In Vitro and In Vivo Investigation of Potential for Complex CYP3A Interaction for PF-00251802 (Dagrocorat), a Novel Dissociated Agonist of the Glucocorticoid Receptor

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Abstract

The dissociated agonists of the glucocorticoid receptor are a novel class of agents in clinical development for rheumatoid arthritis. PF-04171327 (fosdagrocorat) is a phosphate ester prodrug of PF-00251802 (dagrocorat), a selective high-affinity partial agonist of the glucocorticoid receptor, which is further metabolized to PF-04015475. This study evaluated the cytochrome P450 (CYP)–mediated drug–drug interaction (DDI) potential of PF-00251802 and PF-04015475 in vitro and used model-based prediction approaches to estimate clinical impact. PF-00251802 is a reversible inhibitor of several CYPs, but modeling has suggested no clinically relevant interaction. PF-00251802 and PF-04015475 are time-dependent inhibitors and inducers of CYP3A in vitro; PF-00251802 is also a time-dependent inhibitor of CYP2D6. Model-based prediction suggested the potential for weak inhibition of CYP3A in vivo. A clinical DDI study was conducted with midazolam, a sensitive CYP3A substrate. A phase 1 open-label, multiple-dose study evaluated the effect of PF-04171327 on midazolam pharmacokinetics and safety in 12 healthy volunteers. Administration of midazolam alone or concomitantly with PF-04171327 resulted in equivalent pharmacokinetic profiles (AUCinf, 21.17 vs 20.28 ng·h/mL, respectively), indicating that PF-04171327 had no net effect on CYP3A activity in vivo. These findings support the further development of PF-00251802 and PF-04171327 as potential treatments for patients with rheumatoid arthritis (NCT00987038).

Keywords

CYP3A, drug-drug interaction, modeling, PF-00251802, pharmacokinetics, phase 1

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by the presence of swollen and tender joints. An established treatment regimen for RA typically includes the use of glucocorticoids such as prednisone with or without disease-modifying antirheumatic drugs to alleviate inflammation, reduce disease signs and symptoms, and limit structural progression.1–3 However, the use of glucocorticoids in RA is associated with clinically significant adverse effects, including weight gain, diabetes, and hypertension.3 Consequently, new treatments that have efficacy similar to glucocorticoids but improved tolerability are being developed. One novel class of agents that is currently in clinical development for RA is the dissociated agonists of the glucocorticoid receptor (DAGRs). PF-04171327 (fosdagrocorat) is a phosphate ester prodrug of PF-00251802 (dagrocorat), a selective high-affinity partial agonist of the glucocorticoid receptor (Figure 1). Previous preclinical, phase 1, and phase 2 studies have demonstrated the anti-inflammatory effects of PF-04171327.4–10

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After oral administration, PF-04171327 is converted to PF-00251802, presumably by gut phosphatases; PF-00251802 is the principal circulating molecule. PF-04171327 was not detected in systemic circulation (lower limit of quantification, 0.5 ng/mL) in single or multiple ascending-dose phase 1 studies (Pfizer, unpublished data). In preclinical metabolism and excretion studies, PF-00251802 was found to be cleared primarily by metabolism, and the results of in vitro studies using liver microsomes and hepatocytes from preclinical species and from humans indicated that PF-00251802 undergoes both oxidative and conjugative metabolism (Pfizer, unpublished data). With respect to oxidative metabolism, PF-00251802 is metabolized by cytochrome P450 (CYP)3A to an N-oxide metabolite, PF-04015475, which is also present in the circulation of humans.11

PF-04015475 has similar in vitro pharmacologic activity to PF-00251802 and is therefore considered a pharmacologically active metabolite.11

A major class of enzymes involved in drug metabolism is the CYP family, with estimates suggesting its involvement in the metabolism of up to 80% of prescribed drugs.12 Of the CYPs, CYP3A is the major form expressed in hepatocytes and intestinal enterocytes and is the primary form that mediates first-pass metabolism of orally administered drugs.12

During drug development, regulatory authorities recommend that any drug–drug interactions (DDIs) between the drug under investigation and other agents should be routinely assessed.13,14 The Food and Drug Administration acknowledges the importance of CYPs in drug metabolism and recommends stepwise, model-based evaluations of metabolism-based interactions. The evaluations should begin with in vitro assessments of CYP enzymes to determine potential interactions between the investigational drug and these enzymes in terms of inhibition (reversible or time-dependent) or induction (CYP1A2, CYP2B6, and CYP3A4). The potential for any detected in vitro interactions to translate into in vivo interactions can be assessed in a stepwise fashion starting with basic models that incorporate the potency of the in vitro interaction and the expected plasma concentration of drug at therapeutic doses. Depending on results obtained using basic models, subsequent use of more mechanistic models and/or physiologically based pharmacokinetic modeling studies and/or in vivo assessment may be recommended.13 Of note, the in vitro findings can influence decisions on the timing of in vivo assessments within clinical development plans. Therefore, it is important that the in vitro assessments be based on robust, validated methodologies.

Here, we present findings from in vitro investigations of the potential effects of PF-00251802 and PF-04015475 on CYP enzymes, including reversible and time-dependent inhibition and induction, along with the use of model-based prediction approaches to assess the potential for clinically relevant DDIs. Based on a complex CYP3A DDI scenario, a phase 1 open-label, multiple-dose study was conducted to evaluate the effect of the prodrug PF-04171327 on midazolam pharmacokinetics (PK) in healthy volunteers.

Methods

In Vitro: Reversible Inhibition

The potential for PF-00251802 and its metabolite PF-04015475 to reversibly inhibit human drug-metabolizing enzymes in vitro was evaluated by determining half maximal inhibitory concentration (IC₅₀) values. Pooled human liver microsomes were incubated with standard marker activity substrates (at concentrations near their determined Kₘ values) and PK-00251802 (0–30 μM) or PK-04015475 (0–100 μM) in the presence of nicotinamide adenine dinucleotide phosphate (NADPH; 1.3 mM). Human liver microsome concentrations and times were chosen to respect the linearity of the reaction for each probe substrate. At the end of the incubation period, termination solvent containing internal standard was added and the incubation mixture filtered to remove microsomal protein.
Samples were analyzed using high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) and compared with positive control data using isoform-selective inhibitors of each CYP enzyme.

**In Vitro: Time-Dependent Inhibition**

The potential for PF-00251802 and PF-04015475 to produce time-dependent inhibition of CYPs in vitro was determined using midazolam (PF-00251802 and PF-04015475) and dextromethorphan (PF-00251802 only) as marker substrates for CYP3A and CYP2D6, respectively. Pooled human liver microsomes (0.3 mg/mL) were incubated with NADPH (1.3 mM) and PF-00251802 (0.03–5 μM) for 1, 2, 4, 6, 13, and 20 minutes (midazolam assay) or 1, 5, 10, 21, 30, and 41 minutes (dextromethorphan assay) or PF-04015475 (0.3–30 μM) for 1, 5, 10, 20, 30, and 40 minutes (midazolam assay). An aliquot of the primary incubation mixture was then added to a secondary reaction containing NADPH (1.3 mM) and midazolam (23 μM) or dextromethorphan (14.4 μM), resulting in a 20-fold dilution from the primary incubation to minimize reversible inhibition of each investigational agent on CYP enzymes. Following a 6-minute (midazolam assay) or 10-minute (dextromethorphan assay) incubation, the reaction was terminated, and samples were analyzed using HPLC-MS/MS for formation of 1′-hydroxymidazolam or dextrorphan. Data were analyzed using the procedure for time-dependent inhibition of CYP enzymes described by Yates et al (2012).15

**In Vitro: Induction of CYP3A and CYP1A2**

The potential for PF-00251802 and PF-04015475 to induce CYP3A and CYP1A2 activity in vitro was evaluated in cultured, cryopreserved human hepatocytes (CellzDirect, Pittsboro, California). Cells were incubated in collagen-coated 24-well plates with PF-00251802 (0.5–100 μM) or PF-04015475 (0.5–60 μM), rifampin (positive control for CYP3A; 25 and 10 μM in PF-00251802 and PF-04015475 experiments, respectively), omeprazole (positive control for CYP1A2; 50 μM), or a vehicle control for 72 hours. Following a cell wash, testosterone (250 μM, PF-00251802 assay), midazolam (30 μM, PF-04015475 assay), or etoxygenorufin (2 μM) was added for various incubation times. As a measure of CYP3A activity, levels of the metabolites 6β-hydroxytestosterone and 1′-hydroxy-midazolam were determined using HPLC with ultraviolet detection and LC-MS/MS, respectively. As a measure of CYP1A2 activity, etoxygenorufin O-deethylation to resorufin was measured using fluorometry.

Total messenger RNA (mRNA) was extracted from hepatocytes using a mini RNeasy kit (Qiagen, Valencia, California), and CYP3A4 and CYP1A2 mRNA was quantified using a TaqMan 2-step reverse transcription polymerase chain reaction method (Applied Biosystems, Foster City, California), according to the manufacturer’s instructions. A positive in vitro induction result was defined as a ≥2-fold induction based on enzymatic activity and a ≥4-fold induction based on mRNA.16 The concentration at which 50% of a maximum induction effect occurred (EC50) and maximum fold induction observed in vitro (Emax) were estimated based on the observed drug concentration-versus-fold-induction curves.

**Net Effect (Mechanistic Static) Model for CYP Inhibition and Induction**

To predict the impact of reversible inhibition, time-dependent inhibition, and induction on enzyme activity in vivo, variations of the net effect (mechanistic static) model were used.17–19 As a first approach, each interaction component was evaluated separately, that is, reversible inhibition, time-dependent inhibition, and induction were evaluated using equations 1–3, respectively. The net effect of the 3 processes was then evaluated using equation 4. The net effect of equation 4 was modified from the original net effect, in that the fraction unbound in the gut was assumed to be the same as in plasma, and the hepatic CYP3A half-life was assumed to be 23 hours rather than 36 hours. These modifications have been shown to improve DDI predictions, particularly for highly protein bound compounds.20,21

Note that fraction unbound in microsomes and hepatocytes was not applied to these equations for the following reasons. Fraction unbound in microsomes was assessed for PF-00251802 (approximately 0.22 at 0.76 mg/mL microsomal protein [data on file]). PF-04015475 was not measured, but would be expected to have higher microsomal fraction unbound than PF-00251802. The contribution of microsomal unbound fraction, at the low microsomal protein concentrations used in the various assays, would not significantly change the overall results from the in vitro studies (within the variability of the assays). Because incorporation of fraction unbound microsomes is not generally applied to the basic prediction models and given the minor impact it would have in the case of PF-00251802 and PF-04015475, it was not included. The incorporation of unbound fraction in hepatocytes into the DDI prediction, although scientifically justified, has not been fully verified in our laboratory. Work on the best practices for measuring unbound fraction in hepatocytes and induction media, and incorporation of these values into the DDI prediction equations, is ongoing.

Equation 1 — reversible inhibition

\[
R_1 = 1 + \frac{[I]}{IC_{50}/2}
\]
Equation 2 — time-dependent inhibition
\[ R_2 = \frac{(k_{\text{obs}} + k_{\text{deg}})}{k_{\text{deg}}} \]

Equation 3 — induction
\[ R_3 = \frac{1}{1 + \left(\frac{E_{\text{max}} \times [I]}{EC_{50} + [I]}\right)} \]

Equation 4 — modified net effect
\[ R_4 = \left(\frac{R_1 \times R_2 \times R_3 \text{hepatic}}{f_{\text{m, CYP3A}}} + (1 - f_{\text{m, CYP3A4}}) \right) \times \left(\frac{(R_1 \times R_2 \times R_3 \text{intestine} \times F_{\text{g}})}{(1 - F_{\text{g}})}\right) \]

In these models, R indicates the ratio of area under the plasma concentration–time curve (AUC) for a sensitive CYP substrate in the presence/absence of the interacting drug. Inhibitor concentration ([I]) was estimated using the unbound maximum observed plasma concentration during the dosing interval (C_{\text{max}}) concentration of the drug. The fraction unbound in human plasma (f_u) for PF-00251802 is 0.007, and the f_u for PF-04015475 is 0.028. Following a 25-mg steady-state administration of PF-04171327, the estimated total C_{\text{max}} for PF-00251802 is approximately 300 ng/mL (0.61 \mu M), and the unbound C_{\text{max}} is approximately 2.1 ng/mL (4.2 nM) — Miyoshi et al.\textsuperscript{11} Weatherley et al.\textsuperscript{22} and unpublished data. Following a 25-mg steady-state administration of PF-04171327, the estimated total C_{\text{max}} for PF-04015475 is approximately 100 ng/mL (0.20 \mu M), and the unbound C_{\text{max}} is approximately 2.8 ng/mL (5.4 nM)\textsuperscript{11}. For equation 1, the reversible inhibition experiments were run at a substrate concentration near the K_m, and IC_{50}/2 was therefore used as a surrogate for K_i.\textsuperscript{23} The observed rate (k_{\text{obs}}) was defined as maximal inactivation rate (k_{\text{inact}}) \times [I] / (K_i + [I]), where K_i is the apparent inactivation constant at half-maximal rate of inactivation, and k_{\text{deg}} is the approximate degradation rate constant for CYP3A as a surrogate for K_i.\textsuperscript{23} Assuming a hepatic half-life of 23 hours (0.0005 min\textsuperscript{-1}). The hepatic k_{\text{deg}} value for CYP2D6 was 0.0002 min\textsuperscript{-1}.\textsuperscript{24} The term f_{\text{m, CYP3A}} represents the fraction of the substrate drug that is metabolized by CYP3A. F_{\text{g}} represents the fraction of substrate drug that passes through the intestine. For predictions using midazolam as the substrate drug, the f_{\text{m, CYP3A}} used was 0.9, and F_{\text{g}} was 0.51. For predictions using desipramine as the substrate drug, the f_{\text{m, CYP2D6}} used was 0.9, and F_{\text{g}} was 1.00.

**Phase 1 Study in Healthy Volunteers**

The study protocol and informed consent documentation were approved by the institutional review board at the investigational center (New Haven Clinical Research Unit, New Haven, Connecticut). The study was conducted in accordance with applicable legal and regulatory requirements, as well as the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Subjects, International Council for Harmonization Guidelines for Good Clinical Practice, and the Declaration of Helsinki. All subjects provided written informed consent prior to entering the study.

**Study Design.** This phase 1 open-label, single-fixed-sequence, multiple-dose study in healthy volunteers (ClinicalTrials.gov NCT00987038) was conducted between September and December 2009 to evaluate the effect of PF-04171327 on midazolam PK. Healthy volunteers were required to be aged 18–55 years, with a body mass index (BMI) of 17.5 to 30.5 kg/m\textsuperscript{2}, a total body weight of >50 kg, and an absence of clinically relevant abnormalities following a detailed medical history, full physical examination, 12-lead electrocardiogram (ECG), and safety laboratory tests. Cortisol levels were required to be within the normal laboratory reference range. Key exclusion criteria included history of intolerance or significant adverse event (AE) with glucocorticoid therapy; history of intolerance to midazolam or other benzodiazepines; evidence or history of clinically significant hematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, or hepatic disease; presence of any condition affecting drug absorption; and current treatment for or a history of previously untreated infection with *Mycobacterium tuberculosis*.

**Treatment.** All treatment was administered at approximately 8:00 AM (±30 minutes) each day. On day 1, following a 10-hour fast, subjects received a single oral dose of midazolam 2 mg as a syrup formulation. On days 2–16, patients received a once-daily oral dose of PF-04171327 25 mg, which was supplied as 5- or 10-mg immediate-release tablets and was administered ≥2 hours before a meal or ≥4 hours following the last meal. On day 15, subjects received 25 mg of PF-04171327 first, followed immediately by midazolam 2 mg.

Prescription or nonprescription drugs, vitamins, or dietary supplements, and use of CYP3A inhibitors, inducers, or substrates were not permitted within 28 days or 5 half-lives (whichever was longer) prior to the first dose of study medication. Corticosteroid use of prednisone 20 mg or higher equivalent per day was not permitted for ≥3 weeks in the 55 weeks prior to dosing.

**Study Objectives.** The primary objective of the phase 1 study was to evaluate the effect of multiple doses of PF-04171327 on the PK of a single oral dose of midazolam. The primary end point of the study was AUC from time 0 extrapolated to infinity (AUC\textsubscript{inf}); secondary PK end points included C_{\text{max}}, time to obtain C_{\text{max}} (T_{\text{max}}), AUC from time 0 to the time of last quantifiable
concentration (AUC$_{\text{last}}$), time of last quantifiable concentration (T$_{\text{last}}$), and terminal elimination half-life (t$_{1/2}$).

A secondary objective was to evaluate the safety and tolerability of a single oral dose of midazolam when coadministered with PF-04171327.

Assessments. Blood samples for analysis of plasma midazolam concentrations were collected at 0 hours (predose) and 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, and 24 hours after dosing on days 0 and 15. On day 15, blood samples were also obtained 36 and 48 hours after dosing.

Blood samples for analysis of plasma PF-00251802 concentrations were collected at 0 hours (predose) on days 2, 3, 5, 8, 10, 13, 15, and 16 of PF-04171327 dosing. On day 17, a blood sample was obtained 24 hours after day 16 PF-04171327 dosing.

AEs and serious AEs (SAEs) were reported throughout the study. Blood and urine specimens for laboratory evaluations were collected at the screening visit and on days 0 and 17. Vital signs, 12-lead ECGs, and physical examinations were performed at prespecified times throughout the study.

Analytical Methods. Human plasma samples were analyzed for midazolam concentrations at Covance Bioanalytical Services (Shanghai, China) using a validated high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) method. Plasma sample aliquots were fortified with the internal standard (alpha-hydroxytriazolam); midazolam alone was the reference treatment, and midazolam and the internal standard were extracted from plasma by automated liquid–liquid extraction. After evaporation, under nitrogen, the residue was reconstituted and analyzed by HPLC-MS/MS. The HPLC system consisted of a Merck Chromolith SpeedROD RP-18e 50 × 4.6 mm column with a prefilter (Upchurch, 0.5 μm), with mobile phases of 0.15% trifluoroacetic acid in 20 mM ammonium formate (A) and methanol (B). The mass spectrometer was a Sciex API 4000; samples were analyzed using positive ion electrospray in the MRM mode. The m/z transition was 326.0/291.0 and 359.0/176.0 for midazolam and alpha-hydroxytriazolam, respectively. Calibration standard responses were linear over the range of 0.05 to 50 ng/mL, using a 1/concentration$^2$ linear least-squares regression. The between-assay accuracy, expressed as the ratio (%) of the estimated to theoretical QC concentrations, ranged from 9.82% to 14.1% for the low, medium, and high QC samples. Assay precision, expressed as between-day CV% of the estimated concentrations of QC samples, was ≤2.65%.

Analysis. The PK population consisted of all enrolled subjects who were randomized and treated and had ≥1 PK parameter of interest in ≥1 PK assessment period following dosing on days 1 and 15.

The PK parameters C$_{\text{max}}$, AUC$_{\text{inf}}$, and AUC$_{\text{last}}$ were generated via noncompartmental analysis. Natural log-transformed C$_{\text{max}}$, AUC$_{\text{inf}}$, and AUC$_{\text{last}}$ of midazolam were analyzed using a mixed-effects model with treatment as a fixed effect and subject as a random effect. Estimates of the adjusted mean differences (test/reference) and corresponding 90% confidence intervals (CIs) were obtained from the model and were used to obtain estimates of the ratio of adjusted geometric means (test/reference) and 90%CIs for the ratios. Midazolam alone was the reference treatment, and midazolam coadministered with PF-04171327 was the test treatment. Descriptive statistics are presented for all PK data.

All subjects who received at least 1 dose of study treatment were included in the safety analysis set. No formal analyses were planned for safety data.

Results

In Vitro Reversible CYP Inhibition

PF-00251802 (dagrocorat) and its primary metabolite PF-04015475 were examined for potential inhibitory effects on a panel of drug-metabolizing enzymes in human liver microsomes. A summary of IC$_{50}$ values for PF-00251802 and PF-04015475 against several marker substrates is presented in Table 1. PF-00251802 demonstrated little or no inhibition of CYP1A2, CYP2B6, and CYP2C19 (IC$_{50}$ > 30 μM). PF-00251802 demonstrated reversible inhibition of CYP2C8, CYP2C9,
Table 1. Reversible Inhibition of CYP3A Activities in Human Liver Microsomes

| Enzyme                                | PF-00251802 Mean IC50 (μM) | PF-04015475 Mean IC50 (μM) |
|---------------------------------------|---------------------------|---------------------------|
| Felodipine/nifedipine oxidationa      | CYP3A                     | 1.3                       | >100                      |
| Midazolam 1′-hydroxylation            | CYP3A                     | 4.6                       | >100                      |
| Testosterone 6β-hydroxylation         | CYP3A                     | 1.0                       | 58                        |
| Phenacetin O-deethylase               | CYP1A2                    | >30                       | >100                      |
| Bupropion hydroxylase                | CYP2B6                    | >30                       | 49                        |
| Amiodiaquine N-deethylase/paclitaxel 6α-hydroxylase | CYP2C8                   | 0.48                      | 12                        |
| Diclofenac 4′-hydroxylase             | CYP2C9                    | 1.8                       | 21                        |
| S-mephenytoin 4′-hydroxylase          | CYP2C19                   | >30                       | 44                        |
| Dextromethorphan O-demethylase        | CYP2D6                    | 0.57                      | 39                        |

CYP, cytochrome P450; IC50, half maximal inhibitory concentration.
aPF-00251802 (dagrocorat) was tested at concentrations ranging from 0.1 to 30 μM.
bPF-04015475 was tested at a later date than PF-00251802, and some practices had changed (standard test concentrations and some marker substrates; see footnotes d and e). PF-04015475 was tested at concentrations ranging from 0.1 to 100 μM.
cAverage data (ie, percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC50 values.
dFelodipine was used in the PF-00251802 study; nifedipine was used in the PF-04015475 study.
eAmodiaquine was used as a CYP2C8 marker substrate in the PF-00251802 study; paclitaxel was used as a CYP2C8 marker substrate in the PF-04015475 study.

may be time-dependent inhibitors of CYP3A. As a result, time-dependent inhibition of the CYP isoforms was further evaluated, as shown in Table 2.

Using midazolam as a marker substrate, PF-00251802 was shown to be a time-dependent inhibitor of CYP3A at concentrations ≥ 0.08 μM, and PF-04015475 was a time-dependent inhibitor at all concentrations tested (Figure 3). A full concentration–response profile was not achievable because of solubility limitations; therefore, the K1 and k_inact could not be estimated directly from the curve fitting (data not shown). Under these circumstances, the slope of the k_obs-versus-concentration curve can be used to calculate the k_inact/K1 ratio. Using this approach, the k_inact/K1 ratios were 72 and 6.8 mL/(min·μmol) for PF-00251802 and PF-04015475, respectively. Furthermore, the k_inact Values were estimated based on the highest k_obs achieved in the experiment: approximately 0.22 min⁻¹ for PF-00251802 and 0.027 min⁻¹ for PF-04015475. The K1 values were then estimated by dividing the estimated k_inact value by the k_inact/K1 ratio, which gave estimated K1 values of 3.0 and 4.0 μM for PF-00251802 and PF-04015475, respectively (Table 2).

Using dextromethorphan as a probe substrate, PF-00251802 was shown to be a time-dependent inhibitor of CYP2D6 at concentrations ≥ 0.6 μM. The k_inact/K1 ratio was 22 mL/(min·μmol), and the estimated k_inact and K1 values were 0.013 min⁻¹ and 0.57 μM, respectively (Table 2).

In Vitro: Induction Data

The potential for PF-00251802 (dagrocorat) and PF-04015475 to induce CYP3A4 and CYP1A2 was evaluated using cultures of cryopreserved human
Table 2. Time-Dependent CYP Inhibition Data

| Compound    | Enzyme (Substrate)          | K_i (µM) | k_inact (min⁻¹) | k_inact/K_i (mL/min·µmol) | Predicted AUC Ratio (R²) |
|-------------|-----------------------------|----------|-----------------|---------------------------|--------------------------|
| PF-00251802 | CYP3A4/5 (midazolam)        | 3.0      | 0.22            | 72                        | 1.6                      |
| PF-00251802 | CYP2D6 (dextromethorphan)   | 0.57     | 0.013           | 22                        | 1.5                      |
| PF-04015475 | CYP3A4/5 (midazolam)        | 4.0      | 0.027           | 6.8                       | 1.1                      |

AUC, area under the plasma concentration–time curve; CYP, cytochrome P450; I, inhibitor concentration; K_i, apparent inactivation rate; k_inact, maximal inactivation rate; k_obs, observed rate.

*aSaturation of inhibition was not achieved because of solubility limitations. As such, the slope of the linear portion of the k_obs versus [I] curve was reported as a composite slope value (k_inact/K_i). The k_inact was then estimated from the maximum k_obs from the k_obs versus [I] curve, and K_i was calculated from K_i = maximum k_obs/composite slope (k_inact/K_i). Predicted AUC ratio (R²) represents the ratio of AUC for a sensitive CYP substrate in the presence/absence of the interacting drug, as described in the Methods section.

Figure 3. Time-dependent inhibition of CYP3A activity in pooled human liver microsomes by (A) PF-00251802 (dagrocorat) and (B) PF-04015475, using midazolam as a probe substrate.

A hepatocytes (Table 3). No induction of CYP1A2 enzyme activity was observed (data not shown). Induction of CYP3A4 mRNA was observed for both PF-00251802 and PF-04015475 for all lots tested. Induction of CYP3A4 enzyme activity was not observed, which may potentially be because of concomitant time-dependent inhibition.

EC50 and E_max values were estimated from CYP3A4 mRNA concentration–response curves (Table 3). For PF-00251802, complete concentration–response curves were not achieved because of cell viability limits at higher test concentrations; therefore, E_max and EC50 values were estimated from the observed data, without using curve fitting. The E_max was estimated based on the highest fold induction observed, and the EC50 was estimated as the concentration producing half the E_max. The E_max for PF-00251802 ranged from approximately 4.7- to 40-fold and the EC50 value from 3.0 to 5.0 µM. E_max and EC50 values for PF-04015475 were based on curve-fitting the dose–response curves. The E_max for PF-04015475 ranged from 34- to 140-fold and the EC50 value from 20 to 29 µM (Table 3). Relative to the response with the positive control rifampicin, these maximal fold induction values ranged from 21% to 54% of the rifampicin response in the same experiment.

Taken together, the results of in vitro interaction studies indicated a complex CYP3A DDI scenario, with potential for reversible inhibition, time-dependent inhibition, and induction of CYP3A mRNA. Various modeling approaches were used to estimate the potential for these in vitro results to translate to DDI in vivo, as described below.

Model-Based Prediction of DDI
To understand whether the in vitro CYP inhibition and induction results were likely to translate into clinically relevant CYP interactions, various mathematical prediction models were used. As a first approach, each
Table 3. Induction of CYP3A mRNA in Human Hepatocytes

| Compound       | Lot* | Estimated EC<sub>50</sub> (μM) | Estimated E<sub>max</sub> (Fold Induction) | E<sub>max</sub> Relative to Rifampin<sup>b</sup> (%) | R<sub>3</sub>; Predicted AUC Ratio Due to Induction<sup>c</sup> |
|----------------|------|-------------------------------|-------------------------------------------|------------------------------------------------|-------------------------------------------------|
| PF-00251802 (dagrocorat) | Lot 1, expt 1 | 3.0 | 13 | 21 | 0.98 |
|                 | Lot 1, expt 2 | 5.0 | 4.7 | 28 | 1.00 |
|                 | Lot 2, expt 1 | 3.0 | 40 | 53 | 0.95 |
|                 | Lot 2, expt 2 | 5.0 | 22 | 38 | 0.98 |
| PF-04015475 | Lot 1 | 25 | 140 | 53 | 0.97 |
|                 | Lot 2 | 20 | 34 | 42 | 0.99 |
|                 | Lot 3 | 29 | 54 | 46 | 0.99 |

AUC, area under the plasma concentration–time curve; CYP, cytochrome P450; EC<sub>50</sub>, concentration at which 50% of a maximum induction effect occurs; E<sub>max</sub>, maximum fold induction observed in vitro; mRNA, messenger RNA; R<sub>3</sub>, induction.

<sup>a</sup>Lot refers to the different sources of hepatocytes.

<sup>b</sup>Rifampin was used as a positive control at a final concentration of 25 μM in PF-00251802 (dagrocorat) experiments and 10 μM in PF-04015475 experiments.

<sup>c</sup>Predicted AUC ratio of midazolam in the presence of compound using the equation for R<sub>3</sub> described in the Methods section.

R<sub>3</sub> = 1/(1 + (E<sub>max</sub> × [I])/(EC<sub>50</sub> + [I]))

interaction component (reversible inhibition, time-dependent inhibition, and induction) was evaluated separately using basic models, and then the net effect of the 3 processes was evaluated, as described below. In general, no interaction was anticipated if the AUC ratio (R value) in the presence/absence of the interacting drug was >0.8 and <1.25. Predicted interactions were considered weak if the AUC ratio (R value) in the presence/absence of the interacting drug was ≥1.25 but <2.0, moderate if ≥2.0 but <5, and strong if ≥5.0.<sup>13</sup>

Note that unbound C<sub>max</sub> was used, rather than the total C<sub>max</sub>, to reflect the inhibitor concentration. Food and Drug Administration (FDA) guidance recommends use of total C<sub>max</sub> as a conservative starting point and then progressing to more mechanistic, physiologically based models that incorporate unbound C<sub>max</sub>. In most investigations of DDI prediction, unbound C<sub>max</sub> has been shown to produce better predictions, particularly for highly protein-bound compounds.<sup>17</sup> Therefore, unbound C<sub>max</sub> was used as a starting point in the current work, which required a more physiologically based, risk-based approach, as recommended subsequent to basic models in FDA guidance.

With respect to the reversible inhibition of CYPs, K<sub>i</sub> values were estimated as IC<sub>50</sub>/2, and compared with free plasma concentrations of PF-00251802 (dagrocorat) and PF-04015475 at C<sub>max</sub> (approximately 4.2 nM for PF-00251802 and 5.4 nM for PF-04015475, following a 25-mg dose of PF-04171327); see Methods section, equation 1. All R<sub>1</sub> values for reversible inhibition were <1.05 (not shown); hence, the potential for DDIs from reversible inhibition is considered low for all CYPs.

With respect to time-dependent inhibition of CYPs, K<sub>i</sub>, k<sub>inact</sub>, and unbound C<sub>max</sub> values were used to estimate the impact on a sensitive substrate for CYP3A or CYP2D6 using the equation for R<sub>2</sub>, as described in the Methods section. PF-00251802 was predicted to have a weak interaction with both CYP3A (R<sub>2</sub> = 1.6) and CYP2D6 (R<sub>2</sub> = 1.5) using the basic model. PF-04015475 was predicted to not have a significant inhibitory effect on CYP3A in vivo (R<sub>2</sub> = 1.1); see Table 2.

With respect to the CYP3A4 induction results, the EC<sub>50</sub> and E<sub>max</sub> values were used along with the unbound C<sub>max</sub>, as described in the Methods section, equation 3. Based on this assessment, the CYP3A4 induction observed in vitro would be expected to have minimal impact in vivo, based on R<sub>3</sub> values >0.90 for all experiments (Table 3).

Next, the net effect of reversible inhibition, time-dependent inhibition, and induction was considered, as described in the Methods section, equation 4. Using this model, the overall change in AUC of midazolam (a sensitive CYP3A substrate) following a 25-mg dose of PF-04171327 was predicted to be an approximately 1.8-fold increase, and the overall change in AUC of desipramine was predicted to be approximately 1.5-fold. These prediction results are driven by the weak time-dependent inhibition by PF-00251802.

Taken together, the results of the model-based DDI prediction suggested the potential for interaction with CYP3A substrates because of inhibition primarily driven by PF-00251802. Although the predicted interaction was considered weak inhibition, the complex DDI scenario led to a decision to conduct a clinical DDI study with the sensitive CYP3A substrate, midazolam, prior to initiating phase 2 studies.
Mean (SE) plasma PF-00251802 (dagrocorat) concentration, ng/mL

| Study day | 0  | 25 | 50 | 75 | 100 | 125 |
|-----------|----|----|----|----|-----|-----|

Figure 4. Mean plasma PF-00251802 (dagrocorat) trough concentration over time in patients enrolled in the phase 1 study. Arrows indicate midazolam dosing.

Mean (SE) plasma midazolam concentration, ng/mL

Nominal time post-dose, hours

Treatment:
- Midazolam 2 mg
- Midazolam 2 mg + PF-04171327 (fosdagrocorat) 25 mg

Figure 5. Mean plasma concentration–time profile for midazolam ± PF-04171327 (fosdagrocorat).

Phase 1 Study in Healthy Volunteers
Twelve healthy volunteers were enrolled and assigned to study treatment. All subjects were male, with a mean (range) range of 41.6 years (23–55 years). Mean (range) BMI and weight were 25.5 kg/m² (23.0–28.8 kg/m²) and 81.5 kg (62.0–98.0 kg), respectively. Six subjects (50%) were white, 4 (33%) were black, and 2 (17%) were Asian/other. All subjects completed the study and were included in the PK and safety analyses.

PK Analyses. The mean trough concentration–time of PF-00251802 (dagrocorat) is shown in Figure 4. The mean plasma midazolam concentration–time profiles for midazolam alone (reference treatment) and in combination with PF-04171327 (test treatment) are shown in Figure 5. The rate of midazolam absorption was consistent between administration of midazolam with and without PF-04171327. Table 4 presents a summary of PK parameters for midazolam when administered alone and in combination with PF-04171327. Median $T_{\text{max}}$ was approximately 0.50 hours postdose for each treatment. Co-administration of midazolam with PF-04171327 resulted in a 4.20% decrease in $\text{AUC}_{\text{inf}}$ and a 10.34% decrease in $C_{\text{max}}$ versus midazolam alone. The test/reference ratios of the adjusted geometric means (90% CIs) were 104.62% (88.61%–123.53%) for $\text{AUC}_{\text{inf}}$, 105.20% (90.53%–122.25%) for $\text{AUC}_{\text{last}}$, and 92.48% (82.75%–103.34%) for $C_{\text{max}}$. All CIs were within established equivalence limits of 80% and 125%. The test/reference ratios of arithmetic means (Table 4) for $\text{AUC}_{\text{inf}}$, $\text{AUC}_{\text{last}}$ and $C_{\text{max}}$ were similar.

Safety. Seven subjects experienced ≥1 AE. Following treatment with PF-04171327 alone, treatment-emergent AEs (TEAEs) considered related to treatment were insomnia (2 subjects), constipation, dry mouth, dry lips, nausea, paresthesia, feeling hot, feeling of body temperature change, tremor, and blood cortisol decreased (1 subject each). No TEAEs were reported following treatment with midazolam only. Treatment-related TEAEs reported following treatment with midazolam in combination with PF-04171327 were constipation, dry lips, and paresthesia (1 subject each). All TEAEs were mild in severity.

No discontinuations or dose reductions from an AE, SAEs, or deaths occurred. No clinically significant abnormalities in laboratory values, vital signs, or ECG recordings were reported.

Discussion
The investigational DAGR compound PF-00251802 (dagrocorat) and its major metabolite PF-04015475 were initially evaluated in this study for potential interactions with CYP enzymes in vitro, consistent with the recommendations of regulatory authorities to elucidate potential drug interactions during the drug development process.13,14 Preliminary findings from in vitro experiments in human liver microsomes demonstrated that both PF-00251802 and PF-04015475 were reversible inhibitors of multiple CYP enzymes in vitro, including CYP3A, with PF-00251802 being a more potent inhibitor of each CYP enzyme tested. PF-00251802 and PF-04015475 both caused time-dependent inhibition of CYP3A in human liver microsomes and induction of CYP3A4 mRNA in human hepatocytes in vitro.

Consequently, modeling approaches were used to predict the potential clinical impact of DDIs with PF-00251802 and PF-04015475, with predicted interactions considered weak if the AUC ratio (R value) in the presence/absence of the interacting drug was ≥1.25 but <2.0, moderate if ≥2.0 but <5, and strong if ≥5.0.13 Results of modeling suggested potential for weak inhibition of CYP3A and CYP2D6 in vivo, driven primarily by time-dependent inhibition by PF-00251802. Results of the modeling also suggested that interactions from reversible inhibition of CYPs or induction of CYP3A4 were unlikely. The predicted lack of effect is a result of the low unbound drug concentrations of...
Table 4. Effect of PF-04171327 (Fosdagrocorat) on Pharmacokinetics of Midazolam, Mean (SD)

| Parameters (Unit) | Midazolam 2 mg (n = 12) | Midazolam 2 mg + Fosdagrocorat 25 mg (n = 12) |
|-------------------|--------------------------|-----------------------------------------------|
| AUC_{int} (ng·h/mL) | 21.17 (12.1)\(^a\) | 20.28 (6.2) |
| AUC_{last} (ng·h/mL) | 20.53 (11.0) | 19.84 (6.0) |
| C_{max} (ng/mL) | 8.03 (2.61) | 7.20 (1.66) |
| T_{max}\(^b\) (h) | 0.50 (0.50–1.02) | 0.50 (0.50–1.02) |
| t_{1/2} (h) | 3.87 (1.97)\(^a\) | 3.34 (1.53) |

AUC, area under the plasma concentration–time curve; AUC_{int}, AUC from time 0 extrapolated to infinity; AUC_{last}, AUC from time 0 to the time of last quantifiable concentration; C_{max}, maximum observed plasma concentration during the dosing interval; T_{max}, time to obtain C_{max}; T_{last}, time of last quantifiable concentration; t_{1/2}, terminal elimination half-life.

\(^a\)n = 11.

\(^b\)Median (range).

Both PF-00251802 and PF-04015475 at clinically relevant doses.

Although the predicted effect of PF-00251802/PF-04015475 on CYP activity in vivo was relatively weak, there was uncertainty associated with the prediction approaches because of the complexity of the in vitro interactions. More experience and confidence have been gained with prediction approaches for compounds that are either CYP inhibitors or inducers\(^18\) than for those that have a combination of effects. In the current case, there was a combination of time-dependent inhibition and induction for both parent and metabolite molecules. The complexity in prediction led to the decision to conduct a clinical DDI study using a sensitive substrate for CYP3A.

The results of the clinical DDI study between PF-04171327 and midazolam indicated a lack of effect of multiple-dose PF-04171327 on midazolam PK, with 90% CIs for the ratios of geometric means for AUC_{int}, AUC_{last}, and C_{max} within the accepted limits for demonstration of equivalence. These findings support those from a previous phase 1 multiple-dose study of PF-04171327 in healthy volunteers that suggested no in vivo CYP3A interaction based on the lack of observed autoinduction.\(^4\)

The dose of PF-04171327 selected for this study (25 mg once daily) represents the highest PF-04171327 dose currently planned for future clinical studies. The duration of PF-04171327 dosing prior to concomitant midazolam administration (14 days) was based on the time to achieve apparent steady-state concentrations of PF-00251802 in the multiple-dose study (12 days). In addition, the 14-day dosing duration maximized the potential time-dependent effects on the inhibition of CYP3A4 in vivo. The single midazolam dose of 2 mg provided sufficient exposure to characterize midazolam PK in the absence of PF-00251802, while avoiding saturation of first-pass extraction and minimizing adverse effects of midazolam if inhibition of midazolam clearance was observed in the presence of PF-00251802.

The apparent absence of an effect on midazolam PK by PF-04171327 suggests that although PF-00251802 interacts with CYP3A in vitro, this does not translate to the in vivo setting. The reversible inhibition and induction that were observed in vitro were not predicted to translate to an in vivo effect based on modeling of the unbound concentrations of the drug. However, the time-dependent inhibition was predicted to translate to weak inhibition in vivo. The overprediction of DDIs because of time-dependent inhibition is not unusual,\(^18\) and an internal analysis of prediction accuracy for time-dependent inhibition led to a decision to use a less conservative estimate of [I], that is, use of unbound C_{max} rather than total C_{max} in prediction models. In addition, a less conservative estimate of k_{deg} was used. However, even with these less conservative estimates, DDIs were still somewhat overpredicted. Use of unbound average concentration (C_{avg}) would have improved the prediction accuracy. However, this is likely not the only factor influencing the overprediction of the interaction.

The general approach to clinical DDI assessment is to conduct a DDI study using a sensitive substrate for the CYP enzyme predicted to be the most significantly impacted by the test drug, based on in vitro data. If this clinical DDI study shows no or little interaction, then it is presumed that there will be no interaction with less sensitive substrates or with substrates for CYP enzymes that were less impacted in vitro. Based on the in vitro studies with PF-00251802 and PF-04015475, the only interaction that was predicted to translate to a clinical interaction was the time-dependent inhibition with CYP3A and CYP2D6. Because the CYP3A interaction was predicted to be more significant than the CYP2D6 interaction, the clinical DDI study was run with midazolam. It was then presumed that because no interaction was observed with a sensitive CYP3A substrate, then no interaction would be observed with a CYP2D6 substrate or with substrates for any of the other CYPs.

Coadministration of PF-04171327 and midazolam at the doses given in this study demonstrated an
acceptable safety profile. No SAEs or deaths were reported, and all AEs were mild in severity. No clinically significant changes were observed in any laboratory parameters or vital signs. These safety and PK findings are consistent with those from a multiple-dose study in healthy volunteers.4

Approximately 30% of clinically used drugs are predominantly metabolized by the CYP3A enzymes,12 and it is therefore important to rule out any drug interaction with CYP3A substrates or inhibitors. The findings at steady state presented here provide support for the development of PF-04171327 as a treatment for RA. Results from a recent phase 2 randomized, double-blind study of PF-04171327 (10 and 25 mg) in patients with RA demonstrated an acceptable safety profile and superior efficacy relative to placebo and comparable efficacy relative to prednisone in improving signs and symptoms of RA in week 8.25 Additional studies of the long-term safety and efficacy of PF-04171327 in patients with RA are awaited with interest.

Conclusion

In this study, PF-00251802 (dagrocorat) and PF-0015475, reversible inhibitors of CYP enzymes, caused time-dependent inhibition of CYP3A in human liver microsomes and/or induction of CYP3A4 mRNA in human hepatocytes in vitro. Results of model-based predictions suggested no impact from reversible inhibition or induction in vivo, but the potential for weak time-dependent inhibition of CYP3A and CYP2D6 in vivo.

Results of a phase 1 open-label, multiple-dose DDI study conducted to evaluate the effect of PF-04171327 (fosdagrocorat) on midazolam PK and safety in healthy volunteers indicated a lack of interaction with CYP3A. These findings supported the further clinical development of PF-00251802 and its prodrug PF-04171327 as potential treatments for patients with RA.

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Declaration of Conflicting Interests

S.L. Ripp, A. Mukherjee, H. Eng, T. Stock, D. Fleishaker, and B. Tammara are employees and shareholders of Pfizer Inc. T. Checchio was an employee and shareholder of Pfizer Inc at the time of the study.

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References

1. Bijlsma JW. Disease control with glucocorticoid therapy in rheumatoid arthritis. Rheumatology (Oxford). 2012;51(suppl 4):iv9–iv13.
2. Bijlsma JW, Jacobs JW. Glucocorticoids in the treatment of rheumatoid arthritis: still used after 65 years. Ann NY Acad Sci. 2014;1318:27–31.
3. Kavanaugh A, Wells AF. Benefits and risks of low-dose glucocorticoid treatment in the patient with rheumatoid arthritis. Rheumatology (Oxford). 2014;53(10):1742–1751.
4. Stock T, Fleishaker D, Mukherjee A, Le V, Xu J, Mebus C. Evaluation of the safety, pharmacokinetics (PK), and pharmacodynamics (PD) of a dissociated agonist of the glucocorticoid receptor (DAGR), in healthy volunteers [abstract]. Ann Rheum Dis. 2010;69:218.
5. Hu X, Du S, Tunca C, et al. The antagonists but not partial agonists of glucocorticoid receptor ligands show substantial side effect dissociation. Endocrinology. 2011;152(8):3123–3134.
6. Liberman AC, Antunica-Noguerol M, Ferraz-de-Paula V, et al. Compound A, a dissociated glucocorticoid receptor modulator, inhibits T-bet (Th1) and induces GATA-3 (Th2) activity in immune cells. PLoS One. 2012;7(4):e35155.
7. Harcken C, Riether D, Kuzmich D, et al. Identification of highly efficacious glucocorticoid receptor agonists with a potential for reduced clinical bone side effects. J Med Chem. 2014;57(4):1583–1598.
8. Schäcke H, Rehwinkel H, Asadullah K. Dissociated glucocorticoid receptor ligands: compounds with an improved therapeutic index. Curr Opin Investig Drugs. 2005;6(5):503–507.
9. Takahashi H, Razavi H, Thomson D. Recent progress in the discovery of novel glucocorticoid receptor modulators. Curr Top Med Chem. 2008;8(6):521–530.
10. Conrado DJ, Krishnaswami S, Shoji S, et al. Predicting the probability of successful efficacy of a dissociated agonist of the glucocorticoid receptor from dose-response analysis. J Pharmacokinet Pharmacodyn. 2016;43(3):325–341.
11. Miyoshi S, Hey-Hadavi J, Nagaoka M, Tammara B. Pharmacokinetics and food effect of fosdagrocorat (PF-04171327), a dissociated agonist of glucocorticoid
11. Ripp et al. Clinical net drug-drug interaction based on CYP3A4 inhibition, inactivation, and induction determined in vitro. Drug Metab Dispos. 2016;36(6):966–976.
12. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther. 2013;138(1):103–141.
13. Food and Drug Administration. Guidance for Industry: Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations. 2012. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm292362.pdf.
14. European Medicines Agency CIIHMP. Guidance on the investigation of drug interactions. 2012. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf.
15. Yates P, Eng H, Di L, Obach RS. Statistical methods for analysis of time-dependent inhibition of cytochrome p450 enzymes. Drug Metab Dispos. 2012;40(12):2289–2296.
16. Fahmi OA, Kish M, Boldt S, Obach RS. Cytochrome P450 3A4 mRNA is a more reliable marker than CYP3A4 activity for detecting pregnane X receptor-activated induction of drug-metabolizing enzymes. Drug Metab Dispos. 2010;38(9):1605–1611.
17. Fahmi OA, Hurst S, Plowchalk D, et al. Comparison of different algorithms for predicting clinical drug-drug interactions, based on the use of CYP3A4 in vitro data: predictions of compounds as precipitants of interaction. Drug Metab Dispos. 2009;37(8):1658–1666.
18. Vieira ML, Kirby B, Ragueneau-Majlessi I, et al. Evaluation of various static in vitro-in vivo extrapolation models for risk assessment of the CYP3A inhibition potential of an investigational drug. Clin Pharmacol Ther. 2014;95(2):189–198.
19. Fahmi OA, Maurer TS, Kish M, Cardenas E, Boldt S, Nettleton D. A combined model for predicting CYP3A4 clinical net drug-drug interaction based on CYP3A4 inhibition, inactivation, and induction determined in vitro. Drug Metab Dispos. 2008;36(8):1698–1708.
20. Wang YH. Confidence assessment of the Simcyp time-based approach and a static mathematical model in predicting clinical drug-drug interactions for mechanism-based CYP3A inhibitors. Drug Metab Dispos. 2010;38(7):1094–1104.
21. Rowland Yeo K, Walsky RL, Jamei M, Rostami-Hodjegan A, Tucker GT. Prediction of time-dependent CYP3A4 drug-drug interactions by physiologically based pharmacokinetic modelling: impact of inactivation parameters and enzyme turnover. Eur J Pharm Sci. 2011;43(3):160–173.
22. Weatherley B, McFadyen L, Conrado D, Tammar A. The Population Pharmacokinetics of Active Metabolites of a prodrug PF-0417132, (Dissociated Agonist of Glucocorticoid Receptor), in Rheumatoid Arthritis subjects [abstract]. Abstract presented at PAGE 24. 2015. www.page-meeting.org/default.asp?abstract=3381.
23. Haupt LJ, Kazmi F, Ogilvie BW, et al. The reliability of estimating Ki Values for direct, reversible inhibition of cytochrome P450 enzymes from corresponding IC50 values: a retrospective analysis of 343 experiments. Drug Metab Dispos. 2015;43(11):1744–1750.
24. Venkatakrishnan K, Obach RS. In vitro-in vivo extrapolation of CYP2D6 inactivation by paroxetine: prediction of nonstationary pharmacokinetics and drug interaction magnitude. Drug Metab Dispos. 2005;33(6):845–852.
25. Stock T, Fleishaker D, Wang X, Mukherjee A, Mebus C. Improved disease activity with fosdagrocorat (PF-04171327), a partial agonist of the glucocorticoid receptor, in patients with rheumatoid arthritis: a phase 2 randomized study. Int J Rheum Dis. 2017;20(8):960–970.