

## 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	$\lambda_{exc}/\lambda_{em}$	Affinity <sup>a</sup>	Selectivity <sup>a</sup>	Validation
<b>Dopamine Receptor</b>					
<b>D<sub>2</sub></b>	<b>CELT-174</b>	589/616	1.06 nM	<b>Selective</b> K <sub>i</sub> (D <sub>3</sub> )=136.5 nM K <sub>i</sub> (D <sub>4</sub> )=152.7 nM	Fluorescence Microscopy in transfected cells Flow cytometry
	<b>CELT-426</b>	560/571	89.3 nM	<b>Partially Selective</b> K <sub>i</sub> (D <sub>3</sub> )=194.8 nM K <sub>i</sub> (D <sub>4</sub> )=263 nM	Fluorescence polarization Flow cytometry
	<b>CELT-175</b>	748/776	3.15 nM	<b>Selective</b> K <sub>i</sub> (D <sub>3</sub> )=294.6 nM K <sub>i</sub> (D <sub>4</sub> )=220.3 nM	Fluorescence Microscopy in transfected cells (ongoing)
<b>D<sub>3</sub></b>	<b>CELT-429</b>	589/616	75.4 nM	<b>Selective</b> % displ. 1 $\mu$ M D <sub>2</sub> = 6% D <sub>4</sub> = 3%	Fluorescence Microscopy in transfected cells (ongoing)
	<b>CELT-419</b>	560/571	65.6 nM	<b>Partially Selective</b> K <sub>i</sub> (D <sub>2</sub> )=151.4 nM	Fluorescence polarization
<b>D<sub>2</sub>/D<sub>3</sub></b>	<b>CELT-240</b>	589/616	D <sub>3</sub> = 2.14 nM D <sub>2</sub> = 2.34 nM	<b>Selective against D<sub>4</sub></b> % displ. 1 $\mu$ M D <sub>4</sub> = 1%	Flow cytometry
	<b>CELT-241</b>	646/662	D <sub>3</sub> = 4.77 nM D <sub>2</sub> = 5.22 nM	<b>Selective against D<sub>4</sub></b> K <sub>i</sub> (D <sub>4</sub> )=302.55 nM	Fluorescence Microscopy in transfected cells (ongoing)
<b>Adenosine Receptor</b>					
<b>PAN-ADO</b>	<b>CELT-298</b>	646/662	A <sub>1</sub> = 20.9 nM A <sub>2A</sub> = 171 nM A <sub>2B</sub> = 44.7 nM A <sub>3</sub> = 95.2 nM	<b>Non Selective</b>	Fluorescence Microscopy in transfected cells

A <sub>1</sub>	CELT-448	560/571	26.2 nM	<b>Selective</b> % displ.1 μM A <sub>2A</sub> = 11% A <sub>2B</sub> = 22% A <sub>3</sub> = 24%	Fluorescence polarization (ongoing) Fluorescence Microscopy in transfected cells (ongoing)
	CELT-372 (A <sub>1</sub> /A <sub>2B</sub> )	589/616	A <sub>1</sub> = 1.89 nM A <sub>2B</sub> = 24.75 nM	<b>Partially Selective</b> K <sub>i</sub> (A <sub>2A</sub> )=80.33 nM K <sub>i</sub> (A <sub>3</sub> )=967.8 nM	Fluorescence Microscopy in transfected cells
	CELT-360	646/662	8.6 nM	<b>Non Selective</b> K <sub>i</sub> (A <sub>2A</sub> )=98.38 nM K <sub>i</sub> (A <sub>2B</sub> )=72.24 nM K <sub>i</sub> (A <sub>3</sub> )=231.01 nM	Fluorescence Microscopy in transfected cells
A <sub>2A</sub>	CELT-316	589/616	116.1 nM	<b>Selective</b> % displ.1 μM A <sub>1</sub> = 18% A <sub>2B</sub> = 33% A <sub>3</sub> = 31%	Fluorescence Microscopy in native cells
	CELT-300	646/662	8.35 nM	<b>Selective</b> % displ.1 μM A <sub>1</sub> = 31% A <sub>2B</sub> = 18% A <sub>3</sub> = 38%	Fluorescence Microscopy in transfected cells (ongoing)
A <sub>2B</sub> /A <sub>3</sub>	CELT-327	589/616	A <sub>2B</sub> = 35.6 nM A <sub>3</sub> = 45.7 nM	<b>Selective</b> % displ.1 μM A <sub>1</sub> =41% A <sub>2A</sub> =1%	Fluorescence Microscopy in native cells <sup>1</sup>
A <sub>3</sub>	CELT-228	560/571	52.7 nM	<b>Selective</b> % displ.1 μM A <sub>1</sub> = 2% A <sub>2A</sub> = 1% A <sub>2B</sub> = 5%	Fluorescence Microscopy in native cells <sup>1</sup> Fluorescence polarization <sup>2</sup>
	CELT-171	589/616	6.13 nM	<b>Selective</b> % displ.1 μM A <sub>1</sub> =2.1% A <sub>2A</sub> = 2.21% A <sub>2B</sub> = 1.9%	Fluorescence Microscopy in transfected and native cells
	CELT-480	646/662	12 nM	<b>Selective</b> % displ.1 μM A <sub>1</sub> = 38% A <sub>2A</sub> = 23 % A <sub>2B</sub> = 49 %	Fluorescence Microscopy in transfected cells (ongoing)
<b>Serotonin Receptor</b>					
5HT <sub>2B</sub>	CELT-211	589/616	56.32 nM	<b>Selective</b> % displ.1 μM 5HT <sub>2A</sub> =0.94% 5HT <sub>2C</sub> = 1.75%	Fluorescence Microscopy in transfected cells

Cannabinoid Receptor					
PAN-CB	<b>CELT-335</b>	646/662	CB <sub>1</sub> = 44.8 nM CB <sub>2</sub> = 7.4 nM	<b>Non Selective</b>	HTRF in adherent cells <sup>3</sup> High Content screening Fluorescence Microscopy in transfected cells
CB <sub>2</sub>	<b>CELT-331</b>	646/662	75.9 nM	<b>Selective<sup>4</sup></b> % displ.1 μM CB <sub>1</sub> =20%	High Content screening Fluorescence Microscopy in transfected cells
Muscarinic Receptor					
M <sub>1</sub> /M <sub>2</sub>	<b>CELT 195</b>	748/776	M <sub>1</sub> =57.77 nM M <sub>2</sub> =37.7 nM	No data	Fluorescence Microscopy in transfected cells (ongoing)
M <sub>1</sub> /M <sub>2</sub>	<b>CELT-249</b>	589/616	M <sub>1</sub> =133 nM M <sub>2</sub> =11.5 nM	No data	Fluorescence Microscopy in transfected cells (ongoing)
Adrenergic Receptor					
α <sub>1</sub>	<b>CELT-130</b>	646/662	28.3 nM (α <sub>1A</sub> )	<b>Selective</b> K <sub>i</sub> (α <sub>2A</sub> )=1081 nM	Fluorescence Microscopy in transfected cells (ongoing)
α <sub>1</sub>	<b>CELT-133</b>	560/571	5 nM (α <sub>1A</sub> )	<b>Selective</b> % displ.1 μM α <sub>2A</sub> =15%	Fluorescence Microscopy in transfected cells (ongoing)
C5a Receptor					
C5aR	<b>CELT-58</b>	646/662	24.89 nM	Not Defined	Flow cytometry
Angiotensin Receptor					
AT <sub>1</sub>	<b>CELT-145</b>	646/662	160 nM	<b>Selective</b> % displ.1 μM AT <sub>2</sub> =8,4%	Fluorescence Microscopy in transfected cells (ongoing)
AT <sub>1</sub>	<b>CELT-252</b>	560/571	39 nM	<b>Selective</b> % displ.1 μM AT <sub>2</sub> =3,3%	Fluorescence Microscopy in transfected cells (ongoing)
Oxytocin Receptor					
OTR	<b>CELT-501</b>	597/657 (water)	0.54 nM	Not Defined	TR-FRET and confocal microscopy <sup>5</sup>
OTR	<b>CELT-502</b>	650/667	1.59 nM	<b>Selective</b> >1000 nM (V1aR and V1bR), 509 nM (V2R)	TR-FRET and confocal microscopy <sup>6</sup>

GLP1 Receptor					
GLP1	<b>CELT-111 (LUXendin551)</b>	551/576	7.2	<b>Selective<sup>8</sup></b>	Widefield/confocal/2-photon microscopy in live and fixed mammalian cells and tissue, as well as anaesthetized mice <sup>7</sup>
GLP1	<b>CELT-112 (LUXendin645)</b>	645/664	7.5	<b>Selective<sup>9</sup></b>	Widefield/confocal/2-photon microscopy in live and fixed mammalian cells and tissue, as well as anaesthetized mice <sup>8</sup> TR-FRET <sup>9,10,11</sup>
GLP1	<b>CELT-113 (LUXendin762)</b>	762/784	7.0	<b>Selective<sup>9</sup></b>	Widefield/confocal/2-photon microscopy in live and fixed mammalian cells and tissue, as well as anaesthetized mice. <sup>8</sup> Non-invasive fluorescence preclinical imaging

<sup>a</sup>K<sub>i</sub> or % of displacement at 1 μM measured by radioligand binding assay. In the case of the C5a5 ligand corresponds to the EC<sub>50</sub>. In the case of GLP1R ligands corresponds to pIC<sub>50</sub>. In the case of OTR ligands, K<sub>i</sub> was determined by competition experiments against [<sup>3</sup>H]AVP (labelled arginine vasopressin) for CELT-501 and K<sub>d</sub> by TR-FRET saturation binding experiments for CELT-502.

### E3 ligases fluorescent ligands

E3 ligase	Code	λ <sub>exc</sub> /λ <sub>em</sub>	Affinity <sup>a</sup>	Selectivity <sup>a</sup>	Validation
VHL	<b>CELT-150</b>	646/662	96 nM	Not defined	TR-FRET competition binding

<sup>a</sup> K<sub>D</sub> in fluorescence polarization competition binding

### Intracellular receptors fluorescent ligands

Receptor	Code	λ <sub>exc</sub> /λ <sub>em</sub>	Affinity <sup>a</sup>	Selectivity <sup>a</sup>	Validation
<b>SIGMA receptor σ1 / σ2</b>	<b>CELT-483</b>	646/662	σ1=51.3 nM σ2= 30.2 nM	Non Selective	Flow cytometry <sup>12</sup> Confocal microscopy <sup>12</sup> Live cell microscopy <sup>12</sup>
	<b>CELT-483 + Masking agents<sup>b</sup></b>				

<sup>a</sup> K<sub>i</sub> by radioligand binding assay.

<sup>b</sup> We also provide kits of CELT-483 together with potent and selective σ1 and σ2 receptors masking agents (L6 and F390, respectively).

<sup>1</sup>J. Barbazán, M. Majellaro, A. L. Martínez, J. M. Brea, E. Sotelo and M. Abal Identification of A2BAR as a potential target in colorectal cancer using novel fluorescent GPCR ligands. *Biomedicine & Pharmacotherapy* 2022 <https://doi.org/10.1016/j.biopha.2022.113408>

<sup>2</sup>D. Miranda-Pastoriza, R. Bernárdez, J. Azhujaje, R. Prieto-Díaz, M. Majellaro, A. V. Tamhankar, L. Koenekoop, A. González, C. Gioe-Gallo, A. Mallo-Abreu, J. Brea, M. I. Loza, A. García-Rey, X. García-Mera, H. Gutiérrez de Terán and E. Sotelo. *ACS Medicinal Chemistry Letters*. DOI: 10.1021/acsmchemlett.1c00598.

<sup>3</sup>Lu Raich et al. "Similarities and differences upon binding of naturally occurring  $\Delta^9$ -tetrahydrocannabinol-derivatives to cannabinoid CB1 and CB2 receptors" *Pharmacol. Res.*, 2021,174,105970

<sup>4</sup>Development of a CB<sub>1</sub> selective ligand is ongoing.

<sup>5</sup>Karpenko, I. A.; Kreder, R.; Valencia, C.; Villa, P.; Mendre, C.; Mouillac, B.; Mely, 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, 359–363. DOI: 10.1002/cbic.201300738

<sup>6</sup>Karpenko, Iuliia A.; Margathe, Jean-Francois; Rodriguez, Thieric; Pflimlin, Elsa; Dupuis, Elodie; Hibert, Marcel; Durroux, Thierry; Bonnet, Dominique. *Journal of Medicinal Chemistry* 2015, 58(5), 2547-2552. DOI: 10.1021/jm501395b.

<sup>7</sup>J. Ast, A. N. Novak, T. Podewin, N. H. F. Fine, B. Jones, A. Tomas, R. Birke, K. Roßmann, B. Mathes, J. Eichhorst, M. Lehmann, A. K. Linnemann, D. J. Hodson, J. Broichhagen, 2021, DOI 10.33774/chemrxiv-2021-7rngq.

<sup>8</sup>J. Ast, A. Arvaniti, N. H. F. Fine, D. Nasteska, F. B. Ashford, Z. Stamataki, Z. Koszegi, A. Bacon, B. J. Jones, M. A. Lucey, S. Sasaki, D. I. Brierley, B. Hastoy, A. Tomas, G. D'Agostino, F. Reimann, F. C. Lynn, C. A. Reissaus, A. K. Linnemann, E. D'Este, D. Calebiro, S. Trapp, K. Johnsson, T. Podewin, J. Broichhagen, D. J. Hodson, *Nat Commun* 2020, 11, 467.

<sup>9</sup>A. Marzook, S. Chen, P. Pickford, M. Lucey, Y. Wang, I. R. Corrêa Jr, J. Broichhagen, D. J. Hodson, V. Salem, G. A. Rutter, T. M. Tan, S. R. Bloom, A. Tomas, B. Jones, *Biochemical Pharmacology* 2021, 190, 114656.

<sup>10</sup>M. Lucey, T. Ashik, A. Marzook, Y. Wang, J. Goulding, A. Oishi, J. Broichhagen, D. J. Hodson, J. Minnion, Y. Elani, R. Jockers, S. J. Briddon, S. R. Bloom, A. Tomas, B. Jones, *Mol Pharmacol* 2021, DOI 10.1124/molpharm.121.000270.

<sup>11</sup>Z. Fang, S. Chen, P. Pickford, J. Broichhagen, D. J. Hodson, J. Ivan R. Corrêa, S. Kumar, F. Görlitz, C. Dunsby, P. M. W. French, G. A. Rutter, T. Tan, S. R. Bloom, A. Tomas, B. Jones, *ACS Pharmacology & Translational Science* 2020, DOI 10.1021/acspsci.0c00022.

<sup>12</sup>F.S. Abatematteo et al., 2023. Development of Fluorescent 4-[4-(3H-Spiro[isobenzofuran-1,4'-piperidin]-1'-yl)butyl]indolyl Derivatives as High-Affinity Probes to Enable the Study of  $\sigma$  Receptors via Fluorescence-Based Techniques. *J. Med. Chem.* 2023, 66, 6, 3798–3817 <https://doi.org/10.1021/acs.jmedchem.2c01227>

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

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