http://dx.doi.org/10.5012/bkcs.2013.34.7.1953

Synthesis and *Anti*-HCV Activity of 2,6-Bisarylmethyloxy-5-hydroxy-7-phenylchromones

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Received March 7, 2013, Accepted April 10, 2013

Key Words : Hepatitis C virus (HCV), 5-Hydroxychromone, Aryl diketoacid (ADK)

Hepatitis C virus (HCV) was identified to be the causative agent of non-A-non-B viral hepatitis in the late 1989.[1] Approximately 80% of the acute infections become chronic, leading to liver cirrhosis[2] and hepatic cellular carcinoma,[3] but a protective vaccine does not exist yet and the current therapeutic options are very limited.

Previously, we reported potent anti-HCV activity of the 5-hydroxychromone derivatives, 6-arylmethyl-5-hydroxy-7-phenylchromone[4] (**1**, Fig. 1) and 2-arylmethyloxy-6-(3-chlorobenzyloxy)-5-hydroxychromome[5] (**2**, Fig. 1), of which aromatic substituents (R, Fig. 1) were found to play the critical role for antiviral activity. In both cases, electron withdrawing substituents on the aromatic C-3 or C-3' position provided the resulting 5-hydroxychromone derivative with significantly enhanced antiviral activity against HCV. In addition, compound **2** having 3'-substituted arylmethoxy group showed higher anti-HCV activity than the corresponding analogue of **1**. This result suggests that the HCV RdRp might have a binding site specific for the 5-hydroxychromone scaffold around which two hydrophobic pockets are located. In this study, as a part of our ongoing efforts to discover a potent *anti*-HCV compound, we designed a novel 5-hydroxychromone derivative with a combination of structures **1** and **2**. Herein, we report synthesis of 2,6-bis(arylmethyloxy)-5-hydroxy-7-phenylchromone derivatives (**3**) with various *meta*- or *para*- electron withdrawing aromatic substituents (R = F, Cl, Br, I, CN, NO_2, CF_3) and evaluation of their *anti*-HCV activities.

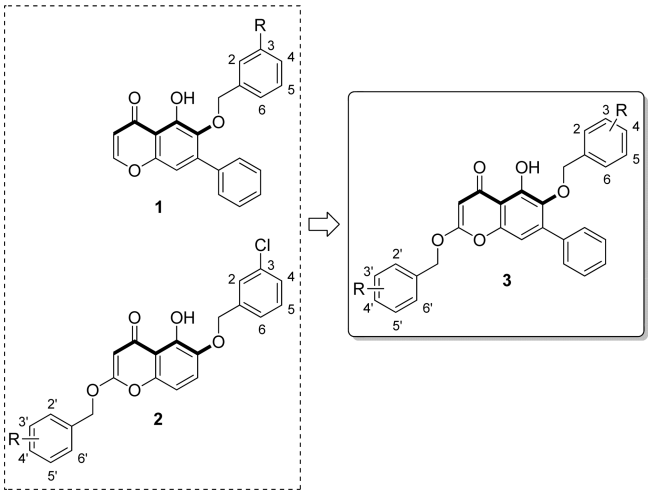
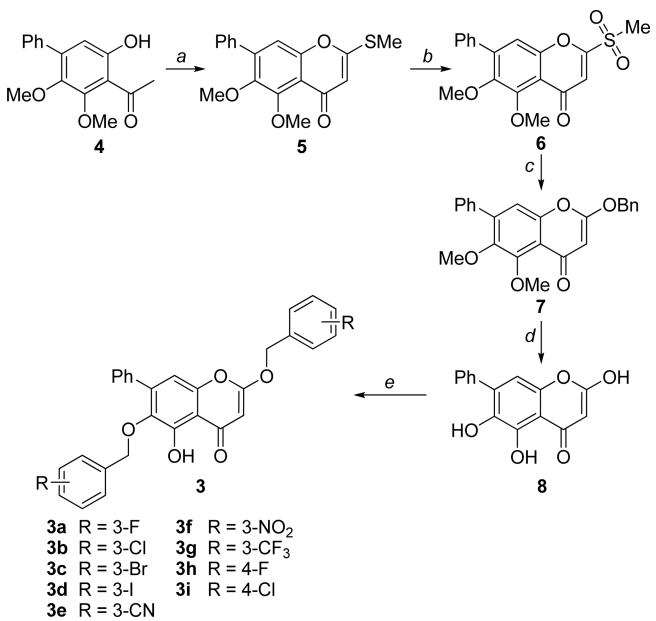


Figure 1. Structures of 6-arylmethyl-5-hydroxy-7-phenylchromone (**1**), 2-arylmethyloxy-6-(3-chlorobenzyloxy)-5-hydroxychromome (**2**) and the title compound of this study (**3**).

The synthetic route to the title compounds, 2,6-bis(arylmethyloxy)-5-hydroxy-7-phenylchromes (**3a-3k**) is outlined in Scheme 1.

Following our previous protocols, 1-[5-hydroxy-2,3-dimethoxy-(1,1'-biphenyl)-4-yl]ethanone (**4**) was prepared from commercially available 5-bromovanilline.[4] Treatment of **4** with a mixture of LiHMDS, CS_2, and MeI in THF provided the corresponding ketene dithioacetal, which was then cyclized under basic conditions to give **5** in 41% yield.[6] Oxidation of **5** was accomplished by mCPBA to **6** (83% yield), of which methanesulfonyl group was displaced with benzyloxy moiety upon treatment with benzyl alcohol and NaH in THF to furnish **7** in 49% yield.[7] Lewis acid-catalyzed cleavage of methyl and benzyl ether linkages of **7** provided the free chromone **8** in 90% yield. Due to the intramolecular hydrogen bonding between 4-keto and 5-OH



3a R = 3-F **3f** R = 3-NO_2
3b R = 3-Cl **3g** R = 3-CF_3
3c R = 3-Br **3h** R = 4-F
3d R = 3-I **3i** R = 4-Cl
3e R = 3-CN

Reagents and conditions: (a) i) LiHMDS, CS_2, MeI, THF, −78 °C to rt; ii) 10 N KOH; (b) mCPBA, toluene; (c) BnOH, NaH, THF, 0 °C to rt; (d) $AlCl_3$, toluene, reflux; (e) K_2CO_3, $RPhCH_2Br$, acetone

Scheme 1. Synthesis of 2,6-bis(arylmethyloxy)-5-hydroxy-7-phenylchromones (**3a-3j**).

Table 1. *Anti*-HCV activity and cytostatic effect of 2,6-bis(arylmethyloxy)-5-hydroxy-7-phenylchromone (**3**) derivatives[a]

Compd	R		EC_{50} (μM)[b,c]	CC_{50} (μM)[c,d]	SI[e]
	Position	Substituent			
3a	3	F	10	19	1.9
3b		Cl	13	42	3.2
3c		Br	52	> 100	> 1.9
3d		I	50	68	1.4
3e		CN	42	> 100	> 2.4
3f		NO_2	> 100	48	< 0.5
3g		CF_3	> 100	31	< 0.3
3h	4	F	14	10	0.7
3i		Cl	9	19	2.1

[a]Interferon α-2b was used as a reference compound at 10000 units/well and reduced the signal in the viral RNA (luciferase) assay to background levels without any cytotoxic activity. [b]Concentration required to inhibit HCV RNA replication by 50% in HCV replicon cell. [c]The values obtained as the average of triplicate determinations. [d]Concentration required to reduce cell proliferation by 50%. [e]Selectivity index = ratio of CC_{50} to EC_{50}.

functionalities, 2-OH and 6-OH of **8** were selectively reacted with variously substituted benzyl bromides in the presence of K_2CO_3 in acetone to give the desired compounds (**3a-3i**) in 60-70% yield.

The synthesized 2,6-bis(arylmethyloxy)-5-hydroxy-7-phenyl-chromone derivatives (**3a-3i**) were evaluated for their activity to inhibit HCV replication in Huh-5-2 cells.[8-10] The cytostatic effect of the test compounds was also evaluated in the same cell line. *Anti*-HCV activity and cytostatic effect were summarized as EC_{50} and CC_{50} values, respectively, in Table 1.

Most 2,6-bis(arylmethyloxy)-5-hydroxy-7-phenylchromone derivatives (**3**) showed moderate *anti*-HCV activity with EC_{50} values of 9-52 μM (Table 1), but 3-NO_2- and 3-CF_3- substituted derivatives, **3f** and **3g**, were not active up to 100 μM. Among the compounds having *meta*-position substituent, the compound **3a**, which has the smallest functional group (R = 3-F), showed the most potent antiviral activity (EC_{50} = 10 μM). By comparison, **3b** (R = 3-Cl) exhibited slightly decreasing activity (EC_{50} = 13 μM), and the *anti*-HCV activity of **3c** with much bigger R group, Br substituent, dramatically decreased (EC_{50} = 52 μM). The compound **3d** (R = 3-I) showed similar activity compared to **3c**. Interestingly, **3e**, of which cyano-substituent is similar in size but more electron withdrawing than the bromo-substituent, showed more potent activity (EC_{50} = 42 μM) than **3c**. Also noteworthy was that the 4-fluoro- and 4-chloro- substituted 5-hydroxychromone derivatives (**3h** and **3i**) showed almost the same *anti*-HCV activity as the corresponding 3-substituted congeners (**3a** and **3b**). Taken together, these results indicate that the *anti*-HCV activity of 2,6-bis(arylmethyloxy)-5-hydroxy-7-phenylchromone derivatives (**3**) was affected by the size as well as the electron withdrawing capacity of the aromatic substituent.

Unfortunately, the title compounds, except **3c** and **3e**, showed general cytotoxicity in the hepatoma cell line with CC_{50} values of 10-68 μM (Table 1). Presumably due to complex mechanisms related to their cytotoxicity, it was not amenable to draw a relationship between structure and cytotoxicity. However, it is of particular interest that 3-Br and 3-CN substituted derivatives (**3c** and **3e**) did not show any cytotoxic effect up to 100 μM.

In summary, a short series of 2,6-bis(arylmethyloxy)-5-hydroxy-7-phenylchromone derivatives (**3**) was prepared and their *anti*-HCV activities were evaluated. The title compounds (**3a-3i**) showed modest to potent *anti*-HCV activity, and the structure-activity relationship was clear in that 2,6-bis(arylmethyloxy)-5-hydroxy-7-phenylchromone derivatives (**3**) with small and electron withdrawing substituent showed potent *anti*-HCV activity. Among the series, 3-Br and 3-CN substituted derivatives (**3c** and **3e**) showed modest but selective *anti*-HCV activity, which warrants further in-depth investigation of the structure-activity relationship of the 5-hydroxychromone derivatives.

Acknowledgments. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0008260), by a grant of the Korea Healthcare technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A08-4628-AA2023-08N1-00010A), by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012-0006686), and by a grant from Next Generation Bio-Green 21 PJ007982 (Korea Rural Development Administration).

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