

# Novel Homodimer Metabolites of GDC-0994 via Cytochrome P450-Catalyzed Radical Coupling<sup>[S]</sup>

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## ABSTRACT

Two novel homodimer metabolites were identified in rat samples collected during the in vivo study of GDC-0994. In this study, we investigated the mechanism of the formation of these metabolites. We generated and isolated the dimer metabolites using a biomimetic oxidation system for NMR structure elucidation to identify a symmetric dimer formed via carbon-carbon bond between two pyrazoles and an asymmetric dimer formed via an aminopyrazole-nitrogen to pyrazole-carbon bond. In vitro experiments demonstrated formation of these dimers was catalyzed by cytochrome P450 enzymes (P450s) with CYP3A4/5 being the most efficient. Using density functional theory, we determined these metabolites share a mechanism of formation, initiated by an N-H hydrogen atom abstraction by the catalytically active iron-oxo of P450s. Molecular modeling studies also show these dimer metabolites fit in the CYP3A4 binding

site in low energy conformations with minimal protein rearrangement. Collectively, the results of these experiments suggest that formation of these two homodimer metabolites is mediated by CYP3A, likely involving activation of two GDC-0994 molecules by a single P450 enzyme and proceeding through a radical coupling mechanism.

## SIGNIFICANCE STATEMENT

These studies identified structures and enzymology for two distinct homodimer metabolites and indicate a novel biotransformation reaction mediated by CYP3A. In it, two molecules may bind within the active site and combine through radical coupling. The mechanism of dimerization was elucidated using density functional theory computations and supported by molecular modeling.

## Introduction

GDC-0994 (structure shown in Fig. 1A) is a selective, potent, orally bioavailable, small molecule inhibitor of extracellular signal-regulated kinase (ERK)1 and ERK2. It has proven antitumor activity in RAS- and RAF-mutant human tumor cell-line models in vitro and in vivo (Blake et al., 2016). Based on its mechanism of action and the high frequency of mitogen-activated protein kinase pathway activation in human cancers, GDC-0994 was in development as an anticancer therapeutic with potential in a broad array of cancer indications (Ren et al., 2015; Kirouac et al., 2017) including its clinical testing in patients with locally advanced or metastatic solid tumors (Varga et al., 2020). While thoroughly evaluating the metabolic profile of GDC-0994 in rats, the nonclinical toxicological species that supported entry into clinical development, we identified two novel dimer metabolites (**M13** and **M14**) that formed via cytochrome P450 (P450) oxidation.

P450 enzymes catalyze a wide range of reactions for the metabolism of xenobiotics. The most common reactions include carbon hydroxylation, heteroatom oxygenation, dealkylation, and epoxidation (Ortiz de Montellano and De Voss, 2005). However, a variety of uncommon reactions by P450s have been observed. These reactions are of particular interest because their products are often not easily predicted (Guengerich and Munro, 2013).

From a research perspective, these case examples provide interesting opportunities to probe the diversity of P450 function. From a drug discovery and development perspective, an unexpected metabolite adds to uncertainties and associated risks of a candidate molecule upon its first administration to human subjects (Schadt et al., 2018). Therefore, understanding reaction mechanisms, structural requirements, and specific isoform(s) responsible for these reactions is critical to anticipate and mitigate metabolic liabilities.

Of the P450 isoforms found in humans, CYP3A4 is most frequently identified as the principal metabolizing enzyme of approved drugs (Cerny, 2016). CYP3A4 has a large and flexible active site that accommodates a variety of ligands, including large substrates such as cyclosporine A (molar mass 1203 g/mol). Understanding the diversity of reactions that CYP3A4 can catalyze provides a foundation to understand the breadth of possible metabolic products it can generate and feed back to the design and optimization of new molecular entities.

In this report, we describe the identification and structure determination of two distinct homodimers of GDC-0994 and provide in vitro evidence that CYP3A catalyzed their formations. We also embarked on a computational study using density functional theory (DFT) to determine the likely mechanism of dimer formation and molecular modeling to evaluate the feasibility of dimer formation within CYP3A4.

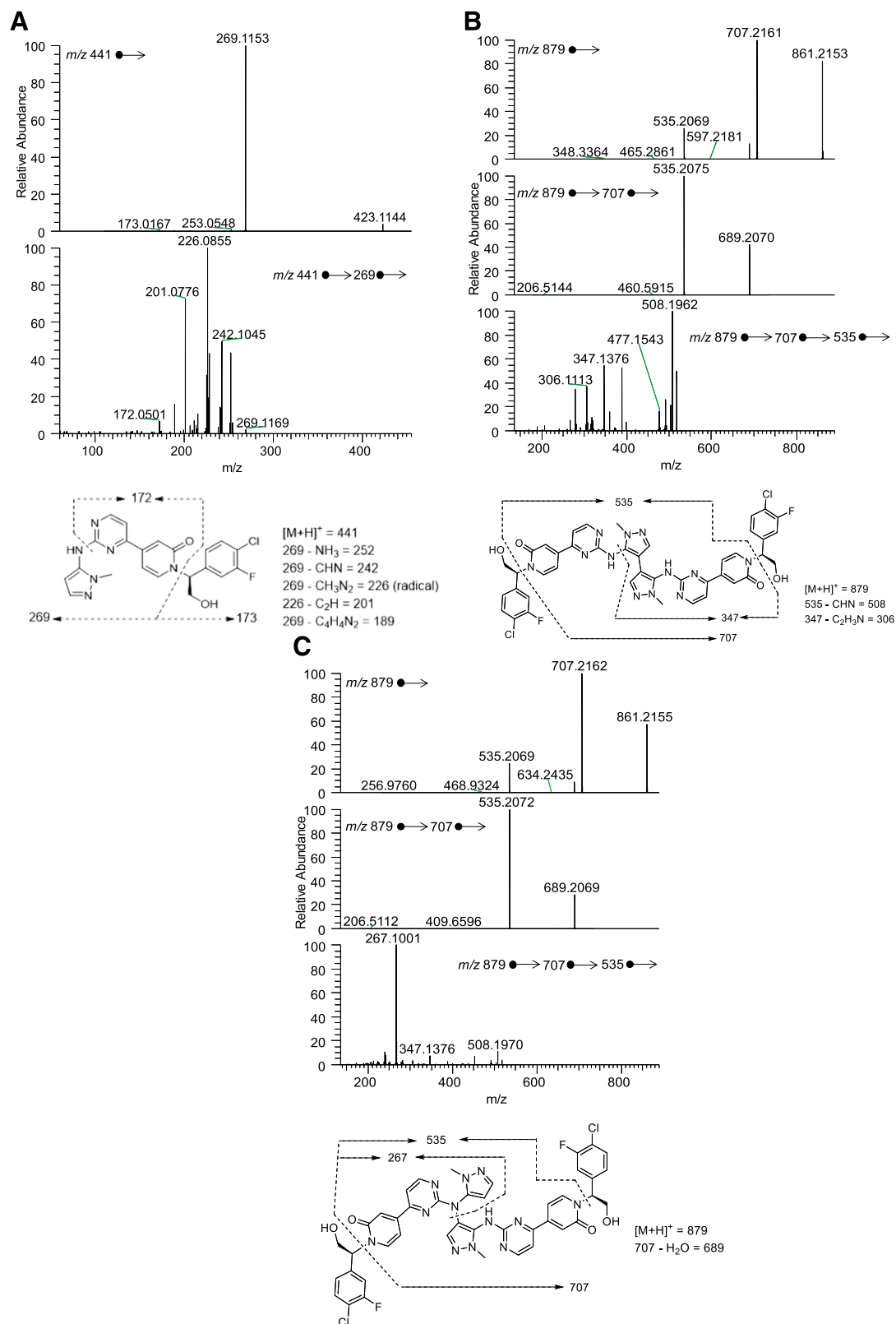
## Materials and Methods

**Chemicals and Reagents.** [<sup>14</sup>C]GDC-0994 was synthesized by Selcia Limited (Essex, UK) with radiochemical purity >98.5% (specific activity 52.23 mCi/mmol), and nonradiolabeled GDC-0994 was synthesized at Genentech

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**Fig. 1.** MS<sup>2</sup> and MS<sup>n</sup> product ion spectra of (A) GDC-0994, (B) M13, and (C) M14.

(South San Francisco, CA). Liver microsomes and recombinant P450 enzymes were purchased from BD Gentest (San Jose, CA) or Bioreclamation IVT (Westbury, NY). Horseradish peroxidase was obtained from Sigma-Aldrich (St Louis, MO). Myeloperoxidase from human leukocytes was purchased from EMD Millipore Co. (Temecula, CA). All chemical reagents and solvents were purchased from commercial sources at the highest purities possible.

**In Vivo Metabolite Identification Study.** Male and female Sprague-Dawley rats were orally administered a single dose of [ $^{14}\text{C}$ ]GDC-0994 at 50 mg/kg (100  $\mu\text{Ci/kg}$ ) in 1% carboxymethylcellulose, 0.5% Tween, and 5 mM citrate solution. In vivo study design, sample collection, and radioanalysis are described in Supplemental Materials. Metabolite profiling and identification was performed using liquid chromatography coupled with simultaneous radiodetection and high-resolution mass spectrometric analysis. Method details are also provided in Supplemental Materials.

**Metabolite Generation Using Biomimetic Enzymes.** BMO Production Kit (HCK1001-03-027-A7-10) (HepatoChem Inc., Beverly, MA) was used to generate, scale up, and isolate two metabolites of interest, **M13** and **M14**. Standard protocols provided by the vendor were followed for screening, optimization, and production of metabolites. The incubations were performed with 6.25 mM of GDC-0994 free base. The dimer metabolites were purified by semipreparative liquid chromatography using a Hypersil Gold C18 column (150  $\times$  10 mm, 5  $\mu\text{m}$  particle size; Thermo Scientific, San Jose, CA) with mobile phases of 0.1% formic acid in water and acetonitrile at a constant flow rate of 4.6 ml/min. The incubation products were separated by gradient elution, and fractions were collected and surveyed by liquid chromatography–mass spectrometry for the analytes of interest. The fractions containing **M13** and **M14** were then separately combined and evaporated to dryness under vacuum.

**Structure Determination by NMR.** All NMR experiments were collected on an Avance II spectrometer (Bruker BioSpin, Billerica, MA) operating at 500 MHz for  $^1\text{H}$ , 125 MHz for  $^{13}\text{C}$  equipped with 5 mm TCI cryoprobe with Z-gradient. Each metabolite sample was dissolved in 180  $\mu\text{l}$  of DMSO- $d_6$  (Cambridge Isotope Laboratories, Tewksbury, MA). One-dimensional  $^1\text{H}$  and  $^{13}\text{C}$  NMR were collected at ambient temperature and 335 K, respectively. Chemical shifts were referenced to tetramethylsilane at  $\delta = 0$  ppm. Two-dimensional correlation spectroscopy, heteronuclear single quantum correlation spectroscopy, heteronuclear multiple bond correlation spectroscopy, and rotating frame nuclear Overhauser effect spectroscopy were collected at 335 K.

**In Vitro Metabolism.** GDC-0994 (10  $\mu\text{M}$ ) was incubated with liver microsomes (male rat, female mouse, male beagle dog, male cynomolgus monkey, or 200 mixed sex donor pooled human, 0.5 mg/ml) in potassium phosphate buffer (100 mM, pH 7.4) containing magnesium chloride (3 mM) supplemented with 1 mM NADPH in an incubation volume of 0.5 ml. Incubations with individually expressed recombinant P450 enzymes (Super-somes) were completed similarly with GDC-0994 (1  $\mu\text{M}$ ) and a constant amount of P450 (40 pmol/ml). Inhibition experiments were conducted with human liver microsomes using standard concentrations of P450 isoform-selective chemical inhibitors: 1-aminobenzotriazole (ABT; 1 mM, pan-P450), furafylline (10  $\mu\text{M}$ , CYP1A2), 2-phenyl-2-(1-piperidinyl)propane (20  $\mu\text{M}$ , CYP2B6), montelukast (2  $\mu\text{M}$ , CYP2C8), sulfaphenazole (10  $\mu\text{M}$ , CYP2C9), 3-benzylirin (2  $\mu\text{M}$ , CYP2C19), quinidine (1  $\mu\text{M}$ , CYP2D6), ketoconazole (1  $\mu\text{M}$ , CYP3A4), or troleanomycin (20  $\mu\text{M}$ , CYP3A4). In the incubations with ABT and troleanomycin, human liver microsomes were preincubated with inhibitors for 15 minutes before GDC-0994 was added to the incubations. At the end of incubation, reactions were stopped, and proteins were removed by adding acetonitrile and centrifuging.

GDC-0994 (100  $\mu\text{M}$ ) was incubated with horseradish peroxidase (40 U/ml), NaCl (150 mM), and  $\text{H}_2\text{O}_2$  (100  $\mu\text{M}$ ) in 100 mM phosphate buffer pH 7.4 at 37°C for 1 hour. GDC-0994 (100  $\mu\text{M}$ ) was separately incubated with myeloperoxidase (0.1 mg/ml), NaCl (150 mM), and  $\text{H}_2\text{O}_2$  (100  $\mu\text{M}$ ) in 100 mM phosphate buffer pH 6.0 at 37°C for 1 hour. Reactions were quenched with three volumes of acetonitrile. The supernatant was diluted with three volumes of water and injected for liquid chromatography–mass spectrometry analysis.

**Computational Methods.** All DFT computations were performed using Gaussian09 (Frisch et al., 2013). Geometry optimizations and frequency calculations were performed at the B3LYP (Becke, 1993) level using LANL2DZ for Fe and 6-31G(d) on all other atoms. Frequencies were used to check for the presence of a local minimum (zero negative frequencies) or a transition structure (exactly one negative frequency, corresponding to the desired transformation).

Frequencies were also used to compute enthalpy and free energy corrections at a standard state of 1 atm and 298.15 K. An additional correction for entropy was used to apply a quasiharmonic approximation, as discussed by Truhlar (Zhao and Truhlar, 2008; Ribeiro et al., 2011). Single point energy calculations were computed for each structure at the B3LYP-D3(BJ) (Grimme et al., 2011) level using LANL2DZ for iron and 6-311+G(d,p) on all other atoms and the SMD (Marenich et al., 2009) implicit solvent models for water.

**Molecular Modeling.** Molecular modeling studies were performed using MOE (Chemical Computing Group ULC, 2018) with the MMFF94x forcefield (Halgren, 1996a,b,c,d; Halgren and Nachbar, 1996; Halgren, 1999a,b), and docking was performed with Glide (Schrödinger LLC, 2016). Conformational analyses of the dimer structures were performed with the sdfMMConfAnalysis module of the Chemlot software package (Lee et al., 2017).

## Results

**Identification of Metabolites.** GDC-0994 was extensively metabolized in rats (Supplemental Fig. 1). A total of 14 metabolites were tentatively identified (Supplemental Fig. 2), primarily consisting of oxidation, *N*-dealkylation, glucuronidation, or a combination of these reactions. Two distinct radiopeaks, **M13** and **M14**, which eluted later than unchanged GDC-0994, were observed in bile and feces profiles (Supplemental Fig. 1). The protonated molecular ions observed in **M13** and **M14** were identical [ $m/z$  879.2343,  $\text{C}_{42}\text{H}_{35}\text{N}_{12}\text{O}_4\text{Cl}_2\text{F}_2$  ( $-0.1$  ppm)], which corresponded to two molecules of GDC-0994 connected by loss of two hydrogens, suggesting they are homodimer metabolites. The  $\text{MS}^2$  and  $\text{MS}^n$  product ion spectra of GDC-0994, **M13**, and **M14** are shown in Fig. 1. **M13** and **M14** shared highly similar  $\text{MS}^2$  and  $\text{MS}^3$  fragmentation spectra (Fig. 1, B and C), which showed product ions at  $m/z$  707 (loss of chloro fluoro phenyl ethanol),  $m/z$  689 (loss of water from  $m/z$  707), and  $m/z$  535 (loss of chloro fluoro phenyl ethanol from  $m/z$  707). However, their  $\text{MS}^4$  spectra were dramatically different, implying the connectivity of the dimers was the structural distinction between **M13** and **M14**. Heterodimers composed of 1 molecule of GDC-0994 and 1 molecule of the *N*-desmethyl metabolite (**M12**) were only detected in bile by mass spectrometry in significantly lower quantities than **M13** or **M14**. Homodimers of **M12** were not detected in any matrices, presumably due to its low overall concentration.

**Dimer Metabolite Generation and Structure Elucidation.** To fully characterize the structures of the proposed GDC-0994 dimers, we attempted to scale up the metabolites with various in vitro systems. The product yields in microsomal or expressed P450 incubations were too low to efficiently obtain sufficient amounts of product. Thus, metabolite generation was surveyed and scaled using organometallic catalysis purchased from HepatoChem. This tool provides biomimetic catalysts to mimic the suite of P450 enzymes present in human hepatocytes, offering the researcher a unique approach to quickly find reaction conditions that generate target drug metabolites and scale them to provide sufficient quantities for structural characterization or pharmacological potency testing (Chen and White, 2007; Cusack et al., 2013). From the biomimetic screening panel, **M13** and **M14** were generated to greatest extent by metalloporphyrin FeTDCIPP and  $\text{H}_2\text{O}_2$  as oxidant. This catalytic system was used to generate sufficient quantities of metabolites for isolation by liquid chromatography and fractionation for definitive structure characterization by NMR.

Based on NMR studies, **M13** is a symmetrical dimer, whereas **M14** is an asymmetric dimer of GDC-0994 (Fig. 2). At room temperature, proton NMR spectra of both **M13** and **M14** showed broad resonances due to restricted rotation. Thus, both **M13** and **M14** were heated to 335 K to obtain well resolved one-dimensional proton NMR and two-dimensional data. Proton NMR of **M13** gave only one set of resonances, which was comparable to those observed for GDC-0994. The distinct differences were that one pyrazole proton H16 at 6.3 ppm was missing

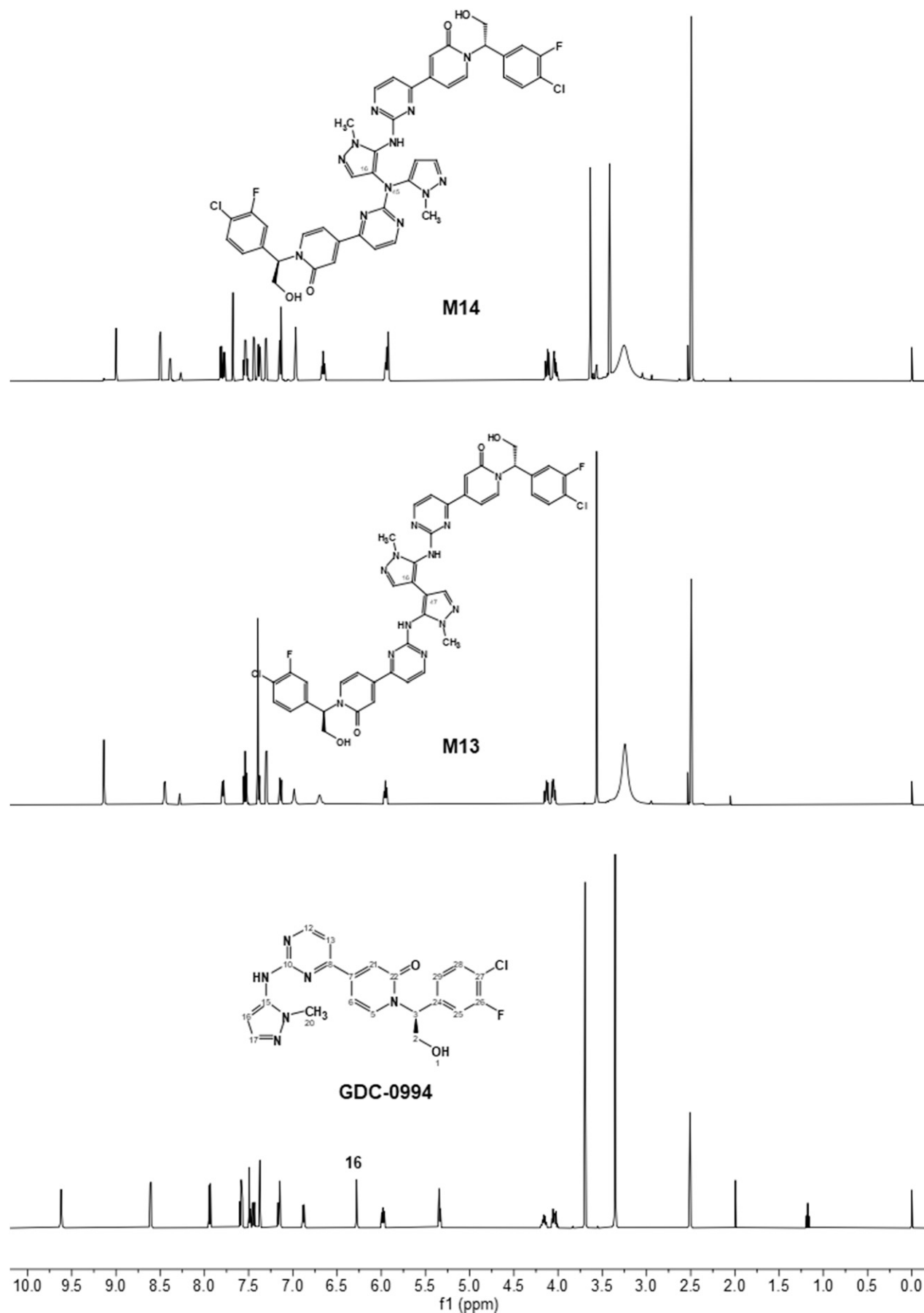
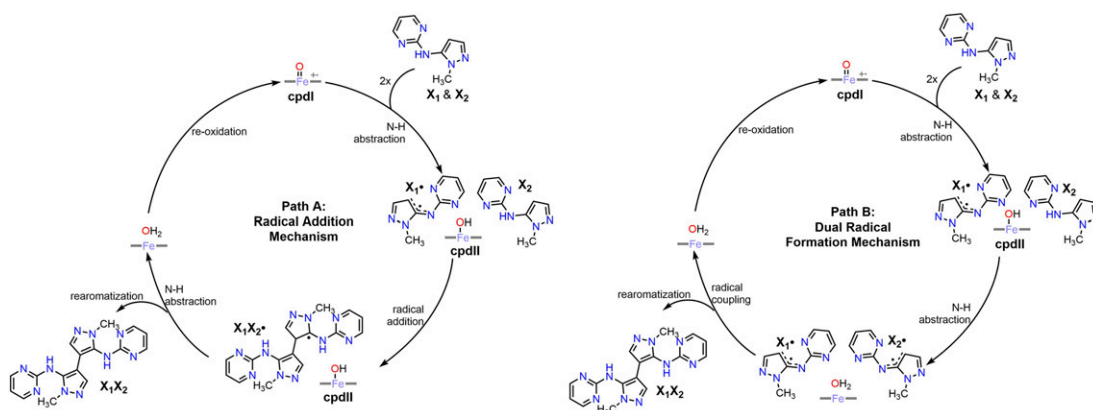


Fig. 2. Proton nuclear magnetic resonance spectroscopy data of GDC-0994 and its homodimer metabolites M13 and M14.



**Fig. 3.** Possible catalytic cycles for formation of C-C dimer and C-N dimer (only formation of C-C dimer is shown for simplicity). Pathway A, left; Pathway B, right. **X** (**X1** and **X2**) is a small model of GDC-0994 used for computational purposes.

and the other pyrazole proton H17 became singlet by losing coupling with H16. There was also an extra quaternary carbon on carbon-13 NMR of **M13**. These structural features were consistent with two GDC-0994 molecules forming a symmetrical dimer at C16 position. The new carbon-carbon bond formed in **M13** was labeled as C16-C47. For **M14**, proton NMR gave two sets of resonances consistent with GDC-0994 with two resonances missing, one pyrazole proton H16 from one GDC-0994 molecule, and H45 from N-H of another GDC-0994 molecule. This evidence concludes that a carbon-nitrogen bond was formed between two GDC-0994 molecules to give an asymmetric dimer with same molecular weight as **M13**. The new carbon-nitrogen bond formed in **M14** was labeled as C16-N45.

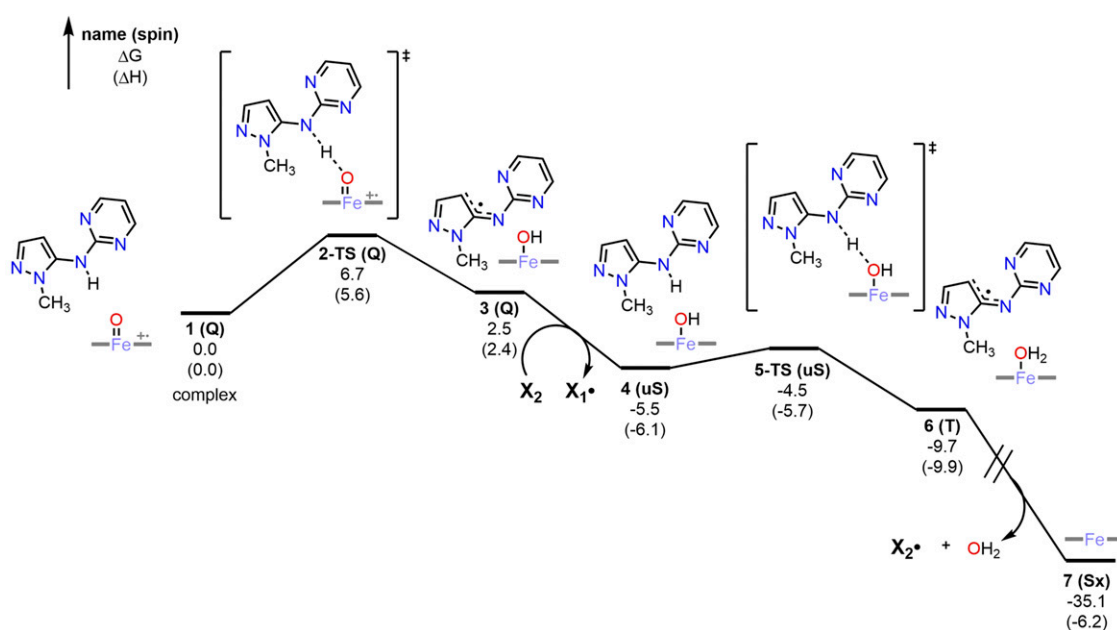
**In Vitro Formation of Dimer Metabolites.** In vitro studies demonstrated dimer formation with liver microsomal incubations for rats, mice, rabbits, dogs, monkeys, and humans, albeit all to low extent. With human liver microsomes, the formations of the dimers were time and NADPH dependent and were abolished when P450 enzymes were inactivated by preincubating with ABT or ketoconazole, pan-P450 and CYP3A inhibitors, respectively (Supplemental Fig. 3). Incubations with

singly expressed recombinant P450s showed CYP3A4 and 3A5 were the most productive in generating **M13** and **M14**.

Incubations of GDC-0994 with horseradish peroxidase in the presence of  $\text{Cl}^-$  and  $\text{H}_2\text{O}_2$  produced both **M13** and **M14** with less than 5% yield (Supplemental Fig. 4), providing evidence of a radical initiation of the mechanism. Dimers were not detected in the incubations of GDC-0994 with myeloperoxidase.

**Mechanism of Dimer Metabolite Formation.** Density functional theory was used to computationally evaluate two mechanisms of dimer formation (Fig. 3, Pathways A and B), using a truncated model of GDC-0994 (**X**). Computations show that the preferred mechanism (Fig. 4) is Pathway B, involving dual radical formation followed by radical coupling. Pathway A is disfavored due to the high energetics of radical addition to a neutral monomer (Fig. 5).

Docking of either dimer product (**M13** or **M14**) to CYP3A4 (Protein Data Bank code 6BD5) did not yield reasonable substrate poses. Suspecting the protein conformation captured in the crystal structure does not adequately capture the flexibility of the binding pocket, we built custom models for each dimer-protein complex using a protocol similar



**Fig. 4.** Computed mechanism for sequential N-H abstraction (Pathway B in Fig. 3B). Lowest energy spin state for each step is indicated in parentheses next to the name. Spin state abbreviations: Q, quartet; Sx, sextet; T, triplet; uS, open-shell singlet.



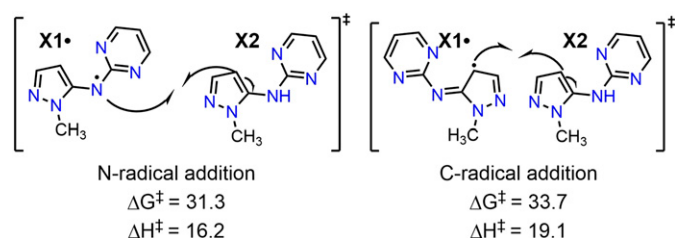


Fig. 5. Barriers to N-radical addition (left) and C-radical addition (right).

to that described by Hayes et al. (2014). Binding pocket residues of the enzyme were mutated to alanine (details in Supplemental Information), and the core of each dimer structure ( $X_1X_2$  in Fig. 3 and the N-linked dimer analog) were positioned such that the dimer bond was in proximity of the iron-heme. We then gradually and iteratively grew each ligand to its full form in the active site by: 1) adding compound substituents to the core in conformations that avoided steric clashes with the backbone and mutated residues of the protein, 2) minimizing the resulting ligand in situ, 3) removing the protein and minimizing the ligand in vacuo to remove ligand strain, and 4) reintroducing the protein and minimizing side chains and backbone with harmonic constraints in the presence of the fixed ligand. After the dimer structures were completely assembled, the mutated sidechains were restored to the native structure in rotamer states that accommodated the dimer-protein complex. Lastly, each completed and restored complex was minimized one additional time to produce the final models (Fig. 6). The final models had minor differences from the original PDBs, with root mean square deviation values of 1.1 and 0.48 Å for the CYP3A4-M13 complex and CYP3A4-M14 complex, respectively. Both M13 and M14 are in low energy conformational states within the complexes (<2 kcal/mol from the conformational global energy minimum), so ligand strain is minimal.

### Discussion

The P450-mediated bioactivation of a small molecule to generate a radical reactive intermediate and initiate dimerization was an unexpected biotransformation route. Some preceding examples of similar reactions have been described in the literature, and diverse mechanistic routes for dimer formation have been proposed. Gillam and coworkers reported P450-mediated oxidation of indole to indoxyl, which was subsequently oxidized and dimerized to form indigoids (Gillam et al., 2000; Isin and Guengerich, 2007). Two diastereoisomeric thiophene *S*-oxide dimers were observed in thiophene metabolism in vitro and in vivo in rats, where the combination of molecules was rationalized to occur via a Diels-Alder reaction (Treiber et al., 1997). Similar dimerization was reported for ticlodipine (Dansette et al., 2005).

Dimerization through free radical intermediates has been described for capsaicin and CJ-047710 (Emoto et al., 2007; Reilly et al., 2013). Homodimerization of raloxifene was also reported to occur via a CYP3A4-mediated oxidative reaction (Davis et al., 2011). Several overlaps exist in the observations for CJ-047710, raloxifene, and GDC-0994, suggesting these dimerization reactions may share common mechanistic features. After identifying that the dimers of GDC-0994 were formed via CYP3A4 using standard in vitro techniques, we sought to determine the mechanism of their formation to glean a deeper understanding of how these and other dimers are formed.

Two possible mechanisms for the formation of the observed dimers were evaluated with DFT (Pathways A and B, Fig. 3). Aromatic C-H abstraction is not a part of either proposed mechanism. C-H hydrogen abstraction from pyrazole was ruled unfavorable given the high bond dissociation energies of the C-H bonds, which were previously computed to be between 118.7 and 122.2 kcal/mol (Feng et al., 2003). In contrast, the bond dissociation energy for aniline N-H has been computed to be significantly lower at 91.4 kcal/mol (Song et al., 2003). The proposed mechanisms in Fig. 3 are consistent with existing precedence for P450-mediated aromatic couplings (Isin and Guengerich, 2007; Makino et al., 2007; Woithe et al., 2007; Belin et al., 2009; Grandner et al., 2016).

Both Pathways A and B initiate with N-H abstraction from model substrate **X** by the iron-oxo complex for P450 (**cpd I**) to provide a radical species ( $X_1^\bullet$ ) and iron-hydroxo (**cpd II**). This radical species ( $X_1^\bullet$ ) is delocalized between the nitrogen and other atoms in conjugated pyrazole via resonance, and thus the radical can form a new bond from the initial nitrogen involved in the abstraction or carbon in the pyrazole ring. For subsequent steps in Fig. 3, only C-C coupling is shown for simplicity, but C-N coupling is proposed to occur in the same manner. In Pathway A, radical addition of  $X_1^\bullet$  to  $X_2$  occurs, and subsequent N-H abstraction by **cpd II** from  $X_1X_2$  forms the final product. The alternate Pathway B describes forming a second radical species ( $X_2^\bullet$ ) via N-H abstraction, facilitated by **cpd II**, and radical coupling of  $X_1^\bullet$  and  $X_2^\bullet$  for the formation of the final dimer product. Thus, Pathways A and B both describe two hydrogen abstraction steps and a coupling reaction but only diverge in the order of the reaction steps.

The proposed mechanisms were computed using DFT and compared to determine the intrinsically favored pathway (Fig. 4). N-H abstraction by **cpd I** from  $X_1$  has a barrier of only 6.7 kcal/mol (**2-TS**, Fig. 4). This very low barrier indicates abstraction would occur rapidly at room temperature upon appropriate substrate binding. For radical additions of  $X_1^\bullet$  to ground-state  $X_2$  (Pathway A) to form either C-N or C-C coupled product are >30 kcal/mol and prohibitively high at room temperature (Fig. 5). Accounting for reduced entropic costs due to precomplexation of  $X_1^\bullet$  and  $X_2$  to CYP3A, this barrier is still rather high and indicates

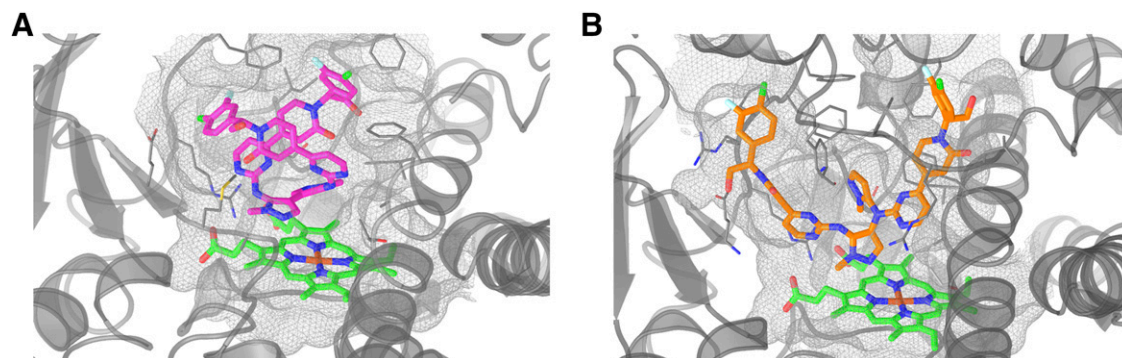


Fig. 6. Molecular models of dimer metabolites M13 (pink, left) and M14 (orange, right) in complex with CYP3A4 (protein and surface in gray, iron-heme in green).

a disfavoring of this radical addition. In the subsequent steps for Pathway B (Fig. 4), ligand exchange to allow the N-H of  $X_2$  to coordinate to the iron-hydroxo **cpd II**, formed by the first abstraction, is favored by  $>5$  kcal/mol (**3** to **4**, Fig. 4). N-H abstraction from  $X_2$  by **cpd II** is nearly barrierless at 1 kcal/mol (**4** to **5-TS**, Fig. 4). This barrier is low due to the release of water in the transition state. Radical combination of the delocalized radicals after formation via the two hydrogen abstractions would be rapid, further facilitated by the forced close proximity in the active site. The mechanism is the same for each metabolite, as formation of the two distinct products is facilitated by the delocalized nature of the radicals formed from N-H abstraction ( $X_1$  and  $X_2$ ).

The molecular modeling studies generated two dimer-CYP3A4 structures that indicate that **M13** or **M14** formation within the active site of the enzyme is sterically feasible with minimal rearrangement of the protein. Although we do not claim that these models are definitive binding modes, they provide supportive evidence for the mechanism we propose for homodimer formation.

In summary, during the in vivo metabolite profiling of GDC-0994 in rats, we discovered two novel dimer metabolites. In vitro studies indicated these metabolites were generated across several animal species and human, with CYP3A4/5 being the most efficient. Biomimetic catalysis produced sufficient quantities of the metabolites to resolve the structures and lead to the identification of a symmetrical and asymmetric homodimer. Computational studies determine that the dimers likely form via tandem N-H abstractions from monomer GDC-0994 units and subsequent radical coupling. Molecular modeling indicates that the CYP3A4 pocket is large and flexible enough to accommodate the dimer in the active site. All together, these studies provide evidence that large dimers can be formed by P450s and the mechanistic analysis can inform other drug discovery programs dealing with metabolism of similar substrates.

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## Authorship Contributions

*Participated in research design:* Takahashi, Grandner, Bobba, Liu, Beroza, Ma.

*Conducted experiments:* Takahashi, Bobba, Liu, Ma.

*Contributed to computational studies:* Grandner (DFT), Beroza (modeling).

*Performed data interpretation:* Takahashi, Grandner, Bobba, Liu, Beroza, Zhang, Ma.

*Contributed to the writing of the manuscript:* Takahashi, Grandner, Bobba, Liu, Beroza, Ma.

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## **Supplemental Information for**

### **Novel Homodimer Metabolites of GDC-0994 via Cytochrome P450-Catalyzed Radical Coupling**

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## **Supplemental Methods**

**In Vivo Study.** Male and female nonsurgicalized and bile duct–cannulated (BDC) Sprague-Dawley rats (n = 3 per group) were orally administered a single dose of [ $^{14}\text{C}$ ]GDC-0994 at 50 mg/kg (100  $\mu\text{Ci/kg}$ ) in 1% carboxymethylcellulose, 0.5% Tween, and 5 mM citrate solution. BDC rats were supplemented with an infusion of taurocholic acid (2.3 mg/ml in 0.9% saline) via the distal (duodenal) cannula. Urine and bile were collected on dry ice at approximately 0–8 h, 8–24 h, and then at 24-h intervals. Feces were collected on dry ice at intervals of 24 h up to 168 and 96 hours postdose from intact and BDC rats, respectively, and homogenized with 3–5 volumes of 50% (by volume) aqueous isopropyl alcohol. Plasma samples at 0 hour (predose) and at 1, 2, 4, 8, and 24 hours post-dose were also collected from another group of male and female rats for metabolite profiling.

**Radioactivity Analysis.** Total radioactivity in each matrix was measured by LSC analyses using a Model 2800TR analyzer (Perkin Elmer) with counting for 5 min. Plasma (~0.1 mL), urine (~0.3 mL) or bile (~0.1 mL) were mixed with Ultima Gold scintillation cocktail (5 mL) for radio counting. Fecal homogenates (~0.5 g) were dried and combusted in a Model 307 sample oxidizer (Perkin Elmer) and the resulting  $^{14}\text{CO}_2$  was trapped in CarboSorb and Perma-Fluor scintillation cocktail was added for LSC counting.

**Sample Preparation for Metabolite Profiling.** Urine, bile, and feces were pooled with an equal percentage of the weight or volume collected to give a single sample that represented >95% of the radioactivity eliminated by each excretion route. Pooled urine and bile samples (~0.4 mL) were centrifuged, and the supernatants were injected directly for metabolite profiling. Plasma samples (1 mL) were extracted twice with three volumes of acetonitrile. Fecal samples (~1 g) were extracted three times with CAN. The extracts were combined, evaporated to dryness by an N-

Evaporator (Caliper Life Sciences, Hopkinton, MA), and reconstituted in 500  $\mu$ L of ACN/H<sub>2</sub>O (v/v, 1:3).

**LC-MS/MS and Radioprofiling of Metabolites.** Chromatography was performed on a Kinetex XB C18 column (150 x 4.6 mm, 2.6  $\mu$ m particle size, Phenomenex, Torrance, CA) with mobile phases A (0.1% formic acid in water), and B (acetonitrile), at a constant flow rate of 1 ml/min. The gradient was as follows: initial holding at 10% B for 5 min, increased to 25% B at 20 min, 35% B at 40 min, 40% at 41 min, 45% at 50 min, and 95% B at 54 min, holding until 57 min and then column re-equilibration. The flow was split 10:1 post-column for radio-measurements and mass spectrometry, respectively. Radio-measurements were completed via fractionation to Deepwell LumaPlate 96 microplates (PerkinElmer) (10s per fraction), evaporation of solvents under vacuum, then counting radioactivity on the plates using a TopCount NXT scintillation and luminescence counter (PerkinElmer) for 5 min at 20 °C. Radioprofiles were reconstructed and integrated using the LSC import function in Laura software (LabLogic Systems; Brandon, FL). Mass spectrometric measurements were made with high resolution accurate mass full scan and tandem mass spectrometry (MS) experiments with an LTQ-Orbitrap with a heated electrospray ionization source (Thermo Scientific, San Jose, CA). The electrospray voltage was set at 4.0 kV and capillary temperature was 270°C. The full-scan mass spectra were obtained at resolving power of 30,000 and corresponding data dependent MS<sup>n</sup> scans following collision induced dissociation were acquired at a resolving power of 7,500.

## **Molecular Modeling Details**

A survey of structures in the protein databank revealed that the structure with PDB code 6BD5 had the largest binding pocket volume of all CYP 3A4 structures (Michael Drummond, personal communication). A protein structure with a larger binding site volume was created by mutating residues bordering the binding site (Hayes et al., 2014). Residues that had a heavy atom within 4.5 Å of the native ligand in the 6BD5 structure were mutated to alanine (residues affected: R105 F107 S119 I120 R212 F213 F215 F241 I301 F304 A305 T309 A370 R372 L373 E374).

Following the creation of the bound ligand protein complexes through iterative growth and structure relaxation as described in the main text, the final protein structures did not deviate significantly from the original structure. For protein C $\alpha$  atoms that were within 10 Å of either modeled dimer (i.e, the  $\alpha$ -carbons near the binding site), the root mean square deviation between their positions in the original CYP3A4 structure and those of the current models were 1.1 Å (CYP3A4-**M13**) and 0.48 Å (CYP3A4-**M14**). The difference between the two is presumably due to a more globular shape to **M13**, which requires greater protein motion to fit in the CYP3A4 pocket compared to **M14**.

## **References**

Hayes C, Ansbro D, and Kontoyianni M (2014) Elucidating substrate promiscuity in the human cytochrome 3A4. *J Chem Inf Model* **54**:857-869.

## Coordinates, Energies and Correction Factors from DFT Calculations

The 3D coordinates for each atom of each step of the reaction coordinate in Figure 4 are listed. The corresponding energies and correction factors for calculation of zero-point energy (ZPE), enthalpy, and entropy are listed below each of the coordinates.

### **cpdI (doublet)**

Fe	0.08985	0.04161	-0.36327
N	1.84727	-0.93077	-0.14071
N	-0.87452	-1.73699	-0.26205
N	1.03648	1.80272	-0.13577
N	-1.68541	0.99525	-0.29118
C	3.09280	-0.36101	-0.04918
C	-2.23303	-1.94178	-0.33421
C	2.05554	-2.28389	-0.20173
C	-0.30141	-2.99012	-0.30345
C	2.38733	2.00299	-0.03687
C	-2.92911	0.42685	-0.37388
C	0.47161	3.05016	-0.16895
C	-1.88852	2.35346	-0.31607
C	4.11144	-1.38345	-0.03976
C	-2.52233	-3.35437	-0.38666
C	3.46749	-2.57748	-0.14054
C	-1.32597	-4.00228	-0.37250
C	2.68449	3.41484	0.00229
C	-3.94560	1.44915	-0.43826
C	1.49276	4.06639	-0.08286
C	-3.29878	2.64628	-0.40306
H	5.17465	-1.19305	0.03266
H	-3.51771	-3.77697	-0.43862
H	3.89073	-3.57352	-0.16558
H	-1.13589	-5.06744	-0.40702
H	3.67959	3.83335	0.08304
H	-5.00922	1.25777	-0.50248
H	1.30380	5.13233	-0.08541
H	-3.72052	3.64291	-0.43229
C	3.34901	1.00054	0.01346
C	-3.19138	-0.93969	-0.38033
C	-0.88974	3.31419	-0.25637
C	1.05876	-3.24847	-0.28863
H	4.38694	1.30975	0.09043
H	-4.23076	-1.24686	-0.44691
H	-1.19619	4.35561	-0.27751
H	1.37117	-4.28724	-0.33207
O	0.16227	0.04880	-1.98261
S	-0.02198	-0.39125	2.22824
C	-1.70731	-0.05746	2.81579
H	-2.40012	-0.74549	2.31539
H	-1.75928	-0.23968	3.89321
H	-2.01484	0.96587	2.58566

SCF energy: -1625.578559 hartree  
zero-point correction: +0.317644 hartree  
enthalpy correction: +0.341313 hartree  
free energy correction: +0.266301 hartree  
quasiharmonic free energy correction:  
+0.268727 hartree

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### **cpdI (quartet)**

Fe	0.08878	0.03912	-0.36679
N	1.85127	-0.92254	-0.14551
N	-0.86400	-1.74016	-0.26736
N	1.02636	1.80770	-0.12872
N	-1.69164	0.98498	-0.29346
C	3.09355	-0.34529	-0.05010
C	-2.22246	-1.95248	-0.33403
C	2.06808	-2.27408	-0.20704
C	-0.28478	-2.99142	-0.30414
C	2.37579	2.01492	-0.03106
C	-2.93238	0.41123	-0.37789
C	0.45474	3.05174	-0.16140
C	-1.90127	2.34249	-0.31490
C	4.11809	-1.36179	-0.04083
C	-2.50412	-3.36645	-0.38038
C	3.48153	-2.55952	-0.14444
C	-1.30448	-4.00833	-0.36682
C	2.66572	3.42842	0.00867
C	-3.95391	1.42853	-0.44200
C	1.47049	4.07361	-0.07526
C	-3.31286	2.62861	-0.40350
H	5.18000	-1.16517	0.03408
H	-3.49749	-3.79419	-0.42845
H	3.91065	-3.55301	-0.16989
H	-1.10919	-5.07264	-0.39792
H	3.65871	3.85208	0.08850
H	-5.01651	1.23212	-0.50773
H	1.27581	5.13851	-0.07766
H	-3.73931	3.62328	-0.43099
C	3.34256	1.01716	0.01710
C	-3.18686	-0.95674	-0.38306
C	-0.90797	3.30833	-0.24982
C	1.07624	-3.24383	-0.29285
H	4.37887	1.33137	0.09552
H	-4.22431	-1.27049	-0.44851

H	-1.22015	4.34809	-0.26883
H	1.39328	-4.28120	-0.33539
O	0.17298	0.08304	-1.98569
S	-0.02032	-0.39498	2.22663
C	-1.70802	-0.07500	2.81567
H	-2.39713	-0.76358	2.31097
H	-1.75851	-0.26585	3.89174
H	-2.02172	0.94833	2.59416

SCF energy: -1625.578152 hartree

zero-point correction: +0.317687 hartree

enthalpy correction: +0.341320 hartree

free energy correction: +0.265742 hartree

quasiharmonic free energy correction:

+0.268181 hartree

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### FeIII-heme (sextet)

Fe	0.06497	-0.00127	0.10880
N	1.67358	-1.28586	-0.27675
N	-1.20941	-1.58021	-0.42516
N	1.38616	1.58040	-0.30082
N	-1.49610	1.28655	-0.43079
C	3.00639	-0.95425	-0.27008
C	-2.57447	-1.52131	-0.57025
C	1.60773	-2.65882	-0.29822
C	-0.86726	-2.91121	-0.43575
C	2.75761	1.52097	-0.29329
C	-2.82273	0.95507	-0.57546
C	1.04882	2.91151	-0.33893
C	-1.42696	2.66011	-0.45597
C	3.80766	-2.15587	-0.28064
C	-3.11217	-2.85870	-0.66717
C	2.94249	-3.21022	-0.29584
C	-2.05636	-3.71813	-0.58157
C	3.30473	2.85843	-0.32442
C	-3.61576	2.15661	-0.68200
C	2.24751	3.71875	-0.35041
C	-2.75283	3.21101	-0.60498
H	4.89019	-2.17859	-0.28300
H	-4.16077	-3.09748	-0.79357
H	3.17600	-4.26732	-0.31527
H	-2.06931	-4.79995	-0.62521
H	4.36124	3.09545	-0.33145
H	-4.69083	2.18033	-0.80935
H	2.26557	4.80082	-0.38469
H	-2.98183	4.26796	-0.65870
C	3.50916	0.34675	-0.26850
C	-3.32373	-0.34574	-0.62801
C	-0.25211	3.40979	-0.39299
C	0.43328	-3.40851	-0.35622

H	4.58981	0.45513	-0.26238
H	-4.39810	-0.45343	-0.74614
H	-0.36068	4.49008	-0.42498
H	0.54463	-4.48880	-0.37536
S	0.02331	-0.03785	2.43647
C	-1.75623	0.05950	2.88333
H	-2.33612	-0.69413	2.34443
H	-1.84498	-0.12319	3.95761
H	-2.15534	1.05116	2.65466

SCF energy: -1550.410452 hartree

zero-point correction: +0.313197 hartree

enthalpy correction: +0.336183 hartree

free energy correction: +0.260862 hartree

quasiharmonic free energy correction:

+0.263772 hartree

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### 3 (doublet)

C	-3.41679	-0.02312	3.04581
H	-4.33162	-0.23197	3.60793
H	-2.55633	-0.49259	3.52945
S	-3.61890	-0.61570	1.33990
O	-0.14368	0.24850	-0.97904
Fe	-1.49188	-0.24493	0.08833
N	-2.30218	1.61909	0.02703
N	-0.48216	0.22593	1.76977
N	-0.84810	-2.13841	0.26260
N	-2.61741	-0.73368	-1.52222
C	-3.14330	2.13885	-0.93120
C	-3.40394	3.53207	-0.65958
C	-2.70311	3.85392	0.46194
C	-2.00572	2.65864	0.87631
C	-0.42323	1.45881	2.36266
C	0.45736	1.42422	3.50680
C	0.92968	0.15042	3.59934
C	0.33831	-0.58995	2.51153
C	0.00545	-2.65362	1.20223
C	0.25585	-4.04999	0.94109
C	-0.45806	-4.37170	-0.17315
C	-1.14174	-3.16991	-0.58870
C	-2.65222	-1.95840	-2.13481
C	-3.49028	-1.90758	-3.31157
C	-3.95318	-0.63175	-3.40463
C	-3.40195	0.09099	-2.28090
C	-3.64875	1.43431	-2.01257
H	-4.29356	1.97116	-2.70186
C	-1.13624	2.58640	1.95730
H	-0.99036	3.49476	2.53481
C	0.56131	-1.93573	2.25635
H	1.23729	-2.46495	2.92020

C	-1.97911	-3.09216	-1.69839
H	-2.10989	-3.99968	-2.28061
H	-4.04029	4.16828	-1.26170
H	-2.64312	4.80949	0.96768
H	0.67252	2.27152	4.14575
H	1.61624	-0.26414	4.32644
H	0.89604	-4.68520	1.54003
H	-0.52597	-5.32648	-0.67916
H	-3.68413	-2.74591	-3.96873
H	-4.60868	-0.20384	-4.15252
H	-3.25560	1.06201	3.03400
H	0.70569	-0.00865	-0.56817
N	2.94369	0.39775	-0.48140
C	3.07888	1.64990	-0.87558
C	3.93948	-0.54658	-0.40723
C	2.00467	2.47443	-1.34386
N	4.20313	2.48423	-0.88656
C	2.57712	3.70347	-1.60915
H	0.98758	2.11989	-1.45553
C	5.53848	2.24650	-0.36136
N	3.89805	3.72084	-1.30825
C	4.64210	-2.49796	0.54202
C	5.78946	-1.55408	-1.29585
H	2.11846	4.60429	-1.99408
H	5.47588	1.71883	0.59488
H	6.12792	1.64871	-1.05861
H	5.99596	3.22447	-0.20888
H	4.50003	-3.25798	1.30998
H	6.57976	-1.53923	-2.04584
N	4.94235	-0.52380	-1.31726
N	3.75666	-1.50521	0.53082
C	5.69260	-2.59438	-0.37374
H	6.38987	-3.42477	-0.36638

SCF energy: -2209.812551 hartree  
 zero-point correction: +0.489856 hartree  
 enthalpy correction: +0.526463 hartree  
 free energy correction: +0.416984 hartree  
 quasiharmonic free energy correction:  
 +0.429549 hartree

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**1 (doublet)**

C	3.30517	-1.94566	2.36126
H	4.05026	-2.07193	3.15220
H	2.33790	-2.30007	2.73823
S	3.16991	-0.18305	1.94527
O	0.15588	0.41213	-0.94060
Fe	1.40591	0.12272	0.05966
N	2.81721	0.39479	-1.34658
N	1.43325	-1.85950	-0.30456

N	0.23946	-0.20550	1.68187
N	1.59393	2.05450	0.62105
C	3.40856	1.57849	-1.69932
C	4.28666	1.38441	-2.82724
C	4.21229	0.06587	-3.15696
C	3.29196	-0.54121	-2.22675
C	2.08341	-2.49512	-1.33481
C	1.73574	-3.89522	-1.35637
C	0.85891	-4.09679	-0.33536
C	0.67465	-2.82027	0.31065
C	-0.34314	-1.39759	2.04954
C	-1.21886	-1.19632	3.17743
C	-1.18287	0.13220	3.47321
C	-0.28146	0.74776	2.53254
C	0.88727	2.70308	1.60023
C	1.20528	4.11039	1.59962
C	2.11502	4.30580	0.60738
C	2.34547	3.01901	-0.00315
C	3.20036	2.80160	-1.07289
H	3.73891	3.65948	-1.46359
C	2.95377	-1.88899	-2.22803
H	3.40473	-2.51635	-2.99078
C	-0.14511	-2.61495	1.41525
H	-0.69859	-3.46950	1.79080
C	0.00572	2.10119	2.48947
H	-0.48645	2.74047	3.21556
H	4.87316	2.16588	-3.29319
H	4.72563	-0.46279	-3.94995
H	2.11912	-4.61387	-2.06944
H	0.36875	-5.01374	-0.03497
H	-1.78599	-1.98118	3.66122
H	-1.71188	0.66380	4.25389
H	0.78165	4.83690	2.28105
H	2.59409	5.22700	0.30131
H	3.57268	-2.54286	1.48586
H	-1.77726	0.47030	-0.77468
N	-2.75972	0.18936	-0.72655
C	-3.01612	-1.18528	-0.81263
C	-3.67628	1.18112	-0.97200
C	-2.50190	-2.13312	-1.68472
N	-3.78540	-1.85963	0.09325
C	-3.03448	-3.35267	-1.22643
H	-1.84047	-1.94688	-2.51775
C	-4.55408	-1.31094	1.19287
N	-3.81944	-3.18886	-0.15908
C	-4.05545	3.40372	-1.26195
C	-5.81738	1.83638	-1.36661
H	-2.88909	-4.34654	-1.63092
H	-3.93241	-0.62025	1.77059
H	-5.43139	-0.77763	0.81686
H	-4.85930	-2.14929	1.81999



H	-3.65038	4.41453	-1.30471
H	-6.86208	1.55378	-1.49444
C	-5.41897	3.16808	-1.45412
H	-6.12111	3.96943	-1.65402
N	-3.17594	2.43544	-1.01956
N	-4.96972	0.83401	-1.12562

SCF energy: -2209.820209 hartree  
zero-point correction: +0.492867 hartree  
enthalpy correction: +0.529296 hartree  
free energy correction: +0.421668 hartree  
quasiharmonic free energy correction:  
+0.432320 hartree

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### 2-TS (doublet)

C	2.91891	-1.53561	2.74569
H	3.56661	-1.46676	3.62591
H	1.93452	-1.88779	3.07031
S	2.81759	0.13686	2.01577
O	0.16469	0.14638	-1.12439
Fe	1.28430	0.10035	0.15259
N	2.81391	0.56265	-1.08211
N	1.64410	-1.86393	-0.13436
N	-0.14854	-0.36317	1.49461
N	1.06809	2.05545	0.60141
C	3.21794	1.81387	-1.45408
C	4.30190	1.72825	-2.40434
C	4.55246	0.40402	-2.59098
C	3.61264	-0.31503	-1.76327
C	2.59811	-2.41256	-0.95148
C	2.46574	-3.84944	-0.97374
C	1.40275	-4.15757	-0.18020
C	0.89765	-2.90936	0.33729
C	-0.64007	-1.61147	1.77137
C	-1.69080	-1.52546	2.75689
C	-1.81964	-0.20803	3.07852
C	-0.85819	0.51351	2.28120
C	0.19382	2.59991	1.49981
C	0.29301	4.04009	1.48363
C	1.22836	4.35717	0.54787
C	1.70929	3.10942	0.00378
C	2.69864	3.00620	-0.96275
H	3.12050	3.92999	-1.34641
C	3.53083	-1.69943	-1.69363
H	4.23163	-2.27128	-2.29417
C	-0.18127	-2.79778	1.20869
H	-0.68257	-3.71463	1.50166
C	-0.69614	1.88875	2.29646
H	-1.33082	2.45929	2.96725
H	4.79818	2.58010	-2.85144

H	5.29460	-0.06081	-3.22738
H	3.10435	-4.51795	-1.53698
H	0.98889	-5.13167	0.04724
H	-2.23694	-2.37389	3.14911
H	-2.49400	0.24964	3.79128
H	-0.28679	4.70378	2.11247
H	1.58104	5.33570	0.24777
H	3.34526	-2.26135	2.04784
H	-1.02394	0.09220	-0.99412
N	-2.30777	-0.07202	-0.95819
C	-2.73677	-1.35471	-1.04867
C	-3.07812	1.05746	-0.99500
C	-2.03785	-2.41137	-1.67767
N	-3.86572	-1.92721	-0.50213
N	-4.37634	0.97578	-1.37313
N	-2.41759	2.20436	-0.70292
C	-2.83892	-3.53672	-1.48443
H	-1.10505	-2.30993	-2.21135
C	-4.84927	-1.35016	0.39906
N	-3.93138	-3.25418	-0.76109
C	-5.03329	2.12997	-1.48246
C	-3.10930	3.33269	-0.81986
H	-2.67935	-4.55067	-1.82710
H	-5.57950	-0.75643	-0.15254
H	-5.33360	-2.18337	0.90931
H	-4.34598	-0.71265	1.13153
C	-4.44753	3.36846	-1.22087
H	-6.07417	2.05808	-1.79718
H	-2.56786	4.24789	-0.58214
H	-4.99632	4.29807	-1.32103

SCF energy: -2209.803080 hartree  
zero-point correction: +0.485930 hartree  
enthalpy correction: +0.521590 hartree  
free energy correction: +0.418128 hartree  
quasiharmonic free energy correction:  
+0.426252 hartree

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### 3 (quartet)

C	-3.86662	0.52585	2.41582
H	-4.78154	0.22210	2.93266
H	-3.09697	0.76914	3.15308
S	-3.33895	-0.86024	1.35272
O	-0.12230	0.23224	-0.91002
Fe	-1.47612	-0.22535	0.15097
N	-2.51478	1.31617	-0.64773
N	-0.82893	0.97082	1.62712
N	-0.44305	-1.76330	0.94713
N	-2.20388	-1.45785	-1.27528
C	-3.34368	1.26831	-1.73638

C	-3.85147	2.58870	-2.02648
C	-3.30409	3.43173	-1.10818
C	-2.47121	2.62493	-0.24698
C	-1.01427	2.33014	1.71844
C	-0.34271	2.83744	2.88879
C	0.24433	1.77205	3.50249
C	-0.05103	0.61172	2.69974
C	0.25383	-1.76140	2.12913
C	0.79878	-3.07335	2.38289
C	0.42645	-3.86290	1.33965
C	-0.36166	-3.04045	0.45221
C	-1.86015	-2.76603	-1.48174
C	-2.55224	-3.28042	-2.63992
C	-3.31511	-2.26374	-3.12603
C	-3.08165	-1.12195	-2.27389
C	-3.63293	0.13388	-2.48347
H	-4.31154	0.24747	-3.32305
C	-1.76161	3.10896	0.84560
H	-1.81950	4.17357	1.05002
C	0.42950	-0.66551	2.95938
H	1.02337	-0.80498	3.85693
C	-0.99406	-3.50915	-0.68987
H	-0.83024	-4.54654	-0.96397
H	-4.53029	2.82226	-2.83693
H	-3.44407	4.50042	-1.00451
H	-0.33403	3.87765	3.18921
H	0.83885	1.75778	4.40722
H	1.38853	-3.33424	3.25253
H	0.63958	-4.91168	1.17504
H	-2.45458	-4.29106	-3.01574
H	-3.97093	-2.26349	-3.98742
H	-4.07258	1.41329	1.81071
H	0.74998	-0.00233	-0.52280
N	2.83180	0.40045	-0.52762
C	3.03680	1.65759	-0.87677
C	3.76910	-0.60263	-0.46418
C	2.01143	2.54089	-1.34510
N	4.19509	2.44137	-0.83632
C	2.64170	3.75081	-1.56230
H	0.98341	2.23900	-1.49118
C	5.50302	2.13647	-0.27566
N	3.95345	3.70139	-1.22827
C	4.27724	-2.66075	0.37827
C	5.61937	-1.65732	-1.29096
H	2.23230	4.68097	-1.93253
H	5.38757	1.57933	0.65837
H	6.09317	1.54051	-0.97328
H	5.98911	3.09141	-0.07395
H	4.02303	-3.46476	1.06780
H	6.46499	-1.63900	-1.97774
N	4.83723	-0.57622	-1.29543

N	3.45863	-1.61315	0.37945
C	5.38960	-2.75440	-0.46268
H	6.03417	-3.62636	-0.47124

SCF energy: -2209.813230 hartree  
 zero-point correction: +0.490060 hartree  
 enthalpy correction: +0.526591 hartree  
 free energy correction: +0.416940 hartree  
 quasiharmonic free energy correction:  
 +0.429276 hartree

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**1 (quartet)**

C	3.30508	-1.94135	2.36340
H	4.04929	-2.06605	3.15546
H	2.33789	-2.29637	2.73998
S	3.16874	-0.17913	1.94538
O	0.16706	0.40787	-0.96672
Fe	1.39503	0.12336	0.06220
N	2.81477	0.39239	-1.34315
N	1.42610	-1.86037	-0.29932
N	0.23616	-0.20194	1.68156
N	1.58266	2.05643	0.61689
C	3.40070	1.57637	-1.70185
C	4.27712	1.38120	-2.83101
C	4.20657	0.06108	-3.15532
C	3.28979	-0.54513	-2.22085
C	2.07959	-2.49728	-1.32717
C	1.73172	-3.89746	-1.34794
C	0.85233	-4.09793	-0.32899
C	0.66745	-2.82067	0.31538
C	-0.34238	-1.39544	2.05539
C	-1.21013	-1.19362	3.18873
C	-1.17348	0.13513	3.48352
C	-0.27977	0.75148	2.53683
C	0.87940	2.70721	1.59657
C	1.19665	4.11456	1.59218
C	2.10384	4.30791	0.59725
C	2.33372	3.01986	-0.01081
C	3.18906	2.80116	-1.07952
H	3.72530	3.65922	-1.47297
C	2.95293	-1.89340	-2.21858
H	3.40555	-2.52247	-2.97894
C	-0.14975	-2.61339	1.42152
H	-0.70258	-3.46676	1.80064
C	0.00278	2.10532	2.49070
H	-0.48706	2.74410	3.21875
H	4.85952	2.16300	-3.30152
H	4.71943	-0.46894	-3.94772
H	2.11704	-4.61692	-2.05916
H	0.36162	-5.01454	-0.02848

H	-1.77332	-1.97869	3.67678
H	-1.69789	0.66664	4.26735
H	0.77482	4.84232	2.27339
H	2.58241	5.22841	0.28832
H	3.57445	-2.53940	1.48917
H	-1.76603	0.46382	-0.78791
N	-2.74869	0.18491	-0.73364
C	-3.00868	-1.18900	-0.81981
C	-3.66386	1.17950	-0.97359
C	-2.49870	-2.13738	-1.69385
N	-3.77757	-1.86221	0.08727
C	-3.03334	-3.35598	-1.23547
H	-1.83870	-1.95203	-2.52823
C	-4.54212	-1.31302	1.18951
N	-3.81551	-3.19114	-0.16627
C	-4.03821	3.40350	-1.25870
C	-5.80490	1.84112	-1.35742
H	-2.89134	-4.34984	-1.64117
H	-3.91787	-0.62332	1.76563
H	-5.41995	-0.77845	0.81655
H	-4.84638	-2.15127	1.81725
H	-3.63054	4.41324	-1.30196
H	-6.85093	1.56153	-1.48085
C	-5.40325	3.17184	-1.44479
H	-6.10414	3.97540	-1.64017
N	-3.16029	2.43244	-1.02176
N	-4.95884	0.83607	-1.12184

SCF energy: -2209.819777 hartree  
 zero-point correction: +0.492904 hartree  
 enthalpy correction: +0.529304 hartree  
 free energy correction: +0.421082 hartree  
 quasiharmonic free energy correction:  
 +0.431762 hartree

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## 2-TS (quartet)

C	2.95421	-1.51792	2.73143
H	3.61516	-1.45814	3.60145
H	1.97982	-1.89574	3.05861
S	2.79172	0.16009	2.03290
O	0.16626	0.15972	-1.10844
Fe	1.28678	0.10101	0.16131
N	2.80676	0.54299	-1.09445
N	1.62757	-1.86578	-0.11735
N	-0.14179	-0.34002	1.51626
N	1.08300	2.06344	0.59681
C	3.22354	1.79023	-1.46808
C	4.29127	1.69060	-2.43542
C	4.51754	0.36323	-2.63138
C	3.58108	-0.34453	-1.78998

C	2.55874	-2.42685	-0.95422
C	2.41536	-3.86277	-0.96206
C	1.37154	-4.15813	-0.13887
C	0.88597	-2.90346	0.38148
C	-0.62289	-1.58673	1.82495
C	-1.66573	-1.48445	2.81734
C	-1.80443	-0.16067	3.10705
C	-0.85374	0.54996	2.28743
C	0.19860	2.62313	1.47775
C	0.30571	4.06224	1.44398
C	1.25601	4.36265	0.51769
C	1.73595	3.10640	-0.00744
C	2.72601	2.98915	-0.97199
H	3.15727	3.90709	-1.35908
C	3.48441	-1.72813	-1.71736
H	4.16662	-2.30969	-2.32976
C	-0.16832	-2.78159	1.28016
H	-0.66229	-3.69451	1.59699
C	-0.69781	1.92603	2.27797
H	-1.33726	2.50663	2.93537
H	4.79281	2.53622	-2.88856
H	5.24147	-0.11062	-3.28204
H	3.03487	-4.53972	-1.53655
H	0.95720	-5.12784	0.10596
H	-2.20290	-2.32744	3.23315
H	-2.47828	0.30847	3.81288
H	-0.27899	4.73692	2.05644
H	1.61707	5.33571	0.20974
H	3.37484	-2.21570	2.00248
H	-1.00650	0.09713	-1.01157
N	-2.30343	-0.07605	-1.01528
C	-2.72693	-1.36152	-1.08260
C	-3.08204	1.04852	-1.02332
C	-2.05159	-2.41438	-1.74122
N	-3.82541	-1.94159	-0.48173
N	-4.38874	0.95640	-1.36843
N	-2.42363	2.19979	-0.74632
C	-2.83123	-3.54515	-1.50549
H	-1.14301	-2.30852	-2.31459
C	-4.78201	-1.36333	0.44743
N	-3.89388	-3.26718	-0.73441
C	-5.05646	2.10601	-1.46314
C	-3.12633	3.32273	-0.84594
H	-2.67995	-4.55945	-1.85067
H	-5.24656	-2.19564	0.97712
H	-4.25864	-0.71995	1.16023
H	-5.53163	-0.77608	-0.08498
C	-4.47399	3.34865	-1.21568
H	-6.10407	2.02607	-1.75252
H	-2.58654	4.24184	-0.61983
H	-5.03201	4.27414	-1.30201

SCF energy: -2209.803206 hartree  
 zero-point correction: +0.486147 hartree  
 enthalpy correction: +0.521675 hartree  
 free energy correction: +0.417926 hartree  
 quasiharmonic free energy correction:  
 +0.425915 hartree

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**6 (triplet)**

C	3.83181	-1.80762	1.79700
H	4.47928	-1.90521	2.67448
H	3.23379	-2.71737	1.69661
S	2.77781	-0.33818	2.07126
O	0.24021	0.32114	-1.39190
Fe	1.46371	-0.04946	0.29002
N	2.98393	-0.20688	-1.01303
N	1.06306	-2.01028	0.02492
N	-0.15949	0.13298	1.49463
N	1.73602	1.94753	0.42396
C	3.82186	0.80630	-1.39927
C	4.80580	0.30948	-2.33548
C	4.54002	-1.01188	-2.51989
C	3.39810	-1.32439	-1.69025
C	1.73213	-2.89863	-0.78380
C	1.16529	-4.21918	-0.64861
C	0.15487	-4.12158	0.25997
C	0.09708	-2.73920	0.67420
C	-0.94061	-0.89689	1.95491
C	-1.93009	-0.39572	2.88157
C	-1.73550	0.95009	2.97345
C	-0.62281	1.26789	2.10916
C	1.01544	2.84963	1.16453
C	1.58706	4.17091	1.03626
C	2.66943	4.05058	0.21975
C	2.75598	2.65725	-0.15495
C	3.72988	2.13280	-0.99644
H	4.47217	2.82253	-1.38716
C	2.81387	-2.58229	-1.59618
H	3.24804	-3.38273	-2.18777
C	-0.82783	-2.23005	1.57980
H	-1.52877	-2.93206	2.02135
C	-0.09080	2.54192	1.94749
H	-0.55720	3.35379	2.49783
H	5.58332	0.91117	-2.78932
H	5.05475	-1.72235	-3.15455
H	1.51148	-5.09660	-1.18060
H	-0.50033	-4.90216	0.62616
H	-2.66018	-1.00932	3.39497
H	-2.27196	1.66992	3.57943
H	1.20528	5.05911	1.52442

H	3.35740	4.82047	-0.10674
H	4.45775	-1.68922	0.90851
H	-0.39174	1.06352	-1.22465
N	-2.75034	-0.17206	-1.31824
C	-3.51251	-1.18441	-0.94620
C	-3.08292	1.15830	-1.30882
C	-3.30197	-2.54257	-1.34617
N	-4.61013	-1.23670	-0.08071
C	-4.28997	-3.27367	-0.71681
H	-2.52650	-2.87072	-2.02201
C	-5.15507	-0.19965	0.78633
N	-5.06259	-2.48900	0.07119
C	-2.27825	3.30947	-1.29328
C	-4.57890	2.85944	-1.61278
H	-4.49803	-4.33383	-0.76565
H	-4.34033	0.32012	1.29929
H	-5.73888	0.51491	0.20557
H	-5.78244	-0.70754	1.51907
H	-1.41891	3.96868	-1.18944
H	-5.61093	3.16027	-1.78849
H	-0.36154	-0.43900	-1.48315
C	-3.56316	3.80931	-1.50821
H	-3.75873	4.87205	-1.59337
N	-4.36341	1.54756	-1.50158
N	-2.02694	2.00479	-1.21317

SCF energy: -2210.482540 hartree  
 zero-point correction: +0.502921 hartree  
 enthalpy correction: +0.539543 hartree  
 free energy correction: +0.433131 hartree  
 quasiharmonic free energy correction:  
 +0.442285 hartree

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**4 (triplet)**

C	2.63916	-1.43751	3.13875
H	3.47913	-1.77094	3.75402
H	2.05648	-0.67812	3.66681
S	3.27991	-0.78609	1.56800
O	0.13284	0.18012	-1.03689
Fe	1.43496	-0.07035	0.17513
N	2.85017	-0.16975	-1.25971
N	1.13051	-2.08242	-0.02754
N	0.16560	-0.02368	1.72613
N	1.76871	1.90088	0.39520
C	3.57402	0.88110	-1.76219
C	4.41456	0.43396	-2.84624
C	4.17077	-0.89570	-3.00789
C	3.18687	-1.26307	-2.01878
C	1.69630	-2.91774	-0.95590
C	1.15192	-4.25132	-0.82502

C	0.24949	-4.20795	0.19109
C	0.24110	-2.84578	0.67945
C	-0.59331	-1.07006	2.19080
C	-1.44942	-0.62401	3.26170
C	-1.21028	0.70791	3.42992
C	-0.21158	1.07652	2.45887
C	1.18980	2.74709	1.30628
C	1.69332	4.09001	1.13581
C	2.59795	4.04099	0.12024
C	2.64534	2.66823	-0.32769
C	3.49049	2.19810	-1.32638
H	4.13361	2.92366	-1.81533
C	2.65298	-2.53984	-1.88698
H	3.01526	-3.30221	-2.56986
C	-0.55880	-2.37886	1.71599
H	-1.22826	-3.09188	2.18755
C	0.27060	2.36680	2.27598
H	-0.11267	3.14064	2.93417
H	5.08733	1.06982	-3.40757
H	4.60361	-1.57798	-3.72837
H	1.43411	-5.09575	-1.44090
H	-0.36738	-5.00734	0.58135
H	-2.13908	-1.25892	3.80344
H	-1.66625	1.39083	4.13553
H	1.39148	4.94263	1.73094
H	3.19333	4.84532	-0.29298
H	1.98511	-2.29370	2.93707
H	-1.66616	-0.14348	-0.91382
N	-2.67809	0.05213	-0.87822
C	-3.56053	-1.02978	-0.98987
C	-3.01486	1.36776	-1.04704
C	-3.56611	-2.07873	-1.89714
N	-4.55380	-1.28554	-0.08625
C	-4.61598	-2.90959	-1.46219
H	-2.90687	-2.19995	-2.74423
C	-4.96123	-0.47988	1.04773
N	-5.22123	-2.42621	-0.37601
C	-2.28898	3.52626	-1.21523
C	-4.58424	2.98990	-1.33724
H	-4.96445	-3.83887	-1.89495
H	-4.07674	-0.12316	1.58393
H	-5.54998	0.38090	0.71816
H	-5.55809	-1.11941	1.69903
H	-1.44996	4.22082	-1.22006
H	-5.63772	3.24652	-1.44605
H	-0.13324	1.12323	-1.01269
N	-1.97757	2.24172	-1.05515
N	-4.31693	1.69363	-1.17589
C	-3.60040	3.97715	-1.36816
H	-3.83808	5.02618	-1.50112

SCF energy: -2210.472727 hartree  
 zero-point correction: +0.504262 hartree  
 enthalpy correction: +0.540744 hartree  
 free energy correction: +0.432626 hartree  
 quasiharmonic free energy correction:  
 +0.443510 hartree

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### 5-TS (triplet)

C	4.41169	-0.54881	1.53432
H	5.09880	-0.47011	2.38224
H	4.30097	-1.60031	1.25524
S	2.81587	0.17599	2.04414
O	0.04609	0.12922	-1.17873
Fe	1.33502	0.07060	0.26923
N	2.61463	1.16000	-0.83456
N	2.14533	-1.61567	-0.51504
N	0.05389	-1.03595	1.37552
N	0.51403	1.74401	1.03427
C	2.68099	2.53409	-0.87128
C	3.71057	2.95647	-1.79225
C	4.25700	1.82696	-2.31739
C	3.56131	0.71120	-1.71783
C	3.15464	-1.70971	-1.44340
C	3.45170	-3.09362	-1.71979
C	2.61674	-3.83565	-0.93816
C	1.80712	-2.90293	-0.19190
C	-0.00022	-2.40667	1.42563
C	-1.04317	-2.82915	2.33317
C	-1.61118	-1.69586	2.82983
C	-0.91208	-0.58661	2.22424
C	-0.51528	1.83507	1.94253
C	-0.80446	3.22167	2.23253
C	0.05354	3.96204	1.48318
C	0.86837	3.02621	0.73708
C	1.87356	3.40055	-0.15215
H	2.04215	4.46433	-0.29033
C	3.81359	-0.62638	-2.01253
H	4.59368	-0.83919	-2.73738
C	0.81586	-3.27451	0.71718
H	0.65678	-4.33813	0.86696
C	-1.18444	0.75879	2.49514
H	-1.97893	0.97217	3.20397
H	3.96472	3.98813	-2.00001
H	5.05209	1.73789	-3.04690
H	4.20453	-3.43742	-2.41784
H	2.54352	-4.91338	-0.86469
H	-1.29727	-3.85991	2.54442
H	-2.42098	-1.60314	3.54235
H	-1.56754	3.56137	2.92121
H	0.14612	5.03943	1.42793

H	4.83635	-0.00220	0.68623
H	-1.15471	0.04428	-1.01454
H	0.33766	-0.49118	-1.86486
N	-2.42088	-0.09760	-0.90886
C	-2.87660	-1.38422	-1.00233
C	-3.20513	1.01839	-1.00794
C	-2.25836	-2.44590	-1.68515
N	-3.97156	-1.93380	-0.37260
N	-4.51675	0.89513	-1.32733
N	-2.55484	2.19285	-0.81187
C	-3.06006	-3.55902	-1.42482
H	-1.37358	-2.37749	-2.30001
C	-4.86154	-1.32867	0.60140
N	-4.08516	-3.25800	-0.61616
C	-5.20252	2.02910	-1.46368
C	-3.27475	3.29914	-0.95382
H	-2.94813	-4.57436	-1.78251
H	-5.22953	-2.13159	1.24132
H	-4.30487	-0.60422	1.20153
H	-5.69149	-0.81886	0.10841
C	-4.63218	3.28984	-1.29092
H	-6.25465	1.92184	-1.72727
H	-2.74375	4.23617	-0.78943
H	-5.20399	4.20308	-1.41136

SCF energy: -2210.458244 hartree  
zero-point correction: +0.497199 hartree  
enthalpy correction: +0.533434 hartree  
free energy correction: +0.427794 hartree  
quasiharmonic free energy correction:  
+0.436809 hartree

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**6 (open-shell singlet)**

C	3.83424	-1.80739	1.79304
H	4.48244	-1.90572	2.66990
H	3.23678	-2.71741	1.69183
S	2.77943	-0.33905	2.07005
O	0.23909	0.32296	-1.39028
Fe	1.46302	-0.04963	0.29037
N	2.98364	-0.20063	-1.01320
N	1.06656	-2.01044	0.02080
N	-0.16046	0.12663	1.49525
N	1.73161	1.94776	0.42927
C	3.81971	0.81513	-1.39674
C	4.80451	0.32258	-2.33431
C	4.54107	-0.99876	-2.52227
C	3.39977	-1.31553	-1.69339
C	1.73712	-2.89535	-0.79051
C	1.17313	-4.21744	-0.65837
C	0.16298	-4.12426	0.25095

C	0.10242	-2.74302	0.66862
C	-0.93933	-0.90586	1.95340
C	-1.93018	-0.40875	2.88076
C	-1.73871	0.93734	2.97521
C	-0.62650	1.25934	2.11186
C	1.00886	2.84668	1.17151
C	1.57782	4.16940	1.04654
C	2.66079	4.05315	0.23019
C	2.75029	2.66089	-0.14771
C	3.72526	2.14039	-0.99045
H	4.46626	2.83252	-1.37938
C	2.81791	-2.57479	-1.60245
H	3.25345	-3.37286	-2.19623
C	-0.82346	-2.23800	1.57558
H	-1.52274	-2.94257	2.01568
C	-0.09716	2.53483	1.95318
H	-0.56559	3.34448	2.50507
H	5.58094	0.92687	-2.78657
H	5.05706	-1.70660	-3.15884
H	1.52091	-5.09277	-1.19274
H	-0.49037	-4.90714	0.61554
H	-2.65909	-1.02496	3.39267
H	-2.27694	1.65477	3.58246
H	1.19401	5.05571	1.53655
H	3.34730	4.82520	-0.09426
H	4.45947	-1.68737	0.90425
H	-0.39134	1.06656	-1.22288
N	-2.74866	-0.17153	-1.31767
C	-3.50989	-1.18437	-0.94498
C	-3.08267	1.15847	-1.30938
C	-3.29789	-2.54271	-1.34360
N	-4.60774	-1.23692	-0.07985
C	-4.28550	-3.27416	-0.71396
H	-2.52186	-2.87078	-2.01883
C	-5.15422	-0.19949	0.78575
N	-5.05921	-2.48950	0.07292
C	-2.28068	3.31066	-1.29444
C	-4.58034	2.85757	-1.61650
H	-4.49253	-4.33456	-0.76191
H	-4.34030	0.32135	1.29892
H	-5.73811	0.51406	0.20383
H	-5.78179	-0.70718	1.51846
H	-1.42230	3.97103	-1.19002
H	-5.61253	3.15699	-1.79371
H	-0.36460	-0.43575	-1.48123
C	-3.56590	3.80878	-1.51145
H	-3.76265	4.87121	-1.59771
N	-4.36340	1.54605	-1.50404
N	-2.02785	2.00634	-1.21284

SCF energy: -2210.482551 hartree



zero-point correction: +0.502918 hartree  
enthalpy correction: +0.539540 hartree  
free energy correction: +0.434161 hartree  
quasiharmonic free energy correction:  
+0.443320 hartree

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**4 (open-shell singlet)**

C	4.35181	-1.40134	1.33740
H	5.22946	-1.39603	1.98996
H	3.76085	-2.30569	1.50510
S	3.37132	0.09271	1.66621
O	0.04815	0.36624	-0.95388
Fe	1.40413	0.09593	0.21974
N	2.79581	0.50098	-1.20033
N	1.55480	-1.85789	-0.24396
N	0.12812	-0.32040	1.71125
N	1.35512	2.04601	0.74525
C	3.21549	1.75509	-1.58600
C	4.16187	1.64760	-2.66990
C	4.31147	0.32102	-2.93730
C	3.44326	-0.38671	-2.02770
C	2.34083	-2.44183	-1.20562
C	2.09070	-3.86072	-1.26480
C	1.13114	-4.12714	-0.33475
C	0.80500	-2.87291	0.29798
C	-0.41464	-1.54513	1.99288
C	-1.32045	-1.44639	3.11364
C	-1.30782	-0.14359	3.51064
C	-0.40601	0.54943	2.62095
C	0.64985	2.61492	1.77407
C	0.82378	4.04879	1.76920
C	1.62743	4.33894	0.71094
C	1.95892	3.07993	0.08263
C	2.80684	2.95054	-1.01311
H	3.21516	3.86484	-1.43318
C	3.24295	-1.76249	-2.02256
H	3.80588	-2.35167	-2.74044
C	-0.11783	-2.72996	1.32789
H	-0.64763	-3.62230	1.64567
C	-0.14797	1.91729	2.67017
H	-0.64636	2.49031	3.44620
H	4.64310	2.48919	-3.15174
H	4.93593	-0.14913	-3.68638
H	2.58579	-4.54780	-1.93927
H	0.67655	-5.07795	-0.08795
H	-1.87814	-2.27529	3.53014
H	-1.85432	0.32108	4.32145
H	0.37314	4.72804	2.48143
H	1.97931	5.30645	0.37623
H	4.69181	-1.40013	0.29465

H	-1.78285	0.37595	-0.77737
N	-2.77847	0.10607	-0.75561
C	-3.04959	-1.26714	-0.79683
C	-3.69257	1.08887	-1.03433
C	-2.54556	-2.24965	-1.63720
N	-3.82740	-1.90619	0.12722
C	-3.09150	-3.44895	-1.14265
H	-1.89047	-2.10005	-2.48287
C	-4.59834	-1.31434	1.20274
N	-3.87557	-3.24253	-0.08253
C	-4.08036	3.30346	-1.37696
C	-5.83122	1.72480	-1.48258
H	-2.95740	-4.45660	-1.51595
H	-3.97600	-0.60573	1.75691
H	-5.47227	-0.79283	0.80290
H	-4.90900	-2.12756	1.85955
H	-3.68027	4.31581	-1.43249
H	-6.87193	1.43345	-1.62399
H	0.27878	-0.10522	-1.77226
N	-3.19967	2.34623	-1.09896
N	-4.98298	0.73273	-1.20599
C	-5.43901	3.05700	-1.58956
H	-6.14173	3.85022	-1.81797

SCF energy: -2210.477210 hartree  
zero-point correction: +0.503507 hartree  
enthalpy correction: +0.540221 hartree  
free energy correction: +0.433641 hartree  
quasiharmonic free energy correction:  
+0.443541 hartree

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**4 (open-shell singlet)**

C	4.18797	-1.03260	1.70420
H	4.86278	-0.98118	2.56464
H	3.86315	-2.06903	1.57511
S	2.77788	0.07708	2.05833
O	0.07430	0.21924	-1.20104
Fe	1.33668	0.08780	0.27214
N	2.84384	0.38190	-1.04413
N	1.51373	-1.90215	-0.04029
N	-0.21013	-0.20346	1.54207
N	1.14166	2.07835	0.56053
C	3.35049	1.59584	-1.42267
C	4.41879	1.41859	-2.37782
C	4.55088	0.07710	-2.57370
C	3.56123	-0.55942	-1.73800
C	2.39922	-2.55800	-0.86156
C	2.18390	-3.98325	-0.79374
C	1.15171	-4.17976	0.07367
C	0.74238	-2.87372	0.53335

C	-0.72979	-1.41485	1.90730
C	-1.79185	-1.23664	2.87217
C	-1.89935	0.10327	3.08877
C	-0.90688	0.73806	2.25208
C	0.26608	2.72879	1.37890
C	0.46293	4.15922	1.29886
C	1.47071	4.36167	0.40881
C	1.88867	3.05324	-0.04574
C	2.90593	2.83454	-0.96502
H	3.40770	3.71227	-1.36212
C	3.35632	-1.93451	-1.65488
H	3.99434	-2.57144	-2.26063
C	-0.30664	-2.65003	1.42677
H	-0.83216	-3.52626	1.79398
C	-0.68831	2.10767	2.18087
H	-1.31531	2.74652	2.79551
H	4.98283	2.22450	-2.83000
H	5.24457	-0.44586	-3.21978
H	2.75317	-4.72200	-1.34362
H	0.69813	-5.11313	0.38189
H	-2.36119	-2.04174	3.31896
H	-2.57410	0.62771	3.75363
H	-0.10420	4.89353	1.85683
H	1.90570	5.29725	0.08088
H	4.73700	-0.71671	0.81245
H	-1.15646	0.07460	-1.06295
H	0.39094	-0.32133	-1.94179
N	-2.39067	-0.09643	-0.97138
C	-2.82592	-1.38591	-0.94544
C	-3.17327	1.02285	-1.06908
C	-2.16370	-2.49612	-1.51266
N	-3.93382	-1.90073	-0.31011
N	-4.47764	0.90143	-1.41347
N	-2.52290	2.19371	-0.86214
C	-2.96503	-3.59572	-1.19780
H	-1.25386	-2.46074	-2.09219
C	-4.88186	-1.24220	0.57279
N	-4.02452	-3.24452	-0.46042
C	-5.15353	2.03803	-1.57765
C	-3.23323	3.30345	-1.03552
H	-2.82452	-4.63536	-1.46345
H	-5.33364	-2.02296	1.18570
H	-4.35320	-0.53171	1.21408
H	-5.64374	-0.71242	-0.00097
C	-4.58017	3.29780	-1.40722
H	-6.20022	1.93309	-1.86269
H	-2.70058	4.23912	-0.86739
H	-5.14422	4.21239	-1.55141

SCF energy: -2210.469885 hartree  
zero-point correction: +0.497252 hartree

enthalpy correction: +0.533452 hartree  
free energy correction: +0.429312 hartree  
quasiharmonic free energy correction:  
+0.437940 hartree

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**C-N X1• X2 radical addition (conformer 1)**

C	0.66303	-2.13674	1.21407
C	0.25532	-0.74662	1.01927
H	0.28078	-0.02262	1.82877
C	-1.00033	-0.93308	0.31098
N	-1.10162	-2.24842	-0.01215
H	1.53135	-2.49225	1.75199
C	-2.02978	-2.92030	-0.90272
H	-1.52787	-3.81682	-1.26893
H	-2.95476	-3.18219	-0.38484
H	-2.27079	-2.26180	-1.74119
N	-0.10108	-2.98856	0.57196
N	-1.82168	0.09875	-0.07701
H	-1.32238	1.00095	-0.07548
C	-3.19814	0.18169	-0.05060
N	-3.68485	1.40319	-0.35691
N	-3.91450	-0.90673	0.27688
C	-5.01006	1.52224	-0.32943
C	-5.24121	-0.74666	0.28906
C	-5.86325	0.46244	-0.01022
H	-6.94101	0.57564	0.00679
H	-5.40603	2.50765	-0.57202
H	-5.82384	-1.62751	0.55556
N	1.49108	0.09588	-0.02723
C	2.61992	-0.62733	-0.39440
C	1.45262	1.45552	-0.06465
C	2.80100	-1.49442	-1.46470
H	2.08776	-1.69732	-2.24986
N	3.76381	-0.71349	0.36389
N	0.26228	2.04903	0.22282
N	2.58213	2.13139	-0.39889
C	4.08809	-2.02864	-1.27977
H	4.61823	-2.74571	-1.89354
C	4.12093	0.06565	1.53269
N	4.67458	-1.54845	-0.18044
C	0.22012	3.38303	0.17115
C	2.48974	3.45597	-0.46033
H	4.92298	-0.46414	2.04798
H	4.45483	1.06655	1.24287
H	3.25662	0.16002	2.19625
C	1.31841	4.16265	-0.17353
H	1.26744	5.24395	-0.22229

H	-0.74403	3.83365	0.40222
H	3.40121	3.98135	-0.74591

SCF energy: -1167.770036 hartree  
zero-point correction: +0.336050 hartree  
enthalpy correction: +0.359093 hartree  
free energy correction: +0.281479 hartree  
quasiharmonic free energy correction:  
+0.286736 hartree

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**C-N X1• X2 radical addition (conformer 2)**

C	-0.80488	-1.94108	-1.38179
C	-0.34797	-0.57348	-1.10184
H	-0.29253	0.16566	-1.89831
C	0.88145	-0.85833	-0.36941
N	0.90990	-2.19170	-0.10589
H	-1.67220	-2.22646	-1.96064
C	1.79909	-2.94879	0.75471
H	1.25867	-3.84328	1.06755
H	2.71841	-3.22026	0.23146
H	2.05842	-2.34662	1.62946
N	-0.10193	-2.85613	-0.75533
N	1.74825	0.10410	0.09399
H	1.29310	1.02813	0.13826
C	3.12706	0.12377	0.06709
N	3.67110	1.30073	0.44448
N	3.79193	-0.97593	-0.32592
C	5.00044	1.35888	0.42035
C	5.12464	-0.87745	-0.33272
C	5.80292	0.28123	0.03591
H	6.88481	0.34461	0.02230
H	5.44238	2.30840	0.72008
H	5.66534	-1.76721	-0.65270
N	-1.57466	0.26224	-0.10344
C	-2.83190	-0.34558	-0.10285
C	-1.46696	1.62872	-0.04899
C	-3.93013	-0.25728	-0.95252
H	-4.05418	0.43161	-1.77460
N	-3.13325	-1.32198	0.81090
N	-2.58152	2.36780	0.15761
N	-0.21802	2.14796	-0.17610
C	-4.83570	-1.21188	-0.45978
H	-5.82561	-1.45505	-0.82480
C	-2.29216	-1.83783	1.87335
N	-4.35152	-1.86288	0.60215
C	-2.42009	3.68414	0.24604
C	-0.09871	3.47657	-0.09929
H	-1.72145	-2.70784	1.53035
H	-1.60125	-1.05486	2.19160
H	-2.93713	-2.12955	2.70386

C	-1.18240	4.32005	0.11390
H	-1.07307	5.39614	0.18089
H	-3.32699	4.26122	0.42706
H	0.91307	3.86586	-0.20195

SCF energy: -1167.770553 hartree  
zero-point correction: +0.336279 hartree  
enthalpy correction: +0.359165 hartree  
free energy correction: +0.282315 hartree  
quasiharmonic free energy correction:  
+0.287167 hartree

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**C-C X1• X2 radical addition**

C	0.12653	-1.72953	1.17031
C	-0.61613	-0.59448	0.58676
H	-0.40029	0.41450	0.93180
C	-1.97697	-1.09811	0.70438
N	-1.90165	-2.41396	1.06980
H	1.19587	-1.76078	1.33825
C	-2.98301	-3.35663	1.24326
H	-3.48061	-3.58254	0.29049
H	-2.54447	-4.27396	1.63560
H	-3.72744	-2.98145	1.95792
N	-0.61387	-2.78389	1.38616
N	-3.18694	-0.54980	0.33254
H	-3.99267	-1.15929	0.26528
C	-3.50819	0.77828	0.13241
N	-4.80373	0.99023	-0.17704
N	-2.54236	1.70149	0.25417
C	-5.14438	2.26185	-0.37769
C	-2.92302	2.96503	0.04272
C	-4.23213	3.31704	-0.28023
H	-4.52517	4.34671	-0.44800
H	-6.18901	2.44281	-0.62640
H	-2.14172	3.71704	0.13694
C	-0.12727	-0.30010	-1.14129
H	-0.76702	0.55002	-1.36241
C	-0.18823	-1.49779	-1.98081
C	1.33635	-0.04641	-1.06157
H	-1.07478	-2.00261	-2.34602
N	0.98468	-1.99722	-2.23281
N	1.91835	-1.14814	-1.65111
N	1.85844	1.00199	-0.49784
C	3.32024	-1.38100	-1.93748
C	3.09332	1.06462	0.08718
H	3.84979	-1.73459	-1.04892
H	3.36694	-2.12634	-2.73258
H	3.79119	-0.45304	-2.27937
N	3.72229	2.26460	0.03156
N	3.55212	-0.01978	0.77132

C	4.86630	2.36826	0.70162
C	4.69771	0.13362	1.43808
C	5.41945	1.32455	1.44765
H	6.34828	1.43344	1.99656
H	5.36672	3.33564	0.64321
H	5.04954	-0.73924	1.98876

SCF energy: -1167.765018 hartree  
zero-point correction: +0.335008 hartree  
enthalpy correction: +0.358282 hartree  
free energy correction: +0.279746 hartree  
quasiharmonic free energy correction:  
+0.285541 hartree

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**pyrimidine**

C	-3.34268	-0.55724	-0.78331
C	-2.22126	-1.38928	-0.60348
H	-2.06150	-2.39831	-0.95518
C	-1.33621	-0.61354	0.12924
N	-1.95230	0.58405	0.35616
H	-4.26623	-0.77318	-1.30484
C	-1.42208	1.74475	1.04546
H	-2.26982	2.36509	1.33756
H	-0.87248	1.42196	1.93390
H	-0.74677	2.30599	0.39351
N	-3.17406	0.63860	-0.21667
N	-0.07969	-0.93242	0.66717
H	0.00582	-1.80326	1.17535
C	1.13056	-0.43357	0.23075
N	1.12079	0.62846	-0.59117
N	2.21501	-1.07584	0.70878
C	2.32112	1.07251	-0.97410
C	3.39269	-0.59916	0.30690
C	3.51632	0.49227	-0.55470
H	4.48168	0.86589	-0.87597
H	2.31813	1.93091	-1.64453
H	4.27215	-1.11556	0.68965

SCF energy: -584.222959 hartree  
zero-point correction: +0.174300 hartree  
enthalpy correction: +0.185962 hartree  
free energy correction: +0.136754 hartree  
quasiharmonic free energy correction:  
+0.137950 hartree

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**pyrimidine radical**

C	-3.56553	-0.37618	-0.30747
C	-2.56152	-1.32020	-0.39831
H	-2.61345	-2.35882	-0.68852
C	-1.35370	-0.63860	-0.04557
N	-1.76415	0.66934	0.24808
H	-4.62436	-0.47688	-0.50417
C	-0.99856	1.77265	0.80899
H	-1.72121	2.49237	1.19414
H	-0.36760	1.41316	1.62718
H	-0.36593	2.22816	0.04562
N	-3.08809	0.82134	0.10789
N	-0.16681	-1.20377	0.02301
C	1.05224	-0.56974	-0.00486
N	1.21448	0.51740	-0.79587
N	2.03253	-1.18346	0.69686
C	2.45814	0.98949	-0.90700
C	3.25056	-0.65883	0.59188
C	3.54051	0.43730	-0.22517
H	4.54474	0.83507	-0.32086
H	2.58661	1.84925	-1.56389
H	4.03265	-1.14349	1.17575

SCF energy: -583.572063 hartree  
zero-point correction: +0.160484 hartree  
enthalpy correction: +0.171874 hartree  
free energy correction: +0.122661 hartree  
quasiharmonic free energy correction:  
+0.123865 hartree

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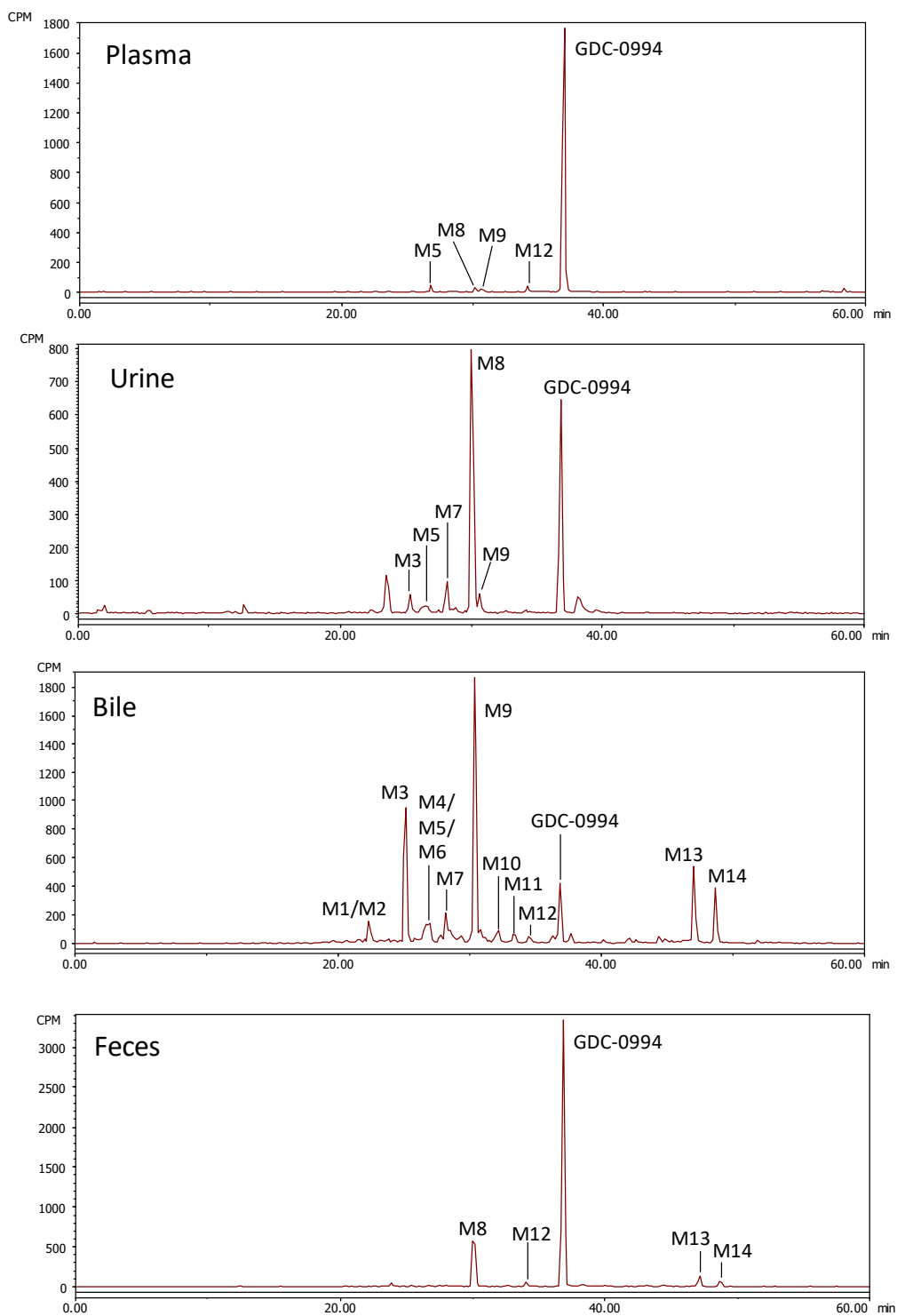
**water**

O	0.00000	-0.00000	0.11943
H	-0.00000	0.76262	-0.47770
H	-0.00000	-0.76262	-0.47770

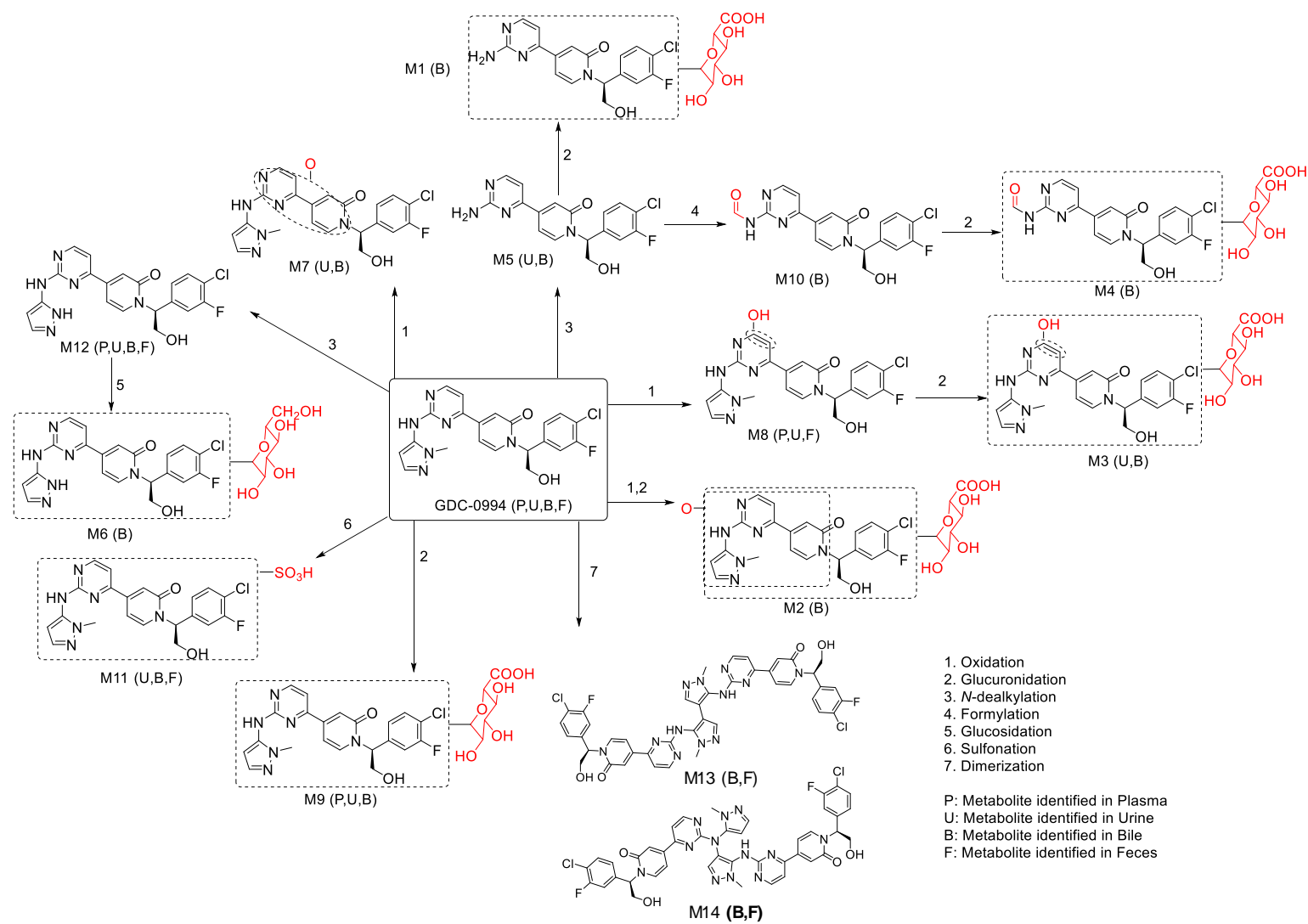
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zero-point correction: +0.021140 hartree  
enthalpy correction: +0.024919 hartree  
free energy correction: +0.003474 hartree  
quasiharmonic free energy correction:  
+0.003474 hartree

## Supplemental Figures

**Figure S1.** Radiochromatogram of male AUC<sub>0-8h</sub> pooled plasma, 0 to 48 hour pooled urine, bile and 0 to 72 hour pooled feces samples

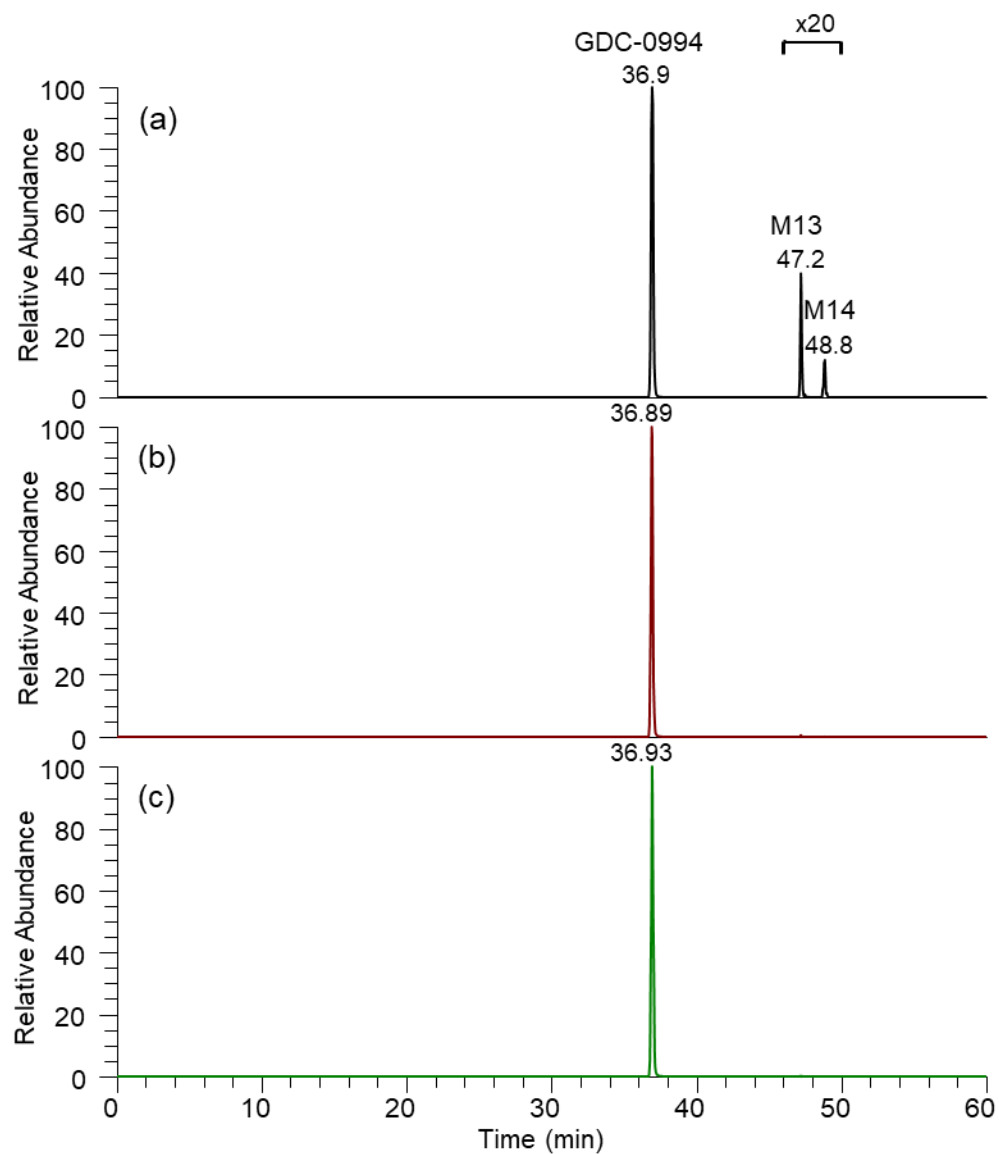


**Figure S2.** Proposed metabolic pathways of GDC-0994 in rats





**Figure S3.** (a) Extracted ion chromatogram of GDC-0994, **M13** and **M14** from the incubation of (a) GDC-0994 with human liver microsomes and NADPH, (b) GDC-0994 with human liver microsomes and NADPH after a preincubation with ABT, a pan P450 inhibitor, and (c) GDC-0994 with human liver microsome and NADPH after a preincubation with ketoconazole, a selective CYP3A inhibitor.



**Figure S4.** (a) Extracted ion chromatogram of GDC-0994, **M13** and **M14** from the incubation of GDC-0994 with horseradish peroxidase. (b) MS<sup>2</sup> and MS<sup>3</sup> product ion spectra of **M13**

