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Convergent synthesis and preliminary biological evaluation of (±)-B-norrhazinal †

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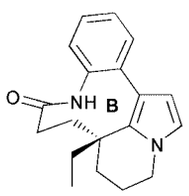
The title compound 3, an analogue of the naturally-derived spindle toxin rhazinilam (1), has been synthesised, characterised crystallographically and subjected to a preliminary biological evaluation.

(−)-Rhazinilam (**1**) was first isolated in 1965 from *Melodinus australia*,[1] then again in 1970 from *Rhazya stricta* (Apocynaceae)[2] and most recently (1987) from the Malaysian plant *Kopsia singapurensis* (Ridlay).[3] In 1998 the formyl-derivative (−)-rhazinal (**2**) was obtained from a related Malayan *Kopsia* species.[4] Compound **1**, at least, is an artefact of the isolation process, and the natural precursor is 5,21-dihydrorhazinilam.[5] Rhazinilam, which can be prepared from the natural product vincadiffomine,[6,7] has been the subject of one successful total synthesis study reported by Smith and co-workers in 1972.[6,7]

Recently, rhazinilam has assumed some importance because of indications that it is an unusual spindle toxin. Thus, the compound reportedly mimics the effects of both vinblastine and Taxol™ by inducing a non-reversible assembly (spiralisation) of tubulin (a vinblastine-type effect) and by inhibiting the cold-induced disassembly of microtubules (a Taxol™-like property).[3,8] In an even more striking similarity to Taxol™, rhazinilam has the ability to induce formation of asters in mitotic cells and microtubule bundles in interphase cells.[8] Such observations have generated renewed interest in compound **1**[9] and its congeners such that a range of (generally less complex) analogues has now been prepared.[10–13] Structure–activity studies deriving from these analogues suggest that the presence of the phenylated-pyrrole unit and the lactam ring as well as restricted rotation about the biaryl axis are required for antimitotic activity.[11] Consequently, we now describe a convergent synthesis of (±)-B-norrhazinal (**3**), a conformationally more constrained analogue of congeners **1** and **2**, and report on a preliminary biological evaluation of this compound.

The synthesis of compound **3** is outlined in Scheme 1. The reaction of the potassium salt **4** of pyrrole with γ-butyrolactone (**5**) at 160 °C according to the procedure of Li and Snyder[14] afforded the previously reported acid **6** (60–90%), which was converted into the corresponding Weinreb amide **7** (87%)‡ using a modified Mukaiyama amide coupling procedure.[15] Treatment of compound **7** with ethylmagnesium bromide followed by careful acidic work up then gave the ketone **8** (≥95%) which was immediately subjected to a Wadsworth–Emmons reaction using methyl diethyl phosphonoacetate. The resulting 1:1 mixture of (*E*)- and (*Z*)-acrylates **9** (91%) engaged in an intramolecular Michael addition on treatment with *ca*. five molar equivalents of aluminium trichloride in diethyl ether at



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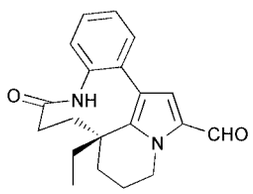

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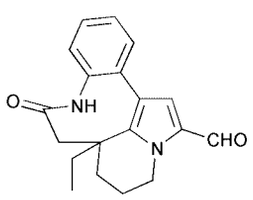

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18 °C, and in this manner the tetrahydroindolizine **10** (83%) was obtained. Vilsmeier–Haack formylation of the last compound afforded, in a completely regioselective manner, the aldehyde **11** (95%), which was reacted with iodine–silver trifluoroacetate to give the C-1 iodinated product **12** (75%) as the exclusive product of the reaction. Suzuki–Miyuara[16] cross-coupling of compound **12** with 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline[17] then gave the C-1 arylated tetrahydroindolizine **13** (30–40%). Saponification of the ester moiety within the latter compound was achieved using ethanolic potassium hydroxide, and the acid salt thus formed was acidified with mineral acid. The resulting amino acid was subjected to reaction with the salt derived from 2-chloro-4,6-dimethoxy-1,3,5-triazine and *N*-methylmorpholine,[18] and in this manner the target compound **3**§ (50%, no mp, decomposition above 150 °C) was obtained. Single crystal X-ray analysis (Fig. 1)¶ of this material revealed that, *inter alia*, the amide unit adopts an s-*cis* conformation and the dihedral angle between the planes of the two aromatic rings is *ca*. 56° (which contrasts with an angle of 96° reported[19] for rhazinilam itself).

(±)-B-Norrhazinal (**3**) was evaluated for its cytotoxic effects on the growth of human CA46 Burkitt lymphoma cells, and an IC_{50} value of 3 μM was observed. Considering that compound **3** was tested as a racemic mixture and that (+)-rhazinilam is inactive[10] then this is almost identical to the values (0.6–2 μM) reported for (−)-rhazinilam in a variety of cell lines.[8,10,12] Compound **3** was also examined for its effects on the polymerisation of purified tubulin dependent either on glutamate or on microtubule-associated proteins. Results similar to those described[8] for (−)-rhazinilam were obtained but we had no (−)-rhazinilam with which to perform a quantitative comparison. The foregoing results suggest that B-norrhazinilam derivatives warrant further evaluation as potential anti-mitotic agents.

† The work described herein is the subject of a patent application (AIPO Patent Office Provisional Application No. PQ5495/00, February 8th 2000).

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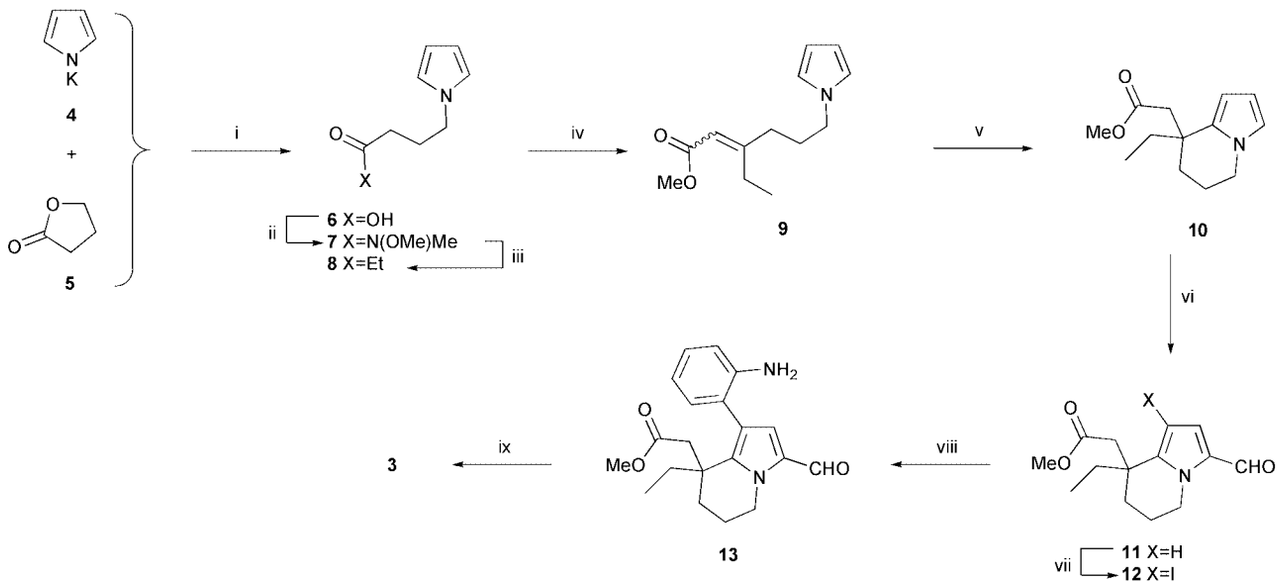

Scheme 1 *Reagents and conditions*: (i) 160 °C, 2 h; (ii) $H_3CNHOCH_3$·HCl (1.2 mol equiv.), Et_3N (1.2 mol equiv.), bis(*N*-oxido-2-pyridyl) disulfide (1.5 mol equiv.), Bu_3P (1.5 mol equiv.), CH_2Cl_2, 18 °C, 16 h; (iii) (a) EtMgBr (3 mol equiv.), Et_2O, 18 °C, 1 h then (b) 0.3 M aq. $KHSO_4$ (excess), −40 °C, 0.1 h then (c) $NaHCO_3$ (excess), −40 °C to 18 °C; (iv) NaH (2 mol equiv.), $(EtO)_2POCH_2CO_2CH_3$ (2 mol equiv.), THF, 18 °C, 48 h; (v) $AlCl_3$ (5 mol equiv.), Et_2O, 18 °C, 5 h; (vi) $POCl_3$ (1.1 mol equiv.), 1 : 33 v/v DMF–Et_2O, 18 °C, 3 h; (vii) I_2 (1.2 mol equiv.), $AgOCOCF_3$ (1.2 mol equiv.), $CHCl_3$, 18 °C, 4 h; (viii) 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (1.5 mol equiv.), $Pd(PPh_3)_4$ (5 mol%), toluene, 2 M aq. Na_2CO_3 (excess), H_3COH, 80 °C, 1.5 h; (ix) (a) KOH (excess), EtOH, 18 °C, 48 h then (b) 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (excess), THF, 18 °C, 2 h.

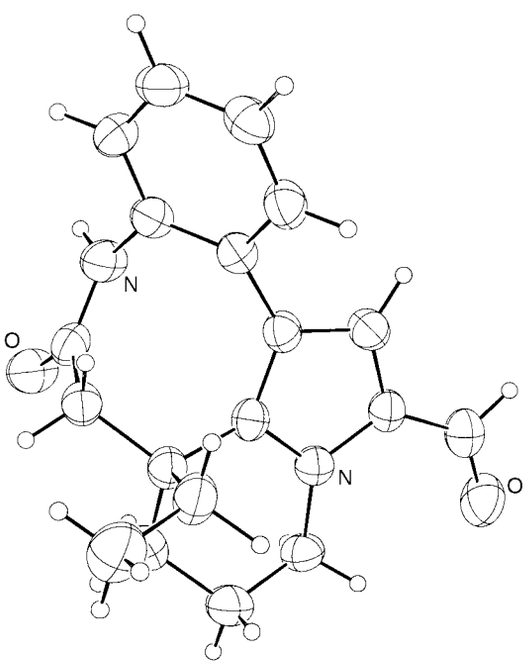

Fig. 1 An ORTEP diagram (with 50% probability ellipsoids) of compound **3** derived from X-ray crystallographic data.

Experimental

Methyl 2-(8-ethyl-5,6,7,8-tetrahydroindolizin-8-yl)acetate (10)

Aluminium chloride (815 mg, 6.1 mmol) was added in small portions to a chilled (ice-bath) and magnetically stirred solution of the α,β-unsaturated ester **9** (270 mg, 1.22 mmol) in diethyl ether (20 cm^3). The reaction mixture was stirred at 18 °C for 5 h then cooled and treated with water (15 cm^3) followed by sulfuric acid (20 cm^3 of a 0.5 M aqueous solution). The separated aqueous phase was extracted with diethyl ether (1 × 25 cm^3) and the combined organic phases were then dried ($MgSO_4$), filtered and concentrated under reduced pressure to give compound **10** (223 mg, 83%) as a light-yellow oil [Found: $M^{+\bullet}$, 221.1413. $C_{13}H_{19}NO_2$ requires $M^{+\bullet}$, 221.1415]. ν_{max}(neat) 2942, 1732, 706 cm^{-1}; ^{1}H NMR (300 MHz, CD_3COCD_3) δ 0.93 (3H, t, J = 7.5 Hz), 1.81–1.96 (3H, m), 2.40–3.20 (3H, complex m), 2.67 (2H, d, J = 3.5 Hz), 3.66 (3H, s), 3.99 (2H, m), 5.90 (1H, q, J = 1.65 Hz), 6.06 (1H, t, J = 3.25 Hz), 6.58 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.0, 20.1, 30.5, 33.0, 37.6, 44.7, 45.3, 51.3, 103.9, 107.3, 110.8, 118.7, 172.1; *m/z* 221 (43%, $M^{+\bullet}$), 192 (70), 148 (100), 132 (82), 118 (17).

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Notes and references

‡ All new and stable compounds had spectroscopic data [IR, UV, NMR, mass spectrum] consistent with the assigned structure. Satisfactory combustion and/or high-resolution mass spectral analytical data were obtained for new compounds and/or suitable derivatives.

§ *Selected spectral data for* **3**: ^{1}H NMR (300 MHz, $CDCl_3$) 0.68 (3H, t, J = 7.5 Hz), 1.44–1.68 (3H, m), 1.87 (1H, dt, J = 13.5 and 3.5 Hz), 1.94–2.03 (1H, m), 2.15 (1H, d, J = 11.5 Hz), 2.40 (1H, td, J = 13.5 and 3.6 Hz), 2.79 (1H, d, J = 12.4 Hz), 4.08 (1H, m), 4.83 (1H, dd, J = 14.3 and 6.0 Hz), 6.71 (1H, s), 7.10–7.16 (1H, m), 7.17 (1H, br s), 7.28–7.42 (3H, m), 9.46 (1H, s); *m/z* 308 (56%, $M^{+\bullet}$), 279 (75), 251 (42), 237 (100).

¶ *Crystal data for* **3**: $C_{19}H_{20}N_2O_2$, M = 308.38, T = 200(1) K, monoclinic, space group $P2_1/n$, Z = 4, a = 10.040(1), b = 12.331(1), c = 12.675(1) Å, β = 99.651(7)°, U = 1547.0(3) Å^3, D_c = 1.324 g cm^{-3}, $F(000)$ = 656, μ(Mo-Kα) = 0.087 mm^{-1}, 3045 unique data ($2\theta_{max}$ = 27.5°), 1610 with $I > 3\sigma(I)$, R = 0.048, R_w = 0.051, S = 1.15. Images were measured on a Nonius Kappa CCD diffractometer (Mo-Kα, graphite monochromator, λ = 0.71073 Å) and data extracted using the DENZO package.[20] Structure solution was by direct methods (SIR92)[21] and refinement was by full matrix least-squares on F using the maXus program package.[22] Atomic coordinates, bond lengths and angles, and displacement parameters have been deposited at the Cambridge Crystallographic Data Centre. CCDC reference number 207/415. See http://www.rsc.org/suppdata/p1/b0/b001731k/ for crystallographic files in .cif format.

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