Epothilone A–D and their thiazole-modified analogs as novel anticancer agents*

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Abstract: Starting from epothilone A–D (1a–2b) obtained by large scale fermentation of the myxobacterium Sorangium cellulosum the thiazole side-chain was extensively modified by substitution, oxidation and replacement. Metallation afforded the C-19 carbanion 4 which was quenched by various carbon and heteroatom electrophiles to give C-19 substituted epothilones 5. Thiazole N-oxides 9 were obtained by treatment of 2a and 2b with m-chloroperbenzoic acid and rearranged by acetic anhydride to 21-acetoxy epothilones 10. Cleavage of epothilones A and B with ozone gave methyl ketones 11 from which carbonyl derivatives 12, 13, 14, and aldol condensation products 16 were prepared. Similarly vinyl boronic acid 17 was obtained and transformed by Suzuki coupling or iodination/Stille coupling to aryl and heteroaryl analogs 15.

INTRODUCTION

During the past four years epothilones [1] have attracted great interest amongst biologists and synthetic chemists after it was discovered by Bollag et al. [2] that their cytotoxic properties are based on the same mode of action as described for paclitaxel (Taxol®) [3], i.e. stabilization of microtubules, arrest of mitosis, and programmed cell death (apoptosis). High activity against multidrug resistant tumor cell lines [2,4] and other favourable properties [1,5] soon promoted epothilones into promising anticancer drug candidates.

In the meantime more than a dozen total syntheses have been published [6,7] and a great variety of structural analogs synthesized en route to the natural epothilones A–E (1a–3a) to elucidate the structure/activity relationships [6–8].

First results of in vivo studies on mouse models were reported by Danishefsky et al. In these studies epothilone B (2b) demonstrated efficacy which was however accompanied by serious toxic side effects [9]. In contrast, the significantly less active epothilone D (1b) showed a promising therapeutic range with good activity against sensitive and multidrug resistant tumors up to the point of complete remission [10].

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Scheme 1 Biosynthesis of epothilones A and B (2a and 2b) by epoxidation of epothilones C and D (1a and 1b) and their hydroxylation to epothilones E and F (3a and 3b) by Sorangium cellulosum.
In the search for possible clinically suitable derivatives, we have concentrated our efforts on the isolation of natural structural variants [11] and the chemical modification of the basic epothilones A–D [12] obtained by large scale fermentation of the myxobacterium Sorangium cellulosum. During these fermentations the metabolites are released into the culture medium and can be easily extracted and isolated by chromatography [1,11]. The abundant epothilones A (2a) and B (2b) are obtained in a crystalline state in multigram quantities. Minor constituents are epothilone C and D (1a, 1b), according to feeding experiments the biosynthetic precursors of 2a and 2b [13]. In addition small amounts of epothilone E (3a) and F (3b) are sometimes observed as microbial hydroxylation products of 2a and 2b (Scheme 1) [13].

This report describes the chemical modification of the thiazole side-chain of epothilone A–B (1a–2b) along three lines: C-19 carbanion formation followed by reaction with electrophiles, N-oxidation and rearrangement to C-21 substituted analogs, and cleavage of the C-16/C-17 double bond and introduction of new side-chains.

**METALLATION OF C-19 AND REACTION WITH ELECTROPHILES**

In the course of functional group manipulations we treated epothilone A (2a) with strong bases followed by quenching with D2O. Surprisingly no deuterium was incorporated in α-position of the carbonyl groups nor was significant addition observed of e.g. butyl lithium to the carbonyl or epoxide groups. Instead with 5 equiv. n-butyl lithium in THF at −90°C only the heteroaryl position C-19, and to a small extent the benzylic position C-21, were metallated in addition to the 3-OH and 7-OH groups. After quenching with a variety of electrophiles C-19 substituted epothilones 5 were obtained [12e]. Although only yields of 15–50% of 5 in addition to 20–40% of starting material were achieved after optimization, this one step procedure is extremely valuable in preparing a broad spectrum of analogs for SAR investigations. As all derivatives 5 were essentially inactive in the cytotoxicity cell assay it may be concluded that the conformation of epothilones in the tubulin binding site is restricted to cis for 19-H and 16-Me. For steric reasons this conformation cannot be attained in 5 with R bigger than proton. Only some C-21 alkylated analogs were obtained as by-products of the metallation/quenching procedure and found to be biologically active provided the residue introduced was not too big. Therefore a search was started for more general access to C-21 substituted analogs.

**N-OXIDATION AND POLONOVSKY-TYPE REARRANGEMENT**

The introduction of heteroatoms at C-21 was achieved by an entirely different route discovered by serendipity. When we investigated the outcome of the epoxidation of epothilone C (1a), the last intermediate in some of the ongoing total syntheses of epothilone A (2a) at that time [6], we observed complex reaction mixtures by HPLC and 1H-NMR. Particularly with m-chloroperbenzoic acid in addition to epothilone A (2a), and its β-epoxide isomer 2a’, we identified two stereoisomers of the 16,17-epoxide 6, four stereoisomers of the bis-epoxide 7 and three compounds 8, 9a and 9b with a strong unexpected chromophore (λ<sub>max</sub> = 236 nm) [14,15]. The same chromophore developed when we attempted to transform the C-5 ketone of epothilone A or B into a lactone by Bayer-Villiger oxidation [12a].

From analytical data including 1H, 15N-correlated NMR spectra (δ<sub>N</sub> = 282 ppm, reference liquid NH<sub>3</sub> = 0 p.p.m) the N-oxide structure of 9a was assigned and confirmed by X-ray crystal structure analysis [16]. An advantage is that the N-oxides 9a and 9b can be prepared in 50% isolated yield directly from epothilone A and B and m-chloroperbenzoic acid.
When the N-oxides 9a and 9b were treated with acetic anhydride at 75 °C a rapid Polonovsky-type rearrangement into the 21-acetoxy epothilones 10a and 10b was observed. Cleavage of the acetate esters with dilute ammonia or pig liver esterase gave good yields of epothilone E and F (3a and 3b). Obviously the hydroxymethyl group in 3 can be extensively modified further using standard chemistry.

REPLACEMENT OF THE THIAZONE SIDE-CHAIN

More deep-seated modifications of the side-chain required cleavage of the C-16, C-17 double bond with ozone to give the central intermediate methyl ketone 11a [12c]. From this common carbonyl derivatives, e.g. O-phenyloxime 14, or, after borohydride reduction, benzoate 13 were obtained. After TMS protection of the hydroxyl groups to 11b olefination with Schlosser’s ‘instant ylid’ gave exo-methylene derivative.
12 in moderate yield. However, in spite of extensive experimentation with various olefination methodologies no higher homologue 15 could be produced.

As this failure was presumably caused by enolisation of the methyl keto group we investigated aldol condensations of 11a. Using lithium tetramethylpiperidid various aromatic and heterocyclic aldehydes could be condensed to α,β-unsaturated ketones 16 in good to moderate yields. The heterocycles in 16 were hoped to mimic the thiazole side-chain of epothilone, whereas the N-methyl urocanyl residue in 16a might mimic the side-chain of the tubulin inhibitor eleutherobin [17]. However, none of the analogs 16 showed significant activity in the cell toxicity or tubulin assay.

Finally a Wittig-type condensation of 11b was achieved with a good yield using the less basic bisboryl methyl lithium reagent [18]. The resulting boronic acid 17 (83% of E,Z mixture 7:3) [19] proved to be a valuable intermediate for chain extension reactions. Thus the E-isomer smoothly underwent Suzuki coupling with iodobenzene to phenyl analog 15a or could be converted with iodosuccinimide into vinyl iodide 18 with retention of configuration [20]. The latter, similarly to published procedures [7b], reacted with tributyltin heterocycles under Stille conditions to e.g. thiophene analog 15b.

CONCLUSION

In summary, it could be shown that the thiazolyl side-chain of epothilones can be extensively modified in the presence of the various sensitive functional groups of the macrocycle. In some reactions not even the 3,7-hydroxyl groups need protection. Although yields are moderate and sometimes low, a variety of analogs could be prepared from the fermentation products in very few steps.

The structure-activity relationships for this part of the molecule are in line with published data obtained from analogs prepared by total synthesis. Only few modifications are tolerated without significant loss of activity, i.e. replacement of the thiazole by an oxazole ring or introduction of small substituents at C-21. A more detailed evaluation of the biological properties of these derivatives is in progress.
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REFERENCES


14 The product ratio is largely independent of the reaction temperature in the range of 0–50°C. However, epoxidation with dimethyl dioxirane produces significantly more epothilone A (2a) and its β-epoxide isomer 2a'. Due to the increased electron density of the C-12, C-13 double bond in epothilone D (16) epoxidation to epothilone B (2b) is even more favoured.


19 E and Z isomers were separated by preparative RP-18 HPLC with MeOH/H2O. The configuration was assigned by NOE between 17-H and 16-Me.