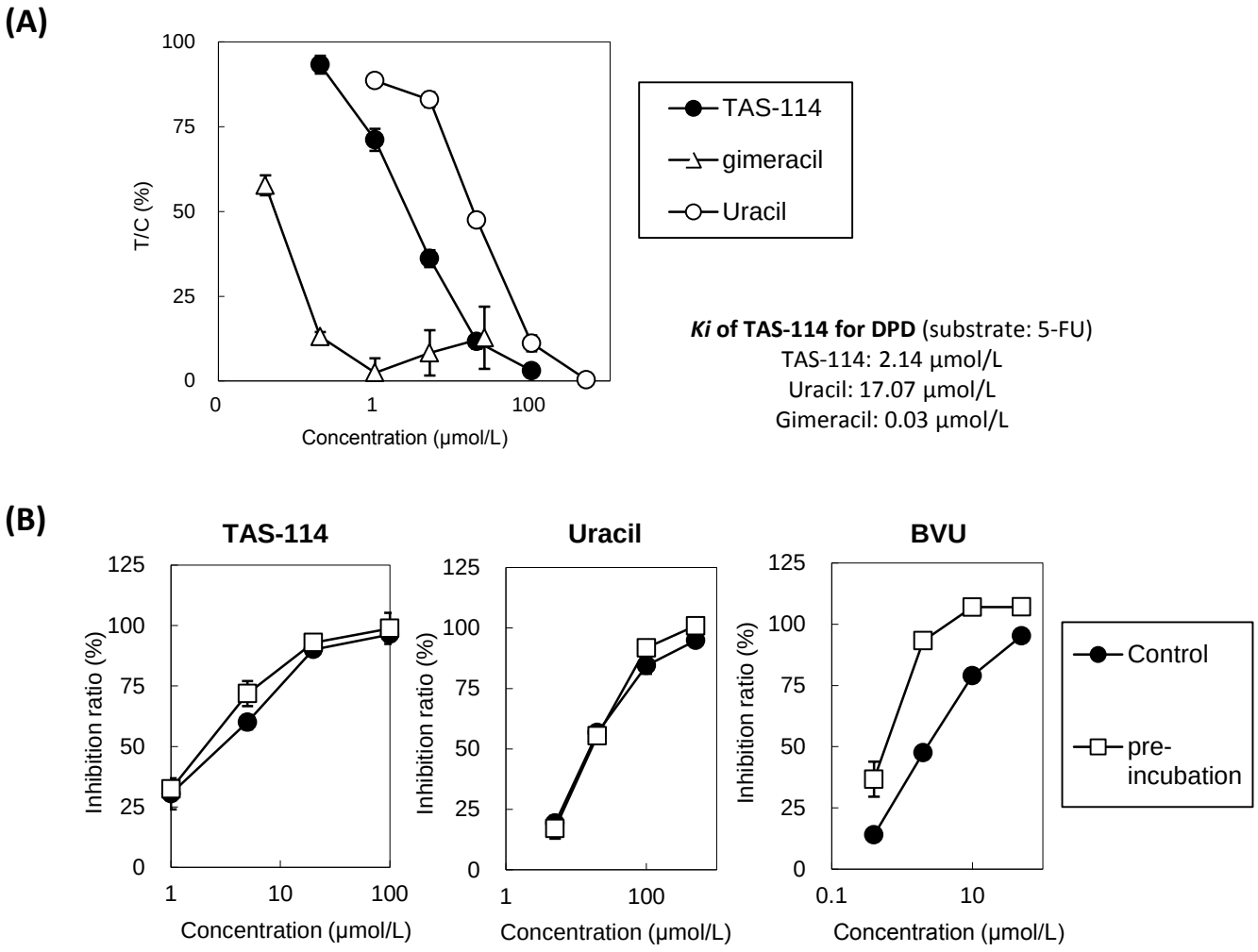
Supplementary Figure S3



Supplementary Figure S3.
TAS-114 possesses moderate dihydropyrimidine dehydrogenase (DPD) inhibitory activity, and its inhibition mode is reversible. (A) Evaluation of direct inhibitory effect of TAS-114 on the metabolism of 5-fluorouracil (5-FU) by DPD. Effects of gimeracil, uracil, and TAS-114 on the conversion of 5-FU (5 μmol/L) to 5,6-dihydrofluorouracil (DHFU) were investigated using the human liver S-9 fraction (1 mg/mL). After pre-incubation of a reaction mixture at 37 °C for 5 min, the reaction was initiated by the addition of an NADPH-generating system (n = 3). Ice-cold acetonitrile was added to terminate the reaction at 60 min from initiation. The concentrations of 5-FU were quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). (B) Evaluation of time-dependent inhibitory effect of TAS-114 on the metabolism of 5-FU. Time-dependent inhibitory effects of TAS-114 were investigated using a human liver cytosol fraction (1 mg/mL). After 20 min of pre-incubation of cytosol at 37 °C with the NADPH-generating system and bromovinyluracil (BVU, a positive control for irreversible DPD inhibition), uracil (a negative control for irreversible DPD inhibition), or TAS-114 at various concentrations, the reactions were initiated by the addition of on 5-FU at a concentration of 4 μmol/L (pre-incubation). In contrast, for control samples, after 20 min pre-incubation of cytosol with 5-FU and BVU, uracil, or TAS-114, the reaction was initiated by the addition of the NADPH-generating system (control). The reactions were stopped by adding ice-cold acetonitrile at 45 min from initiation. The inhibition rates (%) for each concentration of inhibitor were calculated by comparing to 5-FU metabolic activity without each inhibitor.