Supporting Information

Role of extracellular cysteine residues in the adenosine A_{2A} receptor

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Table of Contents

Supplementary Table 1. Summary of main findings of the current study on the A_{2A}AR and comparison with the results of a mutagenesis study on the related A_{2B}AR.

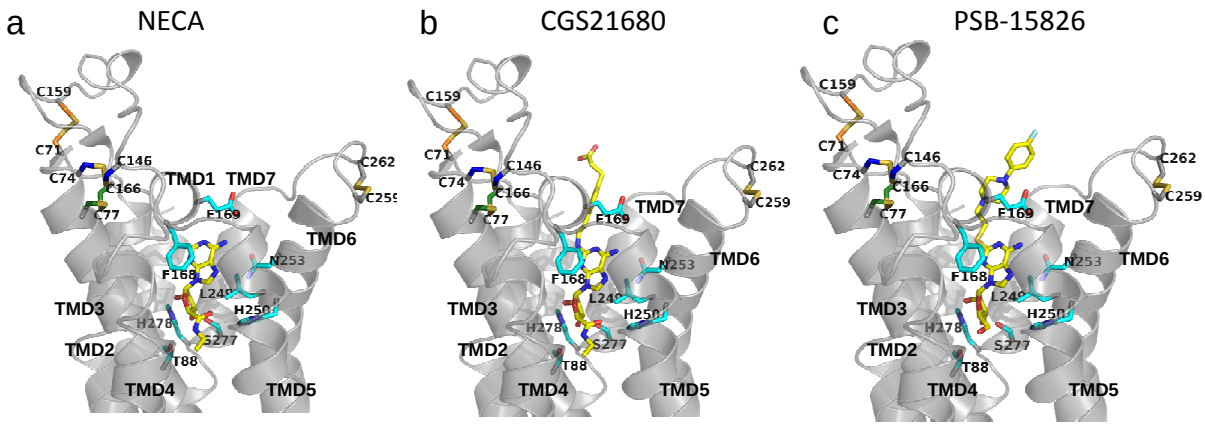
Disulfide bond number	**A_{2A}AR**	**A_{2B}AR** [11]
	All three disulfide bonds are important for the response of the physiological agonist adenosine	
I **C71$^{2.69}$-C159$^{45.43}$**	Required for adenosine potency → potency of adenosine similar to that at the A_{2B}AR Not required for NECA, PSB-15826, CGS21680 binding and function	Not required for binding and function of NECA and BAY60-6583 (Adenosine not investigated)
II **C74$^{3.22}$-C146$^{45.30}$**	Efficacy reduced or abolished Virtually no activation by adenosine and PSB-15826 Reduced efficacy by NECA and CGS21680 → N-ethylcarboxamido group acts as anchor in the orthosteric binding pocket	It cannot be formed (no cysteine residue in ECL1)
III **C77$^{3.25}$-C166$^{45.50}$** conserved in most class A GPCRs	Essential for adenosine potency (efficacy unchanged) → potency of adenosine similar to that at the A_{2B}AR No significant impact on other investigated agonists	Essential for antagonist binding (PSB-603) and for agonist potency (NECA and BAY60-6583)

Supplementary Table 2. Relevance of extracellular disulfide bonds for A_{2A}AR binding and activation by structurally diverse agonists.

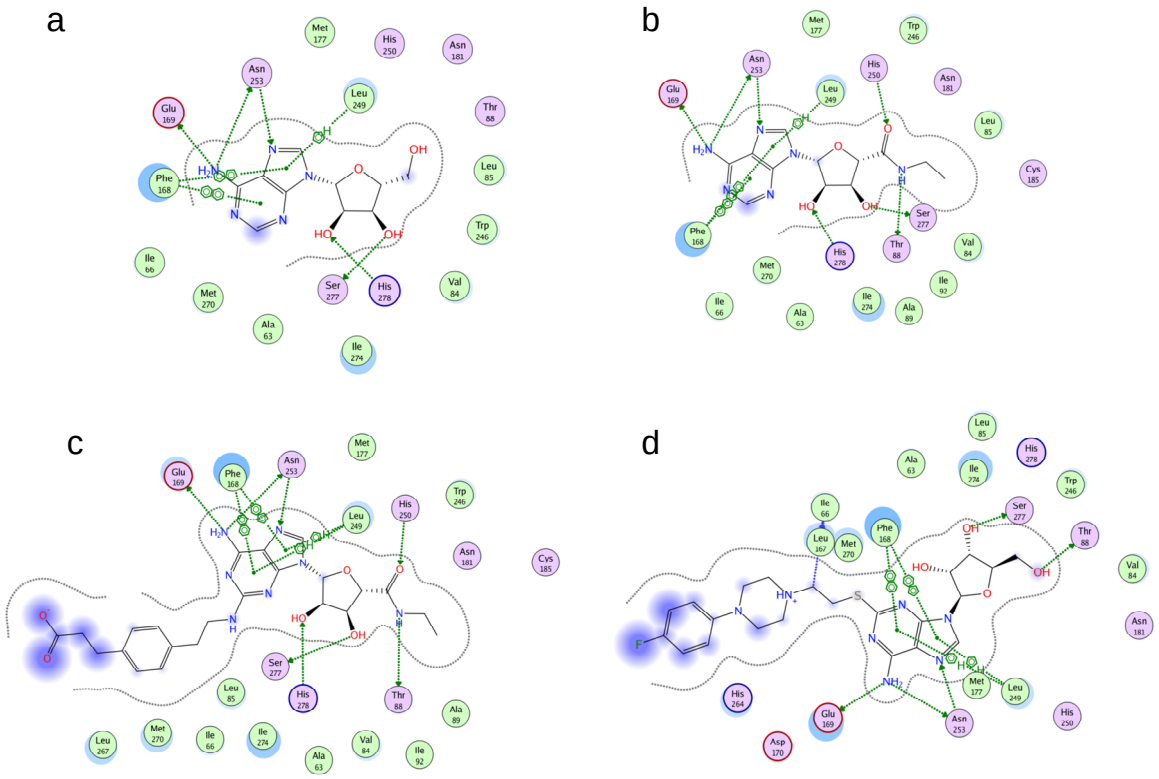
Disruption of disulfide bond		**Adenosine**	**NECA** (carboxamido-sugar)	**PSB-15826** (2-substituted, uncharged)	**CGS21680** (carboxamido-sugar; 2-substituted, negatively charged)
S-S bond No. I - C159S					
Binding		**n.t.**	**+++ (biphasic)**	+	+
Function	**Potency**	--	+	++	+
	Efficacy	**No change**	±	±	±
S-S bond No. II – C146S					
Binding		**n.t.**	-	±	±
Function	**Potency**	**No activation**	+	**No activation**	++
	Efficacy	--	-	---	-
S-S bond No. III – C166S					
Binding		**n.t.**	±	(-)	±
Function	**Potency**	--	±	±	±
	Efficacy	±	±	-	±
S-S bonds No. I plus II – C159S + C146S					
Binding		**n.t.**	--	-	±
Function	**Potency**	---	---	±	+
	Efficacy	+	+	±	±

definition: + (increase), - (decrease)

- no change (< 2-fold): ±
- 2-10-fold: + or -
- 11-30-fold: ++ or --
- > 30-fold: +++ or ---



Supplementary Fig S1 Binding modes of agonists in A_{2A}AR. Crystallographic binding poses of the agonist (a) NECA, (b) CGS21680 and (c) PSB-15826 in the binding pocket of A_{2A}AR (represented as grey ribbon) are shown. All ligands are shows as sticks with carbons in yellow. The sidechains of important residues in the binding pocket are shown as sticks with carbons in cyan. The cysteine residues involved in disulfide bonds are shown as sticks and the carbon atoms are color coded as follows: Cys71$^{2.69}$-Cys159$^{45.43}$ orange, Cys74$^{3.22}$-Cys146$^{45.30}$ blue and Cys77$^{3.25}$-Cys166$^{45.50}$ green.



Supplementary Fig S2 2D interaction diagram for the human A_{2A}AR with selected agonists. (a) adenosine, (b) NECA, (c) CGS21680, and (d) PSB-15826.