Forensic Science International 290 (2018) 146–156

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Proactive response to tackle the threat of emerging drugs: Synthesis and toxicity evaluation of new cathinones

Helena Gaspar[a,c,*], Soraia Bronze[b], Catarina Oliveira[b], Bruno L. Victor[b], Miguel Machuqueiro[a,b], Rita Pacheco[a,d], Maria João Caldeira[e], Susana Santos[b,**]

[a] University of Lisboa, Faculty of Sciences, BioISI – Biosystems & Integrative Sciences Institute, Campo Grande, C8, 1749-016 Lisboa, Portugal
[b] Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[c] MARE, Marine and Environmental Sciences Centre, ESTM, Polytechnic Institute of Leiria, 2520-641 Peniche, Portugal
[d] Área Departamental de Engenharia Química, Instituto Superior de Engenharia de Lisboa, Av. Conselheiro Emídio Navarro, 1959-007 Lisboa, Portugal
[e] Laboratório de Polícia Científica da Polícia Judiciária, Novo edifício-sede da Polícia Judiciária, Rua Gomes Freire, 169-007 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 4 May 2018
Received in revised form 29 June 2018
Accepted 4 July 2018
Available online 11 July 2018

Keywords:
Designer-drugs
Cathinones
NMR
Hepatotoxicity
Membrane permeability
Deprotonation

ABSTRACT

The emergence of potentially dangerous new psychoactive substances (NPS) imposes enormous challenges on forensic laboratories regarding their rapid and unambiguous identification. Access to comprehensive databases is essential for a quick characterization of these substances, allowing them to be categorized according to national and international legislations. In this work, it is reported the synthesis and structural characterization by NMR and MS of a library encompassing 21 cathinones, 4 of which are not yet reported in the literature, but with structural characteristics that make them a target for clandestine laboratories. This in-house library will be an important tool accessible to forensic laboratories, for the quick identification of seized NPS. The *in vitro* cytotoxicity of all cathinones was assessed in HepG2 cells, to have a preliminary but effective indication of their human hepatotoxicity potential. The two new cathinones DMB (**8**) and DMP (**9**) were the more cytotoxic, followed by the already seized mephedrone (**2**), 3,4-DMMC (**3**), 4-MDMC (**7**), NEB (**12**) with EC50 values ranging from 0.81 mM for (**3**) to 1.28 mM for (**2**). Results suggest an increase of cytotoxicity with the increase of the chain length of the acyl moiety and with the substitution (with one or two methyl groups) in the aromatic ring. The nature of the amine moiety seems to play only a minor role in the cytotoxic effect. Molecular dynamics simulations were performed to evaluate the molecular details related with the observed cytotoxicities. Although these studies indicated that cathinones are able to cross lipid bilayers with relative ease, when in their neutral forms, it was observed only a partial correlation between lipophilicity and cytotoxicity, indicating that membrane trafficking may not be the only key factor influencing the bioactivity of these compounds. This work is a valuable contribution to the forensic science field since a quick identification of novel cathinones is urgent to match their rapid increase in the market.

1. Introduction

Over the past decade there has been an increase in the consumption of new psychoactive substances (NPS) across the world. On the market, these substances are known as “designer drugs”, “legal highs”, “bath salts” or “research chemicals”, amongst others. To make the terminology on this subject clear, the European Council uses the catch-all term “new psychoactive substances (NPS)” to define “substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat” [1]. In the European Union, the response system to the emergence of NPS is the European Early Warning System (EWS) administered by the European Monitoring Center for Drugs and Drug Addiction (EMCDDA) and EUROPOL [2]. This system allows the monitoring of NPS for assessment of their risks and for the application of control measures in the EU state members, if necessary. However, the NPS market is resilient, adapting very quickly to changes introduced by legal controls, and new substances are being

* Corresponding author at: University of Lisboa, Faculty of Sciences, BioISI – Biosystems & Integrative Sciences Institute, Campo Grande, C8, 1749-016 Lisboa, Portugal.
** Corresponding author at: Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.
E-mail addresses: hmgaspar@ciencias.ulisboa.pt (H. Gaspar), smsantos@ciencias.ulisboa.pt (S. Santos).

https://doi.org/10.1016/j.forsciint.2018.07.001
0379-0738/

continuously introduced into the market, at an unprecedented rate, in an effort to circumvent legislation. By December 2016, the number of different NPS reported worldwide to UNODOC (739) more than doubled compared to 2012 (260) [3].

Although NPS represent a heterogeneous family of substances, they are all intended to mimic the effects of the main illicitly-used substances controlled under the international drug conventions, like opioids or psychostimulants. According to their pharmacological effects, the majority of NPS can be categorized as synthetic cannabinoid receptor agonists (36%), amphetamine-like stimulants, which include synthetic cathinones and piperazine derivatives (33%), and classic hallucinogens (16%) [2]. Even though NPS are not responsible for as many deaths as cocaine or other illicit drugs, they are a big health concern due to their unknown pharmacological profile, potential off-target sites of action and adverse side reactions [4].

Synthetic cathinones appeared in the drug market in the mid-2000s, as 'legal' replacements for stimulants such as amphetamine, 3,4-methylendioxymethampetamine (MDMA) and cocaine, and they are chemically related to the natural cathinone, the amphetamine-like compound present in the khat plant (*Catha edulis* Forsk) used for chewing amongst communities of the Horn of Africa and Arabian Peninsula [5]. Cathinone has the same core structure as amphetamine, from which differs by the presence of a ketone group in the β position of the side chain (Fig. 1). Synthetic cathinones are β-keto phenethylamines structurally related to natural cathinone and can have substituents in the four positions of the core skeleton: in the α-carbon to the carbonyl function, in the nitrogen (which can be mono or disubstituted) and in any position of the aromatic ring (Fig. 1).

According with the substituent pattern on the nitrogen, synthetic cathinones can be divided in three categories. The first category comprises cathinones with a secondary amine (*N*-mono alkylated cathinones) where R_2 is a hydrogen and R_3 can be either a methyl or ethyl group. The second group is constituted by cathinones with a tertiary amine (*N*,*N*′-dialkylated cathinones), with R_2 and R_3 being independent alkyl groups, usually two methyl or two ethyl groups. The third group is constituted by cathinones where the nitrogen atom is part of a pyrrolidine ring. In all three categories R_1 is an alkyl chain with, typically, 1 to 6 carbon atoms and R_4 is usually a methyl group, a halogen atom or a 3,4-methylenedioxy moiety.

Initially, cathinones were designed as active pharmaceutical ingredients (API) of medicinal products, *e.g.* amfepramone was used as appetite suppressant and pyrovalerone as anti-fatigue medicine [6], but their abusive use as substitutes of conventional drugs is an ongoing problem that causes a real public health issue. Methylone (3,4-methylendioxymethcathinone) was the first cathinone to appear on the market as legal alternative (at the time) to MDMA. Since then, there has been a continuous increase of new cathinones entering the market, which reached its peak in the last three years. Within the EU during the 2014–2016 period, a total of 76 new cathinones were notified to the EWS of EMCDDA and Europol, against only 50 in the nine previous years [7]. The legal status of cathinones depends on the substance and varies between countries, being some of them internationally controlled by the 1971 Convention on Psychotropic Substances: cathinone and methcathinone are listed in Schedule I, mephedrone, methylone and MDPV in Schedule II, cathine in Schedule III and pyrovalerone in Schedule IV [7].

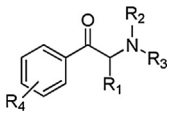

Fig. 1. General structure of a cathinone.

Like many psychostimulants and illicit drugs [8], synthetic cathinones exert their activity interacting with monoamine neurons that possess monoamine transporters (MATs) in the central nervous system (CNS) [6]. According to their structure, synthetic cathinones can increase the synaptic concentration of monoamines either by inhibiting their corresponding transporters, or by rising the pre-synaptic release of the neurotransmitters [9–14].

Several fatalities have been associated with the consumption of these drugs, although, due to the lack of knowledge of pharmacological and toxicological properties of cathinones, their role in overdose death cases is not yet fully understood [15]. Postmortem analysis of blood or other available matrices of several individuals revealed that MDPV, mephedrone, methylone, pyrovalerone, pentylone, α-PVP and methedrone were involved in the cause of death [16–20]. These fatal intoxications involved several factors, including liver failure. Reports of khat-induced hepatotoxicity have been increasing over the years, particularly in the UK, Netherlands and other European countries where *Catha edulis* is legal. Up to date the mechanism of hepatoxicity is not clearly understood, although studies suggested that unbalanced liver enzymes and hepatocellular degeneration could be involved [21]. Since cathinones are chemically and pharmacologically similar to cathinone, the major constituent of khat, and to classic illicit stimulants, it is foreseeable that they pose the same health risks than those compounds. However, since cathinones were recently added to the spectrum of abuse drugs currently used, their effects in the human body, such as hepatotoxicity and long-term side effects, are not yet fully understood.

Luethi et al. has just published the results from a study about the hepatocellular toxicity of several synthetic cathinones (MDPV, mephedrone, methedrone, methylone, naphyrone and bupropion) using two human hepatocyte cell lines (HepG2 and HepaRG) as model [22]. Their results revealed that all cathinones were cytotoxic in both cell lines although HepaRG cells were less sensitive to the cathinones exposure. The toxicity of the cathinones methylone, pentedrone, MDPV and 4-MEC was assessed by Valente et al. [23–25] in primary rat hepatocytes and HepaRG cells. All cathinones were hepatotoxic but at a different extent according with the specific structure. MDPV and pentedrone were the more toxic compounds while methylone was the least toxic. Wojcieszak et al. [26] investigated the effects of pyrovalerone, MDPV, 2,3-MDPV, α-PVP and PV9 using human HepG2 cell line as model of hepatocytes. Pyrovalerone, MDPV, and 2,3-MDPV show low to moderate cytotoxicity, whereas α-PVP and PV6 were the more cytotoxic, at lower concentrations than methamphetamine used as control.

In order to reach human cells and exert their effect, these compounds must cross the biological membranes, indicating a need to retain a high lipophilicity. The cathinones usually have high pK_a values (~8.5) [27] which make them prone to protonation in solution at physiological pH, leading to high aqueous solubility. However, it is expected that cathinones can have their pK_a values shifted when interacting with a lipidic membrane, similarly to what has already been observed for peptides [28]. With these environment-induced pK_a shifts, compounds are able to partitioning into biological membranes and circumvent limitations in solubility, trafficking, bioavailability, and subcellular pharmacokinetics [29]. Molecular dynamics (MD) simulation is a powerful technique yielding atomic details of complex systems which are usually not accessible to experiments. Most compounds usually present thermodynamic and/or kinetic barriers for membrane permeability and crossing, which led to the development of steered MD and umbrella sampling computational protocols to circumvent them [30,31]. However, cathinones can use the amino group (de)protonation event to keep their water and membrane