Research Article

Synthesis and Biological Evaluation of 2,4-Diaminoquinazolines as Potential Antitumor Agents

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In the present study, a set of 2-anilino-4-alkylaminoquinazoline derivatives were synthesized and tested for their antitumor activities *in vitro* against a panel of four human cancer cell lines and for their DNA-binding affinity. Among the synthesized compounds, **4c** and **5b** with 4-substitution at the phenyl ring were found to have the highest inhibitory effects against breast adenocarcinoma (MCF-7), colon cancer (HCT-116), hepatocellular carcinoma (HePG-2), and human skin cancer (HFB4). Further investigation revealed that compounds **4a** and **5d** exhibited better affinity to bind with DNA than other tested compounds.

1. Introduction

Although the development of novel targeted antitumor drugs has made important progress in recent years, cancer remains the major leading cause of death in the world due to drug resistance or undesirable toxic effects [1]. Therefore, continued commitment to the laborious task of discovering new anticancer agents remains critically important [2].

In the course of finding new bioactive molecules that may serve as potent antitumor agents, quinazoline derivatives are of particular interest [3]. These quinazolines have been identified as a new class of cancer chemotherapeutic agents against solid tumors and exert their antitumor activity through the inhibition of the receptor tyrosine kinases (RTKs) [4], specifically epidermal growth factor receptor (EGFR), such as PD153035 [5], and gefitinib [6], or dihydrofolate reductase, such as trimetrexate (TMQ) [7] (Figure 1). Moreover, the anticancer activity of quinazoline-based drugs is related to their DNA-binding affinity to show inhibition of topoisomerases, such as Luotonin A [8], or inhibition of telomerase such as 05^9 (Figure 1), leading to cell death by inhibition of replicative enzymes and DNA repair systems.

In the current study, we have synthesized a series of 2,4-disubstituted quinazolines **4a-d** and **5a-d** (Figure 1), each of which contains a simple alkyl amino side chain at position 4 instead of the diaminopropyl moiety of 05 [9]. In addition, an aryl amino moiety was introduced at position 2 of the quinazoline ring instead of the substituted aryl fragment. This can significantly increase the binding ability of these compounds with the relevant desired receptors, such as EGFR tyrosine kinase or DNA, through the formation of hydrogen bonding. An evaluation of their antiproliferative activity was carried out using four human tumor cell lines, in addition to their DNA-binding affinity.

2. Results and Discussion

2-Arylamino-4-alkylaminoquinazoline derivatives 4a-d and 5a-d were prepared as shown in Figure 2. The starting material 2-aminobenzoic acid was condensed with potassium cyanate conveniently cyclized to quinazoline-2,4-dione as described in the literature. Compound 1 was refluxed with phosphorous oxychloride to corresponding dicloroquinazoline (2) [10] Compoun3d **1** yielded the corresponding 2,4-dichloroquinazoline (**2**) upon refluxing with
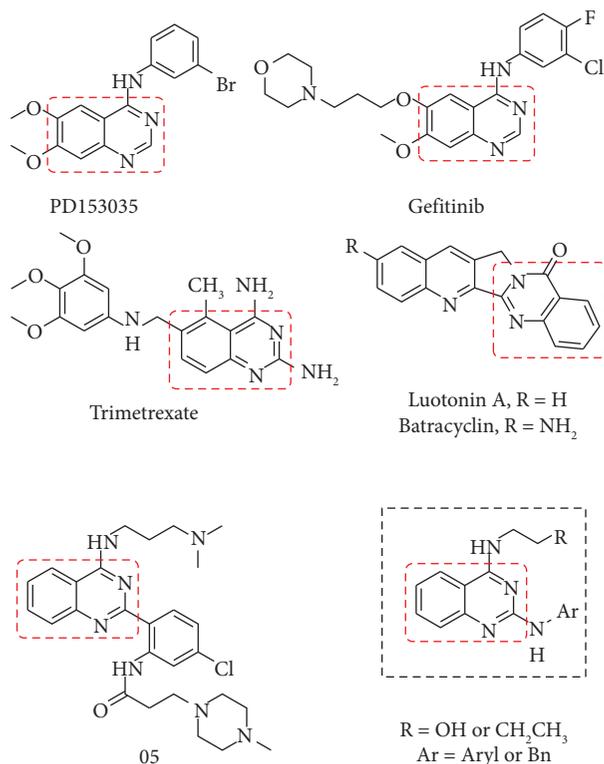
Figure 1: Recently reported anticancer agents with quinazoline moiety and target compounds.

Table 1: Cytotoxic activity (IC$_{50}$, μg/ml) of 2,4-diaminoquinazoline derivatives against human cancer cell lines.[a]

No.	MCF7	HCT 116	HePG2	HFB4
5-FU	5.4 ± 0.20	5.3 ± 0.18	7.9 ± 0.21	8.8 ± 0.52
4a	34.4 ± 3.41	41.4 ± 3.62	48.9 ± 3.60	31.8 ± 3.15
4b	12.7 ± 1.32	36.9 ± 3.04	40.5 ± 3.51	20.4 ± 1.98
4c	**11.1 ± 1.10**	**10.8 ± 0.86**	**12.0 ± 0.97**	**10.8 ± 1.24**
4d	13.6 ± 1.65	19.4 ± 1.50	18.0 ± 1.72	13.7 ± 1.20
5a	27.4 ± 1.97	32.8 ± 2.89	31.2 ± 3.43	30.1 ± 3.36
5b	**10.9 ± 1.03**	**9.1 ± 0.85**	**10.4 ± 1.11**	**9.9 ± 1.07**
5c	16.3 ± 1.56	17.4 ± 1.32	26.2 ± 2.32	15.7 ± 1.61
5d	71.8 ± 4.74	81.4 ± 5.37	88.9 ± 6.41	66.8 ± 4.19

[a]IC$_{50}$: The concentration that causes 50% of cell proliferation inhibition. The data are expressed as the means ± SD of at least three separate experiments.

Table 2: DNA/methyl green colorimetric assay of the DNA-binding compounds.

DNA-active compounds	DNA/methyl green (IC$_{50}$, μg/ml)[a]
4a	64 ± 4
4b	**75 ± 5**
4d	**81 ± 5**
5a	**79 ± 4**
5c	**70 ± 4**
5d	**61 ± 3**

[a]IC$_{50}$ values represent the concentration (mean ± SD, $n = 3$–5 separate determinations) required for a 50% decrease in the initial absorbance of the DNA/methyl green solution.

in a mixture of ethanol and dimethylformamide (for **5a-d**) to give the target quinazolines.

2.1. Biological Evaluation

2.1.1. In Vitro Anticancer Screening.

The newly synthesized compounds **4a-d** and **5a-d** were tested for their potencies for inhibition of breast adenocarcinoma (MCF-7), colon cancer (HCT-116), hepatocellular carcinoma (HePG-2), and human skin cancer (HFB4) by applying the MTT colorimetric assay. 5-Fluorouracil (5-FU) was chosen as a reference cytotoxic agent range of 5.3–8.8 μg/ml. Compounds were tested over a range of concentrations from 1.56 to 100 μg/ml, which we used to determine the inhibitory effects of the compound on cell growth using the MTT assay, this colorimetric is based on the conversion of the yellow MTT to a purple formazan derivative by mitochondrial succinate in viable cells. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Also, the calculated IC$_{50}$ values, that is, the concentration (μg/mL) of a compound that was able to cause 50% growth inhibition with respect to the control culture, were reported differently according to different cancer cells [13].

As shown in Table 1, the results of *in vitro* antitumor activity of the tested compounds indicated that compound **5b** (with 4-NO$_2$ substitution) exhibited the highest cytotoxic activity against MCF-7, HCT-116, HePG-2, and HFB4 with an IC$_{50}$ ranging from 9.1 to 10.9 μM. Moreover, compound **4c** (with 4-NO$_2$ substitution) showed similar activity against the four cancer cell lines, with an IC$_{50}$ ranging from 10.8 to
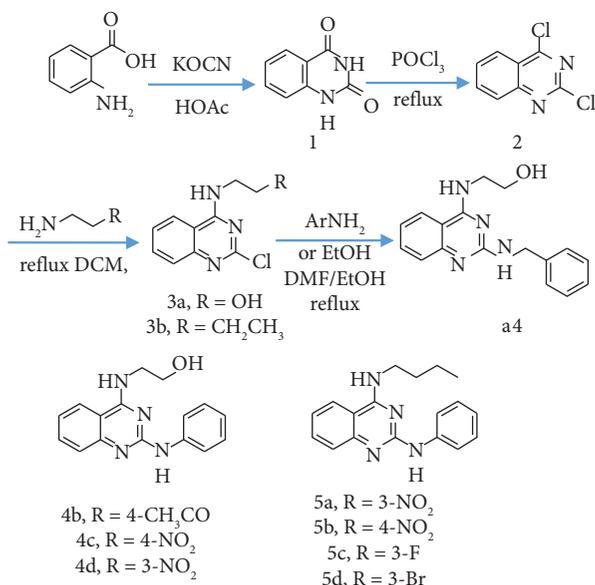

Figure 2: Synthesis of new 2,4-diaminoquinazoline derivatives as anticancer.

phosphorous oxychloride. [11] Selective substitution of the chlorine at the 4-position with ethanolamine and *n*-butylamine in dichloromethane under reflux gave the corresponding 4-alkylamino-2-chloro-quinazoline **3a** and **3b** [12], respectively. Finally, compounds **3a** and **3b** were treated with the appropriate amines in ethanol (for **4a-d**) or

12.0 μM. As shown in Table 2, the replacement of the 4-NO$_2$ substituent in **4c** and **5b** with 3-NO$_2$, F, or Br resulted in compounds **4d**, **5c**, or **5d**, all of which exhibited a decrease in the activity against the four cell lines. These results indicate that the 4-NO$_2$ substitution of the phenyl ring connected to the N_2 position is more favorable for anticancer activity than the 3-substitution.

2.1.2. DNA-Binding Assay. Some anticancer agents block DNA synthesis, leading to cell cycle arrest [14]. We thus sought to determine if these compounds would have an impact on the cell cycle progression through measuring the affinity of these compounds to bind to DNA. A DNA-binding colorimetric assay was applied for the determination of the interaction of the small synthesized molecules with DNA. The results showed that all the tested compounds have a moderate affinity for DNA, which was demonstrated by a decrease in the absorbance of the DNA/methyl green. Compounds **4a** and **5d** exhibited the best DNA-binding affinity among other compounds.

3. Conclusion

A new series of 2,4-diaminoquinazoline compounds **4a–d** and **5a–d** were synthesized and tested against four cell lines belonging to different tumor subpanels. Some of the synthesized compounds **4c** and **5b** showed promising antiproliferative properties against MCF-7, HCT-116, HePG-2, and HFB4 with an IC$_{50}$ range of 9.1–12.0 μg/ml, in comparison to 5-FU with an IC$_{50}$ range of 5.3–8.8 μg/ml as reference agent. From the obtained results, we can conclude that the incorporation of the 4-NO$_2$ group into the phenylamino moiety improved the antitumor activity, which can be considered a valuable lead for future studies to obtain more potent antitumor agents.

4. Experimental

4.1. Chemistry. Melting points (°C uncorrected) were determined using the *Fisher–Johns* melting point apparatus. Column chromatography was performed using 40–60 μm silica gel. NMR spectra were recorded on a Bruker spectrometer (400 MHz for 1H and 100 MHz for 13C) using DMSO or CDCl$_3$ as a solvent, and chemical shifts and coupling constants are presented in parts per million relatives to Me$_4$Si and Hertz, respectively. The abbreviations are as follows: *s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet, *p*, pentet, *m*, multiplet; br, broad. IR spectroscopy was recorded on a Thermo Fisher Scientific Nicolet IS10 spectrophotometer using KBr. The wave numbers of maximum absorption peaks in IR spectroscopy are presented in cm^{-1}. Microanalytical data (C, H, and N) were found within ± 0.4% of the calculated values of the proposed structures. All dried solvents were stored over anhydrous Na$_2$SO$_4$ (EtOH, CH$_2$Cl$_2$, and DMF). Moisture or air-sensitive reactions were conducted under the protection of the CaCl$_2$ tube in oven-dried glassware. Reagents were purchased from commercial suppliers and used without purification. Mass spectra were recorded on a Thermo Scientific DSQ II spectrometer. Elemental analysis was performed at the Microanalytical Center, Cairo University, Egypt.

*4.1.1. Synthesis of Quinazolidine-2,4-Dione (**1**) and 2,4-Dichloroquinazoline (**2**).* A suspension of anthranilic acid (0.073 mol) in dist. water (350 ml) was stirred at room temperature, and acetic acid (0.094 mol) was added dropwise until (pH = 4-5), the mixture changed to a greenish-white suspension. An aqueous solution of KCNO (0.015 mol) was added dropwise to the wall of the breaker for 30 min, and the mixture turned to a heavy white suspension. The reaction was treated with an aqueous solution of NaOH (0.35 mol) till pH was 11-12 and left for stirring for 2 days. Conc. H$_2$SO$_4$ was added to the cooling orange solution to give heavy off-white solids. The solid was filtered, washed with water, and dried to get the pure quinazoline-2,4-dione **1** as a white solid (m.*p* = > 290°C, Yield = 90%). [14] Compound **1** (0.03 mol) and POCl3 (0.85 mol) were refluxed for 13h. A mixture was cooled to room temperature and poured over a mixture of ice and water while stirring. The separated solids were filtered, washed with water, and dried to give a yellow solid product **2** with (m.*p* = 121–123°C, Yield = 90%) [11].

*4.1.2. Synthesis of 2-[(2-chloroquinazolin-4-yl)amino] Ethanol (**3a**).* To a solution of compound **2** (0.005 mol) in DCM, 2-aminoethanol (0.19 mol) was added and the mixture was refluxed for 30 min. After cooling, the separated solids were filtered, washed with water, and dried to get product **3a** as an off-white solid with (m.*p* = 189–192°C, Yield = 88%). [12].

*4.1.3. Synthesis of Compounds **4a–d**.* Synthesis of 2-{[2-(benzylamino)quinazolin-4-yl] amino}ethan-1-ol (**4a**): to a suspension of compound **3a** (1.43 mmol) in absolute EtOH, phenylmethanamine (1.43 mmol) and trimethylamine (1.43 mmol) were added and the reaction mixture was refluxed for 24 h. The separated solids were filtered, washed with EtOH, and purified by column chromatography (SiO$_2$, CH$_2$Cl$_2$/ MeOH = 5 : 1) to give the desired product **4a** (0.16 mg, 50% yield) as white crystals, m.*p* = 243–245°C. ^{1}H NMR (DMSO-d$_6$): δ = 8.57 (s,1 H), 8.35 (d, J = 8.0 Hz, 2 H), 7.77 (s, 1 H), 7.48 (s, 1 H), 7.34 (t, J = 7.4 Hz, 2 H), 7.25 (d, J = 7.0 Hz, 2 H), 4.92 (s, 1 H), 4.68 (s, 2 H), 3.61 (s, 4 H); MS (EI) m/*z*: 294 (4.9, M$^+$), 295 (2.1, M + 1), 296 (1.0, M + 2), 261 (39.0), 234 (43.4), 204 (100.0), 163 (38.1), 90 (44.4), 77 (30.9), 60 (13.7); IR (KBr, cm^{-1}): 3416 (OH), 3273 (NH), 1745 (C=O), 1635 (C=N), 1588 (C=C).

*4.1.4. Synthesis of 1-[4-({4-[(2-Hydroxyethyl)Amino] Quinazolin-2-Yl}Amino)Phenyl]Ethan-1-One(**4b**).* To a suspension of compound **3a** (0.001 mol) in absolute EtOH (15 mL), 4-aminoacetophenone (0.001 mol) and 2–3 drops of acetic acid were added and the reaction mixture was refluxed for 24 h. The separated solids were filtered, washed by EtOH, dried, and purified by recrystallization from absolute EtOH to give the desired product **4b** (0.18 mg, 80% yield) as an off-white crystal, m*p* = 265–268°C. ^{1}H NMR (DMSO-d$_6$): δ = 8.41 (d, J = 8.4 Hz,

1 H), 8.03 (d, $J = 8.4$ Hz, 2 H), 7.86 (t, $J = 7.6$ Hz, 2 H), 7.80 (d, $J = 8.4$ Hz, 1 H), 7.59 (d, $J = 8.3$ Hz, 1 H), 7.51 (t, $J = 7.7$ Hz, 1 H), 3.73 (s, 4 H), 2.58 (s,3 H); ^{13}C NMR (DMSO-d$_6$): $\delta = 194.5$, 175.1, 156.4, 152.3, 143.7, 133.7, 132.1, 128.1, 125.4, 122.3, 119.7, 112.4, 108.6, 62.2, 34.2, 26.8; MS (EI) m/z: 322 (85.5, M$^+$), 323 (16.4, $M + 1$), 324 (2.2, $M + 2$), 291 (21.9), 279 (19.7), 262 (4.6), 138 (40.0), 119 (25.2), 90 (16.6); IR (KBr, cm^{-1}): 3448 (OH), 3241 (NH), 1743 (C=O), 1650 (C=N), 1565 (C=C).

4.1.5. Synthesis of 2-({2-[(4-nitrophenyl)amino]quinazolin-4-yl}amino)ethan-1-ol (4c). To a suspension of compound **3a** (2.24 mmol) in a mixture of absolute EtOH and DMF (18 ml, 5 : 1), 4-nitroaniline (2.24 mmol) was added and the reaction mixture was refluxed for 24 h. The separated solids were filtered, washed with EtOH, and purified by column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH = 5 : 1) to give the desired product **4c** (0.12 mg, 45% yield) as brown solids, m.*p* = 140–142°C. ^{1}H NMR (DMSO-d$_6$): δ 8.31 (d, $J = 8.2$ Hz, 1 H), 7.89 (d, $J = 7.9$, 2 H), 7.80 (t, $J = 7.6$ Hz, 2 H), 7.72 (d, $J = 8.1$ Hz, 1 H), 7.63 (d, $J = 8.1$ Hz, 1 H), 7.50 (t, $J = 7.8$ Hz, 1 H), 4.71 (s, 1 H), 3.59 (s,4 H); MS (EI) m/z: 327 (79.2, $M + 2$), 326 (15.1, $M + 1$), 325 (84.6, M$^+$), 294 (4.4), 280 (37.2), 265 (9.3), 137 (8.5), 90 (49.3), 52 (52.0); IR (KBr, cm^{-1}): 3250 (NH), 1737 (C=O), 1605 (C=N), 1540 (C=C), 1497 (N=O).

4.1.6. Synthesis of 2-({2-[(3-nitrophenyl)amino]quinazolin-4-yl}amino)ethan-1-ol (4d). To a suspension of compound **3a** (2.24 mmol) in a mixture of absolute EtOH and DMF (18 ml, 5 : 1), 3-nitroaniline (2.24 mmol) was added and the reaction mixture was refluxed for 24 h. The separated solids were filtered, washed with EtOH, and purified by column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH = 5 : 1) to give the desired product **4d** (0.24 mg, 48% yield) as brown solids, m.*p*. = 153–155°C. ^{1}H NMR data: (DMSO-d$_6$): $\delta = 8.34$ (d, $J = 8.4$ Hz, 1 H), 7.86 (d, $J = 8.2$, 2 H), 7.77 (t, $J = 7.6$ Hz, 2 H), 7.69 (d, $J = 7.9$ Hz, 1 H), 7.61 (d, $J = 8.1$ Hz, 1 H), 7.53 (t, $J = 8.0$ Hz, 1 H), 4.70 (s, 1 H), 3.57 (s,4 H); IR (KBr, cm^{-1}): 3195 (NH), 1775 (C=O), 1630 (C=N), 1570 (C=C), 1368 (N=O).

4.1.7. Synthesis of N-Butyl-2-Chloroquinazolin-4-Amine (3b). To a solution of compound **3b** (1.5 mmol) in CH$_2$Cl$_2$ (15 ml), *n*-buthylamine (6 mmol) was added and the mixture was refluxed for 6 h. After cooling the solvent was evaporated under reduced pressure to give oil and leave it for 24 h, then added to a mixture of ice and water to give an off-white solid (m.*p* = 109–111°C, Yield = 90%) [12].

4.1.8. Synthesis of Compounds 5c–d. In the solution of compound **3b** (0.43 mmol) in a mixture of absolute EtOH and DMF (18 ml, 5 : 1), the appropriate aryl amine (0.43 mmol) was added, and the mixture was refluxed for 24 h. The separated solids were filtered, washed with EtOH, and the solvent was evaporated under reduced pressure. The crude was purified by column chromatography (SiO$_2$: CH$_2$Cl$_2$/MeOH = 10 : 1) to give the desired products **5a–d**.

4.1.9. Synthesis of Compound N4-Butyl-N2-(3-Nitrophenyl) quinoaniline-2,4-Diamine 5a. 3-Nitroaniline was used to give the desired compound **5a** (0.22 mg, 50% yield) as yellow solids, m.*p* = 183–185°C. ^{1}H NMR (DMSO-d$_6$): $\delta = 10.01$ (s, 1 H), 8.42 (s, 1 H), 8.15 (s, 1 H), 7.86 (d, $J = 8.4$ Hz, 2 H), 7.66 (t, $J = 8.0$ Hz, 2 H), 7.50 (d, $J = 7.9$ Hz, 2 H), 7.30 (t, $J = 8$ Hz, 1 H), 5.75 (s, 1 H), 3.57 (s, 2 H), 1.68 (t, $J = 8.0$ Hz, 2 H), 1.42 (t, $J = 8.0$ Hz, 2 H), 0.95 (t, $J = 8$ Hz, 3 H); IR (KBr, cm^{-1}): 3422 (NH), 1617 (C=N), 1527, 1489 (C=C), 1341 (N=O).

4.1.10. Synthesis of Compound N4-Butyl-N2-(4-Nitrophenyl) quinoaniline-2,4-Diamine 5b. 4-Nitroaniline was used to give the desired compound **5b** (0.22 mg, 50% yield) as yellow solids, m.*p* = 175–177°C. ^{1}H NMR (DMSO-d$_6$): $\delta = 9.58$ (s, 1 H), 8.46 (s, 1 H), 8.01 (s, 1 H), 7.78 (d, $J = 8.1$ Hz, 2 H), 7.70 (t, $J = 8.0$ Hz, 2 H), 7.61 (d, $J = 7.89$ Hz, 2 H), 7.35 (t, $J = 8$ Hz, 1 H), 5.65 (s, 1 H), 3.53 (s, 2 H), 1.65 (t, $J = 8.0$ Hz, 2 H), 1.41 (t, $J = 8.0$ Hz, 2 H), 0.96 (t, $J = 8.0$ Hz, 3 H); ^{13}C NMR (DMSO-d$_6$): $\delta = 180.1$, 160.6, 156.3, 150.1, 148.4, 140.2, 133.4, 125.5, 125.2, 123.4, 123.2, 118.1, 112.4, 41.1, 31.1, 20.2, 14.3; MS (EI) *m/z*: 339 (2.3, $M + 2$), 338 (18.7, $M + 1$), 337 (100, M$^+$), 308 (39.4), 294 (42.0), 280 (59.4), 200 (16.1), 90 (21.4); IR (KBr, cm^{-1}): 3452 (NH), 1624 (C=N), 1538, 1492 (C=C), 1359 (N=O).

4.1.11. Synthesis of Compound N4-Butyl-N2-[3-(Fluoromethyl) phenyl]quinoaniline-2,4-Diamine 5c. 3-Fluoroaniline was used to give the desired compound **5c** (0.22 mg, 74% yield) as off-white solids, m*p* = 167–169°C. ^{1}H NMR (DMSO-d$_6$): $\delta = 10.95$ (s, 1 H), 9.95 (s, 1 H), 8.83 (s, 1 H), 8.42 (s, 1 H), 7.88 (d, $J = 8.4$ Hz, 2 H), 7.67 (t, $J = 8.0$ Hz, 2 H), 7.49 (d, $J = 7.9$ Hz, 2 H), 7.45 (t, $J = 8.0$ Hz, 1 H), 3.62 (s, 2 H), 1.66 (t, $J = 8.0$ Hz, 2 H), 1.36 (t, $J = 8.0$ Hz, 2 H), 0.87 (t, $J = 8.0$ Hz, 3 H); IR (KBr, cm^{-1}): 3422 (NH), 1610 (C=N), 1586, 1541 (C=C).

4.1.12. Synthesis of Compound N2-(3-Bromophenyl)-N4-Butylquinoaniline -2,4-Diamine 5d. 3-Bromoaniline was used to give the desired compound **5d** (0.19 mg, yield = 64%) as off-white solids, m*p* = 186–188°C. ^{1}H NMR (DMSO-d$_6$): $\delta = 9.75$ (s, 2 H), 8.83 (s, 1 H), 8.39 (s, 1 H), 7.77 (d, $J = 8.4$ Hz, 2 H), 7.59 (t, $J = 8.0$ Hz, 2 H), 7.47 (d, $J = 7.9$ Hz, 2 H), 7.36 (t, $J = 8.0$ Hz, 1 H), 3.44 (s, 2 H), 1.57 (t, $J = 8.0$ Hz, 2 H), 1.26 (t, $J = 8.0$ Hz, 2 H), 0.85 (t, $J = 8.0$ Hz, 3 H); MS (EI) m/z: 370 (2.5, M+), 371 (2.1, $M + 1$), 372 (1.4, $M + 2$), 313 (33.1), 304 (78.1), 291 (5.8), 215 (15.4), 200 (22.7), 154 (20.4), 78 (21.6); IR (KBr, cm^{-1}): 3432 (NH), 1617 (C=N), 1564, 1495 (C=C).

4.2. Biological Evaluation

4.2.1. In Vitro Anticancer Screening. The cytotoxicity activity of the synthesized compounds **4a-d,** and **5a-d** are tested on four human tumor cell lines namely; MCF-7 (breast adenocarcinoma), HCT-116 (colon cancer), HePG-2 (hepatocellular carcinoma), and HFB4 (human skin cancer). The cell lines were purchased from ATCC v*ia* a holding

company for biological products and vaccines (VACSERA), Cairo, Egypt. The used reagents are RPMI-1640 medium, MTT, DMSO, and 5-fluorouracil (Sigma Co., St. Louis, USA), and Fetal Bovine serum (GIBCO, UK). 5-fluorouracil (5-FU) was used as a standard anticancer drug for comparison. The previously mentioned cell lines were used to determine the inhibitory effects of the synthesized compounds on the growth of the tested cell lines using the MTT assay [15, 16]. The colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and $100 \mu g/ml$ streptomycin at $37^{\circ}C$ in a 5% CO_2 incubator. The cells were seeded in a 96-well plate at a density of $(1.0 \times 10^4$ cells/well) at $37^{\circ}C$ for 48 h under 5% CO_2. After incubation, the cells were treated with different concentrations of compounds and incubated for 24 h. After 24 h of drug treatment, $20 \mu l$ of MTT solution at 5 mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in a volume of $100 \mu l$ is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at an absorbance of 570 nm using a plate reader (EXL 800, USA). The relative cell viability expressed as a percentage was calculated as (A570 of treated samples/A570 of the untreated sample) X 100 [17].

The relative cell viability % = (A570 of treated samples/ A570 of untreated samples) *100.

4.2.2. DNA-Binding Assay. DNA methyl green (20 mg) was suspended in 100 ml of 0.05 M Tris-HCl buffer (pH = 7.5) containing 7.5 mM $MgSO_4$; the mixture was stirred at $37^{\circ}C$ with a magnetic stirrer for 24 h. Test samples (10,100, and 1000 mg) were dissolved in ethanol in Eppendorf tubes, the solvent was removed under vacuum, and $200 \mu l$ of the DNA/ methyl green solution was added to each tube. Samples were incubated at in the dark at ambient temperature. After 24 h, the final absorbance of the samples was determined at 642.5–645 nm. Readings were corrected for initial absorbance and normalized as the percentage of the untreated standard [18].

Data Availability

All data used to support the findings of the study (NMR, IR, MASS and biological evaluation) are available from the corresponding author upon request (by email).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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