Pharmacokinetic Risks
fu low fraction unbound in plasma
Vd high steady-state volume of distribution

10 Physicochemical and Biological Characterization

10.1 COX-1 and COX-2 Assays

COX-1 and COX-2 IC_{50} determinations were performed by Cerep[4] using published methods (Glaser et al. 1995). The experiments measured the amount of PGE_2 formed from arachidonic acid at pH 8.0 over a 5-minute period using a recombinant enzyme expressed in transfected Sf-9 cells. For basal control measurements, arachidonic acid was omitted from the reaction mixture. The amount of PGE_2 generated was determined by homogeneous time-resolved fluorescence. Results are expressed as a percent inhibition of the control enzyme activity. Diclofenac (Fig. 11) served as the positive control in COX-1 assays and NS-398 served as the positive control in COX-2 assays.

Both standards were tested in each experiment at several concentrations to obtain an inhibition curve from which its IC_{50} value is calculated.

10.2 Thermodynamic Aqueous Solubility Assay

Thermodynamic aqueous solubility assays were also performed by Cerep using a literature protocol (Lipinski et al. 1997) that uses HPLC-MS to quantitate the amount of compound remaining in solution at room temperature after centrifugation at 2,500$\times g$ for 30 min. The dynamic range of the assay is from 1 μM to 2 mM.

10.3 Stability in Human Liver Microsomes

Human liver microsome (HLM) stability assays were performed by Absorptions Systems[5] using human liver microsomes obtained from Xenotech. Compounds were tested at a concentration of 1 μM with a preincubation time of 3 min at 37°C. Testosterone controls were run in parallel. Aliquots (100 μL) of reaction mixture were withdrawn at 0, 10, 20, 30, and 60 min, then quenched, and assayed by HPLC-MS/MS against an internal standard using electrospray ionization detection. Half-lives were estimated by fitting the percent remaining to a single-phase exponential decay curve.

[4] Eurofins Panlabs Inc. 15318 NE 95th Street, Redmond, WA 98052 USA, Tel (425) 895-8666
[5] Absorption Systems, Exton PA 19341, Tel (610) 280-7300