Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 3322–3329

From selective substrate analogue factor Xa inhibitors to dual inhibitors of thrombin and factor Xa. Part 3

Daniel Dönnecke,[a] Andrea Schweinitz,[a] Anne Stürzebecher,[a] Peter Steinmetzer,[a] Maj Schuster,[b] Uta Stürzebecher,[c] Silke Nicklisch,[b] Jörg Stürzebecher[c] and Torsten Steinmetzer[a,*]

[a]*Curacyte Discovery GmbH, Winzerlaer Str. 2, D-07745 Jena, Germany*
[b]*Curacyte Discovery GmbH, Deutscher Platz 5d, D-04103 Leipzig, Germany*
[c]*Institut für Vaskuläre Medizin, Klinikum der Universität Jena, Bachstraße 18, D-07740 Jena, Germany*

Received 22 February 2007; revised 30 March 2007; accepted 31 March 2007
Available online 6 April 2007

Abstract—Highly potent and selective substrate analogue factor Xa inhibitors were obtained by incorporation of non-basic or modestly basic P1 residues known from the development of thrombin inhibitors. The modification of the P2 and P3 amino acids strongly influenced the selectivity and provided potent dual factor Xa and thrombin inhibitors without affecting the fibrinolytic enzymes. Several inhibitors demonstrated excellent anticoagulant efficacy in standard clotting assays in human plasma.

Thrombotic complications are a major reason for mortality and morbidity worldwide. The presently approved anticoagulants have several limitations, in that most can be used only parenterally, and the orally active vitamin-K antagonists require drug monitoring and are influenced by drug and food interactions. Ximelagatran, the first orally available thrombin inhibitor was recently withdrawn from the market due to some cases of hepatotoxicity.

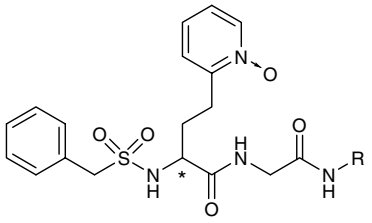
Factor Xa (fXa) is another protease of the clotting cascade and has emerged as an alternative target for the development of new anticoagulants.[1] It is the enzymatically active component of the prothrombinase complex, which is responsible for thrombin generation. During the last decade several groups have described different types of non-substrate like fXa inhibitors.[2,3] Most of the initial inhibitors contained a strongly basic P1 group that interacts with Asp189 at the bottom of the S1 binding site. During optimization it was possible to replace this residue with chloro-substituted aromatics, which make strong interactions to Tyr228 on the back of the specificity pocket.[4–6] Some non-basic fXa inhibitors have recently entered clinical trials, and in preliminary reports it was described that the most advanced compound Bay 59-7939 was well tolerated in healthy male subjects and showed predictable pharmacodynamics and pharmacokinetics.[7,8]

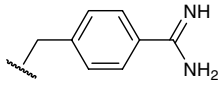
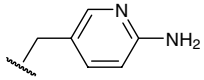
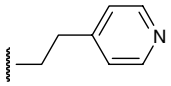
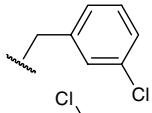
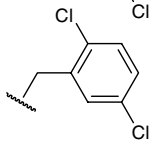
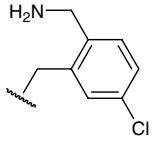
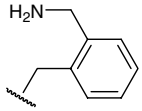
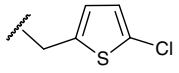
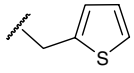
We have recently described several series of substrate analogue fXa inhibitors containing a 4-amidinobenzylamide as the P1 residue.[9] The incorporation of aromatic P3 amino acids with different side-chain lengths revealed serendipitously the strongest fXa affinity for inhibitors containing D-homophenylalanine analogues at this position.[10] Optimized compounds, such as **1** with a D-homo-2-pyridylalanine(*N*-oxide) as P3 residue (Table 1), have excellent selectivity for fXa over a whole set of trypsin-like serine proteases and possess a strong activity in the standard clotting assays and for the inhibition of the prothrombinase complex. However, all pharmacokinetic studies with these benzamidine derived inhibitors, including their hydroxyamidino-, acetylhydroxyamidino- and hexyloxycarbonyl-amidino prodrugs, revealed a rapid elimination from the circulation of rats and an insufficient oral bioavailability. To overcome these pharmacokinetic problems we replaced the 4-amidinobenzylamide in the P1 position with less basic residues, some of which have been previously used for

Keywords: Factor Xa; Thrombin; Protease inhibitor; Anticoagulant; Antithrombotic.
* Corresponding author at present address: Institute of Pharmaceutical Chemistry, Philipps-University Marburg, Marbacher Weg 6, D-35032 Marburg, Germany. Tel.: +49 6421 2825900; fax: +49 6421 2825901; e-mail: torsten.steinmetzer@staff.uni-marburg.de

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.03.105

Table 1. Inhibition of fXa and thrombin by inhibitors with a modified P1 residue



Compound	*	R	K_i[a] (nM)	
			fXa	Thrombin
1	D		0.32	2700
2	D/L		21	88,000
3	D		3900	>1,000,000
4	D		3.3	10,000
5	D/L		2.55	7600
6[b]	D		0.095	170
7	D/L		32	40,000
8	D/L		53	190,000
9	D/L		52	140,000

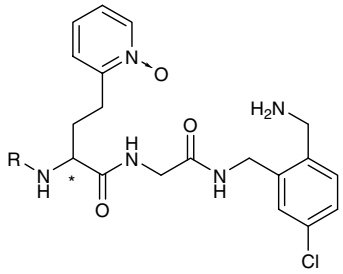
[a] The K_i values were measured as described previously.[9,14a]
[b] Compound **6** inhibits uPA, plasmin, and trypsin with K_i values of 150, 930, and 6.5 μM, respectively.

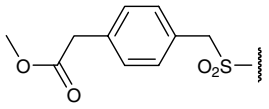
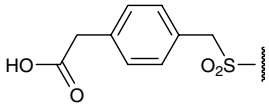
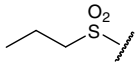
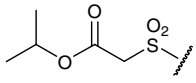
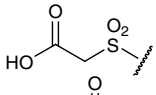
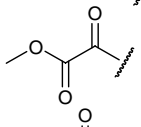
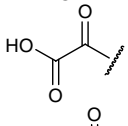
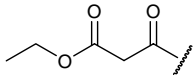
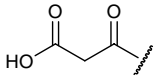
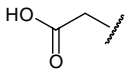
the design of substrate analogue thrombin inhibitors.[11] The results of these studies are presented in this communication.

Although some of the compounds summarized in Table 1 were obtained as racemates, they contain an otherwise constant P4–P2 segment and have a more than 1000-fold selectivity for fXa over thrombin. All inhibitors with a *meta*-chloro-substituted benzylamide group retained high fXa affinity with inhibition constants in the low nanomolar range, as previously found in related thrombin inhibitor series.[11] This was unsurprising, because both sides of the S1 pocket from fXa and thrombin are formed by nearly identical amino acids (segments Asp189-Ser195 and Val213-Cys220, respectively, as well as Tyr228 at its back). The only difference is position 192, which is Gln in case of fXa and Glu in thrombin. Although no X-ray structures of these inhibitors in complex with fXa are available, we assume that in analogy to the related thrombin inhibitors the P1 chloro atom makes a lipophilic contact with the aromatic ring of the fXa residue Tyr228.

Inhibitor **6**, containing a 2-aminomethyl-5-chloro-benzylamide, is an even more potent fXa inhibitor than the benzamidine **1**, despite a moderate decrease in selectivity. This was not expected because in a previously described first X-ray structure with a related thrombin

Table 2. Inhibition of fXa and thrombin by inhibitors with modified P4 residue

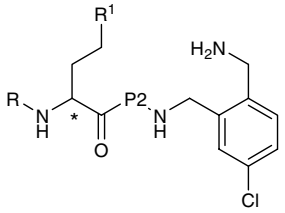


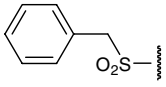
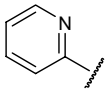
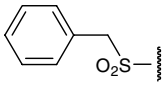
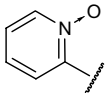
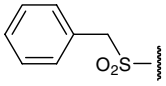
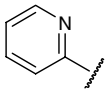
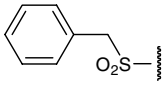
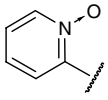
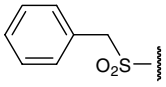
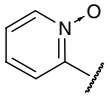
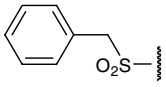
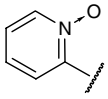
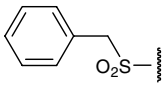
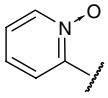
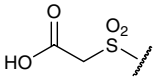
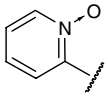
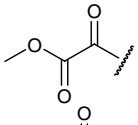
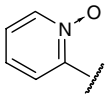
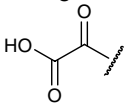
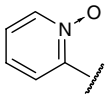
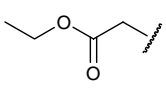
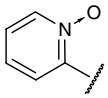
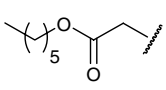
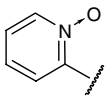
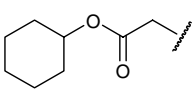
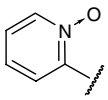
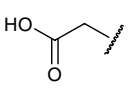
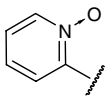
Compound	*	R	K_i (nM)	
			fXa	Thrombin
10	D/L		0.35	1005
11	D/L		0.36	930
12	D/L		3.4	2700
13	D/L		0.76	530
14	D/L		1.6	8300
15	D/L		4.9	6500
16	D/L		4.9	18,000
17	D/L		200	40,000
18	D/L		65	140,000
19	D/L		16	125,000

inhibitor it was found that the interaction between the *ortho*-aminomethyl group of the P1 residue and Glu192 of thrombin is essential for their high potency.[11d] Obviously Gln192 of fXa also contributes to the affinity of this P1 group. The comparison of the fXa K_i values found for inhibitor **7** with those of compounds **4** and **6** (we assumed a K_i-value of 16 nM for the pure D-enantiomer of **7**) reveals a ca. 170- and 35-fold improved potency due to the 5-chloro and 2-aminomethyl substitutions, respectively. We can only speculate that the 2-aminomethyl group might be involved in additional hydrogen bonds, similarly as shown by X-ray crystallography for a second thrombin inhibitor complex containing an identical P1 group (pdb code 1zrb).[12] In this complex the aminomethyl group is in hydrogen bond distance to the carbonyl oxygen of the P2 inhibitor residue, to the carbonyl oxygen of the thrombin residue Gly216 and to a neighbouring water molecule. Interestingly, in this structure the side chain of the thrombin residue Glu192 is rotated away and therefore not involved in inhibitor binding.

In contrast, the 5-chlorothiophene-2-methylamide **8**, which was synthesized analogously to known non-substrate type fXa inhibitors,[6,13] possessed only moderate potency. A similar activity was found for the *des*-chloro thiophene inhibitor **9**; thus both results indicate that the chlorine of compound **8** does not contribute to the binding of fXa. It is also worth mentioning that none of the compounds (except the benzamidine **1**) shown in Tables 1–3 significantly inhibited the fibrinolytic enzymes plasmin and uPA (K_i values >5 μM, data not shown).

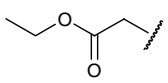
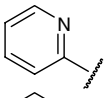
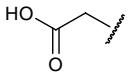
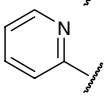
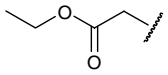
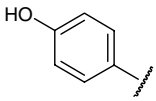
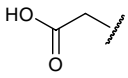
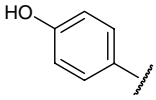
Table 3. Inhibition of fXa and thrombin by inhibitors of the general formula



Compound	*	R	R^1	P2	K_i (nM)	
					fXa	Thrombin
20	D			Ala	0.084	24
21	D			Ala	0.033	170
22	D/L			Ser	0.25	250
23	D			Ser	0.059	860
24	D			Glu(OMe)	0.24	180
25	D/L			Glu	0.52	20,000
26[a]	D			Pro	0.049	0.56
27[a]	D			Pro	0.08	7.7
28	D/L			Pro	0.87	19
29	D			Pro	0.63	17.7
30	D			Pro	1.7	4.9
31	D			Pro	0.92	7.8
32	D			Pro	1.0	9.0
33	D			Pro	0.94	17.3

(*continued on next page*)

Table 3 (*continued*)

Compound	*	R	R^1	P2	K_i (nM)	
					fXa	Thrombin
34	D			Pro	2.9	3.8
35	D			Pro	1.5	3.5
36	D			Pro	17	3.6
37	D			Pro	8.3	3.2

*The diastereomers were separated by preparative HPLC during synthesis, if possible.[17]

[a] The highest inhibitory potency towards trypsin with K_i values of 6.3, 19 and 61 nM was found for the benzamidine derivative **1** and for the newly synthesized inhibitors **26** and **27**, respectively. All other derivatives showed reduced trypsin affinity ($K_i > 0.5$ μM).

Table 4. Anticoagulant activity of selected inhibitors

Compound	IC_{200} (μM)		
	aPTT	PT	TT
6	0.16	0.14	0.5
11	0.49	0.29	1.2
12	0.45	0.6	6.5
14	0.89	1.3	57
16	1.85	1.3	15
19	6.0	3.3	>100
27	0.15	0.39	0.11
29	0.30	0.79	0.13
30	0.18	0.47	0.10
33	0.32	0.61	0.17
34	0.23	0.60	0.09
35	0.16	0.35	0.03
36	0.58	0.85	0.23
37	0.47	0.73	0.15

Although compound **6** had excellent anticoagulant activity (Table 4), it was very rapidly eliminated from the circulation of rats after intravenous injection ($t_{1/2} < 20$ min) and had marginal oral bioavailability ($F < 5\%$, dose 5 mg/kg). Approximately 75% of the given inhibitor dose was found in the bile and ≈15% in the urine, which indicates that the inhibitor is rapidly cleared mainly via the hepatobiliary route. Therefore, further analogues were prepared by modification of the N-terminus to overcome these problems. The replacement of the benzylsulfonyl- by the smaller propylsulfonyl-residue (inhibitor **12**, Table 2) did not change the elimination rate, although we observed a slight shift in the elimination route; that is, similar amounts of approximately 45% of the inhibitor dose were found in the bile and also in the urine. In previous studies on compounds of different structural type we were able to demonstrate a significantly reduced clearance rate after incorporation of carboxyl groups into the inhibitors.[14] Therefore, we synthesized additional inhibitors containing carboxyl groups at the N-terminus while retaining the structure of the rest of the molecule (Table 2).

The racemic 4-(carboxymethyl)benzylsulfonyl derivative **11** inhibits fXa with a subnanomolar K_i value and retains strong anticoagulant activity, however, its elimination rate was not reduced compared to compound **6**. As found previously with the 4-amidinobenzylamide series,[9,10] the replacement of the benzylsulfonyl residue by non-aromatic P4 groups leads to reduced potency although several analogues still inhibit fXa with K_i values in the low nanomolar range. The oxalyl derivative **16** showed a slightly prolonged retention time in the circulation ($t_{1/2} \approx 30$ min), but also a weaker anticoagulant activity, which was further reduced in case of inhibitor **19**, containing a melagatran-like[15] *N*-carboxymethyl group (Table 4).

In recent years several groups have reported the development of dual fXa and thrombin inhibitors and proposed a stronger anticoagulant activity for such compounds compared to monoselective inhibitors.[16] Using this approach, we have designed inhibitors with improved thrombin affinity by replacement of the P2 glycine to recover some loss in anticoagulant activity, which has been observed with some of the compounds listed in Table 2. The incorporation of proline not only enhanced thrombin inhibition, but also improved the affinity towards fXa (Table 3). It is worth mentioning that the ratio between the fXa and thrombin inhibition could also be simply adjusted by the choice of the P3 amino acid: compounds **33** and **35** are superior fXa inhibitors, whereas the D-homotyrosine analogue **37** has higher affinity for thrombin. As expected, these P2 proline derivatives have stronger anticoagulant activity than their glycine analogues, for example, as seen with the inhibitor pairs **14/27** or **19/33** (Table 4).

The *N*-carboxymethylated ethyl ester prodrugs **30**, **34** and **36** were prepared in analogy to the structurally related thrombin inhibitor Ximelagatran, a compound that has sufficient oral bioavailability.[15] A major difference between Ximelagatran and these inhibitors exists in the P1 residues: at physiological pH the prodrug

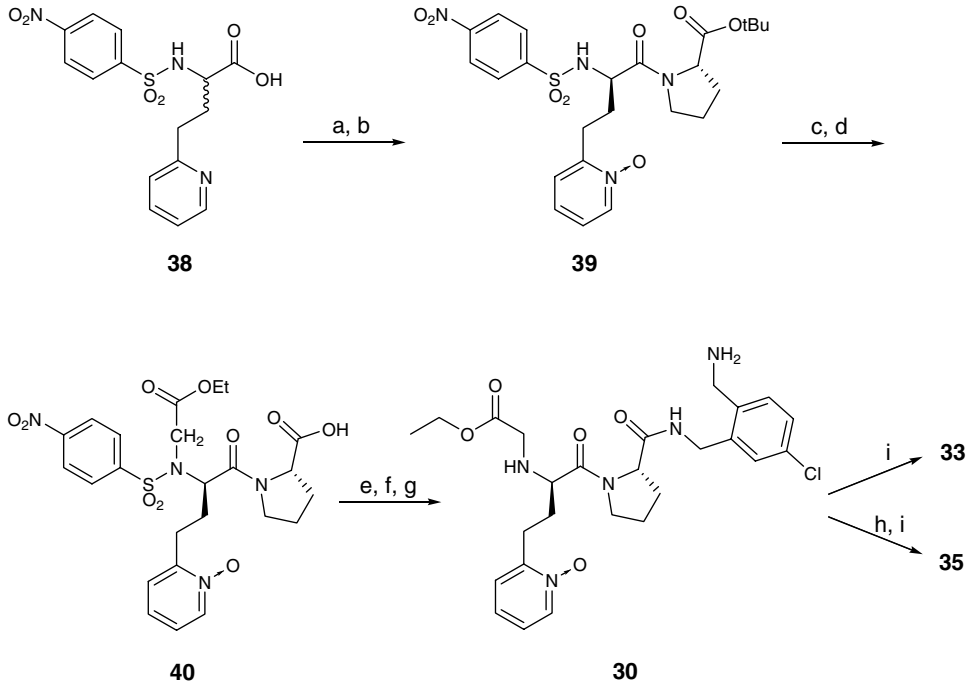
Ximelagatran contains a non-charged 4-hydroxyamidino-benzylamide moiety, whereas the inhibitors of our series posses a weakly basic 2-aminomethyl-5-chlorobenzylamide.

Several compounds listed in Tables 2 and 3 were initially evaluated in a permeability assay across a Caco-2 cell monolayer as a model for intestinal absorption. Compounds that show sufficient oral bioavailability and good absorption by passive diffusion across the intestinal membrane normally have a Caco-2 cell apparent permeability (P_{app}) of $\geqslant$100 nm/s.[18] The highest permeability was found for the ethyl ester of the P3 D-homotyrosine derivative **36** (P_{app} = 89 nm/s), which inhibits thrombin more strongly than fXa. A moderate permeability was also observed for the P3-D-homo-2-pyridylalanine inhibitor **35** (P_{app} = 50 nm/s). Surprisingly, its ethyl ester **34** was transported with reduced efficacy (P_{app} = 24 nm/s). For all other compounds tested, especially the more hydrophilic pyridine-*N*-oxide derivatives, only poor P_{app} values (<50 nm/s) were estimated.

In spite of the moderate Caco-2 cell permeability, we determined the oral bioavailability of selected compounds in rats. Unfortunately, none of the investigated compounds (**6**, **14**, **29**, **35**, **36**) exhibited a sufficient oral bioavailability at a dose of 5 mg/kg (F < 5%).[19]

In conclusion, we have developed highly potent substrate analogue dual inhibitors of the clotting proteases fXa and thrombin, which have strong anticoagulant activity and excellent selectivity towards the fibrinolytic enzymes uPA and plasmin. The highest inhibitory potency towards trypsin with K_i values of 19 and 61 nM was found for inhibitors **26** and **27**, respectively. All other derivatives showed reduced trypsin affinity (K_i > 0.5 μM). However, due to their insufficient pharmacokinetics, that is, low oral bioavailability and short circulation half-life, it is necessary to improve these compounds further.

Non-commercially available P1 residues used for inhibitors **2**, **3** and **6**–**9** were obtained by known procedures. 2-(Boc-amido)-5-aminomethylpyridine for compound **2** was prepared from 2-amino-5-cyanopyridine by Boc protection[20] and subsequent hydrogenation of the nitrile using Pd/C as catalyst. The 4-aminoethylpyridine for analogue **3** was prepared from 4-vinylpyridine by treatment with ammonium chloride.[21] 2-Boc-amidomethyl-5-Cl-benzylamine for inhibitor **6** was prepared as described by Nelson et al.,[22] the 5-chloromethyl-2-chlorothiophene was converted into the amine used for **8** by reaction with sodium azide, followed by brief hydrogenation in ethyl acetate using Pd/C as catalyst. The *des*-chloro P1 residues for inhibitors **7** and **9** were obtained by extended hydrogenation of the chloro aromatics. Most sulfonyl- and acyl chlorides, used for the modification of the P3 amino acid (Table 2), are commercially available or can be purchased as appropriate esters. Chlorosulfonyl-isopropyl acetate for inhibitor **13** was obtained from the bis-chloride by reaction with 1 equiv isopropanol in diethyl ether.[23] The substituted



Scheme 1. Reagents and conditions for the synthesis of inhibitors **30**, **33** and **35**: (a) H-Pro-O^{t}Bu × HCl, PyBOP, 3 equiv DIEA in DMF, 15 min 0 °C, 3 h room temperature; (b) 1.5 equiv mCPBA over 7 h in DCM, separation of the diastereomers by preparative HPLC; (c) 1.1 equiv Cs_2CO_3 in DMF, 15 min; 1.5 equiv $BrCH_2$–COOEt, 12 h, rt; (d) 90% TFA, 1.5 h room temperature; (e) 2-(Boc-amidomethyl)-5-chlorobenzylamine, PyBOP, 2 equiv DIEA in DMF, 15 min 0 °C, 3 h room temperature; (f) 2.25 equiv thiophenol, 4.5 equiv K_2CO_3 in acetonitrile, 5 h 50 °C, 12 h room temperature; (g) 90% TFA, 1 h room temperature, preparative HPLC provides **30** as a TFA salt; (h) zinc dust in acetic acid/1 N HCl 1:1, 1 h room temperature; (i) 1 N LiOH in dioxane (1:1), 1 h room temperature, preparative HPLC.

benzylsulfonylchloride used for **10** and **11** was obtained from methyl-2-(4-(bromomethyl)phenyl)acetate by reaction with sodium sulfite, followed by treatment with phosphorus pentachloride.[24] The *n*-hexyl- and cyclohexyl esters of bromoacetic acid necessary for inhibitors **31** and **32** were prepared from bromoacetyl bromide.[25]

A representative synthesis of the inhibitors is exemplarily described for compounds **30**, **33** and **35** (Scheme 1). Racemic homo-2-pyridylalanine[10] was converted to the 4-nitrobenzylsulfonyl amino acid **38**. PyBOP-mediated coupling of H-Pro-O^{t}Bu, followed by oxidation using mCPBA[26] and separation of the diastereomers by preparative HPLC, provided intermediate **39**, which was alkylated by reaction with ethyl bromoacetate in the presence of Cs_2CO_3 in DMF.[27] After *tert*-butyl ester cleavage and PyBOP-mediated coupling of 2-(Boc-amidomethyl)-5-Cl-benzylamine,[22] the 4-nitrophenylsulfonyl group was removed using thiophenol/K_2CO_3 in acetonitrile.[27] The Boc group was removed and the ethyl ester **30** was saponified using a mixture of 1 N LiOH and dioxane.

Acknowledgments

The authors thank Ute Altmann, Angelika Grau, Claudia Neuwirth, Cathleen Naumann and Martin Korsonewski for their excellent technical help. We thank also Robert Etges for correcting the English version of the manuscript. This work was supported by the 'Thüringer Aufbaubank'.

References and notes

1. Leadley, R. J., Jr. *Curr. Top. Med. Chem.* **2001**, *1*, 151.
2. Maignan, S.; Mikol, V. *Curr. Top. Med. Chem.* **2001**, *1*, 161.
3. Pauls, H. W.; Ewing, W. R.; Choi-Sledeski, Y. M. *Front. Med. Chem.* **2004**, *1*, 129.
4. Adler, M.; Kochanny, M. J.; Ye, B.; Rumennik, G.; Light, D. R.; Biancalana, S.; Whitlow, M. *Biochemistry* **2002**, *41*, 15514.
5. Choi-Sledeski, Y. M.; Kearney, R.; Poli, G.; Pauls, H.; Gardner, C.; Gong, Y.; Becker, M.; Davis, R.; Spada, A.; Liang, G.; Chu, V.; Brown, K.; Collussi, D.; Leadley, R., Jr.; Rebello, S.; Moxey, P.; Morgan, S.; Bentley, R.; Kasiewski, C.; Maignan, S.; Guilloteau, J.-P.; Mikol, V. *J. Med. Chem.* **2003**, *46*, 681.
6. Roehrig, S.; Straub, A.; Pohlmann, J.; Lampe, T.; Pernerstorfer, J.; Schlemmer, K.-H.; Reinemer, P.; Perzborn, E. *J. Med. Chem.* **2005**, *48*, 5900.
7. Kubitza, D.; Becka, M.; Voith, B.; Zuehlsdorf, M.; Wensing, G. *Clin. Pharmacol. Ther.* **2005**, *78*, 412.
8. Kubitza, D.; Haas, S. *Exp. Opin. Invest. Drugs* **2006**, *15*, 843.
9. Schweinitz, A.; Stürzebecher, A.; Stürzebecher, U.; Schuster, O.; Stürzebecher, J.; Steinmetzer, T. *Med. Chem.* **2006**, *2*, 349.
10. Stürzebecher, A.; Dönnecke, D; Schweinitz, A.; Schuster, O.; Steinmetzer, P.; Stürzebecher, U.; Stürzebecher, J.; Steinmetzer, T. *ChemMedChem*, in press.
11. (a) Feng, D.-M.; Gardell, S. J.; Lewis, S. D.; Bock, M. G.; Chen, Z.; Freidinger, R. M.; Naylor-Olsen, A. M.; Ramjit, H. G.; Woltmann, R.; Baskin, E. P.; Lynch, J. J.; Lucas, R.; Shafer, J. A.; Dancheck, K. B.; Chen, I. W.; Mao, S. S.; Krueger, J. A.; Hare, T. R.; Mulichak, A. M.; Vacca, J. P. *J. Med. Chem.* **1997**, *40*, 3726; (b) Lumma, W. C.; Witherup, K. M.; Tucker, T. J.; Brady, S. F.; Sisko, J. T.; Naylor-Olsen, A. M.; Lewis, S. D.; Lucas, B. J.; Vacca, J. P. *J. Med. Chem.* **1998**, *41*, 1011; (c) Tucker, T. J.; Brady, S. F.; Lumma, W. C.; Lewis, S. D.; Gardell, S. J.; Naylor-Olsen, A. M.; Yan, Y.; Sisko, J. T.; Stauffer, K. J.; Lucas, B. J.; Lynch, J. J.; Cook, J. J.; Stranieri, M. T.; Holahan, M. A.; Lyle, E. A.; Baskin, E. P.; Chen, I. W.; Dancheck, K. B.; Krueger, J. A.; Cooper, C. M.; Vacca, J. P. *J. Med. Chem.* **1998**, *41*, 3210; (d) Rittle, K. E.; Barrow, J. C.; Cutrona, K. J.; Glass, K. L.; Krueger, J. A.; Kuo, L. C.; Lewis, D. S.; Lucas, B. J.; McMasters, D. R.; Morrissette, M. M.; Nantermet, P. G.; Newton, C. L.; Sanders, W. M.; Yan, Y.; Vacca, J. P.; Selnick, H. G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3477; (e) Morisette, M. M.; Stauffer, K. J.; Williams, P. D.; Lyle, T. A.; Vacca, J. P.; Krueger, J. A.; Lewis, S. D.; Lucas, B. J.; Wong, B. K.; White, R. B.; Miller-Stein, C.; Lyle, E. A.; Wallace, A. A.; Leonard, Y. M.; Welsh, D. C.; Lynch, J. J.; McMasters, D. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4161.
12. Stauffer, K. J.; Williams, P. D.; Selnick, H. G.; Nantermet, P. G.; Newton, C. L.; Homnick, C. F.; Zrada, M. M.; Lewis, S. D.; Lucas, B. J.; Krueger, J. A.; Pietrak, B. L.; Lyle, E. A.; Singh, R.; Miller-Stein, C.; White, R. B.; Wong, B.; Wallace, A. A.; Sitko, G. R.; Cook, J. J.; Holahan, M. A.; Stranieri-Michener, M.; Leonard, Y. M.; Lynch, J. J., Jr.; McMasters, D. R.; Yan, Y. *J. Med. Chem.* **2005**, *48*, 2282.
13. (a) Mederski, W. W. K. R.; Cezanne, B.; van Amsterdam, C.; Bühring, K.-U.; Dorsch, D.; Gleitz, J.; März, J.; Tsaklakidis, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5817; (b) Bauer, S. M.; Goldman, E. A.; Huang, W.; Su, T.; Wang, L.; Woolfrey, J.; Wu, Y.; Zuckett, J. F.; Arfsten, A.; Huang, B.; Kothule, J.; Lin, J.; May, B.; Sinha, U.; Wong, P. W.; Hutchaleelaha, A.; Scarborough, R. M.; Zhu, B.-Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4045.
14. (a) Stürzebecher, J.; Prasa, D.; Hauptmann, J.; Vieweg, H.; Wikström, P. *J. Med. Chem.* **1997**, *40*, 3091; (b) Schweinitz, A.; Steinmetzer, T.; Banke, I. J.; Arlt, M. J. E.; Stürzebecher, A.; Schuster, O.; Geissler, A.; Giersiefen, H.; Zeslawska, E.; Jacob, U.; Krüger, A.; Stürzebecher, J. *J. Biol. Chem.* **2004**, *279*, 33613; (c) Hauptmann, J.; Steinmetzer, T.; Vieweg, H.; Wikström, P.; Stürzebecher, J. *Pharm. Res.* **2002**, *19*, 1028.
15. Gustafsson, D.; Bylund, R.; Antonsson, T.; Nilsson, I.; Nyström, J.-E.; Eriksson, U.; Bredberg, U.; Teger-Nilsson, A.-C. *Nat. Rev. Drug Disc.* **2004**, *3*, 649.
16. (a) Buijsman, R. C.; Basten, J. E.; van Dinther, T. G.; van der Marel, G. A.; van Boeckel, C. A.; van Boom, J. H. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2013; (b) Hauel, N. H.; Nar, H.; Priepke, H.; Ries, U.; Stassen, J.-M.; Wienen, W. *J. Med. Chem.* **2002**, *45*, 1757; (c) Deng, J. Z.; McMasters, D. R.; Rabbat, P. M.; Williams, P. D.; Coburn, C. A.; Yan, Y.; Kuo, L. C.; Lewis, S. D.; Lucas, B. J.; Krueger, J. A.; Strulovici, B.; Vacca, J. P.; Lyle, T. A.; Burgey, C. S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4411.
17. The P3 stereochemistry of the final inhibitors was assigned by the measurement of their inhibition constants. Based on previously established structure activity relationships within this type of substrate analogue inhibitors it is known that the inhibitory potency is significantly enhanced for compounds with P3 amino acids in D-configuration.
18. Artursson, P.; Karlsson, J. *Biochem. Biophys. Res. Commun.* **1991**, *175*, 880.
19. The pharmacokinetic studies were performed at GenPharmTox Biotech AG, Planegg, Germany, and Aurigon

Life Science GmbH, Tutzing, Germany, according to the guidelines of the German Animal Health and Welfare Act.
20. Krein, D. M.; Lowary, T. L. *J. Org. Chem.* **2002**, *67*, 4965.
21. Steinmetzer, T.; Schweinitz, A.; Stürzebecher, A.; Dönencke, D.; Uhland, K.; Schuster, O.; Steinmetzer, P.; Müller, F.; Friedrich, R.; Than, M. E.; Bode, W.; Stürzebecher, J. *J. Med. Chem.* **2006**, *49*, 4116.
22. Nelson, T. D.; LeBlond, C. R.; Frantz, D. E.; Matty, L.; Mitten, J. V.; Weaver, D. G.; Moore, J. C.; Kim, J. M.; Boyd, R.; Kim, P.-Y.; Gbewonyo, K.; Brower, M.; Sturr, M.; McLaughlin, K.; McMasters, D. R.; Kress, M. H.; McNamara, J. M.; Dolling, U. H. *J. Org. Chem.* **2004**, *69*, 3620.
23. Long, D. D.; Termin, A. P. *Tetrahedron Lett.* **2000**, *41*, 6743.
24. Johnson, T. B.; Ambler, J. A. *J. Am. Chem. Soc.* **1914**, *36*, 372.
25. Skinner, W. A.; Hayford, J.; Shellenberger, T. E.; Colwell, W. T. *J. Med. Chem.* **1966**, *9*, 605.
26. Bremner, D. H.; Sturrock, K. R.; Wishart, G.; Mitchell, S. R.; Nicoll, S. M.; Jones, G. *Synth. Commun.* **1997**, *27*, 1535.
27. Bowman, W. R.; Coghlan, D. R. *Tetrahedron* **1997**, *53*, 15787.