Supporting information

Unraveling the In Vitro Toxicity Profile of Psychedelic 2C Phenethylamines and Their *N*-Benzylphenethylamine (NBOMe) Analogues

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Figures

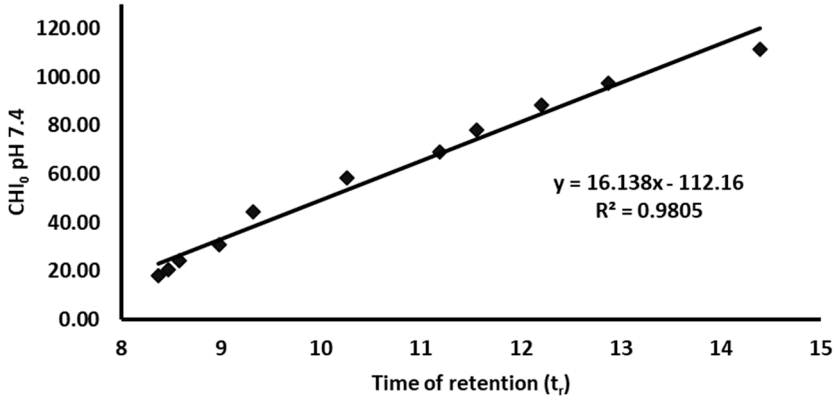

Figure S1. Calibration line obtained after plotting the individual retention times of the standard compounds as a function of their respective literature CHI values at pH 7.4.

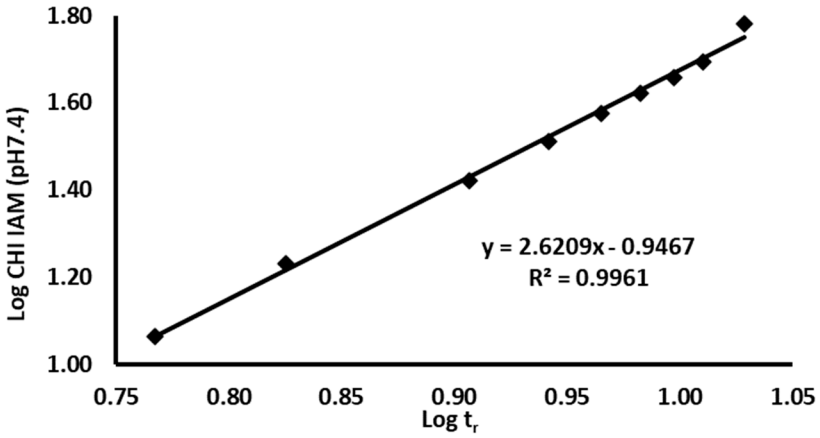

Figure S2. Calibration line obtained after plotting the logarithmic values of the individual retention times of the standard compounds as a function of the logarithmic values of their respective CHI (IAM) values at pH 7.4.

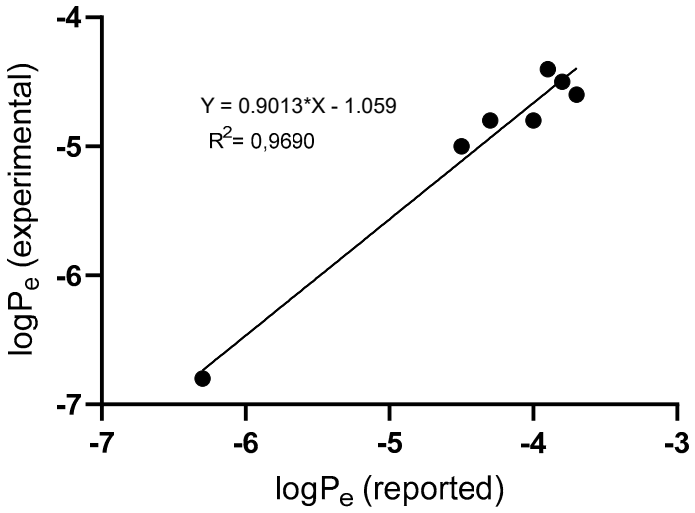

Figure S3. Calibration line obtained after plotting the controls experimental and reported PAMPA-BBB permeability (see data on the Table 2 of the manuscript).

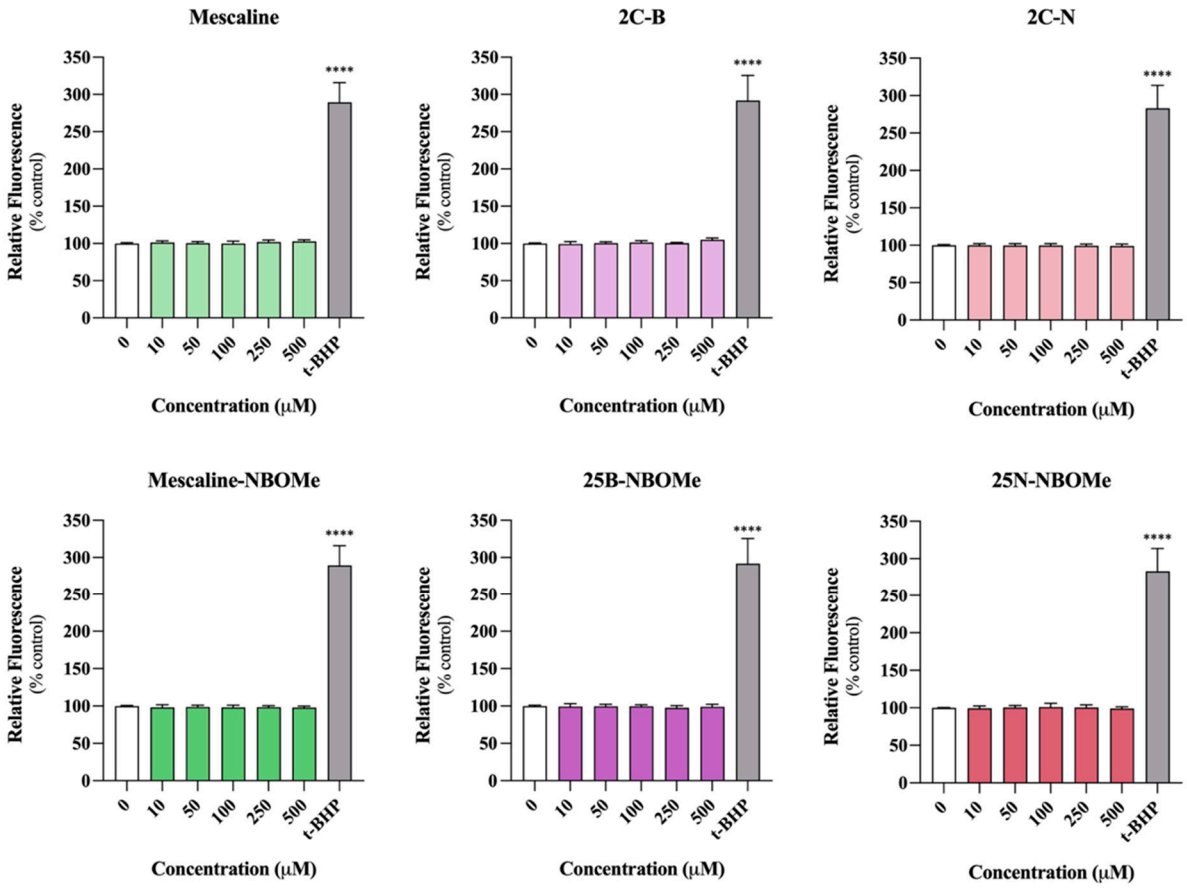

Figure S4. Intracellular reactive oxygen species (ROS) levels evaluated with DCFH-DA probe (10 μM), in differentiated SH-SY5Y cells, 24 hours after exposure to the tested drugs. Results are presented as Mean ± SD of at least 4 independent experiments (performed in triplicate). Tert-butyl hydroperoxide (t-BHP, 200 μM) was used as a positive control. Statistical comparisons were performed using One-way ANOVA, followed by Dunnett's multiple comparison post hoc test. [**** $p < 0.0001$ vs. control (0 μM)]. In all cases, p values < 0.05 were considered statistically significant.

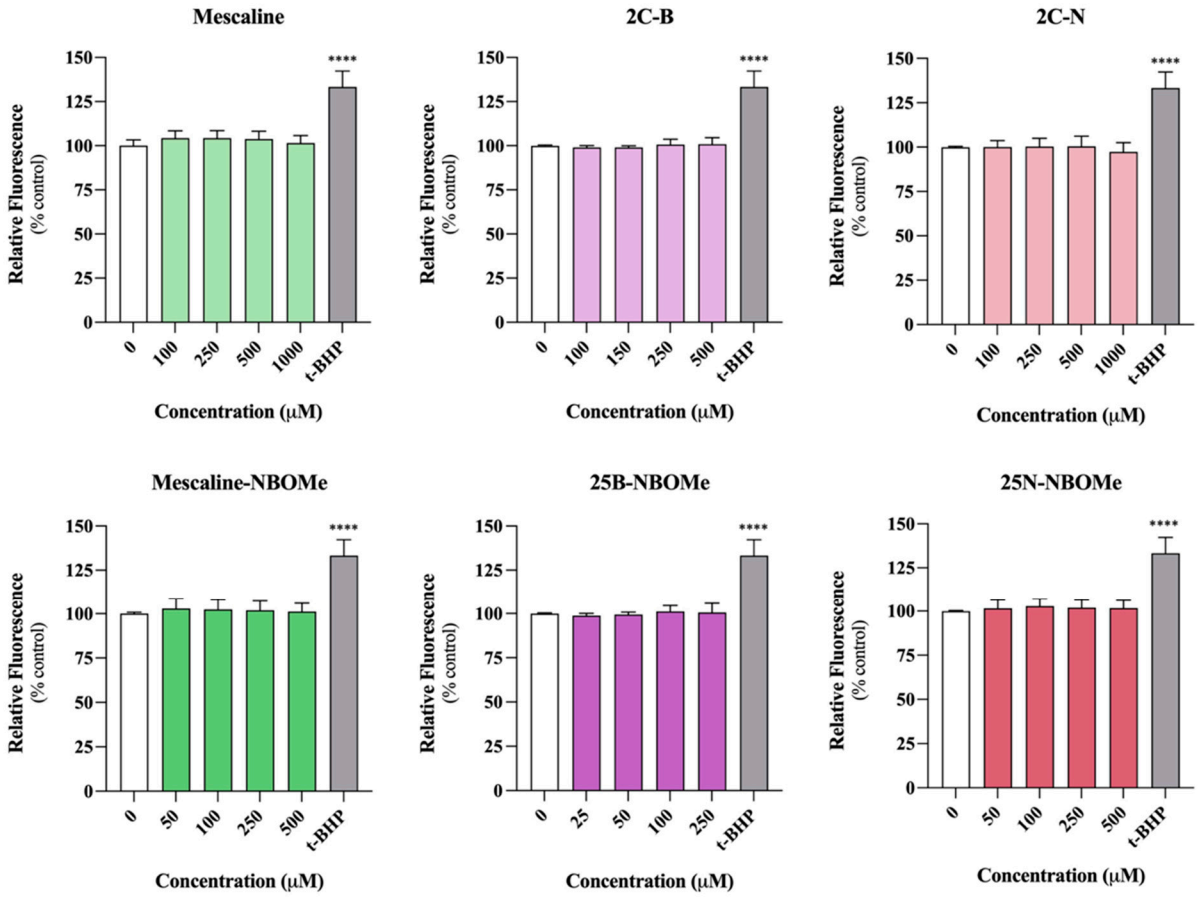

Figure S5. Intracellular reactive oxygen species (ROS) evaluated with DCFH-DA probe (10 μM), 24 hours after exposure of HepG2 cells to the tested drugs. Results are presented as Mean ± SD of at least 4 independent experiments (performed in triplicate). Tert-butyl hydroperoxide (t-BHP, 200 μM) was used as a positive control. Statistical comparisons were performed using One-way ANOVA, followed by Dunnett's multiple comparison post hoc test [**** $p < 0.0001$ vs. control (0 μM)]. In all cases, p values < 0.05 were considered statistically significant.

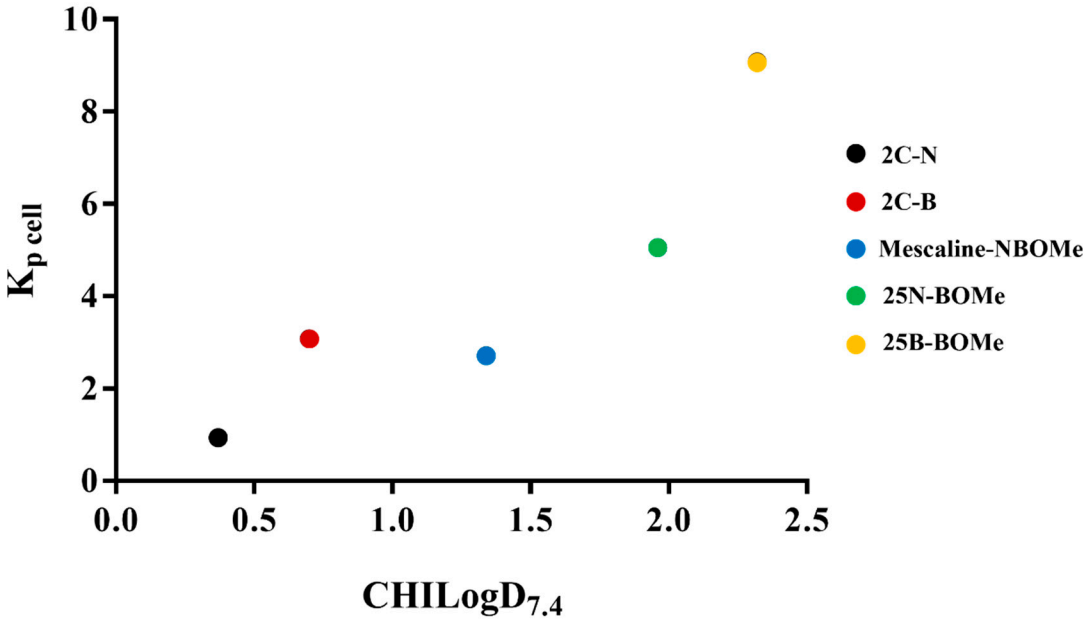

Figure S6. Correlation between the values of K_{pcell} and $CHILogD_{7.4}$ values.

Tables

Table S1. Individual literature CHI values of the of standards at pH 7.4 and their retention times (t_r) obtained by HPLC.

pH 7.4		
Reference compound	**CHI_0**	**t_r**
Theophylline	18.19	8.38
Paracetamol	20.59	8.47
Caffeine	24.12	8.58
Benzimidazole	30.71	8.98
Colchicine	44.32	9.32
Carbamazepine	58.45	10.26
Indole	69.15	11.19
Propiophenone	78.41	11.56
Butyrophenone	88.49	12.21
Valerophenone	97.67	12.88
Heptanophenone	111.8	14.40

Table S2. Individual literature CHI (IAM) values of the standards at pH 7.4, their retention times (t_{rIAM}) experimentally determined by HPLC and their respective logarithmic values.

	pH 7.4			
	t_r (min)	**Log (t_r)**	**CHI (IAM)**	**Log [CHI (IAM)]**
Acetanilide	5.85	0.77	11.57	1.06
Acetophenone	6.69	0.83	17.07	1.23
Propiophenone	8.07	0.91	26.39	1.42
Butyrophenone	8.746	0.94	32.55	1.51
Valerophenone	9.226	0.97	37.71	1.58
Hexanophenone	9.611	0.98	41.91	1.62
Heptanophenone	9.939	1.00	45.49	1.66

Table S3. Evaluation of *h*MAO inhibitory activities of the drugs under study and reference MAO inhibitors.

	% inhibition at 10 µM or IC_{50} (µM)		**SI[a]**
Compound	***h*MAO-A**	***h*MAO-B**	
Mescaline	2.5 %	21.2 %	—
Mescaline-NBOME	3.9 %	89.0 %	—
2CB	17.5 %	27.2 %	—
25B-NBOME	15.0 %	33.9 %	—
2C-N	5.8 %	18.0 %	—
25N-NBOME	8.9 %	25.5 %	—
Clorgyline	0.00260±0.00033	1.93±0.16	0.00135
Rasagiline	3.72 ± 0.38	0.149 ± 0.023	24.9

[a] SI: *h*MAO-B selectivity index = IC_{50}(*h*MAO-A)/IC_{50}(*h*MAO-B).