In vitro metabolism studies on New Psychoactive Substances: N-ethylhexedrone (Hexen) and Buphedrone

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LISBON ADDICTIONS 2019

INTRODUCTION

Over the last two decades synthetic cathinones (SC), often described as "Bath Salts", have emerged as one of the most sought for class of Novel Psychoactive Substances (NPS) among the users. These substances are derivatives of cathinone, a naturally occurring beta-ketone amphetamine analogue found in the leaves of the *Catha edulis* (Khat) plant. Because of their initial legality and effects often similar to cocaine or methylenedioxy-methamphetamine (MDMA), they gained a great popularity on drug markets. As the banning laws on cathinones progressed, new substances with different structures distant from the "mother" molecule, and thus more unpredictable in action, have been synthetized. Emerging SC have been commonly appearing in dark web as research chemicals and adulterants of other drugs or even as substitutes of older synthetic cathinones like mephedrone and MDPV. Having the active dose often lower than those substances and inducing higher desire of redosing, the ingestion of these NPS has often led to psychological distress, toxicity and even to fatalities in USA and in EU countries. Nowadays, over 100 SC are monitored by the EMCDDA, with new ones being added to the list every year. Moreover, since NPS enter recreational markets every week, there is a constant need to monitor the use/consumption of these newly synthesized substances and obtain sufficient knowledge on their metabolites excretion profiles.

OBJECTIVES

- Prediction of the metabolites of Buphedrone (Buph) and N-Ethylhexedrone (NEH) using *in silico* methods and available literature.
- Synthesis of Buph, NEH and selected metabolites
- *in vitro* metabolism studies of the selected SC – liver microsomal incubation
- Preliminary screening of microsomal samples taken at several time-points using high performance liquid chromatography coupled to mass spectrometry (HPLC-MS) in full scan mode, to search for precursor ions corresponding to parent drugs and expected metabolites
- Quantification of the selected SC and their main phase I metabolites by tandem HPLC-MS (HPLC-MS/MS) in multiple reaction monitoring (MRM) mode

METHODS

Metabolism prediction of Buphedrone (Fig.1A) and NEH (Fig. 1B) based on literature [1] and *in silico* methods

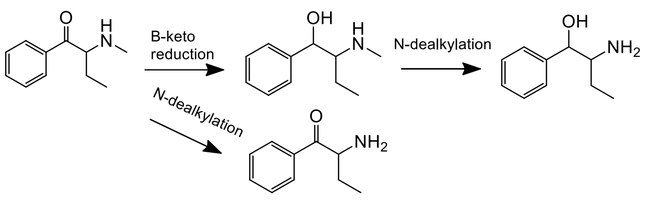

Figure 1A: Buphedrone phase I metabolism prediction

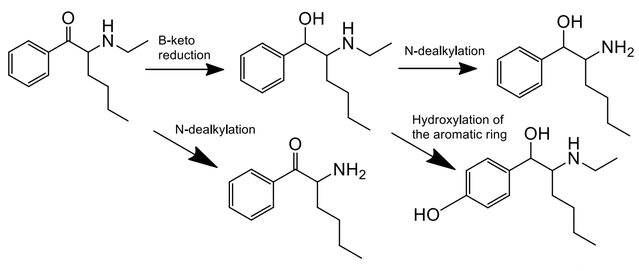

Figure 1B: N-Ethylhexedrone phase I metabolism prediction

Synthesis

- Buphedrone and N-ethylhexedrone were synthesized by bromination of starting butyrophenone and hexanephenone, respectively, followed by reaction of the 2-brominated phenones with the appropriate amine.
- Metabolites were chemically obtained following the synthetic procedures used in the synthesis of the parent drug for introduction of the amine group. Sodium borohydride was used for carbonyl reduction.
- Phenolic metabolites B4, H4 and H5 were obtained by acetylation of the starting 4'-hydroxyphenones to prevent bromination of the activated phenyl rings. This allowed basic hydrolysis during reaction with primary amines and/or sodium borohydride.

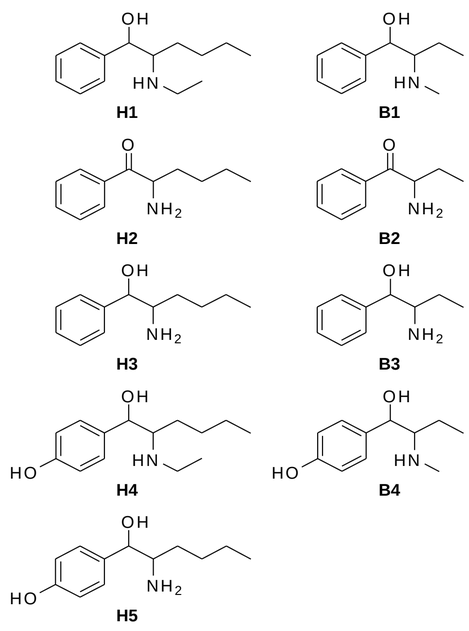

Figure 2: Chemical structures of selected NEH and Buphedrone metabolites

In vitro metabolism studies

- Phenacetine was used as positive control
- Microsomal reaction mixture without cofactors + each SC was used as negative control
- Incubation at 37ºC took 120 min and samples were collected at several time points

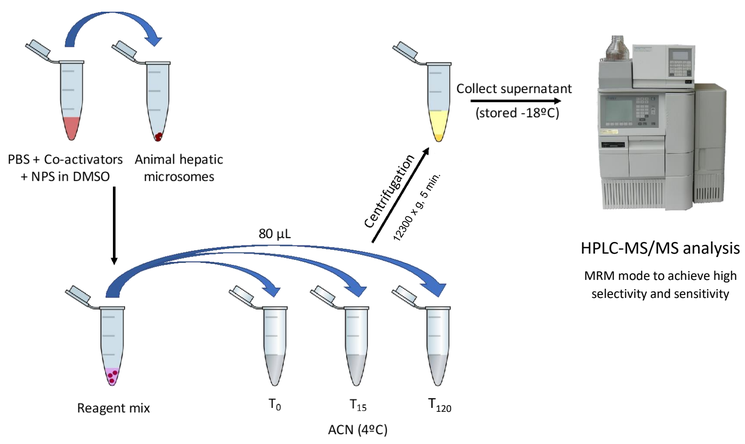

Figure 3: Schematic representation of the microsome assay proceedings

RESULTS

Characterization and optimization of MS/MS parameters

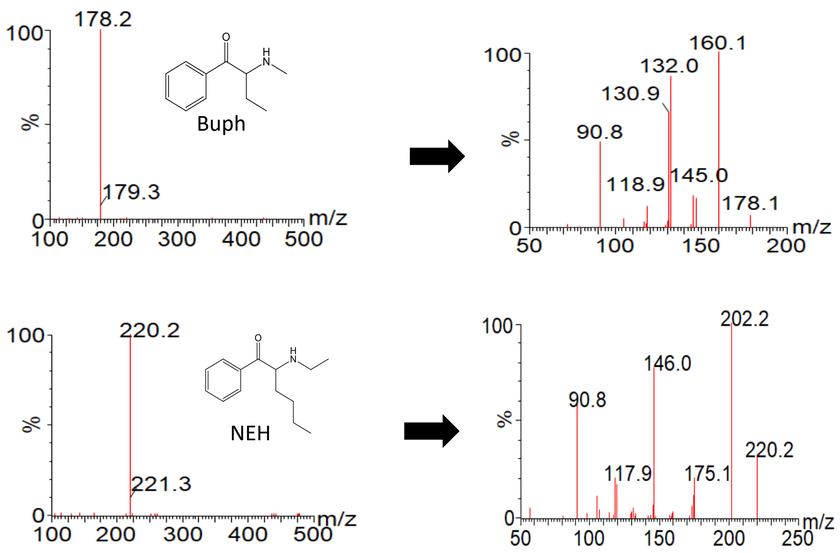

Figure 4: Buphedrone and N-ethylhexedrone fragmentation spectra

Table 1 Optimized MS/MS parameters for NEH and BUPH and metabolites. [M+H]+: precursor ion; MRM1: quantification transition; C.E.: collision energy; MRM2: confirmation transition.

Compound	$[M+H]^+$	Cone Voltage/ V	MRM1 (C.E./ eV)	MRM2 (C.E./ eV)
NEH	220	12	220 > 202 (15)	220 > 175 (15)
H1	222	12	222 > 204 (15)	222 > 147 (15)
H2	192	12	192 > 118 (12)	192 > 91 (12)
H3	194	12	194 > 176 (10)	194 > 117 (10)
H4	238	12	238 > 220 (11)	238 > 163 (15)
H5	210	12	210> 192 (7)	210 > 175 (12)
BUPH	178	12	178 > 160 (15)	178 > 132 (15)
B1	180	12	180 > 162 (12)	180 > 133 (15)
B2	164	12	164 > 118 (12)	164 > 91 (12)
B3	166	12	166 > 148 (10)	166 > 131 (10)
B4	196	12	196 > 178 (9)	196 > 147 (12)

Detection and Quantification of Buphedrone and NEH and their phase I metabolites in microsome studies

The negative controls of either Buph and NEH confirmed that none of the 9 selected metabolites was formed at any sampled time-point, at least above the detection limit (0.002µM)

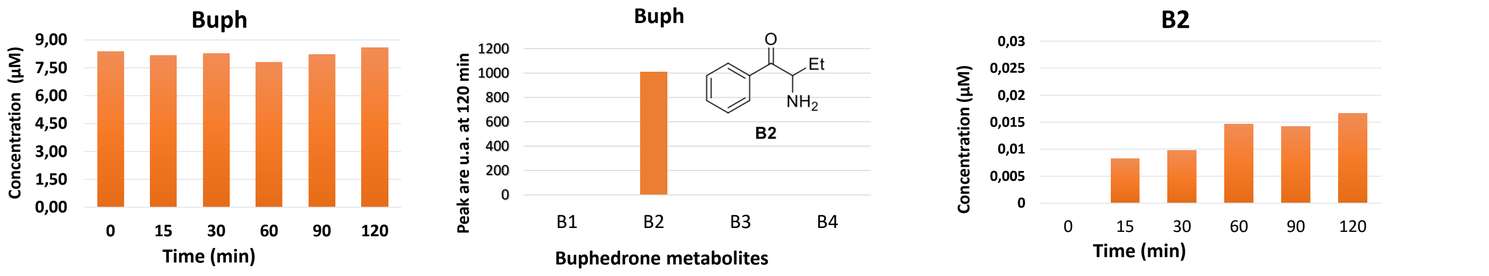

Figure 6. Buphedrone in vitro metabolism studies. From the left: Variations in time of the Buphedrone concentration; Detected buphedrone metabolites (B1, B3 and B4 below the level of detection); Variation in time of the B2 metabolite concentration;

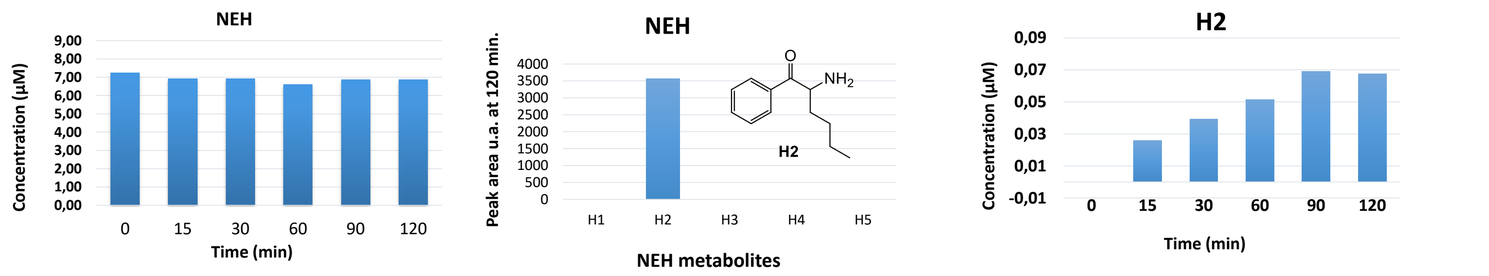

Figure 7. N-Ethylhexedrone in vitro metabolism studies. From the left: Variations in time of the NEH concentration; Detected NEH metabolites (H1, H3, H4 and H5 below the level of detection); Variation in time of the H2 metabolite concentration;

CONCLUSIONS

- Metabolites resulting from primary **N-dealkylation** (B2 and H2) are the only metabolites detected in the microssomal reaction in both cases, Buph and NEH.
- The amount of metabolite formed is 100x smaller than the initial concentration of the substrate, which might explain the maintenance of the parent compounds concentrations along time.
- The concentration of the metabolite H2 is overall higher after 120min than B2 suggesting that N-ethylhexedrone is more metabolised than Buphedrone

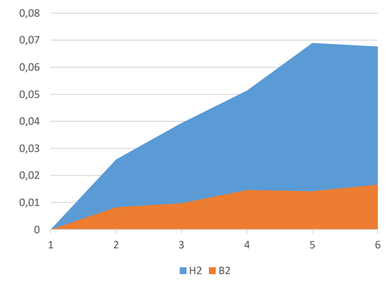

Figure 8: Concentrations of metabolites B2 (orange) and H2 (blue) formed in microsomal reaction

ACKNOWLEDGMENTS

This work was financially supported by the European Commission (HOME/2014/JDRU/AG/DRUG/7086), by the Fundação para a Ciência e a Tecnologia (FCT), through PTDC-SAU-TOX/32515/2017 and, in part, by UID/DTP/04138/2013 (iMed.ULisboa). The authors acknowledge the financial support from FCT and Portugal 2020 to the Portuguese Mass Spectrometry Network (Rede Nacional de Espectrometria de Massa – RNEM; LISBOA-01-0145-FEDER-402-022125). Joana Carrola also acknowledges RNEM for her post-doctoral fellowship. Pedro Florindo acknowledges the European Commission (HOME/2014/JDRU/AG/DRUG/7086) for post-doctoral fellowships.

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[1] Zaitsu K, Metabolism of Synthetic Cathinones. In: Zawilska J. (eds) Synthetic Cathinones. Current Topics in Neurotoxicity, 2018, vol 12. Springer, Cham

DISCLOSURE

The authors declare that they have no conflict of interest