Synthesis and anti-influenza virus activity evaluation of novel andrographolide derivatives

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Abstract
In this paper, using AI78-38, an andrographolide analog with novel skeleton, as the lead compound, we designed and synthesized fifteen amide derivatives at the 17 position of AI78-38. In the synthesis of key intermediate IM4, the low yield of the SeO2 oxidation step impeded the further study. Aiming at improving the yield, White reagent was applied to furnish IM4 by catalyzing the allylic C–H oxidation at the 7, 8, 17 position. It is also firstly reported that White reagent possessed the ability of oxidating the cyclic substrates. Compared with SeO2 oxidative approach, the modified synthetic method utilized by White reagent increased the yield from 32.0 to 53.6%. The anti-influenza A virus (H3N2) results showed that 4-methoxy derivative 10 offered the greatest inhibitory ability, with an IC50 value of 90.2 μg/ml, slightly more potent than ribavirin. Furthermore, the drug-likeness and ADMET properties of compound 10 and AI78-38 were evaluated using the Discovery Studio 2.1 software and the online SwissADME. The results showed that compound 10 offered good bioavailability and overcame the disadvantages of the ADMET properties in AI78-38.

Keywords Andrographolide · White reagent catalysis · Double bond migration · Cyclic substrate · Anti-influenza A virus activity

Introduction

The Influenza virus, which is a single helical RNA virus, can be classified into three categories: A, B, and C. Influenza A virus occurs more frequently than the B and C subtypes [1]. Due to genetic variations caused by antigenic shift and sporadic antigenic drift, influenza A viruses exhibit pathogenic characteristic, resulting in the worldwide spread of these respiratory diseases [2]. According to available statistics, 290,000 to 650,000 patients worldwide die annually of the respiratory diseases caused by influenza virus [3]. To date, vaccines remain one of the most effective strategies to block influenza virus transmission. However, some drawbacks and disadvantages of such vaccines affect their anti-viral abilities. These issues include difficulties in determining key strains,
difficulty in evaluating protective effects, and an inability to estimate the time needed for their design and production. Problematically, only two types of anti-influenza drugs were approved for use in clinic by the FDA: M2 ion channel inhibitors and NA inhibitors [4–6]. Nevertheless, considering the ongoing emergence of novel influenza viruses and the increasing severity of viral resistance, the existing drugs cannot provide full protection for humankind [7, 8]. Thus, there is an extremely urgent demand for developing novel and effective anti-influenza drugs.

Andrographolide (Fig. 1) is the primary constituent in the plant Andrographis paniculata and possesses a variety of biological properties, including anti-inflammatory, antimicrobial, antitumor, anti-influenza-virus, and insecticidal activities [9–15]. As a traditional Chinese medicine, this plant is clinically used for treating various infectious and inflammatory diseases, especially upper respiratory infections [16–18]. Due to this plant’s great therapeutic potential, most related research has been performed on the structural modification of andrographolide. This research has mainly included functionalization of the 3, 14, and 19-hydroxyl groups [19–23]; \( \Delta^8,17 \) double-bond oxidation [24–26]; C-15’s replacement with the alkylidenyl group [27, 28]; \( \Delta^{12,13} \)-alkene isomerization [29]; and the substitution of C-12 with electron-rich groups [30–33]. In recent years, some novel andrographolide analogs were developed via lactone–bioisosteric replacement [34–37], as well as 1, 2-olefination [38] and terpene ring expansion [39]. These methods have enriched the structural diversity of andrographolide and laid a solid foundation for elucidating the accurate SAR of andrographolide.

Although many andrographolide derivatives with various structural characteristics have been successfully synthesized, active analogs with anti-viral activities, especially anti-influenza virus effects, remain scarce. Most of these analogs belong to the 3, 14, and 19-hydroxyl groups’ functional and \( \Delta^{12,13} \)-alkene isomerized derivatives. Among them, only DASS, AL-1, and DAP have presented definite anti-influenza viral activities [40, 41]. Other compounds showed inhibitory capabilities against Zika, hepatitis B, and HIV virus, respectively [26] (Fig. 2). Therefore, it is vital to search for an andrographolide-type anti-influenza viral-active compound with a novel skeleton structure.

In previous work, our group successfully semi-synthesized two microbial transformative products, AI78 and AI89, with new structural fragments in which the \( \Delta^8,17 \) double bond migrated into the ring. Taking AI78 as the lead compound, we synthesized a series of 17-substituted derivatives. Further bioactivity evaluations showed that AI78-38, a 17-benzylamine derivative of AI78, offered the best anti-influenza-virus H3N2 activity among all derivatives and was superior to the positive control drug, Lianbizhi (Fig. 3) [42]. This result indicated that this type of substitution at the
17 position can help enhance anti-influenza-virus effects and suggested that structural optimization at the 17 position in A178 may offer significant advantages in the inhibitory activity of influenza virus.

To further improve the anti-influenza viral potency of A178-38, we expanded our research on the reported active compounds. Based on an extensive literature review, we found that the amide group appeared in most active molecules exhibiting different anti-influenza viral mechanisms of action (Fig. 4) [43–50]. Since the accurate target of action for andrographolide and its analogs is not yet known, a rational strategy would involve introducing the amide group to the 17 position in A178-38. This would also further enrich the structural diversity of A178-type molecules.

Therefore, in this study, fifteen novel amide substituted derivatives at the 17 position of A178 were designed and synthesized. The anti-influenza A virus (H3N2) activities of all derivatives in vitro were then evaluated. The results showed that 4-methoxyl benzamide derivative 10 offered the greatest potency against H3N2 virus and was stronger than the control ribavirin. Compound 10 was predicted to exhibit better drug-likeness and ADMET properties than A178-38 based on the Discovery Studio software and the online SwissADME.

Result and discussion

Chemistry

According to our previous method, IM4 was obtained by three successive steps for SeO2 oxidation, methanesulfonyl chloride to activate the hydroxyl group and S$_2$N$_2'$ substitution reaction (Fig. 1s). However, at the SeO2 oxidation step, the low yield hindered the further study. White reagent is a complex compound composed by Pd(OAc)$_2$ and bisulfoxide ligand in the 1:1 ratio. The White reagent is a highly versatile catalyst for allylic C–H oxidation which allows for the construction of useful C–O, C–N, and C–C bonds directly from relatively inert allylic C–H bonds. However, in published research work, the substrates were mainly chain molecules. We wondered if the White reagent is suitable for the cyclic substrates. Therefore, we firstly investigated the synthesis of IM3a utilized by White reagent. Fortunately, IM2 was successfully converted to IM3a according to classical White condition of 10% mol catalyst with 2 equivalents of BQ in the mixture of dioxane and AcOH in a ratio of 1:1 at 40 °C for 72 h (Scheme 1) [51].

In order to identify the optimal reaction conditions, we performed several experiments to optimize the reaction parameters (Table 1). Initially, eight kinds of solvents were tested under the condition of 40 °C and 72 h. Dioxane proved to be the best solvent, furnished the desired product IM3a in 53% yield (entry 5). AcOH, THF, and toluene afforded the comparable results with the yields of 43%, 45%, and 46%, respectively. IM3a was obtained in the other solvents with less than 25% yields. Secondly, the effect of reaction temperature was investigated. The yield significantly increased to 67% when the temperature was raised to 80 °C. After rising the temperature to 100 °C, the yield is 69% and slightly higher than that of 80 °C. Therefore, we thought that 80 °C was a suitable temperature for the reaction (entry 10). Finally, the influence of the oxidant including BQ, Cu(OAc)$_2$, and BQ(Me) were examined (entry 10, 12, 13). The results showed that BQ was superior to the two other oxidants. In order to examine the scope and generality of White methodology, benzoic acid was chosen to investigate. The result
showed that this method still afforded the target compound in 74% yield. This also demonstrates the efficiency of the White reagent for oxidating cyclic substrates.

Based on it, the modified synthetic plan for 17-N-acylated derivatives of A178 is illustrated in Scheme 2. IM4 was obtained by the further $S_{N}2'$ reaction that occurred between IM3 with potassium phthalimide. Due to stronger leaving ability of 7-benzoyloxy substituent in IM3b, it showed higher yield than IM3a for the synthesis of IM4. Finally, through three sequential reactions, including the Gabriel reaction, acylation, and deacetylation, the target derivatives 1–14 were successfully prepared. The yield of IM4 was increased from 32.0 to 53.6%.

**Evaluation of anti-influenza A virus (H3N2) activity**

We screened the anti-influenza viral activity of all synthesized derivatives against the influenza virus strain A3/JINGKE/30/95—i.e., the inhibition of H3N2 replication in MDCK (Madin–Darby canine kidney) cells. Ribavirin and A178-38 were used as the control, and the results are listed in Table 2.

### Table 1 Optimization of reaction condition

| Entry | Solvent  | Oxidant | Temperature (°C) | Isolated yield |
|-------|----------|---------|-----------------|----------------|
| 1     | AcOH     | BQ      | 40              | 43%            |
| 2     | DMSO     | BQ      | 40              | 10%            |
| 3     | DMF      | BQ      | 40              | 24%            |
| 4     | CH$_3$CN | BQ      | 40              | 17%            |
| 5     | Dioxane  | BQ      | 40              | 53%            |
| 6     | THF      | BQ      | 40              | 45%            |
| 7     | DCM      | BQ      | 40              | 14%            |
| 8     | Toluene  | BQ      | 40              | 46%            |
| 9     | Dioxane  | BQ      | 60              | 58%            |
| 10    | Dioxane  | BQ      | 80              | 67%            |
| 11    | Dioxane  | BQ      | 100             | 69%            |
| 12    | Dioxane  | Cu(OAc)$_2$ | 80       | 25%            |
| 13    | Dioxane  | BQ(Me)  | 80              | 50%            |

Among all derivatives including the control A178-38 and ribavirin, derivatives 1–4 with alkyl and alkenyl substituents showed no anti-viral activity against H3N2. Compared to A178-38, styril analog 7 and benzoyl derivative 8 showed improved anti-viral effects. These results suggest that the aromatic amide moiety at the 17 position is more conducive to anti-viral activity.

We then further investigated the effects of different types of aromatic amide moieties at the 17 position on anti-viral activity. First, we explored the electronic properties of substituents on the phenyl ring. Based on the biological assay results, there was a tendency for the derivatives with the electron-donating group (EDG) to show more pronounced inhibitory effects on the influenza virus than those of the compounds bearing the electron-withdrawing group (EWG). For the EDG series, among all tested compounds, 4-methoxy derivative 10 showed the greatest inhibitory activity, with an IC$_{50}$ value of 90.2 μg/ml—slightly more potent than ribavirin. Next, we explored the impact of the type and length of the linker moiety between phenyl and carbonyl groups on anti-viral activity. Benzoyl derivative 8 displayed more prominent inhibitory effects on the influenza virus (H3N2) and was approximately 1.74- and 1.22-fold stronger, respectively, than the corresponding phenylacetyl 5 and phenylpropyl 6 derivatives. After linker in 6 was replaced with a double bond, the cinnamyl derivative 7 (with an IC$_{50}$ value of 105.4 μg/ml) showed comparable anti-virus activity to compound 8. In addition, the anti-viral activity of the deacetyl product of the intermediate IM4 (compound 15) was also evaluated. The result showed that the inhibitory activity of 15 against H3N2 was about 1.3-fold weaker than that of A1 78-38.

**Examination of cytotoxicity against MDCK cells**

The cytotoxicity and TI (therapeutic index) values of all compounds to host MDCK cells were also evaluated and
analyzed to determine potential toxicity. The results indicated that the TI values of all derivatives were less noteworthy due to their relatively higher cytotoxicity than the control drug ribavirin. These derivatives with EWG showed stronger cytotoxic effects than those of the analogs bearing EDG. Notably, the nitro-substituted group significantly contributed to toxicity, with a value of 590.1 μg/ml and a TI value of 3.1. The phenylpropyl derivative 6 displayed the lowest

### Table 2: The IC₅₀, TC₅₀ and TI values of all compounds against influenza virus strain A3/JINGKE/30/95 (H3N2)

| Compound | Substituent (R) | IC₅₀ b (μg/ml) | TC₅₀ c (μg/ml) | TI d |
|----------|----------------|----------------|----------------|-----|
| 1        | CH₃            | >200           | >200           | –   |
| 2        | ClCH₂-         | >200           | >200           | –   |
| 3        | -CH = CH₂      | >200           | >200           | –   |
| 4        |                |                |                | –   |
| 5        |                | 190.7 ± 2.50   | 2310.2         | 12.1|
| 6        |                | 126.5 ± 0.42   | 2987.8         | 23.6|
| 7        |                | 105.4 ± 5.60   | 788.3          | 7.5 |
| 8        |                | 103.4 ± 3.98   | 2010.1         | 19.4|
| 9        |                | 125.3 ± 1.26   | 2001.6         | 16.0|
| 10       |                | 90.2 ± 3.11    | 1978.9         | 21.9|
| 11       |                | 211.0 ± 5.66   | 688.9          | 3.3 |
| 12       |                | 201.5 ± 4.34   | 781.4          | 3.9 |
| 13       |                | 196.7 ± 0.93   | 798.9          | 4.1 |
| 14       |                | 192.3 ± 3.40   | 590.1          | 3.1 |
| 15       |                | 156.3 ± 3.55   | 1893.2         | 12.1|
| A178-38  |                | 120.9 ± 5.83   | 973.7          | 8.05|
| Ribavirin|                | 91.7 ± 2.45    | 3277.4         | 35.74|

*All data represents average values for three separate experiments

bIC₅₀: compound concentration required to achieve 50% inhibition of replication of H3N2
cTC₅₀: compound concentration required to cause 50% death of uninfected MDCK cells
dTI: therapeutic index (TC₅₀/IC₅₀)
eNo activity
cytotoxicity, which was nearly equivalent to that of ribavirin, with a TI value about 1.5 times lower than that of control ribavirin. The TI value of compound 10, which featured the greatest anti-viral activity, was 21.9—a similar value to that of compound 6. However, compound 10 presented greater cytotoxicity than compound 6, but the former’s TC50 value was 1.5-fold lower than that of the latter.

**Drug-likeness and ADMET prediction**

Lipinski’s rule-of-five is a golden criterion for evaluating the drug-likeness properties of active molecules for oral bioavailability. The Discovery Studio software package was applied to calculate the Lipinski’s rule of control for A178-38 and compound 10. The related parameters were as follows: molecular weight (MW), Log Po/w (average values of all available predictions), topological polar surface area (TPSA), molar refractivity (AMR), hydrogen bond donors (HBD), number of hydrogen bond acceptors (HBA) and rotatable bonds (RB). Two compounds met Lipinski’s rule of five and did not violate any rules (Table 3).

|       | L<sup>a</sup> | G<sup>b</sup> | V | E | M | MW   | LogPo/w | TPSA | AMR | HBA | HBD | RB |
|-------|--------------|--------------|---|---|---|------|---------|------|-----|-----|-----|----|
| 10    | green        | red          |   |   |   | 483.60 | 3.52   | 105.09 | 133.06 | 6   | 3   | 8  |
| A178-38 | green        | red          |   |   |   | 439.59 | 3.71   | 78.79  | 126.15 | 5   | 3   | 8  |

<sup>a</sup>green: meet the requirement; <sup>b</sup>red: violate the rule

The drug likeness of compound 10 and A178-38 based on Lipinski (L), Ghose (G), Veber (V), Egan (E) and Muegge (M) rules

ADMET_AlogP98 properties. Two compounds were placed into the 99% ellipse in the HIA plot. Meanwhile, both predicated values of absorption were found to be 0, indicating that they had ideal absorption levels (Table 4). A178-38 was placed in the 99% ellipse of the BBB plot, indicating that A178-38 likely penetrated BBB and produced nervous system toxicity. Unlike A178-38, compound 10 did not fall into the region of the BBB plot. Other key parameters such as aqueous solubility, plasma protein binding (PPB), cytochrome CYP2D6 enzyme inhibitory capacity, and hepatotoxicity were also calculated. The solubility levels of A178-38 and compound 10 were 2 and 3, respectively. Thus, compound 10 exhibited greater solubility than A178-38. The cytochrome P450 enzymes (CYP), moreover, had a significant effect on drug metabolism. The CYP2D6 subtype participated in the metabolism of about 30% of drugs. The predicated results showed that A178-38 had an inhibitory effect on CYP2D6. Fortunately, compound 10 was not a CYP2D6 inhibitor. The hepatotoxicity screening results indicated that the two compounds displayed no toxicity against the liver. The PPB (plasma protein binding) level is a crucial parameter for evaluating the ability of drug distribution. A178-38 presented a greater binding ability with plasma proteins than the binding ability observed for compound 10.

**Conclusion**

In summary, taken natural andrographolide analog A178-38 as lead compound, fifteen 17-amide substituted derivatives were designed and synthesized. The key intermediate IM4 was successfully obtained by White reagent catalysis. Compared with previous SeO2 oxidative approach, the modified synthetic method utilized by White reagent increased the yield from 32.0 to 53.6%. It is also firstly reported that White reagent possessed the ability of oxidating the cyclic substrates. The anti-influenza A virus (H3N2) activity of all derivatives were screened, and ribavirin and A178-38 were selected for control. The results showed that 4-methoxy derivative 10 showed the greatest inhibitory ability with the IC50 value of 90.2 μg/ml which was slightly more potent than ribavirin. Meanwhile, the phenylpropyl derivative 6 displayed...
the least cytotoxicity nearly equivalent with ribavirin and its 
TI value was about 1.5 times lower than control ribavirin. 
Further, compound 10 exhibited a good drug likeness and 
ADMET properties predicted by the software Discovery 
Studio 2.1 and the online SwissADME.

**Experimental**

**Material and methods**

The melting points were recorded on a Büchi Melting 
Point B-540 apparatus (Büchi Laborteknik, Flawil, 
Switzerland). High resolution accurate mass determina-
tions (HRMS) for all derivatives 1–15 were determined 
on a Bruker Micromass Time of Flight mass spectrometer 
equipped with electrospray ionization (ESI). NMR 
spectra (including {\(^1\)H-NMR and \(^{13}\)C-NMR) were recor-
ded on a Bruker ARX-600 (600 MHz) spectrometer 
(Bruker Bioscience, Billerica, MA, USA), using CDCl$_3$
 as solvent and TMS as an internal standard. Unless 
otherwise indicated, reagents were obtained from com-
mercial suppliers and directly used without purification. 
Organic solvents were dehydrated by classical procedures 
when necessary. The column chromatography was run 
using silica gel (200–300 mesh) from Qingdao Ocean 
Chemicals (Qingdao, Shandong, China) with chloroform/ 
acetone mixture as eluent.

**General procedure for IM3**

A 5 ml round-bottomed flask was charged with the following: 
White reagent (0.048 mmol, 10 mol%) and oxidants (2 eq) 
were dissolved in dioxane (2 ml). A vial was charged with 
IM2 (1.0 g, 2.4 mmol) and AcOH or benzoic acid (5 eq). This 
mixture was taken up in 2 ml of dioxane and sequentially 
transferred via pipette into the round bottomed 
flask. The reaction flask was equipped with a reflux condenser under N$_2$ 
atmosphere and allowed to heat at 40 °C for 72 h. After added 
5 ml saturated NH$_4$Cl solution and stirred for 10 min, the 
mixture was extracted with DCM (3 × 10 ml), the organic 
phase was combined and washed with water (30 ml), and 
brine (30 ml). The organic extracts were dried over anhydrous 
Na$_2$SO$_4$, and concentrated in vacuo, the residue was puri-
fied by silica gel column chromatography (Petroleum ether:ethyl 
acetate = 5:1) to afford the product.

**Table 4** The ADME prediction results for AI78-38 and 
compound 10 obtained from the 
Discovery Studio predictions

|          | BBB level | Absorption | Solubility level | Hepatotoxicity | CYP2D6 | PPB level | PSA-2D |
|----------|-----------|------------|-----------------|---------------|--------|----------|--------|
| AI78-38  | 2         | 0          | 2               | 0             | 1      | 1        | 80.672 |
| 10       | 4         | 0          | 3               | 0             | 0      | 0        | 106.902|

BBB level: blood brain barrier penetration levels. 1: high; 2: medium; 3: low; 4: undefined. Absorption: human intestina absorption level. 0: good; 1: moderate; 2: low. Solubility level: categorical solubility level. 2: low; 3: good. Hepatotoxicity: 0: nontoxic; 1: toxic. CYP2D6: cytochrome P450 2D6. 0: non-inhibitor; 1: inhibitor. PPB level: plasma protein binding levels. 0: binding <90%; 1: binding≥90%; 2: binding≥95%. PSA-2D: the van der Waals surface area of polar nitrogen and oxygen atoms (≤140), fast polar surface area

![Fig. 5](image-url) Plot of the polar surface area (PSA_2D) vs. AlogP
A mixture of **IM3a** or **IM3b** (4 mmol), phthalimide potassium salt (8 equiv.) and NaI (1 equiv.) in 1,2-dichloromethane (20 ml) was stirred at reflux overnight. After the reaction was completed, the reaction mixture was washed continuously with water (20 ml) and brine (20 ml). The residue was purified by silica gel column chromatography (Petroleum ether:ethyl acetate = 1:1) using to furnish the target product **IM3a** or **IM3b** as starting material, the yield is 71 and 80%, respectively. White solid; m.p. 146–150 °C; MS(ESI) m/z: 564.76 [M+Na]+; 1H-NMR (600 MHz, CDCl3): δ 7.87 (2H, dd, J = 5.4, 3.6 Hz, Ar–H), 7.74 (2H, dd, J = 5.4, 3.6 Hz, Ar–H), 7.24 (1H, s, 14-H), 5.34 (1H, s, 7-H), 4.82 (2H, s, 15-H), 4.56–4.59 (1H, m, 3-H), 4.40 (1H, d, J = 12.0 Hz, 19a-H), 4.32 (1H, d, J = 16.2 Hz, 17a-H), 4.19–4.23 (2H, m, 17b, 19b-H), 2.54–2.59 (1H, m, 12a-H), 2.37–2.42 (1H, m, 12b-H), 2.08 (1H, brs, 2a-H), 2.04 (3H, s, CH3COO), 2.02 (3H, s, CH3COO), 2.00–2.02 (1H, dt, J = 13.2, 3.4 Hz, 2b-H), 1.95 (1H, brs, 9-H), 1.85–1.91 (1H, m, 6a-H), 1.69–1.74 (3H, m), 1.45 (1H, dd, J = 10.7, 6.1 Hz, 5-H), 1.22–1.29 (2H, m, 0.96 (3H, s, 18-CH3), 0.86 (3H, s, 20-CH3); 13C-NMR (151 MHz, CDCl3, TMS): δ 174.0 (C-16), 170.7 (C=O)ester, 170.4 (C=O)ester, 168.0 (C=O)amide, 144.7 (C-14), 134.0×2 (C-3’, C-4’), Ar, 134.0 (C-13), 133.8 (C-8), 132.3(C-1’, Ar), 132.0 (C-6’, Ar), 123.3×2 (C-2’, C-5’, Ar), 121.4(C-7), 79.8 (C-3), 70.1 (C-15), 64.3 (C-19), 52.4 (C-5), 50.0 (C-9), 40.6 (C-4), 40.0 (C-17), 36.9 (C-1), 36.5 (C-10), 29.6 (C-2), 27.2 (C-12), 24.6 (C-11), 23.8 (C-6), 22.1 (C-18), 21.1 (CH3CO), 21.0 (CH3CO), 14.3 (C-20).

**General procedure for target products (1–14)**

The amino group of **IM4** was released by classical Gabriel reaction procedure. Hydrazine hydrate (1 ml, 80%) was added to a solution of **IM4** (0.4 g, 0.71 mmol) in ethanol (10 ml) three times with an interval of 30 min. After refluxed for 4 h, the solvent was removed by rotary evaporator. The residue solid was recrystallized in ethyl acetate. The product and DMAP (1 equivalent) were dissolved into DCM (10 ml). Different acyl chlorides (1.05 equivalents) were dropped into the solution in an ice bath and the mixture stirred overnight. The mixture was washed with 20% citric acid solution (5 ml), saturated sodium bicarbonate solution (5 ml), and brine (5 ml), dried by anhydrous Na2SO4. After DCM evaporated off, acylated derivatives (0.2 mmol) were dissolved in MeOH (2 ml), and acetyl chloride (29 µl, 0.4 mmol) was dropped slowly at 0 °C, the solution was stirred for 16 h at room temperature. After reaction, the solution was washed with saturated sodium bicarbonate solution (2 × 2 ml), water (2 ml), and brine (2 ml). The residue was purified by silica gel column chromatography using to furnish the target product (89.8–97.3%).

**Spectroscopy data of compounds 1~15**

\[ N-\{(15S, 5R, 6R, 8aS)-6-hydroxy-5-(hydroxymethyl)-5, 8a-dimethyl-1\-(2\-(2-oxo-2, 5-dihydrofuran-3-yl)ethyl)-1, 4, 4a, 5, 6, 7, 8, 8a-octahydropaphthalen-2-yl)ethyl\}acetamide (1) \]

Yield: 95.2%; White solid; m.p. 164–165 °C; HR-MS(ESI) m/z calc'd for C25H34O5N4Na, 442.2366, found 442.2253 [M +Na]+; 1H-NMR (600 MHz, CDCl3): δ 7.24 (1H, s, 14-H), 6.04 (1H, s, N-H), 5.69 (1H, t, J = 2.4 Hz, 7-H), 4.80 (2H, 1H, s, 15-H), 4.24 (1H, d, J = 11.4 Hz, 19a-H), 3.94 (1H, dd, J = 15.0, 6.0 Hz, 17a-H), 3.74 (1H, dd, J = 15.0, 4.8 Hz, 17b-H), 3.45 (1H, d, J = 10.8 Hz, 19b-H), 3.44–3.45 (1H, m, 3-H), 2.38–2.43 (1H, m, 12a-H), 2.29–2.33 (1H, m, 12b-H), 2.11–2.14 (1H, brd, J = 16.3 Hz, 2a-H), 2.01 (3H, s, CH3CO), 1.92–1.95 (1H, dt, J = 13.4, 3.5 Hz, 2a-H), 1.85–1.90 (1H, brd, J = 16.2 Hz, 6a-H), 1.81–1.84 (1H, brd,
3.44–3.45 (1H, m, 3-H), 2.37–2.42 (1H, m, 12a-H), 2.29–2.34 (1H, m, 12b-H), 2.11–2.15 (1H, brd, J = 16.1 Hz, 2a-H), 1.91–1.94 (1H, dt, J = 13.4, 3.2 Hz, 2b-H), 1.85–1.89 (1H, brd, J = 16.7 Hz, 6a-H), 1.80–1.82 (1H, brd, J = 15.0 Hz, 6b-H), 1.79 (1H, brs, 9-H), 1.74–1.77 (1H, td, J = 13.3, 3.9 Hz, 1a-H), 1.65–1.70 (1H, m, 11a-H), 1.48–1.54 (1H, m, 11b-H), 1.31 (1H, dd, J = 12.5, 4.6 Hz, 5-H), 1.22 (3H, s, 18-CH3); 1H-NMR (600 MHz, CDCl3): δ 1.29 (6H, m, 3 H and 19b-H), 1.25–1.82 (2H, m, 12b-H and 1a-H), 1.22–2.42 (1H, m, 12a-H), 2.21–2.30 (2H, m, 12b-H and 1′-H), 2.08–2.11 (1H, dt, J = 11.9, 3.2 Hz, 2a-H), 1.93–1.97 (1H, dt, J = 13.4, 3.0 Hz, 2b-H), 1.82–1.85 (1H, t, J = 12.8 Hz, 6a-H), 0.79–1.81 (1H, brs, 6b-H), 1.80 (1H, brs, 9-H), 1.75–1.78 (1H, td, J = 13.3, 3.9 Hz, 1a-H), 1.69–1.74 (1H, m, 11a-H), 1.44–1.50 (1H, m, 11b-H), 1.33 (1H, dd, J = 12.5, 4.6 Hz, 5-H), 1.23 (3H, s, 18-CH3), 1.11–1.16 (1H, td, J = 13.3, 3.8 Hz, 1b-H), 0.72 (3H, s, 20-CH3); 13C-NMR (151 MHz, CDCl3, TMS): δ 174.4 (C-16), 165.7 (C=O)amide, 145.0 (C-14), 134.1 (C-13), 133.8 (C-8), 125.4 (C-7), 80.8 (C-3), 70.2 (C-15), 63.9 (C-19), 52.0 (C-5), 50.2 (C-9), 43.4 (C1′CH2CO), 42.6 (C-4), 41.9 (C-17), 37.0 (C-1), 36.3 (C-10), 27.6 (C-2), 27.1 (C-12), 25.0 (C-11), 23.1 (C-6), 22.0 (C-18), 14.6 (C-20). Purity: 98.46%; retention time: 5.61 min.

N-(((15S, 5R, 6R, 8αS)-6-hydroxy-5-(hydroxymethyl)-5, 8a-dimethyl-1-[(2-oxo-2, 5-dihydrofuran-3-yl)ethyl]-1, 4, 4a, 5, 6, 7, 8, 8a-octahydronaphthalen-2-yl)methyl)acrylamide (3)

Yield: 95.0 %; White solid; m.p. 154–157 °C; HR-MS(ESI) m/z calc. for C24H33NO5Na, 426.2256, found 426.2250 [M + Na]+; 1H-NMR (600 MHz, CDCl3): δ 7.25 (1H, s, 14-H), 6.38 (1H, t, J = 5.4 Hz, N-H), 6.29 (1H, dd, J = 16.8, 1.2 Hz, CH = CH2), 6.18 (1H, dd, J = 16.8, 10.2 Hz, CH = CH2), 5.73 (1H, t, J = 2.4 Hz, 7-H), 5.64 (1H, dd, J = 10.2, 1.2 Hz, CH = CH2), 4.80 (2H, s, 15-H), 4.24 (1H, d, J = 10.8 Hz, 19a-H), 4.02 (1H, dd, J = 15.0, 6.0 Hz, 17a-H), 3.84 (1H, d, J = 15.0, 5.4 Hz, 17b-H), 3.45 (1H, d, J = 10.2 Hz, 19b-H), 3.16 (1H, brs, 9-H), 2.37 (1H, brd, J = 10.2 Hz, 10-H), 1.77 (1H, m, 11-H), 1.65–1.74 (1H, m, 11a-H), 1.44–1.50 (1H, m, 11b-H), 1.33 (1H, dd, J = 12.5, 4.6 Hz, 5-H), 1.23 (3H, s, 18-CH3), 1.11–1.16 (1H, td, J = 13.3, 3.8 Hz, 1b-H), 0.72 (3H, s, 20-CH3); 13C-NMR (151 MHz, CDCl3, TMS): δ 174.4 (C-16), 165.7 (C=O)amide, 145.0 (C-14), 134.1 (C-13), 133.8 (C-8), 125.4 (C-7), 80.8 (C-3), 70.2 (C-15), 63.9 (C-19), 52.0 (C-5), 50.2 (C-9), 43.4 (C1′CH2CO), 42.6 (C-4), 41.9 (C-17), 37.0 (C-1), 36.3 (C-10), 27.6 (C-2), 27.1 (C-12), 25.0 (C-11), 23.1 (C-6), 22.0 (C-18), 14.6 (C-20). Purity: 98.46%; retention time: 5.61 min.
\begin{align*}
\text{\textbf{31:1959–1973}}
\end{align*}
N-((1S, 5R, 6R, 8aS)-6-hydroxy-5-(hydroxymethyl)-5, 8a-dimethyl-1-(2-(2-oxo-2, 5-dihydrofuran-3-yl)ethyl)-1, 4, 4a, 5, 6, 7, 8, 8a-octahydropyridazin-2-yl)methyl)-4-methoxybenzamide (9)

Yield: 96.3%; White solid; m.p. 198–199 °C; HR-MS(ESI) m/z calc. for C29H32N2O7Na, 505.2066, found 505.2065 [M +Na]+; 1H-NMR (600 MHz, CDCl3): 8 7.70 (2H, d, J = 7.9 Hz, Ar–H), 7.24 (1H, s, Ar–H), 7.23 (2H, d, J = 8.4 Hz, Ar–H), 6.43 (1H, s, N–H), 5.76 (1H, s, 7-H), 4.77 (2H, s, 15-H), 4.45 (1H, d, J = 10.8 Hz, 19a-H), 4.18 (1H, dd, J = 15.0, 5.4 Hz, 17a-H), 3.92 (1H, dd, J = 15.0, 5.4 Hz, 17b-H), 3.45–3.47 (2H, m, 3H and 19–H), 2.39–2.42 (1H, m, 12a-H), 2.27–2.32 (1H, m, 12b-H), 2.13–2.17 (1H, brd, J = 17.7 Hz, 2a-H), 1.94–1.96 (1H, brd, J = 13.7 Hz, 2b-H), 1.88–1.90 (1H, brd, J = 18.0 Hz, 6a-H), 1.84 (1H, brs, 9-H), 1.77–1.79 (1H, brd, J = 12.3 Hz, 6b-H), 1.71–1.77 (2H, m, 1a-H and 11a-H), 1.52–1.57 (1H, m, 11b-H), 1.34 (1H, dd, J = 12.3, 4.1 Hz, 5-H), 1.22 (3H, s, 18-CH3), 1.11 (1H, t, J = 12.5 Hz, 1b-H), 0.72 (3H, s, 20-CH3); 13C-NMR (151 MHz, CDCl3, TMS): δ 174.6 (C-16), 167.2 (C(O)amide, 145.3 (C-14), 141.9 (C-4', Ar), 135.0 (C-13), 133.8 (C-8), 131.4 (1'C', Ar), 129.2×2 (C-3', C-5'), Ar), 126.8×2 (C-2', C-6', Ar), 125.5 (C-7), 80.8 (C-3), 70.3 (C-15), 63.9 (C-19), 52.1 (C-5), 43.8 (3-C), 41.9 (C-17), 37.1 (C-1), 36.3 (C-10), 27.7 (C-2), 27.2 (C-12), 25.1 (C-11), 23.1 (C-6), 22.1 (C-18), 14.7 (C-20). Purity: 94.46%; retention time: 11.34 min.

4-fluoro-N-((1S, 5R, 6R, 8aS)-6-hydroxy-5-(hydroxymethyl)-5, 8a-dimethyl-1-(2-(2-oxo-2, 5-dihydrofuran-3-yl)ethyl)-1, 4, 4a, 5, 6, 7, 8, 8a-octahydropyridazin-2-yl)methyl benzamide (11)

Yield: 93.4%; White solid; m.p. 176–179 °C; HR-MS(ESI) m/z calc. for C29H32N2O7NaFNa, 494.2319, found 494.2315 [M+Na]+; 1H-NMR (600 MHz, CDCl3): 8 7.84 (2H, dd, J = 8.6, 5.3 Hz, Ar–H), 7.23 (1H, s, 14-H), 7.11 (2H, t, J = 9.0 Hz, F–Ar–H), 6.67 (1H, d, J = 4.8 Hz, 5-H), 5.78 (1H, t, J = 2.4 Hz, 7-H), 4.79 (2H, s, 15-H), 4.25 (1H, d, J = 10.8 Hz, 19a-H), 4.14 (1H, dd, J = 15.0, 5.4 Hz, 17a-H), 3.96 (1H, dd, J = 15.0, 4.8 Hz, 17b-H), 3.45–3.46 (2H, m, 3-H and 19–H), 2.39–2.43 (1H, m, 12a-H), 2.30–2.35 (1H, m, 12b-H), 2.14–2.17 (1H, brd, J = 18.1 Hz, 2a-H), 1.92–1.94 (1H, dt, J = 13.3, 3.2 Hz, 2b-H), 1.87–1.90 (1H, brd, J = 16.7 Hz, 6a-H), 1.85 (1H, brs, 9-H), 1.79–1.81 (1H, brd, J = 14.7 Hz, 6b-H), 1.74–1.79 (1H, td, J = 13.3, 3.8 Hz, 1a-H), 1.67–1.71 (1H, m, 11a-H), 1.54–1.60 (1H, m, 11b-H), 1.34–1.36 (1H, dd, J = 12.2, 4.4 Hz, 5-H), 1.22 (3H, s, 18-CH3), 1.09–1.14 (1H, td, J = 13.3, 3.5 Hz, 1b-H), 0.72 (3H, s, 20-CH3); 13C-NMR (151 MHz, CDCl3, TMS): δ 174.9 (C-16), 166.3 (C=O)amide, 165.5 (C-4', Ar), 145.4 (C-14), 134.8 (C-13), 133.8 (C-8), 130.5 (1'C', Ar), 129.3×2 (C-3', C-5', Ar), 128.5 (C-7), 115.6×2 (C-3', C-5', Ar), 80.7 (C-3), 70.4 (C-15), 63.9 (C-19), 52.1 (C-5), 50.2 (C-9), 44.0 (C-4), 41.9 (C-17), 37.1 (C-1), 36.2 (C-10), 27.6 (C-2), 27.2 (C-12), 25.2 (C-11), 23.2 (C-6), 22.1 (C-18), 14.6 (C-20). Purity: 96.52%; retention time: 7.03 min.
4-bromo-97.36%, retention time: 7.04 min. (C-17), 37.1 (C-1), 36.3 (C-10), 27.7 (C-2), 27.3 (C-12), 25.3 (C-11), 23.2 (C-6), 22.0 (C-18), 14.6 (C-20). Purity: 97.48%, retention time: 7.03 min.

N-([1S,5R,6R,8aS]-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-1-(2-(2-oxo-5-dihydrofuran-3-yl)ethyl)-1,4,4a,5,6,7,8,8a-octahydropyridazine-2-yl)methyl)-4-nitrobenzamide (14)

Yield: 91.4%; White solid; m.p. 176–177 °C; HR-MS(ESI) m/z calcd. for C27H34NO5ClNa, 510.2023, found 510.2020 [M+Na]+; 1H-NMR (600 MHz, CDCl3): δ 8.27 (2H, d, J = 8.4 Hz, Ar–H), 8.03 (2H, d, J = 9.0 Hz, Ar–H), 7.25 (1H, s, 14-H), 5.84 (1H, t, J = 2.4 Hz, 7-H), 4.83 (2H, s, 15-H), 4.24 (1H, d, J = 10.8 Hz, 19a-H), 4.12 (1H, dd, J = 15.0, 5.4 Hz, 17a-H), 4.04 (1H, dd, J = 15.0, 5.4 Hz, 17b-H), 3.45–3.46 (2H, m, 3-H and 19b-H), 2.34–2.45 (1H, m, 12-H), 2.13–2.16 (1H, m, 2a-H), 1.88–1.94 (2H, m, 2b-H and 6a-H), 1.85 (1H, brs, 9-H), 1.81–1.83 (1H, brd, J = 13.4 Hz, 6b-H), 1.74–1.79 (1H, td, J = 13.6, 3.9 Hz, 1a-H), 1.60–1.68 (2H, m, 11-H), 1.34 (1H, dd, J = 12.2, 4.6 Hz, 7-H), 1.21 (3H, s, 18-CH3), 1.08–1.13 (1H, td, J = 13.6, 3.9 Hz, 1b-H), 0.72 (3H, s, 20-CH3); 13C-NMR (151 MHz, CDCl3, TMS): δ 175.3 (C-16), 165.4 (C=O) amide, 149.4 (C-4’, Ar), 145.7 (C-14), 139.9 (C-1’, Ar), 134.4 (C-13), 133.7 (C-8), 128.3 × 2 (C-2’, C-6’, Ar), 126.7 (C-7), 123.6 × 2 (C-3’, C-5’, Ar), 80.6 (C-3), 70.5 (C-15), 63.9 (C-19), 52.0 (C-5), 50.1 (C-9), 44.5 (C-4), 41.9 (C-17), 37.1 (C-1), 36.3 (C-10), 27.6 (C-2), 27.3 (C-12), 25.4 (C-11), 23.2 (C-6), 22.1 (C-18), 14.6 (C-20). Purity: 96.74%, retention time: 8.28 min.

According to the above-mentioned method, removal of acetyl groups in IM4 directly furnished compound 15. Yield: 96.3%; White solid; m.p. 158–162 °C; HR-MS(ESI) m/z calcd. for C28H33NO5Na, 520.2206, found 520.2206 [M+Na]+; 1H-NMR (600 MHz, CDCl3): δ 7.86 (2H, dd, J = 5.4, 3.0 Hz, ArH), 7.74 (2H, dd, J = 5.4, 3.0 Hz, Ar–H), 7.23 (1H, s, 14-H), 5.33 (1H, t, J = 1.8 Hz, 7-H), 4.8 (2H, s, 15-H), 4.30 (1H, d, J = 15.6 Hz, 17a-H), 4.24 (1H, d, J = 10.8, 19a-H), 4.18 (1H, d, J = 15.6 Hz, 17b-H), 3.46 (1H, dd, J = 11.4, 3.6 Hz, 3-H), 3.42 (1H, d, J = 10.8 Hz, 19b-H), 2.52–2.57 (1H, m, 12a-H), 2.36–2.41 (1H, m, 12b-H), 1.97–1.99 (1H, brd, J = 13.4 Hz, 2a-H), 1.91 (1H, brs, 9-H), 1.84–1.87 (2H, m, 2b-H and 6a-H), 1.80–1.83 (1H, brd, J = 13.5 Hz, 6b-H), 1.75–1.79 (1H, td, J = 13.6, 3.9 Hz, 1a-H), 1.63–1.73 (2H, m, 11-H), 1.34 (1H, dd,
$J = 12.4, 4.6$ Hz, $5$–$H_2$, $1.19$ (3$H$, s, $20$–$H$), $1.13$–$1.16$ (1$H$, m, $1b$–$H$), $0.76$ (3$H$, s, $18$–$H$). $^{13}$C-NMR (151 MHz, CDCl$_3$, TMS): $δ$174.1 (C–16), $168.1 \times 2$ (C–O)$_{amide}$, $144.7$ (C–14), $134.0 \times 2$ (C–3', C–4', Ar), $134.0$ (C–13), $133.9$ (C–8), $132.3 \times 2$ (C–1', C–6', Ar), $123.3 \times 2$ (C–2', C–5', Ar), $121.4$ (C–7), $80.8$ (C–3), $70.2$ (C–15), $63.9$ (C–19), $52.5$ (C–5), $50.0$ (C–9), $41.9$ (C–4), $40.0$ (C–17), $37.0$ (C–1), $36.4$ (C–10), $27.6$ (C–2), $27.3$ (C–12), $24.6$ (C–11), $22.7$ (C–6), $22.0$ (C–18), $14.8$ (C–20). Purity: 95.84%, retention time: 10.57 min.

Cells and cell culture

MDCK cells, used as host cells, were provided by Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences (Beijing, China). They were grown in Eagle’s minimum essential medium (EMEM) containing 10% fetal bovine serum (Gibco, USA), penicillin G (100 U/ml) and streptomycin (100 μg/ml). The cells were maintained in a humidified atmosphere containing 5% CO$_2$ at 37 °C.

Anti-H3N2 study

The antiviral activities were determined by CPE inhibition assay [61]. Influenza viruses A3/Beijing/30/95 were provided by Shanghai Municipal Center for Disease Control & Prevention (Shanghai, China). MDCK cells were seeded into 96-well plates and incubated for 24 h at 37 °C in 5% CO$_2$. After infected with 30 TCID$_{50}$ virus solutions, cultures were incubated for another 2 h at 37 °C in 5% CO$_2$, and then the supernatants of H3N2 were discarded. Subsequently, 100 μl various concentrations of compounds were added to quadruplicate culture wells. All cultures were incubated again in 5% CO$_2$ at 37 °C for 72 h. The cells were examined microscopically for CPE and 50% inhibitory concentration (IC$_{50}$) and therapeutic index (TI) were calculated.

Cytotoxicity activity of the derivatives in MDCK cells in vitro

MDCK cells were seeded into 96-well culture plates at a density of $1 \times 10^4$ cells per well. Cells were incubated for 24 h until 90% confluence. The derivative was dissolved in RPMI-1640 medium containing 10% fetal bovine serum, penicillin G (100 U/ml) and streptomycin (100 μg/ml) respectively. These dilutions were incubated with monolayer MDCK cells at 37 °C in 5% CO$_2$ for 72 h. Then cell growth was detected by modified MTT assay and cell viability was expressed as optical density.

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Compliance with ethical standards

Conflict of interest

The authors declare no competing interests.

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