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Macrocyclic ketone analogues of halichondrin B

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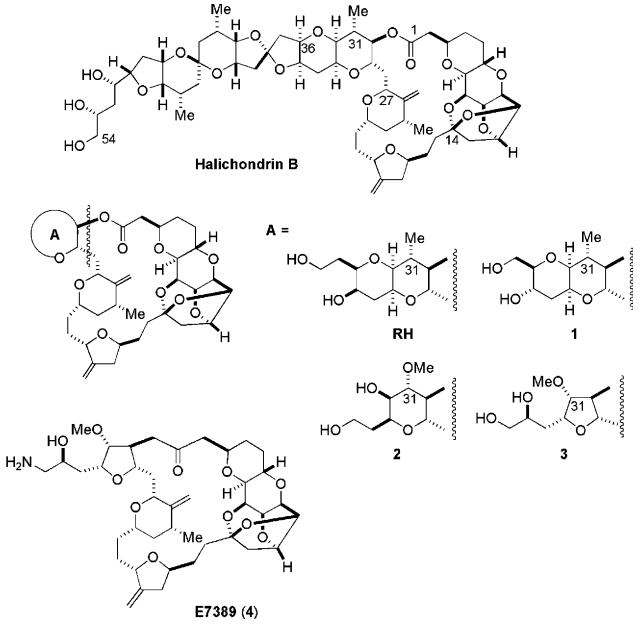
This paper is dedicated to memory of Bruce F. Wels, our friend and colleague

Abstract—Structurally simplified macrocyclic ketone analogues of halichondrin B were prepared by total synthesis and found to retain the potent cell growth inhibitory activity in vitro, stability in mouse serum, and in vivo efficacy of the natural product.

Halichondrin B (HB) is a structurally complex marine natural product.[1,2] Despite extensive synthetic studies, the only successful total synthesis of HB and norhalichondrin B was reported by Kishi and co-workers in 1992.[3] The discovery that the right half (RH) of HB and analogue **1** exhibited potent cell growth inhibitory activity in vitro[4,5] and antitumor efficacy in vivo,[6] respectively, provided a compelling starting point for a drug discovery program. In the preceding paper[7] we reported our SAR investigation and identification of a series of macrolactone analogues (e.g., **2** and **3**) that retained the remarkable in vitro biological profile of HB. However, these compounds failed to demonstrate salutary effects in vivo. Herein, we report our efforts to optimize the in vivo properties of these structurally simplified analogues, culminating in the discovery of E7389 (**4**), a fully synthetic analogue of HB that is currently undergoing Phase I clinical evaluation.

Instability of the macrolactone acyl moiety was regarded as the most likely explanation for the observed lack of in vivo efficacy associated with the structurally simplified macrolactone series. Consistent with this hypothesis, in vitro activity of octahydropyrano[3,2-*b*]pyran analogue **1** was not affected by inclusion of up to 1% mouse serum in the 3–4 day growth inhibition assay, whereas activity of the tetrahydropyran or tetrahydrofuran series of analogues exemplified by **2** and **3**, respectively, were completely abrogated by mouse serum at concentrations as low as 0.01%. Since the seco acid of



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RH was not active, the lack of in vivo efficacy for structurally simplified macrolactone analogues in the mouse xenograft model was most likely attributed to sensitivity of the macrolactone ring to nonspecific esterases present in serum.

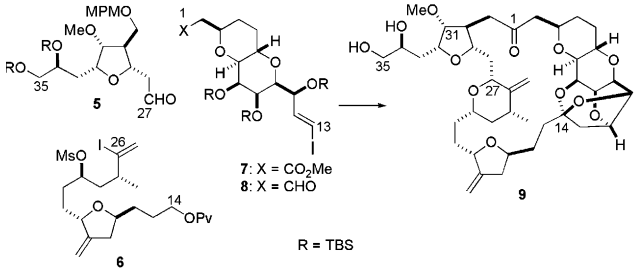
To further test this hypothesis, nonhydrolyzable ester bioisosteres were prepared to identify derivatives that would retain activity in the presence of mouse serum. Of the surrogate groups examined, only the ketone bioisostere provided analogues with sufficient in vitro potency to warrant further investigation.

The macrocyclic ketone analogues were prepared in a convergent manner from three major fragments **5**, **6**,[3a,8] and **8**[9] in a way similar to that developed by Kishi and co-workers for the total synthesis of HB (Scheme 1).

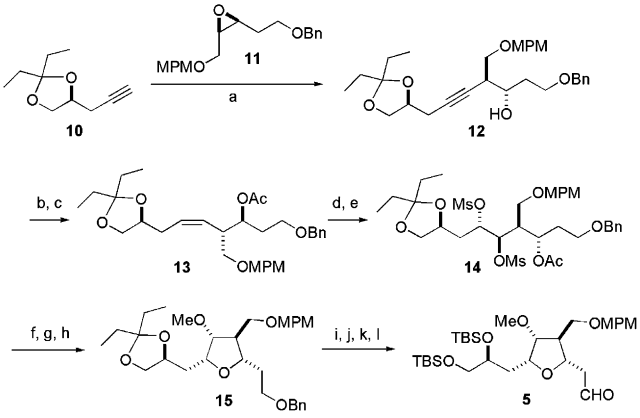
Aldehyde **5** was prepared as outlined in Scheme 2. Regioselective opening of epoxide **11**[10] with the lithium anion of **10**[11] in the presence of $BF_3{\cdot}OEt_2$ furnished a 3:1 mixture of structural isomers favoring desired product **12**.[12] Hydrogenation using Lindlar's catalyst followed by acetylation furnished *cis*-olefin **13**. Alkene dihydroxylation with osmium tetroxide afforded an 8:1 mixture of diastereomers, which was directly converted to the corresponding dimesylates. The desired isomer **14** was separated by column chromatography and subjected to cyclization in the presence of Triton B. Methylmagnesium bromide mediated desulfonylation[13] followed by methylation of the corresponding alcohol furnished **15**. Adjusting protecting groups on the C.32 side chain, selective cleavage of the benzyl ether under Raney-Nickel conditions[14] and Swern oxidation[15] afforded aldehyde **5**.

Nozaki–Hiyama–Kishi coupling[18] of aldehyde **5** with vinyl iodide **6**[3a] and subsequent KHMDS-promoted cyclization afforded a 3:1 mixture of two C.27 diastereomers favoring the desired product (Scheme 3). The MPM-ethers were cleaved using DDQ[19] and two C.27 isomers were separated by flash chromatography. The major product **16** was converted to sulfone **17** in four steps. Coupling of sulfone **17** with aldehyde **8** followed by oxidation resulted in **18**. SmI_2 mediated desulfonylation[20] followed by Nozaki–Hiyama–Kishi macrocyclization[18] and subsequent allylic alcohol oxidation gave enone **19**. Treatment with TBAF and PPTS afforded the desired macrocyclic ketone **9**.

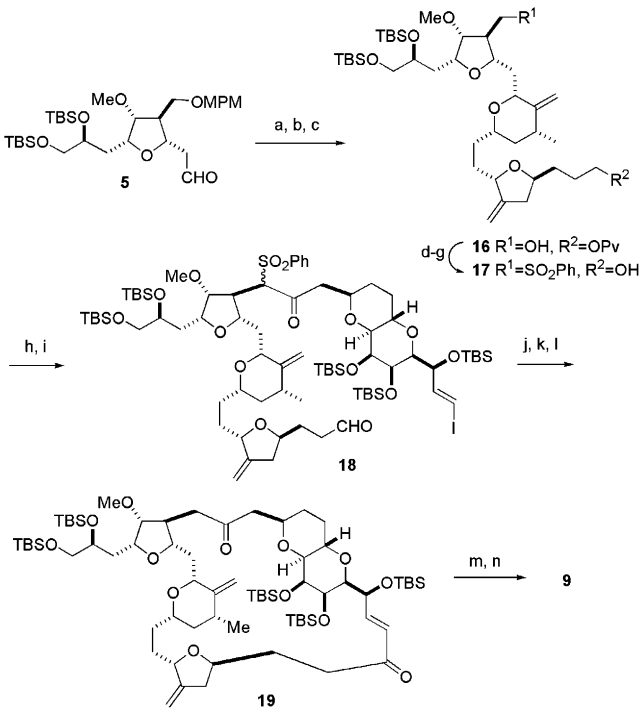
The vicinal diol on the C.32 side chain provided a convenient handle for subsequent derivatization. Analogues



Scheme 1. Final assembly from key fragments.



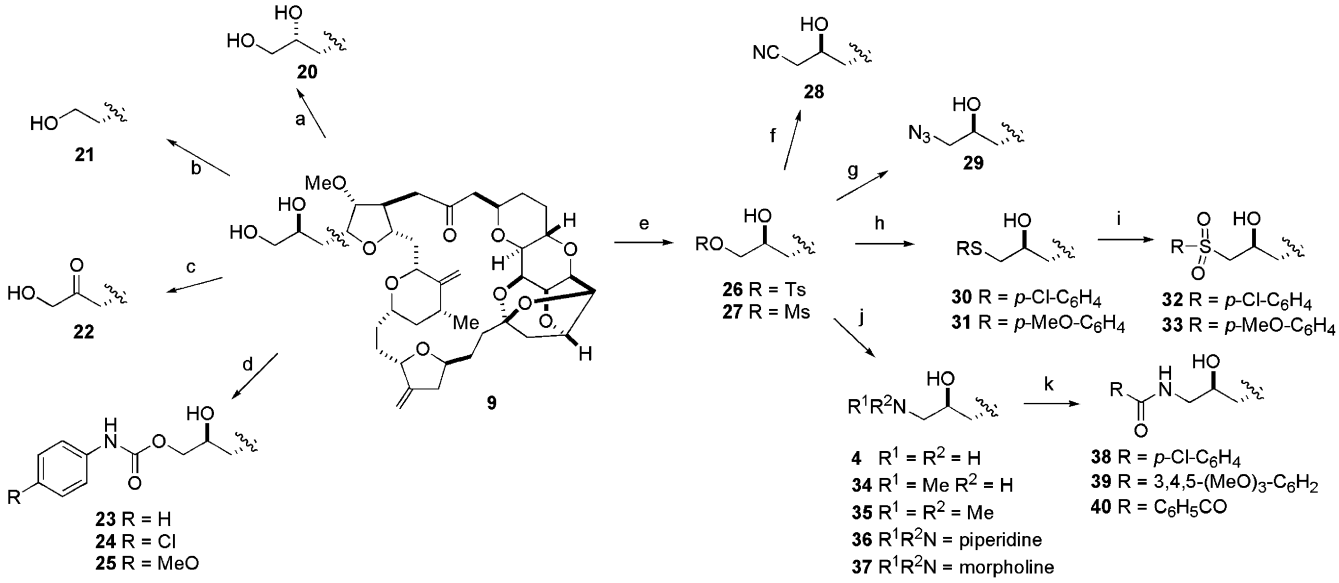
Scheme 2. Synthesis of aldehyde **5**. Reagents and conditions: (a) *n*-BuLi, **11**, $BF_3{\cdot}OEt$, toluene, −50 °C, 69%; (b) H_2, 5% $Pd/CaCO_3$, quinoline, hexanes/CH_2Cl_2, rt, 99%; (c) Ac_2O, pyridine, DMAP, CH_2Cl_2, rt, 99%; (d) OsO_4, NMO, *t*-BuOH/H_2O, rt; (e) MsCl, pyridine, rt, 58%; (f) Triton B, MeOH, rt; (g) MeMgBr, THF, 45 °C; (h) *t*-BuOK, MeI, THF, 0 °C, 84%; (i) HCl, THF/MeOH/H_2O, rt; (j) TBSCl, imidazole, DMF, rt; (k) H_2, Raney Ni, EtOH, 84%; (l) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −60 to 0 °C, 73%.



Scheme 3. Synthesis of macrocyclic ketone **9**. Reagents and conditions: (a) 0.5% $NiCl_2/CrCl_2$, **6**, THF/DMF (4:1), rt; (b) KHMDS, THF, 0 °C, 75%; (c) DDQ, CH_2Cl_2/pH 7 buffer, rt, 59%; (d) MsCl, Et_3N, CH_2Cl_2, 0 °C, 98%; (e) PhSH, *i*-Pr_2NEt, DMF, rt, 96%; (f) TPAP, NMO, CH_3CN, rt, 81%; (g) DIBAL, CH_2Cl_2, −78 °C, 97%; (h) *n*-BuLi, **8**, DME, −40 to −26 °C; (i) Dess–Martin,[16] CH_2Cl_2, rt, 82%; (j) SmI_2, THF/MeOH, −78 °C, 82%; (k) 1% $NiCl_2/CrCl_2$, THF/DMF (4:1), rt; (l) Dess–Martin,[16] CH_2Cl_2, rt, 73%; (m) TBAF/imidazole·HCl, THF, rt; (n) PPTS, CH_2Cl_2, rt, 90%.[17]

20–40 were prepared from diol **9** using standard transformations summarized in Scheme 4.

The compounds were evaluated for cell growth inhibitory activity against DLD-1 human colon cancer cells under continuous exposure conditions, and the ability to maintain a complete mitotic block (CMB) 10 h after drug washout using flow cytometric analysis of U937 human histiocytic lymphoma cells (reversibility assay).[21] In addition, susceptibility to P-glycoprotein (PgP)



Scheme 4. C.32 Side chain modifications. Reagents and conditions: (a) i. DEAD, Ph_3P, *p*-$NO_2C_6H_4CO_2H$, Et_2O, rt; ii. K_2CO_3, MeOH, rt, 60%; (b) i. $NaIO_4$, MeOH–H_2O (4:1), rt, ii. $NaBH_4$, MeOH–CH_2Cl_2 (4:1), −78 °C to rt, 89%; (c) i. TBDPSCl, imidazole, DMF, rt; ii. Dess–Martin reagent, CH_2Cl_2, rt; iii. TBAF, imidazole·HCl, THF, rt, 77%; (d) ArNCO, Et_3N, CH_2Cl_2, rt, 83–92%; (e) TsCl, pyridine, CH_2Cl_2, rt, 72%; or MsCl, collidine, CH_2Cl_2, 0 °C, 90%; (f) KCN, DMSO, 60 °C, 67% from **26**; (g) *n*-Bu_4NN_3, DMF, 80 °C, 85% from **26**; (h) i. NaI, acetone, 60 °C, ii. ArSH, DMF, 59–85% from **26**, rt; (i) *m*CPBA, CH_2Cl_2, 0 °C, 61–74%; (j) R^1R^2NH, MeOH, rt, 90–100% from **27**; (k) RCO_2H, EDCI, CH_2Cl_2, rt, 31–70%.

Table 1. In vitro activity profile for macrocyclic ketone analogues of HB

Compound	DLD-1[a] IC_{50}, nM[a] (*n*)	U937[b] IC_{99}, 0 h, nM	U937[b] IC_{99}, 10 h, nM (*n*)	Reversibility ratio	FR[c]
HB	0.74 ± 0.03 (6)	8	25 (4)	3	2.8
1	3.4 ± 0.40 (4)	9	220 (6)	24	12
2	1.8 ± 0.00 (3)	10	300 (3)	30	10
3	0.67 ± 0.09 (3)	2	15 (4)	8	6.2
4 (E7389)	13.0 ± 0.20 (10)	10	10 (4)	1	15
9 (ER-076349)	1.0 ± 0.10 (18)	3	38 (2)	13	9.7
20	1.9 ± 0.10 (4)	3	100 (1)	33	13
21	0.66 ± 0.11 (4)	3	100 (1)	33	8.5
22	1.1 ± 0.20 (5)	3	30 (2)	10	3.7
23	1.0 ± 0.20 (4)	3	65 (2)	22	5.7
24	1.3 ± 0.06 (3)	3	65 (2)	22	8.6
25	0.86 ± 0.07 (3)	3	30 (3)	10	5.8
26	2.0 ± 0.20 (3)	7	30 (2)	4	2.4
27	0.80 ± 0.01 (3)	3	30 (1)	10	3.0
28	0.49 ± 0.07 (4)	2	30 (2)	15	7.0
29	0.53 ± 0.01 (4)	3	30 (2)	10	1.9
30	1.2 ± 0.10 (3)	10	200 (2)	20	2.0
31	0.71 ± 0.12 (3)	3	30 (2)	10	1.7
32	0.66 ± 0.06 (4)	3	30 (2)	10	3.2
33	0.74 ± 0.03 (3)	3	30 (2)	10	3.4
34	3.3 ± 0.10 (3)	10	10 (2)	1	7.0
35	1.2 ± 0.20 (3)	1	10 (2)	10	22
36	0.47 ± 0.08 (3)	1	10 (2)	10	17
37	0.43 ± 0.03 (3)	3	20 (2)	7	4.7
38	0.69 ± 0.03 (4)	3	20 (2)	7	5.9
39	0.72 ± 0.06 (4)	2	10 (2)	5	20
40	0.87 ± 0.03 (4)	3	10 (2)	3	3.6

[a] Cell growth inhibition under continuous exposure conditions for 3–4 days, IC_{50} ± SEM (*n* = number of experiments).
[b] Mitotic block reversibility assay.
[c] Fold resistance calculated as the IC_{50} ratio between retrovirally transformed P388/VMDRC.04 and parental P388 cells.

mediated drug efflux was determined using murine P388/VMDRC.04 cells[22] (Table 1).

Macrocyclic ketone **9** (ER-076349, NSC 707390) exhibited an in vitro profile very similar to the corresponding macrolactone analogue **3**, thus confirming bioisosteric equivalence of the ketone and ester functional groups. Compound **9** remained active in vitro even after inclusion of 100% mouse serum, and exhibited antitumor efficacy in a variety of human tumor xenograft models.[23]

Modifications to the C.32 side chain had little impact on in vitro biological activity (Table 1). Interestingly, amine **4** (E7389, previously ER-086526, also NSC-707389) exhibited a reversibility ratio of one in U937 cells. Several other amine (**34**–**37**) and amide (**38**–**40**) derivatives were subsequently prepared and also found to exhibit a low reversibility ratio (e.g., **34**). E7389 demonstrated remarkable efficacy (0.05–1 mg/kg) in a variety of human tumor xenograft models.[23] This compound was selected for development as a new potential anticancer chemotherapeutic agent, and is currently in collaborative Phase I clinical trials with the United States NCI.

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