Bioorganic & Medicinal Chemistry Letters 20 (2010) 6408–6410

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Catharanthine C16 substituent effects on the biomimetic coupling with vindoline: Preparation and evaluation of a key series of vinblastine analogues

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ARTICLE INFO

Article history:
Received 30 July 2010
Revised 13 September 2010
Accepted 15 September 2010
Available online 19 September 2010

Dedicated to A. Eschenmoser in honor of his 85th birthday.

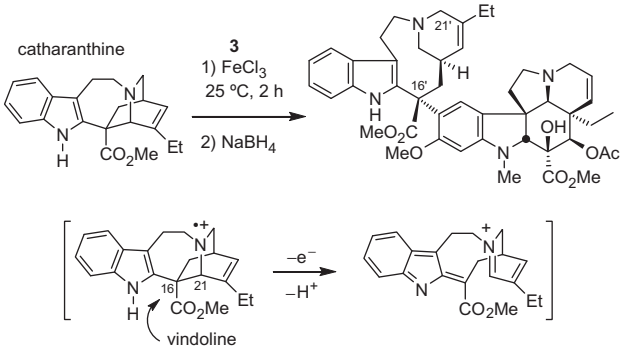
Keywords:
Vinblastine
Catharanthine, Vindoline

ABSTRACT

The examination of the catharanthine C16 substituent effects on the Fe(III)-promoted biomimetic coupling reaction with vindoline is detailed, confirming the importance of the presence of a C16 electron-withdrawing substituent, and establishing an unanticipated unique role (>10-fold) that the C16 methyl ester plays in the expression of the natural product properties. Thus, replacement of the vinblastine C16′ methyl ester with an ethyl ester (10-fold), a cyano group (100-fold), an aldehyde (100-fold), a hydroxymethyl group (1000-fold) or a primary carboxamide (>1000-fold) led to surprisingly large reductions in cytotoxic activity.

Vinblastine (**1**) and vincristine (**2**) represent the most widely recognized members of the vinca alkaloids as a result of their clinical use as antitumor drugs (Fig. 1).[1–3] Originally isolated in trace quantities from *Catharanthus roseus* (L.) G. Don,[1] their biological properties were among the first to be shown to arise from perturbations in microtubule dynamics and inhibition of mitosis that today is still regarded as one of the more successful drug targets for the treatment of cancer.[3–5]

We recently utilized a one-pot, two-step biomimetic Fe(III)-promoted coupling of vindoline (**3**) with catharanthine (**4**) in the total synthesis of vinblastine and reported its extension to the preparation of a series of related natural products including vincristine and key analogues.[6,7] Although key mechanistic insights into this coupling[6–9] and subsequent olefin oxidation[7,10] have been disclosed in the studies to date, the unusual differences in the substrate scope and diastereoselectivity of the Fe(III)-promoted coupling (single natural C16′ diastereomer at 25 °C in aqueous buffer) and the more traditional Polonovski fragmentation[11,12] (5:1 at −78 °C or 1:1 at 0 °C in CH_2Cl_2) or 3-chloroindolenine-based[13] couplings that proceed through azabenzfulvene intermediates suggests that there are mechanistic features of the former reaction that are not yet well understood and that affect the resulting C16′ stereochemistry (Eq. 1). In the case of the Fe(III)-promoted coupling, the attack by vindoline formally occurs with clean inversion of the stereochemistry at the reacting C16 center of the catharanthine C16–C21 bond undergoing cleavage. It has been suggested that the reaction proceeds by two single electron oxidations of catharanthine with the initial radical cation formation occurring at the basic tertiary amine followed by a subsequent oxidative cleavage of the C16–C21 bond.[8,9] Herein, we disclose the first report of the examination of catharanthine C16 substituent effects on the biomimetic coupling reaction, establishing and confirming the importance of the presence of a C16 electron-withdrawing substituent, and further disclose the unanticipated unique role that the C16 methyl ester plays in the expression of the natural product properties.



The electron-withdrawing properties of the C16 methyl ester group were anticipated to be key to the coupling of catharanthine with vindoline. Consequently, only a small series of alternative

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doi:10.1016/j.bmcl.2010.09.091

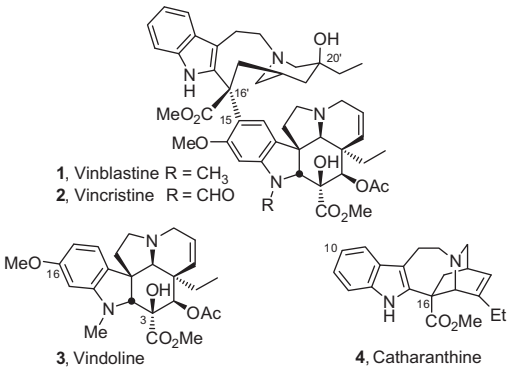
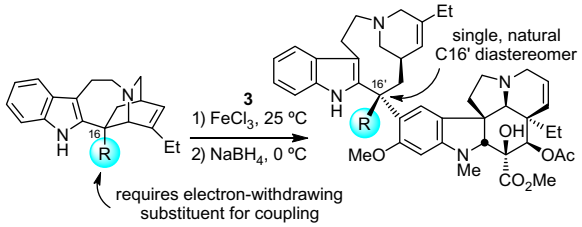
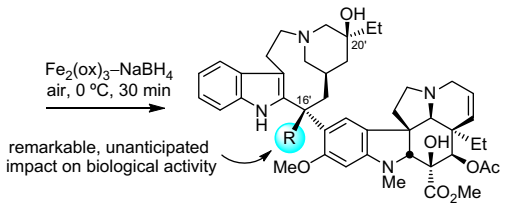


Figure 1. Natural products.

electron-withdrawing substituents were examined (R = CO_2Et, $CONH_2$, CN, CHO, CO_2H) along with derivatives where it was removed (R = H), or replaced with an alkyl (R = CH_2OH, CH_3) or hydroxy (R = OH) group (Fig. 2).[14] As anticipated, the coupling (5 equiv $FeCl_3$, 0.05 N aq HCl–CF_3CH_2OH 10:1, 25 °C, 2 h) required the presence of a C16 electron-withdrawing substituent with **4**–**7** providing comparable superb conversions (79–95% vs 90% for **4**) to the corresponding anhydrovinblastine analogue, and the aldehyde **8** providing a perceptibly lower yield for the generation of product (49%) reflecting some competitive aldehyde reduction upon iminium ion reduction with $NaBH_4$. Interestingly, the carboxylic acid derivative **9** failed to couple with **3**, although this was not investigated in sufficient detail to define the origin of this observation, and the catharanthine analogues **10**–**13** that lack a C16 electron-withdrawing substituent also, but expectedly, did not couple with **3**.



Compd	Product (% yield)	IC_{50} (nM) L1210	HCT116	HCT116/VM46	
4, R = CO_2Me	**4a** (90%)	65	75	600	
5, R = CO_2Et	**5a** (82%)	620	640	4100	10-fold
6, R = CN	**6a** (95%)	6500	6100	6800	100-fold
7, R = $CONH_2$	**7a** (79%)	>10000	>10000	>10000	>100-fold
8, R = CHO	**8a** (49%)	6300	7000	>10000	100-fold
9, R = CO_2H	**9a** (0%)	–	–	–	
10, R = H	**10a** (0%)	–	–	–	
11, R = CH_2OH	**11a** (0%)	–	–	–	
12, R = Me	**12a** (0%)	–	–	–	
13, R = OH	**13a** (0%)	–	–	–	



Compd	Product	IC_{50} (nM) L1210	HCT116	HCT116/VM46	
R = CO_2Me	**1**	6.0	6.8	600	
R = CO_2Et	**5b**	60	70	830	10-fold
R = CN	**6b**	630	670	7400	100-fold
R = $CONH_2$	**7b**	>10000	>10000	>10000	>1000-fold
R = CH_2OH	**11b**[a]	6500	5800	>10000	1000-fold

[a]Obtained from the coupling and oxidation of **8**.

Figure 2. C16 substituent effects.

Since this vinblastine site has not been probed beyond reduction or methyl ester hydrolysis and subsequent decarboxylation,[3,15] each derivative was also converted to the corresponding vinblastine analogue either by exposing the anhydrovinblastine analogue to the conditions developed for oxidation of the C15′–C20′ double bond with installation of the C20′ tertiary alcohol ($Fe_2(ox)_3$–$NaBH_4$, air, 0 °C, 30 min),[7,10] or more directly using the one-pot, two-step procedure of coupling and in situ oxidation.[6,7] In turn, the cell growth inhibition or cytotoxic activity of both the anhydrovinblastine and vinblastine analogues were examined in three cytotoxic assays (72 h exposure) including the matched colon cancer cell lines HCT116 and HCT116/VM46, the latter of which is multidrug resistant by virtue of Pgp overexpression (Fig. 2).[16] Whereas the important role of the C16 methyl ester group in the coupling reaction could be anticipated, the remarkable sensitivity of the properties of the resulting anhydrovinblastine and vinblastine analogues to modifications at this site was unexpected. Each series exhibited the same striking sensitivity to the presence and nature of the C16′ substituent. Simply replacing the C16′ methyl ester group with the corresponding ethyl ester group resulted in a 10-fold loss in activity, a nitrile or aldehyde substitution resulted in a 100-fold loss in activity, incorporation of a hydroxymethyl group led to a 1000-fold loss in activity, and the primary carboxamide replacement produced a >1000-fold loss in activity. Although the importance of this site has been reported previously with more significant modifications (R = H, CH_2OH and $COCH_2CO_2tBu$),[15] it is remarkable that even the more subtle changes detailed herein have such a profound impact on the biological properties of the natural products. Clearly, the role of the C16′ methyl ester extends well beyond facilitating the coupling reaction in the biosynthesis of the natural product; rather it plays an integral role in establishing the biological properties of the natural product presumably stabilizing the interaction of vinblastine with tubulin.[17]

Acknowledgements

We gratefully acknowledge the financial support of the National Institutes of Health (CA115526 and CA042056). We wish to thank Dr. P. Hellier (P. Fabre) for the gift of catharanthine and vindoline, Professor A. Eschenmoser for insightful discussions and NIH (GM087948, A.T.) and JSPS (H.G.) for fellowship support. W.M.R. is a Skaggs Fellow.

Supplementary data

Supplementary data (full experimental details are provided) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.091.

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