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ORIGINAL PAPER

Synthesis and biological evaluation of 2-(3-aminophenyl)-benzothiazoles as antiproliferative and apoptosis-inducing agents

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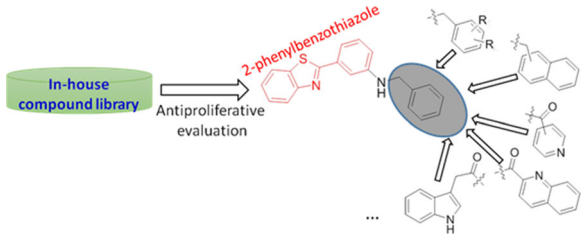
Abstract

A series of new 2-(3-aminophenyl)-benzothiazole derivatives were synthesized and evaluated for their in vitro antiproliferative activity against various human cancer cell lines including A549, HeLa, HepG2, MCF-7, MV4-11, and DB. Among the tested compounds, *N*-[3-(benzo[*d*]thiazol-2-yl)phenyl]nicotinamide displayed significantly improved antiproliferative activity toward A549 and MV4-11 cells with IC_{50} values of 5.42 ± 1.33 and 7.51 ± 0.98 μM, respectively, much stronger than the hit 3-(benzo[*d*]thiazol-2-yl)-*N*-(4-bromobenzyl)aniline. Furthermore, flow cytometric analysis indicated that *N*-[3-(benzo[*d*]thiazol-2-yl)phenyl]nicotinamide induced A549 cell apoptosis with cell cycle arrest at G1 phase in a concentration-dependent manner.

Graphical abstract



Keywords Heterocycles · Cytotoxicity · Antitumor agents · Cell cycle arrest · Structure–activity relationships

Introduction

Cancer is a class of diseases characterized by uncontrolled cell growth, which is one of the most leading death causes worldwide [1]. According to statistics in 2015, about 90.5 million people had cancer and estimated 14.1 million new cases are diagnosed each year [2, 3]. Up to now, chemotherapy is still the main treatment, but most anticancer drugs are limited in the clinical treatment due to their toxicity, resistance, and other side effects [4]. Consequently, developing novel and safer anticancer drugs remains a huge challenge.

Heterocyclic compounds play an extremely important role in the discovery of new chemotherapeutic agents [5–7]. As a characteristic group of heterocycles, the

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benzothiazoles occupy a prominent position in drug design [8]. Benzothiazole derivatives have been proven to possess diverse biological properties [9–13] and been extensively studied for their anticancer activities [14, 15]. However, as an important group of benzothiazole derivatives, only a few 2-phenylbenzothiazoles were reported to have anticancer potential [16–18]. Among them, 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (**1a**, NSC 703786) showed potent in vitro cytotoxicity ($GI_{50} < 1$ nM) against sensitive human breast MCF-7 (ER^+) and MDA 468 (ER^-) cell lines [16], and its prodrug (*S*)-2,6-diamino-*N*-[4-(5-fluorobenzo[*d*]thiazol-2-yl)-2-methylphenyl]-2-methylhexanamide (**1b**, NSC 710305, Phortress) was elected for phase 1 clinical evaluation in 2004 [17]. In our project to discover new anticancer drugs from heterocycles, a new 2-phenylbenzothiazole derivative **2a** with 3-substituted benzylaniline was recently screened out from our in-house compound library and showed weak antiproliferative activity against A549 cells. The anticancer activity of 2-phenylbenzothiazole with 3-substituted amino and its derivatives seems to be rarely studied according to the literature survey [19, 20]. These observations provoked our great interest in preparing 2-phenylbenzothiazole analogs of **2a** by introduction of N-substituents with the aim of increasing its anticancer activity and to extend the range of anticancer application. In this communication, we report the synthesis of a panel of new 2-(3-aminophenyl)-benzothiazole derivatives, and the biological evaluation for their in vitro antiproliferative activities (Fig. 1).

Results and discussion

Chemistry

Based on the substituent diversity-directed synthesis strategy [21], 25 2-(3-aminophenyl)-benzothiazoles were prepared with chemical agents in hand. The synthetic route for **2a**–**2u** and **2q-1** to **2q-4** is shown in Scheme 1. Condensation reaction of 3-nitrobenzaldehyde (**3**) and 2-aminobenzenethiol (**4**) gave 2-(3-nitrophenyl)benzo[*d*]thiazole (**5**), which then underwent reduction reaction catalyzed by iron and acetic acid to generate the 3-(benzo[*d*]thiazol-2-yl)aniline (**6**). Finally, the target compounds **2a**–**2u** and **2q-1** to **2q-4** were smoothly obtained from the reaction of **6** with corresponding bromides or acids.

Pharmacology

All the target compounds were evaluated for in vitro antiproliferative activity in an MTT assay [22], against a panel of human cancer cell lines, including A549 (human lung adenocarcinoma cell), HeLa (human cervical cancer cell), HepG2 (human hepatocellular carcinoma cell), MCF-7 (human breast adenocarcinoma cell), MV4-11 (human acute myeloid leukemia cell), and DB (human lymphoblast cell). Doxorubicin was used as positive control. The bioassay results are shown in Table 1.

From the results, the hit **2a** only showed relatively weak antiproliferative activity toward A549 and HepG2. Compared with **2a**, the 4-F-substituted analog **2d** showed increased activity. Since the fluorinated functionalities were considered to be key pharmacophores and fluorine-containing drugs accounted for about 25% marketed medicines [23], another seven F-substituted analogs **2e**–**2k** were then prepared. However, most of them were inactive, but interesting results were obtained from analog **2i** which showed more extensive anticancer activity against the tested cancer cell lines except for MCF-7, indicating that the introduction of NO_2 at the C-3 position of the benzene ring was pivotal for its activity. Two other compounds (**2l** and **2m**) with electron-withdrawing groups at C-4 position did not show improved activity. Furthermore, the replacement of the benzene ring with naphthalene (**2n** and **2o**), nitrogen- (**2p**–**2t**), or boron-containing heterocycle (**2u**) was driven by the substituent diversity. Two of these analogs, **2q** and **2r** with picolinamide, showed broad-spectrum antiproliferative activity against cancer cells. Particularly, compound **2q** showed more potent cytotoxicity against A549 ($IC_{50} = 5.42 \pm 1.33$ μM) and MV4-11 ($IC_{50} = 7.51 \pm 0.98$ μM) cells in contrast to the hit **2a**. From the bioassay results of **2p**–**2q**, it is obvious that the

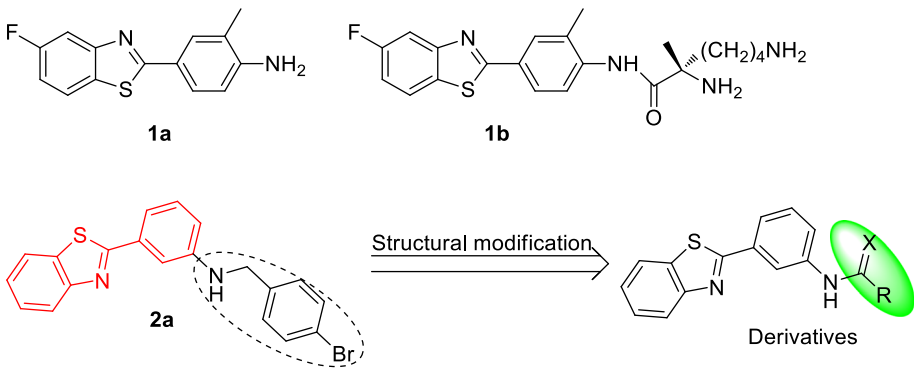
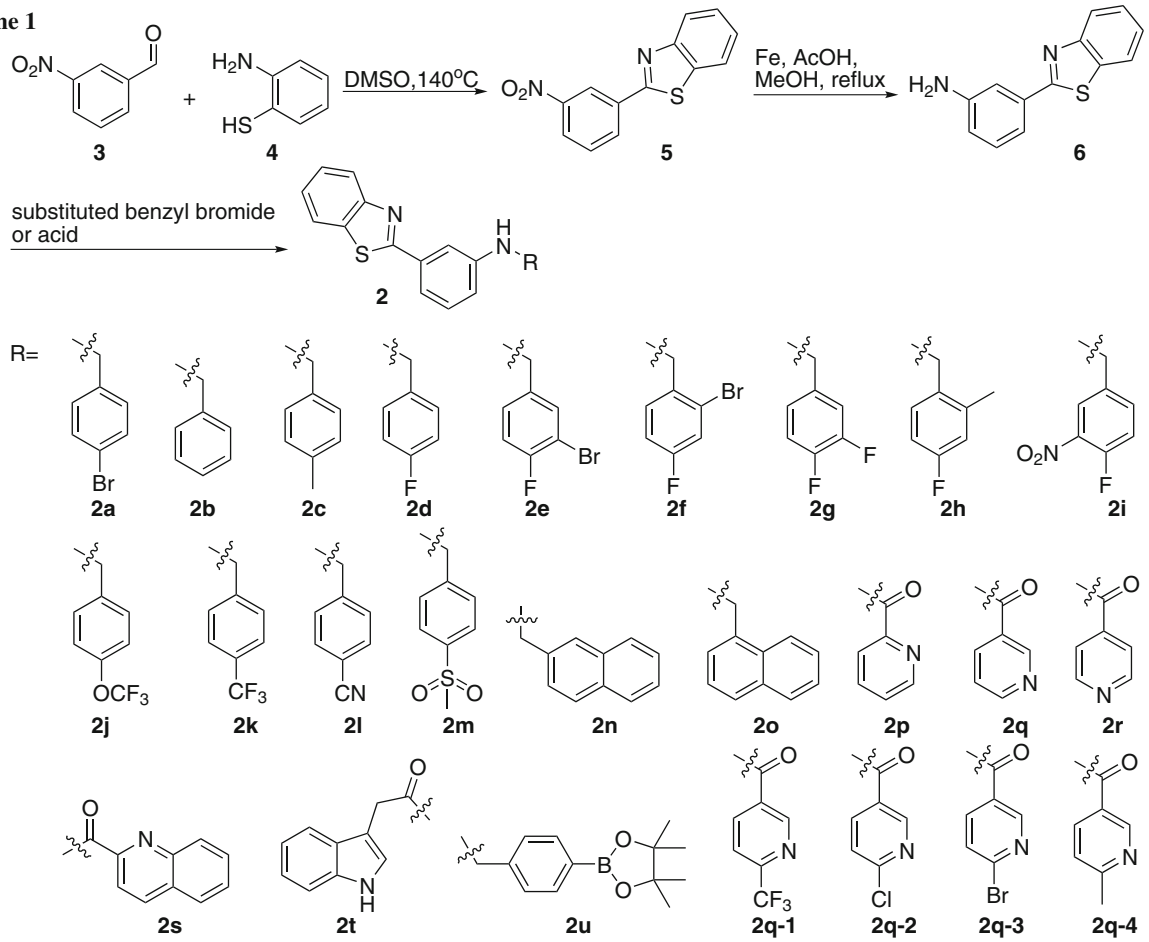


Fig. 1 2-Phenylbenzothiazole derivatives and structural modification of **2a**

Scheme 1



substituent position on the pyridine ring is crucial for their activity and the amide group attached to the C-3 position seems to be optimal. Further, four analogs, **2q-1** to **2q-4**, were prepared and evaluated for their antiproliferative activity; however, none of them showed better activity compared with **2q**, indicating that the substituent on the C-6 position of nicotinic acid is unfavorable for their activity.

Usually, anticancer therapeutics prevent the cancer cell proliferation by blocking the cell cycle at a specific checkpoint [24]. Thus, the blockade of cell cycle progression by antiproliferative agents takes a key role for development of a potential anticancer agent. Since compound **2q** showed the most potent anticancer activity, its effect on distribution of A549 cells cycle phase was studied by cytometric analysis. A549 cells were treated with **2q** at 2, 4, and 8 μM for 48 h, and ethanol-fixed cells were stained with propidium iodide and further subjected to flow cytometry. As shown in Fig. 2, the ratio of cells in G1 phase was increased from 57.09% in the control to 64.55% at 2 μM, 70.72% at 4 μM, and 85.60% at 8 μM, respectively. This result clearly revealed that **2q** could effectively induce A549 cells in G1 phase arrest.

Further, Annexin V–FITC/PI staining assay was performed to evaluate the apoptosis-inducing effect of **2q**. Results from Fig. 3 showed apoptosis of A549 cells in a concentration-dependent manner upon treatment with **2q** at 2, 4, and 8 μM for 48 h. These data indicated that compound **2q** was an apoptosis inducer.

Conclusion

In summary, a series of 2-(3-aminophenyl)-benzothiazole analogs were prepared based on the derivatization of hit compound **2a**. All synthesized compounds were subjected to evaluation of in vitro anticancer potential against a panel of human cancer cell lines, including A549, HeLa, HepG2, MCF-7, MV4-11, and DB. From the in vitro screening results, it was clearly revealed that compound **2q** showed the most potent antiproliferative activity, especially against A549 and MV4-11 cell lines. Flow cytometric analysis indicated that **2q** blocked the G1 phase of the A549 cell

Table 1 Antiproliferative activities of 2-(3-aminophenyl)-benzothiazole derivatives presented as IC_{50}/μM

Comp.	A549	HeLa	HepG2	MCF-7	DB	MV4-11
2a	45.54 ± 4.62	–[a]	39.54 ± 2.88		–	–
2b	–	–	–	–	–	–
2c	–	–	–	–	–	–
2d	33.54 ± 2.31	30.12 ± 3.09	25.54 ± 1.97	–	–	–
2e	–	–	–	–	–	–
2f	–	–	–	–	–	–
2g	–	–	–	–	–	–
2h	–	–	–	–	–	–
2i	25.54 ± 1.24	34.44 ± 3.13	18.12 ± 3.76	–	21.36 ± 3.11	39.50 ± 2.78
2j	–	–	–	–	–	–
2k	–	–	–	–	–	–
2l	31.22 ± 2.15	–	–	–	–	–
2m	–	–	–	–	15.51 ± 1.78	–
2n	–	–	–	–	–	–
2o	–	–	–	–	–	–
2p	–	–	–	–	–	–
2q	5.42 ± 1.33	24.05 ± 1.33	24.28 ± 2.48	21.22 ± 1.56	15.65 ± 3.18	7.51 ± 0.98
2r	–	39.45 ± 3.71	24.04 ± 1.89	31.48 ± 3.21	32.1 ± 2.41	22.48 ± 3.22
2s	–	–	–	–	–	–
2t	–	–	28.16 ± 1.47	–	–	29.80 ± 1.65
2u	–	–	–	–	–	–
2q-1	32.54 ± 1.35	22.67 ± 1.08	32.78 ± 1.65	–	–	–
2q-2	–	23.98 ± 3.45	40.69 ± 4.33	–	34.58 ± 2.45	–
2q-3	–	47.78 ± 2.13	–	27.44 ± 1.77	–	–
2q-4	47.78 ± 2.13	–	42.05 ± 3.45	–	–	–
Doxorubicin	0.16 ± 0.02	0.32 ± 0.15	0.14 ± 0.01	0.25 ± 0.03	0.16 ± 0.02	0.11 ± 0.01

Compounds with inhibition rate < 50% at 50 μM were considered to be inactive and not tested for further IC_{50} values

cycle and was an effective apoptosis-inducing agent. Overall, the present study established **2q** as an antiproliferative agent, and continuing investigation into its potential for the treatment of cancer is warranted.

Experimental

Melting points were measured by a YRY-3 Melting Point apparatus (Tianjin Precision Apparatus Factory, China). Commercially available reagents were used without further purification. Organic solvents were evaporated with reduced pressure using a Büchi R-100 evaporator (Büchi, Switzerland). Silica gel column chromatography was performed on Biotage Isolera One (Biotage, Uppsala, Sweden). NMR spectra were measured on Bruker Avance III 600 MHz spectrometer (Bruker, Fällanden, Switzerland). Chemical shifts were expressed in δ (ppm) and coupling constants (J) in Hz with residual solvent signals as standards ($CDCl_3$, δ_H = 7.26 ppm and δ_C = 77.2 ppm; CD_3OD, δ_H = 3.31 ppm and δ_C = 49.0 ppm). The purity of the samples was determined by an analytical Agilent 1260 HPLC with ZDRBAX SB-C18 column (4.6 mm × 150 mm) using parameters as follows: H_2O/MeOH, 80/20–0/100 in 15 min, plus 10 min isocratic MeOH, flow rate at 1.0 cm^3/min, λ = 280 and 254 nm. Elemental analyses (C, H, N, S) were conducted using the Vario EL III (Elementar, Germany), and their results were found to be in good agreement (± 0.3%) with the calculated values. ESI-MS analyses were performed on an Agilent 1260-6460 Triple Quard LC–MS instrument (Agilent, Waldbronn, Germany).

2-(3-Nitrophenyl)benzo[*d*]thiazole (5) The mixture of 3.02 g 3-nitrobenzaldehyde (20 mmol) and 2.50 g 2-aminobenzenethiol (20 mmol) in 10 cm^3 dimethyl sulfoxide was stirred at 140 °C for 12 h. Then the reaction solvent was diluted in dichloromethane and washed with water. The organic phase was dried over dry magnesium sulfate. The product **5** was purified by flash column chromatography using mixtures of petroleum ether/ethyl acetate. White solid in 56.0% yield; m.p.: 182.2–183.5 °C (Ref. [25] 182–184 °C). ^{1}H NMR and ^{13}C spectra were found to

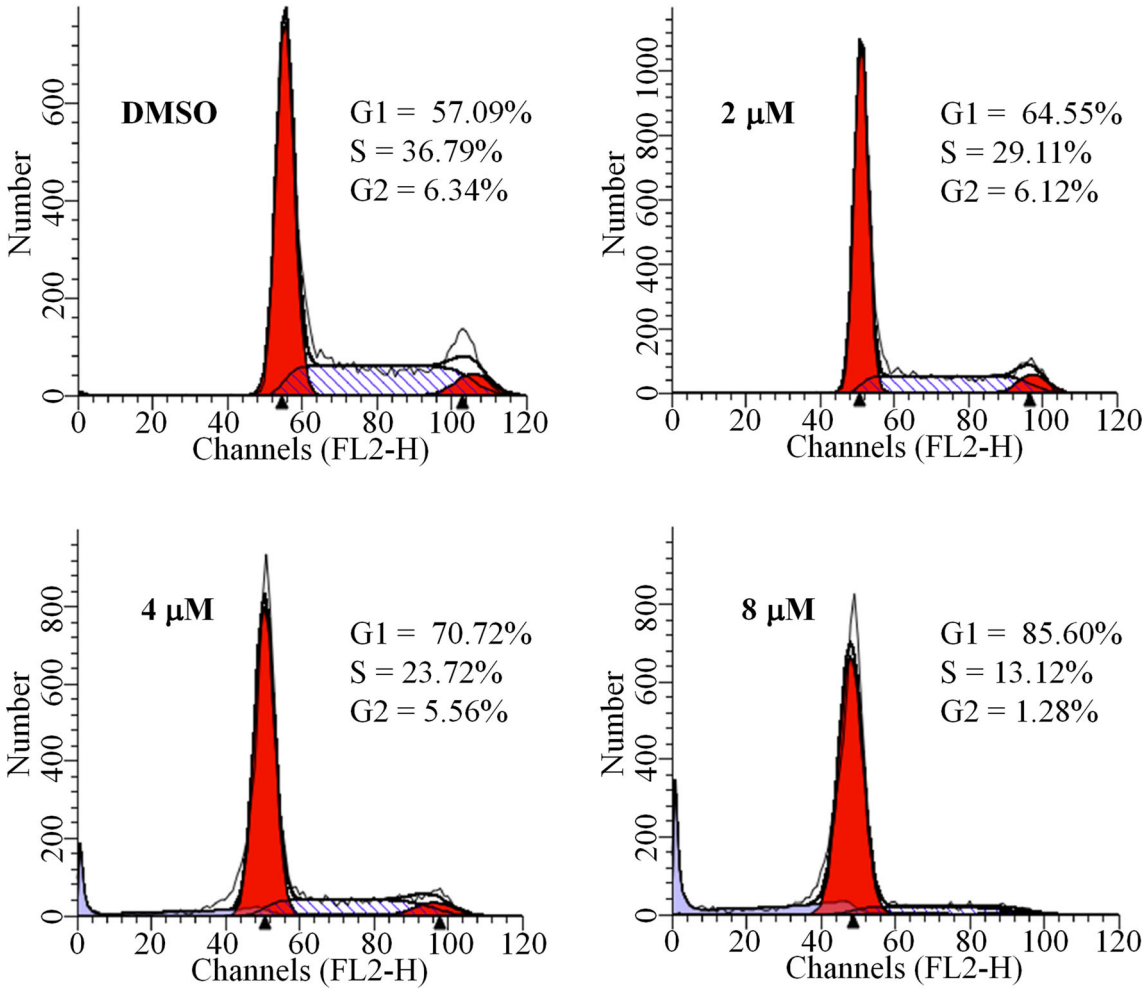


Fig. 2 Treatment with **2q** arrested A549 cells at G1 phase measured at 48 h. Cells were treated with graded concentrations of **2q** for 48 h. Propidium iodide (PI) staining was done to determine the DNA content, and the percentage of cells in different phases of the cell cycle was analyzed by flow cytometry

be identical with the ones described in Ref. [24]. ESI-MS: m/z = 257.0 ($[M+H]^+$).

3-(Benzo[*d*]thiazol-2-yl)aniline (6, $C_{13}H_{10}N_2S$) To a solution of 2.82 g compound **5** (11 mmol) in 20 cm^3 methanol, 1.54 g iron powder (27.5 mmol) and 6.60 g acetic acid (110 mmol) were added. The reaction mixture was stirred at 75 °C overnight. The solution was filtered to remove the catalyst and compound **6** was used in the next step without further purification. Light yellow solid in 77.4% yield; m.p.: 136.4–137.3 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.11 (d, J = 7.9 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.53 (dd, J = 7.9, 7.1 Hz, 1H), 7.44 (dd, J = 8.1, 7.1 Hz, 1H), 7.35 (brs, 1H), 7.24–7.16 (m, 2H), 6.78–6.71 (m, 1H), 5.46 (s, 2H, NH) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.2, 153.6, 149.4, 134.3, 133.4, 129.8, 126.5, 125.3, 122.7, 122.3, 116.8, 114.6, 111.8 ppm; ESI-MS: m/z = 227.0 ($[M+H]^+$).

General procedure for the preparation of target products 2a–2u

To solutions of 50 mg compound **6** (0.22 mmol) in 3 cm^3 DMF, the corresponding bromomethyl benzene (0.22 mmol) and 91.1 mg sodium carbonate (0.66 mmol) or acid (0.22 mmol), EDCI (0.22 mmol), and HOBT (0.22 mmol) were added. The reaction mixtures were stirred at room temperature overnight. The reaction mixtures were extracted with aqueous sodium chloride solution and dichloromethane, and the organic phase dried over anhydrous sodium sulfate. The final products were purified by flash column chromatography using mixtures of petroleum ether/ethyl acetate.

3-(Benzo[*d*]thiazol-2-yl)-*N*-(4-bromobenzyl)aniline (2a, $C_{20}H_{15}BrN_2S$) White solid in 57.2% yield; HPLC purity: 97.47%; t_R = 11.54 min; m.p.: 85.4–86.5 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.11 (d, J = 8.1 Hz,

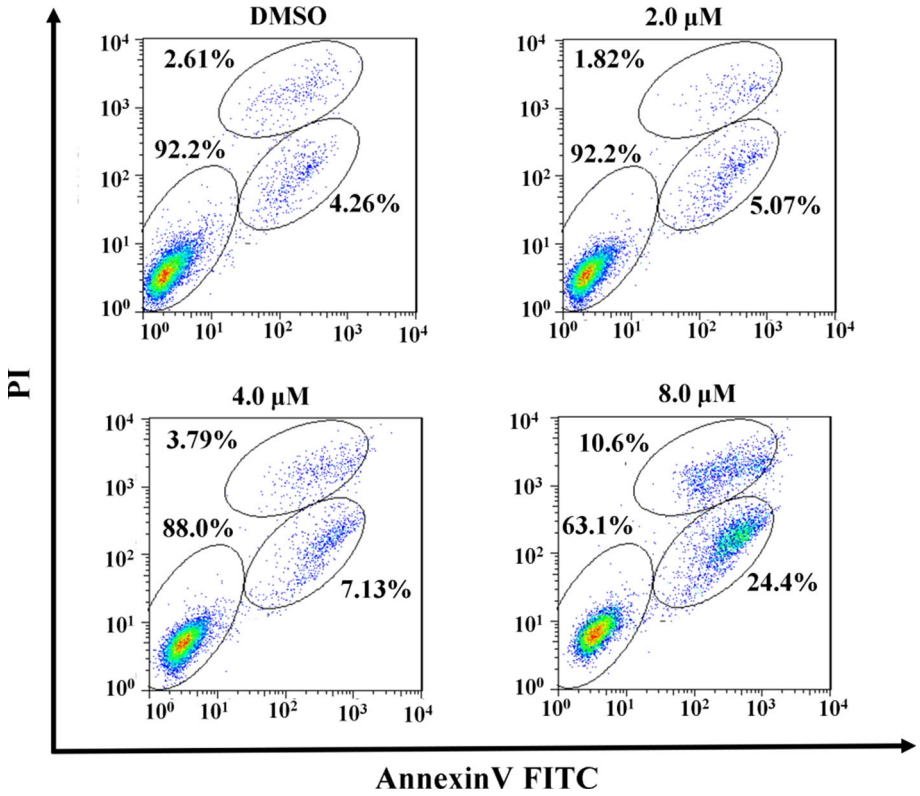


Fig. 3 A549 cells were treated with compound **2q** for 48 h, and apoptosis was determined by Annexin V–FITC/PI staining by flow cytometry

1H), 8.02 (d, J = 8.1 Hz, 1H), 7.53 (d, J = 8.1 Hz, 2H), 7.52 (dd, J = 8.1, 7.2 Hz, 1H), 7.44 (dd, J = 8.1, 7.2 Hz, 1H), 7.35 (d, J = 8.1 Hz, 2H), 7.32 (brs, 1H, NH), 7.25–7.21 (m, 2H), 6.77–6.68 (m, 2H), 4.34 (d, J = 6.1 Hz, 2H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.1, 153.5, 149.0, 139.4, 134.3, 133.5, 131.2, 129.9, 129.4, 126.5, 125.4, 122.7, 122.3, 122.3, 119.7, 115.0, 110.6, 45.6 ppm; ESI-MS: m/z = 394.9 ([M+H]$^+$).

3-(Benzo[*d*]thiazol-2-yl)-*N*-benzylaniline (2b, $C_{20}H_{16}N_2S$) White solid in 58.1% yield; HPLC purity: 98.14%; t_R = 9.55 min; m.p.: 69.1–72.0 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.11 (d, J = 7.9 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.52 (dd, J = 7.9, 7.2 Hz, 1H), 7.44 (dd, J = 8.1, 7.2 Hz, 1H), 7.40 (d, J = 7.4 Hz, 2H), 7.36–7.32 (m, 3H), 7.25–7.20 (m, 3H), 6.78–6.74 (m, 1H), 6.68 (t, J = 6.0 Hz, 1H, NH), 4.36 (d, J = 6.0 Hz, 2H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.2, 153.5, 149.3, 139.8, 134.3, 133.4, 129.9, 128.4, 128.4, 127.2, 127.2, 126.8, 126.5, 125.3, 122.7, 122.3, 115.1, 114.8, 110.5, 46.3 ppm; ESI-MS: m/z = 317.6 ([M+H]$^+$).

3-(Benzo[*d*]thiazol-2-yl)-*N*-(4-methylbenzyl)aniline (2c, $C_{21}H_{18}N_2S$) White solid in 37.3% yield; HPLC purity: 97.29%; t_R = 11.24 min; m.p.: 78.5–79.5 °C; ^{1}H NMR (600 MHz, CD_3OD): δ = 7.96 (d, J = 8.1 Hz, 1H), 7.94 (d, J = 7.9 Hz, 1H), 7.49 (dd, J = 8.1, 7.1 Hz, 1H), 7.39 (dd, J = 7.9, 7.1 Hz, 1H), 7.32 (dd, J = 2.0, 1.8 Hz, 1H), 7.30–7.25 (m, 3H), 7.20 (dd, J = 7.9, 7.8 Hz, 1H), 7.12 (d, J = 7.9 Hz, 2H), 6.77 (dd, J = 7.9, 2.0 Hz, 1H), 4.33 (s, 2H), 2.29 (s, 3H) ppm; ^{13}C NMR (150 MHz, CD_3OD): δ = 171.2, 154.9, 150.8, 137.9, 137.6, 135.9, 135.0, 130.8, 130.1, 128.4, 127.5, 126.4, 123.5, 122.9, 117.0, 116.8, 112.1, 48.2, 21.1 ppm; ESI-MS: m/z = 331.0 ([M +H]$^+$).

3-(Benzo[*d*]thiazol-2-yl)-*N*-(4-fluorobenzyl)aniline (2d, $C_{20}H_{15}FN_2S$) Yellow solid in 43.7% yield; HPLC purity: 98.45%; t_R = 9.23 min; m.p.: 78.9–81.2 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.11 (d, J = 7.9 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.52 (dd, J = 7.9, 7.1 Hz, 1H), 7.46–7.41 (m, 3H), 7.33 (brs, 1H), 7.25–7.21 (m, 2H), 7.17 (dd, J = 8.9, 8.8 Hz, 2H), 6.77–6.74 (m, 1H), 6.68 (t, J = 6.0 Hz, 1H, NH), 4.35 (d, J = 6.0 Hz, 2H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.1, 161.2 (d, J = 241.6 Hz), 153.5, 149.1, 135.9 (d, J = 3.2 Hz), 134.3, 133.4, 129.9, 129.1 (d, J = 8.24 Hz), 126.5, 125.3, 122.7, 122.3, 115.2, 115.0 (d, J = 15.3 Hz), 115.0, 110.6, 45.6 ppm; ESI-MS: m/z = 335.1 ([M+H]$^+$).

3-(Benzo[*d*]thiazol-2-yl)-*N*-(3-bromo-4-fluorobenzyl)aniline (2e, $C_{20}H_{14}BrFN_2S$) White solid in 41.2% yield; HPLC purity: 96.32%; t_R = 11.48 min; m.p.: 98.0–100.0 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.12 (d, J = 7.9 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.67 (dd, J = 7.9, 7.2 Hz, 1H), 7.53 (dd, J = 8.0, 7.2 Hz, 1H), 7.44 (dd, J = 7.8, 7.2 Hz, 1H), 7.38 (dd, J = 7.8, 1.3 Hz, 1H), 7.33 (brs, 1H),

7.27–7.19 (m, 3H), 6.78–6.72 (m, 2H), 4.38 (d, J = 6.1 Hz, 2H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.0, 158.2 (d, J = 244.3 Hz), 153.5, 148.8, 143.0 (d, J = 6.5 Hz), 134.3, 133.5, 133.4, 130.0, 126.5, 125.4, 124.7 (d, J = 3.12 Hz), 122.7, 122.3, 115.3 (d, J = 11.1 Hz), 115.2 (d, J = 10.4 Hz), 115.0, 110.7, 105.6 (d, J = 20.8 Hz), 45.3 ppm; ESI-MS: m/z = 413.0 ([M+H]$^+$).

3-(Benzo[*d*]thiazol-2-yl)-*N*-(2-bromo-4-fluorobenzyl)aniline (2f, $C_{20}H_{14}BrFN_2S$) White solid in 41.7% yield; HPLC purity: 96.21%; t_R = 12.33 min; m.p.: 70.3–71.6 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.11 (d, J = 7.9 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.62 (dd, J = 8.5, 2.5 Hz, 1H), 7.52 (dd, J = 7.9, 7.1 Hz, 1H), 7.48–7.45 (m, 1H), 7.44 (dd, J = 8.1, 7.1 Hz, 1H), 7.32 (brs, 1H), 7.30–7.22 (m, 3H), 6.74 (t, J = 5.9 Hz, 1H, NH), 6.72–6.69 (m, 1H), 4.36 (d, J = 5.9 Hz, 2H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.0, 160.8 (d, J = 246.9 Hz), 153.5, 148.8, 134.4 (d, J = 3.5 Hz), 134.3, 133.6, 130.2 (d, J = 8.1 Hz), 130.1, 126.5, 125.4, 122.8 (d, J = 10.1 Hz), 122.7, 122.3, 119.7 (d, J = 24.0 Hz), 115.3, 114.9 (d, J = 8.35 Hz), 114.8, 110.4, 46.1 ppm; ESI-MS: m/z = 413.0 ([M+H]$^+$).

3-(Benzo[*d*]thiazol-2-yl)-*N*-(3,4-difluorobenzyl)aniline (2g, $C_{20}H_{14}F_2N_2S$) Yellow solid in 43.1% yield; HPLC purity: 97.37%; t_R = 9.50 min; m.p.: 76.4–77.2 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.11 (d, J = 7.9 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.52 (dd, J = 7.9, 7.3 Hz, 1H), 7.46–7.41 (m, 2H), 7.39 (dd, J = 8.1, 7.3 Hz,1H), 7.33 (brs, 1H), 7.27–7.22 (m, 3H), 6.77–6.73 (m, 1H), 6.71 (t, J = 6.0 Hz, 1H, NH), 4.36 (d, J = 6.0 Hz, 2H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.1, 153.6, 148.9, 137.9, 137.9, 134.3, 133.5, 130.0, 126.6, 125.4, 123.8 (d, J = 3.2 Hz), 123.8 (d, J = 3.6 Hz), 122.8, 122.3, 117.5 (d, J = 16.5 Hz), 116.1 (d, J = 16.8 Hz), 115.2, 115.1, 110.7, 45.3 ppm; ESI-MS: m/z = 353.0 ([M+H]$^+$).

3-(Benzo[*d*]thiazol-2-yl)-*N*-(4-fluoro-2-methylbenzyl)aniline (2h, $C_{21}H_{17}FN_2S$) Yellow oil in 46.6% yield; HPLC purity: 98.54%; t_R = 11.05 min; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.11 (d, J = 7.9 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.52 (dd, J = 7.9, 7.2 Hz, 1H), 7.44 (dd, J = 8.1, 7.2 Hz, 1H), 7.36 (brs, 1H), 7.32 (dd, J = 8.4, 6.3 Hz, 1H), 7.27–7.22 (m, 2H), 7.07 (dd, J = 10.0, 2.6 Hz, 1H), 6.97 (ddd, J = 8.7, 8.4, 2.6 Hz, 1H), 6.80–6.73 (m, 1H), 6.49 (t, J = 5.6 Hz, 1H), 4.28 (d, J = 5.6 Hz, 2H), 2.37 (s, 3H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.2, 161.1 (d, J = 242.5 Hz), 153.6, 149.3, 138.8 (d, J = 8.7 Hz), 134.3, 133.5, 133.3 (d, J = 2.6 Hz), 129.9, 129.2 (d, J = 7.2 Hz), 126.5, 125.3, 122.7, 122.3, 116.6 (d, J = 19.6 Hz), 114.9, 114.9, 112.1 (d, J = 20.0 Hz), 110.3, 44.0, 18.64 ppm; ESI-MS: m/z = 349.1 ([M+H]$^+$).

3-(Benzo[*d*]thiazol-2-yl)-*N*-(4-fluoro-3-nitrobenzyl)aniline (2i, $C_{20}H_{14}FN_3O_2S$) Yellow oil in 73.2% yield; HPLC purity: 96.25%; t_R = 7.94 min; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.18 (dd, J = 7.2, 2.0 Hz, 1H), 8.11 (d, J = 8.0 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.84 (ddd, J = 8.4, 4.1, 2.0 Hz, 1H), 7.58 (dd, J = 11.3, 8.6 Hz, 1H), 7.53 (dd, J = 8.0, 7.1 Hz, 1H), 7.44 (dd, J = 8.1, 7.1 Hz, 1H), 7.37–7.34 (m, 1H), 7.28–7.22 (m, 2H), 6.82 (t, J = 6.2 Hz, 1H, NH), 6.78–6.75 (m, 1H), 4.47 (d, J = 6.2 Hz, 2H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.0, 154.4, 153.1 (d, J = 127.8 Hz), 148.7, 137.8 (d, J = 3.7 Hz), 136.7 (d, J = 7.6 Hz), 135.0 (d, J = 8.8 Hz), 134.3, 133.5, 130.1, 126.6, 125.4, 124.4, 122.8, 122.3, 118.5 (d, J = 20.82 Hz), 115.4, 115.0, 110.8, 44.9 ppm; ESI-MS: m/z = 380.0 ([M+H]$^+$).

3-(Benzo[*d*]thiazol-2-yl)-*N*-[4-(trifluoromethoxy)benzyl]aniline (2j, $C_{21}H_{15}F_3N_2OS$) White solid in 55.6% yield; HPLC purity: 97.36%; t_R = 11.14 min; m.p.: 71.0–72.3 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.11 (d, J = 8.0 Hz, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.54–7.50 (m, 3H), 7.44 (dd, J = 8.0, 7.1 Hz, 1H), 7.37–7.32 (m, 3H), 7.26–7.22 (m, 2H), 6.78–6.75 (m, 1H), 6.73 (t, J = 6.0 Hz, 1H, NH), 4.40 (d, J = 6.0 Hz, 2H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.1, 153.5, 149.1, 147.2, 139.4, 134.3, 133.5, 129.9, 129.9, 129.0, 126.5, 125.3, 122.7, 122.3, 121.0, 115.1, 115.0, 110.6, 45.5 ppm; ESI-MS: m/z = 401.0 ([M+H]$^+$).

3-(Benzo[*d*]thiazol-2-yl)-*N*-[4-(trifluoromethyl)benzyl]aniline (2k, $C_{21}H_{15}F_3N_2S$) White solid in 35.7% yield; HPLC purity: 98.59%; t_R = 10.52 min; m.p.: 87.6–89.2 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.11 (d, J = 7.9 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.71 (d, J = 8.1 Hz, 2H), 7.61 (d, J = 8.1 Hz, 2H), 7.52 (dd, J = 7.9, 7.2 Hz, 1H), 7.44 (dd, J = 8.1, 7.2 Hz, 1H), 7.35 (brs, 1H), 7.26–7.21 (m, 2H), 6.81 (t, J = 6.1 Hz, 1H, NH), 6.74–6.71 (m, 1H), 4.48 (d, J = 6.1 Hz, 2H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.1, 153.5, 149.0, 145.1, 134.3, 133.5, 130.0, 127.8, 126.6, 125.3 (q, J = 308.1 Hz), 125.3, 125.3, 125.3, 122.8, 122.3, 115.2, 114.9, 110.6, 45.8 ppm; ESI-MS: m/z = 385.0 ([M +H]$^+$).

4-[[[3-(Benzo[*d*]thiazol-2-yl)phenyl]amino]methyl]benzonitrile (2l, $C_{21}H_{15}N_3S$) Yellow oil in 82.6% yield; HPLC purity: 95.34%; t_R = 7.20 min; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.05 (d, J = 8.0 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.62 (d, J = 8.3 Hz, 2H), 7.50–7.46 (m, 3H), 7.42–7.39 (m, 2H), 7.38 (dd, J = 8.0, 7.0 Hz, 1H), 7.28–7.25 (m, 1H), 6.69–6.65 (m, 1H), 4.50 (d, J = 5.6 Hz, 2H), 4.42 (t, J = 5.6 Hz, 1H, NH) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 168.5, 154.1, 148.0, 144.9, 135.1, 134.7, 132.6, 130.2, 127.9, 126.4, 125.3, 123.3, 121.7, 118.9, 117.8, 115.4, 111.5, 111.2, 47.8 ppm; ESI-MS: m/z = 342.6 ([M+H]$^+$).

3-(Benzo[*d*]thiazol-2-yl)-*N*-[4-(methylsulfonyl)benzyl]aniline (2m, $C_{21}H_{18}N_2O_2S_2$) White solid in 55.2% yield; HPLC purity: 98.26%; t_R = 5.17 min; m.p.: 133.2–135.1 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.05 (d, J = 8.1 Hz, 1H), 7.92 (d, J = 8.3 Hz, 2H), 7.89 (d, J = 8.0 Hz, 1H), 7.59 (d, J = 8.3 Hz, 2H), 7.48 (dd, J = 8.1, 7.2 Hz, 1H), 7.38 (dd, J = 8.0, 7.2 Hz, 1H), 7.43–7.39 (m, 1H), 7.27 (dd, J = 7.8, 7.2 Hz, 1H), 6.68 (dd, J = 7.9, 1.8 Hz, 1H), 4.55 (brs, 2H), 4.45 (brs, 1H, NH), 3.05 (s, 3H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 168.5, 154.1, 148.0, 146.0, 139.6, 135.1, 134.7, 130.2, 128.1, 128.0, 126.4, 125.3, 123.3, 121.7, 117.8, 115.4, 111.5, 47.7, 44.7 ppm; ESI-MS: m/z = 395.0 ($[M+H]^+$).

3-(Benzo[*d*]thiazol-2-yl)-*N*-(naphthalen-2-ylmethyl)aniline (2n, $C_{24}H_{18}N_2S$) Yellow oil in 56.1% yield; HPLC purity: 96.10%; t_R = 12.19 min; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.10 (d, J = 7.6 Hz, 1H), 8.01 (d, J = 7.9 Hz, 1H), 7.93–7.85 (m, 4H), 7.57 (dd, J = 8.5, 1.5 Hz, 1H), 7.52 (dd, J = 7.6, 7.0 Hz, 1H), 7.50–7.45 (m, 2H), 7.43 (dd, J = 7.9, 7.0 Hz, 1H), 7.41 (brs, 1H), 7.25–7.20 (m, 2H), 6.83–6.78 (m, 2H), 4.54 (d, J = 6.0 Hz, 2H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.2, 153.5, 149.3, 137.5, 134.3, 133.4, 133.0, 132.2, 129.9, 128.0, 127.6, 127.5, 126.5, 126.2, 125.9, 125.6, 125.3, 125.3, 122.7, 122.3, 115.1, 114.9, 110.6, 46.6 ppm; ESI-MS: m/z = 367.0 ($[M+H]^+$).

3-(Benzo[*d*]thiazol-2-yl)-*N*-(naphthalen-1-ylmethyl)aniline (2o, $C_{24}H_{18}N_2S$) White solid in 50.7% yield; HPLC purity: 96.96%; t_R = 12.64 min; m.p.: 103.0–105.4 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.19 (d, J = 8.3 Hz, 1H), 8.10 (d, J = 7.7 Hz, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.97 (d, J = 7.3 Hz, 1H), 7.85 (d, J = 8.1 Hz, 1H), 7.60 (dd, J = 8.0, 7.8 Hz, 1H), 7.58–7.55 (m, 2H), 7.51 (dd, J = 8.1, 7.8 Hz, 1H), 7.48 (dd, J = 8.3, 7.2 Hz, 1H), 7.43 (dd, J = 7.9, 7.3 Hz, 1H), 7.42 (brs, 1H), 7.27–7.23 (m, 2H), 6.86–6.81 (m, 1H), 6.69 (t, J = 5.6 Hz, 1H, NH), 4.81 (d, J = 5.6 Hz, 2H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.2, 153.5, 149.5, 134.6, 134.3, 133.5, 133.4, 131.1, 129.9, 128.5, 127.5, 126.5, 126.2, 125.8, 125.5, 125.3, 125.1, 123.7, 122.7, 122.3, 115.0, 114.9, 110.2, 44.5 ppm; ESI-MS: m/z = 367.0 ($[M+H]^+$).

***N*-[3-(Benzo[*d*]thiazol-2-yl)phenyl]picolinamide (2p, $C_{19}H_{13}N_3OS$)** White solid in 69.0% yield; HPLC purity: 98.41%; t_R = 7.41 min; m.p.: 177.7–178.9 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 10.23 (brs, 1H, NH), 8.65 (d, J = 4.3 Hz, 1H), 8.45 (dd, J = 1.7, 1.8 Hz, 1H), 8.33 (d, J = 7.6 Hz, 1H), 8.11 (dd, J = 8.1, 1.8 Hz, 1H), 8.09 (d, J = 8.1 Hz, 1H), 7.96–7.91 (m, 2H), 7.86 (d, J = 7.7 Hz, 1H), 7.55–7.49 (m, 3H), 7.40 (dd, J = 7.7, 7.1 Hz, 1H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 167.8, 162.4, 154.2, 149.7, 148.2, 138.7, 137.9, 135.3, 134.5, 130.1, 126.8, 126.5, 125.4, 123.5, 123.4, 122.6, 122.1, 121.8, 118.4 ppm; ESI-MS: m/z = 332.1 ($[M+H]^+$).

***N*-[3-(Benzo[*d*]thiazol-2-yl)phenyl]nicotinamide (2q, $C_{19}H_{13}N_3OS$)** White solid in 51.7% yield; HPLC purity: 97.92%; t_R = 5.86 min; m.p.: 200.0–200.5 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 10.76 (brs, 1H, NH), 8.84–8.81 (m, 2H), 8.66 (dd, J = 1.8, 1.7 Hz, 1H), 8.17 (d, J = 7.6 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 8.02 (dd, J = 8.1, 1.2 Hz, 1H), 7.94–7.92 (m, 2H), 7.88–7.84 (m, 1H), 7.59 (dd, J = 8.0, 7.9 Hz, 1H), 7.57 (dd, J = 7.6, 7.1 Hz, 1H), 7.49 (dd, J = 8.0, 7.1 Hz, 1H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 167.1, 164.3, 153.5, 150.3, 141.6, 139.6, 134.5, 133.3, 129.9, 126.8, 125.7, 123.0, 122.9, 122.5, 121.6, 118.6 ppm; ESI-MS: m/z = 332.0 ($[M+H]^+$).

***N*-[3-(Benzo[*d*]thiazol-2-yl)phenyl]isonicotinamide (2r, $C_{19}H_{13}N_3OS$)** White solid in 62.3% yield; HPLC purity: 98.13%; t_R = 5.58 min; m.p.: 221.7–222.5 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 10.71 (brs, 1H, NH), 9.17 (d, J = 1.7 Hz, 1H), 8.79 (dd, J = 4.8, 1.7 Hz, 1H), 8.66 (brs, 1H), 8.38–8.34 (m, 1H), 8.17 (d, J = 7.9 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.85 (d, J = 7.9 Hz, 1H), 7.62–7.55 (m, 3H), 7.48 (dd, J = 8.0, 7.1 Hz, 1H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 167.1, 164.4, 153.5, 152.3, 148.8, 139.8, 135.5, 134.5, 133.3, 130.3, 129.9, 126.7, 125.6, 123.5, 122.9, 122.9, 122.7, 122.5, 118.5 ppm; ESI-MS: m/z = 332.1 ($[M+H]^+$).

***N*-[3-(Benzo[*d*]thiazol-2-yl)phenyl]quinoline-2-carboxamide (2s, $C_{23}H_{15}N_3OS$)** White solid in 82.5% yield; HPLC purity: 96.22%; t_R = 11.40 min; m.p.: 194.2–196.1 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 10.44 (brs, 1H, NH), 8.48 (brs, 1H), 8.42 (d, J = 8.8 Hz, 1H), 8.39 (d, J = 8.4 Hz, 1H), 8.27–8.21 (m, 2H), 8.11 (d, J = 8.0 Hz, 1H), 7.96–7.90 (m, 2H), 7.88–7.81 (m, 2H), 7.67 (dd, J = 8.4, 7.8 Hz, 1H), 7.56 (dd, J = 8.8, 7.8 Hz, 1H), 7.52 (dd, J = 7.6, 7.4 Hz, 1H), 7.41 (dd, J = 7.4, 7.4 Hz, 1H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 167.8, 162.6, 154.2, 149.5, 146.5, 138.8, 138.1, 135.3, 134.5, 130.6, 130.1, 129.9, 129.7, 128.4, 128.0, 126.6, 125.5, 123.6, 123.4, 122.2, 121.8, 118.8, 118.3 ppm; ESI-MS: m/z = 382.1 ($[M+H]^+$).

***N*-[3-(Benzo[*d*]thiazol-2-yl)phenyl]-2-(1*H*-indol-3-yl)acetamide (2t, $C_{22}H_{15}N_3OS$)** White solid in 63.2% yield; HPLC purity: 97.81%; t_R = 6.02 min; m.p.: 218.1–219.5 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 10.95 (brs, 1H, NH), 10.40 (brs, 1H, NH), 8.51 (dd, J = 1.8, 1.7 Hz, 1H), 8.14 (d, J = 7.9 Hz, 1H), 8.06 (d, J = 8.1 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.74 (d, J = 7.7 Hz, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.55 (dd, J = 7.9, 7.0 Hz, 1H), 7.49 (dd, J = 8.4, 8.0 Hz, 1H), 7.47 (dd, J = 8.1, 7.0 Hz, 1H), 7.37 (d, J = 8.4 Hz, 1H), 7.30 (d, J = 2.4 Hz, 1H), 7.08 (dd,

J = 7.7, 7.0 Hz, 1H), 7.00 (dd, *J* = 8.1, 7.0 Hz, 1H), 3.79 (s, 2H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 170.2, 167.2, 153.5, 140.3, 136.1, 134.4, 133.3, 129.9, 127.2, 126.7, 125.6, 124.0, 122.9, 122.4, 121.9, 121.7, 121.0, 118.7, 118.5, 117.2, 111.4, 108.3, 33.9 ppm; ESI-MS: *m/z* = 384.1 ([M+ H]$^+$).

3-(Benzo[*d*]thiazol-2-yl)-*N*-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl]aniline (2u, $C_{26}H_{27}BN_2O_2S$) Yellow solid in 63.7% yield; HPLC purity: 98.06%; t_R = 6.86 min; m.p.: 129.3–131.2 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.10 (d, *J* = 7.9 Hz, 1H), 8.02 (d, *J* = 7.9 Hz, 1H), 7.65 (d, *J* = 8.0 Hz, 2H), 7.52 (dd, *J* = 7.9, 7.0 Hz, 1H), 7.43 (dd, *J* = 7.9, 7.0 Hz, 1H), 7.41 (d, *J* = 8.1 Hz, 2H), 7.33 (brs, 1H), 7.23–7.19 (m, 2H), 6.74 (brs, 1H, NH), 6.74–6.71 (m, 1H), 4.39 (d, *J* = 4.8 Hz, 2H), 1.27 (s, 12H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 168.1, 153.5, 149.2, 143.5, 134.6, 134.3, 133.4, 129.9, 126.6, 126.5, 126.1, 125.3, 122.7, 122.3, 115.0, 114.9, 110.5, 83.5, 46.3, 24.7 ppm; ESI-MS: *m/z* = 443.2 ([M+H]$^+$).

***N*-[3-(Benzo[*d*]thiazol-2-yl)phenyl]-6-(trifluoromethyl)nicotinamide (2q-1, $C_{20}H_{12}F_3N_3OS$)** White solid in 45.9% yield; HPLC purity: 97.29%; t_R = 8.68 min; m.p.: 195.4–195.8 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 10.90 (s, 1H, NH), 9.31 (d, *J* = 1.7 Hz, 1H), 8.65 (dd, *J* = 1.7, 1.6 Hz, 1H), 8.63 (dd, *J* = 8.3, 1.7 Hz, 1H), 8.17 (d, *J* = 8.5 Hz, 1H), 8.14 (d, *J* = 8.3 Hz, 1H), 8.09 (d, *J* = 7.7 Hz, 1H), 8.01 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.87 (dd, *J* = 7.9, 1.6 Hz,1H), 7.60 (dd, *J* = 7.9, 7.9 Hz,1H), 7.57 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.49 (dd, *J* = 7.7, 7.3 Hz, 1H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 167.0, 163.2, 153.5, 149.4, 139.5, 138.0, 134.5, 133.5, 133.4, 130.0, 126.8, 125.7, 123.0, 122.9, 122.9, 122.5, 121.6 (q, *J* = 272.0 Hz), 120.7, 120.7, 118.6 ppm; ESI-MS: *m/z* = 400.0 ([M+H]$^+$).

***N*-[3-(Benzo[*d*]thiazol-2-yl)phenyl]-6-chloronicotinamide (2q-2, $C_{19}H_{12}ClN_3OS$)** Yellow oil in 13.7% yield; HPLC purity: 95.04%; t_R = 7.88 min; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 10.90 (s, 1H, NH), 9.01 (d, *J* = 2.0 Hz, 1H), 8.63 (brs, 1H), 8.41 (dd, *J* = 8.3, 2.0 Hz, 1H), 8.17 (d, *J* = 8.0 Hz, 1H), 8.08 (d, *J* = 7.9 Hz, 1H), 8.00 (d, *J* = 7.6 Hz, 1H), 7.85 (d, *J* = 7.7 Hz, 1H), 7.74 (d, *J* = 8.3 Hz, 1H), 7.61–7.54 (m, 2H), 7.48 (dd, *J* = 7.9, 7.4 Hz, 1H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 167.1, 163.2, 153.5, 153.0, 149.4, 139.6, 139.1, 134.4, 133.3, 129.9, 129.7, 126.7, 125.7, 124.2, 122.9, 122.9, 122.9, 122.4, 118.5 ppm; ESI-MS: *m/z* = 388.0 ([M+Na]$^+$).

***N*-[3-(Benzo[*d*]thiazol-2-yl)phenyl]-6-bromonicotinamide (2q-3, $C_{19}H_{12}BrN_3OS$)** White solid in 52.7% yield; HPLC purity: 95.56%; t_R = 9.63 min; m.p.: 180.2–181.8 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 10.75 (s, 1H, NH), 9.12 (brs, 1H), 8.93 (brs, 1H), 8.68–8.56 (m, 2H), 8.17 (d, *J* = 7.6 Hz, 1H), 8.08 (d, *J* = 7.6 Hz, 1H), 8.01 (d, *J* = 7.3 Hz, 1H), 7.85 (d, *J* = 7.6 Hz, 1H), 7.64–7.54 (m, 2H), 7.48 (dd, *J* = 7.6, 6.8 Hz, 1H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 167.0, 162.8, 153.5, 152.9, 147.5, 139.6, 137.8, 134.5, 133.3, 131.8, 129.9, 126.7, 125.7, 122.9, 122.9, 122.8, 122.4, 120.0, 118.5 ppm; ESI-MS: *m/z* = 410.0, 411.9 ([M+H]$^+$).

***N*-[3-(Benzo[*d*]thiazol-2-yl)phenyl]-6-methylnicotinamide (2q-4, $C_{20}H_{15}N_3OS$)** White solid in 39.6% yield; HPLC purity: 95.14%; t_R = 6.39 min; m.p.: 186.4–188.2 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 10.61 (s, 1H, NH), 9.06 (d, *J* = 2.2 Hz, 1H), 8.65 (dd, *J* = 1.8, 1.3 Hz, 1H), 8.26 (dd, *J* = 8.2, 2.2 Hz, 1H), 8.17 (d, *J* = 7.8 Hz, 1H), 8.08 (d, *J* = 7.9 Hz, 1H), 8.02 (dd, *J* = 8.2, 1.3 Hz, 1H), 7.83 (d, *J* = 7.9 Hz, 1H), 7.59–7.55 (m, 2H), 7.48 (ddd, *J* = 7.9, 7.8, 1.0 Hz, 1H), 7.44 (d, *J* = 8.2 Hz, 1H), 2.57 (s, 3H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): δ = 167.2, 164.3, 161.4, 153.5, 148.3, 139.9, 135.8, 134.4, 133.3, 129.8, 127.5, 125.7, 125.5, 122.9, 122.9, 122.7, 122.5, 122.4, 118.5, 24.15 ppm; ESI-MS: *m/z* = 346.1 ([M+H]$^+$).

Cytotoxicity assay

The cancer cell lines including A549, HeLa, HepG2, MCF-7, MV4-11, and DB were cultured in a proper medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 at 37 °C. Cell suspensions were plated in 96-well plates at a density of 2×10^4 cells cm^{-3}. Compounds were solubilized in DMSO at six different concentrations, which were then added to each well. After incubation for 24 h, the cells were treated with various concentrations of tested substances for 48 h and then incubated with 10 mm^3 of MTT at 37 °C for 2 h. The formazan dye product was measured by the absorbance at 490 nm on a Tecan Spark multimode microplate reader (Switzerland), and the IC_{50} values were derived by non-linear regression analysis.

Flow cytometric analysis

A549 cells were plated in six-well plates treated with tested compounds or DMSO (control). Cells were harvested at 48 h and measured using Annexin V–FITC Apoptosis Detection Kit (Vazyme Biotech) according to the manufacturer's instructions for cell apoptosis analysis. For cell cycle analysis, the harvested cells were re-suspended in 70% ethanol overnight at 4 °C for fixation. Then samples were washed with PBS and incubated with propidium iodide/rnase staining buffer (BD Pharmingen) for 0.5 h at room temperature.

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