Full Paper

Synthesis and Biological Evaluation of Novel (Thio)Urea Derivatives as Potential Antitumor Agents

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A series of novel (thio)ureas containing the pyrimidinyl group was designed and synthesized. Their *in-vitro* antitumor activity against different human tumor cells was examined. Some of the compounds showed potential antitumor activity, which provided some hints for further studies on structure modification.

Keywords: Antitumor activity / Pyrimidinyl group / Thiourea / Urea

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Introduction

The urea derivatives such as ureas [1, 2], benzoylureas [3, 4], thioureas [5–7], and diarylsulfonylureas [8, 9] represent one of the generally most useful classes of anticancer agents (Fig. 1), with a wide range of activities against various leukemia and solid tumors. For these reasons, the synthesis of ureas and their functionalized derivatives is a primary objective.

In recent years, many reports have been published on topics about the synthetic and mechanistic aspects of potential anticancer drugs of the urea derivatives. For example, Dai [10] reported a series of thienopyrimidine urea derivatives for receptor tyrosine kinase inhibitors based on a thienopyrimidine scaffold. The antitumor activity studies focused on optimizing activity against KDR (kinase insert domain-containing receptor tyrosine kinase), as this kinase plays an important role in tumor angiogenesis.

The pyrimidinyl group is a highly efficient pharmacophore and is widely used in pesticide [11] and drug [12] molecular design. We wanted to investigate whether there would be some new beneficial properties if the pyrimidinyl group was introduced in the urea derivatives. Herein, a series of novel (thio)ureas containing the pyrimidinyl group were designed and synthesized. The preliminary *in-vitro* cytotoxic activity of the new compounds was evaluated.

Results and discussion

Chemistry

Compound **3** could be synthesized starting from 4-aminophenol **1** and 4,6-dimethoxy-2-(methylsulfonyl)pyrimidine or 4,6-dichloro-2-(methylsulfonyl) pyrimidine **2** in the presence of K_2CO_3 in DMF. Treatment of equimolar amounts of substituted isocyanates or isothiocyanates with compound **3** in dry CH_2Cl_2 afforded the corresponding (thio)urea derivatives (**4a–4l**) (Scheme 1). The preparations are summarized in Table 1. The new compounds were fully characterized by spectroscopic means and their purity established by elemental analysis.

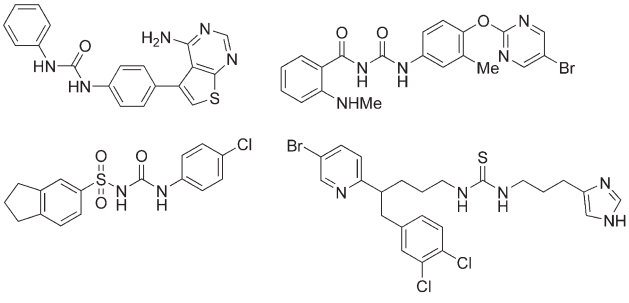

Figure 1. Structures of the uera derivatives.

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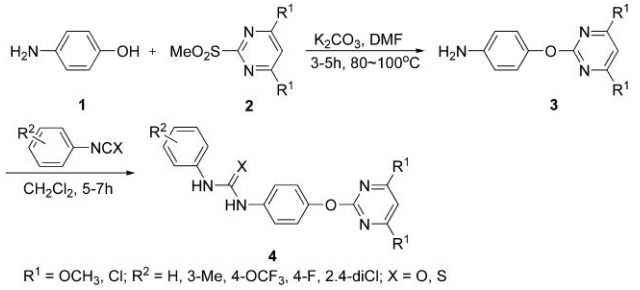


Scheme 1. General synthetic route for compounds **4a–l**.

Table 1. The preparation of 1-(4-(4,6-di-substituted pyrimidin-2-yloxy)phenyl)-3-arylurea derivatives **4a–l**.

Compound	R^1	X	R^2	Yield (%)[a]	Mp (°C)
4a	OMe	O	H	76	211.0∼212.3
4b	OMe	O	3-Me	84	193.6∼194.5
4c	OMe	O	4-OCF_3	84	248.8∼249.7
4d	OMe	O	4-F	88	232.2∼233.1
4e	Cl	O	H	82	240.1∼241.5
4f	Cl	O	3-Me	85	226.5∼227.8
4g	Cl	O	4-OCF_3	67	251.8∼252.6
4h	Cl	O	4-F	86	255.5∼257.6
4i	OMe	S	H	78	65.3∼66.7
4j	OMe	S	2,4-diCl	88	79.1∼81.3
4k	Cl	S	H	80	156.8∼158.4
4l	Cl	S	2,4-diCl	82	179.4∼181.7

[a] Isolated yields

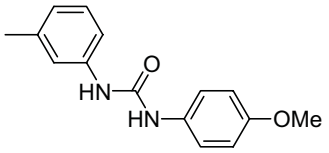
Cytotoxic activity

In-vitro cytotoxicity of the synthesized compounds was screened on different human tumor cell lines using MTT assay. 5-Fluorouracil was used as the reference drug. The results of the cytotoxicity studies are shown in Table 2.

The results indicated that the pyrimidinyl group introduced into the (thio)urea structure was useful for the improvement of antitumor activity. For the compound without pyrimidinyl group displayed very weak antitumor activity against the tumor cells. As could be seen from Table 2, most of the title compounds exhibited moderate cytotoxic activity against the tested tumor cells. Generally, most of the title compounds tended to be more active against KB than against other tested tumor cells. The thiourea derivatives displayed higher activity than urea derivatives.

The compound **4k** displayed more potent cytotoxic activities against KB, MGC-803 and MCF-7 in comparison with 5-FU. The compound **4l** displayed similar potent cytotoxic activities against CNE2 in comparison with 5-FU. The compound **4f** displayed similar potent cytotoxic activities against KB and MCF-7 in comparison with 5-FU. The compounds **4k** showed the best inhibitory activity against KB MGC-803 and MCF-7 with IC_{50} 9.36, 8.80 and 13.0 μM, respectively. The compound **4l** showed the best inhibitory activity against CNE2 with IC_{50} 6.63 μM. We noticed that the structure of the substituent R^1 had great influence on the

Table 2. Cytotoxic activity of urea compounds against human tumor cells.

Compound	*In-vitro* cytotoxicity IC_{50} (μM)[a]			
	KB[a]	CNE2[a]	MGC-803[a]	MCF-7[a]
4a	35.7	>50	34.4	41.7
4b	>50	>50	>50	>50
4c	>50	>50	>50	>50
4d	18.9	–[b]	23.6	43.9
4e	13.5	14.8	>50	>50
4f	12.2	13.3	17.4	14.8
4g	14.8	15.7	15.2	15.3
4h	20.9	27.9	>50	>50
4i	>50	>50	>50	>50
4j	24.7	26.6	>50	>50
4k	9.36	47.4	8.80	13.0
4l	14.4	6.63	24.0	46.5
	>50	>50	–	>50
5-Fluorouracil	12.9	13.1	12.6	15.2

[a] KB cells were the drug sensitive human oral carcinoma cells, CNE2 cells were the drug sensitive human nasopharyngeal carcinoma cells, MGC-803 cells were the drug sensitive human gastric carcinoma cells and MCF-7 cells were the drug sensitive human breast adenocarcinoma cells. [b] –, not tested.

antitumor activity. For some compounds with OMe group (**4b**, **4c** and **4i**) displayed very weak antitumor activity against the tumor cells, whereas some compounds with Cl group (**4f**, **4g** and **4k**) displayed potential antitumor activity against the tumor cells. Still, the change of R^2 group also had a little influence on their activity.

Conclusion

In conclusion, a series of novel (thio)urea derivatives was designed and synthesized. The *in-vitro* preliminary bioassay data showed that some of the synthesized compounds have potential antitumor activities. The compound **4k** was found to have more potent cytotoxic activities against some human tumor cells than 5-FU. Further investigations on structural optimization and biological activities about these derivatives are still underway in our laboratory.

Experimental

General

^{1}H-NMR spectra were recorded at 600 MHz, in $CDCl_3$ or DMSO-d_6 solution on a Varian Mercury-Plus 600 spectrometer and chemical shifts were recorded in parts per million (ppm) with TMS as the internal reference. Melting points were taken on a Buechi B-545 melting point apparatus. Element analysis (EA) was measured on a Vario ELIII CHNSO elemental analyzer. All commercially available solvents and reagents were used as supplied by Acros Organics unless otherwise stated.

Chemistry

*General procedure for the synthesis of compounds **3a,3b***

A solution of 4,6-dimethoxy-2-(methylsulfonyl)pyrimidine or 4,6-dichloro-2-(methylsulfonyl) (18 mmol), 4-aminophenol (18 mmol) and K_2CO_3 (36 mmol) in DMF (50 mL) was stirred at 100°C for 2 h. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with water and saturated brine, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel (PE/EA, 2:1) to give the compound **3a**,**3b**.

*4-(4,6-Dimethoxypyrimidin-2-yloxy)benzenamine **3a***

Yellow solid; yield 85%; mp 84.6∼85.4°C; ^{1}H-NMR ($CDCl_3$, 600 MHz): 3.64 (s, 2H, NH_2), 3.84 (s, 6H, OMe), 5.75 (s, H, CH), 6.68 (d, 2H, J = 12 Hz, ArH), 7.00 (d, 2H, J = 12 Hz, ArH). MS (EI): m/z 247 (M^+, 100), 216 (36), 108 (34), 80 (31).

*4-(4,6-Dichloropyrimidin-2-yloxy)benzenamine **3b***

White solid; yield 45%; mp 258.9∼260.2°C; ^{1}H-NMR ($CDCl_3$, 600 MHz): 3.68 (s, 2H, NH_2), 6.72 (d, 2H, J = 12 Hz, ArH), 6.98 (d, 2H, J = 12 Hz, ArH), 7.10 (s, H, CH). MS (EI): m/z 259 (M+4, 3), 257 (M+2, 10), 255 (M^+, 28), 220 (100), 157 (19), 108 (24), 92 (30), 65 (41).

*General procedure for the synthesis of compounds **4a–4l***

Compound **3** (1 mmol) was dissolved in 15 mL dichloromethane. Temperature was maintained at 0°C, then substituted phenyl isocyanate (1 mmol) was added dropwise with constant stirring. After 1 h, formation of white solid occurred and the resultant solid product was filtered. Recrystallization with ethanol afforded the desired solid urea.

*1-(4-(4,6-Dimethoxypyrimidin-2-yloxy)phenyl)-3-phenylurea **4a***

White solid; ^{1}H-NMR (DMSO-d_6, 600 MHz): 3.78 (s, 6H, OCH_3), 5.98 (s, 1H, CH), 6.97 (m, 1H, ArH), 7.14 (d, 2H, J = 7.8 Hz, ArH), 7.27∼7.30 (m, 2H, ArH), 7.45∼7.49 (m, 4H, ArH), 8.67 (s, 1H, NH), 8.72 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, 125 MHz): 54.2, 83.7, 118.2, 118.9, 121.9, 122.1, 128.8, 136.8, 139.7, 146.9, 152.6, 163.0, 172.7; Anal. calcd. for $C_{19}H_{18}N_4O_4$ (366.4): C, 62.29; H, 4.95; N, 15.29. Found: C, 62.01; H, 4.75; N, 15.26.

*1-(4-(4,6-Dimethoxypyrimidin-2-yloxy)phenyl)-3-m-tolylurea **4b***

White solid; ^{1}H-NMR (DMSO-d_6, 600 MHz): 2.27 (s, 3H, CH_3), 3.78 (s, 6H, OCH_3), 5.98 (s, 1H, CH), 6.79 (d, 1H, J = 7.2 Hz, ArH), 7.13∼7.29 (m, 5H, ArH), 7.47 (d, 2H, J = 9.0 Hz, ArH), 8.60 (s, 1H, NH), 8.71 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, 125 MHz): 20.4, 54.2, 83.8, 115.4, 118.7, 118.9, 121.9, 122.0, 128.6, 136.8, 138.0, 139.6, 146.9, 152.5, 164.0, 172.7; Anal. calcd. for $C_{20}H_{20}N_4O_4$ (380.4): C, 63.15; H, 5.30; N, 14.73. Found: C, 63.38; H, 5.02; N, 14.35.

*1-(4-(4,6-Dimethoxypyrimidin-2-yloxy)phenyl)-3-(4-(trifluoromethoxy)phenyl)urea **4c***

White solid; ^{1}H-NMR (DMSO-d_6, 600 MHz): 3.75 (s, 6H, OCH_3), 5.95 (s, H, CH), 7.12 (d, 2H, J = 8.4 Hz, ArH), 7.26 (d, 2H, J = 8.4 Hz, ArH), 7.46 (d, 2H, J = 9.6 Hz, ArH), 7.53 (d, 2H, J = 8.4 Hz, ArH), 8.75 (s, 1H, NH), 8.86 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, 125 MHz): 54.2, 83.8, 119.1, 119.4, 121.8, 121.9, 136.6, 139.1, 142.6, 147.1, 152.6, 164.0, 172.7; Anal. calcd. for $C_{20}H_{17}F_3N_4O_5$ (450.4): C, 53.34; H, 3.80; N, 12.44. Found: C, 52.91; H, 3.46; N, 12.02.

*1-(4-(4,6-Dimethoxypyrimidin-2-yloxy)phenyl)-3-(4-fluorophenyl)urea **4d***

White solid; ^{1}H-NMR (DMSO-d_6, 600 MHz): 3.78 (s, 6H, OCH_3), 5.98 (s, 1H, CH), 7.12∼7.14 (m, 3H, ArH), 7.46∼7.48 (m, 4H, ArH), 8.71 (s, 1H, NH), 8.72 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, 125 MHz): 54.2, 83.8, 115.4, 119.0, 119.9, 121.9, 136.0, 136.8, 146.9, 152.6, 158.6, 164.0, 172.7; Anal. calcd. for $C_{19}H_{17}FN_4O_4$ (384.4): C, 59.37; H, 4.46; N, 14.58. Found: C, 59.25; H, 4.30; N, 14.73.

*1-(4-(4,6-Dichloropyrimidin-2-yloxy)phenyl)-3-phenylurea **4e***

White solid; ^{1}H-NMR (DMSO-d_6, 600 MHz): 6.95∼6.97 (m, 1H, ArH), 7.16∼7.28 (m, 4H, ArH), 7.44 (d, 2H, J = 7.8 Hz, ArH), 7.50 (d, 2H, J = 9.0 Hz, ArH), 7.74 (s, 1H, CH), 8.70 (s, 1H, NH), 8.76 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, 125 MHz): 115.9, 118.2, 119.3, 119.4, 121.8, 128.8, 137.6, 139.7, 146.4, 152.6, 162.6, 163.8; Anal. calcd. for $C_{17}H_{12}Cl_2N_4O_2$ (375.2): C 54.42, H 3.22, N 14.93. Found: C 54.42, H 3.26, N 14.68.

*1-(4-(4,6-Dichloropyrimidin-2-yloxy)phenyl)-3-m-tolylurea **4f***

White solid; ^{1}H-NMR (DMSO-d_6, 600 MHz): 2.28 (s, 3H, Me), 6.79 (d, 2H, J = 7.2 Hz, ArH), 7.14∼7.30 (m, 4H, ArH), 7.51 (d, 2H,

J = 9.0 Hz, ArH), 7.76 (s,1H, CH), 8.63 (s, 1H, NH), 8.76 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, 125 MHz): 21.2, 115.4, 116.0, 118.7, 119.3, 121.7, 122.6, 128.7, 137.6, 138.0, 139.5, 146.3, 152.5, 162.5, 163.8; Anal. calcd. for $C_{18}H_{14}Cl_2N_4O_2$ (389.2): C 55.54, H 3.63, N 14.39. Found: C 55.18, H 3.31 N 14.12.

1-(4-(4,6-Dichloropyrimidin-2-yloxy)phenyl)-3-(4-(trifluoromethoxy)phenyl)urea ***4g***

White solid; ^{1}H-NMR (DMSO-d_6, 600 MHz): 7.17 (d, 2H, J = 8.4 Hz, ArH), 7.27 (d, 2H, J = 8.4 Hz, ArH), 7.49 (d, 2H, J = 8.4 Hz, ArH), 7.54 (d, 2H, J = 8.4 Hz, ArH), 7.73 (s,1H, CH), 8.81 (s, 1H, NH), 8.90 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, 125 MHz): 116.0, 119.4, 119.5, 120.6, 121.1, 121.8, 137.4, 139.0, 142.6, 146.5, 152.6, 162.6, 163.8; Anal. calcd. for $C_{18}H_{11}Cl_2F_3N_4O_3$ (459.2): C 47.08, H 2.41, N 12.20. Found: C 47.09, H 2.33, N 12.43.

1-(4-(4,6-Dichloropyrimidin-2-yloxy)phenyl)-3-(4-fluorophenyl)urea ***4h***

White solid; ^{1}H-NMR (DMSO-d_6, 600 MHz): 7.10 (d, 2H, J = 8.4 Hz, ArH), 7.17 (d, 2H, J = 8.4 Hz, ArH), 7.45 (d, 2H, J = 7.4 Hz, ArH), 7.49 (d, 2H, J = 8.4 Hz, ArH), 7.74 (s, 1H, CH), 8.74 (s, 1H, NH), 8.76 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, 125 MHz): 115.2, 116.0, 119.5, 121.7, 136.0, 137.6, 146.4, 152.7, 156.6, 158.2, 162.6, 163.8; Anal. calcd. for $C_{17}H_{11}Cl_2F\ N_4O_2$ (393.2): C 51.93, H 2.82, N 14.25. Found: C 51.91, H 2.66, N 13.95.

1-(4-(4,6-Dimethoxypyrimidin-2-yloxy)phenyl)-3-phenylthiourea ***4i***

White solid; ^{1}H-NMR (DMSO-d_6, 600 MHz): 3.78 (s, 6H, OCH_3), 5.99 (s, H, CH), 7.12 (s, 1H, ArH), 7.18 (d, 2H, J = 7.2 Hz, ArH), 7.31∼7.33 (m, 2H, ArH), 7.46 (d, 2H, J = 7.2 Hz, ArH), 7.50 (d, 2H, J = 7.2 Hz, ArH), 9.80 (s, H, NH), 9.82 (s, H, NH); Anal. calcd. for $C_{19}H_{18}N_4O_3S$ (382.4): C 59.67, H 4.74, N 14.65. Found: C 59.65, H 4.62, N 14.65.

1-(2,4-Dichlorophenyl)-3-(4-(4,6-dimethoxypyrimidin-2-yloxy)phenyl)thiourea ***4j***

White solid; ^{1}H-NMR (DMSO-d_6, 600 MHz): 3.77 (s, 6H, OCH_3, 5.99 (s, H, CH), 7.20 (d, 2H, J = 8.4 Hz, ArH), 7.42 (d, 1H, J = 9.0 Hz, ArH), 7.52 (d, 2H, J = 7.8 Hz, ArH), 7.60 (d, 1H, J = 9.0 Hz, ArH), 7.68 (s, 1H, ArH), 9.49 (s, H, NH), 10.04 (s, H, NH); Anal. calcd. for $C_{19}H_{16}Cl_2N_4O_3S$ (451.3): C 50.56, H 3.57, N 12.41. Found: C 50.50, H 3.44, N 12.28.

1-(4-(4,6-Dichloropyrimidin-2-yloxy)phenyl)-3-phenylthiourea ***4k***

White solid; ^{1}H-NMR (DMSO-d_6, 600 MHz): 7.11 (d, 1H, J = 7.8 Hz, ArH), 7.21 (d, 2H, J = 9.0 Hz, ArH), 7.30∼7.33 (m, 2H, ArH), 7.45 (d, 2H, J = 7.2 Hz, ArH), 7.21 (d, 2H, J = 9.0 Hz, ArH), 7.76 (s, 1H, ArH), 9.83 (s, 1H, NH), 9.88 (s, 1H, NH); Anal. calcd. for $C_{17}H_{12}Cl_2N_4OS$ (391.3): C 52.18, H 3.09, N 14.32. Found: C 52.22, H 3.01, N 14.65.

1-(2,4-Dichlorophenyl)-3-(4-(4,6-dichloropyrimidin-2-yloxy)phenyl)thiourea ***4l***

White solid; ^{1}H-NMR (DMSO-d_6, 600 MHz): 7.27 (d, 2H, J = 8.4 Hz, ArH), 7.44 (d, 1H, J = 9.0 Hz, ArH), 7.67∼7.62 (m, 3H, ArH), 7.70 (s, 1H, CH), 7.79 (s, 1H, ArH), 9.58 (s, H, NH), 10.09 (s, H, NH); Anal. calcd. for $C_{17}H_{10}Cl_4N_4OS$ (460.2): C 44.37, H 2.19, N 12.18. Found: C 44.70, H 2.56, N 12.12.

Pharmacology

The *in-vitro* cytotoxicity measurement of the synthesized compounds against different cancer cell lines was performed with the MTT assay according to the Mosmann's method [13]. The MTT assay is based on the reduction of the soluble 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) into a blue-purple formazan product, mainly by mitochondrial reductase activity inside living cells. The cells used in the cytotoxicity assay were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum. Cells suspended in the medium (2Y′ 104/mL) were plated in 96-well culture plates and incubated at 37°C in a 5% CO_2 incubator. After 12 h, the test sample (2 mL) was added to the cells (2Y′ 104) in 96-well plates and cultured at 37°C for 3 days. The cultured cells were mixed with 20 mL of MTT solution and incubated for 4 h at 37°C. The supernatant was carefully removed from each well and 100 mL of DMSO was added to each well to dissolve the formazan crystals which were formed by the cellular reduction of MTT. After mixing with a mechanical plate mixer, the absorbance of each well was measured by a microplate reader using a test wavelength of 570 nm. The results were expressed as the IC_{50}, which is the concentration of the drugs inducing a 50% inhibition of cell growth of treated cells when compared to the growth of control cells. Each experiment was performed at least 3 times. There was a good reproducibility between replicate wells with standard errors below 10%.

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The authors have declared no conflict of interest.

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