A novel allosteric activator of free fatty acid 2 receptor with unique G_i-functional bias

Introduction: Recently, it has become clear that short chain fatty acids (SCFAs) act as ligands for two relatively newly de-orphanised G protein-coupled receptors (GPCRs), FFA2 and FFA3. In this way SCFAs, which can be generated as a by-product of the breakdown of starches by the gut microbiota, regulate a panoply of physiological responses from insulin release to inflammation. However, a general paucity of selective ligands for these receptors has rendered the understanding of their patho-physiological role extremely challenging. Here, we characterise pharmacological effects of AZ12461729, a compound that was initially identified as a regulator for FFA2 in a high-throughput screening program.

Methods: Flp-In™ T-REx™ 293 cells, engineered to inducibly express human FFA2 upon addition of doxycycline, were employed in functional assays measuring inhibition of forskolin-induced cAMP production, inositol monophosphate (IP1) accumulation and [^{35}S]GTPγS incorporation assays, and also radioligand binding assays following previously described protocols (1 and 2).

Results: AZ12461729 acted as an agonist in G_i-dependent assays with pEC_{50} values of 6.90 ± 0.14 in the cAMP assay and 7.23 ± 0.20 in [^{35}S]GTPγS incorporation assays. AZ12461729 was significantly more potent ($p<0.01$) than the SCFA agonist, propionate (C3), in both assays and was also a full agonist reaching an efficacy that was comparable, if not higher, than that of C3. In contrast, AZ12461729 did not show any effect in IP1 accumulation assays, suggesting that this ligand preferentially activates G_i-coupled FFA2. To determine whether AZ12461729 was an orthosteric agonist at FFA2, we performed competition binding experiments with the orthosteric radiolabelled antagonist [^{3}H]GLPG0974 (1). While [^{3}H]GLPG0974 was fully outcompeted by increasing concentrations of C3 (pK_i = 2.78 ± 0.11), this was not the case for AZ12461729 that yielded a maximal displacement of only 16.7 ± 4.4 % of this radioligand with estimated pK_i of 6.77 ± 0.50, indicating that this synthetic ligand likely binds a FFA2 allosteric site. The potential allosteric nature of AZ12461729 was then assessed in a three-way binding experiment (2), in which we assayed the ability of AZ12461729 to modulate the inhibition of [^{3}H]GLPG0974 binding by C3. Increasing concentrations of AZ12461729 enhanced the affinity of C3 to inhibit [^{3}H]GLPG0974 binding, yielding an overall affinity cooperativity factor (α') value for C3 of 4.27 ± 1.17, suggesting that this compound is also a positive allosteric modulator at FFA2. The ability of AZ12461729 to allosterically modulate C3 function was also assessed using cAMP and IP1 accumulation assays. While AZ12461729 was a strong allosteric agonist in a G_i-dependent assay (cAMP), with a net cooperativity factor ($\alpha\beta$) value of 85, this was not the case in a $G_{q/11}$-dependent assay (IP1) where AZ12461729 behaved as an insurmountable/allosteric antagonist by decreasing the maximal efficacy of the C3 dose-response curve.

Conclusions: AZ12461729 is the first potent allosteric modulator of FFA2 with G_i-functional bias. The unique nature of this compound could be a valuable pharmacological tool to understand the relative importance of FFA2 signalling pathway/s and their biological roles.

(1) Sergeev E. et al. (2016) *J. Biol. Chem.* **291**: 303–317.
(2) Langmead CJ (2011) *Methods Mol. Biol.* **746**: 195–209.