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Agonist lead identification for the high affinity niacin receptor GPR109a

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Abstract—A strategy for lead identification of new agonists of GPR109a, starting from known compounds shown to activate the receptor, is described. Early compound triage led to the formulation of a binding hypothesis and eventually to our focus on a series of pyrazole acid derivatives. Further elaboration of these compounds provided a series of 5,5-fused pyrazoles to be used as lead compounds for further optimization.

Niacin (nicotinic acid) is a water-soluble vitamin that at high doses in humans, favourably modulates multiple serum lipid and lipoprotein parameters that are cardiovascular risk factors.[1] Niacin increases high density lipoprotein (HDL) to a greater extent than any other currently used drug and it has been used for many years for the prevention and treatment of cardiovascular disease.[2] Recent mechanistic investigations have shown that niacin interacts with, and may exert its beneficial effects on lipids via, a G-protein coupled receptor (GPCR) expressed on adipocytes.[3] It is postulated that the resultant decrease in intracellular cAMP leads to inhibition of lipolysis via negative modulation of intracellular lipase activity, thereby decreasing plasma free fatty acid (FFA) levels. A further hypothesis suggests that this pathway may ultimately result in elevation of HDL.[4] Two G_i-coupled orphan GPCRs that share 95% identity and which are both expressed in human adipocytes have been identified as possible molecular targets for niacin.[5] GPR109a (HM74a) is the human orthologue of the previously described rodent receptor (PUMA-G)[6] whereas GPR109b (HM74) differs from GPR109a and PUMA-G mainly in the intracellular C-terminal tail portion and has no rodent equivalent.[7] Niacin has been reported to activate GPR109a with an EC_{50} of 250 nM in a GTPγS assay and displaces ^{3}H-niacin from GPR109a expressing CHO cell membranes with an IC_{50} of 81 nM.[5] It is a much weaker ligand for GPR109b.

The use of niacin as a therapeutic is limited by a number of associated side-effects, in particular an uncomfortable cutaneous flushing response which, although tolerance may be acquired, has a negative impact on patient compliance. The development of novel agonists of the niacin receptor that retain the beneficial effect on atherosclerosis but with no flushing side-effect would clearly be of value. In this report we describe our early investigations into the identification of appropriate chemical starting points for the discovery of such compounds.

Following our in-house discovery of potential GPCR targets for niacin,[8] we established high throughput cAMP assays using the stably expressed human

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GPR109a and GPR109b receptors in CHO cells.[9] We initially used these assays to examine whether structurally related small acid analogues of nicotinic acid had any activity at the human receptors in a similar manner to studies with the rodent receptors using rat adipocytes or spleen membranes and mouse macrophages.[3,10]

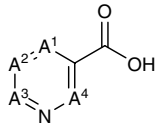
Our initial investigation of commercially available pyridine acids showed that as expected, only the 3-pyridyl carboxylate had any activity at the human cloned GPR109a receptor (EC_{50} = 0.12 μM, n = 216; 95% CI 0.109–0.126 μM, in the cAMP assay; reported K_i = 33 nM in rat spleen membrane ^{3}H-niacin binding assay[3]) whereas the 2- and 4-pyridyl carboxylates were inactive. Nicotinic acid had no activity at the closely related GPR109b in this assay platform at concentrations up to 30 μM. Similar to the observations of Lorenzen et al. in rat adipocytes and spleen membranes, we found that homologation of niacin to 3-pyridyl acetic acid resulted in a significant loss of potency (EC_{50} = 2.8 μM, n = 2; reported K_i = 403 nM in rat spleen binding assay[3]) and further homologation abolished all activity.

We hypothesized early on that, based on their in vivo activity and structural similarity to niacin, several compounds with well documented activity in acutely lowering free fatty acids in rats (and in some cases, human), were likely to be agonists of GPR109a. Thus, a number of 5-membered heterocyclic acids were examined including **13** and **19** (Table 2). The former compound was investigated by Upjohn in the 1960s[11] and in our hands it showed good activity at GPR109a in the cAMP assay and perhaps more surprisingly in view of the high homology between the receptors, essentially no activity (up to 100 μM) at GPR109b. Interestingly, the compound lacking the 5-methyl substituent, that is, pyrazole-3-carboxylate, was an order of magnitude less potent (EC_{50} = 4.2 μM, n = 2; K_i = 594 nM in rat spleen binding assay[12]). A similar observation was also made with the isoxazole analogues. Compound **19**, investigated in the 1960s by workers at Pfizer,[13] was an effective agonist of GPR109a (Table 2) and again had no effect at GPR109b. In this case, the isoxazole analogue lacking the methyl group had barely measurable activity with an EC_{50} for GPR109a in excess of 30 μM. The isomeric isoxazole (5-methylisoxazole-3-carboxylate) also had no effect.

A comparison of the data from our cAMP assay with those from the rat spleen membrane binding assay showed that we had retained approximately the same rank order of potency, providing good validation of our assay, but with an apparent 3- or more fold rightward shift in the dose–response curves. To determine whether this was a species difference or more a reflection of the different types of readout from the two assay platforms, we also established cAMP assays in CHO cells using the cloned rat and mouse receptors. The potency of each of these early compounds tested was essentially identical across species giving us a good correlation between rodent and human receptor activity in the cAMP assays despite the modest difference compared to the endogenous receptor assays.

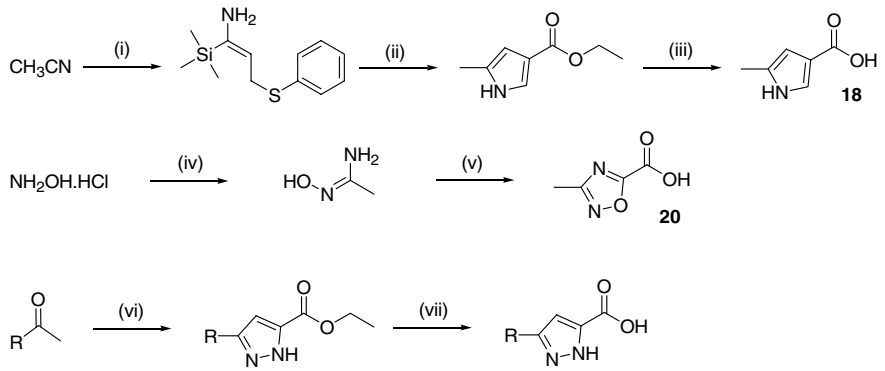
From these early data we formulated a preliminary binding hypothesis in which the acid portion of the agonist molecules would interact with Arg-111, essentially the only available basic residue in the TM domains, on TM3; a common site of interaction for small molecule agonists with GPCRs. The suggestion that Arg-111 is involved in the critical binding interaction is now well supported by compelling mutagenesis and modeling data.[7] In addition to this key bidentate interaction, all of the ligands identified for the receptor thus far had included a hydrogen bond acceptor moiety in the 3-position of the aromatic group relative to the carboxylate function. Other molecules that have been shown to activate GPR109a such as acipimox and acifran[14] also contain these two important pharmacophore features. With this in mind, we selected an extended range of 6-membered ring acids for further testing all of which were commercially available in either the acid or ester form (Table 1). As can be seen from the data, addition of a second nitrogen to the ring resulted in at least a 20-fold loss in activity for GPR109a. To further solidify the requirement for a 1,3-orientation of the carboxylate and the H-bond acceptor nitrogen, we also confirmed that pyrimidine 2-carboxylate and pyrimidine 4-carboxylate had no activity. In contrast to the classical 5-membered ring analogues described above, where a methyl substituent was required for good activity, substitution on the pyridine ring was poorly tolerated which we attributed to a non-favourable steric interaction. Even the small fluoro-substituent was only tolerated in the 5-position and interestingly this compound has also been previously reported to be antilipolytic in the dog.[15] The same authors also reported on a small number of other heterocycles that had moderate antilipolytic activity in the same model from a large selection of 188 compounds.[16] Other halo-substitutions in the 5-position

Table 1. GPR109a agonist activity for a series of 6-membered heterocyclic acids



Compound	A^1	A^2	A^3	A^4	GPR109a EC_{50}[a], μM (n)
1, Niacin	CH	CH	CH	CH	0.12 (216)
2	CH	CH	CH	N	n.e.
3	CH	CH	N	CH	23 (1)
4	CH	N	CH	CH	2.9 (2)
5	N	CH	CH	CH	6.3 (3)
6	CH	CH	CH	CF	n.e.
7	CH	CH	CF	CH	n.e.
8	CH	CF	CH	CH	5.3 (5)
9	CF	CH	CH	CH	n.e.
10	CH	CCl	CH	CH	7.6 (2)
11	CH	CBr	CH	CH	13.6 (2)
12	CH	CCH_3	CH	CH	16 (1)

n.e., less than 50% activity observed at any concentration up to 30 μM.
[a] EC_{50} determination from the cAMP assay.[9] Values are means of multiple determinations. The number of experiments is listed in parentheses. The standard deviations where calculable were <30% of the mean.

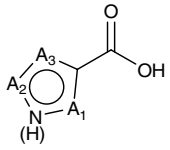


Scheme 1. Preparation of 5-membered heterocyclic acids. Reagents and conditions: (i) a—(trimethylsilyl)methyl triflate, 80 °C; b—thiophenol, benzene, rt; (ii) ethyl propiolate, AgF, MeCN, rt; (iii) aq NaOH, 80 °C, 1 h; (iv) MeCN, Na_2CO_3, H_2O, EtOH, reflux 45 min; (v) ethyl 2-chloro-2-oxoacetate, 120 °C, microwave heating; (vi) a—$(CO_2Et)_2$, EtOH, KO^{t}Bu (or EtONa); b—H_2NNH_2; (vii) 2.5 M NaOH or LiOH.

were not tolerated and no activity was seen with any other commercially available pyridine 3-carboxylate tested. We thus concluded that niacin and the related diazine acids would not make good chemical starting points to identify more potent analogues due to the apparent lack of scope for further expansion.

We next turned our attention to a series of 5-membered heterocyclic acids, again with our focus on those heterocycles containing a nitrogen atom in the 3-position relative to the carboxylate to function as the hydrogen bond acceptor to comply with our binding hypothesis. Again, each of these heterocycles was commercially available as either the ester or the free acid except **18** and **20** which were synthesized as outlined in Scheme 1. As can be seen from Table 2, the 1,3-orientation of the nitrogen relative to the carboxylate appears to be required but is not sufficient for activity at the receptor. The most potent compounds in this series were **13** and the methyl thiazole (**14**) acids. Incorporation of a sulfur in other positions in the ring resulted in a reduction in activity as did incorporation of additional nitrogen atoms.

Table 2. GPR109a agonist activity for a series of 5-membered heterocyclic acids

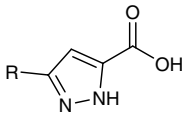


Compound	A^1	A^2	A^3	GPR109a EC_{50}[a] (*n*)
13	NH	CCH_3	CH	0.46 (11)
14	CH	CCH_3	S	0.5 (3)
15	CH	S	CH	1.5 (4)
16	CH	S	N	n.e.
17	N	S	CH	n.e.
18	CH	CCH_3	CH	n.e.
19	O	CCH_3	CH	1.3 (12)
20	O	CCH_3	N	n.e.
21	CH	NCH_3	CH	>20 (3)

n.e., less than 50% activity observed at any concentration up to 30 μM.
[a] EC_{50} determination from the cAMP assay.[9], μM Values are means of multiple determinations. The number of experiments is listed in parentheses. The standard deviations where calculable were <30% of the mean.

With these data in hand we elected to focus further on the pyrazole series both for reasons of the relative ease of synthesis of pyrazoles and that pursuing the thiazole class may be limited by the ring only having one available position for substitution. The known in vivo activity of pyrazole analogues was also attractive. In addition to 5-methylpyrazole carboxylate, the antilipolytic effects of pyrazole acids incorporating longer side chains in the 5-position have been reported.[17] The in vitro activity of series of pyrazoles with a wider variety of substitutents at the 5-position has also been described, with a number of the compounds prepared showing partial agonist activity in a GTPγS functional assay in rat spleen and adipocytes.[12] In that study, the most potent activity was seen with straight chain and branched alkyl substituents of 3 or 4 carbon atoms. We prepared compounds of this type to compare their activities in our human cloned receptor system and in addition designed a number of new analogues with hydrogen bond donors and acceptors in the side chain to try and solicit an additional binding interaction with the receptor to further increase potency. The compounds were in general, prepared by conventional pyrazole synthesis via a keto ester intermediate and cyclization with hydrazine followed by ester hydrolysis, as outlined in Scheme 1. (A number of analogues required more specific synthetic methods including synthesis of the starting ketone as detailed in the supplementary material.) As can be seen from the data in Table 3, a number of such modifications were well tolerated, compared to the simple carbon chain compound of the same length, but did not significantly improve potency in the cAMP assay. The most potent compound in this series was still **13**. Extension of the carbon chain to as few as 3 carbons decreased activity, but no further loss in activity was observed until the carbon chain was extended to 6 carbons. These data are somewhat different to those previously reported for the binding affinity of these compounds ($K_i = 0.143$ and 0.072 μM for compounds **23** and **26**, respectively, in the ^{3}H-niacin rat spleen binding assay[12]) and indeed, our data are more comparable to the functional activity (GTPγS binding) reported in the same study. In our

Table 3. GPR109a agonist activity for a series of 5-substituted pyrazole acids



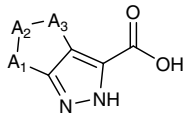
Compound	R	GPR109a EC_{50}[a] (*n*)
13	Me	0.46 (11)
22	Et	0.8 (6)
23	*n*-Pr	3.9 (3)
24	*i*-Pr	8.3 (8)
25	*c*-Pr	1.0 (2)
26	*n*-Bu	1.4 (5)
27	*c*-Bu	16.2 (5)
28	$CH(CH_3)CH_2CH_3$	n.e.
29	CH_2OCH_3	8.7 (2)
30	CH_2SCH_3	3.7 (2)
31	$CH_2S(O)CH_3$	4.9 (2)
32	$CH_2S(O)_2CH_3$	n.e.
33	CH_2NHCH_3	n.e.
34	$CH_2N(CH_3)_2$	n.e.
35	$CH_2CH_2OCH_3$	12.2 (2)
36	CHO	6.1 (3)
37	CH_2OH	2.5 (3)
38	$COCH_3$	n.e.
39	*n*-Pentyl	1.8 (2)
40	*n*-Hexyl	5.6 (5)
41	*t*-Bu	n.e.
42	Ph	n.e.
43	CH_2Ph	8.9 (3)
44	CH_2(3–F–Ph)	2.8 (8)
45	CH_2(3–Cl–Ph)	6.2 (15)
46	CH_2(3–Br–Ph)	10.5 (6)

n.e., less than 50% activity observed at any concentration up to 30 μM.
[a] EC_{50} determination from the cAMP assay.[9], μM Values are means of multiple determinations. The number of experiments is listed in parentheses. The standard deviations where calculable were <30% of the mean.

hands, branching of the side chain was not well tolerated, although interestingly we did observe that cyclopropyl analogue **25**, was more active than the isopropyl compound **24**. However, the *s*-butyl **28** and cyclobutyl **27** analogues were significantly less potent than **13**. Incorporation of ether or thioether groups or a primary alcohol into the side chain was tolerated but did not offer any improvement in activity. Oxidation of the thioether to the sulfoxide, **31**, was also tolerated, but further oxidation to the sulfone **32** was not. The incorporation of basic groups along the side chain was not tolerated in any of the positions tried, further emphasizing the lipophilic nature of the binding pocket. Although only phenyl is shown in the table, a number of other aromatic analogues were prepared including substituted phenyl, thiophene, furan, pyrazole and pyrrole analogues in multiple orientations, but all were devoid of activity. The benzyl analogues, when substituted in the 3-position with halogen atoms, did retain some activity, with the 3-fluoro analogue **44** being the most potent. It has been reported that this compound and a number of other 5-substituted pyrazole-3-carboxylates act as partial agonists in the rat adipocytes and spleen GTPγS assay system.[12] However, in our hands **44** was able to fully reverse the cAMP elevating effect of forskalin in CHO cells overexpressing the cloned human GPR109a receptor. In addition, the efficacy of **44** in this assay was comparable to both niacin and β-hydroxy butyrate, which has been proposed to be a physiologically relevant ligand for the receptor,[18] suggesting that **44** is a full agonist of the receptor under these conditions. It is possible that the differences in the apparent efficacy of compounds in the two systems may be due to the higher level of expression of the receptor in the cloned receptor assay versus the endogenously expressed receptor used in the rat tissue assays. The only clear partial agonists that we identified (showing that our assay system was indeed capable of detecting a partial agonist effect) were compounds **45** and **46** which showed efficacy values of around 70–90% relative to niacin, although a definitive assignment of partial agonism was confounded by the weak potency observed. All the other compounds prepared which had measurable activity and complete dose–response curves, had efficacy values in the range of 95–100% both in the cloned human and (where tested) in the cloned rat receptor assay.

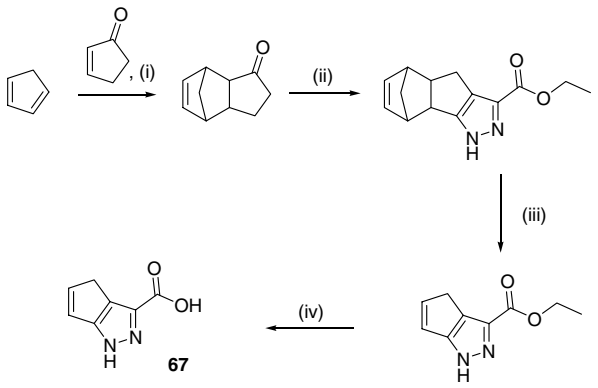
In addition to the 5-substituted pyrazole series, we prepared a number of 4,5-dialkyl substituted analogues. We

Table 4. GPR109a agonist activity for a series of bicyclic pyrazole acids



Compound	A^1	A^2	A^3	GPR109a EC_{50}[a], μM (*n*)
47	CH_2	CH_2	CH_2	0.86 (2)
48	$–CH_2CH_2–$	CH_2	CH_2	n.e.
49	$CH(CH_3)$	CH_2	CH_2	8.3 (3)
50	$CH(CH_3)_2$	CH_2	CH_2	n.e.
51	$CH(CH_2CH_2CH_3)$	CH_2	CH_2	6.6 (2)
52	$CH(OCH_2CH_3)$	CH_2	CH_2	n.e.
53	CH(Ph)	CH_2	CH_2	12 (3)
54	$CH(SCH_3)$	CH_2	CH_2	n.e.
55	CH_2	$CH(CH_3)$	CH_2	n.e.
56	CH_2	O	CH_2	2.7 (2)
57	$–CH_2CH_2–$	O	CH_2	n.e.
58	$CHCH_3$	O	CH_2	2.5 (5)
59	$CH(CH_2CH_3)$	O	CH_2	8.7 (1)
60	$CH(CH_2CH_2CH_3)$	O	CH_2	2.1 (2)
61	CH_2	S	CH_2	5.6 (4)
62	$CH(CH_3)$	S	CH_2	n.e.
63	CH_2	SO_2	CH_2	n.e.
64	CH_2	CH_2	S	2.2 (5)
65	CH_2	NH	CH_2	n.e.
66	CH_2	NCH_2Ph	CH_2	n.e.
67	CH=	=CH	CH_2	0.4 (2)[b]

n.e., less than 50% activity observed at any concentration up to 30 μM.
[a] EC_{50} determination from the cAMP assay.[9] Values are means of multiple determinations. The number of experiments is listed in parentheses. The standard deviations where calculable were <30% of the mean.
[b] Exists as a 1:1 mixture of 2 compounds; 1,4-dihydrocyclopenta[*c*]pyrazole-3-carboxylic acid and 1,6-dihydrocyclopenta[*c*]pyrazole-3-carboxylic acid.



Scheme 2. Synthesis of Compound **67**. Reagents and conditions: (i) $BF_3{\cdot}Et_2O$, reflux; (ii) a—$(CO_2Et)_2$, EtOH, KO^{t}Bu; b—H_2NNH_2; (iii) Ph_2O, 250 °C; (iv) 2.5 M NaOH.

observed however, that activity could only be retained if the two alkyl or substituted alkyl groups were cyclized to form a bicyclic ring system resulting in compounds of the type shown in Table 4. All of these compounds were prepared via our conventional synthesis outlined in Scheme 1, with the exception of **63**, which was prepared by oxidation of the cyclic thioether **61** prior to ester hydrolysis, **65**, which was prepared via debenzylation of **64**, and **67**. This latter compound was prepared using a Diels–Alder and retro Diels–Alder protection strategy (Scheme 2) as the 3,4-cyclohexeneone starting material was prone to isomerization and/or polymerization and we were unable to induce it to react using our conventional procedure. Even following this sequence, the isolated product **67** was found to exist as a mixture of double bond isomers.

The bicyclic ring was relatively tolerant to the incorporation of heteroatoms with the exception of a basic nitrogen group and the somewhat bulky sulfone. Increasing the size of the ring from 5 to 6 atoms however, resulted in complete loss of agonist activity. Methyl substitution was poorly tolerated in the 5-position but several alkyl substitutions were tolerated in the 6-position. Compounds **67** and **47** were among the most potent compounds prepared thus far, despite the former being a mixture of isomers, with activity comparable to **13**. The observation that the partially saturated ring of the bicyclic system could tolerate substitution, suggested to us that there was scope for further improvements in activity leading us to select this 5,5-fused pyrazole series as our lead series of choice for further optimization. We favoured this bicyclic series over the simple 5-substituted pyrazoles, as the latter had somewhat less desirable physicochemical and pharmacokinetic properties, particularly as the lipophilicity of the 5-substituent was increased. Further optimization of this lead series will be detailed in future communications.

In summary, we have outlined our strategy for lead identification of new agonists of GPR109a starting from known small molecule compounds that were shown to activate the receptor. In doing so, we have presented the first comprehensive SAR study of such molecules making use of a recombinant human receptor GPR109a and a cAMP assay. Data with reported compounds in our assay matched well with literature data from previously described endogenous rodent receptor assays. This was further confirmed by testing of selected analogues in the cloned mouse and rat receptor cAMP assays. That these compounds have similar activity across species is of significance for future in vivo studies. Furthermore, an early exploration of the available heterocyclic chemical space allowed us to formulate a binding hypothesis which focused our efforts around aromatic carboxylates with a hydrogen bond acceptor function in the 3-position of the aromatic ring. This spatial arrangement was shown to be required, but not sufficient for receptor activity. From this information we were able eventually to focus on a series of pyrazole acid derivatives. Further elaboration of these compounds provided a series of 5,5-fused pyrazoles which were suitable lead compounds for further optimization.

References and notes

1. Tavintharan, S.; Kashyap, M. L. *Curr. Atheroscler. Rep.* **2001**, *3*, 74.
2. Carlson, L. A. *J. Int. Med.* **2005**, *258*, 94.
3. Lorenzen, A.; Stannek, C.; Lang, H.; Andrianov, V.; Kalvinsh, I.; Schwabe, U. *Mol. Pharmacol.* **2001**, *59*, 349.
4. Offermanns, S. *Trends Pharmacol. Sci.* **2006**, *27*, 384.
5. Wise, A.; Foord, S. M.; Fraser, N. J.; Barnes, A. A.; Elshourbagy, N.; Eilert, M.; Ignar, D. M.; Murdock, P. R.; Steplewski, K.; Green, A.; Brown, A. J.; Dowell, S. J.; Szekeres, P. G.; Hassall, D. G.; Marshall, F. H.; Wilson, S.; Pike, N. B. *J. Biol. Chem.* **2003**, *278*, 9869.
6. Tunaru, S.; Kero, J.; Schaub, A.; Wufka, C.; Blaukat, A.; Pfeffer, K.; Offermanns, S. *Nat. Med.* **2003**, *9*, 352.
7. Tunaru, S.; Lättig, J.; Kero, J.; Krause, G.; Offermans, S. *Mol. Pharmacol.* **2005**, *68*, 1271.
8. Semple, G. Niacin Receptor Agonists. MEDI-224, 233rd A.C.S. National Meeting, Chicago, IL, Mar. 25–29, 2007.
9. Semple, G.; Skinner, P. J.; Cherrier, M. C.; Webb, P. J.; Sage, C. R.; Tamura, S. Y.; Chen, R.; Richman, J. G.; Connolly, D. T. *J. Med. Chem.* **2006**, *49*, 1227, Briefly, GPR109a and GPR109b receptors with an N-terminal HA tag were cloned into pcDNA3.1 (Invitrogen) and stable cell lines were generated by G418 selection. Positive clones were selected by anti-HA immunostaining. Compound potencies were determined by the 96-well adenylyl cyclase activation FlashPlate® assay from Perkin-Elmer as described by the manufacturer. The cAMP assay was optimized for the appropriate receptor stable clone, 5 μM forskolin was used for stimulation and 50,000 cells were used for each well. Positive controls were defined as cAMP generated by cells without forskolin stimulation and negative controls were defined as cAMP generated by cells with 5 μM forskolin stimulation.
10. Lorenzen, A.; Stannek, C.; Burmeister, A.; Kalvinsh, I.; Schwabe, U. *Biochem. Pharmacol.* **2002**, *64*, 645.
11. Kupiecki, F. P.; Marshall, N. B. *J. Pharmacol. Exp. Ther.* **1968**, *160*, 166.
12. van Herk, T.; Brussee, J.; van den Nieuwendijk, A. M. C. H.; van der Klein, P. A. M.; Ijzerman, A. P.; Stannek, C.; Burmeister, A.; Lorenzen, A. *J. Med. Chem.* **2003**, *46*, 3945.
13. Pereira, J. N.; Holland, G. F. *Experientia* **1967**, *23*, 905.

14. Jung, J.-K.; Johnson, B. R.; Duong, T.; Decaire, M.; Uy, J.; Gharbaoui, T.; Boatman, P. D.; Sage, C. R.; Chen, R.; Richman, J. G.; Connolly, D. T.; Semple, G. *J. Med. Chem.* **2007**, *50*, 1445.
15. Carlson, L. A.; Hedbom, C.; Helgstrand, E.; Sjöberg, B.; Stjernström, N. E. *Adv. Exp. Med. Biol.* **1969**, *4*, 85.
16. Carlson, L. A.; Hedbom, C.; Helgstrand, E.; Sjöberg, B.; Stjernström, N. E. *Acta Pharm. Suec.* **1972**, *9*, 289.
17. Seki, K.; Isegawa, J.; Fukuda, M.; Ohki, M. *Chem. Pharm. Bull.* **1984**, *32*, 1568.
18. Taggart, A. K.; Kero, J.; Gan, X.; Cai, T. Q.; Cheng, K.; Ippolito, M.; Ren, N.; Kaplan, R.; Wu, K.; Wu, T. J.; Jin, L.; Liaw, C.; Chen, R.; Richman, J.; Connolly, D.; Offermanns, S.; Wright, S. D.; Waters, M. G. *J. Biol. Chem.* **2005**, *280*, 26649.