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Novel second generation analogs of eribulin. Part I: Compounds containing a lipophilic C32 side chain overcome P-glycoprotein susceptibility

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ABSTRACT

Eribulin mesylate (Halaven™), a totally synthetic analog of the marine polyether macrolide halichondrin B, has recently been approved in the United States as a treatment for breast cancer. It is also currently under regulatory review in Japan and the European Union. Our continuing medicinal chemistry efforts on this scaffold have focused on oral bioavailability, brain penetration and efficacy against multidrug resistant (MDR) tumors by lowering the susceptibility of these compounds to P-glycoprotein (P-gp)-mediated drug efflux. Replacement of the 1,2-amino alcohol C32 side chain of eribulin with fragments neutral at physiologic pH led to the identification of analogs with significantly lower P-gp susceptibility. The analogs maintained low- to sub-nM potency in vitro against both sensitive and MDR cell lines. Within this series, increasing lipophilicity generally led to decreased P-gp susceptibility. In addition to potency in cell culture, these compounds showed in vivo activity in mouse xenograft models.

Eribulin mesylate (Halaven™, Fig. 1) is a fully synthetic analog of the polyether macrolide natural product halichondrin B, which can be isolated from *Halichondria okadai* and other marine sponges.[1] The United States Food and Drug Administration recently approved eribulin as a treatment for locally advanced and metastatic breast cancer based on results of a Phase 3 clinical trial.[2,3] In addition, several other trials evaluating the efficacy of eribulin for sarcoma, non-small cell lung cancer and prostate cancer, have been completed or are ongoing.[4,5] Compared with other clinically used antimitotic drugs targeting microtubule dynamics such as paclitaxel and vinblastine, eribulin possesses a distinct mechanism of action, inhibiting microtubule growth, with little or no effect on microtubule shortening.[6–10] In view of its high potency and a wide therapeutic window in preclinical studies, together with its good physico-chemical and pharmacokinetic properties, eribulin mesylate was selected for further development.

Continuing studies in our laboratories have focused on developing compounds with activity against multidrug resistant (MDR) tumors, and particularly those with activity in animal models. We reasoned that such a preclinical profile would lead to better clinical outcomes, especially in the case of refractory tumors. Moreover, an orally available derivative was desired to facilitate potentially protracted dosing regimens. Our general strategy for addressing both of these properties centered on the susceptibility of the drug to P-glycoprotein (P-gp)-mediated drug efflux. It is believed that one of the mechanisms by which cancer cells can develop MDR is the overexpression of efflux pumps such as P-gp.[11] P-gp expression has also been found to be a negative predictor of clinical response to paclitaxel and other antimitotic therapies.[12] In addition, several studies have demonstrated the effect of P-gp-mediated efflux on the intestinal absorption of drugs.[13,14] Eribulin is a substrate for P-gp,[15] as are paclitaxel, doxorubicin, and other commonly used chemotherapeutic agents.[16,17]

During the discovery and development of eribulin, we noted that changes in the C32 side-chain region of the molecule could significantly affect potency, P-gp susceptibility (expressed as

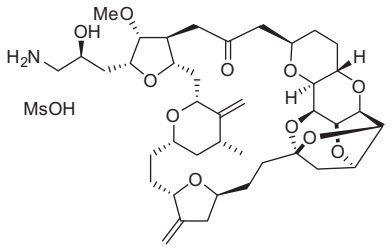

Figure 1. Eribulin mesylate (Halaven™).

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fold-resistance ratio or FR),[18] and physico-chemical properties.[1] This finding allowed us to focus our second generation efforts on a relatively small portion of this highly complex structure, while still targeting a significantly enhanced profile. This late-stage modification approach also curtailed the synthetic transformations required to access each analog, permitting a fairly extensive exploration of chemical space.

Primary amine-containing compounds are often substrates for PgP.[19,20] In designing compounds with decreased susceptibility to P-gp, we sought to eliminate the C35 nitrogen substituent and generate analogs with a neutral C32 side chain. We have previously reported that compound **1**, a closely related analog, had similar in vitro and in vivo activity as eribulin, but diminished P-gp liability.[10,21] In addition, compound **1** is a crystalline intermediate in the production route to eribulin and therefore, available in large quantity and excellent purity. Another advantage is the presence of the vicinal diol as a handle for further manipulation. Therefore, compound **1** was an ideal starting point for the optimization studies described herein.

Our initial focus was to generate a wide variety of non-amine containing C32 side chain analogs to explore the SAR for P-gp susceptibility. Figure 2 summarizes the chemistry utilized to access a number of such analogs. We frequently took advantage of the differential reactivities of the 1° and 2° –OH groups in **1** to introduce the desired side chain functionalities. In addition to **1**, C34 aldehyde **2**, and its corresponding primary alcohol **3** were also useful common intermediates. Briefly, direct alkylation of the diol unit of **1** provided analogs **4**–**7**. Selective mono-protection of the C35 OH as a TBDPS ether, followed by alkylation of the secondary –OH and deprotection gave analogs **8** and **9**. Compounds **10**–**12** were accessed by first conversion of both –OH groups to the corresponding TBDPS ethers, selective deprotection of the 1° –OH and its functionalization, followed by cleavage of the 2° –OTBDPS ether. Dess–Martin oxidation of **10** gave ketone **13**. Selective mono-tosylation of the C35 hydroxyl group and its displacement with NaN_3 or KCN, followed by alkylation of C34 –OH afforded compounds **14**–**16**. $NaClO_2$ oxidation of aldehyde **2** gave acid **17**, from which the corresponding esters **18** and **19** were accessed. Wittig reaction of aldehyde **2** to generate the methyl enol ether, hydrolysis to the corresponding aldehyde and $NaBH_4$ reduction gave alcohol **20**. Alkyne **21** was synthesized from **2** using the Bestmann–Ohira diazophosphonate.[22] Compounds **22**, **23** and **24** were obtained from C34 alcohol **3** via radical deoxygenation, tosylation followed by CN^- displacement, and direct methylation, respectively.[23]

All compounds were tested for cell growth inhibitory activity in four human cancer cell lines (Table 1). The primary assay consisted of measurement of cell growth inhibition against the human sarcoma cell line MES-SA. As a measure of P-gp susceptibility, these compounds were also tested against the P-gp-overexpressing doxorubicin-resistant cell line MES-SA/Dx5-Rx1.[24] The data indi-

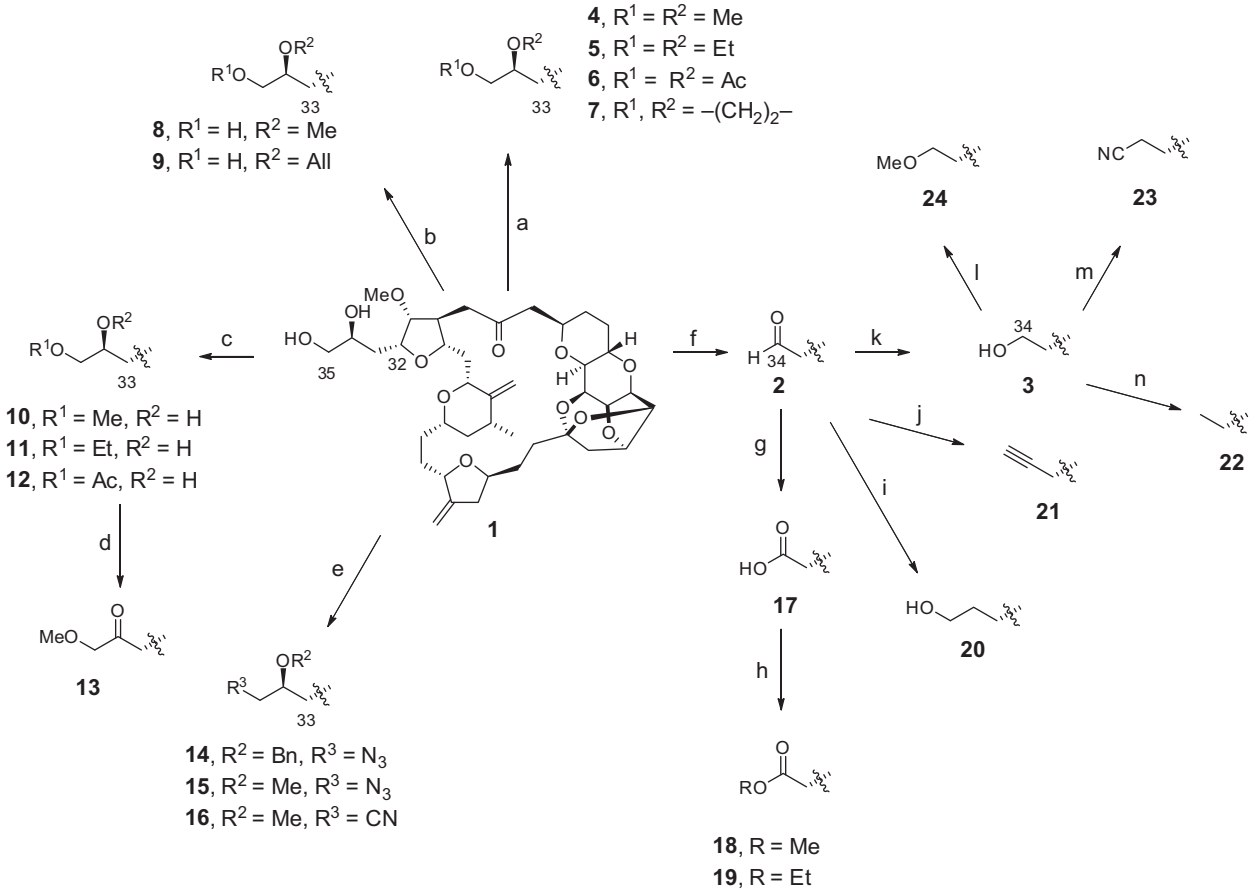

Figure 2. Synthetic transformations to analogs **4**–**24**. Reagents: (a) MeI, Ag_2O, ether, 40 °C; or EtI, Ag_2O, ether, 40 °C; or $BrCH_2CH_2Br$, Ag_2O, ether, 40 °C; or Ac_2O, pyridine, DMAP, CH_2Cl_2. (b) (i) TBDPSCl, imidazole, DMF, 23 °C; (ii) MeI, Ag_2O, ether, 40 °C, or AllylBr, Ag_2O, ether, 23 °C; (iii) TBAF, imidazole·HCl, 23 °C. (c) (i) TBDPSCl, imidazole, DMF, 23 °C; (ii) TBAF, imidazole·HCl, THF, 23 °C; (iii) MeI, Ag_2O, ether, 40 °C; or EtI, Ag_2O, ether, 40 °C; or Ac_2O, pyridine, DMAP, CH_2Cl_2; (iv) TBAF, imidazole·HCl, THF, 23 °C. (d) Dess–Martin periodinane, CH_2Cl_2, 23 °C. (e) (i) TsCl, pyridine, CH_2Cl_2, 23 °C; (ii) NaN_3, DMF, 60 °C; or KCN, DMSO, 60 °C; (iii) MeI, Ag_2O, ether, 40 °C; or BnBr, Ag_2O, ether 40 °C. (f) $NaIO_4$, MeOH·H_2O (4:1), 23 °C. (g) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH·H_2O, 23 °C. (h) $TMSCHN_2$, MeOH, toluene, 23 °C; or EtOH, EDC, DMAP, CH_2Cl_2, 23 °C. (i) (i) $Ph_3P^+CH_2OMe{\cdot}Cl^-$, KO*t*-Bu, THF, ether, 0–23 °C; (ii) PPTS, acetone, H_2O, 23 °C; (iii) $NaBH_4$, EtOH, CH_2Cl_2, −78 to 23 °C. (j) $H_3CCOC(N_2)PO(OMe)_2$, K_2CO_3, MeOH, 23 °C. (k) $NaBH_4$, EtOH, CH_2Cl_2, −78 to 23 °C. (l) MeI, Ag_2O, ether, 40 °C. (m) (i) TsCl, pyridine, CH_2Cl_2, 23 °C; (ii) KCN, DMSO, 60 °C. (n) (i) PhOCOCl, pyridine, 23 °C; (ii) Bu_3SnH, AIBN, toluene, 100 °C.

Table 1
In vitro antiproliferative potency against four human cancer cell lines, and calculated fold-resistance ratio (FR) for compounds synthesized in this study

Compd	C35 subst.	C34 subst.	MES-SA IC_{50} (nM)	MES-SA/Dx5-Rx1 IC_{50} (nM)	FR[a]	DLD-1 IC_{50} (nM)	HCT-15 IC_{50} (nM)
1	OH	OH	0.03	6.50	216.50	0.54	0.22
4	OMe	OMe	0.24	1.86	7.76	0.36	0.21
5	OEt	OEt	0.54	2.38	4.40	0.80	0.55
6	OAc	OAc	0.32	2.77	8.78	0.44	0.32
7	OCH_2CH_2O		1.00	22.96	22.96	0.88	1.24
8	OH	OMe	0.30	11.32	37.73	0.75	0.67
9	OH	OAllyl	0.24	4.13	17.19	0.20	0.22
10	OMe	OH	0.17	2.82	17.09	0.27	0.20
11	OEt	OH	0.20	2.24	11.20	0.27	0.23
12	OAc	OH	0.11	3.47	33.00	nt	nt
13	OMe	C═O	0.44	2.92	6.70	0.67	0.62
14	N_3	OBn	4.83	36.66	7.60	10.57	7.08
15	N_3	OMe	0.44	1.86	4.19	0.78	0.48
16	CN	OMe	0.40	3.90	9.86	1.05	0.61
17	No. C35	CO_2H	12.38	95.55	7.72	17.55	18.30
18	No. C35	CO_2Me	0.35	1.48	4.23	0.83	0.44
19	No. C35	CO_2Et	0.37	1.17	3.21	0.45	0.40
20	OH	None	0.30	5.67	18.88	0.47	0.40
21	C═C		0.73	2.01	2.75	1.46	0.83
22	No. C35	None	1.06	1.36	1.81	nt	nt
23	OMe	None	0.75	2.91	3.88	1.43	0.68
24	No. C35	CN	0.19	0.78	4.08	0.38	0.24
Eribulin	NH_2	OH	1.66	3058	1842	20	204
Doxorubicin	—	—	26.40	2702.33	134.00	nt	153.95
Paclitaxel	—	—	2.96	>1000	>338	nt	nt
Vinblastine	—	—	2.28	439.73	224.18	nt	28.50

IC_{50} values are means of at least two measurements.
nt = not tested.
[a] Defined as ratio of antiproliferative IC_{50} against MES-SA/Dx5-Rx1 cells to IC_{50} against MES-SA cells.

cate that most analogs in this study were highly potent against MES-SA cells, with cell-growth inhibitory activity equal to or better than eribulin. The fold resistance ratio (FR) value is calculated as the ratio of IC_{50} against MES-SA/Dx5-Rx1 to the IC_{50} against MES-SA cells. As seen in Table 1, replacement of the basic amine functionality of eribulin with neutral functional groups led to a decrease in the fold-resistance ratio (FR) from >200 to <20. In addition, capping free –OH groups as ethers or esters was found to decrease FR (e.g., compounds **4** and **8** or **10**, **5** and **11**, etc.). Compounds with simple aliphatic side-chains such as **21** and **22** had the lowest FR, indicating a critical dependence on lipophilicity of the side-chain. The overall dependence of FR on calculated log $D_{7.4}$ values is shown in Figure 3. The clear trend seen within this series is that increasing lipophilicity generally leads to compounds with lower FR.

It is notable that these compounds also showed low- to sub-nM potency against the drug-resistant human colon cancer cell lines DLD-1 and HCT-15 (Table 1), suggesting that these compounds may be suitable for treatment of a wide range of tumor types. A few compounds were tested for cytotoxicity against quiescent

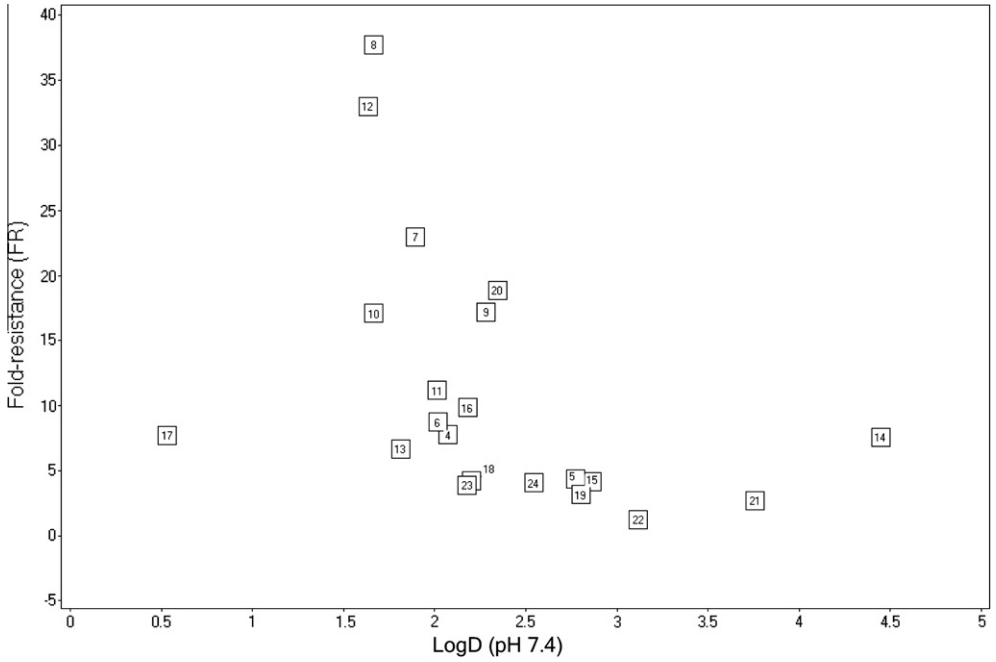

Figure 3. Correlation of fold-resistance (FR) with log $D_{7.4}$ values for analogs synthesized in this study (compound **1** not shown).

IMR-90 human fibroblasts at concentrations up to 1 μM. In these studies, proliferation-independent nonspecific toxicity was not observed (data not shown), indicating that the activity of the compounds in this study is specific for proliferating cells.

Pharmacokinetic studies revealed that many compounds from this series had good exposure levels in mice following intravenous dosing. Specifically, compound concentration over 3–4 h after dosing was found to be higher than the antiproliferative IC_{50} value measured in cell culture (data not shown). Accordingly, a few candidate compounds were selected for in vivo evaluation in tumor xenograft models.

Moderate in vivo antitumor activity was observed with several analogs against LOX human melanoma xenografts in athymic mice. For purposes of direct comparison, all compounds were tested at 0.1 mg/kg using the same Q1D × 5[×2] dosing schedule (once daily, five days a week for two weeks); that is, no attempt was made to optimize the dose or the regimen for each compound. The data for compounds **4** and **10** are shown in Figure 4. Overall, significant inhibition of tumor growth by both compounds was observed, although the levels of activity seen were somewhat less than that of eribulin at the same dose. For compounds **4** and **10**, tumor growth was strongly inhibited as long as compound administration continued, with tumor sizes increasing post dosing. No signs of toxicity such as lowering of body weight or water uptake were evident. Although these in vivo data for non-amine-containing compounds **4** and **10** represented an important starting point, compounds retaining an amine substituent in the C32 side-chain ultimately proved to be the most active in xenograft models. These studies are described in more detail in Part II.[25]

In conclusion, several second generation analogs of eribulin possessing neutral C32 side-chains have been synthesized and evaluated. By limiting the number of transformations required to access each analog, we were able to carry out a wide range of chemistry on this highly complex scaffold. These side chain modifications led to eribulin analogs that possess low- to sub-nM antiproliferative potency against both sensitive and resistant human cancer cell lines in vitro. Furthermore, these compounds displayed extremely low P-gp susceptibility as measured by differential antiproliferative activity against related sensitive and P-gp overexpressing cell lines. Finally, in vivo activity in mouse xenograft models was observed in case of some compounds.

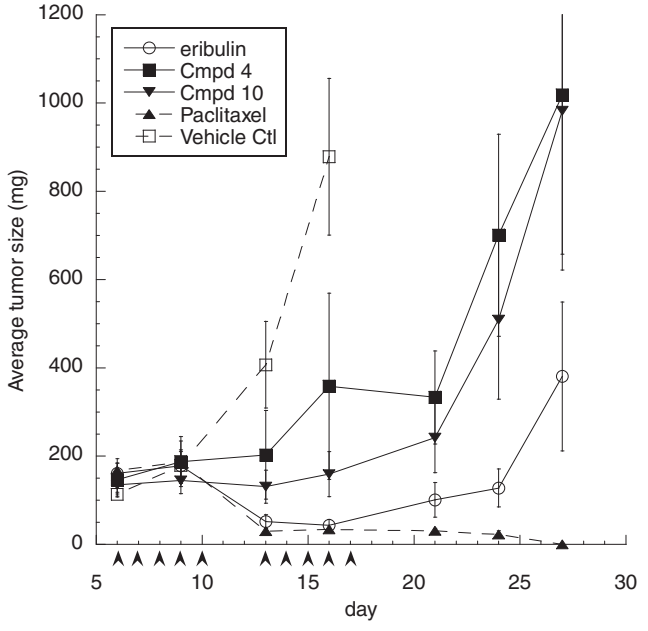

Figure 4. Effect of compounds **4** and **10** on the growth of subcutaneous LOX melanoma xenografts in athymic mice. Compounds were dosed intravenously once daily, 5 days a week, for two weeks. Compounds **4**, **10** and eribulin were dosed at 0.1 mg/kg; the positive control paclitaxel was dosed at 20 mg/kg.

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