

# Identification of small-molecule antagonists targeting the Growth Hormone Releasing Hormone Receptor (GHRHR)

*Minos-Timotheos Matsoukas<sup>1,‡,\*</sup>, Tarryn Radomsky<sup>2,3,‡</sup>, Vasilis Panagiotopoulos<sup>1</sup>, Robin du Preez<sup>2,3,‡</sup>, Michail Papadourakis<sup>4</sup>, Konstantinos Tsianakas<sup>1</sup>, Robert P Millar<sup>2,5,6,7</sup>, Ross C Anderson<sup>2,3</sup>, Georgios A Spyroulias<sup>8</sup>, Claire L Newton<sup>2,5,\*</sup>.*

1 University of West Attica, Department of Biomedical Engineering, Athens, 12243, Greece

2 Centre for Neuroendocrinology, Department of Immunology, Faculty of Health Sciences, University of Pretoria, Private Bag X323, Gezina, Pretoria, 0031, South Africa

3 Department of Physiology, Faculty of Health Sciences, University of Pretoria, Private Bag X323, Gezina, Pretoria, 0031, South Africa

4 Cloudpharm PC, Athens, 15125, Greece

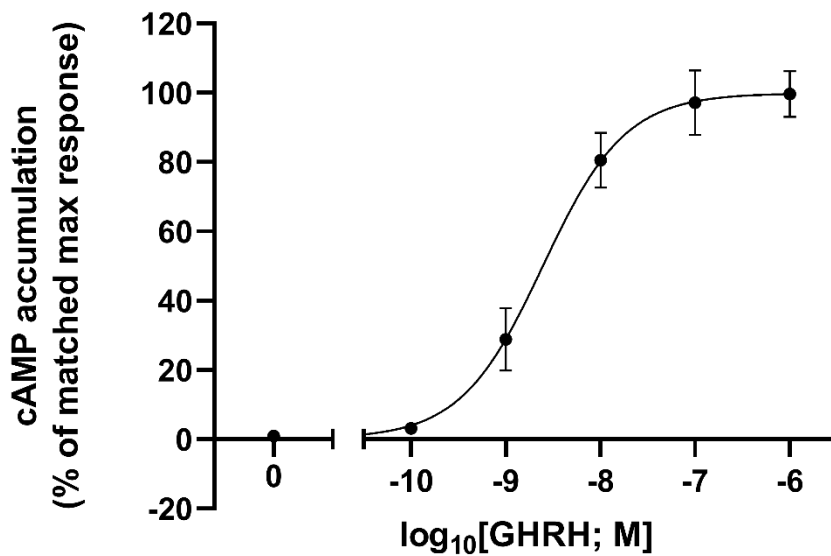
5 Deanery of Biomedical Sciences, University of Edinburgh, Edinburgh, EH8 9JZ, UK

6 Institute of Infectious Diseases and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, 7925, South Africa

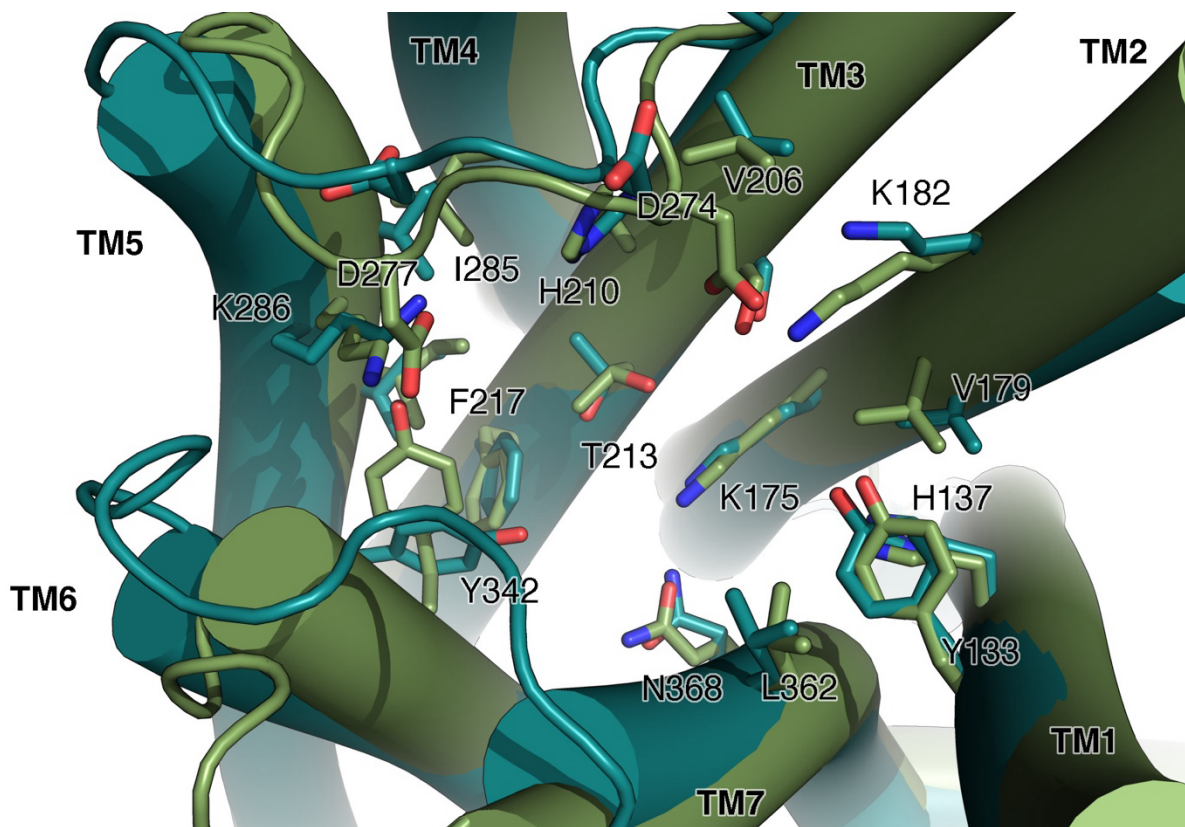
7 School of Medicine, University of St Andrews, St Andrews, KY16 9TF, UK

8 University of Patras, School of Health Sciences, Department of Pharmacy, University Campus, Rion, Patras, 26500, Greece

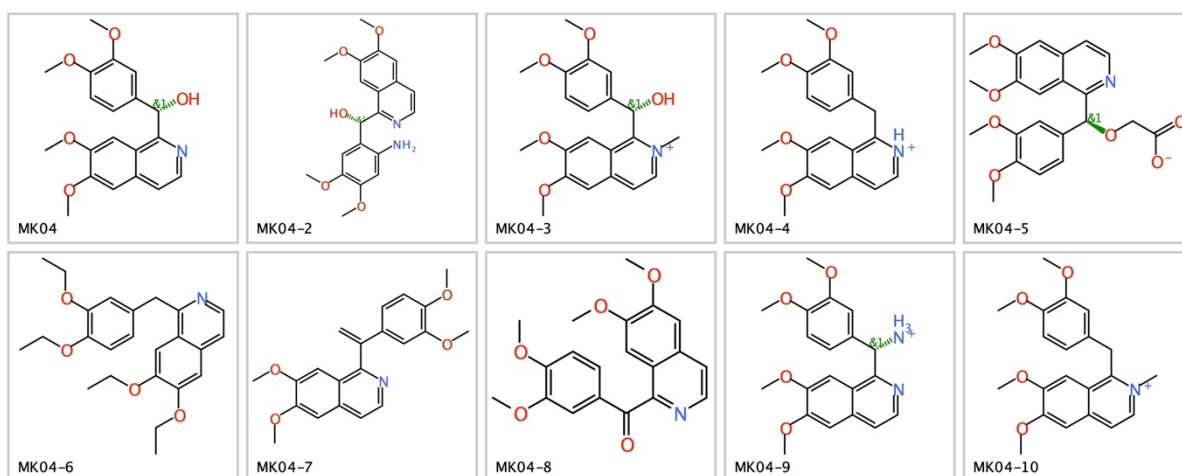




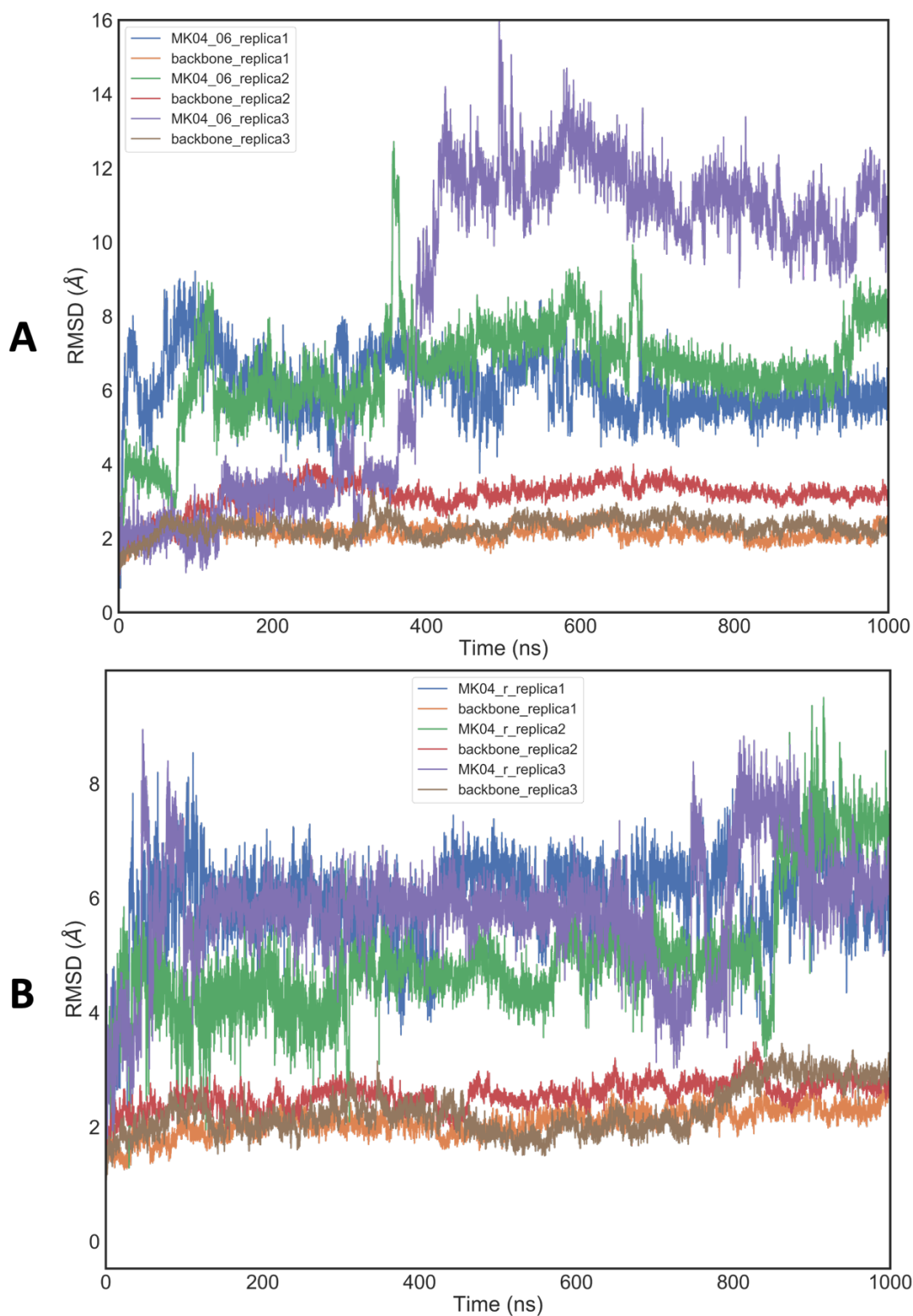
**Supplemental Figure S2. cAMP accumulation upon GHRH stimulation of cells transfected with GHRHR.** cAMP accumulation was measured by cAMP ELISA in HEK 293T cells transiently transfected with GHRHR following stimulation with a range of GHRH concentrations. Data are presented as mean  $\pm$  SEM from three independent experiments (N=3) and as % of the matched maximal responses after subtraction of basal stimulation (measured in the absence of ligand; 0).  $EC_{50} = 3$  nM.



**Supplemental Figure S3. Comparison between initial homology model (cyan) of GHRHR and AlphaFold 2 model (green).**



**Supplemental Figure S4. Hit expansion series (MK04-2 to MK04-10) selected based on the parent compounds MK04.**



**Supplemental Figure S5.** RMSD moving average of **A**) MK04\_06 (blue, green, and magenta for replicas 1, 2 and 3 respectively) and GHRH 7TMD backbone (orange, red and brown for replicas 1, 2 and 3 respectively) **B**) MK04 (blue, green, and magenta for replica 1, 2 and 3 respectively) and GHRH 7TMD backbone (orange, red and brown for replicas 1, 2 and 3 respectively) as a function of time during the MD simulations.