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Structure-based design of fenofibrate amide analogues acting at CB_2 cannabinoid receptors

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Introduction: Cannabinoid receptors have been suggested to have multiple opportunities for therapeutic exploitation (1). The failure of rimonabant in the clinic, however, has drawn attention away from the CB_1 cannabinoid receptor. CB_2 cannabinoid receptors, on the other hand, represent an unexploited target with potential in inflammatory disorders. We recently identified that the PPARα pro-drug fenofibrate, but not fenofibric acid, exhibited a complex profile at cannabinoid receptors, with a 'conventional' agonist profile at CB_2 receptors (2). We set out to identify the mechanism of binding to the CB_2 receptor using *in silico* docking studies and to synthesise and test analogues based on an amide substitution for the ester target for hydrolysis.

Methods: A homology model of the human CB_2 receptor was initially constructed based on the crystal structure of the human A_{2A} adenosine receptor (PDB code 3EML) (3). The model was embedded in a fully hydrated POPC lipid bilayer, and refined using molecular dynamics simulations using the GROMOS 53a6 force field (4). Screening of analogues was conducted using ^{3}H-CP55940 occupancy and ^{35}S-GTPγS enhancement by conventional methods (2).

Results: The predicted binding mode of these compounds was found to be stabilized primarily by hydrogen bonds with W5.43 and C7.42, aromatic stacking with F2.57, F3.36 and W6.48, and hydrophobic interaction with F2.64, V3.32 and I5.47. A series of structural analogues were designed and synthesized based on these modelling data and tested in ^{3}H-CP55940competition binding and ^{35}S-GTPγS binding assays. The pharmacology of these compounds provided experimental evidence which appeared to validate the modelling predictions. A number of these compounds exhibited greater efficacies than fenofibrate, indicating a possible involvement of the polar residues T3.35 and S3.39 in eliciting "full" agonist responses. Additionally, it was found that increasing the hydrophobic bulk of the amide *N*-substituent resulted in compounds with distinct pharmacology, exhibiting an incomplete but saturable displacement of ^{3}H-CP55940 along with inverse agonist activity in the ^{35}S-GTPγS assay.

Conclusions: We conclude that this series of fenofibrate analogues represent a novel structure:activity relationship of CB_2 receptor pharmacology.

References

1 Alexander SPH (2016) *Prog Neuropsychopharmacol Biol Psychiatry* **64**: 157-166

2 Priestley RS et al. (2015) *FASEB J* **29**: 1446-1455

3 Jaakola VP *et al*. (2008) *Science* **322**: 1211-1217

4 Oostenbrink C et al. (2004) *J Comput Chem* **25**: 1656-1676