Piperine is a Mechanism-based inactivator of CYP3A

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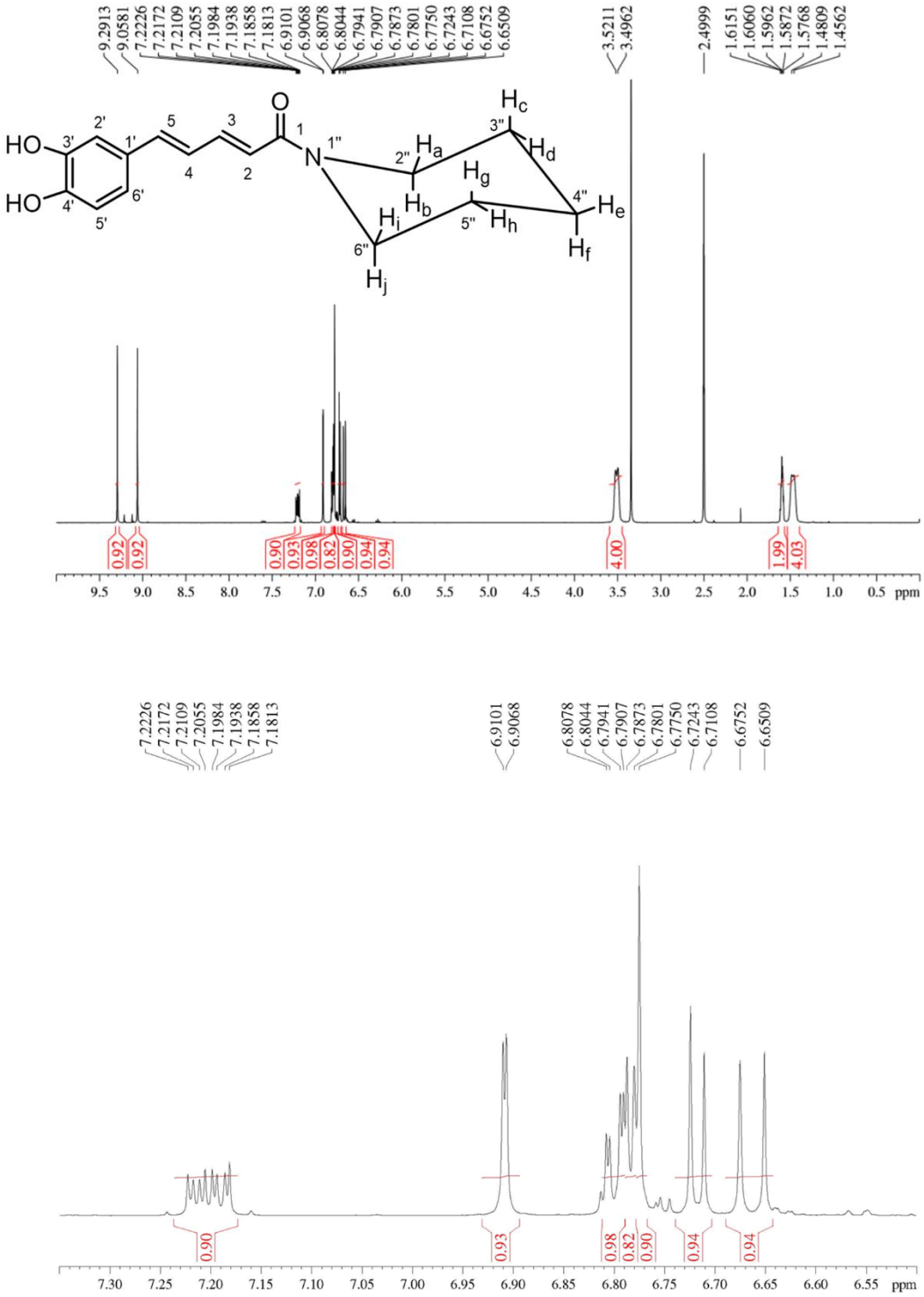
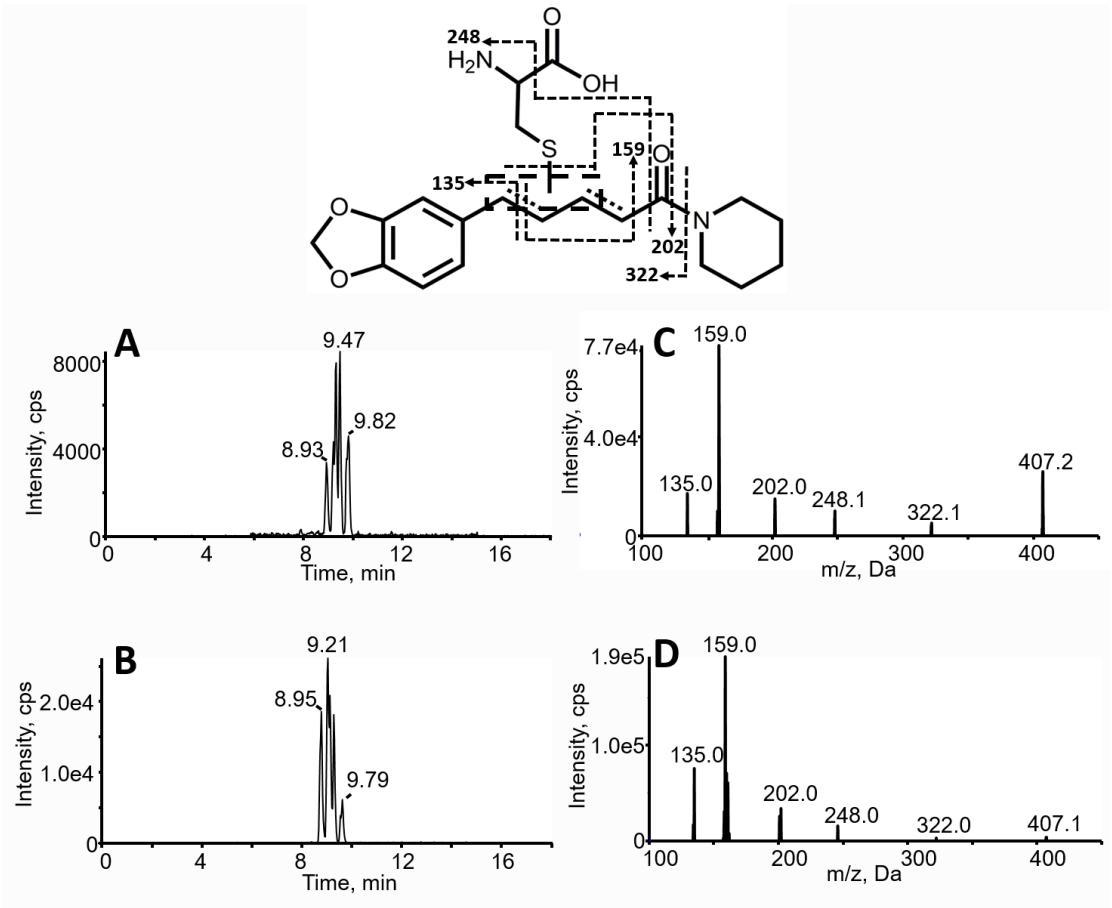
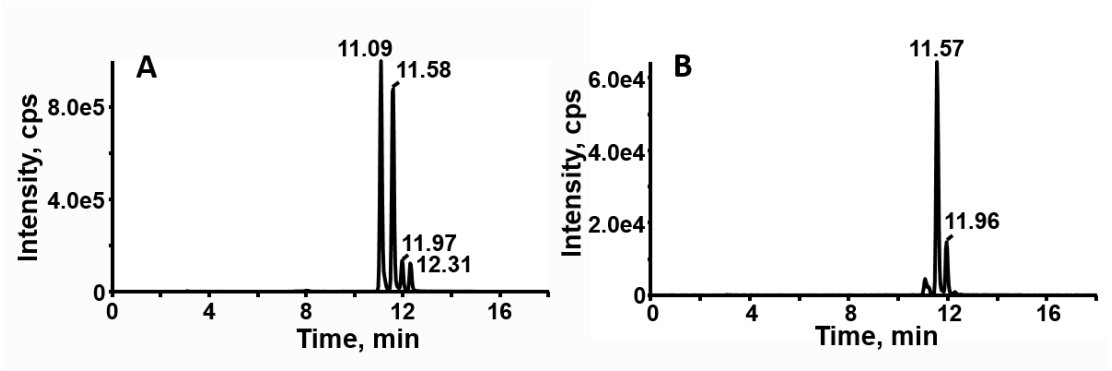
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Table 1

		$[M+H]^+$		error		
Compound	**formula**	**calculated**	**detected**	**ppm**	**mDa**	**mSigma**
DM-PPR	$C_{16}H_{20}NO_3$	274.1438	274.1436	0.5	0.12	22.54

Supplemental method

Determination of protein modification

PPR (100 μM) 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 μL 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 $CaCl_2$ (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 μM) 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.