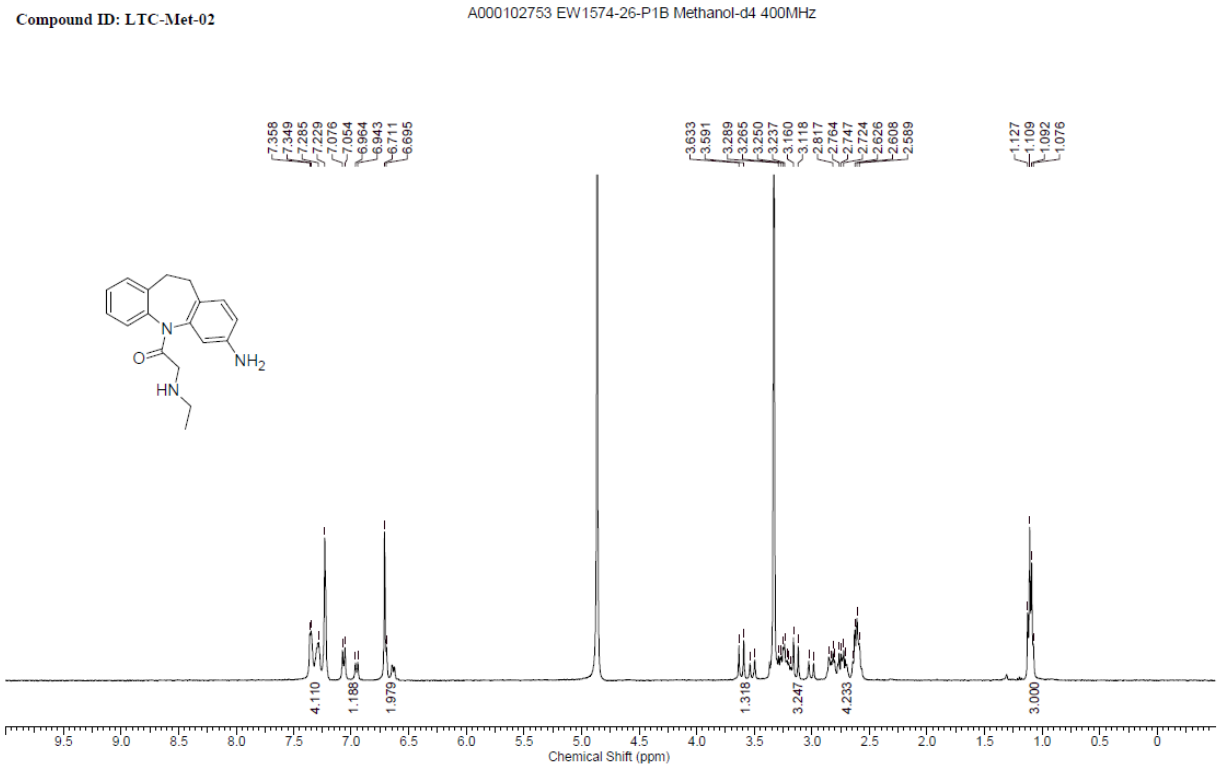
Supplemental Information

Identification and characterization of CINPA1 metabolites facilitates structure-activity studies of the constitutive androstane receptor

Milu T. Cherian, Lei Yang, Sergio C. Chai, Wenwei Lin, and Taosheng Chen

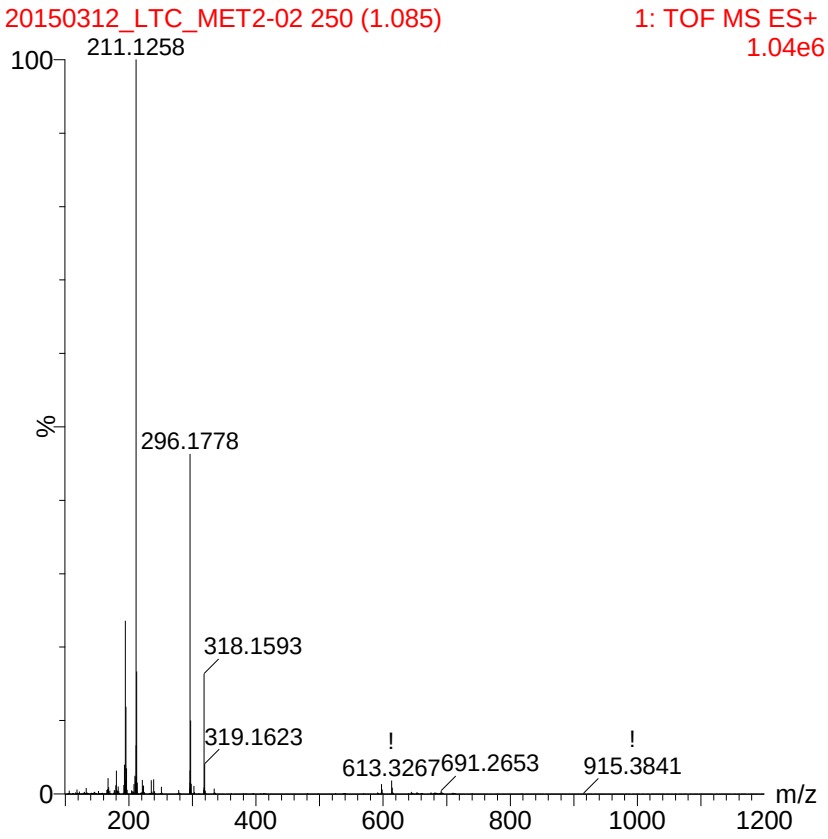
Department of Chemical Biology & Therapeutics, St. Jude Children's Research Hospital, Memphis, Tennessee, United States

Drug Metabolism and Disposition



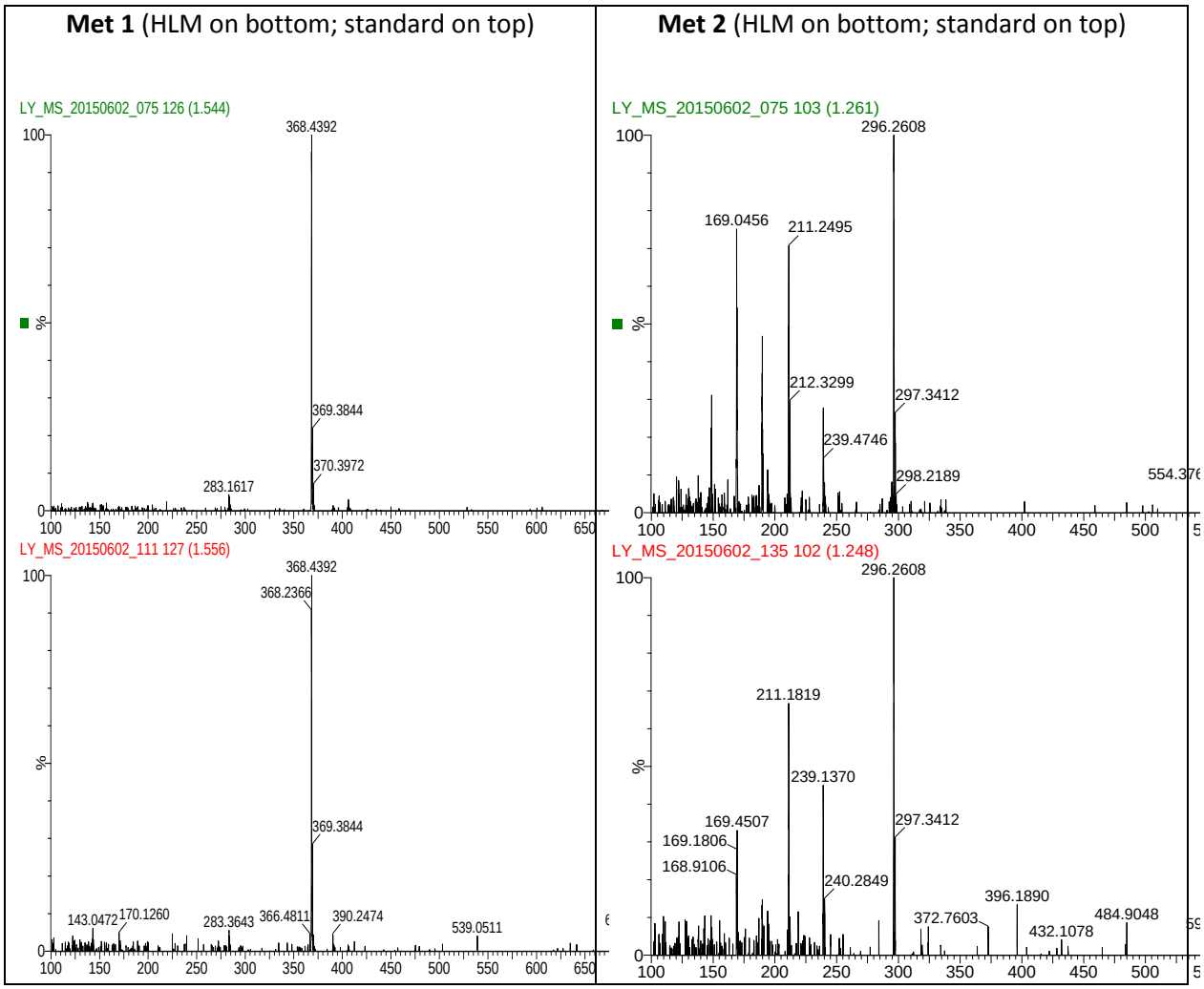
Supplemental Figure 1: ^{1}H NMR spectrum of Met2.

The ^{1}H NMR spectrum was recorded on a Bruker UltraShield 400 PLUS system. The chemical shift values are expressed in ppm relative to tetramethylsilane as the internal standard.



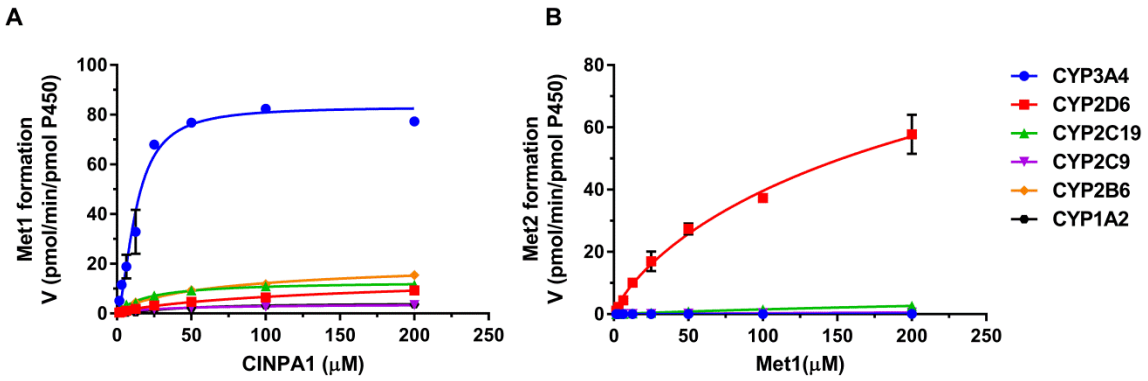
Supplemental Figure 2: ESI-TOF HRMS of Met2.

The ESI-TOF HRMS of Met2 was recorded using Waters Acquity UPLC-Xevo G2 QTOF system.



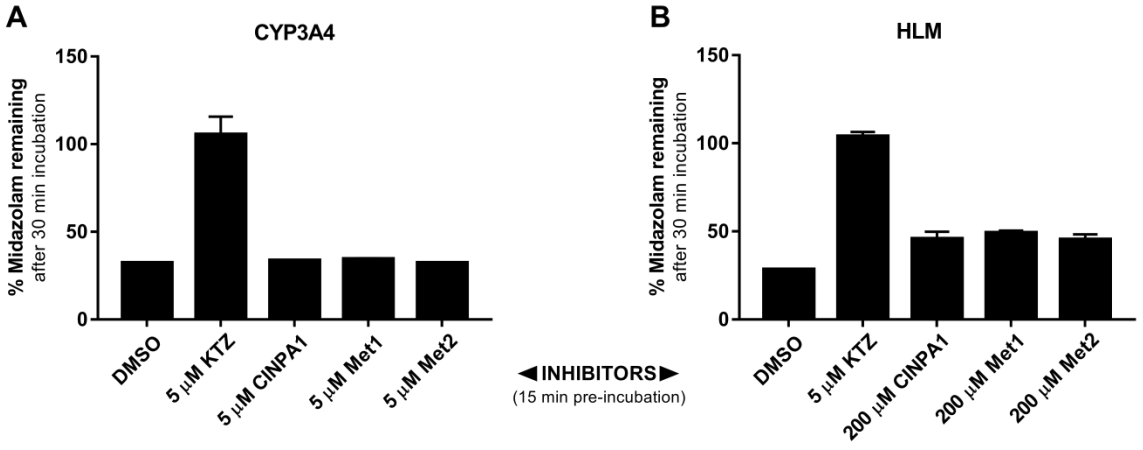
Supplemental Figure 3: MS spectra of the metabolites identified in HLM compared to the analytic standards of Met1 and Met2.

The MS spectra of the metabolites identified from HLM lysates were compared to analytic standards of Met1 (obtained from ChemBridge Corp., San Diego, CA; Catalog # 5214995) and Met2 (synthesized as described in Materials and Methods). MS was performed using a Waters ACQUITY-UPLC–SQD–UV system.



Supplemental Figure 4: Kinetics of metabolite formation from CINPA1 or Met1 in recombinant human P450s.

An increasing concentration of (A) CINPA1 or (B) Met1 (0–200 µM) was incubated with the indicated recombinant human cytochrome P450s and an NADPH regenerating system at 37°C for 30 min. The velocity of metabolite formation (pmol of product/min/pmol of P450) versus the substrate concentration was fitted to a Hill equation (see Materials and Methods section) to obtain the kinetic parameters for each enzyme, as represented in Table 3 of the main text. Each point represents the average ± SD of triplicate reaction wells. Error bars smaller than the data points are not visible.



Supplemental Figure 5: Metabolism of midazolam in human recombinant CYP3A4 or HLM in the presence of inhibitors.

(A) CYP3A4 enzyme (41 pmol/ml) or (B) HLM (0.5 mg/ml) were preincubated with DMSO (control), CYP3A4-specific inhibitor ketoconazole (KTZ), CINPA1, Met1, or Met2 and an NADPH regenerating system at 37°C for 15 min. 5 µM of midazolam was added as substrate to reaction mixtures, which were then incubated for a further 30 min at 37°C. All reactions were terminated with ACN and analyzed by LC/MS. The enzyme activity was calculated by measuring the amount of substrate remaining in the reaction wells after 30 min incubation at 37°C. Midazolam concentrations at 0 min (pre-incubated with DMSO) were set as "100% midazolam remaining", and "% midazolam remaining" for each inhibitor after 30 min incubation at 37°C was calculated as percentage of the midazolam concentration at time 0. Each bar represents the average ± SD of triplicate reaction wells. Some error bars are too small to be visible.