mutation of two further residues flanking the extra-helical binding site, Thr217$^{5.53}$ to leucine and Leu125$^{3.41}$ to phenylalanine (positioned directly above, and one helical turn below Trp213$^{5.49}$) had no effect on NDT9513727 binding, demonstrating that these residues do not provide a crucial contribution to the shape complementarity of C5aR1 to NDT9513727 (Fig. 2d and Extended Data Fig. 6).

NDT9513727 seems to antagonize receptor activation from outside the helical bundle by stabilizing a network of interactions that hold the receptor in the inactive state and by inhibiting the helical movements required to transition to the agonist conformation for downstream signalling (Fig. 3). In the receptor β_2-AR[16,17], activation has been proposed to be initiated by ligand binding causing an approximately 2 Å inward movement of TM5 around Ser$^{5.46}$. The inward movement of TM5 at the proline bulge of Pro$^{5.50}$ disrupts a network of interactions between Pro$^{5.50}$, Ile$^{3.40}$, Phe$^{6.44}$ and Asn$^{7.45}$ that stabilize the receptor in the inactive state. Moreover, the inward movement at the top of TM5 (contributing to a contraction of the orthosteric site) is considered one of the structural signatures of class A receptor activation in general[18]. The recently reported structures of GPR40 in complex with the partial agonist MK-8666, and in ternary complex with the full ago-PAM AP8 site (bound to an analogous extra-helical site between TM3, TM4 and TM5) demonstrate that the positive cooperativity of these compounds is embedded in the 'interlocution' of TM4 and TM5, with ago-PAM binding shifting TM5 along its axis by roughly half a helical turn towards the extracellular side relative to TM4[14]. Superposition of C5aR1 with both the active-state β_2-AR–G_s (PDB code 3SN6) and the GPR40–MK-8666–AP8 (PDB code 5TZY) agonist ternary structure that are themselves in close agreement in terms of TM4–TM5 (Fig. 3), suggests that NDT9513727 acts as a 'molecular wedge' between TM4, TM3 and TM5 of C5a1R. The extended benzodioxolane packs against TM4, the 2-phenyl group then makes packing interactions between TM3 and TM5 involving Ile124$^{3.40}$, Ala128$^{3.44}$ and Pro214$^{5.50}$, and the imidazole core then crucially hydrogen bonds to Trp213$^{5.49}$ (Fig. 2c), and hinders the movement of TM5 relative to TM4 upon activation (Fig. 3). Indeed, molecular dynamics simulations that measure the inter-helical distances between TM3–TM5 and TM4–TM5 across the extra-helical NDT9513727-binding site over a 200-ns time course show that, in the absence of NDT9513727, these distances decrease (Supplementary Videos 1 and 2).

A range of strategies including fluorescence resonance energy transfer (FRET)[19], disulfide trapping (at Cys144 in ICL2)[20] and mutagenesis analysis[21] have shown that C5aR1 dimerizes in both recombinant systems as well as in human neutrophils, with a TM4–TM5 contact interface previously proposed for the C5aR1[20]. The two copies of C5aR1 present in the asymmetric unit assemble in parallel fashion making a network of interactions between TM4–TM4, TM4–TM5, ICL2 and the two copies of NDT9513727 themselves burying a surface area of 3,565.4 Å^2 (1,651.5 Å^2 without contribution from NDT) (Fig. 1 and Extended Data Fig. 7). However, another crystal form of C5aR1 with NDT9513727 was also obtained in initial screening (yet consistently diffracted to lower resolution), revealing a single molecule in the asymmetric unit and signal in the difference density for NDT9513727 with the small molecule mediating no crystallographic or non-crystallographic contacts (data not shown). Furthermore, mutation of Ile155$^{4.44}$ to methionine, Val159$^{4.48}$ to phenylalanine and Gly162$^{4.51}$ to phenylalanine in an attempt to disrupt NDT9513727 binding across the dimeric interface (Extended Data Fig. 7) had no effect on ligand binding in whole-cell functional assays (data not shown). Taken together, this provides evidence that binding of the small molecule is not dependent upon the parallel TM4–TM5-mediated assembly observed between the two copies in the high-resolution crystal structure. In structural terms, evidence for potential modes of homodimerization are available for several class A GPCRs including, for example, β_2-AR[22,23], CXCR4[24], μ-opioid[25], κ-opioid[26] and smoothened (SMO)[27]. The structure of the SMO receptor in complex with the antitumorigenic small-molecule antagonist

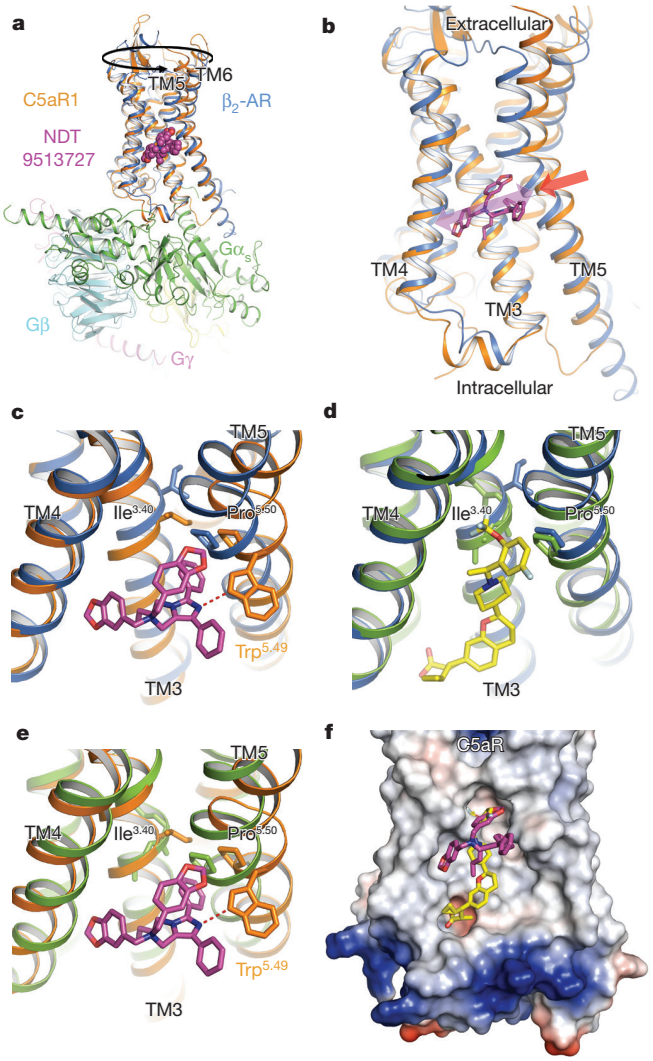

Figure 3 | NDT9513727 extra-helical antagonism and comparison of C5aR1 to β_2-AR–G_S and GPR40 agonist crystal structures. a, The C5aR1 structure (orange ribbon) overlaid with the β_2-AR–G_S structure (β_2-AR coloured blue; PDB code 3SN6). NDT9513727 is in sphere representation coloured as in Fig. 1, viewed from a plane parallel to the membrane. **b**, As in **a**, isolating the receptor seven-transmembrane domains. **c**, Close-up view of the C5aR1 NDT9513727-binding site compared to β_2-AR. **d**, View of the GPR40 (green; PDB code 5TZY) ago-PAM (AP8) (yellow) binding site compared to β_2-AR. **e**, View of the C5aR1 NDT9513727-binding site compared to GPR40 (green). **f**, Molecular surface representation of C5aR1 with NDT9513727 bound and the GPR40 ago-PAM overlaid.

LY2940680 (PDB code 4JKV) displays a TM4–TM5 contact interface most closely resembling that of the C5aR1 (Extended Data Fig. 7). Although it is tempting to speculate that the non-crystallographic dimer of C5aR1 reported here is physiologically relevant, the different crystal forms that can be obtained for C5aR1 highlight the non-trivial nature of deconvoluting physiologically relevant dimerization interfaces from those that simply mediate crystal contacts.

The structure of C5aR1 complexed with NDT9513727 provides the first, to our knowledge, detailed view of a complement component receptor and reveals an extra-helical negative allosteric-binding pocket between TM3, TM4 and TM5. The NDT9513727 ligand seems to act as a sterical wedge that blocks the relative movement of TM5 (as seen in β_2-AR–G_s and GPR40) and thereby inhibits activation of C5aR1. Interestingly, although this pocket centred on Trp213$^{5.49}$ forms the site for NDT9513727 and similar small molecules, previous mutation data[28] together with results from our competition assay (Extended