Understanding the pharmacokinetics of synthetic cathinones: evaluation of the blood-brain barrier permeability of 13 related compounds in rats.

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Abstract

Synthetic cathinones are the second most commonly seized new psychoactive substance family in Europe. These compounds have been related to several intoxication cases, including fatalities. Although the pharmacological effects, metabolism and pharmacokinetics of cathinones have been studied, there is little information about the permeability of these compounds through the blood-brain barrier (BBB). This is an important parameter to understand the behaviour and potency of cathinones. In this work, 13 selected cathinones have been analysed in telencephalon tissue from Sprague-Dawley rats intraperitoneally dosed at 3 mg/kg. Our results revealed a direct relationship between compound polarity and BBB permeability, with higher permeability for the more polar cathinones. The chemical moieties present in the cathinone had an important impact on the BBB permeability, with lengthening of the α-alkyl chain or functionalization of the aromatic ring with alkyl moieties resulting in lower concentration in telencephalon tissue. Our data suggest that transport of cathinones is a carrier-mediated process, similar to cocaine transport across the BBB.

Keywords Blood-brain barrier, new psychoactive substances, synthetic cathinones, pharmacokinetics, pharmacology, toxicological analysis.

Introduction

The consumption of synthetic cathinones represents an important public health problem, according to the most recent report from the United Nations Office on Drugs and Crime [1], which illustrates that this new psychoactive substance (NPS) family is one of the most commonly seized worldwide, together with synthetic cannabinoids [1]. Most of the cathinone seizures are powder, together with pills and similar products. These compounds have also been found as adulterants in "classical" illegal drugs such as cocaine, illustrating that their prevalence of consumption could be underestimated [2,3]. In addition to the data obtained by seizure analysis, the public health problem related to cathinones is also illustrated by numerous intoxication cases related to these substances, and even some fatalities [4–6]. The synthetic cathinones prevalence can also be illustrated by analytical data obtained from wastewater analysis, illustrating that these compounds are being consumed worldwide [7,8].

It is almost impossible to ban all the cathinone derivatives existing nowadays due to the continuous change in structure of new compounds appearing on the market. Besides, new compounds that could replace banned ones surface in mere weeks, in a similar way to what occurs with synthetic cannabinoids [9]. To face this public health problem, the scientific community must be able to provide information about novel compounds, their chemical, pharmacological and toxicological properties. Thus, a notable number of papers have been published, as illustrated by the reviews available in literature about the metabolism of these substances [10–12], the associated pharmacological behavior [13,14], toxicology [5,15], and even their neurotoxicity [16].

An important pharmacological issue to highlight is how cathinones affect endogenous compounds, producing a psychoactive effect. Several studies have demonstrated that cathinones act as non-selective monoamine uptake inhibitors, increasing the levels of

dopamine and serotonin [17,18], producing effects similar to cocaine [19,20]. Thus, the potency of cathinones and other NPS may be studied using *in vitro* approaches [21–23], in a similar way to synthetic cannabinoids [24]. Although *in vitro* studies provide valuable information about the *intrinsic* potency of a compound, the *in vivo* effect must be determined by the extent to which a compound reaches its site of action. One of the key barriers in this context is the blood-brain barrier (BBB), modulating the exchange of compounds between the brain and the blood [25]. The BBB is a complex system that presents different "entry routes" that can be used by drugs or hormones [25], such as passive diffusion (usually used by non-polar compounds such as steroids) and carrier-mediated influx [25] (used by some psychoactive substances such as cocaine [26]), whereby a specific transporter helps the compound to cross the BBB and reach the brain. To complement the *in vitro* data and better understand the pharmacokinetics (and *in vivo* potency) of cathinones, it is therefore essential to generate accurate data on the BBB permeability of these compounds [26].

This work is the first to quantify an extensive series of cathinones in brain samples from rats intraperitoneally injected with these compounds, with the objective of relating the permeability through the BBB with their structure. To this aim, we have developed and validated [27–30] advanced analytical methodology based on ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) for the determination of 13 cathinones (**Figure 1**) in Sprague-Dawley rats' telencephalon tissue. UHPLC coupled to mass spectrometry (MS) plays an essential role in cathinone analysis, using both high-resolution MS (HRMS) and low-resolution MS/MS. Thus, most studies about the identification of novel cathinones [31–33] and the elucidation of their metabolites [34–36] have utilized UHPLC-HRMS, taking profit of the full-spectrum acquisition and high mass accuracy provided by this technique. UHPLC-MS/MS is the preferred technique for the accurate and sensitive determination of a predetermined list of compounds, and has

often been used for studying the pharmacokinetics and pharmacodynamics of cathinones [20,37–40].

As shown in **Figure 1**, taking pentedrone as a template, the cathinones investigated in this study differ in the amine functionalization, aromatic ring substitutions and/or length of the alkyl chain of the alpha carbon atom. The studied compounds were thoroughly selected in order to cover most of the possible combinations of moiety changes usually appearing in cathinones. Using a human *in vitro* BBB permeability model, Simmler *et al.* readily assessed the transendothelial transport of a series of cathinones [17], whereas the permeability of three cathinones with different alkyl chain length (methylone, butylone and pentylone) was evaluated by Grecco *et al.* using Sprague-Dawley rats [41]. Our work further elaborates on this by testing *in vivo* an extensive set of well-chosen cathinones with different changes in the moieties of the molecule.

Materials and methods

Reagents and chemicals

Research chemicals containing cathinones were provided by Energy Control (ABD Foundation, Barcelona, Spain). All the compounds were characterized and purity-tested by UHPLC-HRMS and nuclear magnetic resonance, following the same procedures already reported in literature [42,43]. Cathinone stock solutions were prepared at approximately 1 mg/mL in methanol (0.01 mg accuracy). HPLC-grade water was obtained by purifying demineralized water using a Milli-Q system from Millipore (Bedford, MA, USA). HPLC-grade methanol, HPLC-grade acetonitrile, HPLC-grade ethanol, formic acid and acetone were purchased from Scharlau (Scharlab, Barcelona, Spain). Physiological saline solution was purchased from Laboratorios ERN (Barcelona, Spain).

Animal testing

For animal experiments, dose and brain dissection time were selected based on the information available in literature. In this work, a realistic 3 mg/kg dose [40] was preferred over the very large doses (around 20 mg/kg) used in other pharmacokinetic studies [39,41]. This is relevant because extremely high doses could affect the metabolome, metabolic routes or active transport - among other parameters - that could be involved in the pharmacology and pharmacokinetics of these compounds. It should be noted that, depending on the compound, the doses reported by users are vastly different. For example, in the case of pentedrone, the compound used as reference in this work, 30 to 60 mg has been described as a typical dose when using intravenous route (roughly 0.5 to 1.0 mg/kg) [44]. However, for the aim of this work, it seemed reasonable to use similar doses for all the tested compounds, in order to facilitate the interpretation of the results and avoid the influence of different cathinone concentrations on the data obtained.

Peters and colleagues [40] quantified three cathinones in rat brains by LC-MS/MS, dosing the rats at 3 mg/kg for methylone and mephedrone, and at 1 mg/kg for MDPV. Pharmacokinetic data obtained revealed that the highest concentration was achieved 20 min after administration. Also another study [45], studying the variation of monoamine transporters in brain tissue from rats injected at 1, 3 and 10 mg/kg, reported the highest concentration in brain tissue 20 min after injection. Based on these studies, 3 mg/kg was selected as the dosage to be administered to the rats for all the compounds, and the brain was dissected 20 min post-injection.

Thus, thirty female Sprague-Dawley rats (8 weeks of age), weighing between 280 and 320 g, were purchased from Janvier Labs (Le Genest-Saint-Isle, France). Animals were housed in groups of 3 animals in polypropylene plastic cages under controlled temperature (24 ±2 ºC) and lighting conditions (12h:12h; lights ON at 8

144 am), with *ad libitum* access to food and water. Before drug administration, animals
145 were handled and habituated to the experimental room for one week. Experiments
146 were conducted in accordance with the standard ethical guidelines (European
147 Communities Directive 86/60-EEC) and approved by the Valencian Region
148 government ethical committee (*Generalitat Valenciana, Direcció General*
149 *d'Agricultura, Ganaderia i Pesca,* ref. 2019/VSC/PEA/0048).

150 Two rats were dosed per compound and the brains were pooled in order to avoid
151 possible animal differences. In total, twenty-six rats received intraperitoneal injections
152 of the 13 different cathinones at a dose of 3 mg/kg in 300 µL of physiological saline
153 solution containing 5% ethanol – the latter for increasing the solubility of the
154 synthetic cathinones. Four additional animals were injected with the same volume of
155 the vehicle and used to obtain blank brain tissue samples, to be used to prepare quality
156 control samples (QCs) and matrix-matched calibration curves. After 20 min, rats were
157 anesthetized with CO_2 and decapitated immediately. The brain was dissected
158 (avoiding blood that could contaminate it), and the telencephalon (both cerebral
159 hemispheres) was isolated, quickly frozen in liquid nitrogen and stored at -23 ºC until
160 analysis.

161 **Sample treatment**

162 Brain tissue samples were homogenized and crushed with dry ice (Praxair, Valencia,
163 Spain) using an electric grinder, followed by a -23 ºC overnight storage for CO_2
164 evaporation. After that, approximately 250 mg were accurately weighted (± 0.1 mg) in
165 1.5 mL polypropylene tubes, and 750 µL of acetonitrile containing 1% of formic acid
166 were added. Samples were extracted for 30 min under agitation using a vortex (Velp
167 Scientifica, Usmate Velate, Italy) at 1200 rpm. After keeping extracts for 30 min at -23
168 ºC, samples were centrifuged at 12000 rpm for 10 min. Finally, the supernatant was

diluted with ultrapure water for UHPLC-MS/MS analysis: in the case of samples used for method validation, the supernatant was 10-fold diluted, whereas for the brain samples obtained after administration of cathinones, supernatant was 1000-fold diluted.

The sample treatment procedure was adapted from literature [19], with the only difference being the homogenization procedure. In the present study, homogenization was performed using an electric grinder and dry ice, followed by extraction with acetonitrile and 1% formic acid, a freezing step as clean-up and dilution of the supernatant with HPLC-grade water. Sample weight, extraction volumes and dilutions were designed according to information available in literature [40], with some modifications to improve method sensitivity. For a detailed description on analytical methodology validation and the results obtained, see **Supporting Information**.

Instrumentation

Samples were analyzed using an Acquity UPLC™ H-Class liquid chromatography system (Waters Corp, Mildford, MA, USA) coupled to a Xevo TQ-S mass spectrometer (Waters Corp, Manchester, UK) equipped with a triple quadrupole mass analyzer, using a Z-Spray electrospray interface (ESI). Further information about the UHPLC-MS/MS instrument, the conditions employed, and its optimization can be found in **Supporting Information**.

Results

Cathinone concentrations found in brain displayed wide differences, from 762 ng/g brain tissue for *N*,*N*-dimethylpentylone to 10596 ng/g for N-ethyl-pentylone, the concentrations for most of the remaining compounds ranging between 1000 and 4000 ng/g. In all cases, the concentrations were well above the analytical performance, in terms

of sensitivity and limits of quantification of our methodology. In addition, reliability of the analytical methodology was supported by analysis of quality control (QC) samples in duplicate, spiked at 1 and 10 ng/g, included in the sample batch. Recoveries between 70 and 120% were obtained, confirming the correct quantification of the cathinones in telencephalon tissue.

The concentrations found in telencephalon samples for all the compounds are shown in **Tables 1-3**. The differences observed in the cathinone levels suggest that the BBB permeability of these compounds is structure-dependent, being associated with their polarity, as discussed further.

Discussion

Cathinone penetration through the blood-brain barrier

The main objective of our study was to evaluate the relationship between the structure of cathinones and their BBB permeability, in order to get better acquainted with the pharmacological behaviour of these substances.

Pronounced concentration differences were observed in the telencephalon for different cathinones, ranging from 762 ng/g (*N*,*N*-dimethylpentylone) to 10596 ng/g (*N*-ethyl-pentylone). This difference is surprising given the very high structural similarity of these two compounds, which only differ in the amine functionalization (dimethyl *vs*. ethyl).

Table 1 shows the concentrations found in telencephalon tissue for cathinones that differ by the functionalization of the amine moiety (*N*). In the two groups (those without aromatic ring substitution and those with a 3,4-methylenedioxy substituent), cathinones with an *N*-methyl (pentedrone and pentylone) moiety were found at a higher concentration than those with a pyrrolidine ring (α-PVP and MDPV). Regarding compounds with an *N*-ethyl group, *N*-ethyl-pentedrone had lower permeability than the

N-methyl analogue whereas *N*-ethyl-pentylone had a higher permeability than the *N*-methyl analogue. It is also remarkable that the cathinone with a *N*,*N*-dimethyl group (*N*,*N*-dimethylpentylone) seemed to have the lowest permeability of the BBB.

N-ethyl-pentylone was, by far, the cathinone with the highest concentration in telencephalon tissue. Also known as ephylone or bk-EBDP, this substance is a recently reported cathinone that has been involved in numerous recent intoxication cases [46,47] including 151 deaths between 2014 and 2018 [48], which raises high concerns regarding the toxicity of this compound. The high *N*-ethyl-pentylone concentration found in telencephalon tissue is in line with a recent study about the pharmacokinetic behavior of this cathinone, which also suggested a high BBB permeability [39], which could explain its elevated toxicity.

Another common modification seen in cathinone analogs is altering the length of the alkyl chain. In this study, we evaluated three pairs of cathinones that only differed from each other in the length of the alkyl chain. (**Table 2**): buphedrone and pentedrone, *N*-ethyl-pentedrone and *N*-ethyl-hexedrone, and butylone and pentylone. In all three cases, lengthening the alkyl chain led to a reduction of the BBB permeability, as shown in **Table 2**. These results are in concordance with data reported in a similar study [41], where the permeability of methylone, butylone and pentylone through the BBB was evaluated. The reported concentrations in cerebrospinal fluid were around 13 mg/L for butylone and 7 mg/L for pentylone after dosing Sprague-Dawley rats at 20 mg/kg. These results are coherent with those of the present study, where around 6,000 and 3,700 ng/g butylone and pentylone, respectively, were found in telencephalon for rats dosed at 3 mg/kg. Based on these data, the increment of the non-polarity of the cathinones due to the increase of the alkyl chains produces a reduction of the BBB permeability. Strangely, the most potent cathinone analogs in terms of dose reported by consumers are those that have a three-

carbon alkyl chain: MDPV, pentylone, α-PVP, pentedrone, etc., with the dose being higher if the length is shortened or increased further in most cases [49]. This could indicate that the mechanisms of toxicity of these compounds are not directly linked to their BBB permeability. As can be seen in the case studies for bk-EBDP intoxications, users frequently report a long duration of action for this compound, which is not so common for other cathinones. Perhaps the duration of effects indicates that bk-EBDP lingers in the body for an unusually long amount of time, and some of the toxicity may stem from this phenomenon.

The last typical change in the cathinone structure is functionalization of the aromatic ring. As can be observed in **Table 3**, the functionalizations studied were the addition of a 3,4-methylenedioxy moiety, a methyl group, a 3,4-dimethoxy group, and the addition of an halogen atom (in this case, a fluorine). Three of the four cathinone couples with/without a 3,4-methylenedioxy moiety (buphedrone and butylone, pentedrone and pentylone, and α-PVP and MDPV) presented a reduction of the permeability through the BBB when this moiety was added to the molecule (**Table 3)**, and it could also be related to the increment of the non-polarity of the compound by this modification. The remarkably low brain tissue concentration (860 ng/g) for MDPV is in line with previously reported concentrations for MDPV in rat brain, quantified around 260 ng/g at 30 min when dosing a rat at 1 mg/kg [38]. Only for the couple *N*-ethyl-pentedrone and *N*-ethyl-pentylone, the cathinone with the 3,4-methylenedioxy moiety presented a higher concentration in telencephalon tissue. Similar to the results obtained when analyzing the *N*-functionalization (**Table 1**), *N*-ethyl-pentylone produced an unexpected result when compared to the other cathinones. The higher telencephalon concentration of 4-fluoropentedrone, when compared with pentedrone, suggests that the presence of a halogen atom may increase BBB permeability, potentially due to an increment of the

compound's polarity. The reason for the increment of the permeability observed when adding a methyl group (*N*-ethyl-pentedrone vs *N*-ethyl-4-methylpentedrone) or a 3,4-dimethoxy group (α-PVP vs 3,4-dimethoxy-α-PVP) is unclear. It is possible that the presence of these terminal methyl groups allows for easier passing through the BBB. In order to confirm this, more cathinones with these aromatic ring changes should be evaluated.

The concentration differences discussed above, when changing the *N*-functionalization, alkyl chain length and aromatic ring substitution, point at a positive correlation between polarity and BBB permeability, as also suggested by others [41]. However, based on an *in vitro* model using TY09 conditionally immortalized human brain capillary endothelial cells, Simmler and colleagues suggested the opposite: these authors reported that a decrease in polarity of cathinones produces an increment of the permeability of the BBB, with non-polar cathinones presenting a particularly high transendothelial permeability [17]. Although these *in vitro* data apparently contradict our findings and those of the previous literature [38,41], the use of live animals instead of a cell culture is a closer representation of the real pharmacokinetic behavior of these compounds in a process as complex as BBB permeability.

In fact, the BBB is composed not just of endothelial cells, but also includes associated cell elements such as astrocyte endfeet, pericytes and microglia. There are several important routes of transport across the BBB [25], passive diffusion and the carrier-mediated influx being the most common ones related to psychoactive substances. The coexistence of both influx processes for cocaine through the BBB has been reported in literature using an *in vivo* model with Swiss mice; here, the carrier mediated influx rate was 3.4 times greater than its passive diffusion rate [26]. The same publication indicates that MDPV is also a substrate for the cocaine transporter. Based on this evidence and in line

with the data presented in this work as well as in literature [41], it can be deduced that the penetration of cathinones through the BBB is a carrier-mediated process. An exhaustive study about the solute carrier transporters involved in cathinone transport should be performed in order to confirm this hypothesis.

In addition to compound polarity and moieties present in the distinct structures, different physicochemical properties of the compounds such as logP and logD, topological polar surface area, number of rotatable bonds, fraction of sp3 carbons, heavy atom count, among other parameters, can be also be related to BBB permeability. For the compounds studied in this work, topological polar surface area values (obtained from PubChem) were evaluated for 11 compounds. No relationship between this parameter and BBB permeability was found. No additional parameters were found in compound databases, consequently those should be determined experimentally or theoretically to evaluate their contribution to BBB permeability.

In summary, the relationship between cathinone structure and their ability to cross the blood-brain barrier was studied in this work. To this aim, telencephalon tissues from Sprague-Dawley rats dosed at 3 mg/kg with 13 different cathinones were analyzed by a validated UHPLC-MS/MS procedure. The results obtained showed that permeability of cathinones is related to their polarity, with better crossing of the BBB with increasing polarity. These findings are in accordance with previously published data using rats [41] but differ from those obtained using *in vitro* experiments [17], demonstrating the importance of not solely relying on *in vitro* data. Less polar *N* functionalizations, such as the presence of a pyrrolidine ring, reduced the cathinone transport through the BBB. In a similar way, cathinones with longer alkyl chain were less able to cross the BBB. Non-polar aromatic ring substitutions such as 3,4-methylenedioxy reduced BBB permeability, while the presence of a fluorine atom increased BBB transport. All this data, together with

information available in literature from similar studies [41] and the BBB transport [26] suggest that cathinones cross the BBB through a carrier-mediated process. Additionally, this study shows that studying the pharmacology and pharmacokinetics of cathinones, and NPS as a whole, is crucial for a better understanding of the *in vivo* potency of these compounds, complementing other studies such as dopamine and serotonin uptake inhibition. Our future work will be focused on the study of additional cathinones that will appear on the continuously evolving NPS market, in order to support the role of carrier-mediated processes in the BBB passage of cathinones.

Acknowledgements

D. Fabregat-Safont, J.V. Sancho, F. Hernández and M. Ibáñez acknowledge financial support from the University Jaume I (UJI-B2018-19). D. Fabregat-Safont acknowledges Ministerio de Educación, Cultura y Deporte in Spain for his predoctoral grant (FPU15/02033). F. Martinez-Garcia and M. Barneo-Muñoz acknowledge financial support from Ministerio de Economía y Competitividad-FEDER (BFU2016-77691-C2-1-P), the Generalitat Valenciana (PROMETEO/2016/076) and the Universitat Jaume I de Castelló (UJI-B2016-45). X. Carbón and M. Ventura acknowledge the grants from Subdirecció General de Drogodependències, Departament de Salut, Generalitat de Catalunya and Plan Nacional sobre Drogas. Authors also acknowledge Esther Fuentes-Ferragud for helping during method development.

Competing Interests

The authors declare that they have no competing interests.

Author contribution

D.F-S., J.V.S and M.I. conceived the work. M.B-M. and F.M-G. performed animal experiment and brain dissection. D.F-S. and M.I. performed sample treatment, instrumental analysis and data process. D.F-S., J.V.S, M.B-M., F.M-G, X.C, M.V. and M.I. interpreted and discussed the results. F.H. and F.M-G contributed with new reagents and analytical tools. D.F-S and M.I. wrote the first draft of the manuscript. J.V.S, M.B-M., F.M-G, X.C, M.V., C.S. and F.H. provided useful comments and feedback for the manuscript.

References

1. United Nations Office on Drugs and Crime. *World Drug Report 2020*.; 2020.
2. Oliver CF, Palamar JJ, Salomone A, et al. Synthetic cathinone adulteration of illegal drugs. *Psychopharmacology (Berl)*. 2019;236(3):869-879. doi:10.1007/s00213-018-5066-6
3. Giné CV, Espinosa IF, Vilamala MV. New psychoactive substances as adulterants of controlled drugs. A worrying phenomenon? *Drug Test Anal*. 2014;6(7-8):819-824. doi:10.1002/dta.1610
4. Zaami S, Giorgetti R, Pichini S, Pantano F, Marinelli E, Busardò FP. Synthetic cathinones related fatalities: An update. *Eur Rev Med Pharmacol Sci*. 2018;22(1):268-274. doi:10.26355/eurrev-201801-14129
5. Majchrzak M, Celiński R, Kuś P, Kowalska T, Sajewicz M. The newest cathinone derivatives as designer drugs: an analytical and toxicological review. *Forensic Toxicol*. September 2017. doi:10.1007/s11419-017-0385-6
6. Kraemer M, Boehmer A, Madea B, Maas A. Death cases involving certain new psychoactive substances: A review of the literature. *Forensic Sci Int*. 2019;298:186-267. doi:10.1016/j.forsciint.2019.02.021
7. Bijlsma L, Celma A, López FJ, Hernández F. Monitoring new psychoactive substances use through wastewater analysis: current situation, challenges and limitations. *Curr Opin Environ Sci Heal*. 2019;9:1-12. doi:10.1016/j.coesh.2019.03.002
8. Celma A, Sancho J V., Salgueiro-González N, et al. Simultaneous determination of new psychoactive substances and illicit drugs in sewage: Potential of micro-liquid chromatography tandem mass spectrometry in wastewater-based epidemiology. *J Chromatogr A*. 2019;1602:300-309. doi:10.1016/j.chroma.2019.05.051
9. Bijlsma L, Ibáñez M, Miserez B, et al. Mass spectrometric identification and structural analysis of the third-generation synthetic cannabinoids on the UK market since the 2013 legislative ban. *Forensic Toxicol*. 2017;35(2):376-388. doi:10.1007/s11419-017-0368-7
10. Zawilska JB, Wojcieszak J. Designer cathinones—An emerging class of novel recreational drugs. *Forensic Sci Int*. 2013;231(1-3):42-53. doi:10.1016/j.forsciint.2013.04.015

11. Coppola M, Mondola R. 3,4-Methylenedioxypyrovalerone (MDPV): Chemistry, pharmacology and toxicology of a new designer drug of abuse marketed online. *Toxicol Lett*. 2012;208(1):12-15. doi:10.1016/j.toxlet.2011.10.002
12. Zaitsu K, Katagi M, Tsuchihashi H, Ishii A. Recently abused synthetic cathinones, α-pyrrolidinophenone derivatives: a review of their pharmacology, acute toxicity, and metabolism. *Forensic Toxicol*. 2014;32(1):1-8. doi:10.1007/s11419-013-0218-1
13. Gatch MB, Dolan SB, Forster MJ. Comparative Behavioral Pharmacology of Three Pyrrolidine-Containing Synthetic Cathinone Derivatives. *J Pharmacol Exp Ther*. 2015;354(2):103-110. doi:10.1124/jpet.115.223586
14. Coppola M, Mondola R. Synthetic cathinones: Chemistry, pharmacology and toxicology of a new class of designer drugs of abuse marketed as “bath salts” or “plant food.” *Toxicol Lett*. 2012;211(2):144-149. doi:10.1016/j.toxlet.2012.03.009
15. Kelly JP. Cathinone derivatives: A review of their chemistry, pharmacology and toxicology. *Drug Test Anal*. 2011;3(7-8):439-453. doi:10.1002/dta.313
16. Angoa-Pérez M, Anneken JH, Kuhn DM. Neurotoxicology of Synthetic Cathinone Analogs. In: *Brain Imaging in Behavioral Neuroscience*. ; 2016:209-230. doi:10.1007/7854_2016_21
17. Simmler L, Buser T, Donzelli M, et al. Pharmacological characterization of designer cathinones in vitro. *Br J Pharmacol*. 2013;168(2):458-470. doi:10.1111/j.1476-5381.2012.02145.x
18. Baumann MH, Ayestas MA, Partilla JS, et al. The Designer Methcathinone Analogs, Mephedrone and Methylone, are Substrates for Monoamine Transporters in Brain Tissue. *Neuropsychopharmacology*. 2012;37(5):1192-1203. doi:10.1038/npp.2011.304
19. Olesti E, Rodríguez-Morató J, Gomez-Gomez A, Ramaekers JG, de la Torre R, Pozo OJ. Quantification of endogenous neurotransmitters and related compounds by liquid chromatography coupled to tandem mass spectrometry. *Talanta*. 2019;192(July 2018):93-102. doi:10.1016/j.talanta.2018.09.034
20. Olesti E, De Toma I, Ramaekers JG, et al. Metabolomics predicts the pharmacological profile of new psychoactive substances. *J Psychopharmacol*. 2019;33(3):347-354. doi:10.1177/0269881118812103
21. Simmler LD, Liechti ME. Interactions of Cathinone NPS with Human

Transporters and Receptors in Transfected Cells. In: ; 2016:49-72. doi:10.1007/7854_2016_20

22. Simmler LD, Buchy D, Chaboz S, Hoener MC, Liechti ME. In Vitro Characterization of Psychoactive Substances at Rat, Mouse, and Human Trace Amine-Associated Receptor 1. *J Pharmacol Exp Ther*. 2016;357(1):134-144. doi:10.1124/jpet.115.229765

23. Rickli A, Hoener MC, Liechti ME. Monoamine transporter and receptor interaction profiles of novel psychoactive substances: Para-halogenated amphetamines and pyrovalerone cathinones. *Eur Neuropsychopharmacol*. 2015;25(3):365-376. doi:10.1016/j.euroneuro.2014.12.012

24. Noble C, Cannaert A, Linnet K, Stove CP. Application of an activity-based receptor bioassay to investigate the in vitro activity of selected indole- and indazole-3-carboxamide-based synthetic cannabinoids at CB1 and CB2 receptors. *Drug Test Anal*. 2018;(August):1-11. doi:10.1002/dta.2517

25. Abbott NJ, Patabendige AAK, Dolman DEM, Yusof SR, Begley DJ. Structure and function of the blood–brain barrier. *Neurobiol Dis*. 2010;37(1):13-25. doi:10.1016/j.nbd.2009.07.030

26. Chapy H, Smirnova M, Andre P, et al. Carrier-Mediated Cocaine Transport at the Blood-Brain Barrier as a Putative Mechanism in Addiction Liability. *Int J Neuropsychopharmacol*. 2015;18(1):pyu001-pyu001. doi:10.1093/ijnp/pyu001

27. European Medicines Agency. Guideline on bioanalytical method validation, EMEA/CHMP/EWP/192217/2009. 2011.

28. Food and Drug Administration. Bioanalytical Method Validation, FDA-2013-D-1020. Presented at the: 2018.

29. Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the Assessment of Matrix Effect in Quantitative Bioanalytical Methods Based on HPLC−MS/MS. *Anal Chem*. 2003;75(13):3019-3030. doi:10.1021/ac020361s

30. Constanzer ML, Chavez-Eng CM, Fu I, Woolf EJ, Matuszewski BK. Determination of dextromethorphan and its metabolite dextrorphan in human urine using high performance liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry: a study of selectivity of a tandem mass spectrometric assa. *J Chromatogr B*. 2005;816(1-2):297-308. doi:10.1016/j.jchromb.2004.11.049

31. Fabregat-Safont D, Carbón X, Gil C, et al. Reporting the novel synthetic

cathinone 5-PPDI through its analytical characterization by mass spectrometry and nuclear magnetic resonance. *Forensic Toxicol*. 2018;36(2):447-457. doi:10.1007/s11419-018-0422-0

32. Doi T, Akiko Asada B, Akihiro Takeda B, et al. Identification and characterization of a-PVT, a-PBT, and their bromothienyl analogs found in illicit drug products. *Forensic Toxicol*. 2016;34(1):76-93. doi:10.1007/s11419-015-0288-3

33. Qian Z, Jia W, Li T, Hua Z, Liu C. Identification and analytical characterization of four synthetic cannabinoids ADB-BICA, NNL-1, NNL-2, and PPA(N)-2201. *Drug Test Anal*. 2017;9(1):51-60. doi:10.1002/dta.1990

34. Ibáñez M, Pozo ÓJ, Sancho J V., Orengo T, Haro G, Hernández F. Analytical strategy to investigate 3,4-methylenedioxypyrovalerone (MDPV) metabolites in consumers' urine by high-resolution mass spectrometry. *Anal Bioanal Chem*. 2016;408(1):151-164. doi:10.1007/s00216-015-9088-1

35. Swortwood MJ, Ellefsen KN, Wohlfarth A, et al. First metabolic profile of PV8, a novel synthetic cathinone, in human hepatocytes and urine by high-resolution mass spectrometry. *Anal Bioanal Chem*. 2016;408(18):4845-4856. doi:10.1007/s00216-016-9599-4

36. Helfer AG, Turcant A, Boels D, et al. Elucidation of the metabolites of the novel psychoactive substance 4-methyl- N -ethyl-cathinone (4-MEC) in human urine and pooled liver microsomes by GC-MS and LC-HR-MS/MS techniques and of its detectability by GC-MS or LC-MS n standard screening approach. *Drug Test Anal*. 2015;7(5):368-375. doi:10.1002/dta.1682

37. López-Arnau R, Martínez-Clemente J, Carbó M, Pubill D, Escubedo E, Camarasa J. An integrated pharmacokinetic and pharmacodynamic study of a new drug of abuse, methylone, a synthetic cathinone sold as "bath salts." *Prog Neuro-Psychopharmacology Biol Psychiatry*. 2013;45:64-72. doi:10.1016/j.pnpbp.2013.04.007

38. Horsley RR, Lhotkova E, Hajkova K, et al. Behavioural, Pharmacokinetic, Metabolic, and Hyperthermic Profile of 3,4-Methylenedioxypyrovalerone (MDPV) in the Wistar Rat. *Front Psychiatry*. 2018;9(APR):1-13. doi:10.3389/fpsyt.2018.00144

39. Lin Z, Chen Y, Li J, et al. Pharmacokinetics of N-ethylpentylone and its effect on increasing levels of dopamine and serotonin in the nucleus accumbens of

conscious rats. *Addict Biol*. 2019;(November 2018):e12755. doi:10.1111/adb.12755

40. Peters JR, Keasling R, Brown SD, Pond BB. Quantification of Synthetic Cathinones in Rat Brain Using HILIC–ESI-MS/MS. *J Anal Toxicol*. 2016;(July):718-725. doi:10.1093/jat/bkw074

41. Grecco GG, Kisor DF, Magura JS, Sprague JE. Impact of common clandestine structural modifications on synthetic cathinone "bath salt" pharmacokinetics. *Toxicol Appl Pharmacol*. 2017;328:18-24. doi:10.1016/j.taap.2017.05.010

42. Fabregat-Safont D, Carbón X, Ventura M, et al. Updating the list of known opioids through identification and characterization of the new opioid derivative 3,4-dichloro-N-(2-(diethylamino)cyclohexyl)-N-methylbenzamide (U-49900). *Sci Rep*. 2017;7(1). doi:10.1038/s41598-017-06778-9

43. Fabregat-Safont D, Carbón X, Ventura M, Fornís I, Hernández F, Ibáñez M. Characterization of a recently detected halogenated aminorex derivative: para-fluoro-4-methylaminorex (4′F-4-MAR). *Sci Rep*. 2019;9(1):8314. doi:10.1038/s41598-019-44830-y

44. Expert Committee on Drug Dependence. *Pentedrone Critical Review Report*.; 2016. https://www.who.int/medicines/access/controlled-substances/4.6_Pentedrone_CritReview.pdf?ua=1.

45. Baumann MH, Ayestas MA, Partilla JS, et al. The Designer Methcathinone Analogs, Mephedrone and Methylone, are Substrates for Monoamine Transporters in Brain Tissue. *Neuropsychopharmacology*. 2012;37(5):1192-1203. doi:10.1038/npp.2011.304

46. Krotulski AJ, Papsun DM, De Martinis BS, Mohr ALA, Logan BK. N-Ethyl Pentylone (Ephylone) Intoxications: Quantitative Confirmation and Metabolite Identification in Authentic Human Biological Specimens. *J Anal Toxicol*. 2018;42(7):467-475. doi:10.1093/jat/bky025

47. Thirakul P, S. Hair L, L. Bergen K, M. Pearson J. Clinical Presentation, Autopsy Results and Toxicology Findings in an Acute N-Ethylpentylone Fatality. *J Anal Toxicol*. 2017;41(4):342-346. doi:10.1093/jat/bkx004

48. Drug Enforcement Administration (DEA). *Schedules of Controlled Substances: Temporary Placement of N-Ethylpentylone in Schedule I*. Vol 83.; 2018. https://www.federalregister.gov/documents/2018/06/13/2018-12669/schedules-of-controlled-substances-temporary-placement-of-n-ethylpentylone-in-schedule-

i.

49. Debruyne D, Loilier M, Cesbron A, Le Boisselier R, Bourgine J. Emerging drugs of abuse: current perspectives on substituted cathinones. *Subst Abuse Rehabil*. May 2014:37. doi:10.2147/SAR.S37257

Figure captions

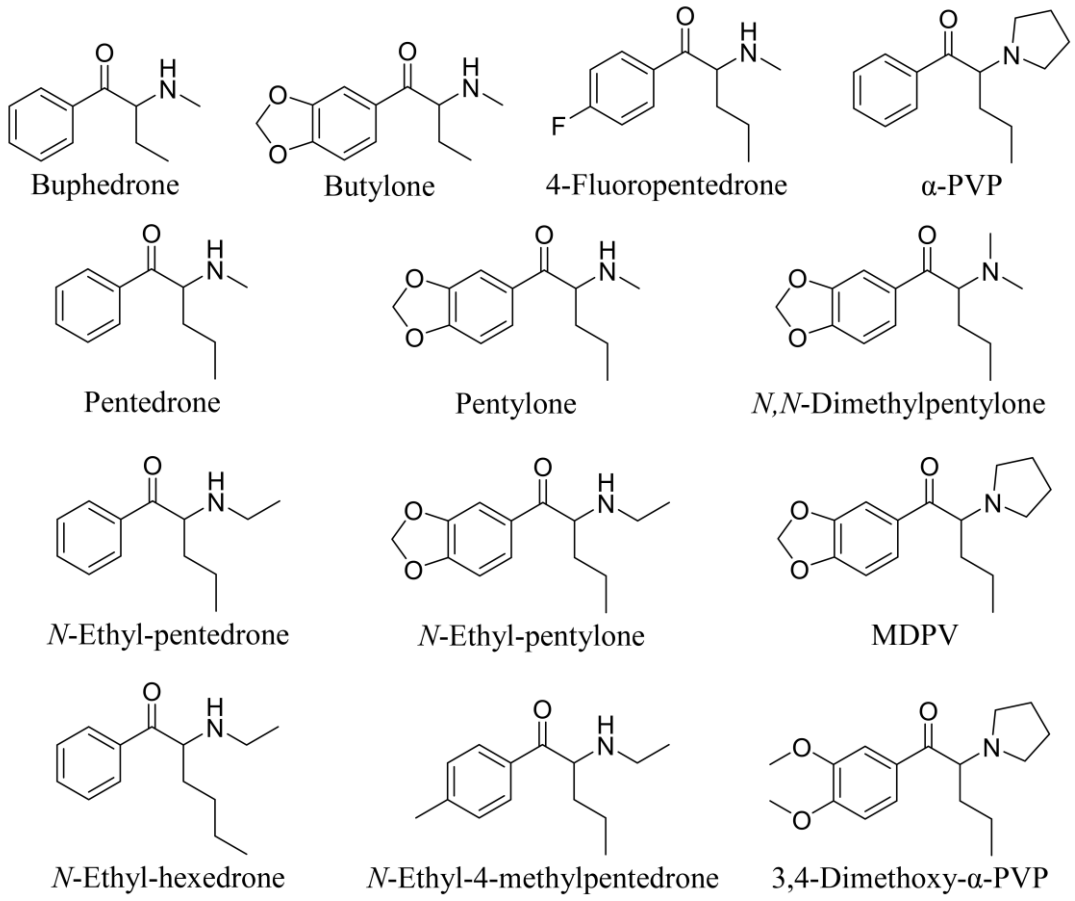
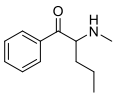
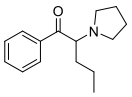
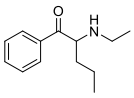
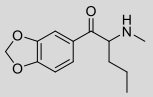
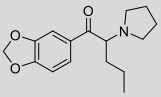
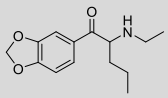
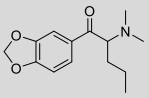


Figure 1. Structures of the 13 synthetic cathinones selected for the study.

Tables

Table 1. Concentration levels found in rat telencephalon tissue for cathinones differing on the amine (*N*) functionalization (rats dosed at 3 mg/kg).

Change in the *N* functionalization			
Compound	**Brain tissue conc. (ng/g)**	**Ratio found/dosed (µg/mg)**	**Structure**
Pentedrone	3,718	1.03	
α-PVP	3,054	0.84	
N-Ethyl-pentedrone	1,432	0.40	
Pentylone	3,113	0.86	
MDPV	863	0.24	
N-Ethyl-pentylone	10,596	2.94	
N,*N*-Dimethylpentylone	762	0.21	

535 **Table 2.** Concentration levels found in rat telencephalon tissue for cathinones differing
536 on the alkyl chain length (rats dosed at 3 mg/kg).

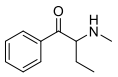
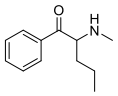
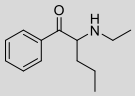
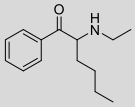
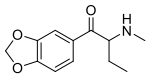
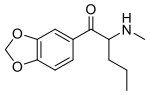
Alkyl chain length			
Compound	**Brain tissue conc. (ng/g)**	**Ratio found/dosed (µg/mg)**	**Structure**
Buphedrone	6,067	1.69	
Pentedrone	3,718	1.03	
N-Ethyl-pentedrone	1,432	0.40	
N-Ethyl-hexedrone	1,160	0.32	
Butylone	4,659	1.29	
Pentylone	3,113	0.86	

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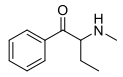
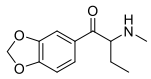
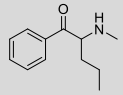
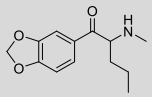
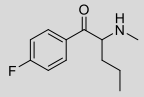
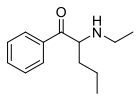
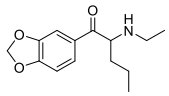
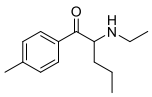
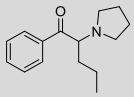
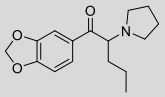
Table 3. Concentration levels found in rat telencephalon tissue for cathinones differing on the aromatic ring substitutions (rats dosed at 3 mg/kg).

Aromatic ring substitution			
Compound	**Brain tissue conc. (ng/g)**	**Ratio found/dosed (µg/mg)**	**Structure**
Buphedrone	6,067	1.69	
Butylone	4,659	1.29	
Pentedrone	3,718	1.03	
Pentylone	3,113	0.86	
4-Fluoropentedrone	4,806	1.34	
N-Ethyl-pentedrone	1,432	0.40	
N-Ethyl-pentylone	10,596	2.94	
N-Ethyl-4-methylpentedrone	2,541	0.71	
α-PVP	3,054	0.85	
MDPV	863	0.24	
3,4-dimethoxy-α-PVP	2,083	0.58