Synthesis and biological evaluation of a new series of 1-aryl-3-[4-(pyridin-2-ylmethoxy)phenyl]urea derivatives as new anticancer agents

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Abstract

The diaryl ureas are very important fragments in medicinal chemistry. By means of computer-aided design, 1-aryl-3-[4-(pyridin-2-ylmethoxy)phenyl]urea derivatives were designed and synthesized, and evaluated for their antiproliferative activity against A549, HCT-116, PC-3 cancer cell lines, and HL7702 human normal liver cell lines in vitro by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric assay. Most of the target compounds demonstrate significant antiproliferative effects on all the selective cancer cell lines. The calculated IC50 values were reported. The target compound 1-(4-chlorophenyl)-3-{4-[(3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methoxy]phenyl}urea (7u) demonstrated the most potent inhibitory activity (IC50 = 2.39 ± 0.10 μM for A549 and IC50 = 3.90 ± 0.33 μM for HCT-116), comparable to the positive-control sorafenib (IC50 = 2.12 ± 0.18 μM for A549 and IC50 = 2.25 ± 0.71 μM for HCT-116). Conclusively, 1-aryl-3-[4-(pyridin-2-ylmethoxy)phenyl]urea derivatives as the new anticancer agents were discovered, and could be used as the potential BRAF inhibitors for further research.

Keywords Anticancer agent · BRAF · Urea · Synthesis · Antiproliferative activity

Introduction

Cancer is a major public health problem in developed countries and will become the most serious life-threatening disease worldwide in the near future (Siegel et al. 2013). Some advances in cancer treatment by targeted drugs, such as imatinib, gefitinib, and trastuzumab, were expected to improve cancer cure rates and also to reduce severe adverse actions because of the high specificity of the targeted drugs, which are expressed and have critical roles in cancer cells, but not in normal cells. However, the clinical effect was found to be limited and did not last for a long period because of the acquired resistance of the tumor cells. Furthermore, these drugs often cause on-target and/or off-target severe toxicity (Keefe and Bateman 2011). Therefore, the development of more target-specific therapy, with minimum toxicity, is warranted to extend disease-free survival and improve the quality of life of cancer patients.

Sorafenib is an orally available drug that acts predominantly by targeting the Ras/Raf/MEK (mitogen-activated protein kinase (MAPK)/ERK kinase)/ERK (extracellular signal-regulated kinase) pathway. In vitro sorafenib inhibits several members of the RAF/MEK/ERK signaling cascade, including wild-type BRAF (B-Raf proto-oncogene), mutant BRAF carrying V600E, and serine–threonine kinases CRAF (C-Raf proto-oncogene) (Davies et al. 2002; Moreau et al. 2012; Vultur et al. 2011; Wan et al. 2004; Wilhelm et al. 2004). BRAF is a member of the RAF family of serine–threonine kinases, which are

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part of the RAS/RAF/MEK/ERK MAPK signaling pathway (Barras 2015; Cheng et al. 2018; Schreck and Rapp 2006). RAF is mainly activated by the G protein, RAS, in response to mutations in cancer cells or by overexpression of different receptor protein tyrosine kinases such as epidermal, platelet derived, and vascular endothelial growth factors (Regad 2015; Sun et al. 2015).

As known, the diaryl urea moiety is widely used in the design of anticancer drugs, such as sorafenib, regorafenib, linifanib, and tivozanib (Fig. 1). And the diaryl ureas are very important fragments in medicinal chemistry and can be used for the synthesis of numerous heterocyclic compounds with diversified biological activities, including antithrombotic (Qiao et al. 2013), antimalarial (Anderson et al. 2013), antibacterial (Keche et al. 2012), and anti-inflammatory (Kulkarni et al. 2013) properties, and they are characterized by their ability to form hydrogen bond interactions with biotargets (Kim et al. 2012; Lu et al. 2014; Xuan et al. 2013). The carbonyl oxygen atom acts as a proton acceptor while the two amide nitrogen atoms are proton donors (Fig. 2). This unique type of structure endows urea derivatives with the ability to bind a variety of enzymes and receptors in the biological systems.

In recent years, proton pump inhibitors (PPIs) as potential anticancer agents were intensively studied in cancer treatment. Lugini et al. compared the antitumor efficacy of different PPIs, including omeprazole, esomeprazole, lansoprazole, rabeprazole, and pantoprazole in vitro and in vivo (Lugini et al. 2016). As shown in Fig. 3, all of the PPIs molecules contain pyridin-2-ylmethyl fragments. It can be predicted that these fragments should play an important role in the antiproliferative activity.

Molecular hybridization strategy is a useful concept in drug design and development based on the combination of pharmacophoric moieties of different bioactive substances to produce a new structure, the affinity and efficacy would be improved, when compared with the parent drugs (Viegas-Junior et al. 2007). These above interesting findings and our continuous quest to identify more potent antiproliferative agents led to the molecular hybridization of diaryl urea and pyridin-2-ylmethyl to integrate them in one molecular platform to generate a new hybrid, and expected that taking this way could get the antiproliferative agents with highly inhibitory activity. The molecular docking results also showed that the urea structure and pyridine can act as a hydrogen bond donor to generate hydrogen bond interactions. Therefore, a novel
series of 1-aryl-3-[4-(pyridin-2-ylmethoxy)phenyl]urea derivatives were designed and synthesized.

**Results and discussion**

**Molecular docking studies**

A docking study aiming at the characterization of the interaction between the target molecule and BRAF at molecular level was carried out and more-effective BRAF inhibitors were expected to be discovered.

In this study, docking model was selected from Protein Data Bank (PDB) of Research Collaborator for Structural Bioinformatics (RCSB), and the docking study was prepared by Molegro Virtual Docker (MVD) 2008 (v3.0) (Thomsen and Christensen 2006). Initially, the docking studies were carried out on the BRAF—sorafenib complex (PDB code 4R5Y) (Tang et al. 2015), using the Discovery Studio 2016 client to remove the small-molecule ligand of erlotinib in 4R5Y to obtain the three-dimensional crystal structure of BRAF, and then the designed compounds were docked to the crystal structure BRAF with MVD software. The detailed docking data will be described below, using docked to the crystal structure BRAF with MVD software.

Table 1 The MolDock score of 7u and sorafenib

| No.     | MolDock score       |
|---------|---------------------|
| 7u      | −141.4 kcal/mol     |
| Sorafenib | −155.922 kcal/mol   |

The general synthetic route is illustrated in Scheme 1. The reaction of the commercially available 4-nitrophenol (1) with 2-(chloromethyl)pyridine derivatives (2) in ethanol at room temperature obtained compounds 3a–3d (Seto et al. 2005), which converted to key intermediates 4a–4d via Pd-C catalytic hydrogenation reduction (Nguyen et al. 2014). The aryl isocyanates 6a–6f were prepared by reaction between aromatic amines and bis(trichloromethyl)carbonate (BTC) (Liu et al. 2013). Finally, treatment of 4a–4d with arylisocyanates 6a–6f in methylene dichloride yielded 1-aryl-3-[4-(pyridin-2-ylmethoxy)phenyl]urea derivatives (7a–7x) as the target compounds (Liu et al. 2013). The structures of the target compounds and intermediates were characterized by infrared (IR) spectra, proton nuclear magnetic resonance spectra (1H NMR), electrospray ionization mass spectra (ESI-MS), and no target compounds were reported in the literatures.

**Bioactivity in cell line**

Using sorafenib as a positive control, all of the target compounds were evaluated for the antiproliferative activity in vitro against cancer cell lines, which include Raf/MEK/ERK pathway cells, including lung cancer A549, and colorectal cancer HCT-116 cell lines, prostate cancer PC-3 cell lines, which Raf/MEK/ERK pathways do not affect (Lee et al. 2005), and human normal liver HL7702 cell lines by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The antiproliferative assay results evaluated as the half maximal inhibitory concentration (IC50) value (Table 2) and demonstrated that several target compounds have shown moderate to excellent potency against A549 and HCT-116 cancer cell lines, and most of the target compounds hardly exhibit any antiproliferative activities against PC-3 cell lines. Among the target compounds 7u showed the similar inhibitory effect against two cancer cell
lines than positive control with IC\textsubscript{50} values of 2.39 ± 0.10 and 3.90 ± 0.33 μM, respectively. All the tested compounds exhibit weak cytotoxic activities against HL7702.

All the target compounds could be divided into three classes according to different substituents on the pyridinyl ring (Fig. 5). The analyses of the structure–activity
Scheme 1 Synthetic route of the target compounds

Table 2 The chemical structures and inhibitory activities of the target compounds

| No. | R               | R<sub>3</sub> | R<sub>4</sub> | R<sub>5</sub> | IC₅₀ (μM)<sup>a</sup> | A549  | HCT-116 | PC-3 | HL7702 |
|-----|-----------------|--------------|--------------|--------------|-------------------------|-------|---------|-----|--------|
| 7a  | 3,5-difluoro    | -CH₃         | -O(CH₂)₂OCH₃ | H            | 44.80 ± 3.18            | 56.61 ± 2.94 | >100   | >100 | >100   |
| 7b  | 3-trifluoromethyl | -CH₃          | -O(CH₂)₂OCH₃ | H            | 4.38 ± 1.03             | 8.81 ± 0.46   | >100   | >100 | >100   |
| 7c  | 4-chloro        | -CH₃         | -O(CH₂)₂OCH₃ | H            | 10.24 ± 1.48            | 9.50 ± 1.90   | >100   | >100 | >100   |
| 7d  | 4-methoxy       | -CH₃         | -O(CH₂)₂OCH₃ | H            | 11.44 ± 0.77            | 12.66 ± 2.17  | 47.18 ± 3.12 | >100 | >100   |
| 7e  | 4-fluoro        | -CH₃         | -O(CH₂)₂OCH₃ | H            | 5.74 ± 0.44             | 6.44 ± 0.71   | >100   | >100 | >100   |
| 7f  | 4-chloro-3-trifluoromethyl | -CH₃       | -O(CH₂)₂OCH₃ | H            | 6.19 ± 0.45             | 10.10 ± 0.19  | >100   | >100 | >100   |
| 7g  | 3,5-difluoro    | -OCH₃        | -OCH₃        | H            | 17.28 ± 4.66            | 25.70 ± 1.20  | >100   | >100 | >100   |
| 7h  | 3-trifluoromethyl | -OCH₃       | -OCH₃        | H            | 6.65 ± 2.16             | 22.88 ± 3.71  | 75.05 ± 1.01 | >100 | >100   |
| 7i  | 4-chloro        | -OCH₃        | -OCH₃        | H            | 10.38 ± 0.21            | 12.85 ± 0.12  | >100   | >100 | >100   |
| 7j  | 4-methoxy       | -OCH₃        | -OCH₃        | H            | 16.72 ± 0.76            | 13.14 ± 1.02  | 46.41 ± 3.10 | >100 | >100   |
| 7k  | 4-fluoro        | -OCH₃        | -OCH₃        | H            | 23.27 ± 1.0             | 22.29 ± 1.78  | 50.11 ± 2.33 | >100 | >100   |
| 7l  | 4-chloro-3-trifluoromethyl | -OCH₃       | -OCH₃        | H            | 15.40 ± 0.67            | 18.13 ± 2.18  | >100   | >100 | >100   |
| 7m  | 3,5-difluoro    | -OCH₃        | -OCH₂CF₃     | H            | 10.16 ± 0.21            | 10.76 ± 0.39  | 57.63 ± 3.18 | >100 | >100   |
| 7n  | 3-trifluoromethyl | -OCH₃       | -OCH₂CF₃     | H            | 20.21 ± 1.55            | 12.51 ± 1.51  | >100   | >100 | >100   |
| 7o  | 4-chloro        | -OCH₃        | -OCH₂CF₃     | H            | 13.72 ± 1.46            | 19.34 ± 2.76  | 47.92 ± 2.98 | >100 | >100   |
| 7p  | 4-methoxy       | -OCH₃        | -OCH₂CF₃     | H            | 12.94 ± 0.34            | >100    | >100   | >100 | >100   |
| 7q  | 4-fluoro        | -OCH₃        | -OCH₂CF₃     | H            | 13.20 ± 0.89            | 12.68 ± 1.20  | 43.32 ± 2.21 | >100 | >100   |
| 7r  | 4-chloro-3-trifluoromethyl | -OCH₃       | -OCH₂CF₃     | H            | 6.63 ± 0.28             | 8.17 ± 0.34   | >100   | >100 | >100   |
| 7s  | 3,5-difluoro    | -OCH₃        | -OCH₂CF₃     | H            | 7.67 ± 0.77             | 51.96 ± 1.22  | >100   | >100 | >100   |
| 7t  | 3-trifluoromethyl | -OCH₃       | -OCH₂CF₃     | H            | 2.65 ± 0.17             | 5.43 ± 0.28   | >100   | >100 | >100   |
| 7u  | 4-chloro        | -OCH₃        | -OCH₂CF₃     | H            | 2.39 ± 0.10             | 3.90 ± 0.33   | >100   | >100 | >100   |
| 7v  | 4-methoxy       | -OCH₃        | -OCH₂CF₃     | H            | 12.46 ± 0.79            | 19.25 ± 0.95  | 89.10 ± 4.00 | >100 | >100   |
| 7w  | 4-fluoro        | -OCH₃        | -OCH₂CF₃     | H            | 5.37 ± 0.14             | 6.35 ± 0.07   | 60.24 ± 3.68 | >100 | >100   |
| 7x  | 4-chloro-3-trifluoromethyl | -OCH₃       | -OCH₂CF₃     | H            | 4.35 ± 0.09             | 5.60 ± 0.15   | 54.19 ± 0.44 | >100 | >100   |

Sorafenib 2.12 ± 0.18 2.25 ± 0.71 3.60 ± 1.08 n.d.

*Sorafenib*
The results of cell inhibitory activity assay showed, when the 4th position \((R_4)\) of the C ring is occupied by the \(-\text{OCH}_2\text{CF}_3\) group, the inhibitory activity is optimal. (2) The substituent on the A ring has a significant effect on the inhibitory activity of the target compounds. When the substituents on the C ring are the same, when the ring A of the compound is substituted by an electron withdrawing group such as \(-\text{F}, -\text{Cl}\) or \(-\text{CF}_3\) or the like, the inhibitory activity is superior to the compound substituted with an electron donating group such as \(-\text{OCH}_3\). In addition, when electron withdrawing group coexist in the A ring, the inhibitory activity is better than of the two electron withdrawing groups. (3) The inhibitory effect of the compound on the three test cells showed different selectivity. Most of the compounds inhibited the activity of A549 cells better than the inhibitory activities of HCT-116 cells, while the inhibitory activity against PC-3 cells was poor.

**Conclusion**

Based on the structure of sorafenib, and hybridization, and bioisosterism, combining structural optimization, a novel series of 1-aryl-3-[4-(pyridin-2-ylmethoxy)phenyl]urea derivatives, was designed as the target compounds. The binding mode of 7u, a representative of the target compounds, to BRAF was simulated by MVD software. A novel series of 1-aryl-3-[4-(pyridin-2-ylmethoxy)phenyl]urea derivatives were evaluated for their antiproliferative activity against A549, HCT-116, PC-3 cancer cell lines and HL7702 human normal liver cell lines in vitro by using the MTT colorimetric assay. Most of the target compounds demonstrate significant antiproliferative effects on all the selective cancer cell lines. Conclusively, 1-aryl-3-[4-(pyridin-2-ylmethoxy)phenyl]urea derivatives as new anticancer agents were discovered, and could be used as potential lead compounds for further research.

**Experimental section**

**Materials and methods**

The reaction process is detected by high performance thin layer chromatography silica gel plate. The melting point was determined by WRX-1S type microscopic melting-point meter, and the thermometer was not corrected. The IR spectra were determined by Bruker IFS55 IR spectrometer (KBr pellets), mass spectrometry data were determined by Waters ACQUITY QDa liquid chromatography-mass spectrometer, and nuclear magnetic resonance spectra were determined by Bruker 400 MHz nuclear magnetic resonance spectrometer with tetramethylsilane as internal standard and perdeuterated dimethyl sulfoxide (DMSO-d<sub>6</sub>) as solvent.

**Preparation of 2-[4-nitrophenoxy) methyl]pyridine derivatives (3a–3d)**

The reaction of the commercially available 4-nitrophenol (0.1 mol) with 2-(chloromethyl)pyridine derivatives (0.1 mol) in ethanol, add 3 mol/L NaOH solution (100 mL) at r.t. and the reaction mixture was stirred for an additional 3 h. After completion of the reaction was monitored TLC, ethanol was removed by vacuum evaporation, the residue was added 200 mL of water, filtered off with suction after 15 min of stirring, placing the filter cake was dried in air, the product was used directly in the next step without purification.

4-(3-methoxypropoxy)-3-methyl-2-[4-nitrophenoxy)methyl]pyridine (3a) Yield 90.5% (white solid). ESI-MS \((m/z)\): 333.4 ([M + H]^+).

3-methyl-2-[4-nitrophenoxy)methyl]-4-(2,2,2-trifluoroethoxy)pyridine (3b) Yield 90.2% (white solid). ESI-MS \((m/z)\): 343.5 ([M + H]^+).

4-methoxy-3,5-dimethyl-2-[4-nitrophenoxy)methyl]pyridine (3c) Yield 92.5% (white solid). ESI-MS \((m/z)\): 289.0 ([M + H]^+).
3,4-dimethoxy-2-[(4-nitrophenoxymethyl)pyridine (3d)
Yield 95.1% (white solid). ESI-MS (m/z): 291.1 ([M + H]+).

Preparation of 4-(pyridin-2-ylmethoxy) aniline derivatives (4a–4d)
The reaction of the commercially available 2-[(4-nitrophenoxymethyl)pyridine derivatives (50.0 mmol) with 10% Pd-C in methanol, catalytic hydrogenation reduction reaction at room temperature for 4 h. After completion of the reaction was monitored TLC, palladium carbon was to be filtered, and then distillation to remove methanol under reduced pressure, the product was used directly in the next step without purification.

4-[(4-(3-methoxypropoxy)-3-methylpyridin-2-yl)methoxy]aniline (4a) Yield 90.0% (yellowish oil). ESI-MS (m/z): 329.4 ([M + H]+).

4-[(3-methyl-4-(2,2,2-trifluoromethoxy)pyridin-2-yl)methoxy]aniline (4b) Yield 91.7% (yellowish oil). ESI-MS (m/z): 313.1 ([M + H]+).

4-[(4-methoxy-3,5-dimethylpyridin-2-yl)methoxy]aniline (4c) Yield 94.8% (yellowish oil). ESI-MS (m/z): 259.3 ([M + H]+).

4-[(3,4-dimethoxypyridin-2-yl)methoxy]aniline (4d) Yield 91.1% (yellowish oil). ESI-MS (m/z): 261.2 ([M + H]+).

Preparation of diaryl urea derivatives (7a–7x)
To a solution of BTC (1 mmol) in CH2Cl2 (20 mL) was added dropwise to primary aromatic amine 5 (1 mmol) in CH2Cl2 (20 mL) followed by the dropwise addition of triethylamine (1 mL) in CH2Cl2 (10 mL). The solvent was evaporated. The resulting residue was dissolved in CH2Cl2 (10 mL) and intermediates (6a–6f) (1 mmol) in CH2Cl2 (10 mL) was added dropwise. The mixture was stirred for about 3 h, monitored by TLC. After the reaction completed, the solvent was washed with water and brine, then dried over anhydrous magnesium sulfate. The mixture was filtered, the filtrate was evaporated and purified by silica gel chromatography (CH2Cl2/Methanol = 60/1, v/v) to obtain target compounds.

1-(3,5-difluorophenyl)-3-[(4-(3-methoxypropoxy)-3-methylpyridin-2-yl)methoxy]phenyl)urea (7a)
Yield 56.4% (white solid). m.p.: 126–127 °C. IR (KBr, cm−1): ν 3422.0, 3279.6, 2921.6. ESI-MS (m/z): 452.3 ([M + H]+).

1-(4-fluorophenyl)-3-[(4-(3-methoxypropoxy)-3-methylpyridin-2-yl)methoxy]phenyl)urea (7b) Yield 47.9% (white solid). m.p.: 118–119 °C. IR(KBr, cm−1): ν 3422.9, 2922.1, 2852.5, 1643.3, 1562.3, 1508.1, 1463.0, 1384.2, 1294.9, 1210.0, 1118.9, 1045.5, 1014.8, 833.3, 619.5, 521.2. 1H NMR (400 MHz, DMSO-d6) δ 8.64 (s, 1H), 8.48 (s, 1H), 7.34 (d, J = 2.2 Hz, 1H), 7.32 (d, J = 2.2 Hz, 1H), 7.18 (d, J = 2.3 Hz, 1H), 7.16 (d, J = 2.3 Hz, 1H), 7.00 (d, J = 5.7 Hz, 1H), 6.97 (d, J = 2.3 Hz, 1H), 6.96 (d, J = 2.2 Hz, 1H), 6.79–6.73 (m, 1H), 5.11 (s, 2H), 4.11 (t, J = 6.3 Hz, 2H), 3.49 (t, J = 6.2 Hz, 2H), 3.25 (s, 3H), 2.18 (s, 3H), 2.02–1.95 (m, 2H). ESI-MS (m/z): 458.4 ([M + H]+).
8.27 (d, J = 5.6 Hz, 1H), 7.46–7.44 (m, 1H), 7.44–7.40 (m, 1H), 7.34 (d, J = 2.2 Hz, 1H), 7.32 (d, J = 2.2 Hz, 1H), 7.11 (d, J = 8.9 Hz, 1H), 7.08 (d, J = 2.2 Hz, 1H), 7.00 (d, J = 5.7 Hz, 1H), 6.96 (d, J = 2.3 Hz, 1H), 6.94 (d, J = 2.1 Hz, 1H), 5.10 (s, 2H), 4.11 (t, J = 6.2 Hz, 2H), 3.49 (t, J = 6.2 Hz, 2H), 3.25 (s, 3H), 2.18 (s, 3H), 2.02–1.95 (m, 2H). ESI-MS (m/z): 440.2 ([M + H]+).

1-[4-chloro-3-(trifluoromethyl)phenyl]-3-[4-[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methoxy]phenylurea (7f)

Yield 37.2% (white solid). m.p.: 179–181 °C. IR(KBr, cm⁻¹): ν 3421.6, 2922.7, 1694.9, 1590.8, 1544.7, 1511.5, 1484.5, 1462.9, 1418.4, 1384.0, 1326.7, 1311.7, 1293.2, 1217.1, 1173.4, 1129.3, 1032.4, 826.5, 662.2. ¹H NMR (400 MHz, DMSO-d₆) δ 9.09 (s, 1H), 8.65 (s, 1H), 8.27 (d, J = 5.6 Hz, 1H), 8.10 (d, J = 2.3 Hz, 1H), 7.64–7.61 (m, 1H), 7.59 (d, J = 8.8 Hz, 1H), 7.36 (d, J = 2.0 Hz, 1H), 7.34 (d, J = 2.3 Hz, 1H), 7.00 (d, J = 5.7 Hz, 1H), 6.98 (d, J = 2.2 Hz, 1H), 6.96 (d, J = 2.2 Hz, 1H), 5.11 (s, 2H), 4.11 (t, J = 6.2 Hz, 2H), 3.49 (t, J = 6.2 Hz, 2H), 3.25 (s, 3H), 2.18 (s, 3H), 2.02–1.96 (m, 2H). ESI-MS (m/z): 524.3 ([M + H]+).
1H NMR (400 MHz, DMSO-d$_6$) δ 8.70 (d, 1H), 8.53 (s, 1H), 7.90 (d, 1H), 7.88 (d, 1H), 7.73 (s, 1H), 7.34 (s, 1H), 7.30 (d, J = 2.2 Hz, 1H), 6.94 (d, J = 2.2 Hz, 1H), 6.86 (d, J = 2.3 Hz, 1H), 6.84 (d, J = 2.1 Hz, 1H), 5.08 (s, 2H), 3.74 (s, 3H), 3.71 (s, 3H), 2.25 (s, 3H), 2.22 (s, 3H). ESI-MS (m/z): 408.3 ([M + H$^+$]); 430.2 ([M + Na$^+$]).

1-(4-chloro-3-(trifluoromethyl)phenyl)-3-(4-[(4-methoxy-3,5-dimethylpyridin-2-yl)oxy]phenyl)urea (7u) Yield 37.4% (white solid). m.p.: 162–163 °C. IR(KBr, cm$^{-1}$): ν 3443.4, 2922.3, 2852.2, 1691.1, 1592.4, 1550.8, 1534.8, 1488.0, 1397.6, 1330.3, 1315.5, 1287.7, 1269.3, 1214.3, 1172.5, 1144.5, 1155.8, 1090.4, 1075.0, 1031.6, 995.3, 916.5, 878.9, 814.8, 747.7, 663.3, 618.1. $^1$H NMR (400 MHz, DMSO-d$_6$) δ 8.98 (s, 1H), 8.61 (s, 1H), 8.35 (d, J = 5.7 Hz, 1H), 7.34 (d, J = 2.1 Hz, 1H), 7.33 (s, 1H), 7.10 (d, J = 7.5 Hz, 1H), 6.90 (d, J = 2.2 Hz, 1H), 5.21–5.13 (m, 1H), 4.81 (d, J = 7.5 Hz, 1H), 3.71 (s, 3H), 2.25 (s, 3H), 2.22 (s, 3H). ESI-MS (m/z): 502.2 ([M + H$^+$]); 522.1 ([M + Na$^+$]).
578.0. 1H NMR (400 MHz, DMSO-d6) δ 8.74 (s, 1H), 8.51 (s, 1H), 8.35 (d, J = 5.6 Hz, 1H), 7.47 (d, J = 2.1 Hz, 1H), 7.46 (d, J = 2.3 Hz, 1H), 7.34 (d, J = 2.2 Hz, 1H), 7.32 (s, 1H), 7.31 (s, 1H), 7.30 (d, J = 2.2 Hz, 1H), 7.14 (d, J = 5.7 Hz, 1H), 6.97 (d, J = 2.1 Hz, 1H), 6.95 (d, J = 2.3 Hz, 1H), 5.14 (s, 2H), 4.92 (q, J = 8.7 Hz, 2H), 2.21 (s, 3H). ESI-MS (m/z): 466.2, 467.2, 468.1, 469.2 ([M + H]+).

1-(4-methoxyphenyl)-3-[4-[[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methoxy]phenyl]urea (7v) Yield 19.9% (yellow solid). m.p.: 174–175 °C. IR(KBr, cm−1): ν 3424.7, 2921.8, 2852.5, 1627.5, 1559.2, 1510.9, 1482.0, 1456.7, 1419.2, 1383.3, 1172.3, 1133.1, 1032.3, 974.7, 828.2, 664.9. 1H NMR (400 MHz, DMSO-d6) δ 8.99 (s, 1H), 8.68 (d, J = 2.4 Hz, 1H), 7.92 (d, J = 8.3 Hz, 2H), 7.83 (d, J = 8.7 Hz, 2H), 7.79 (d, J = 8.7 Hz, 2H), 7.44 (d, J = 2.3 Hz, 1H), 7.44 (d, J = 2.2 Hz, 1H), 7.34 (d, J = 2.2 Hz, 1H), 7.14 (d, J = 5.7 Hz, 1H), 7.11 (d, J = 8.8 Hz, 1H), 7.09 (d, J = 2.2 Hz, 1H), 6.82 (d, J = 2.2 Hz, 1H), 6.82 (d, J = 2.2 Hz, 1H), 6.93 (d, J = 2.2 Hz, 1H), 5.08 (s, 2H), 4.92 (q, J = 8.7 Hz, 2H), 2.21 (s, 3H). ESI-MS (m/z): 462.2, 463.2 ([M + H]+); 484.2, 485.2 ([M + Na]+).

Bioactivity in cell line

The antiproliferative activities of target compounds were determined using a standard MTT assay (Mosmann 1983; Zhang et al. 2017). Exponentially growing cells A549, HCT-116, PC-3, and HL7702 were seeded into 96-well plates and incubated for 24 h to allow the cells to attach. After 24 h of incubation, the culture medium was removed and fresh medium containing various concentrations of the candidate compounds was added to each well. The cells were then incubated for 72 h, thereafter MTT assays were performed and cell viability was assessed at 570 nm by a microplate reader (ThermoFisher Scientific (Shanghai) Instrument Co., Ltd, Shanghai, China). Inhibitory effects were determined as IC50 value.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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