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Synthesis of new heterocyclic compounds containing benzimidazole moiety as inhibitors of breast cancer cell growth

Abstract: A series of new benzimidazole derivatives, namely 2-acylbenzimidazoles **2–9**, a dihydroquinoxaline **10**, a benzoxazine **11**, quinolines **13–15** and fused 1,2,4-triazines **17–24** were synthesized. Structure elucidation of the compounds was conducted using IR, ^{1}H NMR, ^{13}C NMR, mass spectral data and elemental analysis. These products were evaluated for *in vitro* antitumor activity against MCF7 cell line (human breast cancer). Compounds **13–15** and **24** manifested significant antitumor activity.

Keywords: antitumor; benzimidazoles; benzoxazines; quinolines; quinoxalines; 1,2,4-triazines; synthesis.

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Introduction

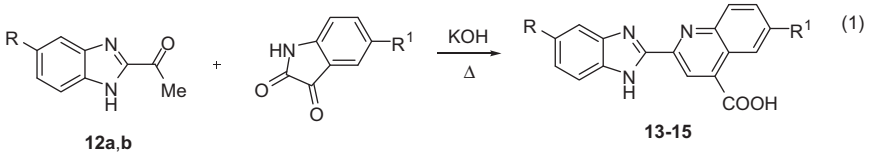
Cancer represents the largest cause of death worldwide [1]. Despite progress in the efficacy of surgical operations to remove tumors, treatment with chemotherapy is considered to be one of the most important approaches. A literature survey has revealed the importance of benzimidazoles as antitumor agents [2–12] including bendamustine, dovitinib, Hoechst 33342 and Hoechst 33258 (Figure 1). Recently, benzoxazines [13, 14] and quinoxalines [15, 16] have been identified as anticancer agents. Certain quinolines [17–20] and 1,2,4-triazines [21–23] are also active. Hence, the aim of the current study was the synthesis of novel benzimidazole derivatives that incorporate benzoxazine, quinoxaline, quinoline and 1,2,4-triazine moieties. It was reasoned that this type of molecular combination might lead to finding compounds with improved antitumor activity.

Results and discussion

Chemistry

The target compounds were synthesized as depicted in Scheme 1 and Equations 1–2. The key intermediate, 1-(1*H*-benzimidazol-2-yl)-2-bromoethanone (**1**), was synthesized as previously reported [24]. Treatment of **1** with the appropriate substituted anilines gave the corresponding products **2–5** (Scheme 1). The reaction of **1** with sodium benzoate or its 2-hydroxy derivative yielded the respective benzoate **6** or **7**. In addition, the desired hydrazides **8** and **9** were synthesized by treatment of the substrate **1** with the appropriate hydrazides. By contrast, the reaction of **1** with 1,2-phenylenediamine or 2-aminophenol gave 3-(1*H*-benzimidazol-2-yl)-1,2-dihydroquinoxaline (**10**) and 3-(1*H*-benzimidazol-2-yl)-2*H*-benzo[*b*][1,4]oxazine (**11**), respectively. All compounds **2–11** were fully characterized by spectroscopic methods and elemental analysis.

Known 2-acetylimidazoles **12a,b** [25] were allowed to react with isatins to furnish the desired 2-(1*H*-benzimidazole-2-yl)-6-substituted quinoline-4-carboxylic acids **13–15** (Equation 1).



(1)

12a,**b**

12a: R=H
12b: R=Me

13-15

13: R= H, R^1= Cl
14: R= Me, R^1= H
15: R= Me, R^1= Me

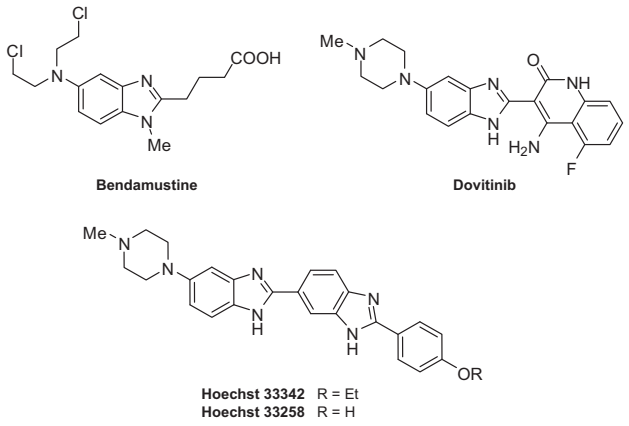
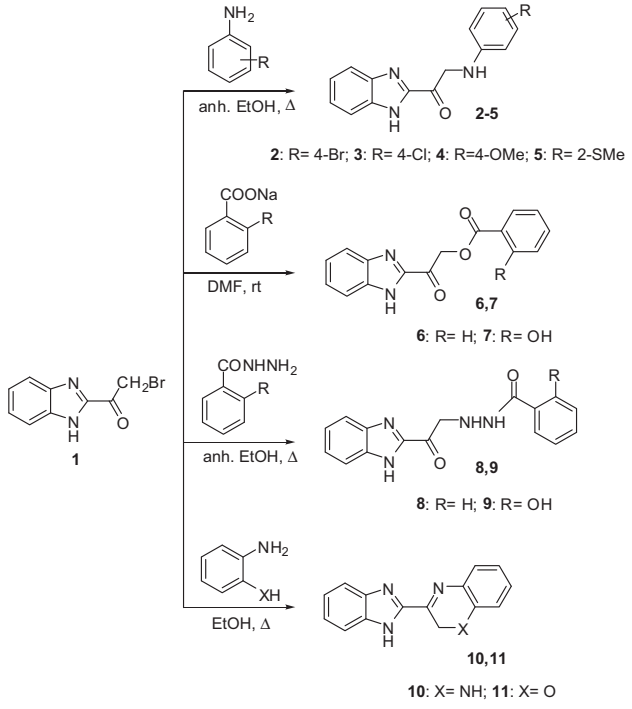
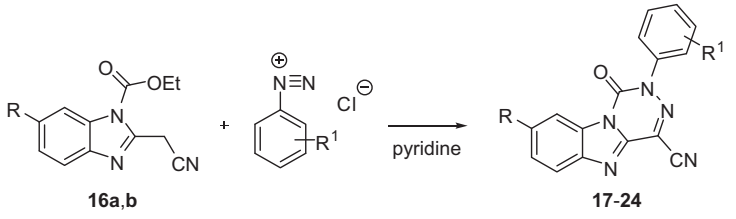


Figure 1 Structures of some antitumor drugs containing benzimidazole moiety.

Finally, the substrates **16a** [26–28] and **16b** were used in the preparation of benzimidazo[1,2-*d*][1,2,4]triazine-4-carbonitriles **17–24** (Equation 2). Again, the given structures of the synthesized compounds **12–24** were fully consistent with the spectroscopic and elemental analysis data.



Scheme 1



(2)

16a: R= H
16b: R= CH_3

17: R= H, R^1= 2-Cl
18: R= H, R^1= 3-Cl
19: R= H, R^1= 4-OMe
20: R= H, R^1= 2-SMe

21: R= Me, R^1= 2-Cl
22: R= Me, R^1= 3-Cl
23: R= Me, R^1= 4-OMe
24: R= Me, R^1= 2-SMe

Biology

All compounds **2–15** and **17–24** were evaluated for their *in vitro* antitumor activity against human MCF7 cell line (breast cancer) using a one dose primary anticancer *in vitro* assay [29, 30]. The results are presented in Table 1. The requirement for antitumor activity set by the National Cancer Institute is that the fraction of surviving tumor cells is 30% or less, which corresponds to inhibition of 70% or more. According to this definition it may be concluded that compounds **13–15** and **24** are active. The IC_{50} values for the active compounds **13–15** and **24** are also given in Table 1.

Conclusion

A series of benzimidazoles **2–11**, **13–15** and **17–24** were synthesized and evaluated for their *in vitro* antitumor activity against MFC7 cell line. Compounds **13–15** and **24** exhibit significant activity.

Table 1 Antitumor activity of compounds **2–11**, **13–15** and **17–24** against MCF7 cell line.

Compound	% Surviving	% Inhibition	IC_{50}[b], μg/mL
2	40.5	59.5	
3	33.2	66.8	
4	32.9	67.1	
5	48.4	51.6	
6	39.5	60.5	
7	34.3	65.7	
8	31.2	68.8	
9	30.8	69.2	
10	47.4	52.6	
11	42.7	57.3	
13	28.1	71.9[a]	16.3
14	26.0	74.0[a]	15.5
15	27.0	73.0[a]	12.7
17	41.4	58.6	
18	32.9	67.1	
19	40.6	59.4	
20	34.4	65.6	
21	37.1	62.9	
22	32.8	67.2	
23	33.2	66.8	
24	29.9	70.1[a]	16.7

[a]Compounds showing significant antitumor activity. [b]IC_{50} for active antitumor compounds.

Experimental

General

Unless otherwise noted, all materials were obtained from commercial suppliers (Aldrich and Merck companies) and used without further purification. Melting points were recorded using an Electrothermal C14500 apparatus and were uncorrected. Microanalyses were performed at the microanalytical unit, Cairo University. IR spectra were recorded on a Mattson 5000 FT-IR spectrometer using KBr disks. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance-400 spectrometer in DMSO-d_6 at Georgia State University, Atlanta, GA, USA and the Korea Institute of Science and Technology, Republic of Korea. Mass spectral analyses were performed on a JOEL JMS-600H spectrometer at Cairo University. Reaction progress was monitored using TLC on silica gel plates 60 F_{245} E. Merck, and the spots were visualized by UV light at 366 nm or 245 nm. Substrates **1** [24], **12a,b** [25] and **16a** [26–28] were synthesized according to reported methods.

1-(1*H*-Benzimidazol-2-yl)-2-[(substituted phenyl)amino]ethanones 2–5

A mixture of 1-(1*H*-benzimidazol-2-yl)-2-bromoethanone **1** [24] (0.5 g, 0.002 mol), a substituted aniline (0.002 mol) and sodium bicarbonate (1.55 g) in anhydrous ethanol (50 mL) was heated under reflux for 4 h. Then the mixture was allowed to cool to room temperature and the resultant precipitate was filtered, washed thoroughly with water, dried and crystallized from absolute ethanol to afford the title compound **2–5**.

1-(1*H*-Benzimidazol-2-yl)-2-[(4-bromophenyl)amino]ethanone (2) Yellow compound; yield 68%; mp 158–160°C; ^{1}H NMR: δ 4.40 (s, 2H, CH_2), 6.28 (s, 1H, NH, D_2O exchangeable), 6.48 (d, *J* = 8, 2H), 7.12 (d, *J* = 8, 2H), 7.24 (d, *J* = 8.7, 2H), 7.66 (d, *J* = 8.7, 2H), 12.81 (s, 1H, NH, D_2O exchangeable); MS: m/z 330 [M^+], 332 [M^++2]. Anal. Calcd for $C_{15}H_{12}BrN_3O$: C, 54.56; H, 3.66; N, 12.73. Found: C, 54.49; H, 3.61; N, 12.82.

1-(1*H*-Benzimidazol-2-yl)-2-[(4-chlorophenyl)amino]ethanone (3) Yellowish brown compound; yield 72%; mp 182–184°C; ^{1}H NMR: δ 5.12 (s, 2H, CH_2), 6.24 (s, 1H, NH, D_2O exchangeable), 6.54 (d, *J* = 7.5, 2H), 7.17 (d, *J* = 7.5, 2H), 7.27 (d, *J* = 8.7, 2H), 7.59 (d, *J* = 8.7, 2H), 12.81 (s, 1H, NH, D_2O exchangeable); MS: m/z 285 [M^+], 287 [M^++2]. Anal. Calcd for $C_{15}H_{12}ClN_3O$: C, 63.05; H, 4.23; N, 14.71. Found: C, 63.11; H, 4.17; N, 14.68.

1-(1*H*-Benzimidazol-2-yl)-2-[(4-methoxyphenyl)amino]ethanone (4) Brown compound; yield 70%; mp 199–201°C; ^{1}H NMR: δ 3.80 (s, 3H, OCH_3), 4.19 (s, 2H, CH_2), 6.50 (s, 1H, NH, D_2O exchangeable), 6.70 (d, *J* = 7.8, 2H), 6.90 (d, *J* = 7.8, 2H), 7.22 (d, *J* = 8.4, 2H), 7.59 (d, *J* = 8.4, 2H), 12.81 (s, 1H, NH, D_2O exchangeable); MS: m/z 281 [M^+]. Anal. Calcd for $C_{16}H_{15}N_3O_2$: C, 68.31; H, 5.37; N, 14.94. Found: C, 68.24; H, 5.45; N, 15.03.

1-(1*H*-Benzimidazol-2-yl)-2-[(2-methylthiophenyl)amino]ethanone (5) Brown compound; yield 79%; mp 151–153°C; ^{1}H NMR: δ 2.33 (s, 3H, SCH_3), 5.18 (s, 2H, CH_2), 6.22 (s, 1H, NH, D_2O exchangeable), 6.28 (t, *J* = 7.8, 1H), 6.57 (t, *J* = 7.8, 1H), 6.82 (d, *J* = 7.8, 1H), 7.10 (d, *J* = 7.8, 1H), 7.22 (d, *J* 8.7, 2H), 7.59 (d, *J* = 8.7, 2H), 12.81 (s, 1H, NH, D_2O exchangeable); MS: m/z 297 [M^+]. Anal. Calcd for $C_{16}H_{15}N_3OS$: C, 64.62; H, 5.08; N, 14.13. Found: C, 64.66; H, 5.13; N, 14.09.

1-(1*H*-Benzimidazol-2-yl)-2-oxoethyl benzoates 6, 7

Sodium benzoate or sodium 2-hydroxybenzoate (0.002 mol) was added at room temperature to a solution of 1-(1*H*-benzimidazol-2-yl)-2-bromoethanone **1** [24] (0.5 g, 0.002 mol) in DMF. Stirring at room temperature was continued for another 8 h. Then the mixture was poured into water and the precipitated product was filtered, washed thoroughly with water, dried and crystallized from DMF to afford the title compound **6, 7**.

1-(1*H*-Benzimidazol-2-yl)-2-oxoethyl benzoate (6) Orange compound; yield 72%; mp 136–138°C; IR: 1660 (-CO-CH_2), 1740 (-COO-CH_2), 2985, 2960 (CH_2), 3150 cm^{-1} (NH); ^{1}H NMR: δ 5.86 (s, 2H, CH_2), 7.22 (d, *J* = 8.7, 2H), 7.46 (t, *J* = 8Hz, 1H), 7.59 (d, *J* = 8.7, 2H), 7.66 (t, *J* = 8Hz, 2H), 8.03 (d, *J* = 8Hz, 2H), 12.01 (s, 1H, NH, D_2O exchangeable); MS: m/z 280 [M^+]. Anal. Calcd for $C_{16}H_{12}N_2O_3$: C, 68.56; H, 4.32; N, 9.99. Found: C, 68.51; H, 4.28; N, 10.11.

1-(1*H*-Benzimidazol-2-yl)-2-oxoethyl 2-hydroxybenzoate (7) Yellow compound; yield 75%; mp 158–160°C; IR: 1650 (-CO-CH_2), 1740 (-COO-CH_2), 2940, 2875 (CH_2), 3100 (NH), 3500 cm^{-1} (OH); ^{1}H NMR: δ 5.89 (s, 2H, CH_2), 6.88 (d, *J* = 7.7 Hz, 1H), 7.01 (t, *J* = 7.7 Hz,

1H), 7.12 (t, *J* = 7.7 Hz, 1H), 7.24 (d, *J* = 8.7 Hz, 2H), 7.59 (d, *J* = 8.7 Hz, 2H), 7.88 (d, *J* = 7.7 Hz, 1H), 10.29 (s, 1H, OH), 12.01 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 60.2, 111.6, 114.3, 116.2, 120.2, 122.1, 129.2, 130.1, 132.4, 150.2, 155.1, 160.9, 163.2; MS: m/z 296 [M^+]. Anal. Calcd for $C_{16}H_{12}N_2O_4$: C, 64.86; H, 4.08; N, 9.46. Found: C, 64.91; H, 4.11; N, 9.39.

N'-[2-(1*H*-Benzimidazol-2-yl)-2-oxoethyl] benzohydrazides 8, 9

A mixture of 1-(1*H*-benzimidazol-2-yl)-2-bromoethanone **1** [24] (0.5 g, 0.002 mol), an acid hydrazide (0.002 mol) and sodium bicarbonate (1.55 g) in absolute ethanol (50 mL) was heated under reflux for 6 h. Then the mixture was allowed to cool to room temperature and the resulting precipitate was filtered, washed thoroughly with water, dried and crystallized from absolute ethanol to afford the title compound **8**, **9**.

***N'*-[2-(1*H*-Benzimidazol-2-yl)-2-oxoethyl]benzohydrazide (8)** Yellow compound; yield 74%; mp 150–152°C; IR: 1662 (-CO-CH_2), 1685 (C=O hydrazino), 2960, 2890 (CH_2), 3150, 3350, 3410 cm^{-1} (NH); ^1H NMR: δ 3.34 (s, 2H, CH_2), 5.0 (s, 1H, NH, D_2O exchangeable), 7.22 (d, *J* = 8.7 Hz, 2H), 7.42 (t, *J* = 8 Hz, 2H), 7.59 (d, *J* = 8.7 Hz, 2H), 7.63 (t, *J* = 8 Hz, 1H), 7.84 (d, *J* = 8 Hz, 2H), 8.0 (s, 1H, NH, D_2O exchangeable), 12.10 (s, 1H, NH, D_2O exchangeable); MS: m/z 294 [M^+]. Anal. Calcd for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.79; N, 19.04. Found: C, 65.37; H, 4.71; N, 19.13.

***N'*-[2-(1*H*-Benzimidazol-2-yl)-2-oxoethyl]-2-hydroxybenzohydrazide (9)** Yellow compound; yield 71%; mp 177–179°C; IR: 1662 (-CO-CH_2), 1685 (C=O hydrazino), 2972, 2860 (CH_2), 3150, 3350, 3410 (NH), 3500 cm^{-1} (OH); ^1H NMR: δ 3.34 (s, 2H, CH_2), 5.0 (s, 1H, NH, D_2O exchangeable), 6.95 (d, *J* = 7.7 Hz, 1H), 7.03 (t, *J* = 7.7 Hz, 1H), 7.22 (d, *J* = 8.7 Hz, 2H), 7.32 (t, *J* = 7.7 Hz, 1H), 7.59 (d, *J* = 8.7 Hz, 2H), 7.86 (d, *J* = 7.7 Hz, 1H), 8.0 (s, 1H, NH, D_2O exchangeable), 11.50 (s, 1H, OH), 12.10 (s, 1H, NH, D_2O exchangeable); MS: m/z 310 [M^+]. Anal. Calcd for $C_{16}H_{14}N_4O_3$: C, 61.93; H,4.55; N, 18.06. Found: C, 61.99; H, 4.50; N, 17.97.

3-(1*H*-Benzimidazol-2-yl)-1,2-dihydroquinoxaline (10) and 3-(1*H*-benzimidazol-2-yl)-2*H*-benzo[*b*][1,4]oxazine (11)

A mixture of 1-(1*H*-benzimidazol-2-yl)-2-bromoethanone **1** [24] (0.01 mol), 1,2-phenylenediamine or 2-aminophenol (0.011 mol), and ethanol (20 mL) was heated under reflux for 3 h. After cooling, the separated solid was filtered and washed with ethanol. The precipitate was then suspended in water, stirred with saturated sodium bicarbonate solution (30 mL, 5%), filtered, washed thoroughly with water, dried and crystallized from ethyl acetate/ethanol (1:1) to afford the title compound **10** or **11**.

3-(1*H*-Benzimidazol-2-yl)-1,2-dihydroquinoxaline (10) Yellow compound; yield 79%; mp 227–229°C; ^1H NMR: δ 3.2 (s, 2H, CH_2), 5.0 (s, 1H, NH, D_2O exchangeable), 6.89 (d, *J* = 8.8 Hz, 1H), 7.01 (t, *J* = 8.8 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 2H), 7.32 (t, *J* = 8.8 Hz, 1H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 2H), 12.0 (s, 1H, NH, D_2O exchangeable); MS: m/z 248 [M^+]. Anal. Calcd for $C_{15}H_{12}N_4$: C, 72.56; H, 4.87; N, 22.57. Found: C, 72.62; H, 4.75; N, 22.69.

3-(1*H*-Benzimidazol-2-yl)-2*H*-benzo[*b*][1,4]oxazine (11) Brown compound; yield 76%; mp 211–213°C; ^1H NMR: δ 4.0 (s, 2H, CH_2), 6.68 (d, *J* = 8.2 Hz, 1H), 7.02 (t, *J* = 8.2 Hz, 1H), 7.22 (d, *J* = 8.7 Hz, 2H), 7.43 (t, *J* = 8.2 Hz, 1H), 7.59 (d, *J* = 8.7 Hz, 2H), 7.81 (d, *J* = 8.2 Hz, 1H), 12.01 (s, 1H, NH, D_2O exchangeable); MS: m/z 249 [M^+]. Anal. Calcd for $C_{15}H_{11}N_3O$: C, 72.28; H, 4.45; N, 16.86. Found: C, 72.21; H, 4.34; N, 16.82.

2-(1*H*-Benzimidazol-2-yl)quinoline-4-carboxylic acids 13–15

A solution of potassium hydroxide (1.02 g, 0.018 mol) in water (5 mL) was added dropwise to the appropriate isatin derivatives (0.003 mol) in ethanol (10 mL) over 15 min. The appropriate benzimidazole derivatives **12a,b** [25] (0.003 mol) were added and the reaction mixture was heated under reflux for 18 h, then cooled to room temperature and the solvent was removed under vacuum. The resulting solid was dissolved in water, washed with diethyl ether, cooled in ice-cold water and acidified with acetic acid. The separated solid was filtered and recrystallized from acetic acid to afford the title compounds **13–15**.

2-(1*H*-Benzimidazol-2-yl)-6-chloroquinoline-4-carboxylic acid (13) Yellow compound; yield 69%; mp 257–259°C; IR: 1725 (CO), 3200 (NH), 3400 cm^{-1} (OH); ^1H NMR: δ 7.22 (d, *J* = 8.4 Hz, 2H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.86 (d, *J* = 8.6 Hz, 1H), 8.26 (d, *J* = 8.6 Hz, 1H), 8.47 (s, 1H), 9.07 (s, 1H), 12.81 (s, 1H, NH, D_2O exchangeable), 13.21 (s, 1H, COOH); ^{13}C NMR: δ 114.8, 122.3, 123.1, 124.0, 124.5, 126.8, 129.0, 130.1, 133.2, 140.2, 145.1, 152.7, 154.2, 162.1; MS: m/z 323 [M^+], 325 [M^++2]. Anal. Calcd for $C_{17}H_{10}ClN_3O_2$: C, 63.07; H, 3.11; N, 12.98. Found: C, 62.96; H, 3.18; N, 13.09.

2-[(5-Methyl)-1*H*-benzimidazol-2-yl]quinoline-4-carboxylic acid (14) Buff compound; yield 70%; mp 133–135°C; IR: 1725 (CO), 3200 (NH), 3500 cm^{-1} (OH); ^1H NMR: δ 2.44 (s, 3H, CH_3), 7.15 (d, *J* = 8.4 Hz, 1H), 7.33 (s, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.86 (t, *J* = 8.6 Hz, 1H), 7.98 (d, *J* = 8.6 Hz, 1H), 8.10 (t, *J* = 8.6 Hz, 1H), 8.20 (d, *J* = 8.6 Hz, 1H), 8.47 (s, 1H), 12.77 (s, 1H, NH, D_2O exchangeable), 13.19 (s, 1H, COOH); ^{13}C NMR: δ 20.8, 114.5, 119.2, 122.8, 124.5, 124.9, 126.5, 128.0, 128.8, 130.0, 135.4, 135.7, 138.2, 145.1, 152.7, 153.2, 162.8; MS: m/z 303 [M^+]. Anal. Calcd for $C_{18}H_{13}N_3O_2$: C, 71.28; H, 4.32; N, 13.85. Found: C, 71.36; H, 4.25; N, 13.71.

2-(5-Methyl-1*H*-benzimidazol-2-yl)-6-methylquinoline-4-carboxylic acid (15) Yellow compound; yield 65%; mp 204–206°C; IR: 1725 (CO), 3200 (NH), 3500 cm^{-1} (OH); ^1H NMR: δ 2.51 (s, 6H, $2CH_3$), 7.12 (d, *J* = 8.4 Hz, 1H), 7.32 (s, 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.76 (d, *J* = 8.6 Hz, 1H), 8.16 (d, *J* = 8.6 Hz, 1H), 8.36 (s, 1H), 8.48 (s, 1H), 12.81 (s, 1H, NH, D_2O exchangeable), 13.21 (s, 1H, COOH); ^{13}C NMR: δ 20.7, 20.9, 114.9, 122.1, 123.0, 124.2, 125.1, 128.2, 129.4, 132.1, 132.4, 134.2, 134.8, 137.8, 144.2, 152.1, 152.4, 163.7; MS: m/z 317 [M^+]. Anal. Calcd for $C_{19}H_{15}N_3O_2$: C, 71.91; H, 4.76; N, 13.24. Found: C, 72.12; H, 4.72; N, 13.28.

Ethyl 2-cyanomethyl-5-methyl-1*H*-benzimidazole-1-carboxylate (16b)

A solution of 5-methyl-2-cyanomethylbenzimidazole [27, 28] (0.01 mol) in pyridine (20 mL) was stirred in an ice bath and treated drop-

wise with ethyl chloroformate (2.7 g, 0.025 mol). Stirring was continued for an additional 10 min and then the mixture was poured on cold water (400 mL). The resultant precipitate of **16b** was collected by filtration, dried and crystallized from ethanol: yellow compound; yield 74%; mp 76–78°C; IR: 2220 (CN), 1715 (CO), 1620 cm^{-1} (C=N); ^{1}H NMR: δ 1.25 (t, *J* = 7 Hz, 3H, CH_2CH_3), 2.34 (s, 3H, CH_3), 3.67 (s, 2H, CH_2-CN), 4.17 (q, *J* = 7 Hz, 2H, CH_2CH_3), 7.12 (d, *J* = 8.7 Hz, 1H), 7.33 (s, 1H), 7.54 (d, *J* = 8.7 Hz, 1H); MS: m/z 243 [M^+]. Anal. Calcd for $C_{13}H_{13}N_3O_2$: C, 64.19; H, 5.39; N, 17.27. Found: C, 64.24; H, 5.45; N, 17.33.

1-Oxo-2-(substituted phenyl)-1,2-dihydrobenzimidazo[1,2-*d*][1,2,4]triazine-4-carbonitriles 17–24

To a solution of **16a** [26] or **16b** (0.01 mol) in pyridine (20 mL) in an ice bath, a solution of a diazonium hydrochloride [amine (0.01 mol), 36% HCl (3 mL), ice water (10 mL) and $NaNO_2$ (0.7 g, 0.01 mol)] was added with stirring. After 24 h, the mixture was diluted with ice-cold water (200 mL), and the resultant precipitate of **17–24** was collected by filtration, dried and crystallized.

1-Oxo-2-(2-chlorophenyl)-1,2-dihydrobenzimidazo[1,2-*d*][1,2,4]triazine-4-carbonitrile (17) Crystallized from absolute ethanol; yellow compound; yield 71%; mp 151–153°C; IR: 1600, 1640 (C=N), 1700 (CO), 2220 cm^{-1} (C≡N); ^{1}H NMR: δ 7.63 (d, *J* = 8.4 Hz, 2H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.92 (t, *J* = 7.5 Hz, 1H), 8.01 (t, *J* = 7.5 Hz, 1H), 8.15 (d, *J* = 7.5 Hz, 1H), 8.35 (d, *J* = 7.5 Hz, 1H); MS: m/z 321 [M^+], 323 [M^++2]. Anal. Calcd for $C_{16}H_8ClN_5O$: C, 59.73; H, 2.51; N, 21.77. Found: C, 59.60; H, 2.55; N, 21.73.

1-Oxo-2-(3-chlorophenyl)-1,2-dihydrobenzimidazo[1,2-*d*][1,2,4]triazine-4-carbonitrile (18) Crystallized from absolute ethanol; orange compound; yield 69%; mp 183–185°C; IR: 1600, 1640 (C=N), 1680 (CO), 2225 cm^{-1} (C≡N); ^{1}H NMR: δ 7.63 (d, *J* = 8.4 Hz, 2H), 7.72 (d, *J* = 8.4 Hz, 2H),7.88 (t, *J* = 7.5 Hz, 1H), 8.11 (d, *J* = 7.5 Hz, 1H), 8.28 (d, *J* = 7.5 Hz, 1H), 8.37 (s, 1H); MS: m/z 321 [M^+], 323 [M^++2]. Anal. Calcd for $C_{16}H_8ClN_5O$: C, 59.73; H, 2.51; N, 21.77. Found: C, 59.79; H, 2.48; N, 21.69.

1-Oxo-2-(4-methoxylphenyl)-1,2-dihydrobenzimidazo[1,2-*d*][1,2,4]triazine-4-carbonitrile (19) Crystallized from methanol; orange compound; yield 75%; mp 200–202°C; IR: 1605, 1640 (C=N), 1680 (CO), 2225 cm^{-1} (C≡N); ^{1}H NMR: δ 3.86 (s, 3H, OCH_3), 7.02 (d, *J* = 7.8 Hz, 2H), 7.62 (d, *J* = 8.7 Hz, 2H), 7.91 (d, *J* = 8.7 Hz, 2H), 8.35 (d, *J* = 7.8 Hz, 2H); MS: m/z 317 [M^+]. Anal. Calcd for $C_{17}H_{11}N_5O_2$: C, 64.35; H, 3.49; N, 22.07. Found: C, 64.44; H, 3.45; N, 22.17.

1-Oxo-2-(2-thiomethylphenyl)-1,2-dihydrobenzimidazo[1,2-*d*][1,2,4]triazine-4-carbonitrile (20) Crystallized from absolute ethanol; yellow compound; yield 66%; mp 194–196°C; IR: 1560, 1614 (C=N), 1743 (C=O), 2220 cm^{-1} (C≡N); ^{1}H NMR: δ 2.51 (s, 3H, S-CH_3), 7.44 (d, *J* = 8.7 Hz, 2H), 7.62 (d, *J* = 7.8 Hz, 1H), 7.80 (t, *J* = 7.8 Hz, 1H), 7.91 (t, *J* = 7.8 Hz, 1H), 8.04 (d, *J* = 8.7 Hz, 2H), 8.36 (d, *J* = 7.8 Hz, 1H); MS: m/z 333 [M^+]. Anal. Calcd for $C_{17}H_{11}N_5OS$: C, 61.25; H, 3.33; N, 21.01. Found: C, 61.33; H, 3.28; N, 21.07.

8-Methyl-1-oxo-2-(2-chlorophenyl)-1,2-dihydrobenzimidazo[1,2-*d*][1,2,4]triazine-4-carbonitrile (21) Crystallized from absolute ethanol; yellow compound; yield 72%; mp 150–152°C; IR: 1600, 1630 (C=N), 1700 (C=O), 2225 cm^{-1} (C≡N); ^{1}H NMR: δ 2.47 (s, 3H, CH_3), 7.39 (d, *J* = 8.4 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.71 (t, *J* = 7.5 Hz, 1H), 7.90 (d, *J* = 7.5 Hz, 1H), 8.0 (d, *J* = 8.4 Hz, 1H), 8.12 (d, *J* = 7.5 Hz, 1H), 8.21 (s, 1H); MS: m/z 335 [M^+], 337 [M^++2]. Anal. Calcd for $C_{17}H_{10}ClN_5O$: C, 60.81; H, 3.00; N, 20.86. Found: C, 60.89; H, 3.12; N, 20.71.

8-Methyl-1-oxo-2-(3-chlorophenyl)-1,2-dihydrobenzimidazo[1,2-*d*][1,2,4]triazine-4-carbonitrile (22) Crystallized from methanol, yellowish brown compound; yield 88%; mp 147–149°C; IR: 1605, 1640 (C=N), 1700 (C=O), 2220 cm^{-1} (C≡N); ^{1}H NMR: δ 2.43 (s, 3H, CH_3), 7.39 (d, *J* = 8.4 Hz, 1H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.70 (t, *J* = 7.5 Hz, 1H), 7.91 (d, *J* = 7.5 Hz, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 8.12 (s, 1H), 8.21 (s, 1H); MS: m/z 335 [M^+], 337 [M^++2]. Anal. Calcd for $C_{17}H_{10}ClN_5O$: C, 60.81; H, 3.00; N, 20.86. Found: C, 60.87; H, 3.09; N, 20.89.

8-Methyl-1-oxo-2-(4-methoxyphenyl)-1,2-dihydrobenzimidazo[1,2-*d*][1,2,4]triazine-4-carbonitrile (23) Crystallized from absolute ethanol, buff compound; yield 85%; mp 166–168°C; IR: 1600, 1640 (C=N), 1680 (C=O), 2220 cm^{-1} (C≡N); ^{1}H NMR: δ 2.56 (s, 3H, CH_3), 3.85 (s, 3H, O-CH_3), 7.12 (d, *J* = 7.8 Hz, 2H), 7.44 (d, *J* = 7.8 Hz, 2H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.84 (d, *J* = 8.4 Hz, 1H), 8.15 (s, 1H); ^{13}C NMR: 20.9, 55.0, 111.7, 113.7, 113.9, 119.9, 127.2, 127.6, 128.4, 131.9, 136.3, 140.7, 159.0; MS: m/z 331 [M^+]. Anal. Calcd for $C_{18}H_{13}N_5O_2$: C, 65.25; H, 3.95; N, 21.14. Found: C, 65.20; H, 3.87; N, 21.18.

8-Methyl-1-oxo-2-(2-thiomethylphenyl)-1,2-dihydrobenzimidazo[1,2-*d*][1,2,4]triazine-4-carbonitrile (24) Crystallized from aqueous ethanol; brown compound; yield 77%; mp 162–164°C; IR: 1600, 1630 (C=N), 1700 (C=O), 2220 cm^{-1} (C≡N); ^{1}H NMR: δ 2.34 (s, 3H, CH_3), 2.53 (s, 3H, S-CH_3), 7.42 (d, *J* = 7.8 Hz, 1H), 7.60 (t, *J* = 7.8 Hz, 1H), 7.78 (t, *J* = 7.8 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.91(d, *J* = 7.8 Hz, 1H), 8.11 (d, *J* = 8.4 Hz, 1H), 8.20 (s, 1H); MS: m/z 347 [M^+]. Anal. Calcd for $C_{18}H_{13}N_5OS$: C, 62.23; H, 3.77; N, 20.16. Found: C, 62.34; H, 3.73; N, 20.19.

Biology

All materials were obtained from Sigma Chemical Co. (USA). Human tumor cell lines were obtained frozen in liquid nitrogen (-180°C) from the American Type Culture Collection. The tumor cell lines were maintained in the National Cancer Institute, Cairo, Egypt, by serial subculturing. *In vitro* antitumor activity against human MCF7 (breast cancer cell line) was determined using the Sulforhodamine B assay [29, 30]. Cells were plated in a 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compounds to allow attachment of the cell to the wall of the plate. Monolayer cells were incubated with the compounds for 48 h at 37°C in a humidified incubator with 5% CO_2. Cells were fixed with trichloroacetic acid and stained for 30 min with 0.4% (wt/vol) Sulforhodamine B (SRB) stain dissolved in 1% acetic acid. Unbound dye was washed with 1% acetic acid and protein bound dye was extracted with Tris EDTA. The optical density (OD) of each well was measured spectrophotometrically at 564 nm with

an ELISA microplate reader (Meter tech. Σ 960, USA). Cell survival was calculated as follows: survival fraction = OD (treated cells)/OD (control cells) (Table 1). For determination of IC_{50}, different concentrations of the compound under test (0, 1, 2.5, 5 and 10 μg/mL) were added to the cell monolayer wells which were prepared for each individual dose. The absorbance of each well was determined by an ELISA reader. The relation between surviving fraction and compound concentration was plotted to obtain the survival curve of the tumor cell line after the specified compound (Table 1).

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