Structure-Affinity Relationships and Structure-Kinetics Relationships of Pyrido[2,1-*f*]purine-2,4-dione Derivatives as Human Adenosine A_3 Receptor Antagonists

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Supporting information

Contents

1. Binding affinities of short RT antagonist (**5**) and long RT antagonist (**27**) at human adenosine A_1 and A_{2A} receptors

Binding affinities of antagonist **5** at hA_1 and hA_{2A} receptors have been reported before,[1] whilst binding affinities of antagonist **27** at hA_1 and hA_{2A} receptors were determined as described previously.[2, 3] Percentage displacement of antagonists (n=2, average) or K_i ± SEM (nM, n=3) at human adenosine A_1 and A_{2A} receptors are listed in Table S1.

2. The KRI values of pyrido[2,1-f]purine-2,4-dione derivatives without methoxy-substitution at C-8 position

The KRI values of pyrido[2,1-f]purine-2,4-dione derivatives without methoxy-substitution at C-8 position were obtained as described under "*the 'dual-point' competition association assays*". Experiments were designed as described previously,[4] where in this case the two time points were selected at 20 (t_1) and 240 min (t_2). The results are listed in Table S2.

3. The comparison between the KRI values of pyrido[2,1-f]purine-2,4-dione derivatives without methoxy-substitution at C-8 position and their methoxy-substituted counterparts
The comparison is presented in Figure S1.

4. The correlation between pk_{off} and LogP for antagonists

Log*P* values were calculated using Chemdraw Professional 15.0 (Cambridge Soft, Perkin Elmer, Waltham, MA, USA). R^2 and *P* values were calculated using the GraphPad Prism linear regression analysis function. The correlations are presented in Figure S2.

Table S1. Binding affinities of short RT antagonist (**5**) and long RT antagonist (**27**) at human adenosine A_1 and A_{2A} receptors.

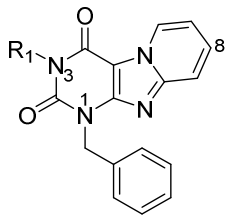
Compound	hA_1 [a]	hA_{2A} [b]
5	**49%**[c]	**10%**[c]
27	**209 ± 16**	**24%**

[a] Displacement of [^{3}H]DPCPX from CHO cell membranes expressing the human adenosine A_1 receptor. Percentage displacement at 10 μM (n=2, average) or K_i ± SEM (nM, n=3).

[b] Displacement of [^{3}H]ZM241385 from CHO cell membranes expressing the human adenosine A_{2A} receptor. Percentage displacement at 10 (**5**) or 1 μM (**27**, n=2, average),

[c] Published data.[1]

Table S2. Kinetic Parameters of Pyrido[2,1-*f*]purine-2,4-dione Derivatives without Methoxy-substitution at *C*-8 Position.



Compound	R_1	KRI[a]
S1	CH_2CH_3	0.61 (0.63,0.59)
S2	$CH_2CH_2CH_3$	0.59 (0.40,0.77)
S3	$CH_2CH(CH_3)_2$	0.88 (0.85,0.90)
S4	$CH_2CH{=}CH_2$	0.88 (0.79,0.96)
S5	$CH_2C{\equiv}CH$	1.16 ± 0.38
S6	$CH_2C_3H_5$	1.01 (1.08,0.93)

[a] KRI ± SEM (n = 3) or KRI (n = 2, individual estimates in parentheses), obtained at 10 °C from dual point competition association assays at 10 °C with [^{3}H]**34** on CHO cell membranes stably expressing the hA_3R.

Supplementary

Figures

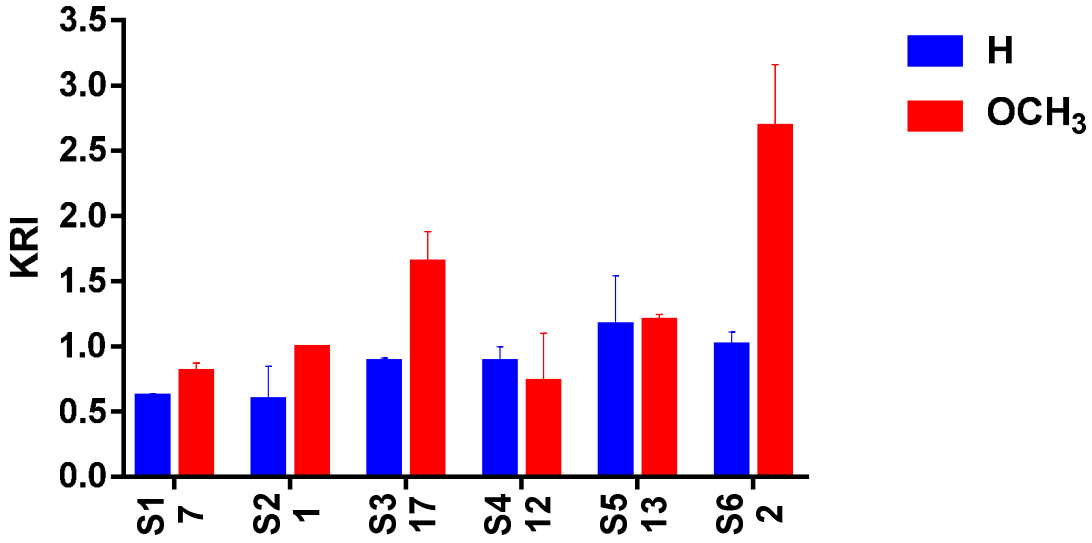


Figure S1: Comparison of KRI values of compounds with (red bars) and without (blue bars) *C*-8 methoxy substitution. KRI values obtained from dual point competition association assay on CHO cells stably expressing hA_3R at 10 °C. Each data point is the average of two or three independent experiments performed in duplicate.

A. **B.**

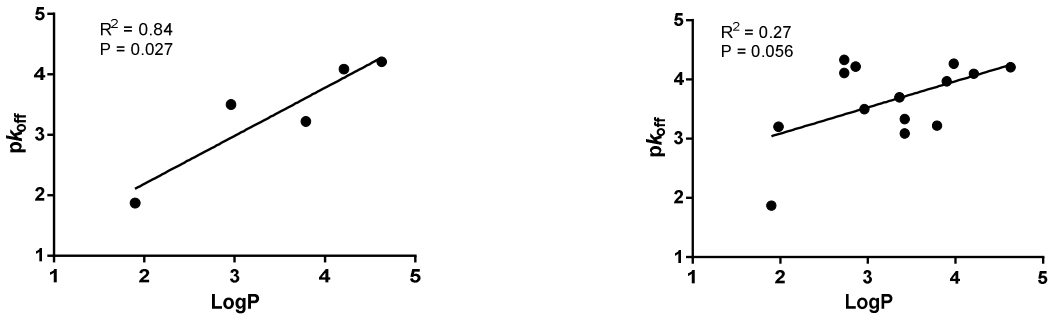


Figure S2: Correlation between pk_{off} and LogP for antagonists with an elongated carbon chain (**1, 5, 9, 10** and **11**) at the R_1 position (A), or for all antagonists (B), obtained from competition association assays.

References

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