STRUCTURE – ACTIVITY RELATIONSHIPS OF NEW SELECTIVE ADENOSINE RECEPTOR ANTAGONISTS USING MOLECULAR DYNAMICS SIMULATIONS

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INTRODUCTION

Over the last decades, pharmaceutical companies and academic research laboratories are involved in an intense effort to develop selective modulators for each Adenosine Receptor (AR) subtype for a possible "soft" treatment of different diseases [1]. In a recent study [2], our lab implemented a combined structure- and ligand- based drug design workflow incorporating docking, similarity search, and Molecular Dynamics (MD) simulation protocols to find new ligands for each receptor subtype. Our approach allowed the identification of novel chemotypes for each receptor subtype (**Scheme 1**). Of particular interest were the 2-amino-thiophene-3-carboxamide (**Class A**) and the 3-amino-thiophene-2-carboxamide (**Class B**) derivatives.

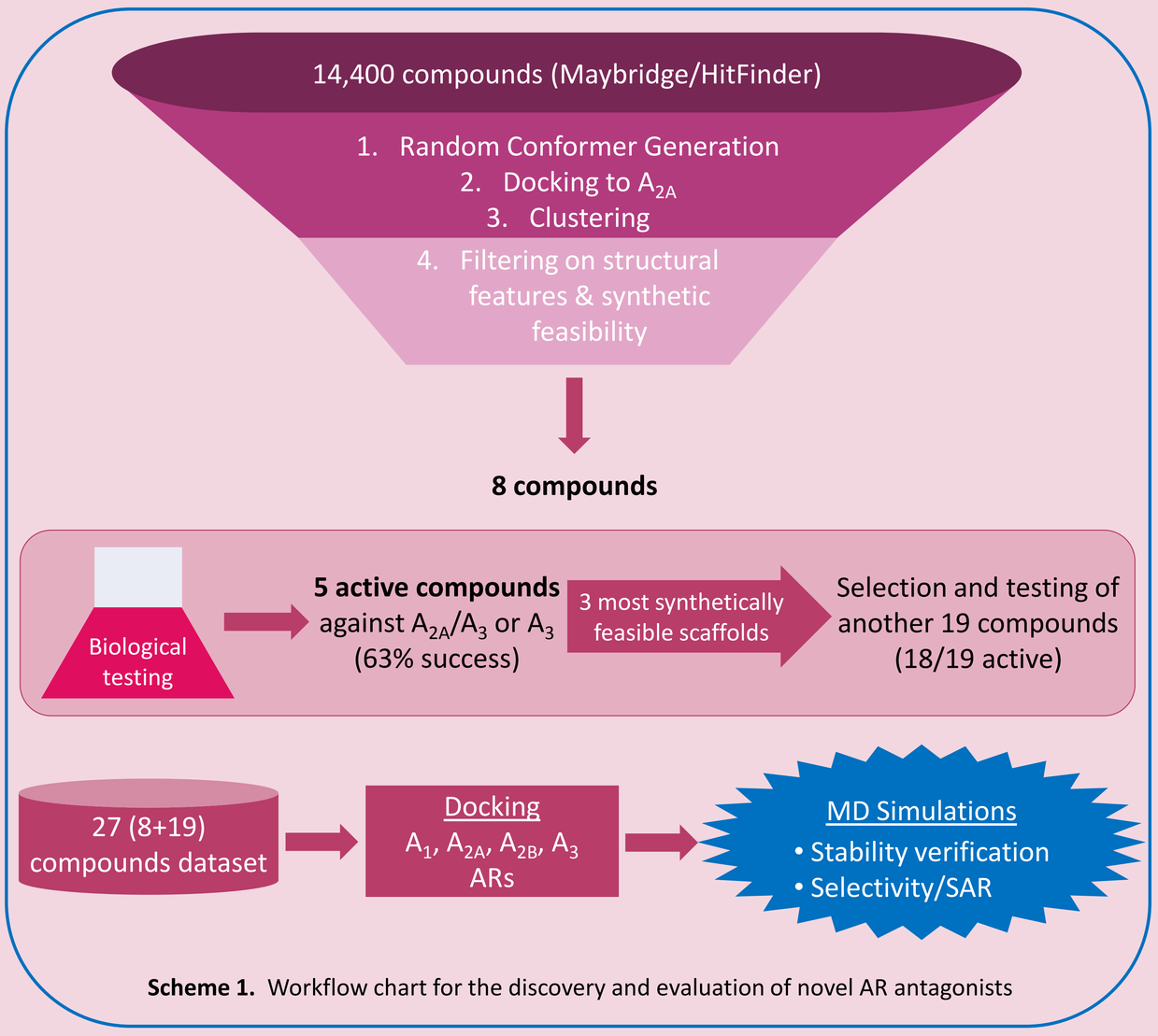
SIMULATION METHODS

➢ The 27 compounds were docked (GOLD 5.2 with GoldScore and ChemPLP) in homology models of ARs based on the crystal structures 5UEN (A_1AR) and 3EML (A_{2A}, A_{2B} and A_3AR), with ZM241385 as reference ligand.

➢ **MD Simulations**

System Builder (Maestro)		Molecular Dynamics (Desmond)	
Lipid	POPE	Minimization	2,000 restrained steps and 10,000 unrestrained steps
Water	TIP3P	Equilibration	400 ps NVT; then 3 ns restrained NPT
Salt	0.150 M NaCl	Production*	10 ns NPT
Box size	15x15x15 Å^3 buffer	Temperature	310 K
Force Field	OPLS_2005	*stable ligands were simulated up to 100 ns	

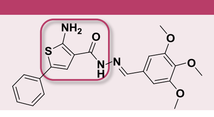
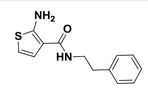
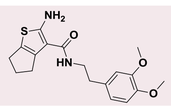
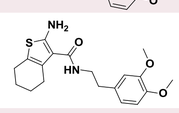
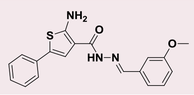
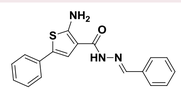
➢ Complexes with ligands **1**, **11**, **13**, and **17** were also simulated using AMBER with ff14SB and lipid14 force fields, using similar conditions and parameters as with Desmond.



Scheme 1. Workflow chart for the discovery and evaluation of novel AR antagonists

RESULTS AND DISCUSSION

Class A Derivatives

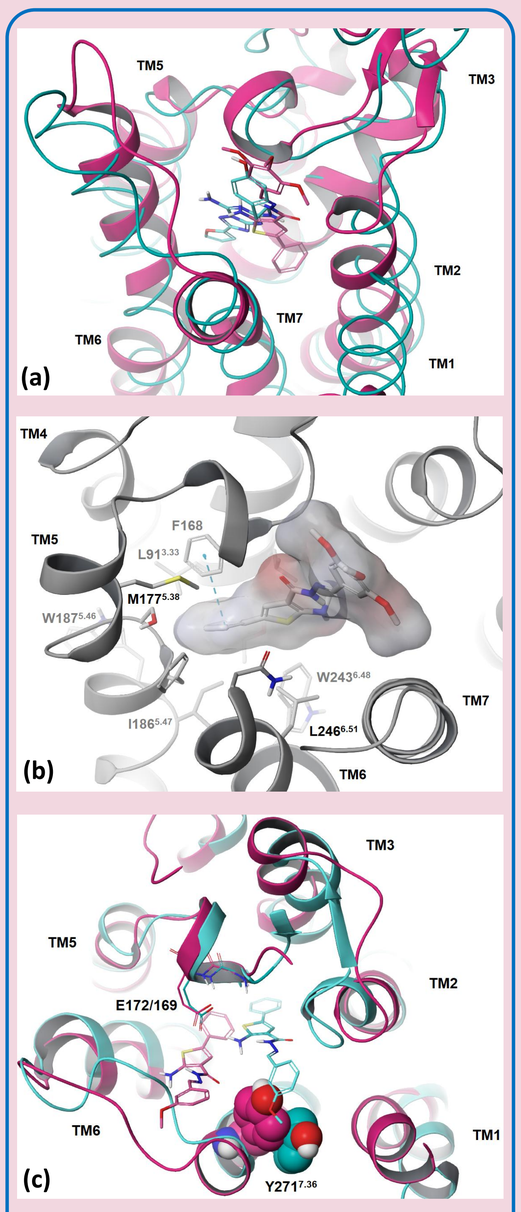
Cmpd	Structure	K_i A_1 (μM)	K_i A_{2A} (μM)	K_i A_3 (μM)
1		> 100	2.67	**3.10**
9		> 100	> 60	37.1
10		> 30	> 60	16.5
11		30	> 60	14.8
12		100	3.93	**5.77**
13		15.2	4.59	**5.16**

K_i A_{2B} values were > 30 μM for all ligands and are thus omitted

The docking calculations and 100 ns MD simulations showed that **1, 12,** and **13** are stabilized inside the binding pocket of A_{2A}AR with similar orientation to ZM241385, resulting in good affinity against the receptor (**Scheme 2a**).

Analysis of the protein-ligand contacts shows that the ligands are stabilized inside the binding sites mainly by hydrophobic (e.g. $A^{2.61}$, $V/V/L^{3.32}$, $I^{7.39}$) and π-π interactions (F171/168/168) through their aromatic groups. The H-bond interactions with E172/169 (A_1/A_{2A}) and $N^{6.55}$ through their 2-amino or 3-carbonyl moieties are also important.

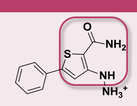
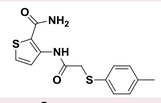
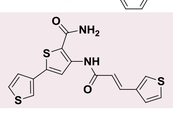
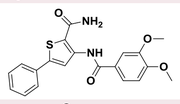
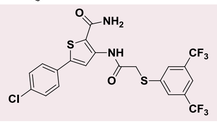
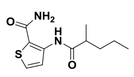
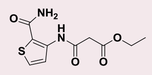
Introduction of the 5-phenyl ring increases the affinity towards all ARs, since the ligands are able to interact with non-polar residues of TM3, TM5 and TM6, like $V/V/L^{3.33}$, F171/168/168, $M^{5.38}$, and $L^{6.51}$ (**Scheme 2b**).



Scheme 2. (a) Average structure of **1** in A_{2A}AR during 100ns simulation (pink) superposed to crystal complex 3EML (cyan); (b) Average structure of **1** in A_3AR during 100ns simulation; (c) Average structures of **12** during 100ns simulation in A_1AR (pink) and A_{2A}AR (cyan).

The presence of the methoxyphenyl group provides selectivity towards A_{2A} and A_3 ARs, as simulations show that this bulky moiety clashes mainly with $Y^{7.36}$ in A_1AR, which force it out of the binding site (**Scheme 2c**).

Class B Derivatives

Cmpd	Structure	K_i A_1 (μM)	K_i A_{2A} (μM)	K_i A_3 (μM)
14		> 30	31.7	19.7
15		7.48	> 100	5.39
16		1.18	4.69	**1.65**
17		1.09	7.29	**0.918**
18		> 100	> 100	**1.55**
26		7.33	> 100	27.4
27		18.0	> 100	> 100

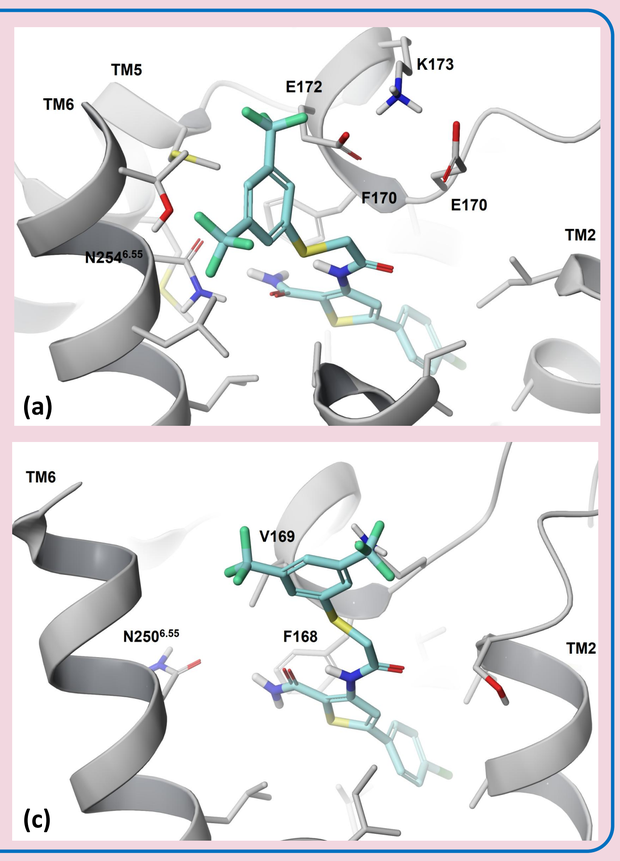
K_i A_{2B} values were > 30 μM for all ligands and are thus omitted

Similarly to **13**, the MD simulations against A_1 and A_{2A} ARs showed that the bulky lipophilic groups of **16** and **17** are likewise oriented close to EL2. Compound **14** lacking the bulky 3-acylamino substituent (compared to **16** and **17**) has a moderate affinity against all ARs.

The 100 ns MD simulations showed that the van der Waals interactions of **16** and **17** inside the binding cavity of A_1, A_{2A}, and A_3 ARs are similar to those of compounds **1**, **12** and **13**.

Compound **18** was the only class B antagonist with affinity solely against A_3AR. In A_1AR, the bulky $-CF_3$ group on the phenyl ring result in clashes with residues of TM2, EL2, and TM6 that drive the non-polar group close to the charged E172. Residues of A_3AR accommodate this group better (**Scheme 3**).

Scheme 3. Average structure of **18** during 100ns simulations inside (a) A_1AR (b) A_{2A}AR and (c) A_3AR. Residues that participate in protein-ligand interactions are displayed as thin tubes. (Parts of the receptors are hidden for clarity)



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1. B.B. Fredholm *et al.*, *Pharmacol. Rev.* **2001**, *53*, 527–552.
2. P. Lagarias *et al.*, *J. Chem. Inf. Model.* **2018**, *58*, 794–815.