SYNTHESIS OF RUPESTONIC ACID AMIDE DERIVATIVES AND THEIR *in vitro* ACTIVITY AGAINST TYPE A_3 AND B FLU VIRUS AND HERPES SIMPLEX I AND II

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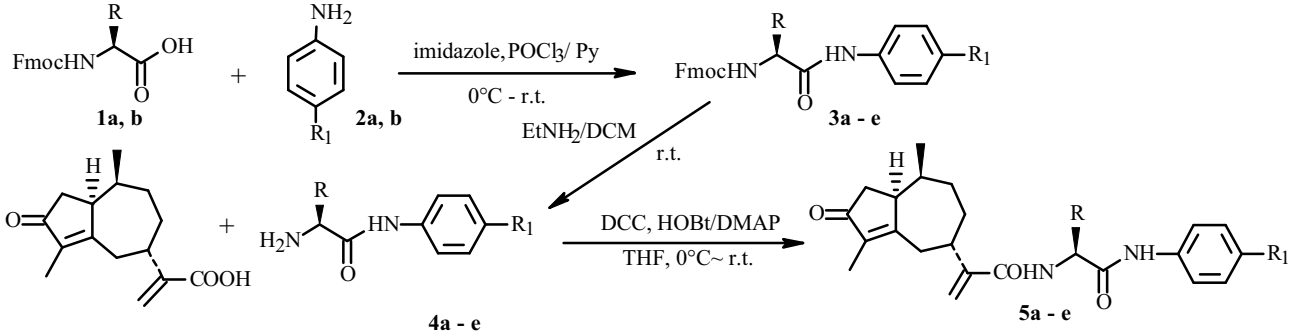
*Derivatives of rupestonic acid (**5a-e**) were synthesized and evaluated preliminarily at the National Center for Drug Screening (PRC) for antiviral activity against type A_3 and B flu virus and HSV-I and HSV-II in order to improve the biological activity of rupestonic acid. It was found that compound **5b** was more active than rupestonic acid against type A_3 flu virus.*

Key words: synthesis, rupestonic acid, amide derivatives, flu virus, herpes simplex virus, activity.

The plant *Artemisia rupestris* L. (Chinese name Yizhihao) is a traditional Chinese medicinal herb that has been used since antiquity in Uigur folk medicine. It is known for its effective antiallergy [1], antitumor [2], anti-inflammatory, and antibacterial activities and as an antitoxin [3, 4].

(5*R*,8*R*)-2-(3,8-Dimethyl-2-oxo-1,2,4,5,6,7,8,8α-octahydroazulen-5-yl)acrylic acid (called rupestonic acid) is a multifunctional sesquiterpene that was isolated from *A. rupestris*. It was noted [5] that certain sesquiterpenes exhibit a powerful cytotoxic activity toward cancer cell lines P-388, A549, and HT-26. It was reported [6] that a sesquiterpene hydroquinone and its acetate derivative exhibit high activity against tumor cell line P-388 and flu strain PR-8. Investigations of the activity of rupestonic acid against viruses HSV-1 and HSV-2 and flu viruses A_3 and B showed that it is highly active against type B flu virus (TC_{50} = 258.7 μg/mL, IC_{50} = 28.7 μg/mL). Due to its unique structure, we attempted to modify it in order to prepare compounds with higher biological activity.

Herein we describe the synthesis of intermediates **3a-e** through the reaction of amines **2** with *N*-Fmoc-L-phenylalanine and *N*-Fmoc-L-Leu-OH in the presence of $POCl_3$ and imidazole.



1a: R = $C_6H_5CH_2$-; **1b:** R = $CH_2CH(CH_3)_2$; **2a:** R_1 = CH_3; **2b:** R_1 = OCH_3

3, 4, 5a: R = C_6H_5-CH_2; R_1 = CH_3; **3, 4, 5b:** R = C_6H_5-CH_2, R_1 = OCH_3; **3, 4, 5c:** R = $C_6H_5CH_2$, R_1 = H

3, 4, 5d: R = $CH_2CH(CH_3)_2$, R_1 = CH_3; **3, 4, 5e:** R = $CH_2CH(CH_3)_2$, R_1 = OCH_3

Scheme 1

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TABLE 1. Antiviral Activity of **5b**, Oseltamivir, and Ribavirin Against Flu Viruses A_3 and B

Compound	[a]TC_{50} (μg/mL)	Against flu virus A_3		Against flu virus B	
		[b]IC_{50} (μg/mL)	[c]SI	[b]IC_{50} (μg/mL)	[c]SI
Rupestonic acid	259	-[c]	-[f]	28.7	9.0
5b	53.4	28.7	1.9	-[c]	-[f]
Oseltamivir	>500	2.1	>242.7	-[c]	-[f]
Ribavirin	384	0.4	936.6	2.9	133.8

[a]50% cytotoxic concentration; [b]50% virus-inhibiting concentration determined by CPE of the inhibiting sample; [c]selectivity index (TC_{50}/IC_{50}); [d]starting material (rupestonic acid, RA); [e]inactive at 50% cytostatic concentration; [f]SI cannot be calculated because the highest tested concentration was $<IC_{50}$.

Then, removal of the Fmoc protecting group from **3a-e** in the presence of ethanolamine and CH_2Cl_2 (DCM) produced intermediates **4a-e**. The target compounds **5a-e** were synthesized by reaction of **4a-e** with rupestonic acid in the presence of dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt), and 4-dimethylaminopyridine (DMAP). Scheme 1 shows the synthetic route.

EXPERIMENTAL

Melting points were determined on a Yanaco MP-300 apparatus equipped with a microscope and are uncorrected. PMR spectra in $CDCl_3$ were recorded on a Varian Inova-400 spectrometer using tetramethylsilane (TMS) as an internal standard. ESI-MS spectra were obtained in an HP1100LC/MS spectrometer. Rupestonic acid was isolated from *A. rupestris* L. (HPLC purity >98%). DCC, HOBt, and DMAP were purchased from ShangHai reagent company (PRC); *N*-Fmoc-L-Leu-OH and *N*-Fmoc-L-phenylalanine, from ShangHai Qiude Biochemical Engineering Co. Ltd., PRC. Other commercially available reagents were used without further purification. We used THF that was freshly distilled over sodium and benzophenone; DCM, over CaH_2. Viruses HSV-1 (VR733) and HSV-II (SAV) were obtained from ATCC; flu viruses, innoculated into chicken embryos in 2006 and stored at 80°C at Beijing Institute of Virology immediately before use in this test.

General Method for Synthesizing Intermediates 4. L-Phenylalanine-*p*-methoxyanilide (4b). A solution of *p*-methoxyaniline (2.1 mmol), *N*-Fmoc-L-phenylalanine (2.1 mmol), and imidazole (0.29 g, 3.4 mmol) in pyridine (10 mL) was treated dropwise with $POCl_3$ (3.4 mmol) at 0°C under N_2 over 1 h [7]. The mixture was stirred at room temperature for 4 h, poured into $NaHCO_3$ solution (100 mL), and extracted with ethylacetate (3 × 50 mL). The combined organic extract was washed with $CuSO_4$ solution (30 mL) and brine and dried over $MgSO_4$. Solvent was removed. The crude product was purified by flash chromatography over a column of silica gel with elution by petroleum ether:ethylacetate (5:1-2:1). Fractions with similar R_f values were combined to afford *N*-Fmoc-L-phenylalanine-*p*-methoxyanilide, **3b**, white solid, yield 76%, mp 170-172°C.

PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.99-1.01 (6H, d, J = 8, 2 × CH_3), 1.64 (1H, m), 1.73-1.82 (2H, m, CH_2), 3.82 (3H, s, OCH_3), 4.23-4.26 (2H, m, O–CH_2–C=O), 4.49 (1H, m), 5.24-5.26 (1H, m), 6.87-6.89 (2H, d, J = 8, Ph–H), 7.29-7.43 (6H, m, Ph–H), 7.59-7.61 (2H, d, J = 8, Ph–H), 7.79-7.81 (2H, m, Ph–H), 7.83-7.85 (2H, br.s, NH–C=O). Other intermediates **3a** and **3c-e** were synthesized analogously to **3b** and were used without further purification. *N*-Fmoc-L-phenylalanine-*p*-methoxyaniline (**3b**) (0.6 g) was dissolved in DCM containing ethanolamine (10 mL, 30%), stirred for 4 h at room temperature, diluted with DCM (20 mL), and washed with water (3 × 10 mL) and brine (20 mL). The organic layer was dried over $MgSO_4$. Solvent was removed. The crude product was purified by flash chromatography over a column of silica gel with elution by petroluem ether:ethylacetate (5:1-2:1). Fractions with similar R_f values were combined to afford L-phenylalanine-*p*-methoxyanilide (**4b**), yellow oil. Products **4a** and **4c-e** were synthesized by the same method as **4b**.

L-Phenylalanine-*p*-methoxyanilide (4b). Light-yellow oil, yield 65.8%. PMR spectrum ($CDCl_3$, δ, ppm): 1.91 (2H, br.s, NH_2), 2.76-2.82 (1H, m), 3.23 (H, s, CH_3), 3.35-3.38 (1H, m), 7.04-7.25 (7H, m), 7.52-7.55 (2H, m), 9.26 (1H, s, NHCO). IR spectrum (ν, cm^{-1}): 3296, 2954, 1674, 1605, 1512, 1246, 829. ESI-MS (*m*/*z*, %): 255 (100) $[M + 1]^+$.

L-Phenylalanine-*p*-methylanilide (4a). Light-yellow oil, yield 75.6%. PMR spectrum ($CDCl_3$, δ, ppm): 1.65 (2H, br.s, NH_2), 2.76-2.82 (1H, m), 3.35-3.40 (1H, m), 3.73-3.76 (1H, m), 3.80 (3H, s, OCH_3), 6.86-6.89 (2H, m), 7.25-7.27 (3H, m), 7.32-7.36 (2H, m), 7.49-7.52 (2H, m), 9.24 (1H, s, NHCO). IR spectrum (ν, cm^{-1}): 3290, 2956, 1666, 1605, 1512, 1246, 829. ESI-MS (*m*/*z*, %): 271 (100) $[M + 1]^+$.

L-Phenylalanineanilide (4c). Light-yellow oil, yield 78.8%. PMR spectrum ($CDCl_3$, δ, ppm): 1.86 (2H, br.s, NH_2), 2.74-2.80 (1H, m), 3.33-3.38 (1H, m), 3.74 (1H, m), 7.08-7.62 (10H, m), 9.42 (1H, s, NHCO). IR spectrum (ν, cm^{-1}): 3296, 2954, 1674, 1605, 1512, 1246, 768. ESI-MS (*m*/*z*, %): 241 (100) $[M + 1]^+$.

L-Leucine-*p*-methylanilide (4d). Light-yellow oil, yield 70.8%. PMR spectrum ($CDCl_3$, δ, ppm): 0.98-1.00 (6H, m, $2CH_3$), 1.42-1.47 (1H, m), 1.76-1.78 (2H, br.s, NH_2), 1.79-1.86 (2H, m, CH_2), 2.33 (3H, s, CH_3), 3.53-3.55 (1H, m), 7.13-7.15 (2H, m), 7.49-7.50 (2H, m), 9.43 (1H, s, NHCO). IR spectrum (ν, cm^{-1}): 3296, 2954, 1674, 1605, 1512, 1246, 831. ESI-MS (*m*/*z*, %): 221 (100) $[M + 1]^+$.

L-Leucine-*p*-methoxyanilide (4e). Light-yellow oil, yield 76.6%. PMR spectrum ($CDCl_3$, δ, ppm): 0.91-0.94 (6H, m, $2CH_3$), 1.43-1.46 (1H, m), 1.75-1.81 (2H, m, CH_2), 1.86-2.01 (2H, br.s, NH_2), 3.43 (1H, m), 3.73 (3H, s, CH_3), 6.81-6.83 (2H, m), 7.46-7.49 (2H, m), 9.37 (1H, s, NHCO). IR spectrum (ν, cm^{-1}): 3296, 3954, 1668, 1605, 1512, 1246, 829. ESI-MS (*m*/*z*, %): 237 (100) $[M + 1]^+$.

General Method for Synthesizing Rupestonic Acid Derivatives (5) [8]. Rupestonic acid (0.124 g, 0.5 mmol), DCC (0.11 g, 0.55 mmol), and THF (dry, 5 mL) were placed in a 25-mL one-necked round-bottomed flask and stirred on an ice bath for ~10 min. HOBt (0.08 g, 0.6 mmol) and DMAP (0.07 g, 0.55 mmol) in THF (2 mL) were added using a syringe. The mixture was stirred on an ice bath for 30 min, treated with **4** (0.6 mmol), and stirred at 0°C for 30 min and then at room temperature for 8 h. The completion of the reaction was monitored using TLC. The solvent was removed in vacuo. The solid was chromatographed over a column of silica gel with elution by petroleum ether:ethylacetate (5:1-2:1). Fractions with similar R_f values were combined to afford **5a-e**.

Amide 5a: white compound, yield 55.8%, mp 50-52°C. PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.62 (3H, d, J = 7.2, CH_3), 1.58 (3H, s, CH_3), 1.72-1.75 (1H, s), 1.78-1.82 (2H, m), 2.01-2.05 (1H, d, J = 16), 2.11-2.12 (1H, m), 2.27-2.29 (1H, m), 2.38-2.43 (1H, m), 2.54-2.56 (1H, m), 2.76 (1H, m), 2.86-2.89 (1H, m), 3.12-3.13 (1H, m), 3.15-3.27 (2H, m), 4.09 (3H, s, OCH_3), 4.76-4.82 (1H, m), 5.36 (1H, s), 5.52 (1H, s), 6.63-6.65 (1H, d, J = 8, NHCO), 7.07-7.33 (9H, m, Ph-H), 7.52 (1H, s, CO–NH–Ph). IR spectrum (ν, cm^{-1}): 3431, 3304, 3066, 2957, 1697, 1647, 1608, 1541, 1514, 1249, 817. ESI-MS (*m*/*z*, %): 501 (100) $[M + 1]^+$.

Amide 5b: white compound, yield 65.8%, mp 78-80°C. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.62 (3H, d, J = 7.2, CH_3), 1.58 (3H, s, CH_3), 1.72-1.75 (1H, s), 1.78-1.82 (2H, m), 2.01-2.05 (1H, d, J = 16), 2.11-2.12 (1H, m), 2.27-2.29 (1H, m), 2.30 (3H, s, CH_3), 2.38-2.43 (1H, m), 2.54-2.56 (1H, m), 2.76 (1H, m), 2.86-2.89 (1H, m), 3.12-3.13 (1H, m), 3.15-3.27 (2H, m), 4.76-4.82 (1H, m), 5.36 (1H, s), 5.52 (1H, s), 6.63-6.65 (1H, d, J = 8, NHCO), 7.07-7.33 (9H, m, Ph–H), 7.52 (1H, s, CO–NH–Ph). IR spectrum (ν, cm^{-1}): 3431, 3304, 3066, 2957, 1697, 1647, 1608, 1541, 1514, 1249, 817. ESI-MS (*m*/*z* %): 507 (100) $[M + 23]^+$.

Amide 5c: white compound, yield 68.8%, mp 89-90°C. PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.62 (3H, d, J = 7.1, CH_3), 1.58 (3H, s, CH_3), 1.72-1.75 (1H, s), 1.78-1.82 (2H, m), 2.01-2.05 (1H, d, J = 8), 2.11-2.12 (1H, m), 2.27-2.32 (1H, m), 2.38-2.43 (1H, m), 2.54-2.56 (1H, m), 2.76 (1H, m), 2.86-2.89 (1H, m), 3.12-3.13 (1H, m), 3.15-3.27 (2H, m), 4.82-4.85 (1H, m), 5.36 (1H, s), 5.52 (1H, s), 6.66-6.68 (1H, d, J = 8, NHCO), 7.07-7.35 (10H, m, Ph–H), 7.84 (1H, s, CO–NH–Ph). IR spectrum (ν, cm^{-1}): 3429, 3138, 3080, 2957, 1695, 1647, 1609, 1545, 1510, 1246, 1172, 829. ESI-MS (*m*/*z*, %): 493 (100) $[M + 23]^+$, 471 (12) $[M + 1]^+$.

Amide 5d: white compound, yield 76.8%, mp 84-86°C. PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.61-0.63 (3H, d, J = 7.2, CH_3), 0.81-0.87 (2H, m, CH_2), 0.89-0.98 (6H, m, $2CH_3$), 1.26-1.30 (1H, m), 1.57 (3H, s, CH_3), 1.72-1.82 (4H, m), 1.99-2.01 (1H, d, J = 12), 2.09 (1H, m), 2.27 (3H, s, Ph–CH_3), 2.38-2.45 (1H, m), 2.51-2.57 (1H, m), 2.75-2.80 (1H, m), 2.88-2.90 (1H, m), 3.06 (1H, br.s), 4.71-4.72 (1H, m), 5.37 (1H, s), 5.62 (1H, s), 6.62 (1H, d, J = 7.8, NH–C=O), 7.05 (2H, d, J = 7.8, Ph–H), 7.36 (2H, d, J = 8.2, Ph–H), 8.59 (1H, s, O=C–NH–Ph). IR spectrum (ν, cm^{-1}): 3431, 3304, 3066, 2957, 1697, 1647, 1608, 1541, 1514, 1249, 817. ESI-MS (*m*/*z*, %): 451 (42) $[M + 1]^+$, 473 (100) $[M + 23]^+$.

Amide 5e: white compound, yield 72.4%, mp 89-91°C. PMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.62 (3H, d, J = 7.2, CH_3), 0.63-0.79 (2H, m), 0.91-1.02 (6H, m, $2CH_3$), 1.24-1.28 (1H, m), 1.54 (3H, s, CH_3), 1.62-1.81 (4H, m, $2CH_2$), 1.99-2.09 (2H, m), 2.42-2.58 (2H, m), 2.75-2.90 (2H, m), 3.07 (1H, m), 3.75 (3H, s, CH_3), 4.69-4.74 (1H, m), 5.40 (1H, s), 5.64 (1H, s), 6.61-6.63 (1H, d, J = 8, NH–C=O), 6.76-6.78 (2H, d, J = 8, Ph–H), 7.36-7.39 (2H, d, J = 12, Ph–H), 8.57 (1H, s,

O=C–NH–Ph). IR spectrum (ν, cm^{-1}): 3293, 3138, 3080, 2957, 1695, 1647, 1609, 1545, 1510, 1246, 1172, 829. ESI-MS (*m*/*z*, %): 489 (100) $[M + 23]^+$.

Biological Studies. The antiviral activity of rupestonic acid derivatives **5a**-**e** against viruses A3/Beijing/90/15 (H3N2), B/Beijing/97/13, HSV-1 (VR733), and HSV-2 (SAV) was evaluated preliminarily *in vitro* at the National Center for Drug Screening (PRC). The inhibiting properties of the compounds were comparable with those of rupestonic acid and commercial preparations such as ribavirin (RBV), acyclovir (ACV), and oseltamivir. The results showed that not one of the compounds **5a**-**e** was an inhibitor of HSV-I and HSV-II. However, **5b** showed higher activity against flu virus A_3 than rupestonic acid (Table 1).

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