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RATIONAL DRUG DESIGN OF POTENT V600E-BRAF KINASE INHIBITORS THROUGH MOLECULAR DOCKING SIMULATION

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ABSTRACT

B-RAF is a one of the RAF protein kinase group that contribute to the development of different types of cancer. V600E-BRAF protein has lot potential for scientific investigation as the therapeutic target owing to its participation in melanoma cancer and is the molecular target of many anticancer compounds like quinolinylaminopyrimidines (QAP) derivatives. In this research, interactions of QAP derivatives with V600E-BRAF kinase were modeled and predicted using molecular docking simulation approach with the help of Autodock vina version 4.0 of Pyrx software. The molecular docking simulation result of this research shows that QAP6 (−11.7 kcalmol^{-1}) best inhibit V600E-BRAF when compared with other QAP analogous within the dataset and was found to be better than the standard V600E-BRAF inhibitor vemurafenib (-11.3 kcalmol^{-1}). This compound (QAP6) were further used in designing novel and potent V600E-BRAF inhibitors by attaching substituents to the quinoline ring of the compound. Moreover, the two newly designed inhibitors N1 and N2 with a binding energy of −12.7 kcalmol^{-1} and –12.9 kcalmol^{-1} respectively were found to be more potent than the parent structure QAP6 (−11.7 kcalmol^{-1}) and the standard V600E-BRAF inhibitor vemurafenib (-11.3 kcalmol^{-1}). Thus; this study provides a valuable approach and new direction to novel drug discovery. There is hope in the future studies to include the synthesis and evaluation of these newly designed inhibitors which can establish them to be the most potent V600E-BRAF inhibitors and efficient ant-melanoma cancer drug.

1. INTRODUCTION

Malignant melanoma is the most dangerous form of skin cancer caused by the abnormal formation of melanocytes (Cummins et al., 2006). It covers only ten percent (10%) of skin cancers, but it is consequences results for most of skin cancer deaths (Jemal et al., 2006). Mutations in various outgrowth genes led to activation of more fold related-cancer signaling pathways, then followed by uncontrollable propagation and spreading to melanocytes. The oncoprotein BRAF, as discovered in 1988 is responsible for nearly sixty six percent (66%) of melanomas and twelve percent (12%) of colorectal cancers (Dhillon et al., 2007). BRAF is the main target of therapies, being it the most regularly mutated protein kinase in human cancers (Bollag et al., 2010). Furthermore, nearly seven percent (7%) of all cancers, BRAF gene mutations can leads to MAP kinase pathway over-activation (Ren et al., 2011). The most frequent mutation of BRAF, among more than 30 mutations of BRAF (Namba et al., 2003), is V600E (Puzanov et al., 2015). The V600E-BRAF mutation ended in 500-fold greater constitutive kinase activity when compared to other BRAF wild kind, and many inhibitors of V600E-BRAF have been designed(Wang et al., 2011, Li et al., 2014).

Several potent drugs are now available in clinical trials against melanoma including kinase inhibitors with different degree of success. Sorafenib one of the multi-kinase inhibitor, which inhibit EGFR tyrosine receptor kinase, BRAF serine and threonine kinase. US FDA approved Sorafenib for the treatment of certain kinds of cancer, among which are hepatocellular carcinoma, advanced renal cell carcinoma and radioactive iodine-resistant advanced thyroid carcinoma (Shi et al., 2015). vemurafenib (Zelboraf 1), a more distinct BRAF inhibitor was approved in 2011 by the FDA for melanoma (metastatic) and is under consideration for thyroid and colorectal cancers (Wu and Ambudkar, 2014). Since almost fifty percent (50%) of BRAF mutations are relate to have the V600E mutation, therefore vemurafenib is extremely important anti-metastatic and anti-melanoma drug due to its explicit inhibition of V600E-BRAF (Robinson et al., 2014).

However, Treatment with the use of BRAF inhibitors can result in the development of inhibitor (drug) resistance which restrict their usage (Zubrilov et al., 2015). Melanoma (Metastatic) is particularly dangerous form of cancer that has a very bad prognosis and is resistant to many standard anti-cancer therapies, this helps these cancer cells to evade the immune system. Mutations (Genetic) can as well accumulate which may activate other signaling pathways (Saini et al., 2013). Majority of patients that were administered vemurafenib (standard V600E-BRAF inhibitor) eventually develop resistance towards it. Therefore, identifying other BRAF inhibitors and development of novel drugs against melanoma is of great importance for cancer (Melanoma) research (Roskoski, 2012).

Furthermore, Molecular docking simulation is a computational technique used to predict the binding ability of the active site residues to specific groups on the receptor and to reveal the strength of interaction (Bollag et al., 2010). Molecular docking is a very useful and popular tool used in the drug discovery arena to evaluate the binding of small molecules (inhibitors) to the receptor (macromolecule) (Abdulfatai et al., 2017). In this research, compounds that are similar structurally to quinolinylaminopyrimidines were assessed to predict the most potent compounds. The aim was to establish V600E-BRAF inhibitors in place of vemurafenib that have the same therapeutic properties and efficacy through a molecular docking simulation.

2.0 Materials and Method

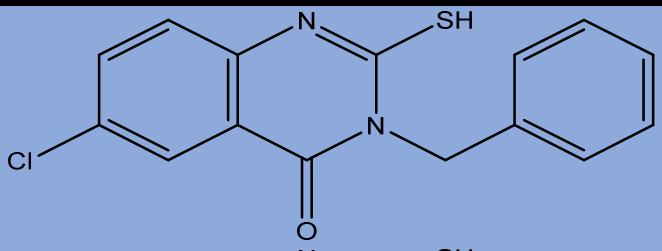
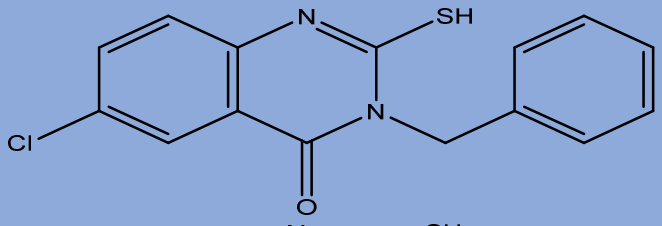
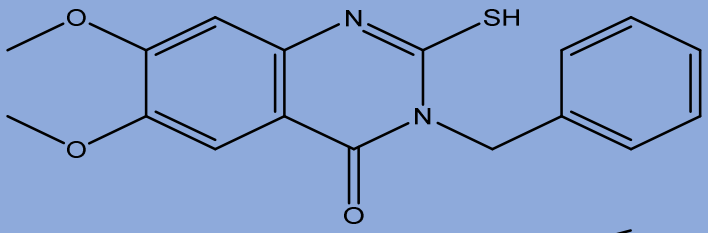
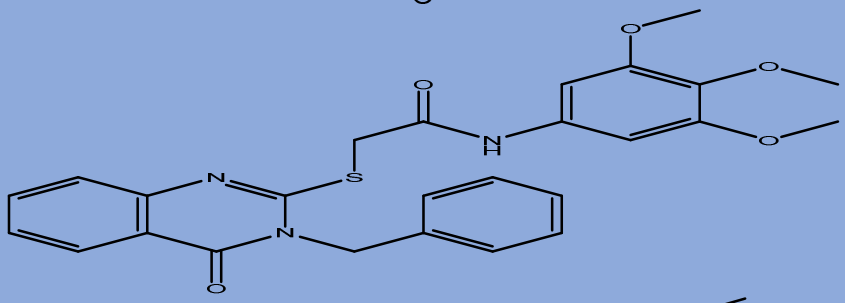
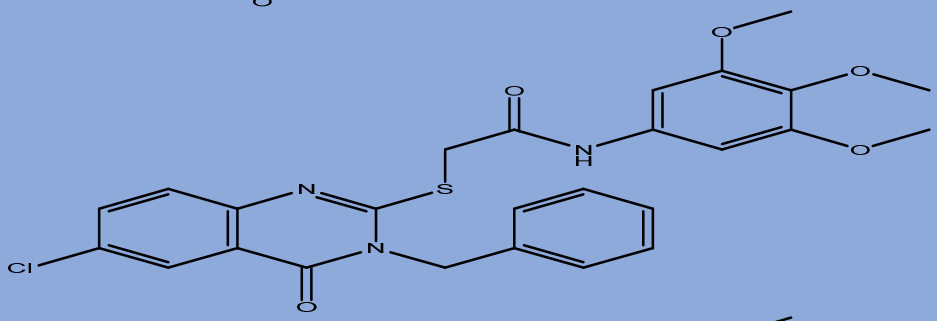
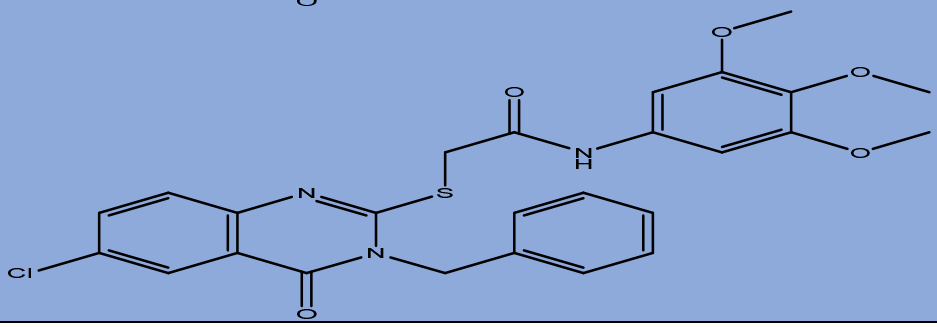
2.1 Hardware and Software Specifications

All the molecular docking studies were carried out on a Dell Intel(R)Core(TM)i7-5500U CPU), 16.00GB RAM @ 2.400GHz 2.400GHz processor, 64-bit Operating system, ×64-based processor on Windows 8.1 Pro). Ligands and receptor preparation was carried out utilizing Discovery Studio and the docking was run by employing Pyryx. Spartan 14(Hehre and Huang, 1995) was employed to perform density functional theory calculations.

2.1.1 Ligand selection

In this research, a data set of 11 quinolinylaminopyrimidines (QAP) analogous and their anti-proliferative activities towards A375P human melanoma cell line were taken from (Lee et al., 2015). Their structure and anti-proliferative activity results as IC_{50} were presented in Table 1.The selected ligands were selected on the bases of chemical properties, thus, are molecular weight, H-bond acceptor, H-bond donor, Log P, and topological polar surface area (Table 2). rule of five (Lipinski's) was checked for the 11 analogous, out of which all the selected ligands has passed the test (Lipinski et al., 2012). In addition to studied ligands, vemurafenib was used as control in the present study.

Table 1-Structure and Antiproliferative activity of quinolinylaminopyrimidines (QAP1-QAP11) against A375P human melanoma cell line

Ligand	STRUCTURE	IC50
QAP1		6.5
QAP2		15.3
QAP3		14.0
QAP4		9.3
QAP5		>20
QAP6		9.2
QAP7		>20

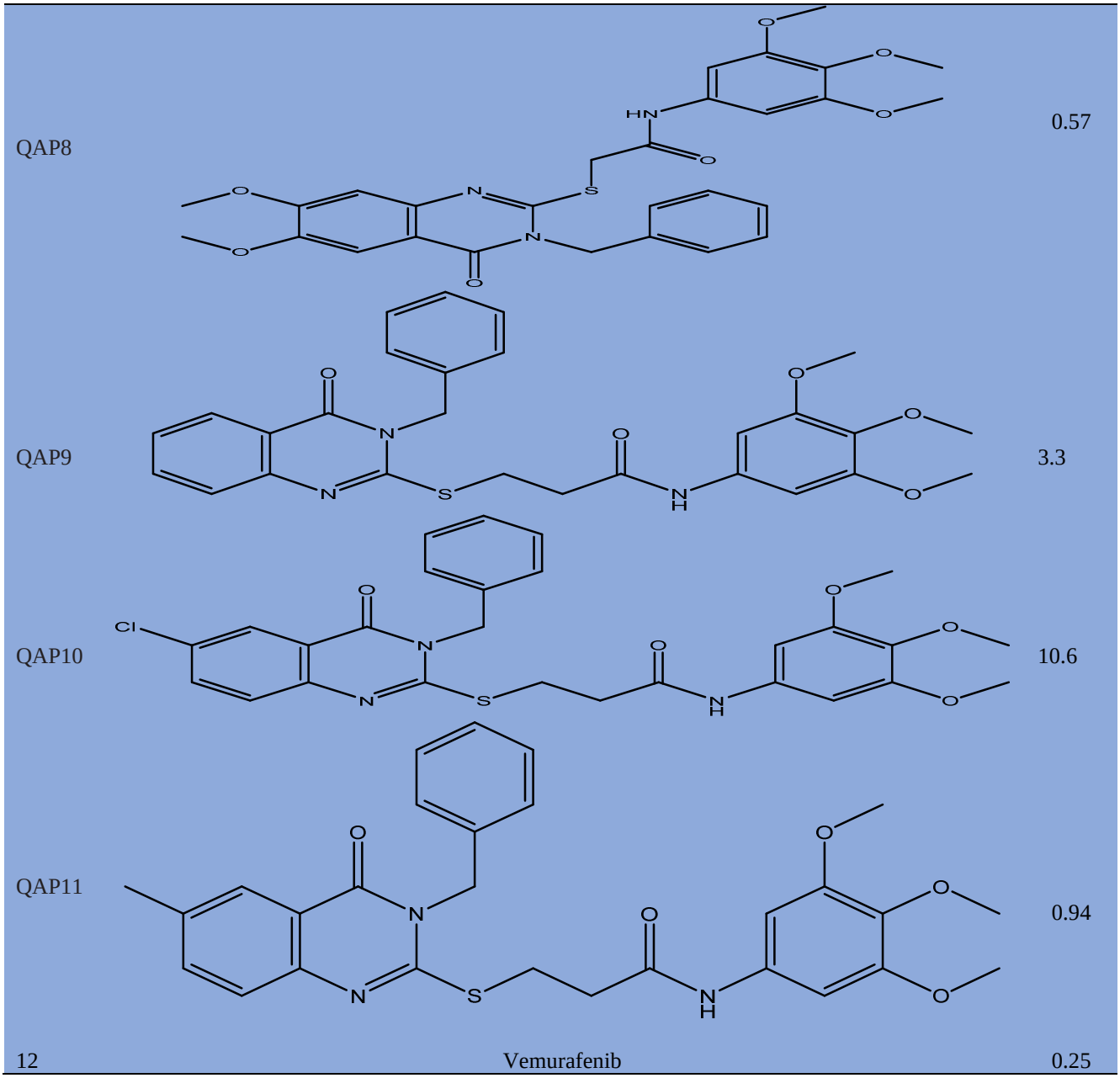


Table 2-Physico-chemical properties of selected ligands.

Ligand	MW (Da)	nHBA	nHBD	LogP	TPSA (Å^2)
QAP1	356.433	5	2	0.06	78.153
QAP2	396.376	5	2	0.32	76.951
QAP3	430.821	5	2	0.18	74.978
QAP4	414.366	5	2	-0.22	76.128
QAP5	464.373	5	2	0.93	72.834
QAP6	420.476	6	2	-0.79	84.952
QAP7	367.416	6	2	-0.40	93.537
QAP8	396.376	5	2	0.32	76.251
QAP9	430.821	5	2	0.18	76.144
QAP10	430.821	5	2	0.18	76.116
QAP11	464.373	5	2	0.93	76.261
Vemurafenib	489.930	7	2	-0.43	81.736

2.1.2 Ligand preparation

The selected ligands was prepared and optimized for docking, the 2D structures was drew using Chemdraw Ultra 12.0 and were converted to the 3D structure using Spartan 14. The structures was cleaned by minimizing and checking using a molecular mechanic force field (MM+) option on Spartan 14, so as to remove all strain from the structure of the

molecule. Additionally, this will guarantee a well-defined and stable conformer relationship within the compounds in the study (Viswanadhan et al., 1989). Geometry optimization was set at the ground state utilizing the density functional theory (DFT) at the Becke88 three-parameter hybrid exchange potentials with Lee-Yang-Parr correlation potential (B3LYP) level of theory and for the basis set 6-311G (d) was selected and all the ligands was formatted to pdb files.

2.1.3 Receptor preparation

The x-ray structure of V600E-BRAF kinase (receptor) in complex with vemurafenib (PLX4032) (PDB-code: 3OG7) (Brose et al., 2002, Bollag et al., 2010, Choi et al., 2011) was retrieved from (www.rcsb.org). V600E-BRAF was imported into Discovery studio and the PDB file was prepared by removing the excess water molecules attached in the x-ray structures and updating the hydrogen atoms. Other amino acids like proline, Histidine, cysteine, glycine, etc., that were found missing chains (side) was as well treated before their used for the docking simulation. This complex structure consists of two homo dimeric chains A and B. Our goal was to target the mutated chain (chain A) of V600E-BRAF. Therefore, the chain B was deleted from the structure of 3OG7 and the bound inhibitor also extracted from chain A.

2.1.4 Docking process

Molecular docking is considered the most appropriate way to stumble on bioactive conformations of compounds with their corresponding receptors. All ligands from the data set were docked into the active kinase domain of V600E-BRAF using Autodock vina of PYRYX docking program software. To illustrate interactions (inter-molecular), for example h-bonds, hydrophobic, halo-bonds and aromatic/π interactions). The 3D and 2D interactions for the docking simulation was obtained by importing the result into the visualizer (Discovery studio visualizer), thus in order to identify the most significant interaction between the inhibitors (ligands) and the corresponding receptor used in the molecular docking simulation.

2.1.5 Designing V600E-BRAF kinase inhibitor

The docking result of the inhibitors (ligands) docked against V600E-BRAF target was reviewed, and the best interactions of the ligands was further studied. Some of Their molecular descriptors and bulky side groups were altered in order to improve their effect (poisonous) on the V600E-BRAF target. This was accomplish by introducing some relevant substituents found to interact strongly on the binding segment of the receptor when docked.

3.0 Result and Discussion.

3.1 Docking results.

All the ligands from the data set were docked into the active kinase domain of V600E-BRAF using PYRYX docking program and the desirable conformations of the studied ligands was identified. Based on the binding energies of the studied ligands with the type of interactions involved, it is found that the ligands were sufficiently bonded to the active site and show similar orientations in some instances and comparable with the standard drug used as control. To further analyze the interaction, the values of the binding energies are leveled from the most active to the least active ligand and were all computed and reported in Table 3.

Table 3-Docking information for the interaction of QAP derivatives docked to kinase domains of BRAF (PDB ID: 3OG7).

Molecular system	Binding Energy(kcal/mol)	Hydrophobic Interaction	Electrostatic Interaction/Others	Hydrogen Bonds	Hydrogen Bond Distance (Å)
BRAF/QAP1	-9.0	TRP531, ALA481, LYS483, ILE527, ILE463 and ALA481		GLY534	2.84388
BRAF/QAP2	-9.8	LYS483, ILE527, VAL471 and ILE463		LYS483	2.80349
QAP3	-10.2	ILE463, LEU505, ILE527, LYS483 and LEU514	LYS483	ASP594, GLY596 and TRP531	2.67905, 2.6292 and 2.97165
BRAF/QAP4	-10.3	TRP531, PHE583, LYS483, ILE527, ILE463 and ALA481		LYS483, LYS483, ASP594, GLY534, CYS532 and GLY534	2.32278,2.55,2.56039,2.62756, 2.504 and 2.69374
BRAF/QAP5	-10.5	ILE463, ALA481, VAL471, LYS483, ILE527 and LEU514	CYS532, ALA481, ALA481, VAL482, ILE527 and LYS483	LYS483, LYS483, THR529, ASP594, CYS53 and TRP531	2.68111, 2.58178, 2.30248, 2.70343, 2.65501 and 2.40611

BRAF/QAP6	**-11.7**	**LEU505, LEU514, THR529, TRP531, ILE463 and LYS483**	**LYS483**	**ASP594, THR529, CYS532 and GLN530**	**2.15618,2.93365, 2.01085 and 3.56215**
BRAF/QAP7	-9.2	LYS483, VAL471, ALA481 and LYS483	ILE527	LYS483, LYS483, ASP594, GLY534 and GLY534	2.41136, 2.8364, 2.48139, 2.71377 and 2.62584
BRAF/QAP8	-10.3	LYS483, LEU505, ILE527, LEU514 and VAL471	LEU514	ASP594	3.01885
BRAF/QAP9	-9.1	ILE463, ALA481, VAL471, LYS483, ILE527 and LEU514	CYS532, ALA481, ALA481, VAL482, ILE527 and LYS483	LYS483, LYS483, THR529, ASP594, CYS53 and TRP531	2.68111, 2.58178, 2.30248, 2.70343, 2.65501 and 2.40611
BRAF/QAP10	-10.2	LEU505, LEU514, LYS483, ILE527 and VAL471	LEU514 and GLY596	GLY596 and ASP594	3.41703 and 2.97842
BRAF/QAP11	-11.0	LEU505, LEU514, LYS483, ILE527 and VAL471	LEU514 and GLY596	GLY596 and ASP594	3.41703 and 2.97842
Vemurafenib	-11.3	TRP531, PHE583, CYS532, ALA481, LEU514, LYS483 and ILE463	LYS483	PHE595, GLY596, CYS532 and GLN530	2.66721, 2.06321, 3.0153 and 2.35105

Based on their obtained excellent binding energies these ligands are arranged in the order; **QAP6 >** QAP11 > QAP5 > QAP4, QAP8 > QAP3, QAP10 > QAP2 > QAP7 > QAP9 > QAP1. The most important residue for hydrogen bond interactions for the studied ligands was LYS483, THR529, ASP594, and GLN530 through the pyrimidine ring of the studied ligands. Also, the most important residue for hydrophobic interactions for these ligands was LYS483, LEU514, and ILE463, which show good similarities and to some extent with the standard V600E-BRAF inhibitor vemurafenib.

QAP6 was selected as the best ligand docked on the active segment of V600E-BRAF kinase with the binding energy of -11.7 kcal/mol (Table 3).This docking simulation research revealed that QAP6 was found to bound in the active segment on the protein dimer due to the formation of four (4) hydrogen bonds with ASP594 (2.15618Å), THR529 (2.93365 Å), CYS532 (2.01085Å) and GLN530 (3.56215 Å). Furthermore, there is one pi-pi interaction which appear between the binding segment of the receptor and the QAP6 ligand which happened between quinoline segment and TRP531. There was also pi-sigma interaction between the ring (aromatic) of the ligand and the aliphatic site of LEU514, LEU505 and THR 529 as shown in Figure 1.The obtained results of this molecular docking simulation suggest that the selected active compound (QAP6) can inhibit the growth of the melanoma cell lines by inhibiting the V600E-BRAF kinase which support the experimental finding in table 1 as this ligand was found to be more potent with IC50 = 9.2 than the standard the V600E-BRAF inhibitor Vemurafenib (IC50 = 0.25).

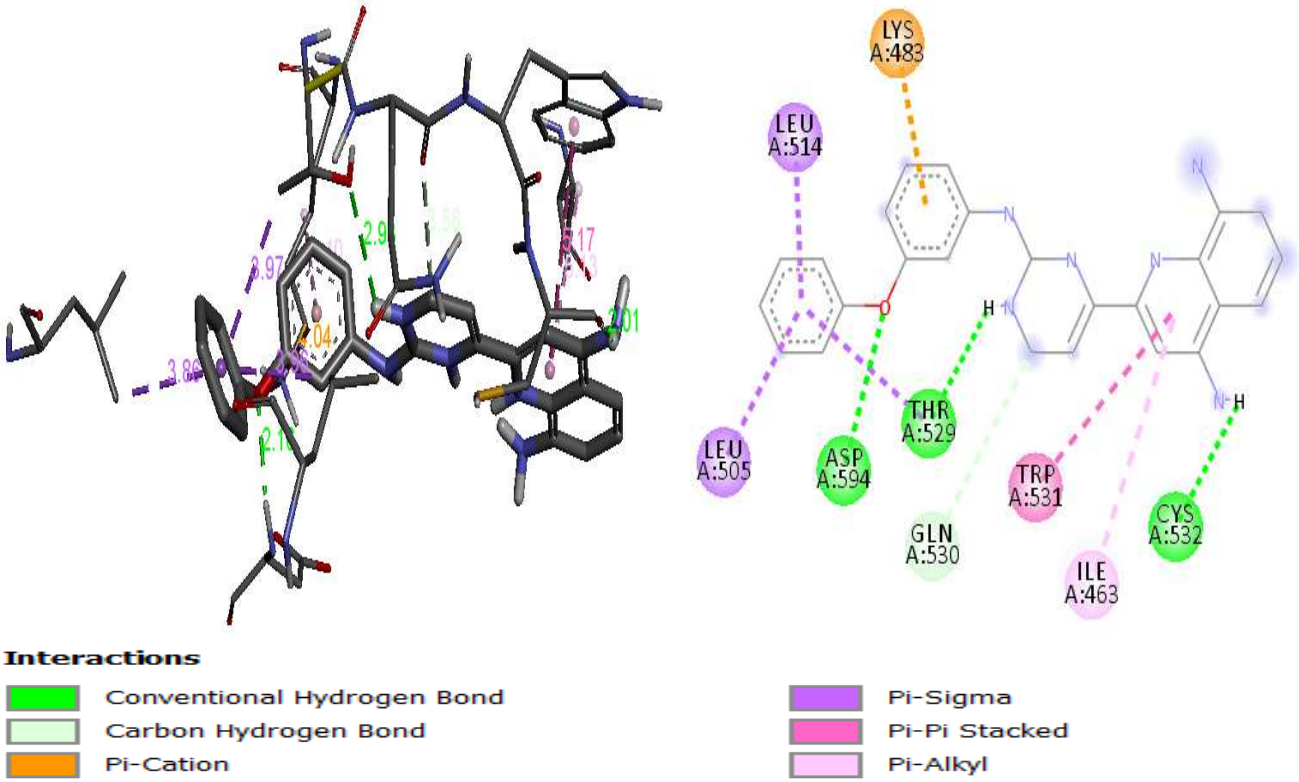


Figure 1-(A) 3D and 2D interaction BRAF/QAP6 molecular system

Vemurafenib the standard V600E-BRAF inhibitor which is the ligand removed from the x-ray structure of V600EB-RAF retrieved from PDB (ID: 3OG7) was docked in order to check the position, orientation, and interaction of the ligand with V600E-BRAF receptor. The comparison of position, orientation, and interaction of the ligand (Vemurafenib) with top most conformation of docked ligand (QAP6) exhibited some good similarities. Three important aromatic residues: ILE 463, TRP 531 and LYS 483. The central pyrrole and pyridine ring of the Vemurafenib ligand also exhibited the same hydrogen bond interaction with GLN 530 (2.35105), and CYS 532 (3.0153) almost similar to the most active QAP6. There was also formation of two hydrogen bonds through the O=S=O moiety of the ligand which are PHE595 (2.66721) and GLY596 (2.06321) respectively as shown in Table 3. The pyrrole moiety of the ligand is involved in two important conserved pi-pi interactions, one is present between nitrogen of ligand and PHE 583 residue while second bonding is formed between the aren of the pyrrole moiety and TRP 531 active side residue of V600EBRAF as depicted in Figure 2.

To identify the optimal V600E-BRAF inhibitors, top ranked conformations of all ligands were considered as best preference for the design. For the structure based design, the binding energy were served, after which the optimal V600E-BRAF inhibitors was selected on the basis of the highest binding energy. This study revealed that all the QAP analogous are effective in targeting V600E-BRAF, especially QAP6 with binding energy better than that of Vemurafenib the standard V600E-BRAF inhibitor. This ligand was chosen and two novel QAP derivatives as N1 and N2 were generated, which were not reported previously.

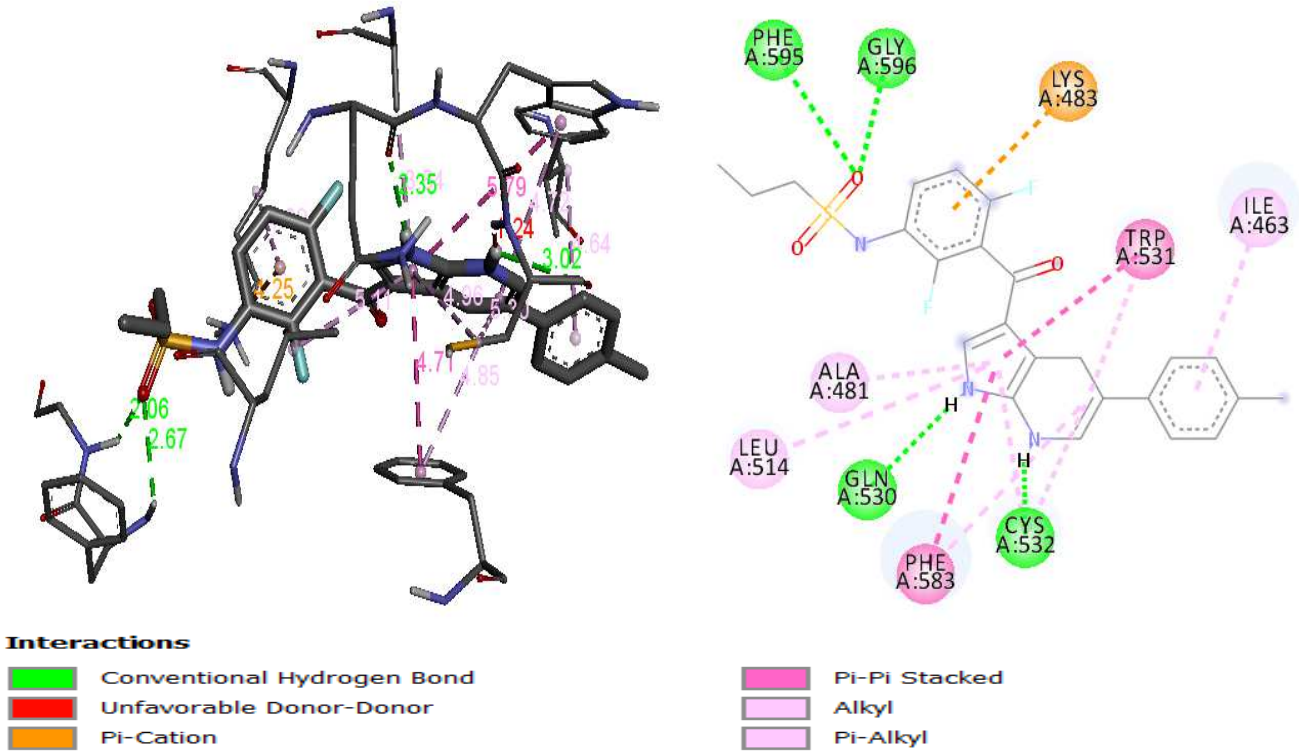


Figure 2-(A) 3D and 2D interaction of BRAF/Vemurafenib molecular system

3.1.1 Design and docking simulation of the new ligands (N1 and N2)

The docking simulation result of QAP6 propose the possibility of improving the activity of the molecule by introducing some new substituents. A library of substituent was imputed into the chemical table and all this substituent were docked within the binding site of the receptor so as to assess the chemical behavior of all these substituent in that site. On comparing the docking results of and molecular descriptors, a group (trifluoromethyl benzyl) was chosen and added to QAP6 at amino group of the quinoline moiety as shown in Scheme 1. This group have two important features, halogen bonds were generated and hydrophobicity is increased. Also, the possibility of interacting with lysine may increase, and through these substituents, new QAP derivatives were designed as N1 and N2 respectively.

The designed compounds were evaluated for drug-likeness by analyzing their properties (physiochemical) and by applying rule of five (Lipinski's) as presented in Table 4. The rule states that molecule must have molecular weight of <650 Da, H-bond acceptors <10, H-bond donors <5, log P of <5 and topological polar surface area (TPSA) < 100 $Å^2$. All the new design compounds passed the test (Lipinski et al., 2012).

After conducting molecular docking simulation for the newly designed compounds, it was found that the binding energy of QAP6 were increased to -12.7 kcal/mol for N1 and -12.9 kcal/mol for N2 respectively as shown in table 5. Therefore, the two new compounds are the novel V600E-BRAF inhibitors, thus, their docking results were compared to the docking result of vemurafenib as a positive control. In Figure 3 and 4, N1 and N2 binding to V600E-BRAF was presented and depict the similarity of interactions to QAP. N1 and N2 were bound to the active site of the receptor with some similar residue involvement. Most of the iterations, LYS483 was the most significant residue associated in vemurafenib-V600E-BRAF and QAP-V600E-BRAF interactions.GLN530, THR529, and ASP594 was associated in H-bond and π-interactions (cation). Aromatic groups of N1 and N2 play important role in hydrophobic interactions. There are two aromatic residues, THR531 and GLN530 which increases the binding stability by providing pi-donor hydrogen bond interactions with QAP aromatic rings. While LEU514, LEU 505 and THR529 form pi-sigma interactions. The newly introduced substituent increases the binding stability by forming two pi-alkyl bonds with PHE 468 and VA471 and also form halogen bond with ASP594 as shown in Figure 3 for N1. For N2 face-face pi-pi interactions or (π-stacking) are the usual form of pi-interactions which occurs between the quinoline moiety and the newly introduced benzene ring with PHE583 residue, T-shaped interactions (pi-pi) produced an edge-face pattern of two (2) aromatic rings as depicted in Figure 4. Furthermore, there is a formation of an additional hydrogen bond with ASN580 from the fluorine atom of the newly introduced substituent. Much more interaction was found in the N2 which make it better V600E-BRAF inhibitor than the QAP6.

Table 4-Physico-chemical properties of N1 and N2.

Ligand	MW (Da)	nHBA	nHBD	LogP	TPSA (Å^2)
N1	564.571	6	2	0.82	72.120
N2	632.568	6	2	1.43	72.344
QAP6	420.476	6	2	-0.79	84.952
Vemurafenib	489.930	7	2	-0.43	81.736

Table 5-Docking information for the interaction of N1 and N2 docked to kinase domains of BRAF (PDB ID: 3OG7).

Molecular system	Binding Energy(kcal/mol)	Hydrophobic Interaction	Electrostatic Interaction/Others	Hydrogen Bonds	Hydrogen Bond Distance (Å)
BRAF/N1	-12.7	LEU505, LEU514, THR529, VAL471, PHE468, ILE463 and LYS483	ASP594 and LYS483	ASP594, THR529, H…F, GLN530 and TRP531	2.25108, 2.97344, 2.43464 and 3.51967
BRAF/N2	-12.9	LEU505, LEU514, THR529, PHE583, PHE583, VAL471, PHE468, PHE583 and LYS483	ASN580, ASP594 and LYS483	ASN580, THR529, H…F, CYS532, CYS532, GLY534 and GLN530	2.52778, 2.52715, 1.84198, 2.69728, 2.51183, 2.64959 and 3.24081
BRAF/QAP6	-11.7	LEU505, LEU514, THR529, TRP531, ILE463 and LYS483	LYS483	ASP594, THR529, CYS532 and GLN530	2.15618,2.93365, 2.01085 and 3.56215
BRAF/Vemurafenib	-11.3	TRP531, PHE583, CYS532, ALA481, LEU514, LYS483 and ILE463	LYS483	PHE595, GLY596, CYS532 and GLN530	2.66721, 2.06321, 3.0153 and 2.35105

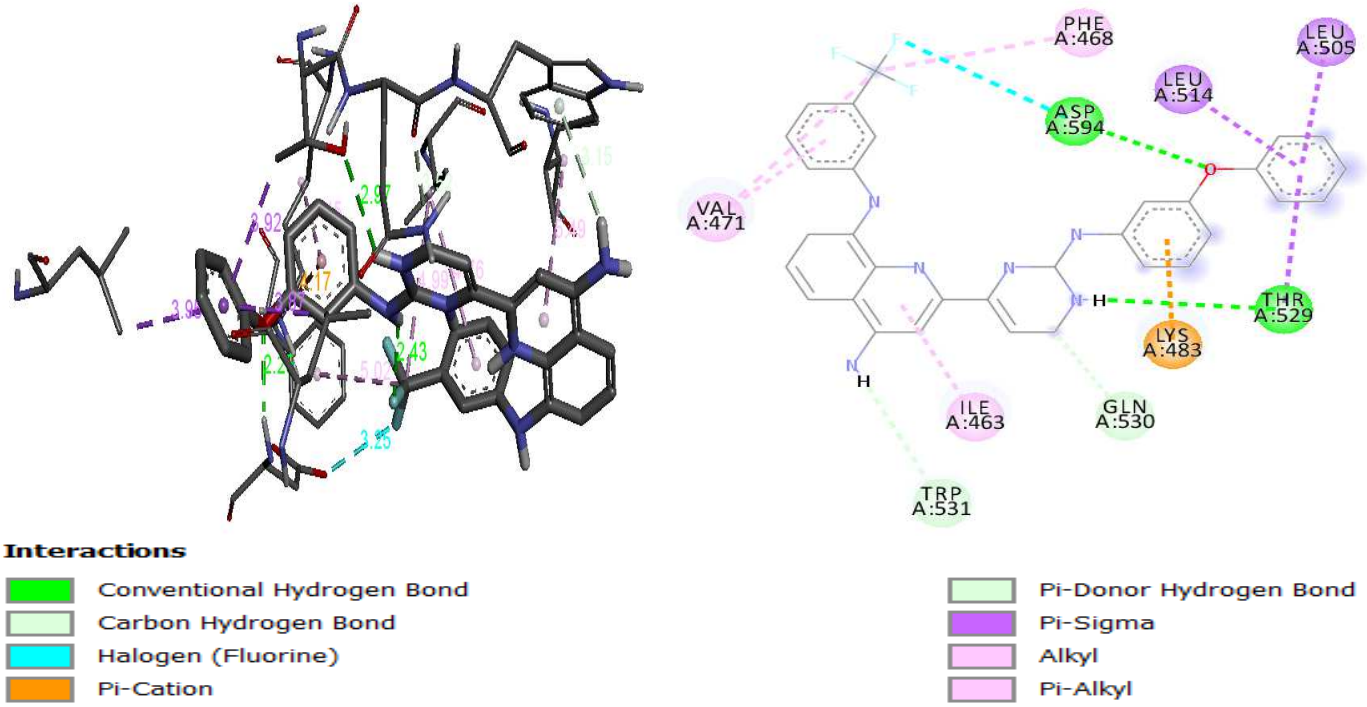


Figure 3-(A) 3D and 2D interaction of BRAF/N1molecular system

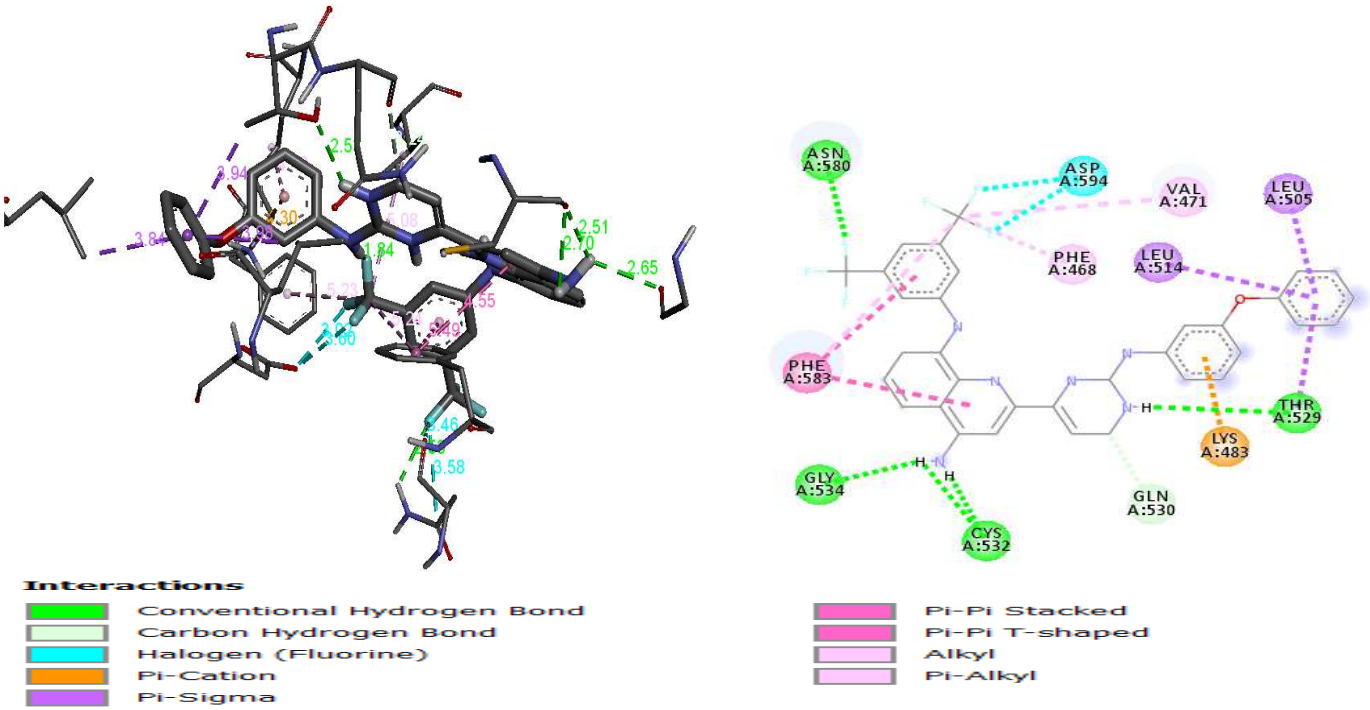
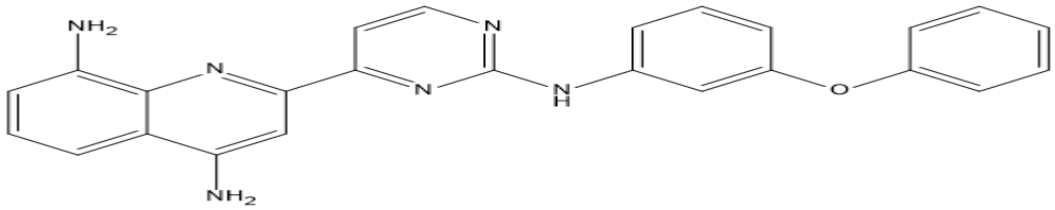
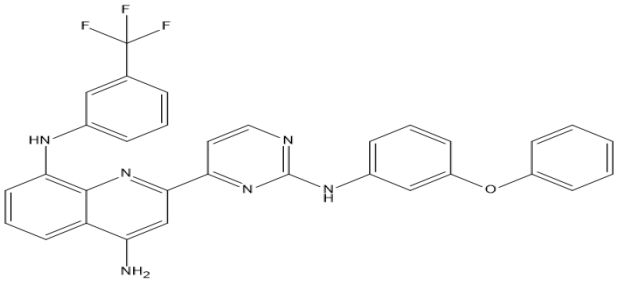


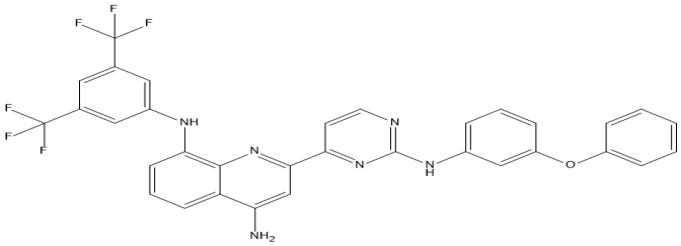
Figure 4-(A) 3D and 2D interaction of BRAF/N2 molecular system



Template QAP6 BE = -11.7 kcal/mol



N1, BE = -12.7 kcal/mol



N2, BE = -12.9 kcal/mol

Scheme 1-Structure and binding energy of new Designed V600E-BRAF inhibitors (N1 and N2)

4.0 Conclusion.

V600E-BRAF is the frequent oncogenic protein kinase whose inhibition can prevent humans from cancers. In this study, molecular docking simulation is applied for V600E-BRAF on quinolinylaminopyrimidines (QAP) derivatives to investigate the proper binding mode. All the studied ligands were able to inhibit the receptor by totally occupying the active segment in the target (receptor). The compounds that have best binding energy for the receptor was utilized to design new derivatives, thereby enhancing the activity of the parent structure. The newly designed QAP analogues as N1 and N2 with the binding energy of −12.7 $kcalmol^{-1}$ and −12.9 $kcalmol^{-1}$ differ significantly in terms of binding energy from their parent structure, QAP6 (−11.7 $kcalmol^{-1}$) and the standard V600E-BRAF inhibitor Vemurafenib (−11.3 $kcalmol^{-1}$) due to the introduction of aromatic and in combination with halogen groups, which have the capability of increasing the overall binding energy by increasing the number of hydrogen bond and hydrophobic interactions shown in their complex. Therefore, in the future studies there is hope to include the synthesis, in vivo and in vitro evaluation of these ligands (inhibitors) which can establish them to be the most potent V600E-BRAF inhibitors to treat melanoma cancer.

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