EXPLORING THE BINDING MODE OF NEW SELECTIVE A_3 ADENOSINE RECEPTOR ANTAGONISTS USING MOLECULAR DYNAMICS SIMULATIONS AND MM-PBSA CALCULATIONS

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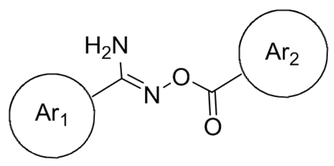
INTRODUCTION

Adenosine receptors (ARs), G protein-coupled receptor family members, comprise four subtypes A_1, A_{2A}, A_{2B} and A_3 and mediate the multiple physiological effects of adenosine.[1] Pharmaceutical companies and academic research laboratories are involved in intense efforts to develop selective antagonists for each AR subtype for a possible "soft" treatment of different diseases.

Previously, an *in silico* virtual screening (VS) of 14400 compounds of Maybridge database against the X-ray structure of $A_{2A}R$ had been explored using a combination of structure- and ligand-based methods.[2] Out of the eight selected and tested in four ARs compounds, five had been found positive hits with low μM affinity against A_1/A_3 or A_{2A}/A_3 ARs, and A_3AR. Nineteen new molecules were selected, based on their similarity to the five active hits, from the e-molecules search engine, purchased and tested.

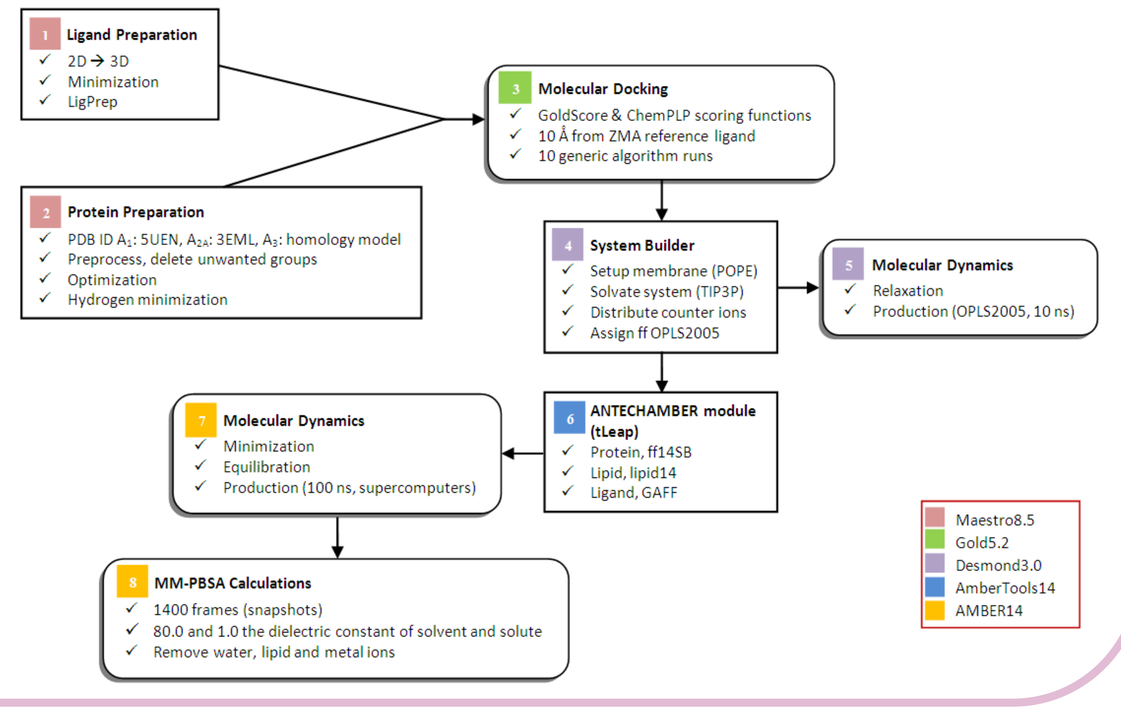
In the present study, the six molecules **20**–**25** were investigated. They were selected based on their structural similarity with the most interesting compound **5**, as regards synthetic feasibility, that identified as a good binder to A_{2A}/A_3 ARs from VS pipeline. The common substructure is a carbonyloxycarboximidamide moiety (**Scheme 1**).

The compounds' testing results are showed in **Table 1**. Their K_i values ranged from 30 μM to submicromolar values. In particular, compound **23** exhibited affinity for A_{2A}/A_3; compound **21** for A_1/A_3; whereas compounds **22**, **24**, and **25** were selective for A_3.

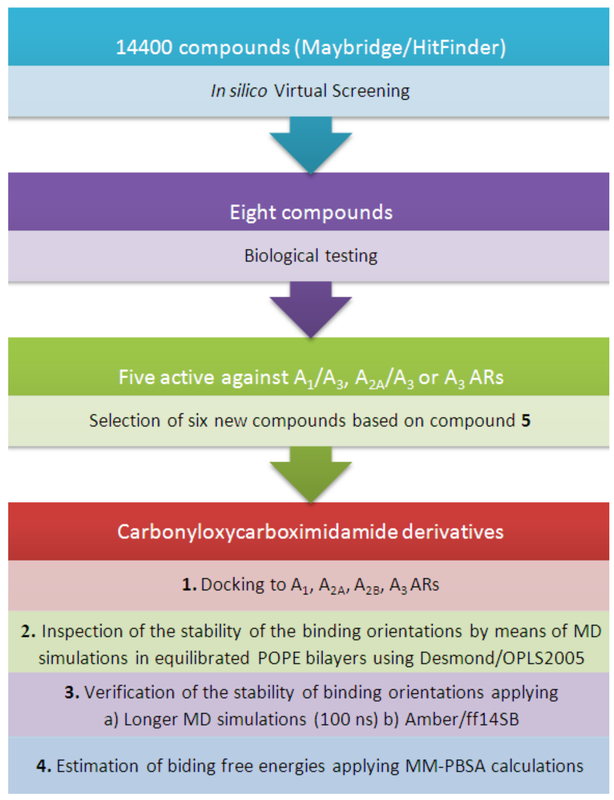


Scheme 1. The carbonyloxycarboximidamide segment presented in studied compounds.

METHODS



RESULTS AND DISCUSSION



Scheme 2. Workflow used in the present study.

A computational workflow was applied in order to investigate the binding mode of ligands into ARs (**Scheme 2**). Molecular docking calculations and molecular dynamics (MD) simulations of 10 ns were employed for ligands against all ARs in order to allow the prediction of stable and unstable complexes which agree with the experimental results.

According to **Table 1**, compounds **22**, **24**, and **25** exhibited selective binding to A_3AR. Longer MD simulations using AMBER were performed in order to investigate further the binding interactions of the active compounds.

Furthermore, Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) calculations were performed in order to calculate the binding free energies and to *in silico* quantitatively estimate the experimental binding affinities of compounds to A_3AR.

Table 1. Binding affinities obtained from radioligand binding assays of studied compounds against A_1, A_{2A}, A_{2B}, A_3 ARs.

Compound	Ar_1	Ar_2	K_i A_1AR (μM)	K_i A_{2A}AR (μM)	K_i A_{2B}AR (μM)	K_i A_3AR (μM)
3			>100	>100	>30	>100
5			>100	21.8	>30	9.45
20			>100	>100	>30	30.9
21			6.91	>100	>30	4.13
22			>60	>60	>30	**4.49**
23			>60	30	>30	5.15
24			>30	>60	>30	**4.16**
25			>100	>100	>30	**0.899**

The MD simulations showed that the bulky biaryl group can be positioned close to V5.30 of the A_3AR orthosteric binding area, where the isoxazole ring can engage in an aromatic π-π stacking interaction with the phenyl group of F5.29. The monoaryl group can be oriented deeper into the receptor favoring interactions with L6.51, and the binding orientation is stabilized due to the H-bonding interactions. In inactive to all ARs **3**, the biaryl group is bulky and cannot be fitted deep into the receptor toward L6.51 and W6.48.

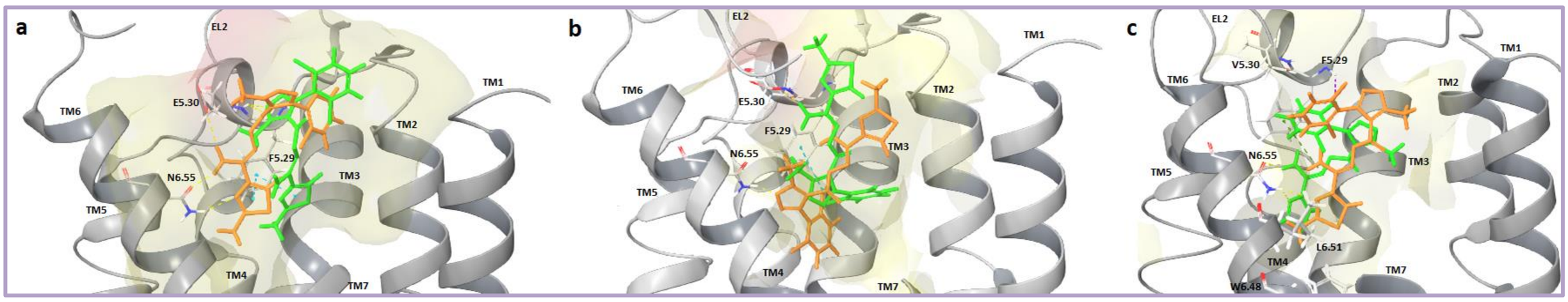


Figure 1. Predicted binding of ligand **25** in the orthosteric binding area of the (a) A_1, (b) A_{2A}, and (c) A_3 ARs using docking calculations (orange) and MD simulations (green).

In A_{2A}AR, where 5.30 is changed from Val to Glu, the phenyl group of 3-phenyl-isoxazole can be oriented toward the TM2 through rotation around the biaryl-carbonyloxy bond to avoid any unfavorable interactions with the E5.30 carboxylate group. In A_1AR, the binding region is wider, and thus, the ligand cannot bind tightly inside the receptor.

The 100 ns MD simulations of **25** showed that the dichlorophenyl ring favors increased vdW interactions with F5.29 and V5.30 which enable the ligand to form stronger H-bonding interactions with the side chain of N6.55 and the backbone of L6.51 (**Figure 1**).

Table 2. MM–PBSA energy calculations for the compounds.

	5	**24**	**25**
ΔG^*_{bind} (kcal mol^{-1})	– 23.0885	– 26.4220	– 28.0126

*the contribution of entropy has been neglected

The MM-PBSA calculations were performed on ligands **5**, **24**, and **25** in A_3AR, as there is an interesting structural difference among them; the presence of chloro substituent in the phenyl ring of 3-phenyl-isoxazole favors A_3AR selectivity, as following 0Cl < 1Cl < 2Cl, i.e. **5** < **24** < **25**. The MM-PBSA results are listed in **Table 2**.

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2. P. Lagarias *et al.*, *J. Chem. Inf. Model.* **2018**, *58*, 794–815.