新型吲哚-嘧啶联芳类化合物的设计、合成及抗肿瘤活性研究

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摘要 设计合成了一系列新型的吲哚-嘧啶联芳类化合物,并评估了它们对组蛋白去甲基化酶(LSD1)的抑制活性和5种肿瘤细胞系(MGC-803, PC-3, EC-109, PC-12 和 MCF-7)的抗增殖活性。探讨了22个新型吲哚-嘧啶联芳类骨架衍生物的主要构效关系。在这些化合物中,1-(4-(4-(1-甲基-1*H*-吲哚-3-基)嘧啶-2-基)哌嗪-1-基)-2-((3,4,5-三甲氧基苯基)氨基)乙-1-酮(**6i**)展示出了潜在的LSD1抑制活性($IC_{50}=1.03\ \mu\text{mol/L}$),而1-(4-(4-(1-甲基-1*H*-吲哚-3-基)嘧啶-2-基)哌嗪-1-基)-2-(间甲苯胺)乙-1-酮(**6c**),2-((4-丁基苯基)氨基)-1-(4-(4-(1-甲基-1*H*-吲哚-3-基)嘧啶-2-基)哌嗪-1-基)乙-1-酮(**6f**)和2-((3-氟苯基)氨基)-1-(4-(4-(1-甲基-1*H*-吲哚-3-基)嘧啶-2-基)哌嗪-1-基)乙-1-酮(**6k**)对PC-3细胞显示出潜在的抗肿瘤活性。其中活性最强的化合物**6k**的对PC-3细胞系 IC_{50} 值为 $2.75\ \mu\text{mol/L}$,可作为生物活性片段和开发更有效抗肿瘤药物的靶点化合物。

关键词 吲哚; 嘧啶; 联芳基; 抗肿瘤活性

Design, Synthesis and Anticancer Activity Studies of Novel Indole-Pyrimidine Biaryl Derivatives

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Abstract A series of novel indole-pyrimidine biaryl derivatives were designed, synthesized and evaluated for inhibitory activity against Lysine Specific Demethylase 1 (LSD1) and antiproliferative activity against five selected cancer cell lines (MGC-803, PC-3, EC-109, PC-12 and MCF-7). The preliminary structure-activity relationship (SAR) for this indole-pyrimidine biaryl scaffold was explored with evaluation of 22 variants of the structural class. Among these analogues, compound 1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)-2-((3,4,5-trimethoxyphenyl)amino)ethan-1-one (**6i**) exhibited the potential inhibitory activity against LSD1 ($IC_{50}=1.03\ \mu\text{mol/L}$), compounds 1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)-2-(*m*-tolylamino)ethan-1-one (**6c**), 2-((4-butylphenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6f**) and 2-((3-fluorophenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6k**) showed the potential inhibitory activity against PC-3 cells. Especially, compound **6k** exhibited the best antitumor activity ($IC_{50}=2.75\ \mu\text{mol/L}$), which was served as bioactive fragment and hit compound for developing more potent antitumor agents.

Keywords indole; pyrimidine; biaryl; antitumor

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1 Introduction

According to new drugs approved by the United States Food and Drug Administration (FDA), cancer remained the dominant therapeutic area in recent years.^[1-2] Although several modalities, such as small molecules, monoclonal antibody (mAb), antibody-drug conjugate (ADC), oligonucleotide and fusion protein, *etc.* have been widely explored, finding new treatments of cancer is still challenging and greatly needed.^[3-4]

Biaryl fragments, especially which containing indole-pyrimidine core structures, exist widely in many drug molecules and natural products with broad and interesting biological activities.^[5-6] For instance, compound **1** (Osimertinib) was the 3rd epidermal growth factor receptor tyrosine kinase inhibitor for the treatment of non-small-cell lung carcinomas,^[7] compound **2** (Meridianin D) was one of the marine derived indole alkaloids isolated from the tunicate aplidium meridianum,^[8] and compound **3** (Alocasin A) was an anti-fungal agent isolated from the giant taro *Alocasia macrorrhiza*.^[9] In addition, some modifications based on this core scaffold may bring unexpected improvement of biological activity. Compound **4** was designed as a GSK-3 β inhibitor for the treatment of inflammation-related diseases with an IC₅₀ value of 0.14 $\mu\text{mol/L}$.^[10] Compound **5** exhibited potent inhibitory activity against Dyrk1A with an IC₅₀ value of 0.5 $\mu\text{mol/L}$ and had promising neuroprotective activity.^[11] Compound **6** displayed antibiofilm activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and increased potency of colistin in Gram-negative bacteria with an IC₅₀ value of 9.6 $\mu\text{mol/L}$ (Figure 1).^[12]

Figure 1 Structures of selected biaryl-containing drugs, natural products and bioactive molecules

The structures of carbonyl piperazines are widely existed as the unique pharmacophores in many lysine specific de-

methylase **1** (LSD1) inhibitors, such as CBB1007, IMG-7289, RN-1 and S2157.^[13-16] Moreover, carbonyl piperazines also work as the hydrophobic linker, and aromatic amines act as the additive to improve bioactivity by occupying protein pocket or increasing liposolubility.^[17] The introduction of these two motifs into one fragment may generate a novel pharmacophore which widely exists in many bioactive molecules as the core structure. Compound **7** (JNJ-40355003), a covalent inhibitor of fatty acid amide hydrolase (FAAH), was found to elevate the plasma levels and brain concentrations.^[18] Compound **8** (UCB9608) was a potent PI4KIII β inhibitor with excellent ADME (Absorption, Distribution, Metabolism, Excretion) properties, which makes it an ideal tool compound to prolong allogeneic organ engraftment *in vivo*.^[19] Compound **9** (JNJ-17203212) was demonstrated as a selective Transient receptor potential vanilloid 1 (TRPV1) antagonist to modulate the signaling of noxious stimuli.^[20] Compound **10** was identified as the antiviral agent for the treatment of human mastadenovirus (HAdV) infections in immunocompromised patients.^[21] Compound **11** was designed as the potential Hepatitis C virus (HCV) inhibitor that worked through binding to the allosteric site "Thumb pocket II" (Figure 2).^[22]

Figure 2 Structures of bioactive molecules having carbonyl piperazine-aromatic amine cores

In our previous work, we found that osimertinib had potential inhibitory activity against LSD1.^[23-25] To develop novel LSD1 inhibitors, we assumed that linking indole-pyrimidine biaryl fragment to the carbonyl piperazine-aromatic amine structure through the principle of molecular hybridization strategy could produce novel bioactive molecules with potential inhibitory activity. Thereby, a series of small molecules containing indole-pyrimidine biaryl fragment and carbonyl piperazine-aromatic amine scaffold

(Figure 3) were designed and synthesized, and the relevant studies about bioactivity of them against LSD1 and five selected cancer cell lines were also completed.

2 Results and discussion

2.1 Chemistry

All the target compounds (**6a**~**6t**) were synthesized in five steps starting from commercially available 2,4-dichloropyrimidine (**1a**) and *N*-methylindole as shown in Scheme 1. The substitution reaction between 2,4-dichloropyrimidine (**1a**) and *N*-methylindole was realized in the presence of aluminium chloride, affording biaryl **2a** as a solid in 78% yield that was readily isolated by filtration. Buchwald-Hartwig reaction between biaryl **2a** and 1-Boc-piperazine in the presence of Pd₂dba₃ and bis(diphenyl-

Figure 3 Design of novel antitumor agents via molecular hybridization

phosphino)methane (DPPM), followed by trifluoroacetic acid (TFA) mediated *N*-Boc deprotection delivered the salt **4a** in 40% yield over the two-step sequence. Treatment of **4a** with linker chloroacetyl chloride in tetrahydrofuran (THF) produced the substitution product **5a** in 90% yield. Finally, treatment of **5a** with aromatic amines, followed by addition of sodium hydride, gave the target compound **6a**~**6v** in 31%~74% yields. All synthesized compounds were fully characterized by ¹H NMR, ¹³C NMR, ESI-MS and melting point analysis.

2.2 Inhibitory activity against LSD1

The inhibitory activity of compounds **6a**~**6v** against LSD1 at the concentration of 10 μmol/L was determined by fluorescence method. The reported LSD1 inhibitor ORY-1001 was used as the positive control. The results are summarized in Table 1. Among them, compounds **6d**, **6i**, **6r** and **6s** exhibited potential inhibitory activity against LSD1 with inhibitory rates more than 30%. Especially, compound **6i** showed potential inhibitory activity against LSD1 with an IC₅₀ value of 1.03 μmol/L.

2.3 Antitumor activity

To the best of our knowledge, LSD1 is overexpressed in a variety of tumor cells. Therefore, the cell viability assays were carried out to verify the effectiveness of these synthetic compounds. All compounds (**6a**~**6v**) were evaluated for their antitumor activities against five human cancer cell lines [Human gastric cancer cell (MGC-803), Human prostate cancer cell (PC-3), Human Esophageal Cancer Cell (EC-109), Rat adrenal pheochromocytoma cell (PC-12) and Human breast cancer cell (MCF-7)] using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method and the anti-tumor drug 5-fluorouracil (5-Fu) as a positive control drug. The results are summarized in Table 2. Among them, compound **6g** showed potential antitumor activity against MGC-803 cells with an IC₅₀ value of 4.35 μmol/L. Compounds **6c** and **6k** exhibited potential antitumor activities against PC-3 cells with IC₅₀ values of 4.10 and 2.75 μmol/L, respectively. Compound **6i** showed potential antitumor activity against EC-109 cells with an IC₅₀ value of 9.34 μmol/L, which also inhibited MGC-803, PC-3, PC-12 and MCF-7 cells proliferation with IC₅₀ values of 14.50, 15.62, 8.49 and 10.55 μmol/L, respectively. Compound **6v** showed potential antitumor activity against PC-12 with an IC₅₀ value of 6.51 μmol/L.

From the antitumor activity results of compounds **6a**~**6v** as shown in Table 2, some compounds displayed moderate activities against five human cancer cell lines. Most of these compounds behaved interesting antitumor activities following certain principles which were dependent on the substituents on the simple aromatic amine. Compound

Scheme 1 Synthesis of the target compounds **6a**~**6v**

Table 1 Inhibitory activities of compounds **6a**~**6v** against LSD1

Compd.	R	Inhibitory rate ^a /%	Compd.	R	Inhibitory rate ^a /%
6a	—	<10	6m	2-Cl	<10
6b	2-Me	<10	6n	3-Cl	<10
6c	3-Me	<10	6o	4-Cl	<10
6d	4-Me	30	6p	2-Br	<10
6e	4-Et	<10	6q	3-Br	<10
6f	4- ⁿ Bu	11	6r	4-Br	32
6g	2-OMe	<10	6s	3-Cl-4-F	41
6h	3-OMe	11	6t	3-Br-4-F	<10
6i	3,4,5-(OMe) ₃	55 (1.03 μmol/L)	6u	2,4-Cl ₂	<10
6j	2-F	<10	6v	4-CN	12
6k	3-F	<10	ORY-1001 ^b		99
6l	4-F	20			

^a Inhibitory activity of the synthesized compounds at 10 μmol/L was evaluated through fluorescence-based assay. ^b IC₅₀=15.63 nmol/L.

Table 2 Antitumor activities of compounds **6a**~**6v** against five human cancer cell lines

Compd.	R	IC ₅₀ ^a /(μmol·L ⁻¹)				
		MGC-803	PC-3	EC-109	PC-12	MCF-7
6a ^b	—	>40	6.80±0.53	>40	27.74±1.00	20.86±2.85
6b	2-Me	13.65±1.13	>40	>40	>40	>40
6c	3-Me	>40	4.10±0.46	>40	25.15±0.34	22.29±0.68
6d	4-Me	>40	29.16±3.15	>40	>40	>40
6e	4-Et	14.68±1.20	10.21±2.13	>40	31.29±1.41	8.19±0.09
6f	4- ⁿ Bu	>40	6.10±1.52	>40	>40	12.51±0.79
6g	2-OMe	4.35±1.33	>40	>40	>40	10.69±1.59
6h	3-OMe	>40	>40	>40	>40	>40
6i	3,4,5-(OMe) ₃	14.50±1.79	15.62±3.14	9.34±2.08	8.49±1.14	10.55±0.21
6j	2-F	23.53±1.03	18.45±2.11	>40	>40	>40
6k	3-F	>40	2.75±0.20	>40	27.74±0.74	24.67±0.46
6l	4-F	>40	13.23±1.32	>40	>40	>40
6m	2-Cl	20.41±0.86	>40	>40	>40	>40
6n	3-Cl	>40	11.56±2.01	>40	>40	>40
6o	4-Cl	>40	25.33±1.26	>40	>40	>40
6p	2-Br	17.12±1.38	26.81±1.32	>40	>40	>40
6q	3-Br	>40	10.20±1.31	>40	>40	>40
6r	4-Br	>40	>40	>40	>40	>40
6s	3-Cl-4-F	26.58±1.65	>40	13.43±1.82	16.47±0.17	30.33±2.36
6t	3-Br-4-F	>40	22.88±3.53	26.02±0.71	31.43±0.17	>40
6u	2,4-Cl ₂	>40	>40	>40	>40	>40
6v	4-CN	6.24±1.44	>40	19.95±1.92	6.51±0.11	>40
5-Fu		7.26±1.21	15.30±1.56	18.42±1.67	20.12±1.68	15.81±1.24

^a Antitumor activity was assayed by exposure for 48 hours; ^b The symbol of ‘—’ means that there was no R group on the desired compounds.

6g with *ortho*-methoxyl group showed the most potent activity against MGC-803 cell lines with an IC₅₀ value of 4.35 μmol·L⁻¹, which exhibited better activity than other compounds substituted at the *ortho*-position of the aromatic amine. Compound **6k** with *meta*-fluoro exhibited the best activity against PC-3 cell lines with an IC₅₀ value of 2.75 μmol·L⁻¹, which showed better activity than other compounds with electron-donating groups and electron-withdrawing groups at the *meta*-position of the aromatic amine. The relationships between the halogen substituents and the antiproliferative activity against PC-3 cell lines were 3-F>3-Br>3-Cl>4-F>2-F. In addition, it

could also be found that the relationships between the electron-donating groups and the antiproliferative activity against PC-3 cell lines were 3-Me>4-ⁿBu>4-Et>4-Me>3-OMe. Multi-substituted compounds exhibited potent activity against EC-109 cell lines, especially, compound **6i** showed potential antitumor activity against five cell lines (MGC-803, PC-3, EC-109, PC-12 and MCF-7) with IC₅₀ values of 14.50, 15.62, 9.34, 8.49 and 10.55 μmol/L. Compound **6v** with *para*-cyano showed the most potent activity against PC-12 cell lines with an IC₅₀ value of 6.51 μmol/L. Compound **6e** with *para*-ethyl showed the most potent activity against MCF-7 cell lines with an IC₅₀ value

of 8.19 $\mu\text{mol/L}$. Furthermore, EC-109 cells were less sensitive to these synthetic compounds than MGC-803 cells, PC-3 cells, PC-12 cells and MCF-7 cells.

3 Conclusions

In conclusion, a series of novel indole-pyrimidine biaryl derivatives were designed and synthesized by molecular hybridization strategy. These chemical structures were characterized by NMR and ESI-MS spectroscopic methods. The evaluation of these compounds against LSD1 was carried out through fluorescence-based assay, and compound **6i** showed the best inhibitory activity against LSD1 with an IC_{50} value of 1.03 $\mu\text{mol}\cdot\text{L}^{-1}$ against LSD1. The anti-tumor activity of these synthetic compounds against MGC-803, PC-3, EC-109, PC-12 and MCF-7 cell lines were evaluated through using MTT assay. All hybrids possessed moderate to good growth inhibition against the tested cancer cells. Particularly, compound **6k** exhibited excellent growth inhibition against PC-3 cells with an IC_{50} value of 2.75 $\mu\text{mol}\cdot\text{L}^{-1}$. The preliminary structure-activity relationship (SAR) illustrated that carbonyl piperazine-aromatic amine scaffold as a reported pharmacophore could play potential synergistic effect for indole-pyrimidine biaryl skeleton. These indole-pyrimidine derivatives in this work might serve as bioactive fragments and hit compounds for developing more potent antitumor agents. The further mechanism investigation of compounds **6i** and **6k** is ongoing in our lab.

4 Experimental section

4.1 Materials

Melting points were determined on an X-5 micromelting apparatus and uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz and 100 MHz spectrometer, respectively. Electrospray ionization mass spectrometry of all derivatives was recorded on a Waters Micromass Q-T of Micromass spectrometer by electrospray ionization (ESI). All commercial materials were used without further purification. Thin layer chromatography (TLC) analysis was carried out on silica gel plates GF254 (Qingdao Haiyang Chemical, China). Silica gel chromatography was carried out on 200~300 mesh gel. Anhydrous solvents and reagents were dried by routine protocols.

Compounds **2a**,^[26] **3a**^[27] and **4a**^[28] were synthesized by reference to literatures.

4.2 Synthesis

4.2.1 Synthesis of 3-(2-chloropyrimidin-4-yl)-1-methyl-1H-indole (**2a**)

To a round bottom flask were added 2,4-dichloropyrimidine (**1a**, 1.49 g, 10 mmol), aluminium chloride (1.37 g, 10 mmol) and 1,2-dimethoxyethane (20 mL). The mixture was stirred at room temperature for 20 min. To this solution was added 1-methyl-1H-indole (1.25 mL, 10 mmol). The resulting solution was stirred at 80 $^{\circ}\text{C}$ for 8 h, then pure water was added to quench the reaction. The

mixture was stirred at room temperature for another 2 h. To this mixture was added EtOH (20 mL), the residue was filtrated and washed with EtOH. The crude product **2a** was obtained in 78% yield and used for the next step without any purification.

4.2.2 Synthesis of *tert*-butyl 4-(4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)piperazine-1-carboxylate (**3a**)

To a round bottom flask were added **2a** (1.22 g, 5 mmol), *tert*-butyl piperazine-1-carboxylate (3.72 g, 20 mmol), Cs_2CO_3 (2.44 g, 7.5 mmol), Pd_2dba_3 (0.46 g, 0.5 mmol), DPPM (0.38 g, 1 mmol) and H_2O (10 mL). The mixture was stirred at 120 $^{\circ}\text{C}$ for 5 h. Upon completion of the reaction (monitored by TLC), the mixture was diluted with 20 mL of ethyl acetate. The organic layer was dried over by Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel chromatography to give the coupling product **3a** in 40% yield.

4.2.3 Synthesis of 1-methyl-3-(2-(piperazin-1-yl)-pyrimidin-4-yl)-1H-indole trifluoroacetate salt (**4a**)

To a round bottom flask were added **3a**, TFA (15 mL) and dichloromethane (DCM) (15 mL). The mixture was stirred at room temperature for 1 h. Upon completion of the reaction (monitored by TLC), the solvent was evaporated. To this mixture was added ether, the residue was filtrated and washed with ether. The crude product **4a** was obtained in 99% yield.

4.2.4 Synthesis of 2-chloro-1-(4-(4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**5a**)

To a round bottom flask were added **4a** (2.93 g, 10 mmol), triethylamine (TEA) (4.16 mL, 30 mmol) and THF (15 mL). To this solution chloroacetyl chloride (0.953 mL, 12 mmol) in THF (5 mL) was added dropwise at 0 $^{\circ}\text{C}$. The mixture was stirred at room temperature for 6 h. Upon completion of the reaction (monitored by TLC), the solvent was evaporated, and the residue was purified by silica gel chromatography to afford the product **5a** in 90% yield.

4.2.5 Synthesis of compounds **6a**~**6v**

To a microwave reaction tube were added **5a** (185 mg, 0.5 mmol), aromatic amines (1 mmol), NaH (36 mg, 1.5 mmol) and *N,N*-dimethylformamide (DMF) (2 mL). After sealing the tube, the mixture was heated to 100 $^{\circ}\text{C}$ in an oil bath and stirred for 8 h. The sealed tube was cooled to room temperature, and the mixture was diluted with 10 mL of EtOAc and then treated with 10 mL of H_2O . The organic layer was dried over by Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel chromatography to give the desired products **6a**~**6v** in 31%~74% yields.

1-(4-(4-(1-Methyl-1H-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)-2-(phenylamino)ethan-1-one (**6a**): Yellow solid, yield 62%. m.p. 126~127 $^{\circ}\text{C}$; ^1H NMR (400 MHz, CDCl_3) δ : 8.42~8.34 (m, 1H), 8.28 (d, $J=5.3$ Hz, 1H), 7.76 (s, 1H), 7.38 (d, $J=7.4$ Hz, 1H), 7.35~7.28 (m, 3H), 7.21 (t, $J=7.9$ Hz, 2H), 6.91 (d, $J=5.3$ Hz, 1H), 6.74 (t, $J=7.3$ Hz, 1H), 6.66 (d, $J=7.8$ Hz, 2H), 4.94 (s, 1H), 4.07~4.01 (m,

2H), 4.01~3.97 (m, 2H), 3.95 (s, 2H), 3.86 (s, 3H), 3.85~3.80 (m, 2H), 3.62~3.56 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ : 167.8, 162.3, 161.7, 157.0, 147.4, 138.0, 131.1, 129.8, 129.3, 129.1, 126.0, 122.7, 121.8, 121.3, 119.9, 119.0, 117.6, 114.2, 113.0, 109.9, 106.3, 45.4, 44.3, 43.9, 43.8, 42.1, 33.3; HRMS (ESI) calcd for $\text{C}_{25}\text{H}_{27}\text{N}_6\text{O}$ $[\text{M} + \text{H}]^+$ 427.2246, found 427.2230.

1-(4-(4-(1-Methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)-2-(*o*-tolylamino)ethan-1-one (**6b**): Yellow solid, yield 53%. m.p. 152~153 °C; ^1H NMR (400 MHz, CDCl_3) δ : 8.42~8.33 (m, 1H), 8.28 (d, $J=5.3$ Hz, 1H), 7.76 (s, 1H), 7.38 (d, $J=7.4$ Hz, 1H), 7.35~7.27 (m, 2H), 7.14 (t, $J=7.7$ Hz, 1H), 7.09 (d, $J=7.1$ Hz, 1H), 6.91 (d, $J=5.3$ Hz, 1H), 6.69 (t, $J=7.3$ Hz, 1H), 6.53 (d, $J=7.9$ Hz, 1H), 4.93 (s, 1H), 4.13~3.95 (m, 6H), 3.90~3.80 (m, 5H), 3.66~3.56 (m, 2H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ : 168.0, 162.3, 161.7, 156.9, 145.4, 138.0, 131.1, 130.2, 127.0, 126.0, 122.7, 122.6, 121.8, 121.3, 117.2, 114.2, 109.9, 109.8, 106.3, 45.4, 44.3, 43.9, 43.8, 42.1, 33.3, 17.4; HRMS (ESI) calcd for $\text{C}_{26}\text{H}_{29}\text{N}_6\text{O}$ $[\text{M} + \text{H}]^+$ 441.2403, found 441.2385.

1-(4-(4-(1-Methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)-2-(*m*-tolylamino)ethan-1-one (**6c**): Yellow solid, yield 57%. m.p. 197~198 °C; ^1H NMR (400 MHz, CDCl_3) δ : 8.46~8.33 (m, 1H), 8.28 (d, $J=5.3$ Hz, 1H), 7.76 (s, 1H), 7.38 (d, $J=7.4$ Hz, 1H), 7.35~7.27 (m, 2H), 7.10 (t, $J=8.0$ Hz, 1H), 6.91 (d, $J=5.3$ Hz, 1H), 6.57 (d, $J=7.5$ Hz, 1H), 6.48 (d, $J=6.7$ Hz, 1H), 4.89 (s, 1H), 4.13~4.01 (m, 2H), 4.01~3.97 (m, 2H), 3.95 (s, 2H), 3.87 (s, 3H), 3.86~3.80 (m, 2H), 3.64~3.55 (m, 2H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ : 167.9, 162.3, 161.8, 157.0, 147.4, 139.1, 138.0, 131.1, 129.2, 126.0, 122.7, 121.8, 121.3, 118.6, 114.2, 113.8, 110.3, 109.8, 106.3, 45.4, 44.3, 43.9, 43.8, 42.0, 33.3, 21.6; HRMS (ESI) calcd for $\text{C}_{26}\text{H}_{29}\text{N}_6\text{O}$ $[\text{M} + \text{H}]^+$ 441.2403, found 441.2387.

1-(4-(4-(1-Methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)-2-(*p*-tolylamino)ethan-1-one (**6d**): Yellow solid, yield 62%. m.p. 191~192 °C; ^1H NMR (400 MHz, CDCl_3) δ : 8.40~8.35 (m, 1H), 8.27 (d, $J=5.3$ Hz, 1H), 7.75 (s, 1H), 7.41~7.35 (m, 1H), 7.35~7.27 (m, 2H), 7.02 (d, $J=8.2$ Hz, 2H), 6.90 (d, $J=5.3$ Hz, 1H), 6.59 (d, $J=8.3$ Hz, 2H), 4.06~4.00 (m, 2H), 4.00~3.96 (m, 2H), 3.94 (s, 2H), 3.86 (s, 3H), 3.84~3.78 (m, 2H), 3.62~3.55 (m, 2H), 2.25 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ : 168.0, 162.3, 161.8, 157.0, 145.2, 138.0, 131.1, 129.8, 126.9, 126.0, 122.7, 121.8, 121.3, 114.2, 113.2, 109.8, 106.3, 45.8, 44.3, 43.9, 43.8, 42.0, 33.3, 20.4; HRMS (ESI) calcd for $\text{C}_{26}\text{H}_{29}\text{N}_6\text{O}$ $[\text{M} + \text{H}]^+$ 441.2403, found 441.2386.

2-((4-Ethylphenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6e**): Yellow solid, yield 63%. m.p. 160~161 °C; ^1H NMR (400 MHz, CDCl_3) δ : 8.43~8.35 (m, 1H), 8.27 (d, $J=5.3$ Hz, 1H), 7.75 (s, 1H), 7.43~7.36 (m, 1H), 7.35~7.26 (m, 2H), 7.05 (d, $J=8.3$ Hz, 2H), 6.90 (d, $J=5.3$ Hz, 1H), 6.61 (d, $J=8.4$ Hz, 2H), 4.80 (s, 1H), 4.06~4.00 (m, 2H), 4.00~3.95 (m, 2H), 3.93 (s, 2H), 3.85 (s, 3H), 3.84~3.78 (m, 2H), 3.62~3.52 (m, 2H), 2.55 (q, $J=7.6$ Hz, 2H), 1.20 (t,

$J=7.6$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ : 168.0, 162.3, 161.8, 157.0, 145.4, 138.0, 133.5, 131.1, 128.6, 126.0, 122.7, 121.8, 121.3, 114.2, 113.2, 109.9, 106.3, 45.8, 44.3, 43.9, 43.8, 42.0, 33.3, 28.0, 16.0; HRMS (ESI) calcd for $\text{C}_{27}\text{H}_{31}\text{N}_6\text{O}$ $[\text{M} + \text{H}]^+$ 455.2559, found 455.2540.

2-((4-Butylphenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6f**): Yellow solid, yield 65%. m.p. 109~110 °C; ^1H NMR (400 MHz, CDCl_3) δ : 8.38 (d, $J=7.1$ Hz, 1H), 8.27 (d, $J=5.3$ Hz, 1H), 7.75 (s, 1H), 7.37 (d, $J=7.3$ Hz, 1H), 7.36~7.26 (m, 2H), 7.02 (d, $J=8.2$ Hz, 2H), 6.90 (d, $J=5.3$ Hz, 1H), 6.60 (d, $J=8.3$ Hz, 2H), 4.79 (s, 1H), 4.13~3.96 (m, 4H), 3.94 (s, 2H), 3.86 (s, 3H), 3.83 (d, $J=5.3$ Hz, 2H), 3.58 (s, 2H), 2.51 (t, $J=7.7$ Hz, 2H), 1.60~1.49 (m, 2H), 1.41~1.28 (m, 2H), 0.91 (t, $J=7.3$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ : 168.0, 162.3, 161.8, 157.0, 145.4, 138.0, 132.2, 131.1, 129.2, 126.0, 122.7, 121.8, 121.3, 114.2, 113.1, 109.8, 106.3, 45.8, 44.3, 43.9, 43.8, 42.0, 34.7, 34.0, 33.3, 22.3, 14.0; HRMS (ESI) calcd for $\text{C}_{29}\text{H}_{35}\text{N}_6\text{O}$ $[\text{M} + \text{H}]^+$ 483.2872, found 483.2853.

2-((2-Methoxyphenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6g**): Yellow solid, yield 31%. m.p. 167~168 °C; ^1H NMR (400 MHz, CDCl_3) δ : 8.40~8.34 (m, 1H), 8.27 (d, $J=5.3$ Hz, 1H), 7.75 (s, 1H), 7.43~7.36 (m, 1H), 7.35~7.26 (m, 2H), 6.94~6.84 (m, 2H), 6.79 (d, $J=7.0$ Hz, 1H), 6.74~6.67 (m, 1H), 6.58~6.50 (m, 1H), 4.04~3.94 (m, 6H), 3.87 (s, 3H), 3.86~3.80 (m, 5H), 3.63~3.55 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ : 168.0, 162.4, 161.7, 156.9, 147.3, 138.0, 137.6, 131.2, 126.0, 122.7, 121.8, 121.3, 121.1, 117.0, 114.2, 110.0, 109.9, 109.6, 106.2, 55.5, 45.4, 44.4, 43.9, 43.8, 42.0, 33.3; HRMS (ESI) calcd for $\text{C}_{26}\text{H}_{29}\text{N}_6\text{O}_2$ $[\text{M} + \text{H}]^+$ 457.2352, found 457.2332.

2-((3-Methoxyphenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6h**): Yellow solid, yield 55%. m.p. 198~199 °C; ^1H NMR (400 MHz, CDCl_3) δ : 8.42~8.35 (m, 1H), 8.28 (d, $J=5.3$ Hz, 1H), 7.76 (s, 1H), 7.38 (d, $J=7.3$ Hz, 1H), 7.35~7.27 (m, 2H), 7.11 (t, $J=8.1$ Hz, 1H), 6.91 (d, $J=5.3$ Hz, 1H), 6.35~6.26 (m, 2H), 6.20 (t, $J=2.2$ Hz, 1H), 4.97 (s, 1H), 4.05~3.96 (m, 4H), 3.94 (s, 2H), 3.86 (s, 3H), 3.85~3.80 (m, 2H), 3.79 (s, 3H), 3.65~3.53 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ : 167.7, 162.3, 160.9, 156.9, 148.7, 138.0, 131.1, 130.1, 126.0, 122.7, 121.8, 121.3, 114.2, 109.9, 106.3, 106.2, 102.7, 99.1, 55.2, 45.3, 44.3, 43.9, 43.8, 42.1, 33.3; HRMS (ESI) calcd for $\text{C}_{26}\text{H}_{29}\text{N}_6\text{O}_2$ $[\text{M} + \text{H}]^+$ 457.2352, found 457.2336.

1-(4-(4-(1-Methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)-2-((3,4,5-trimethoxyphenyl)amino)ethan-1-one (**6i**): Yellow solid, yield 63%. m.p. 175~176 °C; ^1H NMR (400 MHz, CDCl_3) δ : 8.38 (d, $J=7.1$ Hz, 1H), 8.27 (d, $J=5.3$ Hz, 1H), 7.74 (s, 1H), 7.37 (d, $J=7.6$ Hz, 1H), 7.35~7.26 (m, 3H), 6.90 (d, $J=5.3$ Hz, 1H), 5.88 (s, 2H), 4.85 (s, 1H), 4.07~4.01 (m, 2H), 4.01~3.95 (m, 2H), 3.92 (s, 2H), 3.87~3.80 (m, 12H), 3.77 (s, 3H), 3.63~3.55 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ : 167.7, 162.3, 161.7, 157.0, 154.1, 144.3, 138.0, 131.1, 130.5, 126.0, 122.7, 121.8,

121.3, 114.1, 109.9, 106.3, 90.9, 61.1, 56.1, 45.7, 44.3, 43.9, 43.8, 42.1, 33.3; HRMS (ESI) calcd for $C_{28}H_{33}N_6O_4$ $[M+H]^+$ 517.2563, found 517.2547.

2-((2-Fluorophenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6j**): Yellow solid, yield 58%. m.p. 191~192 °C; 1H NMR (400 MHz, $CDCl_3$) δ : 8.38 (d, $J=7.1$ Hz, 1H), 8.28 (d, $J=5.3$ Hz, 1H), 7.76 (s, 1H), 7.38 (d, $J=7.5$ Hz, 1H), 7.35~7.26 (m, 2H), 7.07~6.96 (m, 2H), 6.91 (d, $J=5.3$ Hz, 1H), 6.70~6.60 (m, 2H), 5.15 (s, 1H), 4.06~3.94 (m, 6H), 3.86 (s, 3H), 3.85~3.80 (m, 2H), 3.59 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 167.4, 162.3, 161.8, 157.0, 151.8 (d, $J=239$ Hz), 138.0, 136.0 (d, $J=12$ Hz), 131.1, 126.0, 124.5 (d, $J=4$ Hz), 122.7, 121.8, 121.3, 117.1 (d, $J=7$ Hz), 114.7 (d, $J=19$ Hz), 114.2, 112.4 (d, $J=4$ Hz), 109.9, 106.3, 45.2, 44.3, 43.9, 43.8, 42.1, 33.3; HRMS (ESI) calcd for $C_{25}H_{26}FN_6O$ $[M+H]^+$ 445.2152, found 445.2134.

2-((3-Fluorophenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6k**): Yellow solid, yield 62%. m.p. 199~200 °C; 1H NMR (400 MHz, $CDCl_3$) δ : 8.41~8.34 (m, 1H), 8.28 (d, $J=5.3$ Hz, 1H), 7.75 (s, 1H), 7.42~7.36 (m, 1H), 7.35~7.26 (m, 2H), 7.16~7.08 (m, 1H), 6.91 (d, $J=5.3$ Hz, 1H), 6.46~6.38 (m, 2H), 6.35~6.28 (m, 1H), 5.11 (s, 1H), 4.09~4.01 (m, 2H), 4.00~3.95 (m, 2H), 3.91 (d, $J=4.0$ Hz, 2H), 3.85 (s, 3H), 3.84~3.79 (m, 2H), 3.60~3.54 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 167.3, 164.1 (d, $J=241$ Hz), 162.3, 161.7, 157.0, 149.0 (d, $J=11$ Hz), 138.0, 131.1, 130.4 (d, $J=10$ Hz), 126.0, 122.7, 121.8, 121.3, 114.2, 109.9, 109.0 (d, $J=2$ Hz), 106.3, 103.9 (d, $J=21$ Hz), 99.6 (d, $J=25$ Hz), 45.1, 44.3, 43.8, 43.7, 42.1, 33.3; HRMS (ESI) calcd for $C_{25}H_{25}FN_6O$ $[M+H]^+$ 445.2152, found 445.2132.

2-((4-Fluorophenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6l**): Yellow solid, yield 65%. m.p. 189~190 °C; 1H NMR (400 MHz, $CDCl_3$) δ : 8.38 (d, $J=7.1$ Hz, 1H), 8.27 (d, $J=5.3$ Hz, 1H), 7.75 (s, 1H), 7.38 (d, $J=7.5$ Hz, 1H), 7.35~7.26 (m, 2H), 6.96~6.87 (m, 3H), 6.63~6.54 (m, 2H), 4.83 (s, 1H), 4.05~4.00 (m, 2H), 4.00~3.96 (m, 2H), 3.91 (s, 2H), 3.86 (s, 3H), 3.85~3.79 (m, 2H), 3.61~3.54 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 167.7, 162.3, 161.8, 157.0, 156.0 (d, $J=234$ Hz), 143.9 (d, $J=2$ Hz), 138.0, 131.1, 126.0, 122.7, 121.8, 121.3, 115.7 (d, $J=22$ Hz), 114.2, 113.8 (d, $J=7$ Hz), 109.9, 106.3, 46.0, 44.3, 43.8, 43.8, 42.1, 33.3; HRMS (ESI) calcd for $C_{25}H_{26}FN_6O$ $[M+H]^+$ 445.2152, found 445.2134.

2-((2-Chlorophenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6m**): Yellow solid, yield 61%. m.p. 177~178 °C; 1H NMR (400 MHz, $CDCl_3$) δ : 8.37 (d, $J=7.1$ Hz, 1H), 8.27 (d, $J=5.2$ Hz, 1H), 7.73 (s, 1H), 7.36 (d, $J=7.7$ Hz, 1H), 7.34~7.26 (m, 3H), 7.15 (t, $J=7.7$ Hz, 1H), 6.89 (d, $J=5.2$ Hz, 1H), 6.66 (t, $J=7.4$ Hz, 1H), 6.55 (d, $J=8.0$ Hz, 1H), 5.62 (s, 1H), 4.08~3.90 (m, 6H), 3.88~3.77 (m, 5H), 3.56 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 167.2, 162.3, 161.8, 157.0, 143.3, 138.0, 131.1, 129.3, 127.7, 126.0, 122.7, 121.8, 121.3, 119.7, 117.5, 114.2, 111.3, 109.9, 106.3, 45.1, 44.3,

43.8, 43.7, 42.1, 33.3; HRMS (ESI) calcd for $C_{25}H_{26}ClN_6O$ $[M+H]^+$ 461.1857, found 461.1838.

2-((3-Chlorophenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6n**): Yellow solid, yield 58%. m.p. 237~238 °C; 1H NMR (400 MHz, $CDCl_3$) δ : 8.43~8.35 (m, 1H), 8.28 (d, $J=5.3$ Hz, 1H), 7.76 (s, 1H), 7.38 (d, $J=7.3$ Hz, 1H), 7.35~7.26 (m, 2H), 7.10 (t, $J=8.0$ Hz, 1H), 6.91 (d, $J=5.3$ Hz, 1H), 6.69 (dd, $J=7.8$, 1.1 Hz, 1H), 6.60 (t, $J=1.9$ Hz, 1H), 6.54 (dd, $J=8.1$, 1.7 Hz, 1H), 5.08 (s, 1H), 4.08~4.01 (m, 2H), 4.01~3.96 (m, 2H), 3.91 (d, $J=3.8$ Hz, 2H), 3.86 (s, 3H), 3.85~3.80 (m, 2H), 3.62~3.54 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 167.2, 162.3, 161.7, 157.0, 148.4, 138.0, 135.1, 131.1, 130.2, 126.0, 122.7, 121.8, 121.3, 117.4, 114.2, 112.3, 111.7, 109.9, 106.3, 45.0, 44.3, 43.8, 43.7, 42.1, 33.3; HRMS (ESI) calcd for $C_{25}H_{26}ClN_6O$ $[M+H]^+$ 461.1857, found 461.1837.

2-((4-Chlorophenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6o**): Yellow solid, yield 63%. m.p. 200~201 °C; 1H NMR (400 MHz, $CDCl_3$) δ : 8.40~8.34 (m, 1H), 8.27 (d, $J=5.3$ Hz, 1H), 7.75 (s, 1H), 7.38 (d, $J=7.4$ Hz, 1H), 7.35~7.26 (m, 2H), 7.14 (d, $J=8.8$ Hz, 2H), 6.91 (d, $J=5.3$ Hz, 1H), 6.57 (d, $J=8.8$ Hz, 2H), 4.98 (s, 1H), 4.07~4.00 (m, 2H), 4.00~3.94 (m, 2H), 3.90 (d, $J=3.0$ Hz, 2H), 3.86 (s, 3H), 3.85~3.78 (m, 2H), 3.62~3.53 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 167.4, 162.3, 161.7, 157.0, 145.9, 138.0, 131.1, 129.1, 126.0, 122.7, 122.2, 121.8, 121.3, 114.2, 114.0, 109.9, 106.3, 45.3, 44.3, 43.8, 43.7, 42.1, 33.3; HRMS (ESI) calcd for $C_{25}H_{26}ClN_6O$ $[M+H]^+$ 461.1857, found 461.1839.

2-((2-Bromophenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6p**): Yellow solid, yield 65%. m.p. 112~113 °C; 1H NMR (400 MHz, $CDCl_3$) δ : 8.39~8.35 (m, 1H), 8.26 (d, $J=5.3$ Hz, 1H), 7.73 (s, 1H), 7.45 (dd, $J=7.8$, 1.4 Hz, 1H), 7.39~7.32 (m, 1H), 7.32~7.26 (m, 2H), 7.22~7.15 (m, 1H), 6.89 (d, $J=5.3$ Hz, 1H), 6.63~6.56 (m, 1H), 6.52 (dd, $J=8.1$, 1.1 Hz, 1H), 5.66 (t, $J=3.6$ Hz, 1H), 4.06~3.94 (m, 4H), 3.92 (d, $J=3.9$ Hz, 2H), 3.86~3.77 (m, 5H), 3.58~3.50 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 167.1, 162.3, 161.7, 157.0, 144.2, 138.0, 132.6, 131.2, 128.4, 126.0, 122.7, 121.8, 121.3, 118.0, 114.1, 111.4, 110.0, 109.9, 106.3, 45.2, 44.3, 43.8, 43.7, 42.1, 33.3; HRMS (ESI) calcd for $C_{25}H_{26}BrN_6O$ $[M+H]^+$ 505.1351, found 505.1332.

2-((3-Bromophenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6q**): Yellow solid, yield 68%. m.p. 235~236 °C; 1H NMR (400 MHz, $CDCl_3$) δ : 8.41~8.32 (m, 1H), 8.28 (d, $J=5.3$ Hz, 1H), 7.76 (s, 1H), 7.38 (d, $J=7.4$ Hz, 1H), 7.35~7.26 (m, 2H), 7.04 (t, $J=8.0$ Hz, 1H), 6.91 (d, $J=5.3$ Hz, 1H), 6.84 (d, $J=7.8$ Hz, 1H), 6.76 (d, $J=1.9$ Hz, 1H), 6.59 (dd, $J=8.2$, 1.6 Hz, 1H), 5.08 (s, 1H), 4.12~4.01 (m, 2H), 4.01~3.96 (m, 2H), 3.91 (d, $J=3.8$ Hz, 2H), 3.87 (s, 3H), 3.86~3.80 (m, 2H), 3.63~3.54 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 167.2, 162.3, 161.7, 157.0, 148.5, 138.0, 131.1, 130.5, 126.0, 123.3, 122.7, 121.8, 121.4, 120.3, 115.1,

114.2, 112.1, 109.9, 106.3, 45.0, 44.3, 43.8, 43.7, 42.1, 33.4; HRMS (ESI) calcd for $C_{25}H_{26}BrN_6O$ $[M+H]^+$ 505.1351, found 505.1333.

2-((4-Bromophenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6r**): Yellow solid, yield 67%. m.p. 182~183 °C; 1H NMR (400 MHz, $CDCl_3$) δ : 8.38 (d, $J=7.3$ Hz, 1H), 8.28 (d, $J=5.3$ Hz, 1H), 7.77 (s, 1H), 7.39 (d, $J=7.5$ Hz, 1H), 7.36~7.26 (m, 4H), 6.92 (d, $J=5.3$ Hz, 1H), 6.53 (d, $J=8.5$ Hz, 2H), 5.00 (s, 1H), 4.18~4.02 (m, 2H), 4.02~3.97 (m, 2H), 3.92 (s, 2H), 3.88 (s, 3H), 3.86~3.80 (m, 2H), 3.65~3.54 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 162.4, 138.0, 131.2, 126.0, 122.7, 121.8, 121.4, 114.2, 109.9, 106.4, 44.3, 43.9, 43.8, 42.1, 33.4; HRMS (ESI) calcd for $C_{25}H_{26}BrN_6O$ $[M+H]^+$ 505.1351, found 505.1332.

2-((3-Chloro-4-fluorophenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6s**): Yellow solid, yield 72%. m.p. 267~268 °C; 1H NMR (400 MHz, $CDCl_3$) δ : 8.38 (d, $J=8.6$ Hz, 1H), 8.28 (d, $J=5.5$ Hz, 1H), 7.77 (s, 1H), 7.39 (d, $J=8.0$ Hz, 1H), 7.36~7.28 (m, 2H), 6.97 (t, $J=9.0$ Hz, 1H), 6.92 (d, $J=5.5$ Hz, 1H), 6.65~6.59 (m, 1H), 6.54~6.46 (m, 1H), 4.08~4.02 (m, 2H), 4.02~3.97 (m, 2H), 3.91 (s, 2H), 3.88 (s, 3H), 3.86~3.80 (m, 2H), 3.64~3.56 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 167.3, 155.0 (d, $J=235$ Hz), 138.0, 131.7, 131.2, 126.0, 122.7, 122.2, 121.8, 121.8 (d, $J=8$ Hz), 121.4, 121.1 (d, $J=18$ Hz), 117.0 (d, $J=22$ Hz), 114.2, 113.6, 113.3, 112.5 (d, $J=7$ Hz), 109.9, 106.4, 45.6, 44.3, 43.9, 43.8, 42.1, 33.4; HRMS (ESI) calcd for $C_{25}H_{25}ClFN_6O$ $[M+H]^+$ 479.1762, found 479.1742.

2-((3-Bromo-4-fluorophenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6t**): Yellow solid, yield 65%. m.p. 271~272 °C; 1H NMR (400 MHz, $DMSO-d_6$) δ : 8.43 (d, $J=7.9$ Hz, 1H), 8.31 (s, 1H), 8.27 (d, $J=5.3$ Hz, 1H), 7.54 (d, $J=7.8$ Hz, 1H), 7.31~7.20 (m, 2H), 7.13~7.05 (m, 2H), 6.98 (dd, $J=5.8$, 2.8 Hz, 1H), 6.79~6.71 (m, 1H), 5.88 (t, $J=4.8$ Hz, 1H), 4.00 (d, $J=4.8$ Hz, 2H), 3.97~3.91 (m, 2H), 3.90~3.80 (m, 5H), 3.71~3.60 (m, 4H); ^{13}C NMR (150 MHz, $DMSO-d_6$) δ : 168.2, 162.6, 161.8, 150.8 (d, $J=231$ Hz), 146.5 (d, $J=2$ Hz), 138.1, 133.5, 126.0, 122.7, 122.2, 121.5, 121.5, 117.0 (d, $J=23$ Hz), 115.8, 113.6 (d, $J=6$ Hz), 113.1, 111.0, 108.4 (d, $J=23$ Hz), 106.0, 45.4, 44.2, 44.1, 43.9, 41.8, 33.5; HRMS (ESI) calcd for $C_{25}H_{25}BrFN_6O$ $[M+H]^+$ 523.1257, found 523.1238.

2-((2,4-Dichlorophenyl)amino)-1-(4-(4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (**6u**): Yellow solid, yield 71%. m.p. 212~213 °C; 1H NMR (400 MHz, $CDCl_3$) δ : 8.37 (d, $J=7.1$ Hz, 1H), 8.28 (d, $J=5.3$ Hz, 1H), 7.77 (s, 1H), 7.39 (d, $J=7.6$ Hz, 1H), 7.36~7.27 (m, 3H), 7.12 (dd, $J=8.7$, 2.4 Hz, 1H), 6.92 (d, $J=5.3$ Hz, 1H), 6.50 (d, $J=8.7$ Hz, 1H), 5.64 (s, 1H), 4.08~3.99 (m, 4H), 3.98 (d, $J=4.1$ Hz, 2H), 3.88 (s, 3H), 3.87~3.82 (m, 2H), 3.64~3.57 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 166.8, 162.3, 161.7, 157.0, 142.0, 138.0, 131.1, 129.0, 127.6, 126.0, 122.7, 121.8, 121.5, 121.3, 120.0, 114.2, 111.8, 109.9, 106.4, 45.0, 44.3, 43.8, 43.7, 42.1, 33.3;

HRMS (ESI) calcd for $C_{25}H_{25}Cl_2N_6O$ $[M+H]^+$ 495.1467, found 495.1447.

4-((2-(4-(4-(1-Methyl-1*H*-indol-3-yl)pyrimidin-2-yl)piperazin-1-yl)-2-oxoethyl)amino)benzonitrile (**6v**): Yellow solid, yield 74%. m.p. 114~115 °C; 1H NMR (400 MHz, $CDCl_3$) δ : 8.37 (t, $J=6.9$ Hz, 1H), 8.31~8.25 (m, 1H), 7.76 (s, 1H), 7.46 (d, $J=8.6$ Hz, 1H), 7.41~7.36 (m, 1H), 7.36~7.27 (m, 2H), 6.91 (t, $J=4.9$ Hz, 1H), 6.61 (d, $J=8.7$ Hz, 1H), 5.57 (s, 1H), 4.14 (s, 1H), 4.07~3.97 (m, 4H), 3.95 (d, $J=4.0$ Hz, 1H), 3.87 (d, $J=1.6$ Hz, 3H), 3.86~3.81 (m, 1H), 3.80~3.73 (m, 1H), 3.70~3.62 (m, 1H), 3.62~3.54 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 166.5, 165.4, 162.4, 161.7, 157.0, 150.1, 138.0, 133.8, 131.1, 126.0, 122.7, 122.7, 121.8, 121.8, 121.3, 120.3, 114.2, 114.1, 112.5, 109.9, 109.8, 106.4, 106.3, 99.2, 46.3, 44.2, 44.0, 43.8, 43.7, 43.6, 42.2, 41.0, 33.4; HRMS (ESI) calcd for $C_{26}H_{26}N_7O$ $[M+H]^+$ 452.2199, found 452.2181.

4.3 LSD1 enzymatic assay

Full length LSD1 cDNA encoding LSD1 was obtained by RT-PCR and cloned into pET-28b (pET-28b-LSD1). Then the plasmid pET28b-LSD1 was transfected into BL21 (DE). The recombinant protein was induced with 0.25 mmol/L IPTG at 20 °C and purified following affinity chromatography, ion exchange chromatography and gel filtration. Then the compounds were incubated with 5 nmol/L recombinant LSD1 and 25 mmol/L H3K4me2 peptide in the presence of flavin adenine dinucleotide (FAD) (50 nmol/L), Amplex Red (20 nmol/L) and horseradish peroxidase (5.5 U/mL) for 30 min. After that, the fluorescence was measured at excitation wavelength of 530 nm and emission wavelength of 590 nm as reported in order to evaluate the inhibition rate of the candidate compounds.

4.4 In vitro antitumor activities

4.4.1 Cell culture and cell viability assay

EC-109 cells (Human esophageal cancer cells), PC-3 cells (human prostate cancer) and MGC-803 cells (human gastric cancer) were cultured at 37 °C in an atmosphere containing CO_2 ($\phi=5\%$). RPMI-1640 medium with 10% fetal bovine serum, 100 U/mL penicillin and 0.1 mg/mL streptomycin was used as culture medium. In cell viability assay, cells were seeded at a density of 5×10^3 per well in 96-well plates and treated with compounds for 48 h after 24 h incubation. Then, 20 μ L of MTT solution each well was added, and incubated for 4 h at 37 °C. 100 μ L of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan after removing the supernatant liquid, the absorbance was determined at 570 nm.

Supporting Information The experimental procedure, 1H NMR ^{13}C NMR and ESI-MS of compounds **6a**~**6v**. The Supporting Information is available free of charge via the Internet at <http://sioc-journal.cn/>.

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