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Synthesis and Antiviral Activity of Substituted Quercetins

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Abstract

Influenza viruses are important pathogens that cause respiratory infections in humans and animals. In addition to vaccination, antiviral drugs against influenza virus play a significant role in controlling viral infections by reducing disease progression and virus transmission. Plant derived polyphenols are associated with antioxidant activity, anti-carcinogenic, and cardio- and neuro-protective actions. Some polyphenols, such as resveratrol and epigallocatechin gallate (EGCG), showed significant anti-influenza activity *in vitro* and/or *in vivo*. Recently we showed that quercetin and isoquercetin (quercetin-3- β -D-glucoside), a glucoside form of quercetin, significantly reduced the replication of influenza viruses *in vitro* and *in vivo* (isoquercetin). The antiviral effects of isoquercetin were greater than that of quercetin with lower IC₅₀ values and higher *in vitro* therapeutic index. Thus, we investigated the synthesis and antiviral activities of various quercetin derivatives with substitution of C3, C3', and C5 hydroxyl functions with various phenolic ester, alkoxy, and aminoalkoxy moieties. Among newly synthesized compounds, quercetin-3-gallate which is structurally related to EGCG showed comparable

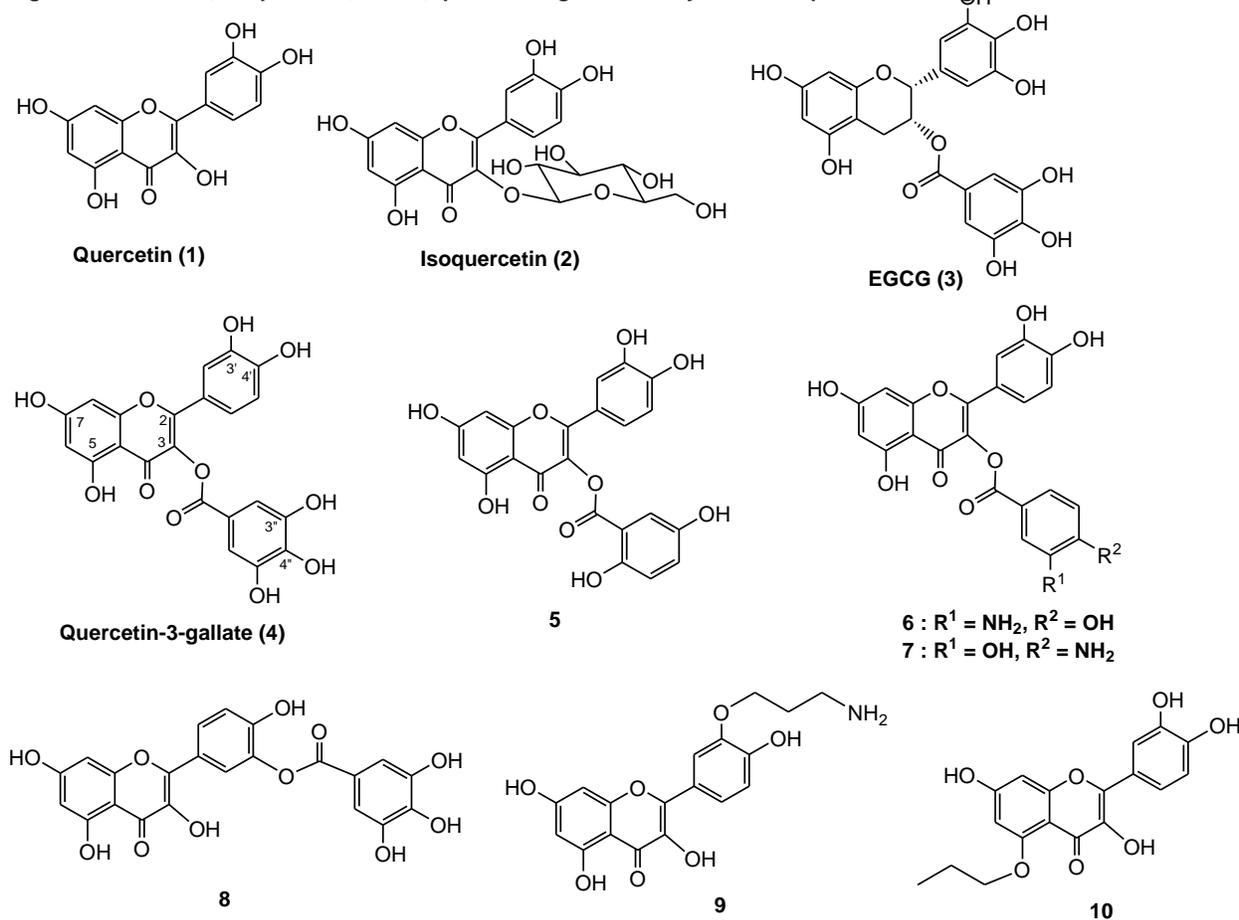
antiviral activity against influenza virus (porcine H1N1 strain) to that of EGCG with improved *in vitro* therapeutic index.

Key words: antiviral activity, influenza virus, porcine H1N1 strain, quercetin-3-gallate, substituted quercetins.

Influenza infections are responsible for over 3 million cases of illness and up to a half of million deaths per year.¹ Although antiviral drugs against influenza infections are available, the emergence of viral resistance to existing antiviral drugs emphasizes the demand for development of new antiviral drugs against influenza infections. In the past few years, polyphenols including quercetin (**1**) and its analogs,²⁻⁴ quercetin-3- β -galactoside,⁵ quercetin 3-rhamnoside,⁶ quercetin 3- β -D-glucoside or isoquercetin (**2**)⁷ (Figure 1) were reported effective against influenza infections. Among them, isoquercetin (**2**) demonstrated a lower IC₅₀ value against influenza viruses and higher therapeutic index compared to that of quercetin.⁷ (-)-Epigallocatechin-3-gallate (EGCG; **3**) is the major polyphenol in green tea and reported to have anti-influenza virus activity^{8,9} and organic anion-transporting enhancing property.¹⁰ Quercetin-3-gallate (**4**) has been reported to be effective for the treatment of inflammatory bowel disease by inhibiting Na⁺-K⁺-ATPase and/or Na⁺/H⁺ exchange activities.¹¹ However, the synthesis and anti-influenza virus activities of **4** and its hydroxyl aryl analogs have not been reported. A hybrid of quercetin and gallate or its analogs may show improved antiviral activities. Therefore antiviral activities of derivatives of quercetin with various substitutions at the hydroxyl functions were investigated. Herein, we report the synthesis and anti-influenza virus activities of compounds **4** - **10** containing C3-dihydroxybenzoate, C3-aminohydroxybenzoates, C3'-gallate, C3'-

aminopropoxy, and C5-propoxy functions. Among the derivatives, quercetin-3-gallate (**4**) which is structurally related to EGCG showed comparable antiviral activity against influenza virus porcine H1N1 as that of EGCG with improved *in vitro* therapeutic index.

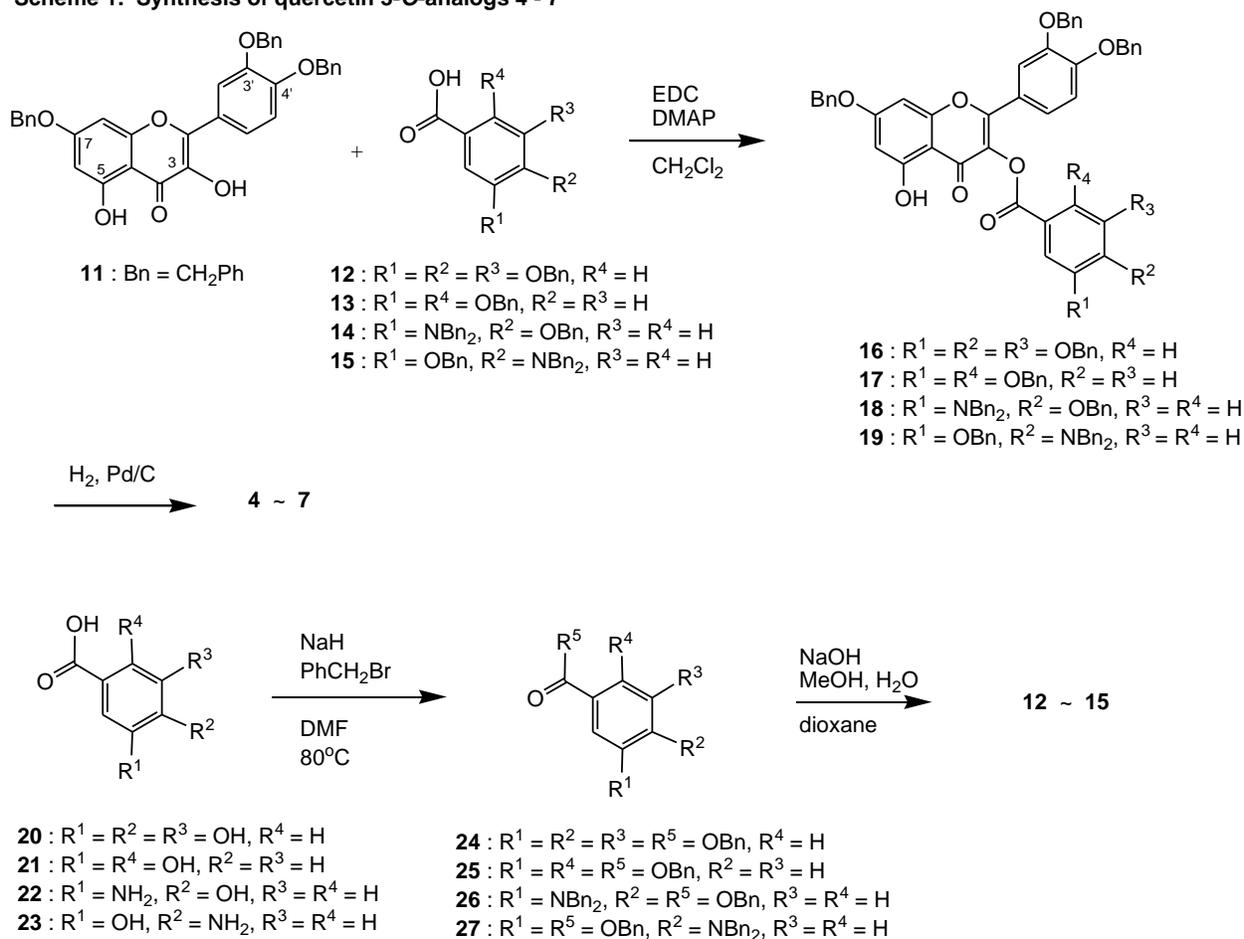
Figure 1. Quercetin, Isoquercetin, EGCG, quercetin-3-gallate and synthesized quercetin analogs



For the synthesis of C3-*O*-ester analogs of quercetin, a reported selective protection of the three most reactive hydroxyl functions at C7, C3', and C4' was adapted.¹² Hence, 3',4',7-tri-*O*-benzylquercetin (**11**), derived from tribenylation of rutin with potassium carbonate and benzyl bromide in DMF followed by removal of the C3-rutinoside with hydrochloric acid and ethanol, was condensed with 3,4,5-tribenzylgallic acid (**12**),¹³ *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide (EDC), and a catalytic amount of 4-dimethylaminopyridine

(DMAP) in dichloromethane to give ester **16** in 64% yield (Scheme 1). Removal of the benzyl ether protecting groups of **16** with hydrogen (15 psi) and 5% palladium over carbon afforded quercetin gallate **4** (68% yield). It should be noted that C5-hydroxyl function is less reactive than C3-hydroxyl due to intramolecular hydrogen bonding and resonance conjugation with C4-keto function.

Scheme 1. Synthesis of quercetin 3-O-analogs 4 - 7



Similarly, condensation of compound **11** with 2,5-dibenzyloxybenzoic acid (**13**),^{14,15} 4-benzyloxy-3-(*N,N*-dibenzylamino)benzoic acid (**14**), and 3-benzyloxy-4-(*N,N*-dibenzylamino)benzoic acid (**15**) separately gave benzyl esters **17**, **18**, and **19** (83%, 75%, and

71% yield), respectively, which upon hydrogenation furnished respectively quercetin analogs **5**, **6**, and **7** (61%, 58%, and 70% yield) (Scheme 1). Compounds **12** and **13** are known and were prepared from a modified procedure of benzylation of gallic acid (**20**) and 2,5-dihydroxybenzoic acid (**21**), respectively, with sodium hydride (or sodium carbonate) and benzyl bromide in DMF^{13,15} followed by basic hydrolysis with sodium hydroxide (Scheme 1). Carboxylic acids **22** and **23** were similarly tetrabenzylated and hydrolyzed to give benzylated arylcarboxylic acids **14** and **15** in 76% and 87% overall yield, respectively.

To access quercetin analogs containing C3'-*O*- and C5-*O*-substituents, we prepared hydroxyl-protected quercetins, compounds **28** and **29**, as described previously¹⁶ from **1** and sodium carbonate and 3 equiv. of benzyl chloride in DMF (Scheme 2). A mixture of tribenzyl **28** (63% yield) and tetrabenzyl **29** (18 % yield) were isolated after column chromatographic separation. As mentioned above, due to intramolecular hydrogen bonding, C5-hydroxyl function is less reactive toward an electrophile than C3'-hydroxyl, hence selective esterification of **28** with tribenzyl gallic acid **12**, EDC, and DMAP afforded C3'-gallic ester **30**. Deprotection of the benzyl ether functions of **30** with hydrogen and palladium gave quercetin C3'-gallate **8** (Scheme 2).

Selective C3'-alkylation can similarly be carried out. Hence, alkylation of **28** with sodium hydride and *N*-(3-iodopropyl)phthalimide (**31**)^{17,18} gave compound **32** (40% yield), which was subjected to reduction with hydrazine followed by hydrogen and palladium afforded 3-aminopropyl quercetin **9** (49% overall yield). The structure of compound **32** was unequivocally determined by a single-crystal x-ray analysis (Figure 2).¹⁸ The crystal is triclinic, space group of P-1, and the R factor of 0.051. A mole of water was revealed in the crystal structure of **32**.

Among five hydroxyl moieties, C5-hydroxyl function is the least reactive hydroxyl group of quercetin. We were unable to esterify C5-hydroxyl function of tetrabenzyl protected quercetin **29** with either tribenzyl protected gallic acid **12** or tribenzyl protected galloyl chloride and pyridine or sodium hydride at elevated temperature. Likely, the bulky 3,4,5-tribenzyloxybenzoyl group could not fit into the C5-hydroxyl function. However, smaller electrophiles such as allyl bromide can be attached to C5-hydroxyl group of **29**. Alkylation of quercetin **29** with sodium hydride and allyl bromide gave C5-*O*-allyl quercetin **33** in 88% yield, which upon reduction with hydrogen and palladium furnished C5-*O*-propyl derivative **10** (90% yield). Allyl bromide was chosen as the alkylating agent instead of propyl iodide because a higher yield was produced.

Scheme 2. Syntheses of quercetin C3' and C5 analogs

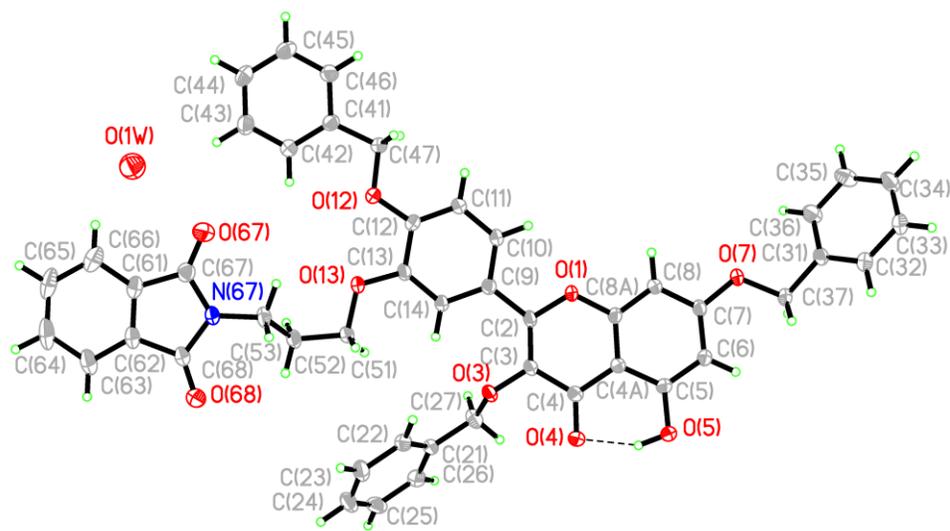
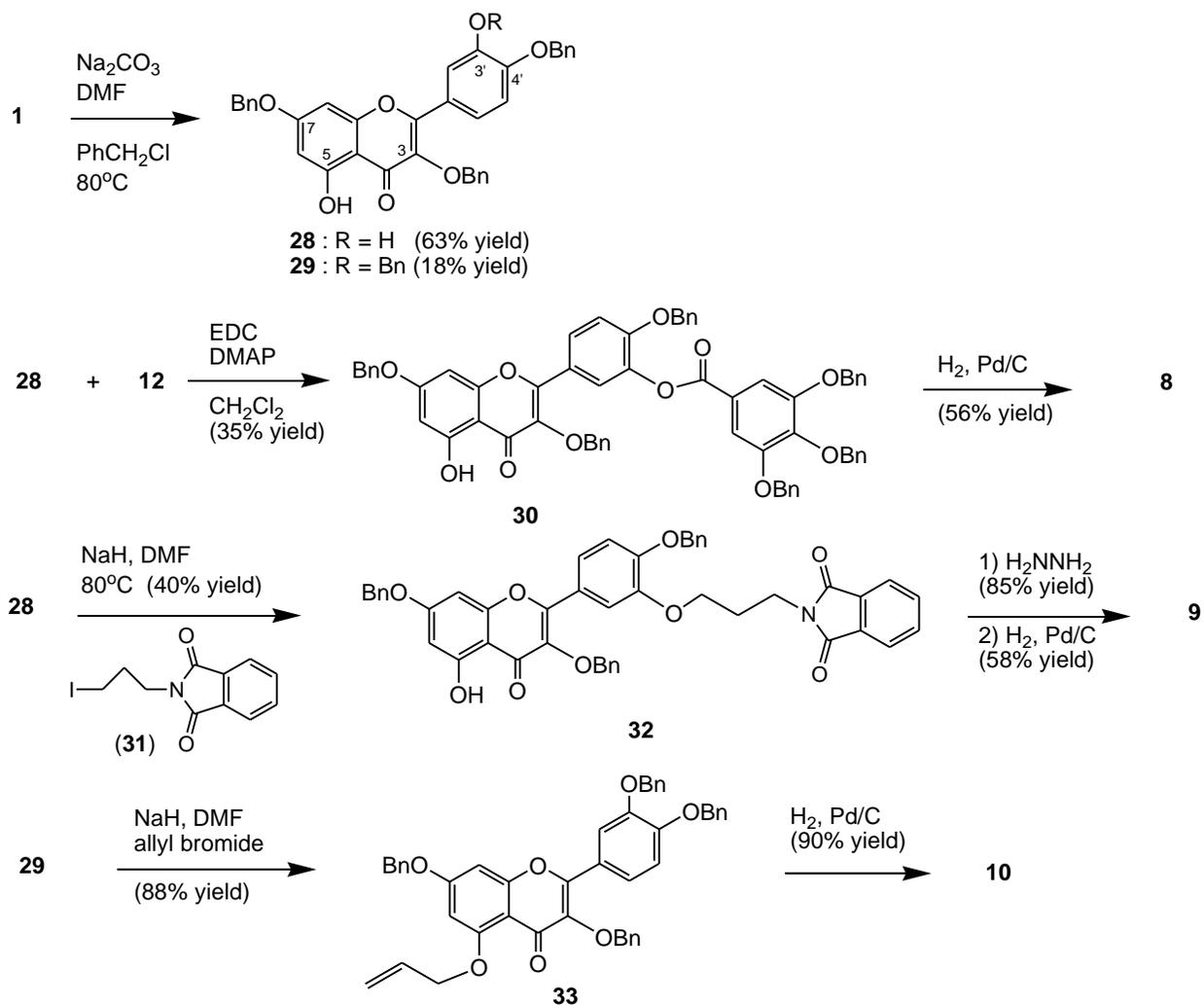


Figure 2. An ORTEP drawing of X-ray crystallographically determined structure of compound **32**.

The **inhibition** of influenza A virus (A/swine/OH/511445/2007[H1N1], Oh7) by compounds **1** – **10** and **29** **was** evaluated following a procedure reported previously.⁷ In brief, Madin-Darby canine kidney (MDCK) cells were infected with influenza virus Oh7 and incubated with each compound at various concentrations from 0.1 – 150 μM in the presence of trypsin (10 $\mu\text{g}/\text{mL}$) for up to 4 days. Cytopathic effects (CPE) of the cells were observed for each compound. The effective doses for 50% virus reduction (ED_{50}) and toxic doses at 50% cell death (TD_{50}) of the above compounds are summarized in Table 1. Viral replication was also confirmed by immunofluorescence assay and Western blot analysis with antibodies to the viral nucleoprotein or whole influenza virus, respectively. Among the tested compounds, isoquercetin, EGCG, quercetin-3-gallate appear to have similar ED_{50} values against H1N1 virus, and isoquercetin (**2**) is the most active compound with a ED_{50} value of 1.2 μM . The ED_{50} of EGCG and quercetin-3-gallate was determined at 8 and 9.5 μM , respectively, and therapeutic index (TI) value of quercetin-3-gallate of 9.9 is slightly better than that of EGCG of 5.5. C3-Dihydroxyl and hydroxylaminobenzoate analogs, compounds **5** – **7**, have ED_{50} values of 20 – 24 μM and similar TD_{50} values as that of EGCG indicating modification of the gallate moiety (of EGCG and quercetin-3-gallate) retains the anti-influenza activity. Quercetin (**1**), without gallate function, is less effective with ED_{50} and TD_{50} values of 48.2 and 83.4 μM , respectively. Despite having a gallate function at C3', compound **8**, and C3'-3-aminopropoxy analog **9** and C5-propoxy compound **10** are not effective up to 50 μM . Benzylated analogs such as compound

29 are not effective up to 50 μM . Therefore the TD_{50} values of compounds **8 – 10** and **29** were not determined. It appears that derivatization of C3' and C5 led to lower antiviral activity.

Table 1. Values of effective dose required to reduce the replication of virus by 50% (ED_{50}) and toxic dose for 50% cell death (TD_{50}) of various natural and synthetic compounds.

Compound	ED_{50} value in μM	TD_{50} value in μM	Therapeutic index (TI)
Quercetin (1)	48.2	83.4	1.7
Isoquercetin (2)	1.2	45.1	37.6
EGCG (3)	8.3	45.5	5.5
Quercetin-3-gallate (4)	9.1	90.2	9.9
5	19.4	45.2	2.3
6	22.6	60.1	2.7
7	24.1	54.8	2.3
8	>50	ND*	-
9	>50	ND	-
10	>50	ND	-
29	>50	ND	-

* ND: not determined due to high ED_{50} values. Note: Quercetin, isoquercetin and EGCG were purchased from Sigma-Aldrich (St. Louis, MO).

In conclusion, various C3, C3', and C5 substituted quercetins were synthesized and their anti-H1N1 activities were examined. C3-Substituted quercetins were derived from a carbodiimide-activated coupling reaction of C3',C4',C7-*O*-tribenzyl-protected quercetin **11** and various arylcarboxylic acids, and C3'- and C5-substituted quercetins were synthesized from C3,C4',C7-*O*-tribenzyl- and C3,C3',C4',C7-*O*-tetrabenzyl-protected quercetin, respectively. Subsequent global removal of benzyl protecting groups afforded substituted quercetins. C5-Esterification with gallic acid **12** failed, but alkylation was possible. The synthetic sequence is short and amenable to large scale synthesis, and the synthesized C3-analogs have comparable antiviral activity as that of EGCG implying further modification at C3 is possible in improving efficacy.

Acknowledgement.

This work has been supported by the National Institutes of Health, National Institute of Allergy and Infectious Diseases (U01 AI081891).

Supplementary data

Synthetic procedure, analysis data, and protocols for antiviral evaluation are included. Supplementary data associate with this article can be found in the online version, at ...

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19. The authors have deposited atomic coordinates for the structures, compound **32** with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 175022. The coordinates can be obtained on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, U.K.

Synthesis and Antiviral Activity of Substituted Quercetins.

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Supplementary Materials

Experimental Section.

General Methods. NMR spectra were obtained from a 400-MHz spectrometer (Varian Inc.), in CDCl₃, unless otherwise indicated, and reported in ppm. Infrared spectra were taken from a Nicolet 380 FT-IR instrument (Thermo Scientific) in solid forms and are reported in wave numbers (cm⁻¹). Low-resolution mass spectra were taken from an API 2000-triple quadrupole ESI-MS/MS mass spectrometer (from Applied Biosystems). High-resolution Mass spectra were obtained from a LCT Premier (Waters Corp., Milford MA) time of flight mass spectrometer. The instrument was operated at 10,000 resolution (W mode) with dynamic range enhancement that attenuates large intensity signals. The cone voltage was 60 eV. Spectra were acquired at 16666 Hz pusher frequency covering the mass range 100 to 1200 μ and accumulating data for 2 seconds per cycle. Mass correction for exact mass determinations was made automatically with the lock mass feature in the MassLynx data system. A reference compound in an auxiliary sprayer is sampled every third cycle by toggling a “shutter” between the analysis and reference needles. The reference mass is used for a linear mass correction of the analytical cycles. Chemicals such rutin, quercetin, and others were purchased from Fisher Scientific and Aldrich Chemical Co.

***O*-Tribenzylgallic acid (12).**¹³ A mixture of 5.0 g (29 mmol) of gallic acid (**20**), 15 g (0.15 mol) of sodium carbonate, 16 g (0.12 mol) of benzyl chloride in 50 mL of dried DMF was stirred under argon at 25°C for 14 h, diluted with 100 mL of water, and extracted with chloroform. The organic layer was washed three times with water, dried (MgSO₄), concentrated, crystallized from hexanes to give 9.1 g (58% yield) of pure tetrabenzyl **24**: ¹H NMR δ 7.42-7.24 (22 H, m), 5.32 (2 H, s), 5.12 (6 H, s); ¹³C NMR δ 166.2, 152.7, 142.7, 137.6, 136.9, 136.3, 128.8, 128.7, 128.4, 128.4, 128.3, 128.2, 128.2, 127.7, 125.3, 109.5, 75.3, 71.5, 67.0.

A solution of 2.0 g (3.5 mmol) of compound **24**, 0.3 g (7.0 mmol) of sodium hydroxide in 45 mL of methanol, water, and dioxane (1:1:1) was stirred under reflux for 14 h, cooled to 25°C, acidified with 1 N HCl, and extract with chloroform. The chloroform extract was washed with water and brine, dried (MgSO₄), concentrated, and crystallized from dichloromethane and diethyl ether to give 1.31 g (78% yield) of pure **12**¹³ as a white solid, mp 190 – 195°C; ¹H NMR δ 7.43 – 7.27 (m, 17 H), 5.15 (s, 4 H, CH₂), 5.14 (s, 2 H, CH₂); ¹³C NMR δ 152.8, 137.6, 136.7, 128.8, 128.7, 128.4, 128.3, 128.2, 127.8, 109.8, 75.4, 71.4.

7-(Benzyloxy)-2-[3,4-bis(benzyloxy)phenyl]-5-hydroxy-4-oxo-4*H*-chromen-3-yl 3,4,5-tris(benzyloxy)benzoate (16). A mixture of 0.10 g (0.17 mmol) of 3',4',7-tri-*O*-benzylquercetin (**11**), 72 mg (0.16 mmol) of *O*-tribenzylgallic acid (**12**), 27 mg (0.17 mmol) of EDC, and 4 mg of DMAP was dried under vacuum and maintained under argon. To it, 8 mL of CH₂Cl₂ was added, and the resulting solution was stirred at 25°C for 12 h. The reaction mixture was diluted with 20 mL of CH₂Cl₂, washed three times with water (30 mL each) and brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 0.11 g (64% yield) of compound **16** as a white solid, mp 98 – 99°C. ¹H NMR δ 12.20 (s, 1 H, OH), 7.57 (s, 2 H), 7.48 – 7.27 (m, 32 H), 6.92 (d, *J* =

8.6 Hz, 1 H), 6.54 (d, $J = 2.2$ Hz, 1 H), 6.48 (d, $J = 2.2$ Hz, 1 H), 5.21 (s, 2 H), 5.16 (s, 2 H), 5.15 (s, 2 H), 5.13 (s, 2 H), 5.12 (s, 2 H), 4.99 (s, 2 H); ^{13}C NMR δ 176.0, 165.11, 163.65, 162.20, 157.16, 156.35, 151.98, 148.76, 143.79, 137.51, 136.72, 136.66, 136.59, 135.86, 131.48, 128.99, 128.86, 128.77, 128.70, 128.65, 128.46, 128.31, 128.25, 128.06, 127.81, 127.73, 127.48, 127.31, 123.32, 122.81, 122.40, 114.62, 114.01, 110.20, 105.87, 99.24, 93.71, 75.43, 71.58, 71.02, 70.76. MS (electrospray) m/z 1017.7 ($\text{M}+\text{Na}^+$), 903.6, 518.5; HRMS calcd for $\text{C}_{64}\text{H}_{51}\text{O}_{11}^+$ ($\text{M}+\text{H}^+$) 995.3431, found 995.3445.

Quercetin 3-gallate [or 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl 3,4,5-trihydroxybenzoate] (4).¹¹ A mixture of 0.11 g (0.11 mmol) of Compound **16**, 11 mg of 10% palladium on carbon in 5 mL each of methanol, dichloromethane, and ethyl acetate was stirred under hydrogen atmosphere at 25°C for 10 h. The mixture was filtered through Celite and concentrated to give 50 mg (68% yield) of compound **4** as a solid, mp >270°C;¹¹ ^1H NMR ($\text{DMSO}-d_6$) δ 12.20 (bs, 1 H, OH), 9.30 (bs, 6 H, OH), 7.36 (d, $J = 2.0$ Hz, 1 H), 7.26 (dd, $J = 8.5, 2.0$ Hz, 1 H), 6.28 (d, $J = 1.8$ Hz, 1 H), 7.12 (s, 1 H), 6.91 (s, 1 H), 6.87 (d, $J = 8.5$ Hz, 1 H), 6.54 (d, $J = 1.8$ Hz, 1 H); ^{13}C NMR δ 175.77, 165.29, 164.00, 161.74, 157.28, 156.75, 149.89, 146.46, 146.35, 146.19, 146.16, 140.36, 130.61, 121.08, 120.44, 117.89, 116.43, 115.74, 110.05, 104.13, 99.71, 94.72; MS (electrospray) m/z 477.2 ($\text{M}+\text{Na}^+$; 100%), 413.4. HRMS calcd for $\text{C}_{22}\text{H}_{15}\text{O}_{11}^+$ ($\text{M}+\text{H}^+$) 455.0614, found 455.0589.

Benzyl 2,5-dibenzyloxybenzoate (25). To a solution of 1.0 g (6.4 mmol) of 2,5-dihydroxybenzoic acid, 3.6 g (26 mmol) of potassium carbonate in 25 mL of DMF under argon at 25°C, was added 3.6 g (21 mmol) of benzyl bromide via syringe. The solution was stirred at 60°C for 12 h, cooled to 25°C, diluted with aqueous ammonium chloride, and extracted with dichloromethane. The organic layer was washed with water three time, and brine, dried

(MgSO₄), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 2.8 g (87% yield) of compound **25**.¹⁵ ¹H NMR δ 7.48 (d, *J* = 3.2 Hz, 1 H), 7.42 - 7.25 (m, 15 H), 7.05 (dd, *J* = 8.8, 3.2 Hz, 1 H), 6.94 (d, *J* = 8.8 Hz, 1 H), 5.34 (s, 2 H), 5.09 (s, 2 H), 5.02 (s, 2 H); ¹³C NMR δ 166.2, 152.9, 152.7, 137.1, 137.0, 128.8, 128.7, 128.7, 128.4, 128.3, 128.3, 128.0, 127.8, 127.4, 121.7, 120.6, 117.7, 72.0, 70.9, 67.1.

2,5-Dibenzyloxybenzoic acid (13).^{14,15} A solution of 1.0 g (2.4 mmol) of ester **25** and 0.19 g (4.7 mmol) of sodium hydroxide in 15 mL of methanol, dioxane, and water (1:1:1) was stirred under reflux for 14 h, cooled to 25°C, and most of the organic solvents were removed from a rotary evaporator. The residue was diluted with water, acidified with 1 N HCl, extracted twice with ethyl acetate (50 mL), and the ethyl acetate layers were combined, washed with water and brine, dried (MgSO₄), concentrated, and column chromatographed using a mixture of hexane and ethyl acetate (1:1) as eluent to give 0.72 g (91% yield) of compound **13**.¹⁵ ¹H NMR δ 11.06 (s, 1 H), 7.81 (d, *J* = 3.1 Hz, 1 H), 7.43 - 7.32 (m, 10 H), 7.17 (dd, *J* = 9.0, 3.1 Hz, 1 H), 7.07 (d, *J* = 9.0 Hz, 1 H), 5.26 (s, 2 H), 5.08 (s, 2 H); ¹³C NMR δ 165.3, 153.9, 151.9, 136.6, 134.6, 129.4, 129.4, 128.8, 128.4, 128.2, 127.8, 123.0, 119.0, 117.8, 115.1, 73.2, 70.9.

2-(3,4-Dibenzyloxyphenyl)-7-benzyloxy-5-hydroxy-4-oxo-4H-chromen-3-yl 2,5-dibenzyloxybenzoate (17). To a solution of 50 mg (0.15 mmol) of 2,5-dibenzyloxybenzoic acid (**13**), 27 mg (0.15 mmol) of EDC, and 4 mg (3 μmol) of DMAP in 3 mL of dichloromethane under argon at 25°C was added a solution of 50 mg (87 μmol) of 3',4',7-tri-*O*-benzylquercetin (**11**) in 5 mL of dichloromethane via syringe. The reaction mixture was stirred at 25°C for 12 h, diluted with 50 mL of water, and extracted three times with 15 mL each of dichloromethane. The combined organic layer was washed with brine, dried (anhydrous MgSO₄), concentrated,

and column chromatographed on silica gel using a mixture of 1:1 hexane and diethyl ether as eluent to give 65 mg (83% yield) of **17** as a white solid, mp 85 - 88°C. ¹H NMR δ 12.3 (s, 1 H, OH), 7.79 (d, *J* = 3.6 Hz, 1 H), 7.56 (d, *J* = 2.0 Hz, 1 H), 7.53 (dd, *J* = 8.6, 2 Hz, 1 H), 7.46 - 7.22 (m, 25 H), 7.15 (dd, *J* = 9.0, 3.6 Hz, 1 H), 7.00 (d, *J* = 9.0 Hz, 1 H), 6.88 (d, *J* = 8.2 Hz, 1 H), 6.54 (d, *J* = 2.0 Hz, 1 H), 6.48 (d, *J* = 2.0 Hz, 1 H), 5.16 (s, 2 H), 5.15 (2, 2 H), 5.12 (s, 2 H) 5.04 (s, 2 H), 4.97 (s, 2 H); ¹³C NMR δ 176.1, 165.1, 162.8, 162.3, 157.2, 156.5, 154.1, 152.9, 151.9, 148.8, 136.9, 136.8, 136.7, 136.0, 131.5, 129.0, 128.9, 128.8, 128.7, 128.6, 128.3, 128.25, 128.2, 128.0, 127.9, 127.8, 127.6, 127.4, 127.34, 127.32, 123.0, 122.6, 122.3, 119.2, 118.4, 116.8, 114.8, 114.0, 106.0, 99.2, 93.7, 72.1, 71.5, 71.03, 71.01, 70.8. MS (electrospray) *m/z* 889.5 (M+H⁺; 100%), 413.2. HRMS calcd for C₅₇H₄₅O₁₀ (M+H⁺) 889.3013, found 889.3035.

2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl 2,5-dihydroxybenzoate (5).

A mixture of 50 mg (56 μmol) of compound **17** and 5 mg of 10% palladium/carbon in 9 ml of ethyl acetate, methanol and dichloromethane (1:1:1) was shaken at 25°C under 25 psi hydrogen atmosphere for 10 h. The reaction mixture was diluted with 10 mL of methanol and filtered through Celite, and the filtrate was concentrated to give 15 mg (60% yield) of **5** as a solid, mp >270°C. ¹H NMR (DMSO-*d*₆) δ 12.14 (s, OH, 1 H), 11.08 (bs, OH, 1 H), 9.98 (bs, OH, 1 H), 9.59 (s, OH, 1 H), 9.48 (bs, OH, 1 H), 9.35 (s, OH, 1 H), 7.36 - 7.28 (m, 3 H), 7.06 (dd, *J* = 8.8, 2.8 Hz, 1 H), 6.89 (dd, *J* = 13.6, 8.4 Hz, 1 H), 6.51 (d, *J* = 1.2 Hz, 1 H), 6.27 (d, *J* = 1.6 Hz, 1 H); ¹³C NMR (DMSO-*d*₆) δ 175.3, 165.4, 161.7, 157.3, 156.9, 154.1, 150.4, 150.1, 146.2, 130.1, 125.4, 121.2, 120.5, 120.1, 119.3, 116.5, 115.8, 115.7, 112.5, 104.0, 99.8, 94.8. MS (electrospray) *m/z* 461.1 (M+Na⁺; 100%), 325.3. HRMS calcd for C₂₂H₁₅O₁₀ (M+H⁺) 439.0665, found 439.0663.

Benzyl 4-(benzyloxy)-3-(dibenzylamino)benzoate (26). To a solution of 1.0 g (6.5 mmol) of 3-amino-4-hydroxybenzoic acid (**22**) and 0.71 g (29 mmol) of sodium hydride (50% oil, which was removed by washing with dried diethyl ether twice) in 20 mL of DMF under argon at 25°C was added 3.9 mL (33 mmol) of benzyl bromide. The reaction solution was stirred at 60°C for 14 h, cooled to 25°C, diluted with 50 mL of aqueous NH₄Cl, and extracted with dichloromethane. The organic layer was washed three times with water, and brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether to give 3.1 g (90% yield) of **26**: Mp 109 – 110°C; ¹H NMR δ 7.66 (dd, *J* = 8.4, 2.1 Hz, 1 H), 7.60 (d, *J* = 2.0 Hz, 1 H), 7.42 (dd, *J* = 8.0, 2.0 Hz, 2 H), 7.37 - 7.16 (m, 18 H), 6.91 (d, *J* = 8.4 Hz, 1 H), 5.26 (s, CH₂O, 2 H), 5.19 (s, CH₂O, 2 H), 4.25 (s, CH₂N, 4 H); ¹³C NMR δ 166.5, 156.5, 140.0, 138.9, 136.7, 136.6, 128.8, 128.7, 128.3, 128.3, 128.2, 128.1, 127.7, 127.0, 125.2, 123.4, 122.8, 112.5, 70.8, 66.5, 55.9. MS (electrospray) *m/z* 514.3 (M+H⁺), 536.4 (M+Na⁺), 332.3. HRMS calcd for C₃₅H₃₂NO₃⁺ (M+H⁺) 514.2382, found 514.2362.

4-(Benzyloxy)-3-(dibenzylamino)benzoic acid (14). A solution of 1.5 g (2.9 mmol) of benzoate **26** and 0.23 g (5.9 mmol) of sodium hydroxide in 15 mL of methanol, dioxane, and water (1:1:1) was stirred under reflux for 3 h, cooled to 25°C, and acidified with 5 mL of 1 N HCl. The solution was extracted with 50 mL of ethyl acetate, and the organic extract was washed three times with water (20 mL each), and brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a mixture of hexane and diethyl ether (7:3) to give 0.70 g (84% yield) of acid **14**: Mp 177 – 180°C; ¹H NMR δ 4.26 (s, 4 H) 5.24 (s, 2 H) 6.97 (d, *J* = 8.4 Hz, 1 H) 7.14 - 7.49 (m, 15 H) 7.61 (s, 1 H) 7.70 (d, *J* = 8.4 Hz, 1 H); ¹³C NMR δ 172.0, 157.1, 140.1, 138.7, 136.6, 128.8, 128.7, 127.7, 127.1, 126.0, 123.9, 121.8, 112.5, 70.9, 55.8.

MS (electrospray) m/z 424.4 ($M+H^+$; 100%), 446.2 ($M+Na^+$), 242.5. HRMS calcd for $C_{28}H_{26}NO_3^+$ ($M+H^+$) 424.1913, found 424.1899.

7-(Benzyloxy)-2-(3,4-dibenzyloxyphenyl)-5-hydroxy-4-oxo-4H-chromen-3-yl 4-(benzyloxy)-3-(dibenzylamino)benzoate (18). To a solution of 0.15 g (0.35 mmol) of acid **14**, 55 mg (0.35 mmol) of EDC, 9 mg (7 μ mol) of DMAP in 3 mL of dichloromethane under argon at 25°C was added a solution of 0.14 g (0.24 mmol) of benzyl quercetin **11** in 5 mL of dichloromethane. The solution was stirred for 14 h, diluted with water and extracted with dichloromethane (30 mL). The organic extract was washed with water and brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 0.18 g (75% yield) of **18** as a solid, mp 114 – 115°C. 1H NMR δ 12.25 (s, 1 H, OH), 7.85 (dd, $J = 8.6, 2.0$ Hz, 1 H), 7.78 (d, $J = 2.0$ Hz, 1 H), 7.12 - 7.48 (m, 32 H), 7.00 (d, $J = 8.6$ Hz, 1 H), 6.90 (d, $J = 9.4$ Hz, 1 H), 6.53 (d, $J = 1.6$ Hz, 1 H), 6.47 (d, $J = 2.0$ Hz, 1 H), 5.20 (s, 2 H), 5.19 (s, 2 H), 5.15 (s, 2 H), 4.81 (s, 2 H), 4.27 (s, 4 H, CH_2N); ^{13}C NMR δ 176.3, 165.0, 163.9, 162.3, 157.4, 157.2, 156.2, 151.8, 148.8, 140.6, 138.8, 136.9, 136.7, 136.4, 135.9, 131.5, 129.0, 128.9, 128.8, 128.7, 128.6, 128.4, 128.37, 128.3, 128.1, 127.8, 127.7, 127.5, 127.3, 127.1, 126.2, 123.9, 122.7, 122.6, 120.8, 114.5, 114.1, 112.7, 106.0, 99.2, 93.6, 71.3, 71.1, 71.0, 70.8, 55.8 (2 C's). MS (electrospray) m/z 1000.6 ($M+Na^+$), 978.6 ($M+H^+$), 224.0 (100%). HRMS calcd for $C_{64}H_{52}NO_9^+$ ($M+H^+$) 978.3642, found 978.3681.

5,7-Dihydroxy-2-(3,4-dihydroxyphenyl)-4-oxo-4H-chromen-3-yl 4-hydroxy-3-aminobenzoate (6). A mixture of 66 mg (0.07 mmol) of compound **18**, 20 mg of 10% palladium over carbon in 9 mL of ethyl acetate, methanol, and dichloromethane (1:1:1) was shaken in a hydrogenator under 36 psi of hydrogen at 25°C for 14 h. The mixture was diluted with 20 mL of methanol and filter through Celite, concentrated to dryness to give 17 mg (58%

yield) of **6** as a solid, mp >270°C. ¹H NMR (DMSO-*d*₆) δ 12.19 (s, 1 H, OH), 11.17 (bs, 1 H, OH), 9.93 (bs, 1 H), 9.47 (bs, 1 H), 7.93 (s, 1 H), 7.75 (d, *J* = 7.6 Hz, 1 H), 7.39 (d, *J* = 2.4 Hz, 1 H), 7.27 (dd, *J* = 8.4, 2.0 Hz, 1 H), 7.11 (d, *J* = 8.8 Hz, 1 H), 6.88 (d, *J* = 8.4 Hz, 1 H), 6.55 (d, *J* = 2.4 Hz, 1 H), 6.29 (d, *J* = 1.6 Hz, 1 H); ¹³C NMR (DMSO-*d*₆) δ 176.0, 165.2, 164.0, 161.8, 157.3, 156.7, 149.8, 146.1, 143.8, 143.7, 130.7, 124.6, 121.1, 120.6, 116.4, 115.8, 115.7, 115.0, 113.6, 104.2, 99.6, 94.6. MS (electrospray) *m/z* 438.3 (M+H⁺), 136.1 (100%). HRMS calcd for C₂₂H₁₆NO₉⁺ (M+H⁺) 438.0825, found 438.0810.

Phenyl 3-(benzyloxy)-4-(dibenzylamino)benzoate (27). A mixture of 1.0 g (6.5 mmol) of 4-amino-3-hydroxybenzoic acid (**23**) and 0.7 g (27 mmol) of sodium hydride was dried under vacuum and maintained under argon. To it 5 mL of dried DMF was added and the solution was stirred at 25°C for 0.5 h. To the reaction solution, 3.1 mL (26 mmol) of benzyl bromide was added, and the resulting solution was stirred at 80°C for 16 h, cooled to 25°C, and 20 mL of saturated aqueous NH₄Cl was added. The mixture was extracted with 100 mL of ethyl acetate, and the organic layer was washed with 50 mL of water three times and brine, dried (anhydrous MgSO₄), concentrated, and column chromatographed on silica gel using a mixture of 5:1 hexane and ethyl acetate as eluent to give 3.0 g (90% yield) of **27** as white solid, mp 105 – 107°C. ¹H NMR δ 7.67 (d, *J* = 2.0 Hz, 1 H), 7.55 (dd, *J* = 6.4, 2.0 Hz, 1 H), 7.42 - 7.16 (m, 20 H), 6.76 (d, *J* = 8.8 Hz, 1 H), 5.31 (s, CH₂O, 2 H), 5.18 (s, CH₂O, 2 H), 4.40 (s, CH₂N, 4 H). ¹³C NMR δ 166.5, 156.4, 139.9, 138.8, 136.6, 129.4, 128.8, 128.7, 128.7, 128.3, 128.23, 128.20, 128.1, 127.7, 127.0, 125.1, 123.3, 122.6, 112.4, 70.7, 66.4, 55.8; MS (electrospray) *m/z* 514.3 (M+H⁺; 100%), 536.4 (M+Na⁺), 332.1. HRMS calcd for C₃₅H₃₂NO₃⁺ (M+H⁺) 514.2382, found 514.2394.

3-(Benzyloxy)-4-(dibenzylamino)benzoic acid (15). A mixture of 1.0 g (2.0 mmol) of **27** and 0.16 g (4.0 mmol) of sodium hydroxide in 15 mL of methanol, dioxane, and water (1:1:1) was

stirred under reflux for 3 h, cooled to 25°C, acidified with 5 mL of 1 N HCl, and extracted with 50 mL of ethyl acetate. The organic layer was washed three times with 20 mL of water, and brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 0.8 g (97% yield) of acid **15** as white solid, mp 185 – 187°C. ¹H NMR δ 7.70 (s, 1 H), 7.59 (d, *J* = 8.2 Hz, 1 H), 7.14 - 7.44 (m, 15 H), 6.80 (d, *J* = 8.2 Hz, 1 H), 5.19 (s, 2 H, CH₂O), 4.44 (s, 4 H, CH₂N); ¹³C NMR δ 172.2, 150.5, 145.8, 138.4, 136.7, 128.7, 128.5, 128.2, 128.17, 128.0, 127.1, 124.6, 121.5, 119.5, 114.7, 71.1, 55.3; MS (electrospray) *m/z* 424.4 (M+H⁺, 100%). HRMS calcd for C₂₈H₂₆NO₃⁺ (M+H⁺) 424.1913, found 424.1914.

7-(Benzyloxy)-2-(3,4-dibenzyloxyphenyl)-5-hydroxy-4-oxo-4H-chromen-2-yl 3-(benzyloxy)-4-(dibenzylamino)benzoate (19). To a solution of 48 mg (0.11 mmol) of **15**, 18 mg (0.11 mmol) of EDC, and 2 mg (18 μmol) of DMAP in 3 mL of dichloromethane under argon at 25°C was added a solution of 50 mg (87 μmol) tribenzyl quercetin **11** in 5 mL of dichloromethane. The reaction solution was stirred for 12 h, diluted with 50 mL of water, and extracted three times with dichloromethane (15 mL each). The combined organic layer was washed with brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a mixture of hexane and diethyl ether (1:1) as eluent to give 60 mg (71% yield) of compound **19** as white solid, mp. 110 – 112°C; ¹H NMR δ 12.22 (s, 1 H, OH), 7.79 (d, *J* = 1.6 Hz, 1 H), 7.73 (dd, *J* = 8.4, 1.8 Hz, 1 H), 7.47 - 7.54 (m, 2 H), 7.14 - 7.45 (m, 30 H), 6.92 (d, *J* = 8.6 Hz, 1 H), 6.82 (d, *J* = 8.6 Hz, 1 H), 6.51 (d, *J* = 2.0 Hz, 1 H), 6.43 (d, *J* = 2.0 Hz, 1 H), 5.16 (s, 2 H), 5.13 (s, 2 H), 5.10 (s, 2 H), 4.93 (s, 2 H), 4.44 (s, 4 H, CH₂N); ¹³C NMR δ 176.7, 164.9, 163.86, 162.1, 157.1, 156.1, 151.7, 150.5, 148.5, 146.1, 138.2, 136.7, 136.6, 136.4, 135.8, 131.5, 128.9, 128.7, 128.6, 128.5, 128.2, 128.17, 128.14, 128.1, 128.0, 127.9, 127.7, 127.4, 127.2, 127.1, 125.0, 122.6, 122.5, 119.9,

119.3, 114.9, 114.4, 113.8, 105.8, 99.1, 93.5, 71.3, 71.1, 70.9, 70.6, 55.2. MS (electrospray) m/z 1000.6 ($M+Na^+$), 978 ($M+H^+$), 224.0 (100%). HRMS calcd for $C_{64}H_{52}NO_9^+$ ($M+H^+$) 978.3642, found 978.3681.

2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl **4-amino-3-**

hydroxybenzoate (7). A mixture of 0.1 g (0.1 mmol) of **19** and 40 mg of 10% palladium over carbon in 9 mL of ethyl acetate, methanol, and dichloromethane (1:1:1) was shaken at 25°C under 36 psi hydrogen atmosphere for 10 h. After removal from the hydrogenator, the mixture was diluted with 10 mL of methanol, filtered through Celite, concentrated to give a solid. This solid was washed with dichloromethane and vacuum dried to obtain 31 mg (70% yield) of **7** as a light yellow solid, mp >270°C (dec.). 1H NMR (DMSO- d_6) δ 7.44 (dd, $J = 8.3, 1.9$ Hz, 1 H), 7.35 (dd, $J = 8.3, 2.0$ Hz, 2 H), 7.27 (dd, $J = 8.3, 2.0$ Hz, 1 H), 6.84 (d, $J = 8.3$ Hz, 1 H), 6.69 (d, $J = 8.3$ Hz, 1 H), 6.49 (d, $J = 2.0$ Hz, 1 H), 6.25 (d, $J = 2.0$ Hz, 1 H); ^{13}C NMR (DMSO- d_6) δ 176.0, 165.2, 164.0, 161.8, 157.3, 156.7, 149.8, 146.1, 143.8, 143.7, 130.7, 124.6, 121.1, 120.6, 116.4, 115.8, 115.7, 115.0, 113.6, 104.2, 99.6, 94.6. MS (electrospray; negative mode) m/z 436.4 ($M-H^+$), 255.4. HRMS calcd for $C_{22}H_{16}NO_9^+$ ($M+H^+$) 438.0825, found 438.0816.

3,7-(Dibenzyloxy)-2-(4-benzyloxy-3-hydroxyphenyl)-5-hydroxy-4-oxo-4H-chromene (28)

and 3,7-(dibenzyloxy)-2-(3,4-dibenzyloxyphenyl)-5-hydroxy-4-oxo-4H-chromene (29).¹⁶ To a solution of 1.0 g (3.3 mmol) of quercetin (**1**) and 1.0 g (9.9 mmol) of sodium carbonate in 30 mL of DMF under argon at 80°C was added 1.3 g (9.9 mmol) of benzyl chloride via syringe. The solution was stirred at 80°C for 20 h and most of the DMF solvent was removed via vacuum distillation at 50 – 60°C/5 mm Hg. The remaining mixture was diluted with dichloromethane, washed with 2 N HCl, water, and brine, dried (anhydrous Na_2SO_4), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and ethyl acetate as eluent to

give 1.2 g (63% yield) of compound **28** and 0.4 g (18% yield) of compound **29**. Compound **28**:¹⁶ Mp 146 – 149°C; ¹H NMR δ 12.71 (s, 1 H, OH), 7.65 (d, *J* = 2 Hz, 1 H), 7.63 (s, 1 H), 7.46 – 7.22 (m, 15 H), 6.97 (d, *J* = 9 Hz, 1 H), 6.51 (d, *J* = 2 Hz, 1 H), 6.45 (d, *J* = 2 Hz, 1 H), 5.72 (s, 1 H, OH), 5.21 (s, 2 H), 5.14 (s, 2 H), 5.08 (s, 2 H); ¹³C NMR δ 179.0, 164.7, 162.2, 157.0, 156.5, 148.0, 145.8, 137.8, 136.0, 135.9, 129.0, 128.5, 127.7, 124.2, 122.1, 115.1, 111.8, 98.9, 93.2, 74.5, 71.4, 70.6. Compound **29**:¹⁶ Mp 110 – 114°C; ¹H NMR δ 12.70 (s, 1 H, OH), 7.72 (d, *J* = 2 Hz, 1 H), 7.56 (dd, *J* = 9, 2 Hz, 1 H), 7.50 – 7.20 (m, 20 H), 6.98 (d, *J* = 9 Hz, 1 H), 6.48 (d, *J* = 2 Hz, 1 H), 6.45 (d, *J* = 2 Hz, 1 H), 5.26 (s, 2 H), 5.15 (s, 2 H), 5.06 (s, 2 H), 5.01 (s, 2 H); ¹³C NMR δ 179.0, 164.7, 162.3, 156.9, 156.5, 151.3, 148.4, 137.7, 136.9, 136.7, 136.0, 129.0, 128.7, 128.5, 127.7, 127.4, 123.7, 122.8, 115.5, 113.9, 106.4, 98.8, 93.3, 74.6, 71.3, 71.1, 70.7.

5-(3,7-Bis(benzyloxy)-5-hydroxy-4-oxo-4H-chromen-2-yl)-2-(benzyloxy)phenyl 3,4,5-tris(benzyloxy)benzoate (30). To a solution of 0.12 g (0.26 mmol) of acid **12**, 40 mg (26 mmol) of EDC and 4 mg (35 μmol) of DMAP in 2 mL of dichloromethane under argon was added a solution of 0.10 g (0.17 mmol) of compound **28**. The reaction solution was stirred at 25°C for 12 h, diluted with 50 mL of dichloromethane, washed three times with water, and brine, dried (anhydrous Na₂SO₄), concentrated and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluent to give 60 mg (35% yield) of compound **30**. ¹H NMR δ 12.7 (s, 1 H, OH), 7.94 (d, *J* = 2.0 Hz, 1 H), 7.92 (s, 1 H), 7.56 (s, 2 H), 7.46 - 7.18 (m, 30 H), 7.04 (d, *J* = 9.2 Hz, 1 H), 6.50 (d, *J* = 2.0 Hz, 1 H), 6.44 (d, *J* = 2.0 Hz, 1 H), 5.19 (s, 2 H), 5.17 (s, 2 H), 5.15 (s, 4 H), 5.12 (s, 2 H), 5.11 (s, 2 H); ¹³C NMR δ 179.0, 164.8, 164.4, 162.3, 156.9, 155.6, 151.9, 151.6, 143.3, 140.4, 137.9, 137.6, 136.8, 136.4, 136.3, 136.0, 129.2, 129.0, 128.8, 128.77, 128.6, 128.54, 128.51, 128.45, 128.32, 128.26, 128.24, 128.0, 127.8, 127.7, 127.6, 127.4, 127.1, 124.4, 123.9, 123.7, 113.8, 110.0, 106.4, 98.9, 93.3, 75.4, 74.7, 71.6, 70.8,

70.7. MS (electrospray) m/z 995.9 ($M+H^+$). HRMS calcd for $C_{64}H_{51}O_{11}^+$ ($M+H^+$) 995.3431, found 995.3475.

2-Hydroxy-5-(3,5,7-trihydroxy-4-oxo-4H-chromen-2-yl)phenyl 3,4,5-trihydroxybenzoate

(8). A mixture of 55 mg (55 μ mol) of compound **30** and 11 mg of 5% palladium over carbon in 11 mL of ethyl acetate, methanol, and dichloromethane (5:3:3) was shaken in a hydrogenator under 36 psi pressure of hydrogen at 25°C for 16 h. The reaction mixture was diluted with ethanol and filtered through Celite, concentrated to dryness to give a solid. The solid was washed with 5 mL of water and 15 mL of diethyl ether, and dried under vacuum to give 14 mg (56% yield) of C3'-gallate **8**. 1H NMR (DMSO-*d*6) δ 12.40 (s, 1 H, OH), 9.10 - 9.90 (bs, 5 H, OH), 7.96 (dd, J = 8.6, 2.2 Hz, 1 H), 7.91 (d, J = 2.4 Hz, 1 H), 7.10 (s, 3 H), 6.47 (d, J = 1.6 Hz, 1 H), 6.19 (d, J = 2.0 Hz, 1 H); ^{13}C NMR δ 176.6, 164.9, 164.8, 164.7, 161.3, 156.8, 151.8, 149.7, 146.4, 146.3, 139.8, 139.2, 137.7, 136.7, 123.5, 122.7, 118.9, 117.5, 109.9, 109.9, 103.7, 99.0. MS (electrospray) m/z 477.1 ($M+Na^+$), 455.2 ($M+H^+$). HRMS calcd for $C_{22}H_{15}O_{11}^+$ ($M+H^+$) 455.0614, found 455.0609.

3,7-(Dibenzyloxy)-2-[4-benzyloxy-3-(3-phthaloylpropyloxy)phenyl]-5-hydroxy-4-oxo-4H-

chromene (32). A solution of 1.1 g (1.9 mmol) of compound **28**, 0.73 g (2.3 mmol) of 3-iodopropylphthalimide (**31**),^{17,18} and 92 mg (3.9 mmol) of sodium hydride (50% oil) in 20 mL of DMF was stirred under argon at 80°C for 48 h. The reaction solution was cooled to 25°C, diluted with aqueous NH_4Cl , and extracted three times with ethyl acetate. The organic layer was washed with water and brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and ethyl acetate as eluent to give 0.58 g (40% yield) of compound **32**. Crystallization of **32** in ethyl acetate and hexane afforded single crystals suitable for X-ray analysis. Mp 168 – 174°C; 1H NMR δ 12.70 (s, 1 H, OH), 7.81 (dd, J = 8, 2 Hz, 2 H),

7.67 (dd, $J = 8, 2$ Hz, 1 H), 7.63 (d, $J = 2$ Hz, 1 H), 7.57 (dd, $J = 8, 2$ Hz, 1 H), 7.45 – 7.23 (m, 15 H), 6.91 (d, $J = 8$ Hz, 1 H), 6.55 (d, $J = 2$ Hz, 1 H), 6.44 (d, $J = 2$ Hz, 1 H), 5.14 (s, 2 H, CH₂OPh), 5.13 (s, 2 H, CH₂OPh), 5.07 (s, 2 H, CH₂OPh), 3.88 (m, 4 H, CH₂O & CH₂N), 2.15 (quin, $J = 7$ Hz, 2 H); ¹³C NMR δ 179.0, 168.5, 164.7, 162.3, 156.9, 156.5, 151.1, 148.6, 137.8, 136.9, 134.1, 132.4, 129.0, 128.5, 127.7, 127.2, 123.7, 123.4, 122.7, 114.6, 113.9, 106.4, 98.8, 93.3, 74.6, 70.9, 70.7, 67.0, 35.64, 28.64. MS (electrospray) m/z 782.3 (M+Na⁺), 760.6 (M+H⁺), 413.1 (100%). HRMS calcd for C₄₇H₃₈NO₉⁺ (M+H⁺) 760.2547, found 760.2571.

3,5,7-(Trihydroxy)-2-[4-hydroxy-3-(3-aminopropoxy)phenyl]-4-oxo-4H-chromene (9). A solution of 100 mg (0.13 mmol) of phthalimide **32** and 99 mg (2.0 mmol) of hydrazine in 3 mL of THF was stirred under argon at 25°C for 11 h. The solution was diluted with 50 mL of dichloromethane, precipitated white solids was removed by filtration, and the filtrate was washed twice with water and brine, dried (anhydrous Na₂SO₄), concentrated to give 70 mg (85% yield) of 3,7-(dibenzoyloxy)-5-hydroxy-2-[4-benzoyloxy-3-(3-aminopropoxy)phenyl]-4-oxo-4H-chromene. ¹H NMR δ 7.50 – 7.10 (m, 17 H), 6.82 (d, $J = 2$ Hz, 2 H), 6.67 (d, $J = 2$ Hz, 1 H), 6.40 (bs, 2 H), 6.22 (s, 1 H), 4.99 (s, 2 H, CH₂O), 4.97 (s, 2 H, CH₂O), 4.69 (s, 2 H, CH₂O), 3.89 (t, $J = 7$ Hz, 2 H, CH₂O), 2.78 (t, $J = 7$ Hz, 2 H, CH₂N), 1.80 (quin, $J = 7$ Hz, 2 H); ¹³C NMR δ 178.9, 164.6, 162.2, 156.8, 156.5, 151.0, 148.7, 137.8, 136.8, 136.7, 135.9, 128.9, 128.8, 128.5, 128.5, 128.4, 128.3, 127.7, 127.4, 123.5, 122.4, 114.2, 113.4, 106.3, 98.7, 93.2, 74.6, 70.9, 70.6, 68.1, 67.7, 29.7. MS (electrospray) m/z 630.3 (M+H⁺). This material was used in next step without purification. A mixture of 50 mg (79 μ mol) of the above benzyl quercetin, 15 mg of 5% palladium over carbon in 2 mL each of THF and methanol along with 5 drops of 2 N HCl, was shaken in a hydrogenator under 36 psi hydrogen for 16 h at 25°C. The mixture was diluted with 10 mL of methanol, filter through Celite, concentrated to give 17 mg (58% yield) of compound

9. ^1H NMR (DMSO-*d*₆) δ 12.45 (s, 1 H, OH), 10.81 (s, 1 H, OH), 9.74 (s, 1 H, OH), 9.46 (s, 1H, OH), 7.75 (d, J = 2.0 Hz, 1 H), 7.71 (dd, J = 8.4, 2.0 Hz, 1 H), 6.98 (d, J = 8.4 Hz, 1 H), 6.47 (d, J = 2.0 Hz, 1 H), 6.21 (d, J = 2.0 Hz, 1 H), 4.13 (t, J = 6.0 Hz, 2 H), 3.05 (bs, 2 H), 2.07 - 2.03 (m, 2 H); ^{13}C NMR δ 176.5, 164.7, 161.3, 156.8, 149.6, 147.1, 147.0, 136.5, 122.7, 122.67, 116.4, 113.8, 103.7, 98.9, 94.3, 66.6, 37.2, 27.6. HRMS calcd for $\text{C}_{18}\text{H}_{18}\text{NO}_7^+$ ($\text{M}+\text{H}^+$) 360.1083, found 360.1100.

3,7-(Dibenzyloxy)-2-(3,4-dibenzyloxyphenyl)-5-allyloxy-4-oxo-4H-chromene (33). To a solution of 54 mg (82 μmol) of compound **29** and 3 mg (0.12 mmol) of sodium hydride in 1 mL of DMF under argon at 25°C was added 12 mg (98 μmol) of allyl bromide via syringe. The reaction solution was stirred for 10 h, diluted with dichloromethane, neutralized with 1 N HCl, washed three times with water, and brine, dried (MgSO_4), concentrated, and column chromatographed on silica gel using 20% ethyl acetate in hexane as eluent to give 50 mg (88% yield) of compound **33** as a white solid, mp 107 – 110°C. ^1H NMR δ 7.72 (d, J = 2.0 Hz, 1 H), 7.54 (dd, J = 8.5, 2.0 Hz, 1 H), 7.46 - 7.21 (m, 20 H), 6.95 (d, J = 8.5 Hz, 1 H), 6.53 (d, J = 2.4 Hz, 1 H), 6.42 (d, J = 2.4 Hz, 1 H), 6.13 - 6.08 (ddt, J = 17.6, 10.4, 6.4 Hz, 1 H, =CH), 5.64 (dd, J = 17.6, 1.6 Hz, 1 H, =CH₂), 5.35 (dd, J = 10.4, 1.6 Hz, 1 H, =CH₂), 5.23 (s, 2 H, CH₂OPh), 5.12 (s, 2 H, CH₂OPh), 5.08 (s, 2 H, CH₂OPh), 4.94 (s, 2 H, CH₂OPh), 4.69 (d, J = 6.4 Hz, 2 H, CH₂O); ^{13}C NMR δ 174.1, 162.9, 160.1, 158.9, 153.2, 150.6, 148.4, 141.5, 140.0, 137.3, 137.2, 137.0, 135.9, 132.5, 129.1, 129.0, 128.8, 128.6, 128.3, 128.2, 128.1, 128.0, 127.8, 127.6, 127.4, 124.1, 122.2, 118.3, 115.3, 113.9, 110.1, 97.9, 93.8, 74.2, 71.2, 71.0, 70.7, 70.1. MS (electrospray) m/z 725.5 ($\text{M}+\text{Na}^+$, 100%), 703.4 ($\text{M}+\text{H}^+$), 396.3. HRMS calcd for $\text{C}_{46}\text{H}_{39}\text{O}_7^+$ ($\text{M}+\text{H}^+$) 703.2696, found 703.2687.

3,7-(Dihydroxy)-2-(3,4-dihydroxyphenyl)-5-propyloxy-4-oxo-4H-chromene (10). A mixture of 80 mg (0.11 mmol) of compound **33** and 30 mg of 10% palladium over carbon in 6 mL of ethyl acetate, acetone, and ethanol (1:1:1) was shaken in a hydrogenator under 36 psi of hydrogen at 25°C for 18 h. The reaction mixture was diluted with ethanol and filtered through Celite, the filtrate was concentrated to dryness giving 35 mg (90% yield) of compound **10**, mp 269 – 272°C. ¹H NMR (DMSO-*d*₆) δ 10.60 (bs, 1 H, OH), 9.40 (bs, 1 H, OH), 9.30 (bs, 1 H, OH), 8.70 (s, 1 H, OH), 7.62 (d, *J* = 2.0 Hz, 1 H), 7.47 (dd, *J* = 9.0, 2.0 Hz, 1 H), 6.86 (d, *J* = 9.0 Hz, 1 H), 6.42 (d, *J* = 2.0 Hz, 1 H), 6.31 (d, *J* = 2.0 Hz, 1 H), 3.98 (t, *J* = 7.0 Hz, 2 H), 1.77 (sextet, *J* = 7.0 Hz, 2 H), 1.08 (t, *J* = 7.0 Hz, 3 H). ¹³C NMR δ 171.0, 162.4, 160.0, 157.9, 146.9, 145.1, 141.7, 137.2, 122.3, 119.1, 115.6, 114.5, 105.2, 104.3, 96.4, 94.4, 69.8, 22.0, 10.5. MS (electrospray) *m/z* 345.5 (M+H⁺; 100%), 303.1. HRMS calcd for C₁₈H₁₇O₇⁺ (M+H⁺) 345.0969, found 345.0972.

Screening of compounds 1 – 10 and 29 for anti-influenza virus activity:⁷ Influenza virus Oh7 was inoculated to two-day-old monolayer of Madin-Darby canine kidney (MDCK) cells in 6-well plates at a multiplicity of infection (MOI) of 0.05 or 5. The virus-infected cells were incubated in the presence of each compound at various concentrations from 0.1 to 150 μM with trypsin (10 μg/mL) for up to 4 days. Amantadine or oseltamivir carboxylate was used as positive controls. Virus replication was assessed with various methods including virus titration with TCID₅₀ method, and real-time qRT-PCR.⁷