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# Cytotoxicity of 6,16-Disubstituted Analogues of (−)-Vincadifformine

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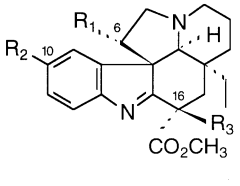
**Abstract**—Eight analogues of (−)-16-chloro-1-dehydro-6*S*-bromovincadifformine **1** were synthesized and evaluated for cytotoxicity in L1210 cell culture. None of the new compounds was more active than **1** but the modulation at C6, C16 and on the aromatic ring at C10 informs about structure–activity relationships within this series. 

In a previous publication,[1] we reported the cytotoxicity of **1** ($IC_{50}$ on L1210 leukemia cells $7\times10^{-7}$ M), a semi-synthetic alkaloid with an aspidospermane skeleton. This compound was tested by the National Cancer Institute in vitro against a total of 60 human tumor cell lines derived from nine cancer types. It revealed selectivity against leukemia (HL-60TB), non-small cell lung cancer (NCI-H522), melanoma (LOX IMVI) and renal cancer (UO-31) cell lines with $GI_{50}$ (concentrations causing 50% cell growth inhibition) between $8\times10^{-7}$ and $8\times10^{-8}$ M. Consequently, compound **1** was then selected by the NCI to continue in vivo assays in the hollow fiber-based screen,[2] but it appeared inactive.



| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **1** | Br | H | Cl |
| **2** | H | H | Cl |
| **13** | Br | Br | Cl |
| **14** | Br | $NO_2$ | Cl |
| **16** | Br | H | $NO_2$ |
| **17** | Br | Br | $NO_2$ |

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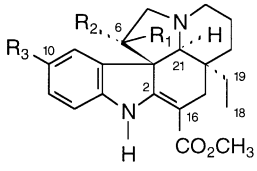
Difunctionalization on C6 and C16 seemed essential for the in vitro activity of **1**, since (−)-16-chloro-1-dehydrovincadifformine **2**[3] and (−)-6*S*-bromovincadifformine **3**[4] were not cytotoxic. This paper relates to new analogues of **1** modified in different ways on C6, C16 and also on C10, the most reactive aromatic carbon. All these compounds were synthesized from **3** with the goal of increasing the cytotoxicity and establishing structure–activity relationships in this series.

## 6-Acyloxy Analogues of 1

Replacement of bromine on C6 by an oxygenated leaving group was undertaken. Conversion of a cyclic sec-



| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **3** | H | Br | H |
| **4** | OAc | H | H |
| **5** | OBz | H | H |
| **6** | H | OBz | H |
| **11** | H | Br | Br |
| **12** | H | Br | $NO_2$ |
| **15** | H, OEt | | $NHCOCF_3$ |

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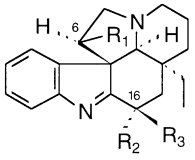
ondary bromide to an acyloxy by an SN2-type substitution is known to be unsatisfactory.[5] Fortunately, the 6-acetoxy and benzoyloxy analogues of **1** could be easily prepared otherwise by heating **3** in $CH_3COOH$ (100 °C, 2.5 h) or in melted $C_6H_5COOH$ (140 °C, 1.5 h). These reactions provided, as main compound, respectively 6*R*-acetoxyvincadifformine **4** (75%) and 6*R*-benzoyloxyvincadifformine **5** (27%). The minor derivative was easy to purify only from the reaction with $C_6H_5COOH$ and was identified as 6*S*-benzoyloxyvincadifformine **6** (6%).[6] The stereochemistry at C6 in **4**, **5** and **6** was inferred from $^1H$ NMR spectra by comparison with **3**. The conclusions were based on: (a) the observation of the H6 signal (dd, $J = 10$ and 6 Hz for **3** and **6**; d, $J = 3$ Hz for **4** and **5** at, respectively, 4.15, 5.17, 4.87 and 4.99 ppm; (b) strong NOE between H21 and H18 and 19 with the four compounds and between H21 and H6 only with **4** and **5**. These results agree with a 6*R* configuration for **4** and **5** (*endo*-6 ester group) and a 6*S* configuration for **6** (*exo*-6 ester group) as in **3**. The mechanism of these substitutions very likely implies a well-known key intermediate indole-iminium and so fits in with the solvolysis of a benzylic bromide (Scheme 1). Attack on C6 by the solvent from the less hindered side (opposite the ethyl chain and methoxycarbonyl group) explains the 6*R* configuration of the major compounds **4** and **5**. Generalization of this reaction to the substitution at C6 by other oxygenated leaving groups proved to be unsuccessful: heating **3** in some other acid solvents (TFA, PTAS in THF, melted phenol) led to complex mixtures.

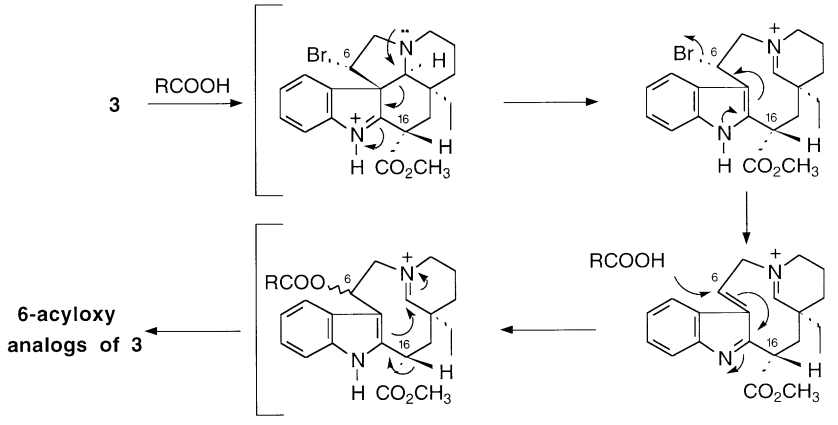
Chlorination of **4** by *t*-BuOCl ($CH_2Cl_2$, triethylamine, 30 min, 0 °C)[3] allowed isolation of two 16-chloroindolenine epimers on C16, **7** (36%) and **8** (31%). In the same manner, **5** led to the epimers **9** (36%) and **10** (31%).[7] While chlorination of vincadifformine or **3** provides only one epimer (respectively, **2** and **1**) because of the attack of C16 by the chloronium species from the less hindered β-face, isolation from **4** or **5** of the pair of epimers in a ratio 54:46 results from an about equal hindrance of α and β faces. This conclusion fully agrees with the *endo* orientation of the 6-ester group in **4** and **5**. The configuration of C16 was proved to be *R* for **7** and **9** (16β-Cl) and *S* for **8** and **10** (16α-Cl) according to mass spectrometry experiments. For all compounds **7**–**10**, EIMS show the same prominent fragment ion at *m*/*z* 370 ($M^+ - RCOOH$, $R = CH_3$ or $C_6H_5$), giving rise in CAD/MS/MS to the same ion at *m*/*z* 335 by loss of $Cl^\cdot$ to the same extent. On the contrary, the specific loss of a chlorine atom from the molecular ion is observed only for **8** and **10** (at, respectively, *m*/*z* 395 and 457), in the source as well as in the collision cell. This suggests for this fragmentation of **8** and **10** an anchimeric assistance of one of the oxygen atoms of the acetate or benzoate group and an SN1-like mechanism which result in a stabilized seven-membered acetal cation (Scheme 2) and, consequently, a *trans* configuration between C16–Cl and C6–OCOR bonds. This means a 16α-configuration of Cl can be proposed for **8** and **10**.



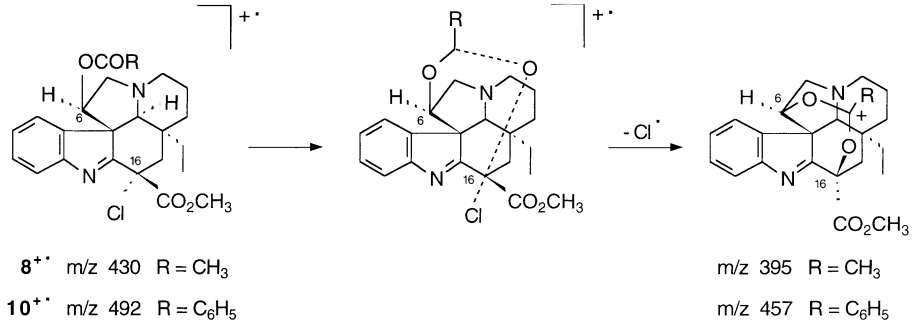
| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **7** | OAc | $CO_2CH_3$ | Cl |
| **8** | OAc | Cl | $CO_2CH_3$ |
| **9** | OBz | $CO_2CH_3$ | Cl |
| **10** | OBz | Cl | $CO_2CH_3$ |

## 10-Substituted Analogues of 1

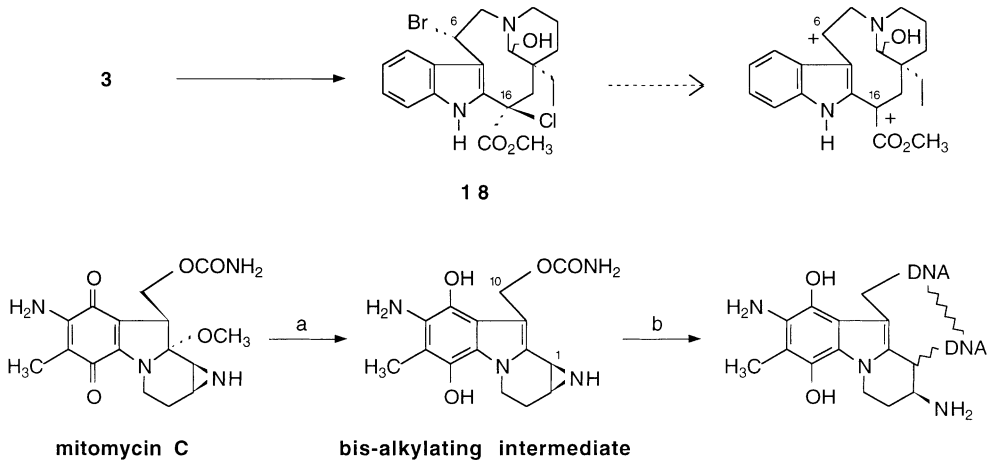
The 10-bromo and 10-nitro analogues of **1** were obtained from **3** in two steps: (a) bromination or nitration of **3** in TFA (1 equiv NBS or $HNO_3$ 65%, 2 h, rt) giving, respectively, **11** (80%) and **12** (40%); (b) chlorination of **11** and **12** by *t*-BuOCl providing, respectively, **13** (88%) and **14** (30%).[8] Synthesis of analogues of **1** 10-substituted by electron-donating groups (amino, hydroxy, alkoxy) was unfruitful: catalytic hydrogenation of the 10-nitro group, though very easy to achieve from 10-nitrovincadifformine,[9] proved to be ineffective with **12**, probably because of the neigboring 6-bromine. In other respects, treatment of **12** by $SnCl_2{\cdot}2H_2O$[10] (5 equiv in EtOH at reflux, 4 h) provoked, at the same time, the reduction of the nitro group and substitution at C6 by the solvent and afforded **15** (32%) by trifluoroacetylation of the crude dry extract (TFAA,




**Scheme 1.**




**Scheme 2.** Hypothetical mechanism for the EI-induced diastereospecific loss of $Cl^{\cdot}$ from molecular ions of **8** and **10**.




**Scheme 3.** (a) Bioreductive activation; (b) cross-linking of DNA.

$CH_2Cl_2$, 15 min, rt). Lastly, 10-methoxylation of 6-bromo-2,16-dihydrovincadifformine by iodobenzene diacetate in MeOH was unsuccessful.[11]

## 16-Nitro Analogues of 1

The previously described easy access to 16-nitroindolenines[9,12] led us to synthesize 16-nitro analogues of **1** and **13** to compare the cytotoxicity of both 16-substituted series. Nitration of **3** in $CH_3COOH$ (1 equiv $HNO_3$ 65%, 2 h, rt) furnished **12** as well as the nitroindolenine **16** (25%) while **11** provided, under the same conditions, **17** (73%) as sole compound of the reaction.[13]

## Biological Results

Though failing in its initial goal of access to more cytotoxic compounds than **1**, this study confirmed our previous suggestion of a cytotoxicity for **1** related to a probable indole intermediate **18** with two potential electrophile centers at C6 and C16.[1] This hypothesis, which has not been verified experimentally, reminds one of the mechanism of mitomycin C, an antitumor drug which leads by a bioreductive activation to a bis-alkylating intermediate with two electrophile carbons C1 and C10 in a similar position with regard to the indole nucleus, as in **18** (Scheme 3).

Analysis of the results (Table 1) demonstrates clearly: (a) a decrease of the cytotoxicity by replacement of any of the two halides at C6 or C16 by a weaker leaving group (**1** vs **7**, **8**, **9**, **10**, **16**); (b) a striking influence of stereochemistry at C16 on the biological assay (**7** vs **8** and **9** vs **10**); (c) an unfavorable effect of 10-deactivating groups upon the cytotoxicity (**1** vs **13** and **14**). Consequently, analogues of **1** with electron-donating group(s)

**Table 1**

| Compd | $IC_{50}$ (μM)[a] |
|---|---|
| **1** | 0.7 |
| **2** | 25.4 |
| **7** | 8.3 |
| **8** | 48.7 |
| **9** | 20.2 |
| **10** | 67.6 |
| **13** | 3.5 |
| **14** | 5.4 |
| **16** | 7.5 |
| **17** | 11.9 |

[a]Inhibition of L1210 cell proliferation measured by the microculture tetrazolium assay.

on the indole nucleus appear as promising derivatives but their semisynthesis implies other strategies with initial aromatic substitution of (−)-vincadifformine.

## Acknowledgements

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6. **4**: mp 140–142 °C (MeOH); $[\alpha]_D$−64 (*c* 0.3, $CHCl_3$); HRMS calcd for $C_{23}H_{28}N_2O_4$ 396.2049, found 396.2049. **5**: amorphous; $[\alpha]_D$ −371 (*c* 0.3, $CHCl_3$); HRMS calcd for $C_{28}H_{30}N_2O_4$ 458.2206, found 458.2206. **6**: amorphous; HRMS calcd for $C_{28}H_{30}N_2O_4$ 458.2206, found 458.2214.
7. **7** and **8**: amorphous; HRMS calcd for $C_{23}H_{27}{}^{35}ClN_2O_4$ 430.1659, found, respectively, 430.1652 and 430.1650. **9** and **10**: amorphous; HRMS calcd for $C_{28}H_{29}{}^{35}ClN_2O_4$ 492.1816, found, respectively, 492.1807 and 492.1808.
8. **11**: amorphous; $[\alpha]_D$ −496 (*c* 1.1, $CHCl_3$); HRMS calcd for $C_{21}H_{24}{}^{79}Br_2N_2O_2$ 494.0205, found 494.0211. **12**: amorphous; $[\alpha]_D$ −173 (*c* 0.3, $CHCl_3$); HRMS calcd for $C_{21}H_{24}{}^{79}BrN_3O_4$ 461.0950, found 461.0983. **13**: mp 143–145 °C (MeOH); $[\alpha]_D$ −51 (*c* 1.4, $CHCl_3$); HRMS calcd for $C_{21}H_{23}{}^{79}Br_2{}^{35}ClN_2O_2$ 527.9815, found 527.9787. **14**: mp 103–105 °C (MeOH); $[\alpha]_D$ +5 (*c* 0.3, $CHCl_3$); HRMS calcd for $C_{21}H_{23}{}^{79}Br^{35}ClN_3O_4$ 495.0561, found 495.0544.
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13. **16**: mp 189–191 °C (MeOH); $[\alpha]_D$ −151 (*c* 0.7, $CHCl_3$); HRMS calcd for $C_{21}H_{24}{}^{79}BrN_3O_4$ 461.0950, found 461.0987. **17**: mp 182–184 °C (MeOH); HRMS calcd for $C_{21}H_{23}{}^{79}Br_2N_3O_4$ 539.0055, found 539.0028.