.1111/mmi.13695.
34. O. Trott, A.J. Olson and A.D. Vina, AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization and Multithreading, *J. Comput. Chem.*, **31**, 455 (2010).
35. E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng and T.E. Ferrin, UCSF Chimera-A Visualization System for Exploratory Research and Analysis, *J. Comput. Chem.*, **25**, 1605 (2004); https://doi.org/10.1002/jcc.20084.
36. A. Bordogna, A. Pandini and L. Bonati, Predicting the Accuracy of Protein–Ligand Docking on Homology Models, *J. Comput. Chem.*, **32**, 81 (2011). https://doi.org/10.1002/jcc.21601.
37. R.A. Laskowski and M.B. Swindells, LigPlot+: Multiple Ligand–Protein Interaction Diagrams for Drug Discovery, *J. Chem. Inf. Model.*, **51**, 2778 (2011); https://doi.org/10.1021/ci200227u.
38. A.C. Wallace, R.A. Laskowski and J.M. Thornton, LIGPLOT: A Program to Generate Schematic Diagrams of Protein-ligand Interactions, *Protein Eng.*, **8**, 127 (1995); https://doi.org/10.1093/protein/8.2.127.
39. S.S. Gholap and S.R. Ugale, A Total Synthesis of the Cyclic Depsipeptide Chaiyaphumine-A, *Chem. Select*, **2**, 7445 (2017); https://doi.org/10.1002/slct.201701520.
40. S.R. Ugale and S.S. Gholap, An Efficient Synthesis of Structurally Diverse 2-Methyl-*N*-[(3-phenylamino)oxetan-3-yl]-2-propanesulfinamide Derivatives under Catalyst Free Conditions, *Chem. Pap.*, **71**, 2435 (2017); https://doi.org/10.1007/s11696-017-0237-1.
41. S.S. Gholap and N. Gunjal, 2,4,6-Trichloro-1,3,5-triazine (TCT) Mediated One Pot Direct Synthesis of N-benzoylthioureas from Carboxylic Acids, *Arab. J. Chem.*, **10**, S2750 (2017); https://doi.org/10.1016/j.arabjc.2013.10.021.
42. S.S. Gholap, Pyrrole: An Emerging Scaffold for Construction of Valuable Therapeutic Agents, *Eur. J. Med. Chem.*, **110**, 13 (2016); https://doi.org/10.1016/j.ejmech.2015.12.017.
43. V.D. Dhakane, S.S. Gholap, U.P. Deshmukh, H.V. Chavan and B.P. Bandgar, An Efficient and Green Method for the Synthesis of [1,3]Oxazine Derivatives Catalyzed by Thiamine Hydrochloride (VB1) in Water, *C.R. Chim.*, **17**, 431 (2014); https://doi.org/10.1016/j.crci.2013.06.002.
44. S.S. Gholap, V.D. Dhakane and S. Sandip, Solid-Supported Dichloro-[1,3,5]-triazine: A Versatile Synthetic Auxiliary for Direct Synthesis of N-Sulfonylamines from Sulphonic Acid and Amine, *Jordan J. Chem.*, **7**, 279 (2012).