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Cytochrome P450 inhibition potential of new psychoactive substances of the tryptamine class

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HIGHLIGHTS

- All tested tryptamines inhibited at least one isoenzyme.
- IC_{50} values were comparable to that of clinically relevant inhibitors.
- Members of the DALT family were strong inhibitors of CYP1A2 and CYP2D6 activity.
- 5-MeO-DALT showed in vivo inhibition against CYP1A2 activity.

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ABSTRACT

New psychoactive substances (NPS) are not tested for their cytochrome P450 (CYP) inhibition potential before consumption. Therefore, this potential was explored for tryptamine-derived NPS (TDNPS) including alpha-methyl tryptamines (AMTs), dimethyl tryptamines (DMTs), diallyl tryptamines (DALTs), and diisopropyl tryptamines (DiPTs) using test substrates preferred by the Food and Drug Administration in a cocktail assay. All tested TDNPS with the exception of DMT inhibited CYP2D6 activity with IC_{50} values below 100 μM. DALTs inhibited CYP2D6 activity similar to paroxetine and quinidine and CYP1A2 activity comparable to fluvoxamine. 5-Methoxy-*N*,*N*-diallyltryptamine reduced in vivo the caffeine metabolism in rats consistent with in vitro results. Five of the AMTs also inhibited CYP1A2 activity comparable to amiodarone. AMT and 6-F-AMT inhibited CYP2A6 activity in the range of the test inhibitor tranylcypromine. CYP2B6 activity was inhibited by 19 tryptamines, but weakly compared to efavirenz. CYP2C8 activity was inhibited by five of the tested TDNPS and three showed values comparable to trimethoprim and gemfibrozil. Six tryptamines inhibited CYP2C9 and seven CYP2C19 activities comparable to fluconazole and chloramphenicol, respectively. Nineteen compounds showed inhibition of CYP2E1 and 18 of CYP3A activity, respectively. These results showed that the CYP inhibition by TDNPS might be clinically relevant, but clinical studies are needed to explore this further.

1. Introduction

The class of *N*,*N*-dialkylated tryptamines has been a constant element of interest within the context of new psychoactive substances (NPS) and this was reflected by notifications to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA, 2015)). Examples of tryptamine-derived NPS (TDNPS) include a number of alpha-methyl tryptamines (AMTs), dimethyl tryptamines (DMTs), diallyl tryptamines (DALTs), and diisopropyl tryptamines (DiPTs). The recently published United Nations Office on Drugs and Crime World Drug Report (United Nations Office on Drugs and Crime (UNODC, 2014)) indicated that up to July 2012 twenty-five new tryptamines were reported by Member States (United Nations Office on Drugs and Crime (UNODC, 2014). The control status varies across the globe but most of them are not yet placed under legislative control. Case reports on intoxications associated with 5-methoxy-*N*,*N*-diisopropyl tryptamine (5-MeO-DiPT) were reported since the 2000s

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(Meatherall and Sharma, 2003; Smolinske et al., 2005; Tanaka et al., 2006; Vorce and Sklerov, 2004; Wilson et al., 2005). Another newer tryptamine, 5-methoxy-*N*,*N*-diallyltryptamine (5-MeO-DALT), was associated with a fatal poisoning (Elliott and Evans, 2014).

In contrast to medicinal products with marketing authorization (http://medicine.iupui.edu/clinpharm/ddis/main-table/), drugs of abuse (DOA) and most NPS, are marketed and consumed without availability of information regarding their pharmacological and toxicological properties (Brandt et al., 2014). One important part of safety testing is the evaluation of cytochrome P450 (CYP) inhibition in order to assess the potential for interactions when more than one drug (of abuse) is taken. Inhibition can lead to prolonged plasma elimination half-life and increased bioavailability, which can lead to increased risks of developing adverse reactions. They can cause increased number and/or duration of hospitalization (Palleria et al., 2013). A systematic screening of methylenedioxy-derived designer drugs showed that these drugs are inhibitors of the main CYP isoenzymes, some with IC_{50} values in the range of clinically relevant inhibitors (Dinger et al., 2014c).

The present study aimed to evaluate TDNPS for their inhibition potential towards relevant CYP isozymes. For this purpose, a two cocktail approach (Dinger et al., 2014a; Dinger et al., 2014b) was used to determine the inhibitor concentrations which reduced the enzyme activity by 50% (IC_{50} value). In addition, the in vitro inhibition potential against CYP1A2 of 5-MeO-DALT was confirmed in vivo using rats and caffeine as test substrate.

2. Materials and methods

2.1. Chemicals and reagents

Alpha-naphthoflavone, amodiaquine 2HCl, caffeine solution 1 mg/mL, coumarin, deethyl amodiaquine, dextrorphan HBr, diclofenac, 5-hydroxy coumarin, isocitrate, isocitrate dehydrogenase (IDH), omeprazole, sulfaphenazole, superoxide dismutase (SOD), trimethoprim, and verapamil HCl were obtained from Sigma–Aldrich (Steinheim, Germany), bupropion HCl and 4-hydroxy bupropion HCl from GlaxoSmithKline (Munich, Germany), 4-hydroxy diclofenac from Pombiotech (Saarbrücken, Germany), phenacetin and dextromethorphan from Roche (Grenzach, Germany), fluconazole and sertraline from Pfizer (Berlin, Germany), paracetamol from Benchemie (Munich, Germany), tranylcypromine from Röhm Pharma (Darmstadt, Germany), quinidine from Chininfabrik Buchler (Braunschweig, Germany), clomethiazole from AstraZeneca (Wedel, Germany), chlorzoxazone from Cilag (Schaffhausen, Swiss), 6-hydroxy chlorzoxazone from Toronto Research Chemicals (Toronto, Canada) and delivered from LGC Standard (Wesel, Germany), 5-hydroxy omeprazole from @rtMolecule (Poitiers, France) and delivered from IBL international (Hamburg, Germany), dimethyltryptamine from THC Pharma (Frankfurt, Germany), NADP+ from Biomol (Hamburg, Germany), formic acid (mass spec grade) and testosterone from Fluka (Neu-Ulm, Germany), paraxanthine solution 1 mg/mL in methanol from LGC Standards, theobromine and theophylline from Knoll AG (Ludwigshafen, Germany), acetonitrile and methanol (all LC–MS grade), and all other chemicals from VWR (Darmstadt, Germany). All non-scheduled tryptamines were prepared following procedures published previously (Brandt et al., 2012; Young, 1958). Pooled human liver microsomes (pHLM, 20 mg microsomal protein/mL, 400 pmol total CYP/mg protein, 25 donor pool, mixed gender, average age 55 years, one Hispanic, two African Americans and 22 Caucasians) were obtained from Gentest and delivered from Corning (Amsterdam, Netherlands). After delivery, the microsomes were thawed at 37 °C, aliquoted, snap-frozen in liquid nitrogen, and stored at −80 °C until use. Whatman 903 filter paper cards (WHA10334885) were from GE Healthcare (Dassel, Germany). S-Monovette mixing aid (1.6 mg EDTA/mL blood) and multi adapter from Sarstedt (Nümbrecht, Germany), BD Microlance 3, sterile needle (0.5 × 25 mm) from BD Medical Systems (Drogheda, Ireland). Indicator silica gel pearls KC were obtained from NeoLab Migge (Heidelberg, Germany).

2.2. LC-HR-MS/MS apparatus for in vitro cocktail incubations

All analytical features associated with instrumentation have been described in detail elsewhere (Dinger et al., 2014a). Briefly, a Thermo Fisher Scientific (TF, Dreieich, Germany) Aria Transcend TLX-I HTLC, together with a TF Accela 1250 pump, a TF HTC PAL autosampler, and a valve interface module were used with built-in switching valves, all controlled by the TF Aria software version 1.6.3. It was coupled to a TF Q-Exactive equipped with a heated electrospray ionization source (HESI-II). The gradient elution was performed on a TF Accucore RP-MS (150 × 2.1 mm, 2.6 μm) column using water containing 0.02% (v/v) formic acid pH 3.1 (eluent A) and acetonitrile containing 0.1% (v/v) formic acid (eluent B) and a flow rate of 600 μL/min. The injection volume for all samples was 10 μL.

The MS conditions were as follows: HESI-II, positive and/or negative mode; sheath gas, nitrogen at a flow rate of 60 arbitrary units (AU); auxiliary gas, nitrogen at a flow rate of 20 AU; vaporizer temperature, 350 °C; spray voltage, 3.00 kV positive and 4.00 kV negative; ion transfer capillary temperature, 380 °C; and S-lens RF level, 50.0.

A targeted single ion monitoring experiment with mass spectrometric multiplexing (msx) and inclusion list was performed using the protonated or deprotonated ions of the metabolites. For the detection of *O*-deethyl phenacetin and *N*-deethyl amodiaquine, fragment ions obtained from in-source collision induced dissociation with 25 eV were used.

The scan parameters were as follows: microscan, 1; resolution, 17,500; AGC target, 1e6; maximum injection time, 250 ms; msx count, 5; isolation window, 1 *m*/*z*; scan range, *m*/*z* 100–400. The scan parameters were the same for positive and negative mode.

TF Xcalibur 2.2 software was used for data handling. The settings for automated peak integration were as follows: peak detection algorithm, ICIS; smoothing, 7; area noise factor, 5; and peak noise factor, 10.

2.3. Preparation of stock solutions and spiking solutions for in vitro inhibition experiments

The specific substrates, their metabolites, and model inhibitors were used and prepared in accordance to Dinger et al. (2014b). All stock solutions (10 mM) for the test inhibitors (tryptamines) were prepared in water/methanol 9/1 (v/v) with the exception of 4,5-ED-DALT, 5-F-2-Me-DALT, 6-F-DALT (water/methanol 8/2 (v/v)), 4-HO-DiPT, 5-MeO-2-Me-DiPT, 5-MeO-DiPT, 5-MeO-2-Me-DMT (water/methanol 1/1 (v/v)). These stock solutions were serially diluted with phosphate buffer for the incubations.

2.4. Conditions for the cocktail incubations

The conditions were already described elsewhere (Dinger et al., 2014b). Briefly, microsomal incubations were performed at 37 °C for 15 min with 0.2 mg/mL pHLM and the test substrates, split in two cocktails. Incubation time and protein content were in the linear range of product formation for all incubated enzymes and corresponding substrates. Cocktail A contained 12 μM of phenacetin, 1.1 μM of coumarin, 3.5 μM of diclofenac, 8.5 μM of dextromethorphan, and 86 μM of testosterone, and cocktail B 30 μM of bupropion, 2 μM of amodiaquine, 21 μM of omeprazole,

and 20 μM of chlorzoxazone so that all concentrations were at or below the K_m value (U.S.Department of Health and Human Services et al., 2006). Besides enzymes and substrates, 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 $NADP^+$, 0.5 U/mL isocitrate dehydrogenase and 200 U/mL superoxide dismutase. Reactions were started by addition of the ice-cold microsomes and stopped with 50 μL of ice-cold acetonitrile containing the internal standards (IS, diphenhydramine 0.1 μM, trimipramine 0.1 μM, and ibuprofen 100 μM). The incubation mixtures were centrifuged for 5 min at 14,000 g, 40 μL of both supernatants (cocktail A and B) were pooled, transferred to an autosampler vial, and 10 μL were injected onto the LC-HR-MS/MS apparatus for analysis under the conditions described above.

2.5. Prescreening and determination of IC_{50} values

2.5.1. Prescreening

According to Dinger et al. (2014c), the general inhibition potential of the TDNPS was tested using the cocktails incubated with 100 μM of each TDNPS (structures given in Fig. 1) in triplicates. In addition to these samples, control samples without inhibitor, positive control samples with model inhibitors (20 μM), and interfering samples were also prepared in triplicates. For the interfering samples, control samples without inhibitor were incubated and the reaction was terminated with ice-cold acetonitrile containing the IS and the tested TDNPS. For sample evaluation, the metabolite formation in the incubation sample was compared to the formation in the control sample. Every inhibition sample was compared with every control sample ($n = 3$ each).

2.5.2. Determination of IC_{50} values

According to Dinger et al. (2014c), the IC_{50} values were determined in duplicate for TDNPS, showing more than 50% inhibition of the activity of one or more isoenzymes. The TDNPS were used at ten different concentrations (0.01, 0.05, 0.1, 1, 10, 50, 100, 200, 400, 800 μM). Control, positive control, and interfering samples were also prepared as described above. The IC_{50} values were calculated by plotting the metabolite formation (relative to the control sample) over the logarithm of the TDNPS (inhibitor) concentration using GraphPad Prism 5.00 (GraphPad Software, San Diego, CA, USA). Relative metabolite formation was calculated by division of every inhibition sample with every control sample ($n = 2$ each). Statistic evaluation of the IC_{50} values were also performed using GraphPad Prism 5.00.

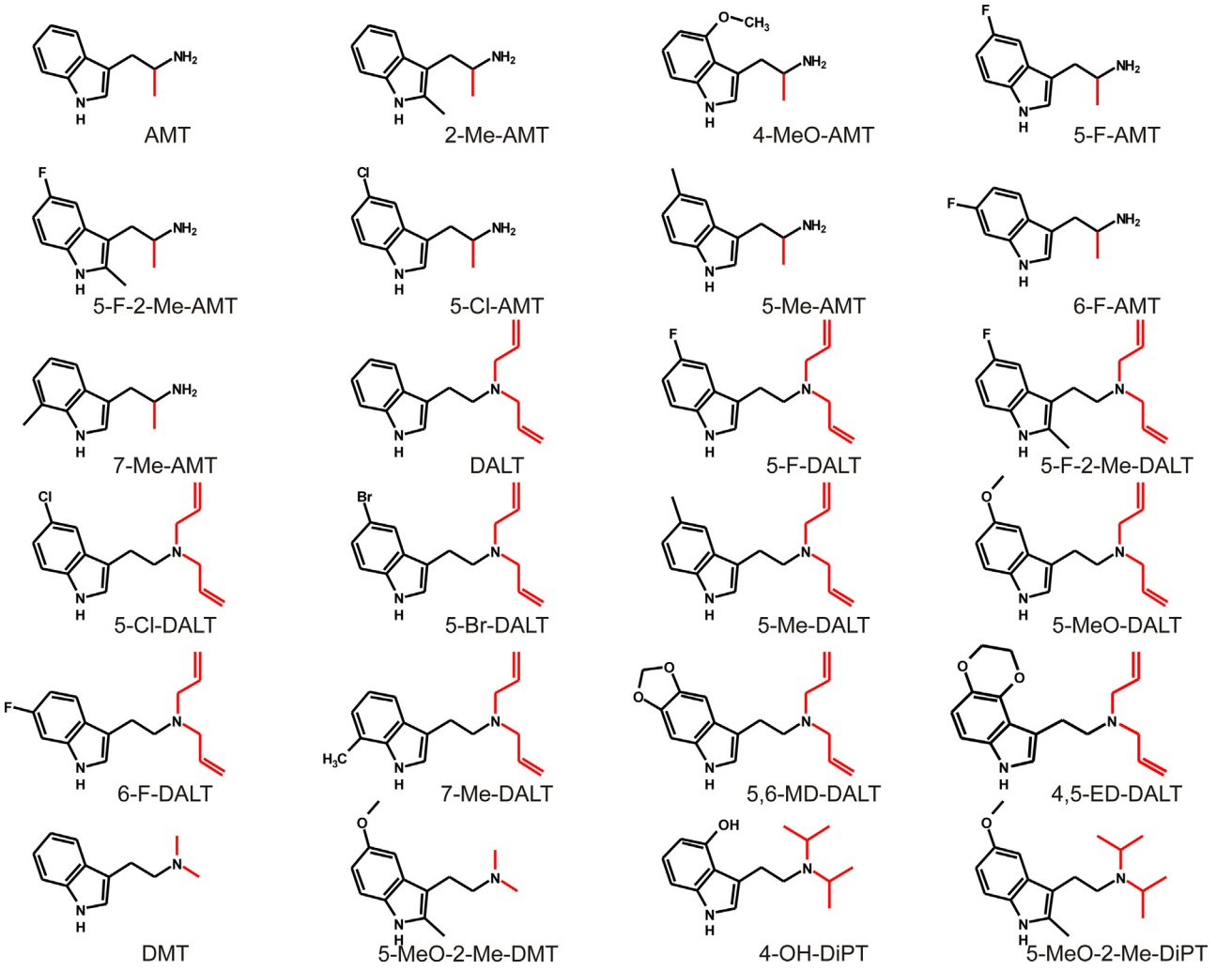

Fig. 1. Structures of the tested tryptamines. Eponymous structural parts are given in red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.6. In vivo CYP1A2 inhibition testing for 5-MeO-DALT

2.6.1. LC–MS/MS apparatus

All samples were analyzed using a TF TSQ Quantum Access mass spectrometer coupled to a TF Accela UHPLC system consisting of a degasser, a quaternary pump, and an autosampler. Gradient elution was performed on a TF Accucore RP-MS column (150 × 2.1 mm, 2.6 μm). The mobile phase consisted of water plus 0.01% formic acid (eluent A) and methanol plus 0.01% formic acid (eluent B). The flow rate was set to 700 μL/min, and the gradient was programmed as follows: 0–1.5 min to 85% A, 1.5–2.5 min to 70% A, 2.5–3.1 min hold, 3.1–4.5 min to 60% A, 4.5–6.5 min to 5% A, 6.5–7.5 min hold, 7.5–7.6 back to 85% A and hold till 10 min.

The MS conditions were as follows: atmospheric pressure chemical ionization (APCI), positive mode, sheath gas, nitrogen at a flow rate of 40 arbitrary units; auxiliary gas, nitrogen at flow rate of ten arbitrary units; collision gas, argon; vaporizer temperature, 300 °C; discharge current, 4.0 μA; ion transfer capillary temperature, 250 °C; and capillary offset, 35 V.

The mass spectrometer was operated in the timed multiple-reaction monitoring (MRM) mode with the following settings: collision pressure, 1.5 mTorr; isolation width Q1, 0.7 units; and scan time, 1 s. Transitions and their particular settings, collision energy, and tube lens were optimized for each analyte. All MRM transitions, collision energies, tube lens settings, and retention times used for the given analytes are summarized in Table 1. For data evaluation, TF Xcalibur 2.1.0 were used to obtain peak areas. The settings were as follows: peak detection algorithm ICIS; smoothing, 5; and area-to-noise factor, 5, peak-to-noise factor 10. GraphPad Prism 5.00 was used for statistical analysis.

2.6.2. Preparation of stock solutions, calibration standards, and control samples for validation of the LC–MS/MS method

Two different methanolic stock solutions (1.0 mg/mL) of caffeine were used. For 8-chlorotheophylline, fluvoxamine, 5-MeO-DALT, theobromine, and theophylline, methanolic solutions (1.0 mg/mL) were prepared and for paraxanthine a standard methanolic solution (1.0 mg/mL) was used.

Working solutions (0.01 and 0.1 mg/mL) of analytes were prepared in methanol by dilution from each stock solution. The spiking solutions for calibration standards and quality control (QC) samples were prepared by adding the appropriate amount of the corresponding stock or working solution to volumetric flasks to obtain the corresponding plasma or spot concentration. Calibration standards were prepared freshly every day spotting 10 μL of the corresponding spiking solution to a 30 μL blank dried blood spots (DBS). QC samples were prepared at two different concentrations (LOW and HIGH) in the same way than the calibration standards and additionally by spiking fresh whole blood with the solution, ten times higher than the recommended concentration, before spotting.

2.6.3. Method validation for caffeine quantification

The method was validated as recommended for single case analysis, including selectivity, recovery, matrix effects, process efficiency, linearity, accuracy and precision, and limit of quantification (Peters et al., 2007). Additionally, it was evaluated whether blank DBS could be used to prepare calibrators and QC samples. Therefore, blank rat blood was spiked at low and high concentration before spotting and blank DBS were spiked with the corresponding concentrations ($n = 6$, each). The peak areas of both sample sets were compared using a two-tailed unpaired t-test using Graphpad prism. Selectivity was determined by analyzing six blank DBS in multiple reaction monitoring mode (MRM) to check for signals that might interfere with caffeine or the IS. In addition, two zero samples (blank spot including IS) were analyzed. Matrix effects were determined according to Matuszewski et al. (1998) analyzing six samples for each sample set at low and high concentration. Sample set 1 consisted of neat standard solution, set 2 of extracted blank DBS spiked after extraction, and set 3 of the extracted spiked DBS. The calibration at six different concentrations (0.05, 1, 3, 5, 7, 10 mg/L) was performed in duplicate and analyzed as described above. The regression line was calculated using a first-order polynomial weighted ($1/x^2$) least-squares model. Six QC samples at low (0.1 mg/L) and high (9 mg/L) concentrations were analyzed and determined based on calibration curves. Accuracy was calculated in terms of bias as the percent deviation of the mean calculated concentration at each concentration from the corresponding theoretical concentration. Precision was calculated as the relative standard deviation (RSD). The lower limit of quantification (LLOQ) of the method was defined as the concentration of the QC low and was checked for the acceptance criterion of a signal-to-noise (S/N) ratio of >10. Theobromine, theophylline, fluvoxamine and 5-MeO-DALT, and paraxanthine were qualitatively monitored to exclude interferences.

2.6.4. Rat plasma sampling

Blood samples from male Wistar rats (Charles River, Sulzfeld, Germany) were taken after administration of the compounds by gastric intubation using an aqueous suspension for toxicological diagnostic reasons according to the corresponding German law. A single 10 mg/kg body mass (BM) dose of caffeine was administered to all rats ($n = 6$). In addition to caffeine, a 6 mg/kg BM dose of fluvoxamine and 0.6 mg/kg BM of 5-MeO-DALT were administered in parallel to two rats, respectively. Doses were calculated according to known human doses (De Kesel et al., 2014; FachInfo Service (www.fachinfo.de), 2013; Shulgin and Shulgin, 2004) using a simple factor approach, considering the species differences in pharmacokinetic parameters (clearance, volume of distribution) (Sharma and McNeill, 2009). Blood samples were taken at eight different time points (0, 1, 2, 3, 4, 5, 6, 24 h after administration) from lateral tail vein using EDTA tubes combined with a sterile needle. A 30-μL aliquot of whole venous blood was directly spotted

Table 1
Compounds, precursor ions, monitored fragment ions for MRM, collision energies, and tube lens settings of caffeine and its metabolites, fluvoxamine, 5-MeO-DALT and the internal standard.

Compounds	Precursor ions, u	Fragment ion 1			Fragment ion 2		
	$[M+H]^+$	$[M+H]^+$	Collision energy (V)	Tube Lense (V)	$[M+H]^+$	Collision energy (V)	Tube Lense (V)
Caffeine	195	110	22	70	138	18	70
Fluvoxamine	319	71	15	95	258	11	95
5-MeO-DALT	271	159	31	42	174	19	42
Paraxanthine	181	124	18	72			
Theobromine	181	138	18	72			
Theophylline	181	124	18	72			
8-Chlorotheophylline (IS)	215	158	20	69			

on Whatman paper cards for DBS as already done by Wickremsinhe (2015). Blank blood samples for calibration standards were collected before drug administration and spotted directly. DBS were stored in exsiccator with silica gel pearls as drying agent at room temperature until analysis.

2.6.5. Extraction procedure for DBS

The DBS were extracted according to De Kesel et al. (2014). In contrast, the whole DBS was punched and divided in 4 parts. These parts were extracted with 280 μL of a mixture of methanol and water 80/20 (v:v) containing 0.01% of formic acid and 1 mg/L of 8-chlorotheophylline as internal standard (IS) for 10 min on a rotary shaker. Afterwards, the samples were centrifuged for 10 min at 14,680 rpm and 200 μL of the upper phase was transferred to a glass vial. The solvent was evaporated under a stream of nitrogen at 70 °C and reconstituted in 100 μL of solvent A. Ten microliters of this extract were injected onto the LC–MS/MS system.

2.6.6. Determination of in vivo CYP1A2 inhibition by 5-MeO-DALT

The inhibition effects on the metabolism of caffeine were determined by calculating the kinetic constants area under the plasma concentration/time curve (AUC), C_{max}, and t_{max} for all groups. The AUC was calculated using the trapezoidal rule. The

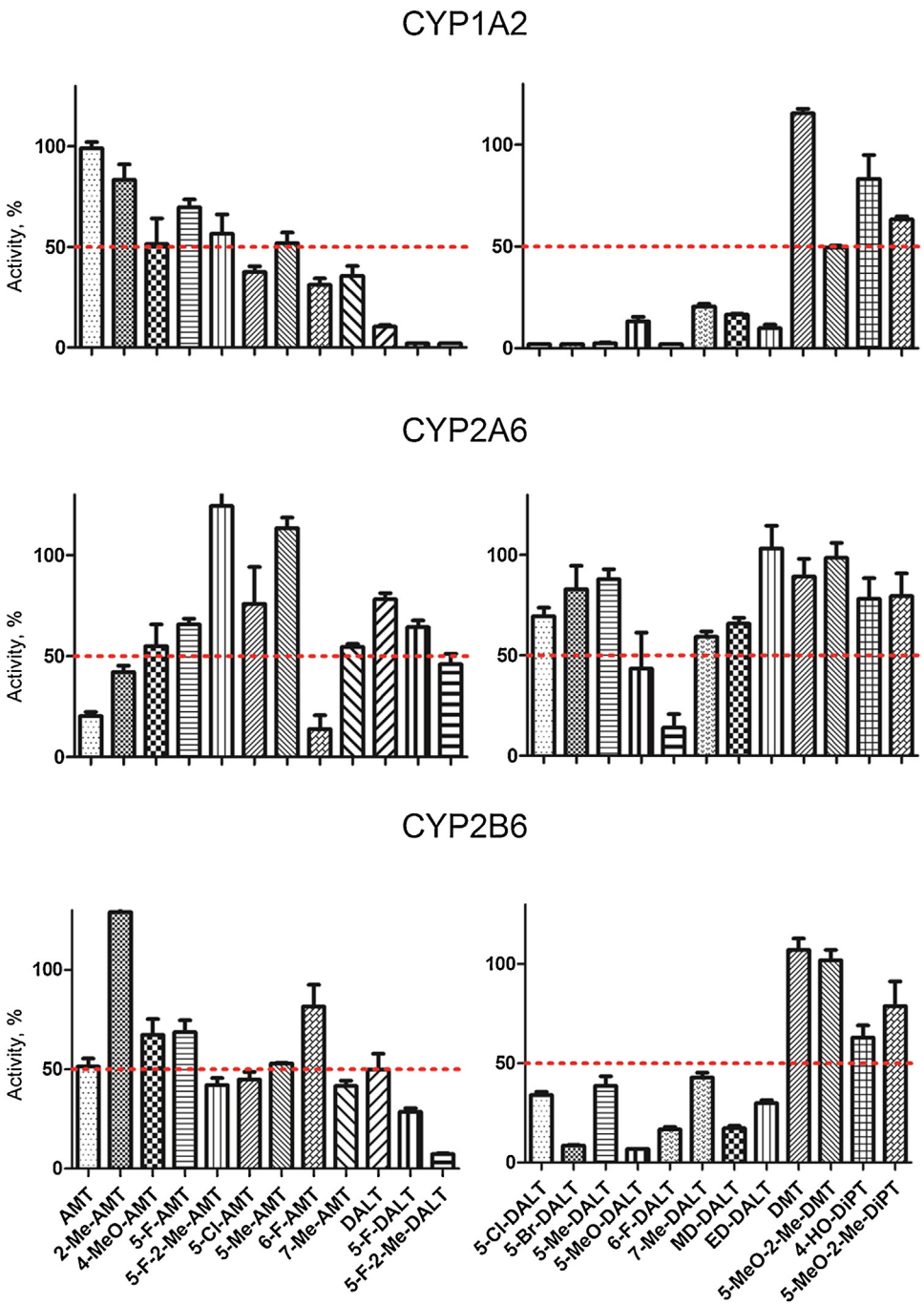

Fig. 2. Prescreening results ($n = 3$ each) using 100 μM each of TDNPS arranged in the order used in Table 1. Percentage of activity was the percentage of metabolite formation in relation to control sample (100%), incubated without inhibitor.

Bateman equation (Eq. (1)) was used for calculating c_{max} and t_{max}.

$$C = f * \frac{D}{V} * \frac{k_a}{k_a - k_e} * (\exp.^{-ke*}t - \exp.^{-ka*t}) \quad (1)$$

3. Results and discussion

3.1. Prescreening and determination of IC_{50} values

For all incubations, protein and time courses were tested for all isoenzyme/substrate reactions, and incubation conditions were chosen accordingly. The protein concentration of 0.2 mg/mL was sufficient for analyzing the specific substrate metabolites and were not expected to show non-specific protein binding (Baranczewski et al., 2006). In addition, the Food and Drug Administration (FDA) guidance advised a protein concentration below 1 mg/mL for in vitro CYP inhibition studies (U.S. Department of Health and Human Services et al., 2006). Microsomes were used instead of hepatocytes or liver tissues, because the enzyme concentrations were much higher in microsomes (Lu, 2014). Thus, smaller sample amounts could be used reducing the risk of unspecific protein bindings. Suitable incubation conditions were monitored incubating positive control samples with a mixture of the model inhibitors (20 μM) of each isoenzyme (Dinger et al., 2014b). Analytical interferences such as ion suppression or enhancement caused by co-eluting TDNPS were tested using incubated control samples (no inhibitor). The incubation was terminated with acetonitrile containing the internal standards and the corresponding TDNPS

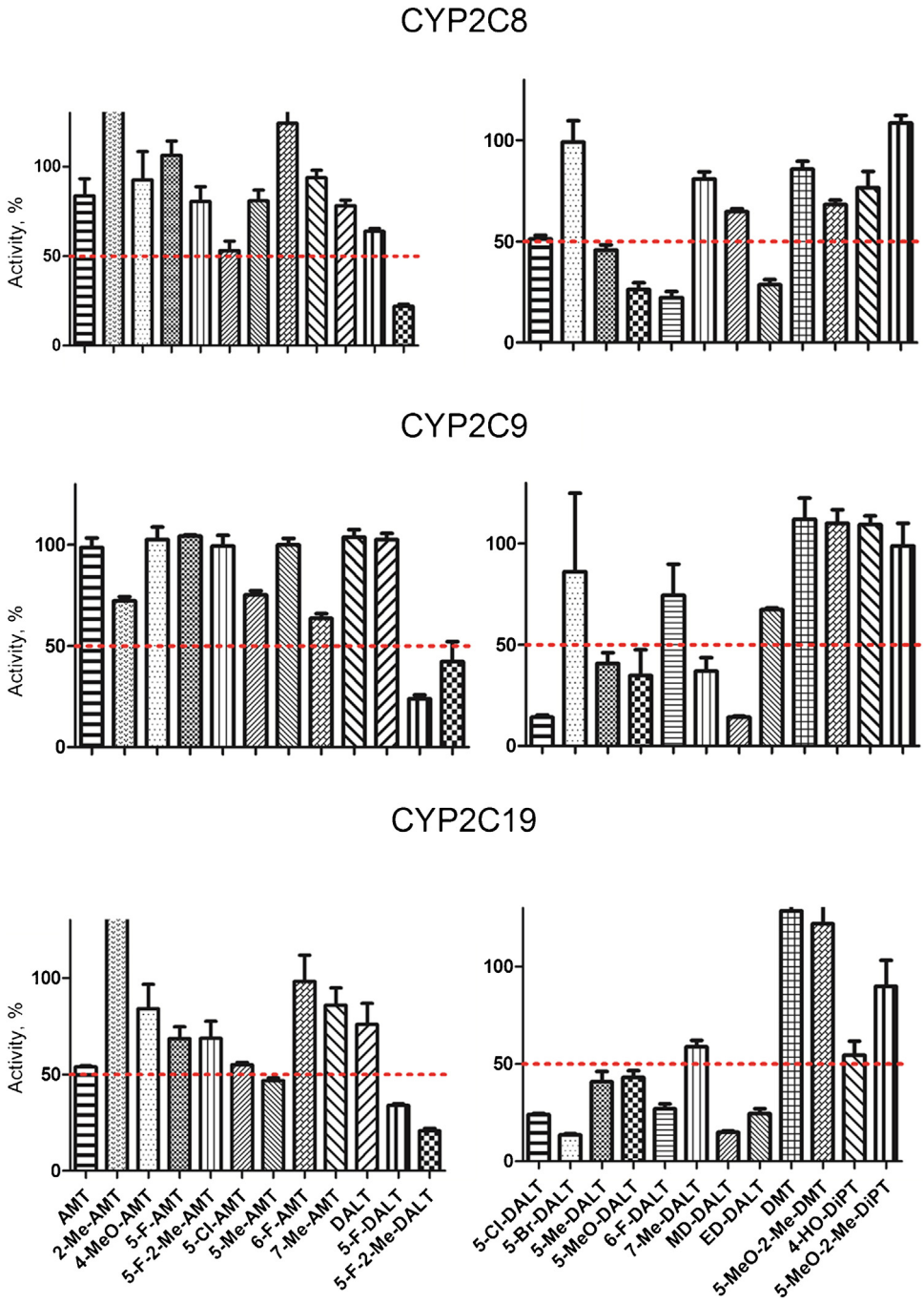

Fig. 2. (Continued)

(Dinger et al., 2014a). These samples were prepared to avoid false positive inhibition results in case of ion suppression or negative results in case of ion enhancement. This was necessary because only the specific metabolites were monitored by the analytical method and possibly co-eluting TDNPS were not detected. All control samples (without inhibitor) were compared to each incubation sample ($n = 3$ each for prescreening, $n = 2$ each for IC_{50} value determination).

As shown in Fig. 2, DMT was the only compound without inhibition of any isoenzyme activities. All others showed inhibition on CYP2D6 resulting in less than 50% activity in the prescreening experiment. All tested members of the DALT family reduced the CYP1A2 activity to less than 20% activity. Analytical interferences could be excluded. All isoenzymes were inhibited by the specific inhibitors in the positive controls. These kind of prescreening experiments were performed to gain a general overview of the inhibition potential for the tested drugs. A tryptamine concentration of 100 μM was chosen because at that concentrations, no or only slight inhibition could be considered as clinically irrelevant. Clinically relevant inhibitors showed IC_{50} values of about 100 μM (Bertelsen et al., 2003; Brosen et al., 1993; Burt et al., 2010; Ewald and Maurer, 2008; Kobayashi et al., 1998; Niwa et al., 2005; Park et al., 2003; von Moltke et al., 1998; Wang et al., 2002; Xu and Desta, 2013). Therefore, the IC_{50} values were only determined for TDNPS that showed inhibition of more than 50%. Performing such pre-experiments was helpful to save time and costs if isoenzymes of only one of both cocktails or no isoenzymes were inhibited. Thus, the IC_{50} values of DMT were not determined and for 2-Me-

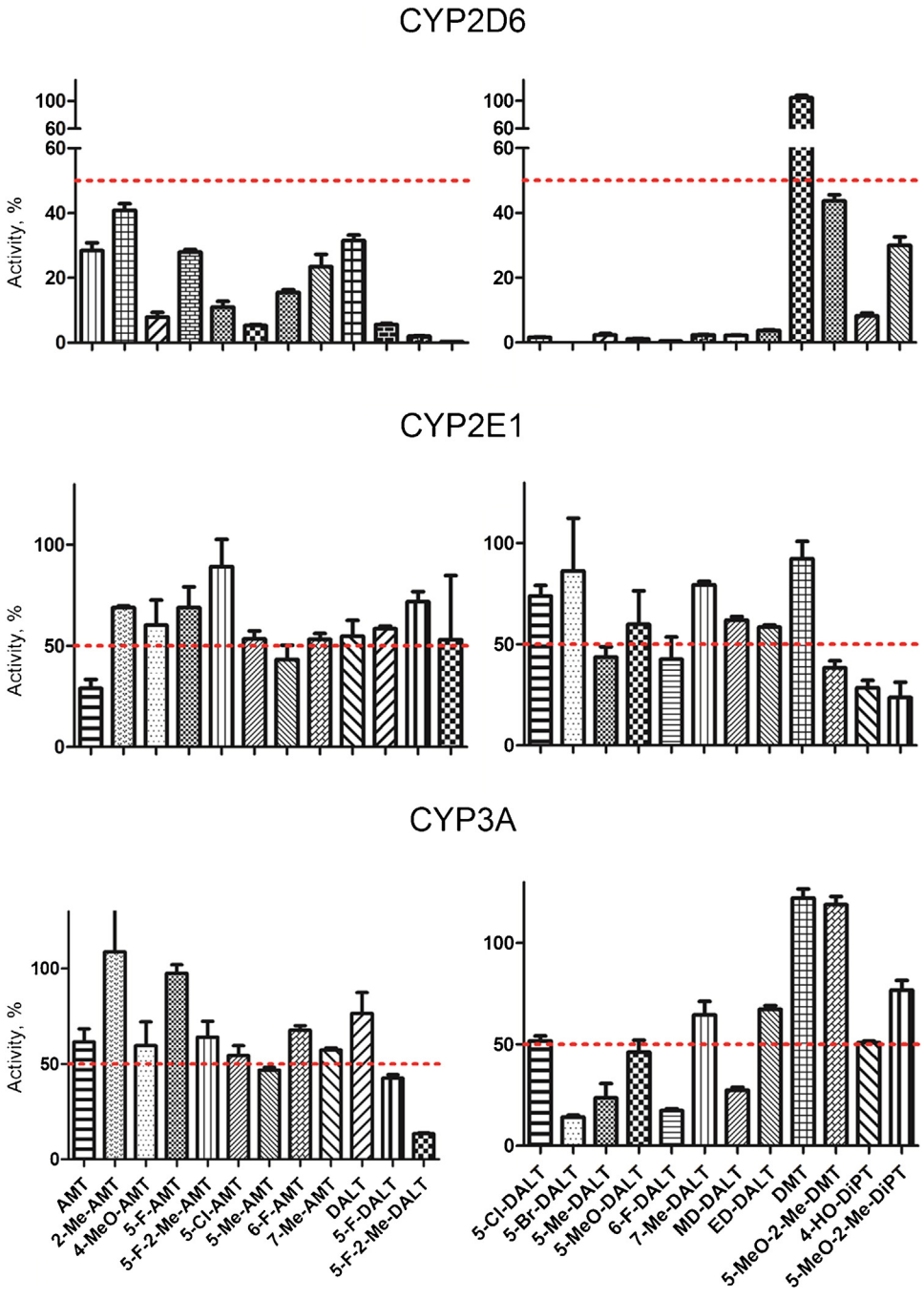

Fig. 2. (Continued)

Table 2
Test compounds, reference plasma concentrations, IC_{50} values of TDNPS and known inhibitors, and inhibited CYP enzyme activities. Plasma concentrations in μM were calculated from the published data in mg/L. Plasma concentrations given in italic were assessed according to the structure-related compounds AMT and 5-MeO-DiPT. Probable clinically relevant IC_{50} values are given in bold.

Test compounds	Reference plasma concentrations		IC_{50} values on CYP (μM)								
	(mg/L)	(μM)	1A2	2A6	2B6	2C8	2C9	2C19	2D6	2E1	3A
AMT-type											
AMT	0.16–1.3 (Elliott and Evans, 2014)	0.9–7.5	336 (±0.8)	**3.2 (±0.1)**	159 (±0.06)			62 (±20)	46 (±12)	292 (±91)	163 (±5.8)
2-Me-AMT	*0.16–1.3* (Elliott and Evans, 2014)	*0.8–6.9*	138 (±0.5)	74 (±51)					56 (±14)		166 (±77)
4-MeO-AMT	*0.16–1.3* (Elliott and Evans, 2014)	*0.8–6.8*	**7.7 (±0.9)**	89 (±22)					**14 (±2.2)**	169 (±20)	136 (±76)
5-F-AMT	*0.16–1.3* (Elliott and Evans, 2014)	*0.8–6.8*	170 (±8.8)	187 (±27)			86 (±0.06)		**19 (±9)**	214 (±79)	
5-F-2-Me-AMT	*0.16–1.3* (Elliott and Evans, 2014)	*0.8–6.3*	**126 (±0.07)**		64 (±14)			300 (±21)	**15 (±5)**		217 (±9)
5-Cl-AMT	*0.16–1.3* (Elliott and Evans, 2014)	*0.8–6.2*	**25 (±0.07)**	101 (±27)	206 (±45)			235 (±53)	**11 (±3)**	181 (±30)	205 (±46)
5-Me-AMT	*0.16–1.3* (Elliott and Evans, 2014)	*0.8–6.9*	**22 (±17)**	395 (±48)	221 (±85)			268 (±103)	34 (±13)	115 (±14)	317 (±46)
6-F-AMT	*0.16–1.3* (Elliott and Evans, 2014)	*0.8–6.7*	**36 (±0.02)**	**2.7 (±0.9)**	345 (±74)				53 (±19)	210 (±6.4)	
7-Me-AMT	*0.16–1.3* (Elliott and Evans, 2014)	*0.8–6.9*	**54 (±8.4)**	261 (±164)	85 (±7.2)			318 (±94)	71 (±22)	181 (±74)	337 (±70)
DALT-type											
DALT	*0.14–1.7* (Elliott and Evans, 2014; Tanaka et al., 2006; Wilson et al., 2005)	*0.6–7.1*	**0.4 (±0.1)**		175 (±105)				**2.6 (±2)**	91(±12)	90 (±12)
5-F-DALT	*0.14–1.7* (Elliott and Evans, 2014; Tanaka et al., 2006; Wilson et al., 2005)	*0.5–6.6*	**0.9 (±0.5)**		64 (±10)		109 (±5.6)	200 (±13)	**4.8 (±0.9)**	390 (±8.7)	**75 (±8.1)**
5-F-2-Me-DALT	*0.14–1.7* (Elliott and Evans, 2014; Tanaka et al., 2006; Wilson et al., 2005)	*0.5–6.3*	**0.8 (±0.3)**		**30 (±1.4)**	199 (±3.3)	63 (±2.2)	33 (±1.8)	**0.8 (±0.1)**	146 (±74)	**31 (±3.1)**
5-Cl-DALT	*0.14–1.7* (Elliott and Evans, 2014; Tanaka et al., 2006; Wilson et al., 2005)	*0.5–6.2*	**0.3 (±0.009)**		**39 (±12)**	190 (±52)	31 (±0.8)	36 (±11)	**1.2 (±0.3)**	135 (±76)	**59 (±23)**
5-Br-DALT	0.14–1.7 (Elliott and Evans, 2014; Tanaka et al., 2006; Wilson et al., 2005)	0.4–5.3	**0.8 (±0.2)**		52 (±0.6)	176 (±15)	23 (±0.04)	52 (±3.4)	**2.5 (±0.4)**	122 (±58)	**60 (±16)**
5-Me-DALT	*0.14–1.7* (Elliott and Evans, 2014; Tanaka et al., 2006; Wilson et al., 2005)	*0.6–6.7*	**0.7 (±0.5)**		138 (±14)		77 (±13)	156 (±3.4)	**3.8 (±0.7)**	**11 (±12)**	**67 (±9.8)**
5-MeO-DALT	*0.14–1.7* (Elliott and Evans, 2014; Tanaka et al., 2006; Wilson et al., 2005)	*0.5–6.3*	**0.4 (±0.1)**		**0.8 (±0.9)**			111 (±128)	**13 (±11)**	**20 (±14)**	114 (±36)
6-F-DALT	*0.14–1.7* (Elliott and Evans, 2014; Tanaka et al., 2006; Wilson et al., 2005)	*0.5–6.6*	**0.3 (±0.02)**		48 (±16)	**75 (±84)**	71 (±8.4)	48 (±14)	**0.3 (±0.05)**	48 (±55)	**42 (±14)**
7-Me-DALT	*0.14–1.7* (Elliott and Evans, 2014; Tanaka et al., 2006; Wilson et al., 2005)	*0.6–6.7*	**0.1 (±0.03)**		135 (±45)		159 (±31)	261 (±79)	**0.2 (±0.2)**	238 (±48)	84 (±12)
5,6-MD-DALT (Dinger et al., 2014c)	*0.14–1.7* (Elliott and Evans, 2014; Tanaka et al., 2006; Wilson et al., 2005)	*0.5–6*	**0.3 (±0.2)**		**26 (±15)**		58 (±0.7)	58 (±17)	**0.4 (±0.3)**		15 (±0.1)
4,5-ED-DALT		*0.5–5.7*	**0.1 (±0.01)**	139 (±121)	66 (±28)	**17 (±20)**	116 (±9.2)	55 (±26)	**2.9 (±0.8)**	168 (±20)	

Table 2 (*Continued*)

Test compounds	Reference plasma concentrations		IC_{50} values on CYP (μM)								
	(mg/L)	(μM)	1A2	2A6	2B6	2C8	2C9	2C19	2D6	2E1	3A
	0.14–1.7 (Elliott and Evans, 2014; Tanaka et al., 2006; Wilson et al., 2005)										
DMT-type											
DMT	*0.14–1.7* (Elliott and Evans, 2014; Tanaka et al., 2006; Wilson et al., 2005)	*0.7–9*									
5-MeO-2-Me-DMT	*0.14–1.7* (Elliott and Evans, 2014; Tanaka et al., 2006; Wilson et al., 2005)	*0.6–7.3*	198 (±107)		377 (±80)				57 (±1)	**20 (±6)**	
DiPT-type											
4-HO-DiPT	*0.14–1.7* (Wilson et al., 2005; Tanaka et al., 2006)	*0.5–6.5*	297 (±3.3)	450 (±175)	271 (±78)			88 (±23)	**9.6 (±2.4)**	60 (±6.2)	115 (±2.2)
5-MeO-2-Me-DiPT	*0.14–1.7* (Wilson et al., 2005; Tanaka et al., 2006)	*0.5–5.9*			135 (±27)			122 (±20)	57 (±0.01)	114 (±83)	198 (±34)
Known inhibitors											
Fluvoxamine	0.06–0.23 (Schulz et al., 2012)	0.2–0.7	0.2 (Brosen et al., 1993)								
Amiodarone	0.5–2.5 (Schulz et al., 2012)	0.7–3.9	86 (Kobayashi et al., 1998)								
Efavirenz	>1 (Schulz et al., 2012)	>3.2			6 (Xu and Desta, 2013)						
Gemfibrozil	25 (Schulz et al., 2012)					91 (Wang et al., 2002)					
Fluconazole	1–5 (Schulz et al., 2012)	4.9–16.3					30.3 (Niwa et al., 2005)				
Chloramphenicol	5–10 (Schulz et al., 2012)	15.5–31						32 (Park et al., 2003)			
Fluoxetine	0.12–0.5 (Schulz et al., 2012)	0.4–1.6							8.2 (K_i) (Ewald and Maurer, 2008)		
Paroxetine	0.01–0.05 (Schulz et al., 2012)	0.03–0.15							2.85 (Bertelsen et al., 2003)		
Clarithromycin	2.5–4 (Schulz et al., 2012)	3.3–5.4									116 (Burt et al., 2010)
Saquinavir	0.1–5 (Schulz et al., 2012)	0.15–0.4									11.5 (von Moltke et al., 1998)
In vitro											
Probe inhibitors (Dinger et al., 2014b)											
Alpha-	naphthoflavone			0.04							
Tranylcypromine	<0.05 (Schulz et al., 2012)	< 0.4		0.4							

Sertraline	0.001–0.01 (Schulz et al., 2012)	0.003–0.03	1						
Trimethoprim	1.5–2.5 (Schulz et al., 2012)	5–8.6		17					
Sulfaphenazole					0.4				
Fluconazole	0.001–0.01 (Schulz et al., 2012)	4.9–16.3				17			
Quinidine	1–5 (Schulz et al., 2012)	3.1–15.4					0.1		
Clomethiazole	0.01–0.05 (Schulz et al., 2012)	0.6–31						10.9	
Verapamil	0.1–0.25 (Schulz et al., 2012)	0.04–0.55							22

AMT, only cocktail A had to be incubated. For all other tested TDNPS, both cocktails were incubated because at least one isoenzyme each was inhibited during the prescreening.

As can be seen in Table 2, all tested TDNPS showed inhibition against at least one isoenzyme of the nine tested with IC_{50} values comparable to corresponding known clinically relevant inhibitors. However, to assess the clinical relevance based on IC_{50} values of known drugs, the plasma levels of these drugs and the tested TDNPS have to be considered (given in Table 2). Plasma concentrations of the tested tryptamines are only published for AMT and 5-MeO-DiPT (0.9–7.5 μM, 0.14–1.7 mg/mL). Common dosage levels associated with a range of psychoactive tryptamines were described to vary considerably although ranges between 5 and 60 mg were not uncommon (Shulgin and Shulgin, 1997). For most of the tested tryptamines, no data were available in the published literature but similar doses and therefore, similar plasma concentrations were considered acceptable (in the range of 0.5–9 μM). All tested AMTs, all DALTs, 5-MeO-2-Me-DMT, and 4-HO-DiPT showed inhibition against CYP1A2 activity. All DALTs showed IC_{50} values against CYP1A2 activity below 1 μM, being in the same range as the strong inhibitor fluvoxamine (IC_{50} value of 0.2 μM). Compared to the probe inhibitor alpha-naphthoflavone, the values were up to 10 times higher. The assumed plasma levels were in the same range as those for fluvoxamine. Based on these data, clinical relevance of the CYP1A2 inhibition by DALTs could not be excluded. This inhibition could especially be relevant for co-administered drugs such as clozapine, which were exclusively metabolized by CYP1A2. Severe clozapine intoxications caused by CYP1A2 inhibition were known as problem under the treatment with clozapine (Heeringa et al., 1999; Pinninti and de, 1997; Van Strater and Bogers, 2012). Five of the tested AMT showed values below 50 μM and the other inhibitors showed higher IC_{50} values (105–336 μM). For the AMTs, clinical relevance could also not be excluded although the values were higher than for the DALTs. However, they were below the range of the IC_{50} value of amiodarone (IC_{50} value of 86 μM), a known CYP1A2 inhibitor, with plasma levels of the AMTs comparable with that of amiodarone. Comparing the structural properties and the IC_{50} values, it seemed that the *N,N*-diallyl group enhanced the inhibition potential in contrast to the alpha-methyl, dimethyl, or diisopropyl groups. However, the substituents in positions 2, 5, 6, or 7 enhanced the potential significantly compared to DALT itself, but no difference between these substituents could be observed. In contrast to that family, the different substituents of the AMTs influenced the inhibition potential. Methylation in position 5 enhanced the potential in contrast to position 2 resulting in a higher IC_{50} value of 138 μM in contrast of 22 μM for 5-Me-AMT. The derivative with 2-methylation and 5-fluorination (IC_{50} value of 126 μM) increased significantly the inhibition potential in contrast to the single substituted analogues (IC_{50} value of 170 μM). In the case of CYP2A6, AMT and 6-F-AMT showed the highest inhibition with IC_{50} values of 3.2 and 2.7 μM, respectively. These values were in the range of the value of the FDA-recommended specific inhibitor tranylcypromine (IC_{50} value of 0.4 μM). Plasma levels for tranylcypromine were described to be lower than those for tryptamines in fatal cases. These data should not be overestimated,

Table 3
Validation data for caffeine quantification.

Validation parameter	QC low	QC high
Recovery, % (CV%)	59 (35)	44 (18)
Matrix effect, % (CV%)	59 (15)	87 (14)
Process efficiency, % (CV%)	36 (33)	38 (26)
Accuracy bias, %	−10.7	7.1
Precision (RSD%)	29.6	31.2

Table 4
Kinetic parameters of the in vivo metabolism of caffeine for control group, positive group, and test group, administered doses and drugs.

Study group	Administered drug	Dose, mg/kg BM	Kinetic parameter of caffeine		
			AUC (h*mg/L)	C_{max} (mg/L)	t_{max} (h)
Control	Caffeine	10	36 (±8)	4.2 (±0.5)	2.5 (±0.7)
	–	–			
Positive	Caffeine	10	103 (±31)	20 (±10)	1.9 (±0.4)
	Fluvoxamine	6			
Test	Caffeine	10	307 (±3)	18.5 (±0.5)	2.3 (±0.3)
	5-MeO-DALT	0.6			

as nothing was known about common users' plasma concentrations. However, clinical relevance cannot be excluded. 2-Me-AMT, 4-MeO-AMT, 5-F-AMT, 5-Cl-AMT, 5-Me-AMT, 7-Me-AMT, 4,5-ED-DALT, and 4-HO-DiPT inhibited also CYP2A6 activity, but with values between 74 and 450 μM, 100 times higher than that of tranylcypromine, so clinical relevance could not be assumed. CYP2B6 activity was inhibited by 19 of the tested TDNPS with IC_{50} values between 0.8 and 377 μM with 5-MeO-DALT as the strongest inhibitor. The value of 5-MeO-DALT was in the same range as the in vitro test inhibitor sertraline and the clinically relevant inhibitor efavirenz. As plasma levels of 5-MeO-DALT were lower than that of efavirenz, the concentrations needed for inhibition should only be reached in overdose cases. However, clinical relevance cannot be excluded as this tryptamine showed lower IC_{50} values than efavirenz. However, interaction could be relevant for polydrug users under substitution with methadone, which is metabolized by CYP2B6 to EDDP, resulting in increased methadone levels (Kharasch et al., 2015). For the other TDNPS, comparably weak inhibition with values higher than 25 μM (5-F-2-Me-AMT, 7-Me-AMT, 5-F-DALT, 5-F-2-Me-DALT, 5-Cl-DALT, 5-Br-DALT, 6-F-DALT, 4,5-ED-DALT,) or higher than 100 μM (AMT, 5-Cl-AMT, 5-Me-AMT, 6-F-AMT, DALT, 5-Me-DALT, 7-Me-DALT, 5-MeO-2-Me-DMT, 4-HO-DiPT, 5-MeO-2-Me-DiPT) could be shown and the clinical relevance of these inhibitions seemed to be negligible. CYP2C8 activity was inhibited by five of the tested tryptamines (5-F-2-Me-DALT, 5-Cl-DALT, 5-Br-DALT, 6-F-DALT, 4,5-ED-DALT) and the value of 4,5-ED-DALT was the lowest with 17 μM. This value was in the range of the specific test inhibitor trimethoprim (IC_{50} value of 17 μM) and the value of 6-F-DALT was in the range of that of gemfibrozil (IC_{50} value of 91 μM), a known inhibitor (Niemi et al., 2003). The described plasma level of trimethoprim was also comparable to that of the tryptamines, so a clinical relevance could not be excluded for 4,5-ED-DALT. In contrast, the plasma levels of gemfibrozil were described as much higher than that of the tryptamines, so relevance cannot be assumed, although the IC_{50} values were in the same range. All other compounds showed a weaker inhibition with values of about 100 μM. Nine compounds inhibited the CYP2C9 activity with IC_{50} values between 23 and 159 μM with 5-Br-DALT and 5-Cl-DALT as strongest inhibitors. Six of these values were comparable to the value of fluconazole (IC_{50} value of 30.3 μM), a clinical relevant CYP2C9 inhibitor (http://medicine.iupui.edu/clinpharm/ddis/clinical-table/). Concerning the specific inhibitor sulfaphenazole (IC_{50} value of 0.4 μM), the values were hundred times higher (Dinger et al., 2014b). The plasma levels of all TDNPS were lower than those of fluconazole and thus, clinical relevance of the inhibition of CYP2C9 activity by these TDNPS seemed to be improbable. CYP2C19 activity was inhibited by AMT, five members of the DALT family, and 4-HO-DiPT with values between 33 and 88 μM. These values were in the range of the IC_{50} value of 32 μM of chloramphenicol, a known inhibitor with clinical relevance (Hafner et al., 2008; Koup et al., 1978) and slightly above the probe inhibitor fluconazole (IC_{50} value of 17 μM). Concerning the plasma levels, the levels of fluconazole and chloramphenicol were much higher than those of the tryptamines (4.9–31 μM), so a clinical relevance of these inhibition potentials seemed also improbable. The other nine tryptamines showing CYP2C19 inhibition had values above 100 μM and these values could be seen to be not really clinically relevant. As already seen during the prescreening experiments, CYP2D6 was inhibited by all tested tryptamines with the exception of DMT. All determined IC_{50} values were below 100 μM. The DALTs showed the strongest inhibition with values below 5 μM with the exception of 5-MeO-DALT (13 μM) and were in the same range as those of the well-known clinically relevant inhibitors paroxetine and fluoxetine (IC_{50} values of 2.85 μM and Ki value of 8.2 μM). The IC_{50} values of 5-F-2-Me-DALT, 6-F-DALT, and 7-Me-DALT were even comparable with the value of the CYP2D6 probe inhibitor quinidine (0.1 μM). Three of the AMTs and one of the tested DiPTs also inhibited CYP2D6 activity in the same range as the known inhibitors. The plasma levels of all tested TDNPS were in comparable ranges as those of paroxetine and fluoxetine. Therefore, the inhibition potential of these drugs might be of clinical relevance. The inhibition of this isoenzyme could be critical because a lot of therapeutic and abused drugs are metabolized by this isoenzyme (http://medicine.iupui.edu/clinpharm/ddis/clinical-table/). The therapeutic effect of prodrugs such as risperidone or tramadol could not be effective. Antidepressive such as amitriptyline, clomipramine, venlafaxine or neuroleptics such as haloperidol are also metabolized by this isoenzyme. Inhibition of their metabolism could result in increased plasma levels and intoxication symptoms. Comparing the structural properties and the determined IC_{50} values, it seemed that the IC_{50} values increased from diallyl, over alpha-methyl and dimethyl to diisopropyl-substituted tryptamines. This could be observed for all tested tryptamines. The second correlation was that the IC_{50} values decreased from drugs with methyl or fluoro substituents in position 5 to those with chloro substituents. In contrast, the substitution by methyl or fluoro group influenced the inhibition potential in the same range. Substitution in position 6 and 7 by a fluoro or methyl group prefer the CYP2D6 inhibition by DALTs in contrast to AMTs. CYP2E1 activity was reduced by 19 tryptamines with 5-Me-DALT, 5-MeO-DALT and 5-MeO-2-Me-DMT showing the highest potential with IC_{50} values of 11, 20, and 20 μM, respectively. Only these IC_{50} values were comparable with the values of the probe inhibitor clomethiazole (IC_{50} value of 10.9 μM) (Dinger et al., 2014b; Gebhardt et al., 1997). The IC_{50} values and the plasma concentrations were in the same range, so a clinical relevance of the CYP2E1 inhibition could not be excluded for these three compounds. However, the values of the other tryptamines were much higher but a clinical relevance could also not be excluded. Eighteen of the tested TDNPS showed also inhibition against CYP3A activity. All DALTs showed inhibition potentials (IC_{50} values 31–114 μM) in the range of the protease inhibitor saquinavir (IC_{50} value of 11.5 μM), the calcium channel blocker verapamil (IC_{50} value of 22 μM), the probe inhibitor for CYP3A, and the time-dependent inhibitor clarithromycin (IC_{50} value of 116 μM). The clinical relevance of verapamil inhibition is well-known. For example, Kandavar and Sander described a multi-organ failure caused by co-administration of the CYP3A substrate atorvastatin

and the CYP3A inhibitor verapamil (Kandavar and Sander, 2010). In addition, the IC_{50} values of 4-MeO-AMT and 4-HO-DiPT were in the range of clarithromycin. The clinical relevance of CYP3A4 inhibition by clarithromycin is well-known. For example, Gasche et al. (2004) described a narcotic syndrome of a patient under therapeutic codeine doses because of the co-administration of clarithromycin inhibiting the CYP3A4-catalyzed detoxification to norcodeine. Other factors fortified the effect. As the plasma levels of saquinavir and verapamil were in the same range as that of the tryptamines, clinically relevant interaction could not be excluded. In contrast, the therapeutic plasma levels of the antibiotic clarithromycin were much higher than for the tested TDNPS with the exception of poisoning cases. Because of these data, the inhibition potentials of these drugs against CYP3A activity could be assessed as weak. Concerning the structural properties and the IC_{50} values of the DALT family, it seemed that introduction of substituents in 5 or 6 positions enhanced the inhibition potential. The values of DALT itself and the substituted analogues, with the exception of 5-Cl-DALT and 5-MeO-DALT, were significantly different. Substitution of both positions with methylenedioxy bridge showed the highest effect (Dinger et al., 2014c).

3.2. In vivo inhibition potential of 5-MeO-DALT

3.2.1. Quantification of caffeine

For determination of the corresponding caffeine concentrations in rat blood by LC–MS/MS, DBS were prepared to save blood volume. Besides common validation parameters, the usage of blank DBS for preparing calibrators and QC samples was evaluated. Using a two-tailed unpaired *t*-test, the peak areas of sample set 1 (whole blood spiked before spotting) and set 2 (blank DBS spiked after spotting) at low and high concentration were not significantly different ($p < 0.05$). The method was selective for caffeine as well as for the IS and no interfering peaks could be detected in the MRM mode. Results for recovery, matrix effect, process efficiency, accuracy, and precision are summarized in Table 3. The calibration curves were linear and the back-calculated calibrators were within the acceptance criteria. The LLOQ was consistent with the lowest calibrator and within the required acceptance criteria. The used blood volume for spotting was 30 μL. According to Wickremsinhe (2015) there was no difference between 10, 20 and 30 μL spot volume. Thus, 30 μL was used for more sensitive determination of caffeine. The whole spot was punched avoiding variations in spread area caused by different hematocrit values (Wickremsinhe, 2015).

3.2.2. Determination of in vivo inhibition potential of 5-MeO-DALT

According to Kot and Daniel, CYP1A2 metabolized over 80% of caffeine in humans and rats and only the metabolite profile differed (Kot and Daniel, 2008). The study design was chosen in accordance to the FDA guidance (U.S. Department of Health and Human Services et al., 2006). To monitor also a rapidly reversible inhibition, the substrate and the inhibitor were given simultaneously because the mode of inhibition was not known for 5-MeO-DALT. As positive control, the known CYP1A2 inhibitor fluvoxamine was tested under the same conditions. Results are summarized in Table 4. The control group (only caffeine administration) showed the lowest AUC of the plasma concentration time curve with 36 h*mg/L, in good correlation with described values (Latini et al., 1978). For the positive control group, the AUC was about three times higher than for the control group, proving the study design. The AUC of test group was 307 h*mg/L. The control group showed the lowest C_{max} value of 4.2 mg/L. For the other two groups, the value increased five times compared to the control group. T_{max} was for all groups in the same range (1.9–2.6 h). The tested kinetic parameters of caffeine increased for the positive and test group instead of the control group as expected according to the in vitro data. Hence, in vivo data seemed to confirm the in vitro data for 5-MeO-DALT. However, further studies with larger groups are needed to confirm this observation.

4. Conclusions

The presented study showed that with the exception of DMT all tested TDNPS showed more or less inhibition potential against the various CYP isoforms. Because of this inhibition potential in the range of clinical relevant inhibitors and similar plasma concentrations, the following drugs might cause clinically relevant interactions with the given CYPs: CYP1A2, 4-MeO-AMT, 5-F-2-Me-AMT, 5-Cl-AMT, 6-F-AMT, 7-Me-AMT, all DALTs; CYP2A6, AMT, 6-F-AMT; CYP2D6, AMT, 5-F-AMT, 5-F-2-Me-AMT, 5-Cl-AMT, all DALTs, 4-HO-DiPT; CYP2E1, 5-Me-DALT, 5-MeO-DALT, 5-MeO-2-Me-DALT; CYP3A, 5-F-2-Me-DALT, 5-Cl-DALT, 5-Br-DALT, 5-Me-DALT, 6-F-DALT.

Thus, these TDNPS could change the pharmacokinetics, namely the plasma levels and the corresponding effects of co-administered therapeutics or NPS. Prodrugs such as tramadol, tilidine, or risperidone, mainly metabolized by one or two isoenzyme, could lose their potency, if bioactivation was blocked (Mannheimer et al., 2008; Stamer et al., 2007; Wustrow et al., 2012). Neuroleptics such as clozapine or haloperidol could accumulate in plasma and lead to toxic concentrations (Murray, 2006). The inhibition of CYP2D6 and CYP3A4 could be important because many endogenous compounds and xenobiotics were metabolized by these two isoenzymes (http://medicine.iupui.edu/clinpharm/ddis/clinical-table/). However, it should be kept in mind that clinical cases or studies would be desirable to finally assess the risks associated with drug–drug interactions.

Conflict of interest

The authors declare that there are no conflicts of interest.

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