Identification and Mechanistic Investigation of Drug–Drug Interactions Associated With Myopathy: A Translational Approach

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Myopathy is a group of muscle diseases that can be induced or exacerbated by drug–drug interactions (DDIs). We sought to identify clinically important myopathic DDIs and elucidate their underlying mechanisms. Five DDIs were found to increase the risk of myopathy based on analysis of observational data from the Indiana Network of Patient Care. Loratadine interacted with simvastatin (relative risk 95% confidence interval [CI] = [1.39, 2.06]), alprazolam (1.50, 2.31), ropinirole (2.06, 5.00), and omeprazole (1.15, 1.38). Promethazine interacted with tegaserod (1.94, 4.64). In vitro investigation showed that these DDIs were unlikely to result from inhibition of drug metabolism by CYP450 enzymes or from inhibition of hepatic uptake via the membrane transporter OATP1B1/1B3. However, we did observe in vitro synergistic myotoxicity of simvastatin and desloratadine, suggesting a role in loratadine–simvastatin interaction. This interaction was epidemiologically confirmed (odds ratio 95% CI = [2.02, 3.65]) using the data from the US Food and Drug Administration Adverse Event Reporting System.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC? Drug-induced myopathy can be exacerbated by DDIs. No study to date has attempted to identify and investigate myopathic DDIs systematically. • WHAT QUESTION DID THIS STUDY ADDRESS? This study identified DDIs that increased risk of myopathy and investigated their underlying mechanisms using a high-throughput, translational approach. • WHAT THIS STUDY ADDS TO OUR KNOWLEDGE Five previously unknown DDIs were identified to increase the risk of myopathy, none of which appeared to result from inhibition of drug metabolism or hepatic uptake via OATP1B1/1B3. Synergistic myotoxicity may contribute to the interaction between loratadine and simvastatin. • HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS Pharmacoepidemiologic screening followed by mechanistic investigations proved to be an efficient approach to identify clinically important DDIs.

Drug-induced myopathy, among the most common causes of muscle disease, has clinical presentations ranging from asymptomatic muscle enzyme elevation to massive rhabdomyolysis with acute renal failure. Among 7 million case reports in the US Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS) from 2001–2010, about 100,000 cases involved myopathy as a suspected adverse drug reaction (ADR). Among various drug classes associated with myopathy, statins have received extensive public and scientific attention. Statin-induced myopathy occurs in 5–20% of patients and is a significant barrier to maximizing the benefits of statin therapy. Considering that more than 18% of Americans aged ≥45 (~127 million) took statins in 2012, 1.1 to 4.6 million patients might have experienced myopathy in 2012 alone.

Drug-induced myopathy can be exacerbated by pharmacokinetic and/or pharmacodynamic drug–drug interactions (DDIs). In a pharmacokinetic myopathic DDI, the object drug induces myopathy, and the precipitant drug modifies the object drug’s myopathic effects by changing its pharmacokinetics. One such example is the interaction between cerivastatin and gemfibrozil that contributed to the withdrawal of cerivastatin from the market. The risk of cerivastatin-induced rhabdomyolysis is 10-fold higher than that of other statins; with concurrent use of gemfibrozil, a drug that substantially inhibits the metabolism of cerivastatin, the risk is 50-fold higher. Although drug-induced myopathy and the role of DDIs as risk factors have been well documented, to our knowledge no study has attempted to identify and investigate unknown myopathic

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DDIs systematically. Research on DDIs has been mostly limited to pharmacokinetic DDIs with identifiable mechanisms, a small scope, a relatively low efficiency, and often a low clinical relevance. Recognizing the need for a translational approach for the study of DDIs, a promising new strategy involves pairing epidemiological studies with mechanistic investigations such as in vitro screening for metabolism-based DDIs. This approach was recently successfully applied to the study of interactions between sulfonylureas and statins/fibrates. Our previous study predicted recently successfully applied to the study of interactions between sulfonylureas and statins/fibrates.

### RESULTS

**DDIs associated with increased risk of myopathy**

We applied the myopathy concept definition (Supplementary Table S1) to a subset (n = 828,905) of the Indiana Network for Patient Care (INPC) database (2004–2009) formatted in the Observational Medical Outcomes Partnership (OMOP) Common Data Model. We identified 59,572 myopathy cases, of which 48,877 (82.8%) had myalgia and myositis, 12,720 (1.5%) had muscle weakness, and 53 (0.0064%) had rhabdomyolysis. For each of the 5.9% had myalgia and myositis, 12,720 (1.5%) had muscle weakness, 53 (0.0064%) had rhabdomyolysis. For each of the drug pairs, we estimated a risk ratio (RR) adjusted for age and sex, both known risk factors of myopathy. An RR greater than 1 indicated that the incidence of myopathy following the prescription for either drug alone. Drug pairs with RRs greater than 1 were therefore considered to be interacting and associated with an increased risk of myopathy. As a small sample size may yield an unreliable estimate of risk ratio, drug pairs with counts of myopathy cases less than 100 were excluded. We identified five DDIs associated with an increased risk of myopathy (Table 1), four of which involved the widely used antihistamine loratadine. The risk of myopathy increased with age at 1.0015 (95% confidence interval [CI] = 1.00148, 1.00152) per year, and was 1.64-fold (95% CI = 1.63, 1.65) higher in females (8.6%) than in males (5.4%) (Supplementary Table S3). Since sicker patients tend to take more medications, we used the number of prescribed medications, including the relevant drug pair, within drug exposure windows to adjust for confounding by morbidity. The average number of prescribed medications was 3.8 ± 2.5. The five DDIs remained significant after adjusting for the number of coprescribed medications (Supplementary Table S4).

### Inhibition of CYP-mediated drug metabolism

Cytochrome P450s (CYPs) are responsible for about 75% of drug metabolism, and their inhibition is a common mechanism of pharmacokinetic DDIs. Since each drug in the five DDIs relies on CYPs for elimination, we examined whether the DDIs were possibly caused by inhibition of CYP drug metabolism. Using fluorometric CYP inhibition screening assays, we assessed the potential of the drugs, and their pharmacologically active metabolites, to inhibit the enzymatic activities of the major human CYPs isoforms CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. The half maximal inhibitory concentration (IC50s) are presented in Supplementary Table S6. It is commonly accepted that a dissociation constant (Kd) is more relevant than an IC50 when predicting the clinical risk of metabolism-based DDIs. We therefore determined Kd for 11 drug-enzyme pairs (Table 2) that showed relatively strong CYP inhibitions (IC50 ≤20 μM).

Following FDA guidelines for drug interaction studies, we applied a stepwise approach to evaluate the risk of clinical DDIs resulting from inhibition of drug metabolism by CYPs. For each of the 11 drug-enzyme pairs for which a Kd was observed, we first used a conservative R-value approach to evaluate each drug's potential to act as a hypothetical precipitant. An R-value represents the predicted ratio of the area under concentration–time curve (AUC) of a hypothetical object drug that is exclusively metabolized by the inhibited CYP in the presence vs. absence of an inhibitor. Table 2 shows the predicted R-values. Consistent with FDA guidelines, an R value ≥1.1 (or ≥11 for CYP3A4 inhibitors administered orally) indicates that the drug could act as a precipitant. With R-values of 1.31 and 1.15, respectively,

### Table 1 Drug–drug interactions associated with increased risk of myopathy after adjusting for age and sex

<table>
<thead>
<tr>
<th>Drug 1</th>
<th>Drug 2</th>
<th>Risk1</th>
<th>Risk2</th>
<th>Risk12</th>
<th>Risk ratio (95% CI)</th>
<th>M1</th>
<th>N1</th>
<th>M2</th>
<th>N2</th>
<th>M12</th>
<th>N12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loratadine</td>
<td>Simvastatin</td>
<td>0.022</td>
<td>0.033</td>
<td>0.093</td>
<td>1.69 (1.39, 2.06)</td>
<td>1,264</td>
<td>44,245</td>
<td>4,197</td>
<td>102,345</td>
<td>137</td>
<td>1,223</td>
</tr>
<tr>
<td>Loratadine</td>
<td>Alprazolam</td>
<td>0.022</td>
<td>0.029</td>
<td>0.095</td>
<td>1.86 (1.50, 2.31)</td>
<td>1,257</td>
<td>43,341</td>
<td>2,251</td>
<td>52,341</td>
<td>176</td>
<td>1,448</td>
</tr>
<tr>
<td>Loratadine</td>
<td>Rapinrole</td>
<td>0.020</td>
<td>0.018</td>
<td>0.122</td>
<td>3.21 (2.06, 5.00)</td>
<td>1,218</td>
<td>43,491</td>
<td>164</td>
<td>6,531</td>
<td>17</td>
<td>123</td>
</tr>
<tr>
<td>Promethazine</td>
<td>Tegaserod</td>
<td>0.011</td>
<td>0.020</td>
<td>0.093</td>
<td>3.00 (1.94, 4.64)</td>
<td>1,332</td>
<td>78,334</td>
<td>109</td>
<td>3,745</td>
<td>23</td>
<td>224</td>
</tr>
<tr>
<td>Loratadine</td>
<td>Omeprazole</td>
<td>0.022</td>
<td>0.059</td>
<td>0.102</td>
<td>1.26 (1.15, 1.38)</td>
<td>1,260</td>
<td>44,207</td>
<td>4,339</td>
<td>70,345</td>
<td>304</td>
<td>2,734</td>
</tr>
</tbody>
</table>

Risk1 and risk2 are myopathy risks for drug 1 and drug 2, respectively. The risk ratios were calculated as risk12/(risk1 + risk2). 95% CIs were calculated using multivariate logistic regression adjusted for age and sex. N1, N2, and N12 is the number of patients who had prescription for drug 1 only, drug 2 only, and both drugs, respectively; and M1, M2, and M3 is the number of myopathy cases who had prescription for drug 1 only, drug 2 only, and both drugs, respectively.
promethazine and ropinirole could potentially interact with drugs exclusively metabolized by CYP2D6, the isof orm most strongly inhibited by both drugs. The predicted potential of the other inhibitor-enzyme pairs was negligible. These determinations suggest that the DDIs not involving promethazine and ropinirole were unlikely to result from inhibition of drug metabolism by CYPs.

A limitation of R values is that they only account for inhibition of a single metabolic pathway without regard to object drugs. In cases where multiple pathways are responsible for the metabolism of an object drug, an AUC ratio (AUCR) taking into account the fractional contribution of inhibited pathways to the overall metabolism is preferred. We thus predicted AUCRs for the interaction between ropinirole and loratadine, and that between promethazine and tegaserod. Accounting for 10% of the hepatic metabolism of loratadine by CYP2D6 that would be inhibited by ropinirole, the AUCR of loratadine in the presence vs. absence of ropinirole was predicted to be 1.01. Consistent with the FDA guidelines, it indicates that loratadine and ropinirole are unlikely to have CYP-based interactions. Because CYP2D6 is insignificant in tegaserod’s elimination, the inhibition of CYP2D6 by promethazine was considered to have no clinical effect on the pharmacokinetics of tegaserod. Overall, our data suggest that CYP inhibition is unlikely the major mechanism underlying the significant DDIs identified previously.

### Inhibition of OATP1B1/1B3-mediated hepatic uptake

It has been increasingly recognized that organic anion-transporting polypeptides (OATPs) represent an important site of DDIs. Particular attention has been paid to OATP1B1 and 1B3, the transporters of the OATP family demonstrated as most engaged in drug disposition. Among their substrates are many clinically important drugs including simvastatin acid, the active metabolite of simvastatin. The risk of simvastatin-induced myopathy was 4.5-fold higher in individuals with a genetic variant of SLCO1B1 (the OATP1B1 gene), compared to those with the wildtype allele.

We hypothesized that the DDIs identified previously may result from, at least in part, the inhibition of OATP1B1/1B3 that leads to impaired hepatic uptake and compromised hepatic clearance. We first evaluated the potential of the drugs, as well as their pharmacologically active metabolites, to inhibit the active uptake of β-estradiol 17-β-D-glucuronide (E217DG) in cryopreserved rat hepatocytes. E217BDG is a relatively specific substrate of OATP1B2, a functional homolog of human OATP1B1/1B3 with very similar substrate specificity. At 100 μM, simvastatin acid, omeprazole, alprazolam, desloratadine (the active metabolite of loratadine), simvastatin, tegaserod, ropinirole, loratadine, and promethazine inhibited E217BDG uptake by 103.3 ± 0.5%, 60.1 ± 4.8%, 54.5 ± 0.3%, 44.9 ± 14.2%, 36.3 ± 6.0%, 24.6 ± 15.3%, 23.7 ± 2.7%, 18.1 ± 10.9%, and 17.7 ± 7.7%, respectively. We then determined the inhibitory potencies of the drugs showing ≥45% inhibition. The IC50 (95% CI) of simvastatin acid, omeprazole, alprazolam, and desloratadine were 4.3 μM (3.5, 5.3), 84.3 μM (49.8, 142.9), 99.5 μM (79.5, 124.6), and 140.5 (111.4, 177.1) μM, respectively (inhibition curves are shown in Supplementary Figure 1).

Following a similar strategy for evaluating CYP-based DDIs, we estimated R-values (from IC50s) to evaluate the drugs’ potential to interact clinically with OATP1B1/1B3 substrates. The R-values of simvastatin acid, omeprazole, alprazolam, and desloratadine were 3.85, 1.23, 1.01, and 1.01, respectively.

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### Table 2 Predicting potential of CYP-based drug–drug interaction

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Pathway</th>
<th>Dissociation constant (K_d μM)</th>
<th>Fraction of unbound (f_u,inc)</th>
<th>Unbound dissociation constant (K_u, unbound μM)</th>
<th>Peak plasma concentration (C_max ng/ml)</th>
<th>Inhibitor concentration ([I], μM)</th>
<th>Predicted R-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simvastatin</td>
<td>CYP3A4</td>
<td>0.51</td>
<td>0.93</td>
<td>0.47</td>
<td>—</td>
<td>0.764</td>
<td>2.61</td>
</tr>
<tr>
<td>Promethazine</td>
<td>CYP2D6</td>
<td>0.25</td>
<td>0.88</td>
<td>0.22</td>
<td>19.3 (36)</td>
<td>0.068</td>
<td>1.31*</td>
</tr>
<tr>
<td>Tegaserod</td>
<td>CYP3A4</td>
<td>5</td>
<td>0.92</td>
<td>4.61</td>
<td>—</td>
<td>0.796</td>
<td>1.17</td>
</tr>
<tr>
<td>Ropinirole</td>
<td>CYP2D6</td>
<td>0.85</td>
<td>0.84</td>
<td>0.71</td>
<td>26.9 (37)</td>
<td>0.103</td>
<td>1.15*</td>
</tr>
<tr>
<td>Loratadine</td>
<td>CYP2D6</td>
<td>0.5</td>
<td>0.93</td>
<td>0.47</td>
<td>4.12 (38)</td>
<td>0.011</td>
<td>1.02</td>
</tr>
<tr>
<td>Tegaserod</td>
<td>CYP2D6</td>
<td>0.51</td>
<td>0.92</td>
<td>0.47</td>
<td>2.7 (39)</td>
<td>0.009</td>
<td>1.02</td>
</tr>
<tr>
<td>Loratadine</td>
<td>CYP2B6</td>
<td>2</td>
<td>0.93</td>
<td>1.86</td>
<td>4.12 (38)</td>
<td>0.011</td>
<td>1.01</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>CYP2C9</td>
<td>18.3</td>
<td>0.93</td>
<td>17.03</td>
<td>25.4 (40)</td>
<td>0.061</td>
<td>1.00</td>
</tr>
<tr>
<td>Loratadine</td>
<td>CYP2C9</td>
<td>7.6</td>
<td>0.93</td>
<td>7.07</td>
<td>4.12 (38)</td>
<td>0.011</td>
<td>1.00</td>
</tr>
<tr>
<td>Tegaserod</td>
<td>CYP2C19</td>
<td>9.2</td>
<td>0.92</td>
<td>8.48</td>
<td>2.7 (39)</td>
<td>0.009</td>
<td>1.00</td>
</tr>
<tr>
<td>Tegaserod</td>
<td>CYP2C9</td>
<td>11.4</td>
<td>0.92</td>
<td>10.51</td>
<td>2.7 (39)</td>
<td>0.009</td>
<td>1.00</td>
</tr>
</tbody>
</table>

K_d is the dissociation constant determined in vitro; f_u,inc is the fraction of unbound drug in the incubation mixture and was predicted using the Hallifax-Houston model; K_u is the unbound dissociation constant estimated as K_d * f_u,inc; C_max is the peak total plasma concentration at the highest clinical dose; [I] is the inhibitor concentration used to predict R values and is equal to C_max, except for CYP3A4 inhibitors administered orally. For simvastatin and tegaserod with CYP3A4, [I] is the estimated gut concentration at the highest proposed clinical dose, 80 mg (191 μM) and 6 mg (19.9 μM), respectively, divided by 250 mL (approximate gut volume); R values were estimated as 1 + [I]/K_u,inc. *R values ≥1.1 (or ≥11 for simvastatin and tegaserod with CYP3A4), indicating a probable clinical CYP450-based DDI.
Table 3. Consistent with the FDA guidelines, simvastatin acid and omeprazole (R value ≥ 1.1) might interact with drugs relying on OATP1B1/1B3 for hepatic uptake. The potential of alprazolam and desloratadine as precipitants was negligible.

**Direct myotoxicity**

Although all the drugs involved in the DDIs have known muscle-related side effects, their direct myotoxicity, except that of simvastatin, has not been examined. We tested whether the DDIs resulted from the direct toxicity of the individual drugs, or their combinations, to muscle cells. We first evaluated the myotoxicity of each individual drug to rat L6 myotubes, a commonly used in vitro skeletal muscle model previously used to study mechanisms of statin-induced myopathy. After treatment of healthy, fully differentiated rat L6 myotubes with each drug individually at 10 μM for 5 days, tegaserod, simvastatin, desloratadine, and simvastatin acid induced 97.9 ± 0.4%, 73.7 ± 2.6%, 73.3 ± 1.1%, and 33.0 ± 2.1% myotube death, respectively, compared to dimethyl...
increased risk of myopathy, with ORs of 2.20 (95% CI 1.28, 1.82) and 1.53 (95% CI 1.28, 1.82) in the FAERS and INPC datasets, respectively. In additional subgroup analyses stratified by sex, age, or myopathy type (muscle weakness or myalgia), the interaction between loratadine and simvastatin remained significant in all subgroups. The IC_{50s} and K_{S} that we reported provide a comprehensive view of the potential of these drugs to cause CYP-based DDIs. These data are consistent with those published previously. To our knowledge, we are the first to describe the potential of these drugs (except simvastatin) to inhibit OATP1B2 in rat hepatocytes and assess their potential OATP-mediated DDIs in humans. We are also likely the first to report myotoxicity of desloratadine and tegaserod, which may underlie their muscle-
related side effects. Of note, simvastatin was much more toxic than simvastatin acid to myotubes in vitro, an observation previously reported, suggesting that simvastatin-induced myopathy is due primarily to simvastatin rather than simvastatin acid. Similarly, the in vitro myotoxicity of desloratadine suggests that myalgia associated with loratadine may be primarily due to its metabolite, desloratadine.

Although inhibition of drug metabolism by CYPs and inhibition of OATP1B1/3 are the most common mechanisms underlying pharmacokinetic DDIs, they are unlikely the major mechanisms for the DDIs that we observed. The results from the R-value approach suggest that simvastatin acid and omeprazole may interact with drugs that rely on OATP1B1/1B3 for hepatic uptake. We suggest that such data be interpreted with caution, as the R-value approach, for both CYPs and transporters, is known to overpredict the risk of clinical DDIs and lead to spurious conclusions that a drug is a precipitant when it is not. It implies, however, that the drug pairs predicted not to interact using this approach in our study are very unlikely to have real interactions.

There are a few limitations to our study. We used a simple cohort design that may be subject to residual confounding and misclassification. The use of the FAERS may not provide a definitive validation for a simvastatin–loratadine interaction. The CYP450 inhibition assays involve fluorogenic substrates and recombinant CYP enzymes that occasionally generate inhibitory potencies very different from those using conventional approaches. Both the R value and AUCR approaches use a single static in vitro concentration of an inhibitor drug, which may overestimate the risk of DDI for drugs, such as simvastatin, with relatively short half-lives and whose circulating concentrations drop rapidly following a dose. We did not evaluate the drugs as direct substrates of OATP1B1/1B3 or other transporters, limiting our understanding of the role of drug transporters in the DDIs. We also used cryopreserved rat hepatocytes and rat L6 myotubes, which are less clinically relevant than human-derived cell models. Future studies are warranted to further evaluate the underlying mechanisms of these DDIs.

**METHODS**

**Evaluation of CYP450 inhibition**

Fluorometric cytochrome P450 inhibition kits (BD Biosciences/Gentest, San Jose, CA) were used to determine the IC50 of the drugs for the major CYPs. The assays were performed following the manufacturer’s instructions under the conditions in Supplementary Table S7. Data were analyzed using GraphPad Prism 5 software (La Jolla, CA).

R values were estimated as 1 + ![expression](https://www.wileyonlinelibrary.com/doi/pdf/10.1002/cpt.326?casa_token=67bb2W0mDKMAAAAAR:6bH6CWht9-r0xu8o1JYc0C0uaD2IYK3dFLrRzn8T9vB2DQa0j5rjOEfZz6xqvUHf9qzC9IaZwMCQ6). Data were analyzed using GraphPad Prism 5 software (La Jolla, CA).

R values were estimated as 1 + ![expression](https://www.wileyonlinelibrary.com/doi/pdf/10.1002/cpt.326?casa_token=67bb2W0mDKMAAAAAR:6bH6CWht9-r0xu8o1JYc0C0uaD2IYK3dFLrRzn8T9vB2DQa0j5rjOEfZz6xqvUHf9qzC9IaZwMCQ6). Data were analyzed using GraphPad Prism 5 software (La Jolla, CA).

R values were estimated as 1 + ![expression](https://www.wileyonlinelibrary.com/doi/pdf/10.1002/cpt.326?casa_token=67bb2W0mDKMAAAAAR:6bH6CWht9-r0xu8o1JYc0C0uaD2IYK3dFLrRzn8T9vB2DQa0j5rjOEfZz6xqvUHf9qzC9IaZwMCQ6). Data were analyzed using GraphPad Prism 5 software (La Jolla, CA).

**Evaluation of inhibition of OATP1B1/1B3**

The drugs (100 μM) were incubated with cryopreserved rat hepatocytes (1 × 10⁶ cells/mL) and [³H]E₂17BDG (1 μM, 0.1 μCi) for 3 minutes at 37°C and 0°C in triplicate. Uptake was stopped with addition of 1 mL ice-cold PBS and immediate centrifugation at 4500 rpm for 1 minute at 4°C. Cells were resuspended in 1 mL ice-cold PBS and centrifuged again. After removing supernatant, cell pellets were lysed with 200 μL of 50% acetonitrile in H₂O₂, followed by vigorous vortexing. The fraction of uptake was the ratio of the radioactivity in hepatocyte lysate to the total radioactivity in both lysate and supernatants. The fraction of active uptake was the difference between the total uptake at 37°C and that at 0°C.

**Evaluation of myotoxicity**

Rat L6 muscle cells were cultured as previously detailed by Klip and colleagues with slight modifications. Cells were maintained in monolayer culture in α-MEM containing 10% fetal bovine serum (FBS) and 1% antibiotic–antimycotic solution (10,000 U/ml penicillin G, 10 mg/ml streptomycin, and 25 mg/ml amphotericin B) in an atmosphere of 5% CO₂ at 37°C. Five days after seeding, myoblasts were differentiated into multinucleated myotubes with 2% FBS. All drug treatments were initiated 5 days after the initiation of differentiation and continued for 5 days. The CellTiter 96 aqueous nonradioactive cell proliferation (MTS/PMS) assay (Promega, Madison, WI) was used to measure cell viability after drug treatment.

Combination index (CI) values were calculated as described by Chou. The fraction of unaffected (f₀), in this case equivalent to cell viability, was calculated as described above. Fractional inhibition (fᵢ) was calculated as 1 – f₀. The slope factor m and IC₅₀ of simvastatin and desloratadine were estimated by fitting the data of each drug when applied alone to Eq. 2:

\[
\log f_{fa} = m \times \log D - m \times \log (D_m)
\]

CI values were then calculated using Eq. 3:

\[
CI = \frac{D_1}{(D_1 + D_2)D_m(1-f_{fa})}1/m1 + D_2/(D_1 + D_2)D_m(2-f_{fa})/m2
\]

A CI - fᵢ plot was constructed by plotting CI values and fᵢ on y and x axes, respectively.

Additional Supporting Information may be found in the online version of this article.

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**AUTHOR CONTRIBUTIONS**

L.L. and X.H. wrote the article; L.L., S.K.Q., J.S.E., and D.F. designed the study.

**CONFLICT OF INTEREST**

The authors declare no competing financial interests.