Multigram Scale Synthesis and Anti-Influenza Activity of 3-Indoleacetonitrile Glucosides

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Abstract
Indole-3-acetonitrile-6-O-β-D-glucopyranoside 1 is a simple alkaloid with anti-influenza A virus activity extracted from Radix isatidis. Herein, a concise and practical total synthetic route of 1 was presented, starting from 6-benzyloxyindole on a multigram scale. The pivotal reaction sequence involved the Mannich reaction, the fluoride ion-induced elimination-addition reaction, and phase-transfer-catalyzed glycosylation reaction as key steps. In addition, compounds 1 and two modified derivatives 8 and 16 were tested for anti-influenza efficacy and cytotoxicity in vitro performed on Madin-Darby canine kidney cells. The results revealed that the compounds exerted antiviral activity against the influenza A virus to a certain extent and displayed no cytotoxicity. These findings could contribute to the development of traditional Chinese medicine R. isatidis for treating the influenza virus.

Keywords
radix isatidis, 3-indoleacetonitrile, synthesis, anti-influenza activity, cytotoxicity

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“Ban langen” (Radix isatidis) is an official crude drug being recorded in Chinese Pharmacopoeia for many times.¹ It has been also used in combination with other botanicals in traditional Chinese medicine and Ayurveda for the treatment of various diseases since ancient times, especially for preventing and treating influenza.² The chemical and biological diversity has been widely investigated and assessed with ethanol extracts or water decoctions of the roots and leaves of Isatis indigotica.³,⁴ It contains various constituents with different structural features, such as alkaloids, lignans, indirubin, clemastin B, ceramides, flavonoids, epigallocatechin, and polysaccharides, etc.⁵-⁷ Meanwhile, the extracts of R. isatidis display extensive biological activity, for instance, antiviral,⁸,⁹ anti-inflammatory,¹⁰ antipyretic,¹¹ neuroprotective,¹² anti-infective,¹³ and anti-diabetic activity.¹⁴ However, it is difficult to fully elucidate which chemical component causes the corresponding pharmacological efficacy in the use of traditional Chinese medicine. The diversity of the chemical constituents from R. isatidis and the ambiguity of real effective ingredients provide a good opportunity for further exploration.

The indole scaffold, as a privileged structure, is considered as one of the most promising heterocycles in drug discovery and embedded within the backbones of numerous pharmaceuticals and biologically important natural alkaloids.¹⁷ Indole derivatives possess versatile biological activities, including antiviral, anti-inflammatory, antibacterial, antimalarial, anticonvulsant, and anticancer, etc.¹⁸-⁲¹ Due to their impressive natural structures and significant biological profiles, indole derivatives constantly call for the development of methods for their innovative synthetic strategies. Since the first synthesis of indole was completed by Adolf von Baeyer in 1866,²² many efficient synthetic approaches have been successfully discovered and applied for the construction of indole and its complex derivatives, such as classical name reactions represented by Fischer indole synthesis,²³ organic electrosynthesis,²⁴ catalytic asymmetric dearomatization reactions,²⁵ organocatalytic asymmetric synthesis,²⁶ transition-metal-catalyzed C-H functionalization,²⁷ aerobic oxidative functionalization,²⁸ and photoredox catalysis.²⁹ Nevertheless, novel strategies for the synthesis of indole derivatives are still urgently needed.

In order to clarify the antiviral active constituents of R. isatidis, numerous researchers had isolated single compound and conducted its bioactivity assay by the extracts of R. isatidis (organic solvents or water).⁷,⁰,³⁰,³² Various indole alkaloids were isolated

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from *R. isatidis* and considered as the main active constituents in free and glycosidic forms. Among them, indole-3-acetonitrile-6-O-β-D-glucopyranoside 1 was an alkaloid with a relatively simple skeleton. Recent studies demonstrated that the single compound could inhibit influenza A virus (H3N2) activity in vitro and significantly cure lung tissue lesions and pneumonia induced by influenza virus in vivo. Meanwhile, this compound also exerted remarkable inhibitory activity on lipopolysaccharide-induced nitric oxide production in macrophages with half-maximal inhibitory concentration (IC50) of 5.87 µM to reduce the development of inflammation.

At present, it is time-consuming, laborious, and inefficient to extract enough amount of this indole alkaloid 1 from crude indigowoad root. The scarcity of material has limited further pharmacological activity evaluation and, more generally, has hampered efforts to obtain potentially superior derivatives. Therefore, in this study, we designed and accomplished a novel synthetic route to meet the supply of this alkaloid for further bioactivity assay and mechanism exploration.

**Results and Discussion**

Our retrosynthetic analysis is illustrated in Figure 1. We envisioned the key glycosylation formation via nucleophilic attack of 6-hydroxyindole-3-acetonitrile 3 to a glycosylating reagent 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide 2 to acquire the target molecule 1 after hydrolysis of the glycosylated product. Compound 3 could be achieved from the deprotection of the benzyl group via Pd-catalyzed hydrogenation. The required precursor 4 should be ingeniously obtained from the nucleophilic displacement of the N,N-dimethylamino group of Mannich base 5 via the fluoride ion-induced elimination-addition reaction. Compound 5 might be easily accessible from commercially available 6-benzyloxyindole 6, formaldehyde, and dimethylamine via Mannich reaction.

Preparation of the target compound 1 commenced with the synthesis of the crucial intermediate 3 from commercially available 6-benzyloxyindole 6 as shown in Figure 2. Initial step to introduce the C-3 side chain of 6-benzyloxyindole involved a
A sequence of a Mannich reaction, quaternization of the Mannich base, substitution of the trimethylamine moiety with a cyanide source to yield the cyanomethyl group. Several conditions (Table 1) were tried to optimize this transformation.

Initial scouting of cyanomethylation was carried out by using sodium cyanide or potassium cyanide as a cyanide source by examining an array of conditions reported in the literature (Table 1, entry 1 and 2). Unfortunately, only 65%-70% yield of the product was obtained, and the common cyanides were highly toxic to exhustedly manipulate. Therefore, trimethylsilyl cyanide (TMS-CN), which was of lower toxicity and easy to handle, turned out to be a better cyanide source affording 4 in 65% yield; the yield was not significantly improved with the prolongation of time (Table 1, entry 3). After screening several solvents (Table 1, entry 3-6), tetrahydrofuran (THF) was found to be the most efficient, and other solvents afforded low yields with the incomplete conversion. To our delight, we found that the yield increased to 92% with elongated reaction time (Table 1, entry 7). Replacing tetrabutylammonium fluoride (TBAF) with other fluorides such as potassium fluoride (KF) or sodium fluoride (NaF) did not improve the reaction (Table 1, entry 8). Consequently, the optimum reaction conditions were determined to be stirring quaternary ammonium salt in the presence of TMS-CN/(n-Bu)4NF in THF at room temperature for 2 hours. Most significantly, the reaction could be reliably scaled up to multigram scales. Namely, aminomethylation derivative 6 was obtained by treatment 7 with dimethylamine/aqueous formaldehyde in 1,4-dioxane at 25 °C in high yield without further purification. The resulting Mannich base was reacted with methyl iodide in dichloromethane (CH2Cl2)/toluene and subsequently subjected to nucleophilic substitution using TMS-CN/(n-Bu)4NF to afford the nitrile 4. Compound 4 was selectively reductive removal of O-benzyl to provide the 6-hydroxyindole-3-acetonitrile 3 using palladium on carbon (Pd/C) as a catalyst.

It was another difficulty for us to perform the crucial glycosylation reaction, although various methods were developed for the construction of O-linked glucosides. At first, Koenigs-Knorr glycosidation reaction was conducted by treating the commercially available glycosylating reagent tetra-O-acetyl-a-d-glucopyranosyl bromide 2 with phenol 3 in the presence of silver (I) oxide, but it did not work well (Table 2, entry 1). Under the reaction conditions using lithium hydroxide (LiOH)/dimethylformamide (DMF) system reported by Somei and co-workers, SN2 reaction took place in 71% yield (Table 2, entry 2). Finally, the phase-transfer catalyzed glycosylation was practical and efficient. Namely, glucoside 1 was prepared by the phase-transfer-catalyzed (benzyltributylammonium chloride) glycosylation of 3 with 2,3,4,6-tetra-O-acetyl-a-d-glucopyranosyl bromide 2 in a biphasic system (chloroform [CHCl3]/potassium carbonate [K2CO3] solution), and subsequently subjected to hydrolysis using LiOH in THF/water (H2O) (Table 2, entry 4 and 5).

Having established an efficient method to synthesize the 3-indoleacetonitrile glucoside as described above, we also had access to the 3-indoleacetonitrile 4-position derivative (Figure 3, Cappariloside A), with an overall yield of 49.2% versus 41% previously reported by Masanori Somei. The pharmacological activity of capparilloside A lay in broad-spectrum antiviral efficacy and impairing the upregulations of proinflammatory factors in host cells induced by the influenza virus.

The synthesized compounds 1, 8, and 16 were selectively evaluated for the anti-influenza virus effects against 2 influenza virus subtypes, namely, A/HK/68 (H3N2) and A/PR/8/34. TheTable 2. Optimizing the Condition of Preparation of O-Linked Glucoside 8.

| Entry | Condition | Time (h) | Temperature (°C) | Yield (%) |
|-------|-----------|----------|------------------|-----------|
| 1     | Ag2O, CH3CN | 6        | 35               | 58        |
| 3     | LiOH, DMF  | 4        | 25               | 71        |
| 4     | K2CO3/THAB/CHCl3/H2O | 48      | 25               | 78        |
| 5     | K2CO3/THAB/CHCl3/H2O | 48      | 25               | 89        |

Abbreviations: Ag2O, silver (I) oxide; CHCl3, chloroform; CH3CN, acetoneitrile; DMF, dimethylformamide; H2O, water; K2CO3, potassium carbonate; LiOH, lithium hydroxide; TBAB, tetrabutylammonium bromide.
(H1N1), on Madin-Darby canine kidney (MDCK) cells utilizing the cytopathic effect (CPE) assay. And cytotoxic effects were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In addition, oseltamivir carboxylate was used as a positive control in parallel. The inhibitory results were expressed as a half-maximal effective concentration (EC50) and half-maximal toxic concentration (TC50) summarized in Table 3.

| Compound | EC50 (µg/mL) | TC50 (µg/mL) |
|----------|--------------|--------------|
| 1         | 106.9 ± 6.1  | 123.8 ± 12.2 |
| 8         |              |              |

Compound 1 possessed inhibitory activity against H3N2 and H1N1 with an EC50 value of 106.9 ± 6.1 µg/mL and 123.8 ± 12.2 µg/mL, respectively. Compared with acetylated product 8, it seemed that the free sugar ring was of importance to maintain anti-influenza activity. Meanwhile, N-arylindole derivative 16 exhibited weaker antiviral efficiency. It should be noted that the 3 compounds also possessed no cytotoxicity toward the MDCK cells.

Conclusions

In conclusion, we accomplished a facile and efficient synthesis of indole-3-acetonitrile-6-O-β-d-glucopyranoside 1 in 5 steps with 56.5% overall yield from the readily available 6-benzyloxyindole. And the phenyl-substituted analog 16 was obtained via an one-step Chan-Lam-Evans coupling reaction. Moreover, compounds 1, 8, and 16 were evaluated for anti-influenza activity and their cytotoxicity in vitro employing the CPE and MTT assay, respectively. The results indicated that simple structural modification of compound 1 could not increase antiviral effectiveness, for example, through acetylation of the glucose unit or N-arylation of the indole-3-acetonitrile moiety. At last, the tested compounds showed no cytotoxicity. This study had laid a solid foundation for further access to various 3-indoleacetonitrile glycosides and structure-activity relationship research.

Experimental

General

The chemicals and all solvents used were of analytical grade without further purification unless otherwise noted. Reactions were monitored by thin-layer chromatography using Qing Dao Hai Yang GF254 silica gel plates (5 × 10 cm); zones were detected visually under ultraviolet (UV) irradiation (254 nm). 1H NMR spectra and 13C NMR were recorded on a Bruker NMR Avance 400 (400 MHz) or a Bruker NMR Avance 500 (500 MHz), using deuterated chloroform (CDCl3) or dimethylsulfoxide.
Table 3. Anti-Influenza Virus Efficacy of the Compounds 1, 8, and 16 In Vitro.

| Compound | Antiviral EC<sub>50</sub><sup>a</sup> (μg/mL) | Cytotoxicity (TC<sub>50</sub>, μg/mL) |
|----------|------------------------------------------|-----------------------------------|
|          | A/HK/6                                   | A/PR/8/34(H1N1)                     |
| 1        | 106.9 ± 6.1                              | 123.8 ± 12.2                       |
| 8        | 232.3 ± 15.1                             | 243.0 ± 10.3                       |
| 16       | 142.7 ± 20.5                             | 155.0 ± 15.7                       |
| Cappariside A<sup>b</sup> | 362.2 ± 9.5                             | 288.0 ± 10.5                       |
| OSC      | 0.054 ± 0.01                             | 0.051 ± 0.01                       |

Abbreviations: CPE, cytopathic effect; EC<sub>50</sub>, half-maximal effective concentration; OSC, oseltamivir carboxylate; TC<sub>50</sub>, 50% cytotoxic concentration.

<sup>a</sup>The effect of compounds was determined by measuring the survival of Madin-Darby canine kidney cells infected with the influenza A virus using the CPE assay.

<sup>b</sup>EC<sub>50</sub> represents 50% effective concentration.

<sup>c</sup>TC<sub>50</sub> represents the maximal nontoxic concentration, determined by CPE assay.

(DMSO)<sub>δ</sub><sub>6</sub> as a solvent and tetramethysilane as an internal standard. Low-resolution mass spectra were recorded on Agilent 6460 triple-quadrupole mass spectrometer with electrospray ionization (ESI). High-resolution mass spectra (HR-MS) were performed on an AB SCIEX TripleTOF 4600 instrument. Melting points (MPs) were determined using a WRS-1C melting point apparatus (Shanghai INESA Physico optical instrument Co. Ltd.) and were uncorrected.

Synthesis of Indole-3-acetonitrile-6-O-β-D-glucopyranoside (Compound 1)

Synthesis of 1-(6-(benzylcyclo)-1H-indol-3-yl)-N,N-dimethylmethanamine (Compound 6). A 500 mL round-bottomed flask was charged with 37% formaldehyde solution (5.32 g, 4.92 mL, 65.59 mmol), dimethylamine 40% w/w in H<sub>2</sub>O (9.5 g, 84.42 mmol), acetic acid (100 mL), and 1,4-dioxane (100 mL). A solution of 6-benzoxynindole (14.5 g, 64.94 mmol) in 1,4-dioxane (100 mL) was added dropwise while keeping the internal temperature between 0 °C and 5 °C. The reaction mixture was then stirred for 2 hours and kept at room temperature for 36 hours. The mixture was diluted with H<sub>2</sub>O (500 mL) and adjusted to pH 8-9 with aqueous sodium hydroxide (NaOH) solution (10%). The precipitate collected by filtration, and the filter cake was washed with ethyl acetate several times, dried in vacuum to afford the compound 6 (15.6 g, 85.7%) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-<sub>δ</sub>6): δ 10.68 (s, 1 H), 7.46-7.40 (m, 5 H), 7.32 (d, J = 5.2 Hz, 1 H), 7.04 (s, 1 H), 6.91 (s, 1 H), 6.71 (d, J = 6.8 Hz, 1 H), 5.10 (s, 2 H), 3.46 (s, 2 H), 2.12 (s, 6 H) (supplemental Figure S1).

Synthesis of 2-(6-hydroxy-1H-indol-3-yl) acetonitrile (Compound 4). Methyl iodide (6.68 g, 29.3 ml, 47.08 mmol) was added to a suspension of compound 6 (6 g, 21.4 mmol) in a mixture of CH<sub>3</sub>Cl<sub>2</sub>/toluene (80/160 mL) under argon atmosphere. The mixture was vigorously stirred at room temperature for 16 hours. The reaction mixture was concentrated under reduced pressure, and THF (120 mL) was added to the residue. TMSCN (3.18 g, 32.1 mmol) and TBAF (1 M in THF, 85 mL, 85 mmol) were added dropwise to the resulted suspension successively. The reaction mixture was stirred at 25 °C for 2 hours and concentrated under reduced pressure. The residue was dissolved in ethyl acetate (180 mL). The mixture was washed with saturated sodium bicarbonate (NaHCO<sub>3</sub>) solution (100 mL), brine (100 mL), dried over anhydrous magnesium sulfate (MgSO<sub>4</sub>), and evaporated. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 5:1-1:1) to give the expected product 4 (5.16 g, 92%) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-<sub>δ</sub>6): δ 10.91 (s, 1 H), 7.52-7.36 (m, 5 H), 7.35-7.28 (m, 1 H), 7.19 (d, J = 2.2 Hz, 1 H), 6.96 (d, J = 2.2 Hz, 1 H), 6.80 (dd, J = 8.5, 2.2 Hz, 1 H), 5.12 (s, 2 H), 3.99 (s, 2 H) (supplemental Figure S2). LC/MS (ESI) m/z: [M + H]<sup>+</sup><sup>c</sup> calcd for C<sub>17</sub>H<sub>14</sub>N<sub>2</sub>O, 263.31; found, 263.3.

Synthesis of 2-(6-hydroxy-1H-indol-3-yl) acetonitrile (Compound 3). A 250 mL single-necked round-bottomed flask was charged with compound 4 (10 g, 38.1 mmol), 10% Pd/C (4.2 g), and methanol (100 mL), evacuated and refilled with hydrogen 3 times. The solution was vigorously stirred at room temperature for 10 hours. After evaporation of the solvent under reduced pressure, the residue was purified by gel column chromatography eluting with petroleum ether/ethyl acetate (4:1) to give compound 3 as a light yellow solid (5.97 g, 91%). <sup>1</sup>H NMR (400 MHz, DMSO-<sub>δ</sub>6): δ 10.69 (s, 1 H), 8.99 (s, 1 H), 7.33 (d, J = 8.5 Hz, 1 H), 7.09 (d, J = 2.2 Hz, 1 H), 6.74 (d, J = 2.2 Hz, 1 H), 6.58 (dd, J = 8.5, 2.2 Hz, 1 H), 3.94 (s, 2 H) (supplemental Figure S3). LC/MS (ESI) m/z: [M + H]<sup>+</sup><sup>c</sup> calcd for C<sub>16</sub>H<sub>14</sub>N<sub>2</sub>O, 263.18; found, 263.1.

Synthesis of (2R, 3R, 5R, 6S)-2-(acetoxymethyl)-6-((3-cyanomethyl)-1H-indol-6-yl)oxy) tetrahydro-2H-pyran-3,4,5-triyrate (Compound 8). A 500 mL round-bottomed flask was charged with compound 3 (5.68g, 33 mmol), 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide 2 (27.1, 66 mmol), CHCl<sub>3</sub> (120 mL), anhydrous K<sub>2</sub>CO<sub>3</sub> (22.8 g, 165 mmol), and distilled water (12 mL). Benzyltributylammonium chloride (2.06 g, 6.6 mmol) was added in portions, and the mixture was stirred at room temperature for 48 hours. After removal of solvent by evaporation, the residue was dissolved in ethyl acetate and washed with H<sub>2</sub>O and brine, dried over anhydrous MgSO<sub>4</sub>, and evaporated. The crude product was purified by column chromatography on silica gel (petroleum ether/ethyl acetate, 1:1) to yield the title compound 8 (14.75g, 89%) as a light yellow solid. MP: 289.8-291.2 °C.

<sup>1</sup>H NMR (500 MHz, DMSO-<sub>δ</sub>6): δ 11.04 (s, 1 H), 7.50 (d, J = 8.6 Hz, 1 H), 7.28 (s, 1 H), 7.02 (s, 1 H), 6.77 (dd, J = 8.6, 2.2 Hz, 1 H), 5.49 (d, J = 8.0 Hz, 1 H), 5.44 (t, J = 9.6 Hz, 1 H), 5.06 (dd, J = 9.6, 8.0 Hz, 1 H), 5.00 (t, J = 9.6 Hz, 1 H), 4.25-4.19 (m, 2 H), 4.09-4.03 (m, 1 H), 4.01 (s, 2 H), 2.04 (s, 3 H), 2.03 (s, 3 H), 2.01 (s, 3 H), 1.97 (s, 3 H) (supplemental Figure S4).
\[ ^{13}C \text{ NMR (125 MHz, DMSO-\text{d}_6)}: \delta 170.4, 170.0, 169.7, 169.5, 153.3, 136.9, 124.3, 122.7, 119.8, 119.1, 111.0, 104.2, 100.0, 99.1, 72.4, 71.4, 71.1, 68.6, 62.1, 20.9, 20.8, 20.7, 13.7 \text{ (supplemental Figure S5).} \]

HRMS (ESI): [M + Na]+ caled for C_{24}H_{27}N_{2}O_{10} Na, 525.1481; found, 525.1485 (supplemental Figure S16).

**Deprotection of Compound 8 (Compound 1).** LiOH-H$_2$O (5.41 g, 129 mmol) was slowly added to a solution of ester (10.8 g, 215 mmol) in THF-MeOH-H$_2$O (180 mL, V$_{\text{THF}}$/V$_{\text{MeOH}}$/V$_{\text{water}}$ = 3:2:1) at 0 °C in portions. The reaction mixture was stirred at room temperature for 2 hours. The solvent was removed in vacuo and neutralized by 1 M hydrochloric acid (HCl) after cooling to 0 °C. The reaction mixture was stirred with H$_2$O and extracted with ethyl acetate (150 mL × 3). The combined organic extract was washed with brine, dried over anhydrous MgSO$_4$, and concentrated under reduced pressure. The residue was dissolved in ethyl acetate (160 mL). The mixture was washed with saturated NaHCO$_3$ solution (100 mL), brine (100 mL), dried over anhydrous MgSO$_4$, and evaporated. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 2:1) to give the desired product 12 (6.8 g, 91.8%) as a colorless viscous liquid. 

\[ ^1H \text{ NMR (400 MHz, DMSO-\text{d}_6)}: \delta 11.11 \text{ (s, 1 H), 7.57 (d, J = 7.6 Hz, 2 H), 7.41 (t, J = 7.2 Hz, 2 H), 7.32 (t, J = 7.2 Hz, 1 H), 7.23 (s, 1 H), 7.05-6.93 (m, 2 H), 6.65-6.52 (m, 1 H), 5.21 (s, 2 H), 4.05 (s, 2 H) (supplemental Figure S9).} \]

A 100 mL single-necked round-bottomed flask was charged with compound 12 (2.62 g, 10 mmol), 10% Pd/C (1 g), and methanol (50 mL), and evacuated and refilled with hydrogen 3 times. The solution was vigorously stirred at room temperature for 4 hours. After evaporation of the solvent under reduced pressure, the residue was purified by silica gel chromatography (petroleum ether/ethyl acetate, 1:1) to give the product 13 (1.36 g, 80%) as a gray solid. 

\[ ^1H \text{ NMR (400 MHz, DMSO-\text{d}_6)}: \delta 10.91 \text{ (s, 1 H), 9.52 (s, 1 H), 7.13 (s, 1 H), 6.87 (t, J = 7.6 Hz, 1 H), 6.81 (d, J = 7.0 Hz, 1 H), 6.35 (d, J = 7.4 Hz, 1 H), 4.04 (s, 2 H) (supplemental Figure S10).} \]

A mixture of compound 13 (1.72 g, 10 mol), 2,3,4,6-tetra-O-acetyl-a-d-glucopyranosyl bromide 2 (8.2 g, 20 mmol), benzyltributylammonium chloride (0.6 g, 2 mmol), anhydrous K$_2$CO$_3$ (6.9 g, 50 mmol), H$_2$O (2 mL), and CHCl$_3$ (20 mL) was stirred at room temperature for 48 hours. After removal of the solvent by evaporation, the residue was dissolved in ethyl acetate and washed with H$_2$O and brine, dried over anhydrous sodium sulfate (Na$_2$SO$_4$), and evaporated. The crude product was purified by column chromatography on silica gel eluted with petroleum ether/ethyl acetate (1:1) to yield the title compound 14 as a white solid (4.3 g, 83%).

\[ ^1H \text{ NMR (400 MHz, DMSO-\text{d}_6)}: \delta 11.20 \text{ (s, 1 H), 7.26 (d, J = 2.3 Hz, 1 H), 7.09-7.01 (m, 7.6 Hz, 2 H), 6.64 (d, J = 6.8 Hz, 1 H), 5.76 (d, J = 8.0 Hz, 1 H), 5.47 (t, J = 9.6 Hz, 1 H), 5.22 (dd, J = 9.6, 8.0 Hz, 1 H), 5.02 (t, J = 9.6 Hz, 1 H), 4.38–4.25 (m, 1 H), 4.21 (dd, J = 12.4, 5.7 Hz, 1 H), 4.06 (dd, J = 12.4, 2.3 Hz, 1 H), 3.92 (s, 2 H), 2.04 (s, 3 H), 2.02 (s, 3 H), 1.99 (s, 3 H), 1.98 (s, 3 H) (supplemental Figure S11).} \]

LiOH-H$_2$O (503.5 mg, 12 mmol) was slowly added to a solution of compound 14 (1 g, 2 mmol) in THF-MeOH-H$_2$O (12 mL, V$_{\text{THF}}$/V$_{\text{MeOH}}$/V$_{\text{water}}$ = 3:2:1) at 0 °C in portions. The reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was evaporated in vacuo and neutralized by 1 M HCl after cooling to 0 °C. The mixture was diluted with H$_2$O and extracted with ethyl acetate (20 mL × 3). The combined organic layers were washed with brine, dried over anhydrous Na$_2$SO$_4$, and evaporated. The residue was purified by flash chromatography (CH$_2$Cl$_2$/MeOH 20:1-10:1) to afford capparilaside A (574 mg, 85.9%) as a white solid. The NMR spectrum coincided with that the previously reported in the literature.

\[ MP: 234.8 °C-236.7 °C. \]

\[ ^1H \text{ NMR (500 MHz, DMSO-\text{d}_6)}: \delta 11.08 \text{ (s, 1 H), 7.20 (d, J = 2.4 Hz, 1 H), 7.04-6.97 (m, 2 H), 6.69 (dd, J = 7.1, 1.2 Hz, 1 H), 5.30 (d, J = 5.6 Hz, 1 H), 5.10 (d, J = 4.9 Hz, 1 H), 5.02 (d,} \]
ular sieves (200 mg) in CH$_2$Cl$_2$ (5 mL) was stirred for 5 minutes with DIPEA (2 equiv.), pyridine (95 mg, 1.2 mmol, 4 equiv.), and 4 Å molecular sieves (200 mg, 0.6 mmol, 2 equiv.), copper (II) acetate (109 mg, 0.6 mmol, 2 equiv.) in THF/MeOH/H$_2$O (3 mL, 3:2:1) at 0 °C. The reaction mixture was stirred at room temperature for 2 hours. The solvent was removed in vacuo and the crude residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 5:1-1:1) to provide the ester (120.6 mg, 69.5%) as a white solid.

Synthesis of 2-(1-phenyl-6-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-1H-indol-3-yl)acetonitrile (Compound 16). A suspension of phenylboronic acid (73.1 mg, 0.6 mmol, 2 equiv.), pyridine (95 mg, 1.2 mmol, 4 equiv.), and 4 Å molecular sieves (200 mg) in CH$_2$Cl$_2$ (5 mL) was stirred for 5 minutes at room temperature. To this stirring suspension was added compound 8 (150.7 mg, 0.3 mmol, 1 equiv.). The mixture was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate (3 mL) and filtered through a plug of silica gel eluting with additional ethyl acetate (20 mL). The filtrate was concentrated, and the resulting residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 5:1-1:1) to provide the ester (120.6 mg, 69.5%) as a white solid.

LiOH·H$_2$O (50.4 mg, 1.2 mmol, 6 equiv.) was slowly added to a solution of the resulting ester (120.6 mg, 0.21 mmol, 1 equiv.) in THF/MeOH/H$_2$O (3 mL, $V_{\text{THF}}/V_{\text{MeOH}}/V_{\text{water}} = 3:2:1$) at 0 °C. The reaction mixture was stirred at room temperature for 2 hours. The solvent was removed in vacuo and neutralized by 1 M HCl after cooling to 0 °C. The mixture was diluted with water and extracted with ethyl acetate (5 mL × 3). The combined organic layers were washed with brine, dried over anhydrous MgSO$_4$, and concentrated. The residue was purified by silica gel column chromatography (CH$_2$Cl$_2$/MeOH, 40:1-20:1) afforded the target compound 9 (76.9 mg, 89.3%) as a white powder.

MP: 256.4 °C-258.2 °C.

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 7.67-7.52 (m, 6 H), 7.39 (s, J = 6 Hz, 1 H), 7.28 (s, 1 H), 6.99 (d, J = 8.6 Hz, 1 H), 5.29 (d, J = 2.4 Hz, 1 H), 5.06 (s, 1 H), 4.99 (d, J = 4.4 Hz, 1 H), 4.79 (d, J = 6.4 Hz, 1 H), 4.62 (s, J = 5.6 Hz, 1 H), 4.11 (s, 2 H), 3.70 (dd, J = 10.8, 4.4 Hz, 1 H), 3.50-3.45 (m, 1 H), 3.30-3.21 (m, 3 H), 3.17–3.12 (m, 1 H) (supplemental Figure S14).

$^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 154.9, 138.6, 135.8, 129.9, 126.4, 126.3, 123.5, 122.6, 119.3, 119.0, 111.9, 106.4, 101.7, 98.2, 77.1, 76.6, 73.3, 69.8, 60.8, 60.3, 13.2 (supplemental Figure S15).

HRMS (ESI): $m/z$ [M + K]$^+$ calef for C$_{32}$H$_{22}$N$_2$KO$_6$: 449.1109; found: 449.1112 (supplemental Figure S18).

CPE Assay Protocol. In CPE assay, MDCK cells were grown to a confluent monolayer in a 96-well culture plate at a concentration of $5 \times 10^3$ cells/well for 24 hours. The medium was removed, and the cells were rinsed twice. For the anti-influenza activity assay, an infectious virus at 100 TCID$_{50}$ was inoculated into the MDCK cells, which were then incubated for 2 hours at 37 °C in 5% carbon dioxide (CO$_2$). The virus supernatant was then removed, followed by the addition of serial 2-fold dilutions of antiviral compounds in Dulbecco’s modified Eagle medium containing 1.5 µg/mL trypsin. After being incubated at 34 °C in 5% CO$_2$ for 48 hours, the infected cells displayed 100% CPE under the microscope, and the CPE percentages in the antiviral compound-treated groups were recorded. The EC$_{50}$ of virus-induced CPE was detected by the Reed-Muench method. All data are obtained for at least 3 independent experiments.

Cytotoxicity Assay

Cytotoxicity of compounds was assessed by MTT assay. MDCK cells on 96-well plates were washed with sterile phosphate-buffered saline. Then 100 µL of compounds in serum-free MEM at 2-fold dilution was added to the cells, and the cells were incubated in CO$_2$ at 37 °C. Cell controls were also performed. After 48 hours, 5 µL/mL of fresh MTT was added to each well, and the plates were incubated at 37 °C for 4 hours. Afterward, the medium was removed and formazan crystal was dissolved in DMSO (100 µL per well). Absorbance of each well at 490 nm was read by a CLARIOstar multi-mode microplate reader (BMG Labtech, Germany). The cell viability (%) = OD of compound well/average OD of control wells. The TC$_{50}$ of each compound was obtained using a nonlinear regression model in GraphPad Prism 6.

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Declaration of Conflicting Interests

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Supplemental Material

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