Current Trends in Biotechnology and Pharmacy
Vol. 16 (1) 14 - 22, Jan 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2022.1.2

*In silico*Evaluation of 2,4-diaminoquinazoline Derivatives as Possible Anticancer agents

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Abstract

The quinazoline nucleus is one of the most privileged scaffolds for the designing of pharmacodynamic compounds.There were several molecules originated from natural as well as synthetic sources.Molecules with quinazolinenucleus have been found not only aspotential anticancer agents butalsofor treating other diseases.In the present study, the designed compounds were subjected to*in silico* screening followed by molecular docking with caspase-9 protein. Initially, compounds were screened for drug-likeness usingSwissADME, Osiris Property Explorer, ProTox-II, and PASS web servers.It has been found that most of the compounds interactwith caspase-9 through H-bonding. During the preliminary screening, the compounds have shown drug-likeness anddevoid of any mutagenicity and hepatotoxicity.Our results indicate that these scaffolds could be further improved for their anticancer efficacy by structural modifications.

Keywords: Quinazoline, *In silico*, Caspase-9, Drug-likeness, Anticancer

Introduction

Quinazoline heterocyclic nucleus is one of the most important pharmacophores in designing novel drug-like molecules. Quinazoline contains the benzene ring fused with a pyrimidine ring also known as 1,3-diazanaphthalene. The pyrimidine ring nitrogens arenot suitable for the electrophilic substitution reactions and the 4thposition of quinazoline is more reactive than the 2ndposition. But the benzene ring is more convenient for electrophilic substitution(1). Many derivatives of quinazolineare biologically active and are being used clinically for treating diversediseases (2). It is an important scaffold and exhibits anticancer, anti-inflammatory, anticonvulsant, diuretic, analgesic, antidepressant, antifungal, anti-microbial, anti-viral, anti-hypertensive, and anti-plasmodial activities. The favorable substitution at C_4, C_6, and C_7positions showed promising effects. The potency of the quinazoline moiety depends on the substituents and their position in one of the cyclic rings(3). Some of the derivatives have been approved by Food and Drug Administration (FDA) as anticancer agents. Quinazoline derivatives like Erlotinib, Gefitinib, Afatinib, Lapatinib, and Vandetanib haveshown promising therapeutic efficacy, specifically against solid tumors.In the current investigation, we have selected some of the novel 2,4-diaminoquinazoline derivatives and carried out*in silico* screening for their potentialanticancer activity using caspase-9 as a target protein (Figure 1).

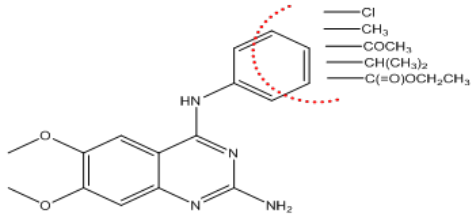

Fig. 1.One of the quinazoline derivatives that has been designed in the lab.

Materials and Methods

ADMET and drug-related properties: Drug related properties and bioactivity (molecular mass, Log P, hydrogen bond acceptor and donors, rotatable bonds, PSA, and Ro5 violations) of selected compounds were predicted by SwissADME. The oral bioavailability of molecules has been predicted by the value of Log *P*. To analyze Lipinski's rule of five, the number of rotatable bonds, H-bond acceptors, and H-bond donors were attained by utilizing SwissADME. According to this rule, molecules that can easily cross the membrane should have molecular weight ≤500, hydrogen bond donor's ≤5, Log P ≤5, and hydrogen bond acceptors ≤10. Further, pharmacokinetic parameters like absorption, distribution, metabolism and excretion (ADME) have also been analyzed by the SwissADME webserver(4).

***Swiss ADME*:** Using this web server, molecules can be estimated for ADME, drug-likeness, pharmacokinetics and medicinal chemistry friendliness properties(5).By applying the web server,molecular and physicochemical descriptors like molecular weight (MW), molecular refractivity (MR), molecular formula, number of heavy atoms, number of aromatic heavy atoms, number of rotatable bonds, number of H-bond acceptors, number of H-bond donors, molar refractivity, count of specific atom types and polar surface area (PSA) have been computed.

The Bioavailability Radar: The Bioavailability Radar wasused for a quick estimation of drug-likeness. The radar considers six physicochemical parameters i.e.,lipophilicity, size, polarity, solubility, saturation, and flexibility. The pink shaded area defines the optimal values of the above six parameters and the range of the individual parameter has been calculated as follows. Lipophilicity: XLOGP3 between −0.7 and +5.0; size: MW between 150 and 500 g/mol; polarity: TPSA between 20 and 130 Å; solubility: molar solubility in water (log S) with less than 6; saturation: fraction of carbons in the sp^3 hybridization with 0.25 or more and flexibility. A maximum of 9 rotatable bonds and a large scale deviation from these parameters indicates that the ligand was not orally bioavailable(6).

The BOILED-Egg Tool: The access of the given compounds to the brain and gastrointestinal tract (GIT) was estimated using the Brain OrIntestinaLEstimateD permeation (BOILED-Egg) method in the same web tool. The method estimated the passive gastrointestinal absorption and ability of brain entrance of small molecules. Compounds with a high probability to permeate through the blood brain barrier (BBB) to enter the central nervous system (CNS) were represented in the yellow ellipse whereas compounds with a high probability to be passively absorbed by the GIT were represented in the white ellipse. Molecules in the grey zone were those not well absorbed by GIT and do not cross BBB. While molecules predicted asthe substratesof the P-glycoprotein (PGP+) have been displayed with blue color points, those that act as the non-substrate of the P-glycoprotein (PGP-) represented in red color (7).

Toxicity studies

***OSIRIS Property Explorer*:** The OSIRIS Property Explorer, calculates various important drug-related properties of a given structure. Predicted results appear with colors and have specific values. Toxicity predictions of likely compounds were run through the prediction of this software and the compounds which havethe potential risk of toxicity (mutagenicity, tumorogenicity, irritant and effects on reproductive physiology) were removed from the list. Properties of the compounds with high risks of undesired effects or toxicity like mutagenicity, tumorogenicity, skin irritations, reproductive effects or poor intestinal absorption have been displayed in red color with a value of 0.6. Yellowcolor indicates that the compound has a medium risk with a value of 0.8 and green color shows drug-conform behavior. Such compounds are safe which have values of 1.0(8).The prediction process relied

on a precomputed set of structural fragments that give rise to toxicity alerts in case they come across in the structure currently drawn or uploaded. These fragment lists were created by rigorously ripping up all compounds of the RTECS (Registry of Toxic Effects of Chemical Substances) database, a collection of toxicity information collected and compiled from the open scientific literature, known to be active in a certain toxicity class (e.g. mutagenicity). It furnished toxic effects and regulatory information in about 133,000 chemicals(9). During the ripping up, any molecule that was first cut at every rotatable bondresults in a set of core fragments. These in turn have been used to rebuild all possible bigger fragments which might be a substructure of the original molecule. Thereafter, a substructure search process determined the occurrence frequency of core and built fragments within all compounds of that toxicity class. Besides, it calculated frequencies of the fragments in about 3000 marketed drugs. Considering the fact that the marketed drugs are largely free of toxic effects, any fragment can be taken as a risk factor if it occurred often as the substructure of harmful compounds but never or rarely occurred in marketed drugs(10).

ProTox-II**:** This web server predicts toxicity and uses a two-dimensional chemical structure as input and the possible toxicity profile of the chemical for 33 models with confidence scores and an overall toxicity radar chart along with the three most similar compounds with known acute toxicity(11).ProTox-II, a virtual lab software, is used for the prediction of toxicities of small molecules. ProTox-II contained computer-based models trained on real data (in vitro or in vivo) to predict the toxic potential of the existing and virtual compounds (Figure 3).ProTox-II incorporates molecular similarity, pharmacophores, fragment propensities, and machine-learning models for the prediction of acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcome pathways (Tox21)and toxicity targets. The predictive models are built on data from both in vitro assays (e.g., Tox21 assays, Ames bacterial mutation assays, hepG2 cytotoxicity assays, immunotoxicity assays) and *in vivo* cases (e.g. carcinogenicity, hepatotoxicity). The models have been validated on independent external sets and have shown strong performance (12).

Pass Online**:**It evaluates the general biological potential of drug-like molecules based on corresponding organic structures. Molecules that exhibit drug-likeness in SwissADME are put forward in Pass Online (using smiles, MOL file format, Marvin JS) for retaining information regarding biological activity along with the stimulatory and inhibitory activity of the molecule on the receptors(13). PASS Online predicts over 4000 kinds of biological activity, including pharmacological effects, mechanisms of action, toxic and adverse effects, interaction with metabolic enzymes and transporters, influence on gene expression, etc. 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 a well-known biologically active substance, it is possible to estimate if a new compound has a specific effect. Multilevel Neighborhoods of Atoms (MNA) structure descriptors of a molecule are generated based on connection table (C) and table of atom types (A) present in the compound.

Molecular Docking Studies

Ligand Preparation:The designed molecule structures were drawn in ChemDraw 18.2 (ChemOffice 2018, Perkin-Elmer Informatics, USA) and saved in the sdf format. Later, the molecules were subjected for energy minimization in Chem3D 18.2 ultra-software until the energy difference reaches 0.001kJ/mol, and a single minimum energy 3D conformer generated for each compound. These 3D structures were opened in Maestro 12.6 (Schrodinger, LLC, New York, USA) and subjected for ligand preparation in LigPrep tool(14) with filtration criteria of Molecular

weight ≤ 150 & ≥ 500, Force Field - OPLS3, and checking the default options: Ionization; Generate possible states at target pH 7.0 ± 2.0; Epik program for ionization states: Desalt; Generate tautomers: Stereoisomers; Retain specified chiralities; Stereoisomers: Generate at most: 32 per ligand; Output format: Maestro. The LigPrep results were verified and one conformation per ligand was selected based on their Epik state penalty (kcal/mol) (15).Structures were excluded based on their molecular weight (>500), and the remaining structures were forwarded for docking calculations. The selected compounds are well under 500 Daltons and considered for molecular docking studies.

Protein Preparation and Active Site Prediction:The crystal structure of caspase-9 (PDB ID:2AR9) protein was imported from Protein Databank (16) into the Maestro protein preparation tool from RCSB PDB and the protein structure was corrected for integrity.Missingresidues were included by employing the prime program. Later, explicit hydrogen atoms were added to the protein and optimization has been performed and energy of the protein minimized. The H-bond assignment section has been used for optimizing hydrogen bonding network i.e., a process thatsamples water orientations and flips Asn, Gln, and/or His side chains at a specified pH value. The protein protonation states were kept at a pH range of 7.0 ± 2.0 and geometry optimized with a maximum RMSD of 0.3 Å by OPLS3 force-field(17,18). The restrained minimization section was used to fix the crashes that occur due to the addition of hydrogen atoms or filling missing sidechains. By default, an RMSD of 0.3 Å was used, minimizing both the hydrogens and heavy atoms via harmonic penalty constraints. As there was no active co-crystallized ligand, the SiteMap tool of the Maestro was employed for detecting the potential binding sites on the protein, based on various parameters (17). SiteMap features the regions surrounded by the binding sites convenient for occupancy by hydrophobic functional units or with ligand H-bond acceptors, donors, or metal chelating functional groups. Out of the predicted five binding sites by SiteMap, one binding site has been chosen for further docking(19,20).

Receptor Grid Generation and Molecular Docking Studies: Around the chosen binding cavity of the protein, a grid was generated by using the Glide program by selecting one residue in the binding cavity with a grid box of 16×16×16 Å with the length of 8 Å corresponding to the x, y, and z coordinates of 11.55, -8.28, and 8.78 respectively. With these coordinates and default parameters, the grid file has been generated and stored for further docking studies(21). In the Glide program, flexible docking has been employed to identify the possible binding interactions and affinity between the designed molecules and the predicted binding site of caspase-9 (PDB ID:2AR9). The generated receptor grid files have been browsed into the Glide and the prepared ligands included from the workspace to study the binding interaction with the protein and with the Extra Precision (XP) docking program employing all default parameters unaltered(22,23).

Results and Discussion

ADMET and Drug-Related Properties: Lipinski's rule of five was used for predicting whether selected and designed compounds have the property of drug-like molecules or not. This is based on the selected compound satisfying the five parameters of this rule *viz.*, having less than 500 Da molecular weight, less than 5 log P, less than 5 H-bond donors, less than 10 H-bond acceptors, 40-130 molar refractivity, and below 140 total polar surface area. All the 2,4-diamnoquinazoline compounds were successfully passed through Lipinski filters with no violations. The values of all the molecules were represented in table 1 and all compounds were predicted to be drug-like molecules(24-27). The compound's molecular weight wasbetween 310.35 to 368.39 Daltons. All the compounds possess less than 5 hydrogen bond donors (HBDs) and 10 hydrogen bond

Table 1. Prediction of physicochemical properties of the selected compounds

Mol	Formula	MW	#Heavy atoms	#Arom. heavy atoms	Fraction Csp^3	#Rotatable bonds	#H-bond acceptor	#H-bond donors	MR	TPSA
5a	$C_{18}H_{18}N_4O_3$	338	25	16	0.17	5	5	2	96.66	99.36
5b	$C_{18}H_{18}N_4O_3$	338	25	16	0.17	5	5	2	96.66	99.36
5c	$C_{16}H_{15}ClN_4O_2$	330	23	16	0.12	4	4	2	91.48	82.29
5d	$C_{16}H_{15}ClN_4O_2$	330	23	16	0.12	4	4	2	91.48	82.29
5e	$C_{17}H_{18}N_4O_2$	310	23	16	0.18	4	4	2	91.44	82.29
5f	$C_{19}H_{22}N_4O_2$	338	25	16	0.26	5	4	2	101.05	82.29
5g	$C_{19}H_{20}N_4O_4$	368	27	16	0.21	7	6	2	102.56	108.59

acceptors (HBAs). The molar refractivity of all the compounds spanned between 91.48 to 102.56 and the total polar surface area was between 82.29 to 108.59. All the compounds have a minimum of 4 rotatable bonds and a maximum of 7. The compounds were found to possess the heteroatoms of 'N' and 'O'. Log P or partition coefficient of any compound plays a major role in its distribution in the biological system while crossing the membranes and dissolving in body fluids. The selected quinazoline derivatives have been subjected to the prediction of LogP based on various algorithms and calculation approaches. The first one is iLogP, which is a physics-based method and has been found that the tested compounds possess the iLogP value that ranges between 2.68 to 3.26 (12). XLOGP3 Atomistic is a knowledge-based method calculated by XLOGP3 program and the compounds have shown an XLOGP3 value of 2.63 to 4.14. Similarly, the WLOGP3 Atomistic methodhas been found to have values between 3.16 to 4.1 (28). MLOGP is a topical method, and the compounds have been found to have a minimum value of 1.39 and a maximum of 2.76 (14, 15). SILICOS-IT is another algorithm based on calculation with hybrid fragmental/ topological method calculated by FILTER-IT program, and it was spanning around 2.4 to 3.07. But each program or algorithm prediction has given different values for the individual compounds, so a consensus Log P was taken as an average of all the five methods, which was between 2.47 to 3.05. It was observed that all the compounds haveLogP value below 5 and satisfied the Lipinski RO5 rule.

The water solubility prediction was carried out by the SwissADME online tool. The ESOL was predicted and that the compounds 5a and 5bhave been found soluble with a LogS value of -3.78 with a solubility of 0.0567 mg/ mL. Compounds 5c to 5g were moderately soluble with a LogSvalue of -4.14 to -4.69 and solubility range of 0.00691 to 0.027 mg/mL. All compounds were moderately soluble with a LogS value between -4.43 to -5.58; and the solubility was 0.0009 to 0.0126 mg/mL. As per the Silicos-IT calculations, all compounds have been predicted to be poorly soluble with a LogS value of -6.17 to -6.58; and the solubility in between 0.000088 to 0.00027 mg/ mL. The results have shown some ambiguity about water solubility and it was assumed that the designed compounds possess moderate solubility in water based on ESOL and Ali predictions (29).The pharmacokinetic properties like absorption, distribution and protein binding along with skin permeation were predicted. All the tested molecules have been found to possess high gastrointestinal absorption as per the BOILLED Egg method. The compounds were not permeable through the BBB and

not a substrate for the p-glycoproteins. Most of the compounds have been predicted to inhibit the CYP enzymes such as CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 responsible for xenobiotic metabolism. The skin permeation has been predicted and was found that the compounds have the logKpvalue of -5.42 to -6.45 cm/s, and they are suitable for topical application on the skin also.None of the compounds have violated the drug-likeness rules such as Lipinski, Ghose, Veber, Egan, and Muegge theories. The bioavailability of all the compounds has been predicted as 0.55. There were no PAINS and Brenk alerts for any of the tested compounds. All compounds were predicted as lead-like compounds and the synthetic feasibility has been defined at 2.7 to 3.04 and practically synthesizable. In the BOILED-Egg method prediction, the passive gastrointestinal absorption and ability of brain entrance of small molecules were assessed. While the compounds that permeate through the BBB to enter the CNS with high probability are represented in yellow ellipse, compounds to be passively absorbed have been represented in white ellipse. Molecules in the grey zone are not well absorbed by GIT and do not cross BBB. The tested compounds arein white space and possess the passive GIT absorptionand may not cross the BBB. The red color points indicate that these compounds do not act as substrates to the P-glycoproteins (PGP).

***Osiris property explorer*:** The quinazoline derivatives have been subjected to the OSIRIS property explorer server to predict their mutagenic, tumorogenic, irritative and reproductive toxicity effects. The results gave the drug-likeness score and total drug score for each molecule. All the quinazoline derivatives did not show any kind of toxic effects, but the standard drug tamoxifen displays reproductive toxicity effect and 5-fluoro uracil (5-FU) exhibits all the toxic effects. While compound 5b has shown mutagenicity, the remaining did not show any toxicity. The drug score of the compounds has been found as 0.29 and 0.55. The drug-likeness score of tamoxifen and 5-FU have been found as 6.3 and -4.5 andthe drug-scores 0.35 and 0.06 respectively. Comparatively, the tested molecules have been found to be better than standard drugs in terms of toxicity and total drug score profile.

Pro Tox-II: The quinazoline derivatives have been subjected to toxicity prediction by ProTox-II and for measuring their LD_{50} and classification. The compounds have been foundin class 3 toxicityand if swallowed they arefatal to human being at a concentration of $50 < LD_{50} > 300$ mg. The lethal dose is 125 mg/kg weight for all the compoundsand the average similarity of the designed compounds ranged between 55.23 to 65.65%. The prediction accuracy was 67.38 for compounds 5a, 5g and 68.07 for the remaining compounds.The ProTox-II platform is divided into five different classification steps: (i) acute toxicity (oral toxicity model with six different toxicity classes); (2) organ toxicity (one model); (3) toxicological endpoints (four models); (4) toxicological pathways (12 models), and (5) toxicity targets (15 models). The probability score for hepatotoxicity of the designed compounds has fluctuated between 0.50 to 0.58. The probability score for carcinogenicity of the compounds has been 0.55 as minimum and 0.61as maximum. Whileimmunotoxicity stretched between 0.89 to 0.99,mutagenicity differed from 0.58 to 0.70. The cytotoxicity probability has extended up to 0.62 to 0.71 for the tested compounds. Certainly, the compounds possess immunotoxicity, but needto be experimentally verified through*in vivo* experiments.For Tox21-nuclear receptor signaling pathways, several parameters such as AhR, AR,AR-LBD, Aro, ER, ER-LBD, and PPAR-gamma were predicted for the designed compounds. Except AhR, for the remaining protein pathways the compounds have shown inactive probability. These resultssuggest that these compounds exhibit not only weak estrogenic, but also antiestrogenic, antiandrogenic, and anti-TH activities via ER, AR, and TR pathways.For Tox21-stress

Table 2. Molecular docking data of the quinazoline compounds by maestro

Compound No	Docking score	Glide energy	XP HBond
5A	-5.994	-60.907	-0.275
5B	-8.539	-69.487	-0.896
5C	-7.032	-62.291	-0.896
5D	-5.81	-43.219	-0.343
5E	-5.773	-35.714	-0.343
5F	-3.644	-29.738	-0.299
5G	-2.525	-35.296	-0.275

response pathways, parameters like nrf2/ARE, HSE, MMP,p53, and ATAD5 have been studied. All the compounds displayed active probability for the nrf2/ARE and HSE stress response. The compounds have also exhibited inactive probability for the rest of the stress response pathways.

***PASS Online and Docking Studies*:** PASS Online predicted over 4000 types of biological activities, such as pharmacological effects, mechanisms of action, toxic and adverse effects, interaction with metabolic enzymes and transporters, influence on gene expression, etc. We predicted the biological activity ofquinazolines by submitting the molecules to the PASS Online server. The results revealed antimitotic and antineoplastic activity.In this study, we selectedcaspase-9as a target protein with the resolution of 2.80 Å crystal structure of the protein (PDB ID: 2AR9) for preliminary docking analysis(30). The structure of the protein comprises 4 chains of 278 amino acid residues complexed with MLT ligand. The MLT is malic acid and found to form H-bonding interaction with Arg 217 and hydrophobic interactions with Trp 216, and ASP218. The docking score of selected molecules, number of hydrogen bonds, amino acid residues, and glide score of the molecules with the protein are shown in Table 2. The compound 5bhas been found to possess a significant binding affinity with the cavity of the protein by forming hydrogen bond interactions with Thr166

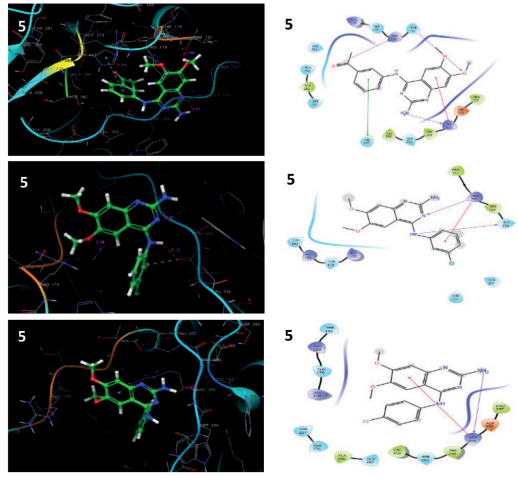

Fig. 2. 3D and 2D representation of H-bond interactions of quinazoline derivatives with caspase-9 (2AR9)

(2.47Å), Leu168(1.92Å), Arg144(2.36Å), and Gly122 (2.33Å) (30). Over and above, it forms hydrophobic interactions with the residues Glu167, Asp169, Cys170, Val189, Tyr203 and formed pi-pi stacking with His121. The hydrophobic interactionbond lengths fluctuate from 3.39 to 3.94 Å with the glide G-score and energy levels of -8.539 and -69.907kcal/ molrespectively (Figure2). The compound 5c has been found to form H-bond interactions with Thr 166 (2.07Å) and hydrophobic interactions with Gly165, Glu167, Leu168, Phe256, Lys259 and Thr255 with bond lengths ranging from 3.52 to 3.79Å. Most of the residues interacting with5b and 5c formed within the active site of the protein. The docking score of the protein 5c complex was -7.032 with the corresponding Glide energy of -62.291kcal/mol. This has been noticed as the best among the three compounds employed in this study. Similarly,the remaining compounds also exhibited reasonably good interactions with the binding site amino acid residues with a good binding score and energy.

Conclusions

Novelquinazoline molecules have been designed in the present study by considering the privileged medicinal scaffold pyrimidine and

with the incorporation of substituted aromatic groups. It is hoped that these compounds interact with caspase-9 protein and display anticancer properties.The designed compounds were subjected to*in silico* screening followed by molecular docking analysis. Most of the compounds have been found to interact with caspase-9 with H-bonding interactions. Results of the preliminary screening show that these compounds have drug-likeness and are devoid of toxic properties such as tumorogenicity, mutagenicity and hepatotoxicity. The results indicate that these scaffolds could be further established as possible anticancer agents by the structural modification in the quinazoline nucleus for enhancing anticancer efficacy.

Acknowledgements

The authors acknowledge Prof. M. Vijjullatha, Department of Chemistry, Osmania University, Hyderabad for providing the compounds. PBK thanks VFSTR, Guntur for providing emeritus fellowship.

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