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Synthesis and Antitumor Activity Evaluation of Pyrimidine Analogues Bearing Urea Moiety

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A series of 4-anilino-6-phenylpyrimidines containing urea moiety were synthesized and the structures of all products were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS. The antiproliferative activities of these compounds were evaluated against three human tumor cell lines (MGC-803, MCF-7 and EC-109) by applying the MTT assay method. compounds **4a**, **4b** and **6a** showed the most effective activity, among which, **6a** was more cytotoxic than 5-fluorouracil against all tested human cancer cell lines with IC_{50} values ranging from 1.80 to 2.72 μmol•L^{-1}.

Keywords synthesis, pyrimidine, urea, antitumor

Introduction

Cancer is a leading cause of death worldwide. The pharmacological fight against cancer has made significant progress in the last twenty years. In 2012 (October 1, 2011—September 30, 2012), 35 novel drugs were approved by the FDA, among which, 10 (28.57%) were used for the treatment of cancer.[1] Although many chemotherapeutic agents have been developed to treat different kinds of cancer effectively, some side effects could happen simultaneously. Therefore novel molecules to fight this disease are still urgently needed.[2,3]

Pyrimidine is found as a core structure in a large variety of compounds that exhibit important biological activity.[4,5] Many papers have reported that pyrimidine derivatives showed impressive anticancer activity[6-9] and several members of this class have earned valued places in chemotherapy as effective agents, like 5-fluorouracil, tegafur, carmofur and fluoroadenosine. Among them, 4-anilino-6-phenyl-pyrimidines (**I**, Figure 1) play an important role in the development of anticancer drugs.[10,11]

On the other hand, urea hybrids with a wide range of activities against various leukemias and solid tumors.[12] 2-Substituted-*N*-(arylcarbamoyl)acetamide derivatives (**II**, Figure 1) are an important class of urea derivatives and have received much attention. Several promising antitumor active agents were found to contain the scaffold.[13,14]

With this in view, here we report the synthesis and biological activity of pyrimidine derivatives bearing 2-substituted-*N*-(arylcarbamoyl)acetamide moiety (**III**, Figure 1). The synthetic strategy to prepare the target compounds is depicted in Scheme 1. The structure-activity relationship (SAR) of these compounds was also discussed.

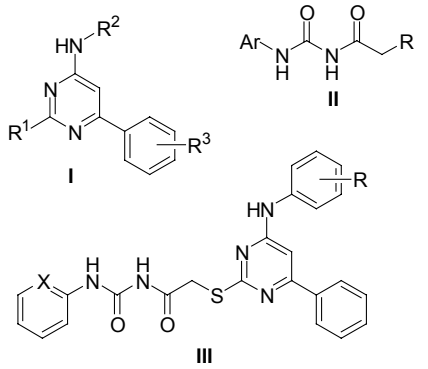

Figure 1 The structures of compounds **I**, **II** and **III**.

Experimental

General

Reagents and solvents were purchased from commercial sources and were used without further purification. Thin-layer chromatography (TLC) was carried out on glass plates coated with silica gel (Qingdao Haiyang Chemical Co., G60F-254) and visualized by UV light (254 nm). The products were purified by column chromatography over silica gel (Qingdao Haiyang Chemical Co., 200—300 mesh). Melting points were determined on an X-5 micromelting apparatus and are uncorrected. All the NMR spectra were recorded with a Bruker

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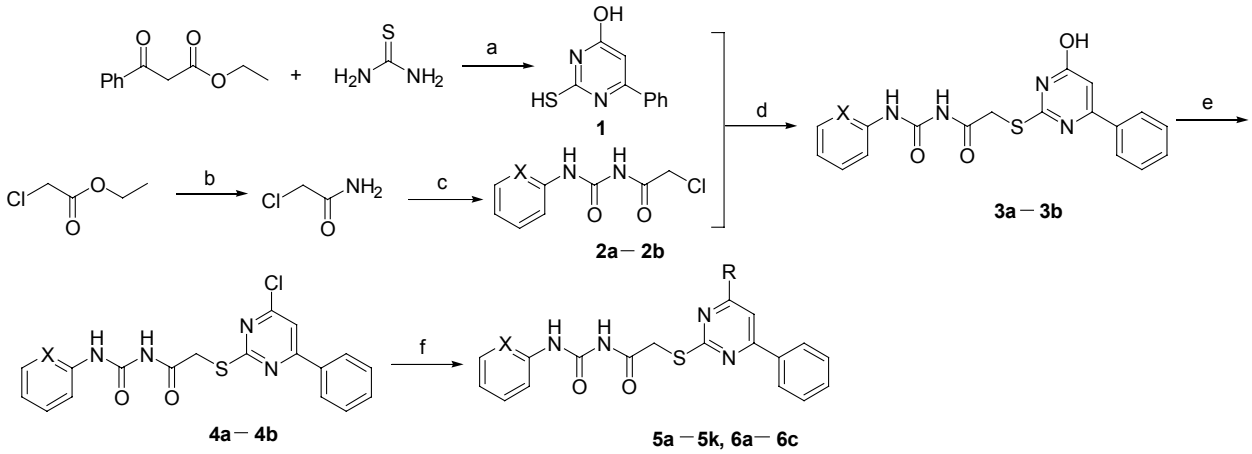
Scheme 1 The synthesis of pyrimidines analogues bearing urea moiety



Reagents and conditions: (a) KOH, EtOH, reflux; (b) $NH_3 \cdot H_2O$, 0 ℃; (c) i) Oxalyl chloride, 1,2-dichloroethane, 90 ℃; ii) 2-aminopyridine or aniline, 0 ℃; (d) KOH, H_2O-acetone (2:1), 55－60 ℃; (e) $POCl_3$, 90 ℃; (f) substituted anilines, AcOH, 90 ℃

DPX 400 MHz spectrometer. Chemical shifts (δ) are given relative to TMS. High-resolution mass spectra (HRMS) were recorded on a Waters Micromass Q-T of Micromass spectrometer by electrospray ionization (ESI). The synthesized compounds were named using ChemDraw Ultra software (v 12.0).

General procedure to synthesize compounds 2a－2b

To a solution of compound **1** (1.8 g, 19.4 mmol) in anhydrous 1,2-dichloroethane (20 mL) was added oxalyl chloride (2 mL) at 0 ℃, then refluxed on oil bath at 90 ℃ for 4 h. The reaction mixture was then cooled to 0 ℃, and 2-aminopyridine or aniline (19.40 mmol) was added into the reaction mixture. The reaction mixture was stirred for another 5 min. Upon completion, the solid was filtrated and washed with 1,2-dichloroethane to yield the pure product.

2-Chloro-*N*-(pyridin-2-ylcarbamoyl)acetamide (2a) Yield 90.7%, white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.63 (s, 1H), 8.78 (s, 1H), 8.36 (d, J=4.1 Hz, 1H), 8.08 (d, J=6.9 Hz, 1H), 7.80－7.68 (m, 1H), 7.10 (dd, J=6.9, 5.2 Hz, 1H), 4.24 (s, 2H). HR-MS (ESI) calcd for $C_8H_8ClN_3O_2$ $[M+H]^+$: 214.0378, found 214.0379.

2-Chloro-*N*-(phenylcarbamoyl)acetamide (2b) Yield 86.3%, white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.92 (s, 1H), 10.18 (s, 1H), 7.57－7.50 (m, 2H), 7.34 (t, J=7.9 Hz, 2H), 7.11 (t, J=7.4 Hz, 1H), 4.41 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 169.0, 150.7, 137.9, 129.4, 124.2, 120.2, 43.7. HR-MS (ESI) calcd for $C_9H_9ClN_2O_2$ $[M+H]^+$: 213.0431, found 213.0430.

General procedure to synthesize compounds 3a－3b

To a solution of compound **1** (2.0 g, 10.0 mmol) and potassium hydroxide (0.6 g, 10.0 mmol) in water (40 mL) was slowly added compound **2a** or **2b** (10.0 mmol) dissolved in acetone (10 mL), and the reaction was stirred at 50 ℃. Upon completion, the solid was filtrated and washed with water then with acetone to yield the pure product.

2-((4-Hydroxy-6-phenylpyrimidin-2-yl)thio)-*N*-(pyridin-2-ylcarbamoyl)acetamide (3a) Yield 85.8%, white solid, m.p. 221－222 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.75 (s, 1H), 11.34 (s, 1H), 10.70 (s, 1H), 8.33－8.20 (m, 1H), 8.04 (d, J=7.2 Hz, 2H), 7.98 (d, J=8.2 Hz, 1H), 7.86－7.78 (m, 1H), 7.44 (q, J=7.3 Hz, 1H), 7.38 (t, J=7.3 Hz, 2H), 7.12 (dd, J=6.9, 5.3 Hz, 1H), 6.72 (s, 1H), 4.26 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.1, 164.8, 162.5, 161.1, 151.2, 150.8, 148.6, 139.0, 136.2, 131.1, 129.0, 127.2, 120.1, 113.5, 104.2, 35.5. HR-MS (ESI) calcd for $C_{18}H_{15}N_5O_3S$ $[M+H]^+$: 382.0974, found 382.0948.

2-((4-Hydroxy-6-phenylpyrimidin-2-yl)thio)-*N*-(phenylcarbamoyl)acetamide (3b) Yield 89.8%, white solid, m.p. 208－209 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.87 (s, 1H), 11.13 (s, 1H), 10.34 (s, 1H), 8.05 (d, J=7.4 Hz, 2H), 7.51 (d, J=7.9 Hz, 2H), 7.45 (d, J=7.1 Hz, 1H), 7.40 (t, J=7.4 Hz, 2H), 7.31 (t, J=7.8 Hz, 2H), 7.08 (t, J=7.4 Hz, 1H), 6.73 (s, 1H), 4.24 (s, 2H). HR-MS (ESI) calcd for $C_{19}H_{16}N_4O_3S$ $[M+H]^+$: 381.1021, found 381.1023.

General procedure to synthesize compounds 4a－4b

In an ice bath, phosphorous oxychloride (10 mL) was added dropwise with gentle stirring to compound **3a** or **3b** (10.12 mmol). The reaction mixture was stirred at room temperature for 30 min, and then stirred at 90℃. Upon completion, after cooling, the reaction mixture was poured with continuous stirring onto crushed ice. The formed solid was collected by vacuum filtration, washed with water to yield the pure product.

2-((4-Chloro-6-phenylpyrimidin-2-yl)thio)-*N*-(pyridin-2-ylcarbamoyl)acetamide (4a) Yield 91.2%, white solid, m.p. 192－193 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.36 (s, 1H), 10.70 (s, 1H), 8.27 (d, J=4.6 Hz, 1H), 8.22 (d, J=7.4 Hz, 2H), 8.02 (s, 1H), 7.98 (d, J=8.3 Hz, 1H), 7.82 (t, J=7.8 Hz, 1H), 7.57 (t, J=7.3 Hz, 1H), 7.49 (t, J=7.5 Hz, 2H), 7.12 (dd, J=6.8, 5.4 Hz, 1H), 4.30 (s, 2H); ^{13}C NMR (100 MHz,

DMSO-d_6) δ: 171.2, 171.1, 165.6, 161.6, 151.2, 150.8, 148.5, 139.1, 134.8, 132.5, 129.4, 128.0, 120.1, 113.5, 113.2, 36.1. HR-MS (ESI) calcd for $C_{18}H_{14}ClN_5O_2S$ [M＋H]$^+$: 400.0635, found 400.0632.

2-((4-Chloro-6-phenylpyrimidin-2-yl)thio)-*N*-(phenylcarbamoyl)acetamide (4b) Yield 92.1%, white solid, m.p. 201－202 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.17 (s, 1H), 10.32 (s, 1H), 8.23 (d, J=7.5 Hz, 2H), 8.03 (s, 1H), 7.58 (t, J=7.3 Hz, 1H), 7.50 (t, J=7.4 Hz, 4H), 7.31 (t, J=7.9 Hz, 2H), 7.08 (t, J=7.4 Hz, 1H), 4.27 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.2, 170.9, 165.6, 161.6, 151.0, 137.9, 134.8, 132.6, 129.4, 128.0, 124.2, 120.1, 113.2, 36.0. HR-MS (ESI) calcd for $C_{19}H_{15}ClN_4O_2S$ [M＋H]$^+$: 399.0682, found 399.0673.

General procedure to synthesize compounds 5a－5k and 6a－6c

To a well stirred solution of the appropriate amine (5.30 mmol) in acetic acid glacial (3 mL), equimolar amount of compound **4a** or **4b** (5.30 mmol) was added. The reaction mixture was stirred at 90 ℃. Upon completion, the solvent was then removed by distillation under reduced pressure and the remained solid was washed with cold water and purified either by recrystallization or by silica gel column chromatography.

2-((4-Phenyl-6-(phenylamino)pyrimidin-2-yl)-thio)-*N*-(pyridin-2-ylcarbamoyl)acetamide (5a) Yield 70.3%, white solid, m.p. 231－232 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.28 (s, 1H), 10.77 (s, 1H), 9.83 (s, 1H), 8.28 (d, J=4.8 Hz, 1H), 8.00 (dd, J=7.7, 1.7 Hz, 3H), 7.83 (t, J=7.8 Hz, 1H), 7.66 (d, J=8.0 Hz, 2H), 7.48 (dd, J=12.5, 6.8 Hz, 3H), 7.36 (t, J=7.9 Hz, 2H), 7.16－7.10 (m, 1H), 7.05 (t, J=7.4 Hz, 1H), 6.99 (s, 1H), 4.20 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.7, 169.5, 161.5, 161.2, 151.2, 150.9, 148.3, 139.9, 139.3, 136.8, 131.0, 129.3, 126.9, 123.2, 120.4, 120.1, 113.6 99.1, 35.9. HR-MS (ESI) calcd for $C_{24}H_{20}N_6O_2S$ [M＋H]$^+$: 457.1447, found 457.1450.

2-((4-Phenyl-6-(*p*-tolylamino)pyrimidin-2-yl)thio)-*N*-(pyridin-2-ylcarbamoyl)acetamide (5b) Yield 82.6%, white solid, m.p. 204－205 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.30 (s, 1H), 10.82 (s, 1H), 9.97 (s, 1H), 8.30 (d, J=4.0 Hz, 1H), 8.07－7.93 (m, 3H), 7.93－7.84 (m, 1H), 7.62－7.40 (m, 5H), 7.16 (dd, J=11.0, 7.8 Hz, 3H), 7.00 (s, 1H), 4.21 (s, 2H), 2.24 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.1, 168.9, 160.9, 150.8, 150.2, 146.3, 141.2, 136.7, 135.6, 132.8, 131.2, 129.6, 129.3, 127.1, 120.9, 120.3, 114.5, 99.4, 36.0, 20.9. HR-MS (ESI) calcd for $C_{25}H_{22}N_6O_2S$ [M＋H]$^+$: 471.1603, found 471.1605.

2-((4-Phenyl-6-(*m*-tolylamino)pyrimidin-2-yl)-thio)-*N*-(pyridin-2-ylcarbamoyl)acetamide (5c) Yield 80.8%, white solid, m.p. 205－206 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.79 (s, 1H), 9.84 (s, 1H), 8.28 (d, J=4.4 Hz, 1H), 7.99 (d, J=6.5 Hz, 3H), 7.84 (t, J=7.8 Hz, 1H), 7.53 (d, J=8.0 Hz, 1H), 7.50－7.42 (m, 3H), 7.40 (s, 1H), 7.24 (t, J=7.8 Hz, 1H), 7.13 (dd, J=11.8, 6.2 Hz, 1H), 7.01 (s, 1H), 6.87 (d, J=7.4 Hz, 1H), 4.20 (s, 2H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.7, 169.4, 161.4, 161.2, 151.0, 150.8, 148.0, 139.7, 139.6, 138.5, 136.7, 131.0, 129.2, 129.1, 126.9, 124.1, 121.1, 120.1, 117.8, 113.8, 99.1, 35.8, 21.6. HR-MS (ESI) calcd for $C_{25}H_{22}N_6O_2S$ [M＋H]$^+$: 471.1603, found 471.1605.

2-((4-((4-Butylphenyl)amino)-6-phenylpyrimidin-2-yl)thio)-*N*-(pyridin-2-ylcarbamoyl)aceta-mide (5d) Yield 89.1%, white solid, m.p. 231－232 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.29 (s, 1H), 10.81 (s, 1H), 9.96 (s, 1H), 8.35－8.23 (m, 1H), 8.06－7.95 (m, 3H), 7.91－7.84 (m, 1H), 7.53 (t, J=8.1 Hz, 2H), 7.52－7.43 (m, 3H), 7.21－7.12 (m, 3H), 7.01 (s, 1H), 4.22 (s, 2H), 1.50 (dt, J=15.2, 7.5 Hz, 2H), 1.27 (dq, J=14.6, 7.3 Hz, 2H), 0.87 (t, J=7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.2, 169.1, 160.9, 160.2, 150.8, 150.5, 146.8, 140.6, 137.6, 137.1, 135.9, 131.2, 129.3, 129.0, 127.1, 120.7, 120.2, 114.5 (s), 99.4, 36.0, 34.7, 33.5, 22.2, 14.2. HR-MS (ESI) calcd for $C_{28}H_{28}N_6O_2S$ [M＋H]$^+$: 513.2073, found 513.2070.

2-((4-((4-Methoxyphenyl)amino)-6-phenylpyrimidin-2-yl)thio)-*N*-(pyridin-2-ylcarbamoyl)acetamide (5e) Yield 84.5%, white solid, m.p. 201－202 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.25 (s, 1H), 10.77 (s, 1H), 9.68 (s, 1H), 8.28 (d, J=4.5 Hz, 1H), 7.98 (t, J=7.3 Hz, 3H), 7.83 (t, J=7.8 Hz, 1H), 7.52 (d, J=8.6 Hz, 2H), 7.47 (t, J=6.3 Hz, 3H), 7.16－7.10 (m, 1H), 6.94 (d, J=8.9 Hz, 2H), 6.89 (s, 1H), 4.18 (s, 2H), 3.73 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.6, 169.5, 161.2, 155.7, 151.2, 150.9, 148.3, 139.3, 136.8, 132.6, 130.9, 129.2, 126.9, 122.5, 120.1, 114.5, 113.6, 98.4, 55.6, 35.9. HR-MS (ESI) calcd for $C_{25}H_{22}N_6O_3S$ [M＋H]$^+$: 487.1552, found 487.1551.

2-((4-((2-Methoxyphenyl)amino)-6-phenylpyrimidin-2-yl)thio)-*N*-(pyridin-2-ylcarbamoyl)acetamide (5f) Yield 88.9%, yellow solid, m.p. 189－190 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.26 (s, 1H), 10.80 (s, 1H), 9.08 (s, 1H), 8.29 (d, J=4.0 Hz, 1H), 8.05－7.95 (m, 3H), 7.92 (d, J=7.7 Hz, 1H), 7.89－7.83 (m, 1H), 7.53－7.39 (m, 3H), 7.15 (dd, J=6.6, 5.5 Hz, 2H), 7.09 (dd, J=9.7, 7.8 Hz, 2H), 7.03－6.93 (m, 1H), 4.17 (s, 2H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.4, 169.0, 161.6, 160.9, 151.3, 150.8, 150.7, 147.3, 140.3, 136.4, 129.3, 127.5, 127.0, 125.3, 123.6, 120.8, 120.2, 114.1, 112.0, 99.2, 56.1, 35.8. HR-MS (ESI) calcd for $C_{25}H_{22}N_6O_3S$ [M＋H]$^+$: 487.1552, found 487.1548.

2-((4-((4-Fluorophenyl)amino)-6-phenylpyrimidin-2-yl)thio)-*N*-(pyridin-2-ylcarbamoyl)acetamide (5g) Yield 80.1%, white solid, m.p. 215－216 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.36 (s, 1H), 10.90 (s, 1H), 10.23 (s, 1H), 8.31 (d, J=4.3 Hz, 1H), 7.99 (t, J=9.4 Hz, 3H), 7.93 (t, J=7.7 Hz, 1H), 7.69 (dd, J=8.1, 4.6 Hz, 3H), 7.48 (d, J=6.5 Hz, 3H), 7.18 (t, J=8.4 Hz, 3H), 7.05 (s, 1H), 4.22 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.2, 169.0, 160.9, 160.3, 159.6, 157.3, 150.8, 149.9, 145.7, 141.7, 135.8, 131.2,

130.0, 129.3, 127.1, 122.5, 120.3, 115.9, 115.7, 114.8, 99.4, 36.0. HR-MS (ESI) calcd for $C_{24}H_{19}FN_6O_2S$ $[M+H]^+$: 475.1352, found 475.1345.

2-((4-((4-Chlorophenyl)amino)-6-phenylpyrimidin-2-yl)thio)-*N*-(pyridin-2-ylcarbamoyl)acetamide (5h) Yield 90.6%, white solid, m.p. 213−214 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.34 (s, 1H), 10.83 (s, 1H), 10.19 (s, 1H), 8.30 (d, J=4.6 Hz, 1H), 8.00 (d, J=5.9 Hz, 3H), 7.89 (t, J=7.2 Hz, 1H), 7.72 (d, J=8.8 Hz, 2H), 7.54−7.44 (m, 3H), 7.40 (d, J=8.7 Hz, 2H), 7.22−7.11 (m, 1H), 7.06 (s, 1H), 4.23 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.3, 169.3, 160.9, 150.8, 150.1, 146.1, 141.4, 138.8, 136.2, 131.2, 129.3, 129.1, 127.0, 126.8, 121.9, 120.3, 114.7, 99.6, 36.0. HR-MS (ESI) calcd for $C_{24}H_{19}ClN_6O_2S$ $[M+H]^+$: 491.1057, found 491.1055.

2-((4-((2-Chlorophenyl)amino)-6-phenylpyrimidin-2-yl)thio)-*N*-(pyridin-2-ylcarbamoyl)acetamide (5i) Yield 81.7%, white solid, m.p. 195−196 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.34 (s, 1H), 10.83 (s, 1H), 10.19 (s, 1H), 8.30 (d, J=4.6 Hz, 1H), 8.00 (d, J=5.9 Hz, 3H), 7.89 (t, J=7.2 Hz, 1H), 7.72 (d, J=8.8 Hz, 2H), 7.54−7.44 (m, 3H), 7.40 (d, J=8.7 Hz, 2H), 7.22−7.11 (m, 1H), 7.06 (s, 1H), 4.23 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.3, 169.3, 160.9, 150.8, 150.1, 146.1, 141.4, 138.8, 136.2, 131.2, 129.3, 129.1, 127.0, 126.8, 121.9, 120.3, 114.7, 99.6, 36.0. HR-MS (ESI) calcd for $C_{24}H_{19}ClN_6O_2S$ $[M+H]^+$: 491.1057, found 491.1053.

2-((4-((3-Chlorophenyl)amino)-6-phenylpyrimidin-2-yl)thio)-*N*-(pyridin-2-ylcarbamoyl)acetamide (5j) Yield 86.1%, white solid, m.p. 231−232 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.28 (s, 1H), 10.76 (s, 1H), 10.00 (s, 1H), 8.27 (d, J=4.1 Hz, 1H), 8.02 (d, J=7.3 Hz, 2H), 7.98 (s, 1H), 7.89−7.78 (m, 2H), 7.61 (d, J=8.0 Hz, 1H), 7.52−7.42 (m, 3H), 7.38 (t, J=8.1 Hz, 1H), 7.11 (dd, J=12.2, 7.2 Hz, 2H), 7.00 (s, 1H), 4.22 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.7, 169.6, 162.0, 161.0, 151.3, 150.9, 148.62, 141.5, 139.0, 136.7, 133.6, 131.1, 130.9, 129.3, 127.0, 122.6, 120.1, 119.6, 118.6, 113.5, 99.5, 35.8. HR-MS (ESI) calcd for $C_{24}H_{19}ClN_6O_2S$ $[M+H]^+$: 491.1057, found 491.1056.

2-((4-((4-Nitrophenyl)amino)-6-phenylpyrimidin-2-yl)thio)-*N*-(pyridin-2-ylcarbamoyl)acetamide (5k) Yield 92.3%, yellow solid, m.p. 198−199 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.35 (s, 1H), 10.76 (s, 1H), 10.71 (s, 1H), 8.27 (t, J=6.3 Hz, 3H), 8.03 (dd, J=7.7, 1.6 Hz, 2H), 7.96 (d, J=9.2 Hz, 3H), 7.86 (dd, J=10.8, 4.9 Hz, 1H), 7.49 (dt, J=8.8, 4.4 Hz, 3H), 7.20 (s, 1H), 7.18−7.12 (m, 1H), 4.28 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.4, 169.8, 162.3, 160.8, 150.8, 150.3, 146.6, 141.6, 140.9, 136.4, 131.3, 129.4, 127.0, 125.5, 120.3, 119.2, 114.4, 100.5, 36.1. HR-MS (ESI) calcd for $C_{24}H_{19}N_7O_4S$ $[M+H]^+$: 502.1297, found 502.1296.

2-((4-Phenyl-6-(*p*-tolylamino)pyrimidin-2-yl)thio)-*N*-(phenylcarbamoyl)acetamide (6a) Yield 79.7%, white solid, m.p. 200−201 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.05 (s, 1H), 10.38 (s, 1H), 9.70 (s, 1H), 8.01 (t, J=10.6 Hz, 2H), 7.49 (dd, J=15.4, 7.2 Hz, 7H), 7.32 (t, J=7.7 Hz, 2H), 7.16 (d, J=8.0 Hz, 2H), 7.08 (t, J=7.3 Hz, 1H), 6.93 (s, 1H), 4.16 (s, 2H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.6, 169.6, 161.6, 161.2, 151.1, 138.0, 137.2, 136.9, 132.3, 130.9, 129.7, 129.3, 126.9, 124.1, 120.7, 120.0, 98.6, 35.7, 20.9. HR-MS (ESI) calcd for $C_{26}H_{23}N_5O_2S$ $[M+H]^+$: 470.1651, found 470.1649.

2-((4-((4-Fluorophenyl)amino)-6-phenylpyrimidin-2-yl)thio)-*N*-(phenylcarbamoyl)acetamide (6b) Yield 81.9%, white solid, m.p. 198−199 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.06 (s, 1H), 10.38 (s, 1H), 9.82 (s, 1H), 8.00 (d, J=6.3 Hz, 2H), 7.71−7.60 (m, 2H), 7.50 (dd, J=11.8, 7.4 Hz, 5H), 7.32 (t, J=7.6 Hz, 2H), 7.20 (t, J=8.7 Hz, 2H), 7.08 (t, J=7.4 Hz, 1H), 6.93 (s, 1H), 4.17 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 172.5, 171.5, 161.7, 161.1, 151.1, 137.9, 136.8, 136.2, 131.0, 129.3, 126.9, 124.1, 122.2, 120.1, 116.3, 116.1, 115.9, 98.7, 35.7. HR-MS (ESI) calcd for $C_{25}H_{20}FN_5O_2S$ $[M+H]^+$: 474.1400, found 474.1405.

2-((4-((4-Chlorophenyl)amino)-6-phenylpyrimidin-2-yl)thio)-*N*-(phenylcarbamoyl)acetamide (6c) Yield 87.1%, white solid, m.p. 230−231 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.08 (s, 1H), 10.38 (s, 1H), 9.98 (s, 1H), 8.11−7.95 (m, 2H), 7.70 (d, J=8.7 Hz, 2H), 7.50 (dd, J=10.0, 7.9 Hz, 5H), 7.41 (d, J=8.8 Hz, 2H), 7.31 (t, J=7.8 Hz, 2H), 7.07 (t, J=7.3 Hz, 1H), 6.99 (s, 1H), 4.19 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.4, 169.6, 161.6, 160.9, 151.1, 138.9, 138.0, 136.6, 131.1, 129.6, 128.9, 127.0, 126.7, 124.1, 121.7, 120.1, 99.3, 35.8. HR-MS (ESI) calcd for $C_{25}H_{20}ClN_5O_2S$ $[M+H]^+$: 490.1104, found 490.1106.

Results and Discussion

Compounds **3a**−**3b**, **4a**−**4b**, **5a**−**5k** and **6a**−**6c** were investigated for their *in vitro* anti-tumor activities against three human cancer cell lines including MCF-7 (human breast cancer cell line), MGC-803 (human gastric cancer cell line) and EC-109 (human esophageal cancer cell line) using MTT assay method according to Mosmann's method.[15] Table 1 reported the IC_{50} (μmol•L^{-1}) values (concentration required to achieve 50% inhibition of the tumor growth) of the tested compounds and the standard. It has been observed that compounds **4a**, **4b** and **6a** showed most effective activity as compared to other compounds. One of the most active compounds was **6a**, with IC_{50} values against the three tested human cancer cell lines ranging from 1.80 to 2.72 μmol•L^{-1}, which was more cytotoxic than 5-fluorouracil.

According to the activity data (**3a**, **4a**, and **5a**−**5k**), it has been observed that the change at 4-position of -pyrimidine may lead to change in the activity. The analogues with −Cl and *para*-substituted anilines were found to be more effective as compared to compounds having −OH and *ortho*- or *meta*-substituted anilines. It

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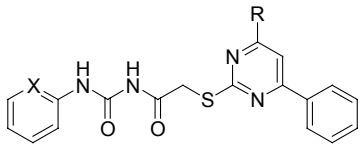
Table 1 Inhibitory results of pyrimidine derivatives against three human cancer cell lines



Compound	X	R	IC_{50}[a]/(μmol•L^{-1})		
			MGC-803	MCF-7	EC-109
3a	N	OH-	＞100	＞100	64.92
3b	CH	OH-	＞100	73.20	45.45
4a	N	Cl-	7.63	8.77	3.71
4b	CH	Cl-	3.03	25.38	11.01
5a	N	C_6H_5-NH-	38.76	42.34	47.72
5b	N	4-CH_3-C_6H_5-NH-	15.69	23.36	28.21
5c	N	3-CH_3-C_6H_5-NH-	73.16	＞100	64.58
5d	N	4-butyl-C_6H_5-NH-	61.82	85.36	70.13
5e	N	4-CH_3O-C_6H_5-NH-	10.33	22.97	26.81
5f	N	2-CH_3O-C_6H_5-NH-	＞100	＞100	＞100
5g	N	4-F-C_6H_5-NH-	52.92	67.73	89.65
5h	N	4-Cl-C_6H_5-NH-	65.77	＞100	＞100
5i	N	2-Cl-C_6H_5-NH-	＞100	＞100	＞100
5j	N	3-Cl-C_6H_5-NH-	＞100	＞100	＞100
5k	N	4-NO_2-C_6H_5-NH-	＞100	＞100	＞100
6a	CH	4-CH_3-C_6H_5-NH-	2.19	1.80	2.72
6b	CH	4-F-C_6H_5-NH-	38.79	10.54	42.38
6c	CH	4-Cl-C_6H_5-NH-	54.21	32.28	68.43
5-Fu			3.80	6.45	5.15

[a] Inhibitory activity was assayed by exposure for 72 h to substances and expressed as concentration required to inhibit tumor cell proliferation by 50% (IC_{50}).

is worthy to mention that electron donating groups (−CH_3, −$CH_2CH_2CH_2CH_3$ and −OCH_3) on benzene ring are more effective than electron withdrawing groups (−F, −Cl, −NO_2). Moreover, reviewing and comparing the activity data (**3a**, **4a**, **5b**, **5g**, **5h** and **3b**, **4b**, **6a**−**6c**), it is worthy to mention that the benzene ring is more effective than the pyridine ring.

Conclusions

In conclusion, we synthesized a series of pyrimidine hybrids containing urea moiety and their antitumor activities were evaluated *in vitro* against three human cancer cell lines. The nature of the aromatic ring attached directly to urea and the substitutions on the phenyl ring influenced the antitumor activities remarkably. Biological activity data revealed that compounds **4a**, **4b** and **6a** showed most effective activity. Among them, **6a** was more cytotoxic than 5-fluorouracil against all tested three human cancer cell lines, which is under further investigation as lead structure. Further modifications and SAR study are undergoing and will be reported elsewhere.

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