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Advances in Studies on the Rupestonic Acid Derivatives as Anti-influenza Agents

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Abstract: Rupestonic acid (isolated from the Chinese traditional medicine *Artemisia rupestris* L.) is a sesquiterpene with multifunctional groups and possess higher activity against influenza virus *B*. In order to improve the biological activity of rupestonic acid, many derivatives have been synthesized and their anti-influenza activity was screened. This review describes the rupestonic acid derivatives and their anti-influenza activity studied by our researching group.

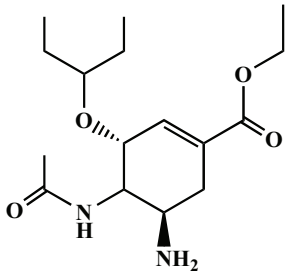
Keywords: Rupestonic acid derivatives, anti-influenza virus, structure-activity relationship.

INTRODUCTION

Influenza is an acute infectious disease caused by a member of the orthomyxovirus family: influenza virus A, B or to a much lesser extent, influenza virus C. Epidemic influenza continues to be associated with significant morbidity in the general population, and mortality in the elderly and other high risk patients. Although the case fatality rate averages less than 0.01%, tens of thousands of deaths occur each year. Control through immunization programs has not been possible due to incomplete protective efficacy and antigenic variations that occur frequently.

In recent years, the persistence of the strain of influenza H5N1 (avian influenza) in many Asian countries and its ability to cause fatal infections in humans has raised serious concerns. And the novel strain of influenza virus H1N1(swine flu) arouse in 2009 and persistently spread globally and caused fatal death. Oseltamivir (Fig. **1**) has been orally used for protection against and treatment of influenza, and regarded as the effective drug in clinical. However, it has been reported that H5N1 influenza virus (avian influenza) has shown resistant to Oseltamivir [1-3]. Thus it is urgent to find new inhibitors, especially to find the novel and more active inhibitors from the natural products.

Artemisia rupestris L. (Yizhihao in Chinese) is one of Chinese traditional herbal medicines, long been used in folk of Kazak nationality of Xinjiang region as anti-cancer [4], anti-inflammatory [5], anti-bacterial [6], antiviral, antidote agents and protecting liver [7]. It is the key ingredient in the Compound Yizhihao granul (Fufang yizhihao Keli, No. Z20026711), which is clinically used to cure the cold in China. Rupestonic acid [(5R,8R)-2-(3,8-dimethyl-2-oxo-1,2,4,5,6,7, 8,8α-octahydroazulen-5-yl)acrylic acid] (Fig. **2**) is a sesquiterpene with multifunctional groups, isolated from *Artemisia rupestris* L [8]. We have tested this compound against influenza virus A3 and B. The results showed that it exhibits higher activity against influenza virus B (TC_{50} = 1044.4 μM, IC_{50} =115.7 μM, SI = 9). In order to improve its anti-influenza activity, many rupestonic acid derivatives have been synthesized and screened anti-influenza activity by our researching group. Some of the recent studies are briefly presented here.



Oseltamivir

Fig. (1).

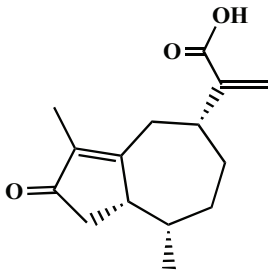
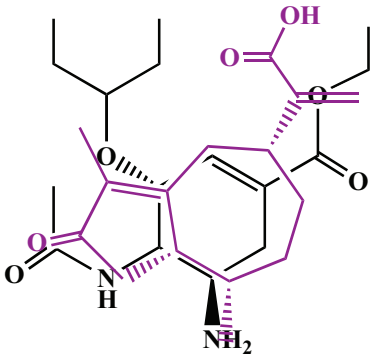


Fig. (2).



Oseltamivir **rupestonic acid**

Fig. (3).

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THE STRUCTURE CHARACTERISTIC BETWEEN THE OSELTAMIVIR AND RUPESTONIC ACID

Firstly, we optimized the structure of Oseltamivir and rupestonic acid at DFT-B3LYP/6-311G basis, overlay the two structures (Fig. **3**) and found there are some similarities. Thus we mainly modified the carboxyl position of rupestonic acid.

ALKYL RUPESTONATES AND ANTI-INFLUENZA ACTIVITY

Yong *et al.* [9] have synthesized a series of alkyl rupestonates and screened their *in vitro* anti-influenza activity (Fig. **4**; Table **1**). The result showed that ethyl rupestonate is the most potent compound among the alkyl rupestonates. Meanwhile, the alkyl chain length of these rupestonates had dramatic impacts on the inhibitory potency, the alkyl chain of rupestonates is longer, the anti-influenza activity is lower.

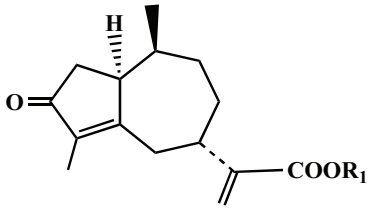


Fig. (4).

Yong *et al.* [9] have synthesized a series of phenyl rupestonates and screened their *in vitro* anti-influenza activity (Fig. **5**; Table **2**). The results showed that most of the phenyl rupestonates possess the higher activity against influenza virus A3 than the parent compound (rupestonic acid), 4-*tert*-butyl-phenyl rupestonate (TC_{50}=18.9μM, IC_{50}=0.5μM) is the most potent compound among the phenyl rupestonates and 10-fold more active than the reference drug Oseltamivir (TC_{50}>1219.5μM, IC_{50}=5.1μM), but the toxicity is more higher than that of the Oseltamivirs'. In addition, we can also conclude from the Table **2** that the inhibition against influenza virus A3 will increase while introducing the big hydrophobic group at the 4-position of phenyl ring. However, there is not much difference in the IC_{50} values of the rupestonic acid phenyl esters (phenyl ring substituted by halogen).

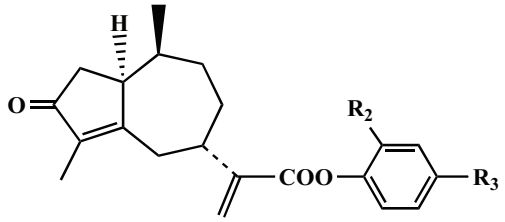


Fig. (5).

Yong *et al.* [10]. have synthesized a series of benzyl rupestonate derivatives and screened their *in vitro* anti-influenza activity (Fig. **6**; Table **3**). The results showed that the anti-influenza activity will reduce while the phenyl ring was substituted by the electron donor group, and the anti-influenza activity will rise while the phenyl ring was substituted by the electron withdraw group.

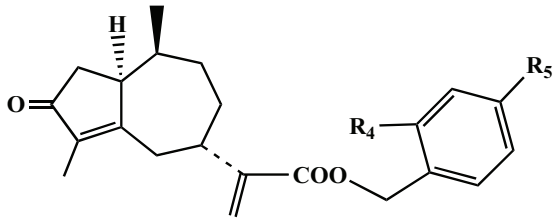


Fig. (6).

Aisa *et al.* [11] have reported a series of piperazine moiety contained rupestonate derivatives (Fig. **7**) and which were bioassayed *in vitro* for their anti-influenza activity, the results (Table **4**) showed that all of these compounds possess dual biological activity both against influenza virus A and B. Entry **7** exhibits the highest inhibition against the strains of H3N2 (IC_{50}=5.22μM), H1N1(IC_{50}=3.72μM) and flu-B(IC_{50}>25.41), respectively which can be a lead compound for anti-influenza drug development.

Table 1. Derivatives of Alkyl Rupestonate and *in vitro* Anti-Influenza Activity.

Entry	R_1	TC_{50} (μM)	Against Influenza Virus A3, IC_{50} (μM)	Against Influenza Virus B, IC_{50} (μM)
1	H	1044.4	—[a]	115.7
2	$-CH_3$	611.8	331.3	329.0
3	$-CH_2CH_3$	193.6	39.4	50.9
4	$-CH_2(CH_2)_3CH_3$	153.1	—[a]	—[a]
5	$-CH_2(CH_2)_6CH_3$	1335.6	308.6	239.4
6	$-CH_2(CH_2)_{10}CH_3$	621.9	—[a]	112.3
7	$-CH_2(CH_2)_{12}CH_3$	127.9	—[a]	—[a]
8	$-CH_2(CH_2)_{14}CH_3$	706.1	—[a]	—[a]
9	$-CH_2(CH_2)_{16}CH_3$	>250	—[a]	—[a]

[a] No anti-viral activity at the 50% cytotoxic concentration.

Table 2. Derivatives of Phenyl Rupestonate and *in vitro* Anti-Influenza Activity.

Entry	R_2	R_3	TC_{50} (μM)	Against Influenza Virus A3, IC_{50} (μM)	Against Influenza Virus B, IC_{50} (μM)
1	H	H	41.4	16.7	19.4
2	H	CH_3	191.3	13.4	—[a]
3	H	$C(CH_3)_3$	18.9	0.5	—[a]
4	H	OH	141.5	43.5	—[a]
5	H	F	520.8	12.0	—[a]
6	H	Cl	240.5	11.5	—[a]
7	Cl	H	49.6	11.5	—[a]
8	Cl	Cl	136.3	—[a]	—[a]
9	H	Br	44.2	10.2	—[a]
10	H	$NHCOCH_3$	189.5	29.4	24.9
11	H	$COOCH_3$	22.5	6.3	—[a]
Oseltamivir			>1219.5	5.1	—[a]

[a] No anti-viral activity at the 50% cytotoxic concentration.

Table 3. Derivatives of Benzyl Rupestonate and *in vitro* Anti-Influenza Activity.

Entry	R_4	R_5	TC_{50} (μM)	Against Influenza Virus A3, IC_{50} (μM)	Against Influenza Virus B, IC_{50} (μM)
1	H	H	109.6	—[a]	28.3
2	H	F	20.0	—[a]	—[a]
3	H	CF_3	3.4	—[a]	—[a]
4	H	Cl	47.7	12.5	19.1
5	H	NO_2	25.0	5.5	5.5
6	H	Br	9.8	—[a]	—[a]
7	Br	H	51.3	—[a]	23.0
8	H	OCH_3	19.4	—[a]	—[a]
9	Cl	Cl	14.6	7.8	—[a]
10	Cl	H	57.3	15.2	25.7

[a] No anti-viral activity at the 50% cytotoxic concentration.

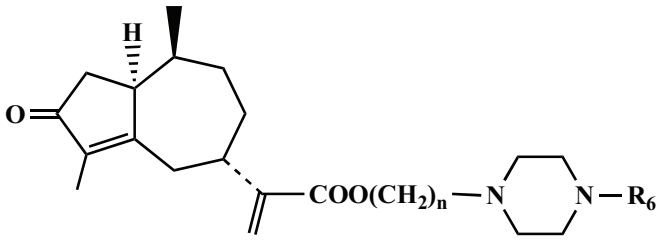


Fig. (7).

Table 4. Derivatives of Piperazine Moiety Contained Rupestonate and *in vitro* Anti-Influenza Activity.

Entry	n	R_6	TC_{50} (μM)	Against Influenza Virus A, IC_{50} (μM)		Against Influenza Virus B, IC_{50} (μM)
				H3N2	H1N1	
1	2	CH_3	230.6	>33.0	128.5	>33.02
2	3	CH_3	666.8	>95.5	>95.5	>95.46
3	4	CH_3	643.5	71.5	10.25	71.49
4	5	CH_3	207.3	20.6	17.14	20.58
5	6	CH_3	86.0	>9.58	>9.58	>9.58
6	8	CH_3	188.3	>26.85	>26.85	>26.86
7	10	CH_3	177.4	5.22	3.72	>25.41
8	2	Ph	445.0	49.44	49.44	>49.44
9	3	Ph	171.1	57.07	63.87	57.07
10	4	Ph	414.9	>79.83	79.83	>79.83
11	5	Ph	111.7	>25.7	20.04	>25.84
12	6	Ph	394.3	>56.46	>56.46	>56.5
13	8	Ph	333.3	>160.2	92.52	>160.19
14	10	Ph	456.2	94.0	2.43	>152.0

Aisa *et al.* [12] have reported a series of 1,2,4-triazole moiety contained rupestonate derivatives (Fig. **8**), the *in vitro* bioassayed (Table **5**) results showed that all of these compounds possess dual biological activity both against influenza virus A and B. Entry **7** showed the highest inhibition to the strains of H3N2 and H1N1, with the respective IC_{50} values of 0.97 and 0.42μM, which were even smaller that those of Oseltamivir. Unfortunately, Entry **7** showed the highest toxicity (TC_{50}=27.1μM) among these rupestonates.

Some sugar esters often exhibit antitumor [13], disinfection [14], and some suger esters are always used as prodrug [15]. Thus Aisa *et al*. [16] have prepared a series of sugar moiety contained rupestonic acid ester derivatives (Fig. **9**) and screened their *in vitro* anti-influenza activity, the result showed that compound **d** and **g** respectively possess higher activity against the strain of influenza H3N2 (IC_{50}=0.35μM), (IC_{50}=0.21μM) than the reference drug Oseltamivir against the strain of influenza H3N2 (IC_{50}=1.11μM), which can be as the lead compounds for developing the anti-influenza drugs.

RUPESTONIC ACID AMIDE DERIVATIVES AND ANTI-INFLUENZA ACTIVITY

Yong *et al* [17] synthesized a series of chiral amino moiety contained intermediates, then introduced these intermediates at the carboxyl position of rupestonic acid and synthesized a series of novel amino acid moiety contained

Table 5. Derivatives of 1,2,4-triazole moiety Contained Rupestonate and *in vitro* Anti-Influenza Virus.

Entry	n	TC_{50} (μM)	Against Influenza Virus A, IC_{50} (μM)		Against Influenza Virus B, IC_{50} (μM)
			H3N2	H1N1	
1	2	224.5	74.9	83.8	>8.0
2	3	311.2	34.6	24.0	>34.6
3	4	386.0	42.9	57.6	77.5
4	5	672.0	17.3	7.4	17.3
5	6	119.5	14.9	48.2	>31.0
6	8	201.9	5.2	7.2	3.2
7	10	27.1	0.97	0.42	11.3
Oseltamivir		1260	1.1	15.5	>500

rupestonic acid amide derivatives (Fig. **10**) and screened their *in vitro* anti-influenza activity, the result showed that compound **j** possess higher anti-influenza virus A3 (IC_{50}=28.7μM) than the parent compound (rupestonic acid).

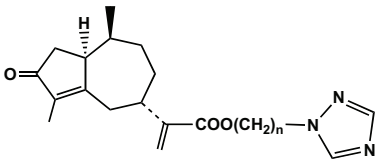
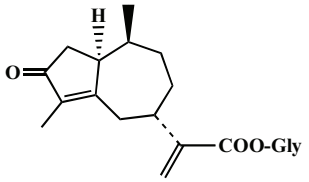


Fig. (8).



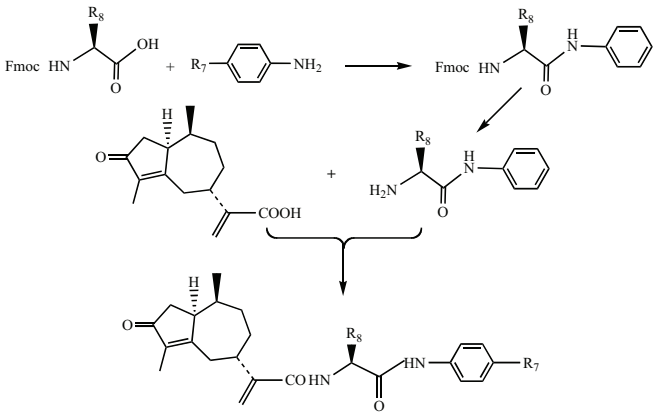
Gly **a :** 2,3,4,6-tetra-O-acetyl-bata-D-glucosyl **b :** 2,3,4,6- tetra-O-acetyl-alfa-D-mannosyl

c : 2,3,4,6-tetra-O-acetyl-bata-D-galactosyl **d :** 2,3,4-tri-O-acetyl-bata-D-xylosyl

e : 2,3,4-tri-O-acetyl-alfa-L-arabinosyl **f :** 2,3,4-tri-O-acetyl-bata-D-ribosyl

g : 2,3,6,2 ,3 ,4 ,6 -hepta-O-acetyllactosyl **h :** 2,3,6,2 ,3 ,4 ,6 -hepta-O-acetylmaltosyl

Fig. (9).



i : $R_8 = C_6H_5CH_2$- , $R_7 = CH_3$ **j :** $R_8 = C_6H_5CH_2$- , $R_7 = OCH_3$

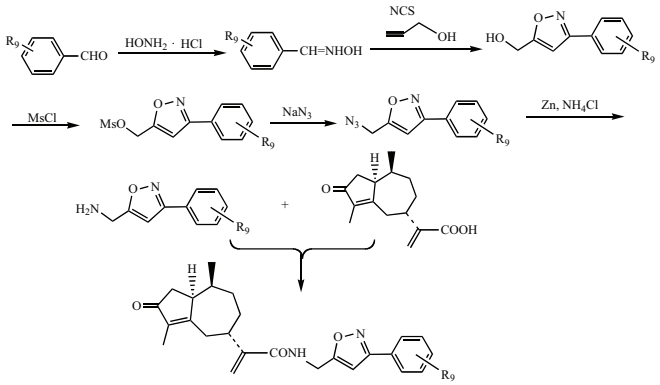
k : $R_8 = C_6H_5CH_2$- , $R_7 = H$ **l :** $R_8 = CH_2CH(CH_3)_2$, $R_7 = CH_3$

m : $R_8 = CH_2CH(CH_3)_2$, $R_7 = OCH_3$

Fig. (10).

Yong *et al.* [18] also have reported a series of rupestonic acid aromatic amide derivatives and screened their *in vitro* anti-influenza activity. However, most of these derivatives do not show anti-influenza activity.

Aisa *et al.* synthesized a series of 3-aryl-5-isoxazole-methylamine intermediates [19], then introduced them at the carboxyl group of rupestonic acid and synthesized a series of rupestonic acid amide derivatives (Fig. **11**) and assayed their *in vitro* anti-influenza activity. The results showed most of these compounds possess higher inhibition to both influenza virus A and B, and compound **q** possess higher inhibition to H3N2 strain with IC_{50}=1.09μM [20].



n : R_9 =H **o :** R_9 = *p*-Cl **p :** R_9= *p*-F

q : R_9 = *o*-OCH_3 **r :** R_9 = *p*-OCH_3 **s :** R_9 = *p*-CH_3

Fig. (11).

In order to study the structure-activity relationship of rupestonic acid, Yong *et al* [21] also synthesized the 3-deoxyrupestonic acid by the Clemmensen reduction and screened its *in vitro* anti-influenza activity. The result showed that 3-deoxyrupestonic acid possessed less anti-influneza virus B activity (TC_{50}=1424.4μM, IC_{50}=417.3μM) than that of rupestonic acid (TC_{50}=1044.4μM, IC_{50}=115.7μM), but the toxicity reduced.

CONCLUSIONS

By now, our researching group takes the lead in synthesizing the rupestonic acid derivatives and has achieved significant results. We find the lead anti-influenza compounds. Based on the previous study of the structure activity relationship of neuraminidase inhibitors [22-28]. More rupestonic acid derivatives will be designed and synthesized soon for the development of new anti-influenza drugs.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

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