Synthesis and Anti-Influenza Virus Activities of a Novel Class of Gastrodin Derivatives

Si-Tu Xue, Wei-Ying He, Lin-Lin Ma, Hui-Qiang Wang, Bo Wang, Guang-Hui Zheng, Xing-Yue Ji, Tian Zhang, Yu-Huan Li *, Jian-Dong Jiang and Zhuo-Rong Li *

Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China

* Authors to whom correspondence should be addressed; E-Mails: yuhuanlibj@126.com (Y.-H.L.); l-z-r@263.net (Z.-R.L.); Tel.: +86-10-6301-0984 (Y.-H.L.); Fax: +86-10-6301-7302 (Y.-H.L.); Tel.: +86-10-6302-7185 (Z.-R.L.).

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Abstract: A series of substituted aryl glycoside analogues of gastrodin have been identified as potential anti-influenza agents. The most potent inhibitor 1a exhibited moderate inhibitory activity against the A/Hanfang/359/95(H3N2) and A/FM/1/47(H1N1) strains of the influenza A virus (IC₅₀ values of 44.40 and 34.45 μM, respectively) and the oseltamivir-null B/Jifang/13/97 strain of influenza B (IC₅₀ value of 33.01 μM). In this article, multiple doses of compound 1a (80 mg/kg/day, oral administration) were used for the treatment of mice infected with influenza A/FM/1/47-MA (H1N1), and surprisingly we found that compound 1a significantly increased the number of survivors and prolonged the mean survival time. The preliminary studies on the mechanism of antiviral activity showed no interaction between compound 1a and the neuraminidase or the M2 protein. The novel target to overcome drug resistance combined with its good in vivo profile support compound 1a to be a new lead for further development of antiviral agents.

Keywords: gastrodin; aryl glycoside; synthesis; influenza virus inhibitors

1. Introduction

Influenza is a respiratory disease caused by the influenza virus. According to the World Health Organization (WHO), as of April 4, 2010, the 2009-H1N1 influenza A virus have spread to over 213
countries and resulted in over 600,000 laboratory-confirmed cases and at least 17,700 deaths (WHO, 2010). These numbers represent the tip of the iceberg with regard to the potential morbidity and mortality associated with infection resulting from this virus [1].

Despite the extensive effort has been invested in attempting to control influenza infection, only two classes of drugs are currently clinically available, namely inhibitors of the M2 protein (e.g., amantadine and rimantadine) and the neuraminidase (e.g., zanamivir and oseltamivir) (Figure 1). Clinical applications of amantadine and rimantadine have been limited as a consequence of the increasing incidence of adamantane-resistant viruses in the general population [2,3]. Moreover, blockers of the M2 ion channel inhibit only the replication of influenza A virus and could cause neurological side effects [4]. The neuraminidase inhibitors, zanamivir and oseltamivir were marketed in 1999 for the treatment and prophylaxis of influenza and have been subsequently stockpiled by many countries for usage in the event of a pandemic. However, significant increases in the frequency of oseltamivir-resistant seasonal influenza A (H1N1) in Europe, the United States, Oceania and South Africa have been identified recently. By April 30, 2010, a total of 285 cases of resistance had been reported worldwide [5], and the widespread emergence of oseltamivir-resistant strains had become increasingly likely [6]. In addition to mutation of H275, an S274N neuraminidase mutation has also been reported [7]. The recent pandemic of influenza A (H1N1) effectively reemphasizes the importance of identifying new anti-influenza drugs.

Figure 1. The chemical structures of some anti-influenza drugs and gastrodin.

Gastrodia elata Blume, which is an orchid plant, is a well-known Traditional Chinese Medicine and has been clinically used for about 2,000 years. Its medicinal part is the dry tuber, and it is widely believed that as a traditional medicine Gastrodia elata Blume has anti-epileptic, anti-palpitation, anti-rheumatic, tranquil and nourishing effect. Gastrodin, which was isolated and identified from the extracts of Gastrodia elata Blume, has been widely used in the clinic in China for the treatment of hypertension, headache and neuralgia [8]. To the best of our knowledge, it has never been used as an antiviral agent in clinic, and there are also no reports about its possible antiviral activities.
In the current work, a series of substituted aryl glycosides were synthesized as analogues of gastrodin and their anti-influenza activity was evaluated. The compounds proved to be a novel class of potential anti-influenza agents. The most potent compound [methyl 4-fluoro-3-((2S,3R,4S,5R,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)-tetrahydro-2H-pyran-2-ylxylo) benzoate (1a)] exhibited significant inhibitory activity against the tested influenza A strains: A/Hanfang/359/95(H3N2) and A/FM/1/47(H1N1), with IC\textsubscript{50} values of 44.4 and 34.4 \(\mu\)M, respectively. Mice infected with influenza A/FM/1/47-MA (H1N1) were treated with multiple doses of compound 1a on a dose regimen of 80 mg/kg/day (oral administration), which led to a significant increase in the number of survivors and a prolonged mean survival time. Herein, we reported the synthesis and biological evaluation of a series of novel gastrodin analogues as new antiviral agents for the first time, along with a discussion of the preliminary structure-activity relationships (SAR) study and the \textit{in vivo} activity.

2. Results and Discussion

2.1. Chemistry

The chemical structures of the target analogues differed in three ways, and the modifications are as follows: (1) changes to the substituents and their positions on the phenyl ring; (2) conversion of the phenyl ring to a pyridyl ring; and (3) changes to the sugar moiety or protection of the hydroxyl group by acetylation.

As shown in the Scheme 1, compound 1a was synthesized from the reaction of methyl 4-fluoro-3-hydroxybenzoate (1) with 2,3,4,6-tetra-O-acetyl-\(\alpha\)-D-glucopyranosyl bromide using potassium carbonate (K\textsubscript{2}CO\textsubscript{3}) as the base. The color of the reaction system darkened slightly as the reaction proceeded. Unfortunately, we were unable to develop conditions for driving the reaction to completion. Consequently, significant levels of the starting material were detected in the reaction mixture, and a low yield of less than 40% was obtained. Compound 13a was obtained from 1a in a two-step procedure involving the initial reduction of the methyl ester in 1a with lithium aluminum hydride (LiAlH\textsubscript{4}) \[9\] followed by acetylation of the resulting primary alcohol with acetic anhydride. Subsequent deprotection of the acetyl groups by saponification with sodium methoxide (CH\textsubscript{3}ONa) in MeOH gave 14a \[10\] in greater than 70% overall yield from 1a (Scheme 1). Compounds 2a–5a, 7a–12a, 1b, 6b–10b were also synthesized by this route, as depicted in Scheme 1.

2.2. Evaluation of Anti-Influenzavirus Activity In Vitro

The antiviral activities of the synthesized glycoside compounds against influenza A/Hanfang/359/95(H3N2) and A/FM/1/47(H1N1) were initially evaluated through their ability to prevent cytopathic effects (CPE) in influenza A virus-infected Madin-Darby canine kidney (MDCK) cells, with oseltamivir and ribavirin (RBV) as the positive controls. Except for the known compounds, 1 (the methyl 3-hydroxy-4-fluorobenzoate starting material), 5a, 7a, 11a, 6b and gastrodin, all compounds tested in the current study were, to the best of our knowledge, synthesized and were reported for the first time in academia. Ultimately, a total of 21 compounds were evaluated, and the results were summarized in Table 1.
Scheme 1. The synthetic route to the target compounds.

Reagents and conditions: (a) K$_2$CO$_3$, anhydrous acetone, 56 °C; (b) LiAlH$_4$, THF, 66 °C, acetic anhydride, pyridine, 80 °C; (c) CH$_3$ONa/CH$_3$OH, CH$_3$OH, room temperature.

As shown in Table 1, compounds 1a, 8a, 11a and 10b exhibited moderate inhibitory activity against the A/Hanfang/359/95(H3N2) strain of the influenza virus, with IC$_{50}$ values of 44.40, 44.40, 40.52 and 48.68 μM, respectively. Nevertheless, they were not as potent as the reference drugs ribavirin (IC$_{50}$ = 11.87 μM) and oseltamivir (IC$_{50}$ = 2.08 μM). Of all the tested compounds, 2a showed the highest level of inhibitory activity against the A/FM/1/47(H1N1) strain of the influenza virus, with an IC$_{50}$ value of 11.58 μM, which was close to that of the reference drug oseltamivir (IC$_{50}$ value of 57.52 μM). Furthermore, compounds 1a, 9a, 11a and 12a also exhibited moderate levels of inhibitory activity against the A/FM/1/47(H1N1) strain of the influenza virus, with IC$_{50}$ values of 34.45, 34.45, 52.23 and
25.24 μM, respectively. Compounds 1a and 11a showed activity against both the A/Hanfang/359/95(H3N2) and A/FM/1/47(H1N1) strains of the influenza virus. Compounds 5a and 1b showed low levels of inhibitory activity against the A/Hanfang/359/95(H3N2) strain of the influenza virus, with IC₅₀ values of 93.64 and 92.38 μM, respectively. Compounds 8a and 9b showed low levels of inhibitory activity against the A/FM/1/47(H1N1) strain of the influenza virus, with IC₅₀ values of 83.72 and 66.56 μM, respectively. In contrast, the remaining compounds (including compound 1 and gastrodin) showed weak or no activity against these strains under the specific conditions used in this paper.

Table 1. Anti-influenza activities of the synthesized compounds.

| Compounds | TC₅₀ (μM) | A/Hanfang/359/95 IC₅₀ (μM) | SI | A/FM/1/47 IC₅₀ (μM) | SI |
|-----------|-----------|--------------------------|----|-------------------|----|
| 1a        | 192.14    | 44.40                    | 4.3| 34.45             | 5.6|
| 2a        | 23.65     | >13.66                   | _  | 11.58             | 2.0|
| 3a        | >362.77   | >362.77                  | _  | 362.77            | _  |
| 4a        | >402.05   | >134.02                  | _  | 119.03            | >3.4|
| 5a        | 232.53    | 93.64                    | 2.5| 134.29            | _  |
| 7a        | 277.10    | 103.39                   | 2.5| 133.23            | _  |
| 8a        | 277.10    | 44.40                    | 6.2| 83.72             | 3.3|
| 9a        | >399.66   | >44.40                   | _  | 34.45             | >11.6|
| 10a       | >438.20   | >146.07                  | _  | 146.07            | _  |
| 11a       | 226.03    | 40.52                    | 5.6| 52.23             | 4.3|
| 12a       | 350.06    | 50.12                    | _  | 25.24             | 13.9|
| 13a       | >388.76   | >129.60                  | _  | 129.60            | _  |
| 14a       | >657.31   | >657.31                  | _  | 657.31            | _  |
| 1b        | >399.66   | 92.38                    | 2.5| 133.23            | _  |
| 6b        | >414.56   | >138.19                  | _  | 138.19            | _  |
| 7b        | >399.66   | >133.22                  | _  | 133.22            | >3.0|
| 8b        | >399.66   | >399.66                  | _  | >399.66           | _  |
| 9b        | 399.66    | >133.22                  | _  | 66.56             | 6.0|
| 10b       | 252.99    | 48.68                    | 5.2| 52.23             | _  |
| 1         | >1175.50  | >1175.50                 | _  | >391.85           | _  |
| Gastrodin | >698.62   | 161.49                   | 4.3| >232.19           | _  |
| Ribavirin | 4766.99   | 11.87                    | 401.4| 13.47            | 353.8|
| Oseltamivir| 4033.29   | 2.08                     | 1938.5| 57.52            | 70.1|

a 316 TCID₅₀; b 280 TCID₅₀; Half maximal (50%) inhibitory concentration (IC) of a substance, TC₅₀ represents for median lethal dose, and SI represents for Selective therapeutic index.

The active compounds against the A/Hanfang/359/95(H3N2) and A/FM/1/47(H1N1) strains of the influenza virus were further evaluated for their potential antiviral activity against an oseltamivir-null strain of the influenza B virus, also with the means of CPE method. As summarized in Table 2, compounds 1a, 2a and 10b also displayed antiviral activity against the influenza B oseltamivir-null strain. Especially, compound 2a was found to be the most effective compound against the oseltamivir-resistant strain of the influenza virus (B/Jifang/13/97), with IC₅₀ and SI values of 6.25 μM and 6.80, respectively.
Table 2. Activities of the synthesized compounds against the oseltamivir-null influenza strain.

| Compounds  | TC<sub>50</sub> (μM) | IC<sub>50</sub> (μM) | SI |
|------------|----------------------|---------------------|----|
| 1a         | 202.75               | 33.01               | 6.14|
| 2a         | 24.63                | 6.25                | 3.94|
| 9a         | 399.66               | >44.40              | _  |
| 11a        | 235.95               | >52.23              | _  |
| 12a        | 322.63               | >50.12              | _  |
| 9b         | 360.25               | >133.22             | _  |
| 10b        | 266.16               | 39.41               | 6.80|
| Ribavirin  | 4921.94              | 11.38               | 432.35|
| Oseltamivir| 3968.32              | >100                | _  |

<sup>a</sup> 100 TCID<sub>50</sub>.

2.3. Structure-Antiviral Activity Relationships

As shown in Table 1, compounds 1a, 8a and 11a showed moderate activity against both the A/Hanfang/359/95(H3N2) and A/FM/1/47(H1N1) strains of the influenza virus, whereas compound 10b only showed moderate activity against the A/Hanfang/359/95(H3N2) strain but no activity against the A/FM/1/47(H1N1) strain. In contrast, compounds 2a, 9a and 12a only showed activity against the A/FM/1/47(H1N1) strain, but no activity against the A/Hanfang/359/95(H3N2) strain. However, all of our active compounds were less potent than the positive controls.

Relative to compound 1a, the corresponding starting material 1, which does not contain the 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl moiety, showed no activity against either of the viral strains. Additionally, when the 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl moiety was changed to the 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl moiety, the antiviral activity decreased in the majority of the derivatives and even disappeared in some cases, with only compound 10b retaining activity against the A/Hanfang/359/95(H3N2). Those results indicated that the 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl moiety was crucial to the antiviral activity of these compounds. When the fluorine substituent in compound 1a was replaced with an amino group (compound 4a), the ester group in compound 1a was replaced with an acetoxyethyl (compound 13a) or hydroxymethyl (compound 14a) group, or the ester group was migrated to the 4-position of the benzene ring (compound 7a), the antiviral activity was significantly reduced or even disappeared completely in some cases. Interestingly, when the positions of the fluorine and ester groups in compound 1a were exchanged with each other (compound 8a), the activity was retained. Taken together, it was possible that possessing hydrophobic substituents (–F, –COOMe) both at the 2- and 5-positions simultaneously enhanced the antiviral activity, yet this need to be confirmed by further experiment. Furthermore, it was also indicated that the presence of a nitrogen-containing heteroaryl ring enhances antiviral activity because compounds 11a and 12a both showed antiviral activity. In summary, this is just our preliminary SAR study, and the detailed structure optimization is ongoing in our lab.
2.4. Anti-Influenza Activity of Compound 1a in Vivo

We proceeded to investigate the effect of compound 1a on influenza A/FM/1/47-MA (H1N1) virus-infected mice, with ribavirin being used as a reference standard. Two doses of 1a (80 and 40 mg/kg/day) were administered intragastrically. The control compound ribavirin was introduced as a single dose (100 mg/kg/day, po). All experiments were administered twice daily over a seven day period.

Against infection of the influenza A/FM/1/47-MA (H1N1) virus, the ribavirin treatment group exhibited significant increases in mean survival time and moderate decreases in mean lung score. In the group of mice receiving the intragastrically administered dose of 80 mg/kg/day of 1a, the numbers of surviving animals (6/10) and the mean survival time (11.3 days) increased significantly, whereas all of the control animals died. By contrast, in the group of mice receiving the lower dosage of 40 mg/kg/day of 1a, only one animal survived (1/10), and the mean survival time was therefore not improved. Therefore, the intragastric administration of 1a provided protection against the lethal effects of the influenza A/FM/1/47-MA (H1N1) virus in a dose-dependent fashion.

The effects of 1a on lung score and on decreasing lung consolidation at day 6 are shown in Table 3. An 80 mg/kg/day dose of 1a provided a clear improvement in both the lung consolidation and mean lung score relative to that of the vehicle.

Table 3. Effects of oral treatment a with 1a on influenza A/FM/1/47-MA (H1N1) virus infections b in mice.

| Compound | Dosage (mg/kg/day) | Treated mice | Mean day to death c | Lung consolidation d | Mean lung score |
|----------|--------------------|--------------|---------------------|---------------------|----------------|
| 1a       | 80 (po)            | 60 (6/10)    | 11.3                | 2.25                | 1.82 ± 0.82    |
|          | 40 (po)            | 10 (1/10)    | 8.0                 | 3.56                | 2.61 ± 0.85    |
| Ribavirin| 100 (po)           | 100 (10/10)  | 14.0 *              | 0.50 *              | 1.31 ± 0.34 *  |
| Vehicle  | 100 (po)           | 10 (1/10)    | 9.0                 | 3.50                | 2.65 ± 1.06    |
| Normal controls | 100 (10/10) | 14.0 *       | 0.00 *              | 1.01 ± 0.12 *      |

a b.i.d × 7, beginning 1 day before viral infection; b The viral infection dose was 16.32 LD50; c Mean survival time of mice dying on or before day 14; d Lungs were removed on day 6 post-virus exposure. Lung scores were determined based on the percentage of the lung displaying signs of consolidation, with 0 being normal and 4 indicative of 100% consolidation. * p < 0.05.

2.5. The Preliminary Studies on Anti-Influenza Mechanism of Compound 1a

Because compound 1a was orally active and showed potential to be developed as antiviral drug, we researched on anti-influenza mechanism of compound 1a by neuraminidase inhibition assay, hemagglutination assay and M2 protein expression inhibition assay.

2.5.1. Hemagglutination Inhibition Assay

The early influenza virus replication steps include virus absorption, internalization of virus to form a virus-containing endosome, and fusion. Hemagglutination-inhibition test was used in which aggregation of chicken erythrocytes was the consequence of the binding between viral hemagglutinin
and sialic acid receptors on the surface of the cells [11]. At 2 fold serial diluted compound 1a (from 40 μg/mL) (Figure 2), the compound 1a did not show an inhibitory effect on hemagglutination (data not shown), suggesting that these compounds do not interfere with virus-receptor interaction.

![Figure 2. Hemagglutination inhibitory activity of 1a.](image)

### 2.5.2. Neuraminidase Inhibition Assay

Neuraminidase inhibition assay were evaluated for in vitro inhibitory actions using the method according to the reference with slight modification [12]. This spectrofluorometric assay (excitation wavelength: 355 nm and emission wavelength: 460 nm) uses 2′-(4-methylumbelliferyl)-α-D-acetylneuraminic acid (MUNANA) as the enzyme substrate, which is converted to a fluorescent product upon cleavage by NA. The intensity of fluorescence can reflect the activity of NA sensitively.

The compound 1a did not have inhibitory effect (IC$_{50}$ > 40 μg/mL) on neuraminidase of the two influenza virus A strain. Oseltamivir has a strong inhibitory effect (IC$_{50}$ = 0.02 μg/mL) on neuraminidase of influenza virus A/Wuhan/359/95.

### 2.5.3. M2 Protein Expression Inhibition Assay

The effect of compounds on M2 protein expression level were detected by immunoblotting assay [13]. The compound 1a had no effect on influenza virus M2 protein expression (Figure 3). Amantadine at concentration of 25 μg/mL significantly inhibited influenza virus M2 protein expression. Oseltamivir at concentration of 10 μg/mL has inhibitory effect on influenza virus M2 protein expression, but not so good as amantadine (25 μg/mL). Ribavirin at concentration of 10 μg/mL has significant inhibitory effect on influenza virus M2 protein expression, because at concentration of 10 μg/mL, ribavirin completely inhibits influenza virus induced cytopathic effect.

![Figure 3. The effect of 1a on M2 protein expression in MDCK cell infected with influenza virus A/Wuhan/359/95.](image)
In summary, compound 1a tested in hemagglutination inhibition assay, neuraminidase inhibition assay and M2 protein expression inhibition assay have no effect on the three antiviral target of influenza virus. Although this series novel compounds were not as potent as reference compounds, they had different mechanism against influenza virus. With these results in hand, the future research became more meaningful. Further mechanism studies are now in progress.

3. Experimental

3.1. Chemistry

1H-NMR spectra were recorded in CDCl3 on a 400 or 500 MHz Varian Inova spectrometer. Chemical shifts have been reported in parts per million (ppm) relative to tetramethylsilane (TMS) as the internal standard. The melting points were determined with an X6 microscope melting point apparatus and have been reported uncorrected. Electrospray ionization (ESI) mass spectra and high-resolution mass spectra (HRMS) were obtained on an MDS SCIEX Q-Trap mass spectrometer.

3.2. Synthesis

Methyl 4-fluoro-3-(((2S,3R,4S,5R,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)-tetrahydro-2H-pyran-2-yloxy)benzoate (1a). Methyl 4-fluoro-3-hydroxybenzoate (1, 1.7 g, 10 mmol), 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (5 g, 12 mmol) and K2CO3 (1.7 g, 12 mmol) were added to anhydrous acetone (75 mL), and the resulting mixture was stirred at 56 °C for 16 h before being cooled to ambient temperature and filtered. The solvent was then evaporated in vacuo to give a sticky residue, which was partitioned between dichloromethane (60 mL) and a 0.1 N NaOH solution (20 mL). The organic phase was collected and concentrated, and the resulting solid was purified by column chromatography on silica gel to yield compound 1a (1.65 g, 36%). White solid; m.p.: 144–146 °C; 1H-NMR (400 MHz, CDCl3) δ (ppm): 7.86 (1H, d, J = 7.6 Hz, PhH), 7.79 (1H, m, PhH), 7.14 (1H, t, J = 8.0 Hz, PhH), 5.15 (4H, m, CH), 4.28 (2H, m, CH), 3.93 (1H, d, J = 4.0 Hz, CH), 3.90 (3H, s, OCH3), 2.10 (3H, s, CH3), 2.07 (3H, s, CH3), 2.02 (6H, s, CH3); ESI-MS (m/z): 523 [M+Na]+; HRMS-ESI (m/z): calculated for C22H26FO12 [M+H]+: 501.14083; measured: 501.14078.

Ethyl 3,5-dichloro-4-(((2S,3R,4S,5R,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)-tetrahydro-2H-pyran-2-yloxy)benzoate (2a). Compound 2a was synthesized using a method similar to that described above for 1a. Yield: 35%, white solid; m.p.: 121–123 °C; 1H-NMR (400 MHz, CDCl3) δ (ppm): 6.50 (2H, s, PhH), 5.28 (3H, m, CH), 5.15 (1H, d, J = 7.2 Hz, CH), 4.22 (1H, m, CH), 4.09 (1H, d, J = 8.4 Hz, CH), 3.89 (3H, s, OCH3), 3.85 (6H, s, OCH3), 3.69 (1H, m, CH), 2.04 (3H, s, CH3), 2.01 (9H, s, CH3); ESI-MS (m/z): 565 [M+Na]+; HRMS-ESI (m/z): calculated for C24H31O14 [M+H]+: 543.17138; measured: 543.17148.

Ethyl 3,5-dichloro-4-(((2S,3R,4S,5R,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)-tetrahydro-2H-pyran-2-yloxy)benzoate (3a). Compound 3a was synthesized using a method similar to that described above for 1a. Yield: 32%, white solid; m.p.: 112–114 °C; 1H-NMR (400 MHz, CDCl3) δ (ppm): 7.93 (2H, s, PhH), 6.39 (1H, s, CH), 5.30 (3H, m, CH), 4.33 (3H, m, CH), 4.11 (2H, m, CH), 2.06 (3H, s, CH3),
Methyl 4-amino-3-((2S,3R,4S,5R,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)-tetrahydro-2H-pyran-2-yloxy)benzoate (4a). Compound 4a was synthesized using a method similar to that described above for 1a. Yield: 29%; brown solid; m.p.: 126–128 °C; 1H-NMR (400 MHz, CDCl₃) δ (ppm): 7.84 (1H, d, J = 6.0 Hz, PhH), 7.77 (1H, m, PhH), 7.10 (1H, t, J = 8.8 Hz, PhH), 5.54 (1H, t, J = 8.4 Hz, CH), 5.44 (1H, d, J = 3.2 Hz, CH), 5.14 (1H, dd, J = 8.8 Hz, J = 3.2 Hz, CH), 5.02 (1H, d, J = 8.4 Hz, CH), 4.21 (2H, m, CH), 4.05 (1H, t, J = 6.4 Hz, CH), 3.90 (3H, s, OCH₃), 3.74 (2H, s, NH₂), 2.19 (3H, s, CH₃), 2.10 (3H, s, CH₃), 2.07 (3H, s, CH₃), 2.00 (3H, s, CH₃); ESI-MS (m/z): 520 [M+Na]⁺; HRMS-ESI (m/z): calculated for C₂₂H₂₈NO₁₂ [M+H]+: 498.16115; measured: 498.16102.

Methyl 4-((2S,3R,4S,5R,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)-tetrahydro-2H-pyran-2-yloxy)benzoate (5a). Compound 5a was synthesized using a method similar to that described above for 1a. Yield: 30%; white solid; m.p.: 147–149 °C; 1H-NMR (400 MHz, CDCl₃) δ (ppm): 8.00 (2H, d, J = 8.4 Hz, PhH), 7.00 (2H, d, J = 8.4 Hz, PhH), 5.53 (1H, d, J = 7.6 Hz, CH), 5.27 (2H, m, CH), 5.14 (1H, t, J = 6.4 Hz, CH), 5.02 (1H, d, J = 7.2 Hz, CH), 4.25 (4H, m, CH), 2.08 (3H, s, CH₃), 2.05 (6H, s, CH₃), 2.04 (3H, s, CH₃), 1.38 (3H, s, CH₃); ESI-MS (m/z): 519 [M+Na]⁺; HRMS-ESI (m/z): calculated for C₂₃H₂₉O₁₂ [M+H]+: 497.16590; measured: 497.16594.

Methyl 3-fluoro-4-((2S,3R,4S,5R,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)-tetrahydro-2H-pyran-2-yloxy)benzoate (7a). Compound 7a was synthesized using a method similar to that described above for 1a. Yield: 31%; white solid; m.p.: 121–123 °C; 1H-NMR (500 MHz, CDCl₃) δ (ppm): 7.83 (1H, t, J = 6.5 Hz, PhH), 6.90 (1H, dd, J = 10.5 Hz, J = 2.5 Hz, PhH), 6.83 (1H, t, J = 8.0 Hz, PhH), 5.57 (1H, d, J = 4.0 Hz, CH), 5.21 (4H, m, CH), 4.37 (2H, m, CH), 3.90 (3H, s, OCH₃), 2.10 (6H, s, CH₃), 2.06 (6H, s, CH₃); ESI-MS (m/z): 523 [M+Na]⁺; HRMS-ESI (m/z): calculated for C₂₂H₂₆FO₁₂ [M+H]⁺: 501.14083; measured: 501.14091.

Ethyl 4-fluoro-2-((2S,3R,4S,5R,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)-tetrahydro-2H-pyran-2-yloxy)benzoate (8a). Compound 8a was synthesized using a method similar to that described above for 1a. Yield: 30%; white solid; m.p.: 136–138 °C; 1H-NMR (500 MHz, CDCl₃) δ (ppm): 7.80 (1H, t, J = 7.5 Hz, PhH), 6.95 (1H, dd, J = 8.0 Hz, J = 2.0 Hz, PhH), 6.84 (1H, m, PhH), 5.92 (1H, t, J = 9.0 Hz, CH), 5.48 (1H, d, J = 3.5 Hz, CH), 5.08 (2H, m, CH), 4.17 (3H, m, CH), 3.89 (3H, s, OCH₃), 2.16 (3H, s, CH₃), 2.09 (3H, s, CH₃), 2.05 (3H, s, CH₃), 2.01 (3H, s, CH₃); ESI-MS (m/z): 523 [M+Na]⁺; HRMS-ESI (m/z): calculated for C₂₂H₂₆FO₁₂ [M+H]⁺: 501.14083; measured: 501.14082.

Methyl 2-fluoro-6-((2S,3R,4S,5R,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)-tetrahydro-2H-pyran-2-yloxy)benzoate (9a). Compound 9a was synthesized using a method similar to that described above for 1a. Yield: 35%; white solid; m.p.: 130–132 °C; 1H-NMR (500 MHz, CDCl₃) δ (ppm): 7.31 (1H, q, J = 8.5 Hz, PhH), 6.90 (1H, d, J = 8.5 Hz, PhH), 6.86 (1H, t, J = 8.0 Hz, PhH), 5.27 (2H, m, CH), 5.18 (1H, t, J = 6.5 Hz, CH), 5.05 (1H, d, J = 7.5 Hz, CH), 4.25 (3H, m, CH), 3.89 (3H, s, OCH₃), 2.11 (3H, s, CH₃), 2.08 (3H, s, CH₃), 2.05 (3H, s, CH₃), 2.02 (3H, s, CH₃); ESI-MS (m/z): 523 [M+Na]⁺; HRMS-ESI (m/z): calculated for C₂₂H₂₆FO₁₂ [M+H]⁺: 501.14083; measured: 501.14077.
(2R,3R,4S,5R,6S)-2-(Acetoxymethyl)-6-(3-fluoro-2-methylphenoxy)-tetrahydro-2H-pyran-3,4,5-triyl triacetate (10a). Compound 10a was synthesized using a method similar to that described above for 1a. Yield: 33%; white solid; m.p.: 127–129 °C; 1H-NMR (500 MHz, CDCl3) δ (ppm): 7.62 (1H, m, PhH), 7.21 (1H, m, PhH), 6.90 (1H, dd, J = 9.5 Hz, J = 3.5 Hz, PhH), 6.33 (1H, d, J = 3.5 Hz, CH), 5.72 (1H, d, J = 8.0 Hz, CH), 5.50 (2H, m, CH), 4.57 (1H, t, J = 7.0 Hz, CH), 4.22 (2H, m, CH), 2.40 (3H, s, CH3), 2.07 (6H, s, CH3), 2.02 (6H, s, CH3); ESI-MS (m/z): 479 [M+Na]+; HRMS-ESI (m/z): calculated for C21H26FO10 [M+H]+: 457.15100; measured: 457.15095.

(2R,3R,4S,5R,6S)-2-(Acetoxymethyl)-6-(pyridin-4-yloxy)-tetrahydro-2H-pyran-3,4,5-triyl triacetate (11a). Compound 11a was synthesized using a method similar to that described above for 1a. Yield: 27%; colorless solid; m.p.: 132–134 °C; 1H-NMR (500 MHz, CDCl3) δ (ppm): 8.30 (2H, d, J = 9.0 Hz, PhH), 6.93 (2H, d, J = 9.0 Hz, PhH), 5.50 (1H, dd, J = 10.5 Hz, J = 3.0 Hz, CH), 5.18 (1H, d, J = 3.0 Hz, CH), 5.05 (1H, t, J = 7.0 Hz, CH), 4.15 (4H, m, CH), 2.08 (3H, s, CH3), 2.04 (3H, s, CH3), 2.02 (6H, s, CH3); ESI-MS (m/z): 448 [M+Na]+; HRMS-ESI (m/z): calculated for C19H24NO10 [M+H]+: 426.14002; measured: 426.14006.

(2R,3R,4S,5R,6S)-2-(Acetoxymethyl)-6-(3-fluoropyridin-2-yloxy)-tetrahydro-2H-pyran-3,4,5-triyl triacetate (12a). Compound 12a was synthesized using a method similar to that described above for 1a. Yield: 31%; pale yellow solid; m.p.: 140–142 °C; 1H-NMR (500 MHz, CDCl3) δ (ppm): 7.92 (1H, d, J = 5.0 Hz, PhH), 7.39 (1H, t, J = 8.5 Hz, PhH), 6.99 (1H, m, PhH), 6.18 (1H, d, J = 7.5 Hz, CH), 5.35 (2H, m, CH), 5.24 (1H, t, J = 9.5 Hz, CH), 4.30 (1H, dd, J = 12.5 Hz, J = 4.5 Hz, CH), 4.14 (1H, d, J = 12.5 Hz, CH), 3.94 (1H, m, CH), 2.10 (3H, s, CH3), 2.07 (3H, s, CH3), 2.04 (3H, s, CH3), 2.02 (3H, s, CH3); ESI-MS (m/z): 466 [M+Na]+; HRMS-ESI (m/z): calculated for C19H23FNO10 [M+H]+: 444.13060; measured: 444.13051.

(2R,3R,4S,5R,6S)-2-(Acetoxymethyl)-6-(5-(acetoxymethyl)-2-fluorophenoxy)-tetrahydro-2H-pyran-3,4,5-triyl triacetate (13a). Compound 13a (500 mg, 1 mmol) was added to THF (25 mL), and solid LiAlH4 was added in a portion-wise manner to the resulting solution. Upon completion of the addition, the mixture was stirred at 66 °C for 3 h before being cooled to ambient temperature and quenched by the dropwise addition of CH3OH (10 mL). The reaction mixture was then stirred at room temperature for 10 min and concentrated to dryness under vacuum. The resulting residue was then dissolved in a mixture of acetic anhydride (20 mL) and pyridine (1 mL). The reaction mixture was stirred at 80 °C for 4 h and then concentrated to dryness in vacuo. The residue was added to ice water and stirred until precipitation occurred. The mixture was then filtered, and the filter-cake was collected and purified by flash chromatography over silica gel, affording the title compound (401 mg, 78%). White solid; m.p.: 125–127 °C; 1H-NMR (500 MHz, CDCl3) δ (ppm): 7.80 (1H, d, J = 8.0 Hz, PhH), 7.74 (1H, m, PhH), 7.10 (1H, t, J = 8.5 Hz, PhH), 5.89 (1H, d, J = 6.5 Hz, CH), 5.30 (1H, dd, J = 10.5, 4.0 Hz, CH), 5.07 (1H, t, J = 11.0 Hz, CH), 5.19 (2H, s, CH), 4.36 (1H, m, CH), 4.14 (3H, m, CH), 2.11 (3H, s, CH3), 2.08 (6H, s, CH3), 2.06 (3H, s, CH3), 2.04 (3H, s, CH3); ESI-MS (m/z): 537 [M+Na]+; HRMS-ESI (m/z): calculated for C23H28FO12 [M+H]+: 515.15648; measured: 515.15652.

(2S,3R,4S,5S,6R)-2-(2-Fluoro-5-(hydroxymethyl)phenoxy)-6-(hydroxymethyl)-tetrahydro-2H-pyran-3,4,5-triol (14a). To a solution of 14a (510 mg, 1 mmol) in CH3OH (10 mL) was added a 0.2 N


solution of CH₃ONa in CH₃OH, and the resulting mixture was stirred at room temperature for 1 h and then allowed to stand at ambient temperature overnight. The reaction mixture was filtered, and the filter cake was collected and recrystallized from anhydrous ethanol to afford the title compound (280 mg, 90%). White solid; m.p.: 134–136 °C; ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 7.15 (1H, t, J = 9.5 Hz, PhH), 6.95 (1H, dd, J = 9.5 Hz, J = 4.0 Hz, PhH), 6.91 (1H, m, PhH), 5.60 (1H, s, CH), 4.66 (2H, s, CH), 4.20 (1H, s, CH), 3.47 (5H, m, CH), 2.76 (1H, s, OH), 2.16 (1H, s, OH), 1.78 (2H, s, OH), 0.99 (1H, s, OH); ESI-MS (m/z): 327 [M+Na]^+; HRMS-ESI (m/z): calculated for C₁₃H₁₈FO₇ [M+H]^+: 305.10366; measured: 305.10362.

Methyl 4-fluoro-3-((2S,3R,4S,5S,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)-tetrahydro-2H-pyran-2-yloxy)benzoate (1b). Compound 1b was synthesized using a method similar to that described above for 1a. Yield: 38%; white solid; m.p.: 143–145 °C; ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 7.88 (1H, d, J = 6.5 Hz, PhH), 7.78 (1H, m, PhH), 7.14 (1H, t, J = 9.0 Hz, PhH), 5.53 (1H, t, J = 8.5 Hz, CH), 5.46 (1H, d, J = 3.0 Hz, CH), 5.11 (1H, dd, J = 10.0, 3.0 Hz, CH), 5.01 (1H, d, J = 8.0 Hz, CH), 4.20 (2H, m, CH), 4.07 (1H, t, J = 6.5 Hz, CH), 3.90 (3H, s, OCH₃), 2.19 (3H, s, CH₃), 2.10 (3H, s, CH₃), 2.07 (3H, s, CH₃); ESI-MS (m/z): 523 [M+Na]^+; HRMS-ESI (m/z): calculated for C₂₂H₂₆FO₁₂ [M+H]^+: 501.14083; measured: 501.14086.

Methyl 3-((2S,3R,4S,5S,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)-tetrahydro-2H-pyran-2-yloxy)benzoate (6b). Compound 6b was synthesized using a method similar to that described above for 1a. Yield: 36%; white solid; m.p.: 154–156 °C; ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 7.75 (1H, d, J = 8.0 Hz, PhH), 7.67 (1H, s, PhH), 7.37 (1H, t, J = 8.0 Hz, PhH), 7.20 (1H, dd, J = 8.0, 2.5 Hz, CH), 5.50 (2H, m, CH), 5.12 (2H, m, CH), 4.16 (3H, m, CH), 3.91 (3H, s, OCH₃), 2.19 (3H, s, CH₃), 2.07 (6H, s, CH₃), 2.00 (3H, s, CH₃); ESI-MS (m/z): 505 [M+Na]^+; HRMS-ESI (m/z): calculated for C₂₂H₂₇O₁₂ [M+H]^+: 483.15025; measured: 483.15030.

Methyl 3-fluoro-4-((2S,3R,4S,5S,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)-tetrahydro-2H-pyran-2-yloxy)benzoate (7b). Compound 7b was synthesized using a method similar to that described above for 1a. Yield: 32%; white solid; m.p.: 121–123 °C; ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 7.77 (2H, m, PhH), 7.21 (1H, t, J = 8.5 Hz, PhH), 5.52 (1H, t, J = 8.0 Hz, CH), 5.45 (1H, d, J = 3.0 Hz, CH), 5.11 (1H, dd, J = 10.5, 3.0 Hz, CH), 5.01 (1H, d, J = 7.5 Hz, CH), 4.20 (2H, m, CH), 4.05 (1H, m, CH), 3.90 (3H, s, OCH₃), 2.15 (3H, s, CH₃), 2.08 (3H, s, CH₃), 2.03 (3H, s, CH₃), 1.99 (3H, s, CH₃); ESI-MS (m/z): 523 [M+Na]^+; HRMS-ESI (m/z): calculated for C₂₂H₂₆FO₁₂ [M+H]^+: 501.14076; measured: 501.14074.
Methyl 2-fluoro-6-((2S,3R,4S,5S,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)-tetrahydro-2H-pyran-2-yloxy)benzoate (9b). Compound 9b was synthesized using a method similar to that described above for 1a. Yield: 35%; white solid; m.p.: 130–132 °C; $^1$H-NMR (500 MHz, CDCl$_3$) $\delta$ (ppm): 7.31 (1H, q, $J = 8.5$ Hz, PhH), 6.92 (1H, d, $J = 8.0$ Hz, PhH), 6.84 (1H, t, $J = 8.5$ Hz, PhH), 5.47 (2H, m, CH), 5.07 (1H, dd, $J = 10.5$ Hz, $J = 3.0$ Hz, CH), 5.00 (1H, d, $J = 8.0$ Hz, CH), 4.16 (3H, m, CH), 3.90 (3H, s, OCH$_3$), 2.21 (3H, s, CH$_3$), 2.12 (3H, s, CH$_3$), 2.06 (3H, s, CH$_3$), 2.03 (3H, s, CH$_3$); ESI-MS ($m/z$): 523 [M+Na$^+$]; HRMS-ESI ($m/z$): calculated for C$_{22}$H$_{26}$FO$_{12}$ [M+H$^+$]: 501.14083; measured: 501.14077.

(2R,3S,4S,5R,6S)-2-(Acetoxymethyl)-6-(3-fluoro-2-methylphenoxy)-tetrahydro-2H-pyran-3,4,5-triyyl triacetate (10b). Compound 10b was synthesized using a method similar to that described above for 1a. Yield: 36%; white solid; m.p.: 127–129 °C; $^1$H-NMR (500 MHz, CDCl$_3$) $\delta$ (ppm): 7.60 (1H, m, PhH), 7.20 (1H, m, PhH), 6.93 (1H, dd, $J = 9.5$, 3.5 Hz, PhH), 6.32 (1H, d, $J = 3.5$ Hz, CH), 5.74 (1H, d, $J = 8.0$ Hz, CH), 5.50 (2H, m, CH), 4.54 (1H, t, $J = 7.0$ Hz, CH), 4.24 (2H, m, CH), 2.40 (3H, s, CH$_3$), 2.09 (3H, s, CH$_3$), 2.07 (3H, s, CH$_3$), 2.04 (3H, s, CH$_3$), 2.02 (3H, s, CH$_3$); ESI-MS ($m/z$): 479 [M+Na$^+$]; HRMS-ESI ($m/z$): calculated for C$_{21}$H$_{26}$FO$_{10}$ [M+H$^+$]: 457.15100; measured: 457.15098.

3.3. Antiviral Assays

MDCK cells, influenza A (A/Hanfang/359/95, H3N2), influenza A (A/FM/1/47, H1N1) and influenza B (B/Jifang/13/97) were obtained from the Institute of Virology of the Chinese Academy of Preventive Medicine (Beijing, China).

3.3.1. Cytotoxicity Assay

The cytotoxicities of the compounds in the presence of MDCK cells were monitored by CPE. MDCK cells (2.5 × 10$^4$/well) were plated into a 96-well plate. After 24 h, the monolayer cells were incubated in the presence of a variety of different concentrations of the test compounds. After 48 h of culture at 37 °C and under a 5% CO$_2$ atmosphere in a carbon dioxide incubator, the cells were monitored by CPE. The median toxic concentrations (TC$_{50}$) were determined using a Reed and Muench calculation.

3.3.2. Anti-Influenza Assays

Confluent MDCK cells grown in 96-well microplates were infected with 100 median tissue culture infective dose units (100TCID$_{50}$) of the test strains. Following 2 h of adsorption at 37 °C, the monolayers were washed with phosphate-buffered saline (PBS) and incubated at 37 °C in the maintenance medium either in the presence or in the absence of different concentrations of the test compounds. The viral CPE was observed when the viral control group reached 4, and the antiviral activities (IC$_{50}$) of the bisaryl amide derivatives were determined using a Reed and Muench calculation. The SI value was calculated from TC$_{50}$/IC$_{50}$.
3.4. Antiviral Assays in Vivo

The effect of compound 1a on influenza A/FM/1/47-MA (H1N1) virus-infected mice was evaluated during the course of the current study, with ribavirin being used as a reference standard. Mice (female, 5–6 weeks of age and 14–16 grams in weight) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

Ether-anesthetized mice were infected intranasally with a 50 times LD$_{50}$ dose of mouse-adapted influenza A/FM/1/47-MA (H1N1) virus. Compound 1a, prepared as a suspension in 5% tween-80, or 5% tween-80 alone (vehicle group), was administered intragastrically twice daily for seven days, with the dose regimen being initiated one day prior to viral infection. The 10 mice involved in each group were observed twice daily for 14 days during the experiment for the occurrence of death. The mean day-to-death and survival times were then calculated (Table 3).

In a separate study, all eight of the mice involved in each group were killed when deaths began to occur in the virus control group (day 6 post-virus exposure), and their lungs were removed aseptically. Visual evidence of lung consolidation was scored on a blind basis. Lung scores were based on the percentage of the lung displaying signs of consolidation, with 0 being normal and 4 being indicative of 100% consolidation. Lung consolidation scores were evaluated using ranked sum analysis (Table 3).

3.5. Mechanism Studies

3.5.1. Hemagglutination Inhibition Assay

Hemagglutination inhibition (HI) assay was employed to evaluate the effect of a compound on virus adsorption to target cells according to the reference with slight modification. Briefly, compound in PBS was mixed with an equal volume of influenza virus that was serially diluted by two-fold in PBS. After 30 min incubation at 4 °C, 100 µL of compound-virus preparation was mixed with an equal volume of 1% chicken erythrocyte suspension in PBS in a U bottom 96-well plate. The mixture was incubated for 1 h at room temperature before observing erythrocyte aggregation on the plate.

3.5.2. Neuraminidase Inhibition Assay

Neuraminidase inhibition assay were evaluated for \emph{in vitro} inhibitory actions using the method according to the reference with slight modification. This spectrofluorometric assay (excitation wavelength: 355 nm and emission wavelength: 460 nm) uses 2'-(4-methylumbelliferyl)-α-D-acetylneuraminic acid (MUNANA) as the enzyme substrate, which is converted to a fluorescent product upon cleavage by NA. The intensity of fluorescence can reflect the activity of NA sensitively.

The enzyme reaction system included 10 µL sample, 30 µL enzyme and 60 µL substrate buffer mix (30 µM MUNAN, 33 mM MES buffer (pH 3.5), 4 mM CaCl$_2$, DDW). The terminal volume was 100 µL. Two-fold serially diluted compound samples and 30 µL enzyme incubated 1 hour at room temperature, and 60 µL substrate buffer mix was added. After 15 min at 37 °C, 100 µL of 14 mM NaOH in 83% ethanol was added to the reaction mixture to terminate the reaction. The intensity of the fluorescence was quantitated in fluorescence read on Enspire (Perkin Elmer, Waltham, MA, USA) (excitation, 355 nm; emission, 460 nm), and substrate blanks were subtracted from the sample readings. The IC$_{50}$
was calculated by plotting percent inhibition versus the inhibitor concentration, and determination of each point was performed in duplicate.

3.5.3. M2 Protein Expression Inhibition Assay

The effect of compounds on M2 protein expression level were detected by immunoblotting assay. The immunoblotting experiment was employed according to the reference with slight modification. MDCK cells were infected with influenza virus A/Wuhan/359/95 at an 300TCID$_{50}$, and treated with the indicated concentration of compounds. 24 hours post infection, the cells were lysed with lysis buffer (1% Triton, 150 mM NaCl, 20 mM HEPES pH7.5, 10% Glycerol, 1 mM EDTA) with protease inhibitor cocktail (Roche, Indianapolis, IN, USA). The lysates were then performed to immunoblotting with M2 and β-actin antibodies (Influenza A m2 Antibody from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA) and β-Actin Mouse mAb from Cell Signaling Technology Inc. (Danvers, MA, USA). Protein was detected using Immobilon Western Chemiluminescent HRP Substrate (Millipore, Inc., Billerica, MA, USA) with ChemiDoc XRS and Image Acquisition (BioRad, Inc., Hercules, CA, USA). Most studies of the activity and drug sensitivity of A/M2 channels have employed electrophysiology techniques, but since establishment of this technology takes time, we just detected M2 protein expression levels.

4. Conclusions

Gastrodin is a traditional Chinese herbal medicine that has been used clinically in China for thousands of years, mainly for the treatment of neurasthenia. To the best of our knowledge, there have been no reports documenting the use of gastrodin or any of its derivatives as antiviral agents. In our study, a novel series of gastrodin analogues was found to exhibit potent anti-influenza activity. A series of substituted aryl glycoside compounds were synthesized and evaluated for their anti-influenza virus activities, and preliminary structure-activity relationships (SAR) were analyzed.

A total of 15 novel glycoside compounds were synthesized and screened for their antiviral activity against influenza A virus, among which the most potent inhibitor 1a exhibited moderate inhibitory activity against the virus. Herein, we also report the first total synthesis of 1a and its associated in vivo anti-influenza data. In this experiment, a dose regimen of 80 mg/kg/day of compound 1a (oral administration) yielded a significant increase in the survival numbers and prolonged the mean survival time of the animals tested.

Furthermore, the data were analyzed to produce a preliminary understanding of the structure-activity relationships. Seven compounds exhibited clear antiviral activity against the tested strain of the influenza A virus. The IC$_{50}$ and SI values of the most effective compound, 12a, against the influenza A (A/FM/1/47(H1N1)) virus were 25.24 μM and 13.9, respectively. Unfortunately, the SI values for all the compounds tested were much lower than those of the reference compounds. Remarkably, compounds 1a, 2a and 10b also displayed antiviral activity against the oseltamivir-null strain of influenza B. In addition, there was an increasing need for the development of antiviral along with novel antiviral mechanism. Compound 1a had a different mechanism of action from oseltamivir and ribavirin against influenza virus, so future research may be more meaningful with this result.
Undoubtedly, further exploration will be needed to produce more potent compounds with higher SI values and to deduce the detailed mechanism of action of this novel class of glycosides compounds against the influenza virus. Besides, since compound 1a has been demonstrated that it has no interaction with NA or M2, it probably has novel targets which might be an important breakthrough in viral study.

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*Sample Availability*: Samples of the compounds 1a–5a, 7a–14a, 1b and 6b–10b are available from the authors.

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