

# Molecular docking studies of 1-(substituted phenyl)-3-(naphtha [1, 2-*d*] thiazol-2-yl) urea/thiourea derivatives with human adenosine A<sub>2A</sub> receptor

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Received June 27, 2011; Accepted June 28, 2011; Published July 19, 2011

## Abstract:

Computational assessment of the binding interactions of drugs is an important component of computer-aided drug design paradigms. In this perspective, a set of 30 1-(substituted phenyl)-3-(naphtha[1, 2-*d*] thiazol-2-yl) urea/thiourea derivatives showing antiparkinsonian activity were docked into inhibitor binding cavity of human adenosine A<sub>2A</sub> receptor (AA<sub>2A</sub>R) to understand their mode of binding interactions *in silico*. Lamarckian genetic algorithm methodology was employed for docking simulations using AutoDock 4.2 program. The results signify that the molecular docking approach is reliable and produces a good correlation coefficient ( $r^2 = 0.483$ ) between docking score and antiparkinsonian activity (in terms of % reduction in catalepsy score). Potent antiparkinsonian agents carried methoxy group in the phenyl ring, exhibited both hydrophilic and lipophilic interactions with lower energy of binding at the AA<sub>2A</sub>R. These molecular docking analyses should, in our view, contribute for further development of selective AA<sub>2A</sub>R antagonists for the treatment of Parkinson's disease.

**Keywords:** Docking; Adenosine A<sub>2A</sub> receptor antagonist; Parkinson's disease; naphtha [1, 2-*d*] thiazol-2-amine; Urea derivatives.

## Background:

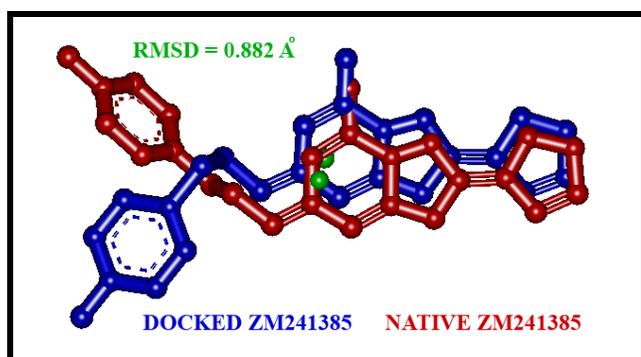
Adenosine A<sub>2A</sub> receptors (AA<sub>2A</sub>R) are highly distributed in the central nervous system and are found in abundance in the basal ganglia, a region of the brain associated with motor function [1]. The colocalization of AA<sub>2A</sub>R and dopamine D<sub>2</sub> receptors in the striatopallidal neurons provides the anatomical basis for the existence of a functional antagonistic interaction between these receptors [2]. Stimulation of the dopamine D<sub>2</sub> receptors with dopamine or other dopamine D<sub>2</sub> receptor agonists enhances motor activity whereas stimulation of AA<sub>2A</sub>Rs reduces this effect [3]. Likewise, AA<sub>2A</sub>R antagonists may provide a novel therapy for the treatment of Parkinson's disease (PD) with lower risk of dyskinesias. In addition, they may exhibit neuroprotective effects [4]. Furthermore, AA<sub>2A</sub>R antagonists were found not only to diminish the symptoms of PD but also to potentiate the effect of levodopa [5], so, it may be possible to reduce the dose of the dopaminergic drugs and therefore the occurrence of side effects. AA<sub>2A</sub>R antagonists are therefore a promising adjunctive to dopamine replacement therapy, and several companies have now advanced selective antagonists of this receptor into clinical development [4]. Significant progress has been made in computer-aided drug design by pharmaceutical companies at different stages of drug discovery such as

identifying new hits, enhancing molecule binding affinity in hit-to-lead, and lead optimization [6]. Moreover, *in silico* approaches are routinely used in modern drug design to help understand drug-receptor interactions. It has been shown in the literature that computational techniques can strongly support and help the design of novel, more potent inhibitors by revealing the mechanism of drug-receptor interactions [7].

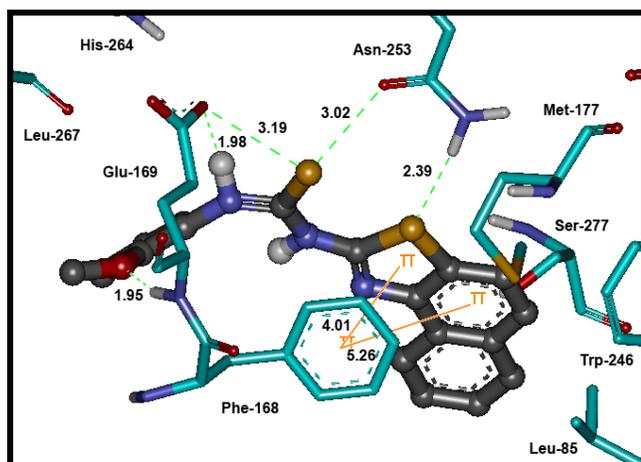
Thiazole derivatives have evoked considerable attention in recent years as these are endowed with wide range of biological activities as well as drugs of PD [8-12]. Hoffmann-La Roche discovered benzothiazole derivatives as novel scaffold for AA<sub>2A</sub>R antagonists [13]. Furthermore, certain thiazoles with a urea moiety [14] have also demonstrated AA<sub>2A</sub>R antagonistic activities for the development of a suitable approach to the treatment of PD that may be the starting point for the future drug design. Recently, we have discovered urea derivatives of naphtha [1, 2-*d*] thiazol-2-amine as novel anti-Parkinsonian agents that cause neuroprotection against haloperidol-induced oxidative stress in mice [10]. These findings have motivated us to reveal their interactions with AA<sub>2A</sub>Rs *in silico* by using AutoDock program and to attest anti-Parkinsonian activity associated with them.

## Materials and Methodology:

For the present study, crystal structure of human AA<sub>2A</sub>R with PDB ID: 3EML [15] was downloaded from the protein data bank ([www.rcsb.org/pdb](http://www.rcsb.org/pdb)). For docking experiments with AutoDock 4.2, antiparkinsonian molecules reported by our group [10] were drawn in ChemBioDraw Ultra 12.0 and converted to their three dimensional structures in ChemBio3D Ultra 12.0, energy minimized by PM3 method using MOPAC Ultra 2009 program (<http://OpenMOPAC.net>) and saved in pdb format. The prepared ligands were used as input files for AutoDock 4.2 in the next step. Lamarckian genetic algorithm method [16] was employed for docking simulations. The standard docking procedure was used for a rigid protein and a flexible ligand whose torsion angles were identified (for 10 independent runs per ligand). A grid of 60, 60, and 60 points in x, y, and z directions was built with a grid spacing of 0.375 Å and a distance-dependent function of the dielectric constant were used for the calculation of the energetic map. The default settings were used for all other parameters. At the end of docking, the best poses were analyzed for hydrogen bonding/ $\pi$ - $\pi$  interactions and root mean square deviation (RMSD) calculations using Discovery Studio Visualizer 2.5 (Accelrys Software Inc.) and Pymol (The PyMOL Molecular Graphics System) programs. From the estimated free energy of ligand binding ( $\Delta G_{\text{binding}}$ , kcal/mol), the inhibition constant ( $K_i$ ) for each ligand was calculated (Table 1 see Supplementary material).



**Figure 1:** The native co-crystallized ligand ZM241385 docked with the active site of AA<sub>2A</sub>R.



**Figure 2:** Docked conformation of compound 22 shown as ball and stick in grey color. The residues of binding pocket are shown as stick in cyan color. Dashed lines in green indicate H-bonds and  $\pi$ - $\pi$  stacking interactions are shown in orange. Bond distances are given in Å.

## Results and Discussion:

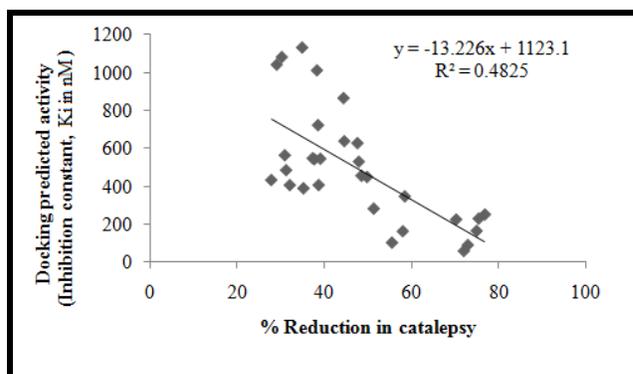
### Validation of the docking protocol:

To evaluate the accuracy of AutoDock 4.2 as an appropriate docking tool for the present purpose, the co-crystallized ligand (ZM241385) was redocked within the inhibitor binding cavity of human AA<sub>2A</sub>R, and the docked position was compared to the crystal structure position by calculating RMSD values (0.88 Å, Figure 1). In general, RMSD values smaller than 2.0 Å indicate that the docking protocol is capable of accurately predicting the binding orientation

of the co-crystallized ligand [17]. In this study, RMSD values were within 2.0 Å, indicating our docking methods are valid for the given structures and AutoDock 4.2, therefore deemed reliable for docking of naphtha [1, 2-*d*]thiazolyl urea/thiourea derivatives into the inhibitor binding cavity of AA<sub>2A</sub>R.

### Correlation between docking scores and antiparkinsonian activity:

The results signify that the molecular docking approach is reliable and produces a good correlation coefficient ( $r^2 = 0.483$ ) between docking score and antiparkinsonian activity (in terms of % reduction in catalepsy score). Removal of 4 compounds (compounds 17, 26, 28 and 30) identified as outliers from the docking dataset and yield a better model with correlation coefficient ( $r^2$ ) of 0.646 with 26 compounds (Figure 3). Outliers in the molecular docking studies are anticipated because, docking calculations simulate the interaction between a compound and a protein's active site and the results are comparable to those of biochemical assays/animal experiments; however, it does not take into account, the bioavailability, toxicity, and other factors present in the body.



**Figure 3:** A correlation for docking predicted activity and experimental antiparkinsonian activity.

### Binding interactions of 1-(substituted phenyl)-3-(naphtha [1, 2-*d*] thiazol-2-yl) urea/thiourea derivatives with human AA<sub>2A</sub>R:

All of the naphtha [1, 2-*d*] thiazolyl urea/thiourea derivatives were docked into the active site of AA<sub>2A</sub>R (PDB code: 3EML) to study the possible mode of their interaction. Docking of these compounds into inhibitor binding cavity of AA<sub>2A</sub>R confirms that these compounds dock in a similar binding mode like native co-crystallized ligand, ZM-241385 (Figure 2). Inhibitor binding cavity of AA<sub>2A</sub>R is outlined by residues Ile-66, Ala-81, Leu-85, Phe-168, Glu-169, Met-177, Trp-246, Leu-249, His-250, Asn-253, His-264, Leu-267, Met-270, Tyr-271, Ser-277 and His-278. Analysis of the receptor/ligand complex models generated after successful docking of the urea and thiourea derivatives was based on parameters such as: 1) Hydrogen bond interactions; 2)  $\pi$ - $\pi$  stacking/hydrophobic interactions; 3) binding energy; 4) RMSD of active site residues; 5) Orientation of the docked compound within the active site. The bicyclic triazolotriazine core of ZM241385, native co-crystallized AA<sub>2A</sub>R antagonist, is anchored by an aromatic stacking interaction with Phe-168 [15], an aliphatic hydrophobic interaction with Ile 274 [18] and a hydrogen bonding interaction with Asn 253 [19]. Likewise, the tricyclic naphthothiazole ring was found to interact with aromatic ring of Phe-168 by  $\pi$ - $\pi$  stacking interactions and compound 22 participated in hydrogen bonding with Asn 253. Adjacent to Phe-168, a polar residue Glu-169 donates a hydrogen bond to oxygen atom of methoxy group substituted in phenyl ring of compounds 4, 6, 16, 21 and 22. Similarly, exocyclic amino group (N-15 atom) of ZM241385 also participates in hydrogen bonding with Glu-169 [15, 20]. As a general rule, in most of the potent and fair antiparkinsonian compounds, both hydrogen bond and  $\pi$ - $\pi$  stacking/hydrophobic interactions between the compound and the active site residues of the receptor have been found to be responsible for mediating the biological activity. In addition to be involved in  $\pi$ - $\pi$  stacking/hydrophobic interactions with most docked compounds, Phe-168 contributes to H-bond interactions in compounds 5, 6, 9, 10, 18 and 29. Very potent antiparkinsonian agents (compounds 4, 5, 6, 7, 21 and 22) carried methoxy group in the phenyl ring and interacted with Phe-168, Glu-169 and His-278 residues for H-bonding. However, replacement of methoxy group with phenoxy group (compound 8) lost H-bond that resulted in decreased antiparkinsonian activity. Generally, compounds substituted with methyl group or halogen in the aromatic ring of urea or thiourea derivatives (compounds 3, 8, 9, 10, 15, 16, 19, 20, 23, 24, 25, 26, 27, 31 and 32) demonstrated poor binding energies as well as both

hydrophilic and lipophilic interactions. These compounds were also noted to be poor antiparkinsonian agents.

### Conclusion:

Molecular docking studies of 1-(substituted phenyl)-3-(naphtha[1,2-*d*]thiazol-2-yl)urea/thiourea derivatives with AA<sub>2A</sub>R exhibited very good binding interactions and warrants further studies to confirm their binding with human AA<sub>2A</sub>R for the design and development of potent antagonists as novel antiparkinsonian agents. These results clearly indicate that before synthesis and biochemical testing of new analogs, one can use molecular docking studies for assessment of relative binding affinities with AA<sub>2A</sub>R for most promising drug candidates.

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Edited by P Kanguane

Citation: Azam *et al.* Bioinformation 6(9): 330-334 (2011)

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## Supplementary material:

**Table 1:** Docking results of 1-(substituted phenyl)-3-(naphtha[1,2-*d*]thiazol-2-yl)urea/thiourea derivatives with AA<sub>23</sub>R. Data of % reduction in catalepsy were taken from reference [10]. Compound number is same as given in the literature. <sup>a</sup>)Binding free energy (kcal/mol); <sup>b</sup>)Inhibition constant estimated by docking (in nM); <sup>c</sup>)Root mean square deviation (in Å); <sup>d</sup>)Amino acid; <sup>e</sup>)Bond distance (in Å); <sup>f</sup>)No H-bond or pi-interaction.

Comp.	R	X	% Reduction in catalepsy	$\Delta G_b^a$	$K_i^b$	RMSD <sup>c</sup>	Hydrogen bonds		$\pi$ -Interactions	
							AA <sup>d</sup>	Dist <sup>e</sup>	AA <sup>d</sup>	Dist <sup>e</sup>
3	H	O	44.44	-8.27	863.78	4.29	<sup>f</sup>	-	Phe-168	4.14
									Phe-168	5.25
									Phe-168	6.43
4	4-OCH <sub>3</sub>	O	58.51	-8.81	346.16	3.99	Glu-169	2.16	Phe-168	4.53
							Tyr-271	1.95	Phe-168	5.26
5	2-OCH <sub>3</sub>	O	76.9	-9.00	250.84	4.21	Phe-168	1.97	Phe-168	4.52
							Tyr-271	1.94	Phe-168	5.24
6	2,4-OCH <sub>3</sub>	O	75	-9.25	164.57	4.03	Phe-168	2.15	Phe-168	4.43
							Glu-169	2.38	Phe-168	5.14
							Tyr-271	1.96		
7	3,4,5-OCH <sub>3</sub>	O	70.29	-9.07	224.23	3.93	His-278	1.87	Phe-168	4.44
									Phe-168	5.65
8	4-Phenoxy	O	55.56	-9.53	102.63	2.52	<sup>f</sup>	-	Phe-168	5.19
9	2-F	O	29.12	-8.16	1040	4.85	Phe-168	2.02	<sup>f</sup>	-
							Tyr-271	2.08		
10	4-F	O	30.3	-8.14	1080	4.83	Phe-168	2.07	<sup>f</sup>	-
							Tyr-271	2.05		
11	2-Br	O	38.74	-8.72	405.86	4.15	Tyr-271	2.18	Phe-168	4.55
									Phe-168	5.26
12	4-Br	O	37.76	-8.55	542.28	4.36	Tyr-271	2.11	Phe-168	4.61
									Phe-168	5.19
13	2-Cl	O	37.37	-8.54	546.60	4.09	Tyr-271	2.22	Phe-168	4.54
									Phe-168	5.28
14	4-Cl	O	38.61	-8.38	721.26	4.34	Tyr-271	2.09	Phe-168	4.60
									Phe-168	5.19
15	2-Cl, 5-CF <sub>3</sub>	O	51.37	-8.94	281.80	4.1	Tyr-271	2.13	<sup>f</sup>	-
16	2-NO <sub>2</sub>	O	72.05	-9.87	58.72	4.69	Glu-169	2.1	<sup>f</sup>	-
							Glu-169	2.1		
17	4-NO <sub>2</sub>	O	31.28	-8.61	484.40	3.93	Val-84	2.04	Phe-168	5.11
18	2-CH <sub>3</sub>	O	47.64	-8.46	626.58	4.01	Phe-168	1.74	Phe-168	4.13
							Tyr-271	1.89	Phe-168	4.57
									Phe-168	6.03
19	4-CH <sub>3</sub>	O	48.56	-8.65	455.03	4.03	<sup>f</sup>	-	Phe-168	4.38
									Phe-168	5.45
									Phe-168	6.38
20	H	S	44.63	-8.45	636.96	3.95	Ala-81	2.17	<sup>f</sup>	-
21	4-OCH <sub>3</sub>	S	58.05	-9.26	162.54	5.02	Glu-169	2.09	Phe-168	4.27
							Tyr-271	1.88	Phe-168	3.76
									Phe-168	3.95
22	2-OCH <sub>3</sub>	S	75.46	-9.06	229.22	2.72	Glu-169	1.95	Phe-168	4.01
							Asn-253	2.39	Phe-168	5.26
							Glu-169	1.98		
							Glu-169	3.19		
							Asn-253	3.02		
23	2-F	S	38.35	-8.18	1010	1.39	<sup>f</sup>	-	Phe-168	3.99
									Phe-168	4.69
									Phe-168	5.91
24	4-F	S	34.95	-8.11	1130	4.24	<sup>f</sup>	-	Phe-168	3.91
									Phe-168	3.81
									Phe-168	4.45
25	2-Br	S	30.96	-8.53	562.85	4.05	<sup>f</sup>	-	Phe-168	3.68
									Phe-168	3.79
									Phe-168	4.49
26	4-Br	S	35.27	-8.75	388.83	4.22	<sup>f</sup>	-	-	-
27	2-Cl	S	39.14	-8.55	544.16	3.1	<sup>f</sup>	-	Phe-168	4.06
									Phe-168	4.80

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28	4-Cl	S	27.88	-8.68	432.38	4.05	Tyr-271	2.22	Phe-168	3.88
									Phe-168	4.92
									Phe-168	4.1
									Phe-168	3.81
29	2-NO <sub>2</sub>	S	72.97	-9.61	90.07	2.63	Phe-168	2.15	-	-
							Phe-168	1.99		
							Ile-66	3.19		
30	4-NO <sub>2</sub>	S	32.13	-8.72	405.62	4.45	His-278	2.05	Phe-168	3.95
									Phe-168	3.99
									Phe-168	4.33
31	2-CH <sub>3</sub>	S	49.87	-8.66	448.49	4.77	f	-	Phe-168	4.12
									Phe-168	4.81
									Phe-168	6.27
32	4-CH <sub>3</sub>	S	47.97	-8.56	529.05	4.30	f	-	Phe-168	4.18
									Phe-168	5.00
									Phe-168	6.26

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