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# Identification of monoamine oxidase and cytochrome P450 isoenzymes involved in the deamination of phenethylamine-derived designer drugs (2C-series)

*Denis S. Theobald, Hans H. Maurer* *

*Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, University of Saarland, D-66421 Homburg (Saar), Germany*

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ABSTRACT

In recent years, several compounds of the phenethylamine-type (2C-series) have entered the illicit drug market as designer drugs. In former studies, the qualitative metabolism of frequently abused 2Cs (2C-B, 2C-I, 2C-D, 2C-E, 2C-T-2, 2C-T-7) was studied using a rat model. Major phase I metabolic steps were deamination and *O*-demethylation. Deamination to the corresponding aldehyde was the reaction, which was observed for all studied compounds. Such reactions could in principal be catalyzed by two enzyme systems: monoamine oxidase (MAO) and cytochrome P450 (CYP). The aim of this study was to determine the human MAO and CYP isoenzymes involved in this major metabolic step and to measure the Michaelis–Menten kinetics of the deamination reactions. For these studies, cDNA-expressed CYPs and MAOs were used. The formation of the aldehyde metabolite was measured using GC–MS after extraction. For all compounds studied, MAO-A and MAO-B were the major enzymes involved in the deamination. For 2C-D, 2C-E, 2C-T-2 and 2C-T-7, CYP2D6 was also involved, but only to a very small extent. Because of the isoenzymes involved, the 2Cs are likely to be susceptible for drug–drug interactions with MAO inhibitors.



## 1. Introduction

The members of the so-called 2C-series belong to a class of designer drugs that are all phenethylamine derivatives. Their chemical structures comprise a primary amine functionality separated from the phenyl ring by two carbon atoms (“2C”), the presence of methoxy groups in positions 2 and 5 of the aromatic ring, and a lipophilic substituent in position 4 of the aromatic ring (alkyl, halogen, alkylthio, etc.) [1]. Typical 2Cs are 4-bromo-2,5-dimethoxy-β-phenethylamine (2C-B), 4-iodo-2,5-dimethoxy-β-phenethylamine (2C-I), 2,5-dimethoxy-4-methyl-β-phenethylamine (2C-D), 4-ethyl-2,5-dimethoxy-β-phenethylamine (2C-E), 4-ethylthio-2,5-dimethoxy-β-phenethylamine (2C-T-2), and 2,5-dimethoxy-4-propylthio-β-

* *Corresponding author*. Tel.: +49 6841 1626050; fax: +49 6841 1626051.
E-mail address: hans.maurer@uniklinikum-saarland.de (H.H. Maurer).

*Abbreviations:* *2C-B, 4-bromo-2,5-dimethoxy-β-phenethylamine; 2C-I, 4-iodo-2,5-dimethoxy-β-phenethylamine; 2C-D, 2,5-dimethoxy-4-methyl-β-phenethylamine; 2C-E, 4-ethyl-2,5-dimethoxy-β-phenethylamine; 2C-T-2, 4-ethylthio-2,5-dimethoxy-β-phenethylamine; 2C-T-7, 2,5-dimethoxy-4-propylthio-β-phenethylamine; 5-HT, 5-hydroxy tryptamine (serotonin); MAO, monoamine oxidase; CYP, cytochrome P450; $K_m$, Michaelis–Menten constant; $V_{max}$, maximal turnover rate; PAR, peak area ratio; SIM, selected-ion monitoring; EI, electron ionization; IS, internal standard; LC–MS, liquid chromatography–mass spectrometry; APCI, atmospheric pressure chemical ionization; HPLC-UV, high performance liquid chromatography ultra violet detection*

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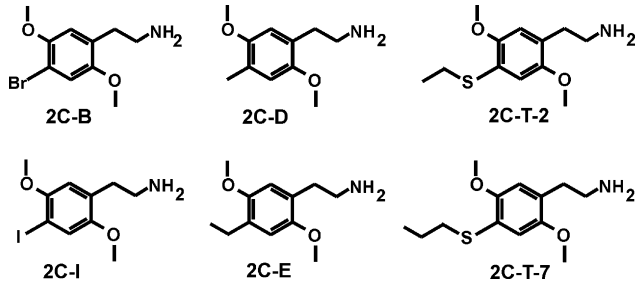


**Fig. 1 – Chemical structures of the studied members of the 2C-series.**

phenethylamine (2C-T-7) [2–5]. Their chemical structures are depicted in Fig. 1.

Most of known members of the 2C-series were synthesized and described by Shulgin during the 1970s and 1980s [1]. Since the 1990s, they have entered the illicit drug market as recreational drugs [3]. Later the 2Cs were sold in so-called ''smart shops'' and were mentioned in scene books and on so-called drug information web sites (http://www.erowid.org, http://www.lycaeum.org June 2006) [3]. Furthermore, seizures by the police of tablets containing 2Cs or combinations of them with other drugs were reported in recent years [6–11]. As a consequence, several 2Cs have been scheduled in many countries [12–14].

Only little information is available on pharmacological properties of the 2Cs, but it is known, that the compounds of the 2C-series show affinity to 5-$HT_2$ receptors, acting as agonists or antagonists at different receptor subtypes [15–23]. For 2C-B, partial agonism at the $\alpha_1$-adrenergic receptor was described [24,25]. Little is known about the toxicology of these compounds, but at least for 2C-T-7 fatal intoxications have been reported during 2000/2001 [4,12,26].

In recent studies, the metabolism of several 2Cs was studied mainly in rats [27–33], but also in humans [34], mice [35], and hepatocytes of different species [36,37]. One major metabolic step was the deamination of the parent compound to the corresponding aldehyde. These aldehydes could not be detected in urine, most probably because they were rapidly reduced or oxidized to the respective alcohols and carboxylic acids, which were present in urine.

The involvement of particular isoenzymes in the biotransformation of a new therapeutic drug has to be thoroughly investigated before it can be marketed. Such investigations allow to predict possible drug–drug-interactions, inter-individual variations in pharmacokinetic profiles and increased appearance of side effects and serious poisonings [38]. Such risk assessment is typically performed for substances intended for therapeutic use, but not for drugs of the illicit market. In addition, there is good evidence that genetic variations in drug metabolism have important behavioral consequences that can alter the risk of drug abuse and dependence [39].

Regarding the above mentioned deamination reaction, isoenzymes of the monoamine oxidase (MAO) and cytochrome P450 (CYP) type might be able to catalyze this reaction. MAO enzymes A and B are outer mitochondrial membrane-bound flavoenzymes that can be found mainly in neuronal and glia cells, but also in the liver. They catalyze the oxidation of primary, secondary, and some tertiary amines to their corresponding protonated imines with further non-enzymatic hydrolysis of the imine products to the corresponding aldehyde [40]. Their physiological substrates are neurotransmitters such as dopamine or noradrenaline, which show structural similarity to the 2Cs [41]. Consistently, phenethylamine is a specific substrate for MAO-B [41]. CYP enzymes are located in membranes, mainly the endoplasmic reticulum, and can be found mainly in the liver. They are also able to catalyze deamination via oxidation of the $\alpha$-carbon atom next to the nitrogen [42].

Therefore, isoenzymes of the MAO- and CYP-type were studied concerning their ability to catalyze deamination of the 2Cs. Furthermore, the enzyme kinetics of these reactions was measured and the kinetic data like Michaelis–Menten constants ($K_m$) and the maximal turnover rates ($V_{max}$) were determined.

## 2. Materials and methods

### 2.1. Materials

For research purposes, hydrochlorides of 2C-D and 2C-E were provided by Dejachem (Schwendi, Germany), 2C-B tartrate by Hessisches Landeskriminalamt (Wiesbaden, Germany), 2C-I hydrochloride by Landeskriminalamt Baden-Württemberg (Stuttgart, Germany), 2C-T-2 hydrochloride by Bundeskriminalamt (Wiesbaden, Germany), and 2C-T-7 hydrochloride by Bayerisches Landeskriminalamt (Munich, Germany).

$NADP^+$ was obtained from Biomol, isocitrate and isocitrate dehydrogenase from Sigma, all other chemicals and reagents from Merck. The following microsomes were from Gentest and delivered by NatuTec: baculovirus-infected insect cell microsomes containing 1 nmol/mL human cDNA-expressed CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 (supersomes), baculovirus-infected insect cell microsomes containing 5 mg/mL human cDNA-expressed MAO-A or MAO-B (supersomes), wild-type baculovirus-infected insect cell microsomes (control supersomes). After delivery, the microsomes were thawed at 37 °C, aliquoted, snap-frozen in liquid nitrogen and stored at −80 °C until use.

### 2.2. Microsomal incubations

For the CYP enzymes, typical incubation mixtures (final volume: 50 μL) consisted of 90 mM phosphate buffer (pH 7.4), 5 mM $Mg^{2+}$, 5 mM isocitrate, 1.2 mM $NADP^+$, 2 U/mL isocitrate dehydrogenase, 200 U/mL superoxide dismutase, and various concentrations of substrate at 37 °C. For the MAO enzymes, typical incubation mixtures (final volume: 50 μL) consisted of 100 mM phosphate buffer (pH 7.4), and various concentrations of substrate at 37 °C. The substrate was added after dilution of a 25 mM aqueous stock solution in buffer. Reactions were started by addition of the ice-cold microsomes and terminated with 5 μL of perchloric acid 60% (w/w).