

## Synthesis, pharmacological evaluation, and molecular modeling of lappaconitine–1,5-benzodiazepine hybrids

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Figure S 1.1.  $^1\text{H}$  NMR spectrum for compound **3** ( $\text{CDCl}_3$ , 400 MHz)

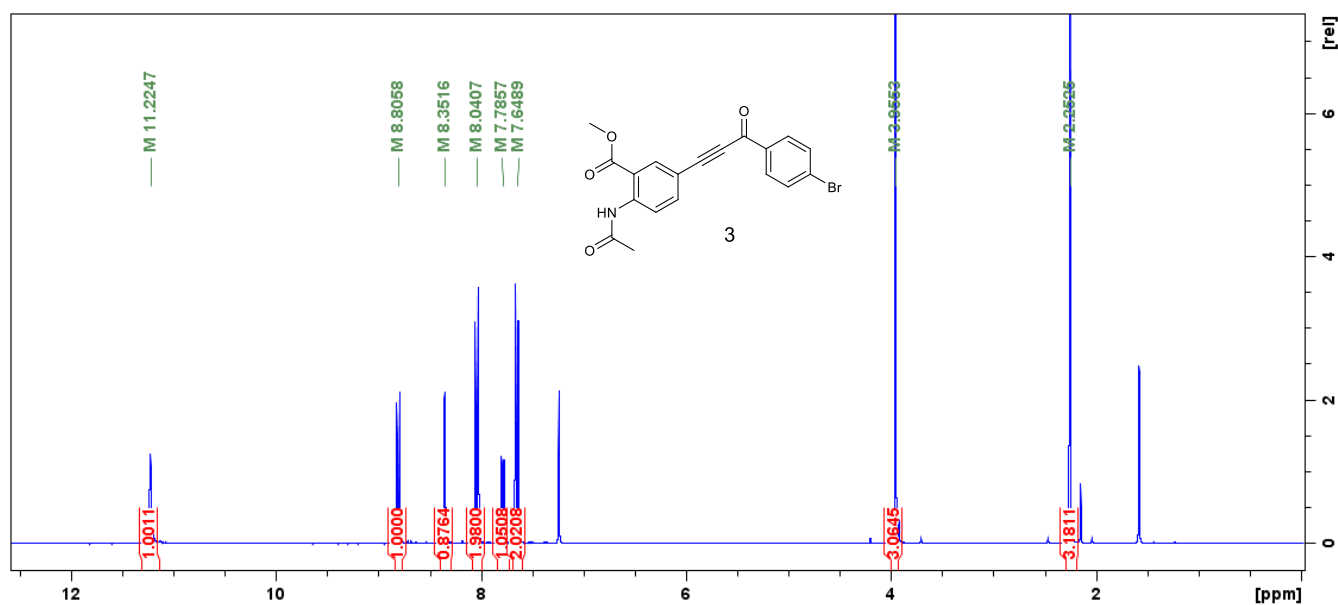


Figure S 1.2.  $^{13}\text{C}$  NMR spectrum for compound **3** ( $\text{CDCl}_3$ , 125 MHz)

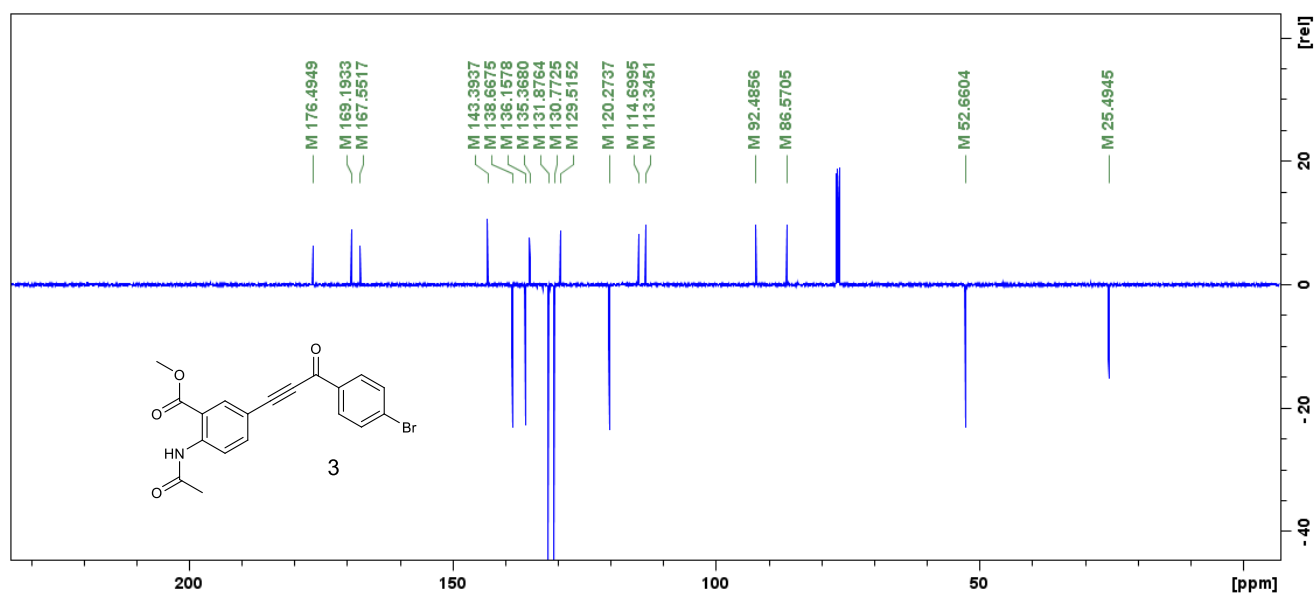


Figure S 2.1.  $^1\text{H}$  NMR spectrum for compound **7** ( $\text{CDCl}_3$ , 500 MHz)

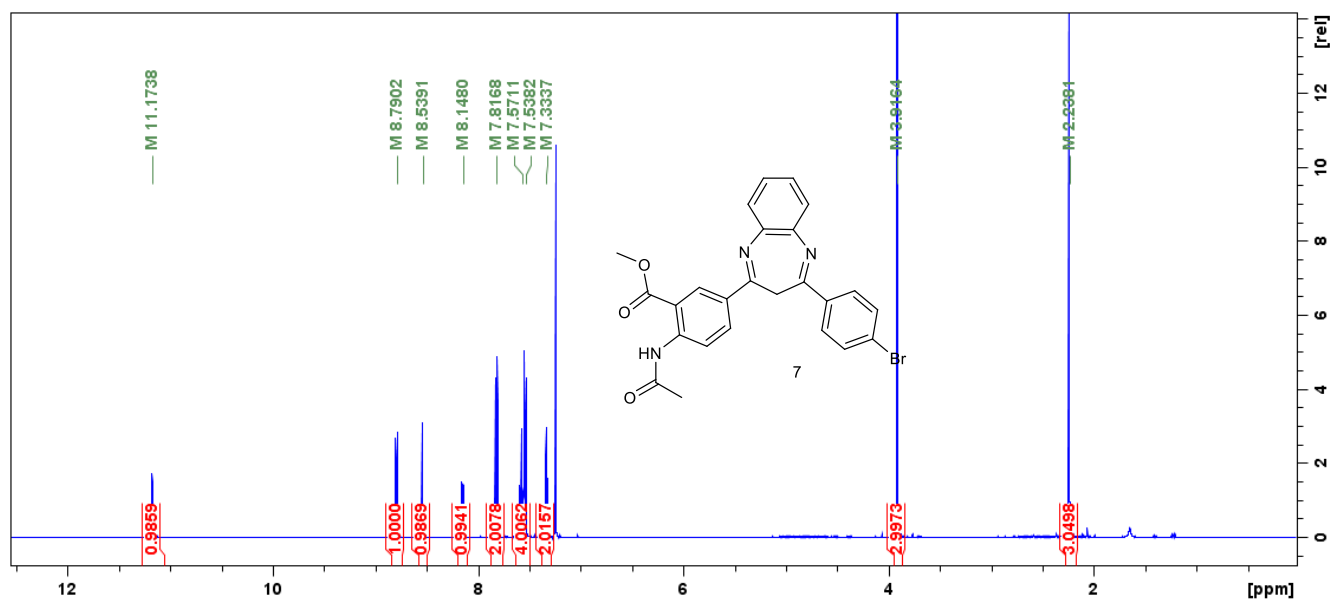


Figure S 2.2. Temperature dependent  $^1\text{H}$  NMR spectrum for compound **7** recorded in  $(\text{CD}_3)_2\text{SO}$ , 400 MHz

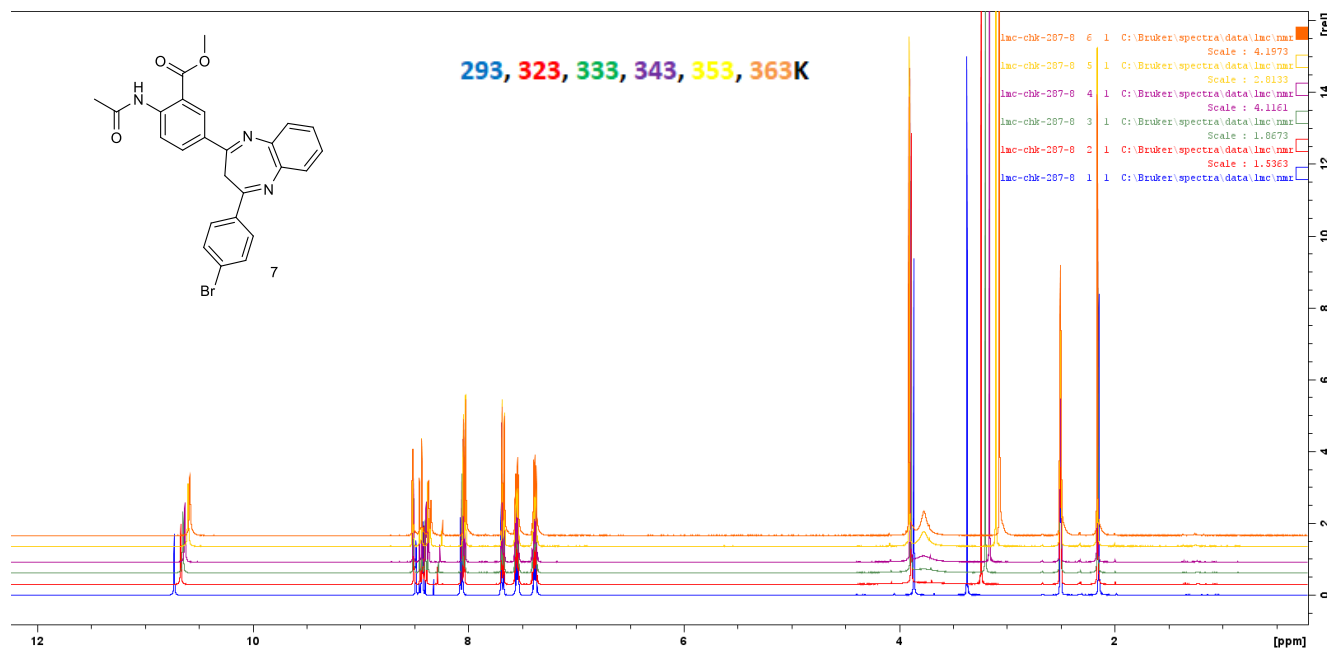


Figure S 2.3. Selected signals from spectra S2.2

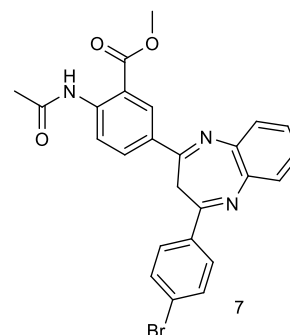
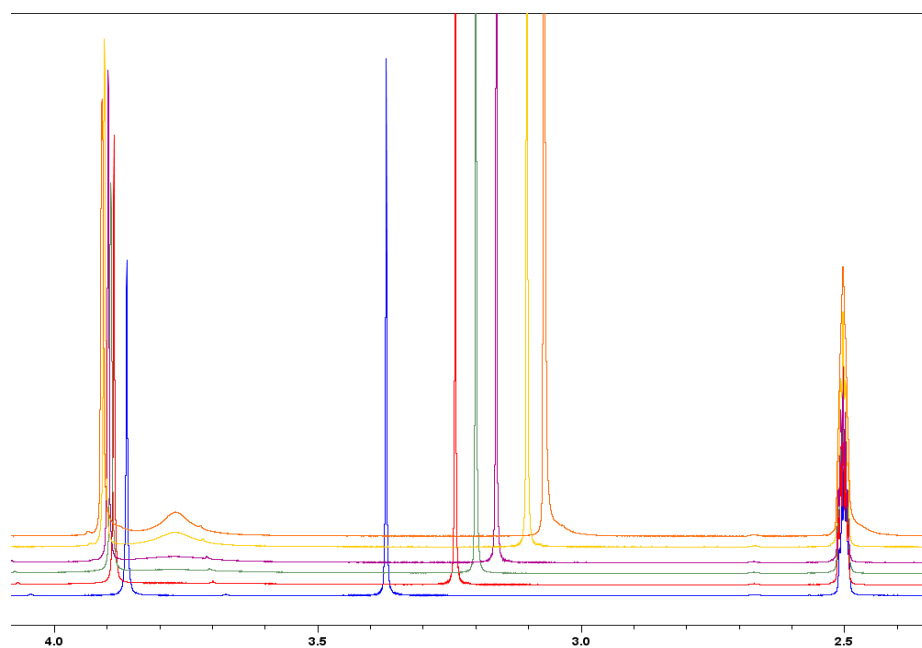


Figure S2.4.  $^{13}\text{C}$  NMR spectrum for compound **7** ( $\text{CDCl}_3$ , 125 MHz)

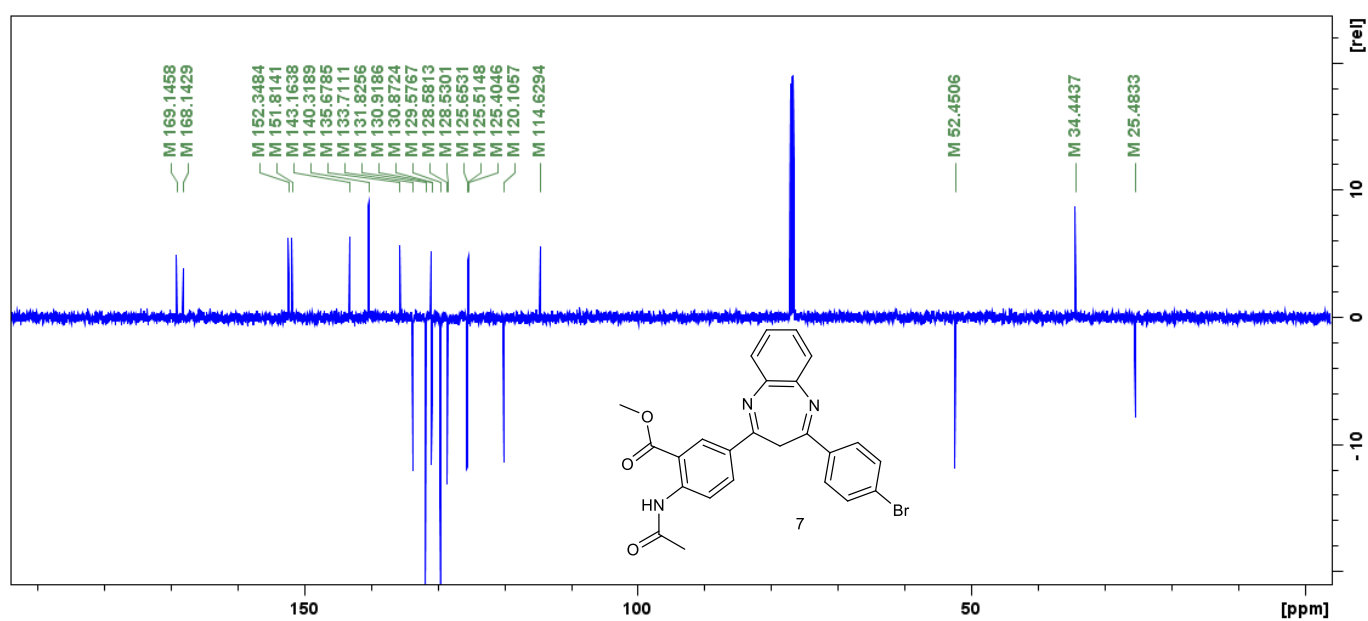
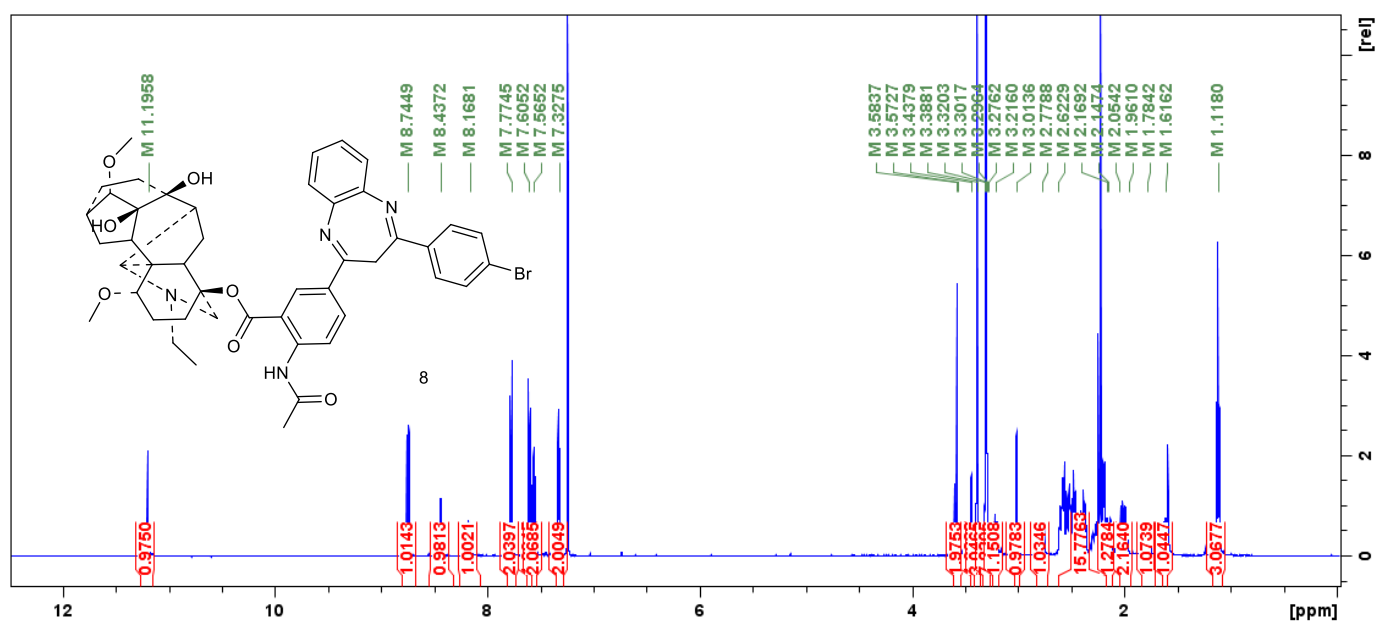
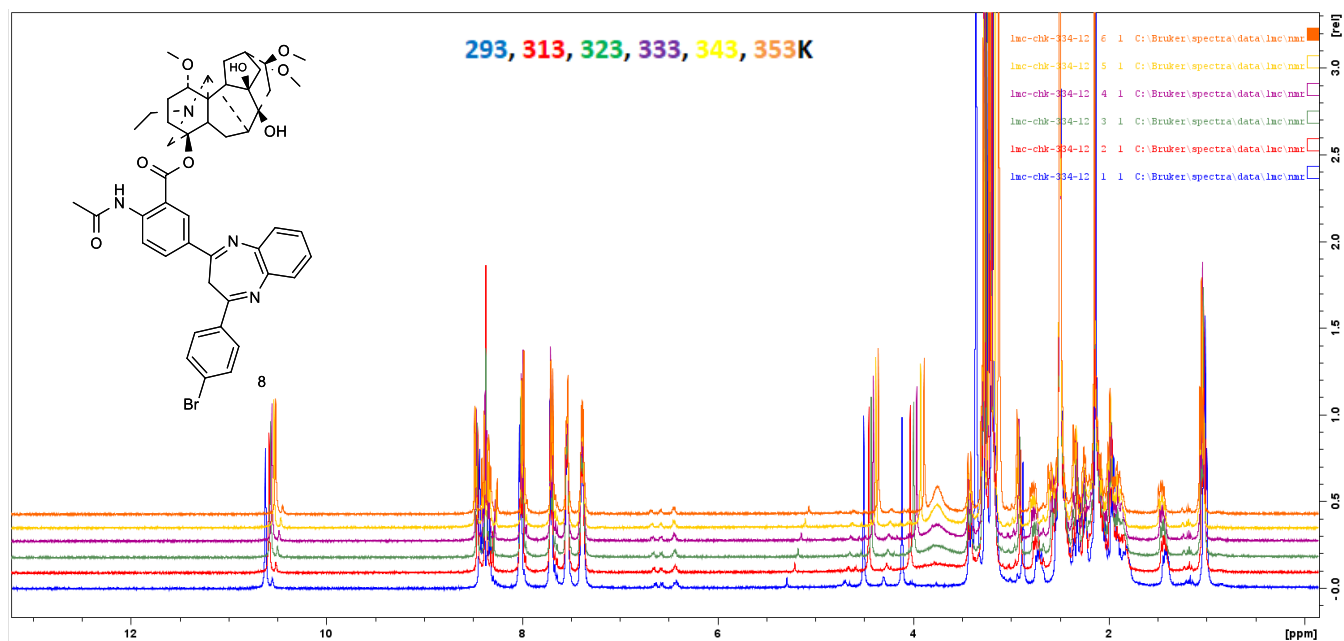


Figure S 3.1.  $^1\text{H}$  NMR spectrum for compound **8** ( $\text{CDCl}_3$ , 500 MHz)



S 3.2. Temperature dependent  $^1\text{H}$  NMR spectrum for compound **8** recorded in  $(\text{CD}_3)_2\text{SO}$ , 400 MHz)



293, 313, 323, 333, 343, 353K

The figure displays a series of <sup>1</sup>H NMR spectra for compound 8, recorded at temperatures of 293, 313, 323, 333, 343, and 353 K. The x-axis represents the chemical shift in ppm, ranging from approximately 1.0 to 5.0. The y-axis represents the relative intensity, ranging from 0.0 to 1.0. The spectra show several distinct peaks, including a broad peak around 4.5 ppm, a sharp peak around 4.1 ppm, and a complex multiplet between 2.5 and 3.5 ppm. The chemical structure of compound 8 is shown below the spectra, featuring a complex polycyclic core with a bromophenyl group and a carbonyl group.

CC(=O)Nc1ccc(cc1C(=O)O[C@H]2[C@@H]3[C@H]4[C@H]2[C@@H](OC)[C@H](OC)[C@H]4O)[C@H]3c5ccccc5C6=CC=CC=C6

8

13C NMR spectrum of compound 10. The x-axis represents chemical shift in ppm, ranging from 0 to 180. The y-axis represents relative intensity in arbitrary units, ranging from -4 to 4. The spectrum shows several sharp peaks, with the most intense ones clustered between 40 and 60 ppm. Numerous peaks are labeled with their corresponding chemical shift values in ppm.

Chemical Shift (ppm)
169.0428
166.7830
152.4812
151.2751
143.4156
140.2987
140.2544
135.9589
133.9216
131.9690
130.4531
130.4206
129.4239
128.6404
128.5199
125.5716
125.3441
125.2570
120.2233
115.4101
89.9678
85.2602
83.9482
82.6892
78.4039
75.3949
61.2995
57.7914
56.4624
55.9533
55.3386
50.8613
49.5711
48.8249
48.3050
47.4336
44.7349
36.0998
33.9261
31.6643
26.6172
26.0457
25.4920
24.0811
13.4072

Figure S 4.1.  $^1\text{H}$  NMR spectrum for compound **9** ( $\text{CDCl}_3$ , 500 MHz)

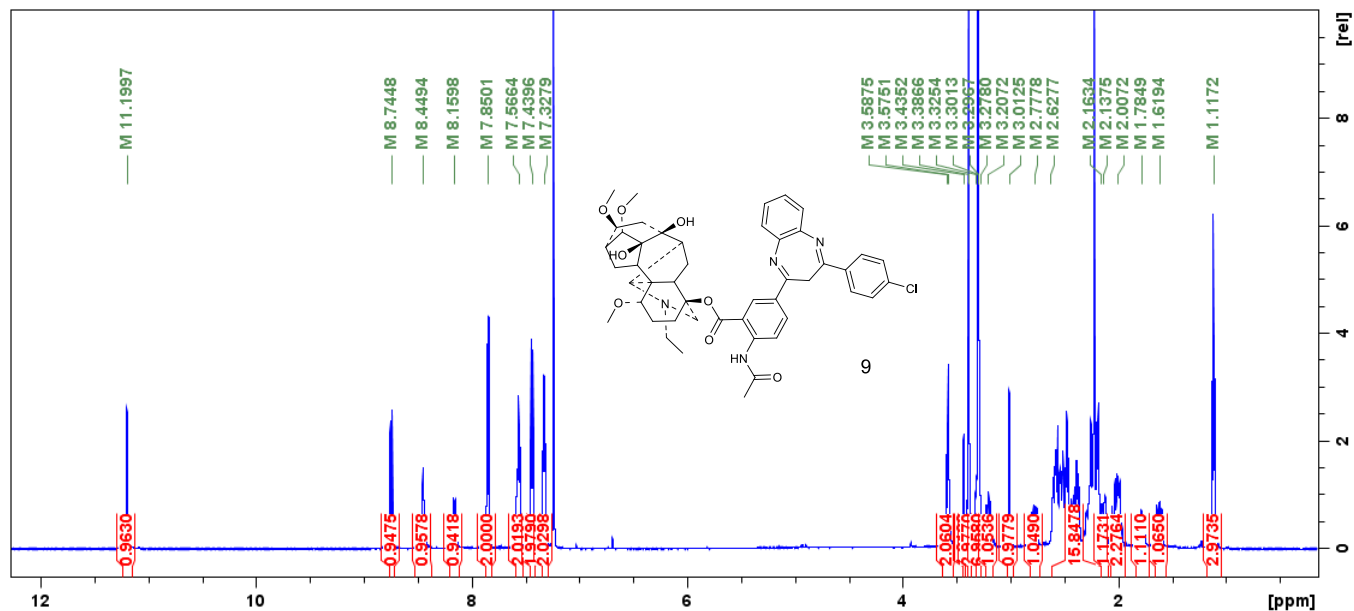


Figure S 4.2.  $^{13}\text{C}$  NMR spectrum for compound **9** ( $\text{CDCl}_3$ , 125 MHz)

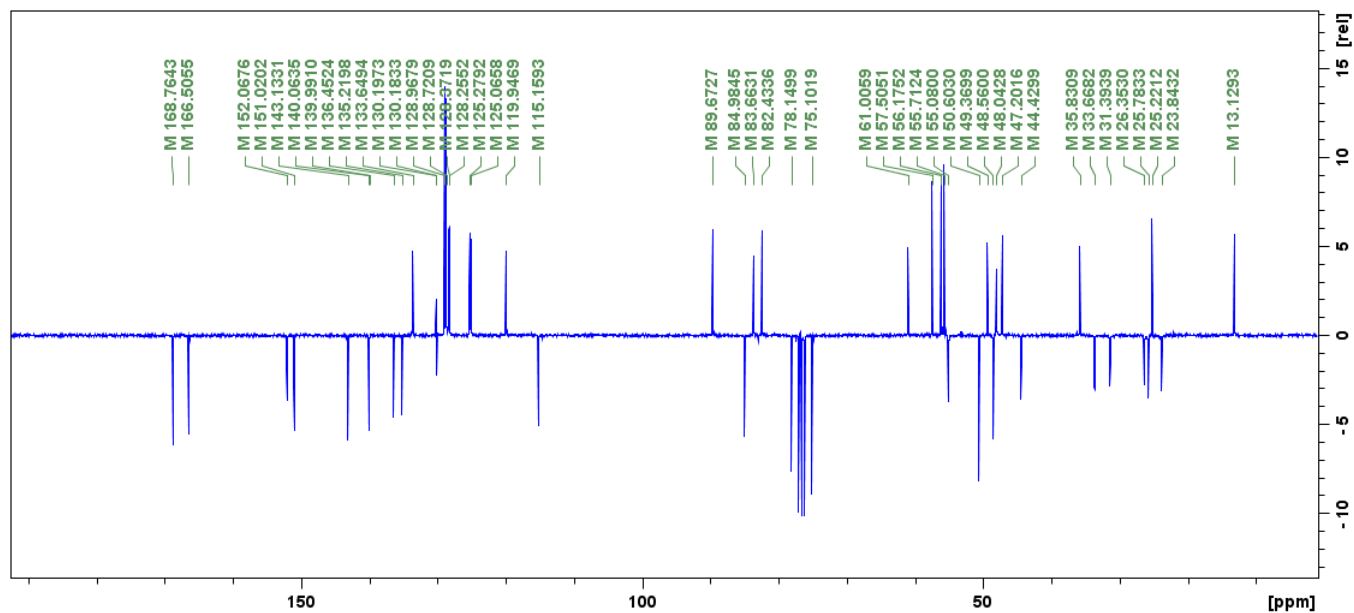


Figure S 5.1.  $^1\text{H}$  NMR spectrum for compound **10** ( $\text{CDCl}_3$ , 500 MHz)

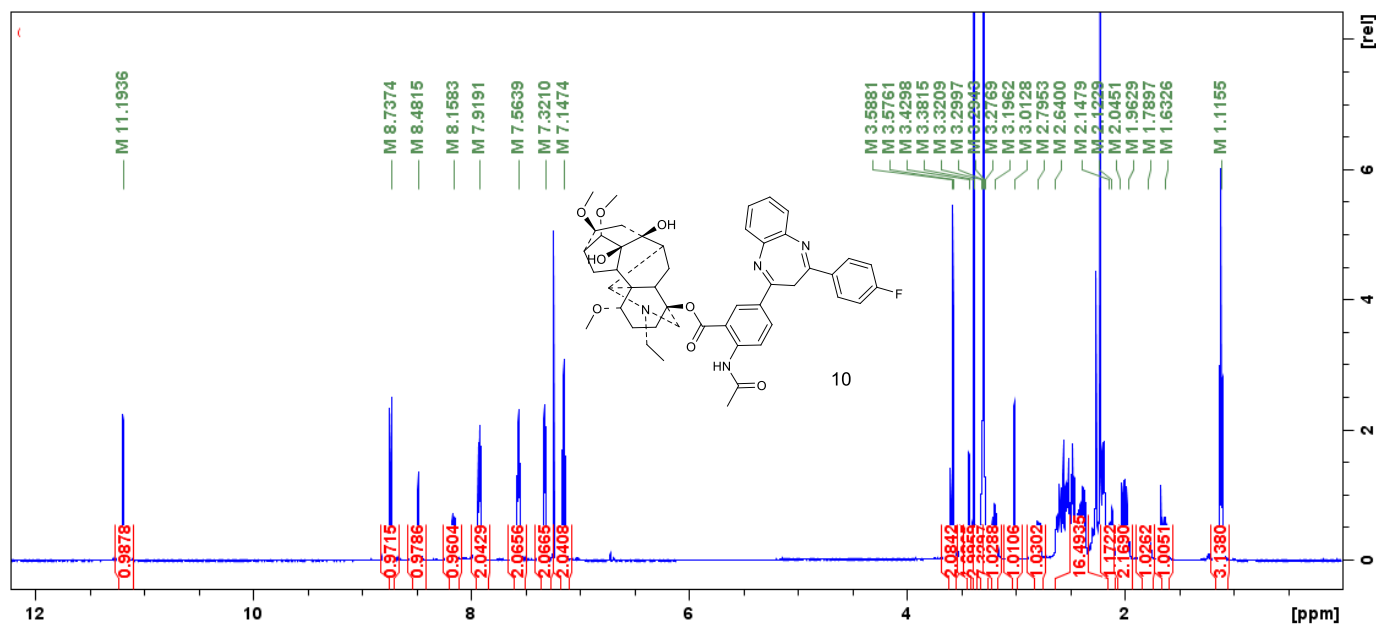


Figure S 5.2.  $^{13}\text{C}$  NMR spectrum for compound **10** ( $\text{CDCl}_3$ , 125 MHz)

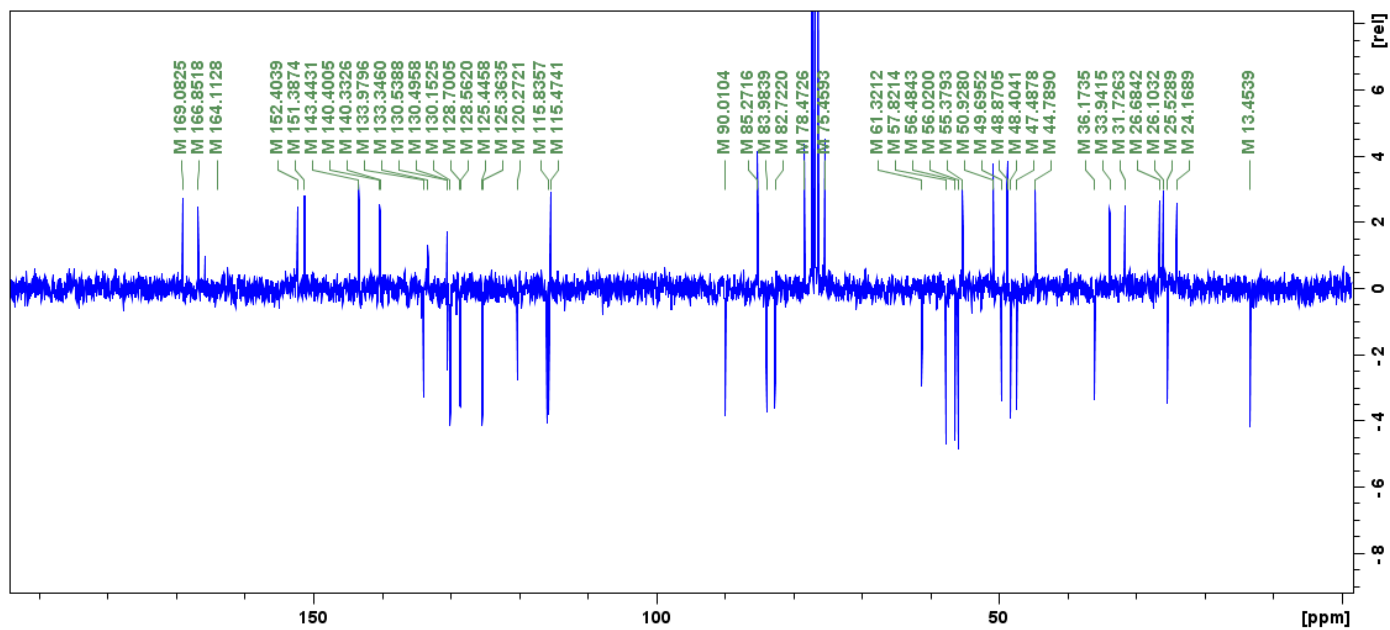




Figure S 6.1.  $^1\text{H}$  NMR spectrum for compound **11** ( $\text{CDCl}_3$ , 400 MHz)

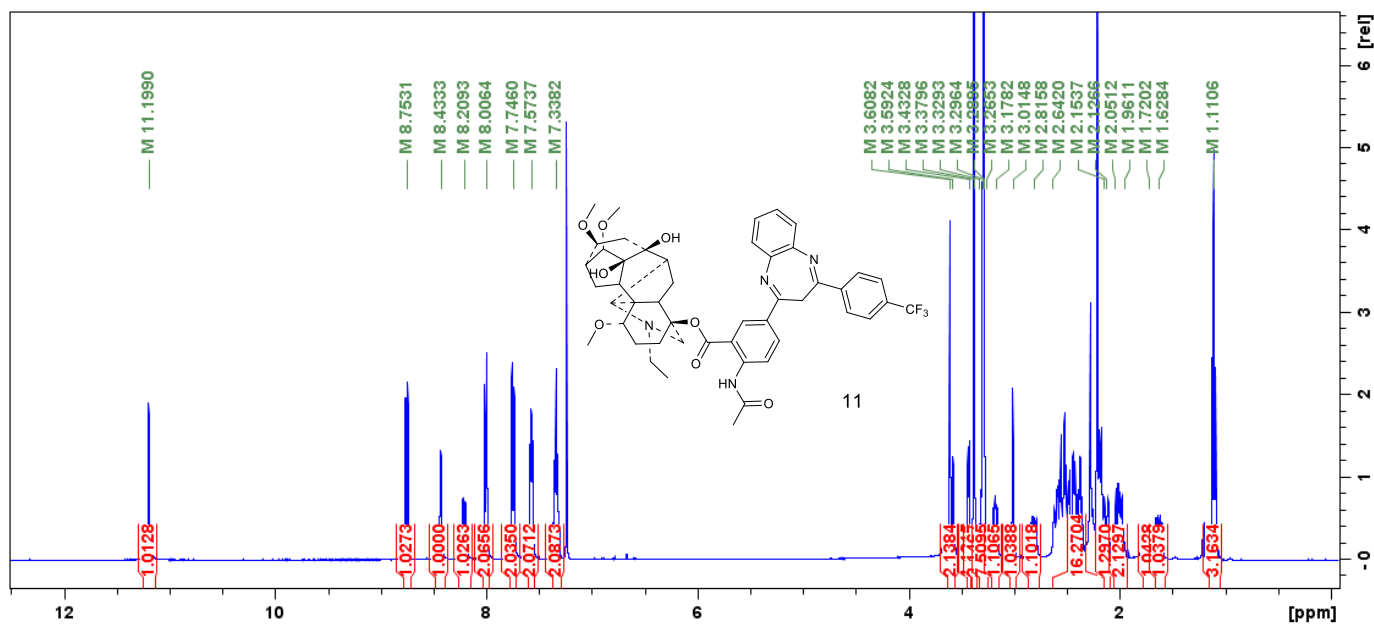


Figure S 6.2.  $^{13}\text{C}$  NMR spectrum for compound **11** ( $\text{CDCl}_3$ , 100 MHz)

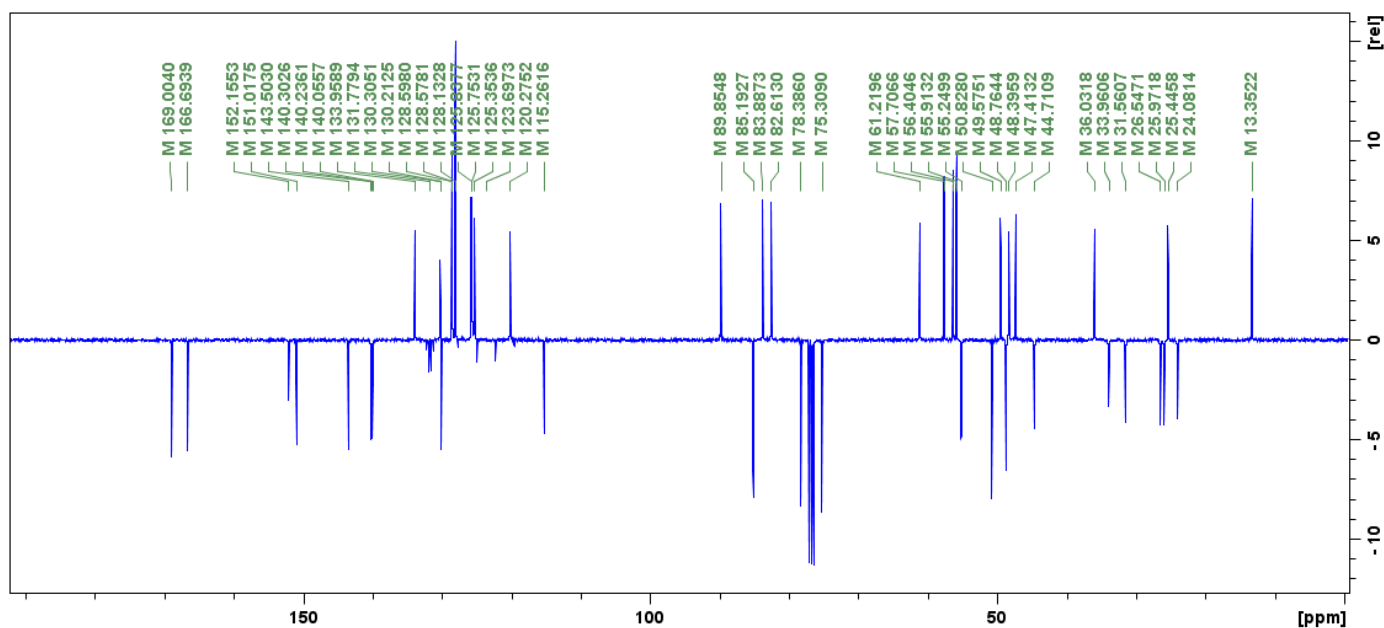


Figure S 7.1.  $^1\text{H}$  NMR spectrum for compound **12** ( $\text{CDCl}_3$ , 400 MHz)

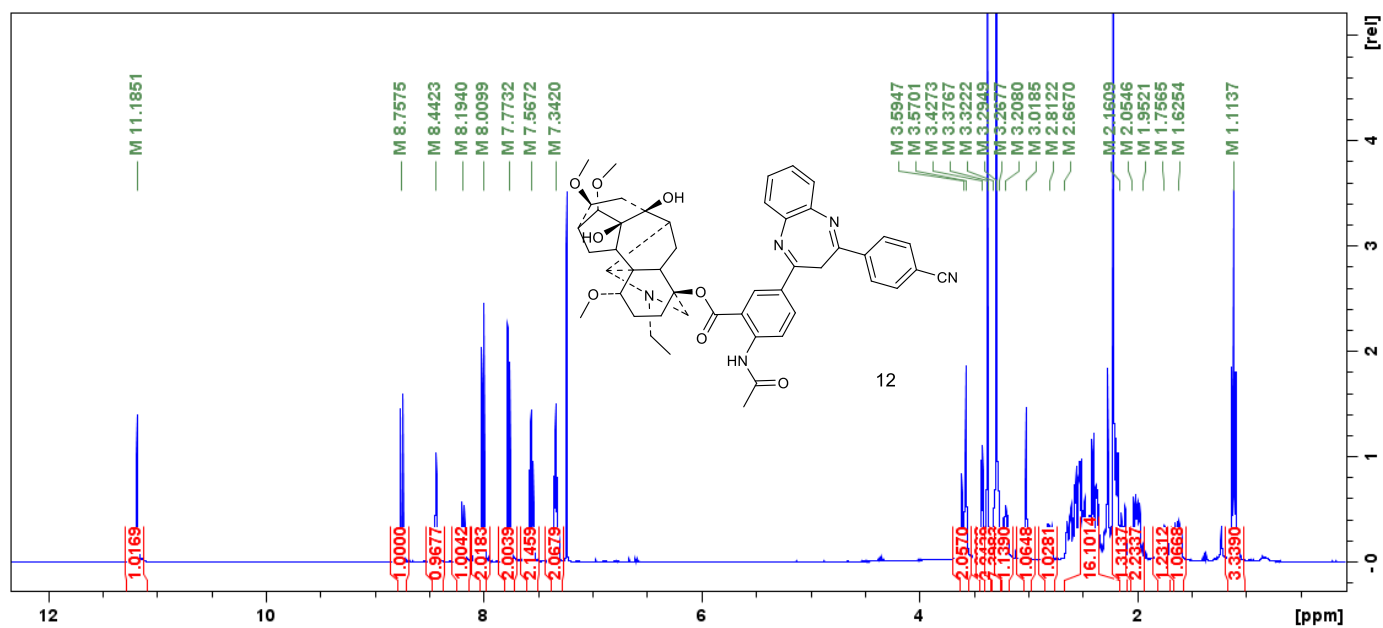


Figure S 7.2.  $^{13}\text{C}$  NMR spectrum for compound **12** ( $\text{CDCl}_3$ , 125 MHz)

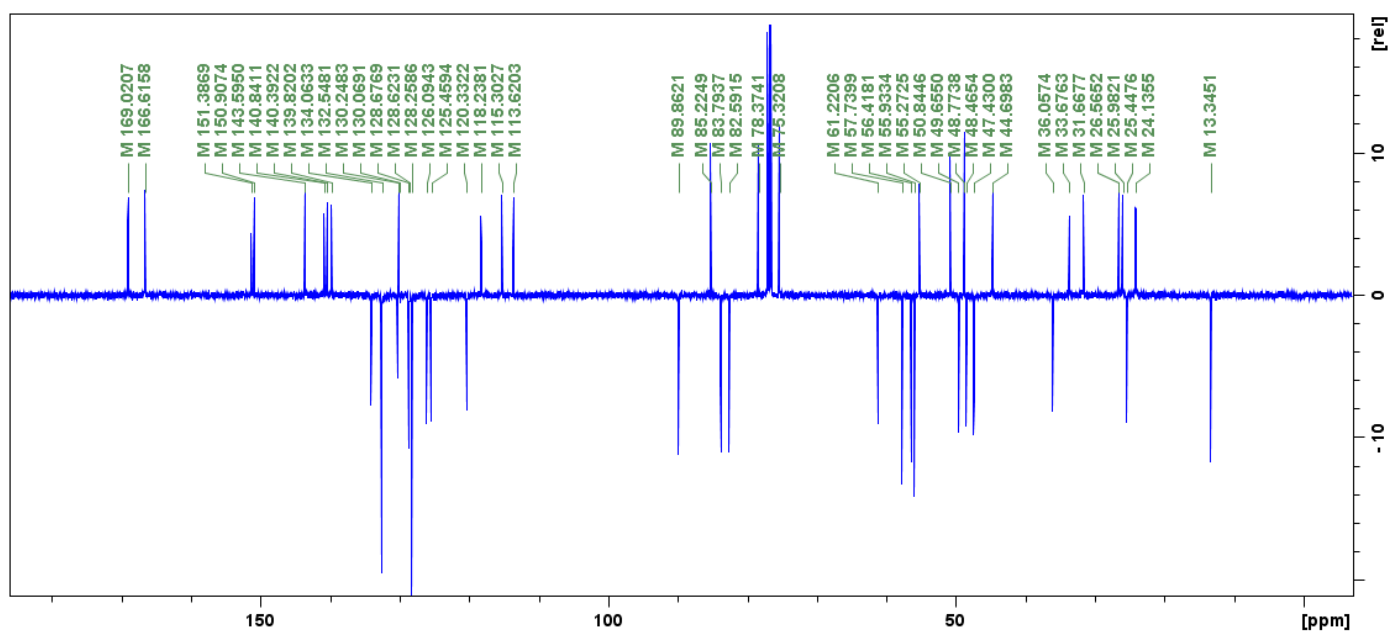


Figure S 8. Effect of compound 8 injection (dose 5mg/kg) on ECG parameters

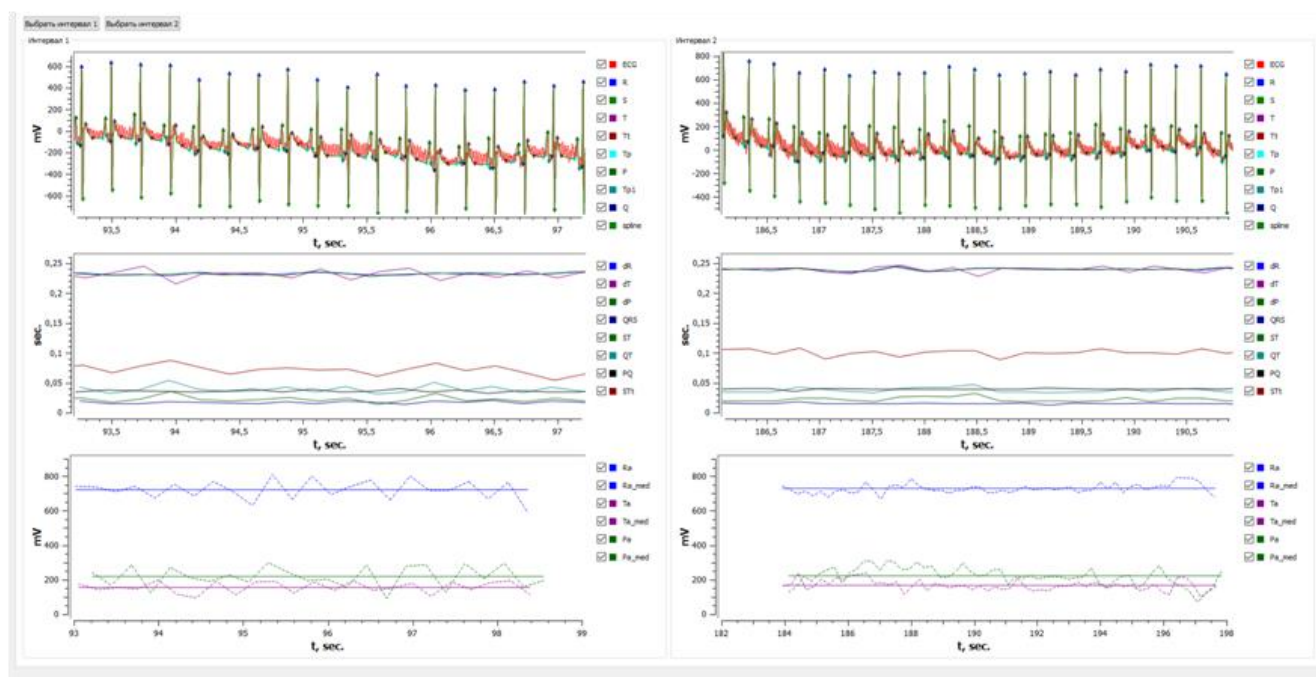


Figure S 9. Effect of compound 10 injection (dose 5mg/kg) on ECG parameters

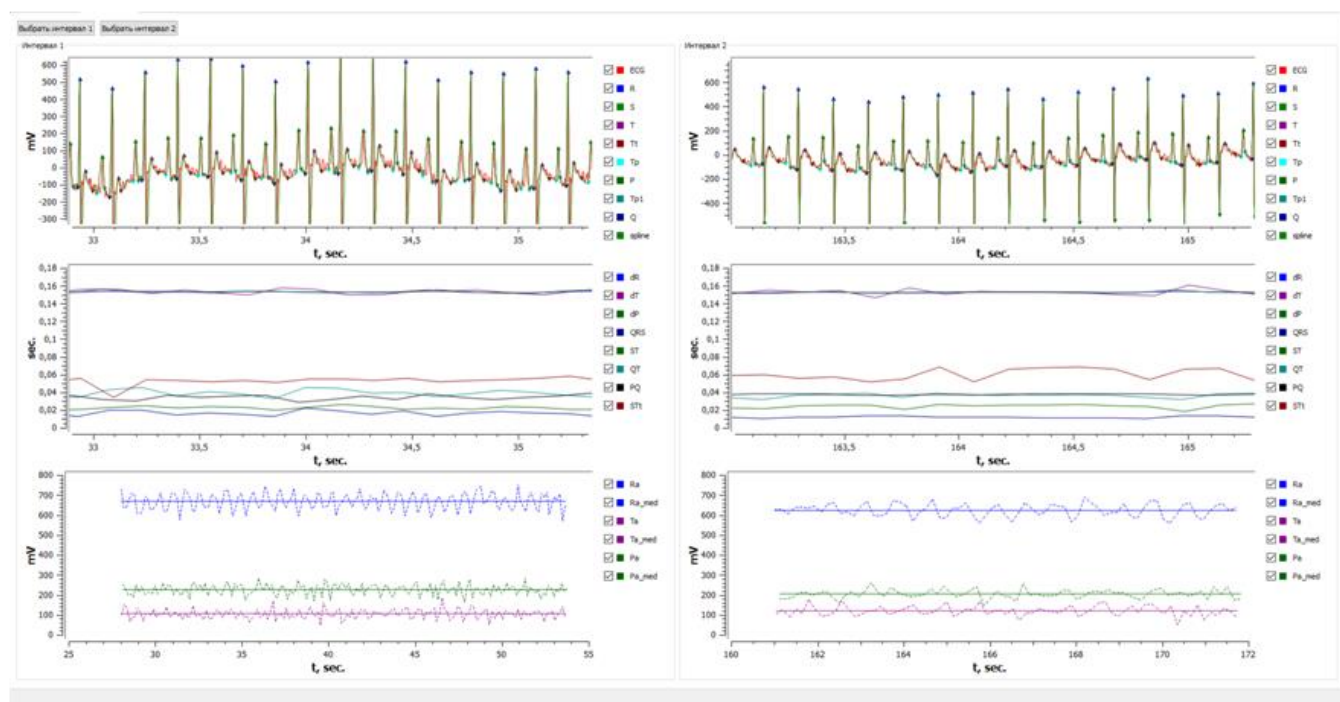


Figure S10. Changes in the rat ECG during administration of compound **8** (10 mg/kg) on a model of calcium chloride arrhythmia.

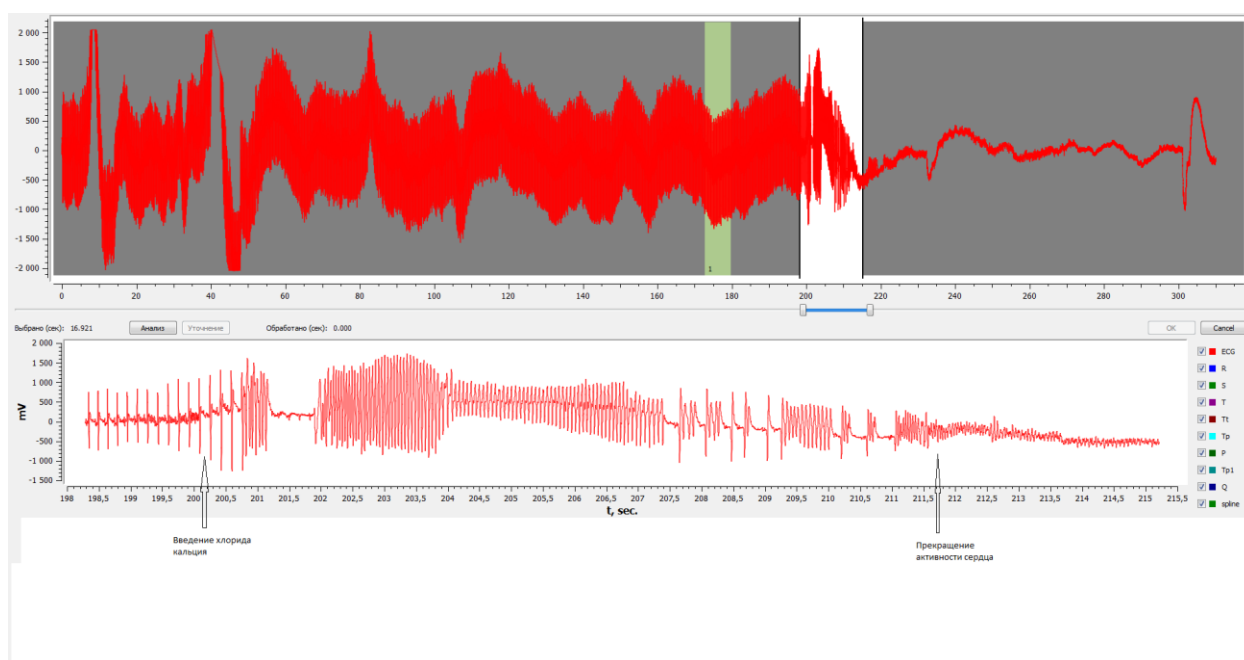


Figure S11. Arrhythmia in rats caused by the introduction of epinephrine and restoration of the rhythm against the background of the administered compound **8** (5 mg/kg). A) native ECG, B) tachycardia after epinephrine administration, C) ECG recovery.

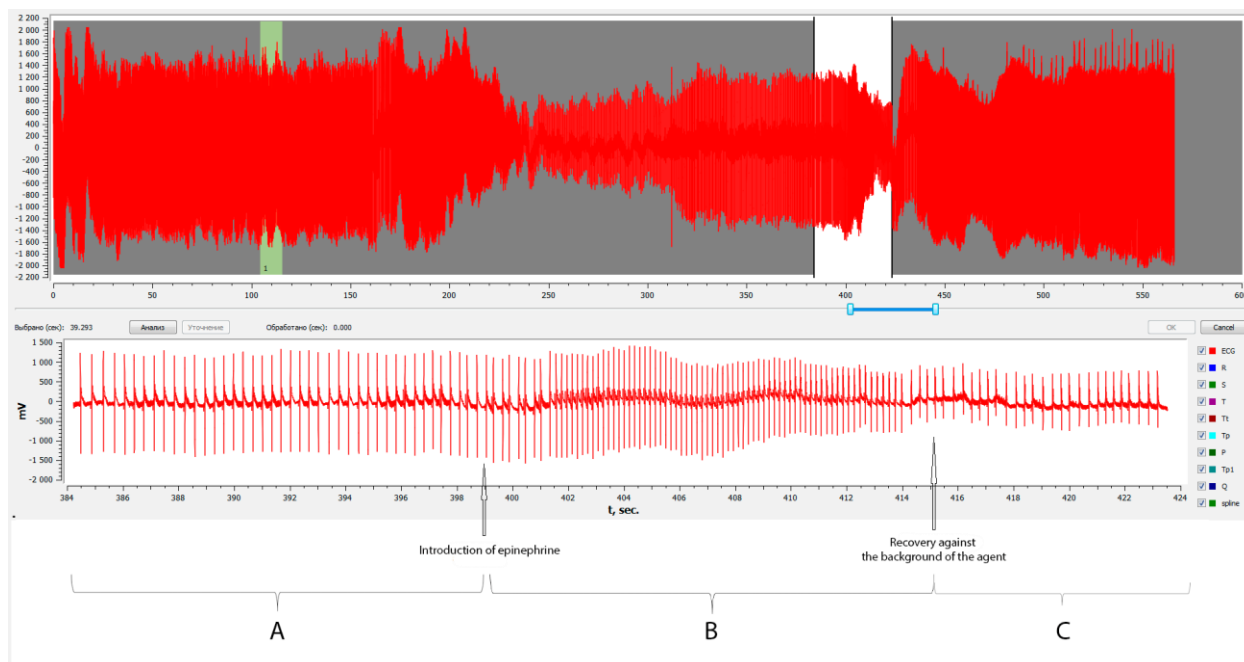


Figure S12. Rat atrium bead alteration after administration of: (1) Epinephrine (2) barium chloride or (3) calcium chloride at the concentration of  $10^{-3}$  M. The peak - injection of the arrhythmogen.

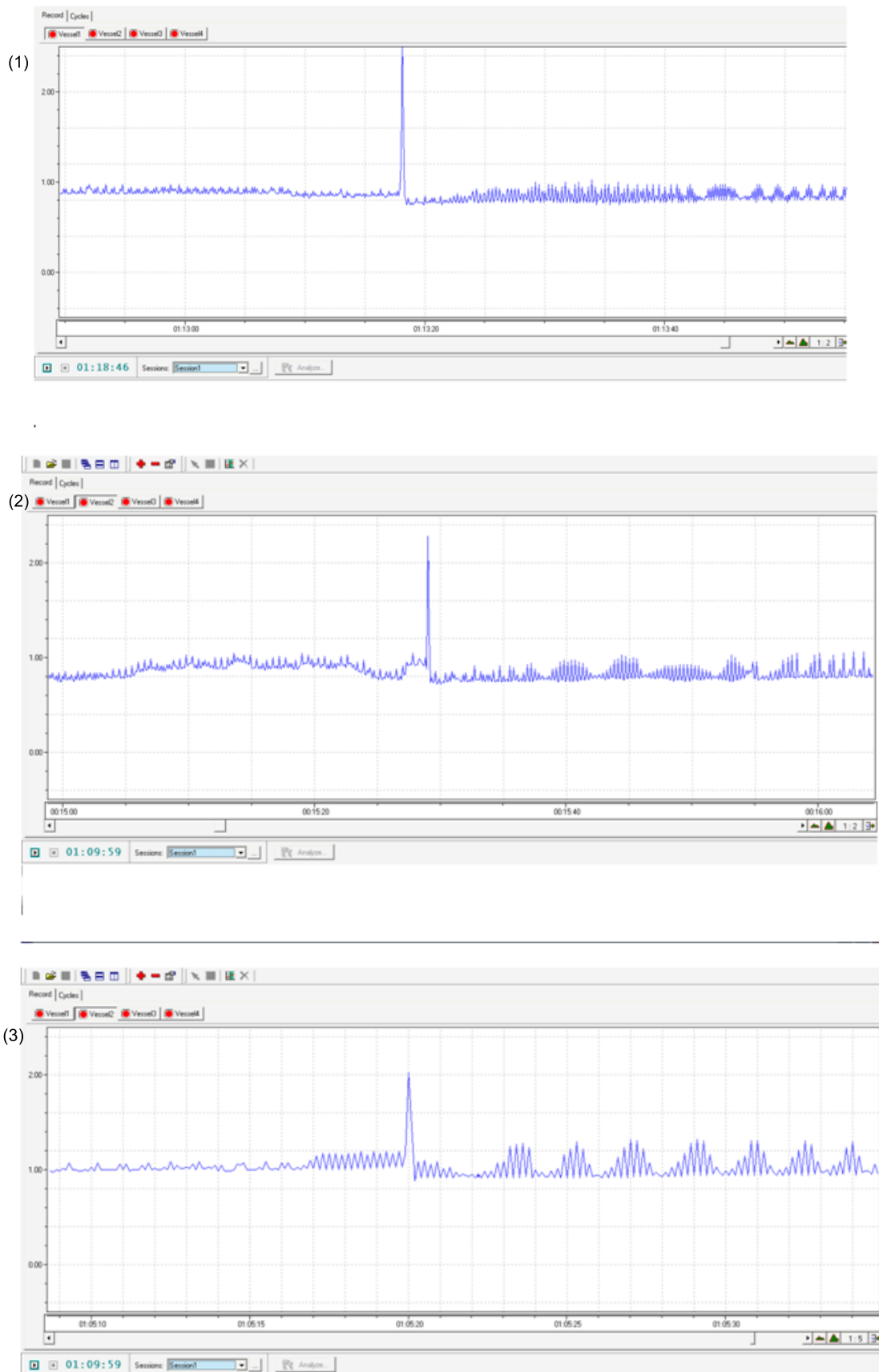
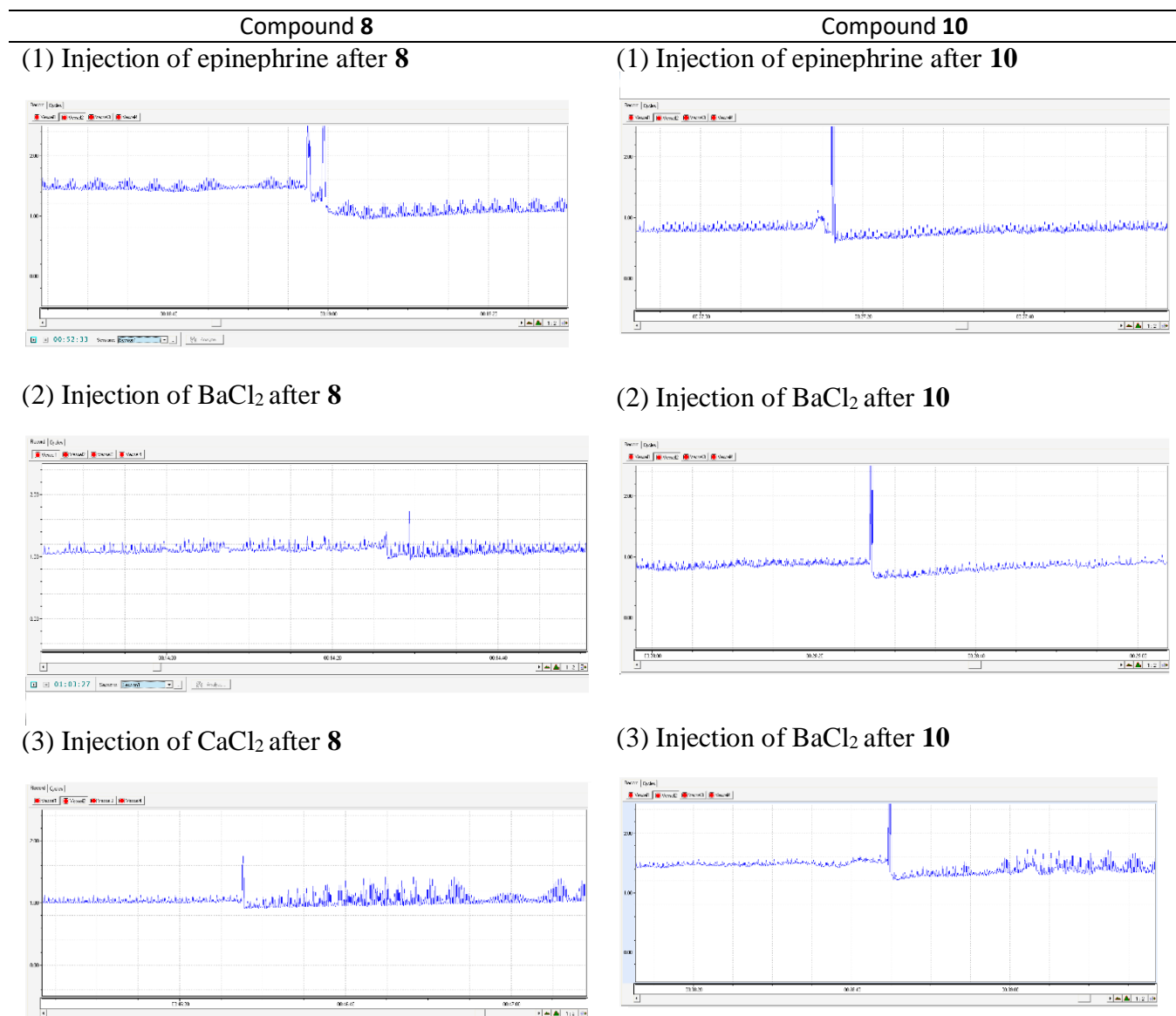


Figure S13. Rat atrium bead alteration after administration of: (1) epinephrine (2) barium chloride or (3) calcium chloride (concentration of  $10^{-3}$  M) against the background of compounds **8** or **10** ( the peak - injection of the arrhythmogen.



**Table S1.** Influence of compounds **8** and **10** on ECG parameters in rats in dose 5mg/kg ( $\pm$  shows the spread of the standard error from the mean data).

ECG parameters	dR	dT	dP	QRS	ST	QT	Ra	Ta	Pa
Starting ECG	0.152 $\pm$ 0.005	0.152 $\pm$ 0.007	0.152 $\pm$ 0.013	0.016 $\pm$ 0.001	0.022 $\pm$ 0.002	0.038 $\pm$ 0.001	667.39 $\pm$ 15.08	104.59 $\pm$ 5.78	226.01 $\pm$ 11.64
With <b>8</b>	0.153 $\pm$ 0.002	0.153 $\pm$ 0.009	0.153 $\pm$ 0.008	0.012 $\pm$ 0.001	0.023 $\pm$ 0.001	0.035 $\pm$ 0.007	624.49 $\pm$ 20.8	119.53 $\pm$ 6.3	204.91 $\pm$ 6.5
Starting ECG	0.232 $\pm$ 0.0008	0.231 $\pm$ 0.052	0.232 $\pm$ 0.012	0.017 $\pm$ 0.0008	0.022 $\pm$ 0.001	0.039 $\pm$ 0.001	721.04 $\pm$ 30.2	156.62 $\pm$ 9.8	218.12 $\pm$ 15.15
With <b>10</b>	0.24 $\pm$ 0.0012	0.24 $\pm$ 0.03	0.24 $\pm$ 0.009	0.015 $\pm$ 0.001	0.022 $\pm$ 0.001	0.037 $\pm$ 0.0001	726.82 $\pm$ 28.8	168.37 $\pm$ 15.128	221.86 $\pm$ 12.8

**Table S2.** Statistical differences in the amplitude and frequency of contraction of the rat right atrium against the background of compounds **8,10** and arrhythmogenes.

Compound	Concentration, M	Amplitude, mV		Frequency, Hz	
		Before	After	Before	After
<b>8</b>	10 <sup>-3</sup>	0.156 $\pm$ 0.018	0.158 $\pm$ 0.004	0.22 $\pm$ 0.04	0.20 $\pm$ 0.001
<b>10</b>	10 <sup>-3</sup>	0.18 $\pm$ 0.015	0.20 $\pm$ 0.009*	0.33 $\pm$ 0.05	0.29 $\pm$ 0.1
Epinephrine	10 <sup>-3</sup>	0.14 $\pm$ 0.023**	0.24 $\pm$ 0.045	2.2	3.4
Barium chloride	10 <sup>-3</sup>	0.10 $\pm$ 0.06**	0.22 $\pm$ 0.01	2.6	3.4
Calcium chloride	10 <sup>-3</sup>	0.18 $\pm$ 0.01**	0.34 $\pm$ 0.01**	2.6	3.2

\*P < 0.05. \*\*P<0.01 before vs. after

**Table S3.** Statistical differences in the amplitude and frequency of contraction of the rat right atrium after the introduction of arrhythmogen against the background of compounds **8, 10**.

Compound	Epinephrine 10 <sup>-3</sup> M				Barium chloride 10 <sup>-3</sup> M				Calcium chloride 10 <sup>-3</sup> M			
	Amplitude, mV		Frequency, Hz		Amplitude, mV		Frequency, Hz		Amplitude, mV		Frequency, Hz	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
<b>8</b>	0.17 $\pm$ 0.01	0.18 $\pm$ 0.02	2.6	2.8	0.095 $\pm$ 0.02*	0.128 $\pm$ 0.01	2.6	1.6	0.095 $\pm$ 0.02*	0.24 $\pm$ 0.03	2.6	3.2
<b>10</b>	0.18 $\pm$ 0.07	0.17 $\pm$ 0.03	2.2	3.4	0.2 $\pm$ 0.018	0.22 $\pm$ 0.04	2.2	3.4	0.38 $\pm$ 0.05*	0.98 $\pm$ 0.01	2.2	3.2

\*P < 0.05





Figure S15. Superposition of sodium channel blockers: green – flecainide **16**, yellow – compound **8**, orange – compound **10**.

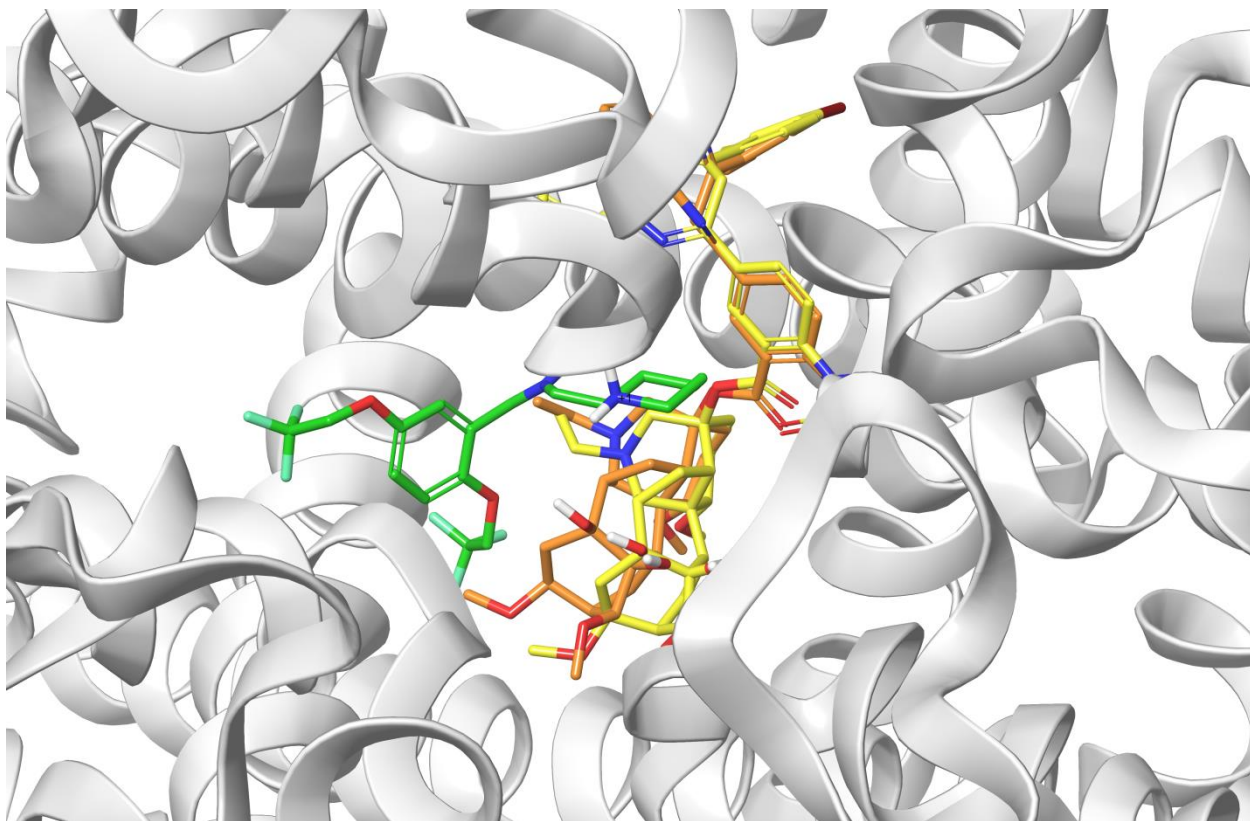
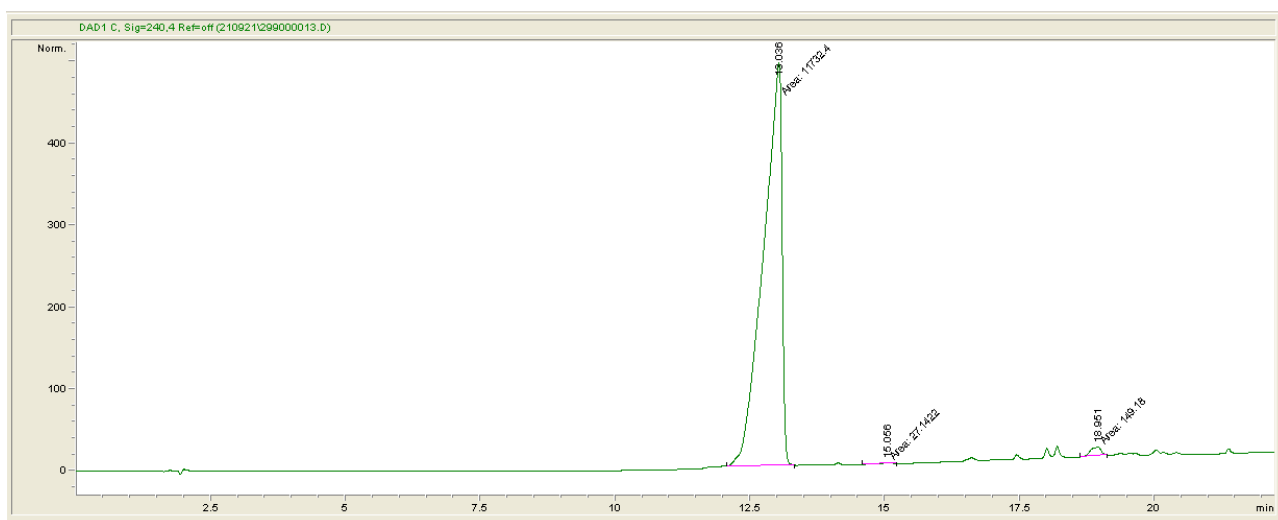


Figure S16. HPLC-UV chromatogram for **4 $\beta$ -{2'-Acetylamino-5'-(4''-(4-bromophenyl)-3H-1,5-benzodiazepine-2''-yl)benzoate}-1 $\alpha$ ,14 $\alpha$ ,16 $\beta$ -trimethoxy-20-ethylaconitane-8,9-diol (8)**



Purity – 98.52%

Retention time - 13,06 мин.

Absorption maximum at wavelength 240 nm.