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2-Arylmethylaminomethyl-5,6-dihydroxychromone derivatives with selective anti-HCV activity

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ABSTRACT

Anti-HCV activity of aryl diketoacid (ADK) has been characterized by its two pharmacophoric elements, α,β-diketo acid moiety and substituted aryl ring. In this study, as a part of our ongoing efforts to discover a novel anti-HCV compound mimicking the ADK scaffold, we designed 2-arylmethylaminomethyl-5,6-dihydroxychromone derivatives of which the dihydroxychromone moiety as well as the arylmethylaminomethyl substituent (R-PhCH$_2$NHCH$_2$–) were anticipated in exact match with the pharmacophore model of the ADK. The dihydroxychromone derivatives (**3a**–**3u**), thus prepared, showed biological activity in a substituent-dependent fashion, thereby leading to selective anti-HCV effect (EC_{50} = 2.0–14.0 μM, CC_{50} >100 μM) with the substituent groups such as Cl, Br, I, and Me specifically at the 3-position of the aromatic ring.

Hepatitis C is an infection of the liver caused by hepatitis C virus (HCV). More than 80% of HCV infected patients develop chronic hepatitis, which damages the liver and eventually leads to liver cirrhosis and hepatocellular carcinoma.[1] Current antiviral treatment for HCV is limited to combination of pegylated interferon α-2a and ribavirin,[2] but low sustained response rate and side effects associated with the therapy[3] necessitates development of more specific antiviral agents.[4]

Aryl diketo acid (ADK), originally identified as an inhibitor of HIV (Human Immunodeficiency Virus) integrase,[5] is reported to be a potent antiviral agent against HCV.[6] Structure–activity relationship study revealed that the ADK analogues with 3-arylmethylamino substituent (**1**, Fig. 1) have potent anti-HCV activity, and the pharmacophore model was updated to include the aromatic substituent of ADK in addition to its characteristic α,β-diketo acid moiety.[7]

However, due to the unfavorable physicochemical property of the terminal carboxylic acid in the pharmacophoric α,β-diketo acid moiety of ADKs, there have been numerous attempts to replace the free carboxylic acid with its bioisosteres such as triazole (S1360),[8] tetrazole (5CITEP),[9] pyridine (L-870,810),[10–12] and a neutral carbonyl group (Raltegravir).[13] Recently, natural flavonoid galangin was also reported to serve as an excellent replacement of the ADK scaffold,[14,15] in particular, 7-*O*-arylmethylgalangins (**2**, Fig. 1) showed anti-HCV activity in the HCV replicon cell-based assay with EC_{50}'s in micromolar range.[14] The superimposed structures of **1** and **2** (Fig. 1) shows that the α,β-diketo acid moiety of ADK is in perfect match with the enol-keto-enol arrangement of the galangin structure (bold lines) while the other pharmacophoric element, the aromatic substituents, (box) is dislocated. In this study, a novel flavonoid with an arylmethylaminomethyl substituent at the dihydroxychromone scaffold (**3**, Fig. 1) was designed, of which two pharmacophoric elements are in match with those of ADK (**1**) in atom-by-atom manner. Herein, we report the synthesis and biological evaluation of the anti-HCV activity of 2-arylmethylaminomethyl-5,6-dihydroxychromone (**3**) derivatives.

Synthesis of the title compounds, 2-arylmethylaminomethyl-5,6-dihydroxychromone derivatives (**3a**–**3u**) was accomplished by the following Scheme 1. Oxidation of the commercially available 2,5-dihydroxyacetophenone (**4**) with PhI(OAc)$_2$ in MeOH furnished the corresponding 6-methoxy derivative in 45% yield,[16] which, upon treatment with BnBr and K_2CO_3 followed by AcCl in pyridine, underwent successive protection with benzyl and acetyl group to provide the intermediate **5**. Intramolecular Claisen condensation of **5** in the presence of *tert*-BuOK resulted in construction of the chromone core (**6**) in 40% yield. The allylic methyl group of **6** was oxidized by SeO_2 to give the corresponding aldehyde, which was converted to the key intermediate **7** by reduction with $NaBH_4$ and successive bromination with CBr_4/PPh_3. Arylmethylamines with various aromatic substituents (R-PhCH$_2$NH$_2$), either commercially available or prepared from the corresponding bromides by Gabriel synthesis, were then substituted into **7** in 40–80% yields. The methyl as well as benzyl protecting groups on the resulting intermediate were removed by treatment with BBr_3 to give the desired

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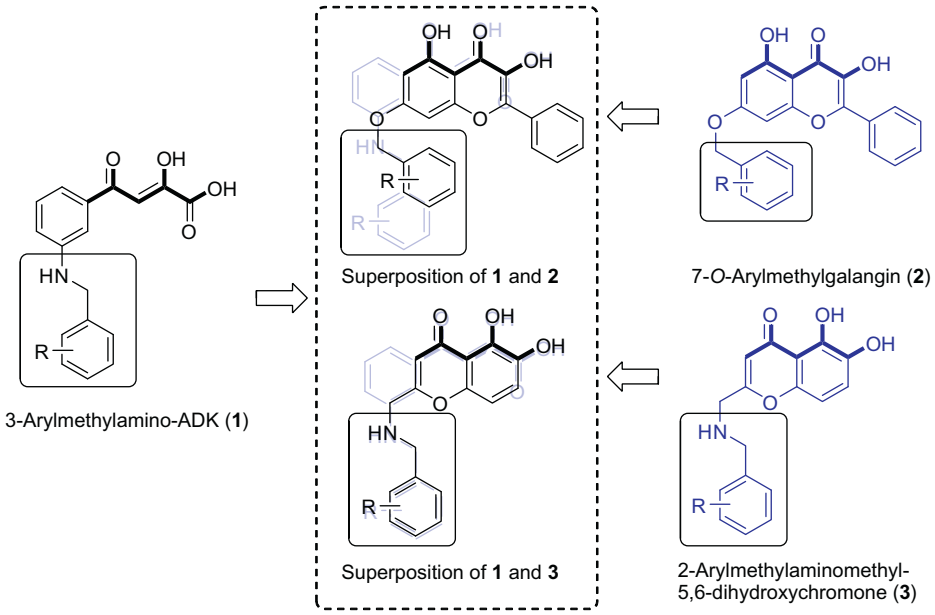
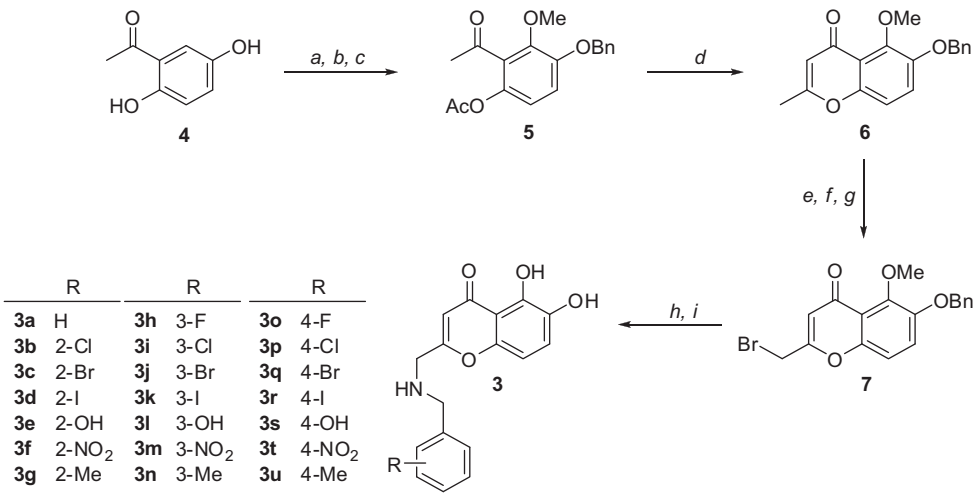


Figure 1. Structures of 3-arylmethylamino-ADK (**1**), 7-*O*-arylmethylgalangin (**2**), and 2-arylmethylaminomethyl-5,6-dihydroxychromone (**3**). The superimposed structures of **1**, **2** and **3** are shown in the middle. The pharmacophoric elements are indicated as thick lines as well as boxes.



	R		R		R
3a	H	**3h**	3-F	**3o**	4-F
3b	2-Cl	**3i**	3-Cl	**3p**	4-Cl
3c	2-Br	**3j**	3-Br	**3q**	4-Br
3d	2-I	**3k**	3-I	**3r**	4-I
3e	2-OH	**3l**	3-OH	**3s**	4-OH
3f	2-NO_2	**3m**	3-NO_2	**3t**	4-NO_2
3g	2-Me	**3n**	3-Me	**3u**	4-Me

Scheme 1. Synthesis of 2-arylmethylaminomethyl-5,6-dihydroxychromone derivatives (**3a–3u**). Reagents and conditions: (a) $PhI(OAc)_2$, MeOH, 60 °C, 45% yield; (b) BnBr, K_2CO_3, acetone, 60 °C, 60% yield; (c) AcCl, Pyr, 60 °C, 90% yield; (d) *tert*-BuOK, THF, 0 °C, then 2N HCl, H_2SO_4, 70 °C, 40% yield; (e) SeO_2, bromobenzene, 160 °C, 78% yield; (f) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH/CH_2Cl_2, 0 °C; (g) CBr_4, PPh_3, CH_2Cl_2, 0 °C, 80% yield (for two steps); (h) R-$PhCH_2NH_2$, DMF, rt, 40–80% yield; (i) BBr_3, CH_2Cl_2, 0 °C, 40% yield.

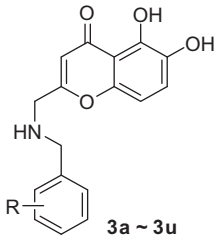
2-arylmethylaminomethyl-5,6-dihydroxychromone derivatives (**3a**–**3u**)[17] in 40% yield.

The synthesized 2-arylmethylaminomethyl-5,6-dihydroxychromone derivatives (**3a**–**3u**) were evaluated for their ability to inhibit HCV replication in Huh-7 cells by using the FRET (Fluorescence Resonance Energy Transfer)-based assay.[18,19] INF-α 2b was included as a positive control at 10000 units/well and reduced the signal in the HCV NS3 protease assay to background levels without any cytotoxic activity. The cytostatic effect of the test compounds was also evaluated in the same cell line. In Table 1, antiviral effect (EC_{50}) and cytostatic effect (CC_{50}) of the dihydroxychromone derivatives (**3a**–**3u**) are summarized and the biological activity data were compared with those of the previously reported 7-*O*-arylmethylgalangins (**2**).[14]

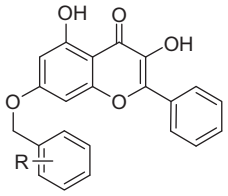
Positional scanning of the aromatic substituents (F, Cl, Br, I, OH, NO_2, and Me) revealed that antiviral activities of the 2-arylmethylaminomethyl-5,6-dihydroxychromone derivatives (**3a**–**3u**) are strongly dependent on the type as well as position of its aromatic substituents. Thus, whereas the dihydroxychromone derivatives with electron withdrawing substituents such as F (**3h** and **3o**), OH (**3e**, **3l**, and **3s**), and NO_2 (**3f**, **3m**, and **3t**) showed no antiviral effect (Table 1), antiviral activity was observed from compounds with I- (**3d**, **3k**, and **3r**) and Me- (**3g**, **3n**, and **3u**) substituents on the aromatic ring regardless of the position of the substituents. In particular, among the iodo- or methyl-derivatives, **3g** (R = 2-Me) and **3n** (R = 3-Me) were the most active compounds with EC_{50} values of 1.2 and 2.0 μM, respectively (Table 1). On the other hand, antiviral activities of the Cl- (**3b**, **3i**, and **3p**) and Br- (**3c**, **3j**, and **3q**) substituted dihydroxychromone derivatives were highly dependent on the position of the substituents, and the 3-substituted chloro- (R = 3-Cl, **3i**) and bromo- (R = 3-Br, **3j**) derivatives were the only active compounds with EC_{50} values of 14.0 and 3.6 μM, respectively (Table 1).

The FRET assay is based on the catalytic activity of HCV NS3 protease which cleaves the fluorescence-quenched substrate to give the FRET signal. Thus, reduced FRET signal indicates the lowered

Table 1
Anti-HCV activity and cytostatic effect of dihydroxychromone derivatives (**3a**–**3u**) in comparison with the previously reported activity data of 7-*O*-arylmethylgalangins (**2**)[a]



3a ~ 3u



2

Compd	R		EC_{50}[b,c] (μM)	CC_{50}[c,d] (μM)	Bioactivity of **2**[a]	
	Position	Substituent			EC_{50} (μM)	CC_{50} (μM)
3a	—	H	>100	>100		
3b	2	Cl	>100	>100	30	>100
3c		Br	>100	>100	10	>100
3d		I	6.3	87.3		
3e		OH	>100	>100		
3f		NO_2	>100	41.3	25	32
3g		Me	1.2	52.2	7	17
3h	3	F	>100	>100		
3i		Cl	14.0	>100	22	>100
3j		Br	3.6	>100	37	>100
3k		I	7.3	>100		
3l		OH	>100	>100		
3m		NO_2	>100	>100	2	76
3n		Me	2.0	>100	27	35
3o	4	F	>100	>100		
3p		Cl	>100	>100	8	>100
3q		Br	>100	>100	6	>100
3r		I	4.1	41.7		
3s		OH	>100	62.7		
3t		NO_2	>100	73.3	10	93
3u		Me	5.7	>100	6	88

[a] Ref. 14.
[b] Concentration required to inhibit HCV RNA replication by 50%.
[c] The values obtained as the average of triplicate determinations.
[d] Concentration required to reduce cell proliferation by 50%.

protease activity due to the inhibition of the HCV replication. By the same token, cytotoxic effect of the test compounds may result in false contribution to reduction of the FRET signal. Unfortunately, 2-iodo- (**3d**, R = 2-I), 2-methyl- (**3g**, R = 2-Me), or 4-iodo- (**3r**, R = 4-I) phenylmethylaminomethyl-5,6-dihydroxychromone derivatives, which showed anti-HCV activity, were also cytotoxic (CC_{50} = 87.3, 52.2, and 41.7 μM, respectively), and this result indicates that their antiviral activities went hand in hand with the cytotoxicity. Interestingly, however, cytotoxicity was dependent on the position of the substituent, and the derivatives with aromatic substituents at the 3-position such as **3i** (R = 3-Cl), **3j** (R = 3-Br), **3k** (R = 3-I), and **3n** (R = 3-Me) showed selective anti-HCV effect with no cytotoxicity up to 100 μM.

It is of another interest to compare the structure–activity relationship of the dihydroxychromone derivatives (**3**) with that of the previously published 7-*O*-arylmethylgalangins (**2**).[14] Compared with 7-*O*-arylmethylgalangins (**2**) of which the antiviral activity was determined by the type of the aromatic substituent (Cl and Br) rather than its position (Table 1),[14] the dihydroxychromone derivatives (**3**) with selective antiviral activity accommodated a broad range of substituents (Cl, Br, I, and Me) specifically at the 3-position of the aromatic ring.

Taken together, the arylmethylaminomethyl functionality installed on the dihydroxychromone core structure, a one-carbon elongation of the corresponding arylmethyl group of 7-*O*-arylmethylgalangins (**2**), endowed the resulting 2-arylmethylaminomethyl-5,6-dihydroxychromones (**3**) with antiviral activity which is specifically controlled by the position of the aromatic substituent.

Acknowledgments

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17. Spectroscopic data for (a) 5,6-dihydroxy-2-[(2-methyl-benzylamino)-methyl]-chromen-4-one (**3g**): ^{1}H NMR (400 MHz, CD_3OD) δ 7.51 (d, J = 7.5 Hz, 1H), 7.28–7.37 (m, 3H), 7.11 (d, J = 8.8 Hz, 1H), 6.78 (d, J = 8.8 Hz, 1H), 6.42 (s, 1H), 4.59 (s, 2H), 4.46 (s, 2H), 2.50 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.0, 152.5, 151.9, 148.5, 147.1, 143.3, 137.5, 136.5, 136.1, 134.8, 131.7, 124.3, 121.3, 113.1, 112.1, 55.3, 54.0, 24.8; HRMS (ESI) m/z Calcd for $C_{18}H_{16}NO_4$ 310.1079 $[M-H]^-$, Found 310.3124. (b) 5,6-Dihydroxy-2-[(3-methyl-benzylamino)-methyl]-chromen-4-one (**3n**): ^{1}H NMR (400 MHz, CD_3OD) δ 7.33–7.39 (m, 3H), 7.27–7.29 (m, 1H), 7.10 (d, J = 8.8 Hz, 1H), 6.77 (d, J = 8.8 Hz, 1H), 6.37 (s, 1H), 4.51 (s, 2H), 4.37 (s, 2H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 152.5, 151.9, 148.5, 147.1, 143.5, 137.0, 136.5, 135.3, 134.2, 133.0, 124.3, 121.2, 113.0, 112.1, 56.6, 55.0, 26.5; HRMS (ESI) m/z calcd for $C_{18}H_{16}NO_4$ 310.1079 $[M-H]^-$, Found 310.3372.
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19. Huh-7 cells with HCV replicon were seeded at a density of 5×10^3 per well in a tissue culture-treated 96-well black plates with clear bottoms plate in complete DMEM supplemented with 500 μg/mL G418. After incubation for 24 h at 37 °C (5% CO_2), medium was refreshed and DMSO stock of test compounds were diluted into seven different concentrations (0.1, 0.5, 1, 5, 10, 50, 100 μM). After 4 days of incubation at 37 °C, medium was removed from culture plates and cells were lysed in lysis buffer. FRET analysis was accomplished according to the protocol provided by the 490 HCV protease assay kit (Anaspec), and the intensity of fluorescence was measured at 340 nm_{ex}/490 nm_{em} on a microplate reader.