10′−Fluorovinblastine and 10′−Fluorovincristine: Synthesis of a Key Series of Modified Vinca Alkaloids

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10’-Fluorovinblastine and 10’-Fluorovincristine: Synthesis of a Key Series of Modified Vinca Alkaloids

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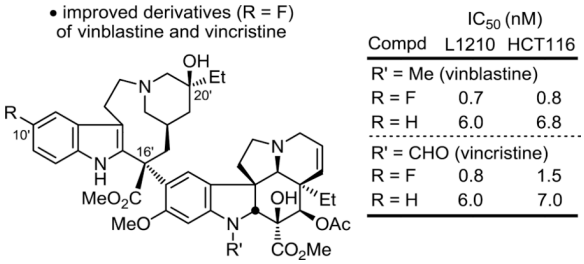
Abstract



	IC_{50} (nM)	
Compd	L1210	HCT116
R' = Me (vinblastine)		
R = F	0.7	0.8
R = H	6.0	6.8
R' = CHO (vincristine)		
R = F	0.8	1.5
R = H	6.0	7.0

A study on the impact of catharanthine C10 and C12 indole substituents on the biomimetic Fe(III)-mediated coupling with vindoline led to the discovery and characterization of two new and substantially more potent derivatives, 10’-fluorovinblastine and 10’-fluorovincristine. In addition to defining a pronounced and unanticipated substituent effect on the biomimetic coupling, fluorine substitution at C10’, which minimally alters the natural products, was found to uniquely enhance the activity 8-fold against both sensitive (IC_{50} = 800 pM, HCT116) and vinblastine-resistant tumor cell lines (IC_{50} = 80 nM, HCT166/VM46). As depicted in the X-ray structure of vinblastine bound to tubulin, this site resides at one end of the upper portion of the T-shaped conformation of the tubulin-bound molecule, suggesting the 10’-fluorine substituent makes critical contacts with the protein at a hydrophobic site uniquely sensitive to steric interactions.

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 (Figure 1).[1–3] Originally isolated in trace quantities from *Cantharanthus roseus* (L.) G. Don,[1] their biological properties were among the first to be shown to arise from inhibition of microtubule formation and 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 biomimetic Fe(III)-promoted coupling of vindoline (**3**) with catharanthine (**4**) in the total synthesis of vinblastine[6] and reported its extension to the total synthesis of a series of related natural products including vincristine and key analogues.[7–10] Although mechanistic insights into this coupling[7,10–14] and subsequent in situ olefin oxidation[7,15] have been disclosed in these and earlier studies, the key differences in the diastereoselectivity of the Fe(III)-promoted coupling, producing exclusively the natural C16’ diastereomer at 25 °C in aqueous buffer, and the more traditional Polonovski

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Supporting Information Available: Full experimental details are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

fragmentation[16,17] (5:1 at −78 °C, 1:1 at 0 °C) or 3-chloroindolenine-based[18] couplings suggest that there are features of the biomimetic coupling reaction that are not yet well understood. Herein, we disclose a study of catharanthine substituent effects on the Fe(III)-mediated coupling reaction that led to the discovery of two new, exciting and more potent synthetic analogues of **1** and **2**.

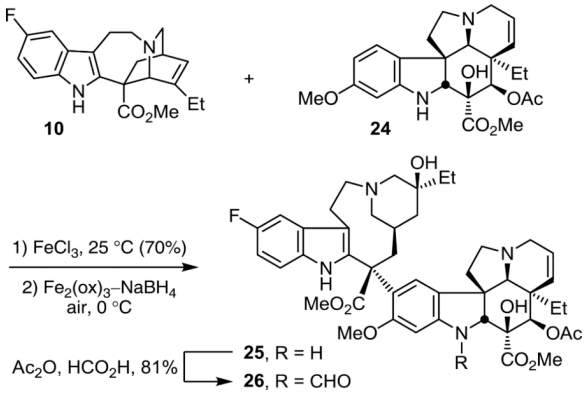
The series examined entailed C10 or C12 substitution in catharanthine, para or ortho to the indole NH. Since the C10' position in **1** is a site of oxidative metabolism, producing 10'-hydroxyvinblastine (**14b**),[19] substitution that blocks formation of such metabolites was viewed as an attribute to such derivatives. More significantly and as depicted in the X-ray structure of vinblastine bound to tubulin (Supporting Information Figure S1),[20] this site resides at one end of the upper portion of the T-shaped conformation of the tubulin-bound molecule, suggesting that it makes critical contacts with the protein at a site sensitive to steric interactions. Consequently, we viewed derivatives with modifications at this site especially interesting to examine.[21] Thus, a systematic series of catharanthine substituents was examined that would provide not only further insight into the Fe(III)-promoted coupling reaction, but also the corresponding vinblastine derivatives for comparative examination (Figure 2).[22]

With notable exceptions, electron-withdrawing substituents were observed to slow or preclude coupling with vindoline, whereas derivatives bearing neutral or electron-donating C10 substituents participated effectively in the reaction (5 equiv $FeCl_3$, 0.05 N aq HCl–CF_3CH_2OH 10:1, 25 °C, 2 h). The exceptions were the amine derivatives (**15–17**) and the phenol **14**, which underwent competitive oxidation (*p*-quinodiimine or *p*-quinoimine formation). Moreover, a smooth trend of decreasing yield and rate of coupling was observed with the derivatives bearing electron-withdrawing substituents [H (90%) > F (65%) > Cl, Br, I (ca. 30%) > CN (ca. 5%) > NO_2 (0%)], those bearing neutral and weakly electron-donating substituents coupled exceptionally well [H (90%), Me (95%), SMe (70%)], and the one derivative containing a strong electron-donating substituent (OMe, 62%) participated effectively in the reaction. Thus, the overall trends are clear and indicate that electron-deficient substituents slow the rate of coupling, suggesting that it is the catharanthine indole and not the previously suggested tertiary amine[11,23] that undergoes the initial single-electron oxidation, initiating the biomimetic coupling. The ramifications of these observations on the mechanism of the Fe(III)-mediated coupling and the requisite catharanthine structural features required are the subject of our continuing investigations.

A smaller series of derivatives bearing substituents ortho to the indole NH was also examined and provided similar results (Figure 3). The derivative bearing a strong electron-withdrawing substituent (NO_2) failed to participate in the coupling reaction, **23** containing an oxidizable electron-donating substituent (NH_2) led to products of competitive indole oxidation, and those containing modestly electron-withdrawing substituents participated slower and less effectively in the coupling reaction [H (90%) > F (62%) > Cl, Br, I (30–44%)].

Without optimization, each derivative containing a C10' or C12' substituent was incorporated into the corresponding vinblastine derivative using the direct oxidation of the corresponding anhydrovinblastine or the one-pot, two-step coupling and oxidation protocol.[7] Analogous to the clear impact of catharanthine substituents on the coupling reaction, their impact on the biological properties of **1** was just as clear. Substitution at C12' was detrimental leading to 10-fold reductions in the potency of the derivatives **19–22**, presumably reflecting destabilizing steric interactions when bound to tubulin (Figure 3). With the provision that polar substituents are not well tolerated, the activity of the vinblastine C10' derivatives **6b–14b** in the cell-based assays (Figure 2) exhibited no

apparent relationship with the electronic character of the substituents, but rather exhibited activity that correlates with their size and shape [R = F > H > Cl > Me, Br >> I, SMe (10-fold) >> CN (100-fold)]. Thus, small hydrophobic C10' substituents are tolerated with one derivative exceeding (R = F) and several matching the potency of **1** (R = H vs Cl, Me, Br), whereas those bearing the larger (R = I, SMe) or rigidly extended (R = CN) substituents proved to be 10–100 fold less potent. Moreover, there is a subtle distinction in the less potent anhydrovinblastine versus vinblastine series, reflecting not only the steric interactions at this site, but their interplay with the disposition of the C20' substituents. The anhydrovinblastine derivatives bearing the small C10' substituents are essentially equipotent with anhydrovinblastine (R = F > H, Cl, Br, Me), albeit 10-fold less active than vinblastine, and exhibited a greater tolerance for the larger substituents at this site (e.g., R = SMe and CN, but not I). These two positions in the upper subunit of vinblastine (C10' and C20') represent the two ends of the upper portion of the T-shaped conformation of the tubulin-bound molecule that is deeply-imbedded in the protein (Figure S1).[20] Presumably, altering the disposition of the C20' ethyl substituent by converting C20' to a sp^2 versus sp^3 center permits some, but not all anhydrovinblastine derivatives with the larger C10' substituents to bind tubulin and exhibit biological activity. However, the most striking observation to emerge from the studies was the behavior of 10'-fluorovinblastine (**10b**). Fluorine substitution at C10' substantially enhances the cell-based activity (8-fold) against both sensitive (L1210 and HCT116) and vinblastine-resistant cell lines (HCT116/VM46). This latter feature, which is derived from overexpression of the cell surface drug efflux pump Pgp,[24] typically limits the effectiveness of vinblastine upon resistance relapse. The more potent activity of **10b** suggests that it represents an attractive alternative drug for both primary care and secondary treatment upon tumor reemergence.



(1)

With a recognition that 10'-fluoro substitution may convey uniquely potent activity to such vinca alkaloids, we additionally prepared 10'-fluorovincristine (**26**) for examination. Thus, Fe(III)-promoted coupling (70%) of synthetic 10-fluorocatharantine (**10**) with synthetic N-demethylvindoline (**24**)[7] and subsequent in situ Fe-mediated oxidation provided N^1-desmethylvinblastine (**25**), which was formylated to provide synthetic 10'-fluorovincristine (**26**), equation 1. Like 10'-fluorovinblastine, 10'-fluorovincristine (**26**) exhibited exceptional activity in the cell-based assays, inhibiting tumor cell growth 5–8 fold more potently than vincristine itself (Figure 4).

Although the enhanced metabolic stability of the 10'-fluoro derivatives may contribute to the increased potency of 10'-fluorovinblastine and 10'-fluorovincristine, the lack of comparable effects with closely related substituents indicate that an effect unique to fluorine

substitution is responsible. We suggest that this is derived from the interaction of a uniquely sized hydrophobic substituent further stabilizing the compound binding with tublin at a site exquisitely sensitive to steric interactions. Comparison models of **1** and **7–10b** built from the X-ray structure of tublin-bound vinblastine[20] illustrate a unique fit for **10b** (Figure 5 and Figure S2).

Although preliminary, an initial in vivo examination of 10'-fluorovinblastine against the vinblastine-resistant HCT116/VM46 human colon cancer cell line (ip, dosed ip at 0.01, 0.1, and 0.5 mg/kg) provided 7/10, 5/10 and 5/10 surviving mice after 110 days comparable to the antitumor efficacy that vinblastine exhibited against sensitive HCT116 at the requisite higher dose of 0.5 mg/kg (6/10 surviving mice).[25]

The detailed profiling of 10'-fluorovinblastine and 10'-fluorovincristine is in progress, continued exploration of the mechanism of the Fe(III)-mediated biomimetic coupling of catharanthine and vindoline building on the substituent effects observed herein is underway, as is the continued examination of vinblastine structural features contributing to its properties and the results of the studies will be reported in due course.[26]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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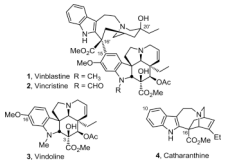


Figure 1.
Natural products.

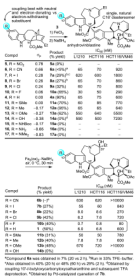
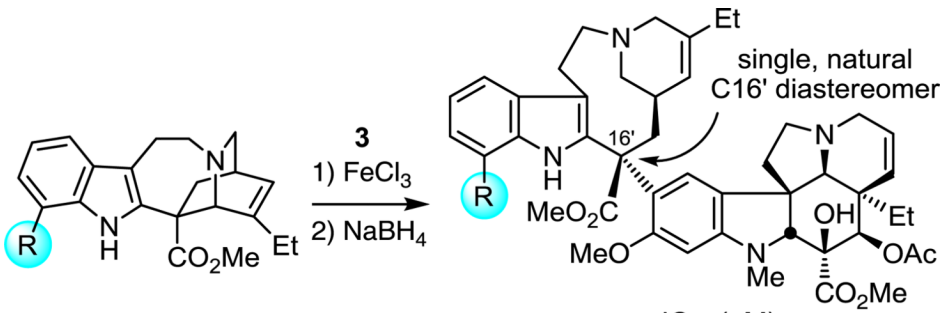
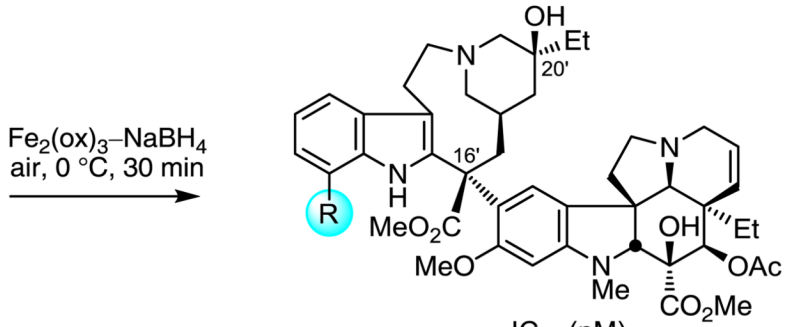


Figure 2.
C10 Substituent effects.



Compd	σ_p	Product (% yield)	IC_{50} (nM) L1210	HCT116	HCT116/VM46
18, R = NO_2	0.78	**18a** (0%)	–	–	–
19, R = I	0.28	**19a** (33%)	690	870	6200
20, R = Br	0.26	**20a** (44%)	600	740	3900
21, R = Cl	0.24	**21a** (27%)	640	880	7300
22, R = F	0.06	**22a** (62%)	650	790	6700
4, R = H	0.00	**4a** (90%)	65	75	600
23, R = NH_2	–0.66	**23a** (0%)	–	–	–



Compd	Product (% yield)	IC_{50} (nM) L1210	HCT116	HCT116/VM46
R = I	**19b** (40%)	55	80	910
R = Br	**20b** (31%)	50	80	910
R = Cl	**21b** (38%)	330	220	5000
R = F	**22b** (37%)	50	65	730
R = H	**1** (50%)	6.0	6.8	600

Figure 3.
C12 Substituent effects

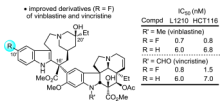


Figure 4.
10’-Fluorovincristine and 10’-fluorovinblastine.

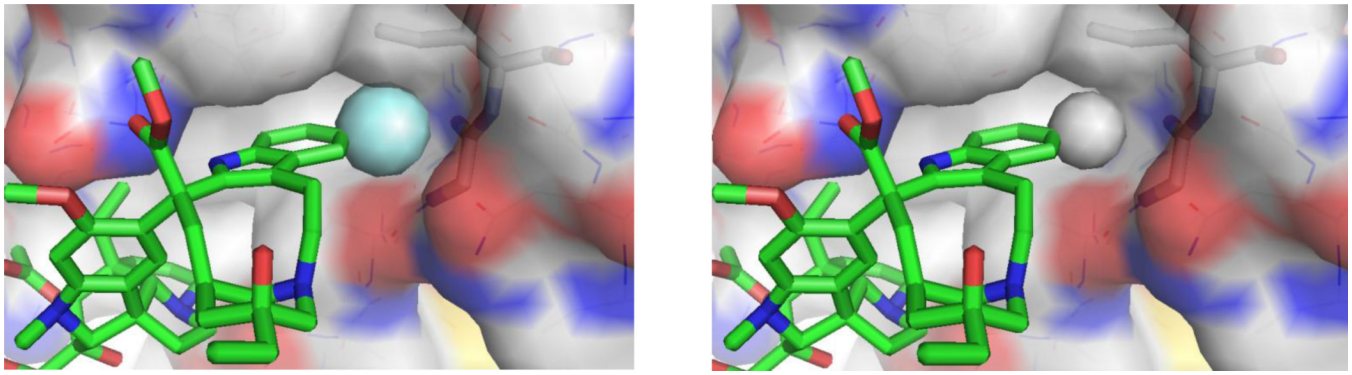


Figure 5.
Space filling model of the 10'-fluoro binding site of **10b** (R = F, left) generated by adding the fluorine substituent to the X-ray structure of tubulin-bound vinblastine[20] (R = H, right). Comparison models of **7b–9b** (R = Cl, Br, and I) are provided in Supporting Information Figure S2 and illustrate the unique fit for **10b** (R = F) and the increasing destabilizing steric interactions at this site as the substituent size progressively increases.