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Interactions of phenethylamine-derived psychoactive substances of the 2C-series with human monoamine oxidases

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Abstract

Psychoactive substances of the 2C-series (2Cs) are phenethylamine-derived designer drugs that can induce psychostimulant and hallucinogenic effects. Chemically, the classic 2Cs contain two methoxy groups in positions 2 and 5 of the phenyl ring, whereas substances of the so-called FLY series contain rigidified methoxy groups integrated in a 2,3,6,7-tetrahydrobenzo[1,2-b:4,5-b']difuran core. One of the pharmacological features that has not been investigated in detail includes the inhibition of monoamine oxidase (MAO). Inhibition of this enzyme can cause elevated monoamine levels that have been associated with adverse events such as agitation, nausea, vomiting, tachycardia, hypertension, or seizures. The aim of this study was to extend the knowledge surrounding the potential of MAO inhibition for 17 test drugs, which consisted of twelve 2Cs (2C-B, 2C-D, 2C-E, 2C-H, 2C-I, 2C-N, 2C-P, 2C-T-2, 2C-T-7, 2C-T-21, bk-2C-B and bk-2C-I) and five FLY analogs (2C-B-FLY, 2C-E-FLY, 2C-EF-FLY, 2C-I-FLY, 2C-T-7-FLY). The extent of MAO inhibition was assessed using an established in vitro procedure based on heterologously expressed enzymes and analysis by hydrophilic interaction liquid chromatography-high resolution tandem mass spectrometry. Thirteen test drugs showed inhibition potential for MAO-A and 11 showed inhibition of MAO-B. In cases where MAO-A IC_{50} values could be determined, values ranged from 10 to 125 μM (7 drugs) and 1.7 to 180 μM for MAO-B (9 drugs). In the absence of detailed clinical information on most test drugs, it is concluded that a pharmacological contribution of MAO inhibition cannot be excluded and that further studies are warranted.

KEYWORDS

Monoamine oxidase inhibition, IC_{50} value, drugs of abuse, HILIC-HRMS/MS, phenethylamines

1 INTRODUCTION

Year after year, more and more so-called new psychoactive substances (NPS) enter the drugs of abuse (DOA) market with 803 different substances being reported to the United Nations Office on Drugs and Crime in the period 2009-2017.[1,2] Many NPS show psychoactive effects similar to drugs under international control and are often sold via the Internet.[1] Although NPS are initially not controlled and easily available, they can pose a significant health risk and reports about adverse effects are frequently available.[3-6] One of the challenges when dealing with this phenomenon is the lack of knowledge concerning pharmacokinetics and toxicity since they are marketed without (pre)clinical safety studies.[7] The 2C-series drugs of abuse (2Cs) are phenethylamine derivatives that commonly exhibit a primary amine functionality separated from the phenyl ring by two carbon atoms, which differs from their amphetamine counterparts that show a methyl group at the alpha-position (3Cs) (Figure 1). Within a 2,5-dimethoxyphenethylamine nucleus, a lipophilic substituent is commonly present in position 4.[8] In the so-called FLY series, the methoxy groups are rigidified into a 2,3,6,7-tetrahydrobenzo[1,2-b:4,5-b']difuran core.[9] A number of 2Cs have shown psychostimulant and hallucinogenic properties and information gathered from casework suggests that intoxicated patients might exhibit either a sympathomimetic toxidrome, serotonin (5-HT) toxicity, hallucinogenesis, or combinations thereof.[10] The available information on the pharmacology of 2Cs indicates that the interaction with serotonin receptor subtypes is one of the pharmacological features linked to these substances.[9,11-14] Partial agonism at alpha-1 adrenergic receptors has also been described for 2C-B.[15] In addition, the re-uptake inhibition of the monoamines norepinephrine and 5-HT into rat brain synaptosomes was described for 2,5-dimethoxy-4-ethylphenetylamine (2C-E) and 2C-I.[16] At the same time, monoamine oxidase (MAO) inhibition may also lead to elevated monoamine levels that have been associated with adverse events described in cases of 2C intoxications, which included agitation, nausea, vomiting, tachycardia, hypertension, or seizures.[10,17-19]

The aim of this study was to extend the knowledge surrounding the potential of MAO inhibition (and to determine their IC_{50} values) for 17 test drugs, which consisted of twelve 2Cs (2C-

B, 2C-D, 2C-E, 2C-H, 2C-I, 2C-N, 2C-P, 2C-T-2, 2C-T-7, 2C-T-21, bk-2C-B and bk-2C-I) and five FLY analogs (2C-B-FLY, 2C-E-FLY, 2C-EF-FLY, 2C-I-FLY, 2C-T-7-FLY) (Figure 1). A previously published MAO inhibition assay based on heterologously expressed enzymes and hydrophilic interaction liquid chromatography-high resolution tandem mass spectrometry (HILIC-HRMS/MS) was applied for this purpose. MAO activity should be assessed using kynuramine (KYN) as the non-selective substrate as the formation of the corresponding aldehyde is catalyzed by MAO-A and B and followed by non-enzymatic condensation to the product 4-hydroxyquinoline (4-OHC).

2 EXPERIMENTAL

2.1 Chemicals and enzymes

The baculovirus-infected insect cell microsomes (Supersomes) containing human complementary DNA-expressed MAO-A or MAO-B (5 mg protein/mL) and wild-type Supersomes without MAO activity as negative control (MAO control, 5 mg protein/mL) were obtained from Corning (Amsterdam, The Netherlands). After delivery, enzyme preparations were thawed at 37°C, aliquoted, snap-frozen in liquid nitrogen, and stored at -80°C until use.

Amphetamine-d_5 was obtained from LGC Standards (Wesel, Germany), selegiline, KYN, 4-OHC, ammonium acetate, potassium dihydrogenphosphate, and dipotassium hydrogenphosphate from Sigma-Aldrich (Taufkirchen, Germany), formic acid (MS grade) from Fluka (Neu-Ulm, Germany), acetonitrile, methanol (both LC-MS grade), and all other chemicals from VWR (Darmstadt, Germany). 5-(2-Aminopropyl)indole (5-IT) was synthesized[21] and provided by the Department of Pharmacology and Therapeutics, Trinity Centre for Health Sciences, St James's Hospital (Dublin, Ireland). 2C-B tartrate was provided for research purposes before scheduled by Hessisches Landeskriminalamt (Wiesbaden, Germany), 2C-I hydrochloride by Landeskriminalamt Baden-Württemberg (Stuttgart, Germany), 2,5-dimethoxy-4-ethylthiophenethylamine (2C-T-2) hydrochloride by Bundeskriminalamt (Wiesbaden, Germany), and 2,5-dimethoxy-phenethylamine (2C-H) and 2,5-dimethoxy-4-nitrophenethylamine (2C-N) by the Department of Forensic

Toxicology, Institute of Forensic Research (Krakow, Poland). 5-IT, 2C-B, 2C-I, and 2C-T-2 were provided before they were scheduled. 2,5-Dimethoxy-4-methylphenethylamine (2C-D) hydrochloride, 2C-E hydrochloride, and 2C-T-7 hydrochloride were purchased from Lipomed AG (Weil am Rhein, Germany), 2,5-dimethoxy-4-propylphenethylamine (2C-P) hydrochloride from Dejachem (Schwendi, Germany), and 2C-B-FLY hydrochloride from Cayman Chemicals (Ann Arbor, USA). beta-Keto-2C-B (bk-2C-B) hydrochloride and bk-2C-I were available from previous work.[22,23] 2,5-Dimethoxy-4-(2-fluoroetylthio)phenethylamine (2C-T-21) hydrochloride was obtained in high purity from a research chemicals supplier. 2-(4-Ethyl-2,3,6,7-tetrahydrofuro[2,3-f][1]benzofuran-8-yl)ethanamine (2C-E-FLY) hydrochloride, 2-(4-(2-fluor)ethyl-2,3,6,7-tetrahydrofuro[2,3-f][1]benzofuran-8-yl)ethanamine (2C-EF-FLY) hydrochloride, 2-(4-iodo-2,3,6,7-tetrahydrofuro[2,3-f][1]benzofuran-8-yl)ethanamine (2C-I-FLY), and 2-(4-propylthio-2,3,6,7-tetrahydrofuro[2,3-f][1]benzofuran-8-yl)ethanamine (2C-T-7-FLY) hydrochloride were provided by Synex Synthetics BV, Maastricht, Netherlands.

Stock solutions were prepared in water (KYN: 6.25 mM, 4-OHC: 0.1 mM) or methanol (5-IT: 5 mM, selegiline: 5 mM, 2Cs: 1 mg/mL, each). Stock solutions were aliquoted and stored at -20°C until use. To obtain the working solutions used for the incubations, stock solutions or enzyme preparations were serially diluted using 100 mM phosphate buffer. Prior to the determinations of IC_{50} values, 2C stock solutions were gently evaporated under nitrogen and dissolved in water/methanol (9:1, v/v) to keep the organic solvent content in the final incubation mixtures constantly below 1%.[24]

2.2 Initial MAO inhibition screening

Final incubation mixtures had a volume of 30 µL and consisted of 1 µg/mL MAO-A or MAO-B, the non-selective MAO substrate KYN at concentrations comparable to its K_m value (MAO-A: 43 µM, MAO-B: 23 µM), and 10 µM of one of the potential inhibitors as described before.[20] Reactions were initiated by addition of the ice-cold enzyme dilution, incubated for 20 min at 37°C, and stopped with 30 µL of ice-cold acetonitrile containing 10 µM amphetamine-d_5 as internal standard

(IS). The mixture was centrifuged for 2 min at 10,000 g, 50 µL of the supernatant were transferred to an autosampler vial, and injected onto the HILIC-HRMS/MS apparatus for analysis. All incubations were performed in triplicate (n = 3). In addition to these samples, reference samples without inhibitor, positive control samples with known inhibitors (MAO-A: 5-IT, MAO-B: selegiline, 10 µM, each), blank samples without MAO activity, and interfering samples were also prepared in triplicate. Interfering samples were incubated reference samples without inhibitor and terminated with ice-cold acetonitrile containing the IS and the test drugs each at a concentration of 10 µM. A simplified scheme of the initial inhibition screening procedure is given in Figure 2. The 4-OHC amount, given as the peak area ratio of 4-OHC and the IS, in blank samples without MAO activity was subtracted from the 4-OHC amount detected in all other samples. The 4-OHC amount detected in reference samples without inhibitor was set at 100% MAO activity and compared to all other incubations. For statistical analysis, a one-way ANOVA followed by Dunnett's multiple comparison test (significance level, $P < 0.001$, 99.9% confidence intervals) by GraphPad Prism 5.00 (GraphPad Software, San Diego, USA) was used.

2.3 Determination of IC_{50} values

MAO-A or B were incubated with the following 10 inhibitor concentrations: 2, 5, 10, 20, 39, 78, 156, 313, 625, 1250 µM, with the exception of 2C-H that was used in lower concentrations (0.04, 0.08, 0.15, 0.3, 0.6, 1, 2, 5, 10, 20 µM). All incubations were performed in duplicate (n = 2). All other incubation conditions were the same as described above. Reference and blank samples were also prepared as described above. The IC_{50} values were calculated by plotting the 4-OHC formation expressed as MAO activity (relative to reference samples) over the logarithm of the inhibitor concentration using GraphPad Prism 5.00.

2.4 HILIC-HRMS/MS conditions

Apparatus and conditions were the same as already described.[20] A Thermo Fisher Scientific (TF, Dreieich, Germany) Dionex UltiMate 3000 Rapid Separation (RS) UHPLC system with a

quaternary UltiMate 3000 RS pump and an UltiMate 3000 RS autosampler was used and controlled by the TF Chromeleon software version 6.80. The chromatographic system was coupled to a TF Q-Exactive Plus equipped with a heated electrospray ionization II source (HESI-II). The gradient elution was performed on a Macherey-Nagel (Düren, Germany) HILIC Nucleodur column (125 x 3 mm, 3 µm) using aqueous ammonium acetate (25 mM, eluent A) and acetonitrile-containing 0.1% (v/v) formic acid (eluent B). The flow rate was set to 500 µL/min and the gradient was programmed as follows: 0– 0.5 min hold 80% B, curve 5; 0.5–3.8 min to 50% B, curve 5; 3.8– 3.9 min to 40% B, curve 5; 3.9–5 min hold 40% B, curve 5; 5–5.1 min to 80% B, curve 5; and 5.1–6 min hold 80% B, curve 5. Chromatography was performed at 60°C maintained by a Dionex UltiMate 3000 RS analytical column heater. The injection volume for all samples was 1 µL. HESI-II conditions were modified according to Helfer et al. due to improved robustness: sheath gas, 60 arbitrary units (AU); auxiliary gas, 10 AU; spray voltage, 4.00 kV; heater temperature, 320°C; ion transfer capillary temperature, 320°C; and S-lens RF level, 60.0.[25] Mass calibration was performed prior to analysis according to the manufacturer's recommendations using external mass calibration. Quantification was performed using targeted single ion monitoring (t-SIM) and a subsequent data-dependent MS^2 (dd-MS^2) mode with an inclusion list containing the exact masses of positively charged KYN (m/z 165.1022), 4-OHC (m/z 146.0600), and the IS (m/z 141.1434). The settings for the t-SIM mode were as follows: micro scan, 1; resolution, 35,000; AGC target, $5e^4$; maximum IT, 100 ms; and isolation window, 4 m/z. The settings for the dd-MS^2 mode were as follows: micro scan, 1; resolution, 35,000; AGC target, $2e^5$; maximum IT, 100 ms; isolation window, 4 m/z; and dynamic exclusion, 4 s. TF Xcalibur Qual Browser 2.2 software was used for data handling. The settings for automated peak integration were as follows: peak detection algorithm, ICIS; area noise factor, 5; and peak noise factor, 300. GraphPad QuickCalcs was used for outlier detection (http://graphpad.com/quickcalcs/grubbs1), while GraphPad Prism 5.00 was used for statistical evaluation.

3 RESULTS AND DISCUSSION

3.1 Initial MAO inhibition screening

Formation of 4-OHC was measured using HILIC-HRMS/MS and the complete analytical procedure was already previously successfully validated.[20] The results of the initial MAO inhibition screening of the 2Cs are summarized in Figure 3. Suitable incubation conditions were verified using positive control samples with known inhibitors (see Figure 2). 5-IT reduced MAO-A activity by more than 90%, while selegiline almost completely inhibited MAO-B activity, which was in agreement with the previous study.[20] As only 4-OHC, KYN, and the IS were detected by the analytical method, it was mandatory to exclude analytical interferences, such as ion suppression or enhancement caused by potentially co-eluting test drugs. Therefore, MAO activity determined from the interfering samples was compared to that in the reference samples. The test compounds caused no analytical interferences.

For thirteen test drugs, MAO-A inhibition was observed to some degree. Seven of these drugs (2C-B, 2C-I, 2C-T-7, 2C-B-FLY, 2C-E-FLY, 2C-I-FLY, and 2C-T-7-FLY) provided a statistically significant reduction of MAO-A activity (***, $P < 0.001$). However, none of these drugs was able to reduce the MAO-A activity by more than 50%, which meant that IC_{50} values could not be determined below 10 µM, as this was the concentration used in the initial inhibition screening. Eleven test drugs resulted in some MAO-B inhibitions and nine substances (2C-B, 2C-D, 2C-E, 2C-H, 2C-I, 2C-N, 2C-T-7, bk-2C-B, and bk-2C-I) induced a reduction in MAO-B activity that was highly significant (***, $P < 0.001$). However, only 2C-H reduced MAO-B activity by more than 50%, which resulted in a corresponding IC_{50} value below 10 µM (Table 1).

3.2 Determination and comparison of IC_{50} values

In order to save time and costs, IC_{50} values for MAO-A and B inhibition were only determined in cases where the test drugs revealed a statistically highly significant (***, $P < 0.001$) inhibition during the initial screening phase. All incubations for the IC_{50} value determinations were performed in duplicate, in contrast to triplicate incubations during the initial MAO inhibition screening, to reduce workload and number of samples in accordance with the previous study.[20] In total, seven IC_{50} values of MAO-A inhibitors and nine IC_{50} values of MAO-B inhibitors were determined and

listed in Table 1. For MAO-A inhibition, IC_{50} values were determined between 10 (2C-T-7-FLY) and 125 μM (2C-B and 2C-I). For MAO-B inhibition, IC_{50} values were found to range between 1.7 (2C-H) and 180 μM (2C-T-7).

As far as the potential clinical relevance of MAO inhibition based on IC_{50} values was concerned, plasma concentrations obtained from the case report literature (Table 1) might be worthy of consideration even though only limited information is available. Usually, case reports involving fatal or non-fatal intoxications are the only information source and difficult to interpret due to individual variation and/or poly-drug intoxication. In those reports, dosage, time of ingestion, and route of application remain often unclear. Postmortem concentrations are particularly problematic as they can be affected by postmortem redistribution.[26,27] Although the presence of some 2Cs on the DOA market is documented,[28,29] few case reports involving drug consumption appear to be available. For example, plasma concentrations from individual patients have only been reported for 2C-P and 2C-T-7.[17,19] In the first case, a 19-year-old male ingested approximately 25 mg 2C-P, which was sold as 2C-B, and was admitted to the emergency department with severe hallucinations, mydriasis, tachycardia, agitation, and confusion.[17] In the second case, a 20-year-old male died after he had been insufflating approximately 35 mg of 2C-T-7 and quantification was subsequently performed in postmortem samples.[19] The IC_{50} values determined in this study were higher than the plasma concentrations reported for 2C-P and 2C-T-7. However, as they were measured in individual cases, their significance remains unclear. Shulgin and Shulgin published their experiences with numerous phenethylamines, which included information about synthesis, dosage and duration of effects.[8] These data are available for all 2Cs tested in this study with exception of the two bk-2Cs and FLY analogs. For some compounds, such as 2C-D or 2C-N, higher doses were described than those estimated in the previously mentioned case reports. Consequently, higher doses are expected to lead to higher plasma concentrations. However, it must be considered that concentrations detectable in certain tissues are often higher than in plasma due to lipophilicity or active transport processes. A brain-to-plasma concentration ratio of 13.9 was described for 2C-B in rats.[30] Elevated concentrations in the liver, the main metabolizing organ, are also more than

likely to be encountered.[31] From this perspective, a contribution to the clinical effects of MAO inhibition can perhaps not be excluded.

2C-B, 2C-I, and 2C-T-7 were identified as moderately potent MAO-A inhibitors with IC_{50} values of 46 (2C-T-7) or 125 µM (2C-B and 2C-I). However, the corresponding FLYs were shown to be more potent MAO-A inhibitors with IC_{50} values of 10 (2C-T-7-FLY), 13 (2C-I-FLY), and 19 µM (2C-B-FLY). As MAO-A is predominantly catalyzing the deamination of 5-HT,[32] its inhibition is likely to contribute to increasing 5-HT levels, which could be clinically relevant. For example, in the case of 2C-I intoxication, 5-HT toxicity has been observed as a clinical feature.[18] The MAO-A activity related to different 2C-B or 2C-B-FLY concentrations is depicted in Figure 4. 2C-E-FLY provided an IC_{50} value of 18 µM comparable to the above-mentioned FLYs, whereas 2C-EF-FLY showed only weak MAO-A inhibition during the initial inhibition screening procedure.

The FLYs were found to be inactive as MAO-B inhibitors with the exception of 2C-E-FLY, which resulted in weak MAO-B inhibition during the initial inhibition screening. In contrast, almost all classic 2Cs (apart from 2C-P and 2C-T-21), and the two bk-2Cs showed MAO-B inhibition potential. While 2C-B and 2C-I moderately inhibited MAO-B with IC_{50} values of 58 and 55 µM, respectively, the inhibition by bk-2C-B and bk-2C-I was found to be more potent resulting in IC_{50} values of 14 and 15 µM, respectively. These findings revealed that the replacement of bromine by iodine did impact on MAO-A or B inhibition. The MAO-B activity related to different 2C-I or bk-2C-I concentrations is depicted in Figure 5.

2C-H was shown to be the most potent MAO-B inhibitor with an IC_{50} value of 1.7 µM. However, Shulgin and Shulgin reported 2C-H to only result in weak psychoactive effects in animal assays.[8] In comparison, 2C-D and 2C-E revealed higher IC_{50} values of 24 and 124 µM, respectively, whereas 2C-P only provided weak MAO-B inhibition potential during the initial inhibition screening. The MAO-B inhibitory potency of 2C-N (IC_{50} value 66 µM) was comparable to that of 2C-B or 2C-I.

A previous study identified the MAO and cytochrome P450 isoforms involved in the deamination of 2C-B, 2C-D, 2C-E, 2C-I, 2C-T-2, and 2C-T-7.[33] MAO-A and B were shown to be

the predominant enzymes involved in formation of their aldehydes and the studied 2Cs had a higher affinity to MAO-A than B.[33] As the present study identified these compounds with exception of 2C-T-2 as inhibitors of MAO-B, a competitive inhibition mechanism might be possible.

Nevertheless, as some 2Cs and 2C-B-FLY were described to have affinities to 5-HT receptor subtypes within the nM range[9,11-14], MAO IC_{50} values within the μM range are expected to play only a minor role in the pharmacological effects after consumption of recreational doses. However, a contribution of MAO inhibition to the clinical effects observed in intoxication cases cannot be excluded.

4 CONCLUSIONS

The presented study identified various 2C-, and FLY-related test drugs as MAO inhibitors. A previously published inhibition assay was successfully applied for initial inhibition screening followed by IC_{50} value determinations. The FLYs were identified as MAO-A inhibitors, whereas the classic 2Cs with or without beta-keto functionality exhibited MAO-B inhibition potential. 2C-T-FLY and 2C-H were identified as the most potent MAO-A or B inhibitors with IC_{50} values of 10 or 1.7 μM, respectively. The estimation of a clinical relevance of MAO inhibition based on IC_{50} values however was challenging given the lack of information regarding plasma concentrations of these test drugs. Nevertheless, the extent to which the clinical pharmacology of the evaluated test drugs involves MAO inhibition warrants further investigation.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

1. World Drug Report 2018. UNODC; 2018. https://www.unodc.org/wdr2018/index.html. Accessed 10/07/2018.
2. World Drug Report 2018. Booklet 3. Analysis of drug markets - opioids, cocaine, cannabis, synthetic drugs. UNODC; 2018. https://www.unodc.org/wdr2018/prelaunch/WDR18_Booklet_3_DRUG_MARKETS.pdf. Accessed 10/07/2018.
3. Wagmann L, Maurer HH. Bioanalytical methods for new psychoactive substances. *Handb Exp Pharmacol.* 2018.
4. Zamengo L, Frison G, Bettin C, Sciarrone R. Understanding the risks associated with the use of new psychoactive substances (NPS): high variability of active ingredients concentration, mislabelled preparations, multiple psychoactive substances in single products. *Toxicol Lett.* 2014;229(1):220-228.
5. Logan BK, Mohr ALA, Friscia M, et al. Reports of adverse events associated with use of novel psychoactive substances, 2013-2016: a review. *J Anal Toxicol.* 2017;41(7):573-610.
6. Fentanils and synthetic cannabinoids: driving greater complexity into the drug situation. An update from the EU Early Warning System. EMCDDA; 2018. http://www.emcdda.europa.eu/system/files/publications/8870/2018-2489-td0118414enn.pdf. Accessed 10/07/2018.
7. Meyer MR. New psychoactive substances: an overview on recent publications on their toxicodynamics and toxicokinetics. *Arch Toxicol.* 2016;90(10):2421-2444.
8. Shulgin A, Shulgin A. Pihkal, a chemical love story. *Transform Press, Berkley (CA).* 1991.
9. Monte AP, Marona-Lewicka D, Parker MA, Wainscott DB, Nelson DL, Nichols DE. Dihydrobenzofuran analogues of hallucinogens. 3. Models of 4-substituted (2,5-dimethoxyphenyl)alkylamine derivatives with rigidified methoxy groups. *J Med Chem.* 1996;39(15):2953-2961.
10. Dean BV, Stellpflug SJ, Burnett AM, Engebretsen KM. 2C or not 2C: phenethylamine designer drug review. *J Med Toxicol.* 2013;9(2):172-178.
11. Johnson MP, Mathis CA, Shulgin AT, Hoffman AJ, Nichols DE. [125I]-2-(2,5-dimethoxy-4-iodophenyl)aminoethane ([125I]-2C-I) as a label for the 5-HT2 receptor in rat frontal cortex. *Pharmacol Biochem Behav.* 1990;35(1):211-217.
12. Villalobos CA, Bull P, Saez P, Cassels BK, Huidobro-Toro JP. 4-Bromo-2,5-dimethoxyphenethylamine (2C-B) and structurally related phenylethylamines are potent 5-HT2A receptor antagonists in Xenopus laevis oocytes. *Br J Pharmacol.* 2004;141(7):1167-1174.
13. Fantegrossi WE, Harrington AW, Eckler JR, et al. Hallucinogen-like actions of 2,5-dimethoxy-4-(n)-propylthiophenethylamine (2C-T-7) in mice and rats. *Psychopharmacology (Berl).* 2005;181(3):496-503.
14. Power JD, Kavanagh P, O'Brien J, et al. Test purchase, identification and synthesis of 2-amino-1-(4-bromo-2, 5-dimethoxyphenyl)ethan-1-one (bk-2C-B). *Drug Test Anal.* 2015;7(6):512-518.
15. Lobos M, Borges Y, Gonzalez E, Cassels BK. The action of the psychoactive drug 2C-B on isolated rat thoracic aorta. *Gen Pharmacol.* 1992;23(6):1139-1142.
16. Nagai F, Nonaka R, Satoh Hisashi Kamimura K. The effects of non-medically used psychoactive drugs on monoamine neurotransmission in rat brain. *Eur J Pharmacol.* 2007;559(2-3):132-137.
17. Stoller A, Dolder PC, Bodmer M, et al. Mistaking 2C-P for 2C-B: what a difference a letter makes. *J Anal Toxicol.* 2017;41(1):77-79.
18. Bosak A, LoVecchio F, Levine M. Recurrent seizures and serotonin syndrome following "2C-I" ingestion. *J Med Toxicol.* 2013;9(2):196-198.

19. Curtis B, Kemp P, Harty L, Choi C, Christensen D. Postmortem identification and quantitation of 2,5-dimethoxy-4-n-propylthiophenethylamine using GC-MSD and GC-NPD. *J Anal Toxicol.* 2003;27(7):493-498.
20. Wagmann L, Brandt SD, Kavanagh PV, Maurer HH, Meyer MR. In vitro monoamine oxidase inhibition potential of alpha-methyltryptamine analog new psychoactive substances for assessing possible toxic risks. *Toxicol Lett.* 2017;272:84-93.
21. Scott KR, Power JD, McDermott SD, et al. Identification of (2-aminopropyl)indole positional isomers in forensic samples. *Drug Test Anal.* 2014;6(7-8):598-606.
22. Texter KB, Waymach R, Kavanagh PV, et al. Identification of pyrolysis products of the new psychoactive substance 2-amino-1-(4-bromo-2,5-dimethoxyphenyl)ethanone hydrochloride (bk-2C-B) and its iodo analogue bk-2C-I. *Drug Test Anal.* 2018;10(1):229-236.
23. Nichols DE. Chemistry and structure-activity relationships of psychedelics. *Curr Top Behav Neurosci.* 2018;36:1-43.
24. Chauret N, Gauthier A, Nicoll-Griffith DA. Effect of common organic solvents on in vitro cytochrome P450-mediated metabolic activities in human liver microsomes. *Drug Metab Dispos.* 1998;26(1):1-4.
25. Helfer AG, Michely JA, Weber AA, Meyer MR, Maurer HH. Orbitrap technology for comprehensive metabolite-based liquid chromatographic-high resolution-tandem mass spectrometric urine drug screening - exemplified for cardiovascular drugs. *Anal Chim Acta.* 2015;891:221-233.
26. Shintani-Ishida K, Saka K, Nakamura M, Yoshida KI, Ikegaya H. Experimental study on the postmortem redistribution of the substituted phenethylamine, 25B-NBOMe. *J Forensic Sci.* 2018;63(2):588-591.
27. Staeheli SN, Gascho D, Ebert LC, Kraemer T, Steuer AE. Time-dependent postmortem redistribution of morphine and its metabolites in blood and alternative matrices-application of CT-guided biopsy sampling. *Int J Legal Med.* 2017;131(2):379-389.
28. King LA. New phenethylamines in Europe. *Drug Test Anal.* 2014;6(7-8):808-818.
29. Report on the risk assessment of 2C-I, 2C-T-2 and 2C-T-7 in the framework of the joint action on new synthetic drugs. EMCDDA; 2004. http://www.emcdda.europa.eu/html.cfm/index33353EN.html. Accessed 10/07/2018.
30. Rohanova M, Palenicek T, Balikova M. Disposition of 4-bromo-2,5-dimethoxyphenethylamine (2C-B) and its metabolite 4-bromo-2-hydroxy-5-methoxyphenethylamine in rats after subcutaneous administration. *Toxicol Lett.* 2008;178(1):29-36.
31. Li R, Niosi M, Johnson N, et al. A Study on pharmacokinetics of bosentan with systems modeling, Part 1: Translating systemic plasma concentration to liver exposure in healthy subjects. *Drug Metab Dispos.* 2018;46(4):346-356.
32. Tipton KF. 90 years of monoamine oxidase: some progress and some confusion. *J Neural Transm (Vienna).* 2018.
33. Theobald DS, Maurer HH. Identification of monoamine oxidase and cytochrome P450 isoenzymes involved in the deamination of phenethylamine-derived designer drugs (2C-series). *Biochem Pharmacol.* 2007;73(2):287-297.
34. Backberg M, Beck O, Hulten P, Rosengren-Holmberg J, Helander A. Intoxications of the new psychoactive substance 5-(2-aminopropyl)indole (5-IT): a case series from the Swedish STRIDA project. *Clin Toxicol (Phila).* 2014;52(6):618-624.
35. Report on the risk assessment of 5-(2-aminopropyl)indole in the framework of the Council Decision on new psychoactive substances. EMCDDA; 2014. http://www.emcdda.europa.eu/publications/risk-assessment/5-IT_en. Accessed 10/07/2018.
36. Barrett JS, Rohatagi S, DeWitt KE, Morales RJ, DiSanto AR. The effect of dosing regimen and food on the bioavailability of the extensively metabolized, highly variable drug eldepryl((R)) (selegiline hydrochloride). *Am J Ther.* 1996;3(4):298-313.

LEGENDS TO THE FIGURES

FIGURE 1 Chemical structures of the investigated test drugs of abuse.

FIGURE 2 Simplified scheme of the initial monoamine oxidases (MAO) inhibition screening procedure. (ACN: acetonitrile, IS: internal standard, 5-IT: 5-(2-aminopropyl)indole, KYN: kynuramine)

FIGURE 3 Initial monoamine oxidases (MAO) inhibition screening results using 10 µM of each test drug (MAO-A: top, MAO-B: bottom). Percentage of MAO activity represented the percentage of 4-hydroxyquinoline (4-OHC) formation in relation to reference incubations without test drug (100%). Values are expressed as mean and were tested for significance (n = 3, ***, $P < 0.001$, **, $P < 0.01$, *, $P < 0.1$ for 4-OHC formation in incubations with the inhibitor versus 4-OHC formation in reference incubations).

FIGURE 4 MAO-A activity related to different test drug concentrations used for IC_{50} value determination. Data points represent the mean value of duplicate measurements (n = 2).

FIGURE 5 MAO-B activity related to different test drug concentrations used for IC_{50} value determination. Data points represent the mean value of duplicate measurements (n = 2).

TABLE 1 IC_{50} values (standard errors) determined for 2C-based drugs of abuse and known monoamine oxidase (MAO) inhibitors. Reference plasma concentrations in μM were calculated from the published data (in μg/L). (PM: post-mortem; n.d.: not determined)

Test compound	Reference plasma concentration,		IC_{50} value, μM	
	μg/L	μM	MAO-A	MAO-B
2C-B	*		125 (1.1)	58 (1.3)
2C-D	*		n.d.	24 (1.4)
2C-E	*		n.d.	124 (1.2)
2C-H	*		n.d.	1.7 (1.1)
2C-I	*		125 (1.2)	55 (1.3)
2C-N	*		n.d.	66 (1.1)
2C-P	18[17]	0.1	n.d.	n.d.
2C-T-2	*		n.d.	n.d.
2C-T-7	57 and 100 (heart and femoral blood, PM)[19]	0.2 and 0.4	46 (1.1)	180 (1.3)
2C-T-21	*		n.d.	n.d.
bk-2C-B	*		n.d.	14 (1.1)
bk-2C-I	*		n.d.	15 (1.1)
2C-B-FLY	*		19 (1.1)	n.d.
2C-E-FLY	*		18 (1.1)	n.d.
2C-EF-FLY	*		n.d.	n.d.
2C-I-FLY	*		13 (1.1)	n.d.
2C-T-7-FLY	*		10 (1.1)	n.d.
Known inhibitors				
5-IT	15-590[34]	0.1-3.4	0.20[20]	
	700-5,100 (PM)[35]	4.0-29		
Selegiline	0.3-1.5[36]	0.002-0.01		0.017[20]

* No plasma concentrations reported

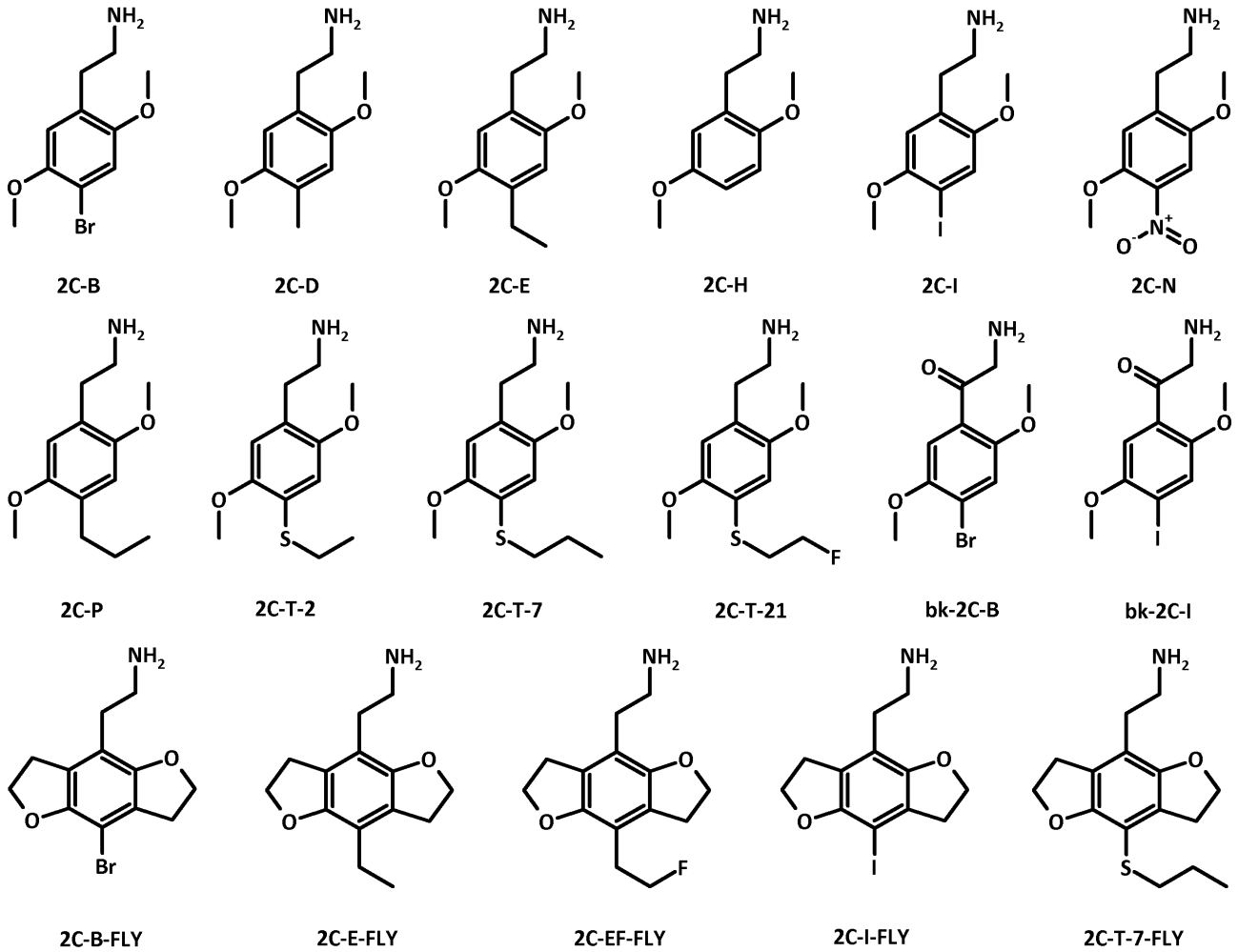
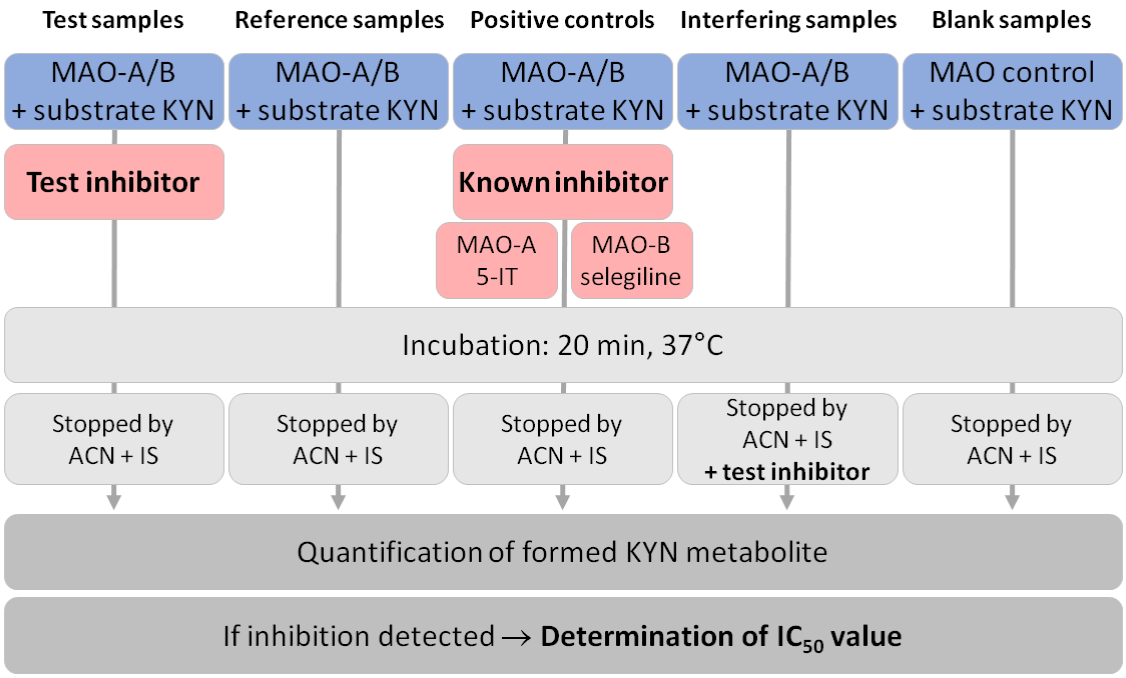


FIGURE 1. Chemical structures of the investigated test drugs of abuse.



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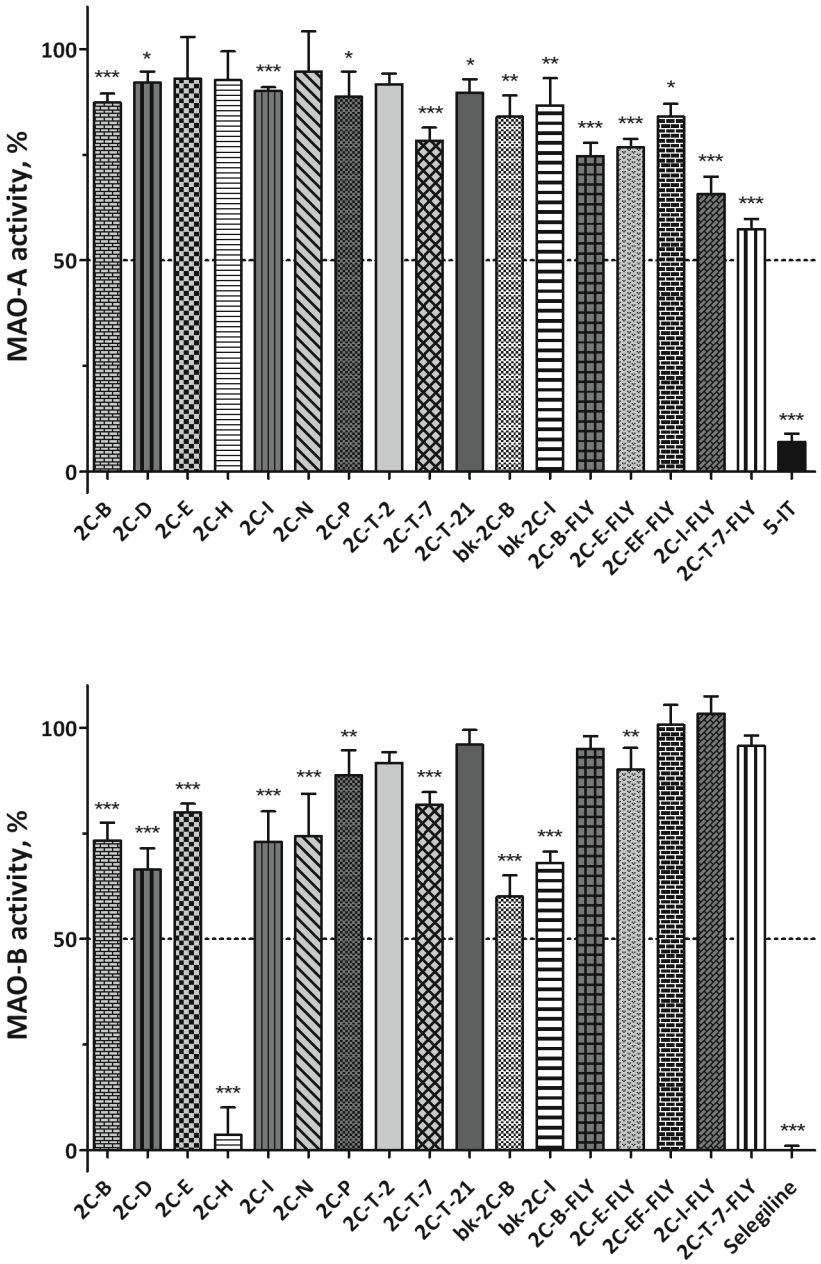

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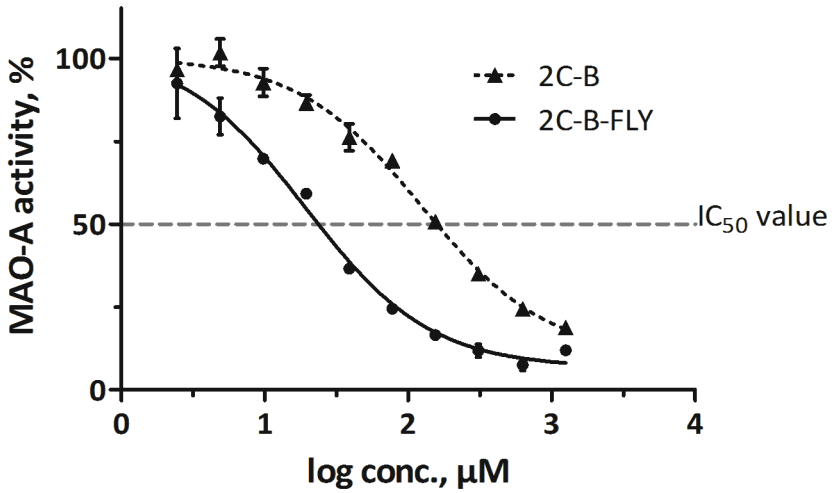

FIGURE 4. MAO-A activity related to different test drug concentrations used for IC50 value determination. Data points represent the mean value of duplicate measurements (n = 2).

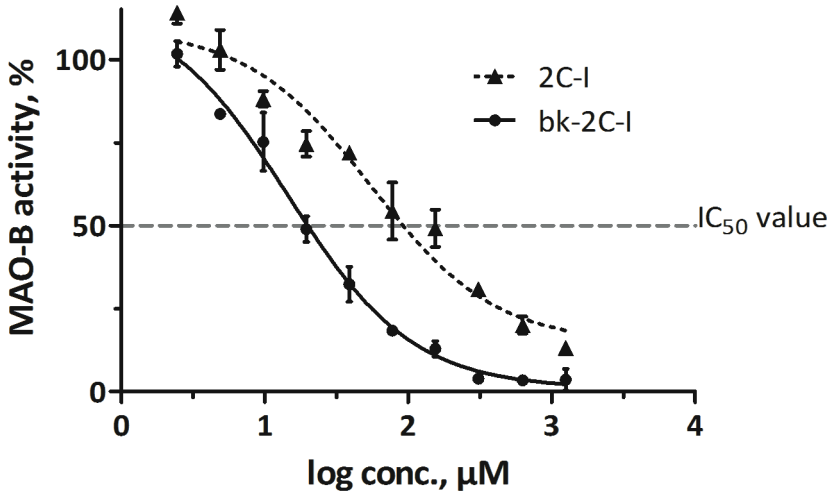

FIGURE 5. MAO-B activity related to different test drug concentrations used for IC50 value determination. Data points represent the mean value of duplicate measurements (n = 2).