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Synthesis and SAR of vinca alkaloid analogues

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ABSTRACT

Versatile intermediates 12′-iodovinblastine, 12′-iodovincristine and 11′-iodovinorelbine were utilized as substrates for transition metal based chemistry which led to the preparation of novel analogues of the vinca alkaloids. The synthesis of key iodo intermediates, their transformation into final products, and the SAR based upon HeLa and MCF-7 cell toxicity assays is presented. Selected analogues **27** and **36** show promising anticancer activity in the P388 murine leukemia model.

Historically, natural products have been a rich source of drug leads and candidates.[1] Vinblastine (**1**) and vincristine (**2**) were isolated in the late 1950s from the periwinkle plant *Cantharanthus roseus*.[2] The vinca alkaloids are antimitotic agents and have been shown to bind tubulin. By interfering with microtubule dynamics the vinca alkaloids prevent cell division and ultimately promote cell death in dividing cells.[3,4]

Structurally vinblastine (**1,** R = CH_3) and vincristine (**2,** R = CHO) are identical except for the substituent found on the indoline nitrogen in the lower vindoline portion of the molecules, Figure 1. Despite the single structural difference both the clinical activity and toxicity profiles of the two molecules differ. Vinblastine's primary use is to treat Hodgkin's disease and its dose limiting toxicity is bone marrow suppression. A major indication for vincristine is acute lymphocytic leukemia in children and the dose limiting toxicity is peripheral neuropathy.[5]

Throughout the 1960s and 1970s new structural analogues of the vinca alkaloids were produced by total synthesis and structural modification of the natural products.[6,7] Of note, ring contraction and dehydration of vinblastine produced vinorelbine (**3**) which

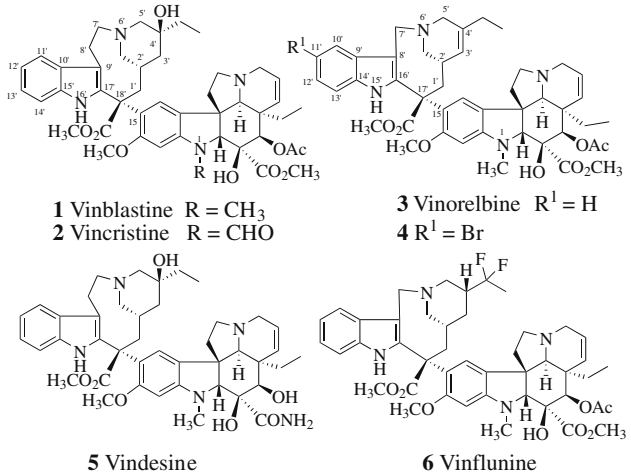

Figure 1. Vinca alkaloids.

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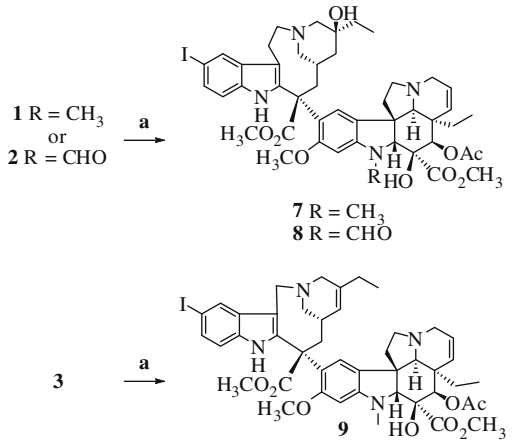


Scheme 1. Reagents and conditions: (a) *N*-Iodosuccinimide, Trifluoroacetic acid/dichloromethane (1:1) −15 °C; 85–92%.

was approved in 1991 for the treatment of non small-cell lung cancer (NSCLC) in Europe and in 1995 for use in the US.[8] Vindesine (**5**), which is approved for use in Europe for treatment of melanoma, is a vinblastine analogue with changes in the lower vindoline portion of the molecule.[9] Lastly, a reduced, bis fluorinated derivative of vinorelbine, vinflunine (**6**), is currently in phase III clinical trials to treat bladder cancer.[10]

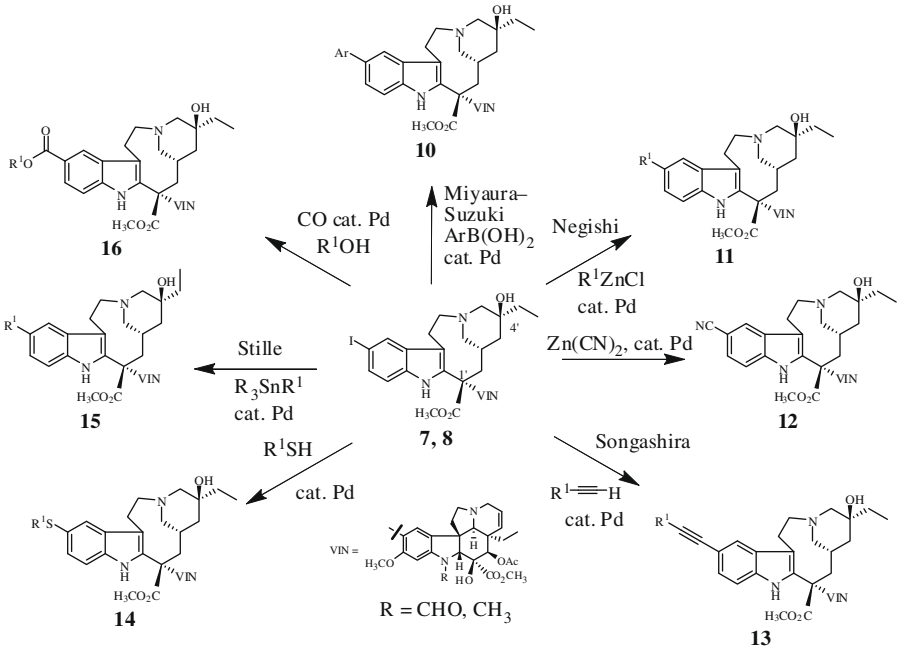
The observation that small changes in structure significantly affected the oncolytic and toxicity profile of vinca alkaloids made these compounds attractive targets for further analogue syntheses. A proven technique to generate unique structures from isolated natural products is enzymatic modification.[11] During enzymatic modification of the vinca alkaloids the chemically novel 11′-bromovinorelbine (**4**) was isolated and tested for its cytotoxic properties.[12] While this compound demonstrated interesting cytotoxic activity, it was particularly inspiring when viewed as a chemical intermediate. The abundance of mild palladium mediated reactions available today was unprecedented during the early era of vinca alkaloid exploration. A review of the literature revealed only a few 12′-vinblastine and vincristine derivatives and no 11′-vinorelbine derivatives had been disclosed.[6a,6b]

Bromination of vincristine and vinblastine with *N*-bromosuccinamide in neat trifluoroacetic acid at room temperature to produce 12′-bromovinblastine and 12′-bromovincristine in moderate yields is reported in the literature.[13,14] In our hands, these procedures produced small amounts of bis-halogenated products which complicated purification attempts and when applied to vinorelbine failed to provide synthetically useful quantities of **4**.

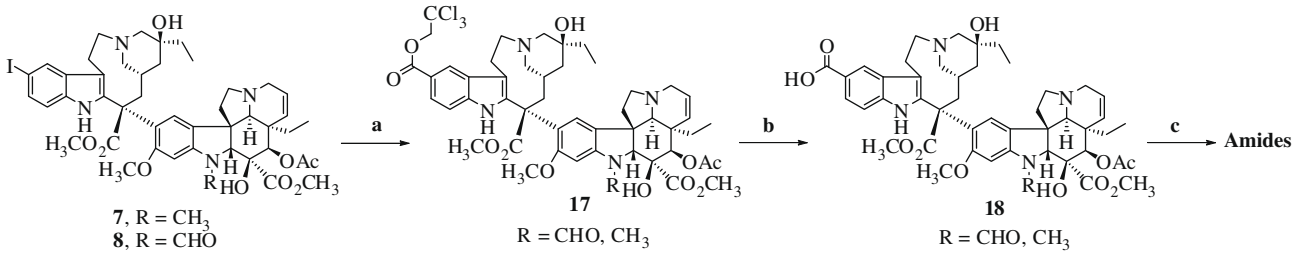
Various halogenation procedures and reagents were investigated. Optimal conditions employed a solution of *N*-iodosuccinimide (1.0 equiv) in dichloromethane, cooled to 0 °C, which was added dropwise to a vinca alkaloid (**1**, **2**, or **3**) in trifluoroacetic acid and dichloromethane (1:1) at −15 °C (Scheme 1).[15] Reactions on scales of 1–5 grams provided products **7**, **8**, **9** with >95% regioselectivity, in 85–92% yields, and were of sufficient purity to use without additional purification.

Aryl iodides **7**, **8**, and **9** were utilized in a wide range of palladium mediated reactions and many analogues could be prepared in a single step without protection or modification of the dimeric alkaloid (Scheme 2).[16] Compounds **10**, **11**, **13**, and **15** were prepared using the Miyaura-Suzuki, Negishi, Songashira, and Stille reactions, respectively. Introduction of the nitrile proved problematic initially but reproducible yields were achieved by adding small amounts of zinc metal and heating at 150 °C for 20 min in a microwave reactor to give **12**. Palladium catalyzed chemistry also facilitated synthesis of thioethers **14** and lastly, palladium catalysis under carbon monoxide atmosphere in the presence of alcohols provided access to esters **16**.

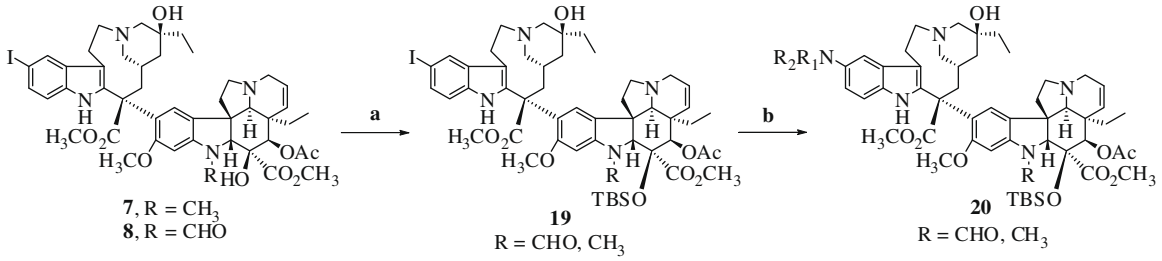
Due to the labile nature of the acetate on the vindoline ring straightforward methods to prepare 11′- or 12′-carboxylic acid



Scheme 2. Reagents and conditions: Compounds **10**—$ArB(OH)_2$, $Pd(PPh_3)_4$, TEA, DMF, 80 °C (entries 2–4, Table 1); compounds **11**—R^1Zn, $Pd(pddf)Cl_2$, dioxane, 50 °C (entries 5, 6, Table 1); compound **12**—$Zn(CN)_2$, Pd_2dba_3, Zn, dppf, DMF, microwave, 120 W, 150 °C 20 min (entry 7, Table 1); compounds **13**—(i) TMSC≡CH, $Pd(Cl)_2(PPh_3)_2$, CuI, TEA, toluene, 60 °C; (ii) 1M TBAF, THF (entry 8, Table 1); compounds **14**—R^1SNa, DMF, $Pd(dppf)Cl_2$, 50 °C (entries 9–10, Table 1) or (i) $HSCH_2CO_2t$-Bu, Pd_2dba_3, dppf, NMP, TEA, 60 °C 2 h; (ii) TFA, DCM, 0 °C, 20 min (entry 11, Table 1); compounds **15**—(i) CH_2=C(OEt)$SnBu_3$, $Pd(PPh_3)_4$, TEA; (ii) 1 N HCl (entry 12, Table 1); compounds **16**—CO, $Pd(Cl)_2(PPh_3)_2$, CH_3OH, DMF, 50 °C 14 h (entry 13, Table 1).

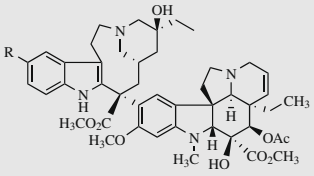


Scheme 3. Reagents and conditions: (a) $PdCl_2(dppf)$, DMF, CO, CCl_3CH_2OH; (b) Zn, THF/AcOH (1:2); (c) HATU, NMM, $NH_2R_1{\cdot}HCl$, DMF.

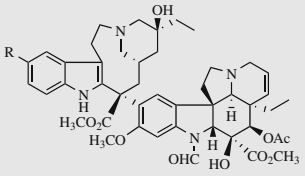


Scheme 4. Reagents and conditions: (a) 1.1 equiv TBSOTf, 10 equiv DIPEA, in CH_2Cl_2; (b) X-phos, $Pd_2(dba)_3$, *t*-BuONa, and HNR_1R_2.

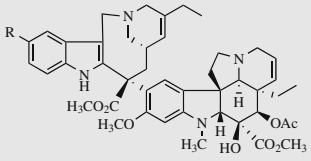
Table 1
Activity of selected vinca analogues



Vinblastine (VBL core)



Vincristine (VCR core)



Vinorelbine (VNB core)

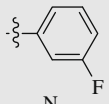
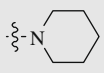
Entry	R	Vinblastine (VBL core) Compound	MCF-7 GI_{50} (nM)	HeLa GI_{50} (nM)	Vincristine (VCR core) Compound	MCF-7 GI_{50} (nM)	HeLa GI_{50} (nM)	Vinorelbine (VNB core) Compound	MCF-7 GI_{50} (nM)	HeLa GI_{50} (nM)
1	–H	**1**	0.4	0.9	**2**	1.5	1.7	**3**	2.4	2.7
2	–Ph	**21**	300	400	**22**	35	50	**23**	300	70
3		**24**	300	300				**25**	>1000	>1000
4		**26**	100	200						
5	$-CH_3$	**27**	0.3	0.3	**28**	3	1			
6	$-CH_2CH_3$	**29**	0.6	0.3	**30**	10	5			
7	–CN	**31**	3	3	**32**	4	10	**33**	6	1
8	–C≡CH	**34**	5	20				**35**	5	0.4
9	$-SCH_3$	**36**	2.0	0.6	**37**	3	3	**38**	1	0.3
10	$-SCH_2CH_3$	**39**	3	2	**40**	8	6	**41**	20	5
11	$-SCH_2CO_2H$	**42**	200	50						
12	$-COCH_3$	**43**	100	200				**44**	20	4
13	$-CO_2CH_3$	**45**	30	20				**46**	50	30
14	$-CO_2H$	**47**	>1000	>1000				**48**	>1000	>1000
15	$-CONH_2$	**49**	>1000	>1000				**50**	>1000	>1000
16	$-NH_2$	**51**	200	200				**52**	600	300
17		**53**	30	20						

Table 2
Summary of in vivo antitumor activity in P388 murine leukemia model

Treatment regimen (i.p., q4d × 3)		Median lifespan (days)	% T/C[a]	Statistical significance[b]
Compound	mg/kg			
Vehicle	—	21.0	—	—
3 (Vinorelbine)	12	24.5	117	ne
	6	24.0	114	ns
27	6	38.0	181	***
	3	28.0	133	**
36	8	51.0	243	***
	4	23.0	110	ns
38	4	26.0	124	*
	2	21.5	102	ns
40	12	22.0	105	ns
	6	22.5	107	ns

Total days of study = 60.
[a] % T/C = (median lifespan for treated animals)/(median lifespan for untreated animals) × 100.
[b] Statistical significance = Logrank test: ne = not evaluable due to >10% mortality rate, ns = not significant.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$, compared to vehicle control group.

analogues through ester hydrolysis were unfruitful. Consequently, the 2-trichloroethylester **17** was prepared as shown in Scheme 3. This material was transformed into the carboxylic acid upon treatment with zinc in acetic acid to afford the desired 11′- or 12′-carboxylic acids **18** (Scheme 3). Transformation of acids **18** to a variety of amides was best accomplished using the coupling reagent HATU (*O*-(7-aza-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium-hexafluorophosphate) and desired amine in DMF.

In order to take advantage of the Buchwald reaction for the installation of amines, the free hydroxyl group on the vindoline ring was protected as a *tert*-butyldimethylsilyl ether **19** (Scheme 4). Recently published conditions[17] using 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (X-phos), tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$), and sodium *tert*-butoxide (*t*-BuONa) in toluene proved to be the most effective allowing the substitution of a wide variety of amines **20** (Scheme 4). Primary amines (entry 16, Table 1) were available through the deprotection of the benzophenone imine adduct.

Due to the interest in finding agents effective against solid tumors, the MCF-7 (breast cancer) and HeLa (cervical cancer) cell lines were selected for initial screening of compounds. Table 1 summarizes the in vitro growth inhibition of a few representative compounds against the MCF-7 and HeLa cell lines. Entry 1 summarizes the data of the parent compounds vinblastine (VBL, **1**), vincristine (VCR, **2**), and vinorelbine (VNB, **3**). Comparison of the new analogues to the parent molecules reveals some basic structure activity relationships. Aryl or heteroaryl substituents (entries 2–4) generally produced weakly to moderately active compounds compared to their parent molecules. Strongly polar or charged functional groups as in entries 14 (carboxylic acid), and 15 (primary amide) gave poorly active analogues. If the polarity and charge of the substituents was reduced, as in entries 13 (methyl ester) and 16 (aniline), or if additional lipophilicity was added (piperdine, entry 17, **53**), moderate to good growth inhibition activity in the cell assays was observed. Additionally, when polar functional groups were attached to the indole ring through a lipophilic linker such as the thioether in entry 11 (**42**) weak to moderate activity was restored. The most active substituents proved to be small, uncharged functional groups. Methyl (entry 5), ethyl (entry 6), nitrile (entry 7), ethynyl (entry 8), and small thioethers (entries 9–10) all produced analogues with ⩽10 nM activity in the cellular assays.

A first line screen of in vivo efficacy as anticancer agents for these new vinca derivatives was performed using the P388 murine leukemia model. The in vivo P388 mouse leukemia model has been used to identify potential oncolytic agents since its development in 1955.[18] Vinorelbine, a member of the vinca alkaloid class, was used for comparison.

Four compounds, chosen for activity and diverse structure were administered by giving three doses intraperitoneally (ip) once every four days (q4d × 3) at their maximum tolerated doses (MTDs) and one-half their MTDs. The compounds were evaluated by their ability to increase the lifespan of mice compared to untreated P388 control mice.

The results from the P388 study are summarized in Table 2. The criteria used to define activity was % T/C ⩾ 125%.[19] From the results below it can be seen that **27** demonstrated activity at its MTD (6 mg/kg) and one-half its MTD (3 mg/kg). A second compound, **36**, showed good activity when dosed at its MTD producing a % T/C of 243% and two 60 day survivors (the only compound in this study to have 60 day survivors). However, when dosed at one-half its MTD **36** did not show significant activity. Compound **38** showed some benefit at 4 mg/kg, but it fell just short of the ⩾125% T/C criterion for activity. Lastly, compound **40** was virtually indistinguishable from control animals.

In summary, various novel vinca alkaloid analogues were prepared by exploiting their aryl halide intermediates. We found that smaller neutral substituents were favored for antiproliferative activity. Profiling a subset of compounds in the P388 murine leukemia model revealed two compounds (**27** and **36**) with activity superior to vinorelbine. Encouraged by the initial data, further studies of these compounds continue and will be published in due course.

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