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Anticancer Activity of Synthetic Analogues of the Phorboxazoles

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Abstract—The phorboxazoles are recently described natural products that are extremely cytostatic towards the National Cancer Institute's panel of 60 tumor cell lines. We report here the results of preliminary structure–activity studies of synthetic analogues of the phorboxazoles against BT-20 breast cancer, NALM-6 B-lineage ALL, U373 brain tumor, and U373 glioblastoma cell lines. These data indicate the importance of several of the natural products' structural moieties for potent anticancer activity.

Phorboxazole A (**1**) and its C13 epimer phorboxazole B represent a new structural class of anticancer agents.[1] The isolation, preliminary structural assignments, and results of initial bioassays of the phorboxazoles were reported by Searle and Molinski in August of 1995.[1a] Using bioassay guided isolation against *Candida albicans*, phorboxazoles A (0.040% dry weight) and B (0.017% dry weight) were isolated as pale-yellow amorphous solids from extracts of the Indian Ocean sponge *Phorbas* sp. In addition to having potent in vitro antifungal activity against *C. albicans* and *S. carlsbergensis*, the phorboxazoles were reported to be extremely cytostatic towards the National Cancer Institute's panel of 60 tumor cell lines. Although the mean GI_{50} values of the phorboxazoles were reported to be less than 1.6×10^{-9} M in these assays, most of the cell lines were 100% inhibited at this lowest test concentration. Hence, the phorboxazoles are among the most potent cytostatic agents yet discovered.[1]

Complete structural assignments for the phorboxazoles have resulted from extensive NMR, derivatization, and degradation/correlation studies,[1] and have been corroborated by total synthesis.[2] Their complex and unique structures distinguish the phorboxazoles as a new class of natural products that contain an unprecedented array of oxane, oxazole, macrolide, and polyene moieties (Fig. 1). In addition, phorboxazoles A and B have also been selected by the National Cancer Institute for in vivo antitumor trials[1b] due to their extraordinary levels of cytostatic activity. In contrast to known potent antimitotic natural products, however, it was shown that **1** did not interact with microtubules, but halted progression of the cell cycle during S phase.[1c] The remarkable biological activities associated with the phorboxazoles' unique molecular structures make these compounds important leads for biotherapeutic development.

A total synthesis of phorboxazole A was completed in February 1998.[2,3] In addition to augmenting the limited supply of the natural products, this synthetic entry to the phorboxazole architecture has since allowed the generation of several unique structural analogues. Reported here are the results of a preliminary structure–activity relationship study based upon these synthetic compounds that provide the first insight into the importance of several of the natural products' structural features for potent anticancer activity.

In a preliminary effort aimed at determining the minimal molecular architecture of phorboxazole A necessary for its biological activity, we studied in a side-by-side comparison the antiproliferative activity of synthetic phorboxazole A and seven distinct synthetic analogues (Fig. 2)

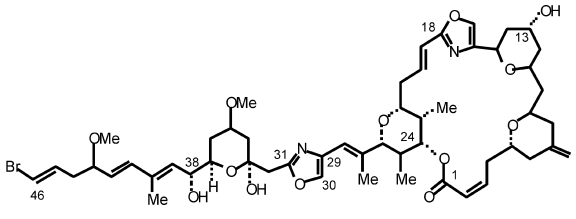

Figure 1. Structure of phorboxazole A.

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against the human B-lineage acute lymphoblastic leukemia cell line NALM-6, human breast cancer cell line BT-20, and human brain tumor (glioblastoma) cell line U373 using standard MTT assays.[4] All three cell lines were inhibited by synthetic phorboxazole A (**1**) as well as its analogues 45,46-dehydrobromo-phorboxazole A (**2**), which bears an alkyne in place of the C45–C46 terminal bromide, and 33-*O*-methyl-phorboxazole A (**3**), which has a mixed methyl ketal instead of the C33 hemiketal, in a concentration-dependent fashion with low nanomolar IC_{50} values (Table 1). NALM-6 cells were more sensitive than BT-20 cells and BT-20 cells were more sensitive than U373 cells against the three active compounds as documented by their lower IC_{50} values. Synthetic phorboxazole A was 2.8-fold more potent against NALM-6 cells (IC_{50} values: 1.7 nM vs 4.8 nM), 3.7-fold more potent against BT-20 cells (IC_{50} values: 3.4 nM vs 12.6 nM), and 4.1-fold more potent against U373 cells (IC_{50} values: 6.7 nM vs 27.4 nM) than its dehydrobromo analogue **2** (Table 1). Phorboxazole A was 3.1-fold more potent against NALM-6 cells (IC_{50} values: 1.7 nM vs 5.2 nM), 3.3-fold more potent against BT-20 cells (IC_{50} values: 3.4 nM vs 11.3 nM), and 4.4-fold more potent against U373 cells (IC_{50} values: 6.7 nM vs 29.2 nM) than its analogue 33-*O*-methyl-phorboxazole **3** (Table 1). The IC_{50} values for the remaining five synthetic analogues (**4**, **5**, **6**, **7**, and **8**) were $>2\,\mu M$ against NALM-6, BT-20 as well as U373 cells. The three active compounds (**1**, **2**, and **3**) also inhibited the clonogenic growth of all three cancer cell lines in a concentration-dependent fashion (Table 2).[4]

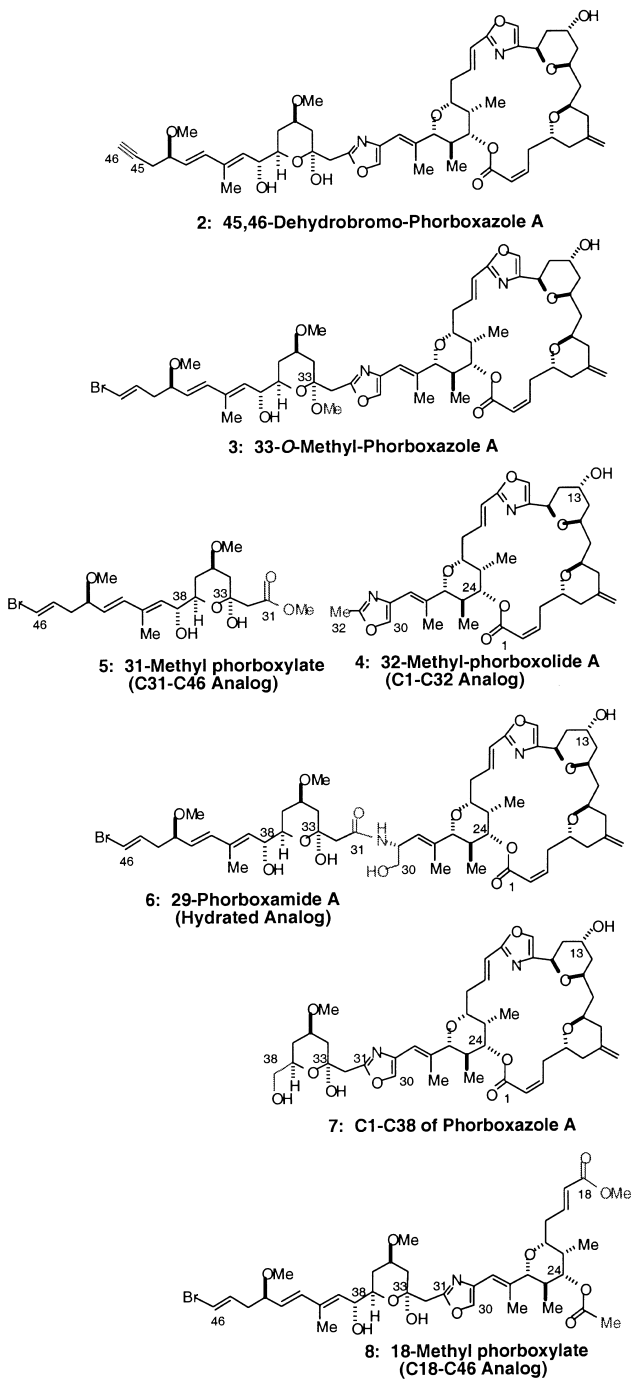

Figure 2. Structures of synthetic analogues of the phorboxazoles. All compounds were prepared by application and extension of the methodology described for the total synthesis of phorboxazole A.[2] All compounds gave physical and spectral data that are fully consistent with the structures assigned.

The data summarized in Table 1 indicate that the simple modifications of replacing the terminal vinyl bromide of **1** with an alkyne (**2**), or the C33 hemiketal with a mixed methyl ketal (**3**) did not result in substantial losses of anticancer activity. However, the data reveal the importance of several key structural features for potent antiproliferative activity. Neither the C1–C32 macrolide-containing domain (**4**), nor the C31–C46 side-chain portion (**5**) were separately sufficient to sustain the potent anticancer activity of **1**. The simple covalent joining of macrolide and side-chain regions of **1** via an amide at C29–C31 (**6**), instead of the planar vinyl-substituted oxazole at this position of **1**, was not sufficient to regain appreciable activity. Interestingly, maintaining the central oxazole, but truncating the side chain by omission of the lipophilic C39–C46 polyene domain (analogue **7**) abolished activity. Finally, deletion of the C2–C17 portion of the phorboxazoles (analogue **8**) that contains oxazole, acrylate, and bispyran moieties similarly resulted in loss of activity.

This study indicates that portions of the macrolide, central oxazole, and polyene side chain of the phorboxazoles are necessary for potent anticancer activity. Maintenance of the levels of activity of **1** was sensitive to the presence of both macrolide (cf. **5**) and side-chain (cf. **4**) domains, an extension from the C38 position (cf. **7**), the hydration state of the C29–C31 oxazole (cf. **6**), and structural features contributed by the C2–C18 portion of the macrolide (cf. **8**). This suggests that at least bimodal interactions of the natural product with key cellular components may occur. Although the conformation of the C1–C26 macrolide-containing domain has been established both in solution[1] and in the solid state,[2] the essential conformation and relative trajectory of the side-chain domain is uncertain. The central C27–C31 vinyl-oxazole moiety may provide an important orientational role or binding contacts. It is not sufficient to simply covalently link C1–C30 and C31–C46 domains together via an amide (**6**).

Table 1. Effects of synthetic phorboxazole A and analogues on proliferation of human cancer cells[a]

Compound	NALM-6 Leukemia IC_{50} (nM)[a]	BT-20 Breast cancer IC_{50} (nM)[a]	U373 Brain tumor IC_{50} (nM)[a]
1	1.7	3.4	6.7
2	4.8	12.6	27.4
3	5.2	11.3	29.2
4	>2000	>2000	>2000
5	>2000	>2000	>2000
6	>2000	>2000	>2000
7	>2000	>2000	>2000
8	>2000	>2000	>2000

[a]Standard MTT assays examined test compounds on human cancer cell lines.[4] Expressed as the average IC_{50} values from 2–5 independent experiments. The SDs were less than 25% of the mean values.

Table 2. Effects of synthetic phorboxazole and analogues on clonogenic growth of human cancer cells[a]

Cell line/treatment	Concn (nM)	Mean no. colonies (10^4 cells)	% Inhibition
NALM-6 B-lineage ALL			
Vehicle (control)	—	2046 (1736, 2356)	—
Compound **1**	1	270 (116, 424)	86.8
1	10	0 (0,0)	>99.9
1	100	0 (0,0)	>99.9
2	1	2116 (1872, 2360)	0.0
2	10	293 (200, 386)	85.7
2	100	0 (0,0)	>99.9
3	1	1760 (1468, 2052)	14.0
3	10	422 (316, 528)	79.4
3	100	0 (0,0)	>99.9
BT-20 Breast cancer			
Vehicle (control)	—	1524 (1256, 1792)	–
Compound **1**	1	1070 (1028,1112)	29.8
1	10	0 (0,0)	>99.9
1	100	0 (0,0)	>99.9
2	1	1540 (1492, 1588)	0.0
2	10	1062 (1020, 1104)	30.3
2	100	0 (0,0)	>99.9
3	1	1824 (1768, 1880)	0.0
3	10	1430 (1264, 1596)	6.2
3	100	0 (0,0)	>99.9
U373 Glioblastoma			
Vehicle (control)	—	656 (576, 736)	—
Compound **1**	10	378 (364, 392)	42.4
1	100	0 (0,0)	>99.8
2	10	586 (528, 644)	10.7
2	100	144 (132, 156)	78.1
3	10	578 (552, 604)	11.9
3	100	0 (0,0)	>99.8

[a]The effects of the test compounds on the in vitro clonogenic growth of human cancer cell lines were evaluated using standard methylcellulose colony assays.[4] Results are expressed as the mean number of tumor cell colonies per 10^4 cells plated from triplicate experiments and the corresponding percent inhibition of colony formation for test cultures.

In addition to determining the importance for the potent antiproliferative activity of major structural domains of **1**, the contributions of several functional groups were also indicated. Clearly, the terminal 45,46-vinyl bromide and free 33-hemiketal are biologically dispensable, whereas the C29–C31 oxazole apparently is not. It remains to be determined whether the loss of activity associated with omission of the C2–C18 portion of the macrolide domain (**8**) is due to the absence of specific functional groups within this deletion, such as acrylate, bis-oxane, or oxazole, or to disruption of essential intermolecular interactions involving larger structural features. The deleterious effect on activity accompanying removal of the C39–C46 domain also requires further detailed investigation.

The potent activities of analogues **2** and **3** allow simplification of the preparative chemistry required to access novel anticancer agents. Complete definition of the minimal pharmacophore will require the generation and assay of a considerably larger array of structural variants than those examined here. Such studies are ongoing and their results will be reported in due course.

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