Synthesis and Cytotoxic Activity of Pyranocarbazole Analogues of Ellipticine and Acronycine

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Various 2,2,5,11-tetramethyl- and 2,2,5,6,11-pentamethyl-2,6-dihydropyrano[3,2-*b*]carbazole derivatives were synthesized by condensation of 3-methylbut-2-enal or 3-chloro-3-methylbut-1-yne with an appropriate hydroxycarbazole. These compounds associate the tricyclic system responsible for the intercalating properties of ellipticine related drugs, with the dimethylpyran pharmacophore of acronycine derivatives. The study of the biological properties of the new pyrano[3,2-*b*]carbazole derivatives was carried out *in vitro* on L1210 murine leukaemia cell line. The three (±)-*cis*-diol diesters 15, 16, and 18 were the most active compounds.

Key words ellipticine; acronycine; 2,6-dihydropyrano[3,2-*b*]carbazole; cytotoxicity

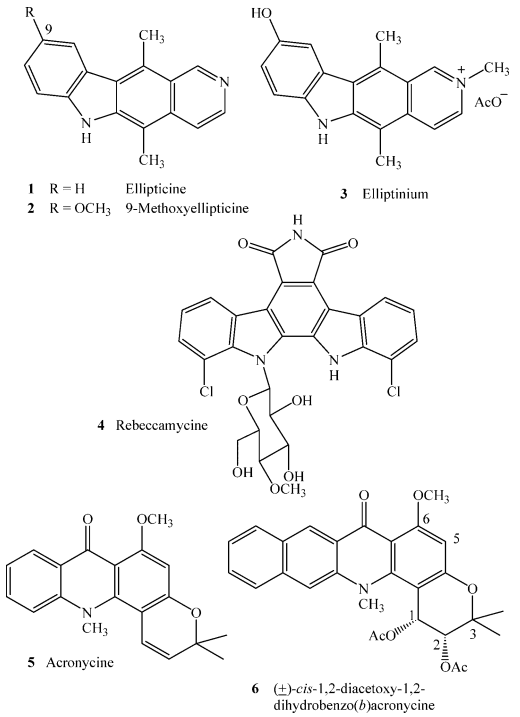
Natural products including a carbazole basic skeleton fused with another heterocyclic system currently receive a strong attention, due to the promising antitumor properties of several of their representatives.[1)] Of particular interest in this field are the pyrido[4,3-*b*]carbazole alkaloids and the indolo[2,3-*a*]carbazole antibiotics. The first series, illustrated by ellipticine (**1**) and 9-methoxyellipticine (**2**), both isolated from *Ochrosia* species (Apocynaceae) include compounds which act by intercalation within the DNA-double strand, followed by inhibition of topoisomerase II.[2)] A derivative of ellipticine, elliptinium (**3**), has been commercialized for the treatment of metastatic breast cancer.[3)] Rebeccamycine (**4**), isolated from *Saccharothrix aerocolonigenes*, is a typical representative of the second series currently under clinical trials. It induces topoisomerase I mediated DNA-cleavage.[4)] The antitumor activity of several related indolocarbazoles has been recently discussed.[5)]

The structurally related pyrano[2,3-*c*]acridone alkaloid acronycine (**5**) isolated from *Achronycia baueri* SCHOTT. (Rutaceae) was shown to exhibit antitumor properties against a broad spectrum of solid tumor models.[6—8)] Nevertheless, its moderate potency and low water solubility rapidly appeared as severe drawbacks for a possible introduction into the clinic. A hypothesis of bioactivation of the 1,2-double bond of the chromene ring system of acronycine into the corresponding epoxide[9)] led to the development of several series of more active structural analogues. The most significant improvements were obtained with diesters of 1,2-dihydroxy-1,2-dihydroacronycine[10)] and, more recently, of 1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine.[11)] Representatives of this latter series, such as diacetate **6**, are considered as valuable candidates for clinical studies.[12)] The mechanism of their action implies alkylation of the 2-amino group of DNA guanine residues by the ester leaving group at position 1 of the drug.[13,14)]

We describe here the synthesis and cytotoxic properties of various 2,2,5,11-tetramethyl- and 2,2,5,6,11-pentamethyl-2,6-dihydropyrano[3,2-*b*]carbazole derivatives. Such compounds associate the 1,4-dimethylcarbazole tricyclic system responsible for the intercalating properties of ellipticine related drugs, with the dimethylpyran pharmacophore of acronycine derivatives.

Chemistry

For the synthesis of 2,2,5,11-tetramethyl-2,6-dihydropyrano[3,2-*b*]carbazole (**7**), the readily available 1,4-dimethyl-9*H*-carbazole-3-carbaldehyde (**8**), previously used as key-intermediate in several ellipticine syntheses,[15)] was chosen as starting material. It was first oxidized by use hydrogen peroxide in acidic medium, into the corresponding 1,4-dimethyl-9*H*-carbazol-3-ol (**9**), according to the Matsumoto's procedure which has been applied by Langendoen *et al.* to the synthesis of related hydroxycarbazoles.[16)] Construction of the fused dimethylpyran ring was ensured by condensation of **9**



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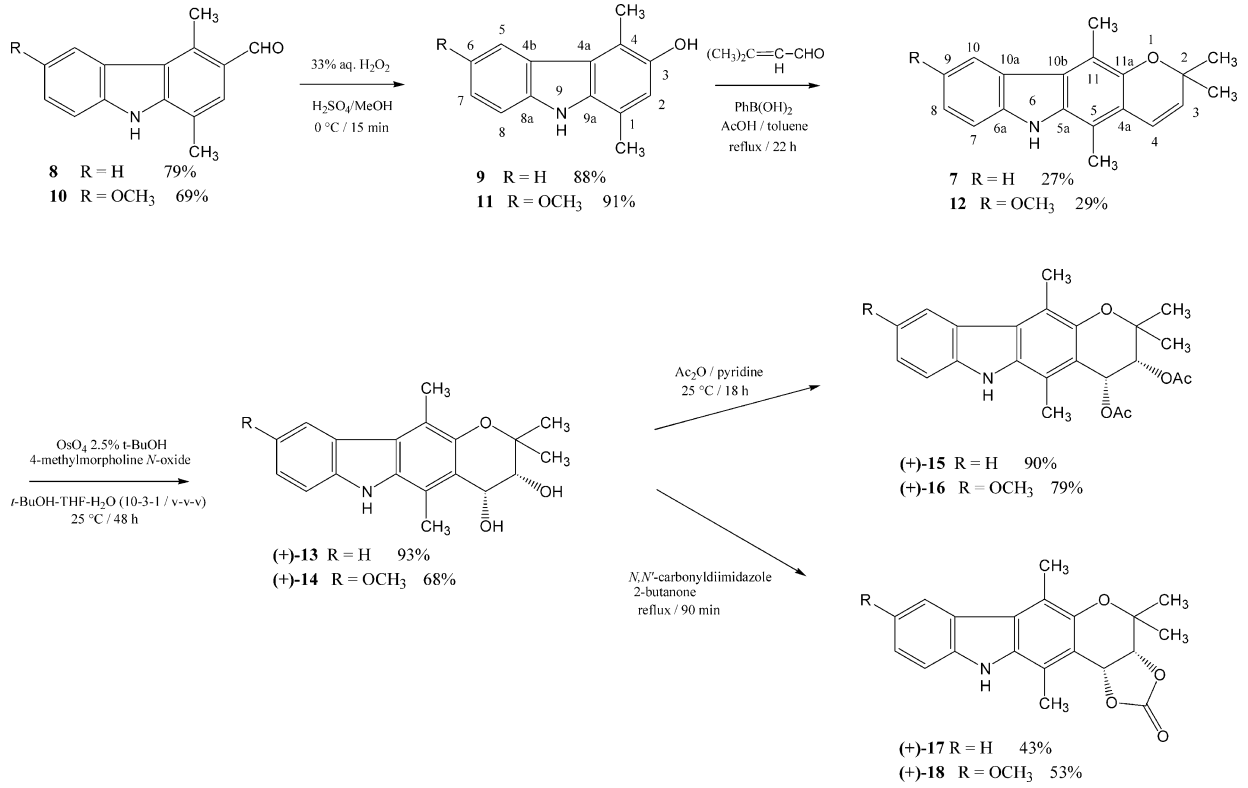


Chart 1

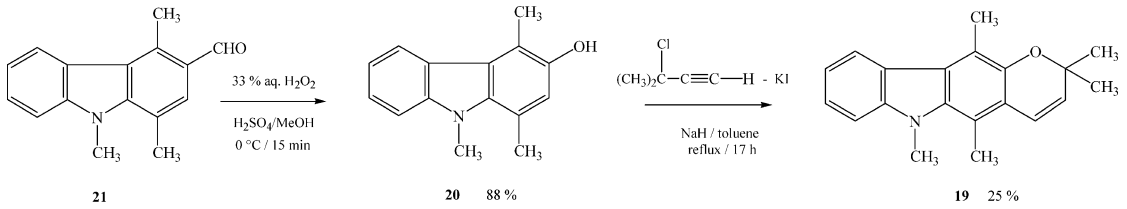


Chart 2

with 3-methylbut-2-enal (senecioaldehyde) in the presence of phenylboronic acid as Lewis acid catalyst[17] which gave the target compound **7**. The same reaction sequence, applied to 6-methoxy-1,4-dimethyl-9*H*-carbazole-3-carbaldehyde (**10**),[15] furnished successively 6-methoxy-1,4-dimethyl-9*H*-carbazol-3-ol (**11**) and 9-methoxy-2,2,5,11-tetramethyl-2,6-dihydropyrano[3,2-*b*]carbazole (**12**) (Chart 1).

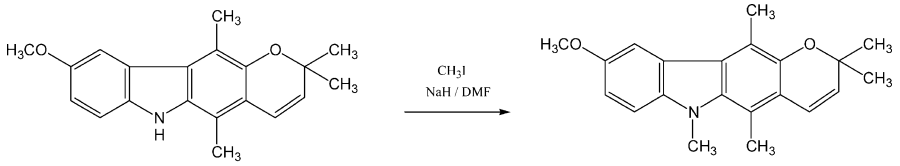
Functionalization of the pyran 3,4-double bond of **7** and **12** in order to prepare corresponding (±)-*cis*-dihydrodiol diesters was further envisaged, since this pharmacomodulation had previously given outstanding results in terms of antitumor potency in the acronycine series.[10]

Accordingly, the racemic *cis*-diols **13** and **14** were conveniently obtained by catalytic osmium tetroxide oxidation of **7** and **12**, using *N*-methylmorpholine *N*-oxide to regenerate the oxidizing agent.[18] Treatment of diols **13** and **14** with excess acetic anhydride afforded the desired diacetates **15** and **16**, respectively. Reaction of the same diols with *N*,*N*′-carbonydiimidazole in 2-butanone under reflux afforded the corresponding cyclic carbamates **17** and **18**.

An alternative approach involving the Claisen rearrangement of an intermediate dimethylpropargylic ether was envisioned to create the dimethylpyran ring of 2,2,5,6,11-pentamethyl-2,6-dihydropyrano[3,2-*b*]carbazole (**19**).[19] The starting 1,4,9-trimethyl-9*H*-carbazol-3-ol (**20**) was first conveniently prepared by hydrogen peroxide oxidation of the known 1,4,9-trimethyl-9*H*-carbazole-3-carbaldehyde (**21**).[15] Further treatment of **20** with 3-chloro-3-methylbut-1-yne[20] in the presence of sodium hydride in refluxing toluene afforded the desired **19** (Chart 2). Nevertheless, the difficulties encountered in both terms of yield and separation of isomers in the course of the preparation of **21** from 1,4,9-trimethylcarbazole severely limited the practical scope of syntheses using *N*-methycarbazoles as starting materials. In contrast, *N*-methylation of a preformed pyranocarbazole gave excellent results, illustrated by the obtainment in 89% yield of 9-methoxy-2,2,5,6,11-pentamethyl-2,6-dihydropyrano[3,2-*b*]carbazole (**22**), when **12** was treated by methyl iodide and sodium hydride in dimethylformamide (Chart 3).

Pharmacology

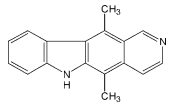
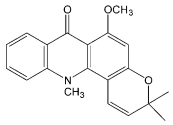
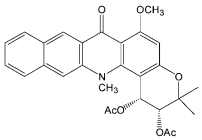
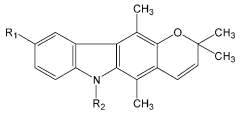
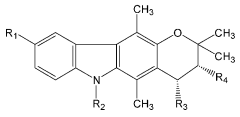
The study of the biological properties of the new pyrano[3,2-*b*]carbazole derivatives was carried out *in vitro* on L1210 murine leukaemia cell line. The results (IC_{50}) are



12 22 89 %

Chart 3

Table 1. Inhibition of L1210 Cell Proliferation

	Compound	R_1	R_2	R_3	R_4	IC_{50} μM
	**1**					0.1
	**5**					10.4
	**6**					0.6
	**7**	H	H	H	H	19.9
	12	OCH_3	H	H	H	19.2
	19	H	CH_3	H	H	31.2
	22	OCH_3	CH_3	H	H	>50
	(±)-**13**	H	H	OH	OH	25.3
	(±)-**15**	H	H	$OCOCH_3$	$OCOCH_3$	16.8
	(±)-**17**	H	H	OCOO	OCOO	25.2
	(±)-**14**	OCH_3	H	OH	OH	20.0
	(±)-**16**	OCH_3	H	$OCOCH_3$	$OCOCH_3$	13.6
	(±)-**18**	OCH_3	H	OCOO	OCOO	16.6

reported in Table 1.

The three (±)-*cis*-diol diesters **15**, **16**, and **18** were the most active compounds.

Results and Discussion

From a chemical point of view, no synthetic method giving a general entry to the pyrano[3,2-*b*]carbazole system had been developed up to now. Consequently, only two compounds deriving from this basic skeleton have been described in the literature to our knowledge. Pyrayaquinone-A is the only natural product belonging to this series. It was first isolated from *Murraya euchrestifolia* HAYATA (Rutaceae) and subsequently synthesized, although in low yield, by palladium-assisted intramolecular ring closure of a suitable chromenylamino-1,4-benzoquinone.[21] The other pyrano[3,2-*b*]carbazole previously described, isoglycomaurin, was obtained as a minor product in the course of the synthesis of glycomaurin, through Claisen rearrangement of the corresponding dimethylpropargylic ether.[22] Therefore, the condensation of a hydroxycarbazole with 3-methylbut-2-enal in presence of a Lewis acid catalyst described here, appears as the first efficient method giving a facile access to the pyrano[3,2-*b*]carbazole series.

Considering the structure–activity relationships, it appears that in both 2,2,5,11-tetramethyl- and 6-methoxy-2,2,5,11-tetramethyl-2,6-dihydropyrano[3,2-*b*]carbazole series, functionalization of the pyran double bond to give (±)-*cis*-dihydro-diol diesters results in an increase of the cytotoxic activity. Nevertheless, none of these esters exhibit an antiproliferative activity within the same range of magnitude as the corresponding acronycine derivatives.

In contrast, *N*-methylation reduces significantly the biological activity in both chemical series. Indeed, **19** only exhibits a marginal activity, whereas **22** was devoid significant cytotoxic activity.

The effect of the substitution by a methoxy group at position 9 seems to be more discrete.

However, methoxy compounds appear, as a whole, slightly more cytotoxic than their unsubstituted counterparts.

Experimental

The melting points were determined on a Leica VM apparatus and are not corrected. IR spectra (ν_{max} in cm^{-1}) with KBr pellets were obtained on a Nicolet FT-IR instrument. UV spectra (λ_{max} in nm) were determined in >99.5% (v/v) EtOH on a Beckman DU® 600 spectrophotometer. ^{1}H-NMR (δ in ppm, J in Hz) and ^{13}C-NMR spectra were recorded at 400 MHz and 100 MHz respectively, using a Bruker Avance 400 spectrometer. When necessary, the signals were unambiguously assigned by 2D NMR techniques: COSY, NOESY, HMQC, and HMBC. These experiments were performed using standard Bruker microprograms. Mass spectra were recorded with a Nermag R-10-10C spectrometer using chemical ionization technique (reagent gas: NH_3) (CI-MS) and with a ZQ 2000 Waters using a Zspray (ESI-MS). Column chromatographies were conducted using silica gel 60 Merck (35—70 μm) with an overpressure of 200 mbars.

1,4-Dimethyl-9*H*-carbazol-3-ol (9) To an ice-cooled solution of 1,4-dimethyl-9*H*-carbazole-3-carbaldehyde[15] (7.8 g, 35.0 mmol) in methanol (400 ml) were added 33% aqueous hydrogen peroxide (5 ml) and 95% sulfuric acid (2.5 ml). After stirring for 15 min at 0 °C, the reaction mixture was diluted with a mixture of crushed ice and water and neutralized with aqueous 1 M NaOH to give a whitish precipitate which was filtered off and washed with water. Crystallization from MeOH gave 1,4-dimethyl-9*H*-carbazol-3-ol as whitish needles (6.5 g, 88%), mp 246—247 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.45 (3H, s, CH_3-1), 2.60 (3H, s, CH_3-4), 6.77 (1H, s, C2-H), 7.07 (1H, t, J=8 Hz, C6-H), 7.30 (1H, t, J=8 Hz, C7-H), 7.44 (1H, d, J=8 Hz, C8-H), 8.08 (1H, d, J=8 Hz, C5-H), 8.65 (1H, D_2O exch, s, OH), 10.80 (1H, D_2O exch, s, NH). ^{13}C-NMR (DMSO-d_6) δ: 13.7 ($\underline{C}H_3$-4), 18.1 ($\underline{C}H_3$-1), 112.0 (C-8), 115.1 (C-4a), 116.8 (C-2), 118.3 (C-4), 119.0 (C-6), 122.8 (C-1), 123.3 (C-5), 124.9 (C-4b), 125.6 (C-7), 134.4 (C-9a), 141.7 (C-8a), 148.7 (C-3). IR ν_{max} cm^{-1}: 3493, 3319, 3056, 2959, 2933, 2917, 1457, 1306, 1090, 756, 735. UV λ_{max} nm (log ε): 222 (4.38), 238 (4.45), 252.5 (4.23), 267.5 (4.23), 295 (4.07). CI-MS *m/z*: 212 ([MH]$^+$). *Anal.* Calcd for $C_{14}H_{13}NO$: C, 79.59; H, 6.20; N, 6.63. Found: C, 79.31; H, 6.18; N, 6.61.

2,2,5,11-Tetramethyl-2,6-dihydropyrano[3,2-*b*]carbazole (7) A solution of **9** (5.0 g, 23.7 mmol), phenylboronic acid (4.3 g, 35.5 mmol), 3-methylbut-2-enal (3.0 g, 35.5 mmol), glacial acetic acid (90 ml) in anhydrous toluene (300 ml) was refluxed for 22 h under N_2 in an apparatus fitted with a Dean-Stark trap. After cooling, the mixture was concentrated *in vacuo* and the residue was extracted with CH_2Cl_2 (4×100 ml). The combined organic phase was washed successively with H_2O (150 ml), 5% $NaHCO_3$ solution (200 ml), and brine (150 ml), dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. Column chromatography of the crude product on silica gel (CH_2Cl_2/cyclohexane: 7/3, v/v) gave **7** as a pale yellow crystalline product (1.76 g, 27%), mp 139—140 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (3H, s, CH_3-2), 1.48 (3H, s, CH_3-2), 2.50 (3H, s, CH_3-5), 2.75 (3H, s, CH_3-11), 5.81 (1H, d , J=10 Hz, C3-H), 6.74 (1H, d, J=10 Hz, C4-H), 7.18 (1H, ddd, J=8, 7, 1.5 Hz, C9-H), 7.37 (1H, td, J=7, 1 Hz, C8-H), 7.43 (1H, dd, J=7, 1.5 Hz, C7-H), 7.78 (1H, D_2O exch, s, NH), 8.17 (1H, dd, J=8, 1 Hz, C10-H). ^{13}C-NMR ($CDCl_3$) δ: 12.0 ($\underline{C}H_3$-5), 12.2 ($\underline{C}H_3$-11), 27.2 (2C, ($\underline{C}H_3$)$_2$-2), 74.5 (C-2), 110.4 (C-7), 112.0 (C-4a), 117.2 (C-10b), 118.8 (C-5), 118.9 (C-9), 120.3 (C-10), 121.7 (C-11), 122.6 (C-4), 124.7 (C-10a), 124.9 (C-8), 131.8 (C-3), 133.8 (C-5a), 140.2 (C-6a), 144.2 (C-11a). IR ν_{max} cm^{-1}: 3412, 3050, 2973, 2915, 1301, 1147, 1135, 746, 725. UV λ_{max} nm (log ε): 258 (4.58), 313 (4.34), 325 (4.44). CI-MS *m/z*: 278 ([MH]$^+$). *Anal.* Calcd for $C_{19}H_{19}NO$: C, 82.28; H, 6.90; N, 5.05. Found: C, 82.28; H, 6.88; N, 5.03.

6-Methoxy-1,4-dimethyl-9*H*-carbazol-3-ol (11) Oxidation of 6-methoxy-1,4-dimethyl-9*H*-carbazole-3-carbaldehyde (**10**)[15] (4.0 g, 15.8 mmol) under conditions identical with those described for the preparation of **9**, afforded **11** as pale green yellow needles (3.5 g, 91%), mp 185—186 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.50 (3H, s, CH_3-1), 2.70 (3H, s, CH_3-4), 3.90 (3H, s, OCH_3), 6.85 (1H, s, C2-H), 7.04 (1H, dd, J=8, 1 Hz, C7-H), 7.43 (1H, d, J=8 Hz, C8-H), 7.67 (1H, d, J=1 Hz, C5-H), 8.60 (1H, D_2O exch, s, OH), 10.68 (1H, D_2O exch, s, NH). ^{13}C-NMR ($CDCl_3$–CD_3OD) δ: 12.1 ($\underline{C}H_3$-4), 16.4 ($\underline{C}H_3$-1), 56.2 ($O\underline{C}H_3$), 106.4 (C-5), 110.9 (C-8), 113.5 (C-7), 115.1 (C-4a), 115.7 (C-2), 117.3 (C-4), 122.3 (C-1), 124.9 (C-4b), 134.6 (C-9a), 135.5 (C-8a), 146.6 (C-3), 153.0 (C-6). IR ν_{max} cm^{-1}: 3452, 3421, 3348, 2967, 2916, 1482, 1215, 1091, 1020. UV λ_{max} nm (log ε): 231 (4.47), 258 (3.05), 271.5 (2.84), 305.5 (3.18), 357 (2.57). CI-MS *m/z*: 242 ([MH]$^+$). *Anal.* Calcd for $C_{15}H_{15}NO_2$: C, 74.67; H, 6.27; N, 5.81. Found: C, 74.48; H, 6.26; N, 5.79.

9-Methoxy-2,2,5,11-tetramethyl-2,6-dihydropyrano[3,2-*b*]carbazole (12) Synthesis of **12** from **11** (3.46 g, 14.4 mmol) carried out under the same conditions as those applied for the preparation of compound **7**, afforded **12** as a pale yellow amorphous product (1.27 g, 29%). ^{1}H-NMR ($CDCl_3$) δ: 1.48 (6H, s, (CH_3)$_2$-2), 2.45 (3H, s, CH_3-5), 2.72 (3H, s, CH_3-11), 3.95 (3H, s, OCH_3), 5.81 (1H, d, J=10 Hz, C3-H), 6.73 (1H, d, J=10 Hz, C4-H), 7.03 (1H, dd, J=9, 2.5 Hz, C8-H), 7.32 (1H, d, J=9 Hz, C7-H), 7.64 (1H, D_2O exch, s, NH), 7.68 (1H, d, J=2.5 Hz, C10-H). ^{13}C-NMR ($CDCl_3$) δ: 12.0 ($\underline{C}H_3$-11), 12.1 ($\underline{C}H_3$-5), 27.2 (2C, ($\underline{C}H_3$)$_2$-2), 56.2 ($O\underline{C}H_3$), 74.4 (C-2), 106.3 (C-10), 110.9 (C-7), 112.1 (C-4a), 113.6 (C-8), 117.0 (C-10b), 118.8 (C-5), 120.3 (C-4), 121.8 (C-11), 125.3 (C-10a), 131.9 (C-3), 134.9 (C-5a), 135.5 (C-6a), 144.0 (C-11a), 153.4 (C-9). IR ν_{max} cm^{-1}: 3431, 3356, 3045, 2961, 2922, 1482, 1314, 1214, 1135. UV λ_{max} nm (log ε): 210 (4.15), 257.5 (4.52), 282 (3.84), 321.5 (4.31), 334 (4.44). CI-MS *m/z*: 307 ([MH]$^+$). *Anal.* Calcd for $C_{20}H_{21}NO_2$: C, 78.15; H, 6.89; N, 4.56. Found: C, 78.02; H, 6.88; N, 4.57.

(3*RS*,4*RS*)-2,2,5,11-Tetramethyl-2,3,4,6-tetrahydropyrano[3,2-*b*]carbazole-3,4-diol (13) Compound **7** (135 mg, 0.49 mmol) was added to a solution of osmium tetroxide (2.5% in 2-methyl-2-propanol) (0.31 ml) and *N*-methylmorpholine *N*-oxide dihydrate (65 mg, 0.54 mmol) in a mixture of *t*-BuOH/THF/H_2O: 10/3/1, v/v/v (3 ml). The reaction mixture was stirred at 25 °C for 48 h. After addition of aqueous saturated $NaHSO_3$ and stirring for 1 h, the mixture was extracted with CH_2Cl_2 (3×50 ml). The combined organic layers were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Column chromatography of the crude product on silica gel (CH_2Cl_2/MeOH: 99/1, v/v) gave **13** as a whitish amorphous product (141 mg, 93%). ^{1}H-NMR (CD_3OD) δ: 1.38 (3H, s, CH_3-2), 1.45 (3H, s, CH_3-2), 2.58 (3H, s, CH_3-5), 2.65 (3H, s, CH_3-11), 3.75 (1H, d, J=5 Hz, C3-H), 4.97 (1H, d, J=5 Hz, C4-H), 7.06 (1H, td, J=8, 1 Hz, C9-H), 7.30 (1H, td, J=8, 1 Hz, C8-H), 7.42 (1H, dd, J=8, 1 Hz, C7-H), 8.10 (1H, dd, J=8, 1 Hz, C10-H). ^{13}C-NMR (CD_3OD) δ: 12.7 ($\underline{C}H_3$-5), 13.4 ($\underline{C}H_3$-11), 21.4 ($\underline{C}H_3$-2), 27.2 ($\underline{C}H_3$-2), 66.1 (C-4), 74.6 (C-3), 77.0 (C-2), 111.6 (2C, C-7 and C-4a), 117.5 (C-10b), 118.6 (C-5), 119.0 (C-9), 121.3 (C-11), 123.5 (C-10), 125.3 (C-10a), 125.9 (C-8), 135.8 (C-5a), 142.6 (C-6a), 144.8 (C-11a). IR ν_{max} cm^{-1}: 3523, 3450, 3362, 3282, 3050, 2971, 2918, 1504, 1458, 1392, 1301, 1149, 1102, 1007, 746. UV λ_{max} nm (log ε): 224 (4.42), 240 (4.57), 257.5 (4.31), 271 (4.09), 290 (4.08), 299 (4.32). CI-MS *m/z*: 312 ([MH]$^+$). *Anal.* Calcd for $C_{19}H_{21}NO_3$: C, 73.29; H, 6.80; N, 4.50. Found: C, 73.12; H, 6.82; N, 4.51.

(3*RS*,4*RS*)-9-Methoxy-2,2,5,11-tetramethyl-2,3,4,6-tetrahydropyrano[3,2-*b*]carbazole-3,4-diol (14) Oxidation of **12** (50 mg, 0.16 mmol) according to the same procedure as that described for the preparation of **13**, afforded diol **14** as a whitish amorphous product (38 mg, 68%). ^{1}H-NMR (DMSO-d_6) δ: 1.30 (3H, s, CH_3-2), 1.41 (3H, s, CH_3-2), 2.52 (3H, s, CH_3-5), 2.60 (3H, s, CH_3-11), 3.65 (1H, dd , J=7, 5 Hz, C3-H), 3.83 (3H, s, OCH_3), 4.78 (1H, D_2O exch, d, J=5 Hz, C4-OH), 4.85 (1H, t, J=5 Hz, C4-H), 5.00 (1H, D_2O exch, d, J=5 Hz, C3-OH), 7.01 (1H, dd, J=9, 2 Hz, C8-H), 7.37 (1H, d, J=9 Hz, C7-H), 7.59 (1H, d, J=2 Hz, C10-H), 10.60 (1H, D_2O exch, s, NH). ^{13}C-NMR (DMSO-d_6) δ: 12.8 ($\underline{C}H_3$-11), 13.7 ($\underline{C}H_3$-5), 21.5 ($\underline{C}H_3$-2), 27.8 ($\underline{C}H_3$-2), 56.2 ($O\underline{C}H_3$), 64.5 (C-4), 73.2 (C-3), 76.3 (C-2), 106.1 (C-10), 111.8 (C-7), 114.2 (C-8), 115.4 (C-10b), 118.2 (C-4a), 121.7 (C-11), 121.9 (C-5), 124.2 (C-10a), 135.5 (C-5a), 136.4 (C-6a), 143.4 (C-11a), 152.9 (C-9). IR ν_{max} cm^{-1}: 3557, 3431, 3271, 2990, 2916, 2870, 1480, 1218, 1145, 1095. UV λ_{max} nm (log ε): 238 (4.52), 250 (4.35), 260.5 (4.08), 274.5 (3.68), 299 (4.09), 308.5 (4.34). ESI-MS *m/z*: 364 ([M+Na]$^+$). *Anal.* Calcd for $C_{20}H_{23}NO_4$: C, 70.36; H, 6.79; N, 4.10. Found: C, 70.35; H, 6.80; N, 4.11.

(3*RS*,4*RS*)-2,2,5,11-Tetramethyl-2,3,4,6-tetrahydropyrano[3,2-*b*]carbazole-3,4-diyle Diacetate (15) Diol **13** (305 mg, 0.98 mmol) was acetylated with acetic anhydride in anhydrous pyridine under Ar at 25 °C for 18 h. The mixture was evaporated under reduced pressure and the residue gave **15** as a pale yellow amorphous product (349 mg, 90%). ^{1}H-NMR (DMSO-d_6) δ: 1.36 (3H, s, CH_3-2), 1.37 (3H, s, CH_3-2), 2.05 (3H, s, $OCOCH_3$) 2.07 (3H, s, $OCOCH_3$), 2.32 (3H, s, CH_3-5), 2.63 (3H, s, CH_3-11), 5.17 (1H, d, J=5 Hz, C3-H), 6.33 (1H, d, J=5 Hz, C4-H), 7.12 (1H, t, J=8 Hz, C9-H), 7.37 (1H, t, J=8 Hz, C8-H), 7.48 (1H, d, J=8 Hz, C7-H), 8.12 (1H, d, J=8 Hz, C10-H), 11.00 (1H, D_2O exch, s, NH). ^{13}C-NMR (DMSO-d_6) δ: 12.8 ($\underline{C}H_3$-11), 13.4 ($\underline{C}H_3$-5), 20.9 ($OCO\underline{C}H_3$), 21.1 ($OCO\underline{C}H_3$), 22.1 ($\underline{C}H_3$-2), 26.4 ($\underline{C}H_3$-2), 64.6 (C-4), 72.2 (C-3), 74.7 (C-2), 111.5 (C-7), 115.9 (C-4a), 116.7 (C-10a), 117.6 (C-5), 118.8 (C-9), 122.8 (C-11), 123.1 (C-10), 123.5 (C-10a), 126.0 (C-8), 134.7 (C-5a), 141.6 (C-6a), 144.0 (C-11a), 170.2 ($\underline{C}OCH_3$), 170,6 ($\underline{C}OCH_3$). IR ν_{max} cm^{-1}: 3406, 3049, 2990, 2940, 1747, 1728, 1369, 1242, 1016, 741. UV λ_{max} nm (log ε): 240 (4.28), 247.5 (4.22), 258.5 (3.99), 270 (3.71), 290.5 (3.73), 299.5 (4.05). ESI-MS *m/z*: 418 ([M+Na]$^+$), 336 ([M−OAc]$^+$). *Anal.* Calcd for $C_{23}H_{25}NO_5$: C, 69.86; H, 6.37; N, 3.54. Found: C, 69.90; H, 6.40; N, 4.53.

(3*RS*,4*RS*)-9-Methoxy-2,2,5,11-tetramethyl-2,3,4,6-tetrahydropyrano[3,2-*b*]carbazole-3,4-diyle Diacetate (16) Diol **14** (80 mg, 0.23

mmol) was acetylated according to the same procedure as that used for the preparation of compound **15**, to afford the diacetate **16** as a pale yellow amorphous product (79.5 mg, 79%). ^{1}H-NMR (DMSO-d_6) δ: 1.35 (3H, s, CH_3-2), 1.37 (3H, s, CH_3-2), 2.05 (3H, s, $OCOCH_3$), 2.07 (3H, s, $OCOCH_3$), 2.30 (3H, s, CH_3-5), 2.64 (3H, s, CH_3-11), 3.84 (3H, s, OCH_3), 5.16 (1H, d, J=5 Hz, C3-H), 6.32 (1H, d, J=5 Hz, C4-H), 7.05 (1H, dd, J=9, 2 Hz, C8-H), 7.38 (1H, d, J=9 Hz, C7-H), 7.60 (1H, d, J=2 Hz, C10-H), 10.80 (1H, D_2O exch, s, NH). ^{13}C-NMR (DMSO-d_6) δ: 12.7 ($\underline{C}H_3$-11), 13.3 ($\underline{C}H_3$-5), 20.9 ($OCO\underline{C}H_3$), 21.1 ($OCO\underline{C}H_3$), 22.1 ($\underline{C}H_3$-2), 26.4 (CH_3-2), 56.2 ($O\underline{C}H_3$), 64.6 (C-4), 72.2 (C-3), 74.7 (C-2), 106.1 (C-10), 112.0 (C-7), 115.0 (C-8), 116.0 (C-4a), 116.6 (C-10b), 117.7 (C-5), 122.8 (C-11), 123.8 (C-10a), 135.5 (C-5a), 136.6 (C-6a), 143.6 (C-11a), 153.1 (C-9), 170.2 ($\underline{C}OCH_3$), 170,6 ($\underline{C}OCH_3$). IR ν_{max} cm^{-1}: 3424, 2979, 2936, 2831, 1734, 1250, 1220, 1018. UV λ_{max} nm (log ε): 239.5 (4.68), 250.5 (4.52), 262.5 (4.24), 273.5 (3.89), 298.5 (4.24), 308.5 (4.48). ESI-MS *m*/*z*: 425 ([M+Na]$^+$). *Anal.* Calcd for $C_{24}H_{27}NO_6$: C, 67.75; H, 6.40; N, 3.29. Found: C, 67.73; H, 6.38; N, 3.29.

(3a*RS*,12b*RS*)-4,4,6,12-Tetramethyl-3a,4,11,12b-tetrahydro-1,3-dioxolo[4′,5′:4,5]pyrano[3,2-*b*]carbazol-2-one (17) To a solution of **13** (103 mg, 0.33 mmol) in 2-butanone (5 ml) was added *N*,*N*′-carbonyldiimidazole (261 mg, 1.61 mmol). The reaction mixture was refluxed under Ar for 1.5 h and after cooling, aqueous 5% $NaHCO_3$ solution (8 ml) was added. The solution was extracted with CH_2Cl_2 (3×20 ml) and the combined organic layers were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Column chromatography of the crude product on silica gel (cyclohexane/EtOAc: 5/5, v/v) gave **17** as a whitish amorphous product (48 mg, 43%). ^{1}H-NMR (DMSO-d_6) δ: 1.13 (3H, s, CH_3-2), 1.51 (3H, s, CH_3-2), 2.56 (3H, s, CH_3-5), 2.63 (3H, s, CH_3-11), 5.07 (1H, d, J=8 Hz, C3-H), 6.36 (1H, d, J=8 Hz, C4-H), 7.13 (1H, t, J=8 Hz, C9-H), 7.39 (1H, t, J=8 Hz, C8-H), 7.52 (1H, d, J=8 Hz, C7-H), 8.13 (1H, d, J=8 Hz, C10-H), 10.14 (1H, D_2O exch, s, NH). ^{13}C-NMR (DMSO-d_6) δ: 12.6 ($\underline{C}H_3$-11), 13.5 ($\underline{C}H_3$-5), 22.4 ($\underline{C}H_3$-2), 24.6 ($\underline{C}H_3$-2), 71.9 (C-4), 75.3 (C-2), 79.4 (C-3), 111.6 (C-7), 115.2 (C-4a), 117.8 (C-10b), 118.5 (C-5), 119.0 (C-9), 123.1 (C-10 and C-11), 123.5 (C-10a), 126.2 (C-8), 134.9 (C-5a), 141.5 (C-6a), 143.7 (C-11a), 154.7 (C=O). IR ν_{max} cm^{-1}: 3365, 3050, 2979, 2970, 1782, 1182, 1006, 744. UV λ_{max} nm (log ε): 241.5 (4.56), 248.5 (4.51), 258 (4.23), 270 (3.84), 290.5 (3.99), 300.5 (4.28). ESI-MS *m*/*z*: 360 ([M+Na]$^+$). *Anal.* Calcd for $C_{20}H_{19}NO_4$: C, 71.20; H, 5.68; N, 4.15. Found: C, 71.16; H, 5.65; N, 4.16.

(3a*RS*,12b*RS*)-8-methoxy-4,4,6,12-tetramethyl-3a,4,11,12b-tetrahydro-1,3-dioxolo[4′,5′:4,5]pyrano[3,2-*b*]carbazol-2-one (18) Compound **18** was obtained from diol **14** (100 mg, 0.29 mmol) according to the same procedure as that described for the preparation of **17**, as a whitish amorphous product (57 mg, 53%). ^{1}H-NMR (DMSO-d_6) δ: 1.28 (3H, s, CH_3-2), 1.51 (3H, s, CH_3-2), 2.53 (3H, s, CH_3-5), 2.63 (3H, s, CH_3-11), 3.84 (3H, s, OCH_3), 5.06 (1H, d, J=8 Hz, C3-H), 6.35 (1H, d, J=8 Hz, C4-H), 7.07 (1H, dd, J=9, 2 Hz, C8-H), 7.42 (1H, d, J=9 Hz, C7-H), 7.60 (1H, d, J=2 Hz, C10-H), 10.93 (1H, D_2O exch, s, NH). ^{13}C-NMR (DMSO-d_6) δ: 12.5 ($\underline{C}H_3$-11), 13.4 ($\underline{C}H_3$-5), 22.5 ($\underline{C}H_3$-2), 24.6 ($\underline{C}H_3$-2), 56.2 ($O\underline{C}H_3$), 71.9 (C-4), 75.3 (C-2), 79.4 (C-3), 106.0 (C-10), 112.2 (C-7), 115.2 (C-4a), 115.4 (C-8), 117.7 (C-10b), 118.6 (C-5), 122.9 (C-11), 123.8 (C-10a), 135.7 (C-5a), 136.5 (C-6a), 143.2 (C-11a), 153.2 (C-9), 154.7 (C=O). IR ν_{max} cm^{-1}: 3381, 2982, 2932, 2823, 1784, 1225, 1181. UV λ_{max} nm (log ε): 242.5 (4.71), 251.5 (4.58), 263.5 (4.24), 301 (4.26), 309.5 (4.47). ESI-MS *m*/*z*: 390 ([M+Na]$^+$). *Anal.* Calcd for $C_{21}H_{21}NO_5$: C, 68.65; H, 5.76; N, 3.81. Found: C, 68.64; H, 5.77; N, 3.81.

1,4,9-Trimethyl-9*H*-carbazol-3-ol (20) To an ice-cooled solution of 1,4,9-trimethyl-9*H*-carbaldehyde[15] (7.8 g, 35.0 mmol) in methanol (400 ml) were added 33% aqueous hydrogen peroxide (5 ml) and 95% sulfuric acid (2.5 ml). After stirring for 15 min at 0 °C, the reaction mixture was diluted with a mixture of crushed ice and water and neutralized with aqueous 1 M NaOH solution to give a whitish precipitate which was filtered off and washed with water. Crystallization from MeOH gave 1,4,9-trimethyl-9*H*-carbazol-3-ol as white needles (6.5 g, 88%), mp 208—209 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.61 (3H, s, CH_3-1), 2.72 (3H, s, CH_3-4), 4.00 (3H, s, NCH_3), 6.79 (1H, s, C2-H), 7.12 (1H, td, J=8, 1 Hz, C6-H), 7.39 (1H, td, J=8, 1 Hz, C7-H), 7.48 (1H, dd, J=8, 1 Hz, C8-H), 8.11 (1H, dd, J=8, 1 Hz, C5-H), 8.73 (1H, D_2O exch, s, OH). ^{13}C-NMR (DMSO-d_6) δ: 13.5 (CH_3-1), 20.8 (CH_3-4), 33.1 ($\underline{N}CH_3$), 109.8 (C-8), 115.5 (C-4a), 118.8 (C-4), 119.0 (2C, C-2 and C-6), 123.2 (C-5), 123.5 (C-1), 124.1 (C-4b), 125.8 (C-7), 134.5 (C-9a), 142.9 (C-8a), 148.6 (C-3). IR ν_{max} cm^{-1}: 3445, 1473, 1394, 1299, 750. UV λ_{max} nm (ε): 227 (4.17), 243.5 (4.26), 255 (3.97), 272 (3.90), 295.5 (3.81). CI-MS *m*/*z*: 226 ([MH]$^+$). *Anal.* Calcd for $C_{15}H_{15}NO$: C, 79.97; H, 6.71; N, 6.22. Found: C, 79.97; H, 6.72; N, 6.22.

2,2,5,6,11-Pentamethyl-2,6-dihydropyrano[3,2-*b*]carbazole (19) Sodium hydride (50% dispersion in oil, 160 mg, 6.66 mmol) was added to a stirred solution of **20** (750 mg, 3.33 mmol) in dry toluene (20 ml) under Ar. 3-Chloro-3-methylbut-1-yne[20] (1.70 g, 16.6 mmol) and potassium iodide (2.75 g, 16.6 mmol) were then added. The reaction mixture was refluxed for 17 h, cooled, washed with water, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Column chromatography of the crude product on silica gel (cyclohexane/CH_2Cl_2: 6/4, v/v) gave **19** which crystallized from the eluent as pale yellow needles (243 mg, 25%), mp 180—181 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (6H, s, $(CH_3)_2$-2), 2.74 (3H, s, CH_3-5), 2.78 (3H, s, CH_3-11), 4.08 (3H, s, NCH_3), 5.82 (1H, d, J=12 Hz, C3-H), 6.81 (1H, d, J=12 Hz, C4-H), 7.18 (1H, dd, J=8, 7 Hz, C9-H), 7.35 (1H, d, J=8 Hz, C7-H), 7.44 (1H, dd, J=8, 7 Hz, C8-H), 8.20 (1H, d, J=8 Hz, C10-H). ^{13}C-NMR ($CDCl_3$) δ: 12.2 ($\underline{C}H_3$-5), 14.3 ($\underline{C}H_3$-11), 27.1 (2C, $(\underline{C}H_3)_2$-2), 33.4 ($N\underline{C}H_3$), 74.1 (C-2), 108.4 (2C, C-7 and C-4a), 113.2 (C-10b), 117.4 (C-5), 118.4 (C-9), 119.6 (C-11), 120.6 (C-10), 122.5 (C-4), 123.9 (C-10a), 124.9 (C-8), 131.8 (C-3), 135.5 (C-5a), 143.2 (C-6a), 144.1 (C-11a). IR ν_{max} cm^{-1}: 3433, 3048, 2968, 2920, 2862, 1467, 1300, 1133, 745, 715. UV λ_{max} nm (log ε): 260.5 (4.53), 302 (3.94), 314 (4.77), 326.5 (4.37). CI-MS *m*/*z*: 292 ([MH]$^+$). *Anal.* Calcd for $C_{20}H_{21}NO$: C, 82.44; H, 7.26; N, 4.81. Found: C, 82.50; H, 7.27; N, 4.82.

9-Methoxy-2,2,5,6,11-pentamethyl-2,6-dihydropyrano[3,2-*b*]carbazole (22) Sodium hydride (50% dispersion in oil, 10 mg, 0.4 mmol) was added to an ice-cooled solution of **12** (25.0 mg, 0.08 mmol) in dry *N*,*N*-dimethylformamide (5 ml). After stirring for 10 min at 0 °C, methyl iodide (17.0 mg, 0.12 mmol) was added and the reaction mixture was stirred for 5 min at 0 °C. The reaction mixture was diluted with ice water, extracted with CH_2Cl_2 (3×10 ml), and the combined organic layers were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Column chromatography of the crude product on silica gel (cyclohexane/EtOAc: 99/1, v/v) gave **22** which crystallized from the eluent as pale yellow needles (23 mg, 89%), mp 166—167 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (6H, s, $(CH_3)_2$-2), 2.70 (3H, s, CH_3-5), 2.75 (3H, s, CH_3-11), 3.94 (3H, s, OCH_3), 4.02 (3H, s, NCH_3), 5.82 (1H, d, J=10 Hz, C3-H), 6.79 (1H, d, J=10 Hz, C4-H), 7.08 (1H, dd, J=9, 2.5 Hz, C8-H), 7.24 (1H, d, J=9 Hz, C7-H), 7.71 (1H, d, J=2.5 Hz, C10-H). ^{13}C-NMR ($CDCl_3$) δ: 12.1 ($\underline{C}H_3$-11), 14.3 ($\underline{C}H_3$-5), 27.1 (2C, $(\underline{C}H_3)_2$-2), 33.7 ($N\underline{C}H_3$), 56.0 ($O\underline{C}H_3$), 74.1 (C-2), 106.4 (C-10), 108.9 (C-7), 113.3 (C-8), 113.5 (C-4a), 117.2 (C-10b), 119.8 (C-5), 120.5 (C-4), 122.5 (C-11), 124.2 (C-10a), 128.8 (C-5a), 130.8 (C-6a), 131.9 (C-3), 144.4 (C-11a), 153.0 (C-9). IR ν_{max} cm^{-1}: 3448, 3045, 2967, 2932, 2827, 1484, 1222, 1156. UV λ_{max} nm (ε): 259.5 (4.53), 322 (4.33), 334.5 (4.44). CI-MS *m*/*z*: 322 ([MH]$^+$). *Anal.* Calcd for $C_{21}H_{23}NO_2$: C, 78.47; H, 7.21; N, 4.36. Found: C, 78.52; H, 7.22; N, 4.35.

Cytotoxicity Murine leukaemia L1210 cells from the American Type Culture Collection (Rockville Pike, MD) were grown in RPMI medium 1640 supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 10 mM HEPES buffer (pH 7.4). The cytotoxicity was measured by microculture tetrazolium assay essentially as described.[23] Cells were exposed for 48 h to nine graded concentrations in triplicate of the test drug. Results are expressed as IC_{50} (mean, n=3), which is defined as the drug concentration inhibiting the absorbance by 50% with respect to that of untreated cells.

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