Synthesis of 11-Amino-substituted-5,6-dimethyl-5*H*-pyrido[3',4':4,5]pyrrolo-[2,3-*g*]isoquinolines as New Ellipticine Analogues

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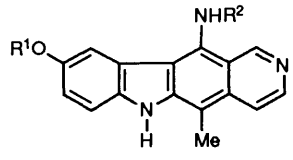
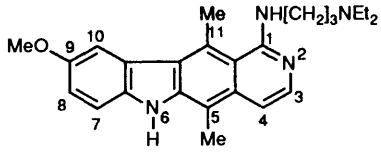
By using 4-acetyl-*N*,*N*-diisopropylnicotinamide and 4-chloro-1-methyl-1*H*-pyrrolo[3,2-*c*]pyridine as starting building blocks, the new ellipticine analogue title compounds have been synthesized through a five-step sequence.

As already stated in the preceding paper,[1] the synthesis of 11-amino-substituted-6*H*-pyrido[4,3-*b*]carbazoles **1** was undertaken with the aim of comparing their possible anti-tumour properties with those of their 1-amino-substituted analogue **2**.[2]

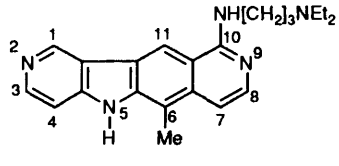


1



2

However, the antitumour properties of the azaellipticine derivative **3** are well established[3,4] and even the tricyclic compounds **4**, which are simplified analogues of compound **3**, also display significant cytotoxicity towards cultured cells and *in vivo* antitumour properties.[5]



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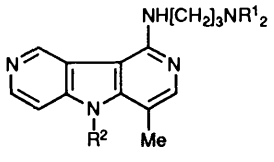
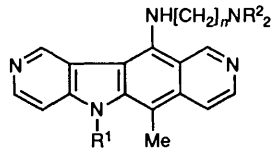


4
R^1 = Me or Et
R^2 = H or Me

Despite the disappointing biological results obtained with compounds **1**,[1] it seemed interesting to us to study the new azaellipticine derivatives corresponding to structure **5**.

Indeed, this would allow us to complete our knowledge of the structure–activity relationships of these series. This paper describes our results in this area.

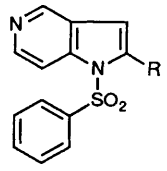
Our initial aim was to synthesize compounds **5** with R^1 = H. In order to specify the optimum experimental conditions, 1-phenylsulphonyl-1*H*-pyrrolo[3,2-*c*]pyridine **6**[6] was lithiated to give compound **7** and this species was set to react with *p*-methoxybenzaldehyde in order to obtain α-(*p*-methoxyphenyl)-



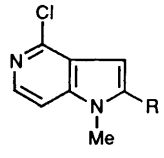
5
n = 2,3: R^1 = H or Me, R^2 = Me or Et

α-(1-phenylsulphonyl-1*H*-pyrrolo[3,2-*c*]pyridin-2-yl)-methanol **8**, as expected.[6] Unfortunately, despite various attempts performed over a large temperature range (−78, −30, −10 and +20 °C) all tests for condensation of the lithio derivative **7** with 4-acetyl-*N*,*N*-diisopropylnicotinamide **11**[1] gave negative results.

In view of this failure, we abandoned the synthesis of the 5-unsubstituted derivatives (**5**; R^1 = H) and kept their N^5-methylated homologues as new target molecules. 4-Chloro-1-methyl-1*H*-pyrrolo[3,2-*c*]pyridine,[7] whose lithiation to **10** and reaction with ketones had been previously studied in our laboratory,[8] appeared therefore to be a suitable starting building block. In order to determine the best conditions, the lithio derivative **10** was treated with the keto-amide **11** using various protocols. When compound **11** was added to a cold solution of lithium compound **10** in tetrahydrofuran (THF) (−50 to −13 °C), the yield of the expected product **12** reached 65%.



6 R = H
7 R = Li
8 R = CH(OH)C_6H_4OMe-*p*

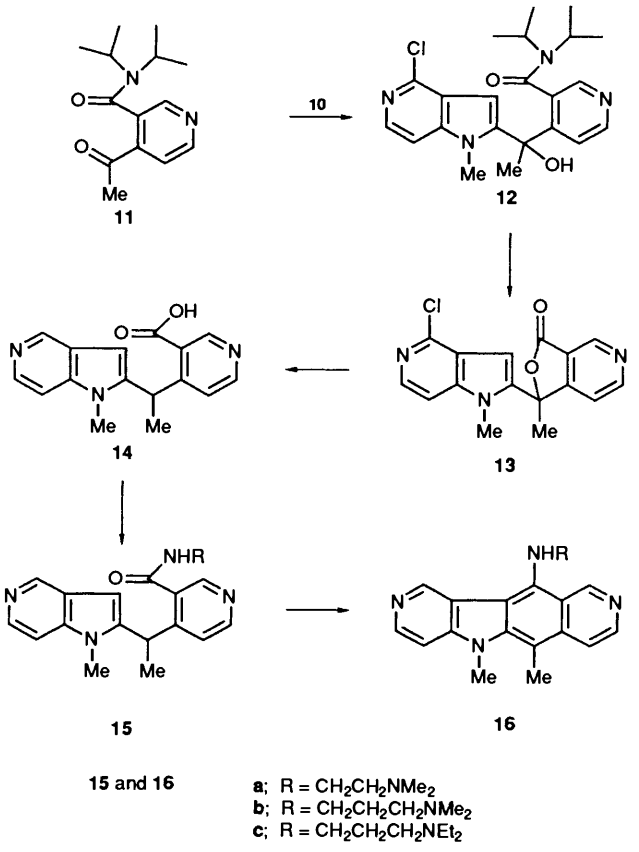


9 R = H
10 R = Li

Hydrolysis of this alcohol amide in 1 mol dm^{-3} hydrochloric acid led to the lactone **13** in high yield (80%). However, reduction of this last compound could not be performed with activated zinc in formic acid, as it could for its indole analogue.[1] Since these conditions provided only a complex mixture, from which no pure compound could be isolated, lactone **13** was submitted to catalytic hydrogenation in the presence of 15% (w/w) of 10% palladized charcoal. Reduction of the lactone,

with simultaneous chlorine-atom elimination,. then gave the acid **14**, characterized as a dihydrate of its hydrochloride salt.

This acid was treated with 2-(dimethylamino)ethylamine, 3-(dimethylamino)propylamine, and 3-(diethylamino)propylamine in the presence of *N,N'*-carbonyldiimidazole. The resulting amides **15a–c** were obtained as oily products that were directly cyclized into their tetracyclic derivatives **16a–c** by using phosphorus trichloride oxide at reflux temperature for 5 h (Scheme 1)



Scheme 1

Cytotoxicity towards L1210 cultured cells and antitumour properties against L1210 leukaemia for these 11-amino-substituted5,6-dimethyl-5*H*-pyrido[3',4':4,5]pyrrolo[2,3-*g*]isoquinolines were determined under the usual testing conditions.[9] Results are given in Table 1.

Table 1 Cytotoxicity and antitumour properties of compounds **16a–c** on L1210 leukaemia

Compound	ID_{50} [a] (10^{-6} mold dm^{-3}) (L1210 cells)	$T/C \times 100$ [b]·L1210 ip (mg kg^{-1}, single dose at day 1, ip)
16	0.03	107 (30)
16b	0.06	105 (30)
16c	0.17	102 (30)
3	0.003	165 (20) [2/10]

[a] ID_{50}: the micromolecular concentration of drug that, when added to cultures of L1210 cells for a period of 48 h, reduces the counted cells to 50% of the control values. [b] $T/C \times 100$: antitumour activity evaluated according to the formula $T/C \times 100$ median day of survival of treated animals at a given dose/median day of survival of control mice. [c] Ref. 3, 4·[]: Long-term survivors (at day 60 after leukaemia cells were inoculated).

These results clearly show that the displacement of the diamino chain from the 10- to the 11-position in these series causes an important decrease in cytotoxicity (at least by a factor of 10) and a total loss of antimour activity.

In conclusion to this paper and the preceding one,[1] 4-acetyl-*N,N*-diisopropylnicotinamide is a good synthon for the building of polyheterocyclic compounds comprising an 8-functionalized isoquinoline nucleus fused to indole systems. From the biological point of view, however, the new ellipticine derivatives and analogues that were prepared are inactive. This point emphasizes the importance of the R–NH–C=N– sequence for the biological activity of compounds **2** and **3**, in which specific interaction(s) with an enzyme's topoisomerase II site could be involved.[10]

Experimental

General comments for m.p.s, ^1H NMR spectra, purification procedures, and elemental analysis are identical with those reported in the preceding paper.[1]

α-(p-*Methoxyphenyl*)-α-(1-*phenylsulphonyl*-1H-*pyrrolo*[3,2-c]*pyridin*-2-*yl*)*methanol* **8**.—A solution of 1-phenylsulphonylpyrrolo[3,2-*c*]pyridine **6**[6] (282 mg, 1.09 mmol) in dry THF (20 cm^3) was placed under argon and cooled to −78 °C. Butyllithium (0.75 cm^3 of the 1.6 mol dm^{-3} commercially available solution, 1.2 mmol) was added all at once, the mixture was stirred for 30 min at the same temperature, and *p*-methoxybenzaldehyde (0.15 cm^3, 1.2 mmol) was added. After 15 h at room temperature the mixture was poured into 3 mol dm^{-3} aq. ammonium chloride (40 cm^3) and extracted with dichloromethane (3 × 50 cm^3) and the extract was treated as usual. The residue was recrystallized twice from ethyl acetate to provide *compound* **8** as crystals (200 mg, 46.6%), m.p. 203 °C (Found: C, 63.7; H, 4.5; N, 7.15; S, 8.1. $C_{21}H_{18}N_2O_4S$ requires C, 63.97; H, 4.6; N, 7.10; S, 8.11%); $\delta_H(CDCl_3)$ 3.76 (3 H, s, OMe), 6.17 (1 H, d, OH), 6.33 (1 H, d, *J* 5.5, C*H*OH), 6.75 (1 H, s, 3-H), 6.88 (2 H, d, $J_{2',3'}$ 8.8, 2'- + 6'-H), 7.26 (2 H, d, 3'- + 5'-H), 7.72 (5 H, m, SO_2Ph), 7.95 (1 H, d, $J_{7,6}$ 5.8, 7-H), 8.42 (1 H, d, 6-H) and 8.86 (1 H, s, 4-H).

4-{1-(4-*Chloro*-1-*methyl*-1H-*pyrrolo*[3,2-c]*pyridin*-2-*yl*)-1-*hydroxyethyl*}-N,N-*diisopropylnicotinamide* **12**.—A solution of 4-chloro-1-methylpyrrolo[3,2-*c*]pyridine **9**[7] (3.33 g, 20 mmol) in dry THF (150 cm^3) under argon was cooled to −78 °C, butyllithium (1.6 mol dm^{-3} solution; 12.5 cm^3, 20 mmol) was added all at once, and the mixture was stirred for 3 h at this temperature. The solid CO_2 cooling bath was replaced by an ice–sodium chloride cooling mixture and a solution of 4-acetyl-*N,N*-diisopropylnicotinamide[1] (4.96 g, 20 mmol) in THF (200 cm^3) was immediately added. Following a temperature rise from −50 to −13 °C, the mixture was allowed to reach room temperature during 15 h and was poured into 3 mol dm^{-3} aq. ammonium chloride (300 cm^3). It was extracted with diethyl ether (2 × 100 cm^3), and the extract was dried over magnesium sulphate and evaporated. The residue was recrystallized from ethyl acetate to give *compound* **12** as crystals (5.35 g, 64.5%), m.p. >280 °C (Found: C, 64.0; H, 6.3; Cl, 8.8; N, 13.6. $C_{22}H_{27}ClN_4O$ requires C, 63.68; H, 6.65; Cl, 8.54; N, 13.50%; $\delta_H[(CD_3)_2SO]$: two rotamers (A + B) in the ratio 2:1 were observed. For A: 1.17, 1.20, 1.46 and 1.5 [12 H, 4 d, $(CHMe_2)_2$], 2.08 (3 H, s, *Me*COH), 3.45 (3 H, s, NMe), 3.57 and 3.69 [2 H, 2 q, $(CHMe_2)_2$], 6.33 (1 H, s, OH), 6.60 (1 H, dd, $J_{5,6}$ 5.5, 5-H), 6.73 (1 H, d, $J_{3',7'}$ 0.6, 3'-H), 7.47 (1 H, dd, $J_{7',6'}$ 5.8, 7'-H), 8.01 (1 H, d, 6'-H), 8.34 (1 H, d, $J_{2,5}$ 0.7, 2-H) and 8.35 (1 H, d, 6-H). For B: 0.76, 1.05, 1.26 and 1.44 [12 H, 4 d $(CHMe_2)_2$], 2.06 (3 H, s, *Me*COH), 3.35 and 3.37 [2 H, 2 q, $(CHMe_2)_2$], 3.57 (3 H, s, NMe), 6.38 (1 H, s, OH), 6.71 (1 H, d, $J_{3',7'}$ 0.6, 3'-H), 7.38 (1 H, dd, $J_{5,6}$ 5.5, 5-H), 7.41 (1 H, dd, $J_{7',6'}$ 5.8, 7'-H), 7.97 (1 H, d, 6'-H), 8.27 (1 H, d, $J_{2,5}$ 0.7, 2-H) and 8.47 (1 H, d, 6-H).

1-(4-*Chloro*-1-*methyl*-1H-*pyrrolo*[3,2-c]*pyridin*-2-*yl*)-1-*methylfuro*[3,4-c]*pyridin*-3(1H)-*one* **13**.—A solution of compound **12** (5 g, 12.06 mmol) in 1 mol dm^{-3} hydrochloric acid (200 cm^3) was heated at reflux for 2 h, cooled, and neutralized to pH 7 with solid sodium hydrogen carbonate. The resulting mixture was extracted with dichloromethane (4 × 50 cm^3) and the combined extracts were dried over magnesium sulphate. Evaporation of solvent provided a residue, which was crystallized from ethanol to give the *lactone* **13** as crystals (3.38 g, 83.5%), m.p. 219 °C (Found: C, 61.5; H, 4.1; Cl, 11.4; N, 13.3. $C_{16}H_{12}ClN_3O_2$ requires C, 61.25; H, 3.86; Cl, 11.30; N, 13.39%); δ_H[$(CD_3)_2SO$] 2.21 (3 H, s, CMe), 3.66 (3 H, s, NMe), 6.75 (1 H, s, 3′-H), 7.61 (1 H, d, $J_{7',6'}$ 5.9, 7′-H), 7.98 (1 H, dd, $J_{7,6}$ 5.2, $J_{7,4}$ 0.8, 7-H), 8.12 (1 H, d, 6′-H), 9.07 (1 H, d, 6-H) and 9.28 (1 H, d, 4-H) (′ numbering for pyrrolo[3,2-*c*]pyridine nucleus).

4-{1-(1-*Methyl*-1H-*pyrrolo*[3,2-c]*pyridin*-2-*yl*)*ethyl*}-*nicotinic Acid* **14**.—A solution of the preceding lactone **13** (2.5 g, 7.97 mmol) in 95% ethanol (300 cm^3) was stirred and heated at 65 °C in the presence of 10% palladized charcoal (380 mg) under hydrogen until disappearance of the starting material **13** (3 h 30 min; TLC monitoring, SiO_2 plates, with ethyl acetate as solvent). After the mixture had cooled the catalyst was filtered off and washed with boiling ethanol (2 × 50 cm^3). Evaporation of the combined filtrates and washings gave a gummy solid, which was dried under reduced pressure overnight. It corresponded to the hydrochloride salt of the expected *compound* **14**, associated with two molecules of water. It was sufficiently pure for use in the following steps (Found: C, 54.7; H, 5.9; H, 11.5; Cl, 9.9. $C_{16}H_{15}N_3O_2$·HCl·$2H_2O$ requires C, 54.32; H, 5.70; N, 11.88; Cl, 10.02%); δ_H[$(CD_3)_2SO$] 1.71 (3 H, d, CHMe), 3.70 (3 H, s, NMe), 5.54 (1 H, q, C*H*Me), 6.98 (1 H, s, 3′-H), 7.18 (1 H, d, $J_{5,6}$ 5.1, 5-H), 8.09 (1 H, d, $J_{7',6'}$ 6.6, 7′-H), 8.48 (1 H, d, 6′-H), 8.67 (1 H, d, 6-H), 9.06 (1 H, s, 2-H) and 9.23 (1 H, s, 4′-H).

11-[(*Dialkylamino*)*alkylamino*]-5,6-*dimethyl*-5H-*pyrido*-[3′,4′:4,5]*pyrrolo*[2,3-g]*isoquinolines* **16a**–**c**.—The crude hydrochloride **14** (1 g, 2.83 mmol) was dissolved in freshly distilled dimethylformamide (10 cm^3) and *N*,*N*′-carbonyldiimidazole (1.83 g, 11.32 mmol) was added. After the mixture had been stirred for 20 min at room temperature the required dialkylaminoalkylamine (17 mmol) was added all at once. The mixture was stirred for 30 min and evaporated to dryness under reduced pressure (1 mmHg). The oily residue was taken up in water, extracted with dichloromethane (6 × 20 cm^3), and the combined extracts were repeatedly washed with water to neutrality (pH 7). The organic layer was dried over magnesium sulphate and evaporated to give the crude amide **15** as a gummy solid, which was directly cyclized by phosphorus trichloride oxide (40 cm^3) at reflux for 5 h. Evaporation of the phosphorus trichloride oxide provided a residue, which was dissolved in water (60 cm^3, stirred for 20 min) and basified with an excess of 5 mol dm^{-3} aq. sodium hydroxide. Extraction with dichloromethane (8 × 50 cm^3) and evaporation of the dried (magnesium sulphate), combined extracts afforded a residue, which was dissolved in the minimum amount of acetone and an excess (1 g) of maleic acid was added. The solid trimaleate salt was filtered off, washed with cold acetone (2 × 10 cm^3) and air dried.

Compound **16a**: 23% overall yield from the lactone **13**; m.p. (progressive from) 180 °C (Found: C, 56.2; H, 5.3; N, 10.2. $C_{20}H_{23}N_5$·$3C_4H_4O_4$ requires C, 56.38; H, 5.18; N, 10.27%). The *free base*, obtained from the trimaleate under the usual conditions, was recrystallized from acetone, m.p. 169 °C (Found: C, 70.2; H, 6.8; N, 20.35. $C_{20}H_{23}N_5$·$0.5H_2O$ requires C, 70.15; H, 7.06; N, 20.45%); δ_H($CDCl_3$; free base) 2.42 (6 H, s, NMe), 2.61 (2 H, m, β-H_2), 3.03 (3 H, s, 6-Me), 3.59 (2 H, t, α-H_2), 4.16 (3 H, s, 5-Me), 5.45 (1 H, br s, 11-H), 7.28 (1 H, 7-H, overlapped by $CDCl_3$ signal), 7.88 (1 H, dd, $J_{7,8}$ 6.3, $J_{7,10}$ 0.8, 7-H), 8.52 (1 H, d, 8-H), 8.64 (1 H, d, $J_{3,4}$ 5.8, 3-H), 9.61 (1 H, d, $J_{1,4}$ 0.7, 1-H) and 9.76 (1 H, d, 10-H) (an NOE study at 400 MHz on the 5-Me signal was used for correct attributions); *m*/*z* 334 (MH^+, 100%) and 275 (10).

Compound **16b**: 8.5% overall yield from the lactone **13**; m.p. progressive from 165 °C. This crystalline *trimaleate salt* remained associated with one molecule of acetone and two molecules of water (Found: C, 54.9; H, 5.75; N, 9.3. $C_{21}H_{25}N_5$·$3C_4H_4O_4$·C_3H_6O·$2H_2O$ requires C, 54.75; H, 6.00; N, 8.87%); δ_H[$(CD_3)_2SO$] 1.94 (2 H, m, β-H_2), 2.67 (6 H, s, NMe_2), 3.01 (3 H, s, 6-Me), 3.10 (2 H, m, γ-H_2), 3.37 (2 H, m, α-H_2), 4.26 (3 H, s, 5-Me), 6.06 (6 H, s, CH=CH maleate), 7.98 (1 H, d, $J_{7,8}$ 6.6, 7-H), 8.13 (1 H, d, $J_{4,3}$ 6.2, 4-H), 8.51 (1 H, d, 8-H), 8.72 (1 H, d, 3-H), 9.34 (1 H, s, 1-H) and 9.84 (1 H, s, 10-H); *m*/*z* 348 (MH^+, 100%) and 289 (30).

Compound **16c**: 18.4% overall yield from the lactone **13**. *Free base* obtained from crude trimaleate, m.p. 144–145 °C (from cyclohexane) (Found: C, 73.6; H, 7.6; N, 18.6. $C_{23}H_{29}N_5$ requires C, 73.57; H, 7.78; N, 18.65%); δ_H($CDCl_3$) 1.14 [6 H, t, $(MeCH_2)_2N$], 2.07 (2 H, m, β-H_2), 2.75 [6 H, m, $N(CH_2Me)_2$ + γ-H_2], 3.02 (3 H, s, 6-Me), 3.50 (2 H, t, α-H_2), 4.14 (3 H, s, 5-Me), 5.35 (1 H, br s, 11-H), 7.26 (1 H, d, $J_{4,3}$ 5.5, 4-H), 7.87 (1 H, dd, $J_{7,8}$ 6.5, $J_{7,10}$ 0.8, 7-H), 8.53 (1 H, d, 8-H), 8.63 (1 H, d, 3-H), 9.49 (1 H, d, 10-H) and 9.72 (1 H, s, 1-H); *m*/*z* 376 (MH^+, 100%) and 275 (16).

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