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Synthesis and antitumor activity of novel pyridino[2,3-*d*] pyrimidine urea derivatives

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Abstract

A series of novel *N*-(3-((6-bromopyrido[2,3-*d*]pyrimidin-4-yl)oxy)phenyl) pyrrolidine-1-carboxamide and 1-(3-((6-bromopyrido[2,3-*d*]pyrimidin-4-yl)oxy) phenyl)-3-propylurea derivatives were synthesized. Their antitumor activities against human breast carcinoma cells (MCF-7) and human colon cancer cells (HCT-116) in vitro were evaluated, using sorafenib as a positive control drug. Anticancer bioassays indicated that several compounds exhibited appreciable anticancer activity against MCF-7 and HCT-116 cells. Particularly, compounds **9g** and **8b** demonstrated the most significant inhibitory effect against HCT-116 and MCF-7 cells, with inhibition ratios of 25.56% and 26.46%, respectively. Additionally, the synthesized pyridine[2,3-*d*]pyrimidine derivatives containing a urea group moieties exhibited antitumor activities against MCF-7 and HCT-116 cells in *vitro*.

1 | INTRODUCTION

As a common malignant tumor, hepatocellular carcinoma and melanoma are devastating diseases and lead to the occurrence of cancer-related deaths [1–3]. Although sorafenib is currently the standard chemotherapy drug available for the treatment of advanced hepatocellular carcinoma (HCC), improvement of its efficacy is necessary [4]. It is essential to develop therapeutic strategies that combine sorafenib with other anticancer agents and to design a series of novel sorafenib derivatives. Nitrogen-containing heterocycles are pharmacologically important scaffold and are present in numerous clinically approved drugs [5–7]. The indazole structural unit is utilized in numerous pharmacologically active agents, including drugs with anticancer properties [8–10]. For example, indazole-based pazopanib and axitinib have been developed for application as multityrosine kinases [11–19]. Halogen bonds play a vital role in drug design, crystal engineering, and other fields because of the noncovalent interaction.

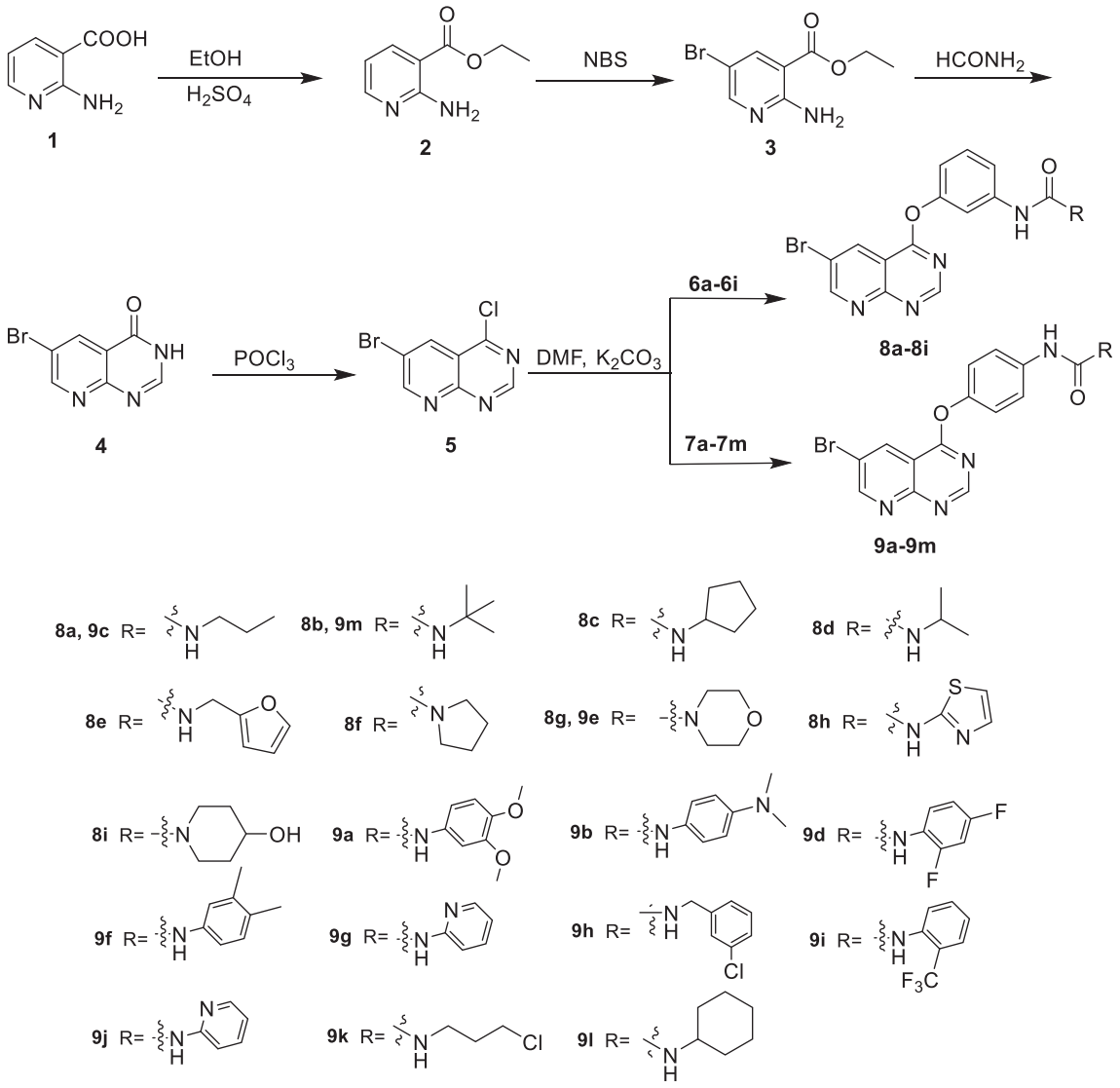
Urea is an organic compound that contains carbonyl groups flanked by two nitrogen atoms with unique hydrogen bonding capabilities, and urea derivatives can be used as modulators of biological targets [20], proteases, and epigenetic enzymes. Additionally, the keratolytic properties of urea are also considered for its applicability in cosmetology and dermatology, such as

utilization as a component of cosmetic formulas for altering skin texture to reduce roughness and discolorations [21,22]. Halogen bonds are essential in medicinal chemistry as the halogenation of drugs, generally, improves both selectivity and efficacy toward active protein sites [23]. For example, the formation of halogen bonds in ligand–target complexes is now recognized as a kind of intermolecular interaction that favorably contributes to the stability of protein–ligand complexes [24]. Therefore, we designed a bromine substituent in the pyridino[2,3-*d*] pyrimidine ring of target compounds to improve the antitumor activity of the target compound and the binding activity with the receptor protein through the interaction of halogen bonds. Furthermore, a series of pyrido[3,4-*d*] pyrimidine inhibitors demonstrated excellent potency and kinase selectivity [25]. B-Raf and c-Abl are vital in cancer treatment, and protein kinases c-Abl, B-Raf, and p38α are recognized as major targets for therapeutic intervention [26]. Owing to the importance of indazoles, sorafenib, and halogen bond in drug development, this study proposed that conjugation of the halogenated pyridine[2,3-*d*]pyrimidine template with phenyl urea would aid the design of novel drug candidates with malignant tumor inhibitors.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The detailed synthetic routes for the preparation of *N*-(3-((6-bromopyrido[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)pyrrolidine-1-carboxamide and 1-(3-((6-bromopyrido[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-3-propylurea derivatives are



SCHEME 1 Synthesis routes of **8a–8i** and **9a–9m**

summarized in Scheme 1. The synthesis route of the target compound was obtained by splicing the parent nucleus (**5**) with the side chain of the phenolic hydroxyl urea structure. First, the parent nucleus (**5**) can be prepared by esterification, bromination, cyclization and chlorination using 2-aminoniacin as the starting material. The specific synthesis route was as follows: the key intermediate ethyl 2-aminonicotinate (**2**) was prepared by esterification using 2-aminonicotinic acid as the starting material. The conditions of esterification are absolute ethyl alcohol and concentrated sulfuric acid. Ethyl 2-aminonicotinate (**2**) was subjected to bromination with *N*-bromosuccinimide (NBS) to obtain ethyl 2-amino-5-bromonicotinate (**3**). 6-Bromopyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**4**) was obtained from ethyl 2-amino-5-bromonicotinate (**3**) and formamide by cyclization. 6-Bromo-4-chloropyrido[2,3-*d*]pyrimidine (**5**) was synthesized from 6-bromopyrido[2,3-*d*]pyrimidin-4(*3H*)-one (**4**) via chlorinated reaction in the presence of phosphorus oxychloride. Second, the formation of the side chain of the phenolic hydroxyl urea structure was prepared by 3-aminophenylboronic acid pinacol ester (**10**)/4-aminophenylboronic acid pinacol ester (**11**) and triphosgene in dichloromethane. Thereafter, 2-(3-iso cyanatophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane was reacted with various aromatic amine to obtain different urea derivatives. The key intermediate phenolic hydroxyl urea compounds were further synthesized from aryl urea and hydrogen dioxide in methanol, as shown in Scheme 2. The structures of the synthesized compounds were confirmed by performing ^{1}H NMR and MS. In addition, a few target compounds were characterized via ^{13}C NMR spectroscopy.

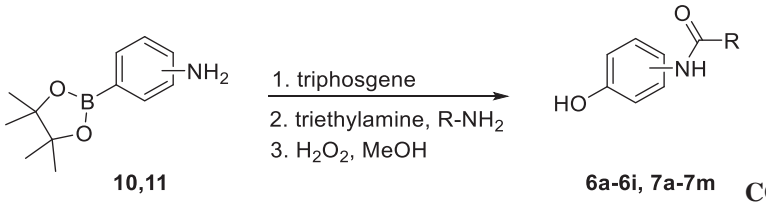
2.2 | Antitumor activity of the synthesized compounds

To evaluate the antitumor properties of *N*-(3-((6-bromopyrido[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)pyrrolidine-1-carboxamide and 1-(3-((6-bromopyrido[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-3-propylurea derivatives, we selected sorafenib as a positive control drug to determine their activities using MTT assay in *vitro*. According to the existing literature, urea derivatives exhibit cytotoxic activities against most cancer cell lines, such as HCT-116 and MCF-7 [27–29]. Additionally, a few compounds were designed and showed higher activities against the HCT-116 and MCF-7 cell lines based on the study of sorafenib. Therefore, MCF-7 and HCT-116 cells were used as the test cell lines in this work. The results of all compounds are listed in Table 1.

The results showed that a few compounds exhibited appreciable inhibitory effects against MCF-7 and HCT-116 cells in vitro, compounds **9g**, **9i**, and **9l** exhibited better anticancer effects on most tested HCT-116 cells, and compounds **9g** demonstrated the best efficacy which was better than that demonstrated by sorafenib. We totally synthesized 22 pyridine[2,3-*d*]pyrimidine derivatives, and the antiproliferative activity of the target compounds was influence by the type of the urea substituent in the *para*-position and *meta*-position of phenyl. Besides, Table 1 showed that the growth of MCF-7 cells was considerably inhabited by **8b**, with an inhibition ratio of 26.46%. The compounds-associated inhibition ratio of MCF-7 cells was in the order **8b** > **8d** > **9f** > sorafenib. The presence of electron-donating and high-steric-hindrance-exhibiting urea groups (3-isopropyl and 3-*tert*-butyl) at the *meta*-position of phenyl such as compounds **8b** and **8d** showed an increase in the inhibitory potency against MCF-7 cells. However, compounds in which the urea group were substituted at the *meta*-position of the benzene ring did not exert a remarkable effect on HCT-116 cells. Furthermore, compound **9i** showed appreciable inhibitory effects on MCF-7 and HCT-116 cells. In summary, the synthesized compounds exhibited inhibitory activity against human breast cancer cells and colon cancer cells.

3 | CONCLUSIONS

A series of novel *N*-(3-((6-bromopyrido[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)pyrrolidine-1-carboxamide and 1-(3-((6-bromopyrido[2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-3-propylu rea derivatives were synthesized. Their antitumor activities against human breast carcinoma cells (MCF-7) and



10,11 6a-6i, 7a-7m

SCHEME 2 Synthesis routes of **6a**–**6i** and **7a**–**7m**

CONCLUSIO

NS

TABLE 1 In vitro inhibitory activity of target compounds against HCT-116 and MCF-7 cells

Compounds	Inhibition rate of HCT-116 (%)	Inhibition rate of MCF-7 (%)
8a	0.69	5.38
8b	5.09	26.46
8c	5.98	4.24
8d	5.09	18.47
8e	—	—
8f	—	8.14
8g	—	4.24
8h	3.08	7.46
8i	5.47	7.63
9a	—	—
9b	8.53	11.85
9c	—	7.85
9d	—	5.76
9e	0.94	3.73
9f	—	15.25
9g	25.56	1.19
9h	7.14	11.19
9i	15.81	10.34
9j	9.57	12.88
9k	—	1.36
9l	17.28	9.54
9m	—	4.46
Sorafenib	17.28	14.77

human colon cancer cells (HCT-116) were evaluated in *vitro*. Anticancer bio-assay highlighted the potency of the synthesized compounds **8b**, **8d**, **9b**, **9f**, **9g**, **9h**, **9i**, **9j**, and **9l** as anticancer agents against MCF-7 and HCT-116 cells. Moreover, compound **8b** with 3-*tert*-butyl urea exhibited the best antiproliferative activity against MCF-7 cancer cell lines, indicating that the use of compounds harboring an electron-donating alkyl urea moiety at the *meta*-position of the phenyl ring can result in an increase in antiproliferative activity. However, compounds **9a–9m** with phenyl and heteroaromatic ring moieties at the terminal urea were found to exhibit certain antitumor properties and showed better activity against the two tested cell lines. Compound **9g**, containing a pyridinyl moiety at the terminal urea, showed better antiproliferative activity against HCT-116 cells than that exhibited by sorafenib. Furthermore, compound **9i** with an electron-withdrawing (2-trifluoromethyl)phenyl) moiety at the terminal urea exhibited influence on HCT-116 and MCF-7 cells. The results of the anticancer bioassay revealed that the type of urea substituent present at the *para*-position and *meta*-position of phenyl and the properties of the group at the terminal urea are responsible for their activity against MCF-7 cells and HCT-116 cells. In summary, the synthesized compounds exhibited antitumor activity against human breast cancer cells and colon cancer cells.

4 | EXPERIMENTAL

All common reagents and solvents were used as obtained from commercial supplies without further purifications, unless otherwise noted. The compounds' melting points were determined on an X-4 binocular microscope (Beijing Tech Instrument Co., China) and were not corrected. MS were determined on an Agilent 1100 LC–MS in ESI mode (Agilent Technologies, Palo Alto, CA, USA). ^{1}H NMR and ^{13}C NMR spectra were recorded using a 400 MHz spectrometer, a Bruker ACF-400 (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. All anhydrous solvents were dried and purified according to standard techniques before using.

4.1 | Synthesis of ethyl 2-aminonicotinate (2)

2-Aminonicotinic acid (100 g, 723.95 mmol) was added to a 500 mL flask, followed by ethanol (300 mL) and concentrated sulfuric acid (95 mL). The temperature of reaction was then increased from room temperature to 90°C, stirring for 12 h. TLC was used to monitor the reaction progress. When the reaction was complete, the reaction liquid was poured into the ice water, and the pH was adjusted to 8–9 using ammonia water to afford solid. Then filtered, and the filter cake was washed by water, the solvent was dried to obtain ethyl 2-aminonicotinate (**2**). White solid; yield 98.08%; ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ: 8.26 (dd, J = 4.8, 1.9 Hz, 1H), 8.19 (dd, J = 7.8, 1.9 Hz, 1H), 6.67 (dd, J = 7.8, 4.8 Hz, 1H), 4.40 (q, J = 7.1 Hz, 2H), 1.44 (t, J = 7.1 Hz, 3H).

4.2 | Synthesis of ethyl 2-aminonicotinate (3)

Ethyl 2-aminonicotinate (**2**) (100 g, 601.76 mmol) was added to a 1000 mL flask, followed by acetonitrile (300 mL) and NBS (139 g, 10.00 mmol) that were added slowly under an ice water bath. The mixture was then allowed to undergo reaction at room temperature for 1 h, and TLC was used to monitor the reaction progress. After the reaction was complete, it was filtered, and the filter

cake was stirred well with ammonia water. The filter cake was washed with water and then dried to obtain ethyl 2-amino-5-bromonicotinate (**3**). Light yellow solid; yield 94.93%; ^{1}H NMR (400 MHz, $CDCl_3$-*d*) *δ*: 8.29 (d, *J* = 2.5 Hz, 1H), 8.27 (d, *J* = 2.4 Hz, 1H), 4.40 (q, *J* = 7.1 Hz, 2H), 1.44 (t, *J* = 7.1 Hz, 3H).

4.3 | Synthesis of 6-bromopyrido[2,3-*d*] pyrimidin-4(3*H*)-one (4)

Ethyl 2-amino-5-bromo nicotinate (**3**) (30 g, 122.41 mmol) and formamide (100 mL) were added to a round-bottom flask. Thereafter, the temperature was increased from 25°C to 155°C and stirring for 20 h. The reaction mixture was poured into ice water when it was complete by TLC. Subsequently, the precipitated solid was filtered. The filter cake was washed with ethyl acetate and dried to afford 6-bromopyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**4**). Yellowish-brown solid, yield 83.15%; ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 12.75 (s, 1H), 9.05 (d, *J* = 2.6 Hz, 1H), 8.62 (d, *J* = 2.6 Hz, 1H), 8.37 (s, 1H).

4.4 | Synthesis of 6-bromo-4-chloropyrido [2,3-*d*]pyrimidine (5)

To a three-necked round-bottom flask containing 6-bromopyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**4**) (20 g, 88.48 mmol), phosphorus oxychloride (22 mL), and toluene (100 mL) were added, after which triethylamine (37 mL) was slowly added using a syringe. The mixture was heated from 25°C to 155°C and stirring for 3 h. Then, the reaction mixture was poured into ice water after the reaction achieved completion. Some insoluble substances were filtered using diatomite, and the filter cake was the filter cake was washed with ethyl acetate (50 mL × 3). After performing vacuum filtration, the filtrate was extracted with ethyl acetate (100 mL × 3). Thereafter, the organic layer was dried with anhydrous sodium sulfate, and the solvent was removed to obtain 6-bromo-4-chloropyrido[2,3-*d*]pyrimidine (**5**). Yellow solid, yield 74.89%. ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 9.07 (s, 1H), 8.66 (s, 1H), 8.47 (s, 1H).

4.5 | General procedure for synthesis of 1-(3-hydroxyphenyl)-3-propylurea (6a–6i and 7a–7m)

(a) Triphosgene (4.74 g, 15.98 mmol) and dichloromethane (100 mL) were added to a 250 mL flask. Next, the mixture was stirred for 10 min. 3-Aminophenylboronic acid pinacol ester was added, and the mixture was stirred continuously for 1 h until the reaction liquid attained transparency. After the reaction achieved completion, it was concentrated under reduced pressure to remove the volatile components. Dichloromethane (100 mL) was added to the concentrated solution, and a mixture of triethylamine (12.7 mL) and *n*-propylamine (7.53 mL) was slowly added to the flask under an ice water bath. Then, the mixture was heated to 48°C and stirred for 2 h. After the reaction was complete via TLC, the solvent was removed by evaporation and 1 mol/L dilute hydrochloric acid (100 mL) was added. Then, the precipitate white solid was filtered, and the filter cake was washed twice with saturated sodium bicarbonate, and the crude product was dried. Finally, the crude product was recrystallized with ethanol (100 mL), and the mixture was pumped, filtered, and dried to obtain compound **10**/**11**. (b) Compound **10**/**11** (5 g 15.14 mmol) and methanol (30 mL) were added to a 100 mL single-mouth flask, and 30% H_2O_2 (4.5 mL) was slowly added. The mixture was reacted for 2 h at room temperature. Thereafter, the reaction mixture was poured into ice water when TLC showed it was complete. Next, the precipitated solid was filtered, and the filter cake was washed with water, 1-(3-hydroxyphenyl)-3-propylurea (**6a–6i**, **7a–7m**) were obtained after the solvent was dried.

1-(3-Hydroxyphenyl)-3-propylurea (***6a***). White solid; yield 81.50%; ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 9.19 (s, 1H), 8.26 (s, 1H), 7.01–6.93 (m, 2H), 6.70 (d, *J* = 8.0 Hz, 1H), 6.28 (d, *J* = 7.4 Hz, 1H), 6.05 (t, *J* = 5.6 Hz, 1H), 3.02 (q, *J* = 6.8 Hz, 2H), 1.43 (h, *J* = 7.3 Hz, 2H), 0.87 (t, *J* = 7.4 Hz, 3H).

1-(tert-Butyl)-3-(3-hydroxyphenyl) urea (***6b***). White solid; yield 82.56%; ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 9.13 (s, 1H), 8.08 (s, 1H), 6.99–6.90 (m, 2H), 6.65 (d, *J* = 7.3 Hz, 1H), 6.27 (d, *J* = 8.1 Hz, 1H), 5.90 (s, 1H), 1.27 (s, 9H).

1-Cyclopentyl-3-(3-hydroxyphenyl) urea (***6c***). White solid; yield 89.82%; ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 9.19 (s, 1H), 8.12 (s, 1H), 7.00–6.92 (m, 2H), 6.69 (d, *J* = 7.3 Hz, 1H), 6.28 (d, *J* = 8.0 Hz, 1H), 6.07 (d, *J* = 7.2 Hz, 1H), 3.91 (q, *J* = 6.6 Hz, 1H), 1.83 (dq, *J* = 12.2, 6.3 Hz, 2H), 1.68–1.47 (m, 4H), 1.33 (dt, *J* = 12.7, 6.2 Hz, 2H).

1-(3-Hydroxyphenyl)-3-isopropyl urea (***6d***). White solid; yield 87.77%; ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 9.19 (s, 1H), 8.15 (s, 1H), 6.99–6.93 (m, 2H), 6.69 (d, *J* = 8.0 Hz, 1H), 6.28 (d, *J* = 7.7 Hz, 1H), 5.91 (d, *J* = 7.5 Hz, 1H), 3.73 (dq, *J* = 13.4, 6.6 Hz, 1H), 1.08 (d, *J* = 6.5 Hz, 6H).

1-(Furan-2-ylmethyl)-3-(3-hydroxyphenyl)urea (***6e***). White solid; yield 94.39%; ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 9.23 (s, 1H), 8.39 (s, 1H), 7.59 (s, 1H), 7.03–6.94 (m,

2H), 6.71 (d, J = 9.8 Hz, 1H), 6.46 (t, J = 5.7 Hz, 1H), 6.40 (d, J = 5.0 Hz, 1H), 6.31 (d, J = 8.0 Hz, 1H), 6.25 (d, J = 3.7 Hz, 1H), 4.27 (d, J = 5.7 Hz, 2H).

N-(3-Hydroxyphenyl) pyrrolidine-1-carboxylamide (***6f***). White solid; yield 85.89%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.17 (s, 1H), 7.94 (s, 1H), 7.07 (t, J = 2.1 Hz, 1H), 6.97 (t, J = 8.0 Hz, 1H), 6.88 (d, J = 7.1 Hz, 1H), 6.32 (d, J = 7.9 Hz, 1H), 3.34 (d, J = 5.5 Hz, 4H), 1.92–1.75 (m, 4H).

N-(3-Hydroxyphenyl) morpholine-4-carboxylamide (***6g***). White solid; yield 95.80%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.37 (s, 1H), 7.03–6.99 (m, 1H), 6.97 (d, J = 8.0 Hz, 1H), 6.84 (d, J = 9.1 Hz, 1H), 6.34 (d, J = 8.0 Hz, 1H), 3.66–3.53 (m, 4H), 3.44–3.37 (m, 4H).

1-(3-Hydroxyphenyl)-3-(thiazole-2-yl) urea (***6h***). White solid; yield 93.84%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.38 (s, 1H), 9.41 (s, 1H), 8.84 (s, 1H), 7.37 (d, J = 3.6 Hz, 1H), 7.14–7.10 (m, 1H), 7.08 (s, 1H), 7.08–7.05 (m, 1H), 6.80 (d, J = 8.5 Hz, 1H), 6.43 (d, J = 8.1 Hz, 1H).

4-Hydroxy-N-(3-hydroxyphenyl)piperidine-1-carboxamide (***6i***). White solid; yield 93.79%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.13 (s, 1H), 8.31 (s, 1H), 7.01 (t, J = 2.1 Hz, 1H), 6.96 (t, J = 8.1 Hz, 1H), 6.83 (d, J = 7.2 Hz, 1H), 6.32 (d, J = 8.0 Hz, 1H), 4.68 (d, J = 4.3 Hz, 1H), 3.80 (dt, J = 13.0, 4.0 Hz, 2H), 3.65 (dq, J = 8.6, 4.2 Hz, 1H), 3.01 (t, J = 10.2 Hz, 2H), 1.72 (dd, J = 13.5, 4.2 Hz, 2H), 1.30 (q, J = 9.1 Hz, 2H).

1-(3,4-Dimethoxyphenyl)-3-(4-hydroxy-phenyl) urea (***7a***). White solid; yield 88.41%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.04 (s, 1H), 8.36 (s, 1H), 8.22 (s, 1H), 7.21 (s, 1H), 7.19 (d, J = 2.1 Hz, 2H), 6.84 (d, J = 2.0 Hz, 2H), 6.68 (d, J = 8.9 Hz, 2H), 3.72 (d, J = 10.8 Hz, 6H).

1-(4-(Dimethylamino)phenyl)-3-(4-hydroxyphenyl) urea (***7b***). White solid; yield 84.32%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.02 (s, 1H), 8.15 (d, J = 5.6 Hz, 2H), 7.23 (d, J = 9.0 Hz, 2H), 7.19 (d, J = 8.8 Hz, 2H), 6.68 (d, J = 6.9 Hz, 2H), 6.66 (d, J = 6.7 Hz, 2H), 2.82 (s, 6H).

1-(4-Hydroxyphenyl)-3-propylurea (***7c***). White solid; yield 87.70%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.88 (s, 1H), 7.99 (s, 1H), 7.12 (d, J = 8.9 Hz, 2H), 6.61 (d, J = 8.9 Hz, 2H), 5.93 (t, J = 5.7 Hz, 1H), 3.00 (q, J = 6.8 Hz, 2H), 1.41 (h, J = 7.4 Hz, 2H), 0.86 (t, J = 7.4 Hz, 3H).

1-(2,4-Difluorophenyl)-3-(4-hydroxyphenyl) urea (***7d***). White solid; yield 96.30%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.10 (s, 1H), 8.69 (s, 1H), 8.37 (s, 1H), 8.08 (td, J = 9.3, 6.2 Hz, 1H), 7.29 (ddd, J = 11.7, 8.9, 2.9 Hz, 1H), 7.21 (d, J = 8.8 Hz, 2H), 7.03 (t, J = 9.5 Hz, 1H), 6.69 (d, J = 8.8 Hz, 2H).

N-(4-Hydroxyphenyl)morpholine-4-carboxylamide (***7e***). White solid; yield 95.67%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.02 (s, 1H), 8.24 (s, 1H), 7.18 (d, J = 8.9 Hz, 2H), 6.64 (d, J = 8.9 Hz, 2H), 3.66–3.55 (m, 4H), 3.44–3.35 (m, 4H).

1-(3,4-Dimethylphenyl)-3-(4-hydroxyphenyl) urea (***7f***). White solid; yield 91.46%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.04 (s, 1H), 8.32 (s, 1H), 8.25 (s, 1H), 7.21 (d, J = 2.5 Hz, 2H), 7.19 (s, 1H), 7.16–7.12 (m, 1H), 7.00 (d, J = 8.2 Hz, 1H), 6.68 (d, J = 8.8 Hz, 2H), 2.18 (s, 3H), 2.15 (s, 3H).

1-(4-Hydroxyphenyl)-3-(pyridine-2-yl) urea (***7g***). White solid; yield 91.74%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.27 (d, J = 4.4 Hz, 1H), 7.74 (t, J = 7.0 Hz, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.30 (d, J = 8.8 Hz, 2H), 7.08–6.95 (m, 1H), 6.74 (d, J = 8.8 Hz, 2H).

1-(3-Chlorobenzyl)-3-(4-hydroxyphenyl)urea (***7h***). White solid; yield 92.23%. *1-(4-Hydroxyphenyl)-3-(2-(trifluoromethyl)phenyl) urea* (***7i***). Gray solid; yield 93.24%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.11 (s, 1H), 9.43 (s, 1H), 7.45 (t, J = 7.7 Hz, 2H), 7.29 (d, J = 6.8 Hz, 1H), 7.24 (d, J = 7.8 Hz, 2H), 7.12 (d, J = 8.1 Hz, 1H), 7.07 (d, J = 10.1 Hz, 1H), 6.93 (d, J = 8.0 Hz, 1H), 6.47 (d, J = 7.8 Hz, 1H).

1-(4-Hydroxyphenyl)-3-(pyrazine-2-yl) urea (***7j***). White solid; yield 85.7%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.97 (s, 1H), 8.34–8.28 (m, 1H), 8.24 (d, J = 2.6 Hz, 1H), 7.29 (d, J = 8.7 Hz, 2H), 6.79–6.71 (m, 2H).

1-(4-Chlorobutyl)-3-(4-hydroxyphenyl) urea (***7k***). White solid; yield 95.90%. *1-Cyclohexyl-3-(4-hydroxyphenyl) urea* (***7l***). White solid; yield 91.10%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.91 (s, 1H), 7.93 (s, 1H), 7.12 (d, J = 8.8 Hz, 2H), 6.62 (d, J = 8.8 Hz, 2H), 5.87 (d, J = 7.9 Hz, 1H), 3.52–3.38 (m, 1H), 1.78 (dd, J = 12.2, 3.2 Hz, 2H), 1.71–1.60 (m, 2H), 1.53 (dd, J = 8.3, 3.9 Hz, 1H), 1.35–1.23 (m, 2H), 1.21–1.06 (m, 3H).

1-(tert-Butyl)-3-(4-hydroxyphenyl) urea (***7m***). Gray solid; yield 92.83%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.91 (s, 1H), 7.89 (s, 1H), 7.22–7.03 (m, 2H), 6.63 (d, J = 8.5 Hz, 2H), 5.82 (s, 1H), 1.29 (s, 9H).

4.6 | General procedure for synthesis of 8a–8i and 9a–9m

(1) Potassium carbonate (0.89 g, 6.44 mmol) and *N,N*-dimethylformamide (10 mL) was added to a 100 mL flask, and then 1-(3-hydroxyphenyl)-3-propylurea (**6a**) was added to the mixture with stirring. After reacting 30 min, 6-bromo-4-chloropyrido[2,3-*d*]pyrimidine (**5**) was slowly added and stirred for 1 h at room temperature. The reaction mixture was poured into ice water when TLC showed it was complete and the some solid was precipitate. Then precipitated solid was filtered, and the filter cake was washed with water and dried to afford 1-(3-((6-bromopyrido [2,3-*d*]pyrimidin-4-yl)oxy)phenyl)-3-propylurea (**8a**).

1-(3-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) -3-propylurea (***8a***). Light solid; yield 67.60%; mp 195–

198°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.35 (d, *J* = 1.6 Hz, 1H), 9.06 (d, *J* = 1.2 Hz, 1H), 8.95 (s, 1H), 8.64 (d, *J* = 9.6 Hz, 1H), 7.58 (s, 1H), 7.34 (t, *J* = 8.0 Hz, 1H), 7.20 (d, *J* = 8.3 Hz, 1H), 6.87 (d, *J* = 7.9 Hz, 1H), 6.22 (t, *J* = 4.8 Hz, 1H), 3.03 (dd, *J* = 12.2, 6.1 Hz, 2H), 1.44 (dd, *J* = 14.2, 7.2 Hz, 2H), 0.87 (t, *J* = 7.3 Hz, 3H). ESI-MS, *m/z*: 424.04[M + H]$^+$.

*1-(3-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) -3-(tert-butyl)urea (**8b**).* Yellow solid; yield 72.04%; mp 215–218°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.33 (d, *J* = 2.4 Hz, 1H), 9.03 (d, *J* = 2.5 Hz, 1H), 8.92 (s, 1H), 7.57 (s, 1H), 7.35 (t, *J* = 8.2 Hz, 1H), 7.13 (d, *J* = 8.0 Hz, 1H), 6.88 (d, *J* = 8.0 Hz, 1H), 6.06 (s, 1H), 1.28 (s, 9H). ESI-MS, *m/z*: 416.07[M − H]$^-$.

*1-(3-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl)- 3-cyclopentylurea (**8c**).* Yellow solid; yield 46.29%; mp 173–176°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.34 (d, *J* = 2.6 Hz, 1H), 9.04 (d, *J* = 2.6 Hz, 1H), 8.93 (s, 1H), 7.55 (t, *J* = 2.1 Hz, 1H), 7.36 (t, *J* = 8.1 Hz, 1H), 7.19 (d, *J* = 9.2 Hz, 1H), 6.89 (dd, *J* = 7.8, 1.8 Hz, 1H), 3.97–3.82 (m, 1H), 1.83 (td, *J* = 12.2, 6.5 Hz, 2H), 1.67–1.58 (m, 2H), 1.57–1.48 (m, 2H), 1.41–1.30 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) *δ*: 167.32, 159.24, 158.27, 157.83, 155.08, 152.76, 142.48, 135.58, 130.14, 118.93, 115.51, 114.52, 112.54, 111.21, 51.35, 33.23, 23.61. ESI-MS, *m/z*: 426.06[M − H]$^{\cdot-}$.

*1-(3-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) -3-isopropylurea (**8d**).* Yellow solid; yield 77.26%; mp 221–224°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.04 (d, *J* = 2.6 Hz, 1H), 8.93 (s, 1H), 7.55 (t, *J* = 2.1 Hz, 1H), 7.35 (t, *J* = 8.1 Hz, 1H), 7.19 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.88 (dd, *J* = 8.0, 2.2 Hz, 1H), 3.73 (dd, *J* = 13.0, 6.5 Hz, 1H), 1.09 (d, *J* = 6.5 Hz, 6H). ESI-MS, *m/z*: 400.04[M − H]$^-$.

*1-(3-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) -3-(furan-2-ylmethyl)urea (**8e**).* Yellow solid; yield 82.29%; mp 197–200°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.37 (s, 1H), 9.08 (d, *J* = 1.6 Hz, 1H), 8.97 (s, 1H), 8.80 (s, 1H), 7.60 (s, 2H), 7.38 (t, *J* = 8.1 Hz, 1H), 7.25 (d, *J* = 7.9 Hz, 1H), 6.92 (d, *J* = 7.8 Hz, 1H), 6.66 (t, *J* = 4.6 Hz, 1H), 6.42 (s, 1H), 6.28 (d, *J* = 2.0 Hz, 1H), 4.30 (d, *J* = 4.9 Hz, 2H). ESI-MS, *m/z*: 462.02 [M − H]$^-$.

*N-(3-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) pyrrolidine-1-carboxamide (**8f**).* Yellow solid; yield 69.70%; mp 215–218°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.34 (d, *J* = 2.6 Hz, 1H), 9.05 (d, *J* = 2.6 Hz, 1H), 8.94 (s, 1H), 7.61 (t, *J* = 2.1 Hz, 1H), 7.44 (d, *J* = 8.3 Hz, 1H), 7.35 (t, *J* = 8.1 Hz, 1H), 6.91 (dd, *J* = 7.9, 2.1 Hz, 1H), 3.36 (t, *J* = 6.5 Hz, 4H), 1.85 (t, *J* = 6.5 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) *δ*: 167.33, 159.27, 158.27, 157.84, 154.12, 152.45, 142.68, 135.55, 129.74, 118.96, 117.22, 114.99, 112.88, 112.50, 46.18, 25.46. ESI-MS, *m/z*: 412.04 [M − H]$^-$.

*N-(3-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) morpholine-4-carboxamide (**8g**).* Pink solid; yield 84.71%; mp 222–224°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.32 (d, *J* = 2.6 Hz, 1H), 9.03 (d, *J* = 2.6 Hz, 1H), 8.92 (s, 1H), 7.53 (s, 1H), 7.37 (s, 1H), 7.36 (d, *J* = 1.5 Hz, 1H), 6.96–6.90 (m, 1H), 3.61–3.56 (m, 4H), 3.43–3.39 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) *δ*: 167.31, 159.27, 158.27, 157.82, 155.36, 152.48, 142.42, 135.57, 129.85, 118.96, 117.39, 115.39, 113.12, 112.52, 66.44, 44.63. ESI-MS, *m/z*: 428.04 [M − H]$^-$.

*1-(3-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) -3-(thiazol-2-yl)urea (**8h**).* Yellow solid; yield 45.64%; mp 230–232°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.34 (d, *J* = 2.5 Hz, 1H), 9.06 (d, *J* = 2.5 Hz, 1H), 8.94 (s, 1H), 7.66 (s, 1H), 7.45 (t, *J* = 8.1 Hz, 1H), 7.37 (d, *J* = 3.4 Hz, 1H), 7.33 (d, *J* = 8.8 Hz, 1H), 7.11 (s, 1H), 7.04 (d, *J* = 9.5 Hz, 1H). ESI-MS, *m/z*: 442.98 [M − H]$^-$.

*N-(3-((6-bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) -4-hydroxypiperidine-1-carboxamide (**8i**).* Brown solid; yield 80.83%; mp 207–208°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:*9.33 (d, *J* = 2.1 Hz, 1H), 9.03 (d, *J* = 2.2 Hz, 1H), 8.92 (s, 1H), 7.54 (s, 1H), 7.41–7.26 (m, 2H), 6.91 (d, *J* = 6.6 Hz, 1H), 3.81 (d, *J* = 12.9 Hz, 2H), 3.66 (s, 1H), 3.04 (t, *J* = 11.7 Hz, 2H), 1.73 (d, *J* = 12.7 Hz, 2H), 1.29 (dd, *J* = 20.8 Hz, H), 1.19 (d, *J* = 23.5 Hz, 2H). ESI-MS, *m/z*: 442.05 [M − H]$^-$.

*1-(4-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) -3-(3,4-dimethoxyphenyl)urea (**9a**).* Gray solid; yield 39.05%; mp 233–235°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.35 (d, *J* = 2.5 Hz, 1H), 9.06 (d, *J* = 2.5 Hz, 1H), 8.93 (d, *J* = 4.3 Hz, 1H), 8.81 (s, 1H), 8.63 (s, 1H), 7.57 (d, *J* = 8.9 Hz, 2H), 7.27 (d, *J* = 9.0 Hz, 2H), 7.23 (s, 1H), 6.88 (t, *J* = 6.0 Hz, 2H), 3.74 (s, 3H), 3.70 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) *δ*: 171.22, 159.21, 157.85, 155.69, 153.26, 149.23, 146.61, 138.50, 135.56, 133.86, 122.46, 119.68, 118.89, 112.95, 112.54, 110.67, 105.78, 104.42, 56.33, 55.83. ESI-MS, *m/z*: 494.05 [M − H]$^-$.

*1-(4-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phe- nyl)-3-(4-(dimethylamino)phenyl)urea (**9b**).* Brown solid; yield 11.32%; mp 229–230°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.30 (s, 1H), 9.02 (s, 1H), 8.90 (s, 1H), 8.65 (s, 1H), 8.32 (s, 1H), 7.52 (d, *J* = 7.9 Hz, 2H), 7.25 (d, *J* = 9.9 Hz, 4H), 6.70 (d, *J* = 8.7 Hz, 2H), 2.81 (s, 6H). ESI-MS, *m/z*: 477.07 [M − H]$^-$.

*1-(4-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) -3-propylurea (**9c**).* Yellow solid; yield 70.05%; mp 200–201°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.33 (d, *J* = 2.6 Hz, 1H), 9.04 (d, *J* = 2.6 Hz, 1H), 8.92 (s, 1H), 8.57 (s, 1H), 7.49 (s, *J* = 9.0 Hz, 2H), 7.22 (d, *J* = 9.0 Hz, 2H), 6.21 (t, *J* = 5.7 Hz, 1H), 3.09–3.03 (dd, 2H), 1.46 (dd, *J* = 7.2 Hz, 2H), 0.89 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) *δ*: 167.60, 159.18, 158.23, 157.86, 155.71, 146.12, 139.12, 135.55, 122.37, 118.93, 112.54, 41.36, 23.47, 11.82. ESI-MS, *m/z*: 424.04 [M + H]$^+$.

*1-(4-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) -3-(2,4-difluorophenyl)urea (**9d**).* Yellow solid; yield 62.67%; mp 240–242°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.34 (d, J = 2.5 Hz, 1H), 9.06 (d, J = 2.5 Hz, 1H), 8.93 (s, 1H), 8.12–8.00 (m, 1H), 7.56 (dd, J = 8.8 Hz, 2H), 7.37–7.30 (m, 2H), 7.30 (s, 1H), 7.08 (t, J = 8.1 Hz, 1H). ESI-MS, m/z: 494.00 $[M - H]^-$.

*N-(4-((6-bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) morpholine-4-carboxamide (**9e**).* Yellow solid; yield 70.25%; mp 224–226°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.34 (d, J = 2.6 Hz, 1H), 9.04 (d, J = 2.6 Hz, 1H), 8.93 (s, 1H), 7.60–7.50 (m, 2H), 7.29–7.21 (m, 2H), 3.66–3.60 (m, 4H), 3.48–3.41 (m, 4H). ESI-MS, m/z: 428.04 $[M - H]^-$.

*1-(4-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) -3-(3,4-dimethylphenyl)urea (**9f**).* Yellow solid; yield 61.28%; mp 249–250°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.34 (d, J = 2.5 Hz, 1H), 9.05 (d, J = 2.5 Hz, 1H), 8.93 (s, 1H), 8.76 (s, 1H), 8.53 (s, 1H), 7.56 (d, J = 8.9 Hz, 2H), 7.29 (d, J = 8.9 Hz, 2H), 7.24 (s, 1H), 7.18 (d, J = 7.6 Hz, 1H), 7.05 (d, J = 8.2 Hz, 1H), 2.20 (s, 3H), 2.16 (s, 3H) ESI-MS, m/z: 462.06 $[M - H]^-$.

*1-(4-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) -3-(pyridin-2-yl)urea (**9g**).* Yellow solid; yield 78.64%; mp 235–238°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.34 (d, J = 2.6 Hz, 1H), 9.05 (d, J = 2.6 Hz, 1H), 8.93 (s, 1H), 8.30 (d, J = 5.7 Hz, 1H), 7.82–7.71 (m, 1H), 7.71–7.57 (m, 2H), 7.49 (dd, J = 8.2, 5.8 Hz, 1H), 7.32 (d, J = 8.9 Hz, 2H), 7.03 (dd, J = 7.1, 5.8 Hz, 1H). ESI-MS, m/z: 435.02 $[M - H]^-$.

*1-(4-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) -3-(3-chlorobenzyl)urea (**9h**).* Light yellow solid; yield 76.50%; mp 249–251°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.33 (s, 1H), 9.04 (s, 1H), 8.92 (s, 1H), 7.71 (s, 1H), 7.56 (d, J = 7.9 Hz, 2H), 7.34–7.19 (m, 4H), 7.03 (d, J = 7.6 Hz, 1H), 1.15 (s, 2H). ESI-MS, m/z: 483.21 $[M - H]^-$.

*1-(4-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) -3-(2-(trifluoromethyl)phenyl)urea (**9i**).* Yellow solid; yield 94.00%; mp 202–205°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.33 (t, J = 2.5 Hz, 1H), 9.04 (dd, J = 4.3, 2.6 Hz, 1H), 8.92 (d, J = 4.1 Hz, 1H), 7.59 (d, J = 1.9 Hz, 1H), 7.57 (d, J = 2.0 Hz, 1H), 7.54–7.49 (m, 1H), 7.34–7.26 (m, 2H), 7.25–7.17 (m, 2H), 6.73–6.66 (m, 1H). ESI-MS, m/z: 504.37 $[M + H]^+$.

*1-(4-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) -3-(pyrazin-2-yl)urea (**9j**).* Yellow solid; yield 80.09%; mp 232–234°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 9.34 (d, J = 2.6 Hz, 1H), 9.05 (d, J = 2.7 Hz, 1H), 9.05 (d, J = 1.4 Hz, 1H), 8.93 (s, 1H), 8.35–8.31 (m, 1H), 8.27 (d, J = 2.6 Hz, 1H), 7.63 (d, J = 8.9 Hz, 2H), 7.34 (d, J = 8.9 Hz, 2H). ESI-MS, m/z: 440.03 $[M + H]^+$.

*1-(4-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) -3-(3-chloropropyl)urea (**9k**).* Yellow solid; yield 84.00%; mp 198–199°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.32 (d, J = 2.6 Hz, 1H), 9.03 (d, J = 2.6 Hz, 1H), 8.92 (s, 1H), 7.53 (d, J = 1.9 Hz, 1H), 7.35 (s, 1H), 7.33 (d, J = 8.1 Hz, 1H), 6.90 (dt, J = 6.3, 2.3 Hz, 1H), 1.62 (d, J = 12.5 Hz, 1H), 1.59 (d, J = 8.2 Hz, 1H), 1.51 (dd, J = 19.1, 7.0 Hz, 3H), 1.12 (d, J = 6.9 Hz, 3H). ESI-MS, m/z: 450.90 $[M + H]^+$.

*1-(4-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) -3-cyclohexylurea (**9l**).* Yellow solid; yield 31.78%; mp 194–195°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.34 (d, J = 2.6 Hz, 1H), 9.04 (d, J = 2.6 Hz, 1H), 8.93 (s, 1H), 8.43 (s, 1H), 7.47 (d, J = 9.0 Hz, 2H), 7.21 (d, J = 9.0 Hz, 2H), 6.11 (d, J = 7.8 Hz, 1H), 3.58–3.38 (m, 1H), 1.92–1.75 (m, 2H), 1.75–1.60 (m, 2H), 1.30 (dd, J = 17.0, 7.1 Hz, 2H), 1.17 (dd, J = 9.3, 4.7 Hz, 4H). ESI-MS, m/z: 440.07 $[M - H]^+$.

*1-(4-((6-Bromopyrido[2,3-d]pyrimidin-4-yl)oxy)phenyl) -3-(tert-butyl)urea (**9m**).* Yellow solid; yield 76.04%; mp 213–215°C; ^{1}H NMR (400 MHz, DMSO-d_6) *δ:* 9.30 (d, J = 2.6 Hz, 1H), 9.01 (d, J = 2.6 Hz, 1H), 8.88 (s, 1H), 8.35 (s, 1H), 7.43 (d, J = 8.9 Hz, 2H), 7.19 (d, J = 8.9 Hz, 2H), 6.01 (s, 1H), 1.28 (s, 9H). ESI-MS, m/z: 416.07 $[M - H]^-$.

4.7 | Antitumor activity

The antiproliferative activity of the target compounds was evaluated against human breast carcinoma cells (MCF-7) and human colon cancer cell (HCT-116) using the standard MTT assay in *vitro*. The title compounds were dissolved in proper DMSO to obtain different diluted solution as blank control and commercial reagent sorafenib was used as positive control to evaluate the antiproliferative activity of the target compounds. The cancer cell line was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Approximately, 4×10^3 cells, suspended in DMEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37°C for 12 h. The tested compound at the final concentration of 100 μmol/L was added to the culture medium, and the cell cultures were continued for 24 h. Fresh MTT (20 μL) was added to each well at a terminal concentration of 5 mg/mL and incubated with cells at 37°C for 4 h but needed keeping in the dark. The formazan crystals were dissolved in 150 mL DMSO for each well, and the absorbancy at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) were measured with an ELISA reader. The compound was tested three times. The OD value of each well at 490 nm was detected by using a microplate reader. The cellular viability of every well (%) = OD dosed/OD normal×100.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. The data that supports the findings of this study are available in the supporting information of this article.

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