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TETRAHEDRON

Absolute Stereochemistry of Cicutoxin and Related Toxic Polyacetylenic Alcohols from *Cicuta virosa*[1]

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Abstract: The absolute stereostructures of cicutoxin (**1**) and related toxic polyacetylenic alcohols, isocicutoxin (**2**), and virols A (**3**) and C (**4**), from *Cicuta virosa* were determined on the basis of spectroscopic analysis. The CD exciton chirality method was successfully applied to 4-methoxybenzoates of diyne-conjugated polyenyl alcohol systems. Fatal doses of these compounds on mice are also reported. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Polyacetylenic alcohols; Optical properties; Stereochemistry.

INTRODUCTION

The toxic effects of the poisonous plant, water hemlock, *Cicuta virosa* (Umberiferae), have been well known for several centuries. Cicutoxin (**1**) was isolated in 1915,[2a] as the lethal constituent causing clonic and tonic convulsions, and Anet *et al.* elucidated its gross structure in 1953.[2b,2c] Recent investigation by Wittstock *et al.* on the constituents of *C. virosa* uncovered more than ten congeners of cicutoxin (**1**).[2e] However, neither stereochemical nor pharmacological studies of cicutoxin and its congeners[3] have been reported. Taking advantage of a case of *Cicuta* poisoning in May 1992 in Sendai, Japan, we planned to uncover the stereostructures of cicutoxin (**1**) and its congeners and the mechanism of *Cicuta* poisoning.

In this paper, we wish to describe the isolation, the absolute stereostructures of toxic polyacetylenic alcohols, cicutoxin (**1**), isocicutoxin (**2**),[3e] virols A (**3**),[3d,3e] and C (**4**)[3d,3e,4] from *C. virosa*, and acute toxicity for some of those congeners.

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RESULTS AND DISCUSSION

The Et_2O soluble fraction (20 g) from the MeOH extract (120 g) of the fresh rhizome of *C. virosa* (2 kg) was repeatedly subjected to column chromatography to give cicutoxin (**1**, 883 mg, 0.044%), isocicutoxin (**2**, 269 mg, 0.013%) and two other optically active congeners, virols A (**3**, 30.0 mg, 0.0015%) and C (**4**, 24.5 mg, 0.0012%) in pure form.

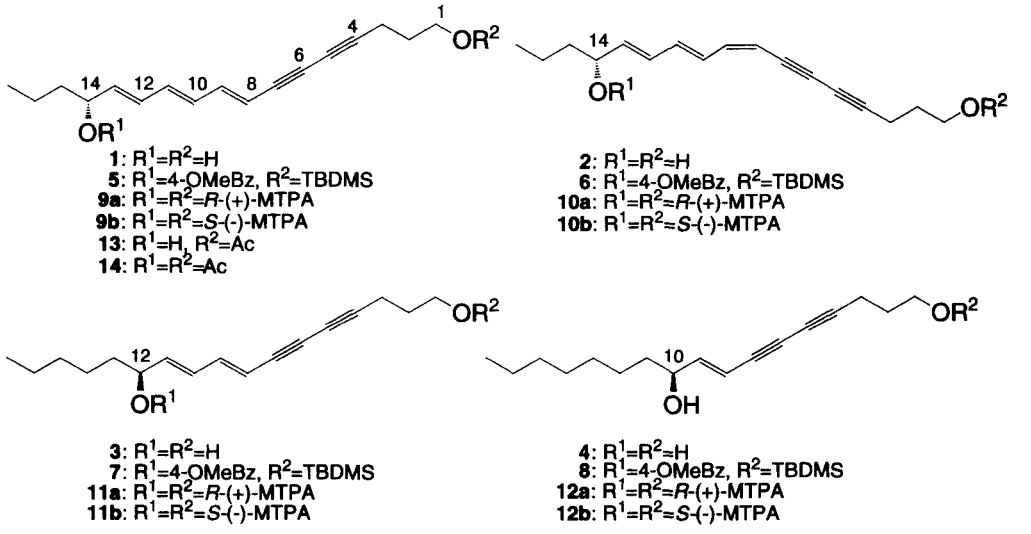

Fig. 1 Structures of cicutoxin (**1**), its congeners (**2**, **3** and **4**) and their derivatives

Structure Analysis

Table 1 showed the ^1H and ^{13}C NMR resonances for cicutoxin (**1**), $[\alpha]_D^{26}$ -13.8° (*c* 1.098, EtOH), isocicutoxin (**2**), $[\alpha]_D^{26}$ -63.7° (*c* 0.609, MeOH), and its congeners unambiguously assigned by 2D HMQC and HMBC experiments. Some of the data are inconsistent with those reported.[2e] Virol A (**3**) and virol C (**4**) showed positive optical rotations, $[\alpha]_D^{20}$ +15.5° (*c* 1.098, MeOH) and $[\alpha]_D^{26}$ +6.4° (*c* 0.820, MeOH), respectively. Although the planar structure obtained for virol A (**3**) and virol C (**4**) were identical with those reported[2d] we named those virols because of their lethal toxicity in mice and optical activities.

Absolute configuration

Although the conjugated π-electron system of cicutoxin (**1**) and its congeners (**2**, **3** and **4**) generates weak Cotton effects between 250-350 nm in their CD spectra which reflect a twisted chromophore of an olefinic bisacetylene system, the unsymmetrical chromophore prevented an

Table 1. ^1H and ^{13}C NMR Spectral Data for Cicutoxin (**1**), Isocicutoxin (**2**), Virols A (**3**) and C (**4**) in $CDCl_3$ [a]

	1		**2**		**3**		**4**	
No.	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	3.76 t (6.0)	61.4 t	3.76 t (6.5)	61.5 t	3.76 t (6.1)	61.4 t	3.74 t (6.9)	61.3 t
2	1.79 m	31.0 t	1.82 m	31.0 t	1.81 m	30.9 t	1.79 quint (6.9)	30.8 t[b]
3	2.48 dt (7.0, 1.0)	16.3 t	2.51 dt (7.0, 0.9)	16.4 t	2.48 t (7.0)	16.2 t	2.46 t (6.9)	16.0 t
4		85.3 s		85.8 t		84.7 s		83.6 s
5		65.9 s		65.9 s		65.8 s		65.6 s
6		77.7 s		81.1 s		77.4 s		74.8 s
7		75.1 s		72.9 s		72.5 s		73.4 s
8	5.61 d (15.5)	110.0 d	5.43 d (11.0)	107.9 d	5.61 d (15.5)	110.5 d	5.72 d (15.4)	108.6 d
9	6.69 dt (15.5, 10.5)	144.4 d	6.52 t (11.0)	143.1 d	6.68 dt (15.5, 11.3)	143.8 d	6.27 dt (15.9, 6.0)	149.2 d
10	6.24 dt (15.0, 10.5)	131.6 d	6.34 dt (13.5, 11.0)	130.1 d	6.27 dt (15.3, 11.3)	129.0 d	4.15 br.-dt	72.1 d
11	6.32 dt (15.5, 10.0)	135.4 d	6.73 dt (13.5, 11.0)	123.0 d	5.84 dt (15.3, 6.3)	140.1 d	1.51 m	36.8 t
12	6.24 dt (15.5, 10.0)	129.8 d	6.39 dt (14.5, 11.0)	136.1 d	4.17 m	72.3 d	1.27 m[d]	25.2 t
13	5.82 dt (15.5, 7.0)	139.3 d	5.82 dt (14.5, 6.5)	139.4 d	1.53 m	37.2 t	1.27 m[d]	29.4 t[b]
14	4.19 m	72.3 d	4.20 m	72.4 d	1.38 m 1.29 m[c]	25.0 t	1.27 m[d]	29.2 t[b]
15	1.54 m	39.4 t	1.55 m	39.4 t	1.29 m[c]	31.7 t	1.27 m[d]	31.7 t[b]
16	1.39 m	18.7 t	1.40 m	18.7 t	1.29 m[c]	22.6 t	1.27 m[d]	22.6 t
17	0.93 t (7.0)	14.0 q	0.94 t (7.0)	14.0 q	0.89 t (6.8)	14.0 q	0.88 t (6.7)	14.0 q

[a] Coupling constants (Hz) are given in parentheses

[b] Signals are interchangeable

[c, d] Signals are not identified

assignment of the Cotton effects. In order to unambiguously determine the absolute configuration at the polyenyl carbinol carbon in cicutoxin (**1**) and related diols (**2**, **3** and **4**), we applied the CD exciton chirality method[5] to their 4-methoxybenzoates (**5**, **6**, **7** and **8**). The 4-methoxybenzoate chromophore (λ_{max} 257 nm) was selected so that the π- π^* transition along the long axis of the olefinic acetylene

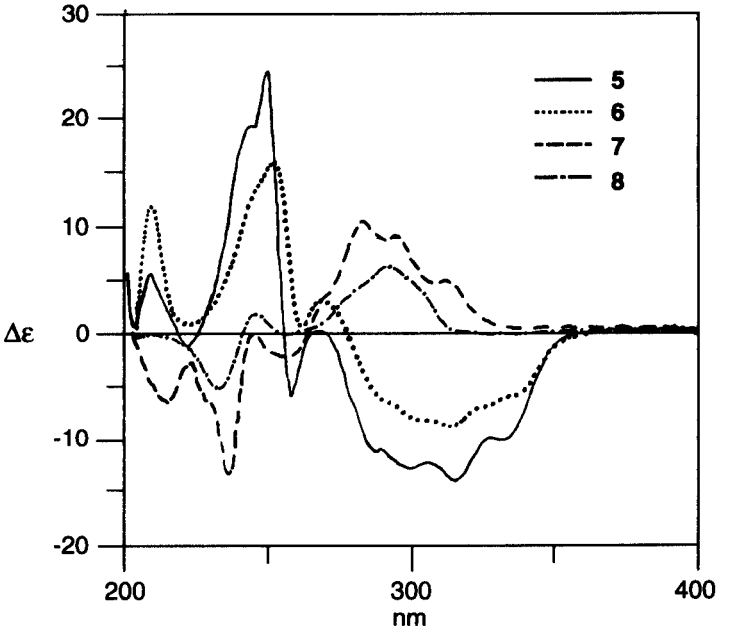

Fig. 2. Difference CD spectra of 4-methoxybenzoates (**5**, **6**, **7** and **8**)

chromophore of the congeners at 250-350 nm effectively couples with that of the benzoate chromophore to generate intense exciton split Cotton effects.

The 4-methoxybenzoate (**5**) was prepared from cicutoxin 1-*O*-TBDMS ether by treatment with 4-methoxybenzoyl chloride, triethylamine and DMAP. The difference CD spectral curve for the methoxybenzoate (**5**) indicated the positive Cotton effect at 243 nm and the overlapped negative Cotton effects between 250 and 350 nm, which are correlated to the UV absorptions. As the UV absorptions from 250 to 350 nm were considered as the bands due to the triene-diyne chromophore system along the long axis of the molecule, the overlapped Cotton effects between 250 and 350 nm could be assigned as a negative first Cotton effect. The observed negative exciton chirality of the 4-methoxybenzoate (**5**) indicated the counterclockwise twist of the electron transition moments for the two chromophores in **5**. The absolute configuration at C-14 of cicutoxin (**1**) was thus determined as *R*.

Three other alcohols (**2**, **3** and **4**) were transformed into 4-methoxybenzoyl derivatives (**6**, **7** and **8**) in a similar manner as above. Like the case of **5**, multiple UV absorptions due to a conjugated chain chromophore led to a broad band first Cotton effect in the difference CD spectral curves for 4-methoxybenzoyl derivatives (**6**, **7** and **8**). 14*R* configuration for isocicutoxin (**2**) suggested by the negative optical rotation value ($[\alpha]_D^{26}$ -63.7°) which was the same sign as that of cicutoxin (**1**) ($[\alpha]_D^{26}$ -13.8°) was confirmed by the negative first Cotton effect as shown in Fig. 2, while positive first Cotton

effects for **7** and **8** indicated *S* configuration at C-12 and C-10 of virols A (**3**) and C (**4**), respectively.

To confirm the absolute configurations deduced from the CD exciton chirality method, we applied the extended Mosher method[6] to these polyacetylenic alcohols (**1**, **2**, **3** and **4**). The di-(*R*-(+) and *S*-(-)) MTPA esters (**9a,b**, **10a,b**, **11a,b** and **12a,b**) were prepared from cicutoxin (**1**), isocicutoxin (**2**) and virols A (**3**) and C (**4**), respectively, by treatment with MTPA chloride, triethylamine and DMAP. As seen in Fig. 3 the anisotropic effect of the phenyl group of the MTPA moiety to the remote protons clearly exerted in the same direction as that on the protons on the β- and β'-carbons without exception. These results strongly supported the absolute configurations suggested by the CD exciton chirality method.

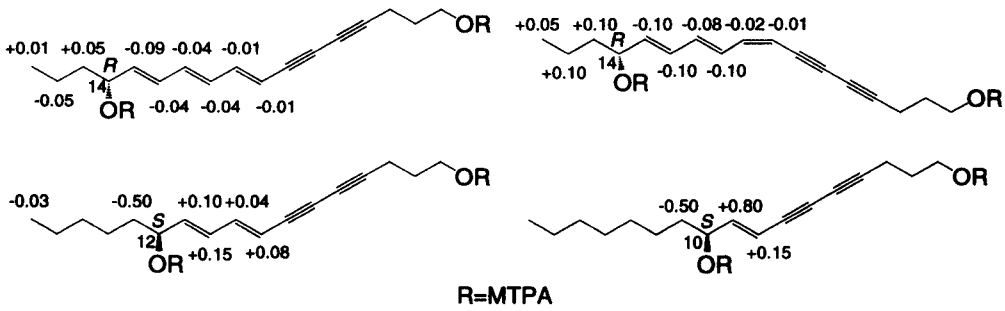

Fig. 3 ^{1}H-NMR chemical shift differences for MTPA derivatives of cicutoxin (**1**), its congeners (**2**, **3** and **4**): Δδ (ppm)=δ (*S*-MTPA ester)-δ (*R*-MTPA ester).

Biological activity

The acute toxicity (LD_{50}) of the *C. virosa* polyacetylenic alcohols, cicutoxin (**1**), isocicutoxin (**2**), virol A (**3**) and some analogues in mice was determined by the Litchfield-Wilcoxon method. Although virol C (**4**) also showed a week toxicity, the lethal dose (LD_{50}) was not calculated because of insufficient amount available. The result demonstrates that cicutoxin (**1**) is the most toxic in the congeners. Table 2 suggested that the size and the geometry of the conjugation has an important role in the toxicity. The fact that the acetates (**13** and **14**) have similar toxicity to that of **1** implies the importance of the hydrophorbicity.

Table 2 Acute toxicity of cicutoxin (**1**) and congeners (**2**, **3**, **13** and **14**) in ddy mice

	cicutoxin (**1**)	acetate (**13**)	acetate (**14**)	isocicutoxin (**2**)	virol A (**3**)
LD_{50} (mg/kg)[a]	2.8	2.8	13.0	38.5	28.0
(μmol/kg)	(10.8)	(9.3)	(38.0)	(149)	(108)

[a] Route: *i.p.*

CONCLUSION

We determined the absolute stereostructures of cicutoxin (**1**) and their related toxic polyacetylenic alcohols, isocicutoxin (**2**), virols A (**3**) and C (**4**) from *C. virosa* based on analysis of physicochemical data. The CD exciton chirality method was successfully applied to 4-methoxybenzoates of diyne-polyenyl alcohol systems which were unsymmetrically conjugated along the long axis direction. The extended Mosher method confirmed their absolute stereochemistry. We could not investigate the toxicity of virols C (**4**) because of insufficient amount available for the *in vivo* experiment. Syntheses of virols A (**3**) and C (**4**), and detailed investigation of the mode of action of polyacetylenic alcohol-induced tonic and clonic convulsions and a systematic study of the structure-activity relationships of polyacetylenic alcohols will be reported elsewhere in the near future.

EXPERIMENTAL SECTION

Optical rotations were recorded on JASCO DIP-370 and DIP-340 polarimeters. Infrared and ultraviolet spectra were recorded on JASCO A-100-S-IR and HITACHI U-3000 spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were measured by a Varian Gemini 2000 (300 and 75 MHz) and a JEOL JMN-GX500 (500 and 125 MHz) spectrometers. Chemical shifts (δ) are reported in ppm relative to tetramethylsilane (TMS), and coupling constants are given in Hz. Multiplicity is indicated as follows: s (singlet); d (doublet); dd (doublet of doublets); ddd (doublet of double doublets); t (triplet); m (multiplet); br (broad). Mass spectra were recorded on JEOL JMS-DX303, JMS-AX-500 and JEOL HX-105 spectrometers.

Isolation of cicutoxin (1), isocicutoxin (2) and virols A (3) and C (4).

C. virosa was collected in Sendai in May, 1992. Flesh rhizome (1.8 kg) was extracted with MeOH at room temperature for 3 days. The MeOH extract was filtrated, concentrated in *vacuo* to yield a residue (200 g) which was partitioned between Et_2O and H_2O. Column chromatography of the concentrated Et_2O layer (20.0 g) on silica gel (200 g) (*n*-hexane and *n*-hexane-acetone (9:1, 4:1, 7:3, 3:2, 1:1 *v/v*)) yielded 18 fractions (fraction A to R). The fraction K (0.65 g) which was eluted with *n*-hexane-acetone (4:1 *v/v*) was repeatedly subjected to column chromatography using *n*-hexane-AcOEt and $CHCl_3$ as eluting solvent to give cicutoxin (**1**: 883 mg), isocicutoxin (**2**: 269 mg), virols A (**3**: 30.0 mg) and C (**4**: 24.5 mg).

Physical properties of cicutoxin (1): a colorless oil, $[\alpha]_D^{26}$ -14.9° (*c* 1.12, MeOH). UV (Et_2O) λ (logε): 335.4 (4.64), 317.8 (4.66), 304.5 (sh), 251.6 (4.22), 241.8 (4.07) nm. IR ($CHCl_3$) ν: 3607, 3445, 2226, 2133, 1603, 997 cm^{-1}. HREI MS: *m/z*: 258.1647 (M^+), Calcd 258.1620 for $C_{17}H_{22}O_2$.

Physical properties of isocicutoxin (2): a colorless oil, $[\alpha]_D^{26}$ -51.9° (*c* 0.784, MeOH). UV (MeOH) λ (logε): 332.0 (4.54), 317.4 (4.56), 302.0 (sh), 254.8 (4.23), 242.2 (4.06) nm. IR ($CHCl_3$) ν: 3607, 3427, 2226, 2131, 1603, 997 cm^{-1}. HREI MS: *m/z*: 258.1643 (M^+), Calcd 258.1620 for $C_{19}H_{22}O_2$.

Physical properties of virol A (3): a colorless oil, $[\alpha]_D^{20}$ +15.5° (*c* 1.10, MeOH). UV (MeOH) λ (logε): 310.6 (4.46), 293.2 (4.53), 280.5 (sh), 235.6 (4.57), 226.2 (4.37) nm. IR ($CHCl_3$) ν: 3352, 2230, 2135, 1636, 986 cm^{-1}. HREI MS: *m/z*: 260.1812, Calcd 260.1776 for $C_{17}H_{24}O_2$.

Physical properties of virol C (4): a colorless crystal, mp 46.5-48.0 °C. $[\alpha]_D^{26}$ +6.4° (*c* 0.82, MeOH). UV (Et_2O) λ (logε): 214 (4.63), 229 (3.56), 240 (3.84), 253 (4.15), 267 (4.32), 283 (4.22) nm. IR ($CHCl_3$) ν: 3609, 3439, 3013, 2932, 2859, 2236, 2141 cm^{-1}. HREI MS: *m/z*: 262.1914 (M^+), Calcd 262.1933 for $C_{17}H_{26}O_2$.

General Procedure for the preparation of p-Methoxybenzoylates of polyacetylenic alcohols (cicutoxin (5), isocicutoxin (6), virols A (7) and C (8)).

In a typical experimental procedure, a mixture of cicutoxin (**1**: 100 mg, 0.39 mmol), *t*-butyldimethylchlorosilane (87.6 mg, 0.58 mmol), Et_3N (0.24 mL, 1.74 mmol) and DMAP (4.7 mg, 38.8 μmol) in CH_2Cl_2 (0.5 mL) was stirred for 3h at 0 °C. Et_2O was added to the reaction mixture and then the resulting solution was washed with saturated solution of aqueous $NaHCO_3$, and brine, successively, dried over $MgSO_4$ and concentrated in *vacuo*. Purification by column chromatography on silica gel (*n*-hexane-AcOEt, 10:1 *v/v*) gave the cicutoxin TBS ether (116 mg, 80 %) as a colorless oil, $[\alpha]_D^{22}$ -13.9°(*c* 1.02, MeOH). UV (MeOH) λ (logε): 332.8 (4.74), 316.2 (4.73), 304.5 (sh), 251.6 (4.24), 242.0 (4.01) nm. CD (MeOH) λ_{ext} (Δε): 215 (+0.4), 241 (-1.0), 253 (-1.3), 295 (+0.3), 310 (+0.2), 318 (-0.7), 340 (-0.2) nm. IR ($CHCl_3$) ν: 3601, 2228, 1603, 997 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.06 (s, 6H), 0.89 (s, 9H), 0.93 (t, 3H, *J*=7.0 Hz), 1.29-1.47 (m, 2H), 1.48-1.64 (m, 2H), 1.70-1.79 (m, 2H), 2.44 (t, 2H, *J*=7.0 Hz), 3.69 (t, 2H, *J*=6.0 Hz), 4.19 (q, 1H, *J*=6.4 Hz), 5.61 (d, 1H, *J*=15.5 Hz), 5.81 (dd, 1H, *J*=15.0, 6.4 Hz), 6.22-6.33 (m, 3H), 6.70 (dd, 1H, *J*=15.5, 10.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ -5.36 (q), 13.97 (q), 16.20 (t), 18.31 (s), 18.59 (t), 25.92 (q), 31.32 (t), 39.39 (t), 61.38 (t), 65.49 (s), 72.27 (d), 74.73 (s), 77.85 (s), 85.88 (s), 110.11 (d), 129.74 (d), 131.62 (d), 135.18 (d), 139.12 (d), 144.12 (d). HREI MS: *m/z*: 372.2493 (M^+), Calcd 372.2483 for $C_{23}H_{36}O_2Si$.

Physical properties of isocicutoxin TBS ether: according to the same procedure for the preparation of cicutoxin TBS ether, isocicutoxin TBS ether was synthesized from isocicutoxin (**2**) as a colorless oil, $[\alpha]_D^{24}$ -47.1° (*c* 0.085, EtOH). UV (EtOH) λ (logε): 334 (4.54), 318 (4.57), 301 (sh), 255 (4.21), 242 (4.06), 204 (4.29) nm. CD (EtOH) λ_{ext} (Δε): 335 (-0.8), 319 (-1.0), 305 (sh), 281 (sh), 255 (-0.8), 245 (sh), 230 (+0.3), 209 (+1.7) nm. IR ($CHCl_3$) ν: 3601, 3503, 2226 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.07 (s, 6H), 0.90 (s, 9H), 0.94 (t, 3H, *J* = 7.1 Hz), 1.30-1.65 (m, 4H), 1.76 (quint., 2H, *J* = 6.6 Hz), 2.46 (t, 2H, *J* = 7.0 Hz), 3.70 (t, 2H, *J* = 5.9 Hz), 4.10-4.26 (m, 1H), 5.44 (d, 1H, *J* = 10.9 Hz), 5.83 (dd, 1H, *J* = 6.9, 14.0 Hz), 6.28-6.44 (m, 2H), 6.52 (t, 1H, *J* = 10.9 Hz), 6.75 (dd, 1H, *J* = 11.8, 13.7 Hz). HREI MS: *m/z*: 372.2495 (M^+), Calcd 372.2483 for $C_{23}H_{36}O_2Si$.

Physical properties of virol A TBS ether: according to the same procedure for the preparation of cicutoxin TBS ether, virol A TBS ether was synthesized from virol A (**3**) as a colorless oil, $[\alpha]_D^{25}$

+10.9° (*c* 0.824, MeOH). UV (MeOH) λ (logε): 310 (4.43), 293 (4.50), 280 (sh), 236 (4.54), 226 (4.33), 215 (sh). CD (MeOH) λ_{ext} (Δε): 315 (+0.4), 292 (+1.0), 237 (+2.1), 228 (+1.6) nm. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.06 (s, 3H), 0.06 (s, 3H), 0.79-0.96 (m, 12H), 1.21-1.35 (m, 5H), 1.35-1.45 (m, 1H), 1.47-1.61 (m, 2H), 1.70-1.77 (m, 2H), 2.43 (t, 2H, *J* = 7.0 Hz), 3.69 (t, 2H, *J* = 6.0 Hz), 4.13-4.20 (m, 1H), 5.61 (d, 1H, *J* = 15.5 Hz), 5.83 (dd, 1H, *J* = 15.5, 6.5 Hz), 6.27 (dd, 1H, *J* = 15.5, 11.0 Hz), 6.67 (dd, 1H, *J* = 15.5, 11.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ -5.36 (q), 14.02 (q), 16.15 (t), 18.31 (s), 22.58 (t), 25.00 (t), 25.92 (q), 31.31 (t), 31.71 (t), 37.20 (t), 61.38 (t), 65.34 (s), 72.31 (d), 74.18 (s), 76.77 (s), 85.37 (s), 110.31 (d), 129.04 (d), 139.89 (d), 143.65 (d). HREI MS: *m/z* 374.2625 (M^+), Calcd 374.2639 for $C_{23}H_{38}O_2Si$.

Physical properties of virol C TBS ether: according to the same procedure for the preparation of cicutoxin TBS ether, virol C TBS ether was synthesized from virol C (**4**) as a colorless oil, $[\alpha]_D^{24}$ +2.21° (*c* 1.448, EtOH). UV (EtOH) λ (logε): 283 (3.84), 267 (3.94), 253 (3.79), 240 (3.52), 229 (3.34), 214 (4.30), 206 (4.23) nm. CD (EtOH) λ_{ext} (Δε): 284 (+0.90), 269 (+0.93), 258 (sh), 254 (+0.70), 214 (+1.88) nm. IR ($CHCl_3$) ν: 3603, 3439, 3007, 2957, 2932, 2858, 2235 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.06 (s, 6H), 0.80-0.98 (m, 3H), 0.89 (s, 9H), 1.18-1.50 (m, 10H), 1.50-1.82 (m, 6H), 2.42 (t, 2H, *J* = 6.7 Hz), 3.68 (t, 2H, *J*=5.9 Hz), 4.17 (quint., 1H, *J*=6.2 Hz), 5.46 (quint., 1H, *J* = 6.9 Hz), 5.73 (d, 1H, *J* = 15.5 Hz), 6.27 (dd, 1H, *J* = 5.8, 15.5 Hz). HREI MS: *m/z* 376.2818, Calcd 376.2797 for $C_{23}H_{40}O_2Si$.

A mixture of cicutoxin TBS ether (33.4 mg, 89.8 μmol), *p*-methoxybenzoyl chloride (22.1 mg, 0.13 mmol), Et_3N (37.5 μL, 269 μmol), DMAP (1.10 mg, 9.0 μmol) in CH_2Cl_2 (1.0 mL) was stirred 3 h at 0 °C. Et_2O was added to the reaction mixture and then the resulting solution was washed with saturated solution of aqueous $NaHCO_3$ and brine, successively, dried over $MgSO_4$ and concentrated in *vacuo*. Purification by column chromatography on silica gel (*n*-hexane-AcOEt, 30:1 *v/v*) gave *p*-methoxybenzoate of cicutoxin (**5**; 42.6 mg, 94%) as a colorless oil, $[\alpha]_D^{24}$ -103.6° (*c* 0.193, MeOH). UV (MeOH) λ (logε): 335.2 (4.63), 318.0 (4.64), 305.5 (4.43), 252.4 (4.35), 245.0 (4.15) nm. CD (MeOH) λ_{ext} (Δε): 335.0 (-11.1), 318.0 (-14.9), 300.0 (-12.7), 267.0 (+3.1), 259.0 (-3.2), 250.0 (+24.7), 243.5 (+19.9) nm. IR ($CHCl_3$) ν: 2330, 1750, 1600, 990 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.02 (s, 6H), 0.84 (s, 9H), 0.90 (t, 3H, *J*=3.5 Hz), 1.36 (m, 2H), 1.65-1.74 (m, 4H), 2.38 (t, 2H, *J*= 7.0 Hz), 3.63 (t, 2H, *J*=6.0 Hz), 3.81 (S, 3H), 5.48 (m, 1H), 5.55 (d, 1H, *J* =15.5 Hz), 5.79 (dd, 1H, *J* = 14.0, 7.0 Hz), 6.20-6.27 (m, 3H), 6.63 (dd, 1H, *J*=15.5, 10.5 Hz), 6.87 (d, 2H, *J*=9.0 Hz), 7.95 (d, 2H, *J*=9.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ -5.36 (q), 13.89 (q), 16.20 (t), 18.31 (s), 18.48 (t), 25.92 (q), 31.32 (t), 36.66 (t), 55.45 (q), 61.37 (t), 65.50 (s), 74.05 (d), 74.14 (s), 77.80 (s), 85.94 (s), 110.41 (d), 113.60 (d), 131.57 (d), 132.30 (d), 134.52 (d), 134.89 (d), 135.63 (d), 142.51 (d), 163.37 (s). HREI MS: *m/z* 506.2892, Calcd 506.2850 for $C_{31}H_{42}O_4Si$.

Physical properties of p-methoxybenzoate of isocicutoxin (6): according to the same procedure for the preparation of *p*-methoxybenzoate of cicutoxin (**5**), *p*-methoxybenzoate of isocicutoxin (**6**) was synthesized from isocicutoxin TBS ether as a colorless oil, $[\alpha]_D^{23}$ -269.3° (*c* 1.13, EtOH). UV (EtOH) λ (logε): 335 (4.57), 319 (4.60), 300 (sh), 273 (sh), 256 (4.48), 244 (sh), 205 (4.31) nm. CD

(EtOH) λ_{ext} ($\Delta\varepsilon$): 393 (+0.1),337 (sh), 326 (sh), 313 (-10.6),300 (sh), 287.9 (sh), 267 (+2.8), 259 (-18.5), 249 (+16.6), 206 (+14.04) nm. IR ($CHCl_3$) v: 2226, 1705 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.07 (s, 6H), 0.90 (s, 9H), 0.95 (t, 3H, *J* = 7.4 Hz), 1.34-1.50 (m, 2H), 1.60-1.88 (m, 4H), 2.46 (t, 2H, *J* = 6.9 Hz), 3.70 (t, 2H, *J* = 5.9 Hz), 3.86 (s, 3H), 5.43 (d, 1H, *J* = 11.0 Hz), 5.55 (br.dt, 1H), 5.85 (dd, 1H, *J* = 6.7, 14.4 Hz), 6.20-6.48 (m, 2H), 6.50 (t, 1H, *J* = 11.0 Hz), 6.76 (dd, 1H, *J* = 11.4, 14.1 Hz), 6.90 (d, 2H, *J* = 9.1 Hz), 8.02 (d, 2H, *J* = 9.1 Hz). HREI MS: *m/z* 506.2857 (M^+), Calcd 506.2852 for $C_{31}H_{42}O_4Si$.

Physical properties of p-methoxybenzoate of virol A (7): according to the same procedure for the preparation of *p*-methoxybenzoate of cicutoxin (**5**), *p*-methoxybenzoate of virol A (**7**) was synthesized from virol A TBS ether as a colorless oil, $[\alpha]_D^{25}$ +52.0° (*c* 0.342, MeOH). UV (MeOH) λ (logε): 312.0 (4.51), 293.8 (4.57), 278.4 (4.48), 260.2 (4.47), 235.2 (4.57), 226.9 (4.41) nm. CD (MeOH) λ_{ext} ($\Delta\varepsilon$): 310.0 (+5.5), 292.0 (+10.1), 280.0 (+11.8), 255.0 (-3.8), 235.0 (-17.7), 228.0 (-8.4) nm. ^1H NMR (500 MHz, $CDCl_3$): δ 0.05 (s, 6H), 0.89 (m, 3H), 0.90 (s, 9H), 1.30-1.31 (m, 5H), 1.39 (m, 1H), 1.72 (m, 1H), 1.79 (m, 1H), 2.42 (t, 2H, *J* = 7.0 Hz), 3.68 (t, 2H, *J*=6.0 Hz), 3.86 (S, 3H), 5.50 (m, 1H), 5.61 (d, 1H, *J* =15.5 Hz), 5.84 (dd, 1H, *J* = 15.5, 6.5 Hz), 6.32 (dd, 1H, *J*=15.5, 11.0 Hz), 6.65 (dd, 1H, *J*=15.5, 11.0 Hz), 6.92 (d, 2H, *J*=8.5 Hz), 8.00 (d, 2H, *J*=8.5 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ -5.36 (q), 13.99 (q), 16.15 (t), 20.32 (s), 22.50 (t), 24.81 (t), 25.92 (q), 31.30 (t), 31.57 (t), 34.46 (t), 55.45 (q), 61.37 (t), 65.38 (s), 74.69 (d), 75.18 (s), 76.19 (s), 85.50 (s), 111.07 (d), 113.63 (d), 130.82 (d), 131.64 (d), 135.31 (d), 143.34 (d), 163.41 (s). HREI MS: *m/z*: 508.2971 (M^+), Calcd 508.3007 for $C_{31}H_{44}O_4Si$.

Physical properties of p-methoxybenzoate of virol C (8): according to the same procedure for the preparation of *p*-methoxybenzoate of cicutoxin (**5**), *p*-methoxybenzoate of virol C (**8**) was synthesized from virol C TBS ether as a colorless oil, $[\alpha]_D^{24}$ +10.8° (*c* 0.0922, EtOH). UV (EtOH) λ (logε): 285 (4.26), 269 (4.47), 255 (4.41), 241 (sh), 213 (4.72), 206 (4.65) nm. CD (EtOH) λ_{ext} ($\Delta\varepsilon$): 272 (+7.17), 268 (sh), 218 (+3.50), 203 (-4.87) nm. IR ($CHCl_3$) v: 3026, 3018, 2957, 2930, 2858, 2235, 1707 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.04 (s, 6H), 0.79-0.90 (m, 3H), 0.88 (s, 9H), 0.87 (t, 3H, *J* = 6.9 Hz), 1.15-1.44 (m, 12H), 1.72 (quint., 2H, *J*=6.0 Hz), 2.40 (t, 2H, *J* = 6.9 Hz), 3.67 (t, 2H, *J*=5.9 Hz), 3.86 (s, 3H), 5.49 (ddt, 1H, *J* = 1.4, 6.3, 6.6 Hz), 5.76 (dd, 1H, *J* =1.4, 15.9 Hz), 6.26 (dd, 1H, *J* = 6.3, 15.9 Hz), 6.92 (d, 2H, *J*=9.1 Hz), 8.0 (d, 2H, *J*=9.1 Hz). HREI MS: *m/z*: 510.3134 (M^+), Calcd 510.3165 for $C_{31}H_{46}O_4Si$.

MTPA esters of cicutoxin (9a and 9b), isocicutoxin (10a and 10b), virols A (11a and 11b) and C (12a and 12b)

In a typical experimental procedure, a mixture of cicutoxin (**1**: 9.9 mg, 38.4 μmol), (*R*)-MTPA chloride (38.8 mg, 153 μmol), Et_3N (26.7 μL, 192 μmol) and DMAP (0.5 mg, 3.8 μmol) in CH_2Cl_2 was stirred for 5h at r.t.. Et_2O was added to the reaction mixture and then the resulting solution was washed with saturated solution of aqueous $NaHCO_3$ and brine, successively, dried over $MgSO_4$ and concentrated in *vacuo*. Purification by column chromatography on silica gel (*n*-hexane-AcOEt, 10:1 *v/v*)

gave (*R*)-MTPA ester of cicutoxin (**9a**; 20.2 mg, 76%) as a colorless oil, $[\alpha]_D^{22}$ +26.9° (*c* 0.876, MeOH). UV (MeOH) λ (logε): 334.4 (4.71), 317.2 (4.74), 305.0 (4.57), 252.0 (4.31), 241.8 (4.19) nm. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.91 (m, 3H), 1.30 (m, 1H), 1.43 (m, 1H), 1.58 (m, 1H), 1.70 (m, 1H), 1.93 (m, 2H), 2.39 (t, 2H, *J* = 6.5 Hz), 3.53 (s, 3H), 3.55 (s, 3H), 4.38 (dt, 1H, *J*=12.0, 6.0 Hz), 4.46 (dt, 1H, *J*=12.0, 6.0 Hz), 5.53 (m, 1H), 5.64 (d, 1H, *J* = 15.5 Hz), 5.74 (dd, 1H, *J* = 15.0, 7.0 Hz), 6.19-6.38 (m, 3H), 6.70 (dd, 1H, *J*=15.5, 10.0 Hz), 7.39 (m, 6H), 7.50 (m, 4H). HREI MS: *m/z* 456.1935 (M^+- $C_{10}H_9O_3F_3$), Calcd 456.1912 for $C_{27}H_{27}O_3F_3$.

*Physical properties of (S)-MTPA ester of cicutoxin (**9b**)*: according to the same procedure for the preparation of (*R*)-MTPA ester of cicutoxin (**9a**), (*S*)-MTPA ester of cicutoxin (**9b**) was synthesized from cicutoxin (**1**) as a colorless oil, $[\alpha]_D^{23}$ -114.7° (*c* 1.172, MeOH). UV (MeOH) λ (logε): 334.8 (4.63), 316.4 (4.67), 304.5 (4.52), 253.4 (4.24), 241.4 (4.12) nm. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.90 (m, 3H), 1.25 (m, 1H), 1.36 (m, 1H), 1.63 (m, 1H), 1.74 (m, 1H), 1.94 (m, 2H), 2.40 (t, 2H, *J* = 6.7 Hz), 3.53 (s, 3H), 3.55 (s, 3H), 4.39 (dt, 1H, *J*=11.6, 5.8 Hz), 4.45 (dt, 1H, *J*=11.6, 5.8 Hz), 5.51 (m, 1H), 5.63 (d, 1H, *J* = 15.3 Hz), 5.65 (dd, 1H, *J* = 15.6, 7.0 Hz), 6.15-6.35 (m, 3H), 6.69 (dd, 1H, *J*=15.3, 9.2 Hz), 7.41 (m, 6H), 7.51 (m, 4H). HREI MS: *m/z* 456.1935 (M^+- $C_{10}H_9O_3F_3$), Calcd 456.1912 for $C_{27}H_{27}O_3F_3$.

*Physical properties of (R)-MTPA ester of isocicutoxin (**10a**)*: according to the same procedure for the preparation of (*R*)-MTPA ester of cicutoxin (**9a**), (*R*)-MTPA ester of isocicutoxin (**10a**) was synthesized from isocicutoxin (**2**) as a colorless oil, $[\alpha]_D^{24}$ -30.9° (*c* 0.298, EtOH). UV (EtOH) λ (logε): 335 (4.53), 318 (4.57), 302 (sh), 255 (4.22), 242 (4.02), 224 (sh), 205 (4.52) nm. IR ($CHCl_3$) ν: 2227, 1747 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H, *J* = 7.3 Hz), 1.15-1.37 (m, 2H), 1.50-1.79 (m, 4H), 1.91 (quint., 2H), 2.43 (t, 2H, *J* = 7.1 Hz), 3.53 (s, 3H), 3.56 (s, 3H), 4.35-4.58 (m, 2H), 5.48 (d, 1H, *J* = 11.0 Hz), 5.54 (dt, 1H, *J* = 6.0, 7.4 Hz), 5.76 (dd, 1H, *J* = 7.4, 14.3 Hz), 6.22-6.43 (m, 2H), 6.53 (t, 1H, *J* = 11.0 Hz), 6.75 (dd, 1H, *J* = 11.5, 14.0 Hz), 7.37-7.45 (m, 6H), 7.47-7.57 (m, 4H). HREI MS: *m/z*: 690.2394 (M^+), Calcd 690.2416 for $C_{37}H_{34}O_6F_6$.

*Physical properties of (S)-MTPA ester of isocicutoxin (**10b**)*: according to the same procedure for the preparation of (*R*)-MTPA ester of cicutoxin (**9a**), (*S*)-MTPA ester of isocicutoxin (**10b**) was synthesized from isocicutoxin (**2**) as a colorless oil, $[\alpha]_D^{24}$ -132.5° (*c* 0.400, EtOH). UV (EtOH) λ (logε): 335 (4.58), 318 (4.61), 300 (sh), 255 (4.25), 242 (4.08), 230 (sh), 205 (4.64) nm. IR ($CHCl_3$) ν: 2229, 1747 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.93 (t, 3H, *J* = 7.3 Hz), 1.29-1.46 (m, 2H), 1.50-1.83 (m, 2H), 1.98 (quint., 2H), 2.44 (t, 2H, *J* = 6.9 Hz), 3.56 (s, 3H), 3.59 (s, 3H), 4.36-4.54 (m, 2H), 5.47 (d, 1H, *J* = 11.0 Hz), 5.52 (dt, 1H, *J* = 6.9, 6.9 Hz), 5.66 (dd, 1H, *J* = 6.9, 14.6 Hz), 6.13-6.36 (m, 2H), 6.51 (t, 1H, *J* = 11.0 Hz), 6.67 (dd, 1H, *J* = 11.5, 14.3 Hz), 7.36-7.45 (m, 6H), 7.46-7.57 (m, 4H). HREI MS: *m/z* 690.2419 (M^+), Calcd 690.2416 for $C_{37}H_{34}O_6F_6$.

*Physical properties of (R)-MTPA ester of virol A (**11a**)*: according to the same procedure for the preparation of (*R*)-MTPA ester of cicutoxin (**9a**), (*R*)-MTPA ester of virol A (**11a**) was synthesized from virol A (**3**) as a colorless oil, $[\alpha]_D^{24}$ +70.3° (*c* 0.336, EtOH). UV (EtOH) λ (logε): 312 (4.45),

294 (4.50), 280 (sh), 270 (sh), 236 (4.53), 226 (4.35), 213 (sh), 202 (3.32) nm. IR (CHCl3) ν: 2231, 1747 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.87 (t, 3H, J = 6.6 Hz), 1.10-1.31 (m, 6H), 1.50-1.77 (m, 2H), 1.94 (quint., 2H, J = 6.3 Hz), 2.39 (t, 2H, J = 6.9 Hz), 3.55 (s, 3H), 3.57 (s, 3H), 4.33-4.52 (m, 2H), 5.48 (dt, 1H, J = 6.6, 7.1 Hz), 5.55 (d, 1H, J = 15.4 Hz), 5.67 (dd, 1H, J = 7.1, 15.4 Hz), 6.16 (dd, 1H, J = 10.9, 15.4 Hz), 6.60 (dd, 1H, J = 10.9, 15.4 Hz), 7.25-7.46 (m, 6H), 7.47-7.56 (m, 4H). HREI MS: *m/z*: 692.2579 (M^+), Calcd 692.2573 for $C_{37}H_{36}O_6F_6$.

*Physical properties of (S)-MTPA ester of virol A (**11b**)*: according to the same procedure for the preparation of (*R*)-MTPA ester of cicutoxin (**9a**), (*S*)-MTPA ester of virol A (**11b**) was synthesized from virol A (**3**) as a colorless oil, $[\alpha]_D^{22}$ -45.3° (*c* 0.791, EtOH). UV (EtOH) λ (logε): 312 (4.48), 294 (4.52), 280 (sh), 270 (sh), 236 (4.57), 226 (4.39), 214 (sh), 205 (4.31) nm. IR (CHCl3) ν: 2231, 1747 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.84 (t, 3H, J = 6.6 Hz), 1.10-1.35 (m, 6H), 1.50-1.75 (m, 2H), 1.94 (quint., 2H, J = 6.3 Hz), 2.39 (t, 2H, J = 6.7 Hz), 3.52 (s, 3H), 3.55 (s, 3H), 4.33-4.52 (m, 2H), 5.50 (dt, 1H, J = 6.0, 7.4 Hz), 5.63 (d, 1H, J = 15.7 Hz), 5.77 (dd, 1H, J = 7.4, 15.4 Hz), 6.31 (dd, 1H, J = 11.0, 15.4 Hz), 6.65 (dd, 1H, J = 11.0, 15.7 Hz), 7.37-7.46 (m, 6H), 7.46-7.56 (m, 4H). HREI MS: *m/z* 692.2596 (M^+), Calcd 692.2573 for $C_{37}H_{36}O_6F_6$.

*Physical properties of (R)-MTPA ester of virol C (**12a**)*: according to the same procedure for the preparation of (*R*)-MTPA ester of cicutoxin (**9a**), (*R*)-MTPA ester of virol C (**12a**) was synthesized from virol C (**4**) as a colorless oil, $[\alpha]_D^{24}$ +39.7° (*c* 0.392, EtOH). UV (EtOH) λ (logε): 339 (2.70), 316 (2.90), 285 (4.09), 269 (4.18), 254 (4.03), 241 (3.74), 229 (3.56), 214 (4.31), 192 (3.94) nm. IR ($CHCl_3$) ν: 3092, 3072, 3034,3013, 2932,2856, 2239, 1747 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.87 (t, 3H, J = 6.9 Hz), 1.13-1.41 (m, 10H), 1.55-1.82 (m, 2H), 1.94 (quint., 2H), 2.38 (t, 2H, J = 6.9 Hz), 3.53 (s, 3H), 3.55 (s, 3H), 4.31-4.52 (m, 2H), 5.46 (quint., 1H, J = 6.9 Hz), 5.65 (d, 1H, J = 15.9 Hz), 6.10 (dd, 1H, J = 6.9, 15.9 Hz), 7.38-7.45 (m, 6H), 7.46-7.55 (m, 4H). HREI MS: *m/z*: 694.2700 (M^+), Calcd 694.2729 for $C_{37}H_{38}O_6F_6$.

*Physical properties of (S)-MTPA ester of virol C (**12b**)*: according to the same procedure for the preparation of (*R*)-MTPA ester of cicutoxin (**9a**), (*S*)-MTPA ester of virol C (**12b**) was synthesized from virol C (**4**) as a colorless oil, $[\alpha]_D^{23}$ -33.0° (*c* 0.297, EtOH). UV (EtOH) λ (logε): 339 (2.85), 316 (3.00), 285 (4.03), 269 (4.13), 254 (3.97), 241 (3.70), 229 (3.52), 214 (4.41), 192 (3.89) nm. IR ($CHCl_3$) ν: 3092, 3072, 3036, 2930, 2239, 1747 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.87 (t, 3H, J = 6.9 Hz), 1.12-1.36 (m, 10 H), 1.51-1.75 (m, 2H), 1.94 (quint., 2H), 2.38 (t, 2H, J = 7.0 Hz), 3.53 (s, 3H), 3.55 (s, 3H), 4.34-4.52 (m, 2H), 5.48 (dt, 1H, J = 7.1, 6.2 Hz), 5.77 (d, 1H, J = 15.9 Hz), 6.18 (dd, 1H, J = 7.1, 15.9 Hz), 7.38-7.45 (m, 6H), 7.46-7.56 (m, 4H). HREI MS: *m/z* 694.2748 (M^+), Calcd 694.2729 for $C_{37}H_{38}O_6F_6$.

Assay for Acute toxicity

Ddy male mice (6 weeks old, SLC JAPAN) were housed in group of 10 per cage (30x30x16), on keeping in air-conditioned room (25±1 °C, 55.5±5% of humidity) with 12 h light cycle, and allowed to take food (CE-2, CLEA JAPAN) and water *ad. lib.* Samples in DMSO (0.1 g/mL) were diluted with

olive oil, and administered in geometric progression between 0-100% of lethal dose by intraperitoneally (10 mL/kg body weight) to the mice. 10 mice per group were used in the same dose. The LD_{50} values were estimated according to the Litchfield-Wilcoxon method.

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