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Research Article

Synthesis and Biological Activity of 2-Arylidene-*N*-(quinolin-6-yl) hydrazine-1-carboxamides

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A series of 2-arylidene-*N*-(quinolin-6-yl)hydrazine-1-carboxamides **5a–5o** were synthesized and characterized. The synthesized compounds (**5a–5o**) were screened *in vitro* against three breast cancer cell lines: SKBR3, MDA-MB-231, and MCF-7 cancer cell lines by the MTT assay. According to MTT results, compounds **5k** and **5l** showed better antiproliferative activities over MCF-7 cell lines with IC_{50} values of 8.50 and 12.51 μM. Colony formation assay indicated **5k/5l** treatment obviously inhibited the growth of MCF-7 cells and **5k/5l**-induced cell cycle was arrested in the G2-M phase. Moreover, **5k/5l** significantly increased the level of cleaved PARP and induced the apoptosis in MCF-7 cells. In addition, compared to Hela cells, MCF-7 cells were more sensitive to **5k/5l** treatment.

1. Introduction

Breast cancer is one of the most common malignant tumors threatening the health of females all over the world, causing 23% of the total cancer cases and 14% of the cancer deaths [1]. It has become the fastest growing cancer in China, with an average annual growth rate of more than 3% [2]. Breast cancer is a heterogeneous disease that shows remarkably different biological characteristics and clinical behaviors, it can be classified into five main subtypes: luminal, basal-like, human epidermal growth factor receptor-2 (HER-2), HER-2 expression, and normal breast-like subtypes [3]. Triple-negative breast cancer (TNBC) patients have the worst prognosis and distant metastasis-free survival among all major subtypes of breast cancer [4]. The standard treatment options for breast cancer include surgery, radiation, and/or anticancer drugs. Despite a number of active agents available today, it is still a huge clinical demand to discover more effective agents [5]. Quinoline ring system is the pharmacologically important structural scaffold displaying a wide range of biological activities [6–10]. Neratinib (Figure 1) is a quinoline derivative which was approved by FDA for the treatment of HER-2 breast cancer in patients [11]. The presence of urea functionality plays an important role in a drug's aqueous solubility and permeability due to its dual nature as a hydrogen bond donor and acceptor; it also stabilizes the binding of compounds in the pocket [12]. Sorafenib, which is a urea derivative, has been used in the treatment of various cancers. Although it has shown limited efficacy in breast cancer as a single agent, sorafenib combined with other drugs in the treatment of breast cancer could be worth looking forward to [13]. The aromatic urea-quinazolines [14] which integrate the structural features of gefitinib and sorafenib and the thiourea derivatives [15] have already showed significant antibreast cancer activities.

Recently, we investigated various urea/thiourea derivatives as potential antitumor agents [16–20]. It was found in our previous studies that indole-substituted urea derivatives

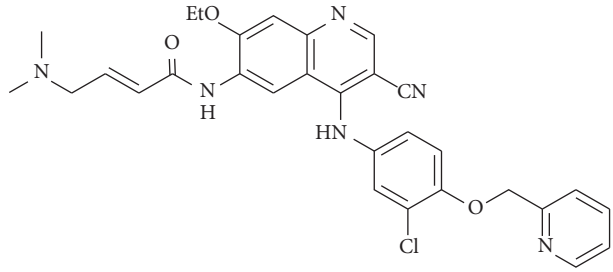


Figure 1: Structure of neratinib.

showed good antitumor activity [20]. A new class of urea derivatives **5a–5o** (Figure 2) was designed by replacing the parent nucleus with a quinoline ring instead of a indole ring. Target compounds **5a–5o** were synthesized and evaluated for their anticancer activity on three breast cancer cell lines: SKBR3, MDA-MB-231, and MCF-7 by MTT assay. The compounds **5k** and **5l** exhibited remarkable activities with IC_{50} values of 8.50 and 12.51 μM over MCF-7 cell lines. On the basis of the results, further structural optimization and research will be carried out.

2. Experimental

All chemicals were analytically pure and purchased from commercial sources. All melting points were determined on a WRS-1B digital melting point apparatus (uncorrected). All new compounds were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS. NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker AV 600 MHz instrument. HRMS spectra were obtained on an Agilent 6230 mass spectrometer. Human breast cell lines SKBR3, MDA-MB-231, and MCF7 were purchased from Shanghai Cell Bank, Chinese Academy of Sciences.

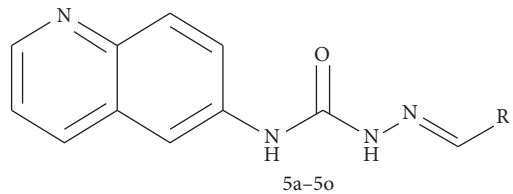
*2.1. Synthesis of 6-Aminoquinoline **2** [21].* The solution of 6-nitroquinoline (174.2 mg, 1 mmol) in methanol (10 mL) was treated with Pd/C (10%, 14.0 mg). The reaction mixture was vigorously stirred under an atmosphere of hydrogen at room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was then filtered and concentrated in vacuo to give the crude product. The crude product was purified by column (ethyl acetate : petroleum ether = 1 : 4) to afford the product 2 (131.1 mg, 91.0%) as white solid. m.p. 116.8–119.0°C. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.64 (d, J = 4.0 Hz, 1H), 7.89 (t, J = 9.7 Hz, 2H), 7.25 (t, J = 4.8 Hz, 1H), 7.14 (dd, J = 9.0 Hz, 2.6 Hz, 1H), 6.88 (d, J = 2.5 Hz, 1H), 3.84 (brs, 2H).

*2.2. Synthesis of Phenyl Quinolin-6-ylcarbamate **3**.* A mixture of **2** (144.2 mg, 1 mmol) and triethylamine (203.8 mg, 2 mmol) in dichloromethane (6 mL) was degassed and flushed with nitrogen. The reaction mixture was cooled down to 5°C, a dichloromethane (6 mL) solution of phenyl chloroformate (156.6 mg, 1 mmol) was added dropwise, and the reaction was stirred overnight. The reaction mixture was washed by water (20 mL) twice, dried over sodium sulfate, filtered, and evaporated. The crude product was purified by column (ethyl acetate : petroleum ether = 1 : 4) to afford the product **3** (226.3 mg, 85.6%) as off-yellow solid. 164.6–167.6°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.64 (brs, 1H), 8.79 (dd, J = 1.3, 4.0 Hz, 1H), 8.27 (d, J = 7.7 Hz, 1H), 8.18 (brs, 1H), 8.02 (d, J = 9.0 Hz, 1H), 7.85 (dd, J = 2.3, 9.0 Hz, 1H), 7.50-7.42 (m, 3H), 7.33-7.24 (m, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 152.3, 150.9, 149.5, 145.1, 137.1, 135.8, 130.2, 129.9, 129.3, 128.8, 127.5, 126.0, 122.4, 122.3; HRMS, m/z calcd. for $C_{16}H_{12}N_2O_2Na$ $[M+Na]^+$: 287.0791; found: 287.0801.

*2.3. Synthesis of N-(Quinolin-6-yl)hydrazine Carboxamide **4**.* A mixture of **3** (264.3 mg, 1 mmol) and hydrazine hydrate solution (80%, 76.9 mmol, 3 mL) in ethanol (95%, 10 mL) was heated to reflux. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled down to room temperature, and a white solid was precipitated out. The reaction mixture was filtered and dried, affording the product **4** (187.6 mg, 92.8%) as white solid. m.p. 200.5–200.8°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.04 (brs, 1H), 8.74 (dd, J = 1.7, 4.2 Hz, 1H), 8.33-8.13 (m, 2H), 7.95-7.91 (m, 1H), 7.91-7.87 (m, 1H), 7.67 (brs, 1H), 7.44 (dd, J = 4.2, 8.3 Hz, 1H), 4.50 (brs, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 157.9, 148.6, 144.5, 138.5, 135.4, 129.7, 129.0, 123.8, 122.0, 113.2; HRMS, m/z calcd. for $C_{10}H_{11}N_4O$ $[M+H]^+$: 203.0927, found: 203.0930.

*General Procedure for the Synthesis of 2-Arylidene-N-(quinolin-6-yl)hydrazine-1-carboxamides **5a–5o**.* A mixture of **4** (202.2 mg, 1 mmol) and aldehyde (1 mmol) in absolute alcohol (15 mL) was heated to reflux. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled down to room temperature, and a white solid was precipitated out. The reaction mixture was filtered and dried, affording the products **5a–5o**.

*2.3.1. 2-Benzylidene-N-(quinolin-6-yl)hydrazine-1-carboxamide (**5a**).* Product **5a** was obtained as white solid, yield 87.9%. m.p. 217.22–17.5°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.97 (s, 1H), 9.29 (s, 1H), 8.79 (dd, J = 1.7, 4.22 Hz, 1H), 8.36 (d, J = 2.4 Hz, 1H), 8.29 (dd, J = 1.0, 8.7 Hz, 1H), 8.11 (dd, J = 2.4, 9.0 Hz, 1H), 8.04 (s, 1H), 8.00 (d, J = 9.0 Hz, 1H), 7.94-7.89 (m, 2H), 7.50-7.45 (m, 3H), 7.44-7.41 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 153.7, 149.1, 145.0, 141.7, 137.7, 135.7, 134.8, 130.0, 129.5, 129.1, 128.8, 127.6, 124.9, 122.1, 115.4; HRMS, m/z calcd. for $C_{17}H_{14}N_4ONa$ $[M+Na]^+$: 313.1060; found: 313.1062.



5a–5o

Figure 2: Structures of target compounds 5a–5o.

*2.3.2. 2-(2-Methylbenzylidene)-N-(quinolin-6-yl)hydrazine-1-carboxamide (**5b**).* Product **5b** was obtained as white solid, yield 82.6%. m.p. 215.1–216.1°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.91 (s, 1H), 9.24 (s, 1H), 8.78 (d, J = 3.1 Hz, 1H), 8.35 (d, J = 1.5 Hz, 1H), 8.33 (s, 1H), 8.27 (d, J = 8.3 Hz, 1H), 8.22-8.18 (m, 1H), 8.08 (dd, J = 1.9,9.0 Hz, 1H), 7.98 (d, J = 9.0 Hz, 1H), 7.47 (dd, J = 4.1, 8.1 Hz, 1H), 7.31-7.25 (m, 2H), 7.25-7.21 (m, 1H), 2.42 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ:153.6, 149.1, 144.9, 140.2, 137.7, 136.7, 135.7, 132.7, 131.1, 129.7, 129.5, 128.8, 126.5, 126.5, 124.8, 122.1, 115.3, 19.4; HRMS, m/z calcd. for $C_{18}H_{16}N_4ONa$ $[M+Na]^+$: 327.1216, found: 327.1215.

*2.3.3. 2-(3-Methylbenzylidene)-N-(quinolin-6-yl)hydrazine-1-carboxamide (**5c**).* Product **5c** was obtained as white solid, yield 84.0%. m.p. 202.0–204.0°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.96 (s, 1H), 9.29 (s, 1H), 8.87-8.69 (m, 1H), 8.36 (brs, 1H), 8.32-8.25 (m, 1H), 8.10 (d, J = 8.1 Hz, 1H), 8.02-7.92 (m, 2H), 7.72–7.64 (m, 2H), 7.53-7.45 (m, 1H), 7.32 (t, J = 7.4 Hz, 1H), 7.21 (d, J = 7.2 Hz, 1H), 2.36 (brs, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 153.6, 148.9, 144.5, 141.9, 138.3, 137.8, 136.1, 134.7, 130.7, 129.2, 129.0, 128.8, 127.9, 125.1, 124.9, 122.1, 115.4, 21.4; HRMS, m/z calcd. for $C_{18}H_{17}N_4O$ $[M+H]^+$: 305.1397, found: 305.1398.

*2.3.4. 2-(4-Methylbenzylidene)-N-(quinolin-6-yl)hydrazine-1-carboxamide (**5d**).* Product **5d** was obtained as white solid, yield 74.9%. m.p. 207.5–208.7°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.89 (s, 1H), 9.24 (s, 1H), 8.77 (dd, J = 1.7, 4.2 Hz, 1H), 8.34 (d, J = 2.4 Hz, 1H), 8.27 (d, J = 8.3 Hz, 1H), 8.09 (dd, J = 2.3, 9.1 Hz, 1H), 8.00-7.96 (m, 2H), 7.78 (d, J = 8.1 Hz, 2H), 7.47 (dd, J = 4.0, 8.3 Hz, 1H), 7.25 (d, J = 8.1 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ:153.7, 149.1, 144.9, 141.8, 139.7, 137.7, 135.7, 132.1, 129.7, 129.5, 128.8, 127.6, 124.9, 122.1, 115.4, 21.5; HRMS, m/z calcd. for $C_{18}H_{17}N_4O$ $[M+H]^+$: 305.1397, found: 305.1401.

*2.3.5. 2-(2-Hydroxybenzylidene)-N-(quinolin-6-yl)hydrazine-1-carboxamide (**5e**).* Product **5e** was obtained as white solid, yield 80.9%. m.p. 210.5–211.6°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 11.09 (brs, 1H), 9.62 (brs, 1H), 9.02 (d, J = 4.8 Hz, 1H), 8.83 (d, J = 8.4 Hz, 1H), 8.58 (d, J = 2.0 Hz, 1H), 8.34 (s, 1H), 8.27 (d, J = 8.4 Hz, 1H), 8.15 (d, J = 9.2 Hz, 1H), 7.99 (brs, 1H), 7.83 (dd, J = 5.0, 8.4 Hz, 1H), 7.32-7.18 (m, 1H), 6.96-6.83 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 159.5, 159.2, 156.8, 153.3, 145.2, 142.1, 139.7, 138.1, 131.3, 129.6, 127.7, 124.5, 122.4, 120.6, 119.6, 116.5, 114.9; HRMS, m/z calcd. for $C_{17}H_{15}N_4O_2$ $[M+H]^+$: 307.1190, found: 307.1193.

*2.3.6. 2-(4-Hydroxybenzylidene)-N-(quinolin-6-yl)hydrazine-1-carboxamide (**5f**).* Product **5f** was obtained as white solid, yield 85.7%. m.p. 266.9–269.7°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.76 (s, 1H), 9.91 (s, 1H), 9.19 (s, 1H), 8.77 (dd, J = 1.7, 4.0 Hz, 1H), 8.35 (d, J = 2.2 Hz, 1H), 8.26 (d, J = 7.7 Hz, 1H), 8.09 (dd, J = 2.4, 9.0 Hz, 1H), 7.98 (d, J = 9.0 Hz, 1H), 7.93 (s, 1H), 7.73 (d, J = 8.6 Hz, 2H), 7.46 (dd, J = 4.2, 8.25 Hz, 1H), 6.86 (d, J = 8.6 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 159.4, 153.7, 149.0, 144.8, 142.0, 137.8, 135.7, 129.5, 129.3, 128.8, 125.9, 124.8, 122.1, 116.0, 115.1; HRMS, m/z calcd. for $C_{17}H_{15}N_4O_2$ $[M+H]^+$: 307.1190, found: 307.1187.

*2.3.7. 2-(4-(Dimethylamino)benzylidene)-N-(quinolin-6-yl)hydrazine-1-carboxamide (**5g**).* Product **5g** was obtained as white solid, yield 83.4%. m.p. 195.1–196.9°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.65 (s, 1H), 9.15 (s, 1H), 8.76 (dd, J = 1.6, 4.13 Hz, 1H), 8.34 (d, J = 2.0 Hz, 1H), 8.26 (d, J = 8.1 Hz, 1H), 8.08 (dd, J = 2.2, 9.0 Hz, 1H), 7.96 (d, J = 9.2 Hz, 1H), 7.88 (s, 1H), 7.69 (d, J = 8.8 Hz, 2H), 7.46 (dd, J = 4.0, 8.2 Hz, 1H), 6.74 (d, J = 8.8 Hz, 2H), 2.97 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 153.7, 151.6, 149.0, 144.8, 142.6, 137.9, 135.6, 132.0, 129.5, 128.9, 124.8, 122.3, 122.1, 114.9, 112.1, 40.3; HRMS, m/z calcd. for $C_{19}H_{19}N_5ONa$ $[M+Na]^+$: 356.1482, found: 356.1494.

*2.3.8. 2-(4-Fluorobenzylidene)-N-(quinolin-6-yl)hydrazine-1-carboxamide (**5h**).* Product **5h** was obtained as white solid, yield 80.5%. m.p. 207.0–208.5°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.96 (s, 1H), 9.29 (s, 1H), 8.77 (d, J = 2.6 Hz, 1H), 8.33 (d, J = 1.8 Hz, 1H), 8.27 (d, J = 8.1 Hz, 1H), 8.08 (dd, J = 2.0, 9.0 Hz, 1H), 8.00 (s, 1H), 7.99-7.95 (m, 3H), 7.47 (dd, J = 4.1, 8.2 Hz, 1H), 7.29 (t, J = 8.7 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 164.1, 162.5, 153.7, 147.0 (d, J = 321.8 Hz), 140.5, 137.7, 135.7, 131.4 (d, J = 1.5 Hz), 129.7 (d, J = 3.8 Hz), 129.5, 128.8, 124.9, 122.1, 116.1 (d, J = 10.5 Hz), 115.0; HRMS, m/z calcd. for $C_{17}H_{14}FN_4O$ $[M+H]^+$: 309.1146; found: 309.1134.

*2.3.9. 2-(4-Methoxybenzylidene)-N-(quinolin-6-yl)hydrazine-1-carboxamide (**5i**).* Product **5i** was obtained as white solid, yield 79.0%. m.p. 188.0–189.1°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.81 (s, 1H), 9.22 (s, 1H), 8.77 (dd, J = 1.3, 3.8 Hz, 1H), 8.33 (d, J = 1.8 Hz, 1H), 8.27 (d, J = 7.9 Hz, 1H), 8.08 (dd, J = 1.9, 9.0 Hz, 1H), 7.99-7.94 (m, 2H), 7.84 (d, J = 8.6 Hz, 2H), 7.47 (dd, J = 4.2, 8.2 Hz, 1H), 7.01 (d, J = 8.6 Hz, 2H), 3.81 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 160.9, 153.7, 149.0, 144.9, 141.6, 137.8, 135.7, 129.5, 129.2, 128.8, 127.4, 124.8, 122.1, 115.2, 114.6, 55.7; HRMS, m/z calcd. for $C_{18}H_{17}N_4O_2$ $[M+H]^+$: 321.1346, found 321.1349.

*2.3.10. 2-(3-Bromobenzylidene)-N-(quinolin-6-yl)hydrazine-1-carboxamide (**5j**).* Product **5j** was obtained as white solid, yield 81.2%. m.p. 237.5–239.9°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 11.21 (s, 1H), 9.34 (s, 1H), 8.78 (dd, J = 1.6, 4.1 Hz, 1H), 8.42 (dd, J = 1.5, 7.9 Hz, 1H), 8.38 (s, 1H), 8.33 (d, J = 2.4 Hz, 1H), 8.28 (d, J = 7.9 Hz, 1H), 8.08 (dd, J = 2.3, 9.1 Hz, 1H), 7.98 (d, J = 9.0 Hz, 1H), 7.67 (dd, J = 0.9, 8.1 Hz, 1H), 7.51-7.44 (m, 2H), 7.34 (dt, J = 1.7, 7.6 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 153.5, 149.2, 145.0, 139.9, 137.5, 135.7, 133.5, 133.4, 131.6, 129.5, 128.8, 128.4, 128.3, 125.0, 123.5, 122.1, 115.7; HRMS, *m/z* calcd. for $C_{17}H_{14}BrN_4O$ $[M+H]^+$: 369.0346, found: 369.0350.

*2.3.11. 2-(3,4-Dimethoxybenzylidene)-N-(quinolin-6-yl) hydrazine-1-carboxamide (**5k**).* Product **5k** was obtained as white solid, yield 85.4%. m.p. 191.1–193.1°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.92 (s, 1H), 9.38 (brs, 1H), 8.83 (d, J = 2.9 Hz, 1H), 8.44 (brs, 1H), 8.40 (d, J = 7.9 Hz, 1H), 8.12 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 9.0 Hz, 1H), 7.97 (s, 1H), 7.60 (brs, 1H), 7.54 (dd, J = 4.0, 7.9 Hz, 1H), 7.27 (d, J = 7.9 Hz, 1H), 6.99 (d, J = 8.1 Hz, 1H), 3.89 (s, 3H), 3.80 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 153.7, 150.8, 149.5, 148.1, 143.3, 142.1, 138.2, 137.3, 129.0, 128.3, 127.6, 125.6, 122.1, 121.9, 115.1, 111.8, 109.6, 56.1, 56.0; HRMS, *m/z* calcd. for $C_{19}H_{18}N_4O_3Na$ $[M+Na]^+$: 373.1271; found: 373.1278.

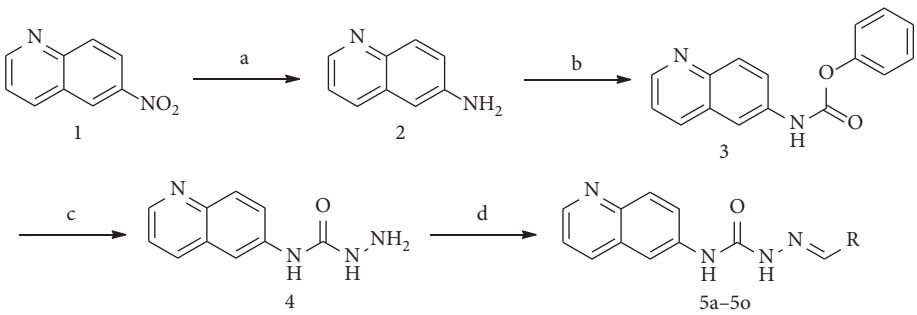
*2.3.12. 2-(2,4-Dimethoxybenzylidene)-N-(quinolin-6-yl) hydrazine-1-carboxamide (**5l**).* Product **5l** was obtained as white solid, yield 90.1%. m.p. 195.–195.7°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.88 (s, 1H), 9.37 (brs, 1H), 8.92 (d, J = 3.7 Hz, 1H), 8.59 (d, J = 8.3 Hz, 1H), 8.49 (d, J = 2.0 Hz, 1H), 8.28 (s, 1H), 8.23 (dd, J = 1.8, 9.0 Hz, 1H), 8.18-8.13 (m, 1H), 8.07 (d, J = 9.0 Hz, 1H), 7.68 (dd, J = 4.6, 8.3 Hz, 1H), 6.61 (dd, J = 2.2, 4.2 Hz, 2H), 3.85 (s, 3H), 3.82 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 162.5, 159.2, 153.6, 146.6, 140.6, 139.7, 139.0, 137.6, 129.3, 127.8, 126.7, 126.3, 122.2, 115.5, 115.0, 106.7, 98.3, 56.1, 55.8; HRMS, *m/z* calcd. for $C_{19}H_{18}N_4O_3Na$ $[M+Na]^+$: 373.1271; found: 373.1278.

*2.3.13. 2-(Pyridin-2-ylmethylene)-N-(quinolin-6-yl)hydrazine-1-carboxamide (**5m**).* Product **5m** was obtained as white solid, yield 78.0%. m.p. 212.5–214.3°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 11.14 (s, 1H), 9.34 (s, 1H), 9.05 (d, J = 1.7 Hz, 1H), 8.78 (dd, J = 1.7, 4.2 Hz, 1H), 8.59 (dd, J = 1.6, 4.7 Hz, 1H), 8.36 (td, J = 1.7, 8.0 Hz, 1H), 8.33 (d, J = 2.2 Hz, 1H), 8.28 (d, J = 8.3 Hz, 1H), 8.08 (dd, J = 2.3, 9.0 Hz, 1H), 8.03 (s, 1H), 7.98 (d, J = 9.0 Hz, 1H), 7.52-7.42 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 153.6, 150.5, 149.2, 149.1, 145.0, 138.7, 137.6, 135.7, 134.2, 130.7, 129.5, 128.7, 125.0, 124.2, 122.1, 115.7; HRMS, *m/z* calcd. for $C_{16}H_{14}N_5O$ $[M+H]^+$: 292.1193; found: 292.1195.

*2.3.14. 2-(Furan-2-ylmethylene)-N-(quinolin-6-yl)hydrazine-1-carboxamide (**5n**).* Product **5n** was obtained as white solid, yield 79.4%. m.p. 205.4–206.0°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.93 (s, 1H), 9.10 (s, 1H), 8.77 (d, J = 2.8 Hz, 1H), 8.31 (d, J = 2.0 Hz, 1H), 8.26 (d, J = 8.1 Hz, 1H), 8.00–8.06 (m, 1H), 7.92–7.99 (m, 2H), 7.83 (s, 1H), 7.46 (dd, J = 4.0, 8.3 Hz, 1H), 7.00 (d, J = 3.3 Hz, 1H), 6.64 (dd, J = 1.7, 3.2 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 153.4, 150.1, 149.1, 145.0, 144.9, 137.6, 135.7, 132.0, 129.6, 128.8, 124.6, 122.1, 115.1, 112.6, 112.5; HRMS, *m/z* calcd. for $C_{15}H_{12}N_4O_2Na$ $[M+Na]^+$: 303.0852; found: 303.0861.

*2.3.15. 2-(Thiophen-2-ylmethylene)-N-(quinolin-6-yl)hydrazine-1-carboxamide (**5o**).* Product **5o** was obtained as white solid, yield 77.9%. m.p. 214.7–217.3°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 11.04 (s, 1H), 9.32 (brs.,1H), 9.03-8.85 (m, 1H), 8.65 (d, J = 8.3 Hz, 1H), 8.46 (d, J = 1.8 Hz, 1H), 8.23 (s, 1H), 8.15 (dd, J = 2.0, 9.0 Hz, 1H), 8.08 (d, J = 9.0 Hz, 1H), 7.72 (dd, J = 4.6, 8.3 Hz, 1H), 7.66 (d, J = 5.0 Hz, 1H), 7.47 (d, J = 3.3 Hz, 1H), 7.13 (dd, J = 3.8, 4.9 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 159.5, 153.2, 146.4, 140.1, 139.3, 139.0, 137.5, 130.3, 129.3, 128.7, 128.2, 126.7, 126.1, 122.3, 114.9; HRMS, *m/z* calcd. for $C_{15}H_{13}N_4OS$ $[M+H]^+$: 297.0805, found: 297.0811.

2.4. Antitumor Activities. The *in vitro* antiproliferation activity of target compounds **5a–5o** against three human breast cancer lines including SKBR3, MDA-MB-231, and MCF-7 cancer cell lines was measured by the MTT colorimetric method. In all experiments, cells in good condition and logarithmic growth phase were taken and digested by 0.25% trypsin. The adherent cells were detached and counted, a 2–4*10^4 cells/mL suspension was prepared and inoculated into a 96-well cell culture plate with 180 μL a well, placed in a constant-temperature carbon dioxide incubator and cultured for 24 h, then was replaced with the dimethyl sulfoxide (DMSO) solution of target compound (20 μL) a well, and added 10 vol% serum culture medium (80 μL), and cultured for 48 h. MTT (20 μL) was added to each well and reacted for 4 h. The supernatant was removed and DMSO (150 μL per well) was added to dissolve MTT. The plate was shaken on a platform rocker for five minutes, and the optical density (OD) of each well was measured on an enzyme-linked immunosorbent assay (ELISA) meter at the wavelength of 570 nm. The inhibition activity of cell proliferation was determined. Dissolvent control group was used as the negative control group, and target compounds **5a**–**5o** were used as the positive control group. The inhibition rate of target compounds on tumor cell growth was calculated according to the following formula: inhibition rate% = (OD value of the negative control–OD value of tested compound)/OD value of the negative control×100%. The IC_{50} value of target compounds was calculated based on the inhibition rates of different concentrations.



Scheme 1: Synthesis of target compounds **5a–5o**. (a) Pt/C, H_2, MeOH; (b) phenyl chloroformate, triethylamine, DCM; (c) 80% hydrazine hydrate, 95% ethanol; (d) aldehydes, ethanol.

2.5. Colony Formation Assay. MCF-7 cells and Hela cells were cultured in a 6-well plate (200 cells/well) and treated with various compounds in 10% serum medium for 12 days and then fixed with methanol and stained with 0.1% crystal violet.

2.6. Annexin V/PI Staining. Cells were cultured in a six-well plate with about 1×10^5 cells in each well and treated with 10 μM **5k** and **5l** for 24 h. Then, the apoptosis analysis was performed following the protocols (BD Bioscience). After stained with FITC annexin V/PI, the apoptosis status was analyzed using a Beckman Epics Altra Culter and data was analyzed with EXP032 software. The status and percentage of cells undergoing apoptosis were defined as early apoptosis (annexin V-positive and PI-negative) and late apoptosis (annexin V-positive and PI-positive).

2.7. Western Blot Analysis. To check the level of cleaved PARP1 after **5k** and **5l** treatment, the western blot was performed using PARP1 antibody (9542, CST, Shanghai, China); meanwhile, the GAPDH antibody (G8795, Sigma) was used as the control. After different concentrations of **5k/5l** treatment for 24 h, the whole cell lysates were used for western blot analysis. Both anti-rabbit and anti-mouse IgG were purchased from Sigma.

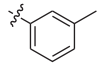
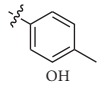
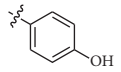
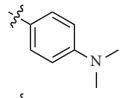
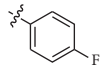
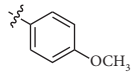
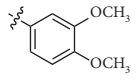
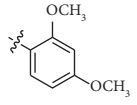
3. Results and Discussion

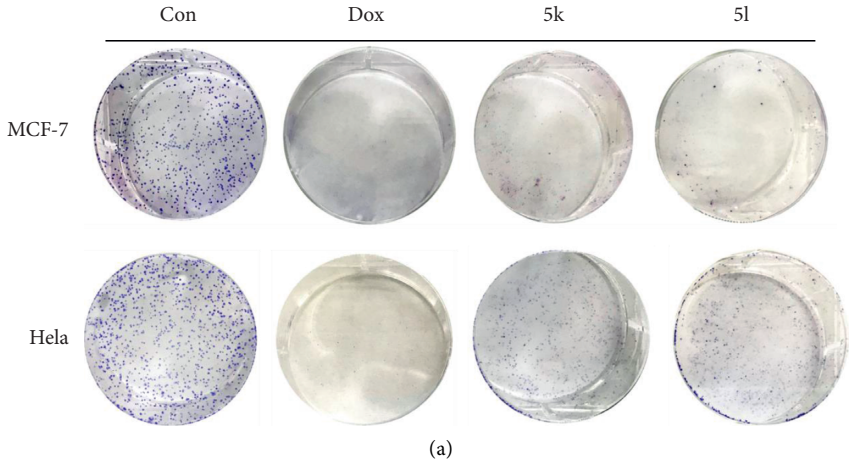
3.1. Chemistry. The synthetic strategy to prepare target compounds is outlined in Scheme 1. The intermediate **2** was obtained from classical reduction of 6-nitroquinoline, compound **3** was prepared by acylation of intermediate **2**, and derivative **4** was generated from substitution reaction between compound **3** and 80% hydrazine hydrate solution. Target products **5a–5o** were synthesized from condensation of derivative **4** with aldehydes. The structures of target compounds **5a–5o** were characterized by ^{1}H NMR,^{13}C NMR, and HRMS.

3.2. Antitumor Activities. The *in vitro* cytotoxic activity of target compounds **5a–5o** was evaluated in three human breast cancer cell lines including SKBR3, MDA-MB-231, and MCF-7 by the MTT assay, and Tamoxifen was used as the positive control. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and is summarized in Table 1. Among the synthesized compounds (**5a–5o**), the cytotoxic activity order is as follows: compounds **5h–5l** bearing methoxy or halogen-substituted phenyl group ≈ compounds **5m–5o** bearing pyridyl, furyl, and thienyl group >compounds **5a–5g** bearing nonsubstituted or other substituted phenyl group. Compound **5k** with the substitution of 3,4-dimethoxyphenyl and **5l** with the substitution of 2,4-dimethoxyphenyl displayed better antiproliferative activities against tested cancer cell lines than other compounds, and both of them exhibited more potential cytotoxicity on MCF-7 cell line than SKBr3 and MDA-MB-231 cell lines with IC_{50} of 8.50 and 12.5 μM. MCF-10A cells (normal breast cell line) were used to evaluate the cytotoxicity of **5k** and **5l** in the normal cell line. The results showed that **5k** and **5l** had no antiproliferative activity on MCF-10A cells with an IC_{50} >100 μmol/L, indicating the good selectivity of both compounds.

*3.3. The Biological Activity of **5k** and **5l** in MCF-7 Cells.* According to the MTT assay of this series of compounds displayed in Table 1, compounds **5k** and **5l** significantly inhibited the proliferation of MCF-7 cells. Therefore, compounds **5k** and **5l** were selected for further cellular experiments. The MTT results showed that **5k** and **5l** had antiproliferation activities with the IC_{50} values 8.50 and 12.51 μM; so, firstly we detected their growth inhibitory activities based on colony formation assay, which indicated **5k** and **5l** significantly inhibited MCF-7 cell growth at the concentration of 10 μM (Figure 3(a)). Besides, colony formation assay was conducted on HeLa cells, the results showed that compared with HeLa cells, **5k** and **5l** seemed to have stronger antiproliferation activities on MCF-7 cells. As acknowledged, cell cycle is essential for the normal proliferation of cells. To figure out the effects of **5k** and **5l** on cell cycle, flow cytometry was performed after 12 h treatment with **5k/5l** at a concentration of 10 μM. The results revealed that **5k/5l** induced cell cycle arrest in the G2-M phase with the ratio increasing from 6.19% to 17.40%/18.66% on MCF-7 cells, suggesting that the growth inhibitory of compounds **5k** and **5l** might be related to cell cycle arrest. Interestingly, when HeLa cells were treated with compounds **5k** and **5l**, contrasting to MCF-7 cells, no significant change of cell cycle

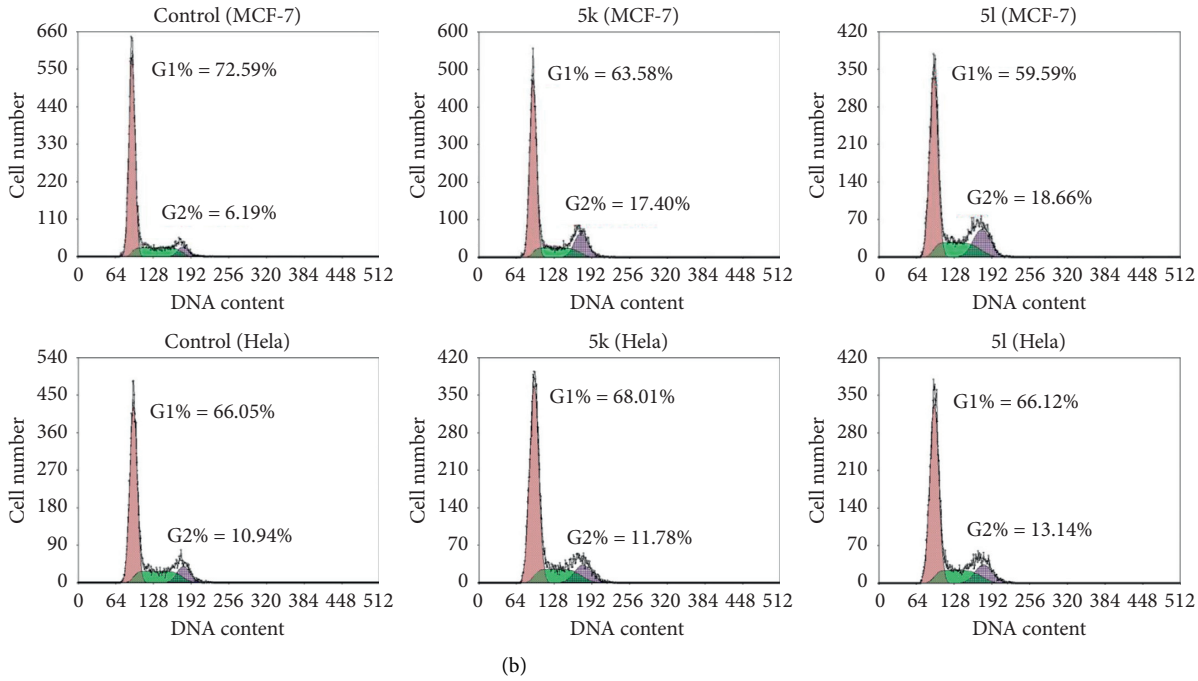
Table 1: The cytotoxicity of target compounds **5a–5o** against SKBr3, MDA-MB-231, and MCF-7 cancer cell lines.

Compounds	Substituents *R*	IC_{50} (μmol/L) SKBr3	MDA-MB-231	MCF-7
5a		47.89 ± 0.44	45.55 ± 0.56	34.34 ± 0.50
5b		43.80 ± 0.12	35.55 ± 0.22	37.34 ± 0.16
5c		33.82 ± 0.33	35.35 ± 0.19	35.87 ± 0.13
5d		43.22 ± 0.26	33.78 ± 0.22	34.32 ± 1.23
5e		23.12 ± 0.16	23.78 ± 0.27	27.54 ± 0.18
5f		23.45 ± 0.26	27.78 ± 0.33	24.50 ± 0.23
5g		23.10 ± 0.26	20.33 ± 0.21	27.51 ± 0.33
5h		17.10 ± 0.26	16.33 ± 0.11	13.51 ± 0.13
5i		15.16 ± 0.11	17.39 ± 0.12	13.51 ± 0.14
5j		17.10 ± 0.20	17.55 ± 0.23	14.52 ± 0.32
5k		9.16 ± 0.11	10.34 ± 0.36	8.50 ± 0.26
5l		16.10 ± 0.11	15.39 ± 0.46	12.51 ± 0.33
5m		19.20 ± 0.11	18.30 ± 0.15	16.33 ± 0.12
5n		16.11 ± 0.12	15.33 ± 0.26	16.51 ± 0.21
5o		14.18 ± 0.22	16.30 ± 0.34	13.56 ± 0.11
Tamoxifen		7.54 ± 0.16	10.68 ± 0.23	9.05 ± 0.22



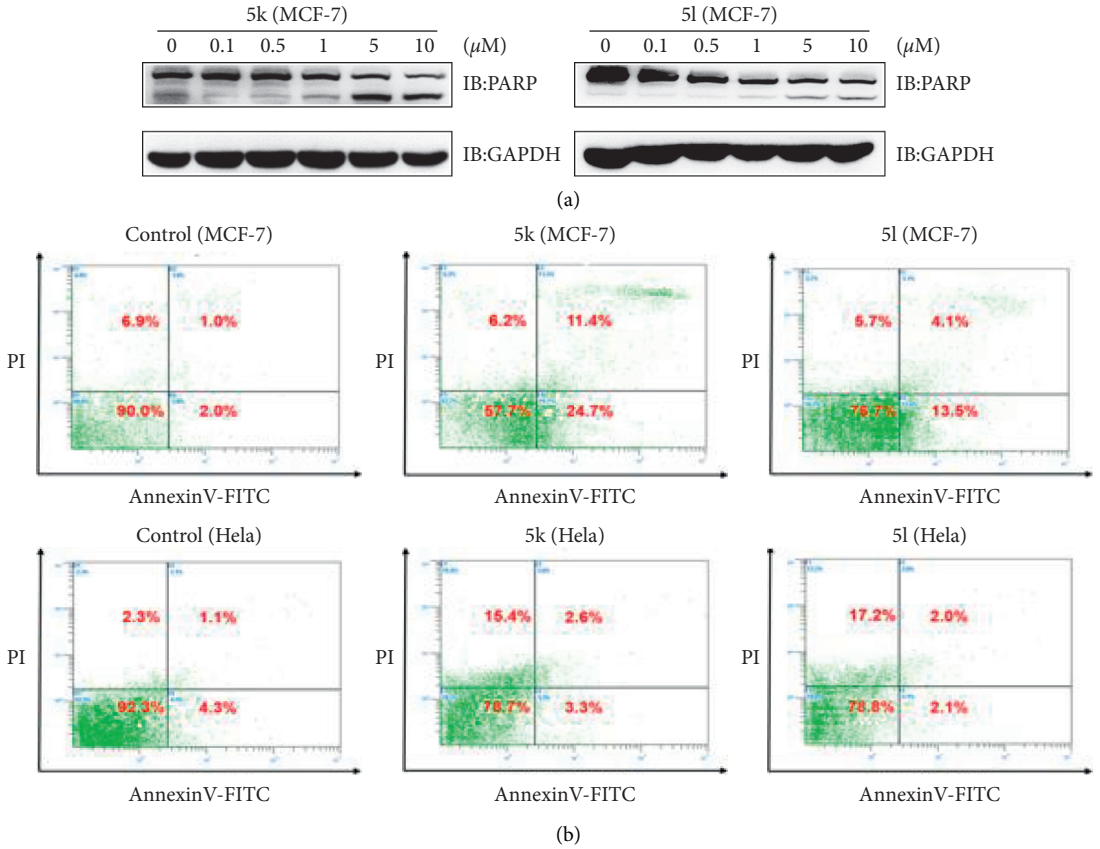
(a)

Figure 3: Continued.



(b)

Figure 3: Effects of compounds **5k** and **5l** on colony formation and cell cycle disruption: (a) compounds **5k** and **5l** inhibited cell growth on MCF-7 cells. (b) Compounds **5k** and **5l** induced G2-M phase arrest.



(b)

Figure 4: Apoptosis induction by compounds **5k** and **5l.** (a) Western blot analysis of PARP in MCF-7 cells treated with different concentrations of compounds **5k** and **5l**. (b) Annexin V/PI staining of MCF-7 cells and Hela cells treated with 10 μM **5k** and **5l** for 24 h.

was observed in HeLa cells. The data implied that **5k** and **5l** were more sensitive to breast cancer cell lines than the Hela cell line (Figure 3(b)).

Subsequently, in order to test whether **5k** and **5l** could induce apoptosis on MCF-7 cells, cleaved PARP (the mark of apoptosis) was detected by western blot assay after 24 h treatment with **5k** and **5l** on MCF-7 cells. The results showed that the PARP cleavage increased in a dose-dependent manner (Figure 4(a)). Moreover, after the treatment with **5k** and **5l** for 24 h, the MCF-7 or HeLa cells were doubly stained with annexin V/PI (propidium iodide). The results suggested that both **5k** and **5l** could notably promote apoptosis. Consistent with the abovementioned hypothesis that **5k** and **5l** had less effect on Hela cells, this result further confirmed that compounds **5k** and **5l** had the specificity on the MCF-7 cell line (Figure 4(b)).

4. Conclusions

A series of 2-arylidene-*N*-(quinolin-6-yl)hydrazine-1-carboxamides **5a**–**5o** were designed and synthesized based on combination of the biological profiles of the quinoline ring and the urea group. The reaction condition was optimized and fifteen target compounds **5a**–**5o** were achieved under best condition. The structures of target compounds **5a**–**5o** were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS. The cytotoxic activity *in vitro* of compounds **5a**–**5o** against SKBR3, MDA-MB-231, and MCF7 cell lines was evaluated by the MTT assay, and compound **5k** displayed the most potential cytotoxic activity with IC_{50} values of 9.16 μmol/L, 10.34 μmol/L, and 8.50 μmol/L against SKBR3, MDA-MB-231, and MCF-7, respectively. Additionally, compounds **5k** and **5l** showed better antiproliferative activities over MCF-7 cell lines with IC_{50} values of 8.50 and 12.51 μM. Colony formation assay showed **5k/5l** had a growth inhibitory effect on MCF-7 cells. Moreover, the results of western blot and flow cytometry indicated that **5k/5l** could significantly induce apoptosis. Interestingly, compared to Hela cells, MCF-7 cells were more susceptible to **5k/5l** treatment. On the basis of the results, further structural optimization and research will be carried out.

Data Availability

The data supporting the findings of the study are already given within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Shengxian Zhao and Yin Cao contributed equally to this work.

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