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Discovery of Potent and Selective Phenylalanine Derived CCR3 Receptor Antagonists. Part 2

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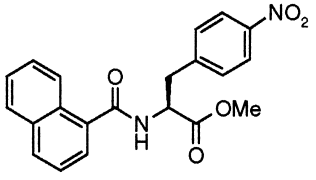
Abstract—Highly potent CCR3 antagonists have been developed from a previously reported series of phenylalanine ester-based leads. Solution-phase, parallel synthesis optimization was utilized to identify highly potent, functional CCR3 antagonists. © 2001 Elsevier Science Ltd. All rights reserved.

The orchestrated infiltration of specific pro-inflammatory leukocytes to sites of inflammation is recognized as one of the hallmarks of a robust immune response.[1] Recently, several CC chemokines have been shown to be key players in this response[2] and amongst these, eotaxin and eotaxin-2 have been reported to be important mediators for the selective recruitment of eosinophils into the lungs of patients suffering from allergic diseases such as asthma.[3] Both of these proteins elicit their biological response by binding to and activating a cell surface, seven-transmembrane spanning G-protein coupled receptor designated CCR3.[4] Under pathophysiological conditions, the recruitment and subsequent activation of eosinophils results in the release of cytotoxic and other mediators which ultimately lead to the clinically observed manifestations of inflammatory disease.[5] Approaches towards the in vivo blockade of CCR3 have included the evaluation of neutralizing antibodies raised against eotaxin,[6] modified chemokines,[7] and also small molecule antagonists.[8] Our interest in this area has been focused on the last of these approaches, that is, the development of selective, small molecule CCR3 antagonists as antiinflammatory agents. We have recently reported the discovery and initial SARs of a series of highly selective and potent phenylalanine derived CCR3 antagonists.[9]

A prototypical member of this class of compound, **1**, was highly effective in blocking both the binding and functional activity of a number of physiologically relevant CCR3 agonists such as eotaxin, eotaxin-2, and MCP-4. The presence of a metabolically labile ester functionality, however, precluded the evaluation of **1** in in vivo models of inflammatory disease and we sought to replace the undesired ester moiety with a more stable isostere. We report herein the successful realization of this goal and describe the SAR of this novel class of CCR3 antagonists.



1
Binding IC_{50} = 5 nM
Ca^{2+} Mobilization IC_{50} = 38 nM

Chemistry[10]

The compounds described in this paper were prepared using the procedures outlined in Schemes 1–7 which are based on previously reported protocols. Compounds **2**–**5** and **8**–**15** (Table 1) were synthesized by the routes in Schemes 1–4 and the tetrazoles **6** and **7** obtained using the chemistry reported by Liskamp[11a] and Moltzen.[11b]

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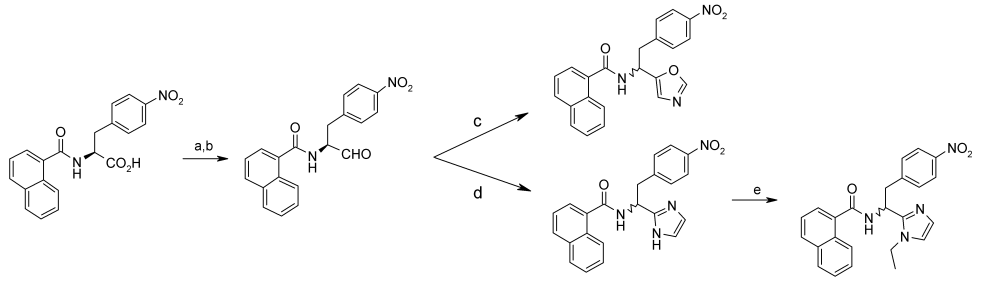
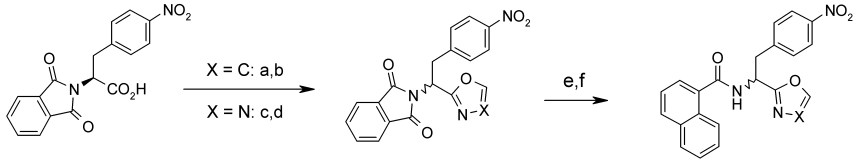
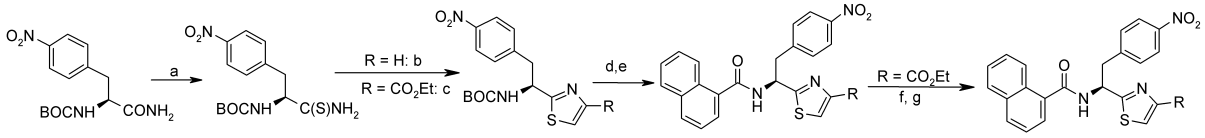


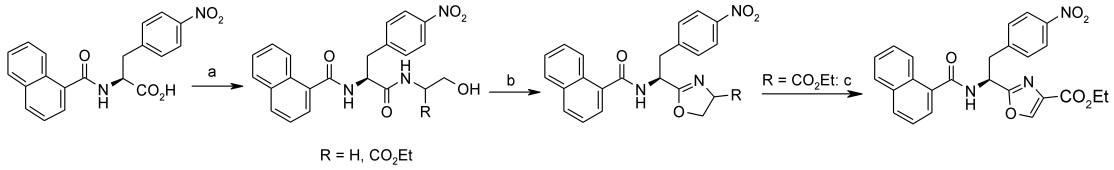
Scheme 1.[12] Reagents: (a) *N,O*-Dimethylhydroxylamine hydrochloride, EDCI, HOBT, NMM, DMF; (b) DIBAL-H, THF, 0 °C; (c) *p*-toluenesulfonyl isocyanate, K_2CO_3, MeOH; (d) ammonia, glyoxal (40%), DMF; (e) aq KOH, EtI, DMF.



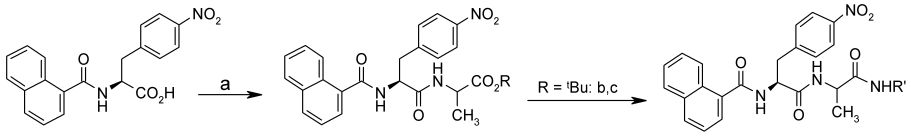
Scheme 2.[13] Reagents: (a) $(COCl)_2$, cat. DMF, CH_2Cl_2; (b) 2-trimethylsilyl-1,2,3-triazole, sulfolane, 140–150 °C; (c) $H_2NNHCHO$, EDCI, HOBT, NMM, DMF; (d) $POCl_3$, 100–105 °C; (e) hydrazine, EtOH, reflux; (f) 1-naphthoic acid, EDCI, HOBT, NMM, DMF.



Scheme 3.[14] Reagents: (a) P_2S_5, Na_2CO_3, THF; (b) $BrCH_2CH(OEt)_2$, HCl, molecular sieves (4 Å), DMF; (c) $BrCH_2(CO)CO_2Et$, EtOH; (d) 4 N HCl in dioxane; (e) 1-naphthoic acid, NMM, HOBT, EDCI, DMF; (f) NaOH, MeOH:H_2O; (g) NHR, DIEA, HOBT, EDCI, DMF.



Scheme 4.[15] Reagents: (a) $NH_2(CHR)CH_2OH$, EDCI, HOBT, NMM, DMF; (b) $CH_3O_2CNSO_2NEt_3$, DMA; (c) $BrCCl_3$, DBU, CH_2Cl_2.

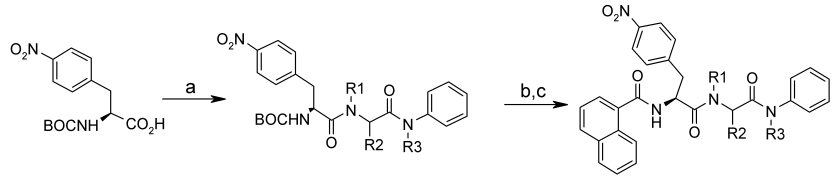


Scheme 5. Reagents: (a) $NH_2CH(CH_3)CO_2R$, EDCI, HOBT, NMM, DMF; (b) 60% TFA in CH_2Cl_2; (c) NH_2R', EDCI, HOBT, NMM, DMF.

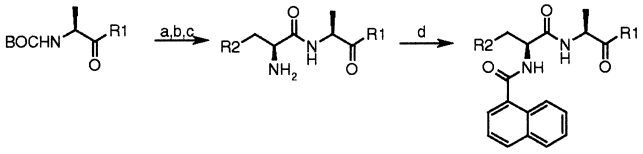
Compounds **17**–**22** (Table 2) were prepared as shown in Scheme 5, with the exception of **21** where the final amide formation was accomplished using the active anhydride method. Scheme 6 details the synthesis of the compounds in Table 3 (**24**–**27**) and the compounds of Table 4 (**28**–**35**) were prepared in a parallel synthesis approach as illustrated in Scheme 7.

Our initial efforts were directed towards simple primary and secondary amides as ester replacements but were unsuccessful and generally resulted in large losses in receptor binding affinity.[9] This prompted us to investigate a variety of heterocycles that have previously been reported to function as effective ester bioisosteres (Table 1).[16] The almost complete loss of CCR3 affinity seen upon replacing the ester group with simple heterocyclic alternatives (e.g., **2**, **4**, **6**, and **8**) was surprising. Nevertheless, the dihydrooxazole **9** did retain some receptor affinity and was found to be a potent functional antagonist as evidenced by its ability to block the eotaxin induced intracellular calcium mobilization in primary human eosinophils ($IC_{50} = 600$ nM).[17] In view of the stereoelectronic similarity between the heterocycles and an ester group, the general lack of activity of the ester mimetics suggested a more subtle role for the ester moiety that the heterocycles were somehow unable to mimic. The exact nature of this role remains unclear but does not appear to involve an irreversible transacylation of a critical residue in the CCR3.[9]

As shown in Table 1, introduction of an ester group into some of the inactive templates resulted in the restoration



Scheme 6. Reagents: (a) $NHR^1(CHR^2)CON(R^3)Ph$, EDCI, HOBT, NMM, DMF or isobutyl chloroformate, NMM, THF, −15 to 25 °C; (b) 4 N HCl in dioxane; (c) 1-naphthoic acid, EDCI, HOBT, NMM, DMF.

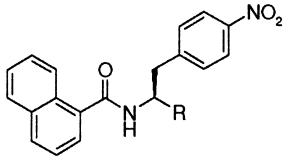


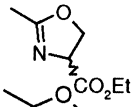
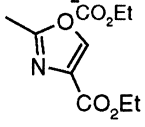
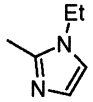
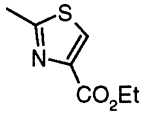
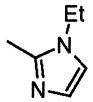
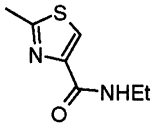
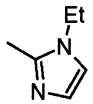
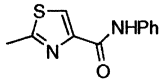
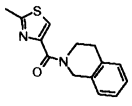
Scheme 7. Reagents: (a) 4 N HCl in dioxane; (b) P-EDC, $R^2CH_2CH(NHBOC)CO_2H$, DCE/DMF; (c) 4 N HCl in dioxane; (d) 1-naphthoic acid, EDCI, HOBT, NMM, DMF.

of submicromolar receptor affinity. The effect was particularly marked for the oxazole **11** and thiazole **12** systems, although these compounds were still ~100-fold less potent than **1**. Consistent with this apparent striking preference for an ester moiety in this part of the molecule, simple modifications to the ester in **12**, that is the corresponding amides **13**–**15**, led to a loss of affinity.

We reasoned that because of conformational constraints inherent in the heterocycle, both the attenuation in the activity of the esters **11** and **12** and the lack of activity of the corresponding amides could be related to an inability of these analogues to access important binding pockets or residues in the receptor. This led initially to the preparation of acyclic derivatives of **10** such as the dehydroserine **16** and alanines **17** and **18** in which the conformational restrictions of the cyclic system were relieved. Encouragingly, as shown in Table 2, these less constrained analogues either retained the activity of or were substantially more potent than their corresponding

Table 1.



Compound	R	IC_{50} (nM)[18]	Compound	R	IC_{50} (nM)
(±)-**2**		22,500	**9**		563
(±)-**3**		>33,000	**10**		213
(±)-**4**		>33,000	**11**		500
(±)-**5**		17,000	**12**		663
(±)-**6**		>33,000	**13**		18,000
(±)-**7**		>33,000	**14**		7700
8		32,000	**15**		12,500

cyclic analogues **11** and **12**. Interestingly, the alanine derivatives showed a marked preference for the natural, L-stereochemistry at both asymmetric centers, consistent with a high degree of enantiospecificity in the receptor and antagonist interaction (**17** and **18**, Table 2). As previously observed, the nature of the alkyl group of the ester did not greatly affect the CCR3 affinity and even the *tert*-butyl ester **19** was a highly potent eotaxin antagonist.

Although the compounds in Table 2 suggested that the phenylalanine ester could be replaced with an amide, the presence of an additional ester moiety in example **18** still precluded in vivo evaluation. We again considered amides as simple ester replacements in these more flexible systems and in this case, found them to retain reasonable CCR3 affinity (**20**, **21**, and **23**, Table 2). In general, secondary amides were preferred over tertiary amides (**20** and **23**) and the presence of an aromatic group was found to be particularly favorable (**21** and **23**).

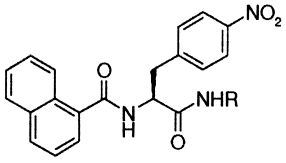
As shown in Table 3, replacement of the alanine methyl group in **23** with either hydrogen (**24**) or phenyl (**25**) did not markedly affect the activity, suggesting that this position was not involved in making critical receptor interactions and, with appropriate substitution, could be useful in modulating the physicochemical properties of the antagonist. *N*-Methylation of the central amide bond (**26**) appeared to be more important and may be related to changes in the geometry around the amide linkage.[19] However, the effect was not observed for the benzamide where *N*-methylation did not affect the activity (**27**).

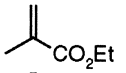
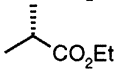
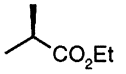
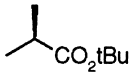
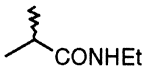
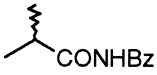
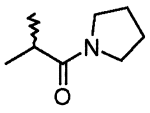
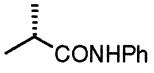
Importantly, amides such as **23** and **26** successfully demonstrated the feasibility of identifying nonester CCR3 antagonists and warranted additional investigation with the objective of further improving the receptor affinity. Given the synthetic amenability of **23**, we chose to accomplish this optimization using solution-phase parallel synthesis (Scheme 7). The most optimal approach in practice was to retain the *N*-acyl residue and vary only the amino acid core and the benzamide group.

A small, explorative library with these variables was prepared for assay versus the CCR3 and representative compounds are shown in Table 4. A number of CCR3 antagonists, significantly more potent than **23**, were identified (Table 4) and the SAR around the molecule further delineated. The nature of the amino acid side chain was clearly important for good affinity. A substituted phenyl group was preferred over both cyclohexyl or 4-thiazoyl (**28** vs **29** and **30**, respectively). As in the related ester series,[9] a range of phenyl substituents was associated with good receptor affinity (**28**, **32**, and **34**). Although the 4-pyridyl derivative **38** was devoid of activity, weak affinity was restored when this group was combined with a different benzamide (**39**). The parallel synthesis approach allowed for the rapid identification of substituents that in combination led to additive, and sometimes surprising, increases in potency. For example, although the activity seemed insensitive to changes in the benzamide part of the molecule (Table 4), the combination of an unsubstituted phenylbenzamide with a 4-chlorophenylalanine moiety was dramatic and provided a compound, **36**, whose CCR3 affinity was comparable to that of **1**.

A survey of alternative *N*-acyl groups was also carried out and quickly led to the identification of potent naphthoyl

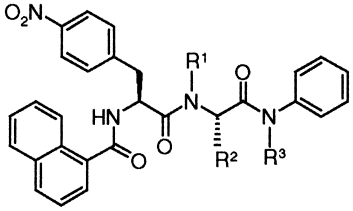
Table 2.



Compound	R	IC_{50} (nM)
16		350
17		383
18		3
19		20
20		666[a]
21		300[a]
22		1125[a]
23		190

[a]Mixture of four diastereomers.

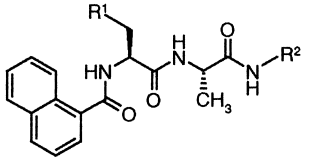
Table 3.



Compound	R^1	R^2	R^3	IC_{50} (nM)
23	H	Me	H	190
24	H	H	H	400
25	H	Ph	H	116
26	Me	Me	H	65
27	H	Me	Me	600[a]

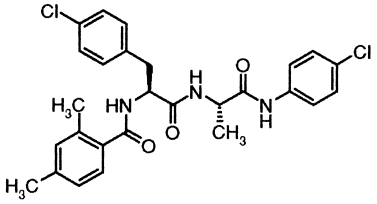
[a]Mixture of four diastereomers.

Table 4.



Compound	R^1	R^2	CCR3 IC_{50} (nM)
23	4-NO_2-Ph	Ph	190
28	4-Cl-Ph	4-OMe-Ph	45
29	Cyclohexyl	4-OMe-Ph	700
30	4-Thiazoyl	4-OMe-Ph	500
31	4-Thiazoyl	4-Cl-Ph	800
32	4-CN-Ph	2-OMe-Ph	125
33	4-CN-Ph	4-OMe-Ph	90
34	4-OMe-Ph	2-OMe-Ph	15
35	4-OMe-Ph	4-CN-Ph	250
36	4-Cl-Ph	Ph	5
37	4-Cl-Ph	3-Pyridyl	27
38	4-Pyridyl	4-OMe-Ph	>1220
39	4-Pyridyl	4-Ph-Ph	600

surrogates as exemplified by **40**. Clearly, our limited survey suggests that there is considerable scope for further optimization to generate compounds with improved CCR3 affinity and different physicochemical properties. However, we concluded that the profile of **36** was suitable for evaluation in functional assays and determined its ability to inhibit the eotaxin induced chemotaxis of primary human eosinophils derived from allergic individuals.[17] In this assay, and consistent with its CCR3 affinity determined in the binding assay, **36** was found to be a potent inhibitor of eosinophil chemotaxis ($IC_{50}=15$ nM). In contrast, in the same assay, **36** had no effect on the C5a induced eosinophil chemotaxis. Taken together with the previously reported ability of related compounds to block the functional responses mediated by eotaxin, MCP-3 or MCP-4, the compounds reported herein appear to be acting via CCR3 antagonism.[9]



40, CCR3 IC_{50} = 45 nM

Highly potent CCR3 antagonists have been developed from a series of phenylalanine ester-based leads. Although classical heterocyclic ester mimetics proved to be ineffective in the present study, conformationally less constrained derivatives gave more promising results. Two-dimensional, solution-phase parallel synthesis optimization was utilized to allow for rapid improvement in the receptor affinity and highly potent, functional CCR3 antagonists have been identified. The hydrolytic stability of these novel antagonists should allow for their in vivo evaluation and the results of these studies will be reported in due course.

References and Notes

1. Gallin, J. I.; Goldstein, I. M.; Syndrman, R. In *Inflammation: Basic Principles and Clinical Correlates*; Raven: New York, 1992.
2. (a) Wells, T. N. C.; Proudfoot, A. E.; Power, C. A. *Immunol. Lett*. **1999**, *65*, 35. (b) Epstein, F. H. *N. Engl. J. Med*. **1998**, *338*, 436. (c) Ward, S. G.; Westwick, J. *Biochem. J*. **1998**, *3333*, 457. (d) Baggiolini, M.; Dewald, B.; Moser, B. *Annu. Rev. Immunol*. **1997**, *15*, 675.
3. (a) Forssmann, U.; Uguccioni, M.; Loetscher, P.; Dahinden, C. A.; Thelen, M.; Baggiolini, M. *J. Exp. Med*. **1997**, *185*, 2171. (b) Williams, T. J. *Eur. Cytokine Netw*. **1999**, *10*, 253. (c) Cook, E. B.; Stahl, J. L.; Graziano, F. M. *Allergy Asthma Proc*. **1998**, *19*, 253. (d) Ponath, P. D.; Qin, S.; Ringler, D. J.; Clark-Lewis, I.; Wang, J.; Kassam, N.; Smith, H.; Xiaojie, S.; Gonzalo, J.-A.; Newman, W.; Guitierrez-Ramos, J.-C.; Mackay, C. R. *J. Clin. Invest*. **1996**, *97*, 604. (e) Garcia-Zepeda, E. A.; Rothenburg, M. C.; Ownbey, R. T.; Celestin, J.; Leder, P.; Luster, A. D. *Nat. Med*. **1996**, *2*, 449. (f) Brown, J. R.; Kliemberg, J.; Marini, M.; Sun, G.; Bellini, A.; Mattoli, S. *Clin. Exp. Immunol*. **1998**, *114*, 137. (g) Rothenburg, M. E.; MacLean, J. A.; Pearlman, E.; Luster, A. D.; Leder, P. J. *J. Exp. Med*. **1997**, *185*, 785.
4. (a) Heath, H.; Qin, S.; Rao, P.; Wu, L.; LaRosa, C.; Kassam, M.; Ponath, P. D.; Mackay, C. R. *J. Clin. Invest*. **1997**, *99*, 178. (b) Ponath, P. D.; Qin, S.; Post, T. W.; Wang, J.; Wu, L.; Gerard, N. P.; Newman, W.; Gerard, C.; Mackay, C. R. *J. Exp. Med*. **1996**, *183*, 2437. (c) Daugherty, B. L.; Siciliano, S. J.; DeMartino, J. A.; Malkowits, L.; Sirotina, A.; Springer, M. S. *J. Exp. Med*. **1996**, *183*, 2349. (d) Uguccioni, M.; Mackay, C. R.; Ochensberger, B.; Loetscher, P.; Rhis, S.; LaRosa, G. L.; Rao, P.; Ponath, P. D.; Baggiolini, M.; Dahinden, C. A. *J. Clin. Invest*. **1997**, *100*, 1137.
5. (a) Gonzalo, J.-A.; Lloyd, C. M.; Wen, D.; Albar, J. P.; Wells, T. N. C.; Proudfoot, A.; Martinez-A, C.; Dorf, M.; Bjerke, T.; Coyle, A. J.; Gutierrez-Ramos, J. C. *J. Exp. Med*. **1998**, *188*, 157. (b) Teixeira, M. M.; Wells, T. N. C.; Lukacs, N. W.; Proudfoot, A. E. I.; Kunkel, S. L. *J. Clin. Invest*. **1997**, *100*, 1657.
6. Sabroe, I.; Conroy, D. M.; Gerard, N. P.; Li, Y.; Collins, P. D.; Post, T. W.; Jose, P. J.; Williams, T. J.; Gerard, C. J.; Ponath, P. D. *J. Immunol*. **1998**, *161*, 6139.
7. Marleau, S.; Griffiths-Johnson, D. A.; Collins, P. D.; Bakhle, T.; Williams, T. J.; Jose, P. J. *J. Immunol*. **1996**, *157*, 4141.
8. (a) Saunders, J.; Tarby, C. M. *DDT* **1999**, *4*, 80. (b) Sabroe, I.; Peck, M. J.; Kenlen, J. V. B.; Jorritsma, A.; Simmons, G.; Clapham, P. R.; William, T. J.; Pease, J. E. *J. Biol. Chem*. **2000**, *275*, 25985.
9. Dhanak, D.; Christmann, L. T.; Darcy, M. G.; Jurewicz, A. J.; Keenan, R. M.; Lee, J.; Sarau, H. M.; Widdowson, K. L.; White, J. R. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 1441.
10. All compounds gave spectroscopic and analytical data consistent with their assigned structure.
11. (a) Boeijen, A.; Liskamp, R. M. J. *Eur. J. Org. Chem*. **1999**, *9*, 2127. (b) Moltzen, E. K.; Pedersen, H.; Bogeso, K. P.; Meier, E.; Frederiksen, K.; Sanchez, C.; Lembol, H. L. *J. Med. Chem*. **1994**, *37*, 4085.
12. (a) Angelastro, M. R.; Mehdi, S.; Burkhart, J. P.; Peet, N. P.; Bey, P. *J. Med. Chem*. **1990**, *33*, 11. (b) Diana, G. D.; Treasurywala, A. M.; Bailey, T. R.; Oglesby, R. C.; Pevear, D. C. *J. Med. Chem*. **1990**, *33*, 1306.
13. (a) Easton, C. J.; Hutton, C. A.; Merrett, M. C.; Tiekink, E. R. T. *Tetrahedron* **1996**, *52*, 7025. (b) Williams, E. L. *Tetrahedron Lett*. **1992**, *33*, 1033. (c) Boesch, R. Ger. Offen. DE 2808842, 1978; *Chem. Abstr*. **1979**, *90*, 23060.
14. (a) Brain, C. T.; Hallett, A.; Ko, S. Y. *J. Org. Chem*. **1997**, *62*, 3808. (b) Houssin, R.; Lohez, M.; Bernier, J.-L.; Henichart, J.-P. *J. Org. Chem*. **1985**, *50*, 2787.

15. (a) Williams, D. R.; Lowder, P. D.; Yu-Gui, G.; Brooks, D. A. *Tetrahedron Lett*. **1997**, *38*, 331. (b) Dunn, D.; Chatterjee, S. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 1273.
16. (a) Brown, J. P.; Davies, D. T.; O'Hanlon, P. J.; Wilson, J. M. *J. Med. Chem*. **1996**, *39*, 446. (b) Forrest, A. K.; O'Hanlon, P. J.; Walker, G. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2657. (c) Bailey, T. R.; Diana, G. D.; Kowalcyzk, P. J.; Akullian, V.; Eissenstat, M. A.; Cutcliffe, D.; Mallano, J. P.; Carabateas, P. M.; Pevear, D. C. *J. Med. Chem*. **1992**, *35*, 4628. (d) Street, L. J.; Baker, R.; Book, T.; Kneen, C. O.; MacLeod, A. M.; Merchant, K. J.; Showell, G. A.; Saunders, J.; Herbert, R. H.; Freedman, S. B.; Harley, E. A. *J. Med. Chem*. **1990**, *33*, 2690. (e) Diana, G. D.; Volkots, D. L.; Nitz, T. J.; Bailey, T. R.; Long, M. A.; Vescio, N.; Aldous, S.; Pevear, D. C.; Dutko, F. J. *J. Med. Chem*. **1994**, *37*, 2421. (f) Bromidge, S. M.; Brown, F.; Cassidy, F.; Clark, M. S. G.; Dabbs, S.; Hadley, M. S.; Loudon, J. M.; Orlek, B. S.; Riley, G. J. *Bioorg. Med. Chem. Lett*. **1992**, *2*, 787.
17. White, J. R.; Lee, J. M.; Dede, K.; Imburgia, C. S.; Jurewicz, A. J.; Dhanak, D.; Christmann, L. T.; Darcy, M. G.; Widdowson, K.; Foley, J. J.; Schmidt, D. B.; Sarau, H. M. *J. Biol. Chem*. **2000**, *275*, 36626.
18. The IC_{50} values reported are the mean of duplicate samples from at least two determinations.[17]
19. Hruby, V. J. *Biopolymers* **1993**, *33*, 1993.