Catalogue of validated fluorescent probes

In the following tables we list the available ligands, for **GPCRs, E3 Ligases** and **Intracellular Receptors** with information about their selectivity, the emission and excitation wavelengths, their affinity measured by a radioligand binding assay and the specific further assays in which they have been validated.

GPCR fluorescent ligands

Receptor	Code	Cat number	λexc/λem	Affinity[a]	Selectivity[a]	Validation
Dopamine Receptor						
D_2	**CELT-074**	DR-589-3	589/616	1.06 nM	**Selective** K_i (D_3)=136.5 nM K_i (D_4)=152.7 nM	Fluorescence Microscopy in transfected cells Flow cytometry
	CELT-426	DR-560-1	560/571	89.3 nM	**Partially Selective** K_i (D_3)=194.8 nM K_i (D_4)=263 nM	Fluorescence polarization Flow cytometry
	CELT-075	DR-743-1	748/776	3.15 nM	**Selective** K_i (D_3)=294.6 nM K_i (D_4)=220.3 nM	Fluorescence Microscopy in transfected cells (ongoing)
D_3	**CELT-429**	DR-589-7	589/616	75.4 nM	**Selective** % displ.1 µM D_2= 6% D_4= 3%	Fluorescence Microscopy in transfected cells (ongoing)
	CELT-419	R-560-2	560/571	65.6 nM	**Partially Selective** K_i (D_2)=151.4 nM	Fluorescence polarization
D_2/D_3	**CELT-240**	DR-589-6	589/616	D_3 = 2.14 nM D_2 = 2.34 nM	**Selective against D_4** % displ.1 µM D_4= 1%	Flow cytometry
	CELT-241	DR-646-1	646/662	D_3 = 4.77 nM D_2 = 5.22 nM	**Selective against D_4** K_i (D_4)=302.55 nM	Fluorescence Microscopy in transfected cells (ongoing)
Adenosine Receptor						
PAN-ADO	**CELT-298**	AORD-646-1	646/662	A_1 = 20.9 nM A_{2A} = 171 nM A_{2B} = 44.7 nM A_3 = 95.2 nM	**Non Selective**	Fluorescence Microscopy in transfected cells
A_1	**CELT-448**	ADOR-560-1	560/571	26.2 nM	**Selective** % displ.1 µM A_{2A}= 11% A_{2B}= 22% A_3= 24%	Fluorescence polarization (ongoing) Fluorescence Microscopy in transfected cells (ongoing)

info@celtarys.com www.celtarys.com
Emprendia Building. Avenida do Mestre Mateo, 2. 15706, Santiago de Compostela (Spain)

	CELT-372 (A_1/A_{2B})	ADOR-589-1	589/616	A_1 = 1.89 nM A_{2B} = 24.75 nM	**Partially Selective** K_i (A_{2A})=80.33 nM K_i (A_3)=967.8 nM	Fluorescence Microscopy in transfected cells
	CELT-360	ADOR-646-2	646/662	8.6 nM	**Non Selective** K_i (A_{2A})=98.38 nM K_i (A_{2B})=72.24 nM K_i (A_3)=231.01 nM	Fluorescence Microscopy in transfected cells
A_{2A}	**CELT-316**	ADOR-589-2	589/616	116.1 nM	**Selective** % displ.1 µM A_1= 18% A_{2B}= 33% A_3= 31%	Fluorescence Microscopy in native cells
	CELT-300	ADOR-646-4	646/662	8.35 nM	**Selective** % displ.1 µM A_1= 31% A_{2B}= 18% A_3= 38%	Fluorescence Microscopy in transfected cells (ongoing)
A_{2B}/A_3	**CELT-327**	ADOR-589-4	589/616	A_{2B} = 35.6 nM A_3 = 45.7 nM	**Selective** % displ.1 µM A_1=41% A_{2A}=1%	Fluorescence Microscopy in native cells[1]
A_3	**CELT-228**	ADOR-560-2	560/571	52.7 nM	**Selective** % displ.1 µM A_1= 2% A_{2A}= 1% A_{2B}= 5%	Fluorescence Microscopy in native cells; Fluorescence polarization[1,2]
	CELT-071	ADOR-589-7	589/616	6.13 nM	**Selective** % displ.1 µM A_1=2.1% A_{2A}= 2.21% A_{2B}= 1.9%	Fluorescence Microscopy in transfected and native cells
	CELT-480	ADOR-646-6	646/662	12 nM	**Selective** % displ.1 µM A_1= 38% A_{2A}= 23 % A_{2B}= 49 %	Fluorescence Microscopy in transfected cells (ongoing)
Serotonin Receptor						
$5HT_{2B}$	**CELT-211**	5HT-589-1	589/616	56.32 nM	**Selective** % displ.1 µM $5HT_{2A}$=0.94% $5HT_{2C}$= 1.75%	Fluorescence Microscopy in transfected cells
Cannabinoid Receptor						
PAN-CB	**CELT-335**	CBR-646-1	646/662	CB_1 = 44.8 nM CB_2 = 7.4 nM	**Non Selective**	HTRF in adherent cells[3,4] High Content screening Fluorescence Microscopy in transfected cells

CB_2	**CELT-331**	CBR-646-3	646/662	75.9 nM	**Selective** % displ.1 µM CB_1=20%	High Content screening Fluorescence Microscopy in transfected cells
Muscarinic Receptor						
M_1/M_2	**CELT-249**	MUSCR-589-2	589/616	M_1=133 nM M_2=11.5 nM	No data	Fluorescence Microscopy in transfected cells (ongoing)
M_1/M_2	**CELT-095**	MUSCR-743-1	748/776	M_1=57.77 nM M_2=37.7 nM	No data	Fluorescence Microscopy in transfected cells (ongoing)
Adrenergic Receptor						
α_1	**CELT-033**	ADR-560-1	560/571	5 nM (α_{1A})	**Selective** % displ.1 µM α_{2A}=15%	Fluorescence Microscopy in transfected cells (ongoing)
α_1	**CELT-030**	ADR-646-1	646/662	28.3 nM (α_{1A})	**Selective** K_i (α_{2A})=1081 nM	Fluorescence Microscopy in transfected cells (ongoing)
C5a Receptor						
C5aR	**CELT-058**	C5aR-646-1	646/662	24.89 nM	Not Defined	Flow cytometry
Angiotensin Receptor						
AT1	**CELT-045**	ATR-646-1	646/662	160 nM	**Selective** % displ.1 µM AT_2=8,4%	Fluorescence Microscopy in transfected cells (ongoing)
AT1	**CELT-252**	ATR-560-1	560/571	39 nM	**Selective** % displ.1 µM AT_2=3,3%	Fluorescence Microscopy in transfected cells (ongoing)

GLP1 Receptor						
GLP1	**CELT-111 (LUXendin551)**	GLP1-551-1	551/576	7.2	**Selective**[8]	Widefield/confocal/2-photon microscopy in live and fixed mammalian cells and tissue, as well as anaesthetized mice[5]
GLP1	**CELT-112 (LUXendin645)**	GLP1-645-1	645/664	7.5	**Selective**[9]	Widefield/confocal/2-photon microscopy in live and fixed mammalian cells and tissue, as well as anaesthetized mice[6] TR-FRET
GLP1	**CELT-113 (LUXendin762)**	GLP1-762-1	762/784	7.0	**Selective**[9]	Widefield/confocal/2-photon microscopy in live and fixed mammalian cells and tissue, as well as anaesthetized mice.[8] Non-invasive fluorescence preclinical imaging[7–9]
Oxytocin Receptor						
OTR	**CELT-114**	OTR-597-1	597/657 (water)	0.54 nM	Not Defined	TR-FRET and confocal microscopy[10]
OTR	**CELT-115**	OTR-650-1	650/667	1.59 nM	**Selective** >1000 nM (V1aR and V1bR), 509 nM (V2R)	TR-FRET and confocal microscopy[11]

[a] K_i or % of displacement at 1 µM measured by radioligand binding assay. In the case of the C5aR ligand corresponds to the EC_{50}. In the case of GLP1R ligands correspond to pIC_{50}. In the case of OTR ligands, K_i was determined by competition experiments against [^{3}H]AVP (labelled arginine vasopressin) for CELT-114 and K_d by TR-FRET saturation binding experiments for CELT-115.

Probes for GPCRs functional assays

target	Code	Cat number	λexc/λem	Description	Validation
G protein	**CELT-503**	G-FUN-589-1	589/616	**fluorescent GTPγS**	G protein activation assays in steady state and kinetic mode

E3 ligases fluorescent ligands

E3 ligase	Code	Cat number	λexc/λem	Affinity[a]	Selectivity[a]	Validation
VHL	**CELT-050**	VHL-646-1	646/662	96 nM	Not defined	TR-FRET competition binding

[a] K_d in fluorescence polarization competition binding.

Intracellular receptors fluorescent ligands

Receptor	Code	Cat number	λexc/λem	Affinity[a]	Selectivity[a]	Validation
SIGMA receptor σ1 / σ2	**CELT-483**	σR-646-1	646/662	σ1=51.3 nM σ2= 30.2 nM	Non Selective	Flow cytometry[12] Confocal microscopy[12] Live cell microscopy[12]
	CELT-483 + Masking agents[b]	σR-BOX-1				

[a] K_i by radioligand binding assay.
[b] We also provide kits of CELT-483 together with potent and selective σ1 and σ2 receptors masking agents (L6 and F390, respectively).

In case you are interested by any of these ligands and they have not been validated yet for the kind of assay you want to test, please let us know: based on our experience we could advise you about which fluorophore works better for each assay, and eventually develop new versions -i.e. keeping the pharmacophore but changing the fluorophore- in a turnaround of 4-6 weeks.

Contact Information

info@celtarys.com

References

(1) Barbazán, J.; Majellaro, M.; Brea, J. M.; Sotelo, E.; Abal, M. Identification of A2BAR as a Potential Target in Colorectal Cancer Using Novel Fluorescent GPCR Ligands. *Biomed. Pharmacother.* **2022**, *153*, 113408. https://doi.org/10.1016/j.biopha.2022.113408.

(2) Miranda-Pastoriza, D.; Bernárdez, R.; Azuaje, J.; Prieto-Díaz, R.; Majellaro, M.; Tamhankar, A. V.; Koenekoop, L.; González, A.; Gioé-Gallo, C.; Mallo-Abreu, A.; Brea, J.; Loza, M. I.; García-Rey, A.; García-Mera, X.; Gutiérrez-de-Terán, H.; Sotelo, E. Exploring Non-Orthosteric Interactions with a Series of Potent and Selective A_3 Antagonists. *ACS Med. Chem. Lett.* **2022**, *13* (2), 243–249. https://doi.org/10.1021/acsmedchemlett.1c00598.

(3) Raïch, I.; Rivas-Santisteban, R.; Lillo, A.; Lillo, J.; Reyes-Resina, I.; Nadal, X.; Ferreiro-Vera, C.; De Medina, V. S.; Majellaro, M.; Sotelo, E.; Navarro, G.; Franco, R. Similarities and Differences upon Binding of Naturally Occurring Δ9-Tetrahydrocannabinol-Derivatives to Cannabinoid CB1 and CB2 Receptors. *Pharmacol. Res.* **2021**, *174*, 105970. https://doi.org/10.1016/j.phrs.2021.105970.

(4) Navarro, G.; Sotelo, E.; Raïch, I.; Loza, M. I.; Brea, J.; Majellaro, M. A Robust and Efficient FRET-Based Assay for Cannabinoid Receptor Ligands Discovery. *Molecules* **2023**, *28* (24), 8107. https://doi.org/10.3390/molecules28248107.

(5) Ast, J.; Novak, A. N.; Podewin, T.; Fine, N. H. F.; Jones, B.; Tomas, A.; Birke, R.; Roßmann, K.; Mathes, B.; Eichhorst, J.; Lehmann, M.; Linnemann, A. K.; Hodson, D. J.; Broichhagen, J. Expanded LUXendin Color Palette for GLP1R Detection and Visualization In Vitro and In Vivo. *JACS Au* **2022**, *2* (4), 1007–1017. https://doi.org/10.1021/jacsau.2c00130.

(6) Ast, J.; Arvaniti, A.; Fine, N. H. F.; Nasteska, D.; Ashford, F. B.; Stamataki, Z.; Koszegi, Z.; Bacon, A.; Jones, B. J.; Lucey, M. A.; Sasaki, S.; Brierley, D. I.; Hastoy, B.; Tomas, A.; D'Agostino, G.; Reimann, F.; Lynn, F. C.; Reissaus, C. A.; Linnemann, A. K.; D'Este, E.; Calebiro, D.; Trapp, S.; Johnsson, K.; Podewin, T.; Broichhagen, J.; Hodson, D. J. Super-Resolution Microscopy Compatible Fluorescent Probes Reveal Endogenous Glucagon-like Peptide-1 Receptor Distribution and Dynamics. *Nat. Commun.* **2020**, *11* (1), 467. https://doi.org/10.1038/s41467-020-14309-w.

(7) Lucey, M.; Ashik, T.; Marzook, A.; Wang, Y.; Goulding, J.; Oishi, A.; Broichhagen, J.; Hodson, D. J.; Minnion, J.; Elani, Y.; Jockers, R.; Briddon, S. J.; Bloom, S. R.; Tomas, A.; Jones, B. Acylation of the Incretin Peptide Exendin-4 Directly Impacts GLP-1 Receptor Signalling and Trafficking. April 1, 2021. https://doi.org/10.1101/2021.04.01.438030.

(8) Marzook, A.; Chen, S.; Pickford, P.; Lucey, M.; Wang, Y.; Corrêa Jr, I. R.; Broichhagen, J.; Hodson, D. J.; Salem, V.; Rutter, G. A.; Tan, T. M.; Bloom, S. R.; Tomas, A.; Jones, B. Evaluation of Efficacy- versus Affinity-Driven Agonism with Biased GLP-1R Ligands P5 and Exendin-F1. *Biochem. Pharmacol.* **2021**, *190*, 114656. https://doi.org/10.1016/j.bcp.2021.114656.

(9) Fang, Z.; Chen, S.; Pickford, P.; Broichhagen, J.; Hodson, D. J.; Corrêa, I. R.; Kumar, S.; Görlitz, F.; Dunsby, C.; French, P. M. W.; Rutter, G. A.; Tan, T.; Bloom, S. R.; Tomas, A.; Jones, B. The Influence of Peptide Context on Signaling and Trafficking of Glucagon-like Peptide-1 Receptor Biased Agonists. *ACS Pharmacol. Transl. Sci.* **2020**, *3* (2), 345–360. https://doi.org/10.1021/acsptsci.0c00022.

(10) Karpenko, I. A.; Kreder, R.; Valencia, C.; Villa, P.; Mendre, C.; Mouillac, B.; Mély, Y.; Hibert, M.; Bonnet, D.; Klymchenko, A. S. Red Fluorescent Turn-On Ligands for Imaging and Quantifying G Protein-Coupled Receptors in Living Cells. *ChemBioChem* **2014**, *15* (3), 359–363. https://doi.org/10.1002/cbic.201300738.

(11) Karpenko, I. A.; Margathe, J.-F.; Rodriguez, T.; Pflimlin, E.; Dupuis, E.; Hibert, M.; Durroux, T.; Bonnet, D. Selective Nonpeptidic Fluorescent Ligands for Oxytocin Receptor: Design, Synthesis, and Application to Time-Resolved FRET Binding Assay. *J. Med. Chem.* **2015**, *58* (5), 2547–2552. https://doi.org/10.1021/jm501395b.

(12) Abatematteo, F. S.; Majellaro, M.; Montsch, B.; Prieto-Díaz, R.; Niso, M.; Contino, M.; Stefanachi, A.; Riganti, C.; Mangiatordi, G. F.; Delre, P.; Heffeter, P.; Sotelo, E.; Abate, C. Development of Fluorescent 4-[4-(3 *H* -Spiro[Isobenzofuran-1,4'-Piperidin]-1'-Yl)Butyl]Indolyl Derivatives as High-Affinity Probes to Enable the Study of σ Receptors via Fluorescence-Based Techniques. *J. Med. Chem.* **2023**, *66* (6), 3798–3817. https://doi.org/10.1021/acs.jmedchem.2c01227.