Casopitant: In Vitro Data and SimCyp Simulation to Predict In Vivo Metabolic Interactions Involving Cytochrome P450 3A4

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ABSTRACT:

Casopitant [1-piperidinecarboxamide,4-(4-acetyl-1-piperazinyl)-N-((1R)-1-(3,5-bis(trifluoromethyl)phenyl)-ethyl)-2-(4-fluoro-2-methyl-phenyl)-N-methyl-(2R,4S), GW679769] has previously been shown to be a potent and selective antagonist of the human neurokinin-1 receptor, the primary receptor of substance P, both in vitro and in vivo, with good brain penetration properties. On the basis of this mode of action it was evaluated for the prevention of chemotherapy-induced and postoperative nausea and vomiting, and for the chronic treatment of anxiety, depression, insomnia, and overactive bladder. Casopitant is shown to be a substrate, an inhibitor, and an inducer of CYP3A4, and, because of this complex behavior, it was difficult to identify the primary mechanism by which it may give rise to drug-drug interactions (DDIs) of clinical relevance. Moreover, the major circulating metabolite is itself an inhibitor of CYP3A4 in vitro. On the basis of the different clinical indications and the various potential comedinations of casopitant, a relevant part of the clinical development plan was focused on the assessment of the importance of clinical DDIs. The present study provides an overview of the DDI potential profile of casopitant, based on in vitro data and clinical evidence of its interaction with CYP3A4 probe substrates midazolam and nifedipine, the strong inhibitor ketoconazole, and the inducer rifampin. Overall, the clinical data confirm the ability of casopitant to interact with CYP3A4 substrates, inhibitors, or inducers. The in vitro data are accurate and robust enough to build a reliable SimCyp population-based model to estimate the potential DDIs of casopitant and to minimize the clinical studies recommended.

Introduction

DDIs have become an important issue in health care. It is now acknowledged that many of the major pharmacokinetic DDIs can be attributed to modulations of the drug-metabolizing enzymes, particularly P450 enzymes, which are present in the liver and extrahepatic tissues (Bibi, 2008). CYP3A4 is the most abundantly expressed P450 enzyme in the liver and gut, and it is involved in the clearance of more than half of the drugs used clinically (Wienkers and Heath, 2005). A number of important drugs have been identified as substrates, inducers, and/or inhibitors of CYP3A4 and the assessment of the potential for CYP3A4-mediated DDIs is an important part of the clinical development program for any new chemical entity (Hewitt et al., 2007).

Casopitant [1-piperidinecarboxamide,4-(4-acetyl-1-piperazinyl)-N-((1R)-1-(3,5-bis(trifluoromethyl)phenyl)-ethyl)-2-(4-fluoro-2-methylphenyl)-N-methyl-(2R,4S), GW679769] (Fig. 1), is a piperidine derivative with potent and selective antagonistic properties toward the human neurokinin-1 receptor and good brain penetration properties (Minthorn et al., 2008). It has been evaluated in humans for chemotherapy-induced nausea and vomiting (Herrstedt et al., 2009), postoperative nausea and vomiting, anxiety, depression, insomnia, and overactive bladder. Casopitant is extensively metabolized, and its metabolism in humans seems to be mediated mainly by CYP3A4 (Pellegatti et al., 2009; Johnson et al., 2010). GSK525060 (Fig. 1), the major circulating metabolite in man, is present at concentrations comparable to those of the parent drug, both after single (Pellegatti et al., 2009) and repeated (Zamuner et al., 2010) oral administration of casopitant.

Given the different therapeutic indications of this drug, many of which may require various comedinations, an assessment of the potential DDI profile for casopitant was important to determine whether it could be safely added to an ongoing therapy and at what dose. To assess the potential for DDIs, knowledge of clearance mechanisms, the enzyme responsible for major metabolic pathways, and modulating capabilities of enzyme activities was essential. As a consequence, several in vitro studies were performed to evaluate the potential of casopitant or GSK525060 as perpetrator and as victim of metabolic interactions involving CYP3A4 and to guide the clinical DDI strategy. This study describes the in vitro data obtained and used to build a reliable SimCyp model to estimate the potential DDIs of casopitant. These simulations were then compared with clinical DDI results already available for casopitant when coadministered with the CYP3A4 probes MID or NIF and with KET or RIF. This retrospective

ABBREVIATIONS: DDI, drug-drug interaction; P450, cytochrome P450; GW679769, 1-piperidinecarboxamide,4-(4-acetyl-1-piperazinyl)-N-((1R)-1-(3,5-bis(trifluoromethyl)phenyl)-ethyl)-2-(4-fluoro-2-methylphenyl)-N-methyl-(2R,4S); MID, midazolam; NIF, nifedipine; KET, ketoconazole; RIF, rifampin; FDA, U.S. Food and Drug Administration; GSK, GlaxoSmithKline; TAO, troleandomycin; HLM, human liver microsomes; 6βT, 6β-hydroxy testosterone; HPLC, high-performance liquid chromatography; MS/MS, tandem mass spectroscopy; AUC, area under the plasma concentration-time curve.
adaptation period, cultures of hepatocytes with a Matrigel overlay were treated as described by Maurel (1996) and LeCluyse (2001). After a 24- to 48-h hepatocytes from three separate donors were isolated and cultured essentially to predict clinical DDIs as reported by Fahmi et al. (2008). Fresh human from baculovirus-infected cells) were obtained from Cypex Ltd. (Dundee, Biosciences (San Jose, CA). Human liver microsomes (HLM) pooled from 15 (Milan, Italy); and 1

6-phosphate, and glucose-6-phosphate dehydrogenase from Sigma-Aldrich Italy); MID, NIF, KET, troleandomycin (TAO), hydroxytriazolam, glucose and all the bioanalytical internal standards from GlaxoSmithKline (Verona, were obtained from the following sources: casopitant, GSK525060 (Fig. 1), available and were obtained from commercial sources. (Durham, NC). All other reagents and solvents were of the highest purity available and were obtained from commercial sources. Materials and Methods Chemicals and Reference Compounds. The test (articles) compounds were obtained from the following sources: casopitant, GSK525060 (Fig. 1), and all the bioanalytical internal standards from GlaxoSmithKline (Verona, Italy); MID, NIF, KET, trolenandomycin (TAO), hydroxytriazolam, glucose 6-phosphate, and glucose-6-phosphate dehydrogenase from Sigma-Aldrich (Milan, Italy); and 1’-hydroxymidazolam and oxidized nifedipine from BD Biosciences (San Jose, CA). Human liver microsomes (HLM) pooled from 15 donors were prepared and characterized at XenoTech, LLC (Lexena, KS). Bactosomes containing individual overexpressed human CYP3A4 (derived from baculovirus-infected cells) were obtained from Cypepx Ltd. (Dundee, UK). RIF, phenobarbital, omeprazole, testosterone, 6β-hydroxytestosterone (6βT), and freshly isolated human hepatocytes were provided by CellDirect (Durham, NC). All other reagents and solvents were of the highest purity available and were obtained from commercial sources. In Vitro Cytochrome P450 Induction. The human hepatocyte model was used as an experimental model for the evaluation of P450 induction potential to predict clinical DDIs as reported by Fahmi et al. (2008). Fresh human hepatocytes from three separate donors were isolated and cultured essentially as described by Maurel (1996) and LeCluyse (2001). After a 24- to 48-h adaptation period, cultures of hepatocytes with a Matrigel overlay were treated for 3 consecutive days with casopitant (1, 5, or 20 μM) once daily, or, alternatively, with one of three prototypical P450 inducers, omeprazole (25 μM), phenobarbital (500 μM), or RIF (10 μM), again given once a day for 3 consecutive days. Casopitant and inducers (positive controls) were dissolved in dimethyl sulfoxide, and hepatocytes treated with dimethyl sulfoxide (final concentration 0.1%, v/v) served as negative controls. Human hepatocytes were harvested after the final treatment to prepare microsomes, which were tested in duplicate for CYP3A4 activity (testosterone 6β-hydroxylation) by high-performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS), according to the procedure reported by Madan et al. (2003). 

Data analysis. Rates of production of 6βT were calculated at each concentration of casopitant or prototypical inducer and are expressed as nanomoles per minute per milligram. Each hepatocyte donor preparation was deemed acceptable when the RIF-positive control elicited a greater than 2-fold increase in the P450 enzyme activity (Sinz et al., 2008). The inductive response of casopitant on CYP3A4 activity was expressed as the mean ratio of treated over vehicle control (fold change) or compared with the appropriate prototypical inducer as a percentage of the positive control (RIF), according to the following equations: In Vitro Cytochrome P450 Inhibition. IC50 determination. Unless indicated otherwise, CYP3A4 activity in HLM was determined according to previously published procedures (Madan et al., 2002), and the concentration that would cause a 50% decrease in P450 activity (IC50) was determined. Incubations were conducted in 250 μl of incubation mixture (pH 7.4) containing 50 mM potassium phosphate buffer, pooled HLM (0.1 mg/ml), probe substrates (2.5 μM MID or 10 μM NIF), and casopitant or GSK525060 (concentration range 0.1–100 μM). All incubations were prewarmed at 37°C for 5 min before the addition of a warmed NADPH-generating system to obtain the following final concentrations: 5.5 mM NADP; 0.4 mM glucose 6-phosphate, and 1.2 units of glucose-6-phosphate dehydrogenase/ml. Reactions were terminated after 5 min for MID or 10 min for NIF by the addition of acetonitrile, and the production of metabolite in each incubation was quantified by HPLC-MS/MS.

To evaluate the potential for metabolism-dependent inhibition, casopitant or GSK525060 (at the same concentrations used to evaluate direct inhibition) were preincubated at 37°C with HLM and a NADPH-generating system (see above) or probe substrates (2.5 μM MID or 10 μM NIF) for 20 min. Reactions were then initiated by the addition of marker substrates or an NADPH-generating system, respectively, and the incubations were continued for 5 or 10 min to measure the residual CYP3A4 activity. Reactions were terminated by the addition of acetonitrile, and the production of metabolite in each incubation was quantified by HPLC-MS/MS. Positive control incubations for both direct (KET) and metabolism-dependent inhibition (TAO) and control incubations without inhibitor (containing methanol only) were also performed. All incubations were performed in duplicate and prepared such that the final concentration of methanol was constant at 2% (v/v). 

Kf and kmax determination. The recommended two-step incubation method was used to determine the kinetics of CYP3A4 inactivation by casopitant (Yang et al., 2005; Polasek and Miners, 2007), the concentration required for half-maximal inactivation (Kf), and the maximum rate of inactivation at saturation (kmax). Various concentrations of casopitant (0.9–90 μM) were prewarmed at 37°C in 0.1 M Tris buffer, pH 7.4, with 0.5 mM EDTA and pooled HLM (0.5 mg/ml). After 5 min of prewarming, the NADPH-generating system was added, and the mixture was incubated for 0 to 20 min. After preincubation, aliquots (25 μl) were transferred to dilution mix wells (225 μl) containing 0.1 M Tris buffer, pH 7.4, with 0.5 mM EDTA, 28 μM MID, and an NADPH-generating system. Reactions were terminated after a 4-min incubation with 250 μl of acetonitrile, and the production of metabolite in each incubation was quantified by HPLC-MS/MS. With the transfer into the dilution mix step, microsomes were diluted 1:10 to a 0.05 mg/ml final concentration of

Fig. 1. Chemical structure of casopitant and its metabolite GSK525060.
protein, and casopitant underwent a similar dilution. Positive control incubations (TAO) and control incubations without inhibitor (containing methanol only) were also performed. All incubations were performed in duplicate and were prepared such that the final concentration of methanol was constant at 2% (v/v).

**Data analysis.** Rates of metabolite production at each concentration of casopitant or GSK525060 or the positive control were expressed as a percentage of the mean uninhibited control rate for each incubation. Data were processed to determine IC_{50} values by nonlinear regression with GraFit (version 5.0; Erithacus Software, Horley, Surrey, UK) according to the following equation:

\[
v = \frac{V_0 I}{1 + \left(\frac{I}{IC_{50}}\right)^s}
\]

where \(V_0\) is the uninhibited control rate of metabolite production, \(v\) is the observed rate of metabolite production, \(I\) is the inhibitor concentration, and \(s\) is the slope factor. Metabolism-dependent inhibition of enzyme activity was inferred from a decrease (>2-fold) in IC_{50} value obtained after the 20-min preincubation period. The observed rate of metabolite production, \(v\), is the inhibitor concentration, and \(s\) is the slope factor. Metabolism-dependent inhibition of enzyme activity was inferred from a decrease (>2-fold) in IC_{50} value obtained after the 20-min NADPH preincubation relative to that obtained with the probe substrate preincubation.

Data for the \(K_i\) and \(k_{max}\) investigation were analyzed to determine the rate of enzyme inactivation at each casopitant concentration tested (Polasek and Miners, 2007), assuming that the loss of enzyme activity was due to inactivation by a first-order process. For each NADPH preincubation period, rates of \(1'\)-hydroxymidazolam production at each concentration of casopitant or of the positive control TAO were expressed as a percentage of the mean uninhibited control rate. Rate constants for loss of CYP3A4 activity, at each inhibitor concentration, were calculated according to a single exponential decay equation using GraFit:

\[
v = V_0 e^{-kt}
\]

where \(v\) is the observed rate of metabolite production, \(V_0\) is the initial rate of metabolite production, \(k_{obs}\) is the observed rate constant, and \(t\) is the NADPH preincubation period. Data were then processed to determine the kinetic constants, \(k_{max}\) and \(K_i\), by nonlinear regression with GraFit according to the following equation:

\[
k_{obs} = \frac{k_{max}I}{K_i + I}
\]

**Metabolism of Casopitant by Human Liver Microsomes and Recombinant Human CYP3A4.** Micromolar incubations (HLM or recombinant CYP3A4) were performed under the conditions described in Madan et al. (2002) (in an incubation mixture containing 50 mM phosphate buffer, pH 7.4, and an NADPH-generating system), and the rate of formation of GSK525060 was determined under conditions in which the reaction was linear with time and protein concentration. Initial studies were conducted in HLM with two concentrations of casopitant (5 and 20 \(\mu M\)) to optimize the incubation time (over the range 0–90 min at 1 mg/ml protein concentration) and protein concentration (over the concentration range 0.05–5 mg/ml at 30 min), with recombinant CYP3A4 and 10 \(\mu M\) casopitant to determine reaction linearity with respect to P450 content (over the concentration range 10–250 pmol/ml). Experiments to determine enzyme kinetics were performed over a 30-min incubation time at a micromolar protein concentration of 1 mg/ml or at 100 pmol of P450/ml with the casopitant final concentration ranging from 0.1 to 50 \(\mu M\). All kinetics incubations were performed in triplicate, and rates of GSK525060 formation were quantified by HPLC-MS/MS.

**Data analysis.** GSK525060 formation was calculated as picomoles of metabolite produced per picomole of P450 per hour. Linear and nonlinear regression analysis of data were performed using GraFit. Kinetic parameters (\(k_{max}\) and \(V_{max}\)) for casopitant metabolism were calculated according to the Michaelis-Menten equation:

\[
V = \frac{V_{max} [S]}{K_m + [S]}
\]

where \(v\) is the observed rate of metabolite production, \(V_{max}\) is the maximal rate of metabolite production, \([S]\) is the casopitant concentration, and \(K_m\) is the concentration of casopitant required to achieve the half-maximal rate. Linearization plots were calculated by using the Lineweaver-Burk linearization equation:

\[
1/v = \frac{V_m}{V_{max}[S]} + 1/V_{max}
\]

**Bioanalytical Methods.** Measurements of casopitant metabolite GSK525060, 6βT, 1'-hydroxyazidolam, and oxidized nifedipine from in vitro incubations were performed with a validated and specific HPLC-MS/MS assay by or on behalf of the Drug Metabolism and Pharmacokinetics Department, GlaxoSmithKline. The bioanalytical methods are based on internal standard addition after protein precipitation with acetonitrile, centrifugation, and subsequent injection onto a HPLC-MS/MS system for analysis using a positive ionization mode. TurboIonSpray ionization and multiple reaction monitoring were conducted on an API-4000 mass spectrometer (Applied Biosystems/MDS Sciex, Concord, ON, Canada) to ensure high selectivity. The characteristic precursor \([M + H]^+\) to product ion transitions monitored in multiple reaction monitoring mode are consistent with the structures of GSK525060, 6βT, 1'-hydroxyazidolam, oxidized nifedipine, and their internal standards.

The selectivity of the methods was confirmed by the inclusion of blank and double blank samples prepared from pooled HLM in validation assays. The precision (relative S.D.) and accuracy (relative error) in pooled HLM were derived from the analysis of replicate validation samples (\(n = 6\)) at five concentrations for each analyte. At all concentrations of these validation samples examined, the intra-day precision value for the casopitant metabolite GSK525060 and for 6βT and the interday precision value for 1'-hydroxyazidolam and oxidized nifedipine were both \(\pm 15\%\) and so was the accuracy of the assay of the casopitant metabolite GSK525060, 6βT, 1'-hydroxyazidolam, and oxidized nifedipine. Both precision and accuracy were therefore considered acceptable (FDA, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf).

For GSK525060 determination in microsomal homogenate, the method was validated over the concentration range 6 to 6000 ng/ml using HPLC-MS/MS. Samples were chromatographed on a Hyclik Gold column (50 × 3 mm, 5 \(\mu M\); Thermo Fisher Scientific, Waltham, MA) with a mobile phase of 5 mM ammonium acetate-acetonitrile (35:65, v/v), at a flow rate of 0.7 ml/min.

The method of 6βT determination in microsomal homogenate was validated over the concentration range 5.00 to 5000 ng/ml using HPLC-MS/MS. Samples were chromatographed on a Hypersil Gold column (150 × 4.6 mm, 5 \(\mu M\); Thermo Fisher Scientific), with mobile phase A (methanol-acetonitrile, 98:2, v/v) and B (water-acetonitrile, 98:2, v/v), at a flow rate of 1 ml/min, using a stepped elution (0–10 min, 43% A/57% B; 10–20 min, 43% A/57% B to 73% A/27% B; 20–30 min, 73% A/27% B; 30–36 min, 73% A/27% B to 43% A/57% B; and 36–41 min, 43% A/57% B).

The methods of analyte determination in microsomal homogenate were validated over the concentration range 1 to 10000 nM for 1'-hydroxyazidolam and 5 to 5000 nM for oxidized nifedipine. Samples were chromatographed on a MetaChem Inertsil ODS-3 column (33 × 3 mm, 3 \(\mu M\); GL Sciences, Inc., Torrance, CA), with mobile phase A (0.1% formic acid, aqueous) and B (acetonitrile), at a flow rate of 0.6 ml/min, using a stepped elution for 1'-hydroxyazidolam (0–0.5 min, 95% A/5% B to 50% A/50% B; 0.50–0.52 min, 50% A/50% B; 0.52–1.10 min, 50% A/50% B to 10% A/90% B; 1.10–1.20 min, 10% A/90% B; 1.20–1.21 min, 10% A/90% B to 95% A/5% B; and 1.21–2.10 min, 95% A/5% B) and an isotropic gradient for oxidized nifedipine (0–2.8 min, 55% A/45% B).

Calibration plots of analyte/intermediate standard peak area ratio versus marker metabolite concentration were constructed and a weighted 1/\(x^2\) linear regression was applied to the data. Quality control samples, prepared at three different analyte concentrations and stored alongside the samples under study, were analyzed with each batch of samples against separately prepared calibration standards. For the analysis to be acceptable, no more than one-third of the total quality control results and no more than half of the results from each concentration level were to deviate from the nominal concentration by more than 15%.

**SimCyp Simulations.** The physicochemical and pharmacokinetic characteristics for casopitant, together with the kinetic parameters generated in vitro, were entered into SimCyp (version 9.03) to create a new “inhibitor” profile.
Physichemical and pharmacokinetic input parameters used in SimCyp simulation for casopitant

$k_{\text{deg}}$ provided by SimCyp was 0.000128 min$^{-1}$ unless indicated otherwise, other inputs are in-house calculated values.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>616.6</td>
</tr>
<tr>
<td>LogP</td>
<td>5.6</td>
</tr>
<tr>
<td>pk'</td>
<td>6.3</td>
</tr>
<tr>
<td>B/P ratio</td>
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</tr>
<tr>
<td>$\phi_{\text{F}}$ (plasma)</td>
<td>0.005</td>
</tr>
<tr>
<td>$F_{\text{u, mic}}$</td>
<td>0.93</td>
</tr>
<tr>
<td>$K_{\text{m}}$ (h$^{-1}$)</td>
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</tr>
<tr>
<td>$Q_{\text{int}}$ (h$^{-1}$)</td>
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</tr>
<tr>
<td>$V_{\text{cmin}}$</td>
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<tr>
<td>$V_{\text{cmax}}$ (kg)</td>
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<tr>
<td>$\text{Cl}_{\text{HLM}}$ ($\mu$L/min/mg protein)</td>
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</tr>
<tr>
<td>$K_{f}$ (µM)</td>
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</tr>
<tr>
<td>CYP3A4 $F_{\text{u, mic}}$</td>
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</tr>
<tr>
<td>CYP3A4 $K_{\text{m}}$ (µM)</td>
<td>3.1</td>
</tr>
<tr>
<td>$f_{\text{u, CYP3A4}}$</td>
<td>$\sim$1</td>
</tr>
</tbody>
</table>

B/P ratio, blood/plasma concentration ratio (in-house data); $F_{\text{u, mic}}$, unbound fraction of drug in plasma; $\phi_{\text{F}}$, fraction of drug absorbed (calculated from the clinical radiolabel study as reported by Pellegrati, 2009); $K_{f}$, scaled from in vivo clearance of clinical intravenous injection, in-house data; $Q_{\text{int}}$, intestinal blood flow predicted by SimCyp; $F_{\text{u, mic}}$, unbound fraction of drug in gut; $V_{\text{cmin}}$, unbound fraction in microsomes (predicted by SimCyp at 1 mg/ml protein); $V_{\text{cmax}}$, assuming a 75 kg volunteer (calculated from a clinical radiolabel study as reported by Pellegrati, 2009); $\text{Cl}_{\text{HLM}}$ calculated as $K_{f} \phi_{\text{F}}$, $K_{f}$ value estimated as $IC_{50}$; calculated value on MID/2, $f_{\text{u, CYP3A4}}$, fraction of drug metabolized by CYP3A4.

Input parameters for casopitant, shown in the Table 1, were calculated in-house from in vitro and in vivo experimental data, whereas the CYP3A4 inhibitor (KET), CYP3A4 inducer (RIF), or CYP3A4 substrate (MID and NIF) input data were supplied by the program.

Before the DDI predictions of casopitant as victim or perpetrator were started, the plasma concentration-time profile and pharmacokinetic parameters for a single oral dose of casopitant at 100 mg of were simulated using a one-compartment distribution model and intrinsic clearance value ($\text{Cl}_{\text{int}}$) (enzyme kinetics options), calculated in-house from the $K_{f}$ and $V_{\text{cmax}}$ values of GSK525060 production, as measured in HLM incubations. The assumptions made were that all casopitant metabolism was due to CYP3A4 and that GSK525060 was the only or the major metabolite of casopitant (Pellegrati et al., 2009), but no parameters for GSK525060 were included in SimCyp for the simulation. For MID and NIF, the model options applied were the oral route, the first order as absorption model, a one-compartment model for distribution, and an enzyme kinetic option for elimination.

The impact of casopitant as perpetrator and victim on the DDI was evaluated. With casopitant as perpetrator, the simulations were performed with two CYP3A4 substrates, MID and NIF, applying the clinical trial design. Virtual trials were run for a single oral dose of 5.0 mg of MID (taken at 10:00 AM, fasted) or 10 mg of NIF (taken at 9:00 AM, fasted) with or without casopitant (given at 9:00 AM, fasted) at a repeated dose of 30 or 120 mg/day. The drug coadministration occurred after 3 or 14 days of casopitant treatment. The magnitude of the interaction was expressed as the increase in exposure of MID or NIF, as the mean area under the plasma concentration-time curve (AUC). To assess the impact of trial design and interindividual variability on the interaction, the simulations were performed using this set of inactivation data: 10 trials of healthy volunteers comprising 10 subjects (20–50 years, 1:1 male/female ratio). The degree of interindividual variability in simulations was investigated using frequency histograms of AUC ratio.

With casopitant as victim, the simulations were performed with KET, a CYP3A4 inhibitor, and RIF, a CYP3A4 inducer, using the same virtual population and trial design described above and both time-dependent and steady-state conditions. The time-dependent model accounts for time- and concentration-dependent enzyme inhibition, applying the actual study design used in the clinical DDI studies. The steady-state simulation was run to evaluate the maximum potential for interaction for which both victim and perpetrator were at steady-state concentrations. Virtual trials were run for repeat doses of KET at 400 mg for 7 days and at day 4, when casopitant was dosed at 100 mg at the same time with KET administration. The simulations with RIF were performed, dosing RIF at 600 mg for 9 days with casopitant also given as a single dose of 150 mg on day 8 at the same time of RIF administration.

**In Vivo Studies: Designs and Treatments. Interaction with midazolam.**

GSK study NKF10011 was a single-center, open-label study to evaluate the potential pharmacokinetic interaction in healthy subjects of repeated daily oral doses of 30 or 120 mg of casopitant and single doses of 5 mg of oral MID. Oral casopitant was administered for 14 days (study days 8–21) to two cohorts of 16 subjects each. Midazolam (Hypnovel; Roche, Hertfordshire, UK, 1 ml of 5 mg/ml solution) was administered alone as single dose on day 1 and 1 h after casopitant on days 10 and 21. The time of administration was chosen to match the maximal plasma concentrations of the two drugs and, thus, maximize the potential pharmacokinetic interaction. This allowed a comparison of the pharmacokinetics of MID when this drug was given on its own or after 3 and 14 days of daily treatment with casopitant (Zamuner et al., 2010).

**Interaction with nifedipine.**

GSK study NKF10012 was a single-center, open-label study to evaluate the potential interaction in healthy subjects between repeated daily oral doses of 30 or 120 mg/day casopitant and a single dose of 10 mg of oral NIF. Oral casopitant was administered for 14 days (study days 8–21) to two cohorts with 13 to 14 subjects each. Both cohorts received a single oral dose of 10 mg of nifedipine (Adalat; Bayer Healthcare AG, Leverkusen, Germany) on day 1. On day 8, all subjects commenced a 14-day treatment with daily oral doses of casopitant. On days 10 and 21, all subjects also received a single 10-mg oral dose of NIF. This administration allowed a comparison of the pharmacokinetics of NIF when this drug was given on its own or after 3 and 14 days of daily treatment with casopitant (Zamuner et al., 2010).

In both studies, blood samples for determination of plasma concentration of MID or NIF, casopitant, and its metabolite GSK525060 were collected at selected days and time points and analyzed by HPLC-MS/MS (Zamuner et al., 2010).

**Interaction with ketoconazole.**

GSK study NKV105093 was an open-label study to evaluate in healthy subjects the potential interaction of repeated daily oral doses of 400 mg of ketoconazole (Nizoral; Janssen Pharmaceutica, New Brunswick, NJ) with a single oral dose of 100 mg of casopitant. Oral KET was administered for 7 days (study days 9–15) to 12 subjects. Oral casopitant was administered alone as single dose of 100 mg on day 1 and administered together with KET on day 12 (Johnson et al., 2010).

**Interaction with rifampin.**

GSK study NKV105091 was an open-label study to evaluate in healthy subjects the potential interaction of repeated single daily doses of 600 mg of oral rifampin (Rifadin, rifampin capsules, USP) with oral casopitant given as a single dose of 150 mg. Oral RIF was administered for 7 days (study days 9–15) to 12 subjects. Oral casopitant was administered alone as single dose of 100 mg on day 1 and administered together with KET on day 12 (Johnson et al., 2010).

**Bioanalytical Methods.**

Measurements of casopitant, its metabolite GSK525060, MID, NIF, KET, and RIF in plasma were performed with validated and specific HPLC-MS/MS assays by or on behalf of the Drug Metabolism and Pharmacokinetics Department, GlaxoSmithKline. All the details of the bioanalytical methods and their specific performances are reported in Zamuner et al. (2010) and Johnson et al. (2010).

**Pharmacokinetic Analysis.**

Pharmacokinetic analysis applied to the concentration-time profiles concerning casopitant, GSK525060, MID, and NIF, is reported in Zamuner et al. (2010) and Johnson et al. (2010).

**Results**

**In Vitro Data. Cytochrome P450 induction.**

The increased CYP3A4 activity (measured as testosterone 6β-hydroxylation) observed in human hepatocytes after exposure to the inducers suggested that greater amounts of enzyme were present as a result of the induction. In particular, the positive control RIF yielded a 2.1-, a 13-, and a 6.0-fold increase in activity, compared with the vehicle control activities, in donors 1, 2, and 3, respectively (Table 2), which is consistent with literature data (Luo et al., 2002; Madan et al., 2003). Although the magnitude of CYP3A4 induction was quite variable...
among the three hepatocyte preparations, these data indicated clearly that the hepatocytes in culture were responding appropriately for at least two of three donors.

A similar, but less potent, pattern of CYP3A4 enzyme induction was observed in hepatocyte cultures treated with casopitant, especially in cells from donors 2 and 3, the ones who had responded more markedly to Rif.

The slight decrease in the CYP3A4 activity observed at 20 μM compared with that at 5 μM may be due to the fact that casopitant and its metabolite are also inhibitors/inactivators of CYP3A4.

Cytotoxicity of casopitant at this concentration was not likely, as this decrease was not observed for the other P450 activities tested on the same human hepatocyte preparations (data not shown).

**Cytochrome P450 inhibition.** The ability of casopitant and its metabolite GSK525060 to inhibit CYP3A4 metabolism of probe substrates (MID and NIF), in a direct and metabolism-dependent manner, was investigated in pooled HLM, and the results are summarized in Table 3. Both casopitant and GSK525060 were shown to inhibit CYP3A4 activity, as measured with both probe substrates, with an IC50 lower than 10 μM. Moreover, the preincubation of casopitant or its metabolite with HLM in the presence of NADPH did increase their inhibitory effects, suggesting that both drugs also behaved as metabolism-dependent inhibitors of CYP3A4. In particular, the decrease in IC50 for casopitant and GSK525060 in the metabolism of MID were characterized by IC50 shifts of approximately 4.3- and 3.0-fold, respectively.

Metabolism-dependent inhibition of CYP3A4 by casopitant was further investigated, and the inactivation parameters k_inact and k_t were determined using MID as probe substrate. The results and associated kinetic plots are shown in Fig. 2. Casopitant showed a progressive increase in inhibitory potency with increasing NADPH preincubation time and concentration. This increase in potency was characteristic of a metabolism-dependent inhibitor, which requires enzymatic activation and, therefore, the presence of NADPH. The kinetic constants for the inactivation of CYP3A4 were determined to be 0.0199 min⁻¹ (k_inact) and 3.10 μM (K_I) by casopitant and 0.0468 min⁻¹ (k_inact) and 0.35 μM (K_I) by TAO. The relative inactivation efficiency on CYP3A4 activity of the test control TAO, presented by the ratio of k_inact/K_I = 0.13, is in agreement with the data reported by Xu et al. (2009) and confirms the validity of this assay.

**Metabolism of casopitant by HLM and recombinant human CYP3A4.** The formation of GSK525060 was found to be linear with time up to 30 min and with the amount of protein up to at least 1.0 mg/ml. The linearity of GSK525060 formation was also linear up to at least 150 pmol CYP3A4/ml when 10 μM casopitant was incubated with Bactosomes expressing human CYP3A4 (data not shown). From these experiments the conditions that gave the best compromise between linearity of reaction and readily detectable amounts of GSK525060 were chosen; namely, an incubation time of 30 min and an enzyme content of 0.1 mg protein/ml or 100 pmol CYP3A4/ml for HLM and recombinant CYP3A4, respectively. Further experiments run under these conditions, with a final concentration of casopitant ranging between 0.1 and 50 μM, indicated that casopitant metabolism followed Michaelis-Menten kinetics. The Michaelis-Menten and Lineweaver-Burk plots are reported in Figs. 3 and 4 for HLM and recombinant CYP3A4, respectively. In HLM, the formation of GSK525060 was characterized by a K_m of 21.1 μM and V_max of 65.4 pmol/pmol P450/h. The formation of GSK525060 and of other minor metabolites of casopitant by other P450 isoforms was negligible (data not shown).

**In Vivo Clinical Data. Interaction of casopitant as DDI perpetrator on CYP3A4 substrates.** The potential of casopitant as perpetrator of CYP3A4 interactions affecting the pharmacokinetics of coadministered drugs was investigated clinically using the two probe substrates, MID and NIF. After repeated-dose administration of casopitant, the plasma exposure to MID increased in a dose-dependent manner, an effect that may result in clinically significant pharmacologic effects, particularly with the 120 mg of casopitant dose (Table 4). Thus, after repeated administration of casopitant at 30 or 120 mg/day, MID exposure as AUC₈₋₁₄ increased 2.0- and 2.7-fold after 3 days of casopitant administration and 1.8- and 3.5-fold with casopitant at steady state, i.e., at 14 days of administration (Zumun et al., 2010).

In the second DDI study, casopitant given at 30 and 120 mg/day significantly increased exposure of NIF by inhibiting its metabolism. The overall effect may be clinically relevant, although mild in magnitude (1.4- to 1.8-fold) (Table 4). The increases in NIF exposures appeared to be nearly comparable, regardless of the casopitant dose

---

**TABLE 2**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Donor 1</th>
<th>Donor 2</th>
<th>Donor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Activity</td>
<td>Inductive Response</td>
<td>Mean Activity</td>
</tr>
<tr>
<td></td>
<td>(nmol per min/μg)</td>
<td>fold change</td>
<td>(nmol per min/μg)</td>
</tr>
<tr>
<td>Control</td>
<td>4.5</td>
<td>1.0</td>
<td>1.7</td>
</tr>
<tr>
<td>Rifampin</td>
<td>9.7</td>
<td>2.1 (100)</td>
<td>22</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>12</td>
<td>2.7 (150)</td>
<td>16</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>4.9</td>
<td>1.1 (6.8)</td>
<td>6.4</td>
</tr>
<tr>
<td>Casopitant</td>
<td>1 μM</td>
<td>4.7</td>
<td>1.0 (4.1)</td>
</tr>
<tr>
<td>5 μM</td>
<td>5.2</td>
<td>1.1 (13)</td>
<td>12</td>
</tr>
<tr>
<td>20 μM</td>
<td>3.1</td>
<td>0.70 (N.A.)</td>
<td>9.6</td>
</tr>
</tbody>
</table>

---

**TABLE 3**

<table>
<thead>
<tr>
<th>Probe Substrate</th>
<th>IC50 (μM)</th>
<th>Direct Inhibition</th>
<th>Metabolism-Dependent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casopitant</td>
<td>9.86 ± 1.78*</td>
<td>4.27</td>
<td>2.37</td>
</tr>
<tr>
<td>Midazolam</td>
<td>9.72 ± 1.18</td>
<td>3.37</td>
<td>3.37</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>8.22 ± 2.76</td>
<td>2.96</td>
<td>2.96</td>
</tr>
<tr>
<td>GSK525060</td>
<td>5.60 ± 0.45</td>
<td>13.7</td>
<td>13.7</td>
</tr>
</tbody>
</table>

---

* S.E. provided by GraFit.
used and the duration of dosing. In contrast, the pharmacokinetics of casopitant or GSK525060 were not altered by coadministration of either MID or NIF.

Interaction of CYP3A4 inhibitor or inducer with casopitant as victim. When coadministered with the potent CYP3A4 inhibitor KET, casopitant elimination was prolonged, resulting in an increase in total exposure after a single dose at 100 mg (Johnson et al., 2010). In particular, the exposure AUC(0–t) was increased at least 12.1-fold and Cmax was increased by approximately 2.7-fold (Table 5). The metabolism of GSK525060 and associated pharmacokinetic parameters were also affected. In particular, metabolite median time to reach Cmax (tmax) was delayed from 2 to 48 h, and its exposure as AUC(0–t) was increased by 3.7-fold. The Cmax of GSK525060 was approximately 45% lower (ratio = 0.55) after administration of KET than when casopitant was administered alone.

Coadministration of casopitant with the CYP3A4 inducer RIF resulted in decreased plasma concentrations of both casopitant and GSK525060. After daily dosing with RIF, casopitant exposure (after a single dose of 150 mg) was greatly decreased with AUC(0–t), and Cmax was reduced by 96% (ratio = 0.039) and 89% (ratio = 0.11), respectively (Table 6). Likewise, systemic exposure to GSK525060 as AUC(0–t) (after casopitant and RIF coadministration) was decreased by 94% (ratio = 0.057), whereas tmax of both casopitant and GSK525060 was not affected by the presence of RIF.

SimCyp Simulations. Simulated plasma concentration-time profiles and pharmacokinetic parameters for a single oral dose of casopitant at 100 mg were consistent with the reported clinical pharmacokinetic studies (Table 7) and support the model and the parameters used for the SimCyp simulations. AUC(0–t) ratios for the simulated interaction between casopitant at 30 and 120 mg and MID or NIF are given in Table 4. The mean AUC(0–t) for MID was predicted to increase from 1.8- to 3.6-fold, whereas for NIF the predicted increase was from 1.6- to 3.5-fold throughout the casopitant regimen. On the basis of these simulations, there were no relevant differences between the two CYP3A4 probes. However, given the different degree of fraction metabolism (fm) by CYP3A4 reported for MID and NIF (Ohno et al., 2007), we would have expected to have a different magnitude of interaction with casopitant. This discrepancy may reflect the inhibition potential of NIF on casopitant metabolism.
olism, NIF being itself a moderate CYP3A4 inhibitor (the SimCyp model includes a $K_i$ value of 24 $\mu$M into the NIF parameters).

Overall, with MID, the simulated mean exposure [AUC$_{0-\infty}$] increases were generally in agreement with mean clinical changes. Nevertheless, in a comparison of simulations with clinical data obtained with casopitant at 30 mg after 3 or 14 days of administration, a slight overestimation of the casopitant inhibition potential by SimCyp was observed. This was probably due to the perpetrator profile introduced into SimCyp lacking any induction parameters for casopitant or any inhibition potential for GSK525060.

The simulated effects of coadministration of KET (400 mg) or RIF (600 mg) on casopitant $C_{\text{max}}$ and AUC$_{0-\infty}$ are given in Table 8. The mean $C_{\text{max}}$ and AUC$_{0-\infty}$ for casopitant at 100 mg were predicted to increase by 2.8- and 9.1-fold, respectively, when coadministered with KET. After repeat dosing of RIF, the systemic exposure to casopitant at 150 mg, as $C_{\text{max}}$ and AUC$_{0-\infty}$, was predicted to decrease by approximately 62% (ratio = 0.38) and 86% (ratio = 0.14), respectively. No simulation has been performed on the effect of KET or RIF on the systemic exposure of GSK525060.

**Discussion**

The assessment of the potential for CYP3A4-mediated DDIs was an important part of the clinical development program for casopitant, due mainly to its in vitro profile. Further complexity was added by the fact that casopitant was under development for different clinical indications in several drug regimens, requiring acute or chronic dosing at different dose strengths, with different potential comedinations.

In vitro studies using HLM have demonstrated that casopitant inhibited CYP3A4 metabolism of the probe substrates MID and NIF with similar potencies. Moreover, casopitant showed metabolism-dependent inhibition of CYP3A4, consistent with the finding that its major metabolite, GSK525060, was itself a CYP3A4 inhibitor. Thus, the overall conclusion of all the in vitro studies was that both casopitant and its metabolite GSK525060 were direct and metabolism-dependent CYP3A4 inhibitors. The metabolite GSK525060 was observed in plasma after single (Pellegatti et al., 2009) and repeated (Zamuner et al., 2010) oral administration of casopitant with an exposure of approximately the same order of magnitude as that of the parent compound. In addition, casopitant showed a moderate induction of CYP3A4 in cultured human hepatocytes, with a response likely to be mediated by the nuclear pregnane X receptor (Luo et al., 2002; Lin, 2006). In vivo these two contrasting effects may vary in time with respect to each other, so that one or the other could prevail, depending on the drug that was coadministered with casopitant and on the casopitant therapeutic regimen.

The in vitro results presented here provided the basis for the strategy to assess the role of casopitant as DDI perpetrator. Two clinical studies were conducted, using MID and NIF as probe substrates. A 3-day dose regimen of oral casopitant increased MID exposure as AUC$_{0-\infty}$, by approximately 2- to 3-fold (from 30 to 120 mg) in a dose-dependent manner. The increase in MID exposure was higher when MID was coadministered with casopitant under steady-state plasma concentrations. This could be the clinical correlation of the time-dependent inhibition observed in vitro. Based on the clinical results obtained at 30 or 120 mg/day, casopitant could be considered as a moderate inhibitor of CYP3A4, according to the FDA classification system (FDA, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072101.pdf).

The increase in exposure caused by casopitant with respect to the CYP3A4 substrate NIF was slightly lower than that observed for MID, i.e., 1.8-fold after a 3-day administration and 1.4-fold after 14 days of oral casopitant (120 mg/day). Moreover, casopitant at steady state increased NIF exposure after less than 3 days of casopitant administration, suggesting that, under conditions of protracted treat-

### Table 4

**Effects of coadministration of casopitant on AUC of CYP3A4 substrates, midazolam (orally dosed at 5 mg), and nifedipine (orally dosed at 10 mg): comparison of clinical results and SimCyp simulations**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Casopitant Dose and Regimen</th>
<th>Clinical Ratio$^a$</th>
<th>Predicted Ratio$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>fold change</td>
<td></td>
</tr>
<tr>
<td>Midazolam</td>
<td>30 mg (once daily for 3 days)</td>
<td>2.02 (1.75–2.32)</td>
<td>1.77 (1.29–2.53)</td>
</tr>
<tr>
<td>Midazolam</td>
<td>30 mg (once daily for 14 days)</td>
<td>1.76 (1.53–2.03)</td>
<td>2.12 (1.34–3.36)</td>
</tr>
<tr>
<td>Midazolam</td>
<td>120 mg (once daily for 3 days)</td>
<td>2.67 (2.18–3.27)</td>
<td>2.46 (1.49–3.96)</td>
</tr>
<tr>
<td>Midazolam</td>
<td>120 mg (once daily for 14 days)</td>
<td>3.49 (2.98–4.08)</td>
<td>3.57 (1.65–7.75)</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>30 mg (once daily for 3 days)</td>
<td>1.56 (1.37–1.78)</td>
<td>1.61 (1.24–2.12)</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>30 mg (once daily for 14 days)</td>
<td>1.61 (1.39–1.87)</td>
<td>1.94 (1.32–2.77)</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>120 mg (once daily for 3 days)</td>
<td>1.77 (1.54–2.04)</td>
<td>2.35 (1.53–3.40)</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>120 mg (once daily for 14 days)</td>
<td>1.42 (1.23–1.65)</td>
<td>3.45 (1.68–6.55)</td>
</tr>
</tbody>
</table>

$^a$ Exposure ratios are given with two decimal places in tables and with at least one decimal place in the text.

$^b$ Ratios from clinical data are expressed as geometric mean ratio with 90% confidence interval given in parentheses. Data from Zamuner et al. (2010).

$^c$ Ratios from simulations are expressed as the geometric mean ratio with the 90% confidence interval given in parentheses.

### Table 5

**Summary statistics of casopitant and GSK525060 pharmacokinetic parameters when casopitant was coadministered with ketoconazole**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Treatment$^a$</th>
<th>AUC$_{0-\infty}$</th>
<th>$C_{\text{max}}$</th>
<th>$t_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ng h/ml</td>
<td>ng/ml</td>
<td>h</td>
</tr>
<tr>
<td>Casopitant</td>
<td>A</td>
<td>2590 ± 572</td>
<td>457 ± 68</td>
<td>1.00 (0.50–2.50)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>31200 ± 7570</td>
<td>1250 ± 254</td>
<td>2.00 (1.00–2.53)</td>
</tr>
<tr>
<td>GSK525060</td>
<td>Ratio treatment B vs. A$^a$</td>
<td>12.10$^b$</td>
<td>2.71</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2470 ± 451</td>
<td>245 ± 40</td>
<td>2.00 (1.00–2.50)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>9150 ± 2250</td>
<td>135 ± 30</td>
<td>47.92 (23.92–96.00)</td>
<td></td>
</tr>
<tr>
<td>Ratio treatment B vs. A$^a$</td>
<td>3.66$^b$</td>
<td>0.55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Treatment A = 100 mg of oral casopitant (n = 13). Treatment B = 100 mg of oral casopitant (day 4) + 400 mg of ketoconazole (q.d., days 1–7) (n = 13).

$^b$ Exposure ratios are given with two decimal places in tables, and with at least one decimal place in the text. Data from Johnson et al. (2010).

$^c$ Comparison based on geometric mean of AUC$_{0-\infty}$. 
ment, the induction potential of casopitant could play a role in the interaction between casopitant and NIF.

Despite the comparable in vitro IC\textsubscript{50} values of casopitant on the CYP3A4-dependent metabolism of NIF compared with that of MID, the clinical results showed a clear difference in probe substrate sensitivity, and this may be explained by different factors: the existence of clearance pathways of the probe substrates not dependent on CYP3A4; the pharmacokinetic clinical variability observed mainly with NIF (Zamuner et al., 2010); or a different metabolism of the probe substrates mediated by intestinal CYP3A4, as reported by Paine et al. (1997) and Foti et al. (2010).

It is well known that MID and NIF are characterized by different kinetics, suggesting the existence of a different preferential binding domain [i.e., hyperbolic inhibition for MID and substrate inhibition for NIF (Galetin et al., 2003)] and by a different fraction of the total dose, which is metabolized by CYP3A4 [\( f_m = 0.92 \) for MID and \( f_m = 0.78 \) for NIF (Ohno et al., 2007)].

Overall, MID is considered to be more sensitive than NIF in providing the best assessment of potential clinical drug-drug interactions (Galetin et al., 2005), and this was also the case for the clinical study reported here with casopitant. Because it was impractical to examine all possible coadministered CYP3A4 substrates for potential interactions, the potential for DDIs between casopitant and other coadministered drugs that are CYP3A4 substrates was estimated from the clinical data just discussed. The extent of interaction is likely to depend on the fractional clearance of the coadministered drugs, which can be attributed to CYP3A4. Thus, MID given orally is largely metabolized by CYP3A4 and will be subjected to a significant interaction by coadministered casopitant (120 mg/day), with a 3.5-fold increase in MID exposure. Interactions may be of lower magnitude by coadministered casopitant (120 mg/day), with a 3.5-fold increase in MID exposure. Interactions may be of lower magnitude

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Treatment\textsuperscript{a}</th>
<th>AUC\textsubscript{(0–t)}</th>
<th>( C_{\text{max}} )</th>
<th>( t_{\text{max}} )</th>
<th>( t_{1/2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{ng} \cdot \text{h/ml} )</td>
<td>( \text{ng/ml} )</td>
<td>( h )</td>
<td>( h )</td>
<td></td>
</tr>
<tr>
<td>Casopitant</td>
<td>A</td>
<td>6570 ± 2180</td>
<td>847 ± 279</td>
<td>1.25 (0.53–6.00)</td>
<td>19.2 ± 7.1</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>273 ± 127</td>
<td>93.2 ± 39.8</td>
<td>1.00 (0.50–2.05)</td>
<td>6.45 ± 2.95</td>
</tr>
<tr>
<td>Ratio treatment B vs. A\textsuperscript{b}</td>
<td>0.04\textsuperscript{a}</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSK525060</td>
<td>A</td>
<td>5260 ± 1380</td>
<td>350 ± 110</td>
<td>2.00 (1.00–8.00)</td>
<td>17.6 ± 7.0</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>311 ± 114</td>
<td>93.0 ± 32.2</td>
<td>1.26 (0.50–2.05)</td>
<td>3.36 ± 1.12</td>
</tr>
<tr>
<td>Ratio treatment B vs. A\textsuperscript{c}</td>
<td>0.06\textsuperscript{a}</td>
<td>0.26</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( a \) Treatment A = 150 mg of oral casopitant (\( n = 18 \)). Treatment B = 150 mg of oral casopitant (day 8) + 600 mg of rifampin (q.d., days 1–9) (\( n = 16 \)).

\( b \) Exposure ratios are given with two decimal places in tables, and with at least one decimal place in the text. Data from Johnson et al. (2010).

\( c \) Comparison based on the geometric mean of AUC\textsubscript{(0–t)}.

A retrospective analysis of the DDI data was performed, simulating the in vivo data using SimCyp, a computer-based DDI tool for estimating the metabolic clearance of drugs and the effects of metabolic interactions in humans using in vitro metabolism and inhibition data (Howgate et al., 2006). Casopitant data were used to assess the potential of this software in the early clinical stages. For this reason, we have not included any data on GSK525060, because it was unavailable in this phase of drug development. The potential of casopitant as a DDI perpetrator was determined with MID and NIF as probe substrates.

SimCyp was able to simulate the different degree of interaction between casopitant and MID observed after repeated administrations of casopitant at doses of 30 or 120 mg/day. On the contrary, simulations with NIF were not in complete agreement with the clinical data, as clinically an interaction lower than that predicted by the model was observed, especially when casopitant was given chronically. This discrepancy is probably due to the high variability observed in the clinical study and casopitant induction potential that was not considered in the model used (Zamuner et al., 2010). Moreover, the DDI predicted by SimCyp seemed to be an overestimation, because the CYP3A4 inhibitor effect of nifedipine included in the model has not been observed in the clinical study.

The retrospective analysis of DDI potential for casopitant as victim was conducted with KET and RIF. The SimCyp analysis on
KET interaction was generally in agreement with the clinically observed changes in casopitant exposure and pharmacokinetics. There was a trend of SimCyp to slightly underestimating by approximately 25% the clinical effect of KET on casopitant exposure as judged by the AUC. In consideration of the higher individual variability of the simulated trials compared with that of clinical study design, this difference may be considered not relevant. The simulation performed with RIF underestimated the magnitude of the interaction by approximately 3.5-fold. However, under steady-state conditions, once the maximum extent of interaction had been reached, the simulated effect of RIF on casopitant became close to the clinical one.

Overall, the clinical data confirmed the ability of casopitant to act as a substrate, as an inhibitor, and possibly as an inducer of CYP3A4. The clinical studies conducted to investigate the potential of casopitant as a DDI perpetrator confirmed that this drug was a moderate inhibitor of CYP3A4 according to the FDA classification system (FDA, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072101.pdf).

The clinical studies conducted to investigate the potential of casopitant as DDI victim suggested that coadministration of casopitant with potent inducers of CYP3A4 was likely to result in decreased casopitant exposure, overcoming the toxicologic cover.

In conclusion, the in vitro data were accurate and robust enough to build reliable SimCyp models to estimate the potential DDI of casopitant and to minimize the clinical studies recommended. The approach based on the prediction of clinical data from in vitro results would eliminate the need of numerous unnecessary clinical DDI studies, especially the need to test different doses and would eventually accelerate the availability of therapy to patients.

Acknowledgments
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Authorship Contributions
Participated in research design: Motta, Pons, Pagliarusco, and Bonomo.
Conducted experiments: Pons.
Performed data analysis: Pons and Bonomo.
Wrote or contributed to the writing of the manuscript: Motta, Pons, Pagliarusco, Pellegratti, and Bonomo.

References


TABLE 8
Effects of coadministration of CYP3A4 inhibitor (ketoconazole orally dosed at 400 mg) or CYP3A4 inducer (rifampin orally dosed at 600 mg/day) on casopitant pharmacokinetics: comparison of clinical results and SimCyp simulations

<table>
<thead>
<tr>
<th>Casopitant</th>
<th>Perpetrator</th>
<th>Casopitant $C_{\text{max}}^a$</th>
<th>Casopitant AUC$^{b}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Clinical Mean$^a$</td>
<td>Predicted Mean$^a$</td>
</tr>
<tr>
<td>100 mg</td>
<td>Ketoconazole</td>
<td>2.71 (2.39–3.06)</td>
<td>12.1 (11.00–13.20)</td>
</tr>
<tr>
<td>150 mg</td>
<td>Rifampin</td>
<td>0.11 (0.08–0.14)</td>
<td>0.38 (0.03–0.47)</td>
</tr>
</tbody>
</table>

SS, steady-state conditions.

$^a$ Exposure ratios are given with two decimal places in tables, and with at least one decimal place in the text.

$^b$ Predicted comparisons are data based on geometric mean of AUC$_{0-\infty}$, with the 90% confidence interval given in parentheses. Data from Johnson et al. (2010).

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