



Celtarys
RESEARCH



Case Study

Development of a fluorescent hMOR ligand for receptor visualization and binding experiments

24th April 2025

We would like to thank Prof. Michael Galko and Dr. Yan Wang from the MD Anderson Center (University of Texas) for sharing their results and supporting us in the preparation of this case study,

Controlled Substances

Controlled substances are drugs or other substances that are tightly controlled by the government because they may be abused or cause addiction. The control applies to the way the substance is made, used, handled, stored, and distributed. Controlled substances include opioids, stimulants, depressants, hallucinogens, and anabolic steroids.

They were initially established in the 1961 Single Convention on Narcotic Drugs, to which modifications and addendums have been made (1972). Their objective is to limit their use to medicinal and research purposes. Both this convention and the 1971 Convention on Psychotropic Substances have served as the basis for modern organisms such as the DEA or EUDA.

Opioids

Among controlled substances, opioids have always been very well known, originally as 'opium' in ancient times for its analgesic and sedative properties. However, the understanding of their mechanism of action and the discovery of specific opioid molecules emerged much later through advancements in pharmacological research. As opioids we refer to substances capable of binding the opioid receptors in the human body. They are divided into three groups: endogenous peptides, natural opioids and synthetic opioids (figure 1). Morphine and its synthetic derivatives such as fentanyl serve as the most effective drugs for pain management, although they present harmful side effects, such as addiction and respiratory depression.¹

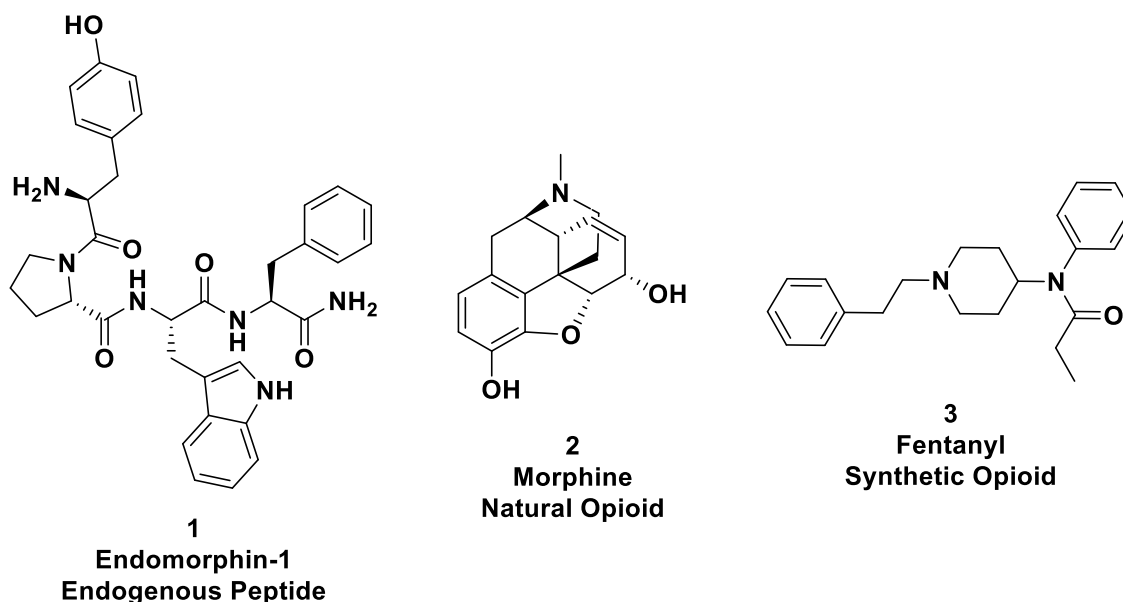


FIGURE 1. EXAMPLE OF MOLECULAR STRUCTURE OF OPIOIDS BELONGING TO THE THREE MAIN FAMILIES.

hMOR: therapeutic vs side effects

μ -OR (or hMOR) is a class A G-protein-coupled receptors (GPCRs) which belongs to the family of opioid receptors (figure 2). Two closely related family members are the δ - and κ -opioid receptors, together with the most recently discovered NOP receptor (3nociception/orphanin FQ opioid peptide receptor).² They are all part of the Endogenous Opioid system (EOS), except for the NOP receptor, whose mechanism opposes those of the other opioid receptors and it is the only one not able to bind to the clinically used morphinan opioids³

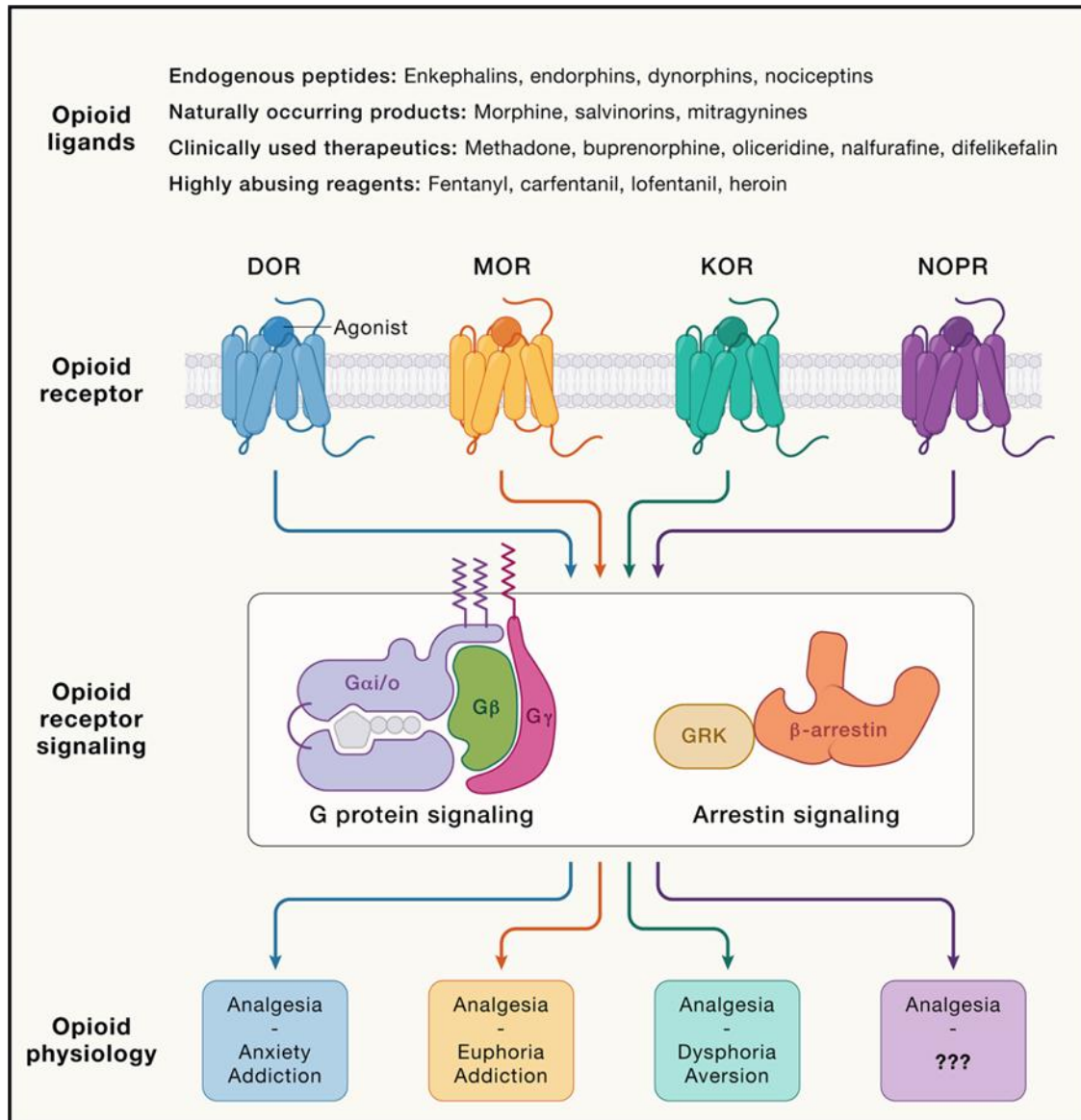


FIGURE 2. OPIOID RECEPTORS: SIGNALLING AND BIOLOGICAL EFFECTS.¹

hMOR receptor is the main responsible for both the analgesic and side-effects. Its analgesic activity has been attributed to G_i signalling while the mechanism behind the other effects is still up for debate. Throughout the years it was thought to be the β -arrestin signalling, thus several bias-ligands were developed, including oliceridine, approved in 2020 for pain management, although later studies indicate that the side-effects persist.⁴

Another proposed mechanism is the GIRK channel (G-protein gated inwardly rectifying potassium channel), which would explain the persistent side-effects in biased ligands. Thus, studying the hMOR receptor as well as the mechanisms surrounding it is key to developing more advantageous analgesics, which lack traditional secondary effects attached to opioids while maintaining their excellent pain management profile.⁴

***Drosophila* fly receptor and hMOR: customised fluorescent morphine development to demonstrate their homology**

Assessing pain response poses a challenge due to its subjective nature in humans, but this subjectiveness can be dodged when testing in other species. Such is the case of *Drosophila* larvae, where several assays determining pain response have been developed. Prof. M. Galko's group is at the forefront of these developments, having found analgesic response to fentanyl in these larvae.^{5,6}

The receptor responsible for the analgesic response to opioids was found in collaboration with Dr. Nate Himmel, who found two similar receptors to the morphine one in the *Drosophila* larvae genome and then established the structural similarities with hMOR through modelling.

Initial biological assays demonstrated that the fly receptor identified is required for fentanyl-induced analgesia in the larvae. The next step is corroborating whether this receptor, needed for opioid-induced analgesia, is the one binding fentanyl. The best method to demonstrate it are binding assays and expression studies using fluorescent probes. In this context, Prof. Galko required a customised fluorescent opioid with specific photophysical and physicochemical properties. Basing on the last insights, we agreed in developing a fluorescent morphine, which should have the following characteristics:

- Full agonistic behaviour (Emax 100%).
- High activity (comparable with morphine).
- High specific interaction.
- Excitation: 510-560 nm Emission: 545 to 685 nm (confocal microscope and stereoscope).

Fluorescent Tools in Opioid Research

There has been previous interest in the development of fluorescent morphine, as shown in several papers. In these papers, the objective is to obtain a fluorescent morphine without the need of maintaining a full agonistic behaviour, since they would be used for receptor visualization (figure 3). While their focus is different from ours, the information is invaluable for synthesizing the required compound.⁷

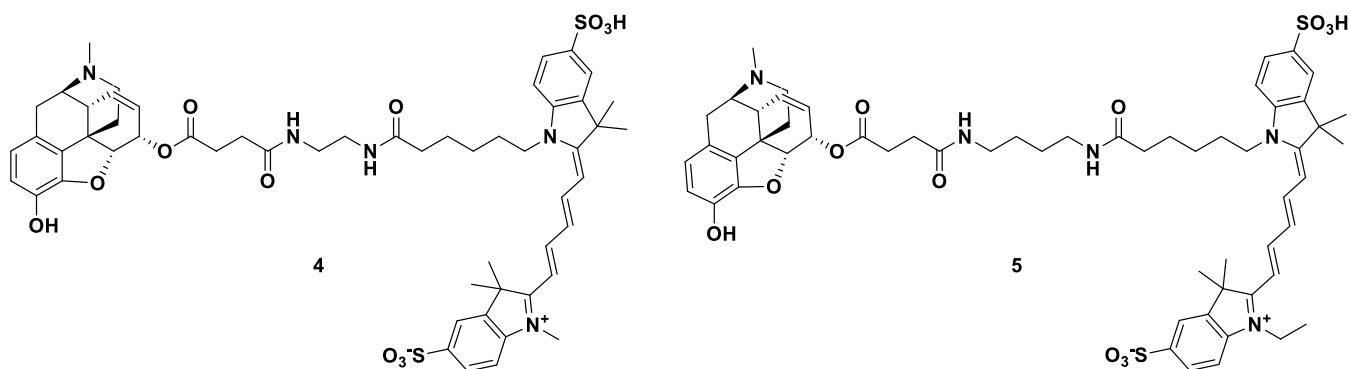


FIGURE 3. EXAMPLES OF FLUORESCENT MORPHINE PUBLISHED IN THE LITERATURE.⁷

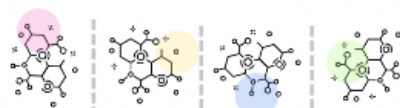
Development of a novel fluorescent morphine

In Celtarys, we offer a customised development of fluorescent ligands, tailored to the specific biological application of interest. These projects are generally divided in 3 main phases (figure 4).

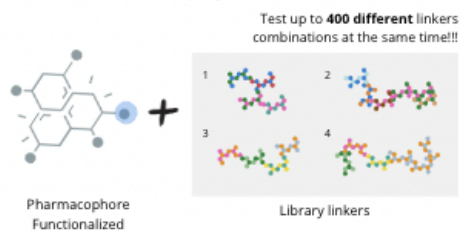
PHASE 1

We start with the identification of the appropriate pharmacophore(s) for each target, using an integrated approach that includes both structure-based and ligand-based drug design approaches for compound functionalization. Thanks to in-silico experimental structure-activity study we identify the optimal position for pharmacophore functionalization and linker conjugation. The optimal pharmacophore(s) are typically identified among a set of 3-5 different chemical scaffolds.

Pharmacophore identification



Linkers library generation



PHASE 2

Once identified the functionalized pharmacophore with the best physicochemical and pharmacological profile, we prepare a library of intermediates (pharmacophore + linker) using our own LEGO-like chemical conjugation technology. The biological evaluation of these compounds allows us to identify the optimal linker(s) for the target of interest and for the desired application.

PHASE 3

In the last phase, we combine the previous identified precursors with fluorophores suitable for the kind of assay of interest. The activity of the final molecules, measured in a binding or functional assay, allows us to select the best one(s).

Fluorescent ligand synthesis

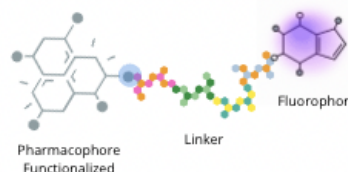


FIGURE 4. CELTARYS WORKFLOW FOR CUSTOM DEVELOPMENT PROJECTS

Phase 1 and 2

In silico studies were not needed in this case, since the information found in bibliography was enough to develop the fluorescent morphine. For phase one we explored 2 different morphine functionalization (P1 and P2), which were used for the following linker conjugation in phase 2. We studied which type of linker was best suited to maintain the relative efficacy of the compounds. The hMOR activity of the corresponding compounds was measured in cAMP functional assays (table 1). As noted in the table below, this objective was accomplished with several linkers: 5 conjugates showed an EC₅₀ lower than 100 nM, keeping the full agonistic behaviour.

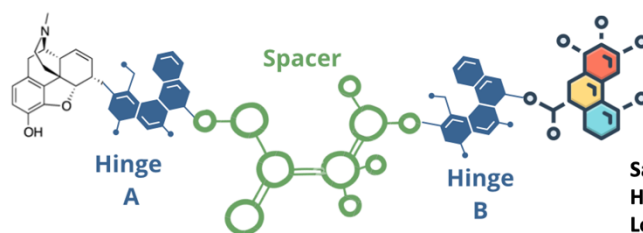
Same potency RP=1
Higher than morphine RP>1
Lower than morphine RP<1

CODE	STRUCTURE	EC50 (AMPc)	pEC50	Relative potency (EC ₅₀ M/EC ₅₀ C)	% E _{max}	Relative efficacy (E _{max} C/E _{max} M)
Morphine	Unlabelled pharmacophore	8.50 nM	8.07	-	101.8 ± 2.74	-
NOS20	P1-L1	4 nM	8.4	2.12	98.4 ± 1.7	0.96
NOS25B	P1-L2	250.2 nM	6.6	0.03	104.6 ± 4.6	1.03
NOS28	P1-L3	8.2 nM	8.09	1.04	99.9 ± 3.5	0.98
NOS29B	P1-L4	69.1 nM	7.16	0.12	97.8 ± 2.2	0.96
NOS30	P2-L1	42.5 nM	7.37	0.2	99.5 ± 1.4	0.98
NOS31	P2-L2	42.8 nM	7.37	0.2	101.8 ± 2.6	1
DAMGO	Ref (3.23 nM)	1.81 nM	8.74	-	100%	-

TABLE 1. MAIN RESULTS OF PHASE 1 AND 2 OF THE PROJECT: FUNCTIONALISED MORPHINE AND CONJUGATES COMPARED WITH PARENT LIGAND (MORPHINE).

Phase 3

In phase 3, the most promising intermediates, selected based on their activity and synthetic accessibility, have been labelled with 2 different fluorescent scaffolds, which are all in the spectrum range requested. The hMOR activity of the corresponding compounds was measured in cAMP functional assays (table 2). The final compounds maintain the **relative efficacy, improve the affinity for hMOR, are selective and fit the excitation/emission criteria.**



Same potency RP=1
Higher than morphine RP>1
Lower than morphine RP<1

CODE	STRUCTURE	λ _{exc/em}	EC50 (AMPc)	pEC50	Relative potency (EC ₅₀ M/EC ₅₀ C)	% E _{max}	Relative efficacy (E _{max} C/E _{max} M)
Morphine	Unlabelled pharmacophore		8.50 nM	8.07	-	101.8 ± 2.74	-
NOS40	Labelled NOS28	560/571	46.4 nM	7.33	0.18	98.02 ± 0.94	0.93
NOS50	Labelled NOS20	565/574	1.13 nM	8.95	7.52	95.08 ± 1.34	0.94
NOS56	Labelled NOS29B	565/574	1.35 nM	8.87	6.3	94.23 ± 0.76	0.93

TABLE 2. MAIN RESULTS OF PHASE 3 OF THE PROJECT: FLUORESCENT MORPHINE DERIVATIVES COMPARED WITH PARENT LIGAND (MORPHINE).

Biological fluorescent assays

After their development and hMOR functional activity assessment, these fluorescent morphine derivatives were shipped to Prof. M. Galko's laboratory, where they were tested in biological assays against both hMOR and the identified fly receptor. Among the different experiments, NOS40 was tested in fluorescence microscopy for hMOR and fly receptor labelling (figure 5). As observed, this fluorescent morphine derivative binds to hMOR, but does not bind to the fly receptor.

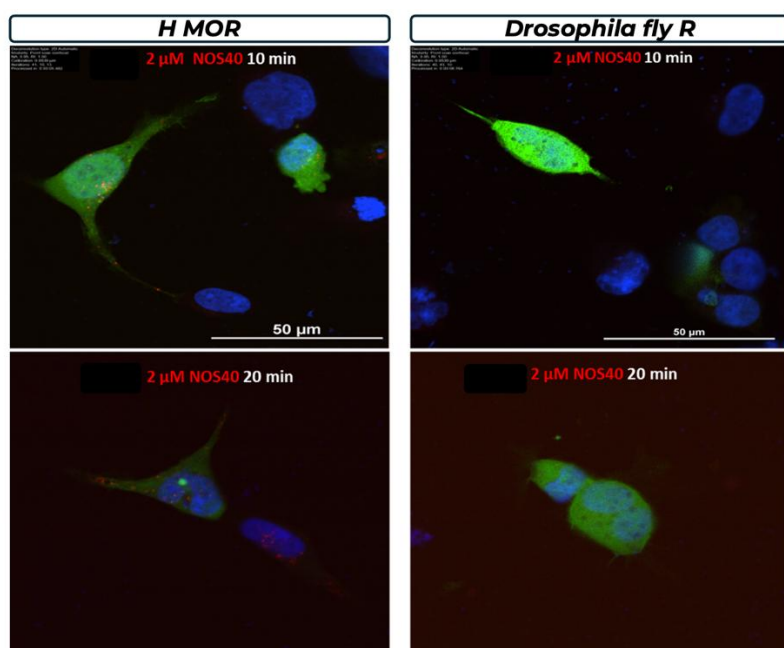


FIGURE 5. FLUORESCENCE MICROSCOPY OF HMOR (LEFT) AND FLY RECEPTOR (RIGHT) EXPRESSING CELLS: NOS40 BINDS HMOR BUT NOT THE IDENTIFIED FLY RECEPTOR.

Further experiments are ongoing, and a new Celtarys customised project started for the development of a fluorescent fentanyl to check if changing the scaffold, we could be able to see binding to the fly receptor of interest.

Conclusions

A set of new fluorescent morphine derivatives was successfully designed and developed, showing high activity and full agonistic properties for the hMOR. These probes have been validated in confocal microscopy, where the hMOR binding was confirmed, while unfortunately no interaction was observed for the fly receptor identified, leading to the need of further investigation.

Methods

cAMP functional assays.

Mu Opioid receptor functional experiments were carried out in CHO-MuOR cell line. The day before the assay, 10.000 cells were seeded in 100µl of medium containing dialyzed FBS (Sigma F0392) on a 96 well half area white plate (Corning 3688). The day assay, medium was replaced by Stim B + 500µM IBMX (Sigma 17018). Test compound was added in their corresponding wells and incubated for 15 minutes at 37°C. Next, 10µM Forskolin (Sigma F6886) was added, and the plate was incubated for 5 minutes at 37°C. Reagents from the kit (#CISBIO 62AM4PEC) were added and after incubation for 1 hour at RT with gentle stirring (90 rpm) and protected from light, HTRF (λEx: 320nm; λEm: 620- 665nm) from each well was measured using a Tecan Infinite M1000 Pro.

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