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Altering Metabolic Profiles of Drugs by Precision Deuteration: Reducing Mechanism-Based Inhibition of CYP2D6 by Paroxetine[S]

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ABSTRACT

Selective deuterium substitution as a means of ameliorating clinically relevant pharmacokinetic drug interactions is demonstrated in this study. Carbon-deuterium bonds are more stable than corresponding carbon-hydrogen bonds. Using a precision deuteration platform, the two hydrogen atoms at the methylenedioxy carbon of paroxetine were substituted with deuterium. The new chemical entity, CTP-347 [(3*S*,4*R*)-3-((2,2-dideuterobenzo[*d*][1,3]dioxol-5-yloxy)methyl)-4-(4-fluorophenyl)piperidine], demonstrated similar selectivity for the serotonin receptor, as well as similar neurotransmitter uptake inhibition in an in vitro rat synaptosome model, as unmodified paroxetine. However, human liver microsomes cleared CTP-347 faster than paroxetine as a result of decreased inactivation of CYP2D6. In phase 1 studies, CTP-347 was metabolized more rapidly in humans and exhibited a lower pharmacokinetic accumulation index than paroxetine. These alterations in the metabolism profile resulted in significantly reduced drug-drug interactions between CTP-347 and two other CYP2D6-metabolized drugs: tamoxifen (in vitro) and dextromethorphan (in humans). Our results show that precision deuteration can improve the metabolism profiles of existing pharmacotherapies without affecting their intrinsic pharmacologies.

Introduction

Compounds labeled with deuterium, a nonradioactive isotope of hydrogen bearing a neutron, have long been used as metabolic probes (Nelson and Trager, 2003), but few studies have assessed the potential of deuterated agents as pharmacotherapies (Harbeson and Tung, 2011; Braman et al., 2013; Gant, 2014). The molecular structures and pharmacologic activities of deuterated compounds (Harbeson and Tung, 2011), and even the structure and function of fully substituted enzymes (Di Costanzo et al., 2007), are extremely similar to their all-hydrogen analogs. Nonetheless, the metabolism of deuterated agents can differ substantially from nondeuterated compounds as a result of a phenomenon known as the deuterium isotope effect (Wiberg, 1955; Shao and Hewitt, 2010), which occurs as a result of the greater atomic mass of deuterium relative to hydrogen, causing deuterium-containing bonds with carbon, oxygen, and nitrogen to have lower vibrational frequencies than corresponding hydrogen-containing bonds. As a result of their lower ground-state energies, deuterium-containing bonds require higher activation energy to reach the transition state for scission and, thus, are more stable than hydrogen-containing bonds. Consequently, as commented on by others (Foster, 1984; Kushner et al., 1999), deuterium modification might be a useful tool to alter beneficially the metabolism of existing pharmacotherapies without affecting their intrinsic pharmacology.

The purpose of this study was to examine whether the metabolism of a widely used drug, paroxetine, could in fact be changed without altering its principal pharmacologic activities via deuterium substitution. It had the additional goal of directly correlating the effects of deuteration at the molecular level with clinically meaningful changes in human pharmacokinetics. Paroxetine (Paxil; GlaxoSmithKline, Research Triangle Park, NC) is a selective serotonin reuptake inhibitor currently indicated for major depressive, obsessive compulsive, panic, social anxiety, general anxiety, and posttraumatic stress disorders (Paxil, 2012). Paroxetine and other serotonin reuptake inhibitors also have proven effective in the treatment of vasomotor symptoms (e.g., hot flashes, night sweats) in women undergoing menopausal transition (Stearns et al., 2005; Deecher and Dorries, 2007) and in patients receiving antiestrogenic cancer therapy (Fisher et al., 1998; Bordeleau et al., 2007). Paroxetine undergoes significant first-pass metabolism (Kaye et al., 1989), catalyzed largely but not exclusively by CYP2D6 (Bloomer et al., 1992; Brosen et al., 1993). Repeat administrations of paroxetine, however, have been shown to inactivate CYP2D6, resulting in nonlinear pharmacokinetics and increased exposure over time (Bourin et al., 2001). The inactivation of CYP2D6 occurs by

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ABBREVIATIONS: AUC, area under the concentration-time curve; CTP-347, (3*S*,4*R*)-3-((2,2-dideuterobenzo[*d*][1,3]dioxol-5-yloxy)methyl)-4-(4-fluorophenyl)piperidine; DM, dextromethorphan; DX, dextrorphan; EM, extensive metabolizer; LC, liquid chromatography; LC-MS/MS, liquid chromatography–tandem mass spectrometry; MBI, mechanism-based inhibition; MIC, metabolic-intermediate complex; MRM, multiple reaction monitoring; MS, mass spectrometry; PM, poor metabolizer.

metabolism or mechanism-based inhibition (MBI) whereby an intermediate metabolite of paroxetine forms a covalent complex known as a metabolic-intermediate complex (MIC) with the cytochrome P450-Fe(II) form of the enzyme and inhibits its activity in a quasi-irreversible fashion (Murray, 2000; Bertelsen et al., 2003; Orr et al., 2012). Since the antidepressant effect of paroxetine exhibits a relatively flat dose-response curve (Bourin et al., 2001), the observed MBI appears to have limited effect on the agent's overall efficacy, but drug-drug interactions of significant clinical relevance have been observed between paroxetine and other coadministered drugs that are also metabolized by CYP2D6 (Paxil, 2012).

CTP-347 [(3*S*,4*R*)-3-((2,2-dideuterobenzo[*d*][1,3]dioxol-5-yloxy)methyl)-4-(4-fluorophenyl)piperidine] is a new chemical entity that is structurally identical to paroxetine except for two deuterium atoms, rather than hydrogen atoms, at the methylenedioxy carbon (Fig. 1). Here we demonstrate using in vitro model systems that CTP-347 abrogated the MBI observed with paroxetine and that this effect reduced drug-drug interactions with other CYP2D6-metabolized drugs. In addition, in phase 1 studies, we showed that CTP-347 was metabolized more rapidly than paroxetine, most likely as a result of the substantial decrease in the inactivation of CYP2D6. These results validate deuterium substitution as a potentially important approach to creating improved therapeutic agents.

Materials and Methods

CTP-347 (hydrochloride salt), paroxetine, *N*-desmethyl tamoxifen, and endoxifen were provided by Concert Pharmaceuticals, Inc. (Lexington, MA). Human liver microsomes were from Xenotech LLC (Lenexa, KS). Human cDNA-expressed CYPD6 supersomes were obtained from Corning Life Sciences (Tewksbury, MA). Dextromethorphan (DM) and NADPH were from Sigma-Aldrich (St. Louis, MO).

Synthetic Procedures for the Synthesis of CTP-347

The synthetic procedures for the synthesis of CTP-347 are shown in Fig. 2 and described in the following sections. The liquid chromatography (LC) purity assessment (Supplemental Fig. 1), mass spectrometry (MS) (Supplemental Fig. 2), and ^{1}H NMR spectra (Supplemental Fig. 3) are shown in the Supplemental Material.

((3*S*,4*R*)-4-(4-Fluorophenyl)-1-Methylpiperidin-3-yl)Methyl Methanesulfonate, HCl Salt (II). ((3*S*,4*R*)-4-(4-Fluorophenyl)-1-methylpiperidin-3-yl)methanol **I** (2.00 g, 8.96 mmol) was dissolved in dichloroethane (20 ml), and methanesulfonyl chloride (0.73 ml) was added. The reaction was stirred for 6 hours at room temperature. The reaction mixture was concentrated on a rotary evaporator to afford **II** as a white solid residue, which was suitable for use in crude form. MS *m*/*z*: 302.1 (M + H).

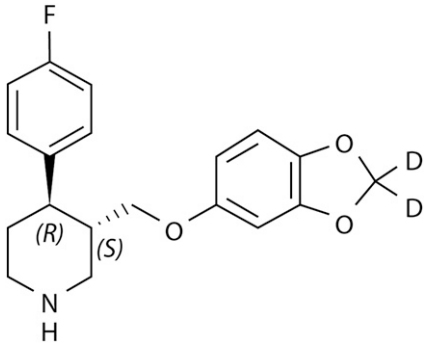

Fig. 1. Structure of CTP-347. D, deuterium atoms.

(3*S*,4*R*)-3-((Benzo[*d*][1,3]Dioxol-(2,2-*d*2)-5-Yloxy)Methyl)-4-(4-Fluorophenyl)-1-Methylpiperidine (IV). To a flask containing crude **II** (approximately 8.96 mmol) were added toluene (45 ml, benzo[*d*][1,3]dioxol-(2,2-*d*2)-5-ol [**III**], 99.7% isotopic purity, 1.26 g, 8.96 mmol), tetra-*n*-octylammonium bromide (245 mg, 0.448 mmol), and 3M aqueous NaOH (22.4 ml, 67.2 mmol) with stirring. The resulting pale yellow turbid bilayer was stirred and heated in a 90°C oil bath under a vented air condenser for 5 hours. The reaction mixture was cooled to room temperature and diluted with water (100 ml) and toluene (50 ml). The mixture was poured into a separatory funnel and shaken, and the layers were separated. The organic layer was washed with saturated aqueous $NaHCO_3$ and with brine, then dried over magnesium sulfate, filtered, and concentrated on a rotary evaporator to afford approximately 4 g of **IV**, which contained some residual toluene. This material was suitable for use in crude form. MS *m*/*z*: 346.3 (M + H).

(3*S*,4*R*)-4-Nitrophenyl 3-((Benzo[*d*][1,3]Dioxol-(2,2-*d*2)-5-Yloxy) Methyl)-4-(4-Fluorophenyl)Piperidine-1-Carboxylate (V). To a flask containing crude **IV** (approximately 8.96 mmol) were added toluene (60 ml), diisopropylethylamine (0.312 ml, 1.79 mmol), and 4-nitrophenylchloroformate (1.81 g, 8.96 mmol). The mixture was stirred and heated in an 80°C oil bath under a vented air condenser for 5 hours. The reaction mixture was cooled to room temperature and diluted with toluene (50 ml). The mixture was poured into a separatory funnel, and the flask was rinsed with an additional 50 ml of toluene. A 100-ml portion of water was added to the separatory funnel, and the layers were shaken and separated. The aqueous layer was extracted with an additional 25 ml of toluene. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated on a rotary evaporator to afford an amber oil. The material was purified via column chromatography (5% –30% EtOAc/hexanes) to provide 2.16 g of **V**. MS *m*/*z*: compound does not ionize well.

(3*S*,4*R*)-3-((Benzo[*d*][1,3]Dioxol-(2,2-*d*2)-5-Yloxy)Methyl)-4-(4-Fluorophenyl)Piperidine, HCl Salt (VI). To a solution of **V** (2.16 g, 4.35 mmol) in dioxane (29 ml) was added 2M aqueous NaOH (43.5 ml, 87.0 mmol), and the mixture was stirred and heated in a 70°C oil bath under a vented air condenser for 3 hours. The reaction mixture was cooled to room temperature and concentrated on a rotary evaporator to remove most of the dioxane. The aqueous residue was poured into a separatory funnel and extracted three times with dimethyl ether (Et_2O). The combined organic layers were washed with 1 N aqueous NaOH, then dried over magnesium sulfate, filtered, and concentrated on a rotary evaporator to afford the free base of **VI** as a pale yellow oil (1.2 g). This material was purified via preparative high-performance LC-MS to provide 710 mg of the free base of **VI**, which was then taken up in a minimal volume of acetone and added slowly to a stirred solution of 1 M HCl/Et_2O (5 ml), Et_2O (15 ml), and hexanes (60 ml). The resulting cloudy white mixture was held at 0°C for 1 hour and then concentrated to a reduced volume on a rotary evaporator. The resulting white solids were filtered, washed with hexanes/Et_2O, and dried in a vacuum oven at 35–40°C overnight to provide 651 mg of the HCl salt of **VI**: MS *m*/*z*: 332.0 (M + H), NMR (300 MHz, dimethylsulfoxide-d_6): δ 9.04 (br s, 2H), 7.25–7.14 (m, 4H), 6.74 (d, 1H, J = 8.3), 6.48 (d, 1H, J = 2.9), 6.18 (dd, 1H, J = 2.4, 8.3), 3.58 (dd, 1H, J = 3.4, 10.2), 3.52–3.47 (m, 2H), 3.39–3.35 (m, 1H), 3.01–2.91 (m, 2H), 2.86 (dt, 1H, J = 3.4, 12.2), 2.47–2.39 (m, 1H), 2.05–1.94 (m, 1H), and 1.88–1.85 (m, 1H).

Dideuterocatechol (VIII). 3,4-Dihydroxybenzaldehyde **VII** (40 g) was dissolved in tetrahydrofuran (160 ml), and then D_2O (160 ml) was added and the resulting mixture stirred overnight at room temperature under nitrogen. The solvent was removed in vacuo and, the residue was obtained dried in vacuo at 40°C overnight to provide the exchanged aldehyde **VIII** as a solid (40 g). Analysis by ^{1}H NMR (dimethylsulfoxide-d_6) showed an H/D exchange level of about 85%.

***d*$_2$-Piperonal (IX).** K_2CO_3 (29.6 g, 0.22 mol) was suspended in *N*-methylpyrollidinone (270 ml) and heated to 110°C under N_2. A solution of 3,4-dideuteroxybenzaldehyde **VIII** (15 g, 0.11 mol) and CD_2Cl_2 (68 ml, 1.1 mol) in *N*-methylpyrollidinone (30 ml) was added dropwise over 45 minutes. The reaction was stirred at 110°C for an additional 90 minutes, after which time analysis (TLC, LC) indicated

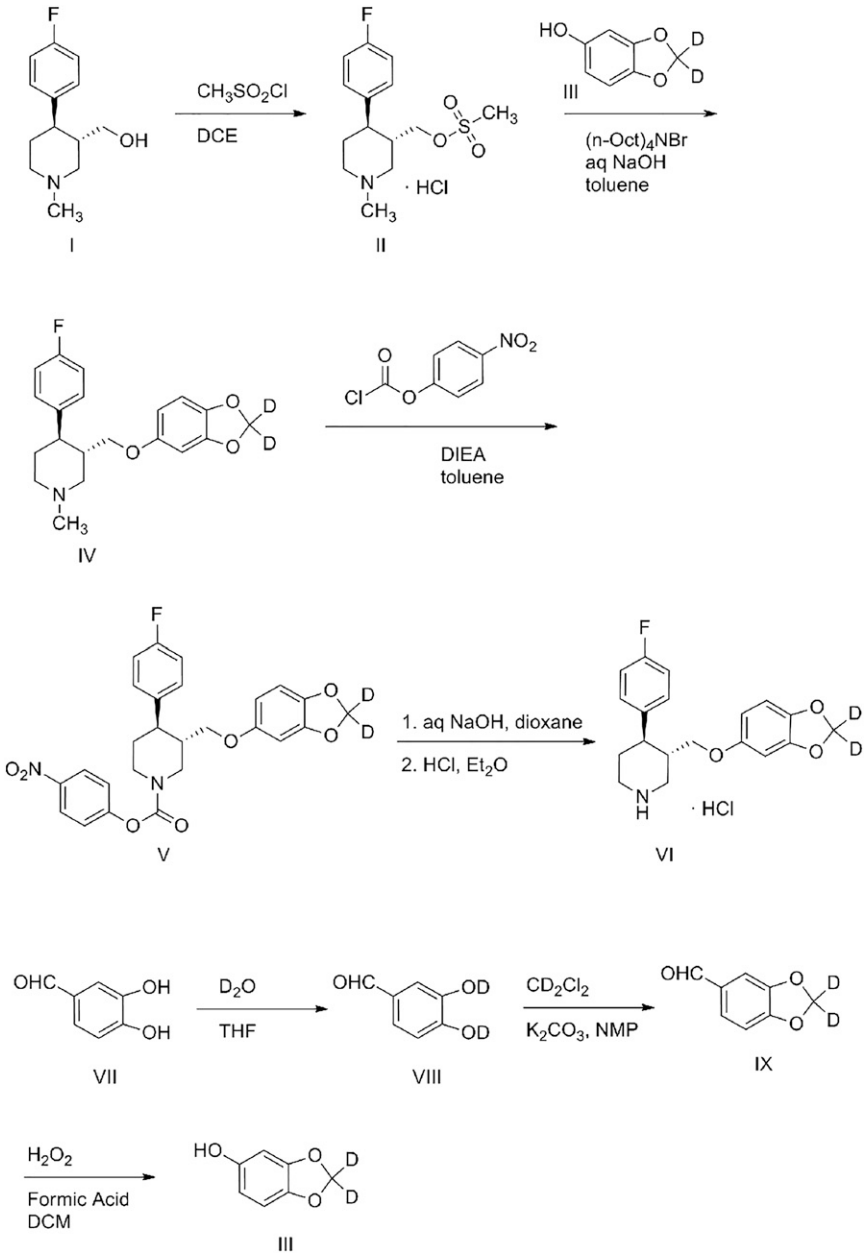

Fig. 2. Synthesis of CTP-347. See *Materials and Methods* for details.

complete reaction. The mixture was allowed to cool, filtered, the filtrate poured into water (900 ml) and extracted with EtOAc (3 × 600 ml). The combined organics were washed with water (2 × 600 ml), dried (magnesium sulfate), filtered, and concentrated in vacuo. The residue was purified by silica-gel chromatography (4/1 hexane/EtOAc) to give **IX** (14.2 g, 87%) of a pale brown oil that solidified on standing.

***d*$_2$-Sesamol (III).** To a stirred solution of d_2-piperonal **IX** (165 g, 1.08 mol, 1.0 eq.) in CH_2Cl_2 (5.5 liters) were added 30% hydrogen peroxide (343 ml, 3.01 mol, 2.75 eq.) and 96% formic acid (182 ml, 4.63 mol, 4.3 eq.), and the resulting mixture was stirred overnight at reflux. Analysis (i.e., by LC) indicated the presence of residual starting material (13%). The reaction mixture was cooled to 0–5°C, 1.5 M NaOH (5.9 liters, 8.85 mol, 8.2 eq.) was added portionwise over 30 minutes (exotherm to 25–30°C), and the mixture stirred for 30 minutes. The layers were separated, the organics were concentrated in vacuo, and the residue was obtained dissolved in methanol (3.6 liters), added to the aqueous, and the mixture stirred at room temperature for 30 minutes. The methanol was removed in vacuo, and the aqueous was washed with CH_2Cl_2 (2 liters and then 1.5 liters), acidified to pH 3 with concentrated aq. HCl, and extracted with CH_2Cl_2 (2 liters, then 2 × 1.5 liters). The combined organics were dried (magnesium sulfate), filtered, and concentrated in vacuo; the residue was combined with material from another 165 g batch and purified by column chromatography (hexane/EtOAc 4/1) to give **III** (185 g, 61% combined yield) as a white solid with a purity of >95% by ^{1}H NMR and 99% by LC.

Bioanalytical Methods

The samples were analyzed by LC–tandem mass spectrometry (LC-MS/MS) using an API 4000 mass spectrometer (Applied Biosystems, Carlsbad, CA) equipped with TurboIonSpray source, Agilent 1200 RR pumps (Agilent Technologies, Santa Clara, CA), and a Leap auto-sampler (Leap Technologies, Carrboro, NC). Analyst Software was used to acquire the data.

CTP-347. Aliquots (10–20 μl) of the in vitro or human plasma samples were injected onto a C8-reverse phase column (SB-C8 Zorbax, 3.5 μm, 2.1 × 30 mm) equilibrated with 95% solvent A (0.1% formic acid in water) and 5% solvent B (0.1% formic acid in acetonitrile), followed

by a linear gradient to 50% solvent B over 5.0 minute and then returned to the original conditions in 1.0 minute. The flow rate was 0.5 ml/min. The multiple reaction monitoring (MRM) transitions for CTP-347 were m/z 332.2 to 192.2 and m/z 377.0 to m/z 293.0 for the internal standard, indiplon. The internal standard for the human plasma bioanalysis of CTP-347 was paroxetine-d6, which had MRM transitions of m/z 336.3 to m/z 198.2.

DM and Dextrorphan. Aliquots (10–20 μl) of the in vitro or human urine samples were injected onto a C18-reverse phase column (Xbridge phenyl, 3.5 μm, 2.1 $\times$ 100 mm) equilibrated with 100% solvent A (0.1% formic acid in water), followed by a linear gradient to 100% solvent B (0.1% formic acid in acetonitrile) over 10 minutes and then returned to the original conditions in 1.0 minute. The flow rate was 0.45 ml/min. The MRM transitions for DM were m/z 272 to m/z 215.3 and for dextrorphan (DX) were m/z 261.2 to m/z 157.1. Indiplon was used as the internal standard.

***N*-Desmethyl Tamoxifen and Endoxifen.** Aliquots (10–20 μl) of the in vitro samples were injected onto a C18-reverse phase column (Xbridge phenyl, 3.5 μm, 2.1 $\times$ 100 mm) equilibrated with 90% solvent A (0.1% formic acid in water), followed by a linear gradient to 90% solvent B (0.1% formic acid in acetonitrile) over 2.0 minutes and then returned to the original conditions in 1.0 minute. The flow rate was 0. 5 ml/min. The multiple MRM transitions for *N*-desmethyl tamoxifen were m/z 358 to m/z 58 and for endoxifen were m/z 374 to m/z 152. Indiplon was used as the internal standard.

In Vitro Pharmacology Assessments

The inhibition of the uptake of serotonin, norepinephrine, and dopamine by CTP-347 and paroxetine was tested in rat brain, hypothalamus, and striatum synaptosomes, respectively. Serial dilutions of CTP-347 and paroxetine were incubated with ^{3}H-labeled serotonin, norepinephrine, or dopamine for 15–20 minutes at 37°C. The concentration range of CTP-347 and paroxetine tested were 0.1–40, 1–400, and 10–4000 nM for inhibition of serotonin, norepinephrine, and dopamine uptake, respectively. The percent of specific activity in the presence of CTP-347 or paroxetine relative to control specific activity was determined. The IC_{50} values for inhibition of the incorporation of ^{3}H-labeled neurotransmitters were determined by nonlinear regression analysis of the percent of control specific activity versus log (concentration) curves using Hill equation curve fitting.

An additional battery of tests against 166 molecular targets was also conducted to characterize other pharmacologic effects of CTP-347. Radioligand binding or enzymatic assays were used, and CTP-347 was tested at concentrations of 10 and 1 μM and compared with that of paroxetine at a concentration of 1 μM.

Metabolic Stability in Human Liver Microsomes

The metabolic stability of CTP-347 in human liver microsomes was evaluated by the in vitro half-life ($t_{1/2}$) method described by Obach et al. (1997). The metabolic stability of paroxetine was also determined similarly in parallel. CTP-347 or paroxetine was incubated with human liver microsomes for 30 minutes at 37°C in triplicate wells of a 96-well plate. Aliquots of the reaction mixtures were removed at 0, 3, 7, 12, 20, and 30 minutes and analyzed for amounts of parent remaining by LC-MS/MS. The experiment was repeated on 4 separate days, and mean values at each time point were reported. The in vitro $t_{1/2}$ values for CTP-347 and paroxetine were calculated from the slopes of the linear regression of % parent remaining (LN) versus the incubation time relationship.

Time-Dependent Inactivation of CYP2D6

This study was conducted in accordance with the procedure established by Bertelsen et al. (2003) for paroxetine. Several concentrations of CTP-347 (0, 0.5, 0.66, 1, 2, 5, 10, 25 μM) were preincubated with human liver microsomes (5 mg/ml) for 0, 10, 25, and 30 minutes in the presence of NADPH in triplicate wells of a 96-well plate. After the preincubation, aliquots of the reaction mixtures were diluted 1:10 in buffer (0.1 M potassium phosphate, pH 7.4 with $MgCl_2$ and NADPH). DM (25 μM) was added to the diluted preincubations, and these mixtures were incubated for another 5 minutes, after which the samples were analyzed by LC-MS/MS. The formation of DX, a CYP2D6-specific metabolite of DM, was monitored as a measure of CYP2D6 activity. Similar reactions were performed with paroxetine in parallel. The experiment was repeated twice, and the results of a representative experiment are shown and discussed.

Reversible Inhibition of CYP2D6

Human liver microsomes were incubated with the CYP2D6-selective marker substrate DM, and 1:3 serial dilutions of CTP-347 (highest concentration used was 10 μM) in 0.1 M potassium phosphate buffer, pH 7.4 with 3 mM $MgCl_2$. The reactions were initiated by the addition of NADPH and were incubated for 15 minutes at 37°C in triplicate wells of a 96-well plate and then analyzed by LC-MS/MS for the amounts of DX formed. Similar incubations were performed with paroxetine and quinidine (positive control inhibitor) in parallel. The percent inhibition relative to blank (no CTP-347 or paroxetine) samples was calculated, and the IC_{50} value was determined by nonlinear regression analysis using the log (inhibitor) versus response sigmoidal fit using GraphPad Prism. The experiment was repeated, and the reported IC_{50} is the mean of two experiments.

Spectral Analysis of Metabolite Intermediate Complex Formation

The formation of a metabolite intermediate complex after preincubation of CYP2D6 supersomes (0.5 nmol/ml) with 10 μM CTP-347 or paroxetine and NADPH (2 mM) was monitored as described by Bertelsen et al. (2003). Difference spectra were obtained by scanning between 500 and 400 nm every 2 minutes for 26 (paroxetine) or 36 (CTP-347) minutes using an Olis DW-2000 spectrophotometer (Olis, Inc., Bogart, GA). The increase in absorption (Abs) at 456 nm over time was observed to determine intermediate complex formation with CTP-347 and paroxetine.

Determination of Binding Affinity K_s for CYP2D6

Type 1 binding difference spectra were determined by spectral scanning (350–500 nM) of a mixture of CYP2D6 supersomes (250 pmol/ml) and several concentrations (0 to 145 μM) of CTP-347 and paroxetine using an Olis DW-2000 spectrophotometer. The difference in absorption (ΔAbs) at 390 nm and 424 nm was calculated, and K_s values were calculated by nonlinear regression analysis.

Assessment of the Drug-Drug Interaction Potential with Tamoxifen

CTP-347 was preincubated in triplicate wells of a 96-well plate with human CYP2D6 supersomes (500 pmol/ml) at concentrations of 0, 0.5, 0.66, 1, 2, 5, 10, and 25 μM for 20 minutes in the presence of NADPH, after which aliquots of the reaction mixture were diluted 1:10 in buffer containing NADPH. *N*-Desmethyl tamoxifen (50 μM) was then added to the diluted preincubation mixtures and further incubated, following which samples were analyzed by LC-MS/MS for amounts of endoxifen formed. Similar incubations with paroxetine were performed in parallel. The percent activity remaining after a 20-minute preincubation with CTP-347 or paroxetine, measured as percent endoxifen formed, was calculated.

Evaluation of Effect of CTP-347 on CYP2D6 Activity in Healthy Subjects

A randomized, double-blind, placebo-controlled, single-center study was conducted in healthy female subjects. The study protocol was approved by the PRACS Institute (Fargo, ND) institutional review board. The study was conducted in accordance with the guidelines set

forth by the International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (ICH Guideline E6), the Code of Federal Regulations for Good Clinical Practice (21 CFR Parts 50 and 56), and the Declaration of Helsinki regarding the treatment of human subjects in a study. Written informed consent was obtained from all subjects at the screening visit.

The primary objectives of the first in-human study with CTP-347 were to investigate the safety, tolerability, and pharmacokinetics of CTP-347 after single and multiple doses. The inactivation of CYP2D6 by CTP-347 after once-daily oral dosing for 14 days to healthy subjects was investigated as a secondary objective, and only the methods and results of this objective are discussed here. CTP-347 5-mg immediate-release tablets were administered to healthy women age 25–65 years. Three cohorts of nine subjects received once-daily doses of 10, 20, or 40 mg, and a fourth cohort of seven subjects received twice-daily doses of 10 mg (20 mg/day) for 14 days. In addition, one cohort of 10 subjects received once-daily paroxetine at a dose of 10 mg for the same time. On the evening before the first CTP-347 dose, 30 mg of DM solution was administered orally to each subject. Urine was collected for 8 hours, and the levels of DM and DX were determined by LC-MS/MS. The urinary DM/DX ratio was used to determine the baseline CYP2D6 phenotype. This procedure was repeated on day 14 of CTP-347 administration to determine the extent of inhibition of CYP2D6 activity. CYPD26 genotyping was performed on blood samples by a contract research organization according to standard validated PCR methods. CTP-347 plasma levels at several time points postdose on day 1 and day 14 were also measured by LC-MS/MS. Each subject was monitored for safety throughout the study including vital signs, physical examinations, clinical laboratory tests, 12-lead electrocardiogram, and cardiac telemetry.

Results

CTP-347 Is a Selective Serotonin Uptake Inhibitor In Vitro. Neurotransmitter uptake inhibition was assessed using an in vitro rat synaptosome model that measured uptake of ^{3}H-labeled neurotransmitter in the presence of CTP-347 or paroxetine (see *Materials and Methods*). In this system, IC_{50} levels for serotonin uptake were similar with CTP-347 (0.89 nM) and paroxetine (0.72 nM) (Table 1). Furthermore, CTP-347 was highly selective for the serotonin transporter, as the IC_{50} for the deuterated agent was 35-fold (31 nM) and 300-fold (270 nM) greater for norepinephrine and dopamine uptake, respectively, again similar to paroxetine. At a concentration of 1 μM, the deuterated and all-hydrogen molecules were approximately equipotent in their inhibitory interactions with other molecular targets, including the human serotonin, norepinephrine, and dopamine transporters; human muscarinic receptors; and rat L-type calcium channels (Table 1). These results are consistent with previous studies indicating that deuterium substitution had little impact on pharmacologic activity and molecular structure relative to their all-hydrogen analogs (Di Costanzo et al., 2007; Harbeson and Tung, 2011).

CTP-347 Exhibits Little or No Mechanism-Based Inhibition of CYP2D6 In Vitro. Metabolic stability of the two drugs was determined by incubating each in the presence of human liver microsomes for 30 minutes. The in vitro $t_{1/2}$ values for CTP-347 and paroxetine were 21 $\pm$ 2 and 49 $\pm$ 8 minutes (n = 4 for both compounds), respectively; that is, the $t_{1/2}$ value for CTP-347 was 57% shorter than that for paroxetine (Fig. 3B). Thus, CTP-347 was cleared faster than paroxetine by human liver microsomal preparations. As measured by type 1 difference spectra (Bertelsen et al., 2003), however, the binding affinity, K_s, of CTP-347 (11.2 μM) was similar to that of paroxetine (8.5 μM) (Fig. 3C), indicating that the higher rate of CTP-347 metabolism was unlikely attributable to differences in binding of the drug to CYP2D6.

A plausible explanation for the rapid metabolism of CTP-347 posits that substitution of deuterium atoms at the methylenedioxy carbon might decrease MIC formation with CYP2D6, thereby allowing for more rapid turnover. To examine this possibility, we analyzed CTP-347 metabolism using a method that was previously applied to paroxetine and takes into account the effects of MBI on reaction kinetics (Kitz and Wilson, 1962; Silverman, 1995; Bertelsen et al., 2003). This method assumes that a dual reactant-inactivator like paroxetine can have three separate fates on binding to CYP2D6: release without further chemical modification (reversible binding), conversion to product (productive catalytic cycle), or formation of an MIC (Fig. 3A). Solution of the mathematical relationships describing these outcomes results in two key kinetic parameters of interest, K_I and k_{inact}. Making simplifying assumptions, K_I represents the equilibrium disassociation constant for paroxetine (reversible binding), whereas k_{inact} represents the rate constant to form inactive MIC from the enzyme-drug complex (Mayhew et al., 2000).

K_I and k_{inact} for MBI are typically determined in a two-step reaction assay (Kitz and Wilson, 1962; Silverman, 1995). In the first step, enzyme is incubated for varying times with

TABLE 1
CTP-347 Pharmacologic effects in vitro

	Species	CTP-347	Paroxetine
Reuptake inhibition, IC_{50} (nM)			
Serotonin	Rat[a]	0.89	0.72
Norepinephrine	Rat[b]	31	43
Dopamine	Rat[c]	270	230
Percent inhibition at 1 μM concentration, %			
Serotonin transporter	Human	88	90
Norepinephrine transporter	Human	87	89
Dopamine transporter	Human	48	67
Muscarinic M_1	Human	50	57
Muscarinic M_3	Human	56	63
Muscariic M_5	Human	51	50
Calcium channel L-type, benzothiazepine	Rat	55	45
Calcium channel L-type, dihydropyridine	Rat	19	47

[a]Tested in rat brain synaptosomes.
[b]Tested in rat hypothalamus synaptosomes.
[c]Tested in rat striatum synaptosomes.

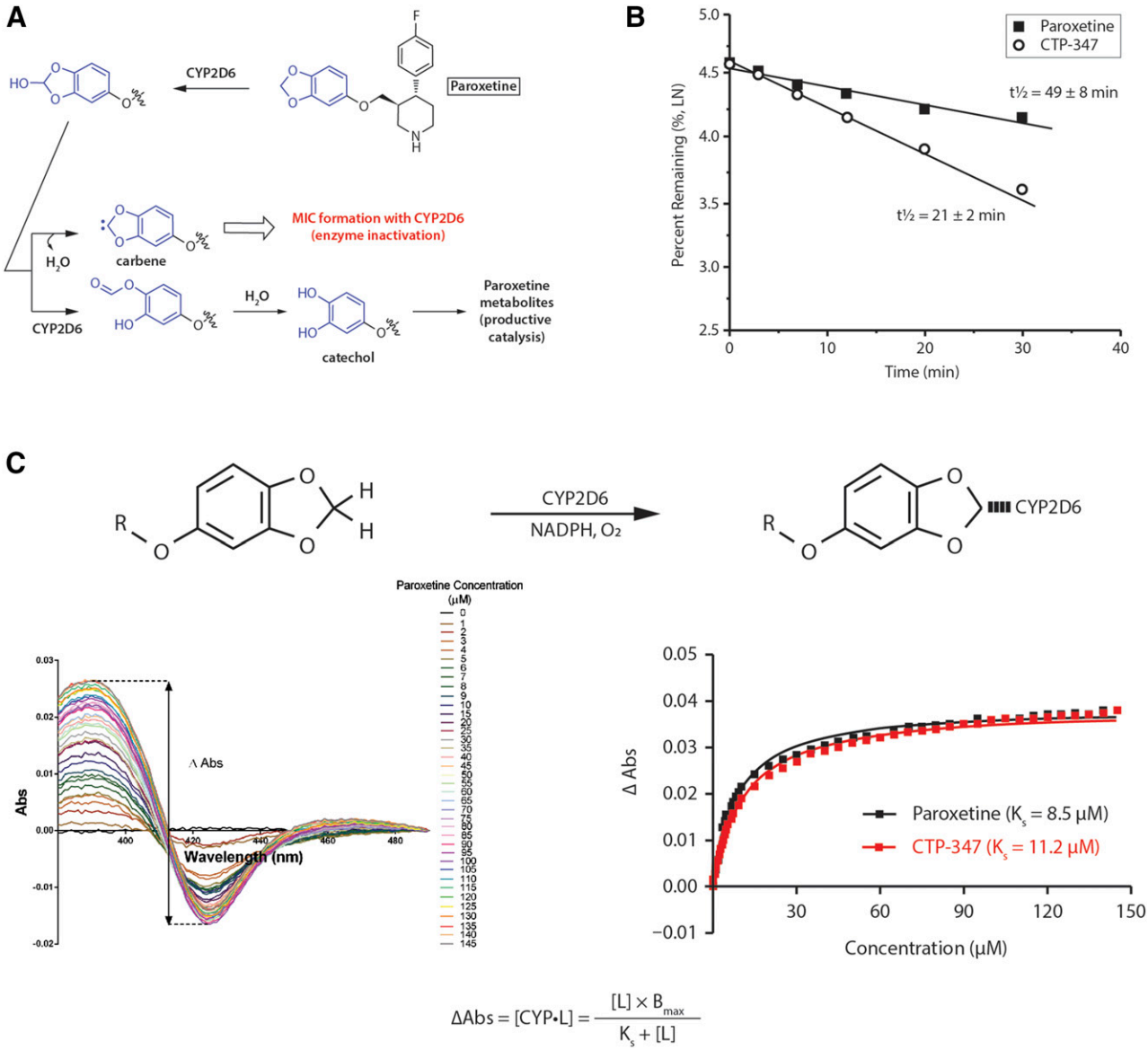

Fig. 3. Metabolism and binding of CTP-347. (A) Early steps of paroxetine metabolism. Paroxetine is metabolized by CYP2D6 to a hydroxylated methylene intermediate that has two potential fates: 1) opening to the formate ester and conversion to the catechol (productive catalysis); or 2) dehydration to form a carbene that can bind to the heme iron of CYP2D6 and form an MIC (enzyme inactivation). (B) Metabolism of CTP-347 and paroxetine by human liver microsomes. (C) Binding affinities of paroxetine and CTP-347 as assessed by type 1 binding spectra (only spectra for paroxetine binding are shown, left). B_{max}, maximum specific binding; [L], ligand concentration; K_s, equilibrium binding constant.

different concentrations of test inactivator, in this case, either CTP-347 or paroxetine, to allow for variable enzymatic inactivation. This step is followed by a second incubation with a probe substrate, in this case DM, to measure the remaining activity. Analysis of the data on a Kitz-Wilson plot determines the key kinetic parameters (Kitz and Wilson, 1962; Silverman, 1995). Using this approach in human liver microsomes (Fig. 4A, right), we found that K_I and k_{inact} for paroxetine were 1.96 μM and 0.08 minute^{-1}, respectively, similar to previously reported values (K_I = 4.85 μM and k_{inact} = 0.17 minute^{-1}) (Bertelsen et al., 2003). By comparison, little if any time-dependent inactivation was observed after preincubation with CTP-347 in the 0.125- to 10-μM concentration range (Fig. 4A, left). Because of the absence of time-dependent inhibition, K_I and k_{inact} could not be calculated for CTP-347, suggesting that the bulk of the in vitro reaction comprised productive catalysis. Limited inhibition of CYP2D6 was observed with CTP-347 at higher concentration (25 μM), although it remains unclear whether this reflected MBI, reversible inhibition (IC_{50} values were approximately 3 and 1 μM for CTP-347 and paroxetine, respectively, in the assay), or a combination of both (Fig. 4A).

Decreased MIC formation with CTP-347 was confirmed by spectral difference scanning. Previous studies showed that the MIC formed between the methylenedioxyphenyl substituent of paroxetine and CYP2D6 was characterized by peaks of absorbance at 430 and 456 nm, referred to as the type 3 binding spectrum (Bertelsen et al., 2003). Consistent with earlier studies, we observed that incubating CYP2D6 with paroxetine over approximately 30 minutes resulted in increasing peaks of absorbance at 455 nm, characteristic of MIC formation (Fig. 4B, right). By contrast, similar absorbance changes were absent when CYP2D6 was incubated with CTP-347 (Fig. 4B, left). In

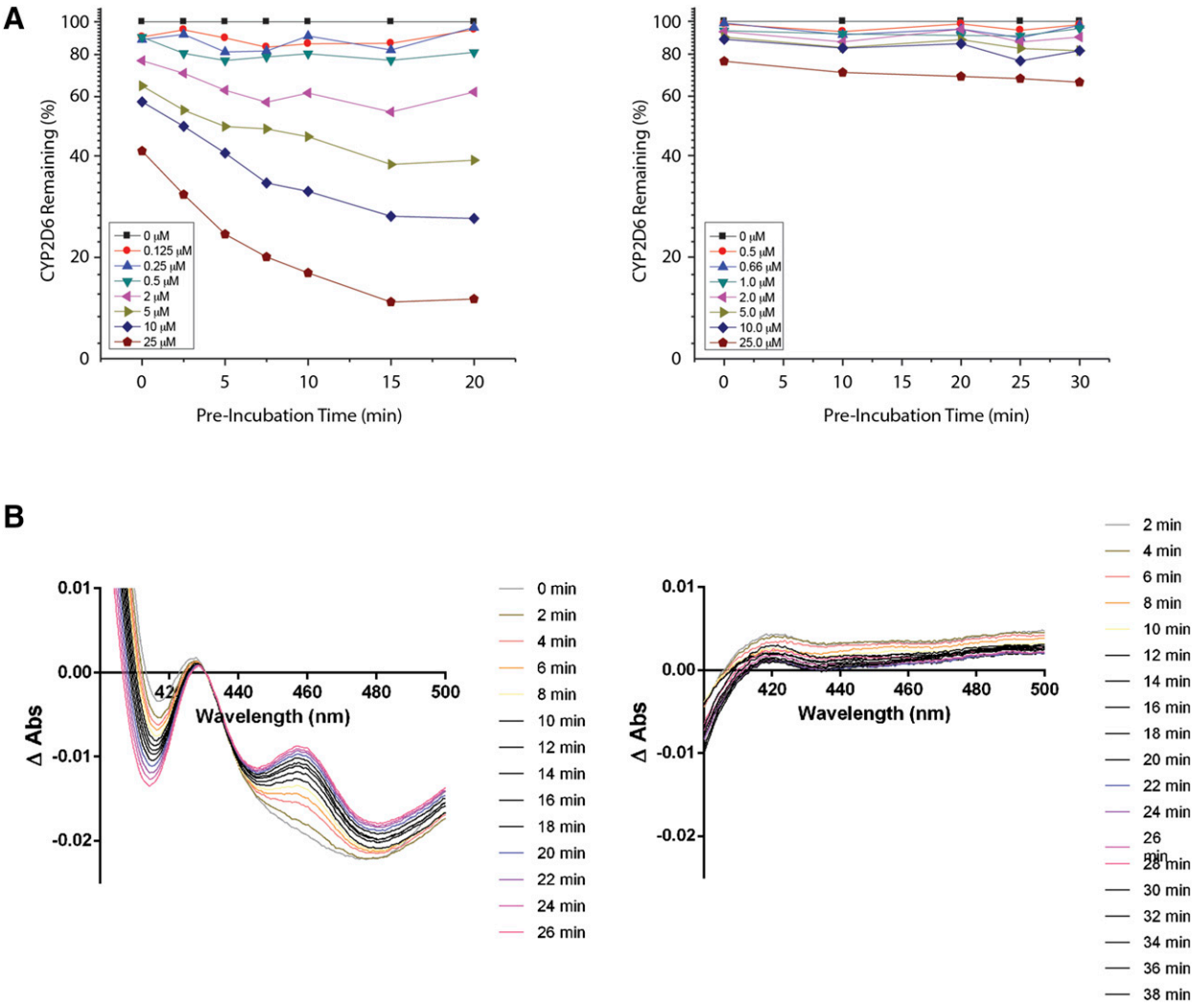

Fig. 4. Reduced mechanism-based inhibition with CTP-347. (A) Metabolism of DM by human liver microsomes after preincubation with paroxetine (left) or CTP-347 (right). (B) Spectral difference scanning of CYP2D6 after incubation with paroxetine (left) or CTP-347 (right). Abs, absorbance.

summary, the previous kinetic and spectral analyses indicated that substituting deuterium for hydrogen at the methylenedioxy carbon significantly decreased the rate of MIC formation and stimulated productive catalysis, although it should be noted that the time frames of these experiments were shorter than what might occur during a typical clinical exposure to the drug (see the following section).

CTP-347 Reduces Drug-Drug Interactions with Tamoxifen. Next, we assessed whether the apparent in vitro metabolic effects of CTP-347 extended beyond the probe substrate DM to encompass a more frequently used drug, tamoxifen (Hertz et al., 2012). Previous studies have shown that tamoxifen is metabolized to *N*-desmethyl tamoxifen, which is further metabolized by CYP2D6 to the primary active metabolite endoxifen (4-OH-*N*-desmethyl-tamoxifen) (Stearns et al., 2003). When *N*-desmethyl tamoxifen was incubated with human liver microsomes in the presence of increasing concentrations of paroxetine, the metabolism of *N*-desmethyl tamoxifen decreased substantially with higher paroxetine concentrations, presumably because of MIC formation with CYP2D6 (Fig. 5). Conversely, little or no change in endoxifen formation was observed over the range of tested CTP-347 concentrations (0–25 μM). At the highest concentration (25 μM), 8-fold more endoxifen was produced in the presence of CTP-347 than in the presence of paroxetine, demonstrating a significantly lower level of drug-drug interaction between CTP-347 and tamoxifen compared with paroxetine and tamoxifen.

CTP-347 Is Metabolized More Efficiently Than Paroxetine in Humans. The reduced MBI and drug-drug interaction of CTP-347 relative to paroxetine might be beneficial in a variety of clinical settings; however, a range of complicating factors in whole organisms may mask or even reverse the theoretical consequences of drug deuteration (Harbeson and Tung, 2011), and it was therefore of interest to determine whether any effects observed in vitro with the deuterated agent translated into the clinical setting.

To examine this issue, we first characterized the multidose pharmacokinetics of paroxetine in humans to provide comparative values for subsequent CTP-347 studies. For these initial analyses, we chose paroxetine 10 mg daily as our test dose. Thus, 10 healthy adult women (Table 2) received paroxetine 10 mg daily for 14 days, blood samples were collected over a 24-hour period on days 1 and 14, and pharmacokinetic parameters were determined (Table 3). The resulting concentration-

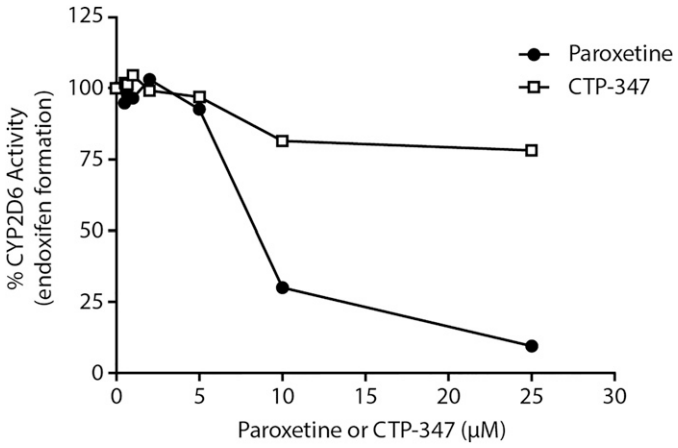

Fig. 5. Reduced drug-drug interaction with CTP-347. Metabolism of *N*-desmethyl tamoxifen to endoxifen by human liver microsomes in the presence of CTP-347 or paroxetine.

TABLE 2
Subject demographics

	CTP-347	Paroxetine
N	34	10
Median age, yr (range)	48 (22–64)	NA
Median weight, kg (range)	68 (51–87)	NA
Median body mass index, kg/m^2 (range)	26 (19–30)	NA
Female, *n* (%)	34 (100)	10 (100)
Race, *n* (%)		
African American	1 (3)	—
Asian	2 (6)	—
White	30 (88)	8 (80)
Native Indian	1 (3)	1 (10)
Unknown	—	1 (10)
CYP2D6 status, *n* (%)		
EM phenotype	33 (97)	10 (100)
PM phenotype	1 (3)	—
Genotype		
*1*1	18 (53)	NA
*1*4	13 (38)	NA
*1*10	1 (3)	NA
*10*10	1 (3)	NA
*1*7(2XN)	1 (3)	NA

—, none; NA, not available.

time curve on day 14 was significantly elevated compared with the curve on day 1 (Fig. 6A), consistent with inactivation of CYP2D6-mediated metabolism over the 2-week study period. The overall exposure to paroxetine on day 14, as assessed by median area under the plasma concentration-time curve during the dosing interval at steady state (AUC_{ss}), was 248.1 hour*ng/ml, whereas the median area under the plasma concentration-time curve during the first 24 hours of exposure on day 1 (AUC_{0-last}) was 17.8 hour*ng/ml (Table 3). In other words, the subjects were exposed to 13.9-fold more paroxetine on day 14 than day 1, despite the equivalent 10-mg oral doses on the 2 days.

We next characterized the multidose pharmacokinetics of CTP-347 in an escalating-dose study. Thirty-four healthy female subjects received once-daily doses of 10 mg ($n = 9$), 20 mg ($n = 9$), or 40 mg ($n = 9$) CTP-347 for 14 days; a fourth cohort received 10-mg doses twice daily ($n = 7$) for the same amount of time. Blood samples were again collected on day 1 and day 14, and pharmacokinetic parameters were determined (Table 3). Subjects receiving CTP-347 10 mg daily had mean concentration-time curves that were very similar on day 1 and day 14, unlike those in the earlier paroxetine 10-mg daily study (Fig. 6A). The median AUC_{ss} in the CTP-347 group was 18.0 hour*ng/ml on day 14, whereas the median AUC_{0-last} was 8.3 hour*ng/ml on day 1, for an accumulation index of 2.9 (Table 3). Thus, not only were the AUC values lower in the CTP-347 10-mg group compared with the paroxetine 10-mg group, but the accumulation of drug over time was significantly reduced. Overall exposure levels to CTP-347 at the 10 mg twice daily and 20 mg daily dosages were also lower than for paroxetine 10 mg daily on day 14 (Table 3). Thus, CTP-347 was metabolized more efficiently than paroxetine in humans and the faster clearance observed for CTP-347 in vitro translated into the clinical setting.

The rise in both AUCs and accumulation indices with increasing CTP-347 dosages nonetheless displayed a small level of nonlinearity (Table 3), suggesting some residual inhibition of CYP2D6. Further insight into this issue was gleaned by examining data on the metabolism of DM, a CYP2D6-metabolized drug, in the same dose-escalation study (Table 4). On the evenings before the first and last doses of CTP-347, 30 mg of DM solution was administered orally to each subject, urine was collected for 8 hours, and the levels of unmetabolized DM versus the DX metabolite were determined by LC-MS/MS. Consistent with the presence of some residual CYPD6 inactivation, urinary DM/DX ratios rose in all patients after 14 days of CTP-347 exposure (i.e., more unmetabolized DM was present in urine on day 14 (Fig. 7; Table 4). Nonetheless, 25 of 32 (78%) subjects who were extensive metabolizers (EMs) at baseline, defined as having DM/DX ratios <0.3 (Schmid et al., 1985; Alfaro et al., 2000), retained their EM phenotype at day 14, and of those who converted from an EM to a poor metabolizer (PM) phenotype, four of seven (57%) were in the 40-mg daily group, two of seven were in the 20-mg daily group, and one of seven was in the 10-mg twice daily group. No EM→PM conversion was observed in the CTP-347 10-mg daily group. Finally, the DM/DX ratios observed in this study were substantially lower than those reported in 12 subjects who had received paroxetine 20 mg daily for 8 days (the mean DM/DX ratios for paroxetine in this study went from 0.017 ± 0.018 to 0.601 ± 0.417; 11 of 12 subjects converted from EM→PM) (Fig. 4B) (Alfaro et al., 2000). The sum of the clinical data is in accord with in vitro results suggesting that deuterium substitution greatly reduced inactivation of CYPD6 in vivo.

Discussion

The current study assessed the potential of precision deuteration of paroxetine as an approach to positively impact the metabolism profiles of drugs. The substitution of two deuterium atoms at the methylenedioxy carbon of paroxetine resulted in a new chemical entity, CTP-347, that demonstrated substantially reduced inactivation of CYP2D6 while retaining selective serotonin uptake inhibition in rat synaptosomes and pharmacologic binding to a battery of receptors. By these criteria, deuterium substitution appeared to have limited effects on the pharmacologic and physicochemical properties of paroxetine. Despite the apparent functional similarities, however, the rate of CYP2D6-mediated metabolism was significantly greater for CTP-347 than for paroxetine, as manifested by shorter $t_{1/2}$ values for metabolism in human liver microsomes. This observation did not appear to

TABLE 3
Pharmacokinetic parameters of paroxetine and CTP-347 on days 1 and 14

	C_{max}		Day 1 AUC_{0-last}	Day 14 AUC_{ss}	Accumulation Index[a]
	Day 1	Day 14			
	ng/ml		*h·ng/ml*		
Paroxetine: Multidose PK					
Paroxetine 10 mg daily					
Patient no.					
1	0.5	4.3	4.3	57.5	13.4
2	0.5	6.9	7.1	122.3	17.2
3	3.2	17.0	40.2	296.5	7.4
4	3.2	15.6	39.1	262.2	6.7
5	1.3	14.0	15.2	261.8	17.2
6	0.3	1.1	2.6	14.8	5.7
7	1.6	25.3	22.9	494.1	21.6
8	0.4	1.0	3.0	12.2	4.1
9	1.6	14.1	20.4	234.5	11.5
10	4.0	35.9	60.0	649.3	10.8
Mean	1.6	13.5	21.5	240.5	11.2
S.D.	1.4	11.0	19.4	207.1	10.7
Median	1.4	14.0	17.8	248.1	13.9
CTP-347: Escalating dose PK					
CTP-347 10 mg daily					
Patient no.					
49	2.8	7.1	31.6	91.0	2.9
52	10.7	34.2	195.7	650.0	3.3
54	1.0	2.3	8.7	29.0	3.3
55	0.2	0.6	1.3	8.0	6.2
56	0.5	0.9	3.6	7.0	1.9
57	0.4	0.5	3.3	5.0	1.5
58	1.6	1.5	15.1	18.0	1.2
59	0.3	0.5	2.1	4.0	1.9
60	0.8	4.7	8.3	65.0	7.8
Mean	2.0	5.8	30.0	97.4	3.3
S.D.	3.36	10.9	62.9	209.4	3.3
Median	0.8	1.5	8.3	18.0	2.2
CTP-347 10 mg twice daily					
Patient no.					
85	1.5	16.9	11.4	164.0	14.4
86	0.8	1.6	5.3	11.0	2.1
88	0.2	0.9	0.6	6.0	10.0
89	0.6	1.3	2.0	11.0	5.5
90	2.3	33.2	20.0	318.0	15.9
91	0.5	5.2	4.0	50.0	12.5
92	0.2	1.0	1.1	5.0	4.5
Mean	0.8	8.6	6.3	80.7	12.8
S.D.	0.8	12.3	7.1	119.2	16.8
Median	0.8	5.2	5.3	50.0	9.4
CTP-347 20 mg daily					
Patient no.					
61	2.0	6.2	21.7	72.0	3.3
62	1.5	29.8	20.4	523.0	25.6
63	12.1	15.6	145.1	233.0	1.6
64	0.6	1.7	4.7	24.0	5.1
66	0.8	7.9	11.8	112.0	9.5
67	3.4	11.6	32.6	136.0	4.2
69	2.6	33.4	33.4	597.0	17.9
71	3.8	37.2	37.5	585.0	15.6
72	1.2	9.0	14.0	134.0	9.6
Mean	3.1	16.9	35.7	268.4	7.5
S.D.	3.6	13.1	42.5	232.6	5.5
Median	2.0	11.6	21.7	136.0	6.3
CTP-347 40 mg daily					
Patient no.					
74	1.0	31.7	11.5	489.0	42.5
75	1.3	24.0	13.7	342.0	25.0
77	2.4	75.4	28.9	1231.0	42.6
78	14.7	124.2	220.0	2170.0	9.9
79	4.7	57.9	60.3	963.0	16.0
81	10.5	132.1	132.0	2090.0	15.8
82	1.1	39.3	15.2	562.0	37.0
83	9.5	122.8	112.4	1924.0	17.1
84	5.9	59.7	66.8	1004.0	15.0
Mean	5.7	74.1	73.4	1197.2	16.3
S.D.	4.9	42.2	70.2	707.2	10.1
Median	4.7	59.7	60.3	1004.0	16.7

ND, not determined; PK, pharmacokinetics.
[a]Day 14 AUC_{ss}/Day 1 AUC_{0-last}.

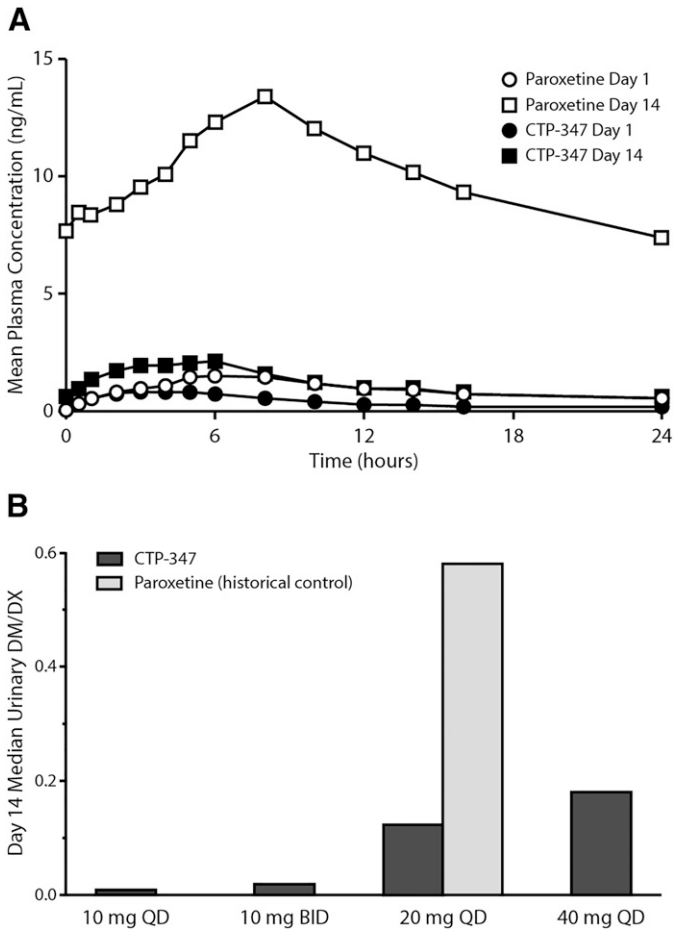

Fig. 6. CTP-347 pharmacokinetics. Mean plasma concentration-time curves after administration of 10 mg CTP-347 or paroxetine (A) and median urinary DM/DX ratios (B) after 14 days of dosing to humans. The paroxetine bar in (B) is an historic control (Alfaro et al., 2000), as assessed 8 days after initiation of 20 mg daily administration.

TABLE 4
Urinary DM/DX ratios for CTP-347 on days 1 and 14

	Urinary DM/DX	
	Baseline	Day 14
CTP-347 10 mg daily		
Patient no.		
49	0.0110	0.0260
52	0.9720[a]	1.4050[a]
54	0.0150	0.0720
55	0.0001	0.0050
56	0.0020	0.0070
57	0.0020	0.0050
58	0.0040	0.0090
59	0.0010	0.0040
60	0.0180	0.1050
Mean	0.1139	0.1820
S.D.	0.3219	0.4600
Median	0.0040	0.0090
CTP-347 10 mg twice daily		
Patient no.		
85	0.0140	0.1640
86	0.0010	0.0020
88	0.0002	0.0010
89	0.0010	0.0050
90	0.0160	0.3530 [a]
91	0.0020	0.0330
92	0.0010	ND
Mean	0.0050	0.0930
S.D.	0.0069	0.1419
Median	0.0020	0.0985
CTP-347 20 mg daily		
Patient no.		
61	0.0030	0.0270
62	0.0050	0.2580
63	0.1020	0.2530
64	0.0020	0.0220
66	0.0040	0.0430
67	0.0380	0.1230
69	0.0090	0.4820[a]
71	0.0070	0.3120[a]
72	0.0060	0.0500
Mean	0.0196	0.1744
S.D.	0.0328	0.1609
Median	0.0060	0.1230
CTP-347 40 mg daily		
Patient no.		
74	0.0040	0.1670
75	0.0030	0.0730
77	0.0010	0.1800
78	0.0380	0.8500[a]
79	0.0010	0.1370
81	0.0020	0.7230[a]
82	0.0010	0.1200
83	0.0010	0.4960[a]
84	0.0110	0.3880[a]
Mean	0.0069	0.3482
S.D.	0.0121	0.2849
Median	0.0020	0.1800

ND, not determined.
[a]Poor metabolizer.

be attributable to differences in binding affinities for CYP2D6 as K_s values for CTP-347 and paroxetine were similar. Moreover, the effect did not appear to be due to changes in metabolism pathways, as studies in human hepatocytes demonstrated that the metabolic profiles of CTP-347 and paroxetine were qualitatively very similar (data not shown). Instead, kinetic analyses indicated that the higher rate of CTP-347 metabolism was due to a decrease in MIC formation with CYP2D6 and a consequent reduction in MBI. Consistent with this, CTP-347 exhibited significantly less drug-drug interaction with tamoxifen, another substrate for CYP2D6 metabolism. To our knowledge, this is the first demonstration of a targeted deuterium substitution approach that greatly diminished MBI liability of an approved drug.

To examine whether the in vitro results translated into the clinical setting, we assessed the steady-state pharmacokinetics of CTP-347 in healthy female volunteers. The results demonstrated that the exposure (AUC_{ss}) of CTP-347 was lower than that of paroxetine after administration of equivalent doses (10 mg daily), establishing that the metabolism of CTP-347 was more rapid in humans than paroxetine. Thus, the in vitro results were recapitulated in humans, a prerequisite for making deuterium substitution a practical approach in drug development. Further, subjects treated with CTP-347 20 mg daily, because of its more rapid metabolism, had an exposure at steady state (AUC_{ss} = 268.4 hour*ng/ml) that was roughly similar to paroxetine 10 mg daily (AUC_{ss} = 240.5 hour*ng/ml). At 20 mg daily, CTP-347 exhibited lower DM/DX ratios and EM→PM conversions than historical paroxetine 20 mg daily data (Alfaro et al., 2000), demonstrating its potential for eliminating drug-drug interactions in vivo and again mirroring the in vitro results.

A closer analysis of the pharmacokinetic results and the urinary DM/DX ratios suggested that there was some residual inactivation of CYP2D6 in human, although it was greatly

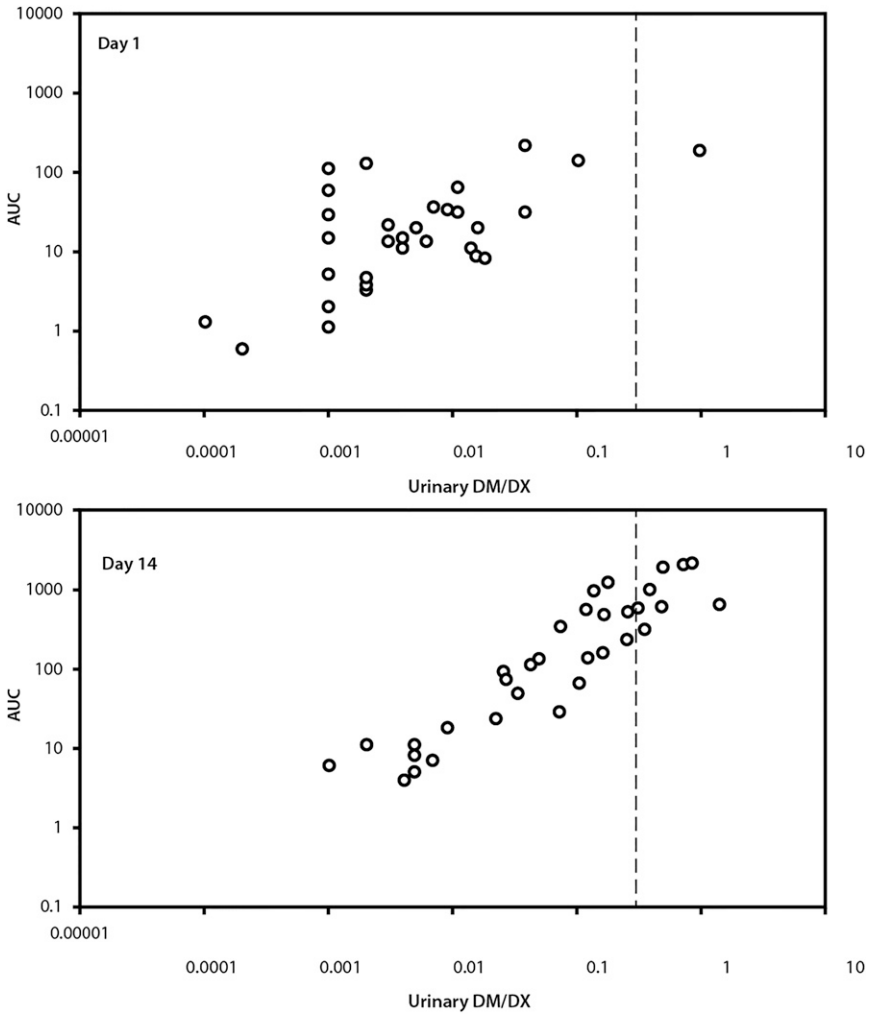

Fig. 7. Exposure (AUC) versus baseline urinary DM/DX ratios for CTP-347 on day 1 and day 14. Extensive metabolizers are to the left, and poor metabolizers are to the right of the dashed lines.

diminished compared with paroxetine. The decrease in ground-state energy for the C-D bond raises the transition energy necessary for its scission (Wiberg, 1955; Shao and Hewitt, 2010). Whether this effect is enough to completely eliminate or, instead, substantially lower, its overall rate is likely to be dependent on the specific context of each reactant-enzyme pair. These effects may become more noticeable in the in vivo setting, where the characteristics of drug distribution, as well as the repeat administrations, may provide conditions whereby a small level of MIC formation can occur. From a practical standpoint, however, it is likely that, even in this case, the overall reduction in MBI could provide substantial clinical benefit.

Heavy water (D_2O) is used as a moderator in nuclear reactors, and multi-ton quantities of deuterium are commercially available at reasonable cost (Marter et al., 1982). Moreover, deuterium exposure in the form of D_2O appears to have low systemic toxicity; 15%–23% deuterium replacement in whole body plasma has been reported to have no evident adverse effects in humans (Blagojevic et al., 1994). The abundant availability, reasonable cost, and low toxicity, coupled with the potential to modify metabolism properties, suggest that deuterium substitution may play a larger role in drug development in the future. One recent report, for instance, described a phase 1 evaluation of another novel deuterium-containing, controlled-release drug candidate under development for diabetic neuropathy, although the effects of this latter agent on metabolic and pharmacokinetic profiles were not detailed (Braman et al., 2013). Other new agents are being investigated (http://investor.auspexpharma.com/release-detail.cfm?releaseid=887958; Harbeson and Tung, 2011; Lu et al., 2015). It is important to note that the effects observed in this study (i.e., reduced MBI and a consequent increase in first-pass metabolism) may be quite different from the metabolism-related effects imparted by deuteration of other drugs. For instance, in other contexts, decreasing bond scission rates could reduce first-pass metabolism, which could be beneficial in certain scenarios. Alternatively, deuteration could affect the proportions of metabolic end products. It is hoped that empirical characterization of this expanding class of drugs, using approaches similar to those described in this report, will provide better tools to predict how deuterium substitution can be leveraged in the future and, as a result, to improve the safety and efficacy of existing therapeutic agents.

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Authorship Contributions

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Conducted experiments: Uttamsingh, Gallegos, Liu, Bridson, Cheng.

Contributed new reagents or analytic tools: Bridson, Cheng, Liu.

Performed data analysis: Uttamsingh, Liu, Graham, Wells, Zelle.

Wrote or contributed to the writing of the manuscript: Uttamsingh, Liu, Harbeson.

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