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Determining the binding site of ORG27569 within the CB1 receptor

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Introduction

- Functional antagonists of the cannabinoid receptor 1 (CB1) receptor are considered to have great potential in obesity management.
- Most neutral antagonists and inverse agonists bind to the main (orthosteric) site of CB1, and are CNS-active, exhibiting adverse neuropsychiatric effects.
- In contrast, negative allosteric modulators (NAMs) block CB1 signaling by binding at a site that is topologically distinct from the orthosteric site.
- NAMs are highly selective. They exhibit a ceiling effect, probe dependence and biased signaling, making them less liable to elicit CNS-mediated adverse effects.
- ORG27569, an intrinsic inverse agonist and negative allosteric modulator (NAM) of CB1, increases the binding affinity of agonist CP55940.
- In this study, we applied computational methods to identify the binding site of ORG27569, amidst contradictory reports in the literature (**Fig. 1**).

Objectives and Significance

- To identify the binding pose of CP55940 within the CB1 X-ray crystal structures.
- To identify potential allosteric sites in the active-state and inactive-state complexes of the CB1 receptor bound to CP55940.
- To identify the preferred binding site of ORG27569 within an inactive-state CB1–CP55940 complex.
- To determine the binding site of ORG27569 will aid rational design of novel NAMs of the CB1 receptor.

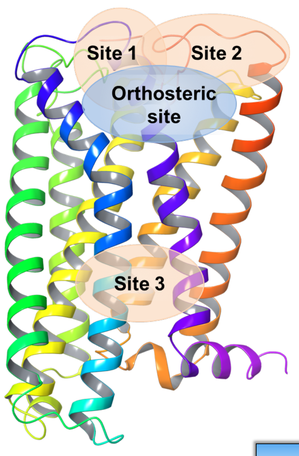
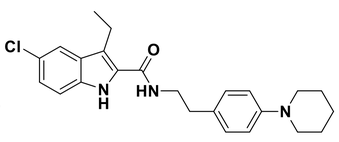
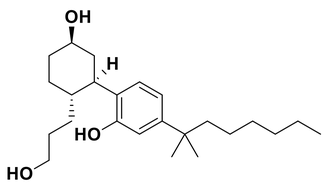


Fig. 1. (Left) CB1 model created from the inactive-state X-ray CB1 crystal structure, 5U09, with the orthosteric site highlighted in blue and previously predicted allosteric sites[1-3] in orange. (Right) CB1 NAM, ORG27569, and CB1/CB2 agonist, CP55940.



ORG27569



CP55940

Methods

- CB1 receptor models were built using each of the four X-ray crystal structures[4-6] of CB1 as a template, with the N-terminus added on through our computational modeling, in order to be able to include the reported C98–C107 disulfide bridge.
- CP55940 was docked into each of the four CB1 receptor models using the Glide[7] Standard Precision (SP) protocol of Schrödinger.
- SiteMap[8], Schrödinger was used to identify the top-ranked potential binding sites in the CB1–CP55940 complexes.
- ORG27569 was docked into all the pockets identified using SiteMap.
- A 50 ns molecular dynamics simulation was performed on the 5U09 model of the CB1 receptor bound to CP55940, using the OPLS3 force field.
- A SiteMap analysis was run on frames extracted every 0.5 ns from the trajectory.
- Molecular docking studies of ORG27569 were performed on six predominant site clusters obtained from the trajectory using the Glide SP protocol, InducedFit docking and Prime[9] free energy calculations.

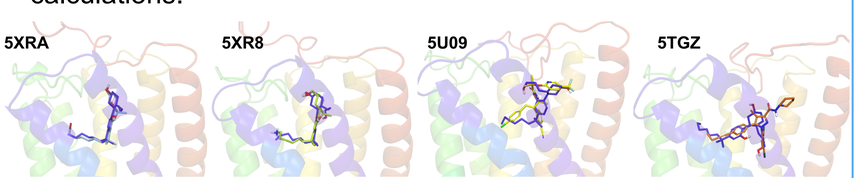


Fig. 2. Docked CP55940 (C purple) overlaid with the co-crystallized ligand within each of the four X-ray crystal structure-based CB1 models.

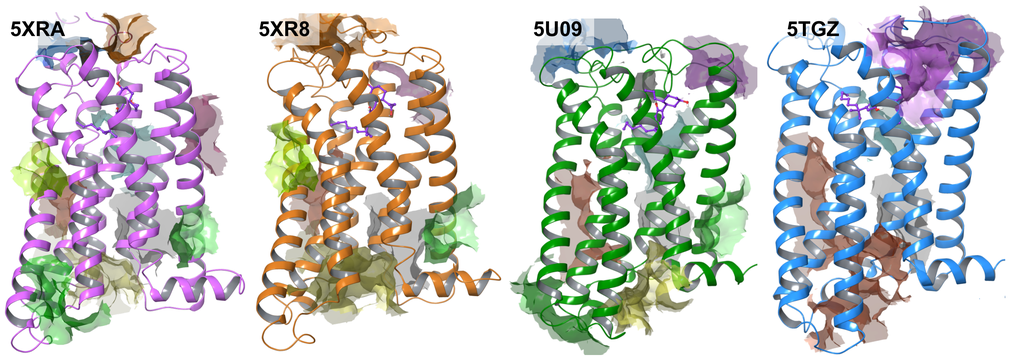


Fig. 3. Maps of identified allosteric binding sites (each site's surface in different color) for the CP55940-bound complex of the CB1 receptor, using X-ray structures of the inactive-state (**A**) 5TGZ and (**B**) 5U09; and active-state (**C**) 5XR8 and (**D**) 5XRA. CP55940 (C magenta; non-polar hydrogen atoms are not shown, for clarity) is shown in the orthosteric site.

Results and Discussion

- The docking orientation of CP55940 in all four models of CB1 was similar (**Fig. 2**).
  - Docked poses of CP55940 obtained in the active-state CB1 X-ray structures were highly similar to the binding poses of the co-crystallized ligands, with an RMSD <1 Å for common heavy atoms.
- SiteMap analysis revealed multiple allosteric sites within the CP55940-bound CB1 complexes (**Fig. 3**).
- Rigid receptor docking within the allosteric sites identified by SiteMap did not provide a consensus docking site for ORG27569 across all structures (**Fig. 4**).

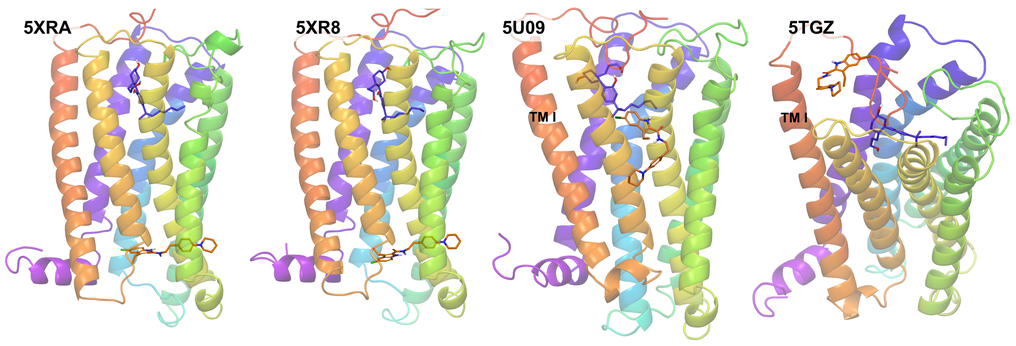


Fig. 4. Most favorable docking sites for ORG27569 (C orange) within the rigid CP55940-bound CB1 complexes. CP55940 (C magenta). Non-polar hydrogen atoms are not shown for clarity.

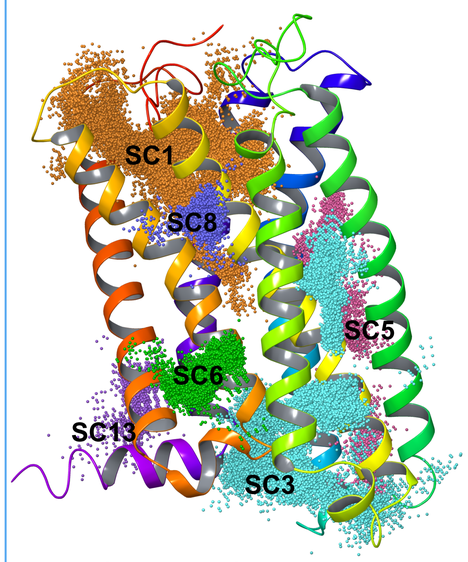


Fig. 5. Site points of six predominant site clusters (SC) in the 5U09-based CB1–CP55940 complex following a 50 ns MD simulation.

- Since the SiteMap and docking protocols consider a rigid receptor conformation that is most suited for docking of small molecules that are very similar to the native ligand, we performed an MD simulation of the CB1–CP55940 complex to generate multiple receptor conformations and performed binding site analysis on them.
- Of thirty site clusters obtained from the MD simulations, six site clusters were present in at least 40 of the frames processed (**Fig. 5**).
- Molecular docking studies of ORG27569 within the site clusters revealed that site cluster 3 was the most favored (**Table 1**).
- ORG27569 docked best at an intracellular site near helices 2, 6 and 7 (SC3) within CB1 (**Fig. 6**).
  - Two hydrogen bond interactions were observed with Y153 and D403, similar to interactions found with the β2AR intracellular antagonist, Cmpd-15PA[10].
  - The close proximity of ORG27569 to the canonical ionic lock residues, R214 and D338, may account for disruption of CB1 signaling.

Table 1. The docking performance of ORG27569 within the predominant site clusters obtained from the CP55940-bound complex of CB1.

Site Cluster	Glide Gscore [a]	IFD Score [a]	Prime Free Energy [a]
1	−6.190	−549.22	−50.00
3	−9.740	−556.08	−86.82
5	−6.542	−489.49	−66.50
6	−8.439	−495.44	−76.66
8	−7.862	−495.47	−65.08
13	−6.862	−493.61	−64.92

[a] Units: kcal/mol

Conclusions

- The orientation of CP55940, which aligned well with native poses of the co-crystallized ligands within the experimental active-state CB1 structures, validated its docking pose within the inactive-state models.
- Binding site analysis and subsequent molecular docking with ORG27569 on the rigid receptor models did not provide a consensus binding site for ORG27569.
- Binding site analysis performed on the flexible receptor model, using a conformational ensemble of the protein, provided more information about the probability of finding a particular site at any time.
- The best docking site for ORG27569 was found at an intracellular site on CB1, near helices 2, 6 and 7.

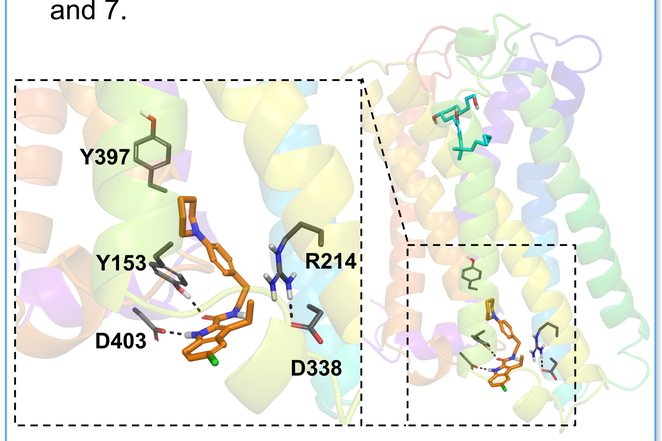


Fig. 6. ORG27569 (C orange) and CP55940 (C cyan) within the 5U09-based CB1 structure. Hydrogen bonds are shown using black dashed lines. Non-polar hydrogen atoms are not shown for clarity.

Future work

- Enhanced MD simulations (such as metadynamics) of the ORG27569−CP55940−CB1 complex using data from previously-reported mutagenesis studies.
- Assessment of the mechanism by which ORG27569 interferes with CP55940-induced CB1 activation.

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Acknowledgements

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