Fluorescent Antagonists For Vasopressin V_2 GPCR: Ligand Screening and Oligomerization Assays

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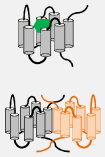
Objectives

G Protein-Coupled Receptor

- Site of action of about 30% of all drugs on the market.
- Drugs target only 60 GPCR (over 390 non-olfactory GPCR): potential to exploit the remaining family members.[1]



1) Accelerate GPCR ligands discovery: new receptor-selective HTS assays.

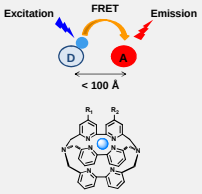
2) Gain a better understanding of their functioning and molecular structure.[2]

(1) Congreve et al. *Trends Pharmacol. Sci.* **2012**, *33*, 249-260; (2) Durroux, T. et al. *Nat. Chem. Biol.* **2010**, *6*, 587-594.

Strategies

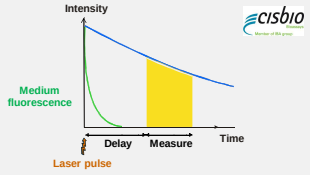
Time Resolved-FRET based assay (principle)

TR-FRET: combines standard FRET with the time resolved measurement of fluorescence



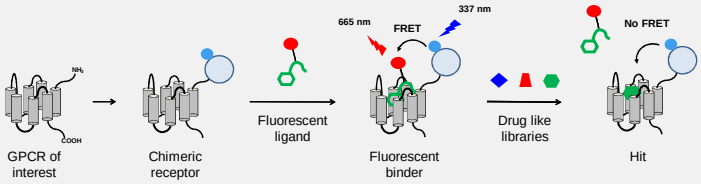
Fluorophore with a long lifetime (ms).

Donor: Eu or Tb cryptate



Short-life fluorescence from biological medium is not measurable after the time delay: increase signal-to-noise ratio.

Application to GPCR for hit discovery

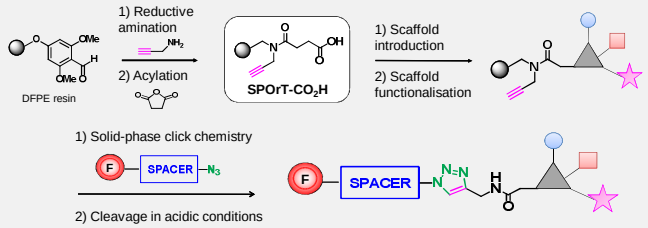


TR-FRET: high sensitivity and reduced environmental safety risk/radioactivity

Challenge: design and synthesis of high affinity fluorescent GPCR ligands

Novel Solid-phase Organic Tagging (SPOrT) resins

Facilitate the labelling of peptides and small organic compounds with fluorescent dyes



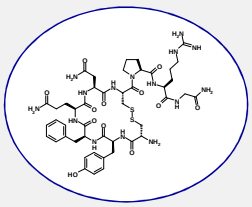
"SPOrT" resin: rapid and high yielding access to fluorescent probes, readily amenable to parallel synthesis.

Bonnet, D. *et al. Chem. Eur. J.* **2008**, *14*, 6247-6254.

Application to Vasopressin V_2 GPCR

Arginine-vasopressin (AVP) hormone

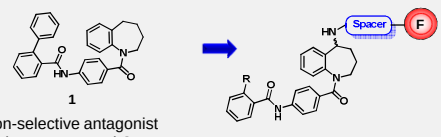
Cardiovascular control, osmoregulation and water homeostasis



Human AVP V_2 receptor (hV_2R)

Potential target for the treatment of congestive heart failure, hypertension, or congenital nephrogenic diabetes insipidus

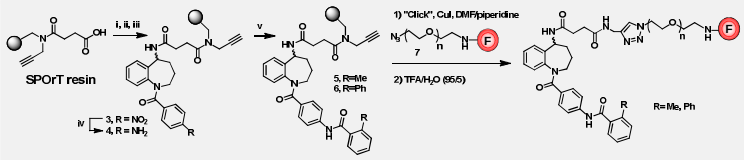
Design of non peptidic fluorescent probes for hV_2R



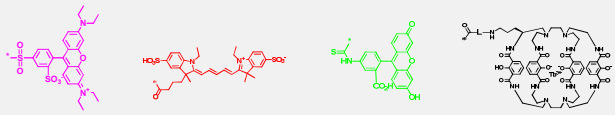
Non-selective antagonist for $hV_{1a}R$, V_2R and OTR
(*Chem. Pharm. Bull.* **1997**, *45*, 1870-1874)

- Non peptidic
- High affinity and selectivity
- Compatible with TR-FRET
- Optimisation: Spacer, Dye, **R** group

V_2R fluorescent probes synthesis



Reagents: (i) 2,3,4,5-tetrahydro-1*H*-benzo[b]azepin-5-amino scaffold, PyBOP, Hünig's base, DMF; (ii) TMSOTf, Et_3N, CH_2Cl_2; (iii) *p*-NO_2BzCl, pyridine, 4-DMAP, CH_2Cl_2; (iv) $SnCl_2.2H_2O$, DMF; (v) Biphenyl or *o*-toluyl acyl chloride, Hünig's base, 4-DMAP, CH_2Cl_2.

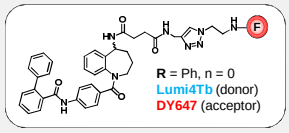


LRB DY647 FLUOR Lumi4-Tb

Biological evaluation

Cpd	R	n		Binding (K_i, nM) V_2	V_{1a}	V_{1b}	OT
AVP	-	-	-	1.48 ± 0.08	0.7 ± 0.17	0.49 ± 0.06	1.65 ± 0.49
1	Ph	-	-	0.57 ± 0.18	2.74 ± 0.19	nd	109 ± 34
8	Me	3	LRB	54.3 ± 6.6	>10000	>10000	495 ± 101
9	Ph	3	LRB	4.0 ± 0.7	>10000	>10000	>10000
40	Ph	3	Fluor	26.1 ± 5.2	nd	nd	nd
44	Ph	0	DY647	5.69 ± 1.05	621 ± 65	>10000	184 ± 31
45	Ph	3	DY647	9.02 ± 0.24	1753 ± 338	>10000	808 ± 61
46	Ph	6	DY647	14.22 ± 0.19	nd	nd	nd
47	Ph	0	Lumi4-Tb	5.86 ± 0.83	180 ± 34	>10000	155 ± 8
48	Ph	3	Lumi4-Tb	16.1 ± 2.5	750 ± 89	>10000	304 ± 70
49	Ph	6	Lumi4-Tb	30.65 ± 5.03	nd	nd	nd

	V_2R affinity	Selectivity
R	Ph>Me	Ph>Me
F	LRB>DY647~ Lumi4Tb>Fluor	LRB>DY647> Lumi4Tb
n	0>3>6	0>3



LRB: not compatible with TR-FRET technics

47

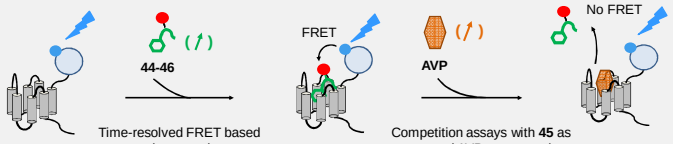
$Lumi_4Tb$ (donor)
K_i = 5.86 nM (hV_2R)
$V_{1a}R/V_2R$ selectivity = 31
antagonist

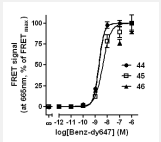
44

DY647 (acceptor)
K_i = 5.69 nM (hV_2R)
$V_{1a}R/V_2R$ selectivity = 109
antagonist

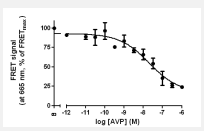
First selective fluorescent non peptidic antagonists for hV_2R

V_2R ligand screening assay





Cpd	Binding [^{3}H]AVP, K_i (nM)	TR-FRET, K_d (nM)
44	5.69 ± 1.05	1.38 ± 0.56
45	9.02 ± 0.24	2.33 ± 1.23
46	14.22 ± 0.19	2.53 ± 1.19



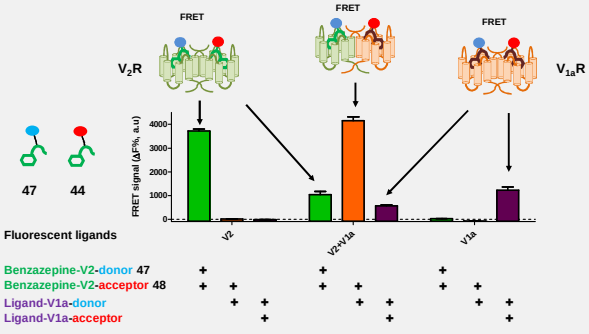
K_i(AVP) 1.93 nM

K_d([^{3}H]AVP) 1.36 nM

Validation: K_i and Kd similar to those determined by radioactive assay

New V_2R TR-FRET based assay readily amenable to HTS

V_2R-$V_{1a}R$ oligomerization assay



V_2R-$V_{1a}R$ heterodimerization at the cell surface

Perspective: studies in native tissues

Loison, S.; Durroux, T.; Mouillac, B.; Hibert, M.; Bonnet, D. Patent FR **2010**/61322; *J. Med. Chem.* **2012**, *55*, 8588-8602.