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Discovery and SAR of hydrazide antagonists of the pituitary adenylate cyclase-activating polypeptide (PACAP) receptor type 1 (PAC_1-R)

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Abstract—Potent small molecule antagonists for the PAC_1-R have been discovered. Previously known antagonists for the PAC_1-R were slightly truncated peptide ligands. The hydrazides reported here are the first small molecule antagonists ever reported for this class B GPCR.

The pituitary adenylate cyclase-activating polypeptide (PACAP) receptor type 1 (PAC_1-R) is a Class B GPCR closely related to the vasoactive intestinal peptide (VIP)/integrin/glucagon family of receptors.[1,2] PACAP binds to the PAC_1-R selectively over VIP, and also binds to the VIP receptors, VPAC1 and VPAC2. The truncated peptide PACAP(6-38) binds selectively to PAC_1-R.[3] Two other peptides with no significant sequence similarity to PACAP also bind to PAC_1-R: maxadilan, a selective agonist, and a shortened version termed M65, acts as a specific PAC_1-R antagonist.[4–6] Recently, the solution structure and binding mode of PACAP(6-38) to the extracellular domain of PAC_1-R_s have been reported.[7]

Identifying drug-like small molecule antagonists for class B GPCRs where the natural agonists are large peptides remains a challenge. Only 5 of the 15 known class B GPCRs have small molecule ligand binders and the class remains largely unexplored with small molecule inhibitors. Discovery of small molecule antagonists for key GPCR protein–protein/peptide interaction remains a significant challenge in the area of drug discovery.[8–10]

This is the first report of any small molecule (non-peptide) antagonists for the PAC_1-R. The PAC_1-R has been implicated in neuroprotection,[11] and is widely dispersed in the central nervous system (CNS). It is also upstream from the MAP-KK signaling system involved in cell cycle regulation. The PAC_1-R presents a potential novel target for small molecule drug discovery in the areas of neuroscience, oncology, and immunoscience.[12,13]

Two lead hydrazides shown in Figure 1 were identified from the Abbott compound library using the ^{125}I-PACAP27 radioligand binding assay to the PAC_1-R expressed in HEK293f membranes.[14] Hydrazide 1 efficiently inhibited radioligand binding with a K_i of 56 nM. This compound was subsequently shown to be a functional antagonist for the PAC_1-R in a calcium influx assay with a potent K_b calculated to be 200 nM.[15] We assumed that this series is competitive with the peptide ligand in both the radioligand competition and the calcium flux assay, however because of the affinity of the PACAP27, equilibrium conditions were difficult to demonstrate. In the radioligand binding assay, hydrazide 2 exhibited similar potency with a K_i of 73 nM. The objec-

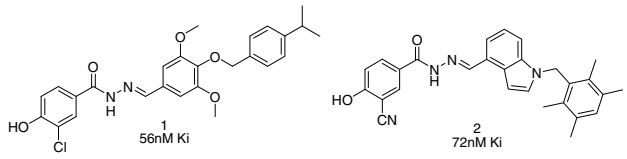

Figure 1. Structures of lead hydrazides that bind to PAC_1-R.

Keywords: PACAP; Class B GPCR; PAC_1-R; Hydrazides.
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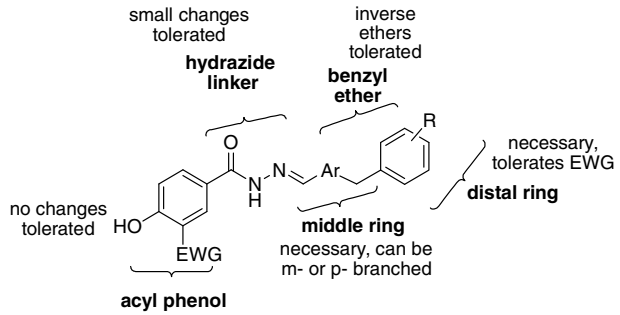
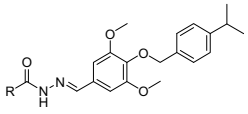


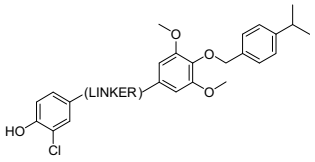
Figure 2. Structural components and SAR trends of hydrazide leads.

tive was to develop SAR around these two potent PAC_1-R antagonists. It is interesting to note that these two hits are potent reference glucagon receptor antagonists.[16] However, other compounds in our compound collection with glucagon activity showed no binding to the PAC_1-R.

The synthesis of new hydrazides was accomplished by known methods[17] via the coupling of acyl hydrazides with aldehydes. The aldehydes were synthesized by reaction of the appropriate phenolic aldehyde and aryl bromide. The hydrazides were synthesized by coupling of

Table 1. Modified phenol and linker hydrazides for PAC_1-R[a]

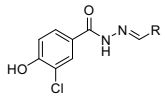


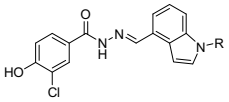


Entry	R	Binding Activity	Entry	LINKER	Binding Activity
3		22%@3μM	**7**		46%@3μM
4		inactive	**8**		40%@3μM
5		44%@3μM	**9**		inactive
6		inactive	**10**		inactive

[a] All binding activity reported herein was measured with an $n = 2$ or greater.

Table 2. PAC_1-R binding affinity of hydrazide distal ring modifications





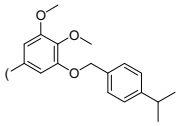
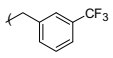
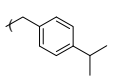
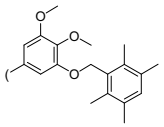
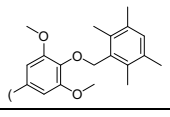
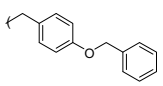
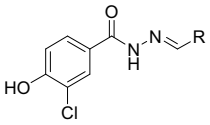
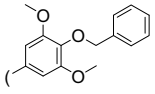
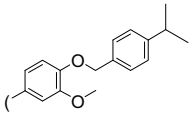
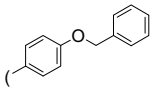
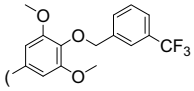
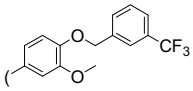
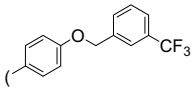
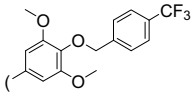
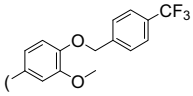
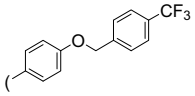
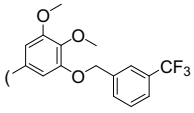
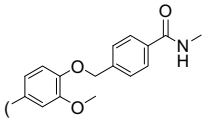
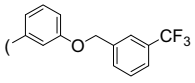
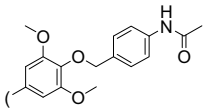
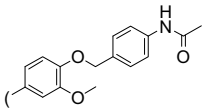
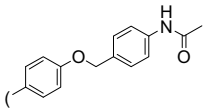
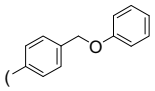
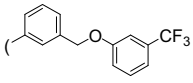
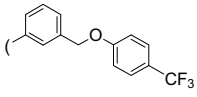
Entry	R	Ki, nM	Entry	R	Ki, nM
11		152	**14**		85
			15		604
12		167	**16**		55%@3μM
			17		49%@3μM
13		insoluble[18]	**18**		51%@3μM

Table 3. PAC_1-R binding activity of middle and distal ring combinations



Entry	R	Ki, nM	Entry	R	Ki, nM	Entry	R	Ki, nM
19		185	**25**		inactive/insoluble	**31**		522
20		204	**26**		404	**32**		537
21		140	**27**		50%@3μM	**33**		43%@3μM
22		inactive	**28**		36%@3μM	**34**		554
23		43%@3μM	**29**		34%@3μM	**35**		inactive
24		41%@3μM	**30**		274	**36**		241

appropriate benzoic acids with Boc-protected hydrazine followed by deprotection. All new compounds were purified by flash chromatography and were characterized by ^{1}H NMR and MS.

For the purposes of systematic SAR studies the lead compounds were divided into four regions and the overall trends are summarized in Figure 2. Both hits were clearly similar having a *p*-acyl phenol with a hydrazide linker to a middle aromatic ring that is further linked via a 2-atom linkage to a distal aromatic ring. The SAR studies were undertaken to investigate the effect of changes in four regions of the hydrazide lead compounds: the phenol portion; the hydrazide linker; the middle aromatic ring and the distal aromatic ring. We found that no changes were tolerated for the acyl phenol. Small changes were tolerated in the hydrazide linker. The middle and distal aromatic rings were necessary for potency, but many small changes to these rings and linkers were acceptable. The distal aromatic ring preferred a lipophilic group appended, but the lipophilic group was not crucial for potency.

Preliminary SAR is shown in Table 1. We discovered that both the *p*-phenol and the *m*-electron-withdrawing group (EWG) were crucial to high PAC_1-R affinity and sequential removal of these substituents resulted in inactive compounds **3** and **4**. Electron donating groups such as *m*-OMe reduced binding activity as shown in entry 5. The 3,4-dioxolane and 2′,2′-difluoro-3,4-dioxolane analogs were completely inactive. A *p*-SO_2NH_2 in place of the *p*-OH group was inactive as was the *m*-OH phenol. Nitrogen and oxygen-containing heterocyclic phenol isosteres similar to compound **6** were inactive. The hydrazide linker was modified as well. Borohydride-mediated reduction of the hydrazide gave compound **7** with the saturated hydrazine linker. This compound showed weak binding to PAC_1-R. Coupling of the acyl hydrazine with the corresponding acid gave compound **8**, which also displayed weak binding to PAC_1-R. Constricting the linker in an oxadiazole or thiadiazole as shown in entries 9 and 10 resulted in loss of PAC_1-R affinity.

We next explored small changes to the distal ring of the hydrazide leads and the results are shown in Table 2. Our approach was to mix and match the substitutents on the two lead compounds to determine which changes were tolerated, if any.

Moving the *p*-isopropyl benzyl ether distal ring to the meta-position of the middle ring maintains PAC_1-R binding affinity as shown for entry 11. Changing to the tetramethyl benzyl ether from hydrazide lead compound **2** results in similar binding affinity as shown in entry 12 or an insoluble[18] compound in entry 13.

We also explored different benzyl groups for the indazole lead compound **2** and these results are summarized in Table 2, entries 14–18. Changes were less tolerated for these indazole compounds, the best being the *m*-trifluoromethyl (CF_3) benzyl group in compound **14** with a binding affinity similar to that of compound **2**. Binding was also observed with the *p*-isopropylbenzyl group in compound **15** and weak binding was observed for compounds simple benzyl (16), 3-pyridyl (17), or extended benzyloxybenzoyl (18). Lipophilic substituents on the distal ring are required for binding to PAC_1-R for both the indazole and dimethoxyphenyl middle rings.

Next, SAR studies focused on middle and distal ring combinations of hydrazide 1 are shown in Table 3. These examples represent the largest changes to the hydrazides that were tolerated. The dimethoxy groups were systematically removed from the middle aryl ring and these changes were combined with different substituents on the distal benzyl ether. Compounds that retain the dimethoxy groups of the middle ring with the distal ring benzyl ether unsubtituted (19), *m*-CF_3 (20), and *p*-CF_3 (21) are potent binders. Interestingly, we lose all activity when the *m*-CF_3 benzyl ether is linked through the *m*-position (22) instead of the *p*-position (20) of the middle ring. The potency drops when the middle dimethoxy groups are removed, as we compare entries 19 and 31. For compounds containing a *m*-CF_3 in the benzyl ether, compared to entry 20, lose two-fold potency when one (26) or both (32) methoxy groups are removed from the middle ring. For compounds with a *p*-CF_3 in the benzyl ether, compared to entry 20, are only weak binders with one (27) or no (33) methoxy groups in the middle ring. Remarkably, entry 22 with the *m*-CF_3 benzyl ether linked through the *m*-position of the middle ring, regains potency when dimethoxy groups are absent as in entry 34. Entries 23, 28, and 29 show that weaker binding is observed upon introduction of an acetamide group to the distal benzyl ether. In general, the most potent compounds contain both dimethoxy groups in the middle aromatic and an aliphatic group on the distal aromatic ring.

Compounds were also synthesized with the reversed benzyl ether linkage between the middle and distal aromatic rings. Entries 30 and 36 show a two-fold improvement in potency when compared to compound 34. This trend is not general, however, because when we compare the unsubstituted benzyl ethers the reversed benzyl ether for entry 24 is weaker in potency than entry 31.

In conclusion, we have synthesized acyl hydrazides that show binding to the PAC_1-R with nM binding affinity. We have developed SAR around the linker, middle, and distal rings of the hydrazides. These compounds represent the only small molecules that bind to this class II GPCR. These acyl hydrazides should be useful in further elucidating the biological significance of PAC1-R.

Acknowledgments

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14. Briefly, membranes prepared from HEK293f cells expressing PAC1R (5 μg) were incubated with 0.2 nM ^{125}I-PACAP27 in 50 mM Tris–HCl, pH7.4, containing 5 mM $MgCl_2$) for 20 min at 37 °C. Non-specific binding was defined by 300 nM unlabeled PACAP38. The assay was terminated by rapid filtration through GF/B filters soaked overnight in 0.5% PEI at 4 °C and washed three times with ice cold harvest buffer (50 mM Tris–HCl, pH 7.4,0.5 mM EDTA and 0.1% BSA). The radioactivity was quantified by a TopCount (Perkin-Elmer). The K_i values were determined from the inhibition data determined using the Cheng–Prusoff equation (Cheng and Prusoff, 1973). All values were calculated using non-linear regression and the Prism Analysis package (GraphPad, San Diego).
15. The hydrazides were assessed as functional antagonists using PACAP-induced Ca^{2+} mobilization in the rat pancreatic acinar cell line AR42J that endogenously expresses PAC_1-R. The cells were loaded with the Ca^{2+} indicator dye Fluo-4AM for 2 h, prior to incubation with the compounds for 30 min at room temperature offline. Following, addition of 2 nM PACAP-27 the changes in calcium concentration were monitored in the FLIPR for

3 min. The K_b values were determined from the inhibition curve using the Cheng-Prusoff method (Cheng and Prusoff 1973). All values were calculated using non-linear regression and the Prism Analysis package (GraphPad, San Diego).

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18. Compound 13 could not be dissolved at 3 μM in DMSO for the binding assay. Similar compounds in this series exhibited aqueous solubility at <3 μM.