ejpmr, 2017,4(5), 384-393

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

INSILICO DEVELOPMENT OF NOVEL RSK2 INHIBITORS USING DOCKING AND PHARMACOPHORE STUDIES

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Article Received on 08/03/2017 **Article Revised on 27/03/2017** **Article Accepted on 18/04/2017**

ABSTRACT

Ribosomal S6 kinase (RSK2) belongs to family of serine/threonine kinases responsible for regulating cellular growth and differentiation. On account of the pathogenic role played by RSK2 in cancer, it has been recognised as a favourable drug target in cancer. Available molecular structure of RSK2 NTKD shows N-terminal and C-terminal lobes and an ATP-binding region residing between the cleft of these lobes. In the present study, an attempt has been made for development of ATP competitive inhibitors using insilico techniques through hybrid molecule concept where distinct functional moeities of either natural or unnatural molecules are constructed with new properties that enhances+ their activity. Pharmacophore mapping and docking studies were performed and new ligands were designed as RSK2 inhibitors.

KEYWORDS: Cancer, Docking, Hybrid Molecules, Pharmacophore mapping, Ribosomal S6 kinase.

INTRODUCTION

90 kDa ribosomal S6 kinase 2 (RSK2) is a widely expressed serine/threonine kinase enzyme stimulated by extracellular signal-regulated protein kinase (ERK1 and ERK2) in respond to several growth factors, peptide hormones and neurotransmitters.[1,2] RSK2 consists of two non identical kinase domains, the ERK-type MAP activated C-terminal kinase domain (CTK) and 3-phosphoinositide dependent protein kinase-1 (PDK1) activated N-terminal kinase domain (NTK) belongs to CamK family and AGC kinase family respectively and these CTK and NTK domains are separated by a linker region.

The mechanism of activation **(Figure 1)**[3-7] involves complex events that includes

(i) binding of ERK to C-terminal kinase domain leads to its activation,

(ii) activated C-terminal kinase domain provides phosphorylation-based docking site for PDK1 to bind and activate N-terminal kinase domain,

(iii) activated N-terminal kinase domain phosphorylate numerous proteins that are involved in cell survival, proliferation and motility.

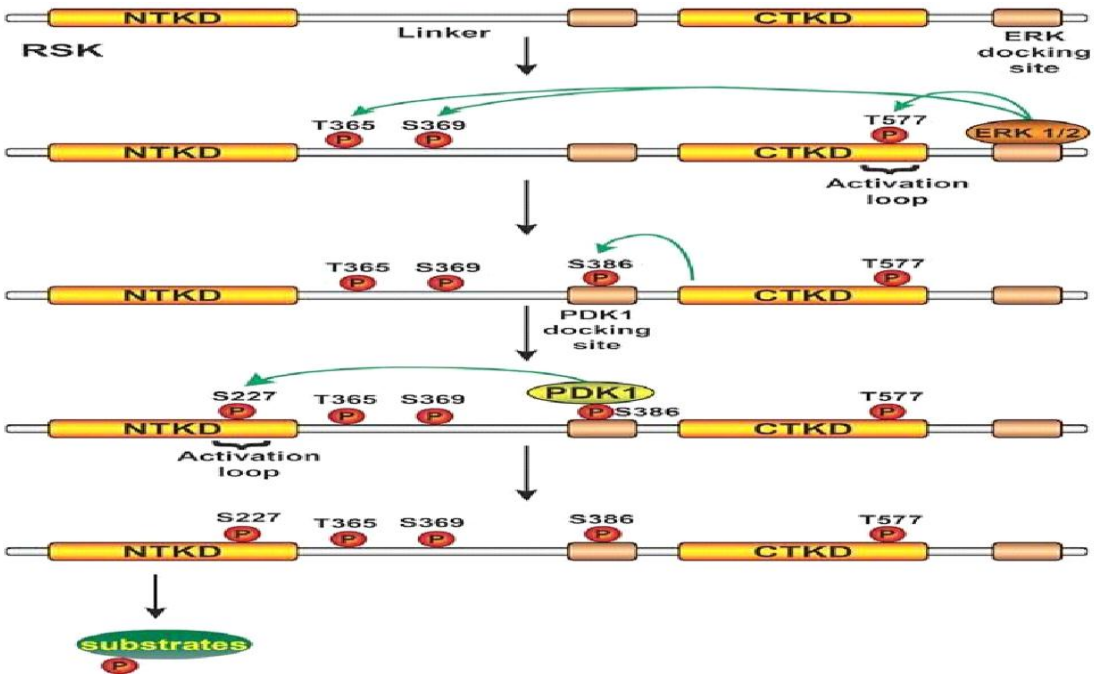

Figure 1: Molecular mechanism of rsk activation

RSK2 phosphorylates numerous cytosolic and nuclear proteins preferentially at the residues serine/threonine which reside in RXRXXS/T or RRXS/T motifs [8]. Up to date the full length molecular structure of RSK2 has not yet determined, but a bilobed molecular model of RSK2 NTKD (residues 68-323) has been reported.[9] The smaller N-terminal lobe comprising beta sheets and P-loop which is a Glycine rich, while the larger C-terminal lobe comprising alpha helices. In between these two lobes, a cleft with an ATP-binding site defined by hinge region that links the two lobes. In between the cleft of these two lobes a binding site for ATP/Mg2+ is present[10] and adjacent to this site DFG motif (Asp211, Phe212 and Gly213 in RSK2) is located below the N-lobe. This motif is a switch between the catalytically active (DFG-in) and inactive (DFG-out) conditions of kinases.[11] In the active conformation of DFG-in, the Asp211 point towards the triphosphate of ATP and side chain of Phe212 shown in reverse direction. The inactive conformation DFG-out occurs either in the absence of ATP or in the presence of inhibitor complexes, in which the Phe212 flips in to the binding pocket of ATP, while the Asp 211 points outside the binding site.[12] ATP binding site is characterized by three regions which include adenine binding site consisting of amino acids Asp 148, Leu 150 and Thr 210; ribose binding site consisting of amino acids Asp 154 and Glu 197; triphosphate binding site consisting of amino acids Ser 78, Phe 79, Gly 80, Lys 100, Lys 195.[9] In addition, a purine motif with hydrophobic clusters on each side which include Leu 74 and Val 82 on one side and Leu 200 on the other side. The Ser/Thr kinases AGC family exist in combination with open-and-close conformations and the relative motion of the two lobes: the open conformation is innate to the free form of nucleotide, while the locked conformation correlate to a binary complex model of bilobed sequester ATP/ Mg2+.[13 -17] In closed form, the P-loop overlays the ATP triphosphate which aids itself in the transfer of phosphate.[18] The tip of flexible P-loop consists of Phe-79 which is found to be responsible for expressing the complete kinase activity where the ATP triphosphate and the phosphorylation site of substrate from solvent is shielded. Because of the P-loop's inherent flexibility, the π-π interactions are formed occasionally between the hydrophobic residue and some inhibitors with aromatic moieties.[19,20] Site directed mutational studies of Lysine 100 to alanine indicated that it is essential for kinase activity of RSK2 through a strong hydrogen bond formation of terminal -NH of Lys 100 of RSK2 NTKD model with alpha phosphate oxygen of ATP.[9] As a member of serine/threonine kinase, both structurally and functionally conserved features of RSK2 enabled identification of its specific inhibitors which can be employed as chemical probes for diagnosing the complex signaling cascades associated with over expression of RSK2 and thus can be used as therapeutic candidates especially in many pathogenic cancers that include breast cancer, prostrate cancer, etc.[21] Development of small molecule inhibitors of RSK2 has gained much attention with the emergence of RSK2 as an anticancer target.

In view of important role played by RSK2 in cancer, here in our study, the hybrid molecule concept was used for generation of RSK2 inhibitors and their efficacy against RSK2 target have been studied. The concept of hybrid molecules, a combination therapy employed by many clinicians for treating unresponsive patients.[22-24] Hybrid molecules are build based on two or more functionally active groups into a single molecule produced by covalent linking which may either increase or modulate the specific activity of distinct components or lead

molecules with novel properties. Based on the activity range reported in various literature resources, few compounds were carefully selected. Molecular docking was performed with the selected ligands to study the binding affinity of the ligand molecules with the drug target protein RSK2. Further, pharmacophore studies are performed to identify the essential features of the ligand molecule responsible for drug activity. With the identified important features of the high active molecule through docking and pharmacophore, new hybrid molecules are designed and analysed for their anti cancer activity through docking and pharmacophore studies.

MATERIALS AND METHODS

Preparation of protein structure

The crystal structure of the RSK2 protein (PDB ID: 4NUS) has been obtained from RCSB Protein Data Bank (http://www.pdb.org). All the water molecules from the target protein were removed and hydrogen atoms were added. Energy minimization of the protein was carried out by applying CHARMm force fields and steepest descent algorithm followed by conjugant gradient algorithm in DS until the convergence gradient is satisfied. **Figure 2** shows the secondary structure of human RSK2.

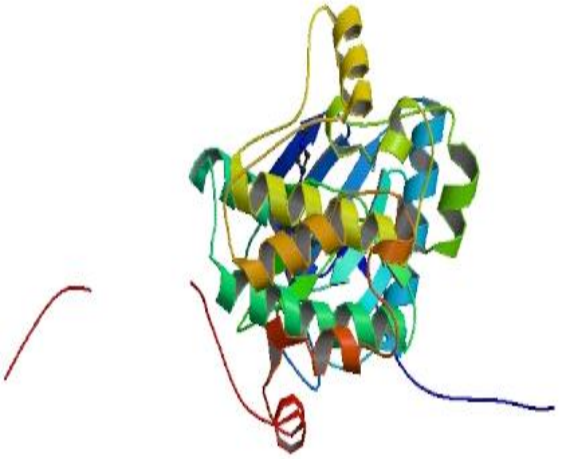

Figure 2: Secondary structure of *human* rsk2

Preparation of ligand structures

From various literature resources[9,25-29] ligand compounds are selected based on their reported activities. The selected ligands are 2-amino-7-substitued benzoxazole analogue (**T-1),** Epigallocatechingallate (EGCG) (**T-2**), kaempferol-alpha-L-diacetyl rhamnoside (SL0101) (**T-3**, Kaempferol (**T-4**) and diamino-niroso-pyrimidine (**T-5**), drawn using ACD/Chem Sketch (12.0). **Figure 3** shows the 2D structure of the sketched compounds. Catalyst algorithm in DS was used for the conversion of 2D to 3D structures.

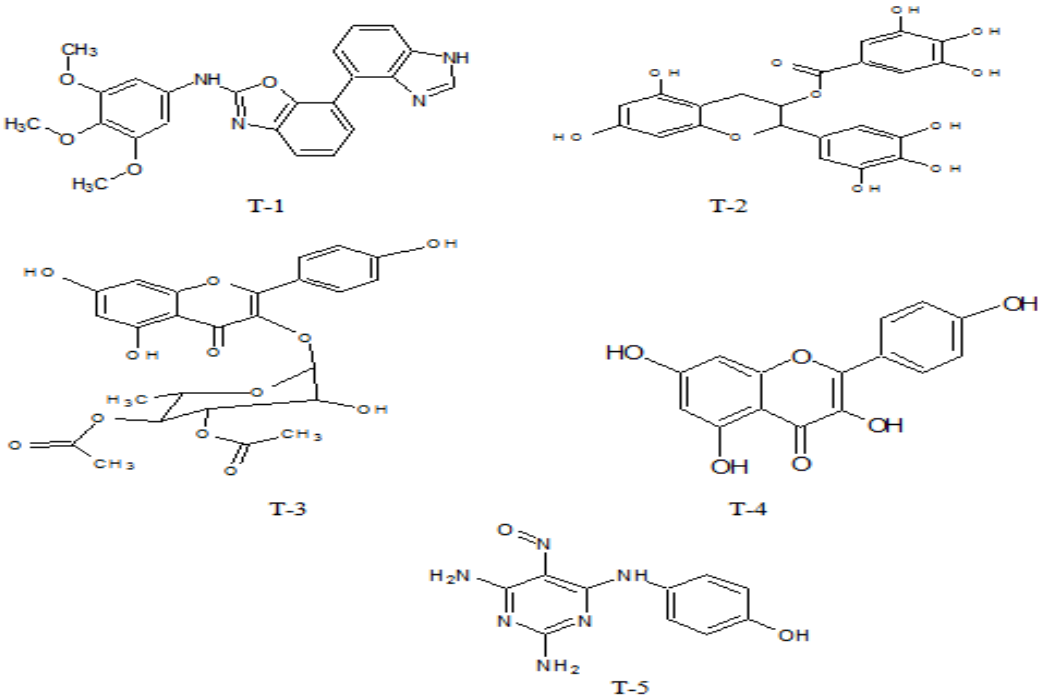

Figure 3: 2d structures of the compounds

Docking

Moleular docking analysis using LibDock7 module of Discovery Studio was carried out to explore the intermolecular interactions of ligands with RSK2 protein. The energy minimized conformation of RSK2 protein was used as receptor for the docking process. The binding sphere was defined with XYZ coordinates of -15.3926, 7.54635 and -24.5639 respectively. LibDock

uses protein site features called hotspots which are of polar and apolar types. The polar hotspot is preferred by` polar ligand atoms like hydrogen bond acceptors (HBA) and apolar hotspots by apolar ligand atoms like carbon. The receptor hotspot file was defined prior to the docking process with 100 hotspots and a docking tolerance of 0.25Å for the best conformer matching. High-quality docking preferences were defined for the quick generation of a set of diverse low energy conformations with an energy threshold of 20 and maximum conformation limit of 255. The ligand poses were placed into the binding sphere and hotspots were matched as triplets. The docked poses were pruned and a final optimization step was performed. Each generated pose was scored by LibDock score and the pose with highest LibDock score was selected and analyzed.

Pharmacophore studies

Pharmacophore studies are conducted to extract a 3D pharmacophore with relevant functional groups that are necessary for inhibitor binding. Interaction Generation protocol in the DS was used in generating the pharmacophore from the active site of the protein that extracts pharmacophore query from the Ludi interaction map created inside the active site sphere and only assigns three main features namely HBA, HBD and HY. All the parameters within this protocol were left as their default values.[30] To the generated pharmacophore, ligand pharmacophore mapping protocol was employed that uses Catalyst algorithm to identify ligands that map to a pharmacophore, and aligns the ligands to the pharmacophore query. Based on the fitvalue which is a measure of how well the ligand fits the pharmacophore, the ligand with highest fit vaue was selected.

RESULTS AND DISCUSSION

Docking and pharmacophore studies of the reported compounds

Docking analysis

To explore the binding modes and interaction analysis of the ligands with the RSK2 protein docking studies are conducted using DS. The results from molecular docking analysis yielded encouraging results with the best docking scores obtained for compound **T-2 (**EGCG**)** which was of natural origin and showed the highest best LibDock score of 119.702 kcal/mol **(Figure 4)**. High dock score observed for **T-2** ligand was due to formation of stable complex with RSK2 involving H-bond interactions with Leu 150 from the hinge region, Lys 100 from the N-lobe, Asp 211 from DFG loop; Hydrophobic interactions involving amino acids Leu 200 from DFG loop and Val 82 from N-lobe; π-π stacking interactions with Phe 79 at the tip of P-loop. Formation of hydrogen bond interactions with Lys-100 of N-lobe and π-π stacking interactions with Phe 79 present at the tip of P-loop are responsible for greater inhibitory activity of this compound (**T-2**). The dock scores of all the ligands are tabulated in **Table 1**. Overall, the compound **T-2** showed the best binding orientations in the N-terminal catalytic site where it formed five hydrogen bonds and three hydrophobic interactions **(Table 2)**.

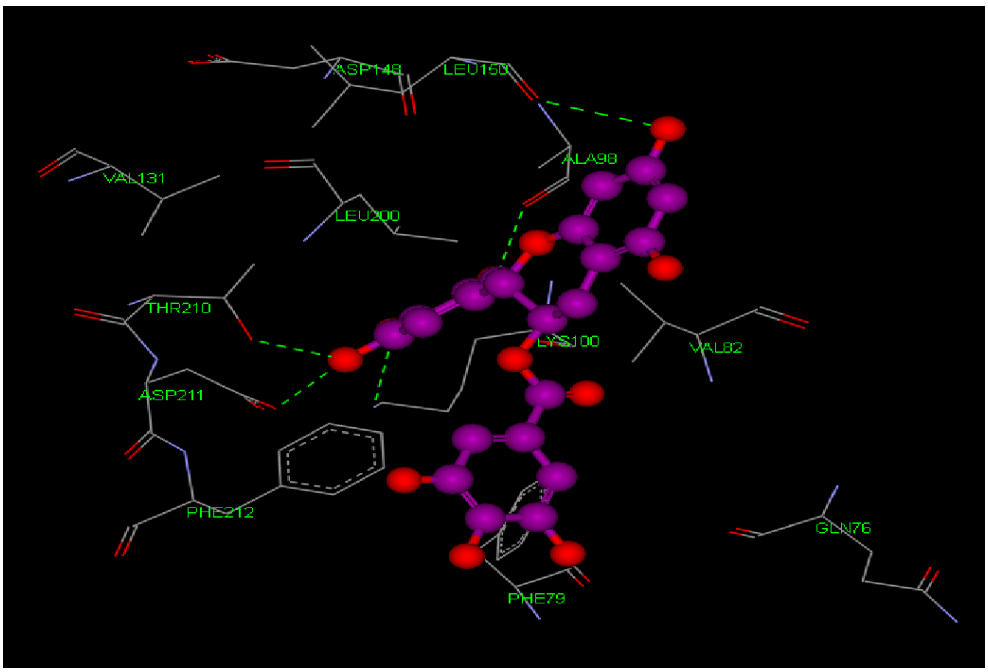

Figure 4. Binding interactions of t-2 (ball and stick model) into the active site of protein rsk2 (thin stick model).

Table 1. Top ranked libdock scores of the reported compounds.

Name of the Ligand	LibDock Score
2-amino-7-substitued benzoxazole analogue (T-1)	114.312
Epigallocatechingallate (EGCG) (T-2)	119.702

Kaempferol-alpha-L-diacetylrhamnoside (SL0101) (T-3)	88.391
Kaempferol (T-4)	95.692
Diamino-nitroso-pyrimidine (T-5)	97.258

Table 2. Libdock score and interacting amino acid residues of t-2 compound with rsk2

Name of the Ligand	LibDock Score	Interacting Residue of RSK2	Type of Bond
Epigallocatechingallate (EGCG) (T-2)	**119.702**	Ala98	Hydrogen
		Lys100	Hydrogen
		Leu150	Hydrogen
		Thr210	Hydrogen
		Asp211	Hydrogen
		Val82	Hydrophobic
		Leu200	Hydrophobic
		Phe79	Hydrophobic

Pharmacophore mapping

The results of Interaction generation generates a pharmacophore model comprising hydrogen bond acceptor (HBD), hydrogen bond donor (HBA) and hydrophobic features. Using Ligand Pharmacophore Mapping protocol, all the compounds are mapped on to the pharmacophore model. Geometric fit values are predicted per compound hit based on the agreeable mapping of the chemical substructures of a compound. Compounds are ranked according to the obtained fit values and the compound with the high fit value is chosen. The pharmacophore mapping studies performed with the selected ligands (T-1 to T-5) revealed that **T-2** ligand **(Table 3, Figure 5)** possessed better pharmacophore feature.

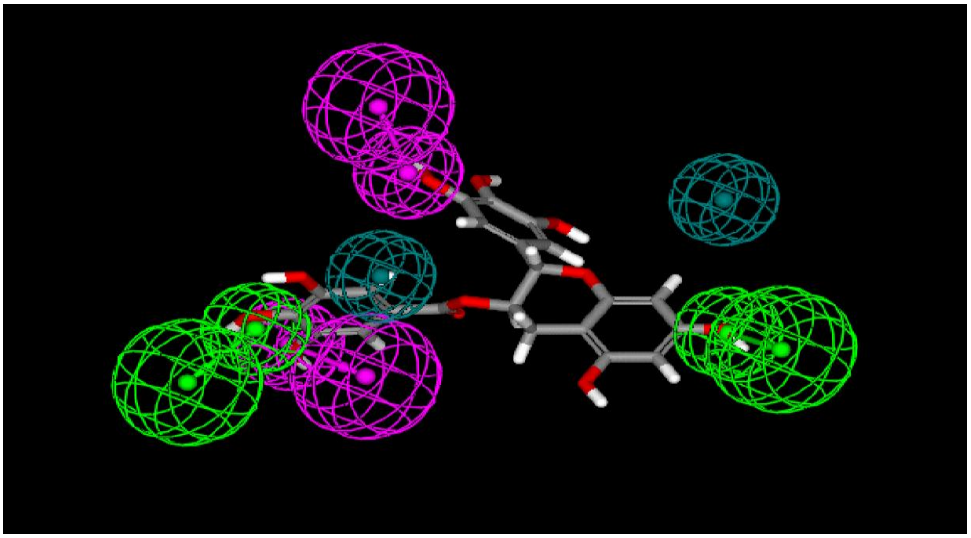

Figure 5. Pharmacophore mapping of egcg (t-2) ligand on rsk2 binding site. Green color (hydrogen bond acceptor), pink (hydrophobic), blue (hydrogen bond donor).

Table 3: Best fit values of the five compounds (t-1 to t-5) from which the pharmacophore models were generated.

Ligand	Fit Value
2-amino-7-substitued benzoxazole analogue (T-1)	2.373
Epigallocatechingallate (EGCG) (T-2)	**3.783**
Kaempferol-alpha-L-diacetylrhamnoside (SL0101) (T-3)	1.895
Kaempferol (T-4)	1.291
Diamino-nitroso-pyrimidine (T-5)	1.038

Docking and pharmacophore studies of the designed hybrid compounds

Design strategy for hybrid compounds

Based on docking studies and fitness values obtained from pharmacophore mapping, the scaffold of **T-2** compound was selected as a base for designing new hybrid molecules as shown in **figure 6**. Using hybrid molecule concept, the **T-2** compound was incorporated in to the next high dock scored synthetic compounds **T-1** and **T-5.** The compounds T-6 to T-10 were designed by covalent coupling of **T-1** with **T-2 (Figure 7)** and

similarly, compounds T-11 to T-16 were designed by covalent coupling of **T-5** with **T-2 (Figure 8)**.

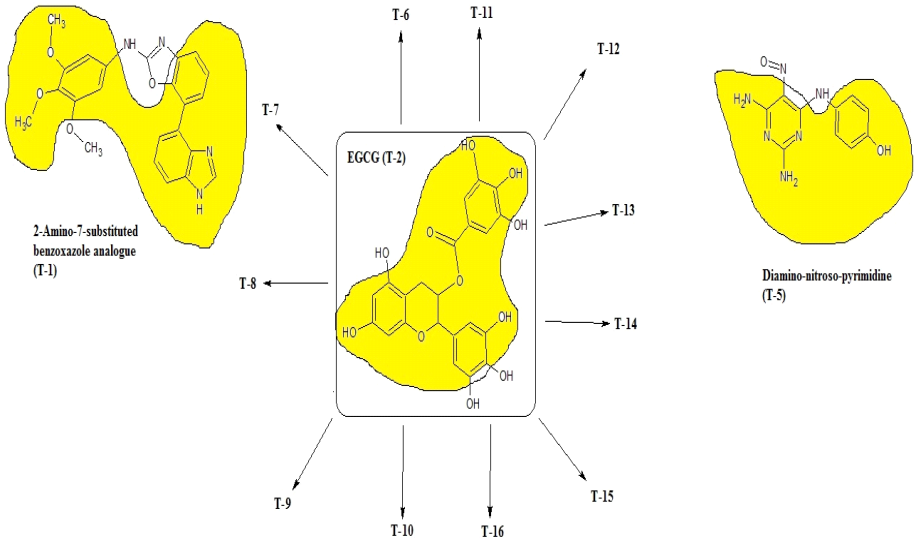

Figure 6. Design strategy for compounds t-6 to t-16

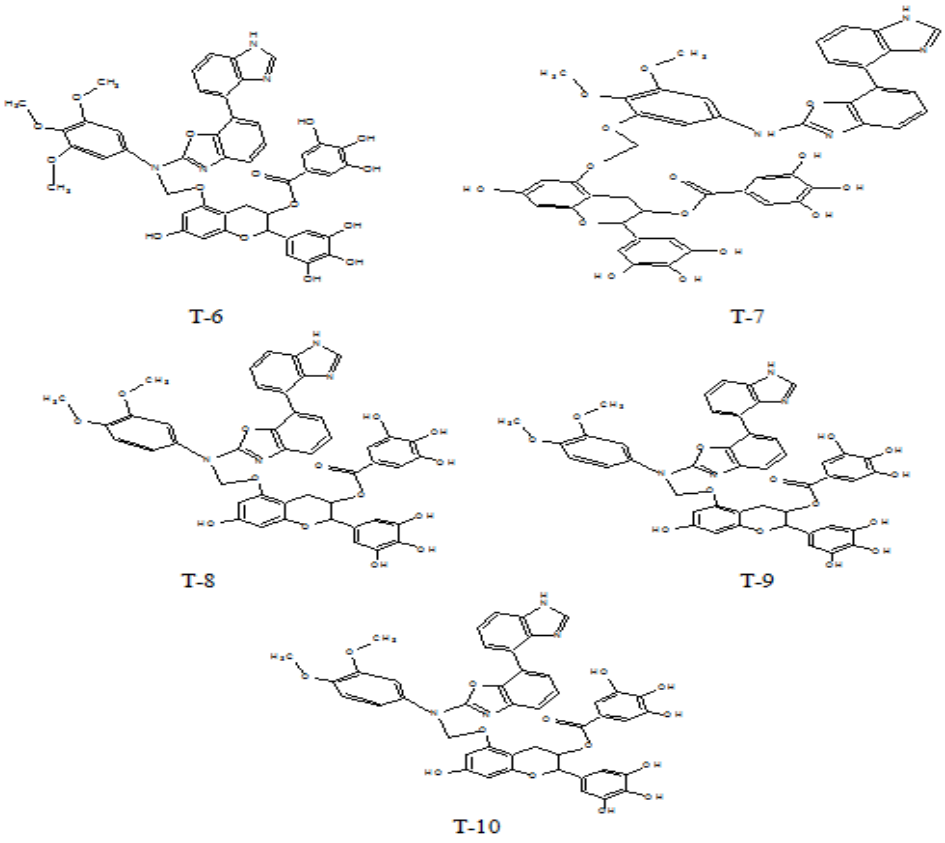

Figure 7: 2d structures of the newly designed hybrid compounds based on the scaffold t-2 and t-1.

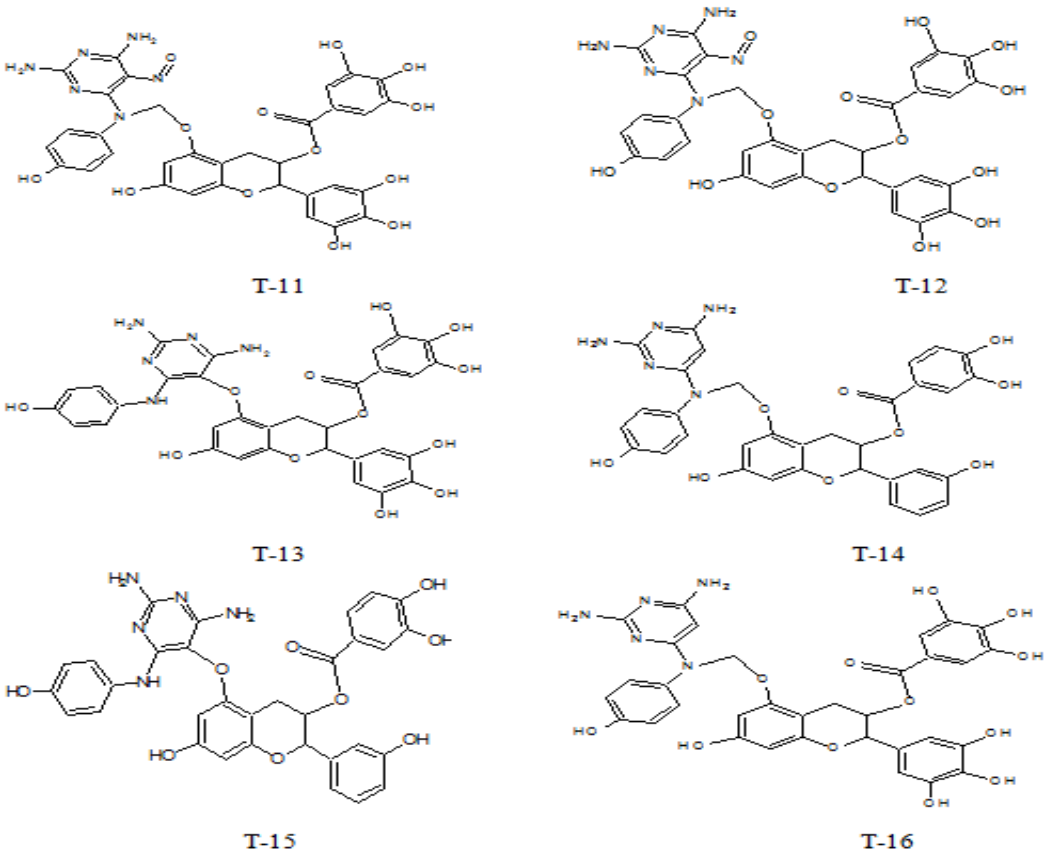

Figure 8: 2d structures of the newly designed hybrid compounds based on the scaffold t-2 and t-5.

Docking analysis

Docking studies are performed for the compounds T-6 to T-16 revealed that **T-6** compound is having a high dock score **194.88 Kcal/mol** (**Table 4**) with the best binding orientations in the N-terminal catalytic site where it formed five hydrogen bonds and three hydrophobic interactions with the RSK2 protein (**Table 5**). The binding interactions of hybrid compound **T-6** with the RSK2 protein are shown in the **Figure 9**. From the results indicate that **T-6** found to possess good inhibitory activity towards RSK2 similarly as the reported potent lead **T-2.**

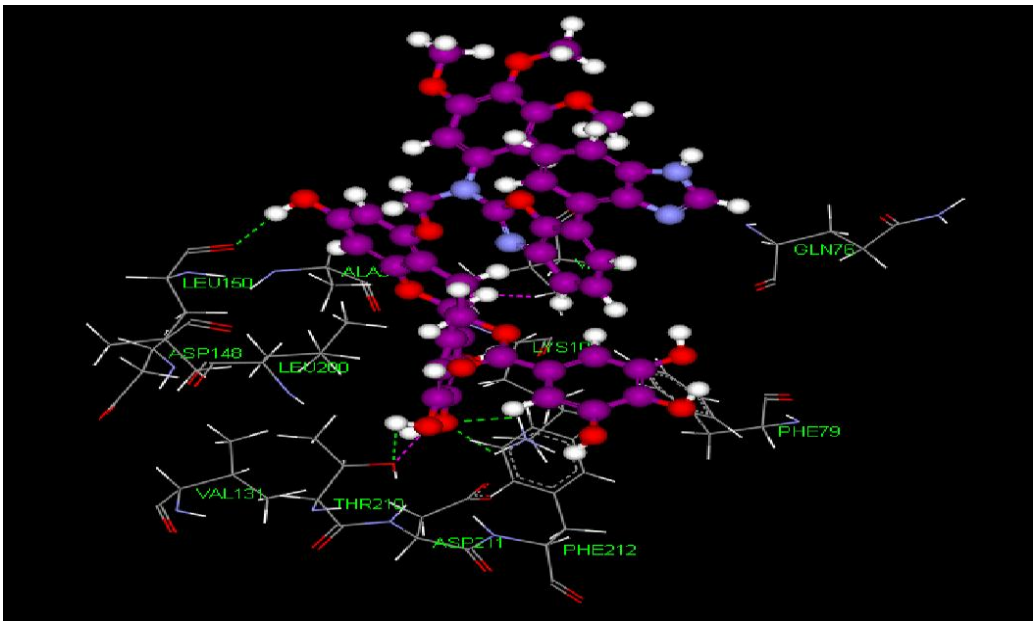

Figure 9: binding interactions of t-6 (ball and stick model) into the active site of protein rsk2 (thin stick model).

Table 4: Top ranked Libdock scores of the newly designed hybrid compounds against rsk2.

Name	LibDockScore
T-6	194.88
T-7	161.692
T-8	181.965
T-9	164.739
T-10	177.339
T-11	162.998
T-12	166.548
T-13	171.353
T-14	145.275
T-15	145.655
T-16	171.015

Table 5. Libdock score and interacting amino acid residues of hybrid compound t-6 with rsk2

Name of the Ligand	LibDock Score	Interacting Residue of RSK2	Type of Bond
Hybrid Compound (T-6)	**194.88**	Gln76	Hydrogen
		Lys100	Hydrogen
		Leu150	Hydrogen
		Thr210	Hydrogen
		Asp211	Hydrogen
		Val82	Hydrophobic
		Phe79	Hydrophobic
		Val131	Hydrophobic
		Leu150	Hydrophobic
		Thr210	Hydrophobic

Pharmacophore mapping

This was further confirmed by pharmacophore mapping studies which revealed that **T-6** ligand possessed better pharmacophore features with a fit value of **4.09 (Table 6, Figure 10).**

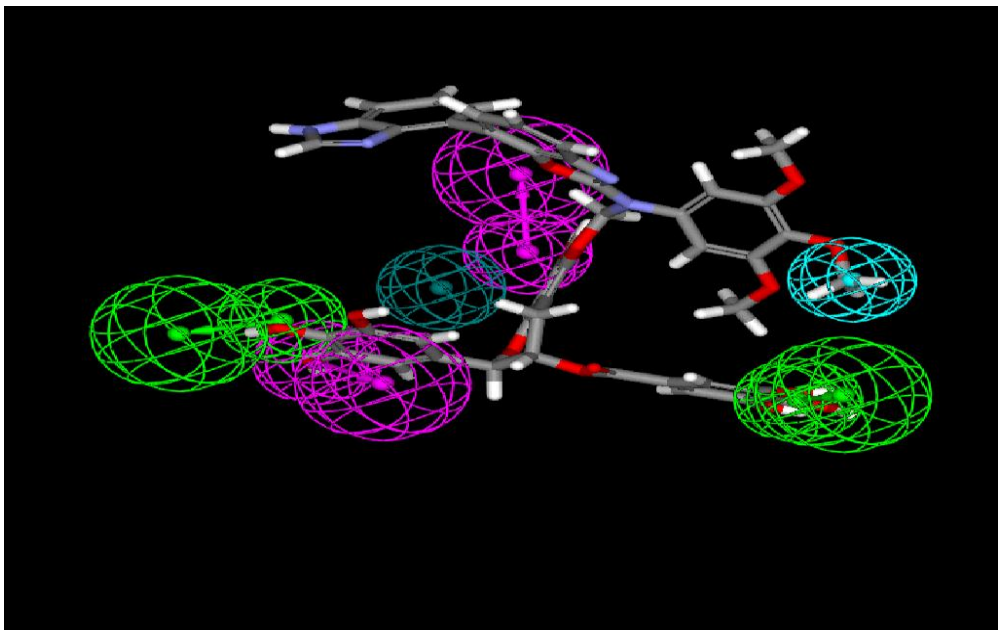

Figure 10: Pharmacophore mapping of hybrid compound t-6 on rsk2 binding site.

CONCLUSIONS

In the present study, molecular docking revealed that the binding affinity of compound EGCG (**T-2**) which is a ATP competitive with the RSK2 NTKD ATP binding site is high and significant interactions with the active site of the drug target RSK2 confirming the inhibitory activity. Further, docking procedures and pharmacophore mapping performed on the new ligands designed based on the scaffold of **T-2** revealed that the compound **T-6** can be used as potent lead for the development of RSK2

inhibitors. Future research would be focussed in this direction.

Conflict of interest

The authors confirm that they do not have any conflict of interest.

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