Supporting Information

A Single Administration of the Atypical Psychedelic Ibogaine and its Metabolite Noribogaine Induce Antidepressant-like Effect in rats

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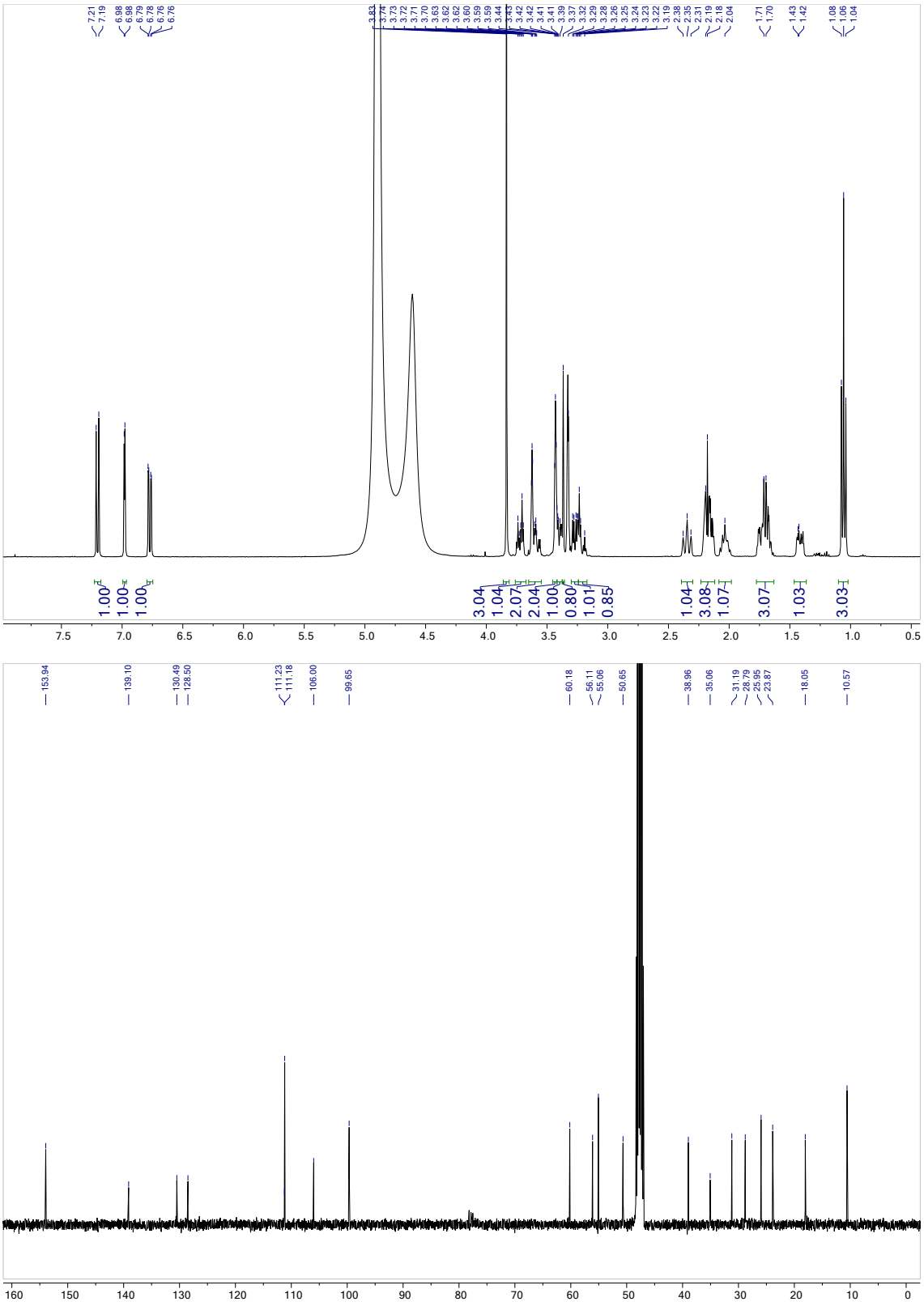
1. Ibogaine-HCl and Noribogane-HCl Preparation. Nuclear Magnetic Resonance spectra were obtained on a Bruker Avance DPX-400 instrument. In both cases samples were characterized as voacangine or ibogaine according to the following NMR data:

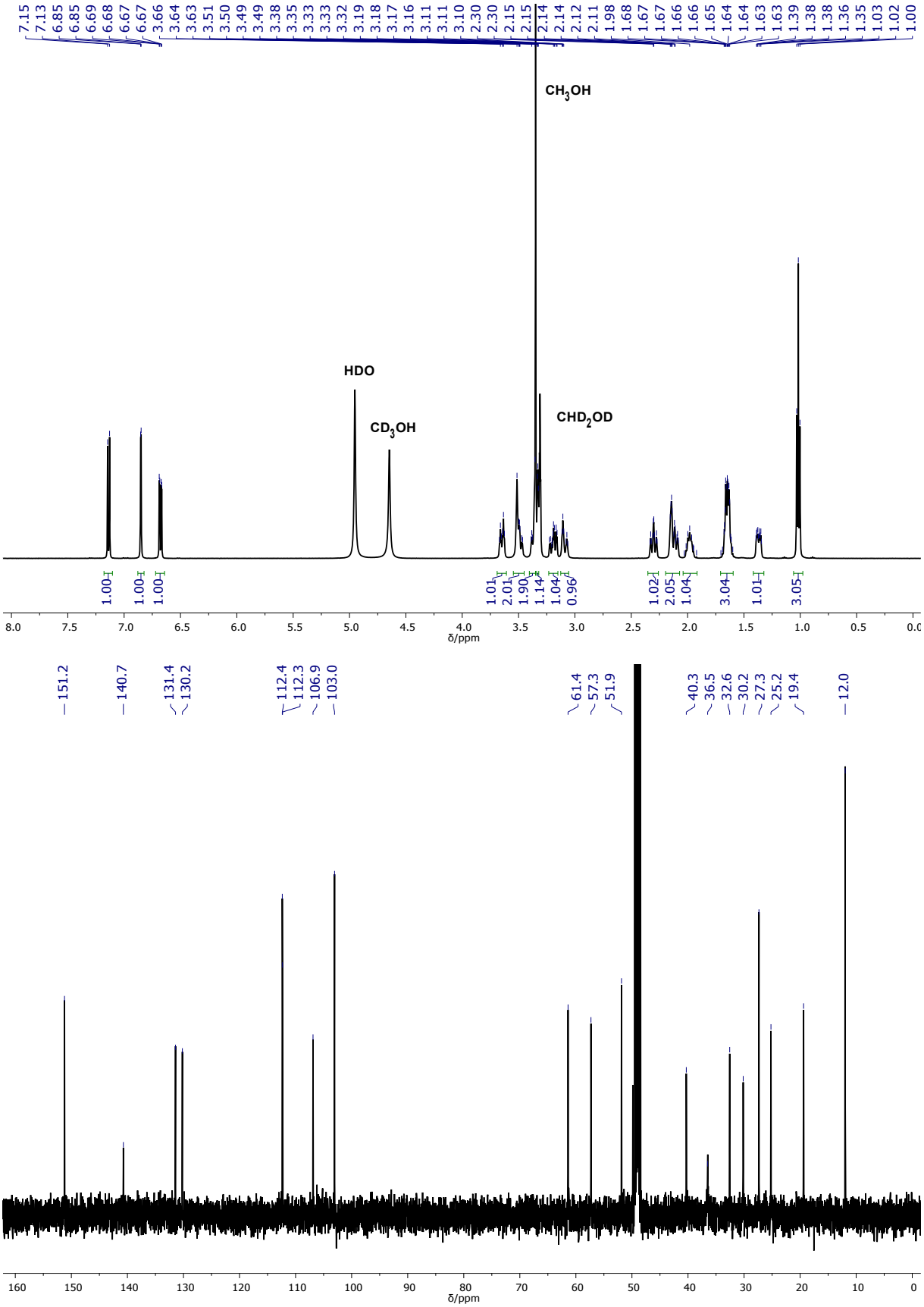
Voacangine (12-methoxy-16-carbomethoxyibogamine) isolated from *Voacanga Africana* root bark (see Material and Methods section in the manuscript)
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.13 (d, *J* = 8.7 Hz, 1H), 6.93 (d, *J* = 2.3 Hz, 1H), 6.80 (dd, J = 8.7, 2.4 Hz, 1H), 3.84 (s, 3H), 3.70 (s, 3H), 3.55 (s, 1H), 3.44 – 3.33 (m, 1H), 3.26 – 3.08 (m, 2H), 3.03 – 2.86 (m, 2H), 2.81 (d, *J* = 8.5 Hz, 1H), 2.58 (dd, *J* = 11.8, 2.1 Hz, 1H), 1.94 – 1.81 (m, 2H), 1.73 (t, *J* = 11.1 Hz, 1H), 1.56 (dt, *J* = 22.0, 7.4 Hz, 1H), 1.49 – 1.39 (m, 1H), 1.37 – 1.28 (m, 2H), 1.15 – 1.08 (m, 1H), 0.90 (t, *J* = 7.3 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 175.71, 153.98, 137.51, 130.53, 129.19, 116.62, 111.81, 111.07, 110.12, 100.77, 99.99, 77.34, 77.03, 76.71, 57.52, 56.04, 55.14, 53.13, 52.59, 51.52, 39.15, 36.56, 32.03, 27.35, 26.76, 22.22, 11.68.

Ibogaine·HCl prepared by decarboxylation of voacangine (see Material and Methods section in the manuscript)
^{1}H NMR (400 MHz, CD_3OD) δ (ppm) =7.19 (d, *J* = 8.9 Hz, 1H), 6.97 (d, *J* = 2.4 Hz, 1H), 6.76 (dd, *J* = 8.8, 2.5 Hz, 1H), 3.87 (s, 3H), 3.70 (dt, *J*= 13.4, 4.2 Hz, 1H), 3.63 – 3.53 (m, 2H), 3.45 – 3.34 (m, 3H), 3.31 – 3.14 (m, 2H), 2.32 (ddt, 13.5, 12.1, 2.7 Hz, 1H), 2.19 – 2.09 (m, 2H), 2.06 (hept, *J* = 7.5 Hz, 1H), 1.74 – 1.65 (m, 3H), 1.46 – 1.34 (m, 1H), 1.03 (t, *J* = 7.3 Hz, 3H) **^{13}C NMR** (100 MHz, CD_3OD) δ(ppm) = 153.0, 139.1, 130.4, 128.5, 111.2, 111.1, 106.0, 99.5, 60.1, 56.0, 54.9, 50.5, 39.0, 35.1, 31.2, 28.8, 26.0, 23.9, 18,0, 10.5

Noribogaine·HCl prepared in Sames laboratory from voacangine by demethylation followed by ester hydrolysis and decarboxylation and in Laboratorio de Síntesis Orgánica (Facultad de Química-UdelaR) by direct demethylation from ibogaine (see Material and Methods section in the manuscript). Spectra were recorded on Bruker Avance III HD 500 MHz. A mixture of CD_3OD+D_2O was used to increase the solubility and slow down the crystallization of the sample.
^{1}H NMR (500 MHz, CD_3OD+D_2O) δ 7.14 (d, *J* = 8.6 Hz, 1H), 6.85 (d, *J* = 2.3 Hz, 1H), 6.68 (dd, *J* = 8.6, 2.4 Hz, 1H), 3.65 (dt, *J* = 13.5, 4.1 Hz, 1H), 3.55 – 3.45 (m, 2H), 3.40 – 3.35 (m, 2H), 3.34 – 3.32 (m, 1H), 3.19 (ddd, *J* = 17.7, 11.7, 4.1 Hz, 1H), 3.09 (dt, *J* = 17.9, 3.9 Hz, 1H), 2.30 (ddt, *J* = 14.4, 12.2, 2.6 Hz, 1H), 2.20 – 2.07 (m, 2H), 1.98 (p, *J* = 7.6 Hz, 1H), 1.71 – 1.60 (m, 3H), 1.37 (ddt, *J* = 13.7, 5.1, 2.4 Hz, 1H), 1.02 (t, *J* = 7.3 Hz, 3H).**^{13}C NMR** (126 MHz, CD_3OD+D_2O) δ 151.2, 140.7, 131.4, 130.2, 112.4, 112.3, 106.9, 103.0, 61.4, 57.3, 51.9, 40.3, 36.5, 32.6, 30.2, 27.3, 25.2, 19.4, 12.0.

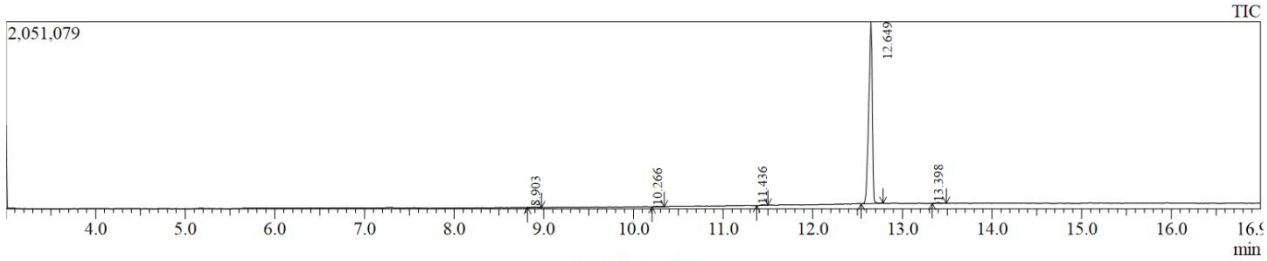


Supplementary Figure 1. Ibogaine-HCl ^{1}H-NMR and ^{13}C-NMR spectra recorded in CD_3OD. Signals at 4.90 and 4.62 corresponds to H_2O found in the CD_3OD, and hydroxylic signal of CD_3OH due to D/H interchange respectively.



Supplementary Figure 2. Noribogaine ^{1}H-NMR and ^{13}C-NMR spectra.

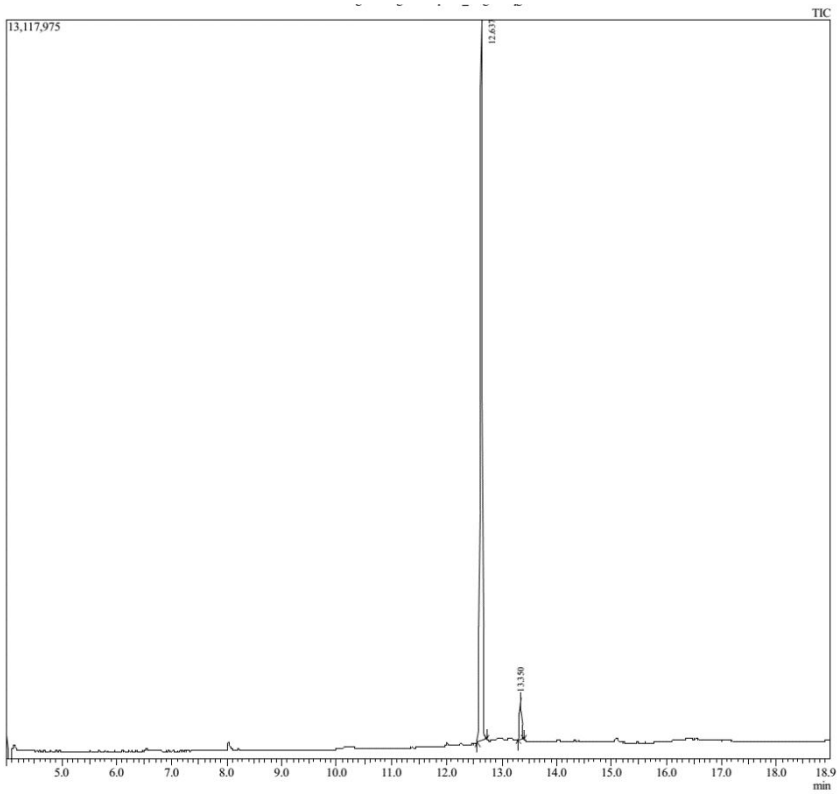
To determine ibogaine-HCl and noribogaine-HCl purity (after crystallization and purification procedures described in the Materials and Methods section of the manuscript) Gas Chromatography analysis was carried out in a GC-MS Shimadzu QP 1100 EX instrument using the electron impact mode, 70 eV. For analysis sample was previously dissolved in aqueous NaOH 10% and extracted with Ethyl Acetate. Conditions: Column HP-5MS (30m x 0.25mm x 0.25um) Temperature Program 200 ºC (Hold time, 2 minutes) to 300 ºC (Hold time, 5 minutes) with a rate of 10 ºC/min. Ibogaine purity was determined as 98.3% and Noribogaine 95.2 %(See chromatograms below)



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	8.903	8.817	8.975	32164	0.58	9633	0.48	3.34	MI	
2	10.266	10.208	10.342	16222	0.29	6610	0.33	2.45	MI	
3	11.436	11.375	11.500	16385	0.30	6125	0.30	2.68	MI	
4	12.649	12.542	12.783	5421252	98.31	1990692	98.49	2.72	MI	
5	13.398	13.333	13.492	28586	0.52	8238	0.41	3.47	MI	
				5514609	100.00	2021298	100.00			

Supplementary Figure 3. Ibogaine-HCl GC-MS chromatogram



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	12.637	12.558	12.725	39608987	95.17	12788798	93.85	3.10		
2	13.350	13.300	13.408	2012179	4.83	837995	6.15	2.40		
				41621166	100.00	13626793	100.00			

Supplementary Figure 4. Noribogaine-HCl GC-MS chromatogram.

2. Pharmacokinetic (PK) Study

Plasma pharmacokinetics and brain distribution of ibogaine and/or its metabolite noribogaine were determined in three study groups of male Wistar rats following a single intravenous (5 mg/kg) and intraperitoneal (40 mg/kg) administration of ibogaine and intraperitoneal (40 mg/kg) administration of noribogaine.

Test Guidelines / SOPs/Compliance

The study was conducted at Sai Life Sciences Limited, Pune, India, with approval of Institutional Animal Ethics Committee (IAEC) in accordance with requirement of The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Sample Formulation

Solution of nor/ibogaine were administered in 10% ethanol and 90% normal saline via intraperitoneal/intravenous route. Formulation strengths for intravenous and intraperitoneal administration were 1 and 4 mg/mL, respectively. A weighed quantity of compound was combined with an appropriate volume of vehicle (10% ethanol and 90% normal saline) and the mixture was vortexed until a clear yellowish solution was obtained. The dosing volume administered was 5 mL/kg for intravenous and 10 mL/kg for intraperitoneal route.

Animal Welfare

All procedures of the present study were in accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) as published in The Gazette of India, December 15, 1998. Prior approval of the Institutional Animal Ethics Committee (IAEC) was obtained before initiation of the study.

Test Subjects

Healthy male Wistar rats (8 - 12 weeks old) weighing between 280 to 300 g were procured from Global, India. Three rats were housed in each cage. Temperature and humidity were maintained at 22 ± 3 ºC and 30 - 70 %, respectively and illumination was controlled to give a sequence of 12 h light and 12 h dark cycle. Temperature and humidity were recorded by auto-controlled data logger system. All the animals were provided laboratory rodent diet (Envigo Research private Ltd, Hyderabad). Reverse osmosis water treated with ultraviolet light was provided *ad libitum*.

Sample Collection

Blood samples (approximately 120 µL) were collected under light isoflurane anesthesia from retro orbital plexus from a set of three rats at specified time intervals. Samples were collected into labeled micro-tubes, containing K_2EDTA solution (10 µL of 20% K_2EDTA of solution per mL of blood) as an anticoagulant. Plasma was immediately harvested from the blood by centrifugation at 4000 rpm for 10 min at 4 ± 2 ºC and stored below -70 ± 10 ºC until bioanalysis.

Immediately, after blood collection animals were euthanized with excess CO_2 and brain was isolated at each time point. Brain was rinsed three times in ice cold PBS (for 5-10 seconds/rinse using ~5-10 mL fresh PBS in disposable petri dish for each rinse) and dried on blotting paper. Brain samples were homogenized using ice-cold phosphate buffer saline (pH 7.4) and homogenates were stored below -70 ± 10 ºC until analysis. Total homogenate volume was three times the tissue weight.

Drug administered	# of animals	Route	Dose (mg/kg)	Time points (h)								
Ibogaine	27	*i.p.*	40	0.08	0.25	0.5	1	2	3	6	12	24
Ibogaine	24	*i.v.*	5	0.017	0.05	0.1	0.5	1	3	6	12	/
Noribogaine	*27*	*i.p.*	40	0.08	0.25	0.5	1	2	3	6	12	24

Bioanalysis

Concentrations of nor/ibogaine in rat plasma and brain samples were determined by fit-for-purpose LC-MS/MS method usingWaters® UPLC-MS\MS-API 4000 equipped with either Triple Quadra or Q-TRAP mass spectrometer (AB Sciex).

Mobile Phase A**:** 0.1% Formic acid in Acetonitrile B**:** 10 mM Ammonium Formate

Column**:** Kinetex EVO, C18, 100A, 100 X 4.6 mm, 5 µm

Injection Volume: 2 µL

Column Oven Temperature: 45 ºC

LC Gradient Used

Time (min)	**Flow Rate (mL/min)**	**PUMP A (%)**	**PUMP B (%)**
Initial	1.0	20	80
1.0		98	2
2.2		98	2
2.4		20	80
3.2		20	80

Mass Conditions

MRM Transitions:

Analyte ID / IS ID	**Q1**	**Q3**	**DP**	**CE**	**CXP**	**Dwell time (msec)**
Noribogaine	297.2	159.7	89	48	10	50
Glipizide	446.3	347.0	40	22	12	50

IS: Internal Standard

Source Parameter:

Polarity	Positive
CAD	8
CUR	25
GS1	40
GS2	60
Ion Spray Voltage	5500
Temperature	550
Interface Heater	ON
EP	10

The extraction procedure for plasma and brain samples and the spiked plasma and brain calibration standards were identical: A 25 µL of study sample or spiked plasma and brain calibration standard was added to individual pre-labeled micro-centrifuge tubes followed by 100 µL of internal standard prepared in Acetonitrile (Glipizide, 500 ng/mL) was added except for blank, where 100 µL of Acetonitrile was added. Samples were vortexed for 5 minutes and then centrifuged for 10 minutes at a speed of 4000 rpm at 4 °C. Following centrifugation, 100 µL of clear supernatant was transferred in 96 well plates and analyzed using LC-MS/MS.

Data Analysis

Non-compartmental analysis tool of Phoenix WinNonlin® (Version 7.0) was used to assess the pharmacokinetic parameters. Peak plasma concentrations (Cmax) and time for the peak plasma concentrations (Tmax) were the observed values. The areas under the concentration time curve (AUC_{last} and AUC_{inf}) were calculated by linear trapezoidal rule. The terminal elimination rate constant, ke was determined by regression analysis of the linear terminal portion of the log plasma concentration-time curve. The terminal half-life ($T_{1/2}$) was estimated by 0.693/ke; $CL_{i.v.}$= Dose/AUC_{inf}; V_{ss}= MRT X $CL_{i.v.}$; %F = [(Mean $AUC_{p.o.}$ × $Dose_{i.v.}$) / (Mean $AUC_{i.v.}$ × $Dose_{p.o.}$)] × 100

Determined Pharmacokinetic Parameters

	Drug administered	Ibogaine		Ibogaine		Noribogaine
	Route	i.p.		i.v.		i.p.
	Dose (mg/kg)	40		5		40
	Detected Compound	Ibo	Noribo	Ibo	Noribo	Noribo
Matrix (Plasma)	**T_{max}(h)**	0.08	0.5	-	-	0.5
	$^{a}C_o/C_{max}$ (ng/mL)	1973.24	1305.64	1331.03	4.31	3754.41
	$T_{1/2}$ (h)	-	-	1.53	2.42	-
	CL (mL/min/kg)	-	-	76.50	116.60	-
	AUClast (hr*ng/mL)	4221.21	8497.58	1086.45	687.76	11448.12
	AUCinf (hr*ng/mL)	4322.39	8535.90	1089.33	714.67	12034.71
	Vss (L/kg)	-	-	6.99	27.27	-
Matrix (Brain)	**T_{max}(h)**	0.08	0.25	-	-	0.25
	$^{a}C_o/C_{max}$ (ng/mL)	19495.65	8588.04	7218.92	32.4	43290.19
	$T_{1/2}$ (h)	-	-	1.22	2.69	-
	CL (mL/min/kg)	-	-	12.85	17.45	-
	AUClast (hr*ng/mL)	29556.15	58459.04	6351.05	4539.95	107427.38
	AUCinf (hr*ng/mL)	30539.21	59286.00	6484.01	4775.00	108327.50
	Vss (L/kg)	-	-	0.81	4.21	-

a - back extrapolated conc. for i.v. group

C_{max}: maximum concentration; **T_{max}**: time to reach maximum concentration; **$T_{1/2}$**: terminal elimination half-life; **CL**: clearance; ***i.p.***: Intraperitoneal; ***i.v.***:Intravenous; **Vss**: Volume of distribution; **AUC**: Area Under the Curve

Mean brain-to-plasma concentration ratio of **ibogaine** following a single intravenous (5 mg/kg) or intraperitoneal (40 mg/kg) dose administration in solution formulation to male Wistar rats

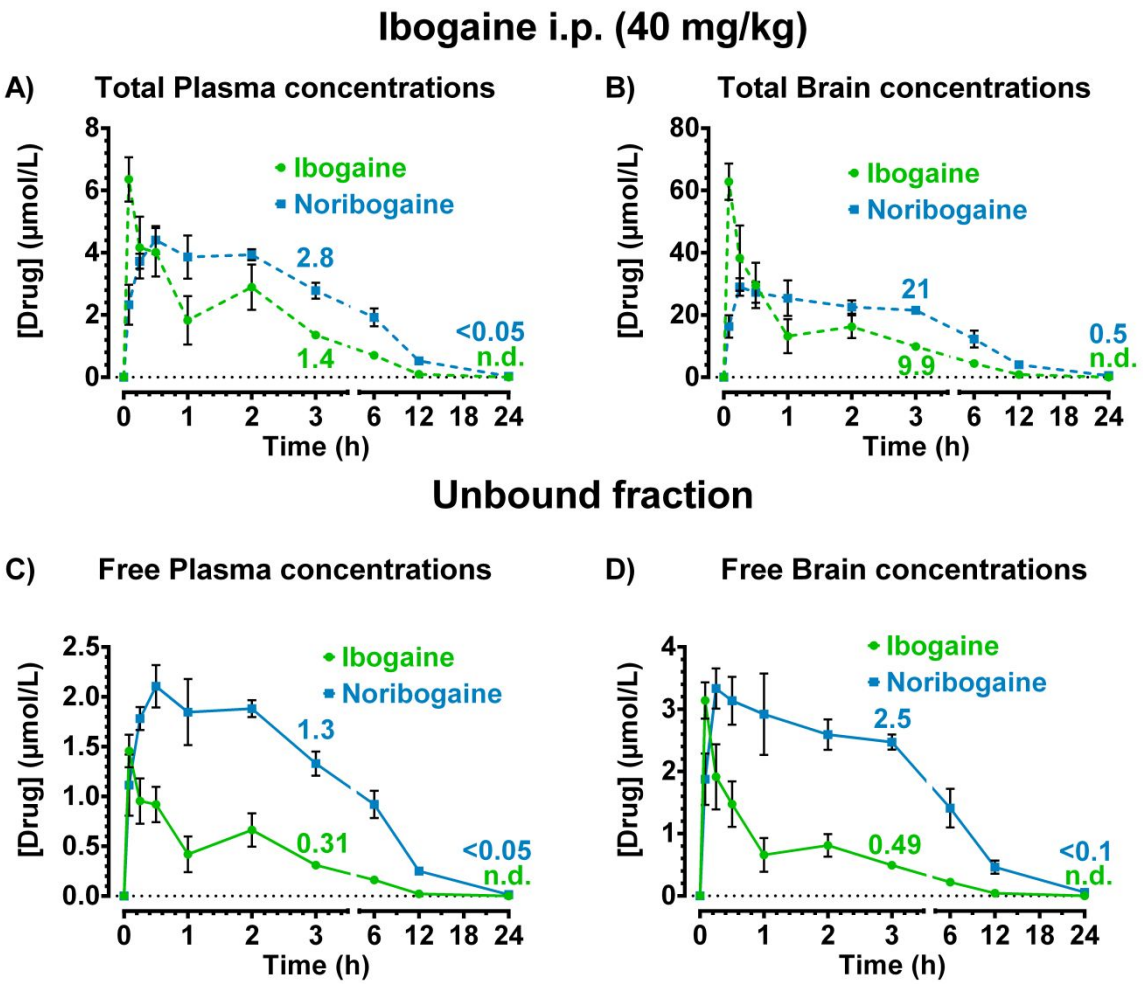
Route	Dose (mg/kg)	Time (h)	Mean plasma Conc. (ng/mL)	SD	#Mean brain Conc. (ng/g)	SD	Brain/Plasma ratio
i.v.	5	0.017	1226.78	95.6	7218.92	2608.24	5.88
		0.05	1047.15	270.49	9588.91	2060.22	9.16
		0.1	929.13	143.08	6853.41	165.05	7.38
		0.5	390.57	51.58	2700.79	279.47	6.91
		1	263.41	19.84	1717.97	113.3	6.52
		3	75.65	28.74	268.15	39.15	3.54
		6	20.87	11.31	90.71	33.4	4.35
		12	1.29	0.24	0.00	0	0.00
i.p.	40	0.08	1973.24	382.65	19495.65	3132.31	9.88
		0.25	1292.48	533.57	11877.71	5626.44	9.19
		0.5	1245.97	417.38	9157.45	3921.78	7.35
		1	567.93	418.5	4088.77	2930.26	7.20
		2	897.91	391	5038.92	1952.93	5.61
		3	420.35	55.3	3059.9	241.29	7.28
		6	218.69	51.16	1370.35	327.99	6.27
		12	29.08	7.67	266.32	210.19	9.16
		24	2.26[c]	NA	0.00	0	0.00

- Brain conc. as ng/g, density of brain tissue was considered as 1 which is equivalent to plasma density (1); **SD**: Standard Deviation; c- Single value reported.

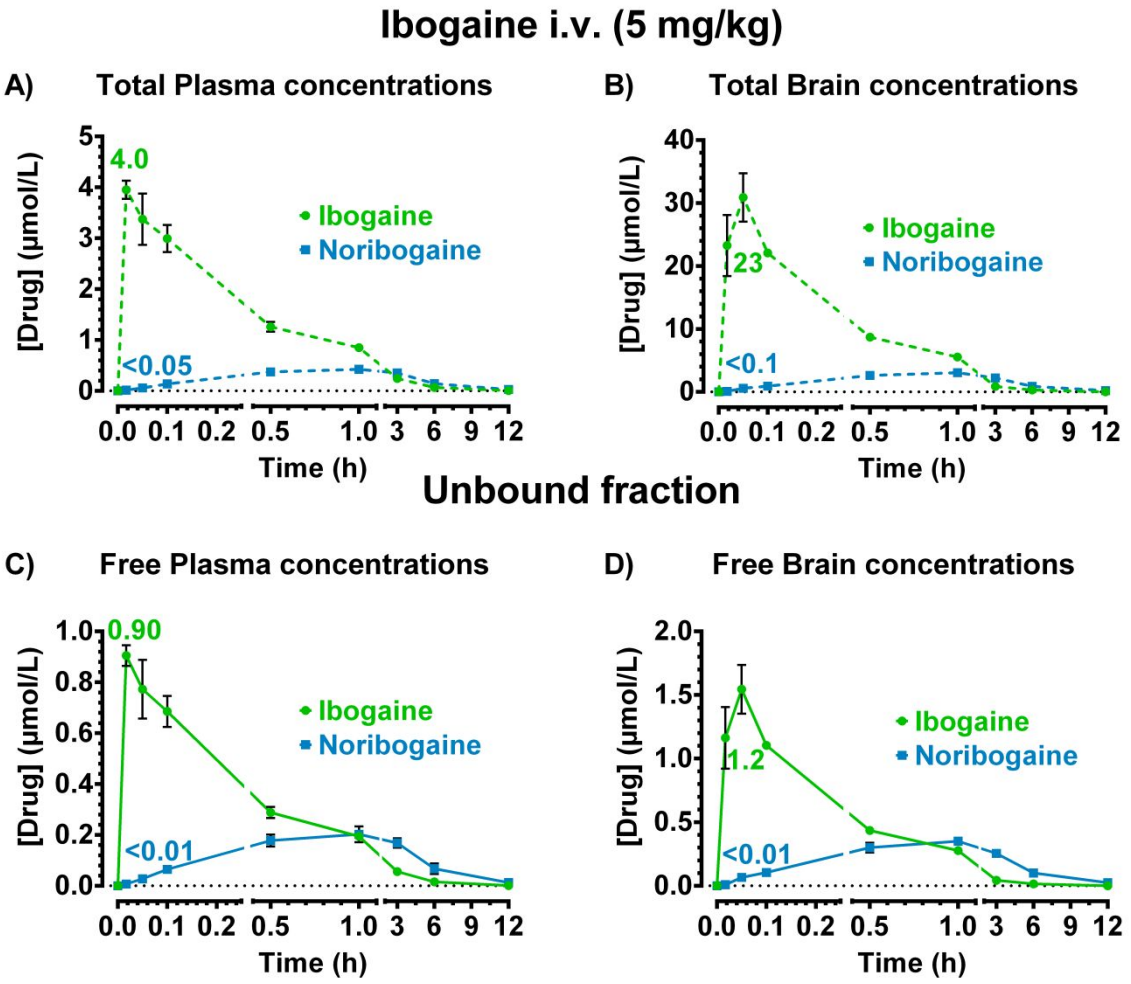
Mean brain-to-plasma concentration ratio of **Noribogaine** (metabolite) following a single intravenous (5 mg/kg) or intraperitoneal (40 mg/kg) dose administration of ibogaine in solution formulation to male Wistar rats

Route	Dose (mg/kg)	Time (h)	Mean plasma Conc. (ng/mL)	SD	#Mean brain Conc. (ng/g)	SD	Brain/Plasma ratio
i.v.	5	0.017	4.31	1.36	32.40[d]	NA	7.52
		0.05	16.99	8.59	169.89	98.57	10.00
		0.1	39.97	15.46	268.35	70.36	6.71
		0.5	110.24	24.83	774.23	171.86	7.02
		1	125.55	33.36	902.36	120.15	7.19
		3	104.38	19.97	656.13	91.41	6.29
		6	41.55	22.26	259.39	105.01	6.24
		12	7.81	0.67	62.5	5.13	8.00
i.p.	40	0.08	690.34	329.23	4830.43	1845.15	7.00
		0.25	1104.47	123.47	8588.04	1443.42	7.78
		0.5	1305.64	228.75	8084.45	1716.31	6.19
		1	1144.62	356.06	7522.75	2924.36	6.57
		2	1166.43	91.39	6681.79	1088.98	5.73
		3	824.26	130.55	6370.13	543.38	7.73
		6	569.93	147.55	3634.25	1388.09	6.38
		12	155.46	21.63	1190.36	470.51	7.66
		24	8.76	2.2	148.10	5.98	16.91

- Brain conc. as ng/g, density of brain tissue was considered as 1 which is equivalent to plasma density (1); **SD**: Standard Deviation
d - Average of two values reported.



Supplementary Figure 5. Total ibogaine and noribogaine concentrationsdetected in A) plasma and B) brain after ibogaine (40 mg/kg) *i.p.* administration, in comparison to unbound fractions in C) plasma and D) brain. Error bars represent mean ± SEM.

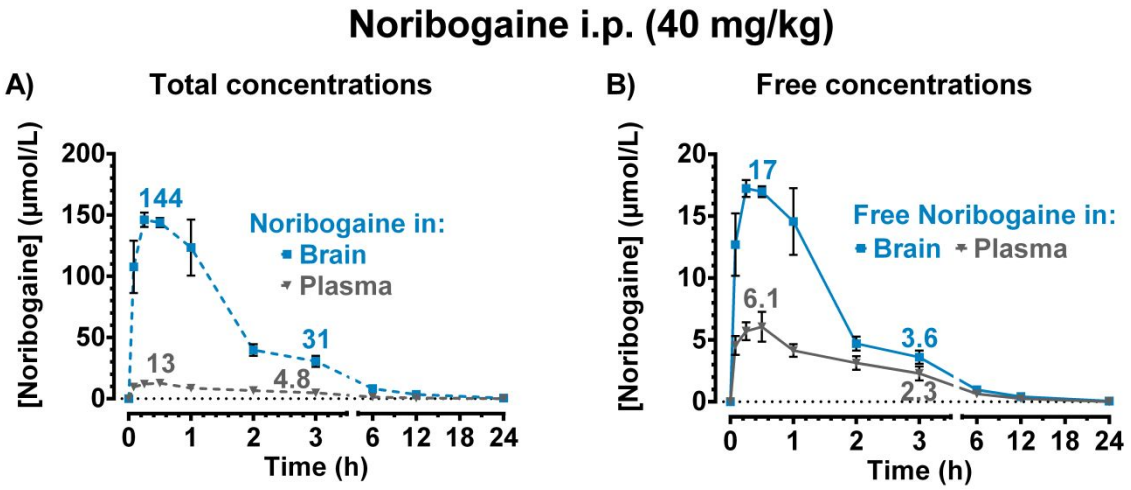


Supplementary Figure 6. Total ibogaine and noribogaine concentrations detected in A) plasma and B) brain after ibogaine (5 mg/kg) *i.v.* administration, in comparison to unbound fractions in C) plasma and D) brain.Error bars represent mean ± SEM.

Mean brain-to-plasma concentration ratio of **noribogaine** following a single intraperitoneal dose administration to male Wistar rats (Dose; 40 mg/kg)

Route	Dose (mg/kg)	Time (h)	Mean plasma Conc. (ng/mL)	SD	# Mean brain Conc. (ng/g)	SD	Brain/Plasma ratio
i.p.	40	0.08	2820.59	466.24	31893.22	6330.53	11.31
		0.25	3537.59	446.57	43290.19	1713.38	12.24
		0.5	3754.41	747.75	42619.47	1105.16	11.35
		1	2566.71	316.03	36570.5	6766.84	14.25
		2	1942.57	344.75	11793.81	1407.37	6.07
		3	1417.81	351.24	9048.06	1340.62	6.38
		6	395.49	88.64	2420.64	264	6.12
		12	153.94	62.11	1034.92	448.47	6.72
		24	0.00	0	140.42	69.3	0.00

- Brain conc. as ng/g, density of brain tissue was considered as 1 which is equivalent to plasma density (1); **SD**: Standard Deviation



Supplementary Figure 7. A) total noribogaine plasma and brain concentrations detected after a single dose of noribogaine (40 mg/kg) *i.p.* administration, in comparison to B) unbound fractions in plasma and brain.Error bars represent mean ± SEM.

3. Plasma Protein and Brain Tissue Binding Study

Ibogaine and noribogaine plasma protein and brain tissue binding were determined using rapid equilibrium dialysis (RED) followed by LC-MS/MS quantification in MRM mode. The study was conducted at Sai Life Sciences Limited, Pune, India.

Preparation of Brain Homogenate

Brain tissue homogenate samples were prepared by diluting one volume of whole brain tissue with three volumes of dialysis buffer (phosphate buffered saline pH 7.4 - 0.1 M sodium phosphate and 0.15 M sodium chloride) to yield 4 times diluted homogenate.

Test Compound Preparation

A 1 mM stock solution of test compounds was prepared in DMSO and diluted 200-folds in rat plasma (Sprague Dawley, male – pool of 5 animals) or brain homogenate to prepare a concentration of 5 µM. The final DMSO concentration was 0.5 %.

Assay Procedures

Rapid equilibrium dialysis was performed with a rapid equilibrium dialysis (RED) device containing dialysis membrane with a molecular weight cut-off of 8,000 Daltons (Thermo Scientific).A 200 µL aliquot of positive controls5 µM and test compound at 5 µM (triplicates) were separately added to the plasma/brain homogenate chamber and 350 µL of phosphate buffer saline (pH 7.4, Thermo Scientific) was added to the buffer chamber of the inserts. After sealing the RED device with an adhesive film, dialysis was performed in incubator at 37 °C with shaking at 100 RPM for 4 hours.

Recovery and stability

A 50 µL aliquot of positive control and test compounds were added to four 0.5 mL microcentrifuge tubes. Two aliquots were frozen immediately (0 minute sample). The other two aliquots were incubated at 37 °C for 4 hours along with the RED device.

Following dialysis, an aliquot of 50 µL was removed from each well (both plasma/brain homogenate and buffer side) and diluted with equal volume of opposite matrix (dialyzed with the other matrix) to nullify the matrix effect. Similarly, 50 µL of buffer was added to recovery and stability samples. An aliquot of 100 µL was submitted for LC-MS/MS analysis.

Sample Preparation and Bio-analysis

A 25 µL aliquot of positive control and test compounds were crashed with 100 µL of acetonitrile containing internal standard and vortexed for 5 minutes. The samples were centrifuged at 4000 RPM at 4 °C for 10 min and 100 µL of supernatant was submitted for LC-MS/MS analysis in MRM mode.

Data Analysis

The samples were run without calibration curve and the peak area ratios (analyte versus internal standard) obtained was used to determine the fraction of compound bound to plasma and brain proteins.

The following equation was used to determine the extent of plasma protein binding:

$$Free\ drug\ (\%) = 100 \times \frac{(\text{peak area ratio in dialysate buffer})}{(\text{Peak area ratio in dialysate plasma})}$$

$$Bound\ drug\ (\%) = 100 - free\ drug(\%)$$

The following equation was used to determine the extent of ***diluted braintissue binding***.

$$fu_{meas} = 1 - \left(\frac{PC-Pf}{PC}\right) \qquad fu_{brain} = \frac{\frac{1}{D}}{\left(\left(\frac{1}{fu_{meas}}\right)-1\right)+\frac{1}{D}}$$

fu_{meas} = fraction unbound using diluted brain tissue
PC = test compound concentration in tissue-containing compartment
PF = test compound concentration in tissue-free compartment
fu_{brain} = fraction unbound in brain
D = dilution factor of brain tissue

Recovery (%): 100 x (response $_{plasma/brain\_homo}$ + response $_{buffer}$) after dialysis / (response $_{plasma/brain\_homo}$) incubated at 37 ºC in tubes along with RED device.

Stability (%):100 x(response$_{plasma/brain_homo}$) incubated at 37 ºC in tubes along with RED device/ (response $_{plasma/brain\_homo}$) samples frozen at 0 min

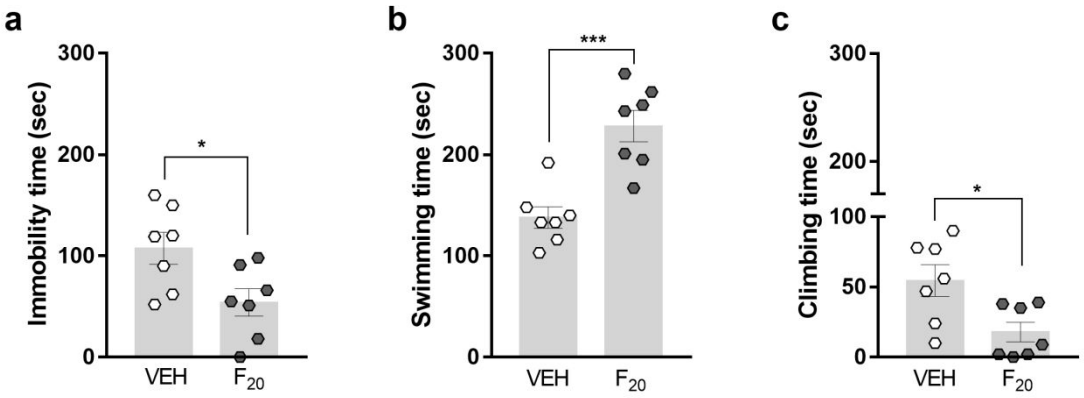
Plasma Protein Binding of Warfarin and Test Compounds in Rat Plasma

Compounds	% Bound				% Unbound	% Recovery	% compound remaining at 4 h
	R1	R2	R3	Mean ± SD			
Warfarin	99.1	99.2	99.2	99.2 ± 0.0	0.08	106	99
Ibogaine	74.6	78.7	78.1	77.1 ± 2.2	22.9	76	104
Noribogaine	49.8	56.3	50.7	52.2 ± 3.5	47.8	74	108

Rat Brain Tissue Binding of Positive Control and Test Compounds

Compounds	% Bound Mean ± SD	% Unbound	% Recovery	% compound remaining at 4 h
Carbamazepine	86.0 ± 0.0	14.0	94	98
Ibogaine	95.0 ± 1.1	5.0	112	83
Noribogaine	88.2 ± 2.7	11.5	87	104

4. Forced Swimming Test after three doses of Fluoxetine.



Supplementary Figure 8. Antidepressant-like effect induced by subacute treatment of fluoxetine 20 mg/kg (F_{20}). Fluoxetine was i.p. administered 24, 5, and 1 h before thetest session. Control group received vehicle (saline) under the same schedule. Error bars represent mean ± SEM of immobility, swimming and climbing time. * $P < 0.05$; *** $P < 0.001$. in comparison to control group. Unpaired Student *t*-test. N = 7 per group.