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# Structure-Activity Relationships of Dimeric *Catharanthus* Alkaloids. 2. Experimental Antitumor Activities of N-Substituted Deacetylvinblastine Amide (Vindesine) Sulfates[1-3]

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While structure–activity relationships for vinblastine (VLB), vincristine, deacetyl-VLB, and deacetyl-VLB amide (vindesine, VDS) in several tumor and leukemia models have been reported previously,[3] the present study explores these relationships for a series of N-substituted vindesine analogues. These compounds were prepared from the reaction of deacetyl-VLB acid azide with the appropriate amines and were characterized by mass spectral analysis, $^1H$ and $^{13}C$ NMR spectra, electrometric titration, and infrared spectra. *N*-Alkylvindesines have reduced activity compared to that of VDS against the Gardner lymphosarcoma (GLS). *N*-β-Hydroxyethyl-VDS surpasses vindesine in its activity against the Ridgway osteogenic sarcoma and the GLS, whereas against the B16 melanoma it is less active than VDS. *N*-β-(4-Hydroxyphenethyl)-VDS, envisaged as a substrate for the enzyme tyrosinase, was shown to be more active than VDS against the B16 melanoma but has only marginal activity against the GLS. In terms of *collective* antitumor activity against the model systems used, vindesine emerges as the congener with optimum qualities. Bis(*N*-ethylidenevindesine) disulfide, the first example of a bridged bisvindesine and comparable to VDS in its antitumor profile, shows evidence of activity against a P388/VCR leukemia strain known to be resistant to maytansine as well as to vincristine.

Despite the relatively "minor" difference between the molecular structures of the *Catharanthus* alkaloids vinblastine (VLB, $N_a$-$CH_3$) and vincristine (VCR, $N_a$-CHO), substantial differences in the clinical usefulness and clinical toxicity of these two oncolytic agents have been noted.[3,5] Vindesine (VDS, 1, $R_1 = R_2 = H$), a chemically modified vinblastine product selected for clinical evaluation, provides an opportunity to explore the consequences of another "minor" structural change.[3]

The selection of VDS for trial in man depended on several factors. VDS possesses an experimental antitumor spectrum[3] which resembles that of VCR rather than that of the parent alkaloid VLB, while its toxicological profile[6] suggested a potential for reduced neurotoxicity relative to that of VCR. The relative ease with which a preparation of adequate purity could be secured also favored this choice.

Phase I and II clinical trial reports[7-13] indicate vindesine to be an active oncolytic agent. Clinically, vindesine appears to be less neurotoxic than VCR, and generally its administration has not had to be discontinued because of neurotoxicity.[13]

These preliminary clinical observations obtained with vindesine provide much needed feedback information for the design of further improved *Catharanthus* alkaloid modification products.

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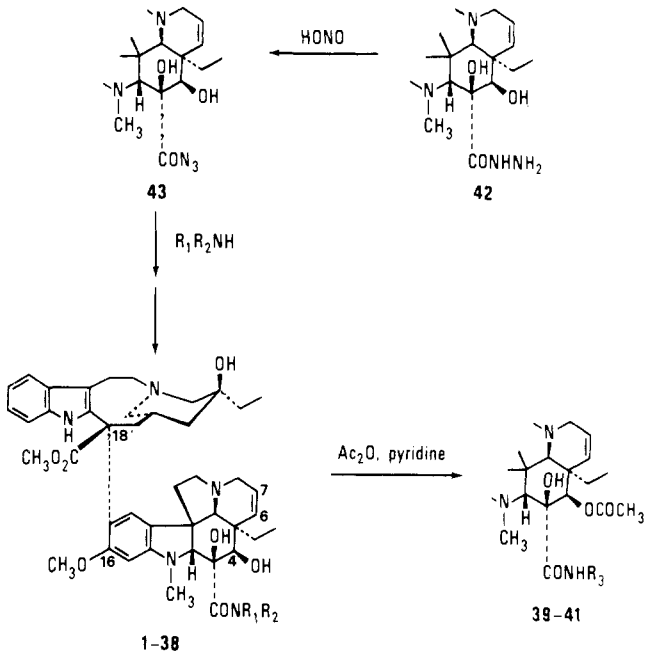
Scheme I




In this paper, we report the preparation and biological evaluation of additional N-substituted VDS congeners (**2**–**41**, see Tables I and II), including *N*-methyl-VDS (**2**), *N*-β-aminoethyl-VDS (**12**), *N*-β-hydroxyethyl-VDS (**17**), *N*-β-mercaptoethyl-VDS (**25**), the latter's oxidation product, the bridged disulfide **37**, and *N*-β-(4-hydroxyphenethyl)-VDS (**34**). In its activity against murine tumor systems and in its toxicological profile, the novel bisvindesine congener **37** occupies a unique position among the alkaloids studied thus far.

Selected VDS congeners (**17**, **25**, **26**, and **37**) were evaluated in ancillary in vitro systems, yielding information concerning tubulin binding constants,[14,15] mitotic arrest of mammalian cells in tissue culture,[16,17] and inhibition of axoplasmic transport in cat sciatic nerve.[3,18,19]

The preparation of a VDS–bovine serum albumin coupling product is described. This product serves as antigen in a radioimmunoassay (RIA) to detect clinical serum levels of VLB, VCR, and VDS, the development of which has been previously reported.[13,20]

**Chemistry.** Of the N-substituted vindesines reported (**2**–**38**), most were prepared by allowing deacetyl-VLB azide **43**[3] to react with an excess of the respective amines in dichloromethane at room temperature (Scheme I, Table I). *N*-Methyl-VDS (**2**), prepared by this method, was also made by aminolysis with methylamine in warm methanol. This latter reaction fails to give a product readily with alkylamines and substituted alkylamines (e.g., β-hydroxyethylamine for **17**). Several other derivatives (**13**, **20**–**23**, **25**, **31**, and **33**) were prepared by subsequent transformations (methods D–G) from N-substituted vindesines formed in the azide reaction.

Product formation was conveniently followed by TLC and by monitoring amide band generation in the infrared region of 1665–1675 $cm^{-1}$. Column chromatography provided the purified product as the free base which was characterized by $R_f$ value and mass spectral and $^1H$ NMR data. $^{13}C$ NMR spectra,[21] electrometric titration, and molecular weight determination (Rast method) were used to identify the disulfide **37**. Elemental analyses, which supported the characterization of VDS itself,[3] only rarely were called upon in the present work (compounds **25**–**27** and **37**). The relatively small amounts of free base product usually obtained (aside from limited physical chemical scrutiny mentioned above) were used for conversion to the sulfate salt.[3]

Acetylation of compounds **1** (VDS), **2**, and **13** using a modification of a published method[22] yielded the corresponding 4-*O*-acetyl derivatives (compounds **39**–**41**, Table II) for biological evaluation (see Table IV).

To explore the effect of sulfur functions on antitumor activity, β-mercaptoethyl-VDS (**25**), β-methylmercaptoethyl-VDS (**26**), bis(*N*-ethylidene-VDS) disulfide (**37**), and others (**27**, **30**, **36**, and **38**) were prepared.

Reaction of the azide **43** with cystamine (2:1 mol ratio) in dichloromethane solution provides a high yield of di-

Scheme II

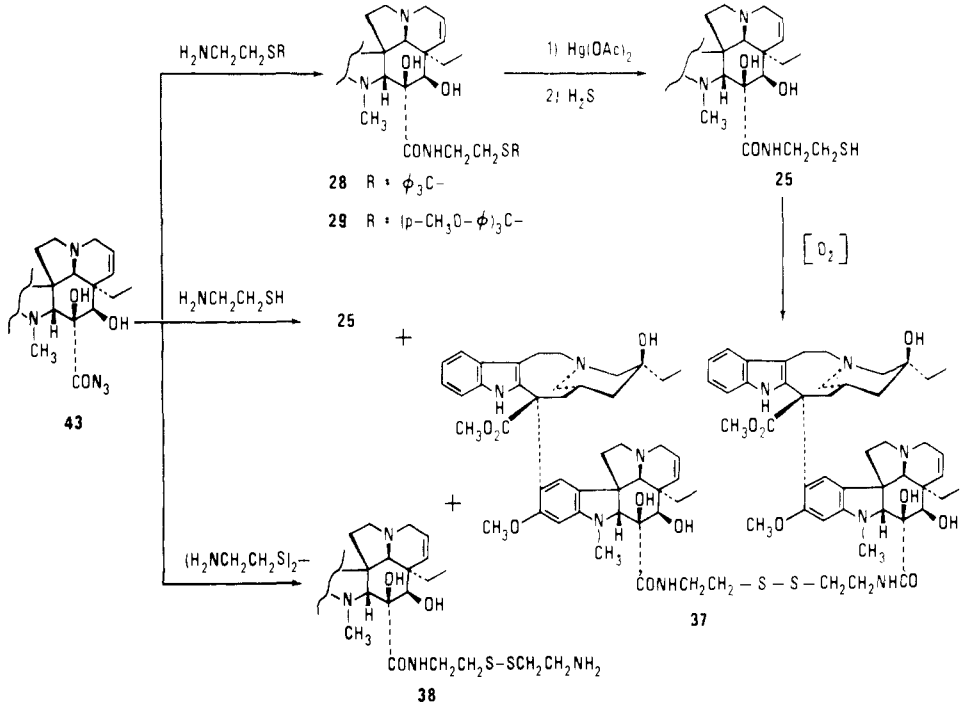
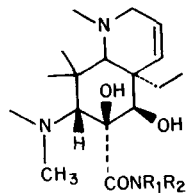
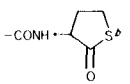


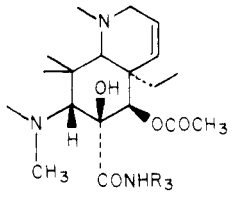
Table I




| no. | -CONR$_1$R$_2$[a] | empirical formula | method[d] | M$^+$ | additional data |
|---|---|---|---|---|---|
| 1 | -CONH$_2$ (vindesine) | $C_{43}H_{55}N_5O_7$ | A, B, C | 753 | see ref 3 |
| 2 | -CONHCH$_3$ | $C_{44}H_{57}N_5O_7$ | A, B | 767 | $^1$H NMR 2.82 (3 H, d, CONHC*H*$_3$, $J$ = 5 Hz) |
| 3 | -CONHCH$_2$CH$_3$ | $C_{45}H_{59}N_5O_7$ | A | 781 | |
| 4 | -CONHCH(CH$_3$)$_2$ | $C_{46}H_{61}N_5O_7$ | A | 795 | $^1$H NMR 1.18, 1.20 [3 H each, d, CH(CH$_3$)$_2$, $J$ = 6.5] |
| 5 | -CONH-*n*-hexyl | $C_{49}H_{67}N_5O_7$ | A | 837 | |
| 6 | -CONH-cyclohexyl | $C_{49}H_{65}N_5O_7$ | A | 835 | |
| 7 | -CONHCH$_2$Ph | $C_{50}H_{61}N_5O_7$ | A | 843 | $^1$H NMR 3.69 (2 H, d, NHC*H*$_2$Ph, $J$ = 7 Hz) |
| 8 | -CONHCH$_2$CH=CH$_2$ | $C_{46}H_{59}N_5O_7$ | A | 793 | |
| 9 | -CONHCH$_2$C≡CH | $C_{46}H_{57}N_5O_7$ | A | 791 | IR 3310 cm$^{-1}$ C≡CH C-H stretch |
| 10 | -CON(CH$_3$)$_2$ | $C_{45}H_{59}N_5O_7$ | A | 781 | $^1$H NMR 2.96, 3.46 [3 H each, s, N(CH$_3$)$_2$] |
| 11 | -CON(c-CH$_2$CH$_2$)$_2$ | $C_{47}H_{61}N_5O_7$ | A | 807 | |
| 12 | -CONHCH$_2$CH$_2$NH$_2$ | $C_{45}H_{60}N_6O_7$ | A | 796 | titr. p$K_a$' = 4.82, 7.12, 9.48 |
| 13 | -CONHCH$_2$CH$_2$NHCOCH$_3$ | $C_{47}H_{62}N_6O_8$ | D | 838 | $^1$H NMR 1.96 (3 H, s, COCH$_3$) |
| 14 | -CONHCH$_2$CH$_2$N(CH$_3$)$_2$ | $C_{47}H_{64}N_6O_7$ | A | 824 | $^1$H NMR 2.23 [6 H, s, N(CH$_3$)$_2$]; titr p$K_a$' = 4.85, 7.0, 8.5 |
| 15 | -CONHCH$_2$CN | $C_{45}H_{56}N_6O_7$ | A | 792 | $^1$H NMR 4.48 (2 H, d, NHC*H*$_2$CN, $J$ = 8 Hz) |
| 16 | -CONHCH$_2$CH$_2$CN | $C_{46}H_{58}N_6O_7$ | A | 806 | IR 2245 cm$^{-1}$ CN stretch |
| 17 | -CONHCH$_2$CH$_2$OH | $C_{45}H_{59}N_5O_8$ | A | 797 | |
| 18 | -CONHCH$_2$CH$_2$CH$_2$OH | $C_{46}H_{61}N_5O_8$ | A | 811 | |
| 19 | -CONHCH$_2$CH(OH)CH$_2$OH | $C_{46}H_{61}N_5O_9$ | A | 827 | |
| 20 | -CONHCH$_2$CH$_2$OCOCH$_3$ | $C_{47}H_{61}N_5O_9$ | D | 839 | $^1$H NMR 2.06 (3 H, s, COCH$_3$) |
| 21 | -CONHCH$_2$CH$_2$OCOCH$_2$CH$_2$CH$_3$ | $C_{49}H_{65}N_5O_9$ | D | 867 | |
| 22 | -CONHCH$_2$CH$_2$OCOC$_{17}$H$_{35}$ | $C_{62}H_{93}N_5O_9$ | D | 1063 | |
| 23 | -CONHCH$_2$CH$_2$OCOCH=CH$_2$[b] | $C_{48}H_{61}N_5O_9$ | D | 851 | |
| 24 | -CONHCH$_2$CH$_2$OCH$_3$ | $C_{46}H_{61}N_5O_8$ | A | 811 | $^1$H NMR 3.34 (3 H, s, -CH$_2$OC*H*$_3$) |
| 25 | -CONHCH$_2$CH$_2$SH | $C_{45}H_{59}N_5O_7S$ | A, E | 827[e] | see text |
| 26 | -CONHCH$_2$CH$_2$SCH$_3$ | $C_{46}H_{61}N_5O_7S$ | A | 827 | see text |
| 27 | -CONHCH$_2$CH$_2$CH$_2$SCH$_3$ | $C_{47}H_{63}N_5O_7S$ | A | 841 | $^1$H NMR 2.08 (3 H, s, SCH$_3$); Anal. S |
| 28 | -CONHCH$_2$CH$_2$SCPh$_3$ | $C_{64}H_{73}N_5O_7S$ | A | 827[f] | |
| 29 | -CONHCH$_2$CH$_2$SC(*p*-CH$_3$OPh)$_3$ | $C_{67}H_{79}N_5O_{10}S$ | A | 827[f] | $^1$H NMR 3.78 [9 H, s, C(PhOCH$_3$)$_3$] |
| 30 | -CONHCH$_2$CH$_2$SSCH$_2$CH$_2$NHCOCH$_3$ | $C_{49}H_{66}N_6O_8S_2$ | A | 827[f] | Anal. S; IR 1670 cm$^{-1}$ (amide twice ester intens) |
| 31 | -CONHCH$_2$CHO | $C_{45}H_{57}N_5O_8$ | F | 795 | $^1$H NMR 9.67 (1 H, s, CHO) |
| 32 | -CONHCH$_2$CH(OCH$_3$)$_2$ | $C_{47}H_{63}N_5O_9$ | A | 841 | see text |
| 33 | -CONHCH$_2$CH$_2$C(=NH)OCH$_3$[c] | $C_{47}H_{62}N_6O_8$ | G | 839[g] | |
| 34 | -CONHCH$_2$CH$_2$Ph-4-OH | $C_{51}H_{63}N_5O_8$ | A | 887[e] | titr p$K_a$' = 5.25, 7.21, 12.82 |
| 35 | -CONHCH$_2$CH$_2$Ph-3,4-(OH)$_2$ | $C_{51}H_{63}N_5O_9$ | A | 903[e] | |
| 36 |  | $C_{47}H_{59}N_5O_8S$ | A | 853 | IR br d 1700 cm$^{-1}$ (thiolactone) |
| 37 | -CONHCH$_2$CH$_2$S)$_2$ | $C_{90}H_{116}N_{10}O_{14}S_2$ | A | 827[f] | see text |
| 38 | -CONHCH$_2$CH$_2$SSCH$_2$CH$_2$NH$_2$ | $C_{47}H_{64}N_6O_7S_2$ | A | 827[f] | |

[a] Except as noted, compounds were tested as their sulfate salts. [b] Free base. [c] HCl salt. [d] See text for experimental: method A, azide plus amine; method B, VLB plus amine; method C, cleavage of hydrazide with Raney nickel; method D, acylation of corresponding derivative 12 or 17; method E, detritylation of **28** and **29**; method F, acid hydrolysis of **32**; method G, treatment of **16** with methanolic HCl. [e] M$^+$ + 14 (transmethylation).[50] [f] Cleavage to the thiol + 14 (transmethylation).[50] [g] M$^+$ + 1.

Table II




| | -CONHR$_3$ | empirical formula | M$^+$ |
|---|---|---|---|
| 39 | -CONH$_2$ | $C_{45}H_{57}N_5O_8$ | 795 |
| 40 | -CONHCH$_3$ | $C_{46}H_{59}N_5O_8$ | 809 |
| 41 | -CONHCH$_2$CH$_2$NHCOCH$_3$ | $C_{49}H_{64}N_6O_9$ | 880 |

sulfide **37** with only small amounts of the asymmetrical monoacylated product **38** (Scheme II). The azide **43** and an excess of cysteamine in dichloromethane react to give β-mercaptoethyl-VDS (**25**) and the bridged bisvindesine disulfide **37** in amounts varying with the reaction conditions. Presumably, air oxidation of **25** yields **37**.

In addition to other physical methods, the presence of a disulfide functionality in **37** was detected through polarographic reduction. Reductive cleavage of **37** to mercaptoethyl amide **25** was accomplished by heating with zinc and acetic acid.

Pure β-mercaptoethyl-VDS (**25**) was obtained by mercuric acetate detritylation of *N*-β-(*S*-trityl)mercaptoethyl-VDS (**28**) or of the corresponding trimethoxytrityl compound **29** and subsequent treatment of the mercuric mercaptide salt with hydrogen sulfide. Upon exposure of free base **25** to air, a new product is formed with an $R_f$ value identical in several TLC systems with that of the disulfide **37**.

Another disulfide derivative (**30**) was conveniently prepared from the azide **43** and *N*-acetylcystamine. Details for the preparation of compounds **13**, **20–23**, **31**, and **33** from **12**, **17**, **32**, and **16**, respectively, are given under the Experimental Section.

In order to develop a RIA sensitive to VLB, VCR, and VDS, the required antigen was prepared by the reaction of the azide **43** with bovine serum albumin—presumably through the ε-amino group of lysine residues—in aqueous dioxane at constant pH 9.0. Isolation and purification of the protein product by Sephadex column chromatography gave an antigen containing approximately 30–40 mol of VDS/mol of BSA.[15,20]

**Biological Results.** When comparing the chemical-modification product vindesine with the parent alkaloid vinblastine in terms of activity against several experimental tumor systems,[3,23] it was stated that it is *collective* activity that is considered significant. Collective activity, in addition to summarizing respective activities against each tumor model, includes an appreciation of the predictive merit[24] of each tumor system. While possible for a few agents, such comparison becomes impractical for the large number of compounds studied here.

One course to follow consists of comparing these compounds first against a single tumor system and selecting a few superior agents for assay against additional tumor systems. The Gardner lymphosarcoma (GLS)[25] served as the primary tumor model (Tables III and IV) and helped to select compounds for further testing against the Ridgway osteogenic sarcoma (ROS)[26] (Table V), the ip B16 melanoma[27,28] (Table VI), the P1534(J) leukemia[17] (Table VII), and the P388/S[27] and the P388/VCR[29] leukemia strains (Table VIII).

Among the N-substituted vindesines (**1–36** and **38–41**), *N*-β-hydroxyethyl-VDS (**17**) appears superior to VDS (**1**) in suppressing the growth of the GLS and the ROS. Activity of the order of that of VDS is noted for com-

Table III. Activity of Vindesine Congeners against the Gardner Lymphosarcoma in C$_3$H Mice

| | dose[b] | | | | |
|---|---|---|---|---|---|
| compd[a] | 0.05 | 0.10 | 0.20 | 0.30 | 0.40 |
| 1 | − | ++ | +++ | +++ | +++ |
| 2 | − | − | +++ | +++ | +++ |
| 3 | | − | +++ | | T |
| 4 | | − | T | | T |
| 5 | | | + | | − |
| 6 | | | − | | + |
| 7 | | | ± | − | − |
| 8 | | − | +++ | | T |
| 9 | | ++ | +++ | | T |
| 10 | | | + | T | T |
| 11 | | | − | | T |
| 12 | | | − | | − |
| 13 | | +++ | +++ | | +++ |
| 14 | | | − | | − |
| 15 | | − | ±++ | T | |
| 16 | − | − | +++ | | +++ |
| 17 | +−+ | +++ | +++ | | +++ |
| 18 | | | ++ | | ++± |
| 19 | | | ± | | ++ |
| 20 | + | ±+ | ±++ | | T |
| 21 | − | ++ | +++ | | T |
| 22 | − | − | ++ | | +++ |
| 23[c] | ++ | +++ | T | | T |
| 24 | | − | − | | − |
| 25 | | − | + | ++ | +++ |
| 26 | | − | − | | T |
| 27 | | − | − | | T |
| 28 | | − | − | | − |
| 29 | | − | − | | − |
| 30 | | − | − | ± | ++ |
| 31 | − | + | +−+ | | +++ |
| 32 | − | +++ | +++ | | T |
| 33[d] | − | − | − | | − |
| 34 | | − | − | | + |
| 35 | | − | − | | + |
| 36[c] | | − | − | T | T |
| 37 | − | − | ++ | +++ | +++ |

[a] Compounds were tested as the sulfate salts, except as noted. For methodology, see ref 17 and 23. [b] Dose in mg/kg ip daily × 8–10 days. Average of one or more tests. Key: −, inhibited tumor growth 0–25%; +, inhibited tumor growth 26–50%; ++, inhibited tumor growth 51–75%; +++, inhibited tumor growth 76–100%; T, lethal to ≥5 of 10 animals per group. [c] Free base. [d] HCl salt.

Table IV. Comparative Activity of Vindesine and 4-*O*-Acetylvindesine Congeners against the Gardner Lymphosarcoma in C$_3$H Mice

| compd[a] | dose[b] | act.[c] | compd | dose | act. |
|---|---|---|---|---|---|
| 1 | 0.5 | T | 39 | 0.5 | T |
| | 0.4 | +±− | | 0.4 | +++ |
| | 0.3 | +++ | | 0.3 | −±− |
| | 0.2 | −±+ | | 0.2 | −++ |
| | 0.1 | ±+ | | 0.1 | − |
| 2 | 0.5 | T | 40 | 0.5 | T |
| | 0.4 | ±++ | | 0.4 | T |
| | 0.3 | ±++ | | 0.3 | T |
| | 0.2 | +±+ | | 0.2 | ±++ |
| | 0.1 | − | | 0.1 | + |
| 13 | 0.5 | ±+− | 41 | 0.5 | +±+ |
| | 0.25 | ±++ | | 0.25 | +− |
| | 0.125 | +± | | 0.125 | − |

[a] See Table III, footnote *a*. [b] Dose in mg/kg ip daily × 8–10 days. Average of one or more tests. [c] See Table III, footnote *b*.

Table V. Activity of Vindesine Congeners against the Ridgway Osteogenic Sarcoma in AKR Mice

| | dose[b] | | | | |
|---|---|---|---|---|---|
| compd[a] | 0.05 | 0.10 | 0.20 | 0.30 | 0.40 |
| 1 | – | – | ++ | +++ | +++ |
| 2 | – | – | ++ | +++ | +++ |
| 3 | | – | T | | T |
| 4 | – | +++ | T | | T |
| 5 | | | – | | – |
| 6 | | | + | | ++ |
| 7 | | – | – | | +++ |
| 10 | | – | T | | T |
| 11 | | | ++ | | T |
| 12 | | – | | – | |
| 14 | | | – | | ++ |
| 15 | | – | | + | |
| 17 | ++ | +++ | +++ | | +++ |
| 18 | + | +++ | +++ | | +++ |
| 19 | | | + | | +++ |
| 20 | | | +++ | | T |
| 21 | | | +++ | | T |

[a] Compounds tested as the sulfate salts. [b] See Table III, footnote *b*. Treatment commenced 8 days after tumor implantation (see ref 17 and 23).

Table VI. Activity of Vindesine Congeners against the B16 (ip) Melanoma in C57BL/6 Mice

| | dose[b] | | | |
|---|---|---|---|---|
| compd[a] | 0.30 | 0.60 | 0.90 | 1.20 |
| 1[c] | | 117 (2) | 144 (2) | 155 (4) |
| 17 | | 51 (1) | 97 (0) | 93 (0) |
| 24 | | T | T | T |
| 25 | | 170 (4) | 141 (1) | T |
| 26 | | 163 (6) | 58 (2) | T |
| 27 | | 101 (1) | 141 (1) | T |
| 28 | | 0 (0) | 1 (0) | 4 (0) |
| 29 | | 16 (0) | 28 (0) | 35 (0) |
| 30 | | 93 (0) | 118 (0) | 127 (0) |
| 34 | | 125 (2) | 152 (4) | 190 (8) |
| 35 | | 28 (0) | 128 (1) | 154 (1) |
| 36[d] | 94 (1) | T | T | |
| 37 | | 115 (1) | 170 (4) | 170 (4) |

[a] Compounds tested as the sulfate salts, except where noted. [b] Dose in mg/kg ip days 1, 5, and 9. $10^6$ cell inoculum. Results are from a single test. Controls average day of death 19.1 days; range 16–22 days. Data recorded as percent prolongation of survival (survivors on day 60/10 animals). Toxic refers to deaths of $\geq 5$ animals of 10 that died before the first control death. [c] See also ref 23. [d] Free base.

Table VII. Activity of Vindesine Congeners against the P1534(J) Leukemia (sc) in $DBA_2$ Mice and Acute $LD_{50}$ Values in Mice

| | dose[b] | | | | | acute $LD_{50}$ (iv) in mice |
|---|---|---|---|---|---|---|
| compd[a] | 0.10 | 0.20 | 0.25 | 0.30 | 0.40 | (mg/kg)[c] |
| 1 | | ++ | +++ | +++ | T | 6.3 ± 0.6 |
| 2 | | | +++ | | | |
| 17 | ++ | | | | | 4.4 ± 0.3 |
| 18 | | +++ | | | +++ | |
| 40 | | ++ | | | | |
| VLB | | – | – | – | ++ | 10.0 ± 0.8 |
| VCR | ++ | +++ | +++ | | | 2.1 ± 0.14 |
| 37[d] | | | | | | 6.9 ± 0.6 |

[a] Sulfate salts. [b] Dose in mg/kg ip daily × 8–10 days. Average of one or more tests. For explanation of activity, see Table III, footnote *b*. [c] Acute $LD_{50}$ values kindly provided by Dr. Glen C. Todd, Toxicology Division, Lilly Research Laboratories.[6] [d] In a single experiment, compound 37, when administered at levels of 1.6, 1.2, 0.9, and 0.6 mg/kg on days 1, 5, and 9, gave activities of +++, +++, ++, and +, respectively, comparable to those shown by VDS.

Table VIII. Activity of Vindesine Congeners against the P388/S, P388/VCR, and P388/VCR/I/63 Leukemias[a]

| | expt I[d] | | | expt II[e] | |
|---|---|---|---|---|---|
| compd[b] | dose[c] | P388/S | P388/VCR | dose[c] | P388/VCR |
| 1 | 3.00 | T (0) | T (0) | 1.20 | 3 (0) |
| | 1.80 | 138 (0) | –8 (0) | 0.90 | 5 (0) |
| | 1.08 | 129 (0) | 0 (0) | 0.60 | 0 (0) |
| | 0.648 | 96 (0) | 4 (0) | | |
| 26 | not done | | | 1.20 | 6 (0) |
| | | | | 0.90 | 42 (0) |
| | | | | 0.60 | 20 (0) |
| 37 | 3.00 | T (0) | T (0) | 1.20 | 22 (0) |
| | 1.80 | 50 (1) | 33 (0) | 0.90 | 54 (0) |
| | 1.08 | 96 (1) | 46 (0) | 0.60 | 35 (0) |
| | 0.648 | 129 (0) | 50 (0) | | |
| VCR | 1.67 | 96 (1) | T (0) | 1.20 | 0 (0) |
| | 1.00 | 138 (1) | 0 (0) | 0.90 | 0 (0) |
| | 0.60 | 83 (0) | 0 (0) | 0.60 | 5 (0) |
| | 0.36 | 83 (0) | 0 (0) | | |
| VLB | 3.00 | T (2) | T (0) | 1.20 | 10 (0) |
| | 1.80 | 92 (0) | 17 (0) | 0.90 | 9 (0) |
| | 1.08 | 96 (0) | 12 (1) | 0.60 | 9 (0) |
| | 0.648 | 75 (0) | 17 (0) | | |

| | expt III[a,f] | | | | |
|---|---|---|---|---|---|
| | | median | | ca. cell kill/ dose, logs | ca. no. of cells alive at end of treat. |
| compd[b] | dose[c] | day of death | % ILS | | |
| 37 | 1.6[g] | 24.5 | +145 | 2.9 | $4.5 \times 10^2$ |
| | 1.0 | 21.5 | +115 | 2.3 | $3.0 \times 10^4$ |
| | 0.7 | 18.5 | +85 | 1.7 | $2.0 \times 10^6$ |
| VDS | 2.0 | 20.0 | +100 | 2.0 | $2.5 \times 10^5$ |
| VCR | 1.5 | 15.0 | +50 | 1.0 | $2.7 \times 10^8$ |

[a] $10^6$ cell inoculum implanted ip on day 0. Data reported as percent increase in lifespan (survivors on day 49). [b] Sulfate salts. [c] Dose in mg/kg ip days 1, 5, and 9. [d] Groups of eight male $CD2F_1$ mice. Data courtesy of Dr. Randall K. Johnson, NCI. [e] Groups of 10 $B6D2F_1$ mice. Test run at the Lilly Laboratories using the cell strain received from the NCI that was used in experiment I. [f] Data courtesy Messrs. W. Russell Laster, Jr., and John A. Montgomery, Southern Research Institute, Birmingham, Al.[36] Groups of 10 male Dublin-$CDF_1$ mice. Control animals mean day of death 10.0 days. Cell doubling time = 0.49 days. [g] $LD_{20}$.

pounds 13 and 31 against the GLS and for compounds 2 and 18 against the ROS.

In contrast to the *N*-β-hydroxyethyl derivative 17, *N*-β-aminoethyl-VDS (12), as well as *N*-β-(*N*′,*N*′-dimethylaminoethyl)-VDS (14), lacks significant activity in the GLS system. Preferential acetylation of 12 yields *N*-β-acetamidoethyl-VDS (13), a highly effective agent in the GLS system. This observation points to the desirability of a hydrogen-bonding function at this site in 13 (as well as in the hydroxy analogue 17) and to the undesirable effect of an ionized amine function in 12 ($pK_a' = 9.48$) and 14 ($pK_a' = 8.5$). Compatible with this notion, good to moderate activity against the GLS is noted for additional *N*-β-substituted ethylvindesines carrying a –CN (16), an *O*-acyl (20–23), a –SH (25), a –CHO (31), or a $-CH(OCH_3)_2$ function (32) but lack of activity for the charged imino ester hydrochloride 33.

While *N*-methylvindesine (2) is comparable to VDS in GLS–ROS activity, gradual loss of such activity and enhanced toxicity accompanies the introduction of increasingly larger alkyl groups. Increased lipophilicity, associated with such alkyl groups lacking hydrogen-bonding functions, is held mainly responsible for the

narrowing or disappearing therapeutic dose range for the *N*-alkylvindesines **2**–**6**, *N*,*N*-dimethyl-VDS (**10**), and *N*,*N*-tetramethylene-VDS (**11**). In contrast, *N*-allyl-VDS (**8**) and *N*-propargyl-VDS (**9**) have fair activity against the GLS. Whether the activity observed for the propargyl derivative **9** is due to hydrogen bonding involving the $-C{\equiv}CH$ function or to other factors[30] cannot be ascertained at this time. Little activity is noted in the GLS system for *N*-benzyl-VDS (**7**) as well as for *N*-β-(4-hydroxyphenethyl)-VDS (**35**).

Of the sulfur-containing compounds prepared, only disulfide **37** shows good activity against the GLS. Methylmercaptoalkylvindesines **26** and **27** were both found to be more toxic than **37** with little, if any, therapeutic effect when dosed daily, whereas *N*-β-mercaptoethyl-VDS (**25**) and another derivative with a disulfide group, the *N*′-acetylcystamine congener **30**, have moderate activity in the GLS system.

While VLB lacks activity against the GLS (Table I in ref 3), 4-*O*-acetylvindesine (**39**) is as active as VDS. 4-*O*-Acetylation of *N*-methyl-VDS (**2**) and of *N*-β-acetamidoethyl-VDS (**13**) results in partial loss of activity in the corresponding 4-*O*-acetyl agents **40** and **41**, respectively (Table IV). The biological activity of deacetyl-VLB in several murine test systems [GLS, ROS, B16 melanoma, P1534(J), etc.],[3] in contrast to the earlier unverifiable report of the inactivity of deacetyl-VLB in the P1534 leukemia,[22] together with the above information would tend to support the idea that the 4-*O*-acetyl function is not essential for biological activity.

To explore whether moderately reactive chemical functions, capable of covalent bond formation with –SH, $-NH_2$ groups, etc. of biological molecules, would enhance antitumor efficacy without a concomitant undue increase of toxicity, we have prepared the acrylyl ester (**23**) of **17**, the *N*-acetaldehyde VDS congener **31** and its dimethyl acetal **32**, methyl VDS-acetimidate hydrochloride (**33**), and the VDS homocysteine thiolactone[31] derivative **36** for assay against the GLS. Except for the aldehyde **31**, the activity of these agents does not surpass that of VDS (**1**), suggesting that—at least in the GLS system—such moderately reactive functions do not improve efficacy.

Against the B16 murine (ip) melanoma (Table VI), *N*-β-hydroxyethyl-VDS (**17**), in contrast to its superiority in the GLS system, is less effective than VDS (**1**). Only *N*-β-(4-hydroxyphenethyl)-VDS (**34**), ineffective against the GLS, and the disulfide **37** equal, or perhaps surpass, VDS in B16 activity. *N*-β-Mercaptoethyl-VDS (**25**) and *N*-β-methylmercaptoethyl-VDS (**26**) appear active and more toxic than VDS. Both *N*-β-(4-hydroxyphenethyl)-(**34**) and *N*-β-(3,4-dihydroxyphenethyl)-VDS (**35**) were prepared as potential substrates for activation by tyrosinase, an enzyme reportedly present at elevated levels in melanoma cells.[32] Hydroxylation of γ-L-glutaminyl-4-hydroxybenzene, the "mushroom factor" of *Agaricus bisporus*, by tyrosinase is reported to yield the corresponding 3,4-dihydroxy factor[32] agaridoxin,[33] while subsequent oxidation is thought to produce quinone-type products capable of reacting with essential –SH groups of cellular polymerases. Dr. F. Stephen Vogel, Duke University School of Medicine, kindly has assayed compound **34** against melanoma inoculae (sc) of human origin in athymic "nude" mice using the dose regimen of Table VI. No tumor size reduction was seen with **34** compared to controls.[34]

Against the P1534(J) leukemia strain, implanted subcutaneously, the activities noted (Table VII) for the substituted vindesines **2**, **17**, **18**, **37**, and **40** are comparable, but not clearly superior, to those seen with VDS and VCR. The activity noted for the disulfide **37** administered ip against the subcutaneous P1534(J) leukemia—as well as the GLS and ROS systems—points to an adequate in vivo distribution of this agent.

Table IX. Mitotic Accumulation of Chinese Hamster Ovary Cells in Culture by Vindesine and Congeners[a]

| alkaloid agent | mitotic arrest, μg/mL[b] 2.0 | 0.2 | 0.02 | 0.002 |
|---|---|---|---|---|
| **1** | | | ++ | + |
| **2** | | | +− | ·· |
| **17** | | ++ | ++ | − |
| **26** | | ++ | ++ | − |
| **37** | | ++ | + | − |
| **39** | | +++ | ++ | − |
| VLB | | +++ | ++ | − |
| VCR | | | +−+[c] | + |
| deacetyl·VLB[d] | | | +++ | − |
| decarbomethoxy·VDS[d] | ± | − | | |
| vindesine $N_b$′·oxide[d] | ++ | ± | | |
| colchicine | | ++ | − | |

[a] Test results kindly provided by George B. Boder, Lilly Research Laboratories. See also ref 23. [b] +++ = increase of 40–50% of cells in mitotic arrest over control value; ++ = 15–40%; + = 10–15%; ± = 7–10%; − = 3–7%. [c] Cytotoxicity noted. [d] The preparation and other biological properties of these compounds are discussed in ref 3.

In two experiments, first at the NCI[35] and then at the Lilly Laboratories (Table VIII), the disulfide **37** was assayed against a P388/VCR[29] strain of leukemia. An increase of about 50% in survival time occurred in mice bearing the P388/VCR strain, shown to be resistant to maytansine[29] as well as to VCR. In order to confirm and verify this observation, indicating that the P388/VCR strain used in these experiments lacks complete resistance to **37**, this agent in a third experiment (Table VIII) was assayed at the Southern Research Institute[36] against the vincristine-resistant leukemia strain designated P388/VCR/I/63. At optimum treatment with **37**, a 115–145% increase of life span of mice over that of the control group was noted, compared (on a mg/kg basis) to 100% ILS for VDS. In a more meaningful expression of antileukemic activity,[37] these data signify that treatment with the disulfide **37** reduces the vincristine-resistant P388 leukemia cell population by 2–3 logs compared to a 1.0 log reduction with VDS.

Auxiliary in vitro assay systems employed for the comparison of VDS and some congeners include the mitotic arrest of Chinese hamster ovary cells in tissue culture[16,17,23] and the binding affinity to (pig brain) tubulin.[14,15] The potency profile of the alkaloids in these systems (Tables IX and X, respectively) generally parallels that seen in the antitumor assays above in a qualitative (semiquantitative) manner.

Development of a radioimmunoassay[13,20,38] using the vindesine–bovine serum albumin product (Experimental Section) as antigen has made possible the determination of alkaloid serum levels down to 0.1 ng/mL in patients receiving VLB, VCR, or VDS. In general, the assay is highly sensitive to alkaloid products with antitumor activity of the order of VLB-VCR-VDS but shows diminished sensitivity toward alkaloids of lesser in vivo activity.[39] The development of a radioimmunoassay has made possible the pharmacokinetic analysis of the alkaloids in patients.[3,14]

**Toxicological Observations.** VDS (**1**) and *N*-methyl-VDS (**2**) show comparable activity against the GLS (Table III), the ROS (Table IV), the P1534(J) leukemia (Table VII), and in the mitotic accumulation assay (Table

Table X. Binding of Vindesine and Congeners to Tubulin[a]

| alkaloid agent | rel binding affinity[b] |
|---|---|
| VLB | 1.0 |
| VDS | 1.8 |
| 8 | 1.1 |
| 13 | 1.0 |
| 17 | 1.4 |
| 26 | 1.3 |
| 34 | 1.0 |
| 42[c] | 0.9 |
| VCR | 3.9 |
| deacetyl-VLB[c] | 1.8 |
| decarbomethoxy-VDS[c] | 0.001 |
| vindesine $N_b$-oxide[c] | 0.07 |

[a] Assay results kindly provided by W. W. Bromer and J. W. Kirk, Lilly Research Laboratories. [b] Binding constant of VLB with pig brain tubulin used is of the order of $10^6$ L/mol. Affinity data determined by competitive exchange of tubulin-bound [$^3$H]VLB by vindesine and congeners. [c] The preparation and other biological properties of these compounds are discussed in ref 3.

IX). To compare the effect of chronic administration of these congeners on the growth of mice, two groups of three mice each (average weight 18.5 ± 0.5 g) were given 1 and 2, respectively, in doses of 0.5 mg/kg ip twice per week for 4 weeks, then three such doses per week for 3 weeks, and finally six doses per week for 2 weeks. While the mice receiving VDS continued to gain weight during the 9-week experiment (average final weight 34.9 ± 0.3 g), those receiving the *N*-methyl derivative 2 gained less weight than those receiving 1 and at the 6-week point started to lose weight (average final weight 24.6 ± 0.5 g).[40]

The acute iv $LD_{50}$ values (Table VII) in mice for two vindesine congeners (17 and 37), like that for vindesine itself, are between those for VCR and VLB. From an inspection of the tumor assays reported above (Tables III–VI), it appears that *N*-alkylvindesines are more toxic than VDS. Thus, unless substituted by a hydrophilic function as in *N*-β-hydroxyethyl-VDS (17), lipophilic derivatization of the vindesine amide functionality appears to have a detrimental effect on activity in these tumor systems.

Vindesine was shown to be less active than vincristine in inhibiting axoplasmic transport in the cat sciatic-nerve model,[3,41,42] indicating a decreased neurotoxicity potential for VDS relative to that of VCR. Subsequent experiments in chickens and monkeys have reinforced this indication.[3,43]

## Discussion

To recommend that a modification product of vinblastine—e.g., vindesine—be evaluated clinically constitutes an occasion of considerable moment. Such a recommendation requires identification of that agent selected from a group of congeners which combines optimum collective experimental activity and minimal toxicity potential, together with chemical accessibility and pharmaceutical acceptability. The latter three aspects, as well as the qualities of VDS in comparison with those of VLB and VCR, have been reported.[3] The present paper is concerned with an evaluation of experimental activities of the vindesine congeners 2–36 and 38–41 and disulfide 37 in comparison with those of VDS.

Of the N-substituted vindesines examined in the ROS–GLS tumor systems (Tables III–V), *N*-β-hydroxyethylvindesine (17) appears superior to VDS in potency. In contrast, against the B16 melanoma (Table VI), 17 is appreciably less active than VDS. Both the ROS[26,44] and the B16 melanoma[24] models reportedly possess varied predictive merit in the selection of new clinical agents. In agreement with Venditti[24] on the strong predictive merit of the B16 melanoma, and because of VDS's optimum *collective* activity, we favor selection of vindesine for clinical evaluation over the hydroxyethyl congener 17. *N*-Acetamidoethyl-VDS (13), while showing good activity against the GLS, was found to be nonpotent against the B16 melanoma.[45]

Activity superior to that of VDS against the B16 melanoma is shown by *N*-β-(4-hydroxyphenethyl)-VDS (34, Table VI), a VDS congener designed as substrate for tyrosinase activation.[32] Negligible activity against the GLS, however, is cause to rank 34 below VDS in terms of collective activity. Lack of activity of 34 against a human melanoma xenograft (sc)[34] in immune depressed mice questions the role of 34 as a specific agent for melanoma control. In considering this dilemma, it is to be noted that vindesine, which shows activity against the ip B16 melanoma as well as against the (sc) P1534(J) leukemia, lacks activity against the sc B16 melanoma.[45]

Thus, among this group of congeners, vindesine itself emerges as having optimum activity against the tumor models used. The favorable preliminary clinical results reported[7,13] appear to support the selection of this vinblastine analogue.

The bisvindesine disulfide 37, because of its novel structure and the indication for its activity against the P388/VCR leukemia strain, represents a unique alkaloid agent worthy of further study.

The question as to whether 37 acts in vivo as the disulfide or as the corresponding sulfhydryl agent 25 cannot be answered conclusively at this time. The ready formation of 37 by air oxidation of 25 and superior activity of 37 in the B16 melanoma as compared with 25 suggest to its acting as the disulfide. Compound 25 exhibits good activity in the B16 melanoma test reported in Table VI, but because of the inherent instability of this derivative and the uncertainty of its oxidation state both before and after injection further testing has not been done. To explore this question, the preparation and biological assay of a number of bridged bisvindesine agents—reported in part elsewhere[46]—is in progress in these laboratories.

Further studies now in progress are aimed at an assessment of the neurotoxic potential of the disulfide 37 in the axoplasmic transport model,[41,42] in the chicken model,[43] and in a new model developed at these laboratories which employs neuronal cells from newborn rat brain in tissue culture.[47] It is hoped that results from these studies will assist in defining the chemotherapeutic merits of bridged bisvindesine 37.

## Experimental Section

Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Microelemental analyses were done on samples heated briefly (block dried) at 120 °C. IR spectra were recorded in $CHCl_3$ solution on a Perkin-Elmer 457A spectrophotometer.

$^1$H NMR 100- and 220-MHz spectra were recorded in $CDCl_3$ solution on Varian Associates HA-100 and HA-220 instruments, respectively. The presence of exchangeable protons, e.g., in -CONHR, $-NH_2$, -OH functions, was confirmed by $D_2O$ shake. $^{13}$C NMR spectra were recorded in $CDCl_3$ solution on a JEOL PS-100 instrument operating at 25.03 MHz. Chemical shifts are recorded in parts per million (δ) relative to $(CH_3)_4Si$ as internal standard. Coupling constants (*J*) are reported in Hz.

Mass spectra (MS) were obtained on a Varian MAT Model 731 double-focusing spectrophotometer. Samples were inserted into the ion source by direct probe. Electrometric titrations were run in 66% aqueous DMF. Thiol titrations were done at pH 4.0 according to the procedure of Grassetti and Murray[48] and are reported as thiol groups per mole.

TLC was performed on EM-reagents precoated silica gel F-254 plates (5 × 10, 5 × 20, and 20 × 20 cm). The presence of alkaloid

materials was detected by fluorescence in shortwave (254 nm) UV light and by spraying with ceric ammonium sulfate reagent.[49] For column chromatography, the absorbent used was Woelm silica gel (activity IV). Solvent systems used in TLC and column chromatography include the following: (A) EtOAc–MeOH (3:1); (B) EtOAc–MeOH (1:1); (C) $CH_2Cl_2$–MeOH–EtOAc (1:1:1); (D) EtOAc–EtOH (3:1).

The amines used for the preparation of the vindesine congeners (Table I) were commercially available, with the exception of those needed for compounds **28–30** as described below.

**General Reaction of Deacetylvinblastine Azide (43) with Amines. Method A.** The preparation of deacetylvinblastine hydrazide (**42**) and its conversion to the acid azide **43** has previously been reported.[3] A typical reaction is as follows.

**Preparation of *N*-β-Methylmercaptoethylvindesine (26).** Deacetylvinblastine hydrazide (**42**, 1.80 g, 2.44 mmol) was dissolved in 100 mL of 1 N HCl and the solution then cooled to 4 °C. Dry $NaNO_2$ (180 mg, 2.60 mmol) was added with stirring. After 5 min, 5% aqueous $NaHCO_3$ was added until the pH was approximately 8. The resulting suspension was then extracted with 3 × 150 mL of $CH_2Cl_2$. The extracts were combined, dried over anhydrous $Na_2SO_4$, and filtered. To this solution was added 4.0 g of β-methylmercaptoethylamine. After stirring in the dark at room temperature for 18 h, the solution was washed once with water, once with 5% aqueous $NaHCO_3$, and again with water. The organic layer was then dried over $Na_2SO_4$ and filtered, and the solvent was evaporated. TLC (system A) revealed the presence of one major component, $R_f$ 0.57, with minor components running slightly before and at the origin. The residue was applied to a silica column and eluted with system A. The appropriate fractions were combined and the solvents removed to yield 540 mg of **26** (27% yield from the hydrazide): MS $M^+$ 827, *m/e* 841 (transmethylation peak[50]), 486; titration $pK_a'$ = 5.17, 7.23; IR 3680, 3600, and 3540 (OH), 3480 (indole NH), 3410 (amide NH), 1740 ($COOCH_3$), 1675 $cm^{-1}$ (CONHR); $^1H$ NMR δ 2.12 (3 H, s, $-S-CH_3$), 2.80 (3 H, s, $N-CH_3$), 3.58 (3 H, s, $COOCH_3$), 3.78 (3 H, s, $C_{16}-OCH_3$). Anal. Calcd for $C_{46}H_{61}N_5O_7S$: C, 66.72; H, 7.43; N, 8.46; S, 3.87. Found: C, 62.58; H, 7.35; N, 7.76; S, 3.47.

Reaction of the azide with gaseous amines (in preparing compounds **1** and **2**) was done as in method C, ref 3. For amines insoluble in $CH_2Cl_2$, THF was added (10–20%, v/v) to effect partial or total solution. Amine salts were sprung to the free amines by conventional techniques. Except for compounds **23** and **33**, the sulfate salts of the amides were prepared as previously reported.[3]

**General Reaction of Vinblastine and Amines. Method B.** The reaction of vinblastine and ammonia to produce deacetylvinblastine amide (vindesine, **1**) has previously been reported (ref 3, method A). An analogous experiment using methylamine yielded deacetylvinblastine *N*-methylamide (**2**). Attempts at reacting other amines under similar conditions failed to yield the products in a desirable state of purity.

**Raney Nickel Cleavage of Deacetylvinblastine Hydrazide. Method C.** The cleavage of deacetylvinblastine hydrazide (**42**) with Raney nickel to yield vindesine (**1**) has previously been reported (ref 3, method B).

**Acylations of *N*-β-Aminoethylvindesine (12) and *N*-β-Hydroxyethylvindesine (17). Method D.** In a typical acylation, 1600 mg (2.01 mmol) of **12** was dissolved in 30 mL of $CH_2Cl_2$ and 5 mL of pyridine. Acetic anhydride (200 mg, 1.96 mmol) was then added and the solution stirred for 22 h. Methanol was added, and the solvents were evaporated. The residue was dissolved in $CH_2Cl_2$ and washed first with dilute $NH_4OH$ and then with water, and the organics were then dried over $Na_2SO_4$. After filtration of the solution, the solvent was evaporated. TLC (system B) revealed one major component ($R_f$ 0.33, starting material **12** $R_f$ 0.05). The residue was chromatographed on a silica column, eluting with system B, and the appropriate fractions were combined to yield 675 mg of **13** (40% yield): IR 3660, 3600, and 3550 (OH), 3460 (indole NH), 3400 (amide NH), 1730 ($COOCH_3$), 1660 (CONHR, twice the intensity of the starting material **12**); $^1H$ NMR δ 1.95 (3 H, s, $NHCOCH_3$), 2.78 (3 H, s, $N-CH_3$), 3.59 (3 H, s, $COOCH_3$), 3.77 (3 H, s, $C_{16}-OCH_3$); MS $M^+$ 838, *m/e* 852 (transmethylation[50]), 779, 571, 497, 355.

The acylations of **17** to give **20–23** were carried out in a similar manner and were characterized by their mass spectral fragmentation and an increased ester absorption in the IR spectra.

**Detritylation of 28 and 29. Method E.** Compounds **28** and **29** were prepared by method A with amines to be described later. The *S*-trityl compound **28** (314 mg, 0.3 mmol) was added to a suspension of 96 mg (0.3 mmol) of $Hg(OAc)_2$ in 6 mL of absolute ethanol at 0 °C. The solution was stirred for 30 min, allowing it to come to room temperature. The solvent was evaporated and the residue washed with ether. The residual matter was dissolved in 20 mL of absolute ethanol, and dry $H_2S$ was bubbled through the solution for 30 min. The resulting suspension was filtered and the solvent evaporated. The residue was chromatographed over silica eluting with system C. The appropriate fractions yielded 84 mg of **25** (34%), identical in physical and biological properties to **25** prepared by method A, to be described later. The dry material as the free base, on standing at refrigerator temperatures, developed a slower moving material after 1 day, with $R_f$ identical to **37**. Detritylation of **29** under these same conditions also gave **25**.

**Hydrolysis of *N*-β,β-Dimethoxyethylvindesine (32) to 31. Method F.** Amide **32** was prepared by method A and was characterized by IR (amide at 1665 $cm^{-1}$), MS ($M^+$ 841), and $^1H$ NMR [singlets of 3 H each at 3.38 and 3.41 for the methyls of the dimethyl acetal and a triplet at 4.42, $J_{ab}$ = 6 Hz for $CH(OCH_3)_2$]. A quantity of **32** was dissolved in 1 N HCl and allowed to react for 4 h at room temperature. The reaction was made basic with $NH_4OH$ and extracted with $CH_2Cl_2$. The $CH_2Cl_2$ solution was dried with $Na_2SO_4$, filtered, and evaporated to dryness. The residue was chromatographed on silica gel eluted with system D, and the desired fractions were combined and evaporated, giving **31** with the following physical characteristics: TLC (system D) $R_f$ 0.43 (starting material **32** $R_f$ 0.50); IR 3420 (amide NH), 1735 ($COOCH_3$ and CHO), 1675 $cm^{-1}$ (CONHR); MS $M^+$ 795; $^1H$ NMR δ 9.67 (1 H, s, CHO).

**Conversion of *N*-β-Cyanoethylvindesine (16) to 33. Method G.** Compound **16** (500 mg, 0.62 mmol) was dissolved in 250 mL of methanol previously saturated with dry HCl gas at 0 °C. The reaction was allowed to come to room temperature, and after 2.5 h the solvents were evaporated, yielding 408 mg of **33** as the trihydrochloride salt (70%). To obtain a small amount of the free base for physical chemistry, a portion was dissolved in pH 7.0 phosphate buffer and extracted with $CH_2Cl_2$, followed by evaporation of the solvent: TLC (system A) $R_f$ 0.41 (starting material **16** $R_f$ 0.47); IR 3415 (amide NH), 1740 ($COOCH_3$), 1670 $cm^{-1}$ (CONHR); MS *m/e* 839 ($M^+$ + 1), 498, 355, 154.

**Preparation of *S*-Trityl-2-mercaptoethylamine.** Trityl chloride, (55.8 g, 0.199 *m*), 2-aminoethanethiol hydrochloride (25.0 g, 0.221 *m*) and 500 mL of DMF were stirred together for 16 h. Ether was added until the solution became turbid. The solution was then extracted twice with 500 mL of water, 50% NaOH was added to bring the combined water extracts to pH 11, and the solution was then extracted with three 300-mL portions of EtOAc. The combined extracts were dried over $Na_2SO_4$ and filtered, and the solvent was evaporated, resulting in an oil (the product plus residual DMF). Water was added, and the resulting white solid was filtered off and washed with water. The solid was then recrystallized from methanol–water, yielding 43.0 g (68%) of *S*-trityl-2-mercaptoethylamine: mp 93–95 °C, lit. 90–93[51]; titration $pK_a'$ = 9.02; $^1H$ NMR (220 MHz) δ 1.22 (2 H, s, $NH_2$), 2.31 (2 H, t, $-CH_2SPh_3$), 2.58 (2 H, t, $-CH_2NH_2$), 7.40 (15 H, m, aromatics). $D_2O$ shake eliminated 1.22 peak. Anal. ($C_{21}H_{21}NS$) C, H, N, S. *S*-(*p,p',p''*-Trimethoxytrityl)-2-mercaptoethylamine was prepared in an analogous manner, giving an oil: titration $pK_a'$ = 8.91; $^1H$ NMR δ 1.25 (2 H, s, $NH_2$), 2.33 (2 H, m, $-CH_2S-$), 2.60 (2 H, m, $-CH_2NH_2$), 3.80 (9 H, s, $ArOCH_3$), doublets at 6.86 and 7.39 (6 H each, aromatics, *J* = 9.5). Anal. ($C_{24}H_{27}NO_3S$) C, H, N, S.

**Preparation of *N*-Acetylcystamine.** Cystamine dihydrochloride (22.5 g, 0.10 *m*) was added to 500 mL of methanol into which had been dissolved 10.8 g (0.20 *m*) of $NaOCH_3$. After stirring the solution for 4 h, the solvent was evaporated. The residue was stirred with ether for 1 h and filtered, and the ether was then evaporated, yielding about 10 g of cystamine base as an oil. Cystamine base (3.04 g, 0.02 *m*) was added to 400 mL of $CH_2Cl_2$. Acetyl chloride (1.56 g, 0.02 *m*) was added at a rate so to effect reflux and the reaction allowed to stir at room temperature for 16 h. The solvent was evaporated and water was added. The oil that did not dissolve was filtered off and the

aqueous layer brought to pH 8 with 5% aqueous $NaHCO_3$. The solution was extracted with $CH_2Cl_2$, the organics were dried with $Na_2SO_4$ and filtered, and the solvent was evaporated, yielding about 2 g of *N*-acetylcystamine (50%) as an oil: titration $pK_a$ = 8.80; IR 3470 and 3400 ($NH_2$), 3430 (amide NH), 1670 $cm^{-1}$ (CONHR); MS $M^+$ 194 *m/e* 152, 150, 147, 118, 76; $^1H$ NMR $\delta$ 1.42 (2 H, s, $NH_2$), 2.00 (3 H, s, $CH_3CO$–), 2.84 (2 H, t, $CONHCH_2CH_2S$), 2.90 (4 H, m, $SCH_2CH_2NH_2$), 3.58 (2 H, q, $CONHCH_2CH_2S$), 6.50 (1 H, br s, CONH). Anal. ($C_6H_{14}N_2OS_2$) C, H, N, S. A $D_2O$ shake eliminated the resonances at $\delta$ 1.42 and 6.50 and resolved the $\delta$ 3.58 peak to a triplet.

**Reaction of Deacetylvinblastine Acid Azide and 2-Mercaptoethylamine.** Deacetylvinblastine hydrazide (**42**, 12.0 g, 15.6 mmol) was converted to the acid azide **43** in the usual manner. To the azide in about 500 mL of $CH_2Cl_2$ was added about 30 g of 2-mercaptoethylamine (prepared from its hydrochloride salt by treatment with 1 equiv of sodium hydroxide solution, solid NaCl, and extraction) in about 100 mL of $CH_2Cl_2$. Immediately a precipitate formed, and in an attempt to effect solution the solution was allowed to reflux on a steam bath for 5 min and then cooled to room temperature. Pyridine (20 mL) was added and the solution stirred in the dark for 16 h. The solution was then washed with 5% aqueous $NaHCO_3$ and with water, dried over $Na_2SO_4$, and evaporated. TLC (system D) revealed **25** and **37** ($R_f$ 0.67 and 0.45, respectively) and a smaller amount of a product at 0.05. The material was chromatographed on silica and eluted with system D. The two major products were collected in the amounts of 3.0 and 1.3 g of **25** and **37**, respectively, identified by the following physical chemistry: *N*-$\beta$-mercaptoethylvindesine (**25**): $R_f$ (system D) 0.67; IR 3660, 3570, 3540 (OH), 3460 (indole NH), 3390 (amide NH), 1730 ($COOCH_3$), 1665 $cm^{-1}$ (CONHR); MS *m/e* 827 ($M^+$ + 14), 769, 768, 651, 571, 486, 395, 355, 295, 154; titration $pK_a'$ = 5.3, 7.38, 11.8; –SH titration = 0.85; $M_r$ (osmotic pressure) 944, calcd $M_r$ 814.073; $^{13}C$ NMR identical with that recorded for vindesine (**1**),[21] except that the resonance of vindesine's amide carbon at 176.7 is moved to 173.6 and extra resonances at 42.3 and 24.2 are noted. $^1H$ NMR consistent with structure. Anal. ($C_{45}H_{59}N_5O_7S$) S. Bis(*N*-ethylidenevindesine) disulfide (**37**): $R_f$ (system D) 0.45; IR identical to **25**; MS identical to **25**; titration $pK_a'$ = 5.2, 7.5; –SH titration = 0.00; $M_r$ (osmotic pressure) 1779, calcd $M_r$ 1626.130; $^{13}C$ NMR identical to **25**, except that the extra resonances are at 38.0 and 37.6; $^1H$ NMR consistent with structure. Anal. Calcd for $C_{90}H_{116}N_{10}O_{14}S_2$: S, 3.94. Found: S, 3.38.

The same products were formed under similar conditions but without reflux and pyridine.

Products **25** and **37** were prepared through alternate routes: **25** as prepared by detritylation of **28** and **29** (method E) and **37** as prepared by method A (described later) had physical characteristics identical to the respective compounds formed in the above reaction.

**Preparation of Bis(*N*-ethylidenevindesine) Disulfide (37).** Deacetylvinblastine hydrazide (**42**, 8.0 g, 10.4 mmol) was converted to the acid azide **43** in the usual manner. To the azide in about 400 mL of $CH_2Cl_2$ was added 780 mg (5.1 mmol) of cystamine free base, followed by about 75 mL of THF. After stirring in the dark for 16 h, the solution was first filtered of precipitated matter, the solvents were evaporated, and the residue was dissolved in $CH_2Cl_2$. The $CH_2Cl_2$ solution was washed with water, dried over $Na_2SO_4$, filtered, and evaporated, yielding a residue which by TLC (system D) revealed two major products. The faster ($R_f$ 0.45) corresponded to **37** obtained in the reaction of the azide with mercaptoethylamine, whereas the second product was of the same polarity ($R_f$ 0.05) as **38** formed when the azide was allowed to react with excess cystamine. The $R_f$ of the precipitate which was filtered off likewise corresponded to pure **38**. The residue was chromatographed over silica eluting with system D. The appropriate fractions yielded 2.60 g of pure **37** (32%) plus additional impure **37**. The physical chemistry of **37** was identical to that of **37** formed in the mercaptoethylamine reaction described earlier.

**Acetylation of Vindesine (1) to Vinblastine Amide (39).** Vindesine (**1**, 2.854 g, 3.79 mmol) was dissolved in 25 mL of pyridine, and 25 mL of acetic anhydride was added at one time. After storing the solution for 3 days, methanol was added and the solution evaporated. The residue was dissolved in $CH_2Cl_2$, washed twice with water, dried with $Na_2SO_4$, filtered, and evaporated. The residue was chromatographed using system D, and 1.06 g of **39** (35%) was recovered: IR 1740 ($COOCH_3$), 1695 $cm^{-1}$ (amides); MS $M^+$ 795 *m/e* 809 (transmethylation[50]), 764, 736, 454; $^1H$ NMR $\delta$ 2.05 (3 H, s, $OCOCH_3$), 2.83 (3 H, s, N-$CH_3$), 3.60 (3 H, s, $COOCH_3$), 3.77 (3 H, s, $ArOCH_3$), 5.28 (1 H, d, $C_6$-H, $J$ = 10 Hz), 5.51 (1 H, s, $C_4$-H), 5.82 (1 H, d, $C_7$-H, $J$ = 10 Hz).

Compounds **40** and **41** were prepared in a similar manner from **2** and **12** or **13**, respectively.

**40**: $^1H$ NMR $\delta$ 2.04 (3 H, s, $OCOCH_3$), 2.75 (3 H, s, N-$CH_3$), 2.80 (3 H, d, $CONHCH_3$, $J$ = 5 Hz), 3.60 (3 H, s, $COOCH_3$), 3.78 (3 H, s, $ArOCH_3$), 5.52 (1 H, s, $C_4$-H); IR 1740 (esters), 1690 $cm^{-1}$ (amide); MS $M^+$ 809 *m/e* 823 (transmethylation[50]), 750, 468.

**41**: $^1H$ NMR $\delta$ 1.94 (3 H, s, $NHCOCH_3$), 2.02 (3 H, s, $OCOCH_3$), 5.51 (1 H, s, $C_4$-H); IR 1730 (esters), 1660 (amides); MS $M^+$ 880, *m/e* 821, 539.

**Coupling of Deacetylvinblastine Acid Azide (43) to Bovine Serum Albumin.** All operations were carried out at room temperature. Crystalline bovine albumin (100 mg) was dissolved in 5 mL of 0.1 N $Na_2HPO_4$ and the pH was adjusted to 9.0 with dilute NaOH. A dried solution of deacetylvinblastine azide (100 mg) in $CH_2Cl_2$ was concentrated in vacuo to 3–4 mL. Two milliliters of dried dioxane was added and the concentration continued until essentially all the $CH_2Cl_2$ was removed.

The dioxane solution of the azide was added dropwise to the stirred protein solution. The slightly turbid mixture grew more turbid within 30 s. The stirred reaction mixture was maintained at pH 9.0 for 2.2 h using a pH-stat and 0.1 N NaOH. Excess azide was consumed by adding 40 $\mu$L of 10% $NH_4OH$ and stirring 1 h.

The reaction mixture was added dropwise with stirring to 6 volumes of ice-cold acetone. The solution was centrifuged and the pellet was washed twice with 5 mL of cold acetone. The crude product (pellet) was dried in vacuo, then dissolved at 10 mg/mL in 1 M HOAc, and applied to a 2.5 × 90 cm Sephadex G-25M column in 1 M HOAc. The excluded peak was lyophilized, giving in high yield VDS–albumin containing about 39% (w/w) VDS, as measured by ultraviolet measurement.

**Biological Testing.** The methodology and parameters used by these Laboratories in running the various bioassays [GLS, ROS, B16 (ip) melanoma, P1534J leukemia, P388/S and P388/VCR leukemias, and mitotic assay] have been previously described in detail.[17,23] The tubulin-binding experiments were done in a manner similar to those previously published.[14,15] $LD_{50}$ determinations were run in the same manner reported for that of vindesine.[6]

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## References and Notes

(1) (a) Presented in part at the 9th International Congress of Chemotherapy, London, July 13–18, 1975, abstract SC-19.

(b) 7th Annual American Pharmacognosy Society Meeting, Telemark Lodge, Cable, Wis., July 11–16, 1976, abstract 4, p 15. (c) 174th American Chemical Society Meeting, Chicago, Ill.
(2) Vindesine is the USAN council approved name for deacetylvinblastine amide (VDS). Other approved nonproprietary names cited herein are vinblastine (VLB) and vincristine (VCR), supplied as Velban® (vinblastine sulfate, Lilly) and Oncovin® (vincristine sulfate, Lilly), respectively. When reference is made in this manuscript to biological or clinical use of these or other agents, the names, numbers, or abbreviations denote the sulfate salts.
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