Eur J Med Chem (1995) 30, 327–332

Short communication

Benzimidazole condensed ring system. IX. Potential antineoplastics. New synthesis of some pyrido[1,2-*a*]benzimidazoles and related derivatives*

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(Received 12 July 1994; accepted 2 November 1994)

Summary — Some pyrido[1,2-*a*]benzimidazoles were prepared in order to investigate their *in vitro* antineoplastic and anti-HIV activities. Two compounds (**9b**, NSC 658526 and **15**, NSC 664715) showed a variable degree of antineoplastic activity against some of the cell lines tested. Compound **9a** (NSC 649900) exhibited a good *in vitro* antineoplastic activity with subpanel disease selectivity, particularly against most of the cell lines of leukemia and some cell lines from colon, melanoma and renal cancer panels.

2-acetylbutyrolactone / pyrido[1,2-*a*]benzimidazole / antineoplastic activity

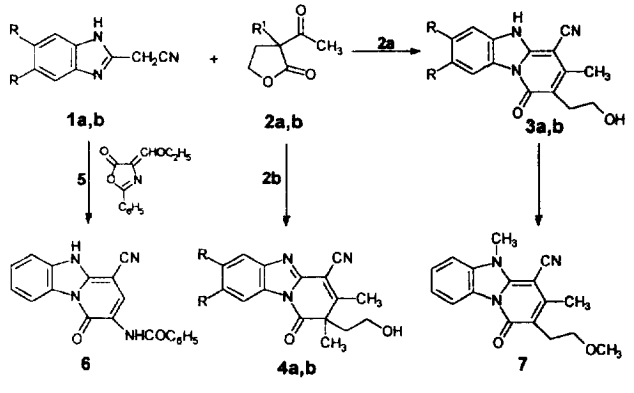
Introduction

Although the synthesis of the pyrido[1,2-*a*]benzimidazole ring system was recognized in 1937 [2], a literature survey indicated that most of its derivatives still have unexplored pharmacotoxicological activities. Few reports concerning the antiviral [3–4], antimicrobial [1, 5], analgesic and antinflammatory [6–8] activities have been recorded. Several years ago we started a project to synthesize and evaluate pyrido[1,2-*a*]-benzimidazoles [9–11] and pyrimido[1,6-*a*]benzimidazoles [12–14] as potential antineoplastic agents. In this report we describe the synthesis of another new series of substituted pyrido[1,2-*a*]benzimidazoles and assess their potential for anticancer and anti-HIV activity.

Chemistry

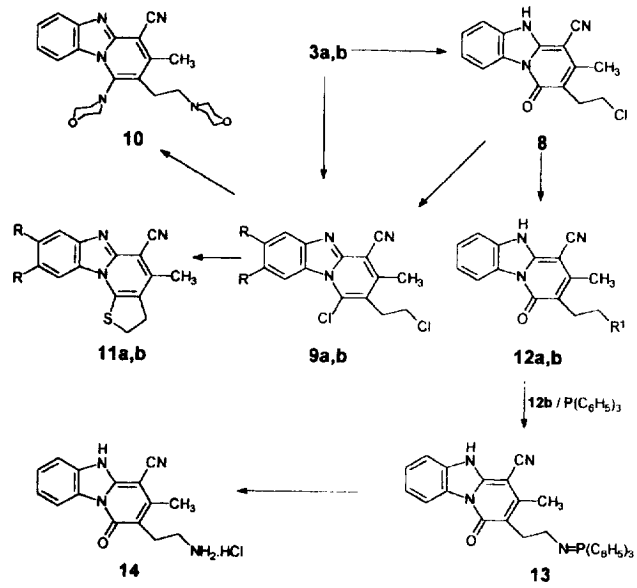
We have previously described a facile one-step synthesis of substituted 1-oxo-1*H*,5*H*-pyrido[1,2-*a*]benzimidazole-4-carbonitriles by fusing 1*H*-benzimidazole-2-acetonitrile (**1a**) with some β-keto esters in the presence of ammonium acetate [10]. We have now extended this cyclocondensation to the synthesis of some substituted 2-(2-hydroxyethyl)-1-oxo-pyrido[1,2-*a*]benzimidazole-4-carbonitriles (**3**, **4**) by reacting **1a,b** with 2-acetylbutyrolactones **2a,b** under similar reaction conditions (scheme 1). The 2-benzamido compound **6** was obtained by reacting **1a** with 4-ethoxymethylene-2-phenyloxazolin-5-one (**5**). Methylation of **3a** with trimethyl phosphate gave compound **7**.

Scheme 2 shows the formation of the mono- and dichloro compounds **8** and **9a,b** by reacting **3a,b** with sulfuryl chloride or phosphorus oxychloride, respectively. Compound **9a** was also obtained from **8** and phosphorus oxychloride. The 2-(morpholino or azido)-ethyl derivatives **12a,b** were obtained by reacting **8**

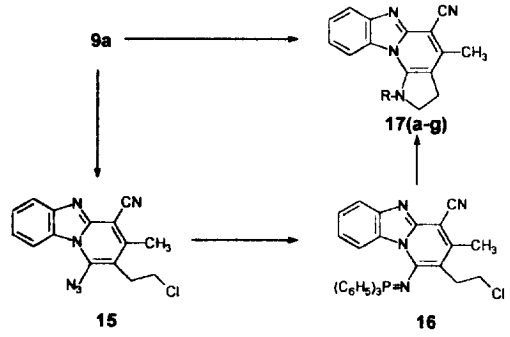


Scheme 1. 1, 3, 4: a: R = H, **b**: R = CH_3; **2a**: R^1 = H, **2b**: R^1 = CH_3.

*For part VIII, see reference [1].



Scheme 2. 3, 9, 11: **a**: R = H, **b**: R = CH_3; **12a**: R^1 = 1-morpholinyl, **12b**: $R^1 = N_3$.



Scheme 3. For R-key see the *Experimental protocols*.

with morpholine or sodium azide, respectively, whereas the dimorpholino compound **10** was obtained by reacting the dichloro derivative **9a** with morpholine. The thieno[2,3-*b*]pyrido[1,2-*a*]benzimidazoles **11a,b** were obtained by reacting **9a,b** with thiourea in refluxing ethanol. Acid hydrolysis of the phosphinimine intermediate **13**, readily available from the parent azido derivative **12b** and triphenylphosphine, resulted in the hydrochloride of the 2-(aminoethyl) compound **14**.

Scheme 3 outlines the chemical transformations of the dichloro compound **9a** to yield the pyrrolo[2,3-*b*]-pyrido[1,2-*a*]benzimidazole-5-carbonitriles **17a–h** *via* two routes. Thus reacting **9a** with sodium azide gave the 1-azido derivative **15** which reacted with triphenylphosphine to give the 1-triphenylphosphoranylideneamino derivative **16**. Acid hydrolysis of the latter compound resulted in the unsubstituted 2,3-dihydro-4-methyl-1*H*-pyrrolo[2,3-*b*]pyrido[1,2-*a*]benzimidazole-5-carbonitrile **17a** (R = H), whereas its substituted derivatives **17b–h** (R = alkyl or aryl) could be obtained by direct reaction of **9a** with the appropriate amine.

Biological investigation and discussion

Antineoplastic activity

Out of the compounds prepared, 17 (table I) were selected by NCI to be screened for their *in vitro* antitumor activity against 60 human cell lines derived from seven clinically isolated cancer types (lung, colon, melanoma, renal, ovarian, brain and leukemia) according to a standard protocol (conducted at the National Cancer Institute, Bethesda, MD, USA). Out of the compounds tested, compound **9a** exhibited a good antineoplastic activity against most of the cell lines of leukemia and some cell lines from colon, melanoma and renal cancers as revealed by its dose–response matrix (fig 1). A similar activity was recorded for **9b**, but with loss of the antileukemic acti-

Table I.

Compound	*NSC*	D_{GI50}[a] (log_{10} conc)	D_{TGI}[a] (log_{10} conc)	$D_{LC_{50}}$[a] (log_{10} conc)	D_H[b] (log_{10} conc)	MDG_H[b]
9a	649900	51.0 (–6.0)	77.0 (–6.0)	47.0 (–6.0)	95.56 (–7.0)	69.70
9b	664715	27.0 (–5.0)	47.0 (–5.0)	48.0 (–5.0)	66.78 (–4.0)	71.97
15	658526	91.0 (–6.0)	14.0 (–6.0)	41.0 (–5.0)	95.45 (–6.0)	95.45

[a,b]Most of the results recorded for these parameters are below 50 and 75, respectively; for the other tested compounds (compound, NSC): (**3a**, 649899); (**4a**, 666275); (**7**, 658525); (**8**, 658524); (**10**, 658530); (**11a**, 658529); (**13**, 658528); (**16**, 658527); (**17a**, 658531); (**17b**, 666580); (**17c**, 666279); (**17d**, 666281); (**17e**, 666277); (**17f**, 666278).

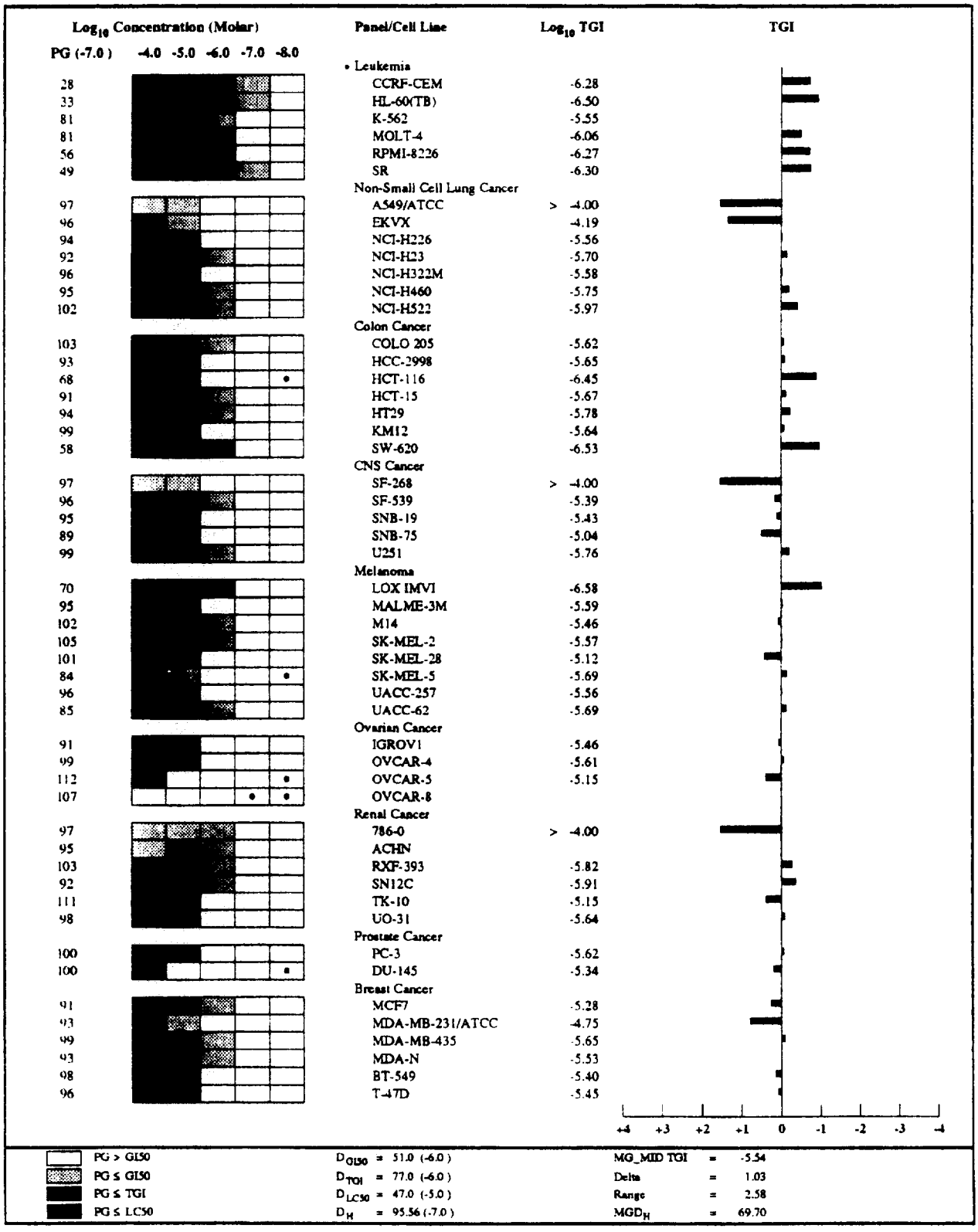


Fig 1. The dose–response matrix from screening of compound **9a** (NSC 649900).

vity. In contrast, compound **15** was only active against the leukemia subpanel. However, only compound **9a** (NSC 649900) was further evaluated for *in vivo* testing because of its marked activity and subpanel disease selectivity as shown from the recorded high values of its subpanel selectivity parameters D_{GI50}, D_{TGI}, $D_{LC_{50}}$, D_H and MGD_H (table I). Computer simulations suggest that a value of D_{GI50}, D_{TGI} or $D_{LC_{50}}$ greater than 50 and values of D_H and MGD_H greater than 75 are statistically significant. The high values of D_{GI50}, D_{TGI} and $D_{LC_{50}}$ recorded for **9a** represent measures of its subpanel selectivity based on the response parameters GI50, TGI and LC_{50}. Moreover the D_H value provides a more general measure of its selective effect and is given primarily as a mean of assigning relative scores of selectivity. In addition, the MGD_H value provides a measure of subpanel selectivity similar to D_H, but is given primarily as a mean of assigning relative scores to the mean graph that represents the sensitivity of the cell line to the test compound in excess of the average sensitivity of all the tested cell lines. The *in vivo* testing indicated that compound **9a** exhibited a weak antineoplastic activity against P388 murine leukemia and lacked the activity against melanoma, SW-620 and HCT-116 colon tumor xenographts.

Anti-HIV activity

The compounds were screened for anti-HIV activity in cultures of CEM cells. Each compound was added in separate cultures at varying concentrations (M) and tested for its ability to counteract the HIV-induced cytopathic effect on CEM cells already infected with HIV. The effect of each compound on cell growth of HIV-infected and uninfected cells was compared with that of untreated uninfected cells. All the compounds failed to counteract the cytopathic effect of HIV on CEM cells, since the cell growth of HIV-infected cells remained between 0–50% of that of uninfected, untreated cultures.

Experimental protocols

Chemistry

Melting points were determined in open-glass capillaries on a Gallenkamp melting point apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer 298 spectrophotometer using samples in potassium bromide discs. The ^{1}H-NMR spectra were recorded on a Varian Gemini 200 at 200 MHz using (DMSO-d_6, unless otherwise stated) with tetramethylsilane (TMS) as the internal standard. ^{13}C-NMR (360 MHz) were performed on a Bruker AM 360 instrument. Microanalyses were performed on a Carlo Erba 1106 analyzer and are within ±0.4% of the theoretical percentages.

*2-(2-Hydroxyethyl)-3-methyl-1-oxo-1H,5H-pyrido[1,2-a]-benzimidazole-4-carbonitrile **3a***
A mixture of 1*H*-benzimidazole-2-acetonitrile **1a** (15.7 g, 100 mmol), 2-acetylbutyrolactone **2a** (10.8 ml, 100 mmol) and ammonium acetate (15.2 g, 200 mmol) was heated in oil bath at 130–140°C for 1 h. During this period, ethanol and ammonia were liberated and the reaction mixture was gradually solidified. After cooling, the product was treated with acetonitrile, filtered and dried; yield: 24.0 g (90%); mp 267°C (DMF). IR ν cm^{-1}: 3500–2500 bm, 2220 s, 1670 s, 1600 m, 1550 s, 1490 w, 1470 m. ^{1}H-NMR: δ 2.4 (s, CH_3), 2.65 (t, CH_2CH_2OH), 3.5 (m, CH_2CH_2OH), 4.65 (bs, O*H*), 7.35 (m, 2 Ar*H*), 7.5 (d, 1 Ar*H* at C-6), 8.6 (d, 1 Ar*H* at C-9). MS *m/z* 267.2, 236 (base peak M/E), 208.4, 182.2, 156.1, 118.4, 92.2, 65.2. Anal $C_{15}H_{13}N_3O_2$ (C, H, N).

*2-(2-Hydroxyethyl)-1-oxo-3,7,8-trimethyl-1H,5H-pyrido[1,2-a]-benzimidazole-4-carbonitrile **3b***
This was prepared from 5,6-dimethyl-1*H*-benzimidazole-2-acetonitrile **1b** (18.5 g, 100 mmol), **2a** (10.8 ml, 100 mmol) and ammonium acetate (15.2 g, 200 mmol) as described for **3a**; yield: 18.0 g (61%); mp 350°C (DMF). IR ν cm^{-1}: 3500–2500 bm, 2205 s, 1660 s, 1610 m, 1590 m, 1540. ^{1}H-NMR: δ 2.35 (2 s, 2 CH_3 at C-7 and C-8), 2.4 (s, CH_3 at C-3), 2.7 (t, CH_2CH_2OH), 3.6 (m, CH_2CH_2OH), 4.6 (bs, 1H, O*H*), 7.25 (s, 1 Ar*H* at C-6), 8.35 (s, 1 Ar*H* at C-9). Anal $C_{17}H_{17}N_3O_2$ (C, H, N).

*1,2-Dihydro-2,3-dimethyl-2-(2-hydroxyethyl)-1-oxopyrido[1,2-a]benzimidazole-4-carbonitrile **4a***
This was prepared from **1a** (1.57 g, 10 mmol), 2-methyl-2-acetylbutyrolactone **2b** (1.23 ml, 10 mmol) and ammonium acetate (1.52 g, 20 mmol) as described for **3a**; yield: 1.5 g (53.3%); mp 220–22°C (ethanol). IR ν cm^{-1}: 3500–3000 s, 2215 s, 1730 s, 1620 m, 1530 s. ^{1}H-NMR: δ 1.6 (s, CH_3 at C-3), 2.2 (t, CH_2CH_2OH), 2.4 (s, CH_3 at C-2), 3.2 (m, CH_2CH_2OH), 4.5 (t, O*H*), 7.5 (m, 2 Ar*H*), 7.7 (d, 1 Ar*H* at C-6), 8.2 (d, 1 Ar*H* at C-9). Anal $C_{16}H_{15}N_3O_2$ (C, H, N).

*1,2-Dihydro-2-(2-hydroxyethyl)-1-oxo-2,3,7,8-tetramethyl-pyrido[1,2-a]benzimidazole-4-carbonitrile **4b***
This was prepared from **1b** (1.85 g, 10 mmol), **2b** (1.23 ml, 10 mmol) and ammonium acetate (1.52 g, 20 mmol) as described for **3a**; yield: 1.15 g (37.2%); mp 240–42°C (DMF). IR ν cm^{-1}: 3500–2500 bm, 2210 s, 1720 s, 1630 m, 1540 w; ^{1}H-NMR: δ 1.55 (s, CH_3 at C-3), 2.2 (t, CH_2CH_2OH) 2.4 (s, CH_3 at C-2 and 2 CH_3 at C-7 and C-8), 3.2 (m, CH_2CH_2OH), 4.5 (t, O*H*), 7.6 (s, 1 Ar*H*), 7.9 (s, 1 Ar*H*). Anal $C_{18}H_{19}N_3O_2$ (C H, N).

*2-Benzamido-1-oxo-1H,5H-pyrido[1,2-a]benzimidazole-4-carbonitrile **6***
The title compound was prepared by refluxing a mixture of **1a** (1.57 g, 10 mmol) and 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (**5**) (2.17 g, 10 mmol) in bromobenzene (20 ml) for 1 h during which time the product separated out; yield: 2.5 g (76.2%); mp 348°C (DMF). IR ν cm^{-1}: 3400–2500 bm, 2210 s, 1665 s, 1665 s, 1630 m, 1600 w, 1530 m. ^{1}H-NMR: δ 7.4–7.7 (m, 6 Ar*H*), 8.05 (d, 2 Ar*H*), 8.4 (s, 1 Ar*H* at C-3), 8.65 (d, 1 Ar*H* at C-9), 9.6 (s, 1 NH). Anal $C_{19}H_{12}N_4O_2$ (C, H, N).

*3,5-Dimethyl-2-(2-methoxyethyl)-1-oxo-1H,5H-pyrido[1,2-a]-benzimidazole-4-carbonitrile **7***
Compound **3a** (1.07 g, 4 mmol) was refluxed with trimethyl phosphate (15 ml) for 1 h in the presence of anhydrous sodium carbonate (0.2 g). After cooling and addition of water, the product was filtered and dried; yield: 1.0 g (85%); mp 195–197°C (DMF). IR ν cm^{-1}: 3000–2800 w, 2200 s, 1650 s, 1600 m, 1550 m, 1480 m. ^{1}H-NMR: δ 2.4 (s, CH_3 at C-3), 2.85 (t, CH_2), 3.2 (s, NCH_3), 3.4 (t,CH_2O), 4.1 (s, OCH_3),7.4 (t, 1 Ar*H*), 7.6 (t, 1 Ar*H*), 7.8 (d, 1 Ar*H* at C-6), 8.7 (d, 1 Ar*H* at C-9). Anal $C_{17}H_{17}N_3O_2$ (C, H, N).

2-(2-Chloroethyl)-3-methyl-1-oxo-1H,5H-pyrido[1,2-a]benzimidazole-4-carbonitrile 8
To a stirred suspension of **3a** (5.4 g, 20 mmol) in benzene (150 ml), thionyl chloride (2.4 ml, 21 mmol) was gradually added and the reaction mixture was refluxed for 2 h. After cooling the product was filtered, washed with benzene, dried and recrystallized from large volume of ethanol; yield: 5.0 g (87.5%); mp 350°C. IR ν cm^{-1}: 3300–2800 bm, 2210 s, 1660 s, 1600 m, 1550 m, 1470 w; ^{1}H-NMR: δ 2.4 (s, CH_3), 3.1 (t, CH_2), 3.8 (t, CH_2Cl), 7.4 (m, 2 Ar*H*), 7.6 (d, 1 Ar*H* at C-6), 8.6 (d, 1 Ar*H* at C-9). Anal $C_{15}H_{12}ClN_3O$ (C, H, N).

1-Chloro-2-(2-chloroethyl)-3-methylpyrido[1,2a]benzimidazole-4-carbonitrile 9a
Compound **3a** or **8** (25 mmol) was refluxed with phosphorus oxychloride (60 ml) for 2–3 h. The excess phosphorus oxychloride was removed under vacuum and the residue was treated with ice-water, neutralized with sodium carbonate. The product was filtered, washed with water and dried; yield: 92%; mp 209°C (DMF). IR ν cm^{-1}: 2220 s, 1630 m, 1600 s, 1470 s, 1450 m. ^{1}H-NMR: δ 2.7 (s, CH_3), 3.4 (t, CH_2), 3.8 (t, CH_2Cl), 7.4 (t, 1 Ar*H*), 7.6 (t, 1 Ar*H*), 7.9 (d, 1 Ar*H* at C-6), 8.7 (d, 1 Ar*H* at C-9). ^{13}C-NMR: δ 19.2 (CH_3), 31.5 (CH_2), 42.0 (CH_2Cl), 98.5, 114.2, 116.0, 118.2, 119.5, 122.0, 126.5, 129.5, 134, 144.0, 146.0, 149.5.0 (11 ArC+*C*N). Anal $C_{15}H_{11}Cl_2N_3$ (C, H, N).

1-Chloro-2-(2-chloroethyl)-3,7,8-trimethylpyrido[1,2-a]benzimidazole-4-carbonitrile 9b
This was similarly prepared from **3b** (2.95 g, 10 mmol); yield: 3.0 g (90.3%); mp 235–236°C (DMF). IR ν cm^{-1}: 2215 s, 1640 w, 1600 m, 1460 s. ^{1}H-NMR: δ 2.4 (2 s, 2 CH_3 *at* C-7 and C-8), 2.7 (s, CH_3 at C-3), 3.3 (t, CH_2), 3.9 (t, CH_2Cl), 7.7 (s, 1 Ar*H* at C-6), 8.4 (s, 1 Ar*H* at C-9). Anal $C_{17}H_{15}Cl_2N_3$ (C, H, Cl, N).

1-Morpholino-2-(2-morpholinoethyl)-3,7,8-trimethylpyrido-[1,2-a]benzimidazole-4-carbonitrile 10
This was prepared by stirring a solution of **9a** (1.22 g, 4 mmol) and morpholine (0.87 ml, 10 mmol) in DMF for 1 h at 60°C. After cooling, the product was filtered, washed with ethanol and dried, yield: 1.2 g (74%), mp: 289–290°C (DMF); IR ν cm^{-1}: 3000–2700 bm, 2220 s, 1630 w, 1600 m, 1500 s, 1445 s. ^{1}H-NMR (360 MHz): δ 2.4 (s, CH_3), 2.9 (t, CH_2CH_2- of ethyl at C-2), 3.2 (t, CH_2NCH_2 of morpholino at C-2), 3.4 (t, CH_2CH_2 of ethyl at C-2), 3.6 (t, CH_2NCH_2 of morpholino at C-1), 3.8 (t, CH_2OCH_2 of morpholino at C-2), 4.0 (t, CH_2OCH_2 of morpholino at C-1), 7.4 (t, 1 Ar*H*), 7.5 (t, 1 Ar*H*), 7.8 (d, 1 Ar*H* at C-6), 8.8 (d, 1 Ar*H* at C-9). Anal $C_{23}H_{27}N_5O_2$ (C, H, N).

2,3-Dihydro-4-methylthieno[2,3-b]pyrido[1,2-a]benzimidazole-5-carbonitrile 11a
A stirred solution of **9a** (1.22 g, 4 mmol) and thiourea (0.38 g, 5 mmol) was refluxed in DMF (15 ml) for 30 min. After cooling, the yellow crystalline product was filtered, washed with ethanol and dried; yield: 1.0 g (94%); mp 250°C (DMF). IR ν cm^{-1}: 2200 s, 1610 w, 1580 m, 1520 w, 1450 s, 1400 w. ^{1}H-NMR: δ 2.6 (s, CH_3), 3.4 (t, CH_2), 3.9 (t, CH_2S), 7.4 (t, 1 Ar*H*), 7.6 (t, 1 Ar*H*), 7.9 (d, 1 Ar*H*), 8.2 (d, 1, Ar*H*). Anal $C_{15}H_{11}N_3S$ (C, H, N).

2,3-Dihydro-4,8,9-trimethylthieno[2,3-b]pyrido[1,2-a]benzimidazole-5-carbonitrile 11b
This was prepared in a similar way as described for **11a**, from **9b** (1.33 g, 4 mmol) and thiourea (0.38 g, 5 mmol); yield: 1.0 g (85.2%); mp: 309–310°C (DMF). IR ν cm^{-1}: 2900 w, 2210 s, 1600 s, 1530 m, 1470 s, 1410 w. Anal $C_{17}H_{15}N_3S$ (C, H, N).

2-(2-Morpholinoethyl)-3-methyl-1-oxo-1H,5H-pyrido[1,2-a]benzimidazole-4-carbonitrile 12a
This was prepared by refluxing **8** (1.14 g, 4 mmol) with morpholine (0.44 ml, 5 mmol) in absolue ethanol (20 ml) for 3 h. After cooling, the product was filtered; yield: 1.0 g (74%); mp 278–280°C (DMF). IR ν cm^{-1}: 3200–2700 bm, 2200 s, 1660 s, 1620 m, 1550 m, 1470 m; ^{1}H-NMR (360 MHz): δ 2.4 (s, CH_3), 2.7 (t, CH_2), 2.9 (s, CH_2-N-CH_2), 3.0 (t, CH_2N), 3.8 (s, CH_2-O-CH_2), 7.2 (t, 1 Ar*H*), 7.4 (t, 1 Ar*H*), 7.5 (d, 1 Ar*H*), 8.6 (d, 1 Ar*H*). Anal $C_{19}H_{20}N_4O_2$ (C, H, N).

2-(2-Azidoethyl)-3-methyl-1-oxo-1H,5H-pyrido[1,2-a]benzimidazole-4-carbonitrile 12b
A solution of **8** (1.14 g, 4 mmol) in DMF (15 ml) was stirred with sodium azide (0.40 g, 6 mmol) for 15 min at 50°C and then at room temperature for 30 min. After addition of water the product was filtered and dried; yield: 1.1 g (94%); mp 223–234°C dec (acetone). IR ν cm^{-1}: 3300–2900 bm, 2200 s, 2100 s, 1650 s, 1630 s, 1570 w, 1525 s, 1460 m. ^{1}H-NMR: δ 2.4 (s, CH_3), 2.75 (t, CH_2), 3.55 (t, CH_2N_3), 7.4 (m, 2 Ar*H*), 7.5 (d, 1 Ar*H* at C-6), 8.6 (d, 1 Ar*H* at C-9). Anal $C_{15}H_{12}N_6O$ (C, H, N).

3-Methyl-2-(2-triphenylphosphoranylideneaminoethyl)-1-oxo-1H,5H-pyrido[1,2-a]benzimidazole-4-carbonitrile 13
This was prepared by refluxing a stirred solution of **12b** (1.46 g, 5 mmol) and triphenylphosphine (1.3 g, 5 mmol) in dry acetone (30 ml) for 5 h, during which time the product was separated. After cooling, the product was filtered and dried; yield: 1.5 g (57%); mp 135°C dec (acetone). IR ν cm^{-1}: 3300–2500 bm, 2200 s, 1710 s,1620 s, 1580 s, 1500 s, 1440 s. ^{1}H-NMR: δ 2.1 (s, CH_3), 2.85 (t, CH_2), 3.15 (t, CH_2-N=P), 7.1 (t, 1 Ar*H*), 7.3 (t, 1 Ar*H*), 7.5 (d, 1 Ar*H* at C-6), 7.6–7.9 (m, 15 Ar*H*), 8.6 (d, 1 Ar*H* at C-9). Anal $C_{33}H_{27}N_4OP$ (C, H, N).

2-(Aminoethyl)-3-methyl-1-oxo-1H,5H-pyridol[1,2-a]benzimidazole-4-carbonitrile hydrochoride 14
This was prepared by refluxing compound **13** (1.58 g, 3 mmol) with a mixture of methanol (30 ml) and hydrochloric acid (2 N) (20 ml) for 5 h during which time the white product was separated out. After cooling the product was filtered and purified by washing with hot ethanol; yield: 0.7 g (77.1%); mp > 350°C. IR ν cm^{-1}: 3300–2300 bm, 2190 s, 1650 w, 1630 s, 1580 s, 1400 s. Anal $C_{15}H_{15}ClN_4O$ (C, H, N).

1-Azido-2-(2-chloroethyl)-3-methylpyrido[1,2-a]benzimidazole-4-carbonitrile 15
A solution of **9a** (1.22 g, 4 mmol) and sodium azide (0.65 g, 10 mmol) in DMF (15 ml) was stirred for 1 h at room temperature. Water was then added, the product was filtered, washed with water and dried away from heat and light; yield: 1.1 g (88.5%); mp 137°C dec (benzene). IR ν cm^{-1}: 2220 s, 2120 s, 1625 m,1590 s, 1520 w, 1474 s, 1450 m. ^{1}H-NMR: δ 2.7 (s, CH_3), 3.35 (t, CH_2), 3.9 (t, CH_2Cl), 7.4 (t, 1 Ar*H*), 7.6 (t, 1 Ar*H*), 7.9 (d, 1 Ar*H* at C-6), 8.6 (d, 1 Ar*H* at C-9). ^{13}C-NMR: δ 19.0 (CH_3), 29 (CH_2), 42 (CH_2Cl), 96, 112.2, 115, 116.2, 119, 122, 126, 129, 138, 144, 146, 150.1 (11 Ar*C* + CN). Anal $C_{15}H_{11}ClN_6$ (C, H, N).

2-(2-Chloroethyl)-3-methyl-1-triphenylphosphoranylideneamino-pyrido[1,2-a]benzimidazole-4-carbonitrile 16
To a stirred solution of **15** (1.24 g, 4 mmol) in benzene (15 ml), a solution of triphenylphosphine (1.05 g, 4 mmol) in benzene (15 ml) was added at room temperature and then the reaction mixture was refluxed for 1 h during which time the product separated; yield: 1.9 g (87%); mp 220–222°C (ethanol/petro-

leum ether. IR ν cm^{-1}: 3200–2500 bm, 2200 s, 1620 s, 1580 s, 1500 s, 1440 s; ^{1}H-NMR: δ 2.1 (s, CH_3), 2.85 (t, CH_2), 3.15 (bs, CH_2-Cl), 7.1 (t, 1 Ar*H*), 7.3 (t, 1 Ar*H*), 7.5 (d, 1 Ar*H* at C-6), 7.6–7.9 (m, 15 Ar*H*), 8.6 (d, 1 Ar*H* at C-9). Anal $C_{33}H_{26}ClN_4P$ (C, H, N).

2,3-Dihydro-4-methyl-1H-pyrrolo[2,3-b]pyrido[1,2-a]benzimidazole-5-carbonitrile ***17a***
Compound **16** (1.64 g, 3 mmol) was refluxed with a mixture of methanol (30 ml) and hydrochloric acid (2 N) (20 ml) for 5 h and the product was obtained after cooling and neutralization with ammonium hydroxide; yield: 0.5 g (68%); mp 249°C dec (DMF). IR ν cm^{-1}: 3350 m, 2200 s, 1630 m, 1600 s, 1480 s, 1400 w; ^{1}H-NMR: δ 2.4 (s, CH_3), 3.2 (t, CH_2), 4.1 (t, CH_2N), 7.3–8.0 (m, 4 Ar*H*). Anal $C_{15}H_{12}N_4$ (C, H, N).

2,3-Dihydro-1-(n-hexyl)-4-methyl-1H-pyrrolo[2,3-b]pyrido-[1,2-a]benzimidazole-5-carbonitrile ***17b***
This was prepared by refluxing a mixture of **9a** (3.04 g, 10 mmol) and *n*-hexylamine (2.0 ml, 20 mmol) in DMF (20 ml) for 3 h. The solvent was removed under vacuum and the residue treated with acetonitrile; yield: 2.0 g (62.0%); mp 170–172°C (DMF); IR ν cm^{-1}: 3000–2700 bm, 2200 s, 1620 s, 1590 s, 1560 m, 1500 w, 1480 m, 1450 m; ^{1}H-NMR: δ 0.9 (t, CH_3 of *n*-hexyl), 1.2 (bs, 3 CH_2), 1.7 (bs, CH_2), 2.45 (s, CH_3 at C-4), 3.1 (t, CH_2), 3.3 (t, CH_2N), 3.9 (t, CH_2N), 7.3–7.9 (m, 4 Ar*H*). Anal $C_{21}H_{24}N_4$ (C, H, N).

2,3-Dihydro-4-methyl-1-(4-tolyl)-1H-pyrrolo[2,3-b]pyrido[1,2-a]benzimidazole-5-carbonitrile ***17c***
This was prepared from **9a** (10 mmol) and *p*-toluidine (2.14 g, 20 mmol) in the same way as for **17b**; yield: 2.9 g (85.7%); mp 280–281% (DMF); IR ν cm^{-1}: 2200 s, 1620 s, 1590 s, 1550 m, 1510 s, 1470 w, 1440 m. ^{1}H-NMR: δ 2.25 (s, CH_3), 2.50 (s, CH_3), 3.2 (t, CH_2), 4.35 (t, CH_2-N), 6.6–7.8 (m, 8 Ar*H*). Anal $C_{22}H_{18}N_4$ (C, H, N).

2,3-Dihydro-1-(3-methoxyphenyl)-4-methyl-1H-pyrrolo[2,3-b]pyrido[1,2-a]benzimidazole-5-carbonitrile ***17d***
This was prepared from **9a** (10 mmol) and *m*-anisidine (2.3 ml, 20 mmol) in the same way as for **17b**; yield: 2.0 g (56.4%); mp 278–280°C (DMF). IR ν cm^{-1}: 2000 s, 1625 s, 1590 s, 1480 m, 1450 w. ^{1}H-NMR: δ 2.5 (s, CH_3), 3.2 (t, CH_2), 3.60 (s, OCH_3), 4.4 (t, CH_2-N), 6.4–7.8 (m, 8 Ar*H*). Anal $C_{22}H_{18}N_4O$ (C, H, N).

1-(4-Chlorophenyl)-2,3-dihydro-4-methyl-1H-pyrrolo[2,3-b]pyrido[1,2-a]benzimidazole-5-carbonitrile ***17e***
This was prepared from **9a** (10 mmol) and *p*-chloroaniline (2.55 g, 20 mmol) in the same way as for **17b**; 3.2 g (89.2%); mp 322–324°C (DMF). IR ν cm^{-1}: 2205 s, 1630 s, 1595 s, 1540 w, 1480 s, 1450 w. ^{1}H-NMR: δ 2.55 (s, CH_3), 3.3 (t, CH_2), 4.45 (t, CH_2-N), 6.8–7.8 (m, 8 Ar*H*). Anal $C_{21}H_{15}ClN_4$ (C, H, N).

2,3-Dihydro-1-(4-fluorophenyl)-4-methyl-1H-pyrrolo[2,3-b]-pyrido[1,2-a]benzimidazole-5-carbonitrile ***17f***
This was prepared from **9a** (10 mmol) and *p*-fluoroaniline (2.22 g, 20 mmol) in the same way as for **17b**; yield: 3.1 g (90.6%); mp 311–313°C (DMF). IR ν cm^{-1}: 2210 s, 1630 s, 1590 s, 1540 m, 1500 s, 1480 s, 1450 w. ^{1}H-NMR: δ 2.55 (s, CH_3), 3.3 (t, CH_2), 4.4 (t, CH_2N), 6.6–7.8 (m, 8 Ar*H*). Anal $C_{21}H_{15}FN_4$ (C, H, N).

2,3-Dihydro-1-(2-phenethyl)-4-methyl-1H-pyrrolo[2,3-b]-pyrido[1,2-a]benzimidazole-5-carbonitrile ***17g***
This was prepared from **9a** (10 mmol) and 2-phenethylamine (2.5 ml, 20 mmol) in the same way as for **17b**; yield: 2.5 g (71%); mp 207–208°C (DMF). IR ν cm^{-1}: 2210 s, 1625 s, 1600 s, 1550 s, 1495 s, 1410 m. ^{1}H-NMR: δ 2.4 (s, CH_3), 3.1 (2t, 2 CH_2), 3.6 (t, CH_2N), 4.0 (t, CH_2N), 7.1–7.8 (m, 9 Ar*H*). Anal $C_{23}H_{20}N_4$ (C, H, N).

Antineoplastic screening

A total of 60 human tumor cell lines, derived from seven cancer types which adequately meet minimal quality assurance criteria were selected for use in pilot-scale screening operation. They were adaptable to a single growth medium and had reproducible profiles for growth and drug sensitivity. All the lines were inoculated onto a series of standard 96-well microtitre plates on day 0, in the majority of cases at 20 000 cells/well, then preincubated in absence of drug for 24 h. The test compounds were then added in five ten-fold dilutions and incubated for a further 48 h and then sulforhodamine b (SRB) protein assay was used to estimate cell viability or growth.

The dose–response matrix
Each column of the matrix corresponds to the drug effect at one of the five concentration levels, and each row corresponds to the effect against each cell line. Thus, each block within a row depicts the effect of compound against the given cell line. The block shading depends on the value of the PG for the given concentration against the given cell line in comparison with the corresponding values for the TGI.

Acknowledgments

The authors are grateful to the Austrian Government for financial support of this research project. Special thanks are due to H Sterk, Institut für Organische Chemie, Karl-Franzens Universität, Graz, Austria, for helpful discussion of NMR spectra. They also thank the staff of the National Cancer Institute, Bethesda MD, USA, for the anticancer and anti-HIV test reports.

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