

# STRUCTURE–ACTIVITY STUDIES RELATED TO ABT-594, A POTENT NONOPIOID ANALGESIC AGENT: EFFECT OF PYRIDINE AND AZETIDINE RING SUBSTITUTIONS ON NICOTINIC ACETYLCHOLINE RECEPTOR BINDING AFFINITY AND ANALGESIC ACTIVITY IN MICE

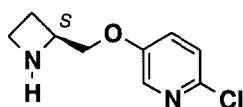
Mark W. Holladay,\* Hao Bai, Yihong Li, Nan-Horng Lin, Jerome F. Daanen, Keith B. Ryther, James T. Wasicak, John F. Kincaid, Yun He, Anne-Marie Hettinger, Peggy Huang, David J. Anderson, Anthony W. Bannon, Michael J. Buckley, Jeffrey E. Campbell, Diana L. Donnelly-Roberts, Karen L. Gunther, David J. B. Kim, Theresa A. Kuntzweiler, James P. Sullivan, Michael W. Decker, and Stephen P. Arneric

*Neurological and Urological Diseases Research, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-3500, U.S.A.*

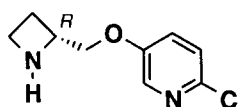
Received 15 July 1998; accepted 28 August 1998

**Abstract:** Analogs of A-98593 (**1**) and its enantiomer ABT-594 (**2**) with diverse substituents on the pyridine ring were prepared and tested for affinity to nicotinic acetylcholine receptor binding sites in rat brain and for analgesic activity in the mouse hot plate assay. Numerous types of modifications were consistent with high affinity for [<sup>3</sup>H]cytisine binding sites. By contrast, only selected modifications resulted in retention of analgesic potency in the same range as **1** and **2**. Analogs of **2** with one or two methyl substituents at the 3-position of the azetidine ring also were prepared and found to be substantially less active in both assays. © 1998 Elsevier Science Ltd. All rights reserved.

Recent reports from these laboratories have described A-98593 (**1**) and its (*R*)-enantiomer (ABT-594, **2**) as novel potent analgesic agents whose activity is mediated by nicotinic acetylcholine receptors (nAChRs).<sup>1–3</sup> During the course of studies culminating in advancement of ABT-594 to human clinical trials, many analogs were prepared in the azetidine and related series to explore structure–activity relationships with respect to interactions with nAChR subtypes in vitro and analgesic action in vivo. In this report, affinity for [<sup>3</sup>H]cytisine binding sites in rat brain and analgesic activity in the mouse hot plate assay are reported for analogs of A-98593 and ABT-594 in the azetidine series.



A-98593 (**1**)



ABT-594 (**2**)

Extensive variations with respect to the substitution pattern on the pyridine ring were undertaken, with representative results compiled in Table 1. Because the (*S*)-2-azetidinedicarboxylic acid precursor to A-98593 was commercially available, early efforts were focused on analogs in the (*S*)-stereochemical series. Analogs in the (*R*)-series were prepared following resolution of issues encountered in the preparation of an appropriate

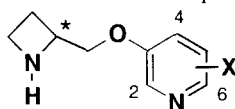
\* Correspondence to: Mark W. Holladay, Ph.D., SIDDCO, Inc., 9000 S. Rita Rd. Bldg. 40, Tucson, AZ 85747

(*R*)-azetidine carboxylic acid precursor in enantiomerically pure form.<sup>4,5</sup> The majority of compounds were prepared, in analogy to previously described procedures, by coupling the corresponding 3-hydroxypyridine to enantiomerically pure *N*-Boc- or *N*-Cbz-protected azetidine-2-methanol using Mitsunobu conditions,<sup>4</sup> or alternatively by displacement of the mesylate or tosylate of *N*-protected azetidine-2-methanol under basic conditions,<sup>5</sup> followed by *N*-deprotection. Routes to commercially unavailable 3-hydroxypyridines are shown in Scheme 1 or have been described elsewhere<sup>6,7</sup> (see Table 1). Some pyridine substituents were incorporated subsequent to the ether forming step via palladium-catalyzed coupling reactions on a halopyridine substrate (Scheme 2 and refs 6–8).

As discussed previously,<sup>1</sup> the relationship between activity at the rat  $\alpha 4\beta 2$  nAChR subtype, represented by the [<sup>3</sup>H]cytisine binding assay,<sup>9</sup> and analgesic activity in mice is tenuous. Functional activity at the human  $\alpha 4\beta 2$  subtype also does not correlate well with data from the mouse hot plate assay.<sup>1</sup> These results are rationalized by postulating that  $\alpha 4\beta 2$  may not be the principal subtype responsible for mouse hot plate activity, although other factors such as species differences, *in vivo* stability, or plasma protein binding must also be considered. The data in Table 1 reinforce the conclusion that binding affinity is not closely correlated to analgesic activity in mice. Thus, replacement of the chloro substituent of **1** with fluoro (**4**), bromo (**5**), or methyl (**6**) affords analogs with potent binding affinity similar to that of **1**. However, whereas the fluoro and methyl substituents are consistent with potent analgesic activity, the bromo compound failed to show an analgesic effect at doses up to 10-fold higher than that for which **1** showed a robust analgesic effect. The 6-ethyl compound (**7**) suffers a steep decline in binding affinity, suggesting considerable steric constraint to substituents at this position for interaction with  $\alpha 4\beta 2$ , whereas substituents with similar or slightly lower steric requirements than ethyl and different electronic effects (-CN, -OMe, vinyl; compounds **8–10**) are somewhat better accommodated with respect to binding affinity. Among compounds **7–10**, an analgesic effect could be demonstrated only for the cyano compound **8**, albeit at a comparatively high dose. Interestingly, the 6-phenyl analog **11** possessed somewhat higher binding affinity than the 6-ethyl compound.

Mono-substitutions on the pyridine ring at the 2-, 4-, and 5-positions, as well as several disubstituted variants, also were examined. Consistent with data reported previously for compounds in the related *N*-methylpyrrolidine series,<sup>10</sup> a variety of substituents at the 5-position are accommodated with respect to high affinity for  $\alpha 4\beta 2$  nAChRs. On the other hand, none showed analgesic activity comparable to that of **1**, although 5-fluoro compound **12** showed an analgesic effect at a 10-fold higher dose, and the 5-OEt (**21**) and 5-NO<sub>2</sub> (**22**) compounds were active at 100-fold higher doses. The 4-chloro compound **26** possesses the lowest affinity (260 nM) for  $\alpha 4\beta 2$  nAChRs among the compounds in Table 1, and is devoid of analgesic activity up to 62  $\mu$ mol/kg. The 2-fluoro compound **27** showed affinity comparable to that of **1**, and analgesic activity at 1.9  $\mu$ mol/kg. By contrast, the 2-chloro (**28**) and 2-methyl (**29**) derivatives were weaker with respect to both binding affinity and analgesic activity. Compounds **30–33** are representative of a number of polysubstituted pyridine analogs that were prepared.<sup>6–8</sup> Whereas the monosubstituted 5-chloro and 5-bromo derivatives (**13** and **14**, respectively) had shown a lack of potent analgesic activity, these substituents in combination with the 6-chloro substituent (**30** and **31**, respectively) were equipotent or only slightly weaker than compound **1** with respect to analgesic activity. On the other hand, the 6-chloro substituent in combination with 5-phenyl (cf. compound **32**) or 2-methyl (cf. compound **33**) substituents failed to exhibit analgesic activity at the doses tested.

Table 1. Neuronal nAChR binding affinities and mouse hot plate activities for azetidine compounds.

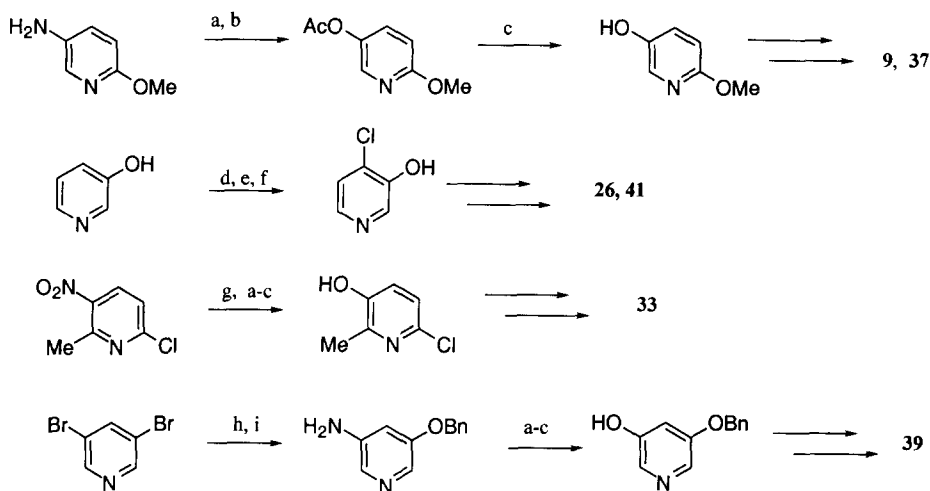


	X	Stereo	[ <sup>3</sup> H]cytisine K <sub>i</sub> (nM) <sup>a</sup>	Hot plate MED μmol/kg <sup>b</sup>	Prep <sup>c</sup>
1	6-Cl	S	0.040 <sup>d</sup>	0.62 <sup>d</sup>	Ref 1
3	H	S	0.052 <sup>e</sup>	>6.2 <sup>d</sup>	Ref 4
4	6-F	S	0.057 ± 0.009	6.2	Ref 7
5	6-Br	S	0.021 ± 0.004	>6.2	Ref 7
6	6-Me	S	0.057 ± 0.003	0.62	Ref 7
7	6-Et	S	19 ± 3	>6.2	Scheme 2
8	6-CN	S	1.9 ± 0.1	62	Ref 7
9	6-OMe	S	1.3 ± 0.1	>62 <sup>f</sup>	Scheme 1
10	6-vinyl	S	1.25 ± 0.09	>62	Scheme 2
11	6-Ph	S	2.3 ± 0.3	>62	Scheme 2
12	5-F	S	0.076 ± 0.009	6.2	Ref 7
13	5-Cl	S	0.042 ± 0.001	>6.2	Ref 7
14	5-Br	S	0.18 ± 0.06	>19	Ref 7
15	5-CF <sub>3</sub>	S	0.49 ± 0.19	>19 <sup>f</sup>	Ref 6
16	5-CN	S	0.025 ± 0.005	>6.2	Ref 6
17	5-NH <sub>2</sub>	S	0.082 ± 0.009	>62	Ref 6
18	5-Me	S	0.047 ± 0.002	>62	Ref 7
19	5-Et	S	0.100 ± 0.005	>62	Ref 7
20	5-n-Pr	S	0.039 ± 0.009	62	Ref 7
21	5-OEt	S	0.039 ± 0.002	62	Ref 7
22	5-NO <sub>2</sub>	S	0.33 ± 0.04	62	Ref 6
23	5-CH <sub>2</sub> NH <sub>2</sub>	S	4.4 ± 0.2	>6.2	Scheme 2

	X	Stereo	[ <sup>3</sup> H]cytisine K <sub>i</sub> (nM) <sup>a</sup>	Hot plate MED μmol/kg <sup>b</sup>	Prep <sup>c</sup>
24	5-CH <sub>2</sub> NHAc	S	0.54 ± 0.06	>62	Scheme 2
25	5-Ph	S	0.046 ± 0.009	>62 <sup>f</sup>	Ref 8
26	4-Cl	S	260 ± 80	>62 <sup>f</sup>	Scheme 1
27	2-F	S	0.048 ± 0.016	1.9	Ref 7
28	2-Cl	S	2.3 ± 0.3	62 <sup>f</sup>	Ref 6
29	2-Me	S	1.36 ± 0.06	>62	Ref 11
30	5-Cl- 6-Cl	S	0.023 ± 0.003	0.62	Ref 7
31	5-Br- 6-Cl	S	0.015 ± 0.002	1.9	Ref 7
32	5-Ph-6-Cl	S	0.022 ± 0.005	>6.2	Ref 8
33	2-Me-6-Cl	S	2.4 ± 0.01	>62	Scheme 1
34	6-F	R	0.066 ± 0.003	1.9	Ref 7
2	6-Cl	R	0.040 <sup>d</sup>	0.62 <sup>d</sup>	Ref 1
35	6-Br	R	0.17 ± 0.04	0.62	Ref 7
36	6-Me	R	0.053 ± 0.009	6.2	Ref 7
37	6-OMe	R	0.67 ± 0.09	>62	Ref 7
38	5-Cl	R	0.12 ± 0.02	62	Ref 7
39	5-OH	R	0.088 ± 0.009	>62	Scheme 1
40	5-Me	R	0.16 ± 0.04	62	Ref 7
41	4-Cl	R	12 ± 2	>6.2	Scheme 1
42	2-Cl	R	85 ± 2	62	Ref 7
43	2-F	R	0.4 ± 0.07	>62	See cpd 27

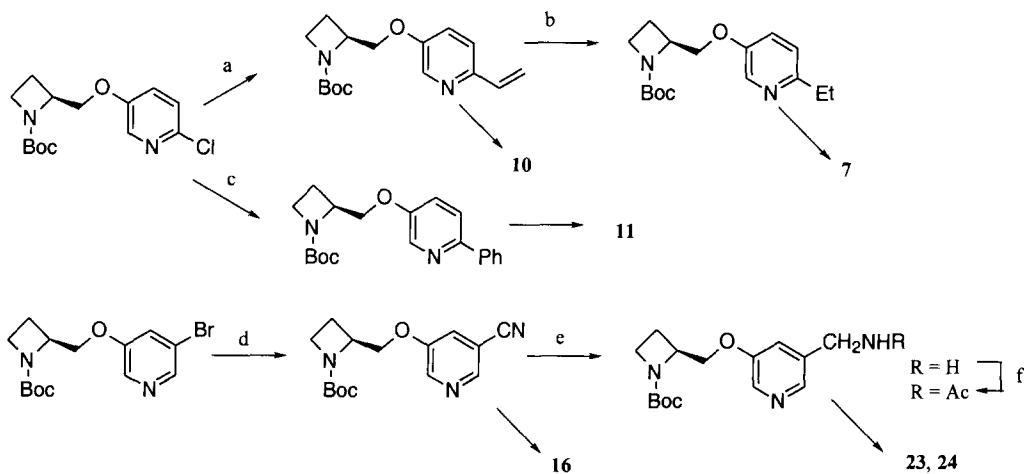
<sup>a</sup>Binding vs. [<sup>3</sup>H]cytisine as described in ref.1 (n≥3). <sup>b</sup>Mouse hot plate assay as described in ref 1 except as noted in footnote f; testing occurred 30 min. after i.p. drug injection. <sup>c</sup>All compounds exhibited satisfactory 300 MHz <sup>1</sup>H-NMR, MS, and microanalytical (±0.4%) data. <sup>d</sup>Ref 1. <sup>e</sup>Ref 4. <sup>f</sup>Locomotor activity and temperature measurements were taken prior to analgesia testing; this additional handling tended to increase sensitivity to drugs in the hot plate assay.

## Scheme 1



a.  $\text{NaNO}_2$ ,  $\text{HBF}_4$  or  $t\text{-BuONO}$ ,  $\text{BF}_3 \cdot \text{Et}_2\text{O}$ ; b.  $\text{Ac}_2\text{O}$ ,  $\Delta$ ; c.  $\text{NaOH}$ ; d.  $\text{CH}_3\text{OCH}_2\text{Cl}$ ,  $\text{NaH}$  (ref 12); e. (i)  $t\text{-BuLi}$ ,  $-78^\circ$  (ii) hexachloroethane; f.  $\text{HCl}$ ,  $\text{EtOH}$ ; g.  $\text{Fe}$ ,  $\text{HOAc}$ ; h.  $\text{BnONa}$ ,  $\text{DMF}$ ; i.  $\text{NH}_3$ ,  $\text{MeOH}$ ,  $\text{CuBr}$ ,  $\Delta$ .

## Scheme 2



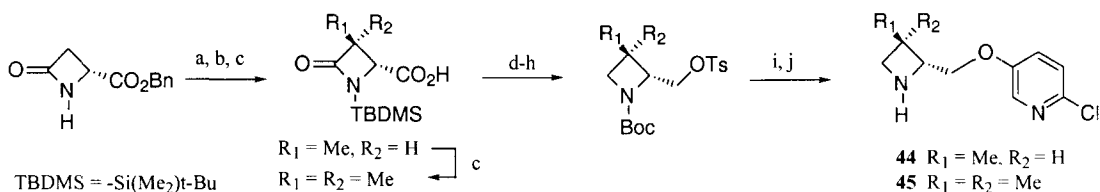
a.  $n\text{-Bu}_3\text{Sn-CH=CH}_2$ ,  $\text{PdCl}_2(\text{dppf})_2$ , toluene; b.  $\text{H}_2$ ,  $\text{Pd-C}$ ,  $\text{MeOH}$ ; c.  $\text{PhB(OH)}_2$ ,  $\text{Pd(PPh}_3)_4$ ; d.  $\text{Zn(CN)}_2$ ,  $\text{Pd(PPh}_3)_4$ ,  $\text{DMF}$ ,  $80^\circ\text{C}$ ; e.  $\text{NaBH}_4$ ,  $\text{CoCl}_2$ ,  $\text{MeOH}$ ; f.  $\text{AcCl}$ ,  $\text{NEt}_3$ ,  $\text{CH}_2\text{Cl}_2$ .

The (*R*)-enantiomers of several analogs that had shown potent analgesic activity in the *S*-enantiomeric series were found to possess comparable activities to the corresponding *S*-enantiomers in both binding and analgesia assays. Thus, the 6-fluoro (**34**), 6-chloro (**2**) and 6-methyl (**36**) analogs in the (*R*)-enantiomeric series possessed binding affinity in the picomolar range and analgesic activity at doses  $\leq 6.2 \mu\text{mol/kg}$ . The (*R*)-6-bromo analog **35** also showed analgesic activity at  $0.62 \mu\text{mol/kg}$ , which is in contrast to the lack of activity observed for the corresponding *S*-enantiomer (**5**) at doses up to 10-fold higher.

In *in vitro* functional assays, the compounds from this study were generally more efficacious than the corresponding analogs in the *N*-methylpyrrolidine series reported earlier.<sup>10</sup> Thus, for example, 5-ethyl compound **19** elicited a maximal response of 90% (at  $10 \mu\text{M}$ ) vs. the maximal response of (*S*)-nicotine in the  $^{86}\text{Rb}^+$  release assay using IMR-32 cells, compared with a 15% maximal response for the corresponding *N*-methylpyrrolidine analog.<sup>12</sup> Although in the azetidine series, 5-phenyl analog **25** elicited only a 45% maximal response (at  $3 \mu\text{M}$ ) in this assay, the 5-phenyl-6-chloro analog (**32**) stimulated a 92% response ( $1 \mu\text{M}$ ). Similarly, **1** had been found to be substantially more efficacious than **3** in this assay (166% vs. 113%), whereas the difference between **2** and its deschloro analog was negligible.<sup>1</sup> Thus, the chloro substituent often, but not always, has the effect of increasing the functional efficacy of compounds in this series.

We also have conducted preliminary studies on the effect of azetidine ring substitution. Specifically, analogs of compound **2** with *trans*-3-methyl (**44**) and 3,3-dimethyl (**45**) substituents were prepared by adaptation of published procedures<sup>5,13,14</sup> (Scheme 3). Compounds **44** and **45** possessed substantially reduced binding affinities ( $K_i = 7.6 \pm 2.2 \text{ nM}$  and  $K_i = 37 \pm 7 \text{ nM}$ , respectively) compared to **2**, and both were devoid of effects in the mouse hot plate assay at doses up to  $62 \mu\text{mol/kg}$ .

### Scheme 3



a.  $t\text{-Bu}(\text{Me})_2\text{SiCl}$ ,  $\text{NEt}_3\text{Pr}_2$ ,  $\text{CH}_2\text{Cl}_2$ ; b.  $\text{H}_2$ ,  $\text{Pd-C}$ ; c. (i)  $\text{LDA}$ ,  $\text{THF}$ ,  $-15^\circ\text{C}$ ; (ii)  $\text{MeI}$ ,  $-15^\circ\text{C}$  to rt; d.  $\text{CH}_2\text{N}_2$ ; e.  $\text{HCl}$ ,  $\text{MeOH}/\text{H}_2\text{O}$ ; f.  $\text{LiAlH}_4$ ,  $0^\circ\text{C}$  to rt; g.  $(\text{Boc})_2\text{O}$ ; h.  $\text{TsCl}$ ,  $\text{NEt}_3$ ,  $\text{CH}_2\text{Cl}_2$ ; i. 2-chloro-5-hydroxypyridine,  $\text{KOH}$ ,  $\text{DMF}$ ,  $80^\circ\text{C}$ ; j.  $\text{CF}_3\text{CO}_2\text{H}$ ,  $\text{CH}_2\text{Cl}_2$

In summary, analogs of A-98593 (**1**) and its enantiomer ABT-594 (**2**) with diverse substituents on the pyridine ring were prepared and tested for affinity to nicotinic acetylcholine receptor binding sites in rat brain and for analgesic activity in the mouse hot plate assay. Numerous types of modifications were consistent with high affinity for  $[^3\text{H}]$ cytisine binding sites, but only selected modifications resulted in retention of analgesic potency in the same range as **1** and **2**. Analogs of **2** with one or two methyl substituents at the 3-position of the azetidine ring also were prepared and found to be markedly less active in both assays.

## References and Notes

1. Holladay, M. W.; Wasicak, J. T.; Lin, N.-H.; He, Y.; Ryther, K. B.; Bannon, A. W.; Buckley, M. J.; Kim, D. J. B.; Decker, M. W.; Anderson, D. J.; Campbell, J. E.; Kuntzweiler, T. A.; Donnelly-Roberts, D. L.; Piattoni-Kaplan, M.; Briggs, C. A.; Williams, M.; Arneric, S. P. *J. Med. Chem.* **1998**, *41*, 407.
2. Bannon, A. W.; Decker, M. W.; Holladay, M. W.; Curzon, P.; Donnelly-Roberts, D.; Puttfarcken, P. S.; Bitner, R. S.; Diaz, A.; Dickenson, A. H.; Porsolt, R. D.; Williams, M.; Arneric, S. P. *Science* **1998**, *279*, 77.
3. Decker, M. W.; Bannon, A. W.; Buckley, M. J.; Kim, D. J. B.; Holladay, M. W.; Ryther, K. B.; Lin, N.-H.; Wasicak, J. T.; Williams, M.; Arneric, S. P. *Eur. J. Pharmacol.* **1998**, *346*, 23.
4. Abreo, M. A.; Lin, N.-H.; Garvey, D. S.; Gunn, D. E.; Hettinger, A.-M.; Wasicak, J. T.; Pavlik, P. A.; Martin, Y. C.; Donnelly-Roberts, D.; Anderson, D. J.; Sullivan, J. P.; Williams, M.; Arneric, S. P.; Holladay, M. W. *J. Med. Chem.* **1996**, *39*, 817.
5. Lynch, J. K.; Holladay, M. W.; Ryther, K. B.; Bai, H.; Hsiao, C.-N.; Morton, H. E.; Dickman, D. A.; Arnold, W.; King, S. A. *Tetrahedron: Asymmetry* **1998**, *in press*.
6. Abbott Laboratories, WO 9640682 (1996).
7. Abbott Laboratories, WO 9825920 (1998).
8. Abbott Laboratories, US 5,629,325 (1997).
9. Pabreza, L. A.; Dhawan, S.; Kellar, K. J. *Mol. Pharm.* **1990**, *39*, 9.
10. (a) Lin, N.-H.; Gunn, D. E.; Li, Y.; He, Y.; Bai, H.; Ryther, K. B.; Kuntzweiler, T.; Donnelly-Roberts, D. L.; Anderson, D. J.; Campbell, J. E.; Sullivan, J. P.; Arneric, S. P.; Holladay, M. W. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 249-254. (b) Lin, N.-H.; Li, Y.; He, Y.; Donnelly-Roberts, D. L.; Anderson, D. J.; Campbell, J. E.; Arneric, S. P.; Holladay, M. W. *Abst. 214th ACS Natl. Meeting*, **1997**, MEDI008.
11. Abbott Laboratories, WO 9408992 (1994).
12. Winkle, M. R.; Ronald, R. C. *J. Org. Chem.* **1982**, *47*, 2101.
13. Finke, P. E.; Shah, S. K.; Fletcher, D. S.; Ashe, B. M.; Brause, K. A.; Chandler, G. O.; Dellea, P. S.; Hand, K. M.; Maycock, A. M.; Osinga, D. G.; Underwood, D. J.; Weston, H.; Davies, P.; Doherty, J. B. *J. Med. Chem.* **1995**, *38*, 2449.
14. Kozikowski, A. P.; Liao, Y.; Tückmantel, W.; Wang, S.; Pshenichkin, S.; Surin, A.; Thomsen, C. Wroblewski, J. T. *Bioorg. Med. Chem. Lett.* **1996**, 2559.