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Identification of a potent non-hydroxamate histone deacetylase inhibitor by mechanism-based drug design

Takayoshi Suzuki,* Azusa Matsuura, Akiyasu Kouketsu, Hidehiko Nakagawa and Naoki Miyata*

Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya, Aichi 467-8603, Japan

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Abstract—In order to find novel non-hydroxamate histone deacetylase (HDAC) inhibitors, we synthesized several suberoylanilide hydroxamic acid (SAHA)-based compounds designed on the basis of the catalytic mechanism of HDACs. Among these compounds, **5b** was found to be as potent as SAHA. Kinetic enzyme assays and molecular modeling suggested that the mercaptoacetamide moiety of **5b** interacts with the zinc in the active site of HDACs and removes a water molecule from the reactive site of the deacetylation.

Histone deacetylases (HDACs) catalyze the deacetylation of the acetylated ε-amino groups of specific histone lysine residues,[1,2] and are involved in the expression of a number of genes.[3] In addition, HDACs have also been implicated in certain disease states such as cancer.[4–7] For this reason, there is a growing interest in the generation of potent small-molecule inhibitors of HDACs. Thus far, several classes of small-molecule HDAC inhibitors have been recognized.[8] Most of these are hydroxamic acid derivatives, typified by suberoylanilide hydroxamic acid (SAHA) (Fig. 3), and are thought to chelate the zinc ion in the active site.[9,10] Although hydroxamic acids are responsible for various potent inhibitors, they generally have many problems associated with their use such as low oral availability, poor in vivo stability, and undesirable side effects.[11,12] Thus, it has become increasingly desirable to find replacement groups that possess strong inhibitory action against HDACs. We and other groups have searched for a suitable hydroxamic acid replacement for HDAC inhibitors by structure-based drug design (SBDD)[13–15] ever since the crystal structure of an archaebacterial HDAC homologue (HDAC-like protein, HDLP)/SAHA complex was first reported.[9] However, SBDD has not yet led to the discovery of potent non-hydroxamate HDAC inhibitors, and the non-hydroxamates found with SBDD are approximately 10–1000-fold less potent than their corresponding hydroxamates. We therefore decided to search for hydroxamic acid replacements by an alternative approach, namely, mechanism-based drug design. In this paper, we report the mechanism-based design, synthesis, enzyme inhibition, and binding mode of non-hydroxamate HDAC inhibitors.

The crystal structures of the HDLP/hydroxamates and HDAC8/hydroxamates complexes have led to a solid understanding of not only the three dimensional structure of the active site of HDACs but also the catalytic mechanism for the deacetylation of acetylated lysine substrate.[9,10] It is proposed that the carbonyl oxygen of this substrate could bind the zinc, and the carbonyl could be attacked by a zinc-chelating water molecule (Fig. 2a), which would result in the production of deacetylated lysine via a tetrahedral carbon-containing transition state (Fig. 1a). On the basis of the proposed catalytic mechanism, we attempted to design non-hydroxamate HDAC inhibitors. First, we designed transition-state (TS) analogues. The TS of HDAC deacetylation was estimated to include a tetrahedral carbon (Fig. 1a) as with other zinc proteases.[16] To date, there has been only one report on TS analogue inhibitors of HDACs, namely, phosphorus-based SAHA analogues.[17] However, these analogues have a potency about 1000-fold less than that of SAHA. We focused attention on sulfone derivative TS analogues because it has been suggested that the sulfonamide moiety has strong similarity with the TS of amide bond hydrolysis,

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* Corresponding authors. Tel./fax: +81 52 836 3407; e-mail addresses: suzuki@phar.nagoya-cu.ac.jp; miyata-n@phar.nagoya-cu.ac.jp

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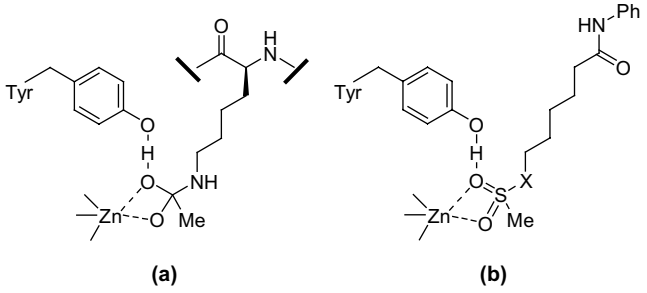
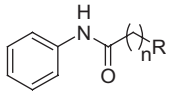


Figure 1. The transition state proposed for HDACs (a), and models for the binding of sulfone derivatives (b).

both from a steric and an electronic point of view.[18] Compounds **1** and **2**, in which a hydroxamic acid of SAHA was replaced by a sulfonamide and a sulfone, respectively, were designed as TS analogues (Figs. 1b, and 3). Our second approach was based on the proposed deacetylation mechanism whereby a zinc-chelating water molecule activated by His142 and His143 (HDAC8 numbering) makes a nucleophilic attack on the carbonyl carbon of acetylated lysine substrate (Fig. 2a). With this mechanism, if the water molecule is forcibly removed from the zinc ion, the HDACs would supposedly be inhibited. We then designed hetero atom containing substrate analogues **3**–**5** (Fig. 3). These analogues would be recognized as substrates by HDACs and would be easily taken into the active site where they could force the water molecule off the zinc ion and the reactive site of the deacetylation by chelation of the hetero atom to the zinc ion, and might behave as HDAC inhibitors (Fig. 2b).

The compounds prepared for this study are shown in Table 1. The routes used for synthesis of the compounds are indicated in Schemes 1–3. Scheme 1 shows the preparation of sulfonamide **1**, a TS analogue. The condensation of dicarboxylic acids **8a**–**c** with an equivalent amount of aniline gave mono-anilides **9a**–**c**. Carboxylic

Table 1. HDAC inhibition data for SAHA, SAHA-based transition state analogues, and substrate analogues[a]



Compd	R	*n*	% Inhbtn at 100 μM	IC_{50} (μM)
SAHA[b]	–CONHOH	6	100	0.28
1	$–NHSO_2Me$	5	10	7500
2	$–SO_2Me$	6	33	230
3	$–NHCOCH_2NH_2$	5	6	>100
4	$–NHCOCH_2OH$	5	0	>100
5a	$–NHCOCH_2SH$	6	96	3.0
5b	$–NHCOCH_2SH$	5	99	0.39
5c	$–NHCOCH_2SH$	4	88	11
6	$–NHCOCH_2SAc$	5	72	22
7	$–NHCOCH_2CH_2SH$	5	78	24

[a] Values are means of at least three experiments.
[b] Prepared as described in Ref. 25.

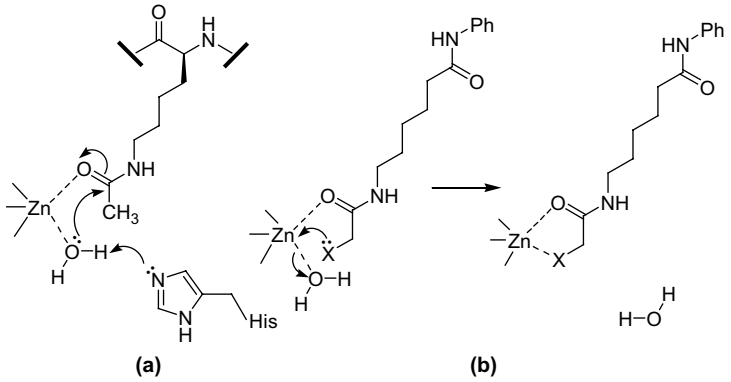
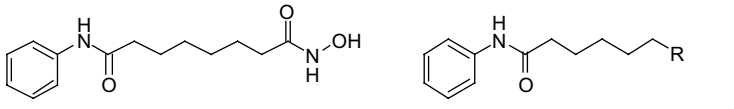
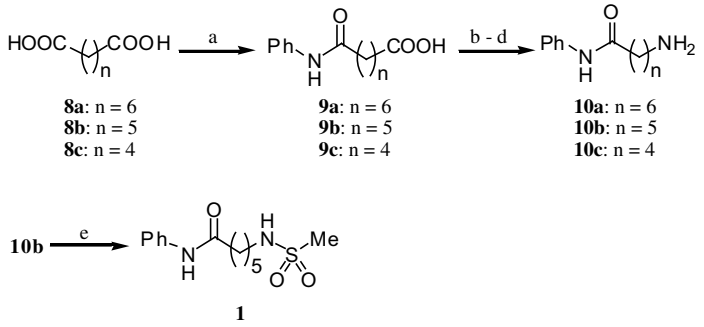


Figure 2. The mechanism proposed for the deacetylation of acetylated lysine substrate (a), and a model for the binding of hetero atom containing substrate analogues to zinc ion (b).

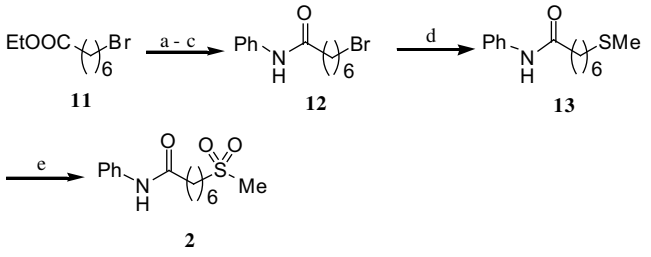


1: R = -$NHSO_2Me$ **3**: R = -$NHCOCH_2NH_2$
2: R = -CH_2SO_2Me **4**: R = -$NHCOCH_2OH$
5: R = -$NHCOCH_2SH$

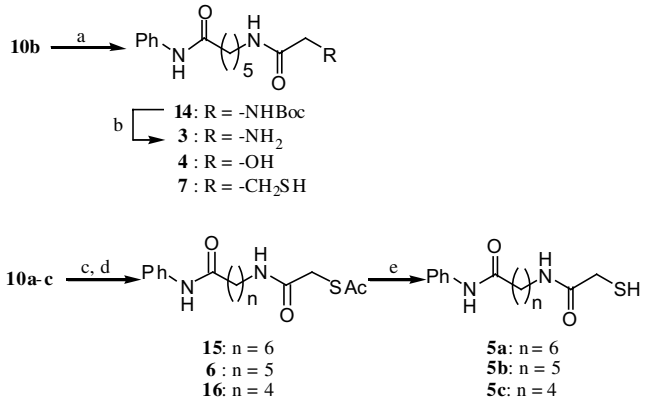
Figure 3. Structures of SAHA, SAHA-based transition state analogues **1** and **2**, and hetero atom containing substrate analogues **3**–**5** designed on the basis of the deacetylation mechanism.



Scheme 1. Reagents and conditions: (a) aniline, 180 °C, 46–54%; (b) diphenylphosphoryl azide, Et_3N, benzene, reflux; (c) BnOH, reflux, 63–94% (two steps); (d) H_2, 5% Pd–C, MeOH, rt, 72–96%; (e) MsCl, pyridine, rt, 71%.



Scheme 2. Reagents and conditions: (a) $LiOH \cdot H_2O$, THF, EtOH, H_2O, rt, 99%; (b) $(COCl)_2$, DMF, CH_2Cl_2, rt; (c) aniline, Et_3N, CH_2Cl_2, rt, 87%; (d) 15% aq NaSMe, EtOH, rt, 99%; (e) *m*-chloroperoxybenzoic acid, CH_2Cl_2, rt, 70%.



Scheme 3. Reagents and conditions: (a) RCH_2COOH, EDCI, HOBt, DMF, rt, 35–99%; (b) trifluoroacetic acid, CH_2Cl_2, rt, 84%; (c) bromoacetyl chloride, Et_3N, CH_2Cl_2, rt, 23–56%; (d) AcSK, EtOH, rt, 50–89%; (e) K_2CO_3, MeOH, rt, 28–75%.

acids **9a**–**c** were converted to amines **10a**–**c** in three steps: Curtius rearrangement of carboxylic acids **9a**–**c**, treatment of the resulting isocyanates with benzyl alcohol, and cleavage of the *Z* group by hydrogenolysis. Coupling between amine **10b** and methanesulfonyl chloride afforded sulfonamide **1**. Preparation of **2**, the other TS analogue, is shown in Scheme 2. 7-Bromoheptanoic acid ethyl ester **11** was converted to **12** in three steps by hydrolysis of the ester of **11**, acid chloride formation by oxalyl chloride, and condensation with aniline. Bromide **12** was allowed to react with sodium methanethiolate to give sulfide **13**, after which treatment with two equivalents of *m*-chloroperoxybenzoic acid gave sulfone **2**. Hetero atom containing substrate analogues **3**–**7** were prepared from amines **10** obtained above by the procedure outlined in Scheme 3. The amine **10b** was reacted with an appropriate carboxylic acid in the presence of EDCI and HOBt in DMF to give compounds **4**, **7** and **14**. The *N*-Boc group of compound **14** was removed by treating with trifluoroacetic acid to give aminoacetamide **3**. Coupling between amines **10a**–**c** and bromoacetyl chloride and subsequent treatment with potassium thioacetate afforded compounds **6**, **15**, and **16** and the deacetylation of these compounds in the presence of K_2CO_3 in MeOH gave mercaptoacetamides **5a**–**c**.

The compounds prepared for this study were evaluated using an HDAC enzyme inhibition assay[19] (Table 1). In the case of TS analogues, sulfone **2** showed anti-HDAC activity and the IC_{50} value was 230 μM, which was greater than those of phosphorus-based SAHA analogues.[17] However, sulfone **2** was approximately 820-fold less effective than SAHA. Next, we examined hetero atom containing substrate analogues. While **3** and **4** did not possess HDAC inhibitory activities,[20] potent inhibition was observed with mercaptoacetamide **5b**. Compound **5b** exhibited an IC_{50} of 0.39 μM, and its activity largely surpassed those of phosphorus compounds[17] and was comparable to those of SAHA and previously reported non-hydroxamates.[21,22] The potency of mercaptoacetamide **5a**–**c** was directly related to chain length, and the most potent compound was **5b**, where $n = 5$. As expected, thiol transformation into thioacetate (**6**) led to a 55-fold less potent inhibitor. This result suggests that thiolate anion generated under physiological conditions has an intimate involvement in the interaction with the zinc ion in the active site. The conversion of mercaptoacetamide to mercaptopropionamide (**7**) reduced potency as compared to compound **5b**.

Next, we studied the inhibition mechanism of mercaptoacetamide **5b**. Although the mercaptoacetamide group of **5b** was designed to make use of its chelation of the zinc ion in the active site, there is a possibility that mercaptoacetamide **5b** inhibits HDACs by forming a covalent disulfide bond with cysteine residues of these enzymes. We examined this possibility using a Lineweaver–Burk plot (a double reciprocal plot of $1/V$ versus 1/[substrate] at varying concentrations of inhibitor **5b**)

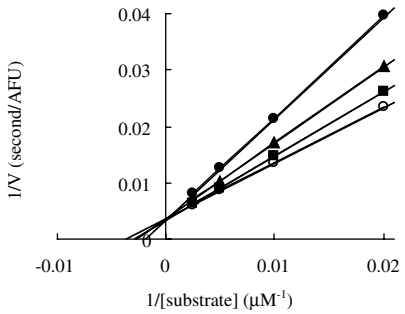


Figure 4. Reciprocal rate versus reciprocal acetyl lysine substrate concentration in the presence of 1 (●), 0.3 (▲), 0.1 (■), and 0 (○) μM of **5b**.

(Fig. 4), and the data from this study established that mercaptoacetamide **5b** engages in competitive inhibition versus acetylated lysine substrate, with an inhibition constant (K_i) of 0.78 μM. Since cysteine is not a component in the construction of the active site of HDACs, the mercaptoacetamide group of **5b** likely interacts with the zinc in the active site.

Since mercaptoacetamide **5b** was proven to act in the HDAC active center, we studied its binding mode in this site. The low energy conformation of **5b** was calculated when docked in the model based on the crystal structure of HDAC8 (PDB code 1T64, 1T67, 1T69, and 1VKG) using Macromodel 8.1 software. An inspection of the HDAC8/**5b** complex showed that the sulfur atom and oxygen atom of **5b** were located 2.44 Å and 2.04 Å from the zinc ion, respectively, and that a water molecule, which is required for the deacetylation of acetylated lysine substrate, was positioned 4.95 Å apart from the zinc ion (Fig. 5). This calculation suggests that **5b** inhibits HDACs by chelating the zinc ion in a bidentate fashion through its sulfur and oxygen atoms, and by removing a water molecule from the zinc and the reactive site of the deacetylation, without being hydrolyzed by HDACs.

In summary, in order to find novel non-hydroxamate HDAC inhibitors, we prepared several SAHA-based compounds whose designs were based on the proposed HDAC catalytic mechanism. Although transition state analogues were weakly active against HDACs, mercaptoacetamide **5b**, one of the hetero atom containing substrate analogues, was found to be as potent as SAHA. Mercaptoacetamide **5b** exhibits strong competitive inhibition versus acetylated lysine substrate. As far as we could determine, this is the first report of HDAC inhibitors with mercaptoacetamide. Since mercaptoacetamides are reported as potent, long-lived, and low-toxic matrix metalloproteinase inhibitors,[23,24] we believe that our findings in this study will provide the basis for the development of ideal HDAC inhibitors free of the problems associated with hydroxamates. Further detailed structure–activity relationship studies are currently under way and the next stage of evaluations pertaining to mercaptoacetamides **5** has begun.

Acknowledgements

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References and notes

1. Grozinger, C. M.; Schreiber, S. L. *Chem. Biol.* **2002**, *9*, 3.
2. Hassig, C. A.; Schreiber, S. L. *Curr. Opin. Chem. Biol.* **1997**, *1*, 300.
3. Taunton, J.; Hassig, C. A.; Schreiber, S. L. *Science* **1996**, *272*, 408.
4. Yoshida, M.; Horinouchi, S.; Beppu, T. *BioEssays* **1995**, *17*, 423.

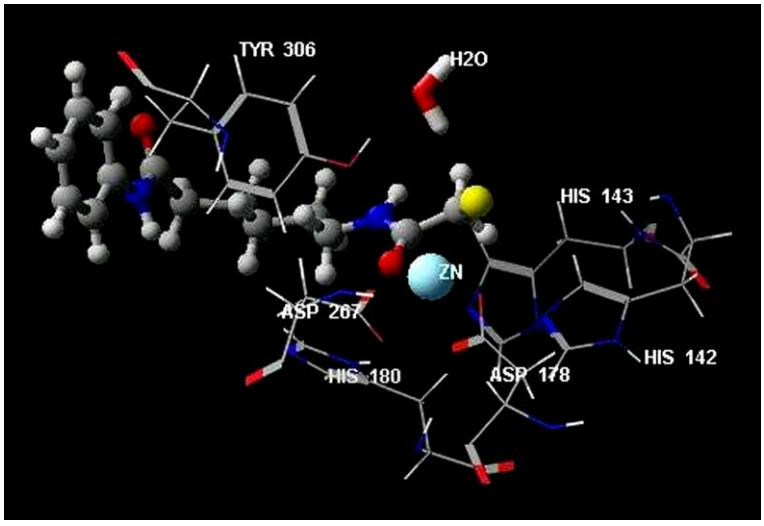

Figure 5. View of the conformation of **5b** (ball and stick) docked in the HDAC8 catalytic core. Residues around the zinc ion and a water molecule are displayed as wires and tubes, respectively.

5. Richon, V. M.; Webb, Y.; Merger, R.; Sheppard, T.; Jursic, B.; Ngo, L.; Civoli, F.; Breslow, R.; Rifkind, R. A.; Marks, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5705.
6. Richon, V. M.; Emiliani, S.; Verdin, E.; Webb, Y.; Breslow, R.; Rifkind, R. A.; Marks, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3003.
7. Cohen, L. A.; Amin, S.; Marks, P. A.; Rifkind, R. A.; Desai, D.; Richon, V. M. *Anticancer Res.* **1999**, *19*, 4999.
8. For a review, see: Miller, T. A.; Witter, D. J.; Belvedere, S. *J. Med. Chem.* **2003**, *46*, 5097.
9. Finnin, M. S.; Donigian, J. R.; Cohen, A.; Richon, V. M.; Rifkind, R. A.; Marks, P. A.; Breslow, R.; Pavletich, N. P. *Nature* **1999**, *401*, 188.
10. Somoza, J. R.; Skene, R. J.; Katz, B. A.; Mol, C.; Ho, J. D.; Jennings, A. J.; Luong, C.; Arvai, A.; Buggy, J. J.; Chi, E.; Tang, J.; Sang, B.-C.; Verner, E.; Wynands, R.; Leahy, E. M.; Dougan, D. R.; Snell, G.; Navre, M.; Knuth, M. W.; Swanson, R. V.; McRee, D. E.; Tari, L. W. *Structure* **2004**, *12*, 1325.
11. Mulder, G. J.; Meerman, J. H. *Environ. Health Perspect.* **1983**, *49*, 27.
12. Vassiliou, S.; Mucha, A.; Cuniasse, P.; Georgiadis, D.; Lucet-Levannier, K.; Beau, F.; Kannan, R.; Murphy, G.; Knaeuper, V.; Rio, M. C.; Basset, P.; Yiotakis, A.; Dive, V. *J. Med. Chem.* **1999**, *42*, 2610.
13. Mai, A.; Massa, S.; Rango, R.; Cerbara, I.; Jesacher, F.; Loidl, P.; Brosch, G. *J. Med. Chem.* **2003**, *46*, 512.
14. Suzuki, T.; Nagano, Y.; Matsuura, A.; Kohara, A.; Ninomiya, S.; Kohda, K.; Miyata, N. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4321.
15. Wu, T. Y. H.; Hassig, C.; Wu, Y.; Ding, S.; Schultz, P. G. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 449.
16. Christianson, D. W.; Lipscomb, W. N. *Acc. Chem. Res.* **1989**, *22*, 62.
17. Kapstin, G. V.; Fejer, G.; Gronlund, J. L.; McCafferty, D. G.; Seto, E.; Etzkorn, F. A. *Org. Lett.* **2003**, *5*, 3053.
18. Moree, W. J.; van der Marel, G. A.; Liskamp, R. M. J. *Tetrahedron Lett.* **1991**, *32*, 409.
19. The HDAC activity assay was performed using an HDAC fluorescent activity assay/drug discovery kit (AK-500, BIOMOL Research Laboratories): HeLa Nuclear Extracts (0.5 μl/well) were incubated (37 °C) with 25 μM of Fluor de Lys™ substrate and various concentrations of samples. Reactions were stopped after 30 min. with Fluor de Lys™ Developer and fluorescence was measured on a fluorometric reader with excitation set at 360 nm and emission detection set at 460 nm.
20. The reason that compounds **3** and **4** were inactive is unclear, but it is probably because the amino group of **3** is not able to chelate zinc ion due to the protonation under the assay conditions, and because the zinc-chelating ability of hydroxyl group (**4**) is less than that of sulfhydryl group (**5b**).
21. Wada, C. K.; Frey, R. R.; Ji, Z.; Curtin, M. L.; Garland, R. B.; Holms, J. H.; Li, J.; Pease, L. J.; Guo, J.; Glaser, K. B.; Marcotte, P. A.; Richardson, P. L.; Murphy, S. S.; Bouska, J. J.; Tapang, P.; Magoc, T. J.; Albert, D. H.; Davidsen, S. K.; Michaelides, M. R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3331.
22. Suzuki, T.; Kouketsu, A.; Matsuura, A.; Kohara, A.; Ninomiya, S.; Kohda, K.; Miyata, N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3313.
23. Rizvi, N. A.; Humphrey, J. S.; Ness, E. A.; Johnson, M. D.; Gupta, E.; Williams, K.; Daly, D. J.; Sonnichsen, D.; Conway, D.; Marshall, J.; Hurwitz, H. *Clin. Cancer Res.* **2004**, *10*, 1963.
24. Baxter, A. D.; Bird, J.; Bhogal, R.; Massil, T.; Minton, K. J.; Montana, J.; Owen, D. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 897.
25. Mai, A.; Esposito, M.; Sbardella, G.; Massa, S. *Org. Prep. Proced. Int.* **2001**, *33*, 391.