CHAPTER **24**

Deuterium in Drug Discovery and Development

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Contents

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Annual Reports in Medicinal Chemistry, Volume 46
ISSN: 0065-7743, DOI: 10.1016/B978-0-12-386009-5.00003-5

ABBREVIATIONS

E	free enzyme
EOS	oxygenating enzyme species with substrate bound
EOS_D	active oxygenating enzyme species for C–D bond oxidation
EOS_H	active oxygenating enzyme species for C–H bond oxidation
EP	enzyme–product complex
ES	enzyme–substrate complex
P	product
P_D	product derived from C–D bond oxidation
P_H	product derived from C–H bond oxidation
S	substrate
S_D	substrate with oxidation of C–D bond
S_H	substrate with oxidation of C–H bond

1. INTRODUCTION

Deuterated compounds have been widely studied in nonclinical settings and have seen broad application as metabolic or pharmacokinetic (PK) probes both *in vitro* and *in vivo* [1,2]. Depending upon a given compound's route of metabolism and the location of the deuterium, deuteration can be metabolically silent, enabling utility as a PK tracer, or it can alter the compound's metabolism allowing use as a mechanistic probe. It is difficult to predict *a priori* which effect deuterium may have on a drug's metabolism. In spite of the potential to alter a compound's metabolic fate, deuterium-containing compounds have rarely been clinically explored in the context of creating new therapeutic agents [3,4]. To date, no deuterated compound has advanced beyond Phase 2 clinical evaluation [5]. The incorporation of deuterium into pharmacologically active agents offers potential benefits such as improved exposure profiles and decreased production of toxic metabolites [6,7], which could yield improvements in efficacy, tolerability, or safety. As noted in a recent review [8], there has been a resurgence of interest in the application of deuterium in medicinal chemistry as evidenced by the emergence of several new companies largely or solely focused on this technology. This chapter will provide a brief review of the use of deuterium to alter the metabolic properties of compounds and will discuss past and current development of potential deuterium-containing drugs.

2. DEUTERIUM BACKGROUND

Deuterium is a naturally occurring, stable, nonradioactive isotope of hydrogen discovered in 1932 [9]. Hydrogen consists of one electron and one proton and has a mass of 1.008 atomic mass units (AMU), whereas deuterium also contains a neutron, which results in a mass of 2.014 AMU. Deuterium occurs at a natural abundance of approximately 1 part in 6400 or 0.015%, which allows large quantities of deuterium to be isolated as heavy water (D_2O) in very high isotopic purity [10]. D_2O can then serve as a direct or indirect source of deuterium for a wide range of chemical reagents and building blocks for preparing deuterated drugs.

2.1. Primary deuterium isotope effect

Due to the greater atomic mass of deuterium, a deuterium–carbon bond has a lower vibrational frequency and, therefore, a lower zero-point energy than a corresponding hydrogen–carbon bond [11]. In contrast, the frequencies associated with scission of C–H and the corresponding C–D bonds in the transition states are similar. Therefore, the lower zero-point energy translates to higher activation energy for C–D bond cleavage and a slower reaction rate (represented by rate constant k). This effect on rate is known as the primary deuterium isotope effect (DIE) and is expressed as k_H/k_D, the ratio of the reaction rate constants of C–H versus C–D bond cleavage with a theoretical limit of about 9 at 37°C in the absence of tunneling effects [12,13]. In principle, the DIE has the potential to affect the biological fate of many drugs that are metabolized by pathways involving hydrogen–carbon bond scission. In practice, the observed DIE, $(k_H/k_D)_{obs}$ for a metabolic reaction is often ''masked'', which means that it is smaller than k_H/k_D or, in some cases, entirely absent [14]. There are also literature examples of inverse DIEs in which $(k_H/k_D)_{obs} < 1$ [15].

A very large number of studies have appeared in the literature over the years reporting DIEs for a great many enzyme-catalyzed reactions. The most important enzymes in drug metabolism are the cytochrome P450s (CYPs), which are responsible for the Phase I metabolism of most drugs. The structures and mechanisms of CYPs have been reviewed in addition to the application of DIEs to the study of CYP-catalyzed reactions [12,16]. Other enzymes in drug metabolism include monoamine oxidase, alcohol dehydrogenase, and aldehyde oxidase. The emerging importance of aldehyde oxidase was recently reviewed, and the potential use of deuterium as a blocking group at aldehyde oxidase metabolic sites was noted [17].

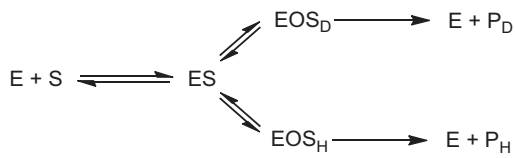
The complexity of biological systems and the number of competing effects that can mask the DIE have made the application of deuterium to drug discovery highly unpredictable and challenging [13]. In order to

observe a DIE, it is necessary that the C–H bond cleavage step is at least partially rate limiting. Enzyme-catalyzed reactions are complex, and catalysis is often dependent upon several partially rate-limiting steps; therefore, the observed DIEs, if any, for deuterium–carbon bond cleavage are often significantly less than the theoretical limit [18].

An irreversible step prior to the C–H bond cleavage in the catalytic sequence can also mask the DIE. As shown for CYP-catalyzed oxidation in Equation (1), an irreversible step, k_3—formation of EOS, the active oxygenating species—occurs prior to the isotopically sensitive C–H bond-cleaving step. In the absence of an alternate pathway, even though the DIE results in $k_{5D} < k_{5H}$, the concentration of EOS_D increases, which compensates for the DIE rate reduction as shown in Eq. (2). This can result in little or no change in the catalytic turnover of the hydrogen versus deuterated substrate [13]. Another mechanism for masking the DIE is ''metabolic switching'' [19], which is illustrated in Eq. (3). In this case, the site of metabolism switches from the site of deuteration (EOS_D) to another site in the molecule (EOS_H). Under these conditions, the rate of substrate turnover may not change, but there will be a decrease in P_D and an increase in P_H. CYP-catalyzed aromatic oxidation generally does not involve direct C–H bond cleavage. In this case, an arene-epoxide intermediate is formed, followed by an intramolecular hydride (deuteride) shift, known as the NIH shift [20], which results in a different DIE from what is observed for aliphatic hydroxylation. As a result of these complexities, the observed magnitude and even direction of the DIEs are unpredictable; DIEs appear to depend on the CYP enzyme involved, the compound being substituted, and the specific substitution pattern.

$$\mathrm{E} + \mathrm{S} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_3} \mathrm{EOS} \xrightarrow{k_5} \mathrm{EP} \xrightarrow{k_7} \mathrm{E} + \mathrm{P} \tag{1}$$

$$k_{5H} \cdot [EOS_H] \approx k_{5D} \cdot [EOS_D] \tag{2}$$



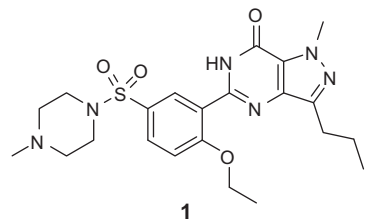
(3)

2.2. Sourcing and properties of deuterium

Deuterium can be sourced through a multistep distillation process that concentrates naturally occurring deuterium from bulk water to produce highly enriched D_2O. Because D_2O is used as a moderator in nuclear

reactors, multiton quantities are commercially available [10]. 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 [21,22]. Deuterium gas, available through electrolysis of D_2O, can also be used for incorporating deuterium into molecules. Catalytic deuteration of olefinic and acetylenic bonds provides a rapid route to incorporation of deuterium [23]. Metal catalysts (*e.g.*, Pd, Pt, and Rh) in the presence of deuterium gas can be used to directly exchange deuterium for hydrogen in functional group containing hydrocarbons [24]. A wide variety of deuterated reagents and synthetic building blocks are now commercially available.

When deuterium is incorporated into molecules in place of hydrogen, in most respects, the deuterated compound is very similar to the all-hydrogen compound. Since the electron clouds of its component atoms define the shape of a molecule, deuterated compounds have shapes and sizes that are essentially indistinguishable from their all-hydrogen analogs [25]. Small physical property changes have been detected in partially or fully deuterated compounds, including reduced hydrophobicity [26,27], decreased acidity of carboxylic acids and phenols [28], and increased basicity of amines [29]. These differences tend to be quite small, and the authors are aware of only one report: deuterated analogs of sildenafil (**1**), in which deuteration of a noncovalent drug appears to change its biochemical potency or selectivity to relevant pharmacological targets [30].



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3. DEUTERIUM SAFETY AND PHARMACOLOGY

3.1. Deuterium exposure in organisms

The availability of sufficient quantities of deuterium-containing compounds has facilitated exploration of the effects of deuterium. In general, deuterium exposure in the form of D_2O has remarkably low systemic toxicity. Single-celled organisms can often be grown in conditions of full deuteration [31]. Lower organisms including fish and tadpoles reportedly survive at least 30% D_2O. Mice and dogs do not display visible effects from long-term

replacement of at least 10–15% of body-fluid hydrogen with deuterium, although concentrations above 25% are broadly toxic to those species [6,32].

Humans can tolerate significant levels of deuterium in body fluids. Acute exposure levels of 15–23% deuterium replacement in whole body plasma levels have been reported with no evident adverse effects [33]. Deuterium (in the form of D_2O or HDO) is excreted by humans *via* the urine with a half-life of about 10 days, which is slightly shorter than the ~14-day half-life of water [34].

3.2. Effects upon metabolism

As discussed previously, deuterium substitution can result in reduced rates of metabolism and/or metabolic switching in which there is a change in the ratio of metabolites formed [35]. In spite of the ability of deuterium to alter metabolism patterns, the authors are not aware of any reports of deuteration resulting in the formation of unique metabolites that were not also observed for the all-hydrogen analog.

3.3. Receptor interactions

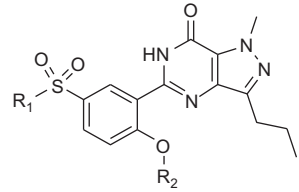
As cited previously [30], deuterated analogs of sildenafil were tested for their inhibitory activity versus phosphodiesterases I–VI. As shown in Table 1, compound **2** was a slightly more potent inhibitor of phosphodiesterase V than sildenafil by 28%. Compound **2** was also reported to be twice as selective for phosphodiesterase V versus phosphodiesterase VI. All three compounds were more potent than sildenafil in an *in vitro* functional assay. Although this appears to be the only example of a binding isotope effect on the pharmacology of a reversible-binding drug with its target enzyme, binding isotope effects are well known and have been recently reviewed [36–38]. Although binding isotope effects have been previously considered negligible, these more recent data support that they are unpredictable and can be insignificant or contribute positively or negatively to measured DIEs.

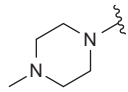
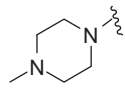
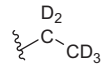
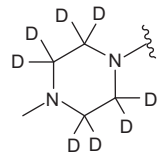
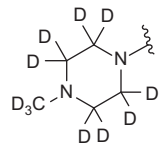
4. DEUTERIUM-CONTAINING DRUGS

4.1. Deuterated tramadol

Deuterated analogs (**5**, **6**, **7**) of tramadol have been evaluated in both *in vitro* and *in vivo* systems [39]. The compounds showed *in vitro* pharmacological activity (mu opioid binding and inhibition of 5-hydroxytryptamine and norepinephrine reuptake) similar to tramadol. When each of the three analogs was incubated in the presence of either human hepatocytes

Table 1 Activities of sildenafil and deuterated analogs

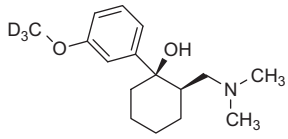


Compounds	R_1	R_2	PDE V IC_{50} (nM)[a]	PDE VI IC_{50} (nM)[a]	ED_{50} (nM)[b]
1			8.73	37.0	245
2			6.31	46.4	85
3			12.3	38.2	91
4			10.0	43.5	121

[a] Inhibition of [^{3}H]cGMP conversion to [^{3}H]guanosine.
[b] Relaxation of phenylephrine-precontracted corpus cavernosum strips (rabbit).

or human liver microsomes (HLM) and the loss of parent was measured, only **7** had a significantly longer half-life ($t_{1/2}$) than tramadol. When metabolic stability versus tramadol was measured by the formation of the *O*-demethylated metabolite, compounds **5** and **7** showed an approximately fivefold reduction in the formation of the metabolite. However,

the increased *in vitro* stability of **7** did not translate to enhanced *in vivo* activity since **7** was no better than tramadol with respect to either potency or duration of analgesia in the rat tail-flick latency model.



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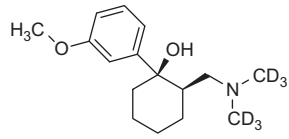

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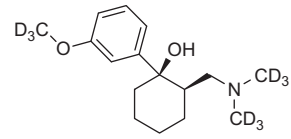

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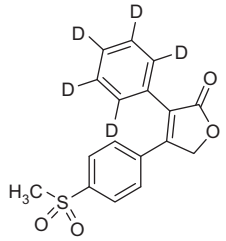
4.2. Deuterated rofecoxib

Improved PK in rats has been reported for **8**, a deuterated analog of rofecoxib [40]. The activities of rofecoxib and **8** for inhibition of cyclooxygenase-1 (COX1) in human platelets were assessed and showed similar IC_{50} values of 169 and 173 nM, respectively. When **8** and rofecoxib were orally dosed individually to rats, the PK parameters C_{max} (peak plasma concentration) and AUC (area under the curve, plasma exposure) for **8** versus rofecoxib were increased by 1.6- and 1.5-fold, respectively; however, **8** showed no increase in $t_{1/2}$.



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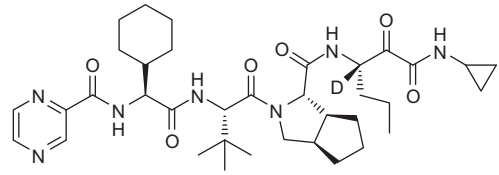

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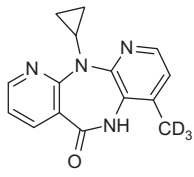
4.3. Deuterated telaprevir

Telaprevir, an inhibitor of hepatitis C viral NS3-4A protease, was recently approved by the FDA for the treatment of HCV and is marketed as Incivek™. Compound **9**, a deuterated analog of telaprevir in which the hydrogen at the chiral center adjacent to the ketoamide moiety was replaced with deuterium, has been reported [41]. The chirality for this center is (*S*) in the active diastereomer of telaprevir, whereas the compound with (*R*) chirality is approximately 30-fold weaker as a protease inhibitor. The deuterium substitution was intended to increase *in vivo* exposure of the active (*S*) compound by decreasing the rate of epimerization at that chiral center. The DIEs observed for the epimerization of **9**

versus telaprevir in plasma (dog, rat, and human) ranged from 4 to 7. Upon oral codosing (1:1) of **9** and telaprevir in rats, a modest 13% increase in AUC for **9** was observed. Compound **9** was equipotent to telaprevir in both a NS3-4A protease inhibition assay and a viral replication assay.

4.4. Deuterated nevirapine

Compound **10**, a deuterated analog of nevirapine, is an example in which the deuterated compound appears to be more rapidly cleared in rats relative to nevirapine [42]. This anomalous observation was attributed to metabolic switching away from a reactive metabolite that inactivates CYP450, resulting in less CYP inhibition by **10** and faster *in vivo* clearance versus nevirapine. Support for this explanation was provided when rats were codosed with compound **10** and a CYP inhibitor and similar plasma levels of **10** and nevirapine were measured.



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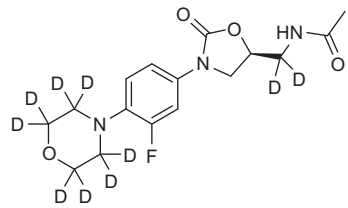

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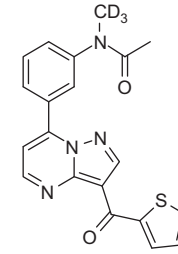


12

4.5. Deuterated linezolid

Compound **11** is a deuterated version of linezolid that shows improved *in vivo* PK in primates [43]. When **11** was codosed with linezolid (IV; 1:1), the $t_{1/2}$s were 6.3 versus 4.5 h, respectively. Compound **11** and linezolid were codosed (1:1) orally to primates and **11** showed enhanced exposure versus linezolid, AUC_{24h} = 16.4 versus 11.3 μg-h/mL, respectively. The improved oral PK translated to improved trough levels as shown by the C_{24h} ratios of **11** to linezolid: 2.99 (female primate) and 2.20 (male primate). The improved PK observed in primates supports the potential use of deuterated linezolid as a once-daily agent in humans [44].

4.6. Deuterated indiplon

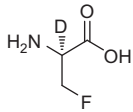
Deuteration of the $GABA_A$-agonist sleep agent indiplon [45] provided compound **12** in which replacement of the N–CH_3 with N–CD_3 resulted in decreased *in vitro* metabolism in both rat and HLM [46]. This *in vitro* result was predictive of the *in vivo* PK in rat. Individual oral dosing of indiplon

or **12** in rats showed a distinct PK advantage for the deuterated molecule since it had both a longer $t_{1/2}$ ($\sim 2\times$) and increased AUC ($>2\times$). The binding of **12** to the central benzodiazepine receptor is reported to be similar to indiplon indicating that deuteration did not alter the pharmacology. Since the $t_{1/2}$ for indiplon in humans is approximately 1.3 h, the doubling of $t_{1/2}$ observed in rat suggests that **12** may have potential as an improved agent for sleep maintenance in humans.

5. DEUTERATED DRUGS AS CLINICAL AGENTS

5.1. Fludalanine

Fludalanine (**13**) appears to be the most extensively studied deuterated-drug candidate to have entered clinical trials. Fludalanine, combined with cycloserine, displays broad and potent antibacterial activity [47]. Its all-hydrogen analog is also a highly effective anti-infective agent; however, preclinical studies demonstrated that it was metabolized by D-aminoacid oxidase to 3-fluoropyruvate followed by L-lactate dehydrogenase reduction to 3-fluorolactate [48]. A recent letter reported that the fluorolactate metabolite caused brain vacuolization in rats and primates [5]. Fludalanine showed a reduced rate of metabolism and produced levels of fluorolactate deemed acceptable in healthy volunteers. The first trial in patients, however, showed higher levels of fluorolactate than expected, and studies on fludalanine were discontinued at Phase 2b. This unexpected result was attributed to metabolic changes associated with the disease state—a not uncommon observation when transitioning from healthy volunteers to patients.



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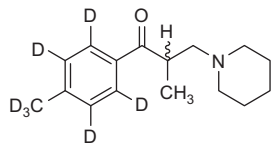

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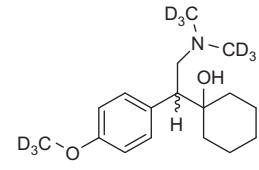

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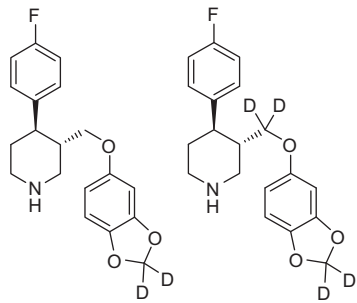
5.2. BDD-10103, deuterated tolperisone

BDD-10103, a deuterated analog of the muscle relaxant tolperisone, has reportedly been dosed in humans [49]; however, neither the structure of BDD-10103 nor the human PK data have been published. The major metabolic path for tolperisone is hydroxylation of the aryl methyl, predominantly *via* CYP2D6 and CYP2C19 [50]. Although the structure of

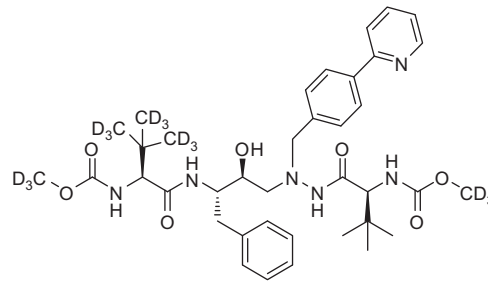
BDD-10103 has not been disclosed, compound **14** has been disclosed [51] and is reported to show a 10-fold decrease in aryl methyl hydroxylation versus tolperisone by both CYP2D6 and CYP2C19. The current clinical status of BDD-10103 is unclear.

5.3. SD-254, deuterated venlafaxine

A selectively deuterated analog of venlafaxine, SD-254 (**15**), has also advanced into clinical development. Although little data are available, the compound has been dosed in a Phase 1 healthy-volunteer study. It was metabolized more slowly than venlafaxine, and maintained an efficacious exposure for a longer time. Development in neuropathic pain is reportedly planned for SD-254 [8,52].



16 **17**



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5.4. CTP-347, deuterated paroxetine

CTP-347, a selectively deuterated analog of paroxetine for the treatment of hot flashes has completed Phase 1 clinical evaluation [53]. While it showed inhibition of serotonin and norepinephrine reuptake similar to paroxetine [54], CTP-347 was reported to be the first example of the use of deuterium to mitigate CYP2D6 inactivation in a clinical setting [7,55]. The structure of CTP-347 has not been disclosed; however, compounds **16** and **17** have been disclosed in the patent literature with data showing *increased* metabolism versus paroxetine in HLM [56]. The percent of parent remaining after 60 min incubation in HLM for paroxetine, **16**, and **17** was 51%, 26%, and 10%, respectively. The greater stability of paroxetine is due to its irreversible inhibition of CYP2D6, whereas the decreased stability of the deuterated compounds results from reduced CYP2D6 inhibition. Metabolism experiments in HLM confirmed that while paroxetine was a mechanism-based inactivator of CYP2D6 ($K_{inact} = 0.08$ min^{-1}), CTP-347 showed little or no CYP2D6 inactivation [7,57]. This was possibly due to metabolic shunting that prevents the formation of a reactive metabolite

that forms an irreversible complex at the active site of CYP2D6 [58]. A Phase 1 study was conducted to assess the effect of CTP-347 on mechanism-based inhibition of CYP2D6 in healthy women [55]. Subjects receiving CTP-347 retained a greater ability to metabolize dextromethorphan (CYP2D6 substrate) than has been reported previously for paroxetine [59,60]. Minor CYP2D6 inhibition was observed at higher CTP-347 doses, which is consistent with the reversible, competitive inhibition seen *in vitro.*

5.5. CTP-518, deuterated atazanavir

CTP-518, a deuterated version of the HIV-protease inhibitor atazanavir, has entered Phase 1 clinical studies [8] with a goal of developing a drug that retains the antiviral potency of atazanavir without the need for ritonavir or another PK-boosting agent [52]. The structure of CTP-518 has not been disclosed. Compound **18** has been disclosed in a patent [61] with data showing increased stability versus atazanavir both *in vitro* (HLM) and *in vivo* (codose studies in primates). The $t_{1/2}$ in HLM for **18** was increased 51% versus atazanavir, which translated to an average 52% increase in $t_{1/2}$ versus atazanavir following an IV codose (1:1) in primates. Atazanavir and **18** showed similar HIV antiviral activity in an *in vitro* viral assay.

6. PATENTABILITY OF DEUTERATED DRUGS

As might be expected from the increase in preclinical and clinical work on deuterated drugs over the past 5 years, there have been a growing number of published patent applications and issued patents in this area. A search from 2005 to 2010 for the terms ''deuterium'' or ''deuterated'' in the claims section and ''pharmaceutical'' in all fields of published U.S. patent applications and issued U.S. patents returns 761 and 142 hits, respectively. For the years 2001–2005, only 77 patents were issued. Figure 1 shows the breakdown for each of the past 6 years. There has been a large overall increase in published U.S. applications for 2008 through 2010 with a smaller but steady increase for issued patents over the same period. This trend shows that patent protection may be obtained for deuterated drugs. As more applications enter prosecution at the USPTO, it will be of great interest to see if the number of issued patents continues to grow and what data may be needed to support patentability.

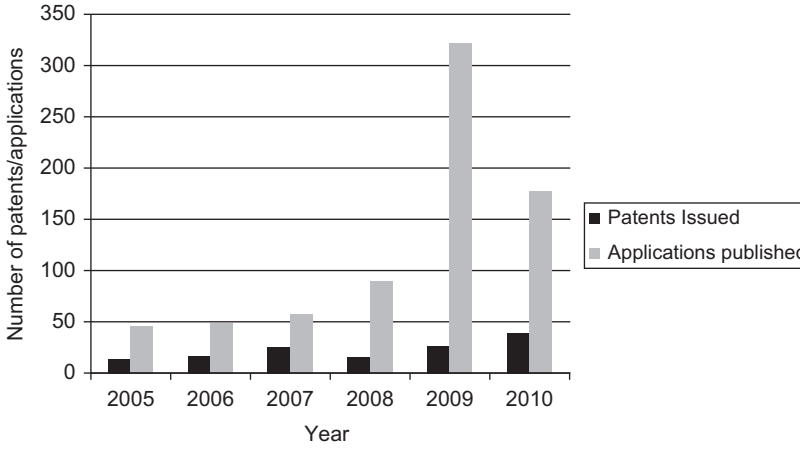

Figure 1 Issued patents and published applications containing "deuterium" or "deuterated" in the claims and "pharmaceutical" in any field for 2005–2010.

7. CONCLUSIONS

Deuterium can be a powerful medicinal chemistry tool that has, until recently, received little attention in the context of new drugs. A majority of the examples that have appeared in the recent literature are deuterated versions of known, well-characterized drugs with established therapeutic utilities. Most deuterated compounds reported-to-date appear to retain full biochemical potency and selectivity. However, in select cases, deuterated drugs show a differentiated PK profile versus the hydrogen-only compounds. In contrast to earlier reviews that were pessimistic about deuterium as an effective strategy for creating new drugs, recent reports suggest the opposite. More scientists in the pharmaceutical industry are now using deuterium as a tool to improve drug properties [7,8,52]. With more deuterium-containing compounds entering clinical evaluation, it appears increasingly likely that the approach will succeed in producing important new medicines if preclinical indications of improvements in safety, tolerability, and/or efficacy are recapitulated in humans.

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