Supplementary Table 1

pK_i and reference values for compounds binding to Nluc-labeled A_1 and A_3 receptors

	Nluc-A_1 receptor				Nluc-A_3 receptor			
	pK_i[a]	*n*	pK_i in literature[b]	Ref[b]	pK_i[a]	*n*	pK_i in literature[b]	Ref[b]
DPCPX	8.17 ± 0.10	5	7.4-9.2	23-27	6.93 ± 0.09	4	5.4-6.6	27-32
SCH 58261	6.65 ± 0.16	4	6.1-6.3	33-35	6.80 ± 0.14	4	5.9	33
MRS 1220	6.89 ± 0.20	4	-	-	9.20 ± 0.08	3	8.2-9.2	36-39
CGS 15943	8.04 ± 0.08	4	8.5	34	8.12 ± 0.10	4	7.0-7.9	30,32,34,37
Propranolol	<5	5	-	-	<5	3	-	-
ZM 241385	5.85 ± 0.20	5	6.1-6.6	33,34	6.63 ± 0.09	3	6.1	33
XAC	7.09 ± 0.19	4	7.6	40	7.70 ± 0.13	3	7.0-7.4	30-32
PSB 603	6.33 ± 0.16	4	<5	41	6.31 ± 0.14	4	<5	41

[a] We obtained pK_i values (mean ± s.e.m.) in the NanoBRET binding assay using whole, live HEK293 cells expressing Nluc-A_1 or Nluc-A_3 receptor and 25 nM CA200645.

[b] Previously published pK_i values and associated references as listed in IUPHAR/BPS Guide to Pharmacology (www.guidetopharmacology.org).

Note: Using ^{3}H-DPCPX in live CHO cells expressing the wild-type A_1 receptor, we have previously published pK_i values for DPCPX, XAC and CGS15943 of 8.37 ± 0.03, 7.25 ± 0.02 and 8.35 ± 0.05 respectively[13]. These are very similar to the respective values quoted for Nluc-A_1 receptor with CA200645 above.

Supplementary Table 2

pK_i values at Nluc-A_1 and Nluc-A_3 receptors using different fluorescent ligands

	Nluc-A_1 receptor		Nluc-A_3 receptor		
	ABEA-X-BY630		ABEA-X-BY630		AV039
	pK_i		pK_i		pK_i
	Co-addition	Preincubation	Co-addition	Preincubation	Co-addition
CGS 15943	8.17 ± 0.25	8.20 ± 0.12	7.54 ± 0.19	7.87 ± 0.04	7.13 ± 0.14
MRS 1220	6.97 ± 0.23	7.31 ± 0.12	8.91 ± 0.14	9.05 ± 0.07	8.26 ± 0.12
SCH 58261	6.54 ± 0.21		6.12 ± 0.06		5.09 ± 0.06
DPCPX	8.49 ± 0.13	8.56 ± 0.11	6.02 ± 0.11	6.54 ± 0.05	5.47 ± 0.17
PSB 603	5.70 ± 0.13		5.93 ± 0.13		5.91 ± 0.09
XAC	7.40 ± 0.12	7.50 ± 0.13	6.96 ± 0.10	7.44 ± 0.06	6.64± 0.11
ZM 241385	6.33 ± 0.12		6.03 ± 0.08		5.49 ± 0.04
propranolol	<5		<5		<5

We obtained pK_i values (mean ± s.e.m.; n = 4) in the NanoBRET binding assay using whole, live HEK293 cells expressing Nluc-A_1 or Nluc-A_3 receptor. We used 250 nM ABEA-X-BY630 in Nluc-A_1 receptor-expressing cells, 50 nM ABEA-X-BY630 in Nluc-A_3 receptor-expressing cells and 10 nM AV039 in Nluc-A_3 receptor-expressing cells. We added unlabeled ligand simultaneously with fluorescent ligand (Co-addition). Alternatively, in order to check for differences in pKi values determined with the fluorescent agonist ABEA-X-BY630 due to simultaneous addition, we also investigated the values obtained for four antagonists following a 30 min preincubation with the antagonist prior to addition of ABEA-X-BY630 (Preincubation).

Note: Using ^{3}H-DPCPX in live CHO cells expressing the wild-type A_1 receptor, we have previously published pK_i values for DPCPX, XAC and CGS15943 of 8.37 ± 0.03, 7.25 ± 0.02 and 8.35 ± 0.05 respectively[13]. These are very similar to the respective values quoted for Nluc-A_1 receptor with ABEA-X-BY630 above.

Supplementary References:

21. Tannous, B.A. & Teng, J. *Biotechnol. Adv.* **29**, 997–1003 (2011).
22. Liu, J, O'Kane D.J., Escher, A. *Gene* **203**, 141–148 (1997).
23. de Ligt, R.A., Rivkees, S.A., Lorenzen, A., Leurs, R. & Ijzerman, A.P. *Eur. J. Pharmacol.* **510**, 1–8 (2005).
24. Iredale, P.A., Alexander, S.P. & Hill, S.J. *Br. J. Pharmacol.* **111**, 1252–1256 (1994).
25. Obiefuna, P.C. *et al. J. Pharmacol. Exp. Ther.* **315**, 329–336 (2005).
26. Rivkees, S.A., Barbhaiya, H. & AP, I.J. *J. Biol. Chem.* **274**, 3617–3621 (1999).
27. Weyler, S. *et al. ChemMedChem* **1**, 891–902 (2006).
28. Auchampach, J.A., Jin, X., Wan, T.C., Caughey, G.H. & Linden, J. *Mol. Pharmacol.* **52**, 846–860 (1997).
29. Hayallah, A.M. *et al. J. Med. Chem.* **45**, 1500–1510 (2002).
30. Klotz, K.N. *et al. Naunyn Schmiedebergs Arch. Pharmacol.* **357**, 1–9 (1998).
31. Salvatore, C.A., Jacobson, M.A., Taylor, H.E., Linden, J. & Johnson, R.G. *Proc. Natl. Acad. Sci. U S A* **90**, 10365–10369 (1993).
32. Varani, K. *et al. Mol. Pharmacol.* **57**, 968–975 (2000).
33. Jacobson, K.A. & Gao, Z.G. *Nat. Rev. Drug. Discov.* **5**, 247–264 (2006).
34. Ongini, E., Dionisotti, S., Gessi, S., Irenius, E. & Fredholm, B.B. *Naunyn Schmiedebergs Arch. Pharmacol.* **359**, 7–10 (1999).
35. Townsend-Nicholson, A. & Schofield, P.R. *J. Biol. Chem.* **269**, 2373–2376 (1994).
36. Jacobson, K.A. *et al. Neuropharmacology* **36**, 1157–1165 (1997).
37. Kim, Y.C., Ji, X.D. & Jacobson, K.A. *J. Med. Chem.* **39**, 4142–4148 (1996).
38. Sullivan, G.W., Rieger, J.M., Scheld, W.M., Macdonald, T.L. & Linden, J. *Br. J. Pharmacol.* **132**, 1017–1026 (2001).
39. Yates, L. *et al. Auton Autacoid Pharmacol.* **26**, 191–200 (2006).
40. Jockers, R. *et al. J. Biol. Chem.* **269**, 32077–32084 (1994).
41. Borrmann, T. *et al. J. Med. Chem.* **52**, 3994–4006 (2009).