LETTER

doi:10.1038/nature25025

Structure of the complement C5a receptor bound to the extra-helical antagonist NDT9513727

Nathan Robertson[1]*, Mathieu Rappas[1]*, Andrew S. Doré[1]*, Jason Brown[1], Giovanni Bottegoni[1], Markus Koglin[1], Julie Cansfield[1], Ali Jazayeri[1], Robert M. Cooke[1] & Fiona H. Marshall[1]

The complement system is a crucial component of the host response to infection and tissue damage. Activation of the complement cascade generates anaphylatoxins including C5a and C3a. C5a exerts a pro-inflammatory effect via the G-protein-coupled receptor C5a anaphylatoxin chemotactic receptor 1 (C5aR1, also known as CD88) that is expressed on cells of myeloid origin[1,2]. Inhibitors of the complement system have long been of interest as potential drugs for the treatment of diseases such as sepsis, rheumatoid arthritis, Crohn's disease and ischaemia-reperfusion injuries[1]. More recently, a role of C5a in neurodegenerative conditions such as Alzheimer's disease has been identified[3]. Peptide antagonists based on the C5a ligand have progressed to phase 2 trials in psoriasis and rheumatoid arthritis; however, these compounds exhibited problems with off-target activity, production costs, potential immunogenicity and poor oral bioavailability. Several small-molecule competitive antagonists for C5aR1, such as W-54011[5] and NDT9513727[6], have been identified by C5a radioligand-binding assays[4]. NDT9513727 is a non-peptide inverse agonist of C5aR1, and is highly selective for the primate and gerbil receptors over those of other species. Here, to study the mechanism of action of C5a antagonists, we determine the structure of a thermostabilized C5aR1 (known as C5aR1 StaR) in complex with NDT9513727. We found that the small molecule bound between transmembrane helices 3, 4 and 5, outside the helical bundle. One key interaction between the small molecule and residue Trp213$^{5.49}$ seems to determine the species selectivity of the compound. The structure demonstrates that NDT9513727 exerts its inverse-agonist activity through an extra-helical mode of action.

To obtain the structure of C5aR1, a thermostabilized receptor (StaR) was generated as described previously[7,8]. C5aR1 was thermostabilized in the presence of the inverse-agonist radioligand [^{3}H] NDT9513727 (*N*,*N*-bis(1,3-benzodioxol-5-ylmethyl)-1-butyl-2, 4-diphenyl-[1]*H*-imidazole-5-methanamine)[6], and contains 11 amino acid substitutions (Extended Data Fig. 1) that had no effect on the pharmacology or ligand binding of the receptor (Extended Data Fig. 2). To promote crystallization further, 29 and 17 residues were removed from the N and C terminus of the receptor, respectively. The C5aR1 was crystallized in lipidic cubic phase and solved at 2.7 Å resolution (Extended Data Fig. 3 and Extended Data Table 1), with two copies of C5aR1 bound to NDT9513727 present in the asymmetric unit (Fig. 1a). The overall structure of C5aR1 is similar to that of other class A receptors crystallized in the inactive state and consists of the canonical seven-transmembrane (TM1–TM7) helix arrangement (Fig. 1a). Cys109$^{3.25}$ (superscripts denote Ballesteros–Weinstein numbering) at the N-terminal end of TM3 forms a conserved disulfide bond with Cys188 in the second extracellular loop (ECL), which itself forms an extended β-hairpin. Continuous density is observed for all extracellular and intracellular loops apart from the C-terminal connection of ECL2 with TM5 and the junction of TM7 with helix 8, whereas intracellular loop 2 (ICL2) adopts a two-turn α-helical structure similar to that of CCR9[9] and other chemokine receptor structures (Fig. 1a, c).

The extracellular portions of the transmembrane α-helices and ECLs have been previously shown to form the peptide-binding vestibule, or orthosteric site, for peptide-binding class A G-protein-coupled receptors (GPCRs), for example, the neurotensin 1 receptor[10] (TM2–TM7 and ECLs). Electron density is well defined across this region in C5aR1; however, it is found to be unoccupied by NDT9513727. Instead, the small molecule binds towards the intracellular side of the receptor and outside the transmembrane helical bundle (Fig. 1b–d). Furthermore, cold competition experiments (Extended Data Fig. 4) demonstrate no displacement of radiolabelled NDT9513727 with the C5a agonist peptide or the PMX53 antagonist macrocycle.

Clear difference density for NDT9513727 (Fig. 2a) is found with one benzodioxolane group in a collapsed conformation making a hydrophobic interaction to the imidazole core and 2,4-phenyl groups, and the other benzodioxolane group in an extended conformation packing against the 1-butyl group from the imidazole core (Fig. 2a, b). Residues on the outside of TM3, TM4 and TM5 form an extensive hydrophobic pocket with shape complementarity for NDT9513727. Ile124$^{3.40}$, Leu125$^{3.41}$ and Ala128$^{3.44}$ on TM3 and Leu209$^{5.45}$, Pro214$^{5.50}$ and Thr217$^{5.53}$ on TM5 supply hydrophobic interactions to the collapsed benzodioxolane group and 2-phenyl ring. The extended benzodioxolane group and 1-butyl component of the ligand sits in a hydrophobic pocket formed between TM3 and TM4 consisting of Leu125$^{3.41}$, Ala128$^{3.44}$, Thr129$^{3.45}$,Val159$^{4.48}$, Ala160$^{4.49}$, Leu156$^{4.45}$ and Leu163$^{4.52}$ (Fig. 2b, c). The extra-helical binding site in C5aR1 between TM3, TM4 and TM5 is distinct from both the more conventional orthosteric sites for GPCR ligands, and other negative allosteric sites identified outside the transmembrane helical bundle (Fig. 1b). For example, *N*-[2-[2-(1,1-dimethylethyl)phenoxy]-3-pyridinyl]-*N*′-[4-(trifluoromethoxy) phenyl]urea (BPTU) is bound to the outside of TM3 on the $P2Y_1$ receptor[11], the site for AZ3451 on PAR2 is centred on a different region of TM3[12], and MK-0893 forms a clamp around the outside of TM6 on the glucagon receptor[13]. Interestingly, however, the NDT9513727-binding site in C5aR1 is analogous to the site of the extra-helical full allosteric agonist AP8 (ago-PAM), which was solved recently in complex with the free fatty acid receptor GPR40[14].

The crucial interaction between C5aR1 and NDT9513727 is a single hydrogen bond supplied by the imidazole core of the ligand to the indole ring of Trp213$^{5.49}$ (Fig. 2c). This residue has been previously reported to confer species selectivity for W-54011 and NDT9520492[15], both of which are chemically similar to NDT9513727 (Extended Data Fig. 2). To confirm that Trp213$^{5.49}$ is crucial for NDT9513727 binding in human C5aR1, we mutated it to leucine (representing the equivalent residue in the mouse and rat C5aR1 sequence) (Extended Data Fig. 1), and subsequently found that this mutation abolishes binding of NDT9513727 to C5aR1. This was further confirmed in molecular dynamics simulations (Extended Data Fig. 5). In addition,

[1]Heptares Therapeutics Ltd, BioPark, Broadwater Road, Welwyn Garden City, Hertfordshire AL7 3AX, UK.
*These authors contributed equally to this work.