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A long-standing mystery solved: The formation of 3-hydroxydesloratadine is catalyzed by CYP2C8 but prior glucuronidation of desloratadine by UGT2B10 is an obligatory requirement

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Running Title: 3-Hydroxydesloratadine formation by UGT2B10 and CYP2C8

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Abbreviations used are: AUC, area under the curve; CHH, cryopreserved human hepatocytes; C_{max}, maximum plasma concentration; DDI, drug-drug interaction; EM, extensive metabolizer; FDA, U.S. Food and Drug Administration; FMO, flavin-containing monooxygenase; HLM, human liver microsomes; HS9, human liver S9 fraction; K_m, substrate concentration that gives a reaction rate equal to half of V_{max}; LC-MS/MS, liquid chromatography tandem mass spectrometry; MIST, metabolites in safety testing; PM, poor metabolizer; UGT, UDP-glucuronosyltransferase.

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ABSTRACT

Desloratadine (Clarinex®), the major active metabolite of loratadine (Claritin®), is a non-sedating long-lasting antihistamine widely used for the treatment of allergic rhinitis and chronic idiopathic urticaria. For over 20 years, it has remained a mystery as to which enzymes are responsible for the formation of 3-hydroxydesloratadine, the major active human metabolite, largely due to the inability of any in vitro system tested thus far to generate this metabolite. In this study, we demonstrated that cryopreserved human hepatocytes (CHH) form 3-hydroxydesloratadine and its corresponding *O*-glucuronide. CHHs catalyzed the formation of 3-hydroxydesloratadine with a K_m of 1.6 μM and V_{max} of 1.3 pmol/min/million cells. Chemical inhibition of cytochrome P450 (CYP) enzymes in CHH demonstrated that gemfibrozil glucuronide (CYP2C8 inhibitor) and 1-aminobenzotriazole (general P450 inhibitor) inhibited 3-hydroxydesloratadine formation by 91% and 98%, respectively. Other inhibitors of CYP2C8 (gemfibrozil, montelukast, clopidogrel glucuronide, repaglinide and cerivastatin) also caused extensive inhibition of 3-hydroxydesloratadine formation (73-100%). Assessment of desloratadine, amodiaquine and paclitaxel metabolism by a panel of individual CHHs demonstrated that CYP2C8 marker activity robustly correlated with 3-hydroxydesloratadine formation (r^2 of 0.70-0.90). Detailed mechanistic studies with sonicated or saponin-treated CHHs, human liver microsomes and S9 fractions showed that both NADPH and UDP-glucuronic acid are both required for 3-hydroxydesloratadine formation, and studies with recombinant UGT and CYP enzymes implicated the specific involvement of UGT2B10 in addition to CYP2C8. Overall, our results demonstrate for the first time that desloratadine glucuronidation by UGT2B10, followed by CYP2C8 oxidation and a de-conjugation event are responsible for the formation of 3-hydroxydesloratadine.

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INTRODUCTION

Desloratadine (Clarinex®) is a second generation, non-sedating selective H1-receptor histamine antagonist with long-acting activity widely used for the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria. Desloratadine is also the major active metabolite of the antihistamine loratadine (Claritin®) and has a half-life of 21-27 h with moderate plasma protein binding (82-87%) permitting once-daily dosing (Henz, 2001; Molimard et al., 2004; Devillier et al., 2008). The major in vivo human active metabolite of desloratadine is 3-hydroxydesloratadine which is subsequently glucuronidated to 3-hydroxydesloratadine *O*-glucuronide. Both are excreted in roughly equal amounts in urine and feces (Ramanathan et al., 2007).

Furthermore, 3-hydroxydesloratadine and its glucuronide were found to be major metabolites in humans, but only trace levels were detectable in nonclinical species such as mice, rats and monkeys (Ramanathan et al., 2006), leading to the concern that nonclinical species may not have been adequately exposed to these metabolites in safety studies.

The conversion of loratadine to desloratadine (a dealkylation reaction leading to loss of a descarboethoxyl moiety) was previously shown to be catalyzed by CYP3A4 and, to a lesser extent, by CYP2D6 (Yumibe et al., 1995; Yumibe et al., 1996; Dridi and Marquet, 2013). However, the enzymology surrounding the conversion of desloratadine to 3-hydroxydesloratadine has remained a mystery both prior to and since its approval by the FDA in 2001 (Clarinex® label; www.accessdata.fda.gov/drugsatfda_docs/label/2001/21165lbl.pdf). Ghosal and colleagues (2009) examined the metabolism of loratadine and further characterized the in vitro enzymology of the metabolites using pooled human liver microsomes (HLM) and recombinant P450 enzymes (rCYPs), demonstrating that desloratadine, 5-hydroxydesloratadine and 6-hydroxydesloratadine formation could be mediated by CYP3A4, CYP2D6 and CYP2C19. However, they were unable to detect 3-hydroxydesloratadine in either in vitro test system and therefore were unable to identify which enzyme or enzymes were involved in its formation.

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However, the subsequent conjugation of 3-hydroxydesloratadine to 3-hydroxydesloratadine-*O*-glucuronide was previously shown to be catalyzed in vitro by recombinant UGT1A1, UGT1A3 and UGT2B15 (Ghosal et al., 2004).

Clinical pharmacology and safety studies demonstrated that some individuals have a phenotypic polymorphism in the metabolism of desloratadine with greatly reduced formation of 3-hydroxydesloratadine, resulting in a 3-hydroxydesloratadine to desloratadine exposure ratio of <0.1 or a desloratadine half-life of >50 h (Prenner et al., 2006). These poor metabolizers (PMs) of desloratadine were found to have a general population frequency of 6% and were most frequent in African American (17%) compared with Caucasian (2%), Native American (8%), Hispanic (2%) and Jordanian populations (3%) (Prenner et al., 2006; Hakooz and Salem, 2012). Exposure to desloratadine in PMs resulted in a 6-fold increase in desloratadine AUC compared with extensive metabolizers (EMs), leaving the FDA unable to rule out an increased risk of adverse events in PMs (Clarinex® label; www.accessdata.fda.gov/drugsatfda_docs/label/2001/21165lbl.pdf). Because the enzymology of 3-hydroxydesloratadine formation has not been elucidated, the genetic basis for the PM phenotype has not been determined.

In the present study, we sought to identify the enzyme or enzymes responsible for the formation of 3-hydroxydesloratadine by identifying an in vitro test system capable of generating the metabolite. In this report, we demonstrate that 3-hydroxydesloratadine can be formed in cryopreserved human hepatocytes. Using reaction phenotyping approaches (correlation analysis, chemical inhibition and studies with recombinant enzymes), we elucidated the main human metabolic enzymes responsible for converting desloratadine to 3-hydroxydesloratadine. We established that the 3-hydroxylation of desloratadine is catalyzed by CYP2C8, but the reaction is unusual because the prior glucuronidation of desloratadine by UGT2B10 is an obligatory step in the reaction.

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MATERIALS AND METHODS

Chemicals and Reagents

1-Aminobenzotriazole, desloratadine, furafylline, gemfibrozil, ketoconazole, mibefradil, paclitaxel, paroxetine, phencyclidine, quinidine and repaglinide were purchased from Sigma-Aldrich (St. Louis, MO); cerivastatin, CYP3cide, esomeprazole, and gemfibrozil glucuronide were purchased from Toronto Research Chemicals (Toronto, ON, Canada); montelukast was purchased from Sequoia Research Products (Pangbourne, UK); tienilic acid was purchased from Cypex (Dundee, Scotland, UK); amodiaquine and troleandomycin were purchased from US Pharmacopeia (Rockville, MD); 3-hydroxydesloratadine and clopidogrel glucuronide were purchased from Santa Cruz Biotechnology (Dallas, TX); 3-hydroxydesloratadine glucuronide, 5-hydroxydesloratadine, 6-hydroxydesloratadine were purchased from TLC PharmaChem (Vaughan, ON, Canada); 3-hydroxydesloratadine-d_4 was purchased from Medical Isotopes, Inc. (Pelham, NH). The sources of all other reagents have been described previously (Ogilvie et al., 2006; Parkinson et al., 2011; Kazmi et al., 2014a).

Test system

Pooled human liver microsomes (HLM, n = 200, mixed gender), pooled human liver S9 fraction (HS9, n = 200, mixed gender) and pooled suspended cryopreserved human hepatocytes (CHH, n = 50 or 100, mixed gender) or individual donor CHHs (see Supplemental Table 1 for donor information) were prepared from non-transplantable livers and characterized at XenoTech, LLC (Lenexa, KS) as described previously (Pearce et al., 1996; Parkinson et al., 2004). Hepatocytes from Sprague-Dawley rat (male, n = 4), Beagle dog (male, n = 3), CD1 mouse (male, n = 7), Rhesus monkey (male, n = 3), New Zealand white rabbit (male, n = 3) and Gottingen minipig (male, n = 3) were prepared and characterized at XenoTech, LLC (Lenexa, KS) as described previously.

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In vitro incubations of desloratadine with HLM, HS9 and CHH

Desloratadine was incubated with HLM, HS9 and CHH to determine if any of these in vitro test systems would support the formation of 3-hydroxydesloratadine. Briefly, 1 or 10 μM desloratadine was incubated at 37°C in 200-μL incubation mixtures containing pooled HLMs (0.1 or 1 mg/mL) or HS9 (0.5 or 5 mg/mL), potassium phosphate buffer (50 mM, pH 7.4), $MgCl_2$ (3 mM), EDTA (1 mM, pH 7.4), an NADPH-generating system (consisting of 1 mM NADP, 5 mM glucose 6-phosphate, and 1 unit/mL glucose-6-phosphate dehydrogenase) for zero, 0.5, 1, 2, and 4 h. For assays with pooled CHH (n = 50), desloratadine incubations were conducted at 37°C with 95% humidity and 5% CO_2 on an orbital shaker (~150 rpm) in 160-μL incubation mixtures containing pooled CHHs (1 million cells/mL) and Williams' E media supplemented with 2 mM glutaMAX (Gibco, Grand Island, NY) and 0.1 mM HEPES. Reactions were initiated by the addition of an NADPH-generating system (for HLM and HS9) or hepatocytes (for CHH assays) and terminated by the addition of 200 μL (160 μL for CHH assays) of acetonitrile containing 3-hydroxydesloratadine-d_4 as an internal standard. Precipitated protein was removed by centrifugation (920*g* for 10 min at 10°C) followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis as described below.

K_m and V_{max} determination of 3-hydroxydesloratadine formation in CHH

Desloratadine was incubated at 0.1, 0.2, 0.5, 1, 2, 5, 10, 20 and 30 μM in 160-μL incubation mixtures containing pooled CHHs (n = 100; 1 million cells/mL) and Williams' E media supplemented with 2 mM glutaMAX (Gibco, Grand Island, NY) and 0.1 mM HEPES. Reactions were initiated with the addition of hepatocytes and conducted for 2 h at 37°C with 95% humidity and 5% CO_2 on an orbital shaker (~150 rpm). An equal volume of acetonitrile containing 3-hydroxydesloratadine-d_4 as an internal standard was added to terminate the reactions, followed by protein precipitation and LC-MS/MS analysis.

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Assessment of 3-hydroxydesloratadine formation in animal hepatocytes

Hepatocytes from rat, dog, mouse, monkey, rabbit, minipig and human (n = 100) were assessed for their ability to form 3-hydroxydesloratadine. Briefly, 1 or 10 µM desloratadine was incubated in 160-µL incubation mixtures containing pooled hepatocytes (1 million cells/mL) and Williams' E media supplemented with 2 mM glutaMAX (Gibco, Grand Island, NY) and 0.1 mM HEPES. Reactions were initiated with the addition of hepatocytes and conducted for 2 h at 37°C with 95% humidity and 5% CO_2 on an orbital shaker (~150 rpm). An equal volume of acetonitrile containing internal standard was added to terminate the reactions, followed by protein precipitation and LC-MS/MS analysis.

Recombinant P450 assessment of 3-hydroxydesloratadine formation

Human recombinant P450 enzymes, namely CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP2J2, CYP3A4, CYP3A5, CYP3A7, CYP4A11, and CYP4F2 Bactosomes® (Cypex, Dundee, Scotland, UK); and CYP4F3a, CYP4F3b, CYP4F12, FMO1, FMO3 and FMO5 Supersomes® (Corning, Woburn, MA) were tested for their ability to form 3-hydroxydesloratadine. Briefly, 1 or 10 µM desloratadine was incubated in 200-µL incubation mixtures containing recombinant enzyme (50 pmol/mL) potassium phosphate buffer (50 mM, pH 7.4), $MgCl_2$ (3 mM), EDTA (1 mM, pH 7.4), an NADPH-generating system (consisting of 1 mM NADP, 5 mM glucose 6-phosphate, and 1 unit/mL glucose-6-phosphate dehydrogenase) for 1 h at 37°C. An additional time course experiment was conducted with rCYP2C8 (both Bactosomes® and Supersomes®) at 1, 2, 4 and 6 h. An equal volume of acetonitrile containing internal standard was added to terminate the reactions, followed by protein precipitation and LC-MS/MS analysis.

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Chemical inhibition of 3-hydroxydesloratadine formation in CHH

P450 involvement was assessed using a chemical inhibition approach as described previously (Kazmi et al., 2014b). Briefly, pooled CHHs (n = 100) at 1 million cells/mL were pre-incubated with P450 inhibitors for 30 min (2 h for the strong CYP2C8 inhibitor panel) at 37°C on an orbital shaker (~150 rpm) with 95% humidity and 5% CO_2 in 120-µL incubation mixtures containing CHH and Williams' E media supplemented with 2 mM glutaMAX (Gibco, Grand Island, NY) and 0.1 mM HEPES. The chemical P450 inhibitors used were furafylline (10 µM), phencyclidine (10 µM), gemfibrozil (100 µM), gemfibrozil glucuronide (100 µM), montelukast (50 µM), clopidogrel glucuronide (100 µM), repaglinide (100 µM), cerivastatin (100 µM), tienilic acid (20 µM), esomeprazole (10 µM), paroxetine (1 µM), quinidine (5 µM), mibefradil (1 µM), CYP3cide (2.5 µM), troleandomycin (50 µM), ketoconazole (4 µM), and 1-aminobenzotriazole (1 mM). Following pre-incubation, 40 µL of desloratadine dissolved in Williams' E media was added to yield a final concentration of 10 µM and the incubation was continued for 2 h; or, for those samples pre-incubated with CYP2C8 inhibitors, additional incubations with amodiaquine (10 µM) and paclitaxel (10 µM) were conducted for 10 and 30 min respectively. Reactions were terminated by the addition of an equal volume of acetonitrile containing internal standard, followed by protein precipitation and LC-MS/MS analysis.

Correlation analysis of CHHs with a range of CYP2C8 activities

Individual donor CHHs that were pre-characterized with a range of CYP2C8 activities were assessed for 3-hydroxydesloratadine formation. Briefly, nine individual lots of hepatocytes were incubated at 1 million cells/mL with 1 or 10 µM desloratadine, amodiaquine or paclitaxel in 160-µL incubation mixtures containing Williams' E media supplemented with 2 mM glutaMAX (Gibco, Grand Island, NY) and 0.1 mM HEPES. Reactions were initiated by the addition of hepatocytes and conducted for 10 min (amodiaquine), 30 min (paclitaxel) or 2 h (desloratadine)

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at 37°C with 95% humidity and 5% CO_2 on an orbital shaker (~150 rpm). Reactions were terminated by the addition of an equal volume of acetonitrile containing internal standard, followed by protein precipitation and LC-MS/MS analysis.

Exogenous cofactor addition with CHH, HLM or HS9

Pooled CHHs (n=100; 1 million cells/mL) were treated with 0.01% (w/v) saponin (5 min) or disrupted with a probe sonicator (45 sec at 40-60% amplitude) followed by incubation in 160-µL incubation mixtures containing 10 µM desloratadine in Williams' E media supplemented with 2 mM glutaMAX (Gibco, Grand Island, NY) and 0.1 mM HEPES. Incubations were conducted in the presence or absence 0.1 mM NADPH and/or 1 mM UDP-GlcUA and conducted for 2 h at 37°C with 95% humidity and 5% CO_2 on an orbital shaker (~150 rpm). For incubations with pooled subcellular fractions, HLM at 0.1 and 1 mg/mL or HS9 at 0.5 and 5 mg/mL were pretreated for 15 min on ice with 25 µg/mg alamethicin followed by incubation with or without 1 mM chemical NADPH and/or 10 mM UDP-GlcUA at 37°C in 200-µL incubation mixtures containing potassium phosphate buffer (50 mM, pH 7.4), $MgCl_2$ (3 mM), EDTA (1 mM, pH 7.4) for zero, 1, 2, 4 and 6 h. Other cofactors such as NADH, FAD, AMP and ATP were also tested (each at 1 mM). All reactions were terminated by the addition of an equal volume of acetonitrile containing internal standard, followed by protein precipitation and LC-MS/MS analysis.

Recombinant UGT panel with recombinant CYP2C8

Recombinant human UGT enzymes, namely UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B10, UGT2B15, and UGT2B17 Supersomes® (Corning, Woburn, MA) supplemented with recombinant CYP2C8 Supersomes® (Corning, Woburn, MA) were evaluated for their ability to form 3-hydroxydesloratadine. Briefly, 0.125 mg/mL recombinant UGT was supplemented with 25 pmol/mL of recombinant CYP2C8, followed by addition of 1 or 10 µM desloratadine and incubation with 1 mM chemical NADPH

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and 10 mM UDP-GlcUA at 37°C in 200-µL incubation mixtures containing potassium phosphate buffer (50 mM, pH 7.4), $MgCl_2$ (3 mM), EDTA (0.5 or 1 mM, pH 7.4) for 2 h. Reactions were terminated by the addition of an equal volume of acetonitrile containing internal standard, followed by protein precipitation and LC-MS/MS analysis.

Analytical methods

Samples were analyzed by LC-MS/MS using a method developed in-house. The LC system comprised a Shimadzu SIL-5000 autosampler, two Shimadzu LC-20AD_{VP} pumps and a Shimadzu DGU-20A3 degasser (Shimadzu, Columbia, MD). An LC gradient employing 0.2% formic acid in water (A) and acetonitrile (B) at 0.6 mL min^{-1} was applied to a Waters XBridge C18 column (5 µm, 4.6 x 100 mm) for separation of desloratadine and its metabolites. The gradient consisted of 10% B for 0.5 min followed by a linear ramp to 95% B at 9.5 min, a 1 min hold at 95% B and then a 2 min re-equilibration period back to 10% B.

Analytes were detected with an AB Sciex API4000 QTrap mass spectrometer (AB Sciex, Foster City, CA) using positive mode and electrospray ionization. A multiple reaction monitoring (MRM) information-dependent acquisition (IDA) detection method was developed based on manually derived transitions for known and predicted metabolites of desloratadine. Twenty-seven MRM transitions with 30 msec dwell times were employed, including 311/259 for desloratadine (5.3 min retention time); 327/275 for 3-hydroxydesloratadine and 331/279 for 3-hydroxydesloratadine-d_4 (5.2 min), 327/275 for 5-hydroxydesloratadine (4.9, 5.0 min) and 327/275 for 6-hydroxydesloratadine (4.7 min); and 503/327 for 3-hydroxydesloratadine glucuronide (5.1 min). Retention times for these 5 analytes were confirmed by comparison with reference standards. The electrospray voltage applied was 4500 V, the collision gas was set to high, the curtain gas was at 30 psi, the source temperature was 600°C, and the collision energy for the MRM scans was 35 eV. The declustering potential applied was 80 V for desloratadine

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and oxidative metabolite transitions and 30 V for labile conjugated metabolite transitions. The IDA criteria were set to acquire product ion spectra across the *m/z* range 80-700 for a peak exceeding 500 counts in the survey MRM scan. The product ion spectra were used to further confirm detection of the 3-hydroxydesloratadine (characteristic neutral loss of 17 amu) versus the 5- and 6-hydroxydesloratadine metabolites (characteristic neutral loss of 18 amu) as reported by Ramanathan et al. (2000). Where applicable, a calibration curve comprising six concentration levels prepared in duplicate across the range 0.005 – 1 μM was used for 3-hydroxydesloratadine.quantitation with quadratic regression and $1/x^2$ weighting.

Data analysis

All data processing and statistical analysis were conducted with Microsoft Excel 2010 (Microsoft, Redmond, WA). Non-linear fitting and determination of K_m and V_{max} were performed with GraFit 7.0.2 (Erithacus Software Ltd., Horley, Surrey, UK).

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RESULTS

Determination of an in vitro test system capable of producing 3-hydroxydesloratadine. To determine whether 3-hydroxydesloratadine could be formed in any conventional in vitro test system, desloratadine (1 and 10 μM) was incubated with HLM (0.1 and 1 mg/mL), HS9 (0.5 and 5 mg/mL) and CHHs (1 million cells/mL) in a time course experiment up to 4 h as described in *Materials and Methods*. The results for 3-hydroxydesloratadine formation with 1 μM desloratadine are shown in Figure 1 (10 μM desloratadine data were similar and not shown). 3-Hydroxydesloratadine formation was not observed in any HLM or HS9 sample, consistent with previous reports (Ghosal et al., 2009). However, 3-hydroxydesloratadine was detected in CHHs as early as 30 min, with linear metabolite formation up to 4 h. Furthermore, 3-hydroxydesloratadine glucuronide was also detected in CHHs as early as 1 h with 10 μM desloratadine, but only at 4 h with 1 μM desloratadine. Additional expected hydroxydesloratadine metabolites such as 5- and 6-hydroxydesloratadine were detected in all three test systems.

Assessment of the kinetics of 3-hydroxydesloratadine formation. Having established that CHHs were the only test system capable of forming 3-hydroxydesloratadine we sought to determine the K_m and V_{max} of 3-hydroxydesloratadine formation from desloratadine. Linearity of 3-hydroxydesloratadine formation was established beyond 2 h. Desloratadine was incubated at nine concentrations with pooled CHHs (1 million cells/mL) for 2 h as described in *Materials and Methods*. As shown in Figure 2, 3-hydroxydesloratadine formation followed Michaelis-Menten kinetics, with K_m and V_{max} values of 1.6 μM and 1.3 pmol/min/million cells, respectively.

Assessment of the species specificity of 3-hydroxydesloratadine formation. We evaluated whether 3-hydroxydesloratadine could be formed by hepatocytes from different species. Mouse, rat, rabbit, dog, minipig, monkey and human hepatocytes (1 million cells/mL) were incubated

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with 1 or 10 μM desloratadine for 2 h as described in *Materials and Methods*. The results for 3-hydroxydesloratadine formation (Figure 3) showed that at 1 μM desloratadine, rabbit and human hepatocytes formed similar amounts of 3-hydroxydesloratadine, whereas dog hepatocytes formed one third as much and monkey hepatocytes formed only a trace amount. In contrast, no 3-hydroxydesloratadine was detected in incubations of 1 μM desloratadine with mouse, rat or minipig hepatocytes. At 10 μM desloratadine, only rabbit and human hepatocytes formed 3-hydroxydesloratadine, with rabbit forming three times as much 3-hydroxydesloratadine as human hepatocytes. No 3-hydroxydesloratadine formation was observed in incubations of 10 μM desloratadine with mouse, rat, dog, minipig or monkey hepatocytes. Formation of both 5- and 6-hydroxydesloratadine in each species was determined simultaneously (shown in Supplemental Figure 1), with all animal species forming higher levels of these metabolites than human hepatocytes. Rabbit hepatocytes formed the greatest amount of 5-hydroxydesloratadine, while rabbit and minipig hepatocytes formed the greatest amounts of 6-hydroxydesloratadine.

Identification of the enzyme responsible for 3-hydroxydesloratadine formation using recombinant enzymes. To determine which specific drug-metabolizing enzymes were responsible for the formation of 3-hydroxdesloratadine, recombinant P450 and FMO enzymes (50 pmol/mL) were incubated with 1 and 10 μM desloratadine and incubated for 1 h as described in *Materials and Methods*. The data are summarized in Table 1. No 3-hydroxydesloratadine was detected in any recombinant P450 or FMO enzyme sample tested, consistent with previously reported findings (Ghosal et al., 2009). Both 5- and 6-hydroxydesloratadine were readily formed by CYP1A1, CYP2D6 and CYP3A4, with trace metabolite formation observed for several other P450 enzymes (Table 1). A time course experiment for up to 6 h with recombinant CYP2C8 also failed to generate any 3-hydroxydesloratadine (data not shown).

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Identification of the enzyme responsible for 3-hydroxydesloratadine formation using chemical inhibitors. Since a recombinant P450/FMO enzyme approach was unable to identify any enzyme capable of forming 3-hydroxydesloratadine, we evaluated the effects of P450-selective inhibitors on the formation of 3-hydroxydesloratasine by CHHs. Initially, a panel of chemical inhibitors specific to different CYP enzymes was used (as summarized in Table 1), namely, furafylline (CYP1A2); phencyclidine (CYP2B6); gemfibrozil glucuronide (CYP2C8); tienilic acid (CYP2C9); esomeprazole (CYP2C19); paroxetine and quinidine (CYP2D6); mibefradil, CYP3cide, troleandomycin and ketoconazole (CYP3A4/5); in addition to the non-specific P450 inhibitor 1-aminobenzotriazole (1-ABT). Desloratadine (10 μM) was incubated with CHHs for 2 h following pre-incubation of the hepatocytes with each individual chemical inhibitor as described in *Materials and Methods*. As shown in Figure 4A and Table 1, the formation of 3-hydroxydesloratadine by CHHs was extensively inhibited by gemfibrozil glucuronide (91.3% inhibition) and 1-ABT (97.8% inhibition). Furthermore, formation of 3-hydroxydesloratadine-*O*-glucuronide was inhibited completely by the CYP2C8 inhibitor gemfibrozil glucuronide and the nonspecific inhibitor 1-ABT, whereas the formation of 5- and 6-hydroxydesloratadine was primarily inhibited by inhibitors of CYP3A4/5 and 1-ABT (see Supplemental Figure 2). To further explore the involvement of CYP2C8 in 3-hydroxydesloratadine formation, the panel of CYP2C8 inhibitors was extended to include montelukast, repaglinide, cerivastatin, clopidogrel glucuronide, gemfibrozil and its acyl glucuronide. As shown in Figure 4B, the formation of 3-hydroxydesloratadine by CHHs was completely inhibited by montelukast, gemfibrozil, and gemfibrozil glucuronide; and extensively inhibited by clopidogrel glucuronide (78.9%), repaglinide (73.3%) and cerivastatin (84.6%). In addition to examining their effects on 3-hydroxdesloratadine formation, the CYP2C8 inhibitors were also examined for their effects on two CYP2C8 marker reactions in CHHs, namely amodiaquine N-dealkylation and paclitaxel 6α-hydroxylation. As expected, inhibition of amodiaquine and paclitaxel metabolism by the

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panel of CYP2C8 inhibitors correlated well each other and with the degree of inhibition of 3-hydroxydesloratadine formation.

Correlation of 3-hydroxydesloratadine formation with known CYP2C8 activities. Hepatocytes from nine individual human donors with a range of CYP2C8 activity towards amodiaquine and paclitaxel were assessed for their ability to form 3-hydroxydesloratadine, as described in *Materials and Methods*. As shown in Figure 5, the sample-to-sample variation in the 3-hydroxylation of desloratadine (1 and 10 μM) correlated well with the 6α-hydroxylation of paclitaxel (1 and 10 μM), r^2 values of 0.84 and 0.90 (at 1 and 10 μM) and with the N-dealkylation of amodiaquine, with r^2 values of 0.84 and 0.70 (at 1 and 10 μM). As expected, amodiaquine and paclitaxel activities highly correlated with each other, with r^2 values of 0.77 and 0.80 (at 1 and 10 μM), as shown in Supplemental Figure 3.

Determining the reason why 3-hydroxydesloratadine forms in hepatocytes but not in subcellular fractions. As a first approach to understand why CYP2C8 in CHHs could convert desloratadine to 3-hydroxydesloratadine whereas HLM, HS9 and recombinant P450 could not, we examined the role of cell integrity and various cofactors involved in xenobiotic metabolism. As described in *Materials and Methods*, CHHs were treated with 0.01% saponin (to permeabilize the plasma membrane) or sonication (to completely disrupt the plasma membrane) in media supplemented with 10 μM desloratadine and various cofactors, NADPH, NADH, FAD, AMP, ATP, and UDP-GlcUA. As shown in Figure 6A, when intact CHHs were treated with 0.01% saponin, the formation of 3-hydroxydesloratadine was reduced by 90%. Addition of exogenous NADPH did not change the rate of 3-hydroxydesloratadine formation; however addition of UDP-GlcUA and NADPH + UDP-GlcUA partially restored 3-hydroxydesloratadine formation to 22% and 37% of that observed in intact CHHs, respectively. Similarly, when CHHs were probe sonicated, 3-hydroxydesloratadine formation was almost completely eliminated with

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only 2.5% activity remaining. Addition of exogenous NADPH here also did not alter the level of 3-hydroxydesloratine formation; however addition of UDP-GlcUA and NADPH + UDP-GlcUA again partially restored 3-hydroxydesloratadine formation to 15% and 34% of that observed in intact CHHs, respectively. To ascertain whether a similar combination of cofactors could confer desloratadine 3-hydroxylase activity on subcellular fractions, HLM (0.1 and 1 mg/mL) and HS9 (0.5 and 5 mg/mL) were incubated with 10 μM desloratadine for up to 6 h with NAPDH and/or UDP-GlcUA, as described in *Materials and Methods*. With either of these subcellular fractions, only the addition of a combination of NADPH + UDP-GlcUA resulted in 3-hydroxydesloratadine formation; addition of NADPH alone or UDP-GlcUA alone did not. Representative data from 1 mg/mL HLM and 5 mg/mL S9 are shown in Figure 6B (0.1 mg/mL HLM and 0.5 mg/mL HS9 data were similar and are shown in Supplemental Figure 4). The addition of several other cofactors (NADH, FAD, AMP and ATP) had no effect on the formation of 3-hydroxydesloratadine in sonicated or permeabilized CHHs or in HLMs or HS9 (data not shown).

Identification of the UGT enzymes involved in 3-hydroxydesloratadine formation. Having established that a combination of both UDP-GlcUA and NADPH was necessary to support 3-hydroxydesloratadine formation by disrupted CHHs or subcellular fractions, we sought to determine the specific UGT enzyme involved in 3-hydroxydesloratadine formation. As described in the *Materials and Methods*, a panel of 13 recombinant UGT enzymes (at 0.125 mg/mL each) supplemented with recombinant CYP2C8 (25 pmol/mL) was incubated for 2 h with 1 or 10 μM desloratadine in the presence of both NADPH with UDP-GlcUA. As shown in Figure 7, 3-hydroxydesloratadine was formed by a combination of UGT2B10 and CYP2C8. Formation of 3-hydroxydesloratadine was not observed when CYP2C8 was incubated with any other recombinant UGT enzyme.

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DISCUSSION

Loratadine (Claritin®), first introduced to the U.S. market in 1993, is metabolized primarily by CYP3A4 and CYP2D6 to its pharmacologically active major metabolite desloratadine (Yumibe et al., 1995; Yumibe et al., 1996; Dridi and Marquet, 2013). As a drug in its own right, desloratadine (Clarinex® in the US, Aerius® in Europe) received FDA approval in 2001. It has long been known that the major circulating metabolite of desloratadine in humans is 3-hydroxydesloratadine, formed by hydroxylation of the pyridine ring (Clarinex® label; www.accessdata.fda.gov/drugsatfda_docs/label/2001/21165lbl.pdf). However, as acknowledged in the package insert, the enzyme or enzymes responsible for forming 3-hydroxydesloratadine was unknown when desloratadine was approved by the FDA in 2001 and has remained unknown since that time. Although presumably formed by P450, conventional in vitro test systems do not convert desloratadine to the 3-hydroxy metabolite (Ghosal et al., 2009). In the present study, we confirmed that human liver microsomes (HLMs), human S9 fraction (HS9) and recombinant human P450 enzymes all failed to convert desloratadine to 3-hydroxydesloratadine but we demonstrated for the first time that cryopreserved human hepatocytes (CHHs) are capable of forming the 3-hydroxy metabolite (Figure 1 and Table 1). In vitro formation of this previously elusive metabolite allowed us to investigate the enzymology surrounding its formation. The formation of 3-hydroxydesloratadine by CHHs conformed to simple Michaelis-Menten kinetics with a V_{max} of 1.26 pmol/min/million cells and a K_m of 1.57 μM (Figure 2), which is consistent with the reported plasma C_{max} of desloratadine in humans (1.3 μM) (Clarinex® label; www.accessdata.fda.gov/drugsatfda_docs/label/2001/21165lbl.pdf). These results strongly suggest that hepatic metabolism is responsible for the formation of 3-hydroxydesloratadine and are consistent with the urinary and biliary excretion of this metabolite (and its *O*-glucuronide) in humans (Ramanathan et al., 2006; Ramanathan et al., 2007). Data on the metabolism of desloratadine in nonclinical species are limited, with 5- and 6-hydroxydesloratadine reported as the major excreted metabolites in mouse, rat and monkey.

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In contrast to the situation in humans, 3-hydroxydesloratadine was found to be a minor or trace plasma, urinary and fecal metabolite in nonclinical species (Ramanathan et al., 2005; Ramanathan et al., 2006). In our assessment of desloratadine metabolism in hepatocytes from different species (Figure 3), 3-hydroxydesloratadine was observed at low levels in mouse, rat and monkey hepatocytes, consistent with previously reported in vivo findings (Ramanathan et al., 2006). However, rabbit, dog and human were able to form the 3-hydroxy metabolite in incubations at a pharmacologically relevant concentration (1 μM desloratadine), whereas only rabbit and human formed it at the high concentration (10 μM desloratadine). It is unclear whether rabbits were evaluated as nonclinical metabolism species during desloratadine development; however, our data suggest they may be appropriate species to model 3-hydroxydesloratadine exposure. Formation of 5- and 6-hydroxydesloratadine was faster in hepatocytes from all nonclinical species tested compared with human hepatocytes (Supplemental Figure 1), consistent with the in vivo data (Ramanathan et al., 2005; Ramanathan et al., 2006). In the present study, formation of 5- and 6-hydroxydesloratadine was primarily mediated by recombinant CYP1A1, CYP2D6 and CYP3A4, confirming previously reported findings (Ghosal et al., 2009). Chemical inhibition experiments in CHHs confirmed the involvement of CYP3A4 in 5-hydroxydesloratadine formation, and likewise confirmed involvement of both CYP2D6 and CYP3A4 in 6-hydroxydesloratadine formation (Table 1 and Supplemental Figure 2).

The non-specific inhibitor 1-ABT markedly inhibited (98%) the formation of 3-hydroxydesloratadine by CHHs, confirming expectations that this reaction is catalyzed by P450 (Figure 4A). Marked inhibition (91%) was also observed with gemfibrozil glucuronide (Figure 4A). Gemfibrozil glucuronide is an irreversible (mechanism-based) inhibitor of CYP2C8 and is widely used as an in vitro diagnostic inhibitor of this enzyme (Ogilvie et al., 2006; Parkinson et al., 2011; Kazmi et al., 2014b). To confirm CYP2C8 involvement in

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3-hydroxydesloratadine formation, a panel of known CYP2C8 inhibitors or substrates (competitive inhibitors) was evaluated (Figure 4B), namely, montelukast, repaglinide, cerivastatin, clopidogrel glucuronide, and both gemfibrozil and gemfibrozil glucuronide (Bidstrup et al., 2003; Walsky et al., 2005; Ogilvie et al., 2006; Tornio et al., 2014). Strong inhibition of 3-hydroxydesloratadine formation was observed with all CYP2C8 inhibitors and correlated well with the degree of inhibition in the metabolism of two CYP2C8 substrates (paclitaxel and amodiaquine), supporting CYP2C8 as the P450 enzyme responsible for 3-hydroxydesloratadine formation. A comparison of CYP2C8 activity in nine individual samples of human hepatocytes demonstrated high correlation (r^2 = 0.7-0.9) between 3-hydroxydesloratadine formation and both amodiaquine N-dealkylation and paclitaxel 6α-hydroxylation (Figure 5).

The results presented so far seem paradoxical. They raise the question: If CYP2C8 is the major enzyme responsible for converting desloratadine to 3-hydroxydesloratadine in human hepatocytes, based on chemical inhibition and correlation analysis, why is no 3-hydroxydesloratadine formed by HLM, HS9 or recombinant CYP2C8? We hypothesized that perhaps cellular integrity or the presence of specific cofactors was critical for 3-hydroxydesloratadine formation. In support of this possibility, we found that permeabilizing the plasma membrane of hepatocytes with saponin or completely disrupting the membrane by sonication greatly reduced 3-hydroxydesloratadine formation (Figure 6A). Addition of various cofactors to permeabilized/sonicated hepatocytes revealed that formation of 3-hydroxydesloratadine could be partially restored by the addition of both NAPDH and UDP-GlcUA. Modest recovery was also observed in permeabilized/sonicated hepatocytes supplemented with only UDP-GlcUA presumably because there was sufficient endogenous NADPH to support some 3-hydroxy metabolite formation. Subsequent experiments with HLM and HS9 (Figure 6B) confirmed the requirement of both NADPH and UDP-GlcUA for the formation of 3-hydroxydesloratadine.

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These results suggested that, in addition to oxidation by CYP2C8, glucuronidation plays a key role in the formation of 3-hydroxydesloratadine. To explore this possibility further, desloratadine was incubated with recombinant CYP2C8 in the absence or presence of a panel of recombinant UGT enzymes (with NAPDH and UDP-GlcUA as cofactors). In the absence of any UGT enzyme, CYP2C8 did not form 3-hydroxydesloratadine but did so in the presence of UGT2B10 (Figure 7). These results suggest that desloratadine is glucuronidated by UGT2B10 and that desloratadine glucuronide, not desloratadine itself, is the substrate that undergoes 3-hydroxylation by CYP2C8. Furthermore, the results suggest that the glucuronide moiety introduced by UGT2B10 is cleaved during or shortly after metabolism by CYP2C8

A proposed metabolic scheme for 3-hydroxydesloratadine formation is shown in Figure 8. The first step is proposed as formation of desloratadine *N*-glucuronide by UGT2B10, followed by hydroxylation to 3-hydroxydesloratadine *N*-glucuronide by CYP2C8, with subsequent de-conjugation to 3-hydroxydesloratadine. Efforts to isolate and characterize the proposed intermediary metabolites are currently underway. An *N*-glucuronide is proposed as the initial metabolite because there are no hydroxyl or thiol groups available for direct conjugation. UGT2B10 is one of two enzymes, the other being UGT1A4, renowned for their ability to catalyze the *N*-glucuronidation of drugs, with UGT2B10 being a high affinity/low capacity enzyme UGT1A4 being a low affinity/high capacity enzyme (Zhou et al., 2010; Parkinson et al., 2013). Ketotifen, a structural analog of desloratadine, is known to be *N*-glucuronidated at the piperidine ring to a quaternary N-glucuronide by UGT2B10 and UGT1A4. Furthermore, *N*-glucuronidation of ketotifen is a prominent reaction in rabbits and humans, the two species whose hepatocytes catalyzed the highest rate of formation of 3-hydroxydesloratadine (Kato et al., 2013; Bolleddula et al., 2014). It has been previously reported that rabbits may be a particularly useful species for nonclinical studies of drugs that undergo N-glucuronidation in humans (Chiu and Huskey, 1998).

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However, *N*-glucuronidation by UGT2B10 on the pyridine moiety of desloratadine cannot be ruled out and has been shown to occur in the case of nicotine and cotinine glucuronidation (Murphy et al., 2014).

The ability of CYP2C8 to metabolize a glucuronide conjugate is well established (Parkinson et al., 2013). For example, whereas the 4′-hydroxylation of diclofenac (parent drug) is catalyzed by CYP2C9, the 4′-hydroxylation of diclofenac acyl glucuronide is catalyzed by CYP2C8 (Kumar et al., 2002). This same pattern, where the aglycone (typically a small acidic substrate) is not metabolized by CYP2C8 (and in some cases is metabolized by CYP2C9) whereas the glucuronide metabolite (a large acidic substrate) is metabolized by CYP2C8, has been reported for estradiol 17-O-β-glucuronide and the acyl glucuronide conjugates of naproxen, the PPARα agonist MRL-C, and gemfibrozil (Delaforge et al., 2005; Kochansky et al., 2005; Ogilvie et al., 2006; Parkinson et al., 2013). In the case of gemfibrozil, the CYP2C8-mediated hydroxylation of its 1-O-β-glucuronide forms a benzyl radical intermediate that causes irreversible inhibition of CYP2C8 (Ogilvie et al., 2006; Baer et al., 2009).

The conversion of desloratadine by UGT2B10 to an *N*-glucuronide that is subsequently hydroxylated by CYP2C8 is consistent with the known properties of these enzymes. Nevertheless, the formation of 3-hydroxydesloratadine is unusual because no 3-hydroxylation is detectable in the absence of glucuronidation and because the *N*-glucuronide is cleaved during or shortly after CYP2C8-dependent hydroxylation. Interestingly, while 3-hydroxydesloratadine is produced from an *N*-glucuronide (formed by UGT2B10 and cleaved following hydroxylation by CYP2C8), 3-hydroxydesloratadine itself is subsequently converted to an *O*-glucuronide (at the 3-hydroxy position) by UGT1A1, UGT1A3 and UGT2B15 (Ghosal et al., 2004).

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It is unclear whether there is any potential for drug-drug interactions (DDIs) with desloratadine as it has a large therapeutic safety margin. As a perpetrator, desloratadine has been shown not to be an inhibitor of CYP1A2, CYP2C9, CYP2C19, CYP2D6 or CYP3A4 (Barecki et al., 2001). However, to our knowledge, inhibition of CYP2C8 and UGT2B10 has not been evaluated, so it is unclear whether desloratadine could cause any clinically-relevant interactions with substrates of these enzymes. A recent clinical study examining the effect of desloratadine on montelukast serum levels found no significant difference in montelukast serum levels in fixed-dose combination with desloratadine (Cingi et al., 2013). All other relevant studies have examined the pharmacodynamic/pharmacokinetic interaction potential of desloratadine with CYP2D6 and CYP3A4 substrates, and desloratadine was found to have limited potential for DDI (Gupta et al., 2001; Banfield et al., 2002a; Banfield et al., 2002b; Gupta et al., 2004). With respect to special populations, patients with moderate hepatic impairment have been shown to have elevated levels of desloratadine (2.4-fold increase in AUC); however it has been reported that 3-hydroxydesloratadine exposure was similar between hepatically-impaired and normal patients (Gupta et al., 2007).

The pharmacogenetic basis for the 3-hydroxydesloratadine poor metabolizer (PM) phenotype has remained a mystery. It has been reported that the polymorphism surrounding 3-hydroxydesloratadine formation occurs in approximately 6% of the general population and at a frequency of 17% in African Americans, with PMs having approximately 6-fold greater systemic exposure than extensive metabolizers (EMs) (Prenner et al., 2006). Our results suggest that CYP2C8 and/or UGT2B10 polymorphism may be responsible for the poor metabolizer phenotype. A large number of CYP2C8 genetic polymorphisms have been identified, with CYP2C8*2, *3, *4, *8 and *14 alleles shown to have decreased functional activity (Dai et al., 2001; Bahadur et al., 2002; Hichiya et al., 2005; Gao et al., 2010; Hanioka et al., 2010; Jiang et al., 2011). However, little is currently known about UGT2B10 polymorphisms, although the

UGT2B10*2 allele has been shown to correspond to a functional decrease in nicotine and cotinine glucuronide formation (Chen et al., 2007). Further studies will be necessary to establish whether genetic polymorphisms of CYP2C8 and/or UGT2B10 can account for the desloratadine PM phenotype.

In summary, the following evidence suggests that the conversion of desloratadine to 3-hydroxydesloratadine is mediated by CYP2C8 in conjunction with UGT2B10:

1. The formation of 3-hydroxydesloratadine by human hepatocytes is inhibited by reversible and irreversible inhibitors of CYP2C8;
2. In human hepatocytes, the sample to sample variation in 3-hydroxydesloratadine formation correlates with CYP2C8 activity towards amodiaquine and paclitaxel;
3. Human liver microsomes and S9 fraction do not form 3-hydroxydesloratadine unless supplemented with both NADPH and UDP-GlcUA;
4. Recombinant CYP2C8 does not form 3-hydroxydesloratadine unless co-incubated with recombinant UGT2B10 and both NADPH and UDP-GlcUA;
5. No other pair of recombinant CYP and UGT enzyme converted desloratadine to 3-hydroxydesloratadine.

We were unable to detect either desloratadine N-glucuronide (formed by UGT2B10) or 3-hydroxydesloratadine N-glucuronide (the initial metabolite formed by CYP2C8). These glucuronides appear to be very unstable, which is a characteristic of certain other *N*-glucuronides (Ciotti et al., 1999). Despite this limitation, the identification of CYP2C8 in combination with UGT2B10 in the formation of 3-hydroxydesloratadine contributes to our understanding of the long-standing mystery surrounding the enzymology of 3-hydroxydesloratadine formation in humans, providing a pathway for future investigation of the genetic basis for the desloratadine poor metabolizer phenotype.

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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Kazmi, Barbara and Parkinson.

Conducted experiments: Kazmi and Yerino.

Contributed new reagents or analytic tools: Barbara.

Performed data analysis: Kazmi, Yerino, Barbara.

Wrote or contributed to the writing of the manuscript: Kazmi, Barbara, and Parkinson.

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FIGURE LEGENDS

Fig. 1: **The formation of 3-hydroxydesloratadine over time in cryopreserved human hepatocytes (CHHs), human liver microsomes (HLM) and human *S9* fraction (HS9).** The time course of formation of 3-hydroxydesloratadine was assessed in hepatocytes (1 million cells/mL), liver microsomes (0.1 and 1 mg/mL) and liver *S9* (0.5 and 5 mg/mL) with 1 μM desloratadine for up to 4h.

Fig. 2: **Determination of the enzyme kinetics for the formation of 3-hydroxydesloratadine from desloratadine in cryopreserved human hepatocytes (CHHs).** As described in *Materials and Methods*, the kinetics for the formation of 3-hydroxydesloratadine were determined in CHH (1 million cells/mL) with 0.1, 0.2, 0.5, 1, 2, 5, 10, 20 and 30 μM desloratadine incubated for 2 h. The left panel represents rate versus substrate concentration, and the right panel represents the Eadie-Hofstee plot.

Fig. 3: **Formation of 3-hydroxydesloratadine in pooled cryopreserved mouse, rat, rabbit, dog, minipig, monkey and human hepatocytes.** Hepatocytes were incubated at 1 million cells/mL with 1 or 10 μM desloratadine for 2 h. Data are represented as the percent of 3-hydroxydesloratadine formation relative to the maximum amount formed.

Fig. 4: **Effect of specific P450 chemical inhibitors on formation of 3-hydroxydesloratadine in pooled cryopreserved human hepatocytes (CHHs).** As shown in panel A, chemical inhibitors towards specific P450 enzymes, namely furafylline (10 μM; CYP1A2), phencyclidine (10 μM; CYP2B6), gemfibrozil glucuronide (100 μM; CYP2C8), tienilic acid (20 μM, CYP2C9), esomeprazole (10 μM; CYP2C19); paroxetine (1 μM; CYP2D6); quinidine (5 μM; CYP2D6), mibefradil (1 μM; CYP3A4/5), CYP3cide (2.5 μM; CYP3A4/5),

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troleandomycin (50 μM; CYP3A4/5), ketoconazole (4 μM, CYP3A4/5), and 1-aminobenzotriazole (1 mM; general CYP inhibitor) were incubated with CHHs (1 million cells/mL) for 30 min, prior to incubation with 10 μM desloratadine for 2 h and analysis by LC-MS/MS as described in *Materials and Methods*. Subsequently, multiple inhibitors of CYP2C8 (shown in panel B) were examined for their ability to inhibit 3-hydrodesloratadine formation, amodiaquine N-dealkylation and paclitaxel 6α-hydroxylation in CHHs; namely montelukast (50 μM), gemfibrozil (100 μM), gemfibrozil glucuronide (100 μM), clopidogrel glucuronide (100 μM), repaglinide (100 μM) and cerivastatin (100 μM) as described in *Materials and Methods*.

Fig. 5: **Correlation between CYP2C8 activity and 3-hydroxydesloratadine formation in individual donor cryopreserved human hepatocytes (CHHs).** As described in *Materials and Methods*, individual donor CHHs from nine donors with varying CYP2C8 activity were incubated (1 million cells/mL) with 1 or 10 μM amodiaquine, paclitaxel, and desloratadine for 10 min, 30 min and 2 h respectively.

Fig. 6: **Formation of 3-hydroxydesloratadine in saponin treated or sonicated cryopreserved human hepatocytes (CHHs) followed by addition of NADPH and/or UDP-GlcUA.** As shown in panel A, CHHs (1 million cells/mL) were either pre-treated with 0.01% saponin or probe sonicated followed by addition of 0.1 mM NADPH and/or 1 mM UDPGA and incubation with 10 μM desloratadine for 2 h. Panel B shows the formation of 3-hydroxydesloratadine in subcellular fractions, namely human liver microsomes (HLM; 1 mg/mL) and human S9 fraction (HS9; 5 mg/mL) as assessed over 6 h with or without 1 mM NADPH and/or 10 mM UDP-GlcUA.

Fig. 7: **Assessment of 3-hydroxydesloratadine formation with a panel of recombinant UGT enzymes supplemented with recombinant CYP2C8.** Thirteen recombinant UGT

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enzymes (at 0.125 mg/mL) were assessed for their ability to form 3-hydroxydesloratadine when supplemented with recombinant CYP2C8 (25 pmol/mL) and 1 mM NADPH with 10 mM UDP-GlcUA, followed by a 2 h incubation with 1 or 10 μM desloratadine.

Fig. 8: **Proposed metabolic scheme for the formation of 3-hydroxydesloratadine and its glucuronide in human hepatocytes and liver subcellular fractions.** The proposed metabolic pathway for desloratadine metabolism based on this study. The conversion of 3-hydroxydesloratadine to 3-hydroxydesloratadine glucuronide was previously described by Ghosal et al. (2004).

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Table 1: Chemical inhibition and formation of 3-hydroxydesloratadine in cryopreserved human hepatocytes (CHHs) and recombinant enzymes

Enzyme	**Inhibitor**	**Inhibition of 3-OH formation (%)**	**Recombinant enzyme activity**		
			3-OH	**5-OH[a]**	**6-OH[a]**
CYP	1-Aminobenzotriazole	98	NA		
CYP1A1	NA	NA	None	Yes	Yes
CYP1A2	Furafylline	0		None[b]	None[c]
CYP1B1	NA	NA			
CYP2A6					
CYP2B6	Phencyclidine	13.3			
CYP2C8	Gemfibrozil glucuronide	91.3-100			
	Gemfibrozil	100			
	Montelukast	100			
	Clopidogrel glucuronide	78.9			
	Repaglinide	73.3			
	Cerivastatin	84.6			
CYP2C9	Tienilic acid	0			
CYP2C18	NA	NA			
CYP2C19	Esomeprazole	22.5			
CYP2D6	Paroxetine, Quinidine	0		Yes	Yes
CYP2E1	NA	NA		None[b]	None[c]
CYP2J2					
CYP3A4/5	Mibefradil	0		Yes (CYP3A4)	Yes (CYP3A4)
	CYP3cide	0			
	Troleandomycin	0			
	Ketoconazole	27.0			
CYP3A7	NA	NA		None[b]	None[c]
CYP4A11					
CYP4F2					
CYP4F3a					
CYP4F3b					
CYP4F12					
FMO1					
FMO3					
FMO5					

[a] Data from 10 uM desloratadine experiments
[b] Trace levels detected for CYP1A2, CYP1B1, CYP2B6, CYP2C8, CYP2C18, CYP2C19, CYP2J2, CYP3A5 and CYP3A7 with 1 µM desloratadine.
[c] Trace levels detected for CYP1A2, CYP1B1, CYP2B6, CYP2C8, CYP2C18, CYP2J2 and CYP3A7 with 1 µM desloratadine.

3-OH: 3-hydroxydesloratadine; 5-OH: 5-hydroxydesloratadine; 6-OH: 6-hydroxydesloratadine; NA: Not applicable

Figure 1

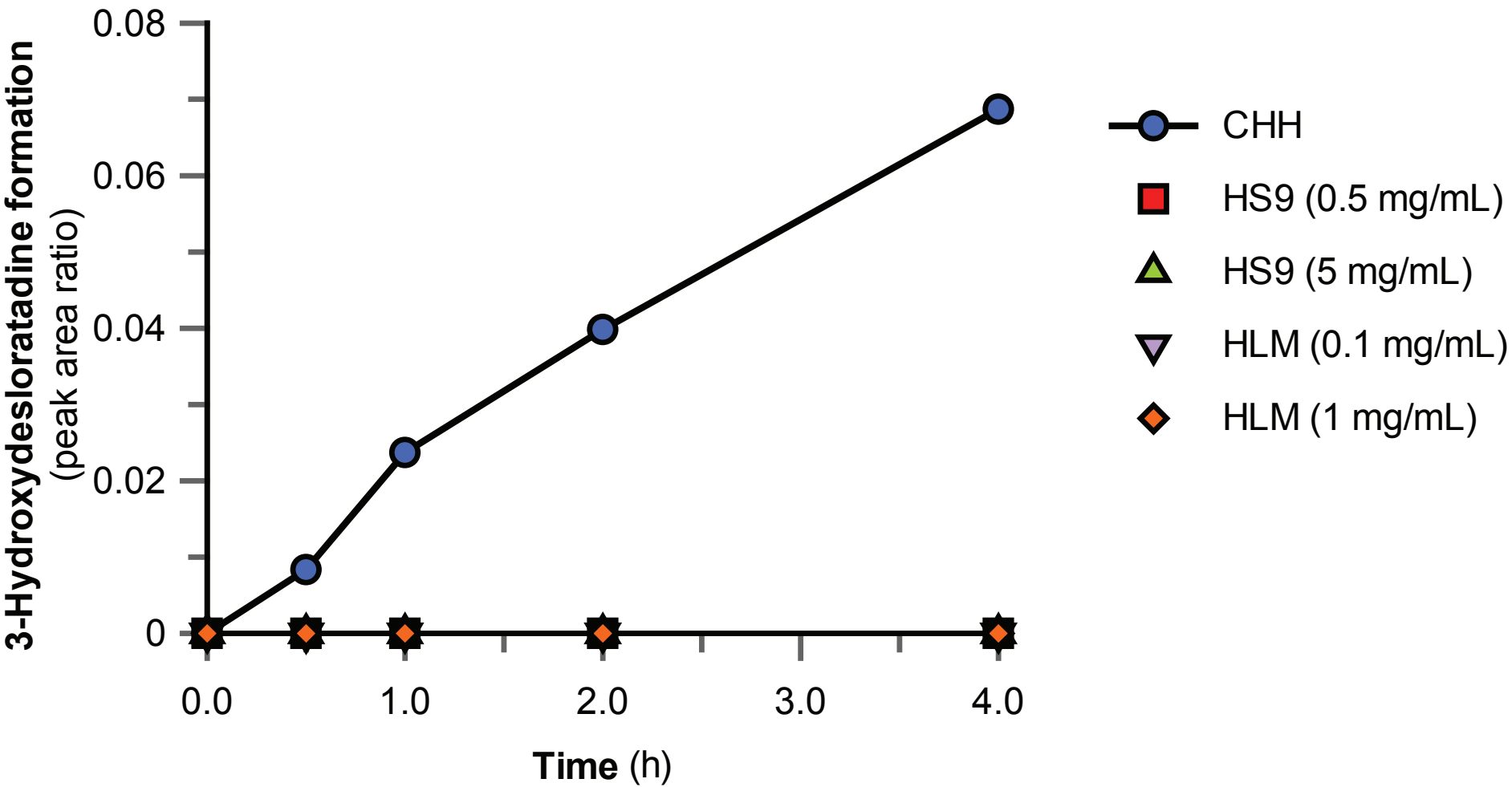
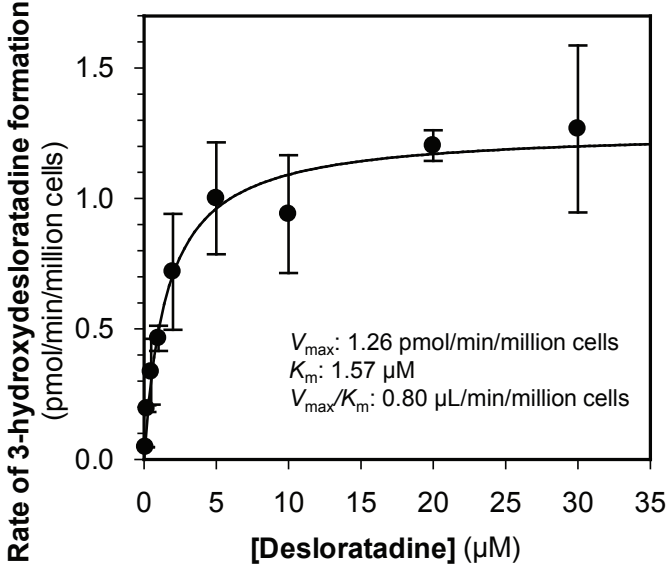

0.08
0.06
0.04
0.02
0
3-Hydroxydesloratadine formation
(peak area ratio)
0.0
1.0
2.0
3.0
4.0
Time (h)
CHH
HS9 (0.5 mg/mL)
HS9 (5 mg/mL)
HLM (0.1 mg/mL)
HLM (1 mg/mL)

Figure 2


Rate of 3-hydroxydesloratadine formation
(pmol/min/million cells)
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
35
[Desloratadine] (μM)
V_{max}: 1.26 pmol/min/million cells
K_m: 1.57 μM
V_{max}/K_m: 0.80 μL/min/million cells

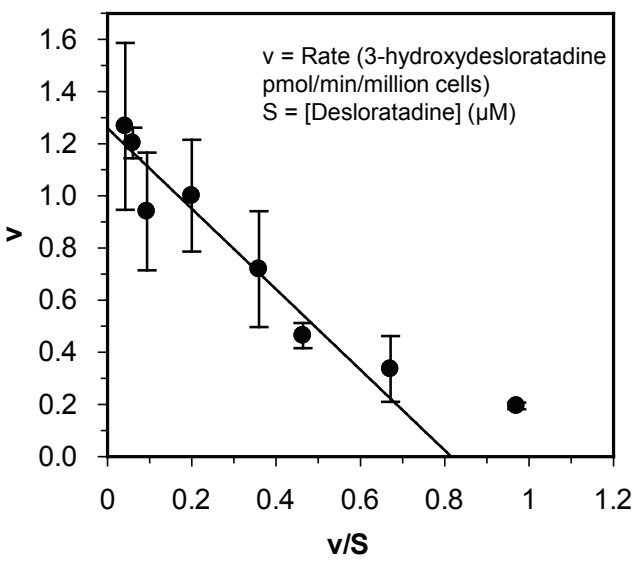

v = Rate (3-hydroxydesloratadine pmol/min/million cells)
S = [Desloratadine] (μM)
v
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
0.2
0.4
0.6
0.8
1
1.2
v/S

Figure 3

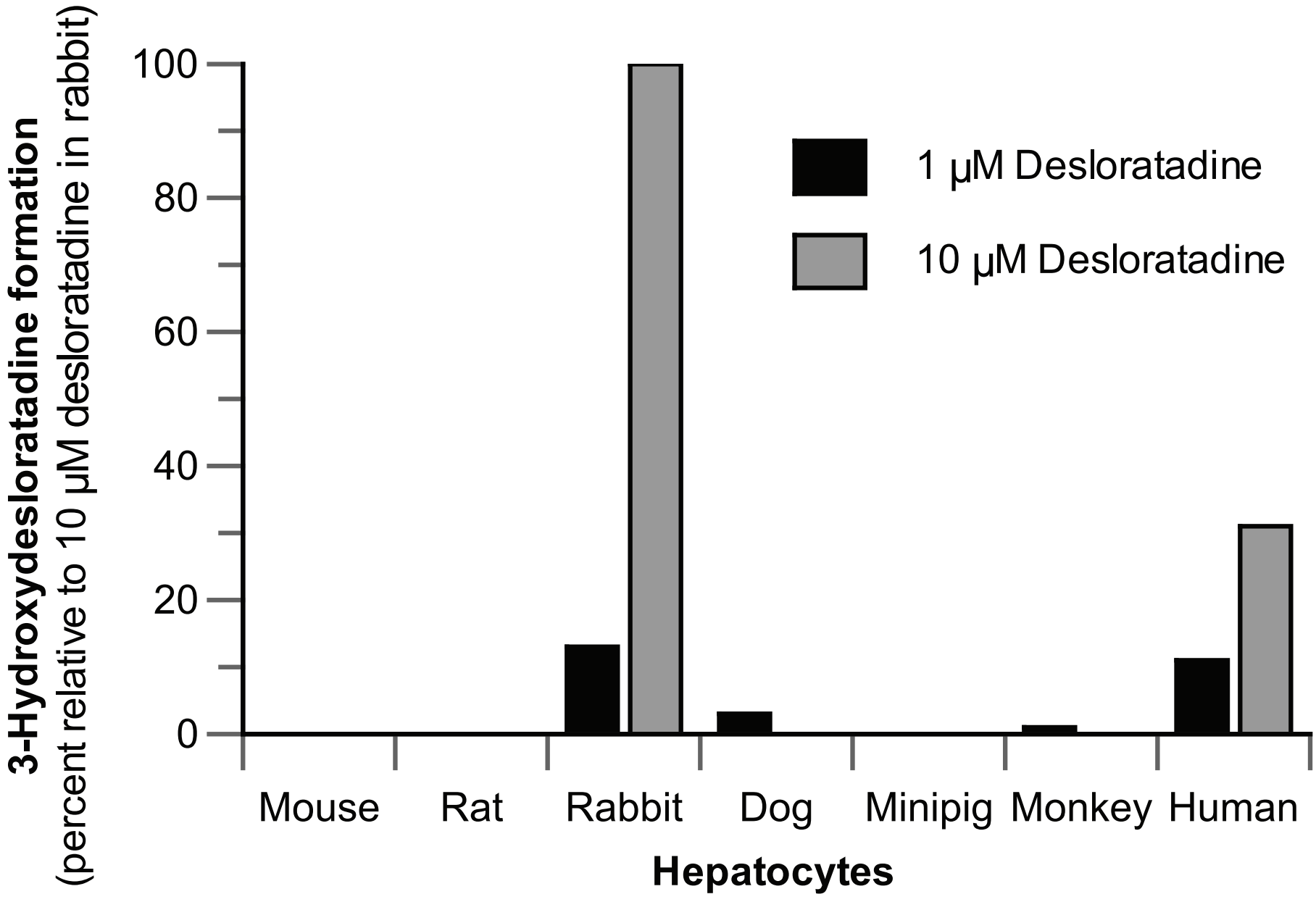

3-Hydroxydesloratadine formation
(percent relative to 10 µM desloratadine in rabbit)
100
80
60
40
20
0
1 µM Desloratadine
10 µM Desloratadine
Mouse
Rat
Rabbit
Dog
Minipig
Monkey
Human
Hepatocytes

Figure 4

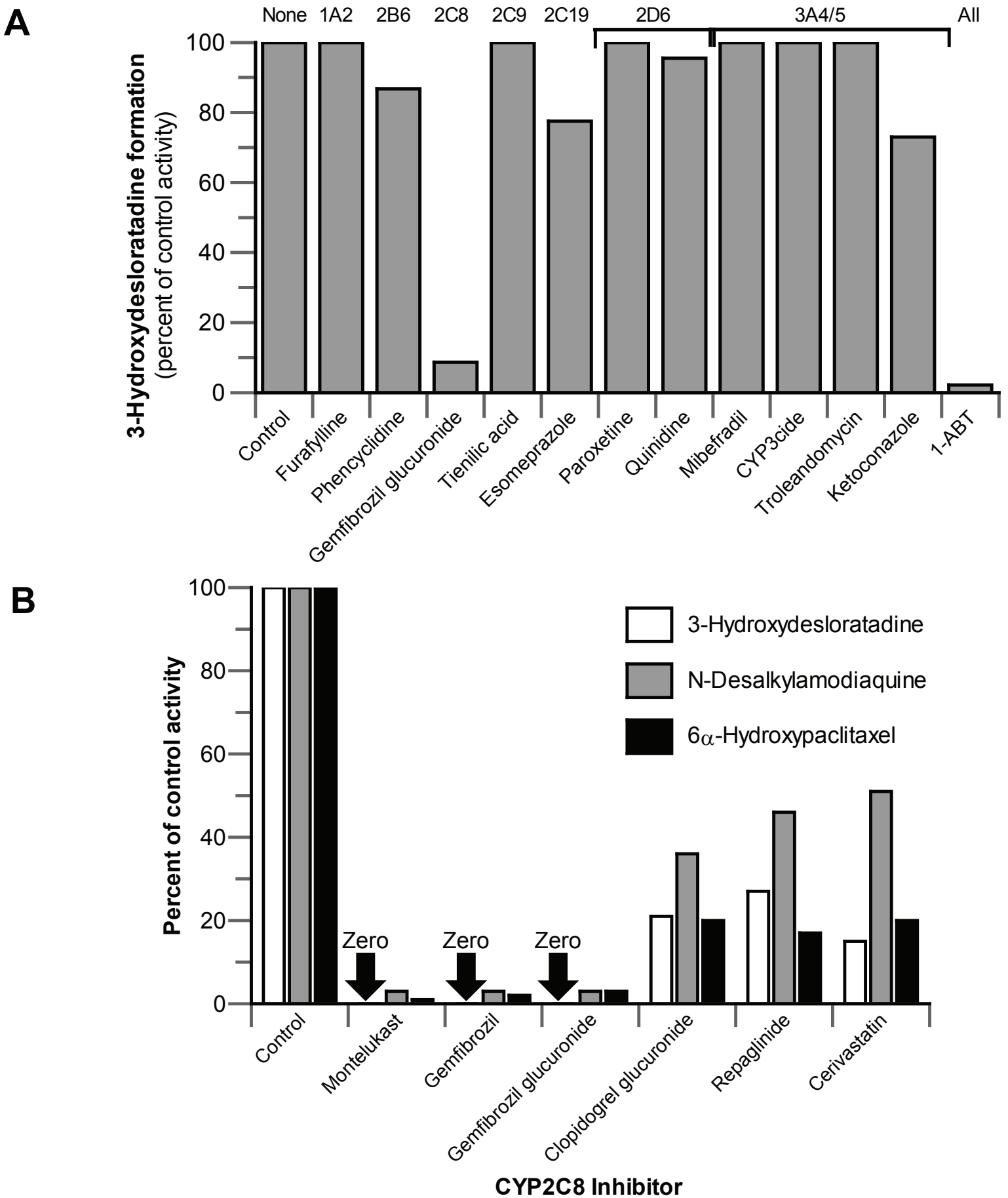
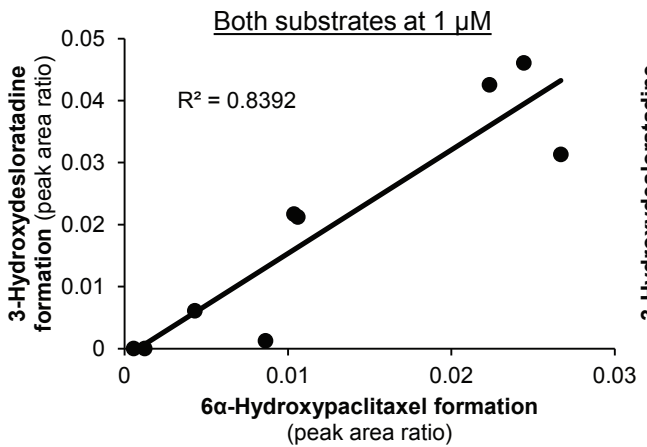
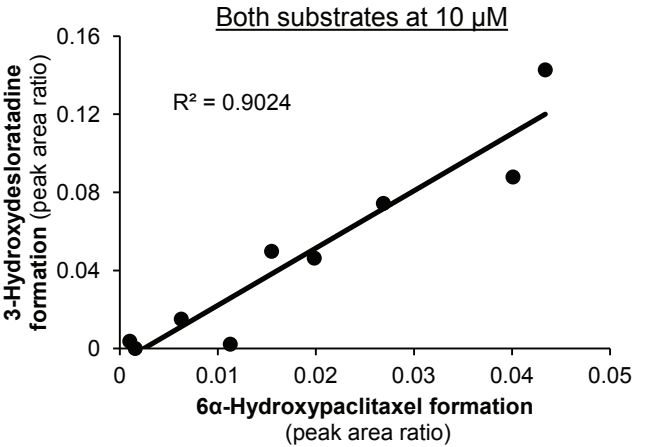
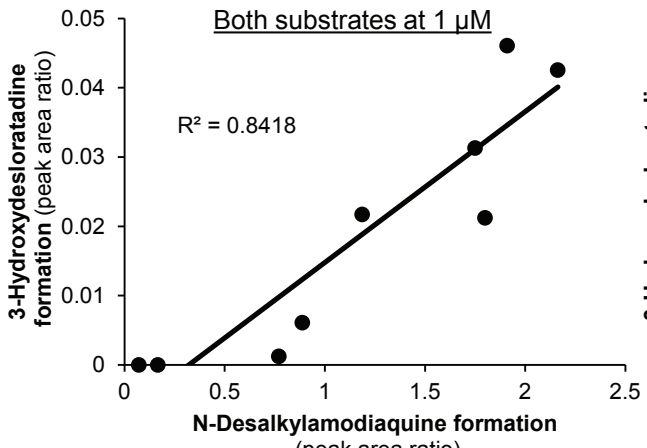

A
None
1A2
2B6
2C8
2C9
2C19
2D6
3A4/5
All
3-Hydroxydesloratadine formation
(percent of control activity)
100
80
60
40
20
0
Control
Furafylline
Phencyclidine
Gemfibrozil glucuronide
Tienilic acid
Esomeprazole
Paroxetine
Quinidine
Mibefradil
CYP3cide
Troleandomycin
Ketoconazole
1-ABT
B
Percent of control activity
3-Hydroxydesloratadine
N-Desalkylamodiaquine
6α-Hydroxypaclitaxel
Zero
Zero
Zero
Control
Montelukast
Gemfibrozil
Gemfibrozil glucuronide
Clopidogrel glucuronide
Repaglinide
Cerivastatin
CYP2C8 Inhibitor

Figure 5


Both substrates at 1 µM
R² = 0.8392
3-Hydroxydesloratadine formation (peak area ratio)
6α-Hydroxypaclitaxel formation (peak area ratio)
0.05
0.04
0.03
0.02
0.01
0
0.01
0.02
0.03


Both substrates at 10 µM
R² = 0.9024
3-Hydroxydesloratadine formation (peak area ratio)
6α-Hydroxypaclitaxel formation (peak area ratio)
0.16
0.12
0.08
0.04
0
0.01
0.02
0.03
0.04
0.05


Both substrates at 1 µM
R² = 0.8418
3-Hydroxydesloratadine formation (peak area ratio)
N-Desalkylamodiaquine formation (peak area ratio)
0.05
0.04
0.03
0.02
0.01
0
0.5
1
1.5
2
2.5

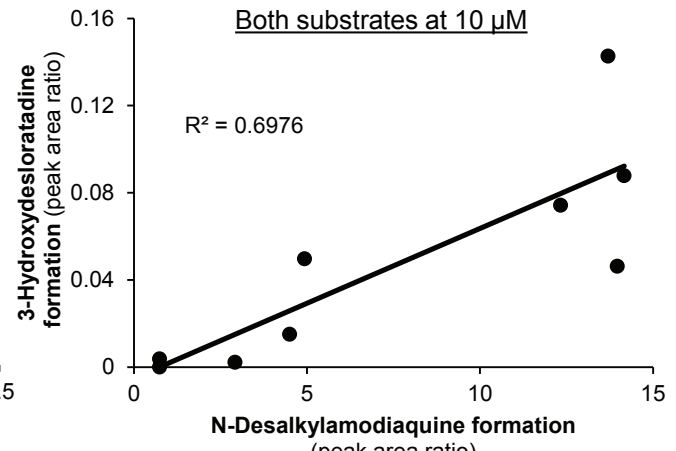

Both substrates at 10 µM
R² = 0.6976
3-Hydroxydesloratadine formation (peak area ratio)
N-Desalkylamodiaquine formation (peak area ratio)
0.16
0.12
0.08
0.04
0
5
10
15

Figure 6

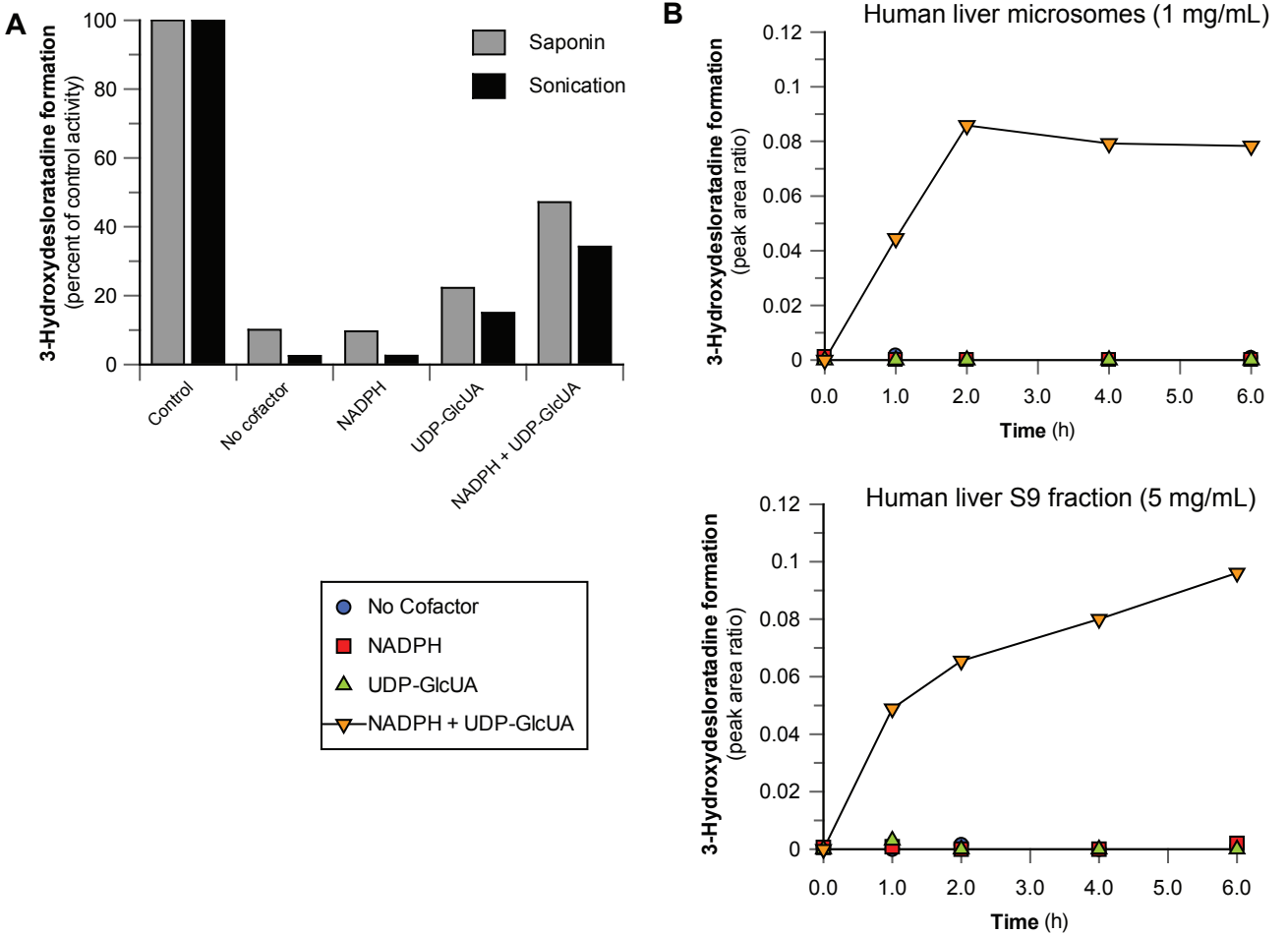

A
3-Hydroxydesloratadine formation
(percent of control activity)
100
80
60
40
20
0
Saponin
Sonication
Control
No cofactor
NADPH
UDP-GlcUA
NADPH + UDP-GlcUA
No Cofactor
NADPH
UDP-GlcUA
NADPH + UDP-GlcUA
B
Human liver microsomes (1 mg/mL)
3-Hydroxydesloratadine formation
(peak area ratio)
0.12
0.1
0.08
0.06
0.04
0.02
0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
Time (h)
Human liver S9 fraction (5 mg/mL)
3-Hydroxydesloratadine formation
(peak area ratio)
0.12
0.1
0.08
0.06
0.04
0.02
0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
Time (h)

Figure 7

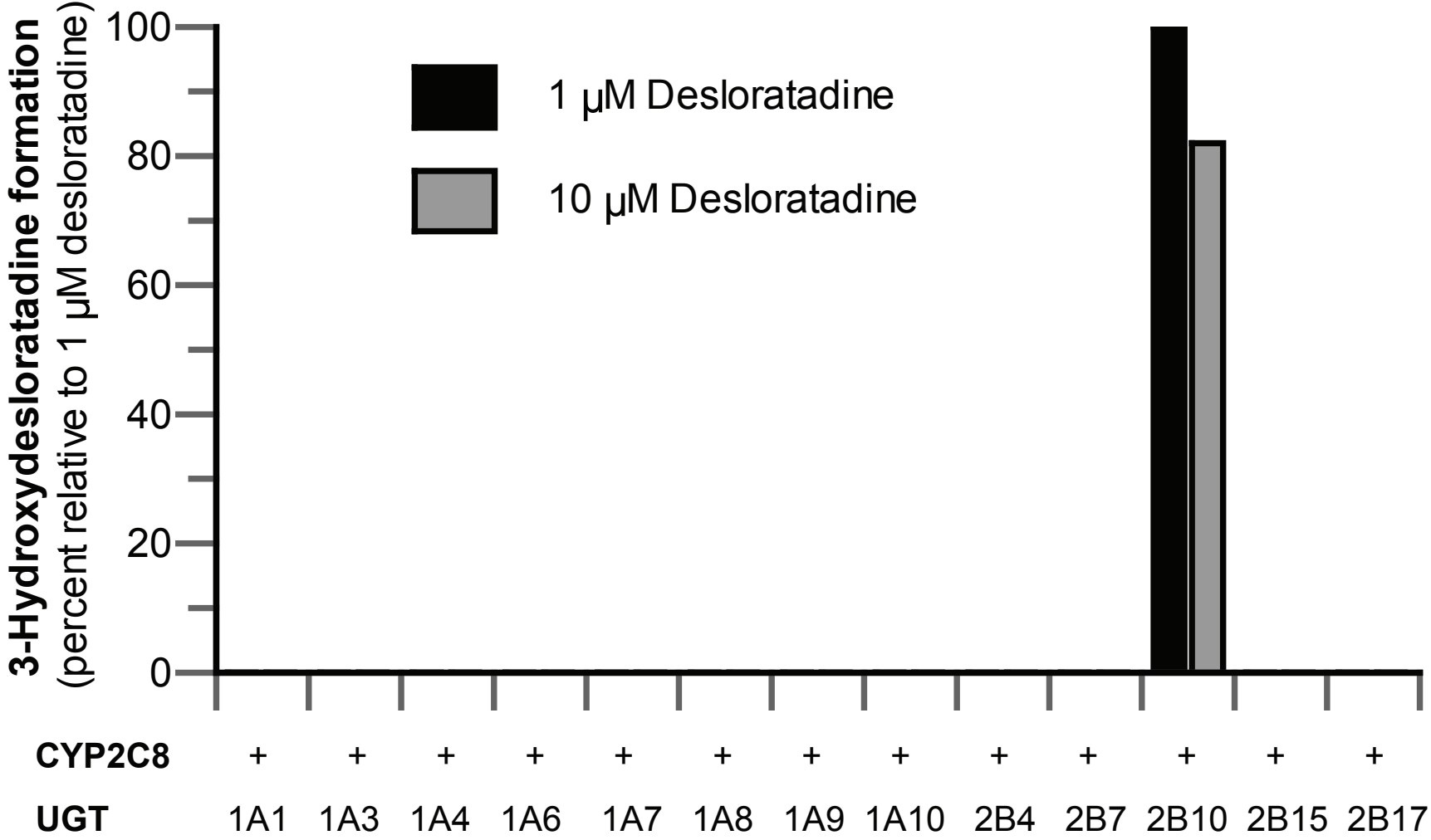
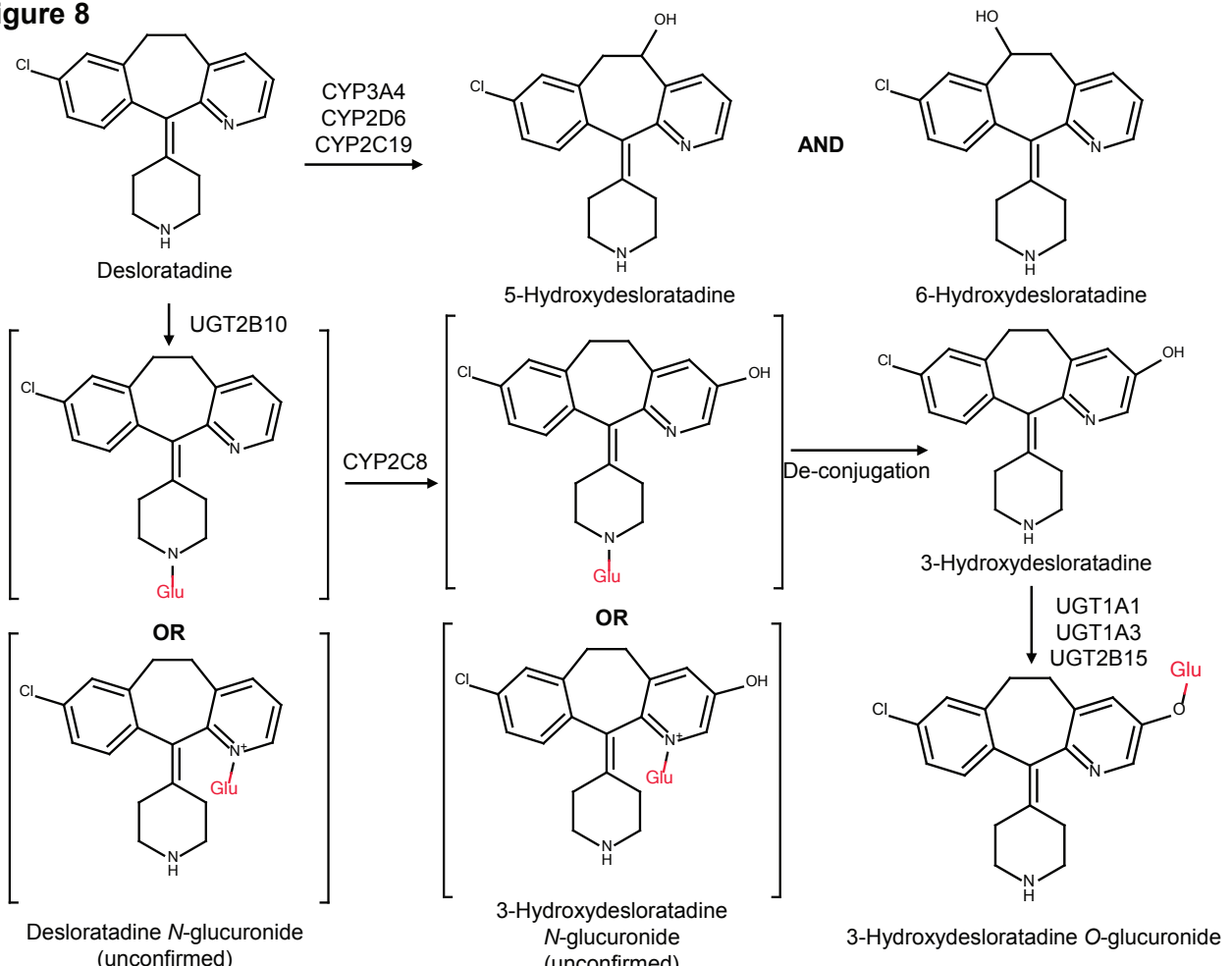

3-Hydroxydesloratadine formation
(percent relative to 1 µM desloratadine)
100
80
60
40
20
0
1 µM Desloratadine
10 µM Desloratadine
CYP2C8 + + + + + + + + + + + + +
UGT 1A1 1A3 1A4 1A6 1A7 1A8 1A9 1A10 2B4 2B7 2B10 2B15 2B17

Figure 8


Cl
N
N
H
Desloratadine
CYP3A4
CYP2D6
CYP2C19
OH
5-Hydroxydesloratadine
AND
HO
6-Hydroxydesloratadine
UGT2B10
Glu
OR
Desloratadine N-glucuronide
(unconfirmed)
CYP2C8
3-Hydroxydesloratadine
N-glucuronide
(unconfirmed)
De-conjugation
3-Hydroxydesloratadine
UGT1A1
UGT1A3
UGT2B15
O
3-Hydroxydesloratadine O-glucuronide