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High-Affinity Thrombin Receptor (PAR-1) Ligands: A New Generation of Indole-Based Peptide Mimetic Antagonists with a Basic Amine at the C-terminus

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Abstract—A new generation of indole-based peptide mimetics, bearing a basic amine at the C-terminus, was developed by the agency of two complementary, multistep, trityl resin-based approaches. Thus, we obtained several high-affinity thrombin receptor (PAR-1) ligands, such as **32** and **34**. Compounds **32** and **34** were found to bind to PAR-1 with excellent affinity ($IC_{50} = 25$ and 35 nM, respectively) and to effectively block platelet aggregation induced by SFLLRN-NH_2 (TRAP-6) and α-thrombin.

Traditionally, serine proteases have been viewed as agents responsible for the degradation of proteins and the formation of peptide messengers. However, more recently, serine proteases such as thrombin, trypsin, and tryptase have been found to serve as cellular signalling molecules by cleaving and activating so-called protease-activated receptors (PARs). PARs represent a unique subtype of 7-transmembrane G-protein-coupled receptor in that they are proteolytically modified to expose a new extracellular N-terminus, which acts as a tethered activating ligand.[1]

Presently, four receptors of this class, PAR-1,[2] PAR-2,[3] PAR-3,[4] and PAR-4[5] are known. PAR-1 is cleaved and activated by α-thrombin, and thereby mediates most of the cellular actions of α-thrombin. Thus, considerable effort has been exerted in the search for potent, selective PAR-1 antagonists, which have therapeutic potential especially as antiplatelet drugs.[6,7] We have described a novel series of indole-based peptide mimetics as PAR-1 antagonists, with good potency against thrombin-induced platelet aggregation and moderate affinity to PAR-1.[7a–e] In seeking to further improve PAR-1 antagonist potency, we have now explored other solid-phase approaches to analogues that were difficult to access via the previous methods. Herein, we disclose a new series of indole-based peptide mimetics, with a basic amine at the C-terminus, which were prepared via a versatile, trityl resin-based, solid-phase parallel synthesis. Several of these compounds have greatly improved binding affinity to PAR-1, and effectively block platelet aggregation induced by both SFLLRN-NH_2 (TRAP-6) and α-thrombin.

In our previous compound-optimization studies,[7b] we attached a secondary amide onto a Sieber or Tentagel S AM resin, then constructed a dipeptide segment followed by introduction of the indole scaffold. Thus, we constructed a library of peptide mimetic PAR-1 antagonists that led to advanced lead RWJ-56110 (**1**, see Table 1).[7b] However, that approach presented some limitations. First, modification of the C-terminus of the dipeptide (determined later to be important for PAR-1 potency) was highly limited due to the difficulty of reductively aminating the resins, and the constraint of introducing the R^6 group (see general structure in Table 1) at the outset of a 10-step process. Second, the final cleaved products typically had only 75–85% purity, and the loadings of the Sieber or Tentagel S AM resins were relatively low. To address these issues, we sought an

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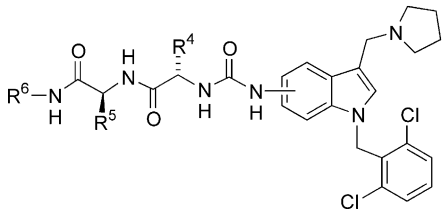
Table 1. PAR-1 binding and platelet aggregation IC_{50} values (μM)[a]



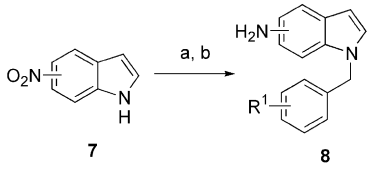
Compd	P[b]	R^4	R^5	R^6	Receptor binding[c]	Gel-filtered platelet aggregation[d]			
						TRAP-6	Thrombin	Collagen	U46619
1	6	3,4-DiF-Bn	$H_2N(CH_2)_2$	Bn	0.44±0.21	0.16±0.05	0.34±0.04	IA	IA
18	6	3,4-DiF-Bn	$H_2N(CH_2)_2$	4-MeOBn	0.48±0.05	0.1±0.01	0.7±0.1	6.7	6.4
19	6	3,4-DiF-Bn	$H_2N(CH_2)_2$	$Ph(CH_2)_2$	0.12	0.16	0.81±0.09	3.0	7.6
20	6	3,4-DiF-Bn	$H_2N(CH_2)_2$	2-Fur-CH_2	0.16	0.07±0.03	0.78±0.02	7.9	IA
21	6	3,4-DiF-Bn	$H_2N(CH_2)_2$	2-Thi-CH_2	0.23	0.16±0.02	0.8±0.06	7.0	IA
22	6	3,4-DiF-Bn	$H_2N(CH_2)_2$	2-Py-CH_2	0.33	0.19±0.02	1.1±0.18	8.4	19.8
23	6	3,4-DiF-Bn	$H_2N(CH_2)_2$	2-Py-$(CH_2)_2$	0.5	0.15±0.01	0.88±0.18	6.6	10.3
24	6	3,4-DiF-Bn	$H_2N(CH_2)_2$	$EtNH(CH_2)_2$	0.09±0.02	0.13	0.41±0.21	IA	13.7
25	6	3,4-DiF-Bn	$H_2N(CH_2)_2$	1-Pyr-$(CH_2)_2$	0.07	0.05±0.00	0.46±0.22	IA	IA
26	4	3,4-DiF-Bn	$H_2N(CH_2)_2$	1-Pyr-$(CH_2)_2$	2.0	16	40±5	IA	IA
27	7	3,4-DiF-Bn	$H_2N(CH_2)_2$	1-Pyr-$(CH_2)_2$	22	4.3	23±4	IA	IA
28	6	3,4-DiF-Bn	$H_2N(CH_2)_3$	1-Pyr-$(CH_2)_2$	0.31	0.06	0.25±0.1	16	22±3
29	6	4-Cl-Bn	$H_2N(CH_2)_2$	$H_2N(CH_2)_3$	0.43±0.001	0.05	0.2±0.02	11	17.3
30	6	4-Cl-Bn	4-Im-CH_2	$H_2N(CH_2)_3$	0.13±0.01	0.07±0.01	0.41±0.1	30±4	18.6
31	6	3,4-DiF-Bn	$MeSCH_2$	$H_2N(CH_2)_2$	0.06	0.06±0.02	1.3±0.2	27	18.6
32	6	3,4-DiF-Bn	$MeS(CH_2)_2$	$H_2N(CH_2)_2$	0.025±0.004	0.07±0.02	0.22±0.07	4.7±1	24±10
33	6	2-F-Bn	4-Py-CH_2	$H_2N(CH_2)_2$	0.07±0.04	0.05±0.01	0.13	IA	IA
34	6	3,4-DiF-Bn	2-Thi-CH_2	$H_2N(CH_2)_2$	0.035±0.004	0.08±0.02	0.27±0.12	16.5	IA

[a]Results are expressed as mean±SEM ($n \geq 2$; $n = 1$ for values without error limits). IA denotes inactivity @ 50 μM of test compound. Abbreviations for R^4, R^5 and R^6: Bn, benzyl. Fur, furyl. Thi, thienyl. Py, pyridyl. Pyr, pyrrolidinyl. Im, imidazolyl.
[b]P denotes the position of indole ring attaching to urea linkage.
[c]Thrombin receptor (PAR-1) binding assay; ligand: [^{3}H]-*S*-(*p*-F-Phe)-homoarginine-L-homoarginine-KY-NH_2, 10 nM (K_d = 15 nM).
[d]Concentrations of agonists for aggregation studies: TRAP-6, 2 μM; α-thrombin, 0.15 nM; collagen, 3 μg/mL; U46619, 0.3 μM.

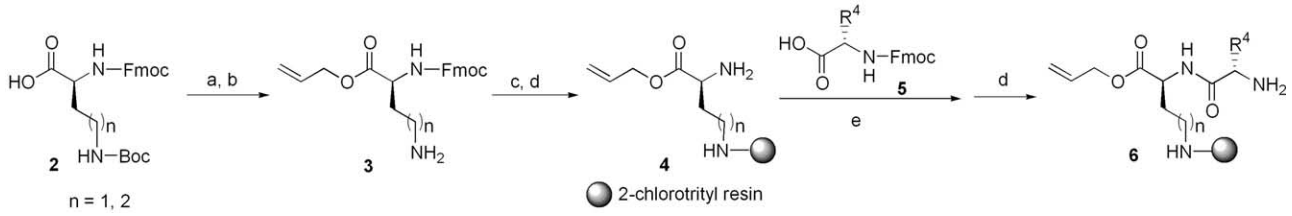
alternative resin-attachment point and designed a new synthetic sequence that permits the introduction of maximum diversity at the C-terminus. Given that a primary amine side chain is well tolerated at the R^5 position, such as for RWJ-56110,[7b] we chose a 2-Cl-trityl resin,[8] which has high loading, is compatible with our multistep reaction conditions, and is easily cleaved, to anchor the key amine side chain.

Fmoc-protected 2,4-diaminobutyric acid or ornithine was coupled with allyl alcohol in the presence of HOBt/DCC followed by removal of the Boc group with TFA/CH_2Cl_2 to give the allyl protected compound **3** (Scheme 1). The amine **3** was loaded onto the 2-Cl-trityl resin (1.5 mmol/g, Advanced ChemTech) in the presence of *i*-Pr_2NEt followed by cleavage of the Fmoc group to give **4**, which was coupled with Fmoc-protected amino acid **5** using HBTU/HOBt and then treated with piperidine to give the corresponding resin-bound dipeptide **6**.

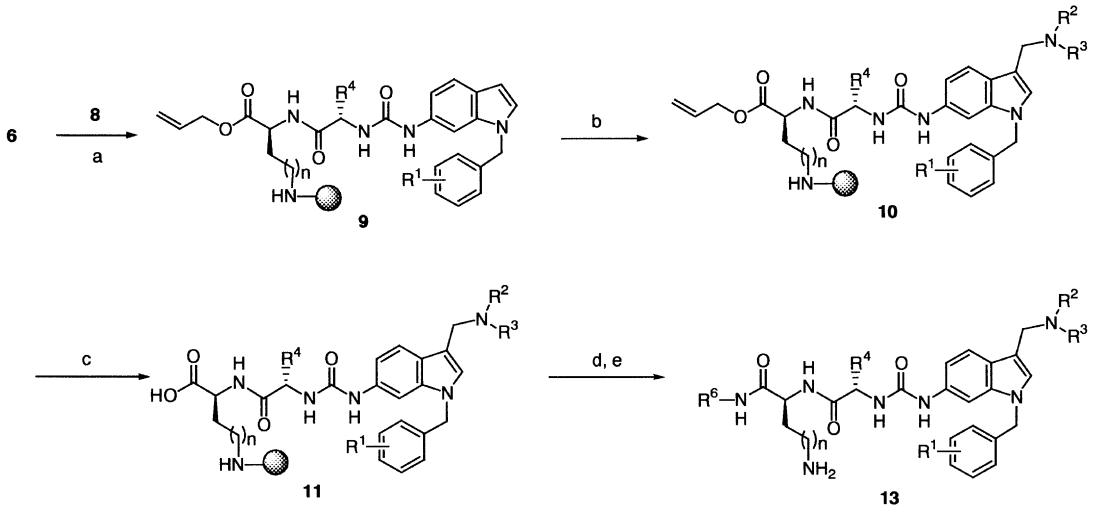
For the other desired segment, various nitroindoles **7** were *N*-alkylated with substituted benzyl bromides in the presence of cesium carbonate and the products were reduced with Me_2NNH_2, $FeCl_3{\cdot}6H_2O$, and charcoal in MeOH to afford (4-7)-aminoindoles **8** (Scheme 2). In a key step of the solid-phase process, aminoindole **8** was



Scheme 2. (a) R^1-$C_6H_4CH_2$-Br, Cs_2CO_3, DMF; (b) Me_2NNH_2, $FeCl_3{\cdot}6H_2O$, charcoal, MeOH, reflux.



Scheme 1. (a) Allyl alcohol, DCC, HOBt, MeCN; (b) TFA, CH_2Cl_2; (c) 2-Cl-Trityl Resin, *i*-Pr_2NEt, CH_2Cl_2-DMF; (d) Piperidine, DMF; (e) HBTU, HOBt, *i*-Pr_2NEt, DMF.

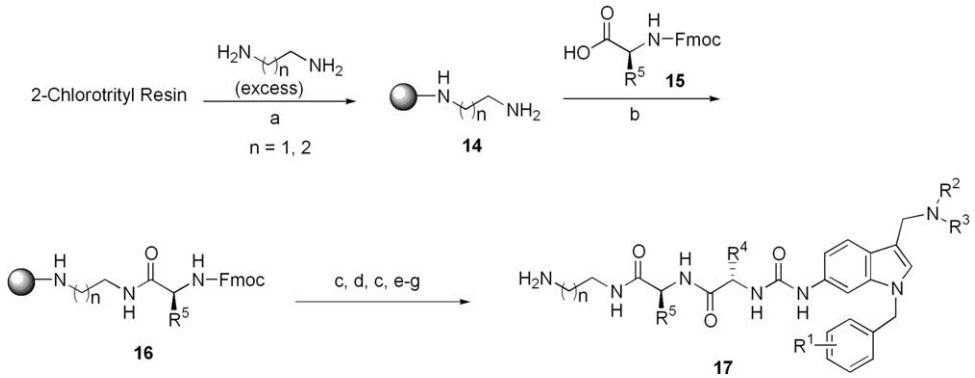


Scheme 3. (a) *p*-$NO_2C_6H_4OCOCl$, *i*-Pr_2NEt, CH_2Cl_2, −20 °C to 23 °C; (b) R^2R^3NH, HCHO, 1,4-dioxane-HOAc (4:1); (c) $Pd(PPh_3)_4$, dimedone, THF; (d) R^6NH_2 (**12**), HOBt, DIC, DMF, 45 °C; (e) TFA, CH_2Cl_2, anisole.

converted with *p*-nitrophenyl chloroformate and *i*-Pr_2NEt at −20 °C to a *p*-nitrophenyl carbamate intermediate, which was coupled with resin-bound dipeptide amine **6** to cleanly afford urea **9** (Scheme 3). Mannich reaction of resin **9** with an amine and formaldehyde using 1,4-dioxane–HOAc (4:1) proceeded smoothly at 23 °C to provide **10**. Removal of allyl protecting group in **10** by using $Pd(PPh_3)_4$/dimedone/THF afforded the key resin-bound precursor **11** for introducing diverse functionalities at the C-terminus. Unfortunately, the coupling of resin **11** with amine **12** under common coupling conditions, such as DCC/HOBt in DMF, resulted in incomplete reaction, and other reagents, such as HBTU/HOBt, BOP-Cl/NMM, or HATU/HOAt, were unsatisfactory too. Complete coupling was ultimately achieved by using DIC/HOBt in DMF at 45 °C. The coupled products were then released from the resin by treatment with 30% TFA in CH_2Cl_2 to give target compounds **13** in ca. 35–40% overall yield from resin **4**.

By using this 7-step solid-phase method, analogues of RWJ-56110 (**1**) were constructed, with diverse modifications at the C-terminus (R^6 in **13**). This method turned out to be more robust than our earlier one that employed the Sieber or Tentagel S AM resin;[7b] thus, it provided target compounds in adequate amounts (starting with higher loading resin) with purities >85%, as determined by reversed-phase HPLC.

Biological evaluation (*vide infra*) of the compounds revealed that replacement of the benzyl group at the C-terminus in **1** with a basic functionality, such as an amine, could significantly improve the binding affinity to PAR-1 and the potency in TRAP-6-induced platelet aggregation. Given that a basic amine group is already present at the C-terminus in these new analogues, we wondered if the basic residue at R^5 position, originally believed to be critical for PAR-1 activity, would still be needed. Therefore, we developed a complementary 2-Cl-trityl resin-based solid-phase method (Scheme 4) to further optimize the series, by modifying R^5 position in **17** with various functionalities. Mono-attachment of 1,2-diaminoethane or 1,3-diaminopropane onto the 2-Cl-trityl resin provided resin-bound amine **14**, which was coupled with Fmoc-protected amino acid **15** in the presence of HBTU/HOBt in DMF to afford **16**. Following the procedures described in Schemes 1–3, **16** was deprotected with piperidine, coupled with another Fmoc-protected amino acid **5**, deprotected again with piperidine, condensed with aminoindole **8** via a urea linkage,



Scheme 4. (a) DMF; (b) HBTU, HOBt, *i*-Pr_2NEt, DMF; (c) Piperidine, DMF; (d) **5**, HBTU, HOBt, *i*-Pr_2NEt, DMF; (e) **8**, *p*-$NO_2C_6H_4OCOCl$, *i*-Pr_2NEt, CH_2Cl_2, −20 °C to 23 °C; (f) R^2R^3NH, HCHO, 1,4-dioxane-HOAc (4:1); (g) TFA, CH_2Cl_2, anisole.

reacted with an amine and formaldehyde to form a Mannich base, and cleaved from the resin with TFA/CH_2Cl_2 to provide the desired targets, **17**. Similar to the approach described in Schemes 1–3, this solid-phase synthesis provided products in good yields (50–60% overall from resin **14**) with excellent purity (>85%).

Compounds prepared via the solid-phase methods were tested for competitive binding versus [^{3}H]-S-(*p*-F-Phe)-homoarginine-L-homoarginine-KY-NH_2 to PAR-1 on the membranes of CHRF-288-11 cells.[7e] We also examined their inhibition of platelet aggregation induced by TRAP-6, α-thrombin, collagen, and U46619 (thromboxane mimetic). Representative compounds with in vitro biological data are presented in Table 1.

Replacement of the benzyl group at the C-terminal (R^6 position) in RWJ-56110 with substituted benzyl (**18**), phenethyl (**19**), and various heteroarylmethyl groups, such as furylmethyl (**20**), thienylmethyl (**21**), pyridylmethyl (**22**), did not significantly affect PAR-1 binding affinity, nor activity in platelet aggregation induced by thrombin and TRAP-6; however, selectivity over collagen and U46619 was generally reduced. Continued screening of the functionality at the C-terminus of RWJ-56110 led to the identification of a new series of amino-containing analogues with a significant improvement of PAR-1 binding affinity, as well as potency in platelet aggregation.[9] For example, **25**, an analogue of **1** with the benzyl group at the C-terminus replaced by a pyrrolidinylethyl group, bound to PAR-1 with excellent affinity (IC_{50} = 70 nM), and inhibited platelet aggregation induced by TRAP-6 and thrombin with IC_{50} values of 50 and 460 nM, respectively. Interestingly, shifting the urea linkage in **25** from the indole 6-position to the indole 4-position (**26**) or 7-position (**27**)[10] resulted in a dramatic loss of potency in both binding and platelet aggregation assays. Thus, the orientation between the dipeptide segment, Mannich base, and benzyl group on the indole nitrogen is critical for PAR-1 activity, which is consistent with our 'three-point model'[7e] for PAR-1 antagonist design. With an aminoethyl or aminopropyl group attached to the C-terminus, a basic residue at R^5 position is no longer required for potent PAR-1 antagonism. In fact, after surveying many functional groups, both basic or nonbasic, a side chain containing a SMe or thienyl group turned out to be optimal at the R^5 position, in combination with an aminoethyl group at the C-terminus. For example, **31**, where R^5 is a CH_2SMe group, had IC_{50} values of 60 nM each in PAR-1 binding and TRAP-6-induced platelet aggregation. Extending the side chain by one carbon further increased the potency: **32** had an IC_{50} value of 25 nM, which is the highest PAR-1 binding affinity observed for any compound from our peptide mimetic PAR-1 antagonist series. High affinity (IC_{50} = 35 nM) was also observed with a 2-thienylmethyl group at the R^5 position (**34**). All of these compounds also effectively blocked platelet aggregation induced by TRAP-6 and thrombin.

By using a robust, trityl resin-based, solid-phase synthesis, we have identified a new generation of indole-based peptide mimetics, bearing a basic amine at the C-terminus, that have high affinity for the thrombin receptor (PAR-1). The ability of these compounds to block TRAP-6-induced platelet aggregation generally correlated well with PAR-1 affinity, though the potency against thrombin-induced platelet aggregation did not correlate as well, perhaps, because of competition with the tethered ligand. These high-affinity PAR-1 ligands should serve as useful tools to further address the physiological roles of PAR-1 and its family members.

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References and Notes

1. (a) Macfarlane, S. R.; Seatter, M. J.; Kanke, T.; Hunter, G. D.; Plevin, R. *Pharmacol. Rev.* **2001**, *53*, 245. (b) Hollenberg, M. D.; Compton, S. J. *Pharmacol. Rev.* **2002**, *54*, 203. (c) Derian, C. K.; Maryanoff, B. E.; Zhang, H.-C.; Andrade-Gordon, P. *Exp. Opin. Invest. Drugs* **2003**, *12*, 209.
2. Vu, T.-K. H.; Hung, D. T.; Wheaton, V. I.; Coughlin, S. R. *Cell* **1991**, *64*, 1057.
3. Nystedt, S.; Emilsson, K.; Wahlestedt, C.; Sundelin, J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9208.
4. Ishihara, H.; Connolly, A. J.; Zeng, D.; Kahn, M. L.; Zheng, Y. W.; Timmons, C.; Tram, T.; Coughlin, S. R. *Nature* **1997**, *386*, 502.
5. (a) Xu, W.-F.; Andersen, H.; Whitmore, T. E.; Presnell, S. R.; Yee, D. P.; Ching, A.; Gilbert, T.; Davie, E. W.; Foster, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6642. (b) Kahn, M. L.; Zheng, Y.-W.; Huang, W.; Bigornia, V.; Zeng, D.; Moff, S.; Farese, R. V., Jr.; Tam, C.; Coughlin, S. R. *Nature* **1998**, *394*, 690.
6. (a) For recent reviews on PAR-1 antagonists, see: Anderluh, M.; Dolenc, S. *Curr. Med. Chem.* **2002**, *9*, 1229. (b) Ahn, H.-S.; Chackalamannil, S. *Drugs Future* **2001**, *26*, 1065. (c) Maryanoff, B. E.; Zhang, H.-C.; Derian, C. K.; Andrade-Gordon, P. *Curr. Med. Chem.-Cardiovasc. Hematol. Agents,* **2003**, *1*, 13.
7. (a) Zhang, H.-C.; Derian, C. K.; Andrade-Gordon, P.; Hoekstra, W. J.; McComsey, D. F.; White, K. B.; Poulter, B. L.; Addo, M. F.; Cheung, W.-M.; Damiano, B. P.; Oksenberg, D.; Reynolds, E. E.; Pandey, A.; Scarborough, R. M.; Maryanoff, B. E. *J. Med. Chem.* **2001**, *44*, 1021. (b) Zhang, H.-C.; McComsey, D. F.; White, K. B.; Addo, M. F.; Derian, C. K.; Andrade-Gordon, P.; Oksenberg, D.; Maryanoff, B. E. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2105. (c) Derian, C. K.; Damiano, B. P.; Addo, M. F.; Darrow, A. L.; D'Andrea, M. R.; Nedelman, M.; Zhang, H.-C.; Maryanoff, B. E.; Andrade-Gordon, P. *J. Pharmacol. Exp. Ther.* **2003**, *304*, 855. (d) Andrade-Gordon, P.; Derian, C. K.; Maryanoff, B. E.; Zhang, H.-C.; Addo, M. F.; Cheung, W.-M.; Damiano, B. P.; D'Andrea, M. R.; Darrow, A. L.; de Garavilla, L.; Eckardt, A. J.; Giardino, E. C.; Haertlein, B. J.; McComsey, D. F. *J. Pharmacol. Exp. Ther.* **2001**, *298*, 34. (e) Andrade-Gordon, P.; Maryanoff, B. E.; Derian, C. K.; Zhang, H.-C.; Addo, M. F.; Darrow, A. L.; Eckardt, A. J.; Hoekstra, W. J.; McComsey, D. F.; Oksenberg, D.; Reynolds, E. E.; Santulli, R. J.; Scarborough, R. M.; Smith, C. E.; White, K. B. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12257. (f) Nantermet, P. G.; Barrow, J. C.; Lundell, G. F.; Pellicore, J. M.; Rittle, K. E.; Young, M. B.; Freidinger, R. M.;

Connolly, T. M.; Condra, C.; Karczewski, J.; Bednar, R. A.; Gaul, S. L.; Gould, R.; Prendergast, K.; Selnick, H. G. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 319. (g) Chackalamanil, S.; Doller, D.; Eagen, K.; Czarniecki, M.; Ahn, H.-S.; Foster, C. J.; Boykow, G. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2851. (h) Barrow, J. C.; Nantermet, P. G.; Selnick, H. G.; Glass, K. L.; Ngo, P. L.; Young, M. B.; Pellicore, J. M.; Breslin, M. J.; Hutchinson, J. H.; Freidinger, R. M.; Condra, C.; Karczewski, J.; Bednar, R. A.; Gaul, S. L.; Stern, A.; Gould, R.; Connolly, T. M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2691. (i) Ahn, H.-S.; Arik, L.; Boykow, G.; Burnett, D. A.; Caplen, M. A.; Czarniecki, M.; Domalski, M. S.; Foster, C.; Manna, M.; Stamford, A. W.; Wu, Y. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2073. (j) McComsey, D. F.; Hawkins, M. J.; Andrade-Gordon, P.; Addo, M. F.; Oksenberg, D.; Maryanoff, B. E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1423. (k) Hoekstra, W. J.; Hulshizer, B. L.; McComsey, D. F.; Andrade-Gordon, P.; Kauffman, J. A.; Addo, M. F.; Oksenberg, D.; Scarborough, R. M.; Maryanoff, B. E. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1649.

8. Hoekstra, W. J.; Greco, M. N.; Yabut, S. C.; Hulshizer, B. L.; Maryanoff, B. E. *Tetrahedron Lett.* **1997**, *38*, 2629.

9. The parallel improvement of potency against thrombin-induced platelet aggregation was not seen with these new analogues. The reason for the difference is not yet understood but may be related to the tether ligand situation or additional ligand-receptor interactions when thrombin is used as an agonist. All PAR-1 antagonists reported from other laboratories have generally not demonstrated parallel activities between PAR-1 receptor binding, TRAP-6-induced platelet aggregation, and thrombin-induced platelet aggregation.

10. 5-Position urea attachment was also found to be unfavorable in a different series (ref 6c).