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Viewpoint

Deuterium-Enabled Chiral Switching (DECS) Yields Chirally Pure Drugs from Chemically Interconverting Racemates

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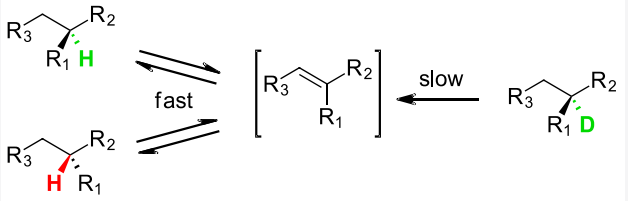
ABSTRACT: Separation of the preferred enantiomer from racemic mixtures, i.e. "chiral switching," often improves efficacy and reduces toxicity. However, this strategy is not applicable for all chiral compounds—particularly for molecules with hydrogen-containing chiral centers, which can be prone to rapid stereoisomerization. Deuterium incorporation can stabilize such chiral centers while retaining the pharmacologic characteristics of the parent racemic mixture, thereby enabling their "chiral switching", changing the drug from a racemate to a single enantiomer. We describe "deuterium-enabled chiral switching" (DECS) as a means of improving on the therapeutic promise of chemically unstable racemic drugs and demonstrate its utility with the isolation and characterization of stable preferred enantiomers of thalidomide and thiazolidinedione (TZD) analogs.



KEYWORDS: *Deuterium, enantiomer, chiral switch, deuterium kinetic isotope effect, thalidomide, pioglitazone*

Stereoisomerism is a critical characteristic of many pharmaceuticals, as it is in other disciplines from food and flavor ingredients to agrochemicals or anywhere else molecular shape determines the interaction of an exogenous molecule with a biological entity. Enantiomeric forms of a drug molecule (i.e., mirror image stereoisomers) may exhibit distinct biological properties, given that biological molecules generally exist in a single stereoisomeric form. Thus, one enantiomer may be responsible for a therapeutic effect, while the other may be inactive, interfere with the therapeutic form, or exhibit toxicity.

Historically, pharmaceuticals were synthesized as racemic mixtures. The advancement of improved synthetic approaches, as well as analytical and separation techniques, dating back to the 1990s, allowed the isolation of the beneficial enantiomer (i.e., "eutomer") from the undesired enantiomer (i.e., "distomer"). This eutomer-focused approach has been termed "chiral switching" and has resulted in a range of new drugs (e.g., Nexium, Lexapro, Lunesta, Xopenex) based on earlier drug approvals of the racemates.[1] However, many racemic drugs were not amenable to this approach. For example, compounds with an acidic hydrogen atom at the chiral center could not be developed as single enantiomers due to the rapid *in vitro* and/or *in vivo* interconversion of their enantiomers.

Replacement of hydrogen with deuterium at the chiral center of these compounds may stabilize the chiral center and enable separation and characterization of each enantiomer. Deuterium exhibits subtle but important differences from hydrogen stemming from the addition of a neutron in its nucleus. While the chemical properties of deuterated compounds remain essentially the same because the number of electrons has not changed, the increased mass of deuterium results in a reduction of the vibrational stretching frequency of the C−D bond relative to a C−H bond. The consequent lowering of the ground state energy results in a greater activation energy of dissociation for a C−D bond compared to the corresponding C−H bond and thus in a slower bond-cleavage reaction rate, manifested in the deuterium kinetic isotope effect (DKIE). Deuterium represents 0.016% of natural hydrogen in the environment. It is stable and nonradioactive. The safety of deuterium has been established through experimental investigations in animals and historical use as a tracer in human metabolic studies.[2] Furthermore, its natural abundance in the environment and, therefore, presence as a natural constituent of biological molecules means that the human body contains roughly ∼1 g of deuterium. In fact, animal studies using deuterium oxide found that a 25% deuterium load in body water was necessary to elicit a toxic effect, which was reversible.[3]

In drug design, the use of deuterated analogs of candidate molecules affords a range of possible improvements, especially turning unworkable molecules into feasible candidates. A review by Pirali et al.[4] highlights five distinct pharmaceutical challenges that can be addressed through the use of deuterated

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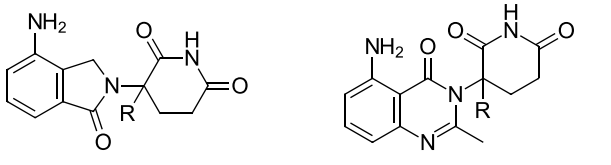
analogs of parent molecules, Deuteration may: (1) improve pharmacokinetics; and, (2) decrease toxicity by slowing down formation of metabolites, some of which may be toxic, or by redirecting metabolism to nontoxic metabolites. Deuterated analogs can also be used: (3) to investigate the mechanism of action, such as contribution of metabolites; (4) as non-radioactive tracers to perform pharmacokinetic and metabolism studies; or, (5) to provide novel, patentable chemical entities. The first deuterated drug approved by the FDA in 2017, Austedo (deutetrabenazine), is an example of using deuterium to improve metabolism.[5] This approach is known as "metabolic switching".

Central to the work we describe here, deuteration can stabilize hydrogen-containing chiral centers through the DKIE, thereby reducing the incidence of stereoisomerization both *in vitro* and *in vivo*. Deuterium presents an opportunity to enable chiral switching for interconverting racemates with minimal impact on pharmacologic properties, unlike earlier attempts to stabilize the chiral center, that included replacement of the hydrogen at the chiral center by a fluorine or a methyl group.[6,7] By contrast to metabolic switching, the chiral center is generally not involved in the metabolism of racemic drugs and therefore little or no change in the metabolite profile or elimination half-life is expected.

Two synthetic approaches have been used, one that employs *de novo* synthesis with deuterated starting materials and/or reagents and another that involves an isotopic exchange of hydrogen for deuterium in a late-stage intermediate or the target molecule itself. Isotopic replacement can be accomplished through reductive deuteration, halogen−deuterium exchange via aromatic dehalogenation, or direct hydrogen−deuterium exchange. The deuterium-substituted stereoisomers of the parent racemic mixture can be obtained in a stereochemically pure form or separated by chiral chromatography or crystallization of a diastereomeric salt. Once stabilization against enantiomerization is confirmed, the stereoisomers can be further characterized both *in vitro* and *in vivo* so as to evaluate their differential pharmacologic and pharmacokinetic properties and assess their potential for further development. These approaches collectively are called "deuterium-enabled chiral switching" or DECS.

The following examples show the potential of DECS in the development of new chemical entities (NCEs) from existing racemic mixtures.

Thalidomide and its analogs (Figure 1) are a class of immunomodulatory racemic drugs with anti-inflammatory and antitumorigenic properties. However, rapid *in vivo* epimerization of the unstable chiral center has limited extensive characterization of their enantiomers.



1a, R = (*R*,*S*)-H (lenalidomide) **2a, R = (*R*,*S*)-H (avadomide)**
1b, R = (*S*)-D (DP-053) **2b, R = (-)-D (DRX-164)**

Figure 1. Structures of thalidomide analogs lenalidomide (**1a**) and avadomide (CC-122) (**2a**) and corresponding preferred deuterated enantiomers (**1b**, **2b**).

Replacing the exchangeable hydrogen at the chiral center with deuterium allowed stabilization of individual enantiomers of lenalidomide (**1a**) and avadomide (**2a**). Different synthetic approaches were used to prepare the deuterium-substituted analogs: stereochemically pure deuterated enantiomers of **1a** were obtained by incorporation of a late-stage synthon with a fixed chiral center while the deuterated enantiomers of **2a** were obtained by chiral chromatography after deuterium oxide quenching of a cyclization reaction. Deuteration had a stabilizing effect on the enantiomers of compounds **1a** and **2a**, as demonstrated by incubating the deuterated enantiomers in buffer and/or plasma at physiologic temperature (37 °C), which showed a significant reduction in the rate of enantiomerization. Unexpectedly, deuteration at the chiral center also showed the added benefit of reducing the degradation rate of **1a** and **2a** in the same medium. The main interest beyond stability is, of course, the distinctions in pharmacokinetic properties and therapeutic effects between the deuterium-stabilized and resolved enantiomers. We used a range of *in vitro* and *in vivo* measures to demonstrate the differences. *In vivo*, deuteration at the chiral center did not affect the pharmacokinetics of the individual enantiomers (same time to maximum plasma concentration and same elimination half-life), even though a single enantiomer was used. Distinct pharmacologic effects between deuterium-stabilized enantiomers were also demonstrated as **1b** is about 10-fold more potent than the deuterated (*R*)-enantiomer at inhibiting tumor necrosis factor α (TNF-α) production in lipopolysaccharide (LPS)-stimulated human peripheral blood monocytes, while **2b** is a 20-fold more potent inhibitor than the corresponding deuterated (+)-enantiomer. *In vivo*, in an H929 myeloma mouse xenograft model, **2b** was antitumorigenic while the deuterated (+)-enantiomer showed limited antitumorigenic activity and perhaps even some tumorigenic properties.[1]

Deuterium substitution at the chiral center was also applied to several members of the class of compounds known as thiazolidinediones (TZDs). TZDs have pharmacologic applications in several disease areas including oncology and metabolic and rare diseases, although they are mostly known for their role as insulin sensitizers in the treatment of type 2 diabetes. All TZDs bear the same labile chiral center at the 5-position of the 1,3-thiazolidine-2,4-dione moiety (Figure 2). In the case of pioglitazone (**3a**) and inolitazone (**4a**), deuteration was effected by taking advantage of this chemical instability of the chiral center, i.e. by hydrogen−deuterium exchange, to form the deuterated racemate, followed by chiral chromatography to separate the pure enantiomers.

However, despite bearing the same chiral center, not all TZDs proved amenable to DECS. Indeed, while deuteration of pioglitazone (**3a**) increased plasma stability of both deuterated enantiomers against enantiomerization,[8] deuterium incorporation at the chiral center of inolitazone only improved the plasma stability of the (+)-enantiomer **4b** (about 2.5-fold) without having any effect on the stability of the (−)-enantiomer.[9] The increased plasma stabilities of the deuterated enantiomers of pioglitazone were confirmed via *in vivo* pharmacokinetic experiments, which showed increased relative exposure to the enantiomer that was dosed.[8] As discussed above, deuteration may improve the stability of the carbon-deuterium bond at the chiral center. However, over time the carbon−deuterium bond can be cleaved and the planar intermediate can be quenched to form both protonated

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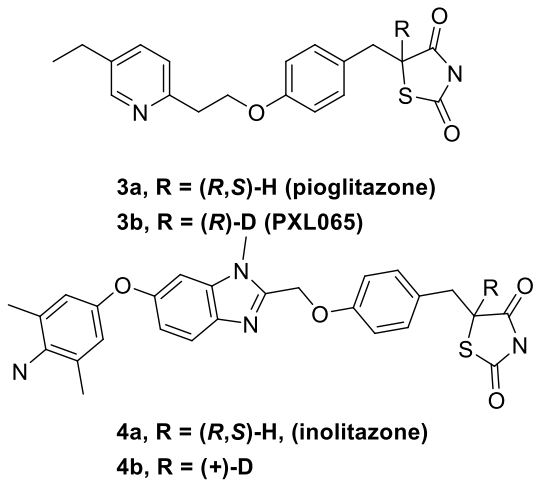

Figure 2. Structures of TZD derivatives pioglitazone (**3a**) and inolitazone (**4a**) and corresponding preferred deuterated enantiomers (**3b**, **4b**).

enantiomers. Therefore, the protonated enantiomers may be observed *in vivo* after dosing of a deuterium-stabilized enantiomer. The degree of deuterium loss observed *in vivo* will be a function of the interplay between the kinetics of deuterium−hydrogen exchange and the elimination rates of the compounds.

The deuterium-stabilized (*R*)-pioglitazone (**3b**) and (*S*)-pioglitazone enabled the *in vitro* and *in vivo* pharmacologic characterization of the enantiomers of pioglitazone.[8] Of significant interest, the (*S*)-enantiomer appears exclusively responsible for the peroxisome proliferator-activated receptor γ (PPARγ) agonist activity typically associated with the TZD class of drugs while both enantiomers inhibit the mitochondrial pyruvate carrier (MPC). The PPARγ effect of a TZD, namely nondeuterated rosiglitazone, was previously attributed to the (*S*)-enantiomer,[10] albeit interconversion of the enantiomers was observed during the *in vitro* study and precluded further evaluation of the enantiomers *in vivo*. It is known that PPARγ agonism-related side effects include weight gain, edema, and bone fracture. In fact, an *in vivo* weight gain study showed that the weight gain observed upon treatment with pioglitazone is recapitulated in animals treated with an equivalent dose of deuterium-stabilized (*S*)-pioglitazone but not with **3b**. Importantly, an *in vivo* mouse model of nonalcoholic steatohepatitis (NASH), a metabolic disease for which pioglitazone has shown efficacy, demonstrated that (*R*)-pioglitazone appears responsible for the efficacy of pioglitazone in NASH, including reduction of hepatic triglycerides, free fatty acids, and cholesterol as well as ballooning, steatosis, inflammation, and fibrosis. The pharmacokinetic observation of increased relative exposure to the enantiomer that is dosed was extended to a Phase 1a clinical trial, where oral dosing of **3b** showed a significant increase in the exposure to the (*R*)-enantiomer and a very limited exposure to the (*S*)-enantiomer, compared to what is observed when dosing racemic pioglitazone. Thus, the deuterium stabilization of (*R*)-pioglitazone occurs *in vivo* in humans. When dosing **3b** vs racemic pioglitazone, the decreased absolute exposure to the (*S*)-enantiomer should result in a much-improved side effect profile. The deuterium-stabilized (*R*)-enantiomer of pioglitazone (**3b**), known as PLXL065, is currently in clinical development for NASH at Poxel S.A.

Many existing and promising drugs are racemic mixtures of enantiomers. For some, interconversion of the enantiomeric forms prevents the isolation of the preferred stereoisomer. Deuterium-enabled chiral switching (DECS) provides an effective means to stabilize, isolate, and administer beneficial enantiomers. As shown above, DECS enabled us to isolate and evaluate *in vitro* and *in vivo* pharmacologically relevant enantiomers of thalidomide analogs and TZDs. This novel use of deuterium may improve the *de novo* synthesis and characterization of numerous other unstable chiral compounds. Of note, the ongoing exploration of numerous racemates, including the analogs described herein, has identified previously unknown differences between enantiomers, expanded our understanding of deuterium chemistry, and identified opportunities to improve the therapeutic profile of drugs and drug candidates. As "chiral switching" was a revolutionary approach for medicinal chemistry in the 1990s, the pioneering use of deuterium to stabilize chiral compounds provides a new tool for medicinal chemists to create superior therapeutics.

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Author Contributions

This manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

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ABBREVIATIONS

LPS, lipopolysaccharide; MPC, mitochondrial pyruvate carrier; NASH, nonalcoholic steatohepatitis; PPAR, peroxisome proliferator activated receptor; TNF-α, tumor necrosis factor α; TZD, thiazolidinedione

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