Published in final edited form as:
ACS Med Chem Lett. ; 4(10): . doi:10.1021/ml400281w.

Potent Vinblastine C20′ Ureas Displaying Additionally Improved Activity Against a Vinblastine-Resistant Cancer Cell Line

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Abstract

A series of disubstituted C20′–urea derivatives of vinblastine were prepared from 20′-aminovinblastine that was made accessible through a unique Fe(III)/$NaBH_4$- mediated alkene functionalization reaction of anhydrovinblastine. Three analogs were examined across a panel of 15 human tumor cell lines, displaying remarkably potent cell growth inhibition activity (avg. IC_{50} = 200–300 pM), being 10–200-fold more potent than vinblastine (avg. IC_{50} = 6.1 nM). Significantly, the analogs also display further improved activity against the vinblastine-resistant HCT116/VM46 cell line that bears the clinically relevant overexpression of Pgp, exhibiting IC_{50} values on par with that of vinblastine against the sensitive HCT116 cell line, 100–200-fold greater than the activity of vinblastine against the resistant HCT116/VM46 cell line, and display a reduced 10–20-fold activity differential between the matched sensitive and resistant cell lines (vs 100-fold for vinblastine).

Vinblastine (**1**) and vincristine (**2**), dimeric Vinca alkaloids, are widely recognized for their use in cancer chemotherapy for the last 50 years (Figure 1).[1–3] Originally isolated from the leaves of *Catharanthus roseus* (L) G. Don,[4–6] vinblastine and vincristine were among the first small molecules shown to bind tubulin and to inhibit microtubule formation and mitosis.[1–3] Vinblastine and vincristine have continued to be studied extensively due to interest in their unique dimeric alkaloid structure, biosynthesis and clinical importance.[7–23]

Both vinblastine and vincristine are superb clinical drugs and are used in combination therapies for treatment of Hodgkin's disease, testicular cancer (80% cure rate), ovarian cancer, breast cancer, head and neck cancer, and non-Hodgkin's lymphoma (vinblastine) or used in the curative treatment regimes for childhood lymphocytic leukemia and Hodgkin's disease (vincristine). The limitation to their continued clinical use is the instances of treatment relapse with the emergence of tumor resistance derived from overexpression of P-glycoprotein (Pgp), a cell surface drug efflux transporter that lowers intracellular concentrations of many chemotherapeutic drugs including vinblastine and vincristine.

Recently, we reported an Fe(III)/$NaBH_4$-mediated free radical oxidation of the anhydrovinblastine trisubstituted alkene used to introduce the vinblastine C20′ tertiary alcohol.[24,25] This reaction was subsequently extended to provide a simple method for functionalization of unactivated alkenes with a number of free radical traps[26,27] and used for late-stage, divergent[28] preparation of otherwise inaccessible vinblastine analogs incorporating alternative C20′ functionality.[26] Although this site is known to be critical to

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The authors declare no competing financial interest.

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

the properties of vinblastine,[29,30] the prior exploration of C20′ substituent effects had been limited.[31–34] In initial studies, we found that incorporation of an amine or azide at the c20′ position provided analogs approximately 100-fold less potent than vinblastine (**1**), but conversion of the amine to a urea (**3**) provided a compound with cell growth inhibition activity equal to vinblastine.[26] The unsubstituted urea **3** approached the potency of vinblastine against the human colon cancer cell line HCT116; however, it exhibited a further decrease in activity against the matched vinblastine-resistant HCT116/VM46 cell line, which overexpresses Pgp.[35,36] Recently, we further defined the key structural features of such ureas contributing to their activity, including the importance of the H-bond donor site on the a C20′ nitrogen substituent, and determined that sterically demanding ureas are well tolerated.[37] In the course of these studies, we also observed a potential trend where further substitution of the urea terminal nitrogen reduced the differential activity of the derivatives against the matched sensitive and resistant HCT116 cell lines (NR2 > NHR > NH2), although the number of such comparisons was limited. Herein, we report the synthesis and evaluation of a key series of disubstituted C20′ urea-based analogs that have provided exceptionally potent derivatives, displaying additionally improved activity against the resistant HCT116/VM46 cell line, based on this knowledge available from our earlier studies.

Previously, we demonstrated that substitution on the terminal nitrogen of the urea resulted in an improvement of activity as seen in compound **4** (Table 1).[37] The enhancement was even more pronounced for the disubstituted ureas against the resistant HCT116/VM46 cell line where the differential in activity from sensitive HCT116 was reduced (30-fold) relative to vinblastine (90-fold) and the unsubstituted urea **3** (600-fold). In these studies, the C20′ urea with a cyclic amine **6** provided an IC_{50} of 50 nM against the resistant HCT116/VM46 cell line and displayed a differential in activity from the sensitive HCT116 cell line of only 13-fold. This compound was the most potent compound against this resistant cancer cell line of the analogs examined in our studies of vinblastine[38–44] and warranted further exploration.

We systematically probed disubstituted C20′ urea analogs, incorporating cyclic amines as the terminal nitrogen (Table 1). Compounds **6–9** exhibited little or no change in the activity against the sensitive HCT116 cell line, but show a clear trend against the resistant HCT116/VM46 cell line with the incorporation of a polar atom in the six-membered ring having a pronounced negative effect on the activity (C = S > O > NMe). After observing this trend, analogs were prepared incorporating additional non-polar functionality on the terminal cyclic amine (**11–14**). C20′ Urea vinblastine analogs in which the terminal nitrogen is allylic (**10**) or benzylic (**12–14**) provided a further enhancement in the activity of approximately 10-fold relative to vinblastine and where the resulting activity against the resistant HCT116/VM46 is 80-fold better than vinblastine and 8-fold better than the saturated piperidine-based urea **6**. Incorporation of a six-membered cyclic amine with a hydrophobic phenyl substituent that was not benzylic (**11**) to the urea nitrogen did not provide the enhanced activity in the HCT116/VM46 cell line observed with the unsaturated piperidine **10** or fused phenyl ring analogs **12–14**. This result suggests that an electronic effect is contributing to the enhanced activity and that it may not simply be the additional van der Waal interactions derived from an added hydrophobic aromatic ring. Addition of a methoxy group to the potent isoindoline (**14**) did not further impact the cell growth activity. Significant in these observations is not only the exceptional activity of the new derivatives, but their reduced differential in activity against the sensitive and resistant tumor cell line (13–16-fold versus 90-fold for **1**). Presumably, this indicates that the new derivatives are not as effective substrates for Pgp efflux as vinblastine itself, whereas the more polar analogs **8** and **9** and especially the unsubstituted urea **3** are effective substrates. Clearly, the c20′ position within vinblastine represents a key site amenable to functionalization capable of simultaneously enhancing potency and presumably decreasing relative Pgp transport central to clinical resistance.

The C20′ urea derivative **13** was examined in a tubulin binding assay conducted by measuring the competitive displacement of ^{3}H-vinblastine from porcine tubulin (Figure 2).[45] The binding studies established that **13** binds tubulin with a higher affinity than vinblastine, establishing that its enhanced potency in the cell growth functional assays correlates directly with its target tubulin binding affinity and suggests that the improved intrinsic activity is a direct result of the inhibition of microtubule formation.

In order to confirm that the exceptional activity observed in our lab would be observed by others, we had vinblastine (**1**) and compounds **10**, **12** and **13** examined offsite[46] in a more comprehensive human tumor 15-cell line panel including cell lines of clinical interest from breast, lung, colon, prostate and ovary tissue (Table 2). Compounds **10**, **12** and **13** exhibited remarkable potency against all cell lines examined with the exception of MCF-7, with all three compounds displaying at least a 10-fold improvement in activity over vinblastine (range of 10–200-fold more potent). Compound **12** exhibited exceptional potency against LNCaP-FGC (20 pM) whereas compound **13** provided the best activity against the resistant HCT116/VM46 cell line (3.5 nM) in this cell line panel and a reduced differential from the sensitive HCT116 cell line of 20-fold. The average IC50 value for vinblastine in this human tumor cell line panel was 6.1 nM, excluding the two cell lines for which it was inactive, and the comparative average IC50 values were 310 pM, 200 pM, and 200 pM for **10**, **12** and **13**, respectively, representing average enhancements of 30-fold for **12** and **13** over the activity of vinblastine.

A series of disubstituted C20′ urea derivatives of vinblastine were prepared and compounds **10** and **12–14** were found to not only possess extraordinary potency, but to exhibit further improved activity against the Pgp overexpressing vinblastine-resistant HCT116/VM46 cell line, displaying a reduced differential in activity against the sensitive and resistant HCT116 cell line of only 10- to 20-fold (vs ca. 100-fold for vinblastine). Compound **12** was found to bind tubulin with a higher affinity than vinblastine, confirming that its enhanced potency observed in the cell growth functional assays correlates with its target tubulin binding affinity. Examination of compounds **10**, **12** and **13** in a human tumor 15-cell line panel revealed that these C20′ urea analogs are on average 20- to 30-fold more potent than vinblastine across a broad spectrum of clinically relevant human cancer cell lines (range of 10–200-fold more potent), displaying low pM IC50 values (40–450 pM for **13**). Clearly, the c20′ position within vinblastine represents a key site amenable to functionalization capable of simultaneously improving tubulin binding affinity, substantially enhancing biological potency, and presumably decreasing relative Pgp transport central to clinical resistance.

Supplementary Material

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Acknowledgments

We gratefully acknowledge the financial support of the National Institutes of Health (CA042056, CA115526). We thank Oliver Gentile for the synthesis of compound **14**. We thank Craig Fairchild, Kathy Johnson and Russell Peterson for conducting and Gregory Vite and Robert Borzilleri for arranging and overseeing the assessment of **1**, **10**, **12**, and **13** at Bristol–Myers Squibb. K.K.D. is a Skaggs Fellow and T.J.B. is a NIH postdoctoral fellow (CA165303).

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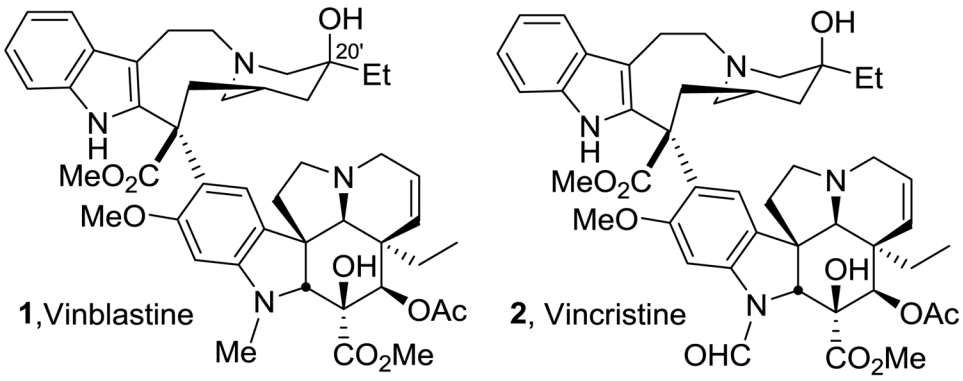
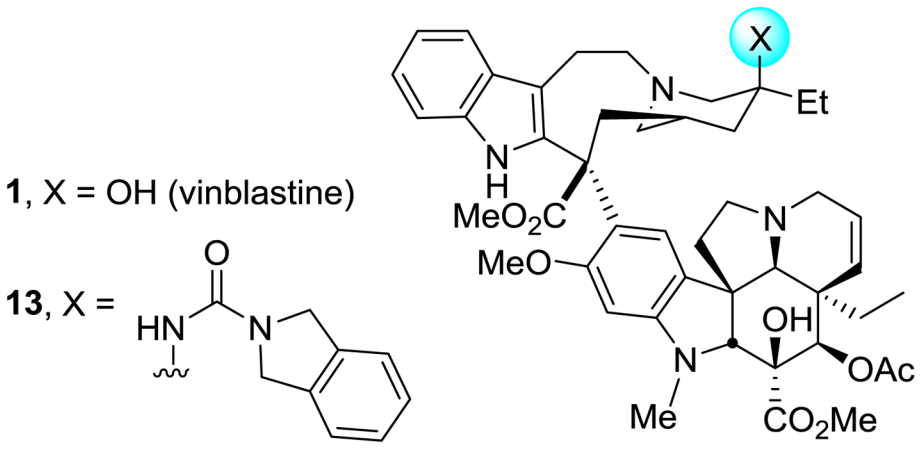


Figure 1.
Structure of vinblastine and vincristine.



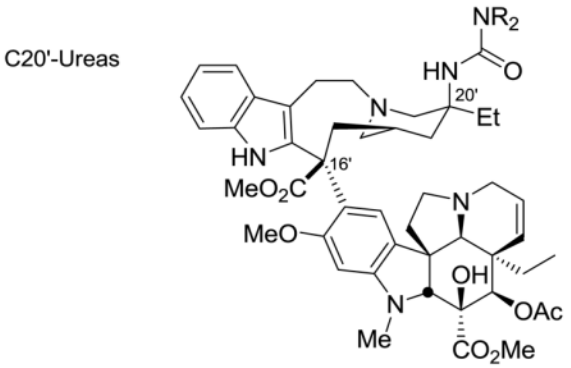
compd	% ^{3}H-vinblastine remaining bound[a]	HCT116 IC_{50} (nM)
1	50.0	6.8
13	41.2	0.60

[a]Competitive binding of ligand versus [^{3}H]VBL (1:1) measuring the remaining bound [^{3}H]VBL. Average of two repeat determinations, normalized to have dpm (25 μL VLB + 25 μL [^{3}H]VLB) = 50.0%.

Figure 2.
Tubulin binding properties.

Table 1

Cell growth inhibition.



	IC$_{50}$ (nM)[a]		
Compound	**L1210**	**HCT116**	**HCT116/VM46**
Vinblastine (**1**)	6.0	6.8	600
R = H (**3**)[b]	40	7.5	4400
R = Me (**4**)[b]	5.9	2.8	80
R = pyrrolidine (**5**)	0.70	0.72	50
R = piperidine (**6**)[b]	5.5	3.9	50
R = thiomorpholine (**7**)	2.1	0.88	50
R = morpholine (**8**)[b]	5.3	4.5	360
R = *N*-methylpiperazine (**9**)	7.7	3.4	710
R = 1,2,5,6- tetrahydropyridine (**10**)	0.52	0.52	8.4
R = 4-phenylpiperidine (**11**)	5.3	3.1	55
R = tetrahydroisoquinoline (**12**)	0.62	0.56	8.7
R = isoindoline (**13**)	0.51	0.60	7.5
R = 5-MeO-isoindoline (14)	0.61	0.69	8.7

[a] L1210 (murine leukemia cell line).

HCT116 (human colon cancer cell line).

HCT116/VM46 (resistant human colon cancer cell line, Pgp overexpression).

Avg IC$_{50}$ (4–16 determinations, SD = ±10%).

[b] Data from reference 37.

Table 2

Cell growth inhibition in 15-cell line panel.

	IC_{50} (nM)			
Cell Line[a]	**1**	**10**	**12**	**13**
AU565	4.0	0.15	0.13	0.11
NCI-H520	4.5	0.17	0.14	0.10
HCC1143	3.8	0.13	0.16	0.09
HCC70	3.5	0.21	0.13	0.04
HCT116	6.8	0.22	0.26	0.16
KPL4	2.9	0.06	0.06	0.04
LNCaP-FGC	5.1	0.45	0.02	0.24
LS174T	19.6	0.46	0.30	0.45
MCF-7	>110[b]	>12.5[b]	2.1	>12.5[b]
MDA-MB-468	4.6	0.40	0.12	0.39
SW403	7.9	0.50	1.2	0.45
T47D	5.0	0.51	0.55	0.41
ZR-75-1	8.0	0.71	0.52	0.45
PA-1	4.6	0.11	0.19	0.11
HCT116/VM46	>110[b]	6.4	6.6	3.5

[a] Cell line identities are provided in Supporting Information.

[b] Highest concentration tested.