# Articles

## Antineoplastic Activity of Didemnin Congeners: Nordidemnin and Modified Chain Analogues[1]

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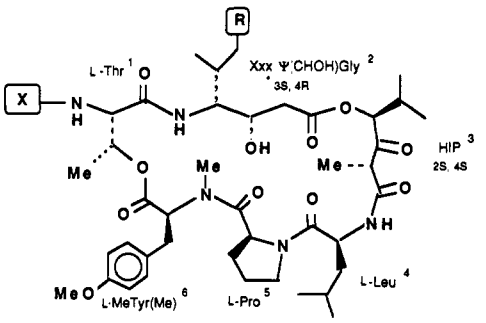
Nordidemnin (**2**), a natural analogue of the marine cyclodepsipeptide didemnin B (**1b**), showed cytotoxic activity against L1210 leukemia and antineoplastic activity against P388 leukemia as well as B16 melanoma; nordidemnin (**2**) was as active as didemnin B (**1b**). The influence of synthetic modifications in the linear peptidic chain on in vitro and in vivo activity was also studied. Replacement of the terminal lactyl residue by mandelyl and 3-(*p*-hydroxyphenyl)propionyl residues in compounds **3** and **4**, respectively, did not affect the cytotoxic activity against L1210 leukemia ($ID_{50}$ of 1.1 nM and 1.2 nM, respectively) or the in vivo activity against P388 leukemia. Unlike these aromatic substituants, the lipophilic palmityl residue induced a dramatic loss in cytotoxic activity. The inverted chirality of the MeLeu joining residue in compound **6** caused a marked reduction in the in vitro activity.

A symbiotic association between an ascidian and a unicellular alga[2] is responsible for the production of a highly potent antineoplastic group of natural compounds, the didemnins.[3] Didemnin B (**1b**), the major component of *Tridemnum solidum*[4] and *Tridemnum cyanophorum*, a tunicate from the Guadeloupean coast,[5] was considered as the more potent derivative of this class.[4] Preliminary studies have shown that didemnin B is active against a variety of DNA viruses and somewhat more active against a number of lethal RNA viruses. This compound was early shown to be effective in vivo against vaginal herpes simplex type 2 infections in mice but unfortunately ineffective against a number of viruses, including the AIDS virus HIV.[6] Didemnin B has also been shown to be active against B16 melanoma, both in vitro and in vivo, P388 leukemia in vivo and L1210 in vitro.[6] Activity against a number of human tumor stem cell lines has also been demonstrated and phase 1 clinical trials have been initiated.[7] Biochemical studies have shown that didemnin B acts as a protein synthesis inhibitor; it cannot be considered as a phase-specific agent since it blocks or freezes cells in all phases.[8] Characterization of the precise mechanism of didemnin B inhibition awaits further investigation.

The cyclodepsipeptide structure of didemnins was published in the early 1980s[9] and the exact determination

**Scheme I**




| | |
|---|---|
| **1a** : R = $CH_3$, X = D-MeLeu | didemnin A |
| **1b** : R = $CH_3$, X = L-Lac-Pro-D-MeLeu | didemnin B |
| **1c** : R = $CH_3$, X = L-Lac-D-MeLeu | didemnin C |
| **1d** : R = $CH_3$, X = L-pGlu-$(Glu)_3$-L-Lac-Pro-D-MeLeu | didemnin D |
| **1e** : R = $CH_3$, X = L-pGlu-$(Glu)_2$-L-Lac-Pro-D-MeLeu | didemnin E |
| **2** : R = H, X = L-Lac-Pro-D-MeLeu | nordidemnin |
| **3** : R = H, X = R-$(C_6H_5)$(HO)CH-CO-Pro-D-MeLeu | Man-nordidemnin |
| **4** : R = H, X = p-$(OH)C_6H_4(CH_2)_2$CO-Pro-D-MeLeu | Hpp-nordidemnin |
| **5** : R = H, X = $CH_3(CH_2)_{14}$CO-Pro-D-MeLeu | Pal-nordidemnin |
| **6** : R = H, X = L-Lac-Pro-L-MeLeu | [MeLeu]7-nordidemnin |

(1) Abbreviations and symbols follow the recommendations of the IUPAC-IUB Joint Comission on Biochemical Nomenclature (Eur. J. Biochem. **1984**, *138*, 9). In addition the following abbreviations are used: BOP, (1*H*-1,2,3-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; Lac, lactyl; Man, mandelyl; Hpp, 3-(*p*-hydroxyphenyl)-propionyl; Pal, palmityl; HIP, (hydroxy-2-isovaleryl)-2-propionic acid; NMM, *N*-methylmorpholine.

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of nonproteinogenic units disclosed more recently.[10] Didemnin B (**1b**) is composed of a lactyl Pro D-MeLeu dipeptide chain branched to the cyclic hexadepsipeptide. Several analogues with a longer chain (didemnins D (**1d**) and E (**1e**)) or shorter chain (didemnins A ((**1a**) and C (**1c**)) have also been isolated from tropical tunicates (Scheme I).[3] Didemnin A (**1a**) and didemnin C (**1c**) were shown to be less potent than didemnin B (**1b**), and the biological profile of the other analogues was not clearly defined.[4] A minor component nordidemnin (**2**) was also extracted from *Trididemnum* species.[11,12] In nordidemnin (**2**), the nonproteinogenic γ-amino β-hydroxy acid residue in position 2 is derived from D-valine instead of from D-*allo*-isoleucine as in didemnin B (**1b**), as has been shown by NMR studies[12] and total synthesis.[13] Herein, we describe the in vivo and in vitro antitumor activities of nordidemnin (**2**) and of the synthetic acyl dipeptide chain modified analogues **3–6**.

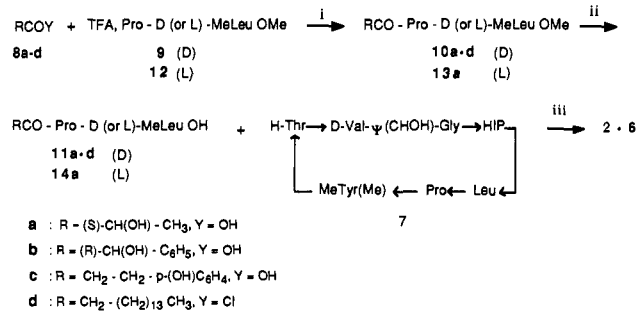
The choice of the target molecules was dictated by the results obtained on structure–activity relationship data for the natural didemnins. The conformational analysis of didemnins in solution has been performed on didemnin B (**1b**) and didemnin A (**1a**).[14] Both molecules showed very similar backbones for different chain length, and the decrease in biological potency was attributed more to the chemical changes than to conformational modifications. We proved likewise the similar conformation of both didemnin B (**1b**) and nordidemnin (**2**) which contains a modified residue in the ring structure.[12] To determine the structural requirements necessary to preserve antineoplastic activity, we replaced the terminal lactyl residue by two aromatic residues, mandelyl in **3** and (*p*-hydroxyphenyl)propionyl in **4**, and the more lipophilic palmityl residue in **5**. The stereochemical requirement for the MeLeu[7] residue was also examined by preparing [L-MeLeu[7]]nordidemnin (**6**).

## Chemistry

The original strategy we described previously for the synthesis of nordidemnin was based on the independent preparation of the cyclodepsipeptide **7** and the lacyl-Pro-D-MeLeu dipeptide **11a**.[13]

The modified acyl dipeptide chains **10b–d** were prepared as described for the lactyl dipeptide **10a**.[13] From the dipeptide Pro-D-MeLeu-OMe (**9**), the mandelyl **10b** and (*p*-hydroxyphenyl)propionyl **10c** derivatives were obtained by using the BOP coupling method,[15] while the palmityl residue was coupled as its commercially available acid chloride to give **10d**. The L-MeLeu isomer **13a** was also obtained by BOP acylation of Pro-MeLeu-OMe (**12**) with lactic acid. The dipeptide Boc-Pro-MeLeu-OMe was prepared in 81% yield by the recently described BroP coupling method.[16] The deprotected *N*-acyl dipeptides **11a–d** and **14a** were obtained by NaOH saponification, with nonobservable epimerization, as assessed by $^1$H NMR analysis of both epimers **11a** and **14a**.[17] The synthesis of compounds **3–6** was achieved by coupling of the appropriate purified acyl dipeptide **11a–d** or **14a** with the common cyclic part **7** with use of the BOP reagent under the usual conditions outlined in Scheme II. HPLC analytical analysis of the coupling reaction between **11a** and the ring system **7** showed the formation of two compounds in a 95:5 ratio.[18] Beside the major expected nordidemnin (**2**), the minor compound was proved to be identical with the [L-MeLeu[7]]nordidemnin (**6**). Compound **6** was prepared independently from **14a** but with 34% epimerization (this discrepancy is under investigation). Synthesis of compounds **3** and **4**, bearing the D-MeLeu joining residue, also led to 5% epimerized compound, as shown by HPLC.[18] Enantiomerically pure material for biological testing was obtained by preparative HPLC, except for compound **5** which was subjected to Lobar column chromatography.

**Scheme II.**[a] Synthesis of Nordidemnin (**2**) and Analogues **3–6**




[a] (i) 4 equiv of $NaHCO_3$, 1 equiv of BOP, DMF, 24 h; except for **8d**, 2 equiv of $Et_3N$, DMF, 24 h. (ii) 3 equiv of NaOH (2 M), MeOH, 90%. (iii) 5 equiv of NMM, 2 equiv of BOP, 2 equiv of **11** or **14**, 3 h.

**Table I.** Effect of Didemnin B (**1b**), Nordidemnin (**2**), and Synthetic Analogues on the Growth of L1210 Cells[a]

| | incubation time | | | |
|---|---|---|---|---|
| | 24 h | | 48 h | |
| compd[b] | ng/mL | nM | ng/mL | nM |
| **1b**[c] | 3.7 | 3.3 | 1.2 | 1.4 |
| **2** | 3.6 ± 1.1 | 3.2 ± 1 | 1.9 ± 0.7 | 1.7 ± 0.7 |
| **3** | 2.0 | 1.8 | 1.3 | 1.1 |
| **4** | 4.8 | 4.1 | 1.5 | 1.2 |
| **5** | >1000 | >790 | >1000 | >790 |
| **6** | 1265 ± 140 | 1151 ± 126 | 589 ± 22 | 536 ± 20 |

[a] ID50 as a function of incubation time with drug. [b] Values are representative of two experiments (2) except for compounds **2** (4) and **6** (3). [c] Lit. 2.2 ng/mL (72-h incubation), ref 4.

## Biological Evaluation

The in vitro cytostatic activity of didemnins was evaluated on L1210 leukemia cells and compared with earlier studies on the naturally occurring derivative **1b** (Table I).

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(12) An extensive NMR study on didemnin B and nordidemnin B, isolated by Dr. M. Guyot, Museum d'Histoire Naturelle, Paris, France, will be described in detail in a future report.
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(14) Kessler, H.; Wil, M.; Antel, J.; Beck, H.; Sheldrick, G. M. *Helv. Chim. Acta* **1989**, *72*, 530.
(15) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219.
(16) Coste, J.; Dufour, M.-N.; Pantaloni, A.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 669.
(17) *N*-Methyl signals are observed at δ 2.97 (major rotamer) and 2.96 (minor rotamer) for **11a**, and at δ 2.92 (major rotamer) and 2.90 (minor rotamer) for **14a**.
(18) The retention time for isomers **2** and **6**, are, respectively, 3.83 min and 3.07 min (system A). The unpurified compounds **3** and **4** gave also two sets of signals in 95:5 ratio: $t_R$ 7.48 min (major) and 6.0 min (minor) for **3**; $t_R$ 7.03 min (major) and 7.70 min (minor) for **4**.

Table II. Antitumor Activity of Nordidemnin (2) against P388 Leukemia[a]

| dose, mg/kg per day | toxicity[b] | body weight change,[c] g | median survival time, days | T/C,[d] % |
|---|---|---|---|---|
| 1.0 | 10/10 | −2.3 | 19.9 | 180 |
| 0.75 | 10/10 | −1.7 | 19.4 | 175 |
| 0.50 | 10/10 | −1.2 | 17.9 | 162 |
| 0.25 | 10/10 | −0.7 | 15.7 | 142 |
| 0.12 | 10/10 | −0.7 | 14.7 | 133 |
| 0.06 | 10/10 | +0.2 | 13.6 | 123 |
| 0.03 | 10/10 | 0 | 12.4 | 112 |
| control | 15/15 | +1.4 | 11.0 | – |

[a] Intraperitoneal graft, intraperitoneal treatment on days 1, 5, and 9. [b] Toxicity was evaluated by the number of survivors on day 5. [c] Average weight change of animals between day 1 and day 5 after tumor implantation. [d] T/C: T being the median survival time of treated mice and C the median survival time of the controls (significant when ≥ 125).

Table III. Antitumor Activity of Nordidemnin (2) against B16 Melanoma[a]

| dose, mg/kg per day | toxicity[b] | body weight change,[c] g | median survival time, days | T/C,[d] % |
|---|---|---|---|---|
| 1.0 | 10/10 | 0 | 29.3 | 154 |
| 0.50 | 10/10 | −0.9 | 28.0 | 147 |
| 0.25 | 10/10 | 0 | 27.0 | 142 |
| 0.06 | 10/10 | 0 | 22.0 | 116 |
| 0.03 | 10/10 | 0 | 19.3 | 101 |
| control | 20/20 | −0.2 | 19.0 | – |

[a-d] See legend Table II.

Table IV. Antitumor Activity of Nordidemnin (2) against B16 Melanoma[a]

| dose, mg/kg per day | toxicity[b] | body weight change,[c] g | median survival time, days | T/C,[d] % |
|---|---|---|---|---|
| 0.50 | 10/10 | −2.3 | 9.9 | 52 |
| 0.25 | 10/10 | −2.1 | 29.8 | 157 |
| 0.06 | 10/10 | −0.3 | 27.8 | 146 |
| 0.03 | 9/10 | −0.1 | 26.5 | 139 |
| control | 20/20 | −0.2 | 19.0 | – |

[a] Intraperitoneal graft, intraperitoneal treatment, repeated injections, day 1 to day 9. [b-d] See legend Table II.

The remarkably low ID50 value of 1.2 ng/mL we obtained for didemnin B (**1b**) after a 48-h incubation time is consistent with the 2.2 ng/mL concentration obtained previously, after a 72-h incubation time.[4] However, after repeated experiments on two different synthetic batches, we measured an ID50 value of 1.9 ± 0.7 ng/mL for nordidemnnin (**2**), which indicated a significantly higher efficiency than the published data (ID50, 7.8 ng/mL).[11] Moreover, nordidemnin (**2**) presented similar cytotoxicity against P388 (ID50, 0.9 ng/mL) as against L1210 (unpublished data).

The same range of in vitro cytotoxic activity against L1210 was also observed for compounds **3** and **4**, bearing an aromatic residue (Table I). In contrast, the activity of nordidemnin was not retained by long aliphatic acyl substitution of the terminal position and only 15% L1210 growth inhibition was measured at a concentration of $10^3$ ng/mL for compound **5**. In addition, only residual potency was preserved by inversion of the D-MeLeu chirality in compound **6**.

Referring to the results of the drug development and clinical trials program of the Division of Cancer Treatment, NCI,[19] we chose leukemia P388 as a reasonable prescreen on the basis of its sensitivity to most classes of clinically

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Table V. Antitumor Activity of Nordidemnin (2) against L1210 Lymphoid Leukemia[a]

| dose, mg/kg per day | toxicity[b] | body weight change,[c] g | median survival time, days | T/C,[d] % |
|---|---|---|---|---|
| 1.0 | 10/10 | −0.5 | 11.2 | 123 |
| 0.50 | 10/10 | −0.9 | 10.7 | 117 |
| 0.25 | 10/10 | 0 | 10.1 | 111 |
| control | 10/10 | +0.4 | 9.1 | – |

[a-d] See legend Table II.

Table VI. Antitumor Activity of Compounds 3 and 4 against P388 Leukemia[a]

| compd | dose, mg/kg per day | toxicity[b] | body weight change,[c] g | median survival time, days | T/C,[d] % |
|---|---|---|---|---|---|
| **3** | 0.75 | 10/10 | −1.5 | 19.0 | 159 |
| | 0.12 | 10/10 | +0.2 | 16.0 | 134 |
| **4** | 0.75 | 10/10 | −1.1 | 18.7 | 157 |
| | 0.12 | 10/10 | +0.2 | 14.7 | 123 |
| control | 0 | 10/10 | +2.4 | 11.9 | – |

[a-d] See legend Table II.

effective drugs. We studied the in vivo activity of nordidemnin on P388 leukemia (Table II) and B16 melanoma (Tables III and IV). Nordidemnin (**2**) exhibited T/C values at approximately the same dosage as that required for didemnin B (**1b**) in both the P388 and B16 ip grafts, in the ip treatment model. In the former model, treatment on days 1–9 (Table IV) was added to the previous schedule of days 1, 5, and 9 (Table III). In that case also we found that the in vivo antitumor activity of the nordidemnin (**2**) was similar to that reported for didemnin B (**1b**). Likewise, we found expectedly low if any in vivo activity against L1210, despite the marked in vitro cell growth inhibition (Table V).

Compounds **3** and **4**, the two analogues of nordidemnin with cytotoxic activity, were also evaluated for P388 ip treatment at doses of 0.12 and 0.75 mg/kg with no significant difference in T/C values at the same dosage as for nordidemnin (**2**) (Table VI). Compounds **5** and **6**, which did not exert cytotoxicity, were not studied in vivo.

## Conclusions

The results we obtained in the P388 assay in vivo and the in vitro cytotoxicity data on L1210 showed that the pure synthetic nordidemnin (**2**) was as potent as didemnin B (**1b**) itself. This is consistent with the high conformational similarities observed between these two closely functionalized structures. Other chemical modifications are needed to define the steric requirement at the γ-amino β-hydroxy acid level.

Substitution of the lactyl residue by an aromatic substituent in compounds **3** and **4** preserved the antineoplastic potency of nordidemnin (**2**). In contrast, the highly lipophilic substitution of the lactyl residue in compound **5** was associated with a dramatic loss in cytotoxic activity. Since no evident modifications in the conformation of the molecule could be deduced from the NMR data, we speculate that this latter result can be explained by an important nonspecific lipophilic interaction between the molecule and the cells.

From these experiments, our results indicate that nordidemnin totally retained antineoplastic activity within a fairly large range of modifications at the terminal position, whenever the D-MeLeu[7] chirality was retained. Otherwise, biological activity was considerably reduced as shown for the [L-MeLeu[7]]nordidemnin **6**. This marked difference was also recently noticed in the didemnin series by Kessler et al., although only minor conformational

modifications could be seen going from didemnin (**1b**) to its L-MeLeu[7] analogue.[20,21] Taking into account this structure–activity relationship data, the preparation of nordidemnin analogues bearing radiolabeled or fluorescent properties is under investigation. Replacement of the lactyl residue by the (*p*-hydroxyphenyl)propionyl gave a molecule ideally substituted for further useful $^{125}I$ radiolabeling. Likewise the potency of other aromatic substitutions will be evaluated prior to developing cell–substrate interaction studies.

## Experimental Section

**Chemistry.** Melting points were determined with use of a Buchi melting point apparatus. NMR data were obtained at 360 MHz on a Bruker WM-360 instrument; chemical shifts (ppm) were reported relative to internal tetramethylsilane. Specific optical rotations were measured on a Schmidt and Haensch Polartronic D apparatus and are at ±1°. Circular dichroism spectra were obtained on a Jobin and Yvon Autodichrograph Mark V instrument; $\lambda_{max}$ values are expressed in nanometers and $\theta$ values in deg cm$^2$/dmol. High-resolution FAB mass measurements were taken with a VG-ZAB E spectrometer. HPLC analyses were performed on a Beckman apparatus (System Gold: pump 126 and detector 167): system A, C8 Ultrabase 5-μm (4.6 × 150 mm) column (Société Française Chromato Colonne) and mixtures of 0.1% trifluoroacetic acid (TFA) in water (solvent A) and 0.1% trifluoroacetic acid in acetonitrile (solvent B) as eluents; system B, Ultrasphere ODS 5-μm (4.6 × 150 mm) column (Beckman) and mixtures of 0.1% trifluoroacetic acid (TFA) in water (solvent A) and 0.1% TFA in methanol (solvent B) as eluents. Preparative HPLC was performed on the previous instrument equiped with a Partisil 10 ODS-3 (25 × 500 mm) column (Whatman). Column chromatoraphies were performed with use of silica gel (70–200 μm, Amicon). Analytic TLC were performed on silica gel F254 aluminium sheets (0.2 mm thick, Merck). Amino acid derivatives were purchased from Bachem or Novabiochem.

**(*R*)-Mandelyl-L-prolyl-*N*-methyl-D-leucine (11b).** The trifluoroacetate salt of L-prolyl-*N*-methyl-D-leucine methyl ester (**9**), (1.8 g, 5 mmol), prepared as described in ref 13, was solubilized in DMF (10 mL). To this stirred solution were added (*R*)-mandelic acid (**8b**) (760 mg, 5 mmol) and $NaHCO_3$ (1.6 g, 19 mmol); BOP reagent (2.2 g, 5 mmol) was added after 5 min. After 24 h at room temperature, the reaction mixture was concentrated under vacuum, solubilized in ethyl acetate (20 mL), and washed with 5% $KHSO_4$ (2 × 5 mL), water (5 mL), 5% $NaHCO_3$ (2 × 5 mL), and saturated brine. The organic phase was dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure. The acyl dipeptide **10b** was chromatographed on silica gel (hexane/acetone, 60:40) to give a colorless oil (1.8 g, 79%), $R_f$ 0.66 (hexane/acetone, 50:50).

To an ice cooled solution of the methyl ester **10b** (1 g, 2.0 mmol) in methanol (3 mL), was added dropwise 2 N NaOH (3 mL) over a 5-min period. Stirring was then continued for 2 h at room temperature. After acidification (pH 2) with 5% $KHSO_4$, the aqueous mixture was saturated with NaCl and extracted with methylene chloride (3 × 30 mL). The combined organic phases were dried ($Na_2SO_4$) and concentrated under reduced pressure, providing **11b** which was crystallized from diethyl ether/hexane (0.80 g, 90%): mp 225 °C; $[\alpha]^{20}_D$ −62° (*c* 1, MeOH); $^1H$ NMR (DMSO-$d_6$) (major conformer) δ 0.83 (dd, *J* = 6.8 Hz, 3 H), 0.89 (dd, *J* = 6.8 Hz, 3 H), 1.31–1.47 (m, 1 H), 1.50–1.66 (m, 1 H), 1.67–1.88 (m, 3 H), 1.93–2.11 (m, 1 H), 2.14–2.32 (m, 1 H), 2.98 (s, 3 H), 3.04–3.20 (m, 1 H), 3.49–3.65 (m, 1 H), 4.82 (dd, $J_1$ = 3.0 Hz, $J_2$ = 8.9 Hz, 1 H), 4.89 (dd, $J_1$ = 4.9 Hz, $J_2$ = 10.8 Hz, 1 H), 5.20 (s, 1 H), 5.40 (s, 1 H), 7.17–7.45 (m, 5 H), 12.3 (s, 1 H); (minor conformers (distinguishable signals)) δ 0.94 (dd, *J* = 5.8 Hz), 0.99 (d, *J* = 5.8 Hz), 2.69 (s), 2.99 (s), 3.34–3.49 (m), 4.17–4.27 (m), 4.53–4.61 (m); HRFABMS *m/e* (MH$^+$) 377.2062 ($C_{20}H_{29}N_2O_5$ requires 377.2076).

**[3-(*p*-Hydroxyphenyl)propionyl]-L-prolyl-*N*-methyl-D-leucine (11c)** was synthesized as described for **11b**, from 3-(*p*-hydroxyphenyl)propionic acid (**8c**) (760 mg, 5 mmol). The intermediate methyl ester **10c** was chromatographied on silica gel (hexane/acetone, 50:50) (1.2 g, 77%); $R_f$ 0.36 (hexane/acetone, 50:50). Saponification of the ester (0.7 g, 1.8 mmol) gave **11c** which was crystallized from diethyl ether/hexane (0.60 g, 85%): mp 248 °C; $[\alpha]^{20}_D$ −34° (*c* 1, MeOH); $^1H$ NMR (DMSO-$d_6$) complex mixture of four conformers; HRFABMS *m/e* (MH$^+$) 391.2238 ($C_{21}H_{31}N_2O_5$ requires 391.2232).

**Palmityl-L-prolyl-*N*-methyl-D-leucine (11d).** The trifluoroacetate salt of L-prolyl-*N*-methyl-D-leucine methyl ester (**9**), (1.8 g, 5 mmol), prepared as described in ref 13, was dissolved in DMF (10 mL). To this stirred solution were added palmityl acid chloride (**8d**) (1.4 g, 5 mmol) and triethylamine (1.7 mL, 10 mmol). After 24 h at room temperature, the reaction mixture was concentrated under vacuum, solubilized in ethyl acetate (20 mL), and washed with 5% $KHSO_4$ (2 × 5 mL), water (5 mL), 5% $NaHCO_3$ (2 × 5 mL), and saturated brine. The organic phase was dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure, and the methyl ester **10d** was chromatographed on silica gel (hexane/acetone, 50:50) (2.1 g, 84%); $R_f$ 0.85 (hexane/acetone, 50:50); $[\alpha]^{20}_D$ −12° (*c* 1, MeOH). Saponification of the ester **10d** (1 g, 3 mmol) gave **11d** which was crystallized from diethyl ether/hexane (0.86 g, 90%): mp 170 °C; $[\alpha]^{20}_D$ −14° (*c* 1, MeOH); $^1H$ NMR (DMSO-$d_6$) (mixture of at least three conformers) δ 0.81–0.98 (m, 9 H), 1.20–1.30 (m, 24 H), 1.32–1.52 (m, 3 H), 1.56–1.90 (m, 5 H), 2.05–2.33 (m, 3 H), 2.66, 2.71, 2.96 (3s, 3 H), 3.34–3.42 (m, 1 H), 3.44–3.53 (m, 1 H), 4.77 (dd, $J_1$ = 3.2 Hz, $J_2$ = 8.6 Hz, 1 H), 4.87 (dd, $J_1$ = 3.5 Hz, $J_2$ = 10.6 Hz, 1 H), 12.7 (s, 1 H). Anal. ($C_{38}H_{52}N_4O_4$) C, H, N.

**L-Lactyl-L-prolyl-*N*-methyl-L-leucine (14a).** Boc-Pro-Leu-OMe (1.07 g, 3 mmol), synthesized according to J. Coste[16] was treated with trifluoroacetic acid (3 mL) for 30 min. The solvent was evaporated under reduce pressure, and the trifluoroacetate salt **12** was precipitated from ether/pentane (1.00 g, 90%). The trifluoroacetate salt **12** was solubilized in dichloromethane (5 mL). To this stirred solution were added successively L-lactic acid (**8a**) (360 mg, 4.05 mmol), DIEA (2.32 mL, 13.5 mmol) and BOP reagent (1.79 g, 4.05 mmol). After 22 h at room temperature, the reaction mixture was concentrated under vacuum, solubilized in ethyl acetate (30 mL), and washed with 5% $KHSO_4$ (5 mL), water (5 mL), 5% $NaHCO_3$ (5 mL), and saturated brine. The organic phase was dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure. The crude acyl dipeptide **13a** (0.8 g, 79%); $R_f$ 0.38 (MeOH/$CH_2Cl_2$, 5:95), was used with no further purification.

To an ice cooled solution of the methyl ester **13a** (0.8 g, 2.0 mmol) in methanol (2.5 mL) was added dropwise 2 N NaOH (2.5 mL) over a 5-min period. Stirring was then continued for 30 min. The solution was acidified to pH 3 with 1 N HCl, saturated with NaCl, and extensively extracted with $CH_2Cl_2$ (6 × 10 mL). The combined organic phases were dried ($Na_2SO_4$) and concentrated under reduced pressure providing **14a** which was crystallized from diethyl ether (0.70 g, 74% overall yield from Boc-Pro-Leu-OMe): mp 125 °C; $[\alpha]^{20}_D$ −148° (*c* 1, pyridine); $^1H$ NMR (DMSO-$d_6$) (major conformer) δ 0.79 (d, *J* = 5.9 Hz, 3 H), 0.89 (d, *J* = 5.9 Hz, 3 H), 1.15 (d, *J* = 6.4 Hz, 3 H), 1.39–1.65 (m, 2 H), 1.65–1.82 (m, 2 H), 1.82–2.00 (m, 2 H), 2.12–2.22 (m, 1 H), 2.92 (s, 3 H), 3.41–3.47 (m, 1 H), 3.65–3.72 (m, 1 H), 4.26–4.31 (m, 1 H), 4.77 (dd, $J_1$ = 4.4 Hz, $J_2$ = 8.8 Hz, 1 H), 5.03 (dd, $J_1$ = 3.9 Hz, $J_2$ = 10.8 Hz, 1 H), 12.55 (m, 1 H); (minor conformer (distinguishable signals)) δ 2.90 (s, 3 H), 3.91–3.95 (m, 1 H); HRFABMS, *m/e* (MH$^+$) 315.1917 ($C_{15}H_{27}N_2O_5$ requires 315.1911).

**General Procedure for Preparation of Nordidemnin B Analogues 3–6.** To a solution of the free amino cycle **7** (81 mg, 100 μmol), prepared as described in ref 13, and **11b–d** or **14a** (200 μmol) in methylene chloride (1 mL) was added BOP reagent (88 mg, 200 μmol), and *N*-methylmorpholine (55 μL). The coupling reaction was monitored by analytical HPLC. After stirring for 3 h at room temperature, usual workup provided a residue which was purified by preparative HPLC, except for compound **5**. After

(20) Kessler, H.; Mronga, S.; Will, M.; Schmidt, U. *Helv. Chem. Acta* **1990**, *73*, 25.

(21) In $C_6D_6$, the 360 $^1H$ 2D NMR spectrum of [L-MeLeu[7]]nordidemnin (**6**) exhibits a complex 2-fold signal set of equal intensity which result from rotamers and differs from the spectrum of nordidemnin (**2**). This result is in agreement with the [L-MeLeu[7]]didemnin $^1H$ NMR study reported by H. Kessler (ref 20). Conformational implications are under investigation.

Table VII. $^{H}$ NMR Data

| residue | H | 2a | 3 | 4 | 5 |
|---|---|---|---|---|---|
| X | | 4.11 (C2-H) | 4.42 (C2-H) | 2.24 (C2-$CH_2$) | 1.70 ($C_2$-$CH_2$) |
| | | 1.26 (C2-$CH_3$) | 7.37–7.16–7.06 | 2.99 (C3-$CH_2$) | 1.23–1.5 ($C_n$-$CH_2$) |
| | | | aromatic | 6.96–7.20 aromatic | 0.90 (C15-$CH_3$) |
| Pro | | αC-H | 4.13 | 4.22 | 4.20 | 4.14 |
| | | βC-$H_2$ | 1.48–1.34 | 1.40–1.14 | 1.63–0.92 | 1.43–1.65 |
| | | γC-$H_2$ | 1.44–1.1 | 1.7–1.06 | 1.70–1.25 | 1.72–1.38 |
| | | δC-$H_2$ | 2.86–2.7 | 3.2–2.65 | 3.28–2.75 | 3.15–2.82 |
| D-MeLeu | | N-$CH_3$ | 2.87 | 2.88 | 2.94 | 2.93 |
| | | αC-H | 5.62 | 5.62 | 5.75 | 5.74 |
| | | βC-$H_2$ | 2.02–1.6 | 1.35–1.64 | 2.03–1.70 | 2.07–1.70 |
| | | γC-H | 1.36 | 1.36 | 1.41 | 1.40 |
| | | γC-$CH_3$ | 0.97–0.85 | 0.94–0.87 | 1.0–0.93 | 1.02–0.90 |
| Thr | | N-H | 8.02 | 7.94 | 8.30 | 8.34 |
| | | αC-H | 4.9 | 5.03 | 4.95 | 4.98 |
| | | βC-H | 5.89 | 5.73 | 5.64 | 5.73 |
| | | βC-$CH_3$ | 1.89 | 1.85 | 1.92 | 1.99 |
| ValΨ(CHOH)Gly | | N-H | 7.51 | 7.80 | 7.47 | 7.48 |
| | | C2-H | 3.92–2.85 | 3.58–2.94 | 3.84–2.92 | 3.95–2.92 |
| | | C3-H | 4.45 | 4.44 | 4.46 | 4.50 |
| | | C4-H | 4.48 | 4.44 | 4.48 | 4.50 |
| | | C5-H | 2.43 | 2.25 | 2.37 | 2.42 |
| | | C5-$CH_3$ | 1.17–1.04 | 1.16–1.02 | 1.15–1.04 | 1.20–1.07 |
| HIP | | C2-H | 4.66 | 4.58 | 4.65 | 4.68 |
| | | C2-$CH_3$ | 1.74 | 1.74 | 1.75 | 1.77 |
| | | C4-H | 5.64 | 5.59 | 5.64 | 5.65 |
| | | C5-H | 2.48 | 2.50 | 2.50 | 2.62 |
| | | C5-$CH_3$ | 0.88 | 0.91 | 0.92–0.86 | 0.93–0.87 |
| Leu | | N-H | 8.17 | 8.27 | 8.26 | 8.30 |
| | | αC-H | 5.1 | 5.1 | 5.12 | 5.1 |
| | | βC-$H_2$ | 1.58–1.45 | 1.81–1.62 | 1.78–1.54 | 1.68–1.50 |
| | | γC-H | 1.8 | 1.78 | 1.77 | 1.72 |
| | | γC-$H_3$ | 0.99 | 1.02 | 0.99–0.85 | 0.98–0.94 |
| Pro | | αC-H | 4.34 | 4.34 | 4.31 | 4.29 |
| | | βC-$H_2$ | 1.24 | 1.30 | 1.32 | 1.30 |
| | | γC-$H_2$ | 1.45–1.17 | 1.54–1.2 | 1.56–1.18 | 1.50–1.15 |
| | | δC-$H_2$ | 3.31–3.09 | 3.30–3.36 | 3.35–3.21 | 3.27–3.10 |
| MeTyr(OMe) | | N-$CH_3$ | 2.16 | 2.13 | 2.20 | 2.20 |
| | | αC-H | 3.38 | 3.36 | 3.35 | 3.45 |
| | | βC-$H_2$ | 3.38–3.16 | 3.34–3.16 | 3.35–3.22 | 3.45–3.23 |
| | | aromatic | 6.77–6.68 | 6.78–6.69 | 6.80–6.72 | 6.81–6.71 |
| | | $OCH_3$ | 3.34 | 3.34 | 3.32 | 3.34 |

concentration of the solvent, the following compounds were obtained. $^1$H NMR data are listed in Table VII.

**Man-nordidemnin (3)** was crystallized from diethyl ether/hexane (45 mg, 39%): mp 166–168 °C; HPLC (system B; gradient A/B, from 30:70 to 0:100 in 15 min at a rate of 1.5 mL/min) $t_R$ = 7.48 min; HRFABMS $m/e$ ($MH^+$) 1160.6507 ($C_{61}H_{90}N_7O_{15}$ requires 1160.6490); CD (methanol): $[\theta]_{235}$ +217, $[\theta]_{295}$ −136.

**Hpp-nordidemnin (4)** was crystallized from diethyl ether/hexane (40 mg, 34%): mp 153–155 °C dec; HPLC (same conditions as described for **3**) $t_R$ = 7.03 min; HRFABMS $m/e$ ($MH^+$) 1174.6621 ($C_{62}H_{92}N_7O_{15}$ requires 1174.6640); CD (methanol) $[\theta]_{218}$ +458, $[\theta]_{295}$ −153.

**Pal-nordidemnin (5)** was purified on a Merck Lichroprep Si 60 silica gel column ($CH_2Cl_2$/acetone, 90:10) and was crystallized from diethyl ether/pentane (54 mg, 43%): mp 105–110 °C; $R_f$ 0.30 ($CH_2Cl_2$/acetone, 90:10); HRFABMS $m/e$ ($MH^+$) 1264.8464 ($C_{69}H_{114}N_7O_{14}$ requires 1264.8416); CD (methanol) $[\theta]_{219}$ +437, $[\theta]_{234}$ +256, $[\theta]_{295}$ −154.

**[L-MeLeu$^7$]nordidemnin (6)** was crystallized from diethyl ether/hexane (28 mg, 26%): mp 135–145 °C; HPLC (system A; A/B, 60:40 at a rate of 2 mL/min) $t_R$ = 3.07 min; HRFABMS $m/e$ ($MH^+$) 1098.6338 ($C_{56}H_{88}N_7O_{15}$ requires 1098.6309); CD (methanol) $[\theta]_{212}$ −171, $[\theta]_{229}$ −283, $[\theta]_{295}$ −60.

**Biological Studies. Cytotoxic Effects.** L1210 leukemia cells in log-phase growth were suspended at an initial concentration of 0.8 × $10^5$ cells/mL in RPMI 1640 medium (Seromed) supplemented with 20% inactivated horse serum (Gibco), 2 mmol/L L-glutamine (Seromed), 50 μg/mL streptomycine (Specia), and 200 units/mL penicillin (Specia). Under these conditions the number of cells in untreated cultures increased exponentially to 2 × $10^6$ cells/mL with a doubling time of 9–12 h.

Drugs were dissolved in ethanol (0.5% final) and were tested at different concentrations. The cytotoxicity of the compounds was evaluated by counting cells 24 and 48 h after incubation and by determining the drug concentrations needed to produce 50% inhibition (ID50) of growth relative to controls. This value was calculated from the regression line as the probit of the percent cell growth inhibition plotted as a function of the logarithm of concentration.

**Antitumor Activity.** Three murine tumors, two leukemias and one solid tumor, were used; they were obtained from the National Cancer Institute (NCI).

**P388 Leukemia.** CDF1 female mice (BALB/c female × DBA/2 male) received $10^6$ cells ip on day 0; treatment was given by the same route on days 1, 5, and 9. Drugs were dissolved in 2% methanol. Seven doses were tested for compounds **2** and two for compounds **3** and **4**. For each group, 10 mice were used and a greater number for the control group.

Body weight and survival were recorded on days 1 and 5 after tumor implantation. Animal mortality was checked for 30 days. Antitumor activity was evaluated from the median survival time of treated mice (T) over that of control mice (C) according to the method of the National Cancer Institute. Activity is significant if T/C ≥ 125%.

**L1210 Leukemia.** The procedure is the same as that described for P388 leukemia except for the number of inoculated cells: $10^5$ cells, instead of $10^6$ cells in the former model, were inoculated ip and mice were treated ip on days 1, 5, and 9.

**Melanoma B16.** B16 was transferred to B6 D2 F1 female mice (C57BL/6 female × DBA/2 male) as a cell suspension obtained by homogenizing 1 g of tumor in 9 mL of saline (0.9%). This cell suspension (0.5 mL) was implanted ip on day 0, and the animals were treated once a day either from day 1 to day 9 or on days 1, 5, and 9. The criteria for activity are as previously described and were based on the determination of T/C value.

**Treatment.** Drugs were administered by the ip route. They

were dissolved in 2% methanol in bidistilled water and injected under 0.1 mL per 10 g of body weight.

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**Registry No.** **16**, 77327-05-0; **2**, 117710-03-9; **3**, 130406-04-1; **4**, 130406-05-2; **5**, 130406-06-3; **6**, 130466-66-9; **7**, 117733-99-0; **8a**, 79-33-4; **8b**, 611-71-2; **8c**, 501-97-3; **8d**, 112-67-4; **9**, 117710-32-4; **10b**, 130406-08-5; **10c**, 130406-09-6; **10d**, 130406-10-9; **11a**, 117710-19-7; **11b**, 130406-11-0; **11c**, 130406-12-1; **11d**, 130406-13-2; **12**, 130406-07-4; **13a**, 130466-67-0; **14a**, 130466-68-1; BOC-Pro-Leu-OMe, 15136-16-0.

# Potential Antitumor Agents. 62. Structure–Activity Relationships for Tricyclic Compounds Related to the Colon Tumor Active Drug 9-Oxo-9*H*-xanthene-4-acetic Acid

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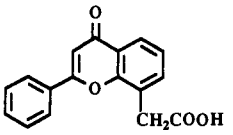
A series of tricyclic analogues of 9-oxo-9*H*-xanthene-4-acetic acid have been prepared and evaluated for their ability to cause hemorrhagic necrosis in subcutaneously implanted colon 38 tumors in mice, in an effort to extend the structure–activity relationships for this series. As was found previously with analogues of flavone-8-acetic acid (FAA) (Atwell et al. *Anti-Cancer Drug Des.* **1989**, *4*, 161), all electronic modifications of the XAA nucleus led to severe decreases or complete abolition of activity, suggesting narrow structure–activity relationships. Dipole moments for many of the compounds were computed, and the degree to which the molecular dipole moment lay out of the plane of the aromatic part of these molecules was found to be determined largely by the contributions from the acetic acid moiety relative to that from the tricyclic ring system. There did not appear to be any general relationship between the *magnitude* of the dipole moment and activity. However, for compounds containing the 9-carbonyl functionality, the *orientation* of the dipole vector may be of significance. In all compounds possessing an ether group peri to the acetic acid side chain, there was a close approach (ca. 2.4 Å) between this and the side chain OH.

Following the discovery of the selective solid tumor activity[1,2] and unusual biological effects[3-5] of the drug flavoneacetic acid (1), there have been a limited number




1

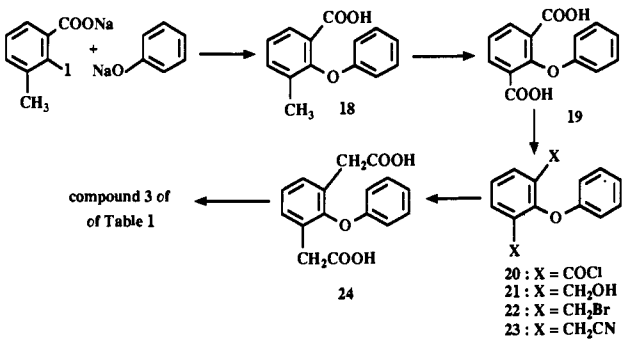
of reports[6-8] of analogue studies. The majority of these have been on compounds closely related to 1, and no clear structure–activity relationships (SAR) are yet apparent, although a limited study of chromophore variations[9] suggested that these are quite narrow. We have recently shown[10] that 9-oxo-9*H*-xanthene-4-acetic acid[11] (**2**, XAA) also has in vivo colon 38 activity and have demonstrated limited SAR for ring-substituted analogues,[12,13] some of which show much better dose-potency than FAA.

In order to further delineate the SAR for this novel type of antitumor agent, we have extended our studies to encompass a wider range of linear tricyclic chromophores and report this work here.

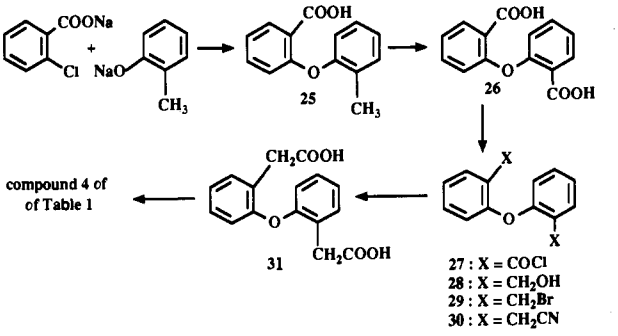
## Chemistry

The 10,11-dihydro-10-oxodibenz[*b*,*f*]oxepin derivative (**3**) was synthesized by the method shown in Scheme I. Ullman condensation of 2-iodo-3-methylbenzoic acid and phenol using the phase-transfer catalyst tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1)[14] gave a 42% yield of 3-methyl-2-phenoxybenzoic acid (**18**). A previous attempt to prepare this compound by the Ullmann route failed,[15] so the present work is a further indication of the usefulness of TDA-1 in such reactions.[12,14] Oxidation of **18** gave dicarboxylic acid **19**, which was elaborated to the corresponding diacetic acid **24** via intermediates **20–23** in an

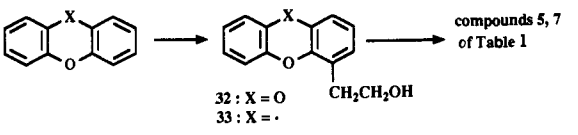
**Scheme I**




**Scheme II**




**Scheme III**

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