Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 1211–1215
Bioorganic & Medicinal Chemistry Letters

Discovery and preliminary evaluation of 5-(4-phenylbenzyl)oxazole-4-carboxamides as prostacyclin receptor antagonists

Marc-Raleigh Brescia,* Laura L. Rokosz, Andrew G. Cole, Tara M. Stauffer, John M. Lehrach, Douglas S. Auld, Ian Henderson and Maria L. Webb

Pharmacopeia Drug Discovery Inc., PO Box 5350, Princeton, NJ 08543, USA

Received 13 October 2006; revised 6 December 2006; accepted 7 December 2006
Available online 12 December 2006

Abstract—The discovery and evaluation of 5-(4-phenylbenzyl)oxazole-4-carboxamides as prostacyclin (IP) receptor antagonists is described. Analogs disclosed showed high affinity for the IP receptor in human platelet membranes with IC_{50} values of 0.05–0.50 μM, demonstrated functional antagonism by inhibiting cAMP production in HEL cells with IC_{50} values of 0.016–0.070 μM, and exhibited significant selectivity versus other prostanoid receptors.

Prostacyclin (PGI_2) is an eicosanoid produced from the cyclooxygenase-mediated metabolism of arachidonic acid.[1] More specifically, it is generated from isomerization of endoperoxide H_2 (PGH_2) via prostacyclin synthase. PGI_2 functions as an inhibitor of platelet aggregation,[2] is a potent vasodilator,[3] and is a regulator of blood flow in kidneys.[4] PGI_2 is also a physiological antagonist of thromboxane A_2 (TXA_2).[1a] PGI_2 mediates its effects through binding and subsequent activation of the prostacyclin (IP) receptor, a G-protein-coupled receptor (GPCR) that signals through both Ca^{2+} and cAMP.[5] A number of studies have shown that PGI_2 activity participates in the etiology of pain and inflammatory disease.[6–11] For example, intraperitonial injection of PGI_2 in mice induces a writhing response,[6] and IP receptor-deficient mice show a distinct reduced response to inflammatory pain.[11] Previously reported IP antagonists (RO1138452 and RO3244794, Fig. 1) have been shown to be orally efficacious in rat acetic-acid writhing and carrageenan-induced paw hyperalgesia models, with no effect on bleeding time in the mouse or cardiovascular effects in the rat.[12]

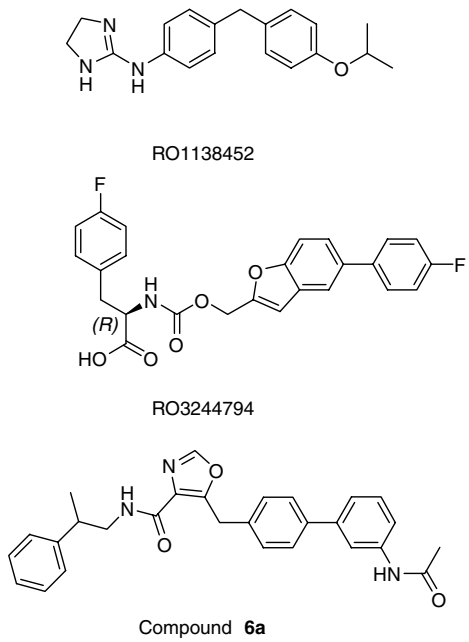


Figure 1. IP receptor antagonists.

We sought to identify a structurally distinct and novel class of IP receptor antagonists by the high-throughput screening of a diverse 2.1 million-member compound collection prepared using ECLiPS™ (Encoded Combinatorial Libraries on Polymeric Support) technology.[13] These compounds were screened using a platelet membrane binding assay with the radioligand, [^{3}H]-Iloprost.[14,15] Of the active structures identified, the 5-(4-phenylbenzyl)oxazole-4-carboxamide **6a** proved to be

Keywords: Prostacyclin; PGI_2; Prostanoid receptors; Pain; Inflammation; ECLiPS™.
* Corresponding author. Tel.: +1 609 452 3799; fax: +1 609 655 4187; e-mail: mbrescia@pcop.com

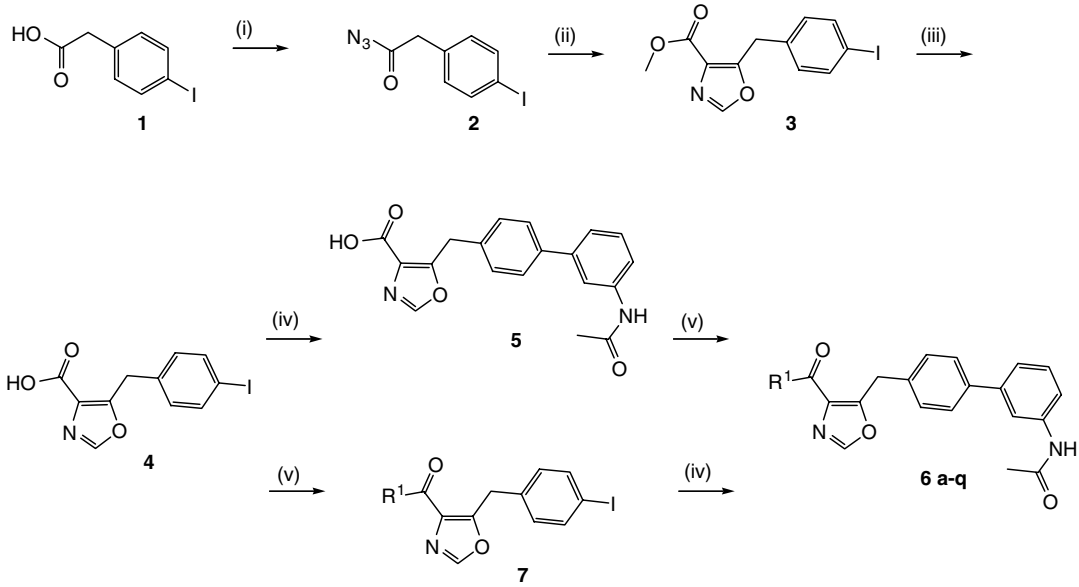
0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.12.025

the most interesting, possessing an IC_{50} value of 0.077 μM (Fig. 1). Interestingly, compound **6a** contains both the aromatic bridging methylene group contained in RO1138452 and the biaryl motif of RO3244794.

Compound **6a** was then supplemented with a series of analogs designed to expand the SAR around the phenethyl-based carboxamide. Compounds were synthesized from 4-iodophenylacetic acid (**1**) in five steps (Scheme 1). Synthesis of acyl azide **2** was accomplished by treatment of **1** with diphenylphosphoryl azide (DPPA), which was then reacted with the anion of methyl isocyanoacetate to afford oxazole **3**. Ester hydrolysis of **3** using lithium hydroxide provided the versatile novel core **4**, which was used in two synthetic pathways to generate IP receptor antagonists **6**. Primarily, compounds **6** were synthesized from **4** via a palladium-catalyzed biaryl formation under standard Suzuki–Miyaura[16] coupling conditions, to afford biaryl **5**, followed by 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC)-mediated amide bond formation. This synthesis provided an efficient way to examine the SAR at the carboxamide position. Alternatively, generation of **7** from **4** by performing the EDC-mediated amide formation first, followed by Suzuki–Miyaura biaryl formation, was also a viable synthesis of **6**. This latter synthesis also provides greater flexibility for the future generation of IP receptor antagonists aimed at examining the SAR around the biaryl motif.

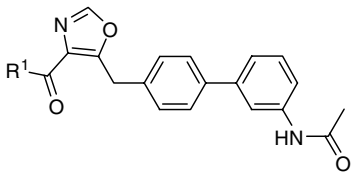
IP receptor binding activity was quantified via a filter binding assay measuring displacement of [^{3}H]-Iloprost binding to human platelet membranes[15] prepared as described.[17] Functional antagonism was quantified by measuring the inhibition of cAMP production induced by Iloprost in human erythroleukemia (HEL) cells using a Perkin-Elmer LANCE™ cAMP detection kit.[18] All analogs were also examined in the cAMP assay in the absence of Iloprost and showed no agonist activity affirming that these analogs are antagonists of the IP receptor.

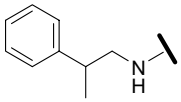
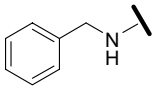
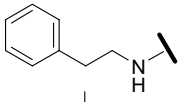
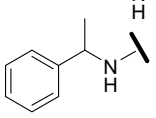
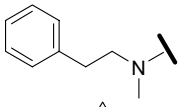
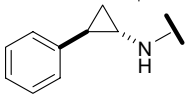
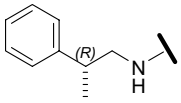
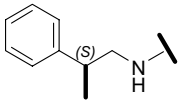
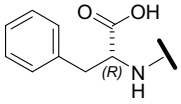
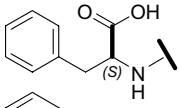
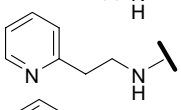
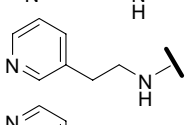
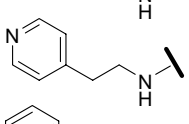
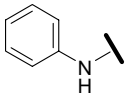
The binding and functional activities of the carboxamide analogs versus the IP receptor are shown in Table 1. In general, all compounds showed greater potency in the functional assay compared to the binding assay. Previously reported IP receptor antagonists have also shown this phenomenon with up to a sevenfold difference in the functional assay versus the binding assay.[12] The functional assay was validated with RO1138452 which showed a potency of 0.00083 μM, which is consistent with the published value of 0.001 μM. The more potent functional activity was used to define SAR trends. Hydrophobic substituents led to potent analogs in this series as seen in benzyl analog **6b** and phenethyl analog **6c**, which showed potencies in the cAMP assay of less than 0.15 μM. α-Methyl substitution of the benzylic methylene of **6b** to provide **6d** was tolerated, but no increase in potency was observed. N-Methylation of **6c** to form tertiary amide **6e** reduced the functional activity 20-fold, thus showing the preference for secondary amides. Substitution of the phenethyl-based analogs was also well-tolerated. For example, the incorporation of a cyclopropyl group to produce **6f** generated an IC_{50} value of 0.36 μM. An increase in potency was also realized by incorporating α-or β-substitution on phenethyl moieties. (*R*)-(+)-β-methylphenethyl **6g** produced an IC_{50} value of 0.051 μM in the functional assay. The *R*-configuration at the β-position was preferred over its enantiomer, as **6h** was sixfold less active in the functional assay than **6g**. Incorporation of a carboxylic acid moiety at the α-position with *S*-configuration to give compound **6j** resulted in the most potent analog in the functional assay with an IC_{50} value of 0.016 μM. The corresponding *R* stereoisomer, **6i**, was significantly less potent with an IC_{50} value of 0.83 μM. It is interesting to note that the *S*-configuration is more potent in this



Scheme 1. Reagents and conditions: (i) DPPA, Et_3N, THF, 0–25 °C; (ii) NaH, methyl isocyanoacetate, DMF, 0–25 °C; (iii) LiOH, THF/H_2O, 25 °C; (iv) $Pd(PPh_3)_4$, K_2CO_3, 3-acetamidophenylboronic acid, dioxane/H_2O, 80 °C; (v) EDC, HOBt, RR′NH, CH_2Cl_2, 25 °C.

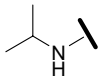
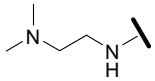
Table 1. Biological activity of IP receptor antagonists



Compound	R^1	IPR IC_{50}[a] (μM)	HEL cAMP IC_{50}[b] (μM)
6a[c]		0.077 ± 0.012	ND
6b		0.561 ± 0.006	0.066 ± 0.016
6c		0.721 ± 0.020	0.138 ± 0.000
6d[c]		1.23 ± 0.08	0.278 ± 0.002
6e		7.16 ± 2.11	2.133 ± 0.435
6f[d]		0.331 ± 0.073	0.358 ± 0.028
6g		0.053 ± 0.003	0.051 ± 0.008
6h		0.916 ± 0.148	0.296 ± 0.026
6i		4.20 ± 0.56	0.831 ± 0.145
6j		0.476 ± 0.193	0.016 ± 0.001
6k		7.60 ± 2.29	1.005 ± 0.157
6l		4.95 ± 2.01	0.741 ± 0.124
6m		4.71 ± 0.21	0.476 ± 0.173
6n		3.48 ± 0.29	0.828 ± 0.072
6o		12.2 ± 0.4	3.306 ± 1.026

(*continued on next page*)

Table 1 (*continued*)

Compound	R^1	IPR IC_{50}[a] (μM)	HEL cAMP IC_{50}[b] (μM)
6p		12.4 ± 4.6	3.584 ± 1.478
6q		34.1 ± 6.3	3.306 ± 1.026

[a] Binding IC_{50} ± standard deviation.[15]
[b] Functional IC_{50} ± standard deviation.[18]
[c] Racemic.
[d] *trans*-Racemic.

series while the *R*-stereochemistry is presented in RO3244794. Pyridylethyl analogs, **6k**–**m**, and the phenyl analog **6n** were all less potent, exhibiting potencies of greater than 0.5 μM. Small alkyl-based analogs (**6o**–**q**) were not tolerated (IC_{50} >3 μM), showing a significant reduction in potency relative to the phenethyl-based antagonists.

Of the initial analogs generated, **6b**, **6g**, and **6j** were the most potent in the cAMP functional assay and were further investigated in in vitro ADMET assays to determine suitability for in vivo evaluation. Metabolic stability was assessed in both human liver microsomes (HLM) and rat liver microsomes (RLM) assays.[19] After a 30-min incubation with 0.3 μM of liver microsomes, **6j** showed substantial stability in both species (HLM 100% remaining and RLM 91% remaining), while **6b** (HLM 63% remaining and RLM 33% remaining) and **6g** (HLM 12% remaining and RLM 11% remaining) were observed to be less stable. Since **6j** showed good stability in the microsome assays, it was further examined against a panel of five cytochrome P450 (CYP) enzymes and showed no significant activity (IC_{50} >20 μM) against 3A4, 2D6, 1A2, and 2C19, and weak activity (IC_{50} = 5.1 μM) versus CYP2C9. Compound **6j** also exhibited reasonable permeability in a Caco-2 assay,[19] with a Papp of 55 nm/s and no efflux. No significant hERG activity was observed for **6j** which showed only 5% inhibition in a rubidium efflux assay at 20 μM. Overall, the in vitro ADMET properties of **6j** are favorable and constitute a lead compound for further optimization against the IP receptor.

Selectivity of **6j** was examined against two other prostanoid receptors, EP2 and EP4, using the cloned recombinant human receptors[20] expressed in a derivative of 293EBNA cells.[21] Significant selectivity over both EP2 and EP4 was observed, with IC_{50} values of 32 and 24 μM, respectively.

In summary, we have identified novel small-molecule functional antagonists of the IP receptor. Specifically, **6j** was shown to be a potent IP receptor antagonist with favorable in vitro ADMET properties and is selective against other prostanoid receptors. This profile bodes well for further pharmaceutical development of compounds in this chemical series. More detailed selectivity studies, SAR expansion, and in vivo pharmacokinetic profiling will be reported in due course.

Acknowledgments

We thank Weiqing Chen, Lynn Rogers, and Hema Desai for assistance in ADME profiling and Robert Swanson and Jingchun Yang for compound analyses in the EP2 and EP4 antagonist assays.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.12.025.

References and notes

1. (a) Dogne, J. M.; de Leval, X.; Delarge, J.; David, J. L.; Masereel, B. *Curr. Med. Chem.* **2000**, *7*, 609; (b) de Leval, X.; Hanson, J.; David, J. L.; Maseree, L. B.; Pirotte, B.; Dogne, J. M. *Curr. Med. Chem.* **2004**, *11*, 1243.
2. Whittle, B. J.; Moncada, S.; Vane, J. R. *Prostaglandins* **1978**, *16*, 373; Weiss, H. J.; Turitto, V. T. *Blood* **1979**, *53*, 244.
3. Moncada, S.; Vane, J. R. *Adv. Prostaglandin Thromboxane Res.* **1980**, *6*, 43.
4. Breyer, M. D. *Exp. Nephrol.* **1998**, *6*, 180.
5. Murray, R.; Furci, L.; FitzGerald, G. A. *FEBS Lett.* **1989**, *255*, 172; Feoktistov, I.; Breyer, R. M.; Biaggioni, I. *Biochem. Pharmacol.* **1997**, *54*, 917.
6. Akarsu, E. S.; Palaoglu, O.; Ayhan, I. H. *Meth. Find. Exp. Clin. Pharmacol.* **1989**, *11*, 273.
7. Schepelmann, K.; Messlinger, K.; Schaible, H. G.; Schmidt, R. F. *Neuroscience* **1992**, *50*, 237.
8. Bley, K. R.; Hunter, J. C.; Eglen, R. M.; Smith, J. A. *Trends Pharmacol. Sci.* **1998**, *19*, 141.
9. Bombardieri, S.; Cattani, P.; Ciabattoni, G.; Di Munno, O.; Pasero, G.; Patrono, C.; Pinca, E.; Pugliese, F. *Br. J. Pharmacol.* **1981**, *73*, 893.
10. Galvani, M.; Bugiardini, R.; Ferrini, D.; Gridelli, C.; Mari, L.; Puddu, P.; Lenzi, S. *Ric. Clin. Lab.* **1985**, *15*, 145.
11. Murata, T.; Ushikubi, F.; Matsuoka, T.; Hirata, M.; Yamasaki, A.; Sugimoto, Y.; Ichikawa, A.; Aze, Y.; Tanaka, T.; Yoshida, N.; Ueno, A.; Oh-ishi, S.; Narumiya, S. *Nature* **1997**, *388*, 678.
12. Clark, R. D.; Jahangir, A.; Severance, D.; Salazar, R.; Chang, T.; Chang, D.; Jett, M. F.; Smith, S.; Bley, K. *Bioorg. Med. Chem. Lett.* **2004**, *16*, 1053; Bley, K. R.; Bhattacharya, A.; Daniels, D. V.; Gever, J.; Jahangir, A.; O'yang, C.; Smith, S.; Srinivasan, D.; Ford, A. P.; Jett, M. F. *Br. J. Pharmacol.* **2006**, *147*, 335.

13. Ohlmeyer, M. H. J.; Swanson, R. N.; Dillard, L.; Reader, J. C.; Asouline, G.; Kobayishi, R.; Wigler, M.; Still, W. C. *Proc. Nat. Acad. Sci. U.S.A.* **1993**, *90*, 10922; Nestler, H. P.; Bartlett, P. A.; Still, W. C. *J. Org. Chem.* **1994**, *59*, 4723.
14. Grant, S. M.; Goa, K. L. *Drugs* **1992**, *43*, 889.
15. *IP receptor filter binding assay*. 0.01 μM [^{3}H]-Iloprost (GE Healthcare) was incubated at room temperature with 50 μg of membranes prepared from human platelets[17] in assay buffer containing 20 mM Hepes, 5 mM $MgCl_2$, pH 7.4, in the presence or absence of compound for 1 h. Compounds were prepared by serially diluting in DMSO, followed by intermediate dilutions in assay buffer. Final DMSO concentrations ranged from 1% to 10%, as indicated. The assay mixture was filtered over a pre-wet 96-well MAFBN0B50 filter plate (Millipore) and washed twice with 1× PBS (Mediatech). Fifty microliters of Optiphase Supermix was added to each well and the assay was counted on a Microbeta® TriLux. Raw data were fit to the Michaelis–Menten equation to calculate IC_{50} values. Data are reported as the average of multiple determinations ± standard deviation.
16. Miyaura, N.; Yanagi, T.; Suzuki, A. *Synth.Comm.* **1979**, *11*, 513; Review: Suzuki, A. *Chem. Comm.* **2005**, *38*, 4759.
17. Kaczmarek, D.; Hohlfeld, T.; Wambach, G.; Schror, K. *Eur. J. Clin. Pharmacol.* **1993**, *45*, 451.
18. *cAMP assay*. HEL 92.1.7 cells were obtained from ATCC (cat # TIB-180). Cells were cultured at a density of 5×10^4 to 1×10^6 cells/mL in RPMI (Invitrogen) media containing 10% FBS, 10 mM Hepes, 1 mM sodium pyruvate, and 1× penicillin/streptomycin solution. 5000 HEL cells were pre-incubated in assay buffer containing 5 mM Hepes, 0.05% BSA, and compound for 15 min at 37 °C in a 384-well plate (Corning # 3710). 0.01 μM of Iloprost was added to stimulate cAMP production and the cells were again incubated for 15 min at 37 °C. Assessment of agonist activity was measured in the absence of Iloprost. cAMP signal was detected using a LANCE™ cAMP 384 assay kit and the EnVision™ multilabel counter, from Perkin-Elmer. Data were normalized relative to maximum and minimum signal controls and the % maximum data were fit to the Michaelis–Menten equation to calculate IC_{50} values.
19. For liver microsome and Caco-2 assay conditions, see: Merritt, J. R.; Rokosz, L. L.; Nelson, K. H.; Kaiser, B.; Wang, W.; Stauffer, T. M.; Ozgur, L. E.; Schilling, A.; Li, G.; Baldwin, J. J.; Taveras, A. G.; Dwyer, M. P.; Chao, J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4107.
20. EP2—Regan, J. W.; Bailey, T. J.; Pepperl, D. J.; Pierce, K. L.; Bogardus, A. M.; Donello, J. E.; Fairbairn, C. E.; Kedzie, K. M.; Woodward, D. F.; Gil, D. W. *Mol. Pharmacol.* **1994**, *46*, 213; EP4—Honda, A.; Sugimoto, Y.; Namba, T.; Watabe, A.; Irie, A.; Negishi, M.; Narumiya, S.; Ichikawa, A. *J. Biol. Chem*. **1993**, *268*, 7759.
21. cAMP signaling was quantified in the presence of 560 pM (EP2) or 170 pM (EP4) PGE2 using a Homogeneous Time Resolved Fluorescence (HTRF) CIS-US cAMP kit, cAMP *dynamic* 2, from CIS-US (Bedford, MA) according to the manufacturer's instructions.