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Reduced Benzimidazo[2,1-*a*]isoquinolines. Synthesis and Cytotoxicity Studies

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6-Butyl-5,6-dihydrobenzimidazo[2,1-*a*]isoquinoline, 3,9- and 3,10-dimethoxy, and 3,9- and 3,10-dihydroxy analogues, and their 12-methyl quaternary salts were prepared by a multistep route. Cytotoxicities against 55 human cancer cell lines were measured in the National Cancer Institute screen. The quaternary salts of the dimethoxy compounds (15b/c) were clearly the most active overall, with a mean graph midpoint (MGM) value of 2 μm.

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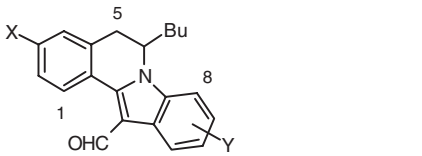
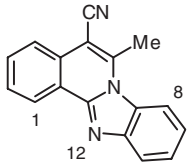
Introduction

Two papers have contained reports of reduced indolo[2,1-*a*]isoquinolines as inhibitors of human mammary carcinoma cells.[1,2] The compounds have form (1). The cyctotoxicity of (1) bearing different substituents at the 12-position was investigated. A formyl group (shown) was found to impart the greatest activity. In addition, hydroxy or methoxy groups at 3,9- or 3,10-positions aided activity, as in examples (1a–d).

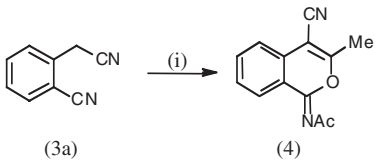




(1a) X = 9-Y = OH; (1b) X = 10-Y = OH
(1c) X = 9-Y = OMe; (1d) X = 10-Y = OMe

(2)

We have previously reported a convenient synthesis of the benzimidazo[2,1-*a*]isoquinoline system (2), by reaction of (4) (readily prepared from α-cyano-*o*-tolunitrile (3a), Scheme 1) with *o*-phenylenediamine.[3,4] There is a recent report of an alternative route to this system.[5]



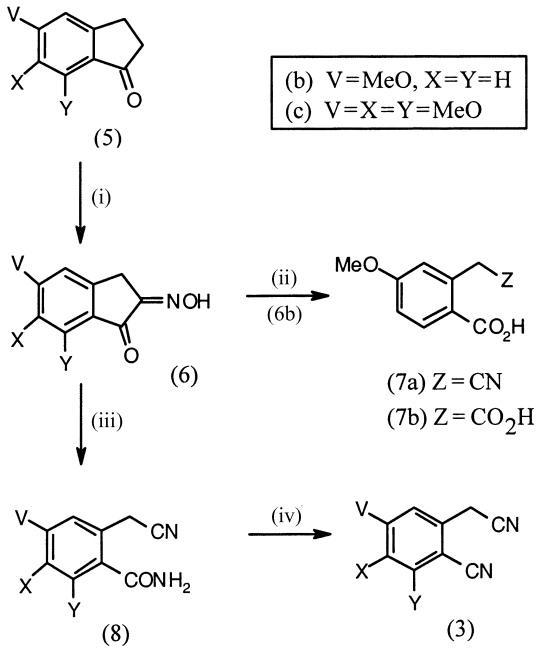
(3a) (4)

Scheme 1. Reagents and conditions: (i) Ac_2O/NaOAc/reflux 2 h.

The key difference between (1) and (2) is the presence in the latter of an additional aza group at the apparently important 12-position.[2] It seemed that our synthesis could be adapted to produce derivatives of (2) which contained the 6-butyl substituent and saturated 5,6-bond of (1). This paper reports the preparation and cytotoxic evaluation against human cancer cell lines of such a series.

Results and Discussion

The parent dinitrile (3a) is commercially available, but a new route was devised to incorporate an oxygen substituent onto the benzene ring to give (3b) and (3c) (Scheme 2).



Scheme 2. Reagents and conditions: (i) *i*-amylnitrite/conc. HCl; (ii) TsCl/10%NaOH/reflux 1 h; (iii) NH_3/PPE; (iv) $SOCl_2$/DMF.

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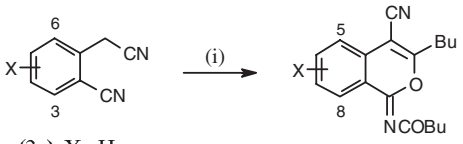
Indan-5-ol was converted by known procedures into the indanone (5b),[6] and derivative (6b).[7] It was reported that the reaction of the 5,6-dimethoxy analogue of (6b) with tosyl chloride in 10% sodium hydroxide gave the corresponding monoacid (7a),[8] but in our hands this treatment of (6b) resulted in formation of the diacid (7b). Ammonia in ethyl polyphosphate (polyphosphate ester, PPE) is recorded as a useful way of converting acids directly into nitriles,[9] but only a low yield of (7a) was obtained from (7b). However, when this process was applied to the precursor (6b), a useful reaction occurred to give (8b) in 56% yield. The remaining amide group was stable in these conditions, and attempted dehydration of (8b) with fresh PPE[10] was not very satisfactory. However, reaction with thionyl choride in dimethylformamide (DMF)[11] gave the target (3b) in a clean reaction (83% yield).

The same sequence of reactions from the trimethoxy indanone (5c) gave (3c) in 42% overall yield, and so it appears to have some general applicability for the preparation of ring-substituted dinitriles of type (3). The acylation of (3) was based on a previously reported acetylation,[3] but used valeric anhydride and sodium valerate (Scheme 3). The use of sodium acetate resulted in the product being contaminated with (4). While the reaction was general, the isolation of each analogue was different. After the residual anhydride was removed by evaporation, the parent (9a) (obtained in 46% yield) was distilled directly from the residue at low pressure, but the boiling points of the others were too high for this method of isolation to be employed. Hot light petroleum (boiling point 90–110°C) extraction gave (9b) in 59% yield, while a black oil separated along with solid (9c) on similar treatment. These could be separated physically, and chromatography of the oil gave a further crop of (9c) (52% total yield).

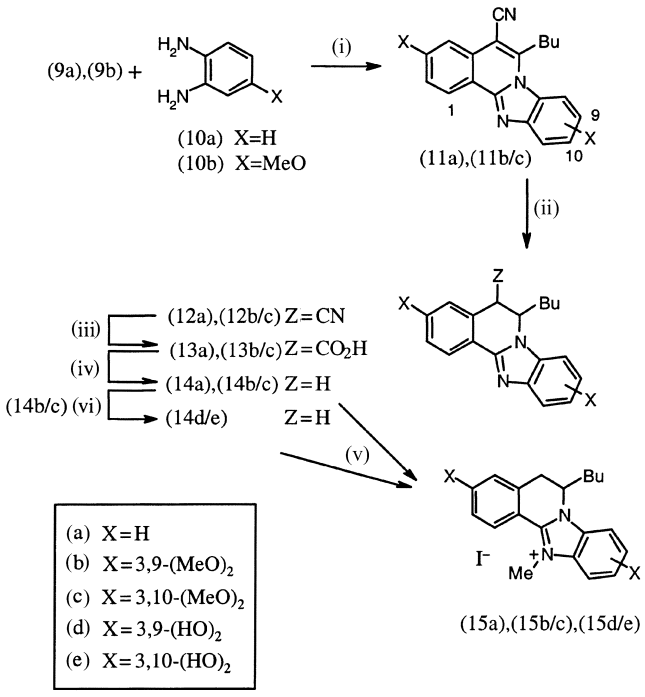


(3a) X=H
(3b) X=5-MeO
(3c) X=3,4,5-$(MeO)_3$

(9a) X=H
(9b) X=6-MeO
(9c) X=6,7,8-$(MeO)_3$

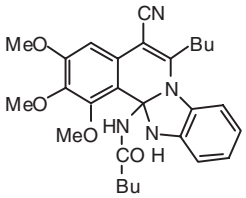
Scheme 3. Reagents and conditions: (i) $(BuCO)_2O/BuCO_2^-$/160°C/2 h.

Reaction of (9) with the *o*-phenylenediamines (10) (see Scheme 4) was carried out under previously devised basic conditions[4] to give the tetracyclic compounds (11a) and (11b/c) in 72 and 41% yields, respectively. Both 9- and 10-methoxy isomers were formed from (10b), and cross-peaks between H8 (a clear doublet in (11c)) and the butyl α-CH_2 protons in a ^{1}H nuclear magnetic resonance (NMR) nuclear Overhauser enhancement spectroscopy (NOESY) spectrum established that the major isomer was the 10-methoxy compound (11c) (ca. 3 : 1, changed to 4 : 1 after recrystallization).



Scheme 4. Reagents and conditions: (i) NEt_3/dioxan/reflux; (ii) $NaBH_4$/EtOH; (iii) 57%H_2SO_4/reflux; (iv) heat 165-180°C; (v) MeI/EtOAc/reflux; (vi) HBr–HOAc/reflux.

The trimethoxy dinitrile (9c) did not react with *o*-phenylenediamine in the same way. We have previously established that there are two competing pathways in the coupling reaction.[3] It now appears that a methoxy group *ortho* to the nitrile group favors the second route. The product isolated was not purified completely, but was assigned structure (16) from an analysis of its spectroscopic data.



(16)

Compounds (11a–c) were readily reduced with sodium borohydride in ethanol to give (12a–c) as a mixture of diastereomers. For (12a) (isolated in 75% yield) there were two compounds present in a ratio of ca. 2 : 1, evident from the H5–H6 coupling patterns observed in the ^{1}H NMR NOESY experiment. The major spectroscopic difference between the two isomers was in the chemical shift and coupling constant for H5 (δ 4.22, $J_{5,6}$ 1 Hz for the major isomer; δ 4.72, $J_{5,6}$ 5 Hz for the other); partial separation of these was achieved by thin-layer chromatography (TLC) on neutral alumina. For (12b/c) (obtained in 85% yield), four diastereomers arose from the 9- and 10-methoxy mixture in the precursor (11b/c)

and this mixture was taken to the next step without further treatment.

Hydrolysis of the nitrile group of (12a–c) to give (13a–c) was achieved in acidic conditions. Interestingly, (13a) appeared to be a single diastereomer, with no coupling observed between H5 and H6 in the ^{1}H NMR spectrum, while the ^{1}H NMR spectrum of the (13b/c) mix (obtained in 50% yield) was too complex to allow the composition to be determined. Facile decarboxylation of compounds (13a–c) was achieved by careful heating of these solids to give the targeted reduced tetracycles (14a) and (14b/c) in 81 and 75% yield, respectively, as oils which slowly solidified. For (14b/c), preparative TLC gave samples of substantially separated 9- and 10-methoxy isomers in milligram quantity, but the isomer mix [(14b)/(14c) 1 : 2) was used for the biological testing and further chemistry. Methylation of (14a–c) in ethyl acetate gave the insoluble methyl iodides (15a) and (15b/c) in 66 and 84% yield, respectively. The ratio of (15b) to (15c) was determined by ^{1}H NMR spectroscopy to be 3 : 5. Strangely, the products that separated from the hot ethyl acetate were readily handled solids but all attempts at the recrystallization of (15b/c) gave sticky products. Demethylation of (14b/c) was achieved with a hot mixture of hydrobromic and acetic acids; careful pH adjustment was required to liberate (14d/e) (obtained in 90% yield in a ratio of 1 : 2). The methyl iodides (15d/e) (obtained in 62% yield in a ratio of 1 : 2) were prepared as before, but this mixture could be recrystallized from water.

In vitro cytotoxicities of the benzimidazo[2,1-*a*]isoquinoline derivatives against 55 human cancer cell lines of diverse types were measured in the National Cancer Institute screen. Results were expressed as GI_{50} values (the concentration corresponding to 50% growth inhibition).

The main interest at the outset was in breast cancer. The limited possible direct comparisons with previous results for the 12-formylindolo[2,1-*a*]isoquinolines (1)[1,2] are shown in Table 1. It was noted that cytotoxicity was aided by oxy substituents, with compounds containing hydroxy groups being generally more active than compounds containing methoxy groups, and this activity further increased when the oxy substituents were in the 9-position. Of the present compounds, the combination of methoxy substituent and quaternized N12, as found in compounds (15b/c), provided the most active derivatives, with results comparable to the best obtained for compounds (1a–d).

The level of general cytotoxity is conveniently summarized by the mean graph midpoint (MGM) values (Table 1). The MGM is based on a calculation of the average GI_{50} for all of the cell lines tested in which GI_{50} values below and above the test range (10^{-4} to 10^{-8} M) are taken as the minimum (10^{-8} M) and maximum (10^{-4} M) drug concentrations used in the screening test.[12]

The present compounds were cytotoxic to most cell lines, although five of the six can be classed as only moderately active (MGM 12–19 μM). The exception is the aforementioned mixture (15b/c), which has a considerably lower MGM of 2 μM, and a consistently enhanced activity towards all types of tumour cells. In the present series, introduction of hydroxy (14d/e) or methoxy (14b/c) substituents to the parent (14a) has no significant effect on increasing cytotoxicity. The interesting combination of a methoxy substituent and quaternized N12 provides the most potent compound, and the presence of $H_3C–N^+$ has a comparable effect on activity to that of the C–CHO of (1). In addition, it is possible that only one of the four regio-/stereo-isomer components of (15b/c) is responsible for the bulk of the activity.

Experimental

Microanalyses were performed at the Campbell Microanalytical Laboratory, University of Otago, New Zealand. NMR spectra were obtained at 300 and 400 MHz and are referenced to Me_4Si. NOESY spectra were recorded on a Bruker DRX-400 spectrometer using the pulse program NOESYTP from the Bruker library. Various standard techniques were used to identify proton-bound carbons in ^{13}C NMR spectra. Electrospray mass spectroscopy (ESMS) was performed in positive-ion mode, and spectra were obtained on a VG Bio-Q triple quadrupole mass spectrometer using a water/methanol/acetic acid (50 : 50 : 1) mobile phase.

2-(Cyanomethyl)-4-methoxybenzonitrile (3b)

Thionyl chloride (4 mL) was added to dimethylformamide (20 mL) and the mixture was heated at 60°C for 5 min. Amide (8b) (2.4 g) was added and the mixture was stirred for 2 h at 60°C. The solution was poured onto ice/water (200 mL) and stirred for 1 h. The solid was filtered off to give (3b) as a light brown solid (1.81 g, 83%), m.p. 108°C. ^{1}H NMR ($CDCl_3$) δ 3.88, s, OCH_3; 3.94, s, CH_2; 6.92, dd, *J* 8.6, 2.3 Hz, H5; 7.12, d, *J* 2.3 Hz, H3; 7.60, d, *J* 8.6 Hz, H6. ^{13}C NMR ($CDCl_3$) δ 22.7, CH_2; 55.8, CH_3; 103.6, C; 114.4, CH; 114.8, CH; 115.9, C; 116.9, C; 134.9, CH; 135.6, C; 163.5, C.

2-(Cyanomethyl)-4,5,6-trimethoxybenzonitrile (3c)

This was prepared from (8c), as for (3b), as a light brown solid (89%), m.p. 91–93°C. ^{1}H NMR ($CDCl_3$) δ 3.84, s, OCH_3; 3.88, s, CH_2; 3.95, s, OCH_3; 4.05, s, OCH_3; 6.48, s, H3.

5-Methoxyindan-1,2-dione 2-Oxime (6b)

5-Methoxyindan-1-one (5b)[6] (20 g) was dissolved in hot methanol (80 mL) and freshly prepared isoamyl nitrite (37 g) was added. Concentrated hydrochloric acid (16 mL) was added slowly at 50°C and the solution was maintained at this temperature for a further 30 min during which a solid precipitated. This was filtered off and washed with

Table 1. Cytotoxicities of indolo- and benzimidazo[2,1-*a*]-isoquinoline derivatives

Cell line	Cytotoxicity (GI_{50} in μM)[A]									
	(1a)[B]	(1b)[B]	(1c)[B]	(1d)[B]	(14a)	(15a)	(14b/c)	(15b/c)	(14d/e)	(15d/e)
MCF7	0.22	0.65	—	—	17.3	8.77	17.2	0.37	19.2	19.8
MDAMB231/ATCC	1.4	0.2	8.7	>10	17.3	22.2	13.1	2.63	13.6	33.9
MGM[C]	—	—	—	—	11.7	13.2	12.6	2.0	15.5	18.6

[A] The cytotoxicity GI_{50} values are the concentrations corresponding to 50% overall growth inhibition. [B] Data from ref. 2.
[C] Mean graph midpoint for growth inhibition of all human cancer cell lines successfully tested.

a little cold ethanol to give (6b) as a cream solid (20 g, 85%), m.p. 206°C. ^{1}H NMR {(D_6)dimethyl sulfoxide [(D_6)DMSO]} δ 3.71, s, CH_2; 3.88, s, OCH_3; 7.01, dd, *J* 8.6, 2.0 Hz, H6; 7.14, d, *J* 2.0 Hz, H4; 7.68, d, *J* 8.6 Hz, H7; 12.44, s, OH.

5,6,7-Trimethoxyindan-1,2-dione 2-Oxime (6c)

This was prepared from 5,6,7-trimethoxyindan-1-one (5c),[13] as for (6b), as a cream solid (73%), m.p. 193–194°C. ^{1}H NMR [(D_6)DMSO] δ 3.63, s, CH_2; 3.69, s, OCH_3; 3.91, s, 2×OCH_3; 6.98, s, H4; 12.35, s, OH.

4-Methoxyhomophthalic Acid (7b)

Toluene-*p*-sulfonyl chloride (8.2 g) was added in small portions to a boiling solution of oxime (6b) (5.0 g) in 10% sodium hydroxide (60 mL) and the solution was heated under reflux for 1 h. The cooled solution was acidified and the precipitate was collected by filtration to give a mixture (2 : 1) of starting material and product (5.2 g). This was treated again with toluene-*p*-sulfonyl chloride and sodium hydroxide to give (7b) as a cream solid (5.1 g, 93%), m.p. 217°C. ^{1}H NMR [(D_6)DMSO] δ 3.79, s, OCH_3; 3.90, s, CH_2; 6.89–6.92, m, 2H; 7.88, d, *J* 8.9 Hz, H6. ^{13}C NMR [(D_6)DMSO] δ 40.2, CH_3; 55.4, CH_2; 111.9, CH; 118.0, CH; 122.4, C; 132.8, CH; 139.3, C; 161.8, C; 167.7, C; 172.3, C. Mass spectrum (ESMS) *m/z* 211 (M + 1).

2-(Cyanomethyl)-4-methoxybenzamide (8b)

A mixture of oxime (6b) (5.0 g), ethyl polyphosphate (PPE)[14] (20 g) and chloroform (75 mL, stored over phosphorus pentoxide) was stirred using a mechanical stirrer at 0°C for 30 min under an atmosphere of ammonia, and then at room temperature for 30 min. 25% Sodium carbonate solution (150 mL) was added, the mixture was extracted many times with chloroform and the combined organic extracts were washed five times with water, dried and the solvent was removed at reduced pressure to give the product as a brown solid (2.78 g, 56%), m.p. 122–124°C. ^{1}H NMR ($CDCl_3$) δ 3.85, s, OCH_3; 4.15, s, CH_2; 5.86, br s, NH_2; 6.84, dd, *J* 8.5, 2.2 Hz, H5; 7.06, d, *J* 2.2 Hz, H3; 7.51, d, *J* 8.5 Hz, H6. ^{13}C NMR ($CDCl_3$) δ 41.4, CH_2; 55.6, OCH_3; 104.3, C; 114.0, CH; 116.2, CH; 118.4, C; 134.4, CH; 140.6, C; 163.1, C; 170.8, C. Mass spectrum (ESMS) *m/z* 191 (M + 1).

2-(Cyanomethyl)-4,5,6-trimethoxybenzamide (8c)

This was prepared from (6c), as for (8b), and was obtained as a brown solid (64%), m.p. 109–110°C. ^{1}H NMR ($CDCl_3$) δ 3.84, s, OCH_3; 3.88, s, OCH_3; 3.89, s, OCH_3; 4.04, s, CH_2; 6.18, br s, NH; 6.80–6.85, m, 2H, H6 and NH. ^{13}C NMR ($CDCl_3$) δ 22.5, CH_2; 55.1, OCH_3; 61.0, OCH_3; 62.0, OCH_3; 109.3, CH; 118.2, C; 120.0, C; 126.7, C; 141.7, C; 152.2, C; 155.0, C; 167.6, C.

*3-Butyl-1-valerylimino-1*H*-2-benzopyran-4-carbonitrile (9a)*

A mixture of dinitrile (3a) (2.0 g), valeric anhydride (8 mL) and sodium valerate (1.8 g) was heated at 210°C until all the solids had dissolved, and the solution was quickly cooled to 160°C and stirred for 2 h. The excess anhydride was removed by distillation at reduced pressure and the remaining mixture was distilled in a Kugelrohr apparatus at 175–180°C/0.03 mmHg to give the product as a pale yellow solid (2.0 g, 46%), m.p. 118°C. This was used in further reactions but a small *sample* was recrystallized from light petroleum (b.p. 90–120°C) for microanalysis (Found: C, 73.5; H, 7.3; N, 9.1. $C_{19}H_{22}N_2O_2$ requires C, 73.5; H, 7.1; N, 9.1%). ^{1}H NMR ($CDCl_3$): δ 0.90–0.95, m, 2×CH_3; 1.38–1.42, m, 2×CH_2; 1.64–1.69, m, 2×CH_2; 2.52, t, *J* 7.5 Hz, CH_2; 2.74, t, *J* 7.4 Hz, CH_2; 7.48, t, 1H, *J* 7.5 Hz; 7.56, d, 1H, *J* 7.8 Hz; 7.69, t, 1H, *J* 7.5 Hz; 8.10, d, 1H, *J* 7.8 Hz. ^{13}C NMR ($CDCl_3$) δ 13.6, 2×CH_3; 22.1, CH_2; 22.3, CH_2; 27.5, CH_2; 29.2, CH_2; 33.4, CH_2; 35.5, CH_2; 92.3, C; 114.1, C; 116.7, C; 123.7, CH; 129.4, CH; 130.0, CH; 133.3, C; 136.0, CH; 159.7, C; 170.0, C; 176.1, C.

*3-Butyl-6-methoxy-1-valerylimino-1*H*-2-benzopyran-4-carbonitrile (9b)*

This was prepared from dinitrile (3b), as for (9a). After removal of the excess anhydride, the residue was extracted with hot light petroleum (b.p. 90–120°C), which, on being cooled, deposited the product as a light brown solid (59%), m.p. 92–100°C. ^{1}H NMR ($CDCl_3$) δ 0.90–0.96, m, 2×CH_3; 1.35–1.44, m, 2×CH_2; 1.62–1.72, m, 2×CH_2; 2.51, t, *J* 7.5 Hz, CH_2; 2.74, t, *J* 7.4 Hz, CH_2; 3.92, s, OCH_3; 6.95, d, *J* 2.4 Hz, H5; 7.01, dd, *J* 8.8, 2.4 Hz, H7; 8.02, d, *J* 8.8 Hz, H8. ^{13}C NMR ($CDCl_3$) δ 13.5, CH_3; 13.7, CH_3; 22.0, CH_2; 22.3, CH_2; 26.5, CH_2; 29.6, CH_2; 33.0, CH_2; 39.6, CH_2; 55.8, CH_3; 91.9, C; 106.2, CH; 112.4, C; 114.3, C; 117.4, CH; 130.2, CH; 132.1, C; 145.9, C; 164.4, C; 167.8, C; 185.5, C.

*3-Butyl-6,7,8-trimethoxy-1-valerylimino-1*H*-2-benzopyran-4-carbonitrile (9c)*

This was prepared from dinitrile (3c), as for (9b). The hot light petroleum (b.p. 90–120°C) extract on being cooled gave a mixture of light brown solid and black oil. These were able to be separated and the oil was chromatographed (silica; ethyl acetate/hexane, 1 : 4) to give a further crop of product (total yield, 52%), m.p. 90–92°C. ^{1}H NMR ($CDCl_3$): δ 0.90–0.96, m, 2×CH_3; 1.35–1.44, m, 2×CH_2; 1.62–1.72, m, 2×CH_2; 2.52, t, *J* 7.5 Hz, CH_2; 2.70, t, *J* 7.4 Hz, CH_2; 3.88, s, 2×OCH_3; 4.00, s, OCH_3; 6.80, s, H5.

*6-Butylbenzimidazo[2,1-*a*]isoquinoline-5-carbonitrile (11a)*

A hot solution of (9a) (1.1 g) in dioxan (4 mL) was added to a refluxing solution of *o*-phenylenediamine (0.42 g) in a mixture of dioxan (4 mL) and triethylamine (4 mL). After being heated under reflux for 1 h, the mixture was poured onto ice and acidified with hydrochloric acid and then adjusted back to pH 8 with 10% sodium carbonate. The solid which formed was filtered off, washed with water and recrystallized from ethanol to give the product as white *needles* (0.76 g, 72%), m.p. 187°C (Found: C, 80.1; H, 5.9; N, 14.1. $C_{20}H_{17}N_3$ requires C, 80.2; H, 5.7; N, 14.0%). ^{1}H NMR ($CDCl_3$) δ 1.03, t, *J* 7.3 Hz, CH_3; 1.66, sextet, *J* 7.6 Hz, CH_2; 1.91, quintet, *J* 7.3 Hz, CH_2; 3.61, t, *J* 7.7 Hz, CH_2; 7.43, t, *J* 8.2 Hz, 1H; 7.55, t, *J* 7.4 Hz, 1H; 7.68, t, *J* 7.9 Hz, 1H; 7.76, t, *J* 7.3 Hz, 1H; 7.85, d, *J* 8.4 Hz, 1H; 7.99, d, *J* 7.8 Hz, 1H; 8.00, d, *J* 8.1 Hz, 1H; 8.77, d, *J* 7.7 Hz, 1H. ^{13}C NMR ($CDCl_3$) δ 13.7, CH_3; 22.3, CH_2; 29.7, CH_2; 32.1, CH_2; 95.9, C; 114.1, CH; 116.1, C; 120.6, CH; 121.4, C; 123.4, CH; 124.3, CH; 125.3, CH; 125.7, CH; 128.3, C; 128.8, CH; 130.1, C; 131.3, CH; 144.4, C; 147.4, C; 149.4, C.

*6-Butyl-3,9-dimethoxybenzimidazo[2,1-*a*]isoquinoline-5-carbonitrile (11b) and 6-Butyl-3,10-dimethoxybenzimidazo[2,1-*a*]isoquinoline-5-carbonitrile (11c)*

A 1 : 4 mixture of these compounds was obtained from the reaction of (9b) with (10b), as for (11a), as white needles (41%), m.p. 155–165°C after recrystallization from ethanol.

^{1}H NMR of (11b) ($CDCl_3$) δ 1.01–1.08, m, CH_3; 1.58–1.70, m, CH_2; 1.87–1.95, m, CH_2; 3.56–3.62, m, CH_2;^{A} 3.92, s, OCH_3; 3.98, s, OCH_3; 7.20, dd, *J* 8.8, 2.4 Hz, H10; 7.25–7.29, m, 2H; 7.34–7.36, m, 1H;^{A} 7.86, d, *J* 9.2 Hz, H11; 8.63–8.68, m, H1. Those signals denoted by a superscripted A indicate signals observed as cross-peaks in the NOESY spectrum.

^{1}H NMR of (11c) ($CDCl_3$) δ 1.01–1.08, m, CH_3; 1.58–1.70, m, CH_2; 1.87–1.95, m, CH_2; 3.56–3.62, m, CH_2;^{A} 3.92, s, OCH_3; 3.98, s, OCH_3; 7.01, dd, *J* 9.1, 2.5 Hz, H9; 7.25–7.29, m, 1H; 7.34–7.36, m, 1H; 7.41, d, *J* 2.5 Hz, H11; 7.71, d, *J* 9.2 Hz, H8;^{A} 8.63–8.68, m, H1. Those signals denoted by a superscripted A indicate signals observed as cross-peaks in the NOESY spectrum.

*6-Butyl-5,6-dihydrobenzimidazo[2,1-*a*]isoquinoline-5-carbonitrile (12a)*

Nitrile (11a) (2.0 g) was dissolved in hot ethanol (100 mL), and sodium borohydride (1.2 g) was added. The mixture was heated under reflux and additional sodium borohydride (1.2 g) was added after 30 min. Heating was continued for another 30 min and the mixture was poured onto water, then concentrated at reduced pressure to a fifth of its volume and extracted three times with chloroform. The combined organic extracts were dried and the solvent was removed at reduced pressure to give the crude product as a semi-solid (1.5 g, 75%) which was used without further purification. TLC of a small sample (neutral

alumina; ethyl acetate/hexane, 1 : 3) gave partial separation of diastereomers ($12a_1$), R_F 0.2 and ($12a_2$) (main), R_F 0.3.

^{1}H NMR of ($12a_1$) ($CDCl_3$) δ 0.71, t, *J* 7.1 Hz, CH_3; 1.17–1.28, m, 2×CH_2; 1.59–1.64, m, CH_2; 4.72, d, *J* 5.0 Hz, H5; 4.86–4.93, m, H6; 7.24–7.53, m, 6H; 7.77–7.80, m, 1H; 8.25–8.28, m, 1H.

^{1}H NMR of ($12a_2$) ($CDCl_3$) δ 0.71, t, *J* 7.1 Hz, CH_3; 1.17–1.28, m, 2×CH_2; 1.59–1.64, m, CH_2; 4.22, d, *J* 1.0 Hz, H5; 4.87–4.93, m, H6; 7.30–7.57, m, 6H; 7.82–7.87, m, 1H; 8.37, d, *J* 7.5 Hz, 1H.

*6-Butyl-3,9-dimethoxy-5,6-dihydrobenzimidazo[2,1-*a*]isoquinoline-5-carbonitrile (12b) and 6-Butyl-3,10-dimethoxy-5,6-dihydrobenzimidazo[2,1-*a*]isoquinoline-5-carbonitrile (12c)*

A mixture of these compounds was obtained from nitriles (11b/c), as for (12a), as a semi-solid (85% yield) which was used without further purification.

*6-Butyl-5,6-dihydrobenzimidazo[2,1-*a*]isoquinoline-5-carboxylic Acid (13a)*

A mixture of crude nitrile (12a) (2.1 g) and sulfuric acid (57% by weight, 20 mL) was heated under reflux for 3 h, then poured onto ice/water (100 mL), basified with sodium hydroxide and the mixture was filtered. The filtrate was acidified with concentrated hydrochloric acid and the precipitate which formed was collected by filtration and washed with water to give a single diastereomer of (13a) as a cream *solid* (1.25 g, 56%), m.p. >160°C (decomposed with decarboxylation). It was used in this state in the next step but a small sample was recrystallized from light petroleum (b.p. 90–120°C)/toluene for microanalysis (Found: C, 74.8; H, 6.5; N, 8.6. $C_{20}H_{20}N_2O_2$ requires C, 75.0; H, 6.3; N, 8.7%). ^{1}H NMR ($CDCl_3$) δ 0.76, t, *J* 7.0 Hz, CH_3; 1.18–1.30, m, 2×CH_2; 1.49–1.56, m, CH_2; 4.00, s, H5; 5.27, t, *J* 7.2 Hz, H6; 6.87, t, *J* 7.5 Hz, 1H; 7.10, t, *J* 7.8 Hz, 1H; 7.17–7.22, m, 2H; 7.34–7.43, m, 3H; 7.75, d, *J* 7.7 Hz, 1H. ^{13}C NMR ($CDCl_3$) δ 13.7, CH_3; 22.2, CH_2; 28.2, CH_2; 33.5, CH_2; 49.9, CH; 55.2, CH; 110.2, CH; 117.4, CH; 121.9, C; 123.9, CH; 124.1, CH; 125.8, CH; 128.1, CH; 130.7, CH; 131.9, CH; 132.6, C; 133.5, C; 137.4, C; 145.7, C; 173.4, C. Mass spectrum (ESMS) *m/z* 321 (M + 1).

*6-Butyl-3,9-dimethoxy-5,6-dihydrobenzimidazo[2,1-*a*]isoquinoline-5-carboxylic Acid (13b) and 6-Butyl-3,10-dimethoxy-5,6-dihydrobenzimidazo[2,1-*a*]isoquinoline-5-carboxylic Acid (13c)*

The crude nitrile mixture (12b/c) was hydrolysed as for (13a) to give a mix of (13b/c), as a cream solid (50%), m.p. >170°C (decomposed with decarboxylation). ^{1}H NMR ($CDCl_3$/(D_6)DMSO) δ 0.73–0.78, m, CH_3; 1.19–1.31, m, 2×CH_2; 1.54–1.57, m, CH_2; 3.79–3.90, m, 7H, 2×OCH_3 and H5; 4.98–5.01, m, H6; 6.79–6.94, m, 4H; 7.15–7.25, m, 1H; 8.04–8.08, m, 1H.

*6-Butyl-5,6-dihydrobenzimidazo[2,1-*a*]isoquinoline (14a)*

Acid (13a) (0.1 g) was heated in a covered Petri dish on a thermostatted hotplate at 165°C for 15 min. The distillate on the cover and the residue were extracted three times into hot hexane and the combined organic extracts were evaporated at reduced pressure to give an oil. Column chromatography (silica; ethyl acetate/hexane, 1 : 3) gave the product as a yellow oil (0.07 g, 81%) which slowly set to a *glass* (Found (EIMS): M^+, 276.1632. $C_{19}H_{20}N_2$ requires M^+, 276.1626). ^{1}H NMR ($CDCl_3$) δ 0.80, t, *J* 7.3 Hz, CH_3; 1.18–1.35, m, 2×CH_2; 1.58–1.65, m, CH_2; 3.07, d, *J* 15.8 Hz, Ha5; 3.48, dd, *J* 15.8, 6.4 Hz, Hb5; 4.65, q, *J* 6.4 Hz, H6; 7.24–7.29, m, 3H; 7.34–7.40, m, 3H; 7.80–7.83, m, 1H; 8.27–8.31, m, 1H. ^{13}C NMR ($CDCl_3$) δ 13.8, CH_3; 22.4, CH_2; 28.6, CH_2; 32.7, CH_2; 33.42, CH_2; 51.9, CH; 109.3, CH; 119.6, CH; 122.4, CH; 122.7, CH; 125.6, CH; 126.2, C; 127.6, CH; 128.8, CH; 130.5, CH; 133.1, C; 134.2, C; 143.5, C; 148.1, C.

*6-Butyl-3,9-dimethoxy-5,6-dihydrobenzimidazo[2,1-*a*]isoquinoline (14b) and 6-Butyl-3,10-dimethoxy-5,6-dihydrobenzimidazo[2,1-*a*]isoquinoline (14c)*

The acid mixture (13b/c) (0.15 g) was heated at 180°C/0.03 mmHg for 90 min and then shaken with a mixture of chloroform and 10% sodium carbonate. The organic layer was dried and the solvent was removed at reduced pressure to give an oil. Column chromatography (silica; ethyl acetate/hexane, 1 : 3) gave (14b/c), in a ratio of ca. 1 : 2, as a yellow *oil* (0.10 g, 75%) which slowly solidified (Found (EIMS): M^+, 336.1839. $C_{21}H_{24}N_2O_2$ requires M^+, 336.1838). ^{1}H NMR ($CDCl_3$) δ 0.77–0.83, m, CH_3; 1.20–1.33, m, 2×CH_2; 1.57–1.62, m, CH_2; 3.00, d, *J* 15.8 Hz, Ha5; 3.45, dd, *J* 15.8, 6.4 Hz, Hb5; 3.85–3.86, 2s, 2×OCH_3; 4.56, q, *J* 6.3 Hz, H6; 6.78–6.80, m, 1H; 6.85–6.93, m, 2H; 7.18, d, *J* 8.8 Hz, H8 (14c); 7.27, d, *J* 2.3 Hz, H11 (14c); 7.64, d, *J* 8.8 Hz, H11 (14b); 8.12–8.17, m, H1.

*6-Butyl-3,9-dihydroxy-5,6-dihydrobenzimidazo[2,1-*a*]isoquinoline (14d) and 6-Butyl-3,10-dihydroxy-5,6-dihydrobenzimidazo[2,1-*a*]isoquinoline (14e)*

A mixture of methoxy compounds (14b/c) (0.06 g), 40% aqueous hydrobromic acid (2 mL) and acetic acid (0.5 mL) was heated under reflux for 3 h, then poured onto ice/water (20 mL) and taken to pH >10 with 10% sodium hydroxide. The solution was washed three times with chloroform and the pH was carefully adjusted to 9.4 with 5% hydrochloric acid. The precipitate that formed was collected by filtration to give the product as a brown solid (0.05 g, 90%). Preparative TLC (alumina; ethyl acetate/triethylamine, 20 : 1) gave (14d/e), in a ratio of ca. 1 : 2, as an off-white solid, m.p. 185°C (darkened >130°C). ^{1}H NMR (CD_3OD) δ 0.78–0.84, m, CH_3; 1.23–1.36, m, 2×CH_2; 1.56–1.61, m, CH_2; 3.01, d, *J* 15.9 Hz, Ha5; 3.39, dd, *J* 16.1, 6.7 Hz, Hb5; 4.62–4.70, m, H6; 6.73–6.77, m; 7.00, d, *J* 2.1 Hz, H11 (14e); 7.24, d, *J* 8.7 Hz, H8 (14e); 7.39, d, *J* 8.7 Hz, H11 (14d); 7.84–7.88, m, H1.

*6-Butyl-12-methyl-5,6-dihydrobenzimidazo[2,1-*a*]isoquinolinium Iodide (15a)*

Iodomethane (2 mL) was added to a solution of tetracycle (14a) (0.1 g) in ethyl acetate (5 mL). The mixture was heated at 80°C for 16 h, during which time a solid separated. This was collected by filtration from the cooled mixture to give the product as a pale yellow *solid* (0.1 g, 66%), m.p. 168°C (from propanol/ethyl acetate) (Found: C, 57.6; H, 5.4; N, 6.7. $C_{20}H_{23}IN_2$ requires C, 57.4; H, 5.5; N, 6.7%). ^{1}H NMR ($CDCl_3$) δ 0.70, t, *J* 6.9 Hz, CH_3; 1.10–1.40, m, 2×CH_2; 1.56, q, *J* 7.3 Hz, CH_2; 3.17, d, *J* 16.4 Hz, Ha5; 3.77, dd, *J* 16.4, 6.3 Hz, Hb5; 4.41, s, H_3CN^+; 5.24, q, *J* 6.9 Hz, H6; 7.47, d, *J* 7.1 Hz, 1H; 7.52–7.62, m, 4H; 7.79–7.86, m, 2H; 8.24, d, *J* 7.3 Hz, 1H. ^{13}C NMR ($CDCl_3$) δ 13.5, CH_3; 22.1, CH_2; 28.0, CH_2; 31.5, CH_2; 33.0, CH_2; 35.9, CH_3; 53.5, CH; 112.8, CH; 113.0, CH; 118.5, C; 127.1, CH; 127.2, CH; 127.8, CH; 128.6, CH; 129.9, C; 130.3, CH; 132.7, C; 134.3, CH; 136.0, C; 143.9, C.

*6-Butyl-3,9-dimethoxy-12-methyl-5,6-dihydrobenzimidazo[2,1-*a*]isoquinolinium Iodide (15b) and 6-Butyl-3,10-dimethoxy-12-methyl-5,6-dihydrobenzimidazo[2,1-*a*]isoquinolinium Iodide (15c)*

The tetracycle mixture (14b/c) was reacted with iodomethane, as in the preparation of (15a), and the mixed methiodides (15b/c) (3 : 5) were obtained as a pale yellow *solid* (84%), m.p. 186–188°C (Found: C, 55.3; H, 5.7; N, 5.9. $C_{22}H_{27}IN_2O_2$ requires C, 55.2; H, 5.7; N, 5.9%). ^{1}H NMR [(D_6)DMSO] δ 0.74–0.79, m, CH_3; 1.09–1.44, m, 2×CH_2; 1.52, q, *J* 7.9 Hz, CH_2; 3.23–3.29, m, Ha5; 3.52, dd, *J* 16.1, 6.1 Hz, Hb5; 3.90–3.92, m, OCH_3; 4.22–4.23, 2s, H_3CN^+; 5.12–5.20, m, H6; 7.13–7.17, m, 1H; 7.24–7.27, m, 2H; 7.58, d, *J* 1.8 Hz, H8 (15b); 7.63, d, *J* 1.8 Hz, H11 (15c); 7.91, d, *J* 9.1 Hz, H8 (15c); 7.97, d, *J* 9.1 Hz, H11 (15b); 8.17–8.21, m, H1.

*6-Butyl-3,9-dihydroxy-12-methyl-5,6-dihydrobenzimidazo[2,1-*a*]isoquinolinium Iodide (15d) and 6-Butyl-3,10-dihydroxy-12-methyl-5,6-dihydrobenzimidazo[2,1-*a*]isoquinolinium Iodide (15e)*

Reaction of (14d/e) with iodomethane in ethyl acetate was carried out as in the preparation of (15a). The solvent was removed at reduced pressure and the residue was extracted with hot water. The product separated from the cooled extract as a cream solid (0.09 g, 62%), m.p. 145–150°C. Microanalysis figures were consistent with $C_{20}H_{23}IN_2O_2$

(C/N found: 20.0 : 2) but the sample was evidently a fractionally hydrated species (Found: C, 50.7; H, 4.9; N, 5.9. $C_{20}H_{23}IN_2O_2 \cdot 1.25H_2O$ requires C, 50.8; H, 5.4; N, 5.9%). ^{1}H NMR (CD_3OD) δ 0.81–0.87, m, CH_3; 1.26–1.47, m, 2×CH_2; 1.61–1.68, m, CH_2; 3.18, d, *J* 16.1 Hz, Ha5; 3.50–3.57, m, Hb5; 4.18–4.24, m, H_3CN^+; 4.92–5.04, m, H6; 6.99–7.26, m, 4H; 7.68–7.72, m, 1H; 8.04–8.13, m, 1H.

6-Butyl-1,2,3-trimethoxy-12a-valerylamino-12,12a-dihydrobenzimidazo[2,1-a]isoquinoline-5-carbonitrile (16)

Reaction of (9c) with *o*-phenylenediamine (10a) was carried out as for the preparation of (11a), and the cream solid obtained after workup was ca. 70% pure (16) (61%) from ^{1}H NMR analysis, and had m.p. 136–140°C. Chromatography (silica; ethyl acetate/hexane, 1 : 2) gave an almost pure sample. ^{1}H NMR ($CDCl_3$) δ 0.78, t, *J* 7.3 Hz, CH_3; 0.90, t, *J* 7.2 Hz, CH_3; 1.23–1.41, m, 2×CH_2; 1.51–1.70, m, 2×CH_2; 2.23, t, *J* 7.4 Hz, CH_2; 2.87, t, *J* 7.5 Hz, CH_2; 3.93, s, OCH_3; 4.03, s, OCH_3; 4.12, s, OCH_3; 7.12, s, H4; 7.16–7.31, m, 2H; 7.38, d, *J* 7.6 Hz, 1H; 7.79, d, *J* 8.1 Hz, 1H; 8.38, s, NH; 9.85, s, NH. Mass spectrum (ESMS) *m/z* 491 (M + 1).

In Vitro Growth Delay Assays

In vitro antitumour activities against 55 human tumour cell lines were tested by the U.S. National Cancer Institute (NCI) using an established protocol.[15]

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