SUPPLEMENTARY DATA

In vitro activity-profiling of Cumyl-PEGACLONE variants at the CB_1 receptor: fluorination *versus* isomer exploration

Liesl Janssens[1], Annelies Cannaert[1], Matthew J. Connolly[2], Huiling Liu[2], Christophe P. Stove[1]

[1] Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, 9000 Ghent, Belgium

[2] Chiron AS, Stiklestadveien 1, N-7041 Trondheim, Norway

Table of contents

Supplementary Data 1: Chemicals for synthesis

Supplementary Table1: Chemicals for synthesis of all Cumyl-PEGACLONE variants

Name	Supplier	Product No.	Lot No.
Sodium hydride (60% in mineral oil)	Sigma-Aldrich	452912	STBG4619V
2-Methylindole	Acros	12702	A0288150
Anhydrous DMF	Sigma-Aldrich	227056	STBG9537
1-Bromo-5-fluoropentane	Fluorochem	050860	FCB059574
Trifluoroacetic anhydride	Acros	14781	A018374101
Methanol	Macron	6712	1634102802
KOH	Roth	6751.1	085224704
Cumylamine	Fluorochem	005015	FCB028595
EDC hydrochloride	Fluorochem	024810	FCB037448
1-Hydroxybenzotriazole	Sigma-Aldrich	54802	BCBV4080
N,*N*-Diisopropylethylamine	Sigma-Aldrich	D125806	STBG7293
n-Butlylithium	Sigma-Aldrich	230707	STBD9156V
Anhydrous THF	Sigma-Aldrich	401757	STBH4810
1-Bromopentane	Avocado	10821	C3101A
(*R*)-1-Phenylpropan-1-amine	Fluorochem	208825	FCC31119
3-Phenyl-1-propylamine	Acros	13088	A0239149

Supplementary Data 2: Characterization of Cumyl-PEGACLONE variants

Cumyl-PEGACLONE

^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm 8.35 (1 H, d, *J*=7.55 Hz), 7.72 (1 H, d, *J*=7.82 Hz), 7.16 - 7.36 (8 H, m), 6.45 (1 H, d, *J*=7.43 Hz), 4.21 (2 H, t, *J*=7.21 Hz), 2.04 (6 H, s), 1.78 - 1.96 (2 H, m), 1.30 - 1.42 (4 H, m), 0.84 - 0.95 (3 H, m); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C ppm 159.9 (s), 147.6 (s), 144.1 (s), 138.3 (s), 131.7 (s), 128.5 (s), 126.2 (s), 124.7 (s), 124.1 (s), 123.9 (s), 122.2 (s), 121.1 (s), 109.0 (s), 108.7 (s), 92.2 (s), 64.3 (s), 43.5 (s), 30.0 (s), 29.3 (s), 29.2 (s), 22.4 (s), 13.9 (s)

5F-Cumyl-PEGACLONE

^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm 8.34 (1 H, d, *J*=7.55 Hz), 7.72 (1 H, d, *J*=7.44 Hz), 7.16 - 7.35 (8 H, m), 6.43 (1 H, d, *J*=7.54 Hz), 4.47 (1 H, t, *J*=5.87 Hz), 4.36 (1 H, t, *J*=5.87 Hz), 4.23 (2 H, t, *J*=7.09 Hz), 2.04 (6 H, s), 1.86 - 1.98 (2 H, m), 1.64 - 1.79 (2 H, m), 1.45 - 1.55 (2 H, m); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C ppm 159.9 (s), 147.5 (s), 144.1 (s), 138.2 (s), 131.9 (s), 128.5 (s), 126.2 (s), 124.7 (s), 124.1 (s), 124.0 (s), 122.2 (s), 121.2 (s), 109.0 (s), 108.6 (s), 92.1 (s), 83.7 (d, *J*=164.3 Hz), 64.4 (s), 43.3 (s), 30.1 (d, *J*=19.8 Hz), 29.9 (s), 29.2 (s), 23.1 (d, *J*=5.1 Hz).

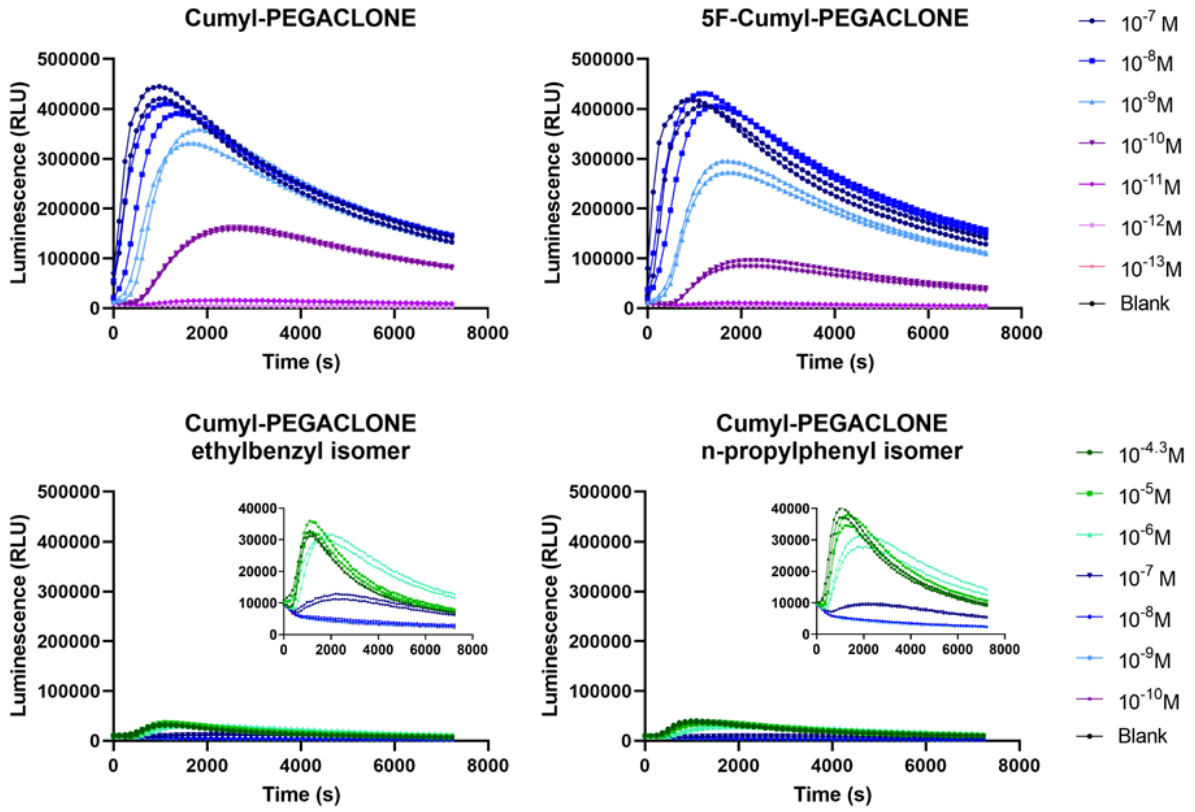
Cumyl-PEGACLONE ethylbenzyl isomer

^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm 8.49 (1 H, d, *J*=7.77 Hz), 7.18 - 7.43 (9 H, m), 6.49 (1 H, dd, *J*=9.05, 6.85 Hz), 6.39 (1 H, d, *J*=7.58 Hz), 4.16 (2 H, t, *J*=7.34 Hz), 2.26 - 2.37 (1 H, m), 2.05 - 2.18 (1 H, m), 1.76 - 1.86 (2 H, m), 1.23 - 1.39 (4 H, m), 1.00 (3 H, t, *J*=7.34 Hz), 0.82 - 0.89 (3 H, m); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C ppm 160.0 (s), 143.7 (s), 140.6 (s), 138.4 (s), 131.2 (s), 128.7 (s), 127.7 (s), 127.6 (s), 124.6 (s), 124.0 (s), 122.0 (s), 121.3 (s), 108.8 (s), 107.2 (s), 93.6 (s), 56.7 (s), 43.5 (s), 29.2 (s), 29.2 (s), 26.5 (s), 22.3 (s), 13.9 (s), 10.9 (s)

Cumyl-PEGACLONE n-propylphenyl isomer

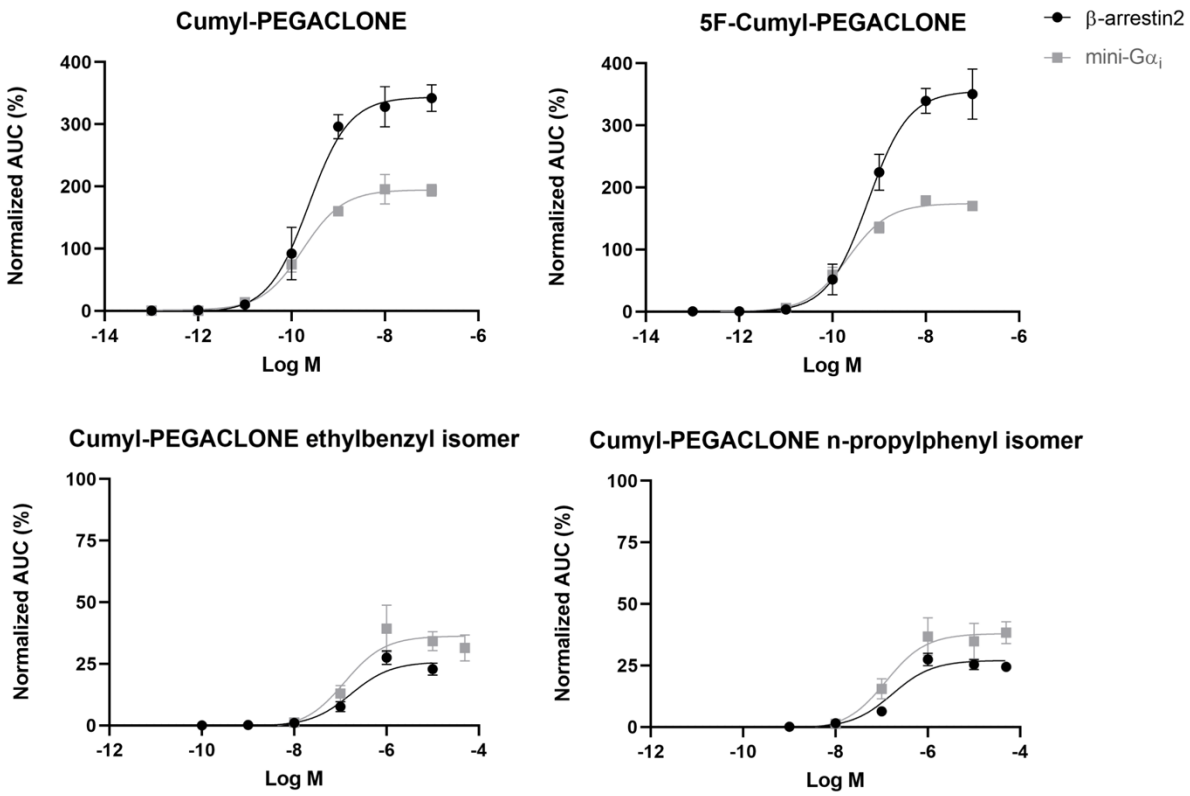
^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm 8.45 (1 H, d, *J*=7.64 Hz), 7.16 - 7.39 (9 H, m), 6.40 (1 H, d, *J*=7.34 Hz), 4.19 (2 H, t, *J*=7.21 Hz), 4.12 (2 H, t, *J*=7.50 Hz), 2.73 (2 H, t, *J*=7.85 Hz), 2.04 - 2.22 (2 H, m), 1.75 - 1.89 (2 H, m), 1.25 - 1.39 (4 H, m), 0.82 - 0.91 (3 H, m); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C ppm 159.6 (s), 144.4 (s), 141.1 (s), 138.3 (s), 134.6 (s), 128.4 (s), 128.3 (s), 126.0 (s), 124.4 (s), 124.0 (s), 122.0 (s), 121.2 (s), 108.8 (s), 107.7 (s), 93.0 (s), 48.4 (s), 43.5 (s), 32.9 (s), 31.3 (s), 29.2 (s), 29.1 (s), 22.4 (s), 13.9 (s)

Supplementary Data 3:



Supplementary Figure 1: CB_1 activation profiles of all tested compounds from one representative experiment in the β-arr2 assay. Note the difference in scale in the zoomed-in graphs of the isomers.

Supplementary Data 4:



Supplementary Figure 2: Concentration-response curves in both the CB_1 bio-assay measuring recruitment of β-arrestin2 and the bio-assay measuring mini-$G\alpha_i$ recruitment, graphically represented per individual compound. Note the difference in scale between the upper and lower panels. AUC: Area Under the Curve (normalized to the maximal receptor activation of JWH-018).