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Novel second generation analogs of eribulin. Part II: Orally available and active against resistant tumors in vivo

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ABSTRACT

Eribulin mesylate is a newly approved treatment for locally advanced and metastatic breast cancer. We targeted oral bioavailability and efficacy against multidrug resistant (MDR) tumors for further work. The design, synthesis and evaluation of novel amine-containing analogs of eribulin mesylate are described in this part. Attenuation of basicity of the amino group(s) in the C32 side-chain region led to compounds with low susceptibility to PgP-mediated drug efflux. These compounds were active against MDR tumor cell lines in vitro and in xenograft models in vivo, in addition to being orally bioavailable.

Eribulin mesylate (Halaven™, Fig. 1) is a novel anti-tubulin agent under development by Eisai as a treatment for breast cancer and other solid tumors.[1,2] A recent Phase 3 study showed that eribulin significantly improved overall survival in patients with locally advanced and metastatic breast cancer.[3] Based on these results it recently won approval in the United States, while it is currently being reviewed by regulatory agencies in Europe and Japan. By virtue of its unique mechanism of action targeting microtubule dynamics as compared to taxanes and the vinca alkaloids,[4–8] as well as its wide therapeutic window in preclinical studies, eribulin represents a significant advance in the arena of chemotherapeutic agents.[9] The current studies represent the second of a series of three articles describing our continuing work on this class of molecules, in particular focusing on development of compounds with oral bioavailability, brain penetration and activity against multidrug resistant (MDR) tumors.

A preclinical profile comprising of activity against a variety of MDR cell lines was selected based on our expectation that such properties could be expected to lead to better clinical outcomes. In addition, although many chemotherapeutic drugs including eribulin are routinely administered as intravenous infusions, an orally available compound may be desirable in certain circumstances. In this Letter, we describe our efforts aimed at optimizing these two properties within the eribulin core structure. P-Glycoprotein (P-gp)-mediated drug efflux is one of the primary mechanisms of drug resistance in a number of cancer cell types.[10,11] Moreover, the role of P-gp in limiting the intestinal absorption of drugs is also well established.[12–14] Therefore, we selected amelioration of cellular efflux by P-gp as a key element of our chosen preclinical profile.

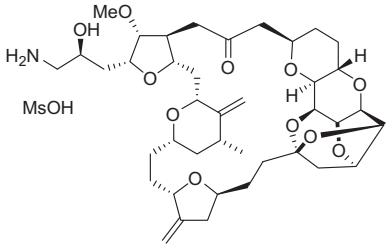

Figure 1. Eribulin mesylate (Halaven™).

We hypothesized that the basic primary amine moiety in the C32 side chain of eribulin played a key role in its P-gp liability, and thus focused on modifying this part of the molecule in our second generation work. In part I, we described the replacement of the C32 amino alcohol side chain of eribulin with functionalities that are neutral at physiologic pH. Since amine basicity is often correlated with P-gp susceptibility,[15,16] we also designed analogs that would still contain an amine, but with attenuated basicity of the nitrogen substituent.

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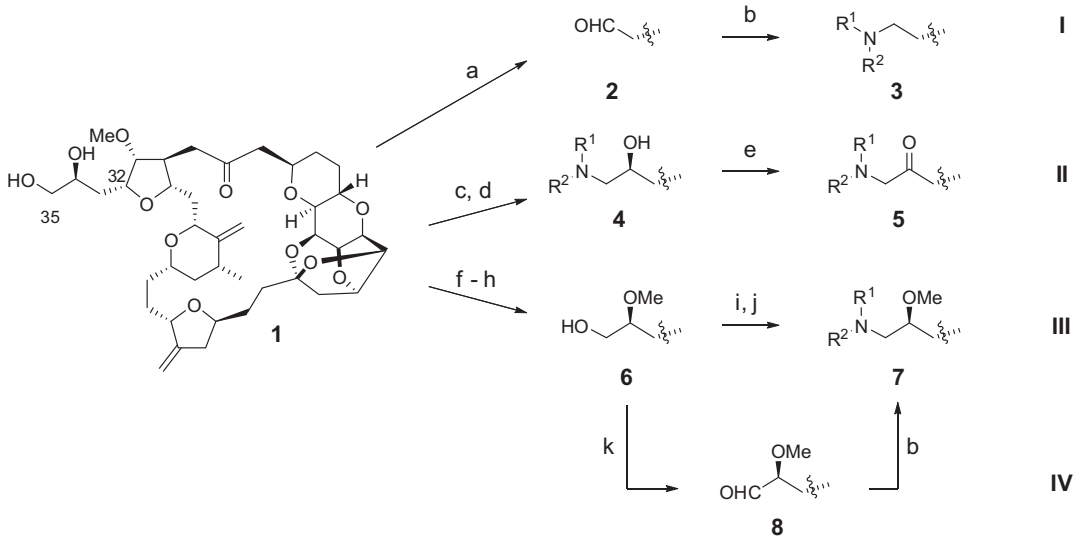

Figure 2. General methods for the synthesis of C34/C35 amine containing analogs. Reagents: (a) $NaIO_4$, $THF{\cdot}H_2O$, 23 °C; (b) R^1R^2NH, $Na(AcO)_3BH$, AcOH, $ClCH_2CH_2Cl$, 23 °C; (c) Ts_2O, DMAP, CH_2Cl_2, 0–23 °C; (d) R^1R^2NH, Et_3N, MeOH; 23–60 °C; (e) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C; (f) TBDPS-Cl, imidazole, DMF, 0 °C; (g) MeI, Ag_2O, Et_2O, 40 °C; (h) Bu_4NF-imidazole.HCl, THF, 23 °C; (i) $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C or TsCl, pyridine, CH_2Cl_2, 23 °C; (j) R^1R^2NH, Et_3N, CH_3CN, 60 °C; (k) Dess–Martin periodinane, CH_2Cl_2, 23 °C.

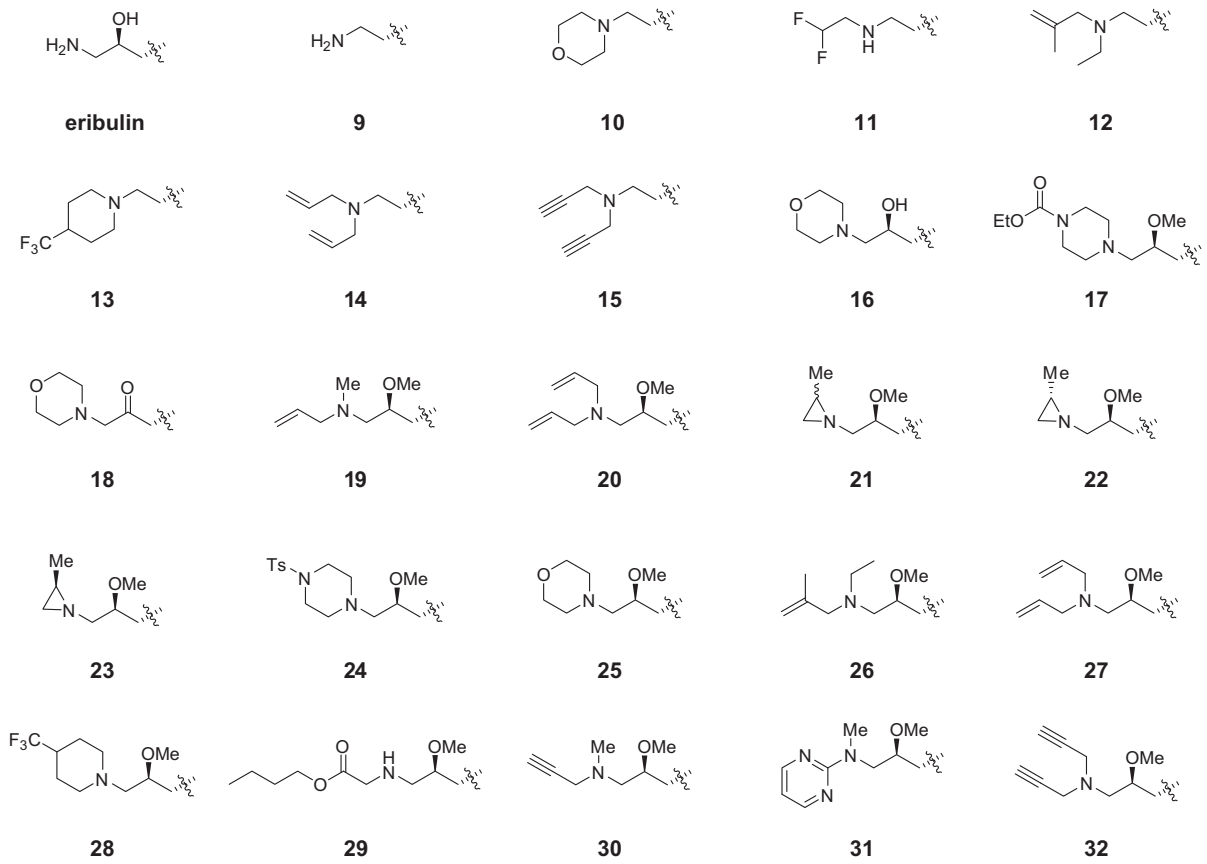

Figure 3. Partial structures (C32 side-chain) of eribulin analogs synthesized in this study.

We report herein a series of eribulin analogs that have low PgP susceptibility (FR ⩽ 25), and show low- to sub-nM antiproliferative potency against resistant tumor cell lines in vitro. These compounds, containing an amine functionality at either C34 or C35, were synthesized starting from a common intermediate. Further exploration led to the identification of compounds that show in vivo efficacy against MDR tumors in xenograft models. Oral efficacy was also demonstrated with some of these compounds.

The compounds in this study were synthesized from previously described[17] diol **1** employing one of four general routes as summa-

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	MES-SA IC_{50} (nM)	MES-SA/Dx5-Rx1 IC_{50} (nM)	FR[a]	DLD-1 IC_{50} (nM)	HCT-15 IC_{50} (nM)
Eribulin	1.66	3058	1842	20	204
9	0.17	47.63	288.17	4.67	8.57
10	0.12	2.52	22.35	0.23	0.16
11	1.91	31.86	16.81	3.16	3.11
12	0.23	2.89	12.35	0.61	0.46
13	0.11	0.75	6.90	0.22	0.14
14	0.17	1.15	6.57	0.31	0.21
15	0.69	3.99	5.65	1.42	0.89
16	0.15	7.26	49.82	0.31	0.36
17	0.08	3.05	38.10	nt	nt
18	0.25	3.12	12.37	0.70	0.46
19	0.07	1.95	29.40	0.23	0.22
20	0.13	2.41	21.59	0.23	0.21
21	0.13	2.55	19.91	0.29	0.40
22	0.13	2.23	18.26	0.47	0.67
23	0.16	2.79	18.04	0.53	0.78
24	3.16	39.10	12.35	0.99	0.62
25	0.16	1.91	11.82	0.23	0.20
26	0.23	2.73	11.87	0.51	0.34
27	0.17	1.01	6.26	0.44	0.27
28	0.14	0.83	6.03	0.17	0.15
29	0.28	1.60	5.83	0.58	0.37
30	0.18	0.95	5.19	0.31	0.19
31	2.17	10.11	4.66	6.90	5.40
32	0.13	0.53	3.99	0.28	0.17
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. Partial structures (C32 side-chain) of compounds **9**–**32** are shown in Figure 3.
nt = not tested.
[a] Defined as ratio of antiproliferative IC_{50} against MES-SA/Dx5-Rx1 cells to IC_{50} against MES-SA cells.

rized in Figure 2. C34 amines were synthesized by oxidative cleavage of **1**, followed by reductive amination of the resulting aldehyde **2** (route I). C35 amino derivatives were obtained by alkylation of the appropriate amine with the 1° mono-tosylate derivative of **1** (route II). C35 amines bearing a C34 methoxy substituent were accessed from alcohol **6**, which in turn was prepared from **1** in a three-step sequence comprising TBDPS protection of the primary alcohol, methylation at the C34 hydroxyl, and TBDPS deprotection (route III). In the case of certain 2° amines which proved recalcitrant to alkylation, route IV involving reductive amination of methoxy aldehyde **8** was employed. These transformations provided access to a variety of analogs with diverse structures at the C32 side chain, and a wide spectrum of physicochemical properties.

All compounds[18] were tested for cell growth inhibitory activity against four human cancer cell lines (Table 1). In addition to the human sarcoma cell line MES-SA, the primary assay system for this study, compounds were also tested against the doxorubicin-resistant subline MES-SA/Dx5-Rx1. The ratio of IC_{50} values between these two cell lines, termed fold-resistance ratio (FR), was used as a measure of Pgp susceptibility.[19] Additionally, compounds were evaluated in the human colon cancer cell lines DLD-1 and HCT-15, both of which also express the MDR phenotype.

In agreement with our prediction, compounds with attenuated basicity of substituents in the C32 side-chain region did indeed lead to significantly reduced FR, while maintaining excellent in vitro potency. The majority of compounds synthesized had FR <20 and low- to sub-nM potency. Amines containing unsaturated alkyl groups or electron-withdrawing substituents generally displayed lower FR, as compared to alkyl substituted or unsubstituted amines. The same decrease in FR was also obtained by introducing other heteroatoms (morpholines, acyl or sulfonyl piperazines), or by way of ring strain (aziridines). Where present, conversion of the C34 hydroxyl to the methyl ether was found to lower the FR. In short, increasing the overall lipophilicity of the molecule led to

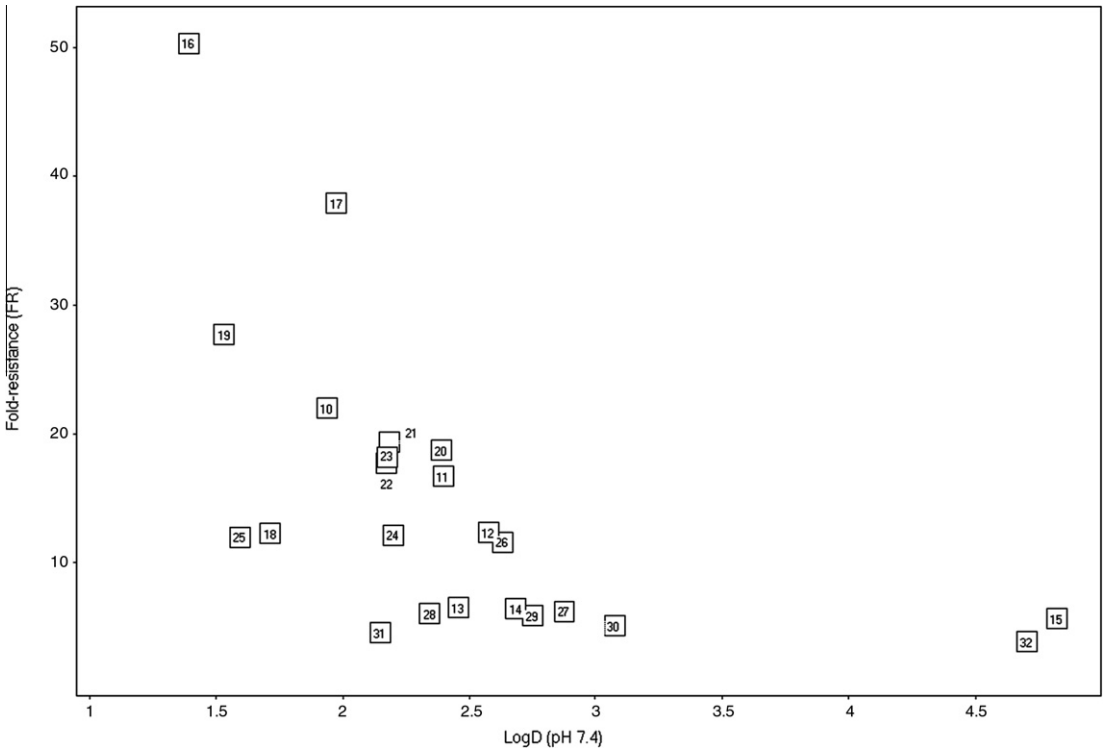

Figure 4. Correlation of fold-resistance (FR) with log $D_{7.4}$ values for the analogs synthesized in this study (compound **9** and eribulin not shown).

lower FR within this series. This trend can be readily seen in Figure 4, which shows the relationship between FR and the calculated log *D* at pH 7.4 of the compounds synthesized in this study. An increase in log *D* from 1.4 to 3 led to a significant lowering of FR from 50 to 5, although log *D* values beyond three did not lead to any further decrease in FR. The observed dependence of FR on amine basicity is in line with observations reported by the Merck group on KSP inhibitors, where P-gp susceptibility was decreased by introducing electron withdrawing groups proximal to an amine.[20]

The most potent compounds exhibited sub-nM potency in all four cell lines, making available a large pool of candidates for further evaluation. We next turned to measuring the pharmacokinetics of compounds from this series in mice. Our aim was to determine if sufficient drug exposure would permit once daily dosing in vivo. Table 2 summarizes pharmacokinetic parameters for eribulin and compounds **11**, **18**, **20**, and **25**. Gratifyingly, for all compounds tested, drug levels were found to be significantly higher than in vitro IC_{50} values over several hours post-administration. In addition, and in contrast to eribulin, several analogs from this study exhibited moderate levels of oral bioavailability. Thus, our premise that optimizing for P-gp susceptibility might lead to improved oral bioavailability appeared to hold true.

Table 2
Pharmacokinetic parameters for selected compounds in BALB-c mice following a single oral dose

Compd	AUC_{0-inf} (ng h/mL)	$t_{1/2}$ (h)	V_{ss}[a](L/kg)	CL[a](L/kg/h)	*F* (%)
Eribulin	161	15.4	4.95	1.19	3.2
11	63	4.1	4.78	2.91	11.3
18	236	1.7	3.64	2.82	13.3
20	229	4.7	12.78	4	18.3
25	1094	0.9	0.92	0.68	14.9

t_{max} = 5 min in all cases. Eribulin was administered at 10 mg/kg; all other compounds were administered at 5 mg/kg.
[a] Measured from a parallel iv experiment.

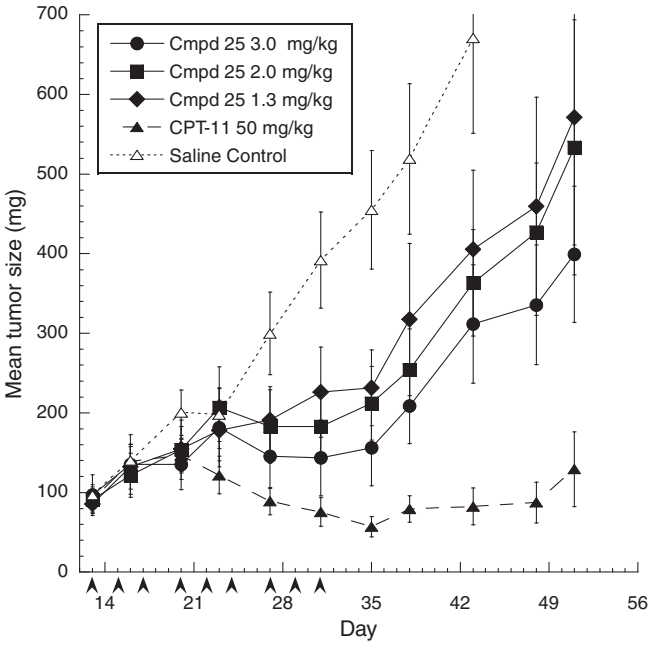

Figure 5. Effect of compound **25** at different doses and CPT-11 on subcutaneously implanted DLD-1 xenografts in athymic mice. Compounds were administered intravenously once daily, on Mondays, Wednesdays and Fridays, for three weeks starting day 13.

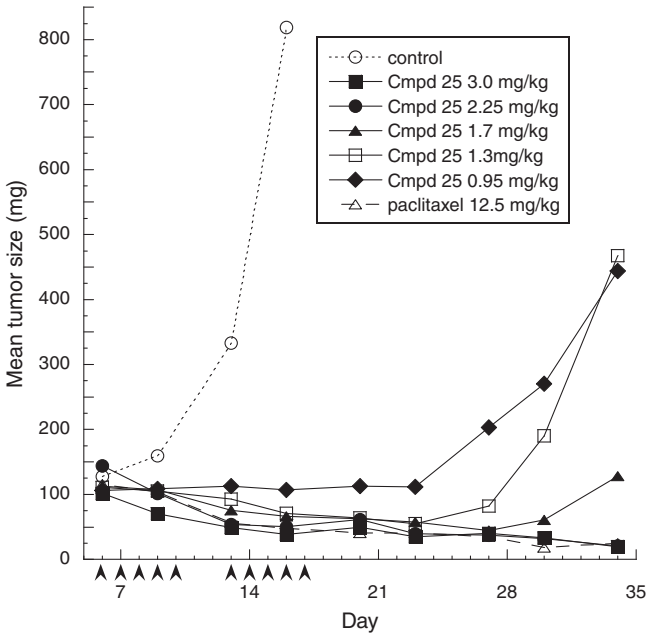

Figure 6. Effect of compound **25** at different doses on the growth of subcutaneously implanted LOX xenografts in athymic mice. Compound **25** was dosed orally, once daily, 5 days a week, for two weeks starting day 6. Paclitaxel was dosed intravenously.

Based on cell growth inhibition data, in vitro distribution, metabolism and pharmacokinetic (DMPK) properties, and in vivo pharmacokinetics, several candidates for were prioritized for efficacy testing in in vivo tumor xenograft models. We chose the LOX human melanoma xenograft as a prototypical drug-sensitive model, while DLD-1 human colon cancer served as a typical MDR tumor model. For purposes of direct comparison, all compounds were tested in similar dosing regimens. In addition, all compounds were administered intravenously so that their relative in vivo activities could be assessed whether or not they exhibited oral bioavailability. Figure 5 shows the effect of compound **25** on tumor growth in a DLD-1 xenograft model in athymic mice. Compound **25** inhibited tumor growth in this model in a dose dependent manner at doses well below 5 mg/kg. After a 2 week initial delay, tumor stasis was observed as long as compound administration was continued, with tumor regrowth occurring after dosing was stopped.

Oral efficacy of compound **25** was evaluated in LOX xenograft models (Fig. 6). Long term tumor regression was observed in this model following oral dosing. Taken together, the data reported herein suggest that compound **25** and its analogs might be active against a wide range of human cancers, in addition to being active against MDR tumors and amenable to oral dosing regimens.

In summary, introduction of amines with low basicity in the C32 side chain, or otherwise increasing lipophilicity in this portion of eribulin, led to the identification of analogs with greatly reduced susceptibility to P-gp mediated drug efflux. These compounds showed moderate oral bioavailability and were orally active in vivo. In addition, this series afforded compounds with activity against resistant tumor cell lines such as DLD-1 in xenograft models. These novel compounds may thus provide candidates for the treatment of a wider variety of human cancers.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.097. These data include MOL files and InChiKeys of the most important compounds described in this article.

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