

## **Piperine is a Mechanism-based inactivator of CYP3A**

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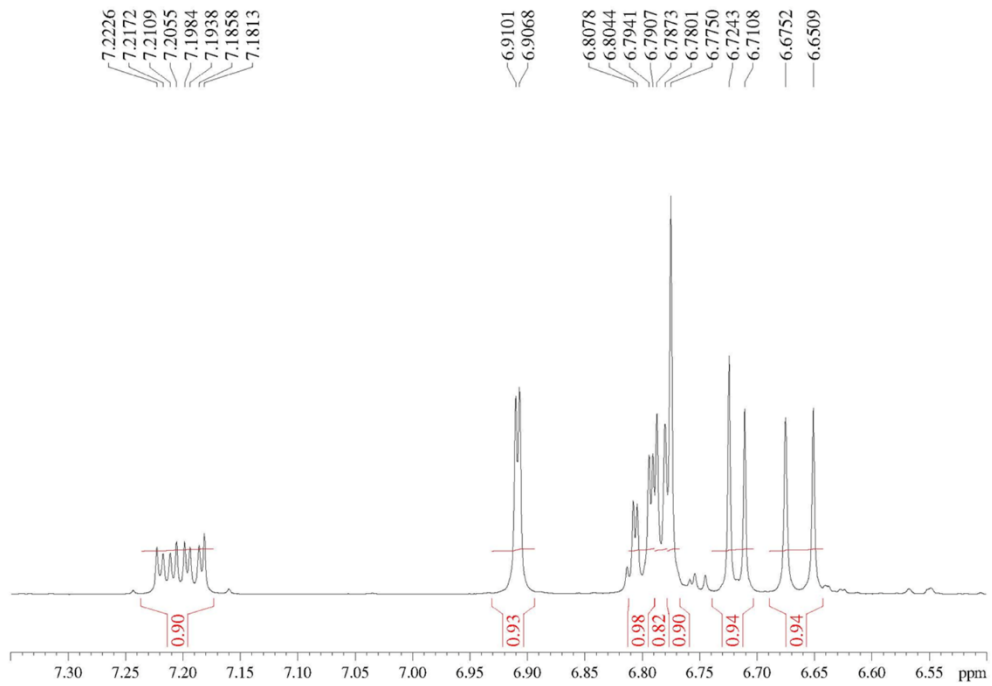
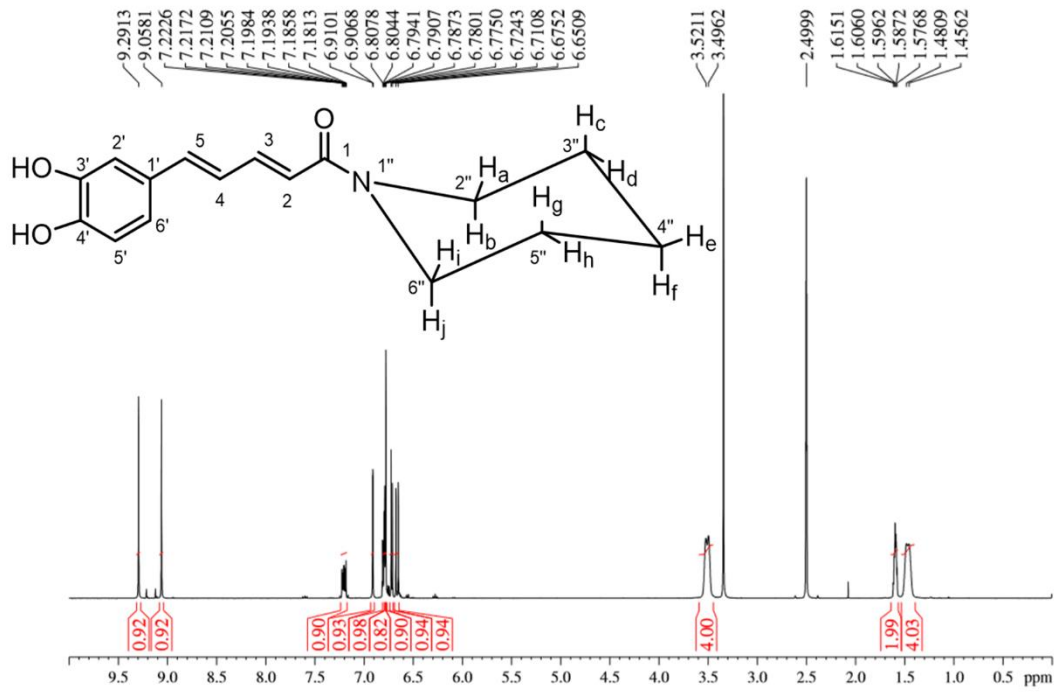
Supplemental Figure 1. <sup>1</sup>H NMR spectrum of synthetic DM-PPR.

Supplemental Figure 2. Characterization of PPR-cysteine adduct. A: Extracted ion ( $m/z$  407.0/159.0) chromatograms obtained from the analysis of proteolytic digestion of recombinant CYP3A4 protein after exposed to PPR in the absence of NADPH. B: Extracted ion chromatogram of MRM scans of  $m/z$  407.0/159.0 obtained from chemical synthesis. MS/MS spectrum of PPR-cysteine adduct generated in proteolytic digestion (C) and chemical synthesis (D) obtained from LC-MS/MS analysis.

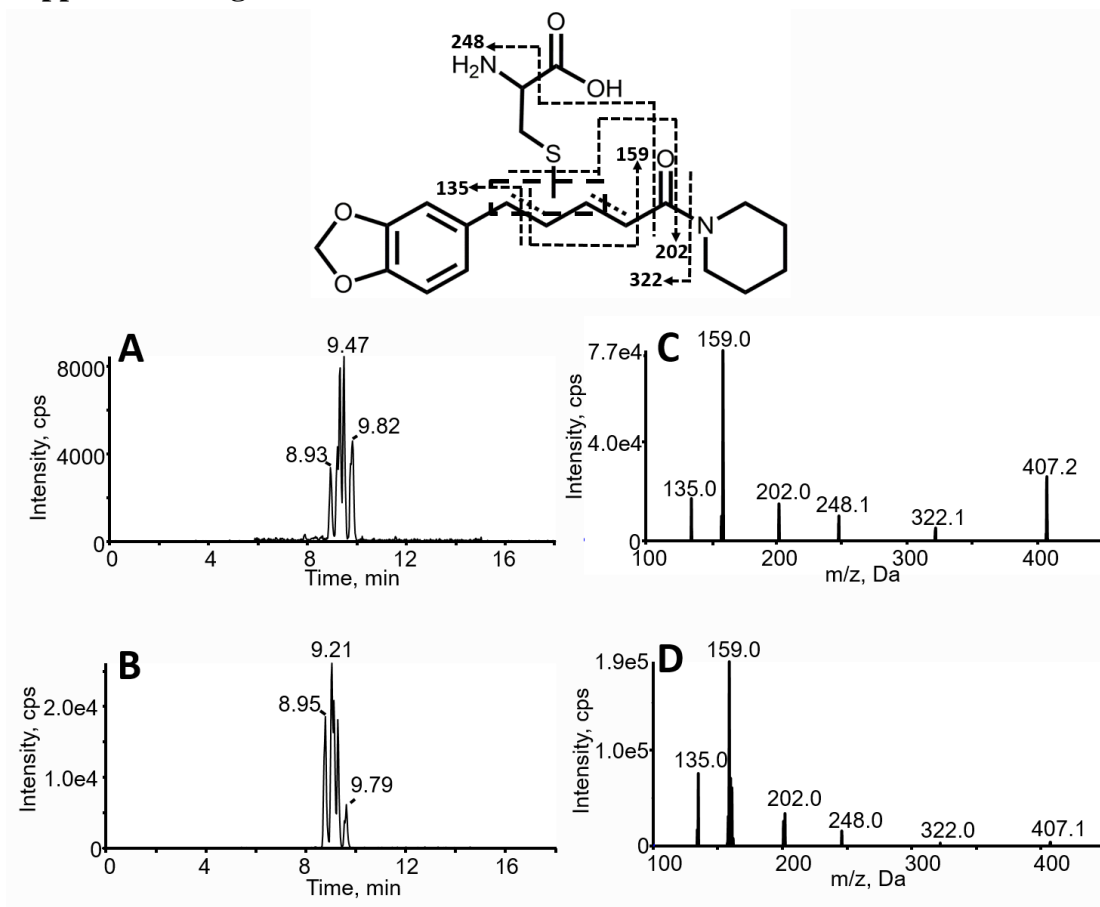
Supplemental Figure 3. Mass spectrometric characterization of DM-PPR-derived NAC conjugates M3-M6 and M7-M8. Extracted ion ( $m/z$  435.0/221.0 for M3-M6) (A) and ( $m/z$  437.0/181.0 for M7 and M8) (B) chromatograms obtained from LC-MS/MS analysis of incubation mixture containing DM-PPR (100  $\mu$ M), human liver microsomes, NAC, and NADPH.

Supplemental Table 1. Mass spectrometric data acquired from high-resolution mass spectrometric analysis of synthetic DM-PPR.

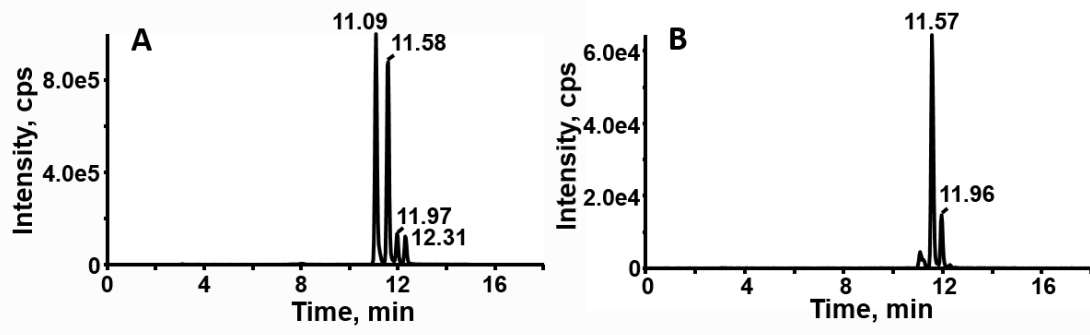
# Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



**Supplemental Table 1**

<b>Compound</b>	<b>formula</b>	<b>[M+H]<sup>+</sup></b>		<b>error</b>		
		<b>calculated</b>	<b>detected</b>	<b>ppm</b>	<b>mDa</b>	<b>mSigma</b>
DM-PPR	C <sub>16</sub> H <sub>20</sub> NO <sub>3</sub>	274.1438	274.1436	0.5	0.12	22.54

## Supplemental method

### Determination of protein modification

PPR (100  $\mu\text{M}$ ) was mixed with human recombinant CYP3A4 (50 nM) in PBS. The final volume of incubation was 0.5 mL. After 20 min of incubation at 37 °C, the protein samples were denatured by 30 min heating at 60 °C in a water bath, followed by centrifugation at 19,000 g for 10 min. The resulting pellets were suspended in 0.1 mL ammonium bicarbonate solution (final concentration: 50 mM, pH 8.0) and then mixed with 20  $\mu\text{L}$  DTT solution (final concentration: 5.0 mM). After incubation at 60 °C for 1 h, the resulting mixture was digested with a mixture of Pronase E (final concentration: 2.5 mg/mL) and chymotrypsin (final concentration: 2.5 mg/mL) in the presence of  $\text{CaCl}_2$  (5.0 mM). After 15 h incubation for 37 °C, the resultant mixtures were centrifuged at 19,000 g for 10 min, and the resulting supernatants were analyzed by LC-MS/MS. In a separate study, PPR (final concentration: 100  $\mu\text{M}$ ) was directly mixed with cysteine (final concentration: 20 mM) in 100 mM PBS with a total volume of 0.5 mL, followed by 30 min incubation and LC-MS/MS analysis.