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Evaluation of the Molecular properties, Bioactivity Score, Pharmacokinetics and Toxicological Analysis of the Novel Quinazoline-Linked Piperazine Derivatives

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ABSTRACT: Quinazoline and Piperazine derivatives have been revealed to exhibit a broad range of biological effects. As a result, substances having such a scaffold have been exploited as a lead in the design of drugs. As such, the objective of this investigation is to synthesize several quinazoline-linked piperazine analogues and execute in silico evaluations of their molecular properties, bioactivity score, pharmacokinetics, and toxicological analysis. The investigation showed that, apart from one molecular weight, the majority of the compounds fitted Lipinski's rule of five, indicating drug-likeness characteristics. The bioactivity data revealed that the N-(4-oxo-2-(4-((4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) methyl) phenyl) quinazolin- 3(4*H*) - yl) benzamide derivatives were moderately active for GPCR ligand, Ion channel modulator, Kinase inhibitor, Nuclear receptor ligand, Protease inhibitor, and Enzyme inhibitor. The analysis revealed that all derivatives had the highest intestinal absorption, was not Blood Brain Barrier permeable, and was simple to remove because they could not inhibit CYP450 1A2. Using ProtTox-II, it was predicted that none of the synthesized molecules were cytotoxic and hepatotoxic. To tackle diseases with multiple drugs resistance, finding new antimicrobial drugs and improving the efficacy of those already in use through structural modification can be extremely important. The study provides information on the drug likeness, bioactivity scores, pharmacokinetics properties and toxicity of novel synthesized substances that can be employed to design and create novel anti-microbial medications that are more effective and have lower toxicity.

Keywords: Quinazolin, *in silico*, Pharmacokinetic, Molecular Properties, Bioactivity, Toxicity.

INTRODUCTION

The World Health Organization reports that as a result of the development and global spread of novel resistance mechanisms, antibiotic resistance is approaching dangerously high levels in every region of the world. Treatment of even common viral infections like pneumonia has become more challenging, and in some cases impossible, as drug efficacy has diminished (World Health Organization, 2020). While more expensive drugs take the role of first-line antibiotics, patient care is increasingly growing more costly. By 2050, the cost of antibiotic resistance-related deaths might rise to 10 million deaths annually, affecting the world's economy with a total of USD 100 trillion (Global Health, 2023). As bacteria adjust to the constant presence of antibiotics, a complex process is known as antibiotic resistance arises (Navon-Venezia *et al.,* 2017). It's much more challenging to control an infection after bacteria develop resistance to antibiotics. Individuals who get a resistant bacterial infection frequently have longer hospital stays and rising death risks. More effective antibiotics are required than ever before, however, few new ones are being developed. New antibiotics have also shown only limited impact against resistant organisms. Antibiotic resistance needs to be kept at a minimum for antibiotics to continue to be an effective therapy for infections both now and in the future. The number of antimicrobials that can be utilized to treat particular species has been diminished because of this enhanced resistance. For specific classes of microorganisms, new antimicrobial drugs are also essential. Antimicrobials that could treat infections brought on by fungus and mycobacteria are extremely

few. The fight against infectious disease must continue with the development of entirely novel drug classes, drugs with fewer side effects, and treatments with shorter periods of time (OhioLINK Electronic Theses & Dissertations Center, 2013). One of the most challenging worldwide health issues of the twenty-first century is bacterial infection. The issue is made worse by the perception that the emergence of resistant germs poses a severe threat. As a result, in recent years more and more work has gone into finding novel antimicrobials (Pradhan *et al*., 2018).

There are numerous different natural, semi-synthetic, and synthetic antimicrobial agents currently available (Baumann *et al*., 2013). Bioinformatics is crucial for the development of drug candidates, virtual screening approaches of therapeutic properties, and the discovery of novel bioactive molecules in the therapeutic context (Srinivasan *et al.,* 2017). One of these methods is the in silico predictive method of absorption, distribution, metabolism, and excretion (ADME), which aims to promote small molecules as new oral medications by performing high precision and reliable theoretical screening of physicochemical and pharmacokinetic properties (Ndombera *et al.,* 2019; Sliwoski *et al.,* 2014); another method is molecular docking, which simulates intermolecular combinational patterns between target molecules and some other molecules (Maryshyla *et al.,* 2020). The search for novel medications has been reinforced by the proliferation in complex synthetic structures. These difficulties manifest in structural limits or by declining to fit biological targets and producing undesirable side effects (Hopkins *et al.,* 2008; Yamanishi *et al.,* 2012). To identify toxic activity in similarity tests and to enhance the design of compounds with the optimum physicochemical properties for synthesis and expected oral bioavailability, in silico prediction approaches thus generate libraries of molecular substructures (Pires et al., 2018 and Lipinski et al., 2004). The current work illustrates the anticipated drug-like, pharmacokinetics properties, their bioactivities with several classes of biological targets, and toxicity studies in this regard.

With their wide range and diverse biological activity, Quinazoline derivatives, which are N-containing heterocyclic compounds, have expressed concerns throughout the globe. Numerous therapeutic actions of Quinazoline derivatives are currently being identified by researchers, including those against cancer (Al-Rashood *et al.,* 2006; Chandregowda *et al.,* 2009; Vasdev *et al*., 2004; Wakeling *et al.,* 2002), inflammation (Alagarsamy *et al.,* 2007; Baba *et al.,* 1996), bacterial infection (Aly *et al*., 2010; Antipenko *et al.,* 2009; Gupta *et al.,* 2008 and Rohini *et al.,* 2010), pain (Alagarsamy *et al.,* 2007), viral infection (Li *et al.,* 1998), cytotoxin (Chandrika *et al*., 2009), spasm (Gupta *et al*., 2006; Paneersalvam *et al.,* 2010), tuberculosis (Nandy *et al*., 2006), oxidation (Saravanan *et al*., 2010), malaria (Lakhan *et al.,* 1987), hypertension (Hess *et al*., 1968), obesity (Sasmal *et al.,* 2012), psychosis (Alvarado *et al.,* 2006), and diabetes (Malamas *et al.,* 1991). By incorporating additional active groups to the Quinazoline moiety using advanced synthetic techniques, medicinal chemists were capable of developing a diversity of Quinazoline molecules with various biological activities. Besides that, research has been done on the possible uses of Quinazoline derivatives in the disciplines of biology, insecticides, and healthcare.

A six-membered ring called Piperazine, a heterocyclic chemical molecular structure, possesses two nitrogen atoms in the first and fourth positions. Piperazine nuclei tend to occur in compounds that are physiologically active and have been designated as preferred structures. The molecules with a Piperazine nucleus have some of these significant biological properties, including antibacterial, anti-tubercular, anti-psychotic, anti-convulsant, anti-depressant, anti-inflammatory, cytotoxic, anti-malarial, anti-arrhythmic, anti-oxidant, and anti-viral properties (Patel *et al*., 2022; Patil *et al.,* 2019; Somashekhar *et al.,* 2013; Subramaniyan *et al*., 2018; Zhang *et al.,* 2019).

Incorporating two or more active moieties into one is a common modification technique, and it could improve activity and eliminate unexpected side effects (Ahmadi, 2017). Such hybridization is planned to look at how such structural variation affects predicted biological activities. Considering the importance of Quinazoline and Piperazine moieties in the field of medicinal chemistry, it was aimed at synthesizing a unique hybrid molecule that contained derivatives of both compounds to assess its molecular properties, bioactivity, and toxicity. Lead compound optimization and virtual screening studies utilize a conventional computational and experimental drug design to discover new compounds with biological impact (Khan *et al.,* 2019).

MATERIAL AND METHODS

Generation of Molecular Structures and Nomenclature of N-(4-oxo-2-(4-((4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) methyl) phenyl) quinazolin- 3(4*H*)- yl) benzamide derivatives. ChemDraw is an easy-to-use program that enables drawing simple two-dimensional illustrations of organic molecules appealingly and effectively. ChemDraw Professional is a drawing program that allows users to draw chemical entities and biological pathways in addition to chemical structures and reactions. It can be used by users to predict properties, analyze spectra, transform chemical names into IUPAC names, view 3D structures, and more (ChemDraw, 2023). The chemical structures and nomenclature of N-(4-oxo-2-(4-((4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) methyl) phenyl) quinazolin- 3(4H) - yl) benzamide derivatives have been created by ChemDraw Ultra 8.0.

Calculation of Molecular Properties of N-(4-oxo-2-(4-((4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) methyl) phenyl) quinazolin- 3(4*H*) - yl) benzamide derivatives and some specific Anti-microbial drugs. Along with SMILES and SDfile conversion, normalization of molecules, tautomer generation, molecule fragmentation, calculation of various molecular properties essential for QSAR, molecular modeling and drug design, high-quality

molecule representation, and molecular database tools supporting substructure and similarity searches. Molinspiration offers an extensive variety of cheminformatics software tools supporting molecule manipulation and processing. Additionally, it supports bioactivity prediction, data visualization, and fragment-based virtual screening. Druglikeness is the result of a complex balancing act between several different chemical characteristics and structural elements that determine whether a given molecule is comparable to recognized medications. These characteristics-primarily hydrophobicity, electronic distribution, hydrogen bonding traits, molecule size, flexibility, and of course the presence of various pharmacophoric features have a consequence on how molecules behave in living things. Such as their bioavailability, transport characteristics, an affinity for proteins, reactivity, toxicity, metabolic stability, and a host of other behaviors (Molinspiration Cheminformatics, 2023). Molinspiration online molecular property computation toolkit (http://www.molinspriration.com) was utilized for calculating the in silico pharmacokinetic characteristics of N-(4-oxo-2-(4-((4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) methyl) phenyl) quinazolin- 3(4H) - yl) benzamide derivatives products along with a few selected anti-microbial drugs according to Lipinski's rule of five. In accordance to the rule, molecules with good membrane permeability have log P values of ≤ 5, a molecular weight of ≤ 500, hydrogen bond acceptors ≤ 10, and hydrogen bond donors ≤ 5. Molecular volume, topological polar surface area, and the amount of rotatable bonds are additional rules that are significant in the computational prediction of drug-likeness. The total number of rotatable bonds shows a compound's conformational flexibility and, in the end, its ability to bind to receptors or ion channels. The total number of rotatable bonds reveals a compound's conformational flexibility and, ultimately, its ability to bind to receptors or ion channels. Molecules' capability to cross the blood-brain barrier or be absorbed into the intestine depends on their molecular volume. TPSA is also acknowledged as a reliable measure of drug penetration through the blood-brain barrier [TPSA <60 $Å^2$] and intestinal drug absorption [TPSA <140 angstroms squared ($Å^2$)]. The percentage of absorption (% ABS), which may be determined using the formula % ABS = 109 - (0.345 × TPSA), can be utilized to express the degree of absorption (Kuchana *et al*., 2020).

Calculation of the Bioactivity Score using selected anti-microbial medicines and N-(4-oxo-2-(4-((4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) methyl) phenyl) quinazolin- 3(4*H*) - yl) benzamide derivatives. Values ascribed to a compound's drug score reveal its general potential as a drug candidate. The bioactivity score of synthesized compounds against common human receptors such GPCRs, ion channels, kinases, nuclear receptors, proteases, and enzymes can be predicted by employing an online tool called Mol Inspiration (Proudfoot, 2002). With the assistance of the online Molinspiration drug-likeness score, all of the parameters were established. (www.molinspiration.com). Each compound's drug-likeness score was determined and compared to the particular body function it affects, and the results have been compared with those of standard medications. For organic molecules, the probability is that they are active if the bioactivity score is greater than 0, moderately active if it is between -5.0 and 0.0, and inactive if it is below -5.0 (Molinspiration Cheminformatics, 2023).

Analyzing the pharmacokinetic characteristics of certain anti-microbial medicines and N-(4-oxo-2-(4-((4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) methyl) phenyl) quinazolin- 3(4*H*) - yl) benzamide derivatives. The Swiss ADME web server (http://www.swissadme.ch/) has received the SMILES of N-(4-oxo-2-(4-((4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) methyl) phenyl) quinazolin-3(4H) - yl) benzamide derivatives for in silico prediction of the absorption, distribution, metabolism, and excretion (ADME) models. The blood-brain barrier (BBB) and P-glycoprotein (Pgp) substrates penetration into the human intestine (HIA) and access to the central nervous system were determined utilizing the statistical graph method of brain or intestinal estimated permeation (BOILED-Egg) through the determined values of lipophilicity and polarity. The similarity test of chemical substances from the potential inhibitor cytochrome P450 (CYP450) isoenzyme inhibitors database 1A2, 2C19, 2C9, 2D6, and 3A4 was carried out to estimate the phase I metabolism and excretion pathway of the substances (Silva *et al.,* 2021).

Evaluation of toxicity predicted properties of N-(4-oxo-2-(4-((4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) methyl) phenyl) quinazolin- 3(4*H*)-yl) benzamide derivatives. ProTox-II, an online lab for the prediction of small molecule toxicities, was utilized for analyzing the N-(4-oxo-2-(4-((4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) methyl) phenyl) quinazolin- 3(4H)- yl) benzamide's toxicity using computational approaches. For the prediction of various toxicity endpoints, which include acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes (Tox21) pathways, and toxicity targets, ProTox-II includes molecular similarity, fragment propensities, most frequent features, and machine learning. The LD_{50} values for toxic dosages are frequently expressed in mg/kg body weight. The median lethal dosage, or LD_{50}, is the dose that causes 50% of test subjects to pass away after being exposed to a substance (ProTox-II, 2023). The SDF file of the derivatives has been uploaded to the ProTox-II web server to conduct an in silico test for similarity of potentially harmful molecular fragments. The server quickly estimates the LD_{50} for acute oral toxicity, where toxic classes range from I (lethal if consumed) to VI. (Non-toxic), further to liver organic toxicity, carcinogenic, immunogenic, mutagenic, and cytotoxic endpoints, the results that are the most trusted belong to those which have a degree of probability of more than 70% for both active and inactive categories of toxicity models (Silva *et al.,* 2021). The LD_{50} values for toxic doses are frequently expressed in mg/kg body weight. The median lethal dosage, or LD_{50}, is the dose

at which 50% of test subjects pass away after being exposed to a substance. According to the globally harmonized system of classification and labeling of substances, toxicity classes are established.

Values for LD_{50} are provided in [mg/kg]:

Class I: Fatal if swallowed ($LD_{50} \leq 5$)

Class II: Fatal if swallowed ($5 < LD_{50} \leq 50$)

Class III: Toxic if swallowed ($50 < LD_{50} \leq 300$)

Class IV: Harmful if swallowed ($300 < LD_{50} \leq 2000$)

Class V: May be harmful if swallowed ($2000 < LD_{50} \leq 5000$)

Class VI: Non-toxic ($LD_{50} > 5000$) (ProTox-II, 2023)

RESULTS AND DISCUSSION

In the discipline of pharmaceutical chemistry, computer-aided drug design is crucial to the development of new compounds. In the arena of computer-aided drug design, computational medicinal chemists can benefit from a wide range of software and tools to find and optimize biologically active molecules. The modern drug development method now includes modifying the chemical structure of lead candidates concerning ADME processes.

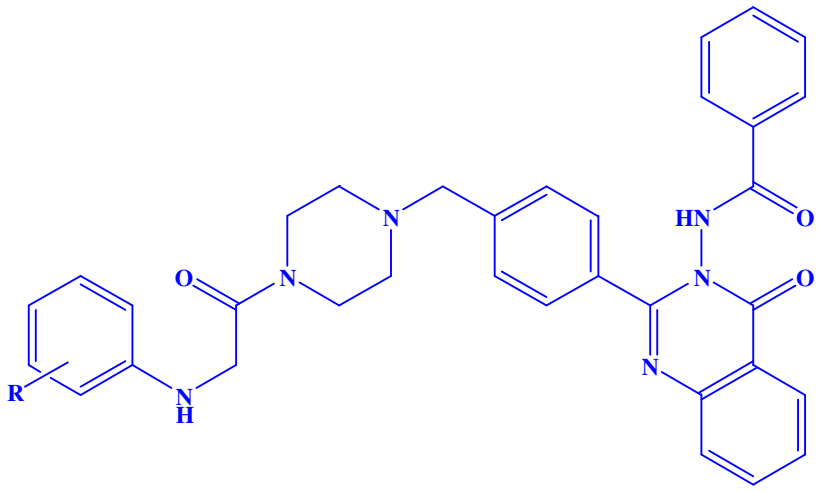

Fig. 1. Structure of N-(4-oxo-2-(4-((4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) methyl) phenyl) quinazolin- 3(4*H*)- yl) benzamide derivatives.

Table 1: Nomenclature and molecular formula of N-(4-oxo-2-(4-((4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) methyl) phenyl) quinazolin- 3(4H)- yl) benzamide derivatives.

Compound No.	Nomenclature	Molecular formula	R
PRP7B1	N-(4-oxo-2-(4-((4-(2-(phenylamino)acetyl)piperazin-1-yl)methyl)phenyl)quinazolin-3(4H)-yl)benzamide	$C_{34}H_{32}N_6O_3$	H
PRP7B2	N-(2-(4-((4-(2-(o-toluidino)acetyl)piperazin-1-yl)methyl)phenyl)-4-oxoquinazolin-3(4H)-yl)benzamide	$C_{35}H_{34}N_6O_3$	2-CH_3
PRP7B3	N-(2-(4-((4-(2-(2-methoxyphenylamino)acetyl)piperazin-1-yl)methyl)phenyl)-4-oxoquinazolin-3(4H)-yl)benzamide	$C_{35}H_{34}N_6O_4$	2-OCH_3
PRP7B4	N-(2-(4-((4-(2-(4-methoxyphenylamino)acetyl)piperazin-1-yl)methyl)phenyl)-4-oxoquinazolin-3(4H)-yl)benzamide	$C_{35}H_{34}N_6O_4$	4-OCH_3
PRP7B5	N-(2-(4-((4-(2-(p-toluidino)acetyl)piperazin-1-yl)methyl)phenyl)-4-oxoquinazolin-3(4H)-yl)benzamide	$C_{35}H_{34}N_6O_3$	4-CH_3
PRP7B6	N-(2-(4-((4-(2-(4-chlorophenylamino)acetyl)piperazin-1-yl)methyl)phenyl)-4-oxoquinazolin-3(4H)-yl)benzamide	$C_{34}H_{31}ClN_6O_3$	4-Cl
PRP7B7	N-(2-(4-((4-(2-(4-hydroxyphenylamino)acetyl)piperazin-1-yl)methyl)phenyl)-4-oxoquinazolin-3(4H)-yl)benzamide	$C_{34}H_{32}N_6O_4$	4-OH
PRP7B8	N-(2-(4-((4-(2-(4-nitrophenylamino)acetyl)piperazin-1-yl)methyl)phenyl)-4-oxoquinazolin-3(4H)-yl)benzamide	$C_{34}H_{31}N_7O_5$	4-NO_2
PRP7B9	N-(2-(4-((4-(2-(m-toluidino)acetyl)piperazin-1-yl)methyl)phenyl)-4-oxoquinazolin-3(4H)-yl)benzamide	$C_{35}H_{34}N_6O_3$	3-CH_3
PRP7B10	N-(2-(4-((4-(2-(3-methoxyphenylamino)acetyl)piperazin-1-yl)methyl)phenyl)-4-oxoquinazolin-3(4H)-yl)benzamide	$C_{35}H_{34}N_6O_4$	3-OCH_3
PRP7B11	N-(2-(4-((4-(2-(2-chlorophenylamino)acetyl)piperazin-1-yl)methyl)phenyl)-4-oxoquinazolin-3(4H)-yl)benzamide	$C_{34}H_{31}ClN_6O_3$	2-Cl

In silico evaluation of "drug-like" or Molecular Properties. Molinspiration Cheminformatics was utilized to calculate the molecular characteristics of all the produced substances, and the results are shown in Table 2. Lipinski's rule of five, which considers four straightforward physicochemical characteristics (log P ≤ 5, molecular weight ≤ 500, number of hydrogen bond acceptors ≤ 10, number of hydrogen bond donors ≤ 5) was used to assess the drug-likeness of all of these compounds. To gain insight into a substance's solubility behavior and, additionally, its oral absorption and bioavailability, one uses the log P measurement. The in silico investigation showed that all of the N-(4-oxo-2-(4-((4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) methyl) phenyl) quinazolin- 3(4*H*)- yl) benzamide derivatives possess log P values between 3.98 and 5.14 (within acceptable range ≤ 5). Except for compounds PRP7B6 and PRP7B11, all the compounds have log P values within the range. PRP7B6 and PRP7B11 both exhibited high log P values (5.14 and 5.09 etc), indicating high lipophilicity or hydrophobicity, accordingly. Consequently, after it has been absorbed, indicates a better distribution of both PRP7B6 and PRP7B11. The molecular weight among all N-(4-oxo-2-(4-((4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) methyl) phenyl) quinazolin- 3(4*H*)- yl) benzamide derivatives is ≤ 618. In comparison with molecules with high molecular weight higher than 500, low molecular weight compounds are more readily absorbed, dispersed, and transported (Kumar *et al*., 2018). A sufficient number of hydrogen bond acceptors and donors are also present in the benzamide derivatives, facilitating a successful interaction with the hydrogen bonding groups of an intractable receptor. The flexibility and conformational alterations that molecules go through to bind to receptors are explained by the number of rotatable bonds. To achieve oral bioavailability, it is generally agreed that there should be less than 10 rotatable bonds (Veber *et al.,* 2002). All of the predicted molecules contain between eight to nine rotatable bonds, which allows for maximum structural flexibility. The TPSA of molecules is highly beneficial physicochemical parameters that reveal the polarity of compound. It is employed to forecast a compound's transport characteristics, including intestinal absorption and blood-brain barrier penetration (Zhao *et al.,* 2002). All of the predicted molecules' TPSA values were found to range between 99.57 to 145.39. These variables were used to determine the % ABS, which is shown in Table 2. In accordance with the details, the of N-(4-oxo-2-(4-((4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) methyl) phenyl) quinazolin- 3(4*H*)- yl) benzamide derivatives had good % absorption, ranging from 58.84 to 74.65. All the compounds have the number of hydrogen bond donors ≤ 5 and hydrogen bond acceptors ≤ 10, as per Lipinski's limit except for PRP7B8 (hydrogen bond acceptors = 12). Every compound had n violations ranging from 1 to 2.

Table 2: Drug likeness score for the compounds.

Compounds	MW	miLogP	TPSA	natoms	nON	nOHNH	nviolations	nrotb	Volume	% ABS
PRP7B1	572.67	4.46	99.57	43	9	2	1	8	520.76	74.65
PRP7B2	586.70	4.86	99.57	44	9	2	1	8	537.33	74.65
PRP7B3	602.70	4.47	108.80	45	10	2	1	9	546.31	71.46
PRP7B4	602.70	4.52	108.80	45	10	2	1	9	546.31	71.46
PRP7B5	586.70	4.91	99.57	44	9	2	1	8	537.33	74.65
PRP7B6	607.11	5.14	99.57	44	9	2	2	8	534.30	74.65
PRP7B7	588.67	3.98	119.80	44	10	3	1	8	528.78	67.67
PRP7B8	617.67	4.42	145.39	46	12	2	2	9	544.10	58.84
PRP7B9	586.70	4.88	99.57	44	9	2	1	8	537.33	74.65
PRP7B10	602.70	4.49	108.80	45	10	2	1	9	546.31	71.46
PRP7B11	607.11	5.09	99.57	44	9	2	2	8	534.30	74.65
Ciprofloxacin	331.35	-0.70	74.57	24	6	2	0	3	285.46	83.27
Fluconazole	306.28	-0.12	81.66	22	7	1	0	5	248.96	80.83

In silico evaluation of Bioactivity Score. The concept of "pharmacological activity" refers to how medications affect living things. The medication has the purpose of being attached to a biological target. Several common proteins, including enzymes, ion channels, and receptors, are considered biological targets. Drug target is another name for biological target. For various characteristics, including binding to G protein-coupled receptor (GPCR) ligand, nuclear receptor ligand, ion channel modulators, Kinase inhibitor, protease inhibitor, and enzyme inhibitor, the bioactivity scores of the synthesized complexes were computed. With the utilization of the online tool Molinspiration (www.molinspiration.com), all the parameters were estimated, and the synthesized compounds were predicted to exhibit moderate biological activity. In Table 3, a bioactivity score is presented. Bioactivity scores, which are divided into three ranges, are utilized to measure biological activity:

1. If the bioactivity score is greater than zero, having considerable highly biological activity.
2. If it is between -5 to 0, it is moderately active.
3. If it is less than -5, it is inactive (Khan *et al.,* 2017).

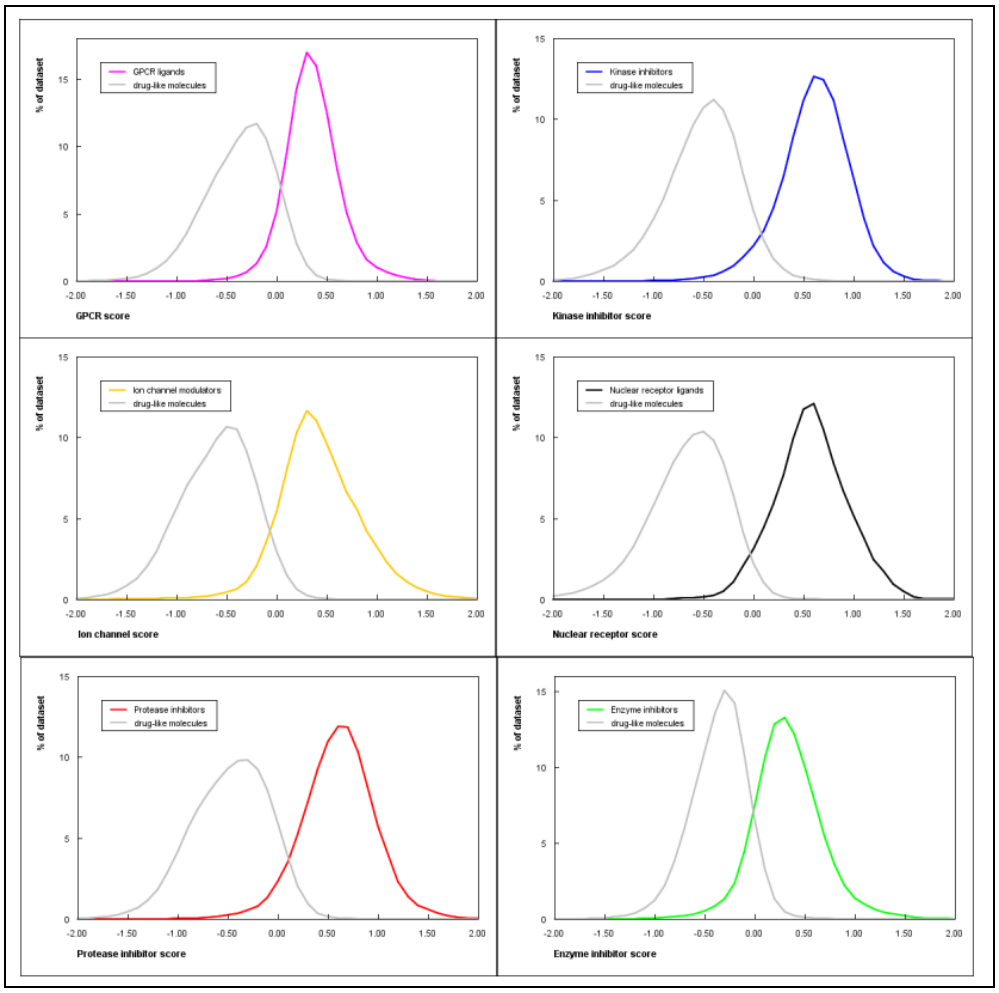

Fig. 2. Bioactivity score graph for various targets.

Table 3 illustrates that all synthetic compounds obtained bioactivity scores ranging from -5.0 to 0.0. All substances were discovered to possess moderately active for GPCR ligand, Ion channel modulator, Kinase inhibitor, Nuclear receptor ligand, Protease inhibitor, and Enzyme Inhibitor.

In silico evaluation of Pharmacokinetics properties. Except for PRP7B8, all of the compounds had high gastrointestinal (GI) absorption, according to Swiss ADME prediction criteria, and none of the compounds could permeate the blood-brain barrier (BBB). P-glycoprotein substrates include PRP7B1, PRP7B3, PRP7B4, PRP7B7, and PRP7B10. P-glycoprotein is not a substrate for the remaining synthetic derivatives, PRP7B2, PRP7B5, PRP7B6, PRP7B8, PRP7B9, and PRP7B11.

Table 3: Bioactivity score of the compounds.

Compounds	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
PRP7B1	-0.17	-0.91	-0.27	-0.83	-0.31	-0.39
PRP7B2	-0.25	-1.05	-0.37	-0.91	-0.38	-0.49
PRP7B3	-0.31	-1.18	-0.46	-1.03	-0.42	-0.58
PRP7B4	-0.30	-1.16	-0.47	-1.00	-0.39	-0.57
PRP7B5	-0.24	-1.05	-0.38	-0.93	-0.36	-0.49
PRP7B6	-0.22	-1.01	-0.36	-0.92	-0.35	-0.48
PRP7B7	-0.19	-0.99	-0.33	-0.83	-0.32	-0.43
PRP7B8	-0.42	-1.26	-0.63	-1.14	-0.47	-0.69
PRP7B9	-0.25	-1.06	-0.37	-0.93	-0.37	-0.50
PRP7B10	-0.31	-1.17	-0.46	-1.01	-0.40	-0.58
PRP7B11	-0.23	-1.02	-0.34	-0.94	-0.39	-0.49
Ciprofloxacin	0.12	-0.04	-0.07	-0.19	-0.20	0.28
Fluconazole	0.04	0.01	-0.09	-0.23	-0.09	0.03

P-glycoprotein has a role in how efficiently drugs are eliminated from the body through the hepatic and renal systems. There may be a chance for drug-drug and drug-food interactions because this active transport pathway is saturable and can be suppressed or activated (Medicines interaction, 2023). For predicting the adverse metabolic effects of oral administration of drug candidates, as well as their half-life in the organism and excretion route, in silico characterization approaches use similarity testing with database substructures. Drugs interact with cytochrome P450 isoenzymes (CYP450), which are oxidases, to lower their plasma concentration, minimize the likelihood of toxicity through metabolic activation, and make them more water soluble for excretion. Furthermore, molecular substructures can have a deleterious impact on ultimate toxicity pathways, such as by inhibiting hERG (human Ether-a-go-go Related Gene) ion transport channels and causing cardiotoxicity (Silva *et al.,* 2021). All of the substances were discovered to be CYP2C19, CYP2C9, and CYP3A4 inhibitors, which points to a rise in their plasma concentrations and a sluggish mode of excretion. The interaction of CYP1A2 with none of the compounds inhibits the compound's rate of metabolism. Furthermore, all of the synthetic compounds, with the exception of PRP7B6, PRP7B8, and PRP7B11, are CYP450 isoenzyme 2D6 inhibitors. Although it does not block CYP450 2D6, the substances PRP7B6, PRP7B8, and PRP7B11 are metabolised by o-demethylation processes, lowering the risk of liver damage brought on by metabolic activation. All synthetic compounds possess a skin permeation coefficient (logKp) ranging from -6.35 to -6.98. The skin's permeability decreases as negative logKp increases.

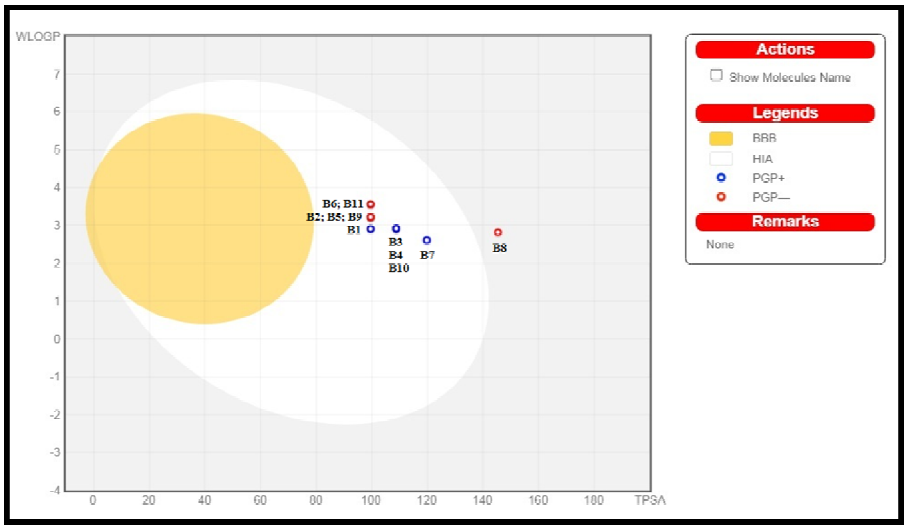

Fig. 3. BOILED-Egg used to estimate human intestinal absorption (HIA) and blood-brain barrier permeation (BBB) through the WlogP and TPSA descriptors (SwissADME, 2023).

Table 4: Pharmacokinetics properties of the compounds.

Compounds	GI absorption	BBB per meant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)
PRP7B1	High	No	Yes	No	Yes	Yes	Yes	Yes	-6.58
PRP7B2	High	No	No	No	Yes	Yes	Yes	Yes	-6.41
PRP7B3	High	No	Yes	No	Yes	Yes	Yes	Yes	-6.79
PRP7B4	High	No	Yes	No	Yes	Yes	Yes	Yes	-6.79
PRP7B5	High	No	No	No	Yes	Yes	Yes	Yes	-6.41
PRP7B6	High	No	No	No	Yes	Yes	No	Yes	-6.35
PRP7B7	High	No	Yes	No	Yes	Yes	Yes	Yes	-6.94
PRP7B8	Low	No	No	No	Yes	Yes	No	Yes	-6.98
PRP7B9	High	No	No	No	Yes	Yes	Yes	Yes	-6.41
PRP7B10	High	No	Yes	No	Yes	Yes	Yes	Yes	-6.79
PRP7B11	High	No	No	No	Yes	Yes	No	Yes	-6.35
Ciprofloxacin	High	No	Yes	No	No	No	No	No	-9.09
Fluconazole	High	No	Yes	No	Yes	No	No	No	-7.92

In silico evaluation of Toxicological Study. Each synthetic compound's toxicity profile was assessed, and the results are shown in Table 5. They were discovered to be similar to standard drugs in terms of toxicity class classification (Banerjee *et al.,* 2018; Garg *et al.,* 2021). All synthetic substances exhibit toxicity class 4, which renders them all practically non-toxic and non-irritating (Wikipedia contributors, 2022). All derivatives exhibited a median lethal dose (LD_{50}) of 1500 mg/kg. Results of endpoints involving hepatotoxicity,

carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity are provided by the toxicological prediction. It was projected that none of the synthesized substances would be cytotoxic and hepatotoxic. The non-carcinogenic properties of the substances PRP7B3, PRP7B4, PRP7B6, PRP7B7, PRP7B10, and PRP7B11 were predicted. It was predicted that the substances PRP7B1, PRP7B2, PRP7B5, PRP7B6, PRP7B7, and PRP7B9 would not be immunotoxic. It was projected that the synthetic substances PRP7B1, PRP7B6, PRP7B7, and PRP7B11 would be non-mutagenic.

Table 5: Toxicity Profile of the compounds.

Compounds	LD_{50} (mg/kg)	Toxicity Class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
PRP7B1	1500	4	Inactive	Active	Inactive	Inactive	Inactive
PRP7B2	1500	4	Inactive	Active	Inactive	Active	Inactive
PRP7B3	1500	4	Inactive	Inactive	Active	Active	Inactive
PRP7B4	1500	4	Inactive	Inactive	Active	Active	Inactive
PRP7B5	1500	4	Inactive	Active	Inactive	Active	Inactive
PRP7B6	1500	4	Inactive	Inactive	Inactive	Inactive	Inactive
PRP7B7	1500	4	Inactive	Inactive	Inactive	Inactive	Inactive
PRP7B8	1500	4	Inactive	Active	Active	Active	Inactive
PRP7B9	1500	4	Inactive	Active	Inactive	Active	Inactive
PRP7B10	1500	4	Inactive	Inactive	Active	Active	Inactive
PRP7B11	1500	4	Inactive	Inactive	Active	Inactive	Inactive
Ciprofloxacin	2000	4	Inactive	Inactive	Inactive	Active	Inactive
Fluconazole	1271	4	Active	Inactive	Inactive	Inactive	Inactive

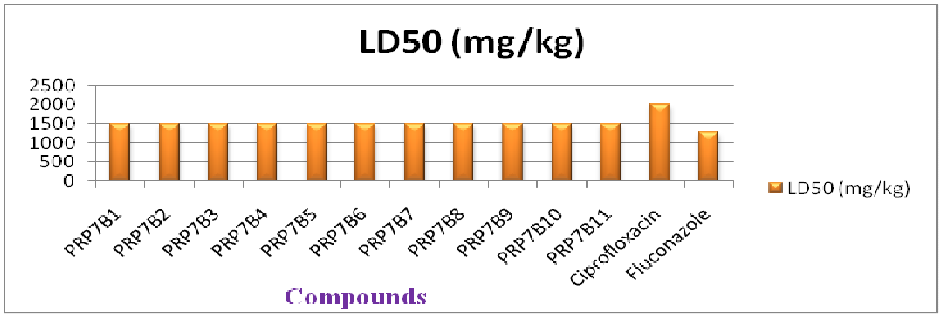

Fig. 4. Distribution of the median lethal dose (LD_{50}) in mg/kg of the synthesized compounds and Standard Drugs.

CONCLUSIONS

In conclusion, every substance complies with Lipinski's rules for molecules' drug-likeness, except for molecular weight. All eleven compounds exhibited moderate bio-activity scores. Compounds have a moderately active for nuclear receptor ligand, kinase inhibitor, GPCR ligand, protease inhibitor, and enzyme inhibitor. All synthesized compounds, except PRP7B8, had significant levels of gastrointestinal absorption and were unable to penetrate the blood-brain barrier, according to results from in silico analyses of pharmacokinetic parameters. The CYP450 isoenzyme 1A2 metabolizes all substances, diminishing their plasma concentrations and toxicity risk. It was predicted that synthetic substances wouldn't be cytotoxic or hepatotoxic. Hence, an attempt was made to compare some specified anti-microbial medicines, such as Ciprofloxacin and Fluconazole, with the molecular properties, bioactivity score, pharmacokinetic parameters and toxicity studies of N-(4-oxo-2-(4-((4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) methyl) phenyl) quinazolin- 3(4H)- yl) benzamide derivatives.

FUTURE SCOPE

The ongoing struggle against infectious diseases depends on the development of innovative drug classes, medications with fewer side effects, and medications with shorter treatment times. Further studies will be detailed on additional alterations of molecules at various places to produce novel molecules with strong anti-microbial properties, more specific and less toxic.

Conflict of Interest. None.

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