



ABSTRACT

Graves' disease is an autoimmune disease characterized by overproduction of thyroid stimulating hormones that materialize into heat intolerance, tremors, weight loss, rapid heartbeat, among many other symptoms.² Through overproduction of thyroid stimulating hormones, the thyroid stimulating hormone receptor (TSH-R) is overstimulated, resulting in the generation of TSH-R specific antibodies that initiate the autoimmune response.⁵

Recently, the cryo-EM structure of TSH-R was solved, revealing the active and inactive states and illuminating a hinge region in the receptor that facilitates movement between them.³ PASSER¹⁷ pocket finding was used to investigate new allosteric sites in the hinge region to stabilize TSH-R in its inactive form. Additionally, molecular docking with SwissDock^{18–23} was used to dock drug-like molecules into the identified pocket. Chemical structures were then explored using fragment-based and rational drug design approaches. Molecules with highly favorable AC Scores (<-300 kcal/mol) were identified as potential TSH-R binders to the hinge region of the inactive receptor. A class of molecules, di(1,3,5-triazin-2-yl)methane, emerged with superior binding affinity, with the optimal binder possessing an AC Score of -354 kcal/mol. Moreover, a fluorescent probe was designed to target the region with an AC Score of -380 kcal/mol.

Here, we identified a new allosteric pocket in the inactive TSH-R. Using molecular docking, we identified potential binders for this region that could exhibit allosteric antagonism of the Graves' disease target. We have also proposed a new fluorescent probe to aid in further investigation of TSH-R activity.

RESEARCH QUESTION

Can an inactive-state-specific hinge pocket be exploited to design small molecules that stabilize TSH-R and enable direct allosteric antagonism?

KEY FINDINGS

A buried pocket found only in inactive TSH-R accommodates highly functionalized bis-triazine ligands, yielding predicted AC scores below -350 kcal/mol.

Keywords: Graves' disease · TSH-R · allostery · docking · fluorescent probe

In Silico Design of Small-Molecule Allosteric Antagonists of the Thyroid-Stimulating Hormone Receptor

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INTRODUCTION

- Graves' disease is the leading cause of hyperthyroidism in developed countries; stimulating autoantibodies keep TSH-R in the activated complex.^{1,3}
- Current treatments reduce hormone synthesis or remove thyroid tissue, but do not directly prevent receptor overstimulation.¹³
- Activation rotates the extracellular domain by ~55°. The hinge couples this motion to the seven-transmembrane domain, creating a structural opportunity for state-selective inhibition.¹⁴

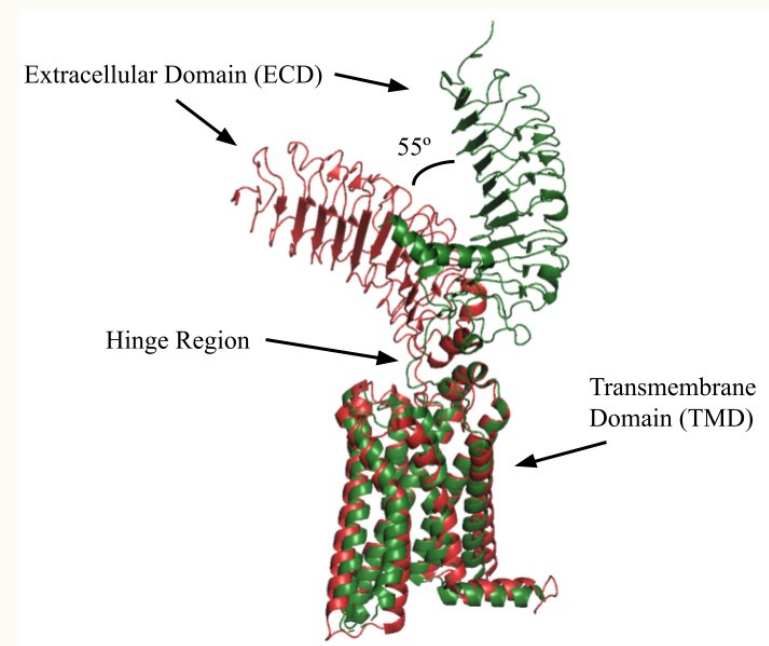


Figure 1. Active (green; 7T9I) versus inactive (red; 7T9M)^{15,16} TSH-R. The extracellular domain pivots around the hinge.

METHODS

1 Structure selection

Active 7T9I and inactive 7T9M^{15,16} TSH-R aligned across Transmembrane domain residues 472–486 to look for pockets that can be the site of docking.

2 Pocket discovery

PASSer¹⁷ identified cavities present in inactive TSH-R that were absent from the active state.

3 Iterative docking

SwissDock^{18–23} screens progressed from common heterocycles²⁴ to fragment-grown analogues.

4 Probe design

Top ligands were conjugated to fluorescein²⁶ through 1–3 PEG units and re-docked.

5 Analysis

AC score (kcal/mol), binding pose, pocket occupancy, and residue-level contacts visualized in PyMOL/SwissDock.

RESULTS

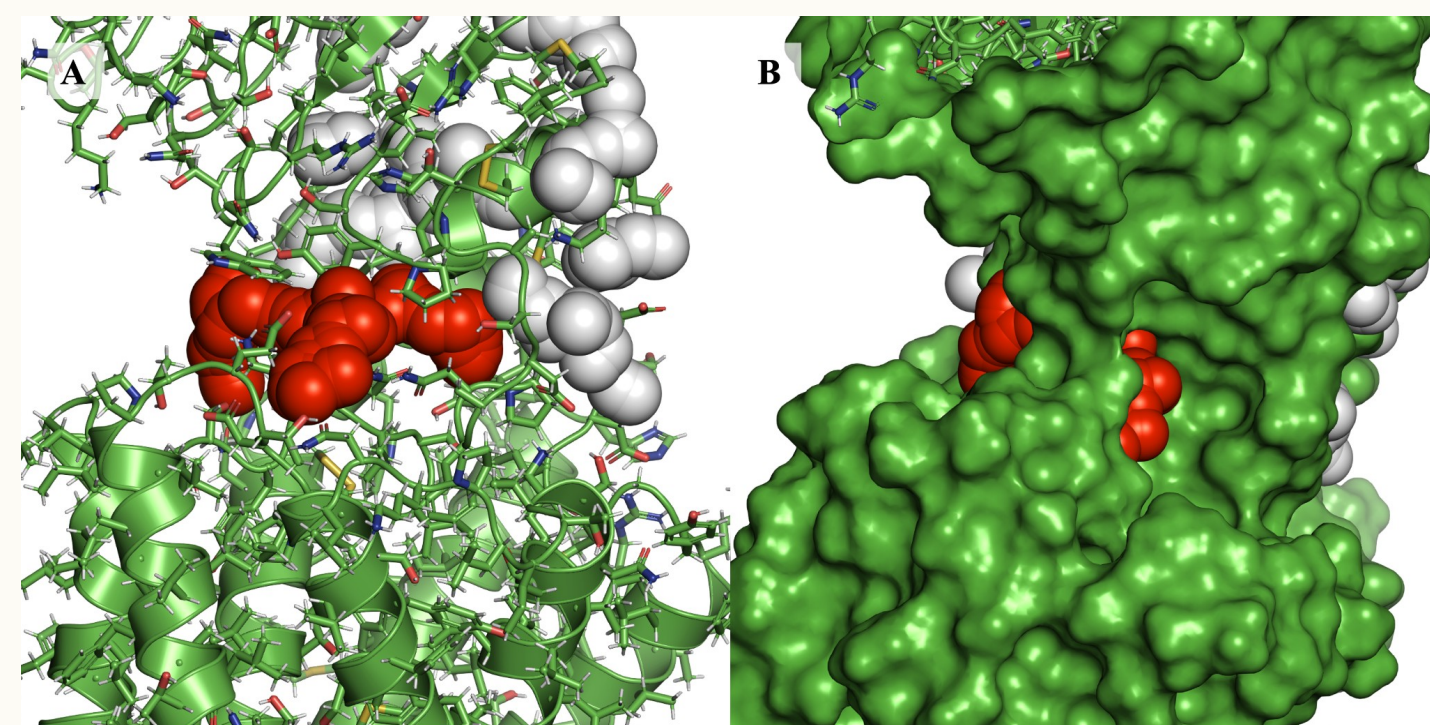


Figure 2. PASSer¹⁷ pocket map. Red spheres mark the buried, inactive-state-specific hinge cavity selected for docking.

POCKET CHEMISTRY

Polar lining: Glu575 · Trp258 · Tyr279 · Lys565 · Ala564 · Gln489 · Thr490 · Asn406

Design implication: combine hydrogen-bonding functionality with sufficient hydrophobic surface to fill the enclosed cavity.

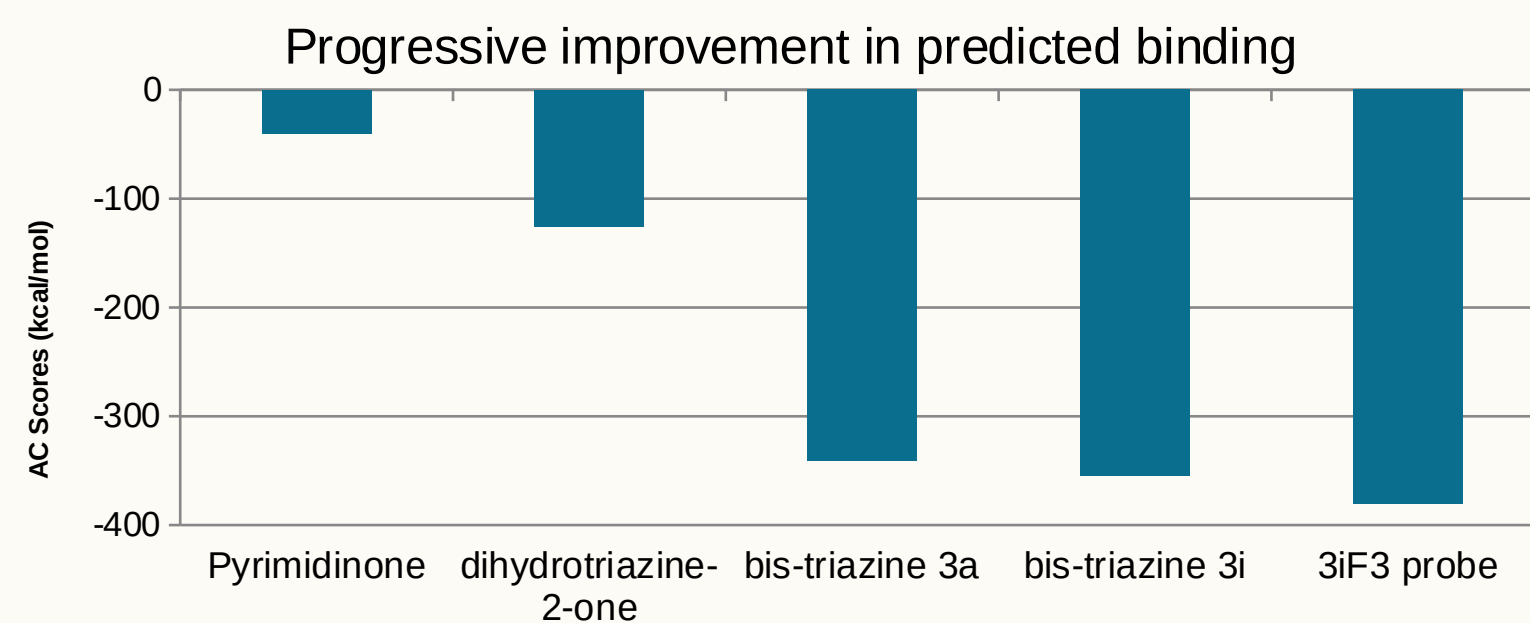


Figure 3. SwissDock^{18–23} AC scores. More negative values indicate stronger predicted cavity attraction; scores are not experimental binding free energies.

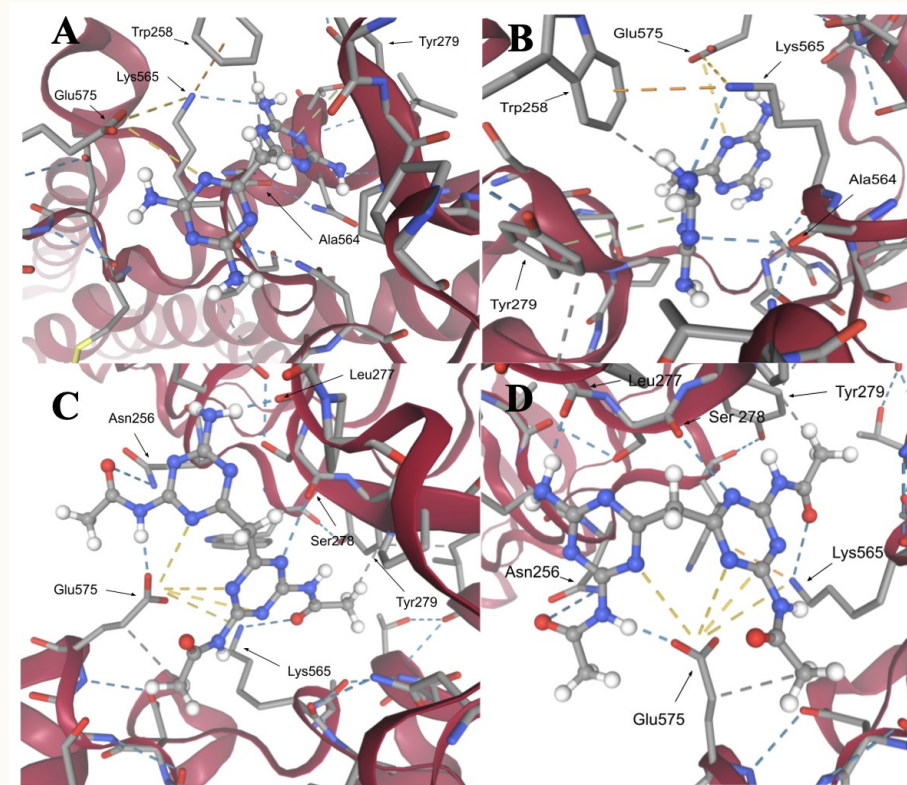


Figure 4. Pocket binding patterns for 3a and 3i. Lead ligands bridge both sides of the hinge pocket through polar, ionic, aromatic, and hydrophobic contacts.

BEST SMALL MOLECULE 3i: -354.861 kcal/mol

DISCUSSION

- State selectivity is structurally plausible: the target cavity is accessible in inactive TSH-R but absent after receptor activation.^{3,14}
- Structure-activity trends favour extended, symmetric bis-triazines with multiple hydrogen-bond donors/acceptors and balanced hydrophobic contacts.
- Structure 3i improves on 3a through acetamide-mediated contacts and broader pocket occupancy; excessive branching reduces entry through sterically constrained regions.
- The docking workflow generates hypotheses, not proof of antagonism. AC scores should be interpreted comparatively within this screen.
- Inhibitors provide expansion with receptor-selective therapeutic strategies.

CONCLUSION

A previously underexplored hinge pocket in inactive TSH-R offers a rational site for allosteric antagonism. Fragment based design transformed weak heterocyclic hits into stronger binding bis-triazine ligands. Compounds 3a and 3i span the buried cavity and engage residues on opposing sides, providing a plausible mechanism for stabilizing the inactive receptor. The 3iF3 probe further suggests that this site can support fluorescence-enabled screening.

NEXT EXPERIMENTS

- Confirm direct binding and selectivity.
- Measure TSH-stimulated cAMP inhibition and determine IC₅₀ and inhibitory constants K_i to test strength of inhibition.²⁵
- Test receptor-state dependence and optimize ADME properties.
- Deploy 3iF3 in competition or high-throughput assays.²⁶

TAKE-HOME: The inactive TSH-R hinge is a druggable allosteric opportunity with experimentally testable chemical leads.

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More references shown in paper.

PDB: 7T9I (active)¹⁵ · 7T9M (inactive)¹⁶ | PASSer¹⁷ · SwissDock^{18–23} · PyMOL