

Supporting information

DREADD AGONIST 21 (C21) IS AN EFFECTIVE AGONIST FOR MUSCARINIC-BASED DREADDs IN VITRO AND IN VIVO

Karen J. Thompson^{1#} Elham Khajehali^{2#}, Sophie J. Bradley¹, Jovana S. Navarrete^{3,4}, Xi Ping Huang⁵, Samuel Slocum⁵, Jian Jin⁶, Jing Liu⁶, Yan Xiong⁶, Reid H. J. Olsen⁵, Jeffrey F. Diberto⁵, Kristen M. Boyt⁵, Melanie M. Pina⁵, Dipanwita Pati⁵, Colin Molloy¹, Christoffer Bundgaard⁷, Patrick M. Sexton², Thomas L. Kash⁵, Michael J. Krashes^{3,4}, Arthur Christopoulos², Bryan L. Roth^{5*}, Andrew B. Tobin^{1*}

¹Centre for Translational Pharmacology, Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, Scotland, G12 8QQ, UK

(email Andrew.tobin@glasgow.ac.uk)

²Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria, 3052 Australia

³Diabetes, Endocrinology, and Obesity Branch, National Institutes of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA.

⁴National Institute on Drug Abuse, National Institutes of Health, Baltimore, Maryland 21224, USA.

⁵Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, North Carolina, NC2751, USA.

(Email: bryan_roth@med.unc.edu)

⁶Center for Chemical Biology and Drug Discovery, Departments of Pharmacological Sciences and Oncological Sciences, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY10029, United States

⁷Eli Lilly & Co, Neuroscience, Erl Wood Manor, Windlesham, Surrey, GU20 6PH, UK

* Corresponding authors:

Andrew B. Tobin

¹Centre for Translational Pharmacology, Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, Scotland, G12 8QQ, UK

(email Andrew.tobin@glasgow.ac.uk)

Bryan L. Roth

Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, North Carolina, NC2751, USA.

(bryan_roth@med.unc.edu)

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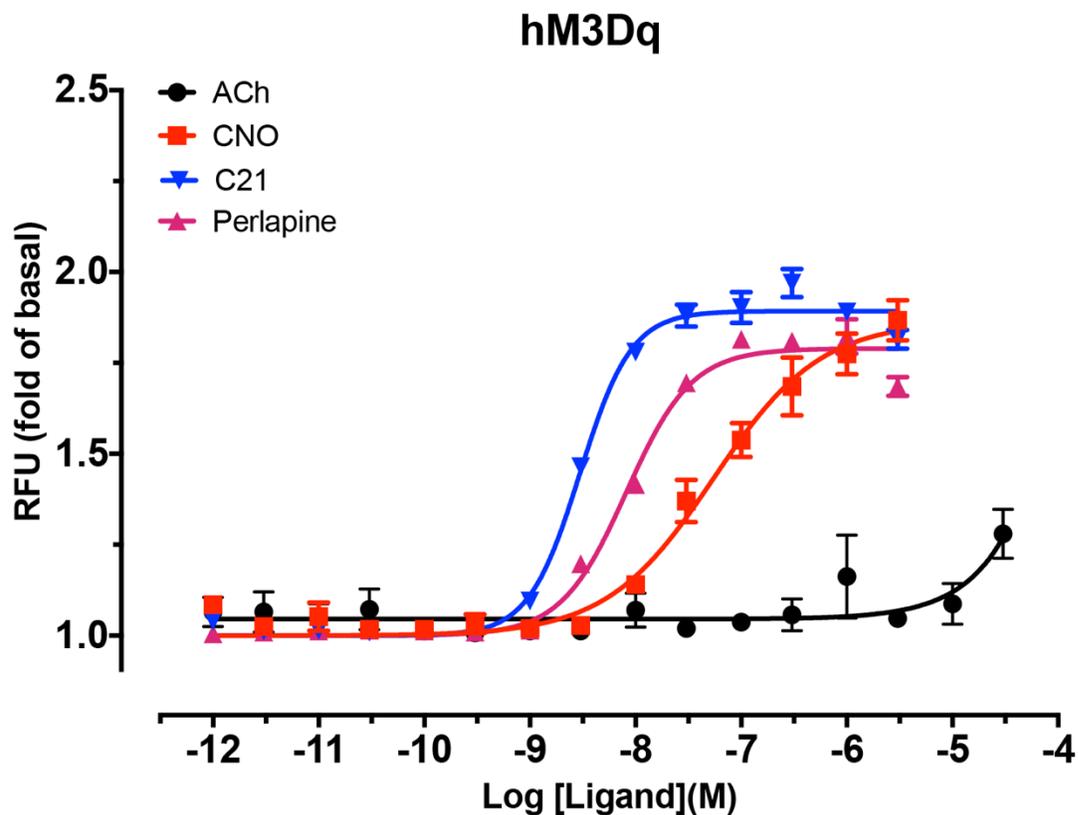


Figure S1. Signaling at hM3Dq mediated by muscarinic DREADD ligands.

Concentration response curves for acetylcholine (ACh), clozapine N-oxide (CNO), C21 and perlapine in calcium mobilization assays. Data represents the mean \pm SEM of at least three experiments performed in duplicate. The pEC_{50} for ACh, CNO, C21 and perlapine was 1.80 ± 0.2 , 7.26 ± 0.07 , 8.48 ± 0.05 and 8.08 ± 0.05 respectively.

Figure S2

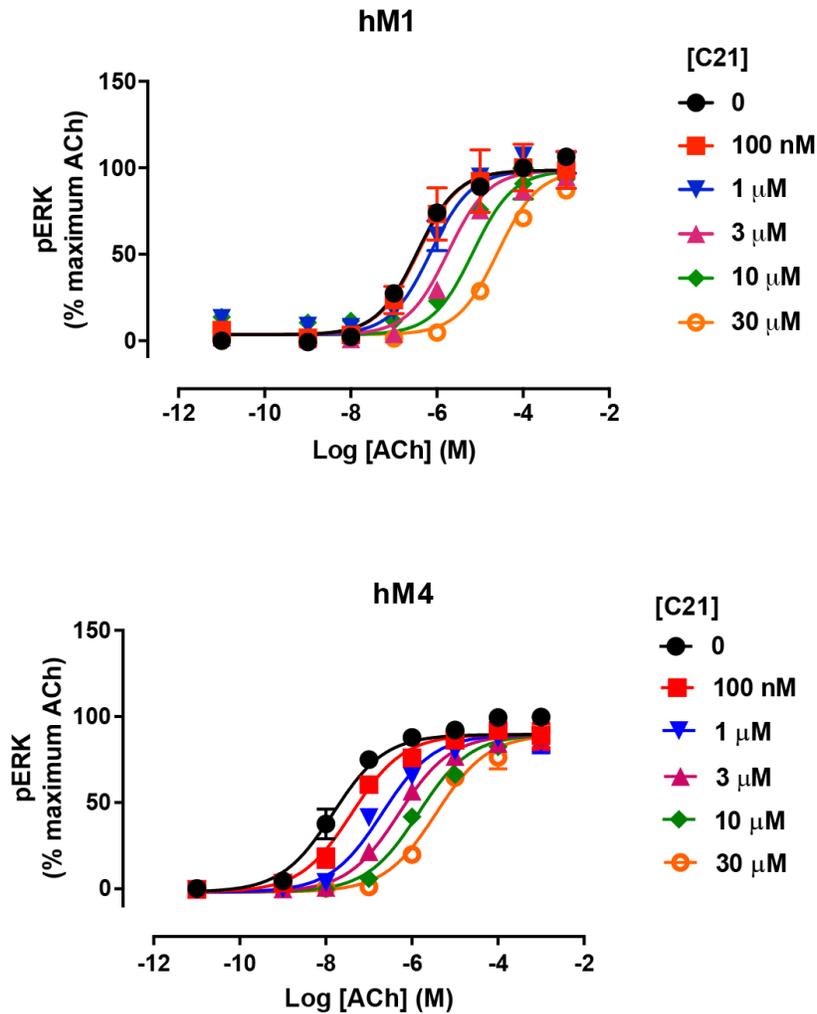


Figure S2: Antagonist activity of C21 at hM1 and hM4

Transfected CHO cells expressing (A) hM1 or (B) hM4 receptors were stimulated with various concentrations of acetylcholine (ACh) in the presence or absence of the indicated concentrations of C21. The pERK response was then determined. Data represents the mean \pm SEM of at least three experiments.

Figure S3

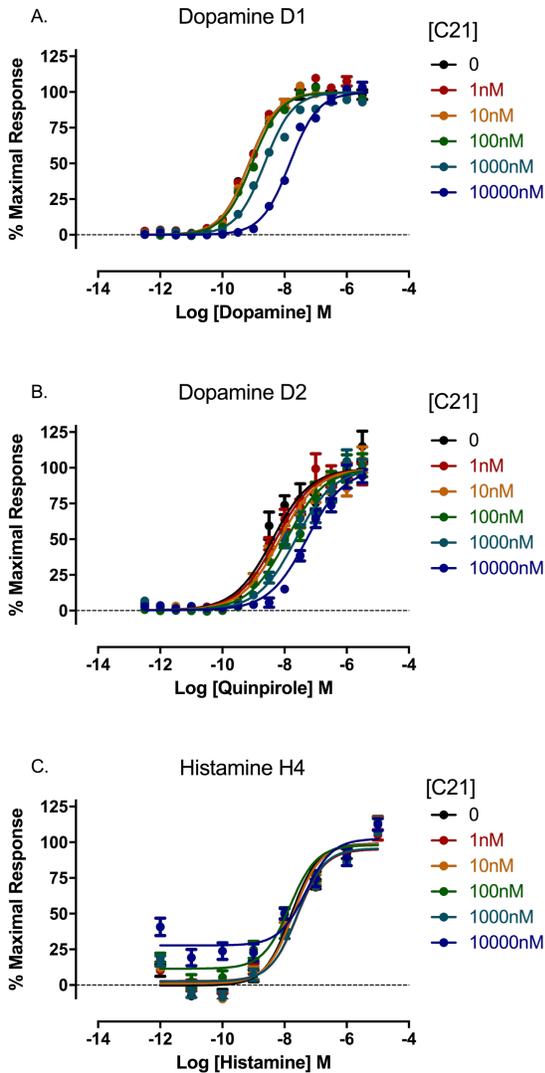


Figure S3: Antagonist activity of C21 at Dopamine D1 and D2 receptors and Histamine H4 receptors

Transfected HEK293t cells expressing **(A)** human D1 or **(B)** human D2 or **(C)** human H4 receptors stimulated with various concentrations of dopamine, quinpirole or histamine as indicated in the presence or absence of the indicated concentrations of C21. The receptor responses (cAMP for Dopamine D1, Gi dissociation for Dopamine D2 and Histamine H4) were then determined. Data represents the mean \pm SEM of at least three experiments.

Table S1: Summary of binding affinities of C21 at indicated receptors and targets.

Radioligand binding assays were carried out by NIMH PDSP as indicated in the Methods. Values were presented in the form of $pK_i \pm SEM$ (K_i nM) from a minimum of 3 independent assays, each in triplicate.

Table S2: Table S2. Assessment of off-target activity of C21 against 318 non-olfactory GPCR targets.

The activation of 318 druggable non-olfactory GPCRs in response to C21 (5 μ M) was tested. The data presented as four independent experiments for each receptor subtype.

