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Synthesis and Initial Structure–Activity Relationships of Modified Salicylihalamides

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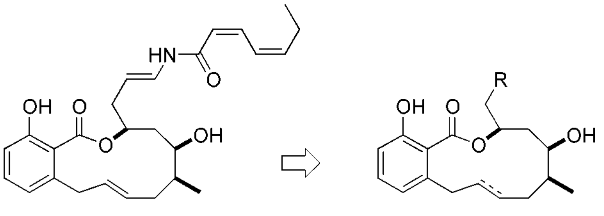
ABSTRACT



1 salicylihalamide A

3

The first stereoselective total synthesis of the potent antitumor compound (−)-salicylihalamide A is presented. The practicality of our approach provides for high material throughput and is highlighted by the rapid construction of a variety of modified congeners. Initial structure–activity relationships are derived from growth inhibition experiments with a human melanoma cancer cell line.

Natural products continue to be the primary source for compounds with unique biological functions. Because such compounds could potentially interact with proteins of unknown function, or interfere with cell-signaling pathways, growth regulation, and differentiation in unique ways, they constitute extremely valuable research tools in discovery biology efforts. In this context, a unique opportunity is provided by the discovery of the marine natural products salicylihalamides A and B (**1** and **2**, Figure 1),[1] the first examples of a growing number of structurally related macrocyclic salicylate natural products.[2] Notably, salicylihalamides displayed a unique signature in the National Cancer Institute's human tumor 60-cell line panel, indicating a potentially novel mechanism of antineoplastic activity.[1] Unfortunately, a limited supply from an unidentified species of the marine sponge *Haliclona* prevents any serious efforts to explore salicylihalamide's molecular pharmacology and develop these compounds as new chemotherapeutic leads for the treatment of cancer.

Our laboratory has offered an initial solution to this problem by exploring reactivity patterns and chemical

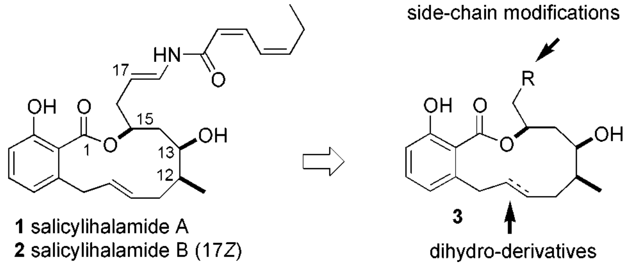


Figure 1. Salicylihalamide A and side chain modified congeners.

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[1] Erickson, K. L.; Beutler, J. A.; Cardellina II, J. H.; Boyd, M. R. *J. Org. Chem.* **1997**, *62*, 8188−8192.

[2] Lobatamides: (a) McKee, T. C.; Galinis, D. L.; Pannell, L. K.; Cardellina, J. H., II; Laakso, J.; Ireland, C. M.; Murray, L.; Capon, R. J.; Boyd, M. R. *J. Org. Chem.* **1998**, *63*, 7805−7810. (b) Lobatamide A is identical to the structure of YM-75518, see: Suzumura, K.-I.; Takahashi, I.; Matsumoto, H.; Nagai, K.; Setiawan, B.; Rantiatmodjo, R. M.; Suzuki, K.-I.; Nagano, N. *Tetrahedron Lett.* **1997**, *38*, 7573−7576. CJ-12,950 and CJ-13,357: (d) Dekker: K. A.; Aiello, R. J.; Hirai, H.; Inagaki, T.; Sakakibara, T.; Suzuki, Y.; Thompson, J. F.; Yamauchi, Y.; Kojima, N. *J. Antibiot.* **1998**, *51*, 14−20. Apicularens: (e) Kunze, B.; Jansen, R.; Sasse, F.; Höfle, G.; Reichenbach, H. *J. Antibiot.* **1998**, *51*, 1075−1080. (f) Jansen, R.; Kunze, B.; Reichenbach, H.; Höfle, G. *Eur. J. Org. Chem.* **2000**, 913−919. Oximidines: (g) Kim, J. W.; Shin-ya, K.; Furihata, K.; Hayakawa, Y.; Seto, H. *J. Org. Chem.* **1999**, *64*, 153−155.

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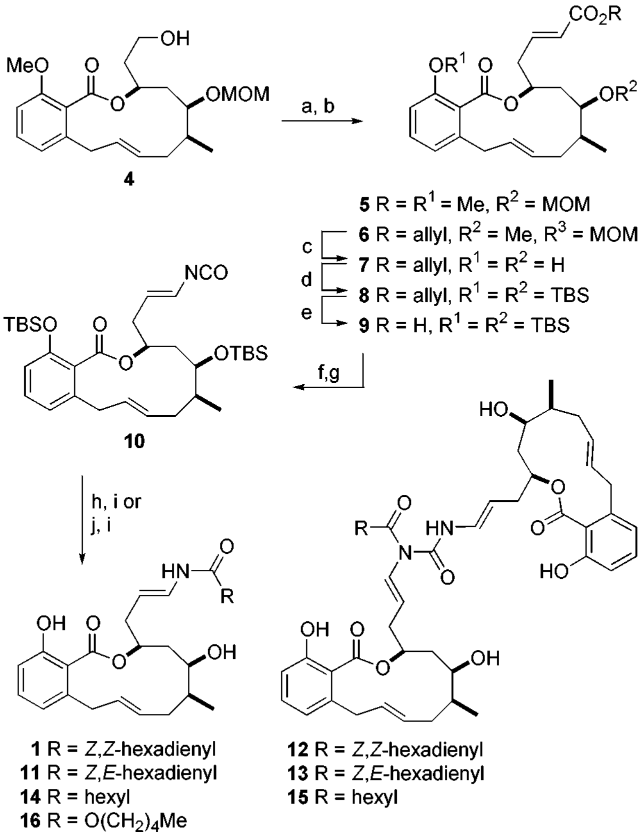
compatibility issues related to salicylihalamides[3] and other macrocyclic salicylate natural products.[4,5] These studies culminated in a revision of the absolute configuration of (−)-salicylihalamide A (**1**) through the first total synthesis of its enantiomer.[3] To facilitate the search for a cellular target, we required structural variants that would allow for the introduction of a suitable reporter without compromising biological activity. Herein, we report a practical synthesis of naturally occurring (−)-salicylihalamide A and a series of modified congeners (generalized structure **3**, Figure 1) to establish the first structure−activity relationships (SAR).

Our first objective was to deliver naturally occurring (−)-salicylihalamide A. Following our initial route,[3] homologation of the aldehyde[6] derived from **4**[7] with trimethyl phosphonoacetate yielded **5** as a 4:1 mixture of *E*/*Z* isomers (Scheme 1). Subsequent routine transformations then delivered isocyanate **10** in 20−30% overall yield from **4**.[3] The moderate yields and stereoselectivity detracted from the efficient chemistry we had developed for the synthesis of **4**. Therefore, a series of optimization experiments were performed that quickly led to the use of allyl diethylphosphonoacetate in the Horner−Wadsworth−Emmons homologation, delivering allyl ester **6** as a single *E* isomer. Subsequent treatment with BBr_3 was followed by bis-silylation of **7**. Isocyanate **10** was now in reach by Pd-catalyzed deprotection of allyl ester **8**,[8] acyl azide formation,[9] and Curtius rearrangement. Importantly, this sequence gave us ∼0.5 g of isocyanate **10** with a dramatically improved overall yield (75% from **4**).

Scheme 1[a]



[a] Reagents and conditions: (a) Dess−Martin periodinane, CH_2Cl_2; (b) allyl diethylphosphonoacetate, NaH, THF, 0 °C, 97% (two steps); (c) BBr_3, CH_2Cl_2, −78 °C, 92%; (d) TBSCl, imidazole, DMF, 95%; (e) cat. Pd(PPh_3)$_4$, morpholine, THF, 97%; (f) $(PhO)_2P(O)N_3$, Et_3N, PhH; (g) PhH, 80 °C, 93% (two steps); (h) 1-bromo-1,3-hexadiene (1:1 mixture of 1*Z*,3*Z* and 1*Z*,3*E* isomers) or 1-bromohexane, *t*-BuLi, Et_2O, −78 °C, then add **10**, −78 °C; (i) HF•pyr., pyr./THF, 20% for **1**/**11**, 10% for **12**, 10% for **13**, 22% for **14**, 14% for **15** (two steps); (j) 1-pentanol, PhH, 75 °C, then step i, 50% (two steps).

Addition of a 1:1 mixture of (1*Z*,3*Z*)- and (1*Z*,3*E*)-1-lithio-1,3-hexadiene to isocyanate **10** and final deprotection afforded a 1:1 mixture of salicylihalamide A (**1**) and C22-*E* isomer **11**.[3,5d−g,10] This mixture was indistinguishable, within the limits of experimental error, from natural salicylihalamide A on the basis of comparative testing in the National Cancer Institute 60-cell screen.[11] Careful examination of the product mixture identified the presence of two additional compounds, which were purified and characterized as salicylihalamide dimers **12** and **13**.[12]

We next turned our attention to a series of side chain modified analogues. The best starting point to initiate these efforts would take advantage of the extremely efficient and high-yielding construction of isocyanate **10**. Carbamate **16** was prepared by heating **10** in the presence of *n*-pentanol followed by deprotection (50%, 2 steps). Tetrahydrosalicylihalamide **14** and the corresponding dimer **15** were obtained in a manner identical to that described for **1**. While lacking the enoyl functionality (potential Michael acceptor), analogues **14** and **16** displayed significant growth inhibitory activity against the human melanoma cell line SK-MEL-5

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[3] Wu, Y.; Esser, L.; De Brabander, J. K. *Angew. Chem., Int. Ed.* **2000**, in press.

[4] For our synthetic efforts toward apicularen A, see: Bhattacharjee, A.; De Brabander, J. K. *Tetrahedron Lett.* **2000**, *41*, 8069−8073.

[5] For other synthetic efforts, see: (a) Georg, G. I.; Blackman, B.; Mossman, C. J.; Yang, K.; Flaherty, P. T. *Abstracts of Papers*, 219th National Meeting of the American Chemical Society, San Francisco, March 2000; American Chemical Society: Washington, DC, 2000; ORGN 807. (b) Fürstner, A.; Thiel, O. R.; Blanda, G. *Org. Lett.* **2000**, *2*, 3731−3734. (c) Feutrill, J. T.; Holloway, G. A.; Hilli, F.; Hügel, H. M.; Rizzacasa, M. A. *Tetrahedron Lett.* **2000**, *41*, 8569−8572. For studies related to the enamide side chain, see: (d) Snider, B. B.; Song, F. *Org. Lett.* **2000**, *2*, 407−408. (e) Kuramochi, K.; Watanabe, H.; Kitahara, T. *Synlett* **2000**, 397−399. (f) Shen, R.; Porco, J. A., Jr. *Org. Lett.* **2000**, *2*, 1333−1336. (g) Stefanuti, I.; Smith, S. A.; Taylor, R. J. K. *Tetrahedron Lett.* **2000**, *41*, 3735−3738.

[6] Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277−7287.

[7] In our previously reported synthesis of (+)-salicylihalamide A, all three stereocenters in *ent*-**4** were introduced by reagent-controlled reactions.[3] The same chemistry was exploited for the preparation of **4**, except for the use of antipodal chiral reagents.

[8] Kunz, H.; Waldmann, H. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 71−72.

[9] Ninomiya, K.; Shioiri, T.; Yamada, S. *Tetrahedron* **1974**, *30*, 2151−2157.

[10] The antipodal geometrical isomers were previously separated and fully characterized individually.[3] The spectroscopic data of the mixture (**1** and **11**) were in full accord with those previously obtained.

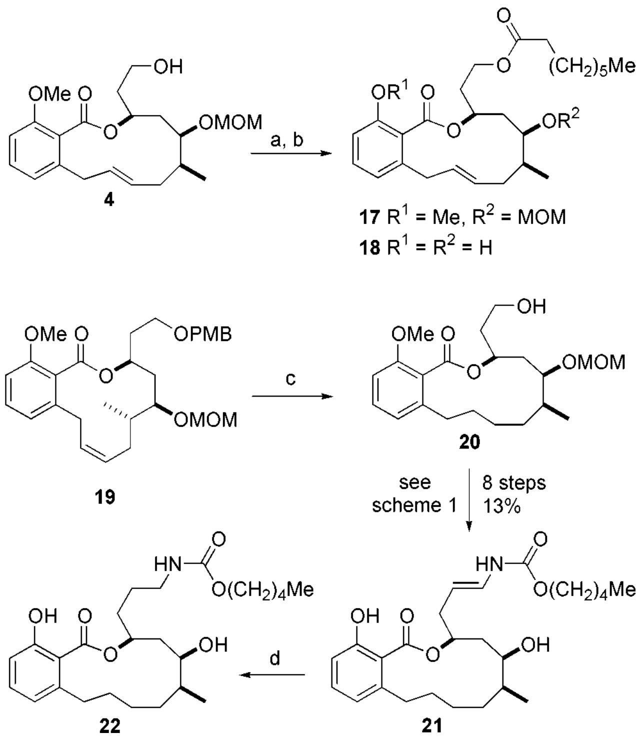
[11] The corresponding enantiomers were completely devoid of activity in the same screen, indicating that biological function is at least partly dependent on a correctly configured macrolactone. We thank Dr. Michael R. Boyd (National Cancer Institute) for testing our compounds in the 60-cell line panel.

[12] These dimers are presumably formed through reaction of the intermediate lithiated amide with a second molecule of isocyanate **10**. For a similar observation, see ref 5e.

[GI_{50} 0.38 μM (**14**); 0.5 μM (**16**)].[13] Interestingly, salicylihalamide dimers **12**, **13**, and **15** were found to be effective at a concentration range similar to that of the parent salicylihalamides [GI_{50} 0.04 μM (**12**); 0.1 μM (**13**); 0.6 μM (**15**)].[13] This observation indicates that substantial sterically demanding modifications can be made without abrogating biological activity and can be exploited for the development of analogues and probe reagents.

Side chain modified analogues that lack salicylihalamide's characteristic *N*-acyl enamine functionality are attractive candidates for the following reasons: (1) they are expected to confer increased acid stability, (2) they can potentially be prepared via shorter sequences, and (3) they would answer an important question related to the functional role of the *N*-acyl enamine moiety. Octanoate **18**, a compound with identical chain length and similar hydrophobicity to that of salicylihalamide, is representative of this class of compounds and was prepared from alcohol **4** via a Mitsunobu esterification[14] followed by deprotection (Scheme 2). An additional member is represented by allyl ester **7**, an intermediate for the synthesis of salicylihalamides (Scheme 1). The inability of analogues **7** and **18** to arrest cell growth[13] indicates a specific role for the side chain, perhaps expressed in the *N*-acyl enamine functionality.[15]

Scheme 2[a]



[a] Reagents and conditions: (a) octanoic acid, DEAD, PPh_3, Et_2O; (b) BBr_3, CH_2Cl_2, −78 °C, 80% (two steps); (c) Pd/C, NH_4O_2CH, MeOH 70%; (d) Pd/C, H_2, MeOH, 30%.

At this point, a more in depth evaluation of the functional role for the acylated enamine was conducted. Indeed, it is possible that salicylihalamides form a covalent complex with a putative binding protein through a protonation/nucleophilic addition mechanism ($RC(O)NHCH{=}CHR \rightarrow RC(O)NH^+{=}CH{-}CH_2R \rightarrow RC(O)NHCHXR'{-}CH_2R$, XR' = nucleophilic amino acid residue).[16] The most straightforward and least intrusive way to knock out the chemical reactivity associated with the acylated enamine would constitute a hydrogenation of salicylihalamide's enamine double bond. It was anticipated, however, that the presence of the endocyclic double bond would pose a serious chemoselectivity problem. For example, direct hydrogenation of biologically active salicylihalamide carbamate **16** is likely to deliver the doubly saturated analogue **22** (Scheme 2). To separate the effect of endocyclic double bond saturation from enamine saturation on biological activity, it was imperative to prepare the saturated macrolactone mutant **21**. Our point of departure entailed a transfer hydrogenation[17] of (*Z*)-cycloalkene **19** with concomitant removal of the *p*-methoxybenzyl (PMB) ether.[18] Subsequent conversion of **20** to **21** took full advantage of the chemistry outlined for the preparation of **16** (Scheme 1) without complication. Hydrogenation of this material then yielded the fully saturated salicylihalamide **22**.

While lacking the endocyclic double bond of carbamate **16** (GI_{50} 0.5 μM), analogue **21** retained a significant, although attenuated, level of growth inhibitory activity (GI_{50} 8 μM).[13] This is of significance because we now have a calibration point for comparing the effect of the enamine to amine permutation (**21** → **22**), which completely abolished the antiproliferative potential of **21**. The question remains, however, if an increased conformational flexibility of the side chain or the inability to form a covalent bond is at the origin of this deleterious (with respect to biological activity) mutation. The final answer will have to come from labeling studies with a yet to be identified cellular target.

In summary, we have synthesized for the first time (−)-salicylihalamide A, a compound that is no longer available from natural sources. Optimization of the chemistry involved has put us in the comfortable position of high material throughput, which was exploited for the synthesis of a variety of synthetic salicylihalamides. Initial SAR studies have revealed an important role for both the side chain and the macrolactone and have indicated possibilities for the development of salicylihalamide-based molecular probes.

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(13) Growth inhibition was determined 2 days after the addition of the compounds by the MTT assay (Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55−63). The GI_{50} values were calculated on the basis of triplicate assays at four different concentrations of the drug. Synthetic salicylihalamide A (**1**/**11**) was used as a positive control (GI_{50} 0.07 μM).

(14) Mitsunobu, O. *Synthesis* **1981**, 1−28.

(15) However, an intact side chain is not sufficient for biological activity; see footnote 11.

(16) For an example of an enamide-containing natural product, and proposed modification of an *N*-acyliminium ion by an enzyme active site nucleophile, see: Gentle, C. A.; Bugg, T. D. H. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1279−1285.

(17) Bieg, T.; Szeja, W. *Synthesis* **1985**, 76−77.

(18) While compound **20** is in principle accessible from **4**, we opted to make a productive use of **19**, a minor isomeric byproduct obtained en route to the synthesis of **4**.[3]

and the University of Texas Southwestern Medical Center are gratefully acknowledged. We also thank Dr. Michael G. Roth and Maria G. Kosfiszer (Department of Biochemistry) for invaluable assistance with the cell-based assays and Dr. Michael R. Boyd (National Cancer Institute) for providing a sample and NMR spectra of natural salicylihalamide A.

Supporting Information Available: ^{1}H NMR spectra of **1**/**11**, *ent*-**1**, and *ent*-**11**, the procedure for the preparation of **1**/**11** and **12−16**, and characterization data and ^{1}H NMR spectra for compounds **6−9**, **12−18**, and **20−22**.

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