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Supplementary Materials for

Identification of a selective small-molecule inhibitor of type 1 adenylyl cyclase activity with analgesic properties

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The PDF file includes:

Fig. S1. Inhibitory activity of ST034307 and NB001 against AC1.
Fig. S2. Inhibitory activity of ST034307 after pertussis toxin treatment or sequestration of Gβγ.
Fig. S3. A23187- and forskolin-stimulated cAMP accumulation in HEK-AC1 cells.
Table S1. Inhibition of A23187-stimulated AC1 activity using two different methods of measuring cAMP accumulation.
Legend for data S1

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencesignaling.org/cgi/content/full/10/467/eaah5381/DC1)

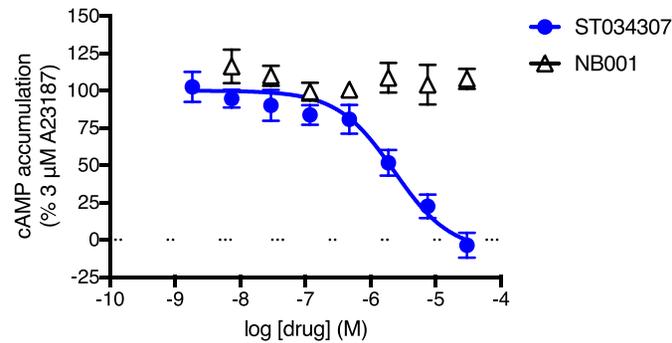Data S1 (Microsoft Excel format). NDL-3000 screen results.

Fig. S1. Inhibitory activity of ST034307 and NB001 against AC1. Inhibition of 3 μM A23187-stimulated cAMP accumulation in stably transfected HEK-AC1 cells by ST034307 or NB001 was done by pretreating the cells with inhibitors for 30 min followed by the addition of A23187 in the presence of IBMX. The data shown represent the average and S.E.M. of four (ST034307) or two (NB001) independent experiments conducted in duplicate. The data for ST034307 is the same that was presented in Fig. 2A and Table 1 (Cisbio cAMP kit).
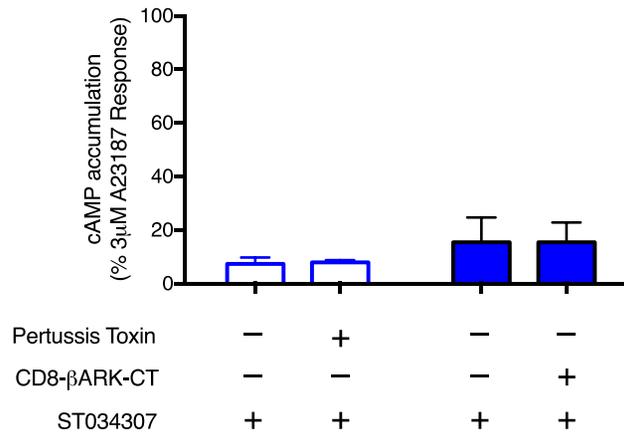

Pertussis Toxin	−	+	−	−
CD8-βARK-CT	−	−	−	+
ST034307	+	+	+	+

Fig. S2. Inhibitory activity of ST034307 after pertussis toxin treatment or sequestration of Gβγ. Inhibition of 3 μM A23187-stimulated cAMP accumulation by ST034307 (30 μM) in HEK cells stably expressing AC1 that were incubated overnight in the absence or presence of 25 ng/ml pertussis toxin; and HEK cells transiently transfected with AC1 plus venus (control) or the Gβγ scavenger, CD8-βARK-CT. All data shown represent the average and S.E.M. of at least three independent experiments conducted in duplicate.
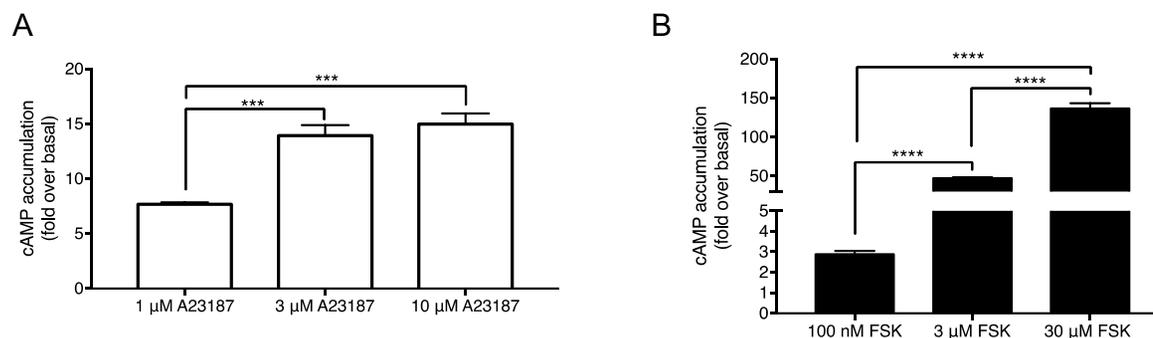
Fig. S3. A23187- and forskolin-stimulated cAMP accumulation in HEK-AC1 cells. (**A**) HEK-AC1 cells were pre-treated with vehicle and stimulated with increasing concentrations of A23187. (**B**) HEK-AC1 cells were pre-treated with vehicle and stimulated with increasing concentrations of forskolin. All data shown represent the average and S.E.M. of at least four independent experiments conducted in duplicate. One-way ANOVA with Tukey's multiple comparison's test was carried out for statistical analyses. ***$p < 0.001$, ****$p < 0.0001$.

Table S1. Inhibition of A23187-stimulated AC1 activity using two different methods of measuring cAMP accumulation. Compounds were tested for inhibition of 3 μM A23187-stimulated cAMP accumulation in HEK-AC1 cells. In the table IC_{50} values with 95% confidence intervals and percent inhibition (with 100% being equal to the basal cAMP amounts) with S.E.M. are reported. The data in the table represent the average of at least three independent experiments conducted in duplicates. *Data for the Cisbio cAMP are also reported in Table 1.

	Cisbio cAMP kit*		DiscoveRx cAMP HitHunter kit	
Compound	IC_{50} - μM (95% C.I.)	% inhibition (SEM)	IC_{50} - μM (95% C.I.)	% inhibition (SEM)
ST072383	0.3 (0.2 - 0.4)	113 (±4)	0.3 (0.1 - 0.8)	140 (±22)
ST034307	2.3 (1.2 – 4.5)	108 (±10)	3.4 (1.8 - 6.7)	114 (±4)

Data S1. NDL-3000 screen results. The chemical ID number, percentage of inhibition of AC1, IUPAC name, and molecular weight are provided in an Excel file.