Supporting Information

Structure-activity relationship studies of tetrahydroquinolone free fatty acid receptor 3 modulators

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Crystallographic data for (*R*)-**1** and (*R,S*)-**1**

(*R,S*)-1

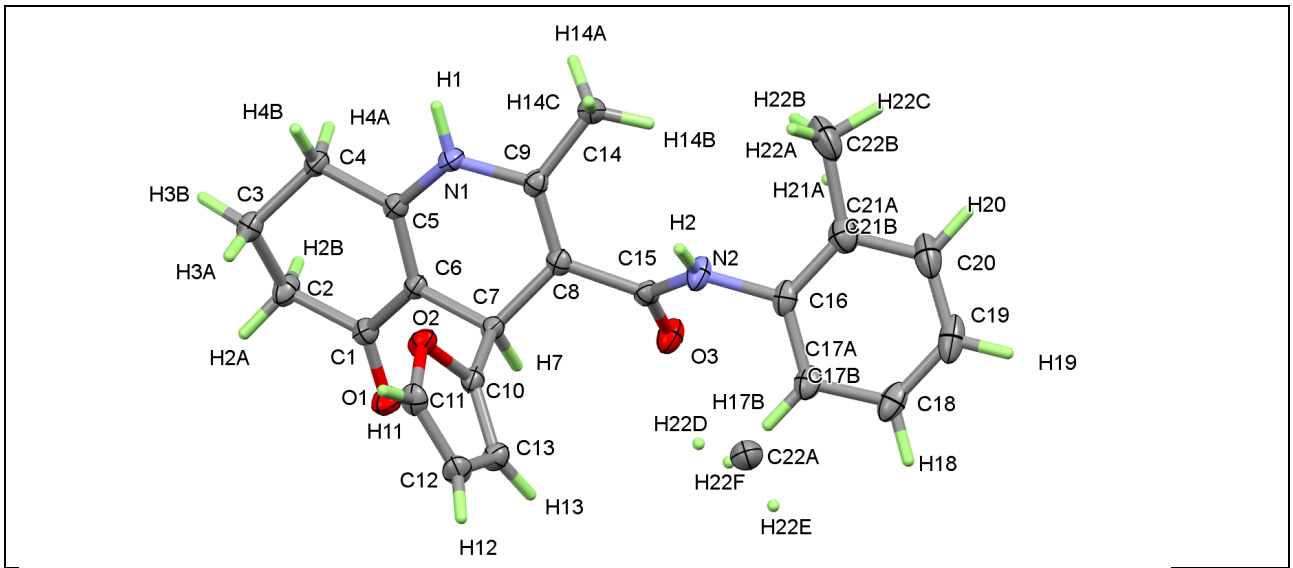


Figure S1. Molecular structure with numbering scheme.

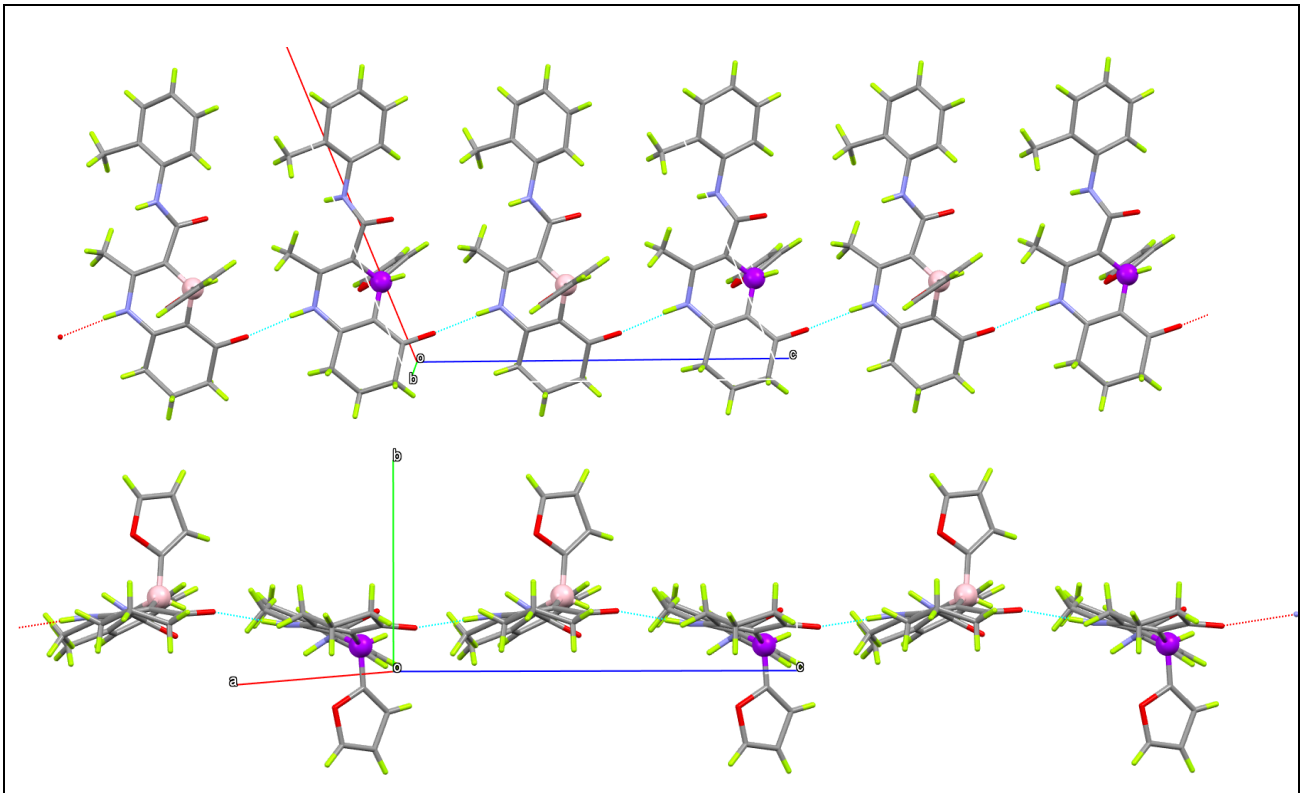


Figure S2. Two views, 90° rotated from each other, of the H-bonded ribbons in (*R,S*)-**1** running parallel with *c*-axis. The *S* centers are colored pink the *R* centers are colored purple. Only the major position (76%) for the disordered aromatic methyl groups was used in the preparation of the images.

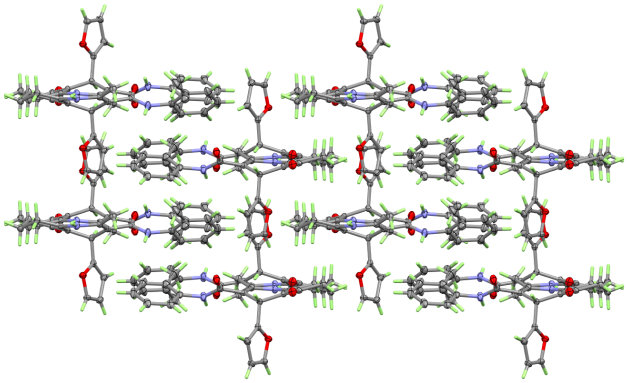


Figure S3. Packing in (*R,S*)-**1** viewed with the molecules in same orientation as those in (b) (down the *c*-axis).

Table S1. Crystal data and structure refinement for (*R,S*)-1

Empirical formula	$C_{22}H_{22}N_2O_3$
Formula weight	362.41
Temperature/K	293(2)
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	17.6367(2)
b/Å	7.53560(10)
c/Å	14.3717(2)
α/°	90
β/°	111.5790(10)
γ/°	90
Volume/Å^3	1776.17(4)
Z	4
ρ_{calc}g/cm^3	1.355
μ/mm^{-1}	0.732
F(000)	768.0
Crystal size/mm^3	0.159 × 0.082 × 0.049
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	10.788 to 133.202
Index ranges	$-20 \le h \le 20$, $-8 \le k \le 8$, $-17 \le l \le 17$
Reflections collected	36815
Independent reflections	3132 [R_{int} = 0.0364, R_{sigma} = 0.0139]
Data/restraints/parameters	3132/0/273
Goodness-of-fit on F^2	0.876
Final R indexes [I>=2σ (I)]	R_1 = 0.0471, wR_2 = 0.1302
Final R indexes [all data]	R_1 = 0.0490, wR_2 = 0.1318
Largest diff. peak/hole / e Å^{-3}	0.20/-0.30

Table S2. Bond Lengths for (*R*,*S*)-1

Atom	Atom	Length/Å	Atom	Atom	Length/Å
O1	C1	1.235(2)	C8	C15	1.498(3)
N1	C5	1.369(2)	C9	C14	1.504(3)
N1	C9	1.404(2)	C10	C13	1.348(3)
C1	C2	1.515(3)	C11	C12	1.344(3)
C1	C6	1.441(3)	C12	C13	1.432(3)
O2	C10	1.373(2)	C16	C17B	1.396(3)
O2	C11	1.375(2)	C16	C21B	1.387(3)
N2	C15	1.347(3)	C16	C17A	1.396(3)
N2	C16	1.433(2)	C16	C21A	1.387(3)
C2	C3	1.520(3)	C18	C19	1.386(3)
O3	C15	1.227(2)	C18	C17B	1.395(3)
C3	C4	1.519(3)	C18	C17A	1.395(3)
C4	C5	1.499(2)	C19	C20	1.381(4)
C5	C6	1.365(3)	C20	C21B	1.386(3)
C6	C7	1.512(2)	C20	C21A	1.386(3)
C7	C8	1.525(3)	C21B	C22B	1.579(11)
C7	C10	1.502(3)	C17A	C22A	1.518(3)
C8	C9	1.343(3)			

Table S3. Bond Angles for (*R*,*S*)-1

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C5	N1	C9	121.80(16)	C13	C10	C7	133.99(18)
O1	C1	C2	120.00(17)	C12	C11	O2	110.52(17)
O1	C1	C6	121.87(17)	C11	C12	C13	106.09(17)
C6	C1	C2	118.11(16)	C10	C13	C12	107.15(17)
C10	O2	C11	106.51(15)	N2	C15	C8	116.82(16)
C15	N2	C16	125.14(17)	O3	C15	N2	122.37(17)
C1	C2	C3	113.01(16)	O3	C15	C8	120.79(17)
C4	C3	C2	110.40(16)	C17B	C16	N2	121.05(18)
C5	C4	C3	111.36(15)	C21B	C16	N2	117.54(19)
N1	C5	C4	116.79(16)	C21B	C16	C17B	121.29(18)
C6	C5	N1	119.85(16)	C17A	C16	N2	121.05(18)
C6	C5	C4	123.26(17)	C21A	C16	N2	117.54(19)
C1	C6	C7	119.66(16)	C21A	C16	C17A	121.29(18)
C5	C6	C1	120.53(16)	C19	C18	C17B	121.1(2)
C5	C6	C7	119.77(16)	C19	C18	C17A	121.1(2)
C6	C7	C8	110.46(15)	C20	C19	C18	120.2(2)
C10	C7	C6	112.11(15)	C19	C20	C21B	119.8(2)
C10	C7	C8	110.40(15)	C19	C20	C21A	119.8(2)
C9	C8	C7	120.32(16)	C18	C17B	C16	117.8(2)
C9	C8	C15	125.53(17)	C16	C21B	C22B	126.2(4)

C15	C8	C7	114.07(15)	C20	C21B	C16	119.8(2)
N1	C9	C14	112.77(16)	C20	C21B	C22B	113.6(4)
C8	C9	N1	119.45(17)	C16	C17A	C22A	124.46(19)
C8	C9	C14	127.67(17)	C18	C17A	C16	117.8(2)
O2	C10	C7	116.24(16)	C18	C17A	C22A	117.7(2)
C13	C10	O2	109.72(17)	C20	C21A	C16	119.8(2)

Table S4. Hydrogen Bonds for (*R*,*S*)-1

D	**H**	**A**	**d(D-H)/Å**	**d(H-A)/Å**	**d(D-A)/Å**	**D-H-A/°**
N1	H1	O1[1]	0.91(3)	1.95(3)	2.857(2)	174(2)

[1]+X,1/2-Y,-1/2+Z

Structure of (*R*)-1

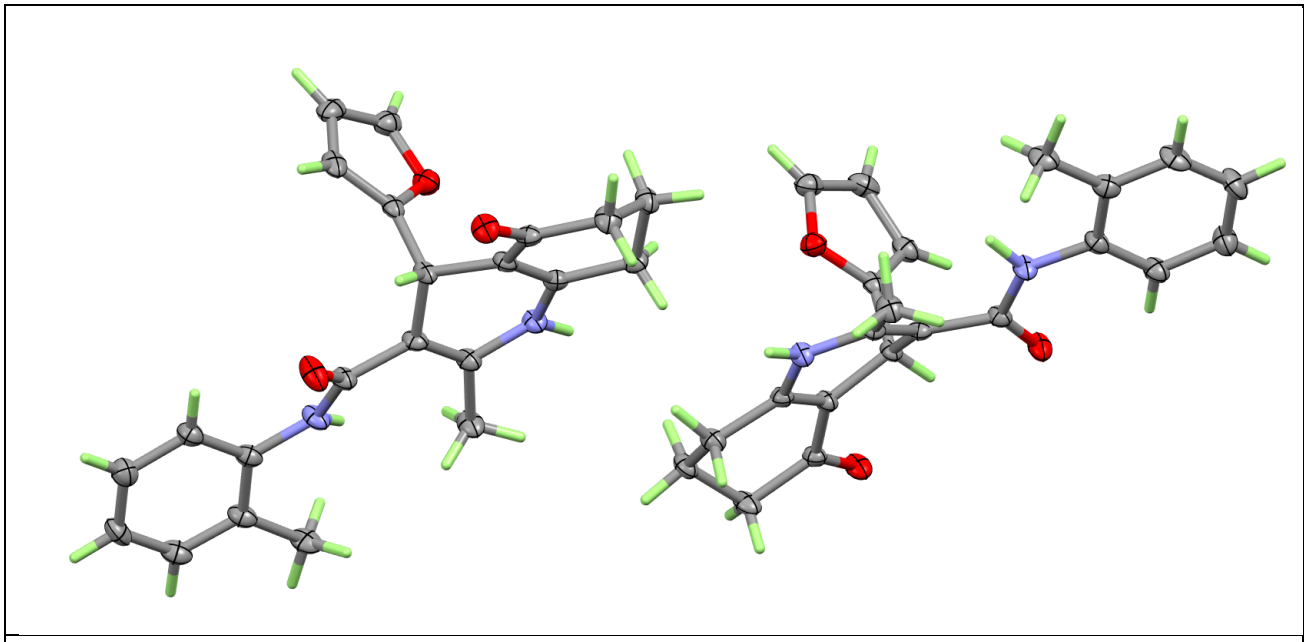


Figure S4. Both molecules of the asymmetric unit, molecule B on left, molecule A on right. The numbering scheme is provided in the manuscript.

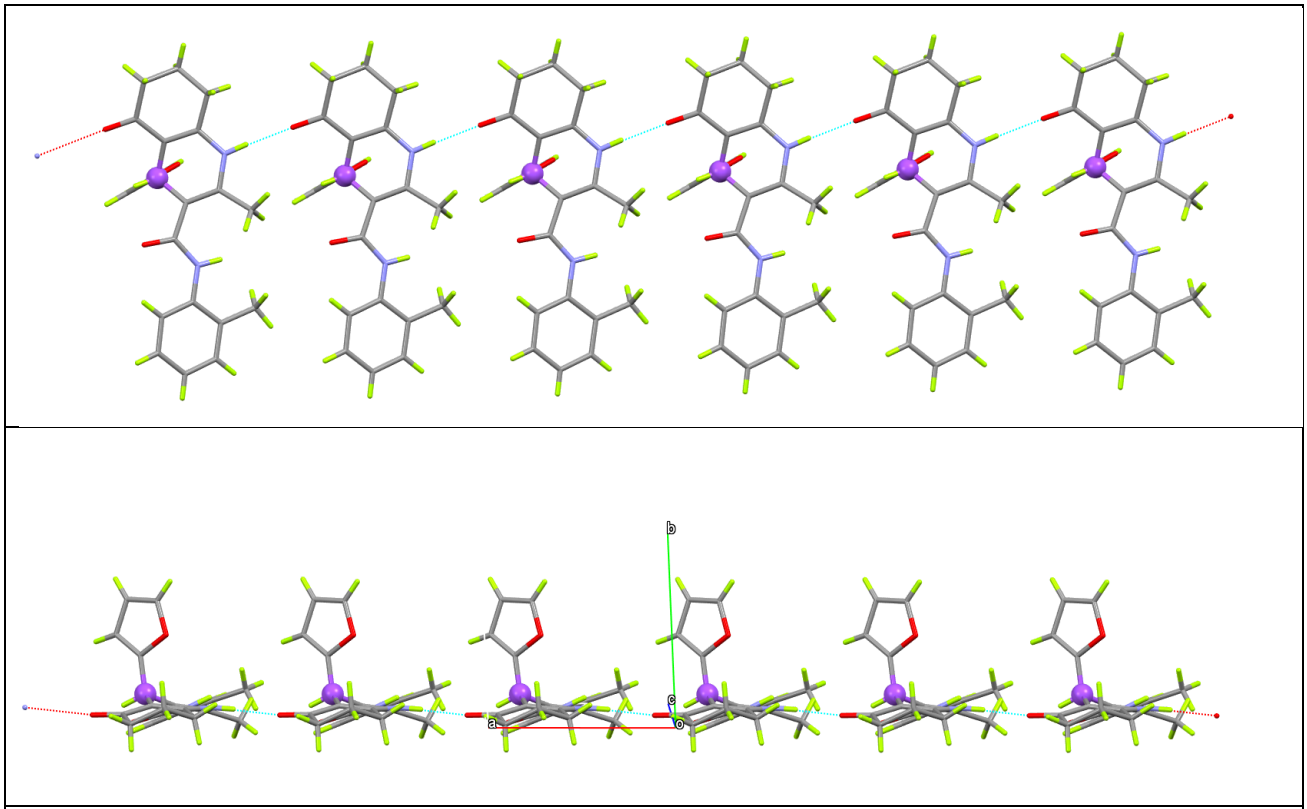


Figure S5. Two views, 90° rotated from each other, of the H-bonded ribbons in (*R*)-**1** running parallel with a-axis. The *R* centers are represented as balls and colored purple. The furanyl rings are all behind the plane of the H-bonded ribbons in the top image.

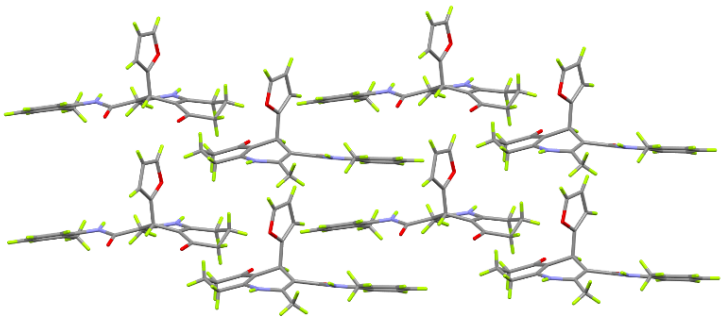


Figure S6. Packing in (*R*)-**1** viewed with the molecules in same orientation as those in (b) (down the *c*-axis).

Table S5 Crystal data and structure refinement for (*R*)-1.

Identification code	cmck2_twin1_hklf4_a
Empirical formula	$C_{22}H_{22}N_2O_3$
Formula weight	362.41
Temperature/K	293(2)
Crystal system	triclinic
Space group	P1
a/Å	7.1817(3)
b/Å	7.4711(3)
c/Å	16.6298(4)
α/°	87.768(3)
β/°	87.408(3)
γ/°	87.615(3)
Volume/Å^3	889.98(6)
Z	2
ρ_{calc}g/cm^3	1.352
μ/mm^{-1}	0.730
F(000)	384.0
Crystal size/mm^3	0.134 × 0.085 × 0.033
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	11.864 to 152.862
Index ranges	-8 ≤ h ≤ 8, -9 ≤ k ≤ 9, -20 ≤ l ≤ 20
Reflections collected	16586
Independent reflections	6283 [R_{int} = 0.0454, R_{sigma} = 0.0384]
Data/restraints/parameters	6283/3/507
Goodness-of-fit on F^2	0.989
Final R indexes [I>=2σ (I)]	R_1 = 0.0371, wR_2 = 0.0936
Final R indexes [all data]	R_1 = 0.0410, wR_2 = 0.0952
Largest diff. peak/hole / e Å^{-3}	0.19/-0.22
Flack parameter	0.04(12)

Table S6. Bond Lengths for (*R*)-1

Atom	Atom	Length/Å	Atom	Atom	Length/Å
C1B	O1B	1.244(3)	C7A	C10A	1.499(4)
C1B	C2B	1.501(4)	C6B	C7B	1.512(4)
C1B	C6B	1.444(4)	C8A	C9A	1.357(4)
N1B	C5B	1.364(4)	C8A	C15A	1.493(4)
N1B	C9B	1.402(4)	C7B	C8B	1.532(3)
C1A	O1A	1.234(3)	C7B	C10B	1.499(4)
C1A	C2A	1.515(4)	C9A	C14A	1.497(4)
C1A	C6A	1.444(4)	C8B	C9B	1.344(4)
N1A	C5A	1.368(3)	C8B	C15B	1.498(4)
N1A	C9A	1.403(3)	C10A	C13A	1.354(4)
C2A	C3A	1.524(4)	C9B	C14B	1.507(4)
N2A	C15A	1.370(4)	C11A	C12A	1.343(5)
N2A	C16A	1.411(4)	C10B	C13B	1.352(4)
O2A	C10A	1.373(4)	C12A	C13A	1.426(4)
O2A	C11A	1.368(3)	C11B	C12B	1.339(5)
C3A	C4A	1.519(4)	C12B	C13B	1.426(4)
O3A	C15A	1.227(3)	C16A	C17A	1.409(4)
C2B	C3B	1.529(4)	C16A	C21A	1.393(4)
N2B	C15B	1.359(4)	C17A	C18A	1.385(4)
N2B	C16B	1.424(4)	C17A	C22A	1.509(4)
O2B	C10B	1.376(3)	C16B	C17B	1.412(4)
O2B	C11B	1.371(4)	C16B	C21B	1.385(4)
C4A	C5A	1.502(4)	C18A	C19A	1.384(5)
C3B	C4B	1.529(4)	C17B	C18B	1.390(4)
O3B	C15B	1.226(3)	C17B	C22B	1.510(4)
C5A	C6A	1.361(4)	C19A	C20A	1.388(4)
C4B	C5B	1.499(4)	C18B	C19B	1.381(5)
C6A	C7A	1.511(3)	C20A	C21A	1.388(4)
C5B	C6B	1.363(4)	C19B	C20B	1.388(4)
C7A	C8A	1.514(4)	C20B	C21B	1.393(4)

Table S7. Bond Angles for (*R*)-1

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
O1B	C1B	C2B	120.2(3)	C9B	C8B	C15B	127.5(2)
O1B	C1B	C6B	121.8(3)	C15B	C8B	C7B	113.4(2)
C6B	C1B	C2B	118.1(2)	O2A	C10A	C7A	116.5(2)
C5B	N1B	C9B	122.2(2)	C13A	C10A	O2A	109.4(2)
O1A	C1A	C2A	119.8(2)	C13A	C10A	C7A	134.1(3)
O1A	C1A	C6A	121.9(2)	N1B	C9B	C14B	112.2(2)
C6A	C1A	C2A	118.3(2)	C8B	C9B	N1B	119.4(2)
C5A	N1A	C9A	122.1(2)	C8B	C9B	C14B	128.1(3)

C1A	C2A	C3A	112.6(2)	C12A	C11A	O2A	110.4(3)
C15A	N2A	C16A	128.8(2)	O2B	C10B	C7B	116.7(2)
C11A	O2A	C10A	106.8(2)	C13B	C10B	O2B	108.9(2)
C4A	C3A	C2A	110.3(3)	C13B	C10B	C7B	134.3(3)
C1B	C2B	C3B	113.0(3)	C11A	C12A	C13A	106.5(2)
C15B	N2B	C16B	126.7(2)	C12B	C11B	O2B	110.1(3)
C11B	O2B	C10B	107.1(2)	C10A	C13A	C12A	106.9(3)
C5A	C4A	C3A	111.0(2)	C11B	C12B	C13B	106.6(3)
C2B	C3B	C4B	109.6(2)	C10B	C13B	C12B	107.2(3)
N1A	C5A	C4A	117.0(2)	N2A	C15A	C8A	117.5(2)
C6A	C5A	N1A	119.4(2)	O3A	C15A	N2A	122.6(3)
C6A	C5A	C4A	123.5(2)	O3A	C15A	C8A	119.8(2)
C5B	C4B	C3B	110.6(2)	C17A	C16A	N2A	116.6(3)
C1A	C6A	C7A	119.8(2)	C21A	C16A	N2A	122.7(3)
C5A	C6A	C1A	120.3(2)	C21A	C16A	C17A	120.7(3)
C5A	C6A	C7A	119.9(2)	N2B	C15B	C8B	117.4(2)
N1B	C5B	C4B	117.2(2)	O3B	C15B	N2B	122.6(3)
C6B	C5B	N1B	118.9(3)	O3B	C15B	C8B	119.9(2)
C6B	C5B	C4B	123.8(2)	C16A	C17A	C22A	121.9(3)
C6A	C7A	C8A	110.5(2)	C18A	C17A	C16A	117.7(3)
C10A	C7A	C6A	111.9(2)	C18A	C17A	C22A	120.3(3)
C10A	C7A	C8A	110.7(2)	C17B	C16B	N2B	117.4(3)
C1B	C6B	C7B	120.7(2)	C21B	C16B	N2B	121.8(2)
C5B	C6B	C1B	119.8(2)	C21B	C16B	C17B	120.7(3)
C5B	C6B	C7B	119.4(2)	C19A	C18A	C17A	122.0(3)
C9A	C8A	C7A	119.9(2)	C16B	C17B	C22B	121.4(3)
C9A	C8A	C15A	126.4(2)	C18B	C17B	C16B	117.6(3)
C15A	C8A	C7A	113.6(2)	C18B	C17B	C22B	121.0(3)
C6B	C7B	C8B	109.8(2)	C18A	C19A	C20A	119.6(3)
C10B	C7B	C6B	111.5(2)	C19B	C18B	C17B	121.9(3)
C10B	C7B	C8B	110.9(2)	C21A	C20A	C19A	120.0(3)
N1A	C9A	C14A	112.5(2)	C18B	C19B	C20B	120.0(3)
C8A	C9A	N1A	118.9(2)	C20A	C21A	C16A	119.9(3)
C8A	C9A	C14A	128.5(3)	C19B	C20B	C21B	119.4(3)
C9B	C8B	C7B	119.1(2)	C16B	C21B	C20B	120.3(3)

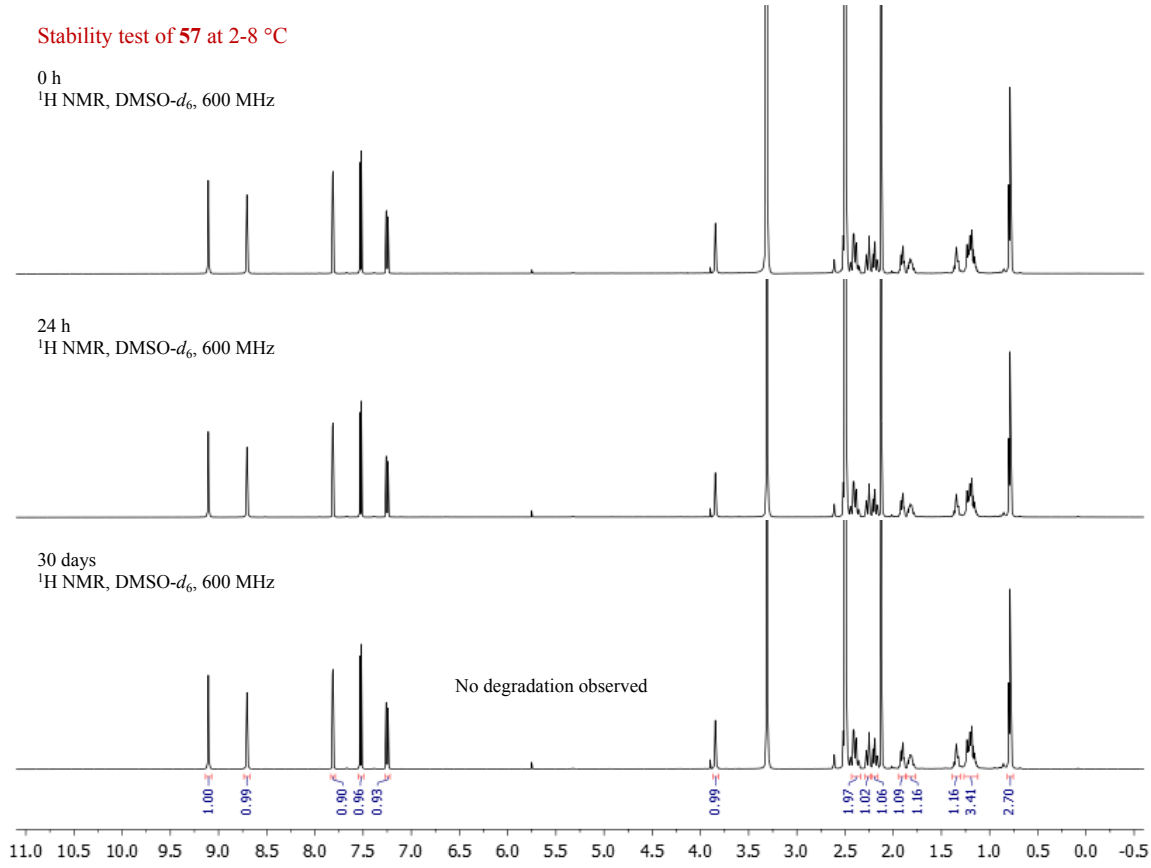
Molecular modeling of hFFA3

A homology model of hFFA3 was constructed using Modeller 9.17 based on the crystal structure of hFFA1 (PDB code 5TZY)[1] using manual alignment (Clustalx coloring):

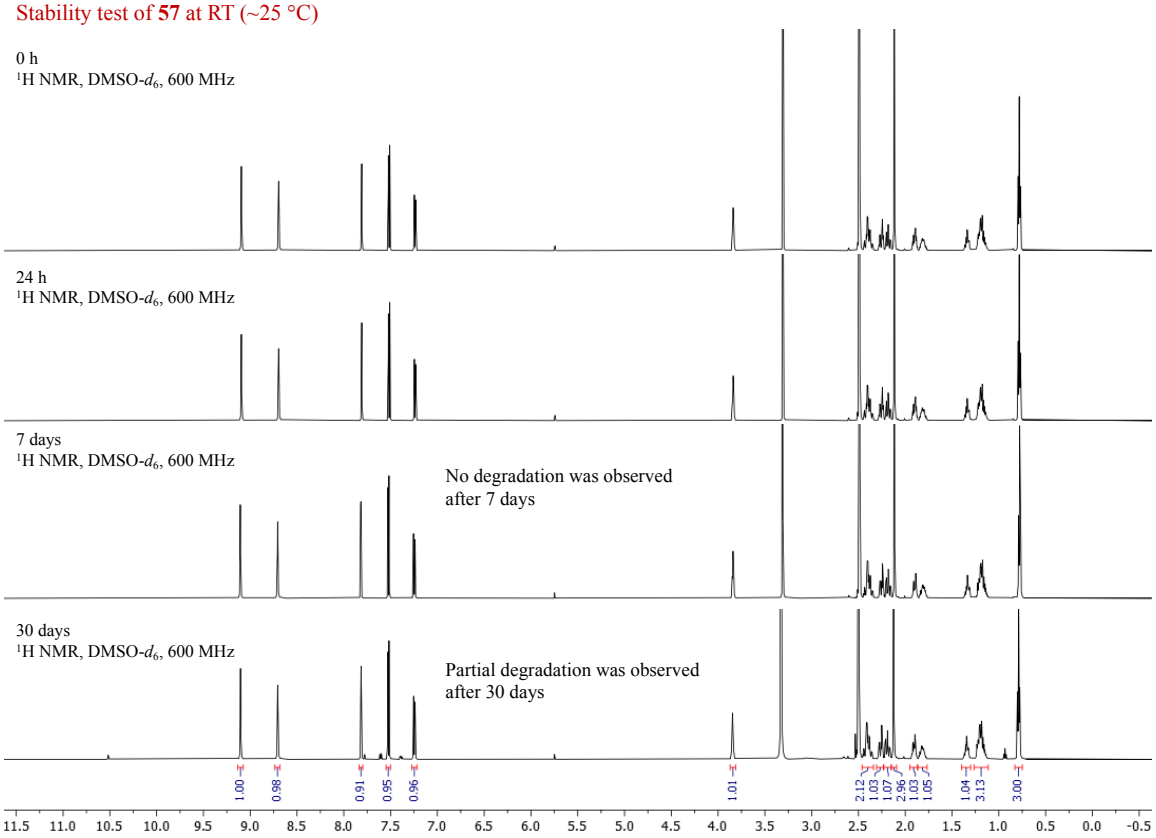
The receptor was prepared using Protein Preparation in Maestro 2015-3 (Schrödinger, LLC) before docking using Induced-fit docking employing default settings with Glide Extra Precision (XP) and OPLS 2005 force field. (*R*)-**1** was prior to docking prepared by LigPrep using default settings. The best pose was selected for redocking without any constraints using Maestro 2019-3 and OPLS3e force field.

Stability of **57**

Stability test of **57** at 2-8 °C



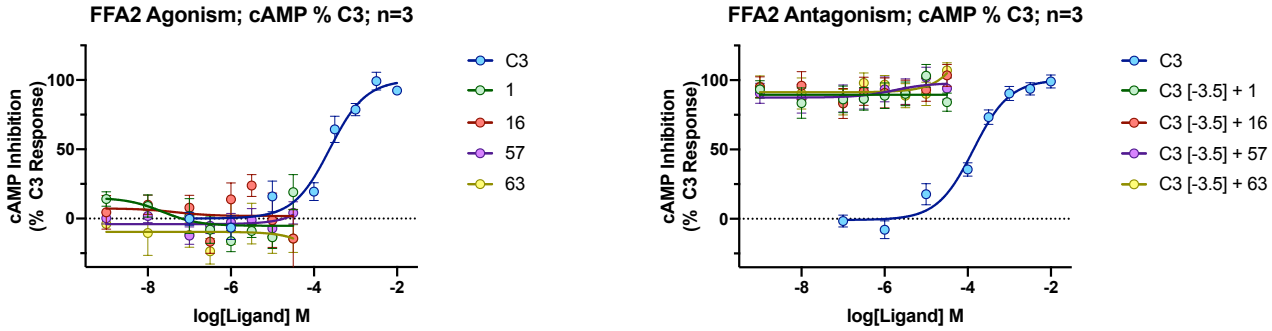
Stability test of **57** at RT (~25 °C)



Counterscreen data on **1**, **16**, **57** and **63**

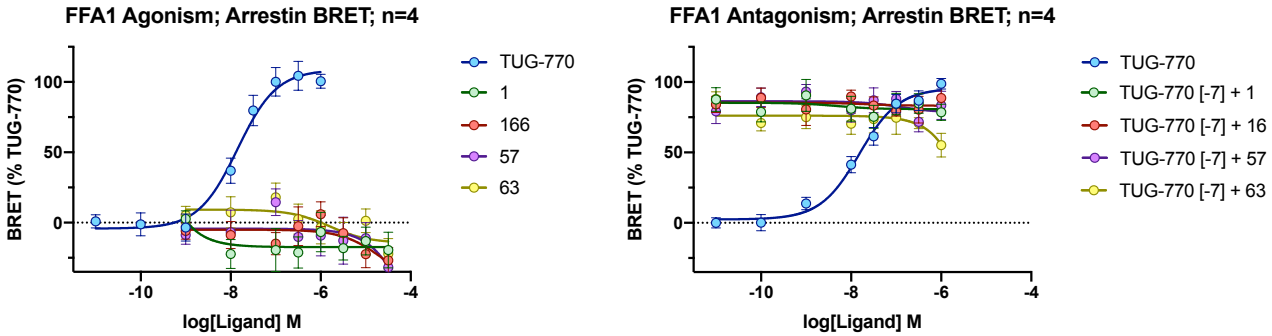
	1	**16**	**57**	**63**
GPR84, agonist, cAMP assay, 10 µM	1±7%	2±4%	1±4%	0±1%
Cav1.2 (L-type) Human Calcium Ion Channel Binding (Antagonist Radioligand) Assay, Panlabs, 10 µM	-20%	13%	12%	1%

FFA2 (cAMP assay)



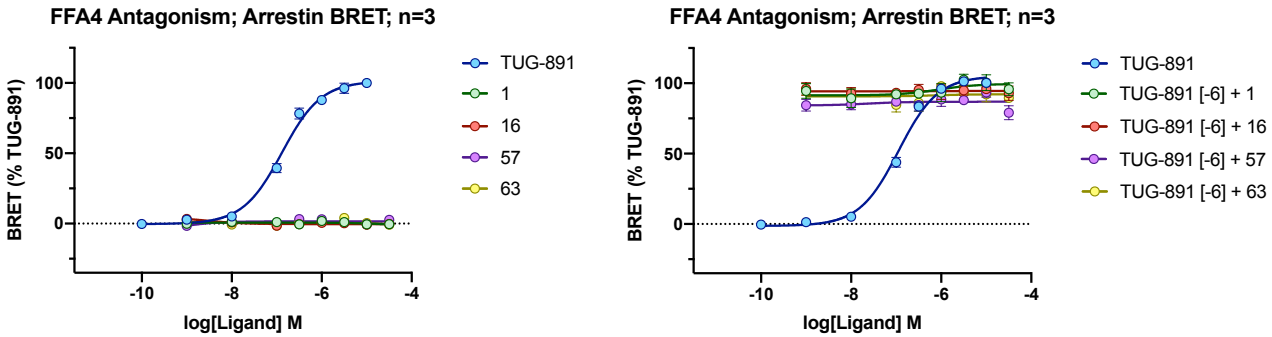
C3: propionate, reference agonist

FFA1 (arrestin BRET assay)



TUG-770: FFA1 agonist reference

FFA4 (arrestin BRET assay)



TUG-891: FFA4 agonist reference

Counter screening against FFA1, FFA2, FFA4 and GPR84

Counter screening against FFA1 and FFA4 was carried out using a modified version of a previously described bioluminescence resonance energy transfer (BRET) designed to assess recruitment of β-arrestin-2 to each receptor.[2] Briefly, HEK293T cells were co-transfected with plasmids encoding either human FFA1 or human FFA4 tagged at their C-termini with eYFP and with a plasmid encoding β-arrestin-2 tagged at its N-terminal with Nanoluciferase. 24 h post transfection cells were seeded into black with clear bottom 96-well plates that had been coated with poly-D-lysine. Cells were then cultured a further 24 h prior to experiments. Cells were then washed twice with Hank's Balanced salt solution supplemented with 10 mM HEPES (HBSSH) then incubated at 37 °C in HBSSH for 30 min prior to running the assay. The Nluc substrate, coelenterazine h was then added to a final concentration of 5 μM and cells were incubated at 37 °C for 10 min prior to adding test agonists. Following a further 5 min incubation, luminescent emission at 475 nm and 535 nm was measured using a Pherastar FS reader (BMG Labtech, Aylesbury, UK) plate reader set for bottom plate optics. The BRET ratio was then calculated as 535/475 emissions and all BRET ratio values were normalized against the maximal BRET ratio obtained using reference agonists for the respective receptors (TUG-891 for FFA4, TUG-770 for FFA1). For antagonism studies, the same protocol was used, however the antagonist was added 15 min prior to the addition of an EC_{80} concentration of the reference agonist for each receptor.

Counter screening for FFA2 was carried out using a cAMP assay. Flp-In T-Rex 293 cells generated to stably express human FFA2 tagged at is C-terminal with eYFP were first treated with doxycycline (100 ng/mL) overnight to induce receptor expression. Cells were then detached and used directly in an HTR-FRET cAMP assay (CisBio) according to the manufacturer's protocol and measured using a Pherastar FS plate reader (BMG Labtech, Aylesbury, UK). For these experiments, 2000 cells were used per well in a low volume 384-well plate and compounds were tested against their ability to inhibit the cAMP response to 1 μM forskolin for 30 min. Propionic acid was used as reference agonist and for antagonism studies the ability of compounds to inhibit the response to and EC_{80} concentration of C3 was tested.

Counter screening for GPR84 was carried out using a cAMP assay. Flp-In T-Rex 293 cells generated to stably express human GPR84 tagged at is C-terminal with Gia were first treated with doxycycline (100 ng/mL) overnight to induce receptor expression. Cells were then detached and used directly in an HTR-FRET cAMP assay (CisBio) according to the manufacturer's protocol and measured using an Envision plate reader (PerkinElmer). For these experiments, 5000 cells were used per well in a low volume 384-well plate and compounds were tested against their ability to inhibit the cAMP response to 1 μM forskolin for 45 min. TUG-1985 was used as reference agonist.

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