

Synthesis and V-ATPase Inhibition of Simplified Lobatamide Analogues

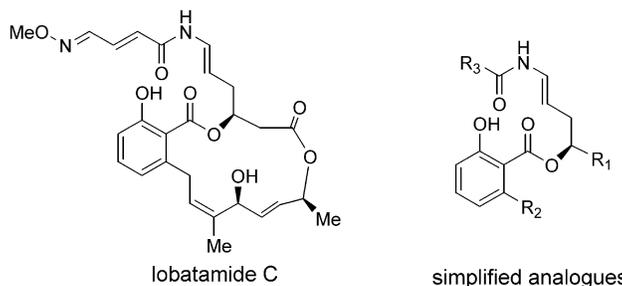
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ABSTRACT



Simplified analogues of the lobatamides have been synthesized and evaluated for inhibition of bovine V-ATPase. The salicylate phenol, enamide NH, and the ortho-substitution of the salicylate ester have been shown to be important for V-ATPase inhibitory activity.

The recently reported salicylate enamide macrolides, including the salicylihalamides,¹ lobatamides,² apicularens,³ CJ-12950, and CJ-13357,⁴ and the oximidines⁵ comprise a unique class of natural products with a common benzolactone core structure and a highly unsaturated enamide side chain. (Figure 1). Two members of the class, salicylihalamides and lobatamides, displayed high potency in the NCI's 60 cell-line human tumor screen (mean panel GI₅₀'s: ~1.6 nM for lobatamides and ~15 nM for salicylihalamide A) along with

unique cellular response profiles.^{1,2} Recent studies have shown that the salicylate enamide macrolides potently inhibit

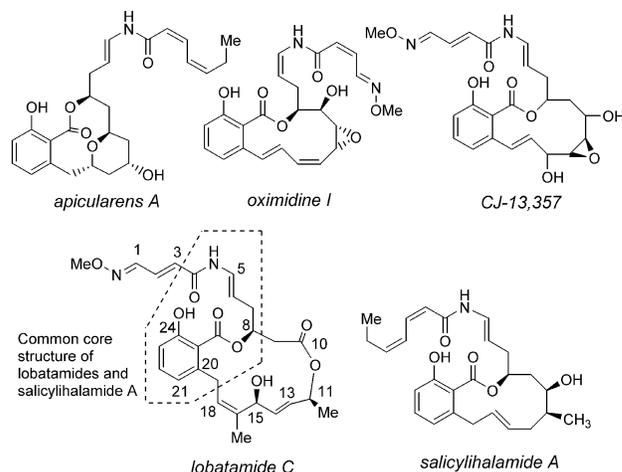


Figure 1. Salicylate enamide natural products.

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(1) (a) Erickson, K. L.; Beutler, J. A.; Cardellina, J. H., II; Boyd, M. R. *J. Org. Chem.* **1997**, *62*, 8188 (b) Erickson, K. L.; Beutler, J. A.; Cardellina, J. H., II; Boyd, M. R. *J. Org. Chem.* **2001**, *66*, 1532.

(2) McKee, T. C.; Galinis, D. L.; Pannell, L. K.; Cardellina, J. H., II; Laasko, J.; Ireland, C. M.; Murray, L.; Capon, R. J.; Boyd, M. R. *J. Org. Chem.* **1998**, *63*, 7805.

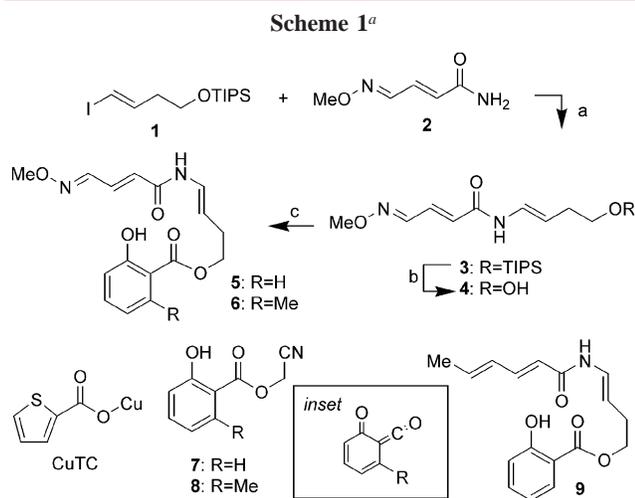
(3) (a) Kunze, B.; Jansen, R.; Sasse, F.; Hofle, G.; Reichenbach, H. *J. Antibiot.* **1998**, *51*, 1075. (b) Jansen, R.; Kunze, B.; Reichenbach, H.; Hofle, G. *Eur. J. Org. Chem.* **2000**, *6*, 913.

(4) Dekker, K. A.; Aiello, R. J.; Hirai, H.; Inagaki, T.; Sakakibara, T.; Suzuki, Y.; Thompson, J. F.; Yamaguchi, Y.; Kojima, N. *J. Antibiot.* **1998**, *51*, 14.

(5) Kim, J. W.; Shin-ya, K.; Furihata, K.; Hayakawa, Y.; Seto, H. *J. Org. Chem.* **1999**, *64*, 153.

mammalian vacuolar-type (H⁺)-ATPases (V-ATPases) which are ubiquitous proton-translocating pumps of eukaryotic cells.⁶ Accordingly, these natural products are exciting new targets for chemical synthesis, lead optimization studies, and preparation of designed analogues to further define interactions with the molecular target. The salicylhalamides have been synthesized in several laboratories,⁷ and De Brabander et al. have reported the preparation and structure–function analysis of a number of promising derivatives.⁸ We recently reported the total synthesis and stereochemical assignment of lobatamide C using Cu(I)-catalyzed amidation for synthesis of the C1–C10 fragment.⁹ In continuation of these studies, we report the synthesis and biological evaluation of simplified analogues of the lobatamides in order to clarify the minimal core structure (pharmacophore) required for V-ATPase inhibition.

Our initial objective was to prepare acyclic lobatamide analogues and determine their ability to inhibit V-ATPase. Since previous studies have shown that the enamide side chain is important for the potent bioactivities of salicylhalamides^{1,8} and apicularen A,¹⁰ we initiated studies employing the C1–C8 subunit of the lobatamides (Scheme 1). TIPS-



protected (*E*)-4-iodo-3-buten-1-ol¹¹ (**1**) underwent copper(I) thiophenecarboxylate (CuTC)-mediated cross coupling with amide **2**¹² to furnish enamide **3**, which was desilylated using

(6) Boyd, M. R.; Farina, C.; Belfiore, P.; Gagliardi, S.; Kim, J. W.; Hayakawa, Y.; Beutler, J. A.; McKee, T. C.; Bowman, B. J.; Bowman, E. J. *J. Pharm. Exp. Ther.* **2001**, *297*, 114.

(7) (a) Wu, Y.; Esser, L.; De Brabander, J. K. *Angew. Chem., Int. Ed.* **2000**, *39*, 4308. (b) Labrecque, D.; Charron, S.; Rej, R.; Blais, C.; Lamothe, S. *Tetrahedron Lett.* **2001**, *42*, 2645. (c) Snider, B. B.; Song, F. *Org. Lett.* **2001**, *3*, 1817. (d) Smith, A. B., III; Zheng, J. *Synlett* **2001**, 1019. (e) Furstner, A.; Dierkes, T.; Thiel, O. R.; Blanda, G. *Chem. Eur. J.* **2001**, *7*, 5286.

(8) Wu, Y.; Liao, X.; Wang, R.; Xie, X.-s.; De Brabander, J. K. *J. Am. Chem. Soc.* **2002**, *124*, 3245.

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TBAF to afford enamide alcohol **4**. Encouraged by literature precedent for esterification of salicylate cyanomethyl esters,¹³ we prepared salicylate enamides **5** and **6** by heating compound **4** with cyanomethyl ester **7** or **8**¹⁴ in the presence of a catalytic amount of K₂CO₃ in DMA (Scheme 1).¹⁵ On the basis of literature reports and lack of acylation using cyanomethyl benzoate, we initially suspected that keto-ketenes (cf. Scheme 1, inset) were the active acylating agents.¹⁶ However, trapping experiments with *N,N*-dimethyl cyanamide, ethyl vinyl ether, ethoxyacetylene, and *N*-benzylmaleimide failed to provide the corresponding Diels–Alder adducts.¹⁷ An alternative mechanistic pathway was further suggested by conformational analysis of the sodium salt of **7**.¹⁸ As shown in Figure 2, the ester carbonyl in the

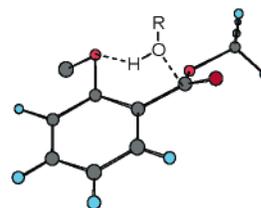


Figure 2. Transesterification using intramolecular general base-catalysis.

conformation shown is out of planarity, which is expected to increase the reactivity of the carbonyl toward transesterification. In addition, the *o*-hydroxyl is suitably oriented to act as a general base-catalyst and direct attack of the alcohol to the carbonyl π*. This mechanism is further substantiated by several literature reports.¹⁹

Two related methylated analogues **10** and **11** were prepared in order to determine the biological importance of the phenol and enamide hydrogens of the lobatamides, respectively (Scheme 2). Enamide **3** was methylated with NaH/MeI and desilylated to afford *N*-methyl enamide alcohol **12**, which was acylated using cyanomethyl ester **7** to afford analogue **10**. Unfortunately, esterification of enamide alcohol **4** with *o*-anisic acid using modified Keck esterification²⁰

(10) Bhattacharjee, A.; Seguil, O. R.; De Brabander, J. K. *Tetrahedron Lett.* **2001**, *42*, 1217.

(11) Posner, G. H.; Weitzberg, M.; Hamill, T. G.; Asirvatham, E. *Tetrahedron* **1986**, *42*, 2919

(12) Shen, R.; Porco, J. A., Jr. *Org. Lett.* **2000**, *2*, 1333.

(13) Sherlock, M. H. S. African Pat. 1968 ZA 6802187; CAN 70: 106224

(14) (a) Byers, J. H.; Baran, R. C.; Craig, M. E.; Jackman, J. T. *Org. Prep. Proced. Int.* **1991**, *23*, 373 (b) Hugel, H. M.; Bhaskar, V.; Longmore, R. W. *Synth. Commun.* **1992**, *22*, 693. For the preparation of **8**, see ref 9.

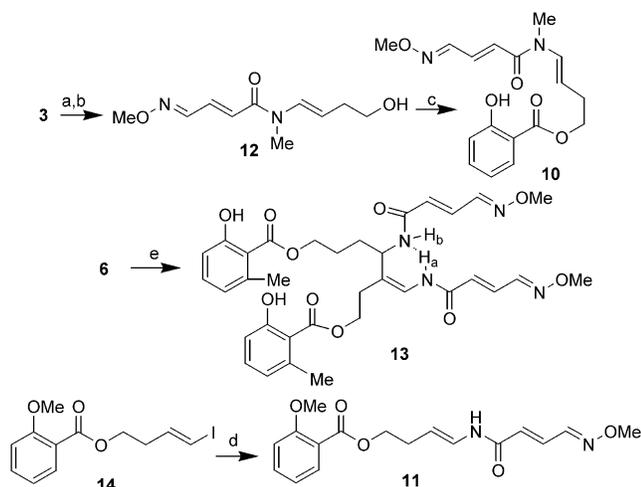
(15) Analogue **9** was synthesized from (*E,E*)-2,4-hexadienamide (Pellegata, R.; Italia, A.; Villa, M. *Synthesis* **1985**, 517) according to the general procedure used to prepare analogue **5**.

(16) For a recent review on keto-ketenes, see: Simion, C.; Costea, I.; Badea, F.; Iordache, F. *Roum. Chem. Quart. Rev.* **2001**, *8*, 131.

(17) (a) Kaneko, C.; Sato, M.; Sakaki, J.-i.; Abe, Y. *J. Heterocycl. Chem.* **1990**, *27*, 25. (b) Stadler, A.; Zangger, K.; Belaj, F.; Kollenz, G. *Tetrahedron* **2001**, *57*, 6757.

(18) A conformational search was performed using PC Spartan Pro version 1.0.7 (Wavefunction, Irvine, CA).

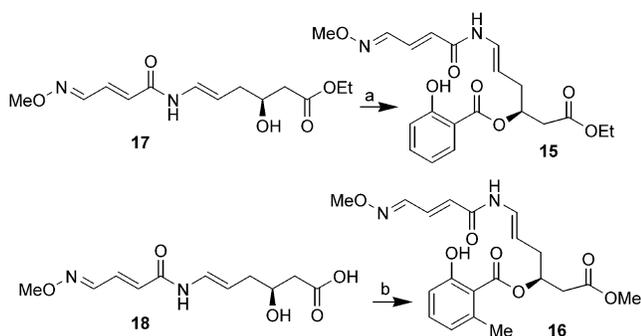
(19) (a) Bender, M. L.; Kezdy, F. J.; Zerner, B. *J. Am. Chem. Soc.* **1963**, *85*, 3017. (b) Khan, M. N. *Int. J. Chem. Kinet.* **1988**, *20*, 443.

Scheme 2^a

^a Reagents and conditions: (a) NaH, DMF, MeI, 98%; (b) TBAF, THF, 90%; (c) **7**, K₂CO₃, DMA, 81%; (d) **2**, CuTC, Cs₂CO₃, DMA, 29%; (e) CSA (50 mol %), CH₂Cl₂, rt, 1 h (78%).

employing DMAP–CSA as a cocatalyst did not afford the desired product **11**. Since we suspected side reactions of the enamide under acidic conditions, enamide **6** was treated with CSA in CH₂Cl₂ to cleanly afford dimer **13** (78%).²¹ Dimerization of enamides by protonation to an *N*-acyliminium ion and reaction with a second equivalent of enamide is preceded in the literature²² and represents a competing side reaction in the presence of anhydrous acid. Analogue **11** was ultimately prepared using Cu-mediated enamide bond construction using amide **2** and vinyl iodide **14**.²³

Additional acyclic, salicylate enamide analogues **15** and **16** were prepared using the C1–C10 fragment of lobatamide C (Scheme 3, **17** and **18**)⁹ to determine the effect of the C8

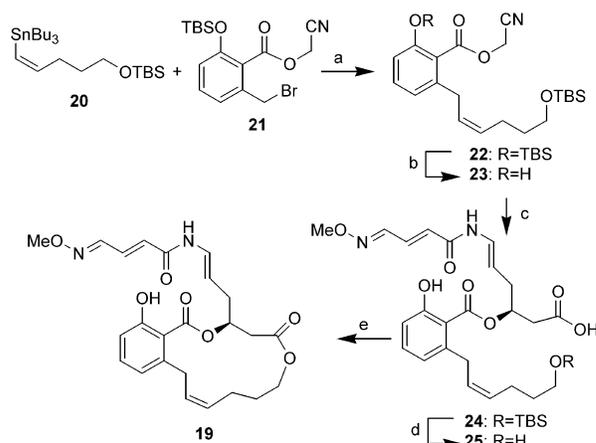
Scheme 3^a

^a Reagents and conditions: (a) **7**, K₂CO₃, DMA, 25%, 60% recovered **17**; (b) (i) Bu₄NOH, MeOH; azeotrope water; Na₂CO₃, **8**, DMF/2-butanone, 80 °C, 2 h, (ii) TMSCHN₂; 34% (two steps).

stereogenic center on V-ATPase inhibition. Although satisfactory yields were obtained for primary alcohols in the synthesis of analogues such as **5** and **6** (Scheme 1), secondary

alcohol **17** exhibited significantly lower reactivity in acylation reactions with cyanomethyl ester **7** to afford **15** (25%). In addition, 15% of a β-elimination product as well as considerable amounts of recovered **17** were obtained. Under similar conditions, acylation of **17** with cyanomethyl ester **8** did not afford the desired salicylate product. However, acid **18**, after neutralization with Bu₄NOH and heating with **8** and Na₂CO₃ (1.0 equiv), furnished the desired salicylate ester. In this reaction, a number of carbonate bases (Li, K, Rb, and Cs) were also evaluated, but interestingly only stoichiometric levels of Na₂CO₃ gave useful results. To facilitate purification, the unstable enamide acid product was methylated with trimethylsilyl diazomethane to afford methyl ester **16** (34% from **18**).

We have also initiated studies employing the C1–C10 fragment of the lobatamides to prepare simplified and potentially more chemically stable macrocyclic analogues (Scheme 4). Our initial target, macrodilactone **19**, replaces

Scheme 4^a

^a Reagents and conditions: (a) Pd₂(dba)₃CHCl₃, AsPh₃, THF, 70 °C, 60%; (b) TBAF, THF, 0 °C, 73%; (c) **18**, Bu₄NOH, MeOH; azeotrope water; Na₂CO₃, **23**, DMF/2-butanone, 80 °C, 2 h; (d) HF-pyridine/pyridine, THF, 36% (2 steps); (e) DIAD, PPh₃, THF, 65%.

the fragile divinylcarbinol moiety with a *Z*-olefin and simplified three-carbon segment. Stille coupling²⁴ of vinyl stannane **20**²⁵ and benzylic bromide **21**⁹ afforded salicylate **22**. Selective desilylation (TBAF) provided cyanomethyl

(20) Sunazuka, T.; Hirose, T.; Harigaya, Y.; Takamatsu, S.; Hayashi, M.; Komiyama, K.; Omura, S.; Sprengeler, P. A.; Smith, A. B. *J. Am. Chem. Soc.* **1997**, *119*, 10247.

(21) Dimer **13** has a hydrogen-bonded amide proton (H_a) and a non-hydrogen-bonded NH proton (H_b); cf. Gardner, R.; Liang, G.-B.; Gellman, S. *J. Am. Chem. Soc.* **1999**, *121*, 1806.

(22) (a) Ben-Ishai, D.; Giger, R. *Tetrahedron Lett.* **1965**, *6*, 4523. (b) Schoemaker, H. E.; Boer-Terpstra, T.; Dijkink, J.; Speckamp, W. N. *Tetrahedron* **1980**, *36*, 143.

(23) Prepared by DIC coupling of *o*-anisic acid and 4-iodo-3-buten-1-ol. See Supporting Information for details.

(24) Kamlage, S.; Sefkow, M.; Peter, M. G. *J. Org. Chem.* **1999**, *64*, 2938.

(25) Lipshutz, B. H.; Keil, R.; Barton, J. C. *Tetrahedron Lett.* **1992**, *33*, 5861.

ester **23**, which was coupled with enamide acid **18** to furnish the salicylate **24**. Desilylation (HF-pyridine/pyridine) provided hydroxy acid **25**, which underwent Mitsunobu macrolactonization⁹ (0.005 M) to afford the target macrocyclic analogue **19** (65%).

The simplified lobatamide analogues were evaluated for activity against bovine V-ATPase (Table 1).²⁶ Enamide **4**

Table 1. Effect of Simplified Analogues of Lobatamides against Bovine V-ATPase

analogue	IC ₅₀ (μM)	analogue	IC ₅₀ (μM)
lobatamide C	0.002	11	no effect
5	<i>b</i>	13	<i>d</i>
6	1.3	15	18
9	<i>c</i>	16	0.1
10	200	19	1.2

^a ATPase activity determined as described in the Supporting Information using 20 μg of membrane protein and the indicated amount of inhibitor.

^b 25% inhibition at 20 μM, higher concentrations not soluble. ^c 25% inhibition at 30 μM, higher concentrations not soluble. ^d 35% inhibition at 30 μM, higher concentrations not soluble.

was found to be inactive, which indicates the requirement for both the salicylate ester and enamide for inhibition. The minimal salicylate enamide compounds **5** and **9** were found to inhibit bovine V-ATPase (25% inhibition at 20 and 30 μM, respectively). Interestingly, permutation of the *ortho* hydrogen to a methyl group substantially increased the activity against bovine ATPase (**6**, IC₅₀ = 1.3 μM). Dimeric compound **13** was considerably less active. Methylated analogues **10** and **11** showed relatively weak inhibition of V-ATPase, which indicates the importance of a free phenol and enamide NH. Macrolactone **19** showed good V-ATPase inhibition (1.2 μM), but not at the nanomolar potency of the lobatamides, which indicates that the ring size and substitution of the macrolactone are important for potent V-ATPase inhibition. The most potent simplified salicylate enamide analogue to emerge from this study was *o*-methyl-

(26) ATPase activity was determined as described in Supporting Information. The assay measures turnover of ATP, which includes ATPase activity of the vacuolar ATPase and other potential ATPase activity present in the membrane preparations. In these assays more than 90% of the activity was inhibited by lobatamide C or by concanamycin A, specific inhibitors of the vacuolar ATPase.

(27) Cf. (a) Pinkus, A. G.; Lin, Ellen Y. *J. Mol. Struct.* **1975**, *24*, 9. (b) Decouzon, M.; Ertl, P.; Exner, O.; Gal, J. F.; Maria, P. C. *J. Am. Chem. Soc.* **1993**, *115*, 12071.

substituted compound **16** (0.1 μM), which was 180 times more active than unsubstituted compound **15**. Conformational analysis¹⁸ of **15** and **16** (Figure 3A) indicates that *ortho*-H-

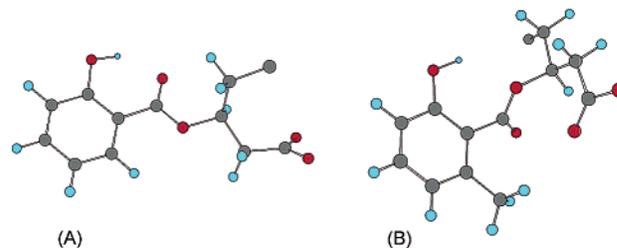


Figure 3. Representative minimum energy conformations of **15** (A) and **16** (B) (Chem 3D, enamide side chain omitted for clarity).

substituted salicylate **15** maintains near planarity of the carbonyl with the aromatic ring. In contrast, the *o*-methyl substituent forces the carbonyl out-of-plane by approximately 60°, presumably because of steric hindrance effects.²⁷ This sterically hindered resonance effect is also seen in the X-ray crystal structure of apicularen A^{3b} (salicylate ester carbonyl out of planarity by 80°) and may be an important configuration of the salicylate moiety in potent V-ATPase inhibitors.

In summary, a series of simplified analogues of the lobatamides have been prepared and evaluated as mammalian V-ATPase inhibitors. Although simplified derivatives are not as potent as the natural products, a number of useful structure–reactivity relationships have been uncovered, including enhancement of V-ATPase inhibition in acyclic analogues by *ortho*-substitution of the salicylate ring. Continued studies on the synthesis of salicylate enamides and analogues are ongoing.

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Supporting Information Available: Experimental procedures and characterization data for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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