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Benzimidazole condensed ring systems. XII. Synthesis and anticancer evaluation of certain pyrido[1,2-*a*]benzimidazole derivatives[3]

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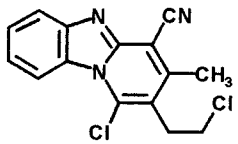
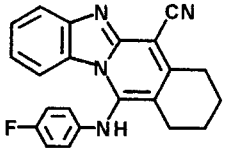
Previously, we have evaluated several pyrido[1,2–*a*]benzimidazoles (PBIs) as potential antineoplastic agents. Among them, NSC 649900 and NSC 682011 revealed good antineoplastic activity against some cell lines of clinically isolated human tumors. For further structure-activity relationship (SAR) studies we report here the synthesis and antineoplastic evaluation of related series of PBIs with similar haloarylamino (**13**–**18, 23**–**28**), haloarylaminomethylene (**29**–**34**) and haloarylazo (**35**–**38**) moieties at position 1 or 2. Some of these derivatives revealed notable activity against some tumor cell lines; the highest activity was recorded for the *p*-fluorophenylamino-3-phenyl-PBI (**23**, NSC 699944) and its *p*-chlorophenyl analog (**24**, NSC 699948). These compounds were selected by the NCI for further testing in a new *in vivo* anticancer hollow fiber assay.

1. Introduction

The pyrido[1,2-*a*]benzimidazole (PBI) ring system was already recognized by Morgan in 1937 [1], but many of its derivatives are still with unexplored pharmacotoxicological properties. However, a few reports describe their potential as antimicrobial [2, 3], antiviral [4, 5], analysis [6–8] and antianxiety agents [9]. In a series of publications, we have evaluated several PBIs for antineoplastic activity [10–15]. Among them, the 1-chloro-2-(2-chloroethyl)-3-methyl-PBI (NSC 649900) displayed good sensitivity and subpanel disease selectivity against leukemia cell lines *in vitro*; however, it revealed only weak activity against P388 murine leukemia *in vivo* [13]. In our search for a cytotoxic candidate with improved antineoplastic profile, some related cycloalkyl-PBIs were prepared and we found that the 5-(4-fluorophenylamino)-tetrahydroisoquino[2,3-*a*]benzimidazole (NSC 682011) exhibited good *in vitro* antineoplastic activity especially against leukemia cell lines [15]. For this reason, this compound was selected by NCI for further testing in a new *in vivo* anticancer hollow fiber assay. In view of these findings [13, 15], we report here the synthesis and antineoplastic evaluation of related series of PBIs which have similar haloarylamino substitutes at position 1 (**13**–**18**, **23**–**28**, Scheme 1). For further information concerning structure-activity-relationships, we also synthesized another series of PBIs with haloarylaminomethylene (**29**–**34**) or haloarylazo (**35**–**38**) moieties at position 2. The reaction sequence leading to these compounds is illustrated in Scheme 1.

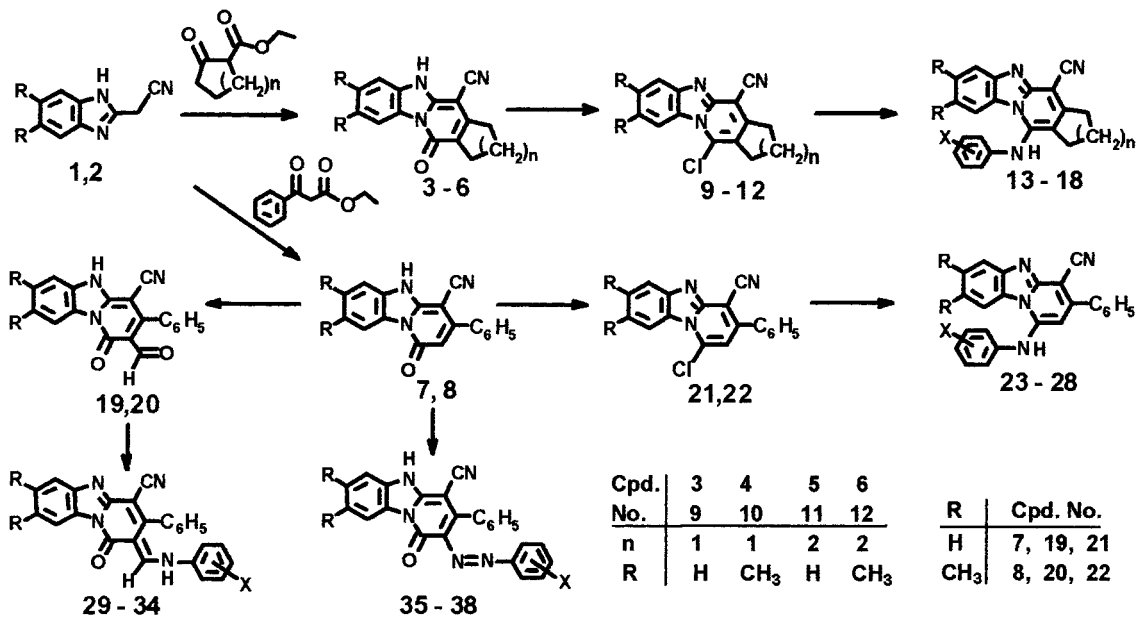


NSC 649900



NSC 682011

Scheme 1

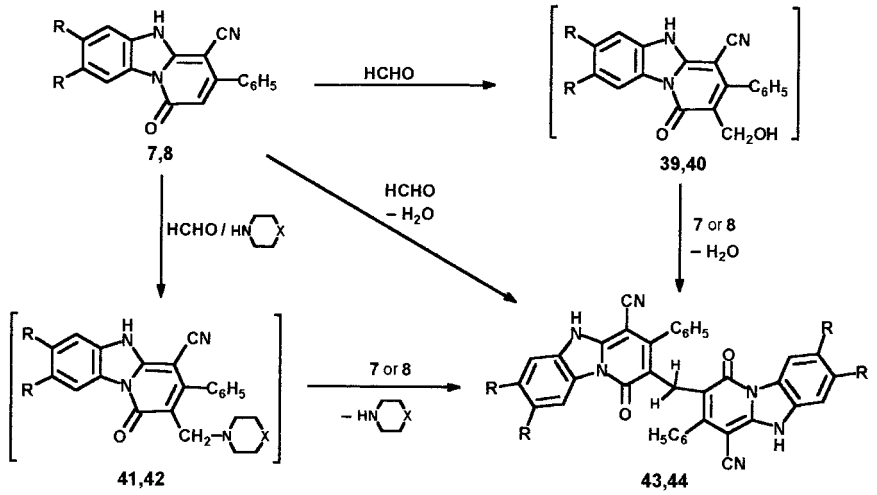


Cpd. No.	3	4	5	6
	9	10	11	12
n	1	1	2	2
R	H	CH_3	H	CH_3

R	Cpd. No.
H	7, 19, 21
CH_3	8, 20, 22

For R,X,n-keys of compounds 13–18, 23–28, 29–34 and 35–34 and 35–38, see Tables 2, 4, 6 and 8

Scheme 2



R = H for 7 and 43; R = CH_3 for 8 and 44; X = O, CH_2

2. Investigations, results and discussion

2.1. Synthesis and characterization

Previously, we have described a facile one step synthesis of some substituted PBIs, such as **3**, **5**, **7**, by fusing 1*H*-benzimidazole-2-acetonitrile (**1**) with the respective β-keto esters at 150 °C in the presence of ammonium acetate [11, 12, 15]. We have now used this reaction conditions for the preparation of the new PBIs **4**, **6**, **8** which are required together with **3**, **5**, **7** for the synthesis of the target compounds **9**–**40** (Scheme 1). Thus, reacting **1** or **2** with ethyl cyclopentanone-2-carboxylate (n = 1) or ethyl cyclohexanone-2-carboxylate (n = 2) gave the corresponding cycloalkyl-PBIs **3**–**6**, while their reaction with ethyl benzoylacetate resulted in the 3-phenyl-PBIs **7**, **8**. Chlorination of the cycloalkyl-PBIs **3**–**6** with phosphoryl chloride resulted in the respective chloro derivatives **9**–**12**. Similarly, the 1-chloro-PBIs **21**, **22** were prepared from their parent 1-oxo-PBIs **7**, **8** and phosphoryl chloride. Reacting the chloro compounds **9**–**12**, or **21**, **22** with the selected aryl amines in dimethylformamide at 80 °C gave the corresponding arylamino derivatives **13**–**18** and **23**–**28**, respectively. Vilsmeir-Haack formylation of **7** or **8** using two molar amounts of phosphoryl chloride in the presence of dimethyl formamide yielded the respective PBI-2-carboxaldehydes **19**, **20** which upon treatment with the selected aryl amines resulted in the Schiff bases **29**–**34**. On the other hand, the 2-arylazo compounds **35**–**38** were obtained by reacting the 3-phenyl-PBIs **7**, **8** with the diazonium salts of the selected aryl amines. Reacting **7** or **8** with a mixture of formaline and piperidine or morpholine gave the dimeric structures **43**, **44** instead of the expected Mannich bases **41**, **42** (Scheme 2). This result indicated that position 2 of the PBIs **7** and **8** is highly nucleophilic, for this reason, the formation of **43** or **44** may proceed through the formation of the 2-hydroxymethyl-intermediates **39**, **40** and/or the Mannich bases **41**, **42** followed by attack with another molecule of the PBIs **7** or **8**, or it may

Table 1: Inhibition of *in vitro* cancer cell lines by compounds 13, 23, 24, 25, 35 and 38

Compd.	NSC	Panel	Cell lines (Cytotoxicity: $\log_{10}$ GI_{50}, M)[a, b]
13	699940	Leukemia	CCRF-CEM (−6.12), RPMI-8226 (−5.41)
		Colon cancer	HCT-116 (−5.70), HT-29 (−5.71), SW-620 (−5.56)
		Renal cancer	786-0 (−5.75), ACHN (−5.55), CAKI-1 (−5.81), RXF 393 (−5.73), SN12C (−5.49), UO-31 (−5.46)
23[c]	699944	Lung cancer	NCI-H322M (−5.20), NCI-H522 (−5.17)
		Colon cancer	COLO 205 (−5.51), HCT-116 (−5.74)
		CNS cancer	SF-295 (−5.06), SNB-75 (−5.18), U251 (−5.42)
		Melanoma	SK-MEL-2 (−5.36), UACC-257 (−5.04), UACC-62 (−4.83)
		Renal cancer	786-0 (−5.01), A498 (−5.01), ACHN (−5.11), SN12C (−5.16), UO-31 (−5.18)
		Breast cancer	MCF7 (−5.22), HS 578T (−5.33), T-47D (−5.24)
24[c]	699948	Leukemia	K-562 (−4.76), Molt-4 (−5.63), SR (−5.56)
		Lung cancer	A549/ATCC (−4.79), EKVX (−4.66), Hop-92 (−5.33)
		Colon cancer	COLO 205 (−5.55), HCT-116 (−5.00), HT-29 (−4.94)
		CNS cancer	SF-295 (−4.95), SNB-75 (−5.01)
		Renal cancer	786-0 (−4.80), CAKI-1 (−5.03), SN12C (−4.98)
25	699946	Lung cancer	HOP-92 (−6.69), CIN-H226 (−6.16), NCI-H23 (−6.47), NCI-H522 (−6.61)
35	699956	Leukemia	CCRF-CEM (−5.90), HL-60TB (5.84), Molt-4 (−5.64), RPMI-8226 (−6.22)
		Colon cancer	COLO 205 (−5.54), HCT-116 (−5.65), HCT-15 (−5.47), HT-29 (−5.53)
38	699959	Leukemia	CCRF-CEM (−5.28), K-562 (7.42), RPMI-8226 (−5.27)

[a] Data obtained from NCI's *in vitro* disease-oriented tumor cell line screen
[b] GI_{50} = compound concentration that inhibits 50% cell growth
[c] Data obtained at $\log_{10}$ TGI level, TGI = compound concentration that inhibits 100% cell growth

Table 2: Experimental data of compounds 13–18

Compd.	Starting material*	n	R	X	Yield (%)	m.p. (°C) Cryst. solvent	Mol. formula Mol. wt.
13	**9**	1	H	p-F	77	292–293 DMF-EtOH	$C_{21}H_{15}N_4F$ 342.4
14	**9**	1	H	m-CF_3	85	251–252 EtOH	$C_{22}H_{15}N_4F_3$ 392.4
15	**10**	1	CH_3	p-F	76	321–322 EtOH	$C_{23}H_{19}N_4F$ 370.4
16	**10**	1	CH_3	p-Cl	97	297–298 DMF-EtOH	$C_{23}H_{19}N_4Cl$ 386.9
17	**11**	2	H	m-CF_3	93	245–246 EtOH	$C_{23}H_{17}N_4F_3$ 368.4
18	**12**	2	CH_3	p-F	88	273–274 DMF-EtOH	$C_{24}H_{19}N_4F$ 384.4

* Compounds **9** and **11** were prepared according to a previously reported procedure [11, 15].

involve the condensation of two molecules of **7** or **8** with one molecule of formaldehyde in the presence of piperidine or morpholine in a one step reaction. The proposed mechanism for this reaction sequences is illustrated in Scheme 2. The ^{1}H NMR of compounds **43**, **44** revealed a singlet at 3.7δ ppm corresponding to the methylene protons. The aromatic protons at C-9 in both PBI rings in structure **43** appeared as a doublet at the same chemical shift (8.2δ ppm); while in case of compound **44**, they appeared as singlet at 8.4δ ppm.

The structures of the compounds illustrated in Scheme 1 were confirmed by microanalyses, IR, ^{1}H NMR and ^{13}C NMR spectral data (see the experimental part). The ^{13}C NMR of the PBI-2-carboxaldehyde (**19**) revealed two signals corresponding to the CO and CHO at 158.56 and 186.26δ ppm, respectively; and another signal corresponding to the CN at 115.14δ ppm. The ^{1}H NMR of compound **29** showed the methylene and NH protons of the arylaminomethylene moiety in a characteristic pattern, where they appeared as two doublets at 7.9 and 12.7δ ppm, respectively. This indicates the presence of the arylaminomethylene moiety in the 3-phenyl-PBI series **29**–**34** in the form of =CH−NH−Ar and not as −CH=N−Ar.

2.2. Biological results

The prepared compounds were evaluated for their *in vitro* antineoplastic activity against 60 human cell lines derived from seven clinically isolated cancer types (lung, colon, melanoma, renal, ovarian, brain and leukemia) according to the NCI standard protocol [16] and the data of the most sensitive cell lines are recorded in Table 1. Among the arylamino-cycloalkyl-PBI series **13**–**18**, the p-fluorophenylamino-compound **13** showed significant activity against most of the cell lines of renal cancer and few cell lines from leukemia and colon cancer panels. Whereas the m-trifluoromethylphenylamino-compounds **14**, **17** showed a marginal activity against few cell lines from the lung cancer panel. Within the 1-arylamino-3-phenyl-PBI series **23** to **28**, the p-fluorophenylamino-compound **23** displayed a notable activity mainly against most of the cell lines of renal cancer and few cell lines from lung, colon, melanoma, CNS and breast cancer panels. On the other hand, the p-chlorophenylamino analog **24** showed significant activity against some cell lines from leukemia, lung, colon, CNS and renal cancer panels; while the m-trifluoromethylphenylamino-derivative **25** was active against most of the cell lines of lung cancer panel. The 2-arylaminomethylene-3-phenyl-PBI series **29**–**34** was almost inactive; whereas in the 2-arylazo-3-phenyl-PBI series **35**–**38** the most pronounced activity was recorded for the p-fluorophenylazo compound **35** particularly against the leukemia cell line. While the p-chlorophenylazo along **36** was less active against renal cancer. In all series, the 7,8-dimethyl derivatives were poorly active; except for the m-trifluoromethylphenylazo derivative **38** which was active against leukemia, particularly against K-562 cell line at $\log_{10}$-GI50 (−7.42).

It can be concluded from these results that a significant antineoplastic activity was only associated with the 4-arylamino-cycloalkyl-PBI, the 1-arylamino- and 2-arylazo-3-phenyl-PBIs which have p-fluoro or p-chloro atoms in the aryl moiety (e.g. **13**, **23**, **24** and **35**). Inspite of the closed structural similarities between these compounds and NSC 682011, they have different antineoplastic profiles. However, both of the 1-(p-fluorophenylamino)-3-phenyl-PBI **23** and its p-chlorophenyl analog **24** are more active than the 4-(p-fluorophenylamino)-cycloalkyl-PBI **13** and 2-(p-fluorophenylazo)-3-phenyl-PBI **35**. These compounds were selected by the NCI for further testing in a new *in vivo* anticancer hollow fiber assay, by virtue of their activity against several cell lines from different cancer panels as recorded in Table 1.

3. Experimental

M.p.'s were determined in open-glass capillaries on a Gallenkamp apparatus and are uncorrected. The IR spectra (KBr) were recorded on a Perkin-Elmer 298 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian

Table 3: ^{1}H NMR spectral data of some compounds from series 13–18

Compd.	$-CH_2-(CH)_n-CH_2-$* (m) + (2t)		Aromatic H	other H
13	2.2	3.2, 3.3	7.0–7.6 (m, 6 H), 7.8, 8.2 (2d, 2 H at C-6 and C-9)	9.5 (s, NH)
14	2.1	3.2, 3.25	7.1–7.0 (m, 6 H), 7.8, 8.2 (2d, H at C-6 and C-9)	9.7 (s, NH)
17	1.8	2.7, 3.2	6.9–7.6 (m, 6 H), 7.9, 8.0 (2d, H at C-7 and C-10)	9.9 (bs, NH)
18	1.8	2.6, 3.1	6.7–7.1 (m, 6 H), 7.6, 7.7 (2s, H at C-7 and C-10)	9.0 (s, NH) 2.2, 2.3 (2s, 2 CH_3)

* For n-key, see Table 2.

Table 4: Experimental data of compounds 23–28

Compd.	R	X	Yield (%)	m.p. (°C) Cryst. solvent	Mol. formula Mol. wt
23	H	p-F	97	282–283 EtOH	$C_{24}H_{15}N_4F$ 378.4
24	H	p-Cl	92	259–260 EtOH	$C_{24}H_{15}N_4Cl$ 394.9
25	H	m-CF_3	95	258–259 EtOH	$C_{25}H_{15}N_4F_3$ 428.4
26	CH_3	p-F	96	311–312 DMF-EtOH	$C_{26}H_{19}N_4F$ 406.5
27	CH_3	p-Cl	89	285–286 DMF-EtOH	$C_{26}H_{19}N_4Cl$ 422.9
28	CH_3	m-CF_3	98	268–269 EtOH	$C_{27}H_{19}N_4F_3$ 456.5

Table 5: ^{1}H NMR spectral data of compounds 23–28

Compd.	H at C-2 (s)	Aromatic H (m)	1 Ar*H* at C-9	NH (bs)	other H
23	6.2	7.2–7.8	8.6 (d)	–	–
24	6.3	7.2–7.9	8.6 (d)	9.8	–
25	6.4	7.2–7.8	8.6 (bs)	9.9	–
26	6.1	7.2–7.7	8.4 (bs)	9.5	2.35, 2.45 (2s, 2 CH_3)
27	6.2	7.3–7.7	8.4 (bs)	9.6	2.40, 2.45 (2s, 2 CH_3)
28	6.3	7.3–7.7	8.4 (bs)	9.7	2.40, 2.45 (2s, 2 CH_3)

Table 6: Experimental data of compounds 29–34

Compd.	R	X	Yield (%)	m.p. (°C) Cryst. solvent	Mol. formula Mol. wt
29	H	p-F	81	311–312 DMF-EtOH	$C_{25}H_{15}N_4FO$ 406.4
30	H	p-Cl	86	310–312 DMF-EtOH	$C_{25}H_{15}ClN_4O$ 422.9
31	H	m-CF_3	76	242–243 EtOH	$C_{26}H_{15}N_4F_3O$ 456.4
32	CH_3	p-F	90	314–315 DMF-EtOH	$C_{27}H_{19}N_4FO$ 434.5
33	CH_3	p-Cl	94	331–332 DMF	$C_{27}H_{19}ClN_4O$ 450.9
34	CH_3	m-CF_3	77	312–313 DMF-EtOH	$C_{28}H_{19}N_4F_3O$ 484.5

Table 7: ^{1}H NMR spectral data of compounds 29–34

Compd.	Methylene H (d)	Aromatic H (m)	1 Ar*H* at C-9	NH (d)	other H
29	7.90	7.2–7.8	8.50 (d)	12.70	–
30	7.90	7.3–7.7	8.45 (d)	12.35	–
31	8.00	7.4–7.9	8.50 (d)	12.70	–
32	7.80	7.3–7.7	8.15 (s)	12.30	2.15, 2.20 (2s, 2 CH_3)
33	7.90	7.3–7.7	8.30 (s)	12.70	2.40, 2.45 (2s, 2 CH_3)
34	7.95	7.5–7.7	8.30 (s)	12.70	2.35, 2.40 (2s, 2 CH_3)

Table 8: Experimental data of compounds 35–38

Compd.	R	X	Yield (%)	m.p. (°C) Cryst. solvent	Mol. formula Mol. wt
35	H	p-F	83	262–263 DMF-EtOH	$C_{24}H_{14}N_5FO$ 407.4
36	H	p-Cl	95	259–260 DMF	$C_{24}H_{14}ClN_5O$ 423.9
37	CH_3	p-F	87	254–255 DMF-EtOH	$C_{26}H_{18}N_5FO$ 435.5
38	CH_3	m-CF_3	94	269–270 DMF	$C_{27}H_{18}N_5F_3O$ 485.5

Table 9: ^{1}H NMR spectral data of compounds 35–38

Compd.	Ar*H* (m)	1 Ar*H* at C-9	NH (s)	other H
35	7.2–7.9	8.70 (d)	–	–
36	7.3–7.9	8.50 (d)	12.10 (s)	–
37	7.2–7.7	8.05 (s)	14.15 (s)	2.15, 2.20 (2s, 2 CH_3)
38	7.5–7.7	8.15 (s)	–	2.35, 2.40 (2s, 2 CH_3)

Gemini 200 at 200 MHz using TMS as the internal standard, the chemical shift is given as δ (ppm), in [D_6]DMSO. Elemental analyses were performed on a Carlo Erba 1106 analyzer and were within ±0.4 of the theoretical percentages.

3.1. Synthesis of the compounds

3.1.1. 7,8-Dimethyl-2,3-dihydro-4-oxo-1H,4H,10H-cyclopenta[4′,5′:2,3]pyrido[1,2-a]benzimidazole-11-carbonitrile (**4**)

Compound **4** was prepared as described for **3** [11] by heating a mixture of **2** (7.4 g, 40 mmol), ethyl cyclopentanone-2-carboxylate (6.5 ml, 44 mmol) and ammonium acetate (6.8 g, 88 mmol) in an oil bath at 140–150 °C for 1 h. After cooling, the separated product was treated with EtOH, filtered, dried and recrystallized from DMF, m.p. >350 °C, yield 70%.
IR (KBr, cm^{-1}): 3300–2500bm, 2210s, 1670s, 1600w, 1590m, 1530s. ^{1}H NMR, (δ ppm): 2.1 (m, 2 H, $-CH_2CH_2CH_2-$), 2.4 & 2.5 (2s, 6 H, 2 CH_3), 2.8 & 3.0 (2t, 4 H, $-CH_2CH_2CH_2-$), 7.3 (s, 1 Ar*H* at C-9), 8.4 (s, 1 Ar*H* at C-6).
$C_{17}H_{15}N_3O$ (277.3)

3.1.2. 8,9-Dimethyl-1,2,3,4-tetrahydro-5-oxo-5H,11H-isoquino[2,3-a]benzimidazole-12-carbonitrile (**6**)

It was similarly prepared from **2** (7.4 g, 40mmol), ethyl cyclohexanone-2-carboxylate (7.0 ml, 44 mmol) and ammonium acetate (6.8 g, 88 mmol), m.p. >350 °C (DMF), yield 65%.
IR (KBr, cm^{-1}): 3300–2600bm, 2210s, 1660s, 1610w, 1590m, 1540s. ^{1}H NMR, (δ ppm): 1.7 (m, 4 H, $-CH_2(CH_2)_2CH_2-$), 2.4 & 2.55 (2s, 6 H, 2 CH_3), 2.45 & 2.7 (2t, 4 H, $-CH_2(CH_2)_2CH_2-$), 7.3 (s, 1 Ar*H* at C-10), 8.4 (s, 1 Ar*H* at C-7).
$C_{18}H_{17}N_3O$ (291.4)

3.1.3. 7,8-Dimethyl-1-oxo-1H,5H-3-phenylpyrido[1,2-a]benzimidazole-4-carbonitrile (**8**)

It was similarly prepared from **2** (1.85 g 10 mmol), ethyl benzoylacetate (1.9 ml, 11 mmol) and ammonium acetate (1.7 g, 22 mmol), m.p. >324 to 325 °C dec. (DMF), yield 58%.
IR (KBr, cm^{-1}): 3200–2300bw, 2205s, 1650s, 1600w, 1520m. ^{1}N NMR, (δ ppm): 2.4 & 2.5 (2s, 6 H, 2 CH_3), 6.0 (s, 1 H at C-2), 7.3 (s, 1 Ar*H* at C-6), 7.6–7.8 (m, 5 H, 5 Ar*H* of phenyl at C-3), 8.4 (s, 1 Ar*H* at C-9), 13.6 (s, N*H*).
$C_{20}H_{15}N_3O$ (313.4)

3.1.4. 4-Chloro-7,8-dimethyl-2,3-dihydro-1H-cyclopenta[4′,5′:2,3]pyrido[1,2-a]benzimidazole-11-carbonitrile (**10**)

This compound was prepared as described for **9** [11] by refluxing **4** (2.77 g, 10 mmol) with $POCl_3$ (30 ml) for 2 h. The excess $POCl_3$ was removed under vacuum and the residue was treated with ice-water, neutralized with Na_2CO_3, the product was filtered, washed with H_2O, dried and recrystallized from DMF, m.p. 236–237 °C, yield 94%.
IR (KBr, cm^{-1}): 3400m, 2980s, 2210s, 1630w, 1600w, 1540m, 1500s, 1450s. ^{1}H NMR, (δ ppm): 2.2 (m, 2 H, $-CH_2CH_2CH_2-$), 2.4 & 2.5 (2s, 6 H, 2 CH_3), 3.1 & 3.3 (2t, 4 H, $-CH_2CH_2CH_2-$), 7.7 (s, 1 Ar*H* at C-9), 8.4 (s, 1 Ar*H* at C-6).
$C_{17}H_{14}ClN_3$ (295.8)

3.1.5. 5-Chloro-8,9-dimethyl-1,2,3,4-tetrahydroisoquino[2,3-a]benzimidazole-12-carbonitrile (**12**)

It was similarly prepared from **6** (2.91 g, 10 mmol) and $POCl_3$ (30 ml), m.p. 239–240 °C (DMF), yield 94%.
IR (KBr, cm^{-1}): 3400m, 2980s, 2210s, 1650w, 1600s, 1520w, 1460s. ^{1}H NMR, (δ ppm): 1.7 (m, 4 H, $-CH_2(CH_2)_2CH_2-$), 2.4 & 2.5 (2s, 6 H, 2 CH_3), 2.8 & 3.0 (2t, 4 H, $-CH_2(CH_2)_2CH_2-$), 7.6 (s, 1 Ar*H* at C-10), 8.4 (s, 1 Ar*H* at C-7).
$C_{18}H_{16}ClN_3$ (309.8)

3.1.6. 4-Arylamino-2,3-dihydro-1H-cyclopenta[4′,5′:2,3]pyrido[1,2-a]benzimidazole-11-carbonitriles (n = 1) (**13–16**)

These compounds were prepared by stirring a solution of **9** or **10** (2 mmol) and the appropriate amine (4 mmol) in DMF (10 ml) for 4 h at 80 °C. After cooling and addition of H_2O, the product was filtered and dried. Experimental data: see Table 2. ^{1}H NMR spectral data: see Table 3.
IR (KBr, cm^{-1}): 2900w, 2210s, 1640–1630s-w, 1610–1590s, 1550s, 1450–1440m.

3.1.7. 5-Arylamino-1,2,3,4-tetrahydroisoquino[2,3-a]benzimidazole-12-carbonitriles (n = 2) (**17**, **18**)

These compounds were similarly prepared from **11** or **12** (2 mmol) and the appropriate amine (4 mmol). Experimental data: see Table 2. ^{1}H NMR spectral data: see Table 3.
IR (KBr, cm^{-1}): 3400–2700bm, 2210s, 1630w, 1600–1590s, 1520–1500s, 1480–1450w.

3.1.8. 4-Cyano-1-oxo-1H,5H-3-phenylpyrido[1,2-a]benzimidazole-2-carboxaldehyde (**19**)

To a stirred suspension of **7** (2.85 g, 10 mmol) in DMF (25 ml), $POCl_3$ (1.8 ml, 20 mmol) was added gradually and the reaction mixture was stirred at 50 °C for 2 h during which clear brown solution was formed followed by separation of yellowish brown product. After cooling and addition of H_2O, the product was filtered, washed with H_2O, dried and recrystallized from DMF, m.p. >350 °C, yield 90%.
IR (KBr, cm^{-1}): 3200–2400bm, 2210s, 1690s, 1630s, 1590w, 1570w, 1480s. ^{1}H NMR, (δ ppm): 7.3–7.7 (m, 8 Ar*H*), 8.7 (d, 1 Ar*H* at C-9), 9.8 (s, 1 H, C*H*O). ^{13}C NMR, (δ ppm): 74.95 (C-4), 115.14 (CN), 135.36 (C-3), 146.39 (C-4a), 158.30 (C-2), 116.32 (2 ArC), 128.08 (4 ArC), 109.92, 112.58, 123.79, 127.04, 128.87, 131.83 (6 ArC), 158.56 (C=O), 186.26 (CHO).
$C_{19}H_{11}N_3O_2$ (313.3)

3.1.9. 4-Cyano-7,8-dimethyl-1-oxo-1H,5H-3-phenylpyrido[1,2-a]benzimidazole-2-carboxaldehyde (**20**)

It was similarly prepared from **8** (3.13 g, 10 mmol) and $POCl_3$ (1.8 ml, 20 mmol) in DMF (25 ml), m.p. >314 °C dec. (DMF-EtOH), yield 75%.
IR (KBr, cm^{-1}): 3100–2300bm, 2200s, 1700s, 1620s, 1590s, 1580s, 1410w. ^{1}H NMR, (δ ppm): 2.40 & 2.45 (2s, 2 CH_3), 7.3–7.6 (m, 6 Ar*H*), 8.5 (s, 1 Ar*H* at C-9), 9.8 (s, 1 H, C*H*O).
$C_{21}H_{15}N_3O_2$ (341.4)

3.1.10. 1-Chloro-7,8-dimethyl-3-phenylpyrido[1,2-a]benzimidazole-4-carbonitrile (**22**)

This compound was prepared from **8** (3.13 g, 10 mmol) and $POCl_3$ (15 ml) as described for **10**, m.p. 260–261 °C (DMF), yield 90%.
IR (KBr, cm^{-1}): 2900w, 2210s, 1590s, 1580s, 1450w. ^{1}H NMR (δ ppm): 2.40 & 2.45 (2s, 2 CH_3), 7.4 (s, 1 Ar*H* at C-2), 7.6–7.8 (m, 5 Ar*H*), 7.75 (s, 1 Ar*H* at C-6), 8.4 (s, 1 Ar*H* at C-9).
$C_{20}H_{14}ClN_3$ (331.8)

3.1.11. 1-Arylamino-3-phenylpyrido[1,2-a]benzimidazole-4-carbonitriles (**23–28**)

These compounds were prepared from **21** or **22** (2 mmol) and the appropriate amine (4 mmol), as described for **13–18**. Experimental data: see Table 4. ^{1}H NMR spectral data: see Table 5.
IR (KBr, cm^{-1}): 3400–3300m-w, 2220–2200s, 1640–1630s-w, 1600 to 1570s, 1540–1520s-w, 1500–1480s-m.

3.1.12. 2-Arylaminomethylene-1-oxo-1H-3-phenylpyrido[1,2-a]-benzimidazole-4-carbonitriles (**29–34**)

The title compounds were prepared by stirring **19** or **20** (2 mmol) with the proper arylamine (2 mmol) in DMF (15 ml) for 30 min. After addition of H_2O, the product was filtered and dried. Experimental data: see Table 6. ^{1}H NMR spectral data: see Table 7.
IR (KBr, cm^{-1}): 3050–3000w, 2210s, 1650–1640s, 1620–1610m-s, 1550–1540s, 1510–1490m.

3.1.13. 2-Arylazo-1-oxo-1H,5H-3-phenylpyrido[1,2-a]benzimidazole-4-carbonitriles (**35–38**)

A solution of the appropriate substituted aniline diazonium acetate, prepared by reacting a cold solution of the selected substituted aniline (3 mmol) in acetic acid (10 ml) with a solution of $NaNO_2$ (0.28 g, 4 mmol) in H_2O (5 ml), was added to a stirred solution of **7** or **8** (2 mmol) in DMF (15 ml). After stirring at room temperature for 1 h, H_2O was added to obtain an orange red product. Experimental data: see Table 8. ^{1}H NMR spectral data: see Table 9.
IR (KBr, cm^{-1}): 3000–2900w, 2210s, 1660–1650s, 1610–1600w-m, 1560m, 1520–1510s, 1450–1440m.

3.1.14. Di-(4-cyano-1-oxo-1H,5H-3-phenylpyrido[1,2-a]-benzimidazol-2-yl)methane (**43**)

To a stirred solution of piperidine (0.27 ml, 2.7 mmol), aqueous formaline 37% (0.23 ml, 2.7 mmol) in dioxane (5 ml) and acetic acid (2 ml), was added a suspension of **7** (0.63 g, 2.2 mmol) in dioxane (10 ml). The reaction mixture was stirred for 5 h. The solvents were evaporated under vacuum and the residue was stirred with ice-H_2O and neutralized with Na_2CO_3 (10%). The product was then filtered, washed with H_2O, dried and recrystallized from DMF-EtOH, m.p. >340–41 °C, yield 45%.
IR (KBr, cm^{-1}): 3300–2500bm, 2200s, 1660s, 1600m, 1520m, 1490w, 1470m. ^{1}H NMR (δ ppm): 3.7 (s, 1 H, −CH=), 7.0–7.6 (m, 16 H, 2 × 8 Ar*H*), 8.2 (d, 2 × 1 Ar*H* at C-9), 13.4 (s, N*H*).
$C_{37}H_{22}N_6O_2$ (582.6)

3.1.15. Di-(4-Cyano-7,8-dimethyl-1-oxo-1H,5H-3-phenylpyrido[1,2-a]-benzimidazol-2-yl)-methane (**44**)

It was similarly prepared from **8** (0.69 g, 2.2 mmol), morpholine (0.25 ml, 2.7 mmol) and aqueous formaline 37% (0.23 ml, 2.7 mmol), m.p. 334 to 335 °C (DMF), yield 50%.
IR (KBr, cm^{-1}): 3300–2600bm, 2200s, 1660s, 1600m, 1520m, 1470m. ^{1}H NMR (δ ppm): 2.35 & 2.40 (2s, 12 H, 2 × 2 *CH*$_3$), 3.7 (s, 1 H, −CH=), 7.2–7.5 (m, 12 H, 2 × 6 Ar*H*), 8.4 (s, 2 × 1 Ar*H* at C-9), 13.2 (s, N*H*).
$C_{41}H_{30}N_6O_2$ (638.7)

3.2. Antineoplastic activity

The prepared compounds were tested for their *in vitro* anticancer activity against 60 human tumor cell lines, derived from seven clinically isolated types of cancer (lung, colon, melanoma, renal, ovarian, brain and leukemia) following the NCI preclinical antitumor drug discovery screen. Each compound was tested at five, tenfold dilutions, a 48 h continuous drug exposure protocol was used and a sulforodamine B (SRB) protein assay was used to estimate cell viability or growth [16]. The results are presented in Table 1.

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