Med Chem Res (2012) 21:1253–1260
DOI 10.1007/s00044-011-9636-y

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of potential anticancer derivatives of pyrido[1,2-a]benzimidazoles

Hanan M. Refaat

Received: 6 January 2011 / Accepted: 25 March 2011 / Published online: 10 April 2011

Abstract In this study, the starting compounds, 2-cyanomethyl benzimidazoles (**1** or **2**) were reacted with ethyl acetoacetate, ethyl benzoylacetate, or 2-acetylbutyrolactone to give the novel series of 4-cyano-3-substituted-1-oxo-1H, 5H-pyrido[1,2-a]benzimidazole (**3**–**6**, **15**, **16**). The latter was chlorinated to give compounds **7**–**10**, **17**, **18** then aminated with 4-(2-fluorophenyl) piperazine to afford compounds **11**–**14**, **19**, **20**. The structures of the new compounds were confirmed by elemental analysis as well as ^{1}H-NMR, IR, and mass data. All the synthesized products were subjected to in vitro anticancer screening that revealed that all the tested compounds exhibited antitumor activity against human breast adenocarcinoma (MCF7) cell line, with IC_{50}'s 3.43–14.70 μg/ml.

Keywords 4-Cyano-1-oxo-pyrido[1,2-a]benzimidazoles · 1-Chloro-4-cyanopyrido[1,2-a]benzimidazoles · 4-Cyano-1-(4-(2-fluorophenyl)piperazin-yl)-3-methylpyrido[1,2-a]benzimidazoles · Anticancer

Introduction

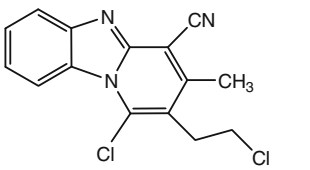
As a part of our ongoing research program on benzimidazole derivatives, which may serve as leads for designing novel chemotherapeutic agents, we have recently reported the synthesis, anticancer evaluation of various series of 2-substituted benzimidazole bearing a chloro or carboxylic acid at the 5 position of the benzimidazole ring (Refaat, 2010). The results obtained from their in vitro screening have shown interesting percent tumor-growth inhibition on various cell lines. All of these compounds exhibited significant values of percent growth inhibition at <10 μg/ml. These results were in accordance with the previously reported papers signifying the 5-chloro or 5-carboxylic acid benzimidazole as potent anticancer agents (Andrzejewska *et al.*, 2002; Pinar *et al.*, 2004; Ram *et al.*, 1992; Galal *et al.*, 2009; Graham *et al.*, 2007).

A literature survey described a variety of substituted pyrido[1,2-a]benzimidazole ring system that received a great deal of attention for their anticancer activity (Soliman *et al.,* 1984; Rida *et al.,* 1988a, b; Badawey and Kappe, 1995, 1999; Badawey *et al.* 1989; EL-Hawash *et al.,* 1999; Demirayak and Mohsen, 1998; Dupuy *et al.,* 1998, 2001). It was concluded from these investigations that the pyrido[1,2-a]benzimidazole nucleus is not the only factor essential for the anticancer activity, and that by proper substitution, good cytotoxic candidates can be obtained. Of particular interest, 1-chloro-2-(2-chloroethyl)-3-methylpyrido[1,2-a]benzimidazole (NSC 649900) (Badawey and Kappe, 1995), 1-(4-fluorophenylamino)-3-phenylpyrido[1,2-a]benzimidazole (NSC 699944), 4-(4-fluorophenylamino)-2,3-dihydro-1H-cyclopenta[4′,5′:2,3]pyrido[1,2-a]benzimidazole (NSC 682011) (EL-Hawash *et al.,* 1999) (Fig. 1) displayed good sensitivity and selectivity against leukemia cell lines in vitro.

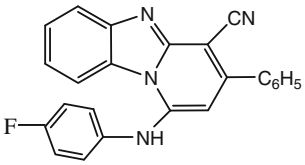
The above-mentioned results prompted us to continue our investigation on benzimidazole in order to achieve new lead compounds for future development as antitumor. In this context, new series of pyrido[1,2-a]benzimidazole having 5-chloro or 5-underivatized carboxylic acid group together with 3-methyl or 3-phenyl were synthesized and their antitumor activity was evaluated.

It is worth mentioning that pyrido[1,2-a]benzimidazole ring system was recently reported as centrally active

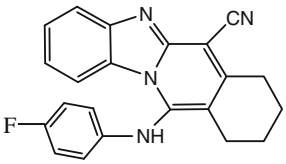
H. M. Refaat (✉)
Department of Organic Chemistry, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt
e-mail: hanan-refaat@hotmail.com



NSC 649900



NSC 699944



NSC 682011

Fig. 1 Structures of potent anticancer pyrido[1,2-a]benzimidazoles

antagonists (Wu *et al.,* 2006; Lazareno *et al.,* 2002; Jordan *et al.,* 2002; Reitz *et al.,* 2004).

Materials and methods

Chemistry

Melting points were obtained on a Griffin apparatus and are uncorrected. Microanalyses for C, H, and N were carried out at the microanalytical center, Cairo University. IR spectra were recorded on a Shimadzu 435 spectrometer, using KBr discs. ^{1}H-NMR spectra were performed on a Varian Mercury-300 (300 MHz) spectrometer, using TMS as the internal standard. Mass spectra were recorded on a GCMP-QP1000 EX Mass spectrometer. Progress of the reactions was monitored by TLC using precoated aluminum sheet silica gel MERCK 60F 254 and was visualized by UV lamp.

*General procedure for the preparation of compounds **3** and **4***

Substituted 2-cyanomethyl benzimidazoles (**1** or **2**) (10 mmol), ammonium acetate (1.54 g, 20 mmol) and ethyl acetoacetate (1.3 g, 10 mmol) were heated at 170–180°C for 7 h. The residue was broken up and extracted with ether. The residue was crystallized from dimethylformamide-water.

8-Chloro-4-cyano-3-methyl-1-oxo-1H, 5H-pyrido [1,2-a]benzimidazole (**3**)

Yield: 82%; mp: 230–232°C; IR (cm^{-1}): 3200–2660, 2212 (C≡N), 1660 (C=O); ^{1}H-NMR (DMSO-d$_6$, δ ppm): 2.35 (s, 3H, CH_3), 5.90 (s, 1H, C_2-*H*), 7.31 (d, 1H, $J = 8.1$ Hz, C_6-*H*), 7.63 (d, 1H, $J = 8.1$ Hz, C_7-*H*), 8.49 (s, 1H, C_9-*H*), 13.62 (brs, 1H, N*H*, D_2O exchangeable); Anal. Calcd. for $C_{13}H_8ClN_3O$ (257.67): C, 60.59, *H*, 3.12, N, 16.30. Found: C, 60.61, *H*, 3.15, N, 16.15.

4-Cyano-3-methyl-1-oxo-1H, 5H-pyrido [1,2-a]benzimidazole-8-carboxylic acid (**4**)

Yield: 70%; mp: 278–280°C; IR (cm^{-1}): 3340–2660, 2210 (C≡N), 1662, 1654 (2C=O); ^{1}H-NMR (DMSO-d$_6$, δ ppm): 2.33 (s, 3H, CH_3), 5.90 (s, 1H, C_2-*H*), 7.84 (d, 1H, $J = 7.5$ Hz, C_6-*H*), 8.09 (d, 1H, $J = 7.5$ Hz, C_7-*H*), 8.35 (s, 1H, C_9-*H*), 12.20–13.42 (brs, 2H, N*H* and COO*H*, D_2O exchangeable); Anal. Calcd. for $C_{14}H_9N_3O_3$ (267.23): C, 62.92, H, 3.39, N, 15.72. Found: C, 62.86, H, 3.42, N, 15.62.

*General procedure for the preparation of compounds **5** and **6***

Substituted 2-cyanomethyl benzimidazoles (**1** or **2**) (10 mmol), ammonium acetate (1.54 g, 20 mmol) and ethyl benzoylacetate (1.9 g, 10 mmol) were heated at 170–180°C for 15 h. The residue was broken up and extracted with ether. The residue was crystallized from dimethylformamide-water.

8-Chloro-4-cyano-3-phenyl-1-oxo-1H, 5H-pyrido [1,2-a]benzimidazole (**5**)

Yield: 68%; mp: 238–240°C; IR (cm^{-1}): 3190–3066, 2210 (C≡N), 1666 (C=O); ^{1}H-NMR (DMSO-d$_6$, δ ppm): 6.1 (s, 1H, C_2-*H*), 7.33 (d, 1H, $J = 8.4$ Hz, C_6-*H*), 7.40–7.53 (m, 5H, Ar-*H*), 7.69 (d, 1H, $J = 8.4$ Hz, C_7-*H*), 7.90 (s, 1H, C_9-*H*), 13.62 (brs, 1H, N*H*, D_2O exchangeable); Anal. Calcd. for $C_{18}H_{10}ClN_3O$ (319.74): C, 67.61, H, 3.15, N, 13.14. Found: C, 67.75, H, 3.18, N, 13.22.

4-Cyano-3-phenyl-1-oxo-1H, 5H-pyrido [1,2-a]benzimidazole-8-carboxylic acid (**6**)

Yield: 91%; mp: >300°C; IR (cm^{-1}): 3400–3066, 2208 (C≡N), 1666, 1654 (2C=O); ^{1}H-NMR (DMSO-d$_6$, δ ppm): 5.95 (s, 1H, C_2-*H*), 7.40–7.67 (m, 5H, Ar-*H*), 7.81 (d, 1H, $J = 8.2$ Hz, C_6-*H*), 7.96 (d, 1H, $J = 8.2$ Hz, C_7-*H*), 8.12 (s, 1H, C_9-*H*), 12.61–13.02 (brs, 2H, N*H*, and COO*H*, D_2O exchangeable); Anal. Calcd. for $C_{19}H_{11}N_3O_3$ (329.30): C,

69.29, H, 3.36, N, 12.76. Found: C, 69.41, H, 3.31, N, 12.84.

*General procedure for preparation of compounds **7–10***

The desired compounds **3–6** (10 mmol) were refluxed with phosphorus oxychloride (15 ml) for 3 h. The excess phosphorus oxychloride was removed under vacuum, and the residue was treated with ice-water (50 ml). The residue was filtered, and crystallized from dimethylformamide-water.

1,8-Dichloro-4-cyano-3-methylpyrido [1,2-a]benzimidazole (**7**)

Yield: 88%; mp: 279–281°C; IR (cm^{-1}): 2229 (C≡N); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.35 (s, 3H, CH_3), 7.51–7.63 (m, 3H, Ar-*H*), 8.50 (s, 1H, C_9-*H*); Anal. Calcd. for $C_{13}H_7Cl_2N_3$ (276.12): C, 56.54, H, 2.55, N, 15.21. Found: C, 56.43, H, 2.61, N, 15.32.

1-Chloro-4-cyano-3-methylpyrido [1,2-a]benzimidazole-8-carboxylic acid (**8**)

Yield: 75%; mp: 285–287°C; IR (cm^{-1}): 2223 (C≡N), 1690 (C=O); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.35 (s, 3H, CH_3), 7.51–7.63 (m, 3H, Ar-*H*), 8.61 (s, 1H, C_9-*H*); Anal. Calcd. for $C_{14}H_8ClN_3O_2$ (285.68): C, 58.85, H, 2.82, N, 14.70. Found: C, 58.97, H, 2.89, N, 14.64.

1,8-Dichloro-4-cyano-3-phenylpyrido [1,2-a]benzimidazole (**9**)

Yield: 75%; mp: 262–264°C; IR (cm^{-1}): 2225(C≡N); ^{1}H-NMR (DMSO-d_6, δ ppm): 7.46–7.58 (m, 5H, Ar-*H*), 7.60–7.85 (m, 3H, Ar-*H*), 8.02 (s, 1H, C_9-*H*); Anal. Calcd. for $C_{18}H_9Cl_2N_3$ (338.19): C, 63.92, H, 2.68, N, 12.42. Found: C, 64.02, H, 2.74, N, 12.49.

1-Chloro 4-cyano-3-phenylpyrido [1,2-a]benzimidazole-8-carboxylic acid (**10**)

Yield: 80%; mp: >300°C; IR (cm^{-1}): 2225(C≡N), 1698 (C=O); ^{1}H-NMR (DMSO-d_6, δ ppm): 7.48–7.60 (m, 5H, Ar-*H*), 7.61–7.92 (m, 3H, Ar-*H*), 8.60 (s, 1H, C_9-*H*); Anal. Calcd. for $C_{19}H_{10}ClN_3O_2$ (347.75): C, 65.62, H, 2.89, N, 12.08. Found: C, 65.75, H, 2.95, N, 12.14.

*General procedure for preparation of compounds **11–14***

A mixture of the appropriate compound **7–10** (10 mmol), 4-(2-fluorophenyl) piperazine (1.8 g, 10 mmol) in dimethylformamide (10 ml) was stirred at room temperature for 72 h. The reaction mixture was poured onto ice-water (50 ml). The residue was filtered, and crystallized from dimethylformamide-water.

8-Chloro-4-cyano-1-(4-(2-fluorophenyl)piperazin-yl)-3-methylpyrido[1,2-a]benzimidazole (**11**)

Yield: 76%; mp: 267–269°C; IR (cm^{-1}): 2222 (C≡N); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.49 (s, 3H, CH_3), 2.95 (t, 2H, $J = 5.4$ Hz, piperazine-*H*), 2.99 (t, 2H, $J = 5.4$ Hz, piperazine-*H*), 3.52 (t, 4H, $J = 5.5$ Hz, piperazine-*H*), 6.81 (s, 1H, C_2-*H*), 7.01–7.17 (m, 3H, Ar-*H*), 7.31 (d, 1H, C_6-*H*), 7.67 (d, 1H, $J = 7.8$ Hz, C_7-*H*), 7.94 (s, 1H, Ar-*H*), 8.06 (s, 1H, C_9-*H*); MS: *m/z* 419.9 (M+, 100%), 422.0 (M + 2, 32.8%); Anal. Calcd. for $C_{23}H_{19}ClFN_5$ (419.89): C, 65.79, H, 4.56, N, 16.68. Found: C, 65.63, H, 4.62, N, 16.79.

4-Cyano-1-(4-(2-fluorophenyl)piperazin-yl)-3-methylpyrido[1,2-a]benzimidazole-8-carboxylic acid (**12**)

Yield: 79%; mp: 236–238°C; IR (cm^{-1}): 2210 (C≡N), 1690 (C=O); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.43 (s, 3H, CH_3), 2.91 (t, 4H, $J = 6.1$ Hz, piperazine-*H*), 3.49 (t, 4H, $J = 6.1$ Hz, piperazine-*H*), 6.89 (s, 1H, C_2-*H*), 6.94–7.12 (m, 3H, Ar-*H*), 7.36 (d, 1H, C_6-*H*), 7.71 (d, 1H, C_7-*H*), 7.94 (s, 1H, Ar-*H*), 8.01 (s, 1H, C_9-*H*), 13.30 (brs, 1H, COO*H*, D_2O exchangeable); MS: *m/z* 429.0 (M+, 0.053%); Anal. Calcd. for $C_{24}H_{20}FN_5O_2$ (429.43): C, 67.12, H, 4.69, N, 16.30. Found: C, 67.23, H, 4.62, N, 16.47.

8-Chloro-4-cyano-1-(4-(2-fluorophenyl)piperazin-yl)-3-phenylpyrido[1,2-a]benzimidazole (**13**)

Yield: 73%; mp: 288–290°C; IR (cm^{-1}): 2212 (C≡N); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.98 (m, 4H, piperazine-*H*), 3.59 (m, 4H, piperazine-*H*), 6.81 (s, 1H, C_2-*H*), 7.00–7.17 (m, 3H, Ar-*H*), 7.31 (d, 1H, C_6-*H*), 7.49–7.52 (m, 5H, Ar-*H*), 7.61 (d, 1H, C_7-*H*), 7.91 (s, 1H, Ar-*H*), 7.94 (s, 1H, C_9-*H*); MS: *m/z* 482.0 (M+, 44.8%), 483.9 (M + 2, 13.6%); Anal. Calcd. for $C_{28}H_{21}Cl\ FN_5$ (481.94): C, 69.78, H, 4.39, N, 14.53. Found: C, 69.65, H, 4.48, N, 14.65.

4-Cyano-1-(4-(2-fluorophenyl)piperazin-yl)-3-phenylpyrido[1,2-a]benzimidazole-8-carboxylic acid (**14**)

Yield: 82%; mp: 296–298°C; IR (cm^{-1}): 2224 (C≡N), 1678 (C=O); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.88 (t, 4H, $J = 6.5$ Hz, piperazine-*H*), 3.53 (m, 4H, $J = 6.5$ Hz, piperazine-*H*), 7.89–7.10 (m, 4H, C_2-*H* and Ar-*H*), 7.48–7.59 (m, 5H, Ar-*H*), 7.79–7.89 (m, 2H, C_6-*H* and C_7-*H*), 8.10 (s, 1H, Ar-*H*), 8.41 (s, 1H, C_9-*H*), 13.15 (brs, 1H, COO*H*, D_2O exchangeable); MS: *m/z* 491.3 (M+, 36.6%);

Anal. Calcd. for $C_{29}H_{22}FN_5O_2$ (491.50): C, 70.86, H, 4.51, N, 14.24. Found: C, 70.01, H, 4.49, N, 14.15.

*General procedure for the preparation of compounds **15** and **16***

Substituted 2-cyanomethyl benzimidazoles (**1** or **2**) (10 mmol), ammonium acetate (1.54 g, 20 mmol) and 2-acetyl butyrolactone (1.3 g, 10 mmol) were heated at 170–180°C for 24 h. The residue was broken up and extracted with ether. The residue was crystallized from dimethylformamide-water.

8-Chloro-4-cyano-2-(2-hydroxyethyl)-3- methyl-1-oxo-1H, 5H-pyrido[1,2-a]benzimidazole (**15**)

Yield: 68%; mp: 238–240°C; IR (cm^{-1}): 2210 (C≡N), 1662 (C=O); ^{1}H-NMR (CF_3COOD, δ ppm): 2.14 (s, 3H, CH_3), 2.38 (m, 2H,CH_2), 3.08 (m, 2H,CH_2), 4.56 (s, 1H,OH), 7.30 (d, 1H, $J = 8.8$ Hz, C_6-*H*), 7.64 (d, 1H, $J = 8.9$ Hz, C_7-*H*), 8.11 (s, 1H, C_9-*H*), 12.96 (brs, 1H, N*H*, D_2O exchangeable); Anal. Calcd. for $C_{15}H_{12}ClN_3O_2$ (301.72): C, 59.71, H, 4.00, N, 13.92. Found: C, 59.89, H, 4.05, N, 14.05.

4-Cyano-2-(2-hydroxyethyl)-3-methyl-1-oxo-1H, 5H-pyrido[1,2-a]benzimidazole-8-carboxylic acid (**16**)

Yield: 93%; mp: >300°C; IR (cm^{-1}): 3400–3066, 2208 (C≡N), 1666, 1654 (2C=O); ^{1}H-NMR (DMSO-d_6, δ ppm): 1.90 (s, 3H, CH_3), 2.30 (t, 2H, $J = 5.2$ Hz, CH_2), 2.74 (t, 2H, $J = 5.2$ Hz, CH_2), 4.55 (brs, 1H, OH), 7.67 (d, 1H, $J = 8.6$ Hz, C_6-*H*), 8.07 (d, 1H, $J = 8.6$ Hz, C_7-*H*), 8.28 (s, 1H, C_9-*H*), 12.67–13.54 (brs, 2H, N*H* and COO*H*, D_2O exchangeable); Anal. Calcd. for $C_{16}H_{13}N_3O_4$ (311.29): C, 61.73, H, 4.20, N, 13.49. Found: C, 61.61, H, 4.05, N, 13.35.

*General procedure for preparation of compounds **17** and **18***

The desired compound **15** or **16** (10 mmol) was refluxed with phosphorus oxychloride (15 ml) for 3 h. The excess phosphorus oxychloride was removed under vacuum, and the residue was treated with ice-water (50 ml). The residue was filtered, and crystallized from dimethylformamide-water.

1,8-Dichloro-4-cyano-2-(2-chloroethyl)-3-methylpyrido[1,2-a]benzimidazole (**17**)

Yield: 87%; mp: 225–227°C; IR (cm^{-1}): 2216 (C≡N); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.48 (s, 3H, CH_3), 2.89 (m, 2H, CH_2), 3.20 (m, 2H, CH_2), 7.59–7.81 (m, 2H, Ar-*H*), 8.10 (s, 1H, C_9-*H*); Anal. Calcd. for $C_{15}H_{10}Cl_3N_3$ (338.61): C, 53.20, H, 2.97, N, 12.40. Found: C, 53.33, H, 3.05, N, 12.35.

1-Chloro-4-cyano-2-(2-chloroethyl)-3- methyl pyrido[1,2-a]benzimidazole-8-carboxylic acid (**18**)

Yield: 93%; mp: 284–286°C; IR (cm^{-1}): 2220 (C≡N), 1685 (C=O); ^{1}H-NMR (DMSO-d_6, δ ppm): 2.49 (s, 3H, CH_3), 2.78 (m, 2H, CH_2), 3.06 (m, 2H, CH_2), 7.69 (d, 1H, $J = 8.1$ Hz, C_6-*H*), 8.01 (d, 1H, $J = 8.1$ Hz, C_7-*H*), 8.32 (s, 1H, C_9-*H*), 13.34 (brs, 1H, COO*H*, D_2O exchangeable); Anal. Calcd. for $C_{16}H_{11}Cl_2N_3O_2$ (348.18): C, 55.19, H, 3.18, N, 12.06. Found: C, 55.28, H, 3.25, N, 12.15.

*General procedure for preparation of compounds **19–20***

The same procedure as described for **11–14** using 4-(2-fluorophenyl) piperazine (3.6 g, 20 mmol).

8-Chloro-4-cyano-1-(4-(2-fluorophenyl)piperazin-yl)-2-[2-(4-(2-fluorophenyl)piperazin-yl)ethyl]-3-methylpyrido[1,2-a]benzimidazole (**19**)

Yield: 72%; mp: 287–289°C; IR (cm^{-1}): 2216 (C≡N); ^{1}H-NMR (CF_3COOD, δ ppm): 2.44 (s, 3H, CH_3), 2.73 (m, 2H, CH_2), 2.93 (t, 4H, $J = 5.1$ Hz, piperazine-*H*), 2.98 (t, 4H, $J = 5.1$ Hz, piperazine-*H*), 3.16 (m, 2H, CH_2), 3.44 (t, 4H, $J = 5.1$ Hz, piperazine-*H*), 3.52 (t, 4H, $J = 5.1$ Hz, piperazine-*H*), 6.90–7.05 (m, 6H, Ar-*H*), 7.53–7.68 (m, 2H, C_6-*H* and C_7-*H*), 7.81–7.83 (m, 2H, Ar-*H*), 7.94 (s, 1H, C_9-*H*); MS: *m/z* 626.2 (M+, 18.65%), 628.3 (M + 2, 6.87%); Anal. Calcd. for $C_{35}H_{34}ClF_2N_7$ (626.12): C, 67.13, H, 5.47, N, 15.65. Found: C, 67.25, H, 5.55, N, 15.79.

4-Cyano-1-(4-(2-fluorophenyl)piperazin-yl)-2-[2-(4-(2-fluorophenyl)piperazin-yl)ethyl]-3-methyl pyrido[1,2-a]benzimidazole-8-carboxylic acid (**20**)

Yield: 72%; mp: >300°C; IR (cm^{-1}): 2216 (C≡N), 1698 (C=O); ^{1}H-NMR (CF_3COOD, δ ppm): 2.46 (s, 3H, CH_3), 2.72 (m, 2H, CH_2), 2.92(t, 4H, $J = 5.4$ Hz, piperazine-*H*), 2.98 (t, 4H, $J = 5.4$ Hz, piperazine-*H*), 3.15 (m, 2H, CH_2), 3.44 (t, 4H, $J = 5.4$ Hz, piperazine-*H*), 3.52 (t, 4H, $J = 5.4$ Hz, piperazine-*H*), 7.00–7.16 (m, 6H, Ar-*H*), 7.58–7.81 (m, 2H, C_6-*H* and C_7-*H*), 7.93–8.05 (m, 2H, Ar-*H*), 8.10 (s, 1H, C_9-*H*), 13.25 (brs, 1H, COO*H*, D_2O exchangeable); MS: *m/z* 635.8 (M+, 41.89%); Anal. Calcd. for $C_{36}H_{35}F_2N_7O_2$ (635.68): C, 68.01, H, 5.54, N, 15.42. Found: C, 68.27, H, 5.39, N, 15.59.

Cytotoxic activity studies

Anticancer activity studies were done at Cairo University, National Cancer Institute, Cancer Biology Department, Pharmacology Unit.

Compounds **3**–**20** were tested at concentrations between 1 and 10 μg/ml using SulfoRhodamine-B (SRB) assay for cytotoxic activity against breast carcinoma cell line (MCF7).

Measurement of potential cytotoxicity by SRB assay

Potential cytotoxicity of the compounds was tested using the method of Skehan *et al.* (1990) as follows:

Cells were plated in 96 multiwell plate (104 cells/well) for 24 h before treatment with the compound(s) to allow attachment to the wall of the plate. Different concentrations of the compounds (0, 1, 2.5, 5, and 10 μg/ml) were added to the cell monolayer triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37°C in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained with SulfoRhodamine-B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader.

The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line after the specified compound.

Statistical analysis

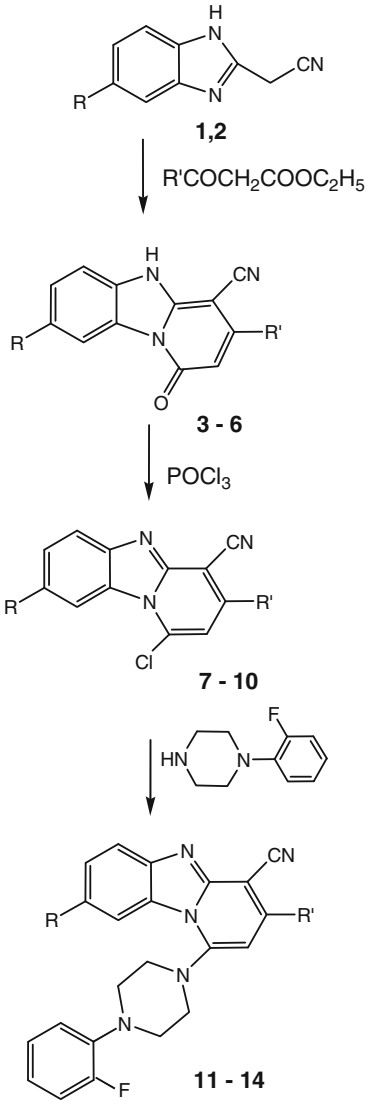
Differences between different treatment groups were analyzed using ANOVA followed by Dunnett *t*-test. *P* values of less than 0.05 were considered to represent a significant difference.

Results and discussion

Chemistry

The reaction sequence employed for synthesis of the target compounds is shown in Schemes 1 and 2. The starting materials, substituted 2-cyanomethyl benzimidazoles (**1** and **2**) were conveniently prepared according to reported methods (Refaat, 2010). Compounds **1** and **2** were cyclized to 4-cyano-3-substituted-1-oxo-1H, 5H-pyrido[1,2-a]benzimidazoles (**3**–**6**, **15**, **16**), by heating it with ethyl acetoacetate, ethyl benzoylacetate or 2-acetyl butyrolactone in presence of ammonium acetate at 170–180°C. The cyclization of 4-cyano-3-substituted-1-oxo-1H,5H-pyrido[1,2-a] benzimidazoles (**3**–**6**, **15**, **16**) was evidenced by its ^{1}H-NMR and IR spectra. The ^{1}H-NMR spectra of compounds **3**–**6** showed a sharp singlet at δ 5.90–6.10 ppm due to C_2 proton, whereas the ^{1}H-NMR spectra of compounds **15** and **16** displayed two multiplet at δ 2.30–2.38 and 2.74–4.08 ppm. On the other hand, the IR spectra of compounds **3**–**6**, **15** and **16** showed the characteristic C=O stretching absorption.

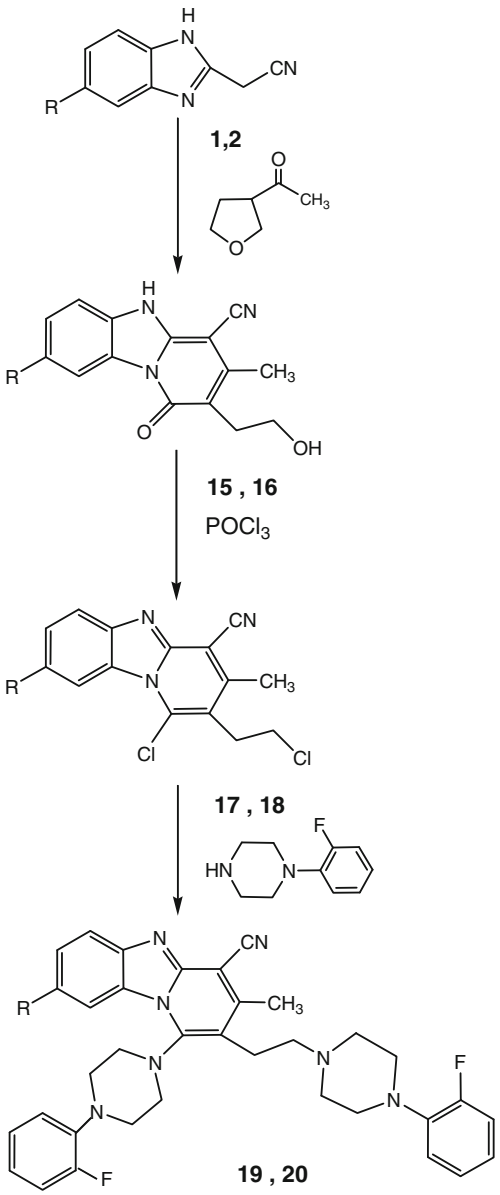
On refluxing 4-cyano-3-substituted-1-oxo-1H, 5H-pyrido[1,2-a]benzimidazole **3**–**6**, **15**, **16**, with phosphorus oxychloride, the corresponding 1-chloro derivatives **7**–**10**, **17**, **18** were produced. The IR spectra of compounds **7**–**10**, **17**, and **18** proved as useful in tracing the disappearance of the C=O



1 R = Cl
2 R = COOH

3, 7, 11	R= Cl	, R' = CH_3
4, 8, 12	R= COOH	, R' = CH_3
5, 9, 13	R= Cl	, R' = C_6H_5
6, 10, 14	R= COOH	, R' = C_6H_5

Scheme 1 Synthesis of compounds **3**–**14**



Scheme 2 Synthesis of compounds **15**–**20**

stretching absorption of the parent compounds **3**–**6**, **15**, and **16**.

On the other hand, the synthesis of 4-cyano-1-(4-(2-fluorophenyl)piperazin-yl)-3-methylpyrido[1,2-a]benzimidazoles **11**–**14** and 4-cyano-1-(4-(2-fluorophenyl) piperazin-yl)-2-[2-(4-(2-fluorophenyl)piperazin-yl)ethyl]-3-methylpyrido[1,2-a]benzimidazoles **19** and **20** was achieved via the reaction of the chloro derivatives **7**–**10**, **17**, **18** with 4-(2-flourophenyl) piperazine. The ^{1}H-NMR and mass spectra were consistent with the proposed structures.

Cytotoxic activity

The cytotoxicity of compounds **3**–**20** was evaluated against human breast adenocarcinoma cell line (MCF7). For comparison purposes, the cytotoxicity of doxorubicin, a standard antitumor drug, was evaluated under the same conditions. The IC_{50} values (dose of the compound which caused a 50% reduction of survival values) are shown in Table 1. The results are represented graphically in Figs. 2, 3, 4, 5, 6, and 7.

All the tested compounds were found to possess potential antitumor activities against all of the tested tumor cell lines, with IC_{50}'s 3.43–14.70 μg/ml (Table 1). Among the 4-cyano-1-oxo-pyrido[1,2-a]benzimidazole series **3**–**6**, **15**, and **16**, compounds **3**, **5**, and **6** showed significant activity, compound **16** was moderately potent, whereas compounds **4** and **15** were the least active. This was of particular interest since all the previously prepared 4-cyano-1-oxo-pyrido [1,2-a]benzimidazoles and their 6,7-dimethyl analogs were inactive as anticancer agents (Rida *et al.,* 1988a, b; Badawey and kappe, 1995, 1999; EL-Hawash *et al.,* 1999). This observation confirms the importance of the structure parameters for the benzimidazole ring substituent in determining the potential antitumor activity.

Table 1 IC_{50} values of compounds **3**–**20** against MCF7

Compd. no	IC_{50} (μg/ml)
3	3.43 ± 0.03
4	14.70 ± 0.07
5	3.58 ± 0.10
6	3.99 ± 0.04
15	14.62 ± 0.07
16	10.55 ± 0.07
7	8.61 ± 0.03
8	12.82 ± 0.04
9	3.89 ± 0.07
10	3.58 ± 0.07
17	7.09 ± 0.04
18	9.07 ± 0.05
11	5.72 ± 0.01
12	12.60 ± 0.04
13	10.40 ± 0.03
14	17.65 ± 0.07
19	14.00 ± 0.08
20	9.90 ± 0.04
DOX	2.97 ± 0.03
P value	0.000*

IC_{50} (μg/ml): dose of the compound which caused a 50% reduction of survival. Values were calculated from dose-response curves done in triplicate for each compound. Values were given ± standard deviation. *MCF7* human breast adenocarcinoma, *Dox* Doxorubicin

* There is a significant difference by using one way ANOVA at $P < 0.05$

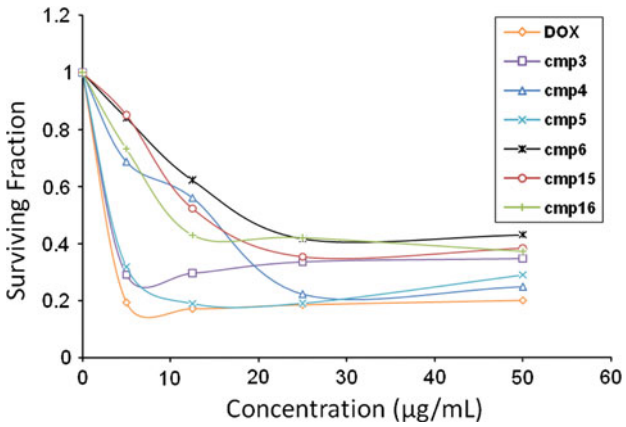


Fig. 2 Cytotoxicity of **3**–**6**, **15**, **16**, and doxorubicin against human breast adenocarcinoma cell line (MCF7)

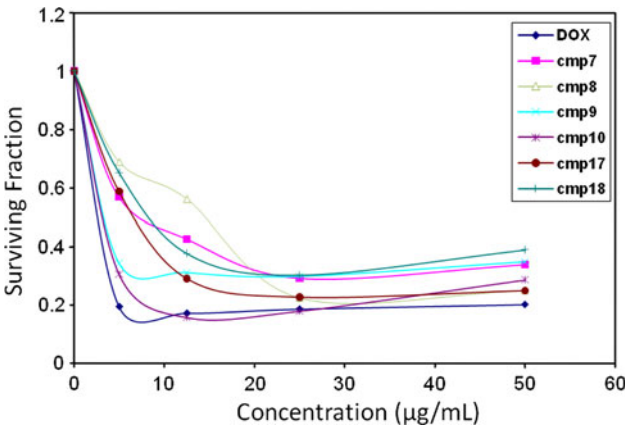


Fig. 3 Cytotoxicity of **7**–**10**, **17**, **18**, and doxorubicin against human breast adenocarcinoma cell line (MCF7)

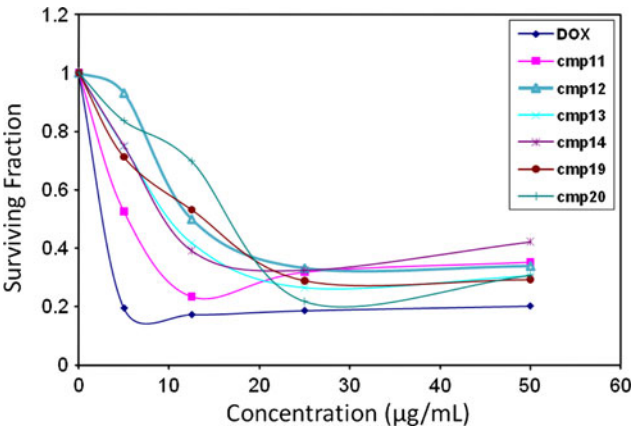


Fig. 4 Cytotoxicity of **11**–**14**, **19**, **20**, and doxorubicin against human breast adenocarcinoma cell line (MCF7)

Within the 1-chloro-4-cyano-pyrido[1,2-a]benzimidazole **7**–**10** and the 1,8-dichloro **17**, **18** series, compounds **9** and**10** displayed a notable potency while compounds **17**, **7**, **18**, and **8** were moderately potent.

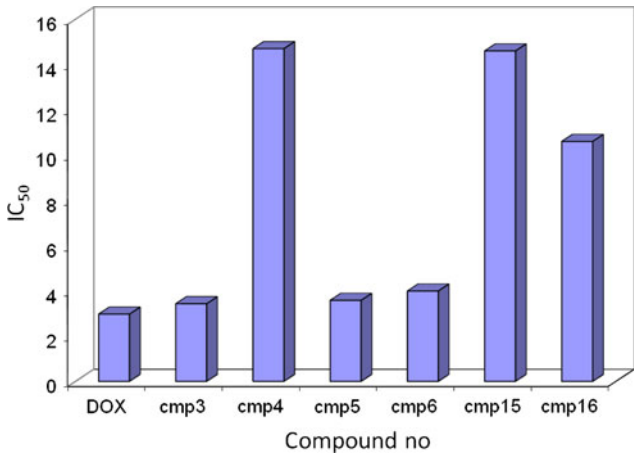


Fig. 5 IC_{50} values of compounds **3**–**6**, **15**, and **16** against MCF7

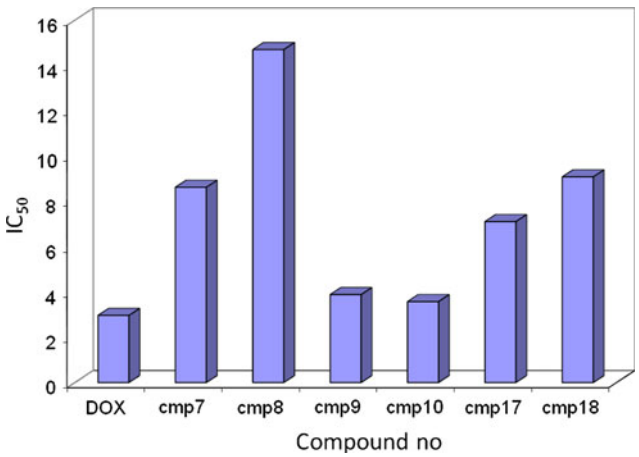


Fig. 6 IC_{50} values of compounds **7**–**10**, **17**, and **18** against MCF7

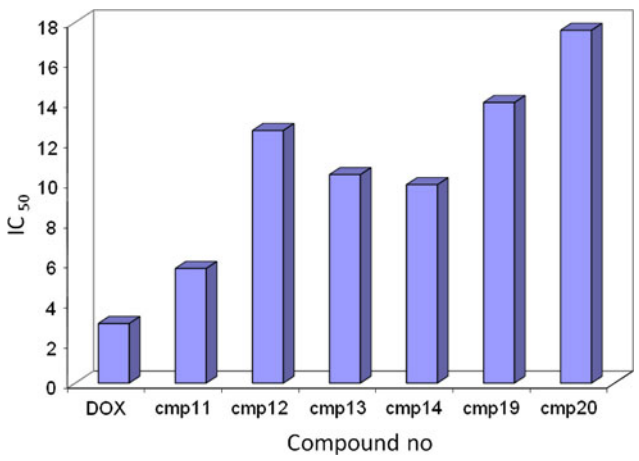


Fig. 7 IC_{50} values of compounds **11**–**14**, **19**, and **20** against MCF7

On the other hand, in the 1-(4-(2-fluorophenyl)piperazin-yl)pyrido[1,2-a]benzimidazole **11**–**14** and 1-(4-(2-fluorophenyl)piperazin-yl)-2-[2-(4-(2-fluorophenyl)piperazin-yl) ethyl]pyrido[1,2-a]benzimidazole **19**, **20** series, the most

pronounced activity was recorded for compound **11**, while compounds **20**, **13**, and **12** were moderately active and compounds **19** and **14** were the least active. Apparently, the activity exhibited by compounds **11**–**14**, **19**, and **20** was not improved by the incorporation of 2-fluorophenylpiperazinyl moiety, as estimated, however, they showed lower activity than their precursor 1-oxo and 1-chloro derivatives.

Conclusion

The present research study reports the successful synthesis, antitumor study of two new series of pyrido[1,2-a]benzimidazoles derivatives. Their screening results revealed that all the compounds showed moderate to very good activities against human breast adenocarcinoma (MCF7) cell line. On this basis, it can be concluded that a combination of 5-chloro or 5-underivatized carboxylic acid group to pyrido[1,2-a]benzimidazoles core showed an increased antitumor activity and hence they are ideally suited for further modifications to obtain more effective antitumor compounds.

References

Andrzejewska M, Ye′pez-Mulia L, Cedillo-Rivera R, Tapia A, Vilpo L, Vilpo J, Kazimierczuk Z (2002) Synthesis, antiprotozoal and anticancer activity of substituted 2-trifluoromethyl-and 2-pentafluoroethylbenzimidazoles. Eur J Med Chem 37:973–978

Badawey EAM, Kappe T (1995) Benzimidazole condensed ring system. IX. Potential antineoplastics. New synthesis of some pyrido[1,2-a]benzimidazoles and related derivatives. Eur J Med Chem 30:327–332

Badawey EAM, Kappe T (1999) Benzimidazole condensed ring systems. XI. Synthesis of some cycloalkyl pyrido[l,2-a]benzimidazoles with anticipated antineoplastic activity. Eur J Med Chem 34:663–667

Badawey EAM, Rida SM, Soliman FSG, Kappe T (1989) Benzimidazole condensed ring systems. III [1]. Synthesis of some substituted 2,3-dihydrocyclopenta-lH-[4′,5′:2,3]pyrido[1, 2-a]benzimidazole-11-carbonitriles. Monatsch Chem 120:73–76

Demirayak S, Mohsen UA (1998) Anticancer and anti-HIV activities of some pyrido/pyrazino-benzimidazole derivatives. Acta Pharm Turcica 40:9–12

Dupuy M, Pinguet F, Blache Y, Chavignon O, Teulade JC, Chapat JP (1998) Regioselective synthesis of pyrazolo[4,5-g]pyrido[1,2-a] benzimidazoles: cytotoxic derivatives of pyrido[1,2-a]benzimidazolic ring system. Chem Pharm Bull 46:1820–1823

Dupuy M, Pinguet F, Chavignon O, Chezal JM, Teulade JC, Chapat JP, Blache Y (2001) Synthesis and in vitro cytotoxic evaluation of new derivatives of pyrido[1,2-a]benzimidazolic ring system: The pyrido[1′,2′:1,2]imidazo[4,5-h]quinazolines. Chem Pharm Bull 49:1061–1065

EL-Hawash SAM, Badawey EAM, Kappe T (1999) Benzimidazole condensed ring systems. XII. Synthesis and anticancer evaluation of certain pyrido[l,2-a]benzimidazole derivatives. Pharmazie 54:341–346

Galal SA, Hegab KH, Kassab AS, Rodriguez ML, Kerwin SM, El-Khamry AA, El Diwani HI (2009) New transition metal ion complexes with benzimidazole-5-carboxylic acid hydrazides with antitumor activity. Eur J Med Chem 44:1500–1508

Graham K, Wang Q, Boy RG, Eisenhut M, Haberkorn U, Mier W (2007) Synthesis and evaluation of intercalating somatostatin receptor binding peptide conjugates for endoradiotherapy. J Pharm Pharm Sci 10:286–297

Jordan AD, Vaidya AH, Rosenthal DI, Dubinsky B, Kordik CP, Sanfilippo PJ, Wu WN, Reitz AB (2002) Potential anxiolytic agents. Part 4: novel orally-active N5-substituted pyrido[1,2-a]benzimidazoles with high GABA-A receptor affinity. Bioorg Med Chem Lett 12:2381–2386

Lazareno S, Popham A, Birdsall NJ (2002) Analogs of WIN 62, 577 define a second allosteric site on muscarinic receptors. Mol Pharmacol 62:1492–1505

Pinar A, Yurdakul P, Yildiz I, Temiz-Arpaci O, Acan NL, Aki-Sener E, Yalcin I (2004) Some fused heterocyclic compounds as eukaryotic topoisomerase II inhibitors. Biochem Biophys Res Commun 317:670–674

Ram S, Wise DS, Wotring LL, McCall JW, Town-send LB (1992) Synthesis and biological activity of certain alkyl 5-(alkoxycarbonyl)-1H-benzimidazole-2-carbamates and related derivatives: a new class of potential antineoplastic and antifilarial agents. J Med Chem 35:539–547

Refaat HM (2010) Synthesis and anticancer activity of some novel 2-substituted benzimidazole derivatives. Eur J Med Chem 45: 2949–2956

Reitz AB, Nortey SO, Sanfilippo P, Scott KM (2004) US Patent 6358946. CN1690044A

Rida SM, Soliman FSG, Badawey EAM, El-Ghazzawi E, Kader O, Kappe T (1988a) Benzimidazole condensed ring systems. 1. Synthesis and biological investigations of some substituted pyrido [l,2-a]benzimidazoles. J Heterocycl Chem 25:1087–1093

Rida SM, Soliman FSG, Badawey EAM, Kappe T (1988b) Benzimidazole condensed ring systems. 2 [1]. New synthesis of substituted 1-oxo-1H, 5H-pyrido[1,2-a]benzimidazole-4-carbonitriles and related derivatives. J Heterocycl Chem 25:1725–1728

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 82:1107–1112

Soliman FSG, Rida SM, Badawey EAM, Kappe T (1984) Synthesis of substituted 3-hydroxy-1H, 5H-pyrido[1,2-a]-Benzimidazol-1-ones as possible antimicrobial and antineoplastic agents. Arch Pharm 317:951–958

Wu WN, McKown LA, Reitz AB (2006) Metabolism of the new anxiolytic agent, a pyrido[1,2-]benzimidazole (PBI) analog (RWJ-53050), in rat and human hepatic S9 fractions, and in dog: identification of cytochrome p450 isoforms mediated in the human microsomal metabolism. Eur J Drug Metab Pharmacokinet 31: 277–283