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3-*O*-Arylmethylgalangin, a novel isostere for anti-HCV 1,3-diketoacids (DKAs)

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ABSTRACT

Through chelation of the metal ions at the enzyme active site, 1,3-diketoacids (DKAs) show potent inhibition of viral enzymes such as HIV integrase and HCV NS5B. In order to optimize the antiviral activity of the DKAs, structural modification of their metal-binding units, keto-enol acids or monoketo acids, have been actively performed. In this study, we proposed 3-*O*-arylmethylgalangin **3** as an alternative to *ortho*-substituted aromatic DKA, a potent inhibitor of HCV NS5B. As a proof-of-concept study, a series of 3-*O*-arylmethylgalangin derivatives (**3a**–**3r**) were prepared and their inhibitory activity against HCV NS5B was estimated. Structure–activity relationship of the 3-*O*-arylmethylgalangin derivatives was in good accordance with that of the *ortho*-substituted aromatic DKA series. In particular, two galangin ethers (**3g** and **3i**) completely superimposable with the most potent *ortho*-substituted aromatic DKA analogue (**2**) in atom-by-atom fashion showed equipotent inhibitory activity to that of **2**. Taken together, this result provides convincing evidence that the 3-*O*-arylmethylgalangin can successfully mimic the chelating function of the DKA pharmacophore to show potent inhibitory activity against the target enzyme, HCV NS5B.

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1. Introduction

Originally, through high-throughput screening, 1,3-diketoacids (DKAs) (**1**, Fig. 1) were identified as HIV (Human Immunodeficiency Virus) integrase inhibitors.[1] Later, the keto-enol acid pharmacophore of DKA was reported to be responsible for its antiviral activity by binding to the magnesium ions present in the active site of the enzyme.[2] Unfortunately, the DKAs are known to suffer from unfavorable physicochemical as well as pharmacokinetical problems such as chemical instability, irreversible covalent binding to protein, and poor stability in plasma.[3] As these problems can be mainly attributed to the free carboxylic acid of DKA, there have been numerous attempts to replace the carboxylic acid with its isosters such as triazole (S1360, Fig. 1),[4] tetrazole (5CITEP, Fig. 1),[5] pyridine (L-870,810, Fig. 1),[6–8] and a neutral carbonyl group (Raltegravir, Fig. 1).[9] Recently, the keto-enol acid pharmacophore model was further refined to identify a monoketo acid as a minimal structural motif for antiviral activity (Elvitegravir, Fig. 1).[10]

HCV (Hepatitis C Virus) NS5B RNA-dependent RNA polymerase has the same feature as HIV integrase of using two metal ions in the active site as a key component of the catalytic machinery. Even though these two enzymes catalyze different types of reaction, they share the common geometry of the active site amino acids as well as the catalytic metal ions.[3] Thus, it is not surprising that DKAs are also potent inhibitors of HCV NS5B.[11] However, in contrast to the active search for novel DKA-based HIV integrase inhibitors,[4–10] modification of the keto-enol acid or monoketo acid pharmacophore of anti-HCV DKAs has rarely been reported. For anti-HCV activity, it was reported that an aryl substituent is specifically required in addition to the keto-enol acid pharmacophore of DKA.[11–14] Moreover, substituents at the aromatic *ortho*-position were shown to significantly improve antiviral activity of the resulting *ortho*-substituted aromatic DKAs with a cyanopropyl ether being the optimum functional group (**2**, Fig. 2a).[11] Based on this information, we reasoned that galangin, a naturally occurring metal chelator,[15–17] might serve as the aforementioned minimal pharmacophore (monoketo acid), and substitution of its 3-OH group with arylmethyl functionality (**3**, Fig. 2a) would successfully mimic the *ortho*-substituted aromatic DKA (**2**). The superimposed structure of **2** and **3** (Fig. 2b) confirms this idea by showing atom-by-atom match of the minimal monoketo acid pharmacophore (shown in thick lines) and aromatic alkyloxy substituents.

In the present proof-of-concept study, we set out to develop novel HCV NS5B inhibitors by isosteric replacement of the DKA core structure with galangin. Herein, we report synthesis and biological evaluation of a series of 3-*O*-arylmethylgalangin.

2. Results and discussions

2.1. Chemistry

In our previous report,[18] we observed unusual desilylative-alkylation of the galangin-3-*O*-silylether (**4**, Fig. 3) to the

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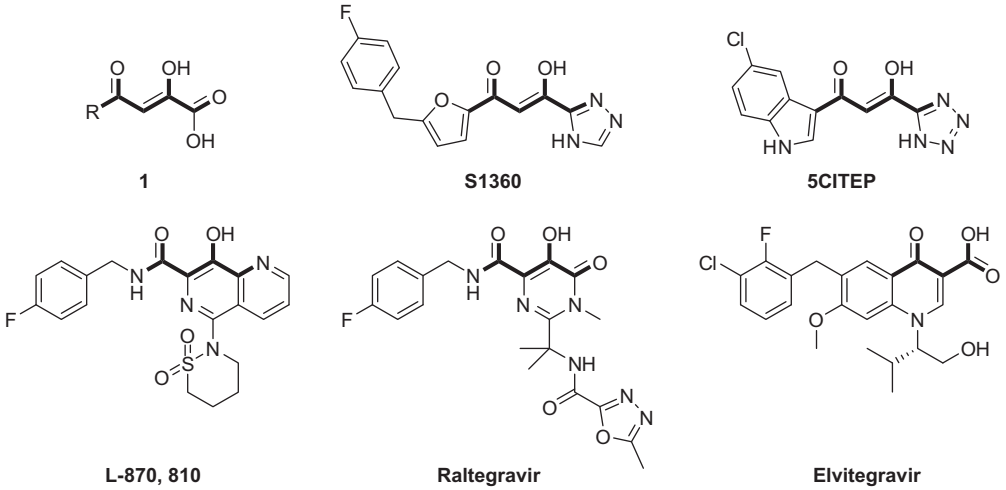


Figure 1. Structures of the DKA (**1**) and its isosteric replacements. Keto-enol acid or monoketo acid motif is shown in thick lines.

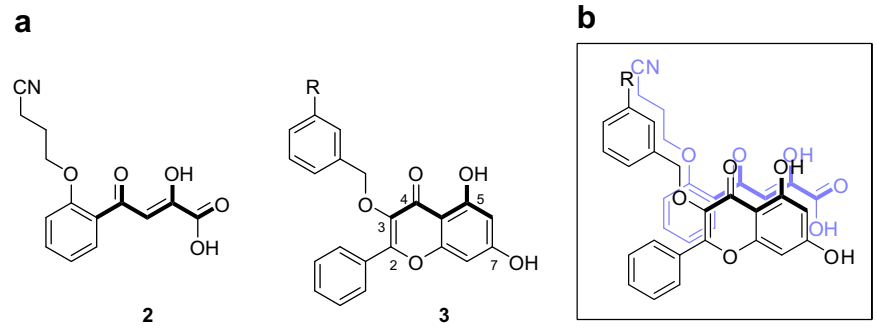


Figure 2. (a) Structures of *ortho*-substituted DKA (**2**) and 3-*O*-arylmethylgalangin (**3**). The pharmacophores are represented in thick lines. (b) Superimposed structures of **2** and **3**.

corresponding alkylgalangin (**5**, Fig. 3), and the nuclear Overhauser effect (NOE) between the protons in the arylmethyl moiety (H_1, Fig. 3) and 2-phenyl ring (H_2, Fig. 3) confirmed the position of the alkyl substituent.

For synthesis of a series of 3-*O*-arylmethylgalangin derivatives, we made use of the desilylative-alkylation of **4** with variously substituted benzyl bromides (Scheme 1). Preparation of the key intermediate **4** was started from the commercially available chrysin (**5**, Scheme 1). Treatment of chrysin (**5**, Scheme 1) with Me_2SO_4 and K_2CO_3 in acetone afforded the dimethylchrysin **6** in 95% yield.[19] Hydroxylation at C-3 of this flavone was accomplished in a highly regioselective manner by treatment with LDA followed by quenching with $B(OMe)_3$ to afford the dimethylgalangin **7** in 60% yield.[20] Protection of the 3-OH group of **7** with TBDMSCl followed by Lewis acid-promoted demethylation provided the key intermediate **4** in 58% combined yield. Arylmethyl bromides with

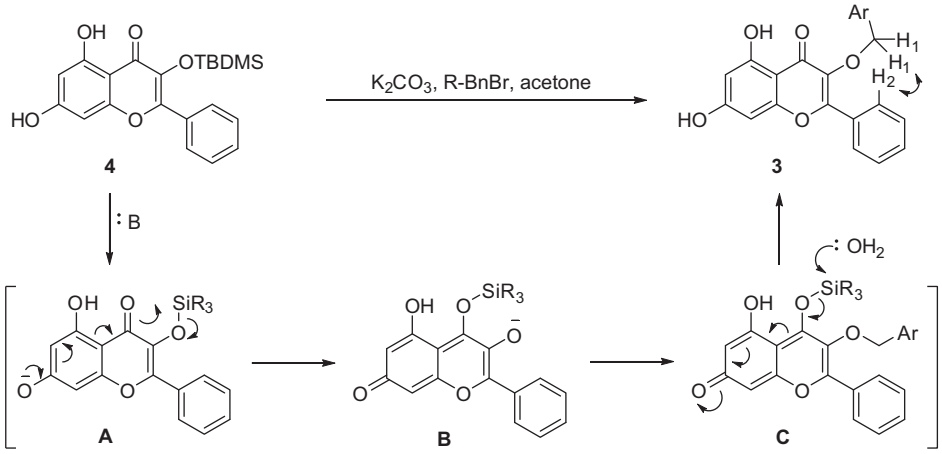
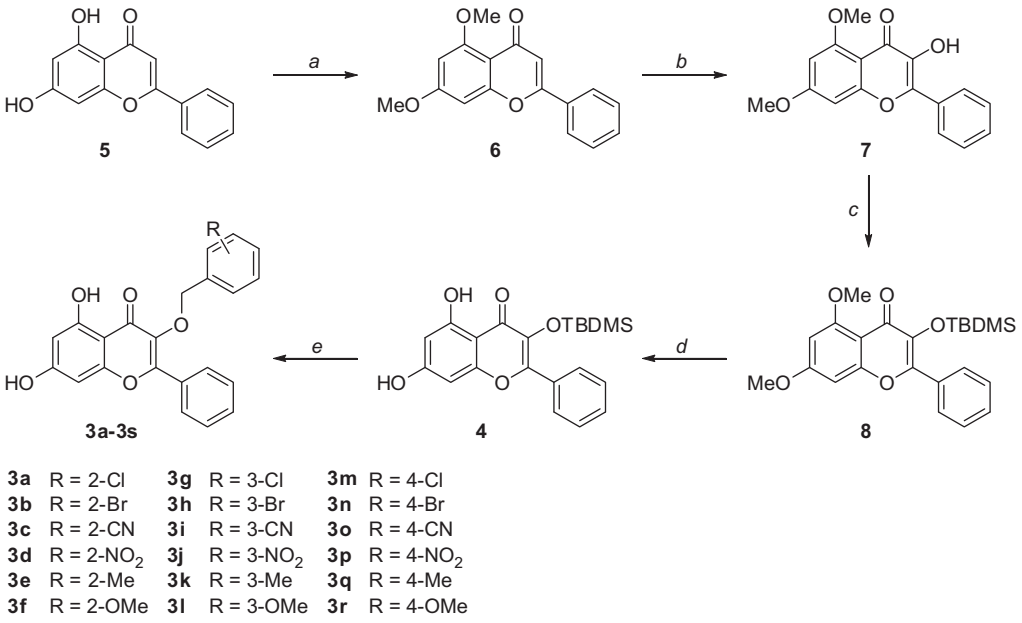


Figure 3. Mechanism for the unusual desilylative-alkylation of **4**.



3a	R = 2-Cl	**3g**	R = 3-Cl	**3m**	R = 4-Cl
3b	R = 2-Br	**3h**	R = 3-Br	**3n**	R = 4-Br
3c	R = 2-CN	**3i**	R = 3-CN	**3o**	R = 4-CN
3d	R = 2-NO_2	**3j**	R = 3-NO_2	**3p**	R = 4-NO_2
3e	R = 2-Me	**3k**	R = 3-Me	**3q**	R = 4-Me
3f	R = 2-OMe	**3l**	R = 3-OMe	**3r**	R = 4-OMe

Scheme 1. Synthesis of 3-*O*-arylmethylgalangin derivatives (**3a**–**3r**). Reagents and conditions: (a) Me_2SO_4, K_2CO_3, acetone, 60 °C, 95% yield; (b) LDA, $B(OMe)_3$, AcOH, H_2O_2, 60% yield; (c) DMAP, TBDMSCI, pyridine, 60 °C, 63% yield; (d) BBr_3, CH_2Cl_2, 92% yield; (e) R-BnBr, K_2CO acetone, 60–80% yield.

various substituents (Cl, Br, CN, NO_2, Me and OMe) at 2-, 3-, or 4-position of the aromatic ring were then reacted with **4** to provide the desired 3-*O*-arylmethylgalangin derivatives (**3a**–**3r**) in 60–80% yield.

2.2. Biological activity

The synthesized galangin arylmethyl ethers (**3a**–**3r**) were evaluated for their ability to inhibit the HCV NS5B enzyme activity (IC_{50}, Table 1).[21] Antiviral activity of the galangin derivatives (EC_{50}, Table 1) was evaluated in the human hepatoma cell line Huh-7, carrying the subgenomic HCV genotype 1 replicon with the luc-ubi-neo (reporter/selective) fusion gene.[22–24] The cytostatic effect of the test compounds (CC_{50}, Table 1) was also evaluated in the same cell line.

As anticipated, the 3-*O*-arylmethylgalangin derivatives showed moderate to potent inhibition of the enzyme activity of HCV NS5B (IC_{50}, Table 1). In general, 2-substituted benzyl ethers (**3a**–**3f**) were not as active as the corresponding regioisomers, 3- or 4-substituted benzyl ethers (**3g**–**3r**). Halogen (Cl, Br) or cyano group substituted at 3- or 4-position of the benzyl ether motif provided the corresponding galangin ethers with potent inhibitory activity against the target enzyme, HCV NS5B. Thus, compounds **3g**, **3i**, and **3m**–**3o** showed potent enzyme inhibition (Table 1), and their IC_{50} values (IC_{50} = 0.1–1.4 μM) were comparable with that of the anti-HCV DKA **2** (IC_{50} = 0.35 μM).[11] It is of particular interest that compounds **3g** and **3i** which are completely superimposable to **2** (Fig. 2b) showed the most potent inhibitory activity. In this regard, it should be pointed out that the compound **2** with a cyanopropyl group attached to the *ortho*-position of aromatic DKA (Fig. 2a) was the most potent inhibitor among the series of the DKA ethers with different length and terminal functionalities of the ether linkage.[11] Therefore, the structure–activity relationship of the 3-*O*-arylmethylgalangin derivatives, in accordance with that of the *ortho*-substituted aromatic DKA series, provides convincing evidence that the galangin ether successfully mimic the chelating function of the DKA pharmacophore.

Unfortunately, the galangin derivatives showed only moderate to marginal antiviral activity in cell-based assay (EC_{50}, Table 1). Even the most potent inhibitors of HCV NS5B (**3g** and **3i**) did not exert significant effects in inhibiting HCV RNA replication inside the cell (EC_{50} = 25 and 36 μM, respectively). Among the series, almost similar antiviral activity was observed regardless of position as well as type of the substituents (EC_{50}, Table 1). This result

Table 1
HCV NS5B inhibition, anti-HCV activity, and cytostatic effect of 3-*O*-arylmethylgalangin derivatives

Compd	R		Activity		CC_{50}[b,e] (μM)	SI[f] (μM)
			Enzyme inhibition[a,b] (IC_{50}, μM)	Cell-based antiviral activity[b,c,d] (EC_{50}, μM)		
3a	2-	Cl	>50	38	>100	—[g]
3b		Br	10.5	9	12	1.3
3c		CN	11.0	5	>100	—[g]
3d		NO_2	>50	12	51	4.3
3e		Me	7.2	34	55	1.6
3f		OMe	>50	28	53	1.9
3g	3-	Cl	0.1	25	46	1.8
3h		Br	4.6	18	15	0.8
3i		CN	0.2	36	21	0.6
3j		NO_2	6.4	5	31	6.2
3k		Me	30.0	28	29	1.0
3l		OMe	21.0	22	28	0.4
3m	4-	Cl	0.3	15	36	2.4
3n		Br	1.4	12	2	0.2
3o		CN	1.3	10	48	4.8
3p		NO_2	>50	7	40	5.7
3q		Me	45.2	14	40	2.9
3r		OMe	29.5	10	57	5.7
2[h]			0.35	—[i]	—[i]	—[i]

[a] Concentration required to inhibit enzyme activity of HCV NS5B by 50%.
[b] The values obtained as the average of triplicate determinations.
[c] Concentration required to inhibit HCV RNA replication by 50%.
[d] 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.
[e] Concentration required to reduce cell proliferation by 50%.
[f] Selectivity index: ratio of CC_{50} to EC_{50}.
[g] Selective antiviral effect.
[h] Ref. 11.
[i] Not available.

indicates that some factors related with the galangin scaffold might be involved in limiting its biological activity in cell-based assay system. Taking the hydrophobic nature of the galangin derivatives into consideration, this finding can probably be explained by a combination of factors such as poor solubility, poor cell permeability, and high protein binding. Adverse hepatic effect is another factor limiting development of the keto-enol acid class of compounds into antiviral drugs.[25] The galangin derivatives synthesized in this study also showed significant cytostatic effect against the hepatoma cell line, Huh-7 (CC_{50}, Table 1). The cytostatic activity was not significantly different from the antiviral effect to result in low selectivity index (SI, Table 1). Among the series, bromobenzyl ethers (**3b**, **3h**, and **3n**) showed significant cytostatic effect irrespective of the position of the bromine substituent on the aromatic ring. With the exception of bromobenzyl ethers, the cytostatic effect of the galangin derivatives was dependent upon the position rather than the type of the aromatic substituent with the most cytostatic derivatives being galangin 3-substituted benzyl ethers (**3g**–**3l**).

3. Conclusion

DKAs show potent inhibition of viral enzymes such as HIV integrase and HCV NS5B through chelation of the metal ions at the active site. There have been many research efforts to optimize the antiviral activity of the DKA derivatives by structural modification of their metal-binding units, keto-enol acids or monoketo acids. However, bioisosteres of anti-HCV DKAs have received relatively little attention compared with those of HIV integrase inhibitors. In this study, in order to obtain a new scaffold as an alternative to monoketo acids, we designed 3-*O*-arylmethylgalangin **3** as an inhibitor against HCV NS5B. Among the series of synthesized galangin arylmethyl ethers (**3a**–**3r**), galangin 3-*O*-chlorobenzyl ether (**3g**) and 3-*O*-cyanobenzyl ether (**3i**) were the most potent HCV NS5B inhibitor with equipotent activity to that of anti-HCV DKA **2**. These two galangin analogues, completely superimposable with **2** in atom-by-atom fashion (Fig. 2b), seem to fully mimic the chelating function of the DKAs. However, most of these inhibitors showed a suboptimal activity in the HCV cell-based assay, which can be mainly attributed to unfavorable physicochemical as well as pharmacokinetic properties such as poor solubility and high protein binding. In addition, the galangin ethers failed to overcome the problem of adverse hepatic effect associated with the DKA analogues.

Taken together, the potent inhibition of HCV NS5B by the 3-*O*-arylmethylgalangin derivatives demonstrates that the galangin can replace the metal-binding function of the DKA pharmacophore but its suboptimal properties need to be tackled by elaborative modification of the galangin scaffold.

4. Experimental

4.1. Materials and general methods

All chemicals including MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide)] were purchased from Sigma-Aldrich. Dulbecco's Modified Eagle Media (DMEM), penicillin, streptomycin and fetal bovine serum (FBS) were purchased from Invitrogen. Nuclear magnetic resonance spectra were recorded on a bruker 400 AMX spectrometer (Karlsruhe, Germany) at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR with tetramethylsilane as the internal standard. Chemical shift are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). Coupling constants are reported in hertz (Hz). The chemical shifts are reported as parts per million (δ) relative to the solvent peak. TLC was performed on Silica Gel 60 F254 purchased from Merck. Column chromatography was performed using either Silica Gel 60 (220–440 mesh) for flash chromatography. Mass spectrometric data (MS) were obtained by MALDI-TOF-TOF mass spectrometer (Ultraflex III, Bruker Daltonik).

4.2. Synthesis of the 3-*O*-arylmethylgalangin derivatives

4.2.1. 5,7-Dimethoxy-2-phenyl-chromen-4-one (6)

Dimethyl sulfate (1.24 g, 9.83 mmol) and K_2CO_3 (4.35 g, 31.47 mmol) were added to a solution of chrysin **5** (1.00 g, 3.93 mmol) in acetone (20 mL), and the reaction mixture was stirred under reflux for 12 h. Volatiles were removed under reduced pressure and then the residue was taken with CH_2Cl_2. The organic layer was washed with water, dried over $MgSO_4$, and concentrated under reduced pressure. Recrystallization from acetone provided **6** (1.06 g, 3.74 mmol, 95% yield) as pale yellow powder. Data of ^{1}H NMR spectra were identical to those in the literature:[16] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87–7.85 (m, 2H), 7.49–7.46 (m, 3H), 6.67 (s, 1H), 6.56 (d, J = 2.3 Hz, 1H), 6.37 (d, J = 2.3 Hz, 1H), 3.91 (s, 3H), 3.84, (s, 3H).

4.2.2. 3-Hydroxy-5,7-dimethoxy-2-phenyl-4*H*-chromen-4-one (7)

The solution of **6** (282 mg, 1 mmol) in anhydrous THF (10 mL) was added dropwise to the solution containing LDA (2.0 M in THF; 2 mL, 4 mmol) in THF (10 mL) at −78 °C. The reaction mixture was stirred for 6 h at −78 °C and then quenched with a solution of trimethyl borate (0.5 mL) in THF (5 mL). The resulting solution was stirred for 1 h at −78 °C. The reaction mixture was acidified with acetic acid (0.4 mL), stirred for 30 min, and then oxidized with 30%-H_2O_2 (1 mL). The solution was allowed to warm slowly to room temperature over 12 h. The reaction mixture was neutralized with saturated aqueous $NaHCO_3$ solution, extracted with CH_2Cl_2 and dried over $MgSO_4$. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, CH_2Cl_2/EtOAc = 5:1) to afford **7** (179 mg, 0.6 mmol, 60% yield) as dark yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J = 5.5 Hz, 2H), 7.48 (t, J = 7.3 Hz, 2H), 7.40 (t, J = 7.3 Hz, 1H), 6.51 (s, 1H), 6.30 (d, J = 1 Hz, 1H), 3.94 (s, 3H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.96, 164.47, 160.50, 158.94, 141.71, 138.14, 131.01, 129.55, 128.45, 127.10, 106.15, 95.68, 92.33, 56.30, 55.75; LC/MS (ESI) m/z Found: 299.3 $[M+H]^+$; calcd for $C_{17}H_{15}O_5$: 299.1.

4.2.3. 3-(*tert*-Butyldimethylsilyloxy)-5,7-dimethoxy-2-phenyl-4*H*-chromen-4-one (8)

TBDMSCl (1.62 g, 10.73 mmol) was added to a solution of **7** (1.60 g, 5.36 mmol) and DMAP (130 mg, 1.10 mmol) in pyridine (30 mL). The reaction mixture was warmed to 60 °C and stirred for 12 h. After reaction, the volatiles were removed under reduced pressure. The residue was dissolved in CH_2Cl_2, washed with water, and dried over $MgSO_4$. The residue was purified by flash column chromatography (SiO_2, CH_2Cl_2/EtOAc = 4:1) to afford **8** (1.38 g, 3.35 mmol, 63% yield) as yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, J = 8.2, 1.8 Hz, 2H), 7.46–7.38 (m, 3H), 6.45 (d, J = 2.2 Hz, 1H), 6.30 (d, J = 2.2 Hz, 1H), 3.93 (s, 3H), 3.85 (s, 3H), 0.77 (s, 9H), 0.19 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.00, 163.60, 161.00, 158.72, 138.47, 131.56, 129.63, 128.63, 128.02, 108.20, 95.55, 92.15, 56.53, 55.66, 25.73, 18.75, −3.74; LC/MS (ESI) m/z Found: 413.4 $[M+H]^+$; calcd for $C_{23}H_{28}O_5Si$: 413.2.

4.2.4. 3-(*tert*-Butyldimethylsilyloxy)-5,7-dihydroxy-2-phenyl-4*H*-chromen-4-one (4)

BBr_3 in dichloromethane (1.0 M, 1 mL) was added to a solution of **8** (100 mg, 0.24 mmol) in dichloromethane (5 mL) dropwise at room temperature. The reaction mixture was stirred for 6 h at room temperature and then quenched with saturated aqueous

K_2CO_3 solution (5 mL). The reaction mixture was diluted with CH_2Cl_2, washed with water, and dried over $MgSO_4$. The residue was purified by flash column chromatography (SiO_2, CH_2Cl_2/EtOAc = 3:1) to afford **4** (85 mg, 0.22 mmol, 92% yield) as yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.7 (s, 1H, –OH), 7.92–7.89 (m, 2H), 7.69–7.65 (m, 3H), 6.39 (s, 1H), 6.31 (s, 1H), 5.73 (s, 1H, –OH), 0.81 (s, 9H), 0.11 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.75, 165.72, 162.23, 157.89, 147.19, 138.58, 132. 43, 131.06, 128.84, 104.69, 99.56, 94.96, 27.26, 26.91, −1.75; LC/MS (ESI) *m/z*; calcd for $C_{21}H_{25}rO_5Si$: 385.1, found: 385.4 $[M+H]^+$.

4.2.5. Desilylative-alkylation of 4

Appropriately substituted benzyl bromide (1.1 equiv) was added dropwise to a solution of **4** (1 equiv) and K_2CO_3 (1.1 equiv) in acetone. The reaction mixture was stirred at room temperature for 48 h. After reaction, the reaction mixture was neutralized with 1N-HCl solution. The reaction mixture was extracted with CH_2Cl_2 and dried over $MgSO_4$. After concentration under reduced pressure, the residue was crystallized from CH_2Cl_2.

4.2.5.1. 3-(2-Chlorobenzyloxy)-5,7-dihydroxy-2-phenyl-4*H*-chromen-4-one (3a). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.70 (s, 1H), 7.98 (d, J = 7.1 Hz, 2H), 7.59–7.61 (m, 1H), 7.47–7.52 (m, 3H), 7.27–7.35 (m, 3H), 6.53 (s, 1H), 6.31 (s, 1H), 5.27 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.41, 164.83, 161.72, 157.03, 156.53, 137.41, 134.29, 132.94, 131.23, 131.07, 130.25, 129.47, 128.78, 128.69, 127.98, 127.55, 104.85, 99.14, 94.19, 70.91; LC/MS (ESI) *m/z*; calcd for $C_{22}H_{16}ClO_5$: 395.1, found: 394.2 $[M+H]^+$.

4.2.5.2. 3-(2-Bromobenzyloxy)-5,7-dihydroxy-2-phenyl-4*H*-chromen-4-one (3b). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.7 (s, 1H, –OH), 7.98–8.01 (m, 2H), 7.62 (d, J = 7.5 Hz, 1H), 7.47–7.53 (m, 4H), 7.34 (t, J = 7.6 Hz, 1H), 7.22 (t, J = 7.7 Hz, 1H), 6.53 (d, J = 1.3 Hz, 1H), 6.31 (d, J = 1.1 Hz, 1H), 5.25 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.25, 166.42, 161.83, 158.85, 157.94, 138.86, 137.98, 132.10, 130.33, 129.81, 129.54, 128.63, 127.95, 127.93, 126.78, 104.58, 96.83, 94.35, 66.78; LC/MS (ESI) *m/z* Found: 440.1 $[M+H]^+$; calcd for $C_{22}H_{16}BrO_5$: 439.1.

4.2.5.3. 2-[(5,7-Dihydroxy-4-oxo-2-phenyl-4*H*-chromen-3-yloxy)-methyl]benzonitrile (3c). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.66 (s, 1H, –OH), 7.94–7.96 (m, 2H), 7.61–7.71 (m, 3H), 7.46–7.54 (m, 4H), 6.52 (d, J = 1.9 Hz, 1H), 6.31 (d, J = 2.0 Hz, 1H), 5.35 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.27, 164.94, 161.70, 157.05, 156.67, 139.59, 137.10, 133.42, 133.06, 130.16, 129.57, 129.45, 129.42, 128.87, 128.73, 117.42, 111.78, 104.84, 98.49, 94.24, 66.39; LC/MS (ESI) *m/z*; calcd for $C_{23}H_{16}NO_5$: 386.1, found: 386.1 $[M+H]^+$.

4.2.5.4. 5,7-Dihydroxy-3-(2-nitrobenzyloxy)-2-phenyl-4*H*-chromen-4-one (3d). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.6 (s, 1H, –OH), 7.99–8.09 (m, 4H), 7.75–7.79 (m, 1H), 7.58–7.61 (m, 1H), 7.49–7.54 (m, 3H), 6.54 (s, 1H), 6.32 (s, 1H), 5.53 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.42, 164.85, 161.65, 157.54, 156.66, 147.45, 137.98, 134.66, 134.34, 130.33, 129.56, 128.99, 128.34, 127.9, 124.98, 104.55, 97.39, 94.05, 66.55; LC/MS (ESI) *m/z*; calcd for $C_{22}H_{16}NO_7$: 406.1, found: 406.1 $[M+H]^+$.

4.2.5.5. 5,7-Dihydroxy-3-(2-methylbenzyloxy)-2-phenyl-4*H*-chromen-4-one (3e). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.77 (s, 1H, –OH), 7.96–7.98 (m, 2H), 7.48–7.59 (m, 3H), 7.11–7.33 (m, 4H), 6.51 (d, J = 1.9 Hz, 1H), 6.31 (d, J = 1.9 Hz, 1H), 5.15 (s, 2H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 178.63, 164.80, 161.73, 157.01, 156.56, 137.66, 137.23, 134.84, 131.27, 130.41, 130.33, 130.25, 129.72, 128.79, 128.73, 125.91, 104.86, 99.16, 94.30, 72.12, 18.63; LC/MS (ESI) *m/z*; calcd for $C_{23}H_{19}O_5$: 375.1, found: 375.1 $[M+H]^+$.

4.2.5.6. 5,7-Dihydroxy-3-(2-methoxybenzyloxy)-2-phenyl-4*H*-chromen-4-one (3f). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.80 (s, 1H, –OH), 7.98–8.00 (m, 2H), 7.39–7.52 (m, 3H), 7.34 (d, J = 7.6 Hz, 1H), 7.24 (t, J = 7.8 Hz, 1H), 6.82–6.86 (m, 2H), 6.51 (s, 1H), 6.30 (s, 1H), 5.22 (s, 2H), 3.64 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.19, 162.27, 159.25, 154.85, 154.47, 153.65, 135.12, 128.57, 128.20, 128.03, 127.94, 127.74, 127.58, 126.25, 117.90, 117.83, 102.29, 96.61, 91.56, 66.43, 55.61; LC/MS (ESI) *m/z*; calcd for $C_{23}H_{19}O_6$: 391.1, found: 391.1 $[M+H]^+$.

4.2.5.7. 3-(3-Chlorobenzyloxy)-5,7-dihydroxy-2-phenyl-4*H*-chromen-4-one (3g). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.7 (s, 1H, –OH), 8.01 (d, J = 7.0 Hz, 2H), 7.54 (d, J = 6.8 Hz, 3H), 7.32 (s, 3H), 7.41 (s, 1H), 6.52 (s, 1H), 6.31 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.89, 166.36, 163.31, 158.48, 157.80, 140.73, 138.92, 134.76, 132.58, 131.82, 131.58, 131.44, 130.09, 129.72, 129.63, 128.36, 106.16, 100.34, 95.42, 74.47; LC/MS (ESI) *m/z*; calcd for $C_{22}H_{16}ClO_5$: 395.1, found: 395.2 $[M+H]^+$.

4.2.5.8. 3-(3-Bromobenzyloxy)-5,7-dihydroxy-2-phenyl-4*H*-chromen-4-one (3h). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.7 (s, 1H, –OH), 8.00–8.02 (m, 2H), 7.52–7.59 (m, 4H), 7.46 (d, J = 7.9 Hz, 1H), 7.37 (d, J = 7.6 Hz, 1H), 7.26 (t, J = 7.8 Hz, 1H), 6.52 (s, 1H), 6.31 (s, 1H), 5.15 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.43, 165.34, 163.55, 158.03, 156.96, 143.66, 137.66, 131.89, 130.56, 129.98, 129.77, 127.89, 126.13, 122.76, 104.69, 96.79, 93.55, 72.66; LC/MS (ESI) *m/z*; calcd for $C_{22}H_{16}BrO_5$: 439.1, found: 438.9 $[M+H]^+$.

4.2.5.9. 3-[(5,7-Dihydroxy-4-oxo-2-phenyl-4*H*-chromen-3-yloxy)methyl]benzonitrile (3i). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.6 (s, 1H, –OH), 8.01 (dd, J = 7.4, 1.4 Hz, 2H), 7.56–7.51 (m, 3H), 7.37 (s, 1H), 7.34–7.30 (m, 3H), 6.51 (d, J = 1.8 Hz, 1H), 6.30 (d, J = 1.8 Hz, 1H), 5.14 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.59, 165.22, 163.31, 158.17, 157.64, 139.57, 138.45, 132.84, 132.71, 132.53, 131.88, 131.44, 130.23, 129.51, 129.39, 119.12, 113.10, 106.14, 99.68, 94.85, 73.68; LC/MS (ESI) *m/z*; calcd for $C_{23}H_{16}NO_5$: 386.1, found: 386.2 $[M+H]^+$.

4.2.5.10. 5,7-Dihydroxy-3-(3-nitrobenzyloxy)-2-phenyl-4*H*-chromen-4-one (3j). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.7 (s, 1H, –OH), 8.26 (s, 1H), 8.15 (d, J = 8.3 Hz, 1H), 7.98–8.00 (m, 2H), 7.83 (d, J = 7.3 Hz, 1H), 7.61 (t, J = 7.9 Hz, 1H), 7.51–7.53 (m, 3H), 6.52 (s, 1H), 6.31 (s, 1H), 5.29 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.24, 164.86, 161.67, 156.93, 156.43, 147.82, 138.94, 137.20, 134.73, 131.05, 130.15, 129.76, 128.56, 122.96, 122.93, 104.63, 98.97, 93.99, 72.58; LC/MS (ESI) *m/z*; calcd for $C_{22}H_{16}NO_7$: 406.1, found: 406.2 $[M+H]^+$.

4.2.5.11. 5,7-Dihydroxy-3-(3-methylbenzyloxy)-2-phenyl-4*H*-chromen-4-one (3k). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.8 (s, 1H, –OH), 8.01–8.03 (m, 2H), 7.51–7.58 (m, 3H), 7.08–7.18 (m, 4H), 6.51 (d, J = 2.0 Hz, 1H), 6.30 (d, J = 2.0 Hz, 1H), 5.12 (s, 2H), 2.86 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.78, 165.38, 163.29, 158.14, 157.40, 138.66, 138.60, 137.64, 131.71, 131.69, 130.32, 129.68, 129.64, 129.30, 128.96, 126.64, 106.04, 99.66, 94.66, 74.93, 21.36; LC/MS (ESI) *m/z* Found: 375.2 $[M+H]^+$; calcd for $C_{23}H_{19}O_5$: 375.1.

4.2.5.12. 5,7-Dihydroxy-3-(3-methoxybenzyloxy)-2-phenyl-4*H*-chromen-4-one (3l). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.7 (s, 1H, –OH), 8.03–8.05 (m, 2H), 7.53–7.55 (m, 3H), 7.20 (t, J = 7.8 Hz, 1H), 6.82–6.93 (m, 3H), 6.51 (d, J = 1.4 Hz, 1H), 6.30 (d, J = 1.1 Hz, 1H), 5.15 (s, 2H), 3.70 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.59, 164.58, 161.31, 159.16, 156.58, 155.82, 137.55, 136.58, 130.78, 130.34, 130.59, 128.97 (br), 128.63,

119.45, 113.94, 113.84, 104.86, 98.35, 94.10, 73.50, 55.59; LC/MS (ESI) *m/z*; calcd for $C_{23}H_{19}O_6$: 391.1, found: 391.1 $[M+H]^+$.

4.2.5.13. 3-(4-Chlorobenzyloxy)-5,7-dihydroxy-2-phenyl-4*H*-chromen-4-one (3m). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.7 (s, 1H, –OH), 9.94 (s, 1H), 8.01 (d, J = 7.2 Hz, 2H), 7.58–7.51 (m, 3H), 7.39 (d, J = 8.2 Hz, 2H), 7.31 (d, J = 8.2 Hz, 2H), 6.52 (s, 1H), 6.31 (s, 1H), 5.13 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.44, 164.81, 161.68, 157.02, 156.39, 137.47, 133.09, 131.31, 130.35, 129.70, 129.63, 129.37, 128.01, 127.70, 104.78, 95.00, 93.30, 73.00; LC/MS (ESI) *m/z*; calcd for $C_{22}H_{16}ClO_5$: 395.1, found: 395.2 $[M+H]^+$.

4.2.5.14. 3-(4-Bromobenzyloxy)-5,7-dihydroxy-2-phenyl-4*H*-chromen-4-one (3n). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.7 (s, 1H, –OH), 7.99–8.01 (m, 2H), 7.53–7.61 (m, 3H), 7.46 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.3 Hz, 2H), 6.52 (s, 1H), 6.30 (s, 1H), 5.12 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.50, 162.91, 159.75, 154.92, 154.47, 135.54, 134.26, 129.60, 129.48, 128.95, 128.82, 128.42, 126.84, 119.57, 102.87, 97.25, 92.08, 71.24; LC/MS (ESI) *m/z*; calcd for $C_{22}H_{16}BrO_5$: 439.1, found: 440.8 $[M+H]^+$.

4.2.5.15. 4-[(5,7-Dihydroxy-4-oxo-2-phenyl-4*H*-chromen-3-yloxy)-methyl]benzonitrile (3o). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.6 (s, 1H, –OH), 7.99–8.02 (m, 2H), 7.75 (d, J = 8.1 Hz, 2H), 7.71 (d, J = 8.2 Hz, 2H), 7.51–7.62 (m, 3H), 6.53 (d, J = 1.8 Hz, 1H), 6.31 (d, J = 1.9 Hz, 1H), 5.24 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.31, 164.88, 161.69, 157.03, 156.43, 142.52, 137.53, 132.49, 131.47, 130.27, 128.93, 128.79, 119.06, 111.03, 104.83, 94.24, 93.03, 73.07; LC/MS (ESI) *m/z*; calcd for $C_{23}H_{16}NO_5$: 386.1, found: 386.3 $[M+H]^+$.

4.2.5.16. 5,7-Dihydroxy-3-(4-nitrobenzyloxy)-2-phenyl-4*H*-chromen-4-one (3p). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.6 (s, 1H, –OH), 8.18 (d, J = 8.7 Hz, 2H), 8.00–8.02 (m, 2H), 7.69 (d, J = 8.7 Hz, 2H), 7.52–7.57 (m, 3H), 6.53 (d, J = 2.0 Hz, 1H), 6.32 (d, J = 2.0 Hz, 1H), 5.30 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.70, 164.29, 161.07, 156.45, 155.91, 146.82, 144.02, 136.91, 130.87, 129.64, 128.62, 128.35, 128.20, 123.07, 104.22, 98.64, 93.70, 72.18; LC/MS (ESI) *m/z*; calcd for $C_{22}H_{16}NO_7$: 406.1, found: 406.1 $[M+H]^+$.

4.2.5.17. 5,7-Dihydroxy-3-(4-methylbenzyloxy)-2-phenyl-4*H*-chromen-4-one (3q). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.8 (s, 1H, –OH), 8.02 (d, J = 7.3 Hz, 2H), 7.52–7.54 (m, 3H), 7.24 (d, J = 7.8 Hz, 2H), 7.09 (d, J = 7.7 Hz, 2H), 6.51 (s, 1H), 6.30 (s, 1H), 5.11 (s, 2H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.59, 164.79, 161.73, 156.98, 156.27, 137.78, 137.58, 133.71, 131.25, 130.46, 129.19, 129.12, 128.83, 128.78, 104.79, 99.14, 94.15, 73.86, 21.12; LC/MS (ESI) *m/z*; calcd for $C_{23}H_{19}O_5$: 375.1, found: 375.1 $[M+H]^+$.

4.2.5.18. 5,7-Dihydroxy-3-(4-methoxybenzyloxy)-2-phenyl-4*H*-chromen-4-one (3r). ^{1}H NMR (400 MHz, acetone-d_6) δ 12.8 (s, 1H, –OH), 8.00–8.03 (m, 2H), 7.50–7.55 (m, 3H), 7.26 (d, J = 8.5 Hz, 2H), 6.82 (d, J = 8.6 Hz, 2H), 6.51 (d, J = 1.7 Hz, 1H), 6.30 (d, J = 1.7 Hz, 1H), 5.09 (s, 2H), 3.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 178.64, 164.78, 161.71, 159.56, 156.98, 156.32, 137.49, 130.68, 130.54, 130.49, 128.77 (br), 128.63, 113.84, 104.82, 99.10, 94.15, 73.70, 55.35; LC/MS (ESI) *m/z*; calcd for $C_{23}H_{19}O_6$: 391.1, found: 391.1 $[M+H]^+$.

4.3. Assay of HCV NS5B inhibitor

As previously reported,[18] recombinant HCV NS5B polymerase was tested for its ability to perform primer/template-directed transcription in assays that contained 30 mM Tris–HCl (pH 7.2), 10 mM $MgCl_2$, 20 mM NaCl, 1 mM dithiothreitol, 0.05% Tween 20, 1% glycerol, 5 pmol of biotin-dG_{12} (primer), 0.5 pmol of poly(rC)$_{300}$ (template), 1 μM GTP, 0.1–0.3 μCi of [α-^{32}P]GTP, and 2.5 pmol (0.15 μg) of HCV polymerase protein in a final volume of 75 μL. Reactions were initiated by addition of enzyme and incubated for 30 min at 30 °C. Reactions were stopped by addition of 33 mM EDTA, and polynucleotide products were collected by filtration through DEAE Filtermat papers (Wallac); unincorporated triphosphate was removed by washing the filters with 5% dibasic sodium phosphate. The filters were counted in a Packard Tri-Lux Microbeta scintillation counter. Compounds to be tested were added at various concentrations from stocks in 10% dimethyl sulfoxide (DMSO)–water (final DMSO concentration = 1% of the reaction mixture). Fifty percent inhibitory concentration (IC_{50}) values were estimated from the primary cpm data (collected in triplicate).

4.4. Anti-HCV assay

The human hepatoma cell line Huh-7, carrying the subgenomic HCV genotype 1 replicon with the luc-ubi-neo (reporter/selective) fusion gene, was kindly provided by Dr. Ralf Bartenschlager (University of Heidelberg, Heidelberg, Germany).[19–21] 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).

4.5. Cytostatic effect

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.

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References and notes

1. Hazuda, D. J.; Felock, P.; Witmer, M.; Wolfe, A.; Stillmock, KI.; Grobler, J. A.; Espeseth, A.; Gabryelski, L.; Schleif, W.; Blau, C.; Miller, M. D. *Science* **2000**, *287*, 646.
2. Grobler, J. A.; Stillmock, K.; Hu, B.; Witmer, M.; Felock, P.; Espeseth, A. S.; Wolfe, A.; Egbertson, M.; Bourgeois, M.; Melamed, J.; Wai, J. S.; Young, S.; Vacca, J.; Hazuda, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 6661.
3. Summa, V.; Petrocchi, A.; Matassa, V. G.; Gardelli, C.; Muraglia, E.; Rowley, M.; Paz, O. G.; Laufer, R.; Monteagudo, E.; Pace, P. *J. Med. Chem.* **2006**, *49*, 6646.
4. Barreca, M. L.; Ferro, S.; Rao, A.; Luca, L. D.; Zappala, M.; Monforte, A. M.; Debyser, Z.; Witvrouw, M.; Chimirri, A. *J. Med. Chem.* **2005**, *48*, 7084.
5. Goldgur, Y.; Craigie, R.; Cohen, G. H.; Fujiwara, T.; Yoshinage, T.; Fujishita, T.; Sugimoto, H.; Endo, T.; Murai, H.; Davies, D. R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13040.
6. Zhuang, L.; Wai, J. S.; Embrey, M. W.; Fisher, T. E.; Egbertson, M. S.; Payne, L. S., ; Guare, J. P., Jr.; Vacca, J. P.; Hazuda, D. J.; Felock, P. J.; Wolfe, A. L.; Stillmock, K. A.; Witmer, M. V.; Moyer, G.; Schleif, W. A.; Gabryelski, L. J.; Leonard, Y. M.; Lynch, J. J., Jr.; Michelson, S. R.; Young, S. D. *J. Med. Chem.* **2003**, *46*, 453.

7. Hazuda, D. J.; Anthony, N. J.; Gomez, R. P.; Jolly, S. M.; Wai, J. S.; Zhuang, L.; Fisher, T. E.; Embrey, M.; Guare, J. P., Jr.; Egbertson, M. S.; Vacca, J. P.; Huff, J. R.; Felock, P. J.; Witmer, M. V.; Stillmock, K. A.; Danovich, R.; Grobler, J.; Miller, M. D.; Espeseth, A. S.; Jin, L.; Chen, I.-W.; Lin, J. H.; Kassahun, K.; Ellis, J. D.; Wong, B. K.; Xu, W.; Pearson, P. G.; Schleif, W. A.; Cortese, R.; Emini, E.; Summa, V.; Holloway, M. K.; Young, S. D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11233.
8. Hazuda, D. J.; Young, S. D.; Guare, J. P.; Anthony, N. J.; Gomez, R. P.; Wai, J. S.; Vacca, J. P.; Handt, L.; Motzel, S. L.; Klein, H. J.; Dornadula, G.; Danovich, R. M.; Witmer, M. V.; Wilson, K. A. A.; Tussey, L.; Schleif, W. A.; Gabryelski, L. S.; Jin, L.; Miller, M. D.; CAsimiro, D. R.; Emini, E. A.; Shiver, J. W. *Science* **2004**, *305*, 528.
9. Summa, V.; Petrocchi, A.; Bonelli, F.; Crescenzi, B.; Donghi, M.; Ferrara, M.; Fiore, F.; Gardelli, C.; Paz, O. G.; Hazuda, D. J.; Jones, P.; Kinzel, O.; Laufer, R.; Monteagudo, E.; Muraglia, E.; Nizi, E.; Orvieto, F.; Pace, P.; Pescatore, G.; Scarpelli, R.; Stillmock, K.; Witmer, M. V.; Rowley, M. *J. Med. Chem.* **2008**, *51*, 5843.
10. Sato, M.; Kawakami, H.; Motomura, T.; Aramaki, H.; Matsuda, T.; Yamashita, M.; Ito, Y.; Matsuzaki, Y.; Yamataka, K.; Ikeda, S.; Shinkai, H. *J. Med. Chem.* **2009**, *52*, 4869.
11. Summa, V.; Petrocchi, A.; Pace, P.; Matassa, V. G.; De Francesco, R.; Altamura, S.; Tomei, L.; Koch, U.; Neuner, P. *J. Med. Chem.* **2004**, *47*, 14.
12. Kim, J.; Han, J. H.; Chong, Y. *Bull. Korean Chem. Soc.* **2006**, *27*, 1919.
13. Kim, J.; Chong, Y. *Mol. Simul.* **2006**, *32*, 1131.
14. Kim, J.; Kim, K.-S.; Lee, H. S.; Park, K.-S.; Park, S. Y.; Kang, S.-Y.; Lee, S. J.; Park, H. S.; Kim, D.-E.; Chong, Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4661.
15. Afanas'ev, I. B.; Dorozhko, A. I.; Brodskii, A. V.; Kostyuk, V. A.; Potapovitch, A. I. *Biochem. Pharmacol.* **1989**, *38*, 1763.
16. Morel, I.; Lescoat, G.; Cillard, P.; Cillard, J. *Methods Enzymol.* **1994**, *234*, 437.
17. Miller, N. J.; Castellucio, C.; Tijburg, L.; Rice-Evans, C. *FEBS Lett.* **1996**, *392*, 40.
18. Kim, J.; Lee, H. S.; Park, K.-S.; Chong, Y. *Bull. Korean Chem. Soc.* **2008**, *29*, 1667.
19. Dao, T. T.; Kim, S. B.; Sin, K.-S.; Kim, S.; Kim, H. P.; Park, H. *Arch. Pharm. Res.* **2004**, *27*, 278.
20. McGarry, L. W.; Detty, M. R. *J. Org. Chem.* **1990**, *55*, 4349.
21. Love, R. A.; Parge, H. E.; Yu, X.; Hickey, M. J.; Diehl, W.; Gao, J.; Wriggers, H.; Ekker, A.; Wang, L.; Thomson, J. A.; Dragovich, P. S.; Fuhrman, S. A. *J. Virol.* **2003**, *77*, 7575.
22. Lohmann, V.; Korner, F.; Koch, J.; Herian, U.; Theilmann, L.; Bartenschlager, R. *Science* **1999**, *285*, 110.
23. Vroljk, J. M.; Kaul, A.; Hansen, B. E.; Lohmann, V.; Haagmans, B. L.; Schalm, S. W.; Bartenschlager, R. *J. Virol. Methods* **2003**, *110*, 201.
24. Gozdek, A.; Zhukov, I.; Polkowska, A.; Poznanski, J.; Stankiewicz-Drogon, A.; Pawlowicz, J. M.; Zagorski-Ostoja, W.; Borowski, P.; Boguszewska-Chachulska, A. *Antimicrob. Agents Chemother.* **2008**, *52*, 393.
25. Kirschberg, T.; Parrish, J. *Curr. Opin. Drug Discov. Dev.* **2007**, *10*, 460.