Acta Pharmaceutica Sinica B 2012;2(6):575–580

Institute of Materia Medica, Chinese Academy of Medical Sciences
Chinese Pharmaceutical Association

Acta Pharmaceutica Sinica B

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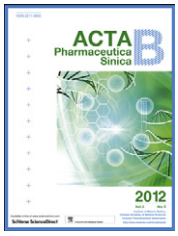

ORIGINAL ARTICLE

Synthesis and antiviral activities of a novel class of thioflavone and flavonoid analogues

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Received 12 July 2012; revised 12 September 2012; accepted 9 October 2012

KEY WORDS

Thioflavones;
Antiviral activity;
Coxsackievirus;
Enterovirus

Abstract A novel class of thioflavone and flavonoid derivatives has been prepared and their antiviral activities against enterovirus 71 (EV71) and the coxsackievirus B3 (CVB3) and B6 (CVB6) were evaluated. Compounds **7d** and **9b** showed potent antiviral activities against EV71 with IC_{50} values of 8.27 and 5.48 μM, respectively. Compound **7f**, which has been synthesized for the first time in this work, showed the highest level of inhibitory activity against both CVB3 and CVB6 with an IC_{50} value of 0.62 and 0.87 μM. Compounds **4b**, **7a**, **9c** and **9e** also showed strong inhibitory activities against both the CVB3 and CVB6 at low concentrations (IC_{50} = 1.42−7.15 μM), whereas compounds **4d**, **7c**, **7e** and **7g** showed strong activity against CVB6 (IC_{50} = 2.91–3.77 μM) together with low levels of activity against CVB3. Compound **7d** exhibited stronger inhibitory activity against CVB3 (IC_{50} = 6.44 μM) than CVB6 (IC_{50} > 8.29 μM). The thioflavone derivatives **7a**, **7c**, **7d**, **7e**, **7f** and **7g**, represent a new class of lead compounds for the development of novel antiviral agents.

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

2211-3835
http://dx.doi.org/10.1016/j.apsb.2012.10.005

1. Introduction

The prevention and treatment of viral infectious diseases has become a global public health problem. The emergence of drug-resistant and unknown viral infections has made the development of new antiviral agents with new mechanisms of action and broad-spectrum levels of activity even more important. Notably, the number of enterovirus 71 (EV71) outbreaks in the Asia-Pacific region has increased significantly in recent years, and group B coxsackieviruses (CVB) are known to be associated with a variety of the acute and chronic forms of several different diseases.

Human enteroviruses are small, single-stranded, positive-sense RNA viruses which belongs to the enteroviruses genus of the picornaviridae family. Like other types of enteroviral infections, EV71 infections may be asymptomatic or may cause diarrhea, rashes, vesicular lesions on the hands, feet, and oral mucosa (hand–foot-and-mouth disease), herpangina, aseptic meningitis, encephalitis, myocarditis, or a combination of these conditions. EV71 infections in children have recently become a significant public health problem in China[1,2]. CVBs are known to be associated with a variety of acute and chronic forms of several different diseases, including myocarditis, meningitis and pancreatitis, especially in neonates, young children and immune-compromised adult patients. Cardiac infection with CVB3 can result in acute myocarditis that spontaneously resolves or chronic myocarditis with prolonged viral persistence[3].

Flavonoids are a group of low molecular weight phenyl benzopyrones that possess a variety of different pharmacological properties, including antioxidant, anticancer, antiviral and anti-inflammatory activities[4–6]. Some naturally occurring and modified flavonoid compounds have been reported to exhibit a broad spectrum of antiviral activity against picornavirus[7,8]. These compounds were reported to interfere with picornavirus replication by preventing the decapsidation of the viral particles and RNA release within the cells. 3-Hydroxyflavone was reported to exhibit antiviral activity against human rhinovirus (HRV)-1B and HRV-14, with IC_{50} values of 1.3 and 2.1 μM, respectively. Although the molecular mechanism of action for flavonoids remains unclear, it has been attributed to interference with the early stages of viral infection, probably represented by viral RNA synthesis.

Thioflavone derivatives are the thio analogues of the flavonoid derivatives, and some thioflavone compounds have been reported to exhibit antimicrobial activities[9]. For example, compounds A and B (Fig. 1) have been reported to exhibit inhibitory activities against *Trichophyton rubrum* IFO5467, with MIC values of 0.19 and 0.05 μg/mL, respectively.

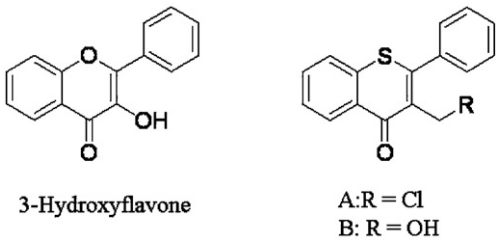


Figure 1 Structures of several known flavone and thioflavone analogues.

However, there have been no reports concerning their antiviral activities.

There are currently no effective antiviral drugs in the clinic for the treatment of EV17 and CVB infections. Treatments for acute EV71 infections with neurological manifestations are principally intended to alleviate the symptoms. With this in mind, herein we describe the synthesis of a series of thioflavone analogues and the subsequent *in vitro* evaluation of their activities against EV71, CVB3 and CVB6. According to the principle of bioisosterism, we synthesized a series of compounds (**7a**–**i**) in which the oxygen atom at position 1 of the flavonoids was replaced with a sulfur atom. To investigate the effects of methoxyl and hydroxyl groups on the activity of these compounds, we obtained compounds **8a**–**c** *via* demethylation of the methoxyl group of **7a**–**c**. Furthermore, several flavonol derivatives were also synthesized in an attempt to improve their antiviral potency and to systemically investigate their antiviral structure activity relationships.

2. Results and discussion

2.1. Chemistry

With the exception of compounds **9a**–**h**, which were purchased from J&K Scientific, all of the target compounds described in this paper were synthesized according to well-established literature procedures[10–12]. The synthetic schemes are discribed below (Schemes 1 and 2).

As shown in Scheme 1, compounds **4a**–**d** were successfully synthesized *via* the condensation reaction of 2-hydroxy acetophenone (**1**) with a variety of substituted benzaldehydes (**2**) in a mixture of MeOH and KOH. The resulting intermediate compounds **3a**–**d** were then reacted with hydrogen peroxide (H_2O_2) in a mixture of MeOH and KOH at 0–5 °C to provide the target compounds **4a**–**d**.

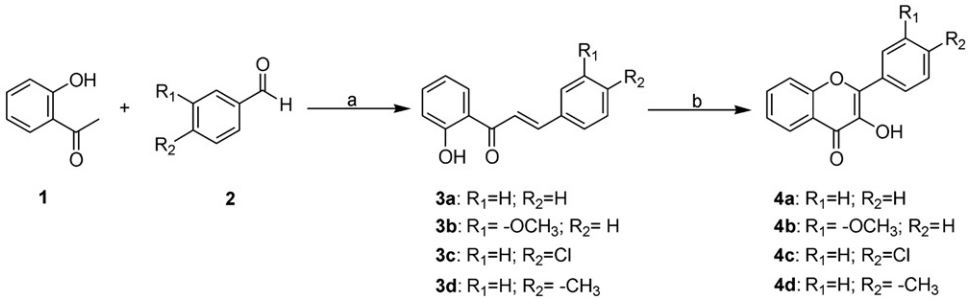
4-Methoxybenzenethiol (**5**) was used as the starting material for the synthesis of the thiochromen-4-one derivatives (**7** and **8**). Compound **5** was condensed with the appropriately substituted ethyl 3-oxopropionates (**6**) in polyphosphoric acid to afford compounds **7a**–**i**, according to a published procedure[10]. These compounds were then treated with boron tribromide in dichloromethane to affect the ether cleavage to generate the substituted thioflavones **8a**–**c**.

2.2. Biological results and discussion

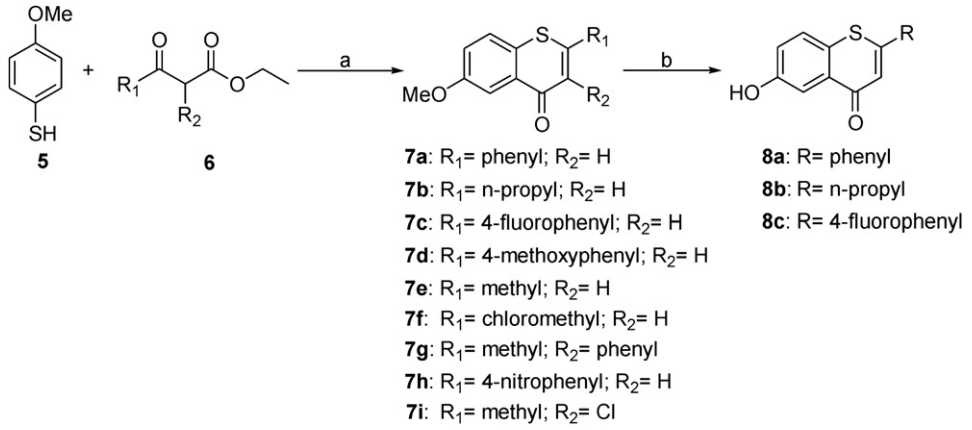
The antiviral activities of the flavonoids against EV71 (SZ-98), CVB3 and CVB6 were evaluated using African green monkey kidney cells (Vero cell) as the virus host. The results are summarized in Table 1.

As shown in Table 1, compounds **7d** and **9b** clearly exhibited the highest levels of inhibitory activity of all of the compounds tested against EV71, with IC_{50} values of 8.27 and 5.48 μM, respectively. Unfortunately, they were not as potent as the reference drug pirodavir, which gave an IC_{50} of 0.32 μM. Compounds **4b** and **7i** showed moderate inhibitory activity against EV71 with IC_{50} values of 16.90 and 39.63 μM, respectively, whereas the remaining compounds showed little to no activity against EV71 under the conditions tested.

The tested compounds showed strong levels of activity against coxsackieviruses. Compound **4a**, for example, showed



Scheme 1 Synthetic route to the flavonoid compounds **4a**–**d**: (a) KOH, CH_3OH; (b) KOH, CH_3OH, 30% H_2O_2.



Scheme 2 Synthetic route to the thioflavone compounds **8a**–**c**: (a) polyphosphoric acid, 90–100 °C; (b) BBr_3, CH_2Cl_2, r.t.

Table 1 Antiviral activities of the tested compounds.

Compd.	EV71 (SZ-98)			CVB3			CVB6		
	TC_{50} μM	IC_{50} μM	SI	TC_{50} μM	IC_{50} μM	SI	TC_{50} μM	IC_{50} μM	SI
4a	280.12	>31.13	–	193.83	13.65	14.20	193.83	31.13	6.23
4b	29.23	16.90	1.73	12.49	1.42	8.80	12.49	2.54	4.92
4c	97.88	>27.24	–	47.06	>9.08	–	47.06	>9.08	–
4d	27.64	>3.05	–	27.64	>3.05	–	27.64	3.05	9.06
7a	39.65	>9.20	–	48.04	7.15	6.72	48.04	3.06	15.70
7b	54.75	>31.62	–	95.11	18.29	5.20	94.11	10.55	9.02
7c	112.06	>25.88	–	77.69	>8.63	–	77.69	3.77	20.60
7d	107.34	8.27	12.98	74.70	6.44	11.60	74.70	>8.29	–
7e	46.05	>11.99	–	46.05	>11.99	–	46.05	3.05	15.10
7f	14.79	>3.40	–	10.23	0.62	16.50	10.23	0.87	11.76
7g	46.22	>28.62	–	55.05	20.39	2.70	45.68	2.91	15.70
7h	102.39	>70.91	–	146.75	>70.99	–	146.75	23.67	6.20
7i	133.15	39.63	3.36	191.80	30.87	6.21	190.80	39.75	4.80
8a	12.51	>3.22	–	12.51	>3.22	–	12.51	>3.22	–
8b	174.41	100.86	1.73	389.79	78.36	4.97	389.79	18.13	21.50
8c	35.07	>9.08	–	35.07	>9.08	–	35.07	9.08	3.90
9a	261.90	>87.48	–	261.90	29.10	9.00	262.19	15.70	16.70
9b	23.75	5.48	4.33	20.83	>3.03	–	20.83	>3.03	–
9c	61.60	>8.81	–	61.60	2.92	21.10	61.61	3.78	16.30
9d	11.66	>1.00	–	11.66	>1.00	–	11.66	>1.00	–
9e	15.07	>8.69	–	11.25	2.25	5.00	11.23	2.88	3.90
9f	35.67	>5.71	–	35.67	>5.71	–	35.67	>5.71	–
9g	111.10	>53.36	–	111.10	>53.41	–	111.10	>53.41	–
9h	198.84	>51.43	–	198.84	>51.43	–	198.84	>51.43	–
Pirodavir	15.39	0.32	48.09	ND	ND	ND	ND	ND	ND
RBV	ND	ND	ND	8205.90	2120.40	3.87	8205.90	911.77	9.00

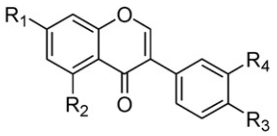
Note: ND means not detected.

activity against both the CVB3 and CVB6 strains of the virus, with IC_{50} values of 13.65 and 31.13 μM, respectively. When the 4′-position of the flavonoid system was functionalized with a methoxy group, as in compound **4b**, the inhibitory activity was more potent against CVB3 and CVB6, with IC_{50} values of 1.42 and 2.54 μM being recorded, respectively. Compound **4c** showed no activity against CVB3 and CVB6, whereas compound **4d** only presented clear inhibitory activity against CVB6, indicating that the presence of an electron donating group on the phenyl ring favored the antiviral activity.

Of the thioflavone analogues tested in the current study, compound **7f** showed the highest level of inhibitory activity against both CVB3 and CVB6 with an IC_{50} value of 0.62 and 0.87 μM, respectively. Compound **7d**, which contained an electron donating group (methoxy) at the 4′-position of the phenyl ring also showed a high level of inhibitory activity against CVB3, with an IC_{50} value of 6.44 μM and a selectivity index (SI) of 11.60. In contrast, compounds **7c** and **7h**, which contained electron withdrawing groups (fluorine and nitro) at the 4′-position of the phenyl ring, showed no activity against CVB3. Taken together, these results indicate that the presence of an electron donating group at the 4′-position of the thioflavone ring system is beneficial to the antiviral activity. Compound **7b** also showed moderate inhibitory activity against CVB3 with an IC_{50} value was 18.29 μM. Compounds **7a**, **7c**, **7e** and **7g** all showed strong levels of inhibitory activity against CVB6 at very low concentrations (IC_{50} = 2.91–3.77 μM). Furthermore, the SI values in these compounds (>15) were much higher than that of the reference drug. Compound **7c** gave the highest SI value of all of the compounds tested against CVB6 at up to 20.6. The ether cleavage compounds **8a**–**c** showed weak to no activity against CVB3, whereas compound **8c** showed a moderate level of activity against CVB6, with an IC_{50} value of 9.08 μM.

As shown in Table 1 and Fig. 2, the isoflavones **9c** and **9e** showed high levels of inhibitory activity against both the CVB3 and CVB6 strains, with IC_{50} values of 2.25–3.78 μM. Furthermore, compound **9c** showed high SI values of 21.1 and 16.3 against CVB3 and CVB6, respectively. Compound **9a** also exhibited inhibitory activity against CVB3 and CVB6. Unfortunately, none of the remaining isoflavones tested showed any activity against CVB3 or CVB6.

In summary, all of the synthesized and purchased compounds were assayed to determine their *in vitro* antiviral activity against EV71, CVB3 and CVB6. The majority of the compounds exhibited promising antiviral activity, especially compound **4b**, which showed activity against all of the viruses tested, indicating that it possessed broad-spectrum antiviral activity. In addition, compounds **7a**, **7f**, **9c** and **9e** showed strong inhibitory activity against both the CVB3 and CVB6 strains. We believe that the active compounds are worthy of further evaluation as novel lead compounds for the development of broad spectrum antiviral agents.



9a: R_1= OH; R_2=H; R_3=OH; R_4=H
9b: R_1= OH; R_2=H; R_3=OH; R_4=OH
9c: R_1= isopropoxy; R_2=H; R_3=H; R_4=H
9d: R_1= OH; R_2=OH; R_3=OH; R_4=H
9e: R_1= OH; R_2=H; R_3=OH; R_4=methoxy
9f: R_1= methoxy; R_2=OH; R_3=OH; R_4=H
9g: R_1= *β-D*-glucopyranosyloxy; R_2=H; R_3=OH; R_4=H
9h: R_1= OH; R_2=OH; R_3=*β-D*-glucopyranosyloxy; R_4=H

Figure 2 Structures of the commercial compounds **9a**–**h**.

3. Experimental procedures

3.1. Chemistry

All solvents and reagents were of chemical or analytical grade. The progress of the reactions was monitored by TLC using solvent systems of different polarities. ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Inova 400/500 MHz spectrometer (Varian, San Francisco, CA, USA). Chemical shift was reported in parts per million relative to a tetramethylsilane internal standard. Melting points were determined with an X-4 microscope melting point apparatus and were uncorrected. All mass spectra (MS, HR-MS) were recorded on a LTQ-Orbitrap linear ion trap high-resolution mass spectrometer (ThermoFisher Scientific, San Jose, CA, USA).

3.1.1. General procedure for the synthesis of compounds *4a–d*

2-Hydroxy acetophenone **1** (1.36 g, 0.01 mol) and benzaldehyde **2** (1.06 g, 0.01 mol) were added to a solution of KOH (1.12 g, 0.02 mol) in methanol (50 mL) at 0–5 °C. The reaction mixture was stirred over night at room temperature and then poured over crushed ice and acidified to pH 6 with 2 M HCl. The resulting yellow solid was filtered and the filter-cake washed with water to give the crude product that could either be recrystallized from ethanol to afford pure 2-hydroxychalcones **3a** or used directly in the next reaction without further purification.

30% H_2O_2 (10 mL) was added to a well-stirred solution of **3a** (1.57 g, 0.007 mol) and 20% (*w*/*w*) aqueous KOH (10 mL) in MeOH (20 mL) at 5–10 °C in a drop-wise manner over 1 h. The resulting reaction mixture was stirred for 10 h and then poured on crushed ice and neutralized with 2 M HCl. EtOAc (50 mL) was added and the organic layer was washed successively with water, a saturated solution of $NaHCO_3$, water and brine and then dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified by column chromatography on silica gel (AcOEt/*n*-hexane = 1/3 to 1/1) to give the title compound **4a** as a white solid.

3-Hydroxy-2-phenyl-4*H*-chromen-4-one (**4a**): yield 55%, mp. 172–173 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.34 (1H, m), 7.40 (4H, m), 7.80 (2H, m), 8.23 (1H, m), 9.61 (1H, s); MS (ESI$^+$) *m*/*z*: 239.1 [M+H]$^+$; HR-MS (ESI$^+$) *m*/*z*: 239.0714 [M+H]$^+$, Calcd. for $C_{15}H_{11}O_3$ 239.0708.

3-Hydroxy-2-(3-methoxyphenyl)-4*H*-chromen-4-one (**4b**): yield 65%, mp. 140–142 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.83 (3H, s), 7.08 (1H, m, J = 2.8 Hz, J = 8.4 Hz), 7.46 (2H, m), 7.80 (4H, m), 8.12 (1H, dd, J = 1.2 Hz, J = 8.4 Hz), 9.63 (1H, s); MS (ESI$^+$) *m*/*z*: 269.1 [M+H]$^+$; HR-MS (ESI$^+$) *m*/*z*: 269.0822 [M+H]$^+$, Calcd. for $C_{16}H_{13}O_4$ 269.0814.

2-(4-Chlorophenyl)-3-hydroxy-4*H*-chromen-4-one (**4c**): yield 62%, mp. 189–191 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.47 (1H, t, J = 7.0 Hz), 7.64 (2H, d, J = 8.5 Hz), 7.79 (2H, m), 8.10 (1H, d, J = 7.0 Hz), 8.25 (2H, d, J = 8.5 Hz), 9.84 (1H, s); MS

(ESI$^+$) m/z: 273.1 [M+H]$^+$; HR-MS (ESI$^+$) m/z: 273.0316 [M+H]$^+$, Calcd. for $C_{15}H_{10}Cl$ 273.0318.

3-Hydroxy-2-*p*-tolyl-4*H*-chromen-4-one (**4d**): yield 59%, mp. 112–114 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.38 (3H, s), 7.37 (2H, d, J=8.0 Hz), 7.46 (1H, m), 8.12 (3H, m), 9.52 (1H, s); MS (ESI$^+$) m/z: 253.1 [M+H]$^+$; HR-MS (ESI$^+$) m/z: 253.0869 [M+H]$^+$, Calcd. for $C_{16}H_{13}O_3$ 253.0865.

*3.1.2. General procedure for the synthesis of compounds **7a–i***

4-Methoxybenzenethiol **5** (1.40 g, 10 mmol) was added to polyphosphoric acid (12 mL) preheated to 90 °C under mechanical stirring. At this temperature, ethyl benzoylacetate **6a** (1.92 g, 10 mmol) was added slowly in a drop-wise manner to the mixture, and stirring was continued for 3 h after the addition. The cooled mixture was vigorously stirred with ice/water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and evaporated *in vacuo*. The crude product was purified by silica gel chromatography (AcOEt/*n*-hexane=1/3 to 1/2) to give the **7a** as colorless crystals.

6-Methoxy-2-phenyl-4*H*-thiochromen-4-one (**7a**): yield 54%, mp. 159–160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.96 (3H, s), 7.30 (1H, dd, J=2.8 Hz, J=8.8 Hz), 7.42 (s, 1H), 7.52 (m, 3H), 7.63 (1H, d, J=8. 8 Hz), 7.72 (2H, m), 8.02 (1H, d, J=2.8 Hz); MS (ESI$^+$) m/z: 269.1 [M+H]$^+$, 291.0 [M+Na]$^+$; HR-MS (ESI$^+$) m/z: 269.0638 [M+H]$^+$, Calcd. for $C_{16}H_{13}O_2S$ 269.0631.

6-Methoxy-2-propyl-4*H*-thiochromen-4-one (**7b**): yield 48%, mp. 59–60 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.01 (t, 3H, J=7.2 Hz), 1.78 (2H, m), 2.67 (2H, t, J=7.6 Hz), 3.92 (3H, s), 6.90 (1H, s), 7.21 (1H, dd, J=2.8 Hz, J=8.8 Hz), 7.48 (1H, d, J=8.8 Hz), 7.96 (1H, d, J=2.8 Hz); MS (ESI$^+$) m/z: 235.2 [M+H]$^+$, 257.1 [M+Na]$^+$; HR-MS (ESI$^+$) m/z: 235.0793 [M+H]$^+$, Calcd. for $C_{13}H_{15}O_2S$ 235.0787.

2-(4-Fluorophenyl)-6-methoxy-4*H*-thiochromen-4-one (**7c**): yield 45%, mp. 139–141 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.96 (3H, s), 7.20 (2H, m), 7.29 (1H, m), 7.27 (1H, s), 7.59 (1H, d, J=8.8 Hz), 7.70 (2H, m), 8.00 (1H, d, J=2.8 Hz); MS (ESI$^+$) m/z: 287.2 [M+H]$^+$, 309.1 [M+Na]$^+$; HR-MS (ESI$^+$) m/z: 287.0544 [M+H]$^+$, Calcd. for $C_{16}H_{12}O_2FS$ 287.0537.

6-Methoxy-2-(4-methoxyphenyl)-4*H*-thiochromen-4-one (**7d**): yield 57%, mp. 162–164 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.88 (3H, s), 3.96 (3H, s), 7.02 (2H, m), 7.28 (1H, dd, J=8.8 Hz, J=2.8 Hz), 7.41 (1H, s), 7.60 (1H, d, J=8.8 Hz), 7.69 (2H, m), 8.00 (1H, d, J=2.8 Hz); MS (ESI$^+$) m/z: 299.1 [M+H]$^+$. HR-MS (ESI$^+$) m/z: 299.0745 [M+H]$^+$, Calcd. for $C_{17}H_{15}O_3S$ 299.0736.

6-Methoxy-2-methyl-4*H*-thiochromen-4-one (**7e**): yield 60%, mp. 100–102 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.49 (3H, s), 3.93 (3H, s), 6.92 (1H, s), 7.23 (1H, dd, J=3.2 Hz, J=8.8 Hz), 7.48 (1H, d, J=8.8 Hz), 7.96 (1H, d, J=3.2 Hz); MS (ESI$^+$) m/z: 207.2 [M+H]$^+$, 229.2 [M+Na]$^+$; HR-MS (ESI$^+$) m/z: 207.0479 [M+H]$^+$, Calcd. for $C_{11}H_{11}O_2S$ 207.0474.

2-(Chloromethyl)-6-methoxy-4*H*-thiochromen-4-one (**7f**): yield 40%, mp. 129–132 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.88 (3H, s), 4.95 (2H, s), 7.12 (1H, s), 7.41 (1H, dd, J=2.8 Hz, J=8.8 Hz), 7.76 (1H, d, J=2.8 Hz), 7.87 (1H, d, J=8.8 Hz); MS (ESI$^+$) m/z: 241.2 [M+H]$^+$, 263.2 [M+Na]$^+$; HR-MS (ESI$^+$) m/z: 241.0082 [M+H]$^+$, Calcd. for $C_{11}H_{10}ClO_2S$ 241.0090.

6-Methoxy-2-methyl-3-phenyl-4*H*-thiochromen-4-one (**7g**): yield 58%, mp. 107–109 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.21 (3H, s), 3.86 (3H, s), 7.18 (2H, d, J=6.8 Hz,), 7.40 (3H, m), 7.38 (1H, dd, J=2.8 Hz, J=8.8 Hz), 7.76 (1H, d, J=2.8 Hz), 7.79 (1H, d, J=8.8 Hz); MS (ESI$^+$) m/z: 283.5 [M+H]$^+$, 305.3 [M+Na]$^+$. HR-MS (ESI$^+$) m/z: 283.0795 [M+H]$^+$, Calcd. for $C_{17}H_{15}O_2S$ 283.0787.

6-Methoxy-2-(4-nitrophenyl)-4*H*-thiochromen-4-one (**7h**): yield 45%, mp. 186–188 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.91 (3H, s), 7.39 (1H, s), 7.46 (1H, dd, J=2.8 Hz, J=8.8 Hz), 7.83 (1H, d, J=2.8 Hz), 7.95 (1H, d, J=8.8 Hz), 8.04 (2H, d, J=8.8 Hz), 8.44 (2H, d, J=8.8 Hz); MS (ESI$^+$) m/z: 314.2 [M+H]$^+$; HR-MS (ESI$^+$) m/z: 314.0491 [M+H]$^+$, Calcd. for $C_{16}H_{12}O_4NS$ 314.0482.

3-Chloro-6-methoxy-2-methyl-4*H*-thiochromen-4-one (**7i**): yield: 40%, mp. 90–93 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.47 (3H, s), 3.86 (3H, s), 7.35 (1H, dd, J=2.8 Hz, J=8.8 Hz), 7.76 (1H, d, J=2.8 Hz), 7.77 (1H, d, J=8.8 Hz); HR-MS(ESI$^+$) m/z: 241.0091 [M+H]$^+$, Calcd. for $C_{11}H_{10}O_2ClS$, 241.0085.

*3.1.3. General procedure for the synthesis of compounds **8a–c***

Compound **7a** (268 mg, 1.0 mmol) was dissolved in dry dichloromethane (30 mL) and treated with a 1 M solution of boron tribromide (4 mL, 4 mmol) in DCM with ice-cooling. The mixture was stirred for 1 h at room temperature and then poured onto ice/water. The organic layer was separated, washed cautiously with aqueous sodium bicarbonate solution, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was purified by chromatography on silica gel (AcOEt/*n*-hexane =1/3 to 1/2) to give the desired product as colorless crystals.

6-Hydroxy-2-phenyl-4*H*-thiochromen-4-one (**8a**): yield 45%, mp.>270 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.71 (1H, s), 7.25 (1H, dd, J=2.8 Hz, J=8.8 Hz), 7.56 (3H, m), 7.72 (1H, d, J=2.8 Hz), 7.79 (3H, m), 10.27 (1H, s); MS (ESI$^+$) m/z: 255.1 [M+H]$^+$, 257.1 [M+Na]$^+$; HR-MS (ESI$^+$) m/z: 255.0481 [M+H]$^+$, Calcd. for $C_{15}H_{11}O_2S$ 255.0474.

6-Hydroxy-2-propyl-4*H*-thiochromen-4-one (**8b**): yield 64%, mp. 147–149 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.93 (3H, t, J=7.2 Hz), 1.68 (2H, m), 2.68 (2H,t, J=7.2 Hz), 6.79 (1H, s), 7.18 (1H, dd, J=2.8 Hz, J=8.8 Hz), 7.67 (2H, m), 10.15 (1H, s); MS (ESI$^+$) m/z: 221.13 [M+H]$^+$, 243.1 [M+Na]$^+$; HR-MS (ESI$^+$) m/z: 221.0635 [M+H]$^+$, Calcd. for $C_{13}H_{13}O_2S$ 221.0631.

2-(4-Fluorophenyl)-6-hydroxy-4*H*-thiochromen-4-one (**8c**): yield 58%, mp. 157–159 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.16 (1H, s), 7.25 (1H, dd, J=2.8 Hz, J=8.4 Hz), 7.40 (2H, m), 7.72 (1H, d, J=2.8 Hz), 7.87 (2H, m), 10.27 (1H, s); MS (ESI$^+$) m/z: 273.1 [M+H]$^+$, 295.0 [M+Na]$^+$; HR-MS (ESI$^+$) m/z: 273.0388 [M+H]$^+$, Calcd. for $C_{15}H_{10}O_2SF$ 273.0380.

3.2. Anti EV71, coxsackie B3 and coxsackie B6 activity assay

The African green monkey kidney cells (Vero), coxsackie viruses B3 (Nancy strain), coxsackie viruses B6 (Schmitt strain) were all acquired from the Institute of Virology at the Chinese Academy of Preventive Medicine. EV71 (SZ-98) was acquired from Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College.

Confluent VERO cells were grown in 96-well microplates and infected with a 100 median tissue culture infective dose ($100TCID_{50}$) of the test strains. After an adsorption period of 1 or 2 h at 37 °C (1 h for EV71 adsorption, and 2 h for

coxsackie B3 and B6 adsorption), the monolayers were washed with phosphate-buffered saline and incubated at 37 °C in the maintenance medium in the absence (control) or presence of different concentrations of the test compounds. The viral CPE was observed when the viral control group reached 4, and the antiviral activities (IC_{50}) of the compounds were determined using Reed and Muench analyses. The SI value was calculated form TC_{50}/IC_{50}.

3.3. Cytotoxicity assay

The cytotoxicities of the compounds toward VERO cells were monitored by CPE. VERO cells (2.5×10^4/well) were plated into a 96-well plate, using pirodavir and ribavirin as positive controls. Following a 24 h hold period the monolayer cells were incubated in the presence of various concentrations of the test compounds. Following 48 h of culture at 37 °C under an atmosphere of 5% CO_2 in an incubator, the cells were monitored by CPE. The median toxic concentration was calculated using Reed and Muench analyses.

Acknowledgment

This work was supported by the National Science and Technology Major Special Project for Major New Drugs Innovation (Item Number: 2012ZX09102-101-001).

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