Structure-Activity Investigations of Analogues of the C15-C26 Phorboxazoles Segment

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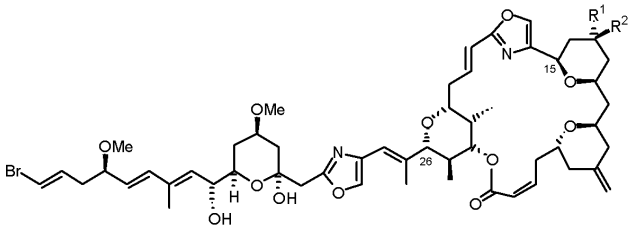
Abstract: A variety of analogues of phorboxazole C15-C26 segment have been prepared *via* three different reaction sequences. Structure activity investigations of the oxazole substitution pattern point to a pharmacophoric lead structure.

Key words: oxazole synthesis, drug discovery, natural products, SAR-studies

During the last decade a wide variety of 2,4-disubstituted oxazoles and thiazoles have been found in biologically active natural products.[1] The biosynthetic origin of 2,4-disubstituted oxazoles has been investigated on the disorazoles by Höfle, Reichenbach and their coworkers who have shown that serine is the nitrogen source for oxazoles in nature.[2] Thus oxazoles are masked amino acids. Unlike conventional zwitterionic α-amino acids the covalent oxazoles are capable of crossing the blood brain barrier. Intensive synthetic and pharmacological studies have also been carried out on the epothilones, containing a 2,4-disubstituted thiazole in their side chain.[3]

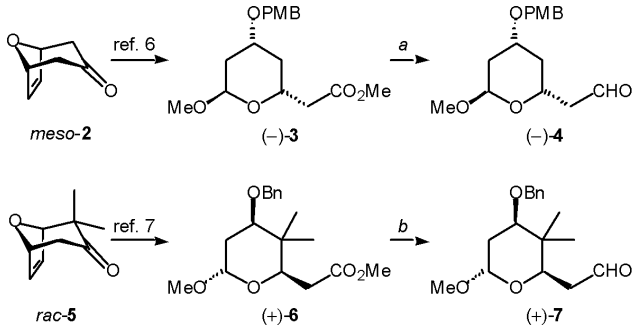


Phorboxazoles A and B A (**1a**): R^1 = OH, R^2 = H; B (**1b**) : R^1 = H, R^2 = OH

Figure 1

The phorboxazoles A and B **1a** and **1b** are two new highly cytotoxic macrolides with two 2,4-disubstituted oxazole rings.[4] Starting from the corresponding C20-C26 aldehyde we have described two new synthetic routes to the C15-C26 segment of the phorboxazoles.[5] Since we were interested in the discovery and design of potential pharmacophores, we synthesized a variety of analogues of the C15-C26 segment of the phorboxazoles. As starting material we required enantiopure tetrahydropyranyl acetaldehydes.

Asymmetric syntheses of the esters (-)-**3**[6] and (+)-**6**[7] in multigram quantities have been described by us starting from the oxabicyclic ketones *meso*-**2** and *rac*-**5**. Under typical reaction conditions (DIBAH reduction and subsequent PCC oxidation) the corresponding aldehydes (-)-**4** and (+)-**7**[7b] were obtained in good yield (Scheme 1).



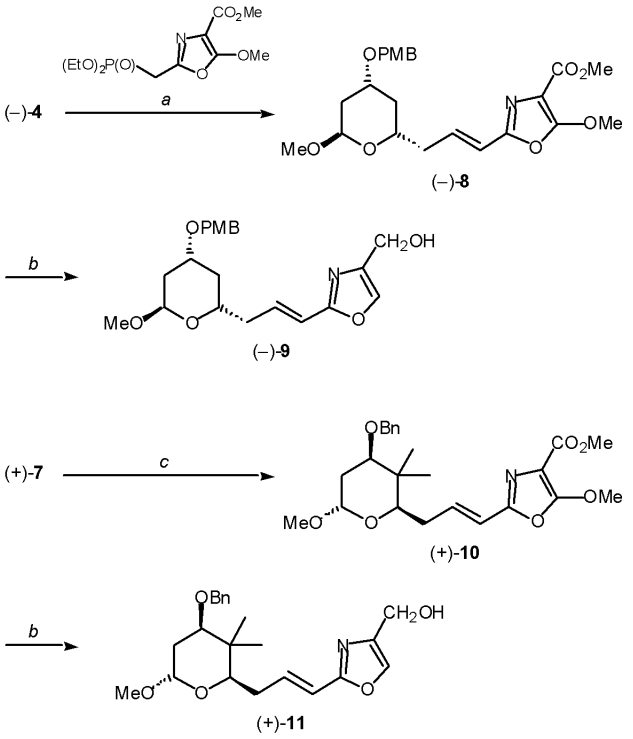
Reaction conditions: *a*) 1. DIBAH, THF, rt, 4 h; 2. PCC, DCM, rt, 15 h, 73% over 2 steps; *b*) DIBAH, toluene, -78 °C, 1 h. 68% (ref. 7b).

Scheme 1

Using the reaction conditions developed earlier[5] aldehyde (-)-**4** was converted into 5-methoxy oxazole ester (-)-**8**. The ester group and the heteroaromatic methoxy group were reduced in one step using $LiAlH_4$ to give oxazolyl methanol (-)-**9**. In contrast, aldehyde (+)-**7** was transformed into the unsaturated nitrile. Rhodium(II) catalyzed cycloaddition with dimethyl diazomalonate gave oxazolyl ester (+)-**10**, which was reduced to the corresponding oxazole methanol (+)-**11** in reasonable yield.

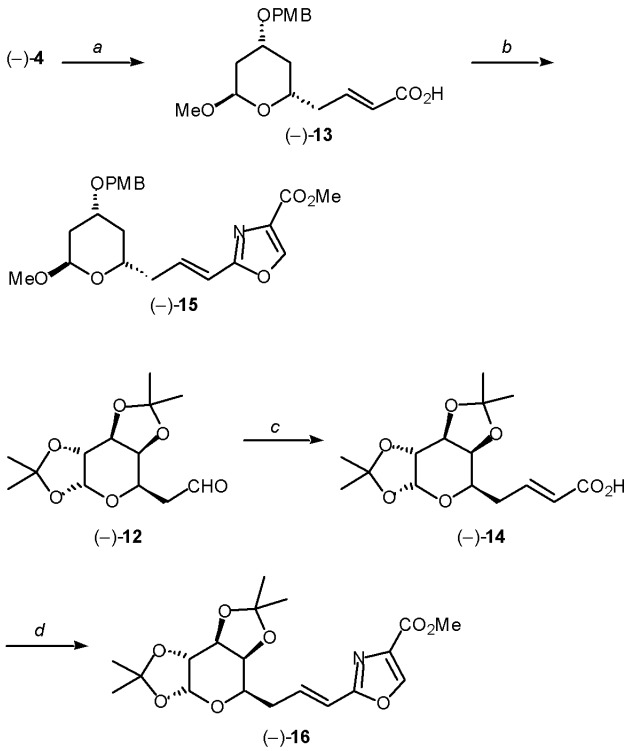
A third pathway towards analogues of the C15-C26 phorboxazole segment is shown in Scheme 3. Starting from aldehydes (-)-**4** and (-)-**12** we prepared α,β-unsaturated esters in excellent yield and with good stereocontrol (>90%; *E:Z* > 20:1) using the protocol of Masamune and Roush.[8] Basic hydrolysis afforded the corresponding α,β-unsaturated acids (-)-**13** and (-)-**14** quantitatively.

The oxazole ring was formed biomimetically using serine as nitrogen source:[9] a peptide coupling of the α,β-unsaturated acids (-)-**13** and (-)-**14** with serine methyl ester using isobutyl chloroformate (IBCF) and N-methyl morpholine (NMM) gave the two corresponding amides, which were



Reaction conditions: *a*) NaH, DCM, rt, 2 h, 83% (*E*:*Z* = 2.7:1); *b*) $LiAlH_4$, THF, -78 °C, 3 h, 45%; *c*) 1. Ph_3PCHCN, toluene, LiCl, rt, 18 h, 94% (*E*:*Z* > 20:1); 2. $(CO_2Me)_2CN_2$, $Rh_2(OAc)_4$, $CHCl_3$, 15 h, reflux, 65%.

Scheme 2



Reaction conditions: *a*) 1. $(EtO)_2P(O)CH_2CO_2Et$, LiCl, DBU, MeCN, rt, 2 h, 91%; 2. LiOH, THF, H_2O, rt, 15 h, 100%; *b*) 1. isobutyl chloroformate, NMM, -25 °C, 15 min, then *L*-serine methylester hydrochloride, rt, 3 h, 75%; 2. $Et_3N^+SO_2N^-CO_2Me$, THF, reflux, 2 h, 71%; 3. $CuBr_2$, DBU, HMTA, DCM, rt, 30 min, 75%; *c*) 1. $(EtO)_2P(O)CH_2CO_2Et$, LiCl, DBU, MeCN, rt, 2 h, 86%; 2. LiOH, THF, H_2O, rt, 15 h, 100%; *d*) 1. isobutyl chloroformate, NMM, -25 °C, 15 min, then *L*-serine methylester hydrochloride, rt, 3 h, 72%; 2. $Et_3N^+SO_2N^-CO_2Me$, THF, reflux, 2 h, 71%; 3. $CuBr_2$, DBU, HMTA, DCM, rt, 75%.

Scheme 3

subsequently cyclodehydrated to the oxazoline esters with Burgess reagent.[10] The oxidation procedure developed at Bristol-Myers Squibb (Cu(II)/DBU/HMTA in DCM)[11] afforded oxazole esters (-)-**15** and (-)-**16**. The three-step reaction sequence (α,β-unsaturated acids → α,β-unsaturated oxazole ester) proceeded in good overall yield (> 20%).

The oxazole containing compounds were tested against pathogenic yeast *Candida albicans* and phytopathogenic fungus *Ustilago zeae*. No antifungal activity was observed. However, some interesting observations were made with regard to cytotoxic activity (Table 1).[12]

Discussion of structure-activity relationship (SAR). All substances show cytotoxic activity. However, the potency of the molecules is significantly dependent on the substitution pattern of the oxazole ring. In the search for a potential lead optimization, the following points should be borne in mind:

- 2,4,5-trisubstituted oxazole esters are potent down to *submicromolar* concentrations [(+)-**10** and (-)-**20** with IC_{50} = 0.1 μg/ml and IC_{50} = 0.5 μg/ml).
- 2,4-disubstituted oxazole methanols (+)-**11** and (+)-**21** are one hundred times less cytotoxic than 2,4,5-trisubstituted oxazole esters (+)-**10** and (-)-**20**.
- 2,4-disubstituted oxazole esters (-)-**15** and (-)-**16** are less active than 2,4,5-trisubstituted oxazole ester.
- less complex 2,4,5-trisubstituted oxazole esters (**17 - 19**) are less cytotoxic than the oxazole esters with tetrahydropyran side chain (+)-**10** and (-)-**20**.

While the biological activity of the most potent substances (+)-**10** and (+)-**21** is considerably lower than that of well known cytotoxic molecules such as the epothilones A and B (IC_{50} = 4 and 1 ng/ml for L929 cells) our compounds are structurally more simple. We think that 5-methoxy oxazole esters containing modified side chains at carbon C2 and C4 are molecules of interest in the area of cancer research.

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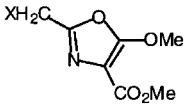
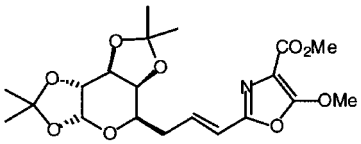
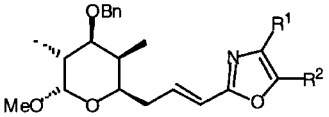
Table 1 Biological Activity of Oxazoles

Oxazole	IC_{50} [µg/ml]
(+)-**10**	0.1
(+)-**11**	12
(–)-**15**	20
(–)-**16**	>10
	
17: X = Cl	40
18: X = Br	12
19: X = SO_2Ph	7
	0.5
(–)-**20**	
	
(+)-**21**: $R^1 = CH_2OH$, $R^2 = H$	25
(+)-**22**: $R^1 = CO_2Me$, $R^2 = OMe$	2

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