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In silico Design of N-Substituted Quinoline-Isatin Hybrids for Multiple Targeted enzymes for Anti-Microbial Activity

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ABSTRACT

The World Health Organization reports that approximately 700,000 people are affected by Antimicrobial Resistance (AMR) each year. This growing concern has accelerated the search for new antibacterial agents. Fluoroquinolones are key broad-spectrum antibiotics and recent interest in Isatin derivatives has revealed promising antimicrobial activity. Combining quinoline and Isatin pharmacophores offers a strategy to develop novel agents with unique mechanisms to overcome resistance. Based on SAR and literature data, a series of N-substituted quinoline-isatin conjugates were designed to target bacterial enzymes such as *Enoyl-ACP reductase, BioA (7,8-diaminopelargonic acid synthase), DNA gyrase.* Docking studies identified QI7f as a lead compound with superior binding scores (-10.35, -12.38, -8.93 kcal/mol) compared to Standards. In silico ADMET analysis predicted favorable pharmacokinetic and toxicity profiles. The compounds were synthesized and structurally confirmed via FTIR, NMR, and mass spectrometry. Q17fa showed notable in-vitro antimicrobial activity (2-10 mg/mL) comparable to gentamicin, likely due to electron-withdrawing groups enhancing target interactions.

Keywords: Antimicrobial resistance; Molecular hybridization; Quinoline; Isatin

INTRODUCTION

Mycobacterium tuberculosis is the primary cause of tuberculosis (TB), a chronic infectious disease. Although it primarily affects the pulmonary system, it can spread to other organs such as the brain, spine, and kidneys. spread by airborne droplets released during a cough, sneeze, or speech by an infected individual. It is one of the most deadly infectious illnesses in the world, especially

in low-income areas.Annually, over 10 million individuals are diagnosed with active TB, resulting in approximately 1.5 million fatalities.[2] Although TB is treatable with antibiotics, the emergence of drug-resistant strains complicates its management. Drug-resistant tuberculosis (DR-TB) presents an escalating public health challenge, underscoring the necessity for novel therapeutic interventions[3]. In 2021, According to the World Health Organization (WHO), there were 1.6 million TB-related fatalities and 11 million new TB infections. Due to resistance to second-line medications, multidrug-resistant TB is a more severe form of the disease with greater fatality rates. 4 Isoniazid, Rifampicin, Pyrazinamide, and Ethambutol are among the first-line drugs for tuberculosis. While XDR-TB shows resistance to these and other important medications, MDR-TB is characterized by resistance to at least isoniazid and rifampicin. Despite the essential role of these first-line drugs in treatment, they can induce significant adverse effects, such as hepatotoxicity, neurotoxicity, and visual impairment, potentially affecting patient adherence and contributing to resistance. Over the past five decades, The USFDA, or US Food and Drug Administration, has authorized only a limited number of new anti-TB agents, highlighting the substantial challenges in developing novel treatments.[5] Bedaquiline, a newer medication for MDR-TB and XDR-TB, disrupts bacterial energy production; however, resistance to bedaquiline has emerged, raising concerns about its efficacy. Bedaquiline should be administered in conjunction with other effective drugs and under close monitoring.[6]

To address the rise of drug-resistant TB, this study targeted on development of innovative Anti-TB medications, specifically N-substituted quinoline-isatin conjugate derivatives. Quinoline and isatin are recognized for their antibacterial properties.[7] By combining these two compounds, there is a promising opportunity to develop new drugs with enhanced efficacy and safety. Molecular hybridization is a strategic approach in drug design, wherein pharmacophoric elements from various bioactive compounds are combined to form new hybrid entities. The objective of these hybrids is to improvise their affinity, efficacy, and selectivity while minimizing adverse drug effects compared to their original counterparts. This approach is particularly advantageous for creating multitarget drugs that can address complex diseases by simultaneously engaging with multiple targets.[8, 9]

Design of N-substituted Quinoline–Isatin hybrids
Quinoline moiety

The quinoline moiety, encompassing both natural and synthetic compounds (Figure 1), has significantly contributed to medicinal advancements over the past five decades. The introduction of various functional groups to the quinoline structure has profoundly influenced its biological activities.[10] Numerous Quinoline and quinolone derivatives exhibit a broad spectrum of biological effect , including antituberculosis,[11] anticancer,[12] antimalarial, [13]antimicrobial,[14] anti-inflammatory, and antiviral properties.[15]

N-Substitution on Quinoline

According to literature and structure-activity relationship (SAR) studies, the Introducing various substituents (alkyl, aryl, or heterocyclic groups) at the nitrogen atom of the quinoline ring can:

1. Enhance lipophilicity or hydrophilicity.
2. Improve cell permeability.
3. Modulate interaction with the biological target.[16]

Isatin moiety

Isatin derivatives have shown potential as inhibitors of *Enoyl-ACP Reductase (InhA),*, an enzyme associated with bacterial "fatty acid metabolic pathway".[22] By attaching isatin scaffold to the quinoline ring, the hybrid molecule could exhibit **dual-target antibacterial activity.** The isatin group may act as a lead compound for targeting InhA. Due to its structural similarity to the enzyme's natural substrate, it is a potential target for drug development, anti-tuberculosis activity, and anti-bacterial activity.[17]

Linker

Schiff bases are versatile compounds widely used as linkers in hybrid molecules for enhancing biological activity. Their imine **(-C=N-)** functional group plays a crucial role in bioactivity, enabling Schiff base hybrids to exhibit diverse pharmacological actions such as antimicrobial, anticancer, antioxidant, and anti-inflammatory effects.[18, 19]

Design strategy

Keep considering the biological importance

of the two chemical moietie isatin and quinoline. Thus, the current work describes the production of new hybrids for anti-tubercular activity.

The development of molecular hybridization technique, combining quinoline's membrane-disrupting properties with isatin's enzymatic inhibition, could accelerate progress towards WHO's 2035 tuberculosis (TB) elimination targets.[20]

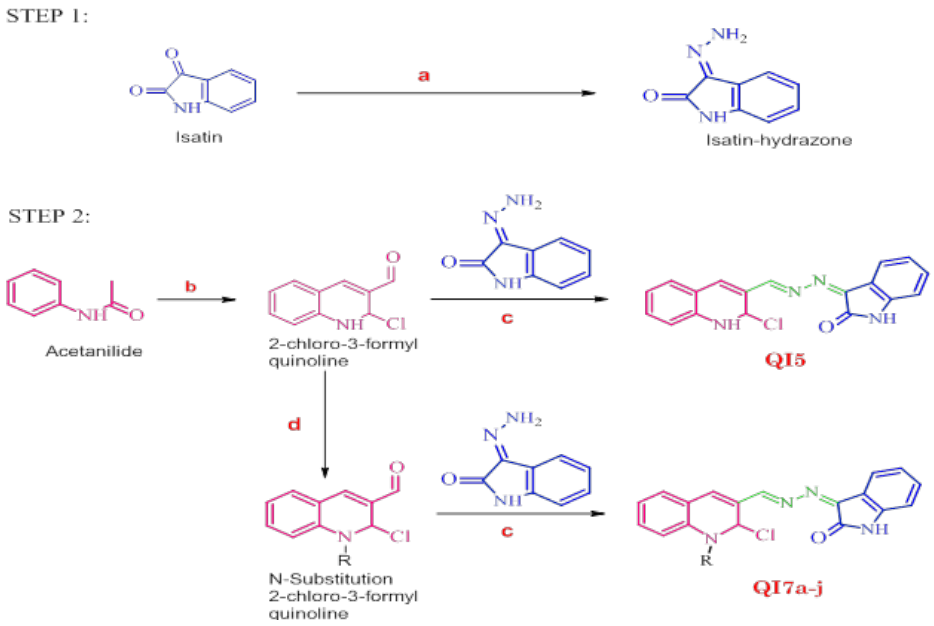
MATERIAL AND METHODS

All compounds were designed using ChemSketch software. Molecular docking analyses of the developed molecules were carried with Molinspiration and Swiss ADME employed to evaluate" drug-likeness ,drug absorption and distribution characteristics parameters.

Experimental section

Design of Compounds

Considering the benefit offered by this hybridization technique in tackling tuberculosis, and based on a comprehensive literature review and assessment of synthetic feasibility, the scaffolds Quinoline and Isatin were selected and designed for further investigation on this research work. (scheme1 and table 1)



Scheme 1: Synthetic route for target quinoline-isatin conjugates QI5 and QI7a-j

Reagents and conditions

a) Ethanol hydrazine hydrate (99%) was heated for 10 min. **b)** DMF, $POCl_3$ 80-90°C reflux 4-16 hrs. **c)** Ethanol, glacial acetic acid, reflux for 2-4 hrs. **d)** DMF, $K2CO_3$, R-X (alkyl/aryl halide), reflux for 3-4 hrs**.**

In silico Studies

Molecular docking is a method that investigates the compatibility of different molecular structures, including drugs, enzymes, or proteins, to gain insights into their interactions and how they fit together. Docking is a molecular modeling technique used to predict the interactions between proteins (enzymes) and small molecules (ligands). The main goal of this approach is to identify the precise locations of ligands within a protein's binding pocket and evaluate the strength of the interactions between the ligand and the protein.[21]

Protein Preparation

This study employed AUTODOCK 1.5.7 to carry out Molecular Docking analysis. Crystallographic structures of the target molecules were saved in a standard 3D format after being taken from the Protein Data Bank (PDB). The Multiple Target of Mycobacterium tuberculosis-InhA protein complexed with NADH (PDB ID: 4DRE) was

the main focus of this investigation. *BioA (DAPA synthase). (PDB-ID-6GE8), DNA gyrase.* (PDB-ID: 3M4I)**.** [22] The active sites of the target proteins were identified using the online Pdbsum online tool.[23] Before initiating the docking procedure, the water molecules and heteroatoms were removed from the target protein.

Ligand Preparation

Marvin sketch/chem Sketch software was used to draw the structure of the Quinoline-isatin hybrids, and a 3D structure was generated and saved in PDB format.[24]

Grid Generation

Utilizing a technique called grid box generation, which functions in three dimensions (3D), molecules are systematically arranged within a cubic grid. This method evaluates interaction energies at each grid point using a predetermined probe. A 3D grid box of 60 × 60 × 60 in with a spacing of 0. A total of 375 points were established, specifying the (x, y, z) coordinates. The docking parameters for the x-, y-, and z-axes are defined as 4. 787, 64. 876, and 56. 214, respectively. The AutoGrid application played a crucial role in selecting macromolecules and ligands in the PDBQT format. Subsequently, a grid parameter file (GPF) was generated, and the associated grid log file (GLG) from AutoGrid was executed.[25, 26]

Docking

Auto Dock Tools is an established program for automated docking known for its effectiveness in conformation searches, especially for ligands with approximately 10 rotatable bonds. The protein and ligand were chosen from the selections provided by AutoGrid and subjected to a docking process. This involved conducting 25 runs of the Genetic Algorithm (GA), which produced a docking parameter file (DPF) and log file for docking (DLG). In order to record the final docked coordinates,AUTODOCK in a docking log file (DLG). [26]

Docking analysis

This process is crucial in drug discovery as it helps predict the binding affinity and mode of interaction between a ligand (small molecule) and a protein receptor is analysed by Discovery Studio of the docked compounds.[27]

Evaluation of Lipinski's rule of five

The Swiss ADME online tool was used to assess the developed compounds' drug-likeness characteristics and Lipinski's rule of five. Various properties, such as TPSA, number of rotatable bonds, log p, molecular weight, hydrogen bond acceptor, and donor were predicted. This rule is applied to assess whether a compound meets specific physicochemical criteria that favor oral absorption.[28]

***in silico* ADME Prediction**

The Swiss ADME online program was used for ADME screening in order to forecast the physicochemical and pharmacokinetic, Lipophilicity and Water solubility of Quinoline-Isatin conjugate derivatives. Whether the compound inhibits cytochrome P450 could also be envisaged. Other attributes including skin penetration, blood-brain barrier penetration, gastrointestinal absorption, and Lipinski, Ghose, and Veber-like drug-likeness properties, can also be predicted.29

Bioactivity

The Molinspiration online platform was used to analyze ion channels, nuclear ligand receptors, proteases, tyrosine kinase-linked receptors, enzyme inhibitors, and G-protein-coupled receptors[30].

Toxicity Assessment

The toxicity profile including mutagenicity, tumorigenicity, irritancy, and reproductive effects of the designed N-Substituted quinoline-isatin hybrids were predicted using Osiris property explorer.31

Antimicrobial Study

The antibacterial activities of the synthesized compounds against Gram-positive (*Staphylococcus aureus and Enterococcus faecalis)* and gram-negative (*Escherichia coli*) bacteria were investigated *in vitro* using the agar well diffusion method.

Media used

For antibacterial screening, nutrient media gelled with 2% agar (bacteriological grade) was utilized. The following components are present in the nutritional media:

Nutrient broth: 13 g/l

Agar: 15 g/l

Final pH at 25°C: 7.4 ± 0.2

Media Preparation and Sterilization

Heat was used to dissolve the components in distilled water, then diluted acid or alkali was used to bring the pH down to 7.4 (±0.2). 20–25 milliliters of nutritional After being moved to Petri plates, the medium was sealed. The medium was autoclaved for 15 psi (121°C) of pressure. 15 min. min (approximately 25 min), respectively.

Microorganisms used: *Gram-negative Escherichia coli, gram-positive Staphylococcus aureus, and gram-positive Enterococcus faecalis.*

Test drug: Synthesized compound used **QI7f**
Standard drug: Gentamicin 10 mg/ml
Concentration used: 2 mg/ml,4 mg/ml, 6 mg/ml and ,10 mg/ml
Control: DMSO
Solvent used: DMSO

Method: Agar well diffusion method

Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* and *Enterococcus faecalis* were used to evaluate the in vitro antibacterial activity of the synthesized compounds using the agar well diffusion method. The synthesized compounds were tested at concentrations of 2, 4, 6, and 10 mg/mL. Compounds that produced zones of inhibition greater than 15 mm were selected for further quantitative evaluation.

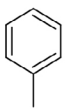
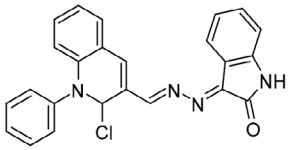
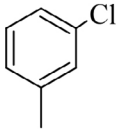
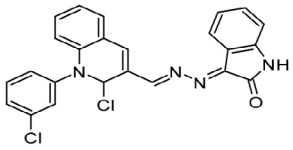
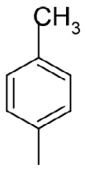
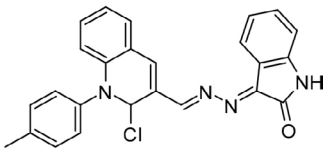
Nutrient agar plates were prepared aseptically and dried at 37°C before inoculation. Gram-negative *Escherichia coli*, Gram-positive *Staphylococcus aureus*, and Gram-positive *Enterococcus faecalis* were inoculated by removing excess inoculum, evenly spreading the bacterial suspension over the agar surface, and allowing the plates to dry at room temperature. Wells were then prepared in the agar, and the test compound (QI7f) was dispensed into the wells. The plates were incubated at 37°C for 18–24 h. The zones of inhibition produced by the test compound were measured and compared with those produced by the standard drug gentamicin (10 mg/mL), following the experimental protocol[32].

RESULTS AND DISSCUSSION

Design of Compounds

A Series of quinolone-isatin conjugate derivatives were desgined successfully. (scheme1 and table1)

Table 1: Structure and scheme details of designed compounds

Compound Code	-R Structure	Hybrid compounds	IUPAC name
QI7a			3-[(E)-2-[(2-Chloro-1-Phenyl-1,2-Dihydroquinolin-3-Yl) Methylidene] Hydrazine
QI7b			3E)-3-[(E)-2-{[2-Chloro-1-(3-Chlorophenyl)-1,2-Dihydroquinolin -3-Yl]Methylidene}Hydrazin-1-Ylidene]-2,3-Dihydro-1H-Indol-2-One
QI7c			3E)-3-[(E)-2-{[2-Chloro-1-(4-Methylphenyl)-1,2-Dihydroquinolin -3-Yl]Methylidene}Hydrazin-1-Ylidene]-2,3-Dihydro-1H-Indol-2-One

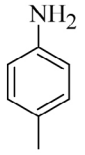
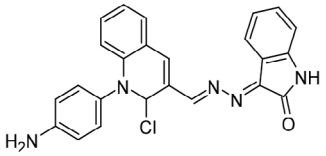
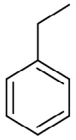
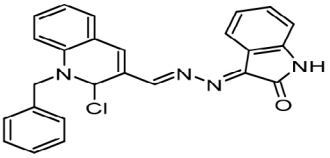
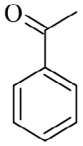
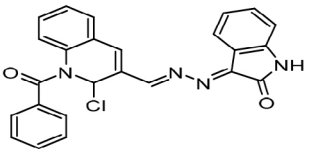
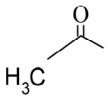
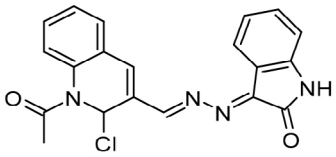
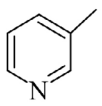
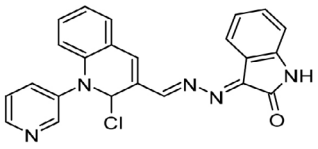
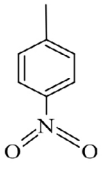
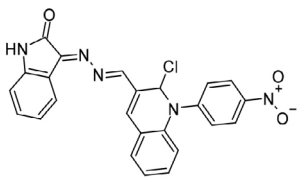
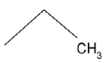
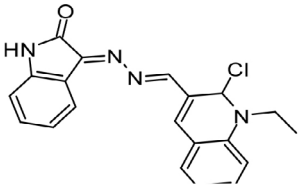
QI7d			(3E)-3-[(E)-2-{[1-(4-Aminophenyl) -2-Chloro-1,2-Dihydroquinolin-3-Yl] Methylidene}Hydrazin-1-Ylidene]-2,3-Dihydro-1H-Indol-2-One
QI7e			(3E)-3-[(E)-2-[(1-Benzyl-2-Chloro-1,2-Dihydroquinolin-3-Yl) Methylidene]Hydrazin-1-Ylidene] -2,3-Dihydro-1H-Indol-2-One
QI7f			3E)-3-[(E)-2-[(1-Benzoyl-2-Chloro-1,2-Dihydroquinolin-3Yl) Methylidene]Hydrazin-1-Ylidene] -2,3-Dihydro-1H-Indol-2-One
QI7g			3E)-3-[(E)-2-[(1-Acetyl-2-Chloro-1,2-Dihydroquinolin-3-Yl) Methylidene]Hydrazin-1-Ylidene] -2,3-Dihydro-1H-Indol-2-One
QI7h			3E)-3-[(E)-2-{[2-Chloro-1-(Pyridin-3-Yl)-1,2-Dihydroquinolin-3-Yl] Methylidene}Hydrazin-1-Ylidene] -2,3-Dihydro-1H-Indol-2-One
QI7i			3E)-3-[(E)-2-{[2-Chloro-1-(4-Nitrophenyl)-1,2-Dihydroquinolin-3-Yl] Methylidene}Hydrazin-1-Ylidene]-2,3-Dihydro-1H-Indol-2-One
QI7j			(3E)-3-[(E)-2-[(2-Chloro-1-Ethyl-1,2-Dihydroquinolin-3-Yl) Methylidene] Hydrazin-1-Ylidene]-2,3-Dihydro-1H-Indol-2-One

Table 2: Molecular interaction of designed ligand with *Mycobacterium tuberculosis* InhA (enoyl-ACP reductase) in associates with NADH. PDB-ID: 4DRE

Compound Code	No. of hydrogen bond formed	H-bond interaction	H-bond distance Å	Binding Energy (kcal/mol)
QI5	1	Val65	2.194	-8.49
QI7a	2	Tyr 158, Ile194	1.905, 1.786	-9.44
QI 7b	2	Tyr 158, Ile194	1.802, 2.055	9.91
QI7c	2	Gly14, Thr39	2.169, 2.072	-9.28
QI7d	2	Try158, Ile194	1.953,2.206	-9.41
QI7e	2	Try158,	1.919, 1.938	-10.13
QI7f	1	Ile194	1.923	-10.35
QI7g	1	Ile194	1.772	-9.29
QI7h	1	Try158	1.921	-9.15
QI7i	3	Ile21, Try158	1.731, 2.232	-9.71
QI7j	1	Ala22	2.076	-9.32
Isoniazid	2	Ile194, Val65	1.769, 2.104	-5.66

Table 3: Molecular interaction of synthesized substances in the active region of the protein *Mycobacterium tuberculosis* BioA (DAPA synthase)(6GE8)

Compound Code	No. of hydrogen bond formed	H-bond interaction	H-bond distance Å	Binding Energy (kcal/mol)
QI5	1	Lys283	2.067	-9.10
QI7a	2	Thr318,Phe319	2.248, 2.226	-11.72
QI7b	1	Phe319	2.139	-10.85
QI7c	1	Ser123	1.804	-1.28
QI7d	1	Phe319	2.187	-11.74
QI7e	2	Lys283, Thr318	2.21, 1.979	-11.25
QI7f	1	Lys283	2.174	-10.92
QI7g	1	Ser125	1.882	-10.18
QI7h	3	Thr318, Phe319, Thr318	2.038, 1.961, 1.9089	-11.82
QI7i	2	Lys283, Leu292	1.993, 2.071	-12.38
QI7j	3	Thr318, Phe319, Thr318	2.015, 2.240, 2.094	-10.98
Isoniazid	3	Lys283, Thr318, Thr318	1.952, 1.827, 2.492	-6.05

Molecular Docking Study

The docking studies were performed for all the designed compounds using AUTODOCK software version 1.5.7. to predict the interaction between the designed ligands and the targeted enzymes - Enoyl-ACP reductase (InhA), BioA (DAPA synthase), DNA gyrase (table 2, table 3 and table 4.)

Enoyl-ACP Reductase (InhA): The binding energies of the synthesized compounds ranged from –8.49 to –10.35 kcal/mol. Among them, compound QI7f exhibited the highest binding affinity (–10.35 kcal/mol) and formed a single hydrogen bond with the amino acid residue Ile194 at an interatomic distance of 1.923 Å.

Compared with the standard drug isoniazid, which exhibited a binding affinity of –5.66 kcal/mol, the standard formed two hydrogen bonds with Ile194 and Val65 at bond distances of 1.769 Å and 2.104 Å, respectively. The results showed that the amino

Table 4: Molecular interaction of ligand compounds in active region of the protein *Mycobacterium tuberculosis* DNA gyrase

Compound Code	No. of hydrogen bond formed	H-bond interaction	H-bond distance A0	Binding Energy (kcal/mol)
QI5	2	Ala531, Ser541	2.213, 1.962	-8.11
QI7a	1	Ser541	2.073	-7.55
QI7b	1	Ser541	2.126	-8.10
QI7c	1	Gln565	2.237	-7.76
QI7d	1	Gln565	2.093	-8.68
QI7e	2	Ala531, Ser541	1.779, 2.189	-7.99
QI7f	1	Gln565	2.048	-8.93
QI7g	1	Gln565	1.907	-8.33
QI7h	1	Ala531	1.972	-7.59
QI7i	1	Ser541	2.009	-7.51
QI7j	1	Ser541	2.0	-7.61
Moxifloxacin	2	Ser541, Gln565	1.866, 1.853	-6.87

Table 5: Lipinski's Rule of Five Analysis using SwissADME

Compound code	Molecular weight	Molecular formula	TPSA	HBA	HBD	RB	LOGP
QI5	316.36	$C_{19}H_{16}N_4O$	65.85 Å²	3	2	2	2.45
QI7a	414.89	$C_{24}H_{17}ClN_4O$	57.06 Å²	3	1	3	2.88
QI7b	449.33	$C_{24}H_{17}Cl_2N_4O$	57.06 Å²	3	1	3	3.3
QI7c	428.91	$C_{25}H_{21}ClN_4O$	57.06 Å²	3	1	3	3.64
QI7d	429.9	$C_{24}H_{20}ClN_5O$	83.08 Å²	3	2	3	2.8
QI7e	426.9	$C_{25}H_{19}ClN_4O$	57.06 Å²	3	1	4	3.3
QI7f	440.88	$C_{25}H_{17}ClN_4O2$	74.13 Å²	4	1	4	2.84
QI7g	380.83	$C_{20}H_{17}ClN_4O$	74.13 Å²	4	1	3	2.6
QI7h	415.87	$C_{23}H_{18}ClN_5O$	69.95 Å²	4	1	3	3
QI7i	457.87	$C_{24}H_{16}ClN_5O_3$	102.88 Å²	5	1	4	2.68
QI7j	366.84	$C_{20}H_{19}ClN_4O$	57.06 Å²	3	1	3	2.89

Table 6: Drug-likeness evaluation of the designed compounds using SwissADME

Compound code	Lipinski	Ghose	Veber	Egan	Muegge	Bio-availability score
QI5	yes	yes	yes	yes	yes	0.55
QI7a	yes	yes	yes	yes	yes	0.55
QI7b	yes	no	yes	yes	No	0.55
QI7c	yes	no	yes	yes	yes	0.55
QI7d	yes	no	yes	yes	yes	0.55
QI7e	yes	no	yes	yes	no	0.55
QI7f	yes	no	yes	yes	yes	0.55
QI7g	yes	yes	yes	yes	yes	0.55
QI7h	yes	yes	yes	yes	yes	0.55
QI7i	yes	no	yes	yes	yes	0.55
QI7j	yes	yes	yes	yes	yes	0.55

Table 7: In silico pharmacokinetic analysis of the designed compounds using SwissADME

Compound code	GI	BBB	P-gp	CYP1A2	CYP2D6	CYP3A4	Log Kp(cm\s)
QI5	High	Yes	No	Yes	No	Yes	-6.04
QI7a	High	Yes	No	Yes	No	Yes	-5.54
QI7b	High	Yes	No	Yes	No	Yes	-5.31
QI7c	High	Yes	No	Yes	No	Yes	-5.37
QI7d	High	Yes	No	Yes	No	Yes	-6.12
QI7e	High	Yes	No	No	No	Yes	-5.32
QI7f	High	Yes	No	No	No	Yes	-5.83
QI7g	High	Yes	No	No	No	Yes	-6.98
QI7h	High	Yes	No	Yes	No	Yes	-6.31
QI7i	High	No	No	No	No	Yes	-5.94
QI7j	High	Yes	No	Yes	No	Yes	-6.10

Table 8: Lipophilicity and water solubility of designed compounds using swiss adme software

Software	Lipophilicity log p (o/w)					Water solubility (logs)			
Compound Code	I log p	X logp3	W log p	M log p	Silics -IT	Cons. Log p	E sol	Ali	Silicos-IT
QI5	2.45	3.09	2.06	2.13	3.68	2.68	-3.99	-4.14	-6.30
QI7a	3.47	4.63	4.63	3.29	4.44	3.98	-5.43	-5.55	-6.91
QI7b	3.77	5.26	4.73	3.76	5.08	4.52	-6.03	-6.21	-7.50
QI7c	3.64	4.99	4.39	3.49	4.96	4.29	-5.73	-5.93	-7.29
QI7d	2.80	3.95	3.67	2.74	3.72	3.38	-5.08	-5.39	-6.54
QI7e	3.30	5.05	3.87	3.42	5.17	4.16	-5.83	-5.99	-8.65
QI7f	3.37	5.68	4.49	3.55	5.40	4.50	-5.53	-5.73	-8.19
QI7g	2.60	2.31	2.42	1.90	3.33	2.51	-3.62	-3.51	-4.76
QI7h	3.00	3.56	3.47	2.26	3.87	3.23	-4.76	-4.71	-6.54
QI7i	2.68	4.94	4.58	2.50	3.00	3.54	-5.93	-6.84	8.08
QI7j	2.89	3.44	2.89	2.38	3.79	3.08	-4.25	-4.32	-5.23

Table 9: Predicted bioactivity of the designed compounds using Molinspiration

Compound code	GPCR Ligand	Ion channel Modulator	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme inhibitor
QI5	-0.49	-0.68	-0.25	-0.78	-0.92	-0.34
QI7a	-0.33	-0.51	-0.13	-0.56	-0.75	-0.20
QI7b	-0.33	-0.50	-0.13	-0.57	-0.78	-0.24
QI7c	-0.35	-0.55	-0.16	-0.57	-0.77	-0.24
QI7d	-0.28	-0.44	-0.05	-0.59	-0.65	-0.12
QI7e	-0.31	-0.49	-0.19	-0.55	-0.68	-0.22
QI7f	-0.33	-0.52	-0.18	-0.60	-0.68	-0.26
QI7g	-0.41	-0.64	-0.29	-0.76	-0.80	-0.32
QI7h	-0.23	-0.43	0.03	-0.61	-0.67	-0.15
QI7i	-0.41	-0.50	-0.23	-0.58	-0.79	-0.26
QI7j	-0.39	-0.62	-0.26	-0.68	-0.85	-0.31

Table 10: In silico toxicity prediction of the designed compounds

Compound code	Mutagenic effect	Tumorigenic Effect	Irritant Effect	Reproductive Effect
QI5				
QI7a				
QI7b				
QI7c				
QI7d				
QI7e				
QI7f				
QI7g				
QI7h				
QI7i				
QI7j				

Low Risk - High-Risk

Table 11: Evaluation of antimicrobial activity of the synthesized compound QI7f

Concentration of Compound QI7f	Zone of inhibition (mm) *Escherichia Coli*	*Staphylococcus aureus*	*Enterococcus Faecalis*
2mg/ml	12mm	16mm	18mm
4mg/ml	13mm	17mm	19mm
6mg/ml	14mm	18mm	20mm
10mg/ml	15mm	19mm	22mm
Standard Gentamicin 10 mg/ml	28	25	27

acid residue Ile194 was involved in interactions with both the test compound and the standard drug, suggesting a similar binding mode and supporting the reliability of the molecular docking results.

BioA: 7,8-Diaminopelargonic acid (DAPA) synthase

The binding energies of all the designed compounds ranged from –9.10 to –12.38 kcal/mol. Compound QI7i exhibited the best binding affinity (–12.38 kcal/mol) and formed two hydrogen bonds with the residues Lys283 and Leu292 at interatomic distances of 1.993 Å and 2.071 Å, respectively. In comparison, the standard drug isoniazid showed a binding affinity of –6.05 kcal/mol and formed three hydrogen bonds with Lys283, Thr318(A), and Thr318(B) at distances of 2.492 Å, 1.952 Å, and 1.827 Å, respectively. The results indicated that the amino acid residue Lys283 was involved in interactions with both the test compound and the

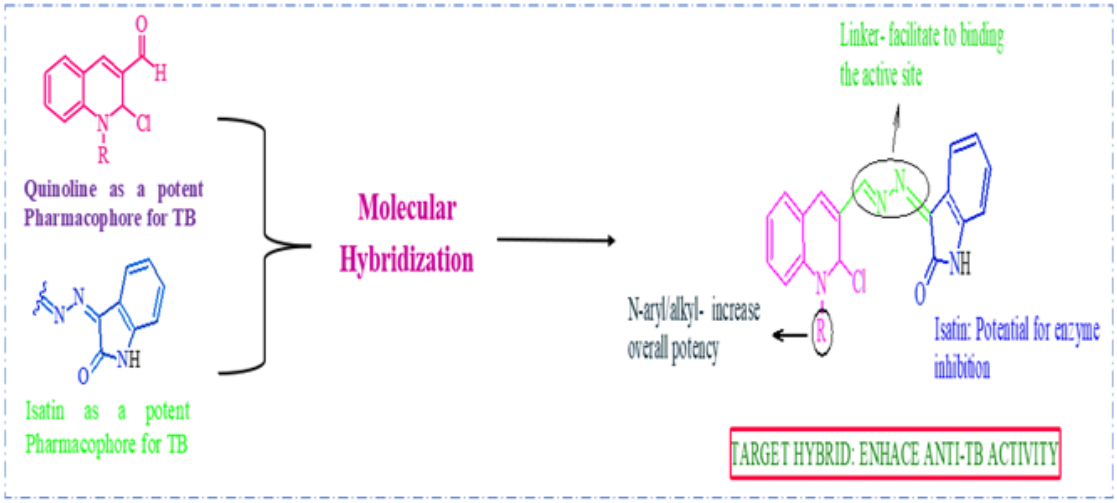


Fig. 1: Design strategy of target molecule

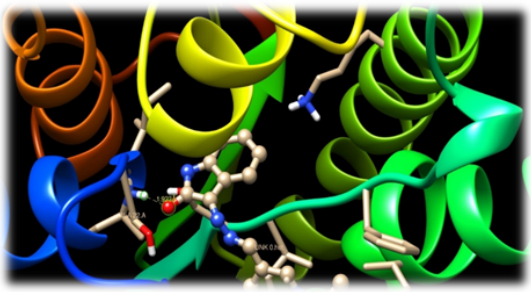


Fig. 2: 3D Binding mode of Compound QI7f in the active site of *Eonyl-ACP reductase* (InhA), along with interacting amino acid viewed through Chimera 1.17 software

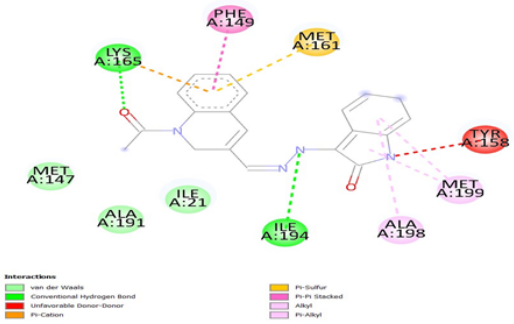


Fig. 3: 2D Binding interaction image of Compound QI7f in the active site of Eonyl-ACP reductase (INHA), along with interacting amino acid viewed through Discovery Studio

standard drug, suggesting a similar binding pattern. DNA gyrase The binding energies of all the designed compounds ranged from –7.51 to –8.93 kcal/mol. Compound QI7f exhibited the best binding affinity (–8.93 kcal/mol) and formed a hydrogen bond with the amino acid residue Gln565 at an interatomic distance of 2.048 Å.

In comparison, the standard drug moxifloxacin showed a binding affinity of –5.66 kcal/mol and formed two hydrogen bonds with Gln565 and Ser541 at distances of 1.866 Å and 1.853 Å, respectively. The results indicated that the amino

BINDING ENERGY: -10.80;
H-BOND INTERACTION: LysA:165, Ile A:194;
H-BOND DISTANCE A^0: 2.169, 2.072

acid residue Gln565 was involved in interactions with both the test compound and the standard drug, suggesting a similar binding pattern.

Evaluation of Lipinski's Rule of Five

The Designed compounds' molecular properties revealed partition coefficient values ranging from 2.45 to 3.64, which suggested they were naturally lipophilic and therefore changed the bioavailability and pharmacokinetic. The molecules

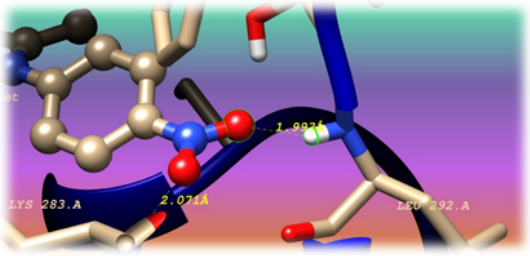


Fig. 4: 3D Binding mode of Compound QI7i in the active site of BioA 7,8-diamino-pelargonic acid (DAPA) synthase along with interacting amino acid viewed through Chimera 1.17 software

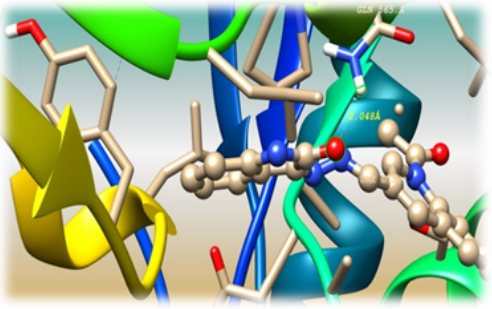


Fig. 6: 3D Binding mode of Compound QI7f in the active site of *DNA-Gyrase* along with interacting amino acid viewed through Chimera 1.17 software

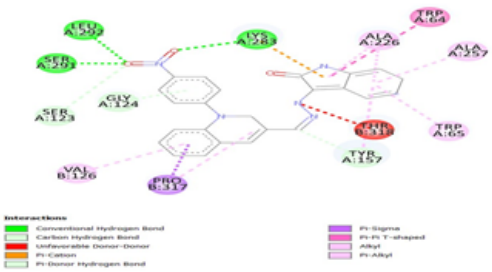


Fig. 5: 2D Binding interaction image of Compound QI7i in the active site of Eonyl-ACP reductase (INHA), along with interacting amino acid viewed through Discovery Studio software.

Binding energy: –12.38 kcal/mol

Hydrogen bond interactions: Lys283 and Leu292

Hydrogen bond distances: 1.993 Å and 2.071 Å

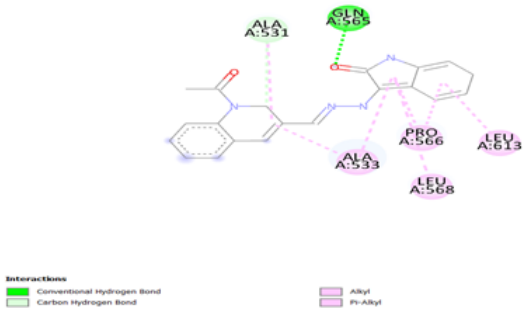


Fig. 7: 2D Binding interaction image of Compound QI7f in the active site of DNA-Gyrase along with interacting amino acid viewed through Discovery Studio software

Binding energy: –8.93 kcal/mol

Hydrogen bond interaction: Gln565

Hydrogen bond distance: 2.048 Å

that are responsible for bioavailability range from strongly to moderately polar, according to the TPSA values, which range from 57.06 Å² to 102.88 Å². The compounds' molar weights range from 352.40 to 405.29 Da. which is smaller than 500 Da indicates that the molecules are more readily absorbed, distributed, and generally bioavailable, making them rapidly soluble and eliminated. The Lipinski Rule of Five states that compounds having flexible linkages, H-bond donors, and H-bond acceptors are within the permissible range for oral delivered medications. When the number of rotatable bonds is within a specific threshold, typically fewer than 10, it indicates that the molecule may still possess satisfactory bioavailability, as they were found to be within the acceptable range. The H bond donors and acceptors have a major impact on oral bioavailability, permeability, and solubility, which results in decreased effectiveness together result in good absorption therefore the compounds shows good bioavailability. The evaluation's findings are listed in Table5.

Evaluation of drug-likeness

The assessment of drug-likeness, including the bioavailability and the Lipinski, Ghose, Veber, and Muegge rules score, indicates that all the designed compounds comply with these criteria and fall within the bioavailability score of 0.055 (Table 6).

***In silico* ADME Prediction**

The ADME characteristics of the

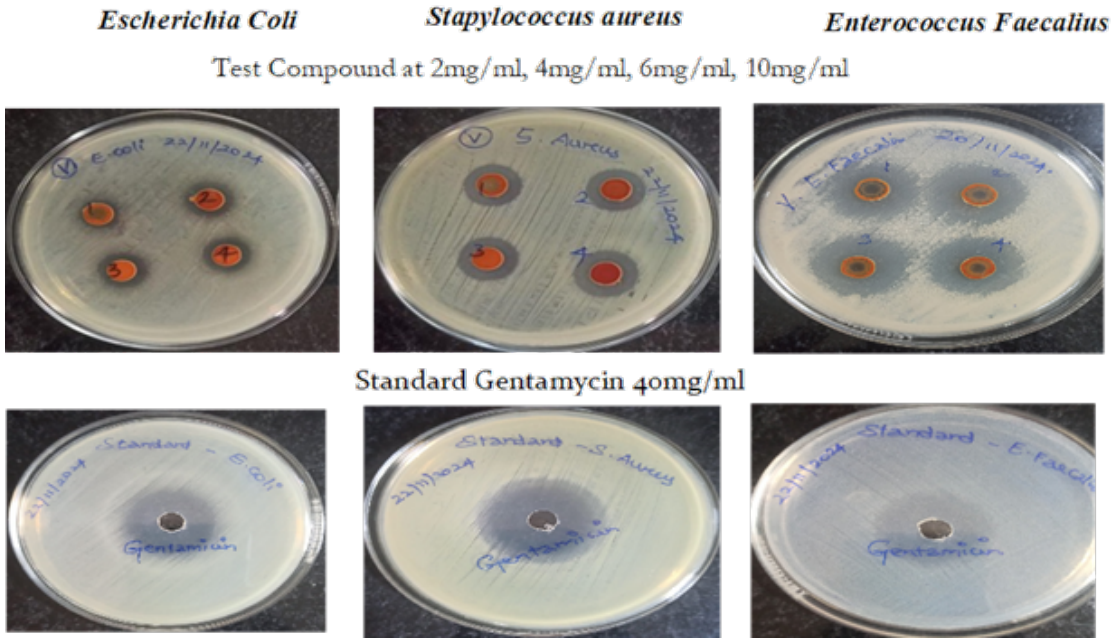


Fig. 8: Antibacterial activity of compound QI7f against *E. coli, S. aureus and E. faecalis*

compounds that were evaluated using the SWISS ADME software. The compounds that were designed exhibited a high gastrointestinal absorption. All compounds developed are less likely to be transported by P-glycoprotein, which may result in improved absorption and distribution. For a drug to be both effective and safe, it should exhibit minimal interactions with cytochrome P450 (CYP) enzymes to prevent drug-drug interactions and potential toxicity. All developed quinolone-isatin conjugates are unlikely to inhibit CYP1A2 and CYP2D6, indicating that they are expected to have a negligible effect on the metabolism of drugs that are substrates for these enzymes. Table 7 presents the results of this assessment**.**

Lipophilicity and water solubility

The lipophilicity and solubility of a compound play a vital part in determining its absorption and bioavailability. The data revealed that each of the developed compounds could successfully penetrate biological membranes and demonstrated moderate solubility. (Table 8)

Bioactivity

The observed range of GPCR ligand values fell within the moderately active category (-0. 23 to 0. 49), suggesting minimal interactions. GPCRs may function as potent antagonists or weak agonists. Similarly, significant interactions were noted between enzyme and kinase inhibition. In contrast, ion channel receptors, nuclear receptor ligands, and protease modulators exhibited moderate to low bioactive effects, indicating that these compounds also demonstrated weak inhibitory properties. Table 9 presents the results of this assessment.

Toxicity Properties

The OSIRIS server serves as an online tool for forecasting toxicity parameters. The findings indicated that all the designed hybrids were falls on Low-risk in terms of Mutagenic, Tumorogenic, Irritant, and Reproductive effects, except for **hybrid QI7d,** which exhibited toxicity in these areas and falls on High-risk. This information is illustrated in the accompanying table 10, where the green circle denotes nontoxicity.

Evaluation of *In Vitro* Antibacterial Activity

Based on the binding energy results, the synthesized compound QI7f was selected for evaluation of its in vitro antibacterial activity against both Gram-negative (Escherichia coli) and Gram-positive (Staphylococcus aureus and Enterococcus faecalis) pathogens. The antibacterial activity was assessed using the well diffusion method at four

concentrations (2, 4, 6, and 10 mg/mL). Among the tested concentrations, QI7f exhibited significant antibacterial activity at 10 mg/mL, producing inhibition zones of 22 mm against Enterococcus faecalis, 15 mm against *Escherichia coli,* and 19 mm against *Staphylococcus aureus*. The results are presented in Table 11 and Figure 8 and were compared with the standard drug gentamicin at a concentration of 10 mg/mL.

CONCLUSION

In this study, quinoline–isatin conjugate derivatives were designed based on SAR analysis and literature surveys, followed by molecular docking studies. The molecular docking results revealed that all the designed compounds exhibited good binding affinities compared with the standard drug. Among the synthesized derivatives, compound QI7f showed the highest binding affinity against the target enzyme Enoyl-ACP reductase (InhA) with a binding energy of –10.23 kcal/mol. Compound QI7i exhibited the strongest binding affinity against BioA (7,8-diaminopelargonic acid [DAPA] synthase) with a binding energy of –12.38 kcal/mol, while compound QI7f showed the highest binding affinity against DNA gyrase with a binding energy of –8.38 kcal/mol. These findings indicate that the designed compounds possess promising binding energies and ligand efficiencies against the selected target enzymes. Furthermore, compounds QI7f and QI7i exhibited low toxicity risk profiles and were predicted to possess good oral absorption and bioavailability characteristics, as evaluated using OSIRIS Property Explorer and SwissADME, respectively. Therefore, these compounds may serve as potential lead molecules for further structural optimization and development of novel anti-tubercular agents. Based on the molecular docking results, the promising compound QI7f was further evaluated for in vitro antimicrobial activity. Compound QI7f demonstrated significant antimicrobial effects, producing inhibition zones of approximately 19 mm against *Staphylococcus aureus,* 22 mm against *Enterococcus faecalis*, and 15 mm against *Escherichia coli* at a concentration of 10 mg/mL. These results were compared with the standard drug gentamicin at the same concentration. The antimicrobial activity results are presented in Table 11 and Figure 8. Overall, compounds QI7f and QI7i showed considerable potential as lead candidates for the development of novel anti-tubercular agents.

Conflict of Interest

Regarding the manuscript's publication, the author states that there are no conflicts of interest. All of the study's investigations and conclusions are based solely on scientific data, and no financial or personal relationships have influenced the result or conclusions drawn.

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REFERENCES

1. Moule, M. G., & Cirillo, J. D., Mycobacterium tuberculosis Dissemination Plays a Critical Role in Pathogenesis. *Frontiers in Cellular and Infection Microbiology*, **2020**; *10*.
2. Barter, D. M., Agboola, S. O., Murray, M. B., & Bärnighausen, T., Tuberculosis and poverty: the contribution of patient costs in sub-Saharan Africa – a systematic review. *BMC Public Health*, **2012b***; 12*(1).
3. Song, H., Tian, J., Song, H., Guo, S., Lin, Y., & Pan, J., Tracking multidrug resistant tuberculosis: a 30-year analysis of global, regional, and national trends. *Frontiers in Public Health*, **2024**; *12*.
4. *Tuberculosis deaths and disease increase during the COVID-19 pandemic*. PAHO/WHO | Pan American Health Organization. https://www.paho.org/en/news/27-10-2022-tuberculosis-deaths-and-disease-increase-during-covid-19-pandemic, **2022**.
5. Bakare, A. A., Moses, V. Y., Beckely, C. T., Oluyemi, T. I., Ogunfeitimi, G. O., Adelaja, A. A., Ayorinde, G. T., Gbadebo, A. M., Fagbenro, O. S., Ogunsuyi, O. I., Ogunsuyi, O. M., & Ige,

O. M., The first-line antituberculosis drugs, and their fixed-dose combination induced abnormal sperm morphology and histological lesions in the testicular cells of male mice. *Frontiers in Cell and Developmental Biology*, **2022**; *10*.

6. Sarathy, J. P., Gruber, G., & Dick, T., Re-Understanding the mechanisms of action of the Anti-Mycobacterial drug bedaquiline. *Antibiotics*, **2019**; *8*(4), 261.
7. Abdelrahman, M. A., Almahli, H., Al-Warhi, T., Majrashi, T. A., Abdel-Aziz, M. M., Eldehna, W. M., & Said, M. A., Development of Novel Isatin-Tethered Quinolines as Anti-Tubercular Agents against Multi and Extensively Drug-Resistant Mycobacterium tuberculosis. *Molecules*, **2022**; *27*(24), 8807.
8. Zampieri, D., & Mamolo, M. G., Hybridization Approach to Drug Discovery Inhibiting Mycobacterium tuberculosis-An Overview. *Current Topics in Medicinal Chemistry*, **2020**; *21*(9), 777–788.
9. Singh, P., Jaiyeola, B., Kerru, N., Ebenezer, O., & Bissessur, A., A review of recent advancements in anti-tubercular molecular hybrids. *Current Medicinal Chemistry*, **2017**; *24*(37).
10. Panchal, N. B., & Vaghela, V. M., From molecules to medicine: the remarkable pharmacological odyssey of quinoline and it's derivatives. *Oriental Journal of Chemistry*, **2023**; *39*(3), 546–567.
11. Tabassum, R., Ashfaq, M., & Oku, H., Current pharmaceutical aspects of synthetic quinoline derivatives. *Mini-Reviews in Medicinal Chemistry*, **2020**; *21*(10), 1152–1172.
12. Yadav, P., & Shah, K., Quinolines, a perpetual, multipurpose scaffold in medicinal chemistry. *Bioorganic Chemistry*, **2021**; *109*, 104639.
13. Ajani, O. O., Iyaye, K. T., & Ademosun, O. T., Recent advances in chemistry and therapeutic potential of functionalized quinoline motifs – a review. *RSC Advances*, **2022**; *12*(29), 18594–18614.
14. Elebiju, O. F., Ajani, O. O., Oduselu, G. O., Ogunnupebi, T. A., & Adebiyi, E., Recent advances in functionalized quinoline scaffolds and hybrids—Exceptional pharmacophore in therapeutic medicine. *Frontiers in Chemistry*, **2023**; *10*.
15. Kaur, R., & Kumar, K., Synthetic and medicinal perspective of quinolines as antiviral agents. *European Journal of Medicinal Chemistry*, **2021**; *215*, 113220. https://doi.org/10.1016/j.ejmech.2021.113220
16. Ökten, S., Aydın, A., Koçyi₃it, Ü. M., Çakmak, O., Erkan, S., Andac, C. A., Taslimi, P., & Gülçin, ₃. (n.d.). Quinoline-based promising anticancer and antibacterial agents, and some metabolic enzyme inhibitors. *Archiv Der Pharmazie*, **2021**; *353*(9).
17. Suvaiv, N., Singh, K., Kumar, P., Hasan, S. M., Kushwaha, S. P., Kumar, A., Ismail, K. S., Mujeeb, S., Maurya, S. K., & Zaidi, S. M. H., Antibacterial potentiality of Isatin-Containing hybrid derivatives. *Asian Journal of Chemistry*, **2023**; *35*(4), 815–827.
18. Belay, Y., Muller, A., Mokoena, F.S. *et al.* 1,2,3-triazole and chiral Schiff base hybrids as potential anticancer agents: DFT, molecular docking and ADME studies. *Sci Rep* **2024**; *14*, 6951.
19. El-Malah, A., Taher, E. S., Angeli, A., Elbaramawi, S. S., Mahmoud, Z., Moustafa, N., Supuran, C.T., & Ibrahim, T. S., Schiff bases as linker in the development of quinoline-sulfonamide hybrids as selective cancer-associated carbonic anhydrase isoforms IX/XII inhibitors: A new regioisomerism tactic. *Bioorganic Chemistry*, **2022**; *131*, 106309.
20. Singh, P., Jaiyeola, B., Kerru, N., Ebenezer, O., & Bissessur, A., A review of recent advancements in anti-tubercular molecular hybrids. *Current Medicinal Chemistry*, **2017b** *; 24*(37).
21. Kitchen, D. B., Decornez, H., Furr, J. R., & Bajorath, J., Docking and scoring in virtual screening for drug discovery: methods and applications. *Nature Reviews Drug Discovery*, **2004**; *3*(11), 935–949.
22. Bank, R. P. D. (n.d.). *Organization of 3D structures in the Protein Data Bank*. https://www.rcsb.org/docs/general-help/organization-of-3d-structures-in-the-protein-data-bank.
23. Team, E. W. (n.d.). *PDBsum home page*. https://www.ebi.ac.uk/thornton-srv/databases/pdbsum.
24. Laxmi, K. L. K., Characterization of Ligand N'-[(1E)-1-phenylethylidene]-1, 3-benzothiazole-2-carbohydrazide by using Marvin Sketch 20.8 Software. *Oriental Journal of Chemistry*,

2022; *38*(1), 77–84.

25. Wilantho, A., Tongsima, S., & Jenwitheesuk, E., Pre-docking filter for protein and ligand 3D structures. *Bioinformation*, **2008**; *3*(5), 189–193.
26. Wang, Y., Guo, Y., Qiang, S., Jin, R., Li, Z., Tang, Y., Leung, E. L. H., Guo, H., & Yao, X., 3D-QSAR, molecular docking, and MD simulations of anthraquinone derivatives as PGAM1 inhibitors. *Frontiers in Pharmacology*, **2021**; *12*.
27. Meng, X., Zhang, H., Mezei, M., & Cui, M., Molecular Docking: a powerful approach for Structure-Based drug discovery. *Current Computer - Aided Drug Design*, **2011**; *7*(2), 146–157.
28. Bayan Abdi, Mona Fekadu and Digafie Zeleke et al. Synthesis and Evaluation of the Antibacterial and Antioxidant Activities of Some Novel Chloroquinoline Analogs. *J Chem.* **2021**; 2021:1-13.
29. Gandhi, P. V., Burande, S. R., Charde, M. S., & Chakole, R. D., Synthesis and Characterization of Isatin-Quinoline Hybrids as Antitubercular Agents. *International Journal of Creative Research Thoughts (IJCRT)*, **2021;** *9(10)*, d348
30. *Shilpa et al., World Journal of Pharmaceutical research 2018*
31. Chodankar, R., & Mahajan, A., Characterization and in-silico toxicity prediction of the oxidative degradation products of Pimozide. *Separation Science Plus*, **2022**; *5*(7), 275–284.
32. Elmongy, E. I., Ahmed, A. a. S., Sayed, I. E. T. E., Fathy, G., Awad, H. M., Salman, A. U., & Hamed, M. A., Synthesis, Biocidal and Antibiofilm Activities of New Isatin–Quinoline Conjugates against Multidrug-Resistant Bacterial Pathogens along with Their In Silico Screening. *Antibiotics*, **2022**; *11*(11), 1507.