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Supporting information

Electrochemically Driven Drug Metabolism and Inhibition *via* Cytochrome P450 2C9 Isozyme Microsomes with Cytochrome P450 Reductase and Indium Tin Oxide Nanoparticle Composites

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Experimental Section

Reagents: Cytochrome P450 2C9 (CYP2C9) isozyme microsomes with CYP reductase (CPR) expressed in baculovirus infected Sf9 cells (≥80 pM/mg protein, human, recombinant), purified CYP2C9 isozyme without CPR produced from over-expressed plasmid in *E. coli.* (≥ 1 nM of P450, human, recombinant), chitosan (CS, 85% deacetylation), indium tin oxide (ITO, nanoparticles, dispersion, 25 wt% in isopropanol), tolbutamide (≥ 99%, powder) and sulfaphenazole (≥ 99%, powder) were obtained from Sigma-Aldrich Chem. Co. Phosphate buffer solution (PBS, 0.1 M, pH 7.4) was prepared by mixing the stock solution of K_2HPO_4 and KH_2PO_4. The microsomes containing CYP2C9 and CPR (CYP2C9/CPR-microsomes) were stored at -70 ^oC in a solution of 0.1 M pH 7.4 PBS containing 0.1 mM ethylene diamine tetraacetic acid (EDTA), 1 mM dithiothreitol (DTT) and 20% (v/v) glycerol until use. The stock solutions of 10 mM drugs were prepared by ethanol. 0.5 wt% CS was obtained by dissolving 50 mg CS in 10 mL of acetic acid (99.5%). Other reagents were of analytical reagent grade and were used as received. Double distilled water was used throughout the study.

Preparation of CYP2C9/CPR-microsome/ITO/CS/GCE: The glassy carbon electrode (GCE) with a diameter of 3 mm was successively polished using 1.0 and 0.3 μm alumina powder, followed by rinsing thoroughly with double distilled water. After successive sonication in 1:1 nitric acid, acetone and double distilled water, the electrode was rinsed with double distilled water

and dried at room temperature.

To prepare CYP2C9 modified electrode, 2 μL of CS (0.5 wt%), 3 μL of ITO nanoparticles (25 wt%) and 5 μL of CYP2C9/CPR-microsomes solution containing 1 μM cyt P450 isozyme or 5 μL of 20 pM CYP2C9 isozyme solution were mixed together. Then 10 μL of this mixture was drop-coated on the surface of the pretreated GCE to obtain CYP2C9/CPR-microsomes modified electrode (CYP2C9/CPR-microsome/ITO/CS/GCE) or pure CYP2C9 modified electrode (CYP2C9/ITO/CS/GCE). For control experiments, only 10 μL of CYP2C9/CPR-microsome/CS (v/v 1 : 1) or ITO/CS (v/v 3 : 2) was directly dropped onto the surface of GCE to produce CYP2C9/CPR-microsome/CS modified electrode (CYP2C9/CPR-microsome/CS/GCE) or ITO/CS modified electrode (ITO/CS/GCE), respectively. All the modified electrodes were dried at 4 ^{o}C overnight to allow the formation of uniform interfaces.

The size and morphology of ITO nanoparticles, CYP2C9/CPR-microsome/ITO/CS were analyzed with a transmission electron microscope (TEM, S-2400N, HITACHI, Japan, at an accelerate voltage of 200 kV). For TEM measurements, 10 μL of ITO nanoparticles or CYP2C9/CPR-microsomes/ITO/CS was dropped onto a copper grid and then dried in vacuum overnight.

Direct Electrochemistry and Electrochemically Driven Drug Metabolism/Inhibition Studies: Electrochemical measurements were performed on a computer-controlled electrochemical workstation (CHI832B, CHI Instrument, Shanghai, China) with a conventional three-electrode system composed of modified GCE as working electrode, platinum wire as counter electrode, and saturated calomel electrode (SCE) as reference electrode, respectively. Rotating disk electrode (RDE) voltammetry was carried out on a speed controller (HP-1A, Jiangsu, China) and CHI 832B electrochemical workstation using a modified GC disk electrode (5 mm diameter glassy carbon core and 9 mm outer diameter) in oxygen atmosphere.

Electrochemically driven drug metabolism experiments were carried out by successive addition of 10 μL of 10 mM tolbutamide into 5.0 mL of air-saturated 0.1 M pH 7.4 PBS at a fixed potential of -0.48 V (vs. SCE) under stirring. To evaluate the metabolic mechanism, enzyme-catalyzed electrolysis was done by CYP2C9/CPR-microsome/ITO/CS/GCE in 5.0 mL of air-saturated 0.1 M pH 7.4 PBS containing 600 μM tolbutamide at an applied potential of -0.48 V (vs. SCE) for 1 h. The reaction mixture from electrochemical biocatalytic conversion of tolbutamide to

4-hydroxytolbutamide was analyzed by high performance liquid chromatography-mass spectrometry (HPLC-MS). An Agilent series 1290 HPLC system (Agilent, Palo Alto, CA, USA) was used in this study. A solution of 5 μL sample was injected into a ZORBAX Eclipse XDB-C18 column (150×2.1 mm I.D., 3.5 μm; Agilent Technology), and the separation was performed using a mobile phase of 40 % methanol and 60 % water at a flow rate of 0.2 mL min^{-1} with UV detection wavelength at 230 nm. For ESI–MS, an Agilent 6460 QQQ mass spectrometer (Agilent, Palo Alto, CA, USA) was operated in the positive ion mode under these conditions: gas temperature 350 ^{o}C, gas flow 8 L min^{-1}, nebulizer gas pressure 45 psi, sheath gas temperature 250 ^{o}C, sheath gas flow 10 L min^{-1}, and capillary voltage 4 kV. The total run time was 30 min. The autosampler needle was rinsed with methanol and water before and after each injection.

Inhibition studies were carried out by adding different amounts of sulfaphenazole into 5.0 mL of air-saturated 0.1 M pH 7.4 PBS containing 200 μM tolbutamide. After each addition, the solution was stirred and allowed to rest for 1 min for substrate binding to the enzyme. Cyclic voltammetry was performed after each addition.

Fig. S1 (A) Transmission electron microscopy (TEM) of ITO nanoparicles. (B) TEM of film CYP2C9/CPR-microsome/ITO/CS.

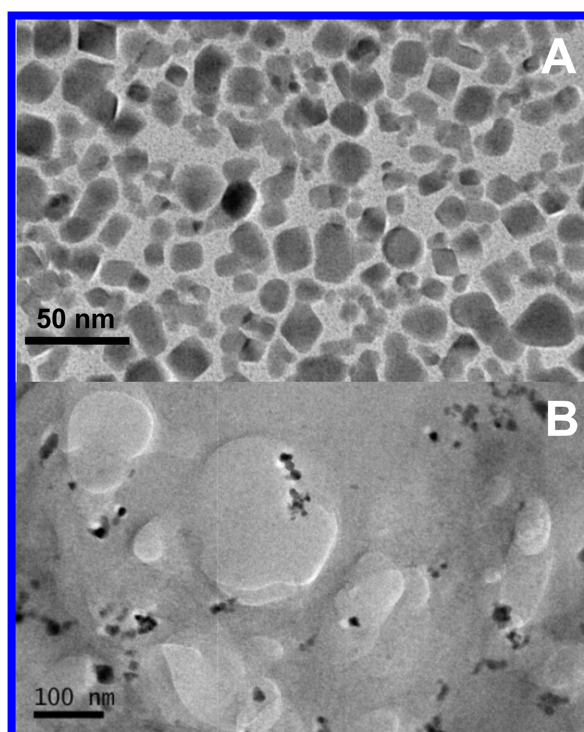

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Fig. S2 CV curves obtained at CYP2C9/CPR-microsome/ITO/CS modified GCE in anaerobic 0.10 M pH 7.4 PBS at different scan rates of 100, 150, 200, 250, 300, 350, 400, 450, 500 mV s^{-1}.

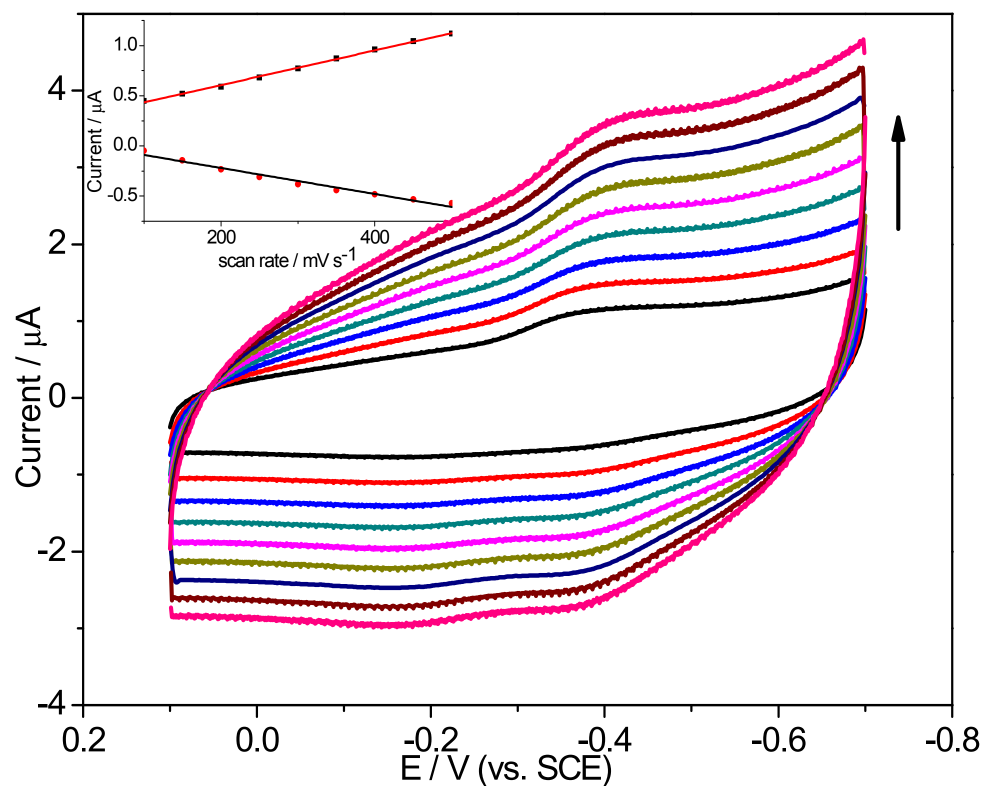

Fig. S3 CV curves obtained at CYP2C9/CPR-microsome/CS/GCE in anaerobic (a) and aerobic (b) 0.1 M pH 7.4 PBS at a scan rate of 200 mV s^{-1}.

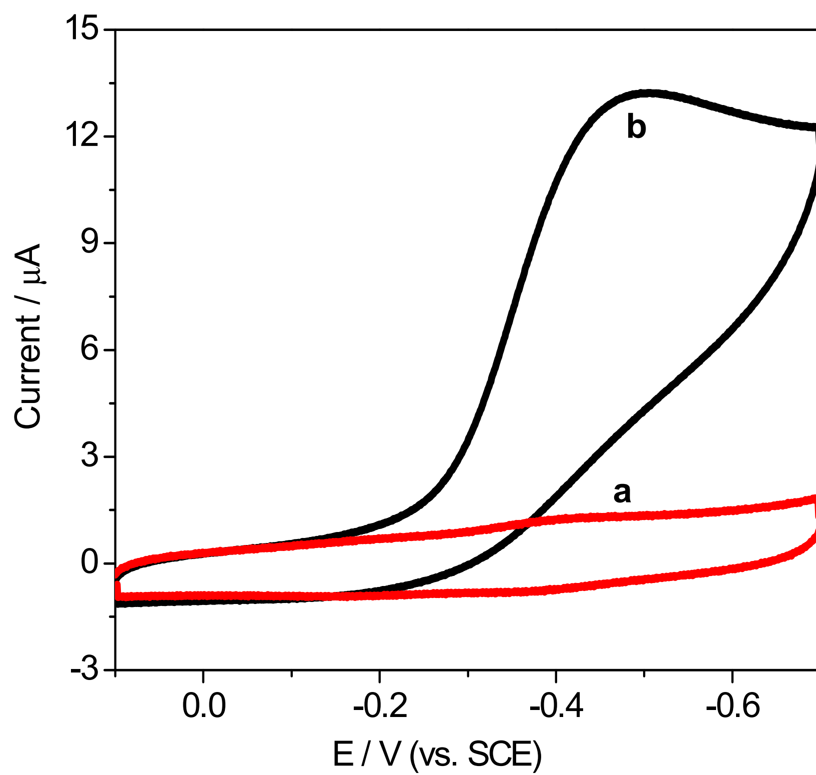

Fig. S4 (A) Capillary HPLC-UV chromatogram (230nm) of reaction mixture after 1h of electrolysis (-0.48 V *vs.* SCE) using CYP2C9/CPR-microsome/ITO/CS/GCE in 5 mL of 0.1 M pH 7.4 PBS with 600 μM tolbutamide. (B) Product ion spectrum of 4-hydroxytobutamide (m/z 287.3, 309) with remaining tolbutamide (m/z 271.1).