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Synthesis and biological evaluation of novel 2,4,5-substituted pyrimidine derivatives for anticancer activity

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ABSTRACT

A series of novel 2,4,5-substituted pyrimidine derivatives were synthesized and evaluated for inhibition against the human hepatocellular carcinoma BEL-7402 cancer cell line. Several compounds showed potent inhibition with an IC_{50} value less than 0.10 μM. Structure–activity relationships for this class of compounds at the 2- and 5-position of the pyrimidine scaffold have been elucidated. The most active compound **7gc** showed good inhibition of several different human cancer cell lines with IC_{50} values from 0.024 to 0.55 μM.

Pyrimidine is found as a core structure in a large variety of compounds that exhibit important biological activity.[1–9]

Specifically, 2,4-disubstituted and 2,4,6-trisubstituted pyrimidines have shown potent anticancer activity as CDK inhibitors,[10] TNF-α inhibitors,[11] Abl tyrosine protein kinase inhibitors,[12] PI-3 kinase inhibitors,[13] Akt kinase inhibitors,[14] and cytokines inhibitors.[15]

The use of combinatorial approaches toward the synthesis of drug-like scaffolds is a powerful tool in helping to speed up drug discovery. Recently, we have developed an efficient method to generate 2,4,5-substituted pyrimidine libraries using a three-component one-pot reaction.[16] Through biological activity screening, compound **1** (Fig. 1) was found to exhibit potent inhibition against the human hepatocellular carcinoma cell line BEL-7402 (IC_{50} = 1.02 μM) and lung cancer cell line A549 (IC_{50} = 2.40 μM). Based on the activity of compound **1**, a series of novel 2,4,5-pyrimidine derivatives were synthesized using modifications at the 2-position and ring B of the pyrimidine moiety. These compounds were evaluated for inhibition against the human hepatocellular carcinoma BEL-7402 cancer cell line. The structure–activity relationships (SARs) for this class of compounds have been clarified.

Initial SAR data indicated aryl or bulky substitution at the 2-position of pyrimidine was unfavorable for anticancer activity.[16] Therefore, we designed and synthesized compounds with less bulky groups and different electronic effects at the 2-position to investigate further SARs (Scheme 1). By the convenient condensation of intermediate **2** with commercially available amidines, compounds **1a–c** were prepared. The subsequent protection of **1** with a Bn group and then oxidation with SeO_2 gave the carboxylic acid **3**. Compound **3** was refluxed with $SOCl_2$ in methanol to afford methyl ester **4**. Both **3** and **4** were hydrogenated in the presence of 10% Pd–C to obtain the desired products **1d** and **1e**. In addition, methyl ester **4** was exchanged with methyl amine and dimethyl amine, followed by deprotection of the Bn group to give pyrimidines **1f** and **1g**.

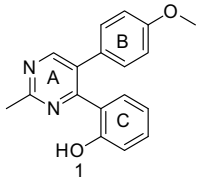

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Figure 1. Molecular structure of active compound **1.**

The activity of compound **1a** is close to the parent compound **1** for against the BEL-7402 cancer cell line. Compound **1c**,[17a] with an amine group, exhibits good inhibition with an IC_{50} of 0.67 μM. Comparing with electron-donating substituted compounds, compounds **1d–g** with electron-withdrawing groups at the 2-position led to poor activity (Table 1).

Based on the preliminary SARs, the focused pyrimidine library with modifications of ring B was designed and prepared from iodochromone **5** using various boronic acids and acetamidine or guanidine by a one-pot reaction (Table 2). Compounds **6a**, **7a**, **7b**, **6c**, **6d**, and **7d** with the different substitutions including methoxy group at the *ortho*- or *meta*-position on the phenyl ring B, did not

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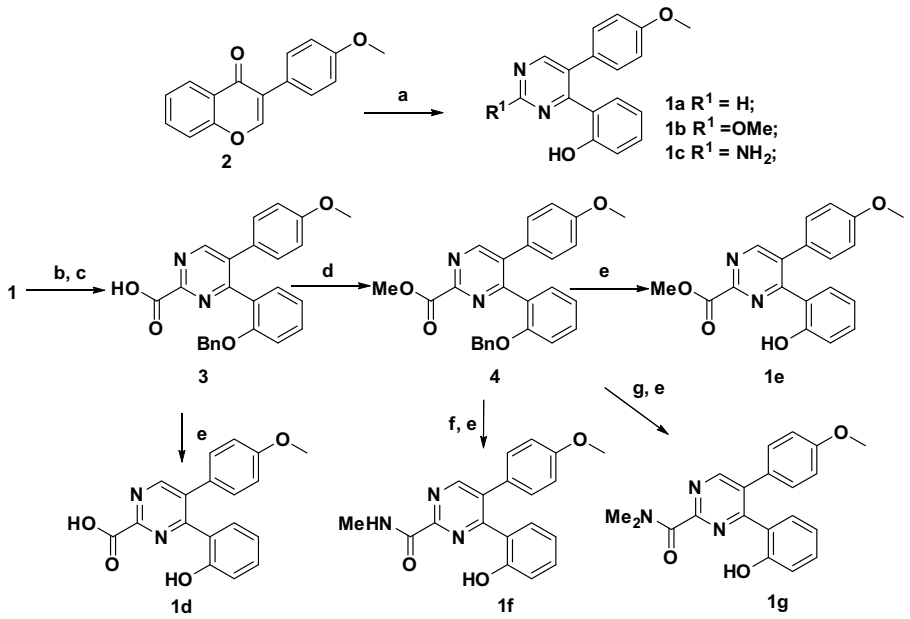
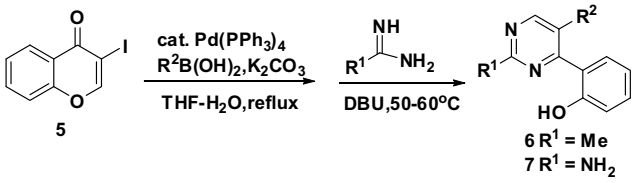


Scheme 1. Reactions and conditions: (a) K_2CO_3, amidine, DMF, 70 °C; (b) BnBr, K_2CO_3, THF, reflux; (c) SeO_2, pyridine, reflux; (d) $SOCl_2$, MeOH, reflux; (e) 10% Pd–C, MeOH, 50 °C; (f) aq $MeNH_2$, MeOH, reflux; (g) aq Me_2NH, MeOH, reflux.

Table 1
2-Substitued effect of pyrimidine derivatives for BEL-7402 inhibition

Compound	R^1	IC $_{50}$ (μM)	Compound	R^1	IC $_{50}$ (μM)
1	Me	1.02	**1d**	CO_2H	>10
1a	H	1.43	**1e**	CO_2Me	>10
1b	OMe	6.00	**1f**	CONHMe	>10
1c	NH_2	0.67	**1g**	$CONMe_2$	>10

Table 2
Study on the SARs of 5-substituted pyrimidine derivatives for BEL-7402 inhibition

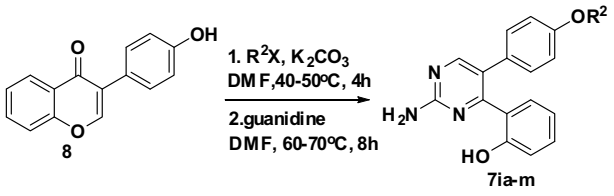


Compound	R^1	R^2	Yield (%)	IC_{50} (μM)
6a	Me	2-FPh	55	>10
7a	NH_2	2-FPh	50	>10
7b	NH_2	2-OMePh	44	>10
6c	Me	3-OMePh	51	>10
6d	Me	3-CF_3Ph	58	>10
7d	NH_2	3-CF_3Ph	52	>10
6e	Me	4-ClPh	53	>10
6f	Me	4-NHBocPh	45	>10
7f	NH_2	4-NHBocPh	41	>10
6g[a]	Me	4-NH_2Ph	85	>10
7g[a]	NH_2	4-NH_2Ph	80	>10
6h	Me	4-OCF_3Ph	57	>10
7h	NH_2	4-OCF_3Ph	55	>10
7i	NH_2	4-OHPh	40	>10
7j	NH_2	4-SMePh	45	0.09
6k	Me	3-Me-4-OMePh	52	0.10
7k	NH_2	3-Me-4-OMePh	49	0.12
7l	NH_2	3,4,-Di-OMePh	55	4.10
6m	Me	3,4,5-Tri-OMePh	53	>10
7m	NH_2	3,4,5-Tri-OMePh	47	>10
6n	Me	3-Pyridinyl	38	>10
7n	NH_2	3-Pyridinyl	35	>10
6o	Me	4-Pyridinyl	39	>10
7o	NH_2	4-Pyridinyl	34	>10

show inhibitory activity. With Cl, Boc-protected amine, amine, hydroxyl and triflurometho xy at the *para*-position of ring B, the compounds also exhibited no activity. Similar to the parent compound **1**, *para*-methylthio substituted compound **7j**[17b] increased the activity with an IC_{50} of 0.09 μM. When bearing a methoxy group at the *para*-position and a methyl group at the *meta*-position of the phenyl ring, compound **6k**[17c] and **7k**[17d] also improved activities with IC_{50}'s of 0.10 and 0.12 μM, respectively. With methoxy substitution at either 3,4-position or 3,4,5-position of the phenyl ring, compounds **7l**, **6m**, and **7m** decrease inhibitory activities. When the aromatic ring was changed to a pyridine ring, the compounds **6n**, **7n**, **6o**, and **7o** did not exhibit inhibition of the BEL-7402 cancer cell line.

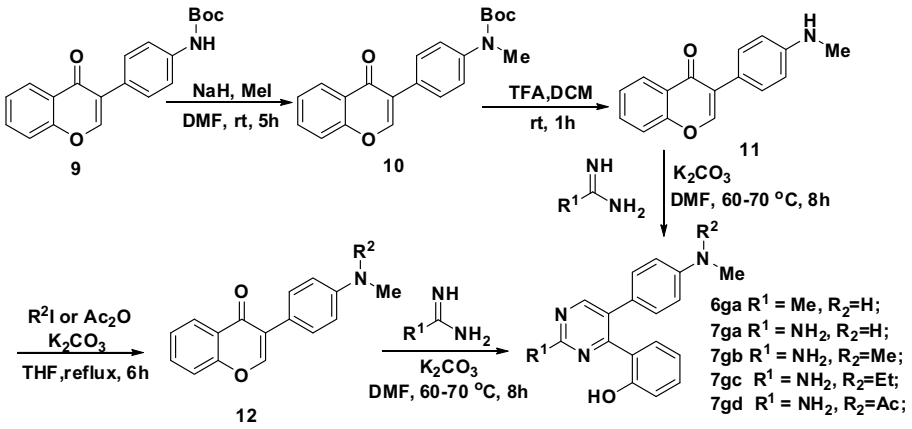
A series of *O*-alkylated derivatives were prepared from intermediate **8** by alkylation with various alkyl halides and then condensa-

Table 3
Synthesis and activity of 4′-alkoxyl pyrimdine derivatives



Compound	R^2	Yield (%)	IC_{50} (μM)
7ia	Et	59	0.24
7ib	Allyl	62	>10
7ic[a]	Pr	89	>10
7id	^{i}Pr	49	>10
7ie	Bu	61	>10
7if	2-Methylallyl	62	>10
7ig[a]	^{i}Bu	85	>10
7ih	3-Methylbut-2-enyl	63	>10
7ii[a]	Isopentyl	92	>10
7ij	CH_2CN	64	>10
7ik	CH_2CO_2Et	52	>10
7il	2-(Pyrrolidin-1-yl)ethyl	43	>10
7im	$CH_2CH_2CH_2OBn$	65	>10

[a] Compounds **7ic**, **7ig**, and **7ii** were prepared from **7ib**, **7if**, and **7ih** by hydrogenation.



Scheme 2.

tion with guanidine (Table 3). Only the ethoxy derivative **7ia**[17e] showed good inhibition with IC_{50} of 0.24 μM. From these results, we suppose a suitable less bulky electron-donating group at the *para*-position of aromatic ring B will improve bioactivity.

Encouraged by the further SARs on the ring B, intermediate **9** was methylated by MeI to give compound **10**. The Boc group of **10** was removed by TFA to produce **11**, which was directly condensed with acetamidine and guanidine to afford **6ga** and **7ga.** Furthermore, compound **11** was alkylated by MeI and EtI and acylated by Ac_2O, followed by condensation with guanidine to obtain compounds **7gb–d** in good yields (Scheme 2).

Compound **7ga** with the NH_2 group at the pyrimidine 2-poistion, showed better inhibition than **6ga** with a methyl group. Further, methylated compound **7gb** maintained good inhibition for the BEL-7402 cancer cell line. The *N*-methyl ethyl substituted compound **7gc**[17f] exhibited excellent inhibitory activity with an IC_{50} of 0.024 μM for the BEL-7402 cancer cell line. However, *N*-acetyl methyl compound **7gd** showed no activity, which is similar to compound **7f** (Table 4).

All active compounds were tested by non-cancerous cell line WI-38 and showed no inhibition ($IC_{50} > 10$ μM). Compound **7gc** was selected for screening against several different human cancer cell lines (Table 5).[18] This compound shows good inhibition for various cancer cell lines with IC_{50} values from 0.09 to 0.55 μM The most sensitive cell lines among them were the human hepatocellular carcinoma cell line BEL-7402 and colon cancer line HT29.

In summary, a novel series of 2,4,5-substituted pyrimidine derivatives were synthesized and evaluated in vitro for inhibition against human hepatocellular carcinoma BEL-7402 cancer cell proliferation. Several compounds showed potent inhibition with an IC_{50} value less than 0.10 μM. From the current investigation, structure–activity relationships of those compounds suggest electron-donating groups at the 2-position of pyrimidine will determine the anticancer activity and *para*-substitution of aromatic ring B with suitable less bulky electron-donating group will increase the anticancer activity. Additional research on the mechanisms of these compounds and modification is underway.

Table 4
Bioactivity of 4′-*N*-substituted pyrimidine derivatives

Compound	R^1	R^2	IC_{50} (μM)
6ga	Me	H	3.40
7ga	NH_2	H	0.32
7gb	NH_2	Me	0.20
7gc	NH_2	Et	0.024
7gd	NH_2	Ac	>10
Adriamycin			0.021

Table 5
In vitro anticancer activity of compound **7gc** on different human cancer cell lines

Cell line	A549	Calu-3	H460	SK-BR3	SGC-7901	HT29
7gc	0.55	0.50	0.12	0.30	0.30	0.090
Control	0.025[a]	0.10[b]	0.0097[b]	0.017[c]	0.0084[b]	0.018[a]

[a] Adriamycin.
[b] Docetaxel.
[c] GW572016.

Acknowledgments

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17. Spectral data for representative compounds. (a) *Compound* ***1c***: Mp 199–200 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.32 (s, 1H), 7.19 (td, J = 7.6, 1.2 Hz, 1H), 7.14 (d, J = 8.7 Hz, 2H), 6.96 (d, J = 7.9 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 6.49 (d, J = 7.8 Hz, 1H), 5.20 (br s, 2H), 3.83 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 162.71, 161.55, 159.69, 158.21, 156.07, 130.51, 129.84, 129.77, 123.50, 122.37, 118.38, 116.55, 113.92, 55.10; MS (EI) *m/z* 293 (M+).(b) *Compound* ***7j***: Mp 223–224 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.31 (s, 1H), 7.28–7.09 (m, 5H), 7.02–6.91 (m, 2H), 6.50 (t, J = 7.6 Hz, 1H), 5.21 (br s, 2H), 2.50 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 162.72, 161.77, 159.33, 155.77,136.35, 134.05, 130.38, 130.30, 128.95, 125.60, 123.69, 122.03, 118.29, 116.35, 14.48; MS (EI) *m/z* 309 (M+).(c) *Compound* ***6k***: Mp 83–84 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.60 (s, 1H), 7.21 (dt, J = 7.7, 1.7 Hz, 1H), 7.08–6.98 (m , 4H), 6.84 (d, J = 9.12 Hz, 1H), 6.50 (dt, J = 7.8, 1.3 Hz, 1H), 3.87 (s, 3H), 2.80 (s, 3H), 2.21 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.81, 161.42, 160.25, 159.63, 157.77, 132.21, 131.22, 130.96, 129.38, 128.44, 127.59, 127.46, 118.41, 118.12, 110.33, 55.30, 25.29, 16.18; MS (EI) *m/z* 306 (M+).(d) *Compound* ***7k***: Mp 204–205 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.31 (s, 1H), 7.18 (t, J = 8.2 Hz, 1H), 7.03–6.93 (m, 4H), 6.80 (d, J = 8.0 Hz, 1H), 6.48 (t, J = 7.6 Hz, 1H), 5.16 (br s, 2H), 3.85 (s, 3H), 2.19 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 162.34, 161.35, 159.70, 156.31, 156.22, 130.64, 130.36, 130.31, 129.26, 127.17, 125.37, 123.18, 122.14, 118.07, 116.53, 110.06, 55.14, 16.05; MS (EI) *m/z* 307 (M+).(e) *Compound* ***7ia***: Mp 209–211 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.32 (s, 1H), 7.18 (dt, J = 7.8, 1.6 Hz, 1H), 7.11 (d, J = 8.5 Hz, 2H), 6.99–6.93 (m, 2H), 6.88 (d, J = 8.6 Hz, 2H), 6.48 (dt, J = 7.5, 1.3 Hz, 1H), 5.24 (br s, 2H), 4.05 (q, J = 7.0 Hz, 2H), 1.43 (t, J = 7.0 Hz, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 162.45, 161.46, 159.61, 157.38, 156.21, 130.36, 130.31, 129.70, 129.55, 123.24, 122.13, 118.12, 116.51, 114.22, 62.90, 14.67; MS (EI) *m/z* 307 (M+).(f) *Compound* ***7gc***: Mp 236–237 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.34 (s, 1H), 7.18 (dt, J = 7.7, 1.6 Hz, 1H), 7.09 (dd, J = 8.1, 1.5 Hz, 2H), 7.05 (d, J = 9.0 Hz, 2H), 697 (dd, J = 8.2, 1.1 Hz, 1H), 6.68 (d, J = 8.8 Hz, 2H), 6.51 (dt, J = 7.6, 1.3 Hz, 1H), 5.06 (br s, 2H), 3.42 (q, J = 7.03 Hz, 2H), 2.93 (s, 3H),1.14 (t, J = 7.15 Hz, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 161.95, 160.91, 159.85, 156.58, 147.52, 130.37, 129.31, 124.28, 122.93, 122.44, 118.01, 116.66, 112.00, 45.81, 36.98, 10.83; MS (EI) *m/z* 320 (M+).
18. The human hepatocellular carcinoma cell line BEL-7402 and gastric adenocarcinoma cell line SGC-7901 were obtained from the Cell Bank of the Shanghai Institute for Biological Sciences, Chinese Academy of Science (Shanghai, China). Human lung adenocarcinoma cell line A549 and Calu-3, colon cancer HT29, large cell lung cancer NCI-H460, breast carcinoma SK-BR3 were all purchased from the American Type Culture Collection (Manassas, USA). The BEL-7402, SGC-7901, NCI-H460, and SK-BR3 cells were cultured in RPMI 1640 medium. Calu-3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM). A549 cells were cultured in Ham's F12K medium. HT29 cells were cultured in McCoy's 5a medium (modified). All media were supplemented with 10% FBS and cells were incubated at 37 °C in an atmosphere of 5% CO_2.*Sulforhodamine B cell proliferation assay:* Cells were seeded in 96-well plates and then treated with either vehicle (DMSO) or different concentrations of compounds. After 72 h of incubation, cells were fixed with 10% trichloroacetic acid for 1 h at 4 °C, washed five times with tap water and air-dried. Cells were stained with 0.4% (w/v) sulforhodamine B (SRB) for 20 min at room temperature and washed five times with 1% acetic acid. Bound SRB was solubilized with 10 mM Tris and absorbance was measured at 540 nm.