Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 767–771
Bioorganic & Medicinal Chemistry Letters

# Acylguanidine inhibitors of β-secretase: Optimization of the pyrrole ring substituents extending into the $S_1'$ substrate binding pocket

Lee D. Jennings,[a,*] Derek C. Cole,[a] Joseph R. Stock,[a] Mohani N. Sukhdeo,[a] John W. Ellingboe,[a] Rebecca Cowling,[a] Guixian Jin,[a] Eric S. Manas,[b] Kristi Y. Fan,[c] Michael S. Malamas,[c] Boyd L. Harrison,[c] Steve Jacobsen,[f] Rajiv Chopra,[d] Peter A. Lohse,[e] William J. Moore,[b] Mary-Margaret O'Donnell,[e] Yun Hu,[f] Albert J. Robichaud,[c] M. James Turner,[f] Erik Wagner[f] and Jonathan Bard[f]

[a]*Chemical & Screening Sciences, Wyeth Research, 401 N. Middletown Road, Pearl River, NY 10965, USA*
[b]*Chemical & Screening Sciences, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, USA*
[c]*Chemical & Screening Sciences, Wyeth Research, 865 Ridge Road, Monmouth Junction, NJ 08852, USA*
[d]*Chemical & Screening Sciences, Wyeth Research, 200 Cambridge Park Drive, Cambridge, MA 02140, USA*
[e]*Arqule, 19 Presidential Way, Woburn, MA 01801, USA*
[f]*Discovery Neuroscience, Wyeth Research, 865 Ridge Road, Monmouth Junction, NJ 08852, USA*

Received 24 September 2007; revised 8 November 2007; accepted 12 November 2007
Available online 17 November 2007

**Abstract**—The proteolytic enzyme β-secretase (BACE-1) produces amyloid β (Aβ) peptide, the primary constituent of neurofibrillary plaques, implicated in Alzheimer's disease, by cleavage of the amyloid precursor protein. A small molecule inhibitor of BACE-1, (diaminomethylene)-2,5-diphenyl-1H-pyrrole-1-acetamide (**1**, BACE-1 $IC_{50}$ = 3.7 μM), was recently described, representing a new small molecule lead. Initial SAR investigation demonstrated the potential of accessing the nearby $S_3$ and $S_1'$ substrate binding pockets of the BACE-1 enzyme by building substituents off one of the phenyl substituents and guanidinyl functional group. We report here the optimization of guanidinyl functional group substituents on **1**, leading to potent submicromolar BACE-1 inhibitors.
© 2007 Elsevier Ltd. All rights reserved.

Alzheimer's disease (AD) is characterized by the deposition of neuritic plaques on the outside of nerve cells in areas of the brain related to memory. These plaques consist predominantly of the abnormal peptide β-amyloid (Aβ), a protein fragment cleaved from amyloid precursor protein (APP), during metabolism.[1] Although neuropathologic and epidemiological studies are inconclusive as to the pathogenic role of Aβ in Alzheimer's disease, studies from many laboratories support the hypothesis that amyloid production plays a key role in the pathogenesis of Alzheimer's disease.[2,3] As a consequence of the widespread acceptance of this hypothesis, vigorous efforts in compound screening and medicinal chemistry have been undertaken to find inhibitors of the two proteases, β- and γ-secretase, responsible for Aβ production.

β-Secretase (BACE-1, Beta Amyloid Cleaving Enzyme 1, also called memapsin 2 and Asp-2) is a membrane-anchored aspartyl protease and a member of the pepsin sub-family of aspartyl proteases. BACE-1 mediates the primary cleavage of APP at residue 671, generating the membrane-bound C-terminal APP fragment C99, which is in turn cleaved by γ-secretase, liberating Aβ.[1,4–6] Peptide-like inhibitors of BACE-1, based on substrate mimicry, have been disclosed[7] and reviewed.[8] Recently, publications disclosing small molecule BACE-1 inhibitors have started to appear.[9] We have recently disclosed the acylguanidine **1** as an inhibitor of BACE-1 with low micromolar affinity (BACE-1 $IC_{50}$ = 3.7 μM, $K_d$ = 2.8 μM) and the key structural features of the enzyme/inhibitor complex, as revealed by X-ray crystallography (Fig. 1).[10] An initial round of SAR investigation using

*Keywords*: BACE-1; Amyloid peptide; Aβ peptide; Aspartyl protease inhibitor; BACE-1 inhibitor; Alzheimer's disease.
* Corresponding author. Tel.: +1 845 602 3618; fax: +1 845 602 3045; e-mail: jenninl@wyeth.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.11.043

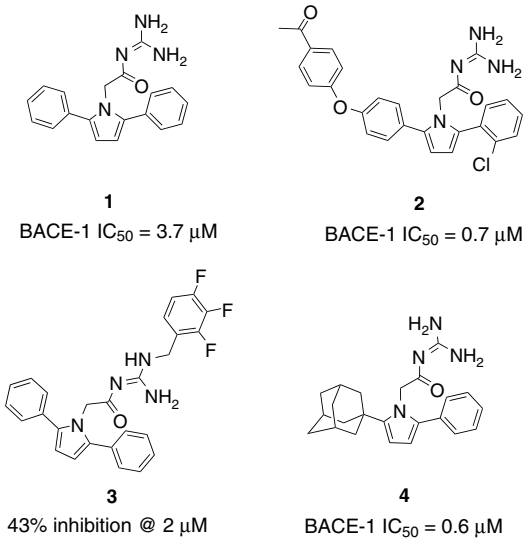
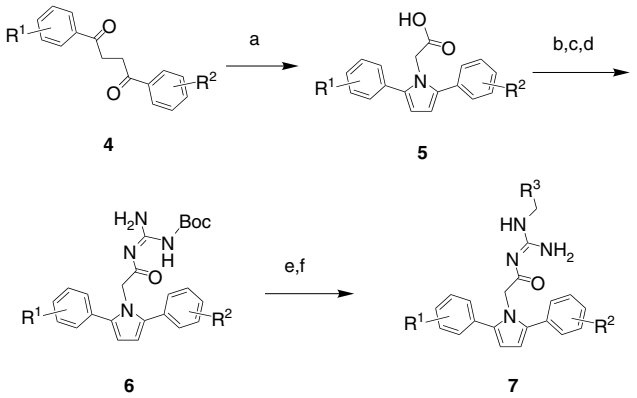



**Figure 1.** Acyl guanidine BACE-1 inhibitors.

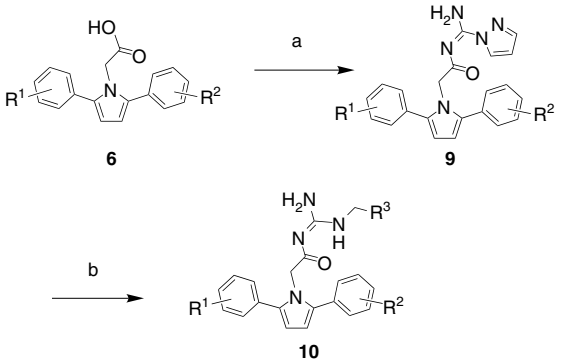
parallel synthesis revealed the opportunity to increase binding potency by adding substituents to the para-position of one of the phenyl groups of **1** extending into the $S_3$ substrate binding pocket. Improved BACE-1 inhibitor **2** was the outcome of this effort.[11]

Molecular modeling also revealed the potential for adding substituents to the guanidinyl functional group of **1** extending into the $S_1'$ pocket, which is occupied by an Asp side chain of the natural substrate. Inhibitors with substituents extending into the $S_1'$ pocket could take advantage of differences in the size and relative hydrophobicity in the $S_1'$ sites of different aspartyl proteases to enhance selectivity for BACE-1 over cathepsin D and renin. Peptide mimetic inhibitors of BACE-1 variously do or do not have substituents directed toward the $S_1'$ pocket.[8] Stachel[9a] and Garino[9b] have separately reported nonpeptidic BACE-1 inhibitors which have substituents that extend into the $S_1'$ pocket, resulting in improved selectivity and potency. We report here the results of our investigation of different substituent groups extending off the guanidinyl group of **1**.

Disubstituted guanidines were synthesized by a number of different routes. In all cases, disubstituted pyrrole intermediates **6** were prepared following our previously disclosed route.[10] Early on, the substituted acyl guanidines were prepared by a protection–deprotection sequence using di-*tert*-butyl dicarbonate. Alkylation of the Boc-protected intermediate was carried out using sodium hydride and was followed by acid mediated deprotection (Scheme 1). Subsequently, a more convenient synthetic procedure was developed allowing the use of amines as building blocks for side-chain synthesis (Scheme 2).[12] Intermediate **6** was reacted with carbonyl diimidazole and pyrazole-1-carboxamidine hydrochloride to afford the iminopyrazole intermediate **9**. The pyrazole could be easily displaced by amines to afford the alkyl substituted acyl guanidines. Alkyl ureido guanidines **11** could be prepared from the Boc-protected acyl guanidine **7** by reaction with amines in DMSO.



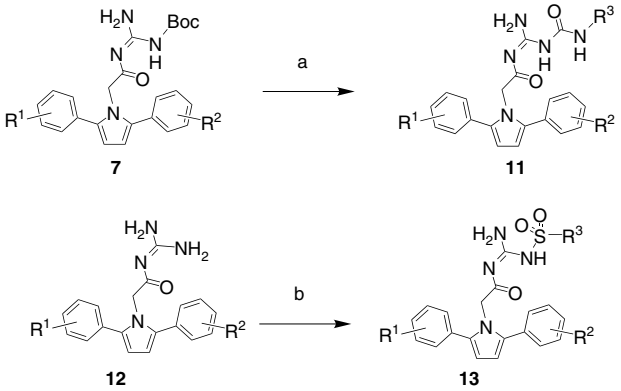

**Scheme 1.** Reagents and conditions: (a) glycine (3 equiv), p-TSA (cat.), EtOH, reflux, 3d, or glycine (2 equiv), AcOH, reflux, 1–5 h; (b) TMSCl, MeOH, reflux, 1–2 h; (c) guanidine (5 equiv), DMSO, rt, 1–4 h; (d) $Boc_2O$, DIPEA, $CH_2Cl_2$, rt, 1–3d; (e) NaH (3 equiv), alkyl halide (2.5 equiv), DMF, 60 °C, 16 h; (f) TFA (1 M in $CH_2Cl_2$), rt, 16 h.




**Scheme 2.** Reagents and conditions: (a) CDI (5 equiv), 1-H-pyrazole-1-carboxamidine HCl (5 equiv), DMAP (0.1 equiv), $CH_2Cl_2$, rt, 2–5 h; (b) alkyl amine (3 equiv), DIPEA (3 equiv), rt, 16 h.

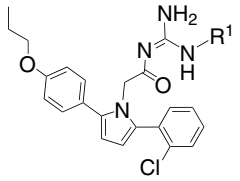
Alkylsulfonyl and arylsulfonyl acyl guanidines **13** could be prepared by the reaction of acyl guanidine intermediates **12** with the appropriate sulfonyl chloride (Scheme 3).

Substituted acyl guanidine **3** (BACE-1 inhibition 43% at 2 μM) (Fig. 1), prepared at an early point in the project,




**Scheme 3.** Reagents and conditions: (a) alkylamine (1.3 equiv), DMSO, 110 °C, 3 h; (b) alkyl or arylsulfonyl chloride (1.3 equiv), $CH_2Cl_2$, rt.

**Table 1.** BACE-1 inhibition by substituted acyl guanidines **14**–**29**



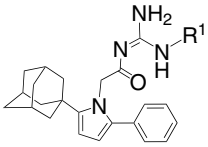

| Example | $R^1$ | BACE-1 inhibition $IC_{50}$ (μM) | Cathepsin D/BACE-1 | Cellular Aβ $ED_{50}$ (μM) |
|---|---|---|---|---|
| **14** | *trans*-4-Carboxy-cyclohexyl | 0.078 | 7.3 | 10.4 |
| **15** | *trans*-4-Hydroxy-cyclohexyl | 0.125 | 110 | 1.28 |
| **16** | 3-Carboxy-propyl | 0.13 | 45 | Inactive |
| **17** | 2-Carboxyethyl | 0.278 | >45 | nt |
| **18** | 2-Carboxamido-ethyl | 0.172 | 55 | nt |
| **19** | 3-Hydroxy-propyl | 0.121 | 85 | 1.18 |
| **20** | 2,3-Dihydroxy-propyl | 0.124 | 81 | 5.27 |
| **21** | 2-Acetamidoethyl | 0.137 | 70 | 4.14 |
| **22** | 2,2-Dimethyl-3-hydroxy-propyl | 0.203 | 15 | 5.27 |
| **23** | 3-Methylthio-propyl | 0.321 | 3.9 | 1.23 |
| **24** | 2-(1,3-Dioxolan-2-yl)-ethyl | 0.269 | 13 | 1.34 |
| **25** | 3-(1H-imidazol-1-yl)propyl | 0.241 | 30 | 3.10 |
| **26** | 2-Thienylmethyl | 0.752 | 0.7 | 10.7 |
| **27** | 2-Furylmethyl | 0.558 | 2.5 | 9.10 |
| **28** | 2,3,4-Trifluorobenzyl | 0.203 | 0.5 | 5.27 |
| **29** | 4-Hydroxy-3-methoxybenzyl | 0.651 | 1.0 | 5.45 |

was approximately as potent as the initial lead. An X-ray crystal structure of the inhibitor bound to BACE-1 clearly showed that the trifluorophenyl substituent was accommodated into the $S_1'$ substrate binding pocket. This was almost certainly a sub-optimal pairing of substituent group with the binding pocket because (1) the $S_1'$ pocket, where the Arg235 and Thr329 residues form part of the pocket wall, was expected to better accommodate polar substituent groups, (2) the volume of the 2,3,4-trifluorobenzyl group was a bit large for the $S_1'$ pocket and, as a consequence, (3) docking experiments indicated that the benzylic linkage of the bound molecule was somewhat strained. Acting on this hypothesis, a library of analogs was prepared to investigate the effect on binding and selectivity of different $S_1'$ substituents (Table 1). Substituent groups were selected (based on docking) for size and polarity complimentarity with the $S_1'$ pocket. All of the analogs made showed a significant (2–9×) boost in potency of BACE-1 inhibition relative to **2**. In order to avoid toxicities caused by inhibition of off-target proteases, selectivity of inhibitors for BACE-1 over other aspartyl proteases was a goal of this project. All compounds were evaluated for inhibition of cathepsin D in a FRET assay. Compounds with polar substituents, whether having a cyclic (e.g. **14** and **15**) or an acyclic (e.g. **16**–**25**) connection to the guanidinyl group, showed high levels of BACE-1/cathepsin D selectivity, whereas analogs with aromatic substituent groups (e.g. **26**–**29**), while potent, showed poor selectivity. Reduction of Aβ production in CHO cells recombinantly expressing human wild-type APP[10] improved 5-fold in some examples ($ED_{50}$ **2** = 5.9 μM, $LD_{50}$ **2** > 25 μM).

An analogous SAR study was done using the adamantyl substituted acyl guanidine inhibitor **4** as a scaffold (Table 2). Addition of $S_1'$ substituents gave a measurable boost to BACE-1 inhibition potency. Substituent groups of sufficient length with hydroxy and cyano groups on the terminus were preferred. Aromatic substituent groups also gave potent inhibitors of BACE-1, although BACE-1/cathepsin D selectivity was diminished. $ED_{50}$ for Aβ production improved in certain examples (**31**, **38**, and **45**) but did not appear to correlate with improving $IC_{50}$ for BACE-1 inhibition. Compounds were generally not toxic to cells ($LD_{50}$) at doses near the $ED_{50}$. Compounds with aromatic $S_1'$ substituents (**41**–**45**) were exceptional in this regard, suggesting that decreased selectivity was associated with increased toxicity. Acyl guanidines with alkyl carbamoyl or alkylsulfonyl substituent groups (not shown in tables) did not inhibit BACE-1 to any appreciable extent at the tested concentrations.

The complex of inhibitor **31** with BACE-1 was crystallized and the resulting crystal structure was diffracted and solved.[10] As anticipated, the *N*-3-hydroxypropyl group extends into the $S_1'$ pocket with little strain, although not displacing tightly held water molecules from the pocket (Fig. 2). These tightly held water molecules effectively become part of the protein with the inhibitor making hydrogen bonding interactions through them. Acyl guanidines substituted with alkyl groups on the guanidine nitrogen provided an improvement in potency of BACE-1 inhibition. A subset of these analogs, particularly aliphatic substituents with polar groups at the end of the chain, exhibited promising levels of selectivity for BACE-1 over cathepsin D, and activity approaching ~1 μM in the cellular Aβ lowering assay. Compounds in this series for which absorption in the Caco-2 drug transport model[13] was assessed (**4**, **30**, and **31**) were found to have poor permeability. The guanidinyl functionality, while involved in important hydrogen bonding interactions with the catalytic aspartic acid residues in the catalytic site, may be responsible for the poor permeability that characterizes

**Table 2.** BACE-1 inhibition by substituted acyl guanidines **4**, **30**–**45**



| Example | $R^1$ | BACE-1 inhibition $IC_{50}$ (μM) | Cathepsin D $IC_{50}$ (μM) | Cathepsin D/BACE-1 (μM) | Cellular Aβ $ED_{50}$ (μM) | Cellular $LD_{50}$ (μM) |
|---|---|---|---|---|---|---|
| **4** | H | 0.6 | 25 | 69 | 8.9 | >25 |
| **30** | 3-Cyanopropyl | 0.255 | 4.25 | 17 | 4.4 | 59 |
| **31** | 3-Hydroxypropyl | 0.239 | 19.9 | 83 | 1.84 | 35 |
| **32** | 2-Hydroxyethyl | 0.68 | 24.7 | 36 | 3.62 | 25 |
| **33** | 2-Cyanoethyl | 0.31 | 12.2 | 39 | 3.44 | 16 |
| **34** | 4-Hydroxybutyl | 0.255 | 6.45 | 25 | 4.15 | 31 |
| **35** | (2*R*)-2-Hydroxypropyl | 0.437 | 12.5 | 28 | 9.51 | 79 |
| **36** | (2*S*)-2-Hydroxypropyl | 0.942 | 54.1 | 57 | 17.8 | 100 |
| **37** | 2,3-Dihydroxypropyl | 0.536 | 73.5 | 137 | 21.1 | 88 |
| **38** | 3-(1H-imidazol-1-yl)propyl | 1.71 | >25 | >15 | 9.16 | 45 |
| **39** | *trans*-4-Hydroxycyclohexyl | 0.283 | n.t. | | 1.44 | 14 |
| **40** | Cyclohexyl | 7.2 | 54.2 | 7.5 | 18.7 | 50 |
| **41** | (5-Methyl-1,3,4-oxadiazol-2-yl)methyl | 0.665 | 3.01 | 4.5 | 9.48 | 22 |
| **42** | (4-Methyl-1,3-thiazol-2-yl)methyl | 0.276 | 0.144 | 0.5 | 3.97 | 8 |
| **43** | 2-(2-Thienyl)ethyl | 0.559 | 0.476 | 0.9 | 2 | 3 |
| **44** | 3-Aminobenzyl | 0.744 | 1.38 | 1.9 | 4.81 | 8 |
| **45** | 2-(3-Thienyl)ethyl | 0.565 | 0.599 | 1.1 | 1.69 | 2 |

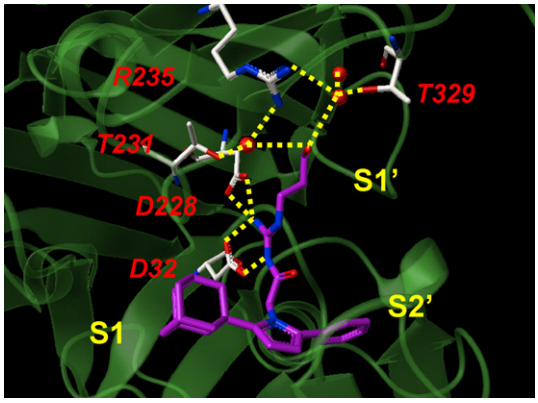



**Figure 2.** X-ray crystal structure of BACE-1 inhibitor **31** with BACE-1. Interactions between the hydroxypropyl group and bound water molecules in the $S_1'$ pocket are indicated with yellow dashed lines.

this series. However, an understanding of the effects of occupation of available substrate binding pockets and a demonstration of the ability of analogs to exploit available protein functionality in the substrate binding pockets for increased BACE-1 potency is an important first step toward the goal of optimizing the lead for oral bioavailability, CNS penetration, and a suitable duration of action. Future publications from these laboratories will disclose the results of further functionalization of the guanidine group and its replacement with bioisosteres.

## References and notes


1. Selkoe, D. J. *Physiol. Rev.* **2001**, *81*, 741.
2. Blass, J. P.; Poirier, J. In *Clinical Diagnosis and Management of Alzheimer's Disease*; Gauthier, S., Ed.; Woburn, Mass: Butterworth-Heinemann, 1996; pp 17–31.
3. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
4. James, M. N.; Sielecki, A. R.; Hayakawa, K.; Gelb, M. H. *Biochemistry* **1992**, *31*, 3872.
5. Pearl, L.; Blundell, T. *FEBS Lett.* **1984**, *174*, 96.
6. Esler, W. P.; Wolfe, M. S. *Science* **2001**, *292*, 1449–1454.
7. (a) Hu, J.; Cwi, C. L.; Smiley, D. L.; Timm, D.; Erickson, J. A.; McGee, J. E.; Yang, H. C.; Mendel, D.; May, P. C.; Shapiro, M.; McCarthy, J. R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4335; (b) Hom, R. K.; Gailunas, A. F.; Mamo, S.; Fang, L. Y.; Tung, J. S.; Walker, D. E.; Davis, D.; Thorsett, E. D.; Jewett, N. E.; Moon, J. B.; John, V. *J. Med. Chem.* **2004**, *47*, 158; (c) Hanessian, S.; Yun, H.; Hou, Y.; Yang, G.; Bayrakdarian, M.; Therrien, E.; Moitessier, N.; Roggo, S.; Veenstra, S.; Tintelnot-Blomley, M.; Rondeau, J. M.; Ostermeier, C.; Strauss, A.; Ramage, P.; Paganetti, P.; Neumann, U.; Betschart, C. *J. Med. Chem.* **2005**, *48*, 5175; (d) Kimura, T.; Hamada, Y.; Stochaj, M.; Ikari, H.; Nagamine, A.; Abdel-Rahman, H.; Igawa, N.; Hidaka, K.; Nguyen, J.-T.; Saito, K.; Hayashi, Y.; Kiso, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2380; (e) Kortuma, S. W.; Bensona, T. E.; Bienkowskia, M. J.; Emmonsa, T. L.; Princea, D. B.; Paddock, D. J.; Tomassellia, A. G.; Moona, J. B.; LaBordea, A.; TenBrinka, R. E. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3378–3383.
8. Hills, I. D.; Vacca, J. P. *Curr. Opin. Drug Discov. Dev.* **2007**, *10*(4), 383–391.
9. (a) Stachel, S. J.; Coburn, C. A.; Steele, T. G.; Jones, K. G.; Loutzenhiser, H. A.; Gregro, A. R.; Rajapakse, H. A.; Lai, M.-T.; Crouthamel, M.-C.; Xu, M.; Tugusheva, K.; Lineberger, J. E.; Pietrak, B. L.; Espeseth, A. S.; Shi, X.-P.; Chen-Dodson, E.; Holloway, M. K.; Munshi, S.; Simon, A. J.; Kuo, L.; Vacca, J. P. *J. Med. Chem.* **2004**, *47*, 6447; (b) Garino, C.; Tomita, T.; Pietranscosta, N.; Laras, Y.; Rosas, R.; Herbette, G.; Maigret, B.; Quelever, G.; Iwatsubo, T.; Kraus, J.-L. *J. Med. Chem.* **2006**, *49*, 4275.
10. Cole, D. C.; Manas, E. S.; Stock, J. R.; Condon, J. S.; Jennings, L. D.; Aulabaugh, A.; Chopra, R.; Cowling, R.; Ellingboe, J. W.; Fan, K. Y.; Harrison, B. L.; Hu, Y.; Jacobsen, S.; Jin, G.; Lin, L.; Lovering, F. E.; Malamas,

M. S.; Stahl, M. L.; Strand, J.; Sukhdeo, M. N.; Svenson, K.; Turner, M. J.; Wagner, E.; Zhou, P.; Bard, J. *J. Med. Chem.* **2006**, *49*, 6158.
11. Cole, D. C.; Stock, J. R.; Chopra, R.; Cowling, R.; Ellingboe, J. W.; Fan, K. Y.; Harrison, B. L.; Hu, Y.; Jacobsen, S.; Jennings, L. D.; Jin, G.; Lohse, P. A.; Malamas, M. S.; Manas, E. S.; Moore, W. J; O'Donnell, M. -M.; Robichaud, A. J.; Svenson, K.; Wu, J. -J.; Wagner, E.; Bard, J. *Bioorg. Med. Chem. Lett*., in press.
12. Cole, D. C.; Stock, J. R., *Tet. Lett.,* submitted.
13. Shah, P.; Jogani, V.; Bagchi, T.; Misra, A. *Biotechnol. Prog.* **2006**, *22*, 186.