MOLECULAR DOCKING STUDIES OF 2-AMINO-4,6-DISUBSTITUTED PYRIDINE-3-CARBONITRILES AGAINST ADENOSINE A2A RECEPTOR AS POTENTIAL ANTI PARKINSONIAN AGENTS.

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Abstract:

Introduction: Parkinson's disease has become one of the most concerned diseases in the recent days. All the Adenosine A2A receptor antagonists were known to exhibit Antiparkinsonian activity. In the present study various 2-Amino-4,6-Disubstituted Pyridine-3-Carbonitriles or 2-amino 3-cyano pyridines were designed and docked against Adenosine A2A receptor for Antiparkinsonian activity. The ligands were compared with that of the standard Adenosine A2A receptor antagonists like Istradefylline and Caffeine.

Materials and methods: Ligands were drawn initially with ChemSketch software in .mol format and then converted to .pdb format using Avogadro software. Molecular docking studies were carried out by using iGEMDOCK software and finally visualized by using Discovery Studio Visualizer.

Results and discussion: Almost all the compounds have shown better binding affinity towards Adenosine A2A receptor. All the ligands have shown better binding energies than that of the standard Adenosine A2A receptor antagonists like Istradefylline (-74.91 kcal/mol) and Caffeine (-69.60 kcal/mol). Compounds C87 (-109.35 kcal/mol) and C67 (-108.14 kcal/mol) were the top compounds and selected for visualization.

Conclusion: 2-Amino-4,6-Disubstituted Pyridine-3-Carbonitriles were having better binding affinity to Adenosine A2A receptor than the standard antagonists and can become promising drug entities for the treatment of Parkinson's disease.

Keywords: 2-amino 3-cyano pyridines, 2-Amino-4,6-Disubstituted Pyridine-3-Carbonitriles, Parkinson's Disease, Adenosine A2A Receptor, ADORA2A, Molecular Docking, iGEMDOCK Software, Discovery Studio Visualizer and Adenosine A2A Receptor Antagonists.

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Introduction:

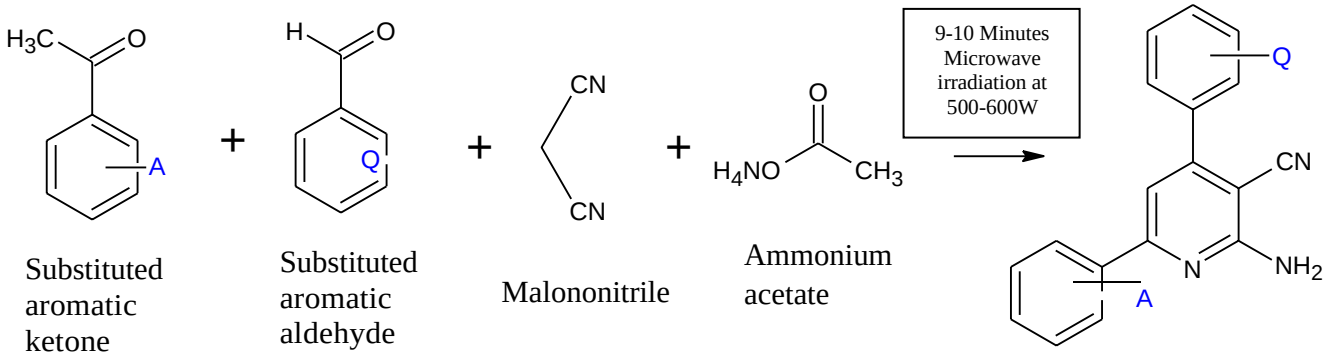
Pyridine containing heterocycles are very important class of drugs for the treatment of many diseases.[1-4]Pyridine ring is present in over thousands of existing drugs used for the treatment of various diseases.[5,6]

2-Amino 3-cyano pyridines were reported of having different activities like antiviral, anti-bacterial and fungicidal activities[7,8], Novel IKK-β inhibitors[9], Inhibitors of HIV-1 integrase[10], cardiotonic activity[11], Antitumor properties[12], Anti-inflammatory, analgesic and antipyretic properties.[13,14]

Present study is based on the fact that 2-Amino-4,6-Disubstituted Pyridine-3-Carbonitriles were Adenosine A2A receptor antagonists which can show Antiparkinsonian activity. It was reported by Mantri et al[15]. The present study aims at Insilco evaluation of various designed 2-Amino-4,6-Disubstituted Pyridine-3-Carbonitriles against Adenosine A2A receptor.

Materials and Methods:

General scheme for the synthesis of 2-Amino-4,6-Disubstituted Pyridine-3-Carbonitriles [16]



Substituted aromatic ketone

Substituted aromatic aldehyde

Malononitrile

Ammonium acetate

2-Amino-4,6 disubstituted Pyridine-3-carbonitrile.

The other methods of synthesis of 2-Amino-4,6-Disubstituted Pyridine-3-Carbonitrile or 2-amino-3-cyano pyridines were also reported[17,18]. From the above mentioned scheme[16] various aromatic aldehydes and various aromatic ketones were selected and final products were designed according to the scheme. A huge library of compounds were obtained and they were screened for the Insilco toxicity by using TopKat software[19] and then their ADME properties were predicted by using SwissADME software[20]. The designed compounds which were predicted as non-toxic, non-carcinogenic, safe and which has good ADME properties were selected for molecular docking studies.

Molecular Docking:

From the library of designed compounds according to the scheme mentioned above,[16] the protein target was chosen based on the Swiss Target Prediction software[21]. All the pool of non-toxic, non-carcinogenic and safe compounds were predicted for the target protein through Swiss Target Prediction software. Most of the compounds have shown Adenosine A2A receptor as potential target. The 2D structure of the ligands were drawn through ChemSketch software and saved in .mol format. The ligand structures in .pdb format were optimized through Avogadro tool [22]. Docking studies for the assessment of binding poses and interactions were done for the designed compounds which were non- carcinogenic, safe and which has good ADME properties. It was done through the iGEMDOCK version 2.1 Software[23]. GEMDOCK stands for **G**enetic **E**volutionary **M**ethod for molecular **D**ocking. iGEMDOCK is a graphical–automatic drug design system for docking, screening and analysis. It is a program for computing ligand conformation and orientation relative to the active site of the protein. Insilico docking simulation studies were performed to evaluate the molecular interactions of the selected safe compounds with the Adenosine A2A receptor (**PDB ID**: **5UIG**, with a co-crystallized ligand inhibitor) downloaded from protein data bank.

The Ligand interactions were visualized and analyzed through the Discovery Studio Visualizer (Biovia). Standard docking protocol was followed and accurate docking method was selected. Based on the scoring function the best docking solutions were analyzed. The scoring function estimates the fitness by combining van der Waals, hydrogen bonding and electrostatic energies. Post docking interaction profile analysis of best poses was conducted to determine the interactions between the ligand and the target protein.

A total of 97 non carcinogenic and safe compounds were identified by Insilco toxicity prediction and were selected for molecular docking along with standard Adenosine A2A receptor antagonists like Istradefylline and Caffeine. Docking simulations for the evaluation of binding affinities and molecular interactions were done. Out of them, the top ten compounds with better binding energies were selected and the top two compounds with better binding energies and molecular interaction profile were chosen for post docking analysis of interactions.

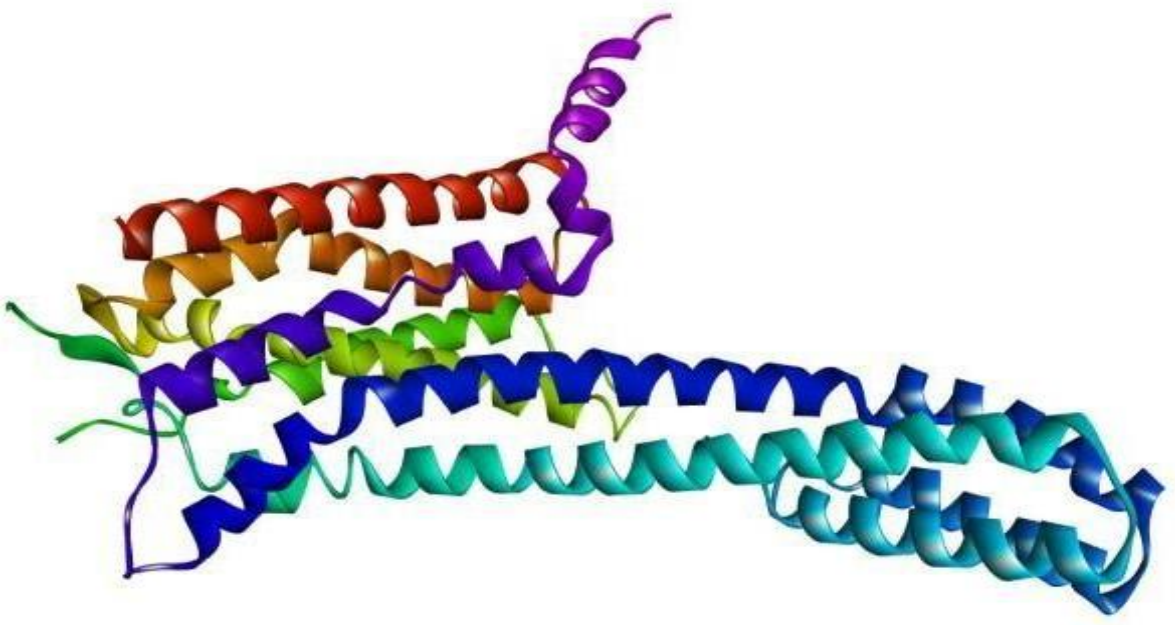
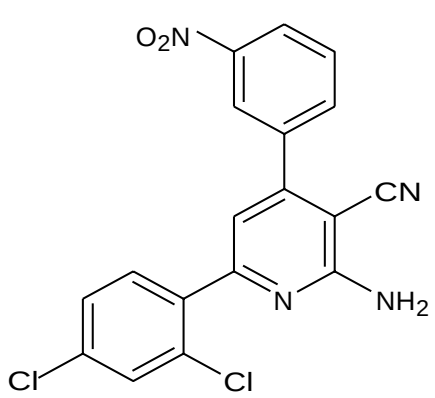
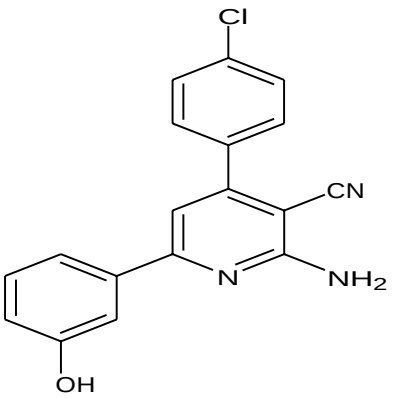


Fig.1- Cleaned Adenosine A2A receptor –PDB ID: 5UIG

Structures of the top two ligands with better binding energies selected for visualization.



C-87:
2-amino-6-(2,4-dichlorophenyl)-4-(3-nitrophenyl)pyridine-3-carbonitrile



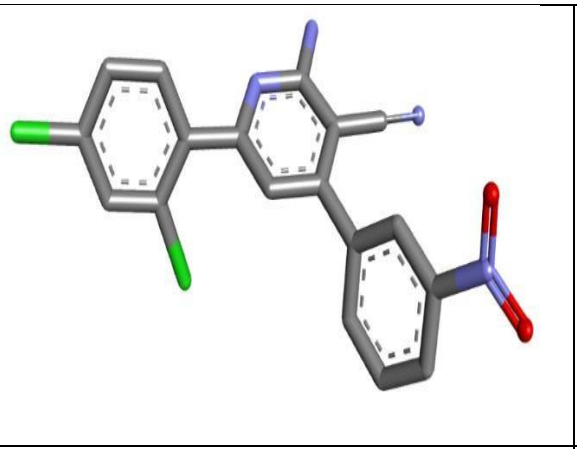
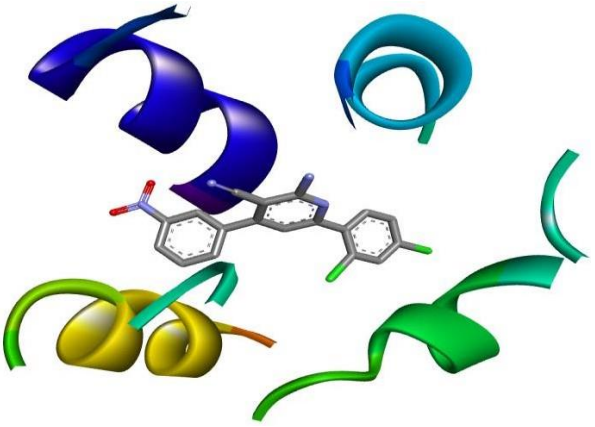
C67:
2-amino-6-(3-hydroxyphenyl)-4-(4-chlorophenyl)pyridine-3-carbonitrile

Table 1: Binding energy and interaction summary of the top ten compounds with Adenosine A2A receptor

Compound Code	Binding Energy K.Cal/mol	Interacting active site amino acid residues
C87	**-109.35**	**GLU:169, ASN:253, SER:67**, THR:88, VAL:84, ALA:81, MET:174, HIS:264, MET:270, TYR:271, PHE:168, ILE:274, MET:177, LEU:85, LEU:249, TRP:246, ILE:66
C67	**-108.14**	**PHE:168**, TYR:271, ILE:66, VAL:84, PHE:62, ILE:274, ALA:81, ALA:63, ALA:59, ILE:80
C18	-107.26	**ASN:253**, ILE:66, TYR:271, MET:177, ILE:274, ALA:63, PHE:168, VAL:172, ALA:81, HIS:264, LEU:267, MET:270
C63	-105.69	**ASN:253, GLU:169**, ILE:274, LEU:249,VAL:84, MET:270, LEU:267,TYR:271
C79	-103.39	**ASN:253, MET:270**, THR:88, ALA:81, ASN:181, VAL:84, LEU:267, GLU:169, HIS:264, LEU:249, MET:177, ILE:274, LEU:85, HIS:250, TRP:246, TYR:271, PHE:168, SER:67
C15	-101.04	**CYS:82, ASN:253, ALA:63**, TYR:271, ALA:81, PHE:168, GLU:169, HIS:264, LEU:267, MET:270, LEU:85, VAL:172, ILE:274, ILE:66,MET:177, MET:174
C66	-99.16	**ASN:253**,ILE:66,CYS:82,PHE:168,MET:270,LEU:267, MET:177,ILE:274,ALA:63,ALA:81,VAL:172
C73	-98.60	**ASN:253, GLU:169,** PHE:168, MET:270, ILE:274, LEU:249, TYR:271, SER:67, ALA:63, ILE:66, LEU:85,VAL:84, TRP:246, LEU:267
C9	-97.35	**GLU:169, ASN:253, ALA:63**, PHE:168,TYR:271, MET:270, ILE:274, VAL:84, LEU:85, MET:177
C16	-96.48	**ALA:63,** PHE:168, GLU:169, ASN:253, MET:177, TYR:271, HIS:264, VAL:84, LEU:249, LEU:267, ILE:274, MET:174, MET:270, **ILE:66, ALA:81**
Istradefylline	**-74.91**	LEU:269, ILE:252, ALA:265, HIS:264
Caffeine	**-69.60**	ILE:274, ALA:63, TYR:9, TYR:271, ILE:66

H-bond interacting residues represented in green color and unfavorable interactions in red color

Table 6082 - Docking and visualization data of C87 against Adenosine A2A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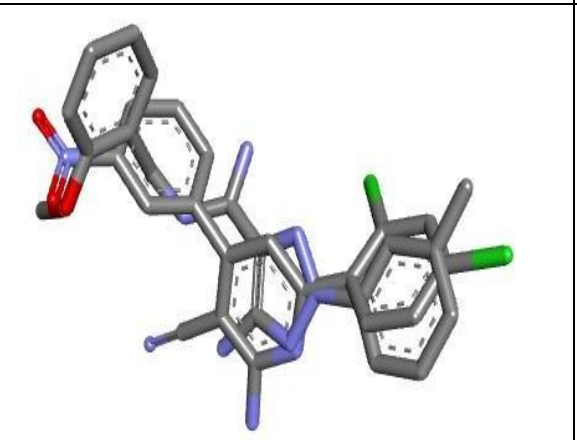
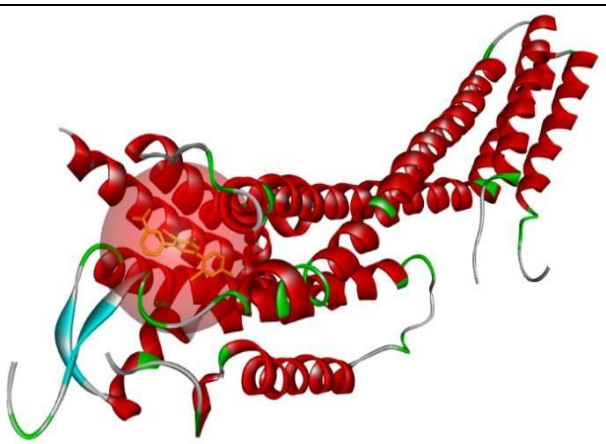
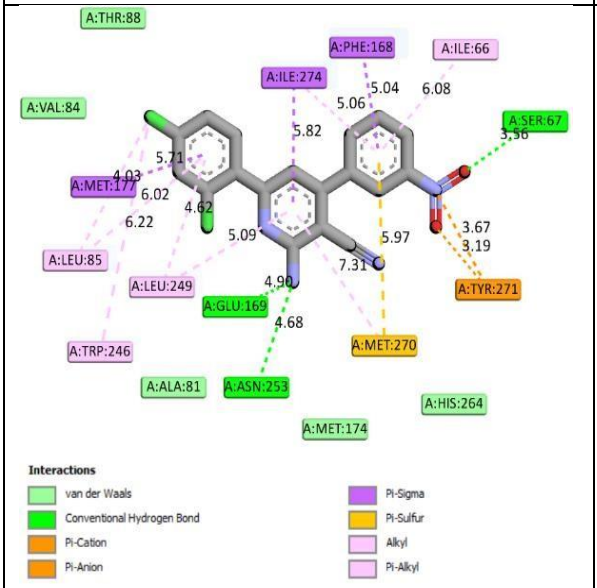
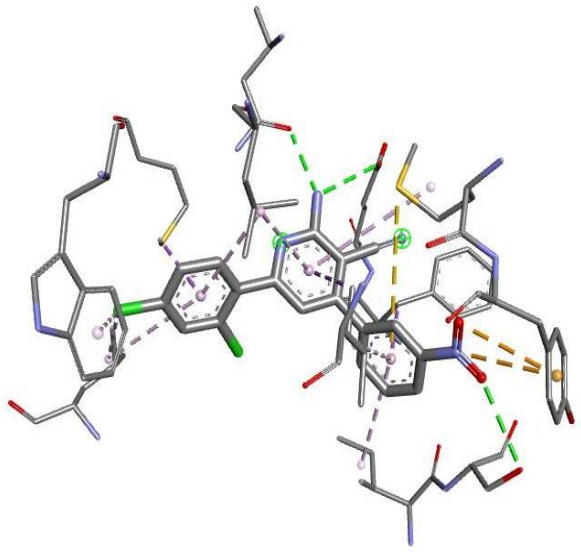
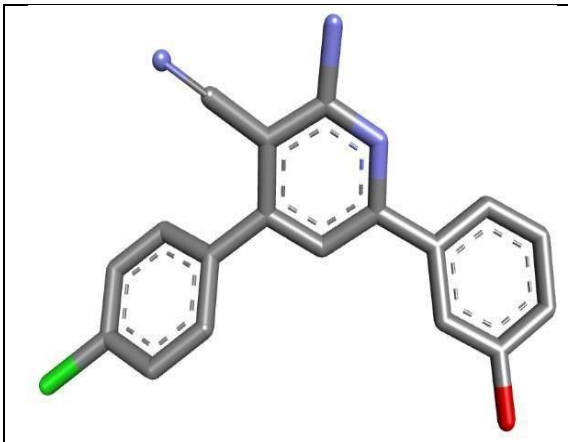
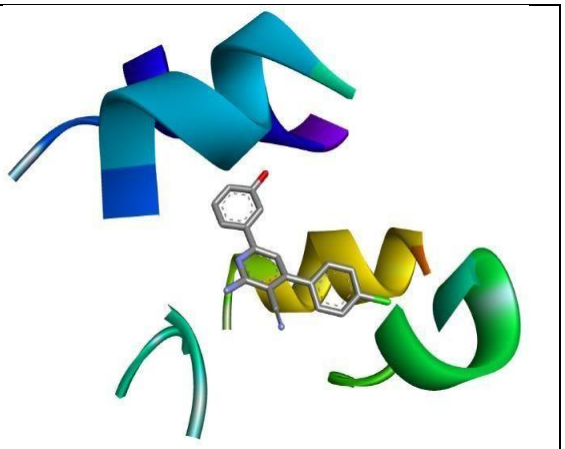
	
3. C87 LIGAND + COCRYSTALLIZED LIGAND COMPLEX	4. C87 LIGAND+ ADENOSINE A2A WHOLE RECEPTOR COMPLEX
	
5. C87 2D INTERACTION WITH ADENOSINE A2A RECEPTOR	6. C87 3D INTERACTION WITH ADENOSINE A2A RECEPTOR

Table 3 - Docking and visualization data of C67 against Adenosine A2A receptor.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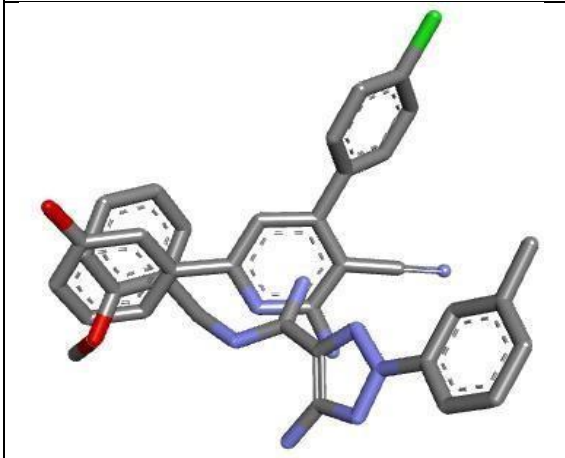
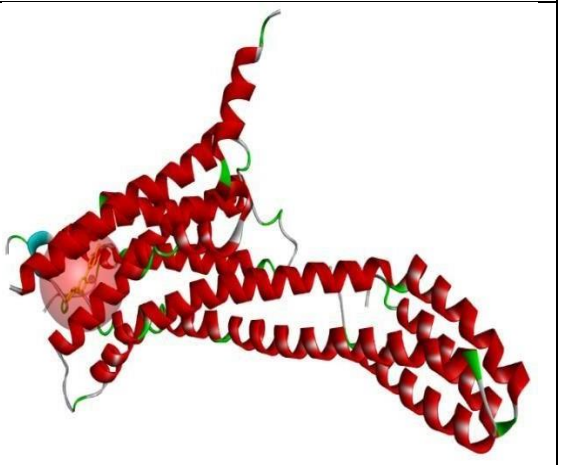
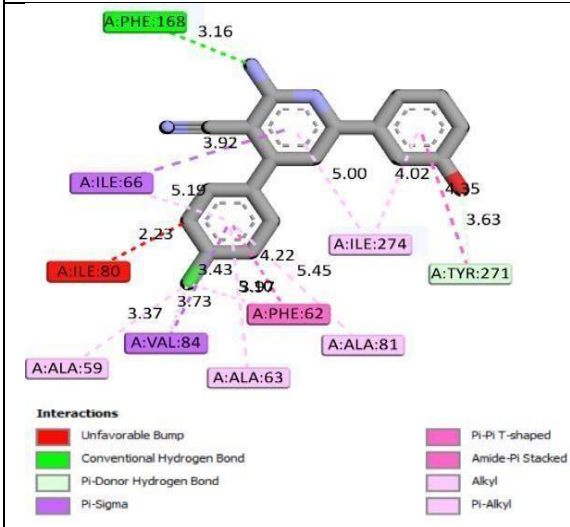
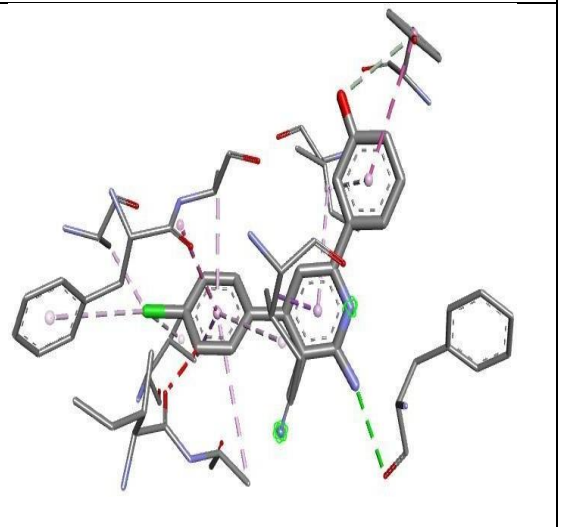
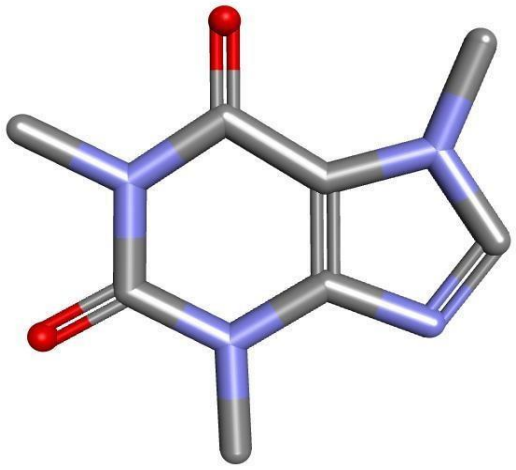
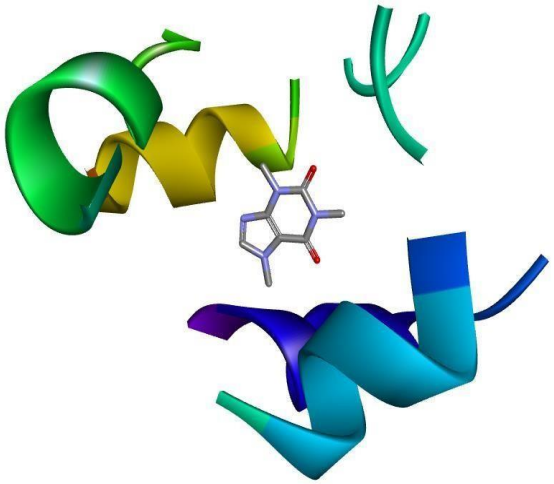
	
3. C67 LIGAND + COCRYSTALLIZED LIGAND COMPLEX	4. C67 LIGAND + ADENOSINE A2A WHOLE RECEPTOR COMPLEX
	
5. C67 2D INTERACTION WITH ADENOSINE A2A RECEPTOR	6. C67 3D INTERACTION WITH ADENOSINE A2A RECEPTOR

Table 4 - Docking and visualization data of standard antagonist caffeine against Adenosine A2A receptor.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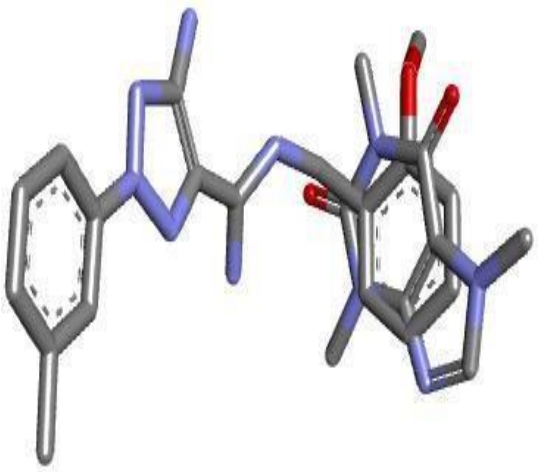
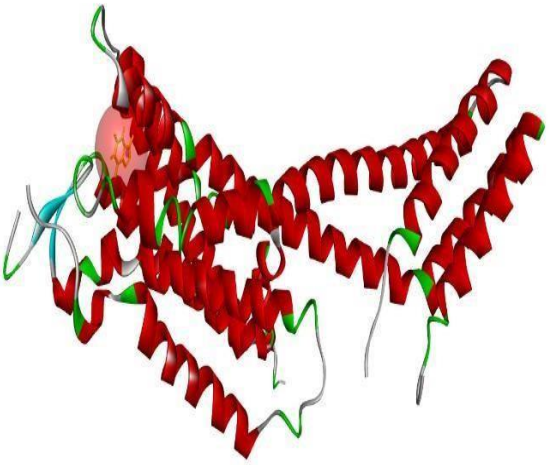
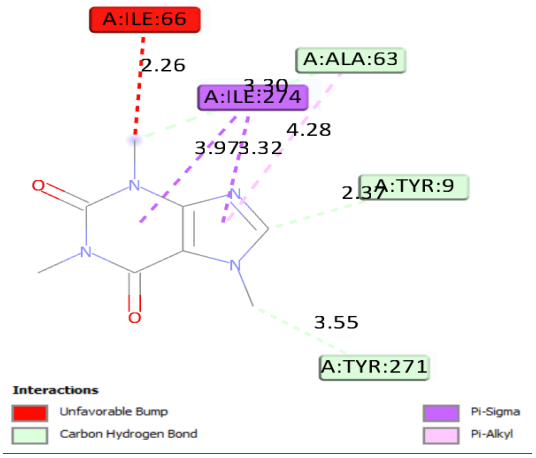
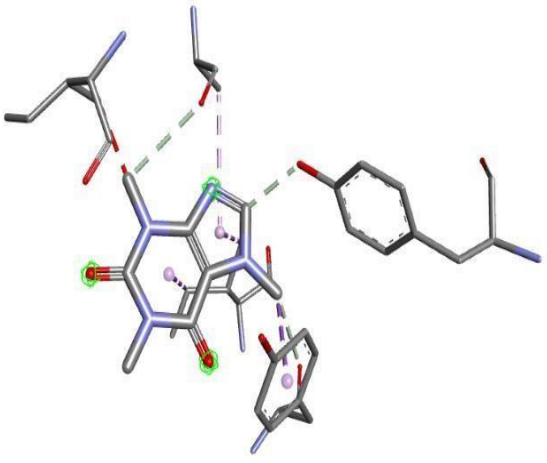
	
3. CAFFEINE LIGAND + COCRYSTALLIZED LIGAND COMPLEX	4. CAFFEINE LIGAND + ADENOSINE A2A WHOLE RECEPTOR COMPLEX
	
5. CAFFEINE 2D INTERACTION WITH ADENOSINE A2A RECEPTOR	6. CAFFEINE 3D INTERACTION WITH ADENOSINE A2A RECEPTOR

Table 5- Docking and visualization data of standard antagonist Istradefylline against Adenosine A2A receptor.

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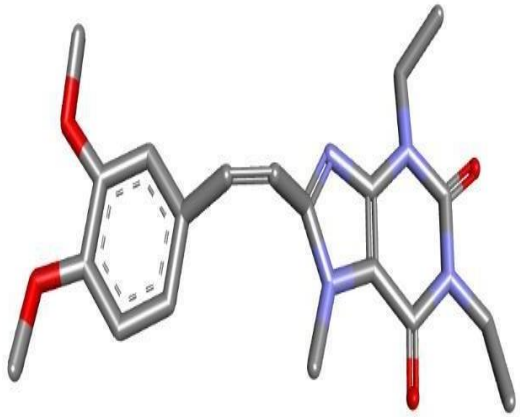
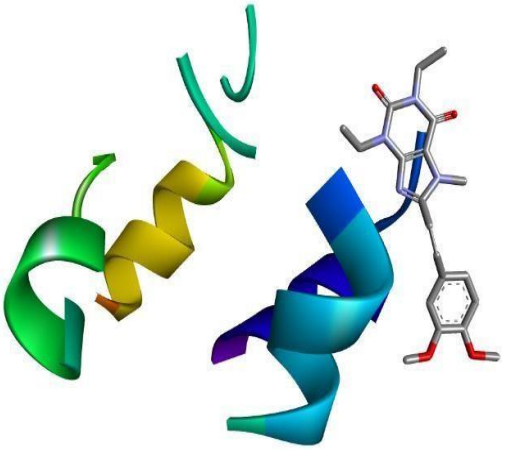
<tr><td>  </td><td>  </td></tr>
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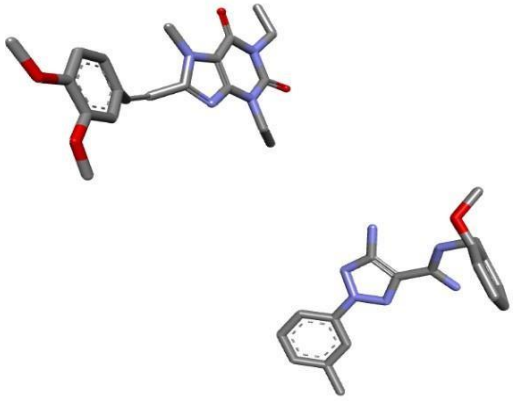
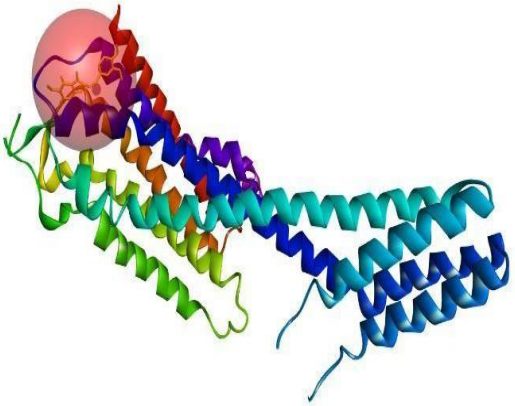
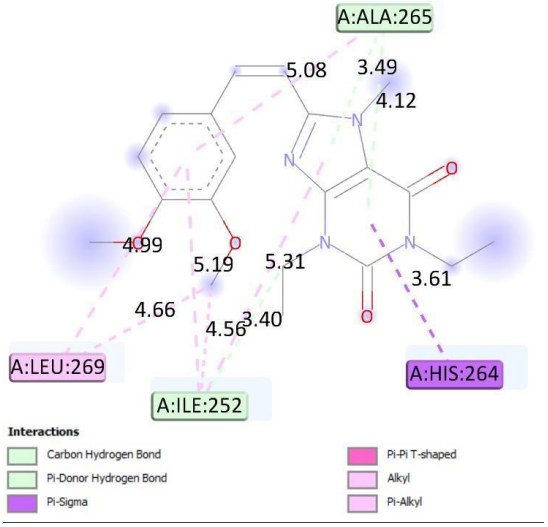
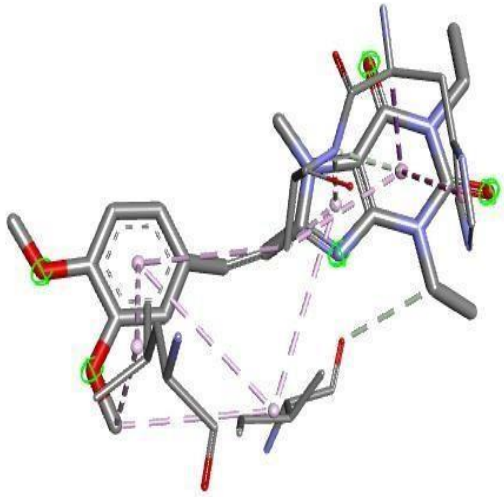
<tr><td>  </td><td>  </td></tr>
<tr><td>3.ISTRADEFYLLINE LIGAND + COCRYSTALLIZED LIGAND COMPLEX</td><td>4. ISTRADEFYLLINE LIGAND + ADENOSINE A2A WHOLE RECEPTOR COMPLEX</td></tr>
<tr><td> 
</td><td>  </td></tr>
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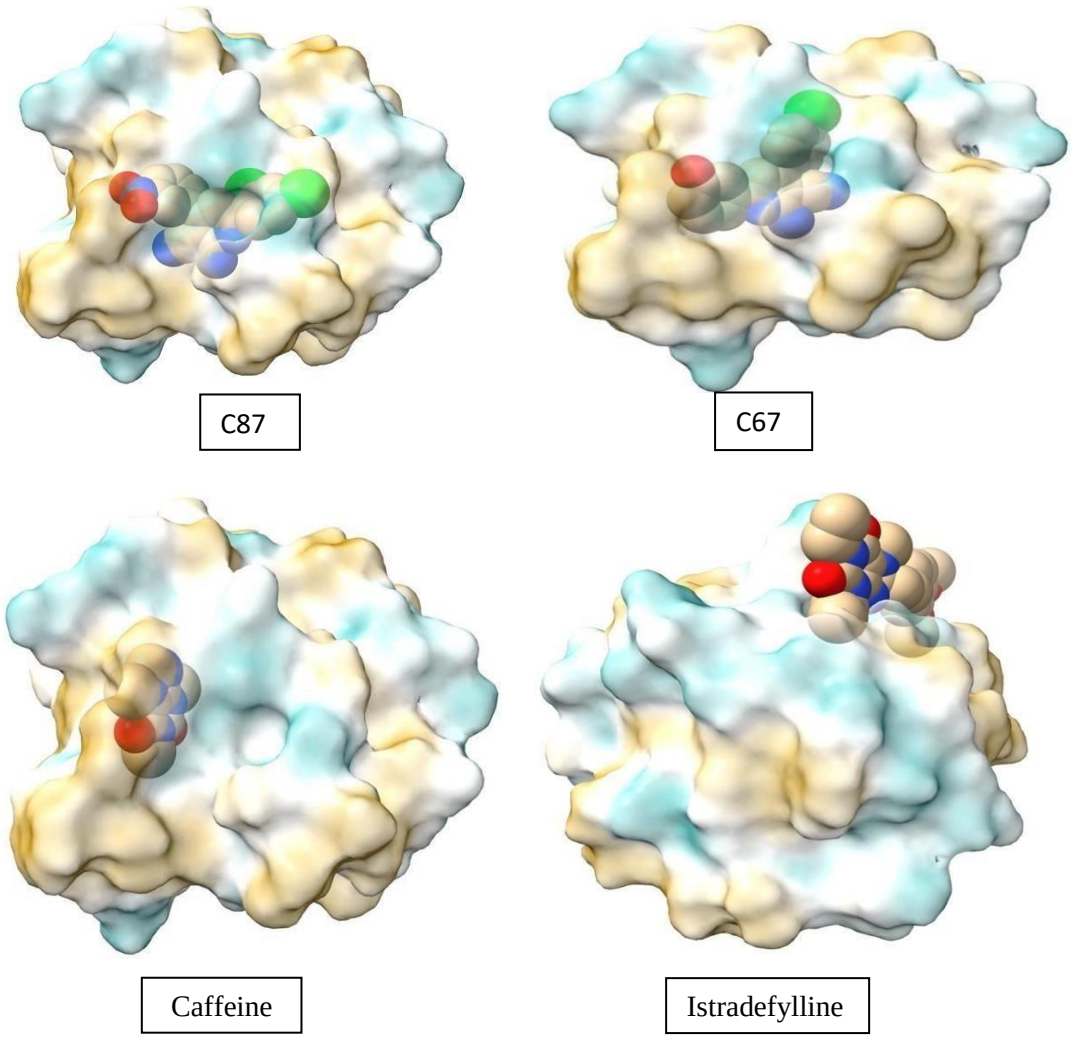

Figure 2: Active site pocket surface and binding modes of C87, C67, Caffeine and Istradefylline with A2A protein.

Conclusion:

Almost all the top ten compounds have better binding energies than that of the standard Adenosine A2A receptor antagonists. The top two compounds C87 and C67 have better binding energy than that of the standard antagonists of Adenosine A2A receptor. Compound C87 has a binding energy of -109.35 k.cal/mol and compound C67 has a binding energy of -108.14 k.cal/mol, which is very good when compared to that of the standard antagonists like Istradefylline[24] with a binding energy of -74.91 k.cal/mol and caffeine[25] with a binding energy of -69.60 k.cal/mol. Compound C87 has three hydrogen bond interactions with the receptor through three amino acid residues **GLU:169, ASN:253, SER:6** and Compound C67 has only one hydrogen bond interaction through the amino acid **PHE:168.** The standard antagonists Istradefylline and Caffeine did not show any hydrogen bond interactions.

C87 and C67 has better orientations compared to that of the standard Adenosine A2A receptor antagonists like Istradefylline and caffeine due to their positioning inside the active site pocket. Istradefylline is binding on to the outer shallow pocket of the active site which can be observed from the figure 2.

The better binding affinity of C87 might be due to the presence of electron withdrawing groups like one NO_2 and two Cl groups. The better binding affinity of C67 might be due to the presence of electron withdrawing one Cl group and electron donating one OH group.

Compounds C87 and C67 can be further synthesized and in vivo activities could be performed as they have better binding affinities and energies than that of the standard Adenosine A2A receptor antagonists like Istradefylline and Caffeine.

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