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Novel 5-Fluorouracil Derivatives: Synthesis and Cytotoxic Activity of 2-Butoxy-4-Substituted 5-Fluoropyrimidines

Jian Sun, Shi-Jie Zhang,[†] Hai-Bo Li,[‡] Wei Zhou,[§] Wei-Xiao Hu,[§] and Shang Shan[*]

College of Chemical Engineering and Materials Science, Zhejiang University of Technology, Hangzhou 310032, China
[*]E-mail: shanshang@mail.hz.zj.cn
[†]Graduate School, Zhejiang Chinese Medical University, Hangzhou 310053, China
[‡]Nantong Center for Disease Control and Prevention, Nantong 226001, China
[§]College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou 310032, China
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Twenty two new 5-fluorouracil (5-FU) derivatives, 2-butoxy-4-substituted 5-fluoropyrimidines, were synthesized and characterized by IR, ^{1}H NMR, MS, HRMS. All compounds were preliminarily evaluated by MTT assay on human liver BEL-7402 cancer cell line *in vitro*. Ten compounds were selected to test their cytotoxic activity against A549, HL-60 and MCF-7 cancer cell lines *in vitro*. These compounds were more sensitive to BEL-7402 than other cell lines, particularly, cytotoxic activity of compounds **6b**, **6d-f**, **6p**, **6s-u** were in sub-micromolar scale. The highest cytotoxic potency against A549, HL-60 and MCF-7 was shown by 2-butoxy-4-chloro-5-fluoropyrimidine (**5**) with IC_{50} values of 0.10, 1.66 and 0.59 μM, respectively. Compounds **6d** and **6e** were effective against MCF-7 with IC_{50} 9.73 μM and HL-60 with IC_{50} 8.83 μM, respectively.

Key Words : 5-Fluorouracil, 2-Butoxy-4-substituted 5-fluoropyrimidine, Synthesis, Cytotoxic activity

Introduction

5-Fluorouracil (5-FU, **1a**), a pyrimidine analogue, has been found as a kind of highly effective antimetabolism drug for the treatment of malignances, ever since it was first synthesized by Duschinsky in 1957.[1] 5-FU was considered as suicide inactivator as it blocked DNA biosynthesis by inhibiting thymidylate synthase, and finally induced cell cycle arrest.[2] However, 5-FU exhibited high toxicity and poor tumor affinity, which limited its therapeutic application and led to high incidence of bone marrow, gastrointestinal tract and central nervous toxicity.[3,4]

Numorous modifications of 5-FU structure have been investigated to improve its pharmacological and pharmacokinetic properties, among which tegafur (**1b**), carmofur (**1c**) and floxuridine (**1d**) have been widely used in clinical with increased bioactivity, selectivity, metabolic stability, absorption or low toxicity (Fig. 1).[5] Other research mainly focused on modifications at N^1 or N^3 positions, such as nucleoside analogues like FdUMP,[6] or conjugation with peptides,[7] amino acids,[8] glucose.[9] Optimization strategies of 5-FU incorporated into synthetic or natural macromolecules, for example pectin-5-FU,[10] porphyran-5-FU,[11] and folic acid-5-FU,[12] were associated with significant anti-tumor activity. Besides, target-oriented 5-FU derivatives were also appreciated, such as liver targeting cholic acid-5-FU conjugate, which delivered the prodrug into liver tissue and released 5-FU in remarkably high concentration,[13] and 5-FU conjugated with sorbitol-based G8 transporter was found to have an affinity toward mitochondria and to readily cross blood-brain barrier.[14]

Hybridization of bioactive natural and unnatural compounds is a niche in the field of drug discovery and regarded as most promising and fundamental novel approaches for the discovery of new and potent drugs.[15,16] In light of the combined use of camptothecin (CPT) and 5-FU in clinical therapy, the conjugation of 5-FU and CPT with dipeptides improved tumor selectivity, efficiency, safety, and were comparable or superior to irinotecan.[17] Other hybrids, incorporating various pharmacophores with antitumor activity including cytarabine-5-FU,[18] cisplatin-5-FU,[19] podophyllotoxin-5-FU,[20] ampelopsin-5-FU,[21] inhanced 5-FU's physical and chemical profiles and overcame its disadvantages.

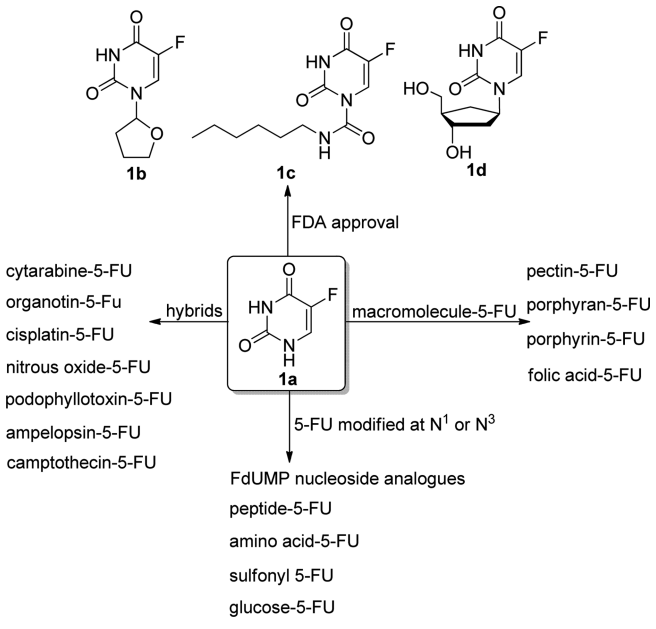


Figure 1. 5-FU and its derivatives.

With these exciting results achieved so far, 5-FU was an attractive lead compound for the development of novel antitumor agents, but few modifications were found at O^2 or O^4 positions. Some *O*-substituted 5-FU derivatives including 2,4-dibenzyloxy-5-fluoropyrimidine, synthesized by Yamashita's group were as effective as tegafur (**1b**) against Ehrlich carcinoma and sarcoma 180 cells, and showed higher blood concentration of 5-FU and higher LD_{50} than tegafur (**1b**).[22] Recently, Benko's group disclosed that some 2-alkoxy-4-substituted amino-5-fluoropyrimidine derivatives possessed good antifungal activity and patented as fungicides.[23] However, there was no further study on whether these compounds have anticancer activity or not.

2-*O*-*n*-Butyl-5-fluorouracil (FD-2) was considered as a low toxic, high efficient antineoplastic drug. *In vivo* study revealed that the toxicity of FD-2 was 1/6 to 1/8 of 5-FU, but the antitumor effect was comparable to 5-FU. In addition, FD-2 would release 5-FU in high concentration in blood by oral administration and maintain longer time course compared to 5-FU and tegadifur *in vivo*.[24]

Inspired with these findings, our group have synthesized a series of new 2-butoxy-4-substituted 5-fluoropyrimidines **5**, **6a-u** by introduction of *n*-butoxy group into the O^2-position and chloro or amino group into the O^4-position to investigate whether proper substituents on the 5-FU could improve the cytotoxic activity against cancer cells.[25]

Experimental

Chemistry. Melting points were determined using an XRC apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Thermo Nicolet 370 FT-IR spectrophotometer. ^{1}H NMR spectra were measured on a Bruker AVANCE spectrometer at 500 MHz using TMS as an internal standard. Mass spectra (MS) were taken on an HP 5989B spectrometer with EI source. High resolution mass spectra (HRMS) were taken on a Varian spectrometer. All the chemicals and solvents were of analytical reagent and commercially available, and used as received unless otherwise stated.

General Procedure for Preparation of Compounds 2-4. 2,4-Dichloro-5-fluoropyrimidine (**2**) was synthesized from 5-FU by treating with phosphorus oxychloride and *N*,*N*-dimethylaniline in good yield. Compound **2** was hydrolyzed in tetrahydrofuran by adding 2 *N* sodium hydroxide to give **3**, which was treated with sodium alkoxide to form 2-butoxy-5-fluoropyrimidine-4(3*H*)-one (**4**). The spectra data of compounds **2-4** are in good agreement with literature.[22,26,27]

Procedure for Preparation of 2-Butoxy-4-chloro-5-fluoropyrimindine 5. A stirred mixture of compound **4** (16.0 g, 86 mmol) and phosphorus oxychloride (39.5 g, 260 mmol) was heated to 95 °C and *N*,*N*-dimethylaniline (20.7 g, 171 mmol) was added dropwise over 0.5 h. The mixture was maintained at 95 °C for 15 h before cooling to room temperature, and cautiously quenched by 3 *N* aqueous hydrochloric acid (45 mL), keeping the temperature below 30 °C over 4 h. The mixture was extracted with dichloromethane (80 mL), and the organic layer was washed with water (30 mL) and concentrated under reduced pressure. The residue was purified through column chromatography to give **5**: colorless oil, yield 92.7%; IR ν_{max}(KBr)/cm^{-1} 2961, 1597, 1548, 1384, 1260, 1193, 1029, 778; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.94 (t, 3H, *J* = 7.4 Hz, CH_3), 1.43-1.50 (m, 2H, CH_2), 1.73-1.78 (m, 2H, CH_2), 4.31 (t, 2H, *J* = 6.6 Hz, CH_2), 8.31 (s, 1H, CH); EIMS *m/z* (%) 204 (M^+, 76), 189 (46), 175 (42), 169 (35), 153 (57), 147 (100), 131 (78), 96 (50); HRMS calcd for $C_8H_{10}ClFN_2O$ 204.0466, found 204.0468.

General Procedure for Preparation of Compounds 6a-u. To a stirred solution of **5** (1.0 g, 4.9 mmol) in ethanol (15 mL) was added sodium carbonate (0.5 g, 4.9 mmol), followed by adding amine (5.9 mmol) under reflux. After completion by TLC detection, ethanol was evaporated. The residue was poured into water (20 mL) and extracted with dichloromethane. The extract was washed with water, dried over sodium sulfate, and evaporated to give the crude product, which was purified by recrystallization or column chromatography to afford **6a-u**.

2-Butoxy-5-fluoro-*N*-phenylpyrimidin-4-amine (6a): brown solid, yield 53.8%, mp 55-58 °C; IR ν_{max}(KBr)/cm^{-1} 3181, 3020, 2957, 1615, 1580, 1376, 1054, 747; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.97 (t, 3H, *J* = 7.4 Hz, CH_3), 1.45-1.52 (m, 2H, CH_2), 1.75-1.81 (m, 2H, CH_2), 4.30 (t, 2H, *J* = 6.8 Hz, CH_2), 6.99 (br, 1H, NH), 7.12 (t, 1H, *J* = 7.4Hz, CH), 7.37 (t, 2H, *J* = 7.7 Hz, 2×CH), 7.67 (d, 2H, *J* = 7.9 Hz, 2×CH), 7.98 (d, 1H, *J* = 2.9 Hz, CH). EIMS *m/z* (%) 261 (M^+, 19), 232 (10), 218 (9), 204 (100), 188 (10), 77 (32), 65 (11), 51 (9); HRMS calcd for $C_{14}H_{16}FN_3O$ 261.1277, found 261.1279.

2-Butoxy-5-fluoro-*N*-phenethylpyrimidin-4-amine (6b): white solid, yield 64.3%, mp 55-57 °C; IR ν_{max}(KBr)/cm^{-1} 3171, 3075, 2957, 1620, 1448, 1383, 1047, 700; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.97 (t, 3H, *J* = 7.4 Hz, CH_3), 1.45-1.52 (m, 2H, CH_2), 1.75-1.81 (m, 2H, CH_2), 2.94 (t, 2H, *J* = 6.9 Hz, CH_2), 3.76-3.80 (m, 2H, CH_2), 4.27 (t, 2H, *J* = 6.8 Hz, CH_2), 5.10 (br, 1H, NH), 7.21-7.28 (m, 3H, 3×CH), 7.33 (t, 2H, *J* = 7.4 Hz, 2×CH), 7.77 (d, 1H, *J* = 2.6 Hz, CH); EIMS *m/z* (%) 289 (M^+, 37), 260 (8), 198 (45), 142 (100), 104 (87), 91 (46), 77 (23), 65 (17); HRMS calcd for $C_{16}H_{20}FN_3O$ 289.1590, found 289.1586.

2-Butoxy-5-fluoro-*N*-(4-methoxyphenyl)pyrimidin-4-amine (6c): oil, yield 52.9%; IR ν_{max}(KBr)/cm^{-1} 3448, 3028, 2958, 1609, 1586, 1383, 1070, 698; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.97 (t, 3H, *J* = 7.4 Hz, CH_3), 1.44-1.52 (m, 2H, CH_2), 1.74-1.80 (m, 2H, CH_2), 3.83 (s, 3H, CH_3), 4.27 (t, 2H, *J* = 6.8 Hz, CH_2), 6.67 (br, 1H, NH), 6.76 (d, 2H, *J* = 8.9 Hz, 2×CH), 7.54 (d, 2H, *J* = 9.0 Hz, 2×CH), 7.95 (d, 1H, *J* = 3.0 Hz, CH); EIMS *m/z* (%) 291 (M^+, 42), 275 (18), 236 (22), 218 (100), 106 (35), 91 (10), 77 (33), 51 (13); HRMS calcd for $C_{15}H_{18}FN_3O_2$ 291.1383, found 291.1382.

2-Butoxy-5-fluoro-*N*-(*m*-tolyl)pyrimidin-4-amine (6d): brown solid, yield 50.0%, mp 51-54 °C; IR ν_{max}(KBr)/cm^{-1} 3185, 3038, 1631, 1593, 1376, 1071, 777, 528; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.98 (t, 3H, *J* = 7.0 Hz, CH_3), 1.46-1.53 (m, 2H, CH_2), 1.76-1.82 (m, 2H, CH_2), 2.34 (s, 3H, CH_3), 4.30 (t, 2H, *J* = 6.8 Hz, CH_2), 6.87 (br, 1H, NH), 6.95

(d, 1H, J = 7.5 Hz, CH), 7.26 (t, 1H, J = 7.9 Hz, CH), 7.46 (s, 1H, CH),7.51 (d, 1H, J = 8.0 Hz, CH), 7.98 (d, 1H, J = 3.0 Hz, CH); EIMS *m/z* (%) 275 (M^+, 45), 246 (17), 218 (100), 203 (20), 106 (13), 91 (35), 77 (13), 65 (16); HRMS calcd for $C_{15}H_{18}FN_3O$ 275.1434, found 275.1436.

2-Butoxy-5-fluoro-*N*-(*p*-tolyl)pyrimidin-4-amine (6e): brown solid, yield 64.7%, mp 62-64 °C; IR ν_{max}(KBr)/cm^{-1} 3185, 3066, 2960, 1639, 1601, 1048, 813, 509; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.98 (t, 3H, J = 7.5 Hz, CH_3), 1.45-1.53 (m, 2H, CH_2), 1.75-1.81 (m, 2H, CH_2), 2.35 (s, 3H, CH_3), 4.29 (t, 2H, J = 6.8 Hz, CH_2), 6.93 (br, 1H, NH), 7.18 (d, 2H, J = 8.3 Hz, 2×CH), 7.54 (d, 2H, J = 8.4 Hz, 2×CH), 7.97 (d, 1H, J = 3.0 Hz, CH); EIMS *m/z* (%) 275 (M^+, 21), 246 (7), 218 (100), 203 (10), 106 (12), 91 (15), 77 (8), 65 (8); HRMS calcd for $C_{15}H_{18}FN_3O$ 275.1434, found 275.1433.

2-Butoxy-*N*-(3-chlorophenyl)-5-fluoropyrimidin-4-amine (6f): white solid, yield 60.2%, mp 69-73 °C; IR ν_{max}(KBr)/cm^{-1} 3180, 3063, 1627, 1591, 1380, 1053, 768, 675; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.98 (t, 3H, J = 7.5 Hz, CH_3), 1.46-1.54 (m, 2H, CH_2), 1.77-1.83 (m, 2H, CH_2), 4.31 (t, 2H, J = 6.9 Hz, CH_2), 6.95 (br, 1H, NH), 7.10 (d, 1H, J = 7.9 Hz, CH), 7.28 (t, 1H, J = 8.1 Hz, CH), 7.47 (d, 1H, J = 8.3 Hz, CH), 7.87 (s, 1H, CH), 8.03 (d, 1H, J = 2.8 Hz, CH); EIMS *m/z* (%) 295 (M^+, 18), 266 (10), 238 (100), 223 (12), 169 (5), 127 (5), 111 (17), 91 (3); HRMS calcd for $C_{14}H_{15}ClFN_3O$ 295.0888, found 295.0885.

***N*-benzyl-2-butoxy-5-fluoropyrimidin-4-amine (6g):** brown solid, yield 69.2%, mp 55-57 °C; IR ν_{max}(KBr)/cm^{-1} 3180, 3031, 2955, 1623, 1494, 1368, 777, 698; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.95 (t, 3H, J = 7.4 Hz, CH_3), 1.44-1.48 (m, 2H, CH_2), 1.72-1.76 (m, 2H, CH_2), 4.25 (t, 2H, J = 6.8 Hz, CH_2), 4.69 (d, 2H, J = 5.7 Hz, CH_2), 5.44 (br, 1H, NH), 7.26-7.38 (m, 5H, 5×CH), 7.81 (d, 1H, J = 3.0 Hz, CH); EIMS *m/z* (%) 275 (M^+, 23), 246 (10), 219 (35), 184 (10), 106 (45), 91 (100), 77 (8), 65 (15); HRMS calcd for $C_{15}H_{18}FN_3O$ 275.1434, found 275.1435.

2-Butoxy-*N*-(3,4-dichlorophenyl)-5-fluoropyrimidin-4-amine (6h): white solid, yield 55.6%, mp 118-120 °C; IR ν_{max}(KBr)/cm^{-1} 3294, 3086, 2960, 1633, 1588, 1052, 776, 685; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.99 (t, 3H, J = 7.5 Hz, CH_3), 1.47-1.54 (m, 2H, CH_2), 1.77-1.84 (m, 2H, CH_2), 4.31 (t, 2H, J = 6.8 Hz, CH_2), 6.90 (br, 1H, NH), 7.42 (d, 1H, J = 8.7 Hz, CH), 7.47 (dd, 1H, J_1 = 2.5 Hz, J_2 = 8.8 Hz, CH), 7.99 (d, 1H, J = 2.5 Hz, CH), 8.05 (d, 1H, J = 2.7 Hz, CH); EIMS *m/z* (%) 329 (M^+, 22), 300 (10), 257 (12), 274 (100), 145 (12), 125 (5), 109 (10), 91 (5); HRMS calcd for $C_{14}H_{14}Cl_2FN_3O$ 329.0498, found 329.0497.

2-Butoxy-*N*-(3-chloro-4-fluorophenyl)-5-fluoropyrimidin-4-amine (6i): yellow crystal, yield 45.5%, mp 77-79 °C; IR ν_{max}(KBr)/cm^{-1} 3296, 3093, 1634, 1587, 1383, 1207, 1052, 813; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.98 (t, 3H, J = 7.4 Hz, CH_3), 1.46-1.53 (m, 2H, CH_2),1.76-1.82 (m, 2H, CH_2), 4.29 (t, 2H, J = 6.8 Hz, CH_2), 6.87 (br, 1H, NH), 7.14 (t, 1H, J = 8.7 Hz, CH), 7.45 (dq, 1H, J_1 = 2.9 Hz, J_2 = 8.9 Hz, CH), 7.89 (d, 1H, J = 9.2 Hz, CH), 8.03 (d, 1H, J = 2.3 Hz, CH); EIMS *m/z* (%) 313 (M^+, 23), 284 (10), 256 (100), 241 (13), 214 (12), 145 (10), 129 (15), 109 (7); HRMS calcd for $C_{14}H_{14}ClF_2N_3O$ 313.0793, found 313.0795.

2-Butoxy-5-fluoro-*N*-(3-(trifluoromethyl)phenyl)pyrimidin-4-amine (6j): brown solid, yield 75.0%, mp 135-136 °C; IR ν_{max}(KBr)/cm^{-1} 3201, 3044, 2962, 1634, 1587, 1117, 800, 698; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.97 (t, 3H, J = 7.4 Hz, CH_3), 1.47-1.51 (m, 2H, CH_2), 1.76-1.82 (m, 2H, CH_2), 4.31 (t, 2H, J = 6.8 Hz, CH_2), 7.07 (br, 1H, NH), 7.38 (d, 1H, J = 7.8 Hz, CH), 7.49 (t, 1H, J = 8.1 Hz, CH), 7.75 (d, 1H, J = 8.1 Hz, CH), 8.06 (d, 1H, J = 2.8 Hz, CH), 8.20 (s, 1H, CH); EIMS *m/z* (%) 329 (M^+, 17), 310 (5), 300 (10), 272 (100), 257 (12), 230 (10), 145 (20), 63 (4); HRMS calcd for $C_{15}H_{15}F_4N_3O$ 329.1151, found 329.1150.

2-Butoxy-*N*-(4-chlorophenyl)-5-fluoropyrimidin-4-amine (6k): brown solid, yield 82.8%, mp 106-108 °C; IR ν_{max} (KBr)/cm^{-1} 3203, 3075, 2959, 1632, 1600, 1378, 825, 506; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.98 (t, 3H, J = 7.4 Hz, CH_3), 1.47-1.52 (m, 2H, CH_2), 1.76-1.82 (m, 2H, CH_2), 4.29 (t, 2H, J = 6.8 Hz, CH_2), 6.85 (br, 1H, NH), 7.34 (d, 2H, J = 8.9 Hz, 2×CH), 7.63 (d, 2H, J = 8.9 Hz, 2×CH), 8.01 (d, 1H, J = 2.9 Hz, CH); EIMS *m/z* (%) 295 (M^+, 17), 266 (10), 238 (100), 223 (10), 169 (5), 111 (13), 99 (7), 75 (10); HRMS calcd for $C_{14}H_{15}ClFN_3O$ 295.0888, found 295.0892.

***N*-(4-Bromophenyl)-2-butoxy-5-fluoropyrimidin-4-amine (6l):** white solid, yield 53.4%, mp 109-111 °C; IR ν_{max}(KBr)/cm^{-1} 3260, 3190, 3075, 1623, 1589, 1371, 823, 504; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.98 (t, 3H, J = 7.4 Hz, CH_3), 1.45-1.53 (m, 2H ,CH_2), 1.76-1.81 (m, 2H, CH_2), 4.29 (t, 2H, J = 6.8 Hz, CH_2), 6.89 (br, 1H, NH), 7.48 (d, 2H, J = 8.8 Hz, 2×CH), 7.58 (d, 2H, J = 8.8 Hz, 2×CH), 8.01 (d, 1H, J = 2.8 Hz, CH); EIMS *m/z* (%) 339 (M^+, 18), 310 (6), 267 (9), 187 (18), 155 (15), 111 (9), 91 (13), 76 (15); HRMS calcd for $C_{14}H_{15}BrFN_3O$ 339.0383, found 339.0380.

4-(2-Butoxy-5-fluoropyrimidin-4-yl)morpholine (6m): white solid, yield 72.0%, mp 45-46 °C; IR ν_{max}(KBr)/cm^{-1} 3190, 3037, 2959, 1638, 1608, 1370, 774, 640; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.92 (t, 3H, J = 7.4 Hz, CH_3), 1.39-1.46 (m, 2H, CH_2), 1.68-1.74 (m, 2H, CH_2), 3.74 (s, 8H, 4×CH_2), 4.19 (t, 2H, J = 6.7 Hz, CH_2), 7.84 (d, 1H, J = 6.1 Hz, CH); EIMS *m/z* (%) 255 (M^+, 71), 239 (10), 226 (42), 200 (32), 183 (35), 168 (45), 154 (33), 142 (100), 86 (41); HRMS calcd for $C_{12}H_{18}FN_3O_2$ 255.1383, found 255.1381.

2-Butoxy-5-fluoro-4-(piperidin-1-yl)pyrimidine (6n): oil, yield 58.3%; IR ν_{max}(KBr)/cm^{-1} 3037, 2960, 1603, 1568, 1378, 1075, 776, 640; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (t, 3H, CH_3), 1.34-1.42 (m, 2H, CH_2), 1.54-1.60 (m, 6H, 3×CH_2), 1.63-1.69 (m, 2H, CH_2), 3.63 (d, 4H, J = 5.1 Hz, 2×CH_2), 4.14 (t, 2H, J = 6.7 Hz, CH_2), 7.72 (d, 1H, J = 6.5 Hz, CH); EIMS *m/z* (%) 253 (M^+, 90), 237 (20), 224 (70), 197 (80), 181 (56), 168 (100), 142 (55), 114 (58); HRMS calcd for $C_{13}H_{20}FN_3O$ 253.1590, found 253.1591.

2-Butoxy-5-fluoro-4-(pyrrolidin-1-yl)pyrimidine (6o): oil, yield 66.7%; IR ν_{max}(KBr)/cm^{-1} 3048, 2936, 1603, 1498, 1420, 1382, 1070, 776; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, 3H, J = 7.4 Hz, CH_3), 1.35-1.43 (m, 2H, CH_2), 1.65-1.70 (m, 2H, CH_2), 1.88 (s, 4H, 2×CH_2), 3.60 (s, 4H, 2×CH_2), 4.16 (t, 2H, J = 6.8 Hz, CH_2), 7.70 (d, 1H, J = 5.4 Hz, CH); EIMS *m/z* (%) 239 (M^+, 97), 223 (10), 210 (80), 197 (35),

183 (97), 167 (75), 128 (80), 113 (28); HRMS calcd for $C_{12}H_{18}FN_3O$ 239.1434, found 239.1433.

2-Butoxy-*N*-butyl-5-fluoropyrimidin-4-amine (6p): oil, yield 66.7%; IR ν_{max}(KBr)/cm^{-1} 3418, 3050, 2958, 1607, 1498, 286, 1073, 780; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.868 (t, 3H, *J* = 7.4 Hz, CH_3), 0.870 (t, 3H, *J* = 7.4 Hz, CH_3), 1.30-1.41 (m, 4H, 2×CH_2), 1.52-1.55 (m, 2H, CH_2), 1.64-1.69 (m, 2H, CH_2), 3.39-3.43 (m, 2H, CH_2), 4.16 (t, 2H, *J* = 6.8 Hz, CH_2), 5.23 (br, 1H, NH), 7.67 (d, 1H, *J* = 3.1 Hz, CH); EIMS *m/z* (%) 241 (M^+, 85), 225 (38), 212 (70), 198 (25), 170 (58), 156 (86), 129 (95), 113 (42); HRMS calcd for $C_{12}H_{20}FN_3O$ 241.1590, found 241.1588.

2-Butoxy-*N*-(*tert*-butyl)-5-fluoropyrimidin-4-amine (6q): oil, yield 55.0%; IR ν_{max}(KBr)/cm^{-1} 3293, 3045, 1616, 1522, 1213, 1374, 1069, 780; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.95 (t, 3H, *J* = 7.5 Hz, CH_3), 1.44-1.49 (m, 11H, CH_2 + 3×CH_3), 1.71-1.77 (m, 2H, CH_2), 4.22 (t, 2H, *J* = 6.7 Hz, CH_2), 4.90 (br, 1H, NH), 7.75 (d, 1H, *J* = 3.2 Hz, CH); EIMS *m/z* (%) 241 (M^+, 75), 226 (31), 212 (22), 185 (42), 170 (100), 156 (36), 113 (38), 57 (55); HRMS calcd for $C_{12}H_{20}FN_3O$ 241.1590, found 241.1583.

2-Butoxy-5-fluoropyrimidin-4-amine (6r): white solid, yield 88.9%, mp 61-63 °C; IR ν_{max}(KBr)/cm^{-1} 3440, 3036, 2961, 1604, 1382, 952, 775, 632; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.95 (t, 3H, *J* = 7.4 Hz, CH_3), 1.42-1.50 (m, 2H, CH_2), 1.70-1.76 (m, 2H, CH_2), 4.22 (t, 2H, *J* = 6.7 Hz, CH_2), 5.33 (br, 2H, NH_2), 7.88 (d, 1H, *J* = 2.7 Hz, CH); EIMS *m/z* (%) 185 (M^+, 21), 156 (28), 143 (18), 130 (100), 113 (33), 101 (35), 86 (52), 73 (15); HRMS calcd for $C_8H_{12}FN_3O$ 185.0964, found 185.0971.

2-Butoxy-*N*-cyclohexyl-5-fluoropyrimidin-4-amine (6s): oil, yield 69.2%; IR ν_{max}(KBr)/cm^{-1} 3185, 3052, 2964, 1616, 1382, 1072, 780, 734; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.89 (t, 3H, *J* = 7.1 Hz, CH_3), 1.13-1.17 (m, 3H , 3×CH), 1.31-1.42 (m, 4H, CH_2 + 2×CH), 1.57-1.59 (m, 1H, CH), 1.67-1.69 (m, 4H, CH_2 + 2×CH), 1.95-1.97 (m, 2H, 2×CH), 3.95-3.97 (m, 1H, CH), 4.17 (t, 2H, *J* = 6.5 Hz, CH_2), 4.92 (d, 1H, *J* = 5.6 Hz, NH), 7.68 (s, 1H, CH); EIMS *m/z* (%) 267 (M^+, 97), 238 (42), 185 (42), 168 (60), 154 (100), 129 (99), 86 (33), 55 (52); HRMS calcd for $C_{14}H_{22}FN_3O$ 267.1747, found 267.1749.

2-Butoxy-*N*-(*sec*-butyl)-5-fluoropyrimidin-4-amine (6t): oil, yield 75.0%; IR ν_{max}(KBr)/cm^{-1} 3440, 3040, 1605, 1580, 1382, 1071, 780, 733; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.842 (t, 3H, *J* = 7.4 Hz, CH_3), 0.845 (t, 3H, *J* = 7.4 Hz, CH_3), 1.12 (d, 3H, *J* = 6.5 Hz, CH_3), 1.32-1.40 (m, 2H, CH_2), 1.43-1.54 (m, 2H, CH_2), 1.62-1.68 (m, 2H, CH_2), 4.06-4.11 (m, 1H, CH), 4.14 (t, 2H, *J* = 6.8 Hz, CH_2), 4.88 (d, 1H, *J* = 7.7 Hz, NH), 7.65 (d, 1H, *J* = 3.0 Hz, CH); EIMS *m/z* (%) 241 (M^+, 60), 212 (70), 186 (54), 170 (72), 156 (100), 130 (98), 113 (33), 86 (24); HRMS calcd for $C_{12}H_{20}FN_3O$ 241.1590, found 241.1589.

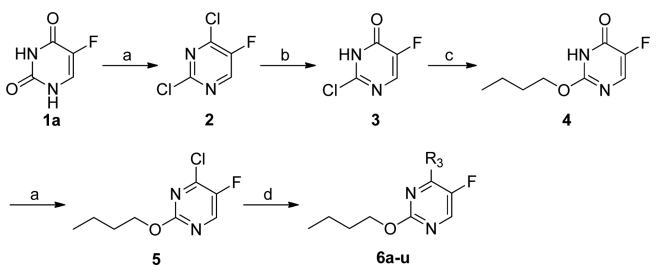
2-Butoxy-5-fluoro-*N*-propylpyrimidin-4-amine (6u): oil, yield 72.7%; IR ν_{max}(KBr)/cm^{-1} 3185, 3050, 2961, 1618, 1383, 1074, 780, 735; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (t, 3H, *J* = 7.4 Hz, CH_3), 0.89 (t, 3H, *J* = 7.4 Hz, CH_3), 1.35-1.40 (m, 2H, CH_2), 1.54-1.59 (m, 2H, CH_2), 1.64-1.69 (m, 2H, CH_2), 3.36-3.40 (m, 2H, CH_2), 4.16 (t, 2H, *J* = 6.8 Hz, CH_2), 5.26 (br, 1H, NH), 7.67 (d, 1H, *J* = 3.1 Hz, CH); EIMS *m/z* (%) 227 (M^+, 58), 198 (45), 184 (18), 176 (100), 156 (76), 142 (99), 129 (97), 113 (38); HRMS calcd for $C_{11}H_{18}FN_3O$ 227.1434, found 227.1433.

Cytotoxic activity against cancer cell lines. Human cancer cell lines (BEL-7402, A549, MCF-7 and HL-60 derived from Shanghai Institutes for Biological Science, Chinese Academy of Sciences) were cultivated at 37 °C, 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM, purchased from Gibco) supplemented with 800 (U/v) penicillin, 0.1% (w/v) streptomycin, 10% (v/v) fetal bovine serum for 3-5 d. Then the human cancer cells, treated with Trypsin-EDTA solution, were dissolved to 10^5 cells/mL by culture medium and seeded into 96-well plates at 100 μL/well and incubated in a 5% CO_2 incubator at 37 °C for 24 h. The cells were treated with compounds synthesized at different concentrations in DMSO solution. Mitochondrial metabolism was measured as a marker for cell growth by adding 10 μL/well MTT (5 mg/mL in medium, Sigma) and incubating 3 h at 37 °C. Crystals formed were dissolved in 150 μL of DMSO. The absorbance was determined using a microplate reader at 570 nm. The absorbance data were converted into a cell proliferation percentage, compared to DMSO treated cells, to determine growth inhibition. Triplicate testing was performed for each test compound.

Results and Discussion

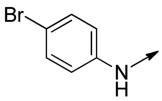
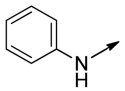
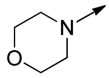
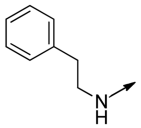
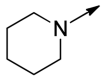
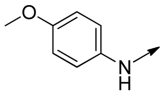
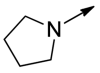
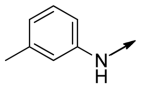
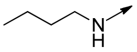
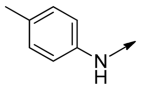
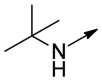
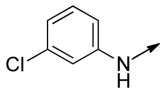
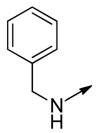
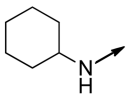
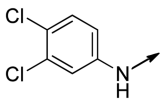
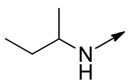
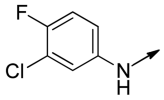
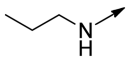
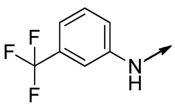
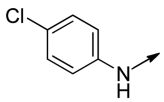
Chemistry. 2,4-Dichloro-5-fluoropyrimidine (**2**) was prepared from 5-FU by reaction with phosphorus oxychloride and *N*,*N*-dimethylaniline in high yield. Compound **2** was hydrolyzed in tetrahydrofuran by adding 2 *N* sodium hydroxide to give 2-chloro-5-fluoropyrimidine-4(3*H*)-one (**3**). 2-Butoxy-5-fluoropyrimidine-4(3*H*)-one (**4**) was prepared by compound **3** and sodium *n*-butanolate refluxing in *n*-butanol. Compound **4** was converted to 2-butoxy-4-chloro-5-fluoropyrimindine (**5**) by reacting with phosphorus oxychloride and *N*,*N*-dimethylaniline for the required time. The desired compounds **6a-u** were obtained by treating compound **5** with different amines, as shown in Scheme 1. The structures of compounds **5**, **6a-u** were confirmed by IR, ^{1}H NMR, MS and HRMS.

Cytotoxic Activity *in vitro*. Preliminary screening of the cytotoxic activity of compounds **5**, **6a-u** was carried out by



Scheme 1. Synthesis of 5-FU derivatives. Reagents and conditions: (a) $POCl_3$, *N*,*N*-dimethylaniline, 95 °C, 15 h; (b) 2 *N* NaOH; (c) Na, *n*-BuOH, reflux, 2 h; (d) amine, EtOH, Na_2CO_3, reflux.

Table 1. Preliminary screening of cytotoxic activity against BEL-7402 cell line[a]

Compd	R_3	MW	clog*P*	IC_{50} (μM)	Compd	R_3	MW	clog*P*	IC_{50} (μM)
5	—	204.6	2.94	**1.26**	**6l**		340.2	5.06	4.41
6a		261.3	4.25	19.7	**6m**		255.3	2.21	**1.32**
6b		289.4	3.92	**0.88**	**6n**		253.3	3.27	13.68
6c		291.3	4.31	**1.17**	**6o**		239.3	2.77	25.30
6d		275.3	4.68	**0.44**	**6p**		241.3	3.56	**0.19**
6e		275.3	4.70	**0.26**	**6q**		241.3	3.30	14.32
6f		295.7	4.91	**0.25**	**6r**		185.2	1.74	> 100
6g		275.3	3.52	19.69	**6s**		267.3	4.02	**0.15**
6h		330.2	5.54	11.10	**6t**		241.3	3.33	**0.10**
6i		313.7	5.02	17.93	**6u**		227.3	3.00	**0.26**
6j		329.3	5.12	> 100	**5-FU**	—	130.1	–0.59	1.49
6k		295.7	4.93	4.41					

[a]IC_{50} values presented are means of three experiments; values in bold are better than that of 5-FU.

MTT assay against human liver BEL-7402 cancer cell line *in vitro*. The results were summarized in Table 1. It suggested that the cytotoxic activity of compounds **5**, **6b-f**, **6m**, **6p**, **6s-u** exhibited superior or equivalent cytotoxic effect to 5-FU against BEL-7402, several of which were in sub-micromolar scale. Some compounds demonstrated dose-dependent cytotoxicity on BEL-7402 cell line that the cytotoxic activity decreased with the concentration declined, which indicated that these compounds were not sensitive to BEL-7402 at lower concentration when O^2, O^4 positions of 5-FU occupied. For example, the cytotoxic activity of compound **6q** decreased rapidly from 10^{-3} M to 10^{-4} M.

Besides, in view of a possible development of these compounds, their druglike properties following the Lipinski's rule of five were examined.[28] Most compounds met the requirements of this rule being HBD < 5, HBA < 10, MW < 500 and log*P* < 5. Evaluation of lipophilicity was performed by measuring partition coefficients (log*P*) with Molinspiration property engine and the results were listed in Table 1.[29]

With these results, ten compounds were selected to

Table 2. Cytotoxic activity against A549, HL-60 and MCF-7 cell lines[a]

Compd	IC_{50} (μM)		
	A549	HL-60	MCF-7
5	**0.10**	**1.66**	**0.59**
6a	39.95	14.08	32.76
6b	73.36	> 100	40.53
6d	23.79	59.50	**9.73**
6e	60.55	**8.83**	60.44
6f	61.55	46.64	56.10
6h	51.12	72.47	57.21
6i	55.31	> 100	11.22
6k	43.76	> 100	21.44
6l	38.04	66.58	48.27
5-FU	0.95	64.49	48.35
cisplatin	28.00	10.86	11.70

[a]Values presented are means of three experiments; values in bold are better than that of 5-FU and cisplatin.

evaluate their cytotoxic activity against human lung A549, leukemia HL-60 and breast MCF-7 cancer cell lines *in vitro*. The results were summarized in Table 2.

Generally, the results showed that these compounds were more sensitive to BEL-7402 cell line. The highest cytotoxic potency was shown by chloro-substituted 2-butoxy-5-fluoropyrimidine **5** with IC_{50} values of 0.10, 1.66, 0.59 μM against A549, HL-60 and MCF-7, respectively. However, compound **5** might not suitable for the potential hit compound due to its reactive chloride functional group. Compounds **6d** and **6e** were effective against MCF-7 with IC_{50} 9.73 μM and HL-60 with IC_{50} 8.83 μM, respectively. Substituent effect of R_3 was not obvious and little structure-activity relationship could be drawn. The calculated log*P* of compounds **5**, **6d** and **6e** are 2.94, 4.68 and 4.70, respectively. The cytotoxicity might be associated with the 5-FU releasing ability in these *O*-substituted derivatives. The lability of some potent compounds as prodrugs should be further investigated.

Conclusion

A series of *O*-substituted 5-FU derivatives were synthesized and their cytotoxic activity was evaluated against BEL-7402, A549, HL-60 and MCF-7 cancer cell lines *in vitro*. The results indicated that the introduction of proper substituents to O^4 position of 2-butoxy-5-fluoropyrimidines could improve their cytotoxic activity. Compound **5** and some compounds **6b-f**, **6m**, **6p**, **6s-u** exhibited potential cytotoxic activity in micro or sub-micromolar scale. Further studies including the 5-FU releasing ability *in vivo* and lability of compounds are being undertaken.

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References

1. Duschinsky, R.; Pleven, E.; Heidelberger, C. *J. Am. Chem. Soc.* **1957**, *79*, 4559.
2. Noordhuis, P.; Holwerda, U.; Van der Wilt, C. L.; Van Groeningen, C. J.; Smid, K.; Meijer, S.; Pinedo, H. M.; Peters, G. J. *Ann. Oncol.* **2004**, *15*, 1025.
3. Kametani, T.; Kigasawa, K.; Hiiragi, M.; Wakisaka, K.; Nakazato, K.; Ichikawa, K.; Fukawa, K.; Irino, O.; Nishimura, N.; Okada, T. *J. Med. Chem.* **1982**, *25*, 1219.
4. Tian, Z. Y.; Du, G. J.; Xie, S. Q.; Zhao, J.; Gao, W. Y.; Wang, C. J. *Molecules* **2007**, *12*, 2450.
5. Pan, X. Y.; Wang, C.; Wang, F.; Li, P. F.; Hu, Z. G.; Shan, Y. Y.; Zhang, J. *Curr. Med. Chem.* **2011**, *18*, 4538.
6. Sun, Y. W.; Chen, K. M.; Kwon, C. H. *Mol. Pharm.* **2006**, *3*, 161.
7. Yin, P.; Hu, M. L.; Hu, L. C. *J. Mol. Struct.* **2008**, *882*, 75.
8. Xiong, J.; Zhu, H. F.; Zhao, Y. J.; Lan, Y. J.; Jiang, J. W.; Yang, J. J.; Zhang, S. F. *Molecules* **2009**, *14*, 3142.
9. Daumar, P.; Decombat, C.; Chezal, J. M.; Debiton, E.; Madesclaire, M.; Coudert, P.; Galmier, M. J. *Eur. J. Med. Chem.* **2011**, *46*, 2867.
10. Wang, Q. W.; Liu, X. Y.; Liu, L.; Feng, J.; Li, Y. H.; Guo, Z. J.; Mei, Q. B. *Med. Chem. Res.* **2007**, *16*, 370.
11. Zhang, Z. S.; Zhang, Q. B.; Wang, J.; Shi, X. L.; Zhang, J. J.; Song, H. F. *Carbohydr. Polym.* **2010**, *79*, 628.
12. Liu, J.; Kolar, C.; Lawson, T. A.; Gmeiner, W. H. *J. Org. Chem.* **2001**, *66*, 5655.
13. Qian, S.; Wu, J. B.; Wu, X. C.; Li, J.; Wu, Y. *Arch. Pharm.* **2009**, *342*, 513.
14. Im, J.; Biswas, G.; Kim, W.; Kim, K. T.; Chung, S. K. *Bull. Korean Chem. Soc.* **2011**, *32*, 873.
15. Meunier, B. *Acc. Chem. Res.* **2008**, *41*, 69.
16. Tsogoeva, S. B. *Mini-Rev. Med. Chem.* **2010**, *10*, 773-793.
17. Liu, Y. Q.; Dai, W.; Yang, L.; Li, H. Y. *Nat. Prod. Res.* **2011**, *25*, 1817.
18. Menger, F. M.; Rourk, M. J. *J. Org. Chem.* **1997**, *62*, 9083.
19. Wang, X. Y.; Lin, J.; Zhang, X. M.; Liu, Q.; Xu, Q.; Tan, R. X.; Guo, Z. J. *J. Inorg. Biochem.* **2003**, *94*, 186.
20. Chen, S. W.; Xiang, R.; Liu, J.; Tian, X. *Bioorg. Med. Chem.* **2009**, *17*, 3111.
21. Zhou, W. M.; He, R. R.; Ye, J. T.; Zhang, N.; Liu, D. Y. *Molecules* **2010**, *15*, 2114.
22. Yamashita, J.; Yamawaki, I.; Ueda, S.; Yasumoto, M.; Unemi, N.; Hashimoto, S. *Chem. Pharm. Bull.* **1982**, *30*, 4258.
23. Benko, Z.; Boebel, T.; Breaux, N.; Bryan, K.; Davis, G.; Epp, J.; Lorsbach, B.; Martin, T.; Meyer, K.; Nader, B.; Owen, W.; Pobanz, M.; Ruiz, J.; Smith, F.; Sullenberger, M.; Webster, J.; Yao, C.; Young, D. (Dow AgroSciences). WO 2009094442. 2009 [*Chem. Abstr.* **2009**, *151*, 198448].
24. Sun, C. J.; Wang, R. C. *Pharm. Ind.* **1986**, *17*, 10.
25. Zhou, W.; Sun, J.; Shan, S.; Hu, W. (Zhejiang Univ Technol). CN 102659689 A. 2012 [*Chem. Abstr.* **2012**, *157*, 492731].
26. Sun, Z.; Wang, H.; Wen, K.; Li, Y.; Fan, E. *J. Org. Chem.* **2011**, *76*, 4149.
27. Kheifets, G. M.; Gindin, V. A.; Studentsov, E. P. *Russ. J. Org. Chem.* **2006**, *42*, 580.
28. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **2001**, *46*, 3.
29. Molinspiration Property Engine v2011.04, http://www.molinspiration.com/cgi-bin/properties, accessed on Oct. 28, 2012.