## **Electronic Supplementary Information**

# Immobilised-enzyme microreactors for the identification and synthesis of conjugated drug metabolite

Bradley Doyle<sup>1</sup>, Leigh A. Madden<sup>2</sup>, Nicole Pamme<sup>\*1,3</sup>, Huw S. Jones<sup>4\*</sup>

<sup>1</sup>School of Natural Sciences, University of Hull, HU6 7RX, UK

<sup>2</sup> Centre for Biomedicine, University of Hull, HU6 7RX, UK

<sup>3</sup>Department of Materials and Environmental Chemistry, Stockholm University, 106-91 Stockholm, Sweden

<sup>4</sup>Institute of Cancer Therapeutics, University of Bradford, BD7 1DP, UK

\*Corresponding author (h.s.jones@bradford.ac.uk)

\*Corresponding author (h.s.jones@bradford.ac.uk)

### ESI 1 – Microfluidic Chip designs



**Figure S1**: **AutoCAD drawing of channel designs.** (a) Chip Design A (parallel channel network) and (b) Chip Design B (serpentine channel).

	Chip Design A	Chip Design B
Channel length (mm)	50	667
Channel width (µm)	300	75
Channel etch (µm)	30	30
Retention time (min) at a flow rate of 0.1 μL min <sup>-1</sup> assuming width at half depth	76	18
Surface area to volume ratio (m <sup>-1</sup> )	5400	150

 Table S1: Specifications for parallel and serpentine chip designs.

#### ESI 2 - Experimental Setup



**Figure S2**: (a) Schematic drawing of the setup featuring the microfluidic chip interfaced to a syringe pump operated under positive pressure via PTFE tubing. A short piece of a silicon capillary was glued onto the microfluidic device. Effluent was collected in Eppendorf tubes. (b) Photograph of the setup.

#### ESI 3 - Surface Immobilisation



**Figure S3**: Surface immobilisation of enzymes on glass channels. Following flushing with sodium hydroxide and methanol, 3-(Aminopropyl) trimethoxy silane (5% v/v in ethanol) was introduced and left to incubate for 5 min. This was washed out with methanol and left to dry at 60 °C for an hour. Next glutaraldehyde (5% v/v in 0.1 M phosphate buffer, pH 7.4) was pumped for 1 h at 3  $\mu$ L min<sup>-1</sup>. Finally, the enzyme solutions, *i.e.* SULT1a1 (10 ng mL<sup>-1</sup>) or UGT1a1 (0.15 mg mL<sup>-1</sup>) were introduced and left to incubate in the fridge overnight.