

**Interaction of halogenated tyrosine/phenylalanine-derivatives with Organic Anion Transporter (OAT) 1 in the renal handling of tumor imaging probes**

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**Supplemental Methods**

***cDNAs and complementary RNA (cRNA) synthesis***

The construction of pcDNA3.1(+)-hOAT1 and pcDNA3.1(+)-hOAT3 was described in the previous study (Wei et al., 2016). *In vitro* transcription was performed to synthesize polyadenylated cRNAs from linearized plasmids using mMESSAGE mMACHINE® Kit and Poly (A) Tailing Kit (Ambion, Austin, TX) following the manufacturer's protocol. cRNAs were then purified with MEGAclear™ Kit (Ambion, Austin, TX).

***Xenopus laevis oocyte expression and uptake and efflux measurements***

Defolliculated oocytes were injected with polyadenylated cRNA (25 ng/oocyte). The oocytes were, then, incubated at 18 °C in Barth's saline. The uptake and efflux experiments were performed 2 to 3 days after injection of cRNA as previously described with minor modifications (Kanai et al., 1998). Na<sup>+</sup>-free ND96 buffer (96 mM choline-Cl, 2 mM KCl, 1.8 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 5 mM HEPES, pH 7.5) was used as the uptake solution. In brief, for the uptake experiments, the oocytes were incubated with 500 µL uptake solution containing 50 µM of <sup>14</sup>C-PAH for 30 min at room temperature. The oocytes were then washed with ice-cold uptake solution, lysed in 10% sodium dodecyl sulfate. The radioactivity was determined by liquid scintillation counting.

For efflux assay, oocytes were preincubated with 50 µM of <sup>14</sup>C-PAH for 30 min. After washed with ice-cold uptake solution for 5 times, the oocytes were transferred individually into separate wells containing 150 µL of uptake solution with or without addition of 500 µM test compounds, and incubated for 10 min to induce efflux of preloaded <sup>14</sup>C-PAH. Then, the radioactivity of the incubation medium and the

corresponding oocyte were counted. The values were expressed as percent of radioactivity (radioactivity of medium/(radioactivity of medium + radioactivity of oocytes))

***Efflux measurement on HEK293-hLAT1 and HEK293-hLAT2 cells***

HEK293 cell lines stably expressing human LAT1 (HEK293-hLAT1 cells) and human LAT2 (HEK293-hLAT2 cells) were generated in the previous study (Khunweeraphong et al., 2012). The cells were seeded on 24-well plates ( $2.0 \times 10^5$  cells/well) and cultured for 2 days. For efflux measurements, the cells were preloaded with  $^{14}\text{C}$ -L-leucine (1  $\mu\text{M}$ ; 2 MBq/mmol) or  $^{14}\text{C}$ -L-alanine (1  $\mu\text{M}$ ; 2 MBq/mmol) by incubating in  $\text{Na}^+$ -free HBSS at 37 °C for 10 min. After washing, the efflux was induced by incubating the cells with or without indicated concentration of test compounds for 1 min at 37 °C. Then, the radioactivity of the medium and that of the cells were counted. The efflux value was expressed as percent of radioactivity (radioactivity of the medium/(radioactivity of the medium + radioactivity of the cells)). L-Leucine and L-alanine were used as model substrates representing the transport mediated by LAT1 and LAT2 in the experimental conditions set for this efflux experiment, respectively (Khunweeraphong et al., 2012),

### Reference for Supplemental Methods

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Khunweeraphong N, Nagamori S, Wiriyasermkul P, Nishinaka Y, Wongthai P, Ohgaki R, Tanaka H, Tominaga H, Sakurai H and Kanai Y (2012) Establishment of stable cell lines with high expression of heterodimers of human 4F2hc and human amino acid transporter LAT1 or LAT2 and delineation of their differential interaction with  $\alpha$ -alkyl moieties. *J Pharmacol Sci.* **119**: 368–380.

Wei L, Tominaga H, Ohgaki R, Wiriyasermkul P, Hagiwara K, Okuda S, Kaira K, Kato Y, Oriuchi N, Nagamori S, and Kanai Y (2016) Transport of 3-fluoro-L- $\alpha$ -methyl-tyrosine (FAMT) by organic ion transporters explains renal background in [ $^{18}\text{F}$ ]FAMT positron emission tomography. *J Pharmacol Sci.* **130**:101-109.