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Graphical Abstract

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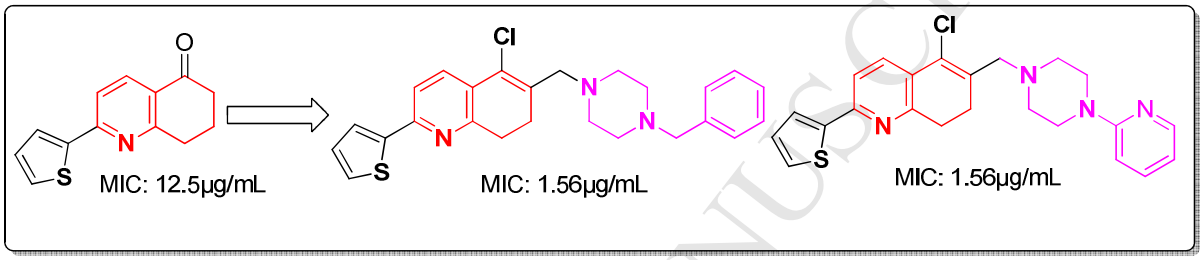

Synthesis of novel morpholine, thiomorpholine and N-substituted piperazine coupled 2-(thiophen-2-yl)dihydroquinolines as potent inhibitors of *Mycobacterium tuberculosis*

Sandeep Kumar Marvadi,[a] Vagolu Siva Krishna,[b] Dharmarajan Sriram,[b] Srinivas Kantevari,[*a,c]

[a] *Fluro & Agrochemicals Division, CSIR- Indian Institute of Chemical Technology, Hyderabad-500007, Telangana, INDIA.*

[b] *Medicinal Chemistry and Antimycobacterial Research Laboratory, Pharmacy Group, Birla Institute of Technology & Science-Pilani, Hyderabad Campus, Jawahar Nagar, Hyderabad-500078, Telangana, India.*

[c] *Academy of Scientific and innovative Research, CSIR- Indian Institute of Chemical Technology, Hyderabad-500 007, Telangana, India.*

Abstract

A series of novel morpholine, thiomorpholine and N-substituted piperazine coupled 2-(thiophen-2-yl)dihydroquinolines **7a–p** was designed and synthesized from 2-acetyl thiophene in six step reaction sequence involving modified Bohlmann-Rahtz and Vilsmeier-Haack-Arnold reactions as key transformations. 2-(Thiophen-2-yl)dihydroquinoline was formylated and subsequently chlorinated using DMF-$POCl_3$. The resulting aldehyde was reduced to give an alcohol and then converted to bromide using PBr_3. Further coupling of bromide with morpholine, thiomorpholine and N-substituted piperazines resulted in the desired quinolines **7a-p** in very good yields. All the new derivatives **7a–p** were characterized by their NMR and mass spectral analysis. *In vitro* screening of new compounds for antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv (MTB), resulted in two derivatives **7f** and **7p** as most potent antitubercular agents (MIC:1.56 µg/mL) with lower cytotoxicity profiles.

Keywords:

Thiophene; Piperazine; Quinoline; Mycobacterium tuberculosis; Vilsmeier-Haack-Arnold reaction

1. Introduction

Tuberculosis (TB) is one of the deadliest airborne infectious disease caused mainly by pathogen *Mycobacterium tuberculosis* (*Mtb*) [1,2]. According to *Global tuberculosis report* 2018 of the World Health Organization (WHO), an estimated 1.3 million deaths and approximately 10.4 million people were affected with tuberculosis in 2017 [3]. Today TB is responsible for a greater number of deaths than HIV and ranked as the leading killer in infectious diseases [3-8]. Further TB threat has acquired a new dimension with the emergence of both multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) [9-13]. In 2017, globally, 558,000 people developed TB that was resistant to first line anti TB drug rifampicin, and of these 82% had MDR & XDR -TB [3,14-16].

After 40 years of long wait, approval of Bedaquiline (Fig. 1), a quinoline based drug for treating multi drug resistant TB [17-19], have gained momentum to investigate various quinoline derivatives as antimycobacterial agents [20-25]. Previously we reported [26-32] that 2-substiuted dihydroquinolones exhibit potential antitubercular activity against H37RV. Also recent reports suggested that the groups like morpholine, piperazine helps improving pharmacological properties [33,34]. Our continued interest [35,36] in developing new antimycobacterial agents led to hybridization of 2-(thiophenyl)dihydroquinolines with morpholine, thiomorpholine, or N-substituted piperazines in one molecular platform to generate a new scaffold for biological evaluation. We herein report an efficient synthesis and evaluation of novel dihydroquinoline derivatives **7a-p** in excellent yields. Screening all sixteen new compounds for *in vitro* activity against *Mycobacterium tuberculosis* H37Rv resulted in two compounds **7f** and **7p** (MIC: 1.56 µg/mL) as most potent antitubercular agents.

< Figure 1 >

Broadly, the designed scaffold (Fig. 2) has two fragments. The first fragment is 2-(thiophen-2-yl)dihydroquinoline pharmacophore having antitubercular activity[36]. The second fragment is morpholine, thiomorpholine and piperazine pharmacophores present in clinical antitubercular drugs **II-VIII** (Figure.1). Combining these two fragments into single molecular frame resulted newer scaffold as depicted in figure 2. Alkyl, aryl, pyridinyl variants appended on piperazine unit could provide lipophilicity control in the proposed scaffold.

< Figure 2 >

2. Results and Discussion

2.1 Chemistry

Initiating the synthesis, (Scheme 1) β-enaminone **1** required was prepared by the condensation of 2-acetylthiophene with N,N-dimethylformamide dimethyl acetal (DMF-DMA) refluxing in xylene[37]. Enaminone **1** was reacted with cyclohexane-1,3-dione and ammonium acetate in the presence of $CeCl_3.7H_2O$-NaI at reflux in propan-2-ol to obtain the compound **2** in very good yield [36-38]. Reaction of **2** was subjected to Vilsmeier-Haack-Arnold reaction ($POCl_3$, dimethylformamide in $CHCl_3$ at 60 ^oC for 4 h) to afford compound **3** in 84% yield. **3** were fully characterized by ^1H and ^{13}C NMR, and mass (ESI-MS and HR-MS) spectral data.

< Scheme 1 >

The aldehyde **3** was reduced with sodium borohydride in methanol to give alcohol **4** in 88% yield. The alcohol **4** was converted to the respective bromide **5** in 82% yield using PBr_3 in diethyl ether at RT for 1 h. Further coupling of **5** with various amines **6a-p** (morpholine, thiomorpholine, N-substituted piperazines) in the presence of K_2CO_3 at RT in acetone resulted **7a-p** in very good yields (72-94%). All of the compounds **7a-p** were purified through silica gel column chromatography (HPLC purity >95%) and were fully characterized by IR, was fully characterized by ^1H and ^{13}C NMR, and mass (ESI-MS and HR-MS) spectral analysis. For example, ^1H NMR spectra of **7a** showed aromatic protons at δ 7.81 - 7.10, morpholine protons at δ 3.74 – 3.69 (4H) as multiplet (N-attached) and δ 2.54 – 2.47 (4H) as multiplet (O-attached), methylene protons ($-CH_2$) at δ 3.35 (2H) as singlet, triplet signals appeared for dihydroquinoline protons at δ 3.04 (J = 8.4 Hz, 2H), 2.66 (t, J = 8.4 Hz, 2H). ^{13}C NMR spectrum showed methylene carbon at δ 53.6, N-attached carbons in morpholine at δ 60.5 and O-attached carbons in morpholine at δ 66.9. HRMS analysis of **7a** displayed a molecular ion peak at m/z 347.09794 $[M+H]^+$ suggesting the molecular formula of $C_{18}H_{20}ON_2ClS$. Additionally, the IR spectra for the target compounds **7a-p** exhibited characteristic absorption bands at 1637-1688 cm^{-1}, 2958-2802 cm^{-1} and 1010 - 1245 cm^{-1} which corresponded to C=O, C-H and C-N respectively.

< Table 1 >

2.2 Pharmacological evaluation

2.2.1 Antimycobacterial activity

All the newly synthesized dihydroquinoline derivatives **7a–p** was screened for *in vitro* antimycobacterial activity against *M. Tuberculosis* H37Rv strain by micro plate alamar blue assay method [39]. Resazurin, used as an oxidation-reduction (REDOX) indicator in alamar

blue® assay undergoes colorimetric change in response to the proliferation of mycobacterium tuberculosis and was measured quantitatively. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound required to completely inhibit the bacterial growth. The MIC values (µg/mL) of **7a-p** along with isoniazid, Rifampicin, ciprofloxacin and ethambutol as standard drugs for comparison are presented in Table 1. All sixteen products **7a-p** screened have shown *in vitro* inhibitory activity against *Mtb* with MIC ranging from 1.56-25.0 µg/mL. With respect to parent compound 2 (MIC: 12.5 µg/mL) six derivatives **7a, 7d, 7f, 7k, 7m** and **7p** are more potent with lower MICs. When compared to first line anti-TB drugs, all the compounds are less potent than Isoniazid (0.1 µg/mL), and Rifampicin (0.2 µg/mL). Two compounds **7f** & **7p** (MIC: 1.56 µg/mL) are more potent than ethambutol and equipotent to another standard drug ciprofloxacin (MIC: 1.56 µg/mL). Compound **7d** (MIC: 3.125 µg/mL) is equipotent to ethambutol. Among other new analogues, three compounds **7a, 7k** and **7m** exhibited MIC 6.25 µg/ mL, and two compounds **7e, 7g** exhibited MIC 12.5 µg/mL, and eight compounds **7b, 7c, 7h, 7i, 7j, 7l, 7n, 7o** exhibited MIC 25.0 µg/mL.

Structure-activity relationship (SAR) of new compounds **7a-p** indicated interesting *in vitro* antimycobacterial activity patterns against *M. tuberculosis* H37Rv (MTB). To note that, thiomorpholine analog **7b is** less potent than parent 2-(thiophen-2-yl)dihydro quinoline **2** (MIC: 12.5 µg/mL) whereas morpholine analog **7a** exhibited better potency than both **2** & **7b**. The best active compounds are N-substituted piperazine analogs. Substation on piperazine played crucial role in the inhibition of MTB. For example, phenyl ring bearing 2-methoxy and 2-fluoro groups **7k & 7m** showed moderate activity (MIC: 6.25 µg/mL) and 1-cyclopropane carbonyl analog **7d** displayed good MTB inhibition (MIC: 3.125 µg/mL). Further replacement of phenyl unit with pyridine and benzyl groups resulted most potent analogs **7p** & **7f** with best MTB inhibition activity and low cytotoxicity.

Lipophilicity (CLogP) is one of the important physicochemical properties analyzed in antitubercular drug discovery to predict drug-like characteristics. In recent years, computational methods are being used in calculating lipophilicity measurements since experimental methods are cost intensive and time consuming. In general, Chemdraw® or quantitative structure–activity databases are used as state-of-the-art software for calculating cLogP values [41].These calculations are straightforward and provide the ClogP for a large number of molecules. Most of

the first line antitubercular drugs show low lipophilicity which includes isoniazid (cLogP = −0.67), ethambutol (cLogP = 0.12), pyrazinamide (cLogP = −0.68), and cycloserine (cLogP = −1.2). However, recent precedents suggested that highly lipophilic compounds should not be discarded in screening programs for antituberculosis drugs. We employed ChemDraw Professional 17.1 to calculate CLogP values for 2-(thiophene-2-yl)dihydroquinolines **7a–p** to assess their lipophilic characteristics. The data (Table 1) suggested that compounds described in this study has cLogP values in a range from 2.92 to 7.09. Compound **7d** (MIC: 3.125 µg/mL) has CLogP 4.20 which is in the limits of Lipinski rule. It is interesting to note that the ClogP values of the active molecules **7f** and **7p** are 6.32 and 5.08 respectively. The success of Bedaquiline and Delaminid as examples with lipophilicity (cLogP values of 7.3 and 5.6, respectively) [41, 42] prompted us to consider active molecules **7f** and **7p** as hit analogues for further *in vivo* and mechanistic evaluations. This information on structure/lipophilicity–antitubercular activity relationship could help further in generating drug like lead analogs.

2.2.2 *In vitro* cytotoxicity

In pre-clinical drug discovery, potential candidates are usually tested against mammalian cell lines such HEK-293T cells in order to assess any cytotoxic effects the compound could exert on the body's own cells. The safety profile of antitubercular active dihydroquinoline derivatives with MIC ≤6.25 µg/mL was assessed by testing *in vitro* cytotoxicity against Human Embryonic Kidney (HEK-293T) cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Reduction of a yellow tetrazolium salt (MTT) gives direct measure to cellular metabolic activity as a proxy for cell viability. The promising antitubercular compounds **7a**, **7d, 7f, 7k, 7m** and **7p** exhibited 34.04%, 28.94%, 19.86%, 36.72%, 32.58% and 20.06% inhibition, respectively, at a 50 µg/mL concentration (Figure 3). The results indicated that most potent analogs **7f** and **7p** are also less toxic compared to other derivatives of this scaffold.

< Figure 3 >

3. Conclusion

In conclusion, we have designed and synthesized a series of novel morpholine, thiomorpholine -and N-substituted piperazine coupled 2-(thiophene-2-yl)dihydroquinolines **7a–p** in excellent yields. All the new analogues **7a–p** was fully characterized by their NMR and mass spectral data and were evaluated for their *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv (*Mtb*). Two compounds **7f** and **7p** (MIC: 1.56 µg/mL) were

found to be most effective inhibiting *Mtb* compared to other evaluated compounds and has low cytotoxicity. Even though both **7f** & **7p** show higher ClogP values (6.32 and 5.08 respectively), the success of Bedaquiline and Delaminid (cLogP values of 7.3 and 5.6, respectively) as drugs treating TB prompted us to consider **7f** and **7p** as hit analogues for further *in vivo* and mechanistic evaluations. With new anti-TB agents desperately needed, we believe that the analogues reported in this work will help global efforts for identification of potential lead antimycobacterial agents for further development.

4. Experimental Section

Melting points were measured by CINTEX programmable melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra of samples in $CDCl_3$ were recorded on AVANCE- 300 MHz, 400 MHz and 500 MHz spectrometers. Chemical shifts (δ) are reported relative to TMS (δ = 0.0) as the internal standard. Spin multiplicities are described as s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Coupling constants are reported in hertz (Hz). Mass spectra were recorded in ESI spectrometers. Analytical HPLC was performed with an Shimadzu LC-20AD series system using waters acquity UPLC BEC 250 mm, C18, 5 µg column (solvent: ACN:H_2O; gradient: 1 ml/min^{-1}; T = 25 ^{0}C). All high resolution mass spectra were recorded on QSTAR XL hybrid ms/ms system (Applied Bio systems/MDS sciex, foster city, USA), equipped with an ESI source (IICT, Hyderabad). IR was recorded on Thermo Nicolet nexus-670 spectrometer with reference to KBr. TLC was performed on Merck 60 F-254 silica gel plates. The chemicals used in this work were obtained from commercial channels and were used without purification.

4.1. Synthesis of 5-chloro-2-(thiophen-2-yl)-7,8-dihydroquinoline-6-carbaldehyde (3)

To a solution of N,N-dimethyl formamide (6 mmol) in anhydrous $CHCl_3$ (15 mL) was cooled to 0 °C. phosphorous oxychloride (4 mmol) was added dropwise over a period of 10 min. The resulting white suspension was warmed to room temperature and stirred for 30 min and a solution of dihydro-6H-quinolin-5-one **2** (1 mmol) in chloroform (15 mL) was added dropwise and mixture was refluxed for 4 h. After completion of the reaction, as monitored by TLC, the reaction mixture was poured in ice water, solid sodium hydrogen carbonate was carefully added to neutralize the acids and extracted with $CHCl_3$.The organic layer was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified on a silica gel column eluting with hexane/ethyl acetate to give 3 as yellow color solid. Yield: 84%; mp: 176-178 °C;

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.37 (s, 1H, CHO), 8.06 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.68 (dd, *J* = 3.8, 0.9 Hz, 1H, Ar-H), 7.61 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.46 (dd, *J* = 5.0, 0.9 Hz, 1H, Ar-H), 7.14 (dd, *J* = 5.0, 3.8 Hz, 1H, Ar-H), 3.08 (t, *J* = 7.7 Hz, 2H, CH_2), 2.78 (t, *J* = 7.7 Hz, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.9, 158.9, 153.5, 143.8, 143.7, 133.7, 131.5, 129.0, 128.3, 125.9, 125.7, 116.9, 29.6, 21.0; IR (KBr) 3449, 3060, 2855, 1660, 1545, 1444, 1262, 1165, 831, 712 cm^{-1}; MS (ESI) *m/z* 276 $[M+H]^+$ HR-MS (ESI) Calcd for $C_{14}H_{11}ONClS$ $[M+H]^+$: 276.02542, found: 276.02444.

4.2. Synthesis of (5-chloro-2-(thiophen-2-yl)-7,8-dihydroquinolin-6-yl)methanol (4)

To a mixture of 5-chloro-2-(thiophen-2-yl)-7,8-dihydroquinoline-6-carbaldehyde **3** (1.0 mmol) in methanol, sodium borohydride (2 mmol) was added at 0 °C and stirred at RT for 1.5 h (Reaction was monitored by TLC).after completion, the reaction mixture was evaporated under vacuum, water and ethyl acetate were added ,and the organic layer was separated washed with brine, dried over anhydrous sodium sulfate and removal of solvent in vacuo. The crude residue was purified by column chromatography over silica gel to afford pure product (5-chloro-2-(thiophen-2-yl)-7,8-dihydroquinolin-6-yl)methanol **4** as colorless solid. Yield: 88%; mp: 114-116 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.59 (dd, *J* = 3.7, 0.9 Hz, 1H, Ar-H), 7.52 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.38 (dd, *J* = 5.0, 0.9 Hz, 1H, Ar-H), 7.11 (dd, *J* = 5.0, 3.7 Hz, 1H, Ar-H), 4.52 (s, 2H, CH_2), 3.09 (t, *J* = 8.3 Hz, 2H, CH_2), 2.71 (t, *J* = 8.3 Hz, 2H, CH_2), 1.76 (bs, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.3, 150.6, 144.1, 136.0, 131.9, 128.0, 127.5, 126.7, 124.7, 124.6, 116.8, 62.7, 30.1, 25.5; IR (KBr) 3336, 2925, 1621, 1557, 1447, 1416, 1229, 1046, 816, 699 cm^{-1}; MS (ESI) *m/z* 278 $[M+H]^+$; HR- MS (ESI) Calcd For $C_{14}H_{13}ONClS$ $[M+H]^+$: 278.0403, found: 278.0401.

4.3. Synthesis of 6-(bromomethyl)-5-chloro-2-(thiophen-2-yl)-7,8-dihydroquinoline (5)

To a solution of (5-chloro-2-(thiophen-2-yl)-7,8-dihydroquinolin-6-yl)methanol **4** (1.0 mmol) in diethyl ether was added PBr_3 (0.5mmol) slowly at 0 °C. The resulting mixture was at RT under stirring for 1 h. The reaction progress was monitored by TLC. After completion, the reaction mixture was quenched with aq. KBr solution. The product was extracted with EtOAc , the combined organic layers washed with brine solution and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the crude residue was subjected to column chromatography to afford pure **5** as colorless solid. Yield: 82%; mp: 128-130 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.61 (dd, *J* = 3.8, 0.8 Hz, 1H, Ar-H), 7.53 (d, *J* =

8.2 Hz, 1H, Ar-H), 7.40 (dd, *J* = 4.9, 0.8 Hz, 1H, Ar-H), 7.12 (dd, *J* = 4.9, 3.8 Hz, 1H, Ar-H), 4.36 (s, 2H, CH_2), 3.12 (t, *J* = 8.5 Hz, 2H, CH_2), 2.73 (t, *J* = 8.5 Hz, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.1, 151.1, 144.1, 132.5, 132.3, 128.1, 127.9, 126.4, 124.9, 116.8, 32.9, 30.2, 27.1; IR (KBr) 3449, 3097, 2926, 1620, 1559, 1443, 1236, 1170, 959, 825, 708 cm^{-1}.

4.4. General Procedure for the synthesis of piperazine-dihydroquinoline derivatives (7a-p)

To a mixture of **6a-p** (1.0 mmol) and K_2CO_3 in acetone (10 mL) was added 6-(bromomethyl)-5-chloro-2-(thiophen-2-yl)-7,8-dihydroquinoline **5** (1 mmol) at room temperature under stirring for 12 h (monitored by TLC), After completion the reaction mixture was evaporated and extracted with ethylacetate and water, the organic layer was separated dried over anhydrous Na_2SO_4. and evaporated under vacuum. The resulting crude product was purified by silica gel column chromatography by using EtOAc/hexane as eluent.

4.4.1. 4-(5-Chloro-2-(thiophen-2-yl)-7,8-dihydroquinolin-6-yl)methyl) morpholine (7a)

Colorless solid, yield: 86%; mp: 88-90 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.58 (dd, *J* = 3.6, 0.9 Hz, 1H, Ar-H), 7.51 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.38 (dd, *J* = 5.0, 0.9 Hz, 1H, Ar-H), 7.10 (dd, *J* = 5.0, 3.6 Hz, 1H, Ar-H), 3.74-3.69 (m, 4H, $2CH_2$), 3.35 (s, 2H, CH_2), 3.04 (t, *J* = 8.4 Hz, 2H, CH_2), 2.66 (t, *J* = 8.4 Hz, 2H, CH_2), 2.54-2.47 (m, 4H, $2CH_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.4, 150.6, 144.4, 134.0, 131.9, 128.0, 127.4, 127.0, 126.4, 124.5, 116.7, 66.9, 60.5, 53.6, 30.4, 27.0; IR (KBr) 3451, 3101, 2958, 2849, 2755, 1446, 1280, 1114, 943, 714 cm^{-1}; MS (ESI) *m/z* 347 $[M+H]^+$; HR-MS (ESI) Calcd for $C_{18}H_{20}ON_2ClS$ $[M+H]^+$: 347.09705, found: 347.09794.

4.4.2. 4-((5-Chloro-2-(thiophen-2-yl)-7,8-dihydroquinolin-6-yl)methyl)thiomorpholine (**7b**)

Colorless solid, yield: 84%; mp: 132-134 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.58 (dd, *J* = 3.6, 0.9 Hz, 1H, Ar-H), 7.51 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.38 (dd, *J* = 5.0, 0.9 Hz, 1H, Ar-H), 7.10 (dd, *J* = 5.0, 3.6 Hz, 1H, Ar-H), 3.35 (s, 2H, CH_2), 3.03 (t, *J* = 8.4 Hz, 2H, CH_2), 2.81-2.59 (m, 10H, $5CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 150.5, 144.4, 134.6, 131.9, 128.0, 127.4, 127.0, 126.2, 124.5, 116.7, 60.7, 54.9, 30.5, 27.9, 26.8; IR (KBr) 3448, 3094, 2912, 2803, 1564, 1444, 1415, 1282, 1102, 956, 830, 722 cm^{-1}; MS (ESI) *m/z* 363 $[M+H]^+$; HR-MS (ESI) Calcd for $C_{18}H_{20}N_2ClS_2$ $[M+H]^+$: 363.07453, found: 363.07509.

4.4.3. *tert*-Butyl4-((5-chloro-2-(thiophen-2-yl)-7,8-dihydroquinolin-6-yl)methyl)piperazine carboxylate (7c)

Colorless solid, yield: 82%; mp: 126-128 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.59-7.56 (m, 1H, Ar-H), 7.51 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.40-7.35 (m, 1H, Ar-H), 7.12-7.08 (m, 1H, Ar-H), 3.49-3.41 (m, 4H, $2CH_2$), 3.36 (s, 2H, CH_2), 3.04 (t, *J* = 8.3 Hz, 2H, CH_2), 2.66 (t, *J* = 8.3 Hz, 2H, CH_2), 2.51-2.40 (m, 4H, $2CH_2$), 1.46 (s, 9H, $3CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4, 154.5, 150.5, 144.3, 133.8, 131.9, 127.9, 127.4, 126.8, 126.5, 124.5, 116.6, 79.5, 60.0, 52.8, 43.3, 30.4, 28.3, 26.9; IR (KBr) 3448, 3004, 2853, 2812, 1688, 1451, 1420, 1245, 1164, 1007, 835, 714 cm^{-1}; MS (ESI) *m/z* 446 $[M+H]^+$; HR-MS (ESI) Calcd for $C_{23}H_{29}O_2N_3ClS$ $[M+H]^+$: 446.16483, found: 446.16635.

4.4.4. (4-((5-Chloro-2-(thiophen-2-yl)-7,8-dihydroquinolin-6-yl)methyl)piperazinyl)(cyclopropyl)methanone (7d)

Colorless solid, yield: 88%; HPLC purity: 97.17%; mp: 138-140 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.58 (dd, *J* = 3.6, 0.9 Hz, 1H, Ar-H), 7.52 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.38 (dd, *J* = 5.0, 0.9 Hz, 1H, Ar-H), 7.11 (dd, *J* = 5.0, 3.6 Hz, 1H, Ar-H), 3.75-3.59 (m, 4H, $2CH_2$), 3.38 (s, 2H, CH_2), 3.06 (t, *J* = 8.3 Hz, 2H, CH_2), 2.68 (t, *J* = 8.3 Hz, 2H, CH_2), 2.59-2.44 (m, 4H, $2CH_2$), 1.76-1.69 (m, 1H, CH), 1.02-0.96 (m, 2H, CH_2), 0.79-0.72 (m, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 156.4, 150.6, 144.3, 133.9, 131.9, 128.0, 127.4, 126.9, 126.5, 124.5, 116.6, 60.0, 53.2, 52.9, 45.3, 42.0, 30.4, 27.0, 10.8, 7.3; IR (KBr) 3446, 2928, 2806, 1637, 1444, 1234, 1132, 1039, 819, 695 cm^{-1}; MS (ESI) *m/z* 414 $[M+H]^+$ HR-MS (ESI) Calcd for $C_{22}H_{25}ON_3ClS$ $[M+H]^+$: 414.13915 found: 414.14014.

4.4.5. 6-((4-((1,3-Dioxolan-2-yl)methyl)piperazinyl)methyl)-5-chloro-2-(thiophen-2-yl)-7,8-dihydro quinoline (7e)

Colorless solid, yield: 92%; mp: 114-116 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.57 (dd, *J* = 3.6, 0.9 Hz, 1H, Ar-H), 7.51 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.37 (dd, *J* = 5.0, 0.9 Hz, 1H, Ar-H), 7.10 (dd, *J* = 5.0, 3.6 Hz, 1H, Ar-H), 5.02 (t, *J* = 4.4 Hz, 1H, CH), 4.02-3.92 (m, 2H, CH_2), 3.90-3.81 (m, 2H, CH_2), 3.36 (s, 2H, CH_2), 3.03 (t, *J* = 8.5 Hz, 2H, CH_2), 2.73-2.46 (m, 12H, $6CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 150.4, 144.5, 134.7, 131.9, 128.0, 127.3, 127.1, 126.0, 124.4, 116.6, 102.6, 64.7, 61.1, 60.1, 53.9, 50.0, 30.5, 26.9; IR (KBr) 3421, 3097, 2939, 2878, 2809, 1449, 1295, 1139, 1064, 824, 705 cm^{-1}; MS (ESI) *m/z* 432 $[M+H]^+$; HR-MS (ESI) Calcd for $C_{22}H_{27}O_2N_3ClS$ $[M+H]^+$: 432.14946, found: 432.15070.

4.4.6. 6-((4-Benzylpiperazinyl)methyl)-5-chloro-2-(thiophen-2-yl)-7,8-dihydroquinoline (7f)

Colorless solid, yield: 94%; HPLC purity: 96.49%; mp: 116-118 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.56 (dd, *J* = 3.6, 0.9 Hz, 1H, Ar-H), 7.50 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.37 (dd, *J* = 5.0, 0.9 Hz, 1H, Ar-H), 7.34-7.28 (m, 4H, Ar-H), 7.25-7.22 (m, 1H, Ar-H), 7.09 (dd, *J* = 5.0, 3.6 Hz, 1H, Ar-H), 3.53 (s, 2H, CH_2), 3.35 (s, 2H, CH_2), 3.02 (t, *J* = 8.3 Hz, 2H, CH_2), 2.64 (t, *J* = 8.3, Hz, 2H, CH_2), 2.59-2.41 (m, 8H, $4CH_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.4, 150.4, 144.4, 137.7, 134.7, 131.8, 129.1, 128.1, 127.9, 127.3, 127.1, 127.0, 126.0, 124.4, 116.6, 62.8, 60.1, 53.1, 52.9, 30.4, 27.0; IR (KBr) 3450, 3097, 2933, 2802, 1629, 1448, 1282, 1140, 1010, 701 cm^{-1}; MS (ESI) *m/z* 436 $[M+H]^+$; HR-MS (ESI) Calcd for $C_{25}H_{27}N_3ClS$ $[M+H]^+$: 436.15969, found: 436.16087.

4.4.7. 5-Chloro-6-((4-(4-chlorobenzyl)piperazinyl)methyl)-2-(thiophen-2-yl)-7,8-dihydro quinoline (7g)

Colorless solid, yield: 88%; mp: 176-178 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.57 (dd, *J* = 3.6, 0.9 Hz, 1H, Ar-H), 7.51 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.37 (dd, *J* = 5.0, 0.9 Hz, 1H, Ar-H), 7.30-7.22 (m, 4H, Ar-H), 7.10 (dd, *J* = 5.0, 3.6 Hz, 1H, Ar-H), 3.48 (s, 2H, CH_2), 3.35 (s, 2H, CH_2), 3.03 (t, *J* = 8.3 Hz, 2H, CH_2), 2.64 (t, *J* = 8.3 Hz, 2H, CH_2), 2.58-2.38 (m, 8H, $4CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4, 150.4, 144.4, 136.5, 134.7, 132.6, 131.8, 130.3, 128.2, 127.9, 127.3, 127.1, 126.0, 124.4, 116.6, 62.0, 60.1, 53.1, 52.9, 30.4, 26.9; IR (KBr) 3450, 3068, 2926, 2804, 1445, 1330, 1279, 1135, 1005, 830, 690 cm^{-1}; MS (ESI) *m/z* 470 $[M+H]^+$; HR-MS (ESI) Calcd for $C_{25}H_{26}N_3Cl_2S$ $[M+H]^+$: 470.12096, found: 470.12190.

4.4.8. 6-((4-(Benzo[d][1,3]dioxol-5-ylmethyl)piperazinyl)methyl)-5-chloro-2-(thiophen-2-yl)-7,8-dihydroquinoline (7h)

Colorless solid, yield: 90%; mp: 112-114 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.57 (dd, *J* = 3.6, 0.9 Hz, 1H, Ar-H), 7.51 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.37 (dd, *J* = 5.0, 0.9 Hz, 1H, Ar-H), 7.10 (dd, *J* = 5.0, 3.6 Hz, 1H, Ar-H), 6.86 (s, 1H, Ar-H), 6.77-6.71 (m, 2H, Ar-H), 5.93 (s, 2H, CH_2), 3.44 (s, 2H, CH_2), 3.35 (s, 2H, CH_2) CH_2, 3.03 (t, *J* = 8.5 Hz, 2H, CH_2), 2.64 (t, *J* = 8.5 Hz, 2H, CH_2), 2.60-2.37 (m, 8H, $4CH_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.5, 150.5, 147.5, 146.6, 144.5, 134.6, 131.9, 131.2, 128.0, 127.4, 127.1, 126.1, 124.5, 122.4, 116.7, 109.6, 107.8, 100.8, 62.5, 60.1, 53.0, 52.7, 30.5, 27.0; IR (KBr) 3422, 3111, 2926, 2809, 1485, 1448, 1245, 1033, 922, 824, 717 cm^{-1}; MS (ESI) *m/z* 480 $[M+H]^+$; HR-MS (ESI) Calcd for $C_{26}H_{27}O_2N_3ClS$ $[M+H]^+$: 480.15205, found: 480.15070.

4.4.9. 5-Chloro-6-((4-phenylpiperazinyl)methyl)-2-(thiophen-2-yl)-7,8-dihydroquinoline (7i)

Colorless solid, yield: 82%; mp: 208-210 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.58 (dd, *J* = 3.6, 0.9 Hz, 1H, Ar-H), 7.52 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.37 (dd, *J* = 5.0, 0.9 Hz, 1H, Ar-H), 7.29-7.23 (m, 2H, Ar-H), 7.10 (dd, *J* = 5.0, 3.6 Hz, 1H, Ar-H), 6.96-6.90 (m, 2H, Ar-H), 6.88-6.82 (m, 1H, Ar-H), 3.41 (s, 2H, CH_2), 3.24-3.17 (m, 4H, $2CH_2$), 3.06 (t, *J* = 8.4 Hz, 2H, CH_2), 2.74-2.61 (m, 6H, $3CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4, 151.1, 150.4, 144.4, 134.5, 131.9, 128.9, 127.9, 127.4, 127.0, 126.1, 124.4, 119.5, 116.6, 115.9, 60.1, 53.1, 49.0, 30.4, 26.9; IR (KBr) 3450, 2939, 2835, 1599, 1501, 1446, 1238, 1137, 1004, 753 cm^{-1}; MS (ESI) *m/z* 422 $[M+H]^+$; HR-MS (ESI) Calcd for $C_{24}H_{25}N_3ClS$ $[M+H]^+$: 422.14403, found: 422.14522.

4.4.10. 5-Chloro-2-(thiophen-2-yl)-6-((4-(*o*-tolyl)piperazinyl)methyl)-7,8-dihydroquinoline (7j)

Colorless solid, yield: 76%; mp: 138-140 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.58 (dd, *J* = 3.6, 0.9 Hz, 1H, Ar-H), 7.52 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.38 (dd, *J* = 5.0, 0.9 Hz, 1H, Ar-H), 7.20-7.13 (m, 2H, Ar-H), 7.10 (dd, *J* = 5.0, 3.6 Hz, 1H, Ar-H), 7.06-7.01 (m, 1H, Ar-H), 7.00-6.95 (m, 1H, Ar-H), 3.43 (s, 2H, CH_2), 3.07 (t, *J*= 8.3 Hz, 2H, CH_2), 2.97-2.91 (m, 4H, $2CH_2$), 2.74-2.64 (m, 6H, $3CH_2$), 2.31 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.6, 151.4, 150.6, 144.5, 132.5, 132.0, 130.9, 128.0, 127.4, 127.1, 126.5, 124.5, 123.0, 118.9, 116.7, 60.2, 53.8, 51.6, 30.5, 27.1, 17.8; IR (KBr) 3447, 3071, 2934, 2820, 1491, 1446, 1222, 1131, 927, 825, 721 cm^{-1}; MS (ESI) *m/z* 436 $[M+H]^+$; HR-MS (ESI) Calcd for $C_{25}H_{27}N_3ClS$ $[M+H]^+$: 436.15950, found: 436.16087.

4.4.11. 5-Chloro-6-((4-(2-methoxyphenyl)piperazinyl)methyl)-2-(thiophen-2-yl)-7,8-di hydro quinoline (7k)

Colorless solid, yield: 80%; mp: 92-94 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.58 (dd, *J* = 3.6, 0.9 Hz, 1H, Ar-H), 7.52 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.38 (dd, *J* = 5.0, 0.9 Hz, 1H, Ar-H), 7.10 (dd, *J* = 5.0, 3.6 Hz, 1H, Ar-H), 7.02-6.84 (m, 4H, Ar-H), 3.86 (s, 3H, OCH_3), 3.44 (s, 2H, CH_2), 3.15-3.03 (m, 6H, $3CH_2$), 2.76-2.66 (m, 6H, $3CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4, 152.0, 150.4, 144.3, 141.1, 134.4, 131.8, 127.9, 127.3, 126.9, 126.2, 124.4, 122.7, 120.8, 118.0, 116.5, 111.0, 60.0, 55.1, 53.3, 50.4, 30.4, 26.9; IR (KBr) 3447, 3062, 2931, 2817, 1582, 1497, 1447, 1237, 1021, 745 cm^{-1}; MS (ESI) *m/z* 452 $[M+H]^+$; HR-MS (ESI) Calcd for $C_{25}H_{27}ON_3ClS$ $[M+H]^+$: 452.15440, found: 452.15579.

4.4.12. 1-(4-(4-((5-Chloro-2-(thiophen-2-yl)-7,8-dihydroquinolin-6-yl)methyl)piperazinyl) phenyl)ethanone (7l)

Colorless solid, yield: 84%; mp: 198-200 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93-7.78 (m, 3H, Ar-H), 7.59 (dd, *J* = 3.6, 0.9 Hz, 1H, Ar-H), 7.53 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.38 (dd, *J* = 5.0, 0.9 Hz, 1H, Ar-H), 7.13-7.09 (m, 1H, Ar-H), 6.91-6.82 (m, 2H, Ar-H), 3.44-3.30 (m, 6H, $3CH_2$), 3.06 (t, *J* = 8.4 Hz, 2H, CH_2), 2.74-2.59 (m, 6H, $3CH_2$), 2.52 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.4, 156.4, 154.0, 150.6, 144.4, 134.0, 132.0, 130.38, 130.30, 128.0, 127.5, 126.9, 126.5, 124.5, 116.7, 113.3, 60.0, 52.8, 47.2, 30.5, 27.0, 26.0; IR (KBr) 3446, 3099, 2930, 2837, 1668, 1597, 1448, 1229, 1193, 821, 701 cm^{-1}; MS (ESI) *m/z* 464 $[M+H]^+$; HR-MS (ESI) Calcd for $C_{26}H_{27}ON_3ClS$ $[M+H]^+$: 464.15676, found: 464.15579.

4.4.13. 5-Chloro-6-((4-(2-fluorophenyl)piperazinyl)methyl)-2-(thiophen-2-yl)-7,8-dihydro quinoline (7m)

Colorless solid, yield: 72%; mp: 186-188 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.58 (dd, *J* = 3.6, 0.9 Hz, 1H, Ar-H), 7.52 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.38 (dd, *J* = 5.0, 0.9 Hz, 1H, Ar-H), 7.10 (dd, *J* = 5.0, 3.6 Hz, 1H, Ar-H), 7.08-6.88 (m, 4H, Ar-H), 3.44 (s, 2H, CH_2), 3.17-3.10 (m, 4H, $2CH_2$), 3.06 (t, *J* = 8.5 Hz, 2H, CH_2), 2.75-2.67 (m, 6H, $3CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 155.6, 150.5, 144.4, 140.0, 134.4, 131.9, 128.0, 127.4, 127.1, 126.3, 124.5, 124.3, 122.3, 118.8, 116.7, 116.0, 60.1, 53.3, 50.4, 30.5, 27.0; IR (KBr) 3449, 3072, 2824, 1499, 1446, 1237, 1134, 928, 719 cm^{-1}; MS (ESI) *m/z* 440 $[M+H]^+$; HR-MS (ESI) Calcd for $C_{24}H_{24}N_3ClFS$ $[M+H]^+$: 440.13434, found: 440.13580.

4.4.14. 5-Chloro-2-(thiophen-2-yl)-6-((4-(2-(trifluoromethyl)phenyl)piperazinyl)methyl)-7,8-dihydroquinoline (7n)

Colorless solid, yield: 76%; mp: 164-166 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.59 (dd, *J* = 3.6, 0.9 Hz, 1H, Ar-H), 7.53 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.50-7.45 (m, 2H, Ar-H), 7.38 (dd, *J* = 5.0, 0.9 Hz, 1H, Ar-H), 7.11 (dd, *J* = 5.0, 3.6 Hz, 1H, Ar-H), 6.94-6.89 (m, 2H, Ar-H), 3.41 (s, 2H, CH_2), 3.32-3.25 (m, 4H, $2CH_2$), 3.06 (t, *J* = 8.3 Hz, 2H, CH_2), 2.72-2.63 (m, 6H, $3CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 153.2, 150.6, 144.4, 134.3, 132.0, 128.0, 127.5, 127.0, 126.33, 126.30, 124.5, 123.3, 116.7, 114.4, 60.1, 52.9, 47.9, 30.5, 27.0; IR (KBr) 3456, 3059, 2883, 2758, 1568, 1294, 1132, 962, 705 cm^{-1}; MS (ESI) *m/z* 490 $[M+H]^+$ Calcd for $C_{25}H_{24}N_3ClF_3S$ $[M+H]^+$: 490.13136, found: 490.13261.

4.4.15. 5-Chloro-6-((4-(4-chlorophenyl)piperazinyl)methyl)-2-(thiophen-2-yl)-7,8-dihydroquinoline (7o)

Colorless solid, yield: 78%; mp: 176-178 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.58 (dd, *J* = 3.6, 1.1 Hz, 1H, Ar-H), 7.53 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.38 (dd, *J* = 5.1, 1.1 Hz, 1H, Ar-H), 7.22-7.17 (m, 2H, Ar-H), 7.11 (dd, *J* = 5.1, 3.6 Hz, 1H, Ar-H), 6.86-6.81 (m, 2H, Ar-H), 3.41 (s, 2H, CH_2), 3.20-3.14 (m, 4H, $2CH_2$), 3.06 (t, *J* = 8.6 Hz, 2H, CH_2), 2.72-2.63 (m, 6H, $3CH_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.5, 150.6, 149.8, 144.4, 134.4, 132.0, 128.8, 128.0, 127.4, 127.1, 126.3, 124.5, 124.3, 117.1, 116.7, 60.1, 53.0, 49.1, 30.5, 27.0; IR (KBr) 3447, 3063, 2940, 2821, 1492, 1446, 1229, 1140, 935, 820, 708 cm^{-1}; MS (ESI) *m/z* 456 $[M+H]^+$ Calcd for $C_{24}H_{24}N_3Cl_2S$ $[M+H]^+$: 456.10557, found: 456.10625.

4.4.16. 5-Chloro-6-((4-(pyridin-2-yl)piperazinyl)methyl)-2-(thiophen-2-yl)-7,8-dihydroquinoline (7p)

Colorless solid, yield: 74%; HPLC purity: 96.02%; mp: 102-104 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21-8.18(m, 1H, Ar-H), 7.83 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.59 (dd, *J* = 3.6, 0.9 Hz, 1H, Ar-H), 7.53 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.50-7.45 (m, 1H, Ar-H), 7.38 (dd, *J* = 5.0, 0.9 Hz, 1H, Ar-H), 7.11 (dd, *J* = 5.0, 3.6 Hz, 1H), 6.67-6.59 (m, 2H, Ar-H), 3.61-3.52 (m, 4H), 3.41 (s, 2H, CH_2), 3.07 (t, *J* = 8.5 Hz, 2H, CH_2), 2.70 (t, *J* = 8.5 Hz, 2H, CH_2), 2.66-2.58 (m, 4H, $2CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.4, 156.5, 150.6, 147.8, 144.4, 137.4, 132.0, 128.0, 127.4, 127.1, 124.5, 116.7, 113.2, 107.0, 60.2, 53.0, 45.1, 30.5, 27.0; IR (KBr) 3448, 3064, 2922, 1634, 1546, 1488, 1256, 1193, 703 cm^{-1}; MS (ESI) *m/z* 423 $[M+H]^+$ Calcd for $C_{23}H_{24}N_4ClS$ $[M+H]^+$: 423.13968, found: 423.14047.

4.5. Antitubercular evaluation assay

All the compounds were further screened for in vitro antimycobacterial activity against *M. Tuberculosis* H37Rv strain by microplate Alamar blue assay method. Briefly, the inoculum was prepared from fresh LJ medium re-suspended in 7H9-S medium (7H9 broth, 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase [OADC]), adjusted to a McFarland tube No. 1, and diluted 1:20; 100 µl was used as inoculum. Each drug stock solution was thawed and diluted in 7H9-S at four-fold the final highest concentration tested. Serial two-fold dilutions of each drug were prepared directly in a sterile 96-well microtiter plate using 100 µl 7H9-S. A growth control containing no antibiotic and a sterile control were also prepared on each plate. Sterile water was added to all perimeter wells to avoid evaporation during the

incubation. The plate was covered, sealed in plastic bags and incubated at 37^{o}C in normal atmosphere. After 7 days incubation, 30 ml of Alomar blue solution was added to each well, and the plate was re-incubated overnight. A change in color from blue (oxidized state) to pink (reduced) indicated the growth of bacteria, and the MIC was defined as the lowest concentration of drug that prevented this change in colour.

4.6 Evaluation of cytotoxicity

Antitubercular active compounds with MIC ≤ 6.25 µg/mL were further examined for toxicity in a HEK-293T cell line at the concentration of 50 µg/mL. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay.

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Figure/Scheme Captions

Scheme 1. Synthesis of 2-(thiophen-2-yl) dihydroquinoline derivatives **7a–p**

Reagents and conditions: (i) DMF-DMA, xylene, reflux, 7 h, 95%; (ii) Cyclohexane-1,3-dione, NH_4OAc, $CeCl_3.7H_2O$-NaI, propan-2-ol, reflux, 4 h, 86%; (iii) $POCl_3$-DMF, $CHCl_3$, 60 ^oC, 4 h,

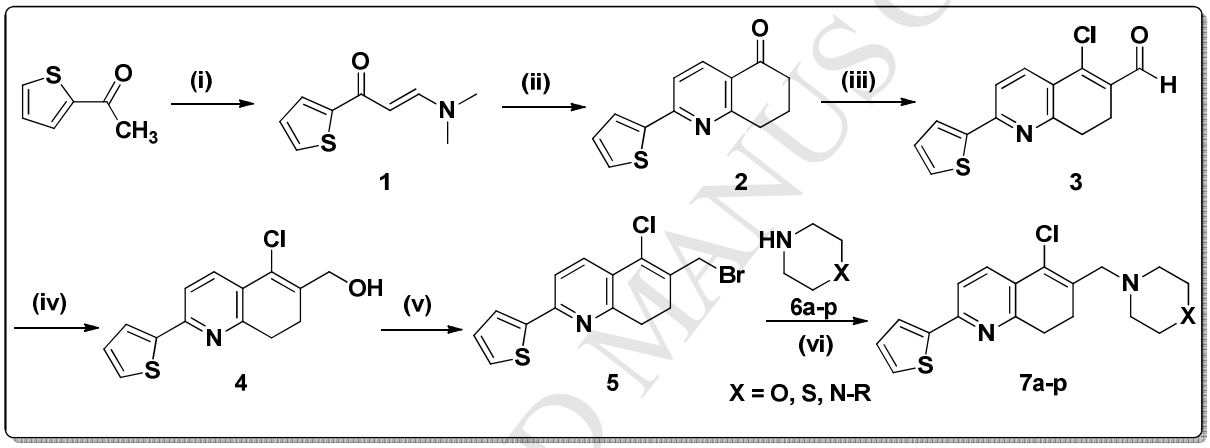
84%; (iv) $NaBH_4$, MeOH, rt, 1.5 h, 88%; (v) PBr_3, diethyl ether, rt, 1 h, 82%; (vi) **6a–p**, K_2CO_3 , acetone, rt, 12 h, 72–94%.

Table 1. Synthesis and *in vitro* activity evaluation (against M. tuberculosis H37Rv) of **7a-p.**

Figure 1. Clinical antitubercular agents containing quinoline, piperazine, morpholine, and thiomorpholine fragments.

Figure 2. Design strategy for new dihydroquinoline derivatives.

Figure 3. Percentage inhibition of HEK-293T cells by new analogs **7a, 7d, 7f, 7k, 7m, 7p** at a concentration of 50 μg/mL.



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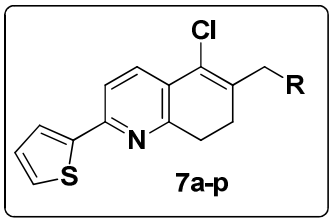
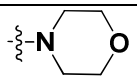
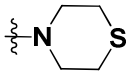
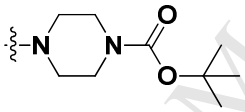
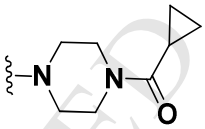
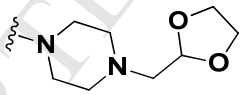
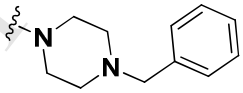
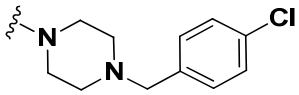
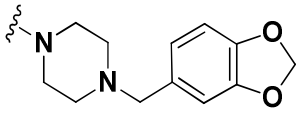
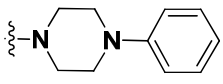
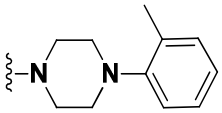
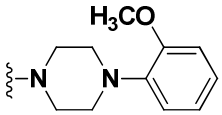
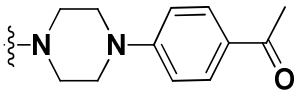
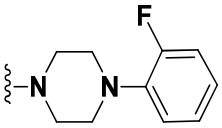
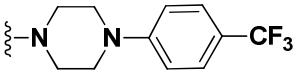
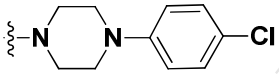
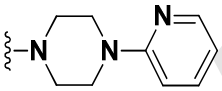


Entry	Product	R	Yield(%)[a]	CLogP[b]	MIC(μg/mL)
1	7a		86	4.04	6.25
2	7b		84	4.77	25
3	7c		82	5.89	25
4	7d		88	4.20	3.125
5	7e		92	2.92	12.5
6	7f		94	6.32	1.56
7	7g		88	7.03	12.5
8	7h		90	6.29	25
9	7i		82	6.03	25

10	**7j**		**76**	**6.41**	**25**
11	**7k**		**80**	**5.93**	**6.25**
12	**7l**		**84**	**5.66**	**25**
13	**7m**		**72**	**6.22**	**6.25**
14	**7n**		**76**	**7.09**	**25**
15	**7o**		**78**	**6.91**	**25**
16	**7p**		**74**	**5.08**	**1.56**
17	Isoniazid	---	---	---	**0.1**
18	Rifampicin	---	---	---	**0.2**
19	Ciprofloxacine	---	---	---	**1.56**
20	Ethambutol	---	---	---	**3.13**

[a] Isolated yield; [b] Calculated using Chemdraw Professional 17.1.

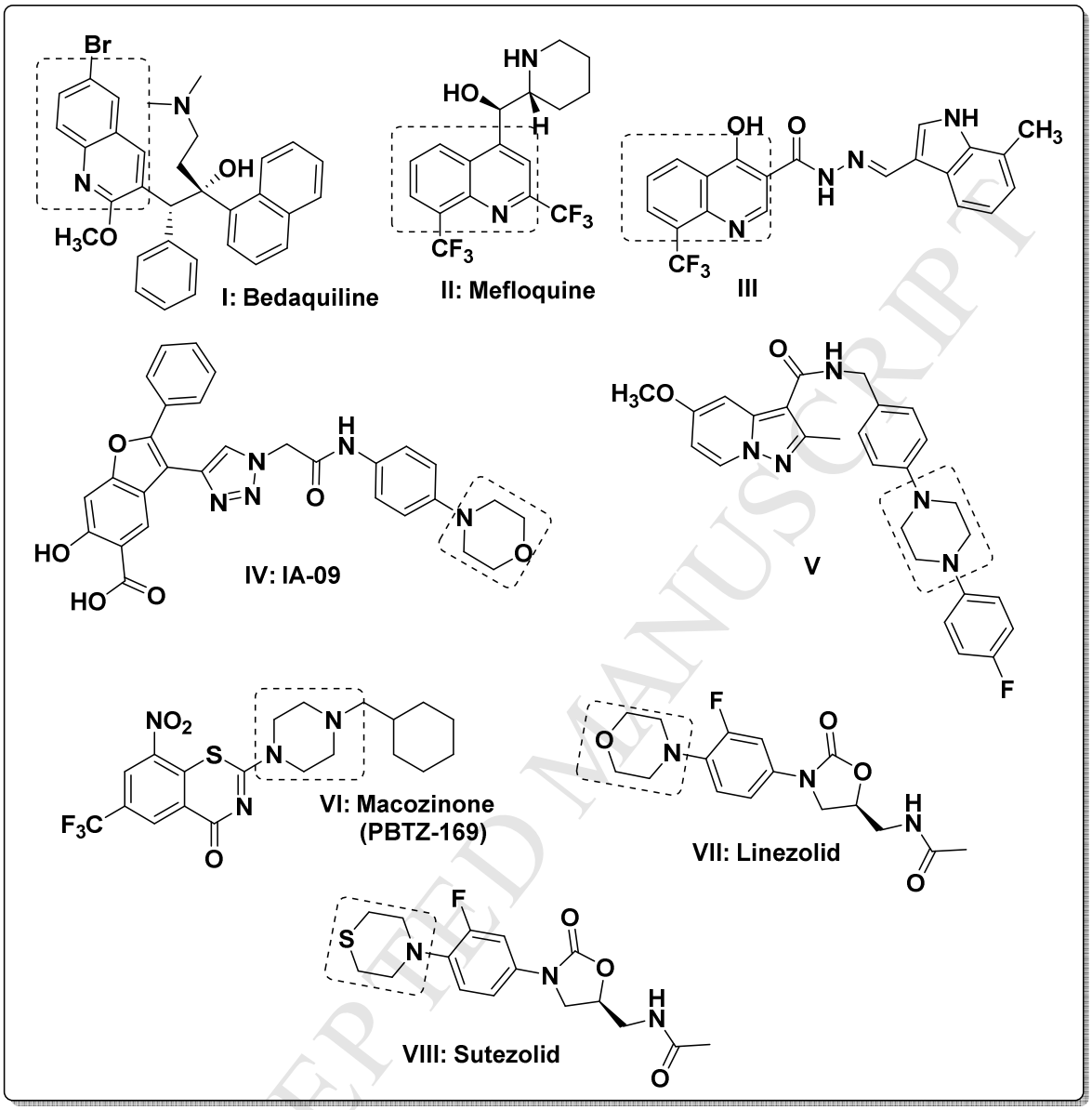


Figure 1. Clinical antitubercular agents containing quinoline, piperazine, morpholine, and thiomorpholine fragments.

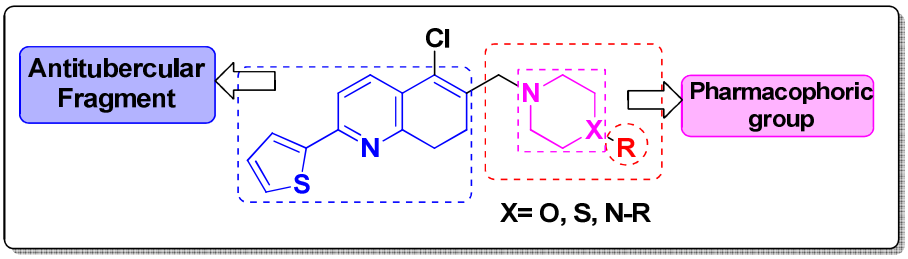


Figure 2. Design strategy for new dihydroquinoline derivatives.

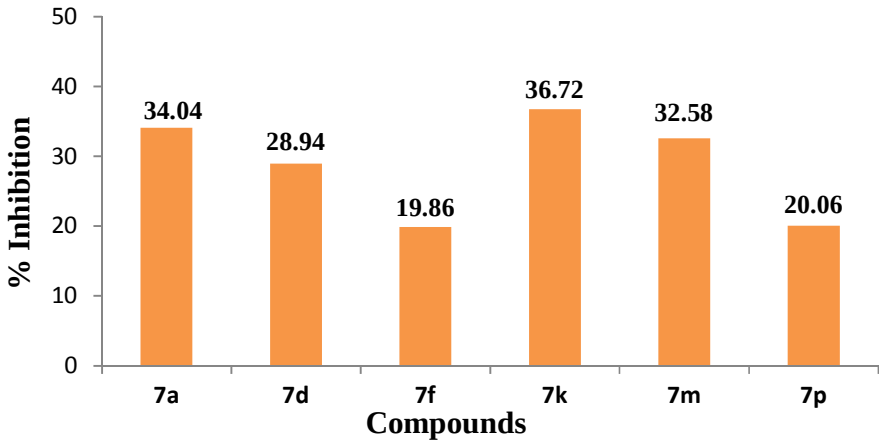


Figure 3. Percentage inhibition of HEK-293T cells by new analogs **7a, 7d, 7f, 7k, 7m, 7p** at a concentration of 50 µg/mL.

Research Highlights

1. A series of novel 2-thiophenyl dihydroquinoline derivatives were designed and synthesized.
2. Morpholine, Thiomorpholine and N-substituted piperazines were coupled to quinoline core.
3. **7f** & **7p** showed most potent *in vitro* antitubercular activity (MIC: 1.56 µg/ml) & low cytotoxicity