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Deuteration and fluorination of 1,3-bis(2-phenylethyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione to improve its pharmacokinetic properties

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ABSTRACT

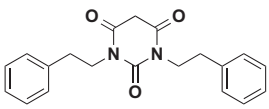
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive loss of motor neurons, leading to muscle weakness, paralysis, and death, most often from respiratory failure. Over 200 pyrimidine-2,4,6-trione (PYT) small molecules, which prevent aggregation and reduce the associated toxicity of mutant superoxide dismutase 1 (SOD1) found in patients with familial ALS, have been synthesized and tested. One of the compounds (1,3-bis(2-phenylethyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione, (**1**) was previously found to have an excellent combination of potency efficacy, and some desirable pharmacokinetic properties. To improve the solubility and metabolic stability properties of this compound, deuterium and fluorine were introduced into **1**. New analogs with better solubility, plasma stability, and human microsome stability were identified.

Amyotrophic lateral sclerosis (ALS), an orphan disease, is estimated to afflict about 87,000 people worldwide, but its prevalence would be much higher were it not for the fact that ALS patients survive only 3–5 years, on average, after diagnosis.[1] Riluzole, which decreases glutamate excitotoxicity,[2] is the only FDA-approved therapeutic drug for ALS, but extends the median survival by only 2–3 months.[3,4]

A cultured cell model, the PC12-G93A-YFP cell line, developed by Morimoto,[5] was utilized in a high throughput assay to identify compounds that protect against mutant SOD1-induced cytotoxicity. Two assays were established, a cytotoxicity protection assay, in which compounds were screened for their ability to protect cells from the cytotoxic effects of aggregated mutant SOD1, and a protein aggregation assay, in which compounds that are active in the cytotoxicity protection screen were tested for their ability to reduce the aggregation of mutant SOD1.[6]

Pyrimidine-2,4,6-triones (PYT) were identified among the active compounds and selected as one of the scaffolds for chemistry optimization.[7] More than 200 PYT compounds were synthesized, and some general observations about the SAR were concluded. As shown in Figure 1, compound **1** from our previous work was identified as one of the best analogues, having good potency, low toxicity (maximum tolerated dose is 100 mg/kg), and good oral and brain absorption; however, the solubility, microsome stability, and plasma stability of this compound was not very satisfactory (see also Supporting information SP-1 for details), and further improvement was needed.

Considering the poor pharmacokinetic properties of **1**, attempts to determine the major metabolites were performed (see Supporting information SP-2 for details). As shown in Figure 2, two major mass spectral peaks were detected, corresponding to the incorporation of an oxygen atom (m/z = 353) and the loss of two hydrogen atoms (m/z = 335), suggesting hydroxylation of either the phenyl ring or the side chain and the oxidation of the side chain to the



1

Potency (EC_{50}) 1.68 μM
Solubility 31.3 μM
Human microsome stability $T_{1/2}$ = 64 min
Mouse microsome stability $T_{1/2}$ = 16 min
Plasma stability $T_{1/2}$ = 63.5 min
Caco-2 A->B = 73.4 x 10^{-6} cm/s
Caco-2 B->A = 26.0 x 10^{-6} cm/s

Figure 1. Pharmacokinetic properties of compound **1**.

Abbreviations: ADME, absorption, distribution, metabolism, excretion; ALS, amyotrophic lateral sclerosis; PYT, pyrimidine 2,4,6-trione.

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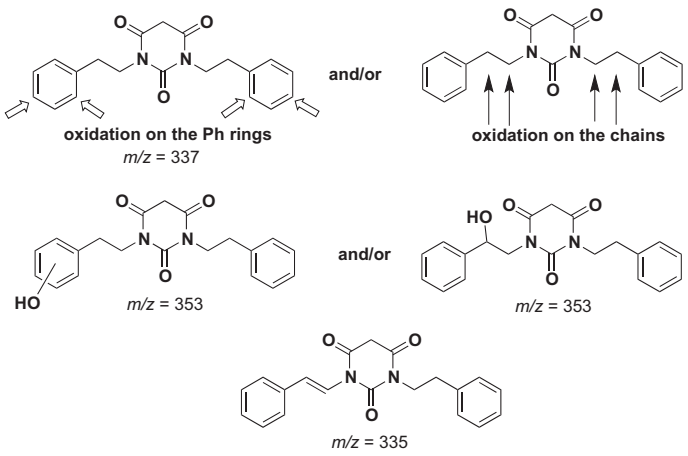


Figure 2. Metabolites of **1** in mouse microsomes.

corresponding alkene; no other major metabolites were observed. The alkene metabolite could have come from the corresponding alcohol by elimination, which supports a metabolite with the hydroxyl group attached to the side chain. The same metabolic products were also observed with human microsomes. With this hypothesis, we directed our efforts at modification of **1** to decrease this potentially harmful metabolism and to increase its solubility.

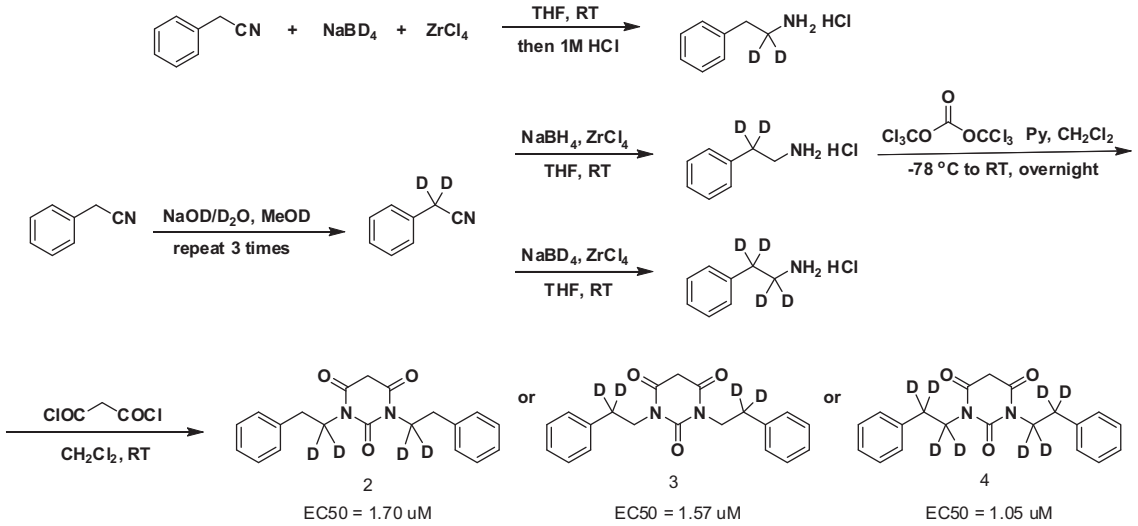
*Deuteration of **1**:* One approach that is exploited to slow cytochrome P450-dependent drug metabolism is deuteration of the suspected site of C—H bond cleavage.[8] The C—D bond has a lower zero point energy and, therefore, is stronger; if the rate-determining step involves C—H bond cleavage, then deuteration at that site should slow the rate of metabolism. The emergence of companies such as Concert Pharmaceuticals and Auspex Pharmaceuticals, which incorporate deuterium into existing drugs with poor metabolic stability, have established this strategy as a viable low-risk approach to drug development.

To protect both carbons on the chain of **1**, several deuterated analogues were synthesized, as shown in Scheme 1. 2-Phenylethanamines, deuterated on either or both carbons, were synthesized from commercially available benzyl cyanide as HCl salts in excellent yields. Following well-established procedures,[9] deuterated compounds **2**, **3**, and **4** were obtained in very high overall yields. As expected, the potencies of all of the compounds were very similar (within the error of the measurement).

Unfortunately, **1** and the three deuterated PYT analogues had very similar solubilities, microsome stabilities, and plasma stabilities (Table 1; see Supporting information SP-1 for details). There was no deuterium isotope effect on the metabolism of **1**, suggesting that side chain C—H(D) cleavage is not a rate-determining step of metabolism.

*Fluorination of **1**:* As deuteration was not effective in slowing metabolism, a more stable bond was sought. Fluorine substitution has been extensively investigated in drug research as a means of enhancing biological activity and increasing chemical or metabolic stability.[10] Important characteristics of fluorine-containing compounds are: (1) the size of the fluorine atom compared to hydrogen; (2) the highly electron-withdrawing character of fluorine; (3) the greater stability of the C—F bond compared to the C—H bond; and (4) the solubility of fluorine-containing compounds.

Despite fluorine's slightly larger van der Waals radius than hydrogen, several studies have demonstrated that it is a reasonable hydrogen mimic and is expected to cause minimal steric perturbations with respect to binding to a receptor or enzyme.[11] Metabolic stability is important to bioavailability of compounds, and the C—F bond is stable to oxidative cleavage. Fluorine substitution also protects adjacent or distal sites from metabolism because of its strong electron-withdrawing properties. Fluorine also can reduce the basicity of compounds, which can result in better membrane permeation of the compound.



Scheme 1. Synthesis of deuterated PYT analogs.