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(+)-Phorboxazole A Synthetic Studies. Identification of a Series of Highly Cytotoxic C(45–46) Analogues

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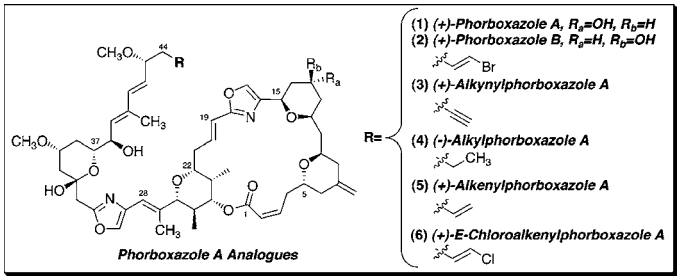
ABSTRACT



Effective, scalable total syntheses and biological evaluation of six phorboxazole A analogues (1–6) have been achieved. Importantly, the C(45–46)-saturated, C(45–46)-alkenyl, and the C(45–46)-*E*-chloroalkenyl congeners (4, 5, and 6, respectively) reveal low nanomolar tumor cell growth inhibitory activity (GI_{50}'s) similar to or, in some cell lines, greater than that of the phorboxazoles across a diverse panel of human cancer cell lines.

Since the isolation of (+)-phorboxazoles A and B (**1** and **2**) in 1995 by Searle and Molinski,[1] five total syntheses,[2] in conjunction with several synthetic approaches targeting these architecturally complex marine natural products, have been reported. Not withstanding these efforts, both the low natural and synthetic availability of the phorboxazoles remains a serious impediment, both to the identification of their precise cellular targets and/or mode of biological action, as well as to clinical development. Thus, the design and synthesis of structurally simplified congeners, possessing significant bioactivity and availability in useful quantities, remains an important goal for both the synthetic and cancer research communities.

In a seminal study, Forsyth identified several (+)-phorboxazole A structural motifs required for retention of high tumor cell growth inhibitory activity.[3] Screening against NALM-6 (leukemia), BT-20 (breast), and U373 (brain) human cancer cell lines, the C(45−46)-alkyne (+)-**3** (Scheme 1) displayed GI_{50} values of 4.8, 12.6, and 27.4 nM, respectively, while the C(33)−(OCH_3) congener revealed values of 5.2, 11.3, and 29.2 nM, respectively. Of equal

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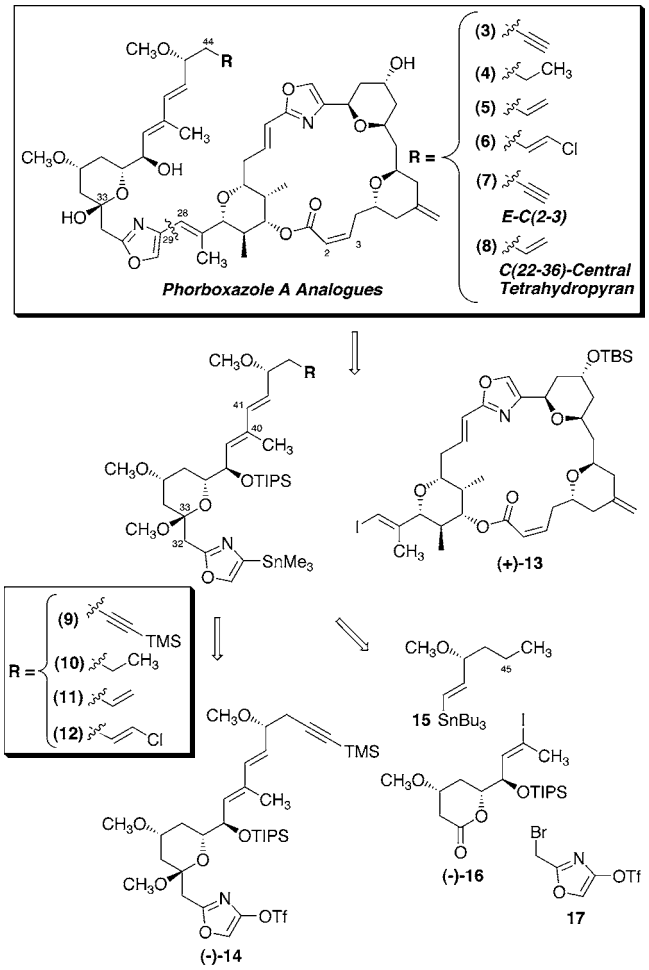
Scheme 1



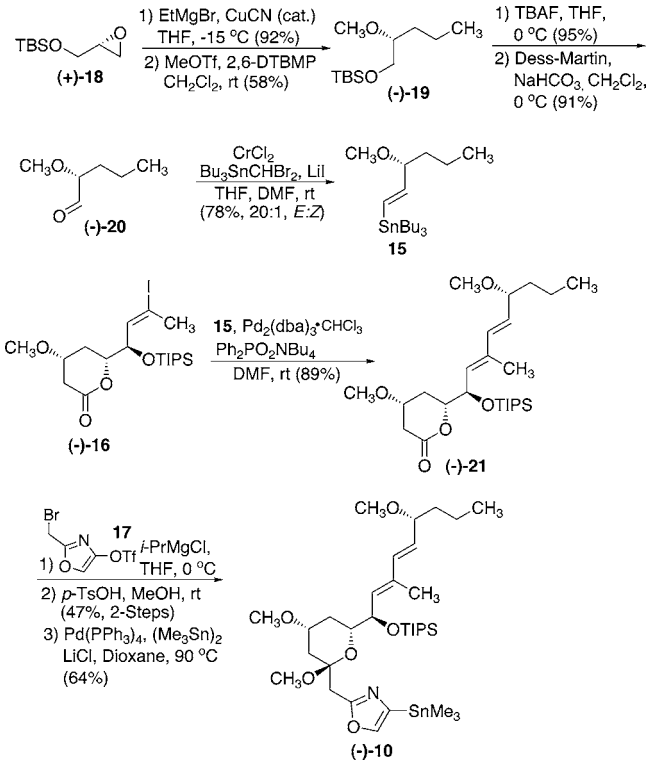
interest, the C(2−3)-saturated and C(29−31)-hydrated oxazole congeners proved to be inactive across the panel of cell lines. Thus, the C(2−3)-enoate and C(29−31)-oxazole moieties appear to be important to retain inhibitory activity, while modification of the C(45−46)- and C(33)-phorboxazole moieties can be tolerated. Forsyth also reported the synthesis of a biotinylated (+)-phorboxazole A analogue; however, the biological utility of this analogue has not as yet been disclosed.[4]

In this, the second Letter of this series, we report convergent, scalable syntheses and biological evaluation of C(45−46)-alkyl (**4**), -alkenyl (**5**), and -*E*-chloroalkene (**6**) congeners of phorboxazole A, as well as the *E*-C(2−3)−C(45−46)-alkynyl analogue (**7**), the C(45−46)-alkenyl−C(22−26)-central tetrahydropyran congener (**8**), and the previously disclosed C(45−46)-alkynyl analogue (**3**).

Our analogue synthesis follows directly from our second generation synthesis of (+)-phorboxazole A (Scheme 1).[5] *It should be emphasized that only by achieving a scalable second generation synthesis of phorboxazole A are we in position with ample quantities of advanced intermediates to achieve the construction of such analogues.* With this in mind, disconnection of analogues **3**−**8** at C(28−29) reveals the common macrocyclic vinyliodide (+)-**13**, prepared in our second generation synthesis, and the oxazole stannane side chains **9**−**12**, which we anticipated could be readily coupled via the Stille protocol developed during our second generation approach.[5] Side chain fragments **9**, **11**, and **12** in turn would be assembled exploiting our first generation Stille coupling partner, oxazole triflate (−)-**14**.[2b,c] For construction of side chain **10**, we will take advantage of the bidirectional oxazole linchpin protocol recently reported by our laboratory.[6] Toward this end, disconnection of fragment **10** at C(32−33) and C(40−41) affords three fragments: C(45−46)-alkyl vinylstannane **15**, vinyliodide lactone (−)-**16**,[2b,c] and oxazole linchpin **17**.

Our study began with the construction of the C(45−46)-alkyl oxazole stannane **10** (Scheme 2). Addition of ethylmagnesium bromide to known epoxide[7] (+)-**18** promoted by CuCN,[8] followed by treatment of the derived secondary alcohol with methyltriflate, furnished methyl ether (−)-**19** in 54% yield for the two steps. Tetrabutylammonium fluoride mediated removal of the TBS group, followed by Dess−Martin oxidation,[9] then led to aldehyde (−)-**20**, which upon chromium(II)-mediated alkenylstannylation[10] furnished vinylstannane **15**, which, due to decomposition upon silica gel chromatography, was employed without purification.

Scheme 2



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(5) Smith, A. B., III; Razler, T. M.; Ciavarri, J. P.; Hirose, T.; Ishikawa, T. *Org. Lett.* **2005**, preceding Letter.

(6) Smith, A. B., III; Minbiole, K. P.; Freeze, S. *Synlett* **2001**, 1739.

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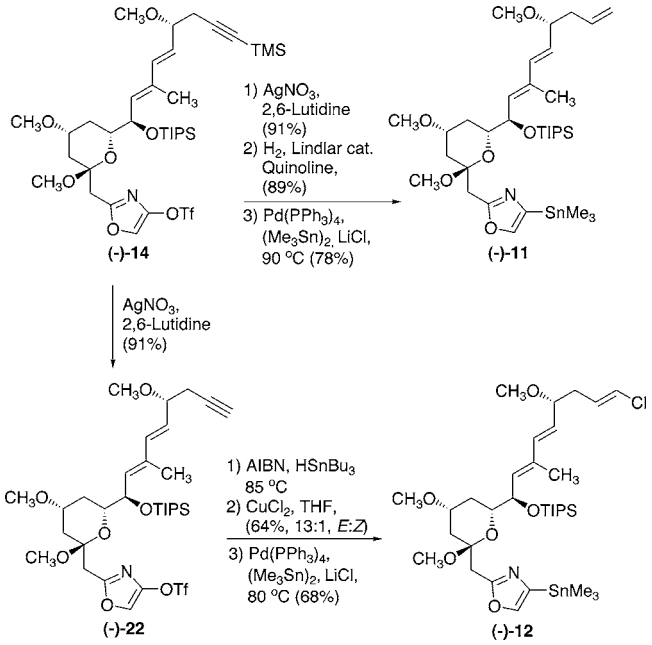
Pleasingly, Stille union of vinyliodide (−)-**16** with vinylstannane **15** afforded lactone (−)-**21** in 89% yield. Completion of the C(45−46)-alkyl side chain then proceeded via addition of the Grignard reagent derived from oxazole **17** to (−)-**21**, followed by conversion to the corresponding mixed methyl ketal with *p*-TsOH and MeOH and, in turn, a palladium-catalyzed trimethylstannane−triflate exchange to furnish the C(45−46)-alkyl stannane (−)-**10**.

Construction of the C(45−46)-alkenyl and C(45−46)-*E*-chloroalkene side chain fragments (**11** and **12**, respectively) began with known oxazole triflate (−)-**14** (Scheme 3).[2b,c]

Scheme 3

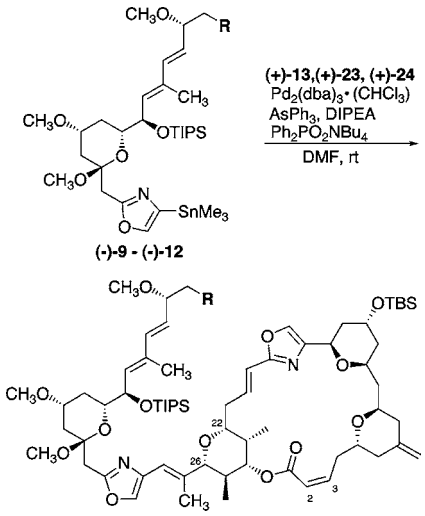


Silver nitrate mediated removal of the terminal trimethylsilane,[11] followed by reduction of the corresponding alkyne, employing Lindlar hydrogenation conditions, furnished the corresponding terminal alkene in high yield. Palladium-catalyzed exchange of the enol triflate for the trimethylstannane moiety led to side chain (−)-**11** in good yield. For the C(45−46)-*E*-chloroalkene side chain **12**, we again began with removal of the trimethylsilyl group mediated with silver nitrate, followed by a radical promoted hydrostannylation[12] of the resultant alkyne (−)-**22** and chlorination[13] to furnish the corresponding vinyl chloride in 64% yield; pleasingly, excellent selectivity (13:1, *E:Z*) with none of the vinyl chloride regioisomer was observed. The straightfoward introduction of the chloride for the C(45−46)-*E*-chloroalkenyl phorboxazole A (**6**), compared to introduction of the bromide in (+)-phorboxazole A (**1**), should be noted (vide infra). Palladium-catalyzed conversion of the triflate to the corresponding trimethylstannane then afforded C(45−46)-*E*-chloroalkene (−)-**12** in 68% yield.

(10) Hodgson, D. M.; Boulton, L. T.; Maw, G. N. *Tetrahedron* **1995**, *51*, 3713.
(11) Carreira, E. M.; Du Bois, J. *J. Am. Chem. Soc.* **1995**, *117*, 8106.
(12) Leusink, A. J.; Budding, H. A.; Drenth, W. *J. Organomet. Chem.* **1968**, *11*, 541.
(13) Madec, D.; Férézou, J.-P. *Tetrahedron Lett.* **1997**, *38*, 6661.

With the requisite side chains in hand, we explored their union with the eastern coupling partners: *Z*-C(2−3)-vinyliodide macrocycle (+)-**13**, *E*-C(2−3)-vinyliodide macrocycle (+)-**23**, and the diminutive C(22−26)-central tetrahydropyran vinyliodide (+)-**24** (Scheme 4).[14] To our delight, coupling

Scheme 4



C(45-46) Sidechain R=	Yield
TMS	**(25)** (+)-*Z-Macrocycle*: 68% **(29)** (+)-*E-Macrocycle*: 66%
CH3	**(26)** (+)-*Z-Macrocycle*: 82%
	(27) (+)-*Z-Macrocycle*: 77% **(30)** (+)-*Central Pyran*: 69%
Cl	**(28)** (+)-*Z-Macrocycle*: 87%

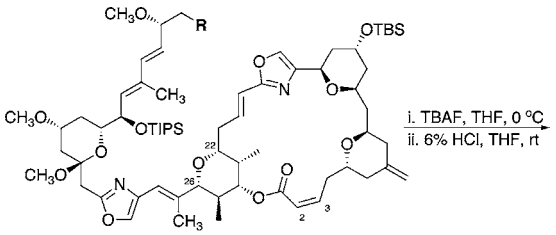
of the *Z*-C(2−3)-macrocycle (+)-**13** in turn with the C(45−46)-TMS-alkynyl (−)-**9**, the alkyl (−)-**10**, the alkenyl (−)-**11**, and the *E*-chloroalkene (−)-**12** oxazole stannane side chains furnished coupled products (+)-**25**−**28** in good to excellent yields (ca. 68, 82, 77, and 87% yield, respectively). In similar fashion, union of the *E*-C(2−3)-macrocycle (+)-**23** with the C(45−46)-TMS-alkynyl side chain (−)-**9** furnished (+)-**29** in 66% yield, while the coupling of the C(22−26)-central tetrahydropyran (+)-**24** with the C(45−46)-alkenyl side chain (−)-**11** led to (+)-**30** in 69% yield.[15]

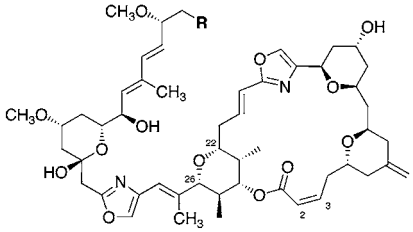
With advanced phorboxazole intermediates (+)-**25**−**30** in hand, we surveyed both global and stepwise deprotection tactics. Optimal conditions proved to entail treatment with 3 equiv of tetrabutylammonium fluoride at 0 °C for 1 h to

(14) The *E*-C(2−3)-macrocycle (+)-**23** is the minor product from the Still−Genari-modified Horner−Emmons olefination. Smith, A. B., III; Razler, T. M.; Ciavarri, J. P.; Hirose, T.; Ishikawa, T. *Org. Lett.* **2005**, preceding Letter.
(15) We note that as the degree of unsaturation at C(45−46) decreased from alkynyl > alkenyl > alkyl the corresponding yields of the Stille reactions increased. This observation may be due to the propensity of palladium to coordinate with more electron-rich functional groups. As the C(45−46) electron density decreases, more palladium would be available to participate in the Stille cross-coupling. Union of the *Z*-C(2−3)-macrocycle (+)-**13** with the C(45−46)-*E*-chloroalkene (−)-**12** side chain proceeded with the highest yield and may reflect the electron-withdrawing capability of chlorine.

effect TIPS, TBS, and TMS group removal, followed by exposure to 6% HCl for 36 h. Purification by silica gel chromatography then furnished the six phorboxazole A analogues (+)-**3**−(−)-**8** in good to excellent yield (Scheme 5).[16]

Scheme 5



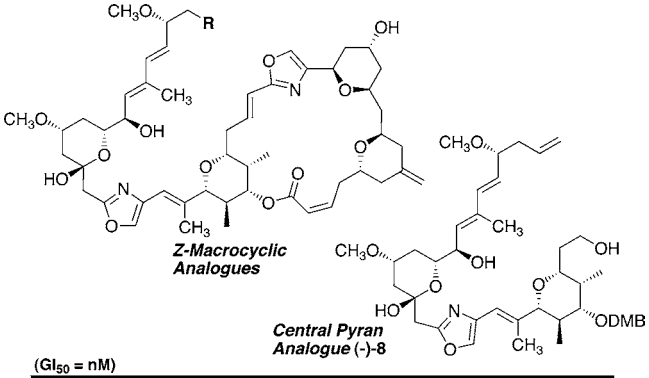


C(45-46) Sidechain R=	Yield
	(3) (+)-*Z-Macrocycle*: 64% **(7)** (+)-*E-Macrocycle*: 69%
CH_3	**(4)** (-)-*Z-Macrocycle*: 86%
	(5) (+)-*Z-Macrocycle*: 67% **(8)** (-)-*Central Pyran*: 66%
Cl	**(6)** (+)-*Z-Macrocycle*: 61%

Biological evaluation of the phorboxazole congeners (+)-**3**−(−)-**8**, in conjunction with (+)-phorboxazole A (**1**), against the human cancer cell line panel, including BXPC-3 (pancreatic), MCF-3 (breast), F-268 (CNS), NCI−H460 (non-small lung), KM20L2 (colon), and DU-145 (prostate), revealed significant tumor cell growth inhibitory activity (Table 1). (+)-Phorboxazole A (**1**) displayed single-digit GI_{50} nanomolar values across the entire cell panel, within experimental error of earlier reported results. The C(45-46)-alkynyl phorboxazole A (+)-**3**,[3] an analogue first reported by Forsyth, as well as C(45−46)-alkyl (−)-**4**, C(45−46)-alkenyl (+)-**5**, and C(45−46)-*E*-chloroalkenyl (+)-**6** phorboxazole congeners were found to have similar and, in several cell lines, enhanced GI_{50} values (cf. 1−2 nM range) against the F-268, NCI-H460, and KM20L2 cell lines to that of (+)-phorboxazole A (**1**). On the other hand, the *E*-C(2−3)-C(45−46)-alkynyl analogue (+)-**7** was 2 orders of magnitude less active than (+)-phorboxazole A (**1**), while diminutive congener (−)-**8** was essentially devoid of activity.

From the structural activity perspective, varying the electronic nature of the side chain at C(45−46) from alkyne > alkene > alkyl > chloroalkene retains potent cytotoxic activity. Taken together with the cytotoxicity or lack thereof observed for congeners (+)-**7** and (−)-**8**, these results indicate that not only is the macrocycle required for activity but also the geometry of the C(2−3) olefin must play a significant role in the conformation and/or interaction of the phorboxazoles with cellular target(s).

Table 1. Human Cancer Cell Line Screening



(GI_{50} = nM)

C(45-46) Sidechain R=	BXPC-3	MCF-3	F-268	NCI-H460	KM20L2	DU-145
Br (+)-**1**	5.8	6.8	5.4	3.8	2.8	4.7
Z-Macrocycle (+)-**3**	4.8	7.4	8.0	4.4	3.2	5.5
E-Macrocycle (+)-**7**	212	243	540	212	148	434
CH_3 (-)-**4**	3.3	4.1	3.7	1.7	1.4	5.2
(+)-**5**	6.1	5.9	5.3	2.9	1.9	3.8
Cl (+)-**6**	4.4	4.3	1.3	2.9	1.5	4.9
Central Pyran (-)-**8**	>10,000	>4,800	>10,000	>10,000	>10,000	>10,000

In summary, we have achieved the total synthesis and evaluation of a series of phorboxazole A analogues which provide considerable insight on the functionality required to retain and/or enhance tumor cell growth inhibitory activity. Importantly, the potent activity of (−)-**4**, (+)-**5**, and (+)-**6**, in conjunction with the efficient introduction of their C(45−46) substituents and facile purification (e.g., flash chromatography), compared with the synthesis of the naturally occurring bromo congener, (+)-phorboxazole A (**1**), suggests the alkyl, alkenyl, and *E*-chloroalkenyl congeners to be ideal candidates for large-scale synthesis, in anticipation of preclinical development. Studies in this regard, as well as to vary the side chain and macrocycle functionality of the phorboxazole skeleton, will be reported in due course.

Acknowledgment. Financial support was provided by the National Institutes of Health through Grant CA-19033.

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

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(16) Global deprotection of central tetrahydropyran analogue (+)-**30** required room temperature TBAF treatment for 2 h to effect BPS removal followed by hydrolysis with 6% HCl.