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2,5-Dimethoxyamphetamine-derived designer drugs: Studies on the identification of cytochrome P450 (CYP) isoenzymes involved in formation of their main metabolites and on their capability to inhibit CYP2D6

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ABSTRACT

The designer drugs 4-methyl-2,5-dimethoxy-amphetamine (DOM), 4-iodo-2,5-dimethoxy-amphetamine (DOI), 4-chloro-2,5-dimethoxy-amphetamine (DOC), 4-bromo-2,5-dimethoxy-amphetamine (DOB), 4-bromo-2,5-dimethoxy-methamphetamine (MDOB), and 2,4,5-trimethoxy-amphetamine (TMA-2) are potent serotonin $5HT_2$ receptor agonists and have appeared on the illicit drug market. These drugs are mainly metabolized by *O*-demethylation or in case of DOM by hydroxylation of the methyl moiety. In an initial activity screening using microsomes of insect cells heterologously expressing human CYPs, CYP2D6 was found to be the only CYP isoenzyme involved in the above-mentioned main metabolic steps whereas the amounts of metabolites formed were very small. As inhibition of CYP2D6 by other amphetamines had been described, the inhibitory effects of the 2,5-dimethoxyamphetamine derivatives were studied using insect cell microsomes with heterologously expressed human CYP2D6 and pooled human liver microsomes (HLM) as enzyme sources and dextromethorphan *O*-demethylation as probe reaction. All studied drugs were observed to be non-mechanism-based competitive inhibitors of CYP2D6 with inhibition constants (K_i) from 7.1 to 296 μM using recombinant CYP2D6 and 2.7–19.9 μM using HLM. For comparison, the K_i values for quinidine and fluoxetine were 0.0092 and 8.2 μM using recombinant CYP2D6 and 0.019 and 0.93 μM using HLM. As the K_i values of the drugs were much higher than that of quinidine and, with the exception of DOI, higher than that of fluoxetine, interactions with other CYP2D6 substrates are possible but rather unlikely.

1. Introduction

The designer drugs 4-methyl-2,5-dimethoxy-amphetamine (DOM), 4-iodo-2,5-dimethoxy-amphetamine (DOI), 4-chloro-2,5-dimethoxy-amphetamine (DOC), 4-bromo-2,5-dimethoxy-amphetamine (DOB), 4-bromo-2,5-dimethoxy-methamphetamine (MDOB), and 2,4,5-trimethoxy-amphetamine (TMA-2) are upcoming substances on the illicit drug market (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 2003; Balikova, 2005; DEAOFS, 2006a,b). These drugs are serotonin $5HT_2$ receptor agonists and have psychoactive properties (Shulgin, 1991). Studies on structure–activity relationship revealed that the hallucinogen-like activity can be attributed to the primary amine function separated from the phenyl ring by two carbon atoms, the presence of methoxy groups in position 2 and 5, and a hydrophobic 4-substituent, especially a halogen atom (Monte et al., 1996; Neuvonen et al., 2006). The methyl moiety in α-position to the nitrogen is reported to be responsible for increased *in vivo* potency and duration of action compared to β-phenethylamines, so-called 2Cs (Monte et al., 1996). Several metabolism studies showed that the studied drugs were mainly metabolized by *O*-demethylation or in case of DOM by hydroxylation of the 4-methyl moiety (Ewald et al., 2006a,b, 2007, 2008a,b; Balikova, 2005; Ho et al., 1971). Involvement of the different cytochrome P450 (CYP) isoenzymes in DOM hydroxylation has been published recently, but so far no information is available concerning involvement of CYP isoenzymes for the main metabolic steps of the other studied 2,5-dimethoxy-amphetamines. However, such studies are the first step for predicting possible risks of pharmacokinetic variations caused by pharmacogenetic variability or interactions with co-administered drugs or food ingredients (Evans and McLeod, 2003; Krishnan and Moncrief, 2007). If more than one isoenzyme is involved, their kinetics must be studied to assess their contribution to the hepatic clearance of the drugs. This is routinely

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performed for substances intended for therapeutic use before registration, but not for drugs of the illicit market. In addition, interactions with other drugs or food ingredients are possible by inhibitory effects on metabolizing enzymes such as described for related amphetamine analogs on CYP2D6. Although the studied drugs are not methylenedioxy amphetamine derivatives which are known to be mechanism-based inhibitors, the mode of inhibition should be studied. Therefore, the aim of the presented study was to investigate the involvement of relevant CYP isoenzymes on the main metabolic steps of the 2,5-dimethoxyamphetamine derivatives as described for DOM (Ewald et al., 2008b), as well as the possible inhibitory effects on recombinant CYP2D6 and pooled human liver microsomes (HLM) in comparison to the clinically relevant CYP2D6 inhibitors quinidine and fluoxetine.

2. Materials and methods

2.1. Chemicals and reagents

DOM was provided by the Bundeskriminalamt (Wiesbaden, Germany), DOI, DOB, and TMA-2 were provided by the Hessisches Landeskriminalamt (Wiesbaden, Germany), DOC by cc chemical consulting (Berlin, Germany), and MDOB by the Landeskriminalamt Berlin (Berlin, Germany) for research purposes. Dextromethorphan (DXM) was obtained from Roche (Grenzach, Germany), dextrorphan (DXO) from MP Biochemicals (Heidelberg, Germany), isocitrate and isocitrate dehydrogenase from Sigma (Taufkirchen, Germany), 3,4-methylenedioxy-metamphetamine (MDMA) from Lipomed (Bad Säckingen, Germany), and $NADP^+$ from Biomol (Hamburg, Germany). All other chemicals and biochemicals used were obtained from Merck (Darmstadt, Germany) and were of analytical grade. The HLM (20 mg/mL protein content) and baculovirus-infected insect cell microsomes containing 1 nmol/mL human cDNA-expressed CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and 2 nmol/mL of CYP2E1 (Supersomes®) were from Gentest and delivered by NatuTec (Frankfurt/Main, Germany). Upon delivery, the microsomes were thawed at 37 °C, aliquoted, shock-frozen in liquid nitrogen and stored at −80 °C until use.

2.2. Microsomal incubations and workup for initial activity screening

Incubation mixtures (final volume: 50 μL, $n = 2$) consisted of 90 mM phosphate buffer (pH 7.4; for CYP1A2, CYP2B6, CYP2C8, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) or Tris buffer (pH 7.4; for CYP2A6 and CYP2C9), 5 mM Mg^{2+}, 5 mM isocitrate, 1.2 mM $NADP^+$, 0.5 U/mL isocitrate dehydrogenase, 200 U/mL superoxide dismutase and substrate at 37 °C. The substrates were added after dilution of 250 mM methanolic stock solutions in respective buffer (final concentration: 250 μM). In none of the samples, the methanol concentration exceeded 0.4%. Reactions were started by adding the ice-cold microsomes (final concentration: 50 pmol/mL) and terminated with 5 μL of 60% (w/w) aqueous $HClO_4$ after 30 min incubation time. After termination, the samples were extracted by liquid–liquid extraction with 1 mL of dichloromethane–isopropanol–ethyl acetate (1:1:3; v/v/v) at pH 8–9. The organic layer was transferred into glass flasks and evaporated under reduced pressure at 70 °C to dryness, acetylated with 20 μL of an acetic anhydride–pyridine mixture (3:2; v/v) for 2 min under microwave irradiation at about 440 W. After evaporation of the derivatization mixture under reduced pressure at 70 °C, the residue was dissolved in 20 μL of methanol and 2 μL were injected into the gas chromatography–mass spectrometry (GC–MS) system. Previous metabolism studies revealed good suitability of this analytical procedure for identification of the respective main metabolites and possible other metabolites (Ewald et al., 2006a,b, 2007, 2008a,b).

2.3. Microsomal incubations for inhibition studies

Incubation mixtures (final volume: 50 μL, $n = 2$) consisted of 90 mM phosphate buffer (pH 7.4), 5 mM Mg^{2+}, 5 mM isocitrate, 1.2 mM $NADP^+$, 0.5 U/mL isocitrate dehydrogenase, 200 U/mL superoxide dismutase and substrate DXM (final concentration: 0.5, 1, 2, 5, 10, 20, 50, 150, 500, 1000, or 1500 μM) in presence or absence of the potential inhibitor (DOM, DOI, DOC, DOB, MDOB or TMA-2, respectively, final concentration: 25, 125, or 250 μM) or the well-known inhibitor MDMA, fluoxetine or quinidine, respectively (final concentration: 25, 125, or 250 μM for MDMA and fluoxetine and 0.025, 0.125, or 0.250 μM for quinidine) at 37 °C for 10 min. The substrate and the corresponding inhibitor were added after dilution of 250 mM methanolic stock solutions in buffer. In none of the samples, the methanol concentration exceeded 0.4%. Reactions were started by adding the ice-cold microsomes and terminated with 50 μL of acetonitrile. Also preincubation studies were performed in a similar manner as described above with the difference that the reaction was started with DXM (final concentration: 25 or 250 μM) in fresh incubation mixture resulting in a 1:1 dilution after 10, 20 or 30 min preincubation of the mixture with or without the respective inhibitor. After termination, 5 μL of a 25-μM codeine solution were added as internal standard. The samples were centrifuged and the supernatants were transferred to autosampler vials and analyzed using liquid chromatography–mass spectrometry (LC–MS).

2.4. Gas chromatography–mass spectrometry

DOM, DOI, DOC, DOB, MDOB, and TMA-2 and their metabolites were analyzed in derivatized extracts (for details see refs. (Ewald et al., 2006a,b, 2007, 2008a,b) using a Hewlett Packard (Agilent, Waldbronn, Germany) 5890 Series II gas chromatograph combined with an HP 5989B MS Engine mass spectrometer and an HP MS ChemStation (DOS series) with HP G1034C software. The GC conditions were as follows: splitless injection mode; column, HP-1 capillary (12 m × 0.2 mm ID), cross linked methyl silicone, 330 nm film thickness; injection port temperature, 280 °C; carrier gas, helium; flow-rate 1 mL/min; column temperature, programmed from 100 to 310 °C at 30 °/min, initial time 3 min, final time 8 min. The MS conditions were as follows: full scan mode, m/z 50–550 μM; EI mode: ionization energy, 70 eV; ion source temperature, 220 °C; capillary direct interface heated at 260 °C.

2.5. Liquid chromatography–mass spectrometry

LC–MS was used for sensitive quantitation without extraction and derivatization steps. DXM, DXO, the designer drugs, and the internal standard codeine were separated and quantified using an Agilent Technologies (AT, Waldbronn, Germany) AT 1100 series atmospheric pressure chemical ionization (APCI) electrospray LC–MSD, SL version and an LC–MSD ChemStation using the A.08.03 software.

2.5.1. LC conditions

Isocratic elution was achieved on an Alltech mixed mode RP-C18/cation column (150 mm × 4.6 mm, 5 μm, Rottenburg-Hailfingen, Germany) and a Wicom cation optiguard column (Heppenheim, Germany). The mobile phase consisted of ammonium formate (50 mM, adjusted to pH 3.5 with formic acid, eluent A) and acetonitrile (eluent B). The mixture of the mobile phase consisted of 45% eluent A and 55% eluent B. The flow rate was 1.0 mL/min and the injection volume was 5 μL.

2.5.2. Electrospray conditions

The following APCI inlet conditions were applied: drying gas (7000 mL/min, 300 °C) and nebulizer pressure (25 psi; 172.3 kPa, both nitrogen); capillary voltage, 4000 V; drying gas temperature set at 300 °C, vaporizer temperature set at 400 °C; corona current was 5.0 μA; positive selected-ion monitoring (SIM) mode; fragmentor voltage 100 V.

2.5.3. MS conditions

For quantification, the following target ions (m/z) were used in the SIM mode: m/z 258 for DXO and m/z 300 for the internal standard codeine.

2.6. Inhibition studies

Duration and protein content for all incubations were in the linear range of metabolite formation (data not shown). The kinetic constants of DXM *O*-demethylation were derived from incubations ($n = 2$) with the following DXM concentrations, protein concentration and incubation time: 0.5, 1, 2, 5, 10, 20, 50, 150, 500, 1000, and 1500 μM DXM with 20 pmol/mL CYP2D6 or 0.8 mg/mL HLM for 10 min. Less than 20% of substrate was metabolized in all incubations. Michaelis–Menten constant (K_m) and maximum velocity (V_{max}) values for CYP2D6 were estimated by nonlinear regression according to the Michaelis–Menten equation using GraphPad Prism (San Diego, CA, USA) software, where V is the velocity at the substrate concentration $[s]$:

$$V = \frac{V_{max}[s]}{K_m + [s]} \quad (1)$$

To determine the inhibition mode of DOM, DOI, DOC, DOB, MDOB, and TMA-2, incubations were performed as mentioned above whereas parts of the phosphate buffer were replaced by solutions of inhibitors in phosphate buffer resulting in three different inhibitor concentrations ($[i] = 250$, 125 and 25 μM, each). The inhibition constants were calculated using apparent Michaelis–Menten constants ($K_{m,app}$) of DXM and the following rearranged equation from Kakkar et al. (1999):

$$K_i = \frac{K_m[i]}{K_{m,app} - K_m} \quad (2)$$

For determination of the inhibition constant of quinidine and fluoxetine phosphate buffer was replaced by the inhibitor leading to an inhibitor concentration of 0.025 and 125 μM, respectively.

3. Results

Among the nine CYP isoenzymes tested, only CYP2D6 was involved in the *O*-demethylation of DOI, DOC, DOB, MDOB, and

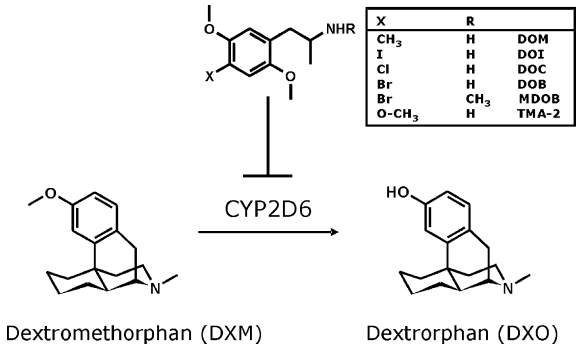

Fig. 1. Chemical structures of 2,5-dimethoxyamphetamine designer drugs acting as inhibitors of the specific CYP2D6 reaction DXM to DXO.

TMA-2. Only very small amounts of *O*-demethyl metabolites were formed and no further metabolites could be detected.

The reaction used for determination of the inhibition constants was the *O*-demethylation of the specific CYP2D6 substrate DXM to DXO in absence or presence of the inhibitors as shown in Fig. 1. The mass fragmentograms in Fig. 2 show that the applied LC–MS conditions provided sufficient separation of DXM, DXO, DOI, and codeine (IS). The other tested inhibitors were also separated from the analytes (data not shown). Matrix effect studies comparing the peak areas of DXO in neat standard solutions with those in spiked incubation mixtures containing the same concentrations of DXO gave no indication of ion suppression or enhancement for any of the compounds tested. The chosen target ions were selective for the analytes under these conditions as proven with blank samples (control microsomes without substrate and IS) and zero samples (control microsomes without substrate, but with IS; data not shown). DXM *O*-demethylation by CYP2D6 showed a typical hyperbolic metabolite formation profile, as shown in Fig. 3. The kinetic parameters for DXM *O*-demethylation were estimated to be 1.7 and 1.1 μM for K_m and 7.5 pmol/(min pmol) and 6.3 pmol/(min mg) for V_{max} in recombinant CYP2D6 (upper part) and HLM (lower part), respectively.

Fig. 4 shows Michaelis–Menten plots and the calculated corresponding apparent K_m values of the formation of DXO in presence of three different concentrations of DOI. Fig. 5 shows secondary plots of these three incubations with different concentrations of DOI and DXM according to Cornish-Bowden (1974). An intersection point of the three extrapolated lines in the upper plot (Dixon plot) and parallel lines in the lower plot revealed a competitive mode of inhibition. Competitive inhibition was also observed for the other tested drugs (data not shown). Fig. 6 shows plots of DXO formation against preincubation time in presence or absence of DOI. No differences in DXO formation depending on preincubation time could be observed also for the other tested drugs (data not shown). Fig. 7 shows plots of three different concentrations

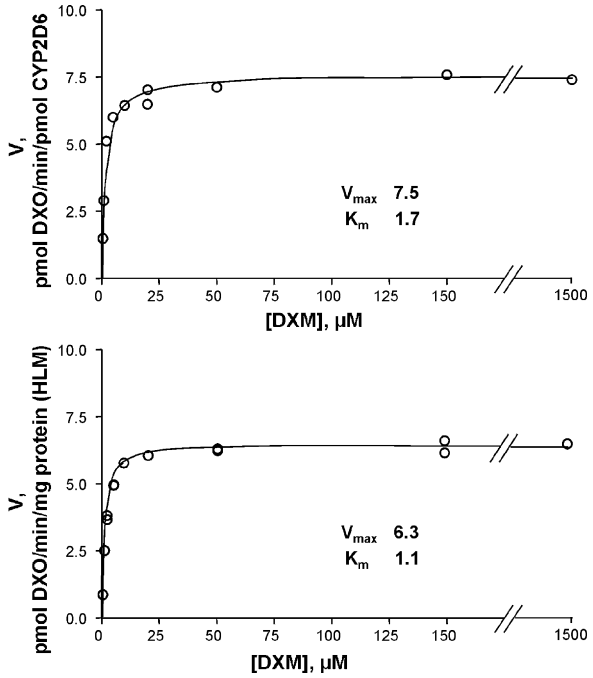

Fig. 3. Michaelis–Menten plot for *O*-demethylation of DXM by recombinant CYP2D6 (upper part) and HLM (lower part). The curves were fitted by nonlinear regression according to Eq. (1).

of each inhibitor against DXO formation with and without 10 min of preincubation. For positive control of mechanism-based inhibition MDMA and for negative control quinidine were used as inhibitors. No mechanism-based inhibition could be observed after preincubation with DOM, DOI, DOC, DOB, MDOB, and TMA-2. For calculation of the inhibition constants, Eq. (2) and the apparent K_m values obtained from the incubations with the three different inhibitor concentrations (Fig. 4) were used. Calculated K_i values using recombinant CYP2D6 and HLM are listed in Table 1. For comparison with clinically relevant CYP2D6 inhibitors, the inhibition constants of quinidine and fluoxetine were also determined using the same incubation model. The K_i values of quinidine and fluoxetine using recombinant CYP2D6 were 0.005 and 0.014 μM and 6.4 and 10 μM, respectively, and 0.014 and 0.024 μM and 0.6 and 1.2 μM, respectively, using HLM.

4. Discussion

One aim of the presented study was to identify the CYP isoenzymes involved in the main metabolic steps of the tested drugs. CYP2D6 was the only CYP catalyzing the *O*-demethylation of the tested drugs. However, even with the high protein concentrations

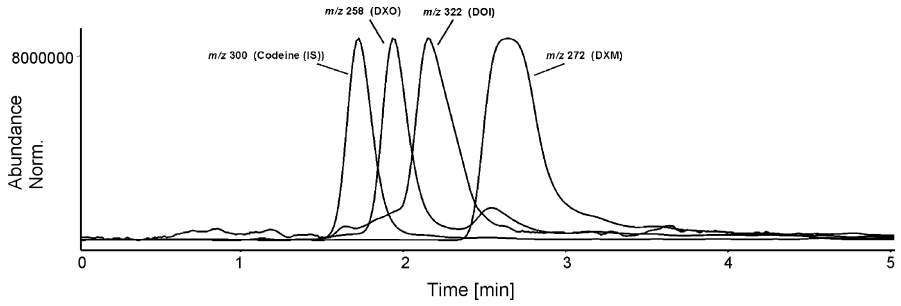

Fig. 2. Typical LC–MS mass fragmentograms of a supernatant of an incubation mixture containing DXM, DXO, DOI, and codeine (*m*/*z* 272, 258, 322, and 300).

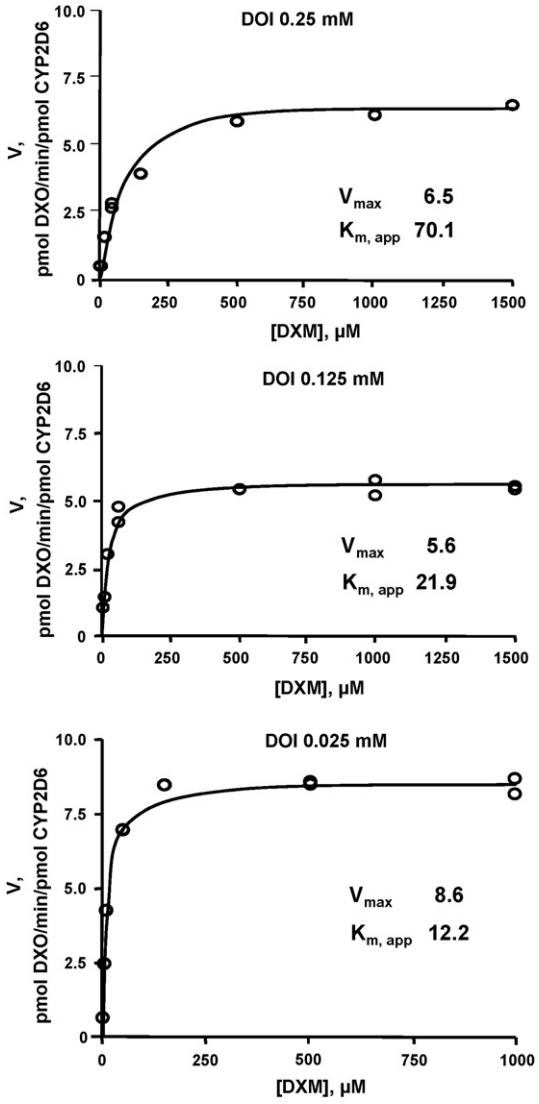

Fig. 4. Michaelis–Menten plots for *O*-demethylation of DXM by CYP2D6 in presence of three different concentrations of DOI for determination of apparent K_m.

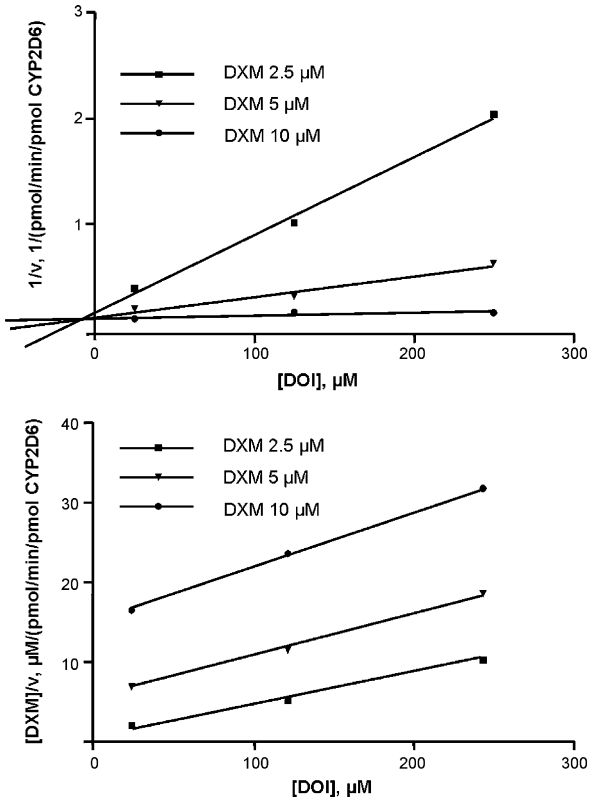

Fig. 5. Dixon plots (upper part) and other secondary plots of incubations with three different DOI concentrations for determination of the inhibition mode of DOI.

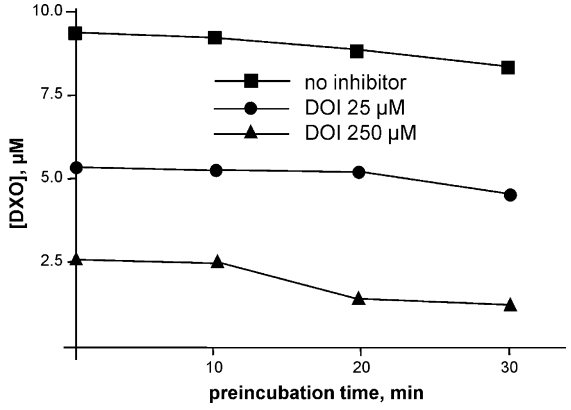

Fig. 6. DXO formation against preincubation time in presence or absence of DOI.

and long incubation times of the initial activity screening study, only very small amounts of *O*-demethyl metabolites were formed and no further metabolites could be detected. Hence, studies on enzyme kinetics were not possible. The differences in turnover rate compared to previous *in vivo* studies in rats (Ewald et al., 2006a,b, 2007, 2008a,b) may be caused by different exposure time of the drugs to the enzyme, the different amounts of enzyme in whole animal compared to *in vitro* incubations and the pos-

Table 1
Kinetic parameters for the inhibition of the formation of DXO from DXM by the designer drugs DOM, DOI, DOC, DOB, MDOB, TMA-2, and by the known CYP2D6 inhibitors quinidine and fluoxetine calculated using Eq. (2) ($n = 2$).

	Recombinant CYP2D6		HLM	
	K_i (µM)	V_{max} (pmol/(min pmol))	K_i (µM)	V_{max} (pmol/(min mg protein) (HLM)
DOI	4.0; 10	5.3; 8.7	2.0; 3.4	7.7; 8.5
MDOB	10; 13	5.7; 9.3	4.5; 10	4.9; 5.5
DOC	23; 31	5.5; 6.7	6.8; 12	5.2; 5.8
DOB	36; 58	5.2; 8.6	7.2; 23	7.8; 9.0
DOM	50; 140	5.9; 8.1	5.0; 7.6	6.1; 6.5
TMA-2	238; 354	6.1; 7.9	16; 24	6.1; 6.7
Quinidine	0.005; 0.014	6.2; 6.6	0.014; 0.024	5.5; 5.9
Fluoxetine	6.4; 10	5.8; 6.6	0.6; 1.2	4.5; 4.9

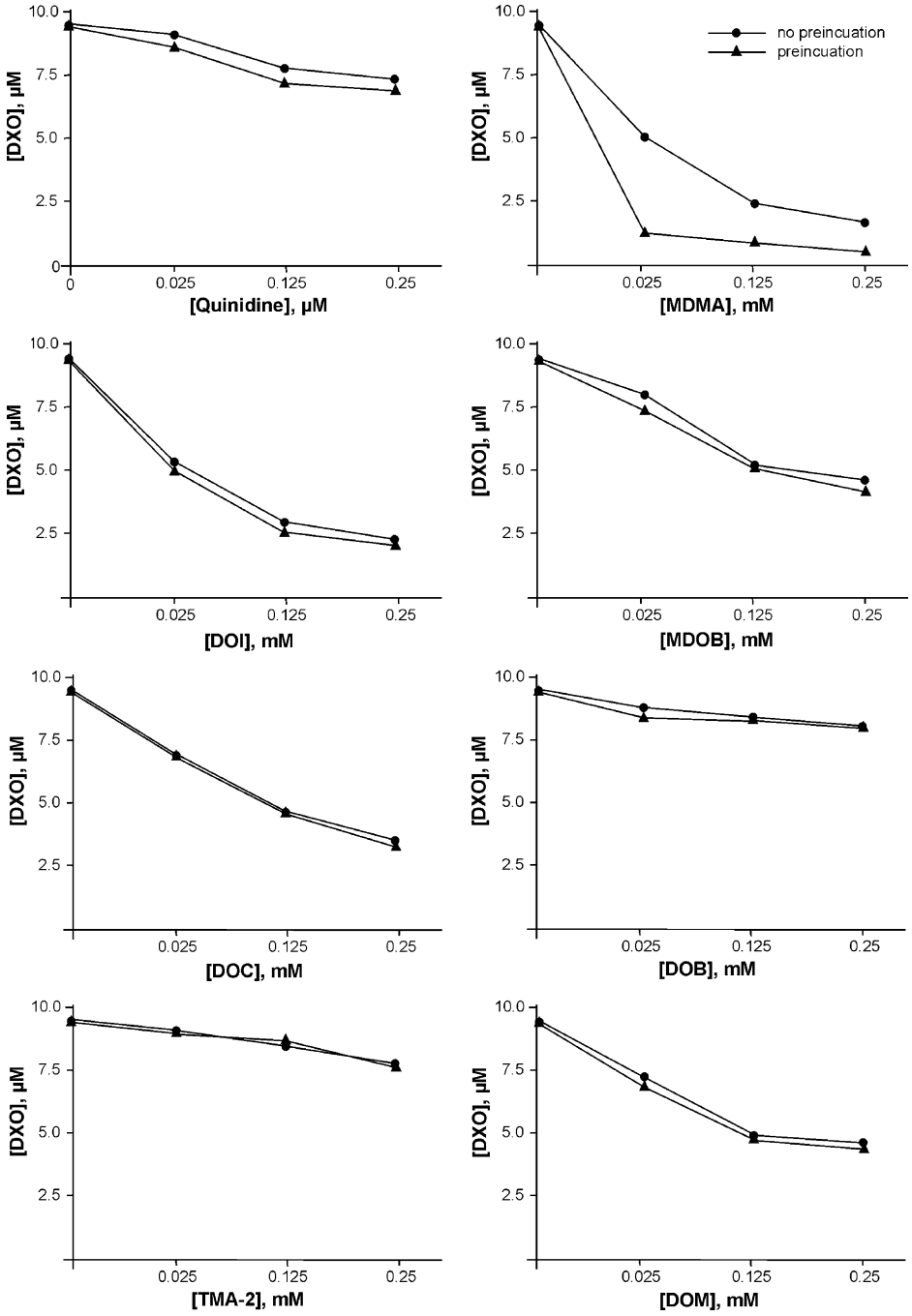

Fig. 7. DXO formation in absence and presence of the given inhibitors at three concentrations, each, after 10 min preincubation and without preincubation.

sibility of conjugation (phase II metabolism) in whole animals. Furthermore, interspecies differences in metabolic properties are a possible source of the different metabolite formation observed here.

The CYP2D6 specific DXM *O*-demethylation was chosen as marker reaction in the inhibition studies. The kinetic parameters of this reaction without inhibitor were similar to those reported by Walsky and Obach (2004). The inhibition constants of quinidine and fluoxetine determined in this study were in a similar range as described in the literature (Shen et al., 2007; Otton et al., 1993).

In accordance with other amphetamine derivatives (Wu et al., 1997; Pritzker et al., 2002), the tested 2,5-dimethoxyamphetamines were found to be competitive inhibitors of CYP2D6. Preincubation studies revealed that none of the tested drugs showed mechanism-based inhibition. This is in contrast to methylenedioxy amphetamine derivatives such as MDMA (Heydari et al., 2004; de la Torre et al., 2005; Yang et al., 2006; Ramamoorthy et al., 2002; Tucker et al., 1994), which inactivate CYPs, quasi-irreversibly, after oxidation of the methylene bridge to species that form a tight, but reversible complex with the heme iron atom (Correia and Ortiz-de-Montellano, 2005). Most probable due to the lack of such a bridge, the 2,5-dimethoxyamphetamine derivatives inhibit CYP2D6 in a different way.

The K_i values determined using HLM are generally lower, for DOM and TMA-2 more than ten times, than those determined using recombinant CYP2D6. Walsky and Obach (2004) described

similar differences for K_m values, but they could not find any reason for that. The K_i values were in the same range as for other amphetamines such as 2-methoxyamphetamine (K_i = 11.5 μM), and 4-methoxyamphetamine (K_i = 24 μM) determined using HLM (Wu et al., 1997). The K_i value of DOI was much higher than that of quinidine, but in the same range as that of fluoxetine so interactions with other CYP2D6 substrates cannot be excluded but are rather unlikely. Clinical studies would be necessary to predict relevant interactions.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

Balikova, M., 2005. Nonfatal and fatal DOB (2,5-dimethoxy-4-bromoamphetamine) overdose. Forensic Sci. Int. 153, 85–91.

Cornish-Bowden, A., 1974. A simple graphical method for determining the inhibition constants of mixed, uncompetitive and non-competitive inhibitors. Biochem. J. 137, 143–144.

Correia, M.A., Ortiz-de-Montellano, P.R., 2005. Inhibition of cytochrome P450 enzymes. In: Ortiz-de-Montellano, P.R. (Ed.), Cytochrome P450—Structure, Mechanism, and Biochemistry, 3rd ed. Kluwer Academic/Plenum Publishers, New York, pp. 247–322.

de la Torre, R., Farre, M., Mathuna, B.O., Roset, P.N., Pizarro, N., Segura, M., Torrens, M., Ortuno, J., Pujadas, M., Cami, J., 2005. MDMA (ecstasy) pharmacokinetics in a CYP2D6 poor metaboliser and in nine CYP2D6 extensive metabolisers. Eur. J. Clin. Pharmacol. 61, 551–554.

Drug Enforcement Administration—Office of Forensic Sciences, 2006. Intelligence alert large fentanyl/MDA/TMA laboratory in Azuza, California—possibly the "OC-80" tablet source. Microgram 39, 45–47.

Drug Enforcement Administration—Office of Forensic Sciences, 2006. Intelligence alert: 4-chloro-2,5-dimethoxyamphetamine (DOC) and 4-iodo-2,5-dimethoxyamphetamine (DOI) in Berkley, Michigan. Microgram 39, 137.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 2003. Report on the risk assessment of TMA-2 in the framework of the joint action on new synthetic drugs. http://www.emcdda.europa. eu/attachements. cfm/att_33358_EN_Risk7.pdf. Accessed August 5, 2008.

Evans, W.E., McLeod, H.L., 2003. Pharmacogenomics-drug disposition, drug targets, and side effects. N. Engl. J. Med. 348, 538–549.

Ewald, A.H., Ehlers, D., Maurer, H.H., 2008a. Metabolism and toxicological detection of the designer drug 4-chloro-2,5-dimethoxyamphetamine in rat urine using gas chromatography–mass spectrometry. Anal. Bioanal. Chem. 390, 1837–1842.

Ewald, A.H., Fritschi, G., Bork, W.R., Maurer, H.H., 2006a. Designer drugs 2,5-dimethoxy-4-bromoamphetamine (DOB) and 2,5-dimethoxy-4-bromomethamphetamine (MDOB): Studies on their metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric techniques. J. Mass Spectrom. 41, 487–498.

Ewald, A.H., Fritschi, G., Maurer, H.H., 2006b. Designer drug 2,4,5-trimethoxyamphetamine (TMA-2): Studies on its metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric techniques. J. Mass Spectrom. 41, 1140–1148.

Ewald, A.H., Fritschi, G., Maurer, H.H., 2007. Metabolism and toxicological detection of the designer drug 4-iodo-2,5-dimethoxy-amphetamine (DOI) in rat urine using gas chromatography–mass spectrometry. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 857, 170–174.

Ewald, A.H., Puetz, M., Maurer, H.H., 2008b. Designer drug 2,5-dimethoxy-4-methylamphetamine (DOM, STP): involvement of the cytochrome P450 isoenzymes in formation of its main metabolite and detection of the latter in rat urine as proof of a drug intake using gas chromatography–mass spectrometry. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 862, 252–256.

Heydari, A., Yeo, K.R., Lennard, M.S., Ellis, S.W., Tucker, G.T., Rostami-Hodjegan, A., 2004. Mechanism-based inactivation of CYP2D6 by methylenedioxymethamphetamine. Drug Metab. Dispos. 32, 1213–1217.

Ho, B.T., Estevez, V., Tansey, L.W., Englert, L.F., Creaven, P.J., McIsaac, W.M., 1971. Analogs of amphetamine. 5. Studies of excretory metabolites of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) in rats. J. Med. Chem. 14, 158–160.

Kakkar, T., Boxenbaum, H., Mayersohn, M., 1999. Estimation of K_i in a competitive enzyme-inhibition model: comparisons among three methods of data analysis. Drug Metab. Dispos. 27, 756–762.

Krishnan, S., Moncrief, S., 2007. An evaluation of the cytochrome p450 inhibition potential of lisdexamfetamine in human liver microsomes. Drug Metab. Dispos. 35, 180–184.

Monte, A.P., Marona-Lewicka, D., Parker, M.A., Wainscott, D.B., Nelson, D.L., Nichols, D.E., 1996. Dihydrobenzofuran analogues of hallucinogens. 3. Models of 4-substituted (2,5-dimethoxyphenyl)alkylamine derivatives with rigidified methoxy groups. J. Med. Chem. 39, 2953–2961.

Neuvonen, K., Neuvonen, H., Fulop, F., 2006. Effect of 4-substitution on psychotomimetic activity of 2,5-dimethoxy amphetamines as studied by means of different substituent parameter scales. Bioorg. Med. Chem. Lett. 16, 3495–3498.

Otton, S.V., Wu, D., Joffe, R.T., Cheung, S.W., Sellers, E.M., 1993. Inhibition by fluoxetine of cytochrome P450 2D6 activity. Clin. Pharmacol. Ther. 53, 401–409.

Pritzker, D., Kanungo, A., Kilicarslan, T., Tyndale, R.F., Sellers, E.M., 2002. Designer drugs that are potent inhibitors of CYP2D6. J. Clin. Psychopharmacol. 22, 330–332.

Ramamoorthy, Y., Yu, A.M., Suh, N., Haining, R.L., Tyndale, R.F., Sellers, E.M., 2002. Reduced(±)-3,4-methylenedioxymethamphetamine ("Ecstasy") metabolism with cytochrome P450 2D6 inhibitors and pharmacogenetic variants in vitro. Biochem. Pharmacol. 63, 2111–2119.

Shen, H., He, M.M., Liu, H., Wrighton, S.A., Wang, L., Guo, B., Li, C., 2007. Comparative metabolic capabilities and inhibitory profiles of CYP2D6.1, CYP2D6.10, and CYP2D6.17. Drug Metab. Dispos. 35, 1292–1300.

Shulgin, A., 1991. Pihkal, A Chemical Love Story, 1st ed. Transform Press, Berkley (CA).

Tucker, G.T., Lennard, M.S., Ellis, S.W., Woods, H.F., Cho, A.K., Lin, L.Y., Hiratsuka, A., Schmitz, D.A., Chu, T.Y., 1994. The demethylenation of methylenedioxymethamphetamine ("ecstasy") by debrisoquine hydroxylase (CYP2D6). Biochem. Pharmacol. 47, 1151–1156.

Walsky, R.L., Obach, R.S., 2004. Validated assays for human cytochrome P450 activities. Drug Metab. Dispos. 32, 647–660.

Wu, D., Otton, S.V., Inaba, T., Kalow, W., Sellers, E.M., 1997. Interactions of amphetamine analogs with human liver CYP2D6. Biochem. Pharmacol. 53, 1605–1612.

Yang, J., Jamei, M., Heydari, A., Yeo, K.R., de la Torre, R., Farre, M., Tucker, G.T., Rostami-Hodjegan, A., 2006. Implications of mechanism-based inhibition of CYP2D6 for the pharmacokinetics and toxicity of MDMA. J. Psychopharmacol. 20, 842–849.