Supplementary Information

Measurement of Inhibitor Potency on sEH and FAAH

Quantification of inhibitor potencies were performed following previously published procedures:

For human sEH enzyme: sEH activity was measured using the substrate cyano(6-methoxynaphthalen-2-yl)methyl ((3-phenyloxiran-2-yl)methyl) carbonate (CMNPC) ($[S]_{final}$ = 5 μM), which generates fluorescent 6-methoxynaphthaldehyde ($\lambda_{excitation}$ = 330 nm, $\lambda_{emission}$ = 465 nm, 25 °C) after hydrolysis. Inhibition was measured in sodium phosphate buffer (0.1 M, pH = 7.4, 0.1 mg/mL bovine serum albumin (BSA)) containing partially purified sEH from baculovirus or partially purified liver extract. (Jones 2005, Rose 2010)

For human FAAH enzyme: FAAH activity was measured using the substrate N-(6-methoxypyridin-3-yl) octanamide (OMP) ($[S]_{final}$ = 5 μM), which generated fluorescent 6-methoxypyridine ($\lambda_{excitation}$ = 303 nm, $\lambda_{emission}$ = 394 nm, 37 °C) after hydrolysis. Inhibition was measured in sodium phosphate buffer (0.1 M, pH = 8, 0.1 mg/mL BSA) containing crude FAAH extract from baculovirus or brain microsomes with a final DMSO concentration of 2%. (Huang 2007, Kodani 2016).

For both enzymes, generated fluorescence was quantified every 30 seconds for 10 minutes and the linear portion of the curve was used to generate the reaction velocity ($v_{inhibitor}$). Values were subtracted from wells containing no enzyme. IC_{50}'s were quantified by simple linear regression of the log [I] vs. % remaining activity ($v_{inhibitor}/v_{DMSO}$) and determining x when y = 0.50.

All measurements were the average of triplicates.

Supplementary References:

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Kodani SD, Overby HB, Morisseau C, Chen J, Zhao L, Hammock BD. Parabens inhibit fatty acid amide hydrolase: A potential role in paraben-enhanced 3T3-L1 adipocyte differentiation. Tox Lett 2016; 262:92-99

Huang H, Nishi K, Tsai HJ, Hammock BD. Development of highly sensitive fluorescent assays for fatty acid amide hydrolase. Anal Biochem 2007; 363:12-21