Scientific Letters

Oral Communication 11

Chiral separation of methylone and pentedrone and synthesis of key metabolites: toward a comprehensive understanding of synthetic cathinone toxicity

Ana Sofia Almeida [1,2,3,4], Paula Guedes de Pinho [3,4], Fernando Remião [3,4] and Carla Fernandes [1,2,*]

[1] Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal
[2] Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Porto, Portugal
[3] UCIBIO – Applied Molecular Biosciences Unit, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal
[4] Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Porto, Portugal

* Correspondence: cfernandes@ff.up.pt

Abstract

Background: Synthetic cathinones, such as methylone and pentedrone, are new psychoactive substances that pose a significant health threat due to their widespread accessibility and limited toxicological data available [1]. For a deeper understanding of their toxicological effects, it is essential to consider synthetic cathinones as a whole, including their stereochemistry and structural modifications resulting from biotransformation. Being chiral compounds, their enantiomers constitute individual distinct chemical entities that can exhibit different properties, as demonstrated in previous studies [2,3]. Likewise, metabolites should be considered alongside the parent compounds, as they may also contribute to overall activity or toxicity [4]. Despite the metabolism of synthetic cathinones being well reported, involving pathways such as β-keto reduction and *N*-demethylation [5,6], the specific effects of their metabolites remain largely underexplored. **Objective:** The primary aim of this study is to investigate the influence of chirality and metabolism on the toxicological profile of methylone and pentedrone. Specifically, this work seeks to isolate both single enantiomers and to synthesize key metabolites. **Methods:** Semi-preparative enantioseparation and evaluation of enantiomeric purity were conducted by chiral liquid chromatography (cLC) using amylose-based columns. Dihydro-metabolites were synthesized by β-keto reduction of parent drugs, while nor-metabolites by 5 synthetic steps starting from benzaldehyde derivatives. Structure elucidation was performed by spectroscopic methods (^{1}H- and ^{13}C-NMR and IR). **Results:** Optimized cLC conditions provided Rs and α values above 1.5 and 1.2, respectively. Enantiomers were obtained with enantiomeric ratios over 95% and recovery rates over 60%. Metabolites and intermediates were synthesized with yields of 48-94%. **Conclusions:** Both enantiomers of methylone and pentedrone were isolated with high enantiomeric purity. Dihydro- and nor-metabolites were successfully synthesized, with good yields. Isolated enantiomers and synthesized metabolites will serve as key tools in future metabolomic studies to evaluate the enantioselective effects and the role of metabolites toward a comprehensive understanding of synthetic cathinones toxicity.

Keywords: enantioselectivity; enantioseparation; metabolism; metabolites; synthetic cathinones

Acknowledgments/Funding

This research was supported by national funds by FCT (UIDB/04423/2020, UIDB/MULTI/04378/2020, UIDP/04423/2020 LA/P/0140/2020, PTDC/CTA-AMB/6686/2020 projects). Ana Sofia Almeida acknowledges her PhD grant provided by FCT (project reference 2023.00262.BD and DOI identifier https://doi.org/10.54499/2023.00262.BD).

References

1. Zawilska, J.B. Legal Highs - An Emerging Epidemic of Novel Psychoactive Substances. *Int Rev Neurobiol* 120: Academic Press 2015, p. 273-300.
2. Almeida, A.S. et al. Synthetic Cathinones: Recent Developments, Enantioselectivity Studies and Enantioseparation Methods *Molecules* **2022,** *27(7)*, 2057, doi:10.3390/molecules27072057.
3. Almeida, A.S. et al. Exploring the Impact of Chirality of Synthetic Cannabinoids and Cathinones: A Systematic Review on Enantioresolution Methods and Enantioselectivity Studies *Int J Mol Sci* **2025,** *26(13)*, 6471, doi:10.3390/ijms26136471.

4. Kirchmair, J. et al. Predicting drug metabolism: experiment and/or computation? *Nat Rev Drug Discov* **2015,** *14(6)*, 387-404, doi:10.1038/nrd4581.
5. Kamata, H.T. et al. Metabolism of the recently encountered designer drug, methylone, in humans and rats. *Xenobiotica* **2006,** *36(8)*, 709-23, doi:10.1080/00498250600780191.
6. Uralets, V. et al. Testing for designer stimulants: metabolic profiles of 16 synthetic cathinones excreted free in human urine *J Anal Toxicol* **2014**, 38(5), 233-41, doi: 10.1093/jat/bku021.