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Selective Anti-HCV Activity of 6,7-Bis-*O*-Arylmethyl-5,6,7-Trihydroxychromone Derivatives

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Recently, we have reported a series of 5-hydroxychromone derivatives (Fig. 1) with interesting antiviral activity.[1-5] Among those, galangin derivatives such as 7-*O*-arylmethyl-galangins[3] (**1**, Fig. 1) and 3-*O*-arylmethylgalangins[4] (**2**, Fig. 1) showed potent anti-HCV (hepatitis C virus) activity, and structure-activity relationship study revealed that the aryl-methyloxy group (dotted circles, Fig. 1) substituted to the core 5-hydroxychromone scaffold played a key role in determining the antiviral activity of both **1** and **2**.[3,4] Position as well as type of the aromatic substituent R also turned out to be the critical determinant for the activity, and the galangin derivatives with 3-Cl or 3-CN substituent showed the most promising antiviral activity.[3,4]

More intriguingly, when the two arylmethyloxy substituents were combined on the 5-hydroxychromone scaffold, the resulting 3,7-bis-*O*-arylmethylgalangin derivatives (**3**, Fig. 2) showed broad spectrum antiviral activity against SCV [Severe Acute Respiratory Syndrome (SARS) Corona Virus, SARS-CoV] as well as HCV.[5] This interesting antiviral profile of the bis-*O*-arylmethyloxy-substituted 5-hydroxy-chromones prompted us to extend the structure-activity relationship study to include positional scanning of the arylmethyloxy substituents on the 5-hydroxychromone core structure. In this study, we designed another bis-*O*-aryl-methyl-5-hydroxychromone scaffold with the arylmethyl-oxy substituents at vicinal 6 and 7 positions. In this pre-

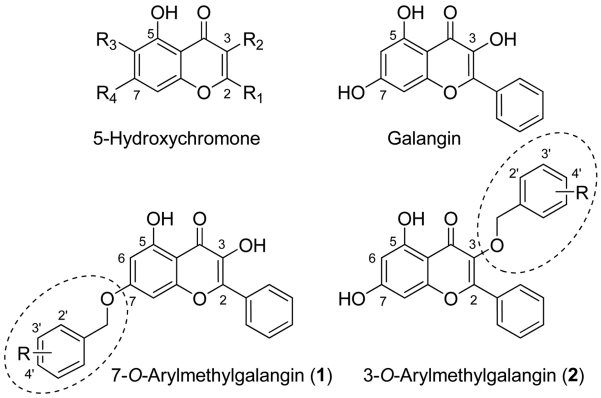


Figure 1. Structures of 5-hydroxychromone, galangin, and substituted galangins (**1** and **2**).

[a]These authors contributed equally to this work.

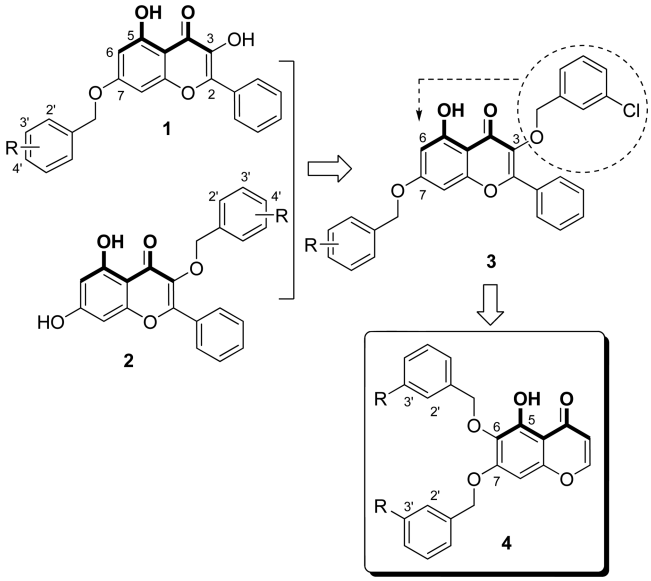
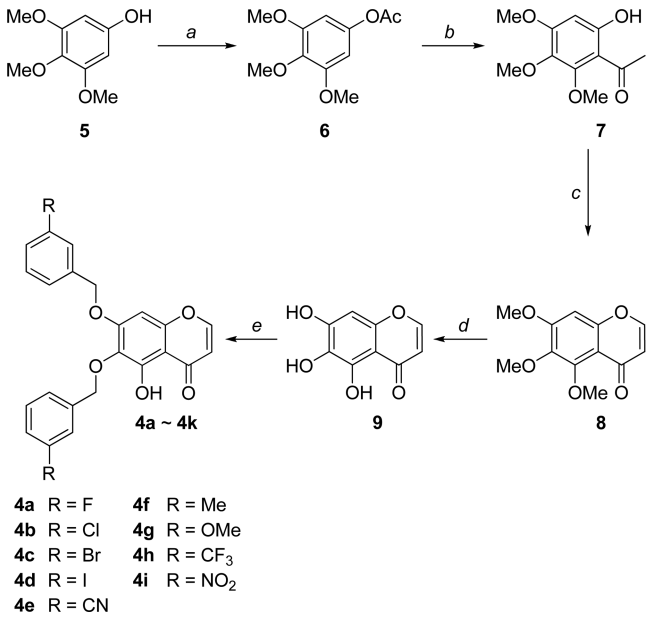


Figure 2. Design of 6,7-bis-*O*-arylmethyl-5,6,7-trihydroxychromone derivatives (**4**).

liminary study, the aromatic substituent R was fixed at 3-position because it was found to be the position of choice for potent antiviral activity in a series of 5-hydroxychromone derivatives.[1-5] Herein, we report synthesis and preliminary evaluation of a series of novel 6,7-bis-*O*-arylmethyl-5,6,7-trihydroxychromone derivatives (**4**, Fig. 2) as potential antiviral agents.

Treatment of a commercially available 3,4,5-trimethoxy-phenol **5** with sodium acetate in acetic anhydride afforded the corresponding *O*-acetyl derivative **6** in 96% yield, which smoothly underwent Lewis acid-catalyzed Fries rearrange-ment to give an acetophenone **7** in 93% yield (Scheme 1).[6] Base-induced condensation of **7** with ethylformate followed by treatment of the resulting intermediate with *p*-toluene-sulfonic acid in boiling benzene afforded 5,6,7-trimethoxy-chrome **8** in 85% of combined yield. The methyl protecting groups of **8** were removed simultaneously by treatment with BBr_3 to give 5,6,7-trihydroxychromone **9** in 92% yield. Dialkylation of **9** with variously substituted benzyl bromide in the presence of K_2CO_3 in acetone proceeded to give the desired products **4a-4i** in 60-70% yield.

All synthesized 6,7-bis-*O*-arylmethyl-5,6,7-trihydroxy-



Reagents and conditions: (a) Ac_2O, NaOAc, 110 °C; (b) $BF_3{\cdot}Et_2O$, AcOH, 70 °C; (c) HCO_2Et, NaH, THF, rt; *p*-TsOH, PhH, reflux; (d) BBr_3, CH_2Cl_2; (e) R-BnBr, K_2CO_3, acetone.

Scheme 1. Synthesis of 6,7-bis-*O*-arylmethyl-5,6,7-trihydroxychromone derivatives (**4**).

chromone derivatives (**4a-4i**) were evaluated for their biological activities on inhibiting the growth of the hepatoma cell lines containing subgenomic HCV genotype 1 replicon with the *luc-ubi-neo* fusion gene.[7,8] The luminescence-based assay protocol[9] was adapted. Anti-SCV activities of the synthesized 5-hydroxychromone derivatives (**4a-4i**) were also tested in terms of inhibition of ATPase[10] as well as duplex DNA-unwinding[11] activities of the SCV helicase.[12] Assays were performed in triplicate and the antiviral activities are summarized as EC_{50} and IC_{50} values in Table 1.

The title compounds (**4a-4i**) showed moderate to potent anti-HCV activity in the HCV replicon cell-based assay (EC_{50} = 0.8-34 μM, Table 1). The halogen-substituted derivatives **4a-4d** showed more potent anti-HCV activity compared with other congeners (**4e-4i**), and 6,7-bis-*O*-(3-iodophenylmethyl)-5,6,7-trihydroxychromone (**4d**) showed the most potent activity (EC_{50} = 0.8 μM) among the series. Other aromatic substituents such as -CN, -Me, -OMe, $-CF_3$, and $-NO_2$ conferred the corresponding 5-hydroxychromone derivatives (**4e-4i**) with less potent anti-HCV activity. However, it is worth to note that the anti-HCV activities of these derivatives are in decreasing order of inductive effect (NO_2 > CN > CF_3 > Me > OMe) of the aromatic substituents, which suggests electron density around the aromatic substituent may be in action to control the antiviral activity. Overall, the anti-HCV activity of the 6,7-bis-*O*-arylmethyl-5,6,7-trihydroxychromone derivatives synthesized in this study (**4**) showed similar anti-HCV activity compared with their mother compounds, 3,7-bis-*O*-arylmethylgalangin derivatives (**3**, Fig. 1).[5] On the contrary, neither ATPase activity nor duplex DNA-unwinding activity of the SCV helicase was inhibited by **4**, which is a clear contrast with the substituent-specific anti-SCV activity of **3**.

Table 1. Antiviral activities of 6,7-bis-*O*-arylmethyl-5,6,7-trihydroxychromone derivatives (**4a-4i**)

Compds	R	HCV (EC_{50}, μM)[a]	SCV (IC_{50}, μM)	
			NTPase[b]	Helicase[c]
4a	F	3	>100	>100
4b	Cl	5	>100	>100
4c	Br	5	>100	>100
4d	I	0.8	>100	>100
4e	CN	19	>100	>100
4f	Me	26	>100	>100
4g	OMe	34	>100	>100
4h	CF_3	26	>100	>100
4i	NO_2	10	>100	>100

[a]Concentration required to inhibit HCV RNA replication by 50% in HCV replicon cell. Interferon α-2b was used as a reference compound at 10000 units/well and reduced the signal to background levels without any cytotoxic activity. [b]Concentration required to inhibit SCV NTPase activity by 50%. [c]Concentration required to inhibit duplex DNA-unwinding activity of SCV helicase by 50%

Taken together, with combination of the broad-spectrum antiviral activity of the 3,7-bis-*O*-arylmethylgalangin derivatives (**3**), this result delineate the difference between the pharmacophore space of anti-HCV and anti-SCV activity of the 5-hydroxychromone derivatives. Further investigations are warranted concerning structure-activity relationship study of the 6,7-bis-*O*-arylmethyl-5,6,7-trihydroxychromone derivatives as well as positional scanning of the aryloxy substituent on the 5-hydroxychromone scaffold.

Experimental Section

Preparation of the Key Intermediate 8. To a stirred mixture of NaH in anhydrous THF, a mixture of **7** (1.1 g, 4.9 mmol) and ethyl formate (0.8 mL, 9.7 mmol) in THF was slowly added. The reaction mixture was stirred overnight at room temperature and then poured into ice-water. After acidification with cold dilute HCl (6 N), the mixture was extracted with ether. The combined organic layers was washed with water, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was dissolved in benzene and treated with *p*-toluenesulfonic acid. After stirring 8 h under reflux, the reaction mixture was cooled, washed with saturated aqueous $NaHCO_3$ solution and water, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (EtOAc:Hexane = 3:1) to afford the desired compound **8** as a light yellow solid (1 g, 87% yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, J = 5.9 Hz, 1H), 6.68 (s, 1H), 6.19 (d, J = 5.9 Hz, 1H), 3.96 (s, 1H), 3.95 (s, 3H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.8, 157.5, 154.34, 154.25, 151.6, 139.8, 113.1, 113.0, 97.1, 61.7, 60.9, 56.4; LC/MS (ESI) *m/z* Found; 237.3 [M + H]$^+$; Calcd for $C_{12}H_{12}O_5$; 236.07.

Preparation of the Trihydroxychromone Derivative 9.

Solution of **8** in anhydrous CH_2Cl_2 was treated with BBr_3 (1.0 M in THF, 3.0 equiv.) at 0 °C and stirred for 12 h at room temperature. After concentration of the reaction mixture under reduced pressure, the residue was purified by column chromatography on silica gel (EtOAc:Hexane = 2:3) to afford **9** in 92% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 5.9 Hz, 1H), 6.52 (s, 1H), 6.20 (d, J = 5.9 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.1, 155.8, 151.9, 110.4, 94.0, 77.4, 41.9, 31.9, 31.6; LC/MS (ESI) *m/z* Found; 195.1 $[M + H]^+$; Calcd for $C_9H_6O_5$; 194.02.

Preparation of the Title Compound 4. Synthetic procedures for compound **4d** are representative. A mixture of **9** (1.0 equiv.), K_2CO_3 (2.2 equiv.), and 3-iodophenylmethyl bromide (2.0 equiv.) in acetone was stirred under reflux for 5 h. After cooling to room temperature, the reaction mixture was extracted with CH_2Cl_2. The combined organic layers was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a residue which was purified by column chromatography on silica gel (EtOAc: Hexanes = 3:1) to give the desired product **4d** in 76% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.63 (s, 1H), 7.85 (s, 1H), 7.77 (s, 1H), 7.75 (d, J = 6.0 Hz, 1H), 7.14 (t, J = 7.8 Hz, 1H), 7.06 (t, J = 7.8 Hz, 1H), 7.69 (d, J = 7.9 Hz, 1H), 7.62 (d, J = 8.3 Hz, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.34 (d, J = 7.8 Hz, 1H), 6.43 (s, 1H), 6.24 (d, J = 5.9 Hz, 1H), 5.07 (s, 2H), 5.05 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.5, 164.7, 163.1, 162.7, 158.8, 158.1, 145.5, 144.1, 137.2, 136.8, 135.6, 135.2, 133.8, 133.2, 116.9, 115.1, 112.1, 100.6, 100.1, 99.1, 97.4, 78.7, 75.1; LC/MS (ESI) *m/z* Found; 627.0 $[M + H]^+$; Calcd for $C_{23}H_{16}I_2O_5$; 625.91.

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