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# Identification and optimization of small molecule antagonists of vasoactive intestinal peptide receptor-1 (VIPR1)

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### ARTICLE INFO

Article history: Received 19 December 2011 Revised 17 January 2012 Accepted 20 January 2012 Available online 2 February 2012 ABSTRACT

Identification, synthesis and structure-activity relationship of small-molecule VIPR1 antagonists encompassing two chemical series are described.

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Keywords: Vasoactive intestinal peptide receptor (VIPR) Vasoactive intestinal peptide (VIP)

Vasoactive intestinal peptide receptor-1 (VIPR1) is a class B GPCR expressed predominantly in lung, small intestine, thymus and brain. VIPR1 and its endogenous agonist VIP (28 AA polypeptide) have been reported to be overexpressed in several tumors.<sup>1,</sup> <sup>2</sup> The density of VIPR1 in certain tumors is so high that labeled ligands like (<sup>125</sup>IVIP) are used in tumor imaging and diagnostic procedures.<sup>3</sup> Thus expression data alone suggests a possible role for VIPR1 in cancer. Consequently, VIPR1 is viewed as a potential therapeutic intervention point for drug discovery efforts. In fact, several peptidic VIP-based VIPR modulators have been reported to exhibit antiproliferative effects on various cancer cell lines<sup>4</sup> including breast,<sup>5</sup> colon,<sup>6</sup> glioblastoma,<sup>7</sup> pancreatic<sup>8</sup> and NSCLC.<sup>9</sup>

In addition to inhibition of cancer cell proliferation, the VIPR1 antagonist SNH has been reported to potentiate the effect of various chemotherapeutic agents on the NSCLC NCI-H727 cell line.<sup>6b</sup> Further, the VIP hybrid antagonist (neurotensin<sub>6-11</sub>VIP<sub>7-28</sub>) has been shown to reduce tumor volume in vivo in mice.<sup>6a</sup> Encouraged by the literature data as well as in-house siRNA experiments,<sup>10</sup> we decided to investigate VIPR1 as an oncology target. All the reported

\* Corresponding author. E-mail address: lalgudi.harikrishnan@bms.com (L.S. Harikrishnan). VIPR1 antagonists are peptide-based with poor drug-like properties. To our knowledge, there are no literature reports on small molecule antagonists of VIPR1. In this communication, identification and initial optimization of two chemical series of VIPR1 antagonists are described.

High throughput screening (HTS) of the Bristol-Myers Squibb compound collection was carried out using a VIPR1 HTRF cAMP assay in 1536 well format.<sup>11</sup> Active compounds were retested in



VIPR1 cAMP IC<sub>50</sub> =  $0.74 \,\mu\text{M}$ 

Figure 1. Representative compounds from biaryl and cyanothiophene series.

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**Scheme 1.** Preparation of biaryl compounds. Reagents and conditions: (a) Ispropylmethylketone, NaOEt, EtOH, rt, 5 h, 40% yield; (b) Diethylmalonate, NaOEt, EtOH, rt, 12 h, 60% yield; (c) KOH, EtOH, reflux, 1 h; (d) Cu<sub>2</sub>O, MeCN, reflux, 1.5 h, 90% yield (two steps); (e) HClO<sub>4</sub>, Ac<sub>2</sub>O, EtOAc, rt, 5 h, 91% yield; (f) cyclohexyl(*i*Pr)NLi, EtOAc, -78 °C, 1.5 h, 79% yield; (g) DDQ, dioxane, reflux, 17 h, 51% yield; (h) LAH, THF, rt, 1 h, 78% yield; (i) DDQ, DCM, rt, 1.5 h, 65% yield; (j) Tf<sub>2</sub>O, pyridine, DCM, 0 °C-rt, 55% yield; (k) ArB(OH)<sub>2</sub>, [Pd(dppf)Cl<sub>2</sub>], K<sub>3</sub>PO<sub>4</sub>, dioxane, 95 °C, 24 h; (l) NaBH<sub>4</sub>, EtOH, rt, 4 h, 10–36% yield (two steps).

dose–response mode in 384 well format.<sup>12</sup> This resulted in the identification of a biaryl series and a cyanothiophene series as exemplified by compounds **1** and **2**, respectively (Fig. 1).<sup>13</sup> Both series were pursued to explore SAR and improve potency against VIPR1.

A variation of a reported synthetic route was utilized to access compounds in the biaryl series having various 2-aryl rings (Scheme 1).<sup>14</sup> Condensation of isopropylmethylketone with aldehyde **3** followed by Michael addition of diethylmalonate gave ketone **4**. Saponification and subsequent decarboxylation of diester **4** gave keto acid **5**. Phenol **8** was then obtained via dehydrative cyclization of acid **5**, followed by ring opening of lactone **6** with the anion of ethyl acetate and subsequent oxidation/aromatization. Reduction of ester **8** to the corresponding primary alcohol followed by Swern oxidation gave aldehyde **9**. Sulfonylation of phenol **9** using triflic anhydride yielded the corresponding aryl triflate **10**. Suzuki coupling of aldehyde **10** with various aryl boronic acids followed by reduction of aldehyde **11** to alcohol **12** was carried out in parallel.<sup>15</sup> All final compounds were purified by reverse phase HPLC.

The SAR of various substitutions on the 2-phenyl ring are summarized in Table 1. Mesomeric or hyperconjugative electron releasing substituents at the 3- and 4-positions of the 2-aryl ring were preferred over substitution at 2-position ( $3 > 4 \gg 2$ ). Overall, 3-chloro and 3,4-dimethyl compounds exhibited the best potency against VIPR1.

To determine the potency of compounds on cell proliferation, three cell lines, namely H727, H1299 and Calu6 were chosen based on high levels of VIPR1 expression. Further, siRNA mediated knock down of VIPR1 in these three cell lines resulted in robust reduction of intracellular cAMP and significant inhibition of cell proliferation (data not shown). In spite of the improvement in potency in the cAMP assay, no significant inhibition of cell proliferation was observed in any of the three cell lines at concentrations of compounds up to 10  $\mu$ M. In order to minimize the impact of compounds being potentially sequestered by albumin, the cell proliferation assays were carried out in as low as 2% serum.<sup>16</sup>

The synthetic approach employed to access various amides in the cyanothiophene series is shown in Scheme 2.<sup>17</sup>

## Table 1

VIPR1 cAMP IC50 data for biaryl series



Compd.	Z	VIPR1 cAMP IC <sub>50</sub> (µM)
13	2-Me	>50
14	3-CF <sub>3</sub>	>50
15	3-CN	>50
16	2-Ph	3.2
17	4-OMe	1.3
18	2-OMe	0.82
1	4-F	0.74
19	4-Ph	0.68
20	Н	0.39
21	3-NMe <sub>2</sub>	0.31
22	4-Me	0.30
23	4-Cl	0.29
24	3-Me	0.24
25	3-Ph	0.21
26	3-OMe	0.20
27	3-SMe	0.17
28	Naphthyl (2,3-fused)	0.18
29	Naphthyl (3,4-fused)	0.12
30	3-Cl	0.10
31	3,4-Dimethyl	0.081

The SAR for various amides in the cyanothiophenes is summarized in Table 2. Larger acyl groups led to improvement in VIPR1 antagonism both in acyclic as well as cyclic examples (see methyl compound **32** to isobutyl compound **34**, as well as cyclopropyl compound **35** to cyclohexyl compound **38**). In addition, moving the phenyl ring farther from the acyl carbon led to significant improvements in potency. Phenethyl compound **41**, the most potent compound in this series, was explored further by substituting



**Scheme 2.** Preparation of cyanothiophene analogs. Reagents and conditions: (a)  $T_3P$ ,  $Et_3N$ , DCM, 0 °C to rt, 5–69% yield; (b) CDI, DIPEA, DCM, rt, 15–30% yield.

Table 2

VIPR1 cAMP IC<sub>50</sub> data for various amides in the cyanothiophene series



the phenyl ring as well as by constraining the ethylene linker. The results are summarized in Table 3. Unfortunately, none of the compounds showed improved potency over the phenethyl compound **41**. Furthermore, despite the improvements in potency, none of the compounds showed significant antiproliferative effects in the cell lines tested (H727, H1299 and Calu6).

In summary, the Bristol-Myers Squibb compound collection was screened, and series of biaryl alcohols and cyanothiophenes were identified as VIPR1 antagonists. SAR studies using parallel synthesis led to modest improvements in potencies (in both series) in the cAMP assay. However, none of the compounds showed significant antiproliferative effects in the cell lines tested. Further improvements in potency may be required to elicit the desired antiproliferative response.

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#### Table 3

VIPR1 cAMP IC<sub>50</sub> data for various substituted and constrained analogs of the phenethyl amide compound  $\mathbf{41}$ 





for distribution of compounds to Lead Discovery and Lead Evaluation groups.

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- High throughput screen (HTS) details: Compounds at 10 μM concentration were screened with 30 pM VIP (EC<sub>80</sub>) in HT29 or RKE cells using an HTRF cAMP assay in 1536-well plates.
- 12. VIPR1 cAMP Assay details: Dose-dependent stimulation of intracellular cAMP accumulation upon treatment with ligand VIP (Cat. 064-16, Phoenix Pharmaceuticals) was measured with an HTRF (high throughput timeresolved fluorescence) cAMP kit (Cat. 62AM4PEC, Cisbio) according to the manufacturer's directions. 384-well assay plates containing test compounds at increasing concentrations (threefold, 11-point serial dilutions) ranging from 10<sup>-10</sup> to 10<sup>-5</sup> M were prepared. RKE-VIPR1 cells were grown in DMEM media (Cat. 11995, Gibco) containing 10% fetal bovine serum (Cat. 16000, Gibco) and 500 µg/mL G418 (Cat. 10131, Gibco). RK3E-VIPR1 cells were detached using versene (Cat. 15040, Gibco) and, subsequently, harvested in assay buffer [Hanks Buffer (Cat. 14025-092, Invitrogen), 20 mM HEPES (Cat. 15630, Gibco), 1 mM IBMX (Cat. I5879, Sigma), 0.1% BSA (Cat. A3059, Sigma)]. Cells were added into compound plates for a final cell density of 4K cells/well and incubated at room temperature for 15 min. Ten nanometer VIP was then added to the assay plates at the final concentration of the EC<sub>50</sub> value; assay plates were incubated for 30 min at room temperature. After VIP stimulation, cells were lysed with lysis buffer (CisBio kit) for 30 min at room temperature. Next, 10 µL of cell lysate was transferred to 384-well proxyplates (Cat. 6008289, Perkin Elmer), mixed with 5 µL of D2 reagent (CisBio kit) and 5 µL of cryptate reagent (CisBio kit). Reactions were incubated for 1 h at room temperature. The HTRF signal was read on an EnVision plate reader (Perkin Elmer) at emission wavelengths of 665 nm and 620 nm. cAMP accumulation was normalized by a cAMP standard curve; IC50s were quantified using the four parameter logistic equation to fit the cAMP accumulation data. The cAMP IC<sub>50</sub> values reported are average readings from two wells, with individual readings reproducible within twofold of each other.
- 13. Both series of compounds were found to bind to VIPR1 with  $\rm IC_{50}$  values that were comparable to the cAMP  $\rm IC_{50}$  values. For VIPR radioligand competition

binding studies, a final concentration of 5 µg/well of RKE-VIPR cell membrane preparations were incubated with 200 µg/well wheat germ agglutinin-coated PVT beads (Cat. 25007078, GE Healthcare) in 100 uL assay buffer [50 mM HEPES, 5 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 0.1% BSA] for 10 min at room temperature. This mixture was then added to 96-well assay plates (Cat. 3912, Costar) containing 1.5 µL test compounds at increasing concentrations (threefold, 11-point serial dilutions) ranging from  $10^{-10}$  to  $10^{-5}$  M. The control for non-specific binding was 1 µM VIP (Cat. V6130, Sigma). A final concentration of 100 pM of [ $^{125}$ I] VIP (Cat. NEX192050UC, Perkin Elmer) in 50 µL assay buffer was then added to the reaction. Sealed assay plates were incubated at room temperature for 3 h then analyzed by TopCount. After correcting for non-specific binding, IC<sub>50</sub> values were determined. The concentration of test compound that inhibited 50% of radioligand bound (IC<sub>50</sub>) was quantified using the four parameter logistic equation to fit the data.

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- 15. Procedure for conversion of aryl triflate 10 to alcohol 12: To an array of vials containing a solution of aryl triflate 10 (30 mg, 1 equiv) in 1,4-dioxane (1 mL) was added various aryl boronic acids (1.1 equiv). bis(diphenylphosphino)ferrocene] dichloropalladium(II) (0.03 equiv) and aqueous K<sub>3</sub>PO<sub>4</sub> (3 equiv). The reaction mixtures were degassed with nitrogen and heated at 95 °C for 24 h. The reaction mixtures were cooled to room temperature and were filtered through a short pad of silica. The filtrates were evaporated in a Genevac™. To the resulting array of crude aldehyde was added a suspension of sodium borohydride (1 equiv) in anhydrous ethanol (1 mL). The reaction mixtures were stirred at room temperature for 4 h. The solvents were evaporated in a Genevac<sup>™</sup> and the residues were purified by reverse phase preparative HPLC.
- 16. Plasma binding determination of a representative compound (compound 1) revealed it was only 90% bound in mouse and human plasma.
- 17. (a) *CDI method*: To an array of carboxylic acids (1.5 equiv) was added a stock solution of CDI (1.5 equiv) and DIPEA (3.0 equiv) in DCM (1 mL). After 1 h, a solution of the primary amine (1.0 equiv) in DCM (1 mL) was added and the reaction mixture was stirred for 18 h at room temperature. The reaction mixture was concentrated, and purified by reverse phase preparative HPLC.
  (b) *T3P method*: To an array of vials containing various carboxylic acids (1.5 equiv) was added a stock solution of the scaffold primary amine (1 equiv) and triethyamine (3.0 equiv) in DCM (1 mL). The reaction mixture was cooled to 0 °C and 0.3 mL of T<sub>3</sub>P (propane phosphonic acid anhydride, 50% solution in EtOAc) was added to each vial. The vials were warmed up to room temperature and stirred overnight. The reaction mixture was then concentrated and the compounds were purified by preparative HPLC.