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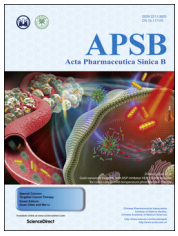

ORIGINAL ARTICLE

Synthesis and biological evaluation of novel tricyclic matrinic derivatives as potential anti-filovirus agents

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Matrinic;
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Abstract Twenty-six novel tricyclic sophoridinic and matrinic derivatives containing a common chlorinated benzene fragment were designed, synthesized and evaluated for their anti-ebolavirus (EBOV) activities. Structure–activity relationship analysis indicated: (i) 12*N*-dichlorobenzyl motif was beneficial for the activity; (ii) the chiral configuration at C5 atom might not affect the activity much. Among the target compounds, compound **7d** exhibited the most potent potency against EBOV with an IC_{50} value of 5.29 μmol/L and an SI value of over 37.8. Further *in vivo* anti-EBOV assay of **7d** identified its high effectiveness, and *in vivo* anti-MARV assay of **7d** suggested its inspiring broad-spectrum anti-filovirus activity. The results provided powerful information on further strategic optimization and development of this kind of compounds against filoviruses.

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1. Introduction

Ebola virus (EBOV), the most well known filamentous virus discovered, along with the marburg virus (MARV), constitutes the filovirus family. Members of the filovirus can cause an acute lethal hemorrhagic fever in humans[1–4]. The 2014–2015 EBOV breakout in West Africa caused more than 28,600 human infections and killed 11,300 people[5,6]. Worse still, new flare-ups have occurred several times after the EBOV breakout was over[6]. Several outbreaks of Marburg hemorrhagic fever were also reported[7], the mortality rate for the 2004–2005 Angola outbreak even reached 90%[8,9]. Although outbreaks have predominantly occurred in central Africa to date, the potential for imported cases or bioterrorism in non-African countries cannot be ignored[10]. In light of this, a lot of efforts have been made to treat EBOV and MARV infections, disappointingly, currently there are still no approved therapeutics (small molecule or biologic agents) for prophylaxis or treatment available, so an approach to effectively treat the infection caused by filovirus is still highly desirable.

Over the past few years, our group has been dedicating to the search and discovery of new antiviral candidates from Tradition Chinese Medicine, such as matrine, sophocarpine and sophoridine (5*R*-matrine) as shown in Fig. 1, and then a compound library of tricyclic matrinic derivatives has been constructed[11–20]. In order to obtain the lead compounds against EBOV, the library was screened in a pseudotyped EBOV virus model (namely pHIV-EBOVGP-Fluc)[21] taking sertraline (Fig. 1) as the positive control[22]. The compound, methyl 12*N*-*p*-chlorobenzyl sophoridinate dihydrochloride (**1**, Fig. 1), displayed a good anti-EBOV activity with an IC_{50} value of 5.07 μmol/L and a CC_{50} value of 22.20 μmol/L.

Interestingly, compared with the structure of sertraline as displayed in Fig. 1, compound **1** also has a similar chlorinated benzene structural fragment at the 12-position, suggesting that chlorinated benzene fragment might be helpful for the potency against EBOV. Based on this strategy, in the present paper, the chlorinated benzene fragment as a pharmacophore was then retained on position 12, and a series of novel tricyclic 12*N*-substituted sophoridinic and matrinic derivatives were generated and evaluated for their activities against EBOV *in vitro*, taking compound **1** the lead. Furthermore, the *in vivo* anti-EBOV and anti-MARV efficacy of the representative compounds were carried out as well.

2. Results and discussion

2.1. Synthetic routes

Totally twenty-six new tricyclic sophoridinic and matrinic derivatives were prepared from commercially available sophoridine, matrine or sophocarpine with purity over 95% as the starting materials as described in Schemes 1–3, respectively. The synthesis of sophoridinic derivatives including methyl sopharidinates (**4a–c**) and sopharidinic acids (**5a–c**) was illustrated in Scheme 1. The key intermediate **3** was obtained by a two-step procedure of hydrolyzation and esterification with sophoridine as the staring material[16]. In the formation of compound **4a**, the condensation of **3** and 3′,4′-dichlorobenzaldehyde achieved a Schiff base, which was then reduced selectively by sodium triacetoxyborohydride (STB)[16]. The desired products **4b** and **4c** were produced from the 12*N*-acylation or 12*N*-sulfonylation of **3** with the corresponding benzoyl chloride or benzenesulfonyl chloride with yields of 55% and 57%, respectively. Products **5a–c** were obtained *via* hydrolysis of **4a–c** in 3 mol/L HCl with yields of 54–55%.

The synthesis of matrinic derivatives including methyl matrinic butyrate compounds (**7a–d**) and matrinic butyric acids (**8a–c**) was illustrated in Scheme 2. The key intermediate methyl butyrate **6** was acquired from matrine through hydrolysis, and methyl esterification in an over yield of 85% as reported previously[13]. The target compounds **7a–d** were acquired from the alkylation, acylation or sulfonylation on the 12 N atom of **6** with yields of 50–62%. The hydrolysis of **7a–c** produced **8a–c** with yields of 55–60% in 3 mol/L HCl.

Scheme 3 depicted the synthesis of matrinic acetic acid derivatives, including methyl matrinic acetates **10a–d** and matrinic acetic acids **11a–c** and **15a–f**. The intermediate **9** was acquired *via* the oxidation and esterification reaction using $KMnO_4$ as an oxidizing agent in acidic condition from sophocarpine[13]. The alkylation, acylation or sulfonylation on the 12 N atom of **9** achieved the target compounds **10a–d** with yields of 45–54%. Compounds **11a–c** were gained from the hydrolysis of **10a–d** in a 48–55% yield in 3 mol/L HCl. Dess-Martin oxidation of commercially available phenethylalcohols or phenylpropanols **12a–f** generated key intermediates **13a–f** as phenylacetaldehydes or phenylpropion aldehydes[23], which were then condensed with **9** in alkaline condition followed by a selective reduction with STB to give intermediates **14a–f**. The final products **15a–f** were obtained *via* acidic hydrolysis of **14a–f** with yields of 42%–50%.

All the final products were purified by flash column chromatography on silica gel with CH_2Cl_2/CH_3OH as the eluents.

2.2. SAR analysis for anti-EBOV activity

All the target compounds were measured for their *in vitro* anti-EBOV activities in human embryonic kidney (HEK) 293 T cells. The potency against EBOV of each tested compound was evaluated by the combination of its IC_{50} and selectivity index (SI) value as the important therapeutic indication. The structures and anti-EBOV activities of all target compounds were displayed in Table 1.

First, mono-chlorobenzene or di-chlorobenzene group was selected as an active substituent at the 12-position, a series of chlorobenzoyl, chlorobenzyl or chlorobenzenesulfonyl sophoridinic derivatives (**4a–c** and **5a–c**) were then generated. As displayed in Table 1, methyl sopharidinates **4a–c** gave 4–29 times lower IC_{50} values than their counterparts sopharidinic acids **5a–c**, consistent with our previous report[11]. Interestingly, among the methyl sopharidinates, 3′,4′-dichlorobenzyl **4a** and *p*-chlorobenzenesulfonyl **4c**, with IC_{50} values of 2.68 and 2.90 μmol/L respectively, displayed comparably higher anti-EBOV activities than *p*-chlorobenzyl **1** and *p*-chlorobenzyl **4b**. Similarly, among the sopharidinic acids, 3′,4′-dichlorobenzyl **5a** and *p*-chlorobenzenesulfonyl **5c** displayed obviously higher anti-EBOV activities than *p*-chlorobenzoyl **5b**. These results hinted that 3′,4′-dichlorobenzyl and *p*-chlorobenzenesulfonyl might be more favorable 12*N*-substitutions than *p*-chlorobenzyl and *p*-chlorobenzoyl groups.

Then, SAR was moved on the effect of chiral configuration on the 5-carbon atom, a group of matrinic compounds with 5*S*-configuration (**7a–d** and **8a–c**) were generated correspondingly. As expected, an obvious advantage of methyl matrinic butyrates over their matrinic butyric acids was revealed in Table 1, **7a–d** gave much higher potencies than their counterparts **8a–c**. *p*-Chlorobenzenesulfonyl **7c** and 3′,4′-dichlorobenzyl **7d** displayed the most potent activities with IC_{50} values of 8.23 and 5.29 μmol/L and SI values of 24.3 and over

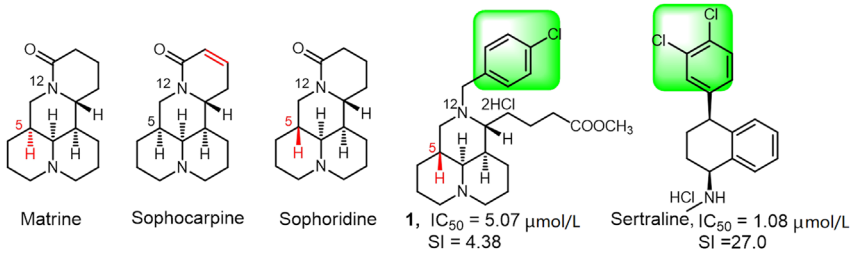
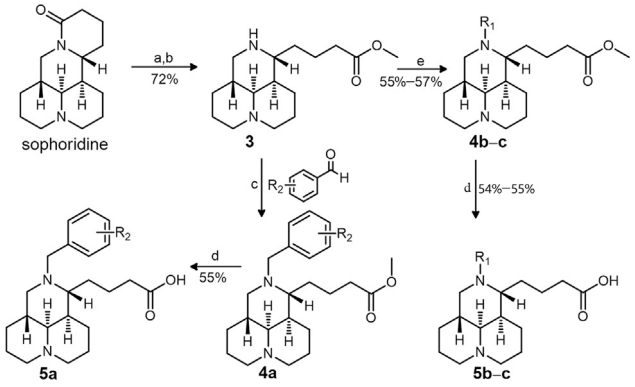
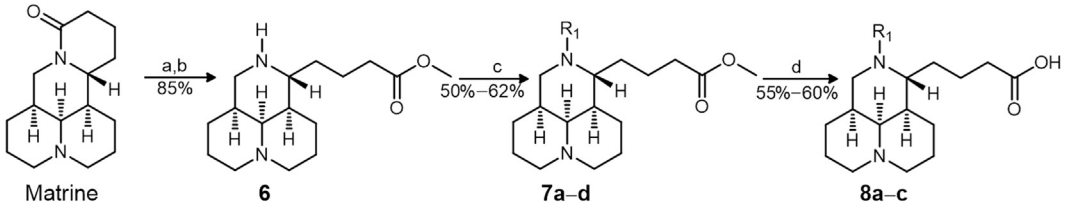


Figure 1 The structures of matrine, sophocarpine, sophoridine, lead **1** and sertraline.



Scheme 1 The synthesis of sophoridinic derivatives including methyl sopharidinates (**4a–c**) and sopharidinic acids (**5a–c**) (a) 6 mol/L HCl, reflux, 6 h; (b) CH_3OH, 2 h; (c) TEA, 1,2-dichloroethane, reflux, 2 h; sodium triacetoxyborohydride (STB), reflux, 2 h; (d) 3 mol/L HCl, reflux, 2 h; (e) $R_1PhSO_2Cl/R_1PhCOCl$, TEA, r.t., 2 h.



Scheme 2 The synthesis of matrinic derivatives including methyl matrinic butyrate compounds (**7a–d**) and matrinic butyric acids (**8a–c**) (a) 20% NaOH, reflux, 9 h, then 12 mol/L HCl, pH = 5–6; (b) 2 mol/L MeOH/HCl, reflux, 2 h; (c) R_1X, K_2CO_3, CH_2Cl_2/CH_3CN, r.t., 4 h; (d) 3 mol/L HCl, reflux, 2 h.

37.8, respectively. These results hinted that the chiral configuration at C5 atom might not affect the potency much.

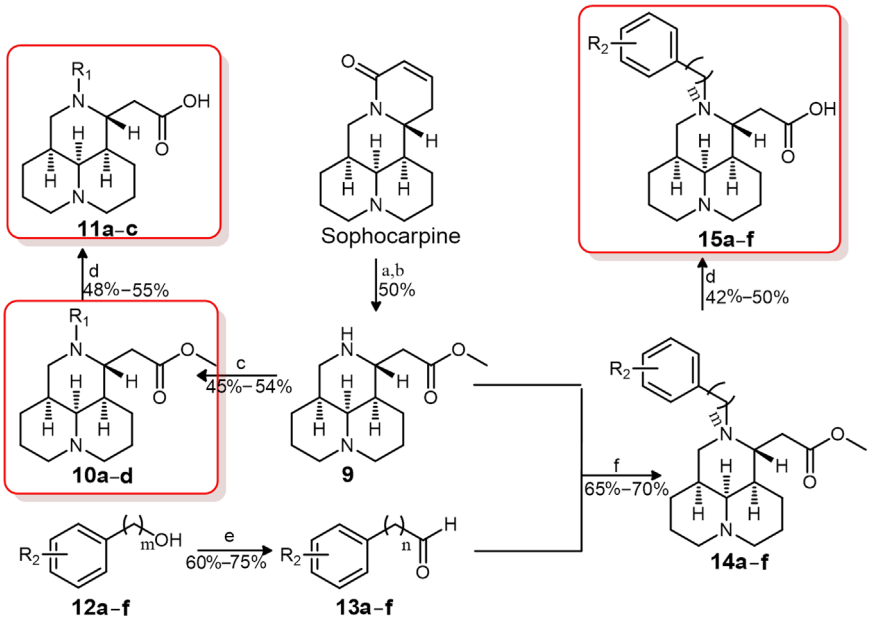
Next, the 5*S*-configuration (matrine series) was retained, SAR study was further focused on the influence of the length of the 11-side chain, and a group of methyl matrinic acetates (**10a–d**) and matrinic acetic acids (**11a–c**) were constructed and evaluated. As anticipated, the esters (**10a–d**) exerted higher activities than the corresponding acids (**11a–c**). The most potent compound 3′,4′-dichlorobenzyl **10d** exhibited a moderate activity with an IC_{50} value of 10.0 μmol/L, which indicated that the shortening the length of 11-side chain to ethyl chain did not affect the activity a lot.

At last, structural variations were concentrated on the most favorable 3′,4′-dichlorobenzyl fragment, and the effects of the methylene linker length and the chlorinated position were investigated, by which monochloro- or dichloro- phenylethyl (**15a–c**), hydrocinnamyl (**15d–f**) matrinic acetic acids were generated. As shown in Table 1, these compounds lost the antiviral activities against EBOV completely, while most of them afforded the higher cytotoxicity. The result indicated that the extention of the methylene linker was not beneficial for the activity.

As reported earlier, our pseudotyped EBOV virus model was composed of EBOV glycoprotein (GP) and envelope-defective strain of HIV-1 containing firefly luciferase reporter gene (pSG3.cmv.Fluc)[21]. To rule out the inhibitory effect of compounds on HIV-1 component(s), a pseudotyped Vesicular Stomatitis Virus (VSV), namely pHIV-VSVGP-Fluc model composed of VSV membrane protein and the same envelope-defective strain of HIV-1 was applied for a comparison[24]. As shown in Fig. 1, the top potent compounds **7c** and **7d** did not show any inhibitory effects to the pseudotyped VSV with the IC_{50} values of over 200 μmol/L, indicating their direct acts on the EBOV virus.

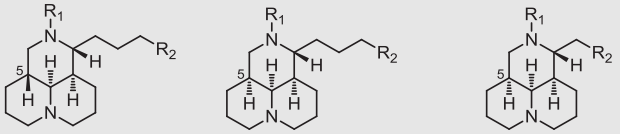
2.3. *Anti-EBOV and anti-MARV activity in vivo of the compound **7d***

Taking sertraline as the positive control, the most potent compound **7d** was selected out for the further *in vivo* anti-EBOV assay in BALB/c mice, which were infected with 5×10^6 $TCID_{50}$ of pHIV-EBOVGP-Fluc virus at Day 0 by IP route. Each compound



Scheme 3 The synthesis of matrinic acetic acid derivatives, including methyl matrinic acetates **10a–d** and matrinic acetic acids **11a–c** and **15a–f**. (a) $KMnO_4$, 10% H_2SO_4, reflux, 2 h; (b) 2 mol/L MeOH/HCl, reflux, 2 h; (c) 3 mol/L HCl, reflux, 2 h; (d) R_1X, K_2CO_3, CH_2Cl_2/CH_3CN, r.t., 4 h; (e) Dess-Martin periodinane, CH_2Cl_2, N_2, 2 h; (f) TEA, 1,2-dichloroethane, reflux, 2 h; sodium triacetoxyborohydride, reflux, 2 h.

Table 1 Structure–activity relationship of newly synthesized compounds against EBOV.



4a–c; 5a–c | 7a–d; 8a–c | 10a–d;11a–c;15a–f

Code	R_1	R_2	Anti-EBOV IC_{50} (μmol/L)	CC_{50} (μmol/L)	SI	Anti-VSV IC_{50} (μmol/L)
1	*p*-ClPhCH_2	$COOCH_3$	5.07	22.2	4.38	14.2
4a	3′,4′-Cl_2PhCH_2	$COOCH_3$	2.68	22.2	8.28	7.52
4b	*p*-ClPhCO	$COOCH_3$	39.1	200	5.12	>200
4c	*p*-ClPhSO_2	$COOCH_3$	2.90	>22.0	>7.59	>22.0
5a	3′,4′-Cl_2PhCH_2	COOH	78.6	200	>2.54	>200
5b	*p*-ClPhCO	COOH	>200	>200	–	>200
5c	*p*-ClPhSO_2	COOH	11.4	200	17.5	>200
7a	*p*-ClPhCH_2	$COOCH_3$	25.4	200	7.87	188
7b	*p*-ClPhCO	$COOCH_3$	88.2	>200	>2.27	>200
7c	*p*-ClPhSO_2	$COOCH_3$	8.23	200	24.3	>200
7d	3′,4′-Cl_2PhCH_2	$COOCH_3$	5.29	>200	>37.8	>200
8a	*p*-ClPhCH_2	COOH	>200	200	–	>200
8b	*p*-ClPhCO	COOH	>200	200	–	>200
8c	*p*-ClPhSO_2	COOH	30.2	200	6.62	>200
10a	*p*-ClPhCH_2	$COOCH_3$	28.1	>200	>7.12	>200
10b	*p*-ClPhCO	$COOCH_3$	88.2	>200	>2.27	>200
10c	*p*-ClPhSO_2	$COOCH_3$	26.8	>200	>7.46	>200
10d	3′,4′-Cl_2PhCH_2	$COOCH_3$	10.0	153	15.3	123
11a	*p*-ClPhCH_2	COOH	>200	>200	–	>200
11b	*p*-ClPhCO	COOH	>200	>200	–	>200
11c	*p*-ClPhSO_2	COOH	>200	>200	–	>200
15a	*p*-ClPh$(CH_2)_2$	COOH	83.3	83.3	–	83.3
15b	3′,4′-Cl_2Ph$(CH_2)_2$	COOH	>200	–	–	–
15c	3′,5′-Cl_2Ph$(CH_2)_2$	COOH	83.3	83.3	–	83.3
15d	*p*-ClPh$(CH_2)_3$	COOH	>200	–	–	–
15e	3′,4′-Cl_2Ph$(CH_2)_3$	COOH	83.3	83.3	–	83.3
15f	3′,5′-Cl_2Ph$(CH_2)_3$	COOH	83.3	83.3	–	83.3
Sertraline			1.08	29.2	27.0	15.6

–Not applicable.

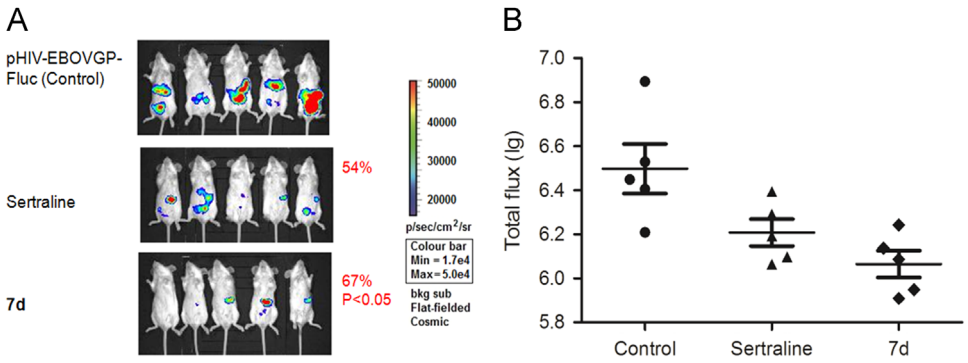


Figure 2 (A) *In vivo* activity of **7d** to pHIV-EBOVGP-Fluc infection. BALB/c mice were injected with 100 µL of saline or 1 mg/mL setraline, **7d**, or 5 × 10^6 $TCID_{50}$ pHIV-EBOVGP-Fluc virus. The images were acquired at Day 4. (B) Measurement of bioluminescence in mice from treatment groups with pHIV-EBOVGP-Fluc infection.

was given by IV and IP ways (half in half) at the dosage of 100 µg, and the activity was measured by bioluminescence. As displayed in Fig. 2, **7d** displayed an exciting anti-EBOV activity by contributing a 65% reduction, significantly higher than that of sertraline (45%), which might result from a better pharmacokinetics profile of compound **7d** *in vivo*.

The *in vivo* antiviral assay of **7d** against another filovirus MARV was also carried out. The experiment was performed in BALB/c mice infected with 9.75 × 10^7 $TCID_{50}$ of pHIV-MARVGP-Fluc virus[25] at Day 0 by IP route following a similar mode of administration and measurement. As displayed in Fig. 3, **7d** contributed to a 50% reduction, while sertraline displayed no activity at all. The result indicated that sertraline was selectively effective to EBOV, while our matrinic compound **7d** showed an inspiring broad-spectrum anti-filovirus activity against both EBOV and MARV, suggesting that its targets against filoviruses might be on the host components, consistent with our previous results[13,18,20].

3. Conclusions

Taken together, twenty-six new tricyclic sophoridinic and matrinic derivatives containing a common chlorinated benzene fragment at the 12-position were designed, synthesized and evaluated for their *in vitro* anti-EBOV activities in a pHIV-EBOVGP-Fluc model. SAR analysis indicated that: (i) 12*N*-dichlorobenzyl motif was beneficial for the anti-EBOV activity; (ii) the chiral configuration at C5 atom might not affect the activity much. Among them, compound **7d** exhibited the most potent activities against EBOV with an IC_{50} value of 5.29 µmol/L and an SI value of over 37.8. Further *in vivo* anti-EBOV assay of **7d** identified its high effectiveness, and *in vivo* anti-MARV assay of **7d** suggested its inspiring broad-spectrum anti-filovirus activity. Overall, this new series of compounds offered powerful information for further strategic optimization and development of this class of compounds against filovirus.

4. Experimental section

4.1. Chemistry

Unless otherwise noted, all reagents and solvents were purchased from the commercial provider without further purification. Melting points (m.p.) were obtained with CXM-300 melting point apparatus (Changfang Optical Instrument Co., Ltd., Shanghai, China) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance III 500 spectrometer or Bruker Avance III 600 spectrometer (Varian, San Francisco, USA) respectively, in DMSO-d_6. ESI high-resolution mass spectrometry (HR-MS) was performed on an Autospec Uitima-TOF mass spectrometer (Micromass UK Ltd., Manchester, UK). Flash chromatography was performed on Combiflash Rf 200 (Teledyne, Nebraska, USA).

4.1.1. *Synthesis of methyl 12N-3′,4′-dichlorobenzyl sophoridinate dihydrochloride (**4a**)*

Sophoridine (5.0 g, 20 mmol) was added in 6 mol/L HCl (50 mL) and heated to reflux for 6 h. The solvent was removed under reduced pressure, the residue was dissolved in CH_3OH (50 mL), and the reaction mixture was then stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure to give **3** in a 72% yield, which was applied directly in the next step without further purification.

To a stirred solution of **3** (2.0 mmol) and triethylamine (6.0 mmol) in 1, 2-dichloroethane (30 mL), *m,p*-dichlorobenzaldehyde (3.0 mmol) in 1, 2-dichloro ethane was added dropwise. The reaction mixture was refluxed for 2 h, cooled to room temperature, and STB (3.0 mmol) was then added into the reaction mixture portionwise. The reaction solution was refluxed for 4 h till TLC analysis showed completion of the reaction. After cooling down, the reaction was quench by the addition of water (20 mL), and the separated organic layer was washed with brine (20 mL), dried over anhydrous Na_2SO_4 and then evaporated under reduced pressure, and the gained residue was purified by flash column chromatography on silica gel with CH_2Cl_2/CH_3OH as eluents and acidified with 2 mol/L HCl/ether (1.0 mL) to give the title compound **4a**. Yield: 60%; yellow solid; m.p. 162–164 °C; ^{1}H NMR (500 MHz) δ 11.56 (br, 1H), 11.16 (br, 1H), 8.30 (s, 1H), 7.90 (d, J = 7.9 Hz, 1H), 7.72 (d, J = 8.1 Hz, 1H), 4.37 (d, J = 11.7 Hz, 2 H), 4.23–4.19 (m, 1H), 3.72 (d, J = 9.6 Hz, 1H), 3.59 (s, 3H), 3.28–3.02 (m, 5H), 2.93–2.88 (m, 1H), 2.82 (t, J = 12.3 Hz, 1H), 2.53–2.58 (m, 1H), 2.46 (d, J = 13.0 Hz, 1H), 2.31–2.18 (m, 2H), 1.90–1.76 (m, 5H), 1.70–1.63 (m, 2H), 1.53 (s, 1H), 1.42 (d, J = 11.6Hz, 2H), 1.22–1.18 (m, 1H); ^{13}C NMR (126 MHz) δ 172.7, 132.9, 132.3, 131.6, 131.1, 131.0, 130.6, 60.2, 55.0, 53.9, 51.6, 51.5, 51.4, 44.2, 33.5, 32.6, 25.6, 24.7, 21.9, 21.7, 21.0, 20.8, 17.2; HR-MS: Calcd. for $C_{23}H_{33}Cl_2N_2O_2 \cdot 2HCl$ $[M - 2HCl + H]^+$: 439.1914, Found: 439.1933.

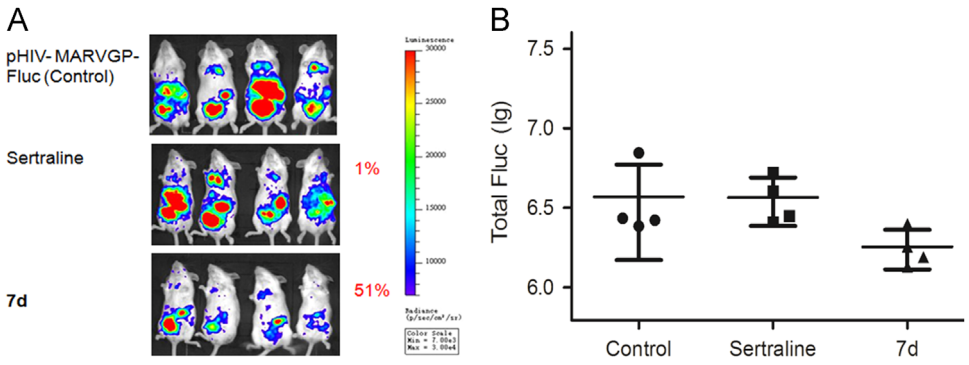


Figure 3 (A) *In vivo* activity of **7d** to pHIV-MARVGP-Fluc infection. BALB/c mice were injected with 100 μL of saline or 1 mg/mL setraline, **7d**, or 9.75×10^7 $TCID_{50}$ pHIV-MARVGP-Fluc virus. The images were acquired at Day 5. (B) Measurement of bioluminescence in mice from treatment groups with pHIV-MARVGP-Fluc infection.

*4.1.2. General procedures for methyl 12N-benzoyl/benzensulfonyl sophoridinates (**4b** and **4c**)*

To a stirred solution of **3** (2.0 mmol) in acetonitrile (30 mL), substituted benzyl/benzensulfonyl chloride (2.4 mmol) and anhydrous triethylamine (6.0 mmol) were added and stirred for 4 h at room temperature. After reaction completed, water (20 mL) was added, and the organic phase was separated, washed with saturated brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure, and the gained residue was purified by flash column chromatography on silica gel with CH_2Cl_2/CH_3OH as eluents to give compounds **4b** and **4c**.

*4.1.2.1. Methyl 12N-p-chlorobenzoyl sophoridinate (**4b**).* Yield: 55%; white solid; m.p. 71–72 °C; ^{1}H NMR (500 MHz) δ 7.54–7.44 (m, 4H), 4.65–4.48 (m, 1H), 3.73–3.54 (m, 1H), 3.54–3.37 (m, 1H), 3.36–3.21 (m, 1H), 3.20–3.04 (m, 1H), 3.03–2.79 (m, 2H), 2.64–2.52 (m, 1H), 2.42–2.04 (m, 3H), 2.00–1.73 (m, 5H), 1.73–1.45 (m, 6H), 1.44–1.26 (m, 3H), 1.26–1.12 (m, 1H); ^{13}C NMR (126 MHz) δ 169.4, 135.4, 134.6, 129.1 (2), 129.0 (2), 59.8, 58.5, 53.5, 52.2, 46.5, 44.4, 40.8, 36.9, 33.6, 28.1, 27.3, 26.0, 25.7, 22.5, 21.4, 17.9; HR-MS: Calcd. for $C_{23}H_{31}ClN_2O_3$ $[M + H]^+$: 419.2096, Found: 419.2092.

*4.1.2.2. Methyl 12N-p-chlorobenzenesulfonyl sophoridinate (**4c**).* Yield: 57%; yellow solid; m.p. 88–90 °C; ^{1}H NMR (500 MHz) δ 7.80 (d, J = 8.6 Hz, 2H), 7.66 (d, J = 8.3 Hz, 2 H), 3.75–3.72 (m, 1H), 3.60 (s, 1H), 3.57 (s, 3H), 3.13 (s, 2H), 2.95 (s, 2H), 2.59 (t, J = 12.4 Hz, 2H), 2.28–2.16 (m, 2H), 2.08 (s, 1H), 1.91 (s, 1H), 1.77–1.80 (m, 1H), 1.65–1.60 (m, 3H), 1.55–1.50 (m, 1H), 1.43–1.22 (m, 6H), 1.15–1.08 (m, 1H); ^{13}C NMR (126 MHz) δ 172.9, 139.8, 137.4, 129.5 (2), 128.4 (2), 58.4, 57.3, 52.6, 51.2, 44.3, 44.2, 36.4, 32.8, 28.4 (2), 26.6, 22.9, 21.6 (2), 17.8; HR-MS: Calcd. for $C_{22}H_{32}ClN_2O_4S$ $[M + H]^+$: 455.1766, Found: 455.1786.

*4.1.3. General procedures for 12N-benzyl/benzoyl/benzensulfonyl sophoridinic acids (**5a–c**)*

Compound **4a–c** (1.0 mmol) were dissolved in 3 mol/L HCl (15 mL), and heated at reflux for 2 h until the TLC showed the completion. After cooling down, the pH value of the reaction was adjusted to 7–8 by addition of 1 mol/L NaOH solution. The solvent was removed under reduced pressure, and the residue was dissolved in MeOH, filtered and evaporated. The gained residue was then purified by flash column chromatography on silica gel with CH_2Cl_2/CH_3OH as the eluents to afford the target compound **5a–c**.

*4.1.3.1. 12N-3′,4′-Dichlorobenzyl sophoridinic acid hydrochloride (**5a**).* The title compound was obtained by a following acidification with 2 mol/L HCl/ether (1.0 mL). Yield: 55%; white solid; m.p. 125–127 °C; ^{1}H NMR (500 MHz) δ 12.10 (br, 1H), 10.82 (br, 1H), 7.57 (d, J = 8.2 Hz, 1H), 7.53 (d, J = 1.3 Hz, 1H), 7.31–7.30 (m, 1H), 3.61 (d, J = 14.4 Hz, 1H), 3.36 (s, 2H), 3.16–3.11 (m, 2H), 2.96 (d, J = 11.5 Hz, 1H), 2.61–2.59 (m, 1H), 2.36–2.30 (m, 2H), 2.24–2.18 (m, 3H), 2.14–2.08 (m, 1H), 1.84–1.79 (m, 3H), 1.61–1.35 (m, 8H), 1.22–1.16 (m, 2H); ^{13}C NMR (126 MHz) δ 174.3, 141.3, 130.9, 130.4, 129.9, 129.2, 128.4, 62.8, 58.5, 56.4, 51.8, 50.0, 44.3, 35.3, 33.8, 27.4, 26.1, 22.5, 22.4 (2), 22.2, 17.7; HR-MS: Calcd. for $C_{22}H_{31}Cl_2N_2O_2$·HCl $[M - HCl + H]^+$: 425.1757, Found: 425.1776.

*4.1.3.2. 12N-p-Chlorobenzoyl sophoridinic acid (**5b**).* Yield: 55%; yellow solid; m.p. 230 °C (dec.); ^{1}H NMR (500 MHz) δ 11.69 (br, 1H), 7.55–7.50 (m, 2H), 7.47–7.28 (m, 2H), 4.63–4.30 (m, 1H), 3.63–3.55 (m, 1H), 3.50–3.42 (m, 2H), 3.35–3.25 (m, 1H), 3.15–3.08 (m, 2H), 2.98–2.87 (m, 2H), 2.42–2.05 (m, 4H), 1.95–1.72 (m, 4H), 1.71–1.14 (m, 8H); ^{13}C NMR (126 MHz) δ 174.4, 168.9, 135.0, 134.1, 128.7 (2), 128.6 (2), 58.1, 53.1, 51.7, 46.1, 44.0, 40.5, 36.5, 33.2, 28.8, 27.7, 25.4, 21.8, 21.0, 17.5; HR-MS: Calcd. for $C_{22}H_{31}ClN_2O_3$ $[M + H]^+$: 405.1940, Found: 405.1954.

*4.1.3.3. 12N-p-Chlorobenzenesulfonyl sophoridinic acid (**5c**).* Yield: 54%; yellow solid; m 214–216 °C; ^{1}H NMR (500 MHz) δ 7.80 (d, J = 8.6 Hz, 2H), 7.65 (d, J = 8.6 Hz, 2H), 3.78 (t, J = 6.6 Hz, 1H), 3.63 –3.59 (m, 1H), 3.41–3.31 (m, 2H), 3.05 (d, J = 7.7 Hz, 2H), 2.86 (d, J = 11.3 Hz, 1H), 2.67–2.62 (m, 1H), 2.20–2.09 (m, 4H), 1.81–1.71 (m, 3H), 1.65–1.51 (m, 3H), 1.42–1.28 (m, 5H), 1.23–1.20 (m, 1H); ^{13}C NMR (126 MHz) δ 174.2, 139.6, 137.6, 129.6 (2), 128.5 (2), 58.3, 57.3, 51.8, 44.1, 43.9, 35.4, 33.3, 28.1, 27.1, 25.5, 22.3, 22.0, 21.5, 17.4; HR-MS: Calcd. for $C_{21}H_{30}ClN_2O_4S$ $[M + H]^+$: 441.1609, Found: 441.1604.

*4.1.4. General procedures for 12N-benzyl/benzoyl/benzensulfonyl matrinates (**7a–d**)*

Matrine (5.0 g, 20 mmol) was added to 5 N NaOH (30 mL), and the reaction mixture was refluxed for 9 h, cooled to the room temperature and then filtered. The collected solid was dissolved in 6 mol/L HCl (25 mL), then the solvent was evaporated, and the residue was dissolved in CH_3OH heated at refluxing for 2 h. The crude intermediate **6** was gained by evaporation under reduced pressure, which was used directly in the next step without further purification.

To the solution of **6** (2.0 mmol) in CH_2Cl_2 or acetonitrile (30 mL), benzoyl/benzyl/sulfonyl chloride (2.4 mmol) and anhydrous K_2CO_3 (7.0 mmol) were added, the reaction solution was then stirred at room temperature until TLC analysis showed completion of the reaction. The mixture was filtered and the filtrate was evaporated. The residue was purified by flash column chromatography on silica gel with CH_2Cl_2/CH_3OH as the eluents to afford the title compound **7**.

*4.1.4.1. Methyl 12N-p-chlorobenzyl matrinate dihydrochloride (**7a**).* The title compound was obtained by a following acidification with 2 mol/L HCl/ether (1.0 mL). Yield: 52%; light yellow solid; m.p. 169–171 °C; ^{1}H NMR (500 MHz) δ 11.65 (br, 1H), 11.05 (br, 1H), 7.65 (d, J = 7.2 Hz, 2H), 7.54 (d, J = 7.2 Hz, 2H), 4.96 (d, J = 12.1 Hz, 1H), 4.21 (s, 1H), 4.01–3.92 (m, 2H), 3.60 (s, 3H), 3.38–3.37 (m, 2H), 3.29–3.23 (m, 2H), 2.97–2.95 (m, 1H), 2.91–2.89 (m, 1H), 2.70 (d, J = 10.2 Hz, 1H), 2.58 (d, J = 8.3 Hz, 1H), 2.47 (s, 1H), 2.01–1.97 (m, 1H), 1.90–1.87 (m, 1H), 1.79–1.70 (m, 5H), 1.66–1.50 (m, 6H); ^{13}C NMR (126 MHz) δ 173.3, 134.4, 134.2, 133.4 (2), 128.8 (2), 60.3, 60.2, 56.4, 54.2, 54.2, 51.4, 48.6, 35.9, 32.4, 30.0, 27.6, 23.9, 23.4, 21.4, 17.9, 17.8; HR-MS: Calcd. for $C_{23}H_{34}ClN_2O_2{\cdot}2HCl$ [M – 2HCl + H]$^+$: 405.2303, Found: 405.2313.

*4.1.4.2. Methyl 12N-p-chlorobenzoyl matrinate (**7b**).* Yield: 60%; light yellow solid; m.p. 120–122 °C; ^{1}H NMR (500 MHz) δ 7.55–7.51 (m, 2H), 7.48–7.47 (m, 2H), 4.15 (s, 1H), 3.86 (t, J = 13.1 Hz, 1H), 3.71 (s, 1H), 3.57 (s, 3H), 3.50 (d, J = 10.3 Hz, 1H), 3.35–3.31 (m, 1H), 3.23–3.16 (m, 2H), 2.92–2.85 (m, 2H), 2.47–2.41 (m, 1H), 2.32–2.23 (m, 3H), 1.95 (s, 1H), 1.87–1.71 (m, 3H), 1.65–1.59 (m, 1H), 1.59–1.48 (m, 5H), 1.35 (d, J = 13.6 Hz, 1H); ^{13}C NMR (126 MHz) δ 173.4, 171.8, 135.5, 134.8, 129.3 (2), 128.8 (2), 63.1, 54.8, 54.5, 54.4, 51.2 (2), 36.3, 35.6, 33.1, 28.2, 24.9, 24.2, 21.4, 18.4, 18.2; HR-MS: Calcd. for $C_{23}H_{32}ClN_2O_3$ [M + H]$^+$: 419.2096, Found: 419.2075.

*4.1.4.3. Methyl 12N-p-chlorobenzenesulfonyl matrinate (**7c**).* Yield: 62%; light yellow solid; m.p. 143–145 °C; ^{1}H NMR (500 MHz) δ 7.83–7.81 (m, 2H), 7.66 (d, J = 6.3 Hz, 2H), 3.57 (s, 3H), 3.42–3.34 (m, 3H), 3.14–3.10 (m, 1H), 2.53 (s, 1H), 2.47–2.39 (m, 1H), 2.25–2.20 (m, 2H), 1.91–1.85 (m, 2H), 1.68–1.61 (m, 5H), 1.52–1.46 (m, 3H), 1.37–1.25 (m, 6H); ^{13}C NMR (126 MHz) δ 173.2, 138.4, 137.4, 129.2 (2), 129.1 (2), 62.0, 56.4, 56.0, 55.9, 51.2, 46.0, 38.7, 33.6, 33.2, 30.8, 27.8, 27.5, 20.5, 20.3, 20.1; HR-MS: Calcd. for $C_{22}H_{32}ClN_2O_4S$ [M + H]$^+$: 455.1766, Found: 455.1779.

*4.1.4.4. Methyl 12N-3′,4′-dichlorobenzyl matrinate dihydrochloride (**7d**).* The title compound was obtained by a following acidification with 2 mol/L HCl/ether (1.0 mL). Yield: 50%; white solid; m.p. 200 °C (dec.); ^{1}H NMR (500 MHz) δ 11.83 (br, 1H), 11.05 (br, 1H), 7.99 (d, J = 1.9 Hz, 1H), 7.73 (d, J = 8.3 Hz, 1H), 7.59–7.57 (m, 1H), 4.97 (d, J = 11.8 Hz, 1H), 4.22–4.18 (m, 1H), 4.03–3.96 (m, 1H), 3.96–3.89 (m, 1H), 3.60 (s, 3H), 3.57 (s, 1H), 3.51 (s, 1H), 3.29–3.23 (m, 2H), 2.99–2.86 (m, 2H), 2.81–2.78 (m, 1H), 2.61–2.58 (m, 1H), 2.48–2.44 (m, 2H), 2.01–1.97 (m, 2H), 1.90–1.72 (m, 6H), 1.69–1.57 (m, 4H); ^{13}C NMR (126 MHz) δ 173.3, 133.4, 132.3, 131.9, 131.3, 130.9, 130.8, 60.4, 60.2, 55.8, 54.2, 54.2, 51.4, 48.7, 36.0, 32.4, 30.0, 27.7, 23.8, 23.4, 21.5, 17.9, 17.8; HR-MS: Calcd. for $C_{23}H_{33}Cl_2N_2O_2{\cdot}2HCl$ [M – 2HCl + H]$^+$: 439.1914, Found: 439.1934.

*4.1.5. General procedures for 12N-benzyl/benzoyl/benzensulfonyl matrinic acids (**8a–c**)*

The product **8** was prepared from **7** using the same methods as **5** from **4**.

*4.1.5.1. 12N-p-Chlorobenzyl matrinic acid dihydrochloride (**8a**).* The title compound was obtained by a following acidification with 2 mol/L HCl/ether (1.0 mL). Yield: 60%; yellow solid; m.p. 188–190 °C; ^{1}H NMR (500 MHz) δ 12.18 (br, 1H), 11.77 (br, 1H), 11.07 (br, 1H), 7.66 (s, 2H), 7.53 (d, J = 6.5 Hz, 2H), 4.99 (d, J = 11.5 Hz, 1H), 4.20 (s, 1H), 3.97 (d, J = 26.1 Hz, 1H), 3.60 (s, 1H), 3.39 (t, J = 6.8 Hz, 2H), 3.25 (d, J = 12.7 Hz, 2H), 3.02–2.81 (m, 2H), 2.70 (d, J = 8.6 Hz, 1H), 2.61 (s, 1H), 2.50–2.44 (m, 1H), 2.38 (s, 1H), 2.00 (d, J = 10.9 Hz, 1H), 1.90 (s, 1H), 1.85–1.68 (m, 5H), 1.66–1.44 (m, 5H); ^{13}C NMR (126 MHz) δ 174.5, 134.2, 133.4, 128.9 (2), 128.8 (2), 60.4, 60.2, 56.4, 54.2, 48.6, 48.6, 36.0, 32.8, 30.0, 27.9, 24.0, 23.4, 21.7, 17.9, 17.8; HR-MS: Calcd. for $C_{22}H_{32}ClN_2O_2{\cdot}2HCl$ [M – 2HCl + H]$^+$: 391.2147, Found: 391.2158.

*4.1.5.2. 12N-p-Chlorobenzoyl matrinic acid hydrochloride (**8b**).* The title compound was obtained by a following acidification with 2 mol/L HCl/ether (1.0 mL). Yield: 50%; light yellow solid; m.p. 194–196 °C; ^{1}H NMR (500 MHz) δ 12.01 (br, 1H), 10.59 (br, 1H), 7.52 (d, J = 8.4 Hz, 2H), 7.47 (d, J = 8.4 Hz, 2H), 4.16 (d, J = 4.1 Hz, 1H), 3.89 (t, J = 13.0 Hz, 1H), 3.51 (d, J = 10.1 Hz, 1H), 3.34–3.30 (m, 1 H), 3.21 (d, J = 11.3 Hz, 1H), 3.15 (d, J = 4.6 Hz, 2H), 2.94–2.83 (m, 2H), 2.32 (d, J = 7.7 Hz, 2H), 2.22–2.17 (m, 2H), 1.92 (s, 1H), 1.86–1.81 (m, 2H), 1.76–1.71 (m, 1H), 1.64–1.60 (m, 1H), 1.57–1.51 (m, 5H), 1.34 (d, J = 12.7 Hz, 1H); ^{13}C NMR (126 MHz) δ 174.5, 171.8, 135.5, 134.8, 129.4 (2), 128.8 (2), 63.2, 55.0, 54.6, 54.4, 48.6, 36.4, 35.9, 33.6, 28.2, 24.9, 24.2, 21.5, 18.4, 18.2; HR-MS: Calcd. for $C_{22}H_{30}ClN_2O_3{\cdot}HCl$ [M – HCl + H]$^+$: 405.1940, Found: 405.1950.

*4.1.5.3. 12N-p-Chlorobenzenesulfonyl matrinic acid (**8c**).* Yield: 55%; light yellow solid; m.p. 210 °C (dec.); ^{1}H NMR (500 MHz) δ 10.71 (br, 1H), 7.88–7.81 (m, 2H), 7.65 (d, J = 8.6 Hz, 2H), 4.14–4.06 (m, 1H), 3.82–3.73 (m, 2H), 3.57–3.55 (m, 1H), 3.18–3.16 (m, 2H), 2.89–2.87 (m, 2H), 2.23–2.22 (m, 2H), 2.16–2.10 (m, 1H), 2.01–1.94 (m, 1H), 1.89–1.86 (m, 2H), 1.72–1.51 (m, 8H), 1.34–1.32 (m, 1H), 1.15–1.11 (m, 1H); ^{13}C NMR (126 MHz) δ 174.6, 140.6, 138.1, 130.0 (2), 129.1 (2), 63.4, 58.0, 55.0, 54.93, 49.0, 38.3, 35.0, 33.7, 27.6, 25.3, 24.7, 21.4, 18.8, 18.5; HR-MS: Calcd. for $C_{21}H_{30}ClN_2O_4S{\cdot}HCl$ [M – HCl + H]$^+$: 441.1609, Found: 441.1623.

*4.1.6. General procedures for methyl 12N-chlorinated benzyl/benzyoyl/benzensulfonyl matrinic acetates (**10a–d**)*

Sophocarpine (5.0 g, 20.0 mmol) was dissolved in a solution of 10% H_2SO_4 (60 mL), $KMnO_4$ (10 g) was then added portionwise slowly in an ice bath and the mixture solution was then heated at refluxing for 2 h. After reaction completed, the solution was cooled to room temperature and CH_3OH (150 mL) was added, the precipitation was filtered off. The filtration was concentrated, and the residue was dissolved in 2 mol/L HCl/MeOH (20 mL) and refluxed for 2 h until TLC analysis showed completion of the reaction. After the reaction mixture was neutralized with ammonia water to pH 6–7, the solvent was removed under reduced pressure and the remaining residue was dissolved with CH_3OH, the precipitation was removed by filtration, the filtrate was concentrated to afford the crude intermediate compound **9** in a 50% yield,

which was applied directly in the next step without further purification.

To a solution of the intermediate compound **9** (2.0 mmol) in acetonitrile or CH_2Cl_2 (50 mL), benzyl/benzoyl/benzensulfonyl chloride (2.4 mmol) and anhydrous triethylamine (6.0 mmol) were added and stirred for 4 h at room temperature. After the reaction completed, water (50 mL) was added, and the organic phase was separated, washed with saturated brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure, and the residue was purified by flash column chromatography on silica gel with CH_2Cl_2/CH_3OH as eluents and acidified with 2 mol/L hydrochloride/ether (1 mL) to give compound **10**.

*4.1.6.1. Methyl 12N-p-chlorobenzyl matrinic acetate dihydrochloride (**10a**).* Yield: 50%; yellow solid; m.p. 148–150 °C; ^{1}H NMR (500 MHz) δ 12.06 (br, 1H), 11.19 (br, 1H), 7.64 (d, J = 8.4 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 4.86 (d, J = 12.2 Hz, 1H), 4.57–4.53 (m, 1H), 4.13–4.07 (m, 2H), 3.69 (s, 3H), 3.59–3.56 (m, 1H), 3.52 (d, J = 2.5 Hz, 1H), 3.27 (t, J = 12.1 Hz, 2H), 2.96–2.87 (m, 3H), 2.70–2.65 (m, 1H), 2.49–2.41 (m, 2H), 1.94–1.90 (m, 1H), 1.83–1.52 (m, 7H); ^{13}C NMR (126 MHz) δ 171.3, 134.8, 133.8 (2), 129.4 (2), 129.2, 60.1, 57.2, 57.1, 54.7, 54.5, 52.8, 48.4, 37.8, 36.2, 30.5, 24.4, 24.1, 18.2, 18.0; HR-MS: Calcd. for $C_{21}H_{30}ClN_2O_2{\cdot}2HCl$ $[M - 2HCl + H]^+$: 377.1990, Found: 377.1999.

*4.1.6.2. Methyl 12N-p-chlorobenzoyl matrinic acetate hydrochloride (**10b**).* Yield: 49%; yellow solid; m.p. 70–72 °C; ^{1}H NMR (500 MHz) δ 10.18 (br, 1H), 7.52 (d, J = 8.4 Hz, 2H), 7.44 (d, J = 8.4 Hz, 2H), 4.40–4.34 (m, 1H), 3.76–3.71 (m, 1H), 3.56 (s, 3 H), 3.54 –3.50 (m, 1H), 3.33–3.30 (m, 1H), 3.25–3.18 (m, 2H), 3.13 –3.08 (m, 1H), 2.92–2.82 (m, 3H), 2.35–2.33 (m, 1H), 2.04 (s, 1H), 1.83–1.73 (m, 3H), 1.64–1.53 (m, 4H), 1.38–1.36 (m, 1H); ^{13}C NMR (126 MHz) δ 171.3, 171.2, 135.5, 134.6, 129.1(2), 128.7 (2), 62.2, 54.6, 54.4, 51.7, 51.4, 48.5, 36.4, 35.7, 34.7, 24.8, 24.1, 18.4, 18.2. HR-MS: Calcd. for $C_{21}H_{28}ClN_2O_3{\cdot}HCl$ $[M - HCl + H]^+$: 391.1783, Found: 391.1794.

*4.1.6.3. Methyl 12N-p-chlorobenzesulfonyl matrinic acetate hydrochloride (**10c**).* Yield: 54%; yellow solid; m.p. 88–90 °C; ^{1}H NMR (600 MHz) δ 10.54 (br, 1H), 7.82–7.79 (m, 2H), 7.68–7.65 (m, 2 H), 4.40–4.36 (m, 1H), 3.78 (t, J = 13.2 Hz, 1H), 3.71–3.68 (m, 1H), 3.61–3.57 (m, 1H), 3.41 (s, 3H), 3.22–3.19 (m, 2H), 2.91–2.86 (m, 3H), 2.82–2.78 (m, 1H), 2.39–2.36 (m, 1H), 2.21–2.17 (m, 1H), 1.88–1.79 (m, 2H), 1.71–1.51 (m, 6H); ^{13}C NMR (151 MHz) δ 170.2, 139.9, 137.7, 129.5 (2), 128.7 (2), 62.4, 54.7, 54.4, 53.7, 51.2, 47.9, 38.1, 34.2, 33.9, 24.7, 24.1, 18.3, 18.0; HR-MS: Calcd. for $C_{20}H_{28}ClN_2O_4S{\cdot}HCl$ $[M - HCl + H]^+$: 427.1453, Found: 427.1467.

*4.1.6.4. Methyl 12N-3′,4′-dichlorobenzyl matrinic acetate dihydrochloride (**10d**).* Yield: 45%; yellow solid; m.p. 196–197 °C; ^{1}H NMR (600 MHz) δ 12.06 (br, 1H), 11.13 (br, 1H), 7.96 (d, J = 2.0 Hz, 1H), 7.74 (d, J = 8.2 Hz, 1H), 7.57–7.55 (m, 1H), 4.88–4.86 (m, 1H), 4.55–4.50 (m, 1H), 4.13–4.07 (m, 2H), 3.69 (s, 3H), 3.58–3.55 (m, 2H), 3.27 (t, J = 13.3 Hz, 2H), 2.97–2.86 (m, 3H), 2.85–2.80 (m, 1H), 2.47–2.42 (m, 2H), 1.93–1.78 (m, 2H), 1.74–1.71 (m, 2H), 1.65–1.58 (m, 4H); ^{13}C NMR (151 MHz) δ 170.8, 133.3, 132.3, 131.8, 131.3, 130.9, 130.7, 59.6, 56.7, 56.0, 54.3, 54.0, 52.3, 48.1, 37.4, 35.8, 30.1, 23.8, 23.7, 17.8, 17.6; HR-MS: Calcd. for $C_{21}H_{29}Cl_2N_2O_2{\cdot}2HCl$ $[M - 2HCl + H]^+$: 411.1601, Found: 411.1596.

*4.1.7. General procedures for 12N-chlorinated benzyl/benzyoyl/benzensulfonyl matrinic acetic acids (**11a–c**)*

The title compound **11** was obtained from **10** using the same methods as **5** from **4**.

*4.1.7.1. 12N-p-Chlorobenzyl matrinic acetic acid (**11a**).* Yield: 48%, yellow solid, m.p. 190–192 °C; ^{1}H NMR (500 MHz) δ 10.15 (br, 1H), 7.55–7.22 (m, 4H), 4.15 (s, 1H), 3.56 (s, 1H), 3.45–3.41 (m, 1H), 3.35–3.30 (m, 1H), 3.16 (s, 2H), 3.16–3.05 (m, 2H), 2.82 (s, 1H), 2.67–2.65 (m, 2H), 2.32 (d, J = 9.4 Hz, 1H), 2.10–2.05 (m, 2H), 1.91–1.75 (m, 3H), 1.60–1.48 (m, 4H), 1.38–1.36(m, 1H); ^{13}C NMR (151 MHz) δ 171.8, 134.2, 133.2, 128.8 (4), 59.6, 57.0, 56.5, 54.3, 54.0, 48.0, 37.6, 36.5, 30.0, 24.0, 23.6, 17.8, 17.6; HR-MS: Calcd. for $C_{20}H_{28}ClN_2O_2$ $[M + H]^+$: 363.1834, Found: 363.1844.

*4.1.7.2. 12N-p-Chlorobenzoyl matrinic acetic acid (**11b**).* Yield: 53%, light yellow solid, m.p. 164–166 °C; ^{1}H NMR (600 MHz) δ 7.44 (d, J = 8.1 Hz, 2H), 7.32 (d, J = 8.1 Hz, 2H), 4.24– 4.19 (m, 1H), 3.60–3.35 (m, 1H), 3.26–3.16 (m, 1H), 2.68–2.60 (m, 3H), 2.55–2.49 (m, 1H), 1.88 (d, J = 3.4 Hz, 1H), 1.80–1.60 (m, 6H), 1.44 (s, 1H), 1.32–1.20 (m, 5H); ^{13}C NMR (151 MHz) δ 173.0, 169.1, 136.6, 133.5, 128.4 (2), 128.2 (2), 62.1, 56.2 (2), 56.0, 51.4, 48.7, 33.9, 28.4, 28.1 (2), 20.6, 20.5; HR-MS: Calcd. for $C_{20}H_{26}ClN_2O_3$ $[M + H]^+$: 377.1626, Found: 377.1638.

*4.1.7.3. 12N-p-Chlorobenzesulfonyl matrinic acetic acid (**11c**).* Yield: 55%, white solid, m.p. 156–158 °C; ^{1}H NMR (600 MHz) δ 7.83–7.79 (m, 2H), 7.65–7.62 (m, 2H), 4.01–3.97 (m, 1H), 3.44–3.40 (m, 1H), 3.27 (t, J = 11.9 Hz, 1H), 2.74–2.71 (m, 1H), 2.59–2.54 (m, 2H), 2.00–1.98 (m, 1H), 1.89–1.85 (m, 1H), 1.80–1.69 (m, 4H), 1.52–1.21 (m, 8H); ^{13}C NMR (151 MHz) δ 172.9, 139.5, 137.4, 129.1 (2), 128.9 (2), 62.4, 56.2 (2), 54.1, 48.6, 47.1, 37.6, 34.2, 27.4, 27.3, 20.3, 20.2; HR-MS: Calcd. for $C_{19}H_{26}ClN_2O_4S$ $[M + H]^+$: 413.1296, Found: 413.1309.

*4.1.8. General procedures for 12N-chlorinated phenylethyl/hydrocinnamyl matrinic acetic acids (**15a–f**)*

Dess-Martin periodinane (2.4 mmol) was diluted in anhydrous CH_2Cl_2 (25 mL) under N_2 and stirred for 10 min, then the substituted phenylethyl/phenylpropyl alcohol **12** (2.0 mmol) was added dropwise. The mixture was stirred for 2 h at room temperature and was then quenched by the addition of 20 mL of saturated $Na_2S_2O_3$ solution. After stirring at room temperature for 15 min, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (50 mL). The organic layer was washed with water and brine (50 mL each), then dried over anhydrous sodium sulfate and concentrated to afford the aldehyde intermediate **13** in a yield of 60%–75% as a yellow oil.

To a stirred solution of **9** (2.0 mmol) and triethylamine (6.0 mmol) in 1,2-dichloroethane (50 mL), aldehyde **13** (3.0 mmol) in 1,2-dichloroethane (5 mL) was added dropwise. The reaction mixture was refluxed for 2 h and STB (3.0 mmol) was added into the reaction mixture slowly. The reaction solution was refluxed till TLC analysis showed completion of the reaction. After cooling down, the mixture was separated and washed successively with water (20 mL), brine (20 mL). The organic layer was evaporated under vacuum to obtain the intermediate **14** in 65%–70% yields. The title compound **15** was obtained from **14** using the same methods as **5** from **4**.

*4.1.8.1. 12N-p-Chlorophenylethyl matrinic acetic acid (**15a**).* Yield: 42%; yellow solid; m.p. 145–147 °C; ^{1}H NMR (500 MHz) δ 7.34 (d, J = 8.4 Hz, 2H), 7.28 (d, J = 8.4 Hz,

2H), 3.45–3.42 (m, 1H), 3.18 (t, J = 11.6 Hz, 1H), 3.04–2.89 (m, 1H), 2.87–2.80 (m, 6H), 2.57–2.51 (m, 1H), 2.49–2.42 (m, 1H), 2.27 (s, 1H), 2.04–1.90 (m, 3H), 1.76–1.74 (m, 2H), 1.68–1.58 (m, 2H), 1.57–1.52 (m, 2H), 1.41–1.35 (m, 3H); ^{13}C NMR (126 MHz) δ 173.3, 138.3, 130.8, 130.7 (2), 128.3 (2), 62.1, 56.1, 54.9, 52.8, 49.9, 37.9, 35.3, 32.3, 30.2, 26.8 (2), 26.1, 20.2, 19.9; HR-MS: Calcd. for $C_{21}H_{30}ClN_2O_2$ $[M + H]^+$:377.1990, Found: 377.1989.

*4.1.8.2. 12N-3′,4′-Dichlorophenylethyl matrinic acetic acid hydrochloride (**15b**).* The title compound was obtained by a following acidification with 2 mol/L HCl/ether (1.0 mL). Yield: 50%; light yellow solid; m.p. 200 °C (dec.); ^{1}H NMR (500 MHz) δ 11.90 (s, 1H), 11.28 (s, 1H), 7.64 (d, J = 2.0 Hz, 1H), 7.61 (d, J = 8.2 Hz, 1H), 7.35–7.33 (m, 1H), 4.38–4.30 (m, 1H), 4.22–4.16 (m, 1H), 3.68–3.62 (m, 1H), 3.56–3.49 (m, 1H), 3.45–3.36 (m, 1H), 3.29–3.21 (m, 4H), 3.19–3.11 (m, 1H), 3.00–2.90 (m, 2H), 2.70–2.64 (m, 2H), 2.41–2.37 (m, 1H), 1.98–1.86 (m, 2H), 1.84–1.58 (m, 6H), 1.24–1.19 (m, 1H); ^{13}C NMR (126 MHz) δ 171.9, 138.4, 131.1, 131.0, 130.8, 129.6, 129.5, 59.6, 55.3, 54.3, 54.1, 53.6, 49.4, 37.8, 35.8, 30.5, 27.8, 24.2, 23.7, 17.9, 17.7; HR-MS: Calcd. for $C_{21}H_{29}Cl_2N_2O_2$·HCl $[M - HCl + H]^+$: 411.1601, Found: 411.1604.

*4.1.8.3. 12N-3′,5′-Dichlorophenylethyl matrinic acetic acid (**15c**).* Yield: 50%; yellow solid; m.p.: 190 °C (dec.); ^{1}H NMR (500 MHz) δ 7.44 (t, J = 1.8 Hz, 1H), 7.38 (d, J = 1.7 Hz, 2H), 3.50–3.48 (m, 1H), 3.24 (t, J = 12.3 Hz, 1H), 3.02 (s, 1H), 2.91–2.83 (m, 7H), 2.55 (s, 2H), 2.06 (d, J = 11.5 Hz, 1H), 1.85 (d, J = 10.1 Hz, 1H), 1.77–1.74 (m, 2H), 1.67–1.65 (m, 1H), 1.60–1.55 (m, 3H), 1.47–1.40 (m, 4H); ^{13}C NMR (126 MHz) δ 173.2, 143.8, 133.9 (2), 127.8 (2), 125.9, 62.0, 55.6 (2), 54.4, 52.5, 49.7, 37.7, 35.5, 31.8, 30.2, 26.3, 25.5, 19.7, 19.0; HR-MS: Calcd. for $C_{21}H_{29}Cl_2N_2O_2$ $[M + H]^+$: 411.1601, Found: 411.1601.

*4.1.8.4. 12N-p-Chlorohydrocinnamyl matrinic acetic acid (**15d**).* Yield: 45%; white solid; m.p. 138–140 °C; ^{1}H NMR (500 MHz) δ 7.34 (d, J = 7.6 Hz, 2H), 7.26 (d, J = 7.6 Hz, 2H), 3.45 (d, J = 9.3 Hz, 1H), 3.16 (t, J = 11.8 Hz, 1H), 3.05–2.98 (m, 1H), 2.88 (d, J = 9.5 Hz, 1H), 2.80–2.72 (m, 3H), 2.63–2.53 (m, 3H), 2.14 (s, 1H), 2.00–1.94 (m, 3H), 1.91–1.79 (m, 4H), 1.72 (d, J = 12.7 Hz, 1H), 1.63–1.57 (m, 2H), 1.54–1.48 (m, 2H), 1.41–1.31 (m, 3H); ^{13}C NMR (126 MHz) δ 174.3, 140.6, 131.5, 131.0 (2), 129.1 (2), 61.5, 57.1, 56.2 (2), 52.0, 49.9, 37.3, 37.2, 34.3, 32.1, 26.2, 26.0, 25.8, 20.0, 19.8; HR-MS: Calcd. for $C_{22}H_{32}ClN_2O_2$ $[M + H]^+$: 391.2147, Found: 391.2144.

*4.1.8.5. 12N-3′,4′-Dichlorohydrocinnamyl matrinic acetic acid (**15e**).* Yield: 48%; yellow solid; m.p. 145–147 °C; ^{1}H NMR (500 MHz) δ 7.55–7.54 (m, 2H), 7.25–7.24 (m, 1H), 3.76 (d, J = 9.3 Hz, 1H), 3.44–3.42 (m, 1H), 3.16 (s, 1H), 3.09–3.07 (m, 2H), 2.98–2.87 (m, 3H), 2.81–2.77 (m, 1H), 2.65–2.57 (m, 4H), 2.25 (d, J = 9.3 Hz, 2H), 2.08 (d, J = 9.9 Hz, 1H), 2.01–1.94 (m, 2H), 1.74–1.71 (m, 2H), 1.69–1.63 (m, 1H), 1.58–1.56 (m, 2H), 1.53–1.42 (m, 3H); ^{13}C NMR (126 MHz) δ 172.5, 142.4, 130.9, 130.4 (2), 128.9, 128.6, 60.8, 55.4 (2), 51.6, 49.7, 48.5, 37.7, 35.0, 31.2 (2), 25.6, 25.0 (2), 19.4, 19.2; HR-MS: Calcd. for $C_{22}H_{31}Cl_2N_2O_2$ $[M + H]^+$: 425.1757, Found: 425.1758.

*4.1.8.6. 12N-3′,5′-Dichlorohydrocinnamyl matrinic acetic acid (**15f**).* Yield: 47%; light yellow solid; m.p. 158–160 °C; ^{1}H NMR (500 MHz) δ 7.43–7.42 (s, 1H), 7.35 (d, J = 1.5 Hz, 2H), 3.87 (s, 1H), 3.56 (s, 1H), 3.17–3.11 (m, 3H), 3.07–2.97 (m, 2H), 2.88–2.84 (m, 1H), 2.68–2.57 (m, 4H), 2.34 (s, 1H), 2.15 (s, 1H), 2.07–1.90 (m, 3H), 1.74–1.72 (m, 3H), 1.60–1.49 (m, 6H); ^{13}C NMR (126 MHz) δ 172.1, 145.4, 133.8 (2), 127.1 (2), 125.60, 60.7 (2), 55.5, 55.1, 51.7, 49.5, 37.6, 35.0, 31.5 (2), 31.0, 25.3, 24.7, 19.0, 18.8; HR-MS: Calcd. for $C_{22}H_{31}Cl_2N_2O_2$ $[M + H]^+$: 425.1757, Found: 425.1756.

4.2. Biology assay

4.2.1. Cell culture

HEK293T (American Type Culture Collection [ATCC], CRL-3216) was grown in Dulbecco's modified Eagle's medium (HyClone, South Logan, UT, USA) supplemented with 10% foetal bovine serum (Gibco, Carlsbad, CA, USA), 1% penicillin-streptomycin solution (Gibco) and 2% 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (Gibco) at 37 °C under 5% CO_2. K-562 cells (ATCC, CCL-243) were grown in RPMI 1640 (HyClone) supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin solution.

4.2.2. Pseudotyped virus

HIV pseudotyped viruses with GP1,2 of EBOV, GP of MARV expressing the firefly luciferase reporter protein (Fluc) were generated as previously described[21,24]. In brief, the lentivirus-based pHIV-EBOVGP-Fluc construct carrying the EBOV GP gene, and pHIV-MARVGP-Fluc construct carrying the MARV GP gene were generated by the co-transfection of 293T cells with pCDNA3.1-EBOV-ZGP-8A and pCDNA3.1-MARVGP respectively, together and HIV-1 containing firefly luciferase reporter gene (pSG3.cmv.Fluc) in a 1:2 ratio using Lipofectamine 3000 (Invitrogen). After incubation for 48 h, the culture supernatant was centrifuged at 210 × g for 5 min, filtered through a 0.45 μmol/L pore-size filter, and concentrated with a 30-kDa ultrafiltration centrifugal tube (Millipore, Boston, MA, USA). All works involving pseudotyped virus were performed in a BSL-2 facility at the National Institutes of Food and Drug Control, Beijing, China.

4.2.3. Animal experiments

The mice used in this study were housed and handled strictly in accordance with the guidelines set by the Association for the Assessment and Accreditation of Laboratory Animal Care (Frederick, MD, USA). The study protocol was approved by the Animal Care and Use Committee at the National Institute for Food and Drug Control (NIFDC, Beijing, China). Four-week-old BALB/c were obtained from the Institute for Laboratory Animal Resources, NIFDC. BALB/c mice were injected with 100 μL of saline or 1 mg/mL sertraline, **7d** at hour+12/Day+2, by IV and IP ways (half in half), and infected with 5 × 10^6 $TCID_{50}$ pHIV–EBOVGP–Fluc or 9.75 × 10^7 $TCID_{50}$ pHIV-MARVGP-Fluc at Day 0 by IP route and monitored for bioluminescent signals at different time points.

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