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In-silico screening of 2,3-diphenylquinozaline derivatives as C-met kinase inhibitors

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Abstract: Quinoxaline, an important class of heterocylic compounds drawn greater attention due to their wide spectrum of biological activities. They are considered as an important chemical scaffold for anticancer drug design due to their potential inhibitory activity against C-met tyrosine kinase. C-met kinase inhibitors are a class of small molecules that having therapeutic potential in the treatment of various types of cancers. The present study aims to focus on the chemistry of quinoxaline derivatives, their potential activities against C-met tyrosine kinase, and in-silico screening of designed compounds. A series of twelve compounds were designed and docked against C-met tyrosine kinase for their binding energy. All compounds were found to be interacting well with the protein. Compound NQ1 was found to have good binding energy showing an estimated Ki value of 1.1µm. SAR study indicated the presence of an electron withdrawing substitution on benzylidine phenyl ring of quinoxaline greatly improves its binding interaction with the protein.

Keywords: Anticancer; Quinoxaline; C-met Kinase; Molecular docking; AutoDock

1. Introduction

Quinoxaline also called as benzopyrazine. It is isomeric with naphthyridines, phthalazine, quinazolines, and cinnoline phthalazine and cinnoline (Figure 1). They are an important class of nitrogen-containing heterocycles as they constitute useful intermediates in organic synthesis.[1] This substructure plays an important role as a basic skeleton for the design of a number of heterocyclic compounds with different biological activities like antitumor[2-3], anticonvulsant[4], antimalarial[5], anti-inflammatory[6-7], antiamoebic[8], antioxidant[6,9], antidepressant[10], antiprotozoal[11], antibacterial[12], antinociceptive agents[13] and anti-HIV agents[14]. Figure 2 lists the drugs with quinoxaline ring that are available in the market for the treatment of human ailments.

C-met is a protein encoded by MET gene. Since it is having tyrosine kinase activity, it is otherwise called as C-met tyrosine kinase and hepatocyte growth factor receptor (HGFR). It is essential for embryonic development, wound healing and organ morphogenesis.[3] Overexpression of C-met has been reported in different cancer cell lines. Inhibition of C-met tyrosine kinase activity was found to inhibit cancer progression. Many C-met inhibitors are in clinical trials, while 2 drugs Crizotinib and Cabozantinib were approved by US-FDA for the treatment of lung cancer and medullary thyroid cancer, respectively in 2012.[15-17] Figure 3 lists the C-met inhibitors approved and that are in clinical trials. 2,3 diphenyl quinoxaline derivatives were reported for their anticancer as well as C-met kinase inhibitory activity.[18-21] With this background, we designed twelve diphenylquinoxaline derivatives as shown in the Figure 4 and subjected them to in silico evaluation for the possible inhibitory activity on C-met tyrosine kinase.

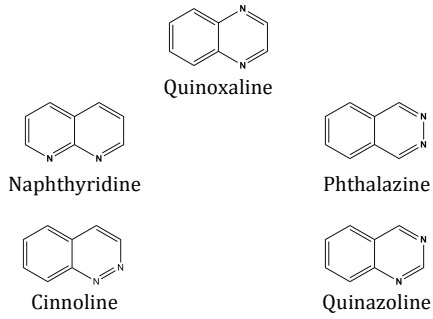
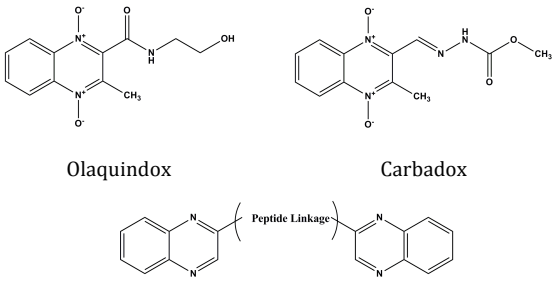


Figure 1. Structure of quinoxaline and their isomers



Quinomycin A (Echinomycin, Levomycin and ActinoleuKin)

Figure 2. Drugs with quinoxaline ring

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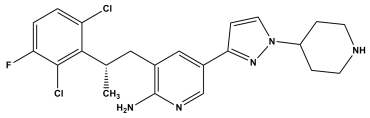
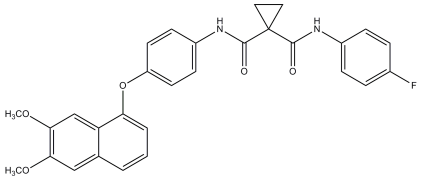
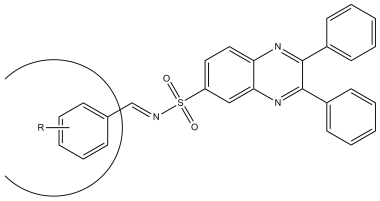


Crizotinib



Cabozantinib

Figure 3. Structure of C-Met inhibitors



R = NO_2, O- Cl, 4-Cl, 4-O-CH_3, 4-CH_3, H, 4-N(CH_3)$_2$, Ar-CH=CH, 4-NH-CO-CH_3, 4-CCl_3, 4-SO_3H, 4-COOH.

Figure 4. Structure of designed library

2. Result and Discussion

Crystal structure of the tyrosine kinase domain of the hepatocyte growth factor receptor C-met (pdb code: 1r1w)[22] was used for simulation studies. The standard drug Nilotinib was used for the validation of docking program and protocol.

The designed compounds were checked for the drug likeness property. Lipinski's Rule of Five (Ro5) violations were calculated through molinspiration online tool (http://www.molinspiartion.com/cgi-bin/properties). All the twelve compounds exhibited drug-like property has no violation was observed for Lipinski's Ro5 parameters (Table 1). The molecules were further subjected to molecular docking studies using Autodock 4.2.

Docking is an approach to rational drug design that seeks to predict the binding mode and binding free energy of ligand-receptor complex. It gives an idea about how the legend is going to bind with the receptor and conformational changes possible upon binding with the receptor. The docking studies of designed analogues NQ(1-12) and Nilotinib were performed using molecular modeling software Autodock 4.2 with Autodock tools-1.5.6.

Estimated Binding Energy (Kcal/mol) and Estimated Ki values (μm) obtained for the compounds and the standard are presented in Table 2. The results reveal that the compounds NQ1 and NQ11 showed better docking score with the highest rank. Among these two compounds, the compound NQ1 interacted with the amino acids of Gly1224, Arg1227, Arg1203, Tyr1230, Asp1222. Meanwhile, the compound NQ11 interacted with the amino acids of Ala1208, Asp1204, Asp1222, Trp1209, and Gly1224. On the other hand, when compared with that of standard Nilotinib, it has shown interaction with Asp1222, Asp1204, Tyr1230, Arg1227, Phe1223, Phe1200, and Arg1203. The key amino acids responsible for interactions are mentioned in Figure 5-8. It was observed from the test set that molecule NQ1 could be a better inhibitor, as it has shown up the better score when compared with that of standard Nilotinib by interacting with the similar amino acid residues Arg1227, Phe1223, Phe1200, Arg1203, which is may be due to the presence of an electron withdrawing group.

3. Conclusion

Quinoxalines represent an important class of nitrogen containing heterocycles and have been publicized to possess a broad spectrum of biological activities including anticancer and kinase inhibitors. The molecular docking simulation with the newly designed molecules have shown that three of them having binding energy comparable with that of standard used in the study. As they have also displayed the drug-like properties without any deviation RO5. Further synthesis and experimental evaluation may provide newer lead compounds with potential anticancer property.

4. Experimental

Materials and methods: In the present investigation, molecular docking methodology was implemented by AutoDock tools-4.2 and Autodock tools 1.5.6 (ADT; The Scripps Research Institute, Molecular Graphics Laboratory, 10550 North Torrey Pines Road, CA, 92037). Construction and energy minimization of ligands were done with ChemDraw Ultra 8.0 and Chem3D ultra 8.0 (Cambridge Soft.Com, 100 Cambridge park drive, Cambridge, MA 02140, USA). Molecular properties were calculated through the molinspiration web-resource. Hardware: HP 64-bit machine with processor- Intel(R) Core(TM) i3-4005U CPU @ 1.70GHZ, RAM- 4.00 GB running on Windows operating system.

Table 1. Lipinski's Rule of Five parameters for compounds NQ1-12

Code	R	MF	MW	Log P	H-bond donor	H-bond acceptor	No of Violations
NQ1	4-NO_2	$C_7H_5NO_3$	494.52	4.82	8	0	0
NQ2	2-Cl	C_7H_5ClO	483.96	4.88	5	0	0
NQ3	4-Cl	C_7H_5ClO	483.96	4.88	5	0	0
NQ4	4-OCH_3	$C_8H_8O_2$	479.56	4.92	6	0	0
NQ5	4-CH_3	C_8H_8O	463.56	4.97	5	0	0
NQ6	-H	C_7H_6O	449.52	4.90	5	0	0
NQ7	4-N(CH_3)$_2$	$C_9H_{11}NO$	492.59	4.95	6	0	0
NQ8	-Ar-CH=CH	C_9H_8O	475.56	4.99	5	1	0
NQ9	4-NH-CO-CH_3	$C_9H_9NO_2$	495.62	5.00	7	1	0
NQ10	4-C(Cl)$_3$	$C_8H_5OCl_3$	499.76	4.85	5	0	0
NQ11	4-SO_3H	$C_7H_6SO_3$	490.23	2.79	8	1	0
NQ12	4-COOH	$C_8H_6O_2$	493.54	4.92	7	1	0

Table 2. Docking results of synthesized quinoxaline derivatives

Code	Binding energy (Kcal/Mol)	Inhibition constant (μM)	No. of H-bonding interaction	Rank
NQ1	-8.04	1.28	2	2
NQ2	-7.72	2.19	0	6
NQ3	-7.29	4.5	1	10
NQ4	-6.89	8.83	0	11
NQ5	-7.76	2.04	0	4
NQ6	-6.59	14.69	0	12
NQ7	-7.74	2.13	0	5
NQ8	-7.68	2.33	1	7
NQ9	-7.36	4.01	0	9
NQ10	-8.01	1.34	1	3
NQ11	-8.13	1.1	1	1
NQ12	-7.41	3.73	1	8
Nilotinib	-8.07	1.22	2	0

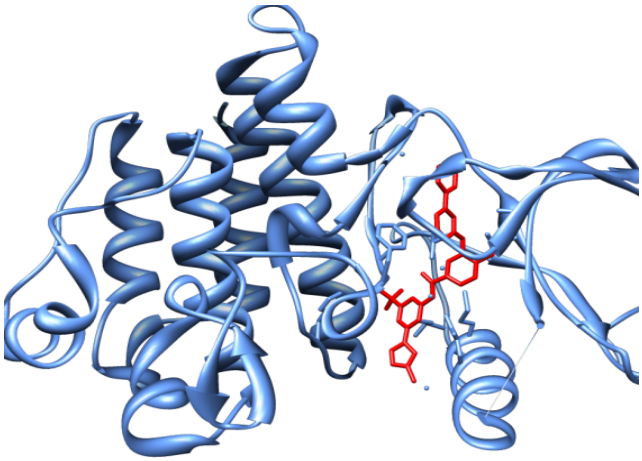


Figure 5. Docking pose of standard **Nilotinib** with the macromolecule (1r1w). The red colour represents the ligand and blue color shows protein.

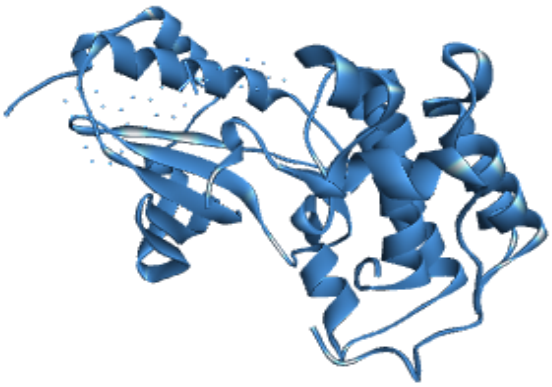


Figure 6. Dockng pose of the quinoxaline derivative **NQ1** with the protein 1r1w. Dotted lines represents the ligand molecule and ribbon represents macromolecule.

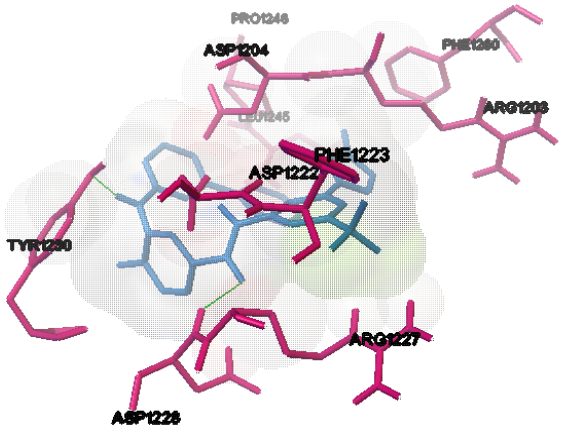


Figure 7. Docking interaction of standard drug Nilotinib with 1r1w. Ligands in blue sticks and aminoacids like Tyr, Asp, Arg, Phe etc., were shown in red sticks. The H – bonding interactions were shown in green dotted lines.

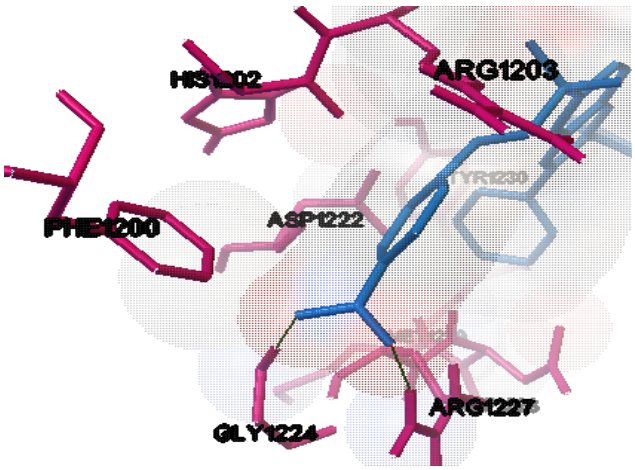


Figure 8. Asp 1222, Gly 1224, Arg 1227 are the important amino acids involved in hydrogen bond interaction which is represented as green lines. Blue sticks represents the ligand molecule NQ_1 and the magenta stick shows the protein.

4.1. Preparation of Enzyme Structure

Crystallographic model of C-met kinase (PDB code: 1r1w) was retrieved from www.pdb.org. In the pdb format so the attack tool can assess it. Protein preparation was done with the minimal options available in the Autodock tools- 1.5.6. Water molecules were deleted, hydrogens were added using polar only option, AD4 atoms types were assigned, gasteiger charges were added and finally saved as protein.pdbqt file.

4.2. Preparation of Ligands

All the designed ligands were built, 3D optimized and energy minimized using ChemDraw Ultra 8.0 version and saved in ligand.pdb formats. The ligands were then imported in ADT for assigning charge and torsion. Finally, it was saved as ligand.pdbqt.

4. 3. Docking Methodology

Map types were generated for the atom types in the ligand library for calculation interaction energy between ligands and protein. It was done by generating grid box covering the active sites with the following parameters: Box dimension of 60x60x60 in xyz with grid spacing of 0.375Å and the grid box was centered on C-met receptor (X-center; 16.431, Y-center; 20.803 & Z-Center; 141.01). The docking parameter file was saved as protein.gpf and maps were generated using autogrid 4.2. The docking parameter file was written with the following parameters. 10 GA runs, 150 GA population and 27000 GA generations keeping all the other parameters at default values. The parameter file was saved as ligand.dpf and docking simulations were executed using autodock 4.2. Analyze option in the AutoDock tools 1.5.6 was used to analyze the dockings. The top-scoring molecule in the highest cluster was examined for their interaction with the active site residues of the protein.

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