1. Introduction

A malignant tumor is a common disease that seriously threatens human health. The number of deaths caused by malignant tumors is second only to cerebrovascular disease among all diseases. General treatment methods for tumors include surgical, radiation, chemical (drug therapy), and biological treatments. However, chemotherapy and surgical treatment remain the most common treatment methods. Antitumor drugs have advanced considerably since the discovery of mechlorethamine in the 1940s, which was used to treat malignant tumors. In the last twenty years, the development of molecular biology and cell biology have further improved understanding of tumor biological mechanisms, and research into antitumor drugs has provided new targets. The batch production of new chemical structures or drugs with rational drug design, with some new high-selectivity drugs produced.

The reversible phosphorylation of protein amino acid side chains is an important mechanism for the regulation of enzyme and signal protein activity. Protein kinase and protein phosphatase are involved in reversible phosphorylation and play key roles in regulating metabolism, gene expression, cell growth, cell division, and cell differentiation. Protein kinase is a phosphotransferase that catalyzes the transfer of phosphate groups from ATP to protein receptor amino acids. Tyrosine kinase is the most important protein kinase, while protein tyrosine kinase is a type of protein that shows tyrosine kinase activity, including receptor-type and nonreceptor-type proteins. Receptor-type proteins include epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), insulin receptor (InsR), and fibroblast growth factor receptor (FGFR). Nonreceptor-type proteins include Src, Abl, Jak, Csk, Fak, and Fes. Protein tyrosine kinase dysfunction can cause many diseases. Some data have shown that over 50% of...
protooncogenes and oncogene products have protein tyrosine kinase activity, and that their abnormal expression leads to cell proliferation and regulation disorders, which cause tumors. Furthermore, abnormal expression of tyrosine kinase is closely related to tumor invasion and metastasis, tumor angiogenesis, and tumor chemotherapy resistance.\textsuperscript{38–41} In recent years, protein tyrosine kinase has become the target of drug action. The design of protein kinase inhibitors that can interfere with the cell signal transduction pathway has been used in the search for disease drug treatments.\textsuperscript{42–45}

Agents that act on the signal transduction pathway mechanism in tumors include protein kinase inhibitors and proteasome inhibitors. Protein kinase inhibitors can be divided into Bcr-Abl protein kinase inhibitors, epidermal growth factor receptor tyrosine kinase inhibitors, and multiple kinase target inhibitors.\textsuperscript{46–50} Currently, protein kinase inhibitors commonly used in clinical practice include imatinib, dasatinib, gefitinib, erlotinib, sorafenib, and sunitinib (Fig. 1). These drugs all contain aniline structures. Herein, using sorafenib as the lead compound, sorafenib analogues and derivatives with core aniline structures were designed (Fig. 2) and synthesized. The results of in vitro and in vivo experiments showed that these compounds had good antitumor activities. In the target molecule design process, bioisosteres and the alkyl principle were used to prepare different compounds. The target compounds were synthesized from 2-aminobenzoic acid (1a) or 6-methyl-2-aminobenzoic acid (1b) by amination and condensation (N-alkylation). The synthetic route involved simple operations and mild reaction conditions, and afforded high total yields (Scheme 1).

2. Results and discussion

2.1. Design and synthesis of sorafenib analogues and derivatives

Sorafenib (protein kinase inhibitor) has a novel structure and is used as an antitumor drug against multiple kinase targets. Herein, the design of protein kinase inhibitors was based on sorafenib as the lead compound. A series of novel sorafenib analogues and derivatives were prepared using bioisosteres and the alkyl principle (Fig. 2). As shown in Fig. 2, the target compounds and sorafenib were found to contain the same aniline structure. According to structure–activity relationship (SAR) studies, this novel series of compounds might have antitumor activities. During structural modification of the lead compound (sorafenib), substituents R\textsuperscript{1}, R\textsuperscript{2}, and R\textsuperscript{3} on the aniline structure were modified. The following substituents were selected and tested: H and CH\textsubscript{3} at R\textsuperscript{1}; pyran, pyridine, pyrimidine, pyrazine, and pyridazine at R\textsuperscript{2}; and morpholine, pyridine, and pyrimidine at R\textsuperscript{3}. These R\textsuperscript{1}, R\textsuperscript{2}, and R\textsuperscript{3} substituents were chosen to alter the physical and chemical properties (log\textsubscript{P} and pK\textsubscript{a}) of the target compounds and achieve good
biological activity. To prepare the target compounds, we chose a synthesis route that involved simple operations and mild reaction conditions, and afforded high total yields (Scheme 1). As shown in Scheme 1, the target compounds were synthesized by amination and condensation [N-alkylation]. Compounds 2a–2j were prepared using a general amination method, with 2-amino-6-bromo-2-methylbenzoic acid (1a) or 6-methyl-2-amino-6-bromo-benzoic acid (1b) as the starting material, tetrahydrofuran (THF) as a solvent, and 1,1′-carbonyldimidazole (CDI) as catalyst under reflux for 3 h to achieve reaction completion. Compounds 3a–3z were prepared using a general condensation [N-alkylation] method with AlCl₃ as a catalyst in EtOH/NaOH under reflux for 5 h. The resulting products were characterized by 1H NMR, 13C NMR, HR-ESI-MS, and elemental analysis.

2.2. In vitro antitumor activity

Biological activity experiments were based on the MTT assay (a colorimetric assay for assessing cell metabolic activity). For the in vitro experiments, human lung cancer cells (H1975 and A549) and cervical cancer cells (Hela) were used as inhibitory targets. The inhibitory activity was measured as the half maximal inhibitory concentration (IC₅₀). Sorafenib and sunitinib were used as positive drugs and DMSO was used as the blank control. The results of the biological activity experiments are shown in Table 1. The data were analyzed by linear regression using statistical software SPSS (version 13.0), with the results showing a linear fit, as shown in Table 1. Compounds 3c, 3d, 3h, 3n, 3r, and 3z showed better inhibitory effects against human cervical cancer cells (Hela), while compounds 3t and 3v showed better inhibitory effects against human lung cancer cells (H1975 and A549). Among these compounds, compound 3d had an inhibitory activity (IC₅₀) of 1.56 ± 0.04 μmol L⁻¹ against Hela cells (human cervical cancer), compound 3t had an IC₅₀ of 2.34 ± 0.07 μmol L⁻¹ against H1975 cells (human lung cancer), and compound 3v had an IC₅₀ of 1.35 ± 0.03 μmol L⁻¹ against A549 cells (human lung cancer). As shown in Table 1, these compounds showed good antitumor activity in vitro. Compounds 3d and 3r showed better biological activity than positive control sunitinib (IC₅₀ = 2.06 ± 0.34 μmol L⁻¹) against Hela cells, compound 3t showed better biological activity than positive control sorafenib (IC₅₀ = 4.20 ± 0.21 μmol L⁻¹) against H1975 cells, and compound 3v showed similar biological activity to positive control sunitinib (IC₅₀ = 1.05 ± 0.04 μmol L⁻¹) against A549 cells.

2.3. In vivo antitumor activity

In vivo antitumor test results are the most important indicators of the effectiveness of candidate antitumor compounds. In vivo experiments were conducted to evaluate the inhibitory effect and intensity of the compounds on the growth of xenograft tumors in nude mice with human carcinoma. During the experiment, human lung cancer (A549) and human cervical cancer (Hela) were inoculated under the right armpit of nude mice. After tumor growth to a certain stage, high (100 mg kg⁻¹ d⁻¹), medium (50 mg kg⁻¹ d⁻¹), and low (10 mg kg⁻¹ d⁻¹) doses of compounds 3c, 3d, 3h, 3n, 3r, 3t, 3v, and 3z were administered (Table 2). Data were analyzed by

| Compounds | Hela IC₅₀ (μmol L⁻¹) ± SD | A549 IC₅₀ (μmol L⁻¹) ± SD | H1975 IC₅₀ (μmol L⁻¹) ± SD |
|------------|---------------------------|---------------------------|---------------------------|
| 3a         | 10.20 ± 0.88              | 12.40 ± 1.20              | 11.13 ± 1.11              |
| 3b         | 7.02 ± 0.85               | 10.31 ± 1.00              | 8.90 ± 0.96               |
| 3c         | 2.01 ± 0.11               | 25.33 ± 2.05              | 43.12 ± 3.22              |
| 3d         | 1.56 ± 0.04               | 20.46 ± 2.20              | 40.39 ± 2.11              |
| 3e         | 34.78 ± 1.55              | 12.66 ± 1.03              | 10.18 ± 1.06              |
| 3f         | 30.69 ± 1.33              | 10.23 ± 1.09              | 9.45 ± 0.55               |
| 3g         | 3.08 ± 0.011              | 10.26 ± 0.65              | 8.02 ± 0.36               |
| 3h         | 2.07 ± 0.33               | 7.45 ± 0.45               | 6.96 ± 0.66               |
| 3i         | 12.67 ± 0.89              | 56.09 ± 3.00              | 45.99 ± 2.98              |
| 3j         | 10.33 ± 1.09              | 54.37 ± 2.16              | 43.56 ± 3.11              |
| 3k         | 56.88 ± 3.12              | 4.60 ± 0.45               | 4.33 ± 0.24               |
| 3l         | 54.70 ± 4.00              | 3.04 ± 0.73               | 2.66 ± 0.29               |
| 3m         | 4.04 ± 0.34               | 24.67 ± 2.23              | 13.56 ± 1.32              |
| 3n         | 2.54 ± 0.23               | 20.45 ± 2.10              | 11.34 ± 1.34              |
| 3o         | 12.45 ± 1.32              | 15.67 ± 1.58              | 20.49 ± 1.99              |
| 3p         | 10.45 ± 0.78              | 13.55 ± 2.10              | 18.48 ± 2.09              |
| 3q         | 4.22 ± 0.26               | 23.47 ± 3.12              | 26.68 ± 2.11              |
| 3r         | 1.90 ± 0.10               | 19.55 ± 3.10              | 23.40 ± 2.89              |
| 3s         | 18.34 ± 2.04              | 4.45 ± 0.21               | 4.36 ± 0.15               |
| 3t         | 16.23 ± 1.23              | 3.78 ± 0.21               | 3.24 ± 0.11               |
| 3u         | 15.04 ± 0.45              | 2.44 ± 0.14               | 4.22 ± 0.22               |
| 3v         | 13.58 ± 1.67              | 1.35 ± 0.02               | 2.67 ± 0.12               |
| 3w         | 25.55 ± 2.55              | 13.33 ± 1.89              | 12.29 ± 2.00              |
| 3x         | 23.68 ± 3.11              | 10.29 ± 0.90              | 10.04 ± 0.89              |
| 3y         | 3.46 ± 0.09               | 46.33 ± 3.11              | 56.48 ± 4.66              |
| 3z         | 2.11 ± 0.23               | 44.60 ± 4.21              | 54.69 ± 5.10              |
| Sorafenib  | 6.02 ± 0.21               | 2.10 ± 0.10               | 4.20 ± 0.21               |
| Sunitinib  | 2.06 ± 0.34               | 1.05 ± 0.04               | 16.02 ± 0.85              |
| DMSO       | None                      | None                      | None                      |

Compounds 3c, 3d, 3h, 3n, 3r, 3t, 3v, and 3z were administered (Table 2). Data were analyzed by linear regression using statistical software SPSS (version 13.0), with the results showing a linear fit, as shown in Table 1. Compounds 3c, 3d, 3h, 3n, 3r, 3t, 3v, and 3z showed better inhibitory activities than the positive controls (Table 2). Data were analyzed by linear regression using statistical software SPSS (version 13.0), with the results showing a linear fit, as shown in Table 1.
linear regression using statistical software SPSS (version 13.0), with the results of data analysis showing a linear fit. As shown in Table 2, the data showed that these compounds had good antitumor effects in vivo and showed low relative tumor proliferation rates (T/C, %). Most of the compounds were effective at high, medium, and low doses (T/C > 40% was ineffective and T/C ≤ 40% was effective). As shown in Table 2, compounds 3c, 3d, 3h, 3n, 3r, and 3z showed general inhibitory activities at low doses (10 mg kg\(^{-1}\) d\(^{-1}\)) against Hela cells. However, at medium doses (50 mg kg\(^{-1}\) d\(^{-1}\)) or high doses (100 mg kg\(^{-1}\) d\(^{-1}\)), their inhibitory activities were significantly increased above those of positive controls sorafenib and sunitinib. Compounds 3t and 3v showed general inhibitory activity at low doses (10 mg kg\(^{-1}\) d\(^{-1}\)) against A549 cells, but better activities than positive controls sorafenib and sunitinib at medium (50 mg kg\(^{-1}\) d\(^{-1}\)) and high doses (100 mg kg\(^{-1}\) d\(^{-1}\)). Therefore good inhibitory activities were obtained at medium doses and high doses against Hela and A549 cells in vivo. We also studied the acute toxicity of these target compounds in vivo (Table 2), which found that target compounds 3c, 3d, 3h, 3n, 3r, 3t, 3v, and 3z had low acute toxicity.

In conclusion, various compounds were designed and prepared using bio-isosteres and the alkyl principle. The target compounds were synthesized from 2-aminobenzoic acid (1a) or 6-methyl-2-aminobenzoic acid (1b) by amination and condensation (N-alkylation). This synthetic route involved simple operations and mild reaction conditions, and afforded high total yields. In vitro activity results showed that compounds 3c, 3d, 3h, 3n, 3r, and 3z had good inhibitory effects on human cervical cancer cells (Hela), and that compounds 3t and 3v had good inhibitory effects on human lung cancer cells (H1975 and A549). Among these compounds, compound 3d had an inhibitory activity (IC\(_{50}\)) of 1.56 ± 0.04 μmol L\(^{-1}\) against Hela cells (human cervical cancer), compound 3t had an IC\(_{50}\) of 2.34 ± 0.07 μmol L\(^{-1}\) against H1975 cells (human lung cancer), and compound 3v had an IC\(_{50}\) of 1.35 ± 0.03 μmol L\(^{-1}\) against A549 cells (human lung cancer). In vivo activity results showed that these compounds had good antitumor effects and low acute toxicity.

### 4. Experimental

#### 4.1. Chemistry

**4.1.1. Materials and general methods.** Reagents were purchased and used without further purification. Nuclear magnetic resonance (NMR) spectroscopy was performed on
a Bruker AMX-400 (TMS as internal standard). Mass spectrometry was performed on an Agilent 6460 spectrometer. HPLC analysis of all final biologically tested compounds was performed on an Agilent 1260 Series HPLC system. Purity was determined using reversed-phase HPLC and was ≥99% for all biologically tested compounds.

4.1.2. Synthesis of compounds 2a–2j. The synthesis of compound 2a is described here as an example. To 1,1-carbonyldiimidazole (CDI; 16.20 g, 0.10 mol) dissolved in THF (30 mL) at around 10 °C was added 4-amino-4H-pyran (8.20 g, 0.10 mol), and the mixture was stirred continuously for 30 min. Next, 2-aminobenzoic acid (1a, 13.70 g, 0.10 mol) dissolved in THF (100 mL) in a 250 mL round bottom flask was added dropwise to the reaction mixture at 10 °C, followed by reflux for 3 h. After reaction completion, the mixture was allowed to cool and the precipitated solid was filtered, washed, and dried in vacuo to afford crude product 2-amino-N-(4H-pyran-4-yl)benzamide (2a).

Crude 2a was recrystallized from acetone, filtered, and dried in vacuo to afford pure 2a as a white crystalline solid. This general procedure was used for the synthesis of compounds 2b–2j.

4.1.3. Synthesis of compounds 3a–3z. The synthesis of compound 3a is described here as an example. To compound 2a (21.10 g, 0.10 mol) in a 250 mL round bottom flask was added anhydrous ethanol (100 mL) and anhydrous aluminum trichloride (13.30 g, 0.01 mol). Under constant pressure conditions, 4-chloromorpholine (12.15 g, 0.10 mol) was added dropwise using a dropping funnel, and then the reaction was refluxed for 5 h. After reaction completion, the mixture was filtered while still hot and ethanol was removed from the filtrate under normal pressure. The mixture was dried in vacuo to afford crude product 3a. Crude 3a was recrystallized from ethanol (volume fraction, 60%), filtered, and dried in vacuo to afford pure 3a as a white crystalline solid. This general procedure was used for the synthesis of compounds 3b–3z.

N-(4H-Pyran-4-yl)-2-(pyridin-4-ylamino)benzamide (3e). 93.4% yield; mp 201–203 °C; 1H NMR (300 MHz, CDCl3) δ: 9.23 (s, 1H, –NH–C), 9.18 (s, 1H, –NH–C), 8.90 (s, 1H, N=CH–N), 8.50 (s, 1H, –CH=N), 8.39 (m, 1H, Ph-H), 7.73 (m, 1H, Ph-H), 7.66 (m, 1H, Ph-H), 7.69 (m, 1H, Ph-H), 6.17 (d, J = 8.4 Hz, 2H, C=C–CH2–O), 4.76 (m, 1H, –CH–), 4.63 (m, 2H, –CH=C–C), 3.65 (m, 4H, C–CH2–O), 3.00 (m, 4H, –CH2–C); 13C NMR (75 MHz, CDCl3) δ: 167.8, 141.0, 140.1, 132.6, 128.0, 119.2, 114.8, 112.1, 104.1, 65.5, 58.4, 51.1; HR-ESI-MS m/z: calculated for C16H14N4O2 ([M + H]+), 294.1116; found, 294.1117; anal. calcld for C16H14N4O2: C, 65.30; H, 4.79; N, 19.03; O, 10.78%.

2-Methyl-N-(4H-pyran-4-yl)-6-(pyrimidin-5-ylamino)benzamide (3f). 94.0% yield, mp 222–223 °C; 1H NMR (300 MHz, CDCl3) δ: 9.23 (s, 1H, –NH–C), 9.18 (s, 1H, –NH–C), 8.90 (s, 1H, N=CH–N), 8.50 (s, 1H, –CH=N), 8.29 (m, 1H, Ph-H), 7.40 (m, 1H, Ph-H), 6.90 (m, 1H, Ph-H), 6.17 (d, J = 8.4 Hz, 2H, C=C–CH2–O), 4.76 (m, 1H, –CH–), 4.63 (m, 2H, –CH=C–C), 3.40 (s, 3H, –CH3); 13C NMR (75 MHz, CDCl3) δ: 167.8, 147.4, 143.8, 147.7, 143.7, 143.1, 141.0, 138.0, 121.5, 120.8, 113.4, 109.0, 104.1, 51.1, 18.1; HR-ESI-MS m/z: calculated for C16H14N4O2 ([M + H]+), 294.3101; found, 294.3117; anal. calcld for C16H14N4O2: C, 65.30; H, 4.79; N, 19.04; O, 10.87%.

2-Methyl-N-(4H-pyran-4-yl)-6-(pyrimidin-5-ylamino)benzamide (3g). 88.4% yield; mp 168–170 °C; 1H NMR (300 MHz, CDCl3) δ: 10.35 (s, 1H, –NH–N), 10.22 (s, 1H, –NH–N), 8.46 (d, J = 7.4 Hz, 2H, –CH=N), 7.69 (m, 1H, Ph-H), 7.50 (m, 1H, Ph-H), 7.36 (d, J = 7.2 Hz, 2H, –CH=C), 7.08 (m, 1H, Ph-H), 3.65 (m, 4H, C–CH2–O), 3.00 (m, 4H, –CH2–C); 13C NMR (75 MHz, CDCl3) δ: 167.5, 155.3, 150.2, 141.0, 132.6, 128.0, 119.2, 114.8, 112.1, 109.0, 65.5, 58.4; HR-ESI-MS m/z: calculated for C16H14N4O2 ([M + H]+), 294.3111; found, 294.3116; anal. calcld for C16H14N4O2: C, 65.30; H, 4.79; N, 19.04; O, 10.87%.

2-Methyl-N-(morphinamo)-N-(4H-pyran-4-yl)benzamide (3h). 90.2% yield; mp 177–178 °C; 1H NMR (300 MHz, CDCl3) δ: 10.35...
2H, 1H, Ph-H), 7.66 (m, 1H, Ph-H), 6.99 (m, 1H, Ph-H), 3.65 (m, 4H, C-CH2-O), 3.00 (m, 4H, -CH=C-C), 2.48 (s, 3H, -CH3); 13C NMR (75 MHz, CDCl3) δ: 164.7, 155.3, 150.2, 149.0, 137.7, 132.5, 121.9, 117.9, 109.1, 109.0, 65.5, 58.4, 18.1; HR-ESI-MS m/z: calculated for C16H13N5O2 [M + H]+, 305.3411; found, 305.3412; analytical calcd for C16H13N5O2: C, 65.97; H, 4.50; N, 10.24; found: C, 66.88; H, 4.95; N, 10.67%.

2-Methyl-6-(pyridin-4-ylamino)-N-(pyrimidin-5-yl)benzamide (3r). 86.3% yield; mp 194–196°C; 1H NMR (300 MHz, CDCl3) δ: 12.32 (s, 1H, -NH-N), 11.17 (s, 1H, -NH-N), 8.59 (s, 1H, -CH=N), 8.40 (s, 1H, -N=CH-N), 8.35 (s, 1H, -N=CH-N), 7.69 (m, 1H, Ph-H), 7.50 (m, 1H, Ph-H), 7.08 (m, 1H, Ph-H), 6.78 (m, 1H, Ph-H), 3.65 (m, 4H, -C=O, CH2-C=O), 2.48 (s, 3H, -CH3); 13C NMR (75 MHz, CDCl3) δ: 164.7, 149.7, 141.9, 139.5, 137.4, 136.1, 132.6, 128.0, 119.2, 114.8, 112.1, 65.5, 58.4; HR-ESI-MS m/z: calculated for C16H13N5O2 [M + H]+, 305.3312; found, 305.3312; analytical calcd for C16H13N5O2: C, 61.09; H, 5.72; N, 23.40; found: C, 61.09; H, 5.73; N, 23.40; O, 10.68%.

2-(Morpholinomethyl)-N-(pyrimidin-5-yl)benzamide (3k). 87.1% yield; mp 178–180°C; 1H NMR (300 MHz, CDCl3) δ: 10.35 (s, 1H, -NH-N), 10.22 (s, 1H, -NH-N), 9.21 (s, 2H, -CH=N), 9.10 (s, 1H, -N=CH-N), 7.69 (m, 1H, Ph-H), 7.50 (m, 1H, Ph-H), 7.08 (m, 1H, Ph-H), 3.65 (m, 4H, C-CH2-O), 3.00 (m, 4H, -CH=C-C); 13C NMR (75 MHz, CDCl3) δ: 167.5, 147.4, 143.7, 141.1, 141.0, 132.6, 128.0, 119.2, 114.8, 112.1, 65.5, 58.4; HR-ESI-MS m/z: calculated for C16H15N5O2 [M + H]+, 313.3631; found, 313.3631; analytical calcd for C16H15N5O2: C, 61.33; H, 6.11; N, 22.35; O, 10.21; found: C, 61.34; H, 6.11; N, 22.35; O, 10.21%.

N-(Pyrido[2,3-d]pyrimidin-4-yl)-2-(pyridin-4-ylamino)benzamide (3g). 89.1% yield; mp 196–198°C; 1H NMR (300 MHz, CDCl3) δ: 11.17 (s, 1H, -NH-N), 11.02 (s, 1H, -NH-N), 8.59 (s, 1H, -CH=N), 8.46 (s, 1H, -N=CH-N), 8.35 (s, 1H, -CH=C-C), 7.73 (m, 1H, Ph-H), 7.66 (m, 1H, Ph-H), 6.99 (m, 1H, Ph-H), 6.99 (m, 1H, -C=CH-N); 13C NMR (75 MHz, CDCl3) δ: 167.5, 155.3, 151.9, 150.2, 149.7, 139.5, 137.4, 136.1, 132.9, 128.3, 118.8, 117.9, 116.4, 109.0; HR-ESI-MS m/z: calculated for C16H13N5O2 [M + H]+, 305.3412; found, 305.3412; analytical calcd for C16H13N5O2: C, 65.98; H, 4.50; N, 10.43; O, 5.45%.

2-Methyl-6-(pyridin-4-ylamino)-N-(pyrimidin-5-yl)benzamide (3m). 90.2% yield; mp 192–193°C; 1H NMR (300 MHz, CDCl3) δ: 11.02 (s, 1H, -NH-N), 10.21 (s, 1H, -NH-N), 9.21 (s, 2H, -CH=N), 9.10 (s, 1H, -N=CH-N), 8.46 (m, 2H, -CH-N), 8.39 (m, 1H, Ph-H), 7.73 (m, 1H, Ph-H), 7.66 (m, 1H, Ph-H), 6.99 (m, 1H, Ph-H), 6.99 (m, 2H, -C=CH-N); 13C NMR (75 MHz, CDCl3) δ: 167.5, 155.3, 151.9, 150.2, 147.4, 143.7, 141.1, 132.9, 128.3, 118.8, 117.9, 116.4, 109.0; HR-ESI-MS m/z: calculated for C16H13N5O2 [M + H]+, 305.3412; found, 305.3412; analytical calcd for C16H13N5O2: C, 65.98; H, 4.50; N, 10.43; O, 5.45%.
cald for C_{17}H_{15}N_{5}O ([M + H]^+), 305.3402; found, 305.1276; anal. calcd for C_{17}H_{15}N_{5}O: C, 65.98; H, 4.50; N, 24.04; O, 5.49; found: C, 65.98; H, 4.51; N, 24.03; O, 5.48.

2-Methyl-N-(pyrazin-2-yl)-6-(pyridin-4-ylamino)benzamide (3z). 91.1% yield; mp 198–200 °C; 1H NMR (300 MHz, CDCl₃) δ: 11.02 (s, 1H, −NH−N), 10.37 (s, 1H, −NH−), 9.61 (s, 1H, −CH−N), 9.30 (m, 1H, −CH−N), 8.29 (m, 1H, Ph-H), 7.69 (m, 1H, Ph-H), 6.99 (m, 1H, Ph-H), 2.48 (s, 3H, −CH₃); 13C NMR (75 MHz, CDCl₃) δ: 164.7, 150.4, 147.4, 134.2, 139.4, 139.8, 134.3, 130.4, 130.0, 128.0, 119.2, 114.8, 114.2, 65.5, 292.1072; found, 292.1072; anal. calcd for C_{16}H_{14}N_{6}O: C, 66.88; H, 4.95; N, 22.93; O, 5.24.

2-Methyl-N-(pyrazin-2-yl)-6-(pyridin-4-ylamino)benzamide (3z). 92.6% yield; mp 223–235 °C; 1H NMR (300 MHz, CDCl₃) δ: 10.37 (s, 1H, −NH−N), 9.61 (s, 1H, −CH−N), 9.30 (m, 1H, −CH−N), 8.29 (m, 1H, Ph-H), 7.69 (m, 1H, Ph-H), 6.99 (m, 1H, Ph-H), 2.48 (s, 3H, −CH₃); 13C NMR (75 MHz, CDCl₃) δ: 167.5, 150.4, 147.4, 134.2, 139.4, 139.8, 134.3, 130.4, 130.0, 128.0, 119.2, 114.8, 114.2, 65.5, 58.4; HR-ESI-MS m/z: calcd for C_{16}H_{14}N_{6}O ([M + H]^+), 292.3012; found, 292.3012; anal. calcd for C_{16}H_{14}N_{6}O: C, 66.88; H, 4.95; N, 22.93; O, 5.24.

4.2. Biological activity

4.2.1. Biological activity screening in vitro. The tumor cells (human lung cancer cells H1975 and A549, and cervical cancer cells Hela; from Chongqing Institute of Chinese Materia Medica) were extended, logarithmic phase cells were collected, the concentration of the cell suspensions was adjusted, and 100 µL was added to each hole so that the test cells were plated to a density modulation of 5000 cell per hole ceiling. The plates were transferred to a CO₂ incubator and incubated at 37 °C under 5% CO₂ and saturated humidity conditions until a cell monolayer covered the hole bottom, using a drug concentration gradient dilution of 5, adding MTT solution (20 µL, 5 mg mL⁻¹; 0.5% MTT) to each hole, and culturing for 3–4 h. After termination of cell culture coloration, the hole broth was carefully aspirated, DMSO (150 µL) was added, and the mixture was subjected to low speed vibration for 10 min on shaking bed to fully dissolve the crystals. The standard deviation of all raw data (OD value) (SD) was analyzed, the mean of each group was used to replace the large deviation data and calculate the inhibition rate, and the IC₅₀ value was derived using specialized software based on the drug concentration and corresponding inhibition. Sorafenib and
sunitinib were used as positive controls in the in vitro experiment. Data were analyzed by linear regression using statistical software SPSS (version 13.0).

4.2.2. Biological activity evaluation in vivo. Logarithmic growth stages of tumor cells (human lung cancer A549 and human cervical cancer Hela) were used to prepare cell suspensions and inoculated under the right axilla of 50 nude mice. Nude mice were randomly assigned to drug feeding after tumor growth of 100–200 mm³. Drug administration included activity screening of compounds in vitro (doses: 10, 50, and 100 mg kg⁻¹ d⁻¹) using positive controls (sorafenib and sunitinib; 50 mg kg⁻¹ d⁻¹) and a blank controls (DMSO, 50 mg kg⁻¹ d⁻¹). Medication was administered orally once a day for 15 consecutive days. The antitumor effect on nude mice was observed dynamically by measuring tumor diameter. The number of tumor diameter measurements (2–3 times a week) was based on growth of the transplanted tumor. The relative tumor proliferation rate (T/C, %) was used as the evaluation index for antitumor activity in vivo. Data were analyzed by linear regression using statistical software SPSS (version 13.0).

Live subject statement

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of “Chongqing Institute of Chinese Materia Medica” and experiments were approved by the Animal Ethics Committee of “Chongqing Institute of Chinese Materia Medica”.

Conflicts of interest

There are no conflicts to declare.

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