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7-*O*-Arylmethylgalangin as a novel scaffold for anti-HCV agents

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ABSTRACT

In spite of potent antiviral activity, suboptimal physicochemical properties of aryl diketo acids (ADKs) necessitates modification of the core 1,3-diketo acid functionality into a novel scaffold. As the metal-binding affinity of the diketo acid is the key to the antiviral activity of ADKs, we anticipated 3,5-dihydroxy-4-oxo arrangement of galangin scaffold would serve as an excellent mimic for the diketo acid functionality. In this study, through synthesis and biological evaluation of various galangin derivatives, we have shown that the diketo acid functionality can be successfully replaced with the galangin scaffold by specific combination of the substituents to result in identification of a novel galangin derivative (**3s**) with anti-HCV activity (EC_{50} = 0.9 μM) comparable to the ADK counterpart.

Infection with hepatitis C virus (HCV) affects more than 170 million people worldwide, and 3–4 million are newly infected each year. If untreated, more than 80% of HCV infected individuals will develop chronic hepatitis, which can lead to liver cirrhosis and eventually to hepatocellular carcinoma.[1] No prophylactic vaccine is available yet and the only approved anti-HCV treatment, combination of pegylated interferon α-2a and ribavirin,[2] suffers from low sustained response and numerous side effects.[3] As a result, there is a compelling need to develop safe and more efficacious antiviral drugs.[4]

Aryl diketo acids (ADKs) have been reported as inhibitors of HCV NS5B polymerase with low-micromolar IC_{50}'s.[5] In an earlier paper,[6] we reported the design, synthesis, and antiviral evaluation of a small series of ADK derivatives, identifying some selective HCV inhibitors. In particular, a 4-chlorophenylmethyloxy or 4-chlorophenylmethylamino substituent provided the resulting ADK derivative with potent antiviral activity [**1**, Fig. 1 (EC_{50} = 0.82 μM)].

However, suboptimal physicochemical properties of ADKs such as chemical instability, irreversible covalent binding to protein and poor stability in plasma[7] necessitates modification of the core 1,3-diketo acid functionality into a novel scaffold with more drug-like properties. Pyrophosphate mimics like ADKs are known to inhibit HCV NS5B through binding the metal ions at the active site while the aryl group forms a specific hydrophobic interaction with an adjacent hydrophobic pocket or surface.[5,8–10] In this context, well-known metal chelating property of naturally occurring flavonoids[11–13] drew our attention. In particular, 3,5-dihydroxy-4-oxo arrangement of galangin (**2**, Fig. 1) is in perfect match with the metal-binding unit of the ADK (**1**). Thus, we reasoned that galangin derivatives with appropriately substituted arylmethyl group (7-*O*-arylmethylgalangin **3**, Fig. 1) would serve as an excellent structural mimic, a bioisostere for ADKs. Herein, we report synthesis of novel 7-*O*-arylmethylgalangin derivatives (**3**) with various aromatic substituents (R^1 = Cl, Br, CN, NO_2, OMe; R^2 = H, CN, OMe) and evaluation of their anti-HCV activities.

Galangin (**2**) and 4′-substituted galangin derivatives (**6a**–**6c**) were prepared from a literature protocol[14–16] using esterification followed by cyclization of the readily available[17] substituted phenol **4** (Scheme 1). Acetylation of **6** with acetic anhydride (Ac_2O) in pyridine afforded the peracetylated galangin derivatives, which underwent regioselective deacethylation of the 7-OAc group with PhSH in NMP[16,18,19] to provide the corresponding galangin 3,5-diacetates **7**. Alkylation of **7** with variously substituted benzyl bromides followed by deacetylation by treatment with methanolic ammonia afforded a series of 7-*O*-arylmethylgalangin **3**.

All synthesized 7-*O*-arylmethylgalangin derivatives (**3a**–**3u**) were evaluated for their ability to inhibit HCV replication in Huh-5-2 cells.[20,21] INF-α was included as a positive control, and the conditions of the luminescence-based assay used to test the antiviral activity of the compounds were previously described.[22,23] The cytostatic effect of the test compounds was also evaluated in the same cell line.[23] Antiviral effect and cytostatic effect are summarized as EC_{50} and CC_{50}, respectively, in Table 1.

When R^1 = H, galangin derivatives (**3a**–**3r**) showed moderate to potent antiviral activity with **3i** and **3j** (EC_{50} = 3 and 2 μM, respectively, Table 1) being most active. As was the case in ADKs,[6] antiviral activity of the galangin derivatives was critically dependent not only on the type, but also on the position of the substituent. Thus, analysis of the anti-HCV activity indicates that the galangin derivatives can be divided into two groups depending upon the R^2

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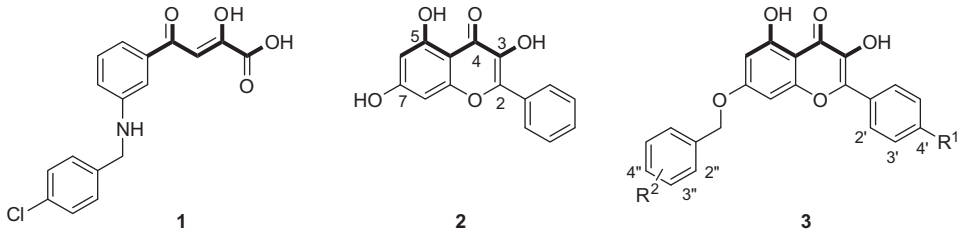
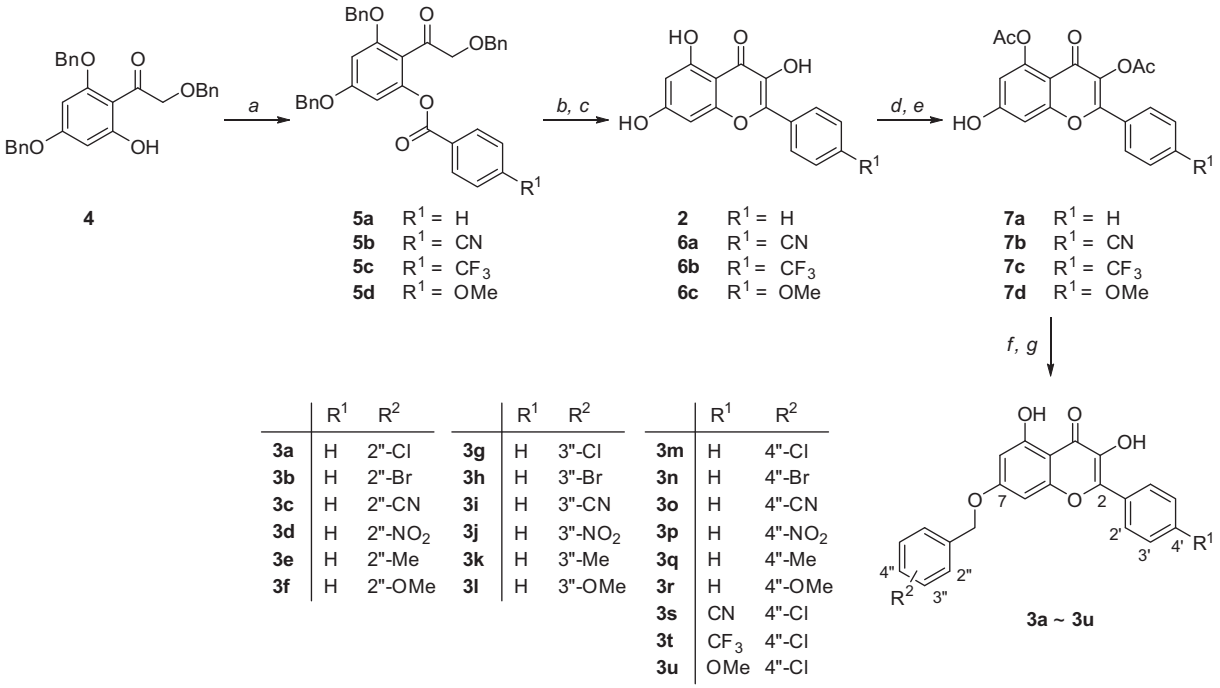


Figure 1. Structures of ADK (**1**), galangin (**2**), and 7-*O*-arylmethylgalangin (**3**) with the numbering system. The common metal-binding unit is represented as thick lines.



	R^1	R^2		R^1	R^2		R^1	R^2
3a	H	2″-Cl	**3g**	H	3″-Cl	**3m**	H	4″-Cl
3b	H	2″-Br	**3h**	H	3″-Br	**3n**	H	4″-Br
3c	H	2″-CN	**3i**	H	3″-CN	**3o**	H	4″-CN
3d	H	2″-NO_2	**3j**	H	3″-NO_2	**3p**	H	4″-NO_2
3e	H	2″-Me	**3k**	H	3″-Me	**3q**	H	4″-Me
3f	H	2″-OMe	**3l**	H	3″-OMe	**3r**	H	4″-OMe
						3s	CN	4″-Cl
						3t	CF_3	4″-Cl
						3u	OMe	4″-Cl

Scheme 1. Synthesis of the 7-*O*-arylmethylgalangin derivatives (**3a**–**3u**). Reagents and conditions: (a) R^1COCl, pyridine, rt, 87–89%; (b) K_2CO_3, Bu_4NBr, toluene, 90 °C; (c) 20% Pd/C, CH_2Cl_2/MeOH (1:1), 62–65%; (d) Ac_2O, pyridine, rt, 77–78%; (e) PhSH, imidazole, NMP, 0 °C, 79–92%; (f) R_2PhCH_2Br, K_2CO_3, acetone, rt, 53–76%; (g) NH_3/MeOH, rt 71–92%.

substituents. Three hydrophobic groups of the similar size (Cl, Br, and Me) substituted at either 2″- or 4″-position provided the corresponding arylmethylgalangin derivatives (**3a**, **3b**, **3e**, **3m**, **3n**, and **3q**) with significant antiviral activity (Table 1, Fig. 2a). On the contrary, site-specific antiviral effects were observed when hydrogen bond acceptors (CN, NO_2, or OMe) were substituted at the 3″-position of the galangin derivatives (**3i**, **3j**, and **3l**; Table 1 and Fig. 2a).

In terms of the cytostatic effect, clear structure–activity relationship was also identified but in different ways. While one group of the galangin derivatives with halogens (Cl and Br) and OMe-substituent (**3a**, **3b**, **3f**, **3g**, **3h**, **3l**, **3m**, **3n**, and **3r**) was not cytostatic at all up to 100 μM, others with CN, NO_2, or Me substituent (**3c**–**3e**, **3i**–**3k**, and **3o**–**3q**) showed cytostatic effect regardless of the substitution position (2″, 3″, and 4″) albeit in different degrees (Table 1, Fig. 2b).

Taken together, the halogen atoms (Cl and Br) substituted at 4″-position of the aromatic ring provided the resulting 7-*O*-arylmethylgalangin derivatives (**3m** and **3n**) with selective (CC_{50} >100 μM) and potent (EC_{50} = 8 and 6 μM, respectively) antiviral activity. These two compounds and the anti-HCV ADK analogue (**1**, Fig. 1)[6] were found to share the same 4-chlorophenylmethyl group directly substituted to the metal-binding unit (galangin and diketo acid, respectively), which provides proof of concept that the galangin scaffold can work as a diketo acid replacement. However, **3m** and **3n** are about 10 times less potent than the ADK analogue **1** (Fig. 1, EC_{50} = 0.8 μM),[6] and the loss of antiviral activity upon replacement of diketo acid with a galangin needs to be overcome.

In this context, it should be reminded that the metal-binding affinity of the diketo acid is the key to the antiviral activity of ADKs, and the lower antiviral activity of the galangin derivative may partly be attributed to its metal-binding capacity different from that of ADK. For these reasons, it was of our interest to vary the R^1 substituent and monitor its effect on antiviral activity of the resulting galangin derivative because the metal-binding capacity of galangin 3-OH can be resonance-controlled by R^1. Thus, with the R^2 substituent fixed with that of the most active **3m** and **3n** (4″-Cl), three functional groups with different electronic property (CN, CF_3, and OMe) were introduced as R^1 to provide three more galangin derivatives (**3s**, **3t**, and **3u**, Scheme 1 and Table 1). Electronic effect of the R^1 substituent was manifested in **3s**. With electron withdrawing cyano group conjugated with the 2-phenyl ring, the galangin derivative **3s** showed significantly increased anti-HCV activity (EC_{50} = 0.9 μM, 8.9 times increase compared with **3m**), which is comparable to that of the ADK analogue **1** (Fig. 1, EC_{50} = 0.8 μM).[6] Through combination of resonance and inductive

Table 1
Anti-HCV activity and cytostatic effect of 7-*O*-arylmethylgalangin derivatives[a]

Compd	R^1	R^2		EC_{50}[b,c] (μM)	CC_{50}[c,d] (μM)	SI[e]
		Position	Substituent			
3a	H	2″	Cl	30	>100	—[f]
3b	H		Br	10	>100	—[f]
3c	H		CN	39	22	0.6
3d	H		NO_2	25	32	1.3
3e	H		Me	7	17	2.4
3f	H		OMe	53	>100	—[f]
3g	H	3″	Cl	22	>100	—[f]
3h	H		Br	37	>100	—[f]
3i	H		CN	3	72	24
3j	H		NO_2	2	76	38
3k	H		Me	27	35	1.3
3l	H		OMe	15	>100	—[f]
3m	H	4″	Cl	8	>100	—[f]
3n	H		Br	6	>100	—[f]
3o	H		CN	19	94	4.9
3p	H		NO_2	10	93	9.3
3q	H		Me	6	88	14.7
3r	H		OMe	38	>100	—[f]
3s	CN	4″	Cl	0.9	67	74.4
3t	CF_3			3	73	24.3
3u	OMe			6	64	10.7

[a] Interferon α-2b was used as a reference compound at 10,000 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%.
[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}.
[f] Select antiviral effect.

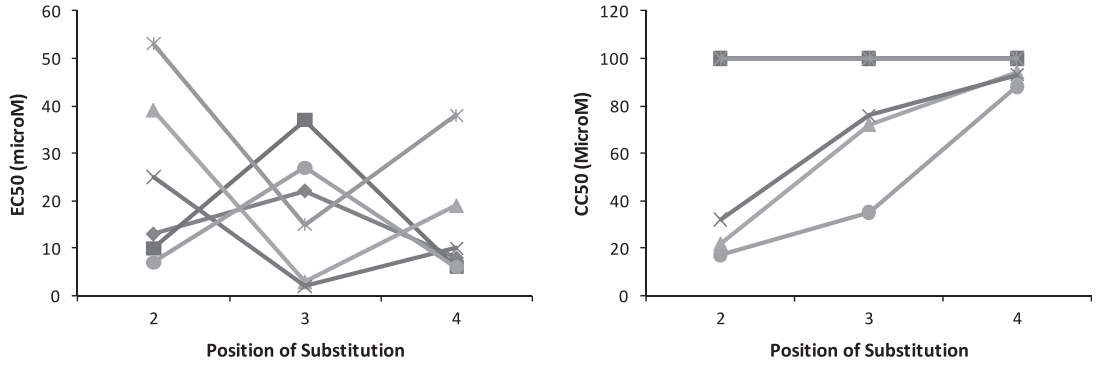


Figure 2. Correlation of the (a) anti-HCV activity (EC_{50}) and (b) cytostatic effect (CC_{50}) with the position of the arylmethyl substituent (R^2) (Cl: ♦, Br: ■, CN: ▲, NO_2: ×, OMe: *, Me: ●).

effect, the cyano group must have dramatically affected the electronic state of the galangin 3-OH and thereby its metal-binding capacity. In comparison, with the lack of resonance effect, substitution with a CF_3 group resulted in only 2.6 times increase in antiviral activity of **3t** (EC_{50} = 3 μM, Table 1). The galangin derivative with electron-donating OMe-substituent (**3u**) showed no significant change (EC_{50} = 6 μM, Table 1).

In summary, as a replacement of the unstable diketo acid core structure, we proposed galangin as a novel anti-HCV scaffold. Synthesis and biological evaluation of the substituted galangin derivatives revealed interesting structure–activity relationship and identified a specific anti-HCV compound 7-*O*-(4″-chlorophenylmethyl)galangin (**3m**) of which antiviral activity was further optimized by introduction of an electron-withdrawing cyano group conjugated with 2-phenyl group (**3s**). The most active galangin derivative **3s** has the same pattern of aromatic substitution with the ADK analogue **1** at the arylmethyl group directly attached to the metal-binding unit, and the anti-HCV activity of **3s** is comparable to that of the ADK analogue **1**. Taken together, the galangin scaffold successfully replaced the diketo acid structure to show potent antiviral activity by specific combination of the aromatic substituents, which warrants extensive structure–activity relationship study around the galangin scaffold.

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23. Huh-5-2 cells were kindly provided by Professor R. Bartenschlager, University of Heidelberg, Germany. Huh-5-2 cells were seeded at a density of 5×10^3 per well in a tissue culture-treated white 96-well view plate in complete DMEM supplemented with 500 μg/mL G418. After incubation for 24 h at 37 °C (5% CO_2), medium was refreshed (with G418) and DMSO stock of test compounds were added. After 4 days of incubation at 37 °C, cell culture medium was removed and luciferase activity was determined using the Steady-Glo luciferase assay system (Promega, Leiden, The Netherlands). In order to estimate the cytostatic effects, Huh-5-2 cells were seeded at a density of 5×10^3 per well of a 96-well plate in complete DMEM with the appropriate concentrations of G418. Serial dilutions of the test compounds in complete DMEM without G418 were added 24 h after seeding. Cells were allowed to proliferate for 3 days at 37 °C, after which the cell number was determined by WST-1 assay.