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Total Synthesis and Evaluation of a Key Series of C5-Substituted Vinblastine Derivatives

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Abstract

A remarkably concise 7–8 step total synthesis of a systematic series of key vinblastine derivatives is detailed and used to characterize the importance and probe the role of the C5 ethyl substituent ($R = H, Me, Pr, CH=CH_2, C\equiv CH, CH_2OH, CHO$ vs Et). The analogues, bearing deep-seated structural changes accessible only by total synthesis, were prepared using a powerful intramolecular [4+2]/[3+2] cycloaddition cascade of 1,3,4-oxadiazoles ideally suited for use in the assemblage of the vindoline-derived lower subunit followed by their incorporation into the vinblastine analogues enlisting a single step biomimetic coupling with catharanthine. The evaluation of the series revealed that the tubulin binding site surrounding this C5 substituent is exquisitely sensitive to the presence (Et > H, 10-fold), size ($Me \leq Et > Pr$, 10-fold), shape (Et > $CH=CH_2$ and $C\equiv CH$, >4-fold), and polarity (Et > CHO > CH_2OH , >10–20-fold) of this substituent, and that on selected occasions only a C5 methyl group may provide analogues that approach the activity observed with the naturally occurring C5 ethyl group.

Introduction

Vinblastine (**1**)¹ and vincristine (**2**) are the most widely recognized members of the class of vinca alkaloids as a result of their clinical use as antitumor drugs (Figure 1). Originally they were isolated in trace quantities from the leaves of *Catharanthus roseus* (L.) G. Don,² and 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 targets for cancer therapeutic intervention.³ Both vinblastine and vincristine possess the identical velbanamine upper subunit and nearly identical vindoline-derived lower subunits differing only in the dihydroindole *N*-substituent. Despite this small structural distinction, vinblastine and vincristine differ in their antitumor properties and dose-limiting toxicities.^{1,3} The major limitation to the use of the vinca alkaloids is the emergence of drug resistance derived principally from over-expression of phosphoglycoprotein (Pgp), an efflux pump that transports many of the major drugs out of the cell.⁴ Thus, in addition to identifying vinblastine and vincristine analogues that may address their dose-limiting toxicities, the development of a modified vinca alkaloid that is not a substrate for Pgp efflux and is efficacious against multidrug resistant (MDR) tumors would constitute a major advance.

Although extensive derivatization of vinblastine has been conducted in exploration of semisynthetic analogues of the natural products,^{3,5} a more limited series of synthetic analogues that contain more deep-seated changes in the structure have been examined.^{3,6}

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Supporting Information. Full experimental details and the full citation 5(a) are provided. This material is available free of charge via the internet at <http://pubs.acs.org>.

This reflects the natural product structural complexity and the intrinsic challenge in preparing such analogues. Recently, we reported the development of a concise total synthesis of vindoline⁷ enlisting a tandem [4+2]/[3+2] cycloaddition cascade of 1,3,4-oxadiazoles⁸ applicable to the preparation of structural analogues,⁹ and the use of a single-pot, two-step biomimetic Fe(III)-promoted coupling with catharanthine and subsequent oxidation for their incorporation into vinblastine and its analogues.^{10,11} Significantly, the approach proved sufficiently concise that systematic explorations of each vinblastine structural feature not accessible by semisynthetic derivatization can be envisioned. One such site that remains unexplored is the C5 ethyl group of the lower vindoline subunit (Figure 2a). The recent X-ray crystal structure of vinblastine bound to tubulin¹² indicates that the tubulin binding pocket surrounding this ethyl substituent is composed of hydrophobic residues (i.e. Leu, Ala, Val) and yet potentially partially exposes the C5 ethyl group to solvent (Figure 2b). As a result, we sought to explore not only the removal (R = H), contraction (R = Me), or extension (R = *n*-Pr) of the C5 ethyl group and the incorporation of other unnatural functional groups at C5 (eg. alkyne, hydroxymethyl, diol), but to examine the incorporation of both polar and hydrophobic functional groups at C5 to further define the subtle features of this binding region. In addition, such changes (e.g., large hydrophobic groups) could impact Pgp export in resistant cell lines addressing the one significant clinical limitation to the class. Access to the targeted series was anticipated to be provided through use of the [4+2]/[3+2] cascade recently introduced for the synthesis of vindoline (Figure 2).

Results and Discussion

The initial modifications were conducted on the 4-desacetoxy-6,7-dihydrovindoline scaffold, which is accessible in 4-steps from oxadiazole **5** and the corresponding 4-substituted 4-pentenoic acids, that in turn may be coupled with catharanthine in a single step to provide the synthetic vinblastine analogue in a remarkably concise 5-steps. Including the synthesis of the common oxadiazole **5** (2–3 steps from *N*-methyl 6-methoxytryptamine) and a direct chromatographic enantiomer resolution of the intermediate cycloadducts, this approach provides the vinblastine analogues in an overall 7–8 step synthetic sequence especially suitable for systematic examination of the importance of the vindoline C5 substituent and highlighting the generality of the synthetic methodology.

The requisite 4-substituted 4-pentenoic acids **6** were coupled to oxadiazole **5** to afford cycloaddition substrates **7–11**, representing the R = H,^{9a} Me, Et,^{7a} Pr, and ethynyl series, respectively (Scheme 1). Warming a solution of the substrates **7–11** in 1,2-dichlorobenzene (DCB) afforded single diastereomers of the [4+2]/[3+2] cycloadducts **12–15**. Consistent with prior studies, the exclusive formation of a single cascade cycloadduct diastereomer is a result of indole endo [3+2] cycloaddition with the intermediate 1,3-dipole directed to the sterically less encumbered face of the stabilized carbonyl ylide opposite the newly formed lactam. The rate of the reaction of the unsubstituted substrate **7** (R = H) was considerably faster than **8–11** (6 h vs 24 h) reflecting the relative ease of the rate determining tethered dienophile initiating [4+2] cycloaddition. Although not examined in detail, all reactions were conducted in refluxing DCB under dilute conditions (1–4 mM) to preclude competitive intermolecular reactions and, in the cases examined, displayed a modest concentration dependence within the narrow range examined (see Supporting Information). Finally, it is notable that only the olefin in the substrate **11**, also bearing a pendant alkyne on the dienophile tether, served to undergo [4+2] cycloaddition with the oxadiazole.¹³ Treatment of cycloadducts **12–15** with Lawessons' reagent afforded thioamides **17–20**. The racemic intermediates were resolved by chiral phase HPLC (2 × 25 cm ChiralCel OD, α = 1.2–1.6) after the cycloaddition for **12–14** (R = H, Me, Et) and **16** (R = alkynyl) or after treatment with Lawessons' reagent for thioamide **20** (R = Pr).¹⁴ Treatment of thioamides **17–20** with Meerwin's salt followed by sodium borohydride reduction of the resulting *S*-methyl iminium

ion served to reductively remove the thioamide with concomitant diastereoselective oxido-bridge opening to afford the C5 substituted vindoline analogues **21–24** (R = H, Me, Et, Pr). Interestingly, treatment of thioamide **16** (R = alkynyl) with Lawesson's reagent afforded the corresponding thioamide in only 25% yield. In this case, a higher yield (67%) was obtained utilizing P₄S₁₀ (Scheme 2), which after treatment with Meerwin's salt and then sodium borohydride gave the C5 alkyne **25**. The corresponding vindoline analogue **26** containing a C5 vinyl substituent was also obtained from **16** utilizing an analogous synthetic sequence following an initial Lindlar reduction of the C5 alkyne (Scheme 2).

Without efforts at optimization of the conversions, the vindoline analogues **21–24** were coupled with catharanthine (**3**) enlisting the Fe(III)-promoted coupling and subsequent oxidation¹⁰ to afford the vinblastine analogues **27, 30, 33** and **36** representing the C5 = H, Me, Et, and Pr series, respectively (Scheme 3). In addition to providing the vinblastine analogues shown, the corresponding C20' epimeric leurosine analogues (**28, 31, 34, 37**) were generated in the now characteristic ca. 2:1 β : α diastereoselectivity for the introduction of the C20' alcohol and the intermediate anhydrovinblastines (**29, 32, 35**, and **38**) were also isolated in yields ranging from 6–22%.

Attempts to prepare the C5 alkynyl (**39**) or vinyl (**40**) vinblastine analogues by direct coupling of the C5 alkynyl (**25**) or vinyl (**26**) vindoline analogues were not successful, due in part to competitive oxidation of the C5 unsaturation during the oxidative stage of the biomimetic coupling (Scheme 4). However, omitting the oxidant (air, Fe₂(ox)₃) and the second step of the direct coupling afforded the C5 vinyl anhydrovinblastine analogue **41** in good yield (54%). Alternatively, protection of the alkyne of **25** as the *t*-butyldimethylsilyl alkyne **42** enabled the two-step coupling with catharanthine and subsequent oxidation to afford the corresponding analogue **43** (Scheme 5). Treatment of **43** with Bu₄NF dried over 4Å molecular sieves afforded the C5 alkyne vinblastine analogue **39**.

The vinyl substituent of the vindoline analogue **26** was converted into a series of additional key functional groups with which we could probe the introduction of polar functionality and explore a unique series of conformational constraints. Treatment of **26** with osmium tetroxide afforded diol **46** as a single diastereomer (Scheme 6). To explore the size of the tubulin binding site and since the incorporation of hydrophobic groups has been shown to overcome Pgp-mediated multidrug resistance with other classes of antitumor drugs,¹⁵ diol **46** was also converted to the dibutyrate **48** for incorporation into a vinblastine analogue. Alternatively, treatment of C5 diol **46** with sodium periodate gave aldehyde **49** and its reduction afforded the primary alcohol **50** (Scheme 7). The incorporation of **49**, as well as **50**, into the vinblastine analogues bearing a C5 hydroxymethyl or formyl substituent (R = CH₂OH or CHO vs Et) was anticipated to address directly the ability of the tubulin C5 ethyl binding site to accommodate polar functionality and the potential solvent accessibility to this site. Just as interesting, the primary alcohol **50** could be converted to lactone **51** by a facile base-catalyzed intramolecular transesterification with the C3 methyl ester. This unique lactonization alters the conformational state of the vindoline subunit. The central six-membered ring characteristically adopts a boat conformation stabilized by a transannular H-bond from the C3 hydroxyl group to N9 placing the C5 ethyl group axial and the C3 methyl ester equatorial on this ring. Not only does this potentially attenuate the basicity of N9, but it strategically places the C3 methyl ester and C4 acetate of vinblastine at the interface of the solvent with the tubulin/vinblastine complex (Figure 2b). The lactonization to provide **51** requires the vindoline central six-membered ring to adopt a chair (vs boat) conformation with both the C3 and C5 substituents axial, disrupting the C3 alcohol/N9 transannular H-bond, and would be expected to only alter the conformational features of vinblastine at the C3/C4 sites forming the interface with solvent in the complex with tubulin without perturbing the relative location of the C5 substituent. Complementary to analogue **51**, the C5

diol **46** could also be closed to give lactone **52** (Scheme 8). Moreover, the detailed ^1H NMR characterization of the **52** was utilized to confidently assign the C5 side chain secondary alcohol stereochemistry obtained through OsO_4 -catalyzed dihydroxylation of olefin **26**. The 2D ^1H - ^1H ROSEY data revealed diagnostic NOE's between H_a - H_b and H_c - H_d , defining the stereochemistry shown. Significantly, this stereochemistry rigidly places the lactone hydroxymethyl substituent in a position to extend toward the solvent interface in the vinblastine/tubulin complex.

The vindoline analogues **46**, **48**, **49**, **51**, and **52** were coupled to catharanthine using the biomimetic Fe(III) coupling and oxidation¹⁰ to afford the corresponding vinblastine analogues without optimization (Scheme 9). Coupling of the C5 aldehyde **49** occurred with concomitant reduction to afford the C5 hydroxymethyl analogue **56** and its subsequent oxidation afforded the C5 aldehyde analogue **58**. Coupling of the conformationally constrained vindoline analogues **51** and **52** gave the vinblastine analogues **59** and **60**, but interestingly failed to provide isolable quantities of the corresponding leurosine (C20' α -OH) product.

The biological activity of the vinblastine analogues was examined in three cytotoxic assays including the matched colon cancer cell lines HCT116 and HCT116/VM46, the latter of which is multidrug resistant by virtue of Pgp overexpression.¹⁵

Consistent with expectations based on past observations, the vinblastine analogues (C20' β -OH) proved more potent (ca. 10-fold) than the corresponding anhydrovinblastine derivatives (C15'-C20' double bond), that in turn were more potent than the corresponding leurosine analogues (C20' α -OH). A full table of the testing results is provided in Supporting Information (Table S1) and highlights such comparisons for the analogues prepared herein. A summary of the key results focusing on the impact of the C5 substituent is provided in Figure 3. The activity proved remarkably sensitive to the presence, size, and polar nature of the C5 substituent. Whereas the analogue bearing the C5 methyl group (**30** or **32**) matched the potency of the corresponding analogue incorporating the natural C5 ethyl substituent (**33** or **35**), the analogues lacking a C5 substituent or bearing a C5 propyl group (**36**) extending the length of this substituent by even one carbon led to ca. 10-fold losses in activity. Even more significant, the introduction of polar functionality with the C5 aldehyde group (**58**) or C5 hydroxymethyl substituent (**56**), two analogues bearing substituents closely approximating the size of the ethyl group, led to progressively larger losses in activity of ca. 10-fold and >20-fold, respectively. This indicates clearly that the tubulin ethyl binding site is exclusively hydrophobic in nature and does not benefit from or permit an interaction with the solvent interface in the bound complex. More subtly, the introduction of unsaturation into the C5 substituent also led to significant losses in activity. The alkyne **39** was found to be >5-fold less active than **33** indicating that either the rigidity of this altered C5 substituent or its π -unsaturation reduces tubulin binding affinity. Similarly, and although we did not prepare the vinblastine analogue bearing a C5 vinyl substituent, the comparison of the corresponding anhydrovinblastine analogues **35** versus **41** indicate that even the substitution of a vinyl group for the C5 ethyl group reduces activity despite the fact that the two hydrophobic substituents would be expected to embody analogous size and conformational characteristics. Finally, the vinblastine analogues possessing even larger C5 substituents (**43**, **53**, and **55**) were inactive as were the lactone derivatives **59** and **60** that alter the conformational characteristics at C3/C4.

As a result of these observations, an analogous but smaller series of comparisons of the effect of replacing the C5 substituent on a more potent vinblastine scaffold was conducted. 4-Desacetoxyvinblastine (**61**) is a naturally occurring vinca alkaloid, but it is an even more minor (ca. 10-fold) constituent of *Cantharanthus roseus* than **1** itself (0.00025% of dry leaf

weight).^{2g} It was reported to possess equally efficacious antitumor activity as **1**, albeit requiring higher doses, but was not pursued at the time of its isolation due to its even lower natural abundance.¹⁶ We recently reported the total synthesis of **61** along with the efforts that provided **1** itself,¹⁰ and its extension to provide the vinblastine analogue **62** lacking the C5 ethyl substituent (C5 R = H).^{9a,10b} The preparation of the corresponding analogue **70**, bearing a C5 methyl substituent, was undertaken and required the additional steps needed to introduce the C6–C7 double bond utilizing the cycloadduct **13**; Scheme 10.⁷ Thus, α -hydroxylation of the lactam **13** and subsequent TIPS ether protection of the free alcohol **63** provided **64**. Thiolactam formation and its reductive removal with Ra–Ni, and diastereoselective oxido bridge cleavage provided **67**. TIPS ether deprotection and subsequent regioselective alcohol elimination⁷ provided the key 4-desacetoxyvindoline analogue **69** bearing the C5 methyl substituent. Without optimization, single-step Fe(III)-mediated coupling of **69** with catharanthine (**3**) and its subsequent in situ oxidation provided the vinblastine analogue **70** (36%), its leurosidine C20' isomer **71** (15%), and the corresponding anhydrovinblastine analogue **72** (28%). Of note, this preparation of the synthetic analogue **70** of the complex natural product **61** containing a deep-seated single site modification required only 10 steps from the oxadiazole **5** with a resolution of the intermediate **13**.

The results of the examination **70** are presented in Figure 4 alongside those of the natural product **61**. Analogous to observations made with **27** versus **33**, removal of the C5 ethyl substituent (**62** vs **61**) resulted in a 10-fold loss in biological activity. However and surprisingly distinct from the observations made with **30** versus **33**, the incorporation of a C5 methyl substituent also provided an analogue that was 10-fold less potent (**70** vs **61**) and roughly equipotent with **62** (R = H). Because of the unusual nature of these results, the activity of both **70** and **30** were examined on multiple occasions with independently prepared materials to insure the accuracy of the results. As a result, we are confident that the comparisons indicate that while on occasion a methyl group may substitute effectively for a C5 ethyl group (e.g., **30** vs **33**), its incorporation may also lead to substantial (10-fold, **70** vs **61**) losses in activity especially within the more potent analogue scaffolds. Thus, not only is the C5 ethyl group uniquely effective, but the magnitude of its effects on the properties of vinblastine is surprisingly large (10-fold).

Conclusions

A concise total synthesis of a series of key vinblastine analogues is detailed that systematically probe and define the importance of the C5 ethyl substituent. The requisite deep-seated structural changes, accessible only by total synthesis, were prepared enlisting an intramolecular tandem [4+2]/[3+2] cycloaddition cascade of 1,3,4-oxadiazoles ideally suited for use in the preparation of the vindoline-derived lower subunit followed by their incorporation into the vinblastine analogues using a single-step biomimetic coupling with catharanthine. The evaluation of the key series of analogues revealed that this site is exquisitely sensitive to the presence (Et > H, 10-fold), size (Me $\leq$ Et > Pr, 10-fold), shape (Et > CH=CH₂ and C $\equiv$ CH, >4-fold), and polarity (Et > CHO > CH₂OH, >10-fold) of the C5 substituent with the corresponding analogues experiencing pronounced losses in activity even with such minor structural changes. The only exception to these observations is the selected equipotent activity observed with a C5 methyl analogue (**30** vs **33**, but not **70** vs **61**), indicating that the C5 ethyl group is surprisingly important to the properties of vinblastine ($\geq$ 10-fold), and that the tubulin binding site surrounding this ethyl substituent is remarkably important, exclusively hydrophobic in nature, restricted in size, and does not access the adjacent solvent interface of the complex. Studies targeting additional sites for single systematic changes enlisting the cycloaddition cascade synthetic strategy are in progress and will be disclosed in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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13. A single crystal X-ray structure determination of **16** confirmed the structural and stereochemical assignment (CCDC 755749).
14. In addition to confirming the structural and relative stereochemical assignments, the absolute configurations were assigned by X-ray structure determinations of a heavy atom derivative of a synthetic intermediate (CCDC 609613) leading to 4-desacetoxy-4-desethylvindoline (for R = H),^{9a} a heavy atom derivative (S) disclosed earlier (R = Et, natural enantiomer of **19**, CCDC 295590)^{7a} or with the heavy atom derivatives prepared herein: R = Me, unnatural enantiomer of **18** (CCDC 755748) and R = Pr, unnatural enantiomer of **20** (CCDC 755747). The absolute configurations of **25** (R = C≡CH), **26** (R = CH=CH₂), and their derivatives were established by conversion of the unnatural enantiomer of **16** (R = C≡CH) to **14** (R = Et) by reduction with Ra-Ni (THF, 25 °C, 15 h, 60%) and correlation of the optical rotation.
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Figure 1.
 Natural products.

Figure 2.
 (a) Synthetic strategy for C5-substituted analogues. (b) X-ray crystal structure of vinblastine bound to tubulin¹² (additional view provided in Supporting Information).

Compound	IC50 (μM)		
	HT-29	HCT-116	HCT-15
11	1.000	1.000	1.000
12	1.000	1.000	1.000
13	1.000	1.000	1.000
14	1.000	1.000	1.000
15	1.000	1.000	1.000
16	1.000	1.000	1.000
17	1.000	1.000	1.000
18	1.000	1.000	1.000
19	1.000	1.000	1.000
20	1.000	1.000	1.000
21	1.000	1.000	1.000
22	1.000	1.000	1.000
23	1.000	1.000	1.000
24	1.000	1.000	1.000
25	1.000	1.000	1.000
26	1.000	1.000	1.000
27	1.000	1.000	1.000
28	1.000	1.000	1.000
29	1.000	1.000	1.000
30	1.000	1.000	1.000
31	1.000	1.000	1.000
32	1.000	1.000	1.000
33	1.000	1.000	1.000
34	1.000	1.000	1.000
35	1.000	1.000	1.000
36	1.000	1.000	1.000
37	1.000	1.000	1.000
38	1.000	1.000	1.000
39	1.000	1.000	1.000
40	1.000	1.000	1.000
41	1.000	1.000	1.000
42	1.000	1.000	1.000
43	1.000	1.000	1.000
44	1.000	1.000	1.000
45	1.000	1.000	1.000
46	1.000	1.000	1.000
47	1.000	1.000	1.000
48	1.000	1.000	1.000
49	1.000	1.000	1.000
50	1.000	1.000	1.000
51	1.000	1.000	1.000
52	1.000	1.000	1.000
53	1.000	1.000	1.000
54	1.000	1.000	1.000
55	1.000	1.000	1.000
56	1.000	1.000	1.000
57	1.000	1.000	1.000
58	1.000	1.000	1.000
59	1.000	1.000	1.000
60	1.000	1.000	1.000
61	1.000	1.000	1.000
62	1.000	1.000	1.000
63	1.000	1.000	1.000
64	1.000	1.000	1.000
65	1.000	1.000	1.000
66	1.000	1.000	1.000
67	1.000	1.000	1.000
68	1.000	1.000	1.000
69	1.000	1.000	1.000
70	1.000	1.000	1.000
71	1.000	1.000	1.000
72	1.000	1.000	1.000
73	1.000	1.000	1.000
74	1.000	1.000	1.000
75	1.000	1.000	1.000
76	1.000	1.000	1.000
77	1.000	1.000	1.000
78	1.000	1.000	1.000
79	1.000	1.000	1.000
80	1.000	1.000	1.000
81	1.000	1.000	1.000
82	1.000	1.000	1.000
83	1.000	1.000	1.000
84	1.000	1.000	1.000
85	1.000	1.000	1.000
86	1.000	1.000	1.000
87	1.000	1.000	1.000
88	1.000	1.000	1.000
89	1.000	1.000	1.000
90	1.000	1.000	1.000
91	1.000	1.000	1.000
92	1.000	1.000	1.000
93	1.000	1.000	1.000
94	1.000	1.000	1.000
95	1.000	1.000	1.000
96	1.000	1.000	1.000
97	1.000	1.000	1.000
98	1.000	1.000	1.000
99	1.000	1.000	1.000
100	1.000	1.000	1.000

Figure 3.
Cytotoxic activity.

Compound	IC ₅₀ (nM)		
	L1210	HCT116	HCT116/VM46
62 , R = H	910	450	3500
70 , R = Me	550	560	5300
61 , R = Et	58	60	660

Anhydrovinblastine analogue

R = H	>10000	>10000	>10000
72 , R = Me	4800	7100	7900
R = Et	3900	4700	6000

Figure 4.
Cytotoxic activity.

Scheme 1.

Scheme 2.

anhydrovinblastine derivatives not shown (29, 32, 35, and 38)

Scheme 3.

Scheme 4.

Scheme 5.

Scheme 6.

Scheme 7.

Scheme 9.

Scheme 10.