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Synthesis, Docking Study and In-vitro Evaluation of Anti-Tuberculosis Activity of Tri Substituted Imidazoles Containing Quinoline Moiety

S. Sahana, G. R. Vijayakumar*, R. Sivakumar†, D. Sriram‡, and D. V. Saiprasad§

Department of Chemistry, University College of Science, Tumkur University, Tumakuru, Karnataka 572103, India.
**E-mail: vijaykumargr18@gmail.com*
†*Department of Pharmaceutical Chemistry, Geethanjali College of Pharmacy, Cheeryal, Hyderabad 500068, India.*
‡*Department of Pharmacy, Birla Institute of Technology & Science-Pilani, Hyderabad Campus, Jawahar Nagar, Hyderabad 500078, India.*
§*Drug Discovery Research Laboratory, Department of Pharmaceutical Science, BITS-Hyderabad 500078, India.*
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ABSTRACT. A simple, efficient, and cost-effective method has been employed for the synthesis of 2,4,5-trisubstituted imidazole derivatives **(3a-j)** containing quinoline substituent at 2nd position. Title compounds were obtained by multicomponent reaction (MCR), involving aryl substituted 1,2-diketone, quinoline carbaldehyde and ammonium acetate in the presence of acetic acid solvent under mild reaction conditions. The newly synthesized quinoline containing imidazole derivatives were confirmed through FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral analysis. *In-vitro* microplate alamar blue assay (MABA) to determine the MIC (minimum inhibitory concentration) values against *Mycobacterium tuberculosis* H37Rv was performed for the synthesized compounds. The synthesized compounds exhibited activity against *Mycobacterium tuberculosis* and among which compounds, **3d, 3f** and **3i** showed good activity. The highest activity was showed with compound **3i**. The anti-mycobacterial activity results are well correlated with the computational molecular docking analysis, which was performed for the synthesized compounds prior to the evaluation of the activity.

Key words: 2,4,5-trisubstituted imidazole, Aryl substituted 1,2-diketone, Quinoline aldehyde, Anti-tuberculosis activity, Computational molecular docking

INTRODUCTION

Tuberculosis (TB) is caused by a single infectious agent *Mycobacterium tuberculosis*, and it is the reason for one of the top ten causes of death.[1] Millions of people fall sick due to TB each year. TB is a lung infection disease and is considered to be one of the most contagious and deadly diseases hence it is a major threat to public health. Global TB end strategy cannot be achieved without TB research and development. All the existing drugs have acquired resistance, cross-resistance and further, they induce toxicity. It is vital to develop new drugs for the complete evacuation of this deadly disease. The recalcitrant nature of persistent infection and increase in multi- and extensively drug-resistant strains (MDR-TB and XDR-TB) are the main challenges for effective treatment of TB with the currently available anti-TB drugs. New TB drugs are needed because of the complexity and toxicity of the current TB drug regimens. It is an urgent need to develop potently and cost-effective anti-TB drugs. New TB drugs need to provide shorter, simpler, affordable, more effective, less toxic, multi-drug regimens for drug-sensitive TB and safe regimens for latent TB.[2]

It has been known from the literature that heterocyclic compounds, quinoline and isoquinoline analogues play an important role in medicinal chemistry applications. Quinoline and isoquinoline moieties are present in many naturally occurring compounds.[3] The compounds containing these scaffolds are significant because of their wide spectrum of biological activities such as antimalarial,[4] antibiotic,[5] anticancer,[6] antiinflammatory,[7] antihypertensive,[8] tyrokinase,[9] PDGF-RTK inhibition[10] and anti-HIV[11] activities. It is revealed from the literature that, quinoline scaffolds containing derivatives with varied substituents were found to exhibit potent anti-TB activity.

Imidazoles are another class of heterocyclic compounds and molecules containing imidazole moiety have found to be useful in many biological applications like anticancer activity,[12] antimicrobial[13–16] and antifungal activity[17] including antitubercular activity.[18,19] Substituted imidazoles might be obtained by multicomponent synthesis involvingaryl-1,2-diketone or α-hydroxyketone or α-ketomonoxime with an aldehyde and ammonium acetate, which comprise the use of ionic liquids,[20] refluxing in acetic acid,[21] silica-sup-

ported sulfuric acid,[22] $InCl_3 \cdot 3H_2O$,[23] ceric ammonium nitrate (CAN),[24] $NiCl_2 \cdot 6H_2O/Al_2O_3$[25] and microwave irradiation.[26]

In continuation with our earlier report,[27] herein an attempt has been made to synthesize 2,4,5-trisubstituted imidazole containing quinoline substituent by the effective method via one-pot three-component reaction involving aryl-1,2-diketone, quinoline aldehyde and ammonium acetate in presence of an acetic acid solvent. The products were afforded in very good yields. The synthesized compounds were subjected to docking studies followed by the determination of anti-tuberculosis activity.

EXPERIMENTAL

All chemicals used in the synthesis were purchased from Sigma-Aldrich (USA) and TCI Chemicals (India) Pvt. Ltd. Analytical grade solvents were acquired from commercial sources and are used without further purification unless otherwise stated. The progress of the reaction was monitored by TLC using petroleum ether and ethyl acetate (7:3) as mobile phase and it was performed on TLC Silica gel 60 F254 (Merck) and spots were visualized by using ultraviolet light of 254 nm. Melting points were determined using an open capillary and are uncorrected. IR spectra were recorded by using Perkin Elmer Spectrophotometer by the KBr pellet method. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Agilent-400 MHz NMR instrument using $CDCl_3$/DMSO-d_6 as solvent. Chemical shift values were expressed in δ (ppm) relative to TMS as an internal standard. All the products are analytically pure and MS spectra of final compounds were recorded on Waters Alliance Micromass ZQ 2000 LCMS.

Preparation of 2-Chloroquinoline-3-Carbaldehyde (1)

2-Chloroquinoline-3-carbaldehyde was synthesized by a well-known Vilsmeier-Haack reaction.[28] Acetanilide (13.5 g, 0.1 mol) was dissolved in dimethylformamide (23 mL, 0.3 mol) and was added with phosphorus oxychloride (65 mL, 0.7 mol) by maintaining the temperature at 0°C. The reaction mixture was taken in a 250 mL round bottom flask, fitted with a water condenser and refluxed for 5–6 h on an oil bath. The solution was cooled to room temperature and then poured into 250 mL ice water. The precipitate was collected by filtration and recrystallized from ethyl acetate which resulted in pure product with 88% yield.

Preparation of 2-Hydroxyquinoline-3-carbaldehyde (2a)

2-Chloroquinoline-3-carbaldehyde (1.91 g, 10 mmol) and 70% acetic acid (50 mL) was taken in an RB flask and refluxed for 4 h on an oil bath. After completion of the reaction, the mixture was poured into ice water. The separated solid was filtered, washed with water and dried. Recrystallization was carried out using ethyl acetate to get the pure product with 85% yield.

General Procedure for the Synthesis of 2,4,5-Trisubstituted Imidazoles (3a-j)

A mixture of aryl-1,2-diketone (1 mmol), quinoline aldehyde (1 mmol), NH_4OAc (10 mmol), and 7 mL of acetic acid was taken in a 100 mL round bottom flask and stirred on a magnetic stirrer at reflux temperature. The progress of the reaction was monitored by TLC using petroleum ether and ethyl acetate (7:3) as mobile phase. After completion of the reaction, the reaction mixture was cooled to room temperature and poured into ice-cold water. The resulting solid product was filtered under suction and washed thoroughly with water. The product was further purified by column chromatography using petroleum ether and ethyl acetate (80:20) as eluent.

Spectral Data

3-(4,5-Diphenyl-1*H*-Imidazol-2-yl) quinolin-2-ol (3a): Pale yellow solid; Yield: 95%; m.p: 308-310°C; FT-IR (KBr, cm^{-1}) ν_{max}: 3332.6 (N-H), 2851.8 (C-H, alkane), 1651.6 (C=C), 1570.1 (C=N), 1219.0 (C-O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ(ppm): 7.23-7.25 (m, 2H, ArH), 7.29-7.30 (m, 4H, ArH), 7.41-7.42 (m, 2H, ArH), 7.47 (d, J = 7.6 Hz, 2H, ArH), 7.52-7.54 (m, 3H, ArH), 7.90 (d, J = 7.6 Hz, 1H, ArH), 8.58 (s, 1H, ArH), 12.21 (s, 1H, -OH), 12.39 (s, 1H, -NH); ^{13}C-NMR (400 MHz, DMSO-d_6) δ(ppm): 115.7, 119.8, 120.2, 123.1, 127.3, 127.5, 127.8, 128.2, 128.7, 129.0, 129.3, 131.2, 135.4, 135.8, 137.8, 138.4, 142.7, 161.4; (ESI-MS) *m/z*: Calculated for $C_{24}H_{17}N_3O$ $[M+H]^+$: 364.41; Found 364.36.

3-(4,5-Bis(3-methoxyphenyl)-1*H*-imidazol-2-yl) quinolin-2-ol (3b): Pale yellow solid; Yield: 95%; m.p: 248-250°C; FT-IR (KBr, cm^{-1}) ν_{max}: 3359.2 (O-H), 2966.7 (m) (C-H, alkane), 1641.9 (C=C), 1606.0 (C=C), 1580.5 (C=N), 1499 (C=N), 1201.7 (C-O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 3.75 (s, 3H,-OCH_3), 3.79 (s, 3H, -OCH_3), 6.84-6.86 (m, 2H, ArH), 7.07 (s, 1H, ArH), 7.11 (d, J = 7.6 Hz, 1H, ArH), 7.21-7.23 (m, 6H, ArH), 7.45 (t, J = 7.6 Hz, 1H, ArH), 7.67 (d, J = 8.0 Hz, 1H, ArH), 8.94 (s,1H,ArH), 12.04 (s, 1H,-OH), 12.11 (s, 1H,-NH); ^{13}C-NMR (400 MHz, $CDCl_3$) δ(ppm): 55.2, 112.7, 113.2, 113.4, 113.5, 115.4, 119.1, 119.7, 120.4, 120.8, 123.6, 127.1, 128.6, 129.3, 129.8, 130.8, 132.3, 136.2, 136.3, 137.1, 138.7, 142.2, 159.6, 159.8,

162.8; (ESI-MS) *m/z*: Calculated for $C_{26}H_{21}N_3O_3$ $[M+H]^+$: 424.46; Found 424.40.

3-(5-(2-Chlorophenyl)-4-(3,4-dimethoxyphenyl)-1*H*-imidazol-2-yl) quinolin-2-ol (3c): Pale yellow solid; Yield: 89%; m.p: 210-212°C; FT-IR (KBr, cm^{-1}) ν_{max}: 3282.2 (N-H), 2955.4 (C-H, alkane), 1644.4 (C=C), 1587.4 (C=C), 1513.6 (C=N), 1242.2 (C-O), 743.9 (C-Cl); ^{1}H-NMR (400 MHz, DMSO-d_6) δ(ppm): 3.52 (s, 3H, -OCH_3), 3.70 (s, 3H, -OCH_3), 6.88 (m, 2H, ArH), 7.01(m, 1H, ArH), 7.26 (m, 1H, ArH), 7.41-7.47 (m, 6H, ArH), 7.89 (dd, J = 7.2, 28.8 Hz, 1H, ArH), 8.77 (s, 1H, ArH), 12.36 (s, 1H, -OH), 12.37 (s, 1H, -NH); ^{13}C-NMR (400 MHz, DMSO-d_6) δ(ppm): 56.0, 110.4, 110.7, 112.5, 112.8, 116.0, 118.9, 119.3, 120.2, 120.7, 123.4, 124.1, 128.0, 128.9, 129.3, 130.4, 131.1, 131.5, 133.6, 134.2, 135.9, 138.8, 142.5, 149.1, 161.6, 243.5; (ESI-MS) *m/z*: Calculated for $C_{26}H_{20}ClN_3O_3$ $[M+H]^+$: 458.91; Found 458.36.

3-(4,5-Bis(4-fluorophenyl)-1*H*-imidazol-2-yl) quinolin-2-ol (3d): Pale yellow solid; Yield: 93%; m.p: 320-322°C; FT-IR (KBr, cm^{-1}) ν_{max}: 3350.9 (O-H), 3345.0 (N-H), 2854.6 (CH, alkane), 1606 (C=C), 1648.3 (C=C), 1511.3 (C=N), 1571.0 (C=N), 1224.9 (C-O), 1241.9 (C-F); ^{1}H-NMR (400 MHz, DMSO-d_6) δ(ppm): 7.15 (t, J = 8.8 Hz, 2H, ArH), 7.25 (t, J = 8.4 Hz, 3H, ArH), 7.41 (d, J = 8.4 Hz, 1H, ArH), 7.48-7.58 (m, 5H, ArH), 7.89 (d,J = 7.6 Hz, 1H, ArH), 8.71 (s, 1H, ArH), 12.29 (s, 1H, -OH), 12.34 (s, 1H, -NH); ^{13}C-NMR (400 MHz, DMSO-d_6) δ(ppm): 115.8, 116.0, 116.5, 116.7, 120.1, 120.5, 123.4, 126.8, 127.7, 129, 129.91, 130.0, 130.8, 131.6, 132.0, 136.3, 137.2, 138.7, 143.0, 160.8, 161.2, 161.6, 163.2, 163.7; (ESI-MS) *m/z*: Calculated for $C_{24}H_{15}F_2N_3O$ $[M+H]^+$: 400.39; Found 400.27.

3-(4,5-Bis(4-methoxyphenyl)-1*H*-imidazol-2-yl) quinolin-2-ol (3e): Pale brown solid; Yield: 86%; m.p: 274-276°C; FT-IR (KBr, cm^{-1}) ν_{max}: 3324.6 (N-H), 2929.4 (C-H, alkane), 1643.0 (C=C), 1612.2 (C=C), 1571.0 (C=N), 1493.3 (C=N), 1246.7 (C-O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ(ppm): 3.71 (s, 6H, -OCH_3), 6.95-6.91 (m, 4H, ArH), 7.26-7.22 (m, 1H, ArH), 7.38-7.54 (m, 6H, ArH), 7.87 (d, J = 8.4 Hz, 1H, ArH), 8.75 (s, 1H, ArH), 12.11 (s, 1H, -OH), 12.38 (s, 1H, -NH); ^{13}C-NMR (400 MHz, DMSO-d_6) δ(ppm): 55.5, 114.0, 114.7, 115.6, 119.8, 120.2, 123.0, 128.32, 128.9, 129.5, 131.0, 135.2, 138.2, 142.0, 161.4, 163.1; (ESI-MS) *m/z*: Calculated for $C_{26}H_{21}N_3O_3$ $[M+H]^+$: 424.46; Found 424.34.

3-(4-(4-Chlorophenyl)-5-phenyl-1*H*-imidazol-2-yl) quinolin-2-ol (3f): Pale brown solid; Yield: 80%; m.p: 298-300°C; FT-IR (KBr, cm^{-1}) ν_{max}: 3330.3 (N-H), 3030.3 (C-H, aromatic), 1650.7 (C=C), 1573 (C=C), 1499.6 (C=N), 1218.1 (C-O), 760.5 (C-Cl); ^{1}H-NMR(400 MHz, DMSO-d_6) δ(ppm): 7.07 (m, J = 4.2 Hz, 1H, ArH), 7.16 (m, J = 7.6 Hz, 1H, ArH), 7.24-7.31 (m, 2H, ArH), 7.38-7.90 (m, 7H, ArH), 7.91 (d, J = 7.6 Hz, 1H, ArH), 8.36 (m, 1H, ArH), 8.79 (s, 1H, ArH), 12.41 (s, 1H, -OH), 12.34 (s, 1H, -NH); ^{13}C-NMR (400 MHz, DMSO-d_6) δ(ppm): 115.7, 119.7, 120.1, 123.0, 126.5, 127.9, 128.3, 128.8, 129.0, 129.2, 129.6, 130.8, 131.2, 136.0, 138.4, 142.9, 161.3; (ESI-MS) *m/z:* Calculated for $C_{24}H_{16}ClN_3O$ $[M+H]^+$: 398.86; Found 398.09.

4-(4,5-Diphenyl-1*H*-imidazol-2-yl) quinoline (3g): Off white solid; Yield: 94%; m.p: 324-326°C; FT-IR (KBr, cm^{-1}) ν_{max}: 3413.1 (N-H), 3060.9 (C-H, aromatic), 1592.3 (C=C), 1507.1 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ(ppm): 7.26-7.34 (m, 6H, ArH), 7.59 (s, 4H, ArH), 7.70 (t, J = 7.6 Hz, 1H, ArH), 7.80 (t, J = 1.2 Hz, 1H, ArH), 7.98 (d, J = 4.4 Hz, 1H, ArH), 8.07 (d, J = 8.4 Hz, 1H, ArH), 8.98 (d, J = 4.4 Hz, 1H, ArH), 9.48 (d, J = 8.4 Hz, 1H, ArH), 13.13 (s, 1H, N-H); ^{13}C-NMR (400 MHz, DMSO-d_6) δ(ppm): 120.2, 125.3, 127.6, 127.9, 128.0, 129.0, 129.1, 129.4, 129.5, 130.2, 130.2, 131.3, 134., 135.6, 139.0, 144.1, 149.5, 150.8; (ESI-MS) *m/z*: Calculated for $C_{24}H_{17}N_3$ $[M+H]^+$: 348.41; Found 348.3.

4-(4,5-Bis(4-methoxyphenyl)-1*H*-imidazol-2-yl) quinoline (3h): Pale yellow solid; Yield: 83%; m.p: 160-162°C; FT-IR (KBr, cm^{-1}) ν_{max}: 3400 (br) (N-H), 2954.6 (C-H, alkane), 1610 (C=C), 1517.9 (C=N), 1248.6 (C-O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ(ppm): 3.79 (s, 3H), 3.82 (s, 3H), 7.01-7.03 (m, 5H, ArH), 7.61-7.62 (m, 4H, ArH), 7.76 (m, 1H, ArH), 7.85 (m, 1H, ArH), 8.11 (d, J = 8.0 Hz, 1H, ArH), 8.16 (d, J = 4.4 Hz, 1H, ArH), 9.03 (d, J = 4.4 Hz, 1H, ArH), 13.19 (s, 1H, -NH); ^{13}C-NMR (400 MHz, DMSO-d_6) δ(ppm): 60.7, 60.8, 119.8, 120.1, 120.3, 125.5, 125.8, 129.5, 131.6, 133.6, 133.8, 134.5, 135.3, 135.4, 137.6, 141.0, 151.0, 154.1, 155.8, 162.2, 165.0, 165.5; (ESI-MS) *m/z*: Calculated for $C_{26}H_{21}N_3O_2$ $[M+H]^+$: 408.46; Found 409.13.

4-(4,5-Bis(4-fluorophenyl)-1*H*-imidazol-2-yl) quinoline (3i): Off white solid; Yield: 80%; m.p: 172-174°C; FT-IR (KBr, cm^{-1}) ν_{max}: 3400 (br) (N-H), 3069.9 (C-H, aromatic), 1587.6 (C=C), 1516.2 (C=N), 1225.9 (C-F); ^{1}H-NMR (400 MHz, DMSO-d_6) δ(ppm): 7.19-7.31 (m, 4H, ArH), 7.58-7.59 (m, 4H, ArH), 7.70 (m, 1H, ArH), 7.80 (m, 1H, ArH), 7.96 (d, J = 4.4 Hz, 1H, ArH), 8.07 (d, J = 8.0 Hz, 1H, ArH), 8.99 (d, J = 4.4 Hz, 1H, ArH), 9.45 (d, J = 8.4 Hz, 1H, ArH), 13.19 (s, 1H, -NH); ^{13}C-NMR (400 MHz, DMSO-d_6) δ(ppm): 115.9, 119.8, 124.9, 127.5, 128.6, 129.5, 129.8, 129.9, 131.2, 134.3, 137.8, 143.7, 149.1, 150.4; (ESI-MS) *m/z*: Calculated for $C_{24}H_{15}F_2N_3$ $[M+H]^+$

: 383.39; Found 383.25.

4-(5-(2-Chlorophenyl)-4-(3,4-dimethoxyphenyl)-1*H*-imidazol-2-yl) quinoline (3j): Pale yellow solid; Yield: 83%; m.p: 200-202°C; FT-IR (KBr, cm^{-1}) ν_{max}: 3400 (N-H), 3060.8 (C-H,aromatic), 2953.9 (CH, alkane), 1583.8 (C=C), 1513.9 (C=N), 1253.7 (C-O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ(ppm): 3.55 (d, J = 20.80 Hz, 3H), 3.72 (d, J = 7.20 Hz, 3H), 6.88-6.90 (m, 3H, ArH), 7.41-7.42 (m, 1H, ArH), 7.51-7.54 (m, 2H, ArH), 7.62-7.82 (m, 3H, ArH), 7.89-7.90(m, 2H, ArH), 8.99 (dd, J = 4.80, 18.60 Hz, 1H, ArH), 9.46 (dd, J = 8.40, 78.40 Hz, 1H, ArH), 13.16 (s, 1H, -NH); ^{13}C-NMR (400 MHz, DMSO-d_6) δ(ppm): 55.9, 110.0, 110.8, 112.3, 118.6, 119.4, 119.9, 122.8, 124.9, 125.5, 127.5, 127.7, 129.9, 130.7, 131.2, 133.1, 133.5, 133.8, 134.6, 135.2, 136.5, 139.3, 143.2,148.2, 149.1, 150.4; (ESI-MS) *m/z*: Calculated for $C_{26}H_{20}ClN_3O_2$ $[M+H]^+$: 442.91; Found 442.30.

Anti-tubercular Activity

Synthesized compounds **(3a-j)** were screened for anti-tubercular activity against *M. tuberculosis* H37Rv strain by determining their minimum inhibitory concentration (MIC) using the microplate alamar blue assay method (MABA). The MIC values of test compounds and positive controls (Isoniazid, Rifampicin, and Ethambutol) are shown in *Table* 1. 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 OD590 1.0, 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 con-

***Table* 1.** MIC values, docking scores and residues of 4TZK interacting with compounds **3a-j**

Compounds	MIC (µg/mL)[a]	Docking Score (kcal/mol)	Interacted Amino Acid Residue	Hydrogen Bond Properties	
				Hydrogen Bonds	Bond Length (Å)
3a	25	-10.2	Ile16, Ile21, Phe41, Ile95, Gly96, Ile122	Gly96	2.45
				Ile95	3.69
3b	25	-10.2	Ile21, Phe41, Val65, Ser94, Ile95, Gly96, Ile122, Met147, Thr196, Ala198	Thr196	2.15
				Gly96	2.47
				Ser94	3.09
				Ile95	3.63
3c	25	-10.2	Gly14, Ile16, Ser20, Ile21, Phe41, Asp64, Val65, Ile95, Gly96, Ile122	Gly14	2.11
				Ser20	2.69
				Asp64	3.12
				Val65	2.50
				Ile95	3.56
				Gly96	2.51
3d	12.5	-10.5	Ile16, Ser20, Ile21, Phe41, Arg43, Asp64, Ile95, Ile122	Ser20	2.88
				Gly96	2.40
				Arg43	3.67
3e	25	-10.5	Gly14, Ile16, Ser20, Ile21, Phe41, Asp64, Val65, Ile95, Gly96, Ile122	Asp64	3.20
				Val65	2.50
				Ile95	3.69
				Gly96	2.53
3f	12.5	-10.5	Ile16, Ile21, Phe41, Val65, Ile95, Gly96, Ile122	Gly96	2.41
3g	>25	-9.6	Ile16, Ile21, Phe41, Ser94, Ile95	Ser94	2.55
3h	>25	-9.7	Ile16, Ile21, Phe41, Val65, Arg43, Ile122	Ser94	2.54
3i	6.25	-10.6	Ile16, Ile21, Phe41, Val65, Ile95, Gly96, Ile122	Gly96	2.55
				Val65	2.67
3j	>25	-9.6	Ile16, Asp64, Val65, Ile95, Gly96, Ile122	Gly96	2.98
				Asp64	3.55
Isoniazid[b]	0.05	-5.8			
Rifampicin[b]	0.1	-8.0			
Ethambutol[b]	1.56	-4.5			

[a]Minimum inhibitory concentration for *in-vitro* activity against *M. tuberculosis* H37Rv strain; [b]Positive control

trol was 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°C in the normal atmosphere. After 7 days of incubation, 30 μL of alamar blue solution was added to each well, and the plate was re-incubated overnight. A change in colour 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.[29,30]

In Silico Molecular Docking Studies

The structures of the newly synthesized ligands were drawn in Chem Draw Ultra 6.0. Molecular docking analysis was performed with the Auto Dock tools 1.5.6. against *Mycobacterium tuberculosis enoyl reductase* (INHA) and the 3D structure of receptor (PDB code: 4TZK, resolution 1.62 Å) was chosen as a target protein. The crystal structure of the target protein was retrieved from the RCSB protein databank (http://www.rcsb.org) as a PDB file. Then, using Auto Dock the PDBQT file of the target protein was prepared by removing all water molecules and heteroatoms. Polar hydrogen atoms and kollman charges were added to complete the protein preparation as shown in *Fig.* 1. The optimized structures of ligands initially saved as SDF files were converted to PDB files using Online Smiles Translator and ligands were prepared and saved as PDBQT files. A grid was generated to identify xyz coordinates around the binding site of the enzyme and saved in a config file. Log-file and output files are obtained on performing docking using the command prompt. The docking score and H-bond interactions were estimated for all the synthesized compounds are given in *Table* 1. The interactions of the docked results were visualized in 2D and 3D and analyzed using Discovery Studio Visualizer as shown in *Fig.* 2.

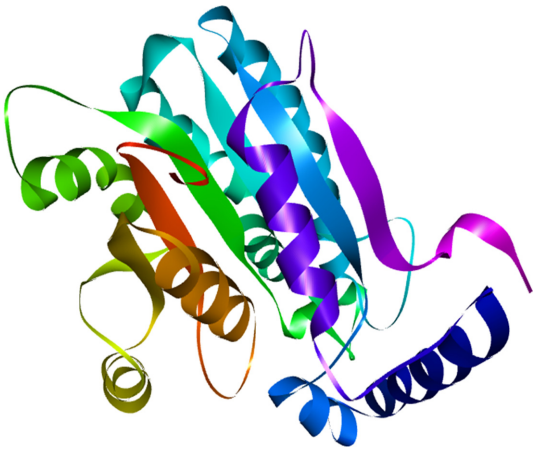


***Figure* 1.** Target protein (PDB: 4TZK) structure.

RESULTS AND DISCUSSION

Chemistry

2,4,5-Trisubstituted imidazoles containing quinoline moiety **(3a-j)** were synthesized via one-pot multi-component condensation reaction involving benzil derivative, quinoline aldehyde and ammonium acetate in acetic acid solvent at reflux temperature (*Scheme* 1). Synthesis of compounds **3a-f** involves 2-hydroxyquinoline-3-carbaldehyde (**2a**) which was obtained by the conversion of 2-chloroquinoline-3-carbaldehyde (**1**) in 70% acetic acid medium. 4-Quinoline carbaldehyde was used to get **3g-j** series of compounds. TLC was used to monitor the progress and completion of the reaction which clearly indicated the formation of products. Column chromatography was used to purify the crude compounds which afford yields ranges from 80 to 95%. All final compounds were confirmed through IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral analysis. Analytical and spectral data of the synthesized compounds were in full agreement with the proposed structures. The standard stretching frequencies in IR spectra of the compounds clearly indicated the presence of N-H group of imidazole ring, C=N, C=C and aromatic C-H functional groups. The number of signals with splitting patterns in the ^{1}H-NMR spectra and carbon signals in ^{13}C-NMR spectra of the compounds were well correlation with the proposed structures. Further, the molecular mass of the compounds was obtained from the mass spectral analysis. Complete characterization data of the compounds is given in the experimental section.

Molecular Docking

In silico molecular docking study of the newly synthesized compounds (**3a-j**) was done with the AutoDock tools 1.5.6. against *Mycobacterium tuberculosis enoyl reductase* (INHA) receptor (PDB code: 4TZK, X-ray diffraction resolution 1.62 Å). Visualization of the possible binding interactions obtained from the docked results was carried out using Discovery Studio Visualizer. The molecular docking score, interacted amino acid residues, hydrogen bond properties are listed in *Table* 1. *In silico* studies revealed that all the synthesized molecules showed good binding energy towards the target protein ranging from -10.6 to -9.6 (kcal/mol). Docking results of the compounds **3d**, **3f** and **3i** showed less binding energy with the docking score (kcal/mol) -10.5, -10.5, -10.6 respectively. The lesser docking

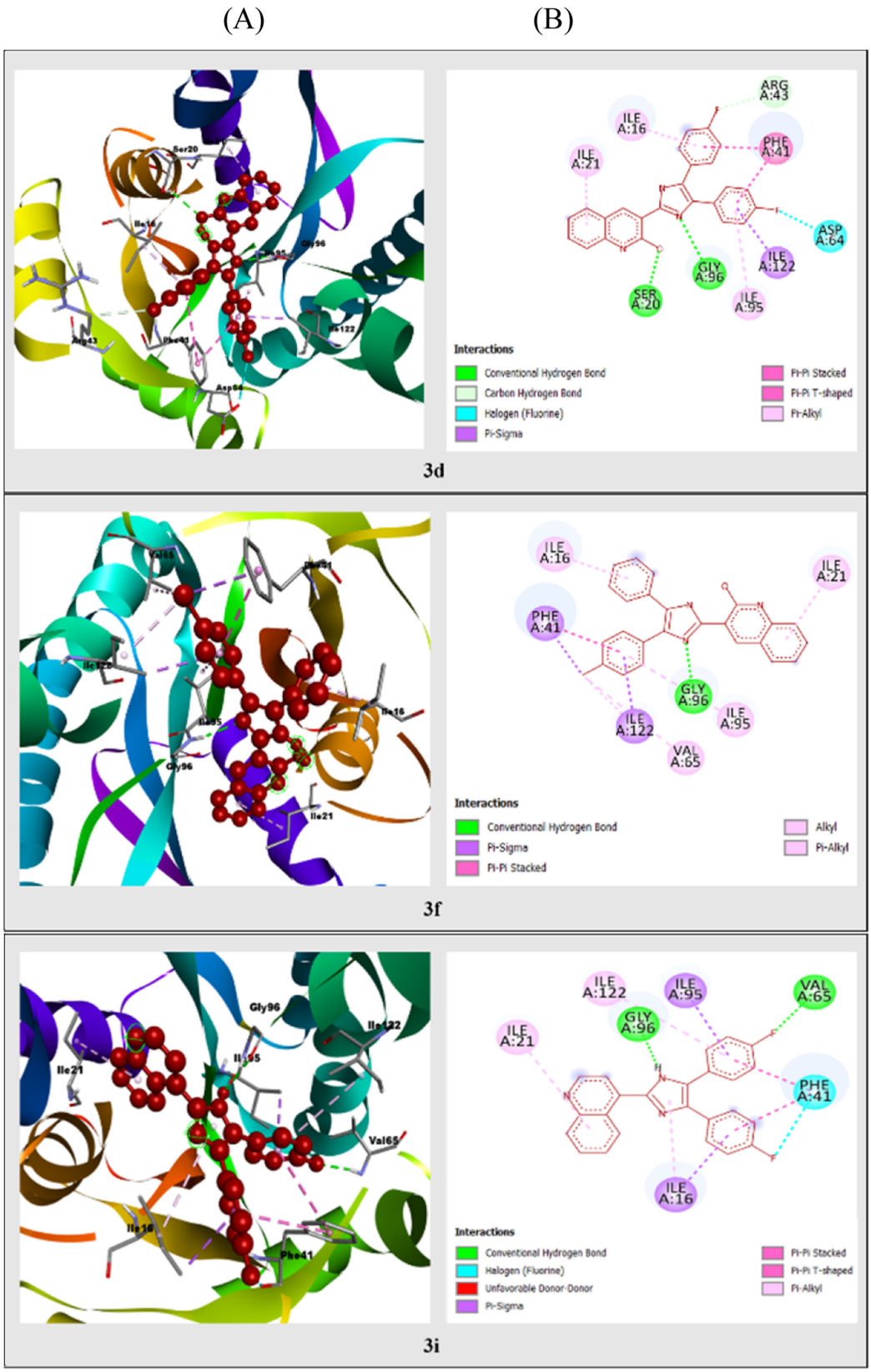
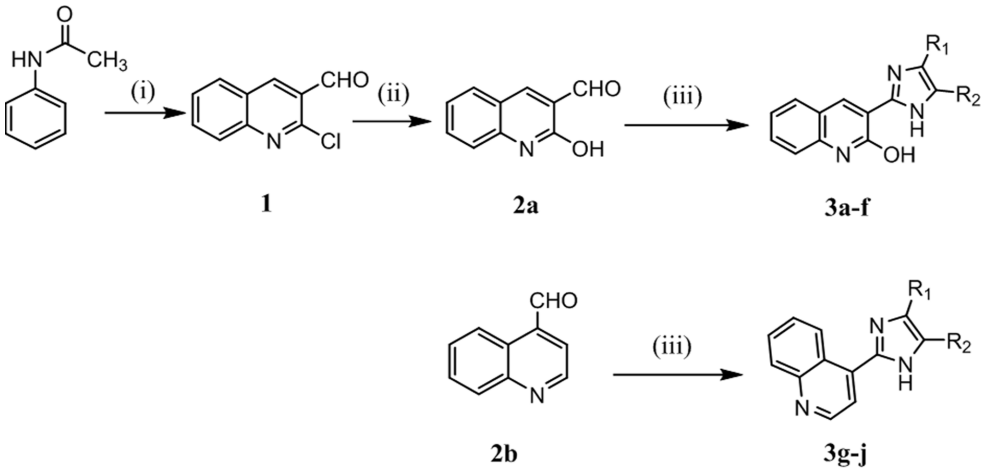
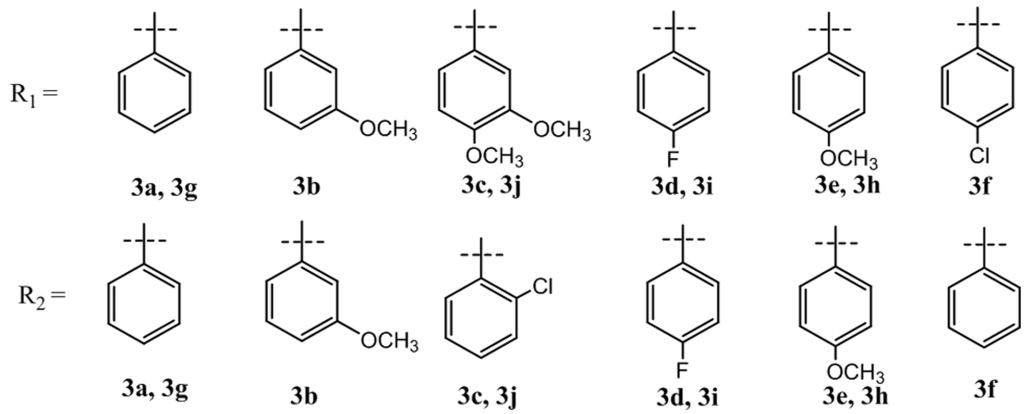


***Figure* 2.** (A) Three-dimension binding interaction (B) Two-dimension binding interaction of the compounds **3d**, **3f** and **3i** with ligands of receptor 4TZK.



Scheme 1

Reaction condition: (i) $POCl_3$, DMF/reflux, (ii) 70% CH_3COOH/reflux, (iii) Aryl-1,2-diketone, CH_3COONH_4, CH_3COOH/reflux



***Scheme* 1.** Synthesis of 2,4,5-trisubstituted imidazole derivatives containing quinoline moiety (**3a-j**).

values indicates the strong binding potency of these molecules towards the target protein. The various types of binding interactions have been observed for the synthesized molecules with the target protein. The compound **3i** showed least binding energy among the tested molecules. It may be due to the presence of fluorine group that could interact much stronger with target protein. Apart from fluorine interaction, the various other interactions found for this molecule are conventional hydrogen bonding, pi-sigma, pi-pi stacked, pi-alkyl and pi-pi T-shaped (*Fig.* 2). Among all the interactions, the hydrogen bond interactions are significant in influencing the action of drug molecules. The hydrogen bonding residues for the compounds, **3d:** Ser20, Gly96; **3f:** Gly96 and **3i:** Gly96 have been observed.

Biological Activity Results

Minimum inhibitory concentration (MIC) values of all newly synthesized compounds which were screened against *M. tuberculosis* H37Rv strain using microplate alamar blue assay method (MABA) indicated that these compounds exhibit anti-tuberculosis activity. Among the prepared quinoline containing imidazole derivatives **3d**, **3f** and **3i** compounds showed moderate to good activity. The highest activity was shown by compound **3i** with MIC value 6.25 μg/mL. The enhanced activity of **3d**, **3f** and **3i** compounds might be due to the presence of halogen substituent on the aryl rings of benzyl derivative. The overall results of *in-vitro* studies revealed that these synthesized quinoline containing imidazoles are effective compounds against *M. tuberculosis* H37Rv.

CONCLUSION

A series of 2,4,5-trisubstituted imidazoles containing quinoline substituent were synthesized via one-pot, three-component reaction of aryl-1,2-diketone, quinoline aldehyde and ammonium acetate in acetic acid at reflux temperature. The products were obtained in a good yield and were confirmed through IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral analysis. Molecular docking was carried out for the newly synthesized derivatives against *Mycobacterium tuberculosis enoyl reductase* (INHA) (PDB: 4TZK). Through molecular docking studies, it is revealed that the synthesized compounds have the lowest binding energy and possess greater stability. The synthesized analogues were tested for antimycobacterial activity against *M. tuberculosis* H37Rv, *in-vitro* using the MABA method. Among the screened samples, compounds **3d**, **3f** and **3i** exhibited good activity with MIC values 12.5, 12.5 and 6.25 μg/mL respectively. The obtained MIC values were correlated with the molecular docking results that, the docking scores for these compounds were found to have lowest binding energy. From the current study, it is concluded that these compounds become prominent anti-tuberculosis agents.

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Supplementary Information. FT-IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectra and docking images of the title compounds are available at in the online version of this article.

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