Three-Component, One-Pot Synthesis of 2,4,5-Substituted Pyrimidines Library for Screening against Human Hepatocellular Carcinoma BEL-7402 Cells

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Received September 8, 2006

Pyrimidine is found widely as a core structure in a large variety of compounds that exhibit important biological activity.[1] It is convenient to synthesize substituted pyrimidines by reaction of amidines or guanidine with α,*β*-unsaturated ketones, *β*-diketones, *β*-alkoxy- and *β*-aminovinyl ketones, and *N*-aryl acetyleneic imines.[2] The use of combinatorial approaches to the high-throughput synthesis of this druglike scaffold would be a powerful advance in helping to speed up drug discovery. Recently, Nie et al. have reported a new method for the preparation of a 2,4,6-substituted pyrimidines library using a microwave-assisted reaction of 2′-hydroxychalcones with amidines or guanidine.[3] To the best of our knowledge, no method was explored to generate the 2,4,5-substitued pyrimidines for combinatorial synthesis. Here, we report (1) a combinatorial synthesis of a 2,4,5-substituted pyrimidine library using a sequential three-component, one-pot reaction and (2) its antitumor activities.

Chromone as a 1,3-diketone equivalent can be condensed with amidine to form *o*-hydroxyphenyl pyrimidine.[5] Since substitution of pyrimidine at the 4-position will block Suzuki coupling of a 5-iodo-4-substituted pyrimidine, we thus designed a one-pot process to form 2,4,5-substitued pyrimidines by Suzuki coupling[6] that applies diversified commercial available boronic acids to the chromone core, followed by condensation with a variety of amidines (Scheme 1).

According to our reported method,[4] a mixture of iodochromone (1.2 mmol) and aryl boronic acids (1.1 equiv) in the presence of 2% $Pd(PPh_3)_4$ and 2.0 equiv K_2CO_3 in 5 mL THF−H_2O (4:1) was refluxed overnight and then split into six portions, to which was added 1.5 equiv of amidines **f**−**k** (0.3 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.3 mmol or 0.6 mmol) for each portion. The mixture was stirred at 50−60 °C for about 10 h, and the corresponding products were obtained by flash chromatography. Using five boronic acids and six amidines (Table 1), we successfully synthesized a small quantity of a pyrimidine library including 30 diversified compounds. The electronic variations on both the aryl group of the boronic acid and the substitution of amidine gave the desired product in moderate to good yield (Table 2).

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Scheme 1. Synthesis of Diversified 2,4,5-Substituted Pyrimidines via Suzuki Coupling and Condensation

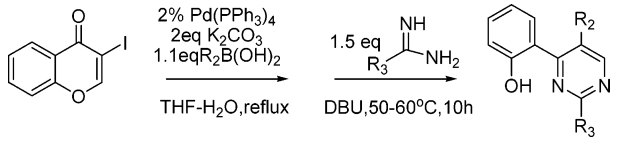


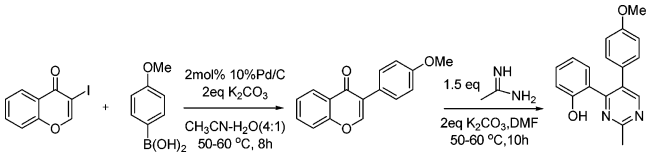
Table 1. Arylboronic Acids and Amidines Used in Library Synthesis (Scheme 1)

$R_2B(OH)_2$		$R_3C(NH)NH_2$	
4-methoxyphenyl	**a**	phenyl	**f**
4-trifluoromethylphenyl	**b**	4-pyrimidinyl	**g**
4-fluorophenyl	**c**	4-aminophenyl	**h**
4-tertbutylphenyl	**d**	4-chlorophenyl	**i**
3-thiophenyl	**e**	tertbutyl	**j**
		CH_3	**k**

Table 2. Library of 2,4,5-Substituted Pyrimidines and Its Inhibition (%) of BEL-7402 Cells Growth at 15 μM Concentration

	substituent		yield	BEL-7402 inhibition		substituent		yield	BEL-7402 inhibition
compd	R_1	R_2	%	%	compd	R_1	R_2	%	%
af	**a**	**f**	46	12	**ci**	**c**	**i**	46	15
ag	**a**	**g**	48	19	**cj**	**c**	**j**	41	8
ah	**a**	**h**	52	15	**ck**	**c**	**k**	53	6
ai	**a**	**i**	50	19	**df**	**d**	**f**	46	35
aj	**a**	**j**	49	20	**dg**	**d**	**g**	40	10
ak	**a**	**k**	47	84	**dh**	**d**	**h**	45	87
bf	**b**	**f**	49	42	**di**	**d**	**i**	52	18
bg	**b**	**g**	45	14	**dj**	**d**	**j**	46	16
bh	**b**	**h**	44	24	**dk**	**d**	**k**	43	14
bi	**b**	**i**	46	23	**ef**	**e**	**f**	47	7
bj	**b**	**j**	49	10	**eg**	**e**	**g**	40	7
bk	**b**	**k**	55	7	**eh**	**e**	**h**	44	2
cf	**c**	**f**	61	17	**ei**	**e**	**i**	46	7
cg	**c**	**g**	51	26	**ej**	**e**	**j**	41	6
ch	**c**	**h**	52	0	**ek**	**e**	**k**	42	2

Scheme 2. Large-Scale Synthesis of Compound **ak**



The compounds were assayed for the inhibition of human hepatocellular carcinoma cell line BEL-7402 (Table 2). Two compounds, **ak** and **dh**, exhibited a high degree of inhibition, more than 80% against BEL-7402 cell growth at 15 μM, with an IC_{50} value of 1.02 and 5.08 μM, respectively.

In the process of preparation of a large amount of **ak**, we tested Felpin's reported method,[7] which applied catalytic 10% Pd/C as an inexpensive catalyst for Suzuki coupling in aqueous acetonitrile, followed by condensation with acetamidine in DMF separately. The compound **ak** was obtained in 77% yield over two steps. (Scheme 2).

According to the bioassay result, we then kept the building blocks **a**, **k** and **d**, **h** to synthesize several derivatives of **ak**

10.1021/cc060128y CCC: $37.00 © 2007 American Chemical Society
Published on Web 12/06/2006

Table 3. Derivative of **ak** and **dh** and Inhibition (%) of BEL-7402 Cell Growth at 15 μM Concentration

	substituent				
compd	R_1	R_2	R_3	yield%	BEL−7402 inhibition %
1ak	OMe	**a**	**k**	50	10
2ak	Cl	**a**	**k**	46	43
3ak	NO_2	**a**	**k**	35	7
4ak	CH_3	**a**	**k**	44	25
1dh	OMe	**d**	**h**	45	10
2dh	Cl	**d**	**h**	42	10
3dh	NO_2	**d**	**h**	33	7
4dh	CH_3	**d**	**h**	55	8

and **dh** using different substituted iodochromones. However, all these substituents, including OMe, Cl, NO_2, and CH_3, at the para position of OH resulted in less inhibition against the growth of BEL-7402 cells (Table 3) than did compounds **ak** and **dh**. It indicates that substitution at that position is unfavorable to improve the activity.

In conclusion, we developed an efficient method to generate a diversified pyrimidine library via a sequential one-pot reaction of iodochromone, arylboronic acid, and amidine by Suzuki coupling and condensation. Through biological activity screening, we have obtained two novel compounds, **ak** and **dh**, which exhibited potent inhibition against BEL-7402 cells, with an IC_{50} value of 1.02 μM and 5.08 μM, respectively. Additional research on the mechanisms and SARs of these compounds is in progress in our group.

Acknowledgment. The Shanghai Commission of Science and Technology (06PJ14112) supported this work.

Supporting Information Available. Experimental procedures, ^{1}H NMR spectra of all compounds, and ^{13}C NMR spectra of compounds **af**, **ak**, **bf**, **bk**, **cf**, **ck**, **df**, **dh**, **dk**, **ef**, and **ek**. This material is available free of charge via the Internet at http://pubs.acs.org.

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CC060128Y