Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1423–1428

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

HETEROCYCLE-PEPTIDE HYBRID COMPOUNDS. AMINOTRIAZOLE-CONTAINING AGONISTS OF THE THROMBIN RECEPTOR (PAR-1)

David F. McComsey, Michael J. Hawkins, Patricia Andrade-Gordon, Michael F. Addo, Donna Oksenberg,† and Bruce E. Maryanoff*

Drug Discovery, The R. W. Johnson Pharmaceutical Research Institute, Spring House, PA 19477, U.S.A. and
†COR Therapeutics, Inc., South San Francisco, CA 94080, U.S.A.

Received 22 January 1999; accepted 8 April 1999

Abstract: The thrombin receptor PAR-1 is activated by α-thrombin to stimulate cells, including platelets, through the tethered-ligand sequence SFLLRN. We have discovered a novel series of heterocycle-peptide hybrids comprised of a tripeptide segment, such as Cha-Arg-Phe, and an N-terminal heterocyclic group, many of which behave as full PAR-1 agonists. Certain compounds with an aminotriazole group, such as **4** and **16**, are nearly as potent as SFLLRN-NH_2 in inducing platelet aggregation. Also, some arylethenoyl "N-capped" compounds, such as **52** and **57**, exhibit mixed PAR-1 agonist-antagonist activity. © 1999 Elsevier Science Ltd. All rights reserved.

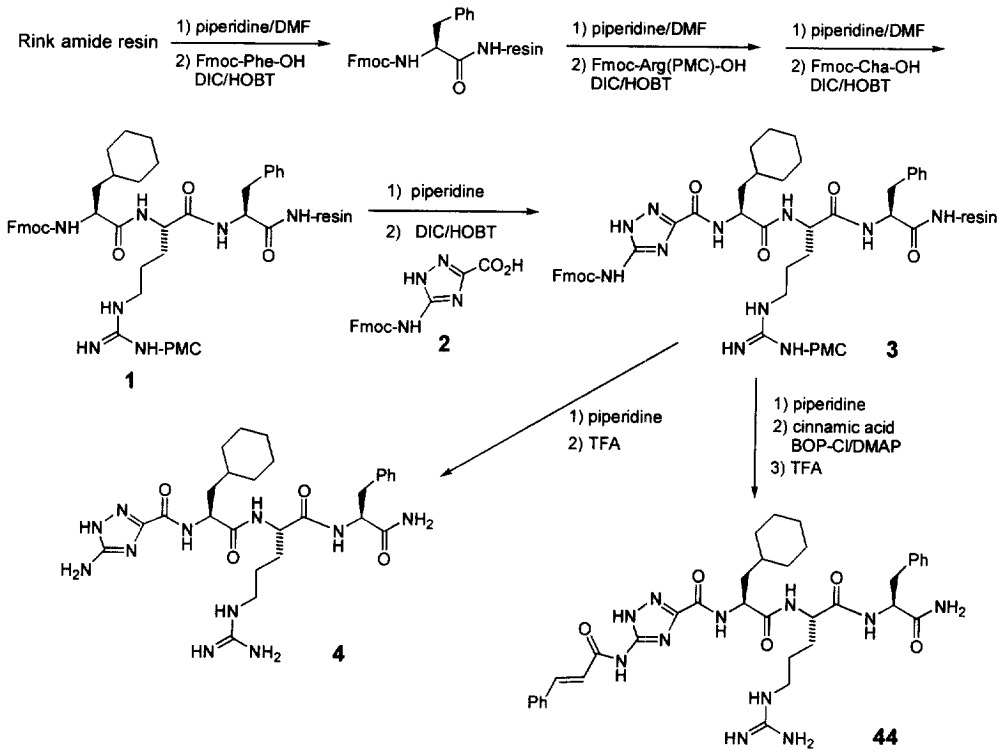
The thrombin receptor, first identified in 1991 from human platelet progenitor cells,[1] is a new type of transmembrane G-protein coupled receptor (GPCR) that is activated by a protease and thus referred to as a protease-activated receptor (PAR).[2] Subsequently, three additional members of this unusual class of GPCRs, PAR-2, PAR-3, and PAR-4, have been discovered.[3] The original thrombin receptor (PAR-1) mediates the cellular actions of the serine protease α-thrombin by undergoing proteolytic cleavage of its extracellular N-terminus, between Arg-41 and Ser-42, to expose a new N-terminus bearing the sequence SFLLRN, which has been referred to as a "tethered ligand".[1,2] Since synthetic peptides containing this motif, such as SFLLRN-NH_2, exhibit full agonist properties for thrombin receptor activation (independent of proteolysis), it is clear that the receptor-linked SFLLRN sequence serves as an activating ligand.[1,4] In a similar vein, PAR-2 can be activated by the serine protease trypsin or its N-terminal recognition peptide SLIGKV-NH_2 (human)[3a] and PAR-4 can be activated by thrombin or its N-terminal recognition peptide GYPGQV-NH_2 (human).[3c]

Structure-activity studies have revealed several critical components of PAR-1 peptide agonists.[2e,4-7] The minimum sequence length is five amino acids, with SFLLR-NH_2 being a good agonist but SFLL-NH_2 not.[4,5] The phenylalanine at position 2 is crucial for activity, while an N-terminal amino group, a basic or aromatic residue at position 5, and a bulky aliphatic residue at position 4 are moderately important.[4,5b-d,6] Positions 3 and 6 are highly tolerant of diverse substitution and positions 1 and 3 tolerate substitution with proline,[4,5b-d,6,7] and a *p*-fluorophenylalanine at position 2 enhances agonist potency by ca. 5 fold.[6a,7,8] Among the large body of structure-activity results for thrombin receptor-activating peptides (TRAPs), no one has yet described agonist molecules that are significantly truncated or that contain a heterocycle within the

0960-894X/99/$ - see front matter © 1999 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(99)00197-3

peptide backbone as a peptidomimetic unit.[9] We now report novel heterocycle-peptide hybrid structures that exhibit agonist activity and reasonable potency relative to SFLLRN-NH_2 (TRAP-6).

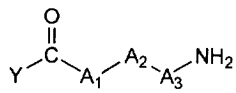
Synthesis. During our studies of heterocycle-based peptide antagonists of PAR-1,[9] we examined some intermediate compounds and found that aminotriazole-peptide **4** possesses significant PAR-1 *agonist* activity in a platelet aggregation assay. This intriguing observation led us to prepare a mini-library of substituted tripeptides by parallel solid-phase chemistry. Except for ca. 12 changes in the peptide portion, most of our changes involved replacement of the aminotriazole carboxylic acid unit with commercially available aromatic and cyclic carboxylic acids. The compounds in this study were prepared via solid-phase chemistry according to the protocol exemplified in the scheme below. Rink-amide resin (Advanced ChemTech; Novabiochem) was loaded by using a classical Fmoc solid-phase protocol[10] (hour-glass bubbler apparatus from Peptides International). After initial Fmoc deprotection with 20% piperidine in DMF (20 min, 23 °C), Fmoc-Phe-OH was coupled to the resin by using *N,N'*-diisopropylcarbodiimide (DIC) and hydroxybenzotriazole (HOBT) in DMF. Elaboration of the peptide was accomplished in the same manner via piperidine deprotection and DIC-coupling to afford a resin bearing the Fmoc-Cha-Arg(PMC)-Phe peptide, **1**.[9] Deprotection was followed by DIC/HOBT coupling of the triazolecarboxylic acid **2** to give **3**, which was Fmoc-deprotected. Cleavage of the final product, such as **4**, from the resin was accomplished with trifluoroacetic acid:anisole (99:1; 1 h).[11] Alternatively, following deprotection of **3**, the N-terminus was acylated with an *E*-cinnamic acid by using BOP-Cl/DMAP,[12] and cleaved as above to give **44**. The compounds in the Table were similarly prepared.



Biological Evaluation. The heterocycle-peptide hybrids were screened for agonist activity by using human gel-filtered platelet aggregation[13] and for binding to the thrombin receptor (PAR-1) on membranes from CHRF cells[9] (see Table). We were impressed by the fact that our initial lead compound, **4**, exhibits agonist activity comparable to the reference hexapeptide SFLLRN-NH_2 (TRAP-6), which contains the native tethered-ligand sequence. In an alanine scan of the four positions in the molecule (**4**), there was a nearly complete loss of activity (**5-8**). Replacement of the Cha at A_1 with a Phe (**16**), and Arg at A_2 by Lys (**15**), retained potency. At A_3, the Phe could be replaced with Cha (**9**) and Phg (**14**) with maintenance of potency; however, there was a modest loss of potency with 2-thienylalanine (**10**) and Tyr (**12**), while homoPhe (**11**) and His (**13**) led to nearly complete loss of agonist activity. In changing the aminotriazole moiety, we found that activity could be retained with a variety of heterocycles. 3-Pyridyl, **23**, and substituted 3-pyridyl (**29-31**) were nearly equipotent with **4**, yet the isomeric 2- and 4-pyridyl compounds (**20** and **24**) and other simple heterocycles, such as pyrrole (**21**) and furan (**22**), were much less potent. Size did not seem to impair agonist potency much as noted with benzimidazole (**36**), 4-oxobenzopyran (**38**), and quinoxaline (**40**). Extending the pyridyl group away from the tripeptide, as in **43**, did not diminish activity (cf. **23**), yet the *E*-styryl derivative (**42**), or various aryl-substituted *E*-styryl derivatives (not shown), possessed little activity.

Bernatowicz et al. reported that introduction of cinnamoyl (*E*-arylethenoyl) groups onto the N-terminus of certain peptide agonists could afford antagonist properties.[14] Cinnamoylation of **4** gave **44**, which was less potent as an agonist, but showed antagonist activity (IC_{50} = 9.8 μM).[15] Some substituted analogues of **44** showed improved agonist potency, and some retained antagonist attributes. The *para*-substituted cinnamoyl compounds were about 10-fold less potent as agonists, while the *ortho*- and *meta*-substituted analogues, **50-53**, were nearly equipotent to **4**. Of **50-53**, only **52** possessed antagonist activity, with an IC_{50} value of 3.6 μM. Vinyl substitution with a fluoro or methyl group, as in compounds **54** and **56**, gave agonist potency similar to **4**, as well as moderate antagonist activity (IC_{50} values of 28 and 14 μM, respectively). Replacement of the phenyl in these cinnamoyl analogues with a thienyl or naphthyl group served to enhance agonist potency (**57**, **59**), with **57**, but not **59**, showing antagonist activity (IC_{50} = 5.4 μM).

Table. Biological Data[a]



#	Y[b]	A_1	A_2	A_3	EC_{50} (μM)[c]	Bndg (μM)[d]
4	5-H_2N-1,2,4-triazol-3-yl[e]	Cha	Arg	Phe	0.76 ± 0.1 (13)	1.9
5	(*S*)-1-H_2N-ethyl	Cha	Arg	Phe	30% ± 2% (2)	9.0
6	5-H_2N-1,2,4-triazol-3-yl	Ala	Arg	Phe	<20% (2)	46
7	5-H_2N-1,2,4-triazol-3-yl	Cha	Ala	Phe	<20% (1)	18
8	5-H_2N-1,2,4-triazol-3-yl	Cha	Arg	Ala	24% ± 3% (2)	10
9	5-H_2N-1,2,4-triazol-3-yl	Cha	Arg	Cha	2.1 ± 0.9 (4)	2.0
10	5-H_2N-1,2,4-triazol-3-yl	Cha	Arg	2-Thi	10.5 ± 7.8 (3)	4.0

Table. Contd.

#	Y[b]	A_1	A_2	A_3	EC_{50} (μM)[c]	Bndg (μM)[d]
11	5-H_2N-1,2,4-triazol-3-yl	Cha	Arg	hPhe	<20% (2)	10
12	5-H_2N-1,2,4-triazol-3-yl	Cha	Arg	Tyr	9.7 ± 7.3 (2)	6.0
13	5-H_2N-1,2,4-triazol-3-yl	Cha	Arg	His	<20% (2)	27
14	5-H_2N-1,2,4-triazol-3-yl	Cha	Arg	Phg	3.1 ± 1.8 (2)	7.0
15	5-H_2N-1,2,4-triazol-3-yl	Cha	Lys	Phe	3.9 ± 1.3 (2)	5.4
16	5-H_2N-1,2,4-triazol-3-yl	Phe	Arg	Phe	1.0 ± 0.1 (3)	2.0
17	3-H_2N-pyrazin-2-yl	Cha	Arg	Phe	4.2 ± 3.7 (2)	8.5
18	3-H_2N-phenyl	Cha	Arg	Phe	2.9 ± 0.2 (3)	2.0
19	4-H_2N-phenyl	Cha	Arg	Phe	40% ± 1% (2)	2.9
20	2-pyridyl	Cha	Arg	Phe	14 ± 2.6 (2)	4.4
21	2-pyrrolyl	Cha	Arg	Phe	55% ± 27% (2)	10
22	3-furyl	Cha	Arg	Phe	38% ± 1% (2)	25
23	3-pyridyl	Cha	Arg	Phe	2.0 ± 0.9 (3)	1.3
24	4-pyridyl	Cha	Arg	Phe	26% ± 6% (2)	7.0
25	1,2,3-thiadiazol-4-yl	Cha	Arg	Phe	1.3 (1)	9.4
26	2-oxopyridin-3-yl	Cha	Arg	Phe	26% ± 7% (4)	16
27	2-aminopyridin-3-yl	Cha	Arg	Phe	8.0 ± 1.5 (2)	2.3
28	2-chloropyridin-3-yl	Cha	Arg	Phe	<20% (2)	7.6
29	6-chloropyridin-3-yl	Cha	Arg	Phe	4.6 (1)	2.4
30	6-aminopyridin-3-yl[e]	Cha	Arg	Phe	1.2 ± 0.3 (5)	2.0
31	5-bromopyridin-3-yl[e]	Cha	Arg	Phe	0.46 ± 0.1 (7)	1.7
32	5,6-dichloropyridin-3-yl	Cha	Arg	Phe	32% ± 7% (2)	2.6
33	benzofuran-2-yl	Cha	Arg	Phe	12.5 ± 0.6 (2)	3.0
34	benzothien-2-yl	Cha	Arg	Phe	8.3 ± 2.8 (2)	2.3
35	1-biphenyl	Cha	Arg	Phe	56% ± 1% (2)	13
36	benzimidazol-5-yl	Cha	Arg	Phe	4.9 ± 0.5 (2)	4.0
37	1,4-benzodioxan-2-yl	Cha	Arg	Phe	<20% (2)	16
38	4-oxobenzopyran-2-yl[e]	Cha	Arg	Phe	0.51 ± 0.1 (7)	1.6
39	2-biphenylenyl[e]	Cha	Arg	Phe	1.2 ± 0 (5)	2.4
40	quinoxalin-2-yl[e]	Cha	Arg	Phe	1.0 ± 0.2 (5)	3.1
41	indolin-2-yl	Cha	Arg	Phe	21% ± 4% (2)	56
42	*E*-styryl	Cha	Arg	Phe	20% ± 16% (2)	5.0
43	*E*-2-(3-pyridyl)ethenyl	Cha	Arg	Phe	2.6 ± 0.9 (3)	5.5
44	5-(PhCH=CHCONH)-1,2,4-triazol-3-yl	Cha	Arg	Phe	21 ± 7 (2)	6.4
45	5-(*p*-F-PhCH=CHCONH)-1,2,4-triazol-3-yl	Cha	Arg	Phe	7.9 ± 1.9 (2)	1.2
46	5-(*p*-MeO-PhCH=CHCONH)-1,2,4-triazol-3-yl	Cha	Arg	Phe	5.6 ± 0.6 (2)	4.0
47	5-(*p*-Cl-PhCH=CHCONH)-1,2,4-triazol-3-yl	Cha	Arg	Phe	55 ± (1)	1.4

Table. Contd.

#	Y[b]	A_1	A_2	A_3	EC_{50} (μM)[c]	Bndg (μM)[d]
48	5-(*p*-CN-PhCH=CHCONH)-1,2,4-triazol-3-yl	Cha	Arg	Phe	5.8 ± 0.4 (2)	31
49	5-(*p*-CF_3-PhCH=CHCONH)-1,2,4-triazol-3-yl	Cha	Arg	Phe	5.0 (1)	30
50	5-(*m*-Cl-PhCH=CHCONH)-1,2,4-triazol-3-yl	Cha	Arg	Phe	2.4 (1)	15
51	5-(*m*-NO_2-PhCH=CHCONH)-1,2,4-triazol-3-yl	Cha	Arg	Phe	1.9 (1)	8.5
52	5-(*o*-Cl-PhCH=CHCONH)-1,2,4-triazol-3-yl	Cha	Arg	Phe	1.1 (1)	1.4
53	5-(*o*-NO_2-PhCH=CHCONH)-1,2,4-triazol-3-yl	Cha	Arg	Phe	1.9 (1)	1.6
54	5-(PhCH=CFCONH)-1,2,4-triazol-3-yl[e]	Cha	Arg	Phe	1.7 (1)	2.2
55	5-(PhCH=CPhCONH)-1,2,4-triazol-3-yl	Cha	Arg	Phe	28 (1)	3.1
56	5-(PhCH=CMeCONH)-1,2,4-triazol-3-yl[e]	Cha	Arg	Phe	0.76 (1)	4.2
57	5-(2-thienyl)acrylamido-1,2,4-triazol-3-yl	Cha	Arg	Phe	2.9 (1)	4.4
58	5-(3-indolyl)acrylamido-1,2,4-triazol-3-yl	Cha	Arg	Phe	14 (1)	100
59	5-(1-naphthyl)acrylamido-1,2,4-triazol-3-yl	Cha	Arg	Phe	1.0 (1)	16
std[f]					0.28 ± 0.02 (10)	0.55

a. Abbreviations: Cha = cyclohexylalanine; 2-Thi = 2-thienylalanine; hPhe = homophenylalanine. b. All cinnamoyl and analogous groups have the *E* geometry. c. Activation of human platelet aggregation (gel-filtered platelets), expressed as an EC_{50} value in μM or as percent aggregation induced at 50 μM (performed as described in ref 13); the number of experiments, n, is given in parentheses. d. Inhibition of [^{3}H] S-(*p*-F-Phe)-Har-L-Har-KY-NH_2 binding to a thrombin receptor CHRF membrane preparation (IC_{50} value).[9] e. Purified to homogeneity (>98% purity) by reverse-phase HPLC. f. Reference standard, SFLLRN-NH_2 (TRAP-6).

In summary, we identified aminotriazole tripeptide **4** as a PAR-1 agonist with comparable potency to SFLLRN-NH_2 (human platelet aggregation). The Cha-Arg-Phe motif of **4**, or a modest variant thereof, was important for potency. The aminotriazole portion could be replaced with other substituted heterocycles, while maintaining agonist potency (e.g., **31** and **38**). An arylethenoyl unit on the N-terminus led to analogues with mixed agonist-antagonist activity. Overall, eight heterocycle-peptide hybrid compounds, **4**, **16**, **30**, **31**, **38**, **52**, **56**, and **59**, were found to exhibit agonist potency (EC_{50}) in the range of ca. 0.5-1 μM, which is comparable to TRAP-6 (EC_{50} = 0.28 μM).[16]

Acknowledgment. We thank Drs. Michael Greco and William Hoekstra for advice and assistance.

References and Notes

1. Vu, T.-K. H.; Hung, D. T.; Wheaton, V. I.; Coughlin, S. R. *Cell* **1991**, *64*, 1057.
2. Reviews on the original thrombin receptor (PAR-1): (a) Van Obberghen-Schilling, E.; Chambard, J.-C.; Vouret-Craviari, V.; Chen, Y.-H.; Grall, D.; Pouysségur, J. *Eur. J. Med. Chem.* **1995**, *30S*, 117. (b) Dennington, P. M.; Berndt, M. C. *Clin. Exp. Pharmacol. Physiol.* **1994**, *21*, 349. (c) Coughlin, S. R. *Thromb. Haemostasis* **1993**, *66*, 184. (d) Coughlin, S. R. *Trends Cardiovasc. Med.* **1994**, *4*, 77. (e) Ogletree, M. L.; Natajaran, S.; Seiler, S. M. *Persp. Drug Discovery Des.* **1994**, *1*, 527.
3. (a) For PAR-2: Nystedt, S., Emilsson, K., Wahlestedt, C., Sundelin, J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9208. Nystedt, S.; Emilsson, K.; Larsson, A.-K.; Stroembeck, B.; Sundelin, J. *Eur. J.*

Biochem. **1995**, *232*, 84. Nystedt, S.; Larsson, A.-K.; Aberg, H.; Sundelin, J. *J. Biol. Chem.* **1995**, *270*, 5950. (b) For PAR-3: Ishihara, H.; Connolly, A, J.; Zeng, D.; Kahn, M. L.; Zheng, Y. W.; Timmons, C.; Tram, T.; Coughlin, S. R. *Nature* **1997**, *386*, 502. (c) For PAR-4: 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. 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.

4. Scarborough, R. M.; Naughton, M. A.; Teng, W.; Hung, D. T.; Rose, J. W.; Vu, T.-K.; Wheaton, V. I.; Turck, C. W.; Coughlin, S. R. *J. Biol. Chem.* **1992**, *267*, 13146.
5. (a) Hui, K. Y.; Jakubowski, J. A.; Wyss, V. L.; Angleton, E. L. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 790. (b) Sabo, T.; Gurwitz, D.; Motola, L.; Brodt, P.; Barak, R.; Elhanaty, E. *Ibid.* **1992**, *188*, 604. (c) Chao, B. H.; Kalkunte, S.; Maraganore, J. M.; Stone, S. R. *Biochemistry* **1992**, *31*, 6175. (d) Vassallo, R. R., Jr.; Kieber-Emmons T.; Cichowski, K.; Brass, L. F. *J. Biol. Chem.* **1992**, *267*, 6081.
6. (a) Feng, D.-M.; Veber, D. F.; Connolly, T. M.; Condra, C.; Tang, M.-J.; Nutt, R. F. *J. Med. Chem.* **1995**, *38*, 4125. (b) Natarajan, S.; Riexinger, D.; Peluso, M.; Seiler, S. M. *Int. J. Peptide Protein Res.* **1995**, *45*, 145.
7. Seiler, S. M.; Peluso, M.; Tuttle, J. G.; Pryor, K.; Klimas, C.; Matsueda, G. R.; Bernatowicz, M. S. *Mol. Pharmacol.* **1996**, *49*, 190.
8. Nose, T.; Shimohigashi, Y.; Ohno, M.; Costa, T.; Shimizu, N.; Ogino, Y. *Biochem. Biophys. Res. Commun.* **1993**, *193*, 694.
9. We have reported antagonists of PAR-1 containing a heterocyclic template, see: 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*, 649.
10. (a) Fields, G. B.; Noble, R. L. *Int. J. Pept. Protein Res.* **1990**, *35*, 161. (b) Atherton, E.; Logan, C. J.; Sheppard, R. C. *J. Chem. Soc., Perkin Trans. 1* **1981**, 538. (c) Penke, B.; Rivier, J. *J. Org. Chem.* **1987**, *52*, 1197.
11. (a) Compound **4** was fully characterized. The material was purified by reverse-phase HPLC with 0.16% TFA/acetonitrile:0.20% TFA/water (35:65 isocratic) and was lyophilized to a white solid, which was characterized by EI-MS and 300-MHz ^{1}H NMR. Anal. Calcd for $C_{27}H_{41}N_{11}O_4$•2.25 CF_3CO_2H• 1.0 H_2O: C, 44.08; H, 5.31; N, 17.95; F, 14.94. Found: C, 43.82; H, 5.27; N, 17.91; F, 14.50. (b) Other target compounds were characterized by EI-MS and 300-MHz ^{1}H NMR; they were >90% pure by NMR. Seven compounds besides **4** were purified by HPLC (see Table).
12. Greco, M. N.; Powell, E. T.; Hecker, L. R.; Andrade-Gordon, P.; Kauffman, J. A.; Lewis, J. M.; Ganesh, V.; Tulinsky, A.; Maryanoff, B. E. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2947.
13. Hoekstra, W. J.; Beavers, M. P.; Andrade-Gordon, P.; Evangelisto, M. E.; Keane, P. M.; Press, J. B.; Tomko, K. A.; Fan, F.; Kloczewiak, M.; Mayo, K. H.; Durkin, K. A.; Liotta, D. C. *J. Med. Chem.* **1995**, *38*, 1582.
14. Bernatowitz, M. S.; Klimas, C. E.; Hartl, K. S.; Peluso, M.; Allegretto, N. J.; Seiler, S. M. *J. Med. Chem.* **1996**, *39*, 4879.
15. Inhibition of human platelet aggregation induced by human α-thrombin (see ref 13).
16. Abbreviations not defined earlier in this paper: Fmoc = 9-fluorenylmethoxycarbonyl; BOP-Cl = *N,N'*-bis(2-oxo-3-oxazolidinyl)phosphinyl chloride; DMAP = 4-(dimethylamino)pyridine; TFA = trifluoroacetic acid; PMC = 2,2,5,7,8-pentamethylchroman-6-sulfonyl; Har = homoarginine.