Bioorganic & Medicinal Chemistry Letters 18 (2008) 4154–4158

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Inhibitors of dipeptidyl peptidase 8 and dipeptidyl peptidase 9. Part 1: Identification of dipeptide derived leads

Pieter Van der Veken [a,*], Ingrid De Meester [b], Véronique Dubois [b], Anna Soroka [a], Sebastiaan Van Goethem [a], Marie-Berthe Maes [b], Inger Brandt [b], Anne-Marie Lambeir [b], Xin Chen [c], Achiel Haemers [a], Simon Scharpé [b], Koen Augustyns [a]

[a] Department of Medicinal Chemistry, University of Antwerp (UA), Universiteitsplein 1, B-2610 Antwerp, Belgium
[b] Medical Biochemistry, University of Antwerp (UA), Universiteitsplein 1, B-2610 Antwerp, Belgium
[c] Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Zhu Nan, Miaoli 350, Taiwan, ROC

ARTICLE INFO

Article history:
Received 23 April 2008
Revised 19 May 2008
Accepted 20 May 2008
Available online 24 May 2008

Keywords:
DPP8
DPP9
DPP IV
DPP4
DPP II
DPP2
Inhibitors
Selectivity
Dipeptide-derived
Cyanopyrrolidines
Nitriles

ABSTRACT

Dipeptide derivatives bearing various P2 residues and pyrrolidine derivatives as P1 mimics were evaluated in order to identify lead structures for the development of DPP8 and DPP9 inhibitors. Structure–activity-relationship data obtained in this way led to the preparation of a series of α-aminoacyl ((*2S*, *4S*)-4-azido-2-cyanopyrrolidines). These compounds were shown to be nanomolar DPP8/9 inhibitors with modest overall selectivity toward DPP IV and DPP II.

Proline-selective dipeptidyl-peptidases (DPPs) have garnered intense research interest since it has become clear that several metabolically important peptides, for example, the insulin-releasing incretin hormone glucagon-like peptide I, are substrates of dipeptidyl-peptidase IV (DPP IV, EC 3.4.14.5).[1] Currently, two inhibitors of this enzyme (sitagliptin/januvia and vildagliptin/galvus) have been approved for the treatment of type 2 diabetes.[2,3] Several other small molecule inhibitors have reached different stages of clinical investigation (e.g. BI 1356, saxagliptin, alogliptin,).[4–6] A number of catalytically active DPPs distinct from DPP IV (DPP II, FAP, DPP8, and DPP9) has been described so far, but these enzymes remain poorly characterized and their natural substrates have not yet been identified.[7–11]

Recently, attention was drawn to the two closely related monomeric cytosolic proteases DPP8 and 9. Inhibition of these enzymes was reported by Lankas et al. to be associated with severe in vivo toxicity in animal models and to provoke attenuation of T-cell proliferation in human in vitro models.[12] In addition, another study claims the observation that DPP8 and DPP9 are up-regulated in experimentally induced asthma and that these peptidases specifically respond to the inflammatory stimulus.[13] In order to verify whether the reported toxicity observations are directly related to DPP8/9 inhibition and to further study the clinical relevance of potential enzyme involvement in pathologies, there is a need for new, structurally distinct inhibitors of these enzymes.[14]

The high degrees of sequence homology between DPP8 and DPP9 on one side and DPP IV on the other, makes selective DPP8/9 inhibitor design a challenging task that hitherto has been the subject of only a limited number of publications.[15,16] Moreover, successful accounts of inhibitor development studies leading to compounds with DPP8 or DPP9 selectivity have not been reported to date.

The aim of this study was to evaluate a structurally diverse library of dipeptide-derived compounds, in order to identify possible leads for the development of inhibitors with maximal affinity and selectivity for DPP 8 and 9. The concept of developing substrate-inspired, dipeptide-derived compounds is still being

* Corresponding author.
E-mail address: pieter.vanderveken@ua.ac.be (P. Van der Veken).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.05.080

explored intensively for DPP IV and DPP II and has led to the majority of inhibitors reported for these enzymes. Furthermore, these compounds possess validated potential for in vivo applicability and efficacy.[17] As suggested by the bimodular structure of dipeptides, compounds prepared and evaluated for this study were divided in P2- and P1-modified variants of the α-aminoacyl pyrrolidine template structure generally used in DPP inhibitor design. In addition, a range of relevant reference inhibitors was tested to support interpretation of the biochemical evaluation results of the library compounds (Fig. 1).

Biochemical evaluation results for the reference inhibitors are summarized in Table 1. Our data confirm the poor selectivity profiles that have been suggested or published (e.g. Val-*boro*Pro) in literature for structures **1**–**4**.[17,18] These compounds were developed before the discussion on potential toxicity of particularly DPP8/9 targeting compounds that reached the forefront of DPP inhibitor development programs. Biological effects reported for these compounds, all of which were investigated pharmacologically, might therefore result from the concomitant functional abrogation of several DPPs.

Table 2 outlines results of the biochemical evaluation of a series of α-aminoacyl pyrrolidines. With these compounds, we wanted to probe the P2 structural requirements for DPP8/9 affinity. Sterically and electronically differing natural (L-)amino acids and closely related analogues or derivatives were selected at this position.[19]

In general, all compounds within the pyrrolidine series share poor selectivity for a specific DPP. For DPP8 and DPP9, binding preference is observed for (a) aliphatic and (b) basic residues. Compounds **1** (Val), **11** (Ile), **12** (*allo*-Ile) and **31** (Nle) can serve to illustrate the former statement. The *allo*-Ile residue seems to be accommodated slightly better by DPP8 compared to DPP9, a factor that was recognized earlier for the P1-thiazolidine analogue of **12** by Xu et al.[20] Among the basic amino acids, the side chain of Lys (**24**) is preferred over the shorter Orn (**26**). In analogy with DPP II and DPP IV, DPP8 and DPP9 also accept the Z-blocked basic amino acid side chains in P2, indicating that larger groups can be tolerated at this position (compounds **25**, **27**, **29**). [19b] Finally, the propylguanidine residue of compound **28** (P2 = Arg) might be regarded as a structural element that offers the possibility to maximize selectivity for DPP8 over DPP9. In conclusion, the side chains of Ile, *allo*-Ile, Lys, Lys(Z) and Arg can be selected as useful P2-fragments for dipeptide derived DPP8 or DPP9 inhibitors. Evidently, they should be combined with a P1 fragment or derivatised with substituents (e.g. at the ε-amine function of lysine) that confer acceptable selectivity ratios with regard to the non-targeted DPPs.

In order to identify selectivity conferring P1 fragments, various pyrrolidine analogues or derivatives were evaluated in combination with a P2 Ile, Lys or Lys(Z) residue. The results are outlined in Table 3.

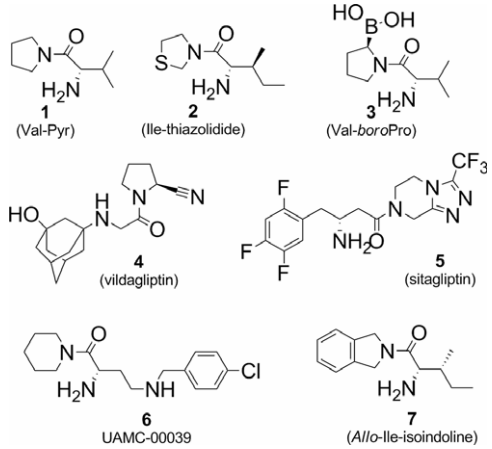


Figure 1. Reference compounds used in this study.

Table 1
IC_{50} values for reference compounds determined in the conditions of this study

Compound	IC_{50} (μM)			
	DPP8	DPP9	DPP IV	DPP II
1	5.2 ± 0.3	11.3 ± 0.48	5.4 ± 0.3	153 ± 12
2	6.0 ± 3.7	6.6 ± 0.4	1.7 ± 0.1	28 ± 9
3	0.051 ± 0.002	n.d.[a]	0.022 ± 0.001	0.086 ± 0.007
4	9.0 ± 0.1	0.68 ± 0.02	0.12 ± 0.01[b]	>1000
5	>50	>100	0.04 ± 0.001	>100
6	142 ± 27	76.6 ± 6.1	165 ± 9	<0.0005[c]
7	0.12 ± 0.01	0.29 ± 0.02	90 ± 4	29 ± 1

[a] n.d., not determined.
[b] K_i = 17 ± 2 nM.
[c] K_i = 0.082 ± 0.048 nM.

Table 2
Evaluation of P2 residues in a series of pyrrolidine derived dipeptides

P2-N

Compound	P2	IC_{50} (μM)			
		DPP8	DPP9	DPP IV	DPP II
8	Gly	>1000	>1000	>1000	>1000
9	Ala	411 ± 38	n.d.[a]	41 ± 6	179 ± 15
10	Pro	50 ± 2	n.d.	15 ± 2	>500
11	Ile	6.6 ± 0.4	13.7 ± 2	4 ± 1	110 ± 7
12	*allo*-Ile	0.55 ± 0.04	2.1 ± 0.1	3.9 ± 0.1	145 ± 33
13	Leu	23 ± 2	68.1 ± 5.9	24 ± 1	122 ± 14
14	Cha[b]	10.5 ± 0.5	n.d.	17 ± 2	42 ± 3
15	Trp	242 ± 37	>100	286 ± 31	49 ± 3
16	Phe	115 ± 8	n.d.	21 ± 4	79 ± 29
17	Glu	>1000	>100	>1000	>1000
18	Asp	>1000	n.d.	122 ± 2	>500
19	Asn	>500	n.d.	188 ± 6	152 ± 50
20	Thr	41 ± 3	34.2 ± 1.5	98 ± 3	230 ± 46
21	Cys	6.8 ± 0.7	62.4 ± 3.8	37 ± 3	19 ± 0.5
22	Ser	>500	n.d.	190 ± 120	65 ± 30
23	Tyr	95 ± 14	76.9 ± 4.2	43.5 ± 2.3	15.7 ± 0.8
24	Lys	4.7 ± 0.2	5.1 ± 0.25	39 ± 2	9.9 ± 1.3
25	Lys(Z)	1.1 ± 0.5	2.79 ± 0.17	15.6 ± 5.7	5.7 ± 0.3
26	Orn	19 ± 1	n.d.	118 ± 17	9.8 ± 0.5
27	Orn(Z)	5.7 ± 0.6	14.5 ± 0.5	9.7 ± 0.5	3.7 ± 0.3
28	Arg	1.9 ± 0.3	10.9 ± 0.7	12.7 ± 0.7	3.1 ± 0.9
29	Arg(diZ)	12 ± 1	n.d.	16.8 ± 0.6	0.86 ± 0.04
30	His	>500	>100	23.1 ± 1.0	1.2 ± 0.1
31	Nle[c]	5.6 ± 0.2	15.9 ± 0.28	15 ± 0.8	60 ± 5

[a] n.d., not determined.
[b] Cha, L-cyclohexylalanine.

Changing the P1 ring size to azetidine, piperidine, tetrahydroisoquinoline or azepine (**32**–**34**, **36**, **44**, **49**, **51**, **55**) or replacing pyrrolidine by cyclopentylamine (**42**) did not result in more potent DPP8 or DPP9 inhibitors. The five-membered pyrrolidine ring seems to be optimal for inhibition with substitutions (**37**–**40**, **45**–**47**) rigidification (**35**) and even ringfusions (iso-indolines **43**, **50** and **54**) being accepted. The potential of these isoindolines to discern between DPP8/9 and DPP IV, is in line with earlier reports by Jiaang et al. and Lankas et al.[12,16b] For the same reason, azido-substituted pyrrolidines **38** and **47** deserve mentioning. The latter fragment was selected for the further development of DPP8/9 inhibitors (*vide infra*). Finally, similar to what was shown earlier for DPP IV, the introduction of an electrophilic (*2S*)-carbonitrile function on the pyrrolidine ring leads to compounds with at least one order of magnitude higher potency for DPP8 and 9 (compounds **48**, **49** and **53**).[21]

The DPP8/9 selectivity potential of azide substituted products mentioned earlier, together with the enhanced potency observed

Table 3
Evaluation of P1 residues in dipeptide-derived DPP inhibitors

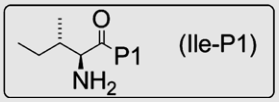
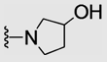
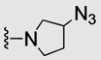
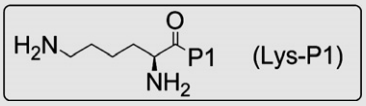
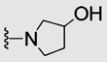
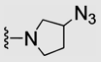
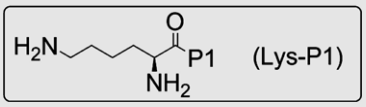
Compound	P1	IC_{50} (μM)			
		DPP8	DPP9	DPP IV	DPP II
					
32		127 ± 8	>100	50.4 ± 2.3	730 ± 140
11		6.6 ± 0.4	13.7 ± 2	4 ± 1	110 ± 7
33		76 ± 4	76 ± 4	67 ± 11	59 ± 15
34		612 ± 69	>100	374 ± 62	228 ± 16
35		14 ± 2	38.4 ± 2.8	15 ± 1.2	288 ± 33
2		6.0 ± 3.7	6.6 ± 0.4	1.7 ± 0.1	28 ± 9
36		23 ± 8	>50	52 ± 3	52 ± 3
37		130 ± 21	>100	93 ± 6	299 ± 2
38		10.3 ± 0.5	16 ± 2	107 ± 6	43 ± 5
39		3.8 ± 1.3	11.2 ± 0.7	3.5 ± 0.2	111 ± 10
40		26 ± 2	50 ± 5	94 ± 6	30 ± 1
42		>1000	>100	>1000	>100
43		1.32 ± 0.25	4.3 ± 0.1	115 ± 11	43 ± 11
					
44		40.5 ± 1.9	28 ± 4	247 ± 20	1.6 ± 0.3
45		3.1 ± 0.1	5.1 ± 0.25	23.0 ± 1.4	2.9 ± 0.2
46		139 ± 9	n.d.	500 ± 25	48 ± 3
47		12.5 ± 1	26.4 ± 5.8	>1000	4.9 ± 0.5
48		0.34 ± 0.02	0.118 ± 0.003	0.32 ± 0.03	0.577 ± 0.625
49		5.2 ± 0.3	n.d.	51 ± 2	1.5 ± 0.09
50		0.20 ± 0.01	0.5 ± 0.03	161 ± 8	1.78 ± 0.08
51		312 ± 22	>100	>1000	393 ± 76
					
52		0.52 ± 0.025	1.42 ± 0.05	3.7 ± 0.33	1.02 ± 0.05

Table 3 (*continued*)

Compound	P1	IC_{50} (μM)			
		DPP8	DPP9	DPP IV	DPP II
53		0.24 ± 0.02	0.212 ± 0.009	0.035 ± 0.002	0.84 ± 0.04
54		0.20 ± 0.02	n.d.	245 ± 85	2.5 ± 0.3
55		>500	>100	>1000	390 ± 50

Table 4
Evaluation of inhibitors containing a P1 4-azido-2-cyanopyrrolidine fragment

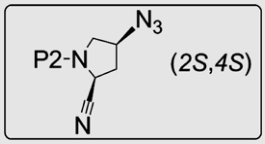
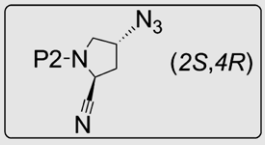
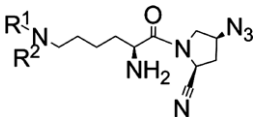
Compound	P2	IC_{50} (μM)			
		DPP8	DPP9	DPP IV	DPP II
					
56	Ile	0.072 ± 0.004	0.035 ± 0.001	0.34 ± 0.01	38 ± 9
57	*Allo*-Ile	0.011 ± 0.0004	0.0063 ± 0.0003	0.29 ± 0.01	24 ± 2
58	Lys	0.071 ± 0.002	0.025 ± 0.002	2.2 ± 0.2	0.31 ± 0.03
59	Lys(Z)	0.021 ± 0.001	n.d.	0.53 ± 0.05	0.35 ± 0.02
					
60	*Allo*-Ile	22 ± 1.8	9.2 ± 0.4	52 ± 2	90 ± 13

Table 5
Evaluation of dipeptide 4-azido-2-cyanopyrroli dines containing a P2 ε-substituted lysine residue



Compound	R^1	R^2	IC_{50} (μM)			
			DPP8	DPP9	DPPIV	DPP II
61	1-Naphthyl	1-Naphthyl	0.187 ± 0.012	0.082 ± 0.003	2.5 ± 0.2	0.025 ± 0.001
62	2-Naphthyl	H–	0.0138 ± 0.0006	0.0046 ± 0.0001	0.56 ± 0.04	0.034 ± 0.001
63	2-Naphthyl	2-Naphthyl	0.202 ± 0.009	0.088 ± 0.007	n.d.	0.068 ± 0.002

for 2-cyanopyrrolidines incited us to prepare α-aminoacyl (4-azido-2-cyanopyrrolidines) **57**–**60** (Table 4). The synthesis of both the (*2S,4S*) and (*2S,4R*) P1-fragments of these compounds was accomplished by an amidation–dehydration sequence on the corresponding Boc-protected (*4R*)- and (*4S*)-azidoprolines, identical to the literature preparation of 2-cyanopyrrolidine.[21,22] Upon biochemical evaluation, these compounds showed excellent potency toward DPP8 and DPP9, accompanied however by limited selectivity with respect to DPP IV and DPP II. The most promising compound of this series was found to be **57**, containing a P2 *allo*-Ile. It combines low nanomolar DPP8/9 inhibitory activity with a selectivity index toward DPP IV of approximately 30 and toward DPP II of approximately 1000. Comparison of the potencies of **57** and **60** demonstrates the preference of all four DPPs for a (*2S,4S*) configuration of the P1 building block.

In an attempt to further optimize the activity/selectivity profiles of these compounds, analogues containing P2 ε-*N*-substituted lysine residues were prepared (Table 5) using a reductive amination protocol optimized previously.[23] Only bulky naphthyl substituents were at this stage selected as they were considered appropriate to provide information on the amount of space available for ligand binding in the active centers of the target

enzymes. While the sterically most demanding bis(naphthyl) compounds (**61** and **63**) displayed a small decrease in both DPP8 and DPP9 potency as compared to parent structure **58** (Table 4), all three ε-substituted structures exhibited a markedly increased DPP II inhibitory activity. Since these results indicate that substitution of the terminal amine function of lysine without simultaneous modification of its linear butyl sidechain has the propensity to increase DPP II activity, no additional analogues of compounds **61**–**63** were planned.

In summary, a library of dipeptide derived compounds were evaluated in order to identify leads for the development of inhibitors of DPP8 and DPP9. The data generated, suggest significant overall similarity in the architecture of the active centers of all DPPs. Using the obtained SAR information, a series of α-aminoacyl ((*2S*,*4S*)-4-azido-2-cyanopyrroli-dines) were prepared, yielding nanomolar DPP8/9 inhibitors with modest overall selectivity toward DPP IV and DPP II. Further structural modification of the P2 residue is currently being investigated as a means to obtain compounds with maximal affinity and selectivity for DPP8 and/or DPP9.

Acknowledgments

The research reported here is financially supported by the Fund for Scientific Research Flanders. P. Van der Veken and M.B. Maes are fellows of the Fund for Scientic Research Flanders.

Supporting information

Enzymatic assay conditions are described in the supplementary information to this article. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.05.080.

References and notes

1. Lambeir, A. M.; Durinx, C.; Scharpé, S.; De Meester, I. *Crit. Rev. Clin. Lab. Med.* **2003**, *40*, 209.
2. Amori, R. E.; Lau, J.; Pittas, A. G. *JAMA* **2007**, *298*, 194.
3. Utzschneider, K. M.; Tong, J.; Montgomery, M.; Udayasankar, J.; Gerchman, F.; Marcovina, S.; Watson, C. E.; Ligueros-Saylan, M. A.; Foley, J. E.; Holst, J. J.; Deacon, C. F.; Kahn, S. E. *Diabetes Care* **2008**, *31*, 108.
4. Eckhardt, M.; Langkopf, M.; Mark, M.; Taddayon, M.; Thomas, L.; Nar, H.; Pfrengle, W.; Guth, B.; Lotz, R.; Sieger, P.; Fuchs, H.; Himmelsbach, F. *J. Med. Chem.* **2007**, *50*, 6450.
5. Augeri, D. J.; Robl, J. A.; Betebenner, D. A.; Magnin, D. R., et al *J. Med. Chem.* **2005**, *48*, 5025.
6. Feng, J.; Zhang, Z.; Wallace, M. B., et al *J. Med. Chem.* **2007**, *50*, 2297.
7. Maes, M. B.; Scharpé, S.; De Meester, I. *Clin. Chim. Acta* **2007**, *380*, 31.
8. Henry, L. R.; Lee, H. O.; Lee, J. S.; Klein-Saznto, S.; Watts, P.; Ross, E.-A.; Chen, W. T.; Cheng, J. D. *Clin. Cancer Res.* **2007**, *13*, 1736.
9. Van der Veken, P.; Haemers, A.; Augustyns, K. *Curr. Topics Med. Chem.* **2007**, *7*, 621.
10. Abbott, C. A.; Yu, D. M.; Woollat, E.; Sutherland, G. R.; McCaughan, G. W.; Gorrell, M. D. *Eur. J. Biochem./FEBS* **2000**, *267*, 6140.
11. Olsen, C.; Wagtmann, N. *Gene* **2002**, *299*, 185.
12. Lankas, G. R.; Leiting, B.; Roy, R. S.; Eiermann, G. J.; Beconi, M. G.; Biftu, T.; Chan, C. C.; Edmondson, S.; Feeney, W. P., et al *Diabetes* **2005**, *54*, 2988.
13. Schade, J.; Stephan, M.; Schmiedl, A.; Wagner, L.; Niestroj, A. J.; Demuth, H.-U.; Frerker, N.; Klemann, C.; Raber, K. A.; Pabst, R.; von Hörsten, S. *J. Histochem. Cytochem.* **2008**, *56*, 147.
14. Rosemblum, J. S.; Minimo, L. C.; Liu, Y.; Wu, J.; Kozarich, J. W. National meeting of the American Diabetes Association, 2007. *Diabetes* **2007**, *56*, A138.
15. Dubois, V.; Lambeir, A. M.; Van der Veken, P.; Augustyns, K.; Creemers, J.; Chen, X.; Scharpé, S.; De Meester, I. *Front. Biosci.* **2008**, *13*, 3558.
16. (a) Van der Veken, P.; Soroka, A.; Brandt, I.; Chen, Y. S.; Maes, M. B.; Lambeir, A. M.; Chen, X.; Haemers, A.; Scharpé, S.; Augustyns, K.; De Meester, I. *J. Med. Chem.* **2007**, *50*, 5568; (b) Jiaang, W.-T.; Chen, Y.-S.; Hsu, T.; Wu, S.-H.; Chien, C.-H.; Chang, C.-N.; Chang, S.-P.; Lee, S.-J.; Chen, X. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 687; (c) Lu, I. L.; Lee, S. J.; Tsu, H.; Kao, K. H.; Chien, C. H.; Chang, Y. Y.; Chen, Y. S.; Cheng, J. H.; Chang, C. N.; Chen, T. W.; Chang, S. P.; Chen, X.; Jiaang, W. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3271.
17. Augustyns, K.; Van der Veken, P.; Haemers, A. *Expert Opin. Ther. Patents* **2005**, *15*, 1397.
18. For a discussion on the discrepancy between the IC_{50} and Ki values for vildagliptin (**4**), see: Brandt, I.; Joossens, J.; Chen, X.; Maes, M. B.; Scharpé, S.; De Meester, I.; Lambeir, A. M. *Biochem. Pharmacol.* **2005**, *70*, 134.
19. Compounds **8**–**55** were synthesised as reported previously (a) Augustyns, K.; Lambeir, A.; Borloo, M.; De Meester, I.; Vedernikova, I.; Vanhoof, G.; Hendriks, D.; Scharpé, S.; Haemers, A. *Eur. J. Med. Chem.* **1997**, *32*, 301; (b) Senten, K.; Van der Veken, P.; Bal, G.; De Meester, I.; Lambeir, A. M.; Scharpé, S.; Beauvois, B.; Haemers, A.; Augustyns, K. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2825.
20. Xu, J.; Wei, L.; Mathvink, R.; He, J.; Park, Y-J.; HE, H.; Leiting, B.; Lyons, K.; Marsilio, F.; Patel, R. A.; Wu, J. K.; Thornberry, N.; Weber, A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2533.
21. Ashworth, D. A.; Atrash, B.; Baker, G. A.; Baxter, A. J.; Jenkins, P. D.; Jones, M. D.; Szelke, M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1163.
22. Gomez-Vidal, J. A.; Silverman, R. B. *Org. Lett.* **2000**, *2*, 3743.
23. Soroka, A.; Van der Veken, P.; De Meester, I.; Lambeir, A.; Maes, M. B.; Scharpé, S.; Haemers, A.; Augustyns, K. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4777.