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Benzylamide antagonists of protease activated receptor 2 with anti-inflammatory activity

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ABSTRACT

Activation of protease activated receptor 2 (PAR2) has been implicated in inflammatory and metabolic disorders and its inhibition may yield novel therapeutics. Here, we report a series of PAR2 antagonists based on C-terminal capping of 5-isoxazolyl-L-cyclohexylalanine-L-isoleucine, with benzylamine analogues being effective new PAR2 antagonists. 5-Isoxazolyl-L-cyclohexylalanine-L-isoleucine-2-methoxybenzylamine (**10**) inhibited PAR2-, but not PAR1-, induced release of Ca^{2+} (IC_{50} 0.5 μM) in human colon cells, IL-6 and TNFα secretion (IC_{50} 1–5 μM) from human kidney cells, and was anti-inflammatory in acute rat paw inflammation (ED_{50} 5 mg/kg sc). These findings show that new benzylamide antagonists of PAR2 have anti-inflammatory activity.

Protease activated receptor 2 (PAR2) was the second member discovered of the protease activated receptors, a unique group of class A G protein coupled receptors.[1–4] PAR2 is expressed in immune and inflammatory cells including T-cells, monocytes, macrophages as well as in many types of cancer cells.[3–6] There has been some controversy about PAR2 activation being pro- and/or anti-inflammatory.[4,7] However PAR2 activation is clearly associated with inflammatory diseases such as arthritis[8,9] and inflammatory bowel disease,[10–12] stimulating growth and invasion of pro-inflammatory and cancer cells, producing TNF-α and IL-6, and inducing joint swelling, mechanical and thermal hyper-algesia.[9,13,14] Effective PAR2 antagonists could be beneficial for probing PAR2 function in vivo and for treating disease.

GPCR antagonists have often been derived from their corresponding endogenous agonists through minor changes.[15,16] 2f-LIGRLO-NH_2 (**1**) and GB110 (**2**) have been reported as equipotent PAR2 agonists (Fig. 1),[16,17] for example, in activating human colon adenocarcinoma (HT29) cells to release intracellular calcium (iCa^{2+}, EC_{50} 0.2 μM).[16] Compound **1** is unstable in vivo due to proteolysis, but the non-peptidic agonist **2** is a promising template for elaborating to a plasma stable PAR2 antagonist. We previously reported that replacement of the C-terminal primary amine in **2** with a morpholine group produced a weak antagonist (**3**, IC_{50} 57 μM, iCa^{2+}, HT29 cells, Fig. 1).[16]

Structure–activity relationship (SAR) studies on the PAR2 antagonist GB88 (**4**) suggested that the isoxazolyl, cyclohexyl-alanine and isoleucine residues were intolerant of other substitution for antagonist potency (unpublished results). Our PAR2 homology model (derived from ORL-1 receptor crystal structure 4EA3)[18] also suggested that the C-terminal spiro[indene-1,4′-piperidine] ring of **4** may be involved in a π-stacking interaction with the receptor in a hydrophobic pocket created by $Y156^{3.33}$ and $F300^{6.48}$. As the structures of **2** and **4** are similar, it is possible that the phenyl ring of **2** binds to the same hydrophobic pocket as the spiroindene phenyl ring of **4**. Furthermore, the benzylamine core of **2** replaces the piperidine of **4**, while presenting a phenyl ring closer to the amide linker than in **4**. The ready availability of benzylamine derivatives provided easy access to a focused library around the common core of **2**–**4**.

Truncation of agonist **2** by removing the piperidine methylamine gave the parent benzylamine derivative **5** (Table 1). Diverse functional groups were attached to the benzyl ring with varying electronic and steric properties. The benzylamine/alkylamine templates were either commercially available or synthesized in-house. The antagonist/agonist potency of all compounds on HT29 cells was assessed using an intracellular calcium (iCa^{2+}) mobilization assay. Compounds that showed agonist activity ~50% or less at 10 μM were screened for potential antagonist activity. Compound

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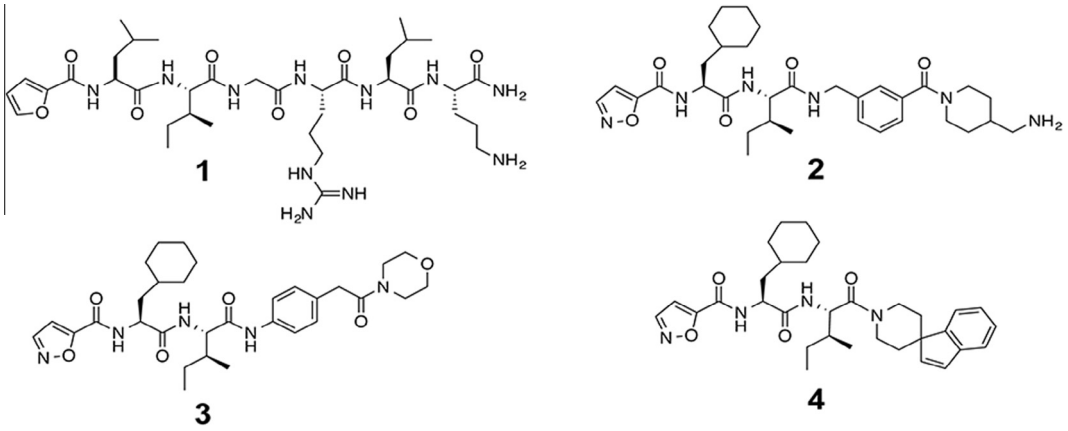
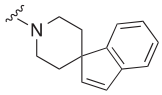
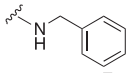
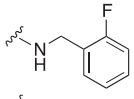
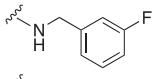
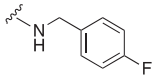
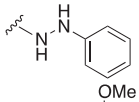
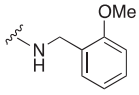
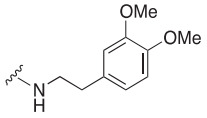
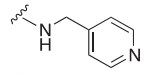
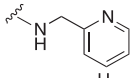
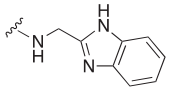
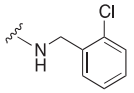
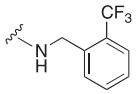


Figure 1. Chemical structures of PAR2 agonists (**1**, **2**) and antagonists (**3**, **4**).

Table 1
Ca^{2+} release induced or inhibited in HT29 cells by isoxazolyl-L-cyclohexylalanine-L-isoleucine-NR_1R_2

Entry	NR_1R_2	% Activation (at 10 μM)[a]	% Inhibition (at 1 μM)[b]
4		16	54
5		23	76
6		40	93
7		28	74
8		28	82
9		41	33
10		20	100
11		39	32
12		87	–
13		83	–
14		86	–
15		43	85
16		42	57

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