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Synthesis and anti-influenza virus activity of dihydrofuran-fused perhydrophenanthrenes with a benzyloxy-type side-chain

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ABSTRACT

As one of our ongoing research project concerning development of a novel anti-influenza virus agent, dihydrofuran-fused perhydrophenanthrenes were derivatized by means of Williamson ether synthesis and Suzuki–Miyaura cross coupling reactions. Newly synthesized compounds were subjected to evaluation of anti-influenza virus activity using influenza A/Aichi/2/68 (H3N2 subtype) virus strain by a plaque titration method. These investigations revealed that incorporation of benzyl-type ether substituents was effective for exerting the inhibition activity of influenza virus proliferation.

1. Introduction

Among a number of infectious diseases, influenza has been one of the most serious medical problems and incessantly exerted deep influence on human health. Consequently, considerable efforts have been devoted to a new anti-influenza drug discovery for the purpose of improved therapeutic option.[1] Development of new agents with novel structure and mode of action has been particularly hastened providing for emergence of mutant viral antigens and drug resistance. In this context, we have reported that a series of dihydrofuran-fused perhydrophenanthrenes can be a promising candidate for a new anti-influenza agent, having a unique chemical structure characterized by a rigid cage-type conformation (Fig. 1).[2] Among them, the compound **1**, possessing a trifluoromethoxy substituent on the aromatic ring, suppressed virus proliferation (influenza A/Aichi/2/68, H3N2 subtype) to ca. 30% of control at 10 μM drug concentration, with low cytotoxicity and broad applicability for various influenza virus strains.[3] Several mechanistic studies suggested that this compound affected influenza virus growth at an early stage in the replication process, probably before mRNA synthesis.[3] This means that these compounds have a different mode of action from popular neuraminidase inhibitors such as oseltamivir and zanamivir. Moreover, other mechanisms of action dissimilar to amantadine were also suggested because of high efficiency of these compounds against influenza B viruses. Considering high potential of the dihydrofuran-fused perhydrophenanthrenes as a new type of anti-influenza agent, we planned further derivatization of this series of compounds in search for a new derivative with much more potent activity. In this paper, we wish to report synthesis and biological evaluation of several designed compounds with respect to the parent lead compound **1**.

2. Results and discussion

2.1. Chemistry

Our successive studies on the series of dihydrofuran-fused perhydrophenanthrenes have suggested that a substituent, which can bring the molecule a suitable lipophilicity, may be crucial for exerting potent biological activities.[2–4] For example, several derivatives having a triisopropylsilyl substituent on the aromatic ring exhibited the most potent growth inhibitory activity against Hemagglutinating Virus of Japan (HVJ)[2] as well as the enhancing effect for hyperthermia-induced apoptosis of human lymphoma U937 cells.[4] In addition, the fluoro-containing derivative **1** was the most effective on the inhibition of influenza virus proliferation, as mentioned

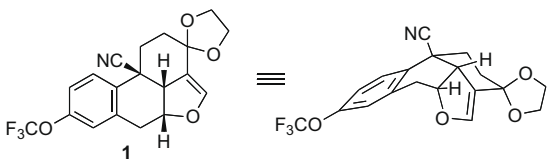

Figure 1. General view of dihydrofuran-fused perhydrophenanthrenes.

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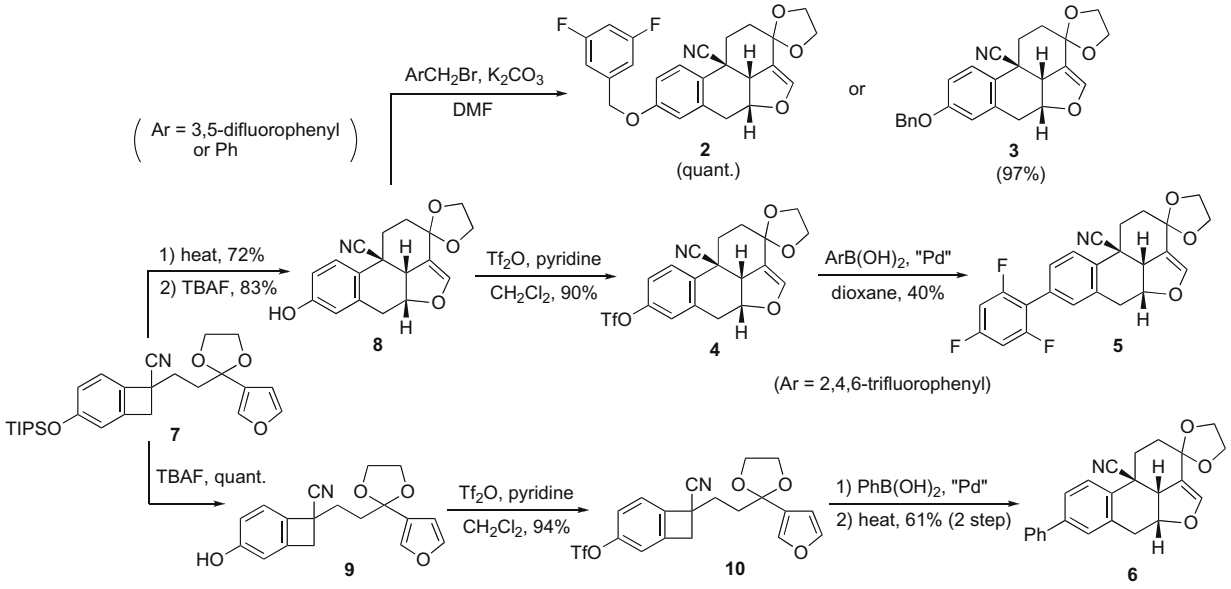


Scheme 1. Synthesis of compounds **2**–**6**.

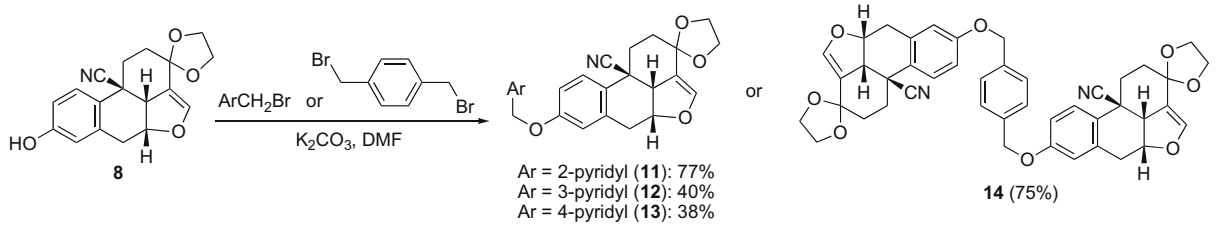
above.[3] With these findings in mind, we decided to synthesize compounds **2**–**6** possessing a hydrophobic aromatic and/or fluorine substituent, by means of Suzuki–Miyaura cross coupling reaction and Williamson ether synthesis.

Synthetic pathway to the target compounds **2**–**6** is outlined in Scheme 1. A starting material, phenolic compound **8**, was obtained according to the previously reported procedure,[2,3] involving stereoselective intramolecular cycloaddition of *o*-quinodimethane generated by a thermal electrocyclic ring-opening reaction of benzocyclobutene derivative **7**.[5] This compound was treated with difluorobenzyl bromide in the presence of K_2CO_3 to afford corresponding benzyl ether **2** in a quantitative yield. For comparison of effects of the fluorine substituent, simple benzyl ether **3** without fluorine was also prepared under the same conditions. On the other hand, introduction of aryl substituents could be achieved via Suzuki–Miyaura cross coupling reaction of triflate **4**, which was easily prepared from **8** upon treatment with triflic anhydride and pyridine. The triflate **4** was allowed to react with 2,4,6-trifluorophenylboronic acid and catalytic amount of $Pd(PPh_3)_4$ in the presence of K_3PO_4 and KBr in refluxing 1,4-dioxane to give the requisite arylated compound **5** in a moderate yield. Similarly, phenyl derivative **6** with no fluorine substituent was synthesized from **4**. However, we encountered a difficulty that the product **6** was inseparable from several side-products in this case. We then synthesized the compound **6** through another route, in which the cross coupling reaction was performed prior to the construction of the tetracyclic system. Namely, triflate **10** prepared from the compound **7** was subjected to the Suzuki–Miyaura coupling reaction, followed by the thermal electrocyclic reactions, to afford the phenyl derivative **6** as a pure form.

We further went on with the syntheses of several benzylated derivatives with structural modification, in the light of high efficacy of the simple benzyl ether **3** against virus proliferation (vide infra). As shown in Scheme 2, three pyridyl analogues (**11**–**13**) were synthesized from **8** under the same reaction conditions as the syntheses of **2** and **3**. In addition, homo-dimer compound **14** bridged by a benzyl ether chain was also prepared utilizing dibromo-*p*-xylene as a reagent.

2.2. Biology

Anti-influenza virus activity of the synthesized compounds was evaluated using influenza A/Aichi/2/68 (H3N2 subtype) virus strain. The virus yields as a percent of control were estimated by a plaque titration method.[3,6] Initially, the compounds **2**–**6** were surveyed at 10 μM drug concentration, using the trifluoromethylated derivative **1** as a reference compound, and the results are shown in Figure 2 (A). Triflate derivative **4** did not show the activity, and the compounds **5** and **6** having aryl substituents with a C–C bond exhibited moderate inhibitory effects on the virus growth (ca. 70% of control). On the other hand, the compounds **2** and **3** showed potent anti-influenza activity (ca. 30–40% of control), which reached a comparable level with the reference compound **1**. These results suggested that an ethereal linkage and a lipophilic aromatic substituent were crucial for exerting the anti-influenza effect. Contrary to our expectation, incorporation of the fluorine substituent did not influence the activity (**2** vs **3**, and **5** vs **6**). With these results in hand, several modified benzyl ether derivatives, including pyridyl analogues **11**–**13** and homo-dimer compound **14**, were also examined on their anti-influenza virus activity. As shown in Figure



Scheme 2. Synthesis of compounds **11**–**14**.

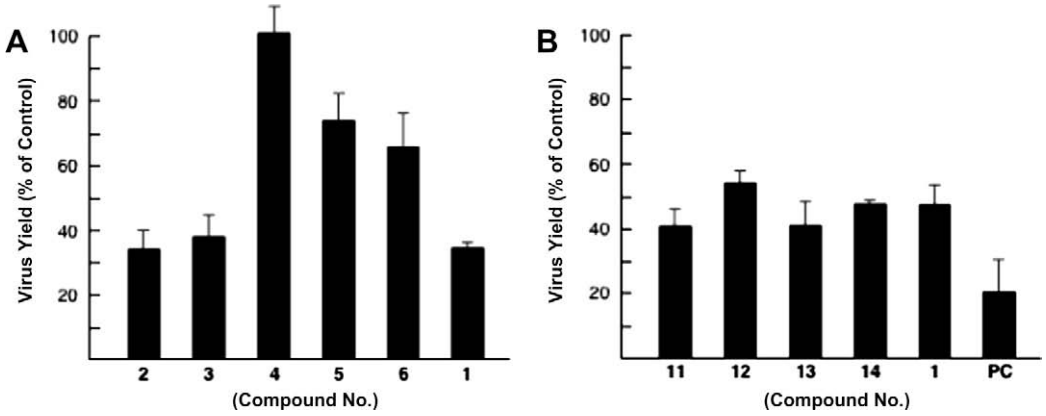


Figure 2. Inhibitory effects of the test compounds on the growth of influenza viruses. The assay was performed using influenza A/Aichi/2/68 (H3N2 subtype) virus strain in Madin–Darby canine kidney (MDCK) cells at 10 μM drug concentration. Amantadine was used as a positive control (**PC**). Data are expressed as means ± SD of three experiments (percent of control yielding 1.06×10^4 plaque forming unit (PFU)/mL).

2 (B), all of these compounds were proved to have almost equal activity to the compound **1**, though not up to clinically used amantadine.[7] These findings indicate that the benzyl-type substituents on the benzene ring of the tetracyclic framework can enhance the antivirus activity and be a suitable choice for further SAR studies of dihydrofuran-fused perhydrophenanthrenes as a novel anti-influenza virus agent.

3. Conclusion

In this paper, we described synthesis of dihydrofuran-fused perhydrophenanthrenes having various aromatic substituents and evaluation of their anti-influenza virus activity. It was found that the ethereal linkage of the substituents was necessary to preserve the potent activity, and that the benzyl ether side-chain or its modification was an effective appendage for the activity. Further studies aimed at developing an efficient anti-influenza virus candidate based on this series of compounds are underway in our laboratory.

4. Experimental

4.1. Chemistry

Reagents were purchased from commercial sources and used as received. Anhydrous solvents were obtained from commercial sources or prepared by distillation over CaH_2 or P_2O_5. ^1H and ^{13}C NMR spectra were obtained on a Varian Gemini 300 (300 MHz for ^1H and 75.46 MHz for ^{13}C), using chloroform as an internal reference. Mass spectra were measured on a JEOL D-200 or a JEOL AX 505 mass spectrometer, and the ionization method was electron impact (EI, 70 eV). IR spectra were recorded on a JASCO FT/IR-460Plus spectrometer. Melting points were taken with a Yanagimoto micro melting point apparatus and are uncorrected. Column chromatography was carried out by employing Cica Silica Gel 60N (spherical, neutral, 40–50 μm). Synthetic procedures for the compounds **7** and **8** have already been reported previously.[2,3]

4.1.1. General procedure for etherification of compound 8: Syntheses of 2, 3, and 11–14

To a stirred solution of the starting material **8** in DMF, potassium carbonate and alkyl bromide were added successively, and the mixture was stirred at room temperature for 24 h. Water was added, and the resulting aqueous mixture was extracted with Et_2O. The combined organic layer was washed with brine, and dried over $MgSO_4$. The residue obtained by evaporation of the solvent was subjected to column chromatography to afford the ether products.

4.1.1.1. 3,5-Difluorobenzyl ether 2. Compound **8** (15.7 mg, 0.05 mmol), 3,5-difluorobenzyl bromide (20.9 mg, 0.1 mmol), and K_2CO_3 (34.8 mg, 0.25 mmol) afforded the ether **2** (22.5 mg, quant.) as a colorless solid. Mp 163–164 °C; ^1H NMR ($CDCl_3$): δ 1.74–1.88 (2H, m), 2.46 (1H, ddd, J = 5.9, 12, 15 Hz), 2.76–2.84 (1H, m), 3.04 (1H, dd, J = 2.3, 16 Hz), 3.22 (1H, dd, J = 3.3, 16 Hz), 3.78–3.90 (4H, m), 3.92–3.98 (1H, m), 4.97 (2H, s), 5.23 (1H, ddd, J = 3.3, 4.3, 8.9 Hz), 5.96 (1H, d, J = 1.3 Hz), 6.69 (1H, dd, J = 2.3, 8.9 Hz), 6.75–6.84 (2H, m), 6.86–6.93 (2H, m), 7.15–7.19 (1H, m); ^{13}C NMR ($CDCl_3$): δ 29.7, 31.3, 34.1, 35.3, 51.1, 63.7, 65.3, 68.7, 79.7, 103.3 (t, J = 25 Hz), 104.6, 109.8 (m), 110.4, 113.4, 116.8, 122.2, 123.7, 127.0, 137.2, 140.8 (t, J = 9 Hz), 143.1, 158.3, 163.2 (dd, J = 13, 249 Hz); IR (KBr): 2233 cm^{-1}; MS: m/z 437 (M^+); HRMS calcd for $C_{25}H_{21}F_2NO_4$: 437.1439 (M^+), found: 437.1442; Anal. Calcd for $C_{25}H_{21}F_2NO_4 \cdot 1/2H_2O$: C, 67.26; H, 4.97; N, 3.14. Found: C, 66.98; H, 5.02; N, 3.16.

4.1.1.2. Benzyl ether 3. Compound **8** (20.3 mg, 0.065 mmol), benzyl bromide (22.3 mg, 0.13 mmol), and K_2CO_3 (45.1 mg, 0.326 mmol) afforded the ether **3** (25.5 mg, 97%) as a colorless solid. Mp 148–150 °C; ^1H NMR ($CDCl_3$): δ 1.71–1.89 (2H, m), 2.48 (1H, ddd, J = 5.2, 13, 15 Hz), 2.76–2.82 (1H, m), 3.04 (1H, dd, J = 2.3, 16 Hz), 3.21 (1H, dd, J = 3.0, 16 Hz), 3.76–3.85 (4H, m), 3.86–3.97 (1H, m), 4.99 (2H, s), 5.23 (1H, ddd, J = 2.3, 3.6, 11 Hz), 5.96 (1H, d, J = 1.3 Hz), 6.78–6.85 (2H, m), 7.13–7.18 (1H, m), 7.24–7.37 (5H, m); ^{13}C NMR ($CDCl_3$): δ 29.7, 31.3, 34.1, 35.2, 51.1, 63.7, 65.3, 70.0, 79.7, 104.7, 104.6, 110.4, 113.5, 116.8, 122.3, 123.0, 126.9, 127.5, 128.1, 128.6, 136.7, 136.9, 143.9, 158.9; IR (KBr): 2227 cm^{-1}; MS: m/z 401 (M^+); HRMS calcd for $C_{25}H_{23}NO_4$: 401.1627 (M^+), found: 401.1637; Anal. Calcd for $C_{25}H_{23}NO_4 \cdot 1/2H_2O$: C, 73.15; H, 5.89; N, 3.41. Found: C, 73.02; H, 5.62; N, 3.51.

4.1.1.3. 2-Pyridylmethyl ether 11. Compound **8** (30.1 mg, 0.097 mmol), 2-(bromomethyl)pyridine hydrobromide (48.9 mg, 0.193 mmol), and K_2CO_3 (133.6 mg, 0.967 mmol) afforded the ether **11** (29.8 mg, 77%) as a colorless oil. ^1H NMR ($CDCl_3$): δ 1.68–2.02 (2H, m), 2.50 (1H, ddd, J = 5.4, 13, 15 Hz), 2.76–2.84 (1H, m), 3.09 (1H, dd, J = 2.3, 16 Hz), 3.25 (1H, dd, J = 3.0, 16 Hz), 3.61–3.93 (4H, m), 3.93–4.06 (1H, m), 5.22 (2H, s), 5.28 (1H, ddd, J = 2.4, 3.5, 11 Hz), 6.00 (1H, d, J = 1.9 Hz), 6.82–7.00 (2H, m), 7.15–7.21 (2H, m), 7.48–7.52 (1H, m), 7.68–7.74 (1H, m), 8.50–8.69 (1H, m); ^{13}C NMR ($CDCl_3$): δ 29.7, 31.3, 34.1, 35.2, 51.1, 63.7, 65.3, 70.2, 79.7, 104.6, 110.3, 113.3, 117.0, 121.5, 122.2, 122.9, 123.4,

127.0, 137.1, 137.5, 143.1, 148.7, 156.7, 158.3; IR (neat): 2232 cm^{-1}; MS: *m/z* 402 (M^+); HRMS calcd for $C_{24}H_{22}N_2O_4$: 402.1580 (M^+), found: 402.1580.

4.1.1.4. 3-Pyridylmethyl ether 12. Compound **8** (30.5 mg, 0.098 mmol), 3-(bromomethyl)pyridine hydrobromide (49.6 mg, 0.196 mmol), and K_2CO_3 (135.4 mg, 0.98 mmol) afforded the ether **12** (15.6 mg, 40%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.65–1.92 (2H, m), 2.52 (1H, ddd, J = 6.5, 11, 15 Hz), 2.70–2.93 (1H, m), 3.10 (1H, dd, J = 2.3, 16 Hz), 3.27 (1H, dd, J = 3.4, 16 Hz), 3.79–3.96 (4H, m), 3.96–4.13 (1H, m), 5.01 (2H, s), 5.29 (1H, ddd, J = 2.4, 3.5, 11 Hz), 6.00 (1H, s), 6.79–6.99 (2H, m), 7.18–7.31 (1H, m), 7.32–7.57 (1H, m), 7.76–7.89 (1H, m), 8.61 (1H, s), 8.72 (1H, s); ^{13}C NMR ($CDCl_3$): δ 29.7, 31.3, 34.1, 35.2, 51.1, 63.7, 65.3, 67.2, 79.6, 104.6, 110.4, 113.4, 116.7, 122.2, 123.8, 124.2, 127.1, 136.8, 137.3, 143.1, 147.2, 147.8, 158.2; IR (neat): 2228 cm^{-1}; MS: *m/z* 402 (M^+); HRMS calcd for $C_{24}H_{22}N_2O_4$: 402.1580 (M^+), found: 402.1586.

4.1.1.5. 4-Pyridylmethyl ether 13. Compound **8** (31.0 mg, 0.10 mmol), 4-(bromomethyl)pyridine hydrobromide (50.4 mg, 0.199 mmol), and K_2CO_3 (137.6 mg, 0.996 mmol) afforded the ether **13** (15.1 mg, 38%) as a colorless solid. Mp 200–201 °C; ^{1}H NMR ($CDCl_3$): δ 1.75–1.95 (2H, m), 2.51 (1H, ddd, J = 6.2, 11, 14 Hz), 2.76–2.99 (1H, m), 3.09 (1H, dd, J = 2.3, 16 Hz), 3.26 (1H, dd, J = 3.2, 16 Hz), 3.60–3.95 (4H, m), 3.95–4.08 (1H, m), 5.09 (2H, s), 5.28 (1H, ddd, J = 2.4, 2.6, 11 Hz), 6.00 (1H, d, J = 2.2 Hz), 6.77–6.94 (2H, m), 7.17–7.25 (1H, m), 7.39 (2H, d, J = 5.7 Hz), 8.62 (2H, d, J = 5.7 Hz); ^{13}C NMR ($CDCl_3$): δ 29.7, 31.3, 34.1, 35.2, 51.1, 63.7, 65.3, 68.2, 79.6, 104.6, 110.4, 113.4, 116.8, 121.9, 122.1, 124.0, 127.1, 137.3, 143.1, 147.9, 148.5, 158.1; IR (KBr): 2233 cm^{-1}; MS: *m/z* 402 (M^+); HRMS calcd for $C_{24}H_{22}N_2O_4$: 402.1580 (M^+), found: 402.1578; Anal. Calcd for $C_{24}H_{22}N_2O_4{\cdot}H_2O$: C, 68.56; H, 5.75; N, 6.66. Found: C, 68.66; H, 5.57; N, 6.66.

4.1.1.6. Homo-dimer compound 14. Compound **8** (30.2 mg, 0.097 mmol), a,a'-dibromo-*p*-xylene (12.8 mg, 0.049 mmol), and K_2CO_3 (134.1 mg, 0.970 mmol) afforded the ether **14** (26.2 mg, 75%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.68–1.95 (4H, m), 2.51 (2H, ddd, J = 5.1, 13, 15 Hz), 2.71–2.90 (2H, m), 3.09 (2H, dd, J = 1.9, 16 Hz), 3.26 (2H, dd, J = 3.4, 16 Hz), 3.70–3.91 (8H, m), 3.91–4.12 (2H, m), 5.06 (4H, s), 5.28 (2H, ddd, J = 2.2, 3.5, 11 Hz), 6.01 (2H, d, J = 1.4 Hz), 6.74–6.92 (4H, m), 7.15–7.24 (2H, m), 7.44 (4H, s); ^{13}C NMR ($CDCl_3$): δ 29.3, 29.7, 31.3, 34.1, 35.2, 51.1, 63.7, 65.3, 69.7, 79.7, 104.6, 110.4, 113.5, 116.8, 122.3, 123.1, 126.9, 127.8, 136.6, 137.0, 143.1, 158.8; IR (neat): 2229 cm^{-1}; MS: *m/z* 725 (M^++H); HRMS Calcd for $C_{44}H_{41}N_2O_8$: 725.2863 (M^++H), found: 725.2844.

4.1.1.7. Synthesis of triflate compound 4. To a stirred solution of compound **8** (52.6 mg, 0.181 mmol) and pyridine (44 mL, 0.542 mmol) in CH_2Cl_2 (3 mL), triflic anhydride (61 mL, 0.361 mmol) was added dropwise at 0 °C, and the reaction mixture was stirred at the same temperature for 20 min. The reaction was stopped by addition of water, and the resulting mixture was extracted with CH_2Cl_2 and then dried over $MgSO_4$. Evaporation of the solvent gave a residue, which was purified by column chromatography to afford triflate **4** (72.1 mg, 90%) as a colorless solid. Mp 127–129 °C; ^{1}H NMR ($CDCl_3$): δ 1.69–1.87 (2H, m), 2.53 (1H, td, J = 4.6, 15 Hz), 2.78–2.86 (1H, m), 3.14 (1H, dd, J = 2.3, 16 Hz), 3.25 (1H, dd, J = 3.3, 16 Hz), 3.78–3.99 (5H, m), 5.26 (1H, ddd, J = 2.6, 3.3, 11 Hz), 5.94 (1H, d, J = 2.0 Hz), 7.16–7.19 (2H, m), 7.33–7.37 (1H, m); ^{13}C NMR ($CDCl_3$): δ 29.6, 31.3, 33.9, 35.5, 51.1, 63.8, 65.4, 79.1, 104.3, 110.2, 118.7 (q, J = 321 Hz), 120.1, 121.2, 123.2, 127.7, 131.6, 138.7, 143.2, 149.3; IR (KBr): 2231 cm^{-1}; MS: *m/z* 443 (M^+); HRMS calcd for $C_{19}H_{16}F_3NO_6S$: 443.0650 (M^+), found: 443.0664; Anal. Calcd for $C_{19}H_{16}F_3NO_6S$: C, 51.47; H, 3.64; N, 3.16. Found: C, 51.46; H, 3.43; N, 3.11.

4.1.1.8. Suzuki–Miyaura coupling reaction of 4: Synthesis of compound 5. A solution of triflate **4** (158 mg, 0.356 mmol), 2,4,6-trifluorophenylboronic acid (68.8 mg, 0.391 mmol), KBr (46.5 mg, 0.391 mmol), K_3PO_4 hexahydrate (142 mg, 0.533 mmol), and $Pd(PPh_3)_4$ (41.1 mg, 0.0356 mmol) in 1,4-dioxane (3 mL) was refluxed for 5 h. After completion of the reaction, the mixture was diluted with CH_2Cl_2, washed with satd $NaHCO_3$, and then dried over $MgSO_4$. Evaporation of the solvent gave a residue, which was purified by column chromatography to afford the compound **5** (60.5 mg, 40%) as a colorless solid. Mp 136–137 °C; ^{1}H NMR ($CDCl_3$): δ 1.82–1.97 (2H, m), 2.56 (1H, ddd, J = 5.2, 13, 15 Hz), 2.89–2.96 (1H, m), 3.17 (1H, dd, J = 2.5, 16 Hz), 3.30 (1H, dd, J = 3.6, 16 Hz), 3.84–4.07 (5H, m), 5.33 (1H, m), 6.01 (1H, d, J = 1.4 Hz), 7.26–7.40 (5H, m); ^{13}C NMR ($CDCl_3$): δ 29.9, 31.6, 34.1, 35.9, 51.4, 63.9, 65.5, 79.9, 104.8, 110.5, 122.2, 125.7, 127.5, 128.7, 130.6, 131.0, 135.5, 143.2; IR (KBr): 2228 cm^{-1}; MS: *m/z* 425 (M^+); HRMS calcd for $C_{24}H_{18}F_3NO_3$: 425.1239 (M^+), found: 425.1262; Anal. Calcd for $C_{24}H_{18}F_3NO_3{\cdot}H_2O$: C, 65.01; H, 4.55; N, 3.16. Found: C, 65.13; H, 4.80; N, 3.31.

4.1.1.9. Synthesis of compound 9. To a stirred solution of compound **7** (542 mg, 1.16 mmol) in THF (50 mL), tetra-*n*-butylammonium fluoride (1 M solution in THF, 1.17 mL, 1.27 mmol) was added dropwise at 0 °C, and the reaction mixture was stirred at room temperature for 30 min. The reaction mixture was diluted with satd NH_4Cl, and the aqueous solution was extracted with $CHCl_3$ and then dried over $MgSO_4$. Evaporation of the solvent gave a residue, which was purified by column chromatography to afford the compound **9** (362 mg, quant.) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.94–2.10 (2H, m), 2.14–2.24 (2H, m), 3.14 (1H, d, J = 14 Hz), 3.59 (1H, d, J = 14 Hz), 3.85–4.05 (4H, m), 6.31 (1H, dd, J = 1.0, 1.7 Hz), 6.60–6.64 (1H, m), 6.67 (1H, dd, J = 2.3, 8.2 Hz), 6.99 (1H, d, J = 8.2 Hz), 7.34–7.38 (2H, m); ^{13}C NMR ($CDCl_3$): δ 31.5, 36.0, 41.4, 42.1, 63.6, 64.8, 106.9, 108.5, 111.1, 115.7, 121.8, 123.0, 127.1, 134.8, 140.1, 142.1, 143.6, 157.3; IR (neat): 3389, 2234 cm^{-1}; MS: *m/z* 311 (M^+); HRMS calcd for $C_{18}H_{17}NO_4$: 311.1158 (M^+), found: 311.1135.

4.1.1.10. Synthesis of triflate compound 10. In accordance with the procedure for the synthesis of **4**, compound **9** (362 mg, 1.16 mmol), pyridine (281 mL, 3.48 mmol), and triflic anhydride (390 mL, 2.32 mmol) gave triflate **10** (484 mg, 94%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 2.02–2.12 (2H, m), 2.13–2.25 (2H, m), 3.28 (1H, d, J = 15 Hz), 3.71 (1H, d, J = 15 Hz), 3.78–4.00 (4H, m), 6.30–6.32 (1H, m), 7.09–6.12 (1H, m), 7.17 (1H, dd, J = 2.3, 8.2 Hz), 7.28 (1H, d, J = 8.2 Hz), 7.35–7.38 (2H, m); ^{13}C NMR ($CDCl_3$): δ 31.2, 36.1, 42.1, 42.3, 64.8, 106.6, 108.4, 118.0, 118.7 (q, J = 321 Hz), 120.4, 122.0, 124.0, 127.1, 140.1, 143.0, 143.61, 143.63, 150.4; IR (neat): 2236 cm^{-1}; MS: *m/z* 443 (M^+); HRMS calcd for $C_{19}H_{16}F_3NO_6S$: 443.0650 (M^+), found: 443.0621.

4.1.1.11. Synthesis of compound 6. In accordance with the procedure for the synthesis of **5**, triflate **10** (203 mg, 0.457 mmol), phenylboronic acid (61.3 mg, 0.503 mmol), KBr (59.9 mg, 0.503 mmol), K_3PO_4 hexahydrate (183 mg, 0.686 mmol), and $Pd(PPh_3)_4$ (52.9 mg, 0.0457 mmol) gave coupling product (167 mg) contaminated with several inseparable materials. This crude product was dissolved in *o*-dichlorobenzene (25 mL) and refluxed for 1 h. After evaporation of the solvent, the residue was purified by column chromatography to afford the compound **6** (104 mg, 61% in 2 steps) as a colorless solid. Mp 178–179 °C; ^{1}H NMR ($CDCl_3$): δ 1.83–2.00 (2H, m), 2.57 (1H, ddd, J = 4.9, 13, 15 Hz), 2.90–2.97 (1H, m), 3.22 (1H, dd, J = 2.3, 16 Hz), 3.34 (1H,

dd, J = 3.0, 16 Hz), 3.80–4.01 (5H, m), 5.34 (1H, ddd, J = 2.3, 3.3, 11 Hz), 6.03 (1H, d, J = 1.7 Hz), 7.29–7.59 (8H, m); ^{13}C NMR ($CDCl_3$): δ 29.7, 31.4, 34.0, 35.5, 51.1, 63.7, 65.3, 79.7, 104.6, 110.3, 122.0, 126.1, 127.1, 127.6, 128.8, 129.1, 129.9, 135.8, 140.2, 141.5, 143.2; IR (KBr): 2226 cm^{-1}; MS: m/z 371 (M^+); HRMS calcd for $C_{24}H_{21}NO_3$: 371.1522 (M^+), found: 371.1518; Anal. Calcd for $C_{24}H_{21}NO_3 \cdot H_2O$: C, 74.02; H, 5.95; N, 3.60. Found: C, 74.21; H, 5.71; N, 3.75.

4.2. Biology

4.2.1. Preparation of the drug solutions

The test compounds were dissolved in dimethyl sulfoxide (DMSO) and then diluted with suitable medium to become a final concentration of DMSO to less than 1%.

4.2.2. Virus

Influenza virus was propagated for 3 days at 35 °C in chorioallantoic cavities of 10-day old embryonated hen eggs. The infected allantoic fluids were clarified by centrifugation at 1000 g for 20 min and stored in small portions at −80 °C as a virus stock solution.

4.2.3. Cells

Madin–Darby canine kidney (MDCK) cells were cultured as monolayers in Eagle's minimum essential medium (MEM) supplemented with 8% fetal bovine serum in a humidified atmosphere containing 5% CO_2 at 37 °C.

4.2.4. Virus growth assay

A confluent monolayer of MDCK cells in a 24-well plate was washed once with phosphate buffered saline (PBS) and then infected with influenza A (Aichi/2/68) virus at a multiplicity of infection (MOI) of 5 plaque forming unit (PFU)/cell for 45 min at room temperature under a drug-free condition. After adsorption, the cells were washed three times with PBS and then cultured in serum free MEM with various drugs or 1% DMSO (as a control) at 37 °C. At 24 h post-infection, the culture fluids were collected and centrifuged at 500 g for 5 min. The virus yield in the supernatants was assayed by plaque titration on MDCK cells.[6]

References and notes

1. (a) Service, R. F. *Science* **1997**, *275*, 756–757; (b) Roberts, N. A. *Prog. Drug Res.* **2001**, *56*, 195–238; (c) Abdel-Magid, A. F.; Maryanoff, C. A.; Mehrman, S. J. *Curr. Opin. Drug Discov. Dev.* **2001**, *4*, 776–791; (d) McKimm-Breschkin, J. L. *Expert Opin. Pharmacother.* **2002**, *3*, 103–112.
2. (a) Matsuya, Y.; Sasaki, K.; Nagaoka, M.; Kakuda, H.; Toyooka, N.; Imanishi, N.; Ochiai, H.; Nemoto, H. *J. Org. Chem.* **2004**, *69*, 7989–7993; (b) Matsuya, Y.; Qin, H.; Nagaoka, M.; Nemoto, H. *Heterocycles* **2004**, *62*, 207–211.
3. (a) Matsuya, Y.; Sasaki, K.; Ochiai, H.; Nemoto, H. *Bioorg. Med. Chem.* **2007**, *15*, 424–432; (b) Matsuya, Y.; Imamura, Y.; Miyahara, T.; Ochiai, H.; Nemoto, H. *Eur. J. Org. Chem.* **2008**, 1426–1430.
4. Yu, D.-Y.; Matsuya, Y.; Zhao, Q.-L.; Ahmed, K.; Wei, Z.-L.; Nemoto, H.; Kondo, T. *Apoptosis* **2007**, *12*, 1523–1532.
5. (a) Toyooka, N.; Nagaoka, M.; Nemoto, H. *Synlett* **2001**, 1123–1124; (b) Toyooka, N.; Nagaoka, M.; Sasaki, E.; Qin, H.; Kakuda, H.; Nemoto, H. *Tetrahedron* **2002**, *58*, 6097–6101.
6. (a) Tobita, K. *Med. Microbiol. Immunol.* **1975**, *62*, 23–27; (b) Mantani, N.; Imanishi, N.; Kawamata, H.; Terasawa, K.; Ochiai, H. *Planta Med.* **2001**, *67*, 240–243.
7. (a) Pinto, L. H.; Holsinger, L. J.; Lamb, R. A. *Cell* **1992**, *69*, 517–528; (b) Hayden, F. G.; Hay, A. J. *Curr. Top. Microbiol. Immunol.* **1992**, *176*, 119–130.