Deuterium Medicinal Chemistry: A New Approach to Drug Discovery and Development

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Introduction

Selective replacement of hydrogen atoms with deuterium (deuteration) has the unique benefit of retaining the pharmacologic profile of physiologically active compounds while, in certain instances, positively impacting their metabolic fate. In these favorable cases, deuterium substitution can in principle improve the safety, efficacy, and/or tolerability of a therapeutic agent. Selective deuteration of compounds such as approved drugs with well-defined human pharmacological effects can potentially provide an efficient and accelerated approach to creating significantly differentiated, patentable new medicines that address important unmet medical needs.

Deuterated compounds have been extensively studied in non-clinical settings and have been used clinically as metabolic or pharmacokinetic probes; however, no deuterated compound has been approved as a human medicine.[1,2] It is unclear as to why a deuterated agent has not been marketed since there do not appear to be any regulatory or economic impediments that would prevent the registration of a deuterated drug. An increased recent interest in deuterium substituted medicines is reflected by a greater number of patent filings, the emergence of new companies focused on this area, and the recent entry of several compounds into clinical trials.[3] Early clinical results have been encouraging, which supports the potential for important new deuterated drugs to reach the market.

Deuterium Background

Deuterium is a naturally-occurring, stable, non-radioactive isotope of hydrogen discovered in 1932.[4] Hydrogen consists of one electron and one proton and has an atomic mass of approximately 1.0 atomic mass unit (AMU). Deuterium also has a single electron but its nucleus contains one neutron and one proton, resulting in an atomic mass of approximately 2.0 AMU.

Deuterium occurs at a natural abundance of approximately 0.0015%, which allows it to be sourced from bulk water to produce highly enriched D_2O (heavy water). D_2O is available on a large scale due to the need for multi-

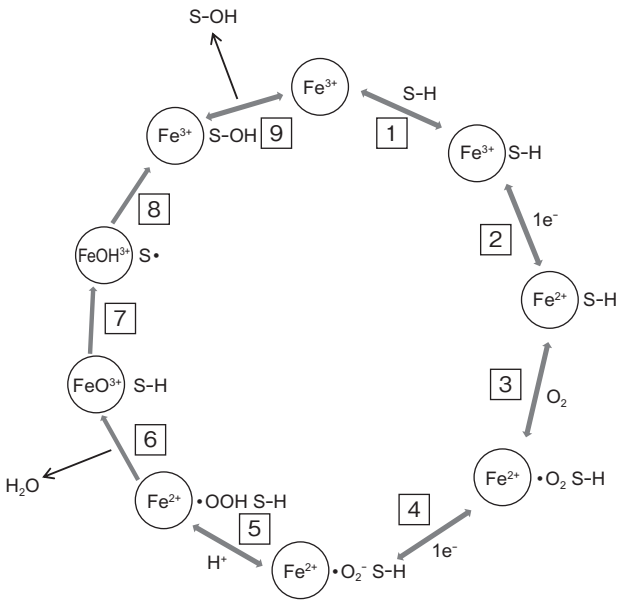
ton quantities of heavy water used in nuclear reactors.[5] Depending on the desired sites of deuteration, in some cases deuterium from D_2O can be exchanged directly into finished drug compounds or into reagents that are useful for synthesizing drug molecules.[6,7] Deuterium gas is also a useful starting material for incorporating deuterium into molecules. Catalytic deuteration of olefinic and acetylenic bonds is a rapid route for incorporation of deuterium.[8] Metal catalysts (i.e., Pd, Pt, and Rh) in the presence of deuterium gas can be used to directly exchange deuterium for hydrogen in functional groups containing hydrocarbons.[9] It is of particular note that a fairly wide variety of deuterated reagents and synthetic building blocks are commercially available from companies such as C/D/N Isotopes, Quebec, Canada (www.cdnisotopes.com); Cambridge Isotope Laboratories Inc., Andover, MA, USA (www.isotope.com); and CombiPhos Catalysts, Inc., Princeton, NJ, USA (www.combiphos.com).

With regard to the shape and size of a molecule, deuterium substitution for hydrogen yields a deuterated compound that is quite similar to the all-hydrogen compound.[10] However, minor physical property changes have been measured in partially or fully deuterated compounds, which include reduced hydrophobicity,[11,12] decreased acidity of carboxylic acids and phenols,[13] and increased basicity of amines.[14] These differences tend to be quite small and in most cases, deuteration of a non-covalent drug has negligible effects upon biochemical potency or selectivity for relevant pharmacological targets. However, binding isotope effects are well known and recent data support their unpredictability in that they can contribute positively or negatively to a measured deuterium kinetic isotope effect.[15-17]

Primary Deuterium Kinetic Isotope Effect (DIE)

Due to the greater atomic mass of deuterium, cleavage of the carbon-deuterium (C–D) covalent bond requires greater energy than the carbon-hydrogen (C–H) bond. C–D bonds have a lower vibrational frequency and, therefore, lower zero-point energy than a corresponding C–H bond.[18] The lower zero-point energy results in a higher activation energy and a slower rate (k) for C–D bond cleavage. This rate effect is the primary deuterium isotope effect (DIE) and is expressed as k_H/k_D, the ratio of the rate of C–H vs. C–D bond-cleavage and has a theoretical limit of 9 at 37℃ in the absence of quantum mechanical tunneling.[19,20] The DIE could potentially affect the pharmacokinetics of many drugs that are metabolized by pathways involving C–H bond scission. However, the observed DIE – $(k_H/k_D)_{obs}$ – for metabolic reactions is often "masked", which means that it can be much smaller than k_H/k_D or, in some cases, altogether absent.[21,22] There are even reports of inverse deuterium isotope effects [$(k_H/k_D)_{obs} < 1$].[23]

The cytochrome P450s (CYPs) represent the most important enzymes in drug metabolism and catalyze the Phase 1 metabolism of most drugs. There are a number of reviews on the structure, function, mechanism and use of the DIE to study CYP-catalyzed reactions.[19,24-28] A proposed catalytic mechanism of the CYP450s is shown in **Scheme 1**. This complexity, in addition to potential alternative clearance mechanisms or sites of metabolism, can result in a number of competing effects that mask the



Scheme 1. General catalytic cycle for CYP catalyzed oxidative metabolism. Adapted from reference 25, S-H: substrate; S-OH: oxidized substrate.

DIE, thereby making the application of deuterium to drug discovery highly unpredictable and challenging.[20] When hydrogen is replaced by deuterium, the C–H bond cleavage step must be at least partially rate-limiting in order to observe a DIE. The masking of k_H/k_D can arise via several mechanisms, including the rate of C–D bond cleavage (Step 7, **Scheme 1**) contributing little or nothing to the rate limiting step, product release (Step 9, **Scheme 1**) contributing significantly to the rate limiting step, the presence of irreversible steps prior to the bond cleavage in the catalytic sequence (Step 6, **Scheme 1**), and metabolic switching, which is a change from one metabolic site to a different site.[19,20,29,30] Proton abstraction in some cases is partially or largely due to quantum tunneling, which is highly sensitive to atomic mass and can result in unusually large deuterium isotope effects.[21] Due to these complexities, the impact of deuterium substitution for hydrogen upon the rate of CYP450 metabolism is highly unpredictable and will be dependent upon the specific compound and the deuterium substitution pattern on the molecule.

Deuterium Safety and Pharmacology

The ready availability of D_2O and deuterated compounds has facilitated the exploration of deuterium's effects upon a variety of organisms. The available literature supports that deuterium has remarkably low systemic toxicity. Single-celled organisms can often be grown in an environment that is fully deuterated and lower organisms, such as fish and tadpoles, have been reported to survive in at least 30% D_2O.[31] Mice and dogs tolerate long-term replacement of at least 10–15% of body fluid hydrogen with deuterium; however, toxicity is observed with sub-acute or chronic exposure above 25%.[32]

As observed for non-human species, humans can also tolerate high exposure to deuterium in body fluids. No evident toxicity was observed upon acute exposure to levels of 15–23% deuterium replacement in whole body plasma.[33] D_2O is excreted by humans via the urine with a half-life of about 10 days, similar to that of H_2O.[34]

Deuterium Effects upon Pharmacokinetics

As noted earlier, deuterium incorporation can sometimes significantly alter the metabolic profile of a molecule, thereby resulting in changes in the ratio of parent drug to metabolites and changes in the amounts of metabolites formed. Importantly, we are not aware of deuteration resulting in unique metabolites that have not been observed for the all-hydrogen analog. However, reduced rates of metabolism and metabolic switching, where the ratio of metabolites is changed, have been reported.[35,36] These changes in the exposure to parent drug and metabolites can have important ramifications with respect to the pharmacodynamics, tolerability and efficacy of a deuterated drug. The panels in **Figure 1**

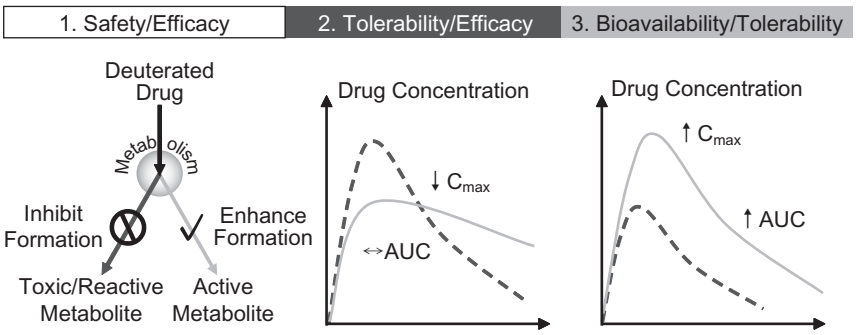


Figure 1. Pharmacological expressions of drug deuteration effects. Panel 1: Metabolic shunting resulting in reduced exposure to undesirable metabolites or increased exposure to desired active metabolites. Panel 2: Reduced systemic clearance resulting in increased half-life. Panel 3: Decreased pre-systemic metabolism resulting in higher bioavailability of unmetabolized drug. AUC is area under the curve and represents drug exposure over time; Cmax is the maximum or peak concentration of a drug.

show three general categories for the potential effects of deuterium on the pharmacokinetics and pharmacodynamics of a drug. Each of these will be discussed separately along with literature examples.

Many drugs are metabolized in complex patterns, resulting in the formation of both active and innocuous metabolites in addition to reactive or toxic metabolites. Figure 1, panel 1 illustrates metabolic shunting in which deuterium substitution reduces the formation of an undesired or toxic metabolite as well as enhancing the formation of a desired metabolite. The potential benefits of metabolic shunting in terms of human medicines will be discussed for two preclinical examples – nevirapine and efavirenz – and, in a later section, a clinical example – CTP-347.

Nevirapine (**1**, Figure 2) provides an example of metabolite shunting in which a deuterated analog (**2**, Figure 2) was prepared that showed effects upon both the metabolic clearance and toxicity. **1** Is a non-nucleoside reverse transcriptase inhibitor for the treatment of HIV infection that is associated with a relatively high incidence of skin rash and hepatotoxicity in humans.[37] The proposed pathways to the hepatotoxicity and skin rash of **1** are shown in Scheme 2 in which CYP metabolism produces a radical intermediate that can either lose a hydrogen atom to produce reactive metabolite **6** or undergo hydroxylation to metabolite **5**, which can be sulfated to reactive metabolite **7**.[38] The deuterium substitution pattern of **2** significantly reduces (approx. 5-fold) covalent binding to hepatic proteins in both mouse and rat hepatic microsomes. Incubation of **1** with human expressed CYP3A4 supersomes also resulted in covalent binding, which was greatly reduced for **2**. These data support the formation of reactive intermediate **6** (Scheme 2) that not only inactivates CYP metabolic enzymes but may

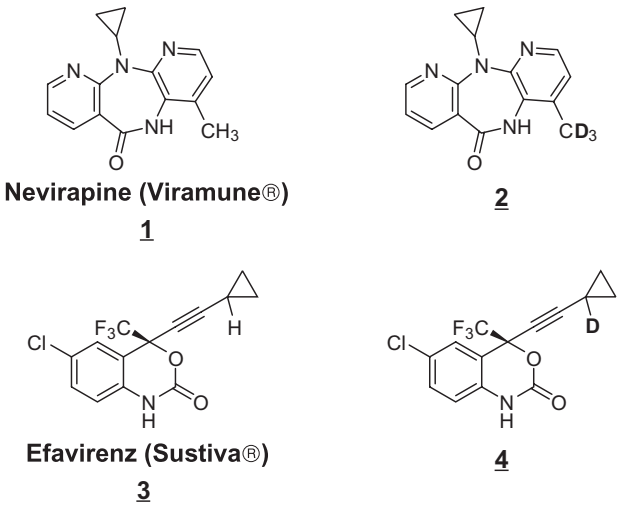
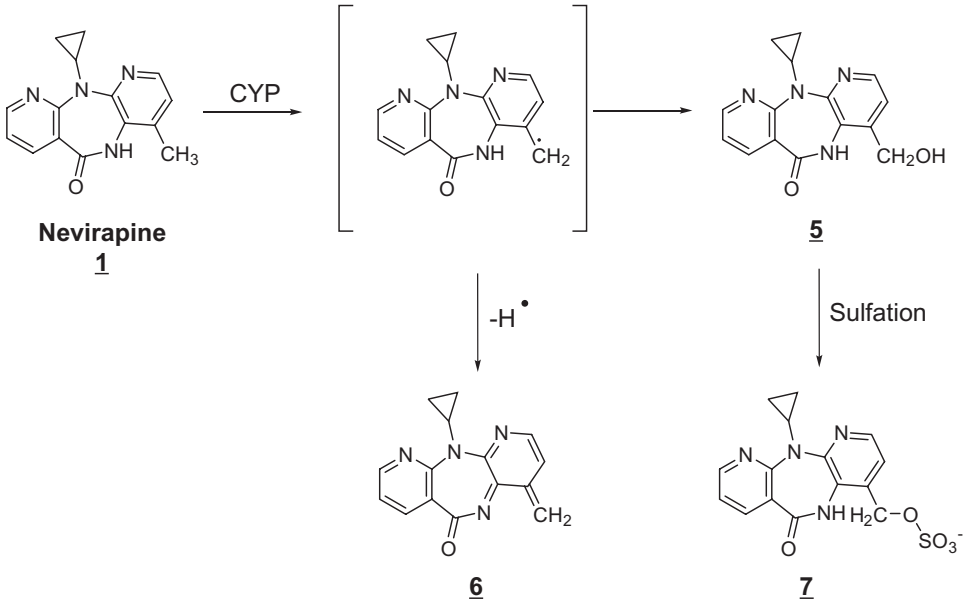


Figure 2. Structures of compounds and deuterated analogs that exhibit effects of deuteration as shown in **Figure 1**, panel 1.



Scheme 2. Proposed mechanisms for nevirapine mediated CYP inhibition, hepatic injury and skin rash. Adapted from reference 38.

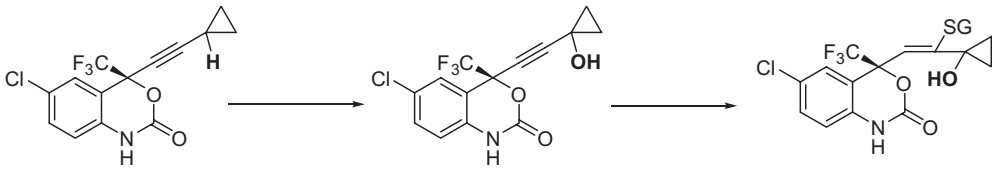
also produce the hepatotoxicity observed for **1**. After oral dosing in rats, **2** was rapidly cleared, resulting in plasma levels that were much lower than observed with the same dose of **1**. This increase in clearance for **2** is attributed to metabolic switching away from the postulated reactive metabolite **6** that inactivates CYP450, which results in less CYP inhibition and faster *in vivo* clearance.

The metabolic shunting proposed for **2** that results in the decreased CYP inhibition and reduced covalent modification of hepatic proteins has also been used to understand the mechanism for skin rash induced by **1**.[39] Using a rat model for the skin rash, **2** was shown to produce much less 12-hydroxy-metabolite **5**, which reduced the incidence and severity of the rash vs. **1**. As shown in Scheme 2, the authors propose that **1** is oxidized by CYP enzymes to the 12-hydroxy metabolite **5**, which is then activated by sulfotransferases in the skin to the sulfate ester **7**. This sulfate ester may be the metabolite that covalently modifies skin proteins thereby resulting in an immune-mediated rash.

Deuterated efavirenz (**4**, Figure 2) is an example in which metabolic shunting was used to elucidate the mechanism for nephrotoxicity in rodents.[36] Efavirenz (**3**, Figure 2) is a non-nucleoside reverse transcriptase inhibitor used for the treatment of HIV infection.[40] During safety assessment in preclinical studies, **3** was found to produce nephrotoxicity in rats but not in other species. The metabolite profile produced in rat liver and kidney preparations showed the formation of a glutathione adduct (**9**, Scheme 3) that was not observed in similar tissue preparations from humans and monkeys.[41] Since the nephrotoxicity was only observed in rats, the rat specific metabolic pathway shown in Scheme 3 leading to **9** was proposed as producing the reactive metabolite responsible for the toxicity. By replacing the cyclopropyl methine hydrogen with deuterium, thereby reducing the oxidative metabolism to **8** (Scheme 3), it was shown that both the incidence and severity of nephrotoxicity was greatly reduced. Deuterium substitution, therefore, shunted metabolism away from the reactive metabolite and established that the nephrotoxicity observed in rats was specific to a rat metabolite that was not observed in humans.

Figure 1, Panel 2 illustrates cases where the major effect of deuteration is to reduce the rate of systemic clearance. As a result, the biological half-life of the compound is increased. The potential clinical benefits shown in Panel 2 would include the ability to maintain similar systemic exposure with decreased peak levels and increased trough levels. This could result in lower side effects and enhanced efficacy, depending on the particular drug's pharmacokinetic/pharmacodynamic relationship. Indiplon, ML-337, and odanacatib, shown in Figure 3, are examples of this deuterium effect.

The effect of deuteration on the metabolism of the $GABA_A$ agonist sleep agent indiplon (**10**, Figure 3) provides an example of improved PK resulting from a decrease in systemic clearance and longer half-life.[42] In this example, replacement of the N-CH_3 with N-CD_3 (**11**, Figure 3) resulted in increases in half-life in both rat and human liver microsomes by 30% and 20%, respectively. This *in vitro* result was predictive of the *in vivo* pharmacokinetics in rat. Individual oral dosing of **10** (20 mg/kg) or **11** (20 mg/kg) in rats showed a distinct pharmacokinetic advantage for the deuterated molecule since the half-life increased 2-fold and the exposure increased 2.6-fold. The binding of **11** to the $GABA_A$ receptor was found to be similar to **10** indicating that the receptor affinity of



Efavirenz **3** **8** **9**

Scheme 3. Proposed metabolic pathway for efavirenz mediated renal toxicity in rats. Adapted from reference 36.

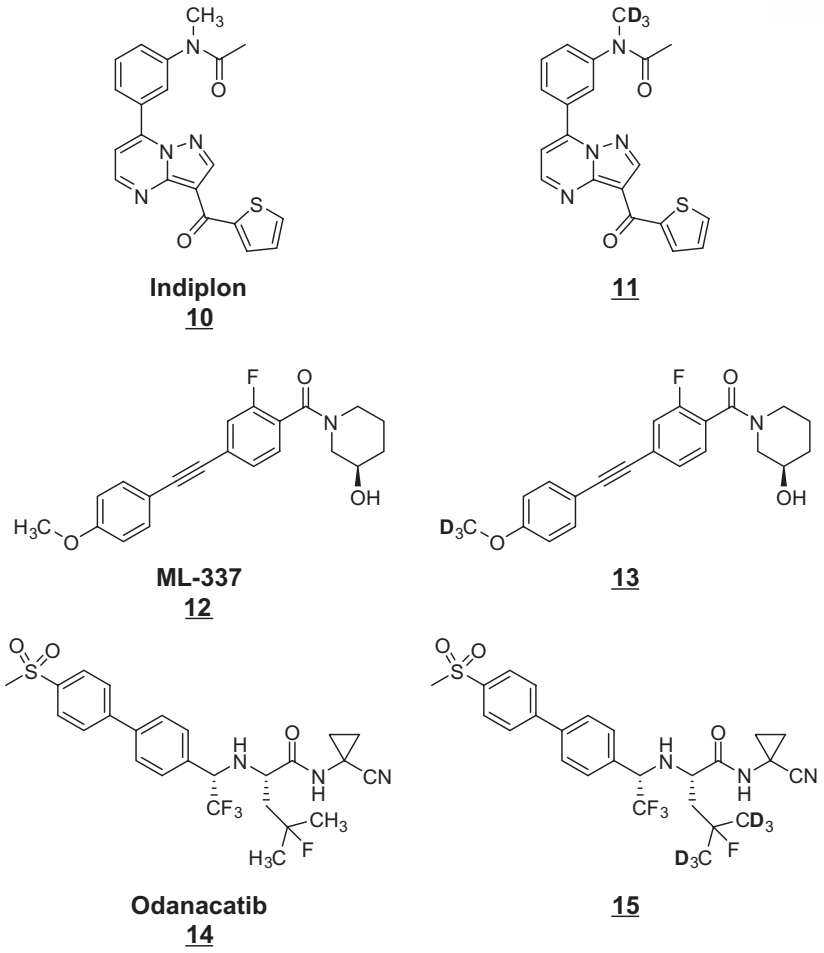
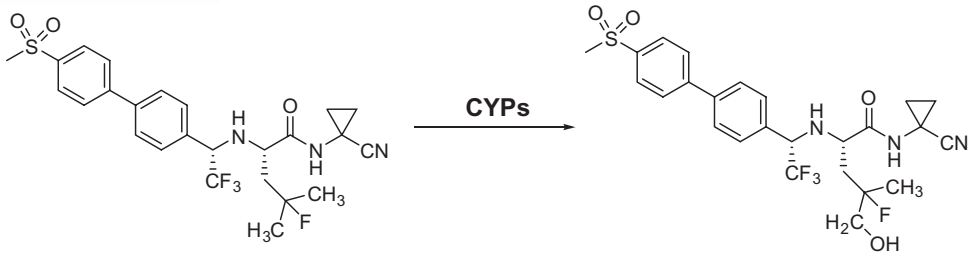


Figure 3. Structures of compounds and deuterated analogs that exhibit effects of deuteration as shown in **Figure 1**, panel 2.

the deuterated agent had not been altered.

ML-337 (**12**, Figure 3) is a negative allosteric modulator at the sub-type 3 G-protein coupled metabotropic glutamate receptor.[43] It is believed that agents of this class could have therapeutic utility for the treatment of cognitive disorders, schizophrenia, depression and Alzheimer's disease. The structure-activity relationships developed during the discovery of **12** showed that the para-methoxy substituent was required for the desired pharmacology; however, CYP mediated demethylation was identified as a major metabolic pathway. Since the para-methoxy substituent of **12** was required for activity, the authors prepared the deuterated methoxy compound **13** (Figure 3) to improve the metabolic stability. The pharmacology was not significantly impacted by deuteration; however, the metabolic stability was positively impacted. In rat liver microsomes, the intrinsic clearance (mL/min/kg) was 73.7 for **13** vs. 239 for **12**. The observed improvement of *in vitro* metabolic stability translated to an *in vivo* study in rats. An intravenous pharmacokinetic codose study (0.2 mg/kg each compound) in rats showed **13** to have a slower plasma clearance (2.9 mL/min/kg) than **12** (5.2 mL/min/kg). Importantly, no new metabolites were observed.

Odanacatib (**14**, Figure 3) is another example of deuterium substitution resulting in decreased *in vivo* clearance. **14** is a cathepsin K inhibitor in Phase 3 clinical studies for the treatment of post menopausal osteoporosis.[44] Cathepsin K appears to be the primary enzyme involved in osteoclast mediated bone resorption. One of the major metabolic pathways identified in rats, dogs and monkeys was hydroxylation of the fluoro-isobutyl sidechain to produce **16** as shown in Scheme 4.[45] A deuterated analog (**15**, Figure 3), was prepared and the



Scheme 4. A major metabolic pathway for odanacatib in rats, dogs and rhesus monkeys.

pharmacokinetics were studied in monkeys.[46] When a 1:1 mixture of **14** and **15** (0.75 mg/kg each) was dosed intravenously, the deuterated compound had 3-fold greater exposure (17,700 vs. 5,726 nM*hr) and 3-fold lower clearance (1.4 vs. 4.6 mL/min/kg), which translated to a longer half-life for **15** vs. **14**: 16.0 vs 3.7 hr, respectively. A similar improvement in PK was also observed when a 1:1 mixture of **14** and **15** (2.5 mg/kg each) was dosed orally to monkeys. The half-life of **15** was increased 2.5-fold vs. **14**.

Figure 1, Panel 3 illustrates a predominantly presystemic effect of deuteration, which has been reported for the compounds in Figure 4: rofecoxib (**17**) and telaprevir (**19**). In these cases, reduced rates of metabolism result in an increase in exposure of the drug without changing the rate of systemic clearance. Deuterated drugs showing this effect may have reduced dosing requirements and produce lower metabolite loads. Since gastrointestinal irritation has been related to the amount of dosed compound rather than blood concentration for certain drugs, this effect could allow enhanced tolerability due to the need for less dosed drug to achieve the same exposure.

Rofecoxib (**17**, Figure 4) is a COX-2 selective nonsteroidal anti-inflammatory drug (NSAID) approved by the FDA in 1999 but withdrawn from the market in 2004 because of safety concerns about increased risk of heart attack and stroke.[47] Deuterated rofecoxib (**18**, Figure 4),

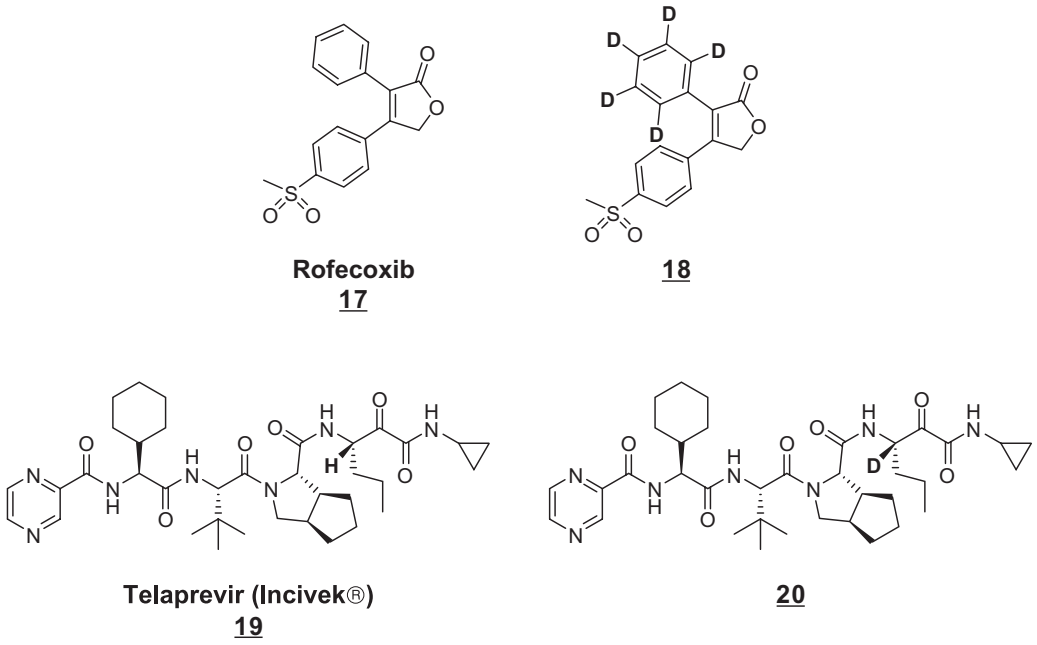
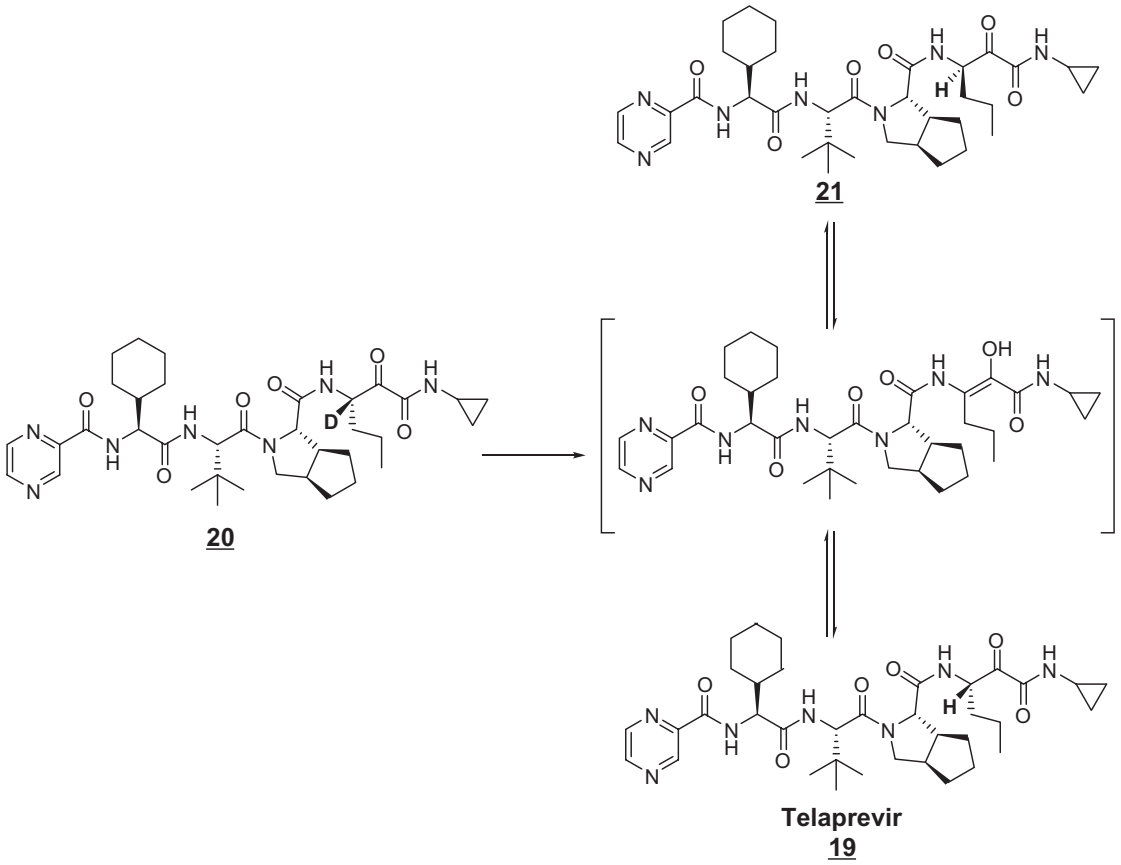


Figure 4. Structures of compounds and deuterated analogs that exhibit effects of deuteration as shown in **Figure 1**, panel 3.

also known as BDD-11602, was reported to have an improved pharmacokinetic profile vs. rofecoxib in rats.[48] Parallel groups of rats (8/group; 4 males and 4 females) were dosed orally with either **18** or **17** at 3 different doses (0.1, 1.0 and 10 mg/kg) and the plasma levels were measured. The mean Cmax value for all doses of the deuterated compound **18** was increased 1.6-fold vs. **17** and the mean exposure was increased 1.5-fold. No improvement in oral half-life was observed. The inhibition of COX-2 enzyme activity was unchanged for **18** (IC_{50}=173 nM) vs. **17** (IC_{50}=169 nM). These data again show that deuterium can significantly impact the pharmacokinetics of a compound without changing the intrinsic pharmacology.

Telaprevir (**19**, **Figure 4**) is an inhibitor of hepatitis C viral NS3-4A protease that was approved as Incivek® in 2011 for the treatment of hepatitis C infection.[49] **19** is the S-diastereomer in which the chiral center adjacent to the ketoamide moiety has the S-chirality. As shown in **Scheme 5**, **19** epimerizes *in vivo* via an enol intermediate to the R-diasteromer **21**, which is the major circulating metabolite in plasma and is a 30-fold less active protease inhibitor than **19**. The hydrogen at this chiral center was replaced with deuterium to provide deuterated telaprevir (**20**, **Figure 4**), which should slow the rate of conversion to the enol and the resulting epimerization to the R-diastereomer **21**.[50] Upon incubation in dog, rat and human plasma, deuterium isotope effects for the rate of epimerization of **20** ranged from 4- to 7-fold slower than for **19**. When **20** and **19** were orally co-dosed to rats (5 mg/kg of each compound) a modest 13% increase in exposure for deuterated compound **20** vs. **19** was observed. As noted for other examples, deuterium substitution had a negligible effect on the pharmacology in that the antiviral activity in the replicon assay was not significantly altered: IC_{99}=4.0 uM and 3.3 uM for **20** and **19**, respectively.



Scheme 5. Reaction scheme for the interconversion of telaprevir (**19**) to its diastereomer **21** and the conversion of **20** to **19** and **21** via an enol intermediate.

Clinical Experience with Deuterium Containing Drugs

Fludalanine (**22**, Figure 5), which was advanced into development by Merck, appears to be one of the earliest deuterated drug candidates to enter the clinic.[51] The combination of **22** with cycloserine provides a broad-spectrum and potent antibacterial. The hydrogen analog is also an effective antibacterial, but preclinical studies reportedly demonstrated that it was metabolized to form L-3-fluorolactate (**24**, Scheme 6), a toxin that caused brain vacuolization. Kahan reported in a recent letter that the DIE reduced production of **24** to what were deemed acceptable levels in healthy volunteers.[52] However, these reduced levels were not observed in patients; therefore, studies on fludalanine were discontinued at Phase IIb.

Phase 1 clinical results have been reported by Concert Pharmaceuticals for CTP-347 (**25**, Figure 5), a compound advanced to development for the treatment of hot flashes.[53] **25** is a selectively deuterated analog of paroxetine (**26**, Figure 5), a centrally acting SSRI (selective serotonin reuptake inhibitor) for the treatment of major depressive disorder, panic disorder, social anxiety disorder, and premenstrual dysphoric disorder.[54] Low doses of **26** have been reported to have good efficacy in treating hot flashes.[55] However, **26** is not only metabolized by CYP2D6 but also potently inhibits its own metabolism by

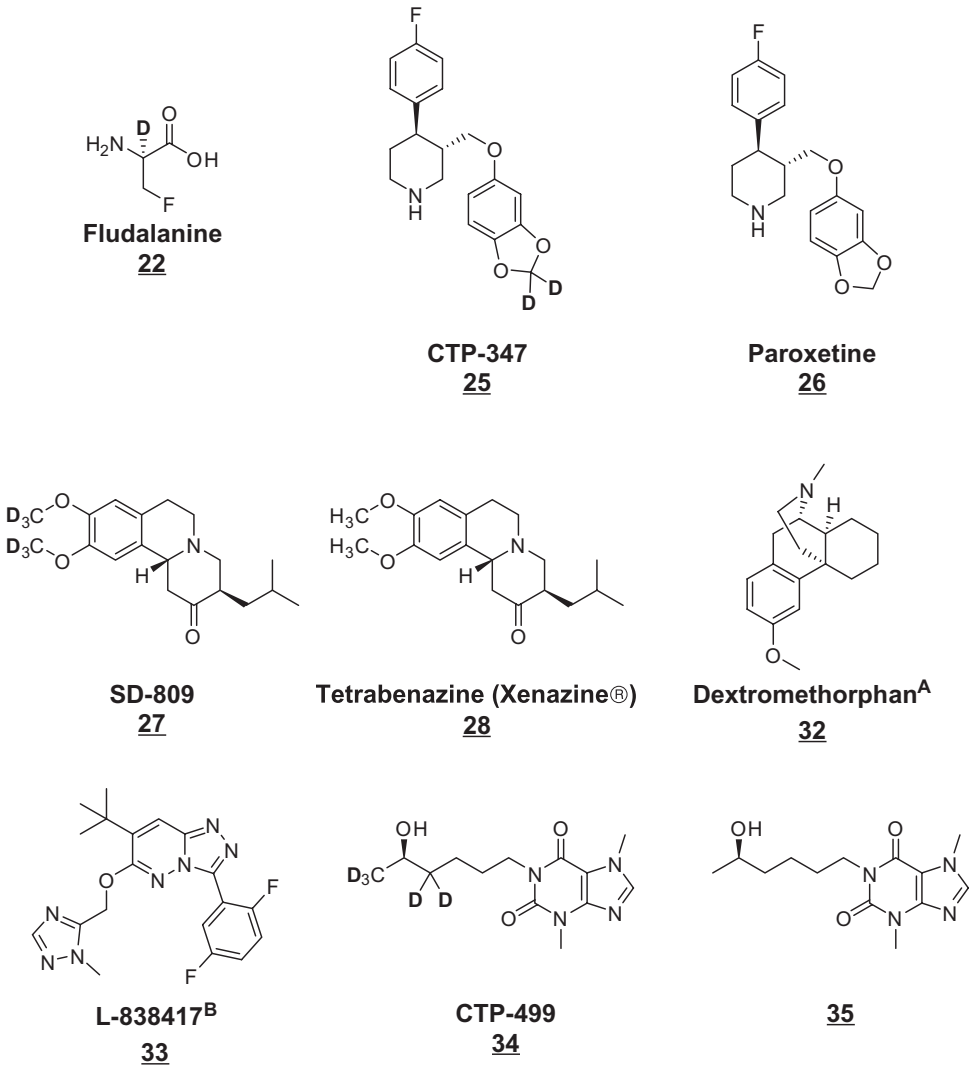
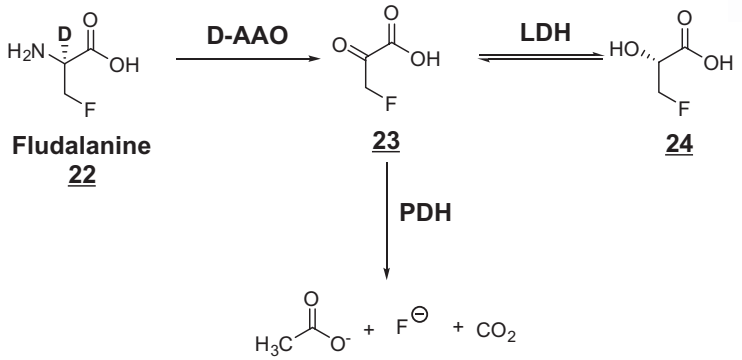
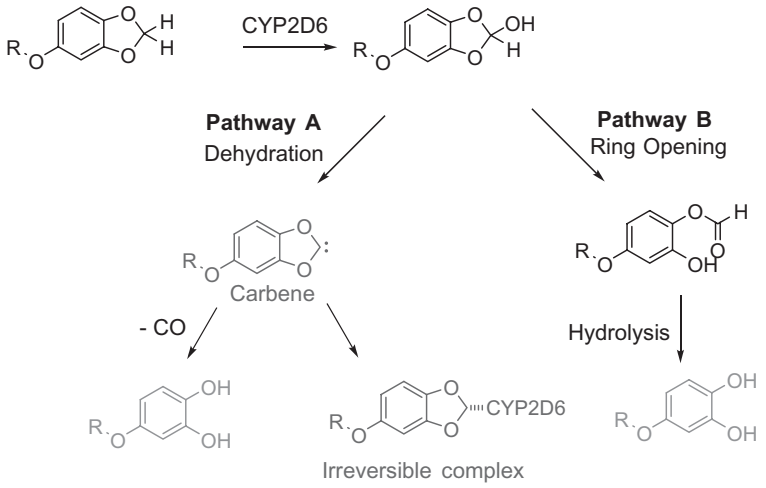


Figure 5. Structures of deuterated compounds or the all-hydrogen versions of deuterated compounds that have entered clinical development. [A]AVP-786 is deuterium modified dextromethorphan. [B]CTP-354 is deuterium modified L-838417.



Scheme 6. Proposed metabolism of fludalanine (**22**) to produce L-fluorolactate (**24**). D-amino acid oxidase (D-AAO); lactate dehydrogenase (LDH), and pyruvate dehydrogenase (PDH).

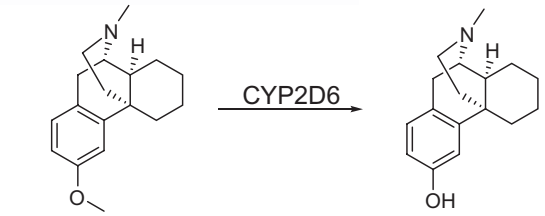


Scheme 7. Proposed inactivation pathway for CYP2D6 by a paroxetine metabolite. Pathway A produces a putative reactive metabolite that results in inactivated enzyme. Pathway B rapidly cleared catechol metabolite via formate ester hydrolysis. Some of the carbene metabolite may also convert to the catechol via decarbonylation.

irreversibly inactivating that enzyme. Its use, therefore, can be complicated in patients potentially benefiting from this agent due to possible drug-drug interactions with other drugs metabolized by CYP2D6. In the case of thioridazine, coadministration of paroxetine is contra-indicated.[54]

The drug-drug interactions observed for **26** are believed to be predominantly due to irreversible inactivation of CYP2D6 by the proposed mechanism shown in Scheme 7. Metabolism of **26** via pathway A is believed to form a highly reactive carbene metabolite that can complex the catalytic iron at the active site of CYP2D6.[56] *In vitro* metabolism experiments with **25** demonstrated little or no CYP2D6 inactivation, presumably due to metabolic shunting away from Pathway A to Pathway B, thereby favoring the ring opening pathway to yield innocuous metabolites.[57] The pharmacology profile of **25** was indistinguishable from paroxetine for serotonin reuptake inhibition and in a battery of receptor and enzyme assays.

25 entered a Phase 1 single- and multiple-ascending dose study in healthy female volunteers to assess its effect on mechanism-based inhibition.[53] Subjects were dosed for 14 days at doses of 10, 20, and 40 mg once-daily and 10 mg twice-daily. Subjects received an oral 30 mg dose of dextromethorphan (**33**, Scheme 8) on days 1 and 14. As shown in Scheme 8, **33** can serve as a selective probe for CYP2D6 activity by measuring urinary levels



Scheme 8. Metabolism of dextromethorphan to dextrorphan by CYP2D6.

of dextrorphan (**34**, Scheme 8), the CYP2D6 specific metabolite.[58] As shown in Figure 6, the ratio of dextromethorphan to dextrorphan (**33**/**34**) in subjects receiving **25** was greatly reduced when compared to historical data for a 20 mg dose of paroxetine.[59] The data in Figure 6 indicate that subjects dosed with **25** retained greater CYP2D6 activity and, therefore, greater ability to metabolize dextromethorphan than has been reported previously for **26**, correlating well with *in vitro* data. Minor CYP2D6 inhibition was observed at higher doses of **25**, consistent with reversible, competitive inhibition seen *in vitro*. The study was also the first clinical demonstration of the use of deuterium substitution to ameliorate drug-drug interactions in humans.

SD-809 (**27**, Figure 5) is being developed by Auspex Pharmaceuticals and is currently in a Phase 3 registration trial for the treatment of chorea associated with Huntington's disease.[60] This agent is a deuterated version of tetrabenazine (**28**, Figure 5), which is currently the

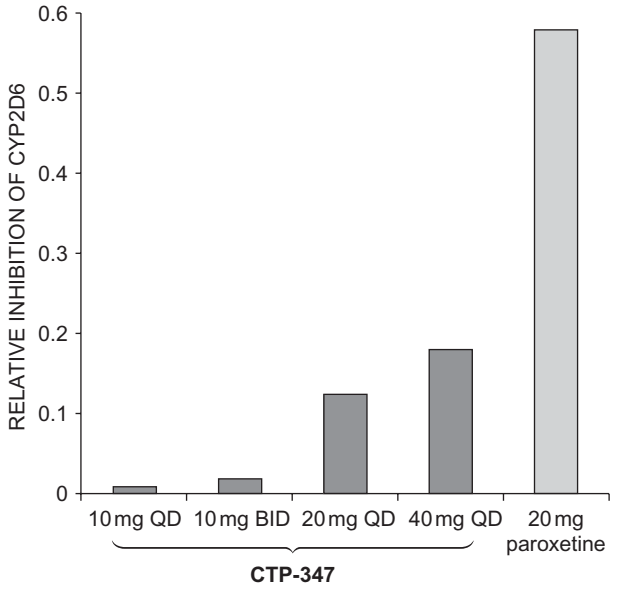
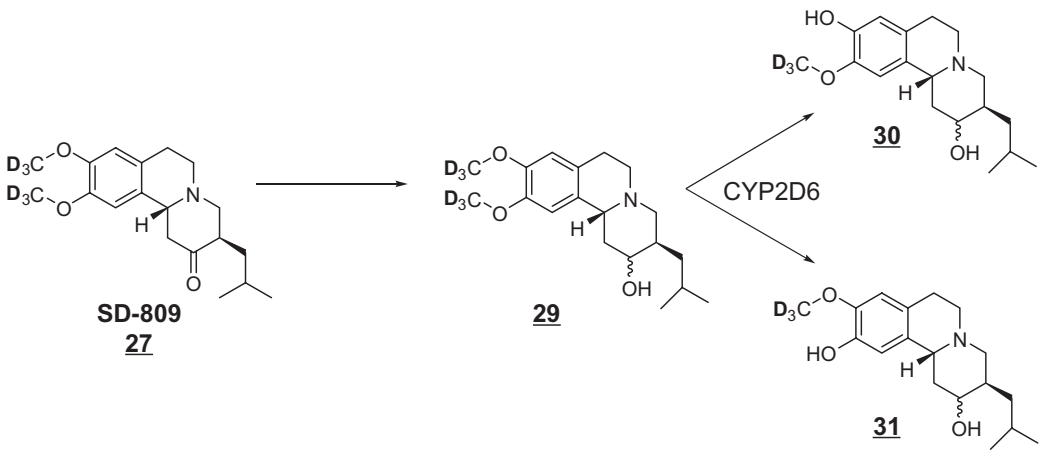


Figure 6. Drug-drug interaction between CTP-347 and dextromethorphan from Phase 1b study. Y-axis shows ratio of intact excreted dextromethorphan vs. dextrorphan metabolite. X-axis shows the dosing amount and frequency, BID (2 times per day) and QD (once per day)

only FDA-approved treatment for this indication.[61] After oral dosing of **27** or **28**, the compounds undergo extensive and rapid metabolism to the major circulating active metabolites shown for **27** in Scheme 9. The active metabolites (**29**, Scheme 9) are then further metabolized by CYP2D6 to **30** and **31**, also shown in Scheme 9. **28** is associated with high rates of adverse events, which include sedation/somnolence (31%), fatigue (22%), insomnia (22%), depression (19%), akathisia (19%), and nausea (13%) that may be associated with not only the



Scheme 9. SD-809 metabolism adapted from reference 62.

Cmax levels of the parent but also the variable levels of active metabolites due to inter-patient variability in CYP2D6 activity. Dosing of **28** requires careful titration to identify a dose (up to three-times daily) that reduces chorea and minimizes the adverse events mentioned above.

Oral doses of **27** (25 mg) have been studied vs. **28** (25 mg) in a single-dose Phase 1 cross-over study in healthy volunteers.[62] This study showed that the half-lives of the active metabolites of **27** vs. **28** were almost doubled, which resulted in more than a doubling of the exposure with only a slight increase in Cmax. This pharmacokinetic profile may allow **27** to achieve similar exposures with a lower Cmax at a lower dose than tetrabenazine. If the adverse events observed for **28** correlate with Cmax, then **27** may exhibit an improved tolerability profile.

AVP-786 is a combination of a deuterated dextromethorphan and ultra-low dose quinidine that is planned for clinical development by Avanir Pharmaceuticals in treatment-resistant major depressive disorder.[63] Avanir currently markets Nuedexta®, which is a fixed-dose combination of dextromethorphan hydrobromide and quinidine sulfate (20 mg/10 mg) for the treatment of Pseudobulbar Affect (PBA).[64] PBA is a neurologic condition resulting in sudden and exaggerated episodes of laughing or crying. Combinations of dextromethorphan and quinidine, referred to as AVP-923, are also in clinical development for other neurologic indications, including agitation in Alzheimer's disease and levodopa-induced dyskinesia. The quinidine in Nuedexta and AVP-923 acts as a pharmacokinetic booster by inhibiting CYP2D6, the major metabolic pathway in humans that converts dextromethorphan to dextrorphan (**Scheme 8**). The deuterated dextromethorphan in AVP-786 maintains the pharmacologic profile of dextromethorphan but is less susceptible to CYP2D6 catalyzed metabolism due to the deuterium isotope effect. In a Phase 1 trial comparing AVP-923 to AVP-786, which has a substantially lower dose of quinidine but equivalent doses of dextromethorphan and deuterated dextromethorphan, AVP-786 provided essentially identical plasma levels of deuterated dextromethorphan compared to (nondeuterated) dextromethorphan from AVP-923.[65] Subsequently, Avanir announced that the FDA has agreed to an expedited development pathway for AVP-786, which would allow referencing of AVP-923 data to support AVP-786 development.[66]

CTP-354 is a potentially non-sedating oral therapy for the treatment of spasticity that is being developed by Concert Pharmaceuticals.[67] CTP-354 is a sub-type selective $GABA_A$ modulator that is a deuterated analog of L-838417 (**33**, **Figure 5**), a preclinical compound discovered at Merck & Co. **33** was extensively characterized by both industry and academic investigators and has been shown to possess promising pharmacology due to its unique $GABA_A$ sub-type selective profile.[68] CTP-354 binds to the $GABA_A$ benzodiazepine binding site, as do both benzodiazepines and non-benzodiazepine sedatives such as zolpidem and eszopiclone. Unlike those drugs, CTP-354 selectively lacks agonist activity at $GABA_A$ receptors containing the $\alpha 1$ subunit and as a result has the potential to avoid side effects associated with those receptors.[69] Preclinical studies with **33** in rodent and monkey models support a profile with greatly reduced sedation, ataxia, and memory loss while retaining a number of positive aspects of benzodiazepine pharmacology: anxiolysis, muscle relaxation, anti-seizure effects, and reduction of inflammatory and neuropathic pain.[70,71] CTP-354 has been reported to show substantially improved pharmacokinetics in rats and dogs relative to **33** in addition to positive activity in a rat model of neuropathic pain – the Chung sciatic nerve ligation model.[67] In this model, CTP-354 showed a longer duration of action than **33** and equivalent efficacy to gabapentin.

CTP-354 has progressed to Phase 1 clinical development.[72] Brain-receptor occupancy of CTP-354 is being determined by Positron Emission Tomography in healthy volunteers with study results anticipated in 2014 (unpublished data, Concert Pharmaceuticals, Inc.).

Concert Pharmaceutical's most advanced development candidate is CTP-499 (**34**, **Figure 5**), which is in Phase 2 development for the treatment of chronic kidney disease in type 2 diabetics who are receiving stable therapy with angiotensin receptor blockers or angiotensin

converting enzyme inhibitors.[73] CTP-499 is a deuterated analog of **35**, which is an active metabolite of pentoxifylline (Trental®). Pentoxifylline is approved in the US for the treatment of intermittent claudication; however, several clinical studies have reported that it also has potential renoprotective effects, particularly for reduction of urinary albumin excretion.[74] It appears that this positive pharmacology for pentoxifylline may be due in large part to the circulating metabolite **35**, which Concert has deuterated to provide **34** with the result of providing high plasma levels of pharmacologically active species - **34** and active metabolites (unpublished data, Concert Pharmaceuticals, Inc.).

A Phase 1 study of **34** that determined the range of tolerated doses and the food effects on exposure has been completed and contributed to the selection of the dose for further Phase 2 development.[75] A Phase 2 study was conducted to evaluate the safety and efficacy of **34** in type 2 diabetic patients with mild to moderate chronic kidney disease and macroalbuminuria, or high levels of albumin in their urine. These patients are at an elevated risk for progression to end-stage renal disease and cardiovascular morbidity.[73] The primary endpoint of the study was the change in urinary albumin excretion, measured as urinary albumin to creatinine ratio (UACR) with secondary endpoints related to safety and tolerability in addition to effects on renal function and various biomarkers. A 24 week blinded extension study (48 weeks total treatment with **34** or placebo), designed to assess renal function endpoints including serum creatinine concentrations and estimated glomerular filtration rates in addition to UACR, safety, and tolerability, has also been recently completed (unpublished data, Concert Pharmaceuticals, Inc.). Results from these studies will be presented in 2014.[76]

Conclusion

Deuterium medicinal chemistry offers a subtle, but at times powerful tool that is only recently achieving widespread attention in the context of new therapeutic agents. The examples presented in this review show the varied effects that deuterium substitution can have on the overall pharmacological profile of a compound. As deuterium containing compounds continue to advance to the clinical setting, more data will become available describing the preclinical to clinical translation of deuterium isotope effects. It is important to note, however, that the deuterium effects on the metabolic profile of a therapeutic are not predictable and must be explored for each compound as one would typically approach medicinal chemistry optimization. Uses of deuterium for the potential improvement of the safety, tolerability, efficacy and dosing of compounds have been emphasized. The application of deuterium medicinal chemistry to compounds with well understood therapeutic utility can potentially provide a risk-reduced approach to creating new drugs that address important unmet medical needs. The clinical examples presented – CTP-347, SD-809, CTP-354, and AVP-786 – emphasize the breadth of this approach. Continued clinical advancement of these and other deuterated agents to registration trials and marketing approval will provide the validation of the approach.

References

1. R. Shoenheimer and D. Rittenberg, *Physiol. Rev.* **1940**, *20*, 218.
2. T. A. Baillie, *Pharmacol. Rev.* **1981**, *33*, 81.
3. S. L. Harbeson and R. D. Tung, (ed. J. E. Macor), in *Ann. Rep. Med. Chem.*, **46**, Elsevier, Oxford, UK, **2011**, p. 403.
4. H. C. Urey, F. G. Brickwedde and G. M. Murphy, *Phys. Rev.*, **1932**, *39*, 164.
5. W. L. Marter, D. W. Hayes and D. W. Jones, (eds. W. A. Cunningham and J. C. McKetta), in *Encyclopedia of Chemical Processing and Design, Issue 15*, Marcel Dekker, Inc., New York, NY, **1982**, p. 308.
6. H. Esaki, N. Ito, S. Sakai, T. Maegawa, Y. Monguchi and H. Sajiki, *Tetrahedron*, **2006**, *62*, 10954.
7. H. Esaki, F. Aoki, M. Umemura, M. Kato, T. Maegawa, Y. Monguchi and H. Sajiki, *Chem. Eur. J.*, **2007**, *13*, 4052.
8. H. J. Leis, G. Fauler and W. Windischhofer, *Curr. Org. Chem.*, **1998**, *2*, 131.
9. J. G. Atkinson and M. O. Luke, *US Patent 3, 966, 781*, **1976**.
10. L. Di Costanzo, M. Moulin, M. Haertlein, F. Meilleur and D. W. Christianson, *Arch. Biochem. Biophys.*, **2007**, *465*, 82.
11. N. E. Tayar, Hvd. Waterbeemd, M. Gryllaki, B. Testa and

W. F. Trager, *Int. J. Pharm.*, **1984**, *19*, 272.
12. M. Turowski, N. Yamakawa, J. Meller, K. Kimata, T. Ikegami, K. Hosoya, N. Tanaka and E. R. Thornton, *J. Am. Chem. Soc.*, **2003**, *125*, 13836.
13. C. L. Perrin and Y. Dong, *J. Am. Chem. Soc.*, **2007**, *129*, 4490.
14. C. L. Perrin, B. K. Ohta, J. Liberman and M. Erdelyi, *J. Am. Chem. Soc.*, **2005**, *127*, 9641.
15. M. W. Ruszczycky and V. E. Anderson, *J. Theor. Biol.*, **2006**, *243*, 328.
16. B. E. Lewis and V. L. Schramm, (eds. A. Kohen and H.-H. Limbach), in *Isotope effects in Chemistry and Biology*, CRC Taylor & Francis, Boca Raton, FL, **2006**, p. 1019.
17. V. L. Schramm, *Curr. Opin. Chem. Biol.*, **2007**, *11*, 529.
18. K. B. Wiberg, *Chem. Rev.*, **1955**, *55*, 713.
19. S. D. Nelson and W. F. Trager, *Drug Metab. Disp.*, **2003**, *31*, 1498.
20. M. B. Fisher, K. R. Henne and J. Boer, *Curr. Opin. Drug Discov. Devel.*, **2006**, *9*, 101.
21. W. P. Jenks, *Catalysis in Chemistry and Enzymology*, Dover Publications Inc., Mineola, NY, **1987**, p. 243.
22. D. B. Northrop, *Biochemistry*, **1975**, *14*, 2644.
23. K. J. Ling and R. P. Hanzlik, *Biochem. Biophys. Res. Comm.*, **1989**, *169*, 844.
24. B. Meunier, S. P. de Visser and S. Shaik, *Chem. Rev.*, **2004**, *104*, 3947.
25. F. P. Guengerich, *J. Label. Compd. Radiopharm.*, **2013**, *56*, 428.
26. L.-A. Higgins, G. A. Bennett, M. Shimoji and J. P. Jones, *Biochemistry*, **1998**, *37*, 7039.
27. G. T. Miwa, W. A. Garland, B. J. Hodshon, A. Y. H. Lu and D. B. Northrop, *J. Biol. Chem.*, **1980**, *255*, 6049.
28. P. R. Ortiz de Montellano (ed.), in *Cytochrome P450: Structure, Mechanism and Biochemistry, 3rd ed.*, Kluwer Academic/Plenum Publishers, New York, NY, **2005**.
29. R. P. Bell, *Chem. Soc. Rev.*, **1974**, *3*, 513.
30. J. A. Krausner and F. P. Guengerich, *J. Biol. Chem.*, **2005**, *280*, 19496.
31. E. Flaumenhaft, S. Bose, H. L. Crespi and J. J. Katz, *Int. Rev. Cytol.*, **1965**, *18*, 313.
32. P. J. H. Jones and S. T. Leatherdale, *Clin. Sci.*, **1991**, *80*, 277.
33. N. Blagojevic, G. Storr, J. B. Allen, H. Hatanaka and H. Nakagawa, (eds. R. Zamenhof, G. Solares and O. Harling), in *Dosimetry & Treatment Planning for Neutron Capture Therapy*, Advanced Medical Publishing, Madison, WI, **1994**, p. 125.
34. P. R. Schloerb, B. J. Friis-Hansen, I. S. Edelman, A. K. Solomon and F. D. Moore, *J. Clin. Invest.*, **1950**, *29*, 1296.
35. D. J. Kushner, A. Baker and T. G. Dunstall, Can. *J. Physiol. Pharmacol.*, **1999**, *77*, 79.
36. A. E. Mutlib, R. J.Gerson, P. C. Meunier, P. J. Haley, H. Chen, L. S. Gan, M. H. Davies, B. Gemzik, D. D. Christ, D. F. Krahn, J. A. Markwalder, S. P. Seitz, R. T. Robertson and G. T. Miwa, *Toxicol. Appl. Pharmacol.*, **2000**, *169*, 102.
37. Viramune [Package insert] Boehringer Ingelheim Pharmaceuticals, Inc.. Ridgefield, CT; January **2014**. http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/020636s044,020933s035lbl.pdf
38. A. M. Sharma, Y. Li, M. Novalen, M. A. Hayes and J. Uetrecht, *Chem. Res. Toxicol.*, **2012**, *25*, 1708.
39. A. M. Sharma, K. Klarskov and J. Uetrecht, *Chem. Res. Toxicol.*, **2013**, *26*, 410.
40. Sustiva [Package insert] Bristol-Myers Squibb Company, Princeton, NJ; May **2013**. http://http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/020972s043,021360s031lbl.pdf
41. A. E. Mutlib, H. Chen, G. A. Nemeth, J. A. Markwalder, S. P. Seitz, L. S. Gan and D. D. Christ, *Drug Metab. Disp.*, **1999**, *27*, 1319.
42. A. J. Morales, R. Gallegos, V. Uttamsingh, C. Cheng, S. Harbeson, R. Zelle, R. Tung and D. Wells, Abstract 285, *The 15th North American Meeting of the International Society of Xenobiotics*, San Diego, CA, October 12-16, **2008**.
43. C. J. Wenthur, R. Morrison, A. S. Felts, K. A. Smith, J. L. Engers, F. W. Byers, J. S. Daniels, K. A. Emmitte, P. J. Conn and C. W. Lindsley, *J. Med. Chem.*, **2013**, *56*, 5208.
44. S. A. Stoch, S. Zajic, J. A. Stone, D. L. Miller, L. van Bortel, K. C. Lasseter, B. Pramanik, C. Cilissen, Q. Liu, L. Liu, B. B. Scott, D. Panebianco, Y. Ding, K. Gottesdiener and J. A. Wagner, *Br. J. Pharmacol.*, **2013**, *75*, 1240.
45. K. Kassahun, W. C. Black, D. Nicol-Griffith, I. McIntosh, N. Chauret, S. Day, E. Rosenberg and K. Koeplinger, *Drug Metab. Disp.*, **2011**, *39*, 1079.
46. K. Kassahun, C. Fandozzi, D. R. Gauthier and L. A. Shipley, *Patent Application WO2012/112363*, **2012**.
47. P. Jüni, L. Nartey, S. Reichenbach, R. Sterchi, P. A. Dieppe and M. Egger, *Lancet*, **2004**, *364*, 2021.
48. F. Schneider, M. Hillgenberg, R. Koytchev and R.-G. Alken, *Arzneim. Forsch. Drug. Res.*, **2006**, *56*, 295.
49. Incivek [Package insert] Vertex Pharmaceuticals, Inc., Cambridge, MA; October **2013**. http://http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/201917s012lbl.pdf
50. F. Maltais, Y. C. Jung, J. Tanoury, R. B. Perni, M. Mani, L. Laitinen, H. Huang, S. Liao, H. Gao, H. Tsao, E. Block, C. Ma, R. S. Shawgo, C. Town, C. L. Brummel, D. Howe, S. Pazhanisamy, S. Raybuck, M. Namchuk and Y. L. Bennani, *J. Med. Chem.*, **2009**, *52*, 7993.
51. G. K. Darland, R. Hajdu, H. Kropp, F. M. Kahan, R. W. Walker and W. J. Vandenheuvel, *Drug Metab. Disp.*, **1986**, *14*, 668.
52. F. M. Kahan, *Chem. Engin. News*, **2009**, *87*, 4.

53. V. Uttamsingh, D. Wells, D. Soergel and R. Zelle, Abstract 11552, *The 38th American College of Clinical Pharmacology*, San Antonio, TX, **2009**.
54. Paxil CR [Package insert] GlaxoSmithKline, Research Triangle Park, NC; December **2012**. http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/0290936s045lbl.pdf
55. V. Stearns, K. L. Beebe, M. Iyengar and E. Dube, *JAMA*, **2003**, *289*, 2827.
56. M. Murray, *Curr. Drug Metab.*, **2000**, *1*, 67.
57. R. Gallegos, V. Uttamsingh, A. Morales, C. Cheng, G. Bridson, J. F. Liu, R. Tung, R. Zelle, S. Harbeson and D. S. Wells, Abstract 188, *The 16th North American Meeting of the International Society of Xenobiotics*, Baltimore MD, **2009**.
58. B. Schmid, J. Bircher, R. Preisig and A. Kupfer, *Clin. Pharmacol. Ther.*, **1985**, *38*, 618.
59. C. L. Alfaro, Y. W. Lam, J. Simpson and L. Ereshefsky, *J. Clin. Pharmacol.*, **2000**, *40*, 58.
60. Auspex Pharmaceuticals, Inc. "Auspex Initiates Phase 3 Trial of SD-809 in Huntington's Disease." *Press Release*, July 11, **2013**. http://www.auspexpharma.com
61. Xenazine [Package insert] Lundbeck, Inc. Deerfield, IL, April **2011**. http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021894s004lbl.pdf
62. Auspex Pharmaceuticals, Inc., *Form S1 Registration Document*. Filed December 20, **2013**, pp. 73-81. SEC website. http://www.sec.gov/Archives/edgar/data/1454189/000119312513481239/d627086ds1.htm
63. Avanir Pharmaceuticals, Inc. "Avanir Pharmaceuticals Reports Fiscal 2014 First Quarter Financial and Business Results." *Press Release*. February 5, **2014**. http://ir.avanir.com
64. Nuedexta [Package insert] Avanir Pharmaceuticals, Inc., Aliso Viejo, CA, October **2010**. http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021879s000lbl.pdf
65. Avanir Pharmaceuticals, Inc., "Avanir Pharmaceuticals Announces Positive Interim Data From Pharmacokinetic Study With Next Generation Compound AVP-786." *Press Release*. February 7, **2013**. http://ir.avanir.com
66. Avanir Pharmaceuticals, Inc., "Avanir Pharmaceuticals Announces Accelerated Development Path for AVP-786 Following Successful Pre-IND Meeting with FDA." *Press Release*. June 5, **2013**. http://ir.avanir.com
67. P. Graham, J. Liu and D. Turnquist, *Drug Dev.*, **2012**, *12*, 67.
68. R. M. McKernan, T. W. Rosahl, D. S. Reynolds, C. Sur, K. A. Wafford , J. R. Atack, S. Farrar, J. Myers, G. Cook, P. Ferris, L. Garrett, L. Bristow, G. Marshall, A. Macaulay, N. Brown, O. Howell, K. W. Moore, R. W. Carling, L. J. Street, J. L. Castro, C. I. Ragan, G. R. Dawson and P. J. Whiting, *Nat. Neurosci.*, **2000**, *3*, 587.
69. U. Rudolph and F. Knoflach, *Nat. Rev. Drug. Disc.*, **2011**, *10*, 685.
70. J. K. Rowlett, D. M. Platt, S. Lelas, J. R. Atack and G. R. Dawson, *Proc. Nat. Acad. Sci.*, **2005**, *102*, 915.
71. J. Knabl, R. Witschi, K. Hosl, H. Reinold, U. B. Zeilhofer, S. Ahmadi, J. Brockhaus, M. Sergejeva, A. Hess, K. Brune, J.-M. Fritschy, U. Rudolph, H. Mohler and H. U. Zeilhofer, *Nature*, **2008**, *45*, 330.
72. Concert Pharmaceuticals, Inc. "Concert Pharmaceuticals Initiates Phase 1 Clinical Trial of CTP-354, a Potential Treatment for Spasticity and Pain." *Press Release*. June 10, **2013**. http://www.concertpharma.com
73. Concert Pharmaceuticals, Inc. "Concert Pharmaceuticals Initiates Phase 2 Clinical Trial Evaluating CTP-499 for the Treatment of Diabetic Nephropathy." *Press Release*. March 5, **2012**. http://www.concertpharma.com
74. M. Rodriguez-Moran, G. Gonzalez-Gonzalez, M. V. Bermudez-Barba, C. E. Medina de la Garza, H. E. Tamez-Perex, F. J. Martinez-Martinez and F. Guerrero-Romero, *Clin. Nephrol.*, **2006**, *66*, 3.
75. V. Braman, P. Graham, C. Cheng, D. Turnquist, M. Harnett, L. Sabounjian and J. Shipley, *Clin. Pharmacol. Drug. Dev.*, **2013**, *2*, 53.
76. B. Singh, S. Diamond, P. Pergola, J. Shipley, L. Wu, L. Sabounjian, and P. Graham, Poster No. 430, *National Kidney Foundation Spring Clinical Meeting*, Las Vegas, NV April 22-26, **2014**.