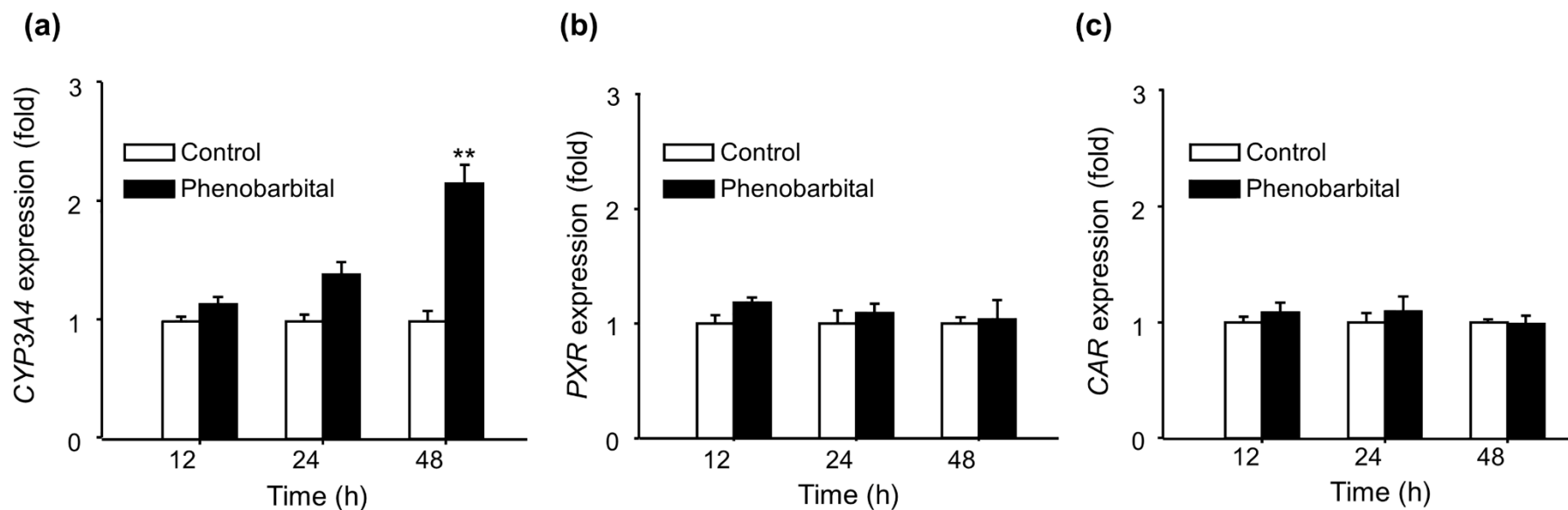
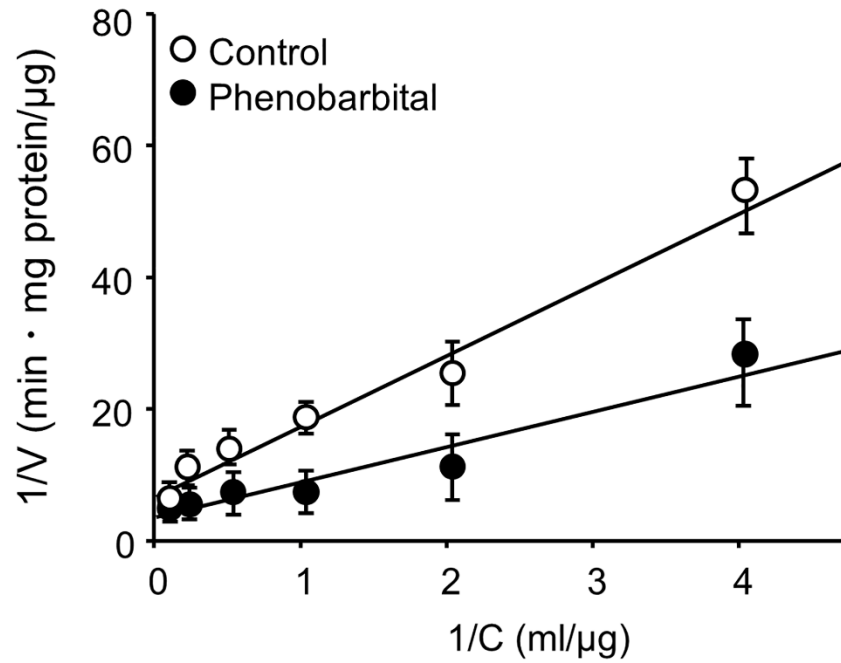


Supplemental Figure 1. Lineweaver-Burk plot of MDZ (a) and 4-NP (b) in 2D or 3D cultured HepG2 cells. Each bar represents the mean \pm S.D. for four experiments.

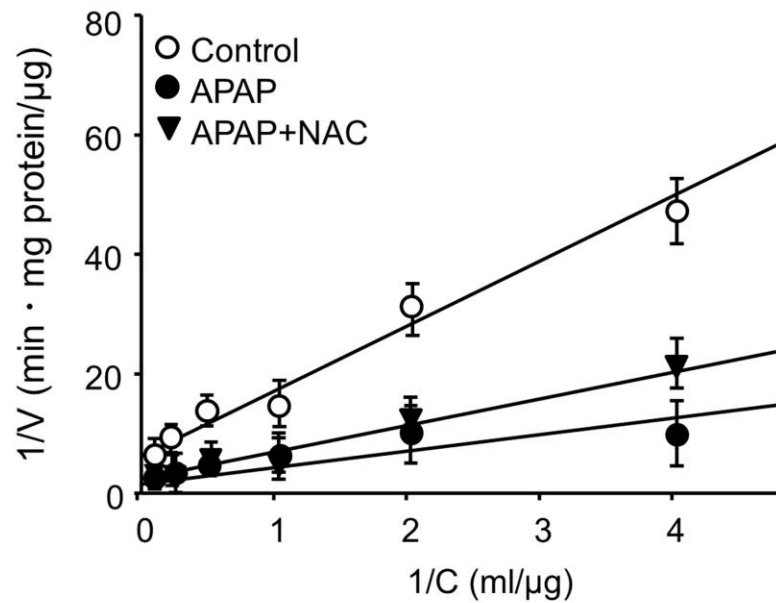


Supplemental Figure 2. Effect of phenobarbital on relative mRNA expression of CYP3A4 (a), PXR (b) and CAR (c) in 3D cultures of HepG2 cells. HepG2 cells were treated with phenobarbital (250 μM) for 12, 24 and 48 hr. Each bar represents the mean + S.D. for four experiments. ** $P < 0.01$ compared to control.

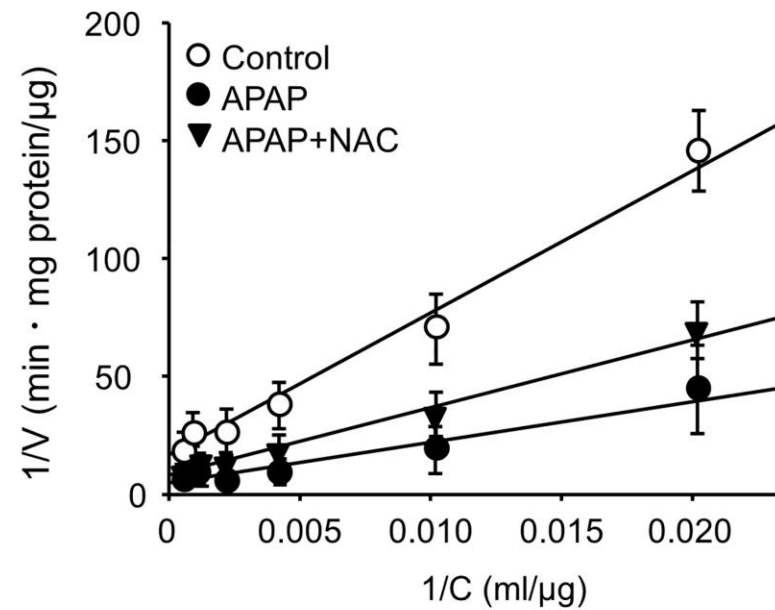


Supplemental Figure 3. The effect of phenobarbital treatment on Lineweaver-Burk plot of MDZ. 3D culture cells were treated with phenobarbital (250 μM) for 60 hr. Each bar represents the mean \pm S.D. for four experiments.

(a)

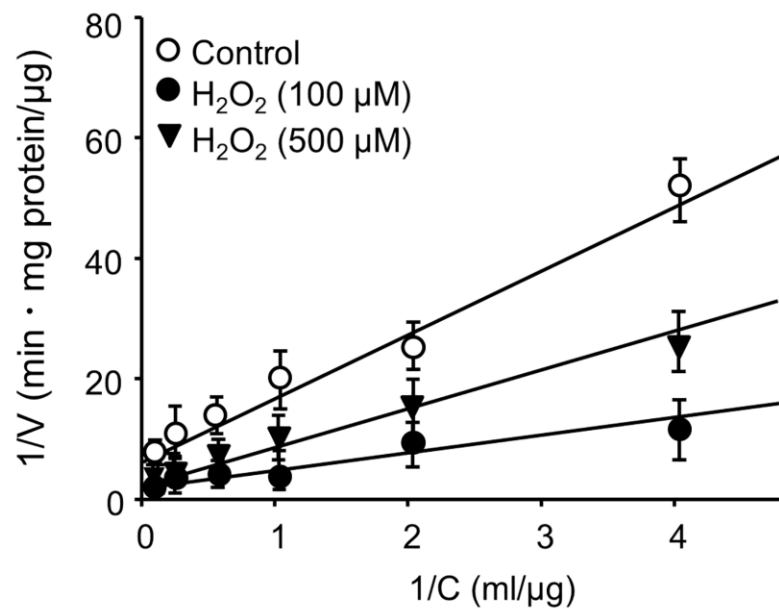


(b)

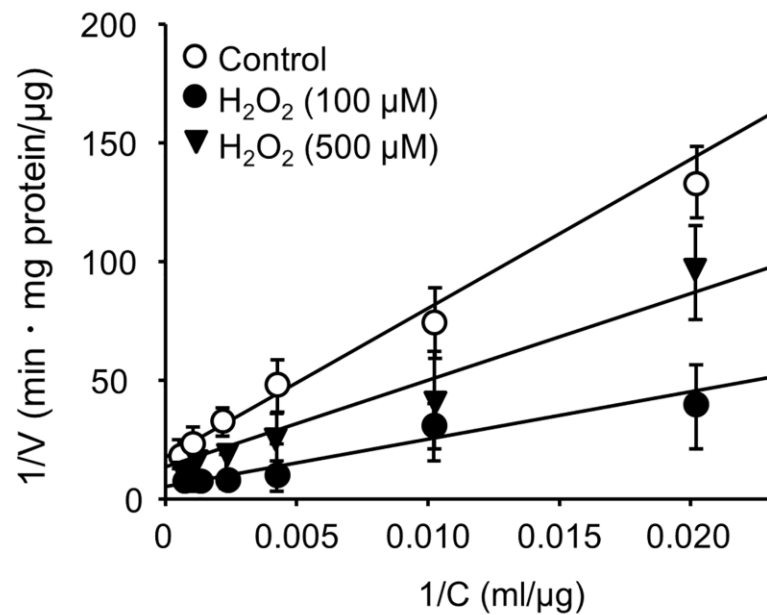


Supplemental Figure 4. The effect of APAP and NAC on Lineweaver-Burk plot of MDZ (a) and 4-NP (b) 3D cultured HepG2 cells were treated with 25 mM APAP in the presence or absence of 100 μ M NAC for 60 hr. Each bar represents the mean \pm S.D. for four experiments

(a)



(b)



Supplemental Figure 5. The effect of H_2O_2 on Lineweaver-Burk plot of MDZ (a) and 4-NP (b). 3D culture HepG2 cells treated with H_2O_2 (100 or 500 μM) for 36 hr. Each bar represents the mean \pm S.D. for four experiments

Supplemental Table 1. Primer sequences of the target genes used in this study.

Genes	Primer	Sequence
<i>β-actin</i>	Forward Reverse	5'-TGGACTTCGAGCAAGAGATGG-3' 5'-GGAAGGAAGGCTGGAAGAGTG-3'
<i>CYP3A4</i>	Forward Reverse	5'-CAATGGACTGCATAAATAACCG-3' 5'-GAGCCAAATCTACCTCCTCACA-3'
<i>CYP2E1</i>	Forward Reverse	5'-TTCAGCGGTTTCATCACCCCT-3' 5'-GAGGTATCCTCTGAAAATGGTGTC-3'
<i>UGT1A6</i>	Forward Reverse	5'-CATGATTGTTATTGGCCTGTAC-3' 5'-TCTGTGAAAAGAGCATCAAAC-3'
<i>PXR</i>	Forward Reverse	5'-GCAAGGGCTTTTTTCAGGA-3' 5'-TCTTCCGCTTGATCAAGG-3'
<i>CAR</i>	Forward Reverse	5'-ACATCAACACTTTCATGGTA-3' 5'-TCAGCTGCAGATCTCCTGGA-3'

Supplemental Table 2. Michaelis Menten enzyme kinetic parameters of MDZ and 4-NP in 2D or 3D cultured HepG2 cells.

	MDZ		4-NP	
	2D	3D	2D	3D
K_m ($\mu\text{g/ml}$)	1.84 ± 0.51	1.77 ± 0.57	360.3 ± 86	352.1 ± 77
V_{max} ($\mu\text{g/min/mg protein}$)	0.04 ± 0.01	0.16** ± 0.03	0.01 ± 0.005	0.05** ± 0.006
CL_{int} (ml/min/mg protein)	0.02 ± 0.006	0.09** ± 0.02	0.03×10^{-3} $\pm 0.007 \times 10^{-3}$	0.14×10^{-3} ** $\pm 0.02 \times 10^{-3}$

Each value is the mean S.D. of four experiments. ** $P < 0.01$ as compared with 2D cultures of HepG2 cells.

Supplemental Table 3. Michaelis Menten enzyme kinetic parameters of MDZ in 3D cultured HepG2 cells treated with phenobarbital.

	Control	Phenobarbital
K_m ($\mu\text{g/ml}$)	1.76 ± 0.51	1.77 ± 0.68
V_{max} ($\mu\text{g/min/mg protein}$)	0.163 ± 0.03	0.335* ± 0.06
CL_{int} (ml/min/mg protein)	0.09 ± 0.01	0.19* ± 0.03

Each value is the mean S.D. of four experiments. * $P < 0.05$ as compared with control.

Supplemental Table 4. Michaelis Menten enzyme kinetic parameters of MDZ and 4-NP in 3D cultured HepG2 cells treated with APAP in presence or absence of NAC.

	MDZ			4-NP		
	Control	APAP	APAP+NAC	Control	APAP	APAP+NAC
K_m ($\mu\text{g/ml}$)	1.75 ± 0.34	1.77 ± 0.51	1.79 ± 0.45	359.3 ± 81	348.22 ± 85	350.3 ± 90
V_{max} ($\mu\text{g/min/mg protein}$)	0.16 ± 0.02	0.64** ± 0.06	0.40** ± 0.05	0.06 ± 0.006	0.20** ± 0.03	0.12* ± 0.02
CL_{int} (ml/min/mg protein)	0.09 ± 0.01	0.36** ± 0.05	0.23* ± 0.04	0.17×10^{-3} $\pm 0.02 \times 10^{-3}$	0.58×10^{-3} ** $\pm 0.06 \times 10^{-3}$	$0.35 \times 10^{-3}\dagger$ $\pm 0.05 \times 10^{-3}$

Each value is the mean \pm S.D. of four experiments. * $P < 0.05$, ** $P < 0.01$ as compared with control. † $P < 0.05$ as compared with APAP-treated group.

Supplemental Table 5. Michaelis Menten enzyme kinetic parameters of MDZ and 4-NP in 3D cultured HepG2 cells treated with H₂O₂

	MDZ			4-NP		
	Control	H ₂ O ₂ (100 μM)	H ₂ O ₂ (500 μM)	Control	H ₂ O ₂ (100 μM)	H ₂ O ₂ (500 μM)
K _m (μg/ml)	1.76 ± 0.42	1.79 ± 0.55	1.77 ± 0.51	356.3 ± 79	360.0 ± 95	363.1 ± 88
V _{max} (μg/min/mg protein)	0.17 ± 0.02	0.36* ± 0.05	0.61** ± 0.06	0.06 ± 0.008	0.10 ± 0.01	0.20** ± 0.03
CL _{int} (ml/min/mg protein)	0.09 ± 0.01	0.20* ± 0.03	0.34** ± 0.05	0.16 × 10 ⁻³ ± 0.03 × 10 ⁻³	0.27 × 10 ⁻³ ± 0.07 × 10 ⁻³	0.55 × 10 ^{-3**} ± 0.06 × 10 ⁻³

Each value is the mean S.D. of four experiments. **P* < 0.05, ***P* < 0.01 as compared with control.