Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 3683–3688

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

ALKOXIDE-CATALYZED RING-OPENING OF A NOVEL HOMOSACCHARIN DERIVATIVE: SYNTHESIS OF POTENT, SELECTIVE P_3-LACTAM THROMBIN INHIBITORS CONTAINING P_4-*o*-ALKOXYCARBONYLBENZYLSULFONAMIDE RESIDUES[1]

Timothy D. Owens[†] and J. Edward Semple*

Department of Medicinal Chemistry, Corvas International, Inc.
3030 Science Park Road, San Diego, CA 92121, U.S.A.

Received 25 September 1998; accepted 4 November 1998

Abstract: A series of lactam derivatives **1b–g** featuring P_4-*o*-alkoxycarbonylbenzylsulfonamide residues along with the potential P_4-homosaccharin prodrug candidate **1h** was prepared in order to probe the thrombin S_3 specificity pocket. The synthesis and alkoxide-catalyzed ring opening of the novel homosaccharin intermediate **7** followed by subsequent elaboration delivered the targets **1b–h** which were potent and selective thrombin inhibitors. The design, synthesis, and biological activity of these targets will be presented.

Thrombin, a multifunctional serine protease with trypsin-like specificity, plays a central role in the blood coagulation cascade.[2,3] Serving as the terminal enzyme of this pathway, thrombin (FIIa) cleaves fibrinogen to fibrin, which in turn aggregates to a gel-like matrix and ultimately forms blood clots. Because of this role and other key regulatory functions, it has continued to attract considerable attention as a therapeutic target. Accordingly, the discovery of novel thrombin inhibitors is a very active research area in the pharmaceutical industry.[4] We have recently described several new classes of peptidomimetic P_1-argininals as thrombin inhibitors which feature P_3-azapeptide,[5] -lactam,[6] -bicyclic lactam,[7] -pyridone,[8] -pyrimidinone,[8] -uracil,[8] and P_3,P_4-quaternary lactam scaffolds.[9] From such systems a wide variety of potent, selective, and orally bioavailable transition-state thrombin inhibitors have emerged.[10] In connection with our further investigations on the monocyclic lactam family, the design, synthesis, and biological activity of a novel series of 7-membered lactam derivatives **1b–g** which feature hydrophobic P_4-o-alkoxycarbonylbenzyl sulfonamide residues, will be presented herein. The P_4-1*H*-2,3-Benzothiazin-4(3*H*)-one, 2,2 dioxide ("homosaccharin") target **1h**, a potential prodrug, is also described.

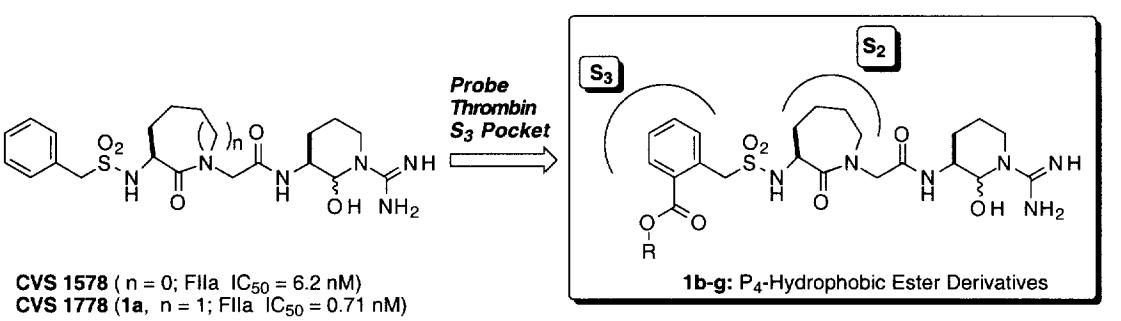


Figure 1: Design of Lactam Thrombin Inhibitors **1b–g** Featuring P_4-Benzylsulfonamide ortho-Ester Residues.

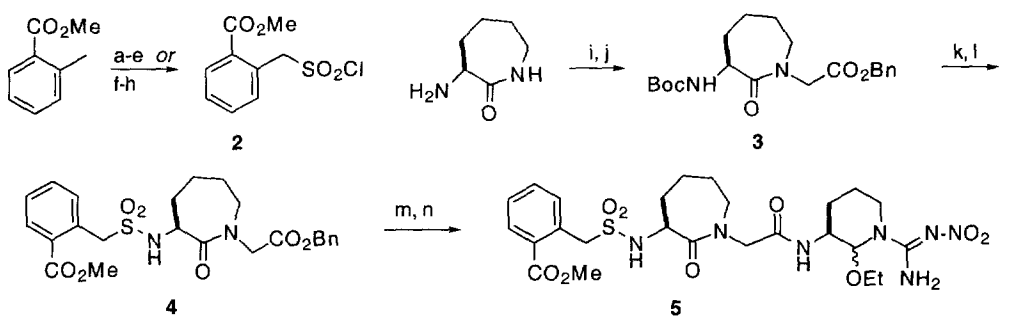
0960-894X/98/$ - see front matter
PII: S0960-894X(98)00667-2

Inhibitor Design Strategy

Crystal structure information from the six-membered lactam CVS 1578–thrombin complex[6a,c] and related rigidified P_1-arginine surrogates[6b,c] suggested the hypothesis that P_3 7-membered lactam homologs may more efficiently pack the S_2 pocket due to improved van der Waals contacts and optimal hydrophobic interactions with the thrombin 60-loop.[6c] Our synthetic work quickly confirmed this hypothesis and demonstrated that the 7-membered homolog CVS 1778 (**1a**) expressed superior activity against thrombin (Figure 1). With interesting potency, selectivity, and ~66% oral bioavailability profiles in dogs, we were encouraged to further pursue this series.[6d] Probing SAR at the P_4-aromatic ring positions with a range of substituents resulted in less active compounds. However, relative potency and selectivity were retained with certain ester substituents. New P_4-ortho-benzylic ester targets were then prepared to both probe SAR at the thrombin S_3 pocket and to deliver candidates with potentially superior PK profiles. We envisioned construction of a late stage homosaccharin intermediate (cf. *1H*-2,3-Benzothiazin-4(3*H*)-one, 2,2 dioxide derivative **7**) that would undergo ring-opening reactions[11] with alkoxide nucleophiles and thereafter rapidly afford the final target inhibitor candidates. Herein, we report new chemistry which proceeds through such an intermediate and efficiently delivered a series of lactam derivatives **1b–h**.

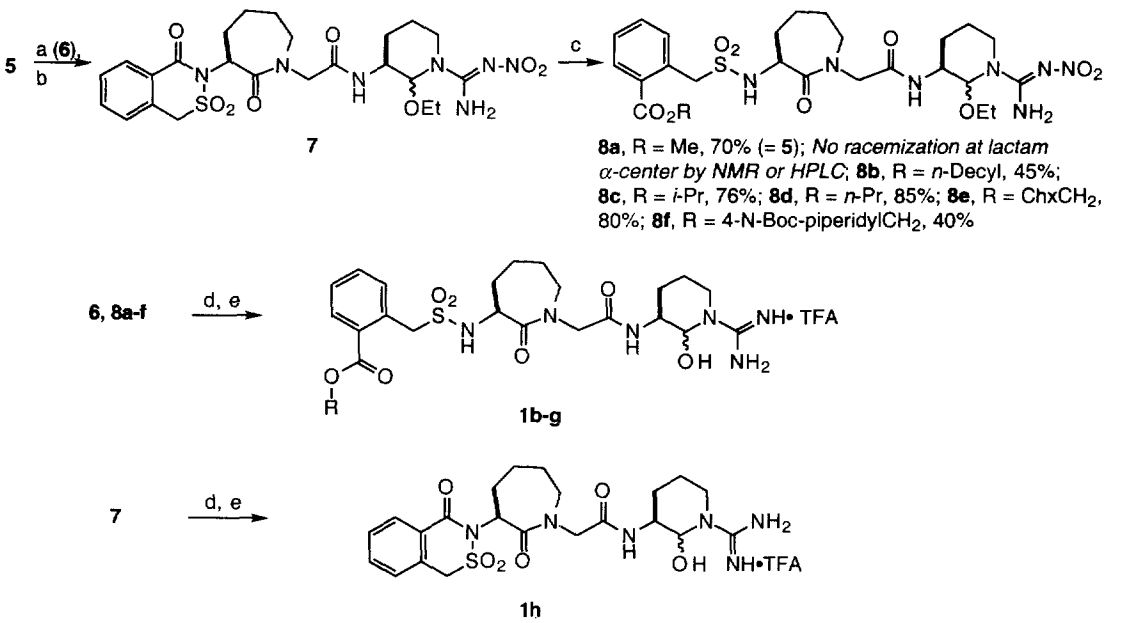
Synthetic Routes to Lactam Targets 1b–h

The sulfonyl chloride **2** was obtained from methyl o-toluate by two different routes as outlined in Scheme 1.[12] Synthesis of ca. 15–20 gram quantities of **2** was possible via the former route. For larger scale work, we found the latter thiouronium salt oxidation protocol to be superior. Synthesis of the lactam sulfonamide intermediate **5** proceeded smoothly from commercially available (l)-α-amino-ε-caprolactam. Intermediate **3** was efficiently prepared in up to 150 g quantities by regioselective alkylation of α-N-Boc-amino-ε-caprolactam with benzyl bromoacetate. $LiN(TMS)_2$ was the preferred base, and the reaction proceeded very smoothly with related electrophiles (alkyl bromoacetates, allyl bromide, ethyl acrylate) providing alkylated products in 65–95% yields. A two-step elaboration of intermediate **3** to the d-Phe-l-Pro dipeptide surrogate **4** was followed by hydrogenolysis and coupling with our recently described P_1-argininal precursor[13] to deliver intermediate **5**.



Scheme 1: *Reagents and Conditions;* (a) NBS, $(PhCO_2)_2$, CCl_4, hυ, reflux, 69-72%; (b) KSAc, DMF, rt,1 h, 96-100%; (c) H_2O_2, HOAc, 60-110 °C, 2 h; (d) NaOH, lyophilize, ~quant, (~13-20% di-Na salt); (e) $POCl_3$, rt to 40 °C, 60-80%; (f) Cl_2, CCl_4, reflux, 60-63%; (g) thiourea, MeOH, reflux, ~quant.; (h) Cl_2, H_2O, 0 °C, 75%; (i) Boc_2O, $NaHCO_3$, THF, H_2O, 0 °C to rt, 95-99%; (j) $LiN(TMS)_2$,THF, $BrCH_2CO_2Bn$, 0 °C to rt, 86-91%; (k) HCl, EtOAc, 0 °C to rt, 99%; (l) **2**, Et_3N, CH_3CN, 0 °C to rt, 87%; (m) H_2, Pd/C, MeOH, 45 psi, ~quant.; (n) HCl•Arg(NO_2)-H O-ethyl aminal, EDC, HOBt, DIEA, CH_3CN, rt, 61-66%.

Formation and alkoxide-catalyzed ring-opening reactions of the key homosaccharin intermediate **7** are outlined in Scheme 2. Lithium hydroxide hydrolysis of intermediate **5** was very slow, requiring three days for completion, but quantitatively produced the carboxylic acid **6**. Mild intramolecular dehydration of **6** generated the novel homosaccharin intermediate **7** in high yield. Alkoxide-catalyzed ring-opening of **7** with a variety of alcohols provided the corresponding esters **8a–f** in 40–85% unoptimized yields. Surprisingly, alkoxide-catalyzed ring-opening reactions of homosaccharin systems were without literature precedent.[11] However, similar examples of alkoxide ring-opening of saccharin derivatives have recently been reported.[11c,d] Although not investigated exhaustively, attempted ring-opening reaction with amines, including benzylamine under various conditions, failed to deliver the analogous amide-type products. As a stringent lactam racemization test case, reaction of the substrate **7** with the sterically small and highly basic nucleophile sodium methoxide was investigated and cleanly led to the product **8a** (= intermediate **5**) whose NMR and HPLC profiles showed complete retention of chiral integrity. Standard deprotection, hydrolysis, and RP-HPLC steps led to the targets **1b–g**. The potential prodrug target **1h** was obtained from **7** in a similar fashion.



Scheme 2: *Reagents and Conditions:* (a) LiOH, EtOH, H_2O, rt, 3 days; HOAc, ~quant. (**6**); (b) EDC, HOBt, NMM, CH_3CN, 0 °C to rt, 80%; (c) ROH, NaH, THF, 0 °C to rt; HOAc, 40-85%; (d) H_2, Pd/C, EtOH, H_2O, HOAc, 45psi, ~quant.; (e) 3-4 N HCl, CH_3CN, rt, 2-4 h; HPLC, 55-75%

Biological Activity

The in vitro biological activity of the targets **1b–h** along with the standards CVS 1578 and CVS 1778 (**1a**) is shown in Table 1.[14] In general, the targets were highly selective against the thrombolytic enzyme plasmin. Selectivity on FXa ranged from modest to excellent. Activity levels on thrombin ranged from 0.56–39.1 nM, with larger branched hydrophobic P_4-esters expressing optimal in vitro activity and demonstrating potentially useful selectivity profiles. It therefore appears that the S_3 specificity pocket of thrombin can readily accommodate a range

of hydrophobic alkyl derivatives when they are specifically tethered from the P_4-ortho-benzylic carboxylate function. In the new 7-membered lactam series, activity decreased in the following order: $ChxCH_2$ (**1g**, 0.56 nM) > **1a** (CVS 1778, 0.70 nM) ≡ Me (**1b**, 0.75 nM) > *i*-Pr (**1e**, 0.94 nM) ≡ *n*-Pr (**1f**, 0.96 nM) > *n*-Decyl (**1d**, 7.03 nM) > H (**1c**, 39.1 nM) >> Homosaccharin prodrug (**1h**, 244 nM). The derivative **1g** expressed optimal thrombin inhibitory potency, being slightly more active than the standard CVS 1778, and showed excellent FXa and trypsin selectivity. Likewise esters **1b** and **1f** demonstrated attractive activity/selectivity profiles. As expected, the potential prodrug **1h** was the least active in vitro.

Based upon our evolving modeling and crystal structure studies, numerous important interactions commonly found in small molecule thrombin inhibitors are present at the active, S_1, S_2, and S_3 subsites in the novel series **1b–h**. The P_1-P_4 lactam argininal motifs appear to provide a full complement of important backbone and side-chain interactions at the active site, including antiparallel β-sheet hydrogen bonds to Gly-216, salt bridges, hydrophobic, edge-to-face and van der Waals interactions. In this series, tethering of the aromatic ring to the S_3 site is most efficiently accomplished employing a tetrahedral benzylic sulfonamide linker. Additionally, we have now demonstrated that appropriately substituted P_4-hydrophobic ortho-esters are well tolerated at the thrombin S_3 specificity pocket. Based on topographical modeling considerations, we surmise that such P_4 ester residues undergo favorable hydrophobic interactions with the S_3 Leu-99 and Ile-174 residues . These additional active site interactions possibly contribute to the increased inhibitor activity profiles. Other important backbone and sidechain inhibitor-active site interactions were conserved.

Table 1. In vitro IC_{50} values (nM) of lactam argininals **1b–h** and reference standards against a range of important serine proteases.[a,b]

Cmpd.	MOLNAME	FIIa	FXa	Plasmin	Hu Tryp	FXa/FIIa	Tryp/FIIa
	Reference Compounds:						
CVS 1578	BnSO2-6Lac-G-R-al	6.2	>2500	Inact.	1271	>403	205.0
CVS 1778 (1a)	BnSO2-7Lac-G-R-al	0.7	39.6	>2500	101	53.6	136.7
	New P_4-Ester Targets:						
1b	(2CO2Me)BnSO2-7Lac-G-R-al	0.75	333	>2500	118	444.0	157.3
1c	(2CO2H)BnSO2-7Lac-G-R-al	39.1	>2500	>2500	247	>64	6.3
1d	(2CO2Decyl)BnSO2-7Lac-G-R-al	7.03	>2500	>2500	143	>356	20.3
1e	(2CO2iPr)BnSO2-7Lac-G-R-al	0.94	369	>2500	41.3	392.6	43.9
1f	(2CO2Pr)BnSO2-7Lac-G-R-al	0.96	451	>2500	79.8	469.8	83.1
1g	(2CO2CH2Chx)Bn SO2-7Lac-G-R-al	0.56	627	1760	135	1119.6	241.1
1h	homosaccharin-7Lac-G-R-al	244	>2500	>2500	2500	>10.2	10.2

[a]Concentration of **1b–g** and standards necessary to inhibit thrombin (FIIa), FXa, plasmin, and human trypsin cleavage of the chromogenic substrates described in ref. 6a,b by 50%. Reported value for each compound is from one to three IC_{50} determinations which confirmed initial range values. [b]All target compounds were characterized by ^{1}H-NMR, RPHPLC, low/high resolution mass spectroscopy.

Conclusions

Starting with the reference P_3–P_4 lactam sulfonamides CVS 1578, CVS 1778 (**1a**) , and related serine protease inhibitors, a new series of P_4-ester derivatives **1b–g** and the prodrug **1h** were designed to probe the S_3 thrombin active-site. During the course of our synthetic work on these targets, the novel homosaccharin derivative **7** was efficiently prepared in high overall yield. Late-stage base-catalyzed ring-opening with alcohols afforded a range of advanced P_4-hydrophobic ester intermediates which were elaborated to the final targets. In vitro evaluation against serine proteases involved in the blood coagulation cascade and trypsin revealed several potent, selective candidates. **1b** and **1g** were of highest interest and have been evaluated in animal studies. Apparently, the S_3 pocket of thrombin prefers hydrophobic ester groups in the ortho-benzylic position, and can accommodate quite a variety of alkyl groups without loss of potency.[15] This series of esters comprises representative prototypes; we have not optimized SAR in this family. Further permutations could lead to targets with altered selectivity towards other serine proteases of interest.

Acknowledgments: We gratefully acknowledge Susanne M. Anderson for all in vitro pharmacological studies, and Drs. T. K. Brunck, R. F. Nutt, W. C. Ripka, and S. Y. Tamura for stimulating discussions regarding thrombin inhibitor targets.

References and Notes

† Current Address: Department of Chemistry, University of California Berkeley, Berkeley, CA 94720, U.S.A.

1. Dedicated with great admiration and respect to our illustrious chemistry department consultant, Professor Henry Rapoport, Department of Chemistry, University of California at Berkeley, on the occasion of his 80th birthday.

2. Portions of this work were presented at the 216th National American Chemical Society Meeting, Boston, MA, August 23-27, 1998; Medicinal Chemistry Section, MEDI-073.

3. Recent Reviews: (a) Vacca, J. P. In *Ann. Rep. Med. Chem.* Bristol, J. A., Ed.; Academic: San Diego, 1998; Vol. 33, 81. (b) Ripka, W. C. *Curr. Opin. Chem. Biol.* **1997**, *1*, 242. (c) Ripka, W. C.; Vlasuk G. P. In *Ann. Rep. Med. Chem.* Bristol, J. A., Ed.; Academic Press: San Diego, 1997; Vol. 32, 71. (d) Edmunds, J. J.; Rapundalo, S. T.; Siddiqui, M. A. *ibid.*, 1996, Vol. 31, 51. (e) Wiley, M. R. ; Fisher, M. J. *Expert Opin. Ther. Pat.* **1997**, *7* , 1265. (f) Lee, K. *Kor. J. Med. Chem.* **1997**,*7*, 127.

4. Recent Thrombin Inhibitors: (a) Gustafsson, D.; Antonsson, T.; Bylund, R.; Eriksson, U.; Gyzander, E.; Nilsson, I.; Elg, M.; Mattson, C.; Deinum, J.; Pehrsson, S.; Karlsson, O.; Nilsson, A.; Sorensen, H. *Thromb. Haemost.* **1998**, *79*, 110. (b) Brady, S. F.; Stauffer, K. J.; Lumma, W. C.; Smith, G. M.; Ramjit, H. G.; Lewis, S. D.; Lucas, B. J.; Gardell, S. J.; Lyle, E. A.; Appleby, S. D.; Cook, J. J.; Holahan, M. A.; Stranieri, M. T.; Lynch, J. J.; Lin, J. H.; Chen, I.-W.; Vastag, K.; Naylor-Olsen, A. M; Vacca, J. P. *J. Med. Chem.* **1998**, *41*, 401. (c) Fevig, J. M.; Buriak, J.; Cacciola, J.; Alexander, R. S.; Kettner, C. A.; Knabb, R. M.; Pruitt, J. R.; Weber, P. C.; Wexler, R. R. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 301. (d) Obst, U.; Banner, D. W.; Weber, L.; Diederich, F. *Chem. Biol.* **1997**, *4*, 287.

5. Semple, J. E.; Rowley, D. C.; Brunck, T. K.; Ripka, W. C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 315.

6. Lactam sulfonamide thrombin inhibitors: (a) Semple, J. E.; Rowley, D. C.; Brunck, T. K.; Ha-Uong, T.; Minami, N. K.; Owens, T. D.; Tamura, S. Y.; Göldman, E. A.; Siev, D. V.; Ardecky, R. J.; Carpenter, S. H.; Ge, Y.; Richard, B. M.; Nolan, T. G.; Håkanson, K.; Tulinsky, A.; Nutt, R. F.; Ripka, W. C. *J. Med. Chem.* **1996**, *39*, 4531. (b) Levy, O. E.; Semple, J. E. ; Lim, M. L.; Reiner, J.; Rote, W. E.; Dempsey, E.; Richard, B.

M.; Zhang, E.; Tulinsky, A.; Ripka, W. C.; Nutt, R. F. *J. Med. Chem.* **1996**, *39*, 4527. (c) Krishnan, R.; Zhang, E.; Hakansson, K.; Arni, R. V.; Tulinsky, A.; Lim-Wilby M. S. L.; Levy, O. E.; Semple, J. E.; Brunck, T. K. *Biochemistry* **1998**, *37*, 12094. (d) Semple, J. E.; Ardecky, R. J.; Nutt, R. F.; Ripka, W. C.; Rowley, D. C.; Lim-Wilby, M. S.; Brunck, T. K. US Patent 5703208, 1997. (*Chem. Abstr.* **1998**, *128:* 102391).

7. Tamura, S. Y.; Goldman, E. G.; Brunck, T. K.; Ripka, W. C.; Semple, J. E. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 331.

8. Tamura, S. Y.; Semple, J. E.; Reiner, J. E.; Goldman, E. A.; Brunck, T. K.; Lim-Wilby, M.; Carpenter, S. H.; Rote, W. E.; Oldschulte, G. L. Richard, B. M.; Nolan, T. G.; Nutt, R. F.; Ripka, W. C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1543.

9. (a) Semple, J. E. *Tetrahedron Lett.* **1998**, *39*, 6645. (b) Semple, J. E. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2501. (c) Semple, J. E.; Minami, N. K.; Tamura, S. Y.; Brunck, T. K.; Nutt, R. F.; Ripka, W. C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2421.

10. For our other recent serine protease inhibitor studies see: (a) Tamura, S. Y.; Shamblin, B. M.; Brunck, T. K.; Ripka, W. C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1359. (b) Nutt, R. F.; Semple, J. E.; Levy, O. E.; Nolan, T. G.; Tamura, S. Y.; Ardecky, R. J.; Rowley, D. C.; Lim, M. L.; Ha-Uong, T. Richard, B. M.; Bergum, P. W.; Ripka, W. C. In *Peptides 1996. Proc. 24th Eur. Pept. Symp.*; Ramage, R. Ed.; Mayflower Scientific, **1997**. (c) Tamura, S. Y.; Semple, J. E.; Ripka, W. C.; Ardecky, R. J.; Carpenter S. H.; Ge, Y.; Brunck, T. K.; Nutt, R. F. US Patent 5658930, 1997 (*Chem Abstr.* **1997**, *127:* 234607).

11. Recent on-line searches in both Sci-Finder® and STN Express® databases employing structural and verbal queries on the preparation and/or ring-opening reactions of homosaccharin derivatives (1*H*-2,3-Benzothiazin-4(3*H*)-one 2,2 dioxide systems) failed to unearth the reaction of such systems with alkoxide or amine-type nucleophiles. However, the preparation of such heterocycles by $SOCl_2$ or PCl_5-mediated intramolecular dehydration using rather vigorous conditions as well as the strong protic acid or base hydrolytic ring-opening reactions under drastic conditions have been described. See (a) Sianesi, E.; Redaelli, R.; Magistretti, M. J.; Massarani, E. *J. Med. Chem.* **1973**, *16*, 1122. (b) Sianesi, E.; Redaelli, R.; Bertani, M.; Da Re, P. *Chem. Ber.* **1970**, *103*, 1992. JES acknowledges a reviewer for providing the following precedents of alkoxide-catalyzed ring-opening of saccharin derivatives, see: (c) Manharrez, N.; Perez, H. I.; Solis, A.; Luna, H. *Synth. Commun.* **1996**, *26*, 585. (d) Bianco, M.; Perillo, I. A.; Schapira, C. B. *J. Heterocycl. Chem.* **1995**, *32*, 145.

12. All new compounds were characterized by full spectroscopic (NMR, low/high resolution MS) data. Yields refer to spectroscopically and chromatographically homogenous (≥95% by HPLC, TLC) materials.

13. (a) Tamura, S. Y.; Semple, J. E.; Ardecky, R. J.; Leon, P.; Carpenter, S. H.; Ge, Y.; Shamblin, B. M.; Weinhouse, M. I.; Ripka, W. C.; Nutt, R. F. *Tetrahedron Lett.* **1996**, *37*, 4109.

14. The in vitro enzyme assays were performed according to the protocols described in refs 3 and 4.

15. (a) Engh, R. A.; Brandstetter, H.; Sucher, G.; Eichinger, A.; Baumann, U.; Bode, W.; Huber, R.; Poll, T.; Rudolph, R.; von der Saal, W. *Structure* **1996**, *4*, 1353. (b) Malley, M. F.; Tabernero, L.; Chang, C. Y.; Ohringer, S. L.; Roberts, D. G. M.; Das, J.; Sack, J. S. *Protein Sci.* **1996**, *5*, 221. (c) Håkansson, K.; Tulinsky, A.; Abelman, M. M.; Miller, T. A.; Vlasuk, G. P.; Bergum, P. W.; Lim-Wilby, M. S. L.; Brunck, T. K. *Bioorg. Med. Chem.* **1995**, *3*, 1009.