

Supporting Information

An Affinity-Based Probe for the Human Adenosine A_{2A} Receptor

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Table S1. Apparent Sffinities of **4** at the Human A₁ and A₃ Adenosine Receptor Subtypes^a.

compound	p <i>K</i> _i		
	hA ₁ ^b	hA _{2A} ^c	hA ₃ ^d
4	7.72 ± 0.05	8.82 ± 0.02	7.22 ± 0.01

^aData are expressed as p*K*_i values (means ± SEM) of three separate experiments each performed in duplicate.

^bAffinity determined from displacement of specific [³H]DPCPX binding on CHO cell membranes stably expressing human adenosine A₁ receptors at 25°C during 3 h incubation.

^cAffinity determined from displacement of specific [³H]ZM241385 binding from the hA_{2A}R at 25 °C during 3 h incubation.

^dAffinity determined from displacement of specific [³H]PSB-11 binding on CHO cell membranes stably expressing human adenosine A₃ receptors at 25°C during 3 h incubation.

Supplementary data

Table S2. Affinities of ZM241385 on hA_{2A}R Preincubated with Different Concentrations of the Indicated Compounds^a

Preincubated Compound	p <i>K</i> _i IC ₅₀	p <i>K</i> _i 0.3IC ₅₀	p <i>K</i> _i control
4 ^b	9.01 ± 0.05 ^{ns}	8.84 ± 0.04 ^{ns}	9.03 ± 0.10
ZM241385 ^c	8.94 ± 0.07 ^{ns}	8.84 ± 0.03 ^{ns}	8.77 ± 0.04

^aData are expressed as means ± SEM of three separate experiments each performed in duplicate. ns indicates a non-significant difference with p>0.05 when compared with the p*K*_i values in control groups; One-way ANOVA test.

^bAffinity of ZM241385, expressed as p*K*_i value, determined from displacement of specific [³H]ZM241385 binding from the hA_{2A}R cell membranes preincubated with compound **4** at indicated concentrations for 3 h at 25 °C and then treated with a three-cycle washing step.

^cAffinity of ZM241385, expressed as p*K*_i value, determined from displacement of specific [³H]ZM241385 binding from the hA_{2A}R cell membranes preincubated with ZM241385 at indicated concentrations for 3 h at 25 °C and then treated with a three-cycle washing step.