Elsevier Editorial System(tm) for Toxicology Letters

Manuscript Draft

Manuscript Number: TOXLET-D-17-00086R1

Title: In vitro monoamine oxidase inhibition potential of alpha-methyltryptamine analog new psychoactive substances for assessing possible toxic risks

Article Type: Full Length Article

Keywords: Alpha-methyltryptamine analog new psychoactive substances, drugs of abuse, MAO inhibition, LC-HR-MS/MS, IC50 value

Corresponding Author: Professor Markus R Meyer, PhD

Corresponding Author's Institution: Saarland University

First Author: Lea Wagmann, MSc

Order of Authors: Lea Wagmann, MSc; Simon D Brandt, PhD; Pierce V Kavanagh, PhD; Hans H Maurer, PhD; Markus R Meyer, PhD

Abstract: Tryptamines have emerged as new psychoactive substances (NPS), which are distributed and consumed recreationally without preclinical studies or safety tests. Within the alpha-methylated tryptamines, some of the psychoactive effects of the prototypical alpha-methyltryptamine (AMT) have been described decades ago and a contributing factor of its acute toxicity appears to involve the inhibition of monoamine oxidase (MAO). However, detailed information about analogs is scarce. Therefore, thirteen AMT analogs were investigated for their potential to inhibit MAO. An in vitro assay analyzed using hydrophilic interaction liquid chromatography-high resolution-tandem mass spectrometry was developed and validated. The AMT analogs were incubated with recombinant human MAO-A or B and kynuramine, a non-selective MAO substrate to determine the IC50 values. The known MAO-A inhibitors 5-(2-aminopropyl)indole (5-IT), harmine, harmaline, yohimbine, and the MAO-B inhibitor selegiline were tested for comparison. AMT and all analogs showed MAO-A inhibition properties with IC50 values between 0.049 and 166 µM, whereas four analogs inhibited also MAO-B with IC50 values between 82 and 376 µM. 7-Me-AMT provided the lowest IC50 value against MAO-A comparable to harmine and harmaline and was identified as a competitive MAO-A inhibitor. Furthermore, AMT, 7-Me-AMT, and nine further analogs inhibited MAO activity in human hepatic S9 fraction used as model for the human liver which expresses both isoforms. The obtained results suggested that MAO inhibition induced by alpha-methylated tryptamines might be clinically relevant concerning possible serotonergic and adrenergic effects and interactions with drugs (of abuse) particularly acting as monoamine reuptake inhibitors. However, as in vitro assays have only limited conclusiveness, further studies are needed.

In vitro monoamine oxidase inhibition potential of alpha-methyltryptamine analog new psychoactive substances for assessing possible toxic risks

Highlights

- Study provided comprehensive in vitro data on the inhibitory potential of tryptamine-like new psychoactive substances (NPS) towards human MAO-A/B
- Studied compounds were shown to inhibit at least one MAO isoform
- 7-Me-AMT was identified to have the lowest IC50 value against MAO-A and to act as competitive inhibitor
- MAO inhibition by tested NPS was shown to be of possible clinical relevance concerning serotonergic and adrenergic effects
- Interactions with drugs and drugs of abuse particularly acting as monoamine reuptake inhibitors were shown to be likely

In vitro monoamine oxidase inhibition potential of alpha-methyltryptamine analog new psychoactive substances for assessing possible toxic risks

Lea Wagmann[a], Simon D. Brandt[b], Pierce V. Kavanagh[c], Hans H. Maurer[a], and Markus R. Meyer[a,*]

[a] *Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, Saarland University, Homburg (Saar), Germany*

[b] *School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK*

[c] *Department of Pharmacology and Therapeutics, Trinity Centre for Health and Sciences, St. James's Hospital, Dublin, Ireland*

*Corresponding author.

E-mail address: markus.meyer@uks.eu (M.R. Meyer).

Abstract

Tryptamines have emerged as new psychoactive substances (NPS), which are distributed and consumed recreationally without preclinical studies or safety tests. Within the alpha-methylated tryptamines, some of the psychoactive effects of the prototypical alpha-methyltryptamine (AMT) have been described decades ago and a contributing factor of its acute toxicity appears to involve the inhibition of monoamine oxidase (MAO). However, detailed information about analogs is scarce. Therefore, thirteen AMT analogs were investigated for their potential to inhibit MAO. An in vitro assay analyzed using hydrophilic interaction liquid chromatography-high resolution-tandem mass spectrometry was developed and validated. The AMT analogs were incubated with recombinant human MAO-A or B and kynuramine, a non-selective MAO substrate to determine the IC_{50} values. The known MAO-A inhibitors 5-(2-aminopropyl)indole (5-IT), harmine, harmaline, yohimbine, and the MAO-B inhibitor selegiline were tested for comparison. AMT and all analogs showed MAO-A inhibition properties with IC_{50} values between 0.049 and 166 µM, whereas four analogs inhibited also MAO-B with IC_{50} values between 82 and 376 µM. 7-Me-AMT provided the lowest IC_{50} value against MAO-A comparable to harmine and harmaline and was identified as a competitive MAO-A inhibitor. Furthermore, AMT, 7-Me-AMT, and nine further analogs inhibited MAO activity in human hepatic S9 fraction used as model for the human liver which expresses both isoforms. The obtained results suggested that MAO inhibition induced by alpha-methylated tryptamines might be clinically relevant concerning possible serotonergic and adrenergic effects and interactions with drugs (of abuse) particularly acting as monoamine reuptake inhibitors. However, as in vitro assays have only limited conclusiveness, further studies are needed.

Keywords Alpha-methyltryptamine analog new psychoactive substances, drugs of abuse, MAO inhibition, LC-HR-MS/MS, IC_{50} value

1. Introduction

New psychoactive substances (NPS) are emerging drugs that are mainly consumed as legal and easy available substitutes for traditional and controlled drugs of abuse (Brandt et al. 2014; Meyer 2016). The detection of synthetic tryptamines including alpha-methyltryptamine (AMT) has been frequently reported to the EU Early Warning System coordinated by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and continuously monitored as part of the EMCDDA's toxicovigilance system (EMCDDA 2015). AMT (α-MT, 3-IT, IT-290) has been investigated in the 1960s as a potential antidepressant and its popularity as a drug of abuse came to light in the 1990s due to its hallucinogenic and stimulant properties (Araujo et al. 2015). In late 2011, an isomer of AMT, 5-(2-aminopropyl)indole (5-IT, 5-API), appeared on the European drug market (EMCDDA 2014). After intake of AMT or 5-IT, symptoms related to monoaminergic toxicity were described, such as restlessness, agitation, disorientation, shivering, sweating, mydriasis, vomiting, tachycardia, or hyperthermia (EMCDDA 2015; Shulgin and Shulgin 1997). Both were also involved in several fatalities (Boland et al. 2005; Elliott and Evans 2014). A key factor involved in the occurrence of clinical features included the inhibition of monoamine oxidase (MAO) enzymes, followed by increased monoamine levels inducing a serotonin syndrome (Boyer and Shannon 2005; EMCDDA 2014). In 1968, AMT, 5-IT, and four positional isomers were identified as inhibitors of guinea pig MAO (Cerletti et al. 1968). More recently, 5-IT was confirmed as highly selective and potent inhibitor of recombinant human MAO-A (Herraiz and Brandt 2014).

Furthermore, tryptamines were identified in NPS products combining different NPS groups (UNODC 2016). Thus, the risk of encountering monoaminergic (side) effects and drug-drug interactions is very likely although the extent to which this might occur is difficult to predict, given that systematic data are not available. In contrast to authorized medicines, NPS are marketed and consumed without preclinical or clinical studies. Procedures employed for various substrates, mostly therapeutic drugs, have been described for in vitro monitoring of MAO activity (Tipton et al. 2006). Examples of drugs of abuse tested for human MAO inhibition activity include MDMA and its metabolites, and 5-IT (Herraiz and Brandt 2014; Steuer et al. 2016).

Therefore, the aim of the present study was to develop a MAO inhibition assay based on hydrophilic interaction liquid chromatography-high resolution-tandem mass

spectrometry (HILIC-HR-MS/MS), to investigate the in vitro inhibition potential of AMT and 13 ring-substituted analogs (Fig. 1) on recombinant human MAO-A or B, and to determine their IC_{50} values.

2. Materials and methods

2.1. Chemicals and enzymes

Harmine was obtained from THC Pharm (Frankfurt, Germany), amphetamine-d_5 and AMT succinate from LGC Standards (Wesel, Germany), harmaline, yohimbine, selegiline, kynuramine (KYN), 4-hydroxyquinoline (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-IT was synthesized (Scott et al. 2014) and kindly provided by the Department of Pharmacology and Therapeutics, Trinity Centre for Health Sciences, St James's Hospital, Dublin 8, Ireland, before it was scheduled. All non-scheduled AMT analogs were prepared following procedures published previously (Young 1958). Details can also be found in (Brandt et al. 2004).

The baculovirus-infected insect cell microsomes (Supersomes) containing human complementary DNA-expressed MAO-A or MAO-B (5 mg protein/mL), wild-type Supersomes as negative control without MAO activity (MAO control, 5 mg protein/mL), and pooled human liver S9 (20 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.

2.2. HILIC-HR-MS/MS apparatus

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. It 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 × 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 already described before (Richter et al. 2016): sheath gas, 53 arbitrary units (AU); auxiliary gas, 14 AU; sweep gas, 3 AU; spray voltage, 3.50 kV; heater temperature, 438 °C; ion transfer capillary temperature, 269 °C; and S-lens RF level, 60.0. Mass calibration was done prior to analysis according to the manufacturer's recommendations using external mass calibration. For evaluating the chromatographic separation, a full scan experiment was used with the following scan parameters: polarity, positive; micro scan, 1; resolution, 35,000; automatic gain control (AGC) target, 3e6; maximum injection time (IT), 200 ms; and scan range, 50-750. The final quantification was performed using a 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 internal standard (IS) amphetamine-d_5 (*m/z* 141.1434). The settings for the t-SIM mode were as follows: micro scan, 1; resolution, 35,000; AGC target, 5e4; 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, 2e5; 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 (GraphPad Software, San Diego, USA) was used for outlier detection (http://graphpad.com/quickcalcs/grubbs1), while GraphPad Prism 5.00 (GraphPad Software) was used for statistical evaluation.

2.3. Preparation of stock solutions

Stock solutions of the MAO substrate KYN (6.25 mM) or its metabolite 4-OHC (0.1 mM) were prepared in water. Stock solutions of harmine, harmaline, 5-IT, selegiline, AMT (5 mM, respectively), or yohimbine (3 mM) were used in methanol. Stock solutions of the AMT analogs (5 mM, respectively) were prepared in DMSO. Afterwards, they were diluted 1:5 (*v/v*) with methanol, gently evaporated under nitrogen, and resolved in water/methanol (9:1, *v/v*). To obtain the solutions for incubation, the stock solutions were diluted in phosphate buffer. The organic solvent content in the final incubation mixtures was always below 2.5% (Chauret et al. 1998). Stock solutions were aliquoted and stored at -20 °C.

2.4. Method validation

The method for quantification of 4-OHC was validated in accordance to the "Guideline on bioanalytical method validation" published by the European Medicines Agency (EMA) (EMA 2011). For validation, 30 µL samples containing 1 µg/mL MAO control in phosphate buffer were used. If needed, 10 µL of the phosphate buffer was replaced by appropriate amounts of the calibration or quality control (QC) stock solution. These samples were diluted with the same volume of acetonitrile with or without IS. Briefly, the method was tested for selectivity (using ten blank samples without IS), carry-over (using a blank sample without IS following the high QC), lower limit of quantification (LLOQ) defined as lowest calibration standard, within-run accuracy and precision (analyzed in a single run five samples per level at four concentration levels: LLOQ QC, low QC, medium QC, and high QC), between-run accuracy and precision (analyzed in three different runs on two different days six samples per level at four concentration levels: LLOQ QC, low QC, medium QC, and high QC), matrix effect (using six samples with matrix and six samples without matrix at two concentration levels: low QC and high QC), and stability of processed samples in the autosampler. The calibration consisted of six concentration points (50, 200, 400, 600, 800, and 1000 nM) equally distributed over the whole range. The concentrations of LLOQ QC, low QC, medium QC, and high QC were as follows: 50, 100, 500, and 900 nM. Instead of MAO-A or B, MAO control was used for sample preparation. For quantification, the ratios of 4-OHC versus IS were used. The analytical runs consisted of a blank sample without IS, a blank sample with IS, calibration standards, three levels of QC samples (low, medium, and high) in

duplicate, and the study samples. All calculations were done using GraphPad Prism 5.00.

2.5. Determination of K_m values

KYN deamination is depicted in Fig. 2. The kinetic constants were derived from incubations with MAO-A, MAO-B, or S9. Incubations were performed at 37 °C for 20 min using 1 µg/mL MAO-A or MAO-B, respectively, or 100 µg/mL S9, and the MAO substrate KYN at concentrations of 0.1, 0.5, 1, 5, 10, 25, 50, 100, 150, 200, 250, and 500 µM. In addition, blank incubations with 1 µg/mL MAO control were prepared as negative control. Besides enzyme preparations and substrate, the incubation mixtures (final volume 30 µL) contained 100 mM phosphate buffer. Reactions were initiated by addition of the ice-cold enzyme preparation and stopped with 30 µL of ice-cold acetonitrile, containing 10 µM amphetamine-d_5 as IS. The solution 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-HR-MS/MS apparatus for analysis. Enzyme kinetic constants were estimated by non-linear curve fitting using GraphPad Prism 5.00 after validated quantification of the formed metabolite 4-OHC via six-point calibration. The Michaelis-Menten equation (Eq. (1)) was used to calculate apparent K_m and V_{max} values for MAO-A, MAO-B, or S9, where v is the initial reaction velocity, S the substrate concentration, V_{max} the maximal reaction velocity, and K_m the substrate concentration at half V_{max}.

(1) $$v = \frac{V_{\max} \times S}{K_m + S}$$

2.6. Initial inhibition screening and determination of IC_{50} values and inhibition constants

2.6.1. Initial inhibition screening

For inhibition studies, 10 µL of the phosphate buffer was replaced by appropriate amounts of the potential inhibitor solution. KYN was used in concentrations comparable to its K_m value. All other incubation conditions were the

same as described above. To test for inhibition capability, MAO-A or B was incubated with 10 µM of the potential inhibitor in triplicate (*n* = 3). In addition to these samples, control samples without inhibitor, positive control samples with model inhibitors (10 µM 5-IT for MAO-A or 10 µM selegiline for MAO-B), blank samples without MAO activity, and interfering samples were also prepared in triplicates. For preparation of the interfering samples, control samples without inhibitor were incubated and the reaction was terminated with ice-cold acetonitrile containing the IS and the potential inhibitors. The 4-OHC formation in test samples was then compared to metabolite formation in control samples. For statistical analysis of data, 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 was used.

2.6.2. Determination of IC_{50} values

Inhibitors were incubated at least at ten different concentrations (0.00004, 0.0002, 0.0006, 0.002, 0.01, 0.04, 0.2, 0.6, 2.5, 10, 40 160 640, 2560 µM), depending on their expected inhibition strengths after initial inhibition screening. All other incubation conditions were the same as described above. Control samples were also prepared as described above. The IC_{50} values were calculated by plotting the metabolite formation (relative to the control samples) over the logarithm of the inhibitor concentration using GraphPad Prism 5.00.

2.6.3. Determination of inhibition constants (K_i values)

For experimental determination, four concentration levels of the test inhibitor were incubated with KYN at five different concentrations (0.5, 5, 25, 100, and 500 µM). Inhibitor concentration levels depended on the previously determined IC_{50} value. For selegiline and 7-Me-AMT, inhibitor concentration levels were: 0, 0.01, 0.05, and 0.1 µM, for 5-IT, 0, 0.1, 0.5, and 1 µM. All other incubation conditions were the same as described above. 7-Me-AMT and 5-IT were incubated with MAO-A, whereas MAO-B was used in the case of selegiline. The inhibition constants (K_i values) were calculated using GraphPad Prism 5.00. Michaelis-Menten plots were transferred to Lineweaver-Burk plots (1/*V* versus 1/*S)*, and fit factors for different inhibition models

were compared to determine the most probable inhibition mode. If competitive inhibition was identified as most probable inhibition model, the equation of Cheng and Prusoff (Eq. (2)) (Cheng and Prusoff 1973) was additionally used to calculate K_i from IC_{50} and K_m values.

$$IC_{50} = K_i \, x \, (1 + \frac{S}{K_m}) \quad (2)$$

2.7. MAO inhibition in S9 fraction

Three concentration levels of the inhibitors were incubated with KYN at concentrations at its K_m value. Inhibitor concentration level one was the lowest concentration and chosen based on plasma concentrations reported after intake (Table 1). Concentration levels two and three were calculated to be ten and one hundred times concentration level one, respectively. For selegiline, inhibitor concentration levels were 0.005, 0.05, and 0.5 µM; for harmine, harmaline, and yohimbine, 0.1, 1, and 10 µM inhibitor were chosen, and for all other compounds, 1, 10, and 100 µM. All other incubation conditions were the same as described above. In addition, control samples without inhibitor and blank samples were prepared. All incubations were performed in triplicate (n = 3). For evaluation, the metabolite formation in test samples was compared to metabolite formation in control samples. For statistical analysis of the data, a one-way ANOVA followed by Dunnett's multiple comparison test (significance level, $P < 0.05$, 95% confidence intervals) by GraphPad Prism 5.00 was used.

3. Results

3.1. Method validation

The analytical procedure was based on HILIC-HR-MS/MS in t-SIM mode with a subsequent dd-MS^2 mode. The method was successfully validated in accordance to the criteria of the EMA (EMA 2011). The method was selective at LLOQ levels, with a response of interfering components less than 20% or 5% compared to the response

of the analyte at the LLOQ and the response of the IS, respectively. No carry-over problems could be observed and the LLOQ for 4-OHC was defined as 50 nM. The mean for within-run and between-run accuracies ranged from 3% to 19% but were within 20% of the nominal values for the LLOQ QC and within 15% for the low, medium, and high QC samples. The mean within-run and between-run precisions ranged from 1% to 13%. Precisions were within 20% for the LLOQ QC and within 15% for the low, medium, and high QC samples. The coefficients of variation (CVs) of matrix factors were 9% and 11% for the analytes and the IS at low QC level and 3% and 7% at high QC level. The CVs of IS-normalized matrix factor were 14% for low and 9% at high QC level and thus not greater than 15%. Processed samples showed sufficient stability in the autosampler for at least 10 h, corresponding to the maximum duration of the analytical runs.

3.2. Determination of K_m values

The kinetics for KYN deamination using MAO-A, MAO-B, or S9 followed classic Michaelis-Menten behavior. Calculated K_m values were 43 ± 5 µM, 23 ± 4 µM, and 34 ± 2 µM for MAO-A, MAO-B, and S9, respectively. V_{max} values were 75 ± 2 nmol/min/mg, 56 ± 2 nmol/min/mg, and 7 ± 0.1 nmol/min/mg for MAO-A, MAO-B, and S9, respectively. R^2 values were 0.9910, 0.9760, and 0.9980, respectively.

3.3. Initial inhibition screening results

The results are summarized in Fig. 3. All test tryptamines showed significant inhibition of MAO-A resulting in residual activities below 50%. MAO-B was additionally inhibited by 5-F-AMT, 6-F-AMT, 5-Cl-AMT, and 5-F-2-Me-AMT, resulting in residual activities between 60 and 80%. Harmine, harmaline, and yohimbine also showed inhibition of MAO-A in this assay. Whereas harmine and harmaline inhibited MAO-A activity almost completely, yohimbine inhibition resulted in residual activities of about 25%. No analytical interferences could be detected for all incubation sets and the positive control samples showed almost complete inhibition of MAO-A or B with residual activities of about 5%.

3.4. Determination of IC_{50} values

All results are given in Table 1. IC_{50} values of AMT and its analogs for MAO-A inhibition were between 0.049 (7-Me-AMT) and 166 µM (1-Me-2-Ph-AMT), for MAO-B inhibition, IC_{50} values of 5-Cl-AMT, 6-F-AMT, 5-F-2-Me-AMT, and 5-F-AMT were 82, 126, 223, and 376 µM, respectively. The known MAO-A inhibitors harmine, harmaline, 5-IT, and yohimbine resulted in IC_{50} values of 0.006, 0.011, 0.20, and 6.0 µM, respectively, while the IC_{50} value of selegiline towards MAO-B was 0.017 µM.

3.5. Determination of K_i values

Michaelis-Menten and Lineweaver-Burk plots after incubation of 7-Me-AMT are given in Fig. 4. The K_i value was 0.026 µM. A competitive inhibition model was preferred, which was in line with the visual inspection of the intersection in the Lineweaver-Burk plot. K_i value calculated from IC_{50} and K_m values was 0.025 µM. For 5-IT and selegiline, experimentally determined K_i values were 0.18 and 0.011 µM, respectively. Calculated K_i values were 0.10 and 0.009 µM, respectively. Visual inspection and software based evaluation preferred competitive inhibition model for both compounds.

3.6. MAO inhibition in S9 fraction

Results are summarized in Fig. 5. 7-Me-AMT exerted significant inhibition of KYN deamination by MAO in S9 for all concentration levels. AMT, 5-Cl-AMT, and 5-Br-AMT inhibited MAO activity when incubated at concentration levels two or three. 4-MeO-AMT, 2-Me-AMT, 5-Me-AMT, 5-F-AMT, 6-F-AMT, 5-F-2-Me-AMT, and 2-Ph-AMT showed significant inhibition only when incubated in concentration level three. 5-MeO-AMT, 1-Me-AMT, and 1-Me-2-Ph-AMT showed no MAO inhibition in S9, even at the highest concentration level. Harmine and harmaline showed significant inhibition of MAO-A at all concentration levels, while in case of 5-IT and selegiline concentration levels two or three led to significant inhibition, and for yohimbine only concentration level three.

4. Discussion

MAO activity was assessed using KYN, a non-selective substrate for MAO-A and B, which was transformed to the corresponding aldehyde, followed by non-enzymatic condensation to 4-OHC (Fig. 2) (Weissbach et al. 1960). 4-OHC formation was analyzed using HILIC-HR-MS/MS. Although literature described many MAO activity assays (Tipton et al. 2006), Herraiz and Chaparro found that the needed selectivity for analyzing 4-OHC could only be achieved by chromatographic separation before detection (Herraiz and Chaparro 2006). Since HILIC was shown to provide sufficient retention and separation of small and polar analytes (Steuer et al. 2016), it was anticipated that HILIC separation would be a suitable method in the present study. Due to its high flexibility and sensitivity, HR-MS/MS required comparatively low levels of recombinant MAO enzymes and thus reduction of the total protein content, which can lead to non-specific protein binding of analytes (Baranczewski et al. 2006). The t-SIM mode with a subsequent dd-MS^2 acquisition allowed simultaneous quantification and identification, respectively. The t-SIM provided approximately 50 scans per peak by far sufficient for quantification, whereas the dd-MS^2 provided additional selectivity for further studies without the need for revalidation. Experimental variability during sample preparation and analysis was corrected by addition of the internal standard amphetamine-d_5, which provided comparable properties to 4-OHC. The whole analytical procedure was successfully validated in accordance with international guidelines (EMA 2011).

For determination of K_i values, the substrate concentration should be used below or at its K_m value. Different K_m values of KYN deamination by MAO-A or B derived from diverse enzyme sources were published (Herraiz and Brandt 2014; Naoi and Nagatsu 1988; Parikh et al. 2002; Ro et al. 2001) and none for S9. Therefore, K_m values were also determined in this study. The question as to whether the S9 fraction would show MAO activity remained inconsistently answered. As enzymes of the outer mitochondrial membrane, MAO was expected to be removed by centrifugation at 9,000x*g* during production of S9 fraction (Lee and Zhu 2011). However, Salva et al. reported NADPH-independent formation of metabolites even in pooled human liver microsomes, which could be abolished by MAO inhibitors. They ascribed this MAO activity to a contamination with mitochondrial enzymes (Salva et al. 2003).

Furthermore, the guidance for industry by the Food and Drug Administration recommended the addition of the MAO inhibitor pargyline to the S9 fraction to identify MAO contribution in the oxidative biotransformation of any given tested drug (FDA 2006). As 4-OHC formation in S9 was also observed in the present study, the K_m value of KYN transformation in S9 was determined. Resulting K_m values for MAO-A and MAO-B were similar, as expected for a non-selective substrate, and comparable to published values (Herraiz and Brandt 2014; Naoi and Nagatsu 1988; Parikh et al. 2002; Ro et al. 2001). Initial experiments were conducted to choose incubation time and enzyme concentration (data not shown) to be within the linear range of metabolite formation. Substrate concentrations between 0.1 and 500 µM KYN allowed modeling of enzyme kinetics. Less than 20% of substrate was metabolized in all incubations, except of the lowest substrate concentrations. To avoid non-specific protein binding, the protein concentrations were chosen as low as analytically possible as recommended by (Baranczewski et al. 2006). Blank incubations should be used to consider MAO-independent formation of 4-OHC.

Applicability of the initial inhibition screening procedure to assess the inhibition by either MAO-A or MAO-B was tested using positive control samples containing 5-IT or selegiline, known for inhibition of MAO-A or B, respectively. A one-way ANOVA followed by Dunnett's multiple comparison test was used to decide whether 4-OHC formation in test samples was statistically significantly different from 4-OHC formation in control samples. Such an initial inhibition screening strategy was already described by Dinger et al. for studying the cytochrome P450 (CYP) inhibition potential of 3,4-methylenedioxy-derived designer drugs (Dinger et al. 2016a). In the present study, a set of blank samples was also analyzed. A lower inhibitor concentration (10 µM) in the positive control samples was chosen because plasma concentrations of known MAO inhibitors were expected to be lower than those of CYP inhibitors. No significant inhibition at 10 µM was thus considered as weak and probably not clinically relevant. Interfering samples were used to exclude mass spectral ion suppression or enhancement effects, caused by co-eluting analytes (Remane et al. 2010). These samples were mandatory as only KYN, 4-OHC, and the IS were monitored by the analytical method and co-eluting compounds could lead to false positive (in case of ion suppression) or false negative (in case of ion enhancement) results.

In accordance to Cerletti et al. and Zirkle and Kaiser (Cerletti et al. 1968; Zirkle and Kaiser 1964), AMT was identified as MAO-A inhibitor. All tested analogs also

revealed MAO-A inhibition properties, whereas 5-F-AMT, 6-F-AMT, 5-Cl-AMT, and 5-F-2-Me-AMT inhibited MAO-B as well. Furthermore, harmine, harmaline, and yohimbine showed MAO-A inhibition consistent with previous investigations e.g. (Dos Santos et al. 2013). The initial inhibition screening was useful to realize that most tested compounds inhibited only one MAO isoform. Hence, time and costs for further IC_{50} determinations could be saved. Consequently, only for compounds showing a statistically significant inhibition in the initial inhibition screening, IC_{50} values were determined.

To predict a potential clinical relevance of the MAO inhibition based on IC_{50} values, the expected plasma levels of inhibitors (given in Table 1) of inhibitors should be considered. In case of NPS, only scarce information is available, concerning plasma concentrations after intake. The only information source was case reports of fatal or non-fatal intoxications but particularly postmortem data is difficult to interpret, due to postmortem redistribution and unclear cause of death. But also data of non-fatal cases is problematic, as dosage and time of ingestion remain often unclear.

7-Me-AMT showed an IC_{50} value against MAO-A activity comparable to the strong inhibitors harmine and harmaline. For 7-Me-AMT, plasma levels were not yet published, but described plasma concentrations for AMT (Elliott and Evans 2014; Ferec et al. 2015) were much higher than published harmine and harmaline plasma concentrations (Baselt 2008; Oliveira et al. 2012). For AMT, Shulgin and Shulgin (Shulgin and Shulgin 1997) described oral intake of 15 to 30 mg or smoking of 5 to 20 mg as common dosage. These amounts were comparable to oral dosages of 5-IT (Shulgin and Shulgin 1997). In comparison, 5-MeO-AMT, however, was found to be significantly more potent with an active oral dose in the 1.5-4.5 mg range (Kantor et al. 1980; Shulgin 1979; Shulgin and Nichols 1978; Shulgin and Shulgin 1997). Concerning other AMT analogs, no data about dosage or plasma concentrations are available so far, but similar doses followed by similar plasma concentrations may be expected. Considering assumed plasma levels, clinical relevance of the MAO-A inhibition after intake of 7-Me-AMT could not be excluded. The same should be true for 5-Cl-AMT, AMT, and 5-F-AMT, as their IC_{50} values against MAO-A were in the same range as the IC_{50} value of 5-IT. Given that the other AMT analogs showed higher IC_{50} values against MAO-A, clinical relevance should be unlikely. Comparing the structural properties and IC_{50} values, a methylation in the 7-position of the indole ring appeared to result in increased potency whereas compounds with a methylation

in position 1, 2, or 5 provided a higher IC_{50} value than AMT itself. The two compounds carrying a methyl group in position 1 provided the highest IC_{50} values. Therefore, an unchanged position 1 might be essential for the unhindered binding to MAO-A and especially the combination of a methyl group in position 1 with a phenyl group in position 2 reduced the inhibition potential. Furthermore, a methoxy group in the 5-position led to an increased IC_{50} value, whereas the methoxy group located at position 4, or insertion of a halogen atom in position 5, had nearly no effect on the IC_{50} value, compared to AMT. Concerning MAO-B inhibition, determined IC_{50} values of four AMT analogs were hundred to one thousand times higher than the IC_{50} value of selegiline but also expected plasma concentrations of the AMT analogs were hundred to thousand times higher than published selegiline plasma concentrations. Additionally, these AMT analogs inhibited both MAO isoforms and thus, clinical relevance could not be excluded.

Furthermore, the K_i values of 7-Me-AMT, 5-IT, and selegiline were experimentally determined and additionally calculated from the IC_{50} values resulting in comparable K_i values. Both K_i values for 5-IT were similar to those published by Herraiz and Brandt (Herraiz and Brandt 2014). In the current study, all three compounds were identified as competitive inhibitors and 7-Me-AMT confirmed as potent MAO-A inhibitor, far stronger than 5-IT.

Moreover, MAO inhibition was tested in human hepatic S9 fraction given that MAO-B is slightly overexpressed in human liver (Nishimura and Naito 2006; Wang et al. 2013). In contrast to the artificial system of recombinant human MAO expressed in baculovirus-infected insect cells, the S9 fraction represents an in vitro model of the human liver, containing both isoforms in their natural environment and different abundances. The test drugs were used at three different concentration levels. Concentration level one represented expected plasma concentrations, as actual concentrations in the liver were difficult to estimate. Being the main metabolizing organ, a higher concentration than in plasma is more than likely (concentration levels two and three). Obtained results correlated well with the determined IC_{50} values. 7-Me-AMT, with the lowest IC_{50} value, showed significant inhibition of MAO activity for all concentration levels, while 5-MeO-AMT, 1-Me-AMT, and 1-Me-2-Ph-AMT, with the highest IC_{50} values towards MAO-A, showed no inhibition at all. Residual MAO activity towards KYN in presence of the test inhibitor could be explained by inhibition of only one isoform, which means that the effect might be more pronounced for a

MAO-A specific substrate such as serotonin or noradrenalin. As tryptamines were often identified in NPS products containing different NPS groups (UNODC 2016), an intake with substances that increase monoamine levels or inhibit MAO could lead to severe intoxications as described by (Brush et al. 2004). They reported about an adolescent who suffered from a severe intoxication exhibiting hyperthermia, tachycardia, and massive agitation after consumption of the combination of a hallucinogenic tryptamine and the monoamine oxidase inhibitor harmaline (Brush et al. 2004). Therefore, clinical effects due to MAO inhibition by AMT or its analogs could not be excluded for most of the tested compounds.

As controlled human trials for NPS are not feasible due to ethical reasons, in vitro approaches for studying their toxicokinetics have to be used (Caspar et al. 2015; Dinger et al. 2016b; Meyer 2016; Meyer et al. 2015; Michely et al. 2015; Wagmann et al. 2016). Concerning MAO inhibition by drugs of abuse, only few studies were published. Cerletti et al. identified AMT, 5-IT, and four positional isomers as inhibitors of guinea pig MAO in an assay based on the manometric determination of the oxygen uptake of guinea pig liver homogenates with serotonin as substrate (Cerletti et al. 1968). Herraiz and Brandt confirmed 5-IT as highly selective and potent inhibitor of recombinant human MAO-A by measuring the KYN deamination using high-performance liquid chromatography coupled to diode array detection (Herraiz and Brandt 2014). Steuer et al. investigated the MAO inhibition potential of MDMA and its metabolites on the deamination of the neurotransmitters dopamine and serotonin using HILIC-MS/MS. MDMA and MDA were identified as inhibitors of recombinant human MAO-A (Steuer et al. 2016). However, MAO inhibition is not always an effect of the abused drug itself. Users also intentionally consume MAO inhibitors in order to enhance the activity of substances such as *N,N*-dimethyltryptamine (DMT) that otherwise would be metabolically inactivated. Ayahuasca for example, a hallucinogenic beverage, is the combination of DMT and the β-carbolines MAO inhibitors harmine and harmaline (Araujo et al. 2015).

5. Conclusion

The presented study was the first to describe the MAO inhibition for a broad range of NPS of the alpha-methylated tryptamine type. The workup and analysis

based on HILIC-HR-MS/MS were validated according to international guidelines. Due to its high sensitivity, only minimal amounts of recombinant MAO enzymes were needed, thus, reducing the risk of non-specific protein binding, as well as material costs. All tested AMT analogs inhibited MAO-A activity, whereas four compounds inhibited MAO-B as well. 7-Me-AMT was identified as potent and competitive inhibitor of MAO-A. MAO inhibition by AMT and its analogs are expected to be clinically relevant. Plasma levels of other MAO substrates, for example neurotransmitters or drugs, could increase, especially if these substances were selective MAO-A substrates. Further clinical studies are warranted to facilitate a more complete assessment.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

The authors like to thank Achim T. Caspar, Julia Dinger, Andreas G. Helfer, Sascha K. Manier, Julian A. Michely, Lilian H. J. Richter, and Armin A. Weber for their support and fruitful discussion.

Table 1 Test compounds, reference plasma concentrations, and IC_{50} values (percentage errors in brackets) of AMT-type new psychoactive substances (NPS) and known MAO inhibitors. Plasma concentrations in µM were calculated from the published data (in mg/L or µg/L). Probable clinically relevant IC_{50} values for AMT-type NPS are given in bold. PM: post-mortem cases

Test compounds	Reference plasma concentrations,		IC_{50} values, µM	
	µg/L	µM	MAO-A	MAO-B
AMT-type NPS				
AMT	440 (Ferec et al. 2015) 160-1,300 (PM) (Elliott and Evans 2014)	2.5 0.9-7.5	**0.38** (8)	
4-MeO-AMT	*	0.8-6.4	1.4 (11)	
5-MeO-AMT	*	0.8-6.4	31 (4)	
1-Me-AMT	*	0.9-6.9	51 (3)	
2-Me-AMT	*	0.9-6.9	11 (7)	
5-Me-AMT	*	0.9-6.9	1.5 (13)	
7-Me-AMT	*	0.9-6.9	**0.049** (2)	
5-F-AMT	*	0.8-6.8	**0.45** (23)	376 (1)
6-F-AMT	*	0.8-6.8	1.8 (19)	126 (2)
5-Cl-AMT	*	0.8-6.2	**0.25** (7)	82 (2)
5-Br-AMT	*	0.6-5.2	1.3 (7)	
5-F-2-Me-AMT	*	0.8-6.3	4.1 (7)	223 (3)
1-Me-2-Ph-AMT	*	0.6-4.9	166 (2)	
2-Ph-AMT	*	0.6-5.2	3.7 (18)	
Known inhibitors				
5-IT	15-590 (Backberg et al. 2014) 700-5,100 (PM) (EMCDDA 2014)	0.1-3.4 4.0-29	0.20 (1)	
Selegiline	0.3-1.5 (Barrett et al. 1996)	0.002-0.01		0.017 (2)
Harmine	36-222 (Baselt 2008) 1.0-15.6 (Oliveira et al. 2012)	0.2-1.0 0.005-0.07	0.006 (2)	

Harmaline	2.7-15.7 (Oliveira et al. 2012)	0.01-0.07	0.011 (2)
Yohimbine	3.7-171 (Baselt 2008)	0.01-0.5	6.0 (7)

* No plasma concentrations reported, thus concentrations estimated according to the structure-related compound AMT.

References

Araujo AM, Carvalho F, Bastos Mde L, Guedes de Pinho P, Carvalho M (2015) The hallucinogenic world of tryptamines: an updated review. Arch Toxicol 89(8):1151-73

Backberg M, Beck O, Hulten P, Rosengren-Holmberg J, Helander A (2014) Intoxications of the new psychoactive substance 5-(2-aminopropyl)indole (5-IT): a case series from the Swedish STRIDA project. Clinical toxicology 52(6):618-24

Baranczewski P, Stanczak A, Sundberg K, et al. (2006) Introduction to in vitro estimation of metabolic stability and drug interactions of new chemical entities in drug discovery and development. Pharmacological reports : PR 58(4):453-72

Barrett JS, Rohatagi S, DeWitt KE, Morales RJ, DiSanto AR (1996) The Effect of Dosing Regimen and Food on the Bioavailability of the Extensively Metabolized, Highly Variable Drug Eldepryl((R)) (Selegiline Hydrochloride). Am J Ther 3(4):298-313

Baselt RC (2008) Disposition of toxic drugs and chemicals in man, 8th ed. edn. Biomedical Publications, Seal Beach, CA

Boland DM, Andollo W, Hime GW, Hearn WL (2005) Fatality due to acute alpha-methyltryptamine intoxication. Journal of Analytical Toxicology 29(5):394-397

Boyer EW, Shannon M (2005) The serotonin syndrome. N Engl J Med 352(11):1112-20

Brandt SD, Freeman S, McGagh P, Abdul-Halim N, Alder JF (2004) An analytical perspective on favoured synthetic routes to the psychoactive tryptamines. Journal of pharmaceutical and biomedical analysis 36(4):675-91

Brandt SD, King LA, Evans-Brown M (2014) The new drug phenomenon. Drug testing and analysis 6(7-8):587-97

Brush DE, Bird SB, Boyer EW (2004) Monoamine oxidase inhibitor poisoning resulting from Internet misinformation on illicit substances. J Toxicol Clin Toxicol 42(2):191-5

Caspar AT, Helfer AG, Michely JA, et al. (2015) Studies on the metabolism and toxicological detection of the new psychoactive designer drug 2-(4-iodo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine (25I-NBOMe) in human and rat urine using GC-MS, LC-MS(n), and LC-HR-MS/MS. Analytical and bioanalytical chemistry 407(22):6697-719

Cerletti A, Taeschler M, Weidmann H (1968) Pharmacologic studies on the structure-activity relationship of hydroxyindole alkylamines. Adv Pharmacol 6(Pt B):233-46

Chauret N, Gauthier A, Nicoll-Griffith DA (1998) Effect of common organic solvents on in vitro cytochrome P450-mediated metabolic activities in human liver microsomes. Drug Metab Dispos 26(1):1-4

Cheng Y, Prusoff WH (1973) Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. Biochem Pharmacol 22(23):3099-3108

Dinger J, Meyer MR, Maurer HH (2016a) In vitro cytochrome P450 inhibition potential of methylenedioxy-derived designer drugs studied with a two-cocktail approach. Archives of toxicology 90(2):305-18

Dinger J, Woods C, Brandt SD, Meyer MR, Maurer HH (2016b) Cytochrome P450 inhibition potential of new psychoactive substances of the tryptamine class. Toxicology letters 241:82-94

Dos Santos PC, Soldi TC, Torres AR, et al. (2013) Monoamine oxidase inhibition by monoterpene indole alkaloids and fractions obtained from Psychotria suterella and Psychotria laciniata. J Enzyme Inhib Med Chem 28(3):611-618

Elliott S, Evans J (2014) A 3-year review of new psychoactive substances in casework. Forensic Sci Int 243:55-60

EMA (2011) Guideline on bioanalytical method validation. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf

EMCDDA (2014) Report on the risk assessment of 5-(2-aminopropyl)indole in the framework of the Council Decision on new psychoactive substances.

http://www.emcdda.europa.eu/attachements.cfm/att_222688_EN_TDAK13002ENN-1_.pdf
EMCDDA (2015) New psychoactive substances in Europe. An update from the EU Early Warning System. http://www.emcdda.europa.eu/attachements.cfm/att_235958_EN_TD0415135ENN.pdf
FDA (2006) Guidance for Industry: Drug Interaction Studies - Study Design, Data Analysis, and Implications for Dosing and Labeling. http://www.fda.gov/OHRMS/DOCKETS/98fr/06d-0344-gdl0001.pdf
Ferec S, Leborgne I, Bruneau C, et al. (2015) Syndrome sérotoninergique sévère après ingestion d'a-méthyl-tryptamine (AMT). Toxicol Anal Clin 27(2):25
Herraiz T, Brandt SD (2014) 5-(2-Aminopropyl)indole (5-IT): a psychoactive substance used for recreational purposes is an inhibitor of human monoamine oxidase (MAO). Drug Test Anal 6(7-8):607-13
Herraiz T, Chaparro C (2006) Analysis of monoamine oxidase enzymatic activity by reversed-phase high performance liquid chromatography and inhibition by beta-carboline alkaloids occurring in foods and plants. J Chromatogr A 1120(1-2):237-43
Kantor RE, Dudlettes SD, Shulgin AT (1980) 5-Methoxy-α-methyltryptamine (α,O-dimethylserotonin), a hallucinogenic homolog of serotonin. Biol Psychiatry 15(2):349-358
Lee MS, Zhu M (2011) Mass spectrometry in drug metabolism and disposition. Wiley, Hoboken (NJ)
Meyer MR (2016) New psychoactive substances: an overview on recent publications on their toxicodynamics and toxicokinetics. Archives of toxicology 90(10):2421-44
Meyer MR, Wagmann L, Schneider-Daum N, et al. (2015) P-glycoprotein interactions of novel psychoactive substances - stimulation of ATP consumption and transport across Caco-2 monolayers. Biochemical pharmacology 94(3):220-6
Michely JA, Helfer AG, Brandt SD, Meyer MR, Maurer HH (2015) Metabolism of the new psychoactive substances N,N-diallyltryptamine (DALT) and 5-methoxy-DALT and their detectability in urine by GC-MS, LC-MSn, and LC-HR-MS/MS. Anal Bioanal Chem 407(25):7831-7842
Naoi M, Nagatsu T (1988) Inhibition of type A monoamine oxidase by methylquinolines and structurally related compounds. J Neurochem 50(4):1105-10
Nishimura M, Naito S (2006) Tissue-specific mRNA expression profiles of human phase I metabolizing enzymes except for cytochrome P450 and phase II metabolizing enzymes. Drug Metab Pharmacokinet 21(5):357-74
Oliveira CD, Okai GG, da Costa JL, de Almeida RM, Oliveira-Silva D, Yonamine M (2012) Determination of dimethyltryptamine and beta-carbolines (ayahuasca alkaloids) in plasma samples by LC-MS/MS. Bioanalysis 4(14):1731-8
Parikh SN, Hanscom SR, Gagne PV, Crespi CL, Patten CJ (2002) A fluorescent-based, high-throughput assay for detecting inhibitors of human monoamine oxidase A and B. Drug Metabolism Reviews 34:164-164
Remane D, Meyer MR, Wissenbach DK, Maurer HH (2010) Ion suppression and enhancement effects of co-eluting analytes in multi-analyte approaches: Systematic investigation using ultra-high-performance liquid chromatography/mass spectrometry with atmospheric-pressure chemical ionization or electrospray ionization. Rapid Commun Mass Spectrom 24(21):3103-3108
Richter LH, Kaminski YR, Noor F, Meyer MR, Maurer HH (2016) Metabolic fate of desomorphine elucidated using rat urine, pooled human liver preparations, and human hepatocyte cultures as well as its detectability using standard urine screening approaches. Analytical and bioanalytical chemistry 408(23):6283-94
Ro JS, Lee SS, Lee KS, Lee MK (2001) Inhibition of type A monoamine oxidase by coptisine in mouse brain. Life Sci 70(6):639-45
Salva M, Jansat JM, Martinez-Tobed A, Palacios JM (2003) Identification of the human liver enzymes involved in the metabolism of the antimigraine agent almotriptan. Drug Metab Dispos 31(4):404-11

Scott KR, Power JD, McDermott SD, et al. (2014) Identification of (2-aminopropyl)indole positional isomers in forensic samples. Drug Test Anal 6(7-8):598-606
Shulgin AT (1979) Profiles of psychedelic drugs. J Psychedelic Drugs 11(4):355
Shulgin AT, Nichols DE (1978) Characterization of three new psychotomimetics. In: Stillman RC, Willette RE (eds) The Psychopharmacology of Hallucinogens. New York, p 74-84
Shulgin AT, Shulgin A (1997) Tihkal, The Continuation. Transform Press, Berkley, CA
Steuer AE, Boxler MI, Stock L, Kraemer T (2016) Inhibition potential of 3,4-methylenedioxymethamphetamine (MDMA) and its metabolites on the in vitro monoamine oxidase (MAO)-catalyzed deamination of the neurotransmitters serotonin and dopamine. Toxicology letters 243:48-55
Tipton KF, Davey G, Motherway M (2006) Monoamine oxidase assays. Curr Protoc Toxicol Chapter 4:Unit4 21
UNODC (2016) World Drug Report 2016. https://www.unodc.org/doc/wdr2016/WORLD_DRUG_REPORT_2016_web.pdf
Wagmann L, Meyer MR, Maurer HH (2016) What is the contribution of human FMO3 in the N-oxygenation of selected therapeutic drugs and drugs of abuse? Toxicol Lett 258:55-70
Wang CC, Billett E, Borchert A, Kuhn H, Ufer C (2013) Monoamine oxidases in development. Cell Mol Life Sci 70(4):599-630
Weissbach H, Smith TE, Daly JW, Witkop B, Udenfriend S (1960) A rapid spectrophotometric assay of mono-amine oxidase based on the rate of disappearance of kynuramine. J Biol Chem 235:1160-3
Young EHP (1958) The Synthesis of 5-Hydroxytryptamine (Serotonin) and Related Tryptamines. Journal of the Chemical Society 3(Oct):3493-3496
Zirkle CL, Kaiser C (1964) Monamine Oxidase Inhibitors (nonhydrazines). In: Gordon M (ed) Psychopharmacological Agents Vol 1. Academic Press, New York

Legends to the figures

Fig. 1 Chemical structures of tested alpha-methyltryptamines. Structural variations in comparison to AMT were given in red (For interpretation of the reference to color in this figure legend, the reader is referred to the web version of the article).

Fig. 2 Deamination of kynuramine catalyzed by MAO-A or MAO-B providing an aldehyde, followed by non-enzymatic condensation to 4-hydroxyquinoline.

Fig. 3 Initial inhibition screening results using 10 µM each of the inhibitor. Percentage of activity represented the percentage of metabolite formation in relation to control incubations without inhibitor (100%). Values are expressed as mean and were tested for significance (n = 3, ***, $P < 0.001$, **, $P < 0.01$, *. $P < 0.1$ for 4-hydroxyquinoline formation in incubations with the test inhibitor versus 4-hydroxyquinoline formation in control incubations).

Fig. 4 Michaelis-Menten plot (left part) of the deamination of kynuramine in absence or presence of three given concentrations of 7-Me-AMT for determination of the inhibition constant. Data points represent means and ranges (error bars) of duplicate measurements. Transformation of data to a Lineweaver-Burk plot (right part) for determination of the inhibition mode.

Fig. 5 Inhibition of MAO activity in S9 fraction by different inhibitor concentration levels (level 3 represented highest inhibitor concentration). Percentage of activity represented the percentage of metabolite formation in relation to control incubations without inhibitor (100%). Values are expressed as mean and were tested for significance (n = 3, ***, $P < 0.001$, **, $P < 0.01$, *. $P < 0.1$ for 4-hydroxyquinoline formation in incubations with the test inhibitor versus 4-hydroxyquinoline formation in control incubations).

Figure 1

Click here to download high resolution image

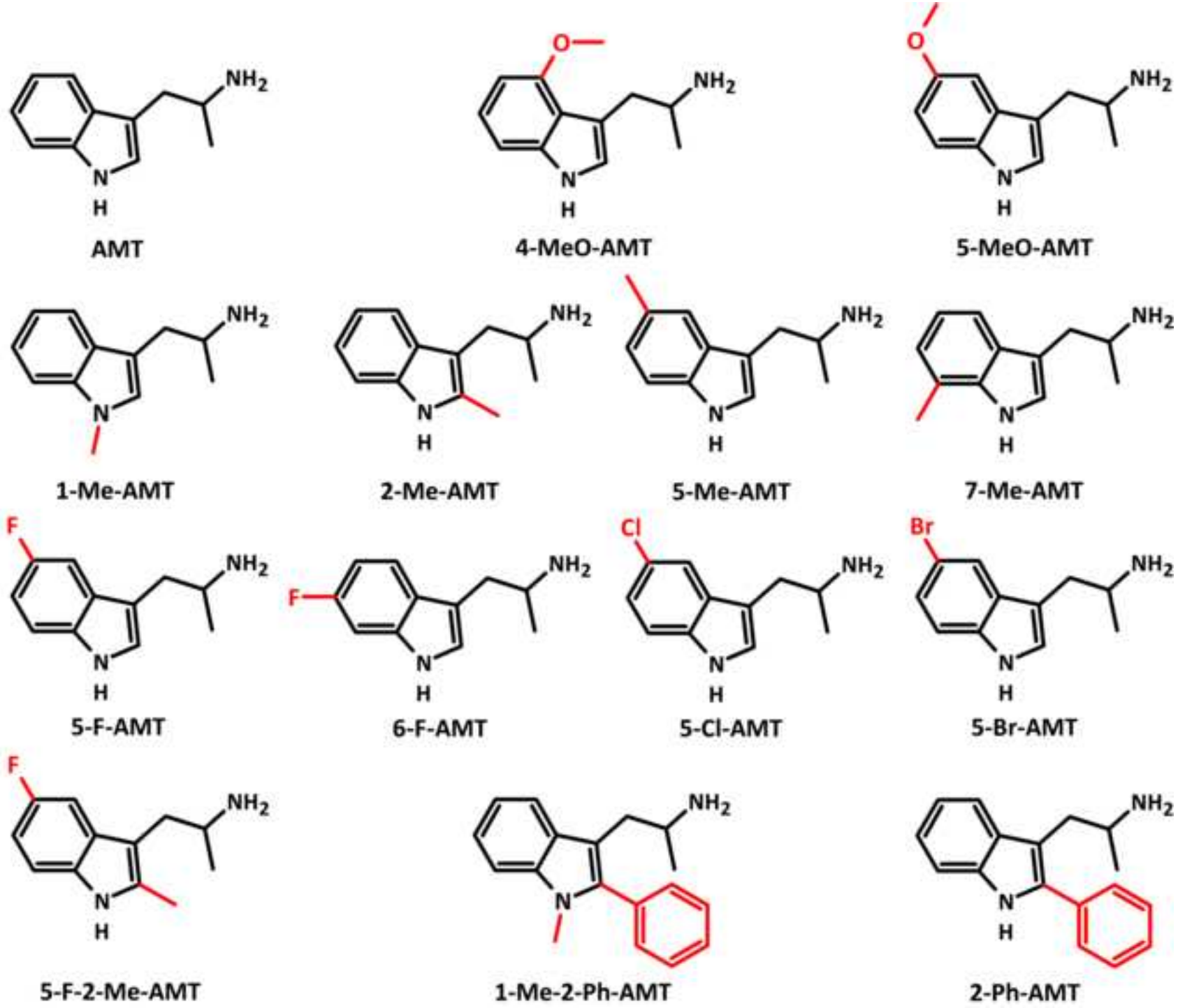


Figure 2

Click here to download high resolution image

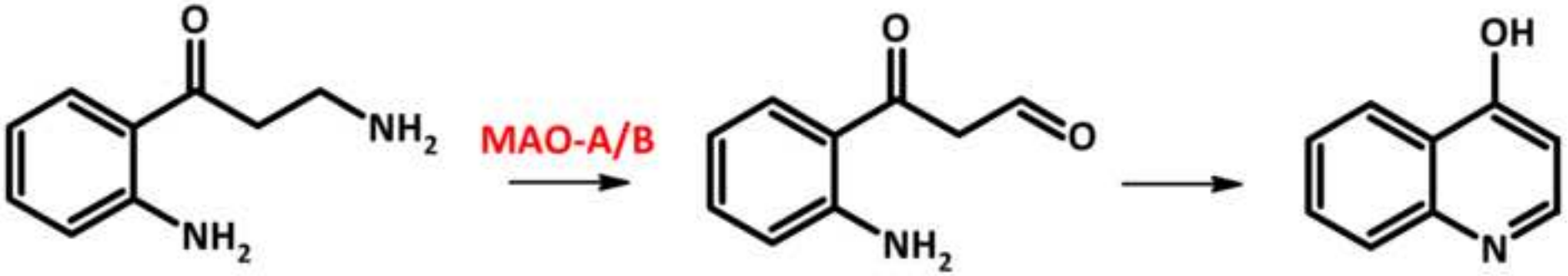


Figure 3

Click here to download high resolution image

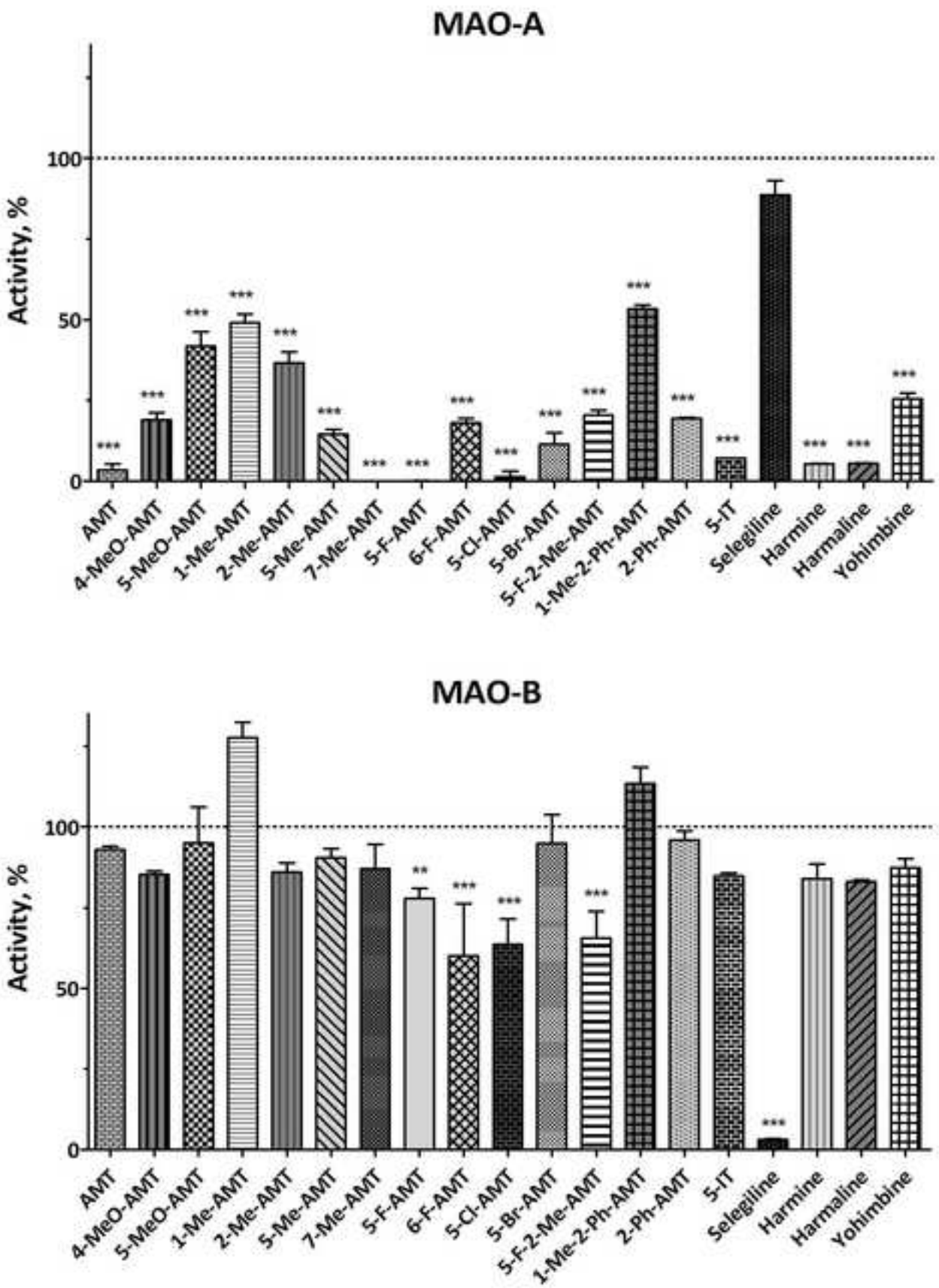
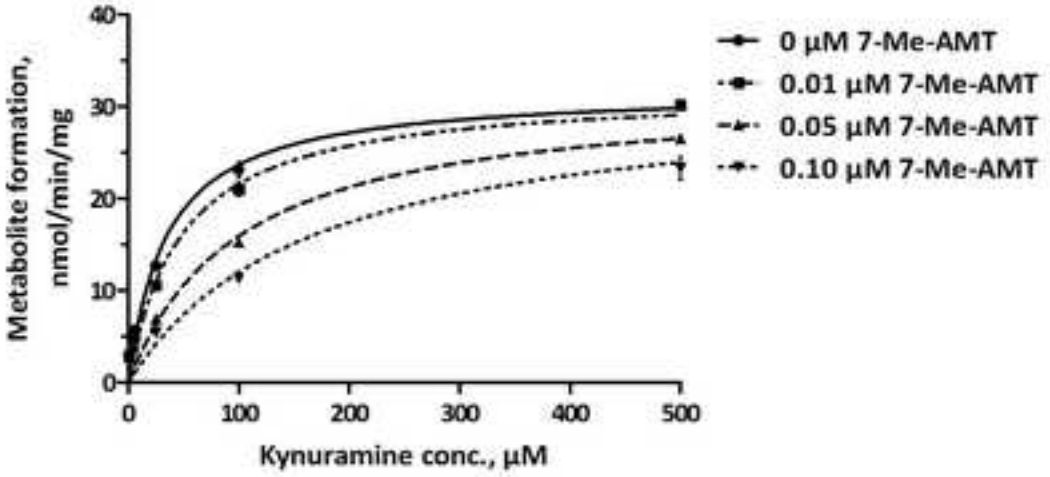


Figure 4

Click here to download high resolution image



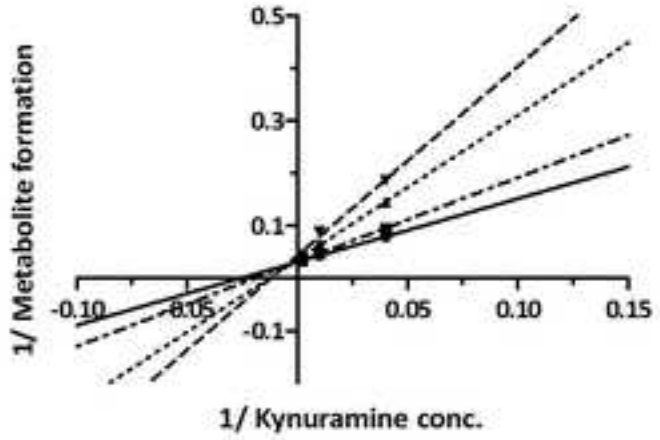


Figure 5

Click here to download high resolution image