IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 Journal UGC CARE Listed (Group-I) Volume 11, Issue 2- Dec 2022

Design, Synthesis and Biological Evaluation of Quinoline Derivatives as Anti-Tubercular Agents

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Abstract:

Molecular hybridization is a developing approach to design novel medicinal compounds by merger of two or more biological subunits of known therapeutic agents. The use of current antimycobacterial drugs has been questioned due to their ineffectiveness cause by overuse of them which ultimately leads to antimycobacterial resistance. In the present research work, with the aim of design, synthesize and biological evaluation of quinoline analogues as potential antimycobacterial agents, we have designed various substituted quinoline derivatives by using VLife MDS 4.6 softwere. From that the five compounds R1, R2, R3, R4 and R5 showed the Hydrogen bonding with the significant dock score compared to standard drug Isoniazid (INH). Compound R1 has a dock score of -6.8746, R2 has -6.7128, R3, R4, & R5 has -6.6633, -6.4582, and -6.2401, respectively. These selected compounds were synthesized. All the synthesized compounds were characterized by IR and ^{1}H-NMR spectroscopy, and selected compounds were confirmed via LC-MS. Further, all the synthesized compounds were evaluated for in vitro antimycobacterial activity against Mycobacterium tuberculosis *H37Rv strain* by using Microplate Alamar Blue Assay. The investigation of antimycobacterial screening data revealed that all the tested compounds show significant activity. The compounds R1-R5 were screened for antimycobacterial activity against *Mtb H37Rv* strain. DMSO was used as the negative control, and isoniazid (INH) was used as the positive control. The compound R2 showed the significant activity than the other four compounds.

Keywords: Mycobacterium tuberculosis, Quinoline, docking

INTRODUCTION

Tuberculosis (TB), a prevalent chronic communicable disease has emerged as a major health threat to the entire population worldwide. It is an airborne disease, mainly spread through the causative agent *Mycobacterium tuberculosis (MTB),* among other species of the *MTB* complex like *Mycobacterium bovis, Mycobacterium africanum, Mycobacterium caprae, Mycobacterium canetti, Mycobacterium microti and Mycobacterium pinnipedii. MTB* was first isolated by scientist Robert Koch in 1882, for which he received a Nobel Prize. Mycobacteria are intercellular pathogens which distinctively infect the oxygen rich macrophages of lungs (termed as pulmonary TB), and may also infect other vital parts of the body (extrapulmonary TB).[1,2]

Tuberculosis is one of the first identified infectious diseases and remains a major health problem all over the globe till date. Being one of the top 10 causes of mortality, TB is an alarming problem and is killing increasing number of populations every year. The epidemic of

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TB is particularly quite rampant in India, as nearly one-fourth (23%) of the global TB incident cases and 21% of the world TB deaths occur in India.[3,4] Although several antitubercular drugs are available since many decades, many TB infections are resistant to frontline treatment (like rifampicin and isoniazid) and also resistant to backup drugs (like kanamycin, capreomycin or amikacin). Moreover, there is a lack of preventive vaccines against TB, making it practically untreatable. Thus, there is an urgent need for new antitubercular agents and in the present study, we aim for the design and development of new potential drug candidates for management of spreading of *Mycobacterium tuberculosis*.

Rationale for exploiting quinolines as antitubercular agents:

With the aim of finding newer drug therapy for curbing TB, the current research work involves synthesis of various quinoline derivatives. Quinoline nucleus has been selected as a target for synthesis, based on the following facts. Fluoroquinolone containing drugs like ciprofloxacin, gatifloxacin, ofloxacin and moxifloxacin are widely recognized drugs for tuberculosis. Bedaquiline, a quinoline based drug, is the first new antitubercular drug since past forty years. It was approved by the U.S. Food and Drug Administration (FDA) with the name Sirturo in 2012, and by the European Medicine Agency (EMA) in 2014 for the treatment of TB patients. Its unique mechanism of action proposed an alternative approach to current antimycobacterial killing by directly inhibiting adenosine triphosphate (ATP) synthase.[5,6] Owing to the vast research carried out on development of new antitubercular agents, many synthesized heterocyclic compounds have proficiently displayed antitubercular activity. Various studies have identified quinoline scaffolds as potential antitubercular agents.[7-9] In this context, we have investigated quinolines and their derivatives as mycobacterial inhibitors. Quinoline nucleus is one of the most versatile and widely exploited heterocycle for the development of bioactive molecules. It is occurring naturally in plants as quinoline alkaloids like quinine, quinidine, cinchonine, cinchonidine; and synthetically in numerous quinoline analogues. It is non-toxic in nature and can be safely inhaled or orally consumed by humans. Quinoline and its analogues have demonstrated a variety of biological activities like antimycobacterial, antimalarial, anticancer, antibacterial, antifungal, antiviral, anti-inflammatory, analgesic, antiprotozoal, anthelmintic, hypoglycemic, cardiovascular, and CNS activities. Certain quinoline analogues have also been used in treatment of chronic obstructive pulmonary disorder, as selective estrogen receptor modulators, and anti-Alzheimer agent. Several pharmaceutical drugs comprise a quinoline nucleus (Fig. 1) including fluoroquinolones like ciprofloxacin, sparfoxacin, gatifloxacin as antibacterial, clioquinol as antifungal-antiprotozoal, quinine as antipyretic, antimalarial, anti-inflammatory, and analgesic, quinidine, chloroquine, mefloquine, amodiaquinine and primaquine as antimalarial, saquinavir as anti-retroviral, camptothecin, irinotecan, topotecan as anticancer, dibucaine as local anesthetic, montelukast as antiasthmatic, aripiprazole, brexpiprazole as antipsychotic, cartiolol as antiglaucoma and vesnarinone as cardiotonic drug.[7-9]

New compounds displaying various biological profiles are designed and synthesized via the technique of molecular hybridization which involves the merger of two or more medicinal moieties. The current research work is based on synthesis of quinoline based hybrid molecules. All the aforementioned facts have clearly proved the strength of quinoline motif as a bioactive

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 Journal UGC CARE Listed (Group-I) Volume 11, Issue 2- Dec 2022

molecule and its ability to efficiently act as an antitubercular agent. This has hence prompted us to develop our synthesized quinoline derivatives as inhibitors of Mycobacterium tuberculosis. We have herein reported the synthesis of quinoline based thiazoles Schiff bases through different synthetic routes, as potential antimycobacterial agents.

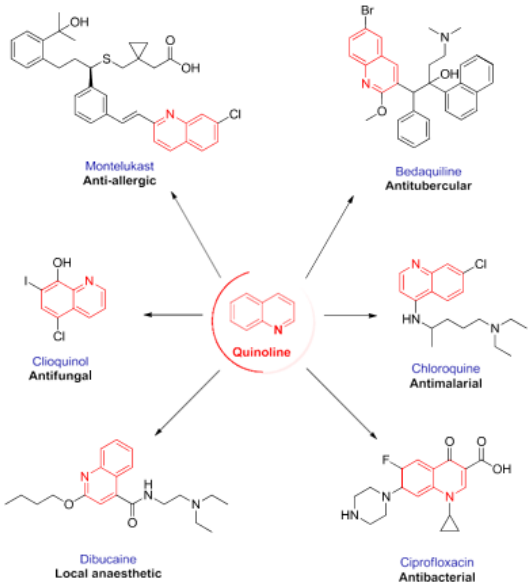

Figure: Examples of some drugs possessing quinoline nucleus

RESULT AND DISCUSSION:

Docking study of designed compounds (R1-R5):

The molecular docking study of Schiff's base of Quinoline was carried out using the VLife MDS 4.6 software. The crystal structure was obtained from the protein data bank (PDB ID: 4TZK). The study was carried out on a selected set of fifteen compounds with variations in their structure. The Quinoline derivatives tested against InhA receptor gave docking score ranging from -6.1239 to -6.8746. Selected Quinoline derivatives R1-R5 tested against InhA receptor gave docking score ranging from -6.2401 to – 6.8746. All the Quinoline derivatives have shown the significant affinity towards the InhA reductase as compared to the standard drug Isoniazid (Table 7.1) Standard drugs Isoniazid and 641 were docked at active site of InhA reductase receptor and further used to compare its dock score with selected compounds and its interactions. It was noticed that the compound having electron donating (-OH) group and electron withdrawing (-Cl) group in it has good affinity and dock score.

Based on the literature survey selected compound may act through InhA reductase. The binding poses of each compound were analyzed for its interaction with active site residues and by examining their dock score and binding energy. It was noticed that the compound having electron donating (-OH) group and electron withdrawing (-Cl) group it has good affinity and dock score. Isoniazid is forming the three-hydrogen bond interaction with ILE194A, THR196A and THR196A **(Figure 7.1),** 641 is forming one-hydrogen bond with THR196A and twenty-one-hydrophobic bonds with ALA198A, ILE202A, LEU207A **(Figure 7.2),** R1 is forming three-hydrogen bond interaction with SER94A and two hydrophobic interactions with ALA198A **(Figure 7.3)**

ISSN PRINT 2319 1775 Online 2320 7876
 Journal UGC CARE Listed (Group-I) Volume 11, Issue 2- Dec 2022

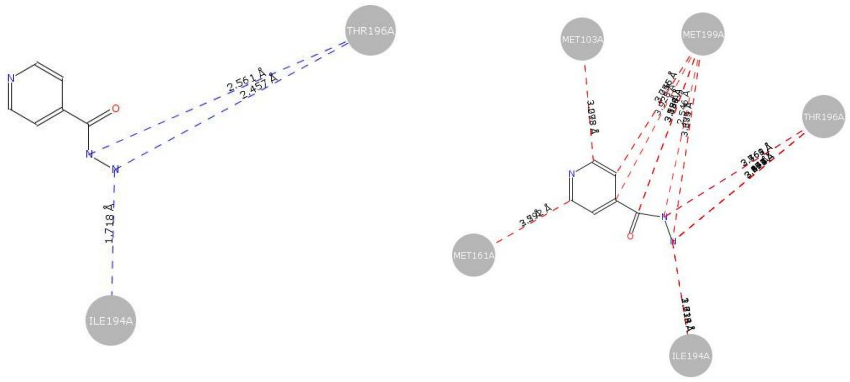

Figure 7.1 Interaction of Isoniazid

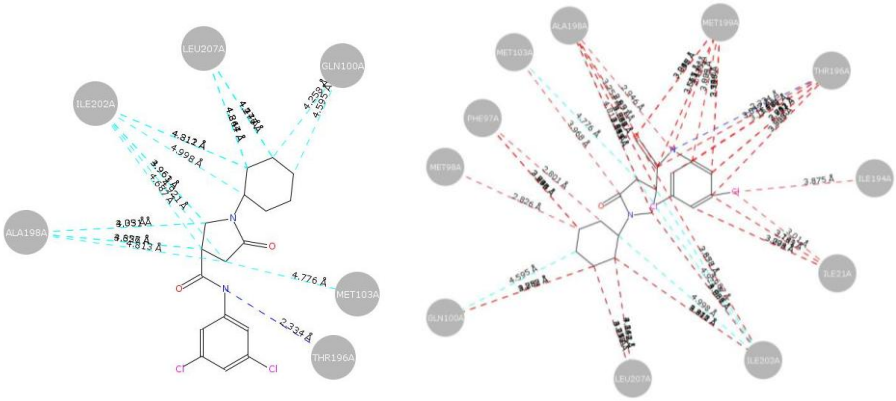

Figure 7.2 Interaction of 641

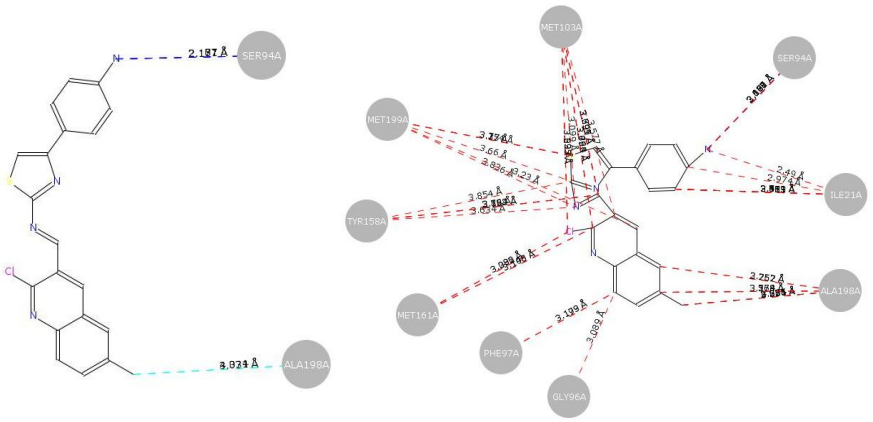

Figure 7.3 Interaction of R1

Table 7.1 Docking study of Schiff's base of Quinoline Derivatives

Compounds	No. of conformers	Dock score	Binding energy	Interactions
Standard	12	-5.4396	-60.9201	HB : ILE194A, THR196A, THR196A Vdw :MET103A, ILE194A, THR196A, MET199A

641	12	-5.6919	-75.9959	HB : THR196A HP : ALA198A, ILE202A, LEU207A Vdw : ILE21A, PHE97A, MET98A, THR196A
R1	12	-6.8746	-91.0049	HB : SER94A Aromatic : TYR158A Vdw: ILE21A, PHE97A, MET98A, MET103A
R2	12	-6.7128	-70.8335	HB : MET98A, MET98A Aromatic : TYR158A Vdw : ILE21A, PHE97A, MET98A, TYR158A
R3	12	-6.6633	-69.0612	HB : THR196A HP : ILE16A Vdw : ILE16A, GLY96A, MET103A, THR196A
R4	12	-6.4582	-64.5387	HB : THR196A Aromatic : PHE149A, TYR158A Vdw : SER20A, PHE97A, MET98A, PHE149A
R5	12	-6.2401	-86.7467	HB : MET98A, MET98A HP : ILE16A Vdw : ILE16A, SER94A, GLY96A, PHE97A

Chemistry:

The starting precursor substituted acetanilide 1 was prepared by the mixture of aniline and acetic acid in presence of benzophenone reflux for the 1 hr. Further this substituted acetanilide treated with the dimethylformamide in phosphorous oxy-trichloride under mild conditions gives the 2-choloro-6-substitutedquinoline-3- carbaldehyde 2[17]. Another precursor 2-amino-4-substituted phenyl thiazole 3 was prepared according to a reported procedure through the reaction of substituted acetophenone with iodine in presence of thiourea[18]. The compound 2 on further nucleophilic addition with 2-amino-4-substituted phenyl thiazole 3 in the presence of glacial acetic acid in ethanol furnished Schiff bases, R1-R4 (Scheme). The structures of prepared congeners have been confirmed by FTIR, 1 H NMR, HRMS. In the 1 H NMR/FTIR, the board signal of NH2 and frequency of NH_2 stretch present in the spectrum respectively in the compound 3 had disappeared in the Schiff base. In the FT-IR spectra of compounds 3a-b, the aldehydic carbonyl stretching at 1689 cm^{-1} disappeared, which clearly suggested that the formation of Schiff base by condensation of CQC with appropriate amine. The new frequency band appeared at 1612-1604 cm^{-1} which was assigned to azomethine –C=N-str group in the compound R1-R4. Further, the 1 H NMR spectral data of the synthesized Schiff base R1-R4 had appeared sharp singlet protons at δ 9.00 which was assigned to azomethine proton.

The predicted molecular weight of the synthesized compounds was confirmed by HR-MS. The compound (4-(2-(((2-chloroquinolin-3-yl)-methylene)amino)thiazol-4-yl)aniline R2 having molecular ion peak at m/z 364.0 (M^+) strongly reveals its predicted molecular formula

ISSN PRINT 2319 1775 Online 2320 7876
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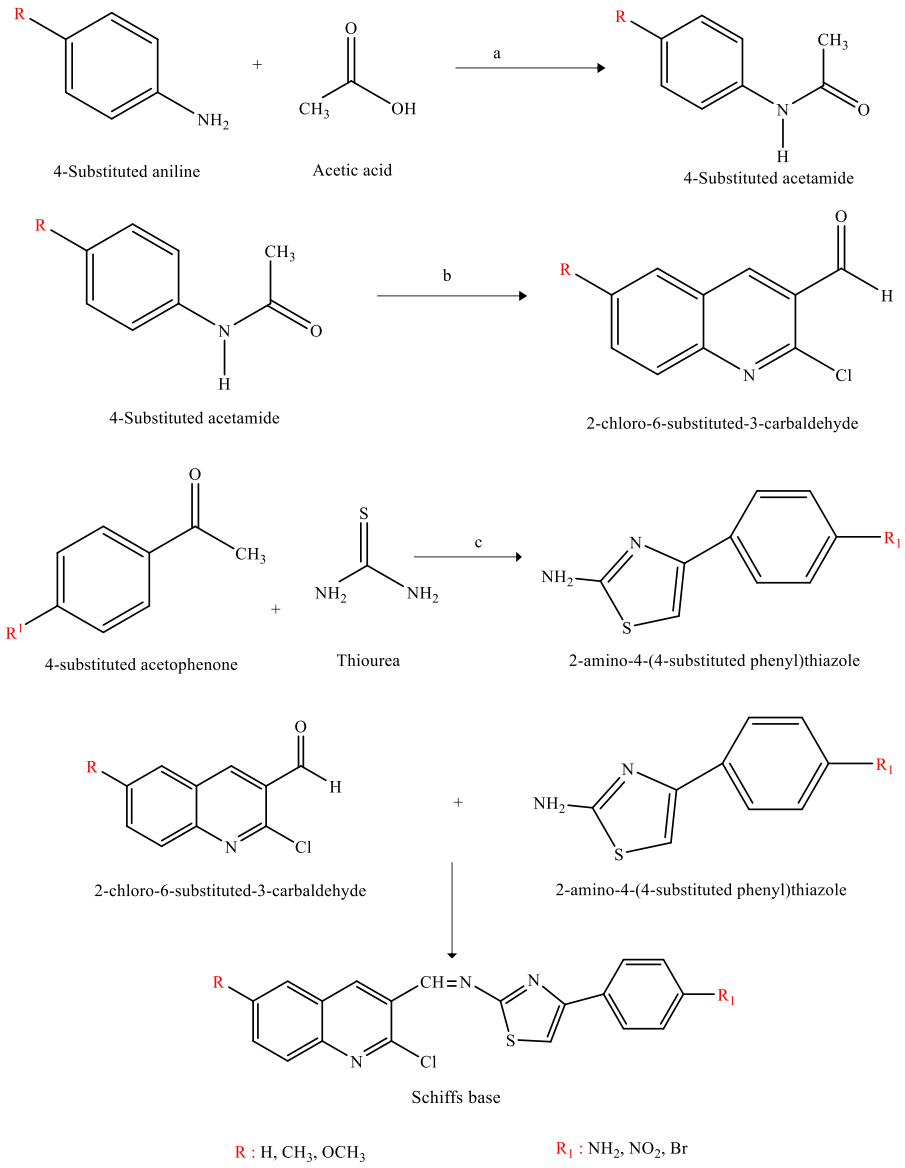
$C_{19}H_{13}N_4SCl$.



Reagents and Conditions :
a) Benzophenone, 12 hrs Stirring at 80˚C ; b) DMF, $POCl_3$, Reflux 20 hrs ; c)Iodine, Reflux 4 hrs ; d) Ethanol/GAA, Reflux 2 hrs

Scheme: Proposed Scheme of synthetic work

In vitro anti-mycobacterial activity:

The investigation of antimycobacterial screening data revealed that all the tested compounds show significant activity. The in vitro antimycobacterial activity was evaluated by the microplate alamar blue assay method. The compounds R1-R5 were screened for antimycobacterial activity against *Mtb H37Rv* strain. DMSO was used as the negative control, and isoniazid (INH) was used as the positive control. The anti-TB profile of sample code R1, R2, R3 and R4 was analyzed by Almar blue assay, on the basis of colour observation and percentage of inhibition the sample code R2 has good anti-TB activity against *Mtb H37Rv* as compared to the standard drug isoniazid (INH). (Table 1)

ISSN PRINT 2319 1775 Online 2320 7876
 Journal UGC CARE Listed (Group-I) Volume 11, Issue 2- Dec 2022

Table 1: Anti-TB activity of the sample R1, R2, R3, R4

SR NO	SAMPLE CODE	Concentration (µg/ml)	Absorbance at 600nm			Mean	% Of Growth Inhibition
			Test 1	Test 2	Test 3		
1	Growth C		1.603	1.603	1.603	1.603	-
2	Standard	7.8	1.593	1.594	1.594	NE	NE
	(Isoniazid)	15.6	1.538	1.538	1.538	NE	NE
		31.2	1.421	1.421	1.421	1.421	11.35%
		62.5	1.397	1.398	1.397	1.397	12.85%
		125	0.834	0.834	0.834	0.834	47.97%
		250	0.611	0.611	0.611	0.611	61.88%
		500	0.531	0.531	0.531	0.531	66.87%
		1000	0.422	0.422	0.422	0.422	73.67%
3	R1	7.8	NE	NE	NE	NE	NE
		15.6	NE	NE	NE	NE	NE
		31.2	NE	NE	NE	NE	NE
		62.5	1.541	1.542	1.542	1.541	3.86%
		125	1.289	1.262	1.257	1.269	20.83%
		250	1.032	1.025	1.025	1.027	35.93%
		500	0.956	0.956	0.956	0.956	40.36%
		1000	0.732	0.732	0.732	0.732	54.33%
4	R2	7.8	1.474	1.474	1.474	1.474	8.04%
		15.6	1.460	1.460	1.460	1.460	8.92%
		31.2	1.420	1.420	1.420	1.420	11.41%
		62.5	1.235	1.235	1.235	1.235	22.95%
		125	1.574	1.574	1.574	1.574	1.80%
		250	0.943	0.943	0.943	0.943	41.17%
		500	0.730	0.730	0.730	0.730	54.46%
		1000	0.648	0.648	0.648	0.648	59.57%
5	R3	7.8	-	-	-	-	-
		15.6	-	-	-	-	-
		31.2	1.330	1.330	1.330	1.330	17.03%
		62.5	1.112	1.112	1.112	1.112	30.63%
		125	1.576	1.576	1.576	1.576	1.68%
		250	0.960	0.960	0.960	0.960	40.11%
		500	0.737	0.737	0.737	0.737	54.02%
		1000	0.650	0.650	0.650	0.650	59.45%
6	R4	7.8	-	-	-	-	-
		15.6	-	-	-	-	-
		31.2	1.320	1.320	1.320	1.320	17.65%
		62.5	1.105	1.105	1.105	1.105	31.06%
		125	1.578	1.578	1.578	1.578	1.55%
		250	0.941	0.941	0.941	0.941	41.29%
		500	0.739	0.739	0.739	0.739	53.89%

ISSN PRINT 2319 1775 Online 2320 7876
 Journal UGC CARE Listed (Group-I) Volume 11, Issue 2- Dec 2022

		1000	0.647	0.647	0.647	0.647	59.63%

Experimental Section:

Molecular Docking:

Docking study is an important tool for checking the type of interactions that exists between the enzyme and the ligand. The finding of major binding modes of a ligand with a protein of known three-dimensional structure is one of the important goals of ligand-protein docking.

V Life MDS 4.6 software was used for docking purpose.

The molecular docking was performed on the InhA reductase receptor to check the possible interactions between newly synthesized compounds and the selected cavity of the InhA receptor. The study was carried out on a selected set of fifteen compounds with variations in their structure. The docking study shows that the different bond interactions, such as hydrogen bond interactions, van der Waals interactions, Pi bond interactions, as well as the hydrophobic interactions between ligand and receptor, are responsible for the inhibition activity.3D representation of PDB ID: 4TZK[10] is represented in Figure 2.

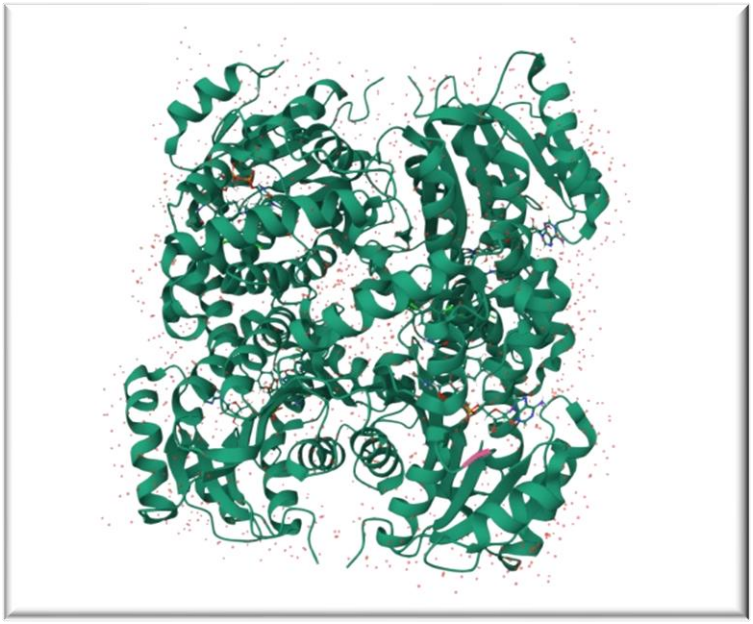


Figure 2: 3D representation of PDB ID: 4TZK

According to the Pandey et al. concept, we designed fifteen compounds of Quinoline derivatives. Molecular docking was carried out for all the designed Quinoline derivatives to check probable interactions with InhA by using PDB-4TZK (Table 2)

From the series of Quinoline derivatives, only five compounds showed hydrogen bonding with the protein. Compounds R1, R2, R3, R4 and R5 showed significant dock score compared with Isoniazid and 641 dock score, which is 5.4396 and -5.6919, respectively. Compound R1 has a dock score of -6.8746, R2 has -6.7128, R3, R4, & R5 has -6.6633, -6.4582, and -6.2401, respectively.

ISSN PRINT 2319 1775 Online 2320 7876
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Table 2: Docking Study of Schiff's Base of Quinoline Derivatives

Compounds	R	R_1	No. of conformers	Dock score	Binding energy
Standard	-	-	12	-5.4396	-60.9201
641	-	-	12	-5.6919	-75.9959
A1	H	H	12	-6.4359	-80.4473
A2	H	NH_2	12	-6.7128	-70.8335
A3	H	NO_2	12	-6.4582	-64.5387
A4	H	Br	12	-6.6920	-91.6926
A5	H	Cl	12	-6.6136	-59.9097
A6	CH_3	H	12	-6.4869	-96.3026
A7	CH_3	NH_2	12	-6.8746	-91.0049
A8	CH_3	NO_2	12	-6.5154	-59.3229
A9	CH_3	Br	12	-6.6633	-69.0612
A10	CH_3	Cl	12	-6.6358	-81.2953
A11	OCH_3	H	12	-6.1239	-100.352
A12	OCH_3	NH_2	12	-6.2401	-86.7467
A13	OCH_3	NO_2	12	-6.5154	-81.9726
A14	OCH_3	Br	12	-6.4683	-101.477
A15	OCH_3	Cl	12	-6.3460	-86.1955

Based on docking results, we selected five compounds for further synthesis, which were A7, A2, A9, A3, and A12, which is namely R1, R2, R3, R4, and R5, respectively. (Table 3)

Table 3: Docking Study of Selected Schiff's Base of Quinoline Derivatives

Compounds	R	R_1	Interactions
Standard	-	-	HB : ILE194A, THR196A, THR196A
641	-	-	HB : THR196A
R1	CH_3	NH_2	HB : MET98A
R2	H	NH_2	HB : MET98A, MET98A
R3	CH_3	Br	HB : THR196A
R4	H	NO_2	HB : THR196A
R5	OCH_3	NH_2	HB : MET98A, MET98A

Material and Methods:

Molecular Docking and Pharmacophore mapping were carried out using VLife MDS 4.6 software. All the chemicals used in the synthesis were of laboratory grade. Melting points were determined in an open capillary with Equiptronics melting point apparatus and are uncorrected. Thin Layer Chromatography (TLC) was performed on precoated aluminum sheets (silica gel 60F254, 6x2.5 cm) using appropriate solvent systems. The spots were visualized under ultra-

violet light. The IR spectra of the synthesized compounds were recorded on a Shimadzu 8400-S FT-IR spectrophotometer using potassium bromide. The 1H NMR spectra were recorded in DMSO using NMR BRUKER 500 MHz, and chemical shifts are given in units as parts per million, downfield from Tetra Methyl Silane (TMS) as an internal standard. The mass spectra were recorded on a mass analyzer.

General procedure for synthesis of N-(p-substituted phenyl)acetamide:[11]

The RBF charged with 5.35gm P-substituted aniline (50mmol) in 50ml of Acetic Acid and stirred continuously until solid completely dissolved. Then added 9.11gm Benzophenone (50mmol) and stirred continuously. The mixture was refluxed for 12 hrs at 80˚C. The reaction was monitored by TLC. The reaction mixture was poured into distilled water, extracted with ether and dried over sodium sulphate. The desired product was purified by recrystallization from hot water and appeared to be off white solid crystal.

N-phenylacetamide (1a) : Off white colored crystals. Mol. Formula: C_8H_9NO. Mol. Wt.: 135.17gm. Yield 88.27%. M.P. 117-120°C. R_f: 0.45; Ir (KBr): 3229.53 (N-H) cm^{-1}, 3140.22(Ar C-H) cm^{-1}, 2924.18(Ali C-H) cm^{-1}, 1658.84(C=O) cm^{-1}, 1550.82(Ar C=C) cm^{-1}, 1319.35(C-N) cm^{-1}.

N-(p-tolyl)acetamide (1b) : Off white colored crystals. Mol. Formula: $C_9H_{11}NO$. Mol. Wt.: 149.19gm. Yield 86.20%. M.P. 146-148°C. R_f: 0.45; Ir (KBr): 3256.81 (N-H) cm^{-1}, 3124.79(Ar C-H) cm^{-1}, 2970.48(Ali C-H) cm^{-1}, 1658.84(C=O) cm^{-1}, 1512.24(Ar C=C) cm^{-1}, 1319.35(C-N) cm^{-1}.

N-(4-methoxyphenyl)acetamide (1c) : Off white colored crystals. Mol. Formula: $C_9H_{11}NO_2$. Mol. Wt.: 165.19gm. Yield 76.11%. M.P. 124-131°C. R_f: 0.5; Ir (KBr): 3248.23(N-H) cm^{-1}, 3132.50(Ar C-H) cm^{-1}, 2839.31(Ali C-H) cm^{-1}, 1651.12(C=O) cm^{-1}, 1512.24(Ar C=C) cm^{-1}, 1319.35(C-N) cm^{-1}, 1242.20(C-O) cm^{-1}.

General procedure for synthesis of 2-chloro-6-substituted quinoline-3-carbaldehyde:[12]

The 2.2 ml of $POCl_3$ added dropwise into the RBF which containing 0.8 ml DMF with continuous stirring. The temperature of the reaction maintained at 0°C. After the addition of $POCl_3$ was done 0.5 gm of P-substituted phenyl acetamide added in it. Heated upto 90°C using water bath & refluxed for 20 hours. The reaction mixture cooled at room temperature then poured into the crushed ice. The product was filtered by whattman filter paper & recrystallized by EtAc.

2-chloroquinoline-3-carbaldehyde (2a) : Orange colored powder. Mol. Formula: $C_{10}H_6NOCl$. Mol. Wt.: 191.64gm. Yield: 33.89%. M.P. 146-149˚C. R_f: 0.6; Ir (KBr): 3039.91 (Ar C-H) cm^{-1}, 2870.17(Aldehydic C-H) cm^{-1}, 1689.70(C=O) cm^{-1}, 1612.54(C=N)cm^{-1}, 1573.97(Ar C=C) cm^{-1}, 1373.36(C-N) cm^{-1}, 810.13(C-Cl) cm^{-1}.

2-chloro-6-methylquinoline-3-carbaldehyde (2b) : Orange colored powder. Mol. Formula: $C_{11}H_8NOCl$. Mol. Wt.: 205.64gm. Yield: 36.28%. M.P. 124-128˚C. R_f: 0.6; Ir (KBr): 3047.63(Ar C-H) cm^{-1}, 2924.18(Ali C-H) cm^{-1}, 2885.60(Aldehydic C-H) cm^{-1}, 1689.70(C=O) cm^{-1}, 1581.68(C=N) cm^{-1}, 1504.53(Ar C=C) cm^{-1}, 1373.36(C-N) cm^{-1}, 810.13(C-Cl) cm^{-1}.

2-chloro-6-methoxyquinoline-3-carbaldehyde (2c) : Orange colored powder. Mol. Formula: $C_{11}H_8NO_2Cl$. Mol. Wt.: 221.64gm. Yield: 23.88%. M.P. 152-156˚C. R_f: 0.68; Ir (KBr): 3055.35(Ar C-H) cm^{-1}, 2955.04(Ali C-H) cm^{-1}, 2831.60(Aldehydic C-H) cm^{-1}, 1681.96(C=O) cm^{-1}, 1620.25(C=N) cm^{-1}, 1573.97(Ar C=C) cm^{-1}, 1334.73(C-N) cm^{-1}, 1226.77(C-O) cm^{-1}, 810.13(C-Cl) cm^{-1}.

General procedure for synthesis of 2-Amino-4-substitutedphenyl thiazole:[13]

The 2 mol of Thiourea & 1 mol of Iodine crushed in crucible. The mixture was transferred into RBF which contain 1 mol of substituted acetophenone & reflux for 4 hours. The reaction mixture cooled at room temperature then transferred into ice cold water. The product was filtered and recrystallized by aqueous ethanol.

2-amino-4-(4-aminophenyl) thiazole (3a): Orange colored crystals. Mol. Formula: $C_9H_9N_3S$. Mol. Wt.: 191.24gm. Yield: 84.86%. M.P. 174-180˚C. Rf: 0.45; Ir (KBr): 3271.38 (Ar N-H) cm^{-1}, 3117.07(Ar C-H) cm^{-1}, 1612.54(C=N) cm^{-1}, 1543.10(C=C) cm^{-1}, 1334.78(C-N) cm^{-1}, 1041.60(C-S) cm^{-1}.

2-amino-4-(4-nitrophenyl) thiazole (3b): Corn yellow-colored crystals. Mol. Formula: $C_9H_7N_3O_2S$. Mol. Wt.: 221.24gm. Yield: 93.42%. M.P. 279-281˚C. Rf: 0.70; Ir (KBr): 3271.38 (Ar N-H) cm^{-1}, 3117.07(Ar C-H) cm^{-1}, 1612.54(C=N) cm^{-1}, 1543.10(C=C) cm^{-1}, 1334.78(C-N) cm^{-1}, 1041.60(C-S) cm^{-1}.

2-amino-4-(4-bromophenyl) thiazole (3c): Off white colored crystals. Mol. Formula: $C_9H_7N_2SBr$. Mol. Wt.: 255.13gm. Yield: 85.93%. M.P. 181-188˚C. Rf: 0.70; Ir (KBr): 3294.53(Ar N-H) cm^{-1}, 3124.79(Ar C-H) cm^{-1}, 1627.97(C=N) cm^{-1}, 1595.25(C=C) cm^{-1}, 1273.06(C-N) cm^{-1}, 1072.46(C-S) cm^{-1}, 462.93(C-Br) cm^{-1}.

General procedure for synthesis of Schiff's Base of Quinoline Derivatives:[14]

Equimolar quantities (0.01 mol) of 4-amino-4-(4-substituted phenyl) thiazole & 2-chloro-6-substitutedquinoline-3-carbaldehyde was taken. Each of reactant was dissolved in minimum 10 ml of Ethanol & mixed together followed by addition of 2 ml of GAA. Then refluxed for 2 hrs. The solid separated was filtered & recrystallized by using Ethanol.

(4-(2-(((2-chloro-6-methylquinolin-3-yl)-methylene)-amino)-thiazol-4-yl)-aniline (R1): White colored crystals. Mol. Formula: $C_{20}H_{15}N_4SCl$. Mol. Wt.: 378.45gm. Yield: 86.86%. R_f: 0.48; Ir (KBr): 3371.68(N-H) cm^{-1}, 3163.36(Ar C-H) cm^{-1}, 2901.04(Ali C-H) cm^{-1}, 1612.54(C=N) cm^{-1}, 1527.54(C=C) cm^{-1}, 1334.78(C-N) cm^{-1}, 1057.03(C-S) cm^{-1}, 825.56(C-Cl) cm^{-1}. ^{1}H NMR (500 MHz, DMSO) δ 9.00 (s, 1H), 8.89 (s, 1H), 8.82(s, 1H), 8.46 (s, 1H), 7.99 (d, J = 7.0 Hz, 2H), 7.36 (d, 2H), 7.22 (d, 2H), 5.17 (s, 2H), 2.43 (s, 3H).

(4-(2-(((2-chloroquinolin-3-yl)-methylene)-amino)-thiazol-4-yl)-aniline (R2): Orange colored crystals. Mol. Formula: $C_{19}H_{13}N_4SCl$. Mol. Wt.: 364.0gm. Yield: 84.86%. R_f: 0.67; Ir (KBr): 3363.97(N-H) cm^{-1}, 3163.36(Ar C-H) cm^{-1},1604.83(C=N) cm^{-1}, 1527.67(C=C) cm^{-1}, 1327.07(C-N) cm^{-1}, 1057.03(C-S) cm^{-1}, 833.28(C-Cl) cm^{-1}; ^{1}H NMR (500 MHz, DMSO) δ 9.04 (s, 1H), 8.56 (s, 1H), 8.36 (s, 1H), 8.19 – 8.11 (m, 4H), 8.07 (d, J = 7.9 Hz, 2H), 7.97 (d, J = 8.5 Hz, 2H), 5.13 (s, 2H); HRMS (ESI): m/z calculated for $C_{19}H_{13}N_4SCl$ 364.0 & found 364.06 [M^+].

ISSN PRINT 2319 1775 Online 2320 7876
 Journal UGC CARE Listed (Group-I) Volume 11, Issue 2- Dec 2022

N-(4-(4-bromophenyl)-thiazol-2-yl)-1-(2-chloro-6-methylquinolin-3-yl)-methanimine (R3): Corn yellow-colored crystals. Mol. Formula: $C_{20}H_{13}N_3SClBr$. Mol. Wt.: 442.45gm. Yield: 93.42%. Rf: 0.35; Ir (KBr): 3194.23(Ar C-H) cm^{-1}, 2924.18(Ali C-H) cm^{-1}, 1604.83(C=N) cm^{-1}, 1519.96(C=C) cm^{-1}, 1334.78(C-N) cm^{-1}, 1072.96(C-S) cm^{-1}, 825.56(C-Cl) cm^{-1}, 516.94(C-Br) cm^{-1}; ^{1}H NMR (500 MHz, DMSO) δ 8.44 (s, 1H), 8.35 (s, 2H), 7.96 (s, 1H), 7.93 – 7.81 (m, 4H), 7.16 (d, *J* = 8.5 Hz, 2H), 2.49 (s, 3H).

1-(2-chloroquinolin-3-yl)-N-(4-(4-nitrophenyl)-thiazol-2-yl)-methanimine (R4): Off white colored crystals. Mol. Formula: $C_{19}H_{11}N_4O_2SCl$. Mol. Wt.: 394.45gm. Yield: 84.93%. Rf: 0.45; Ir (KBr): 3140.22(Ar C-H) cm^{-1},1643.41(C=N) cm^{-1}, 1597.11(N-O) cm^{-1}, 1519.96(C=C) cm^{-1}, 1342.50(C-N) cm^{-1}, 1111.03(C-S) cm^{-1}, 848.71(C-Cl) cm^{-1}; ^{1}H NMR (500 MHz, DMSO) δ 8.56 (s, 1H), 8.35 (d, *J* = 6.7 Hz, 2H), 8.24 (s, 1H), 8.23 – 8.14 (m, 4H), 8.05 (d, *J* = 9.0 Hz, 2H), 7.41 (s, 1H).

In-vitro Anti-mycobacterial Activity:

Mycobacterium tuberculosis H37Rv was cultured to mid-log phase, with an optical density (OD) of 0.6 (~ 5 × 107 colony-forming units [CFU]/ml) at 600 nm. One hundred microliters (5×10^4 CFUs) of bacterial suspension were added to each well of 96-well microplates. Then, 10 µM of the compound was added to each well. DMSO was used as the negative control, and isoniazid (INH) was used as the positive control. The plates were sealed and incubated at 37°C for 6 days.A 10% (v/v) solution of Alamar Blue was then added to each well. The anti-*Mtb H37Rv* effect of each compound was determined based on the color change. The compound was considered to be active against *Mtb H37Rv* if the color changed from blue to pink. The absorbance of the plate was measured at 600nm by using micro plate reader.

Conclusion:

In-silico molecular docking studies revealed that the compound bearing CH_3 at R and NH_2 at R1 i.e. substitution site have good binding capacity towards the enoyl acyl carrier protein reductase receptor (PDB-4TZK).

The target compounds R1-R5 were synthesized by simple and convenient methods in comparison with the reported methods for preparation of Quinoline and thiazole derivatives. The spectral data supports the formation of synthesized compounds. From results of anti-tubercular activity, it may be concluded that hydrogen (least electron donating) group at R and amino (electron withdrawing) group at R1 position of compounds have shown significant activity against *Mtb H37Rv* strain. In conclusion, Quinoline-thiazole derivatives of less electron donating group (-CH_3) and electron withdrawing group (-NH_2) can serve as template for further optimization of anti-tubercular activity.

Acknowledgments

Authors thank Seth Govind Raghunath Sable College of Pharmacy, Saswad, Punrander, Pune, Maharashtra for providing computational facilities and laboratory support and access to library facilities.

Conflict of Interest

ISSN PRINT 2319 1775 Online 2320 7876
 Journal UGC CARE Listed (Group-I) Volume 11, Issue 2- Dec 2022

The authors declare that there are no commercial or financial relationships that could be construed as a potential conflict of interest.

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