

Evidence that Diclofenac and Celecoxib are Thyroid Hormone Receptor Beta Antagonists; use of *in silico*, Molecular and Pharmacological Techniques

Long term use of NSAIDs is linked to several detrimental side effects, inducing gastric bleeding and myocardial infarction. In order to understand the mechanisms by which NSAIDs induce detrimental effects in patients, many have studied the direct activity of NSAIDs on COX enzymes and developed novel models to explain the phenomenon. Since it is common for drugs to bind to multiple proteins, we used computational chemistry methods to investigate the potential for NSAIDs diclofenac and celecoxib to bind to nuclear receptors.

In silico screening predicted that both diclofenac and celecoxib could bind to a number of different nuclear receptors. We have chosen to investigate if the thyroid hormone receptor beta (TR β) could be one of the novel targets for these NSAIDs. Results from TR β luciferase reporter assays confirmed that both NSAIDs lack agonist activity, although display TR β antagonistic properties; diclofenac IC $_{50}$ 6.3×10^{-5} M and celecoxib IC $_{50}$ 4.9×10^{-6} M. In order to determine the effects of NSAIDs in whole organ *in vitro*, we used isometric wire myography to measure the changes to triiodothyronine (T3) induced vasodilation of rat mesenteric arteries. Male Wistar rats (350-450g) were killed by CO $_2$ asphyxiation in accordance with the UK Home Office regulations. Mesenteric arteries were dissected in Krebs's solution and incubated in the presence of the TR β antagonist MLS000389544 (10^{-5} M), as well as diclofenac (10^{-5} M) and celecoxib (10^{-5} M). Results from myography showed a significant inhibition of T3 induced vasodilation compared to controls (Figure 1).

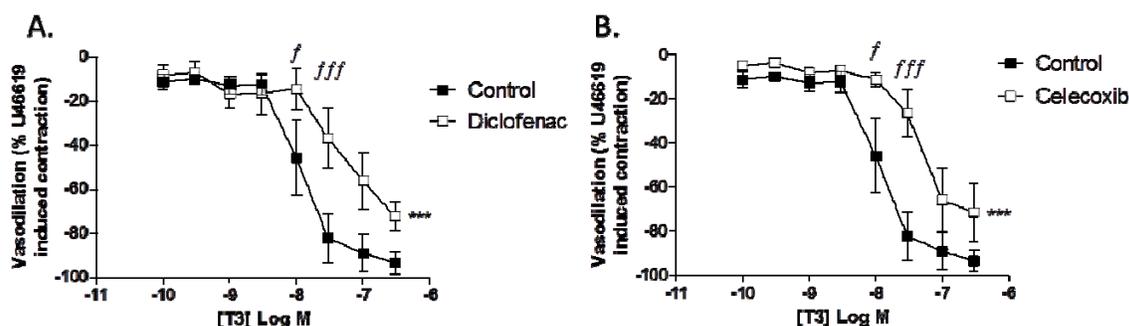


Figure 1. L-Triiodothyronine (T3) induced vasodilation in mesenteric arteries pre-contracted with EC $_{80}$ U46619 (3×10^{-7} M). T3 vasodilation in the presence of A. 10^{-5} M Diclofenac and B. 10^{-5} M celecoxib. Data is presented as mean \pm SEM of an n=6, and significance is represented as *** $p < 0.001$ by two way ANOVA and $f = p < 0.05$ and $fff = p < 0.001$ by Bonferroni post hoc test.

These results highlight the benefits of computational chemistry methods used to retrospectively analyse well known drugs for side effects. Using *in silico* and *in vitro* methods we have shown that both celecoxib and diclofenac exhibit off-target TR β antagonist behaviour, which may be linked to their detrimental side effects.