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Pentedrone and methylone enantiomers: cytotoxicity studies in dopaminergic SH-SY5Y cell line

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Introduction

The consumption of synthetic cathinones, as pentedrone and methylone (Figure 1), has increased exponentially as drug of abuse. Being chiral molecules, each enantiomer may have different binding to proteins, or other chiral biomolecules, leading to many kinetic or dynamic variations. Beside a recent study described by our group [1], there is a lack of scientific information about the enantioselectivity of these substances at toxicity level. Therefore, the aim of this study was to develop a resolution method to obtain pure enantiomers of pentedrone and methylone, and to evaluate their biological/toxicological enantioselectivity.

Experimental

For this purpose an optimized liquid chromatography (LC) chiral method using a semi-preparative Chiralpak AS-H® column, under normal phase elution conditions was carried out to isolate the enantiomers of pentedrone and methylone. For enantioselectivity evaluation, *in vitro* studies were performed in dopaminergic SH-SY5Y cells by incubating each enantiomer of both cathinones.

LC Conditions

Chiralpak® AS-H column (250 × 10 mm i.d., 5 µm particles size). Semipreparative chromatographic was achieved through multiple injections fitted with a 500 µL loop using different proportions of Hex:2-PrOH (*v/v*) as mobile phase. Analyses were performed at 25 ± 1 °C, in isocratic mode under UV detection at a wavelength of 254 nm.

In vitro Conditions

SH-SY5Y cell were routinely cultured in 25 cm2 flasks using DMEM medium supplemented with 10% FBS and 1% of antibiotic. Cells were cultured at a density of 25 000 cells/cm² and differentiated into a dopaminergic phenotype. SH-SY5Y cell differentiation was induced with 10 µM RA for 72 h, followed by 80 nM TPA for 72 h more.

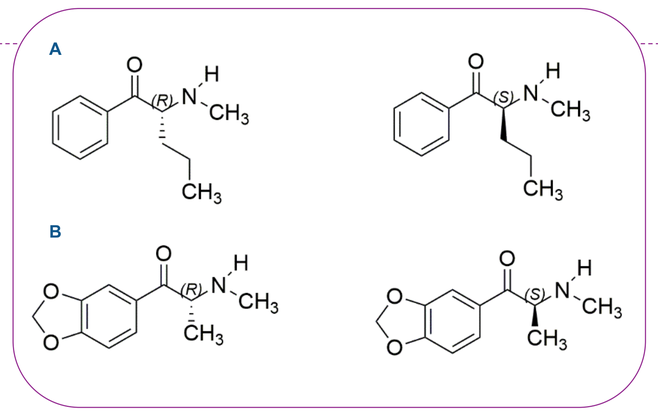

Figure 1. Chemical structures of pentedrone (A) and methylone (B) enantiomers

Results

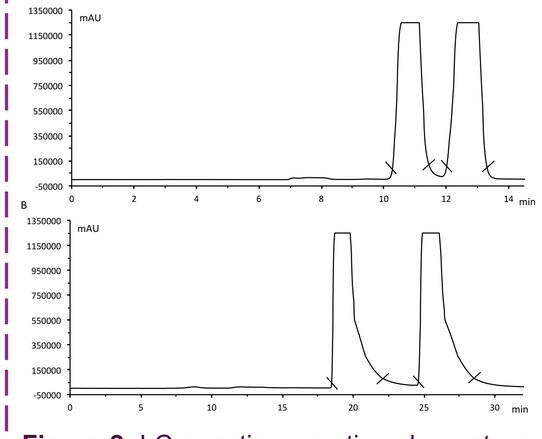

Figure 2. LC enantioseparation chromatogram of pentedrone (A) and methylone (B). Chromatographic conditions: Chiralpak® AS-H column, mobile phase Hex/2-PrOH (97:3, *v/v*) for A and Hex/2-PrOH (85:15, *v/v*) for B, flow rate 2 mL/min, UV detection 254 nm.

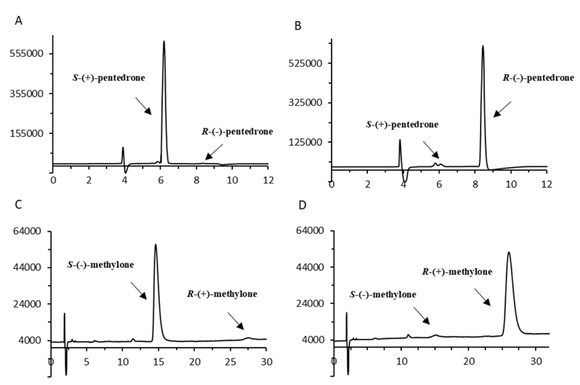

Figure 3. Analytical chromatograms for enantiomeric ratio (e.r.) determination of collected fractions recovered from semipreparative resolution. (A) pentedrone's P1 fraction, (B) pentedrone's fraction P2, (C) methylone's M1fraction and (D) methylone's M2 fraction.

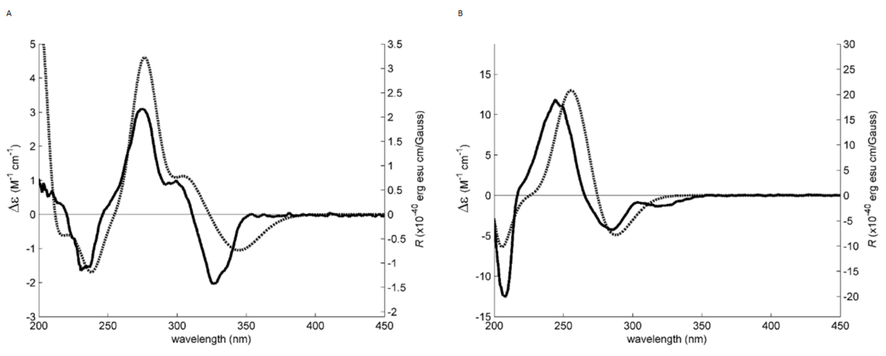

Figure 4. Experimental ECD spectra (solid lines) of (A) M1 fraction and (B) P1 fraction, and simulated ECD spectra (dotted lines) of (A) methylone's C-2(S) and (B) pentedrone's C-2(S) model configurations.

Table 1. Elution order, specific rotation and enantiomeric ratios of pentedrone and methylone enantiomers at 25ºC.

Enantiomer	Elution order	e.r. (%)	$[\alpha]_D$ (c)[a]	Recovery (%)
S-(+)-pentedrone	First	98.4	+ 16 (2.5)	72
R-(-)-pentedrone	Second	97.8	- 12 (2.5)	71
S-(-)-methylone	First	98.3	- 20 (2.5)	80
R-(+)-methylone	Second	97.1	+ 24 (2.5)	79

[a] Specific rotation in EtOH (degrees mL/mg/dm) with c = concentration in mg/mL

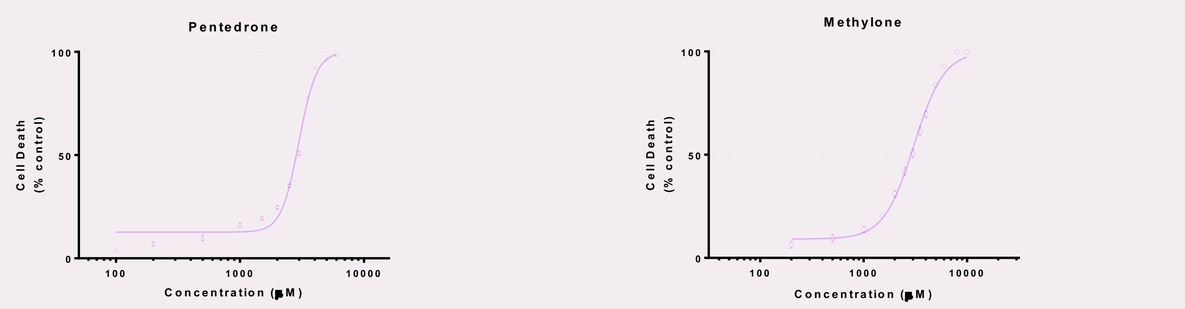

Figure 5. Nonlinear regression model for the cell death induced by pentedrone and methylone in dopaminergic SH-SY5Y cells, evaluated by MTT reduction assay, 24 h after exposure. Results are obtained from five independent experiments (performed in triplicate)

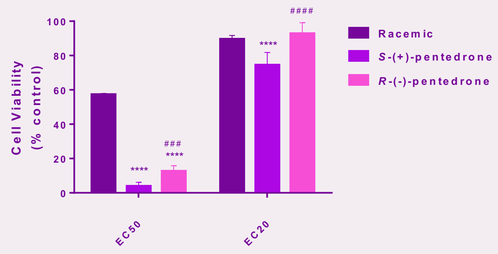

Figure 6. Citotoxicity in dopaminergic SH-SY5Y cells exposed to EC50 and EC20 Pentedrone for 24 h. Results were obtained from three independent experiment, performed in quadriplicate. ****p < 0.0001 vs control ####p < 0.0001 vs. *S*-(+)-Pentedrone.

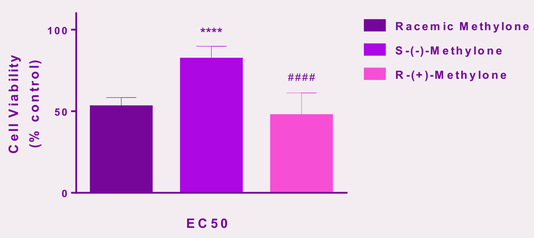

Figure 7. Citotoxicity in dopaminergic SH-SY5Y cells exposed to EC50 Methylone for 24 h. Results were obtained from three independent experiment, performed in quadriplicate. ****p < 0.0001 vs control ####p < 0.0001 vs. (+)-Pentedrone.

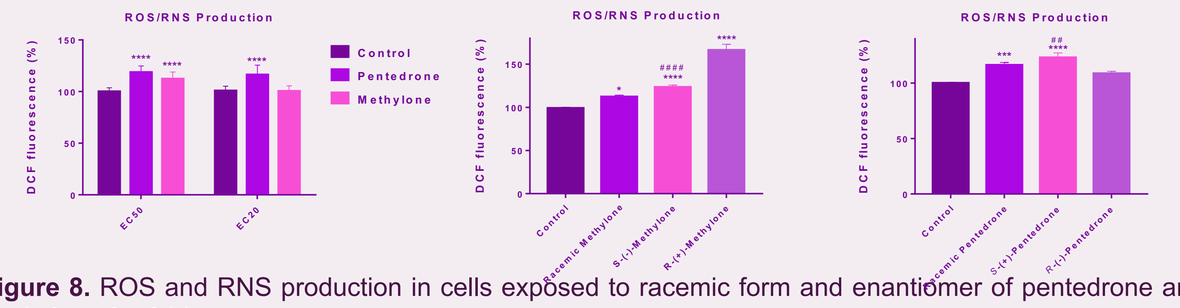

Figure 8. ROS and RNS production in cells exposed to racemic form and enantiomer of pentedrone and methylone for 24 h. Results were obtained from three independent experiment, performed in quadriplicate. ****p < 0.0001 vs control ####p < 0.0001 vs. (+)-Pentedrone.

Conclusions

Enantioresolution of pentedrone and methylone was performed in a multi-milligram scale, using a polysaccharide-based chiral stationary phase, achieving enantiomeric ratio values higher than 98%. The absolute configuration was established by a combined ECD spectroscopy and ECD spectral simulation, as (+)-(*S*) and (-)-(*R*)-pentedrone, and (-)-(*S*) and (+)-(*R*)-methylone. The performed studies in dopaminergic SH-SY5Y cell line with both enantiomers of pentedrone and methylone revealed enantioselectivity in cytotoxic effect as well as in ROS production. It was possible to verify, that *S*-(+)-pentedrone and *R*-(+)-methylone have more potent cytotoxic effect and induce higher reactive oxygen species (ROS) production.

References

1. Silva, B., et al., *Chiral enantioresolution of cathinone derivatives present in "legal highs", and enantioselectivity evaluation on cytotoxicity of 3,4-methylenedioxypyrovalerone (MDPV)*. Forensic Toxic., 2016. **34**(2): p. 372-385.

Acknowledgments

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