PAR2 ANTAGONISTS

Novel, selective, orally active small molecule antagonists (and agonists) of the human protease-activated receptor 2 (PAR2) have been developed for the potential treatment of inflammatory diseases, metabolic syndrome and cancer.

Applications

PAR2 antagonists have the potential to be developed as first-in-class treatments for:
- Arthritis
- Inflammatory Bowel Disease
- Psoriasis
- Obesity, diabetes and cardiovascular disease
- Cancer (breast, lung, colon, stomach, prostate)
- Pancreatitis

There is also the potential to develop PAR2 agonists to treat asthma, fibrosis and gastric ulcers. There are no PAR2 modulators on the market so this is a first-in-class opportunity.

Technology

Distributed widely throughout the body, the G protein coupled receptor, protease-activated receptor 2 (PAR2) has been implicated as a pro-inflammatory mediator in chronic inflammatory diseases including arthritis, psoriasis, inflammatory bowel disease, pancreatitis, and cardiovascular disease. PAR2 has also been reported as anti-inflammatory and protective in some conditions such as gastric ulcer and asthma. PAR2 activation has also been linked to proliferation, metastasis and angiogenesis in many cancers including cancers of the stomach, colon, breast and pancreas. In this context, small-molecule modulators of PAR2 are of potential interest as a new class of anti-inflammatory and pro-inflammatory, and/or anti-proliferative or proliferative agents.
Discovered in the 1990s, there are four PAR family members (designated PAR1, PAR2, PAR3 and PAR4). A defining feature of these receptors is their irreversible activation by proteases; mainly serine. In contrast with other GPCRs, PARs are not activated in vivo by binding of a soluble endogenous extracellular ligand but, instead, are triggered by proteases which cleave extracellularly the N-terminus of the receptor. This cleavage exposes a new amino terminus (tethered ligand; TL) that then binds intramolecularly to activate the receptor (self activation) and induces intracellular signal transduction (Figure opposite). PAR2 is emerging as a viable therapeutic target in a diverse range of diseases.

Short synthetic peptides corresponding to the newly exposed N-terminal tethered ligand can activate three of the four known PARs (PAR1, 2 and 4 and possibly PAR3) in the absence of proteases, and such PAR activating peptides (PAR-APs) have served as tools for agonist/antagonist development. In fact much of the evidence for involvement of PARs in diseases initially relied upon use of PAR-APs, often of low potency and uncertain selectivity. Use of PAR-APs is particularly informative in settings where more than one PAR is expressed and activated by the same protease, such as in human platelets where both PAR1 and PAR4 are expressed and activated by thrombin. There is now extensive knockout data in support of essential roles for PAR2 in disease.

Most PAR antagonists and agonists identified to date have been peptides corresponding to, or derived from, the TL sequence unique to each PAR. Using structure-activity approaches, PAR2-APs have been designed to mimic the natural TL sequence from the human (SLIGKV) and mouse (SLIGRL) receptors. Although some potent PAR2-APs have been developed, the typically poor bioavailability of these molecules is a major limitation for in vivo studies.

To improve drug-like properties, our research team has developed novel classes of non-peptidic PAR2 agonists and antagonists from hexapeptides. Using intracellular Ca\(^{2+}\) release as a measure of PAR2 activation, agonist GB110 (EC\(_{50}\) 0.28\(\mu\)M) was discovered. GB110 selectively activates PAR2 expressed by a range of cell lines. It is equipotent with the most potent synthetic peptide agonists reported for PAR2 as well as being selective for PAR2 over PAR1, and has no effect on PAR1 activation by thrombin or on intracellular Ca\(^{2+}\) release in cells desensitized to PAR2 activation.

Subsequently, our research team has identified a novel PAR2 antagonist, GB88 derived from the agonist GB110. It was the first PAR2 antagonist to reversibly inhibit activation of this receptor by endogenous ligands (e.g. trypsin, tryptase), synthetic peptides (e.g. SLIGRL-NH\(_2\), 2F-LIGRLO-NH\(_2\)) and non-peptide (e.g. GB110) agonists at low concentrations (IC\(_{50}\)~1\(\mu\)M). Furthermore, GB88 is stable in serum, orally active and has been investigated for PAR2 modulation in vivo in a range of animal models of human inflammatory and proliferative diseases. Administered orally GB88 inhibits rat paw oedema elicited by PAR2 agonists but not PAR1 agonists, and substantially inhibits collagen-induced arthritis in rats. The research team recently developed more potent, more PAR2-selective, chemically stable and orally bioavailable small molecule compounds that modulate PAR2, and have been evaluating them as agonists or antagonists in multiple human cells for pro-inflammatory, anti-inflammatory or proliferative activities. A range of novel agonists and antagonists have been identified with greater potency than GB110 or GB88 (EC\(_{50}\) or IC\(_{50}\) between 100 nM to 1\(\mu\)M) in multiple human cells (both cultured and primary cell types). Computer-assisted molecular modeling has been used to further refine antagonist series’ using homology models developed via
sequence alignment, X-ray crystallography and receptor mutations. The table below shows a series of PAR2 antagonists that are being explored in a range of disease models to exploit their therapeutic potential.

<table>
<thead>
<tr>
<th>PAR2 Antagonist</th>
<th>Mol. Wt</th>
<th>IC50 (µM, Ca2+ release)</th>
<th>PAR2 selective vs PAR1</th>
<th>In vivo activity (paw oedema, p.o.)</th>
<th>Stability – serum (min)</th>
<th>Stability – liver microsomes (min)</th>
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</thead>
<tbody>
<tr>
<td>GB88</td>
<td>546</td>
<td>1.1</td>
<td>Yes</td>
<td>10 &amp; 5 mg/kg</td>
<td>&gt;180</td>
<td>60</td>
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<td>AY117</td>
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<tr>
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<tr>
<td>KW007</td>
<td>341</td>
<td>5.2</td>
<td>Yes</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

**Selected PAR antagonists (GB series, AY series and KW series)**

We have demonstrated potent anti-inflammatory and anti-obesity activity for PAR2-selective antagonists in rat models of, for example, TNBS-induced colitis, collagen-induced arthritis and diet-induced obesity, metabolic and cardiovascular dysfunction including cardiac fibrosis.

**Proof of concept models**

In acute models of paw edema initiated by PAR2 agonists (trypsin, tryptase, peptide agonists, or non-peptide agonists), orally administered antagonists attenuate experimental paw edema in rats at doses of 5-10 mg/kg.

Selected compounds have been evaluated in rat models of chronic inflammatory disease, including collagen induced arthritis, inflammatory bowel disease, fibrosis and metabolic disease. The antagonists have shown potent anti-inflammatory activity in these models and exhibit superior efficacy (10-100 fold greater potency) to drugs currently used in the clinic. Compounds have been administered daily by oral gavage for up to 100 days in rats without any signs of adverse toxicity. There have been no indications of overt toxicities in rats or mice at doses up to 10 fold higher than efficacious doses.

**Inflammatory animal models**

GB88 has been shown to be orally active and anti-inflammatory in a 2h acute rat paw oedema model (see below), which was used as a rapid evaluation of in vivo selectivity and function. PAR2 knockout mice have been shown to be resistant to arthritis, and we have shown that GB88 inhibits collagen-induced arthritis in rats (see below). PAR2 ‘agonists’ 2fLIGRLO-NH2 and GB110 are pro-inflammatory in these assays. The team routinely uses these models and monitors paw swelling, inflammatory lesions (paws, ears, tail), pannus formation, synovial hyperplasia, collagen loss, immune cell infiltration to the joint, inflammatory cytokines in synovial fluid, tissue & serum, full histopathology profiles and effects on global human gene expression.
New, improved PAR2 antagonists have been designed and tested and have been shown to also be selective for PAR2 over PAR1, stable in rat plasma and effective in the acute inflammation model (paw oedema).

Examples of a few PAR2 antagonists that are stable in rat plasma (18, 30, 42, 44) and one (40) that is not. For reference the native PAR2 agonist peptide (SLIGRL-NH2) is also shown. Activity of different PAR2 antagonists in PAR2 induced rat paw oedema.

IBD

GB88 (10mg/kg/day p.o.) has been shown to reduce TNBS-induced disease-like symptoms (Fairlie, et al, The Journal of Pharmacology and Experimental Therapeutics, 340 (2), 256 – 265). GB88 was tested in a TNBS-induced model of colitis in Wistar rats. Disease progression (disease activity index (DAI), weight loss and mortality) and postmortem colonic histopathology (inflammation, bowel wall thickness and myeloperoxidase) were measured. Acute colonic inflammation induced in rates by a PAR2 agonist was inhibited by oral administration of GB88 (10mg/kg) with a marked reduction in edema, mucin depletion, PAR2 receptor internalization and mastocytosis. Chronis TNBS-induced colitis in rats was ameliorated by GB88 (10mg/kg/day, p.o.) with reduced mortality and pathology (including colon obstruction, ulceration, wall thickness, and myeloperoxidase release) than the clinically used drug sulfasalazine (100mg/kg/day, p.o.). The data generates strongly supports a disease-modifying roles for the PAR2 antagonists in inflammatory diseases of the colon.
PAR2 Antagonism: TNBS-Induced Colitis with GB88

GB88 reduces TNBS-induced disease-like symptoms. A and B; both GB88 (10mg/kg/day p.o. n=11) and sulfasalazine (100mg/kg/day p.o. n=12) treatments showed distinct improvements in DAI (A) and weight regain as observed in TNBS-control rats (n=11, 8). C; the mortality rate observed in TNBS-controls (55%) was lower when treated with GB88 (8.3%) than sulfasalazine (33.3%).

Metabolic dysfunction

PAR2 knockout mice are protected from weight gain, insulin resistance and adipose tissue macrophage inflammation induced by a high-fat diet. Rats fed a high carbohydrate high fat (HCHF) diet for 16 weeks and develop obesity, abdominal fat deposits, adipose inflammation, glucose/insulin intolerance, lipid abnormalities, liver and cardiovascular dysfunction (steatosis in liver, collagen deposits in left ventricle) relative to rats on a corn starch (CS) diet, GB88 (5 mg/kg/day p.o.), given weeks 8-16 prevents visceral fat deposition, reduces adipose inflammation, improves glucose/insulin tolerance, normalizes liver metabolism, cardiovascular remodeling and inhibits cardiac fibrosis and pancreatic dysfunction.

GB88 (5 mg/kg/day/p.o. Weeks 8-16) reduces body weight by 10% (left), abdominal fat (centre) and glucose intolerance (right) in Wistar rats on a HCHF vs CS diet over 16 weeks.

In summary, the researchers have designed several classes of small molecule modulators for the Protease Activated Receptor 2 (PAR2) and have demonstrated their general oral activity and efficacy in collagen-
induced arthritis in rats; TNBS-induced colitis (inflammatory bowel disease) in rats; ischemia-reperfusion injury in rats; diet-induced obesity, insulin and glucose tolerance and cardiovascular remodeling including cardiac fibrosis in rats. One PAR antagonist has been given orally at doses up to 10 mg/kg/day for over 100 days to rats without evidence of any adverse toxicology and with beneficial effects in diet-induced obesity and metabolic dysfunction.

**PK data**

PAR2 antagonists have been administered at 5 or 10 mg/kg single dose to Wistar rats with Tmax 4h, Cmax 2-8 µM, and can be detected in blood and adipose tissue after 24h. They are stable in rat plasma for several hours, with only traces of metabolites detected in LCMS analysis over 6-12h.

**Research Leader**

Professor David Fairlie is an Australian National Health & Medical Research Senior Principal Research Fellow and Head of the Division of Chemistry and Structural Biology at the Institute for Molecular Bioscience. Professor Fairlie’s research lies at the interface of chemistry, biology, and disease. The group works on the design, discovery, and characterisation of therapeutics for inflammatory and metabolic diseases, cancer and neurodegenerative conditions. Studies extend through computer modeling, solution and solid phase chemical synthesis, medicinal chemistry, NMR structure determination to biochemistry, protein-protein interactions, molecular and animal pharmacology. They study mechanisms of disease development and drug action.

**Intellectual Property**

We have filed a composition of matter patent application for PAR2 modulators (both agonists and antagonists) and uses thereof with a priority date of 28th July 2010 that has just entered national phase (in early 2013) in Australia, Europe, Japan and the United States. It has the following International Patent Number: WO 2012/012843

A second application which is directed to use of PAR2 antagonists for the treatment of metabolic syndrome (Type II diabetes and obesity), fibrosis and cardiovascular diseases was filed on 7th November 2011. It claims priority from the PCT application listed above and is currently in the PCT stage of patent prosecution. It has the following International Patent Number: WO 2013/013273
Key Publications

4. Adams, M., Ramachandran, R., Yau, M-K., Suen, J., Fairlie, D., Holllenberg, M., Hooper, J. (2011); Structure, function and pathophysiology of protease activated receptors; Pharmacology and Therapeutics, 130, 248-282 (a review summarizing structures of PAR agonists and antagonists)
5. Barry, G., Le, G., Fairlie, D. (2006); Agonists and antagonists of protease activated receptors (PARs); Current Medicinal Chemistry, 13, 243-265

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