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% (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 dicyclo-oquinazoline (2) [10] Compound 3d 1 yielded the corresponding 2,4-dichloroquinazoline (2) upon refluxing with
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.

Table 1: Cytotoxic activity (IC50 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 |
| 5c | 11.1 ± 1.10 | 10.8 ± 0.86 | 12.0 ± 0.97 | 10.8 ± 1.24 |
| 5d | 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 |

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

| DNA-active compounds | DNA/methyl green (IC50 μg/ml) a |
|----------------------|--------------------------------|
| 4a | 64 ± 4 |
| 4b | 75 ± 5 |
| 4d | 81 ± 5 |
| 5a | 79 ± 4 |
| 5c | 70 ± 4 |
| 5d | 61 ± 3 |

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 IC50 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-NO2 substitution) exhibited the highest cytotoxic activity against MCF-7, HCT-116, HePG-2, and HFB4 with an IC50 ranging from 9.1 to 10.9 μM. Moreover, compound 4c (with 4-NO2 substitution) showed similar activity against the four cancer cell lines, with an IC50 ranging from 10.8 to
12.0 μM. As shown in Table 2, the replacement of the 4-NO₂ substituent in 4c and 5b with 3-NO₂, 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-NO₂ substitution of the phenyl ring connected to the N₂ 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. Some of the synthesized compounds decrease in the absorbance of the DNA/methyl green.

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 anti-proliferative properties against MCF-7, HCT-116, HePG-2, and HFB4 with an IC₅₀ range of 9.1–12.0 μg/ml in comparison to 5-FU with an IC₅₀ range of 5.3–8.8 μg/ml as reference agent. From the obtained results, we can conclude that the incorporation of the 4-NO₂ 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.

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 anti-proliferative properties against MCF-7, HCT-116, HePG-2, and HFB4 with an IC₅₀ range of 9.1–12.0 μg/ml in comparison to 5-FU with an IC₅₀ range of 5.3–8.8 μg/ml as reference agent. From the obtained results, we can conclude that the incorporation of the 4-NO₂ 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 CDCl₃ as a solvent, and chemical shifts and coupling constants are presented in parts per million relative to Me₄Si 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 Na₂SO₄ (EtOH, CH₂Cl₂, and DMF). Moisture or air-sensitive reactions were conducted under the protection of the CaCl₂ 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. H₂SO₄ 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, phenylmethylamine (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 (SiO₂, CH₂Cl₂/MeOH = 5:1) to give the desired product 4a (0.16 mg, 50% yield) as white crystals, m, p = 243–245°C. ¹H NMR (DMSO-d₆): 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=O), 1588 (C=N). The melting point was 243–245°C, and the yield was 50%.

4.1.4. Synthesis of 1-[(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 with 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. ¹H NMR (DMSO-d₆): δ = 8.41 (d, J = 8.4 Hz, 2H), 7.48 (s, 1H), 7.35 (t, J = 7.4 Hz, 2H), 7.25 (d, J = 7.0 Hz, 2H), 4.92 (s, 1H), 4.68 (s, 2H), 3.61 (s, 4H); 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=N). The melting point was 243–245°C, and the yield was 50%.
In the solution of \( \text{compound } 3b \) (0.22 mg), \( 50\% \) yield as yellow solids, \( m.p=167–169^\circ C \). \(^1\)H NMR (DMSO-\( \text{d}_6 \)): \( \delta =10.95 \) (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=O), 1586, 1541 (C=C), 1359 (N=O).

4.1.12. Synthesis of Compound N2-(3-Bromophenyl)-N4-Butylquinazoline-2,4-Diamine 5d. 3-Bromoaniline was used to give the desired compound 5d (0.19 mg, yield = 64%) as off-white solids, \( m.p=186–188^\circ C \). \(^1\)H NMR (DMSO-\( \text{d}_6 \)): \( \delta =9.75 \) (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 (58.6), 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 cytotoxic 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 via 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 µg/ml streptomycin at 37°C in a 5% CO₂ incubator. The cells were seeded in a 96-well plate at a density of (1.0 × 10⁴ cells/well) at 37°C for 48 h under 5% CO₂. After incubation, the cells were treated with different concentrations of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5 mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in a volume of 100 µl 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 MgSO₄; the mixture was stirred at 37°C in a 5% CO₂ incubator. The cells were seeded in a 96-well plate at a density of (1.0 × 10⁴ cells/well) at 37°C for 48 h under 5% CO₂. After incubation, the cells were treated with different concentrations of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5 mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in a volume of 100 µl 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.

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.

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