Synthesis and Profiling of CNS Prodrugs of 5-lipoxygenase (5-LO) Inhibitors
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Purpose
Pharmacological blockade of 5-lipoxygenase (5-LO) through inhibitors like zileuton has been shown to reduce both gamma secretase-catalyzed misprocessing of amyloid precursor protein and over-phosphorylation of tau protein in transgenic mice, two hallmarks of Alzheimer’s disease (AD), through a novel non-inflammatory mechanism. However, zileuton suffers from relatively poor brain penetration due to its polar hydroxyurea group. Higher doses of zileuton have been associated with elevated liver enzymes and gastrointestinal side effects which hamper its development as a disease-modifying treatment for AD. Prodrugs of zileuton that deliver high concentrations of the parent molecule to the brain at lower plasma concentrations could overcome this problem.

A prodrug is a biologically inactive compound that is metabolized in the target tissue to release the parent drug. CNS prodrug strategies include masking polar groups with lipophilic moieties that promote brain penetration (TL prodrugs), chemical delivery systems (CDS) that trap prodrugs in the brain, and incorporating enzymatically removed groups that promote transport into the brain (TM prodrugs). We have pursued all three strategies to mask the hydroxylurea/hydroxamic acid functionality of one class of 5-LO inhibitors.

Methods
1. Prodrug synthesis: TL prodrugs were prepared by incorporating lipophilic pro-moieties into the drug through ester, carbonate, and carbamate bonds. The CDS prodrugs were prepared by incorporating linked dihydropyridine or dihydroquinoline into the drug. The TM prodrugs were prepared by adding transporter-recognized small molecules such as glucose, taurine, cysteine, methionine, and gallic acid onto the drug.
2. Solubility and stability assays: Maximum aqueous solubility was assessed at a concentration of 200 μM and pH 7.4 with the commercially available Millipore MultiScreen Solubility filter system. Aqueous and plasma stability was tested at 1 μM in 2.5% DMSO/PBS (control) or in 2.5% DMSO/male C57BL/6 mouse plasma at 37 °C for 1 hr. GI tract stability assay was tested in 10 μM in simulated gastric fluid (SGF) for 1 hour and in simulated intestinal fluid (SIF) for 3 hours. Analysis was performed by LC/MS/MS on an API 4000 or Waters Xevo TQ instrument.

Results
We synthesized 100 prodrugs (75 TLs, 11 CDS’s, and 14 TMs). Almost all prodrugs tested demonstrated good aqueous solubility (>10μM). 19 TLs, 5 CDSs, and at least 1 TM showed good plasma stability (t1/2>1h). 19 TLs and 1 CDS showed good gastrointestinal (GI) tract stability (t1/2>1h for SGF, and t1/2>3h for SIF).

Conclusion
The TL prodrugs with carbamate bonds were found to be more stable than esters and carbonates. The dihydroquinoline CDS prodrugs were more stable than the dihydropyridines. The Stability testing on the TM prodrugs is still in progress, but one gallic acid-derived TM prodrug was stable in plasma. These data will be used to prioritize prodrugs for CNS stability studies, in vivo pharmacokinetic assessment and, ultimately, in vivo pharmacological evaluation.

Figure 1. Prodrug strategy for 5-LO inhibitors. TL: traditional lipophilic prodrugs, CDS: chemical delivery system, TM: transporter mediated prodrugs; T: pro-moietly