Bioorganic & Medicinal Chemistry Letters 25 (2015) 3034–3038

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of a novel *trans*-1,4-dioxycyclohexane GPR119 agonist series

Sangdon Han *, Sanju Narayanan, Sun Hee Kim, Imelda Calderon, Xiuwen Zhu, Andrew Kawasaki, Dawei Yue, Juerg Lehmann, Amy Wong, Daniel J. Buzard, Graeme Semple, Chris Carroll, Zhi-Liang Chu, Hussein Al-Sharmma, Hsin-Hui Shu, Shiu-Feng Tung, David J. Unett, Dominic P. Behan, Woo Hyun Yoon, Michael Morgan, Khawja A. Usmani, Chuan Chen, Abu Sadeque, James N. Leonard, Robert M. Jones

Arena Pharmaceuticals, 6154 Nancy Ridge Drive, San Diego, CA 92121, USA

ARTICLE INFO

Article history:
Received 31 March 2015
Revised 23 April 2015
Accepted 30 April 2015
Available online 23 May 2015

Keywords:
T2DM
GPR119 agonist
GDIR
Trans-1,4-dioxycyclohexane

ABSTRACT

The design and optimization of a novel *trans*-1,4-dioxycyclohexane GPR119 agonist series is described. A lead compound **21** was found to be a potent and efficacious GPR119 agonist across species, and possessed overall favorable pharmaceutical properties. Compound **21** demonstrated robust acute and chronic regulatory effects on glycemic parameters in the diabetic or non-diabetic rodent models.

Type 2 diabetes mellitus (T2DM) caused by insulin resistance and loss of β-cell function is a serious metabolic disorder that has reached pandemic proportions worldwide.[1] Due to a lack of effective and safe long-term treatments for this chronic disease, there is a strong demand for novel therapeutic approaches. GPR119, a class A GPCR, is highly expressed in insulin producing pancreatic β-cells and incretin releasing intestinal endocrine cells. Activation of GPR119 elevates intracellular cAMP levels, triggering glucose-dependent insulin secretion from β-cells and incretin (e.g., GLP-1 and GIP) release from intestinal cells.[2,3] GLP-1 and GIP promote β-cell viability and also stimulate insulin secretory activity by interacting with their receptors expressed on β-cells.[4,5] Hence, orally acting GPR119 agonists may constitute a new therapy for improvement of glucose tolerance in patients with T2DM.[6] Importantly, GPR119 mediated glucose control is expected to be associated with a low risk of hypoglycemia due to its glucose-dependent mechanism of action.

We previously disclosed the discovery of APD597 (**1**) and its favorable preclinical and clinical profile.[7,8] Following this, we recently reported a 5-fluoro-4,6-dialkoxypyrimidine series (e.g., **2**) that exhibited improved in vitro agonist efficacy and possessed reduced CYP2C9 inhibitory potential.[9] Thus far, our prototypical GPR119 agonists have had a central pyrimidine pharmacophore as a preferred structural feature. With the goal of identifying alternatives to this preferred motif, we replaced the pyrimidine with a cyclohexane ring (e.g., **3**, **4**, Fig. 1). The 1,3-diether derivative (**3**) was devoid of activity at the human GPR119 receptor (EC_{50} >100 μM), however, the 1,4-diether analogue (**4**, a mixture *cis*/*trans*) possessed modest agonist activity in a *cyclase assay* (EC_{50} >200 nM). Encouraged by this result, we separated the two geometric isomers (*cis*- and *trans*-**4**) to determine if the orientation of the functional groups was important for receptor activity. The superior potency of the *trans*-**4** isomer verified the importance of this geometric configuration (Table 1), and led us to the discovery of a novel *trans*-1,4-dioxycyclohexane GPR119 agonist series.

SAR studies began with an investigation of the aryloxy group in an attempt to identify a motif with the best balance of potency, efficacy, and physicochemical properties (Table 1). The *trans*-isomers depicted in Table 1 were prepared in a similar manner as described in Scheme 1. Alternatively, a Mitsunobu reaction employing *cis*-4-((1-methylpiperidin-4-yl)oxy)cyclohexanol (*cis*-**41**) and a corresponding aromatic alcohol was also effective. Several aromatic rings such as phenyl (**4**, **5**, and **7**), picoline (**10**), and pyrazine (**12**, **14**) were well tolerated in conjunction with a methyl sulfone or nitrile. Presumably, these substituents provide a favorable hydrogen bonding acceptor interaction with GPR119 and contribute to the potency observed. Of the more potent

* Corresponding author.
E-mail address: shan@arenapharm.com (S. Han).

http://dx.doi.org/10.1016/j.bmcl.2015.04.102
0960-894X/

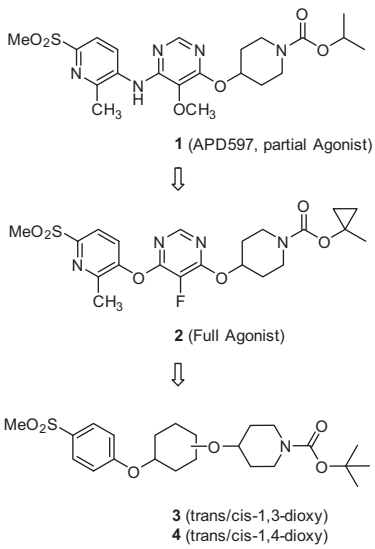
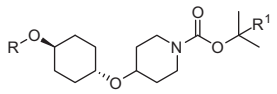
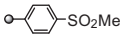
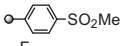
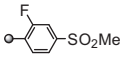
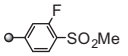
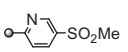
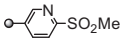
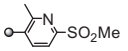
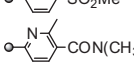
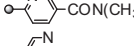
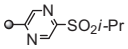
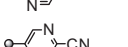
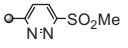


Figure 1. Design of a novel dioxycyclohexane GPR119 series.

Table 1
Aryloxy SAR



Compd	R	R^1	HTRF cAMP (nM)		$c\log P$[c]
			hGPR119 EC_{50}[a] (E_{max})	rGPR119 EC_{50}[a] (E_{max})	
1	—	—	46.0 (75)	421.0 (89)	3.52
2	—	—	13.8 (94)	169.0 (93)	2.45
4		CH_3	16.1 (115)	94.0 (120)	2.91
***cis*-4**		CH_3	266.0 (120)	1390.0 (121)	2.91
5		CH_3	8.5 (113), 227.0[b]	61.0 (119)	2.89
6		CH_3	52.0 (106)	167.0 (109)	3.09
7		CH_3	8.6 (116), 334.0[b]	45.0 (106)	3.88
8		CH_3	47.0 (111)	110.0 (108)	2.46
9		H	13.0 (101)	201.0 (125)	2.46
10		H	11.0 (108)	89.0 (114)	2.96
11		CH_3	262.0 (121)	400.0 (111)	2.84
12		CH_3	5.9 (110), 94.0[b]	64.0 (110)	2.07
13		CH_3	18.5 (109)	54.0 (128)	2.91
14		CH_3	8.3 (110), 144.0[b]	28.4 (100)	2.19
15		CH_3	651.0 (97)	1160.0 (86)	1.21

[a] E_{max} are the mean of three or more replicates, and relative to our reference standard, AR231453.
[b] Serum shift assay (Ref. 10).
[c] ChemBioDraw Ultra 12.0.

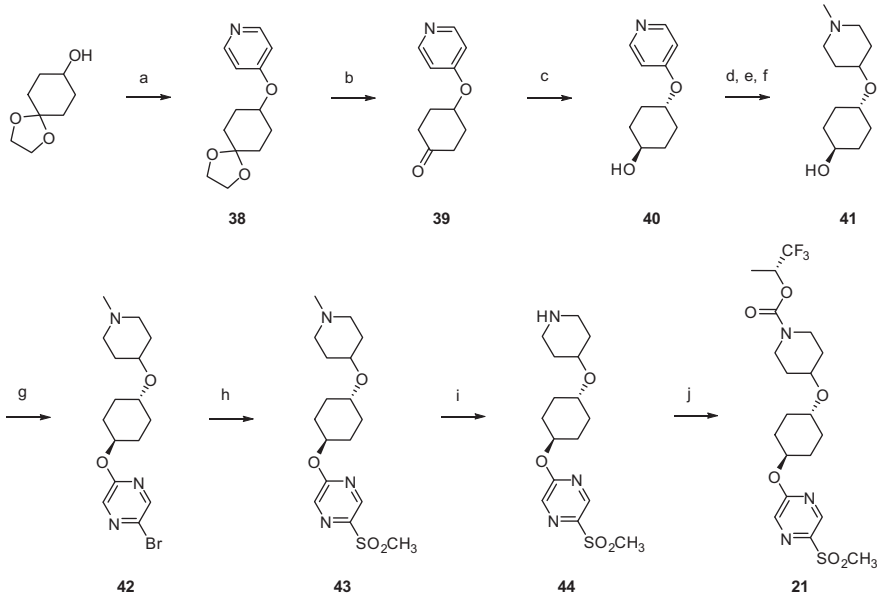
compounds prepared, **12** exhibited the smallest potency shift in the presence of serum,[10] correlating well with the order of $c\log P$ (**12** < **14** < **5** < **7**). Notably, this new series was associated with a very high intrinsic activity (E_{max} >100) at both human and rat GPR119 receptors. Ultimately, the 5-(methylsulfonyl)pyrazine (**12**) was selected as the preferred aryloxy group for further SAR investigation.

We next turned our SAR effort toward exploration of the piperidine substitution (Table 2). Piperidine motifs incorporating carbamates (**12**, **16**–**25**), amides (**26**–**28**), fluorinated alkyl groups (**29**–**33**), and heteroaromatic rings (**34**–**37**) were prepared. These were introduced in the final steps of the synthesis as illustrated in Scheme 1. Trifluoromethyl analogues (**21**–**23**), in particular, displayed significantly improved potency relative to our clinical compound (**1**) and were stable in human, rat, and mouse liver microsomes. Amide **26** attained good potency and had a low shift in the serum shift assay, but suffered from significantly reduced microsomal stability in mouse. Tertiary amines (**29**–**33**) comprised of fluorinated alkyls led to a reduction in GPR119 activity and reduced microsomal stability (e.g., **30**). Based on the combination of in vitro potency and microsomal stability, a handful of compounds were selected for further consideration. Compound **22** possessed a similar potency and PK profile relative to **21**, however this enantiomer was less effective in increasing glucose excursion in an oGTT experiment performed in 129SVE mice. The exceedingly potent hexafluoro analogue (**23**) was removed from further consideration as it impaired physical mobility of rodents in a dose dependent manner. Additionally, we were concerned that the activated *O*-hexafluoroisopropyl (HFIP) carbamate may form a covalent bond with serine hydrolases.[11] Oxadiazole analogues **34**–**36** showed reduced GIP release in normoglycemic 129SVE mice relative to **21**, despite their overall good potency, stability, and pharmacokinetic properties. For the reasons described above, **21** selected for further analysis.

Starting from commercially available 1,4-dioxaspiro[4.5]decan-8-ol, **21** was prepared by the synthetic sequence illustrated in Scheme 1. Aromatic nucleophilic substitution of 1,4-dioxaspiro[4.5]decan-8-ol, followed by an acid catalyzed deprotection efficiently gave **39**. A *cis*- enriched mixture of **40** (*cis*/*trans* = ~3/2) was obtained from the $NaBH_4$ reduction, which was separated efficiently to afford isomerically pure (>98%) *cis* and *trans* material on >100 g scale. The separation was carried out by an initial crystallization, followed by trituration with acetone. Stepwise reduction of the pyridinium salt of **40** subsequently offered the key intermediate, *trans*-4-((1-methylpiperidin-4-yl)oxy)cyclohexanol (**41**). The final intermediate **44** was straightforwardly prepared by nucleophilic substitution, copper promoted sulfonylation, and demethylation. Intermediate **44** was then converted to **21** via a standard carbamate formation.

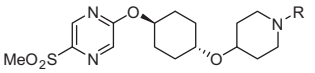
More extensive characterization entailed expanded in vitro screening and pharmacokinetic analysis. Compound **21** was found to maintain agonist activity at canine, monkey, and mouse GPR119 receptors (6.1 nM, 144%; 3.4 nM, 73%; 20.5 nM, 130%, respectively). It was stable in liver microsomes across species (human, rat, mouse half-life ($t_{1/2}$) >60 min), and also demonstrated good stability in human hepatocytes ($t_{1/2}$ >120 min). It was highly bound to both human and rat plasma proteins (human 97.5%, rat 96.0%), but less so for mouse (93.5%). In a subsequent Sprague-Dawley rat PK study, **21** exhibited a low systemic clearance and low steady-state volume of distribution, while possessing excellent systemic exposure, terminal half-life, and oral bioavailability (Table 3). In an abbreviated CNS study, **21** demonstrated a brain to plasma (b/p) ratio of approximately 1 (brain 820 ng/g, plasma 841 ng/mL) at 2 h post-dose, indicating virtually unrestricted blood-brain barrier (BBB) penetration.

In vivo GPR119 activation and corresponding glucose control was assessed for **21** in normoglycemic 129SVE mice after oral administration (Figs. 2 and 3a). Since GIP release is expected upon activation of GPR119, plasma GIP levels were measured 45 min post compound administration (Fig. 2). Two different doses of **21**



Scheme 1. Synthesis of **21**. Reagents and conditions: (a) 4-chloropyridine, *t*-BuONa, 94%, (b) HCl, 92%, (c) $NaBH_4$, 36%, (d) MeI, (e) $NaBH_4$, (f) H_2, Pd/C, 80% (d–f), (g) 2,5-dibromopyrazine, *t*-BuOK, 74%, (h) $NaSO_2Me$, $(CuOTf)_2PhH$, *N*,*N*-dimethylethane-1,2,-diamine, 70%, (i) 1-chloroethyl carbonochloridate, 90%, (j) (*R*)-1,1,1-trifluoropropan-2-ol, CDI, 87%.

Table 2
Piperidine SAR



Compd	R	HTRF cAMP (nM)		LM stability[b] (h, r, m)
		hGPR119EC$_{50}$ (E_{max})	rGPR119EC$_{50}$ (E_{max})	
12	CO_2t-Bu	5.9 (110)	64.0 (110)	
16	CO_2i-Pr	13.7 (110), 92.7[a]	119.0 (120)	>60, >60, >60
17	CO_2(1-methylcyclopropan-1-yl)	25.9 (118)	138.0 (123)	
18	$CO_2CH(Me)CH_2F$ (*R*)	36.8 (108)	317.0 (115)	
19	$CO_2CH(Me)CH_2F$ (*S*)	31.1 (105)	356.0 (123)	
20	$CO_2CH(CH_2F)_2$	65.1 (114)	467.0 (128)	>60, >60, >60
21	$CO_2CH(Me)CF_3$ (*R*)	3.0 (106), 38.0[a]	28.8 (120)	>60, >60, >60
22	$CO_2CH(Me)CF_3$ (*S*)	4.8 (112), 53.0[a]	49.9 (125)	>60, >60, >60
23	$CO_2CH(CF_3)_2$	1.2 (104), 45.2[a]	10.9 (121)	>60, >60, >60
24	CO_2(tetrahydrofuran-3-yl)	427.0 (105)	1500.0 (109)	
25	CO_2Ph	9.6 (116)	71.8 (104)	
26	$COCF_2Et$	5.3 (107), 28.7[a]	66.9 (126)	>60, >60, 3
27	CO(1-CF_3)cyclopentan-1-yl	38.4 (95)	108.0 (124)	
28	CO(1-CF_3)cyclopropan-1-yl	107.0 (107)	1380.0 (140)	
29	CH_2CF_3	776.0 (91)	1960.0 (105)	
30	1-((1-CF_3)cyclopropyl)methyl	62.6 (116)	163.0 (124)	21, >60, 37
31	1-((1-CF_3)cyclobutyl)methyl	54.8 (96)	80.0 (99)	
32	1-((1-CF_3)cyclopentyl)methyl	48.8 (37)	101.0 (88)	
33	1-(1-Fluorocyclopentyl)methyl	291.0 (105)	922.0 (119)	
34	3-Isopropyl-1,2,4-oxadiazol-5-yl	14.0 (102), 64.9[a]	97.9 (115)	>60, >60, >60
35	5-Isopropyl-1,2,4-oxadiazol-3-yl	9.1 (100), 56.3[a]	60.0 (108)	>60, >60, >60
36	3-(2-Fluoropropan-2-yl)-1,2,4-oxadiazol-5-yl	10.7 (103), 59.5[a]	64.9 (114)	>60, 59, 56
37	5-(2-Fluoropropan-2-yl)-1,2,4-oxadiazol-3-yl	30.3 (97), 276.0[a]	267.0 (114)	>60, >60, >60

[a] Serum shift assay (Ref. 10).
[b] Half life ($t_{1/2}$, min).

Table 3
Pharmacokinetics of **21**

Compd	Male Sprague-Dawley rat PK[a]						b/p @ 2h[b]
	Cl (L/h/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	C_{max} (μg/mL)	AUC_{last} (h*μg/mL)	%*F*	
21	0.40	2.94	6.45	0.77	5.21	100	0.98

[a] 2 mg/kg (IV)/2 mg/kg in 20% hp-β-CD (PO).
[b] Male SD rats, 2 mg/kg in 20% hp-β-CD (PO).

were administered and demonstrated a significant increase in GIP levels relative to vehicle treated controls. The 30 mg/kg dose was as efficacious as our tool compound (AR231453)[12] in this study. In an acute oral glucose tolerance test (oGTT), **21** was dosed orally 30 min prior to glucose challenge and blood glucose levels were measured over 120 min time course (Fig. 3a). Notably, all three doses produced an equivalent response as that observed for a 3 mg/kg dose of a representative DPP-4 inhibitor (Sitagliptin), and glycemic suppression relative to vehicle was approximately 40% in an area under curve (AUC) analysis.

Acute glucose control was also evaluated in Zucker Diabetic Fatty (ZDF) rats (Fig. 3b). Compound **21** was administered 60 min prior to glucose challenge and demonstrated markedly reduced blood glucose levels in a dose-dependent manner. It showed comparable efficacy to a 3 mg/kg dose of Sitagliptin when dosed at a 3 and 10 mg/kg dose (43% and 58% relative to 57% (Sitagliptin) in an AUC analysis).

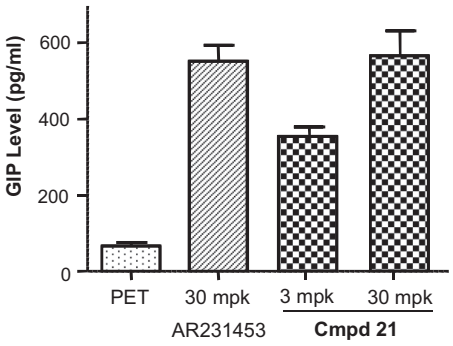


Figure 2. Effects of **21** on GIP release in male 129SVE mice.

With encouraging acute in vivo data, we investigated the effect of chronic treatment in Zucker Diabetic Fatty (ZDF) rats (Fig. 4). Compound **21** was dosed once daily over 4 weeks, and fed glucose and HbA1c levels were measured at the time of dosing. **21** demonstrated a significant fed glucose lowering effect after 2 weeks treatment (Fig. 4a) and markedly improved glycated hemoglobin (HbA1c) levels at day 28 in a dose-dependent manner (1.63%, 1.85%, and 2.58% relative to vehicle) (Fig. 4b). Furthermore, no loss of the acute drug response over the study period was observed, thus validating chronic use of a selective GPR119 agonist for glucose control. Lastly, we did not observe any significant drug effect on body weight irrespective of dose.

Further characterization of **21** revealed no interaction at the hERG channel in either the [^{3}H]-Astemizole binding assay (IC_{50} >1000 μM) or patch clamp assay (IC_{50} >30 μM). In human hepatic microsomal cytochrome P450s, **21** exhibited no significant inhibition of any of the five major isoforms (2C9, 27.5 μM; 2C19, 23.4 μM; 1A2, 3A4, and 2D6, >50 μM) and no inhibitory activity in CYP time-dependent inhibition (K_{inact}/K_i) assay. Glutathione (GSH) conjugates were not detected in the GSH trapping assay, indicating no apparent metabolite-mediated hepatotoxicity. Further, **21** showed no cytotoxic potential in an Essential Cell Function (ECF) panel measuring sensitive cellular parameters in live HepG2 cells.

In summary, we have designed and optimized a new series of *trans*-1,4-dioxycyclohexane GPR119 agonists. SAR studies led to the discovery of the preferred molecule **21** that has potent and efficacious GPR119 activity across species. This lead compound exhibited an excellent ADME and safety profile, and demonstrated robust regulatory effects on glycemic parameters in the acute and chronic diabetic or non-diabetic rodent models. Subsequent investigation of **21** and further development of this novel series will be disclosed elsewhere.

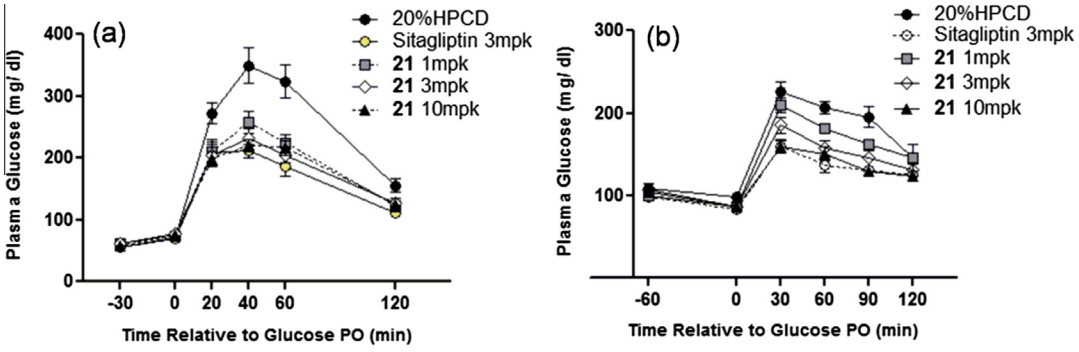


Figure 3. (a) Oral glucose tolerance test (oGTT) of **21** in male 129SVE mice. (b) oGTT in Zucker Diabetic Fatty (ZDF) rats.

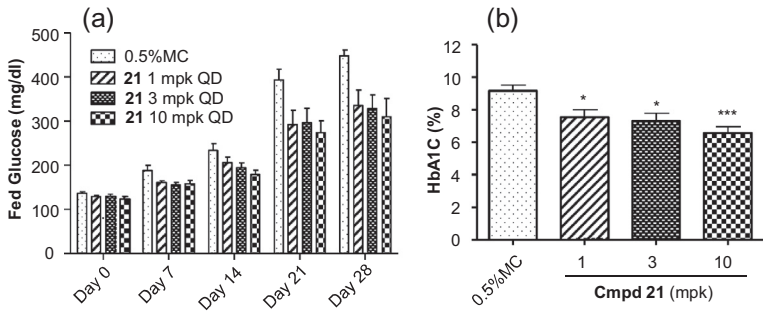


Figure 4. (a) Fed glucose control effect of chronic 28-days administration of **21** in Zucker Diabetic Fatty (ZDF) rats. (b) HbA1c levels in ZDF rats after 28 days.

References and notes

1. (a) http://www.who.int/mediacentre/factsheets/fs312/en/.; (b) Zimmet, P. Z. *Medicographia* **2011**, *33*, 15.
2. Chu, Z.-L.; Jones, R. M.; He, H.; Carroll, C.; Gutierrez, V.; Lucman, A.; Moloney, M.; Gao, H.; Mondala, H.; Bagnol, D.; Unett, D.; Liang, Y.; Demarest, K.; Semple, G.; Behan, D. P.; Leonard, J. *Endocrinology* **2007**, *148*, 2601.
3. Chu, Z.-L.; Carroll, C.; Alfonso, J.; Gutierrez, V.; He, H.; Lucman, A.; Pedraza, M.; Mondala, H.; Gao, H.; Bagnol, D.; Chen, R.; Jones, R. M.; Behan, D. P.; Leonard, J. *Endocrinology* **2008**, *149*, 2038.
4. Jia, X.; Brown, J. C.; Ma, P.; Pederson, R. A.; McIntosh, C. H. *Am. J. Physiol.* **1995**, *268*, E645.
5. Seino, Y.; Fukushima, M.; Yabe, D. J. *Diabetes Investig.* **2010**, *1*, 8.
6. Buzard, D. J.; Lehmann, J.; Han, S.; Jones, R. M. *Pat. Pharm. Anal.* **2012**, *1*, 285.
7. Semple, G.; Lehmann, J.; Wong, A.; Ren, A.; Bruce, M.; Shin, Y.-J.; Sage, C. R.; Morgan, M.; Chen, W.-C.; Sebring, K.; Chu, Z.-L.; Leonard, J. N.; Al-Shamma, H.; Grottick, A. J.; Du, F.; Liang, Y.; Demarest, K.; Jones, R. M. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1750.
8. Katz, L. B.; Gambale, J. J.; Rothenberg, P. L.; Vanapalli, S. R.; Vaccaro, N.; Xi, L.; Polidori, D. C.; Vets, E.; Sarich, T. C.; Stein, P. P. *Clin. Pharmacol. Ther.* **2011**, *90*, 685.
9. Buzard, D. J.; Kim, S.-H.; Lehmann, J.; Han, S.; Calderon, I.; Wong, A.; Kawasaki, A.; Narayanan, S.; Bhat, R.; Gharbaoui, T.; Lopez, L.; Yue, D.; Whelan, K.; Al-Shamma, H.; Unett, D. J.; Shu, H.-H.; Tung, S.-F.; Chang, S.; Chuang, C.-F.; Morgan, M.; Sadeque, A.; Chu, Z.-L.; Leonard, J. N.; Jones, R. M. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 4332.
10. HTRF cAMP assays (Cisbio) were performed following the manufacturer's instructions. For serum shift assays, cells were plated overnight in 384-well plates. On the day of the assay, growth medium was aspirated from the plates and replaced with fetal bovine serum (10 μL). Test compounds were serially diluted in DMSO and then diluted in assay buffer (PBS with 200 μM IBMX & 0.1% BSA) to achieve a 2× concentration containing 1% DMSO prior to addition to assay plates (10 μL).
11. Chang, J.-W.; Cognetta, A. B., III; Niphakis, M. J.; Cravatt, B. F. *ACS Chem. Biol.* **2013**, *8*(7), 1590.
12. Semple, G.; Fioravanti, B.; Pereira, G.; Calderon, I.; Uy, J.; Choi, K.; Xiong, Y.; Ren, A.; Morgan, M.; Dave, V.; Thomsen, W.; Unett, D. J.; Xing, C.; Bossie, S.; Carroll, C.; Chu, Z.-L.; Grottick, A. J.; Hauser, E. K.; Leonard, J. N.; Jones, R. M. *J. Med. Chem.* **2008**, *51*, 5172.