Molecular Pharmacology

Supplemental Information

CINPA1 Is an Inhibitor of Constitutive Androstane Receptor (CAR) that Does Not Activate Pregnane X Receptor (PXR)

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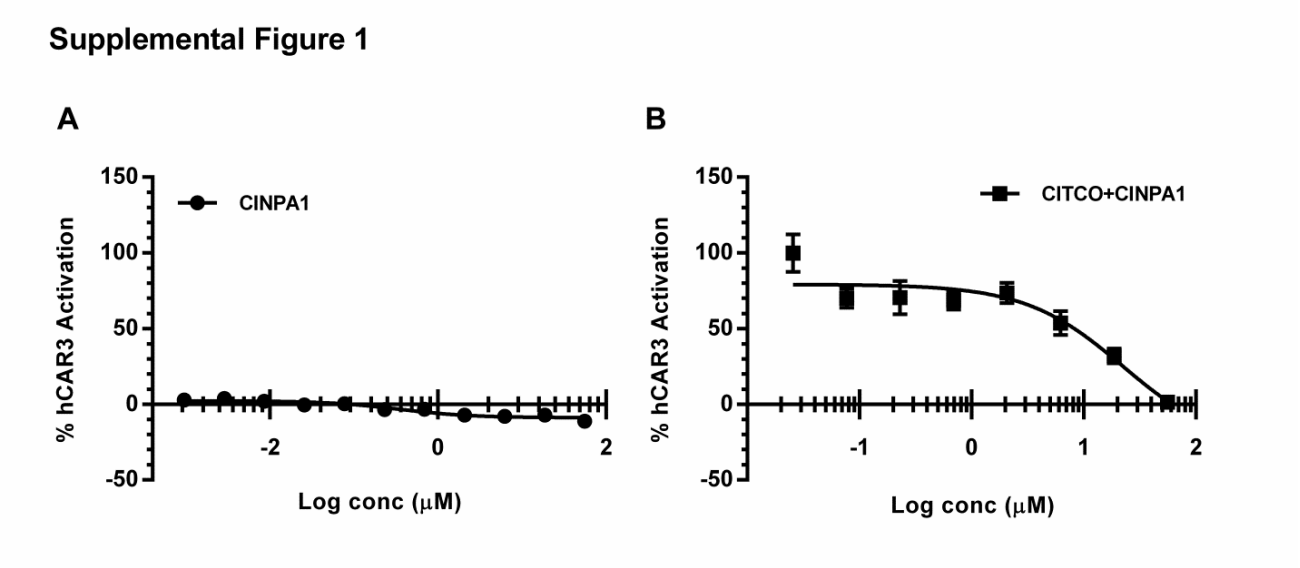
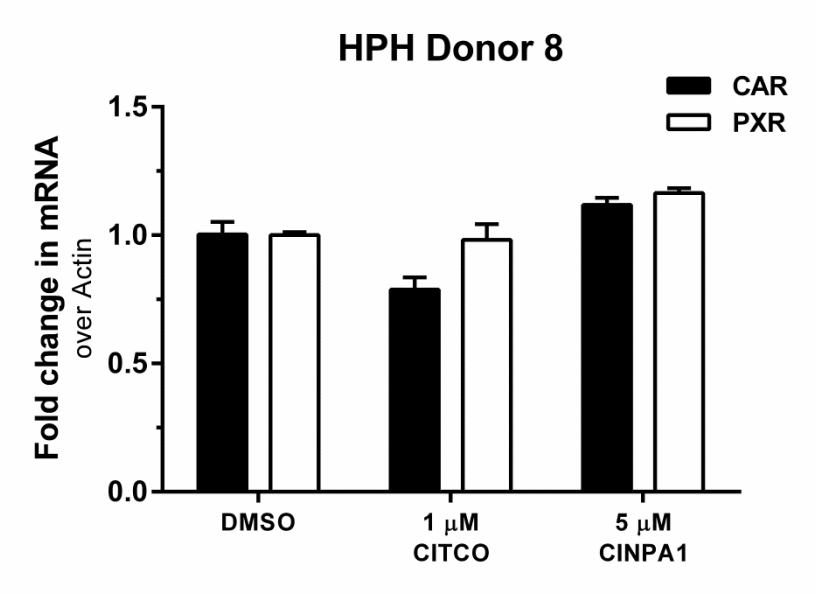
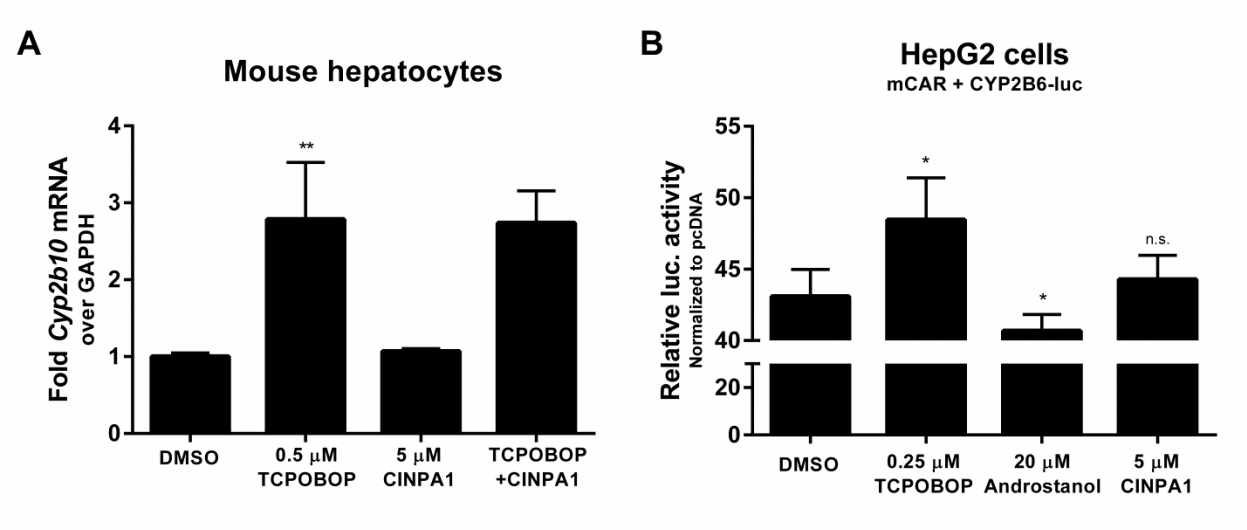


Supplemental Figure 1. CINPA1 acts as a weak antagonist of the hCAR3 isoform.
An hCAR3 expression construct was generated by inserting 5 amino acids (APYLT) at position 271 in the pcDNA-FLAG-hCAR1 plasmid. HepG2 cells were transfected with plasmids expressing hCAR3, RXRα and CYP2B6-luc reporter. After 24 h incubation, cells were treated with indicated concentrations of CINPA1, in the absence (A) or presence (B) of 2 μM CITCO. DMSO, 50 μM PK11195, or 2 μM CITCO was used as controls. Luciferase activity was measured 24 h after treatment by using SteadyLite reagent (PerkinElmer). A. Activity in DMSO-treated samples was set as 0% activation; activity in samples treated with 2 μM CITCO was set as 100% activation. B. Activity in samples treated with 50 μM PK11195 was set as 0% CAR activation and samples treated with 2 μM CITCO was set as 100% activation. GraphPad Prism was used to fit the data into a dose-response stimulation equation to derive IC_{50} values. CINPA1 inhibits hCAR3 with an IC_{50} of 18 μM and shows no agonistic effect on hCAR3 activation.



Supplemental Figure 2. CINPA1 does not alter CAR or PXR mRNA levels in human hepatocytes.

Human primary hepatocytes (Donor 8) were treated with DMSO, 1 μM CITCO, or 5 μM CINPA1 for 48 h. RNA was extracted and used for cDNA synthesis, followed by quantitative real-time PCR assays with Taqman probes. β-actin was used as the internal control. No significant change was observed in CINPA1 treated hepatocytes when compared to DMSO treated samples.



Supplemental Figure 3. CINPA1 does not affect mouse CAR.

A. Mouse hepatocytes were isolated from C57Bl/6 mouse liver (TRL Cat # MBF06OL or MBFS1M; Triangle Research Labs, LLC, Research Triangle Park, NC) and plated in 6-well plates in Hepatocyte Maintenance Medium (TRL Cat # MM250). Cells were treated with DMSO, 0.5 μM TCPOBOP (mouse CAR agonist), 5 μM CINPA1, or 0.5 μM TCPOBOP + 5 μM CINPA1 for 24 h. RNA was extracted and used for cDNA synthesis, followed by performing quantitative real-time PCR assays with mouse-specific Taqman probes. GAPDH was used as the internal control. Statistical analysis was performed using one-way ANOVA for multiple comparisons and **, $p < 0.01$. While TCPOBOP significantly induced Cyp2b10 mRNA, no significant change was detected with 5 μM CINPA1 treatment on basal (DMSO) or TCPOBOP-induced Cyp2b10. B. Plasmids expressing CYP2B6-luc reporter and pTK-Renilla-luciferase were cotransfected with pcDNA (control plasmid) or pCR3-mCAR [kind gift from Dr. Hongbing Wang, (Li et al., 2008)] in HepG2 cells. After 24 h incubation, cells were treated with DMSO, 0.25 μM TCPOBOP (mCAR agonist), 20 μM androstanol, or 5 μM CINPA1. Luciferase activity was measured 24 h after treatment by using Dual-Glo luciferase reagents (Promega, Madison, WI). Relative luciferase activity was calculated as the firefly to renilla luciferase ratio for each treatment condition and normalized to pcDNA (control plasmid) transfected samples. *, $p < 0.05$. CINPA1 does not inhibit mCAR function in these assays.

Reference:

Li L, Chen T, Stanton JD, Sueyoshi T, Negishi M and Wang H (2008) The peripheral benzodiazepine receptor ligand 1-(2-chlorophenyl-methylpropyl)-3-isoquinoline-carboxamide is a novel antagonist of human constitutive androstane receptor. *Mol Pharmacol* **74**(2): 443-453.