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RESEARCH ARTICLE

New Psychoactive Substances 3-Methoxyphencyclidine (3-MeO-PCP) and 3-Methoxyrolicyclidine (3-MeO-PCPy): Metabolic Fate Elucidated with Rat Urine and Human Liver Preparations and their Detectability in Urine by GC-MS, "LC-(High Resolution)-MS^n" and "LC-(High Resolution)-MS/MS"

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Abstract: ***Background*:** 3-Methoxyphencyclidine (3-MeO-PCP) and 3-methoxyrolicyclidine (3-MeO-PCPy) are two new psychoactive substances (NPS). The aims of the present study were the elucidation of their metabolic fate in rat and pooled human liver microsomes (pHLM), the identification of the cytochrome P450 (CYP) isoenzymes involved, and the detectability using standard urine screening approaches (SUSA) after intake of common users' doses using gas chromatography-mass spectrometry (GC-MS), liquid chromatography-multi-stage mass spectrometry ($LC\text{-}MS^n$), and liquid chromatography-high-resolution tandem mass spectrometry (LC-HR-MS/MS).

***Methods*:** For metabolism studies, rat urine samples were treated by solid phase extraction or simple precipitation with or without previous enzymatic conjugate cleavage. After analyses *via* $LC\text{-}HR\text{-}MS^n$, the phase I and II metabolites were identified.

***Results*:** Both drugs showed multiple aliphatic hydroxylations at the cyclohexyl ring and the heterocyclic ring, single aromatic hydroxylation, carboxylation after ring opening, *O*-demethylation, and glucuronidation. The transferability from rat to human was investigated by pHLM incubations, where *O*-demethylation and hydroxylation were observed. The involvement of the individual CYP enzymes in the initial metabolic steps was investigated after single CYP incubations. For 3-MeO-PCP, CYP 2B6 was responsible for aliphatic hydroxylations and CYP 2C19 and CYP 2D6 for *O*-demethylation. For 3-MeO-PCPy, aliphatic hydroxylation was again catalyzed by CYP 2B6 and *O*-demethylation by CYP 2C9 and CYP 2D6.

***Conclusions*:** As only polymorphically expressed enzymes were involved, pharmacogenomic variations might occur, but clinical data are needed to confirm the relevance. The detectability studies showed that the authors' SUSAs were suitable for monitoring the intake of both drugs using the identified metabolites.

Keywords: New psychoactive substances, 3-MeO-PCP, 3-MeO-PCPy, metabolism, $LC\text{-}HR\text{-}MS^n$, screening.

INTRODUCTION

The new psychoactive substances (NPS) 3-methoxyphencyclidine (3-MeO-PCP, Fig. **1a**) and 3-methoxyrolicyclidine (3-MeO-PCPy, Fig. **1b**) are derivatives of phencyclidine (PCP, Fig. **1c**), which is known for over 60 years. As reviewed by Morris and Wallach [1], PCP was synthesized first in 1956 and approved in the following years as a non-narcotic anesthetic in veterinary medicine under the trade names Sernyl or Sernylan. The use for human treatment was short-lived and limited due to unfavorable side effects such as agitation, hallucination, and delirium-like conditions. In 1967, phencyclidine appeared as a street drug in the USA under names such as "Peace Pill", "Angle Dust", "Blue Dust", or "Killer Weed", and in Germany, recreational use appeared in 1977. To overcome national narcotic laws, the structure of phencyclidine was continuously modified and included modifications of the amine moiety, the phenyl, or the piperidine ring [2, 3]. Since the derivative phenylcyclohexylpyrrolidine (also known as rolicyclidine or PCPy, Fig. **1d**) has been scheduled in the USA in the 1970s, other modifications were encountered that included alkoxy derivatives [1, 4]. According to reports from the European Monitoring Center for Drugs and Drug Addiction (EMCDDA), 3-MeO-PCP [5] and 4-MeO-PCP have been identified in 2012 and 2011 as NPS, respectively [6, 7]. 3-MeO-PCP and 3-MeO-PCPy are usually snorted, smoked as a PCP-laced marijuana cigarette, ingested as a tablet, or injected intravenously or subcutaneously [1].

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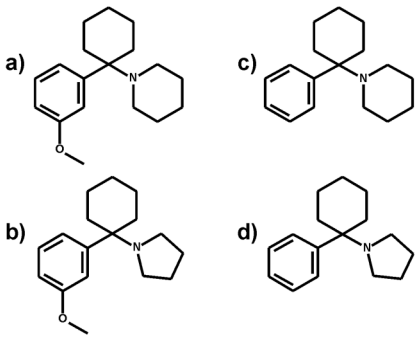

Fig. (1). Structures of 3-MeO-PCP (**a**), 3-MeO-PCPy (**b**), PCP (**c**), and PCPy (**d**).

Effects such as euphoria and analgesia might share some similarities with PCP [8, 9] although some differences might also exist regarding their psychopharmacological profile [1]. Mechanisms of action include uncompetitive antagonism of the excitatory *N*-methyl-D-aspartate (NMDA) receptor and reuptake inhibition of noradrenaline, serotonin, and dopamine [10, 11].

However, a current challenge is that such NPS cannot be detected reliably in toxicological urine screening procedures as excretion products are usually unknown. Therefore, the aims of our study were the elucidation of the phase I metabolism of 3-MeO-PCP and 3-MeO-PCPy in rat and human liver microsomes using high resolution LC-MS^n (LC-HR-MS^n), the identification of the involved CYP isoenzymes, and the investigation of the detectability of common users' doses in standard urine screening approaches (SUSA) using gas chromatography-mass spectrometry (GC-MS), liquid chromatography-multi-stage mass spectrometry (LC-MS^n), and liquid chromatography-high-resolution tandem mass spectrometry (LC-HR-MS/MS).

EXPERIMANTAL PROCEDURES

Chemicals and Reagents

3-MeO-PCP and 3-MeO-PCPy were synthesized and characterized as reported previously [12]. $NADP^+$ and isocitrate dehydrogenase were obtained from Biomol (Hamburg, Germany), Isolute Confirm HCX cartridges (130 mg, 3 mL) from Biotage (Uppsala, Sweden), ammonium sulfate from Fluka (Buches, Switzerland), acetonitrile (LC-MS grade), ammonium formate (analytical grade), dichloromethane, diethyl ether, formic acid (LC-MS grade), isocitrate, magnesium chloride, pyridine, superoxide dismutase, sodium hydroxide, and Tris buffer from Sigma-Aldrich (Taufkirchen, Germany), acetic anhydride, acetic acid, ethyl acetate, glucuronidase/ arylsulfatase, hydrochloric acid (37%), isopropanol, methanol (LC-MS grade), aqueous ammonia (32%), and sodium dihydrogen phosphate and all other chemicals and biochemicals from VWR (Darmstadt, Germany). The baculovirus-infected insect cell microsomes (Supersomes), containing 1 nmol/mL of human cDNA-expressed CYP 1A2, CYP 2A6, CYP 2B6, CYP 2C8, CYP 2C9, CYP 2C19, CYP 2D6, or 2 nmol/mL CYP 2E1, CYP 3A4, CYP 3A5, and pooled human liver microsomes (pHLM, 20 mg microsomal protein/mL, 400 pmol total CYP/mg protein), were obtained from BD Biosciences (Heidelberg, Germany). After delivery, the microsomes were thawed at 37°C, aliquoted, snap-frozen in liquid nitrogen, and stored at -80°C until use.

Urine Samples

Studies were performed using rat urine samples from male Wistar rats (Charles River, Sulzfeld, Germany) for toxicological diagnostic reasons according to the corresponding German law. The compounds were administered once in an aqueous suspension by gastric intubation in a dose of 10 mg/kg body mass (BM) for the identification of the metabolites and once in a 1 mg/kg BM representing common users' doses, calculated and downscaled using an allometric dose-by-factor approach described by Sharma and McNeill [13].

The rats were housed in metabolism cages for 24 h, having water ad libitum. Urine was collected separately from feces over a 24-h period. Blank urine samples were collected before drug administration to confirm the absence of interfering compounds. The samples were directly analyzed and then stored at -20°C.

Sample Preparation for Identification of Phase I and II Metabolites by LC-HR-MS^n

The first of two preparations of high dosed rat urine samples was performed in analogy to a published procedure [14]. A 2-mL aliquot of urine sample was adjusted to pH 5.2 with 1 M acetic acid, a 50-µL aliquot of a mixture (100,000 Fishman units per mL) of glucuronidase (EC No. 3.2.1.31) and arylsulfatase (EC No. 3.1.6.1) from Helix pomatia L was added, and subsequently incubated at 50°C for 2 h. Afterwards, the sample was centrifuged at 5,000 rpm for 5 min, the supernatant loaded onto a cation exchange Confirm HCX solid phase extraction (SPE) cartridge, previously conditioned with 1 mL methanol and 1 mL water. After passing the SPE cartridge, washing steps were performed with 1 mL water, 1 mL 0.01 M hydrochloric acid, 1 mL water, and 1 mL methanol. The retained basic compounds were eluted using 1 mL of a freshly prepared mixture of methanol/32% aqueous ammonia (98:2, *v/v*). The eluate was subsequently evaporated to dryness under a stream of nitrogen at 70°C and reconstituted with 50 µL of methanol. A 10-µL aliquot was injected for LC-HR-MS^n analysis. Conditions were as described below.

The second sample preparation was performed according to Wissenbach *et al*. [15]. A 100-µL aliquot of the high dosed rat urine sample was mixed with 500 µL of acetonitrile and vortexed for 2 min. Afterwards, the sample was centrifuged for 2 min at 10,000 *g*, the supernatant evaporated to dryness under a gentle stream of nitrogen at 70°C, and the residue reconstituted with a mixture of mobile phase A and B (1:1, *v/v*) described below for LC-MS^n. A 5-µL aliquot was injected onto the LC-HR-MS^n system. Conditions were as described below.

Microsomal Incubations for pHLM and Initial CYP Activity Screening Studies

The incubations were performed according to a published procedure [14] using CYP 1A2, CYP 2A6, CYP 2B6, CYP 2C8, CYP 2C9, CYP 2C19, CYP 2D6, CYP 2E1, CYP 3A4,

CYP 3A5, or pHLM for 30 min at 37°C. The final incubation mixture consisted of 90 mM buffer (Tris buffer for CYP 2A6 and CYP 2C9, phosphate buffer for all other incubations), 25 µM drug as substrate, 200 U/mL superoxide dismutase, 75 pmol/mL CYP isoenzyme, and regenerating system consisted of 5 mM magnesium chloride, 5 mM isocitrate, 1.5 mM $NADP^+$, and 0.5 U/mL isocitrate dehydrogenase in a final volume of 50 µL. Reaction was initiated by addition of regenerating system and stopped with 50 µL ice-cold acetonitrile. Afterwards, the sample was centrifuged for 5 min at 10,000 *g*, the supernatant transferred to a glass vial, and a 10-µL aliquot was injected onto the LC-MS^n system. Conditions were as described below.

Identification of Phase I and II Metabolites in Urine by LC-HR-MS^n

Phase I and II metabolites in urine were identified by a system consisted of an Orbitrap Velos Pro (Thermo Fischer Scientific, Dreieich, Germany) equipped with a heated electrospray ionization (HESI)-II source coupled to a Dionex Ultimate 3000 LC system. The used conditions and gradient elution were according to a published procedure [14], gradient program was composed according to Wissenbach *et al*. [15]. A parent mass list was used containing proposed metabolites whereby the list was divided into two separate experiments to reduce the amount of preferred ions for monitoring. The system was running Thermo Scientific Xcalibur 2.2.

Analysis of the Metabolites in Microsomal Incubations by LC-MS^n

LC-MS^n analysis of the metabolites in microsomal incubations was performed according to a published procedure [14]. The system consisted of a LXQ linear ion trap MS equipped with an HESI-II source coupled to an Accela LC system consisting of a degasser, a quaternary pump, and an autosampler (all from ThermoFisher Scientific, Dreieich, Germany). The gradient program was composed according to Wissenbach *et al*. [15] and the same as described for LC-HR-MS^n. The system was running Thermo Scientific Xcalibur 2.0.7.

GC-MS SUSA

According to a published procedure [16], a 5-mL aliquot of the low dosed rat urine sample was divided into two aliquots, the first was submitted to acid hydrolysis for 15 min and basified to pH 9, followed by combining with the second and subsequent extraction with a dichloromethane-isopropanol-ethyl acetate mixture (1:1:3, *v/v/v*). After evaporation, the residue was acetylated with an acetic anhydride-pyridine mixture (3:2, *v/v*) under microwave irradiation, again evaporated and reconstituted in 100 µL of methanol. A 1-µL aliquot was injected onto the GC-MS system, consisted of a Hewlett-Packard (HP; Agilent, Waldbronn, Germany) 5890 Series II gas chromatograph combined with an Agilent 5972 MSD mass spectrometer running HP MS ChemStation (DOS series) B.02.05. GC and MS conditions were according to a published procedure [16].

The full scan data files were evaluated by use of the automated mass spectral deconvolution and identification system (AMDIS, http://chemdata.nist.gov/mass-spc/amdis/) in simple mode. The target library was a modified version of the Maurer/Pfleger/Weber MPW_2016 library [17]. The deconvolution settings were according to Meyer *et al*. [18].

LC-MS^n SUSA

In accordance to Wissenbach *et al*. [19], a 100-µL aliquot of the low dosed urine sample was worked up as described for the identification of phase I and II metabolites in urine. A 10-µL aliquot was injected onto the LC-MS^n system. Conditions were as described for analysis of the metabolites in microsomal incubations.

For data acquisition, ThermoFisher ToxID 2.1.1 for automatic target screening in the MS^2 screening mode was used. The settings were according to Wissenbach *et al*. [19]. ToxID was run automatically after file acquisition by using an Xcalibur processing method starting the software tool. The target library was a modified version of the Maurer/ Wissenbach/Weber MWW_2014 library [20].

LC-HR-MS/MS SUSA

In accordance to Helfer *et al*. [21], a 100-µL aliquot of the low dosed rat urine sample was mixed with 500 µL of acetonitrile and vortexed for 2 min. After centrifugation for 2 min at 10,000 *g*, the supernatant was evaporated to dryness under a gentle stream of nitrogen at 70°C and the residue reconstituted with a mixture of mobile phase A and B (1:1, *v/v*) described below. A 10-µL aliquot was injected onto the LC-HR-MS/MS system, consisted of a Q-Exactive system equipped with an HESI-II source (ThermoFisher Scientific, Dreieich, Germany) coupled to an Accela LC system, consisting of a degasser, a quaternary pump, and an HTC PAL auto-sampler (CTC Analytics AG, Zwingen, Switzerland). Gradient elution and MS conditions were according to a published procedure [21]. The system was running Thermo Scientific Xcalibur 3.0.63. For data acquisition, ThermoFisher Trace Finder Clinical Research 3.2 software was used as described by Helfer *et al*. [21].

Determination of the Detection Limits of the Parent Drugs in Urine for SUSA

For assessing the general performance of the SUSA, the determination of the limits of detection (LOD) for the parent drugs were determined [22]. For this purpose, 3-MeO-PCP and 3-MeO-PCPy were spiked in rat urine in increasing concentrations, respectively, and analyzed *via* GC-MS, LC-MS^n, and LC-HR-MS/MS SUSA. The concentration level with a signal-to-noise of three was defined as LOD.

RESULTS AND DISCUSSION

Identification of Phase I and II Metabolites by LC-HR-MS^n

With the SPE-based sample preparation, only basic compounds were retained leading to cleaner extracts. For the non-basic compounds and the phase II metabolites, the

second sample preparation was performed after simple precipitation.

The proposed structural formulas were deduced by comparing the spectra of the metabolites with those of the parent compounds. Precursor masses (PM) are from MS^1 spectra, fragment ions from MS^2 spectra. All masses are given with the calculated exact masses. The phase I metabolites found for 3-MeO-PCP are shown in Table **1**, those for 3-MeO-PCPy in Table **2**. The phase II metabolites found for 3-MeO-PCP are shown in Table **3**, those for 3-MeO-PCPy in Table **4**. Overall, for 3-MeO-PCP, 30 phase I and seven phase II metabolites, and for 3-MeO-PCPy, 26 phase I and eight phase II metabolites were detected.

Proposed Fragmentation Patterns for Identification of the Phase I and II Metabolites by LC-HR-MS^n

3-MeO-PCP

In general, the spectrum of the parent compound (no. 1 in Table **1**) revealed a characteristic fragmentation pattern, whereby a benzylic cleavage led to a 3-methoxy-phenyl-cyclohexyl fragment represented by the fragment ion of *m/z* 189.1279 and a piperidine fragment represented by the fragment ion of *m/z* 86.0967. In cases where the 3-methoxy-phenylcyclohexyl fragment was unchanged, fragment ions could be observed at *m/z* 189.1279 (nos. 6, 13, 20, and 30), if monohydroxylated at *m/z* 205.1229 (nos. 7, 14, 15, and 24-29), if *O*-demethylated at *m/z* 175.1123 (nos. 2, 3, 8-10, and 16), and if hydroxylated and *O*-demethylated at *m/z* 191.1072 (nos. 4, 5, 11, 12, 17-19, and 21-23). In cases where the piperidine fragment was unchanged, fragment ions could be observed at *m/z* 86.0970 (nos. 2, 4, and 7), if monohydroxylated at *m/z* 102.0919 (nos. 3, 6, 11, 12, 14, and 15), and if dihydroxylated at *m/z* 118.0868 (nos. 8-10, 13, 17-19, and 24). Aliphatic hydroxylations were proposed by the elimination of water resulting in unsaturated fragments at *m/z* 187.1123 (205.1229 – 18.0100 u; nos. 7, 14, 15, and 24-29), 173.0966 (191.1072 – 18.0100 u; nos. 4, 5, 11, 12, 17, 19, and 21-23), and 84.0813 (102.0919 – 18.0100 u; nos. 3, 6, 11, 12, and 14).

A metabolite with PM of *m/z* 260.2014 revealed a spectrum of an *O*-demethyl metabolite (no. 2) with a fragment ion of *m/z* 175.1123. Two hydroxy metabolites were revealed by PM of *m/z* 290.2120. One isomer revealed a piperidine-hydroxy metabolite (no. 6) and one a cyclohexyl-hydroxy metabolite (no. 7). Four dihydroxy metabolites were revealed by PM of *m/z* 306.2069, one piperidine-dihydroxy (no. 13) and two isomeric cyclohexyl-hydroxy piperidine-hydroxy metabolites (nos. 14 and 15). One trihydroxy metabolite was revealed by PM of *m/z* 322.2018 representing a cyclohexyl-hydroxy piperidine-dihydroxy metabolite (no. 24). In addition, a hydroxylation at the α-position to the amine at the piperidine ring would led to a rather instable hemiaminal species followed by ring opening and oxidation to an aliphatic carboxy metabolite. This mechanism had already been described for PCP [23] and for diverse PCP derivatives by Sauer *et al*. [24-29]. The observed corresponding metabolites were a carboxy metabolite (no. 20) with PM of *m/z* 320. 1862, five isomeric methyl artifacts of carboxy cyclohexyl-hydroxy metabolites (nos. 25-29) with PM of *m/z* 336.2175, and one carboxy alkyl-hydroxy metabolite (no. 30) with PM of *m/z* 336.2175. For metabolite no. 7, only one isomer was detected most probably due to the lower formation rate in contrast to the metabolites nos. 25-29. Moreover, in combination with *O*-demethylations ring opened methyl artifacts of carboxy metabolites could be observed with PM of *m/z* 306.2069 (no. 16) and after additional cyclohexyl-hydroxylation (methyl artifact) with PM of *m/z* 322.2018 (nos. 21-23). The existence of some carboxy metabolites was proposed after finding artificially formed methyl ester structures. One explanation for these formations could be a methylation of the carboxy group and nucleophilic attack during the work-up procedure, where the samples were evaporated in methanol at 70°C. Further confirmation was obtained when methanol was replaced with ethanol, leading to ethyl ester structures giving spectra with methylene shifts of the corresponding fragment ions.

Combinations of *O*-demethylation and hydroxylation could also be proposed. Two *O*-demethyl hydroxy isomers occurred with PM of *m/z* 276.1964, in particular one piperidine- (no. 3) and one cyclohexyl-hydroxy metabolite (no. 4), and five *O*-demethyl dihydroxy metabolites with PM of *m/z* 292.1913. Three isomers were observed with both hydroxy groups at the piperidine ring (nos. 8-10) and two isomers with one hydroxy group at the piperidine and the cyclohexyl ring, respectively (nos. 11 and 12). Spectra with PM of *m/z* 308.1862 were revealed after threefold hydroxylation, represented by three *O*-demethyl cyclohexyl-hydroxy piperidine-dihydroxy metabolites (nos. 17-19). One product of the metabolites nos. 8-10 after oxidation and elimination of water could be observed with PM of *m/z* 288.1600 (no. 5).

Concerning phase II metabolism, seven glucuronides were found (nos. 57-63 in Table **3**). These conjugates were identified by the PM shift of +176.0322 u. The fragment ions were identical to those of the underlying phase I metabolites. One of these glucuronides was postulated as *O*-demethyl aryl-hydroxy glucuronide (no. 58). The corresponding phase I metabolite could not be detected, either due to low concentrations or insufficient conjugate cleavage during work-up procedure for this particular metabolite. The position in the aromatic ring system could be confirmed with performed LC-HR-MS/MS analysis, revealing a spectrum with an additional fragment ion of *m/z* 123.0440 ($C_7H_7O_2$), representing a dihydroxy tropylium ion.

3-MeO-PCPy

The parent compound spectrum (no. 31 in Table **2**) showed a characteristic fragmentation pattern analogous to 3-MeO-PCP. The 3-methoxy-phenyl-cyclohexyl fragment was represented by the fragment ion of *m/z* 189.1279, but the corresponding pyrrolidine fragment at *m/z* 72.0813 was not detected. In analogy to 3-MeO-PCP, an unchanged 3-methoxy-phenyl-cyclohexyl fragment was represented at *m/z* 189. 1279 (nos. 37, 39, 44, and 51), after monohydroxylation at *m/z* 205.1229 (nos. 45, 46, and 52-55), after *O*-demethylation at *m/z* 175.1123 (nos. 32, 33, 38, and 40), and after hydroxylation and *O*-demethylation at *m/z* 191.1072 (nos. 34-36, 41-43, and 47-49). Although the pyrrolidine fragment was not detectable in the spectrum of the unchanged molecule, the corresponding fragments were observed in the spectra of

Table 1. 3-MeO-PCP and its phase I metabolites detected in rat urine by LC-HR-MS^n with protonated precursor mass (PM), characteristic fragment ions (FI), calculated exact masses, proposed elemental composition, mass error, relative intensity, and retention times (RT).

No.	Metabolite	Measured Accurate Mass (*m/z*)	Calculated Exact Mass (*m/z*)	Error (ppm)	Elemental Composition	Relative Intensity (%)	RT (min)
1	3-MeO-PCP						10.6
		274.2166	274.2171	-1.82	$C_{18}H_{28}NO$	100	
		86.0963	86.0970	-8.13	$C_5H_{12}N$	100	
		189.1277	189.1279	-1.06	$C_{13}H_{17}O$	40	
2	3-MeO-PCP-M (*O*-demethyl-)						7.3
		260.2006	260.2014	-3.07	$C_{17}H_{26}NO$	100	
		86.0964	86.0970	-6.97	$C_5H_{12}N$	100	
		175.1122	175.1123	-0.57	$C_{12}H_{15}O$	26	
3	3-MeO-PCP-M (*O*-demethyl-piperidine-HO-)						6.1
		276.1955	276.1964	-3.26	$C_{17}H_{26}NO_2$	100	
		84.0808	84.0813	-5.95	$C_5H_{10}N$	8	
		102.0914	102.0919	-4.90	$C_5H_{12}NO$	100	
		175.1122	175.1123	-0.57	$C_{12}H_{15}O$	30	
4	3-MeO-PCP-M (*O*-demethyl-cyclohexyl-HO-)						5.1
		276.1959	276.1964	-1.81	$C_{17}H_{26}NO_2$	100	
		86.0964	86.0970	-6.97	$C_5H_{12}N$	100	
		173.0965	173.0966	-0.58	$C_{12}H_{13}O$	30	
		191.1071	191.1072	-0.52	$C_{12}H_{15}O_2$	6	
5	3-MeO-PCP-M (*O*-demethyl-cyclohexyl-HO-dehydro-oxo-piperidine-)						5.7
		288.1598	288.1600	-0.69	$C_{17}H_{22}NO_3$	100	
		80.0497	80.0500	-3.75	C_5H_6N	5	
		98.0601	98.0606	-5.10	C_5H_8NO	100	
		173.0965	173.0966	-0.58	$C_{12}H_{13}O$	6	
		191.1073	191.1072	0.52	$C_{12}H_{15}O_2$	1	
6	3-MeO-PCP-M (piperidine-HO-)						8.9
		290.2115	290.2120	-1.72	$C_{18}H_{28}NO_2$	28	
		84.0808	84.0813	-5.95	$C_5H_{10}N$	1	
		102.0914	102.0919	-4.90	$C_5H_{12}NO$	18	
		189.1278	189.1279	-0.53	$C_{13}H_{17}O$	8	
7	3-MeO-PCP-M (cyclohexyl-HO-)						7.6
		290.2177	290.2170	2.41	$C_{18}H_{28}NO_2$	60	
		86.0964	86.0970	-6.97	$C_5H_{12}N$	100	
		187.1122	187.1123	-0.53	$C_{13}H_{15}O$	52	
		205.1227	205.1229	-0.98	$C_{13}H_{17}O_2$	14	

(Table 1) contd….

No.	Metabolite	Measured Accurate Mass (*m/z*)	Calculated Exact Mass (*m/z*)	Error (ppm)	Elemental Composition	Relative Intensity (%)	RT (min)
8	3-MeO-PCP-M (*O*-demethyl-piperidine-di-HO-) isomer 1						7.3
		292.1906	292.1913	-2.40	$C_{17}H_{26}NO_3$	30	
		101.0598	101.0603	-4.95	$C_5H_9O_2$	18	
		118.0864	118.0868	-3.39	$C_5H_{12}NO_2$	100	
		175.1121	175.1123	-1.14	$C_{12}H_{15}O$	20	
9	3-MeO-PCP-M (*O*-demethyl-piperidine-di-HO-) isomer 2						8.1
		292.1911	292.1913	-0.68	$C_{17}H_{26}NO_3$	100	
		101.0598	101.0603	-4.95	$C_5H_9O_2$	38	
		118.0865	118.0868	-2.54	$C_5H_{12}NO_2$	100	
		175.1121	175.1123	-1.14	$C_{12}H_{15}O$	28	
10	3-MeO-PCP-M (*O*-demethyl-piperidine-di-HO-) isomer 3						9.2
		292.1912	292.1913	-0.34	$C_{17}H_{26}NO_3$	26	
		101.0600	101.0603	-2.97	$C_5H_9O_2$	1	
		118.0865	118.0868	-2.54	$C_5H_{12}NO_2$	1	
		175.1119	175.1123	-2.28	$C_{12}H_{15}O$	1	
11	3-MeO-PCP-M (*O*-demethyl-cyclohexyl-HO-piperidine-HO-) isomer 1						2.4
		292.1909	292.1913	-1.37	$C_{17}H_{26}NO_3$	100	
		84.0807	84.0813	-7.14	$C_5H_{10}N$	1	
		102.0914	102.0919	-4.90	$C_5H_{12}NO$	100	
		173.0965	173.0966	-0.58	$C_{12}H_{13}O$	14	
		191.1071	191.1072	-0.52	$C_{12}H_{15}O_2$	2	
12	3-MeO-PCP-M (*O*-demethyl-cyclohexyl-HO-piperidine-HO-) isomer 2						4.1
		292.1909	292.1913	-1.37	$C_{17}H_{26}NO_3$	78	
		84.0808	84.0813	-5.95	$C_5H_{10}N$	1	
		102.0913	102.0919	-5.88	$C_5H_{12}NO$	100	
		173.0963	173.0966	-1.73	$C_{12}H_{13}O$	20	
		191.1070	191.1072	-1.05	$C_{12}H_{15}O_2$	3	
13	3-MeO-PCP-M (piperidine-di-HO-)						10.2
		306.2064	306.2069	-1.63	$C_{18}H_{28}NO_3$	50	
		101.0597	101.0603	-5.94	$C_5H_9O_2$	16	
		118.0864	118.0868	-3.39	$C_5H_{12}NO_2$	100	
		189.1276	189.1279	-1.59	$C_{13}H_{17}O$	56	
14	3-MeO-PCP-M (cyclohexyl-HO-piperidine-HO-) isomer 1						4.2
		306.2065	306.2069	-1.31	$C_{18}H_{28}NO_3$	100	
		84.0806	84.0813	-8.33	$C_5H_{10}N$	1	
		102.0913	102.0919	-5.88	$C_5H_{12}NO$	100	
		187.1120	187.1123	-1.60	$C_{13}H_{15}O$	24	
		205.1226	205.1229	-1.46	$C_{13}H_{17}O_2$	8	

(Table 1) contd….

No.	Metabolite	Measured Accurate Mass (*m/z*)	Calculated Exact Mass (*m/z*)	Error (ppm)	Elemental Composition	Relative Intensity (%)	RT (min)
15	3-MeO-PCP-M (cyclohexyl-HO-piperidine-HO-) isomer 2						5.8
		306.2065	306.2069	-1.31	$C_{18}H_{28}NO_3$	38	
		102.0914	102.0919	-4.90	$C_5H_{12}NO$	100	
		187.1122	187.1123	-0.53	$C_{13}H_{15}O$	30	
		205.1228	205.1229	-0.49	$C_{13}H_{17}O_2$	10	
16	3-MeO-PCP-M (*O*-demethyl-carboxy-) methyl artifact						8.7
		306.2062	306.2069	-2.29	$C_{18}H_{28}NO_3$	100	
		115.0756	115.0759	-2.61	$C_6H_{11}O_2$	30	
		132.1022	132.1025	-2.27	$C_6H_{14}NO_2$	100	
		175.1122	175.1123	-0.57	$C_{12}H_{15}O$	18	
17	3-MeO-PCP-M (*O*-demethyl-cyclohexyl-HO-piperidine-di-HO-) isomer 1						4.9
		308.1856	308.1862	-1.95	$C_{17}H_{26}NO_4$	100	
		101.0598	101.0603	-4.95	$C_5H_9O_2$	1	
		118.0864	118.0868	-3.39	$C_5H_{12}NO_2$	100	
		173.0965	173.0966	-0.58	$C_{12}H_{13}O$	14	
		191.1070	191.1072	-1.05	$C_{12}H_{15}O_2$	2	
18	3-MeO-PCP-M (*O*-demethyl-cyclohexyl-HO-piperidine-di-HO-) isomer 2						5.1
		308.1858	308.1862	-1.30	$C_{17}H_{26}NO_4$	20	
		101.0598	101.0603	-4.95	$C_5H_9O_2$	1	
		118.0864	118.0868	-3.39	$C_5H_{12}NO_2$	4	
		191.1068	191.1072	-2.09	$C_{12}H_{15}O_2$	1	
19	3-MeO-PCP-M (*O*-demethyl-cyclohexyl-HO-piperidine-di-HO-) isomer 3						5.5
		308.1857	308.1862	-1.62	$C_{17}H_{26}NO_4$	78	
		101.0597	101.0603	-5.94	$C_5H_9O_2$	1	
		118.0864	118.0868	-3.39	$C_5H_{12}NO_2$	100	
		173.0964	173.0966	-1.16	$C_{12}H_{13}O$	20	
		191.1070	191.1072	-1.05	$C_{12}H_{15}O_2$	3	
20	3-MeO-PCP-M (carboxy-) methyl artifact						11.7
		320.2216	320.2226	-3.12	$C_{19}H_{39}NO_3$	100	
		115.0756	115.0759	-2.61	$C_6H_{11}O_2$	30	
		132.1022	132.1025	-2.27	$C_6H_{14}NO_2$	100	
		189.1278	189.1279	-0.53	$C_{13}H_{17}O$	40	
21	3-MeO-PCP-M (*O*-demethyl-carboxy-cyclohexyl-HO-) methyl artifact isomer 1						5.2
		322.2016	322.2018	-0.62	$C_{18}H_{28}NO_4$	100	
		115.0755	115.0759	-3.48	$C_6H_{11}O_2$	28	
		132.1021	132.1025	-3.03	$C_6H_{14}NO_2$	100	
		173.0963	173.0966	-1.73	$C_{12}H_{13}O$	6	
		191.1069	191.1072	-1.57	$C_{12}H_{15}O_2$	1	

(Table 1) contd….

No.	Metabolite	Measured Accurate Mass (*m/z*)	Calculated Exact Mass (*m/z*)	Error (ppm)	Elemental Composition	Relative Intensity (%)	RT (min)
22	3-MeO-PCP-M (*O*-demethyl-carboxy-cyclohexyl-HO-) methyl artifact isomer 2						5.8
		322.2012	322.2018	-1.86	$C_{18}H_{28}NO_4$	100	
		115.0755	115.0759	-3.48	$C_6H_{11}O_2$	26	
		132.1021	132.1025	-3.03	$C_6H_{14}NO_2$	100	
		173.0964	173.0966	-1.16	$C_{12}H_{13}O$	14	
		191.1070	191.1072	-1.05	$C_{12}H_{15}O_2$	2	
23	3-MeO-PCP-M (*O*-demethyl-carboxy-cyclohexyl-HO-) methyl artifact isomer 3						6.2
		322.2011	322.2018	-2.17	$C_{18}H_{28}NO_4$	58	
		115.0755	115.0759	-3.48	$C_6H_{11}O_2$	30	
		132.1022	132.1025	-2.27	$C_6H_{14}NO_2$	100	
		173.0965	173.0966	-0.58	$C_{12}H_{13}O$	20	
		191.1071	191.1072	-0.52	$C_{12}H_{15}O_2$	2	
24	3-MeO-PCP-M (cyclohexyl-HO-piperidine-di-HO-)						3.8
		322.2016	322.2018	-0.62	$C_{18}H_{28}NO_4$	100	
		100.0758	100.0762	-4.00	$C_5H_{10}NO$	1	
		118.0864	118.0868	-3.39	$C_5H_{12}NO_2$	100	
		187.1120	187.1123	-1.60	$C_{13}H_{15}O$	24	
		205.1226	205.1229	-1.46	$C_{13}H_{17}O_2$	8	
25	3-MeO-PCP-M (carboxy-cyclohexyl-HO-) methyl artifact isomer 1						7.2
		336.2175	336.2175	0.00	$C_{19}H_{30}NO_4$	36	
		115.0756	115.0759	-2.61	$C_6H_{11}O_2$	34	
		132.1023	132.1025	-1.51	$C_6H_{14}NO_2$	100	
		187.1124	187.1123	0.53	$C_{13}H_{15}O$	20	
		205.1229	205.1229	0.00	$C_{13}H_{17}O_2$	4	
26	3-MeO-PCP-M (carboxy-cyclohexyl-HO-) methyl artifact isomer 2						8.0
		336.2173	336.2175	-0.59	$C_{19}H_{30}NO_4$	52	
		115.0755	115.0759	-3.48	$C_6H_{11}O_2$	30	
		132.1021	132.1025	-3.03	$C_6H_{14}NO_2$	100	
		187.1121	187.1123	-1.07	$C_{13}H_{17}O$	24	
		205.1226	205.1229	-1.46	$C_{13}H_{17}O_2$	8	
27	3-MeO-PCP-M (carboxy-cyclohexyl-HO-) methyl artifact isomer 3						8.5
		336.2172	336.2175	-0.89	$C_{19}H_{30}NO_4$	80	
		115.0756	115.0759	-2.61	$C_6H_{11}O_2$	10	
		132.1022	132.1025	-2.27	$C_6H_{14}NO_2$	34	
		187.1122	187.1123	-0.53	$C_{13}H_{15}O$	20	
		205.1228	205.1229	-0.49	$C_{13}H_{17}O_2$	4	

(Table 1) contd….

No.	Metabolite	Measured Accurate Mass (*m/z*)	Calculated Exact Mass (*m/z*)	Error (ppm)	Elemental Composition	Relative Intensity (%)	RT (min)
28	3-MeO-PCP-M (carboxy-cyclohexyl-HO-) methyl artifact isomer 4						8.9
		336.2172	336.2175	-0.89	$C_{19}H_{30}NO_4$	24	
		115.0755	115.0759	-3.48	$C_6H_{11}O_2$	32	
		132.1022	132.1025	-2.27	$C_6H_{14}NO_2$	100	
		187.1122	187.1123	-0.53	$C_{13}H_{15}O$	18	
		205.1227	205.1229	-0.98	$C_{13}H_{17}O_2$	2	
29	3-MeO-PCP-M (carboxy-cyclohexyl-HO-) methyl artifact isomer 5						9.1
		336.2171	336.2175	-1.19	$C_{19}H_{30}NO_4$	45	
		115.0755	115.0759	-3.48	$C_6H_{11}O_2$	20	
		132.1021	132.1025	-3.03	$C_6H_{14}NO_2$	60	
		187.1122	187.1123	-0.53	$C_{13}H_{15}O$	8	
		205.1226	205.1229	-1.46	$C_{13}H_{17}O_2$	100	
30	3-MeO-PCP-M (carboxy-alkyl-HO-)						10.5
		336.2172	336.2175	-0.89	$C_{19}H_{30}NO_4$	50	
		99.0439	99.0446	-7.07	$C_5H_7O_2$	1	
		131.0704	131.0708	-3.05	$C_6H_{11}O_3$	6	
		148.0970	148.0974	-2.70	$C_6H_{14}NO_3$	100	
		189.1277	189.1279	-1.06	$C_{13}H_{17}O$	28	

the metabolites. When the pyrrolidine fragment was unchanged, fragment ions could be observed at *m/z* 72.0813 (no. 32), if monohydroxylated at *m/z* 88.0762 (nos. 33 and 37), and if dihydroxylated at *m/z* 104.0712 (nos. 38, 39, 41-43, 45, and 46). Again, an aliphatic hydroxylation was proposed if water elimination was observed. These fragments were represented at *m/z* 187.1123 (205.1229 – 18.0100 u; nos. 45, 46, and 52-55), 173.0966 (191.1072 – 18.0100 u; nos. 34-36, 41-43, and 47-50), and 70.0657 (88.0762 – 18.0100 u; nos. 33 and 37).

A metabolite with PM of *m/z* 246.1858 revealed an *O*-demethyl metabolite (no. 32). One pyrrolidine-hydroxy metabolite was revealed by PM of *m/z* 276.1958 (no. 37), one pyrrolidine-dihydroxy metabolite by PM of *m/z* 292. 1913 (no. 39), and two trihydroxy metabolite by PM of *m/z* 308.1862, both representing cyclohexyl-hydroxy pyrrolidine-dihydroxy isomers (nos. 45 and 46). In contrast to 3-MeO-PCP, monohydroxylation at the cyclohexyl ring could not be detected being an intermediate to further steps. In accordance to 3-MeO-PCP metabolism, aliphatic carboxy metabolites could be observed as carboxy metabolite (no. 44) with PM of *m/z* 306.2069, as four isomeric methyl artifacts of carboxy cyclohexyl-hydroxy metabolites (nos. 52-55) with PM of *m/z* 322.2018, as one methyl artifact of a carboxy cyclohexyl-dihydroxy metabolite (no. 56) with PM of *m/z* 338.1967, and as one carboxy alkyl-hydroxy metabolite (no. 51) with PM of *m/z* 322.2018. *O*-Demethylation and hydroxylation reactions could be observed for four *O*-demethyl hydroxy isomers with PM of *m/z* 262.1807, whereby one isomer is postulated as the *O*-demethyl pyrrolidine-hydroxy metabolite (no. 33) and three isomers as *O*-demethyl cyclohexyl-hydroxy metabolites (nos. 34-36). Moreover, after dihydroxylation, one *O*-demethyl pyrrolidine-dihydroxy metabolite with PM of *m/z* 278.1756 (no. 38) and after trihydroxylation three *O*-demethyl cyclohexyl-hydroxy pyrrolidine-dihydroxy metabolites with PM of *m/z* 294.1705 (nos. 41-43) could be observed. Furthermore, one methyl artifact of an *O*-demethylated carboxy metabolite could be observed with PM of *m/z* 292.1949 (no. 40) and four isomers after additional cyclohexyl-hydroxylation with PM of *m/z* 308.1862 (nos. 47-50). In analogy to 3-MeO-PCP metabolism studies, the methyl artifacts, postulated as methyl ester structures, were confirmed as described above.

Concerning phase II metabolism, eight glucuronides were found, whereby corresponding phase I metabolites of five of them could not be detected (nos. 66-70 in Table **4**). The reason for the lack of corresponding phase I metabolites for the two aryl hydroxylated metabolites (nos. 66 and 67) were the same already described above. Again, the position of the hydroxy groups were confirmed by additional fragment ions

Table 2. 3-MeO-PCPy and its phase I metabolites detected in rat urine by LC-HR-MS^n with protonated precursor mass (PM), characteristic fragment ions (FI), calculated exact masses, proposed elemental composition, mass error, relative intensity, and retention times (RT).

No.	Metabolite	Measured Accurate Mass (*m/z*)	Calculated Exact Mass (*m/z*)	Error (ppm)	Elemental Composition	Relative Intensity (%)	RT (min)
31	3-MeO-PCPy						10.0
		260.2010	260.2014	-1.54	$C_{17}H_{26}NO$	42	
		189.1276	189.1279	-1.59	$C_{13}H_{17}O$	100	
32	3-MeO-PCPy-M (*O*-demethyl-)						6.6
		246.1850	246.1858	-3.25	$C_{16}H_{24}NO$	100	
		72.0807	72.0813	-8.32	$C_4H_{10}N$	68	
		175.1121	175.1123	-1.14	$C_{12}H_{15}O$	100	
33	3-MeO-PCPy-M (*O*-demethyl-pyrrolidine-HO-)						6.1
		262.1802	262.1807	-1.91	$C_{16}H_{24}NO_2$	100	
		70.0651	70.0657	-8.56	C_4H_8N	1	
		88.0756	88.0762	-6.81	$C_4H_{10}NO$	100	
		175.1120	175.1123	-1.71	$C_{12}H_{15}O$	24	
34	3-MeO-PCPy-M (*O*-demethyl-cyclohexyl-HO-) isomer 1						4.4
		262.1801	262.1807	-2.29	$C_{16}H_{24}NO_2$	70	
		173.0963	173.0966	-1.73	$C_{12}H_{13}O$	100	
		191.1069	191.1072	-1.57	$C_{12}H_{15}O_2$	18	
35	3-MeO-PCPy-M (*O*-demethyl-cyclohexyl-HO-) isomer 2						4.6
		262.1801	262.1807	-2.29	$C_{16}H_{24}NO_2$	100	
		173.0963	173.0966	-1.73	$C_{12}H_{13}O$	100	
		191.1062	191.1072	-5.23	$C_{12}H_{15}O_2$	14	
36	3-MeO-PCPy-M (*O*-demethyl-cyclohexyl-HO-) isomer 3						5.6
		262.1806	262.1807	-0.38	$C_{16}H_{24}NO_2$	64	
		173.0966	173.0966	0.00	$C_{12}H_{13}O$	4	
		191.1070	191.1072	-1.05	$C_{12}H_{15}O_2$	20	
37	3-MeO-PCPy-M (pyrrolidine-HO-)						8.8
		276.1958	276.1964	-2.17	$C_{17}H_{26}NO_2$	60	
		70.0659	70.0657	2.85	C_4H_8N	2	
		88.0756	88.0762	-6.81	$C_4H_{10}NO$	100	
		189.1277	189.1279	-1.06	$C_{13}H_{17}O$	70	
38	3-MeO-PCPy-M (*O*-demethyl-pyrrolidine-di-HO-)						6.5
		278.1754	278.1756	-0.72	$C_{16}H_{24}NO_3$	84	
		87.0440	87.0446	-6.89	$C_4H_7O_2$	14	
		104.0706	104.0712	-5.77	$C_4H_{10}NO_2$	100	
		175.1120	175.1123	-1.71	$C_{12}H_{15}O$	50	

(Table 2) contd….

No.	Metabolite	Measured Accurate Mass (*m/z*)	Calculated Exact Mass (*m/z*)	Error (ppm)	Elemental Composition	Relative Intensity (%)	RT (min)
39	3-MeO-PCPy-M (pyrrolidine-di-HO-)						9.5
		292.1905	292.1913	-2.74	$C_{17}H_{26}NO_3$	18	
		87.0440	87.0446	-6.89	$C_4H_7O_2$	10	
		104.0707	104.0712	-4.80	$C_4H_{10}NO_2$	94	
		189.1277	189.1279	-1.06	$C_{13}H_{17}O$	100	
40	3-MeO-PCPy-M (*O*-demethyl-carboxy-) methyl artifact						8.0
		292.1907	292.1913	-2.05	$C_{17}H_{26}NO_3$	100	
		101.0598	101.0603	-4.95	$C_5H_9O_2$	38	
		118.0865	118,0868	-2.54	$C_5H_{12}NO_2$	100	
		175.1122	175.1123	-0.57	$C_{12}H_{15}O$	30	
41	3-MeO-PCPy-M (*O*-demethyl-cyclohexyl-HO-pyrrolidine-di-HO-) isomer 1						3.0
		294.1710	294.1705	1.70	$C_{16}H_{24}NO_4$	22	
		87.0440	87.0446	-6.89	$C_4H_7O_2$	10	
		104.0706	104.0712	-5.77	$C_4H_{10}NO_2$	100	
		173.0963	173.0966	-1.73	$C_{12}H_{13}O$	20	
		191.1071	191.1072	-0.52	$C_{12}H_{15}O_2$	4	
42	3-MeO-PCPy-M (*O*-demethyl-cyclohexyl-HO-pyrrolidine-di-HO-) isomer 2						4.0
		294.1703	294.1705	-0.68	$C_{16}H_{24}NO_4$	94	
		87.0440	87.0446	-6.89	$C_4H_7O_2$	10	
		104.0706	104.0712	-5.77	$C_4H_{10}NO_2$	100	
		173.0964	173.0966	-1.16	$C_{12}H_{13}O$	44	
		191.1069	191.1072	-1.57	$C_{12}H_{15}O_2$	12	
43	3-MeO-PCPy-M (*O*-demethyl-cyclohexyl-HO-pyrrolidine-di-HO-) isomer 3						4.4
		294.1701	294.1705	-1.36	$C_{16}H_{24}NO_4$	92	
		87.0441	87.0446	-5.74	$C_4H_7O_2$	12	
		104.0707	104.0712	-4.80	$C_4H_{10}NO_2$	100	
		173.0965	173.0966	-0.58	$C_{12}H_{13}O$	30	
		191.1072	191.1072	0.00	$C_{12}H_{15}O_2$	6	
44	3-MeO-PCPy-M (carboxy-)						11.0
		306.2058	306.2069	-3.59	$C_{18}H_{28}NO_3$	100	
		101.0597	101.0603	-5.94	$C_5H_9O_2$	40	
		118.0864	118.0868	-3.39	$C_5H_{12}NO_2$	100	
		189.1277	189.1279	-1.06	$C_{13}H_{17}O$	70	
45	3-MeO-PCPy-M (cyclohexyl-HO-pyrrolidine-di-HO-) isomer 1						4.7
		308.1856	308.1862	-1,95	$C_{17}H_{26}NO_4$	20	
		86.0440	87.0446	-6,89	$C_4H_7O_2$	6	

(Table 2) contd….

No.	Metabolite	Measured Accurate Mass (*m/z*)	Calculated Exact Mass (*m/z*)	Error (ppm)	Elemental Composition	Relative Intensity (%)	RT (min)
45	3-MeO-PCPy-M (cyclohexyl-HO-pyrrolidine-di-HO-) isomer 1						4.7
		104.0707	104.0712	-4,80	$C_4H_{10}NO_2$	100	
		187.1121	187.1123	-1,07	$C_{13}H_{15}O$	42	
		205.1227	205.1229	-0,98	$C_{13}H_{17}O_2$	14	
46	3-MeO-PCPy-M (cyclohexyl-HO-pyrrolidine-di-HO-) isomer 2						6.3
		308.1858	308.1862	-1.30	$C_{17}H_{26}NO_4$	82	
		87.0441	87.0446	-5.74	$C_4H_7O_2$	2	
		104.0707	104.0712	-4.80	$C_4H_{10}NO_2$	44	
		187.1121	187.1123	-1.07	$C_{13}H_{15}O$	20	
		205.1227	205.1229	-0.98	$C_{13}H_{17}O_2$	6	
47	3-MeO-PCPy-M (*O*-demethyl-carboxy-cyclohexyl-HO-) methyl artifact isomer 1						5.0
		308.1855	308.1862	-2.27	$C_{17}H_{26}NO_4$	100	
		101.0598	101.0603	-4.95	$C_5H_9O_2$	30	
		118.0865	118.0868	-2.54	$C_5H_{12}NO_2$	100	
		173.0966	173.0966	0.00	$C_{12}H_{13}O$	20	
		191.1072	191.1072	0.00	$C_{12}H_{15}O_2$	4	
48	3-MeO-PCPy-M (*O*-demethyl-carboxy-cyclohexyl-HO-) methyl artifact isomer 2						5.4
		308.1852	308.1862	-3.24	$C_{17}H_{26}NO_4$	100	
		101.0597	101.0603	-5.94	$C_5H_9O_2$	40	
		118.0864	118.0868	-3.39	$C_5H_{12}NO_2$	100	
		173.0964	173.0966	-1.16	$C_{12}H_{13}O$	18	
		191.1070	191.1072	-1.05	$C_{12}H_{15}O_2$	2	
49	3-MeO-PCPy-M (*O*-demethyl-carboxy-cyclohexyl-HO-) methyl artifact isomer 3						6.3
		308.1857	308.1862	-1.62	$C_{17}H_{26}NO_4$	88	
		87.0440	87.0446	-6.89	$C_4H_7O_2$	1	
		101.0598	101.0603	-4.95	$C_5H_9O_2$	38	
		118.0864	118.0868	-3.39	$C_5H_{12}NO_2$	100	
		173.0963	173.0966	-1.73	$C_{12}H_{13}O$	6	
		191.1070	191.1072	-1.05	$C_{12}H_{15}O_2$	16	
50	3-MeO-PCPy-M (*O*-demethyl-carboxy-cyclohexyl-HO-) methyl artifact isomer 4						10.2
		308.1859	308.1862	-0.97	$C_{17}H_{26}NO_4$	34	
		101.0599	101.0603	-3.96	$C_5H_9O_2$	8	
		118.0864	118.0868	-3.39	$C_5H_{12}NO_2$	24	
		173.0965	173.0966	-0.58	$C_{12}H_{13}O$	4	

(Table 2) contd….

No.	Metabolite	Measured Accurate Mass (*m/z*)	Calculated Exact Mass (*m/z*)	Error (ppm)	Elemental Composition	Relative Intensity (%)	RT (min)
51	3-MeO-PCPy-M (carboxy-alkyl-HO-) methyl artifact						9.7
		322.2015	322.2018	-0.93	$C_{18}H_{28}NO_4$	80	
		102.0553	102.0555	-1.96	$C_4H_8NO_2$	1	
		117.0548	117.0551	-2.56	$C_5H_9O_3$	26	
		134.0814	134.0817	-2.24	$C_5H_{12}NO_3$	100	
		189.1278	189.1279	-0.53	$C_{13}H_{17}O$	30	
52	3-MeO-PCPy-M (carboxy-cyclohexyl-HO-) methyl artifact isomer 1						6.1
		322.2012	322.2018	-1.86	$C_{18}H_{28}NO_4$	34	
		101.0597	101.0603	-5.94	$C_5H_9O_2$	30	
		118.0863	118.0868	-4.23	$C_5H_{12}NO_2$	100	
		187.1119	187.1123	-2.14	$C_{13}H_{15}O$	20	
		205.1225	205.1229	-1.95	$C_{13}H_{17}O_2$	6	
53	3-MeO-PCPy-M carboxy-cyclohexyl-HO-) methyl artifact isomer 2						7.1
		322.2014	322.2018	-1.24	$C_{17}H_{28}NO_4$	32	
		101.0597	101.0603	-5.94	$C_5H_9O_2$	30	
		118.0863	118.0868	-4.23	$C_5H_{12}NO_2$	100	
		187.1119	187.1123	-2.14	$C_{13}H_{15}O$	48	
		205.1225	205.1229	-1.95	$C_{13}H_{17}O_2$	12	
54	3-MeO-PCPy-M (carboxy-cyclohexyl-HO-) methyl artifact isomer 3						7.8
		322.2016	322.2018	-0.62	$C_{18}H_{28}NO_4$	100	
		101.0598	101.0603	-4.95	$C_5H_9O_2$	32	
		118.0865	118.0868	-2.54	$C_5H_{12}NO_2$	100	
		187.1122	187.1123	-0.53	$C_{13}H_{15}O$	30	
		205.1228	205.1229	-0.49	$C_{13}H_{17}O_2$	6	
55	3-MeO-PCPy-M (carboxy-cyclohexyl-HO-) methyl artifact isomer 4						8.3
		322.2016	322.2018	-0.62	$C_{18}H_{28}NO_4$	100	
		101.0598	101.0603	-4.95	$C_5H_9O_2$	10	
		118.0865	118.0868	-2.54	$C_5H_{12}NO_2$	30	
		187.1120	187.1123	-1.60	$C_{13}H_{15}O$	8	
		205.1227	205.1229	-0.98	$C_{13}H_{17}O_2$	100	
56	3-MeO-PCPy-M (carboxy-cyclohexyl-di-HO-) methyl artifact						5.4
		338.1958	338.1967	-2.66	$C_{18}H_{28}NO_5$	4	
		101.0597	101.0603	-5.94	$C_5H_9O_2$	24	
		118.0863	118.0868	-4.23	$C_5H_{12}NO_2$	100	
		203.1069	203.1072	-1.48	$C_{13}H_{15}O_2$	40	
		221.1174	221.1178	-1.81	$C_{13}H_{17}O_3$	70	

Table 3. 3-MeO-PCP and its phase II metabolites detected in rat urine by LC-HR-MS^n with protonated precursor mass (PM), characteristic fragment ions (FI), calculated exact masses, proposed elemental composition, mass error, relative intensity, and retention times (RT).

No.	Metabolite	Measured Accurate Mass (*m/z*)	Calculated Exact Mass (*m/z*)	Error (ppm)	Elemental Composition	Relative Intensity (%)	RT (min)
57	3-MeO-PCP-M (*O*-demethyl-) glucuronide						5.3
		436.2320	436.2335	-3.44	$C_{23}H_{34}NO_7$	100	
		175.1117	175.1123	-3.43	$C_{12}H_{15}O$	100	
		351.1436	351.1444	-2.28	$C_{18}H_{23}O_7$	3	
58	3-MeO-PCP-M (*O*-demethyl-aryl-HO-) glucuronide						4.6
		452.2272	452.2284	-2.65	$C_{23}H_{34}NO_8$	100	
		175.1117	175.1123	-3.43	$C_{12}H_{15}O$	100	
		191.1066	191.1072	-3.14	$C_{12}H_{15}O_2$	30	
		367.1386	367.1393	-1.91	$C_{18}H_{23}O_8$	60	
59	3-MeO-PCP-M (*O*-demethyl-piperidine-HO-) glucuronide isomer 1						5.7
		452.2272	452.2284	-2.65	$C_{23}H_{24}NO_8$	100	
		175.1119	175.1123	-2.28	$C_{12}H_{15}O$	3	
		278.1235	278.1240	-1.80	$C_{11}H_{20}NO_7$	100	
60	3-MeO-PCP-M (*O*-demethyl-piperidine-HO-) glucuronide isomer 2						5.9
		452.2275	452.2284	-1.99	$C_{23}H_{34}NO_8$	100	
		175.1118	175.1123	-2.86	$C_{12}H_{15}O$	3	
		278.1234	278.1240	-2.16	$C_{11}H_{20}NO_7$	100	
61	3-MeO-PCP-M (piperidine-HO-) glucuronide						8.1
		466.2432	466.2441	-1.93	$C_{24}H_{36}NO_8$	100	
		189.1275	189.1279	-2.11	$C_{13}H_{17}O$	6	
		278.1235	278.1240	-1.80	$C_{11}H_{20}NO_7$	100	
62	3-MeO-PCP-M (*O*-demethyl-piperidine-di-HO-) glucuronide						5.5
		468.2226	468.2234	-1.71	$C_{23}H_{34}NO_9$	10	
		175.1117	175.1123	-3.43	$C_{12}H_{15}O$	2	
		351.1440	351.1444	-1.14	$C_{18}H_{23}O_7$	100	
63	3-MeO-PCP-M (*O*-demethyl-cyclohexyl-HO-piperidine-HO-) glucuronide						4.3
		468.2263	468.2234	6.19	$C_{23}H_{34}NO_9$	14	
		191.1067	191.1072	-2.62	$C_{12}H_{15}O_2$	90	
		367.1389	367.1393	-1.09	$C_{18}H_{23}O_8$	100	

of *m/z* 123.0440 ($C_7H_7O_2$) in LC-HR-MS/MS. Furthermore, the other three glucuronides (nos. 68-70) showed keto groups at the pyrrolidine ring. The fact, that the corresponding phase I metabolites could not be detected after conjugate cleavage with subsequent SPE, non-basic structures could be proposed, which led to the suggestion of lactam ring formation following hydroxylation at the α-position and further oxidation.

Proposed Metabolic Pathways

The metabolic pathways, which could be proposed according to the identified metabolites, are given in Fig. (**2**) for

Table 4. 3-MeO-PCPy and its phase II metabolites detected in rat urine by LC-HR-MS^n with protonated precursor mass (PM), characteristic fragment ions (FI), calculated exact masses, proposed elemental composition, mass error, relative intensity, and retention times (RT).

No.	Metabolite	Measured Accurate Mass (*m/z*)	Calculated Exact Mass (*m/z*)	Error (ppm)	Elemental Composition	Relative Intensity (%)	RT (min)
64	3-MeO-PCPy-M (*O*-demethyl-) glucuronide						5.1
		422.2169	422.2179	-2.37	$C_{22}H_{32}NO_7$	100	
		175.1118	175.1123	-2.86	$C_{12}H_{15}O$	100	
		351.1411	351.1444	-9.40	$C_{18}H_{23}O_7$	1	
65	3-MeO-PCPy-M (*O*-demethyl-pyrrolidine-HO-) glucuronide						5.8
		438.2121	438.2128	-1.60	$C_{22}H_{32}NO_8$	60	
		175.1118	175.1123	-2.86	$C_{12}H_{15}O$	1	
		264.1078	264.1083	-1.89	$C_{10}H_{18}NO_7$	100	
66	3-MeO-PCPy-M (*O*-demethyl-aryl-HO-) glucuronide						4.5
		438.2119	438.2128	-2.05	$C_{22}H_{32}NO_8$	100	
		191.1067	191.1072	-2.62	$C_{12}H_{15}O_2$	50	
		367.1388	367.1393	-1.36	$C_{18}H_{23}O_8$	100	
67	3-MeO-PCPy-M (*O*-demethyl-aryl-HO-pyrrolidine-2-oxo-) glucuronide						10.1
		452.1915	452.1920	-1.11	$C_{22}H_{30}NO_9$	30	
		191.1067	191.1072	-2.62	$C_{12}H_{15}O_2$	100	
		367.1387	367.1393	-1.63	$C_{18}H_{23}O_8$	72	
68	3-MeO-PCPy-M (cyclohexyl-HO-pyrrolidine-2-oxo-) glucuronide						10.8
		466.2072	466.2077	-1.07	$C_{23}H_{32}NO_9$	50	
		205.1222	205.1229	-3.41	$C_{13}H_{17}O_2$	100	
		381.1543	381.1549	-1.57	$C_{19}H_{25}O_8$	12	
69	3-MeO-PCPy-M (cyclohexyl-di-HO-pyrrolidine-2-oxo-) glucuronide isomer 1						4.6
		482.2020	482.2026	-1.24	$C_23H_{32}NO_{10}$	10	
		203.1068	203.1072	-1.97	$C_{13}H_{15}O_2$	40	
		221.1173	221.1178	-2.26	$C_{13}H_{17}O_3$	100	
		397.1492	397.1499	-1.76	$C_{19}H_{25}O_9$	8	
70	3-MeO-PCPy-M (cyclohexyl-di-HO-pyrrolidine-2-oxo-) glucuronide isomer 2						5.2
		482.2020	482.2026	-1.24	$C_{23}H_{32}NO_{10}$	14	
		203.1065	203.1072	-3.45	$C_{13}H_{15}O_2$	44	
		221.1170	221.1178	-3.62	$C_{13}H_{17}O_3$	100	
		397.1482	397.1499	-4.28	$C_{19}H_{25}O_9$	4	
71	3-MeO-PCPy-M (cyclohexyl-HO-pyrrolidine-di-HO-) glucuronide						8.0
		484.2178	484.2183	-1.03	$C_{23}H_{34}NO_{10}$	60	
		205.1224	205.1229	-2.44	$C_{13}H_{17}O_2$	100	
		381.1545	381.1549	-1.05	$C_{19}H_{25}O_8$	20	

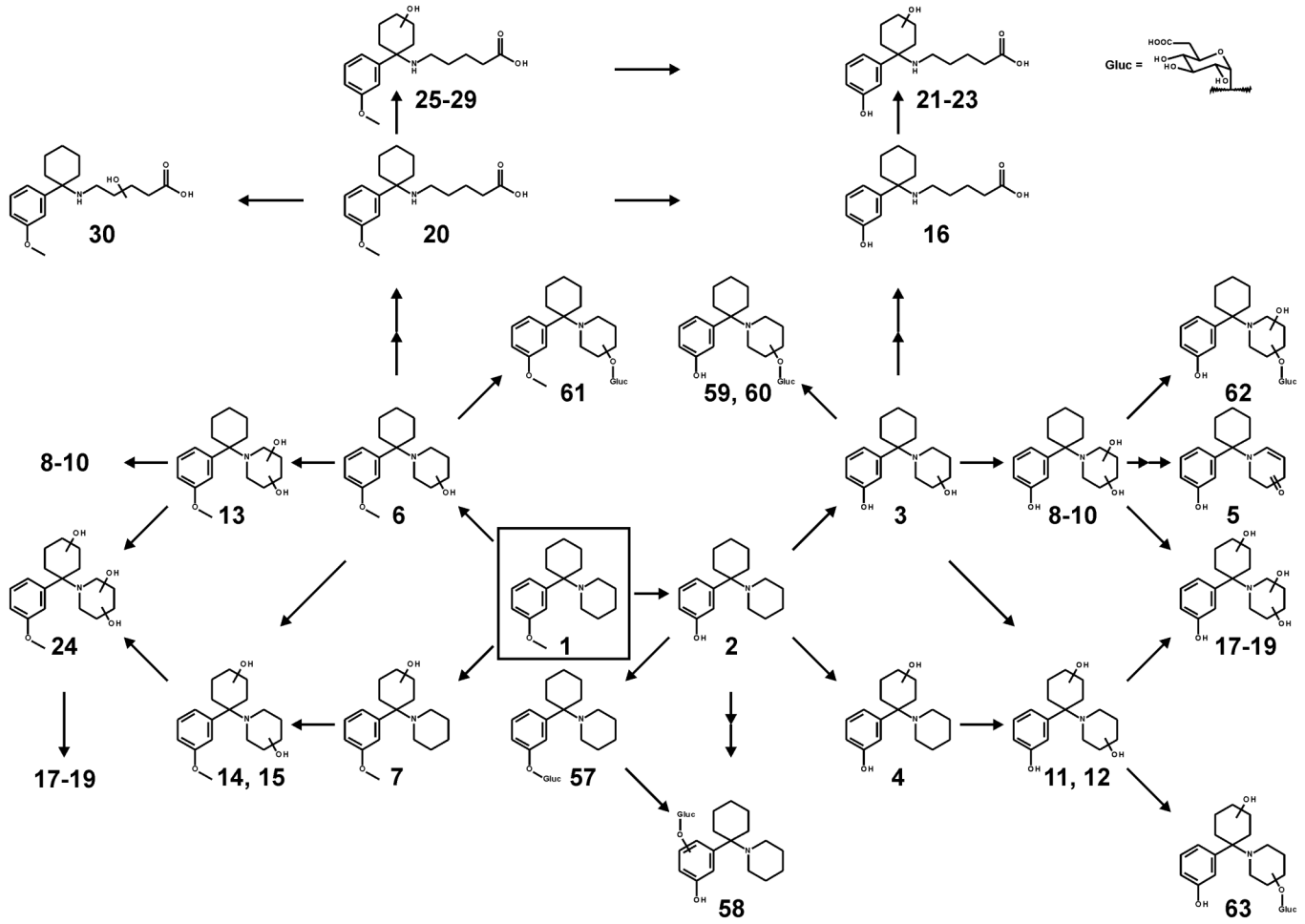

Fig. (2). Metabolic pathways of 3-MeO-PCP. Undefined positions of hydroxylation are indicated by unspecific bonds. Parent compound is marked by a box. Two arrows indicate a pathway that contains multiple metabolism steps.

3-MeO-PCP and in Fig. (**3**) for 3-MeO-PCPy, respectively. Numbers correspond to those given in Tables **1**-**4**.

3-MeO-PCP

Hydroxylation steps could be detected at the cyclohexyl ring (no. 7 in Fig. **2**), at the piperidine ring (no. 6), or at both rings forming two isomeric metabolites (nos. 14 and 15). The metabolite with monohydroxylated piperidine ring (no. 6) as well as both isomers of cyclohexyl-hydroxy piperidine-hydroxy (nos. 14 and 15) could further get hydroxylated at the piperidine ring to a piperidine-dihydroxy metabolite (no. 13) or to isomers of cyclohexyl-hydroxy piperidine-dihydroxy metabolite, whereby only one isomer was detected (no. 24). If hydroxylation of metabolite no. 6 took place at α-position to the amine, the resulting hemiaminal could perform a ring opening, and the formed aldehyde could be further oxidized to the corresponding carboxylic acid (no. 20). Further hydroxylation of this metabolite at the alkyl side chain (no. 30) or at the cyclohexyl ring to five isomeric structures (nos. 25-29) could be observed. *O*-Demethylation (no. 2) could be detected as another pathway, followed by hydroxylation at the cyclohexyl ring (no. 4), at the piperidine ring (no. 3), or at both rings, again into two isomeric metabolites (nos. 11 and 12). In analogy to the pathway without *O*-demethylation, the metabolite with the monohydroxylated piperidine ring (no. 3) as well as both cyclohexyl-hydroxy piperidine-hydroxy isomers (nos. 11 and 12) could further get hydroxylated at the piperidine ring to three isomeric piperidine-dihydroxy metabolites (nos. 8-10) or to three isomeric cyclohexyl-hydroxy piperidine-dihydroxy metabolites (nos. 17-19). Metabolite no. 5 could be explained as a product of one of the metabolites nos. 17-19 after oxidation of one hydroxy group and elimination of water. If hydroxylation of metabolite no. 3 led to a hemiaminal, one ring opened carboxy metabolite (no. 16) and after further hydroxylation at the cyclohexyl ring three isomers (nos. 21-23) could be detected.

Glucuronidation could be observed for metabolites nos. 6 (to 61), 2 (to 57), 3 (to 59 or 60), 11 or 12 (to 63), and 8-10 (to 62). For the glucuronide no. 58, the corresponding precursor should be an aryl-hydroxy of metabolite no. 2, which could not be detected possibly for reasons already described above.

3-MeO-PCPy

The metabolic pathways were similar to those of 3-MeO-PCP. Hydroxylation could be detected at the pyrrolidine ring

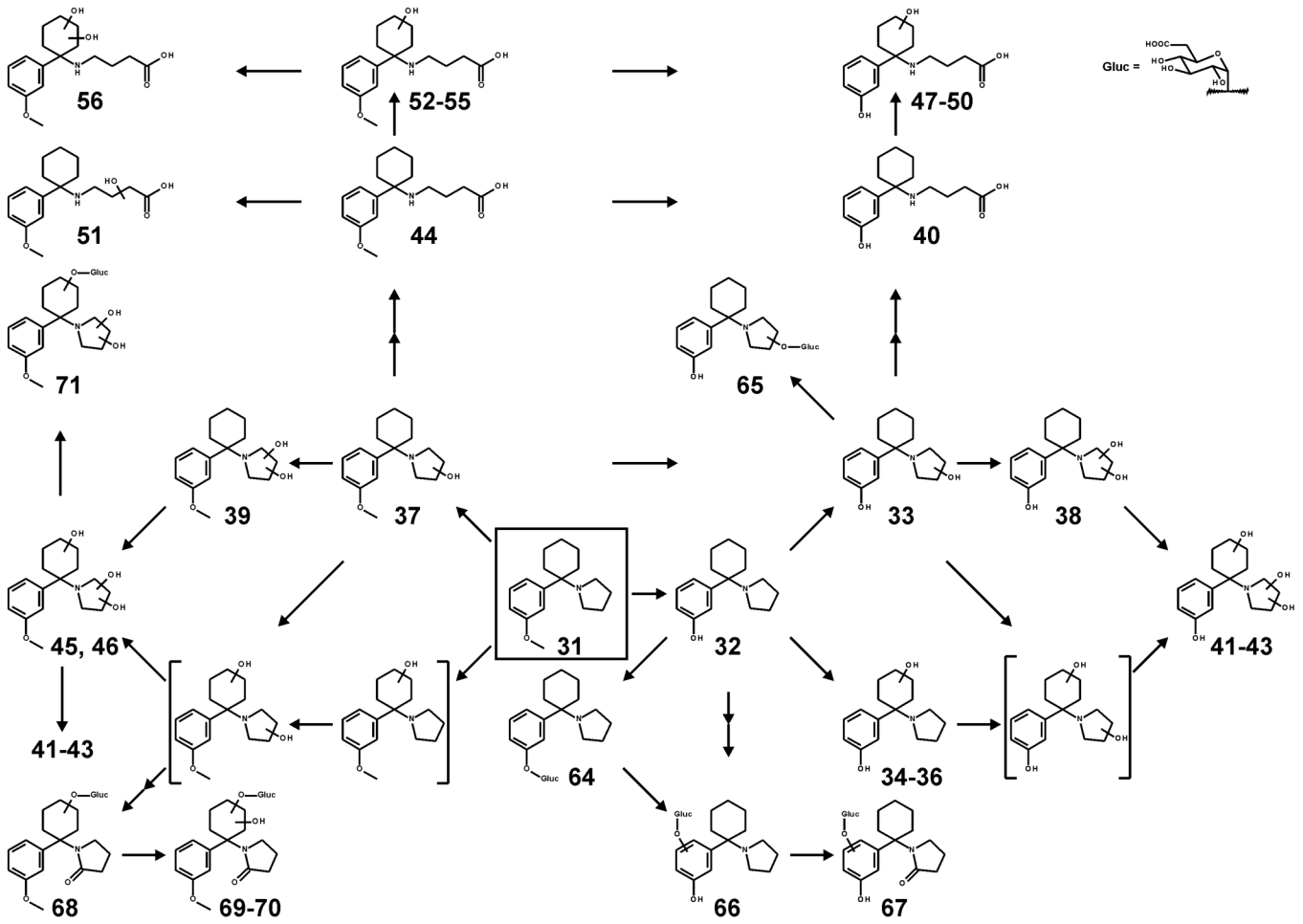

Fig. (3). Metabolic pathways of 3-MeO-PCPy. Undefined positions of hydroxylation are indicated by unspecific bonds. Parent compound is marked by a box. Two arrows indicate a pathway that contains multiple metabolism steps. Structures in brackets are postulated intermediate metabolites.

after monohydroxylation (no. 37 in Fig. (**3**)) and dihydroxylation (no. 39). At the cyclohexyl ring, monohydroxylation was not observed but two isomers in combination with a dihydroxy pyrrolidine ring (nos. 45 and 46). Again, after ring opening, one carboxylic acid metabolite (no. 44), one after further hydroxylation at the alkyl side chain (no. 51), four cyclohexyl-monohydroxy isomers (nos. 52-55), and one cyclohexyl-dihydroxy isomer (no. 56) were detected. *O*-Demethylation (no. 32) followed by hydroxylation at the piperidine ring led to one monohydroxy (no. 33) and one dihydroxy isomer (no. 38). At the cyclohexyl ring, three monohydroxy (nos. 34-36), but no dihydroxy isomers were detected. Combinations only occurred with dihydroxy pyrrolidine ring, again resulting in three isomeric structures (nos. 41-43) fitting with the three precursor isomers nos. 34-36. *O*-Demethylation in combination with ring opening led to metabolite no. 40 and to the three isomers nos. 47-50 after further cyclohexyl-monohydroxylation.

Glucuronidation could be observed for metabolites nos. 45 or 46 (to 71), 32 (to 64), and 33 (to 65). The precursor of no. 68 was a pyrrolidine-monohydroxy metabolite in α-position (no. 37) followed by oxidation to the lactams, those of nos. 69 and 70 after further cyclohexyl-hydroxylation. Metabolite no. 66 resulted by aryl-hydroxylation of the *O*-demethyl metabolite no. 32 followed by glucuronidation. Metabolite no. 67 resulted from metabolite no. 66 after additional lactam formation.

Microsomal Incubations and Initial CYP Activity Screening

Incubations with pHLM were carried out for comparison of the formed rat phase I metabolites with those of humans. For 3-MeO-PCP, the metabolites nos. 2, 3, 4, 7, and 13 from Table **1**, for 3-MeO-PCPy, the metabolites nos. 32 and 39 from Table **2** were detected after pHLM incubation. Essentially, *O*-demethylation and hydroxylation at the pyrrolidine and/or the cyclohexyl ring were observed for both species. However, ring opening steps could not be detected in pHLM, what could either be explained by low formation rates or by interindividual isomeric variability that could lead to the absence of hydroxylation products in the α-position in humans, and thus, absence of detectable carboxy metabolites.

To test the involvement of single CYP enzymes in the initial metabolic steps, the proposed metabolites were detected after incubations with the ten most important human hepatic CYP enzymes. The involvement of the corresponding CYPs

is shown in Table **5** for 3-MeO-PCP and for 3-MeO-PCPy. The relative involvement of individual CYPs was defined in relation to the highest peak abundances during precursor ion monitoring of the formed metabolites. CYPs forming the relative highest peak abundances are given with "++" and all others with "+".

For 3-MeO-PCP, CYP 2B6 was involved in the formation of hydroxylation at the cyclohexyl ring as well as at the piperidine ring. *O*-Demethylation was performed by CYP 2C19, 2B6, and CYP 2D6. Regarding 3-MeO-PCPy, hydroxylation at the pyrrolidine ring was again catalyzed by CYP 2B6. However, cyclohexyl-hydroxylation was not detected.

Table 5. General involvement of human CYP isoenzymes in initial metabolic steps of 3-MeO-PCP and 3-MeO-PCPy.

	CYP 1A2	CYP 2A6	CYP 2B6	CYP 2C8	CYP 2C9	CYP 2C19	CYP 2D6	CYP 2E1	CYP 3A4	CYP 3A5
3-MeO-PCP										
O-Demethylation			+			++	+			
Piperidine-hydroxylation			++			+				
Cyclohexyl-hydroxylation			++							
3-MeO-PCPy										
O-Demethylation			+		+		++			
Pyrrolidine-hydroxylation			++							

Table 6. Proposed targets for GC-MS SUSA monitoring 3-MeO-PCP or 3-MeO-PCPy, with molecular masses, most abundant fragment ions, their relative intensities, and retention indices (RI) according to Kovats [16]. The numbers correspond to those in Figs. (2 and 3).

No.	Target for SUSA	Molecular Mass (u)	GC-MS Fragment Ions (*m/z*), and Relative Intensity (%)	RI
2	3-MeO-PCP	273	273 (40), 230 (100), 161 (52), 121 (41)	2120
2	3-MeO-PCP-M (*O*-demethyl-) AC	301	84 (18), 166 (21), 244 (16), 258 (100), 301 (28)	2210
3	3-MeO-PCP-M (*O*-demethyl-piperidine-HO-) 2 AC	359	164 (16), 258 (21), 300 (100), 316 (12), 359 (15)	2510
32	3-MeO-PCPy-M (*O*-demethyl-) AC	287	70 (38), 107 (60), 152 (38), 244 (100), 287 (22)	2160

Table 7. Proposed targets for LC-MS^n SUSA monitoring 3-MeO-PCP or 3-MeO-PCPy, with protonated precursor ions, characteristic MS^2 and MS^3 fragment ions, and retention times (RT). The numbers correspond to those in Figs. (2 and 3).

No.	Target for SUSA	Precursor Ions (*m/z*)	MS^2 Fragment Ions (*m/z*), and Relative Intensity (%)	MS^3 Fragment Ions (*m/z*), and Relative Intensity (%)	RT (min)
8	3-MeO-PCP-M (*O*-demethyl-piperidine-di-HO-)	292	101 (20), 118, (100), 175 (25)	175: 81 (10), 107 (100)	8.6
13	3-MeO-PCP-M (piperidine-di-HO-)	306	101 (20), 118 (100), 189 (56)	189: 81 (20), 121 (100)	11.6
57	3-MeO-PCP-M (*O*-demethyl-) glucuronide	436	175 (100), 315 (70), 391 (10)	175: 81 (20), 107 (100)	5.5
58	3-MeO-PCP-M (*O*-demethyl-aryl-HO-) glucuronide	452	175 (20), 191 (90), 367 (100)	191: 81 (6), 123 (100)	4.6
61	3-MeO-PCP-M (piperidine-HO-) glucuronide	466	189 (6), 278 (100)	278: 84 (53), 102 (90), 242 (100)	9.0
32	3-MeO-PCPy-M (*O*-demethyl-)	246	72 (100), 175 (60)	175: 81 (15), 107 (100)	8.2
38	3-MeO-PCPy-M (*O*-demethyl-pyrrolidine-di-HO-)	278	87 (14), 104 (100), 175 (50), 232 (30)	175: 81 (20), 107 (100)	7.3
39	3-MeO-PCPy-M (pyrrolidine-di-HO-)	292	87 (10), 104 (94), 189 (100)	189: 81 (20), 121 (100)	10.9
64	3-MeO-PCPy-M (*O*-demethyl-) glucuronide	422	175 (100), 315 (1)	175: 81 (30), 107 (100)	4.9
67	3-MeO-PCPy-M (*O*-demethyl-aryl-HO-pyrrolidine-2-oxo-) glucuronide	452	191 (70), 367 (100)	191: 81 (6), 123 (100)	10.6

Concerning *O*-demethylation, CYP 2C9, 2B6, and CYP 2D6 were involved. As most involved enzymes were polymorphically expressed, pharmacogenomic variations might occur, but clinical data are needed for further studies.

Toxicological Detection by GC-MS, LC-MS^n, and LC-HR-MS/MS

To test for toxicological detectability after common users' doses of approximately 10 mg (https://www.erowid.org, http://bluelight.org), rat urines were screened after administration of compound doses scaled by the dose-by-factor approach of Sharma and McNeill [13] *via* GC-MS, LC-MS^n, and LC-HR-MS/MS SUSA. The detected targets for both compounds are given in Tables **6-8**.

For 3-MeO-PCP, detection was possible *via* the parent compound (no. 1) and metabolites nos. 2 and 3 by GC-MS, *via* metabolites nos. 8, 13, 57, 58, and 61 by LC-MS^n, and nos. 2, 3, 8, 13, 24, 57, 58, 59, and 61 by LC-HR-MS/MS. Administration of 3-MeO-PCPy could be monitored *via* the metabolites no. 32 by GC-MS, nos. 32, 38, 39, 64, and 67 by LC-MS^n, and nos. 32, 38, 39, 45, 64, 66, and 67 by LC-HR-MS/MS. The reason why not all types of metabolites were found for both drugs could be explained by different formation rates, influence of ion suppression, chromato-

Table 8. Proposed targets for LC-HR-MS/MS SUSA monitoring 3-MeO-PCP or 3-MeO-PCPy, with protonated precursor ions, characteristic MS^2 fragment ions, and retention times (RT). The numbers correspond to those in Figs. (2 and 3).

No.	Target for SUSA	Precursor Ions (*m/z*)	MS^2 Fragment Ions (*m/z*) and Relative Intensity (%)	RT (min)
2	3-MeO-PCP-M (*O*-demethyl-)	260.2014	81.0701 (15), 86.0966 (100), 107.0490 (73), 175.1111 (37), 260.2001 (4)	4.4
3	3-MeO-PCP-M (*O*-demethyl-piperidine-HO-)	276.1964	81.0701 (20), 84.0810 (18), 102.0913 (99), 107.0490 (100), 175.1111 (54)	4.0
8	3-MeO-PCP-M (*O*-demethyl-piperidine-di-HO-)	292.1913	79.0545 (16), 84.0810 (18), 102.0914 (100), 107.0491 (41), 173.0954 (58)	3.1
13	3-MeO-PCP-M (piperidine-di-HO-)	306.2069	81.0701 (23), 101.0597 (14), 118.0860 (25), 121.0645 (100), 189.1266 (48)	5.1
24	3-MeO-PCP-M (cyclohexyl-HO-piperidine-di-HO-)	322.2018	79.0545 (41.88), 101.0597 (34), 118.0861 (66), 121.0645 (68), 187.1111 (100)	4.3
57	3-MeO-PCP-M (*O*-demethyl-) glucuronide	436.2335	81.0702 (10), 86.0966 (100), 107.0491 (23), 141.0176 (9), 175.1112 (22)	4.0
58	3-MeO-PCP-M (*O*-demethyl-aryl-HO-) glucuronide	452.2284	81.0701 (8), 86.0966 (55), 123.0438 (100), 191.1059 (76), 367.1369 (3)	3.8
59	3-MeO-PCP-M (*O*-demethyl-piperidine-HO-) glucuronide	452.2284	84.0810 (56), 102.0914 (52), 107.0491 (90), 175.1111 (37), 278.1225 (100)	4.0
61	3-MeO-PCP-M (piperidine-HO-) glucuronide	466.2441	84.0810 (45), 102.0914 (38), 121.0646 (100), 189.1268 (41), 278.1225 (79)	4.7
32	3-MeO-PCPy-M (*O*-demethyl-)	246.1858	72.0812 (100), 81.0702 (16), 107.0491 (86), 175.1112 (45), 246.1844 (4)	4.3
38	3-MeO-PCPy-M (*O*-demethyl-pyrrolidine-di-HO-)	278.1756	70.0656 (14), 79.0546 (15), 88.0759 (100), 107.0491 (38), 173.0956 (64)	3.1
39	3-MeO-PCPy-M (pyrrolidine-di-HO-)	292.1913	81.0702 (24), 87.0443 (9), 104.0706 (18), 121.0646 (100), 189.1268 (46)	5.0
45	3-MeO-PCPy-M (cyclohexyl-HO-pyrrolidine-di-HO-)	308.1862	79.0545 (42), 87.0443 (29), 104.0706 (57), 121.0646 (66), 187.1111 (100)	4.1
64	3-MeO-PCPy-M (*O*-demethyl-) glucuronide	422.2179	72.0812 (100), 81.0701 (11), 107.0491 (27), 175.1112 (26), 422.2160 (13)	3.7
66	3-MeO-PCPy-M (*O*-demethyl-aryl-HO-) glucuronide	438.2128	72.0812 (43), 81.0701 (8), 123.0438 (100), 191.1059 (78), 367.1375 (3)	3.5
67	3-MeO-PCPy-M (*O*-demethyl-aryl-HO-pyrrolidine-2-oxo-) glucuronide	452.1920	81.0702 (8), 86.0603 (22), 123.0439 (100), 149,0592 (9), 191.1060 (78)	6.2

graphic and/or ionization properties. The risk of overlooking a drug consumption caused by ion suppression could be minimized by screening for several targets *e.g.* metabolites.

For general performance of the SUSAs, increasing concentrations of the parent drugs were analyzed although they were not the main targets chosen for urine analysis. The LODs were determined at a signal-to-noise ratio of 3. In GC-MS, LC-MS^n, and LC-HR-MS/MS, the LODs were 5, 10, and 0.1 ng/mL for 3-MeO-PCP and 10, 10, and 0.1 ng/mL for 3-MeO-PCPy, respectively.

CONCLUSION

The PCP analogues 3-MeO-PCP and 3-MeO-PCPy were extensively metabolized in rats *via* aliphatic and aromatic hydroxylation, carboxylation after ring opening, *O*-demethylation, and glucuronidation. The initial steps could be confirmed by detection of the corresponding metabolites in pHLM incubations. The CYP enzymes involved in the metabolism of both compounds were CYP 2B6 and CYP 2D6. In addition, CYP 2C19 was involved in 3-MeO-PCP *O*-demethylation and piperidine-hydroxylation whereas CYP 2C9 in 3-MeO-PCPy *O*-demethylation. As only polymorphically expressed enzymes were involved, pharmacogenomic variations might occur, but clinical data are needed to confirm the relevance. Detectability studies showed that all tested SUSAs were able to monitor consumptions of both drugs considering that metabolites were the main targets.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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