Supporting information

Design of Small non-Peptidic Ligands that Alter Heteromerization between Cannabinoid CB_1 and Serotonin $5HT_{2A}$ Receptors

Minos-Timotheos Matsoukas[1,#], Marc Ciruela-Jardí[2,3,#], Maria Gallo[4], Sergi Ferre[5], David Andreu[4], Vicent Casadó[3], Leonardo Pardo[2,*], and Estefanía Moreno[3,*]

[1]Department of Biomedical Engineering, University of West Attica, Ag. Spyridonos, Egaleo, 12243, Greece

[2]Laboratori de Medicina Computacional, Unitat de Bioestadística, Facultat de Medicina, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

[3]Department of Biochemistry and Molecular Biomedicine, Faculty of Biology, Institute of Biomedicine of the University of Barcelona (IBUB), University of Barcelona, 08028 Barcelona, Spain

[4]Department of Medicine and Life Sciences (MELIS-UPF), Universitat Pompeu Fabra, 08003 Barcelona, Spain

[5]Integrative Neurobiology Section, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, Baltimore, Maryland 21224, United States

*To whom correspondence should be addressed
Email: leonardo.pardo@uab.es, estefaniamoreno@ub.edu

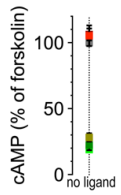
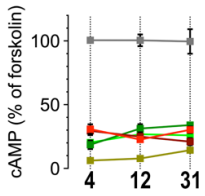
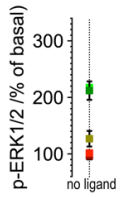
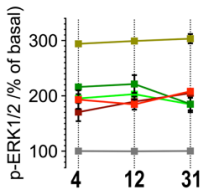
Table of Contents

Table S1. Compounds **1**-**41** obtained in the Virtual Screening exercise of the ZINC20 database that were experimentally tested in BiFC assays.

Number	ZINC ID	logP	Weight (kDa)	# rotatable bonds
1	ZINC000008074648	3.9075	309	6
2	ZINC000012324308	4.2478	313	11
3	ZINC000058184768	4.5072	335	9
4	ZINC000004902363	4.6516	295	10
5	ZINC000019793093	3.8287	314	7
6	ZINC000004906251	3.8782	332	9
7	ZINC000065605933	3.2092	288	10
8	ZINC000012404787	5.5438	340	6
9	ZINC000012382832	3.8889	310	7
10	ZINC000008598990	5.1261	351	9
11	ZINC000003252442	4.7422	334	9
12	ZINC000002645797	4.7885	323	9
13	ZINC000036349658	6.4771	389	8
14	ZINC000067713635	3.3279	332	7
15	ZINC000000092389	4.583	334	9
16	ZINC000002195478	6.42548	359	8
17	ZINC000005505999	4.6738	347	9
18	ZINC000004789866	3.2698	337	9
19	ZINC000032753650	4.6933	351	9
20	ZINC000096343799	3.0973	337	9
21	ZINC000004844985	4.5269	340	11
22	ZINC000044085459	6.4057	377	7
23	ZINC000095429525	3.5823	324	6
24	ZINC000065393706	3.9998	343	7
25	ZINC000037398243	4.5495	349	11
26	ZINC000009267181	4.1349	348	9
27	ZINC000005727162	5.32208	328	7
28	ZINC000035562334	4.5783	349	10
29	ZINC000037525736	5.5466	380	9
30	ZINC000044895984	3.4823	335	9
31	ZINC000036360428	4.1358	322	9
32	ZINC000012785569	5.2243	375	9
33	ZINC000097949683	6.0341	371	9
34	ZINC000002708440	6.473	395	7

35	ZINC000100424414	5.3653	379	9
36	ZINC000005545395	6.3784	352	7
37	ZINC000000755201	5.2825	390	9
38	ZINC000006736386	6.2603	402	11
39	ZINC000036004541	5.0556	366	10
40	ZINC000036041977	4.723	418	10
41	ZINC000004783745	5.747	418	11

Table S2. Statistical analyses used in signaling experiments

	Factor	p-value	Conclusions
	treatment	<0.0001	$5HT_{2A}$Rago < FK (p<0.0001) CB_1Rago < FK (p<0.0001) $5HT_{2A}$Rago+CB_1Rago < $5HT_{2A}$Rago (p=0.659) $5HT_{2A}$Rago+CB_1Rago < CB_1Rago (p=0.930) $5HT_{2A}$Rago+CB_1Ranta < FK (p=0.907) CB_1Rago+$5HT_{2A}$Ranta < FK (p=0.953)
	treatment ligand interaction	<0.0001 0.239 0.075	$5HT_{2A}$Rago < FK (p<0.0001) CB_1Rago < FK (p<0.0001) $5HT_{2A}$Rago+CB_1Rago < $5HT_{2A}$Rago (p<0.0001) $5HT_{2A}$Rago+CB_1Rago < CB_1Rago (p<0.0001) $5HT_{2A}$Rago+CB_1Ranta < FK (p<0.0001) CB_1Rago+$5HT_{2A}$Ranta < FK (p<0.0001)
	treatment	<0.0001	$5HT_{2A}$Rago > basal (p<0.0001) CB_1Rago > basal (p<0.0001) $5HT_{2A}$Rago+CB_1Rago > $5HT_{2A}$Rago (p>0.999) $5HT_{2A}$Rago+CB_1Rago > CB_1Rago (p>0.999) $5HT_{2A}$Rago+CB_1Ranta > basal (p=0.999) CB_1Rago+$5HT_{2A}$Ranta > basal (p=0.999)
	treatment ligand interaction	<0.0001 0.677 0.06	$5HT_{2A}$Rago > basal (p<0.0001) CB_1Rago > basal (p<0.0001) $5HT_{2A}$Rago+CB_1Rago > $5HT_{2A}$Rago (p<0.0001) $5HT_{2A}$Rago+CB_1Rago > CB_1Rago (p<0.0001) $5HT_{2A}$Rago+CB_1Ranta > basal (p<0.0001) CB_1Rago+$5HT_{2A}$Ranta > basal (p<0.0001)

One way or two-way ANOVA, followed by Tukey's multiple comparison tests, was used to analyse the data depicted in the left column

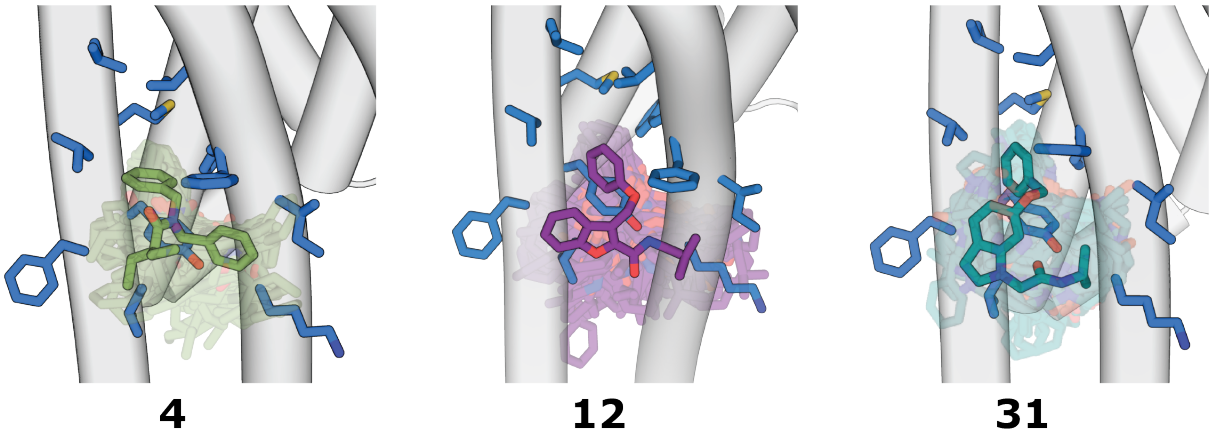
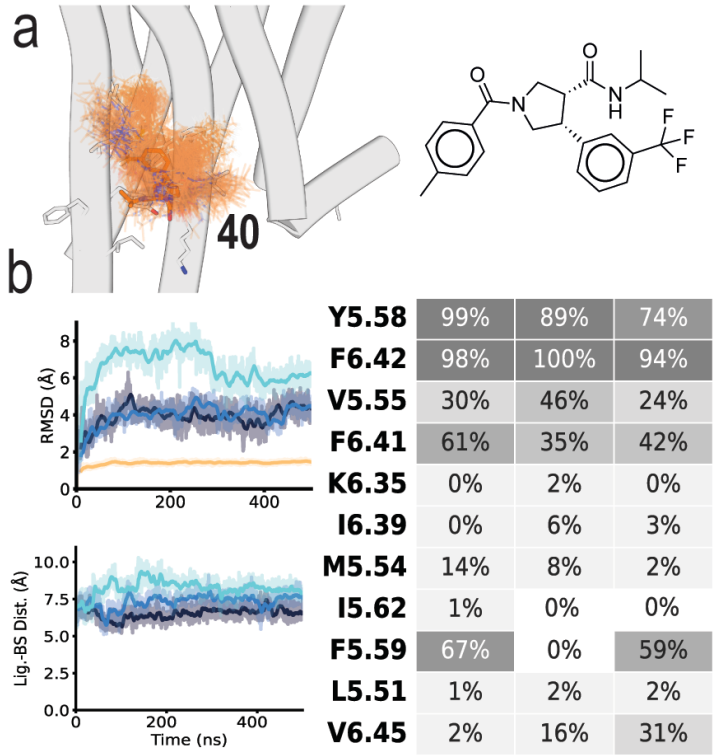


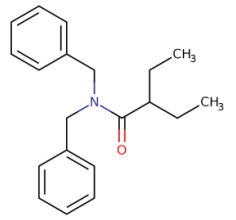
Figure S1. Superposition of the docking poses of compounds **4**, **12**, and **31** (light colored sticks) and the final predicted binding mode (dark colored sticks) in the membrane-facing cavity between TMs 5 and 6 (grey cylinders) of $5HT_{2A}R$. The amino acids predicted to contact the ligands are shown in blue sticks.

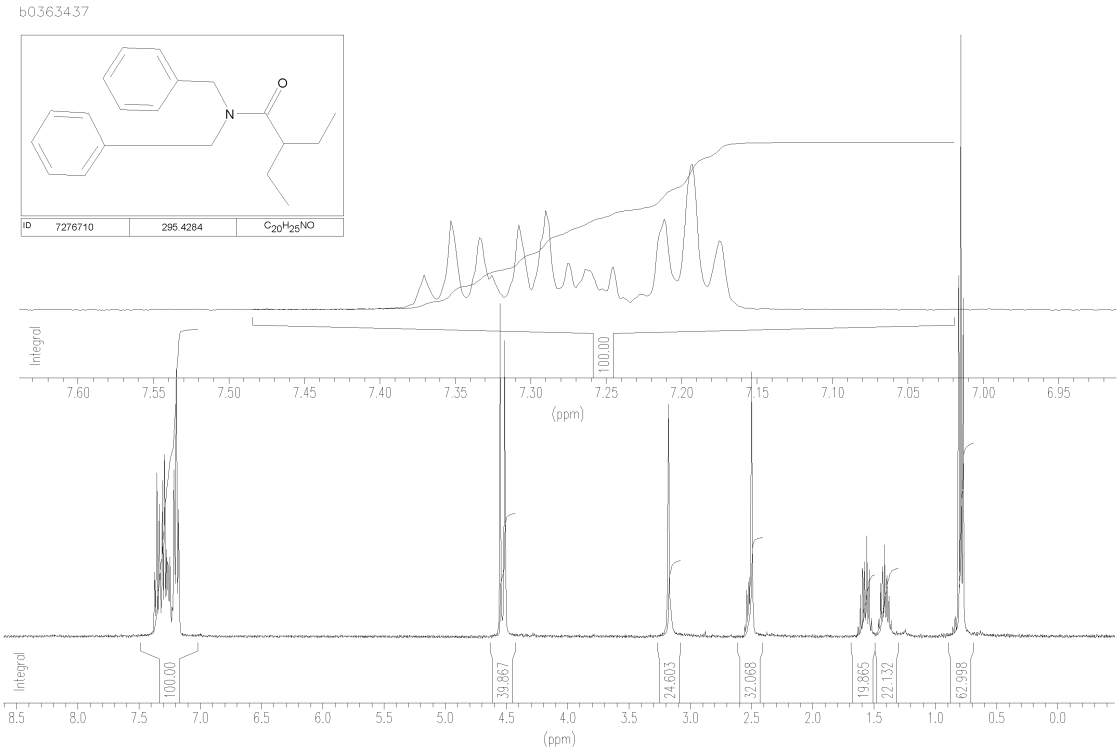


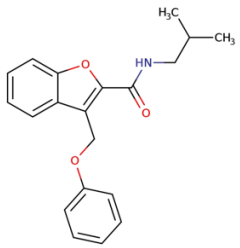
Y5.58	99%	89%	74%
F6.42	98%	100%	94%
V5.55	30%	46%	24%
F6.41	61%	35%	42%
K6.35	0%	2%	0%
I6.39	0%	6%	3%
M5.54	14%	8%	2%
I5.62	1%	0%	0%
F5.59	67%	0%	59%
L5.51	1%	2%	2%
V6.45	2%	16%	31%

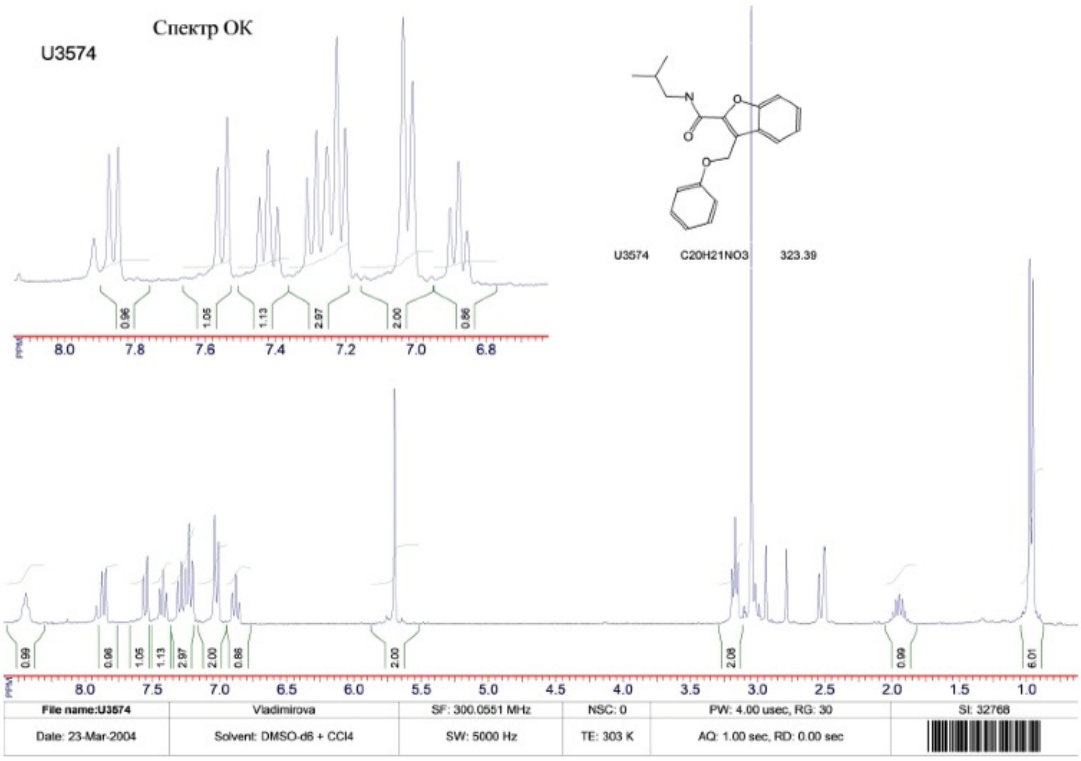
Figure S2. Computational model between inactive compound **40** (negative control) and $5HT_{2A}R$ (PDB id 7WC4). (a) Representative structure (solid sticks) and evolution (lines) of compound **40** (in orange) in complex with $5HT_{2A}R$ (gray cylinders, only the initial structure is shown) during MD simulations (one replica is displayed for better visualization although additional replicas showed consistent behavior). (b) The stability of the ligand-receptor complex was analyzed via root mean-square deviations (RMSD) of the ligand heavy atoms as devised from three replicas of unbiased 500 ns MD simulations (blue colours), and the stability of $5HT_{2A}R$ was analyzed via RMSD of the receptor Cα atoms (orange) (top panel). Evolution of the distance between the center of mass (COM) of the ligand and the residues in the binding site, along the trajectories (blue colours) (bottom panel). Detailed views and heatmaps (calculated with GetContacts, https://getcontacts.github.io/interactions.html) depicting the predicted interactions between compound **40** and $5HT_{2A}R$ during three replicas of unbiased 500 ns MD simulations.

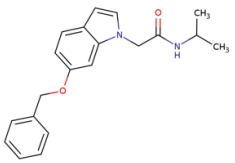
NMR of compounds **4**, **12**, and **31** as provided by the vendors.

	**4**	ZINC000004902363	Molport-001-500-854	STK447807	Vitas



	**12**	ZINC000002645797	Molport-004-061-257	Z30868285	Enamine



	**31**	ZINC000036360428	Molport-008-320-659	STK659895	Vitas