

Protease-Activated Receptor-2 Antagonists and Agonists

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Abstract: Interest in the development of specific antagonists of the protease-activated receptors are significant, however, achieving such goals remain extremely challenging. Considerable efforts have been directed at developing specific antagonists of the first elucidated member of this receptor family, namely the thrombin receptor, PAR-1. However, significantly less effort has been directed at the second member of the family, PAR-2 due in part to lack of clarity concerning its activating protease(s), and uncertainty concerning its physiological and pathophysiological roles in disease pathways. This review will briefly summarize what is known about the activating protease(s), the potential (patho)physiological roles for PAR-2 and structure-activity relationships that have been developed for PAR-2 agonists and antagonists in relationship to agonists and antagonists developed for the other protease-activated receptors.

INTRODUCTION

The protease-activated receptor family is one of the newest and more intriguing members of the larger G-protein coupled receptor family. The discovery and biology of this family has been extensively reviewed [1]. From the earliest days following the cloning of the first family member, PAR-1 there has been great interest in these receptors because the first member of the receptor family was the elusive thrombin receptor postulated to be present on platelets. This thrombin receptor appeared to be critical for signal transduction by thrombin, and thus played an important role in thrombosis and as a potential target for developing novel antithrombotic agents. Shortly after this discovery, the second family member, PAR-2 was discovered [2], and a search for its physiological activator(s) and therapeutic relevance ensued. Since then, the protease receptor family has grown to include PARs 1-4 with indications that additional members are likely to exist.

ACTIVATING PROTEASE (S)

Analysis of the amino terminus of the PAR-2 deduced sequence revealed a slightly shorter N-terminus than PAR-1 by 29 amino acids and the absence of the stretch of acidic residues in PAR-1 that appear to interact with thrombin. However, there was a clear serine protease cleavable site with the strong likelihood that trypsin could cleave and activate this receptor [2]. Consistent with this analysis, when expressed in *Xenopus* oocytes, PAR-2 could not be activated by thrombin, but was stimulated with low concentrations of trypsin ($EC_{50} = 1$ nM) [2]. In spite of these observations, the search for other relevant serine proteases capable of activating PAR-2 was initiated, since the receptor was found to be expressed in many other tissues outside of the small intestine, colon and stomach where trypsin could play a role as a physiological activator. Such a role for trypsin in tissues

other than the pancreas, intestine, colon and stomach have appeared unlikely to many, and this point is still somewhat controversial. Thus, while many other serine proteases have been investigated for their ability to activate PAR-2, no one enzyme is as potent as trypsin to date. Of those studied, - and -tryptases are strong candidates in tissues and cells where mast cells can deploy tryptase such as skin, intestine and lung [3,4,5,6,7,8,9,10,11].

Other proteolytic enzymes which have been investigated that can activate PAR-2 are the sperm enzyme acrosin [4], coagulation factors Xa or VIIa [12,13], a tissue factor-VIIa-Xa complex [14], a EPR-1 factor Xa complex [15] a brain-derived trypsin-like protease (P22) [16], pancreatic trypsin and trypsin-2 [17], gingipain-R from *Porphyromonas gingivalis* [18] and a membrane-type serine protease 1 [19]. Based on these studies, it is reasonable to assume that PAR-2 may not have a single protease responsible for activation, such as appears for PAR-1 and thrombin, but may rely on a variety of enzymes such as trypsin and tryptase in specific cells and tissues. One interesting observation related to protease activation is the occurrence of a polymorphic form of PAR-2 with a Phe²⁴⁰Ser mutation which renders this species ~3.7 fold less sensitive to activation by trypsin [20]. This observation is consistent with earlier observations that extracellular loop 2 of PAR-2 is critically important for interactions with its tethered ligand, where Phe²⁴⁰ resides [21,22], and that the reduced sensitivity to trypsin is likely to be related to tethered ligand interactions rather than decreased cleavage by trypsin.

TISSUE AND CELLULAR RESPONSES OF PAR-2

Since the molecular cloning of PAR-2, and early localization of the receptor in a variety of cell types and tissues [23], many groups have investigated the cellular and tissue responses of PAR-2 to agonist peptides and activating proteases. Of note have been numerous investigations in vascular tissues, gastrointestinal tissues, lung, bone, epidermis, salivary, leukocytes, kidney, glia, astrocytes, neurons, and the pancreas. (Table 1) lists studies of PAR-2 in various tissues, and the various effects mediated by proteases

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Table 1. Proteinase-activated Receptor-2 Tissue and Cellular Responses

Tissue or Cell Type	Effect	Agonist	References
Vascular			
Endothelial cells (HUVEC)	mitogenesis	SLIGRL/trypsin	Mirza <i>et al.</i> , 1996 [28]
	vWf release	SLIGRL/trypsin	Storck <i>et al.</i> , 1996 [29]
	stimulates coagulation/ -TF	SLIGKVD	Alm <i>et al.</i> , 1999 [30]
Smooth muscle cells (RASMC/BPAF)	MAP kinase activation	SLIGRL/trypsin	Belham <i>et al.</i> , 1996 [31]
Bovine coronary smooth muscle cells	mitogenesis	SLIGRL/trypsin	Bretschneider <i>et al.</i> , 1999 [32]
Rat aortic rings	relaxation	SLIGRL/trypsin	Saifeddine <i>et al.</i> , 1996 [33]
	relaxation	SLIGRL/analogs	Hollenberg <i>et al.</i> , 1996 [34]
	relaxation	SLIGRL/analogs	Hollenberg <i>et al.</i> , 1997 [35]
	relaxation	SLIGRL/trypsin	Vergnolle <i>et al.</i> , 1998 [36]
Rat femoral artery	relaxation	SLIGRLETQPPI	Emilsson <i>et al.</i> , 1997 [37]
Porcine pulmonary artery	relaxation	SLIGRL/trypsin	Glusa <i>et al.</i> , 1997 [38]
Human aortic smooth muscle cells	proliferation	SLIGKV/trypsin	Bono <i>et al.</i> , 1997 [39]
Rat hind paw	vascular permeability	SLIGRL-NH ₂	Kawabata <i>et al.</i> , 1998 [40]
	oedema and granulocyte infiltration	SLIGRL	Vergnolle <i>et al.</i> , 1999 [41]
	hyperalgesia	SLIGRL-NH ₂	Vergnolle <i>et al.</i> , 2001 [42]
Human umbilical vein rings	contraction	SLIGRL/trypsin	Saifeddine <i>et al.</i> , 1998 [43]
Rat basilar artery	vasodilatation	SLIGRL/trypsin	Sobey <i>et al.</i> , 1998 [44]
SHR and WKY basilar artery	vasodilation in SHR	SLIGRL/trypsin	Sobey <i>et al.</i> , 1999 [44]
Intact rat	hypotension	SLIGRL/SFLLRN	Cheung <i>et al.</i> , 1998 [46]
Intact rat / endotoxemic rat	hypotension	SLIGRL/trypsin	Cicala <i>et al.</i> , 1999 [47]
Rat mesenteric venules	leukocyte rolling	SLIGRL	Vergnolle <i>et al.</i> , 1999 [48]
Murine cremaster muscle	leukocyte rolling/ microvascular inflame	SLIGRL	Lindner <i>et al.</i> , 2000 [49]
Intact anesthetized rat	hypotension/hypertension	SLIGRL	Cicala <i>et al.</i> , 2001 [50]
Rat heart	ischemia-reperfusion injury	SLIGRL	Napoli <i>et al.</i> , 2000 [51]
Murine tracheal segments	relaxation	SLIGRL	Lan <i>et al.</i> , 2001 [52]
Perfused rat kidney	vasodilation of afferent arteriole	SLIGRL	Trottier <i>et al.</i> , 2002 [53]
Langendorff-perfused rat heart	cardiac inflammation, preconditioning	SLIGRL	Napoli <i>et al.</i> , 2002 [54]
Murine mesenteric arteriole rings	relaxation	SLIGRL/trypsin	McGuire <i>et al.</i> , 2002 [55]
Isolated perfused rat heart	EDHF dependent coronary vasodilatation	SLIGRL	McLean <i>et al.</i> , 2002 [56]
Gastrointestinal			
Rat gastric smooth muscle	contraction	SLIGRL	Al-Ani <i>et al.</i> , 1995 [57]
Murine gastric smooth muscle	contraction	SLIGRL/trypsin	Saifeddine <i>et al.</i> , 1996 [33]
Human intestinal epithelial cells	intracellular Ca ⁺⁺	SLIGRL/trypsin	Bohm <i>et al.</i> , 1996 [58]
Rat jejunum segments	active chloride transport	SLIGRL/trypsin	Vergnolle <i>et al.</i> , 1998 [36]
Rat small intestinal enterocytes	IPs, AA, PG secretion	SLIGRL/trypsin	Kong <i>et al.</i> , 1997 [59]

(Table 1) contd....

Tissue or Cell Type	Effect	Agonist	References
Myenteric plexus neurons of guinea-pig	[Ca ⁺⁺]I	SLIGRL/trypsin/tryptase	Corvera <i>et al.</i> , 1999 [7]
Human gastric carcinoma cells MKN-1	proliferation, adhesion to fibronectin	SLIGKV	Miyata <i>et al.</i> , 2000 [60]
Rat stomach and duodenum	gastric mucus secretion facilitation of pepsin(ogen) secretion	SLIGRL SLIGRL	Kawabata <i>et al.</i> , 2001a; [61] Kawao <i>et al.</i> , 2002 [62]
Intact rat	IP administrated GI transit	SLIGRL	Kawabata <i>et al.</i> , 2001b [63]
Murine and guinea pig GI smooth muscle	relaxation	SLIGRL	Cocks <i>et al.</i> , 1999 [64]
Murine TNBS mediated colitis	antiflammatory	SLIGRL-NH ₂	Fiorucci <i>et al.</i> , 2001 [65]
Guinea-pig gastric smooth muscle	contraction	SLIGRL/analogs	Saifeddine <i>et al.</i> , 2001 [66]
Rat colon	hyperalgesia	SLIGRL-NH ₂	Coelho <i>et al.</i> , 2002 [67]
Rat isolated colonic segments	contraction in circular muscle	SLIGRL-NH ₂	Mule <i>et al.</i> , 2002 [68]
Lung			
Rat trachea, intrapulmonary bronchi	relaxation	SLIGRK/trypsin	Chow <i>et al.</i> , 2000 [69]
Human lung fibroblast	proliferation	SLIGKV/SLIGRL/ tryptase	Akers <i>et al.</i> , 2000 [70]
Guinea-pig (intact)	bronchoprotection to histamine	SLIGRL	Cicala <i>et al.</i> , 2001 [71]
Human airway smooth muscle cells	Ca ⁺⁺ signaling Proliferation	SLIGRL/tryptase SLIGRL/tryptase	Berger <i>et al.</i> , 2001a [72] Berger <i>et al.</i> , 2001b [73]
Human bronchial rings	Contractions relaxation	SLIGRL/tryptase SLIRGL/trypsin	Chambers <i>et al.</i> , 2001 [74] Cocks <i>et al.</i> , 1999 [64]
Human respiratory epithelial cells	cytokine release/ intracellular Ca ⁺⁺	SLIGKV-NH ₂	Asokanathan <i>et al.</i> , 2002 [75]
Miscellaneous			
Human colon cancer cells	Proliferation intracellular Ca ⁺⁺	SLIGKV SLIGKV/trypsin	Darmoul <i>et al.</i> , 2001 [76] Ducroc <i>et al.</i> , 2002 [77]
Keratinocytes/dermal fibroblasts	intracellular Ca ⁺⁺ keratinocyte-melanocyte pigment regulation	Trypsin SLIGRL	Santulli <i>et al.</i> , 1995 [78] Seiberg <i>et al.</i> , 2000 [79]
Salivary and parotid glands	saliva and amylase secretion	tryptase/SLIGRL	Kawabata <i>et al.</i> , 2000 [80]

and agonist peptides. From these studies there is the possibility that PAR-2 plays novel role in cardiovascular function [24], gastrointestinal function [25], pulmonary [26] and dermal function and in inflammation [27].

STRUCTURE-ACTIVITY RELATIONSHIPS OF AGONISTS AND ANTAGONISTS

Three reasons exist for developing PAR-2 agonist SAR. First, since PAR-2 agonist ligands are relatively weak in potency ($EC_{50} > 1 \mu M$), it would be useful to have potent agonists to serve as pharmacological tools and to form the basis for a radioligand binding assay, which does not exist at the moment. Second, there is the potential to use this SAR

information to design novel antagonists of PAR-2, as has been previously done in the PAR-1 system [87]. Third, PAR-2 agonists have the potential utility as therapeutic agents where preliminary evidence suggests they could be used as airway bronchoprotectives [64], [85] and [86], [70], as anti-inflammatory agents to treat inflammatory bowel disease [65], [85], and as depigmentation agents [79].

Similar to the thrombin receptor PAR-1, early on it was recognized that synthetic peptides that utilize the cleaved receptor's amino terminus can function as PAR-2 agonists [2]. The first peptides prepared were based on the rodent and human sequences such as SLIGRL and SLIGKV. A number of different assay systems have been established for evaluating PAR-2 agonist peptide SAR including intact

vascular tissue preparations [57], [34], [41] *Xenopus* oocytes expressing PAR-2 [2], [82], in Kirsten virus-transformed rat kidney cells (KNRK) expressing rat PAR-2 [36], [81], in HEK293 cells expressing PAR-1 and PAR-2 capable of cross-desensitization [83] or finally utilizing immortalized murine PAR-1 (-/-) cells transfected with human PAR-2 [84].

As was previously shown with the PAR-1 agonist peptide SAR studies, full agonist activity appeared most potent in pentapeptide and hexapeptide sequences (Table 2). Fairly good agreement for agonist EC₅₀ values have appeared from these various studies of peptides, although the degree of quantitation appears to be greater in the immortalized PAR-1 (-/-) cell line transfected with human PAR-2 [84]. Similar to previous experience with PAR-1 agonist peptides, carboxyl-

Table 2. Proteinase-activated Receptor-2 Agonist Peptide SAR

Compound	Structure	EC ₅₀ (PAR-2) μM	EC ₅₀ (PAR-1) (μM)	K _i (μM)	References
Truncation					
1	LIGR-NH ₂	low ^a			Hollenberg (1996) [34]
2	SLIGR	~ 10 ^a			Hollenberg (1996) [34]
3	SLIGR-NH ₂	~8 ^d		2.3 ^b	Al-Ani (1999) [81]
4	SLIGRL	~5 ^b 2-5 ^a			Nystedt (1994) [2] Hollenberg (1996) [34]
5	SLIGRL-NH ₂	0.5-2.0 ^c 1-2 ^a 5 ^e 2-5 ^d 2-5 ^m 5.7 ^e	inactive ^f	0.26 ^h	Blackhart (1996) [82] Hollenberg (1996) [34] Hollenberg (1997) [35] Al-Ani (1999) [81] Vergnolle (1998) [36] Maryanoff (2001) [84]
6	SLIGKVD-NH ₂	1-5 ^c	>100		Blackhart (1996) [82]
7	SLIGKV-NH ₂	4-6 ^d 11.8 ^e 30-40 ^a	inactive ^f		Al-Ani (1999) [81] Maryanoff (2001) [84] Hollenberg (1997) [35]
Ala-Scan					
8	ΔLIGRL	>10 ^c	inactive ^f		Blackhart (1996) [82]
9	ΔLIGRL-NH ₂	22 ^e			Maryanoff (2001) [84]
10	SΔLIGRL	>100 ^c >100 ^a			Blackhart (1996) [82] Hollenberg (1996) [34]
11	SΔLIGRL-NH ₂	>300 ^e	>300 ^f		Maryanoff (2001) [84]
12	SLΔGRL	>100 ^c			Blackhart (1996) [82]
13	SLΔGRL-NH ₂	20 ^e >100 ^a	inactive ^f		Maryanoff (2001) [84] Hollenberg (1997) [35]
14	SLIARL	1-10 ^c	90 ^f		Blackhart (1996) [82]
15	SLIARL-NH ₂	5.8 ^e 10-20 ^a			Maryanoff (2001) [84] Hollenberg (1997) [35]
16	SLIGAL	10-100 ^c >100			Blackhart (1996) [82] Hollenberg (1996) [34]
17	SLIGAL-NH ₂	19 ^e	inactive ^f		Maryanoff (2001) [84]
18	SLIGRA	1-10 ^c			Blackhart (1996) [82]
19	SLIGRA-NH ₂	5.6 ^e	inactive ^f		Maryanoff (2001) [84]

^arat aorta relaxation assay; ^bCa²⁺ efflux assay using murine PAR-2 expressed in *X. laevis* oocytes; ^cCa²⁺ efflux assay using human PAR-2 expressed in *X. laevis* oocytes; ^dCa²⁺ signalling assay using rat PAR-2 expressed in KNRK cells; ^eCa²⁺ mobilization assay using human PAR-2 expressed in murine lung fibroblasts from PAR-1(-/-)mice; ^fCa²⁺ mobilization assay using human PAR-1 expressed in murine lung fibroblasts from PAR-1(-/-)mice; ^gHEK cell Ca²⁺ fluorescence receptor desensitization assay; ^hligand binding assay using [³H]-tc-LIGRLOm-NH₂ (29) in KNRK cells expressing rat PAR-2.

Table 3. Proteinase-activated Receptor-2 Agonist Peptide SAR

Compound	Structure	EC ₅₀ (PAR-2) (μ M)	EC ₅₀ (PAR-1) (μ M)	K _i (μ M)	References
Miscellaneous					
20	Ac-SLIGRL-NH ₂	inactive ^c inactive ^a inactive ^e	inactive ^c inactive ^f		Blackhart (1996) [82] Hollenberg (1997) [35] Maryanoff (2001) [84]
21	3-OH-Pr-LIGKV-NH ₂	23 ^c	inactive ^c		Maryanoff (2001) [84]
22	SFLLRNP-NH ₂	0.1-0.5 ^c	0.1 ^c		Blackhart(1996) [82]
23	SFLLRN-NH ₂	8 ^e	0.53 ^f		Maryanoff (2001) [84]
24	SFLLR-NH ₂	8.7 ^g 5-10 ^d	2.2 ^g	2.3 ^h	Kawabata (1999) [83] Al-Ani (1999) [81]
25	TFRIFD-NH ₂	inactive ^c inactive ^g inactive ^d	1 ^c		Blackhart (1996) [82] Hollenberg (1997) [35] Al-Ani (1999) [81]
26	TFLLR-NH ₂	540 ^g	2.5 ^g		Kawabata (1999) [83]
27	TLIGRL-NH ₂	100-200 ^g	inactive ^g		Hollenberg (1997) [35]
28	SFLLED-NH ₂	inactive ^g	~20 ^g		Hollenberg (1997) [35]
29	tc-LIGRLOm-NH ₂	2-5 ^d	inactive		Vergnolle (1998) [36]
30	Mpr-FChaChaRK- -PNDKY-NH ₂	33 ^g	17 ^g		Kawabata (1999) [83]
31	S-pFPheHarLRK-NH ₂	5-10 ^c	0.05-0.10 ^c		Blackhart (1996) [82]
32	S-pFPheCha- -ChaRK-NH ₂	2.0 ^e	0.11 ^f		Maryanoff (2001) [84]
33	SLIGFL-NH ₂	0.5-2.0 ^c	>50		Blackhart (1996) [82]
34	SLIA-pCIPhe-L-NH ₂	7.6 ^c	partial agonist		Maryanoff (2001) [84]

^arat aorta relaxation assay; ^bCa²⁺ efflux assay using murine PAR-2 expressed in *X. laevis* oocytes; ^cCa²⁺ efflux assay using human PAR-2 expressed in *X. laevis* oocytes; ^dCa²⁺ signalling assay using rat PAR-2 expressed in KNRK cells; ^eCa²⁺ mobilization assay using human PAR-2 expressed in murine lung fibroblasts from PAR-1(-/-)mice; ^fCa²⁺ mobilization assay using human PAR-1 expressed in murine lung fibroblasts from PAR-1(-/-)mice; ^gHEK cell Ca²⁺ fluorescence receptor desensitization assay; ^hligand binding assay using [³H]-tc-LIGRLOm-NH₂ (29) in KNRK cells expressing rat PAR-2.

terminal amidation of the peptides appears to afford slightly more potent agonists [82], [35]. Removal of the N-terminal serine residue from the SLIGR-NH₂ (3) sequence leads to (1) and a dramatic reduction in agonist potency, yet analog (1) still retains a low level of measurable agonist activity [34]. Of interest is the observation that SLIGRL-NH₂ (5) is inactive against PAR-1 [82], [35], [84]. Thus, the first five amino acids of the newly exposed N-terminus following protease cleavage appear required for full potent agonist activation of PAR-2, an observation that is similar to PAR-1 activation by PAR-1 agonist peptides. Additional studies of Ala-scanning peptides have been conducted by several groups to elucidate the importance of individual residues of the SLIGRL sequence. Again, fairly good agreement between these studies support the observation that the side chains of second residue Leu, the third residue Ile and the fifth residue Arg are most important for agonist activity. Again, similar to the reported PAR-1 agonist peptide SAR, the second residue Leu appears to be most critical side chain in the agonists [88]. Interestingly, one of the Ala-scan

peptides, SLIARL-NH₂ (15) appeared to have improved activity against PAR-1 (Table 2) [84]. The importance of the free amino group has been explored by acetylation (analog 20) and removal of the -amino group ([3-OH-Propionyl]-LIGKV-NH₂) (21). The acetylated hexapeptide is inactive [82], [84] but the des-amino hexapeptide (21) displayed an IC₅₀ value of 23 μ M in the Ca²⁺ mobilization assay [84] leading to the conclusion that the free -amino group is not absolutely required, but that an N-terminal amide is undesirable.

In the course of conducting SAR studies for PAR-2 it was speculated that similarities in the PAR-1 and PAR-2 agonist peptide sequences might lead to receptor cross-reactivity. For the PAR-2 agonist peptides, this has been clearly demonstrated not to be the case [82], [84]. However, the PAR-1 agonists heptapeptide (22), hexapeptide (23) and pentapeptide (24) are all potent agonists of PAR-1 as well as PAR-2 [82], [35], [83], [84]. These observations are quite important since not only do they point out the potential for

misleading interpretation of the location or activity of various PARs in tissues when using agonist peptides. They also relate to the need in setting up cellular bioassays to control for PAR-family receptors in the cell of choice since many native cells contain multiple similar PARs. These observations also point to the possibility that when antagonists of each of the PARs are identified they need to be evaluated in the PAR-family member assays to demonstrate selective antagonist/agonist activity.

PAR peptides from other species have also been evaluated for PAR-2 activation with the goal of identifying specific PAR-1 and PAR-2 agonists. Notably, the *Xenopus* PAR-1 agonist TFRIFD-NH₂ (**25**) has been shown to be a potent agonist of human PAR-1 and to be inactive against human PAR-2 expressed in *Xenopus* oocytes [82] and was inactive as a PAR-2 agonist or to desensitize PAR-2 in HEK293 cells [35]. Similarly, the PAR-1 derived peptide, TFLLR-NH₂ (**26**), is >250-fold more active as a PAR-1 agonist than a PAR-2 agonist suggesting the role of the Thr¹ residue as an important determinant of PAR-1 selectivity [83]. When Thr¹ is introduced into the PAR-2 sequence the corresponding peptide TLIGRL-NH₂ (**27**) retains its high specificity for PAR-2, mostly because of the Leu² residue. Additionally, the nonselective agonist SFLLRN-NH₂ (**24**) can be made PAR-1 selective simply by replacement of the Arg⁵-Asn⁶ with Phe-Asp yielding SFLLFD-NH₂ (**28**) [35]. Another highly PAR-2 specific compound, *trans*-cinnamoyl-LIGRL-Orn-NH₂ (**29**) has also been developed which has found utility as a radioligand for PAR-2 [36], [81]. Of interest to the issue of selectivity, is the observation that one of the first reported PAR-1 antagonists, Mpr-FChaCha-RKDNPKY-NH₂ (**30**) [89,90], was unexpectedly found to cause a calcium signal in HEK cells with an EC₅₀ of approximately 20 μM and the response was found to be desensitized upon exposure of the cells to high

concentrations of SLIGRL, but not the PAR-1 agonist TFLLR-NH₂ (**26**) [83]. These observations again point out the possibility of cross-reactivity of agonists/antagonists of the PAR-family members.

Attempts to improve the activity of PAR-2 agonist peptides have examined substitutions that have been important in obtaining highly potent PAR-1 agonists. Specifically, substitution of p-FPhe for Leu², replacement of Ile³ with either cyclohexylalanine (Cha) or homoarginine (Har) and substitution of Lys for Leu⁶. Peptides containing these substitutions such as (**31** and **32**) are slightly improved agonists for PAR-2 but pale in comparison to their improved activity as PAR-1 agonists [82], [84]. One interesting series of substitutions of the SLIGRL sequence is the reported improvement or retention of activity in analogs where hydrophobic amino acids such as Phe, p-CIPhe, or 1-Nal are substituted for Arg⁵ such (c.f. analogs **33** and **34**). These results suggest that PAR-2 has an interaction pocket that not only accepts positively charged side chains but is also capable of accepting other rather large hydrophobic residues [84].

With the lack of ability to improve significantly the agonist activity of PAR-2 peptides, one group has resorted to a combinatorial approach making a “directed” library of more than 100 analogs. The results of the analysis of the SAR for these peptides and overall SAR investigations are depicted in (Fig. 1).

A general conclusion that can be drawn from these studies is that similar to the PAR-1 agonist peptide SAR, only a few residues of SLIGRL are severely restricted in their degree of substitution allowed. The Leu² residue appears to be the most critical with respect to retaining specific PAR-2 interactions. However, Cha or Phe

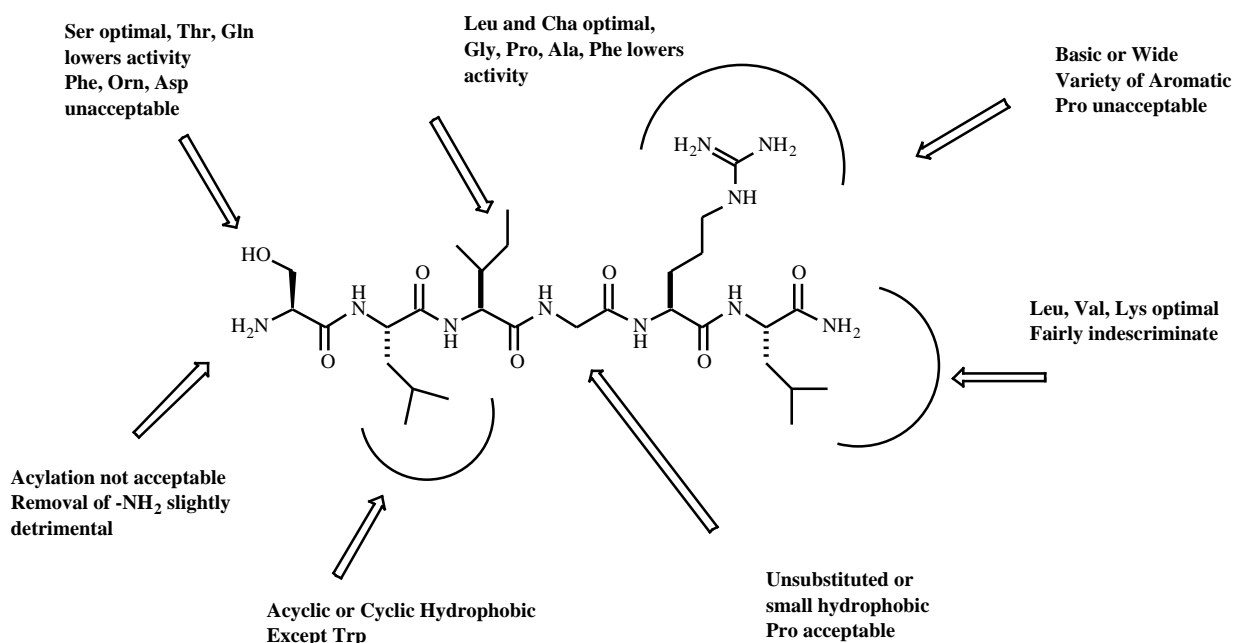


Fig. (1). SAR Summary of PAR-2 Agonist SLIGRL.

substitutions for Leu² still afford active PAR-2 agonists but these peptides are now non-selective and can also activate PAR-1. The N-terminal Ser¹ residue appears optimal, with lower activities observed with Thr and Gln substitution, and significant elimination of activity with other substitution. Ile³ appears to require acyclic and cyclic nonaromatic hydrophobic residues such as Ile, Leu or Cha. The Gly⁴ residue appears to be relatively indiscriminate since Ala substitution appears to enhance activity [84], whereas Leu or Cha substitution appears to be well accepted and even Pro substitution affords a modestly potent peptide. The fifth residue Arg⁵ is also indiscriminate and is accepting of a wide variety of bulky aromatic residues as well as basic sidechains Arg and Lys. Finally the sixth residue Leu⁶, while not required for full agonist potency, is again fairly indiscriminate in which many residues are acceptable but is optimal with Leu, Val or Lys and also gives reasonable potency with Ala and Tyr substitution. These observations at the sixth residue are not surprising in light of the potent activity observed with the pentapeptide sequence.

A preliminary study describing peptide SAR using the classical rat aorta relaxation assay has described peptides derived from the SLIGRL sequence, as small as C-terminal dipeptides as PAR-2 agonists [91]. Specifically, N-benzoyl-Arg(NO₂)-Leu-NH₂ (EC₅₀ = 400 μM) and *p*-CF₃O-benzoyl-Arg(NO₂)-Leu-NH₂ (EC₅₀ = 20 μM) were reported as PAR-2 antagonists. However, these conclusions concerning their PAR-2 agonist activity based solely on the aorta assay should be viewed as very preliminary, until they can be confirmed in other more specific cellular assays or binding assays that have been described for evaluating PAR-2 agonist activity.

A polymorphic PAR-2 (F240S) displaying a significantly reduced sensitivity to trypsin and various PAR-2 activating peptides has been recently reported [20]. Interestingly, this polymorphic receptor displays a 2.5-fold reduced sensitivity to SLIGKV-NH₂ (7) and a 2.8-fold reduced sensitivity to SLIGRL-NH₂ but increased sensitivity to the selective PAR-2 agonists TFLLR-NH₂ (26) (7-fold) and trans-cinnamoyl-LIGRLOrn-NH₂ (29) (4-fold). Based on this information the authors have suggested that this may have important implications for agonist/antagonist design and the occurrence of this polymorph may be a predictor of certain disease states.

While the SAR of PAR-2 peptides has elucidated a fair degree of information concerning the residues involved in interaction with PAR-2, little progress in preparing highly potent analogs has been reported to date, in contrast to PAR-1. Similarly, the information generated has not yet been useful for generating antagonists of this receptor. No reports have appeared in the literature to date predicting the conformation of the PAR-2 agonists peptides. Additional investigations will be required to determine if potent agonists can be obtained and whether they will have utility in various disease indications.

PAR-2 ANTAGONISTS

Little work has been published to date on PAR-2 antagonists. A single report describes two peptides

FSLLRY-NH₂ (35) and LSIGRL-NH₂ (36) which are N-terminal reversed sequences of PAR-1 and PAR-2 agonist peptide sequences respectively, previously shown to lack agonist activity at their respective receptors [92]. Surprisingly, these two peptides can antagonize trypsin (2 nM) induced responses in KNRK cell lines expressing rat or human PAR-2 with IC₅₀ values of 50 μM for 35 and 200 μM for 36. FSLLRY-NH₂ (35) can also antagonize trypsin relaxant activity in rat aorta. However, these peptides are unable to antagonize SFLLRN-NH₂ or SLIGRL-NH₂ agonist responses in these same assays. This discrepancy cannot be accounted for by the ability of these peptides to specifically inhibit the enzymatic activity of trypsin, since they are inactive in this respect. Based on these observations, the authors speculate that these peptides bind to a region on PAR-2 which is specific for the tethered ligands, distinct from the site at which the soluble agonist peptides bind. If in fact this is true, then peptides 35 and 36 represent very interesting lead molecules for antagonist design and would negate the potential entropic disadvantage of compounds that antagonize the soluble peptide agonists. Further SAR of 35 or 36 is eagerly anticipated.

A number of PAR-1 antagonists of the peptide-mimetic and nonpeptide classes have been disclosed since 1999. RWJ-56110 (37) (Fig. 2) is reported to have IC₅₀ = 0.1-0.5 μM as a PAR-1 antagonist in a variety of bioassays but is inactive as a PAR-2 antagonist [87]. A second PAR-1 antagonist SCH 79797 a pyrrolo [3,2-f]quinazoline (38) (Fig. 2), similarly does not antagonize SLIGKV-NH₂ induced calcium transients in vascular smooth muscle cells [93]. Thus, initial indications are that antagonists developed as PAR-1 antagonists are highly specific and unfortunately

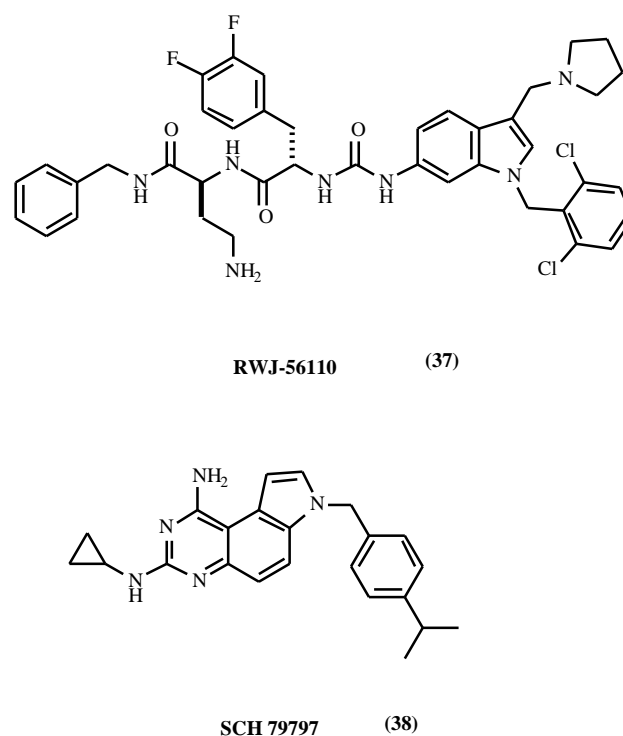


Fig. (2). PAR-1 Antagonists that do not inhibit PAR-2.

cannot be used as leads is the search for PAR-2 antagonists. No reports of specific small molecule screens for PAR-2 have appeared to date but this approach is likely to be explored.

CONCLUSIONS

Since the initial cloning of PAR-2 in 1994, slow but steady progress in understanding the peptide SAR of the agonist peptide sequence for PAR-2 has unfolded. Although requirements for peptide ligand binding are fairly well understood, no real advancement in improving the agonist activity has occurred during this time frame. Likewise, efforts to obtain antagonists have been minimal or have not been reported due to lack of success. The PAR receptors remain potentially interesting drug targets with an added bonus that agonists of PAR-2 may also be therapeutically useful. Obviously, the protease-activated receptors are a unique class of GPCRs due to the tethered-ligand mechanism by which they are activated. So far it has proven difficult to develop PAR agonists and antagonists with appropriate properties for pharmaceutical development. Unless a significant breakthrough is achieved, exploiting these novel receptors may remain elusive. Time will tell.

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