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Synthesis and antiviral activity of lycorine derivatives

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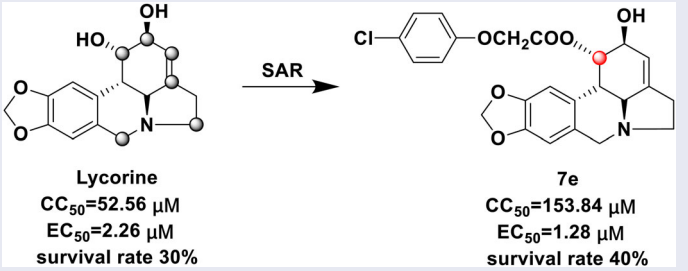
ABSTRACT

There are no effective antiviral drugs to treat hand, foot, and mouth disease. In this study, a series of lycorine derivatives were synthesized and evaluated against enterovirus 71 and coxsackievirus A16 *in vitro*. Derivatives **7c-m** with the phenoxyacyl group at the C-1 position showed higher efficacy and lower toxicity than lycorine. In addition, derivative **7e** enhanced the survival rate to 40% in the mouse model of the lethal EV71 infection.



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Lycorine; hand; foot; and mouth disease; enterovirus 71; coxsackievirus A16

1. Introduction

After severe outbreaks in the past two decades, hand, foot, and mouth disease (HFMD) has become a highly infectious illness for global public health [1, 2]. This disease was common in children, who are younger than five years old, and could be diagnosed in adults occasionally [3]. HFMD were usually benign, mild, and self-limiting. However, serious cardiovascular and neurological complications could also occur in some cases, which might be fatal to children [4]. Coxsackievirus A16 (CAV16) and enterovirus 71 (EV71) were two major causes of HFMD [5]. There was an evidence that EV71 was associated with serious complications [6]. Since 2007, HFMD has been prevalent in China. More than 20 million cases and 3000 deaths were reported

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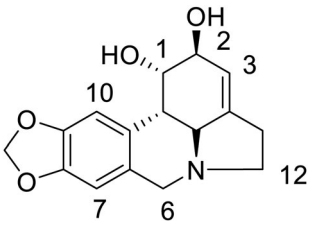


Lycorine

Figure 1. The structure of lycorine.

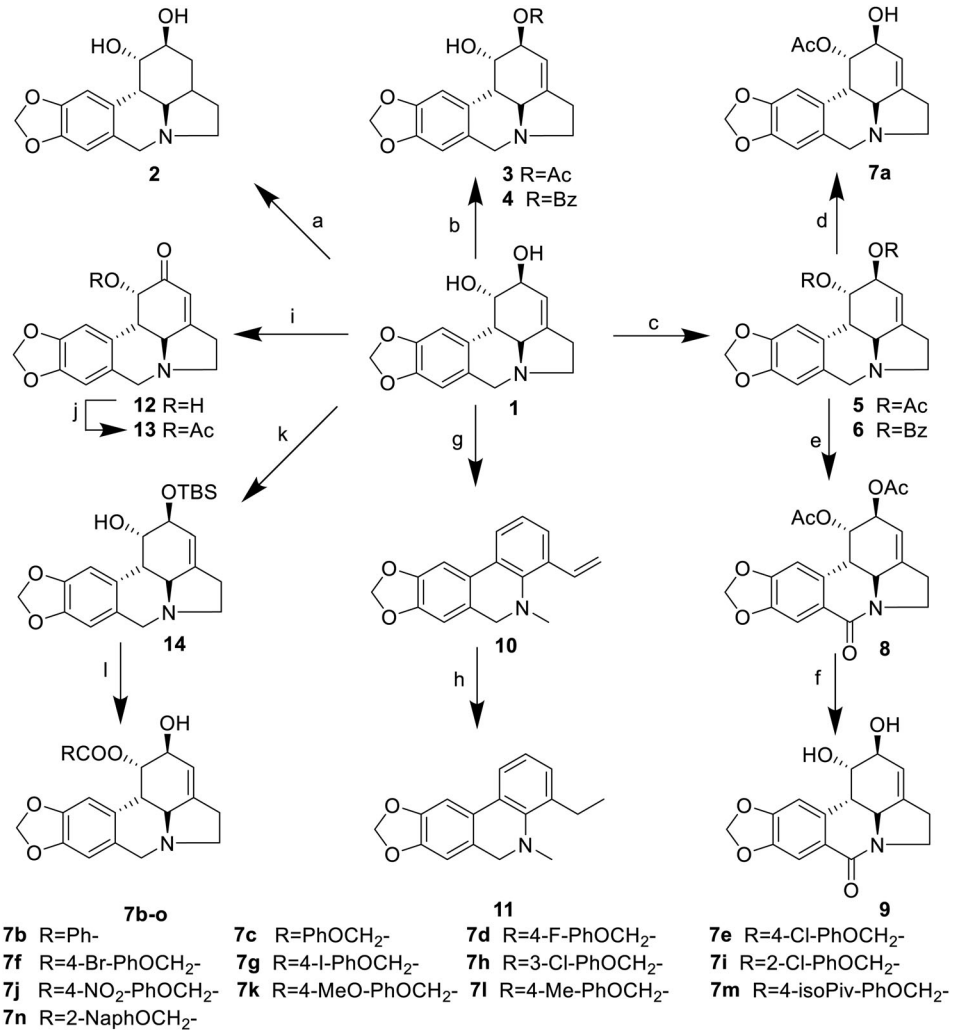
between 2008 and 2018 [7]. An EV71 vaccine was approved for outbreaks of HFMD in 2016 [8]. It not only did not treated infected patients but also not offer cross-protection caused by other pathogens. Up to now, there are no effective antiviral drugs to treat HFMD.

In order to discover novel lead compounds for the treatment of HFMD, we carried out the screening study of natural products according to an in-house collection. Some tannins and alkaloids were identified to possess anti-EV71 activity in both *in vitro* and *in vivo*, including geraniin [9], punicalagin [10], chebulagic acid [11], deferoxamine [12], matrine [13], oxysophocarpine [14], and lycorine [15]. Among them, lycorine (Figure 1) displayed the most effective potency against EV71, and moderate cytotoxicity was also observed. Furthermore, its mechanism was associated with the inhibition of viral proteases and the down-regulation of autophagy [15–17]. Encouraged by these results, we attempted to investigate the structure-activity relationship (SAR) study of lycorine to discover more potent derivatives with less cytotoxicity. In this work, a series of lycorine derivatives were designed and synthesized, and their inhibitory activities against EV71 and CAV16 were evaluated both in *in vitro* and *in vivo*.

2. Results and discussion

2.1. Chemistry

Starting from a commercially available lycorine (**1**), the target compounds were synthesized according to the reported procedures (Scheme 1). Briefly, the hydrogenation of lycorine hydrochloride afforded dihydrolycorine (**2**) [18]. Considering the difference of the C1- and C2-hydroxyls, 2-acetyllycorine **3**, and 2-benzoyllycorine **4** were synthesized by 1 eq acetic anhydride or benzoyl chloride, respectively. By more than 2 eq acylation reagents, the diesters **5** and **6** were achieved [19]. 1-acetyllycorine **7a** was prepared by the selective removal of the acetyl group at the C-2 position [20]. The oxidation of **5a** with $PhI(OAc)_2$ led to lactam **8**, which was further hydrolyzed to yield **9** [19]. The phenanthridine derivative **10** was obtained by the methylation and Hoffman degradation of **1** [21]. The hydrogenation of compound **10** in reflux MeOH (Methanol) afforded **11**. The selective oxidation of **5a** using the Dess–Martin reagent afforded 2-O-lycorine **12**, which was subsequently acylated to provide **13** [19]. The treatment of **1** with TBSCl (tert-Butyldimethylsilyl chloride) afforded 2-TBS-lycorine



Scheme 1. The synthesis of lycorine derivatives. Reagents and conditions: **a**, 10% Pd/C, H_2, H_2O, rt; **b**, Ac_2O or PhCOCl (1.0 eq), Py, rt; **c**, Ac_2O or PhCOCl (2.5 eq), Py, rt; **d**, HCl, MeOH, 60 °C; **e**, PhIO, TBAI, CH_3CN/H_2O 9:1, rt; **f**, K_2CO_3, MeOH/H_2O 9:1, 60 °C; **g**, MeI, DMF, rt then t-BuOK, t-BuOH, reflux; **h**, 10% Pd/C, H_2, MeOH, reflux; **i**, DMP, DMF, rt; **j**, Ac_2O, Py, rt; **k**, TBSCl, $AgNO_3$, TEA, DMF, rt; **l**, acids, EDCI, DMAP, DCM, rt then TBAF, THF, rt.

14 with regioselectivity [19], which could be easily transformed to the monosubstituted ethers **7b–o** at the C-1 position.

2.2. The cytotoxicity and antiviral activity in vitro

The synthesized lycorine derivatives were evaluated against EV71 and CAV16 in human rhabdomyosarcoma (RD) cells. The CC_{50} (50% cytotoxic concentration) values for cytotoxicity and EC_{50} (50% effective concentration) values for antiviral activity were measured as described previously. The selectivity index (SI) was calculated using the ratio of CC_{50} and EC_{50}. As a positive control, lycorine (**1**) showed potent

Table 1. The antiviral activity of lycorine derivatives against EV71 and CAV16 *in vitro*.

		EV71		CAV16	
Comp.	CC_{50} (μM)	EC_{50} (μM)	SI	EC_{50} (μM)	SI
1	52.26	2.26	23.12	1.57	33.27
2	>100	>100	–	31.14	>3.21
3	>100	6.08	>16.45	>100	–
4	>100	51.15	>1.96	>100	–
5	>100	28.30	>3.53	>100	–
6	>100	>100	–	>100	–
7a	>100	2.42	>41.32	2.58	>38.76
7b	>100	12.79	>7.82	12.79	>7.82
7c	>100	1.64	>60.98	1.19	>84.03
8	>100	>100	–	>100	–
9	>100	>100	–	63.12	>1.58
10	>100	>100	–	>100	–
11	>100	>100	–	>100	–
12	>100	70.18	>1.42	70.18	>1.42
13	>100	30.58	>3.27	30.58	>3.27

inhibitory activity against EV71 and CAV16 with EC_{50} values of 2.26 and 1.57 μM, respectively. Meanwhile, its cytotoxicity was 52.26 μM, leading to moderate SIs. Except **7g**, significantly decreased cytotoxicity (> 100 μM) was observed for the synthesized lycorine derivatives, suggesting that these derivatives might be less toxicity than lycorine. As shown in Table 1, for inhibitory activity against EV71, dihydrolycorine (**2**) displayed a loss of activity, indicating that the double bond at the C-3 position played an important role. Unexpectedly, a moderate activity for **2** was observed against CAV16. In general, 2-esters (**3** and **4**) and 1,2-diesters (**5** and **6**) resulted in a reduction or a loss of activity. The derivatives (**8** and **9**) oxidized at the C-6 position were totally inactive. Unfortunately, the activity of phenanthridine derivatives (**10** and **11**) also decreased. The 2-ketone derivatives (**12** and **13**) were reported to increase in an antiviral activity against dengue virus [20], but the poor activity was observed for them against EV71 and CAV16. Compared with lycorine, 1-acetyllycorine **7a** showed similar activity against EV71 and CAV16 with improved SI, but derivative **7b** showed weaker activity. It suggested that the C-1 position might be sensitive to the antiviral activity. Notably, when the phenoxyacyl group (**7c**) was introduced at the C-1 position, an increase in both antiviral activity and SI was observed with EC_{50} values of 1.19 μM for CAV16 and 1.64 μM for EV71.

To further investigate the effects of substitutions in the phenoxyacyl group, derivatives **7d–n** were designed and synthesized. Moreover, both antiviral activity and SI were improved again (Table 2). The introduction of electro-withdrawing groups (**7d**, **7e**, **7j**) was usually superior to electron-donating groups (**7k**, **7l**, **7m**). The para-substitution of benzene ring (**7d**) was more suitable than ortho- (**7i**) and meta-substitution (**7h**). The change of benzene ring (**7c**) to naphthalene ring (**7n**) led to a slight decrease. Among them, derivatives **7d**, **7e,** and **7j** displayed good inhibitory effects against EV71 with EC_{50} values in the range between 1.23 and 1.28 μM.

On the basis of above results, a preliminary SAR could be described. Modifications on the double bond at the C-3 position and the methylene group at the C-6 position were unfavorable for the activity. The maintenance of molecular scaffold and C2-hydroxyl was also important for activity. It was suggested that further modification was focused on the C1-hydroxyl.

Table 2. The antiviral activity of lycorine derivatives against EV71 *in vitro*.

		EV71	
Comp.	CC_{50} (μM)	EC_{50} (μM)	SI
7d	131.19	1.27	103.30
7e	153.84	1.28	139.85
7f	103.43	1.47	70.36
7g	99.64	1.84	54.15
7h	151.56	1.73	87.61
7i	145.24	1.63	89.10
7j	105.38	1.23	85.67
7k	161.12	1.65	97.65
7l	134.22	1.53	87.73
7m	101.25	1.67	60.63
7n	163.41	1.97	82.94

2.3. The treatment effect of 7e in the mouse model

Considering the good inhibitory effect and high SI *in vitro*, derivative **7e** was selected to further investigate the effect in the mouse model of the lethal EV71 infection (Figure 2). All mice died within 10-day post infection (dpi) in the saline-treated group. Ribavirin and lycorine, as control drugs, prolonged the survival rate of mice to 10% and 30%, respectively. As expected, derivative **7e** enhanced the survival time of infected mice to 40% under the treatment of 1.0 mg/kg dose. Compared to the saline-treated group, treatment with **7e** reduced the clinical scores by delaying the appearance of symptoms, including weakness and paralysis in hind limbs.

3. Conclusion

In this study, a series of lycorine derivatives were synthesized and evaluated against EV71 and CAV16 *in vitro*. Compared to lycorine, derivatives **7c–m** with the phenoxyacyl group at the C-1 position showed higher efficacy and lower toxicity. In addition, derivative **7e** enhanced the survival rate to 40% in the mouse model of the lethal EV71 infection. These results provided a potential lead for the treatment of HFMD.

4. Experimental

4.1. Chemistry

All chemical reagents and solvents were commercially available without further purification. With tetramethylsilane (TMS) as internal standard, ^{1}H-NMR spectra were recorded on Varian Mercury 400 MHz spectrometers (California, USA). The API-TOFMS 10000 instrument was used to measure mass spectra with HR-API mode (Guangzhou, China). Compounds **2–14** were synthesized according to reported methods [18–21].

A solution of **14** (1 mmol), acids (1.2 mmol), EDCI (1-Ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1.2 mmol), and DMAP (Dimethylaminopyridine, 0.12 mmol) in DCM (Dichloromethane, 15 ml) was stirred at room temperature for 12 h. Then, 50 ml DCM was added. The organic phase was washed with saturated

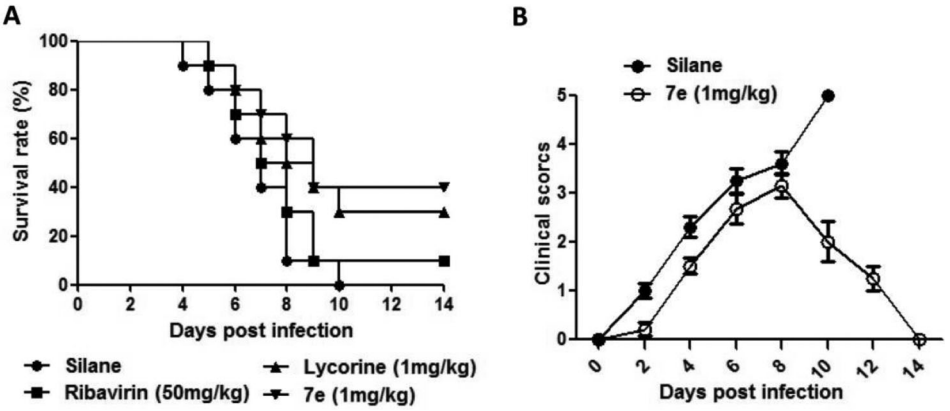


Figure 2. The treatment effect of **7e** in the mouse model. (A) The survival rates. (B) The clinical scores.

$NaHCO_3$ (20 ml), H_2O (20 ml), and brine (20 ml). Dried over Na_2SO_4, the solvent was evaporated. The residue was dissolved in 15 ml THF (Tetrahydrofuran) and 2 mmol TBAF (Tetrabutylammonium fluoride) was added. After the reaction, 50 ml DCM was added. The organic phase was washed with saturated $NaHCO_3$ (20 ml), H_2O (20 ml), and brine (20 ml). After evaporation under reduced pressure, the residue was purified using silica gel column chromatography to afford compounds **7c–n**.

4.1.1. 1-(Phenoxyacyl)-lycorine (7c)

Yield: 53.4%. ^{1}H NMR ($CDCl_3$): δ 7.16–7.12 (m, 2H), 6.93–6.89 (m, 1H), 6.68–6.66 (m, 3H), 6.53 (s, 1H), 5.93 (d, $J = 8.0$ Hz, 2H), 5.75 (s, 1H), 5.51 (s, 1H), 4.51 (s, 2H), 4.21 (s, 1H), 4.08 (d, $J = 16$ Hz, 1H), 3.35–3.30 (m, 2H), 2.85 (d, $J = 8.0$ Hz, 1H), 2.59 (m, 2H), 2.52 (d, $J = 8.0$ Hz, 1H), 2.27 (q, $J = 8.0$ Hz, 1H). HR-API-MS: *m/z* 422.1606 $[M + H]^+$ (calcd for $C_{24}H_{24}NO_6$, 422.1598).

4.1.2. 1-(4-F-Phenoxyacyl)-lycorine (7d)

Yield: 45.6%. ^{1}H NMR ($CDCl_3$): δ 6.82–6.78 (m, 2H), 6.60–6.54 (m, 4H), 5.96 (s, 1H), 5.91 (s, 1H), 5.69 (s, 1H), 5.49 (s, 1H), 4.47 (s, 2H), 4.16 (s, 1H), 4.13–4.11 (m, 1H), 3.34–3.31 (m, 2H), 2.84 (d, $J = 8.0$ Hz, 1H), 2.57 (m, 2H), 2.51 (d, $J = 12.0$ Hz, 1H), 2.28 (q, $J = 8.0$ Hz, 1H). HR-API-MS: *m/z* 440.1517 $[M + H]^+$ (calcd for $C_{24}H_{23}FNO_6$, 440.1504).

4.1.3. 1-(4-Cl-phenoxyacyl)-lycorine (7e)

Yield: 84.1%. ^{1}H NMR ($CDCl_3$): δ 7.08–7.04 (m, 2H), 6.66 (s, 1H), 6.58–6.54 (m, 3H), 6.00 (d, $J = 4.0$ Hz, 1H), 5.94 (d, $J = 4.0$ Hz, 1H), 5.76 (s, 1H), 5.52 (s, 1H), 4.52 (s, 2H), 4.20 (s, 1H), 4.10 (d, $J = 12.0$ Hz, 1H), 3.36–3.28 (m, 2H), 2.85 (d, $J = 12.0$ Hz, 1H), 2.59 (m, 2H), 2.41 (d, $J = 8.0$ Hz, 1H), 2.27 (q, $J = 8.0$ Hz, 1H). HR-API-MS: *m/z* 456.1222 $[M + H]^+$ (calcd for $C_{24}H_{23}ClNO_6$, 456.1214).

4.1.4. 1-(4-Br-phenoxyacyl)-lycorine (7f)

Yield: 48.6%. ^{1}H NMR ($CDCl_3$): δ 7.17 (d, $J = 8.0$ Hz, 2H), 6.60 (s, 1H), 6.57 (s, 1H), 6.49 (d, $J = 8.0$ Hz, 2H), 5.99 (s, 1H), 5.93 (s, 1H), 5.72 (s, 1H), 5.50 (s, 1H), 4.50 (s, 2H), 4.17 (s, 1H), 4.08 (d, $J = 8.0$ Hz, 1H), 3.32–3.26 (m, 2H), 2.83 (d, $J = 12.0$ Hz, 1H), 2.58 (s, 2H), 2.39 (s, 1H), 2.25 (s, 1H). HR-API-MS: m/z 500.0691 $[M + H]^+$ (calcd for $C_{24}H_{23}BrNO_6$, 500.0703).

4.1.5. 1-(4-I-Phenoxyacyl)-lycorine (7g)

Yield: 48.4%. ^{1}H NMR ($CDCl_3$): δ 7.34 (d, $J = 8.0$ Hz, 2H), 6.59 (s, 2H), 6.37 (d, $J = 8.0$ Hz, 2H), 5.99 (s, 1H), 5.93 (s, 1H), 5.70 (s, 1H), 5.49 (s, 1H), 4.49 (s, 2H), 4.15 (s, 1H), 4.08 (d, $J = 12.0$ Hz, 1H), 3.33–3.23 (m, 2H), 2.81 (d, $J = 8.0$ Hz, 1H), 2.57 (s, 2H), 2.36 (m, 1H), 2.24 (m, 1H). HR-API-MS: m/z 548.0568 $[M + H]^+$ (calcd for $C_{24}H_{23}INO_6$, 548.0565).

4.1.6. 1-(3-Cl-phenoxyacyl)-lycorine (7h)

Yield: 56.4%. ^{1}H NMR ($CDCl_3$): δ 7.04–7.01 (m, 1H), 6.89–6.88 (m, 1H), 6.73 (s, 1H), 6.60 (s, 1H), 6.53–6.51 (m, 2H), 5.94 (s, 1H), 5.92 (s, 1H), 5.70 (s, 1H), 5.50 (s, 1H), 4.51 (s, 2H), 4.18 (s, 1H), 4.09 (d, $J = 8.0$ Hz, 1H), 3.35–3.32 (m, 2H), 2.85 (d, $J = 8.0$ Hz, 1H), 2.59 (s, 2H), 2.50 (s, 1H), 2.31 (s, 1H). HR-API-MS: m/z 456.1219 $[M + H]^+$ (calcd for $C_{24}H_{23}ClNO_6$, 456.1214).

4.1.7. 1-(2-Cl-phenoxyacyl)-lycorine (7i)

Yield: 64.3%. ^{1}H NMR ($CDCl_3$): δ 7.28 (m, 1H), 6.97–6.94 (m, 1H), 6.85–6.82 (m, 1H), 6.57 (s, 1H), 6.50–6.47 (m, 2H), 5.95 (s, 1H), 5.91 (s, 1H), 5.68 (s, 1H), 5.46 (s, 1H), 4.61 (d, 2H), 4.19 (s, 1H), 4.14–4.06 (m, 2H), 3.33–3.32 (m, 2H), 2.83 (d, $J = 8.0$ Hz, 1H), 2.57 (s, 4H), 2.31 (s, 1H). HR-API-MS: m/z 456.1224 $[M + H]^+$ (calcd for $C_{24}H_{23}ClNO_6$, 456.1214).

4.1.8. 1-(4-No$_2$-phenoxyacyl)-lycorine (7j)

Yield: 43.5%. ^{1}H NMR ($CDCl_3$): δ 7.02 (d, $J = 4.0$ Hz, 2H), 6.67 (d, $J = 8.0$ Hz, 2H), 6.55 (s, 1H), 6.51 (s, 1H), 6.02 (s, 1H), 5.92 (s, 1H), 5.71 (s, 1H), 5.51 (s, 1H), 4.63 (d, $J = 16.0$, 12.0 Hz, 2H), 4.19 (s, 1H), 4.08 (d, $J = 12.0$ Hz, 1H), 3.33–3.31 (m, 1H), 3.24 (d, $J = 12.0$ Hz, 1H), 2.83 (d, $J = 8.0$ Hz, 1H), 2.59 (s, 2H), 2.43 (s, 1H), 2.24 (s, 1H). HR-API-MS: m/z 467.1453 $[M + H]^+$ (calcd for $C_{24}H_{23}N_2O_8$, 467.1449).

4.1.9. 1-(4-Meo-phenoxyacyl)-lycorine (7k)

Yield: 84.5%. ^{1}H NMR ($CDCl_3$): δ 6.67–6.58 (m, 5H), 6.53 (s, 1H), 5.94 (s, 1H), 5.91 (s, 1H), 5.71 (s, 1H), 5.50 (s, 1H), 4.46 (s, 2H), 4.18 (s, 1H), 4.09 (d, $J = 8.0$ Hz, 1H), 3.73 (s, 3H), 3.36–3.31 (m, 2H), 2.84 (d, $J = 8.0$ Hz, 1H), 2.59 (s, 2H), 2.55 (s, 1H), 2.29 (s, 1H). HR-API-MS: m/z 452.1706 $[M + H]^+$ (calcd for $C_{25}H_{26}NO_7$, 452.1709).

4.1.10. 1-(4-Me-phenoxyacyl)-lycorine (7l)

Yield: 55.3%. ^{1}H NMR ($CDCl_3$): δ 6.91 (d, $J = 8.0$ Hz, 2H), 6.62 (s, 1H), 6.55–6.63 (m, 3H), 5.94 (s, 1H), 5.92 (s, 1H), 5.71 (s, 1H), 5.50 (s, 1H), 4.48 (s, 2H), 4.16 (s, 1H), 4.08 (d, $J = 12.0$ Hz, 1H), 3.32–3.31 (m, 2H), 2.84 (d, $J = 8.0$ Hz, 1H), 2.58 (s,

2H), 2.51 (s, 1H), 2.28–2.27 (m, 1H), 2.24 (s, 3H). HR-API-MS: *m/z* 436.1753 $[M+H]^+$ (calcd for $C_{25}H_{26}NO_6$, 436.1760).

4.1.11. 1-(4-Iso-Piv-phenoxyacyl)-lycorine (7m)

Yield: 59.8%. ^{1}H NMR ($CDCl_3$): δ 7.00 (d, $J=8.0$ Hz, 2H), 6.66 (s, 1H), 6.59 (d, $J=8.0$ Hz, 2H), 6.55 (s, 1H), 5.94 (s, 1H), 5.93 (s, 1H), 5.73 (s, 1H), 5.50 (s, 1H), 4.46 (s, 2H), 4.19 (s, 1H), 4.10 (d, $J=8.0$ Hz, 1H), 3.36–3.32 (m, 2H), 2.95–2.80 (m, 2H), 2.59 (m, 2H), 2.29 (s, 1H), 2.04 (s, 1H), 2.20 (d, 6H). HR-API-MS: *m/z* 464.2053 $[M+H]^+$ (calcd for $C_{27}H_{30}NO_6$, 464.2040).

4.1.12. 1-(2-Naphoxyacyl)-lycorine (7n)

Yield: 59.6%. ^{1}H NMR ($CDCl_3$): δ 7.73 (d, $J=4.0$ Hz, 1H), 7.65 (d, $J=4.0$ Hz, 1H), 7.49–7.47 (m, 1H), 7.42–7.39 (m, 1H), 7.35–7.32 (m, 1H), 7.06–7.04 (m, 1H), 6.78 (s, 1H), 6.63 (s, 1H), 6.38 (s, 1H), 5.88 (s, 1H), 5.87 (s, 1H), 5.73 (s, 1H), 5.46 (s, 1H), 4.66 (d, 2H), 4.20 (s, 1H), 3.95 (d, $J=8.0$ Hz, 1H), 3.25–3.22 (m, 1H), 3.05–3.02 (m, 1H), 2.80 (d, $J=8.0$ Hz, 1H), 2.52–2.36 (m, 3H), 2.10 (s, 1H). HR-API-MS: *m/z* 472.1767 $[M+H]^+$ (calcd for $C_{28}H_{26}NO_6$, 472.1760).

4.2. Antiviral and cytotoxicity assay in RD cells

An EV71 strain FY0805 (HQ882182) and a CAV16 strain Shzh05-1 (262658) were used in RD cells. The CC_{50} (50% cytotoxic concentration) values for cytotoxicity and EC_{50} (50% effective concentration) values for the antiviral activity were measured as described previously [10].

4.3. Mouse protection assay

Each ICR 10-day-old mouse (n = 40) was intraperitoneally (i.p.) inoculated with 1×10^7 $TCID_{50}$ of MP10 (HQ712020). After 2 h, different concentrations of ribavirin, lycorine, and **7e** in saline were injected to the infected mice with once daily for 6 days. The survival rates and symptoms were monitored daily for 2 weeks. The clinical scores of infected mice were graded according to previously described methods [10].

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] J. Huang, Q.H. Liao, M.H. Ooi, B.J. Cowling, Z.R. Chang, P. Wu, F.F. Liu, Y. Li, L. Luo, S.B. Yu, H.J. Yu, and S. Wei, *Emerg. Infect. Dis.* **24**, 432 (2018).

[2] S. Esposito and N. Principi, *Eur. J. Clin. Microbiol. Infect. Dis.* **37**, 391 (2018).

[3] C. Murase and M. Akiyama, *N Engl. J. Med.* **378**, e20 (2018).

[4] K. Shekhar, M.S. Lye, O. Norlijah, O. Flora, L.M. Looi, R. Khuzaiah, I. Marzuki, I. Hussein, S.L. Wong, J. Mohan, M. Sinniah, and S.A. Bakar, *Med. J. Malays.* **60**, 297 (2005).

[5] Z.C. Zhuang, Z.Q. Kou, Y.J. Bai, X. Cong, L.H. Wang, C. Li, L. Zhao, X.J. Yu, Z.Y. Wang, and H.L. Wen, *Viruses.* **7**, 6400 (2015).

[6] T. Solomon, P. Lewthwaite, D. Perera, M.J. Cardosa, P. Mcminn, and M.H. Ooi, *Lancet. Infect. Dis.* **10**, 778 (2010).

[7] J.R. Head, P.A. Collender, J.A. Lewnard, N.K. Skaff, L. Li, Q. Cheng, J.M. Baker, C. Li, D.H. Chen, A. Ohringer, S. Liang, C.H. Yang, A. Hubbard, B. Lopman, and J.V. Remais, *Clin. Infect. Dis.* **71**, ciz1188 (2019).

[8] M. Zhang, Y. Wang, W.L. He, Y. Sun, Y. Guo, W.L. Zhong, Q. Gao, M.Y. Liao, X.X. Wang, Y. Cai, Y. Guo, and Z.H. Rao, *J. Med. Chem.* **63**, 1233 (2020).

[9] Y.J. Yang, L.F. Zhang, X.X. Fan, C. Qin, and J.N. Liu, *Bioorg. Med. Chem. Lett.* **22**, 2209 (2012).

[10] Y.J. Yang, J.H. Xiu, L.F. Zhang, C. Qin, and J.N. Liu, *Phytomedicine.* **20**, 67 (2012).

[11] Y.J. Yang, J.H. Xiu, J.N. Liu, L. Zhang, X.Y. Li, Y.F. Xu, C. Qin, and L.F. Zhang, *Int. J. Mol. Sci.* **14**, 9618 (2013).

[12] Y.J. Yang, J. Ma, J.H. Xiu, L. Bai, F.F. Guan, L. Zhang, J.N. Liu, and L.F. Zhang, *Mar. Drugs.* **12**, 4086 (2014).

[13] Y.J. Yang, J.H. Xiu, X. Zhang, L.F. Zhang, K. Yan, C. Qin, and J.N. Liu, *Molecules.* **17**, 10370 (2012).

[14] Y.J. Yang, F.F. Guan, L. Bai, L. Zhang, J.N. Liu, X.D. Pan, and L.F. Zhang, *Bioorg. Med. Chem. Lett.* **25**, 3526 (2015).

[15] J.N. Liu, Y.J. Yang, Y.F. Xu, C.M. Ma, C. Qin, and L.F. Zhang, *Virol. J.* **8**, 483 (2011).

[16] H.Q. Wang, T.T. Guo, Y.J. Yang, L. Yu, X.D. Pan, and Y.H. Li, *Front. Cell. Infect. Microbiol.* **9**, 277 (2019).

[17] Y. Guo, Y.X. Wang, L. Cao, P. Wang, J. Qing, Q.Z. Zheng, L.Q. Shang, Z. Yin, and Y. Sun, *Antimicrob. Agents Chemother.* **60**, 913 (2016).

[18] D.Z. Chen, J.Y. Cai, J.J. Cheng, C.X. Jing, J.L. Yin, J.D. Jiang, Z.G. Peng, and X.J. Hao, *Sci. Rep.* **5**, 14972 (2015).

[19] N.M. Evdokimov, D. Lamoral-Theys, V. Mathieu, A. Andolfi, L.V. Frolova, S.C. Pelly, W.A. van Otterlo, I.V. Magedov, R. Kiss, A. Evidente, and A. Kornienko, *Bioorg. Med. Chem.* **19**, 7252 (2011).

[20] P. Wang, L.F. Li, Q.Y. Wang, L.Q. Shang, P.Y. Shi, and Z. Yin, *Chem. Med. Chem.* **9**, 1522 (2014).

[21] D.Z. Chen, J.Y. Cai, J.L. Yin, J.D. Jiang, C.X. Jing, Y.P. Zhu, J.J. Cheng, Y.T. Di, Y. Zhang, M.M. Cao, S.L. Li, Z.G. Peng, and X.J. Hao, *Future Med. Chem.* **7**, 561 (2015).