Finanziato dall'Unione europea NextGenerationEU

MNESYS

Ministero dell'Università e della Ricerca

MNESYS – SPOKE 6 Meeting
30 Gennaio 2024
Università degli Studi di Genova,
Palazzo della Meridiana
Salita San Francesco 4, 16100 – GENOVA

Poster presentation

DESIGN OF NOVEL MELATONIN RECEPTOR LIGANDS WITH NEUROPROTECTIVE ACTIVITY

Laura Scalvini,[1] Federica Vacondio,[1] Alessio Lodola,[1] Silvia Rivara,[1] Annalida Bedini,[2] Gilberto Spadoni,[2] Fabrizio Vincenzi,[3] Katia Varani,[3] Mariarosaria Cammarota,[4] Francesca Boscia,[4] Marco Mor[1]

1 Dipartimento di Scienze degli Alimenti e del Farmaco, Università degli Studi di Parma, Parco Area delle Scienze 27/A, 43124 Parma, Italy
2 Dipartimento di Scienze Biomolecolari, Università degli Studi di Urbino Carlo Bo, Piazza Rinascimento 6, 61029 Urbino, Italy
3 Dipartimento di Medicina Traslazionale, Università degli Studi di Ferrara, Via Luigi Borsari 46, 44121, Ferrara, Italy
4 Dipartimento di Neuroscienze e Scienze Riproduttive ed Odontostomatologiche, Università degli Studi di Napoli, Via Pansini 5, 44121, Ferrara, Italy

ALIFAR
DIPARTIMENTO DI ECCELLENZA
MUR 2023-2027
UNIVERSITÀ DI PARMA

Finanziato dall'Unione europea
NextGenerationEU

MNESYS

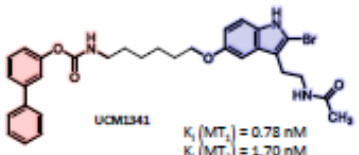
Ministero dell'Università e della Ricerca

Design of novel melatonin receptor ligands with neuroprotective activity

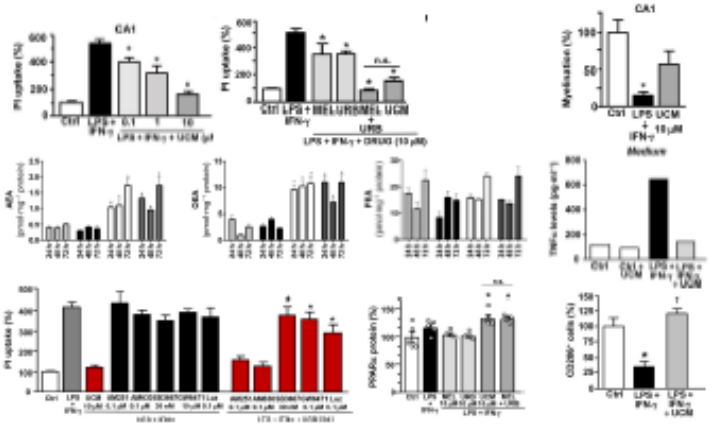
Laura Scalvini,[1] Federica Vacondio,[1] Alessio Lodola,[1] Silvia Rivara,[1] Annalida Bedini,[2] Gilberto Spadoni,[2] Fabrizio Vincenzi,[3] Katia Varani,[3] Mariarosaria Cammarota,[4] Francesca Boscia,[4] Marco Mor[1]

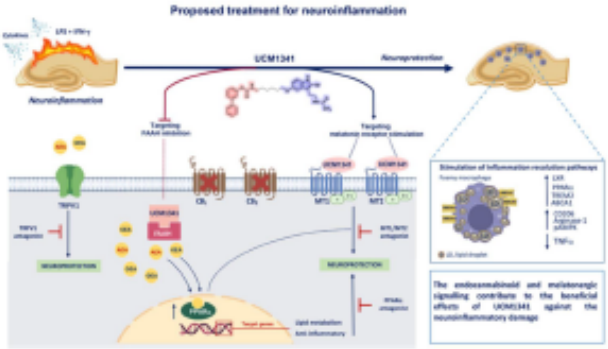
[1] Dipartimento di Scienze degli Alimenti e del Farmaco, Università degli Studi di Parma, Parco Area delle Scienze 27/A, 43124 Parma, Italy
[2] Dipartimento di Scienze Biomolecolari, Università degli Studi di Urbino Carlo Bo, Piazza Rinascimento 6, 61029 Urbino, Italy
[3] Dipartimento di Medicina Traslazionale, Università degli Studi di Ferrara, Via Luigi Borsari 46, 44121, Ferrara, Italy
[4] Dipartimento di Neuroscienze e Scienze Riproduttive ed Odontostomatologiche, Università degli Studi di Napoli, Via Pansini 5, 44121, Ferrara, Italy

- Melatonin, the neurohormone mainly secreted by the pineal gland, binds and activates two GPCRs, MT_1 and MT_2, exerts antioxidant and radical scavenging effects and has neuroprotective activity.[1]
- Fatty acid amide hydrolase (FAAH) is responsible for the inactivating hydrolysis of N-acylethanolamines, including the endocannabinoid anandamide (AEA), and the lipid modulators oleoylethanolamide (OEA) and palmitoylethanolamide (PEA).[2]
- Enhancing the endocannabinoid and melatonergic tone has therapeutic potential to treat neuroinflammatory diseases.
- UCM1341 is a dual-acting compound with FAAH inhibitory action and agonist activity on melatonin receptors.[3]
- Aim: to evaluate the protective effects of UCM1341 against neuroinflammation-induced degeneration.[4]



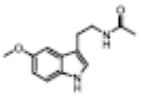
$K_i(MT_1) = 0.78$ nM
$K_i(MT_2) = 1.70$ nM
IC_{50} (FAAH)= 0.85 nM



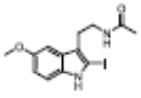


Unbinding simulations of 2-iodomelatonin from MT_1 and MT_2 receptors

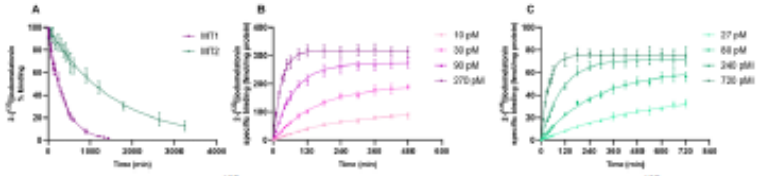
- Determination of ligand binding affinity to melatonin receptors relies on displacement binding assays with the radioligand 2-[^{125}I]iodomelatonin (2-[^{125}I]IMLT) which is characterized by slow dissociation rate.
- Aim: to evaluate the impact of the slow dissociation of 2-[^{125}I]IMLT on K_i values obtained for ligands in standard experimental conditions;
  to provide a mechanistic explanation of the different dissociation half-life observed for 2-iodomelatonin.[5]



melatonin
$pK_i(MT_1) = 9.65$
$pK_i(MT_2) = 9.27$



2-iodomelatonin
$pK_i(MT_1) = 10.71$
$pK_i(MT_2) = 9.83$



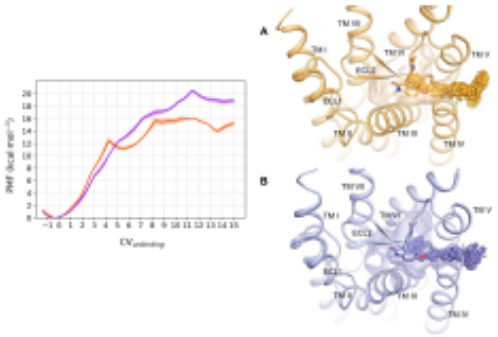
Dissociation curves (A) of 2-[^{125}I]IMLT for MT_1 and MT_2 receptors. Association curves of 2-[^{125}I]IMLT for MT_1 (B) and MT_2 (C) receptors.

	k_{on} (M^{-1} min^{-1})	k_{off} (min^{-1})	Dissociation $t_{1/2}$ (min)
MT_1	1.2 ± 0.1 x 10^6	0.0028 ± 0.0002	248 ± 20
MT_2	3.7 ± 0.3 x 10^7	0.000639 ± 0.000054	1085 ± 98

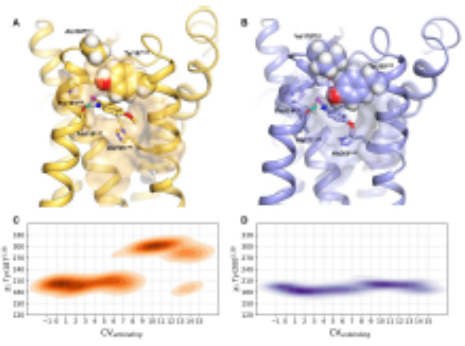
Receptor	Incubation time	K_i (pM)	Bmax (fmol mg^{-1} protein)
MT_1	2 h	26 ± 2	342 ± 26
MT_1	20 h	19 ± 2	329 ± 31
MT_2	2 h	78 ± 5	76 ± 7
MT_2	20 h	65 ± 4	74 ± 6

Results

- Incubation time affected only weakly the binding affinity constants.
- Structure-activity relationships are conserved when binding data are collected at shorter incubation times.



Left: potential of mean force (PMF) profiles calculated from umbrella sampling simulations for 2-iodomelatonin unbinding from the MT_1 (red) and MT_2 receptor (purple). Right: the unbinding pathway of 2-iodomelatonin at the MT_1 (A, orange) and MT_2 (B, purple) receptor is represented through the position of the center of mass of the ligand.



Top: 2-iodomelatonin unbinding from the MT_1 (A) and MT_2 (B) receptors (snapshot corresponding to CV_{unb} ≈6 Å). Bottom: density estimation plots showing χ1 dihedral angle of Tyr187/$200^{5.38}$ in US simulations at the MT_1 (C) and MT_2 (D) receptors (χ1~180°, closed conformation; χ1~300°, open conformation).

Results

- Energy barriers consistent with the longer dissociation half-life at the MT_2 receptor.
- Restricted mobility of a gatekeeper tyrosine along a lipophilic path at the MT_2 receptor.

References

1. Liu J, Clough SJ, Hutchinson AJ, et al. MT1 and MT2 Melatonin Receptors: A Therapeutic Perspective. *Annu Rev Pharmacol Toxicol.* 2016;56:361-83.
2. Mazzarrone M, Di Marzo V, Gertsch J, et al. Goods and Bads of the Endocannabinoid System as a Therapeutic Target: Lessons Learned after 30 Years. *Pharmacol Rev.* 2023;75:905-958.
3. Spadoni G, Bedini A, Furtassi L, et al. Identification of Bivalent Ligands with Melatonin Receptor Agonist and Fatty Acid Amide Hydrolase (FAAH) Inhibitory Activity That Exhibit Ocular Hypotensive Effect in the Rabbit. *J Med Chem.* 2016;59:7902-7916.
4. Cammarota M, Ferlenghi F, Vacondio F, et al. Combined targeting of fatty acid amide hydrolase and melatonin receptors promotes neuroprotection and stimulates inflammation resolution in rats. *Br J Pharmacol.* 2023;180:1316-1336.
5. Bedini A, Elati GM, Fabiola Panini F, et al. Binding and unbinding of potent melatonin receptor ligands: mechanistic simulations and experimental evidence. *J Pineal Res.* 2024, in press.