Development of C5aR1 negative allosteric modulators with potential benefits for neuroinflammation

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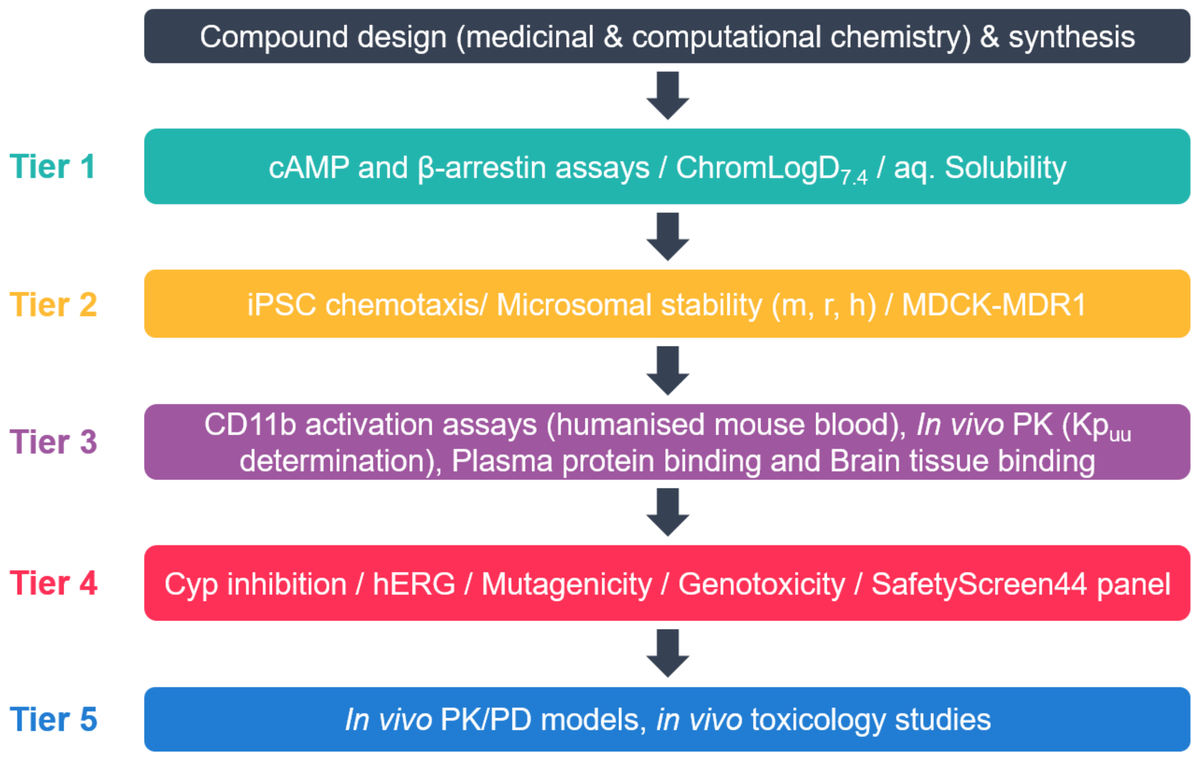
Introduction

- C5a is an inflammatory peptide produced upon complement activation, elevated levels initiate a feed-forward loop of inflammation via recruitment of microglia to sites of injury, leading to neuronal damage and death [1]
- C5a exerts its effects via binding and activation of the Complement 5a receptor 1 (C5aR1), a G protein-coupled receptor, which has been shown to couple to $Ga_{i/o}$, recruit β-arrestins and modulate chemotaxis
- Preventing C5aR1 activation may therefore reduce neuroinflammation resulting in potential disease modifying effects
- Here we present a drug discovery program utilising medicinal and computational chemistry, in vitro pharmacology and ADME/PK, which successfully identified and characterised lead-like negative allosteric modulators (NAMs) of the C5aR1, with data for our most promising compound presented

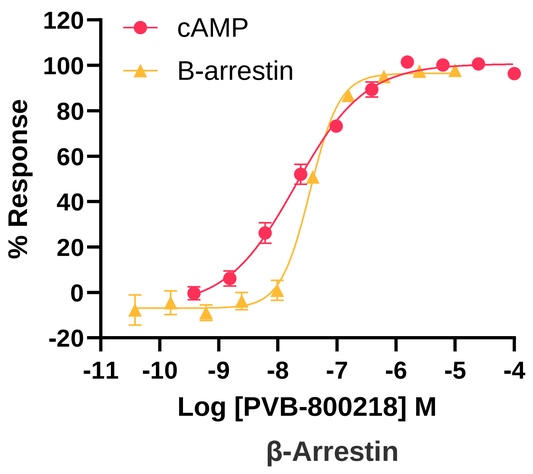
Screening Cascade

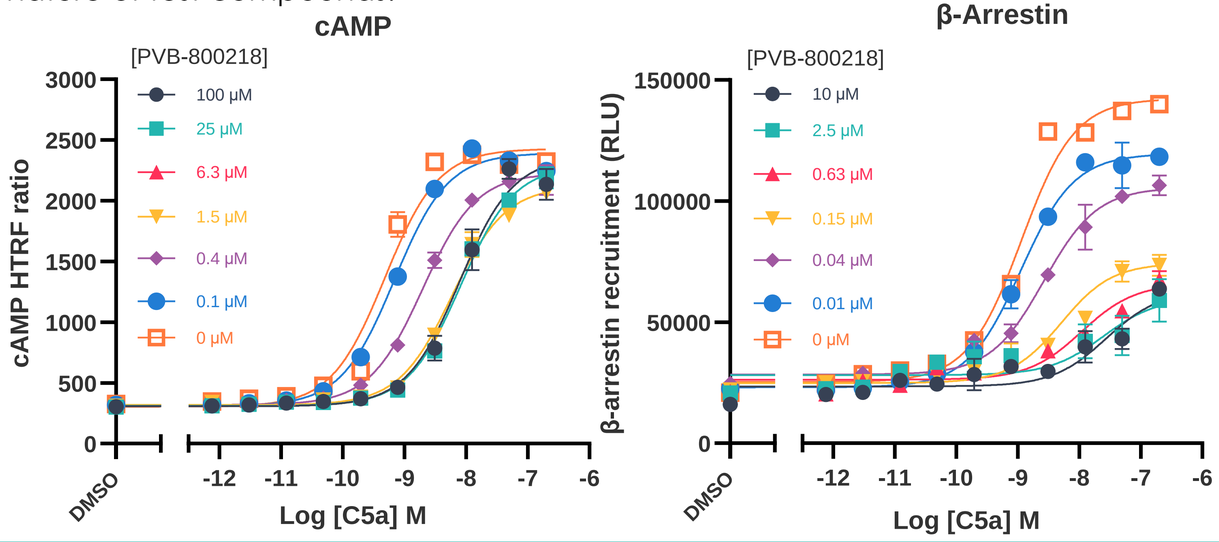


We developed a 5-tier screening cascade suitable for the testing of novel compounds that were designed via robust SAR analysis and further validated via computational chemistry efforts, and then pharmacologically validated to be C5aR1 NAMs via a series of assays utilising a range of recombinant, hiPSC and ex *vivo* models.

Tier 1 Screening – Functional Effects

CHO-K1 cells overexpressing hC5aR1 were utilised for cAMP inhibition assays whilst the DiscoverX PathHunter® assay was employed for investigating β-arrestin recruitment. These assays, utilising C5a CRCs tested in the presence of increasing concentrations of test compound, were analysed with the operational model of allosteric modulation of pharmacological agonism [2], to confirm the allosteric nature of test compounds.

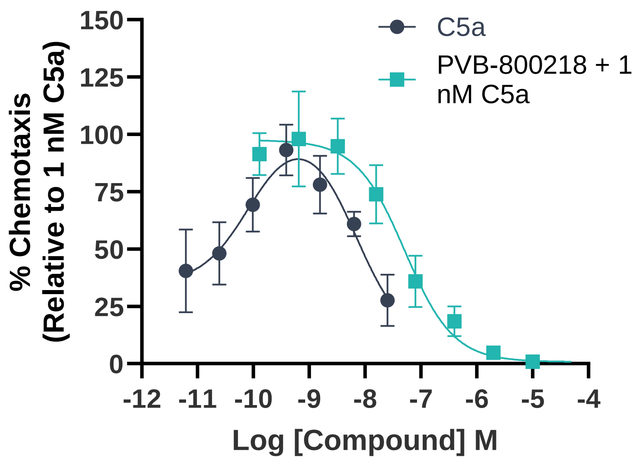




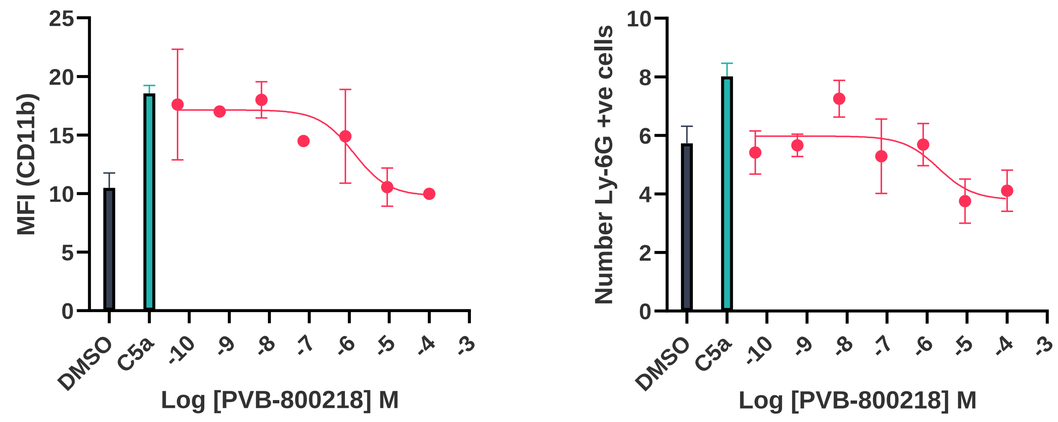
Tier 2 Screening – System Effects

One of the main physiological actions of C5a is as a chemoattractant. We thus tested our C5aR1 NAMs in a chemotaxis assay using human iPSC microglia, investigating their ability to inhibit C5a-induced chemotaxis.

Numerous compounds tested displayed inhibitory activity in this assay, with our lead compound having an IC_{50} of 27 nM.



Tier 3 Mouse Model Validation and *Ex Vivo* Profiling



The C5aR1 allosteric site is not present in the mouse isoform. Thus, a humanised mouse model was developed. To validate this model, initial data identified the ability of our compounds to inhibit C5a-induced neutrophil activation (reduced CD11b expression) as well as reducing the expression of Ly6G, a marker of chemotactic cells, confirming our chemotaxis data with hiPSC microglia.

Lead Compound Profile

Our drug discovery programme has allowed us to develop a lead compound with nM potencies in both recombinant systems and hiPSC models, as well as proven effects in ex *vivo* blood studies using humanised mice. Operational modelling of our data has identified the allosteric nature of our compounds with both α and β values <1.

Initial profiling has also indicated a good candidate for further development, focusing on improving metabolic stability, whilst retaining on target potency and efficacy

Property	PVB-800218
Mwt, TPSA, MPO	561.6, 78, 4.5
β-arrestin IC_{50} (nM), α and β	35, 0.11, 0.76
cAMP IC_{50} (nM), α and β	20, 0.13, 1.02
Chemotaxis IC_{50} (nM)	27
CD11b IC_{50} (µM)	2.2
$ChromLogD_{(7.4)}$	4.7
Kinetic solubility (µM, 60 mins)	26
Mouse microsomal clearance ($t_{1/2}$ (mins), Clint (µL/min/mg protein)	16, 90
$t_{1/2}$ (Hr)	1.6
Cl (ml/min/kg)	8.6
Vd (L/Kg)	0.9
Brain:Plasma (5 min)	0.4
Time [Brain] above β-arrestin IC_{50} (mins)	30

Summary

- We have utilised a combined team of disciplines to deliver a fully integrated drug discovery programme that has successfully identified lead-like negative allosteric modulators of the C5aR1
- Our lead compound will progress through more advanced ADME/DMPK assays, selectivity screening and *in vivo* studies, whilst trying to improve metabolic stability

References

[1] Schartz et al. C5aR1 antagonism suppresses inflammatory glial responses and alters cellular signaling in an Alzheimer's disease mouse model. Nat. Commun. 2024 15: 7028, [2] Carvalho et al. Modulation of C5a-C5aR1 signaling alters the dynamics of AD progression. J. Neuroinflammation. 2022; 19(1):178, [3] Jakubik et al. The operation model of allosteric modulation of pharmacological agonism. Scientific Reports. 2020; 10: 14421

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