Differential Sensitivity and Mechanism of Inhibition of COX-2 Oxygenation of Arachidonic Acid and 2-Arachidonoylglycerol by Ibuprofen and Mefenamic Acid

Jeffery J. Prusakiewicz, Kelsey C. Duggan, Carol A. Rouzer, and Lawrence J. Marnett*

Departments of Biochemistry, Chemistry, and Pharmacology, Vanderbilt Institute of Chemical Biology, Center in Molecular Toxicology, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville TN, 37232-0146

Supporting Information

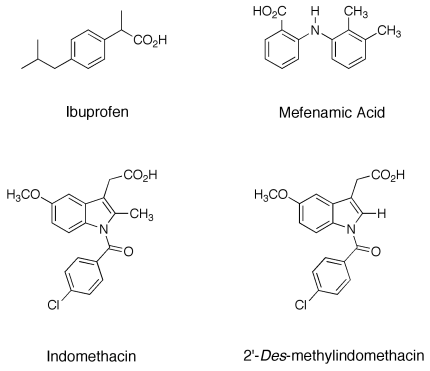

Figure 1. Inhibition of mCOX-2 Oxygenation of AA and 2-AG by Mefenamic Acid. Mefenamate and substrate were mixed in an oxygraph cell and the reaction initiated by addition of mCOX-2. The initial velocity of O_2 uptake was determined from a tangent to the most rapidly descending portion of the curve. A) Instantaneous COX-2 inhibition of AA metabolism by mefenamic acid at 0 μM (■), 20 μM (▼), 40 μM (▲), and 50 μM (□). B) Instantaneous COX-2 inhibition of 2-AG metabolism by mefenamic acid at 0 μM (■), 0.1 μM (●), 0.2 μM (▲), 0.3 μM (▼), and 0.4 μM (♦).

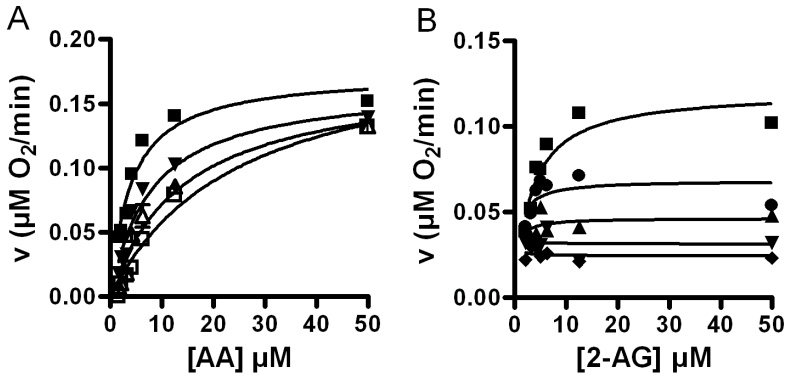

Figure 2. Determination of K_I for Ibuprofen and Mefenamic Acid Inhibition of mCOX-2 Oxygenation of AA. Secondary plots of K_m^{app} vs [I] were used to determine the K_I (-x-intercept) for inhibition of mCOX-2 AA oxygenation by A) Ibuprofen, and B) Mefenamic Acid.

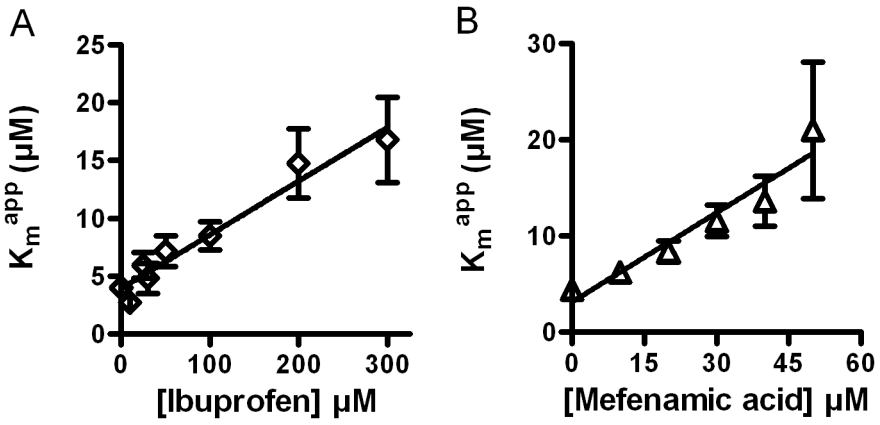

Figure 3. Determination of K_I for Ibuprofen Inhibition of mCOX-2 Oxygenation of 2-AG. Secondary plots of $1/V_{max}^{app}$ vs [I] were used to determine the K_I (-x-intercept) for inhibition of mCOX-2 2-AG oxygenation by ibuprofen.

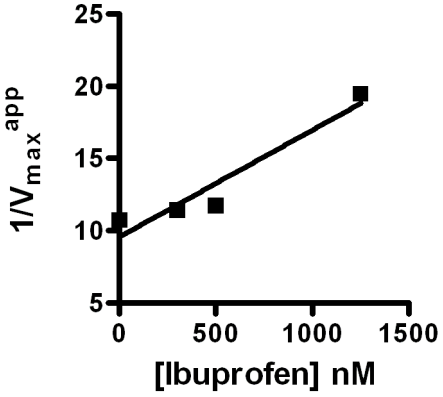

Figure 4. Fluorescence Quenching Titration of mCOX-2 with Mefenamic Acid. A) The quenching of mCOX-2 intrinsic protein fluorescence by mefenamic acid was monitored in a fluorescence cuvette at 37º C. Data are the average of at least two independent determinations. Mefenamic acid exhibited a K_d^{app} of 32 ± 2 nM towards mCOX-2 (100 nM), which was calculated from non-linear regression of the sigmoidal dose-response curve with a variable slope. B) Secondary plot of K_d^{app} dependence on protein concentration. The y-axis intercept is equal to the K_d (4 nM) for mefenamic acid binding to mCOX-2.