Synthesis of (5R,8R)-2-(3,8-Dimethyl-2-oxo-1,2,4,5,6,7,8,8α-octahydroazulen-5-yl) Acrylic Acid (Rupestonic Acid) Amide Derivatives and *in vitro* Inhibitive Activities against Influenza A_3,B and Herpes Simplex Type 1 and 2 Virus

Jian-ping Yong,[†,‡] Qiao-ying Lv,[†] and Haji Akber Aisa[†,*]

[†]*Xinjiang Technical Institute of Physics and Chemistry, The Key Laboratory of Plant Resources and Natural Products Chemistry, Chinese Academy of Science, Urumqi 830011, China. *E-mail: haji@ms.xjb.ac.cn*

[‡]*Graduate School of Chinese Academy of Science, Beijing 100039, China*

Received September 17, 2008, Accepted January 12, 2009

19 Aromatic ring and L-amino acid ester contained rupestonic acid amide derivatives **2a~2l, 3a~3g** were synthesized and preliminarily evaluated *in vitro* against influenza virus A_3,B and herpes simplex virus type 1 (HSV-1), 2(HSV-2) by the national center for drug screening of China. The rusults showed that **2i** possessed the highest inhibition against both influenza virus A_3 (TC_{50} = 120.6 μmol/L, IC_{50} = 19.2 μmol/L, SI = 6.3) and B (TC_{50} = 120.6 μmol/L, IC_{50} = 29.9 μmol/L, SI = 4.0); **2g** was more active against influenza A_3 virus at very low cytotoxicity (TC_{50} > 2092.1 μmol/L, IC_{50} = 143.7 μmol/L, SI > 14.6) than the parent compound; Compounds **2b, 2c, 2f** showed higher activities both against HSV-1 and HSV-2 than that of the parent compound, and **2f** was the most potent inhibitor of HSV-1 (TC_{50} = 200.0 μmol/L, IC_{50} = 11.3 μmol/L, SI = 17.7) and HSV-2 (TC_{50} = 200.0 μmol/L, IC_{50} = 20.7 μmol/L , SI = 9.7).

Key Words: Synthesis. Rupestonic acid amide derivative. Anti-viral activities

Introduction

Artemisia rupestris L.(Chinese name is Yizhihao) is one kind of Chinese traditional herbal medicines long been used in folk of Xinjiang of China. It is known to be effective as antiallergic,[1] antitumour,[2] antiinflammatory, antibacterial,[3] antidote agents.[4,5] (5R,8R)-2-(3,8-Dimethyl-2-oxo-1,2,4,5,6,7,8,8α-octahydroazulen-5-yl) acrylic acid (Rupestonic acid, compound **1**)[6] is a sesquiterpene with multifunctional groups, isolated from the *Artemisia rupestris* L. Sesquiterpenes always exhibit considerable biological activities. Sheu Jyh-Horng and his coworkers have reported that some sesquiterpeneses exhibit potent cytotoxicity toward P-388, A549 and HT-26 cancer cell lines.[7] Amy E. Wright and her coworkers have reported that the sesquiterpeneses hydroquinone and its acetate derivative exhibit higher activities against the P-388 tumor cell line and influenza strain PR-8.[8] We also have tested the rupestonic acid against influenza A_3/Jifang/90/15, B/Jifang/97/13, HSV-1 (VR733) and HSV-2(SAV) viruses. The results showed that it exhibits higher inhibition against influenza B virus (TC_{50} = 1044.4 μmol/L, IC_{50} = 115.7 μmol/L, SI = 9.0). Because of its special structure, we take the lead in modifying its structure with the aim of obtaining the more biological significance.

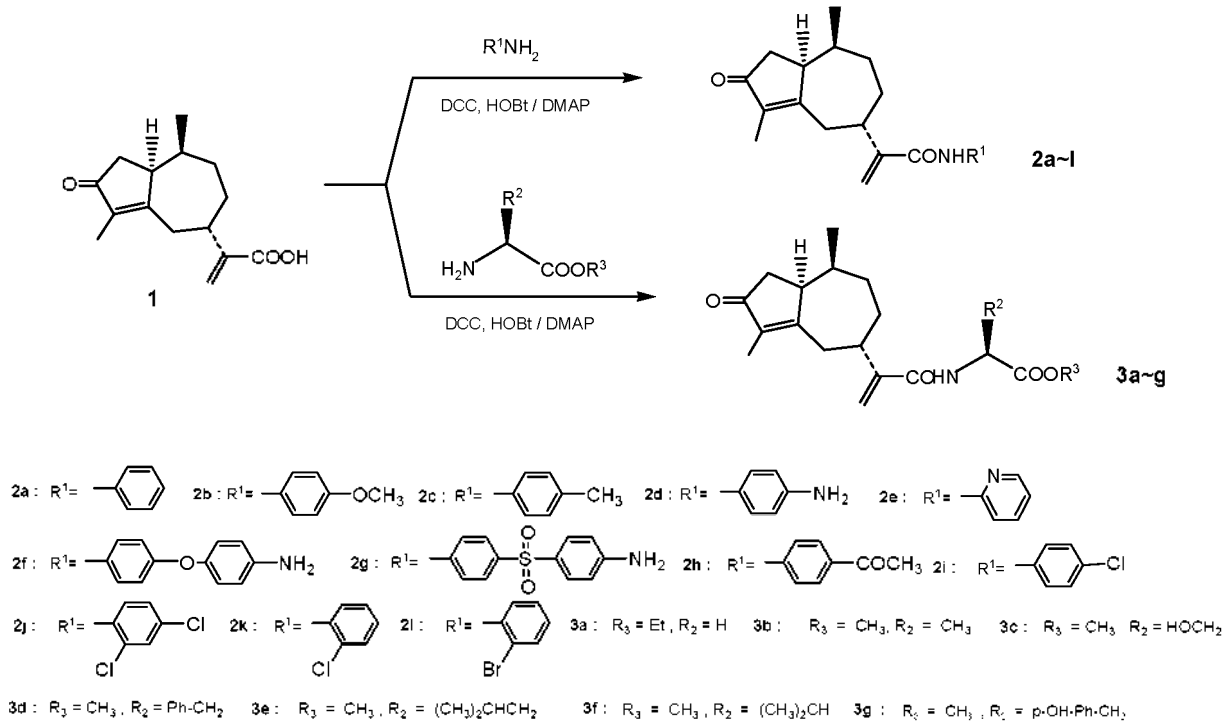
In this study, we mainly modified the carboxyl group of rupestonic acid and synthesized 19 rupestonic acid amide derivatives. All compounds were confirmed by IR, ^{1}H NMR and ESI-MS spectral datas, and preliminarily assayed their in vitro activities against influenza virus A_3,B and HSV-1 and HSV-2 comparing the positive drugs Ribavirin(RBV), Oseltamivir and Acyclovir(ACV).

Experimental Section

Chemistry. All melting points were determined on Yanaco MP-300 micro melting points apparatus and values are uncorrected; ^{1}H NMR spectra datas were recorded on a varian inova-400 spectrometer, using the tetramethylsilane (TMS) as an internal reference and $CDCl_3$ as the solvent; ESI-MS were performed on a HP1100LC/MS; Rupestonic acid was isolated from the *Artemisia rupestris* L (purity: over 98%); Dicyclohexyl carbodiimide(DCC), 1-hydroxybenzotriazole(HOBt), 4-dimethylaminopyridine(DMAP) were purchased from Shanghai reagent Company, China; Other chemicals are commercially available and used without further purification. THF was distilled from sodium and benzophenone before used.

General synthetic procedure for the preparation of rupestonic acid derivatives (2a-2l, 3a-3g, Scheme 1).[9] Compound **1** 0.124 g (0.5mmol), DCC 0.11 g (0.55mmol) and HOBt 0.08 g (0.6mmol) were added into a 25 mL one naked round bottle flask with 5 mL dry THF, the mixture was stirred at 0 °C for about 10 min, then DMAP 0.07 g (0.55mmol) was added and the mixture continued stirring at 0 °C for 30 min. Subsequently, 0.6 mmol aromatic amine (amino acid ester hydrochlorate) was added, the mixture stirred for 30 min under cold bath then rised naturally to room temperature. The completion of reaction was judged from the simple TLC analysis. The mixture was evaporated under reduced pressure, and the residual purified directly by column chromatography (EtOAc/Petroleum ether: 5:1→2:1) to give the desired compounds **2a-2l, 3a-3g.**

Compound 2a: White solid, yield: 75.8%, mp.182~183 °C, IR(KBr)υ: 3300, 2951, 2916, 1693, 1658, 1627, 1595, 1537, 1440, 1321, 759 cm^{-1}; ^{1}H NMR(600 MHz, $CDCl_3$): δ 0.66(d, *J* = 7.2 Hz, 3H, CH_3), 1.67-1.69(s, 4H, $2CH_2$), 1.87(s, 3H, CH_3), 2.03-2.04(m, 1H), 2.14-2.16(m, 1H), 2.52-2.62 (m, 2H, CH_2), 2.91-2.95(m, 1H), 3.01-3.05(m, 1H), 3.20-3.22(m, 1H), 5.48(s, 1H), 5.69(s, 1H), 7.13-7.14(m, 1H, Ph-H), 7.37-7.38(m, 2H, Ph-H), 7.57-5.77(m, 2H, Ph-H), 7.62(s, 1H, CONH); ESI-MS (m/z, 100%): 324([M+1], 13), 346([M+23], 100).



Scheme 1

Compound 2b: White solid, yield: 78.4%, mp.152~153 °C. IR(KBr)υ: 3259, 2961, 2918, 1694, 1653, 1624, 1597, 1537, 1510, 1327, 825 cm^{-1}; ^1H NMR(600 MHz, $CDCl_3$): δ 0.66(d, J = 7.2 Hz, 3H, CH_3), 1.67(s, 4H, $2CH_2$), 1.83(s, 3H, CH_3), 2.05-2.07(m, 1H), 2.13-2.15(m, 1H), 2.53-2.61(m, 2H, CH_2), 2.90-2.94(m, 1H), 2.99-3.01(m, 1H), 3.18(m, 1H), 3.80(s, 3H, OCH_3), 5.44(s, 1H), 5.66(s, 1H), 6.87 (d, J = 8.4 Hz, 2H, Ph-H), 7.48 (d, J = 9.4 Hz, 2H, Ph-H), 7.65(s, 1H, CONH); ESI-MS(m/z, 100%): 354([M+1], 7), 376([M+23], 100).

Compound 2c: White solid, yield: 78.6%, mp.143~145 °C. IR(KBr)υ: 3283, 2961, 2916, 1693, 1657, 1628, 1593, 1514, 1317, 813 cm^{-1}; ^1H NMR(600 MHz, $CDCl_3$): δ 0.66(d, J = 7.2 Hz, 3H, CH_3), 1.66-1.69(s, 4H, $2CH_2$), 1.87(s, 3H, CH_3), 2.05-2.07(m, 1H), 2.14-2.15(m, 1H), 2.33(s, 3H, Ph-CH_3), 2.51-2.61(m, 2H, CH_2), 2.91-2.94(m, 1H), 3.00-3.04(m, 1H), 3.19-3.21(m, 1H), 5.45(s, 1H), 5.67(s, 1H), 7.16 (d, J = 7.8 Hz, 2H, Ph-H), 7.45 (d, J = 8.4 Hz, 2H, Ph-H), 7.60(s, 1H, CONH); ESI-MS(m/z, 100%): 338([M+1], 9), 360([M+23], 100).

Compound 2d: White solid, yield: 97.04%, mp.83~85 °C. IR(KBr)υ: 3340, 3232, 2954, 2920, 1684, 1655, 1622, 1599, 1514, 1429, 1383, 1321, 912, 743 cm^{-1}; ^1H NMR(600 MHz, $CDCl_3$): δ 0.66(d, J = 7.3 Hz, 3H, CH_3), 1.65-1.68(s, 4H, $2CH_2$), 1.87(s, 3H, CH_3), 2.12-2.13(m, 1H), 2.14-2.15(m, 1H), 2.50-2.61(m, 2H, CH_2), 2.91-2.94(m, 1H), 2.99-3.01(m, 1H), 3.18-3.19(m, 1H), 3.69(brs, 2H, NH_2), 5.42(s, 1H), 5.64(s, 1H), 6.66-6.68(m, 2H, Ph-H), 7.32-7.34(m, 2H, Ph-H), 7.51(s, 1H, CONH); ESI-MS(m/z , 100%) : 339([M+1], 12), 361([M+23], 100).

Compound 2e: Light yellow solid, yield: 90.1%, mp.70~71 °C. IR(KBr)υ: 3339, 2955, 2918, 1694, 1609, 1568, 1531, 1470, 1454, 1366, 912, 745 cm^{-1}; ^1H NMR(600 MHz, $CDCl_3$): δ 0.65(d, J = 7.3 Hz, 3H, CH_3), 1.66-1.69(s, 4H, $2CH_2$), 1.88(s, 3H, CH_3), 2.12-2.13(m, 1H), 2.14-2.16(m, 1H), 2.50-2.61(m, 2H, CH_2), 2.91-2.94(m, 1H), 2.99-3.01(m, 1H), 3.18-3.19(m, 1H), 5.42(s, 1H), 5.66(s, 1H), 6.37(s, 1H, Ar-H), 6.54(s, 1H, Ar-H), 7.52-7.55(m, 1H, Ar-H), 8.12-8.14(m, 1H, Ar-H), 8.64 (s, 1H, CONH); ESI-MS(m/z, 100%): 325([M+1], 10), 348 ([M+23], 100).

Compound 2f: White solid, yield: 93.02%, mp.80~81 °C, IR(KBr)υ: 3337, 2957, 2918, 1684, 1624, 1533, 1499, 1221, 912, 743 cm^{-1}; ^1H NMR(600 MHz, $CDCl_3$): δ 0.67(d, J = 7.3Hz, 3H, CH_3), 1.66-1.71(m, 4H, $2CH_2$), 1.87-1.88(m, 3H, CH_3), 2.00-2.06(m, 1H), 2.16-2.18(m, 1H), 2.50-2.66(m, 2H, CH_2), 2.90-2.97(m, 1H), 3.08-3.10(m, 1H), 3.21-3.22(m, 1H), 3.60(brs, 2H, Ph-NH_2), 5.47(s, 1H), 5.68(s, 1H), 6.00-6.01(m, 2H, Ph-H), 6.02-6.04(m, 2H, Ph-H), 6.13-6.14(m, 2H, Ph-H), 6.54-6.56(m, 2H, Ph-H), 7.68-7.70(m, 1H, NHCO); ESI-MS(m/z, 100%): 431([M+1], 6), 453([M+23], 27).

Compound 2g: White solid, yield: 42.26%, mp. 68~70 °C, IR(KBr)υ: 3468, 3340, 1744, 1692, 1603, 1533, 1467, 1447, 1367, 1205, 1115, 912, 743 cm^{-1}; ^1H NMR(600 MHz, $CDCl_3$): δ 0.65(d, J = 7.3 Hz, 3H, CH_3), 1.64-1.69(brs, 4H, $2CH_2$), 1.86(s, 3H, CH_3), 2.03-2.05(m, 1H), 2.14-2.15(m, 1H), 2.51-2.62(m, 2H, CH_2), 2.91-2.94(m, 1H), 2.99-3.01(m, 1H), 3.19-3.20(m, 1H), 3.60(brs, 2H, Ar-NH_2), 5.47(s, 1H), 5.68(s, 1H), 6.68-6.70(m, 2H, Ph-H), 6.85-6.87(m, 2H, Ph-H), 6.93-6.99(m, 2H, Ph-H), 7.57-7.54(m, 2H, Ph-H), 7.57-7.60(m, 1H, Ph-NHCO); ESI-MS (m/z, 100%): 479([M+1], 10), 501([M+23], 100).

Compound 2h: Light yellow oil, yield: 18.2 %, IR(KBr)υ: 3348, 2958, 2920, 1751, 1720, 1705, 1683, 1665, 1541, 1201, 828 cm^{-1}; ^1HNMR(600 MHz, $CDCl_3$): δ 0.68(d, J = 7.2Hz, 3H, CH_3), 1.67(s, 4H, $2CH_2$), 1.83(s, 3H, CH_3), 2.05-2.06(m, 1H), 2.13-2.14(m, 1H), 2.53-2.61(m, 5H, O=C-CH_3, CH_2), 2.90-2.94(m, 1H), 2.99-3.01(m, 1H), 3.19-3.21(m, 1H), 5.44(s, 1H), 5.66(s, 1H), 7.75 (d, J = 8.4 Hz, 2H, Ph-H), 7.83 (d, J = 8.3 Hz, 2H,

Ph-H), 8.16 (s, 1H, CONH); ESI-MS (m/z, 100%): 366([M+1], 9), 388([M+23], 100).

Compound 2i: Colorless solid, yield: 75.5%, m.p: 57-58 °C; IR(KBr) / cm^{-1} 3300, 2951, 2916, 1693, 1658, 1627, 1595, 1537, 1440, 1321, 825; ^{1}H NMR($CDCl_3$): δ 0.66(d, J = 7.2 Hz, 3H, CH_3), 1.67-1.69(s, 4H, $2CH_2$), 1.87(s, 3H, CH_3), 2.03-2.04(m, 1H), 2.14-2.15(m, 1H), 2.52-2.62(m, 2H, CH_2), 2.91-2.95(m, 1H), 3.01-3.05(m, 1H), 3.20-3.21(m, 1H), 5.60(s, 1H), 6.13 (s, 1H), 6.51(brs, 1H, CONH-), 7.01-7.05(m, 2H), 7.50-7.54(m, 2H); MS (*m/z*, %) : 358.8 ($[M+1]^+$, 80), 380.6 ($[M+23]^-$, 90).

Compound 2j: Light yellow oil, yield: 46.8%, IR (KBr) / cm^{-1} 3320, 2951, 2916, 1693, 1658, 1627, 1595, 1537, 1440, 1321, 835 ; ^{1}H NMR($CDCl_3$) δ 0.66 (d, J = 7.2 Hz, 3H, CH_3), 1.65-1.71(s, 4H, $2CH_2$), 1.87(s, 3H, CH_3), 2.05-2.07(m, 1H), 2.16-2.18(m, 1H), 2.52-2.62(m, 2H, CH_2), 2.91-2.95(m, 1H), 3.01-3.05(m, 1H), 3.20-3.21(m, 1H), 5.65(s, 1H), 6.12(s, 1H), 6.55(brs, 1H, CONH-), 7.14-7.16(d, J = 8.0 Hz,1H), 7.28-7.31 (m, 1H), 7.48(d, J = 7.6 Hz,1H); MS (*m/z*, %): 393.1 ($[M+1]^-$, 80).

Compound 2k: Light ceraceous solid, yield: 40.6%, IR(KBr)/ cm^{-1} 3320, 2951, 2916, 1693, 1658, 1627, 1595, 1537, 1440, 1321, 835; ^{1}H NMR($CDCl_3$): δ 0.66(d, J = 7.2 Hz, 3H, CH_3), 1.67-1.69(s, 4H, $2CH_2$), 1.87(s, 3H, CH_3), 2.03-2.04(m, 1H), 2.14-2.16(m, 1H), 2.52-2.62(m, 2H, CH_2), 2.91-2.95(m, 1H), 3.01-3.05(m, 1H), 3.20-3.22(m, 1H), 5.56(s, 1H), 6.18(s, 1H), 6.55(brs, 1H, CONH-), 7.19-7.26(m, 2H), 7.30-7.34(m, 1H), 7.46-7.48(d,d, J = 7.26 Hz, J = 7.8 Hz,1H); MS (*m/z*, %): 358.6 ($[M+1]^-$, 80).

Compound 2l: Light solid, yield: 36.2%, m.p: 128-129 °C ; IR(KBr) / cm^{-1} 3320, 2951, 2916, 1693, 1658, 1627, 1595, 1537, 1440, 1321, 835; ^{1}H NMR($CDCl_3$): δ 0.66(d, J = 7.2 Hz, 3H, CH_3), 1.65-1.71(s, 4H, $2CH_2$), 1.87(s, 3H, CH_3), 2.05-2.07(m, 1H), 2.16-2.17(m, 1H), 2.52-2.62(m, 2H, CH_2), 2.91-2.95(m, 1H), 3.01-3.05(m, 1H), 3.20-3.22(m, 1H), 5.66(s, 1H), 6.13(s, 1H), 6.53(brs, 1H, CONH-), 7.17-7.28(m, 2H), 7.31-7.35(m, 1H), 7.48-7.50(m, 1H); MS(*m/z*, %): 403.1 ($[M+1]^-$, 80).

Compound 3a: Yellow oil, yield: 90.1 %, IR(KBr)υ: 3348, 2958, 2920, 1751, 1720, 1705, 1683, 1665, 1541, 1201, 828 cm^{-1}; ^{1}H NMR(600 MHz, $CDCl_3$): δ 0.67(d, J = 7.2Hz, 3H, CH_3), 1.29(t, J = 8.5Hz, 3H, CH_3), 1.60-1.61(m, 3H, CH_3), 1.78-1.79(m, 4H, $2CH_2$), 1.84-1.86(m, 1H), 2.13-2.14(m, 1H), 2.51-2.52(m, 1H), 2.56-2.57(m, 1H), 2.60-2.97(m, 2H, CH_2), 3.20(s, 1H), 3.75(s, 1H), 4.05-4.08(m, 2H, CH_2), 5.40(s, 1H), 5.7(s, 1H), 6.94 (s, 1H, NH); ESI-MS(*m/z*, 100%): 334([M+1], 13), 356([M+23], 100), 372([M+39], 20).

Compound 3b: White solid, yield: 87.4%, mp.126~128 °C, IR(KBr)υ: 3327, 2954, 2920, 1747(br), 1693, 1681, 1660, 1622, 1531, 1213, 813 cm^{-1}; ^{1}H NMR(600 MHz, $CDCl_3$): δ 0.66(d, J = 7.2Hz, 3H, CH_3), 1.47(d, J = 2.4Hz, 3H, CH_3), 1.61-1.65(m, 4H), 1.82(s, 3H, CH_3), 2.03-2.04(m, 1H), 2.13-2.15(m, 1H), 2.43-2.48(m, 1H), 2.57-2.61(m, 1H), 2.86-2.95(m, 2H), 3.18-3.19(m, 1H), 3.78(s, 3H, CH_3), 4.63-4.65(m, 1H), 5.39 (s, 1H), 5.61(s, 1H), 6.42(d, J = 6.0Hz, 1H, NH); ESI-MS (*m/z*, 100%): 334 ([M+1], 14), 356 ([M+23]).

Compound 3c: White solid, yield: 94.6%, mp. 58~60 °C, IR(KBr)υ: 3445(brs), 2954, 2927, 1747, 1732, 1693, 1681, 1666, 1622, 1531, 1211, 852 cm^{-1}; ^{1}H NMR(600 MHz, $CDCl_3$): δ 0.66(d, J = 7.2Hz, 3H, CH_3), 1.60-1.64(m, 4H), 1.86(d, J = 10.8Hz, 3H, CH_3), 2.03-2.06(m, 1H), 2.14-2.15(m, 1H), 2.44-2.50(m, 1H), 2.57-2.61(m, 1H), 2.90-2.96(m, 2H), 3.20-3.21(m, 1H), 3.78(s, CH_3), 3.94(t, J = 2.4 Hz, OH), 4.07-4.11(m, 2H, CH_2-OH), 4.69(t, J = 4.2 Hz, 1H), 5.42(s, 1H), 5.73 (s,1H), 7.11(d, J = 7.8 Hz, 1H, NH); ESI-MS(*m/z*, 100%): ([350[M+1], 10), 372(M+23], 100).

Compound 3d: White solid, yield: 90.7%, mp. 48~50 °C, IR(KBr)υ: 3325, 3028, 2954, 2920, 1746, 1732, 1693, 1681, 1666, 1622, 1545, 1531, 1215, 735 cm^{-1}; ^{1}H NMR(600 MHz, $CDCl_3$): δ 0.72(d, J = 7.2Hz, 3H, CH_3), 1.61-1.71(m, 4H), 1.80-1.87(m, 3H, CH_3), 2.09-2.10(m, 1H), 2.19-2.20(m, 1H), 2.47-2.49(m, 1H), 2.52-2.53(m, 1H), 2.86-2.96(m, 2H), 3.22-3.23(m, 2H, Ph-CH_2), 3.33-3.35(m, 1H), 3.84(s, 3H, CH_3), 4.99-5.01(m, 1H), 5.40-5.52(m, 1H), 5.57-5.58(m, 1H), 6.59-6.60(m, 1H, NH), 7.21-7.22(m, 2H, Ph-H), 7.36-7.37(m, 3H, Ph-H), ESI-MS(m/z, 100%): 410([M+1], 10), 432([M+23], 100).

Compound 3e: Yellow oil, yield: 95.2%, IR(KBr)υ: 3325, 2957, 2926, 1745, 1693, 1681, 1660, 1625, 1525, 1203, 929 cm^{-1}; ^{1}H NMR(600 MHz, $CDCl_3$): δ 0.66(d, J = 7.2Hz, 3H, CH_3), 0.89-0.97(m, 6H, $2CH_3$), 1.54-1.82(m, 8H), 1.63-1.64(m, 3H, CH_3), 1.87-1.88(m, 1H), 2.55-2.56(m, 1H), 2.87-2.88(m, 2H, CH_2), 3.21(s, 1H), 3.75(s, 1H), 3.78(s, 3H, OCH_3), 4.67-4.68(m, 1H), 5.39-5.40(m, 1H), 5.65-5.67(m, 1H), 6.65-6.68(m, 1H, NH); ESI-MS(*m/z*, 100%): 376([M+1], 15), 398([M+23], 100).

Compound 3f: Colorless oil, yield: 90.3%, IR(KBr)υ: 3343, 2962, 2930, 1746, 1725, 1709, 1693, 1666, 1633, 1519, 1382, 1323, 1201 cm^{-1}; ^{1}H NMR(600 MHz, $CDCl_3$): δ 0.66(d, J = 7.3Hz, 3H, CH_3), 0.92-0.93(m, 1H), 0.97-0.99(m, 6H, $2CH_3$), 1.63-1.68(m, 4H), 1.81-1.82(m, 3H, CH_3), 2.02-2.03(m, 1H), 2.14-2.15(m, 1H), 2.25-2.27(m, 1H), 2.49-2.52(m, 1H), 2.56-2.61(m, 1H), 2.89-2.95(m, 2H), 3.19-3.20(m, 1H), 3.77(s, 3H, OCH_3), 4.60-4.61(m, 1H), 5.39-5.40(m, 1H), 5.64-5.66(m, 1H), 6.48-6.49(m, 1H, NH), ESI-MS(m/z, 100%): 362([M+1], 11), 384([M+23], 100).

Compound 3g: White solid, yield: 82.9 %, mp. 84~86 °C, IR(KBr)υ: 3308(brs), 3017, 2954, 2920, 1744(br), 1687, 1678, 1614, 1516, 1466, 1382, 1221, 837 cm^{-1}; ^{1}H NMR(600 MHz, $CDCl_3$): 0.64(d, J = 7.3Hz, 3H, CH_3), 1.63-1.76(m, 4H), 1.81-1.82(m, 3H, CH_3), 2.05-2.06(m, 1H), 2.07-2.09(m, 1H), 2.45-2.47 (m, 1H), 2.60-2.61(m, 1H), 2.78-2.85(m, 2H), 3.07-3.08(m, 1H), 3.15-3.17(m, 2H, CH_2-Ph), 3.77(s, 3H, OCH_3), 4.89(s, 1H), 5.33-5.35(m, 1H), 5.50-5.51(m, 1H), 6.25-6.27(m, 1H, NH), 6.76-6.77(m, 2H), 6.97-6.98(m, 2H), 7.30 (s, 1H, Ph-OH), ESI-MS (*m/z*, 100%): 409 ($[M^-]$, 8), 448 ([M+39], 15).

Biological activity. The *in vitro* anti-viral activities of the synthesized compounds **2a-2l, 3a-3g** were assayed against influenza virus A_3,B, HSV-1 and HSV-2. Briefly, each compound for assaying was dissolved in DMSO at a concentration of 1000 μg/mL, then diluted successively with 3-fold amount of nutrient mixture (composed with 10-15% fetal calf serum, 1% nonessential amino acid, and 0.66 mM L-glutamine) for 8 different concentrations (250, 62.5, 15.6, 3.9, 1.0, 0.2, 0.06, and 0.02 μg/mL respectively) as stock solution for following experiment.

The procedure for anti-influenza virus assay. Madin-darby canine kidney cells (MDCK) were seeded in 96-well trays and cultured at 37 °C in a humidified CO_2 incubator (95% air, 5%

CO_2) for 24h, then which were infected with influenza A3 virus with 1×10^{-3} [100-fold of the 50% tissue culture infective dose($TCID_{50}$)] and with influenza B virus with 1×10^{-2} [30-fold of the 50% tissue culture infective dose($TCID_{50}$)] respectively. All infected tissue culture plates (96 wells) were incubated at 37 °C for 2h, medium was removed. Subsequently, 100 μL solution of different concentration of each compound was added to each well(every concentration was replicative for 3 times), the plates were incubated again for 36h at 37 °C. Then the inhibition of virus-induced cytopathic effect(CPE) for each sample was recorded referring to the cell control and virus control according to the literature.[8,10] and 50% cell-inhibitory concentration (IC_{50}) values of active compounds were calculated accordingly. The inhibitory potentials of rupestonic acid derivatives were comparable to parent compound, the commercial drug ribavirin(RBV) and Oseltamivir.

The procedure for anti-HSV-1 and HSV-2 assay. Vero cells were seeded in 96-well trays and cultured for 24h at 37 °C in 5% CO_2, which were infected with the HSV-1(VR733) virus with 1×10^{-3} (50-fold of the $TCID_{50}$) and with the HSV-2 (SAV) with 1×10^{-4} (17-fold of the $TCID_{50}$) respectively. All infected tissue culture plates (96 wells) were incubated again at 37 °C for 2h, medium was removed. Subsequently, 100 μL solution of different concentration of each compound was added to each well (every concentration was replicative for 3 times). Then the plates were incubated for 48h at 37 °C, and the inhibition of virus-induced cytopathic effect (CPE) for each sample was recorded referring to the cell control and virus control according to literature.[8] and 50% cell-inhibitory concentration (IC_{50}) values of active compounds were calculated accordingly through Reed-Muench method.[11] The inhibitory potentials of rupestonic acid derivatives were comparable to parent compound and the commercial drug acyclovir (ACV).

Table 1. The results of compounds **2a~2l, 3a~3g** against influenza A3 and B virus

Compd.	TC_{50} (μmol/L)[a]	Against influenza A3 virus		Against influenza B virus	
		IC_{50} (μmol/L)[b]	SI[c]	IC_{50} (μmol/L)[b]	SI[c]
1[d]	1044.4	—[e]	—[f]	115.7	9.0
2a	89.8	—[e]	—[f]	—[e]	—[f]
2b	733.7	—[e]	—[f]	—[e]	—[f]
2c	1427.3	—[e]	—[f]	—[e]	—[f]
2d	> 2958.6	769.5	4.9	569.5	5.2
2e	172.8	—[e]	—[f]	—[e]	—[f]
2f	200.0	—[e]	—[f]	—[e]	—[f]
2g	> 2092.1	143.7	> 14.6	—[e]	—[f]
2h	528.8	—[e]	—[f]	—[e]	—[f]
2i	120.6	19.2	6.3	29.9	4.0
2j	136.3	—[e]	—[f]	—[e]	—[f]
2k	430.7	—[e]	—[f]	—[e]	—[f]
2l	159.6	49.0	3.3	—[e]	—[f]
3a	1255.3	—[e]	—[f]	—[e]	—[f]
3b	579.6	—[e]	—[f]	—[e]	—[f]
3c	1653.3	—[e]	—[f]	—[e]	—[f]
3e	> 1222.5	—[e]	—[f]	—[e]	—[f]
3f	1598.3	—[e]	—[f]	533.2	3.0
3g	> 2445.0	143.7	> 14.6	—[e]	—[f]
Ribavirin	1573.8	1.6	983.6	11.9	132.3
Oseltamivir	> 1219.5	5.1	> 242.7	—[e]	—[f]

[a]50% cytotoxic concentration. [b]50% virus-inhibitory concentration, determined by CPE inhibition assay. [c]Selectivity Index (TC_{50}/IC_{50}). [d]Parent compound. [e]No anti-flu viral activity at the 50% cytotoxic concentration. [f]The SI can't be calculated,since the highest concentration tested was less than the IC_{50}.

Results and Discussion

In this study, we used the common coupling reagent DCC, HOBt/DMAP to activate the carboxyl group of rupestonic acid and synthesized the desired compounds **2a-2l, 3a-3g.**

All the synthesized compounds **2a~2l, 3a~3g** were preliminarily assayed *in vitro* against influenza A_3/Jifang/90/15, B/Jifang/97/13, HSV-1(VR733) and HSV-2(SAV). Their inhibitory activity(IC_{50}) values are listed in Table 1 and Table 2 respectively. The inhibition of active compounds(possessing IC_{50} values) against influenza A_3, B virus and HSV-1, HSV-2 at different concentrations were shown in Figure 1, Figure 2, Figure 2 and Figure 3 respectively. The results showed that compound **2i** possessed the highest inhibition against both influenza virus A_3(TC_{50} = 120.6 μmol/L, IC_{50} = 19.2 μmol/L, SI = 6.3) and B(TC_{50} = 120.6 μmol/L, IC_{50} = 29.9 μmol/L, SI

Table 2. The results of compounds **2a~2l, 3a~3g** against HSV-1 and HSV-2

Compd.	TC_{50} (μmol/L)[a]	Against HSV-1		Against HSV-2	
		IC_{50} (μmol/L)[b]	SI[c]	IC_{50} (μmol/L)[b]	SI[c]
1[d]	> 4032.3	—[e]	—[f]	—[e]	—[f]
2a	266.3	—[e]	—[f]	—[e]	—[f]
2b	733.7	244.2	3.0	184.6	3.8
2c	329.4	73.3	4.5	73.3	4.5
2d	766.3	—[e]	—[f]	—[e]	—[f]
2e	58.6	—[e]	—[f]	—[e]	—[f]
2f	200.0	17.7	11.3	20.7	9.7
2g	117.2	—[e]	—[f]	—[e]	—[f]
2h	145.2	—[e]	—[f]	—[e]	—[f]
2i~2l	NA[g]	NA[g]	NA[g]	NA[g]	NA[g]
3a	333.3	—[e]	—[f]	—[e]	—[f]
3b	333.3	—[e]	—[f]	—[e]	—[f]
3c	550.1	—[e]	—[f]	—[e]	—[f]
3d	105.1	—[e]	—[f]	—[e]	—[f]
3e	690.7	600.2	1.2	478.2	1.4
3f	307.5	—[e]	—[f]	—[e]	—[f]
3g	291.2	—[e]	—[f]	—[e]	—[f]
Acyclovir	> 444.0	7.1	> 62.5	10.2	> 43.5

[a]50% cytotoxic concentration. [b]50% virus-inhibitory concentration, determined by CPE inhibition assay. [c]Selectivity Index (TC_{50}/IC_{50}). [d]Parent compound. [e]No anti-HSV activity at the 50% cytotoxic concentration. [f]The SI can't be calculated, since the highest concentration tested was less than the IC_{50}. [g]The anti-HSV of compounds **2i~2l** don't assayed.

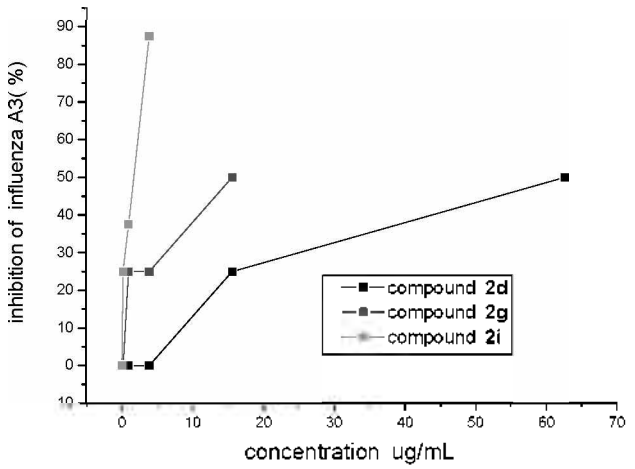


Figure 1. Inhibition and concentration-response curves of **2d, 2g** and **2i** against influenza A_3 virus. Curves represent the average of three separate experiments.

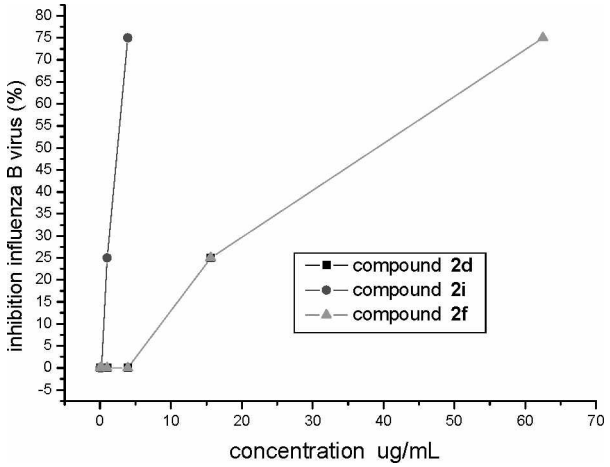


Figure 2. Inhibition and concentration-response curves of **2d, 2i** and **3f** against influenza A_3 virus. Curves represent the average of three separate experiments.

= 4.0), the inhibition both to Flu-A3 and Flu-B virus incerased sharply and showed concentration-dependent at 0.02-15.6 μg/mL, the inhibition to Flu-A3 and Flu-B is 87.5% and 75% respectively; **2g** was more active against influenza A_3 virus at very low cytotoxicity (TC_{50} > 2092.1 μmol/L, IC_{50} = 143.7 μmol/L, SI > 14.6) than the parent compound and the inhibition increase in concentration-dependent manner at 1.0-15.6 μg/mL. **2d** showed comperatively weaker activities both against influenza A_3 and B virus at very low cytotoxicity, but more active than the parent compound. Compounds **2b, 2c, 2f** exhibited higher activities against both HSV-1 and HSV-2 than that of the parent compound. **2b** exhibited inhibition against HSV-1 (TC_{50} = 733.7 μmol/L, IC_{50} = 244.2 μmol/L, SI = 3.0), against HSV-2 (TC_{50} = 733.7 μmol/L, IC_{50} = 184.6 μmol/L, SI = 3.8) and the inhibition both to HSV-1 and HSV-2 show concentration-dependent at 1.0-15.6 μg/mL; **2c** exhibited inhibition against HSV-1 (TC_{50} = 329.4 μg/mL, IC_{50} = 73.3 μmol/L, SI = 4.5), against HSV-2 (TC_{50} = 329.43 μmol/L, IC_{50} = 73.3 μmol/L, SI = 4.5); **2f** exhibited high inhibition against HSV-1 (TC_{50} = 200.0 μmol/L, IC_{50} = 17.7 μmol/L, SI = 11.3), and against HSV-2 (TC_{50} = 200.0 μmol/L, IC_{50} = 20.7 μmol/L,

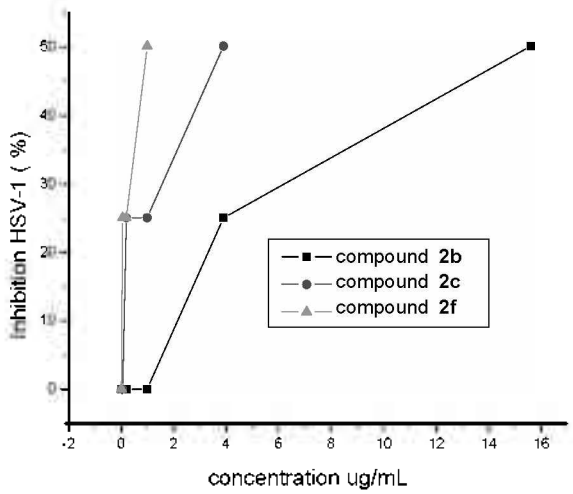


Figure 3. Inhibition and concentration-response curves of **2b, 2c, 2f** against HSV-1 virus. Curves represent the average of three separate experiments.

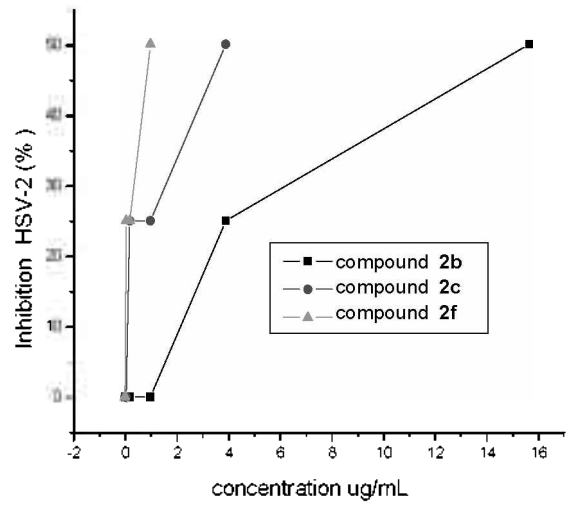


Figure 4. Inhibition and concentration-response curves of **2b, 2c, 2f** against HSV-2 virus. Curves represent the average of three separate experiments.

SI = 9.7), and the inhibition both to HSV-1 and HSV-2 in crease sharply in concentration-dependent manner at 0.02-1.0 μg/mL. On the whole. **2f** is the most potent inhibitor of anti-herpes simplex virus and can be as a lead compound for anti-herpes simplex virus.

In addition, it seems that the introduction the aromatic ring with electron-attracting group to the carboxyl group of rupestonic acid will improve its anti-HSV activities (eg. **2f** is the most potent inhibitor of anti-herpes simplex virus among the active compounds). These results provided guidance for us and encouraged us to synthesize more rupestonic acid derivatives for the development of new anti-influenza virus. HSV-1 and HSV-2 virus drugs.

Acknowledgments. This work is financially supported by National Nature and Science Fundation (No.20872174). We are also thankful for the members of the national center for drug screening of China for the biological activities screening of the synthesized compounds.

References and notes

1. Chen, X. Y.; Wang, S. H. *Chin. Trad. & Herb. Drugs* **1981**, *12*, 25.
2. Siirafil, E. B.; Askar, E. Y.; Ilhamjan, W. F. E. *Chin. J. Biochem. & Mol. Biol.* **2001**, *17*, 226.
3. Zhan, B. H.; Wang, Y. N.; Zhang, Y. Q. *Chin. J. of Modern Med.* **2005**, *15*, 1968.
4. Srapil, E. B.; Abdiryim, Y. S. F.; Gulnar, D. W. T. *Chin. J. Integrated Trad. West. Med.* **2002**, *22*, 126.
5. Srapil, E. B.; Gulnar, D. W. T.; Liu, F. *Chin. J. Trad. Drugs* **1996**, *2*, 35.
6. (a) Compound 1: colorless column crystal, mp. 132~134 °C, IR(KBr)ν: 3230, 2970-2860, 1720, 1680, 1635, 1415, 1390, 1238, 958 cm^{-1}; ^1H NMR(600 MHz, $CDCl_3$): 0.67(d, J = 7.2Hz, 3H, CH_3), 1.63(m, 1H), 1.64(m, 1H), 1.81 (m, 1H), 1.84(m, 1H), 1.88(m, 1H), 2.06(m, 1H), 2.14(m, 1H), 2.46(m, 1H), 2.64(m, 1H), 2.86(m, 1H), 2.90(m, 1H), 3.22(m, 1H), 5.76(s, 1H), 6.40(s, 1H); ^{13}C NMR(150MHz, $CDCl_3$): 7.9, 12.0, 31.4, 35.1, 36.4, 37.5, 38.2, 41.1, 45.9, 125.2, 137.6, 145.6, 171.3, 175.2, 208.8; ESI-MS(m/z): 519[2M+23]$^+$, 497[2M+1]$^-$, 249[M+1]$^+$. (b) Aisa, H. A.; Yong, J. P.; Lv, Q. Y.; Wu, T. *Acta Cryst. E* **2008**, *64*, o479.
7. Sheu, J. H.; Hung, K. C.; Wang, G. H.; Duh, C. Y. *J. Nat. Prod.* **2000**, *63*, 1603.
8. Wright, A. E.; Rueth, S. A.; Cross, S. S. *J. Nat. Prod.* **1991**, *4*, 1108.
9. (a) Liu, L. J.; Yong, J. P.; Wang, J. W. *Chem. J. Chin. Universities* **2006**, *27*, 1669.
   (b) Um, S. J.; Park, M. S.; Park, S. H.; Han, H. S.; Kwon, Y. J.; Sin, H. S. *Bioorg. Med. Chem.* **2003**, *11*, 5345.
   (c) Byung, J. M.; Jong, W. C.; Oh, S. K. *J. Korean Chem. Soc.* **1991**, *35*, 78.
   (d) Soon, U. K.; Nam, J. H. *J. Korean Chem. Soc.* **1987**, *31*, 475.
10. Smee, D. F.; Huffman, J. H.; Morrison, A. C.; Barnard, D. L.; Sidwell, R. W. *Antimicrob. Agents Chemotherapy* **2001**, *45*, 743.
11. Reed, L. J.; Muench, H. *Am. J. Hyg.* **1938**, *27*, 493.