Supporting Information

Pharmacokinetics and *In Vitro* Blood-Brain Barrier Screening of the Plant-Derived Alkaloid Tryptanthrin

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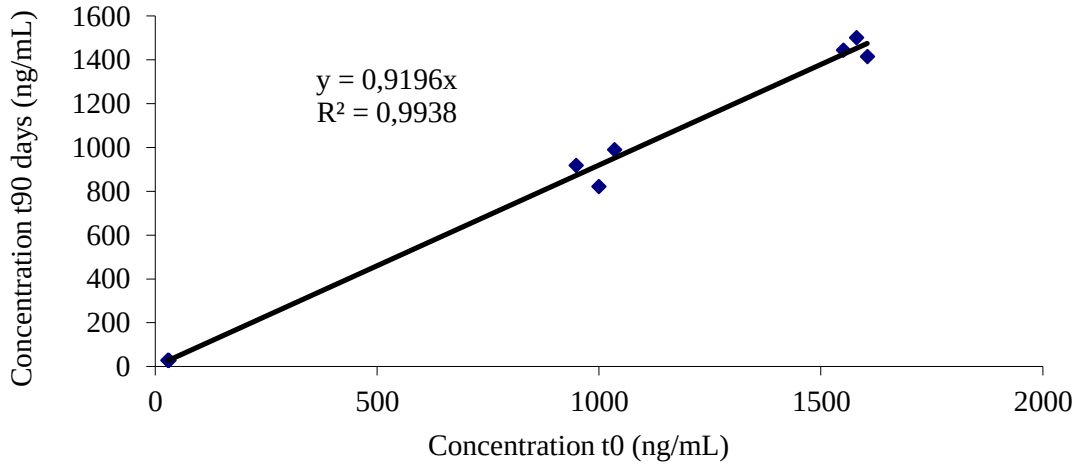

Fig. 1S Long-term stability (LTS) of tryptanthrin in lithium heparinized rat plasma for 90 days below -65°C (n = 3).

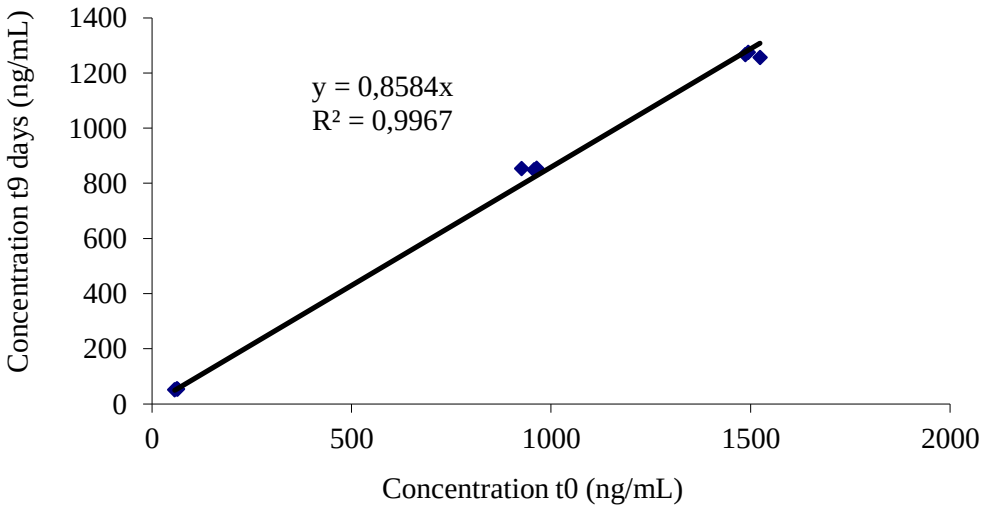

Fig. 2S LTS of tryptanthrin in Ringer HEPES buffer for 9 days below -65°C (n = 3).

Table 1S Calibrators and calibration curve parameters for lithium heparinized rat plasma (n = 10).

Run number	Nominal level (ng/mL)							Regression parameters			
	10.0	**50.0**	**100**	**250**	**500**	**1000**	**2000**	**A**	**B**	**C**	**R^2**
1	10.9	49.0	106	251	511	1048	2072	-0.000000100	0.00183	0.00153	0.998
	10.9	43.5	*84.1	*211	471	976	1920				
2	10.0	52.8	108	271	519	981	1933	-0.0000000904	0.00191	0.00283	0.998
	9.88	45.7	108	236	467	927	2184				
3	11.4	47.5	110	263	511	1023	2011	0.0000000198	0.00122	0.00268	0.999
	11.0	50.1	93.7	242	472	989	1986				
4	10.5	53.8	106	269	489	1021	2062	-0.0000000236	0.00239	0.00452	0.999
	9.88	50.2	99.1	254	481	961	1957				
5	10.1	52.5	109	232	507	1021	2035	-0.000000191	0.00221	0.00450	0.999
	10.2	51.4	106	255	494	957	1980				
Mean	10.5	49.6	105	253	492	990	2014	-0.0000000771	0.00191	0.00321	0.999
S.D.	0.544	3.30	5.34	13.7	19.1	37.4	78.7	0.0000000805	0.000450	-	-
CV%	**5.19**	**6.66**	**5.08**	**5.41**	**3.88**	**3.78**	**3.91**				
RE%	**4.84**	**-0.724**	**5.19**	**1.03**	**-1.54**	**-0.959**	**0.705**				

Response = A × $Conc.^2$ + B × Conc. + C
Quadratic regression, 1/X weighting, origin: included
*> 15.0% outside acceptance criteria, not used for calculations

Table 2S Calibrators and calibration curve parameters for Ringer HEPES buffer (n = 12).

Run number	Nominal level (ng/mL)							Regression parameters			
	20.0	**50.0**	**100**	**250**	**500**	**1000**	**2000**	**A**	**B**	**C**	**R^2**
1	18.9	43.2	95.2	250	477	939	1873	-0.000000675	0.005543	-0.00485	0.996
	21.9	47.8	107	271	542	*1156	2171				
2	18.0	43.8	99.6	252	469	864	1815	-0.000000797	0.000526	-0.00415	0.993
	20.7	49.8	110	264	566	1038	2299				
3	21.7	44.2	101	256	528	979	2101	-0.00000101	0.000593	-0.0130	0.999
	19.2	45.9	99.9	244	510	974	1932				
4	18.1	46.2	94.8	261	541	925	2224	-0.000000973	0.00487	-0.00805	0.995
	20.8	49.7	96.8	262	557	871	2037				
5	19.3	49.0	102	259	506	1009	2132	-0.000000586	0.000284	-0.00311	0.999
	17.9	49.2	106	262	495	927	1984				
6	18.1	48.9	108	246	522	955	1921	-0.000000349	0.000207	0.000934	0.998
	19.0	52.3	106	256	516	939	2183				
Mean	19.5	47.5	102	257	519	947	2056	-0.000000732	0.00200	-0.00537	0.997
S.D.	1.45	2.83	5.15	7.86	29.9	52.7	152	0.000000250	0.00249	-	-
CV%	**7.43**	**5.97**	**5.04**	**3.06**	**5.76**	**5.56**	**7.41**				
RE%	**-2.64**	**-5.04**	**2.14**	**2.78**	**3.78**	**-5.28**	**2.80**				

Response = A × $Conc.^2$ + B × Conc. + C
Quadratic regression, 1/X weighting, origin: included
*> 15.0% outside acceptance criteria, not used for calculations

Table 3S Intra-run (n = 6) and inter-run (n = 18) imprecision (expressed as CV%) and inaccuracy (expressed as RE%) of lithium heparinized rat plasma QC samples, based on 3 series of 6 replicates for each level.

	LLOQ	QCL	QCM	QCH	ULOQ
Nominal levels (ng/mL)	**10.0**	**30.0**	**1000**	**1600**	**2000**
Intra-run Mean	10.9	31.2	963	1464	1736
Intra-run S.D.	0.176	1.97	69.4	38.8	32.4
Intra-run CV%	**1.61**	**6.30**	**7.21**	**2.65**	**1.86**
Intra-run RE%	**9.41**	**4.10**	**-3.73**	**-8.52**	**-13.2**
Inter-run Mean	10.8	31.2	999	1411	1785
Inter-run S.D.	0.494	2.22	64.9	43.8	68.6
Inter-run CV%	**4.59**	**7.10**	**6.50**	**3.11**	**3.84**
Inter-run RE%	**7.74**	**4.05**	**-0.0702**	**-11.8**	**-10.8**

Table 4S Intra-run (n = 6) and inter-run (n = 18) imprecision (expressed as CV%) and inaccuracy (expressed as RE%) of RHB QC samples, based on 3 series of 6 replicates for each level.

	LLOQ	QCL	QCM	QCH	ULOQ
Nominal levels (ng/mL)	**20.0**	**60.0**	**1000**	**1600**	**2000**
Intra-run Mean	18.9	61.3	908	1405	2056
Intra-run S.D.	2.42	2.26	25.3	31.4	22.7
Intra-run CV%	**12.8**	**3.68**	**2.79**	**2.23**	**1.10**
Intra-run RE%	**-5.52**	**2.18**	**-9.17**	**-12.2**	**2.82**
Inter-run Mean	18.5	59.2	1015	1559	2073
Inter-run S.D.	1.67	2.85	25.7	37.6	71.9
Inter-run CV%	**9.04**	**4.82**	**2.53**	**2.41**	**3.47**
Inter-run RE%	**-7.54**	**-1.28**	**1.55**	**-2.55**	**3.66**

Table 5S Carryover assessment for tryptanthrin as the analyte, and for (*E*,*Z*)-3-(benzylidenyl)-indolin-2-one as the I.S. in lithium heparinized rat plasma (n = 10).

Run number	Replicate	Peak response (counts)				Carryover (%)		Mean Carryover (%)	
		Blank sample		LLOQ					
		Analyte	IS	Analyte	IS	Analyte	IS	Analyte	IS
1	1	214	*84649	1894	88280	11.3	*-	12.4	*-
	2	229	*75240	1691	78955	13.6	*-		
2	1	256	0.00	2087	94992	12.3	0.00	10.5	0.00
	2	170	0.00	1963	95081	8.67	0.00		
3	1	55.6	0.00	2558	154281	2.17	0.00	2.36	0.0361
	2	49.5	87.0	1936	120559	2.55	0.0722		
4	1	184	0.00	2796	94349	6.60	0.00	5.81	0.00
	2	125	0.00	2488	88433	5.02	0.00		
5	1	438	192	2666	99474	16.4	0.193	18.5	0.242
	2	539	283	2624	97209	20.5	0.291		
					Mean	**9.91**	**0.0696**		

*Contamination, carryover of the I.S. not calculated

Table 6S Carryover assessment for tryptanthrin as the analyte, and for (*E*,*Z*)-3-(benzylidenyl)-indolin-2-one as the I.S in Ringer HEPES buffer (n = 12).

Run number	Replicate	Peak response (counts)				Carryover (%)		Mean Carryover (%)	
		Blank sample		LLOQ					
		Analyte	IS	Analyte	IS	Analyte	IS	Analyte	IS
1	1	847	471	9387	94179	9.02	0.500	8.61	0.465
	2	897	405	10941	94087	8.20	0.431		
2	1	1032	0.00	8137	90033	12.7	0.00	13.2	0.00
	2	1014	0.00	7405	70891	13.7	0.00		
3	1	513	0.00	4783	41605	10.7	0.00	9.66	0.00
	2	382	0.00	4441	44148	8.60	0.00		
4	1	240	0.00	4684	58675	5.12	0.00	5.95	0.00
	2	345	0.00	5098	54825	6.77	0.00		
5	1	159	0.00	6004	116645	2.65	0.00	2.83	0.0104
	2	149	21.6	4935	103819	3.02	0.0210		
6	1	233	0.00	5842	152568	0.00	0.00	0.00	0.00
	2	254	0.00	5397	134481	0.00	0.00		
					Mean	**6.71**	**0.0793**		

Table 7S Selectivity test at the LLOQ (10.0 ng/mL) for tryptanthrin, based on three different lithium heparinized rat plasma batches (n = 6).

Nominal level (ng/mL)	**10.0**
Mean	10.4
S.D.	0.365
CV%	3.51
RE%	4.02

Table 8S Selectivity test at the LLOQ (20.0 ng/mL) for tryptanthrin based on three different Ringer HEPES buffer batches (n = 6).

Nominal level (ng/mL)	**20.0**
Mean	18.5
S.D.	0.993
CV%	5.37
RE%	-7.62

Table 9S Absolute extraction yields of tryptanthrin and the I.S. (*E*,*Z*)-3-(benzylidenyl)-indolin-2-one for lithium heparinized rat plasma (n = 6).

Tryptanthrin nominal levels (ng/mL)	**30.0**	**1000**	**1600**
Absolute recovery (%)	**79.4**	**77.5**	**77.6**
CV%	8.73	4.90	4.80
SD	6.93	3.80	3.72

Internal final level (ng/mL)	**750**
Absolute recovery (%)	**110**
CV%	3.98
SD	4.38

Table 10S Absolute extraction yields of tryptanthrin and the I.S. (*E*,*Z*)-3-(benzylidenyl)-indolin-2-one for Ringer HEPES buffer (n = 6).

Tryptanthrin nominal levels (ng/mL)	**60.0**	**1000**	**1600**
Absolute recovery (%)	**60.8**	**78.6**	**88.0**
CV%	3.77	3.26	2.68
SD	2.29	2.56	2.36

Internal final level (ng/mL)	**433**
Absolute recovery (%)	**94.4**
CV%	1.81
SD	1.71

Table 11S Dilution test in lithium heparinized rat plasma (n = 6).

Nominal level (ng/mL)	**4000**
Dilution factor	**10X**
Mean	3833
S.D.	100
CV%	**2.62**
RE%	**-4.19**
Dilution factor	**100X**
Mean	4314
S.D.	104
CV%	**2.41**
RE%	**7.85**

Table 12S Dilution test in Ringer HEPES buffer (n = 6).

Nominal level (ng/mL)	**4000**
Dilution factor	**10X**
Mean	3961
S.D.	130
CV%	**3.29**
RE%	**-0.970**
Dilution factor	**100X**
Mean	4186
S.D.	105
CV%	**2.52**
RE%	**4.66**

Table 13S Short-term stabilities in lithium heparinized rat plasma during storage in various conditions (expressed as RE%) (n = 6).

Nominal levels (ng/mL)	30.0	1600
Three successive freeze/thaw cycles below -65°C	-1.60	-13.5
Stored rat plasma at RT for 4 h	4.70	-12.5
Processed rat plasma at 10°C for 36 h	-6.58	-12.8

Table 14S Short-term stabilities in Ringer HEPES buffer during storage in various conditions (expressed as RE%) (n = 6).

Nominal levels (ng/mL)	60.0	1600
Three successive freeze/thaw cycles below -65°C	9.74	3.24
Stored RHB at RT for 4 h	-1.32	3.27
Processed RHB at 10°C for 19 h	8.12	-2.29

Table 15S Stability of tryptanthrin stock solution in DMSO stored below -65°C for 427 days and 6 h at RT (n = 6).

	Peak area ratios
Stock solutions tested	**New (t = 0) SS tryptanthrin + New SS I.S.**
Mean	4.09
S.D.	0.0716
CV%	1.75
Stock solutions tested	**Old (t = 427 days) SS tryptanthrin + New SS I.S.**
Mean	4.03
S.D.	0.0211
CV%	0.524
Difference %	**-1.59**

Table 16S Mean values of the most relevant *in silico* descriptors for permeability.

Tryptanthrin (MW: 248.24 g/mol)	
QikProp descriptors (3D)	**Chemaxon Marvin (2D)**
donorHB: 0.00	LogPo/w: 2.40
accptHB: 6.00	LogD7.4: 2.40
LogPo/w: 1.01	PSA [$Å^2$]: 49.7
LogBB: -0.450	
Human Oral Absorption [%]: 84.4	
PSA [$Å^2$]: 73.1	