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Novel second generation analogs of eribulin. Part III: Blood–brain barrier permeability and in vivo activity in a brain tumor model

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ABSTRACT

Novel second generation analogs of eribulin mesylate, a tubulin agent recently approved for the treatment of breast cancer, are reported. Our recent efforts have focused on expanding the target indications for this class of compounds to other tumor types. Herein, we describe the design, synthesis and evaluation of eribulin analogs active against brain tumor cell lines in vitro and corresponding brain tumor models in mice. Attenuation of basicity of the amino group(s) in the C32 side-chain region led to compounds with lower susceptibility to P-gp mediated drug efflux, allowing these compounds to permeate through the blood–brain barrier. In preclinical in vivo studies, these compounds showed significantly higher levels in the brain and cerebrospinal fluid as compared to eribulin. In addition, analogs within this series showed antitumor activity in an orthotopic murine model of human glioblastoma.

Eribulin mesylate (Halaven™, Fig. 1), an analog of the marine natural product halichondrin B, is under clinical development by Eisai.[1–3] Following successful completion of a Phase 3 trial in which a survival benefit was demonstrated in late-stage metastatic breast cancer patients, eribulin was approved by the United States Food and Drug Administration. Additionally, it is undergoing review by regulatory authorities in Japan, and the European Union for full approval in this disease.[4] Several clinical trials evaluating eribulin in other cancer types are also underway.[5] Although classified as an anti-tubulin agent, eribulin inhibits microtubule dynamics via a mechanism of action that is distinct from vinblastine or paclitaxel.[6–9] Continuing studies in our laboratories have focused on developing compounds with activity against multidrug resistant (MDR) tumors in animal models, with an ultimate goal of expanding the scope of target indications for the eribulin class of compounds. Our studies are summarized in a series of three articles, with this third part focusing on an area of particularly high unmet medical need: the treatment of central nervous system (CNS) and brain cancer.[10]

Despite considerable progress in treating and managing various types of cancers, the treatment of brain cancer remains a significant challenge in contemporary oncology.[11] Both primary and metastatic brain tumors are particularly difficult to treat, with 5-year survival rates of around 30% in adults. In the case of children, primary brain tumors account for up to 25% of all cancers and are a leading cause of mortality. The most common treatment paradigm for CNS and brain tumors involves surgery and radiation, followed by adjunctive chemotherapy.[12] Although surgical resection is effective at clearing portions of brain tumors, large numbers of viable cancer cells inevitably remain at the surgical site. Control of the residual disease is therefore highly dependent on chemotherapy.

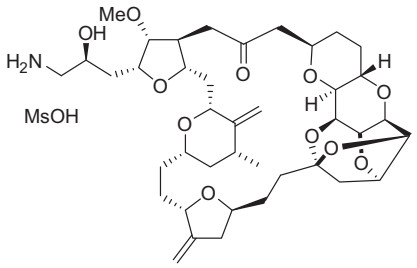

Figure 1. Eribulin mesylate (Halaven™).

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However, current chemotherapy options are only of modest efficacy in brain and CNS tumors, with the most effective treatments involving alkylating agents such as nitrogen mustards, platinum compounds, and temozolomide.[13] In addition, tubulin agents such as taxanes and vinblastine have generally proven to be ineffective at treating this class of cancers. One significant challenge in designing brain tumor chemotherapeutics is the inability of most drugs to traverse the blood–brain barrier (BBB).[14] There is thus a singular need for new highly efficacious chemotherapeutics for brain tumors.

Our efforts at expanding the target drug profile of eribulin, in part by lowering its P-glycoprotein (P-gp) susceptibility, are described in the first two parts of this three-part series.[15] Recently, the role of P-gp in modulating BBB penetration has become clear.[16] P-gp is found in the microvasculature in tissue sections in isolated brain capillaries.[17,18] Moreover, substantially higher levels of paclitaxel and vinblastine have been observed in the brains of *mdr1* knockout mice compared to wild type.[19] Interestingly, both human and animal studies have shown that the BBB is often compromised in the presence of a brain tumor, leading to leaky vasculature through which drugs might reasonably be expected to pass. Nevertheless, P-gp mediated efflux is often active even in such cases.[20] Due to the significant role of P-gp in CNS drug disposition, we have synthesized and evaluated several second generation eribulin analogs for their brain and cerebrospinal fluid (CSF) distribution.

As before, our efforts focused on the C32 side chain region of eribulin, since this allowed us to synthesize analogs with diverse physico-chemical properties while maintaining the exquisite potency and antitumor efficacy of this class of compounds. The compounds utilized in this study comprised a cross-section of analogs that were synthesized while targeting antiproliferative activity

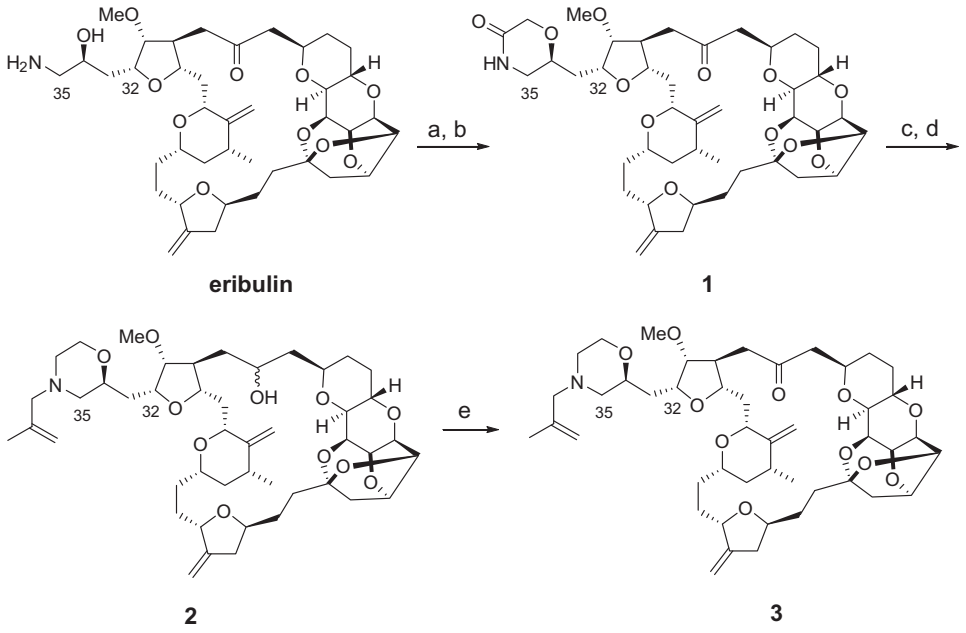

Figure 2. Synthesis of compound **3** from eribulin: Reagents: (a) $ClCH_2COCl$, Et_3N, CH_2Cl_2, 23 °C, 76%; (b) KO^{t}Bu, ^{t}BuOH, THF, 23 °C, 96%; (c) $CH_2{=}C(CH_3)CH_2Cl$, NaH, DMF, 23 °C, 86%; (d) $LiAlH_4$, THF, 40 °C, 97%; (e) Dess–Martin periodinane, CH_2Cl_2, 23 °C, 48%.

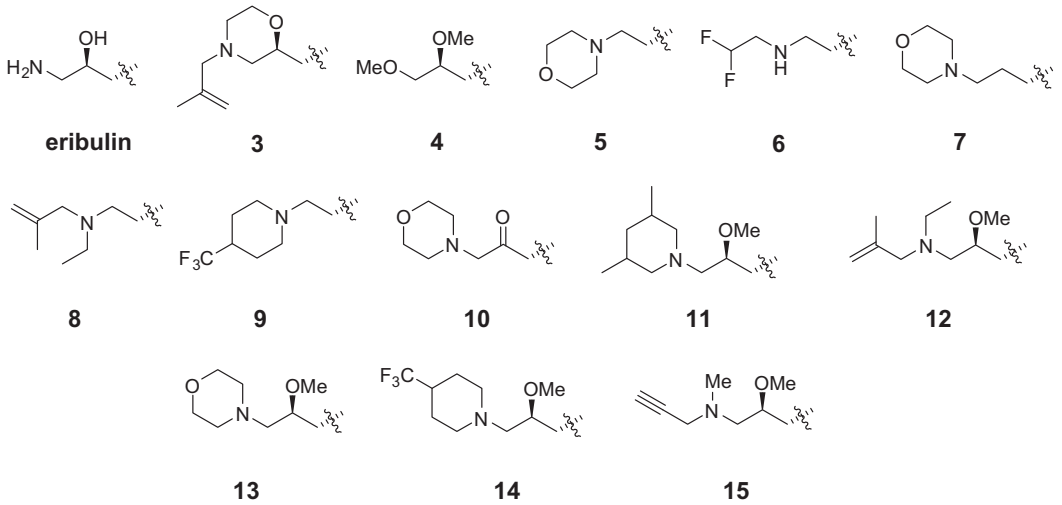

Figure 3. Partial structures (C32 side-chain) of compounds in Table 1.

Table 1
In vitro antiproliferative potency against four human cancer cell lines, and calculated fold-resistance ratio (FR) for compounds synthesized in this study. Partial structures are shown in Figure 3

Compd	MES-SA IC_{50} (nM)	MES-SA/Dx5-Rx1 IC_{50} (nM)	FR[a]	U-251 IC_{50} (nM)	SF-295 IC_{50} (nM)
Eribulin	1.66	3058	1842	5.87	27.85
3	0.25	1.53	6.2	0.50	0.26
4	0.24	1.86	7.76	0.74	nt
5	0.12	2.52	22.35	1.26	nt
6	1.91	31.86	16.81	4.05	nt
7	0.083	1.3	16.0	1.44	nt
8	0.23	2.89	12.35	0.61	nt
9	0.11	0.75	6.90	0.28	nt
10	0.25	3.12	12.37	0.88	nt
11	0.13	2.41	21.59	2.55	0.10
12	0.23	2.73	11.87	3.74	nt
13	0.16	1.91	11.82	1.97	nt
14	0.14	0.83	6.03	0.33	nt
15	0.18	0.95	5.19	0.18	0.41
Paclitaxel	2.96	>1000	>338	nt	24.55

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.

against MDR tumor cell lines. Their syntheses followed methods summarized in the preceding papers.[15] A few analogs were unique to this series, such as the morpholine derivative **3**, which was synthesized from eribulin as shown in Figure 2.[21]

All compounds were tested for cell growth inhibitory activity in three human cancer cell lines (Table 1). The human sarcoma cell lines MES-SA and its doxorubicin-resistant subline MES-SA Dx5/Rx1, which overexpresses P-gp, were used as the primary screening system. The fold resistance ratio (FR) is calculated as the ratio of IC_{50} against MES-SA/Dx5-Rx1 to the IC_{50} against MES-SA cells. Compounds with low resistance ratios ($FR \leqslant 25$) were screened for cell growth inhibitory activity against the human glioblastoma cell line U-251. Certain compounds were also evaluated against SF-295 human glioblastoma cells. We have previously discussed the relationship of structure, physico-chemistry and FR within this series.[15] Of special note is that in almost all cases, these second generation analogs were significantly more potent than eribulin against both U-251 and SF-295 cells. In addition, activity against U-251 cells in vitro also roughly correlated with FR, with lower IC_{50} values observed for compounds with lower FR (Fig. 4).

Since the major goal of this study was to optimize compounds for brain penetration, we evaluated the brain distribution of selected compounds in BALB-c mice in pharmacokinetic studies. Compared to eribulin, significantly higher fractions of drug in the brain were observed for these analogs. The brain penetration index (ratio of dose-normalized AUC in the brain to plasma) was >1 in all cases, with estimates as high as 7.7. Table 2 summarizes various pharmacokinetic parameters for selected compounds. To get an estimate of free drug concentration in the brain compartment, an in vitro assay was performed to measure drug binding to homogenized mouse brain tissue.[22] Based on these experiments, unbound drug concentrations were still predicted to be well above their in vitro antiproliferative IC_{50} values. Since it is easier to obtain, the CSF is often used as a surrogate for the measurement of unbound brain concentration.[23–25] We evaluated the pharmacokinetics of compound **13** in rats and found that it penetrated well into

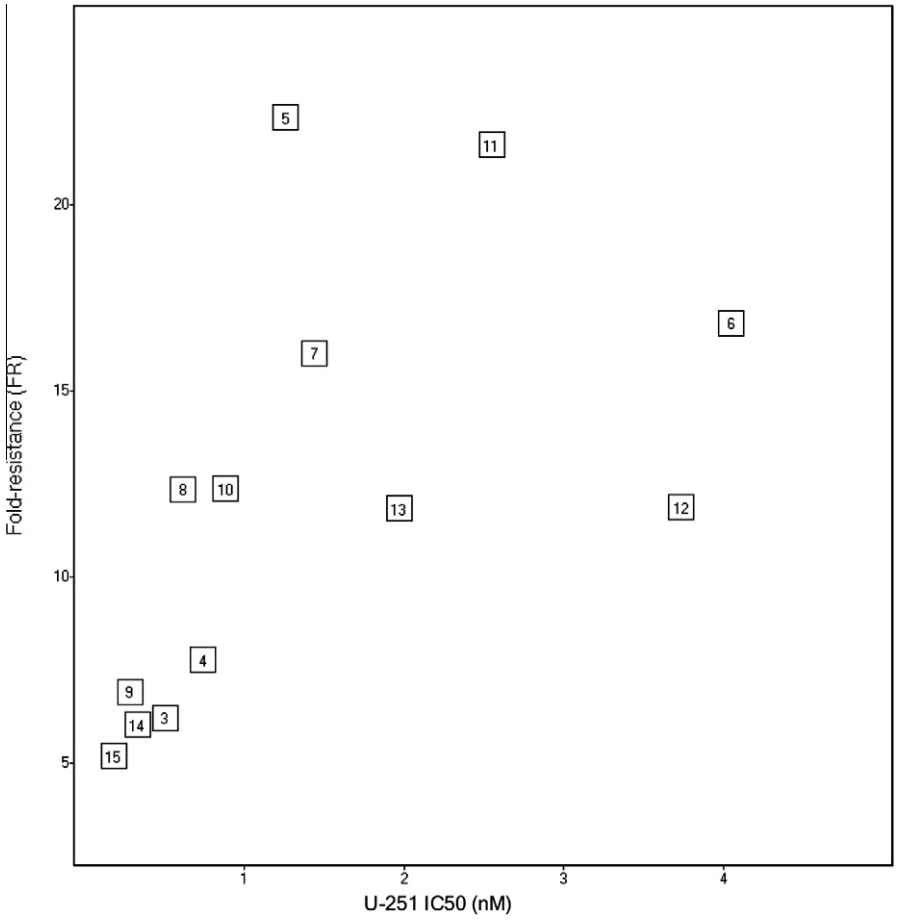

Figure 4. Dependence of potency against U-251 cells on fold-resistance for compounds in Table 1. Eribulin and paclitaxel not shown.

Table 2
Brain pharmacokinetic parameters for selected compounds in BALB-c mice

Compd	AUC_{0-inf} (ng·h/g)	$t_{1/2}$ (h)	t_{max} (h)	$f_{unbound}$[a] (brain tissue)	BPI[b] (mL/g)	FR (Table 1)
Eribulin	435	46	0.5	nt	0.55	1842
3	1362	1.2	0.08	0.024	1.8	6.2
7	10,200	4.6	0.25	0.101	7.7	16.0
11	3108	5.3	0.08	0.034	1.7	21.59
13	1351	0.9	0.08	0.113	1.4	11.82

Concentrations were measured at various times after a single 5 mg/kg intravenous administration.
nt = not tested.
[a] Unbound fraction measured in an in vitro assay of binding to homogenized mouse brain tissue.
[b] Defined as AUC_{0-inf} (brain)/AUC_{0-inf} (plasma).

the CSF, with levels approximately 100-times the in vitro antiproliferative IC_{50} (Fig. 5). The CSF penetration index (ratio of AUC_{CSF} to AUC_{plasma}) was determined to be 2.2. Thus, amelioration of P-gp mediated efflux indeed led to increased brain penetration as compared to eribulin. Taken together, these data suggest that the compounds synthesized in this study provide significantly high levels of brain exposure, and may therefore be suitable for treatment of brain tumors.

Several compounds from this study were evaluated in orthotopic mouse models of human brain tumors. In the first model,

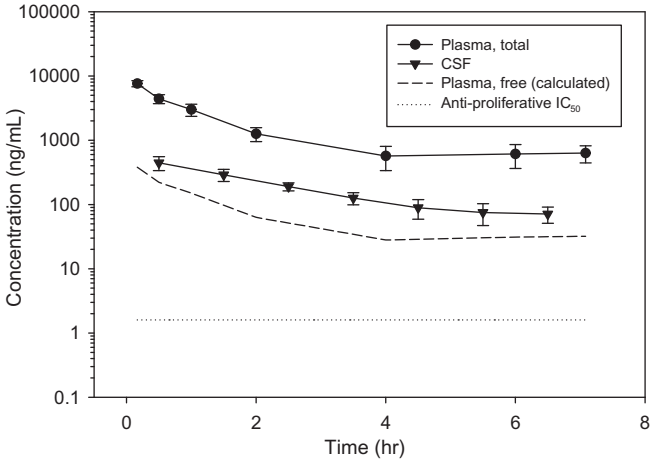

Figure 5. Mean plasma and CSF concentration versus time profile of compound **13** in male Sprague Dawley rats following a single 4 mg/kg intravenous administration ($n = 4$).

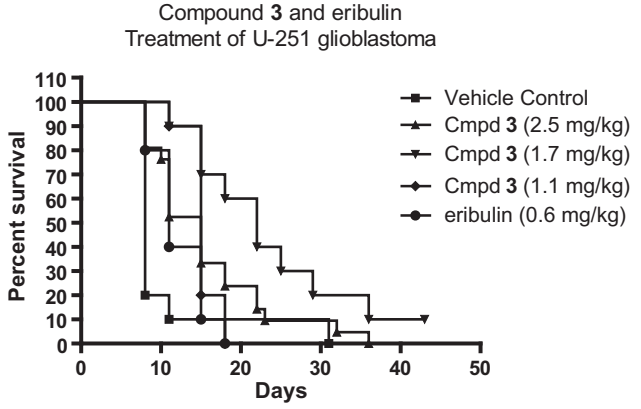

Figure 6. Effect of compound **3** administration on overall survival of athymic mice implanted with orthotopic U-251 human glioblastoma brain tumors.

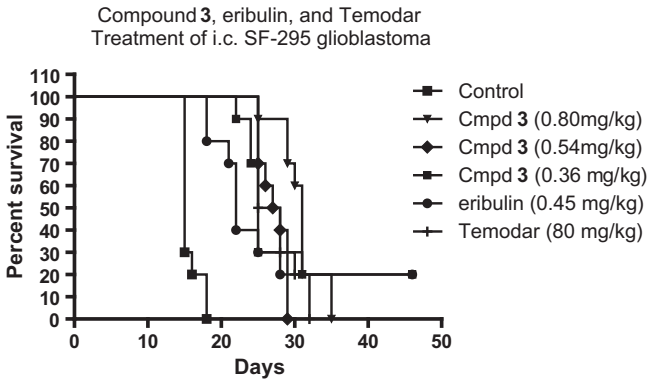

Figure 7. Effect of compound **3** administration on overall survival of athymic mice implanted with orthotopic SF-295 human glioblastoma brain tumors.

U-251 human brain tumor cells were intracranially implanted into athymic mice to engender orthotopic tumors. Mice were then randomly assigned to five groups comprising of vehicle, eribulin and three doses of compound **3**, and the overall survival measured. PRISM software was used to generate Kaplan–Meier survival curves, and to determine whether a significant difference existed between treated and untreated groups. The results are summarized in Figure 6. Compound **3** resulted in significantly increased survival (>175% median group increase) over untreated group with $p = 0.0085$. By comparison, eribulin increased lifespan over untreated by 33%. In a similar manner, compound **3** was evaluated in an orthotopically implanted SF-295 human glioblastoma tumor model in athymic mice (Fig. 7). In this model too, treatment with compound **3** led to increased survival.

In summary, modifications in the C32 side chain region of eribulin led to the identification of compounds with significantly reduced susceptibility to P-gp-mediated drug efflux. The analogs generated showed highly potent activity against U-251 and SF-295 human brain tumor cell lines in culture. Several compounds were also found to have high levels of exposure in brain and CSF. Finally, in vivo activity in multiple orthotopic brain tumor xenograft models was observed, suggesting that these novel analogs may have utility in the treatment of brain and other CNS 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.2011.01.096. These data include MOL files and InChiKeys of the most important compounds described in this article.

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