Cav2.2 and Cav3.1 Calcium Channel Inhibitors from *Valeriana jatamansi* Jones

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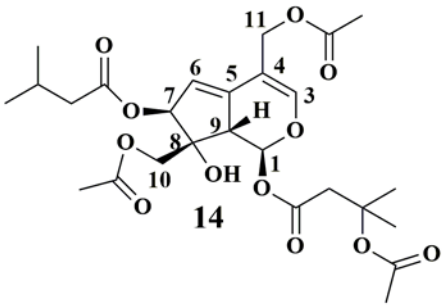
Supporting Information List



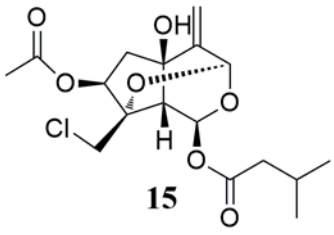


Figure S1 Structures of compounds **14** and **15**

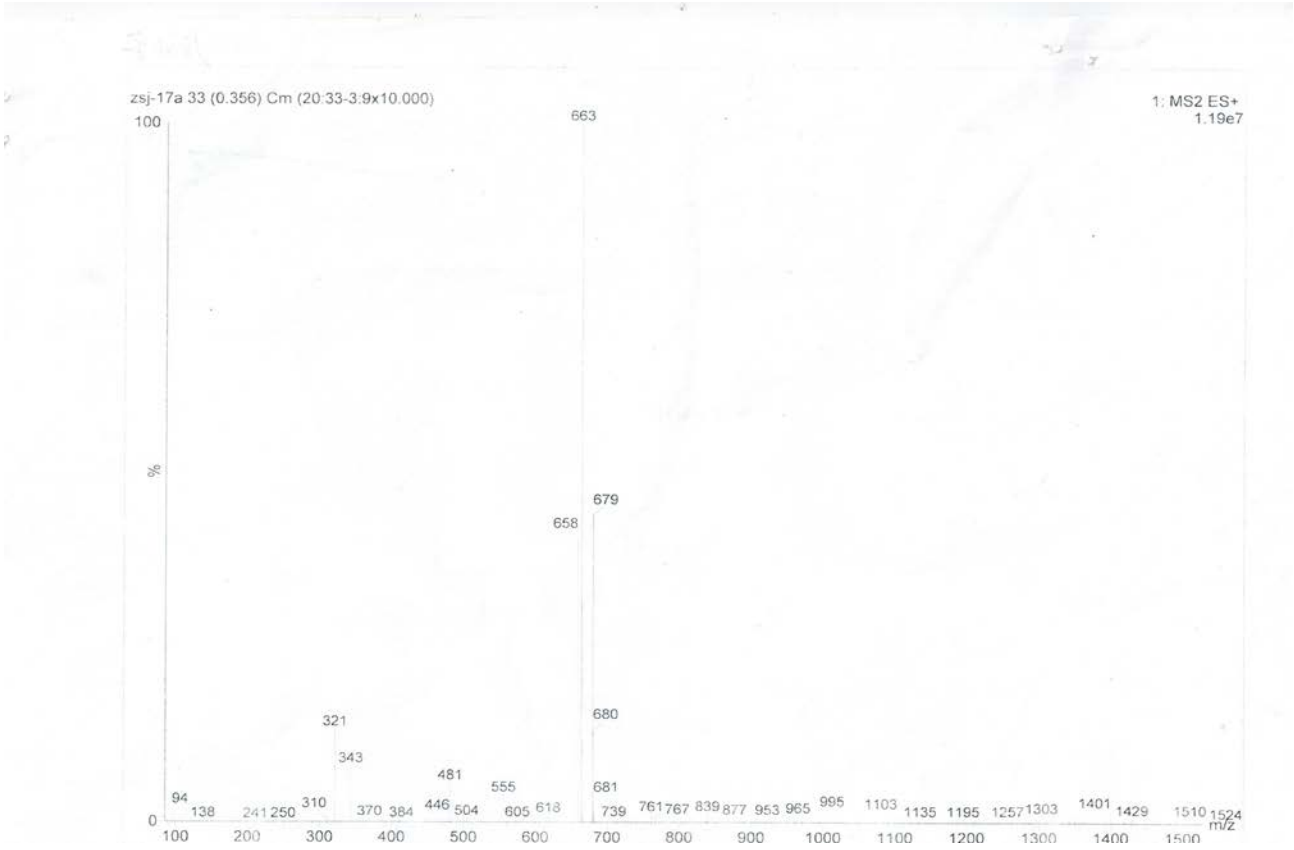
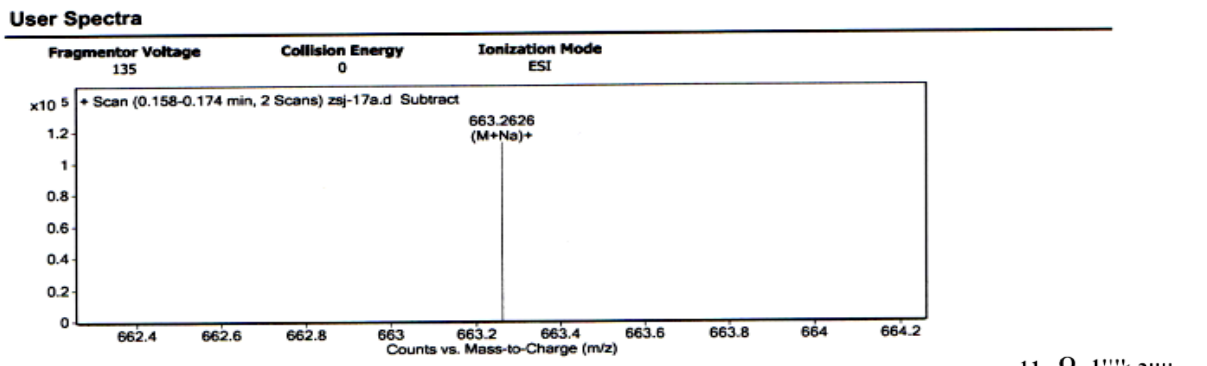


Figure S2. ESIMS of velerivaltrate A (**1**).

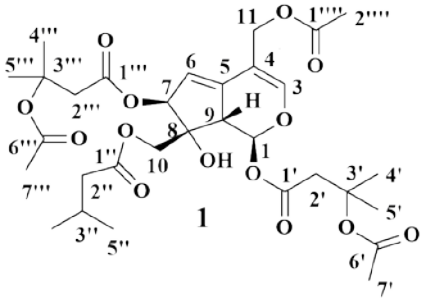


Peak List

m/z	z	Abund	Formula	Ion
658.3068	1	8117.62		
663.2626	1	113270.66	C31 H44 O14	(M+Na)+
664.2657	1	34874.75	C31 H44 O14	(M+Na)+
679.2365	1	92935.27		
680.2396	1	30632.13		
681.239	1	13419.81		

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	20



Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C31 H44 O14	640.2731	663.2623	663.2626	-0.2	-0.3	10.0000

Figure S3. HRESIMS of velerivaltrate A (**1**).

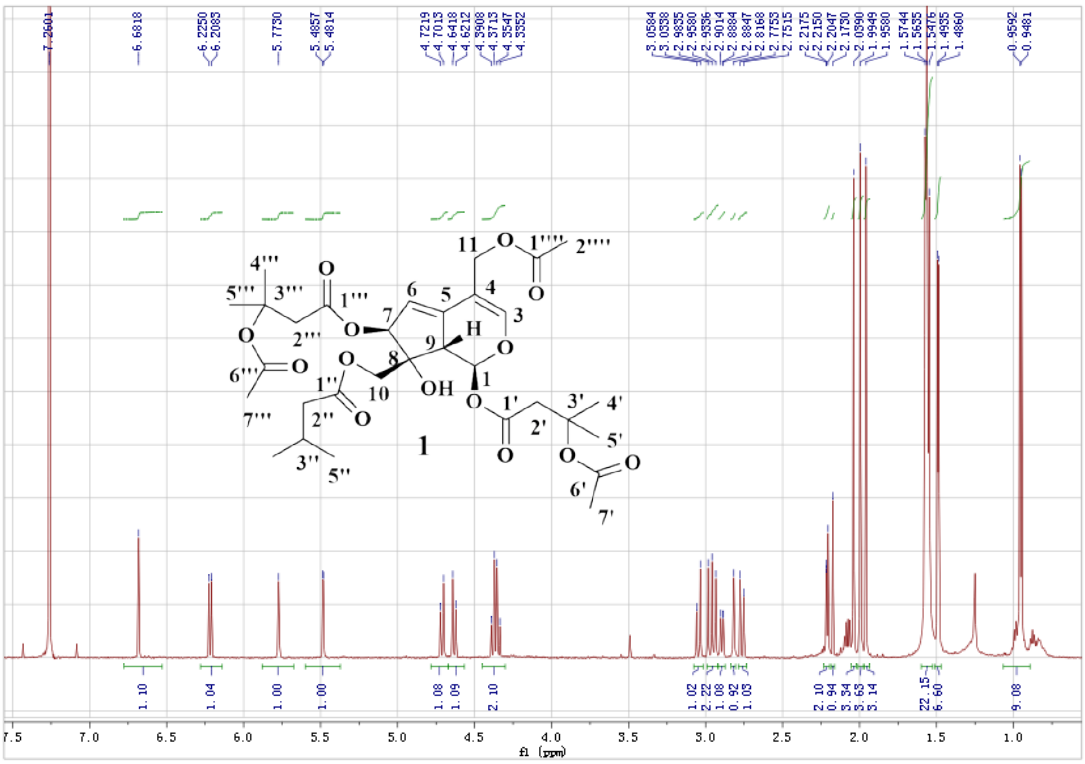


Figure S4. ^{1}H NMR (600 MHz, $CDCl_3$) of velerivaltrate A (**1**).

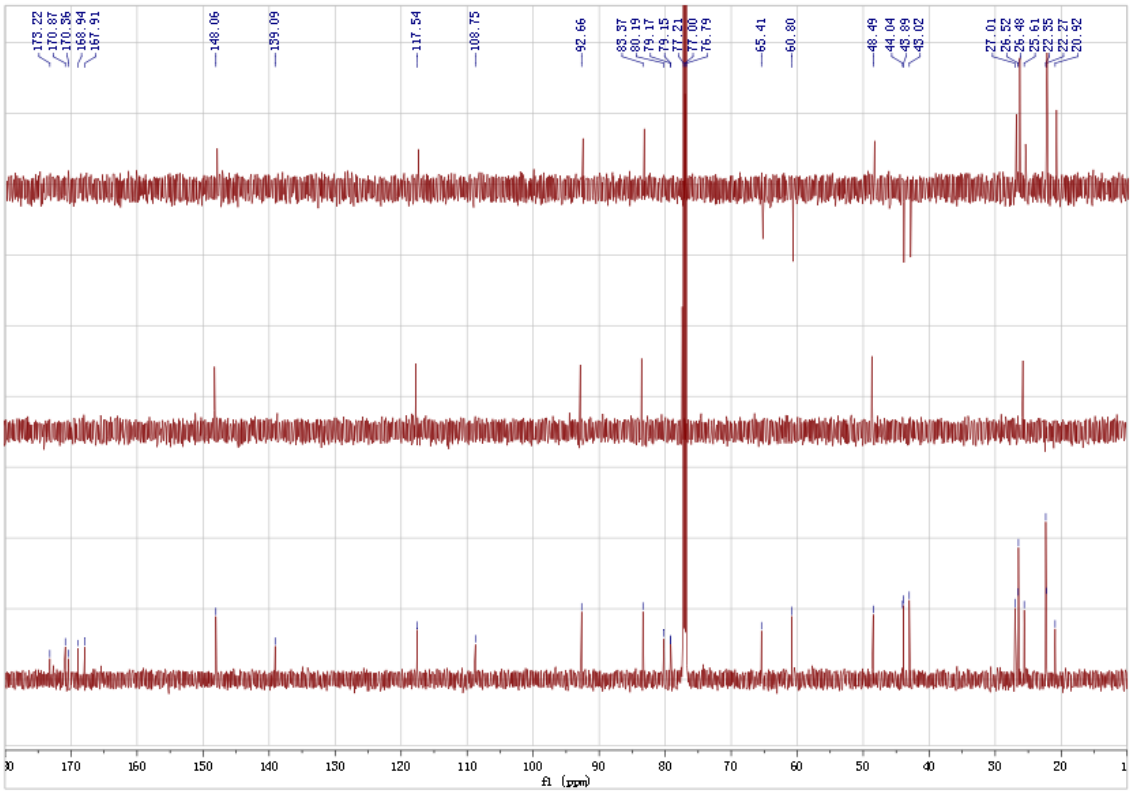


Figure S5. ^{13}C NMR (100 MHz, $CDCl_3$) of velerivaltrate A (**1**).

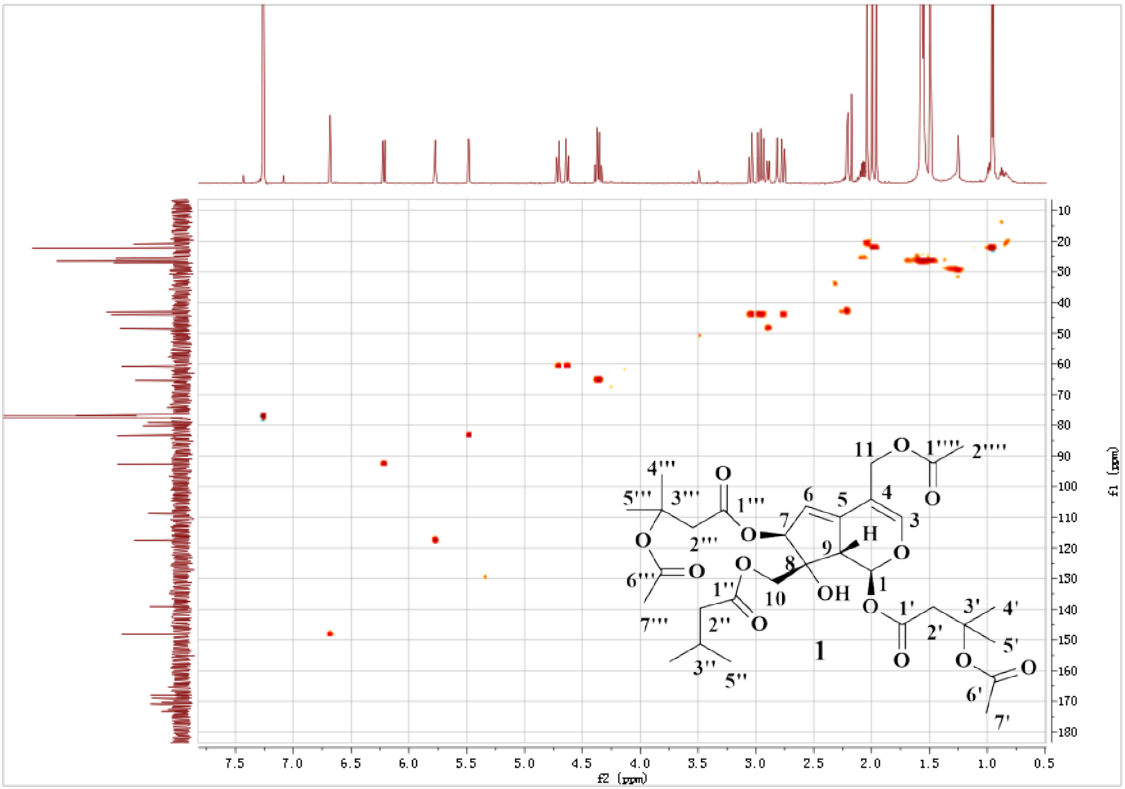


Figure S6. HSQC (600 MHz, $CDCl_3$) of velerivaltrate A (**1**).

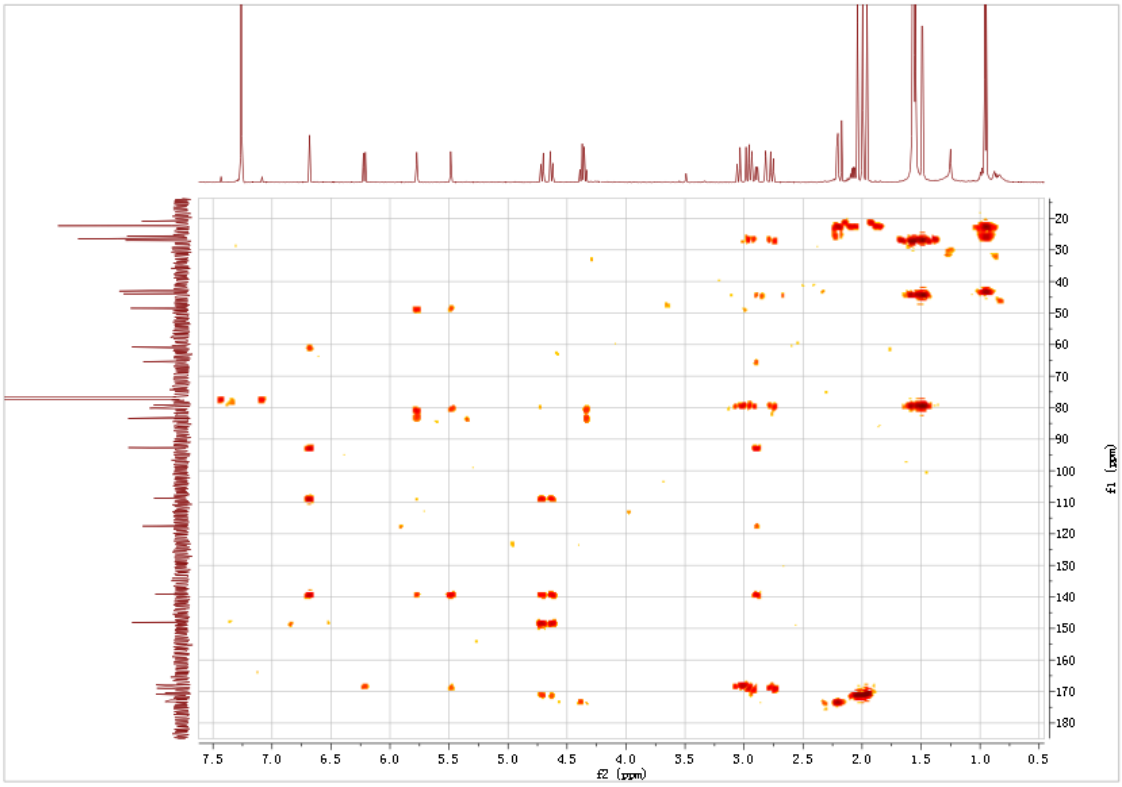


Figure S7. HMBC (600 MHz, $CDCl_3$) of velerivaltrate A (**1**).

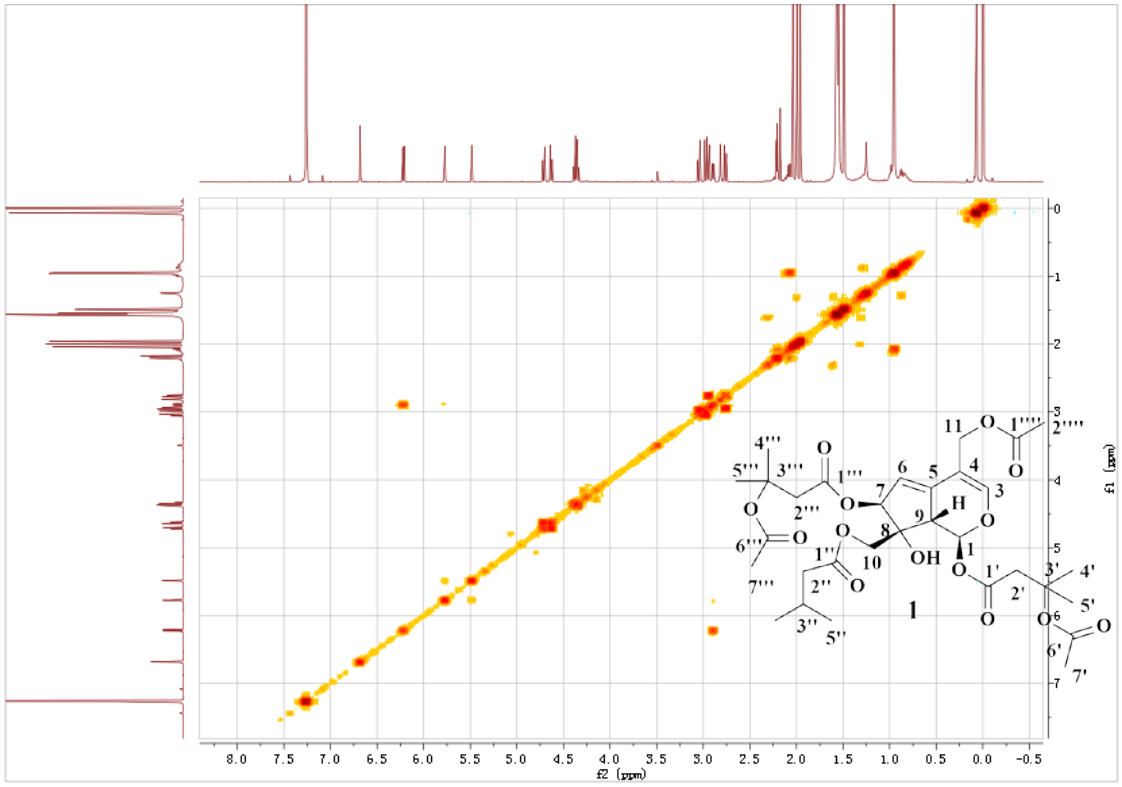


Figure S8. ^{1}H-^{1}H COSY (600 MHz, $CDCl_3$) of velerivaltrate A (**1**).

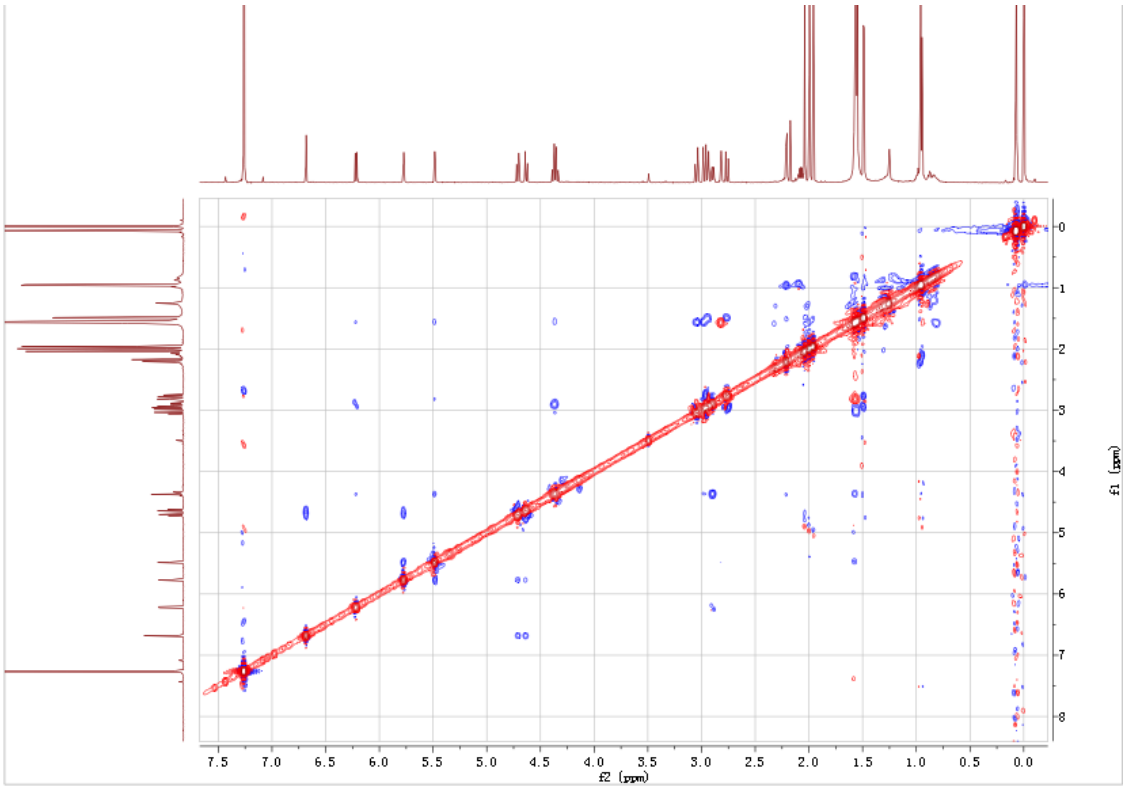


Figure S9. ROESY (600 MHz, $CDCl_3$) of velerivaltrate A (**1**).

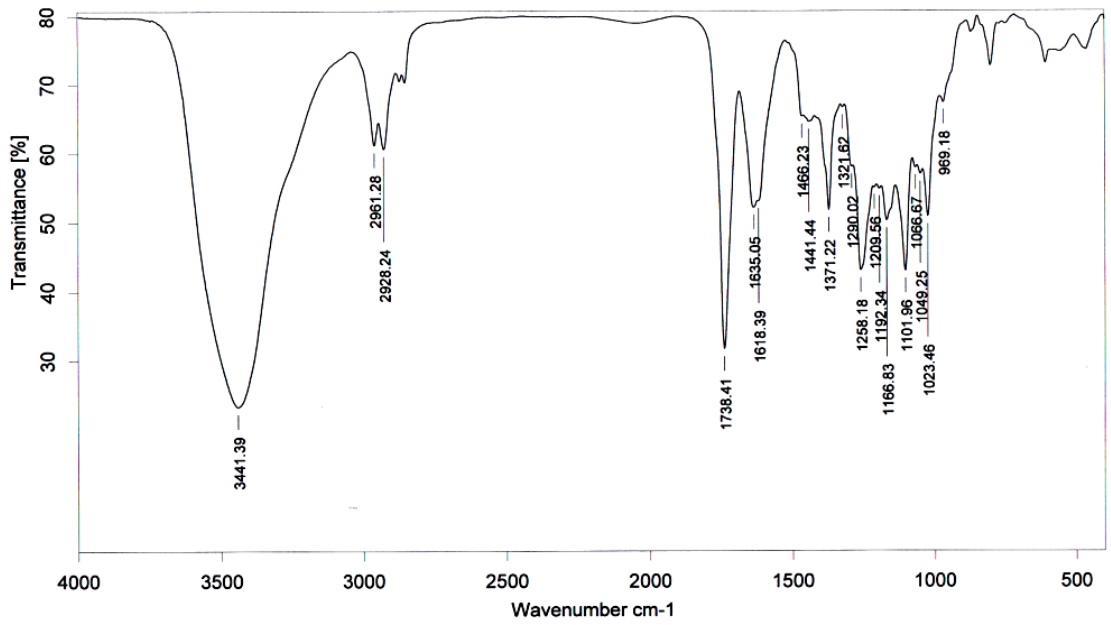


Figure S10. IR spectrum of velerivaltrate A (**1**).

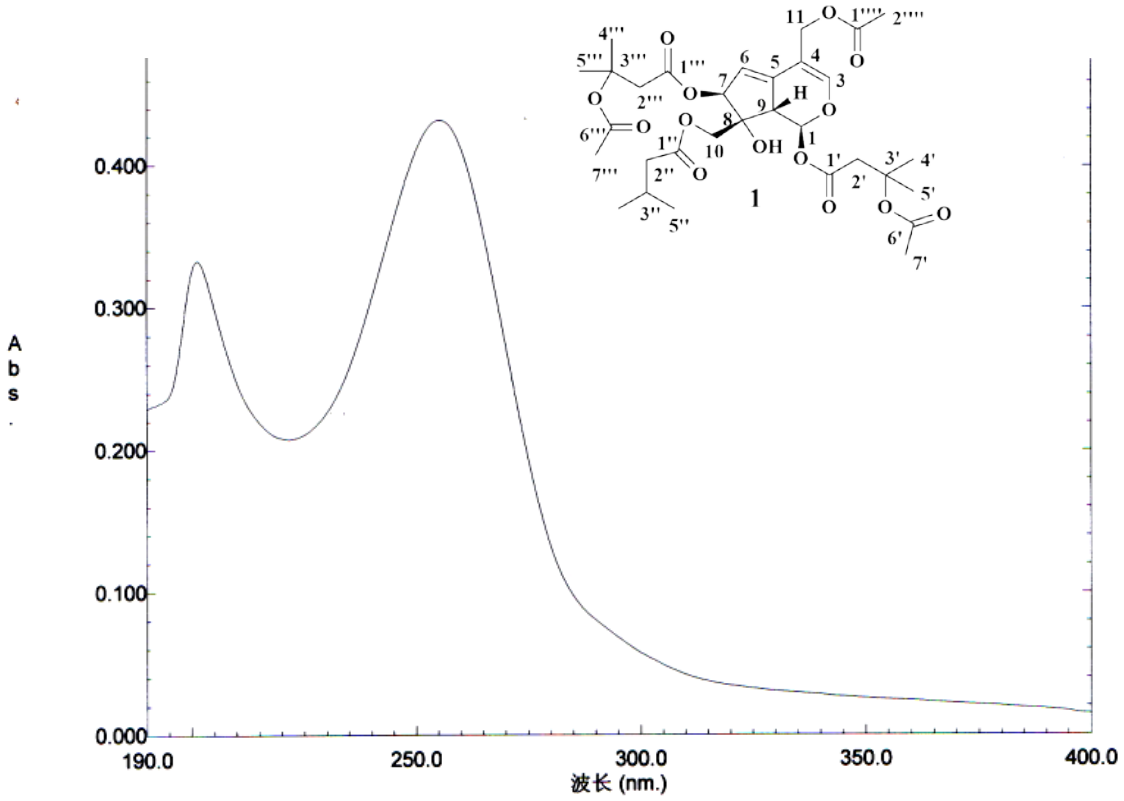


Figure S11. UV spectrum of velerivaltrate A (**1**).

Optical rotation measurement

Model : P-1020 (A060460638)

No.	Sample	Mode	Data	Monitor Blank	Temp. Cell Temp Point	Date Comment Sample Name	Light Filter Operator	Cycle Time Integ Time
No.1	6 (1/3)	Sp.Rot	59.7560	0.0049 0.0000	23.6 10.00 Cell	Tue Mar 17 20:16:57 2015 0.00082g/mL MeOH ZSJ-17A	Na 589nm	2 sec 10 sec
No.2	6 (2/3)	Sp.Rot	53.6590	0.0044 0.0000	23.6 10.00 Cell	Tue Mar 17 20:17:10 2015 0.00082g/mL MeOH ZSJ-17A	Na 589nm	2 sec 10 sec
No.3	6 (3/3)	Sp.Rot	57.3170	0.0047 0.0000	23.6 10.00 Cell	Tue Mar 17 20:17:24 2015 0.00082g/mL MeOH ZSJ-17A	Na 589nm	2 sec 10 sec
No.4	7 (1/3)	Sp.Rot	64.6340	0.0053 0.0000	23.5 10.00 Cell	Tue Mar 17 20:20:53 2015 0.00082g/mL MeOH ZSJ-17A	Na 589nm	2 sec 10 sec
No.5	7 (2/3)	Sp.Rot	62.1950	0.0051 0.0000	23.5 10.00 Cell	Tue Mar 17 20:21:07 2015 0.00082g/mL MeOH ZSJ-17A	Na 589nm	2 sec 10 sec
No.6	7 (3/3)	Sp.Rot	59.7560	0.0049 0.0000	23.5 10.00 Cell	Tue Mar 17 20:21:20 2015 0.00082g/mL MeOH ZSJ-17A	Na 589nm	2 sec 10 sec

+59.773°

Figure S12. $[a]_D$ spectrum of velerivaltrate A (**1**).

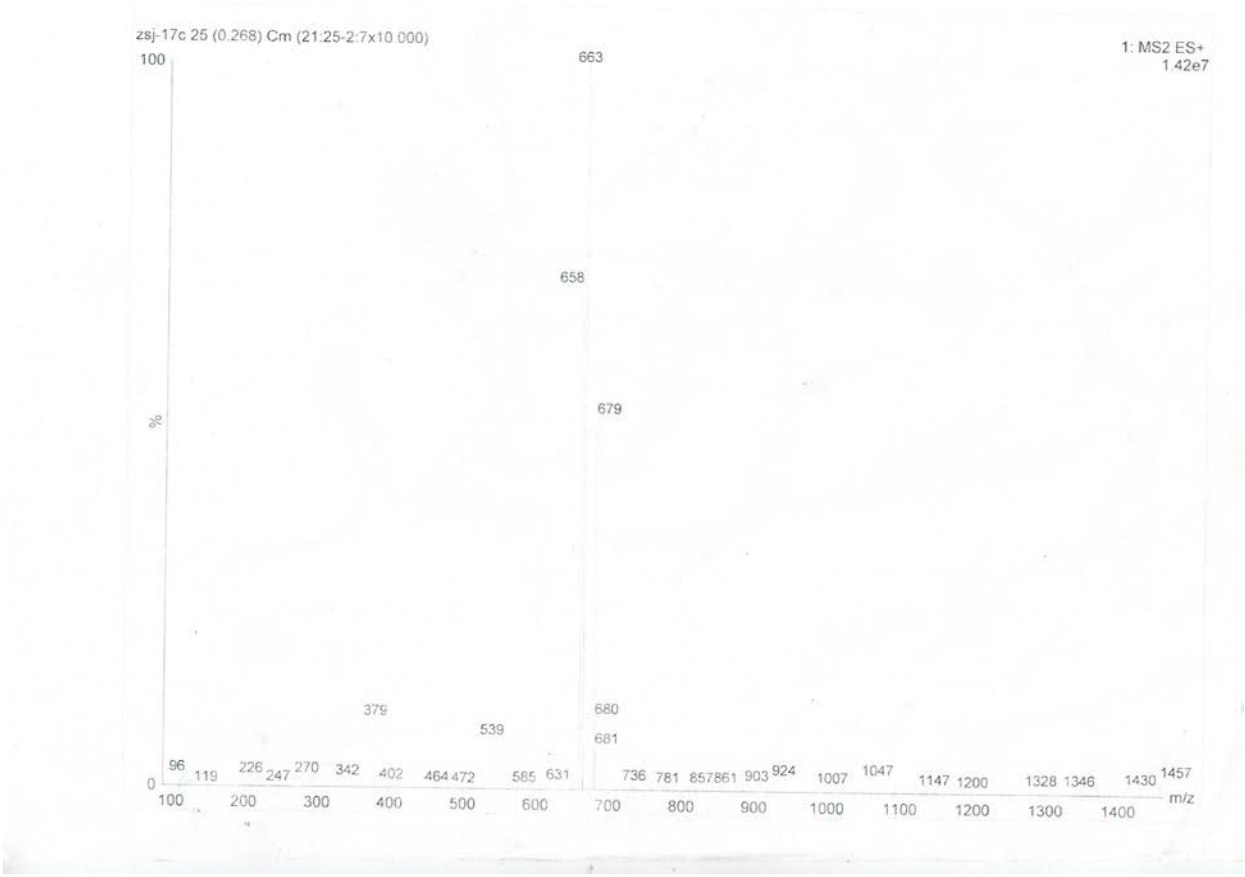
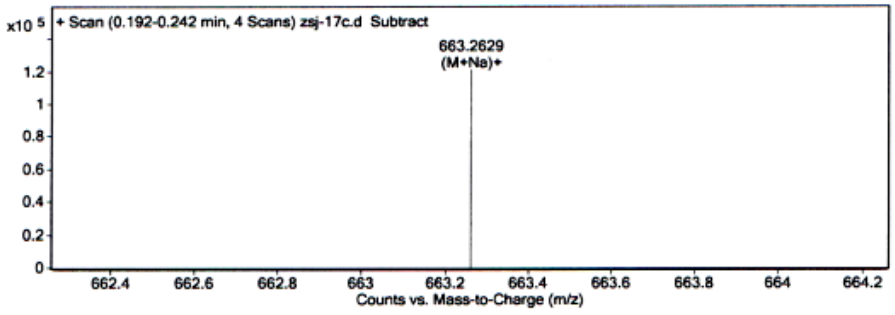


Figure S13. ESIMS of velerivaltrate B (**2**).

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	ESI

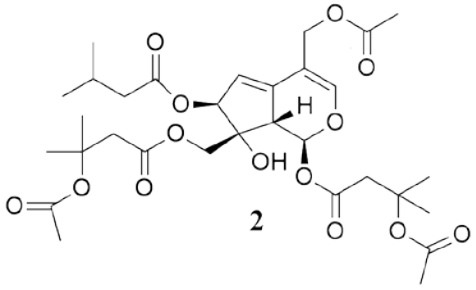


Peak List

m/z	z	Abund	Formula	Ion
98.9757		15997.56		
658.3077	1	33192.18		
663.2629	1	121395.2	C31 H44 O14	(M+Na)+
664.2662	1	39953.23	C31 H44 O14	(M+Na)+
679.237	1	69500.72		
680.2405	1	22352.55		

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	30



Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C31 H44 O14	640.2731	663.2623	663.2629	-0.6	-0.9	10.0000

Figure S14. HRESIMS of velerivaltrate B (**2**).

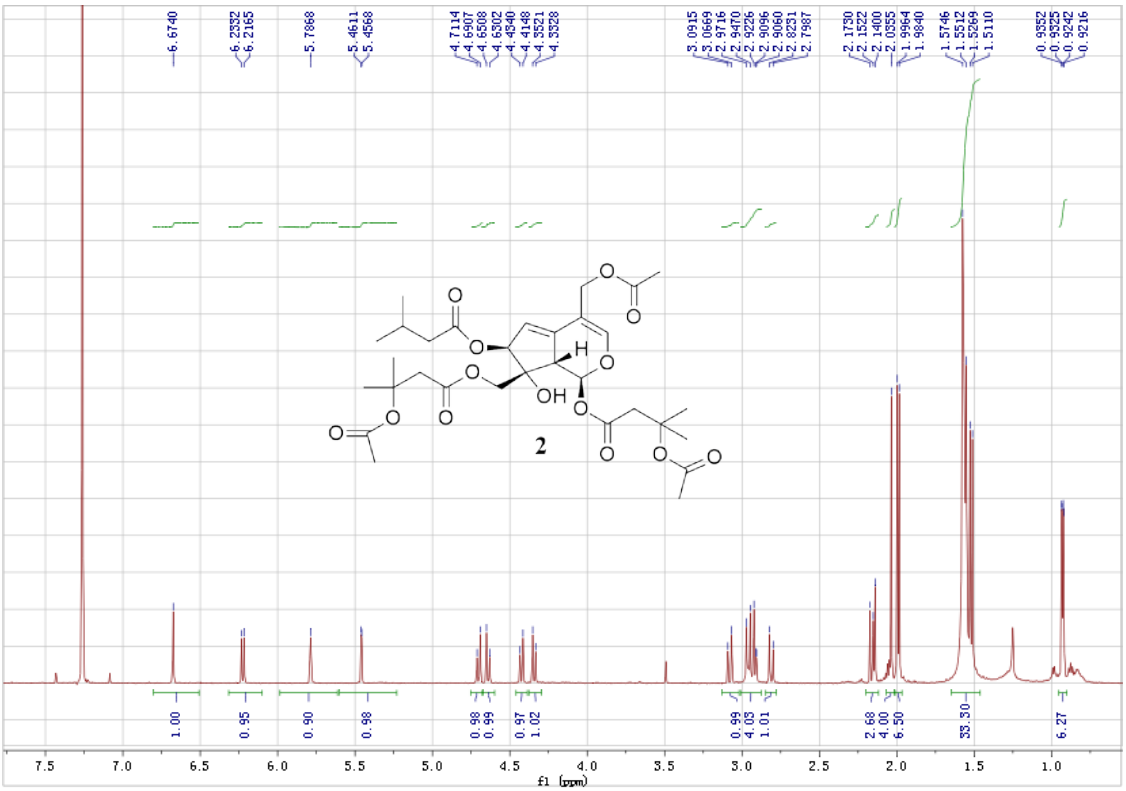


Figure S15. ^{1}H NMR (600 MHz, $CDCl_3$) of velerivaltrate B (**2**).

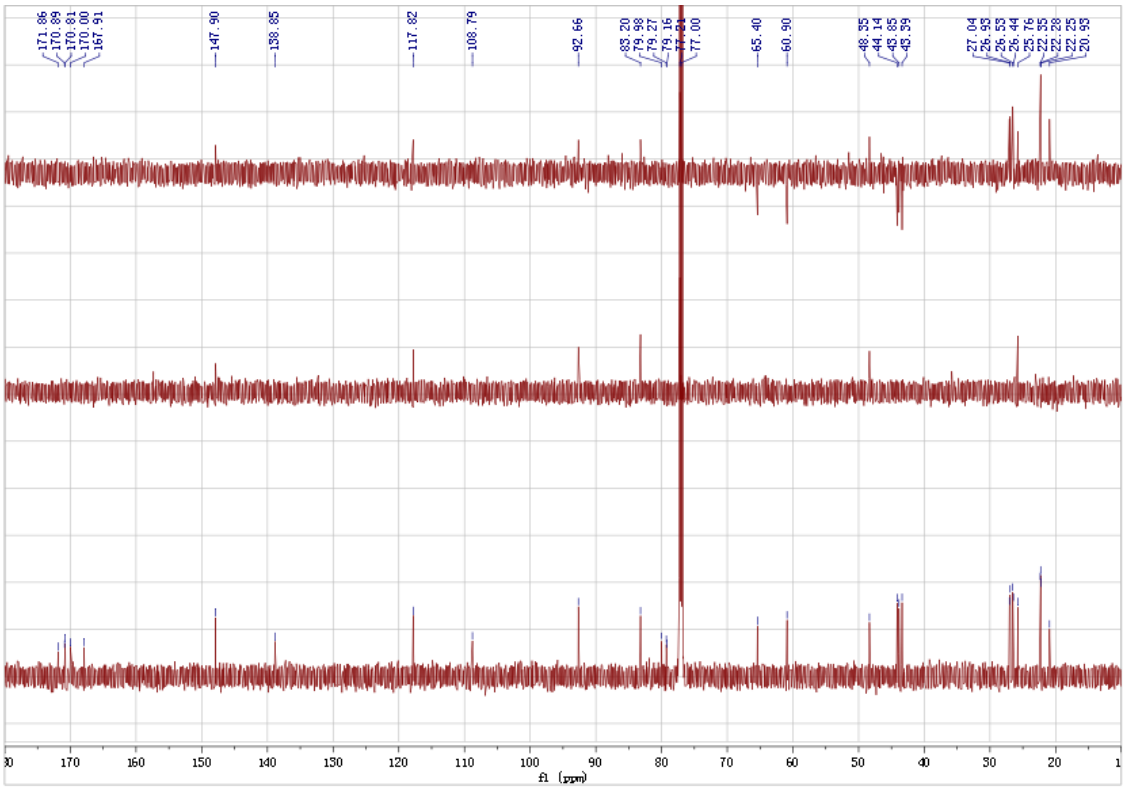


Figure S16. ^{13}C NMR (100 MHz, $CDCl_3$) of velerivaltrate B (**2**).

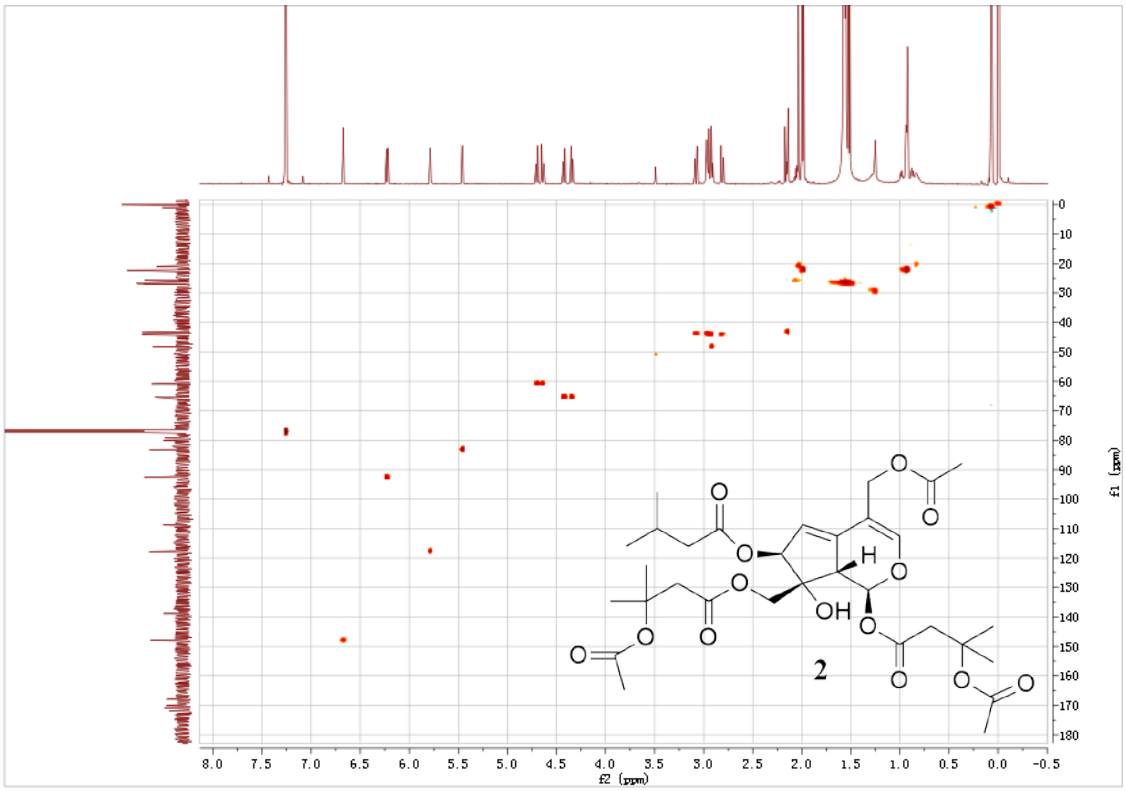


Figure S17. HSQC (600 MHz, $CDCl_3$) of velerivaltrate B (**2**).

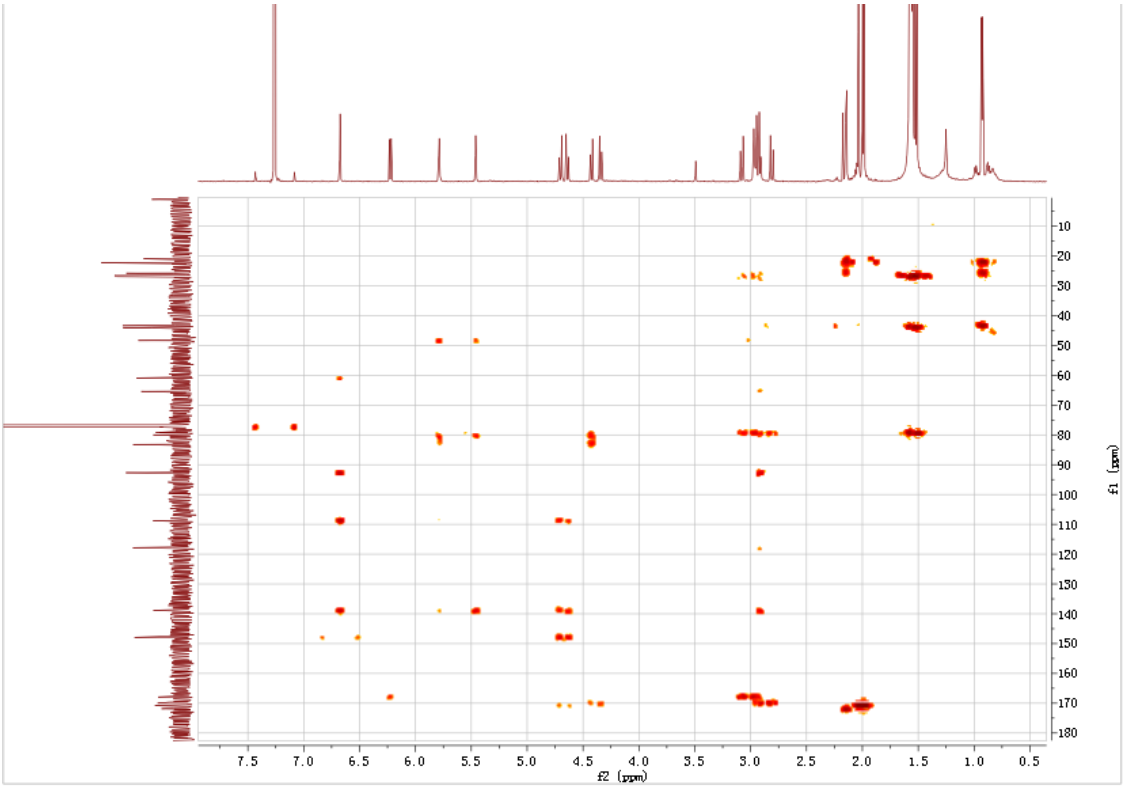


Figure S18. HMBC (600 MHz, $CDCl_3$) of velerivaltrate B (**2**).

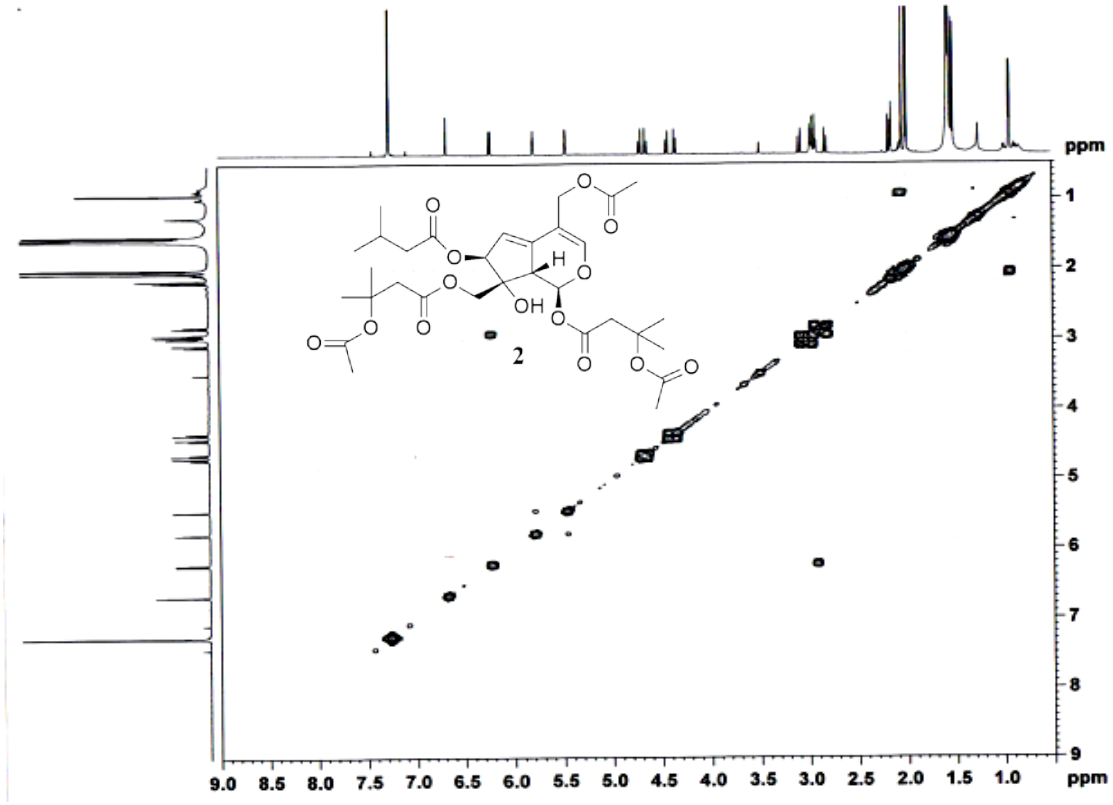


Figure S19. ^{1}H-^{1}H COSY (600 MHz, $CDCl_3$) of velerivaltrate B (**2**).

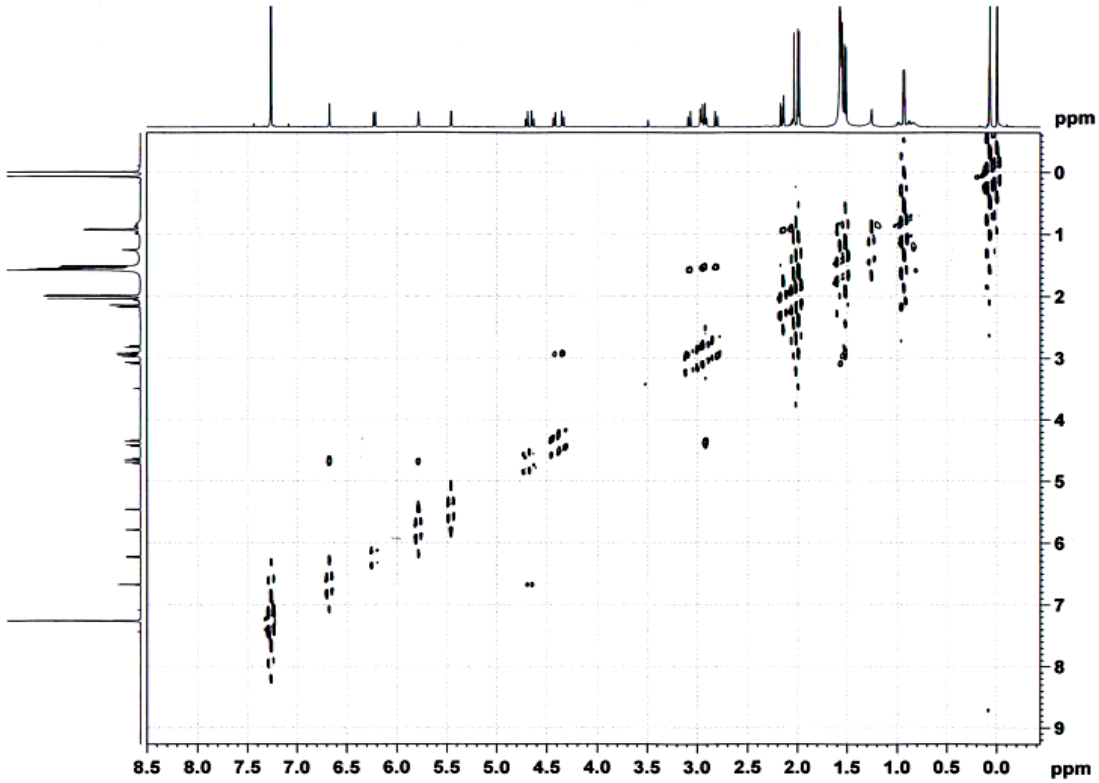


Figure S20. ROESY (600 MHz, $CDCl_3$) of velerivaltrate B (**2**).

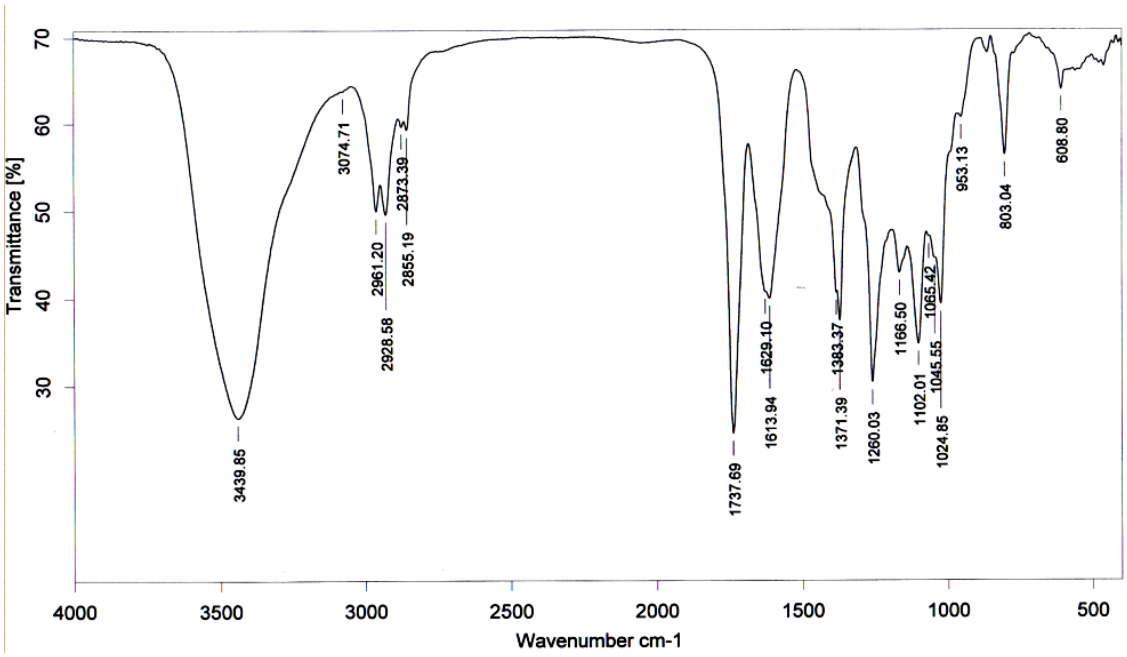


Figure S21. IR spectrum of velerivaltrate B (**2**).

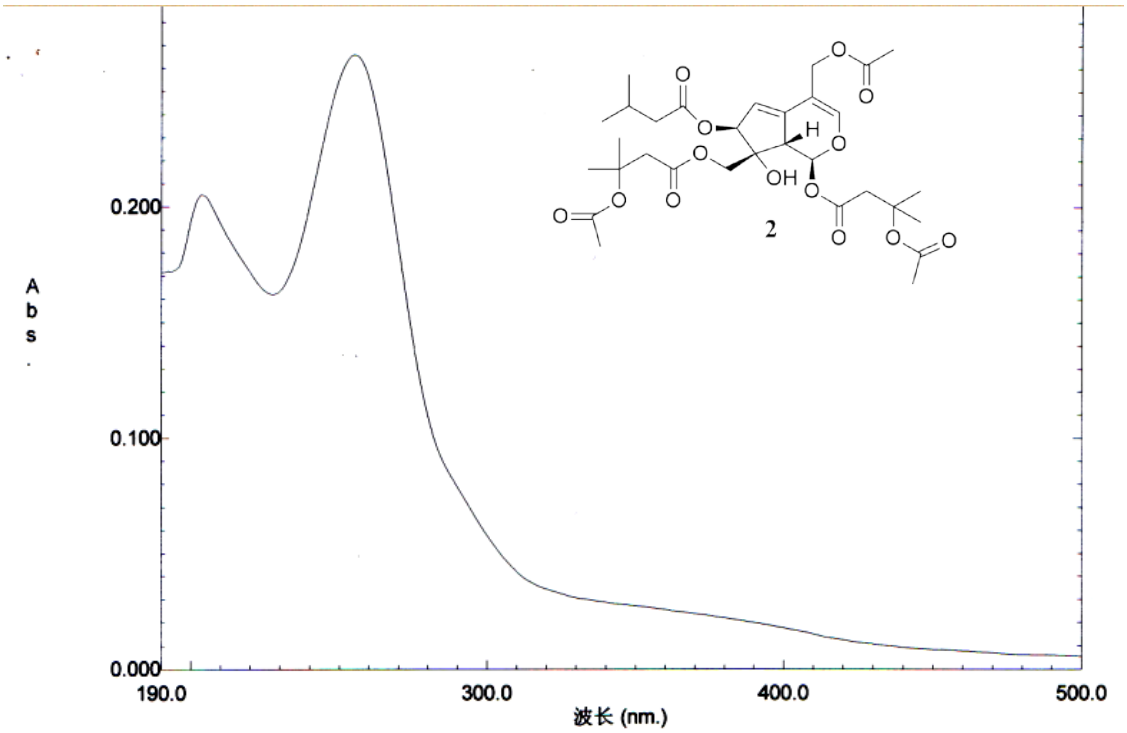


Figure S22. UV spectrum of velerivaltrate B (**2**).

Optical rotation measurement

Model : P-1020 (A060460638)

No.	Sample	Mode	Data	Monitor Blank	Temp. Cell Temp Point	Date Comment Sample Name	Light Filter Operator	Cycle Time Integ Time
No.1	13 (1/3)	Sp.Rot	38.8060	0.0052 0.0000	23.3 10.00 Cell	Fri Mar 20 19:46:49 2015 0.00134g/mL MeOH ZSJ-17C	Na 589nm	2 sec 10 sec
No.2	13 (2/3)	Sp.Rot	35.8210	0.0048 0.0000	23.3 10.00 Cell	Fri Mar 20 19:47:02 2015 0.00134g/mL MeOH ZSJ-17C	Na 589nm	2 sec 10 sec
No.3	13 (3/3)	Sp.Rot	36.5670	0.0049 0.0000	23.3 10.00 Cell	Fri Mar 20 19:47:16 2015 0.00134g/mL MeOH ZSJ-17C	Na 589nm	2 sec 10 sec

+3).064)

Figure S23. $[a]_D$ spectrum of velerivaltrate B (**2**).

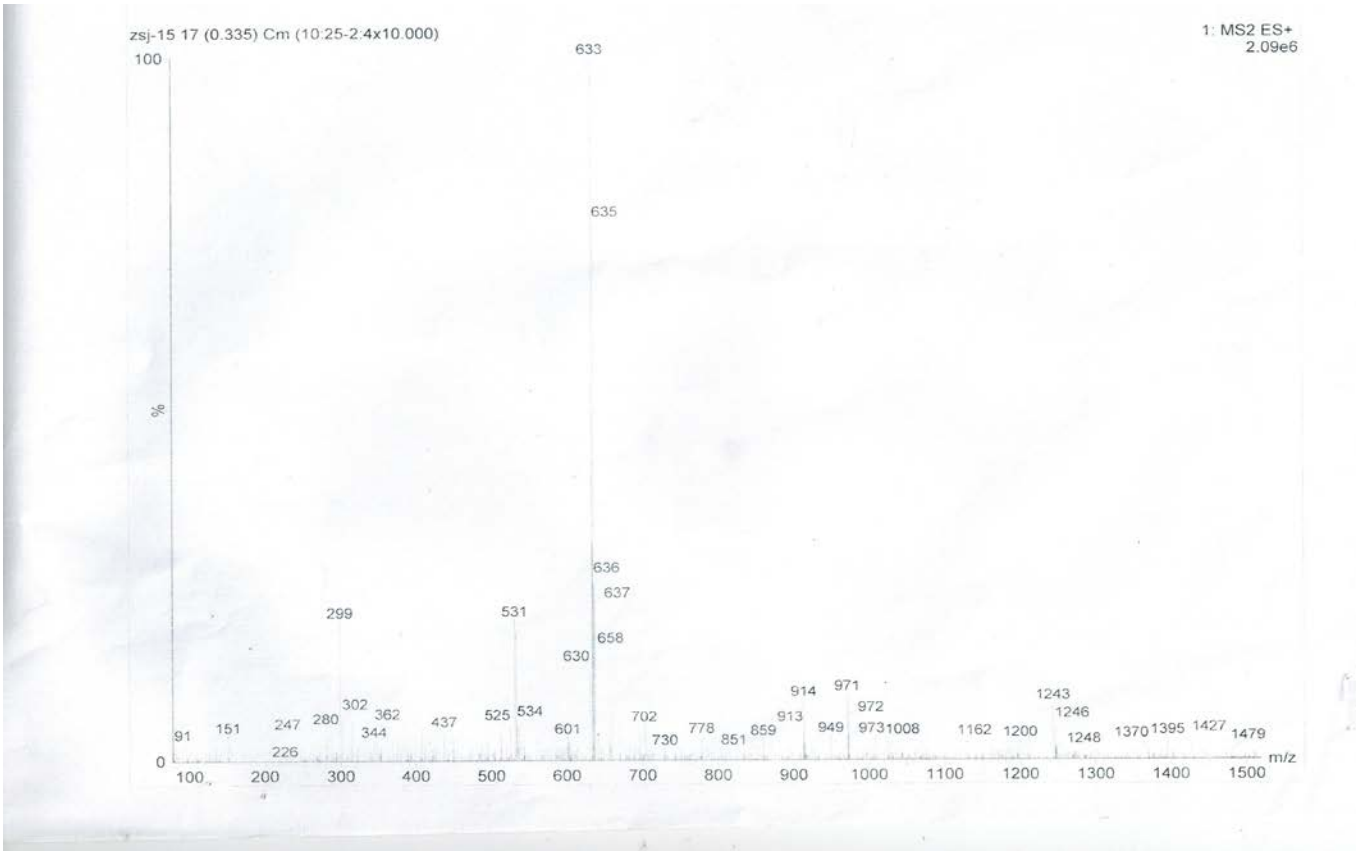
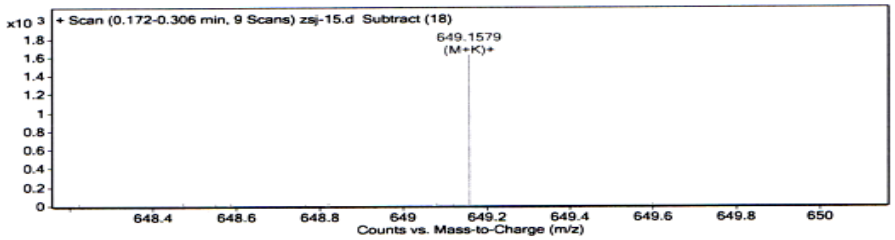


Figure S24. ESIMS of velerivaltrate C (**3**).

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	ESI

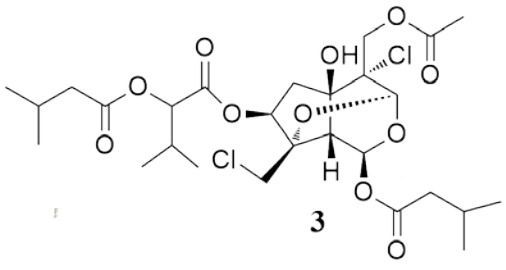


Peak List

m/z	z	Abund	Formula	Ion
158.0389		681.6		
177.0138		965.13		
338.0772		493.73		
412.0962		664.48		
427.0681		1032.35		
503.2979	1	849.02		
520.2757		596.98		
633.1851	1	838.37		
649.1579		1622.7	C27 H40 Cl2 O11	(M+K)+
651.1561		1003.32		

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	15
N	0	10
Cl	0	5



Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C27 H40 Cl2 O11	610.1948	649.1579	649.1579	0.1	0.1	7.0000

Figure S25. HRESIMS of velerivaltrate C (**3**).

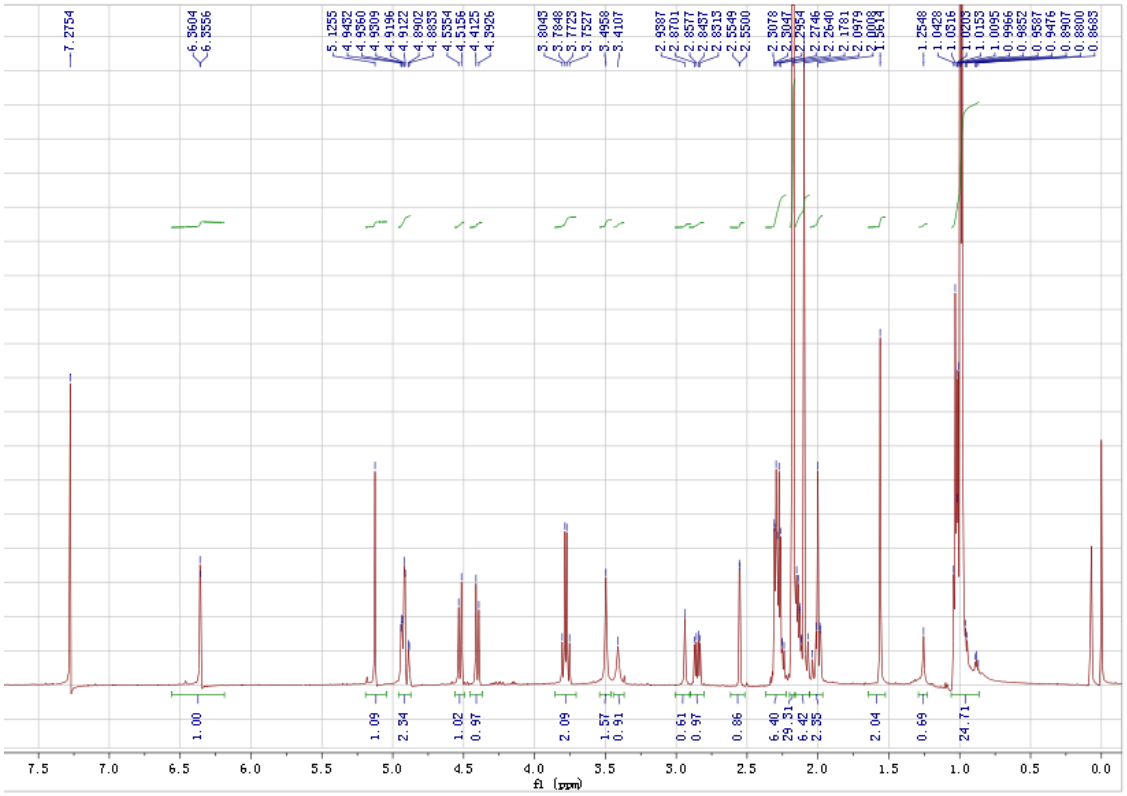


Figure S26. ^{1}H NMR (600 MHz, $CDCl_3$) of velerivaltrate C (**3**).

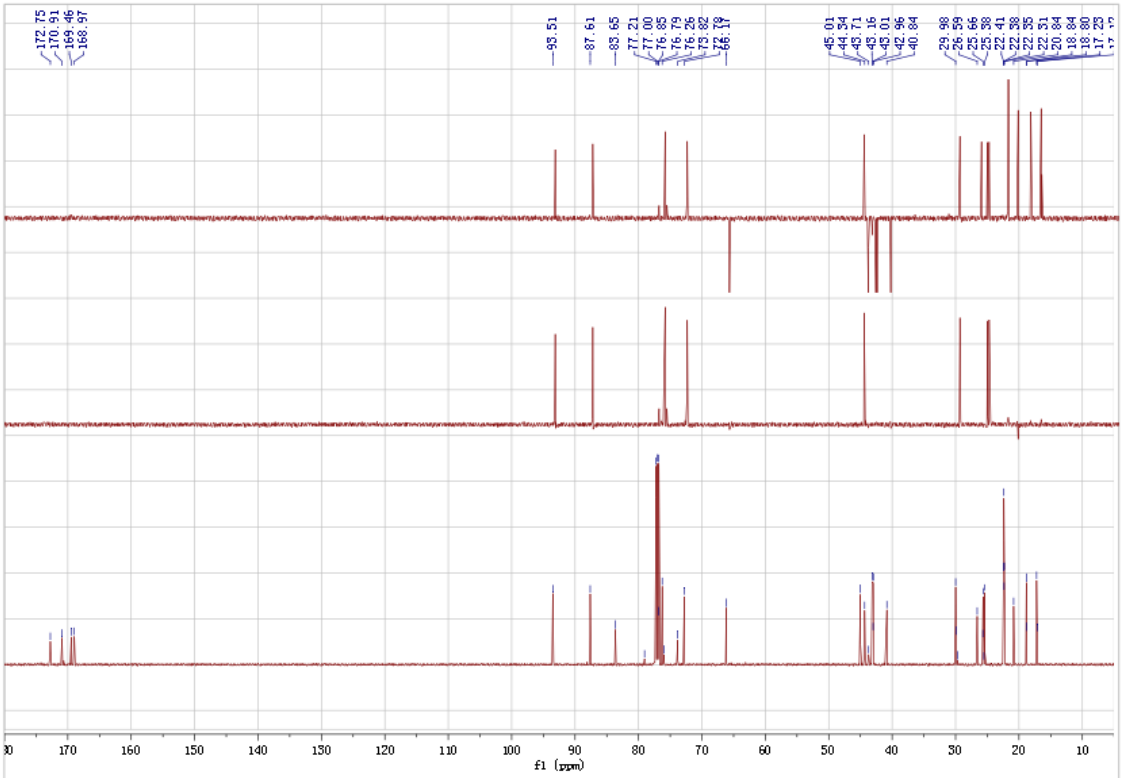


Figure S27. ^{13}C NMR (100 MHz, $CDCl_3$) of velerivaltrate C (**3**).

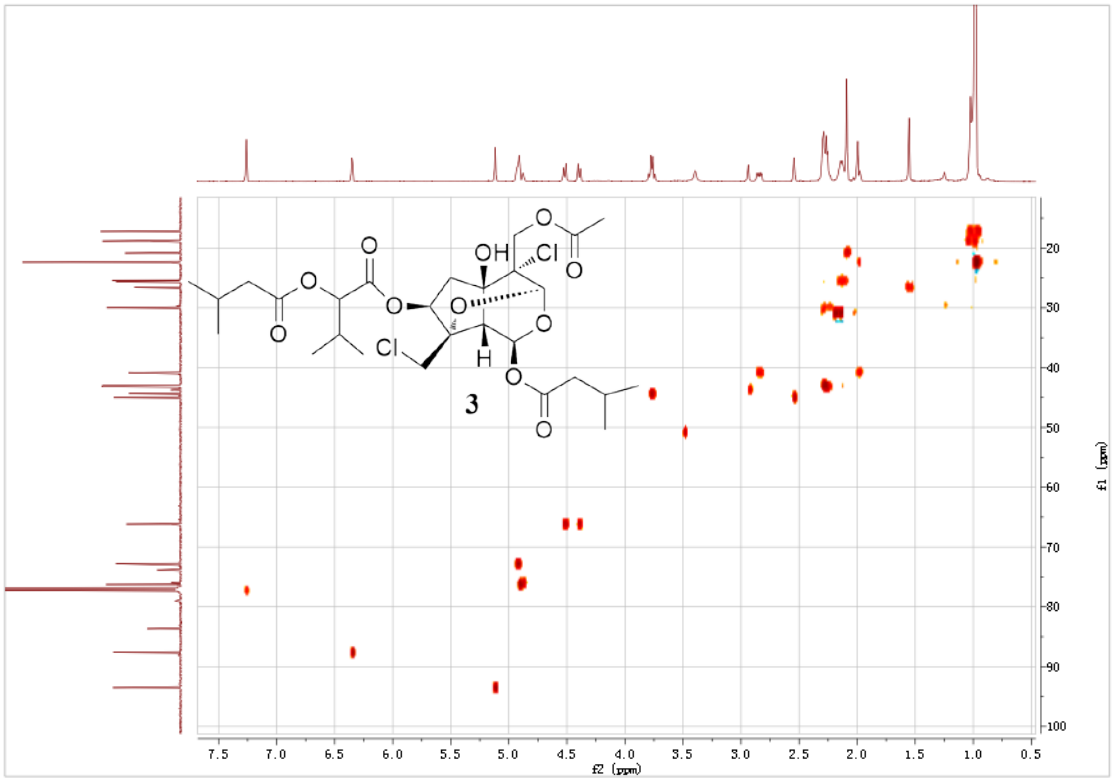


Figure S28. HSQC (600 MHz, $CDCl_3$) of velerivaltrate C (**3**).

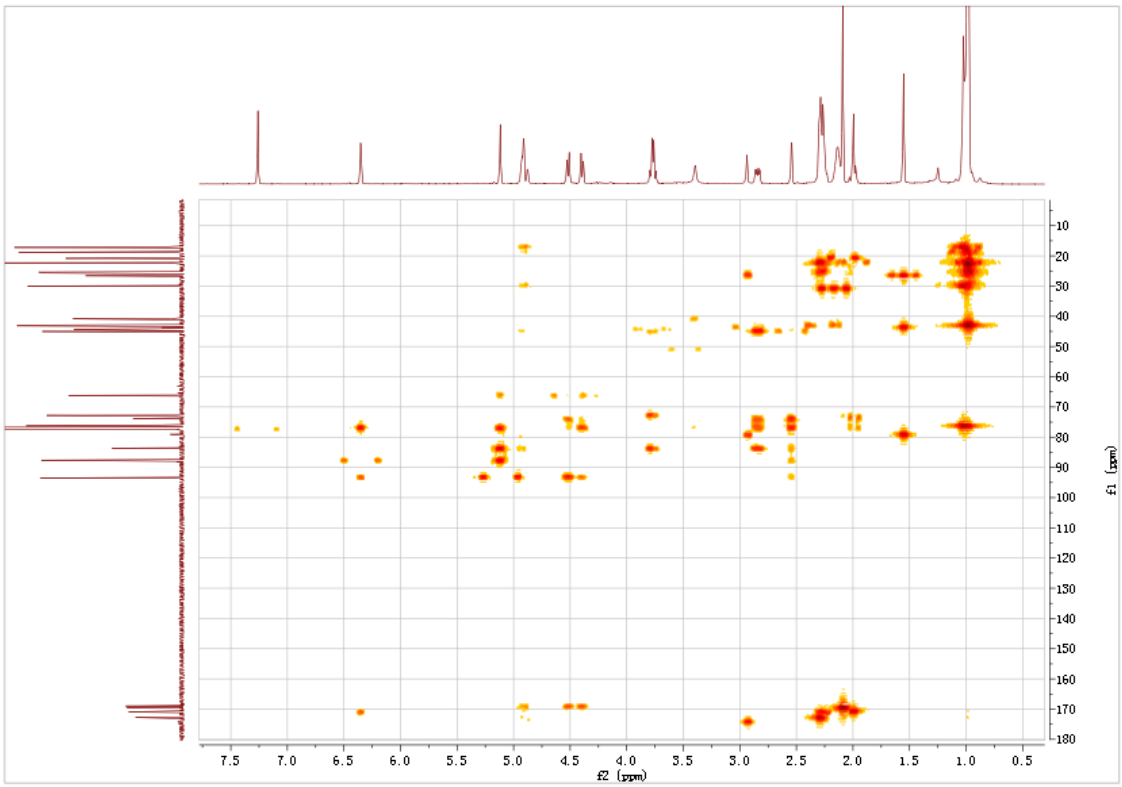


Figure S29. HMBC (600 MHz, $CDCl_3$) of velerivaltrate C (**3**).

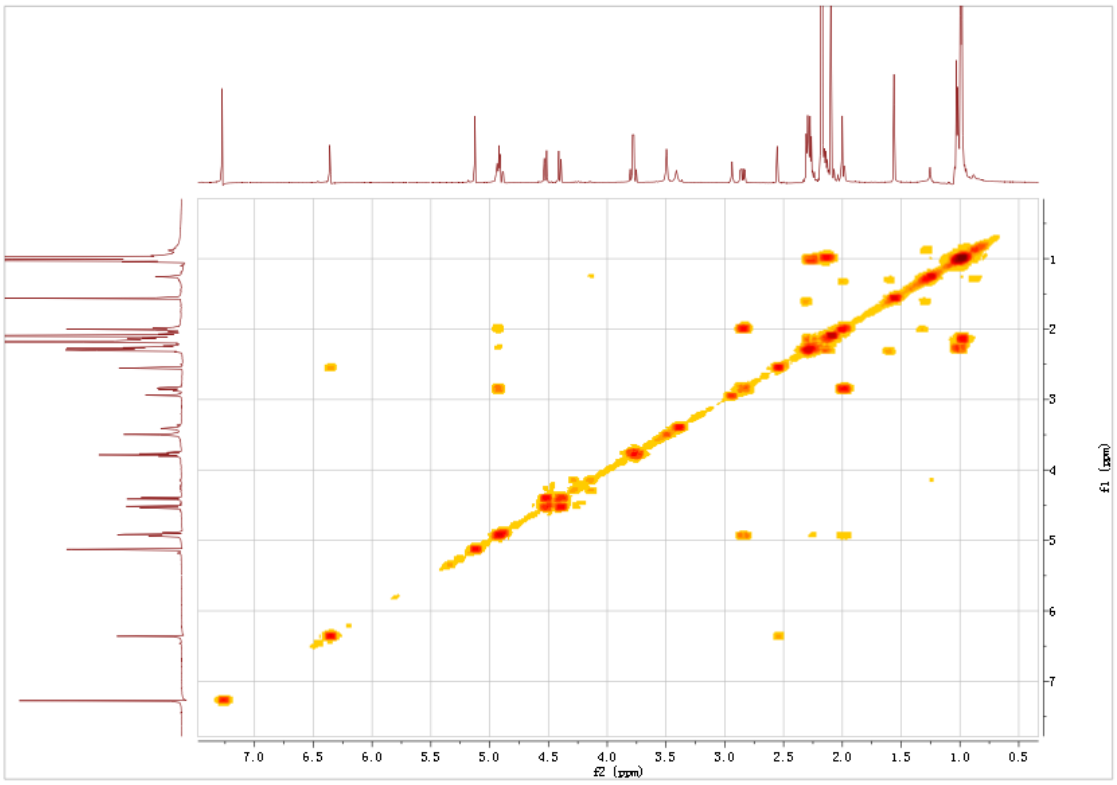


Figure S30. ^{1}H-^{1}H COSY (600 MHz, $CDCl_3$) of velerivaltrate C (**3**).

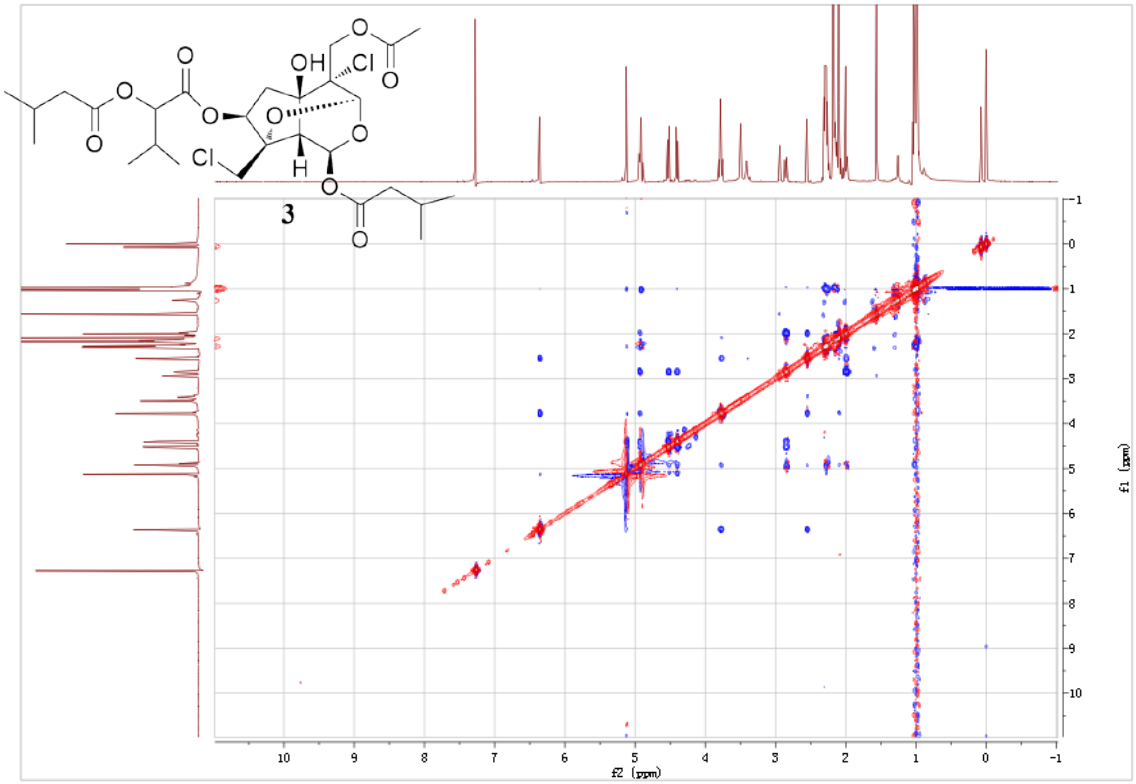


Figure S31. ROESY (600 MHz, $CDCl_3$) of velerivaltrate C (**3**).

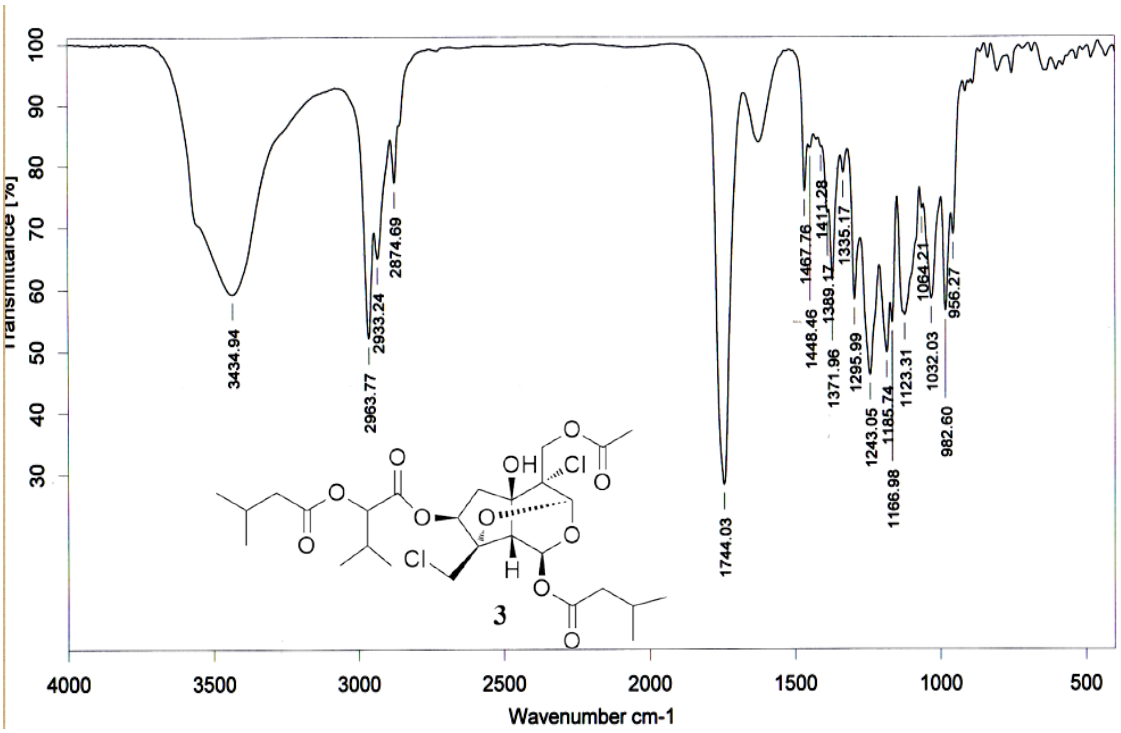


Figure S32. IR spectrum of velerivaltrate C (**3**).

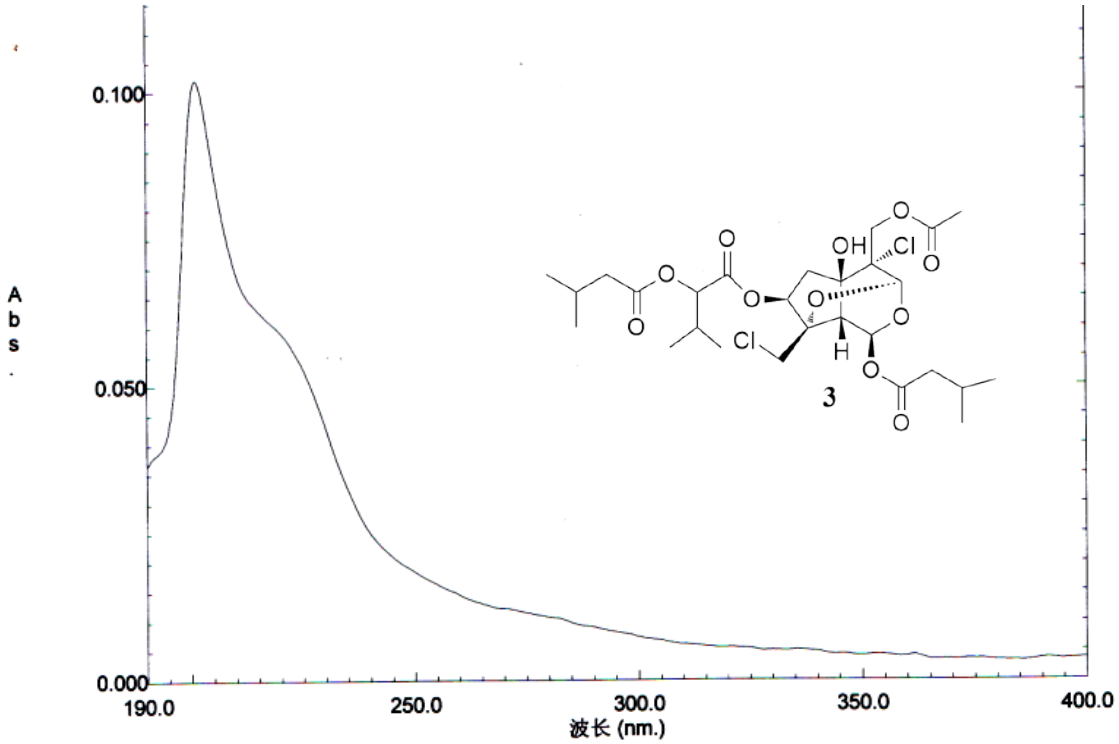


Figure S33. UV spectrum of velerivaltrate C (**3**).

Optical rotation measurement

Model : P-1020 (A060460638)

No.	Sample	Mode	Data	Monitor Blank	Temp. Cell Temp Point	Date Comment Sample Name	Light Filter Operator	Cycle Time Integ Time
No.1	5 (1/3)	Sp.Rot	29.1080	0.0093 0.0000	23.6 10.00 Cell	Tue Mar 17 20:09:36 2015 0.00320g/mL MeOH ZSJ-15	Na 589nm	2 sec 10 sec
No.2	5 (2/3)	Sp.Rot	29.4210	0.0094 0.0000	23.6 10.00 Cell	Tue Mar 17 20:09:50 2015 0.00320g/mL MeOH ZSJ-15	Na 589nm	2 sec 10 sec
No.3	5 (3/3)	Sp.Rot	31.6120	0.0101 0.0000	23.6 10.00 Cell	Tue Mar 17 20:10:03 2015 0.00320g/mL MeOH ZSJ-15	Na 589nm	2 sec 10 sec

+30.046°

Figure S34. $[a]_D$ spectrum of velerivaltrate C (**3**).

Table S1. Dose-related effects of compounds **1**, **6**, **7**, **11**, and **12** on peak currents of $Ca_v2.2$.

compounds	concentration (μM)	Inhibitory ratio (%)
Blank control	0.1% DMSO	1.2±0.8
1	1	7.3±3.1*
	3	21.7±7. 3*
	10	55.9±3.3*
	30	59.9±5.2*
	60	64.3±3.9**
6	0.6	9.5±2.5*
	1	23.3±2.9**
	3	38.2±2.8**
	10	56.8±5.7**
	30	61.1±7.8*
7	0.3	2.5±1.4
	1	30.6±4.4**
	3	51.4±8.4**
	10	59.3±5.4*
	30	57.2±7.1*
11	0.3	3.6±2.10
	1	9.5±0.9*
	3	36.1±1.9**
	10	63.1±1.0**
	30	63.9±3.9**
12	0.6	3.9±2.7
	3	17.7±7.7*
	10	37.0±6.9**
	30	61.8±6.7**
	60	47.9±1.7**

All the data were analyzed with two-tailed student T test and represented as mean ± *SEM* (n=3) compared with the blank control, *$P < 0.01$, **$P < 0.01$.

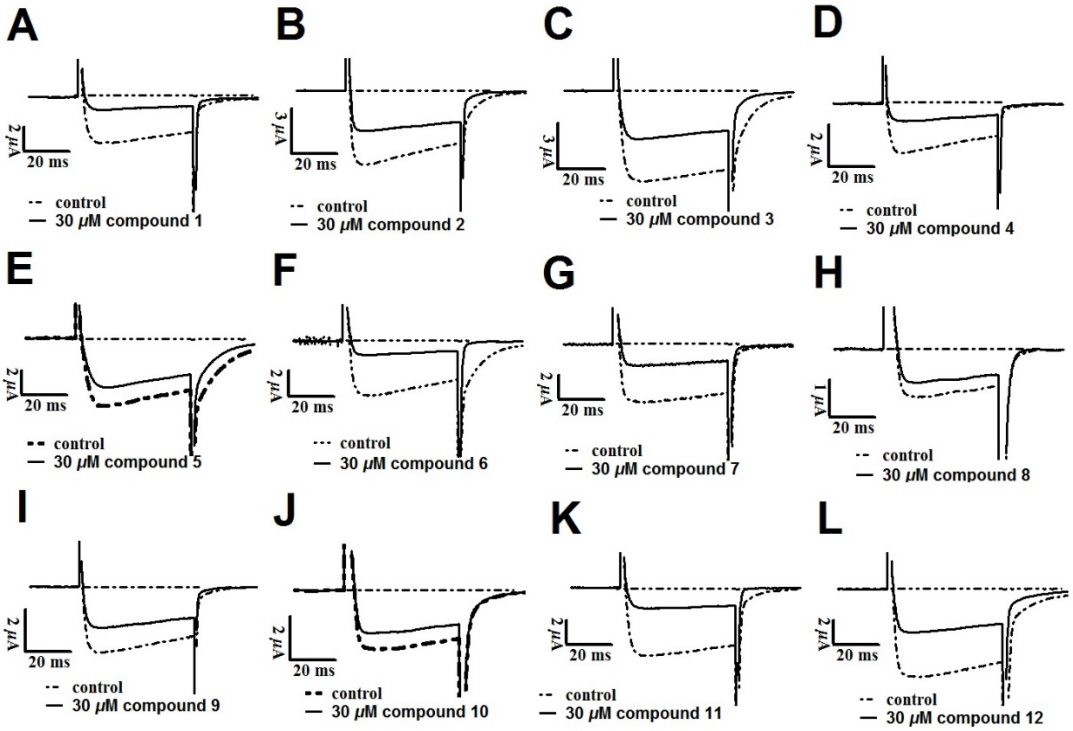


Figure S35. Inhibitory effects of compounds **1-12** on $Ca_v2.2$ at 30 μM. Representative peak current of $Ca_v2.2$ were evoked from a holding potential (HP) of -80 mV by 50 ms depolarization to + 20 mV at 3 s intervals in the absence or presence of the indicated compounds.

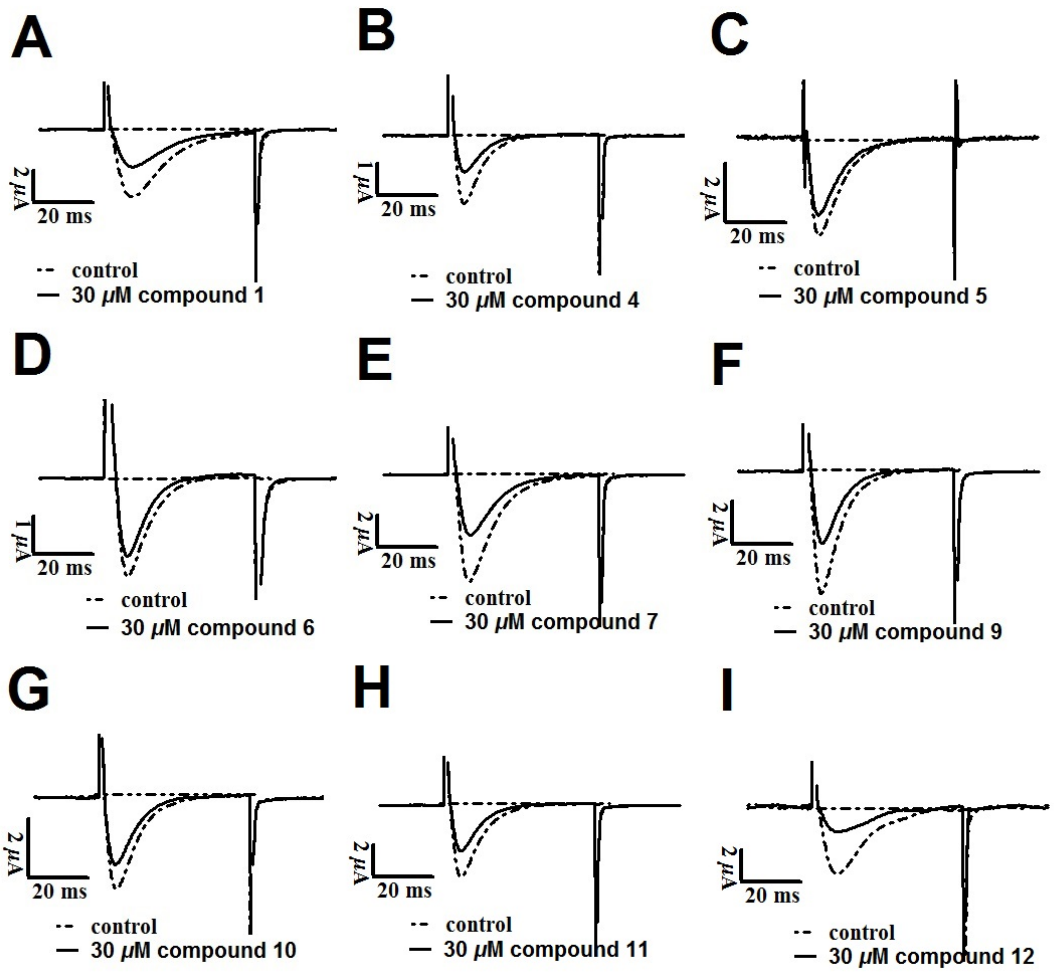


Figure S36. Inhibitory effects of compounds **1**, **4**, **5**, **6**, **7**, **9**, **10**, **11,** and **12** on $Ca_v3.1$ at 30 μM. Representative peak current of $Ca_v3.1$ were evoked from a holding potential (HP) of -80 mV by 50 ms depolarization to -10 mV at 3 s intervals in the absence or presence of the indicated compounds.

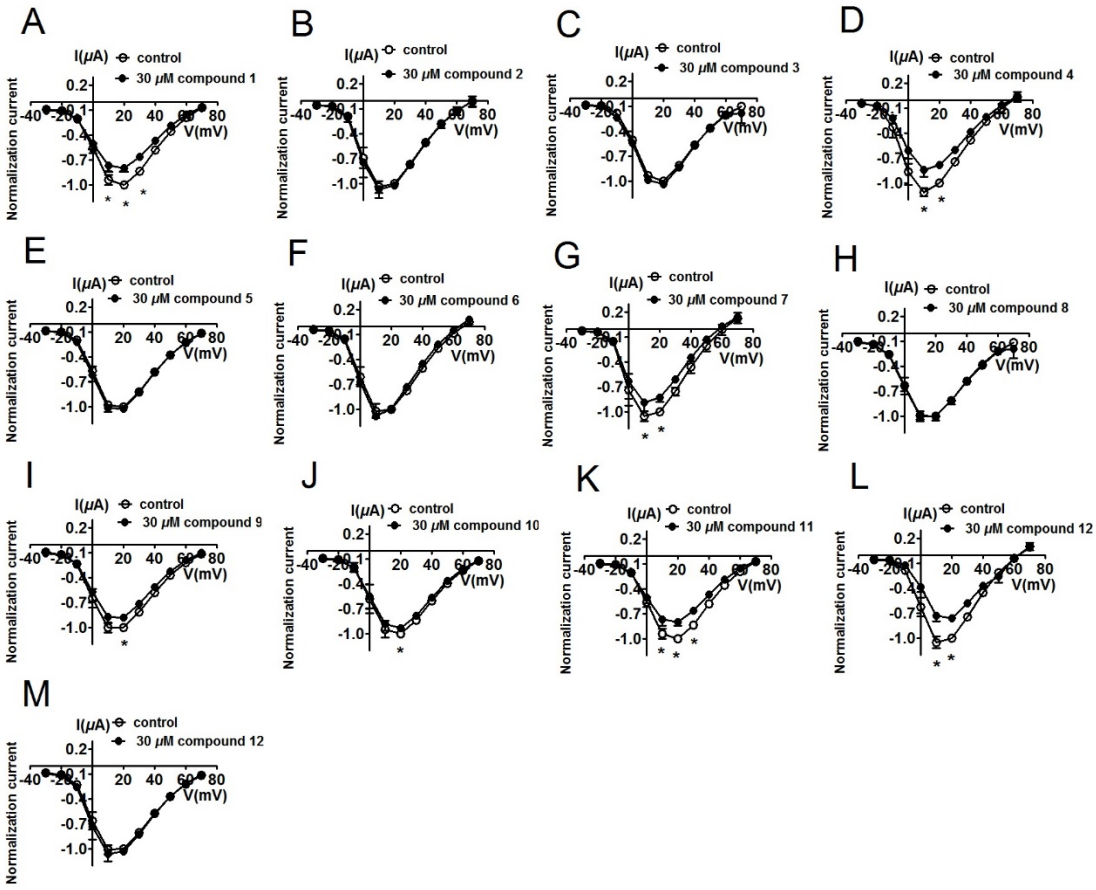


Figure S37. Normalized current-voltage (I-V) curves of $Ca_v1.2$ in the absence or presence of compounds **1-13**. $Ca_v1.2$ Currents were evoked from a holding potential (HP) of -80 mV by 50 ms depolarization from -30 mV to + 70 mV at 3 s intervals. All the data were analyzed with two-tailed student T test and represented as mean ± SEM (n=3). *P<0.05, compared with the absence of the indicated compounds.

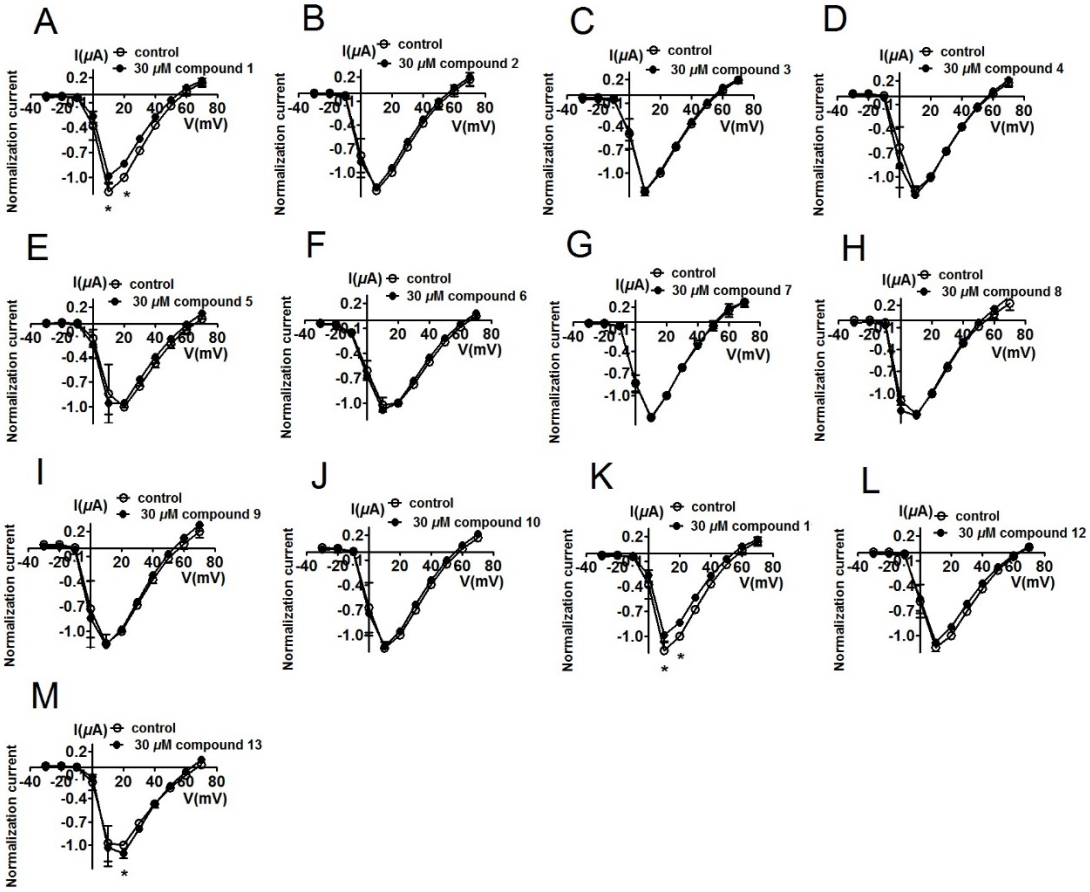


Figure S38. Normalized current-voltage (I-V) curves of $Ca_v2.1$ in the absence or presence of compounds **1-13**. $Ca_v2.1$ currents were evoked from a holding potential (HP) of -80 mV by 50 ms depolarization from -30 mV to + 70 mV at 3 s intervals. All the data were analyzed with tow-tailed student T test and represented as mean ± SEM (n=3). *$P<0.05$, compared with the absence of the indicated compounds.

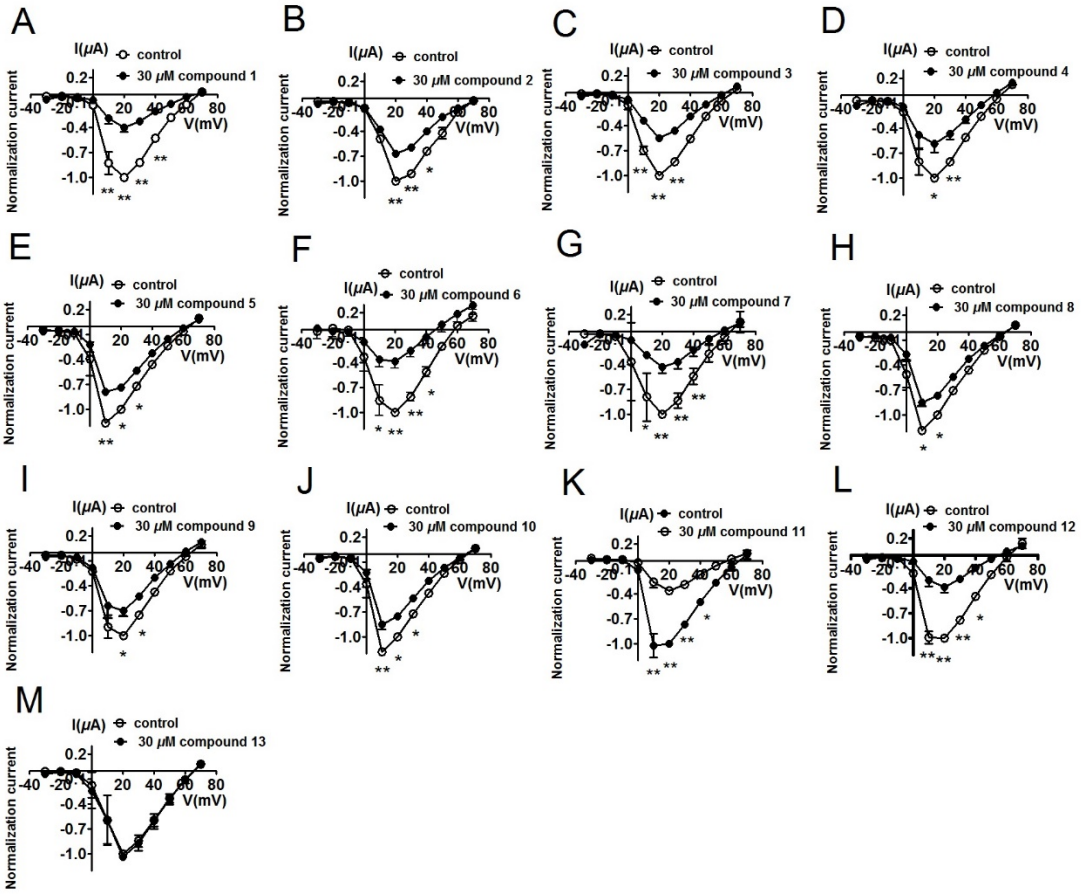


Figure S39. Normalized current-voltage (I-V) curves of $Ca_v2.2$ in the absence or presence of compounds **1-13**. $Ca_v2.2$ currents were evoked from a holding potential (HP) of -80 mV by 50 ms depolarization from -30 mV to + 70 mV at 3 s intervals. All the data were analyzed with tow-tailed student T test and represented as mean ± SEM (n=3). ***P<0.01, *P<0.05*, compared with the absence of the indicated compounds.

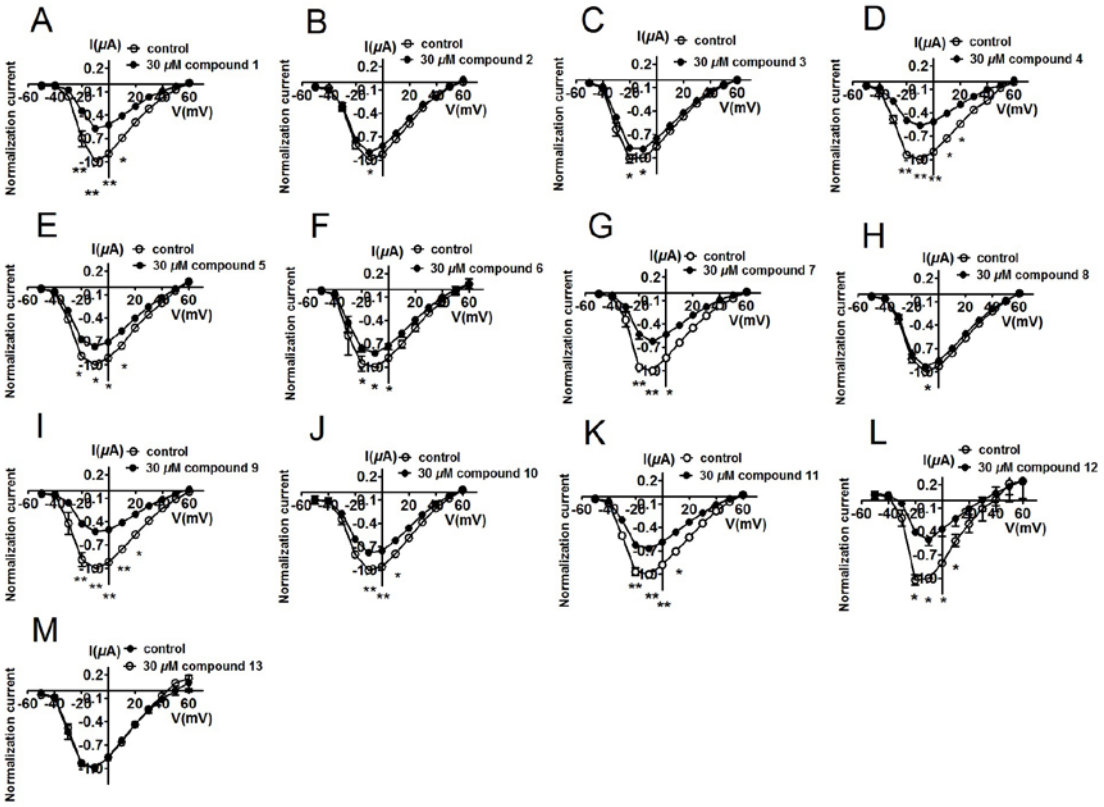


Figure S40. Normalized current-voltage (I-V) curves of $Ca_v3.1$ in the absence or presence of compounds **1-13**. $Ca_v3.1$ currents were evoked from a holding potential (HP) of -80 mV by 50 ms depolarization from -50 mV to + 60 mV at 3 s intervals. All the data were analyzed with tow-tailed student T test and represented as mean ± SEM (n=3). ***P<0.01*, **P<0.05*, compared with the absence of the indicated compounds.

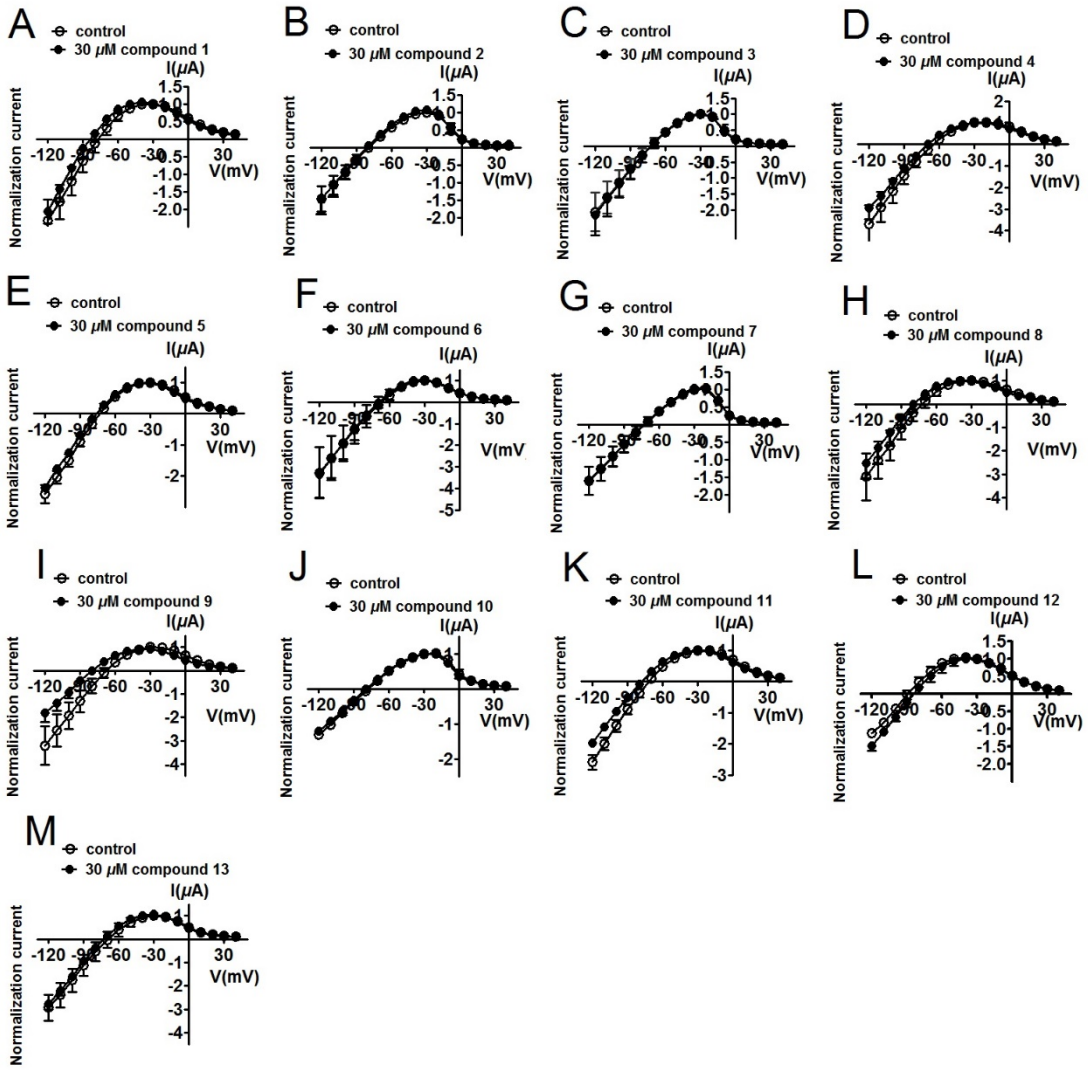


Figure S41. Normalized current-voltage (I-V) curves of KNCH2 in the absence or presence of compounds **1-13**. KCNH2 were pretreated by indicated compounds for 1 s and then currents were evoked from a holding potential (HP) of -80 mV by 3 s depolarization from -120 mV to + 40 mV at 30 s intervals. All the data were analyzed with tow-tailed student T test and represented as mean ± SEM (n=3), compared with the absence of the indicated compounds.

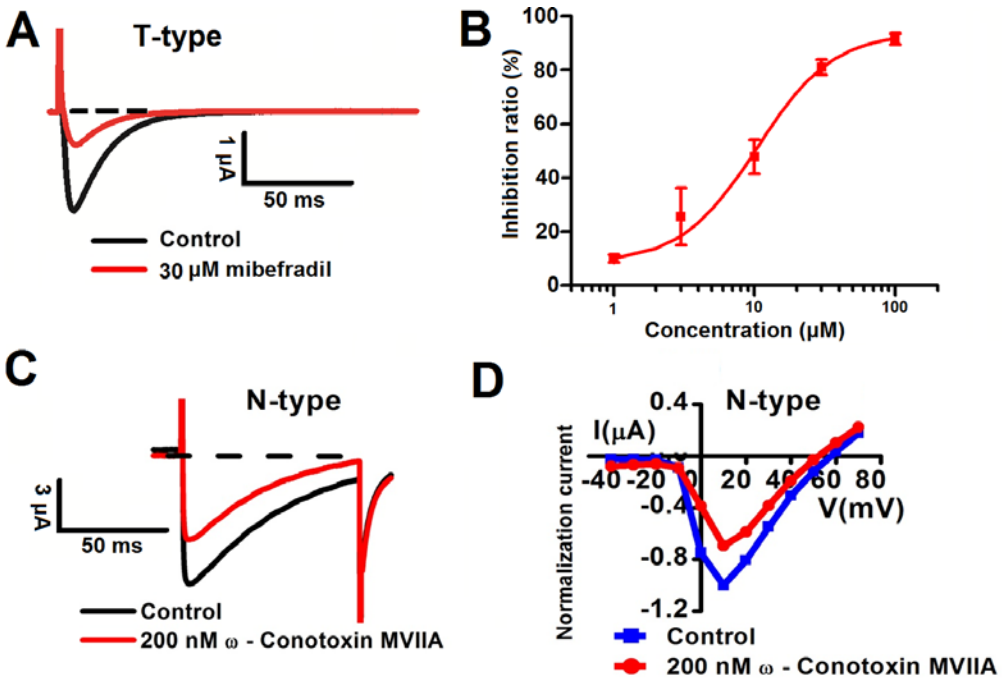


Figure S42. The inhibitory effects of Mibefradil (the commercial T-type calcium channel inhibitor) and ω-Conotoxin MVIIA (the commercial N-type calcium channel inhibitor) on $Ca_v3.1$ and $Ca_v2.2$, respectively. (**A**) Representative $Ca_v3.1$ current trace evoked by 50-ms depolarizations to –10 mV at 3-s intervals from a holding potential (HP) of –80 mV in the absence and presence of mibefradil. $Ca_v3.1$ was expressed in *Xenopus* oocytes; (**B**) Dose-response relationship of mibefradil inhibition of $Ca_v3.1$. Data points represent mean±S.E.M. of three measurements at HP of –80 mV. Solid curve represents a fit to the Hill equation with an IC_{50}value of 10.4μM and a Hill coefficient of 1.6; (**C**) Representative $Ca_v2.2$ current trace evoked by 50-ms depolarizations to +20 mV at 3-s intervals from a holding potential (HP) of –80 mV in the absence and presence of ω-Conotoxin MVIIA. $Ca_v2.2$ was expressed in *Xenopus* oocytes; (**D**) Normalized current-voltage (I-V) curves of $Ca_v2.2$ in the absence or presence of the indicated concentrations of ω-conotoxin MVIIA. Results were obtained in 3 cells.