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Cytotoxic Activities of Novel Hexahydroindolizino[8,7-b]indole Derivatives Prepared by 1,3-Dipolar Cycloaddition Reactions of 3,4-Dihydro-β-carboline Ylides

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Abstract—A series of 1-cyano and 2-cyanohexahydroindolizino[8,7-b]indole derivatives was prepared by 1,3-dipolar cycloaddition of acrylonitrile with ylides derived from 3,4-dihydro-β-carboline and its 6-methoxy, 6-benzyloxy, 9-methyl and 9-benzyl analogues. The products, together with their reduced 1- or 2-aminomethyl derivatives, were evaluated for cytotoxic activity in L1210 cancer cells. Compounds derived from 6-benzyloxy or 9-benzyl-3,4-dihydro-β-carboline were found to be the most active, with IC_{50}'s in the 2–50 μM range. Of these, two compounds, the 1- and 2-cyano 8-benzyloxyindolizino[8,7-b]indole derivatives **20a** and **20c**, respectively, were found by cytometric flux analysis to stop cancer cell growth at the G_2M and 8N ($>G_2M$) stage of the cell cycle. These two compounds also showed no loss of cytotoxic activity in K562R cancer cells resistant to doxorubicin.

The β-carboline nucleus is a recurring motif in a wide variety of cytotoxic compounds, both natural and synthetic. In addition to simple, substituted harman and norharman derivatives,[1] more complex structures such as the manzamines (e.g., **1**),[2] eudistomine K (**2**),[3] azatoxin (**3**),[4] fascaplysine (**4**),[5] picrasidine L (**5**)[6] and javacarboline (**6**)[7] display cytotoxic activities in various cancer cell lines. Structural analogues of javacarboline in the form of 1- and/or 2-methyl (or phenyl)indolizino[8,7-b]indoles (e.g., **7**) were recently synthesized by Nikaido and coworkers and shown to possess activity against P-388 and PC-6 cell lines.[8] The compounds of type **7** were prepared by base-promoted cyclizations of 1-alkyl-2-acyl-β-carbolinium bromides.

We ourselves have reported the synthesis of substituted indolizino[8,7-b]indoles by 1,3-dipolar cycloadditions of β-carboline ylides, generated from *N*-2-trimethylsilylmethyl-β-carbolinium salts, with electron-deficient dipolarophiles.[9–11] In this communication, we wish to report the synthesis and activity against L1210 murine leukemia cells of several indolizino[8,7-b]indoles prepared by our cycloaddition methodology. Some of these compounds were shown to induce significant accumulation of the L1210 cells in the G_2M and 8N ($>G_2M$) phases of the cell cycle and, moreover, to be active against resistant strains of K562 cancer cells.

The starting materials for this study were 3,4-dihydro-β-carboline (**8**) and its 6-methoxy and 6-benzyloxy analogues (**9** and **10**, respectively) prepared from the corresponding N_b-formyltryptamine derivatives by a Bischler–Napieralski reaction.[12] The N-9 position of compound **8** was further methylated (**11**)[13] or benzylated (**12**)[14] by the action of sodium hydride in DMF followed by addition of the appropriate alkylating agent (methyl tosylate or benzyl bromide). The dihydro-β-carbolines **8**–**12** were then efficiently transformed into their corresponding 2-*N*-trimethylsilylmethyl-β-carbolinium triflate salts **13**–**17**,[15] respectively, by treatment with 1.1 equiv of trimethylsilylmethyl triflate in dichloromethane.[11] In the presence of cesium fluoride, the

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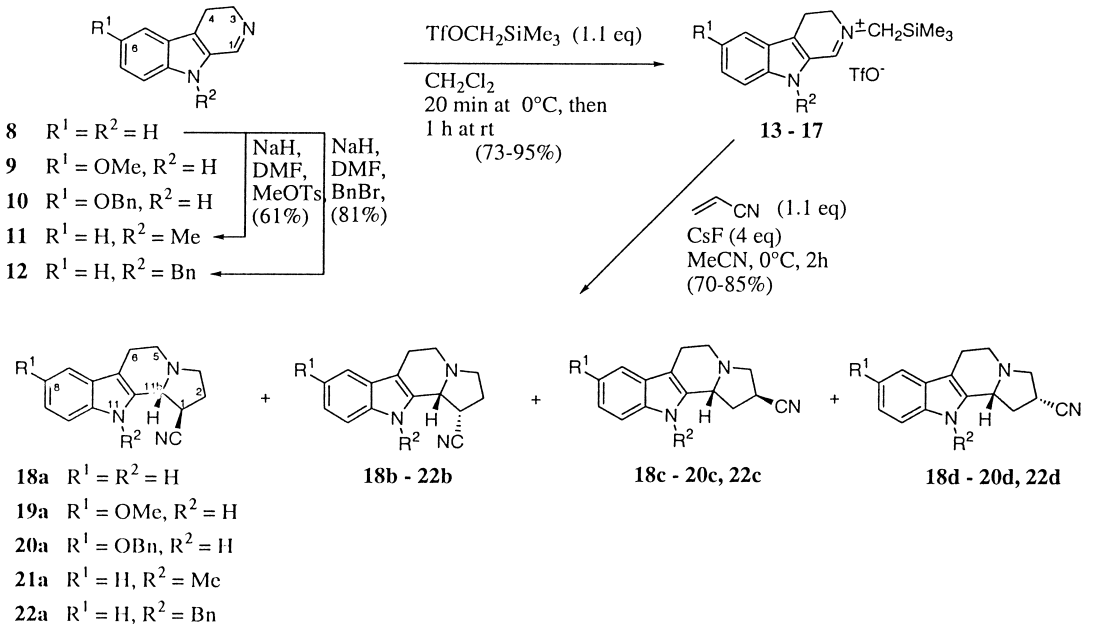
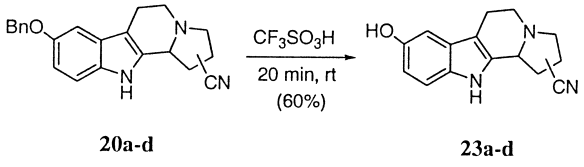


Scheme 1.

triflate salts were converted into the ylides which reacted in situ with acrylonitrile to afford the products of 1,3-dipolar cycloaddition **18**–**22** (Scheme 1).[15,16]

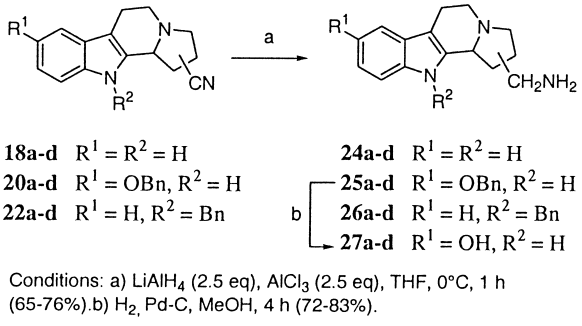
In practically all cases (except for **16**) both possible C-1 and C-2 regioisomers were obtained. Each regioisomer was, in turn accompanied by both diastereomeric forms (**a**, **b** and **c**, **d**). The four isomers produced in each reaction could be easily separated either by fractional crystallization or by chromatography on silica gel. Regio- and relative stereochemistry were determined by ^{1}H- and ^{13}C NMR spectroscopy correlated with the structure of compound **18c** determined by X-ray crystallography. Overall yields were in the 70–85% range for each cycloaddition reaction. All compounds shown are racemic mixtures.

Some of the products of cycloaddition were subjected to further chemical transformations. Thus, treatment of the 8-benzyloxy derivatives **20a–d** with triflic acid in the presence of thioanisole provided approximately 60% of the corresponding analogues (**23a–d**) (Scheme 2). The nitrile functions of compounds **18a–d**, **20a–d** and **22a–d** could be selectively reduced to the primary amines (**24a–d**, **25a–d** and **26a–d**, respectively) in 65–76% yields by the action of aluminum hydride (prepared in situ from lithium aluminum hydride and aluminum chloride) in THF at 0 °C (Scheme 3). Use of lithium aluminum hydride alone led to epimerization of the substrates. Finally, catalytic hydrogenolysis of the 8-benzyloxy-1-(and 2-) aminomethyl derivatives **25a–d** afforded the corresponding 8-hydroxy compounds **27a–d** in 72–83% yield.



Scheme 2.

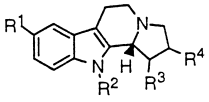
The hexahydroindolizino[8,7-b]indole derivatives synthesized were then tested for cytotoxic activity in vitro in L1210 cancer cells.[17,18] As seen from Table 1, none of the four cyano derivatives having an unsubstituted indole nucleus (**18a–d**) or the two cyano derivatives having an *N*-methyl substituent (**21a,b**) inhibited the growth of L1210 cells at 100 μM concentrations. However, replacement of the *N*-methyl group by an *N*-benzyl group (compounds **22a–d**) produced cytotoxic compounds having IC_{50}'s in the 45–56 μM range. Interestingly, all four isomers showed approximately equivalent cytotoxicities. While introduction of an 8-methoxy group (compounds **19a–d**) also led to inactive compounds, the 8-benzyloxy group had a favorable effect on cytotoxicities with compounds **20a** and **20c** displaying IC_{50}'s of 15.6 and 16.9 μM, respectively. Both these *cis* regioisomeric cyano derivatives were 2–3 times more active than their *trans* isomers (**20b** and **20d**). Removal of the 8-benzyl group to give, by analogy with the ellipticine family of cytotoxic compounds, the 8-hydroxy derivatives **23a–d** led to complete loss of activity for all four isomers.



Scheme 3.

Attention was then turned to the primary amine derivatives corresponding to reduction of the nitrile functions. In contrast to the unsubstituted 1- and 2-cyanoindolizino[8,7-b]indoles **18a–d**, which were completely inactive, the amino counterparts **24a–d** showed significant cytotoxic activities, with the most potent isomer being the *cis*-1-aminomethyl derivative **24a** which displayed an IC_{50} of 17.5 μM. Even more active were the C-8 benzyloxy 1- and 2-aminomethyl derivatives **25a–d** as well as the N-11 benzyl analogues **26a–d** which all displayed IC_{50} values in the 2–5 μM range. Again, in both series of molecules, activity was little affected by the regio- or stereochemistry of the aminomethyl group. In contrast, in the 8-hydroxy series **27a–d**, only the *cis*-1-aminomethyl derivative **27a** showed cytotoxic activity ($IC_{50} = 7.6$ μM), the other three isomers being completely inactive.

Table 1. Antiproliferative activity of the indolizino[8,7-*b*]indoles with respect to L1210 tumor cells



Compound	R^1	R^2	R^3	R^4	IC_{50} (μM)
Adriamycin	—	—	—	—	0.021
18a	H	H	*c*-CN[a]	H	>100
18b	H	H	*t*-CN	H	>100
18c	H	H	H	*c*-CN	>100
18d	H	H	H	*t*-CN	>100
19a	OMe	H	*c*-CN	H	>100
19b	OMe	H	*t*-CN	H	>100
19c	OMe	H	H	*c*-CN	>100
19d	OMe	H	H	*t*-CN	>100
20a	OBn	H	*c*-CN	H	15.6
20b	OBn	H	*t*-CN	H	49.5
20c	OBn	H	H	*c*-CN	16.9
20d	OBn	H	H	*t*-CN	36.6
21a	H	Me	*c*-CN	H	>100
21b	H	Me	*t*-CN	H	>100
22a	H	Bn	*c*-CN	H	55.8
22b	H	Bn	*t*-CN	H	50.0
22c	H	Bn	H	*c*-CN	45.1
22d	H	Bn	H	*t*-CN	47.6
23a	OH	H	*c*-CN	H	>100
23b	OH	H	*t*-CN	H	>100
23c	OH	H	H	*c*-CN	>100
23d	OH	H	H	*t*-CN	>100
24a	H	H	*c*-CN_2NH_2	H	17.5
24b	H	H	*t*-CN_2NH_2	H	37.3
24c	H	H	H	*c*-CN_2NH_2	42.3
24d	H	H	H	*t*-CN_2NH_2	32.1
25a	OBn	H	*c*-CN_2NH_2	H	2.5
25b	OBn	H	*t*-CN_2NH_2	H	2.5
25c	OBn	H	H	*c*-CN_2NH_2	2.1
25d	OBn	H	H	*t*-CN_2NH_2	2.5
26a	H	Bn	*c*-CN_2NH_2	H	4.4
26b	H	Bn	*t*-CN_2NH_2	H	4.85
26c	H	Bn	H	*c*-CN_2NH_2	1.8
26d	H	Bn	H	*t*-CN_2NH_2	2.4
27a	OH	H	*c*-CN_2NH_2	H	7.6
27b	OH	H	*t*-CN_2NH_2	H	>100
27c	OH	H	H	*c*-CN_2NH_2	>100
27d	OH	H	H	*t*-CN_2NH_2	>100

[a]*a*-CN and *c*-CH_2NH_2 refer to these groups being *cis* to H-11b while *t*-CN and *t*-CH_2NH_2 refer to these groups being *trans* to H-11b.

The effects of the most active compounds on the different phases of the cell cycle were then determined using cytometric flux. L1210 cells, in contact with the test substances, could thus be sorted according to their levels of nuclear DNA, thereby indicating at which stage of the cell cycle growth was inhibited.[19] All the aminomethyl derivatives tested (the unsubstituted derivative **24a**, the C-8 benzyloxy derivatives **25a–d,** the N-11 benzyl derivatives **26a–d** and the C-8 hydroxy derivative **27a**) showed non-selective action against L1210 cells (data not shown). In the case of the two most active members of the 1- and 2-cyano derivatives (**20a** and **20c**), these were found to induce significant accumulation of the cells in the G_2M and 8N (>G_2M) phases of the cell cycle at a concentration of 25 μM. Approximately 45% of the L1210 cells were in the G_2M phase and between 17 and 33% in the 8N phase (tetraploid cells) compared to 28 and 1%, respectively, for untreated controls (Table 2). The cytotoxic activity of these two nitrile compounds thus appears to be due to an interaction with specific cellular components, unlike the aminomethyl derivatives which are non-specifically toxic.

In view of this apparently selective action observed with nitriles **20a** and **20c**, these compounds were further investigated for cytotoxic activity in a multidrug resistant cancer cell line. Thus, in K562R human erythroleukemia cells resistant to doxorubicin, both compounds **20a** and **20b** displayed cytotoxic activities (IC_{50} 9.0 and 8.2 μM, respectively) similar or identical to activities in the doxorubicin-sensitive K562S strain (IC_{50} 8.0 and 8.2 μM, respectively; Table 3).[20,21] By comparison, doxorubicin undergoes a 2000-fold loss of potency in the K562R cells with respect to the K562S cells (IC_{50} 20 and 0.01 μM, respectively).

In summary, the present results show that substituted hexahydroindolizino[8,7-*b*]indoles should be a profitable

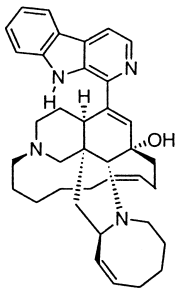
Table 2. Effect of compounds **20a** and **20c** on the L1210 cell cycle

Compound	Effect on cell cycle[a,b]		
	G_2M	8N (>G_2M)	c (μM)
Adriamycin	83	6	0.1
20a	47	17	25
20c	46	33	25

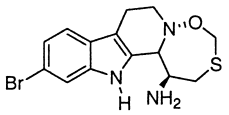
[a]Results expressed as percentage of the cells found in the G_2M and 8N (>G_2M) phases of the cell cycle.
[b]Untreated control L1210 cells: 40% G_1; 31% S; 28% G_2M; 1% 8N (>G_2M).

Table 3. Comparison of the growth inhibitory potency of compounds **20a** and **20c** in K562 tumor cells sensitive (S) and resistant (R) to doxorubicin

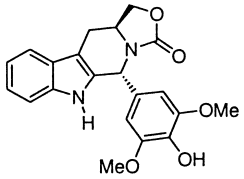
Compound	IC_{50} (μM)	
	K562S	K562R
Doxorubicin	0.01	20
20a	8.0	9.0
20c	8.2	8.2



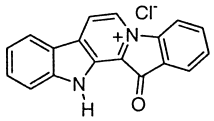
1 Manzamine A



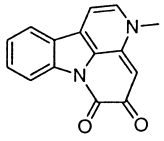
2 Eudistomine K



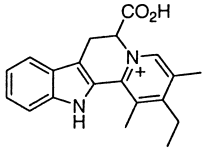
3 Azatoxin



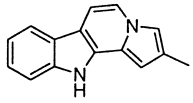
4 Fascaplysine



5 Picrasidine L



6 Javacarboline



7 2-Methylindolizino[8,7-b]indole

Chart 1.

class of compounds to exploit in the search for new antitumor agents. While cytotoxic activities of the compounds presented here remain relatively modest (i.e., with IC_{50}'s in the low μM range) they are comparable to those of the compounds shown in Chart 1, many of which have served as starting points for the development of more active compounds. More significant, however, is the observation that some of the compounds prepared in this study (e.g., **20a**, **20c**) appear to act on specific (though as yet unknown) cell targets rather than via generalized non-specific toxic mechanisms. Finally the fact that both these compounds are also active in a multidrug resistant cancer cell line lends strong impetus to further structure–activity relationship studies in this class of compounds, using the dipolar cycloaddition methodology which we have developed. The results of these studies will be reported in due course.

Experimental

Chemistry

General. Melting points were determined on a Büchi apparatus and are uncorrected. IR spectra of samples were obtained either as KBr pellets or as films with a Nicolet 205 FT-IR spectrometer. ^1H NMR and ^{13}C NMR were determined on a Bruker 200, 250 or 300 MHz instrument. Chemical shifts are given as δ values with reference to Me_4Si as internal standard. Electron impact (EI) and chemical ionization (CI) mass spectra were recorded on an AEI MS-50 nd AEI MS-9 spectrometer, respectively. High-resolution (HR), fast atom bombardment (FAB) and liquid secondary ion (L-SI) mass spectra were obtained using a Kratos MS-80 spectrometer. Thin-layer chromatography was performed on Merck silica gel plates with fluorescent indicator. The plates were visualized with UV light (254 nm) or with a 3.5% solution of phosphomolybdic acid in ethanol. Column chromatography was conducted on Merck 60 silica gel (230–400 mesh) at medium pressure (200 mbar). Reagents were purchased from the Aldrich Chemical Co. and were used without further purification. All solvents were distilled and stored over 4 Å molecular sieves before use. Elemental analyses were performed at the ICNS, CNRS, Gif-sur-Yvette.

General procedure for the preparation of 3,4-dihydro-β-carbolines 8, 9 and 10. To phosphorus oxychloride (5 mL) was added, in small portions with rapid stirring under argon, the appropriate solid N_b-formyltryptamine (10 mmol), the reaction mixture being kept at room temperature by means of an ice-water bath. Stirring was continued until complete disappearance of the starting material (20–60 min). The bright yellow suspension was slowly poured into anhydrous diethyl ether (70 mL) with rapid stirring. The hydrochloride salt of the dihydro-β-carboline which precipitated was then collected by filtration and washed several times with ether. Recrystallization of the solid in a mixture of ethyl acetate and 95% ethanol afforded the pure 3,4-dihydro-β-carboline hydrochloride (which, for **10**, was used for elemental analysis). The latter was dissolved in water (45 mL) and the solution was made basic by slow addition of aqueous sodium hydroxide (1 M), leading to precipitation of the β-carboline. The mixture was extracted with ether (3×20 mL), the organic extracts were combined, washed with saturated aqueous NaCl (30 mL), dried over sodium sulfate and the solvent removed under reduced pressure, leaving a pale yellow amorphous solid which was crystallized in the solvent specified below. The following 3,4-dihydro-β-carbolines were prepared in this manner.

3,4-Dihydro-β-carboline (8). Obtained in 82% yield from N_b-formyltryptamine[22] after crystallization in

ethyl acetate : mp 87–90 °C (lit. mp 93–97 °C)[21]; ^{1}H NMR (200 MHz, $CDCl_3$ + 10% CD_3OD) δ 2.89 (t, J = 8.6 Hz, 2H), 3.87 (t, J = 8.6 Hz, 2H), 7.12 (t, J = 7.4 Hz, 1H), 7.26 (t, J = 7.4 Hz, 1H), 7.38 (d, J = 8.1 Hz, 1H), 7.57 (d, J = 8.1 Hz, 1H), 8.29 (s, 1H); IR (KBr) 3185, 1634 cm^{-1}; L-SIMS m/z 171 $(MH)^+$.

3,4-Dihydro-6-methoxy-β-carboline (9). Obtained in 91% yield from 5-methoxy-N_b-formyltryptamine[23] after crystallization in ethyl acetate: mp 204 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.81 (t, J = 6.0 Hz, 2H), 3.83 (br s, 5H), 6.91 (d, J = 8.4 Hz, 1H), 6.93 (s, 1H), 7.28 (d, J = 8.4 Hz, 1H), 8.35 (s, 1H), 8.56 (br s, 1H, exchangeable with D_2O); IR (KBr): 3300, 1625 cm^{-1}; HREIMS calcd for $C_{12}H_{12}N_2O$ m/z 200.0947, found 200.0959.

6-Benzyloxy-3,4-dihydro-β-carboline (10). Obtained in 72% yield from 5-benzyloxy-N_b-formyltryptamine[24] after crystallization in ethyl acetate–95% ethanol : mp 82–83 °C (hygroscopic); ^{1}H NMR (250 MHz, CD_3OD) δ 2.75 (t, J = 8.5 Hz, 2H), 3.74 (t, J = 8.5 Hz, 2H), 4.96 (s, 2H), 6.93–7.00 (m, 2H), 7.25–7.40 (m, 6H), 8.22 (s, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 19.2, 48.3, 70.8, 102.3, 112.2, 113.1, 115.5, 116.3, 125.5, 127.6, 127.9, 128.5, 132.7, 137.4, 152.2, 153.6; IR (KBr) 3398, 1602 cm^{-1}; CIMS m/z 277 $(MH)^+$. Anal. calcd for $C_{18}H_{16}N_2O.HCl.H_2O$: C, 65.35; H, 5.79; N, 8.47; Cl, 10.72. Found: C, 65.08; H, 5.99; N, 8.31; Cl, 10.68.

3,4-Dihydro-9-*N*-methyl-β-carboline (11). To a suspension of sodium hydride (1.13 g of a 60% dispersion in oil; 28.2 mmol) in anhydrous DMF (20 mL) was added dropwise with stirring at −10 °C under argon a solution of compound **8** (2.84 g, 16.7 mmol) in DMF (10 mL). The mixture was stirred for 1 h at −10 °C and then cooled to −60 °C before dropwise addition of a solution of methyl tosylate (3.41 g, 18.4 mmol) in DMF (10 mL). Stirring was maintained for 2 h at −60 °C and methanol (5 mL), water (5 mL) and saturated aqueous sodium chloride (250 mL) were added successively. The solution was extracted with dichloromethane (3×75 mL), the organic extracts were combined, washed with water (30 mL) and dried over sodium sulfate. The solvents were removed under reduced pressure, the residue was taken up in dichloromethane and the solution was filtered through a pad of silica gel. Evaporation of the filtrate afforded compound **11** which was crystallized in ethyl acetate–95% ethanol (1.87 g, 61%). For analysis, the hydrochloride salt was prepared by dissolving **11** in ethyl acetate and adding ethanolic HCl until the solution turned acid. Addition of diethyl ether to the solution then led to precipitation of the hydrochloride salt which was collected by filtration and washed with ether: mp 148–150 °C (lit. mp 153–154 °C),[13] hydrochloride, 220–222 °C; ^{1}H NMR (hydrochloride, 250 MHz, DMSO-d_6) δ 3.37 (t, J = 9.0 Hz, 2H), 4.05 (s, 3H), 4.06 (t, J = 9.0 Hz, 2H), 7.33 (t, J = 7.5 Hz, 1H), 7.67 (t, J = 7.5 Hz, 1H), 7.75 (d, J = 8.2 Hz, 1H), 7.89 (d, J = 8.2 Hz, 1H), 9.46 (br s, 1H), 13.67 (br s, 1H); ^{13}C NMR (hydrochloride, 62.5 MHz, DMSO-d_6) δ 18.5, 30.1, 41.2, 111.6, 121.4, 121.9, 123.3, 126.9, 128.5, 141.5, 154.1; IR (KBr) 1639 cm^{-1}; CIMS m/z 185 $(MH)^+$. Anal. calcd for $C_{12}H_{12}N_2.HCl.0.15\ H_2O$: C, 64.41; H, 5.99; N, 12.52; Cl, 16.00. Found: C, 64.66; H, 6.29; N, 12.27; Cl, 16.15.

9-*N*-Benzyl-3,4-dihydro-β-carboline (12). Following the same procedure as for the preparation of compound **11**, treatment of compound **8** (3.0 g, 17.6 mmol) with sodium hydride (1.2 g of a 60% dispersion in oil; 29.9 mmols) and then with benzyl bromide (3.32 g, 19.4 mmol) afforded, after workup, compound **12** as a colorless amorphous solid (3.7 g, 81%): mp 132–133 °C (lit. mp 138–139 °C);[14] ^{1}H NMR (200 MHz, CD_3OD) δ 2.92 (t, J = 8.0 Hz, 2H), 3.75 (t, J = 8.0 Hz, 2H), 5.42 (s, 2H), 7.03–7.66 (m, 9H), 8.34 (br s, 1H). Anal. calcd for $C_{18}H_{16}N_2$:C, 81.91; H, 6.26; N, 10.26. Found: C, 82.01; H, 6.53; N, 10.35.

6-Benzyloxy-3,4-dihydro-2-*N*-[(trimethylsilyl)methyl]-β-carbolinium triflate (15). To a solution of compound **10** (2.76 g, 10 mmol) in anhydrous dichloromethane (30 mL) was added dropwise at 0 °C under argon a solution of (trimethylsilyl)methyl triflate (2.2 mL, 11 mmol) in dichloromethane (10 mL). The reaction mixture was stirred for 20 min at 0 °C and then for 1 h at rt. The solvent was removed under reduced pressure and the residue was crystallized in ethanol, affording compound **15** as bright yellow needles (3.78 g, 95%) : mp 168–170 °C; ^{1}H NMR (250 MHz, acetone-d_6) δ 0.30 (s, 9H), 3.42 (m, 2H), 3.56 (s, 2H), 4.25 (m, 2H), 5.15 (s; 2H), 7.20–7.57 (m, 8H), 8.94 (s, 1H), 11.29 (br s, 1H); ^{13}C NMR (62.5 MHz, acetone-d_6) δ-2.6, 20.5, 53.2, 53.6, 70.9, 102.5, 115.6, 122.8, 127.2, 128.5, 128.7, 129.4, 130.7, 132.4, 143.5, 144.1, 158.1, 160.5; IR (KBr) 3206, 1616, 1289 cm^{-1}; L-SIMS m/z 363 $(MH)^+$. Anal. calcd for $C_{23}H_{27}F_3N_2O_4SSi.0.25H_2O$:C, 53.42; H, 5.36; N, 5.42; S, 6.20. Found: C, 53.23; H, 5.22; N, 5.19; S, 5.93.

3,4-Dihydro-9-*N*-methyl-2-*N*-[(trimethylsilyl)methyl]-β-carbolinium triflate (16). Following the same procedure as for the preparation of compound **15**, reaction of compound **11** (1.84 g, 10 mmol) with (trimethylsilyl)methyl triflate (2.2 mL, 11 mmol) afforded, after crystallization in ethanol, compound **16** as bright yellow needles (2.54 g, 94%) : mp 174–175 °C; ^{1}H NMR (250 MHz, CD_3OD) δ 0.28 (s, 9H), 3.37 (t, J = 8.8 Hz, 2H), 3.80 (s, 2H), 3.92 (s, 3H), 4.03 (t, J = 8.8 Hz, 2H), 7.23 (t, J = 7.5 Hz, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.50 (t, J = 7.5 Hz, 1H), 7.65 (d, J = 8.6 Hz, 1H), 9.07 (s, 1H); ^{13}C NMR (200 MHz, DMSO-d_6) δ-2.7, 19.1, 23.0, 51.4, 52.4, 111.4, 120.9, 121.4, 121.7, 123.3, 127.5, 128.2, 141.4, 151.3; IR (KBr) 1630, 1264 cm^{-1}; L-SIMS m/z 271 $(MH)^+$. Anal. calcd for $C_{17}H_{23}F_3N_2O_3SSi$:C, 48.55; H, 5.51; N, 6.66. Found: C, 48.67; H, 5.38; N, 6.61.

(1*R*,11b*R*)- and (1*S*, 11b*S*)-1,2,3,5,6,11b-Hexahydro-8-methoxyindolizino[8,7-*b*]indole-1-carbonitrile [(±)-19a], (1*S*,11b*R*)- and (1*R*,11b*S*)-1,2,3,5,6,11b-hexahydro-8-methoxyindolizino[8,7-*b*]indole-1-carbonitrile [(±)-19b], (2*S*,11b*R*)- and (2*R*,11b*S*)-1,2,3,5,6,11b-hexahydro-8-methoxyindolizino[8,7-*b*]indole-2-carbonitrile [(±)-19c] and (2*R*,11b*R*)- and (2*S*,11b*S*)-1,2,3,5,6,11b-hexahydro-8-methoxyindolizino[8,7-*b*]indole-2-carbonitrile [(±)-19d]. Cesium fluoride (0.61 g, 4 mmol) was placed in a round bottom flask and heated under vacuum at 300–400 °C

for 1–2 min. The flask was cooled and, under a stream of dry argon, anhydrous acetonitrile (3 mL) was introduced through a septum followed by a solution of acrylonitrile (58 mg, 1.1 mmol) in acetonitrile (2 mL). The mixture was cooled to 0 °C and a solution of compound **14**[11] (316 mg, 1.1 mmol) in acetonitrile (2 mL) was added dropwise. The reaction mixture was stirred for 30 min at 0 °C and then for 1 h at rt. The solvent was removed under reduced pressure, the residue was taken up in dichloromethane (50 mL) and the mixture was washed with water (50 mL). The organic phase was then extracted with aqueous hydrochloric acid (2×50 mL of a 1 M solution), the aqueous extracts were combined, washed with dichloromethane (50 mL) and neutralized by addition of aqueous sodium hydroxide (10 M). The white precipitate which formed was extracted with dichloromethane (2×50 mL), the organic extracts were combined, washed with water (50 mL), dried over sodium sulfate and evaporated under reduced pressure. The residue was first purified by filtration through a pad of silica gel (dichloromethane containing 5% methanol), affording, after evaporation of the filtrate, a mixture of **19a**–**d** (250 mg, 85% overall yield), the R_f's of each compound on silica gel being: 0.61 (**19a**), 0.51 (**19c**), 0.36 (**19b**) and 0.30 (**19d**) (CH_2Cl_2/MeOH 95:5). Crystallization of the mixture in ethyl acetate provided **19b** (59 mg, 20%): mp 144–145 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.09–2.18 (m, 1H), 2.21–2.39 (m, 1H), 2.59–2.72 (m, 1H), 2.88–3.39 (m, 6H), 3.86 (s, 3H), 4.35 (d, J=6.5 Hz, 1H), 6.85 (d, J=8.7 Hz, 1H), 6.95 (s, 1H), 7.24 (d, J=8.7 Hz, 1H), 8.23 (br s, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 18.2, 28.4, 31.2, 46.1, 48.5, 56.1, 60.0, 100.6, 110.8, 112.2, 112.4, 121.1, 127.3, 130.5, 131.6, 154.3; IR (KBr) 3547, 2238 cm^{-1}; CIMS m/z 268 $(MH)^+$. Anal. calcd for $C_{16}H_{17}N_3O$. 0.8 H_2O: C, 68.23; H, 6.61; N, 14.92. Found: C, 68.53; H, 6.82; N, 14.63.

The mother liquor was concentrated and the residue was purified by preparative TLC on silica gel (CH_2Cl_2/MeOH 98:2) affording, in order of elution.

19a. (70 mg, 24%): mp 136–138 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.18–2.23 (m, 1H), 2.35–2.41 (m, 1H), 2.75–3.09 (m, 7H), 3.85 (s, 3H), 4.23 (d, J=7.1 Hz, 1H), 6.83 (d, J=8.8 Hz, 1H), 6.94 (s, 1H), 7.23 (d, J=8.8 Hz, 1H), 8.20 (br s, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 20.1, 29.1, 32.2, 47.1, 51.2, 56.0, 62.4, 100.7, 109.1, 112.0, 112.2, 121.9, 127.1, 131.5, 132.3, 154.3; IR (KBr) 3372, 2241 cm^{-1}; CIMS m/z 268 $(MH)^+$. Anal. calcd for $C_{16}H_{17}N_3O$: C, 71.89; H, 6.41; N, 15.72. Found: C, 71.81; H, 6.41; N, 15.75.

19c. (53 mg, 18%): mp 170–172 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.25–2.29 (m, 1H), 2.52–3.27 (m, 8H), 3.85 (s, 3H), 4.33 (t, J=6.8 Hz, 1H), 6.81 (d, J=8.7 Hz, 1H), 6.93 (s, 1H), 7.20 (d, J=8.7 Hz, 1H), 7.69 (br s, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 18.4, 26.6, 34.8, 45.9, 53.9, 56.1, 56.7, 100.7, 108.4, 111.7, 111.9, 122.3, 127.1, 131.3, 134.0, 154.4; IR (KBr) 3150, 2233 cm^{-1}; CIMS m/z 268 $(MH)^+$. Anal. calcd for $C_{16}H_{17}N_3O$. 0.3 $CH_3CO_2C_2H_5$: C, 70.35; H, 6.61; N, 14.31. Found: C, 70.12; H, 6.51; N, 14.67.

19d. 68 mg, 23%); ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.05–2.16 (m, 1H), 2.60–3.31 (m, 7H), 3.85 (s, 3H), 4.22 (t, J=6.8 Hz, 1H), 6.80 (d, J=8.7 Hz, 1H), 6.93 (s, 1H), 7.17 (d, J=8.7 Hz, 1H), 7.97 (br s, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 18.0, 26.5, 34.6, 45.7, 53.3, 56.1, 56.4, 100.7, 107.6, 111.8, 112.9, 121.4, 127.4, 131.4, 134.9, 154.2; IR (KBr) 3386, 2239 cm^{-1}; CIMS m/z 268 $(MH)^+$. Anal. calcd for $C_{16}H_{17}N_3O$. 0.6 $CH_3CO_2C_2H_5$: C, 69.04; H, 6.82; N, 13.13. Found: C, 68.96; H, 6.72; N, 12.95.

(1*R*,11b*R*)- and (1*S*, 11b*S*)-8-Benzyloxy-1,2,3,5,6,11b-hexahydroindolizino[8,7-*b*]indole-1-carbonitrile [(±)-20a], (1*S*,11b*R*)- and (1*R*,11b*S*)-8-benzyloxy-1,2,3,5,6,11b-hexahydroindolizino[8,7-*b*]indole-1-carbonitrile [(±)-20b], (2*S*,11b*R*)- and (2*R*,11b*S*)-8-benzyloxy-1,2,3,5,6,11b-hexahydroindolizino[8,7-*b*]indole-2-carbonitrile [(±)-20c] and (2*R*,11b*R*)- and (2*S*,11b*S*)-8-benzyloxy-1,2,3,5,6,11b-hexahydroindolizino[8,7-*b*]indole-2-carbonitrile [(±)-20d]. Following the same procedure as for the preparation of compounds **19a**–**d**, compound **15** (398 mg, 1.1 mmol) was reacted with acrylonitrile (58 mg, 1.1 mmol) in the presence of cesium fluoride (0.61 g, 4 mmol) in acetonitrile to afford compounds **20a**–**d** (309 mg, 82%) whose R_f's on silica gel (CH_2Cl_2/MeOH 98:2) were 0.45 (**20a**), 0.33 (**20c**), 0.24 (**20b**) and 0.15 (**20d**). Compound **20b** was isolated by crystallization of the crude product in ethyl acetate (75 mg, 20%) : mp 198–199 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 2.37–2.56 (m, 2H), 2.72–2.81 (m, 3H), 2.89–2.98 (m, 1H), 3.22–3.29 (m, 2H), 3.49–3.52 (m, 1H), 3.89 (d, J=6.3 Hz, 1H), 5.09 (s, 2H), 6.84 (d, J=8.7 Hz, 1H), 7.04 (s, 1H), 7.21 (d, J=8.7 Hz, 1H), 7.28–7.38 (m, 4H), 7.47 (d, J=7.6 Hz, 1H), 9.64 (br s, 1H); ^{13}C NMR (75 MHz, acetone-d_6) δ 18.1, 26.3, 34.5, 45.7, 53.6, 56.5, 70.9, 102.2, 108.0, 111.5, 112.3, 122.1, 127.3, 127.4, 127.7, 128.4, 132.7, 133.8, 153.2; IR (KBr) 3363, 2237 cm^{-1}; CIMS m/z 344 $(MH)^+$. Anal. calcd for $C_{22}H_{21}N_3O$: C, 76.94; H, 6.16; N, 12.24. Found: C, 76.93; H, 6.25; N, 12.16.

The mother liquor was then purified by preparative TLC on silica gel (CH_2Cl_2/MeOH 98:2) to provide, in order of elution:

Compound **20a** (98 mg, 26%): mp 172–173 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 2.15–2.22 (m, 1H), 2.24–2.34 (m, 1H), 2.67–3.17 (m, 7H), 4.34 (d, J=7.64 Hz, 1H), 5.10 (s, 2H), 6.88–7.47 (m, 8H), 8.97 (br s, 1H); ^{13}C NMR (75 MHz, acetone-d_6) δ 19.1, 29.1, 32.0, 46.5, 50.2, 62.1, 70.8, 102.1, 109.6, 111.8, 112.5, 126.9, 127.4, 127.6, 128.3, 131.7, 132.4, 137.6, 153.4; IR (KBr) 3352, 2246 cm^{-1}; CIMS m/z 344 $(MH)^+$. Anal. calcd for $C_{22}H_{21}N_3O$: C, 76.94; H, 6.16; N, 12.24. Found: C, 76.69; H, 6.11; N, 12.04.

Compound **20c** (79 mg, 21%): mp 171–174 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.18–2.24 (m, 1H), 2.43–2.51 (m, 1H), 2.52–3.25 (m, 7H), 4.29 (t, J=6.4 Hz, 1H), 5.09 (s, 2H), 6.88 (dd, J=8.7 Hz and 2.3 Hz, 1H), 6.90 (s, 1H), 7.16 (d, J=8.7 Hz, 1H), 7.30–7.47 (m, 5H), 7.80 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.4, 26.5, 34.7, 45.9, 53.8, 56.7, 71.1, 102.4, 108.2, 111.7, 112.5, 122.3, 127.6, 127.9, 128.6, 131.5, 134.1, 137.7, 153.4; IR (KBr) 3360, 2239 cm^{-1}; CIMS m/z 344 $(MH)^+$. Anal.

calcd for $C_{22}H_{21}N_3O$: C, 76.94; H, 6.16; N, 12.24. Found: C, 77.10; H, 6.18; N, 11.96.

Compound **20d** (57 mg, 15%): mp 168–170 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.04–2.19 (m, 1H), 2.60–3.30 (m, 8H), 4.30 (t, J = 6.5 Hz, 1H), 5.11 (s, 2H), 6.90 (dd, J = 8.7 Hz and 2.4 Hz, 1H), 7.04 (d, J = 2.4 Hz, 1H), 7.22–7.49 (m, 6H), 7.68 (br s, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 17.9, 26.6, 34.7, 45.7, 53.2, 56.3, 71.2, 102.4, 107.7, 111.7, 112.6, 121.4, 127.7, 127.9, 128.6, 131.6, 134.3, 139.2, 153.6; IR (KBr) 3371, 2241 cm^{-1}; CIMS m/z 344 $(MH)^+$. Anal. calcd for $C_{22}H_{21}N_3O.0.7\ H_2O$: C, 74.22; H, 6.34; N, 11.80. Found: C, 74.27; H, 6.15; N, 11.62.

(1*R*,11b*R*)- and (1*S*, 11b*S*)-1,2,3,5,6,11b-Hexahydro-11-*N*-methylindolizino[8,7-*b*]indole-1-carbonitrile [(±)-21a], (1*S*,11b*R*)- and (1*R*,11b*S*)-1,2,3,5,6,11b-hexahydro-11-*N*-methylindolizino[8,7-*b*]indole-1-carbonitrile [(±)-21b]. Following the same procedure as for the preparation of compounds **19a**–**d**, compound **16** (300 mg, 1.1 mmol) was reacted with acrylonitrile (58 mg, 1.1 mmol) in the presence of cesium fluoride (0.61 g, 4 mmol) in acetonitrile to afford compounds **21a**,**b** (200 mg, 70%) whose R_f's on silica gel (ethyl acetate/heptane 3:1) were 0.50 (**21a**) and 0.18 (**21b**). Compound **21b** was isolated by crystallization of the crude product in ethyl acetate and recrystallization in ethyl acetate 95% ethanol (85 mg, 30%): mp 185–187 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.23–2.45 (m, 2H), 2.80–2.99 (m, 4H), 3.15–3.31 (m, 2H), 3.33–3.40 (m, 1H), 3.69 (s, 3H), 4.04 (d, J = 7.1 Hz, 1H), 7.07 (t, J = 7.1 Hz, 1H), 7.21 (t, J = 7.1 Hz, 1H), 7.29 (d, J = 7.9 Hz, 1H), 7.51 (d, J = 7.1 Hz, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 21.5, 29.6, 30.1, 32.2, 47.5, 52.5, 60.7, 109.1, 109.9, 118.5, 119.1, 120.7, 121.6, 126.4, 131.7, 137.5; IR (KBr) 2235 cm^{-1}; CIMS m/z 252 $(MH)^+$. Anal. calcd for $C_{16}H_{17}N_3.0.1\ H_2O$: C, 75.92; H, 6.85; N, 16.60. Found: C, 75.85; H, 6.96; N, 16.81.

The mother liquor was purified by preparative TLC on silica gel (ethyl acetate/heptane 3:1) to provide compound **21a** which was crystallized in ethyl acetate/95% ethanol (85 mg, 30%): mp 140–141 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.23–2.39 (m, 1H), 2.41–2.48 (m, 1H), 2.71–3.18 (m, 7H), 3.78 (s, 3H), 4.56 (d, J = 6.4 Hz, 1H), 7.11 (t, J = 6.9 Hz, 1H), 7.23 (t, J = 6.9 Hz, 1H), 7.30 (d, J = 7.9 Hz, 1H), 7.51 (d, J = 7.9 Hz, 1H); IR (KBr) 2237 cm^{-1}; CIMS m/z 252 $(MH)^+$. Anal. calcd for $C_{16}H_{17}N_3$: C, 76.46; H, 6.82; N, 16.72. Found: C, 76.54; H, 6.86; N, 16.65.

(1*R*,11b*R*)- and (1*S*, 11b*S*)-1,2,3,5,6,11b-Hexahydro-8-hydroxyindolizino[8,7-*b*]indole-1-carbonitrile [(±)-23a], (1*S*,11b*R*)- and (1*R*,11b*S*)-1,2,3,5,6,11b-hexahydro-8-hydroxyindolizino[8,7-*b*]indole-1-carbonitrile [(±)-23b], (2*S*,11b*R*)- and (2*R*,11b*S*)-1,2,3,5,6,11b-hexahydro-8-hydroxyindolizino[8,7-*b*]indole-2-carbonitrile [(±)-23c] and (2*R*,11b*R*)- and (2*S*,11b*S*)-1,2,3,5,6,11b-hexahydro-8-hydroxyindolizino[8,7-*b*]indole-2-carbonitrile [(±)-23d]. A solution of the 8-benzyloxy derivative **20a**, **20b**, **20c** or **20d** (50 mg, 0.15 mmol) and thioanisole (0.30 mL, 2.5 mmol) in triflic acid (0.70 mL) was stirred at rt for 20 min. The reaction mixture was then slowly neutralized by addition of saturated aqueous sodium hydrogen carbonate, the resulting emulsion was taken up in saturated aqueous sodium chloride (15 mL) and the mixture was extracted with diethyl ether (3×10 mL). The organic extracts were combined, washed with saturated aqueous sodium chloride (15 mL), dried over sodium sulfate and evaporated under reduced pressure. The residue was taken up in dichloromethane and filtered through a pad of silica gel. After evaporation of the filtrate under reduced pressure, the crude product was crystallized in ethyl acetate/95% ethanol. The following compounds were prepared in this manner:

Compound **23a** (from **20a**) (23 mg, 61%): mp >250 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.03–2.17 (m, 1H), 2.27–2.41 (m, 1H), 2.44–3.26 (m, 7H), 4.37 (d, J = 5.1 Hz, 1H), 6.67 (d, J = 8.6 Hz, 1H), 6.83 (s, 1H), 7.18 (d, J = 8.6 Hz, 1H), 7.66 (br s, 1H), 9.79 (br s, 1H); IR (KBr) 3344, 2244 cm^{-1}; CIMS m/z 254 $(MH)^+$. Anal. calcd for $C_{15}H_{15}N_3O$. 0.25 H_2O: C, 69.88; H, 6.06; N, 16.30. Found: C, 69.75; H, 6.21; N, 16.06.

Compound **23b** (from **20b**) (22 mg, 60%): mp 205–208 °C (decomp); ^{1}H NMR (250 MHz, acetone-d_6) δ 2.04–2.35 (m, 2H), 2.62–2.84 (m, 4H), 3.17–3.27 (m, 2H), 3.51–3.57 (m, 1H), 3.77 (d, J = 6.3 Hz, 1H), 6.67 (dd, J = 8.3 Hz and 2.4 Hz, 1H), 6.85 (d, J = 2.4 Hz, 1H), 7.14 (d, J = 8.3 Hz, 1H), 9.78 (br s, 1H); IR (KBr) 3386, 2239 cm^{-1}; CIMS m/z 254 $(MH)^+$.

Compound **23c** (from **20c**) (21 mg, 55%): mp 235–240 °C (decomp); ^{1}H NMR (250 MHz, acetone-d_6) δ 2.02–2.35 (m, 1H), 2.36–3.29 (m, 8H), 4.30 (d, J = 6.0 Hz, 1H), 6.65 (d, J = 8.6 Hz, 1H), 6.83 (s, 1H), 7.12 (d, J = 8.6 Hz, 1H), 7.59 (br s, 1H), 9.67 (br s, 1H); IR (KBr) 3381, 2240 cm^{-1}; CIMS m/z 254 $(MH)^+$

Compound **23d** (from **20d**) (24 mg, 64%): mp >250 °C; ^{1}H NMR (250 MHz, CD_3OD) δ 2.12–2.34 (m, 2H), 2.68–3.32 (m, 7H), 4.15 (d, J = 6.6 Hz, 1H), 6.68–6.72 (m, 1H), 6.86–6.90 (m, 1H), 7.13–7.17 (m, 1H), 7.13 (s, 1H); IR (KBr) 3387, 2234 cm^{-1}; CIMS m/z 254 $(MH)^+$.

General procedure for the reduction of the nitrile derivatives 18, 20, 22 to the aminomethyl derivatives 24, 25, 26. A stirring suspension of lithium aluminum hydride (41 mg, 1.1 mmol) in anhydrous THF (2 mL) was treated dropwise under argon with aluminum chloride (122 mg, 0.92 mmol) in THF (2 mL). The reaction mixture was maintained at rt during the addition by means of a water bath. After addition was complete, the reaction mixture was stirred vigorously for 20 min and then cooled to 0 °C before adding dropwise a solution of the nitrile (1 mmol) in THF (2 mL). After complete addition, stirring was continued for an additional hour and methanol followed by water were slowly added to the reaction mixture. The mixture was then concentrated under reduced pressure, the residue was taken up in dichloromethane (20 mL) and the mixture was washed with water (20 mL). The organic phase was extracted with aqueous HCl (2×20 mL of a 1 M solution) and the aqueous phases were combined, washed with dichloro-

methane (20 mL) and neutralized by addition of aqueous sodium hydroxide (10 M). The mixture was extracted with dichloromethane (2×20 mL), the organic phases were combined, washed with water (20 mL), dried over sodium sulfate and evaporated under reduced pressure. The residue was filtered through a pad of silica gel, the filtrate was evaporated and the crude amine was crystallized in the solvent indicated and/or was characterized as the L-(+)-tartrate salt, crystallized in 95% ethanol. The following compounds were prepared in this manner.

(1*R*,11b*R*)- and (1*S*, 11b*S*)-1-Aminomethyl-1,2,3,5,6,11b-hexahydroindolizino[8,7-*b*]indole (24a). Prepared from **18a**[11] in 75% yield as described above and was crystallized in ethyl acetate : mp 132–133 °C (tartrate: 170–180 °C, decomp); ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.39–1.47 (m, 1H), 1.84–2.14 (m, 4H), 2.50–2.92 (m, 5H), 3.14–3.29 (m, 1H), 3.29–3.50 (br s, 2H), 3.69 (d, J = 5.5 Hz, 1H), 6.88–7.02 (m, 2H), 7.28–7.36 (m, 2H), 10.85 (br s, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 18.7, 28.4, 45.2, 46.7, 47.1, 65.6, 106.4, 111.2, 118.1, 119.0, 122.3, 127.3, 136.1; IR (film) 3342, 1576 cm^{-1}; CIMS m/z 242 $(MH)^+$. Anal. calcd for $C_{15}H_{19}N_3$: C, 74.65; H, 7.94; N, 17.41. Found: C, 74.83; H, 7.94; N, 17.22.

(1*S*,11b*R*)- and (1*R*, 11b*S*)-1-Aminomethyl-1,2,3,5,6,11b-hexahydroindolizino[8,7-*b*]indole (24b). Prepared from **18b**[11] in 68% yield as described above and was characterized as the tartrate salt: mp 170–180 °C (decomp); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.25–1.38 (m, 1H), 1.82 (br s, 2H), 2.01–2.20 (m, 1H), 2.54–3.29 (m, 9H), 4.41 (d, J = 5.5 Hz, 1H), 7.04–7.14 (m, 2H), 7.32 (d, J = 7.9 H, 1H), 7.49 (d, J = 7.1 Hz, 1H), 10.85 (br s, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 17.2, 28.7, 43.6, 44.1, 46.6, 48.0, 60.5, 108.6, 111.0, 117.8, 118.6, 120.9, 128.0, 133.1, 136.0; IR (film) 3407, 1544 cm^{-1}; FABMS m/z 242 $(MH)^+$. Anal. calcd for $C_{15}H_{19}N_3$. 1.9 $C_4H_6O_6$: C, 51.56; H, 5.78; N, 7.98. Found: C, 51.62; H, 5.61; N, 7.91.

(2*S*,11b*R*)- and (2*R*, 11b*S*)-2-Aminomethyl-1,2,3,5,6,11b-hexahydroindolizino[8,7-*b*]indole (24c). Prepared fom **18c**[11] in 72% yield as described above and was characterized as the tartrate salt: mp 170–180 °C (decomp); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.91–3.26 (m, 13H), 4.19 (m, 1H), 7.03–7.14 (m, 2H), 7.26 (d, J = 7.0 Hz, 1H), 7.46 (d, J = 6.3 Hz, 1H), 8.51 (br s, 1H); IR (KBr) 3410, 1580 cm^{-1}; CIMS m/z 242 $(MH)^+$. Anal. calcd for $C_{15}H_{19}N_3$. 2$C_4H_6O_6$. 0.5 H_2O: C, 50.18; H, 5.86; N, 7.63. Found: C, 50.11; H, 5.89; N, 7.53.

(2*R*,11b*R*)- and (2*S*, 11b*S*)-2-Aminomethyl-1,2,3,5,6,11b-hexahydroindolizino[8,7-*b*]indole (24d). Prepared from **18d**[11] in 65% yield as described above and was characterized as the tartrate salt: mp 185 °C (decomp); IR (KBr) 3330, 1576 cm^{-1}; FABMS m/z 242 $(MH)^+$.

(1*R*,11b*R*)- and (1*S*, 11b*S*)-1-Aminomethyl-8-benzyloxy-1,2,3,5,6,11b-hexahydro-indolizino[8,7-*b*]indole (25a). Prepared from **20a** in 74% yield as described above and was characterized as the tartrate salt: mp 114–116 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.36–1.41 (m, 1H), 1.59 (br s, 2H), 1.93–2.04 (m, 2H), 2.50–3.09 (m, 7H), 3.21–3.27 (m, 1H), 3.42 (d, J = 6.7 Hz, 1H), 5.07 (s, 2H), 6.82 (dd, J = 8.6 Hz and 2.3 Hz, 1H), 7.01 (d, J = 2.3 Hz, 1H), 7.26 (d, J = 7.1 Hz, 1H), 7.28–7.38 (m, 4H), 7.45 (d, J = 7.3 Hz, 1H), 10.41 (br s, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 20.2, 28.2, 45.9, 46.4, 48.1, 51.1, 65.4, 70.9, 102.0, 106.2, 111.1, 111.5, 127.5, 127.6, 127.8, 128.4, 130.8, 136.8, 137.9, 152.7; IR (KBr) 3148, 1587 cm^{-1}; CIMS m/z 348 $(MH)^+$. Anal. calcd for $C_{22}H_{25}N_3O$. 2.3 $C_4H_6O_6$. 0.5 C_2H_5OH: C, 54.04; H, 5.89; N, 5.87. Found: C, 54.09; H, 5.99; N, 5.95.

(1*S*,11b*R*)- and (1*R*, 11b*S*)-1-Aminomethyl-8-benzyloxy-1,2,3,5,6,11b-hexahydro-indolizino[8,7-*b*]indole (25b). Prepared from **20b** in 69% yield as described above and was characterized as the tartrate salt: mp 140 °C (decomp); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.25 (br s, 2H), 1.61–1.89 (m, 1H), 1.89–2.29 (m, 1H), 2.34–3.29 (m, 9H), 4.32 (d, J = 5.8 Hz, 1H), 5.07 (s, 2H), 6.84 (t, J = 7.5 Hz, 1H), 7.00–7.11 (m, 2H), 7.19 (d, J = 8.7 Hz, 1H), 7.28–7.38 (m, 3H), 7.45 (d, J = 6.5 Hz, 1H), 8.36 (br s, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 17.4, 28.6, 43.4, 44.0, 46.7, 48.2, 60.7, 71.2, 101.9, 108.4, 111.5, 111.7, 127.6, 127.7, 128.5, 131.1, 134.0, 137.9, 152.8; IR (KBr) 3402, 1588 cm^{-1}; FABMS m/z 348 $(MH)^+$. Anal. calcd for $C_{22}H_{25}N_3O$. 2.3 $C_4H_6O_6$. 0.5 C_2H_5OH: C, 54.04; H, 5.89; N, 5.87. Found: C, 54.12; H, 6.01; N, 6.05.

(2*S*,11b*R*)- and (2*R*, 11b*S*)-2-Aminomethyl-8-benzyloxy-1,2,3,5,6,11b-hexahydro-indolizino[8,7-*b*]indole (25c). Prepared from **20c** in 76% yield as described above and was characterized as the tartrate salt: mp 128 °C (decomp); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.25 (br s, 2H), 1.80–2.12 (m, 4H), 2.87–3.22 (m, 7H), 4.05–4.15 (m, 1H), 5.06 (s, 2H), 6.82 (d, J = 8.4 Hz, 1H), 7.00–7.37 (m, 6H), 7.44 (d, J = 6.8 Hz, 1H), 8.61 (br s, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 17.8, 33.8, 40.0, 46.0, 46.7, 53.3, 57.5, 71.1, 102.1, 107.6, 111.5, 111.6, 111.7, 127.5, 127.7, 128.4, 131.4, 136.0, 137.8, 153.1; IR (KBr) 3403, 1589 cm^{-1}; FABMS m/z 348 $(MH)^+$. Anal. calcd for $C_{22}H_{25}N_3O$. 2.5 $C_4H_6O_6$. 0.5 C_2H_5OH: C, 54.13; H, 5.81; N, 5.63. Found: C, 54.33; H, 6.06; N, 5.75.

(2*R*,11b*R*)- and (2*S*, 11b*S*)-2-Aminomethyl-8-benzyloxy-1,2,3,5,6,11b-hexahydro-indolizino[8,7-*b*]indole (25d). Prepared from **20d** in 66% yield as described above and was characterized as the tartrate salt: mp 137 °C (decomp); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.17–1.25 (m, 1H), 1.44–1.51 (m, 1H), 1.66 (br s, 2H), 2.26–3.29 (m, 9H), 4.12–4.22 (m, 1H), 5.07 (s, 2H), 6.83 (d, J = 8.5 Hz, 1H), 7.01 (s, 1H), 7.10 (d, J = 8.6 Hz, 1H), 7.28–7.38 (m, 4H), 7.44 (d, J = 7.1 Hz, 1H), 8.40 (br s, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 17.6, 33.4, 41.2, 46.0, 46.6, 53.4, 56.9, 71.1, 102.2, 107.0, 111.5, 111.7, 127.6, 127.7, 128.5, 131.5, 136.7, 137.8, 153.2; IR (KBr) 3402, 1589 cm^{-1}; FABMS m/z 348 $(MH)^+$. Anal. calcd for $C_{22}H_{25}N_3O$. 2.2 $C_4H_6O_6$. 0.5 C_2H_5OH: C, 54.51; H, 5.93; N, 6.00. Found: C, 54.63; H, 5.84; N, 6.13.

(1*R*,11b*R*)- and (1*S*, 11b*S*)-1-Aminomethyl-11-*N*-benzyl-1,2,3,5,6,11b-hexahydro-indolizino[8,7-*b*]indole (26a).

Prepared from **22a**[11] in 71% yield as described above and was characterized as the tartrate salt: mp 80 °C (decomp); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.16 (s, 2H), 1.80–1.95 (m, 2H), 2.14–2.22 (m, 1H), 2.60–3.07 (m, 7H), 3.27–3.40 (m, 1H), 4.20 (d, J = 1.6 Hz, 1H), 5.26–5.47 (m, 2H), 6.92 (d, J = 6.6 Hz, 1H), 7.07–7.26 (m, 7H), 7.50–7.54 (m, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 16.3, 28.7, 45.3, 46.2, 47.1, 47.4, 48.2, 59.8, 108.0, 109.5, 117.9, 119.2, 121.3, 125.5, 127.0, 127.1, 128.7, 136.3, 137.3, 137.9; IR (KBr) 3369, 1605 cm^{-1}; CIMS m/z 332 $(MH)^+$. Anal. calcd for $C_{22}H_{25}N_3$.2.2 $C_4H_6O_6$. 0.8 C_2H_5OH: C, 55.71; H, 6.05; N, 6.02. Found: C, 56.12; H, 6.16; N, 5.77.

(1*S*,11b*R*)- and (1*R*, 11b*S*)-1-Aminomethyl-11-*N*-benzyl-1,2,3,5,6,11b-hexahydro-indolizino[8,7-*b*]indole (26b). Prepared from **22b**[11] in 67% yield as described above and was characterized as the tartrate salt: mp 101 °C (decomp); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.00 (br s, 2H), 1.79–1.86 (m, 1H), 1.94–1.99 (m, 1H), 2.30–3.16 (m, 9H), 3.62 (d, J = 4.9 Hz, 1H), 5.16–5.37 (m, 2H), 6.93 (d, J = 6.2 Hz, 1H), 7.05–7.25 (m, 7H), 7.49–7.53 (m, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 22.1, 27.5, 43.6, 44.7, 47.4, 48.2, 51.9, 61.3, 109.4, 109.8, 118.1, 119.1, 121.2, 126.0, 127.1, 127.2, 128.6, 134.7, 137.0, 137.6; IR (KBr) 3368, 1609 cm^{-1}; CIMS m/z 332 $(MH)^+$. Anal. calcd for $C_{22}H_{25}N_3$. 3.1 $C_4H_6O_6$. C_2H_5OH: C, 51.88; H, 5.89; N, 4.98. Found: C, 51.86; H, 5.86; N, 4.68.

(2*S*,11b*R*)- and (2*R*, 11b*S*)-2-Aminomethyl-11-*N*-benzyl-1,2,3,5,6,11b-hexahydro-indolizino[8,7-*b*]indole (26c). Prepared from **22c**[11] in 73% yield as described above and was characterized as the tartrate salt: mp 74 °C (decomp); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.42 (br s, 2H), 1.77–2.01 (m, 2H), 2.17–2.32 (m, 1H), 2.54–3.21 (m, 8H), 4.01 (t, J = 7.1 Hz, 1H), 5.16–5.33 (m, 2H), 6.92 (d, J = 7.7 Hz, 1H), 7.06–7.26 (m, 7H), 7.50–7.53 (m, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 19.6, 34.3, 40.3, 46.5, 46.9, 47.2, 55.7, 56.9, 107.9, 109.5, 118.1, 119.2, 121.3, 125.6, 125.8, 126.9, 127.2, 128.7, 136.8, 137.8; IR (KBr) 3360, 1602 cm^{-1}; CIMS m/z 332 $(MH)^+$. Anal. calcd for $C_{22}H_{25}N_3$.2.2 $C_4H_6O_6$. C_2H_5OH: C, 55.67; H, 6.30; N, 5.94. Found: C, 55.58; H, 6.38; N, 5.91.

(2*R*,11b*R*)- and (2*S*, 11b*S*)-2-Aminomethyl-11-*N*-benzyl-1,2,3,5,6,11b-hexahydro-indolizino[8,7-*b*]indole (26d). Prepared from **22d**[11] in 66% yield as described above and was characterized as the tartrate salt: mp 82 °C (decomp); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.25 (br s, 2H), 1.38–1.44 (m, 2H), 2.26–2.99 (m, 8H), 3.27–3.30 (m, 1H), 4.16 (t, J = 7.3 Hz, 1H), 5.18–5.28 (m, 2H), 6.94 (d, J = 6.2 Hz, 1H), 7.03–7.28 (m, 7H), 7.51–7.54 (m, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 18.4, 35.5, 41.5, 46.5, 46.7, 47.3, 54.3, 56.4, 106.2, 107.5, 109.5, 118.2, 119.3, 121.3, 125.9, 127.1, 127.3, 128.8, 137.4, 137.8; IR (KBr) 3362, 1605 cm^{-1}; CIMS m/z 332 $(MH)^+$. Anal. calcd for $C_{22}H_{25}N_3$.2.2 $C_4H_6O_6$. C_2H_5OH: C, 55.67; H, 6.30; N, 5.94. Found: C, 55.86; H, 6.18; N, 6.01.

(1*R*,11b*R*)- and (1*S*, 11b*S*)-1-Aminomethyl-1,2,3,5,6,11b-hexahydro-8-hydroxy-indolizino[8,7-*b*]indole (27a). A solution of compound **25a** (150 mg, 0.43 mmol) in methanol (2 mL) was hydrogenated at atmospheric pressure for 4 h in the presence of 10% palladium on carbon (15 mg). The reaction mixture was filtered through Celite, the filter pad was washed with methanol and the collected filtrates were evaporated under reduced pressure, affording compound **27a** (90 mg, 82%). The latter was transformed into its tartrate salt and crystallized in 95% ethanol: mp 112 °C (decomp); ^{1}H NMR (250 MHz, CD_3OD) δ 1.41–1.57 (m, 1H), 1.91–3.25 (m, 9H), 3.45–3.51 (m, 2H), 6.60 (dd, J = 8.4 Hz and 2.2 Hz, 1H), 6.79 (d, J = 2.2 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H); IR (KBr) 3330, 1590 cm^{-1}; L-SIMS m/z 258 $(MH)^+$. Anal. calcd for $C_{15}H_{19}N_3O$. 2.5 $C_4H_6O_6$. H_2O: C, 46.16; H, 5.58; N, 6.46. Found: C, 46.22; H, 5.64; N, 6.76.

(1*S*,11b*R*)- and (1*R*, 11b*S*)-1-Aminomethyl-1,2,3,5,6,11b-hexahydro-8-hydroxy-indolizino[8,7-*b*]indole (27b). Following the same procedure as for the preparation of **27a**, hydrogenation of **25b** (150 mg, 0.43 mmol) afforded compound **27b** (85 mg, 77%) which was characterized as the tartrate salt: mp 135 °C (decomp); ^{1}H NMR (250 MHz, CD_3OD) δ 1.50–1.78 (m, 1H), 1.98–2.23 (m, 1H), 2.44–3.16 (m, 9H), 3.89 (d, J = 5.6 Hz, 1H), 6.65 (dd, J = 8.5 Hz and 2.3 Hz, 1H), 6.79 (s, 1H), 7.10 (m, 1H); IR (KBr) 3349, 1592 cm^{-1}; L-SIMS m/z 258 $(MH)^+$. Anal. calcd for $C_{15}H_{19}N_3O$. 1.8 $C_4H_6O_6$. 0.5 C_2H_5OH: C, 50.62; H, 6.01; N, 7.63. Found: C, 50.63; H, 6.28; N, 7.74.

(2*S*,11b*R*)- and (2*R*, 11b*S*)-2-Aminomethyl-1,2,3,5,6,11b-hexahydro-8-hydroxy-indolizino[8,7-*b*]indole (27c). Following the same procedure as for the preparation of **27a**, hydrogenation of **25c** (150 mg, 0.43 mmol) afforded compound **27c** (92 mg, 83%) which was characterized as the tartrate salt: mp 140 °C (decomp); ^{1}H NMR (250 MHz, CD_3OD) δ 1.85–1.92 (m, 1H), 2.13–2.22 (m, 1H), 2.49–3.36 (m, 9H), 4.12–4.18 (m, 1H), 6.63 (dd, J = 8.5 Hz and 2.1 Hz, 1H), 6.80 (d, J = 2.1 Hz, 1H), 7.11 (d, J = 8.5 Hz, 1H); IR (KBr) 3389, 1629 cm^{-1}; L-SIMS m/z 258 $(MH)^+$. Anal. calcd for $C_{15}H_{19}N_3O$. 1.7 $C_4H_6O_6$ 0.7 C_2H_5OH: C, 51.16; H, 6.14; N, 7.72. Found: C, 51.12; H, 6.42; N, 7.74.

(2*R*,11b*R*)- and (2*S*, 11b*S*)-2-Aminomethyl-1,2,3,5,6,11b-hexahydro-8-hydroxy-indolizino[8,7-*b*]indole (27d). Following the same procedure as for the preparation of **27a**, hydrogenation of **25d** (150 mg, 0.43 mmol) afforded compound **27d** (80 mg, 72%) which was characterized as the tartrate salt: mp 149 °C (decomp); ^{1}H NMR (250 MHz, CD_3OD) δ 1.97–3.27 (m, 11H), 4.23–4.33 (m, 1H), 6.67–6.72 (m, 1H), 6.83 (s, 1H), 7.12–7.20 (m, 1H); IR (KBr) 3385, 1593 cm^{-1}; L-SIMS m/z 258 $(MH)^+$. Anal. calcd for $C_{15}H_{19}N_3O$ 1.7 $C_4H_6O_6$ C_2H_5OH: C, 51.18; H, 6.31; N, 7.53. Found: C, 51.39; H, 6.35; N, 7.71.

Bioassays

Determination of cytotoxic activity on L1210 cells. L1210 cells (murine leukemia) provided by the NCI, Frederick, USA were cultivated in RPMI 1640 medium

(Gibco) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES buffer (pH = 7.4). Cytotoxicity was measured by the microculture tetrazolium (MTT) assay as described.[19] 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).

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 (Brucker, France) using an argon laser (Spectra-Physics) emitting 400 nW at 488 nm. The fluorescence of propidium iodide was collected through a 615 nm long-pass filter. Results are expressed as the percentage of cells found in the phases of the cell cycle.

Determination of cytotoxic activities on K562 cells. Both K562-S (sensitive to doxorubicin) and K562-R (resistant to doxorubicin, stock cultures being kept in the presence of 10^{-6} M doxorubicin in order to maintain selection pressure) erythroleukemia cells[20,21] were grown as described above for L1210 cells except that 40 μg/mL of gentamycin were added to the culture medium and that bicarbonate was used as buffer. Cells (25,000) in 1 mL of medium were seeded in each well of 24-well Nunc microplates and various concentrations of the test compounds in 10 μL of medium were added immediately to each well. Cultures were incubated for 3 days at 37 °C in a 5% CO_2–95% air incubator. Cell viability was determined by the MTT assay as described above.[17,18] Values are reported as the concentration of test substance inhibiting 50% of cell proliferation compared to untreated cells (IC_{50}).

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12. Because the benzyloxy group of compound **10** did not survive the highly acidic medium attained during the typical work up of a Bischler–Napieralski reaction (ex: ref 21), the product was precipitated as its hydrochloride salt at the end of the reaction period, thus protecting it from acid hydrolysis. This procedure was also extended to the preparation of β-carbolines **8** and **9**. See Experimental.
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