Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5552–5557

A refined agonist pharmacophore for protease activated receptor 2

Grant D. Barry, Jacky Y. Suen, Heng Boon Low, Bernhard Pfeiffer, Bernadine Flanagan, Maria Halili, Giang T. Le and David P. Fairlie*

Centre for Drug Design and Development, Institute for Molecular Bioscience, University of Queensland, Brisbane, Qld. 4072, Australia

Received 28 June 2007; revised 10 August 2007; accepted 13 August 2007
Available online 16 August 2007

Abstract—Protease activated receptor 2 (PAR_2) is a G protein-coupled receptor implicated in inflammation and cancer. Only a few peptide agonists are known with greater potency than the native agonist SLIGRL-NH_2. Here we report 52 peptide agonists of PAR_2, 26 with activity at sub-micromolar concentrations, and one being iodinated for radioligand experiments. Potency was highest when the N- or C-termini of SLIGRL-NH_2 were modified, pointing to a new ligand pharmacophore model that may aid development of drug-like PAR_2 modulators.

Protease activated receptor-2 (PAR_2) belongs to an unusual family of G protein-coupled receptors (GPCRs) consisting of PAR 1 through 4 distinguished by their unique mechanism of activation. PARs are not yet known to be directly activated by any extracellular endogenous ligands, but are cleaved by (mainly serine) proteases at a specific site in the extracellular amino terminus which exposes a new N-terminus. Intramolecular binding of this newly formed N-terminus back onto conserved regions of the receptor adjacent to extracellular loops (and possibly to transmembrane helices) results in activation of PAR_2. Among proteases known to activate PAR_2 are trypsin, tryptase, cathepsin G, factor Xa, but not thrombin.[1]

PAR_2 is widely expressed on many cell types and has pro-inflammatory and proliferative functions.[2–4] In endothelial cells PAR_2 is upregulated in response to inflammatory mediators such as interleukin-1 and TNFα.[5] Increased PAR_2 expression in proliferating myofibroblasts and smooth muscle cells has been associated with tissue injury.[6] PAR_2 appears to be involved in nociception,[7] and neuroinflammation,[8] has been linked to tumor progression and fibrosis via upregulation of IL-8 in pancreatic cancer,[9] and contributes to migratory and invasive functions in breast cancer cells.[10–13] Paradoxically, PAR_2 is also thought to be anti-inflammatory in some cases, being bronchoprotective in the respiratory tract via the PAR_2-prostaglandin E_2-prostanoid EP receptor axis,[11] and anti-inflammatory in mouse models of pancreatitis where it suppresses pancreatitis-related abdominal hyperalgesia.[14]

Regulation of PAR_2 in various disease models is generally accomplished by one or more of the following methods; the use of PAR_2 knockout mice,[15] serine proteases,[16] or more commonly the application of fairly weak peptide agonists[17–19] which, although selective for PAR_2 over PAR_1, are now known to be promiscuous in their recognition of other receptors.[20–22] Thus results implicating PAR_2 in mammalian physiology need to be interpreted carefully, since such peptides at high concentrations may be acting through non-PAR receptors. An example where caution is needed in interpreting PAR_2 involvement is the report of a very weak (mM) nonpeptide PAR_2 antagonist[23] that dose-dependently attenuated joint inflammation in a mouse model of arthritis. There is clearly a need for new agonists and antagonists with much higher potency and selectivity.

To date most agonists developed for PAR_2 have relied upon modifications to short peptides corresponding to the newly exposed N-terminus of the receptor created following cleavage by serine proteases. These PAR_2–Activating Peptides (PAR_2–APs) include the human (SLIGKV-NH_2) and murine (SLIGRL-NH_2) sequences (EC_{50} 1–12 μM). There have been relatively few SAR studies leading to agonists with improved potency or selectivity for PAR_2 over PAR_1.[24] The most potent ago-

Keywords: PAR_2; Agonist; PAR_1; Fluorescence; Inflammation.
* Corresponding author. Tel.: +61 7 3346 1989; fax: +61 7 3346 1990; e-mail: d.fairlie@imb.uq.edu.au

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.08.026

nist reported for PAR_2 has a furoyl moiety replacing serine at the N-terminus (2-furoyl-LIGRL-NH_2)[25] and is ~10-fold more potent than SLIGRL-NH_2 for PAR_2 and selective over PAR_1. Herein we report a series of potent PAR_2 agonists derived through different modifications within SLIGRL-NH_2 but all amidated at the C-terminus.

Peptides were synthesized via standard Fmoc chemistry on Rink-amide MBHA resin. Amino acids were obtained commercially except 4-nitrophenoxy-alanine, synthesized from serine on resin via a Mitsunobu reaction with *p*-nitro phenol using triphenyl phosphine and diisopropyl azo-dicarboxylate in anhydrous THF. Peptides were cleaved from resin (TFA) and purified by rpHPLC to >95% purity. All compounds gave satisfactory ^1H NMR and electrospray mass spectral data. Calcium release was monitored from intact human embryonic kidney cells (HEK-293) or colon carcinoma cells (HT-29), incubated overnight in a 96-well clear-bottomed black-walled assay plate (Corning). Adhered cells were pre-treated 1 h before assay by incubating with fluorescent calcium indicator Fluo-3-AM (Bioscientific), washed with buffer before adding 100 μL HBBS buffer (probenecid, Hepes, pH 7.4). Calcium efflux was measured (excitation 495 nm, emission 520 nm) from the bottom of the plate for 60 s using a Polarstar (BMG LABTECH) fluorescent plate reader, with agonist delivered after 10 s.[26]

In studies on structure–activity relationships of SLIGRL-NH_2 (EC_{50} 4.2 μM), we found that adding a seventh residue to the C-terminus increased agonist potency as measured by intracellular calcium efflux. Notably, aromatic side-chains like tyrosine (**1**, EC_{50} 1.2 μM), homophenylalanine (**2**, EC_{50} 1.0 μM), 1-naphthylalanine, (**3**, EC_{50} 1.1 μM), 4-fluorophenylalanine, (**4**, EC_{50} 2.0 μM), and homotyrosine (**5**, EC_{50} 0.6 μM) improved activity by 2- to 6-fold. Replacing aromatic side-chains with the small aliphatic substituent of leucine (**6**, EC_{50} 1.2 μM) maintained potency, isoleucine increased potency further (**7**, EC_{50} 0.7 μM), and the positive charged ornithine (**8**, EC_{50} 1.2 μM) had little further influence. In contrast, the bulkier cyclohexylalanine (**9**, EC_{50} 3.6 μM) led to a several fold decrease in potency. These results indicated that addition of a seventh residue at the C-terminus of SLIGRL-NH_2 is beneficial, producing agonists with sub-micromolar agonist potency (Fig. 1).

We hypothesized that PAR_2 might similarly tolerate bulkier residues at position 6 of the C-terminus of seven-residue peptides. A small compound library designed to probe electronic versus steric effects of aryl and alkyl substitutions in the active heptapeptide **7** (Table 1) shows this to be the case, even for the different cell line, HT-29 (human carcinoma cells). Substitution of leucine at position 6 in compound **7** with phenylalanine (**10**), homophenylalanine (**11**), 4-pyridylalanine (**12**) or 3-pyridylalanine (**13**) had only a marginal effect on activity. But both electron-rich (tyrosine **14)** and electron-poor (4-nitro-phenylalanine **15**) substitutions increased potency (EC_{50} 0.3–0.4 μM), suggesting that steric rather than electronic effects may be important determinants of agonist activity. The potencies of **14** and **15** are comparable to that of the most active reported PAR_2 agonist, the furan capped PAR_2–AP. Other structurally diverse substitutions at the para position of phenylalanine, including cyano, chloro, *tert*-butyl, and iodo, resulted in good agonist potency (**16**–**19**).

Space filling or charge modifying substituents in Table 1 like 4-azido (**20**), 4-diethylphosphonomethyl (**21**), 4-car-

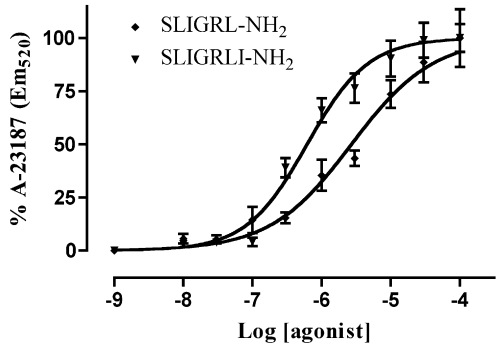
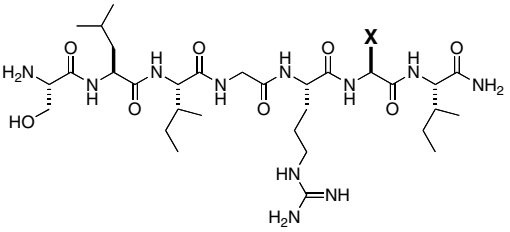


Figure 1. Concentration curves derived from calcium release assays performed on whole HEK-293 cells. Each data point represents mean ± SEM. ♦SLIGRL-NH_2 $n = 7$, ▼SLIGRLI-NH_2 $n = 5$.

Table 1. Intracellular Ca^{2+} release in HT-29 cells by C-terminal amidated heptapeptides SLIGR-X-I-NH_2, with a varying residue X



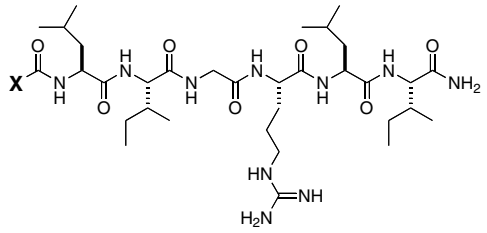
Compound	X	EC_{50}[a] (μM)
7	Leu	0.7 ± 0.06 (3)
10	Phe	1.0 ± 0.03 (2)
11	HomoPhe	1.8 ± 0.4 (3)
12	4-Pyridyl-Ala	1.9
13	3-Pyridyl-Ala	1.1 ± 0.2 (2)
14	Tyr	0.4 ± 0.1 (2)
15	4-Nitro-Phe	0.4 ± 0.1 (3)
16	4-Cyano-Phe	0.9 + 0.05 (3)
17	4-Chloro-Phe	0.5 ± 0.1 (2)
18	4-*t*-Butyl-Phe	0.6 ± 0.03 (3)
19	4-Iodo-Phe	0.6 ± 0.05 (3)
20	4-Azido-Phe	2.5
21	4-Diethylphosphonomethyl-Phe	1.3
22	4-COOH-Phe	5.0
23	Homo-Tyr	3.0
24	4-Nitrophenox-Ala	2.8
25	3-Nitro-Tyr	1.4 ± 0.3 (2)
26	3,4-Dichloro-Phe	0.3 ± 0.03 (3)
27	3,4-Dimethoxy-Phe	1.3 ± 0.01 (2)
28	3,5-Dinitro-Tyr	1.4 ± 0.01 (2)
29	3-Methoxy Phe	3.3
30	3-Cyano-Phe	1.7
31	3-Benzothienyl-Ala	0.3 ± 0.05 (2)

[a] Data from Ca^{2+} release assay. SEM is shown with *n* in parentheses.

boxy (**22**) reduced potency and rendered the compounds only as effective as SLIGRL-NH_2. Extending the aromatic side chain with a methylene bridge (**23**) or a phenoxy group (**24**) had a negative impact on potency (**14** vs **23**; **15** vs **24**), conceivably due to steric restraints within the binding pocket. Unlike the para position, meta substituents were not particularly favorable for agonist activity. Apart from the 3,4-dichloro derivative (**26**), meta-substituted aromatic side chains were 2- to 10-fold less potent than Tyr or *p*-nitro-Phe congeners, with the 3-methoxy (**29**) or 3-cyano rather than 4-cyano (**30** vs **16**) being less active. Results support the hypothesis that the most important role of these side-chains is to fill an implied space in the binding pocket of the receptor for these activating peptides, with steric factors being dominant over electronic factors in determining binding affinity. Consistent with this notion, both aliphatic leucine **7** and bicyclic benzothienyl **31** were effective side chains on the sixth residue, yielding agonists with nanomolar potency. The most active compounds (**14**, **15**, **26**, **31**) in this series were equipotent with the best agonist reported to date (2-furoyl-LIGRL-NH_2).

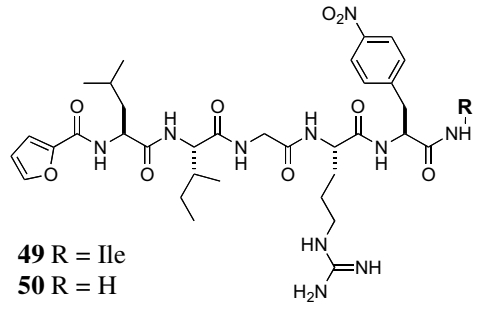
Turning to the N-terminus, where serine has been shown to be important for agonist potency, and substitution with other amino acids or acetylation of the N-terminus reportedly results in decreased activity.[27] Among few reported changes at this position have been unnatural amino acids, a *trans*-cinnamoyl moiety that was found to be equipotent to serine, and a furoyl group that improved potency by a factor of ten.[26,28] No structure–activity relationship studies have been reported in which systematic substitutions of 2-furoyl or cinnamoyl groups have been made. To investigate requirements of the likely binding pocket for 2-furoyl or cinnamoyl moieties, we constructed a library of agonist peptides based on **7** with nonpeptidic aromatic and aliphatic groups replacing serine (Table 2). This study revealed a number of substitutions capable of eliciting similar results to the furan moiety, with bulky bicyclic moieties such as benzofuran (**33**), naphthalene (**34**), benzothiophene (**35**), and indole (**36**) all tolerated and displaying activity comparable to substitution with furan. However, the similarly bulky 1-isoquinoline (**37**) and biphenyl (**38**) substituents were poorly tolerated, perhaps because of the ortho-orientation of the aromatic group in the former and the size of the latter. Simple aliphatic substituents like the bulky cyclohexyl (**39**) or the smaller isobutyl (**40**) were ineffective replacements for serine at position 1, whereas the phenyl substituent in a benzoyl cap (**41**) was tolerated.

Table 2. Intracellular Ca^{2+} release in HT-29 cells by N-terminal capped heptapeptides, X-LIGRLI-NH_2

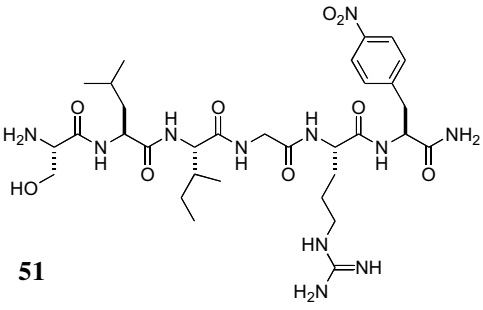


Compound	X	EC_{50}[a] (μM)
32	2-Furanyl	0.16 ± 0.03 (2)
33	2-Benzofuranyl	0.25
34	2-Naphthyl	0.36
35	2-Benzothienyl	0.37
36	2-Indolyl	0.59
37	1-Isoquinolyl	14.9
38	4-Biphenyl	15.8
39	Cyclohexyl	4.8
40	Isobutyl	10.3
41	Phenyl	0.80
42	2-Pyridyl	0.26
43	3-Pyridyl	0.22
44	2-Pyrazyl	0.29 ± 0.01 (2)
45	2-Hydroxy-phenyl	1.6
46	5-Isoxazolyl	0.22 ± 0.02 (3)
47	4-(2-Methyloxazolyl)	0.18 ± 0.004 (2)
48	3-[5-(4-Methylphenyl)isoxazolyl]	4.3

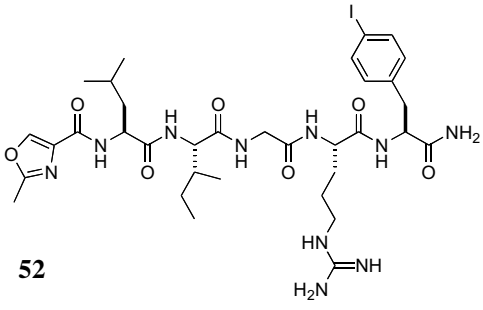
[a] Results from Ca^{2+} release assay. SEM is shown with *n* in parentheses.



49 R = Ile
50 R = H



51



52

Figure 2. Compound **49** features substitution of both serine and leucine **6** with furoyl and 4-nitro phenylalanine, respectively, (EC_{50} 0.2 μM). **50**, **51**: Truncation of seventh residue isoleucine of **49** and **15**, respectively (**50**: EC_{50} 0.17 μM; **51**: EC_{50} 0.6 μM). **52**: Substitution of serine for 4-(2-methyloxazole) and Leu[6] for 4-iodo phenylalanine yields EC_{50} 0.32 ± 0.04 μM.

Generally, most other monocyclic aromatic substituents were well tolerated (**42**–**44**, **46**, **47**), possibly because aromatic functionality is important for participating in π-stacking interactions in the receptor. Addition of a heteroatom to the aromatic ring improved potency; with little difference irrespective of whether the nitrogen was ortho (**42**) or meta (**43**) or both (**44**). Retaining the hydroxy substituent of serine and adding aromaticity, as in the 2-hydroxybenzoic acid (**45**) did not bring any significant advantage over serine. Maintaining the ring size of furan while adding an additional H-bond acceptor through 5-isoxazole carboxylic acid (**46**) and 4-(2-methyloxazole) carboxylic acid (**47**) gave ligands of equal potency to the furoyl substituent. Addition of further bulk to isoxazole with a 4-methylphenyl (**48**) was, similar to the biphenyl derivative (**38**), detrimental for agonist potency.

Finally, we report the concurrent replacement of the first (Ser) and sixth (Leu) residues of compound **7** with furoyl and 4-nitro phenylalanine, respectively, but there was no additive effect in **49** (Fig. 2), which was equipotent (EC_{50} 0.2 ± 0.03 μM) to **32**. Interestingly, truncating the C-terminal Ile residue of **49** to the N-capped pentapeptide (**50**) did not change agonist activity, whereas similar truncation of **15** led to a 2-fold decrease of potency (**51**). When serine in **19** was replaced by 4-(2-methyloxazole) carboxylic acid, activity was improved 2-fold, while truncating the C-terminal Ile was tolerated (**52**, EC_{50} 0.32 μM). The latter compound could potentially be used for a ligand affinity assay by radiolabeling with iodine.

We were able to correlate the above inductions of intracellular Ca^{2+} (Fig. 3) in HEK-293 and HT-29 cells with PAR_2 agonist activity using desensitization experiments. For example, Figure 4 shows desensitization experiments performed for compound **15**. On HT-29 cells, which have been shown to express both PAR_1 and PAR_2 receptors, peptides that have previously been reported to be selective for either PAR_1 or PAR_2 were used to desensitize the receptor, in that successive administration of either compound to the cells fails to cause Ca^{2+} release after the first addition and there is no cross-reaction. Thus Figure 4a demonstrates that 5 min after treating cells with 133 μM of PAR_1 selective TFLLR-NH_2, no further response can be gained from PAR_1 activation by retreating the cells with additional PAR_1 selective agonist. However, treatment of the cells after a 5-min interval with the PAR_2 selective agonist 2-furoyl-LIGRLI-NH_2 elicits a secondary, PAR_2 mediated, calcium response (Fig. 4b). Like 2-furoyl-LIGRLI-NH_2, compound **15** (Fig. 4c) is also capable of eliciting a response at 5 min. Furthermore, when the procedure is reversed and the cells are first treated and desensitized with the PAR_2 selective 2-furoyl-LIGRLI-NH_2 (Fig. 4d), compound **15** was incapable of producing a secondary calcium response (Fig. 4f), unlike the PAR_1 selective TFLLR-NH_2 (Fig. 4e). These results support selectivity for PAR_2 over PAR_1. It is worth noting that other GPCR agonists also cause calcium responses after pre-treatment with μM concentrations of either TFLLR-NH_2, 2-furoyl-LIGRLI-NH_2, or our new peptide agonists, supporting our assertion that the latter are PAR_2 agonists at sub-micromolar concentrations.

In conclusion, we have demonstrated that there are several means of improving activity for the PAR_2 activating peptide, SLIGRL-NH_2. Addition of a seventh residue at

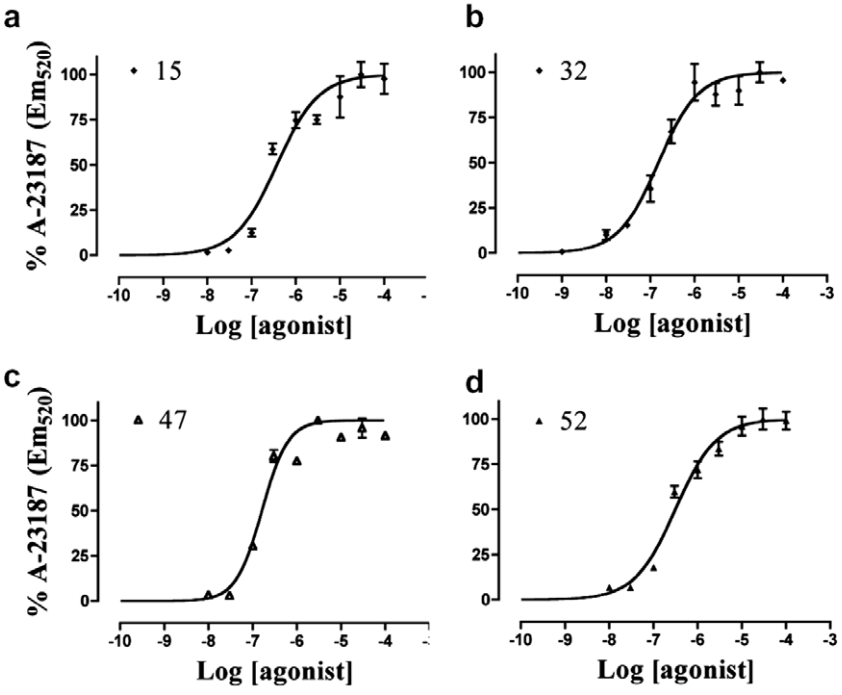


Figure 3. Concentration curves of **15**, **32**, **47**, and **52** derived from calcium release assays performed on whole HT-29 cells. Each data point represents mean ± SEM.

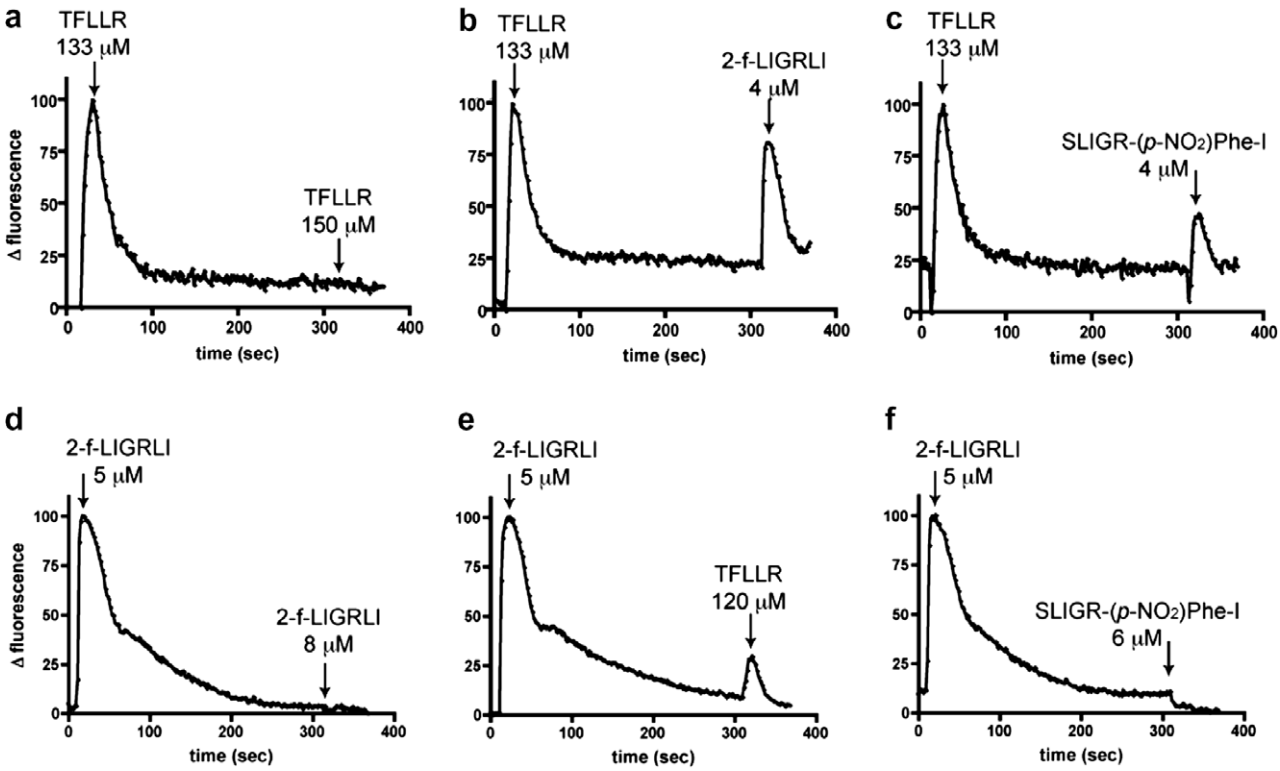


Figure 4. Desensitization curves for compound **15**. Cells were first treated with the selective PAR_1 agonist TFLLR-NH_2 (a–c) then either additional TFLLR-NH_2 (a) the selective PAR_2 agonist 2-furoyl-LIGRLI-NH_2 (b) or compound **15** (c) at 5 min. Conversely the cells were first treated with the PAR_2 agonist 2-furoyl-LIGRLI-NH_2 then with either additional 2-furoyl-LIGRLI-NH_2 (d) the PAR_1 agonist TFLLR-NH_2 (e) or compound **15** (f).

the C-terminus can enhance agonist activity via PAR_2 by at least 5- to 10-fold, with sub-micromolar potencies being observed in a calcium mobilization assay. Certain substituted aromatic residues at position 6 in the C-terminus of SLIGRLI-NH_2 confer substantial PAR_2 agonist activity, with nanomolar potencies for PAR_2 and selectivity over PAR_1 being obtained (e.g. **15**, **26**, **31**) without the need for capping the N-terminus with a furoyl or other ring. The results suggest that C-terminal residues at positions 6 and 7 of SLIGRLI-NH_2 may bind in a large hydrophobic pocket of the PAR_2 receptor, and that receptor-agonist interactions are predominantly steric in nature. We also found that steric fit and aromaticity are important characteristics of an N-terminal capping group used in place of the first residue serine to confer agonist potency. Apart from the already reported furoyl cap, other small aromatic moieties like isoxazole, oxazole, and pyridine are found to be excellent alternatives to serine at the N-terminus and can confer 10- to 20-fold increases in agonist potency of PAR_2 activating peptides. Combining these advances, one can introduce 4-iodo-phenylalanine at position 6 and a small heterocyclic substitute for serine at position 1 to produce a potent PAR_2 activating peptide that is suitable for radiolabeling with iodine for binding assays. Compounds described herein are among the most potent and selective PAR_2 agonists known ($EC_{50} \sim 160$–200 nM), and importantly provide valuable new information for development of potent, more drug-like modulators of PAR_2.

Acknowledgments

We thank the National Health and Medical Research Council and the Australian Research Council for supporting the research and researchers.

Supplementary data

Details of chemical synthesis, compound characterization (high resolution mass spectral data, HPLC retention times and traces, selected NMR spectral data), and the assay method are provided as supporting material (19 pages). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.08.026.

References and notes

1. Macfarlane, S. R.; Seatter, M. J.; Kanke, T.; Hunter, G. D. R. P. *Pharmacol. Rev.* **2001**, *53*, 245.
2. Takizawa, T.; Tamiya, M.; Hara, T.; Matsumoto, J.; Saito, N.; Kanke, T.; Kawagoe, J.; Hattori, Y. *J. Pharmacol. Sci.* **2005**, *98*, 99.
3. De Campo, B. A.; Henry, P. J. *Br. J. Pharmacol.* **2005**, *144*, 1100.
4. Ferrell, W. R.; Lockhart, J. C.; Kelso, E. B.; Dunning, L.; Plevin, R.; Meek, S. E.; Smith, A. J. H.; Hunter, G. D.; McLean, J. S.; McGarry, F.; Ramage, R.; Jiang, L.; Kanke, T.; Kawagoe, J. *J. Clin. Invest.* **2003**, *111*, 35.

5. Vergnolle, N. *Drug Dev. Res.* **2003**, *59*, 375.
6. Damiano, B. P.; D'Andrea, M. R.; de Garavilla, L.; Cheung, W. M.; Andrade-Gordon, P. *Thromb. Haemost.* **1999**, *81*, 808.
7. Hoogerwerf, W. A.; Zou, L.; Shenoy, M.; Sun, D.; Micci, M. A.; Lee-Hellmich, H.; Xiao, S. Y.; Winston, J. H.; Pasricha, P. J. *J. Neurosci.* **2001**, *21*, 9036.
8. Duryea, J.; Neumann, G.; Brem, M. H.; Koh, W.; Noorbakhsh, F.; Jackson, R. D.; Yu, J.; Eaton, C. B.; Lang, P. *Osteoarthritis Cartilage* **2006**.
9. Ikeda, O.; Egami, H.; Ishiko, T.; Ishikawa, S.; Kamohara, H.; Hidaka, H.; Takahashi, M.; Ogawa, M. *Int. J. Oncol.* **2006**, *28*, 939.
10. Shimamoto, R.; Sawada, T.; Uchima, Y.; Inoue, M.; Kimura, K.; Yamashita, Y.; Yamada, N.; Nishihara, T.; Ohira, M.; Hirakawa, K. *Int. J. Oncol.* **2004**, *24*, 1401.
11. Henry, P. J. *Eur. J. Pharmacol.* **2006**, *533*, 156.
12. Hjortoe, G. M.; Petersen, L. C.; Albrektsen, T.; Sorensen, B. B.; Norby, P. L.; Mandal, S. K.; Pendurthi, U. R.; Rao, L. V. *Blood* **2004**, *103*, 3029.
13. Ge, L.; Shenoy, S. K.; Lefkowitz, R. J.; DeFea, K. *J. Biol. Chem.* **2004**, *279*, 55419.
14. Kawabata, A.; Matsunami, M.; Tsutsumi, M.; Ishiki, T.; Fukushima, O.; Sekiguchi, F.; Kawao, N.; Minami, T.; Kanke, T.; Saito, N. *Br. J. Pharmacol.* **2006**, *148*, 54.
15. Uusitalo-Jarvinen, H.; Kurokawa, T.; Mueller, B. M.; Andrade-Gordon, P.; Friedlander, M.; Ruf, W. *Arterioscler. Thromb. Vasc. Biol.* **2007**, *27*, 1456.
16. Cottrell, G. S.; Amadesi, S.; Pikios, S.; Camerer, E.; Willardsen, J. A.; Murphy, B. R.; Caughey, G. H.; Wolters, P. J.; Coughlin, S. R.; Peterson, A.; Knecht, W.; Pothoulakis, C.; Bunnett, N. W.; Grady, E. F. *Gastroenterology* **2007**, *132*, 2422.
17. Miles, R. D.; Butcher, G. D.; Henry, P. R.; Littell, R. C. *Poult Sci.* **2006**, *85*, 476.
18. Wang, H.; He, S. *Cell Biol. Int.* **2006**, *30*, 688.
19. Caruso, R.; Pallone, F.; Fina, D.; Gioia, V.; Peluso, I.; Caprioli, F.; Stolfi, C.; Perfetti, A.; Spagnoli, L. G.; Palmieri, G.; Macdonald, T. T.; Monteleone, G. *Am. J. Pathol.* **2006**, *169*, 268.
20. Abey, H. T.; Fairlie, D. P.; Moffatt, J. D.; Balzary, R. W.; Cocks, T. M. *J. Pharmacol. Exp. Ther.* **2006**, *317*, 598.
21. Moffatt, J. D. *Br. J. Pharmacol.* **2004**, *143*, 441.
22. Stenton, G. R.; Nohara, O.; Dery, R. E.; Vliagoftis, H.; Gilchrist, M.; Johri, A.; Wallace, J. L.; Hollenberg, M. D.; Moqbel, R.; Befus, A. D. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 466.
23. Kelso, E. B.; Lockhart, J. C.; Hembrough, T.; Dunning, L.; Plevin, R.; Hollenberg, M. D.; Sommerhoff, C. P.; McLean, J. S.; Ferrell, W. R. *J. Pharmacol. Exp. Ther.* **2006**, *316*, 1017.
24. Barry, G. D.; Le, G. T.; Fairlie, D. P. *Curr. Med. Chem.* **2006**, *13*, 243.
25. Kanke, T.; Ishiwata, H.; Kabeya, M.; Saka, M.; Doi, T.; Hattori, Y.; Kawabata, A.; Plevin, R. *Br. J. Pharmacol.* **2005**.
26. Kawabata, A.; Kanke, T.; Yonezawa, D.; Ishiki, T.; Saka, M.; Kabeya, M.; Sekiguchi, F.; Kubo, S.; Kuroda, R.; Iwaki, M.; Katsura, K.; Plevin, R. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 1098.
27. Maryanoff, B. E.; Santulli, R. J.; McComsey, D. F.; Hoekstra, W. J.; Hoey, K.; Smith, C. E.; Addo, M.; Darrow, A. L.; Andrade-Gordon, P. *Arch. Biochem. Biophys.* **2001**, *386*, 195.
28. Vergnolle, N.; MacNaughton, W. K.; Al-Ani, B.; Saifeddine, M.; Wallace, J. L.; Hollenburg, M. D. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 7766.