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Three-component, one-pot synthesis of novel 2,4-substituted 5-azolylthiopyrimidine library for screening against anti-influenza virus A

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Abstract—A novel one-pot synthesis of 2,4-substituted 5-azolylthiopyrimidines is achieved by sequential Michael-addition of 3-iodochromones with mercaptoazole (or mercaptotriazoles) and then condensation with a variety of amidines. Compound $\mathbf{A_1B_6C_1}$ exhibits a potent anti-influenza virus A activity with an IC_{50} value of 21.56 mg/mL and SI value of 9.

Pyrimidine is widely found as a core structure in a large variety of compounds that exhibit important biological activity.[1] The use of combinatorial approaches to the high-throughput synthesis of this drug-like scaffold would be a powerful advance in helping to speed up drug discovery. Recently we have developed efficient methods to generate heterocycle library by three-component one-pot reaction.[2] Since antiviral activity can be associated with the presence of nitrogen heterocycle (pyrimidine derivatives)[3] or bis-heterocycle compounds,[4] this strategy leads us to explore the convenient methodology to construct novel bis-heterocycle library containing pyrimidine scaffold for antiviral screening. Here, we report a combinatorial synthesis of 2,4-substituted-5-azolylthiopyrimidine library using a sequential three-component, one-pot reaction and its anti-influenza virus activity.

According to Yokoe's method,[5] 3-azolylthiochromone **E** could be readily prepared by treatment of 3-iodochromone with mercaptoazole under the basic conditions (K_2CO_3). We envision the resulting 3-azolylthiochromone **E** as 1,3-diketone equivalent[6] could be further condensed with amidines in situ to form 2,4-substituted-5-azolylthiopyrimidines. This protocol avoids the nucleophilic substitution of 5-iodopyrimidine with thiols using copper, palladium chemistry or harsh condition.[7]

Initially, a consecutive one-pot process of iodochromones (**A-1**), mercaptoazole (**C-1**), and acetamidine (**B-2**) in the presence of K_2CO_3 as base in DMF only gave the product **D** which is the directly condensed product of iodochromone with acetamidine (Scheme 1, Eq. 1). Refluxing of the mixture could not generate the desired product ($\mathbf{A_1B_1C_1}$). This indicates acetamidine is a stronger nucleophile than mercaptoazole. Then a sequential process was applied. Iodochromone was first reacted with mercaptoazole, followed by addition of acetamidine to give the desired product ($\mathbf{A_1B_1C_1}$) in 68% yield (Scheme 1, Eq. 2).[8] Solvent systems were investigated, THF and CH_3CN as solvent led to low yields.

Through this powerful procedure, the bis-heterocycles library containing pyrimidine scaffold can be generated by parallel split-pool protocol. First a 18-compound library was synthesized by 2 different iodochromones, 9 amidines, and mercaptoazole in moderate to excellent yield (Fig. 1).

Among them, we selected the universal compounds to evaluate for inhibition of influenza virus H3N2 (A3 China/15/90) replication in Madin-Darby canine Kidney (MDCK) cells.[9] The result is given in Table 1. Aryl substitutions of pyrimidine at 2-position did not show the activity. Compounds $\mathbf{A_1B_2C_1}$ and $\mathbf{A_1B_6C_1}$ exhibit the activity against influenza A virus, with IC_{50} values of

Keywords: One-pot synthesis; Michael-addition; Condensation; 5-Azolylthiopyridines; Heterocycle libraries; Anti-influenza.
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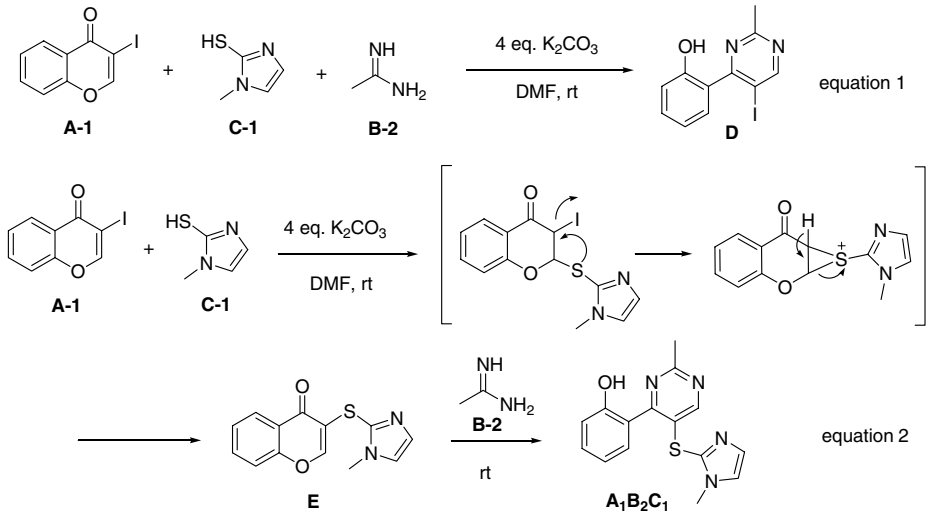
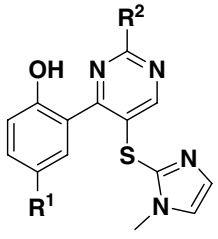


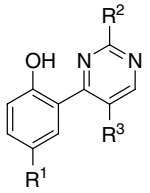
Scheme 1. Sequential one-pot synthesis of 5-azolylthiopyrimidine.



$A_1B_1C_1$	R^1 = H, R^2 = H,	85%	**$A_2B_1C_1$**	R^1 = CH_3O, R^2 = H,	94%
$A_1B_2C_1$	R^1 = H, R^2 = CH_3,	68%	**$A_2B_2C_1$**	R^1 = CH_3O, R^2 = CH_3,	85%
$A_1B_3C_1$	R^1 = H, R^2 = C_6H_5,	54%	**$A_2B_3C_1$**	R^1 = CH_3O, R^2 = C_6H_5,	89%
$A_1B_4C_1$	R^1 = H, R^2 = 4-pyridinyl,	95%	**$A_2B_4C_1$**	R^1 = CH_3O, R^2 = 4-pyridinyl,	90%
$A_1B_5C_1$	R^1 = H, R^2 = 4-ClC_6H_4,	19%	**$A_2B_5C_1$**	R^1 = CH_3O, R^2 = 4-ClC_6H_4,	85%
$A_1B_6C_1$	R^1 = H, R^2 = *tert*-butyl,	70%	**$A_2B_6C_1$**	R^1 = CH_3O, R^2 = *tert*-butyl,	91%
$A_1B_7C_1$	R^1 = H, R^2 = 4-$NH_2C_6H_4$,	60%	**$A_2B_7C_1$**	R^1 = CH_3O, R^2 = 4-$NH_2C_6H_4$,	89%
$A_1B_8C_1$	R^1 = H, R^2 = methylthio,	48%	**$A_2B_8C_1$**	R^1 = CH_3O, R^2 = methylthio,	40%
$A_1B_9C_1$	R^1 = H, R^2 = NH_2,	92%	**$A_2B_9C_1$**	R^1 = CH_3O, R^2 = NH_2,	70%

Figure 1. First 18-compound library.

Table 1. Anti-influenza virus A, B activity and cytotoxicity of substituted pyrimidines in MDCK cells[a]



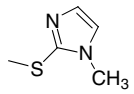
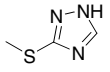
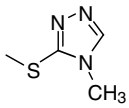
Compound	Substituent			CC_{50}[b] (mg/mL)	Virus A	Virus B
	R^1	R^2	R^3		IC_{50}[c] (mg/mL)	IC_{50}[c] (mg/mL)
$A_1B_2C_1$	H	CH_3	 (**C_1**)	577.35	258.69	—
$A_1B_3C_1$	H	C_6H_5	**C_1**	120.19	—	—
$A_1B_4C_1$	H	4-Pyridinyl	**C_1**	64.67	—	—
$A_1B_5C_1$	H	4-ClC_6H_4	**C_1**	80.12	—	—
$A_1B_6C_1$	H	*tert*-Butyl	**C_1**	194.01	21.56	—
$A_1B_7C_1$	H	4-$NH_2C_6H_4$	**C_1**	80.12	—	—
$A_1B_9C_1$	H	NH_2	**C_1**	388.03	—	—
$A_2B_1C_1$	CH_3O	H	**C_1**	618.39	—	—
$A_2B_6C_1$	CH_3O	*tert*-Butyl	**C_1**	115.56	—	—
$A_2B_8C_1$	CH_3O	Methylthio	**C_1**	7.19	—	—
$A_3B_6C_1$	Cl	*tert*-Butyl	**C_1**	111.11	48.74	86.23

Table 1 (*continued*)

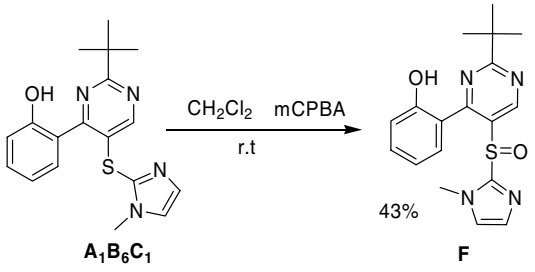
Compound	Substituent			CC_{50}[b] (mg/mL)	Virus A	Virus B
	R^1	R^2	R^3		IC_{50}[c] (mg/mL)	IC_{50}[c] (mg/mL)
$A_4B_6C_1$	CH_3	*tert*-Butyl	**C_1**	115.56	—	—
$A_1B_6C_2$	H	*tert*-Butyl	 **C_2**	160.25	—	—
$A_1B_6C_3$	H	*tert*-Butyl	 **C_3**	160.25	—	—
$A_1B_6C_4$	H	*tert*-Butyl	**H**	16.25	—	—
$A_3B_6C_2$	Cl	*tert*-Butyl	**C_2**	9.58	—	—
Ribavirin				>500	2.06	4.27

[a] Abbeviations and strains used: MDCK, Madin-Darby canine kidney cells, influenza A H3N2 viruses (A3 China/15/90).
[b] Concentrations that cause microscopically detectable toxicity in virus-infected cultures.
[c] Concentrations required to reduce virus-induced CPE in MDCK cells by 50%.

258.69 and 21.56 mg/mL, respectively. These results indicated that the steric hindrance and electron-donating group in 2-position is favorable for activity. Based on **$A_1B_6C_1$**, compounds with thiotriazoles (**$A_1B_6C_2$** and **$A_1B_6C_3$**) or hydrogen (**$A_1B_6C_4$**) substituted at 5-position were synthesized and exhibited no inhibition for influenza A virus.

We investigated the electron effect on substitution of aromatic ring at 5′-position. Only compound **$A_3B_6C_1$** showed the weaker activity (IC_{50} = 48.74 mg/mL) than **$A_1B_6C_1$**. Oxidation of **$A_1B_6C_1$** gave compound **F** which showed no activity against influenza A. In addition, this series of compounds did not exhibit the potent activity against influenza B virus (see Scheme 2).

In summary, we have developed a mild and convenient method for the synthesis of 5-azolylthiopyridines based upon a consecutive Michael-addition, condensation sequence. This method provides facile construction of these bis-heterocycle libraries that are applicable for biological screening. Biological responses of these 5-azolylthiopyridines against influenza virus A were preliminarily evaluated, and the results showed that compound **$A_1B_6C_1$** has inhibitory potency as lead for the development of de novo antiviral agents. Further studies on their structure–activity relationship and optimization of these compounds are underway in our group.



Scheme 2. Oxidation of compound **$A_1B_6C_1$**.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.11.117.

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37, 307; (c) Sosnovskikh, V. Y.; Usachev, B. I.; Sizov, A. Y.; Barabanov, M. A. *Synthesis* **2004**, *6*, 942.
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8. Typical procedure for the synthesis of 2,4-substituted 5-azolylthiopyrimidines: a mixture of substrate **A-1** (0.2 mmol), **C-1** (0.22 mmol), and K_2CO_3 (0.8 mmol) in DMF (3 mL) was stirred at room temperature for 8 h, then acetamidine (0.3 mmol) was added to the reaction mixture and stirred at room temperature for 8 h. The reaction mixture was concentrated. The residue was purified by flash chromatography (silica gel, 30:1 $CHCl_3/CH_3OH$) to afford 40 mg (68%) of compound $\mathbf{A_1B_2C_1}$.
9. MDCK cells were grown as specified in Eagle's minimum essential medium with 10% heat-inactivated fetal bovine serum (FBS) plus antibiotics (penicillin, 100 U/mL; streptomycin, 100 U/mL). Influenza A H3N2 viruses (A3 China/15/90) were propagated in the allantoic cavities of 10-day-old embryonated eggs. Virus titers were determined by hemagglutinin titration, according to standard procedures. Confluent MDCK monolayers were infected with Influenza A viruses for 2 h at 37, after which the viral inoculum was removed and cells were treated with different concentrations of compound. When CPE result of the viral control group reached 4+, the result of compound treated group was observed. The dilution that gives 50% cytopathic effect was determined by the interpolating procedure of Reed and Muench.