catalysts

MDPI

Supplementary Material

Supplementary material 1

The protocol of the expression and purifications of 7α-HSDH or 7β-HSDH.

The *E. coli* BL_{21} (DE_3) strains with 7α-HSDH or 7β-HSDH recombinant plasmids were cultured at 37 °C, 220 rpm in Luria-Bertani (LB) medium containing 100 mg mL^{-1} ampicillin. IPTG was added to a final concentration of 0.2 mM until the OD_{600} reached 0.7-0.8. The recombinant cells were harvested by centrifugation and wall-cracking achieved by sonication. Target protein in supernatant bound with Glutathione Sepharose 4B using GST tag. 7α-HSDH or 7β-HSDH was eluted from columns after prescission protease digestion. The purity of the recombinant protein was confirmed by 12 % polyacrylamide gel electrophoresis (SDS-PAGE). Protein concentration was determined using the BCA protein assay Kit.

Supplementary material 2

The synthetic procedure of T-7-KLCA

To a magnetically stirred solution of 7-KLCA (0.25 mmol) in dry dimethylformamide (DMF; 1 mL) were added successively powdered taurine (0.5 mmol), diethyl phosphorocyanidate (DEPC 0.3 mmol), and anhydrous triethylamine (Et_3N 0.4 mL), and the resulting suspension was stirred at room temperature for 60 min (the reaction was monitored by HPLC). The reaction mixture was adjusted to pH 12–14 with 1 M NaOH and then to pH 7–8 with 10% HCl. The solution was diluted with water (9 mL), passed through a preconditioned Sep-PakVac tC18 cartridge, and eluted successively with water (20 mL), 25% CH_3CH_2OH (20 mL), and CH_3CH_2OH (25 mL). The last fraction containing the desired taurine conjugate sodium salt was evaporated to dryness under a nitrogen stream, and the residue was recrystallized from an appropriate solvent. (Reference: T. Momose, T. Tsubaki, T. Iida, T. Nambara, An improved synthesis of taurine- and glycine-conjugated bile acids, Lipids, 32 (1997) 775-778.)

Supplementary material 3

The fitting formulas for TCDCA, T-7-KLCA and TUDCA

Table S1 The fitting formulas for TCDCA, T-7-KLCA and TUDCA

Bile acid	**Fitting formula**	R^2
TCDCA	$y = 1.60x + 3.80$	0.9998
T-7-KLCA	$y = 1.53x + 4.73$	0.9999
TUDCA	$y = 1.61x + 4.46$	0.9989

TCDCA, T-7-KLCA and TUDCA were dissolved in methanol and analyzed by HPLC-ELSD with at following different concentrations: 0.12 mg·mL^{-1}, 0.24 mg·mL^{-1}, 0.36 mg·mL^{-1}, 0.48 mg·mL^{-1} and 0.60 mg·mL^{-1}. The constant flow rate was 0.8 mL·min^{-1}, and a linear gradient elution was used.

Supplementary material 4

Table S2 The low energy comformation of combination between bilirubin and 7α-HSDH

Number	Binding energy (kcal/mol)	Van der Waals force + Hydrogen bond + Desolvation energy (kcal/mol)	Electrostatic interaction (kcal/mol)
1	-4.71	-6.17	-0.77
2	-4.67	-5.03	-1.64
3	-4.6	-5.17	-1.9
4	-4.55	-4.46	-2.33
5	-4.39	-5.65	-0.01
6	-4.35	-5.27	-1.31
7	-4.31	-4.38	-1.25
8	-4.27	-4.21	-1.57
9	-4.16	-5.3	-0.96
10	-4.07	-4.87	-1.13

Table S3 The low energy comformation of combination between bilirubin and 7β-HSDH

Number	Binding energy (kcal/mol)	Van der Waals force + Hydrogen bond + Desolvation energy	Electrostatic interaction

		(kcal/mol)	(kcal/mol)
1	-5.79	-5.57	-1.69
2	-5.62	-4.72	-1.58
3	-5.39	-5.41	-1.55
4	-4.97	-5.41	-1.61
5	-4.91	-5.27	-1.18
6	-4.68	-5.42	-0.86
7	-4.83	-4.81	-2.31
8	-4.8	-4.66	-2.18
9	-4.74	-4.32	-2.6
10	-4.32	-3.67	-2.63

Supplementary material 5

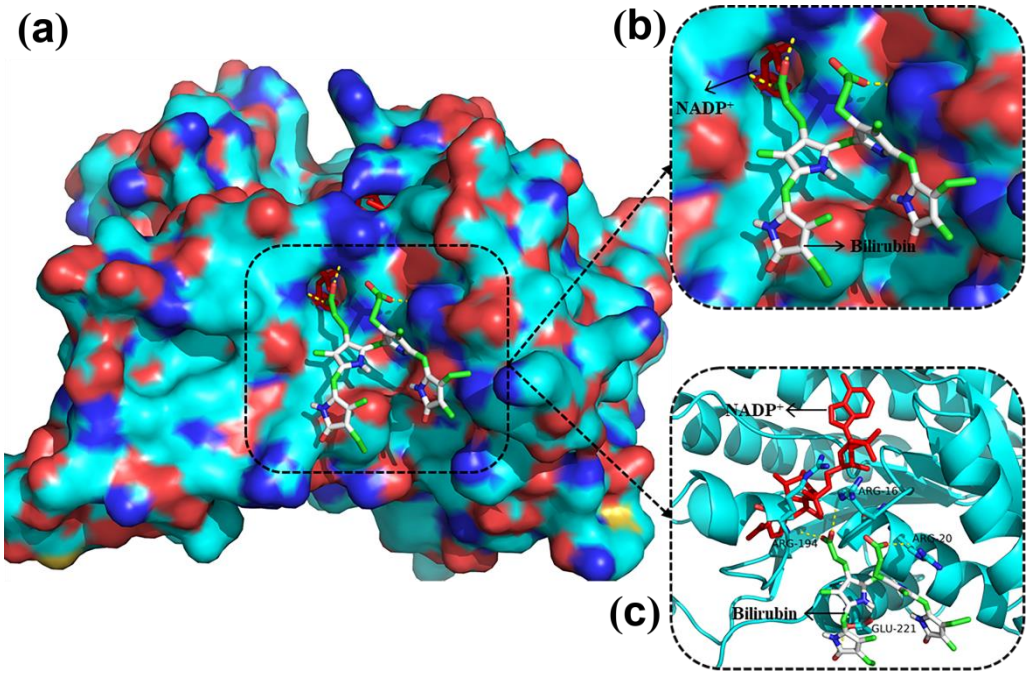

Figure S1. The docking simulation of bilirubin and 7α-HSDH. **(a)** The overview of combination between bilirubin and 7α-HSDH; **(b)** The relative positions of cofactor $NADP^+$ and bilirubin; **(c)** The specific amino acid residues of 7α-HSDH

combined with bilirubin. Molecular docking was performed on autodock 4.2 and the results were analyzed by VMD1.8.3.

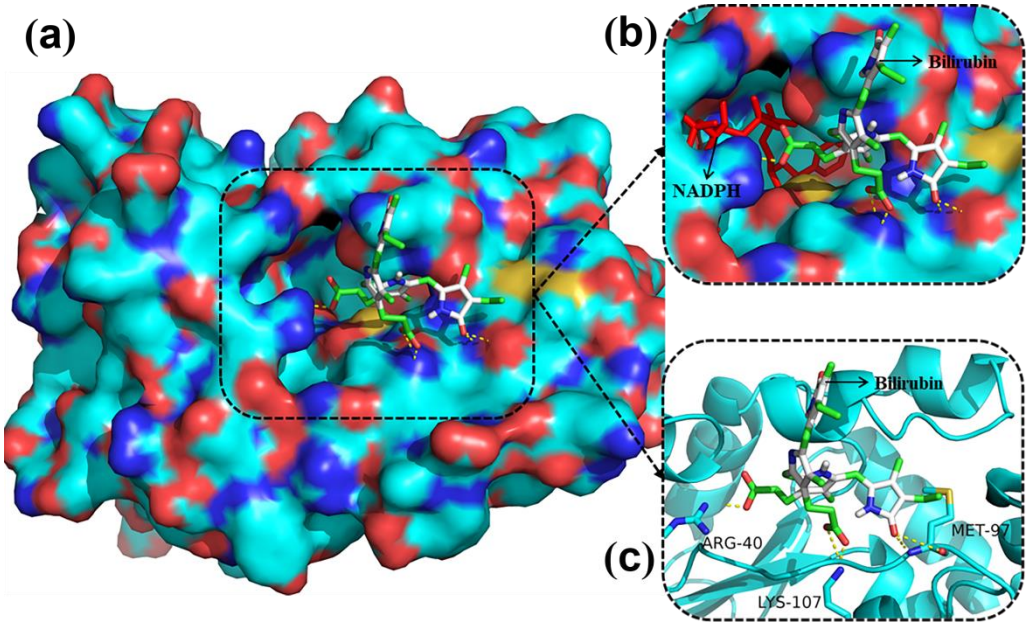

Figure S2. The docking simulation of bilirubin and 7β-HSDH. **(a)** The overview of combination between bilirubin and 7β-HSDH; **(b)** The relative positions of cofactor NADPH and bilirubin; **(c)** the specific amino acid residues of 7β-HSDH combined with bilirubin. Molecular docking was performed on autodock 4.2 and the results were analyzed by VMD1.8.3.