Synthesis and Biological Activity of 12-Aza-Epothilones (Azathilones) – Non-Natural Natural Products with Potent Antiproliferative Activity

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§METTLER TOLEDO Awards 2006 (Oral Presentation)

Abstract: 12-Aza-epothilones ('Azathilones') **1** and **2** have been prepared through ring-closing olefin metathesis or macrolactonization-based cyclization reactions. While RCM of the respective dienes **9** and **12** was found to be very effective and produced macrocyclic olefins with high *E* selectivity, the subsequent reduction of the 9,10-double bond proved to be unexpectedly difficult and low-yielding. Preparation of azathilone **2** was also accomplished *via* macrolactonization and this approach was found to be more effective. Compound **2** is a highly potent inhibitor of human cancer cell growth *in vitro*. The activity of this analog is comparable with that of Epo A, both in terms of cytotoxicity against drug-sensitive human cancer cells as well as its tubulin-polymerizing activity. However, in contrast to Epo A, **2** is considerably less potent against multidrug-resistant cancer cells.

Keywords: 12-Aza-epothilones

1. Introduction

Natural products are a unique and highly productive source of lead structures for drug discovery and development, with approximately 50% of currently marketed drug molecules being derived, either directly or indirectly, from naturally occurring substances.[1] Traditionally, natural products-based drug development has principally relied on the immediate exploitation of compounds obtained directly from natural sources (bacteria, fungi, plants) or structurally related semi-synthetic derivatives thereof. More recently, these approaches have been complemented by novel chemistry-based concepts which aim at expanding the structural scope of natural prod-ucts-based drug discovery. Examples are the *de novo* construction of libraries of natural-products-like compounds through diversity-oriented-synthesis (DOS),[2,3] as pioneered by Schreiber, or the design of natural-products-based libraries, a concept introduced by Waldmann in 2002.[4,5] Our own research in the area of natural-products-based lead generation has focused on the development of new biologically active scaffolds through extensive structural modification (rather than simple peripheral derivatization) of existing natural products.[6] Based on our long-standing interest in epothilones as lead structures for anticancer drug discovery (Fig.), this research has also involved the synthesis and biological investigation of different types of aza-epothilones ('azathilones'), which are characterized by the replacement of a backbone carbon atom by nitrogen in the epothilone macrocycle.[6c,d,7] We have referred to the resulting analogs as 'non-natural' natural products,[8,9] as they still retain most of the (two-dimensional) structural features of the natural product lead; at the same time they are structurally unique, as the incorporation of a backbone nitrogen atom places them outside of the general scope of the natural biosynthetic machinery for polyketide synthesis.[10] As a result of these studies we discovered that 12-aza-epothilone (**1**) (Fig.) exhibits significant antiproliferative activity, which is still in the 100 nM range (*i.e.* only 15–50-fold lower than for Epo A).[6d]
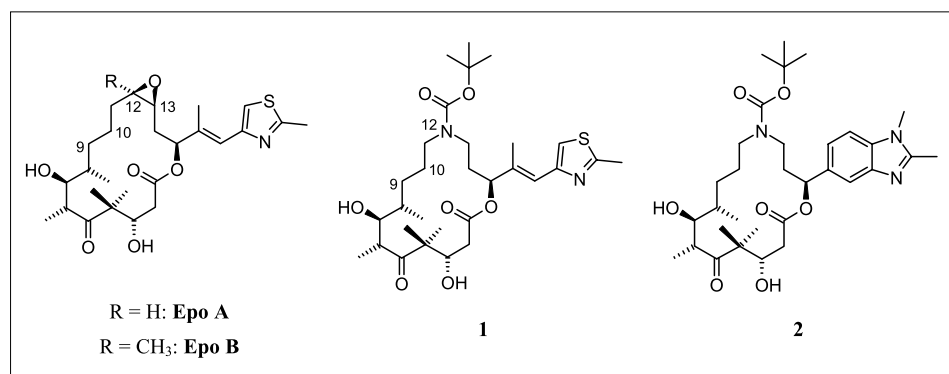
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R = H: **Epo A**
R = CH₃: **Epo B**

Fig.

Unfortunately, our first generation synthesis of **1** and related analogs was relatively inefficient and tedious,[6d] which led us to investigate alternative synthetic routes to this class of compounds. At the same time, we have also investigated the effects of the replacement of the natural epothilone side chain in **1** with a dimethylbenzimidazole moiety, leading to analog **2** as an additional target structure for synthesis (Fig.). For the latter modification we have previously shown that it produces a significant enhancement in antiproliferative activity in combination with the natural epothilone macrocycle.[6a,b,11]

2. Results and Discussion

Macrocycle formation through ring-closing olefin metathesis (RCM) featured as a particularly attractive approach to the target azathilones **1** and **2**, as it would also provide specific unsaturated analogs (as the immediate cyclization products), which could be interesting new antiproliferative agents in their own right.[12] As shown in Schemes 1 and 2, our RCM-based synthesis of target structure **1** and its 9,10-didehydro derivative **10** involved three key strategic steps, namely

i)   the stereoselective aldol reaction between aldehyde **3**[13] and ketone **4**[14] (*dr* = 8:1) (Scheme 1),

ii) esterification of carboxylic acid **7** with the unsaturated alcohol **8**,[15] (Scheme 2) and

iii) RCM with diene **9**.

Initial attempts to cyclize **9** employing the first-generation Grubbs catalyst[16] met with complete failure and no conversion was observed. In contrast, the use of the dihydroimidazol-2-ylidene-based second-generation catalyst[16] produced the cyclic olefin in good yield (68%) and with high *E* selectivity (>20:1). Similar observations were made in the cyclization of diene **12**, which gave macrolactone **13** in excellent yield and with exclusive *E* selectivity (Scheme 3). No trace of the corresponding *Z* product could be isolated. Unfortunately, the efficiency of the cyclization reaction in both cases was thwarted by serious difficulties with the subsequent reduction of the 9,10-double bond, which proved to be extremely sluggish under all experimental conditions investigated (thus leading to low yields and also side reactions such as reductive ester cleavage with H_2/Pd-C without reduction of the double bond in the case of **13**). The most effective method for transforming **10** and **13** into **1** and **2**, respectively, involved the use of *in situ* generated diimide[17] (Schemes 2 and 3). However, even under these conditions **1** was obtained in only 15% isolated yield (30% based on recovered starting material) at 40% conver-
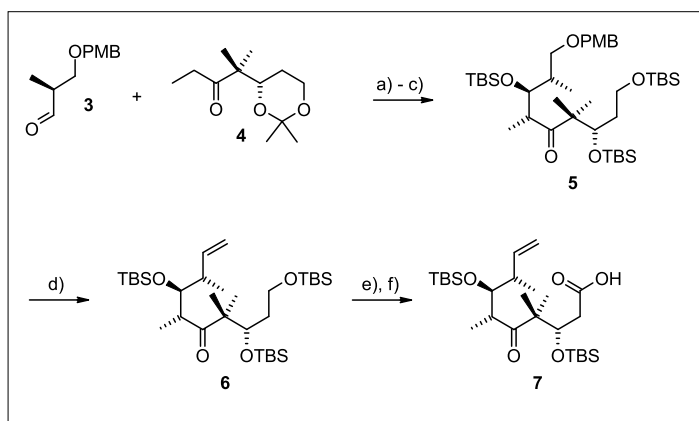
Scheme 1. a) **4**, LDA, −78 °C, 5 h, then addition of **3**, −90 °C, 75 min, 76%, *dr* = 8:1; b) PPTS, MeOH, RT, 20 h, 86%; c) (i) TBSOTf, 2,6-lutidine, −78 °C → RT, 1.5 h; (ii) flash chromatography, 76%; d) (i) H_2/Pd-C, MeOH, RT, 20 h; (ii) TPAP, NMO, 4-Å MS, CH_2Cl_2, RT, 1 h; (iii) MePPh$_3$Br, LiHMDS, THF, 0 °C, 1.5 h, 79% (three steps); e) CSA (1.0 equiv.), CH_2Cl_2/MeOH 1:1, 0 °C, 1 h, 87%; f) PDC (11 equiv.), DMF, RT, 64 h, 85%.
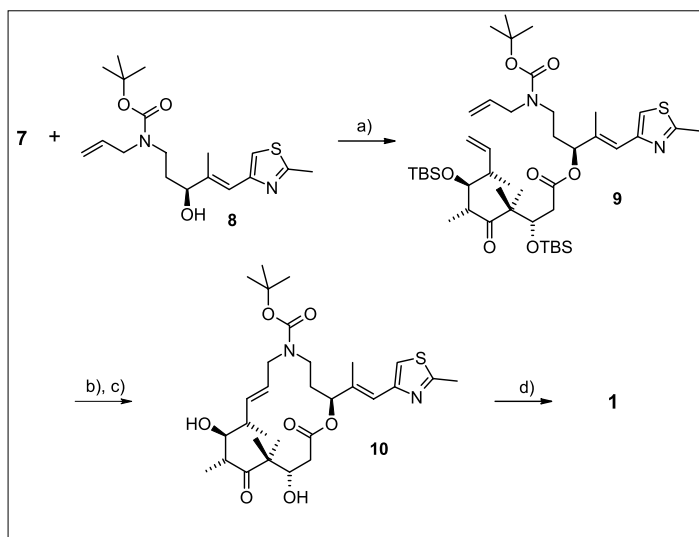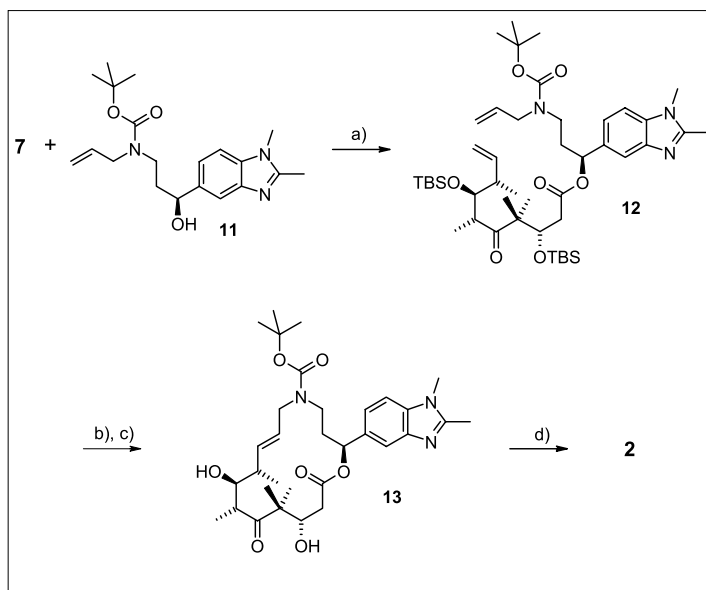

Scheme 2. a) **8** (1.2 equiv.), DCC (1.3 equiv.), DMAP (0.3 equiv.), CH_2Cl_2, 0 °C, 30 min, RT, 6 h, 77%; b) 2nd generation Grubbs catalyst (0.09 equiv., incremental addition), CH_2Cl_2, refl., 24 h, 68% (pure *E* isomer); c) HF•pyridine, pyridine, THF, RT, 4.5 h, 65%; d) KO$_2$C-N=N-CO$_2$K (excess), AcOH, CH_2Cl_2, refl., 15% (30% based on recovered starting material); pure **1** obtained through purification by preparative HPLC.

sion and after purification by preparative HPLC. Macrocycle **2** was obtained in 31% yield from **13** after HPLC purification.

The above RCM approach provided sufficient amounts of **2** for initial biological testing, but it was clear that more extensive profiling of this compound (including, perhaps, *in vivo* studies) would require the development of an alternative synthetic route, in order to facilitate the preparation of larger quantities of material. In light of highly promising early biological data (*vide infra*), we thus embarked on the elaboration of an alternative route to **2** that would be based on macrocyclization through ester

bond formation rather than RCM. As illustrated in Scheme 4, this approach employed the reductive amination of aldehyde **16** with amine **15** (obtained in three steps from the known protected tetrol **14**[14a]) to assemble the hetero-aliphatic skeleton of **2**. As the highly polar reductive amination product was difficult to purify, it was directly converted to the corresponding N-*tert*-butoxycarbonyl derivative (60% yield, based on amine **15**). Selective cleavage of the primary TBS-ether with CSA, oxidation of the resulting free alcohol with PDC, and removal of the TBS-protecting group from C^{15}-O with TBAF furnished *seco*

Scheme 3. a) **11** DCC (1.2 equiv.), DMAP (0.3 equiv.), CH$_2$Cl$_2$, 0 °C, 15 min, RT, 15 h, 60%; b) 2nd generation Grubbs catalyst (0.15 equiv., incremental addition), CH$_2$Cl$_2$, refl., 8 h, 85%; c) HF•pyridine, pyridine, THF, RT, 4 h, 70%; d) KO$_2$C-N=N-CO$_2$K (excess), AcOH, CH$_2$Cl$_2$, refl., 31%; pure **2** obtained through purification by preparative HPLC.
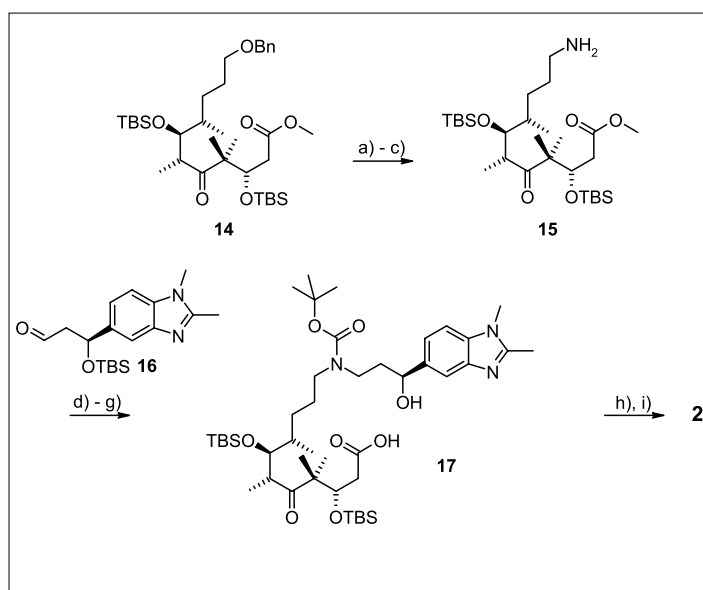
Scheme 4. a) H$_2$/Pd-C, EtOAc, RT, 62 h, 86%; b) HN$_3$, DEAD, PPh$_3$, THF, 0 °C, 25 min, RT, 30 min, 96%; c) H$_2$/Pd-C, MeOH, RT, 3 h, 92%; d) (i) **16** (1.1 equiv.), NaBH(OAc)$_3$ (1.6 equiv.), AcOH (2.0 equiv.), 4-Å MS, RT, 2.5 h; (ii) BOC$_2$O, Et$_3$N, THF, 0 °C, 45 min, 60% (two steps); e) CSA (1.1 equiv.), CH$_2$Cl$_2$/MeOH 1:1, 0 °C, 3 h, 80%; f) PDC (15 equiv.), DMF, RT, 24 h, 50%; g) TBAF (6 equiv.), THF, RT, 24 h; h) 2,4,6-Cl$_3$C$_6$H$_2$C(O)Cl, Et$_3$N, THF, 0 °C, 20 min, then diluted with toluene and added to a solution of DMAP in toluene, 75 °C, 1 h, 44% (two steps); i) HF•pyridine, pyridine, THF, RT, 2.5 h, then preparative HPLC, 40%.

acid **17**. Cyclization of **17** under Yamaguchi conditions[18] produced fully protected **2** (44% based on C^{15}-O-TBS-protected **17**). Subsequent selective removal of the TBS-protecting groups with HF•pyridine gave target structure **2** in 40% yield (after HPLC purification).

As demonstrated by the data summarized in the Table, azathilone **2** is a highly potent antiproliferative agent, which inhibits the growth of different types of drug-sensitive human cancer cell lines (A549, HCT-116, PC-3M, KB-31) with low nM IC$_{50}$-values. The antiproliferative activity of the compound against these cell lines is thus comparable with that of Epo A. Similarly, **2** induces tubulin polymerization *in vitro* with similar potency as Epo A (Table), which strongly suggests that inhibition of human cancer cell proliferation by **2**, as for natural epothilones, is a consequence of interference with microtubule functionality. This view is also in line with the fact that treatment of cancer cells with azathilone **2** results in cell cycle arrest at G2/M,[8] which mirrors the effects on the cell cycle observed upon treatment with Epo A or B.[7] Compound **2** is >60-fold more potent against drug-sensitive human cancer cells than the corresponding parent (natural side-chain-containing) azathilone **1** (Fig.); this potency increase dramatically exceeds the potency-enhancing effects previously observed for the dimethylbenzimidazole side chain in combination with polyketide-based macrocycles (2–15-fold).[6a,b,11] Equally in-

triguing is the observation that compounds **10** and **13**, both of which incorporate a *trans* double bond between C9 and C10, are significantly less potent than the fully saturated azathilones **1** and **2**, respectively (both at the level of tubulin polymerization as well as cellular activity; Table, data for **10** not shown). These findings are in marked contrast to the effects observed for Epo B and D, where the introduction of a *trans* double bond between C9 and C10 results in enhanced (!) cellular potency,[17] and they may be indicative of differences in the bioactive conformation between azathilone-type analogs and natural epothilones.[19]

Unfortunately, azathilone **2** is significantly less potent against the multidrug-re-

sistant cervix carcinoma cell line KB-8511 than the drug-sensitive parental KB-31 line, which indicates that **2** is a substrate for the P-gp efflux pump. However, we have recently shown that the susceptibility of polyketide-based epothilone analogs to P-gp-mediated drug efflux can be modulated through adjustments in compound lipophilicity [20] and this strategy will also be explored for lead structure **2**.

3. Conclusions

In summary, we have achieved the total synthesis of two representative examples of a new class of highly potent microtubule-

Table. Tubulin-polymerizing and antiproliferative activity of azathilones **1**, **2**, and **13**

Compound	EC$_{50}$ Tubulin polymerization [μM]^a	IC$_{50}$ [nM]^b				
		A549	HCT-116	PC-3M	KB-31	KB-8511
1	5.6 ± 0.4	130 ± 24	110 ± 19	126 ± 22	31	105
2	3.9 ± 0.6	1.9 ± 0.4	1.6 ± 0.5	2.3 ± 0.6	0.34 ± 0.15	222 ± 48
13	9.1 ± 0.7	920 ± 85	1009 ± 71	973 ± 64	ND^a	ND^c
Epo A	4.6 ± 0.5	3.2 ± 0.5	2.2 ± 0.3	3.4 ± 0.4	2.15	1.91

^{a}Concentration required to induce 50% of the maximum tubulin polymerization achievable with the respective compound (10 μM of porcine brain tubulin). ^{b}IC$_{50}$-values for human cancer cell growth inhibition. KB-31, KB-8511: cervix; A549: lung; HCT-116: colon; PC-3M: prostate. KB-8511 is a P-glycoprotein 170 (P-gp170)-overexpressing multidrug-resistant subline of the KB-31 parental line. ^{c}Not determined. Data are from ref. 8.

stabilizing agents, which are based on an aza-macrolide backbone and which we have termed azathilones. While the conception of these compounds is closely connected to the structure of natural epothilones (hence the name 'azathilones'), given the degree of structural divergence from the natural epothilone template, in particular in the case of **2**, they may be considered as members of a distinct group of 'non-natural' natural products with unique structural features and, as indicated by some preliminary SAR data, a unique SAR profile.

Received: February 2, 2007

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