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First Synthesis and Pharmacological Evaluation of Benzoindolizidine and Benzoquinolizidine Analogues of α- and β-Peltatin

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Abstract—The benzoindolizidine and-quinolizidine analogues of α- and β-peltatin were designed and synthesized by two different synthetic routes involving as the key step the Bischler–Napieralski cyclization of suitably substituted *N*-acyl-2-arylmethylpyrrolidine and -piperidine derivatives. The in vitro biological activity of these analogues as well as some of their derivatives was subsequently evaluated.

Introduction

Podophyllotoxin **1** and related compounds, including α and β-peltatin **2** and **3** (Fig. 1) are a class of cyclolignan type compounds characterized by several vicinal oxygen functions on the differently substituted aromatic moieties and/or in the central carbocyclic unit.[1] They have been obtained from the rhizoma resin of *Podophyllum peltatum L*[2] and have long been known to display anti-tumour[3] and mitotoxic activities.[4,5] However due to toxic side-effects they have in most cases failed to give satisfactory clinical trial results.[6–9] In fact, the clinical utility of these compounds is compromised by the highly reactive nature of the fused γ-lactone moiety present in these molecules and by epimerization at C2 under physiological conditions.[10] This has been shown to be deleterious to the therapeutic action by giving rise to products devoid of anti-tumour activity.[11] Consequently, a number of groups have carried out modifications at this stereogenic centre and new analogues which are incapable of loss of configurational integrity at the C2 centre, which encompass a broad spectrum of antineoplastic activities and thus overcome clinical limitations, have been explored.[12–17] In particular, ingenious variations have been proposed by several groups which have notably designed and synthesized a variety of analogues in which the sp3 carbon is replaced by a sp2 nitrogen, anticipating configurational integrity and avoiding any problems of epimerization.[18–24] Paradoxically, despite the fact that they retain anti-tumour activities, to our knowledge, such modifications have been mainly confined to the aza-analogues of podophyllotoxin **4–6**[1,18–26] (Fig. 1) and the study of the α and β-peltatin congeners appears to be an unexplored area. We wish therefore to report in this paper the first synthesis and the in vitro biological evaluation of hexahydropyrrolo[1,2-*b*] and hexahydropyrido[1,2-*b*]isoquinoline analogues of α and β-peltatin **7–10**.

Results and Discussion

Chemistry

Two complementary and undoubtedly transposable methods were adopted for the synthesis of the benzoindolizidine and -quinolizidine analogues of α and β-peltatin **7, 8** and **9, 10** respectively (Fig. 1). The first one which is depicted in the retrosynthetic Scheme 1 involved as the key step the formation of the enamides **13, 14** which could be derived from the Horner reaction between the appropriate *O*-benzyl protected aromatic carboxaldehyde **15** and the phosphorylated *N*-acyl pyrrolidine

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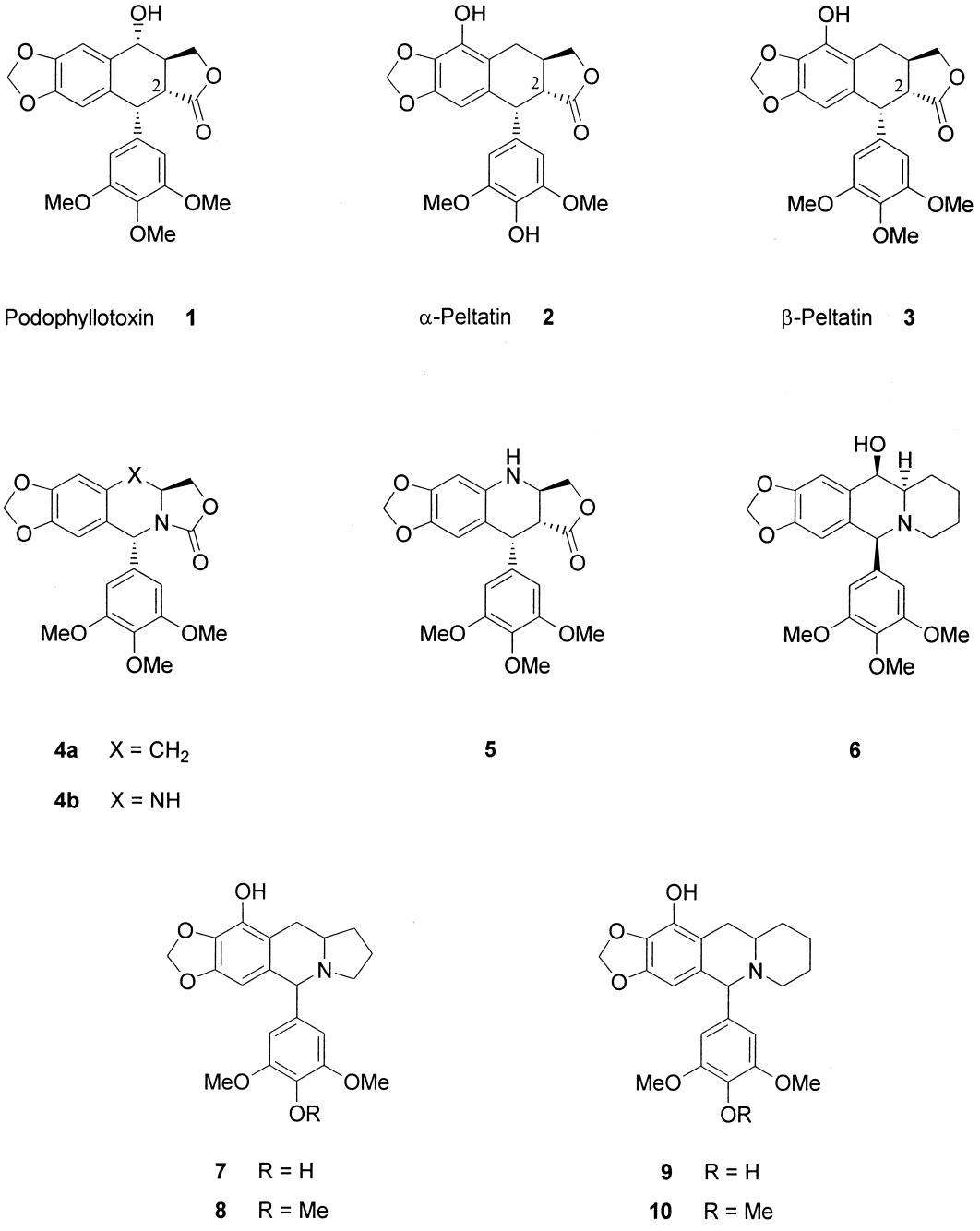


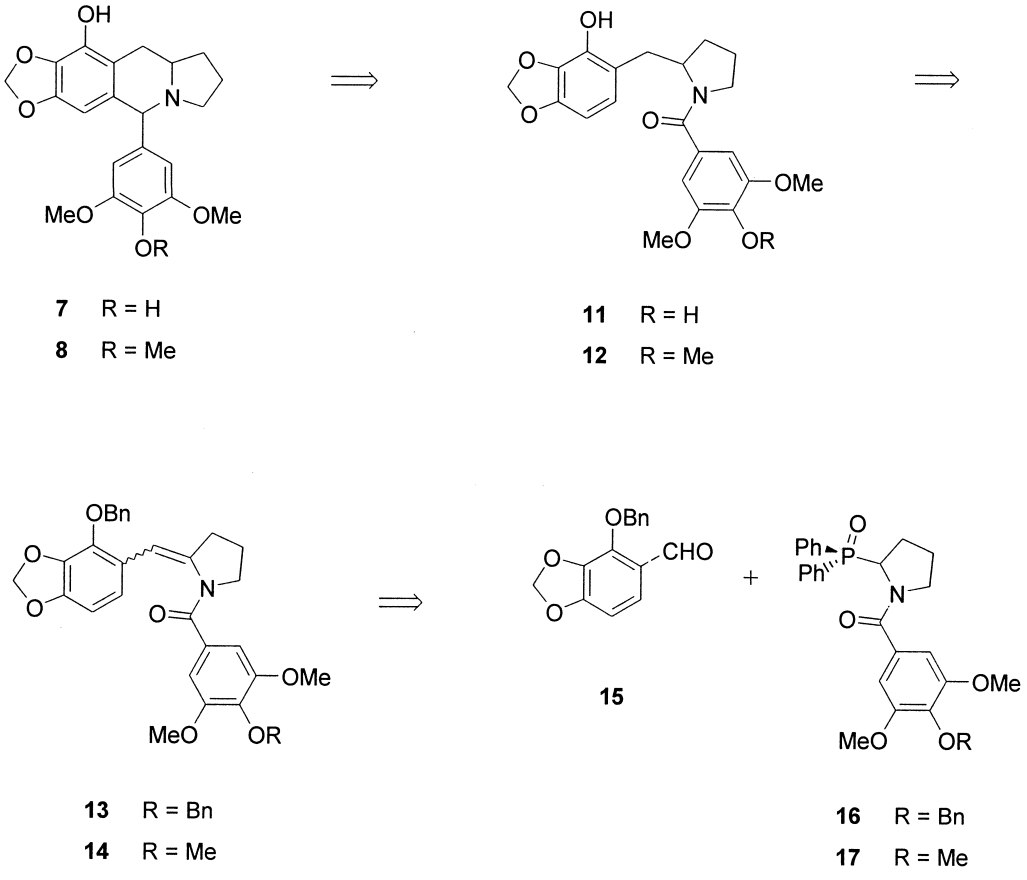
Figure 1.

derivatives **16, 17** respectively. Reduction and subsequent Bischler–Napieralski cyclization then should complete the synthesis of the target compounds **7, 8**.

It was obvious that this synthetic strategy would be fraught with difficulties associated with the presence of several hydroxy phenolic functions at different sites on the environmentally different aromatic moieties of the final compounds **7, 8**. Simple retrosynthetic analysis indicates that the hydroxy phenolic function at the western part of the molecule should be incorporated via the trialkoxybenzaldehyde derivative **15** whereas the corresponding function on the pendant aromatic unit of **7** should be integrated into the models via the protected 1-aroyl-2-diphenylphosphinoylpyrrolidine derivative **16**.

The synthesis started with the preparation of the benzyl protected 4-hydroxybenzo[*d*][1,3]dioxole-5-carbaldehyde **15**. To reach this protected phenol derivative, the metallation and subsequent hydroxylation of piperonal was investigated. Critical to the success of this strategy was the ability to identify a masked carbonyl synthon that was capable not only of retaining the formyl functionality but also of directing subsequent lithiation at the 'in between' ring position. The metallation conditions described by Comins for various aldehydes by using lithium *N,N,N′*-trimethylethylenediamine[27] were tested on piperonal but failed. After considerable experimentation we found that adding 2 equiv of *t*-butyllithium in pentane to 1 equiv of imidazolidine **18**[28] in Et_2O at −78 °C and then quenching with $B(OMe)_3$ at 0 °C followed by

hydrogen peroxide led to the target compound after regeneration of the formyl functionality and ultimate benzyl protection (Scheme 2). The second partners, **16** and **17**, involved in the Horner reaction were easily obtained by coupling the phosphorylated amine **20** with the aromatic carboxylic acids **22**, **23**, the former being obtained by sequential protection-oxidation of syringaldehyde (Scheme 3). Initially the phosphorylated pyrrolidine **20** was readily obtained by addition of diphenylphosphine oxide **26** to the triazine **24**.[29,30] This protocol delivered the phosphorylated carboxamides **16, 17** in 84 and 78% yield respectively. Horner reaction between compounds **16, 17**



7 R = H
8 R = Me

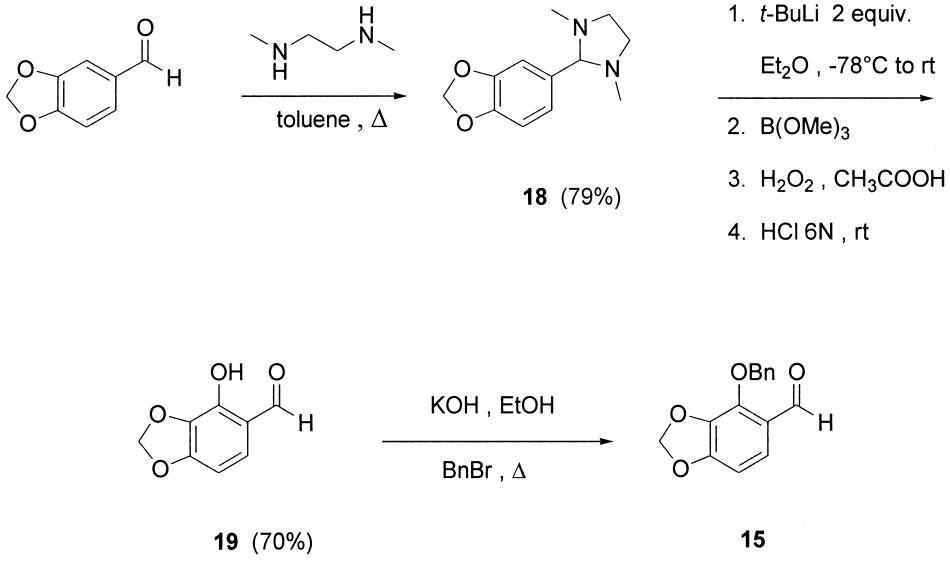
11 R = H
12 R = Me

13 R = Bn
14 R = Me

15

16 R = Bn
17 R = Me

Scheme 1.

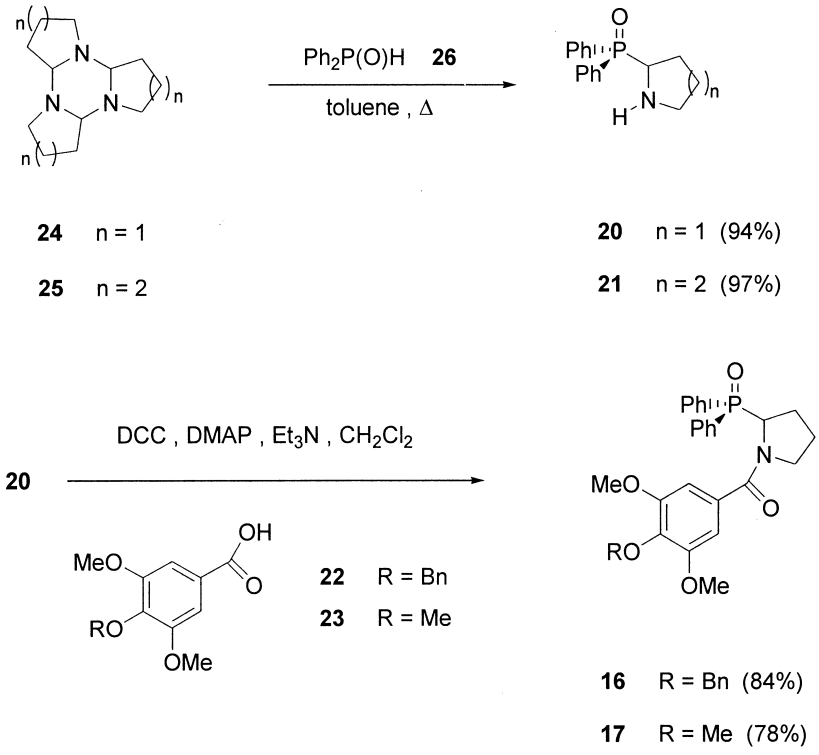


18 (79%)

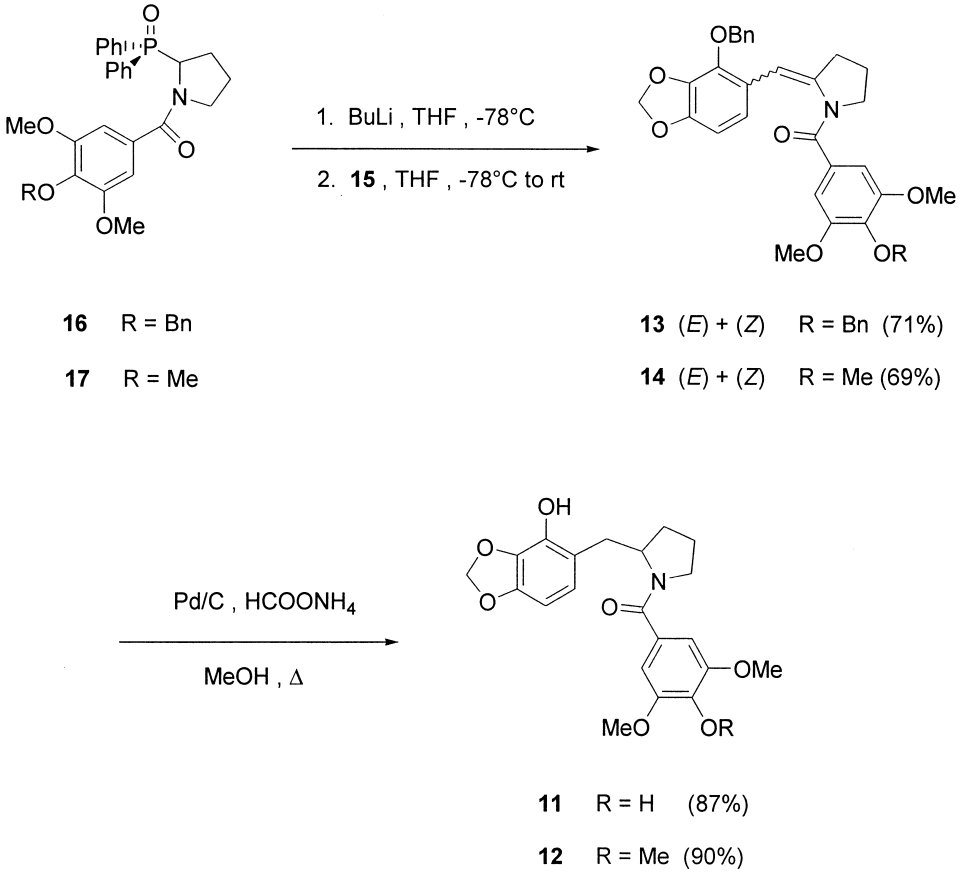
19 (70%)

15

Scheme 2.



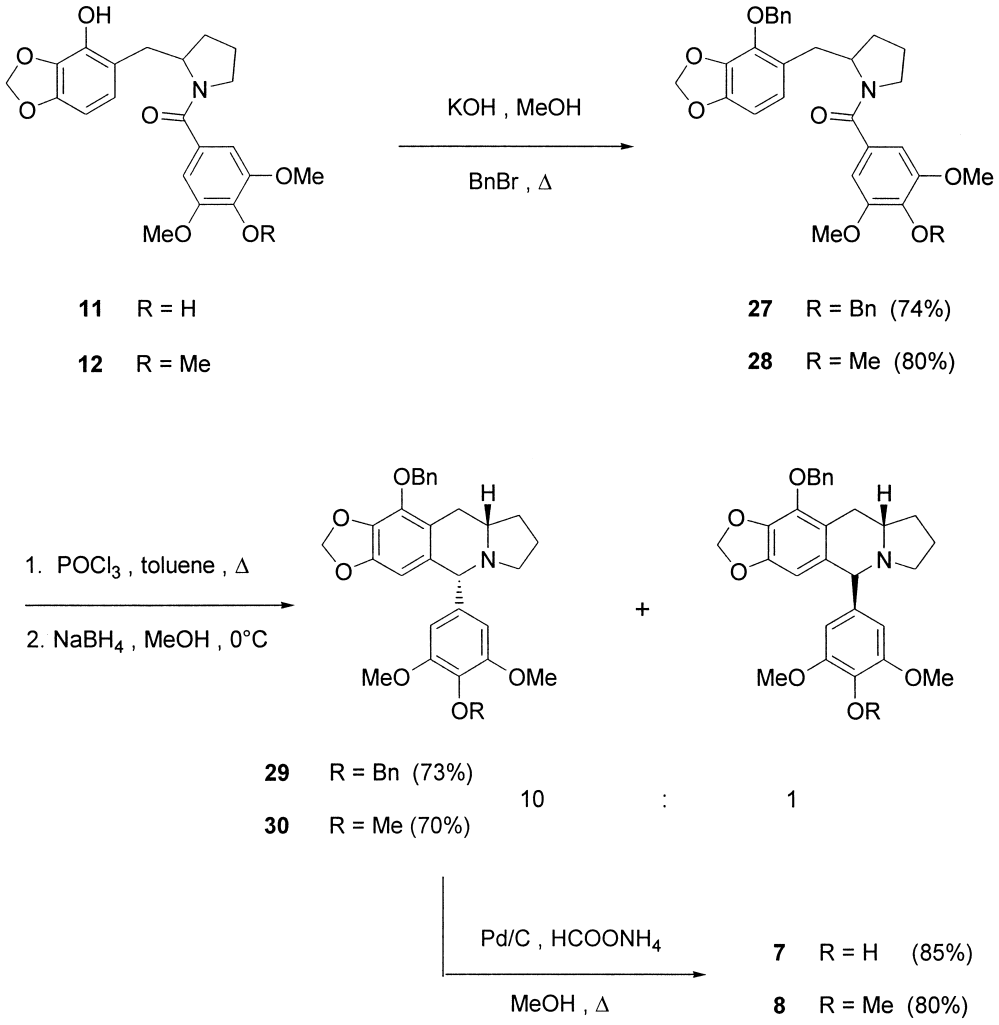
Scheme 3.



Scheme 4.

and **15** was then carried out by smooth deprotonation at −78 °C with *n*-BuLi in THF and subsequent treatment with the suitably substituted aldehyde **15**. Warming the reaction mixture to room temperature ensured the completion of the reaction and the *N*-acylenamides **13, 14** were isolated in high yields (71% and 69% respectively) as a mixture of *Z*- and *E*-isomers with *E*-isomers predominating (40:60 for **13**, 45:55 for **14**) (Scheme 4). The stereochemistry was unambiguously assigned from the ^{1}H NMR spectra and further confirmed by a one-dimensional difference nuclear Overhauser experiment. Thus, in contrast to the *E*-isomer, irradiation of the vinylic proton of the *Z*-form led to an increased intensity of the allylic protons of the pyrrolidine ring.

We then focused on the hydrogenation of the polysubstituted enamides **13** and **14**. The rarely employed method making use of Pd on C and ammonium formate[31,32] could offer, if feasible, a double advantage by effecting simultaneously the reduction of the enamine function with concomitant removal of the benzyloxy protecting group thus giving straightforward access to direct candidates for the Bischler–Napieralski reaction. As anticipated, treatment of enamides **13**, **14** delivered the reduced di- and monophenolic compounds **11** and **12** with excellent yields (87 and 90% respectively) (Scheme 4). Compounds **11**, **12** were then subjected to Bischler–Napieralski conditions by standard treatment with phosphorus oxichloride ($POCl_3$) in toluene at reflux and subsequent reduction with sodium borohydride ($NaBH_4$) in methanol. Unfortunately all attempts to obtain the cyclocondensed products by this protocol, even under mild conditions,[23,33] were unrewarding probably due to the presence of the hydroxy phenolic groups in the opened compounds, a precedented complicating factor in related systems.[17] Assuming that a reprotection step might be a prerequisite to the carboannulation reaction to give the target molecules **7** and **8**, compounds **11** and **12** were then fully benzyl protected prior to effecting the Bischler–Napieralski reaction (Scheme 5). Gratifyingly, this protocol afforded the expected cyclized products **29** and **30**, in 73 and 70% yield respectively, as a mixture of diastereomers in the ratio 10:1. The structure of the major diastereoisomer was deduced from the ^{1}H NMR spectrum, namely by the presence of long range coupling between the mono and dibenzylic protons attributable to their axial relationship.[34,35] Final removal of the benzyl protecting group of the major isomers delivered



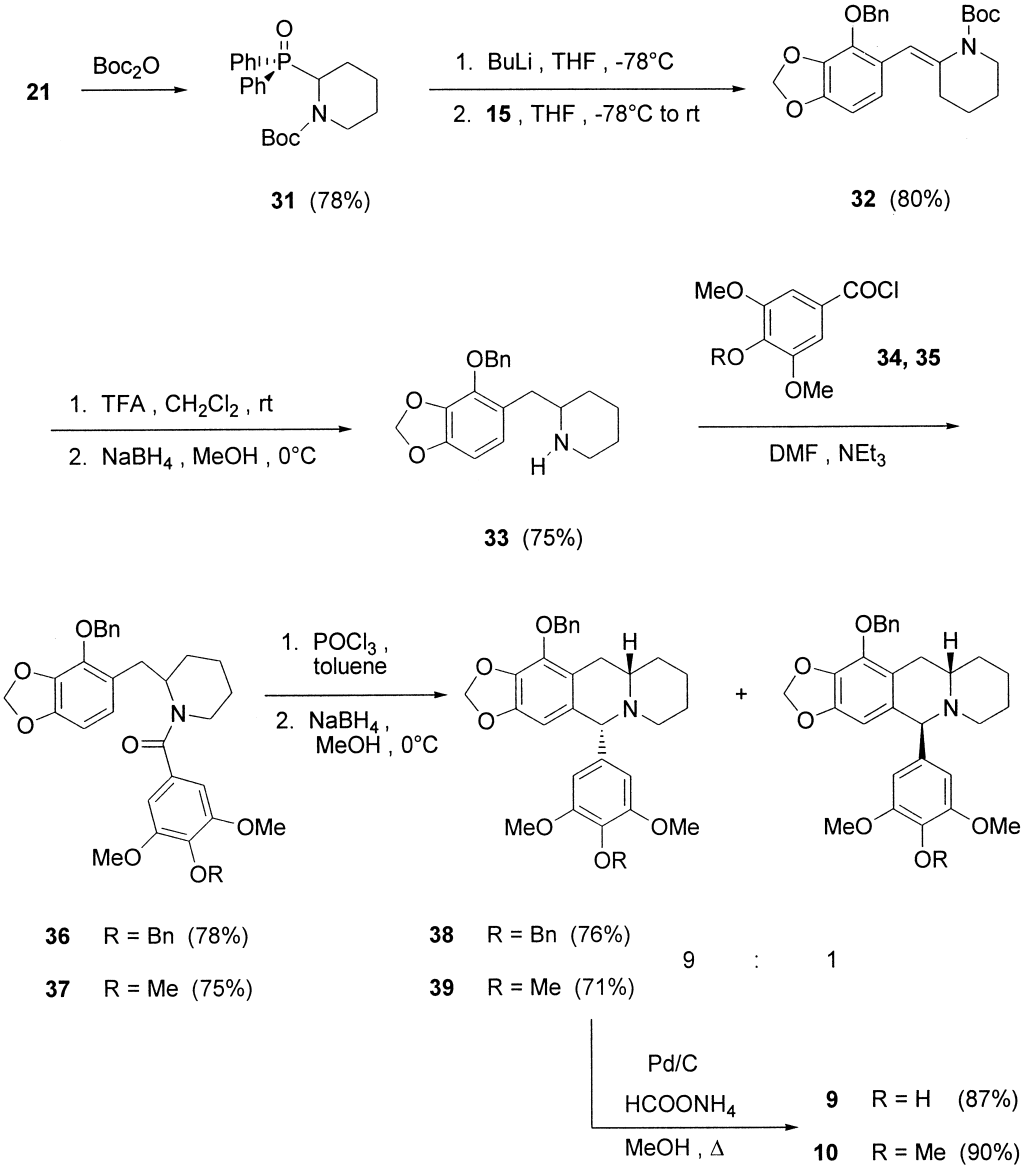
Scheme 5.

the benzoindolizidine analogues of α- and β-peltatin **7** and **8** with very acceptable yields (46 and 45% respectively over the last three steps).

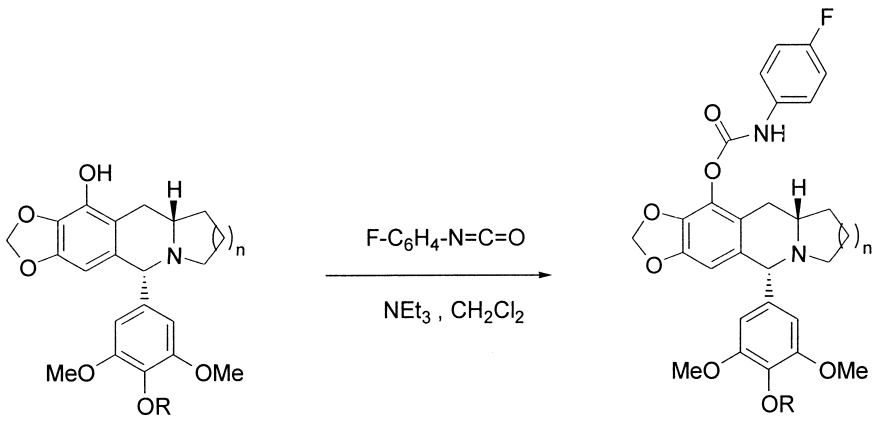
For the synthesis of the benzoquinolizidine analogues of α- and β-peltatin **9** and **10** (Fig. 1) we decided to adopt an alternative strategy which hinges upon the same synthetic principles but precludes the rather unsatisfactory and tedious deprotection-reprotection steps. This alternative approach which is depicted in the Scheme 6 involves as the key step the formation of the 2-arylmethylpiperidine derivative **33** readily obtained after deprotection and subsequent reduction of enecarbamate **32**. This *N*-protected enamine **32** was derived from the Horner reaction between the aromatic carboxaldehyde **15** and the phosphorylated cyclic carbamate **31**. Initially compound **31** was prepared by treatment of the corresponding phosphorylated cyclic amine **21** (Scheme 2) with di-*tert*-butyldicarbonate.[29,30] Horner reaction proceeded uneventfully to afford the *N*-*tert*-butoxycarbonyl-2-arylmethylpiperidine **32** as a mixture of *Z*- and *E*-isomer (5:95). *N*-Deprotection of the enecarbamate **32** with trifluoroacetic acid and subsequent reduction of the transient iminium ion was performed as a single, one-pot reaction and this procedure afforded straightforwardly the 2-arylmethylpiperidine **33** in fairly good yield. Schotten–Baumann reaction between amine **33** and the appropriate carboxylic acid chlorides **34, 35** furnished the amides **36, 37** candidates for the Bischler–Napieralski reaction. The *O*-benzyl protections in **36, 37** survived the cyclization conditions since treatment with $POCl_3$ followed by $NaBH_4$ delivered the cyclocondensed products **38** and **39** as a mixture of diastereoisomers (90:10 for **38** and 95:5 for **39**). Final removal of the benzyl protection in **38, 39** completed the synthesis of the target products **9, 10**.

Pharmacological evaluation

Eleven analogues of α and β-peltatin and particularly their urethane derivatives (Scheme 7) were prepared in



Scheme 6.



7	R = H	n = 1	**40**	R = $CONHC_6H_4F(p)$	n = 1 (75%)
8	R = Me	n = 1	**41**	R = Me	n = 1 (70%)
10	R = Me	n = 2	**42**	R = Me	n = 2 (78%)

Scheme 7.

both the hexahydropyrrolo[1,2-*b*] and in the hexahydropyrido[1,2-*b*]isoquinoline series and evaluated in vitro on L1210 leukemia cell line. The results expressed as IC_{50} (concentration reducing by 50% the cell proliferation) are reported in Table 1. The perturbation of the cell cycle induced by the most active compounds were also investigated on the same cell line (Figure 2 and Table 2).

Surprisingly, the strict analogue of α-peltatin is more active in the pyrido[1,2-*b*]isoquinoline series (IC_{50} (L1210) = 2.3 μM for compound **9** versus 5.4 μM for compound **7**) whilst the strict analogue of β-peltatin is 15 times more potent in the pyrrolo[1,2-*b*]isoquinoline series (IC_{50} (L1210) = 0.6 μM for compound **8** versus 9.5 μM for compound **10**).

Without any exception, all the mono or di *O*-benzylated intermediates were found inactive or significantly less active than their deprotected analogues (IC_{50} between 9.7 and 19.3 μM for compounds **29, 30, 38, 39**).

Table 1. Antiproliferative activity of the target compounds **7**–**10** and some of their derivatives

Compound	IC_{50} (μM)[a]
Podophyllotoxin **1**	0.025
7	5.4
8	0.6
9	2.3
10	9.5
29	9.7
30	17.3
38	18.4
39	19.3
40	4.1
41	0.86
42	9.9

[a]Inhibition of L1210 cell proliferation measured by the MTT assay (mean of at least 2 values obtained in separate experiments).

By contrast, introduction of a *para*-fluorophenylcarbamate group on the β-peltatin analogues has no influence in both the pyrido[1,2-*b*]isoquinoline (IC_{50} (L1210) = 9.9 μM versus 9.5 μM for compound **42** and **10**) and the pyrrolo[1,2-*b*]isoquinoline (IC_{50} (L1210) = 0.86 μM versus 0.6 μM for compound **41** and **8**) series. This is the same thing for the disubstituted analogue of α-peltatin **40**.

Due to their good cytotoxicity, compounds **8** and **41** were selected for investigation of their interaction with the cell cycle of the L1210 cells.

Compared to untreated control, both induced a partial accumulation of the cells on the $G_2 + M$ and $> G_2 + M$ (probably 8N) phases of the cell cycle at 2.5 μM as reported on Figure 2 and Table 2. Between 65% and 69% of the cells are in the G_2M phase of the cell cycle compared to 24% for untreated control. About 20% are in the 8N phase (tetraploid cells) compared to 1% for untreated control. The DNA histogram of compounds **8** and **41** is comparable to that obtained with podophyllotoxin at 0.025 μM. Accumulation of the cell in the tetraploid state could be compatible with antimitotic properties.

Table 2. Typical DNA histogram and distribution into the different phases of the cell cycle of untreated (light) or treated (heavy) L1210 cells

	% of L1210 cells in cell cycle phases	
Compound	$G_2 + M$ (μM)	$> G_2 + M$ (8N) (μM)
Untreated control	24%	1%
Podophyllotoxin	55% (0.025)	26% (0.025)
8	65% (2.5)	21% (2.5)
41	69% (2.5)	20% (2.5)

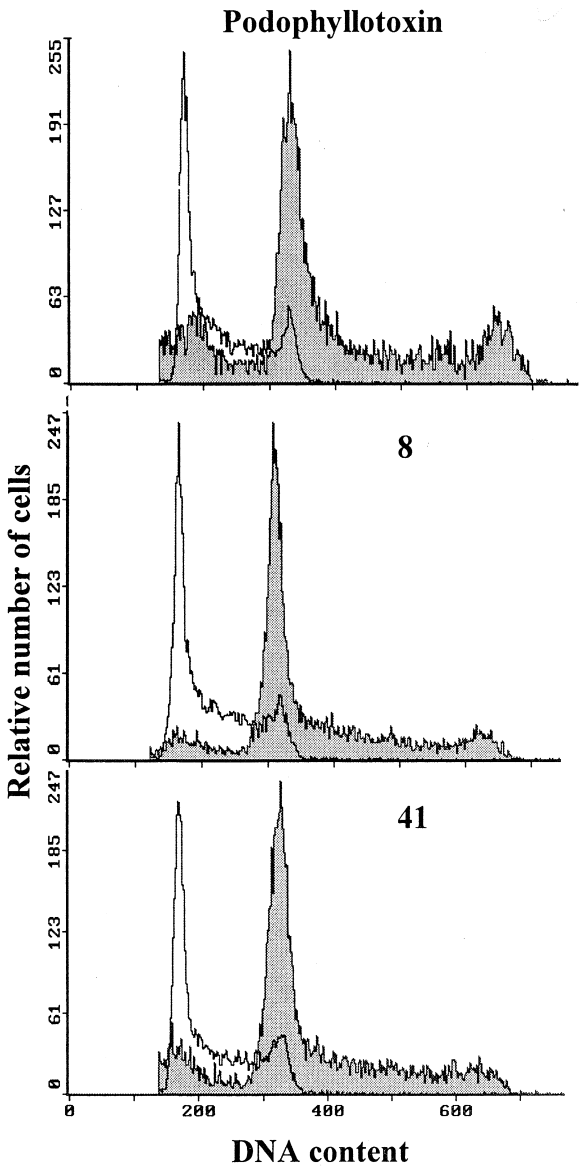


Figure 2. Typical DNA histogram and distribution into the different phases of the cell cycle of untreated (light) or treated (heavy) L1210 cells.

Experimental

General information

Methanol was distilled from magnesium turnings. Tetrahydrofuran (THF) and ether (Et_2O) were pre-dried with anhydrous Na_2SO_4 and distilled over sodium benzophenone ketyl under Ar before use. CH_2Cl_2, NEt_3, toluene were distilled from CaH_2. Dry glassware was obtained by oven-drying and assembly under dry Ar. The glassware was equipped with rubber septa and reagent transfer was performed by syringe techniques. For flash chromatography, Merck silica gel 60 (230-400 mesh ASTM) was used. The melting points were taken on a Reichert-Thermopan apparatus and are not corrected. Unless otherwise stated, proton, carbon and phosphorus NMR spectra were taken with $CDCl_3$ as solvent at 300, 75 and 121 MHz respectively on a Bruker AM 300 spectrometer. Microanalyses were performed by the CNRS microanalysis centre.

Starting materials

Triazines **24**,[33] **25**[29] and diphenylphosphine oxide **26**[36] were prepared according to the literature methods. The phosphorylated amines **20**,[30] **21**,[29] amides **16, 17**[29] and carbamate **31**[29,30] were synthesized according to already reported procedures. The benzyl protected aromatic carboxylic acid **22**[37] was obtained by oxidation with Jones reagent of the corresponding aldehyde[38] deriving from syringaldehyde. The carboxylic acid chloride **34**[39] was easily obtained by treatment of the corresponding acid **22** with thionyl chloride and was used directly without further purification.

Phosphorylated amide 16. Prepared in 84% yield as described;[29] mp 153–154 °C; IR (KBr) ν: 1646 (CO) and 1161 cm^{-1} (PO); ^1H NMR: δ 1.79–1.94 (m, 1H, CH_2), 1.96–2.17 (m, 1H, CH_2), 2.18–2.33 (m, 1H, CH_2), 2.47–2.63 (m, 1H, CH_2), 3.33–3.45 (m, 2H, NCH_2), 3.72 (s, 6H, OCH_3), 4.95 (s, 2H, OCH_2Ph), 5.43–5.52 (m, 1H, CH-P), 6.40 (s, 2H, H_{arom}), 7.21–7.43 (m, 7H, H_{arom}), 7.48–7.58 (m, 4H, H_{arom}), 7.79–7.91 (m, 2H, H_{arom}), 8.04–8.16 (m, 2H, H_{arom}); ^{13}C NMR: δ C 170.0 (CO), 153.1, 137.4, 132.2, 131.8, 131.1, 130.5 (d, $J_{CP}=92.5$ Hz), CH 131.4 (d, $J_{CP}=9$ Hz), 128.8 (d, $J_{CP}=11.5$ Hz), 128.7, 128.4, 128.1, 128.0 (d, $J_{CP}=8$ Hz), 104.6, 56.1 (d, $J_{CP}=71.5$ Hz), CH_2 74.9, 50.7, 25.6, 24.9, CH_3 56.1; ^{31}P NMR: δ 34.6. Anal. calcd for $C_{32}H_{32}NO_5P$: C, 70.97; H, 5.96; N, 2.59. Found: C, 71.12; H, 5.88; N, 2.63.

Phosphorylated amide 17. Prepared in 78% yield as described;[29] mp 148–149 °C; IR (KBr) ν: 1638 (CO) and 1168 cm^{-1} (PO); ^1H NMR: δ 1.82–1.94 (m, 1H, CH_2), 1.97–2.15 (m, 1H, CH_2), 2.19–2.30 (m, 1H, CH_2), 2.45–2.60 (m, 1H, CH_2), 3.31–3.59 (m, 2H, NCH_2), 3.73 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 5.53–5.56 (m, 1H, CH-P), 6.28 (s, 2H, H_{arom}), 7.31–7.45 (m, 3H, H_{arom}), 7.47–7.55 (m, 3H, H_{arom}), 7.82–7.90 (m, 2H, H_{arom}), 8.05–8.11 (m, 2H, H_{arom}); ^{13}C NMR: δ C 170.1 (CO), 152.8, 146.3, 131.5 (d, $J_{CP}=94$ Hz), 130.6, CH 131.8 (d, $J_{CP}=8$ Hz), 131.4 (d, $J_{CP}=9$ Hz), 128.9 (d, $J_{CP}=9$ Hz), 128.2 (d, $J_{CP}=10$ Hz), 104.6, 56.0 (d, $J_{CP}=79$ Hz), CH_2 50.6, 25.5, 24.9, CH_3 60.8, 55.9; ^{31}P NMR: δ 34.6. Anal. calcd for $C_{26}H_{28}NO_5P$: C, 67.09; H, 6.02; N, 3.01. Found: C, 67.18; H, 5.93; N, 2.88.

Synthesis of the benzaldehyde derivative 15. The synthesis of benzaldehyde **15** was carried out as follows. A solution of piperonal (5 g, 33.3 mmol) and *N,N′*-dimethylethylenediamine (3.22 g, 36.6 mmol) in toluene (100 mL) was refluxed for 5 h. The resulting mixture was washed with H_2O. The organic layer was dried over $MgSO_4$ and the solvent was removed in vacuo to leave an oil which was purified by distillation under reduced pressure to afford the imidazolidine **18** (5.8 g, 79%, bp 108 °C/ 5 10^{-3} torr).

Imidazolidine 18. ^1H NMR: δ 2.15 (s, 6H, NCH_3), 2.46–2.53 (m, 2H, NCH_2), 3.14 (s, 1H, NCH), 3.32–3.38 (m, 2H, NCH_2), 5.92 (s, 2H, OCH_2O), 6.73 (d, $J=7.8$ Hz, 1H, H_{arom}), 6.81 (dd, $J=7.8$, 1.6 Hz, 1H, H_{arom}), 6.99 (d, $J=1.6$ Hz, 1H, H_{arom}); ^{13}C NMR: δ C 147.9, 147.8, 133.8, CH 122.5, 108.4, 107.5, 92.1, CH_2 100.9, 53.1,

CH_3 39.4. Anal. calcd for $C_{12}H_{16}N_2O_2$: C, 65.43; H, 7.32; N, 12.72. Found: C, 65.56; H, 7.31; N, 12.61.

To a stirred solution of imidazolidine **18** (2 g, 9.1 mmol) in Et_2O (50 mL) was added *t*-BuLi (10.7 mL, 1.7 M in pentane, 18.2 mmol) with stirring under Ar at −78 °C. The solution was stirred at −78 °C for 15 min, then warmed to rt over a period of 30 min and re-cooled to −78 °C. A solution of trimethyl borate (0.62 g, 10.9 mmol) was added dropwise by syringe and the mixture was warmed to 0 °C over a period of 1 h. Glacial AcOH (0.5 mL) was added followed by hydrogen peroxide (30%, 1.2 mL) and the reaction mixture was stirred at room temperature for 16 h. The crude product was then poured onto an aqueous 6N HCl solution (50 mL) and the mixture was stirred for an additional 30 min. The aqueous layer was extracted twice with CH_2Cl_2 (2×30 mL) and the organic extract subsequently washed with aqueous NaOH (10%, 2×40 mL). Acidification with dilute HCl was followed by extraction with CH_2Cl_2 (2×50 mL). The organic layer was then washed with water and brine and dried ($MgSO_4$). The dried extract was concentrated in vacuo to afford **19** (1.08 g, 70%) as a yellow solid, mp 115–116 °C (lit.:[40] 115–116 °C). Benzylation of **19** under standard procedure furnished quantitatively the benzyl protected benzaldehyde derivative **15**.[41]

Synthesis of the *N*-acyl enamines 13, 14. A solution of *n*-BuLi (1.6 M in hexanes, 1.7 mL, 2.75 mmol) was added dropwise to a solution of the phosphorylated amide **16, 17** (2.5 mmol) in THF (30 mL) at −78 °C with stirring under Ar. The orange solution was stirred for an additional 15 min and a solution of the appropriate aldehyde **15** (2.5 mmol) in THF (5 mL) was then added slowly. After being stirred at −78 °C for 15 min the reaction mixture was allowed to come to room temperature over 2 h. Aqueous NH_4Cl was added and the organic layer was separated, rinsed with brine, dried ($MgSO_4$) and concentrated to dryness. The crude products were analyzed by ^1H NMR in order to determine the *E*/*Z*-isomers ratio and compounds **13, 14** (*E*- and *Z*-isomers) were separated by flash column chromatography using AcOEt:hexane (60:40) as eluent and finally recrystallized from hexane-toluene.

***N*-Acyl enamine 13.** (71%); (*E*)-Isomer, mp 125–126 °C; ^1H NMR: δ 1.78–1.91 (m, 2H, CH_2), 2.73 (td, J=7.6, 1.9 Hz, 2H, =CCH_2), 3.70 (t, J=7.0 Hz, 2H, NCH_2), 3.79 (s, 6H, OCH_3), 5.02 (s, 2H, OCH_2Ph), 5.17 (s, 2H, OCH_2Ph), 5.92 (s, 2H, OCH_2O), 6.52 (d, J=8.1 Hz, 1H, H_{arom}), 6.67 (d, J=8.1 Hz, 1H, H_{arom}), 6.77 (s, 2H, H_{arom}), 7.25–7.51 (m, 11H, $10H_{arom}+H_{vinyl}$); ^{13}C NMR: δ C 169.1 (CO), 153.4, 147.7, 139.9, 139.7, 138.1, 137.5, 137.2, 133.0, 130.6, 124.9, CH 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 121.8, 108.9, 104.6, 102.1, CH_2 101.0, 75.0, 73.6, 50.4, 30.1, 22.4, CH_3 56.2; (*Z*)-Isomer, mp 117–118 °C; ^1H NMR: δ 1.92–2.20 (m, 2H, CH_2), 2.63 (t, J=7.4 Hz, 2H, =CCH_2), 3.61 (s, 6H, OCH_3), 3.78 (t, J=7.1 Hz, 2H, NCH_2), 4.94 (s, 2H, OCH_2Ph), 4.98 (s, 2H, OCH_2Ph), 5.69 (s, 1H, H_{vinyl}), 5.78 (s, 2H, OCH_2O), 6.35 (s, 2H, H_{arom}), 6.38 (d, J=8.1 Hz, 1H, H_{arom}), 6.57 (d, J=8.1 Hz, 1H, H_{arom}), 7.28–7.49 (m, 10H, H_{arom}); ^{13}C NMR: δ C 168.6 (CO), 152.4, 147.7, 139.1, 138.6, 138.1, 137.5, 136.1, 130.9, 130.5, 123.3, CH 128.6, 128.4, 128.3, 128.2 127.8, 127.7, 123.3, 108.0, 104.5, 102.7, CH_2 100.7, 74.9, 73.0, 48.6, 32.2, 20.7, CH_3 55.6. Anal. calcd for $C_{35}H_{33}NO_7$: C, 72.54; H, 5.70; N, 2.42. Found: C, 72.36; H, 5.58; N, 2.63.

***N*-Acyl enamine 14.** (69%); (*E*)-Isomer, mp 116–117 °C; ^1H NMR: δ 1.79–1.88 (m, 2H, CH_2), 2.72 (td, J=7.3, 2.2 Hz, 2H, =CCH_2), 3.69 (t, J=6.8 Hz, 2H, NCH_2), 3.82 (s, 6H, OCH_3), 3.84 (s, 3H, OCH_3), 5.18 (s, 2H, OCH_2Ph), 5.92 (s, 2H, OCH_2O), 6.49 (d, J=8.1 Hz, 1H, H_{arom}), 6.65 (d, J=8.1 Hz, 1H, H_{arom}), 6.74 (s, 2H, H_{arom}), 7.32–7.46 (m, 6H, $5H_{arom}+H_{vinyl}$); ^{13}C NMR: δ C 169.8 (CO), 153.1, 147.7, 139.9, 139.8, 137.2, 133.6, 130.5, 128.6, 124.8, CH 128.3, 128.0, 127.9, 121.8, 108.9, 104.5, 102.7, CH_2 101.0, 73.6, 50.9, 30.2, 22.4, CH_3 60.9, 56.2; (*Z*)-isomer, mp 96–97 °C; ^1H NMR: δ 1.92–2.13 (m, 2H, CH_2), 2.64 (t, J=7.0 Hz, 2H, =CCH_2), 3.63 (s, 6H, OCH_3), 3.82 (s, 3H, OCH_3), 3.84 (t, J=7.3 Hz, 2H, NCH_2), 4.95 (s, 2H, OCH_2Ph), 5.67 (s, 1H, H_{vinyl}), 5.83 (s, 2H, OCH_2O), 6.33 (s, 2H, H_{arom}), 6.37 (d, J=8.0 Hz, 1H, H_{arom}), 6.53 (d, J=8.0 Hz, 1H, H_{arom}), 7.32–7.41 (m, 5H, H_{arom}); ^{13}C NMR: δ C 169.8 (CO), 152.8, 147.8, 145.4, 141.8, 136.1, 133.7, 131.0, 130.1, 129.5, CH 127.5, 121.1, 112.9, 108.0, 104.7, 102.6, CH_2 100.6, 73.7, 49.8, 31.9, 21.1, CH_3 60.8, 56.3. Anal. calcd for $C_{29}H_{29}NO_7$: C, 69.17; H, 5.80; N, 2.78. Found: C, 68.91; H, 5.89; N, 2.60.

Synthesis of the *N*-acyl pyrrolidine derivatives 11, 12. A suspension of compounds **13, 14** (2 mmol) in methanol (30 mL) was stirred with activated Pd/C (10%, 20 mg) and a solution of $HCOONH_4$ (640 mg, 10 mmol) in distilled water (5 mL) was slowly added. The reaction mixture was refluxed for 2 h, filtered on Celite® and water was added. Extraction with CH_2Cl_2 (3×20 mL), drying over $MgSO_4$ and concentration in vacuo left an oily product which was purified by flash chromatography using AcOEt:hexane (1:1) as eluent.

***N*-Acyl pyrrolidine 11.** (87%); mp 168–169 °C; ^1H NMR: δ 1.75–1.87 (m, 2H, CH_2), 1.93–2.17 (m, 2H, CH_2), 2.36 (dd, J=13.9, 9.6 Hz, 1H, CH_2Ar), 3.44 (d, J=13.9 Hz, 1H, CH_2Ar), 3.55–3.66 (m, 2H, NCH_2), 3.87 (s, 6H, OCH_3), 3.96–4.05 (m, 1H, NCH), 5.93 (s, 2H, OCH_2O), 6.31 (d, J=7.8 Hz, 1H, H_{arom}), 6.48 (d, J=7.8 Hz, 1H, H_{arom}), 6.84 (s, 2H, H_{arom}); ^{13}C NMR: δ C 170.4 (CO), 148.0, 146.7, 140.8, 137.1, 134.9, 126.5, 99.8, CH 121.9, 120.0, 105.1, 59.5, CH_2 101.2, 51.0, 36.8, 31.3, 25.0, CH_3 56.5. Anal. calcd for $C_{27}H_{23}NO_7$: C, 62.84; H, 5.73; N, 3.49. Found: C, 62.92; H, 5.79; N, 3.39.

***N*-Acyl pyrrolidine 12.** (90%); mp 185–186 °C; ^1H NMR: δ 1.68–1.79 (m, 2H, CH_2), 1.83–2.01 (m, 2H, CH_2), 2.33 (dd, J=14.1, 9.5 Hz, 1H, CH_2Ar), 3.35 (d, J=14.1 Hz, 1H, CH_2Ar), 3.42–3.51 (m, 2H, NCH_2), 3.79 (s, 6H, OCH_3), 3.85 (s, 3H, OCH_3), 3.75–3.83 (m, 1H, NCH), 5.89 (s, 2H, OCH_2O), 6.25 (d, J=8.0 Hz, 1H, H_{arom}), 6.41 (d, J=8.0 Hz, 1H, H_{arom}), 6.80 (s, 2H, H_{arom}); ^{13}C NMR: δ C 170.0 (CO), 147.8, 146.2, 140.4, 137.9, 134.2, 127.1, 100.0, CH 122.3, 121.6, 104.9, 58.9, CH_2 100.9, 50.1, 37.5, 29.6, 23.2, CH_3 56.8. Anal. calcd for $C_{22}H_{25}NO_7$: C, 63.61; H, 6.07; N, 3.37. Found: C, 63.50; H, 6.18; N, 3.31.

Synthesis of the benzylated compounds 27, 28. Compounds **27, 28** were prepared by benzylation of the parent phenolic compounds **11, 12** under standard conditions (BnBr, KOH, MeOH).

Compound 27. (74%); oil; ^{1}H NMR: δ (mixture of two rotational isomers A and B, 70:30) 1.51–1.98 (m, 4H, CH_2), 2.31–2.46 (m, 3H, CH_2Ar + 1H NCH_2, B), 2.59–2.66 (m, 1H, NCH_2, B), 2.88 (dd, J = 13.2, 7.9 Hz, 1H, CH_2Ar, A), 3.15 (dd, J = 13.2, 3.7 Hz, 1H, CH_2Ar, A), 3.26–3.38 (m, 2H, NCH_2, A), 3.67 (s, 6H, OCH_3, B), 3.74 (s, 6H, OCH_3, A), 4.32–4.39 (m, 1H, NCH, B), 4.46–4.57 (m, 1H, NCH, A), 5.01 (dd, J = 15.9, 10.8 Hz, 4H, OCH_2Ph, A), 5.26 (dd, J = 14.9, 11.3 Hz, 4H, OCH_2Ph, B), 5.86 (s, 2H, OCH_2O, B), 5.92 (s, 2H, OCH_2O, A), 6.14 (d, J = 7.7 Hz, 1H, H_{arom}, B), 6.36 (d, J = 7.7 Hz, 1H, H_{arom}, B), 6.41–6.53 (m, 2H, H_{arom}, A), 6.73 (s, 2H, H_{arom}, A), 6.75 (s, 2H, H_{arom}, B), 7.18–7.49 (m, 10H, H_{arom}); ^{13}C NMR: δ (mixture of two rotational isomers A and B, 70:30) C 169.6 (CO), 153.2, 148.2, 147.7, 141.6, 137.6, 137.4, 132.9, 132.8, 129.4, CH 128.9, 128.1, 128.0, 127.9, 127.4, 124.5, 123.9, 123.2, 104.8, 104.2, 102.9, 102.6, 58.3 (A), 57.1 (B), CH_2 100.9 (A), 100.2 (B), 75.0, 73.6, 50.4 (A), 45.6 (B), 34.9 (B), 32.1 (A), 29.3 (B), 29.2 (A), 24.8 (A), 22.7 (B), CH_3 56.2 (A), 55.2 (B). Anal. calcd for $C_{35}H_{35}NO_7$: C, 71.79; H, 5.98; N, 2.39. Found: C, 71.66; H, 6.09; N, 2.51.

Compound 28. (80%); mp 61–62 °C; ^{1}H NMR: δ (mixture of two rotational isomers A and B, 77:23) 1.46–2.05 (m, 4H, CH_2), 2.28–2.43 (m, 3H, CH_2Ar + 1H NCH_2, B), 2.52–2.64 (m, 1H, NCH_2, B), 2.84 (dd, J = 13.2, 8.0 Hz, 1H, CH_2Ar, A), 3.09 (dd, J = 13.2, 3.4 Hz, 1H, CH_2Ar, A), 3.17–3.35 (m, 2H, NCH_2, A), 3.56–3.61 (m, 1H, NCH, B), 3.71 (s, 3H, OCH_3), 3.78 (s, 6H, OCH_3), 4.34–4.52 (m, 1H, NCH, A), 4.81 (dd, J = 11.9 Hz, 2H, OCH_2Ph, A), 5.58 (dd, J = 15.9, 11.6 Hz, 2H, OCH_2Ph, B), 5.78 (s, 2H, OCH_2O, B), 5.81 (s, 2H, OCH_2O, A), 6.13 (d, J = 7.6 Hz, 1H, H_{arom}, B), 6.29 (d, J = 7.6 Hz, 1H, H_{arom}, B), 6.33–6.47 (m, 2H, H_{arom}, A), 6.66 (s, 2H, H_{arom}, B), 6.68 (s, 2H, H_{arom}, A), 7.13–7.45 (m, 5H, H_{arom}); ^{13}C NMR: δ (mixture of two rotational isomers A and B, 77:23) C 168.9 (CO), 152.4, 147.5, 140.6, 136.9, 136.7, 134.8, 132.4, 132.3, CH 128.4, 127.9, 127.3, 123.8 (A), 123.4 (B), 107.7 (A), 107.6 (B), 102.9 (A), 102.2 (B), 59.1 (B), 58.2 (A), CH_2 101.6 (B), 100.9 (A), 73.6 (A), 72.6 (B), 50.4 (A), 45.2 (B), 35.3 (B), 32.1 (A), 29.7 (B), 29.1 (A), 24.8 (A), 22.1 (B), CH_3 60.8, 56.2. Anal. calcd for $C_{29}H_{31}NO_7$: C, 68.91; H, 6.14; N, 2.77. Found: C, 68.78; H, 6.18; N, 2.90.

Synthesis of the cyclocondensed products 29, 30. A mixture of compound **27, 28** (2 mmol), $POCl_3$ (1.53 g, 10 mmol) in dry toluene (30 mL) was refluxed for 6 h under Ar with stirring. The solvent and excess reagent were removed under vacuum and the residue was dissolved in dry methanol (20 mL). Sodium borohydride (0.38 g, 10 mmol) was then added portionwise until pH 9, AcOEt (50 mL) was added and the organic layer washed with aqueous NaOH (10%) and dried ($MgSO_4$). Evaporation of the solvent left an oily residue which was analyzed by ^{1}H NMR spectroscopy. Major isomer was finally purified by flash column chromatography using acetone:petroleum ether (1:1) as eluent and by recrystallization from Et_2O:hexane.

Compound 29 (major isomer). Mp 122–123 °C; ^{1}H NMR: δ 1.59–1.87 (m, 3H, CH_2), 2.03–2.16 (m, 2H, CH_2), 2.34–2.56 (m, 2H, 1H NCH_2 + 1H CH_2Ar), 2.87 (td, J = 9.4, 2.4 Hz, 1H, NCH_2), 3.12 (dd, J = 15.9, 2.4 Hz, 1H, NCH), 3.78 (s, 6H, OCH_3), 4.05 (s, 1H, NCHAr), 5.03 (s, 2H, OCH_2Ph), 5.29 (dd, J = 16.8, 11.7 Hz, 1H, OCH_2Ph), 5.85 (dd, J = 7.6, 1.3 Hz, 2H, OCH_2O), 5.96 (s, 1H, H_{arom}), 6.52 (s, 2H, H_{arom}), 7.27–7.51 (m, 10H, H_{arom}); ^{13}C NMR: δ C 153.4, 147.0, 139.7, 139.0, 138.0, 137.7, 136.0, 134.4, 133.5, 121.1, CH 128.5, 128.4, 128.0, 127.9, 127.8, 127.3, 106.2, 102.3, 72.3, 60.8, CH_2 100.6, 74.9, 73.2, 53.8, 31.2, 30.7, 21.3, CH_3 56.2. Anal. calcd for $C_{35}H_{35}NO_6$: C, 74.34; H, 6.19; N, 2.48. Found: C, 74.18; H, 6.03; N, 2.55.

Compound 30 (major isomer). Mp 172–173 °C; ^{1}H NMR: δ 1.51–1.89 (m, 3H, CH_2), 2.01–2.17 (m, 2H, CH_2), 2.33–2.56 (m, 2H), 2.86 (t, J = 7.9 Hz, 1H), 3.09 (d, J = 14.9 Hz, 1H, NCH), 3.82 (s, 6H, OCH_3), 3.85 (s, 3H, OCH_3), 4.04 (s, 1H, NCHAr), 5.28 (dd, J = 16.8, 11.2 Hz, 2H, OCH_2Ph), 5.84 (d, J = 5.6 Hz, 2H, OCH_2O), 5.94 (s, 1H, H_{arom}), 6.52 (s, 2H, H_{arom}), 7.32–7.48 (m, 5H, H_{arom}); ^{13}C NMR: δ C 153.1, 147.0, 139.6, 139.0, 137.6, 137.1, 134.4, 133.4, 121.2, CH 128.4, 128.0, 127.8, 106.0, 102.2, 72.2, 60.8, CH_2 100.7, 73.1, 53.8, 31.2, 30.7, 21.2, CH_3 60.9, 56.2. Anal. calcd for $C_{29}H_{31}NO_6$: C, 71.16; H, 6.34; N, 2.86. Found: C, 71.39; H, 6.51; N, 2.73.

Synthesis of the benzoindolizidine analogues of α- and β-peltatin 7, 8. A suspension of compounds **29, 30** (major isomer, 0.5 mmol) and Pd/C (10%, 15 mg) in dry MeOH (20 mL) was treated with a solution of ammonium formate (320 mg, 5 mmol) in distilled water (3 mL). The mixture was refluxed for 2 h, filtered on Celite® and concentrated in vacuo. CH_2Cl_2 (30 mL) and water (30 mL) were added, the organic layer was rinsed with brine and dried (Na_2SO_4). The crude residue was purified by recrystallization from EtOH to afford compounds **7, 8** as pale yellow crystals.

Compound 7. (85%); mp 270–271 °C; ^{1}H NMR (d_6-DMSO): δ 1.51–1.85 (m, 3H, CH_2), 2.00–2.21 (m, 2H, CH_2), 2.31–2.75 (m, 3H, NCH_2 + 1H CH_2Ar), 3.17 (d, J = 15.8 Hz, 1H, NCH), 3.77 (s, 6H, OCH_3), 4.04 (s, 1H, NCHAr), 5.88 (s, 2H, OCH_2O), 6.01 (s, 1H, H_{arom}), 6.50 (s, 2H, H_{arom}); ^{13}C NMR: δ C 153.3, 146.7, 139.0, 138.2, 135.4, 134.2, 132.8, 99.3, CH 118.1, 105.8, 72.0, 60.3, CH_2 100.8, 53.4, 30.9, 30.5, 21.0, CH_3 56.1. Anal. calcd for $C_{21}H_{23}NO_6$: C, 65.45; H, 5.97; N, 3.64. Found: C, 65.56; H, 5.89; N, 3.80.

Compound 8. (80%); mp 257–258 °C; ^{1}H NMR (d_6-DMSO): δ 1.51–1.86 (m, 3H, CH_2), 2.05–2.19 (m, 2H, CH_2), 2.28–2.50 (m, 2H), 2.68–281 (m, 1H), 2.97–3.02 (m, 1H, NCH), 3.81 (s, 6H, OCH_3), 3.84 (s, 3H, OCH_3), 4.07 (s, 1H, NCHAr), 5.68 (s, 1H, H_{arom}), 5.86 (s, 1H, OCH_2O), 6.56 (s, 2H, H_{arom}); ^{13}C NMR: δ C 152.7, 145.9, 139.6, 137.2, 136.1, 132.2, 99.0, CH 117.9, 105.7, 70.9, 60.1, CH_2 100.4, 53.1, 30.8, 30.1, 20.8, CH_3 60.0, 55.8. Anal. calcd for $C_{22}H_{25}NO_6$: C, 66.16; H, 6.27; N, 3.51. Found: C, 66.01; H, 6.36; N, 3.62.

Synthesis of the enecarbamate 32. Enecarbamate **32** was prepared by metallation of the *N*-protected phosphorylated piperidine **31**[30] and subsequent treatment with aromatic aldehyde **15** as already described for enamides **13, 14** and obtained in 80% yield as a mixture of (*E*)- and (*Z*)-isomers (95:5). (*E*)-Isomer; mp 101–102 °C; ^{1}H NMR (C_6D_6): δ 1.27–1.38 (m, 4H, CH_2), 1.42 (s, 9H, CH_3), 2.21–2.27 (m, 2H, CH_2), 3.51–3.58 (m, 2H, CH_2), 5.17 (s, 2H, OCH_2Ph), 5.37 (s, 2H, OCH_2O), 6.44 (d, J = 8.0 Hz, 1H, H_{arom}), 6.60 (s, 1H, H_{vinyl}), 6.67 (d, J = 8.0 Hz, 1H, H_{arom}), 7.02–7.18 (m, 3H, H_{arom}), 7.33–7.37 (m, 2H, H_{arom}); ^{13}C NMR (C_6D_6): δ C 154.3, 148.9, 141.0, 139.7, 138.2, 124.1, 79.2, CH 128.6, 128.3, 123.5, 120.5, 103.1, CH_2 101.1, 73.8, 47.1, 28.3, 26.0, 25.6, CH_3 28.5. Anal. calcd for $C_{25}H_{29}NO_5$: C, 70.90; H, 6.90; N, 3.31. Found: C, 70.96; H, 6.78; N, 3.26.

Synthesis of the 2-arylmethylpiperidine derivative 33. Trifluoroacetic acid (1.8 mL, 23 mmol) was added under Ar with stirring to a solution of the enecarbamate **32** (0.973 g, 2.3 mmol) in anhydrous CH_2Cl_2 (5 mL) and stirring was maintained overnight. Evaporation of the solvent and excess reagent under vacuum left a residue which was dissolved in anhydrous MeOH (50 mL). Sodium borohydride (885 mg, 23.3 mmol) was then added portionwise at 0 °C and the reaction mixture was stirred at this temperature for an additional 1 h. EtOAc (60 mL) was then added and the organic solution was washed twice with aqueous NH_4OH (2×50 mL) then with water and brine and finally dried over Na_2SO_4. The solvent was removed under vacuum to leave an oily residue of the compound **33** (0.56 g, 75%) which was used without further purification; ^{1}H NMR (C_6D_6): δ 1.61–1.80 (m, 7H, CH_2 + NH), 2.63–2.81 (m, 2H, CH_2), 2.92–2.98 (m, 1H, CH_2), 3.21 (br. t, J = 6.7 Hz, 1H, CH_2), 3.83 (dd, J = 8.9, 3.1 Hz, 1H, CH), 5.23 (s, 2H, OCH_2Ph), 5.89 (s, 2H, OCH_2O), 6.46 (d, J = 7.9 Hz, 1H, H_{arom}), 6.62 (d, J = 7.9 Hz, 1H, H_{arom}), 7.21–7.48 (m, 5H, H_{arom}); ^{13}C NMR (C_6D_6): δ C 147.8, 140.5, 137.5, 128.6, 125.9, CH 128.3, 128.0, 127.8, 123.0, 102.8, 59.5, CH_2 100.8, 73.4, 45.9, 36.1, 31.1, 25.5, 24.7. Anal. calcd for $C_{20}H_{23}NO_3$: C, 73.82; H, 7.12; N, 4.30. Found: C, 73.63; H, 6.98; N, 4.44.

Synthesis of the *N*-acyl piperidines 36,37. The Schotten–Baumann reaction between amine **33** and the appropriate carboxylic acid chlorides **34, 35** was carried out under standard conditions.

***N*-Acyl piperidine 36.** (78%); yellow oil; ^{1}H NMR: δ 1.30–1.96 (m, 6H, CH_2), 2.43–2.57 (m, 2H, CH_2), 2.94 (dd, J = 13.5, 8.0 Hz, 1H, CH_2), 3.16–3.31 (m, 2H, CH_2), 3.86 (s, 6H, OCH_3), 5.45 (s, 2H, OCH_2Ph), 5.50 (s, 2H, OCH_2Ph), 5.90 (s, 2H, OCH_2O), 6.21 (s, 1H, H_{arom}), 6.72 (s, 2H, H_{arom}), 7.15–7.43 (m, 10H, H_{arom}); ^{13}C NMR: δ C 169.2 (CO), 153.4, 148.1, 147.6, 140.3, 138.0, 137.1, 134.2, 132.1, 131.8, 129.5, CH 129.0, 128.2, 128.1, 128.0, 127.5, 124.5, 107.7, 55.2, CH_2 100.8, 74.1, 73.2, 45.4, 36.0, 31.1, 25.4, 19.3, CH_3 56.2. Anal. calcd for $C_{36}H_{37}NO_7$: C, 72.59; H, 6.26; N, 2.35. Found: C, 72.71; H, 6.46; N, 2.17.

***N*-Acyl piperidine 37.** (75%); yellow oil; ^{1}H NMR: δ 1.34–1.98 (m, 6H, CH_2), 2.40–2.56 (m, 2H, CH_2), 2.96 (dd, J = 13.4, 8.1 Hz, 1H, CH_2), 3.12–3.26 (m, 2H, CH_2), 3.79 (s, 6H, OCH_3), 3.82 (s, 3H, OCH_3), 5.51 (s, 2H, OCH_2Ph), 5.91 (s, 2H, OCH_2O), 6.18 (s, 1H, H_{arom}), 6.67 (s, 2H, H_{arom}), 7.12–7.39 (m, 5H, H_{arom}); ^{13}C NMR: δ C 169.1 (CO), 152.1, 147.8, 140.7, 137.1, 134.5, 132.1, 131.7, CH 128.5, 128.0, 127.5, 123.9, 107.5, 55.1, CH_2 100.9, 73.1, 45.2, 35.3, 30.9, 25.3, 19.2, CH_3 60.8, 56.3. Anal. calcd for $C_{30}H_{33}NO_7$: C, 69.35; H, 6.40; N, 2.70. Found: C, 69.13; H, 6.36; N, 2.64.

Synthesis of the cyclocondensed products 38, 39. The Bischler–Napieralski cyclization reaction of amides **36, 37** was performed as previously described for the corresponding pyrrolidine derivatives **27, 28** to give **38**, **39** in 76 and 71% yield respectively.

Compound 38 (major isomer). Mp 149–150 °C; ^{1}H NMR: δ 1.23–1.88 (m, 7H, CH_2), 2.24 (br. t, J = 9.7 Hz, 1H, CH_2), 2.47 (dd, J = 16.4, 11.0 Hz, 1H, CH_2), 2.79–2.88 (m, 2H, CH_2), 3.77 (s, 6H, OCH_3), 3.96 (s, 1H, NCH), 5.00 (s, 2H, OCH_2Ph), 5.27 (dd, J = 16.0, 11.7 Hz, 2H, OCH_2Ph), 5.82 (dd, J = 5.2, 1.2 Hz, 2H, OCH_2O) 5.87 (s, 1H, H_{arom}), 6.50 (s, 2H, H_{arom}), 7.25–7.49 (m, 10H, H_{arom}); ^{13}C NMR: δ C 153.4, 146.8, 140.5, 138.3, 138.0, 137.6, 134.0, 132.3, 127.7, 119.9, CH 128.5, 128.4, 128.0, 127.8, 106.4, 102.3, 72.6, 57.8, CH_2 100.5, 74.9, 73.1, 54.1, 34.0, 31.8, 26.0, 24.4, CH_3 56.2. Anal. calcd for $C_{36}H_{37}NO_6$: C, 74.59; H, 6.43; N, 2.42. Found: C, 74.65; H, 6.32; N, 2.30.

Compound 39 (major isomer). Mp 173–174 °C; ^{1}H NMR: δ 1.26–1.85 (m, 7H, CH_2), 2.26 (t, J = 10.5 Hz, 1H, CH_2), 2.45 (dd, J = 16.1, 10.5 Hz, 1H, CH_2), 2.75–2.92 (m, 2H, CH_2), 3.82 (s, 6H, OCH_3), 3.84 (s, 3H, OCH_3), 3.96 (s, 1H, NCH), 5.27 (dd, J = 16.2, 11.6 Hz, 2H, OCH_2Ph), 5.80 (s, 2H, OCH_2O), 5.91 (s, 1H, H_{arom}), 6.52 (s, 2H, H_{arom}), 7.26–7.44 (m, 5H, H_{arom}); ^{13}C NMR: δ C 153.0, 146.8, 140.5, 138.3, 137.6, 136.3, 134.0, 132.2, 119.9, CH 128.4, 128.0, 127.8, 106.2, 102.3, 72.5, 57.7, CH_2 100.5, 73.1, 54.1, 34.0, 31.7, 26.0, 24.4, CH_3 60.8, 56.1. Anal. calcd for $C_{30}H_{33}NO_6$: C, 71.55; H, 6.61; N, 2.78. Found: C, 71.61; H, 6.69; N, 3.73.

Synthesis of the benzoquinolizidine analogues of α- and β-peltatin 9, 10. The final deprotection of the annulated compounds **38, 39** (major isomer) was carried out as described previously for the indolizidine analogues.

Compound 9. (87%); mp 290–291 °C; ^{1}H NMR: δ 1.50–2.10 (m, 7H, CH_2), 2.85–3.18 (m, 3H, CH_2), 3.46–3.52 (m, 1H, CH_2), 3.75 (s, 6H, OCH_3), 3.83 (s, 1H, NCH), 5.50 (s, 1H, H_{arom}), 5.91 (s, 2H, OCH_2O), 6.95 (s, 2H, H_{arom}); ^{13}C NMR: δ C 146.5, 136.7, 136.1, 133.4, 126.8, 124.8, 98.0, CH 115.5, 106.1, 70.8, 60.8, CH_2 100.9, 52.0, 30.4, 28.2, 22.4, 21.7, CH_3 56.1. Anal. calcd for $C_{23}H_{27}NO_6$: C, 66.15; H, 6.31; N, 3.51. Found: C, 66.21; H, 6.36; N, 3.40.

Compound 10. (90%); mp 308–309 °C; ^{1}H NMR: δ 1.56–2.18 (m, 7H, CH_2), 2.78–3.09 (m, 3H, CH_2), 3.35–3.54 (m, 1H, CH_2), 3.69 (s, 3H, OCH_3), 3.77 (s, 6H, OCH_3), 3.81 (s, 1H, NCH), 5.49 (s, 1H, H_{arom}), 5.92 (s, 2H, OCH_2O), 7.02 (s, 2H, H_{arom}); ^{13}C NMR: δ C 146.4, 136.8, 136.1, 133.6, 126.6, 125.0, 98.2, CH 115.6, 106.2,

70.8, 60.8, CH_2 100.9, 52.0, 34.0, 28.2, 22.4, 21.7, CH_3 56.1. Anal. calcd for $C_{23}H_{27}NO_6$: C, 66.81; H, 6.58; N, 3.39. Found: C, 66.74; H, 6.65; N, 3.36.

Synthesis of the urethanes 40–42. Urethanes **40–42** were prepared according to the literature methods.[42]

Compound 40. (75%); mp 115–116 °C; ^{1}H NMR: δ 1.51–1.82 (m, 5H, CH_2), 1.96–2.12 (m, 2H, CH_2), 2.33–2.59 (m, 1H, CH_2), 2.75–2.97 (m, 1H, CH), 3.81 (s, 6H, OCH_3), 4.11 (s, 1H, NCH), 5.90 (d, J = 5.6 Hz, 2H, OCH_2O), 6.15 (s, 1H, H_{arom}), 6.61 (s, 2H, H_{arom}), 6.96–7.43 (m, 10H, $8H_{arom}$ + 2NH); ^{13}C NMR: δ C 156.2 (d, J_{CF} = 249 Hz, CF), 154.1 (d, J_{CF} = 249 Hz, CF), 152.7, 150.6, 147.1, 142.2, 137.7, 133.9, 133.2, 133.0, 130.5, 122.5, 120.7, 120.5, CH 116.0, 115.8, 115.7, 115.5, 106.0, 105.7, 72.1, 60.3, CH_2 101.9, 53.7, 31.1, 30.2, 21.2, CH_3 56.3. Anal. calcd for $C_{35}H_{31}F_2N_3O_8$: C, 63.73; H, 4.74; N, 6.37. Found: C, 63.87; H, 4.58; N, 6.15.

Compound 41. (70%); mp 157–158 °C; ^{1}H NMR: δ 1.48–1.79 (m, 5H, CH_2), 1.98–2.11 (m, 2H, CH_2), 2.36–2.65 (m, 1H, CH_2), 2.80–3.03 (m, 1H, CH), 3.82 (s, 6H, OCH_3), 3.85 (s, 3H, OCH_3), 4.06 (s, 1H, NCH), 5.92 (s, 2H, OCH_2O), 6.12 (s, 1H, H_{arom}), 6.54 (s, 2H, H_{arom}), 6.97–7.04 (m, 2H, H_{arom}), 7.27–7.43 (m, 3H, $2H_{arom}$ + NH); ^{13}C NMR: δ C 155.9 (d, J_{CF} = 240 Hz, CF), 153.1, 150.5, 147.0, 139.2, 133.4, 130.5, 122.5, 120.7, 120.6, CH 115.9, 115.7, 106.1, 105.9, 72.1, 60.4, CH_2 101.8, 53.7, 31.2, 30.2, 21.1, CH_3 60.9, 56.2. Anal. calcd for $C_{29}H_{29}FN_2O_7$: C, 64.92; H, 5.45; N, 5.22. Found: C, 67.17; H, 5.65; N, 5.06.

Compound 42. (78%); mp 121–122 °C; ^{1}H NMR: δ 1.28–1.57 (m, 4H, CH_2), 1.71–1.86 (m, 3H, CH_2), 2.30–2.28 (m, 1H, CH_2), 2.51–2.60 (m, 1H, CH_2), 2.67 (dd, J = 14.3, 3.5 Hz, 1H), 2.79–2.83 (m, 1H, CH), 3.82 (s, 6H, OCH_3), 3.85 (s, 3H, OCH_3), 3.98 (s, 1H, NCH), 5.86 (dd, J = 6.1, 1.4 Hz, 2H, OCH_2O), 6.08 (s, 1H, H_{arom}), 6.55 (s, 2H, H_{arom}), 6.95–7.03 (m, 2H, H_{arom}), 7.36–7.44 (m, 3H, $2H_{arom}$ + NH); ^{13}C NMR: δ C 157.7 (d, J = 245 Hz, CF), 153.2, 150.6, 146.8, 140.0, 137.2, 133.3, 132.2, 129.7, 121.3, 120.6, CH 115.9, 115.6, 106.2, 106.0, 72.4, 57.5, CH_2 101.6, 54.0, 34.0, 31.2, 25.9, 24.3, CH_3 60.9, 56.2. Anal. calcd for $C_{30}H_{31}FN_2O_7$: C, 65.45; H, 5.68; N, 5.09. Found: C, 65.71; H, 5.60; N, 5.30.

Biological materials: cell culture and cytotoxicity

L1210 cells (Murine Leukemia) provide by the NCI, Frederik, USA were cultivated in RPMI 1640 medium (Gibco) supplemented with 10% foetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10mM HEPES buffer (pH = 7.4).

Cytotoxicity was measured by the microculture tetrazolium assay as described.[43] Cells were exposed to graded concentrations of the compounds for 48 h and results expressed as IC_{50} (concentration which reduced by 50% the optical density of treated cells with respect to untreated controls).

For the cell cycle analysis, L1210 cells (2.5 10^5 cells/mL) were incubated for 21 h with various concentrations of the compounds, then fixed by 70% ethanol (v/v), washed and incubated in PBS containing 100 μg/mL RNAse and 25 μg/mL propidium iodide for 30 min at 20 °C. For each sample, 10^4 cells were analyzed on an ATC3000 flow cytometer (Bruker, France) using an argon laser (Spectra-Physics) emitting 400 mW at 488 nM. The fluorescence of propidium iodide was collected through a 615 nM long-pass filter. Data are displayed as linear histograms and results are expressed as the percentage of cells found in the G_2 + M phase of the cell cycle.

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