Allosteric modulation of the human $GABA_B$ receptor

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✓ INTRODUCTION

- Ɣ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system (CNS), and plays highly important roles in regulating the release of other neurotransmitters and excitation of neurons. GABA exerts its function by activating ionotropic $GABA_A$ and $GABA_c$ receptors and the metabotropic $GABA_B$ receptors. The $GABA_B$ receptor is a class C G protein coupled receptor (GPCR) and ligands targeting the receptor may be novel drug therapies for multiple conditions, including addiction, epilepsy, nociception, and depression.
- The $GABA_B$ receptor is a functional heterodimer ($GABA_{B1}$ and $GABA_{B2s}$ subunits). Each subunit consists of an extracellular N-terminal (Venus Fly Trap (VTF)) domain, a transmembrane (7TM) domain , and the intracellular C-terminus. The orthosteric binding site is located in the $GABA_{B1}$ VFT while an allosteric binding site is found in the 7TM domain of $GABA_{B2}$ (Figure 1).
- Binding of compounds to the allosteric site may affect the affinity and/or efficacy of the orthosteric ligands. Allosteric binding sites are usually less conserved than the orthosteric sites among GPCRs. Due to this, allosteric modulators are highly pharmacologically interesting as they may act more specifically and trigger fewer side effects.
- Currently, the only marketed $GABA_B$ drug is the agonist baclofen, a muscle relaxant and antispastic agent. During the last decade, increasing efforts have been put into development of allosteric $GABA_B$ modulators and currently, several positive allosteric modulators (PAMs, Figure 2) and three negative allosteric modulators (NAMs) are known.

✓AIM

The 7TM domain of the $GABA_B$ receptor has not yet been solved by x-ray crystallography, and the lack of 3D structure is hampering structure-based development of new allosteric modulators. In the present study, a ligand-guided homology modelling approach has been undertaken to predict the 3D structure of the $GABA_{B2}$ subunit and the allosteric site of the $GABA_B$ receptor.

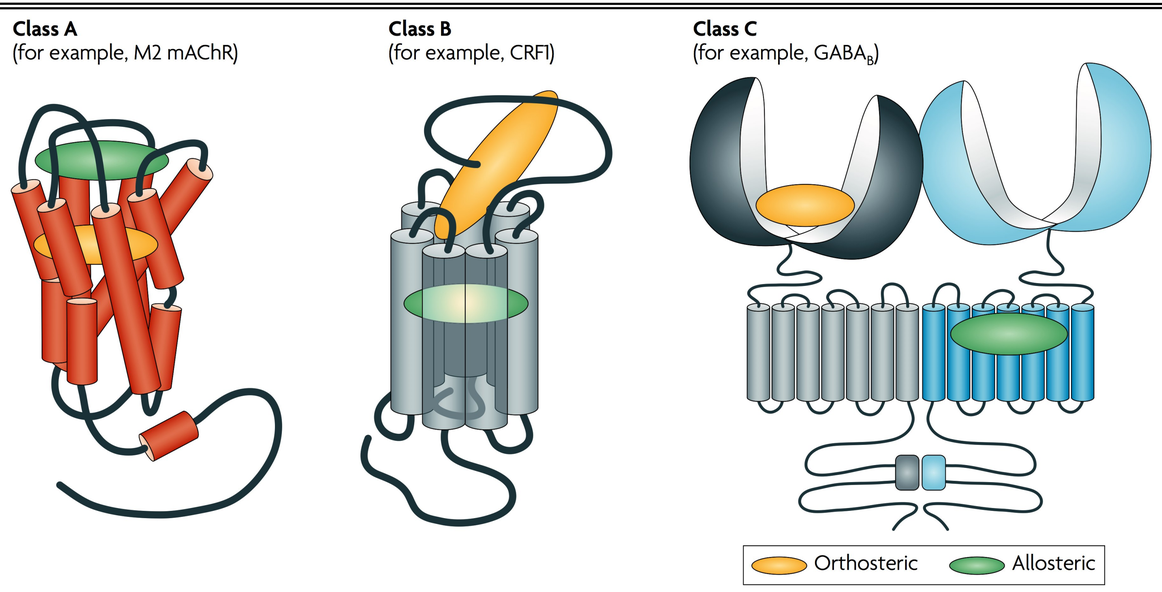

Figure 1: Schematic representation of the location of both orthosteric and allosteric binding sites for the GPCRs families A, B and C. From *Nature Reviews Drug Discovery* 8, 41-54 (January 2009).

✓METHODS

- The following templates were used to construct the $GABA_{B2}$ homology models: class A (PDB ids 1U19 and 2RH1), class B (PDB ids 4K5Y and 4L6R), and class C (PDB ids 4OR2 and 4OO9).
- 100 $GABA_{B2}$ homology models per template were constructed using MODELLER software[1].
- To select models that enriched known active ligands, 72 known PAMs and approx. 2500 property-matched decoys (assumed inactive compounds) were assembled and docked into the $GABA_{B2}$ models using Schrödinger software[2]. To analyse the docking results, the BEDROC method [3] was used.
- 25 $GABA_{B2}$-PAM complexes were optimised using the induced-fit docking protocol of Schrödinger software followed by re-docking of the PAMs and decoys. The orientations of the PAMs in the best-ranked models according to the BEDROC scores were visually inspected and used for selection of the final $GABA_{B2}$ homology models.
- The workflow used is displayed in Figure 3.

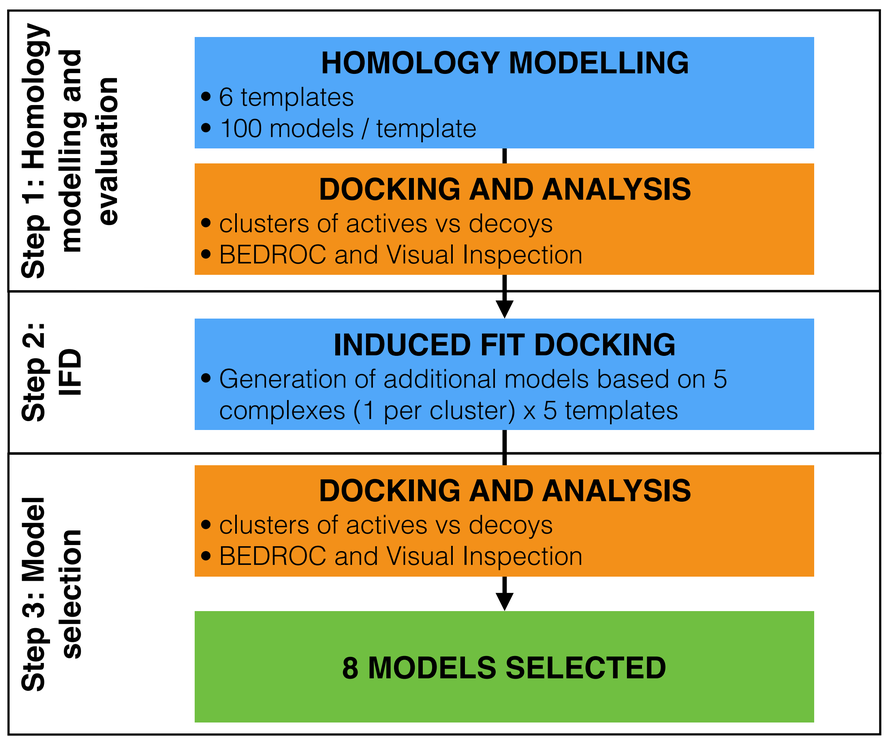

Figure 3: Project workflow.

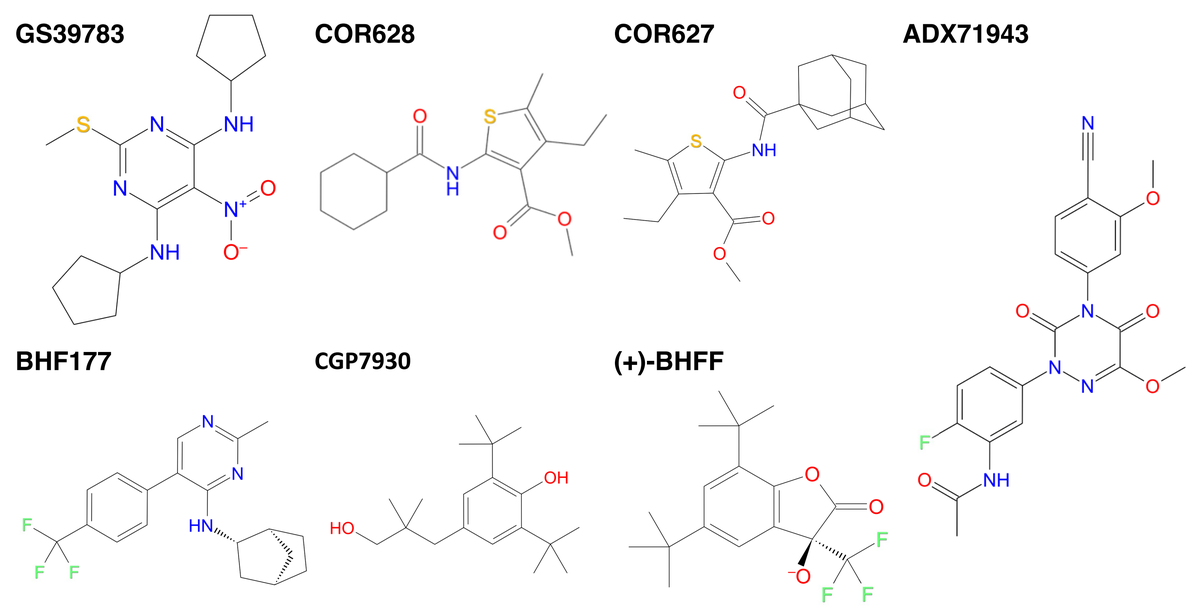

Figure 2: Structure of selected PAMs.

✓RESULTS

- 8 final $GABA_{B2}$ homology models that enriched the known PAMs were selected based on docking of PAMs vs property-matched decoys and visual inspection of ligand orientations in the allosteric binding site.
- The location of the predicted allosteric binding site in one of the final $GABA_{B2}$ homology model is shown in Figure 4.
- The residues constituting the allosteric binding pocket predicted by docking of the PAMs in were in accordance with the mutagenesis data available for the $GABA_B$ receptor and other class C GPCRs.

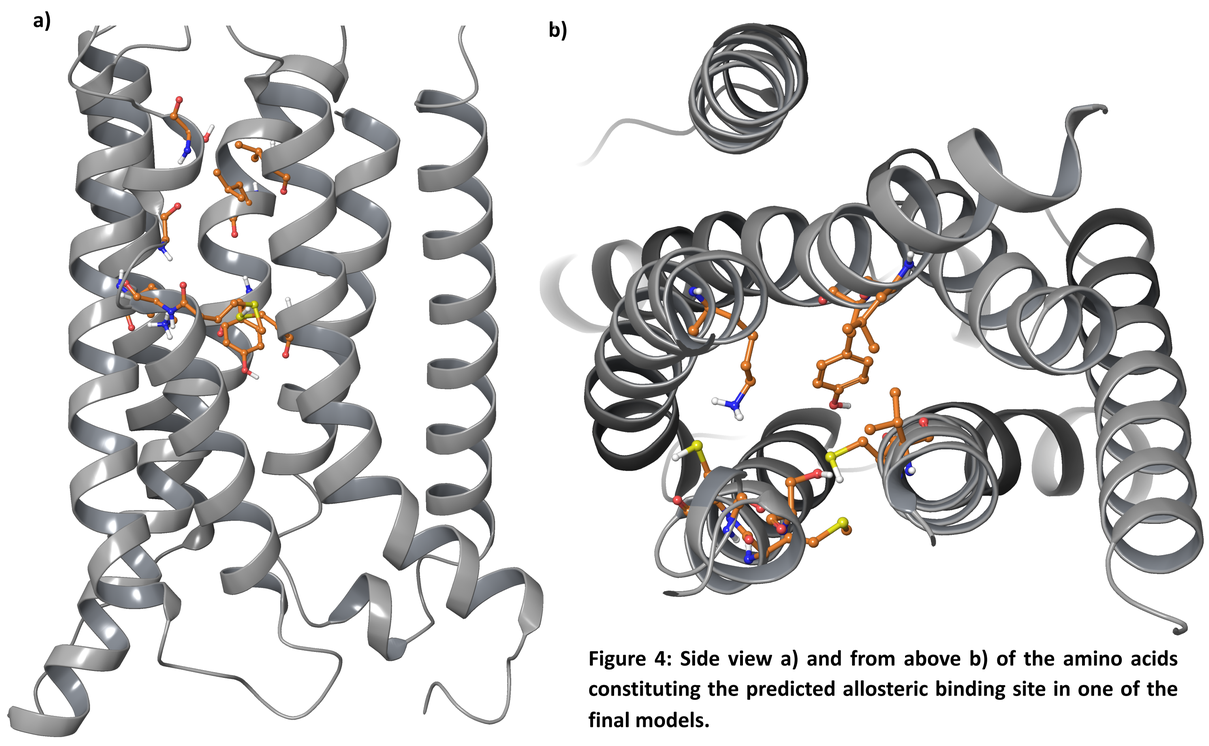

Figure 4: Side view a) and from above b) of the amino acids constituting the predicted allosteric binding site in one of the final models.

✓CONCLUSION

8 $GABA_{B2}$ homology models containing the allosteric binding site of the $GABA_B$ receptor have been constructed. The selected models enrich known PAMs and may be uses as tools for future structure-based studies to identify new allosteric modulators of the $GABA_B$ receptor.

✓BIBLIOGRAPHY

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✓ACKNOWLEDGEMENTS

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