Fighting Obesity with a Sugar-Based Library: Discovery of Novel MCH-1R Antagonists by a New Computational-VAST Approach for Exploration of GPCR Binding Sites

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SUPPLEMENTARY METHODS

Calculation of AMBER MCH-1R-ligand interaction energy

The MM_PBSA/GBSA approach [1] is post-processing method to evaluate free energies of binding between protein and ligand. The all-atom AMBER molecular mechanics force field [1-3] was used to calculate interactions energy[1,4] between MCH-1R and ligand. The calculations of interaction energy were performed using ANAL program of AMBER 8 (2004) molecular simulation package and represent the internal energy (bond, angle and dihedral), and van der Waals and electrostatic interactions. The MM_PBSA script calculates the energies of the complex and of all interacting components and deducts them from each other to obtain free energies of binding. The MM_PBSA/GBSA method combines the molecular mechanical energies with continuum solvent approaches. The MM_PBSA/GBSA approach has been successfully applied to study protein-ligand complexes [5].

Shape comparison method

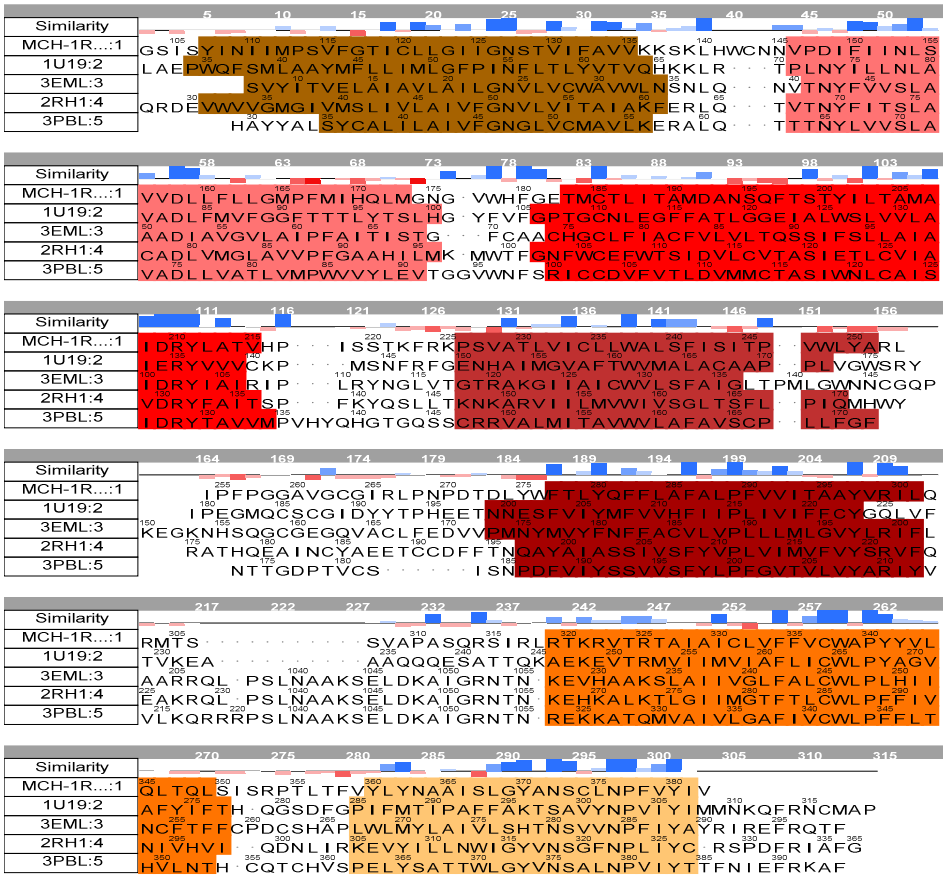
The shape comparison and scoring was performed using the OMEGA-ROCS tool as implemented in the OpenEye software package (version 3.0.0) [6]. Prior to shape comparison, we used OMEGA to generate potential bioactive and energetically accessible conformations for each molecule. A Gaussian description of molecular shape is used to compare the shapes of two molecules by maximization of their volume intersection [6]. ROCS - is a shape-based superposition method. Molecules are superposed by a solid-body optimization process that maximizes the overlap volume between them. Volume overlap is not the hard-sphere overlap volume, but rather a Gaussian-based overlap parameterized to reproduce hard-sphere volumes.

ROCS uses only the heavy atoms of a ligand, hydrogens are ignored. Since shape and volume in this context are so closely related, a volume overlap maximization procedure is an excellent method for gaining insights into similar shapes.

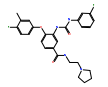
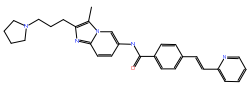
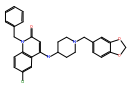
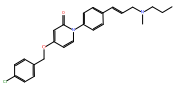
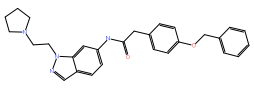
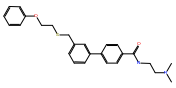
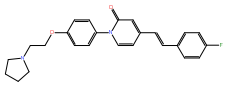
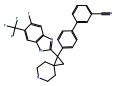
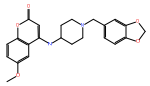
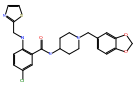
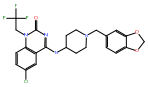
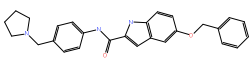
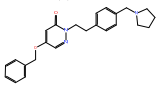
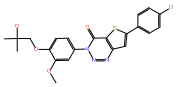
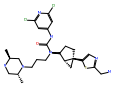
Statistical comparison of sub-pocket occupancy effects

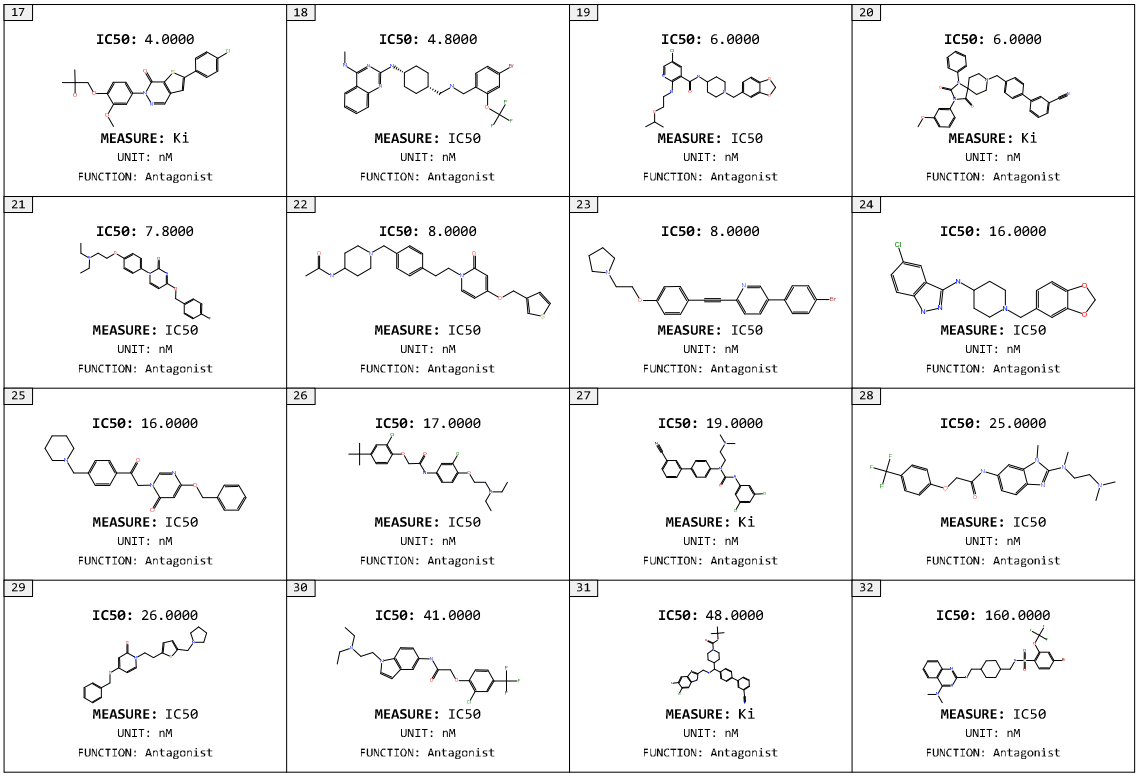
For comparison of the effect of individual sub-pocket occupancy on antagonist activity we performed Welch's *t*-test (Welch,B.L.[7] The generalization of "Student's" problem when several different population variances are involved).This test can be used to estimate the significance of differences in activity between two groups (i.e. where one group of compounds occupies a given pocket, and the other does not). This particular test was chosen as neither the number of members of each group, nor the variance of activities within each group need be the same. Calculations were performed online using GraphPad [http://www.graphpad.com/quickcalcs/ttest1.cfm] server.

Supplementary Figure S1: Shows multiple amino-acid sequence alignment of MCH-1R aligned with four GPCR crystal structures [human dopamine D3 receptor (D3, PDB entry 3PBL), β2-adrenergic receptor (β2AR, PDB entry 2RH1), human A2A adenosine receptor (A2A, PDB entry 3EML) and bovine rhodopsin (PDB entry 1U19)], the colour coding for TM 1 to 7 is dark orange, pink, red, purple, dark red, orange and light yellow respectively, the similarity or dissimilarity of each amino-acid compare to MCH-1R is shown by the bar, the strength of similarity are represented by the spectrum from blue (max similarity) to red (max dissimilarity), bar length and its directionality (up – similarity, down dissimilarity)



Supplementary Table S1: Representative set of known 32 potent MCH-1R antagonists

1 **IC50:** 0.1000  **MEASURE:** IC50 UNIT: nM FUNCTION: Antagonist	2 **IC50:** 0.4700  **MEASURE:** IC50 UNIT: nM FUNCTION: Antagonist	3 **IC50:** 0.8000  **MEASURE:** IC50 UNIT: nM FUNCTION: Antagonist	4 **IC50:** 1.0000  **MEASURE:** Ki UNIT: nM FUNCTION: Antagonist
5 **IC50:** 1.2000  **MEASURE:** IC50 UNIT: nM FUNCTION: Antagonist	6 **IC50:** 1.4000  **MEASURE:** IC50 UNIT: nM FUNCTION: Antagonist	7 **IC50:** 1.4700  **MEASURE:** Ki UNIT: nM FUNCTION: Antagonist	8 **IC50:** 1.5000  **MEASURE:** IC50 UNIT: nM FUNCTION: Antagonist
9 **IC50:** 1.7000  **MEASURE:** Ki UNIT: nM FUNCTION: Antagonist	10 **IC50:** 2.0000  **MEASURE:** IC50 UNIT: nM FUNCTION: Antagonist	11 **IC50:** 2.0000  **MEASURE:** IC50 UNIT: nM FUNCTION: Antagonist	12 **IC50:** 2.0000  **MEASURE:** IC50 UNIT: nM FUNCTION: Antagonist
13 **IC50:** 2.6000  **MEASURE:** IC50 UNIT: nM FUNCTION: Antagonist	14 **IC50:** 3.0000  **MEASURE:** IC50 UNIT: nM FUNCTION: Antagonist	15 **IC50:** 3.0000  **MEASURE:** Ki UNIT: nM FUNCTION: Antagonist	16 **IC50:** 4.0000  **MEASURE:** Ki UNIT: nM FUNCTION: Antagonist



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