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Synthesis and SAR of C12–C13-oxazoline derivatives of epothilone A

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ABSTRACT

The SAR of a series of new epothilone A derivatives with a 2-substituted-1,3-oxazoline moiety *trans*-fused to the C12–C13 bond of the deoxy macrocycle have been investigated with regard to tubulin polymerization induction and cancer cell growth inhibition. Significant differences in antiproliferative activity were observed between different analogs, depending on the nature of the substituent at the 2-position of the oxazoline ring. The most potent compounds showed comparable activity with the natural product epothilone A. Modeling studies provide a preliminary rationale for the observed SAR.

Epothilones A and B (Epo A and B; Fig. 1) are naturally occurring microtubule-stabilizing macrolides that were first isolated from the myxobacterium *Sorangium cellulosum* by Reichenbach, Höfle, and co-workers in 1987.[1,2] Epothilones are potent inhibitors of human cancer cell growth in vitro and in vivo[2,3] and they have served as successful lead structures for the development of new anticancer drugs.[4] Thus, at least seven epothilone-derived agents have entered clinical evaluation in humans (including the natural product Epo B) and the most advanced of these analogs, the Epo B lactam BMS-247550 (ixabepilone), has recently obtained FDA approval for clinical use in cancer patients.[5]

Extensive studies on the epothilone SAR have led to a detailed (empirical) understanding of the structural requirements for biological activity and modifications have been identified that are associated with enhanced potency (relative to the natural products).[4] These studies also revealed that deoxyepothilones (i.e., Epo C and Epo D; Fig. 1), which incorporate a *Z* double bond between C12 and C13 in place of a *cis*-epoxide moiety, possess similar antiproliferative activity as the epoxide-based parent compounds Epo A and B.[6,7] In contrast, Epo C/D analogs with a phenyl[8] or imidazole[9] moiety fused to the C12–C13 bond have been reported to be virtually inactive, thus indicating that conformational restriction of the C12–C13 bond to a *cis*-geometry by itself does not suffice to maintain potent biological activity. Surprisingly, however, the additional steric bulk associated with C12–C13-fused rings appears to be much better tolerated for *non-planar* structures, as the activity of cyclic acetals **2** (Fig. 2) is reduced less than 10-fold compared with Epo A.[9,10] In addition, the activity of these analogs is independent of the geometry of the ring fusion (*cis* (**2a**) or *trans* (**2b**)), if C13 is present in the natural *S*-configuration; the corresponding 13*R*-isomers are substantially less potent.[9,10]

Rather surprisingly, these findings have not been followed up in any systematic fashion and little is known at this point about the SAR of bicyclic epothilone analogs related to **2**. In light of this

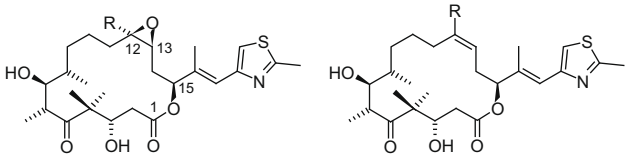

Figure 1.

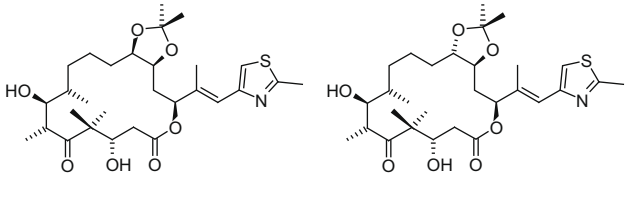

Figure 2.

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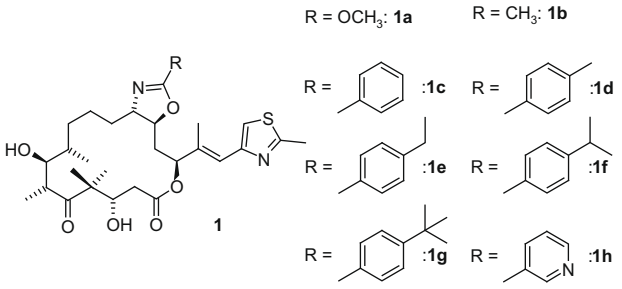
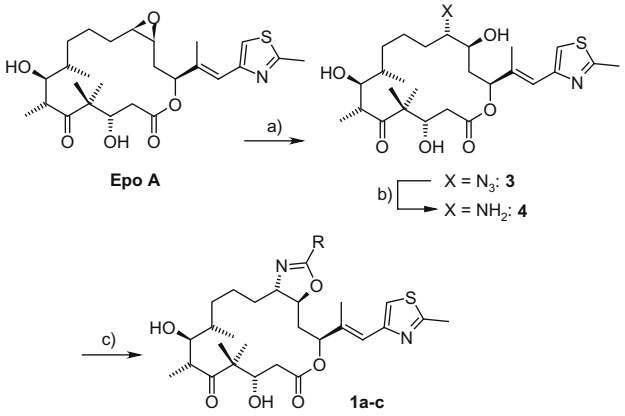


Figure 3.



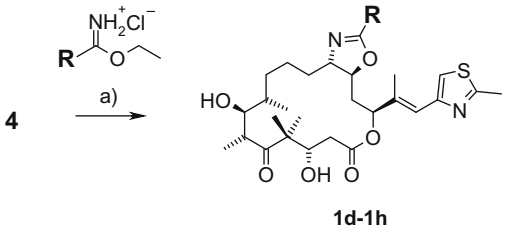
Scheme 1. Reagents and conditions: (a) LiN_3, NH_4Cl, DMF, 85 °C, 24 h, 38%; (b) Ph_3P, THF/H_2O 15/1, rt, 88 h, 50%; (c) compound **1a**: $C(OCH_3)_4$, DCE, 90 °C, 21%. Compound **1b**: $CH_3(OC_2H_5)_3$, DCE, TFA, 90 °C, 23%. Compound **1c**: $PhC(OC_2H_5)_3$, DCE, 90 °C, 69%.

situation and in order to delineate the biological effects of other five-membered heterocycles fused to C12–C13 in a non-planar arrangement and, in particular, to assess the impact of substituents on the five-membered ring, we have embarked on the synthesis and biological evaluation of bicyclic epothilone analogs of the general structure **1** (Fig. 3).

The synthesis of Epo A-derived oxazolines **1** was based on amino alcohol **4** as the central intermediate (Scheme 1). As illustrated in Scheme 1, **4** was obtained through nucleophilic ring-opening of the epoxide moiety in Epo A with azide anion (to produce **3**) and subsequent reduction of the azide group under Staudinger conditions (Ph_3P/H_2O).

The structure of azido alcohol **3** (with a 12-azido group) as the major product of the reaction between Epo A and LiN_3 in DMF in the presence of NH_4Cl (the conditions employed in this work) was firmly established by means of NMR spectroscopy.[11] The regiochemical course of the epoxide opening reaction is thus identical with that reported for the reaction of 12,13-bis-*epi*-Epo A with NaN_3 in EtOH.[12]

The elaboration of amino alcohol **4** into oxazolines **1a-c** involved reaction of this intermediate with commercially available tetramethyl orthocarbonate, triethyl orthoacetate, and triethyl orthobenzoate, respectively. Thus, heating of **4** (either as the free base, for **1b**, or as the hydroacetate, for **1a** and **1c**) produced analogs **1a–c** in yields between 21% and 69% (Scheme 1). Analogs **1d–h** were obtained from amino alcohol **4** (as the hydroacetate) and the appropriate crude imino ester hydrochlorides (prepared from the corresponding nitriles by HCl-catalyzed methanolysis) in refluxing dichloroethane (DCE) in the presence of catalytic amounts of EtOH (Scheme 2; for structures cf. Fig. 3).



Scheme 2. Reagents and conditions: (a) Compound **1d–f**: DCE, EtOH (cat.), 90 °C, 2–6 h, 17–23%. Compound **1g**: DCE, EtOH (cat.), mw, 150 °C, 15 min, 21%. Compound **1h**: DCE, EtOH (cat.), Et_3N, mw, 150 °C, 15 min, 21%.

Using this procedure **1d–h** were obtained in moderate yields (17–23%), but without the need for prior ortho ester preparation (which had proven to be tedious). In the case of **1g** and **1h** microwave heating was employed in an attempt to improve the yield of the desired oxazolines. While no yield improvement could be achieved, microwave heating at least led to significantly reduced reaction times.

Epothilone analogs **1a–h** were evaluated for their ability to induce the assembly of soluble tubulin heterodimers and to inhibit human cancer cell proliferation in vitro. Compounds **1a** and **1b**, which incorporate a small methoxy or methyl substituent at the 2-position of the oxazoline ring, respectively, did not show any profound antiproliferative activity and were not further investigated (IC_{50} values >1 μM against the human cervix carcinoma cell line KB-31; data not shown). In contrast, phenyl derivative **1c** was found to inhibit human cancer cell growth in vitro with IC_{50} values around 20 nM (Table 1); thus, the activity of this analog is within a 10-fold range of the activity of Epo A and it is comparable with the activity of cyclic acetals **2a** and **2b** (Fig. 2; IC_{50} values of 30 nM and 23 nM against the human cervix carcinoma cell line KB-31 have been reported for **2a** and **2b**, respectively.[9] The IC_{50} of **1c** against this cell line is 30 nM). The tubulin-polymerizing activity of **1c** is comparable with that of Epo A, at least under the experimental conditions employed in this work (Table 1). It should be remembered, however, that tubulin polymerization data of the type shown in Table 1 are a relatively crude measure for the interaction of ligands with the tubulin/microtubule system and do not allow to differentiate between compounds with potent tubulin-polymerizing activity.

The introduction of a methyl substituent at the *p*-position of the phenyl moiety leads to a substantial increase in cellular potency and the antiproliferative activity of the corresponding analog **1d** is now comparable with that of Epo A (Table 1). A further increase in the size of the *p*-substituent, however, is associated with a gradual loss in biological potency, at the level of tubulin polymerization as well as cancer cell growth inhibition, such that the *p*-*tert*-butyl phenyl derivative **1g** no longer exhibits any meaningful antiprolif-

Table 1
Tubulin-polymerizing and antiproliferative activity of epothilone analogs **1c–h**

Compound	% Tub-Pol.[a] (5 μM/2 μM)	IC_{50} A549[b] (nM)	IC_{50} MCF-7[b] (nM)
Epo A	89/83	2.2 ± 0.3	2.6 ± 0.3
1c	80/65	16.4 ± 2.7	19.2 ± 1.4
1d	88/71	2.9 ± 0.3	1.9 ± 0.1
1e	36/32	378.5 ± 14.8	297 ± 5.2
1f	15/14	4873 ± 121	5144 ± 207
1g	7/0	>10,000	>10,000
1h	93/78	0.92 ± 0.01	1.3 ± 0.1

[a] Induction of polymerization of porcine brain microtubule protein by 5 μM or 2 μM of test compound relative to the effect of 25 μM Epo B.
[b] Concentration required for 50% growth inhibition of the human lung and breast carcinoma cells lines A549 and MCF-7, respectively (72 h exposure).

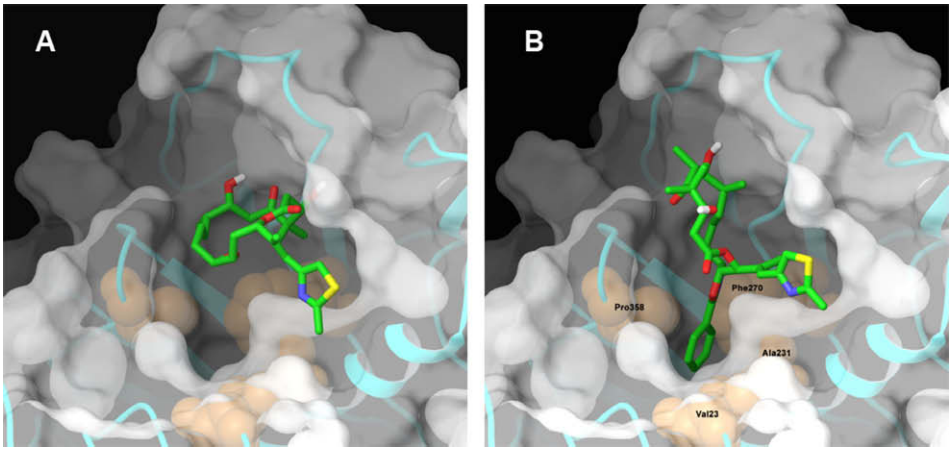


Figure 4. (A) Structure of the taxol/epothilone binding cavity in β-tubulin with bound Epo A, as determined by electron crystallography (1TVK);[14] (B) **1c** docked into β-tubulin of 1TVK using Glide™.[13] For β-tubulin: surface (white), ribbon (cyan), V23, A231, F270, P358 as CPK (brown). For ligand structures: C, green; O, red; N, blue; S, yellow; polar H, white. The figure was produced with Maestro™.[17]

erative activity (Table 1). Likewise, the compound has no significant effect on tubulin assembly, which points to the existence of a spatially restricted, well defined tubulin binding pocket for the phenyl moiety at the 2-position of the oxazoline ring (vide infra).

The most potent analog investigated in this study appears to be pyridine derivative **1h**, whose antiproliferative activity is at least comparable with that of Epo A or analog **1d**. Thus, the replacement of the phenyl ring in **1c** by a 3-pyridyl moiety leads to a >10-fold increase in cellular activity. As would be expected, **1h** is also a potent inducer of tubulin assembly, but the difference in polymerization efficiency between **1c** and **1h** is clearly less pronounced than the difference in antiproliferative activity (at least under the specific conditions of our experiments). It is, therefore, unclear to what extent (if at all) the enhanced cellular activity of **1h** (over **1c**) may be a result of higher affinity interactions with the tubulin/microtubule system (possibly through H-bond formation between the pyridine nitrogen and a donor group on the protein). Other parameters, such as cellular uptake or intracellular distribution might be equally (or even more) important for the difference in cellular potency between **1c** and **1h**.

In an attempt to elaborate a rationale for the observed SAR in the 2-phenyl-oxazoline series at the level of tubulin binding, analogs **1c–g** were docked into the taxol/epothilone binding cavity on β-tubulin using the program Glide™ [13] and the β-tubulin structure that has been derived from an Epo A/β-tubulin complex (as part of a two-dimensional tubulin polymer sheet) by means of electron crystallography (1TVK; Fig. 4A).[14]

For each ligand a set of 100–150 distinct low energy structures were generated by means of MD simulations, energy minimization, and conformational averaging that were individually submitted to the Glide™ [13] docking procedure.[15] The docking itself was restricted by the requirement for a <3 Å deviation between the positions of the thiazole ring in the docked structures and the experimental Epo A/β-tubulin structure 1TVK.[14]

For analogs **1c–f** the lowest energy scores yielded ligand orientations as that depicted in Figure 4B for **1c**, with the phenyl moiety entering into a hydrophobic pocket that is defined by residues V23, A231, F270, and P358 of β-tubulin. The same pocket has been found to be occupied by the C-3′ phenyl group of taxol in a previously reported electron crystallography structure of a taxol/β-tubulin complex.[16] With increasing steric bulk of the *p*-alkyl substituent on the phenyl ring, steric congestion within the pocket would be predicted to increase, thus leading to a gradual loss in binding affinity. If one assumes that the tubulin polymerization data shown in Table 1 are a direct measure of ligand affinity for the tubulin/microtubule system, this is what is experimentally observed. In agreement with this structural hypothesis, and quite intriguingly, the inactive analog **1g** could not be docked into the taxol/epothilone binding pocket on β-tubulin with the aryl group pointing into the hydrophobic cleft that is utilized by **1c–f**.

In conclusion, we have prepared a series of new epothilone analogs with a 2-substituted 1,3-oxazoline ring *trans*-fused to the C12–C13 bond of the deoxy macrocycle. Two of these analogs show cellular potencies that are at least comparable with Epo A, thus confirming and extending previous findings on cyclic acetals **2**. A clear SAR could be discerned in the 2-phenyl oxazoline series with regard to the size of the *para*-substituent and a preliminary structural model has been established that accounts for the observed changes in tubulin polymerization activity. Future work will focus on the investigation of a broader range of substituents on the oxazoline ring and an improved understanding of the interactions of these analogs with tubulin by computational and experimental approaches.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.112.

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