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Research Article

DESIGN, SYNTHESIS AND SCREENING OF NEWER-8-HYDROXYQUINOLINE DERIVATIVES AS NOVEL ANTI TUBERCULAR AGENTS

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Abstract

A series of 8-hydroxyquinolines are synthesized and their Antitubercular, antifungal, and antibacterial activities are tested. Quinolines are found to possess antibacterial, antifungal, immunosuppressive, analgesic, vasorelaxing, Antiplasmodial, anticancer and PDE_4 inhibitory activities. Aryloxypropanolamines were reported to be associated with β-adrenergic blocking, CNS depressant and hypotensive activities. In view of the potential nature of these moieties it was considered worthwhile to study the effects of two pharmacophoric moieties such as quinoline and propanolamines/amino ethane in a single molecule. In the present study the synthesis, antitubercular, antifungal, and antibacterial activities and structure activity relationship of aryloxypropanolamine substituted 8-hydroxy quinolines are reported. The compounds were characterized by IR, H-NMR, spectral and Elemental analysis.

Keywords: 8-hydroxyquinolines; docking; antitubercular; antibacterial; antifungal activity

Introduction

Drug discovery is the process by which drugs are discovered and/or designed. The process of drug discovery involves the identification of candidates, synthesis, characterization, screening, and assays for therapeutic efficacy. In the past most drugs have been discovered either by identifying the active ingredient from traditional remedies or by serendipitous discovery. A new approach has been to understand how disease and infection are controlled at the molecular and physiological level and to target specific entities based on this knowledge. The process of finding a new drug against a chosen target for a particular disease usually involves high-throughput screening (HTS), wherein large libraries of chemicals are tested for their ability to modify the target (Drews, 2000).

8-Hydroxyquinoline is an organic compound of molecular formula C_9H_7NO. It is a derivative of heterocyclic quinoline by the placement of OH group on carbon number 8. This colorless compound widely used commercially, although under variety of names. It is usually prepared from quinoline 8- sulphonicacid acid and from a skraup synthesis from 2-amino phenol. 8-Hydroxyquinoline is a monoprotic bidentate chelating agent. Related ligands are the Schiff bases derived from salicylaldehyde, such as salicylaldoxime and salen. In neutral solution, the hydroxyl is in the protonated form (pKa=9.89) and the nitrogen is not protonated (pKa=5.13). However, an excited-state zwitterionic isomer exists in which H+ is transferred from the oxygen (giving an oxygen anion) to the nitrogen (giving a protonated nitrogencation).The complexes as well as the heterocyclic itself exhibit antiseptic, disinfectant, and pesticide properties. Its solution in alcohol is used as liquid bandages. It once was of interest as an anti-cancer drug (Charles *et al.*, 1956).

Materials and methods

All the chemicals and reagents used in this research work were of analytical or synthetic grade. Compounds procured were purified and dried using standard methods before use, wherever necessary.3Aqueous ethanol (CDH Ltd),8-hydroxy quinoline (SD Fine Chem. Ltd.),1,2-dichloro ethane (Loba Chemie),Anhydrous potassium carbonate (SD Fine Chem. Ltd.),Anhydrous sodium carbonate (Nice Chemicals Ltd),Chloroform (CDH Ltd),Diethyl ether (CDH Ltd),Diphenyl amine (CDH Ltd),Phenyl amine (CDH Ltd),Ortho Nitro phenyl amine (CDH Ltd),Ethyl amine (CDH Ltd),Ortho bromophenyl amine (CDH Ltd),Ortho nitrophenylamine (SD Fine Chem. Ltd.),Methanol (SD Fine Chem. Ltd.),Methylamine h**.** Molinspiration ydrochloride (Himedia),3- Nitro phenyl amine(CDH Ltd),3-Bromo phenyl amine (CDH Ltd),Para chloro phenyl amine (CDH Ltd),Para hydroxyl phenyl amine (CDH Ltd),Ortho chlorophenyl amine (CDH Ltd)

Methods

a) Screening of proposed derivatives for different physicochemical properties using different software. ACD Labs Chemsketch 10.00 software is generally used for drawing, 3D optimizing and calculating various physicochemical properties of the proposed molecules.

The values obtained for the novel molecules will be then compared with that of the standard drugs.

ACD Chemsketch 10.00 gives readings for a wide range of properties including log P, which is a measure of lipophilicity of the molecule software is used to calculate the "Lipinski Rule of Five" and drug likeness analysis. All of these in silico properties will be closely evaluated and compared. Only the analogues with desired physicochemical properties, obeying Lipinski Rule of Five and those with not more than two violations will be selected for wet lab synthesis.

b) Estimation of the biological activity of the proposed molecules by comparing the structure of the new compound with structures of well-known biologically active substances using PASS software. The approach used in PASS is based on the suggestion that Activity = *f* (structure). Thus, it is possible to predict if a new compound may have a particular effect.

Docking studies

To select and prepare target of interest and carry out the docking studies of the analogues using SCHRODINGER software to obtain the GLIDE Score.

In silico adme property prediction;

To predict the ADME profile of the designed molecules using the application QikProp in Maestro Molecular modeling environment.

Synthesis of the novel analogues;

To synthesize the lead molecule 8-hydroxy quinolines in two steps;

In the first step, 8-hydroxy quinoline reacted with 1,2-dichloroethane to give 8-(2'-chloroethanoxy)quinoline. This quinoline derivative then reacts with amines to give amine derivative of the respective compounds. This is the second step and derivative preparing step of the reaction. Percentage yield of the compounds will be calculated and characterized by different analytical techniques.

Characterization

Characterizations of the synthesized compounds will be done by various analytical techniques like Melting point determination,Determination of R_f value by TLC,FTIR and NMR spectroscopy. Data are shown in Table 1

Antitubercular screening

The ant tubercular activity of the selected novel analogues will be screened using Resazurin reduction assay, using $H_{37}Rv$ strain of Mycobacterium tuberculosis with Rifampicin as the standard.

Anti –microbial screening

Two compounds from the synthesized series of analogues will be screened for anti-microbial activity.

Antifungal screening

Two compounds from the synthesized series of analogues will be screened for antifungal activity.

Experimental section

Synthetic procedure

Step 1: Synthesis of 8-(2-chloroethanoxy) quinoline

A mixture of 8-hydroxy quinoline (0.13 mol),1,2 dichloroethane (0.167mol),and anhydrous potassium carbonate (0.195mol) was refluxed in dry acetone (420 ml),for 40 hrs.the reaction mixture was filtered ,and the filtrate on concentration yielded the product. The 2. Synthesis of N-[2-(quinolin-8-yloxy) ethyl]aniline (HQ1b).Product was filtered dried under vacuum and recrystallised by using chloroform: ether (1:1),Yield 50%, melting point 67^0C.

Step 2: 1 .Synthesis of 2-(quinolin-8-yloxy) ethane amine (HQ1a)

A mixture of 8-(2-chloroethanoxy) quinoline (0.01mol), ethane amine (0.012mol), anhydrous sodium carbonate (0.007mol). And sodium iodide (0.0034mol), was refluxed in dry acetone (40ml) for 65 hrs. The reaction mixture was filtered and the filterate on concentration yielded the product. Product was filterd, dried under vacuum and recrystallised using Acetone: diethyl ether (1:1).Yield 52%, melting point64^0C.

Synthesis of N- (phenyl)-N-[2-quinoline-8-yloxyl]amine (HQ1b)

A mixture of 8-(2-chloroethanoxy) quinoline (0.01mol), aniline(0.012mol), anhydrous sodium carbonate(0.007mol) . And sodium iodide (0.0034mol), was refluxed in dry acetone (40ml) for 65 hrs. The reaction mixture was filtered and the filtrate on concentration yielded the product. Product was filtered, dried under vacuum and recrystallised using acetone: diethyl ether (1:1) Yield 36%, melting point 57^0C.

Synthesis of N-(4-nitrophenyl)-N-[2-(quinolin-8-yloxy)ethyl]amine (HQ1c)

A mixture of 8-(2-chloroethanoxy) quinoline (0.01mol),4-nitro aniline (0.012mol), anhydrous sodium carbonate(0.007mol) and sodium iodide (0.0034mol),was refluxed in dry acetone(40ml) for 65 hrs. The reaction mixture was filtered and the filtrate on concentration yielded the product. Product was filtered, dried under vaccum and recrystallised using acetone: diethyl ether (1:1).Yield 43%, melting point 65^0C.

Synthesis of 4-{[2-(quinolin-8-yloxy)ethyl]amino}phenol(HQ1d)

A mixture of 8-(2-chloroethanoxy) quinoline (0.01mol),4-hydroxy aniline 0.012mol), anhydrous sodium carbonate(0.007mol) and sodium iodide (0.0034mol),was refluxed in dry acetone(40ml) for 65 hrs. The reaction mixture was filtered and the filtrates on concentration yielded the product. Product was filtered, dried under vacuum and recrystallised using chloroform: diethyl ether (1:1).Yield 61%, melting point 72^0C

Synthesis of N-(4-bromophenyl)-N-[2-(quinolin-8-yloxy) ethyl]amine(HQ1e)

A mixture of 8-(2-chloroethanoxy) quinoline (0.01mol), 4-bromo aniline (0.012mol), anhydrous sodium carbonate(0.007mol) and sodium iodide (0.0034mol),was refluxed in dry acetone(40ml) for 65 hrs. The reaction mixture was filtered and the filtrate on concentration yielded the product. Product was filtered, dried under vacuum and recrystallised using acetone: diethyl ether (1:1).Yield 60%, 110^{o}C.

Synthesis of N-(4-chlorophenyl)-N-[2-(quinolin-8-yloxy) ethyl]amine(HQ1f)

A mixture of 8-(2-chloroethanoxy) quinoline (0.01mol), 4-chloro aniline (0.012mol), anhydrous sodium carbonate (0.007mol) and sodium iodide (0.0034mol),was refluxed in dry acetone(40ml) for 65 hrs. The reaction mixture was filtered and the filtrates on concentration yielded the product. Product was filtered, dried under vaccum and recrystallised using acetone: diethyl ether (1:1).

Synthesis of N-(2-bromophenyl)-N-[2-(quinolin-8-yloxy)ethyl]amine(HQ2a)

A mixture of 8-(2-chloroethanoxy) quinoline (0.01mol),ortho bromo aniline (0.012mol), anhydrous sodium carbonate(0.007mol) and sodium iodide (0.0034mol),was refluxed in dry acetone(40ml) for 65 hrs. The reaction mixture was filtered and the filterate on concentration yielded the product. Product was filterd, dried under vaccum and recrystallised using acetone: diethyl ether (1:1).Yield 50%, melting point 117^{0}C.

Synthesis of N-(2-nitrophenyl)-N-[2-(quinolin-8-yloxy) ethyl]amine(HQ2b)

A mixture of 8-(2-chloroethanoxy) quinoline (0.01mol),ortho nitro aniline (0.012mol), anhydrous sodium carbonate(0.007mol) and sodium iodide (0.0034mol),was refluxed in dry acetone(40ml) for 65 hrs. The reaction mixture was filtered and the filterate on concentration yielded the product. Product was filterd, dried under vaccum and recrystallised using acetone:diethyl ether (1:1).Yield 52%,melting point 64^{0}C

Synthesis of N-(2-chloro phenyl)-N-[2-(quinolin-8-yloxy) ethyl]amine (HQ2c)

A mixture of 8-(2-chloroethanoxy) quinolone (0.01mol),ortho chloro aniline (0.012mol), anhydrous sodium carbonate(0.007mol) and sodium iodide (0.0034mol),was refluxed in dry acetone(40ml) for 65 hrs. The reaction mixture was filtered and the filtrate on concentration yielded the product. Product was filtered, dried under vaccum and recrystallised using acetone: diethyl ether (1:1).Yield 49%, melting point 64^{0}C.

Synthesis of N-ethyl-N-[2-(quinolin-8-yloxy) ethyl]amine(HQ3b)

A mixture of 8-(2-chloroethanoxy) quinoline (0.01mol),ethyl amine (0.012mol), anhydrous sodium carbonate(0.007mol) and sodium iodide (0.0034mol),was refluxed in dry acetone(40ml) for 65 hrs The reaction mixture was filtered and the filtrate on concentration yielded the product. Product was filtered, dried under vaccum and recrystallised using acetone: diethyl ether (1:1). Yield65% .melting point 82^{0}C.

Purification

To achieve purified synthesized compounds by recrystallisation using suitable solvents.

Recrystallisation of step 1 products using chloroform: ether (1:1).

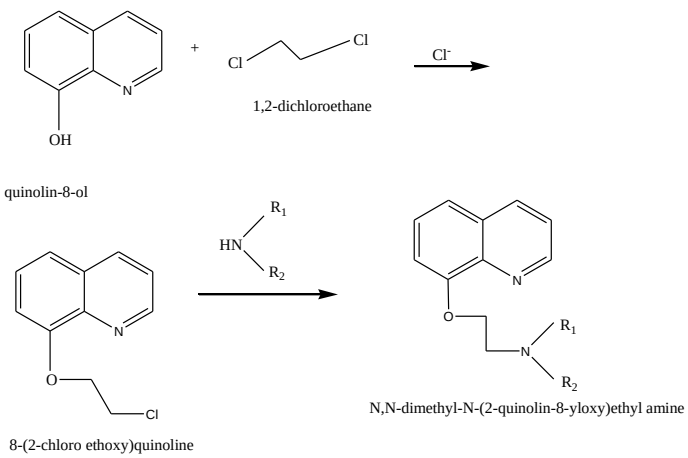
Recrystallisation of step 2 products using Acetone: diethyl ether(1:1).

Results and discussion

Present research work involved the preliminary in silico screening of various novel analogues for quantifying their drug likeness using Molinspiration software. The candidates with not more than one violation for Lipinski Rule of Five were taken for wet lab synthesis. Ten different analogues were synthesized. Purity of the compounds thus synthesized was ascertained by consistency in melting point and R_f value and characterized by IR and NMR spectral studies.

A preliminary antitubercular screening of the synthesized compounds was performed. The analogues namely HQ1a, HQ1b, HQ1d, HQ2a, HQ2b which showed better glide scores were screened for activity towards Mycobacterium H_{37}Rv strain. Their activity was compared with that of Rifampicin as standard. And the better glide score compounds namely HQ1a, HQ1b, HQ1d were screened for anti-bacterial activity and show moderate activity when compared to gentamycin as standard. Antifungal activities were also carried out in same compounds and got good results.

Scheme of work



Prediction of activity spectra of substances

It is possible with computer program PASS (Prediction of Activity Spectra for Substances), which predicts the biological activity spectrum for a compound based on its structural formula. The approach used in PASS is based on the suggestion that *Activity= f (Structure)*. Thus, by "comparing" the structure of a new compound with

structures of well-known biologically active substance it is possible to estimate if a new compound may have a particular effect. PASS operates with many thousands of substances from the training set, so provides more objective estimate if a compound is active or not for any kind of activity as compared with any researcher. PASS training set consists of over 260,000 of drug-like biologically active compounds. They include about drugs, drug-candidates, lead compounds and toxic compounds.

Activity Description

Biological activity is the result of chemical compound's interaction with biological entity. In clinical study, biological entity is represented by human organism. In preclinical testing, it is the experimental animals (in vivo) and experimental models (in vitro). Biological activity depends on peculiarities of compound (structure and physico-chemical properties), biological entity (species, sex, age, etc.), mode of treatment (dose, route, etc.).

Any biologically active compound reveals wide spectrum of different effects. Some of them are useful in treatment of definite diseases but the others cause various side and toxic effects. Total complex of activities caused by the compound in biological entities is called the "biological activity spectrum of the substance". "Biological activity spectrum" is defined as the "intrinsic" property of compound depending only on its structure and physico-chemical characteristics.

PASS training set covers 6825 kinds of biological activities included basic pharmacological effects, biochemical mechanisms of action, specific toxicities, metabolic terms, influence on gene expression and transporters.

PASS Inet predicts 3678 pharmacological effects, mechanisms of action, mutagenicity, carcinogenicity, teratogenicity and embryotoxicity. Their values vary from 0.0 to 1.0.

If Pa > 0.7 the compound is very likely to reveal this activity in experiments, but in this case the chance of being the analogue of the known pharmaceutical agents for this compound is also high.

If 0.5< Pa < 0.7, the compound is likely to reveal its activity in experiments, but this probability is less, and the compound is not so similar to the known pharmaceutical agents.

If Pa < 0.5, the compound is unlikely to reveal its activity in experiments, but if the presence of this activity is confirmed in the compound, it might be a new chemical entity.

Methodolgy of docking

Target Identification and Retrieval,Crystallographic structures of the targets of interest were obtained from PD.

Docking software – Schrodinger

Schrodinger Molecular Modeling Package (Schrodinger, 2012)

Docking, the computational simulation of a candidate ligand and binding to a receptor helps to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. Docking has an important role in the rational design of drugs. Schrodinger develops state-of-the-art chemical simulation software for use in pharmacy, biotechnology and material science research. Since its founding in 1990, Schrodinger has earned a reputation for its leadership in scientific development.

Maestro

Maestro is a powerful, all-purpose molecular modeling environment. It is the unified interface for all Schrodinger software. Impressive rendering capabilities, a powerful selection of analysis tools, and an easy-to-use design combine to make Maestro a versatile modeling environment for all researchers.

Sitemap

Sitemap can treat entire proteins to locate binding sites whose size, functionality, and extent of solvent exposure meet user specifications. Site score, the scoring function used to assess a site's probability for ligand binding, accurately ranks possible binding sites to eliminate those not likely to be pharmaceutically relevant. Sitemap fits perfectly into the Schrodinger structure based drug design workflow**.**

Glide

Glide is a ligand-binding program provided by Schrodinger for predicting protein-ligand binding modes. It provides a complete solution for ligand-receptor docking. Glide offers the full solution for virtual screening from HTVS (High Throughput Virtual Screening) to SP (Standard Precision) to XP (eXtra Precision).

Preparing a working directory

A working directory is created to keep all the input and output files. The commands include Defining the receptor, Defining the active site, Receptor grid generation, Importing the prepared structures, Setting up glide constraints, Starting and monitoring grid calculation,Ligand docking, Specifying a set of grid files and basic options, Specifying ligands to dock, Starting ligand docking job, Examining the output files, Examining glide data, Importing pose data, Viewing poses, Visualizing glide XPdescriptor,Displayingato

Pharmacological screening

Antimicrobial screening

1. Antitubercular screening
2. Antibacterial screening
3. Antifungal screening

Antitubercular screening

Antitubercular study was performed by using Alamar blue assay method (REMA – Resazurin Microtitre Assay). Resazurin, an oxidation reduction indicator, has been used to assess viability and bacterial contamination and to test for antimicrobial activity since Alamar blue has been recently identified as Resazurin in cell cytotoxicity studies.

There are two methods of susceptibility testing

Direct method

This method is done if acid-fast bacilli are seen on the smear of the concentrated clinical specimen. Further dilutions are made and inoculated.

Indirect method

Bacterial culture is suspended in Middle brook 7H9 broth containing three or four small sterile glass beads. Mixture is placed on a vortex mixer and precautions were taken to prevent aerosol production. Tube is allowed to stand for 15 minutes. The stock suspension is diluted and 0.1 ml was inoculated onto the control and the drug containing media.

Alamar blue assay method (REMA)

Test organism: *Mycobacterium tuberculosis* $H_{37}Rv$ maintained in Lowenstein Jensen medium was used as the test organism for antimycobacterial screening studies.

Protocol for antimicrobial activity screening by Resazurin Microtitre assay (REMA)

The bacterial cultures were grown till mid-log phase in the Middle brook 7H9 broth for *Mycobacterium tuberculosis* $H_{37}Rv$., Stock solutions of the test compounds were prepared at a concentration of 2 mg/ml.50 µL of the mid-log phase culture was added to 150µL of the media taken in Microtitre plates.From the stock solution of the compounds [HQ1a, HQ1b, HQ1d, HQ2a, HQ2b, HQ2c**] (Fig 1. A, B, C, D, E and F)** were added to the wells to final concentration of 100, 250, 500 µg/mL. The control wells contained culture without any compound. All the tests were done in duplicates.The plates were then incubated at 37°C for 7 days.After incubation 20µL of Resazurin dye was added and change of color, if any was noted.The control wells showed no change of color from pink. Those compounds which prevented the change of colour of the dye from blue to pink were considered to be inhibitory (Table 16).

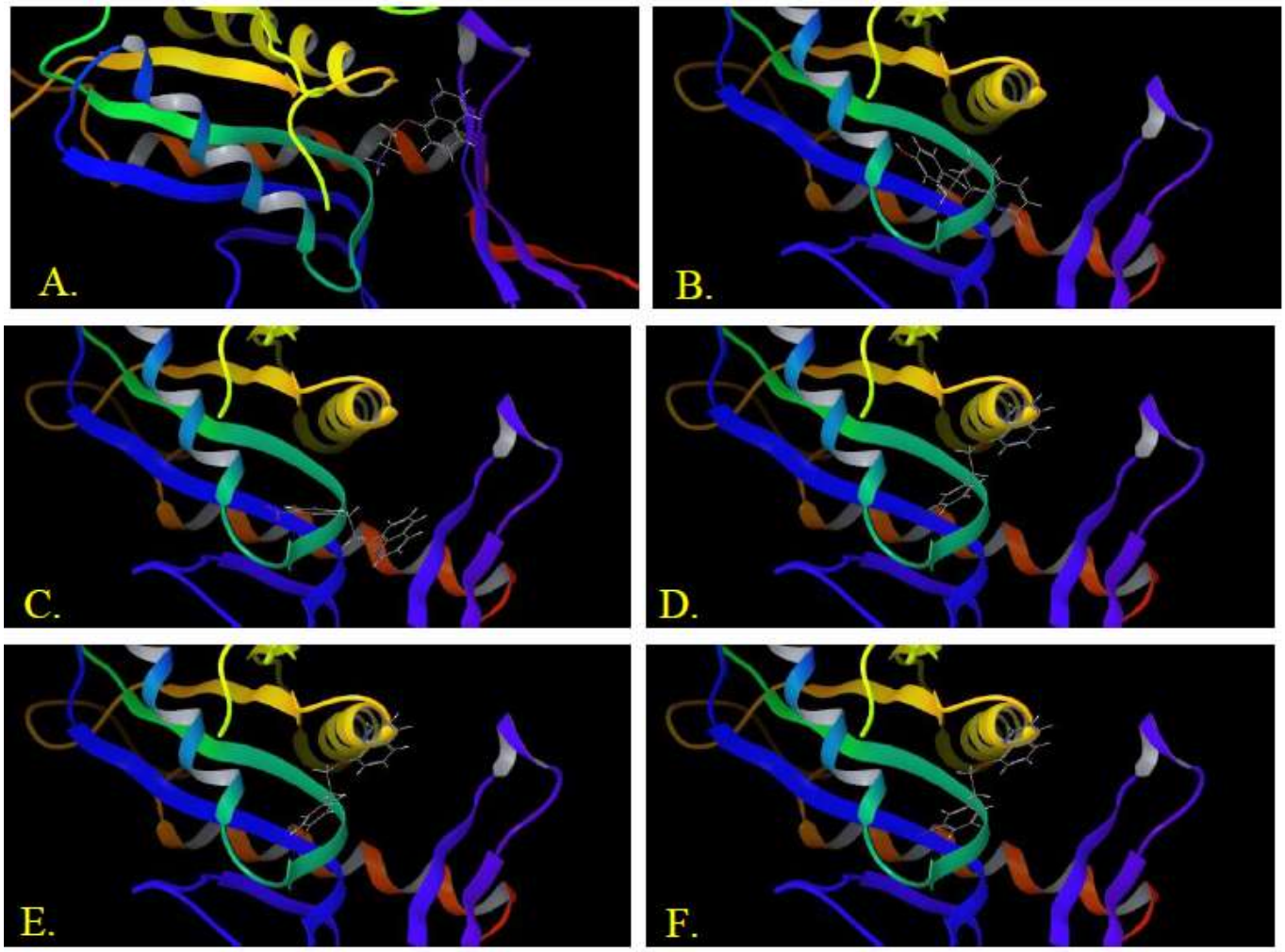


Fig 1: Images of compounds of the study. A. HQ1a; B. HQ1b; C. HQ1d; D. HQ2A; E. HQ2b; F. HQ2c

Antibacterial screening

Antibacterial screening was done on randomly selected novel synthesized Quinoline analogues. Gentamicin was used as standard for both gram positive and gram negative organisms. The screening was carried out in the microbiological laboratory of College of Pharmaceutical Sciences, Medical College, and Thiruvananthapuram. (Table 17)

Test micro-organisms

The organisms used were Staphylococcus aureus ATCC 25923 (Gram positive) and Escherichia coli ATCC 25922 (Gram negative). The organisms were obtained from the Department of Microbiology, Medical College, and Thiruvananthapuram.

Culture Media

Nutrient agar (Hi-media) was used for culturing the bacteria. 28 g of the above culture medium was suspended in 1000 mL of distilled water and boiled to dissolve the media completely. The solution was sterilized by autoclaving at 121 ^{0}C for 20 minutes.All the bacteria were sub cultured on sterile nutrient agar slants and incubated at 37 ± 0.5 ^{o} C for 24 hours. Inoculated 5 ml each of sterile nutrient broth with loop full of each organism was added to the sterilized nutrient agar medium.The sterile inoculated media were poured into previously sterilized petri dishes and marked to distinguish the organism and allowed to settle. All these stages were done under aseptic conditions.

Preparation of the test solution (HQ1a, HQ1b, HQ1d)

The test solutions were prepared in ethanol. The concentrations used for antibacterial screening were 250, 500 µg/mL.Standard drug solution of Gentamicin (100µg/mL) was prepared in distilled water. Using a sterile cork borer of about 5 mm diameters, 4 wells were made in each petridish. Numbers were marked on the bottom of petri dish to identify each cup. The test solutions (single and double strength), standard solution and the vehicle control (ethanol) were placed in each cup of each petri dish and incubated at 37 ± 0.5^{o}C for 24 hrs. The presence of a definite zone of inhibition of any size was observed and compared with that of standard drug solution.

Antifungal activity

Antifungal screening was done on randomly selected novel 8-hydroxy quinoline analogues (HQ1a, HQ1b,and HQ1d)). Clotrimazole was used as standard. The screening was carried out in the Microbiological laboratory of College of Pharmaceutical Sciences, Medical College, and Thiruvananthapuram.

Test micro-organisms

Fungal strain of *Candida albicans(NCIM 3100)* was obtained from the Department of Microbiology, Medical College Thiruvananthapuram

Culture media

Sabouraud dextrose agar media was used for culturing the organism.

Glide scores

Fourteen analogues were docked with various receptors and the compounds having great glide score was selected for synthesis. Docking scores obtained are represented in table

Antibacterial activity

The antibacterial activity was performed on randomly selected synthesized 8-hydroxy quinoline analogues. Gentamicin was used as standard for both gram positive (*Staphylococcus aureus* ATCC 25923) and gram negative organism (*Escherichia coli* ATCC 25922).

Antifungal activity

Randomly selected 8-hydrxy quinoline analogues were subjected to Antifungal activity using *Candida albicans (NCIM 3100)* comparing with the standard Clotrimazole.The percentage human oral absorption in GI less than 25 % is considered poor. QP log S for aqueous solubility can have values from -6.5 to 0.5. QP log P for octanol/water ranges from -2.0 to 6.5. QP log K hsa for Serum Protein Binding ranges from -1.5 to 1.5. The synthesized compounds were found to have poor aqueous solubility but have found to be with excellent human oral absorption.

Antitubercular activity

The synthesized analogues that exhibited good glide score on docking were selected for antitubercular activity. *Mycobacterium tuberculosis* $H_{37}Rv$ maintained in Lowenstein Jensen medium was used as the test organism for antimycobacterial screening studies (Table 16)

Data collected during study are shown in Table 1-17.

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Table 1: Molecular properties of standard drugs

Standard drugs	Molecular formula	Smiles	
Isoniazid	$C_6H_7N_3O$	O=C(NN)c1ccncc1	
Pyrazinamide	$C_5H_5N_3O$	NC(=O)c1cnccn1	
Ethambutol	$C_{10}H_{24}N_2O_2$	CCC(CO)NCCNC(CO)CC	
Ethionamide	$C_8H_{10}N_2S$	CCc1cc(ccn1)C(N)=S	
PAS	$C_7H_7NO_3$	OC(=O)c1ccc(N)cc1O	
standard drugs	Molar volume (cm^3)	TPSA (cm^2)	Polarizability ($10^{-24}cm^3$)
Isoniazid	110.1	68.01	14.61
Pyrazinamide	94.5	68.87	12.43
Ethambutol	207.0	64.51	23.21
Ethionamide	142.0	38.91	19.85
PAS	102.6	83.55	15.57

Table 2: Molecular properties of proposed analogues

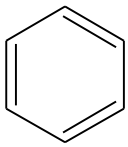
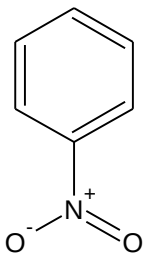
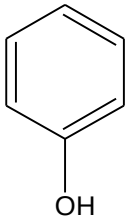
Compounds	Nr1	R2	Smiles notation
HQ1a	NH	H	c12cccc(c2nccc1)OCCN
HQ1b	NH		c12cccc(c2nccc1)OCCCc3ccccc3
HQ1c	NH		c12cccc(c2nccc1)OCCNc3ccc(cc3)[N+]([O-])=O
HQ1d	NH		c12cccc(c2nccc1)OCCNc3ccc(cc3)O

Table 2: Molecular properties of proposed analogues

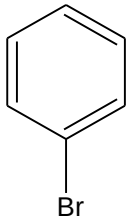
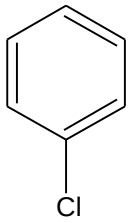
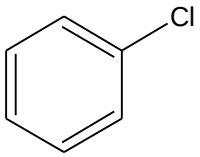
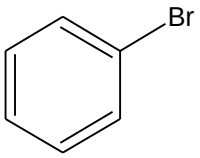
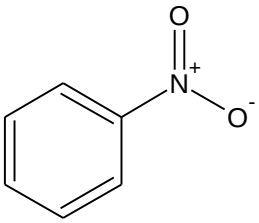
Compounds	Nr1	R2	Smiles notation
HQ1e	NH		c12cccc(c2nccc1)OCCNc3ccc(cc3)Br
HQ1f	NH		c12cccc(c2nccc1)OCCNc3ccc(cc3)Cl
HQ2a	NH		c12cccc(c2nccc1)OCCNc3c(cccc3)Cl
HQ2b	NH		c12cccc(c2nccc1)OCCNc3c(cccc3)Br
HQ2c	NH		c12cccc(c2nccc1)OCCNc3c(cccc3)[N+]([O-])=O
HQ3a	NH	CH3	c12cccc(c2nccc1)OCCNC
HQ3b	NH	C2H5	c12cccc(c2nccc1)OCCNCC
HQ3c	NH	C3H7	c12cccc(c2nccc1)OCCNCCC
HQ3d	NH	C4H9	c12cccc(c2nccc1)OCCNCCCC
HQ3e	NH	C5H11	c12cccc(c2nccc1)OCCNCCCCC

Table 3: Molecular properties of proposed analogues

Compounds	Mf	M. wt.	Refr. index	Polarizability
HQ1a	$C_{11}H_{12}N_2O$	188.226	1.626±0.02	22.60±0.5
HQ1b	C17H16N2O	264.322	1.670±0.02	32.77±0.05
HQ1c	C17H15N3O3	309.319	1.691±0.02	35.37±0.05
HQ1d	C17H16N2O2	280.321	1.696±0.02	33.52±0.05
HQ1e	C17H15BrN2O	343.218	1.686±0.02	35.82±0.05
HQ1f	C17H15ClN2O	298.767	1.674±0.02	34.71±0.05
HQ2a	C17H15BrN2O	343.218	1.686±0.02	35.82±0.05
HQ2b	C17H15N3O3	309.319	1.691±0.02	35.37±0.05
HQ2c	C17H15BrN2O	298.767	1.674±0.02	35.37±O.O5
HQ3a	C12H14N2O	202.252	1.591±0.02	24.47±O.O5
HQ3b	C13H16N2O	216.279	1.581±0.02	26.30±0.05
HQ3c	C14H18N2O	230.306	1.572±0.02	28.14±0.05
HQ3d	C15H20N2O	244.302	1.558±0.02	29.97±0.05

Table 4: Lipinski rule analysis of standard drugs by Molinspiration

Std drugs	C log P	MW	nON	nOHNH	nrotb	Nviol
Isoniazid	-0.916	137.142	4	3	1	0
Pyrazinamide	-0.711	123.11	4	2	1	0
Ethambutol	0.35	204.31	4	4	9	0
Ethionamide	1.46	166.25	2	2	2	0
PAS	0.922	153.13	4	4	1	0

Table shows no violations in lipinsky rule of five.

Table 5: Lipinski rule analysis of novel compounds by Molinspiration

Novel compounds	C log P	MW	nON	nOHNH	nrotb	nviol
HQ1a	o.756	188.23	3	2	3	0
HQ1b	4.273	263.34	2	0	5	0
HQ1c	4.232	308.337	5	0	6	0
HQ1d	3.794	279.339	3	1	5	0
HQ1e	5.082	342.236	2	0	0	1
HQ1f	4.951	297.785	2	0	5	0
HQ2a	5.034	342.236	2	0	5	1
HQ2b	3.345	309.325	6	1	6	0
HQ2c	4.064	298.773	3	1	5	0
HQ3a	3.834	278.355	3	1	5	0
HQ3b	4.3	292.382	3	1	6	0
HQ3c	4.691	306.409	3	1	7	0
HQ3d	5.25	320.436	3	1	8	1
HQ3e	5.755	334.463	3	1	9	1

This table shows the synthesized compounds lipinsky rule properties

Table 6: Drug Likeness Analysis of Standard drugs

Standard drugs	G-protein coupled receptor	Ion channel	Kinase linked	Nuclear receptor
Isoniazid	-1.36	-1.45	-0.96	-2.79
Pyrazinamide	-1.51	-1.64	-2.14	-3.29
Ethambutol	0.21	0.07	-0.27	-0.9
Ethionamide	-1.06	-1.28	-1.34	-2.31
PAS	-0.27	-0.01	-0.41	-1.35

Table 7: Drug Likeness Analysis of Novel Compounds

Novel compounds	G-protein coupled receptor	Ion channel	Kinase linked	Nuclear receptor
HQ1a	-0.21	-0.40	-0.35	-0.24
HQ1b	-0.21	-0.46	-0.35	-0.18
HQ1c	-0.14	-0.48	-0.37	-0.17
HQ1d	-0.30	-0.49	-0.36	-0.48
HQ1e	-0.29	-0.54	-0.35	-0.41
HQ1f	-0.23	-0.56	-0.38	-0.40
HQ2a	-0.32	-0.59	-0.39	-0.42
HQ2b	-0.31	-0.67	-0.42	-0.38
HQ2c	-0.27	-0.71	-0.46	-0.40
HQ3a	-0.18	-0.38	-0.23	-0.32
HQ3b	-0.17	-0.43	-0.23	-0.25
HQ3c	-0.40	-0.45	-0.26	-0.24
HQ3d	-0.31	-0.58	-0.39	-0.42
HQ3e	-0.31	-0.66	-0.42	-0.39

Table 8: PASS of Novel Compounds

Proposed analogues	Activity	P_a	P_i
HQ1a	Immunosuppressive analgesic Anti-inflammatory vasorelaxing	0.613 0.570 0.881 0.606	0.067 0.111 0.017 0.019
HQ1b	Immunosuppressive analgesic Anti-inflammatory vasorelaxing	0.590 0.677 0.855 0.568	0.080 0.048 0.025 0.035
HQ1c	Immunosuppressive analgesic Anti-inflammatory vasorelaxing	0.617 0.680 0.853 0.558	0.065 0.047 0.025 0.040
HQ1d	Immunosuppressive	0.669	0.037

Table 8: PASS of Novel Compounds

Proposed analogues	Activity	P_a	P_i
	analgesic	0.617	0.081
	Anti-inflammatory	0.749	0.051
	vasorelaxing	0.601	0.021
HQ1e	Immunosuppressive	0.652	0.045
	analgesic	0.714	0.035
	Anti-inflammatory	0.650	0.068
	vasorelaxing	0.563	0.038
HQ1f	Immunosuppressive	0.672	0.035
	analgesic	0.718	0.034
	Anti-inflammatory	0.641	0.070
	vasorelaxing	0.553	0.043
HQ2a	Immunosuppressive	0.678	0.032
	analgesic	0.675	0.049
	Anti-inflammatory	0.580	0.115
	vasorelaxing	0.529	0.059
HQ2b	Immunosuppressive	0.662	0.040
	analgesic	0.766	0.022
	Anti-inflammatory	0.601	0.101
	vasorelaxing	0.550	0.128
HQ2c	Immunosuppressive	0.681	0.031
	analgesic	0.769	0.021
	Anti-inflammatory	0.485	0.178
	vasorelaxing	0.477	0.105
HQ3a	Immunosuppressive	0.531	0.112
	analgesic	0.565	0.115
	Anti-inflammatory	0.620	0.073
	vasorelaxing	0.671	0.005
HQ3b	Immunosuppressive	0.613	0.067
	analgesic	0.673	0.050
	Anti-inflammatory	0.529	0.095
	vasorelaxing	0.637	0.050
HQ3c	Immunosuppressive	0.537	0.109
	analgesic	0.676	0.048
	Anti-inflammatory	0.522	0.097

Table 8: PASS of Novel Compounds

Proposed analogues	Activity	P_a	P_i
	vasorelaxing	0.628	0.013
HQ3d	Immunosuppressive	0.701	0.023
	analgesic	0.666	0.053
	Anti-inflammatory	0.580	0.115
	vasorelaxing	0.549	0.046
HQ3e	Immunosuppressive	0.687	0.029
	analgesic	0.758	0.023
	Anti-inflammatory	0.601	0.101
	vasorelaxing	0.509	0.075

Table 9: Targets intended to be docked with the synthesized analogues

Sl. No	Targets	Pdb id
1.	PyrR (pyramidine synthatase)	IW30

Table 10: Glide Scores for Antitubercular Activity

Compounds	Glide score	Glide hbonds	Glide Evdw
HQ1a	**-10.89**	-0.96	-6.27
HQ1b	**-10.03**	-1.58	-6.12
HQ1c	-6.1	0	-5.85
HQ1d	-8.3	0	-6.24
HQIe	-5.9	0	-6.87
HQ1f	-5.76	-0.6	-6.76
HQ2a	-8.97	-0.32	-5.57
HQ2b	-7.73	-0.26	-6.05
HQ2c	-6.68	-0.54	-6.54
HQ3a	-6.53	0	-5.92
HQ3b	-6.52	-1.21	-5.36
HQ3c	-6.70	0	-6.09

Table 10: Glide Scores for Antitubercular Activity

Compounds	Glide score	Glide hbonds	Glide Evdw
HQ3d	-6.03	0	-6.71
HQ3e	-6.48	0	-7.4

Table 11: Physico chemical data of newly synthesized compounds

COMPOUNDS	Molecular Formula	MW	M.P	R_f
HQ1a	$C_{11}H_{12}N_2O$	188.22	50 ^{0}C	0.90
HQ1b	$C_{17}H_{16}N_2O$	264.3	57 ^{0}C	0.92
HQ1c	$C_{17}H_{15}N_3O_3$	309.3	65 ^{0}C	0.75
HQ1d	$C_{17}H_{16}N_2O_2$	280.3	72 ^{0}C	0.78
HQ1e	C_{17} H $_{15}$ BrN$_2$O	343.2	110 ^{0}C	0.89
HQ1f	$C_{17}H_{15}$ Cl N_2O	298.7	64 ^{0}C	0.74
HQ2a	$C_{17}H_{15}$ Br N_2O	343.2	117 ^{0}C	0.77
HQ2b	$C_{17}H_{15}N_3O_3$	309.3	64 ^{0}C	0.88
HQ2c	$C_{17}H_{15}N_4O_2$	480.6	64 ^{0}C	0.91
HQ3b	$C_{31}H_{34}N_4O_2$	494.6	82 ^{0}C	0.93

Table 12: Characteristic IR absorption peaks of newly synthesized compounds

Compounds	IR peaks (KBr) cm $^{-1}$
HQ1c	1482 (C-N),1300(N-H),1112(C-O),840,698(Ar-H)
HQ1e	1444(C-H),1394(C-N),1007(C-O),781,742(Ar-H)
HQ2a	1470 (C-N),1308(N-H),1069(C-O),710,819(Ar-H)
HQ2b	1445(C-H),1394(C-N),1182(C-O), 840,698(Ar-H)

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Table 13: Characteristic Chemical Shifts of DP 2B

Compound	^{1}HNMR ($CDCl_3$) δ ppm
HQ	7.21 – 7.26 (m, Ar-H, 6H), 3.32 (s, CH, 1H) 4.05- (Ar C-NH).

HQ1a	7.9-8.23(m6H,2,3,4,5)5.25-5.45(m,H,NH)
HQ1b	8.11-8.34(m6h,2,3,4,)5.19-5.35(m1H,NH)

Table 14: In Silico ADME Prediction by QikProp

Compounds	**QP log P Octanol/water**	**QP log S**	**No: of 1^0 metabolites**	**Oral absorption %**
HQ1a	5.48	-7.04	8	100
HQ1b	5.62	-6.84	8	100
HQ1c	6.63	-7.81	7	100
HQ1d	5.35	-7.715	7	100
HQ1e	5.51	-7.52	8	91.71
HQ1f	6.36	-8.05	6	100
HQ2a	6.79	-8.67	6	100
HQ2b	5.36	-7.53	7	91.708
HQ2c	6.56	-8.41	7	100
HQ3a	6.91	-8.50	7	100
HA3b	5.88	-7.01	7	100
HQ3c	5.35	-7.20	7	100
HQ3d	6.62	-8.33	6	100
HQ3e	6.07	-7.47	7	100

Table 15: In Silico ADME Prediction by QikProp

Compounds	**QPlogKhsa (serum protein binding)**	**QPlogBB (brain/blood)**	**Predicted CNS action**	**QPlogHERG (K^+ Channel blockage)**
HQ1a	0.961	-0.486	-1	-7.83
HQ1b	1.023	-0.609	-1	-6.99
HQ1c	1.262	0.091	1	-7.28
HQ1d	1.312	-0.038	0	-7.03
HQ1e	1.149	-1.317	-2	-7.19

Table 15: In Silico ADME Prediction by QikProp

Compounds	**QPlogKhsa (serum protein binding)**	**QPlogBB (brain/blood)**	**Predicted CNS action**	**QPlogHERG (K^+ Channel blockage)**
HQIf	1.243	-0.088	0	-7.50
HQ2a	1.389	-0.204	0	-7.788
HQ2b	1.15	-1.32	-2	-7.201
HQ2c	1.333	-0.172	0	-7.566
HQ3a	1.429	-0.166	0	-7.47
HQ3b	1.132	-0.176	0	-6.944
HQ3c	0.972	-0.728	-1	-7.437
HQ3d	1.29	0.075	1	-7.506
HQ3e	1.163	-0.227	0	-7.114

Table 16: Antitubercular activity of selected 8-HTDROXYQUINOLINE analogues (Alamar Blue Assay Method - REMA)

Compound	**Concentration (µg/ml)**		
	100	**250**	**500**
HQ1a	B	B	B
HQ1b	P	P	B
HQ1d	P	P	B
HQ2a	P	P	P
HQ2b	P	P	P

P = Pink (Resistant) B = Blue (Sensitive)

Table 17: Antibacterial activity of selected 8-hydroxyquinoline analogues

Sample	**Zone of inhibition (mm)**					
	Gram - ve (*E. coli*)			**Gram + ve (*S. aureus*)**		
	100 (µg)	**250 (µg)**	**500 (µg)**	**100 (µg)**	**250 (µg)**	**500 (µg)**
Control	-	-	-	-	-	-
Standard (Gentamicin)	19	-	-	20	-	-

Table 17: Antibacterial activity of selected 8-hydroxyquinoline analogues

Sample	Zone of inhibition (mm)					
	Gram - ve (*E. coli*)			Gram + ve (*S. aureus*)		
	100 (µg)	250 (µg)	500 (µg)	100 (µg)	250 (µg)	500 (µg)
HQ1a	-	16	18	-	17	19
HQ1b	-	12	14	-	14	16
HQId	-	15	16	-	12	14

Summary and conclusion

This research work was focused on the rational approach in design and development of 8-hydroxy quinoline as novel antitubercular agents. Quinolines have established themselves as one of the most potent antimalarialdrug. And it possess, immunosuppressive, analgesic, vasorelaxing, antiplasmodial, anticancer, activities. This research work proves that the Aryl propanolamine substituted 8-hydroxy quinolines can act as a lead molecule to develop newer antitubercular agents.

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