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C-15 Thiazol-4-yl Analogues of (*E*)-9,10-Didehydroepothilone D: Synthesis and Cytotoxicity

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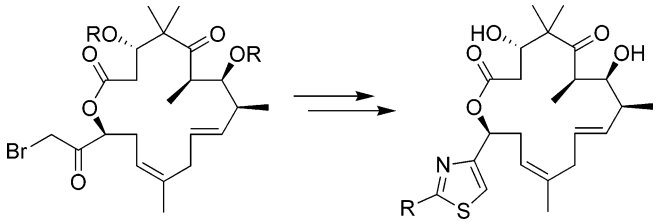
ABSTRACT



The syntheses and biological evaluation of six epothilone D analogues are reported. These side-chain variants of the (*E*)-9,10-didehydroepothilone scaffold contain C-15 thiazole appendages that are derived from bromomethyl ketone intermediates. Although each of these analogues is less cytotoxic than the parent (*E*)-9,10-didehydroepothilone D, three maintain IC_{50} values in the double-digit nanomolar range against both susceptible and resistant cell lines.

The epothilones are naturally occurring polyketides that exert antiproliferative activity through the stabilization of microtubules, a mode of action shared with paclitaxel.[1] Unlike the taxanes, however, the epothilones maintain potency vs multidrug resistant cell lines.[2] The intense scientific research that followed this finding led to the development of an extensive epothilone SAR[3] and, ultimately, to the evaluation of several analogues as anticancer agents in human clinical trials.[4]

During the search for analogues with improved therapeutic properties, two noteworthy aspects of the epothilone SAR were uncovered. First, Danishefsky and co-workers discovered that installation of a C-9−C-10 (*E*)-olefin on the epothilone scaffold increased cytotoxicity against several human cancer cell lines by approximately 5−10-fold relative to the parent compound.[5] This minor structural change was also associated with increased aqueous solubility and plasma stability as well as improved in vivo antitumor activity.[6] Second, we,[7] as well as Altmann,[8] noted that replacement of the epothilone C-15 side chain with aryl groups resulted in compounds that maintained potent cytotoxicity. These two findings served as the basis of our own program to identify structurally novel epothilone analogues with improved pharmacokinetic profiles.

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The C-15 thiazol-4-yl-9,10-didehydroepothilone D analogues described herein were designed to evaluate C-15 thiazole replacements of the C-16−C-17 trisubstituted olefin of **1** and are readily available via modifications to the Danishefsky total synthesis of **1** (Figure 1).[5a] The simplest

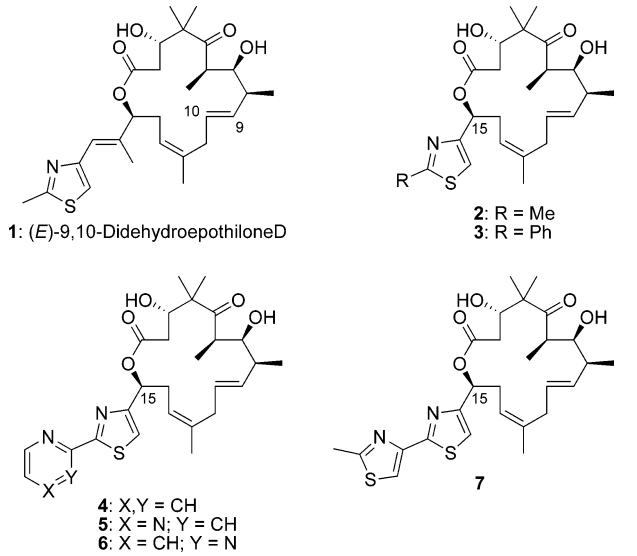
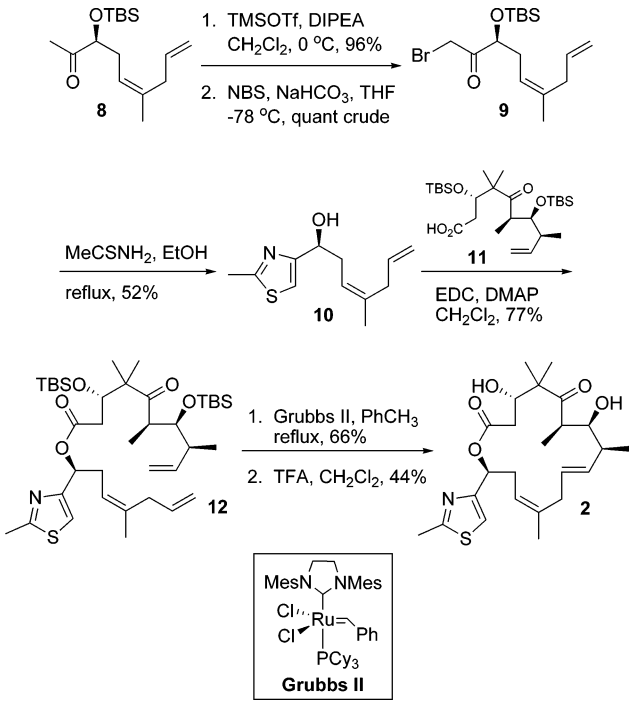


Figure 1. Structures of (*E*)-9,10-didehydroepothilones.

of these analogues (**2**) contains a 2-methylthiazol-4-yl substituent attached directly to the C-15 position of the epothilone scaffold.[9] This structural alteration results in not only a reduction of the steric bulk of the side chain relative to **1** but also a repositioning of the thiazole basic nitrogen. Functionalization at the 2-position of the thiazole can be used to simply increase the steric bulk at C-15 as in **3** or to probe the hydrogen bonding requirements of the side chain as well (**4**−**7**). Nicolaou and co-workers have reported that appropriate positioning of a side-chain basic nitrogen is vital to the cytotoxic potency of epothilone B analogues.[10,11] Furthermore, a recent model of epothilone A binding to tubulin reported by Snyder and Downing, based in part on the Nicolaou work, suggests that the thiazole nitrogen of the natural product may accept a hydrogen bond from His227 of β-tubulin.[12] In hopes of preserving this interaction, the four biaryl analogues (**4**−**7**), each of which contains a nitrogen-bearing distal ring, were targeted for total synthesis.

These epothilone analogues were generated by two distinct strategies that rely on the formation of C-15 bromomethyl ketones derived from intermediates in the Danishefsky total synthesis of 9,10-didehydroepothilone D (**1**).[5a] The first approach involves an early-stage functionalization of the relatively simple methyl ketone **8**. By conserving the more complex fragment **11**, this route is valuable for material throughput of a selected candidate (Scheme 1). The second

Scheme 1. Synthesis of C-15-(2-Methylthiazol-4-yl)-9,10-didehydroepothilone D (**2**)



strategy is characterized by a late-stage divergence, useful for multiple analogue generation, as detailed in Scheme 2.

The synthesis of analogue **2** as described in Scheme 1 required conversion of methyl ketone **8** to its bromomethyl counterpart **9**. This two-step procedure utilized silyl enol ether formation with TMS triflate followed by NBS-promoted electrophilic bromination. The reaction of the resulting bromomethyl ketone (**9**) with thioacetamide generated the desired thiazole with concomitant removal of the TBS protecting group. This cyclization product (**10**) was acylated with carboxylic acid **11**[5a] to provide the desired ester **12**, which was then treated with Grubbs' second-generation ruthenium catalyst[13] to afford the intermediate macrocycle. Final acidic deprotection yielded the desired C-15 thiazole analogue **2** by a synthetic route that was beneficial for the development of our synthetic strategy, namely, bromination and cyclization, but was not optimal for the synthesis of multiple analogues.

A more efficient route for the rapid generation of structural diversity is detailed in Scheme 2. Because macrocyclic

(9) C-15-(2-Methylthiazol-4-yl)epothilone D is a known compound with micromolar cytotoxicity: Su, D.-S.; Balog, A.; Meng, D.; Bertinato, P.; Danishefsky, S. J.; Zheng, Y.-H.; Chou, T.-C.; He, L.; Horwitz, S. B. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2093.

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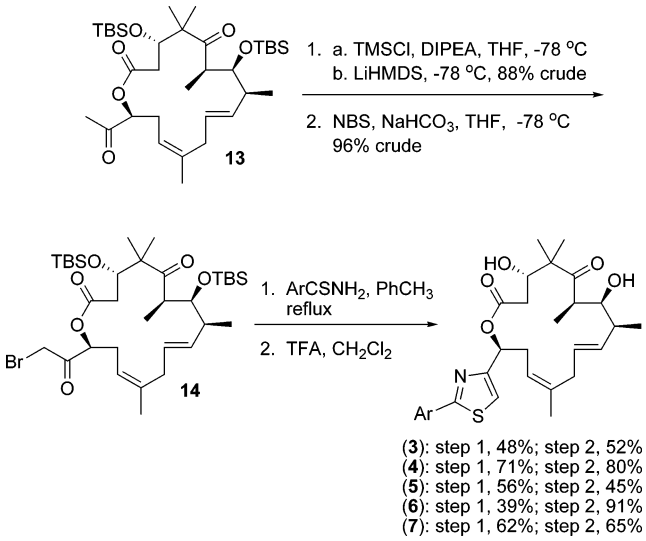
Scheme 2. Synthesis of C-15-(2-Arylthiazol-4-yl)-9,10-didehydroepothilone D Analogues (**3**−**7**)



(**3**): step 1, 48%; step 2, 52%
(**4**): step 1, 71%; step 2, 80%
(**5**): step 1, 56%; step 2, 45%
(**6**): step 1, 39%; step 2, 91%
(**7**): step 1, 62%; step 2, 65%

methyl ketone **13**[5a] contains three enolizable carbonyl groups, a modified bromination protocol was required to achieve high levels of selectivity. Treatment of macrocycle **13** with the kinetic base LiHMDS resulted in selective deprotonation and subsequent silylation of the methyl ketone. After exposing the intermediate silyl enol ether to NBS, the resulting bromomethyl ketone **14** was treated with a series of aromatic thioamides to provide intermediate thiazoles in modest to good yield. These silyl protected intermediates were then desilylated to afford the targeted C-15 thiazole analogues. Using this strategy, the syntheses of analogues **3**−**7** required four steps each from known ketone **13** but only two steps from the C-15 bromomethyl ketone common intermediate **14**.

Analysis of the cytotoxicity data presented in Table 1 provides several conclusions. Three members of this series (**4**, **6**, and **7**) are potent cytotoxins, having IC_{50} values in the double-digit nanomolar range. These results indicate that the C-15 thiazole is a reasonable, but not optimal, replacement of the C-16−C-17 olefin (specifically, **1** vs **7**). Compounds **2** and **3** demonstrate substantially diminished cytotoxicity relative to **1**. This loss of potency is attributed to the absence of an appropriately positioned side-chain basic nitrogen but not simply to variations in the size of the C-15 side chain (compare **1**−**4**). Little change in the level of cytotoxicity results from the incorporation of a redundant nitrogen in the C_2 symmetric pyrimidine (pyridine **4** vs pyrimidine **6**), but the second, non-C_2 symmetric nitrogen of pyrazine **5** is clearly detrimental. Although each of the more promising biaryl analogues (**4**, **6**, and **7**) is a less potent cytotoxin in vitro than either epothilone D or 9,10-didehydroepothilone D (**1**), these compounds retain antiproliferative potency in the NCI/ADR multidrug resistant cell line.

Table 1. Cytotoxicity of C-15-Thiazole-9,10-didehydroepothilone D Analogues

	cell line, IC_{50} (nM)			
compd	MCF-7	NCI/ADR	A549	SKOV-3
Epo D	4	10	6.6	12
1	0.6	3	1.3	4.9
2	380	510	470	750
3	220	380	450	690
4	40	87	53	49
5	52	210	200	320
6	34	68	40	58
7	38	55	49	68

In conclusion, we have utilized bromomethyl ketone intermediates for the synthesis of epothilone analogues containing substituted C-15 thiazole side chains. This synthetic strategy could be easily adapted, however, to the synthesis of various other side chains including imidazoles and oxazoles. The thiazole-containing analogues described herein retain potent cytotoxicity, with the potential for improved pharmacokinetic properties.

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Supporting Information Available: Experimental procedures and characterization data, including copies of ^1H and ^{13}C NMR spectra, are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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