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PATENT HIGHLIGHT

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Inhibitors of the PAR-2 Signaling Pathway May Treat Pain and Inflammation

Ahmed F. Abdel-Magid*

Therachem Research Medilab (India) Pvt. Ltd., Jaipur, India

Title:	Imidazopyridazines Useful as Inhibitors of the PAR-2 Signaling Pathway		
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Priority Application:	US 61/882,173	**Priority date:**	25 September 2013
	US 61/901,617		8 November 2013
Inventors:	Farmer, L. J.; Fournier, P.-A.; Lessard, S.; Liu, B.; St-Onge, M.; Sturino, C.; Szychowski, J.; Yannopoulos, C.		
Assignee Company:	Vertex Pharmaceuticals Inc.; 50 Northern Avenue, 15th Floor, Boston, MA 02210, USA		
Disease Area:	Inflammation and pain caused by inflammation, cancer, or injury	**Biological Target:**	Protease-Activated Receptor 2 (PAR-2) Signaling Pathway

Summary:

The invention in this patent application relates to novel imidazopyridazine derivatives represented generally by formula (I). These compounds are selective inhibitors of the PAR-2 signaling pathway and may potentially be used for the treatment of inflammation and nociception (pain) caused by inflammation, cancer, or injury.

Protease-activated receptors 1, 2, 3, and 4 (PARs-1, -2, -3, and -4) constitute a family of G-protein coupled receptors (GPCRs). They are activated by serine proteases (such as thrombin or trypsin) via cleaving a portion of their N-terminal region to expose a region of the N-terminal extracellular domain called the "tethered ligand." It is believed that the tethered ligand binds to residues contained within a second extracellular loop of the PAR receptor to stabilize an active conformation. Several short peptides mimicking this tethered ligand sequence were successfully synthesized and used to activate PARs-1, -2, and -4 but not PAR-3.

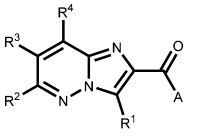
The protease-activated receptor 2 (PAR-2) is important in mediating inflammation, pain, and itch. It is activated by several host and pathogen-derived serine proteases, including trypsin, mast cell tryptase, tissue kallikreins, and members of the coagulation cascade TF-FVIIa and FVa-FXa. Additionally, synthetic peptide agonists such as Ser-Leu-Ile-Gly-Lys-Val-NH_2 (SLIGKV-NH_2) and 2-furoyl-Leu-Ile-Gly-Arg-Leu-Orn-NH_2 (2-fuoryl-LIGRLO-NH_2) can selectively activate PAR-2.

PAR-2 activation has been implicated in mediating neurogenic inflammation, nociception, and in transmission of pain. The administration of PAR-2 activating proteases and synthetic agonists *in vivo* is reported to induce inflammatory responses. Studies have shown that both direct activation of PAR-2 on nerve endings and indirect effects of PAR-2 on resident cells including keratinocytes play roles in pruritus and contribute to itch.

In vitro and *in vivo* studies have demonstrated that activation of PAR-2 plays a role in tissue remodeling. It promotes fibroblast and myofibroblast proliferation, and the secretion of growth factors such as CTGF and extracellular matrix components including collagen. PAR-2 activation was also implicated in cellular migration that may promote tumor growth and metastasis. There is also evidence that links the activation of PAR-2 to the pathophysiology of a variety of disorders including asthma, chronic pain, rheumatoid arthritis, periodontitis, inflammatory bowel diseases, irritable bowel syndrome, skin diseases, cancer, fibrotic diseases, and neurological disease. Other studies have shown that PAR-2 antagonism attenuates some disorders that correlate with PAR-2 expression such as diet-induced obesity, adipose inflammation, and metabolic dysfunction.

The inhibition of the PAR-2 signaling pathway is thus a feasible biological target for the treatment of inflammation and nociception resulting from different disorders. The above-mentioned data emphasize the need to develop potent and selective inhibitors of PAR-2 such as the compounds described in this patent application as possible effective treatment for these conditions.

Important Compound Classes:



Formula (I)

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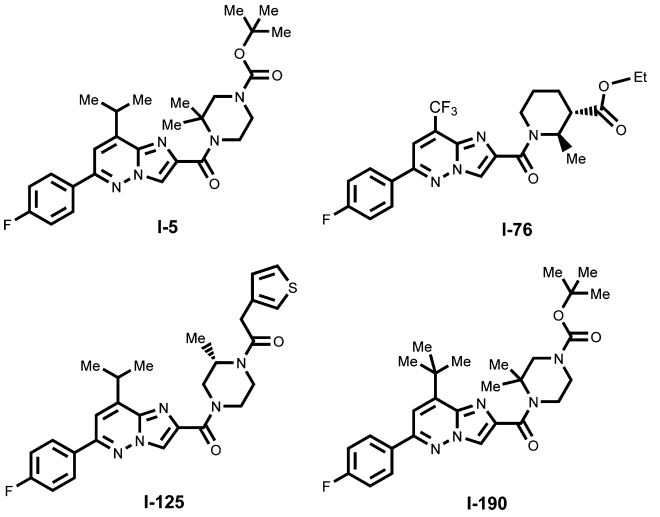
Key Structures: The inventors mentioned 591 compounds of formula (I) and listed the specific structures of 577 compounds including the following four representative examples:



Biological Assay: The following biological assays were described for testing the compounds of formula (I):

1. Protocol for testing PAR-2 compounds in ANTAGONIST mode using the Ca FLIPR^{TETRA} assay (384 well)
2. Protocol for testing PAR-2 compounds *in vivo* rat pharmacokinetics experiments
3. *In vivo* pharmacological evaluation of PAR-2 pathway inhibitor subjects and housing
4. Rat carrageenan-induced paw edema model
5. Rat tryptase-induced mechanical hypersensitivity model
6. Mouse TNBS-induced colitis model

Biological Data: The inventors reported biological data from all the six assays mentioned above for many of the examples of formula (I). The data obtained from testing the above four representative examples using assay 1 are listed in the following table. The assay determines the inhibition of either synthetic activators of PAR-2 such as SLIGKV-NH_2 or protease-dependent activators such as trypsin.

Compound	SLIGKV IC_{50} (μM)	Trypsin IC_{50} (μM)	Thrombin IC_{50} (μM)	UTP IC_{50} (μM)
I-5	0.002	0.005	51	97
I-76	3.3	4.8	5.5	4.4
I-125	0.002	0.005	>0.5	>0.5
I-190	0.0042	0.0048	>50	>50

Recent Review Articles:

1. Kularathna, P. K.; Pagel, C. N.; Mackie, E. J. *Int. J. Biochem. Cell Biol.* **2014**, *57*, 149−156.
2. Bao, Y.; Hou, W.; Hua, B. *Expert Opin. Ther. Targets* **2014**, *18* (1), 15−27.
3. Yau, M.-K.; Liu, L.; Fairlie, D. P. *J. Med. Chem.* **2013**, *56* (19), 7477−7497.

AUTHOR INFORMATION

Corresponding Author

*Address: 1383 Jasper Drive, Ambler, Pennsylvania 19002, United States. Tel: 215-913-7202. E-mail: afmagid@comcast.net.

Notes

The authors declare no competing financial interest.