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Synthesis, Molecular Docking and Antimycobacterial Evaluation of Imidazo Quinoline-5-Carboxylic Acid as Inhibitors of *Mycobacterium Tuberculosis* Pantothenate Synthetase

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ABSTRACT

A Series of Novel (E)-8-fluoro-6-oxo-9-(piperazin-1-yl)-2-styryl-2,6-dihydro-1H-imidazo[4,5,1-ij]quinoline-5-carboxylic acid and its derivatives are synthesised by cyclisation of 3-chloro-4-fluoroaniline in high yield using green methodologies. The molecular structures of target compounds (7a-7i) were confirmed by ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry. Newly synthesised compounds are screened for Anti-mycobacterial activity and MIC was determined. Majority of compounds (the compound 7c) shows better activity and the most active inhibitor of tuberculosis 5f exhibited a promising inhibition of M. tuberculosis with good MIC value. These compounds docked into the active site of nitrate reductase (PDB code, 3IVX) using autodock 4.2 software, which showed good affinity for the enzyme when compared with the kinetic energies of standard drugs such as amoxcillin (-34.28) and ciprofloxacin (-28.20). Among all the designed compounds, the compound 7 g shows highest binding energy with interactions of ARG200, THR85 and GLU189.

Keywords: 3-chloro-4-fluoroaniline; Quinoline-5-carboxylic acid; Antibacterial activity; Molecular docking studies (PDB id: 3IVX)

INTRODUCTION

Tuberculosis (TB) along with HIV ranks as a leading cause of death worldwide. In 2015, tuberculosis killed 1.4 million people and 10.4 million people are estimated to have fallen ill with TB [1]. TB mortality has fallen since 1990; however, the rise of multidrug-resistant (MDR) and extremely drug-resistant (XDR) strains of *Mycobacterium tuberculosis* represents a serious health challenge. Whereas drug-sensitive TB can be treated by 6 months of chemotherapy with the current four-drug frontline regimen, to cure MDR-TB at least 18 to 24 months of therapy with four to six drugs, including a fluoroquinolone and one injectable agent, is required (2,3]. MDR-TB is defined as resistance to at least two of the four current frontline antibacterials; XDR strains of *M. tuberculosis* additionally are resistant to fluoroquinolones and at least one second-line drug [1]. About 3% of new cases and 20% of treated tuberculosis patients are infected with MDR-TB; among these, about 9% are XDR cases [1-3]. To achieve global control of this epidemic, there is an urgent need for new anti-TB drugs that can target MDR and XDR strains and shorten treatment duration for both drug-sensitive and drug-resistant TB [4-6]. To address this unmet medical need, new anti-TB drugs have been discovered and new treatment regimens currently are being evaluated in (pre)clinical trials [5,7]. Two of these agents, the diarylquinoline bedaquiline (BDQ) and the nitroimidazo-oxazole delamanid, have received accelerated regulatory approval by the U.S. Food and Drug Administration (FDA) [7].

It is known that most of the currently existing tubercular medications are contained by the group of nitrogen heterocyclic compounds such as isoniazid, pyrazinamide etc. Further, most of them are derived from pyridine and

pyrazines [8]. In an attempt to look for better bioactive heterocyclic compounds containing nitrogen,sulphur hetero atom (since most of antituberculosis compounds are based on either pyridine or pyrazines) our consideration curved in the direction of benzimidazole derivatives,Quinoline compounds,quinoline cyclic compounds,quinoline carboxylic acid compounds,Benzimidazoles and its derivatives, as these compounds exhibit a wide spectrum of biological activities including antituberculous activity [9]. Specifically, this nucleus is a constituent of vitamin-B12 and many currently existing medications [10].

The start point for the preparation of the quinoline compounds and its derivatives to be evaluated against TB were malaria drugs, such as quinine, chloroquine, mefloquine, primaquine, and amodiaquine (Figures 1 and 2), which shows moderate biological activity against TB, also being evaluate by us using the Microplate Alamar Blue Assay (MABA) [11,12]. Molecular docking studies were performed on binding site of 3IVX protein to study the binding mode of compounds. The results of both *in vitro* and *in silico* studies clearly indicated that 2-heterostyrylbenzimidazole may serve as new drug candidates in the combat against *Mycobacterium tuberculosis* protein (3IVX). In continuation to our efforts in this drug design paradigm, a library of (E)-8-fluoro-2-(4-fluorostyryl)-6-oxo-9-(piperazin-1-yl)-2,6-dihydro-1H-imidazo[4,5,1-ij]quinoline-5-carboxylic derivatives has been synthesized and evaluated for its biological activity, Molecular docking studies using this protein target have not yet been reported. Hence, molecular docking studies of the synthesized 1H-benzimidazoles were performed on protein PDB code 3IVX by means of molecular operating environment (MOE) software (Autodock 4.2). In the combat against multi-drug resistance, such *in silico* studies have played a key role in the identification of new drug targets and the designing of new scaffolds as novel drug candidates. In view of this data we reported the synthesis of (E)-8-fluoro-6-oxo-9-(piperazin-1-yl)-2-styryl-2,6-dihydro-1H-imidazo[4,5,1-ij]quinoline-5-carboxylic acid and its derivatives) which possessed wide variety of biological activity encouraging antitubercular activity, a library of (E)-8-fluoro-6-oxo-9-(piperazin-1-yl)-2-styryl-2,6-dihydro-1H-imidazo[4,5,1-ij]quinoline-5-carboxylic acid and its derivatives has been synthesized and evaluated for its molecular docking studies using the protein.

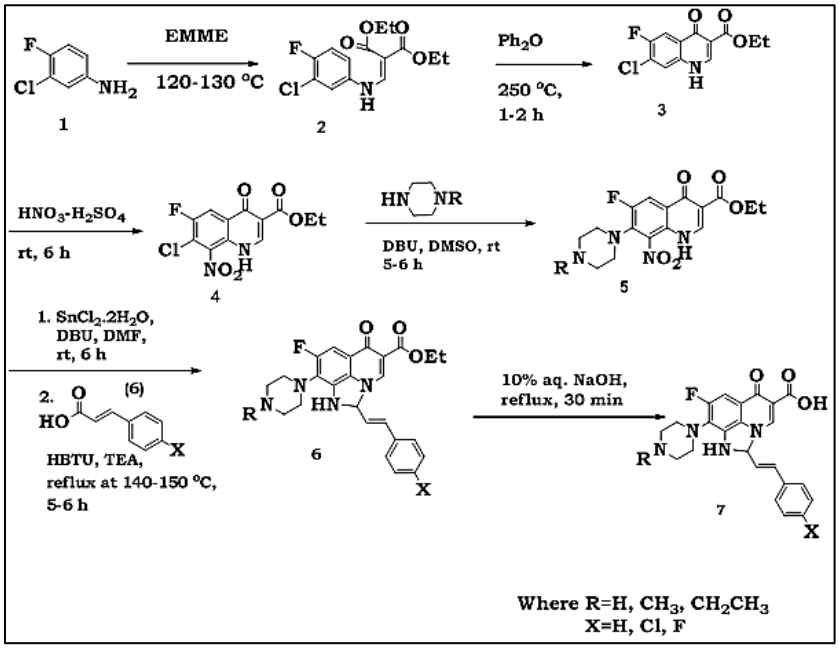

Figure 1: Synthesis of target compounds (7a-7i)

EXPERIMENTAL SECTION

All the reagents used in the present study were obtained from commercial suppliers. All the solvents were freshly distilled before being used. Melting points were determined using a buchi melting point B-545 apparatus and are uncorrected. TLC analyses were done on glass plates coated with silica gel GF-254 and spotting was done using lodine/UV lamp. IR spectra were recorded on a Perkin-Elmer model 446 instrument in KBr phase. H NMR were recorded on LC-MS spectrometer, model HP5989A. ^{13}C NMR was recorded in DMSO using 100 MHZ spectrometer.

General Procedure for the Synthesis of Compound (3) from 3-Chloro-4-Fluoroaniline

A mixture of 1 (10 mmol) and EMME (10 mmol) were heated at 120-130°C for 1-2 h. The reaction mixture was added to diphenyl ether in refluxing conditions at 250°C and allowed to reflux it for 1 h. At the end of the period, the reaction mixture was cooled to rt and washed with hexane (25 mL). The separated solid was filtered and washed it again with diethyl ether (25 mL) and dried to obtain 3.

Preparation of Ethyl 7-Chloro-6-Fluoro-8-Nitro-4-oxo-1,4-Dihydroquinoline-3-Carboxylate (*4*) from Ethyl 7-Chloro-6-Fluoro-4-oxo-1,4-Dihydroquinoline-3-Carboxylate (3):

The nitration mixture by adding HNO_3 (10 mmol) to an ice cold H_2SO_4 (30 mmol) (1:3 ratio) and allowed to stir it for 30 min from 0°C to RT. To this mixture, **3**(10 mmol) was added and allowed to stir it for RT for overnight. At the end of the period, the reaction mixture was added to crush ice and stirs it for 10 min. To this aq. NH_3 was added till the pH of the solution adjusted to ~8.0. The separated solid was filtered and dried to obtain **4**.

Preparation of 5 from 4:

A mixture of 4 (10 mmol), N-alkyl piperzine (10 mmol), DBU (1 mol%) and DMF (25 mL) was allowed stir at RT for 5-6 h. The completion of the reaction was monitored by checking TLC. At the end of the period, the reaction mixture was poured into ice cold water. The separated solid was filtered and dried followed by recrystallization using suitable solvent to obtain pure **5.**

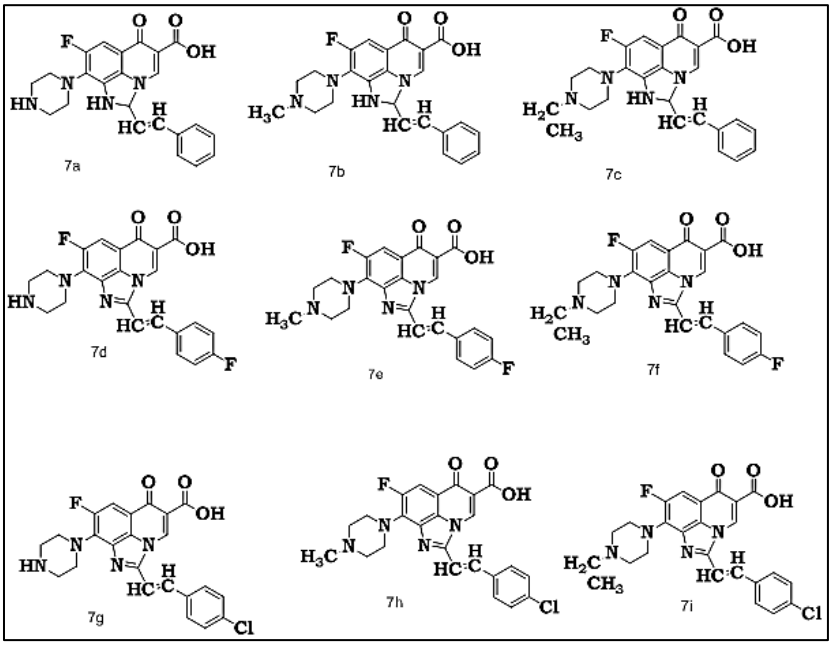

Figure 2: Synthesis of target compounds (7a-7i)

Preparation of 6 from 5 using cinnamic acid derivaties:

A mixture of **5** (10 mmol), aq. $SnCl_2.2H_2O$ (30 mmol), DBU (1 mol %) in DMF (20 mL) was allowed to stir for 6 h at RT. The completion of the reaction was monitored by checking TLC. The disappearance of starting material was observed in TLC, then the reaction mixture was filtered on a celite bed and the collected filtrate was added to cinnamic acid derivaties (10 mmol), HBTU (10 mmol) and triethylamine (1 mol%) and allowed to reflux 140-150°C for 5-6 h. The completion of the reaction was monitored by checking TLC. At the end of the period, the reaction mixture was cooled to rt and poured into ice cold water. The separated solid was filtered and dried followed by recrystalization using suitable solvent to obtain pure **6**.

Preparation of 7 from 6:

A mixture of 6 (10 mmol), 10% aq. NaOH (30 mL) was allowed to heat on water bath for 30 min. At the end of the period, the reaction mixture was cooled to RT followed by the addition of ice cold water (30 mL) and was treated with Conc. HCl and the pH was adjusted to ~8.0. The separated solid was filtered, dried and recrystallized using suitable solvent to obtain the pure compound (**7).**

(E)-8-Fluoro-6-Oxo-9-(piperazin-1-yl)-2-Styryl-2,6-DiHydro-1H-Imidazo[4,5,1-ij]Quinoline-5-Carboxylic Acid:(7a):

7a (i.e., 7a, R=H, X=H): Yield = 1.6 gm (42%), M. P. = 240°C, IR (KBr): 3459 cm^{-1} (small, sharp,-OH stretchings), 3381 and 3018 cm^{-1} (small, sharp, -NH stretching vibrations), 2789-2792 cm^{-1} (small, sharp, enamine –CH stretching vibrations), 1737 cm^{-1} (strong, sharp, -C=O stretching), 1614 cm^{-1} (small, sharp, -O-C=O stretching), 1466 cm^{-1} (aromatic *trans*- C=C stretching), 1290-1370 cm^{-1} (strong, sharp, aromatic –C-F), ^1H – NMR spectrum (DMSO/d_6/TMS, 400 MHz): δ 1.1-1.3 (m, 9H, -NH of Piperazine and its –CH protons), 4.0 (s, 1H, quaternatry –CH), 6.9 (d, 1H, phenyl proton), 7.13 (d, 1H, phenyl proton), 7.23 (d, 1H, vinyl proton, *J* = 16Hz), 7.26 (s, 1H, Ar-H), 7.45-7.47 (m, 3H, phenyl protons), 7.63 (s, 1H, enamine –CH), 8.44 (d, 1H, vinylic proton, *J* = 16Hz), 9.9 (s, 1H, imidazole –NH), 10.8 (d, 1H, tautomeric-OH), ^{13}C NMR (DMSO-d_6, 100 MHz) δ in ppm: 13.73, 13.77, 13.90, 14.01, 14.22, 14.27, 14.61, 15.06, 55.75, 59.26, 59.45, 60.64, 60.70, 60.83, 67.63, 88.03, 91.79, 100.05, 112.47, 113.33, 114.27, 133.90, 139.39, 149.17, 152.23, 152.54, 165.01, 165.56, 166.37, 166.93, 167.64, MS: *m/z* 422.13 ($M^{+\cdot}$).

(E)-8-Fluoro-9-(4-Methylpiperazin-1-yl)-6-oxo-2-Styryl-2,6-Dihydro-1H-Imidazo[4,5,1-ij]Quinoline-5-Carboxylic Acid(7b):

7b (i.e. 7, R=CH_3, X=H): Yield = 1.6 gm (42%), M. P. = >240°C, IR (KBr): 3459 cm^{-1} (small, sharp, -OH stretchings), 3018 cm^{-1} (small, sharp, -NH stretching vibration), 2789-2792 cm^{-1} (small, sharp, enamine –CH stretching vibrations), 1737 cm^{-1} (strong, sharp, -C=O stretching), 1614 cm^{-1} (small, sharp, -O-C=O stretching), 1466 cm^{-1} (aromatic *trans*- C=C stretching), 1290-1370 cm^{-1} (strong, sharp, aromatic –C-F), ^1H – NMR spectrum (DMSO/d_6/TMS, 400 MHz): δ 1.1-1.3 (m, 8H, Piperazine and its –CH protons), 2.26 (s, 3H, Piperazine –N-CH_3), 6.4 (s, 1H, quaternatry –CH), 6.9 (d, 1H, phenyl proton), 7.13 (d, 1H, phenyl proton), 7.23 (d, 1H, vinyl proton, *J* = 16Hz), 7.26 (s, 1H, Ar-H), 7.45-7.47 (m, 3H, phenyl protons), 7.63 (s, 1H, enamine –CH), 8.44 (d, 1H, vinylic proton, *J* = 16Hz), 9.9 (s, 1H, imidazole –NH), 10.8 (d, 1H, tautomeric-OH), MS: *m/z* 423.18 ($M^{+\cdot}$).

(E)-9-(4-Ethylpiperazin-1-yl)-8-Fluoro-6-Oxo-2-Styryl-2,6-DiHydro-1H-Imidazo[4,5,1-ij]Quinoline-5-Carboxylic Acid(7c):

7c (i.e. 7, R=CH_2-CH_3, X=H): Yield = 1.6 gm (42%), M. P. = >240°C, IR (KBr): 3459 cm^{-1} (small, sharp, -OH stretchings), 3381 and 3018 cm^{-1} (small, sharp, -NH stretching vibrations), 2789-2792 cm^{-1} (small, sharp, enamine –CH stretching vibrations), 1737 cm^{-1} (strong, sharp, -C=O stretching), 1614 cm^{-1} (small, sharp, -O-C=O stretching), 1466 cm^{-1} (aromatic *trans*- C=C stretching), 1290-1370 cm^{-1} (strong, sharp, aromatic –C-F), ^1H – NMR spectrum (DMSO/d_6/TMS, 400 MHz): δ 1.23-1.30 (m, 5H, N-CH_2-CH_3), 2.54 (d, 2H, Piperazine –CH), 3.03 (d, 2H, Piperazine –CH), 3.6 (d, 2H, Piperazine –CH), 4.3 (d, 2H, Piperazine –CH), 6.48 (s, 1H, quaternary –CH), 6.9 (d, 1H, phenyl proton), 7.13 (d, 1H, phenyl proton), 7.23 (d, 1H, vinyl proton, *J* = 16Hz), 7.26 (s, 1H, Ar-H), 7.45-7.47 (m, 3H, phenyl protons), 7.63 (s, 1H, enamine –CH), 8.44 (d, 1H, vinylic proton, *J* = 16Hz), 9.9 (s, 1H, imidazole –NH), 10.8 (d, 1H, tautomeric-OH), MS: *m/z* 449.19 ($M^{+\cdot}$).

(E)-8-Fluoro-2-(4-Fluorostyryl)-6-oxo-9-(Piperazin-1-yl)-6H-Imidazo[4,5,1-ij]Quinoline-5-Carboxylic Acid:(7d):

7d (i.e. 7, R=H, X=F): Yield = 1.7 gm (40%), M. P. = >240°C, IR (KBr): 3459 cm^{-1} (small, sharp, -OH stretchings), 3381 and 3018 cm^{-1} (small, sharp, -NH stretching vibrations), 2789-2792 cm^{-1} (small, sharp, enamine –CH stretching vibrations), 1737 cm^{-1} (strong, sharp, -C=O stretching), 1614 cm^{-1} (small, sharp, -O-C=O stretching), 1466 cm^{-1} (aromatic *trans*- C=C stretching), 1290-1370 cm^{-1} (strong, sharp, aromatic –C-F), ^1H – NMR spectrum (DMSO/d_6/TMS, 400 MHz): δ 1.1-1.3 (m, 9H, -NH of Piperazine and its –CH protons), 6.48 (s, 1H, quaternary –CH), 7.01 (d, 1H, phenyl proton), 7.27 (d, 1H, phenyl proton), 7.76 (d, 1H, vinyl proton, *J* = 16Hz), 7.96 (d, 1H, vinyl proton, *J* = 16Hz), 8.05 (d, 1H, phenyl proton), 8.14 (d, 1H, phenyl proton), 8.76 (s, 1H, Ar-H), 8.99 (s, 1H, enamine –CH), 13.42 (s, 1H, imidazole –NH), 14.93 (s, 1H, -OH), MS: *m/z* 437.14 ($M^{+\cdot}$).

(E)-8-Fluoro-2-(4-Fluorostyryl)-9-(4-Methylpiperazin-1-yl)-6-oxo-6H-Imidazo[4,5,1-ij]Quinoline-5-Carboxylic Acid(7e):

7e (i.e. 7, R=CH_3, X=F): Yield = 1.9 gm (44%), M. P. = >240°C, IR (KBr): 3459 cm^{-1} (small, sharp, -OH stretchings), 3381 and 3018 cm^{-1} (small, sharp, -NH stretching vibrations), 2789-2792 cm^{-1} (small, sharp, enamine –CH stretching vibrations), 1737 cm^{-1} (strong, sharp, -C=O stretching), 1614 cm^{-1} (small, sharp, -O-C=O stretching), 1466 cm^{-1} (aromatic *trans*- C=C stretching), 1290-1370 cm^{-1} (strong, sharp, aromatic –C-F), ^1H – NMR spectrum (DMSO/d_6/TMS, 400 MHz): δ 1.1-1.3 (m, 8H, Piperazine and its –CH protons), 2.26 (s, 3H, Piperazine –N-CH_3), 6.4 (s, 1H, quaternatry –CH), 7.01 (d, 1H, phenyl proton), 7.27 (d, 1H, phenyl proton), 7.76 (d, 1H, vinyl proton, *J* =

16Hz), 7.96 (d, 1H, vinyl proton, *J* = 16Hz), 8.05 (d, 1H, phenyl proton), 8.14 (d, 1H, phenyl proton), 8.76 (s, 1H, Ar-H), 8.99 (s, 1H, enamine –CH), 13.42 (s, 1H, imidazole –NH), 14.93 (s, 1H, -OH), MS: *m/z* 451.15 (M^+).

(E)-9-(4-Ethylpiperazin-1-yl)-8-Fluoro-2-(4-Fluorostyryl)-6-oxo-6H-Imidazo[4,5,1-ij]Quinoline-5-Carboxylic Acid(7f):

7f(i.e. 7, R=CH_2-CH_3, X=F): Yield = 1.9 gm (42%), M. P. = 240°C, IR (KBr): 3459 cm^{-1} (small, sharp, -OH stretchings), 3381 and 3018 cm^{-1} (small, sharp, -NH stretching vibrations), 2789-2792 cm^{-1} (small, sharp, enamine –CH stretching vibrations), 1737 cm^{-1} (strong, sharp, -C=O stretching), 1614 cm^{-1} (small, sharp, -O-C=O stretching), 1466 cm^{-1} (aromatic *trans*- C=C stretching), 1290-1370 cm^{-1} (strong, sharp, aromatic –C-F), ^{1}H – NMR spectrum (DMSO/d_6/TMS, 400 MHz): δ 1.23-1.30 (m, 5H, N-CH_2-CH_3), 2.54 (d, 2H, Piperazine –CH), 3.03 (d, 2H, Piperazine –CH), 3.6 (d, 2H, Piperazine –CH), 4.3 (d, 2H, Piperazine –CH), 6.48 (s, 1H, quaternary –CH), 7.01 (d, 1H, phenyl proton), 7.27 (d, 1H, phenyl proton), 7.76 (d, 1H, vinyl proton, *J* = 16Hz), 7.96 (d, 1H, vinyl proton, *J* = 16Hz), 8.05 (d, 1H, phenyl proton), 8.14 (d, 1H, phenyl proton), 8.76 (s, 1H, Ar-H), 8.99 (s, 1H, enamine –CH), 13.42 (s, 1H, imidazole –NH), 14.93 (s, 1H, -OH), MS: *m/z* 464.03 ($M^{+\cdot}$).

(E)-2-(4-Chlorostyryl)-8-Fluoro-6-oxo-9-(Piperazin-1-yl)-6H-Imidazo[4,5,1-ij]Quinoline-5-Carboxylic Acid(7g):

7g (i.e. 7, R=H, X=Cl): Yield = 1.6 gm (42%), M. P. = >240°C, IR (KBr): 3459 cm^{-1} (small, sharp, -OH stretchings), 3381 and 3018 cm^{-1} (small, sharp, -NH stretching vibrations), 2789-2792 cm^{-1} (small, sharp, enamine –CH stretching vibrations), 1737 cm^{-1} (strong, sharp, -C=O stretching), 1614 cm^{-1} (small, sharp, -O-C=O stretching), 1466 cm^{-1} (aromatic *trans*- C=C stretching), 1290-1370 cm^{-1} (strong, sharp, aromatic –C-F), ^{1}H – NMR spectrum (DMSO/d_6/TMS, 400 MHz): δ 1.1-1.3 (m, 9H, -NH of Piperazine and its –CH protons), 6.48 (s, 1H, quaternary –CH), 7.01 (d, 1H, phenyl proton), 7.27 (d, 1H, phenyl proton), 7.76 (d, 1H, vinyl proton, *J* = 16Hz), 7.96 (d, 1H, vinyl proton, *J* = 16Hz), 8.05 (d, 1H, phenyl proton), 8.14 (d, 1H, phenyl proton), 8.76 (s, 1H, Ar-H), 8.99 (s, 1H, enamine –CH), 13.42 (s, 1H, imidazole –NH), 14.93 (s, 1H, -OH), MS: *m/z* 453.11 ($M^{+\cdot}$).

(E)-2-(4-Chlorostyryl)-8-Fluoro-9-(4-Methylpiperazin-1-yl)-6-Oxo-6H-Imidazo[4,5,1-ij]Quinoline-5-Carboxylic Acid(7h):

7H (i.e. 7, R=CH_3, X=Cl): Yield = 1.8 gm (40%), M. P. = >240°C, IR (KBr): 3459 cm^{-1} (small, sharp, -OH stretchings), 3381 and 3018 cm^{-1} (small, sharp, -NH stretching vibrations), 2789-2792 cm^{-1} (small, sharp, enamine –CH stretching vibrations), 1737 cm^{-1} (strong, sharp, -C=O stretching), 1614 cm^{-1} (small, sharp, -O-C=O stretching), 1466 cm^{-1} (aromatic *trans*- C=C stretching), 1290-1370 cm^{-1} (strong, sharp, aromatic –C-F), ^{1}H – NMR spectrum (DMSO/d_6/TMS, 400 MHz): δ 1.1-1.3 (m, 8H, Piperazine and its –CH protons), 2.26 (s, 3H, Piperazine –N-CH_3), 6.4 (s, 1H, quaternatry –CH), 7.01 (d, 1H, phenyl proton), 7.27 (d, 1H, phenyl proton), 7.76 (d, 1H, vinyl proton, *J* = 16Hz), 7.96 (d, 1H, vinyl proton, *J* = 16Hz), 8.05 (d, 1H, phenyl proton), 8.14 (d, 1H, phenyl proton), 8.76 (s, 1H, Ar-H), 8.99 (s, 1H, enamine –CH), 13.42 (s, 1H, imidazole –NH), 14.93 (s, 1H, -OH), MS: *m/z* 467.12 ($M^{+\cdot}$).

(E)-2-(4-Chlorostyryl)-9-(4-Ethylpiperazin-1-yl)-8-Fluoro-6-Oxo-6H-Imidazo[4,5,1-ij]Quinoline-5-Carboxylic Acid(7i):

7i (i.e. 7, R=CH_2-CH_3, X=Cl): Yield = 2.1 gm (44%), M. P. = >240°C, IR (KBr): 3459 cm^{-1} (small, sharp, -OH stretchings), 3381 and 3018 cm^{-1} (small, sharp, -NH stretching vibrations), 2789-2792 cm^{-1} (small, sharp, enamine –CH stretching vibrations), 1737 cm^{-1} (strong, sharp, -C=O stretching), 1614 cm^{-1} (small, sharp, -O-C=O stretching), 1466 cm^{-1} (aromatic *trans*- C=C stretching), 1290-1370 cm^{-1} (strong, sharp, aromatic –C-F), ^{1}H – NMR spectrum (DMSO/d_6/TMS, 400 MHz): δ 1.23-1.30 (m, 5H, N-CH_2-CH_3), 2.54 (d, 2H, Piperazine –CH), 3.03 (d, 2H, Piperazine –CH), 3.6 (d, 2H, Piperazine –CH), 4.3 (d, 2H, Piperazine –CH), 6.48 (s, 1H, quaternary –CH), 7.01 (d, 1H, phenyl proton), 7.27 (d, 1H, phenyl proton), 7.76 (d, 1H, vinyl proton, *J* = 16Hz), 7.96 (d, 1H, vinyl proton, *J* = 16Hz), 8.05 (d, 1H, phenyl proton), 8.14 (d, 1H, phenyl proton), 8.76 (s, 1H, Ar-H), 8.99 (s, 1H, enamine –CH), 13.42 (s, 1H, imidazole –NH), 14.93 (s, 1H, -OH), MS: *m/z* 423.18 (M^+).

RESULTS AND DISCUSSION

Anti-Mycobacterial Activity

The susceptibility test was accomplished in 96 microplates (wells) using the resazurin as an indicator of cellular viability or growth inhibition. Working solutions of the tested extracts were diluted in Middle Brook 7H9 broth supplemented with OADC to obtain final sample concentrations that ranges from 0.78 μg/mL to 100 μg/mL. Rifampicin was dissolved in DMSO and used as positive control drug and extracts/ drug free medium with strain

suspensions were used as negative control. One hundred micro liters of Middle Brook 7H9 broth and the test inoculum were added to all testing wells, as well as to the drug/extract-free control wells. Then, one hundred micro liters working extract solutions were poured into the first well of each row from which two-fold dilution series were made through the micro plate column. Each extract concentration was assayed in duplicate. Each micro plate was then sealed with parafilm and incubated for 5-7 days at 37°C in normal atmosphere. After the incubation period, 25 µL of resazurin 0.02% w/v was added to each wells and reincubated at 37°C for 24 h for color development. The visual MIC was defined as the lowest drug/extract concentration that prevented the color change of resazurin reagent from blue to pink. Blue color in the well was interpreted as there is no mycobacterial growth and pink color was scored as growth occurrence [13,14].

Determination of the Minimum Inhibitory Concentration (MIC):

MIC was determined by using the REMA in 96 well micro titer plates. One hundred micro liters of Middle Brook 7H9 broth and *M. tuberculosi*s and *M. bovis* strains were dispensed into all wells of a sterile 96-well microtitre plate. In the first column (no.1 well of all plate), one hundred, micro liters of extracts were added to each first wells using a unique pipette for each extracts. The extracts were mixed thoroughly and fifty micro liters of extracts were transferred to well 2 to well 8 from which fifty micro liters were discarded. Well 9 up to well 12 were served as sterility and negative controls. The working solution of extracts (100 µg/mL) were diluted out across a 96-well in a two-fold serial dilution to give final testing concentrations of 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 µg/mL. The same procedure was followed for rifampicin with the initial concentration of 32 µg/mL with subsequent dilution to the final testing concentrations 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 and 0.06 µg/mL. The plates were then incubated for 5-7 days at 37°C. After 7th day, 25 µL of resazurin was added to all wells and re-incubated overnight for development. The MIC was defined as the lowest concentration of the extracts/drugs that prevented a colour of resazurin to be changed from blue to pink (visual determination) [15-17]. According to Ramos [12], extracts were considered as active if they inhibited growth of mycobacterium at MIC ≤ 100 µg/mL. Each extract was tested in duplicate against each strain.

Molecular docking:

All the ligands were sketched in Sybyl6.7 and saved it in .mol2 format. All the molecules were energy minimized by adding Gasteiger-Huckel charges for stability (Figure 3). Each and every molecule was docked separately. Initially the molecule was loaded; torsions were set and saved it in PDBQT format. All the heteroatoms were removed from the 3IVX.PDB (Crystal structure of pantothenatesynthetase in complex with 2-(2-(benzofuran-2-ylsulfonylcarbamoyl)-5-methoxy-1H-indol-1-yl) acetic acid), to make complex receptor free of any ligand before docking [18]. The PDB was also saved in PDBQT format. All calculations for protein-ligand flexible docking were performed using the Lamarckian Genetic Algorithm (LGA) method. A grid box with the dimensions of X: 15.137, Y: 17.850 and Z: -3.573 Å, with a default grid spacing of 0.375 Å was used.

Table 1: Physicochemical properties and antimycobacterial activity of synthesized imidazole quinoline-5-carboxylic acid derivatives (7a-7g)

Compound	M.F	Mol.Wt	M.p. (°C)	% yield	Antimycobacterial MIC (µM) *M.smegmatis*
7a	C23H21FN4O3	420.44	240	42	44.2
7b	C24H23FN4O3	434.46	> 240	45	32.42
7c	C25H25FN4O3	448.49	> 240	46	>100
7d	C23H18F2N4O3	436.41	> 240	40	74.2
7e	C24H20F2N4O3	450.44	240	44	35.55
7f	C25H22F2N4O3	464.46	240	42	52.2
7g	C23H18ClFN4O3	452.87	> 240	43	28.62
7H	C24H20ClFN4O3	466.89	240	40	40.2
7i	C25H22ClFN4O3	480.92	> 240	44	[c]
Ref					a
					b

[a]Ethambutol dihydrochloride 5-10 µg/mL, [b]isoniazid—0.2-1 µg/mL, [c]Not determined

The best conformation was chosen with the lowest docked energy [19], after the docking search was completed. The interactions of 3IVX protein and ligand conformations, including hydrogen bonds and the bond lengths were analyzed. Molecular docking study was performed by using AUTODOCK 4.2 which was a suite of automated docking tools and was used to predict the affinity, activity, binding orientation of ligand with the target protein and to analyze best conformations, the protein with all the 5 compounds were loaded individually into ADT and evaluate ten

finest conformations. In the present investigation we focused mainly on the binding energy, hydrogen bonds, and distance between the protein and ligand. Compound 7g shows highest binding energy of -6.92 kCal/mol with three interactions (Arg200, Thr85 and Glu189). Out of 5 compounds three compounds are interacting with Arg200 and Glu189. All the compounds binding energy and interacting aminoacids are given in Tables 1 and 2.

Table 2: Interactions of compounds (7b,7d,7f,7g,7e) with crystal structure of pantothenate synthetase in complex with (PDB id: 3IVX)

C.NO	Coordinates	Interacting Amino Acids	Binding Energy	KI(μM)
7b	15.137, 17.850, -3.573	ASN199, MET195	-5.8	56.28
7d	15.137, 17.850, -3.573	SER196, GLU189	-6.34	22.36
7e	15.137, 17.850, -3.573	ARG200, GLU189	-6.24	26.65
7f	15.137, 17.850, -3.573	ARG200, THR85	-6.36	21.75
7g	15.137, 17.850, -3.573	ARG200, THR85, GLU189	-6.92	8.45

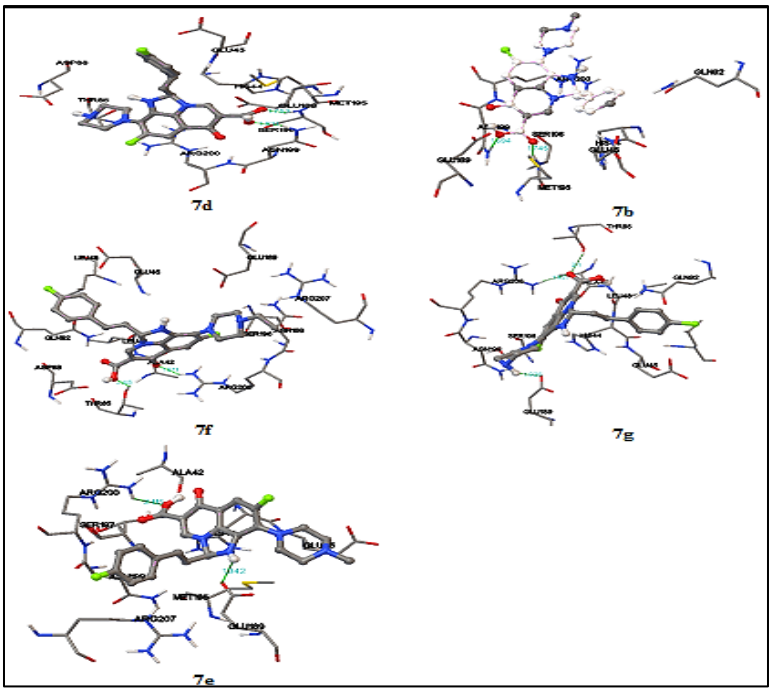

Figure 3: Docked confirmations of target compounds with 3IVX

CONCLUSION

In summary, the synthesis and characterization of new series of imido Quinoline-5-carboxylic acid and its derivatives (7a-7i). *In vitro mycobacterium tuberculosis* by microdilution method showed that 7b, 7d, ,7e,7f and 7g were slightly more active than Ethambutol dihydrochlorid, isoniazid. Whereas, compound 7c displayed the greatest activity.The substituent in the aromatic ring has an important role in the biological activity and generally compounds having electron-withdrawing groups, such as carboxylic acid are active. Further investigation, the imido Quinoline-5-carboxylic acid and its derivatives 7b, 7d, ,7e,7f and 7g were docked and exhibited good binding energy, Π-Π interactions, vanderwall interactions against the same receptor, the energy values are less than the standards (amoxicillin, ciprofloxacin) by employing PDB code 3IVX, and software is autodock 4.2. So, it can be concluded that the designed compounds can be potent antitubercular agents. In future research, these 1*H*-benzimidazole derivatives will be synthesized and screened for their *in vitro* anti-tuberculosis activity.

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