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Design and Synthesis of a Potent Phorboxazole C(11–15) Acetal Analogue

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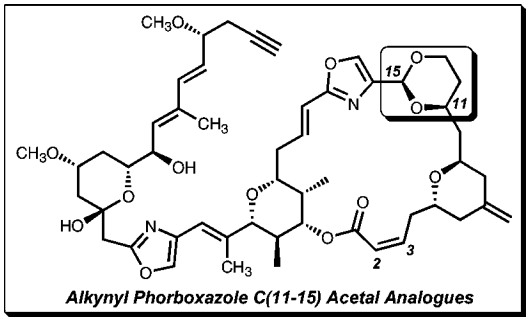
ABSTRACT



We disclose here the design, synthesis, and biological evaluation of simplified *Z*- and *E*-C(2–3) alkynyl phorboxazole C(11–15) acetals (+)-7*Z* and (+)-7*E*, wherein the *Z*-isomer proved to be a potent nanomolar cytotoxic agent. Reevaluation of (+)-C(45–46) *E*-chloroalkenyl phorboxazole A (6) confirms subnanomolar activity across a broad panel of human cancer cell lines.

(+)-Phorboxazole A and B (**1** and **2**), two architecturally complex cytotoxic macrolides, isolated by Searle and Molinski in 1995, have attracted considerable attention from the synthetic community.[1,2] However, because of their low natural as well as synthetic availability, biological studies aimed at defining their mechanism of action and/or cellular targets remain seriously impaired. In conjunction with our second generation total synthesis of (+)-phorboxazole A (**1**),[2f] we recently disclosed SAR studies which identified the C(45−46) alkynyl (**3**),[3] alkenyl (**4**), alkyl (**5**), and *E*-chloroalkenyl (**6**) analogues as highly potent congeners, with activity greater than (+)-phorboxazole A (**1**) in several human cancer cell lines (Figure 1).[4] Consequently, our goal became the design and synthesis of congeners also possessing a simplified, more readily constructed macrocyclic domain, wherein the C(11−15) tetrahydropyran is replaced with a conformationally similar acetal, a tactic exploited to great advantage by the Wender bryostatin program.[5] This goal has now been achieved (vide infra). Moreover, reevaluation of the C(45−46) *E*-chloroalkenyl congener (**6**) reveals subnanomolar activity in several human cancer cell lines, rendering **6** to be one of the most potent cytotoxic agents known to date.[6]

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(1) (a) Searle, P. A.; Molinski, T. F. *J. Am. Chem. Soc.* **1995**, *117*, 8126. (b) Searle, P. A.; Molinski, T. F.; Brzezinski, L.; Leahy, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 9422.

(2) (a) Forsyth, C. J.; Ahmed, F.; Cink, R. D.; Lee, C. S. *J. Am. Chem. Soc.* **1998**, *120*, 5597. (b) Evans, D. A.; Fitch, D. M.; Smith, T. E.; Cee, V. J. *J. Am. Chem. Soc.* **2000**, *122*, 10033. (c) Smith, A. B., III; Minbiole, K. P.; Verhoest, P. R.; Schelhaas, M. *J. Am. Chem. Soc.* **2001**, *123*, 10942. (d) González, M. A.; Pattenden, G. *Angew. Chem., Int. Ed.* **2003**, *42*, 1255. (e) Williams, D. R.; Kiryanov, A. A.; Emde, U.; Clark, M. P.; Berliner, M. A.; Reeves, J. T. *Angew Chem., Int. Ed.* **2003**, *42*, 1258. (f) Smith, A. B., III; Razler, T. M.; Ciavarri, J. P.; Hirose, T.; Ishikawa, T. *Org. Lett.* **2005**, *7*, 4399.

(3) The C(45-46) alkynyl congener (**3**), first reported by the Forsyth laboratory, was shown to have potent cytotoxicity. Uckun, F. M.; Forsyth, C. J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1181.

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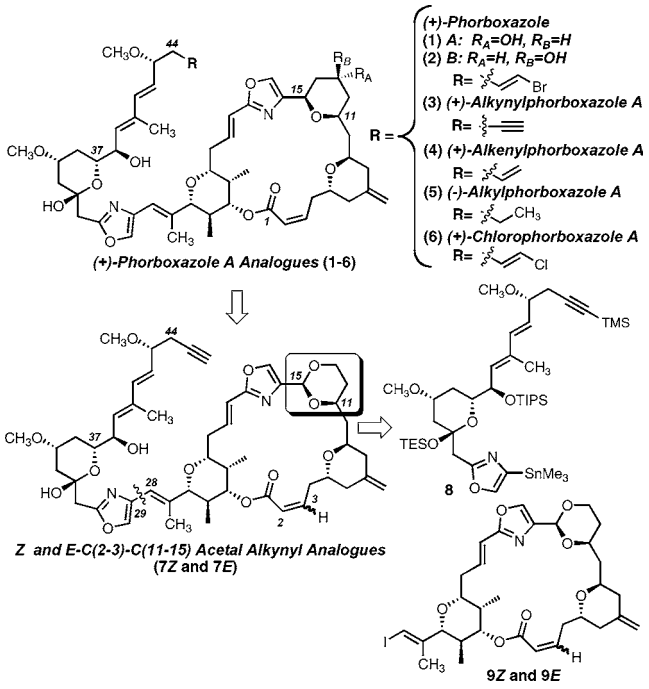
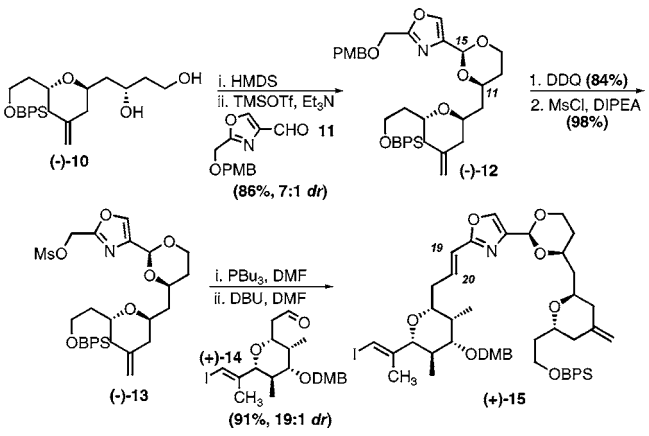


Figure 1. Phorboxazole analogues.

The *Z*- and *E*-C(2−3)-C(11−15) acetal alkynyl analogues (**7***Z* and **7***E*) were envisioned to arise via Stille union of the advanced alkynyl side chain stannane **8** and the corresponding *Z*- and *E*-macrocycles **9***Z* and **9***E*, similar to the strategy employed in our second generation total synthesis of (+)-phorboxazole A.[2f]

We began with construction of the vinyl iodides **9***Z* and **9***E* exploiting the TMSOTf-promoted condensation of the silylated diol derived from (−)-**10** with oxazole aldehyde **11** (Scheme 1) to furnish (−)-**12** as a separable diastereo-

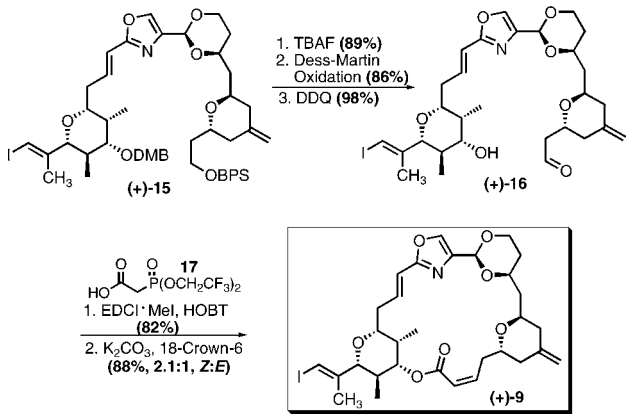
Scheme 1



meric mixture at C(15) (ca. 7:1).[7] Removal of the *p*-methoxybenzyl (PMB) group with DDQ, followed by mesylation, afforded tricycle (−)-**13** in 82% yield (two steps). A one-flask Wittig salt formation/olefination,[2b] involving exposure of (−)-**13** to tri-*n*-butylphosphine, followed by introduction of aldehyde (+)-**14** and DBU generated the C(19−20) *E*-olefin (+)-**15** in 91% yield with excellent configurational control (ca. 19:1, *E*/*Z*).

Completion of macrocycles **9** entailed removal of the *tert*-butyldiphenylsilyl (BPS) group (TBAF), Dess−Martin oxidation, and removal of the 3,4-dimethoxybenzyl (DMB) group (DDQ) to furnish alcohol (+)-**16** in 75% yield (three steps; Scheme 2). Union with phosphonate acid **17** promoted

Scheme 2



by EDCI·MeI/HOBT, followed by an intramolecular Stille modified Horner−Emmons olefination, led to (+)-**9** in 88% yield as a mixture (2.1:1, *Z*/*E*), which proved readily separable via column chromatography (only the *Z*-isomer is shown).[8]

Recognizing that acidic hydrolysis of a C(33) mixed methyl acetal, as employed in our recently reported phorboxazole analogue synthesis, would prove problematic given the C(11−15) acetal functionality,[4] we opted to protect this position as the triethylsilyl (TES) ether.[2b] Global deprotection employing a fluoride source would then permit the C(11−15) acetal to remain intact.

Construction of the requisite stannyl side chain began with addition of the Grignard reagent derived from oxazole **19** to dienyl-lactone (−)-**18** to furnish the corresponding *hemi*-acetal (Scheme 3). Protection as the corresponding TES ether (buffered TESOTf) proceeded in modest yield. Final elaboration of the stannyl side chain (−)-**8** entailed a palladium-catalyzed exchange of the oxazole enol-triflate for trimethylstannane; the overall yield for the three steps was 30%.

Pleasingly, Stille union of both the *Z*- and *E*-acetals (**9***Z* and **9***E*) with side chain (−)-**8** furnished the corresponding *Z*- and *E*-macrocycles in good yield (Scheme 3). Global deprotection employing 4 equiv of tetrabutylammonium

(6) Previous biological screening of (+)-**6** on two occasions revealed subnanomolar activity across the panel of human cancer cell lines; however, one assay resulted in only low nanomolar activity. This discrepancy is now known to be due to sample degradation.

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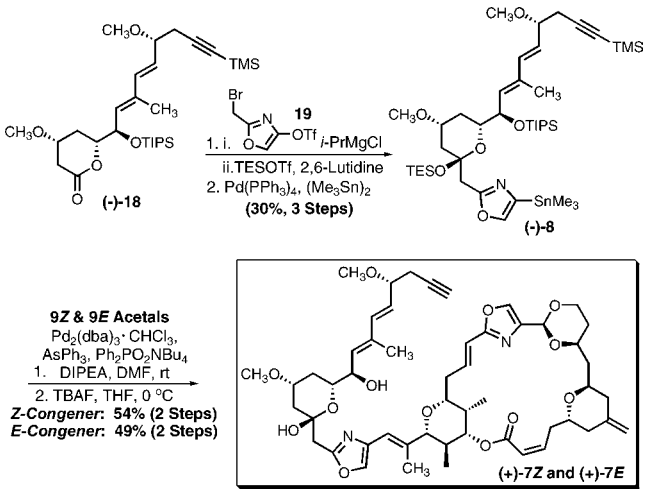
Scheme 3



fluoride (TBAF) removed the protecting groups to furnish alkynyl phorboxazole analogues (+)-**7***Z* and (+)-**7***E* in 54% and 49% yields, respectively (Scheme 3, only the *Z*-isomer is shown).

Biological evaluation of (+)-**7***Z* and (+)-**7***E*, along with the previously reported C(45-46) alkyl phorboxazole A (−)-**5** as a control and the *E*-chloroalkenyl congener (+)-**6**, against a panel of six human tumor cancer cell lines, [BXP-3 (pancreatic), MCF-3 (breast), F-268 (CNS), NCI−H460 (nonsmall lung), KM20L2 (colon), and DU-145 (prostate)] revealed impressive levels of cytotoxicity (Table 1). The

Table 1. Human Cancer Cell Line Screening

GI_{50}=nM						
C(45-46) Sidechain R=	BXP-3	MCF-3	F-268	NCI-H460	KM20L2	DU-145
 (-)-5	5.6	5.8	5.1	4.1	3.1	8.1
 (+)-6	0.62	1.7	0.49	0.64	0.38	2.5
 *Z* -Acetal (+)-7*Z*	31.2	18.3	44.1	27.9	11.8	74.2
E-Acetal (+)-7*E*	>1076	883	560	>1076	990	>1076

control C(45−46) alkyl analogue (−)-**5**, as previosuly reported, displayed an average GI_{50} value of 5.3 nM across the cancer cell panel. Importantly, the simplified, *Z*-acetal analogue (+)-**7***Z* displayed significant activity across the six cell lines with potent activities of 11.8 and 18.3 nM, respectively, against the KM20L2 and MCF-3 cell lines. As expected from our earlier results, the isomeric *E*-acetal analogue (+)-**7***E* was significantly less active. The enhanced activity of the *Z*-acetal congener (+)-**7***Z* clearly reinforces both the importance of the *Z*-geometry imparted by the macrocyclic enoate and the ability of the acetal to mimic the conformation of the (+)-phorboxazole A C(11−15) tetrahydropyran. Equally pleasing, the C(45−46) chloroalkenyl congener (+)-**6** displayed subnanomolar activity when screened against the six cancer cell lines. These biological data place (+)-**6** within the potency range of the spongistatins,[9] bryostatins,[10] and halichondrins[11] as one of the most cytotoxic agents known to date.

In summary, we have achieved the design, synthesis, and biological evaluation of the simplified *Z*- and *E*-phorboxazole acetals (+)-**7***Z* and (+)-**7***E*, possessing the terminal C(45−46) side chain alkyne. The *Z*-isomer proved to be a potent cytotoxic agent, due presumably to the ability of the C(11−15) acetal to mimic the conformation imparted by the corresponding tetrahydropyran functionality in (+)-phorboxazole A (**1**). Importantly, (+)-**7Z** represents the first macrocycle-modified phorboxazole analogue to retain potent tumor cell growth inhibitory activity. Reevaluation of (+)-**6**, the C(45−46) *E*-chloroalkenyl congener of phorboxazole A, revealed subnanomolar activity across a broad panel of human tumor cell lines. Studies to identify additional simplified side chain/macrocycle constructs, which possess significant cytotoxic activity, as well as a campaign to secure large quantities of the chloro congener (+)-**6** by total synthesis in anticipation of further biological studies continue in our laboratory.

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Supporting Information Available: Experimental data and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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