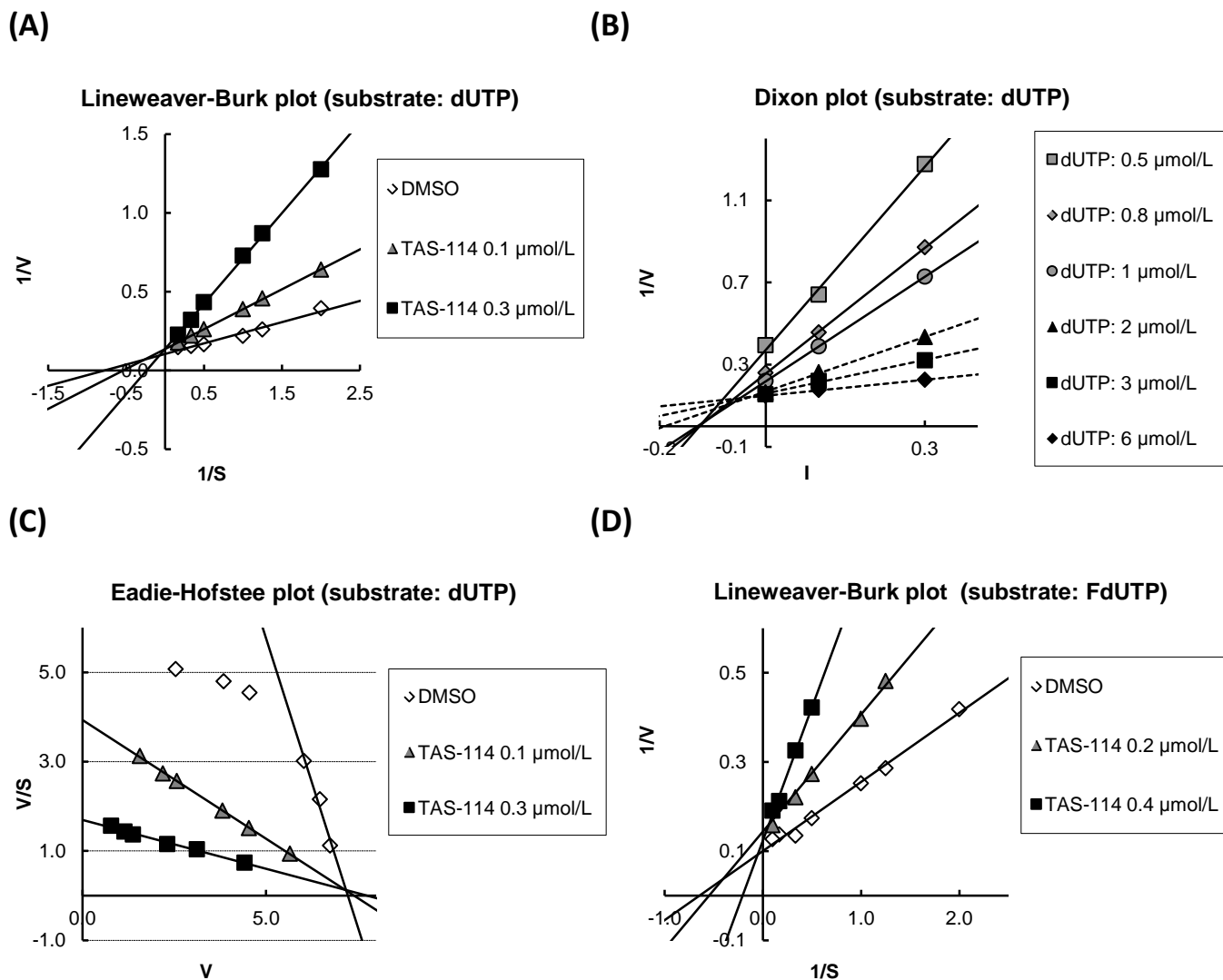


Supplementary Figure S2



Supplementary Figure S2.

Enzyme kinetic analysis of human dUTPase inhibition by TAS-114 and calculation of K_m and K_i values. A substrate ($[^3\text{H}]$ -dUTP) was incubated at 37°C for 30 min in a reaction buffer containing 50 mmol/L Tris-HCl (pH 7.4), 4 mmol/L MgCl_2 , 2 mmol/L 2-mercaptoethanol, 0.1% bovine serum albumin, and 0.005 $\mu\text{g/mL}$ human dUTPase in the absence or presence of TAS-114. $[^3\text{H}]$ -dUMP levels were quantified by radio-high-performance liquid chromatography. The K_m value of dUTP against human dUTPase was calculated from the Lineweaver-Burk plot (A), and the K_i value of TAS-114 was calculated from the Dixon plot (B). The mechanism of enzyme inhibition was determined from the Lineweaver-Burk and Eadie-Hofstee plots (C). Lineweaver-Burk plot of the substrate analysis of FdUTP (D).