9 - 11 December 2024

6th General Assembly Meeting

QSPainRelief

Bologna, Italy

Exploring the multidimensional complexity of opioid receptors to develop improved therapeutics: From innovative peptides to Quantitative Systems Pharmacology platforms

Andrea Bedini, PhD,

Associate Professor of Pharmacology

UNIBO Team Leader within QSPainRelief

Coordinator of Molecular and Cellular Pharmacology Unit

Degree Programme Director of MSc in Pharmaceutical Biotechnology

Department of Pharmacy and Biotechnology, University of Bologna – Bologna (Italy)

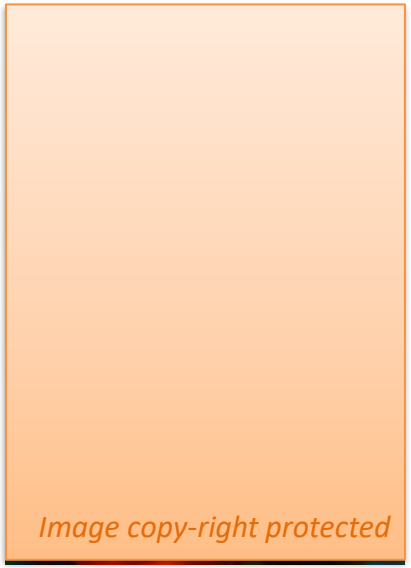
andrea.bedini@unibo.it

ALMA MATER STUDIORUM UNIVERSITÀ DI BOLOGNA | DEPARTMENT OF PHARMACY AND BIOTECHNOLOGY

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

PRESENTATION OUTLINE

- GPCRs and the **evolving view** of their pharmacology: **experimental strategies** to investigate new GPCR ligands with potentially **improved pharmacological profile**.
- Background information on **opioid receptors** and **ligands**.
- Promises and pitfalls in the quest for more effective and safer analgesics: **biased agonists** vs **low intrinsic efficacy** agonists.
- **Kappa opioid receptor:** an intriguing pharmacological target for improved therapeutics to treat **pain** and **psychiatric disorders**.
- **Quantitative Systems Pharmacology** as an innovative avenue for more effective and safer therapeutics: **molecular pathway analysis** to implement **predictive QSP platforms.**

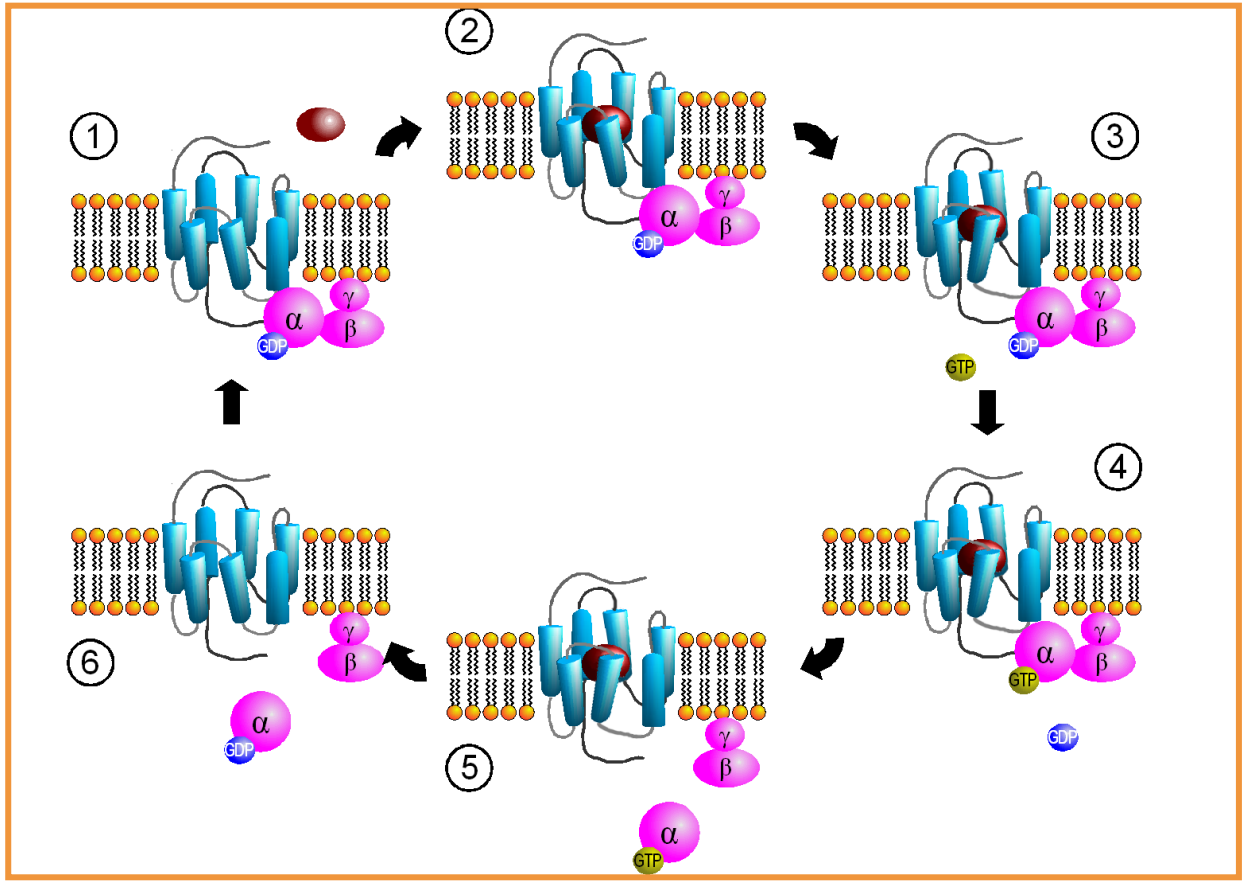


This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

GPCRs AND DRUG DEVELOPMENT

Classic view of GPCR pharmacology



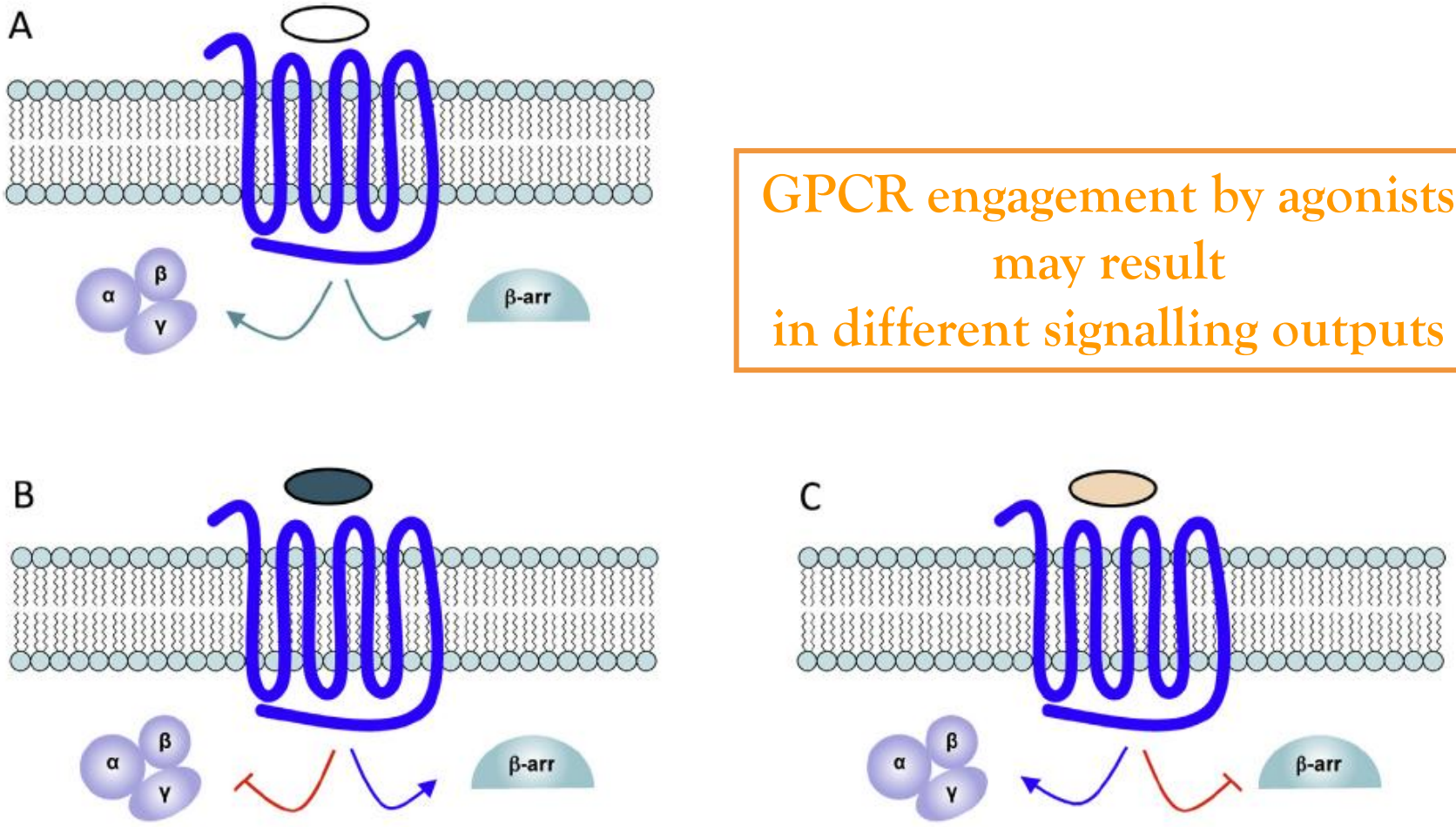
- For decades believed to couple only to G proteins in order to elicit cellular responses *[Pupo et al., 2016].*

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

GPCRs AND DRUG DEVELOPMENT

Present view of GPCR pharmacology



GPCR engagement by agonists
may result
in different signalling outputs

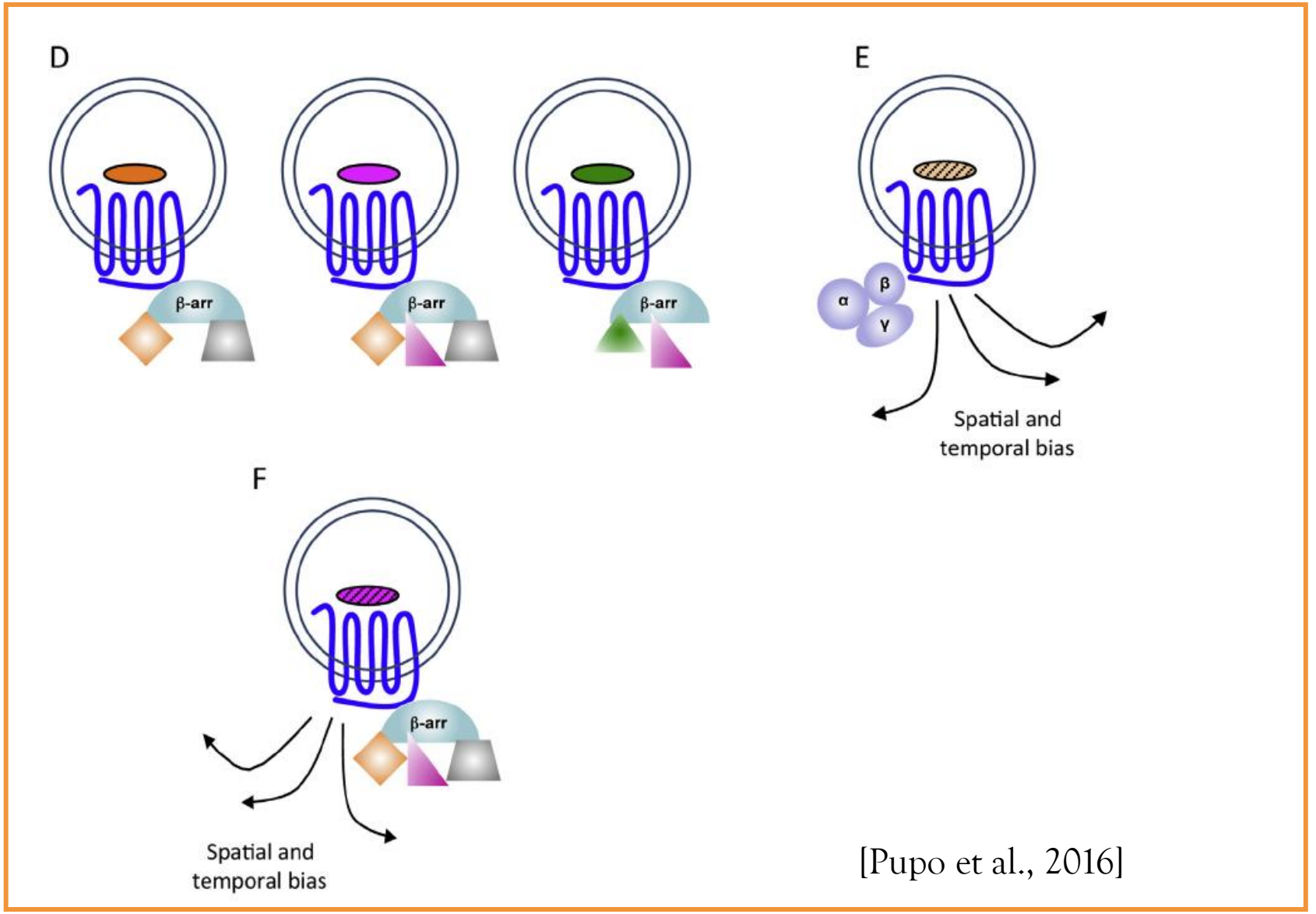
[Pupo et al., 2016]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

GPCRs AND DRUG DEVELOPMENT

Present view of GPCR pharmacology



[Pupo et al., 2016]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

GPCRs AND DRUG DEVELOPMENT

Intrinsic efficacy was the linchpin of GPCR pharmacology

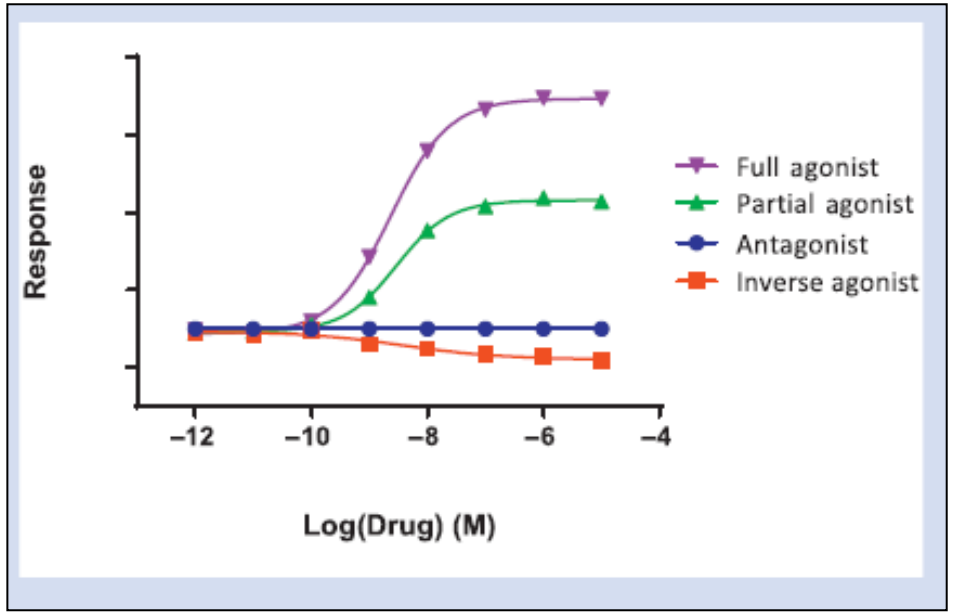
AFFINITY
&
EFFICACY

FULL AGONIST

PARTIAL AGONIST

ANTAGONIST

INVERSE AGONIST



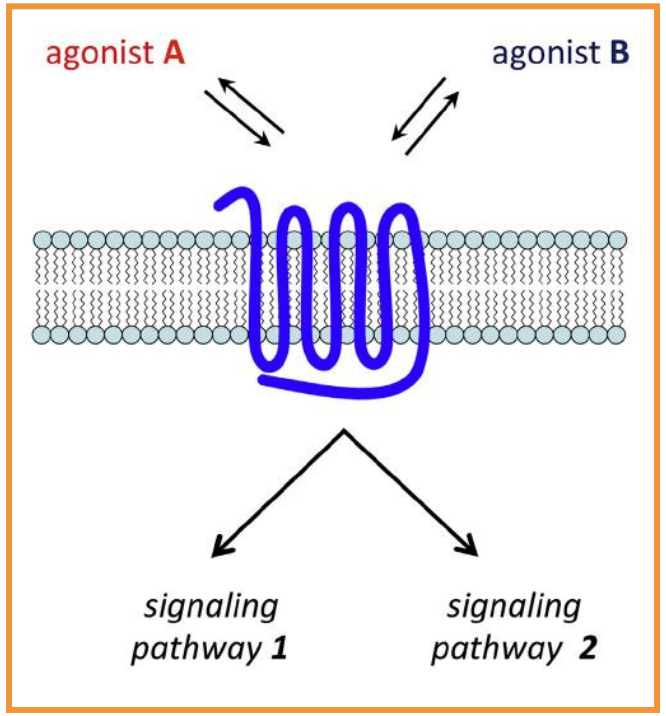
Activity of a ligand at a GPCR described in terms of
INTRINSIC EFFICACY
(e.g.: full agonists activate all the signalling pathways linked to a receptor to the same degree as the endogenous ligand for that receptor)

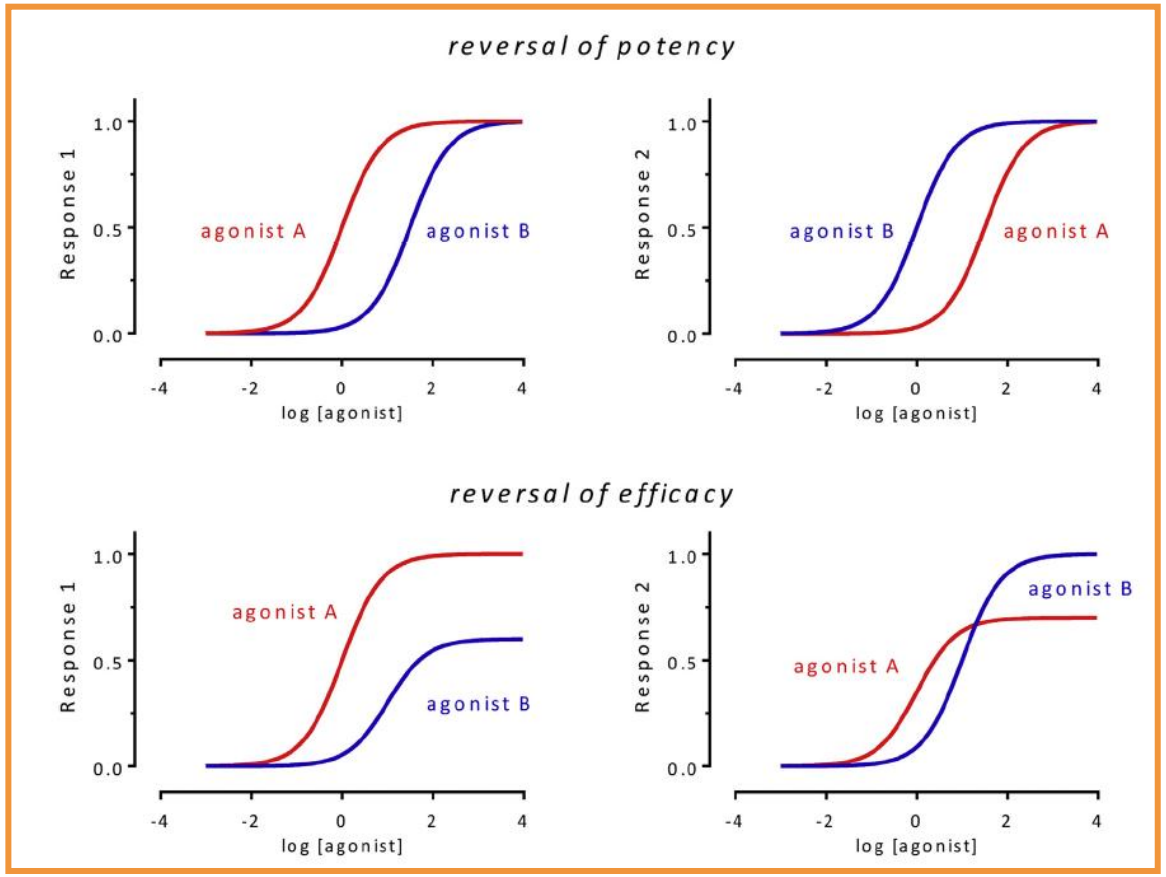
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

GPCRs AND DRUG DEVELOPMENT

From intrinsic efficacy to functional selectivity





Different agonists at a same GPCR do not activate all the responses to the same extent

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

GPCRs AND DRUG DEVELOPMENT

The dawn of functional selectivity

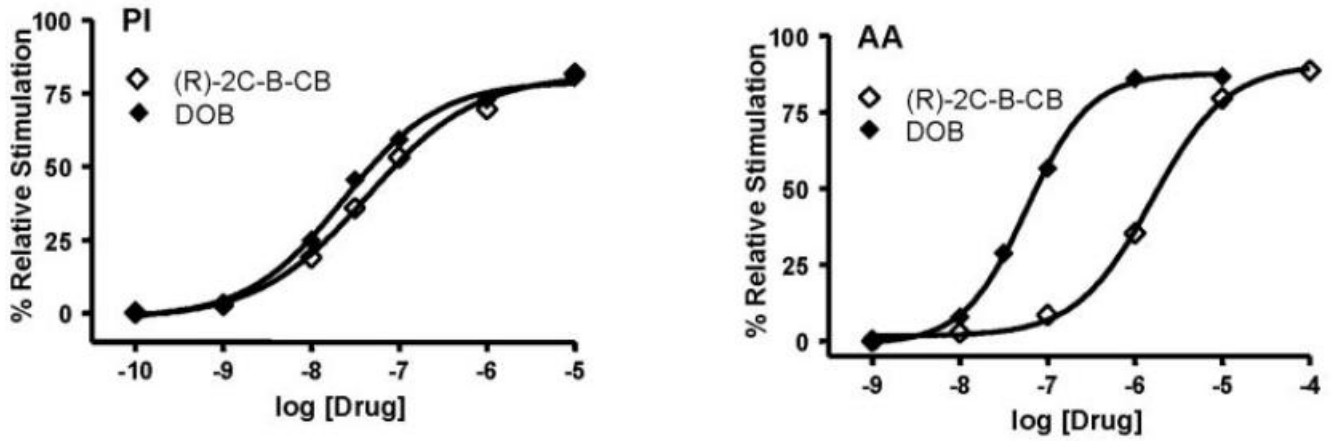
0022-3565/07/3201-1–13$20.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Copyright © 2007 by The American Society for Pharmacology and Experimental Therapeutics
JPET 320:1–13, 2007

Vol. 320, No. 1
104463/3137516
Printed in U.S.A.

Perspectives in Pharmacology

Functional Selectivity and Classical Concepts of Quantitative Pharmacology

Jonathan D. Urban, William P. Clarke, Mark von Zastrow, David E. Nichols, Brian Kobilka, Harel Weinstein, Jonathan A. Javitch, Bryan L. Roth, Arthur Christopoulos Patrick M. Sexton, Keith J. Miller, Michael Spedding, and Richard B. Mailman



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

GPCRs AND DRUG DEVELOPMENT

The dawn of functional selectivity

0022-3565/07/3201-1–13$20.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Copyright © 2007 by The American Society for Pharmacology and Experimental Therapeutics
JPET 320:1–13, 2007

Vol. 320, No. 1
104463/3137516
Printed in U.S.A.

ntal Therapeutics

Perspectives in Pharmacology

Functional Selectivity and Classical Concepts of Quantitative Pharmacology

Jonathan D. Urban, William P. Clarke, Mark von Zastrow, David E. Nichols, Brian Kobilka, Harel Weinstein, Jonathan A. Javitch, Bryan L. Roth, Arthur Christopoulos Patrick M. Sexton, Keith J. Miller, Michael Spedding, and Richard B. Mailman

NEED TO EXPAND RESEARCH TO ELUCIDATE

- how functionally selective ligands determine differential signalling
- which effects are elicited at the level of target tissues and organisms
- what are the physiological and pharmacological consequences of functional selectivity

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

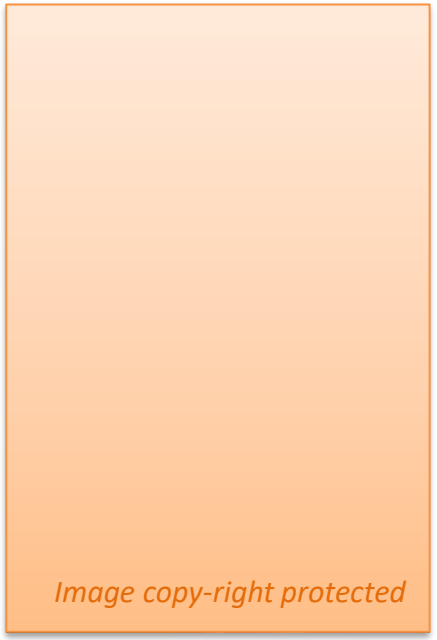
ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

FUNCTIONAL SELECTIVITY AND DRUG DEVELOPMENT

How to investigate functional selectivity in vitro

- Employ different cell models (with both heterologous and endogenous expression of the desired GPCR)
- Always include the appropriate reference balanced agonist
- Employ multiple assays to determine the activation of G protein-dependent (GTPγS assay, cAMP assay) vs arrestin-mediated (BRET, enzyme complementation assay) intracellular signalling
- Possibly investigate more highly complex patterns of functional selectivity and not only the simple dichotomy between G protein- or arrestin-dependent signalling

Quantification of agonist bias
to guide structure-activity studies,
compare different ligands and select drug candidates



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

FUNCTIONAL SELECTIVITY AND DRUG DEVELOPMENT

Beyond G protein-dependent vs arrestin-mediated signalling

G-protein-dependent vs Arrestin-mediated signalling is considered the first branching point within functional selectivity

More highly complex patterns of FUNCTIONAL SELECTIVITY

- **DIFFERENTIAL ACTIVATION OF SIGNAL TRANSDUCERS (e.g.: MAPKs)**
- **DIFFERENTIAL MODULATION OF GENE EXPRESSION**
- **LIGAND-DEPENDENT MODULATION OF CELLULAR RESPONSES (e.g.: cell proliferation)**

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

FUNCTIONAL SELECTIVITY AND DRUG DEVELOPMENT

The challenge of translating functional selectivity to in vivo settings

- Ligand bias has been studied *in vitro* mainly in heterologous expression systems.
- Many of the limitations for evaluating downstream behavioural effects are that the responses may be due to agonist acting non-selectively *[Zhou & Bohn, 2014].*

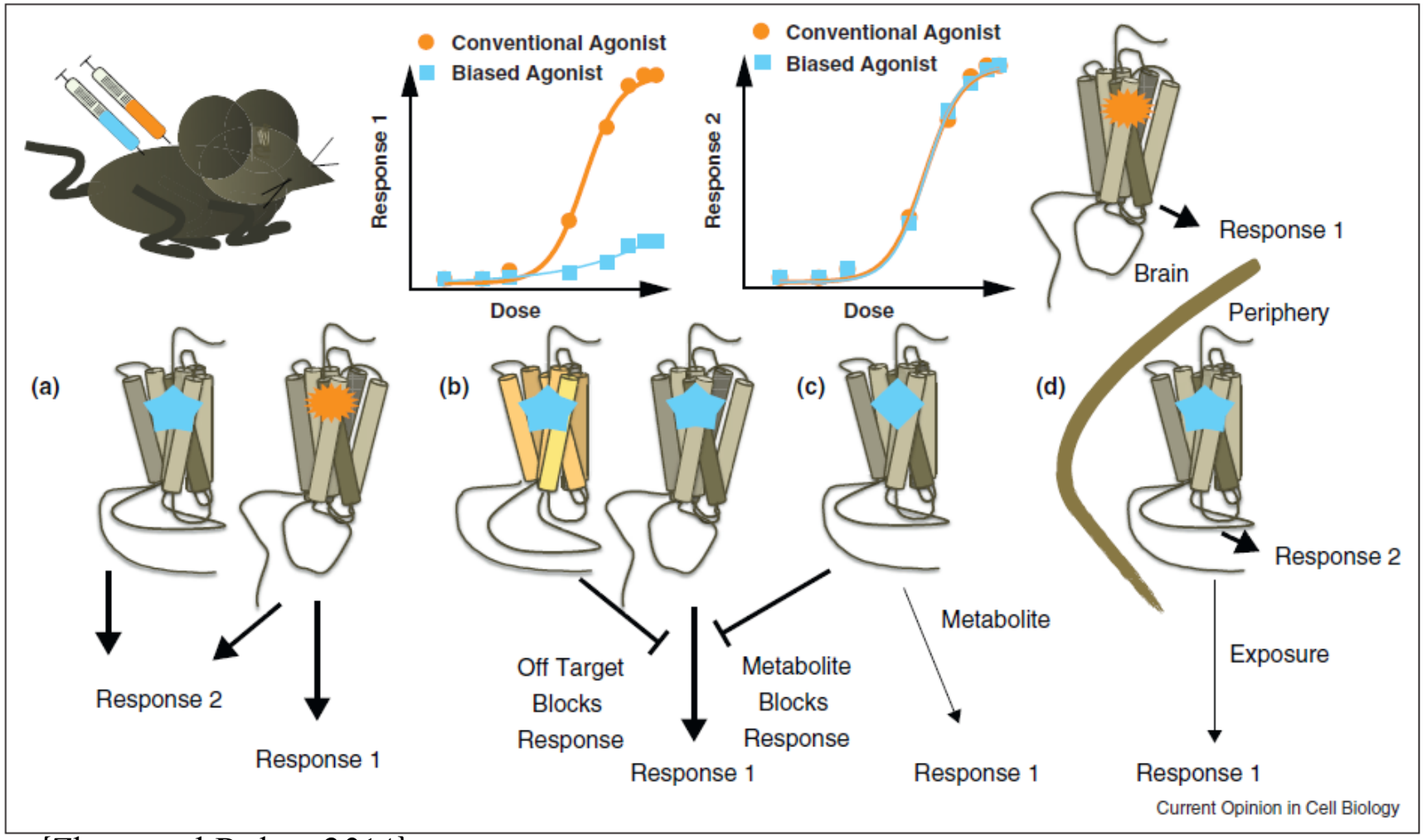
IT IS CRUCIAL TO EMPLOY PRIMARY CULTURES, TISSUE PREPARATION, PRECLINICAL MODELS

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

FUNCTIONAL SELECTIVITY AND DRUG DEVELOPMENT

The complex scenario of functional selectivity in vivo



[Zhou and Bohn, 2014]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

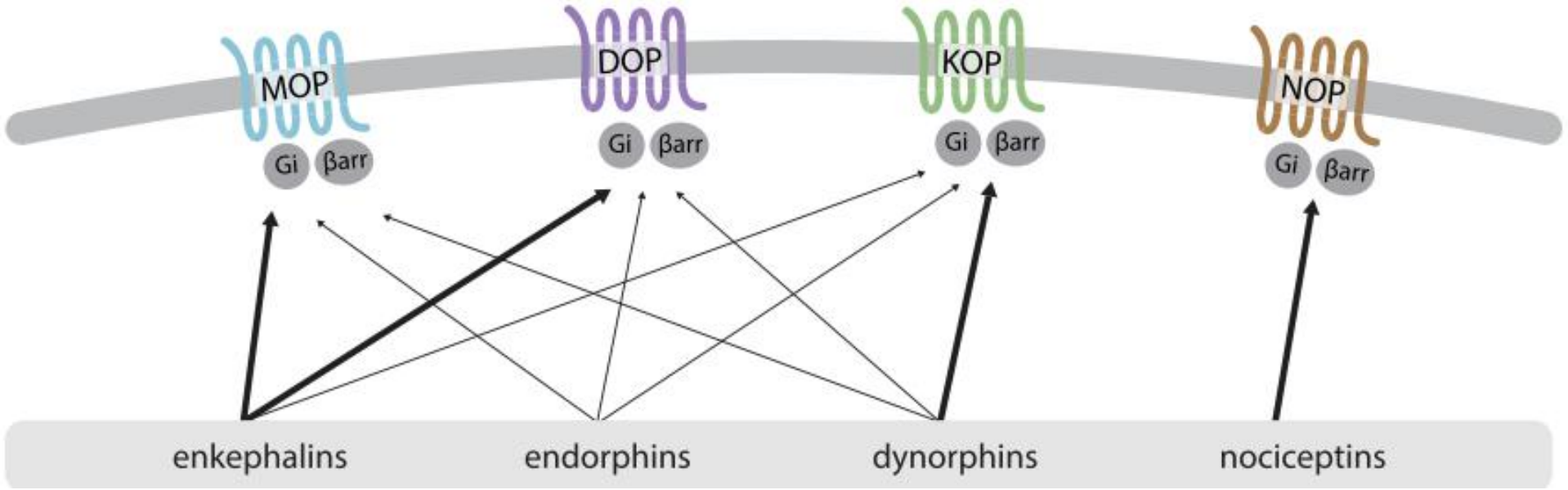
ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

ENDOGENOUS OPIOID SYSTEM

Main receptors and mediators

- Opioid Receptors
  - Mu opioid receptor (MOR or MOP)
  - Delta opioid receptor (DOR or DOP)
  - Kappa opioid receptor (KOR or KOP)
  - Nociceptin/Orphanin FQ Receptor (NOP)

- Endogenous Opioid Peptides
  - Endorphins (END)
  - Enkephalins (ENK)
  - Dynorphin (DYN)
  - Nociceptin

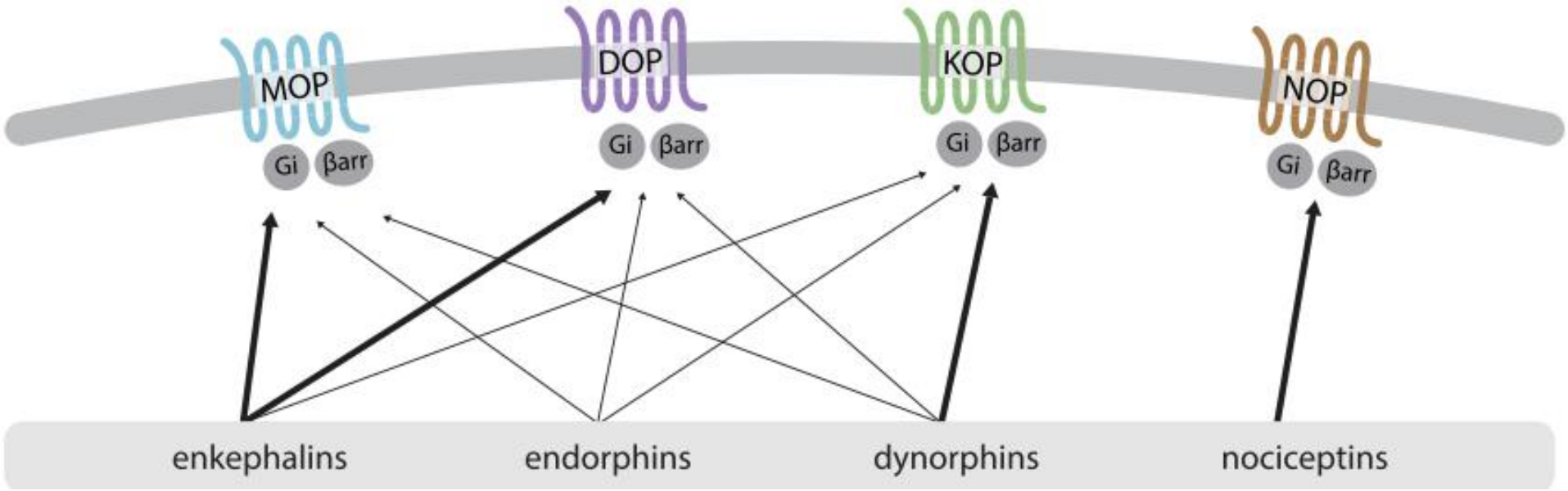


[Palmer et al., 2021]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ENDOGENOUS OPIOID SYSTEM

Main receptors and mediators



- Class A G protein-coupled receptors (GPCRs)
- Involved in a variety of physiological and pathophysiological events, including but not limited to pain modulation, immune function and emotional response
- Typically coupled to Ga_i

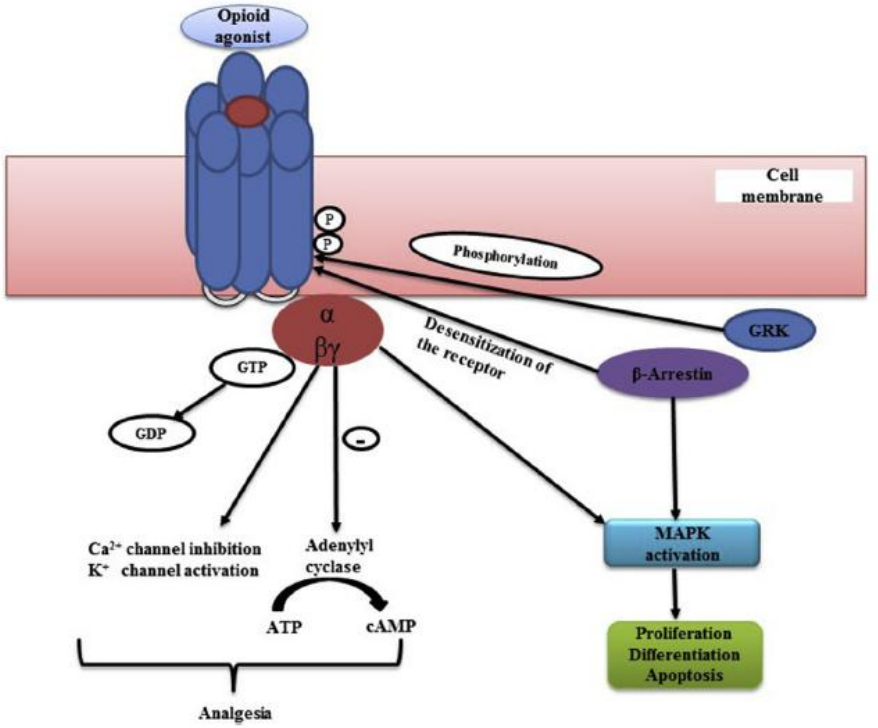
[Palmer et al., 2021]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

ENDOGENOUS OPIOID SYSTEM

Main receptors and mediators



- $G\alpha_{i/o}$-dependent inhibition of adenylyl cyclase
- $G\beta\gamma$-dependent activation of post-synaptic GIRK and inhibition of pre-synaptic VGCC
- MAPKs activation (i.e.: ERK1/2, p38MAPK, JNK)
- GRK activation, arrestin recruitment leading to internalization, desensitization, arrestin-dependent signalling

[Palmer et al., 2021]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

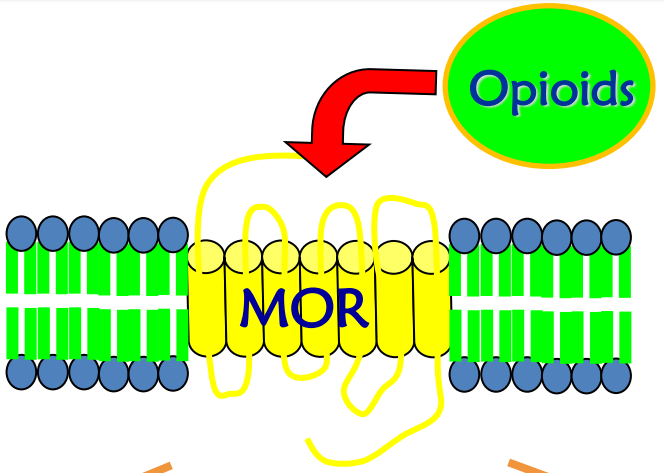
HUMAN μ-OPIOID RECEPTOR (MOR)

Main target of currently available opioid analgesics

ENDOGENOUS

Endorphins

Endomorphins



EXOGENOUS

Morphine

Fentanyl

Oxycodone

Inhibition of adenylyl cyclase

↓ cAMP

↓ PKA

CREB

↓ CRE-mediated gene transcription

Modulation of ionic channels:

↓ pre-synaptic Ca^{2+} influx

↓ pro-nociceptive neurotransmitters release

↑ post-synaptic K^{+} influx

PLC activation

↑ PKA

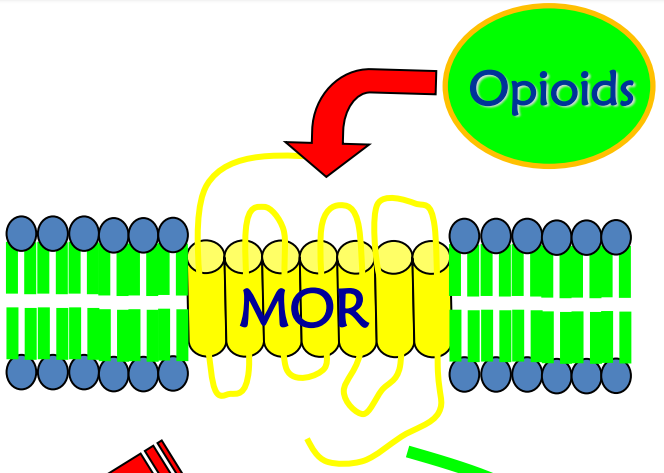
↑ P-MAPK

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

HUMAN μ-OPIOID RECEPTOR (MOR)

Main target of currently available opioid analgesics



Side effects

Respiratory depression
IMMUNOMODULATION
TOLERANCE
ADDICTION

ANALGESIA

Blockade of pre-synaptic Ca^{2+} channels:
Inhibition of neurotransmitters release

Activation of post-synaptic K^{+} channels:
hyperpolarization => blockade of neurotransmission

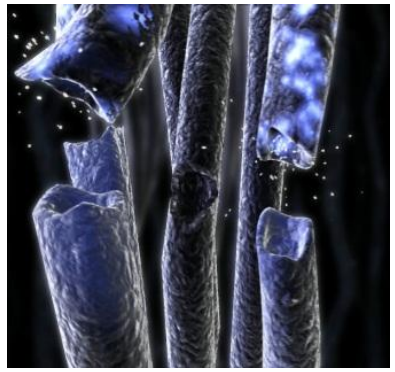
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

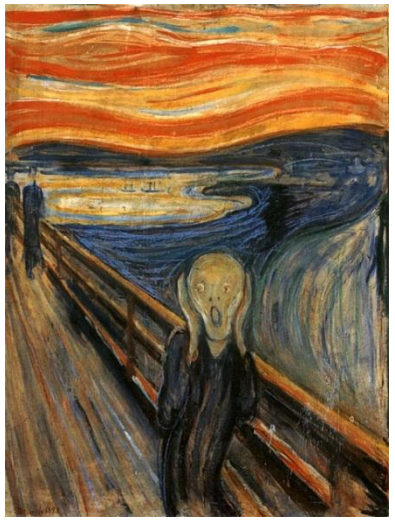
ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

PATHOLOGICAL PAIN

Clinical relevance, treatment options and unmet needs

- **Opioid analgesics** (e.g.: morphine, fentanyl, oxycodone) are still the **mainstay in the treatment** of moderate to severe pain, both acute and chronic; however, their clinical use may be limited due to their **relevant side effects** and their **abuse liability**.
- **60% of patients treated for chronic pain responds poorly** [Van Hecke et al, 2013] and **opioids may be even detrimental** in some chronic pain states
- Opioid over-prescription and misuse led to an exponential increase in **addicted people** and **deaths** due to opioid use disorders and opioid abuse (i.e.: «**opioid crisis**» or «**epidemics**»)





This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

PATHOLOGICAL PAIN

Clinical relevance, treatment options and unmet needs

- **Opioid analgesics** (e.g.: morphine, fentanyl, oxycodone) are still the **mainstay in the treatment** of moderate to severe pain, both acute and chronic; however, their clinical use may be limited due to their **relevant side effects** and their **abuse liability**.
- **60% of patients treated for chronic pain responds poorly** [Van Hecke et al, 2013] and **opioids may be even detrimental** in some chronic pain states
- Opioid over-prescription and misuse led to an exponential increase in **addicted people** and **deaths** due to opioid use disorders and opioid abuse (i.e.: «**opioid crisis**» or «**epidemics**»)

URGENT NEED FOR MORE EFFECTIVE AND SAFER ANALGESICS

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

IDENTIFYING MORE EFFECTIVE AND SAFER ANALESICS

The quest for the Holy Grail or an achievable goal?

- Molecules provided with higher affinity, selectivity, potency
- Molecules provided with "mixed pharmacology" (e.g.: MOR agonist/DOR antagonist, MOR agonist/NOP agonist, etc.)
- **Biased agonists**
- **Low intrinsic efficacy agonists**
- **Combination treatments (e.g.: analgesic + "augmentation" drug aimed at potentiating effects and/or attenuating toxicity)**

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

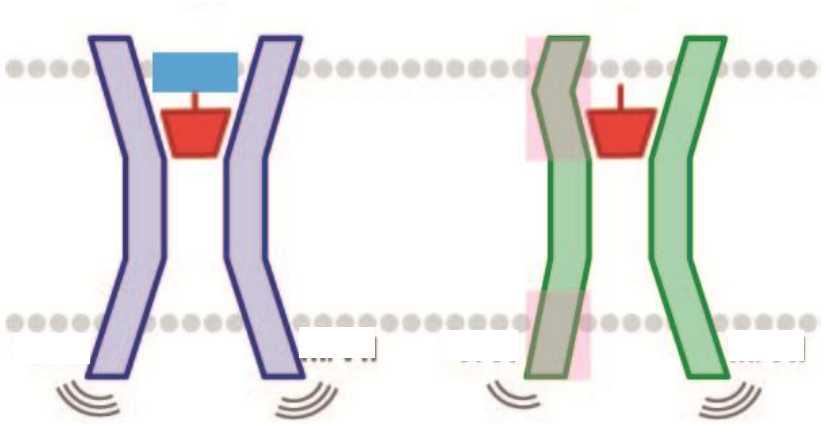
ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

BIASED AGONISM AT GPCRs

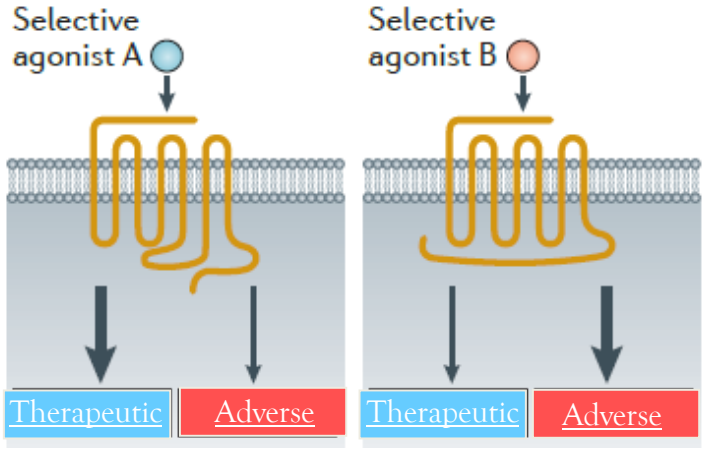
Definition, overview and pharmacological implications

Ability of a ligand at a G-protein coupled receptor to selectively activate particular cell signalling pathways over others

- GPCRs are dynamic entities that exist in multiple conformations: diverse ligands stabilize different active states and elicit distinct conformational changes within the receptor, thus resulting in a selective modulation of intracellular signalling *[Kenakin and Christopoulos., 2013]*
- Ligand-directed signalling represents an intriguing opportunity to design tailor-made therapeutics



Wracker et al., Science 2013



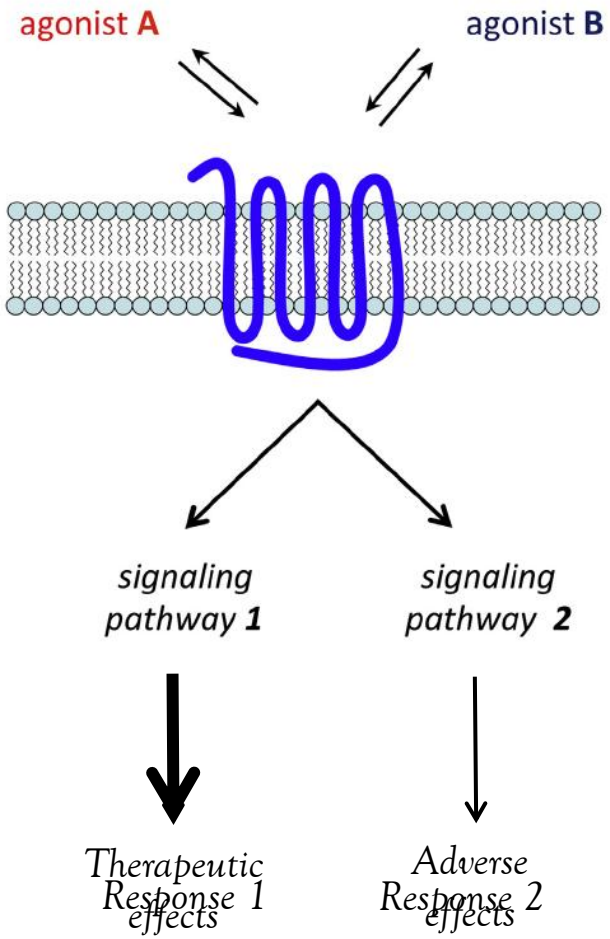
Kenakin and Christopoulos, Nature reviews 2013

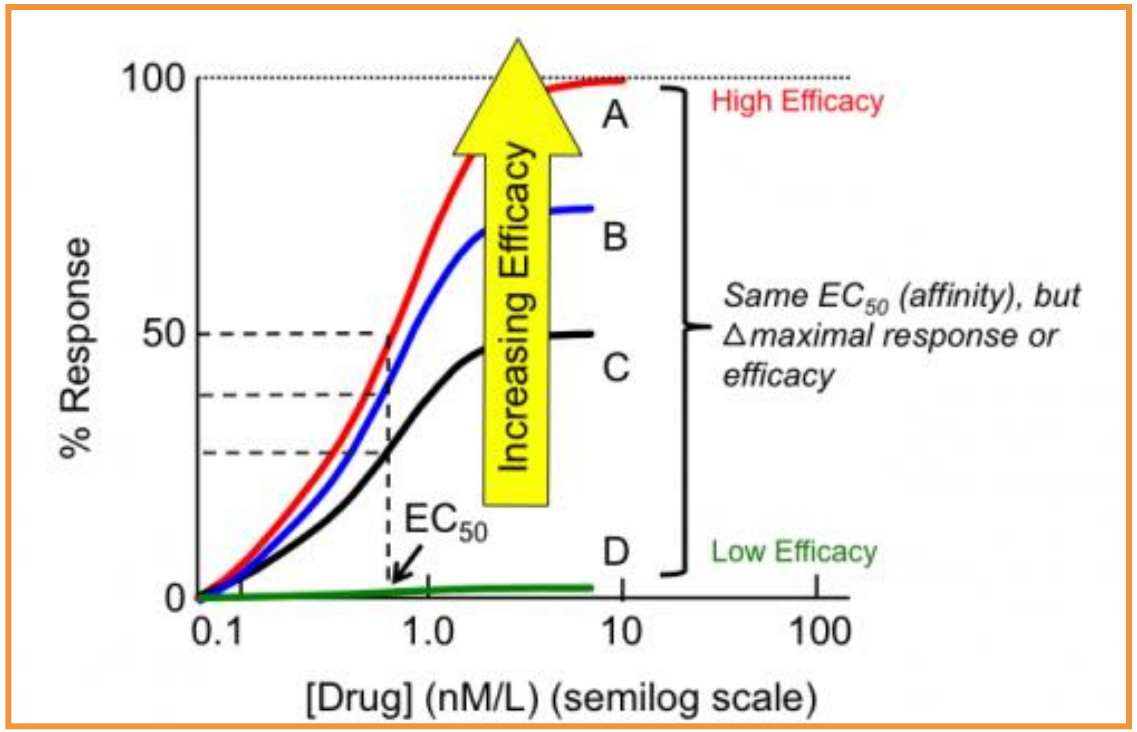
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

POTENCY, EFFICACY AND GPCR MODULATION

Overview and potential pharmacological implications



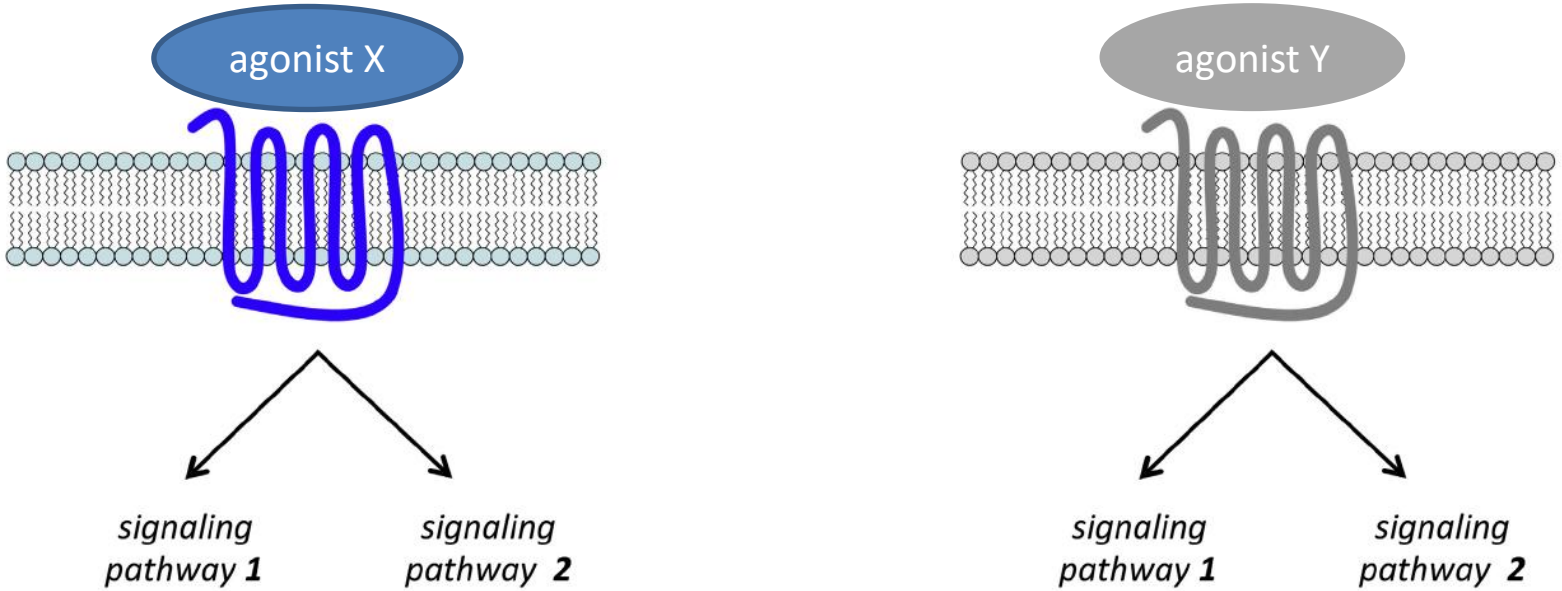


This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

COMBINATION TREATMENTS

A strategy to potentiate effects and/or mitigate toxicity



Analgesia

Sedation
Cognitive impairment
Abuse liability

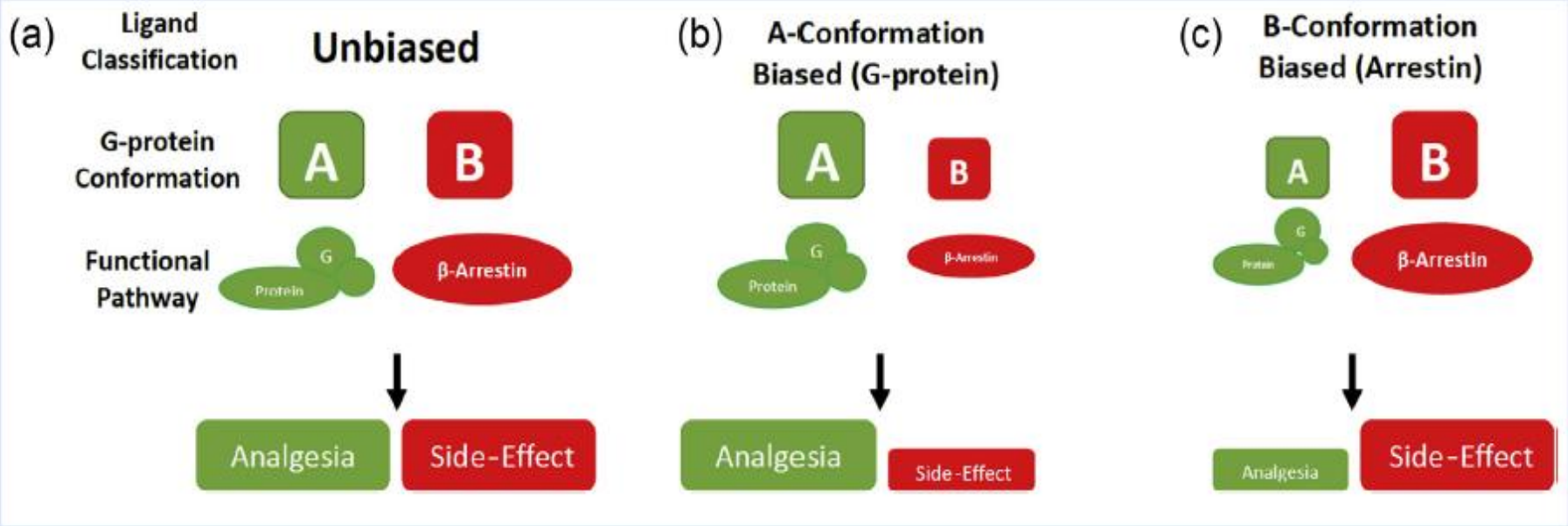
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

BIASED AGONISM AT MOR

A promising avenue for more effective and safer analgesics

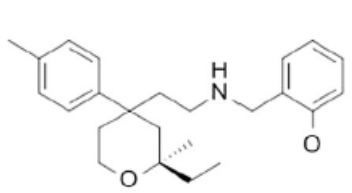
- Hypothesis-driven => Morphine administered to arrestin 3 KO mice produced enhanced analgesia with reduced tolerance and fewer adverse events as compared to *wild-type* mice *[Bohn et al., 2000; Raehal et al., 2005].*
- Similar improved morphine potency with reduced side effects in mice and rats treated with siRNAs targeting arrestin 3 expression *[Li et al., 2009; Yang et al., 2011].*
- Numerous MOR agonists have been developed over the years in a global effort to improve opioid safety and tolerability, but all these structurally diverse opioids display morphine-like side effects (tolerance, nausea, vomiting, sedation, constipation, respiratory depression).



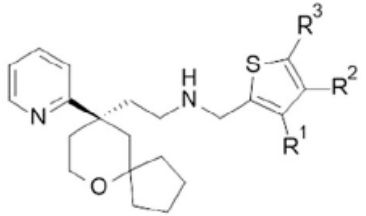
BIASED AGONISM AT MOR

Promises and pitfalls: the paradigmatic story of TRV130

Table 1
G protein-biased MOR agonists[90]

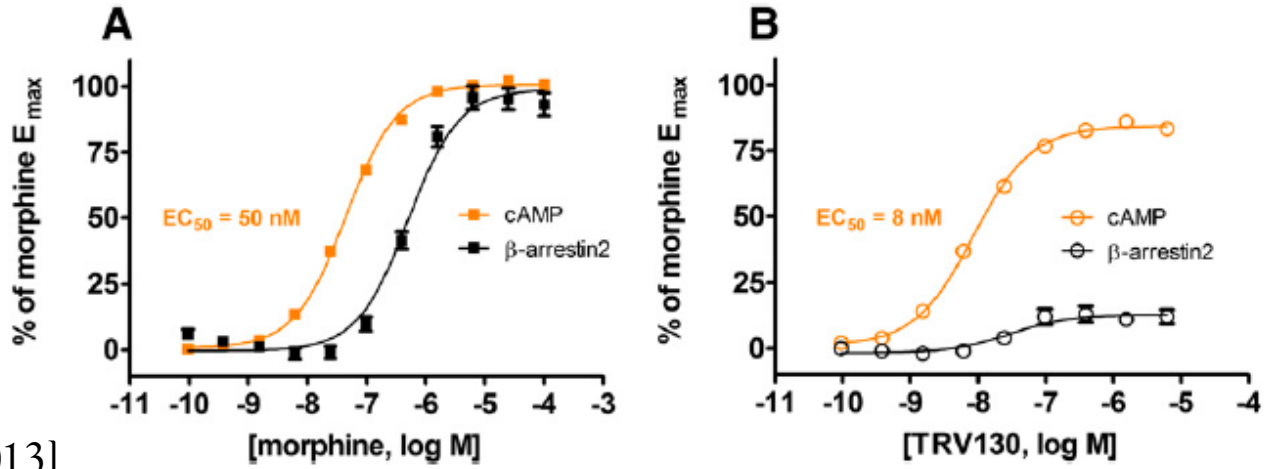


1



2 - 4

Compound	R^1, R^2, R^3	MOR cAMP pEC_{50}	MOR cAMP E_{max} (%)	MOR βarr2 pEC_{50}	MOR βarr2 E_{max} (%)
Morphine	NA	7.4	100	6.3	100
1	NA	6.3	74	5.7	32
2	H, H, H	7.8	95	6.6	15
3	H, Me, Me	8.3	104	6.3	197
4 (TRV-130)	OMe, H, H	8.1	84	7.3	15



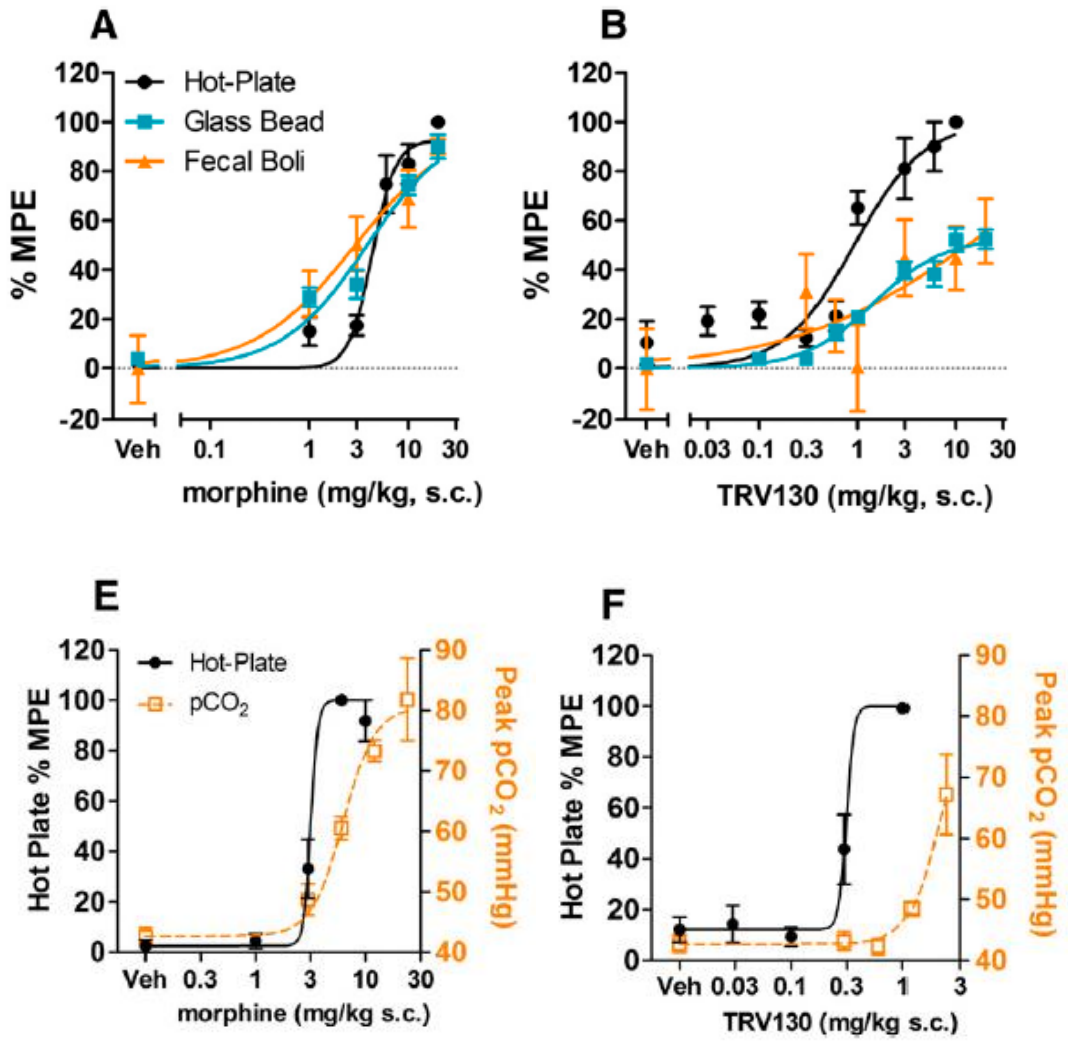
[De Wire et al., 2013]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

BIASED AGONISM AT MOR

Promises and pitfalls: the paradigmatic story of TRV130



[De Wire et al., 2013]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

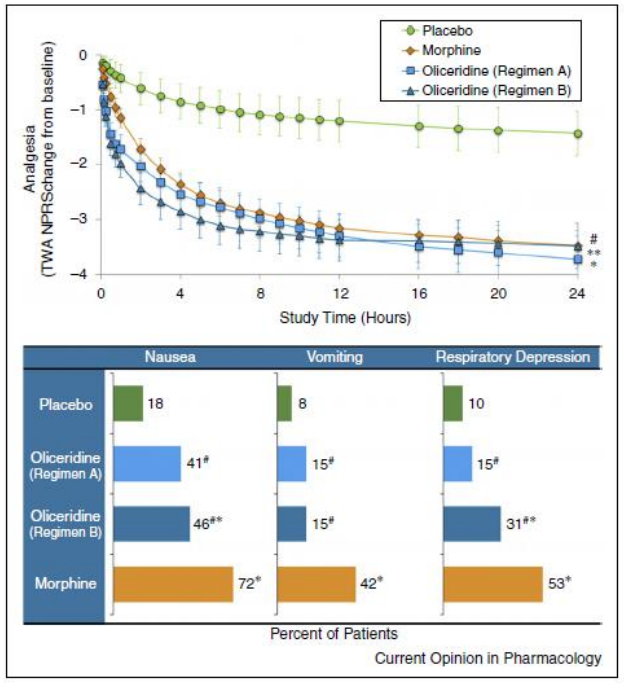
ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

BIASED AGONISM AT MOR

Promises and pitfalls: the paradigmatic story of TRV130

- In healthy volunteers TRV130 produced analgesia with less reduction in respiratory drive and less severe nausea as compared to morphine *[Soergel et al., 2014]*.
- In a phase 2, randomized, placebo- and active-controlled study in acute pain following bunionectomy: i.v. administration of TRV130 determined greater categorical pain relief as compared to morphine; however, no improvement in respiratory effects as compared to morphine *[Viscusi et al., 2015]*.

Figure 2



Top-line results of a randomized, double-blind, placebo-controlled and active-controlled study of oliceridine in patients following abdominoplasty (ClinicalTrials.gov Identifier: NCT02335294). Patients experiencing pain of at least 5 on a numeric pain rating scale (NPRS) of 0–10 following surgery were randomized to receive oliceridine, morphine, or placebo administered by patient-controlled analgesia. Dosing regimens were as follows: morphine: 1.5 mg loading dose followed by on-demand patient-administered doses of 1.0 mg no more frequently than every 6 min ($n = 83$); oliceridine A: 1.5 mg loading dose, 0.1 mg on-demand dose ($n = 39$); oliceridine regimen B: 1.5 mg loading dose, 0.35 mg on-demand dose ($n = 39$); placebo was volume matched ($n = 39$). **(a)** Analgesic effects of oliceridine, morphine, and placebo in patients following abdominoplasty surgery over 24 hours. Analgesia was measured as the time-weighted average (TWA) of NPRS change from baseline. Data are least squares mean ± standard error. $^{*}p = 0.0001$ versus placebo; $^{**}p = 0.0005$ versus placebo; $^{\#}p < 0.0001$ versus placebo. **(b)** Percentage of patients with key opioid related adverse events. Nausea and vomiting are spontaneously reported adverse events; respiratory depression was clinically apparent and persistently decreased respiratory rate, respiratory effort or oxygen saturation ($^{*}p < 0.05$ versus placebo; $^{\#}p < 0.05$ versus morphine).
Figures adapted from Singla N *et al.* American Society of Regional Anesthesia 2016 Regional Anesthesiology and Acute Pain Medicine Meeting Poster Presentation.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

BIASED AGONISM AT MOR

Promises and pitfalls: the paradigmatic story of TRV130

OLINVO™ (oliceridine injection)

The first μ receptor G protein Pathway Selective modulator (μGPS)

	Target	Indication	Lead Optimization	Preclinical Development	Phase 1	Phase 2	Phase 3
OLINVO™ (oliceridine injection)	Mu-receptor	Moderate to Severe Pain	intravenous				

Trevena®

Trevena Announces FDA Acceptance for Review of New Drug Application for OLINVO™ (oliceridine) Injection

G+ Like 0 Share Tweet Condividi

January 02, 2018 16:01 ET | **Source:** Trevena Inc.

CHESTERBROOK, Pa., Jan. 02, 2018 (GLOBE NEWSWIRE) -- Trevena, Inc. (NASDAQ:TRVN) today announced that the U.S. Food and Drug Administration (FDA) has accepted the Company's New Drug Application (NDA) for OLINVO™ (oliceridine) Injection. The Company expects that the PDUFA date for the NDA will be in the fourth quarter of 2018. OLINVO is an investigational product for the management of moderate to severe acute pain. It is the first G protein biased ligand of the mu receptor designed to provide IV opioid pain relief with fewer associated adverse effects.

"The NDA file acceptance represents another major step in our progress towards delivering OLINVO to patients and healthcare providers in need of new options for managing moderate to severe acute pain in the hospital setting," said Maxine Gowen, Ph.D., chief executive officer. "We look forward to working with the FDA as they evaluate the OLINVO application."

Profile

Trevena Inc.

Subscribe via RSS

Subscribe via ATOM

Javascript

Chesterbrook, Pennsylvania, UNITED STATES

http://www.trevena.com/

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

BIASED AGONISM AT MOR

Promises and pitfalls: the paradigmatic story of TRV130

OLINVO™ (oliceridine injection)

The first μ receptor G protein Pathway Selective modulator (μGPS)

	Target	Indication	Lead Optimization	Preclinical Development	Phase 1	Phase 2	Phase 3
OLINVO™ (oliceridine injection)	Mu-receptor	Moderate to Severe Pain	intravenous				

IN OCTOBER 2018, US FDA VOTED 8 to 7 AGAINST OLICERIDINE APPROVAL *[Azzam et al., 2019]*; THUS, DAMPENING THE ENTHUSIASM AROUND BIASED AGONISTS TARGETING MOR

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

BIASED AGONISM AT MOR

Promises and pitfalls: the paradigmatic story of TRV130

- Oliceridine retained **undesirable constipating** and **abuse-related effects** in rodents following repeated treatment, despite its bias for G-protein signalling *[Altarifi et al., 2017]*.
- Oliceridine was shown to elicit **reinforcing** and **antinociceptive effects comparable to oxycodone** in rats; thus, pointing out that a biased-signalling profile at MOR does not necessarily reduce abuse potential *[Zamarripa et al, 2018]*.
- Two further phase 3 clinical studies were carried out to assess oliceridine efficacy for treating moderate to severe acute pain following bunionectomy and abdominoplasty (i.e., APOLLO-1 and APOLLO-2 clinical trials)[*Viscusi et al., 2019; Singla et al., 2019*].

IN 2020, US FDA GRANTED OLICERIDINE APPROVAL, ALTHOUGH AS NON-SUPERIOR TO CLASSIC OPIOIDS AS REGARDS ITS EFFICACY/SAFETY PROFILE

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

BIASED AGONISM AT MOR

The sunset of biased opioid agonists?

- **Respiratory depression** induced **through MOR** has been shown to be at least partially mediated by receptor coupling to **GIRK channels** through the activation of **Gβγ proteins** *[Montandon et al., 2016].*
- Neurons in several regions of the **brainstem respiratory network** are hyperpolarized by activation of classical, **arrestin-independent MOR signalling** pathway *[Levitt et al, 2015].*
- **Robust physiological evidence** for arrestin signal from MOR affecting respiratory function is **absent** [*Raheal and Bohn, 2014*].
- Opioid side effect profile is not improved in a knock-in mouse expressing phosphorylation deficient, **G protein biased MOR** *[Kliewer et al., 2019].*
- **Several laboratories** have been **unable to repeat the primary result** of reduced morphine respiratory depression in arrestin knock-out mice *[Kliewer et al., 2020].*
- Different levels of **signal amplification** between experimentally measured G-protein-dependent vs arrestin-mediated events may represent **a relevant confounding factor** *[Gillis et al., 2020].*

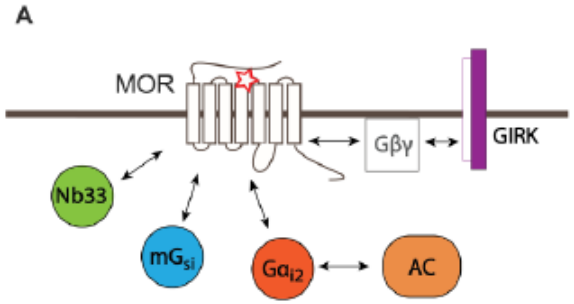
HYPOTHESIS OF G PROTEIN BIAS AS DETERMINANT OF IMPROVED PHARAMCOLOGY HAS BEEN CHALLENGED

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

BIASED AGONISM AT MOR

The sunset of biased opioid agonists?



DAMGO
Fentanyl
Methadone
Morphine
Oxycodone
Oliceridine
PZM21
SR-17018
Buprenorphine

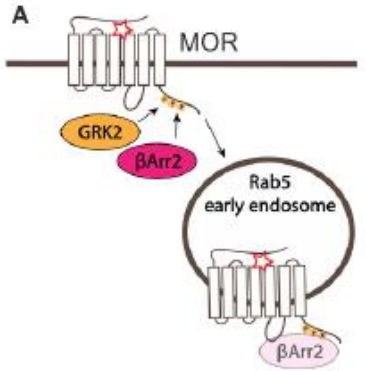


Table 1. Determination of the efficacies (τ) of selected agonists for all the pathways measured at the MOR. Values are expressed as means ± SEM from 3 to 14 independent experiments. Efficacy of oliceridine, PZM21, SR-17018, and buprenorphine were compared to morphine in a two-way analysis of variance (ANOVA) with a Holm-Sidak multiple comparison–corrected post hoc test. ND, not determined.

Log(τ)	Nb33	mG_{si}	$G\alpha_{i2}$ activation	cAMP	GIRK
Morphine	0.42 ± 0.08	0.70 ± 0.07	1.74 ± 0.22	2.00 ± 0.31	0.09 ± 0.05
Oxycodone	0.56 ± 0.12	0.65 ± 0.09	1.86 ± 0.26	1.87 ± 0.22	ND
Oliceridine	−0.34 ± 0.12***	−0.12 ± 0.05**	1.15 ± 0.25	1.29 ± 0.23**	−0.24 ± 0.05
PZM21	−0.33 ± 0.07***	−0.08 ± 0.08**	1.18 ± 0.28	1.44 ± 0.39*	−0.18 ± 0.04
SR-17018	−0.86 ± 0.21***	−0.37 ± 0.16***	0.66 ± 0.37***	1.04 ± 0.28**	−0.28 ± 0.12
Buprenorphine	−0.62 ± 0.09***	−0.40 ± 0.05***	0.63 ± 0.25***	1.35 ± 0.39*	−0.61 ± 0.10**

Log(τ)	GRK2	βArr2 (GRK2)	Rab5 (GRK2)
Morphine	0.21 ± 0.07	0.34 ± 0.07	0.45 ± 0.18
Oxycodone	0.22 ± 0.14	0.35 ± 0.09	0.11 ± 0.16
Oliceridine	−0.30 ± 0.18	0.13 ± 0.03	−0.08 ± 0.13*
PZM21	−0.37 ± 0.11	0.11 ± 0.06	−0.06 ± 0.18
SR-17018	−0.30 ± 0.09	−0.04 ± 0.15	0.04 ± 0.15
Buprenorphine	−0.57 ± 0.08	−0.30 ± 0.09*	−0.84 ± 0.32***

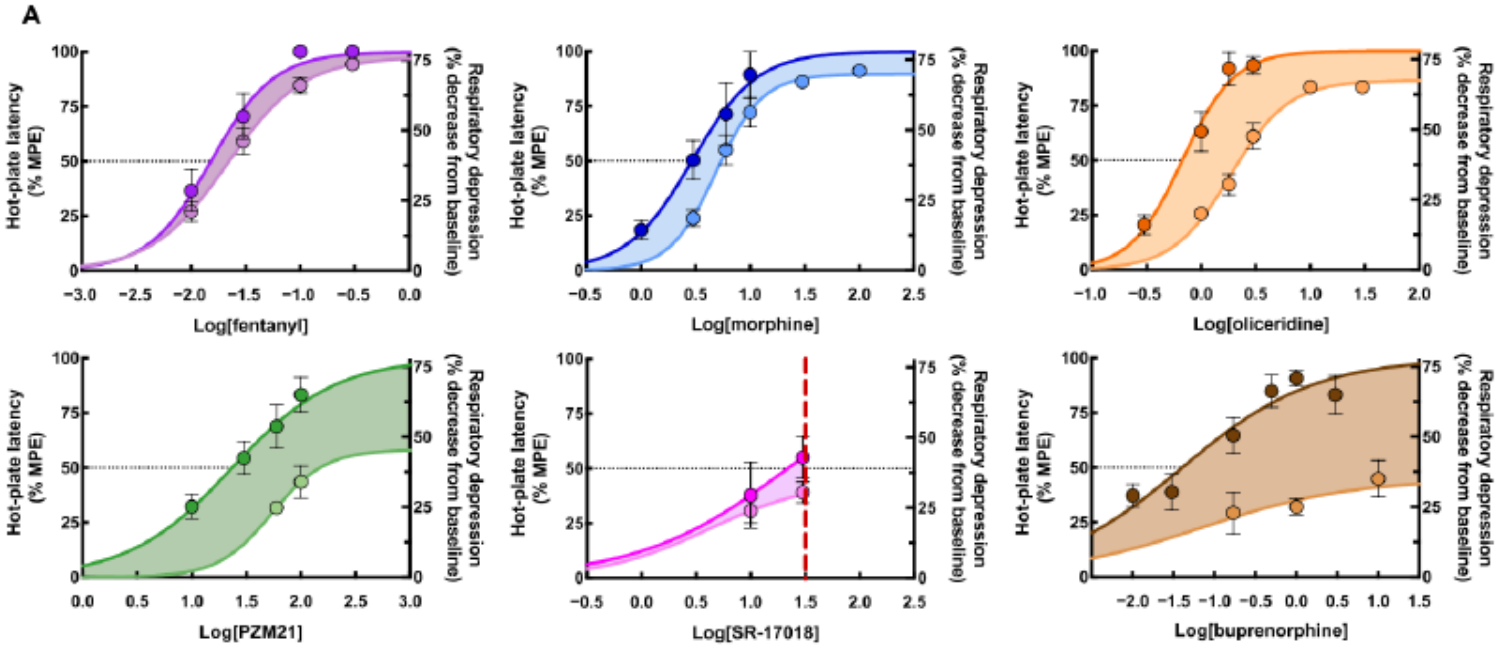
*$P < 0.05$. **$P < 0.01$. ***$P < 0.001$.

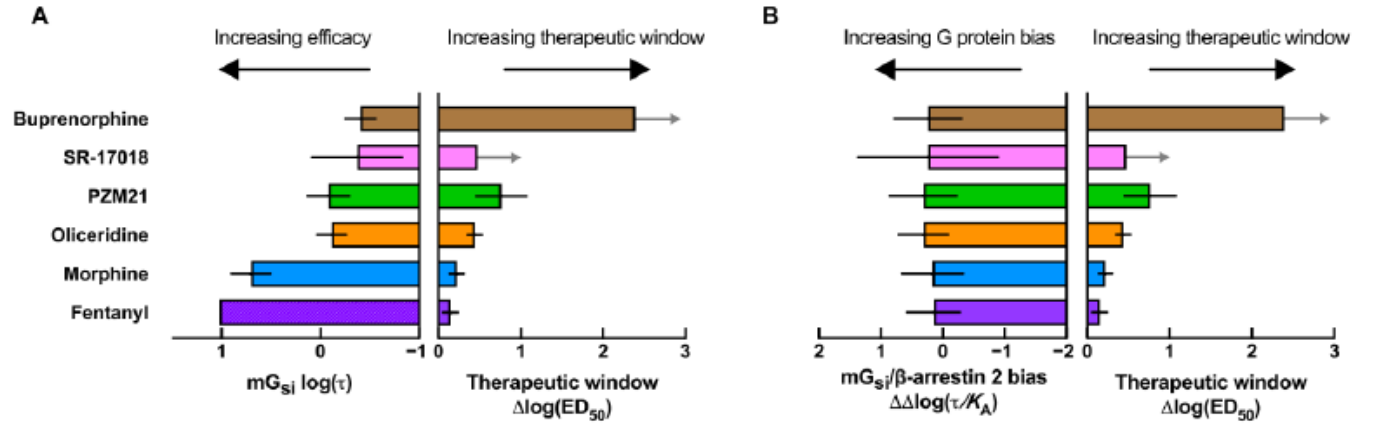
[Gillis et al., 2020]

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

BIASED AGONISM AT MOR

The sunset of biased opioid agonists?





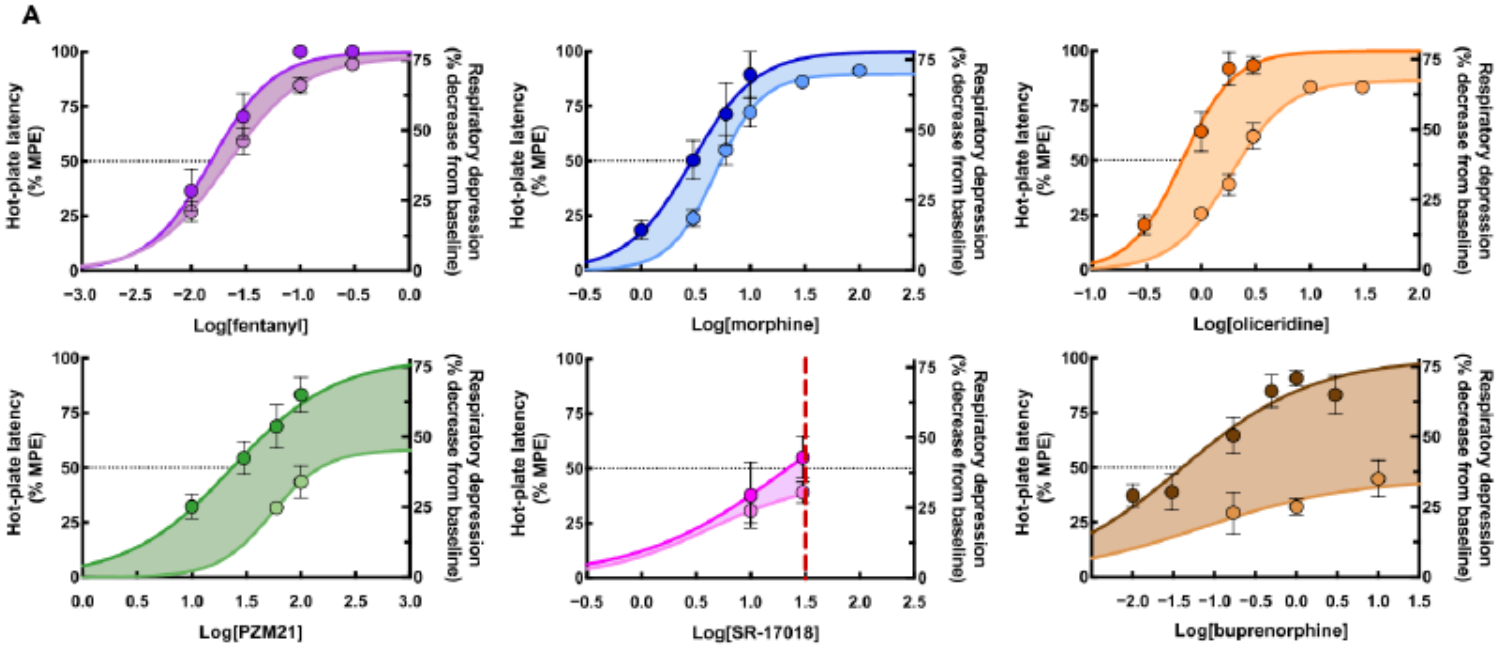
[Gillis et al., 2020]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

BIASED AGONISM AT MOR

The sunset of biased opioid agonists?



[Gillis et al., 2020]

WITH REGARD TO ANALGESIA AND RESPIRATORY DEPRESSION LOW G PROTEIN EFFICACY MAY ALSO PLAUSIBLY UNDERLIE THE FAVORABLE THERAPEUTIC WINDOW

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

BIASED AGONISM VS LOW EFFICACY

Which mechanism is responsible for improved pharmacological profile?

- **Extremely low-efficacy opioid**, buprenorphine, induces **reasonable analgesia** with **reduced side effects** and overdose liability *[Gillis et al., 2020]*.
- **Oliceridine** (TRV130) **failed** to show improvement over an active comparator (morphine), albeit with just a **slim majority against approval** *[Azzam et al., 2019]*; more recent studies pointed out **TRV130 as a low efficacy** rather than a biased agonist and showed an **inverse correlation between efficacy and therapeutic window** *[Gillis et al., 2020]*.
- **Functional selectivity** at receptors **other than MOR** was clearly connected to **improved pharmacological profiles** (e.g.: kappa opioid receptor, 5HT1 receptor, beta-adrenergic receptor).
- **Different endogenous opioid peptides** were shown to favour **particular signalling pathways** at the three opioid receptors leading to biased signalling *[Gomes et al., 2020]*.

Potential utility of biased agonists at opioid receptors is still highly debated and cannot be completely ruled out

Further studies are necessary to fully understand if, and to what extent, biased agonism may be exploited to develop more effective and safer analgesics

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

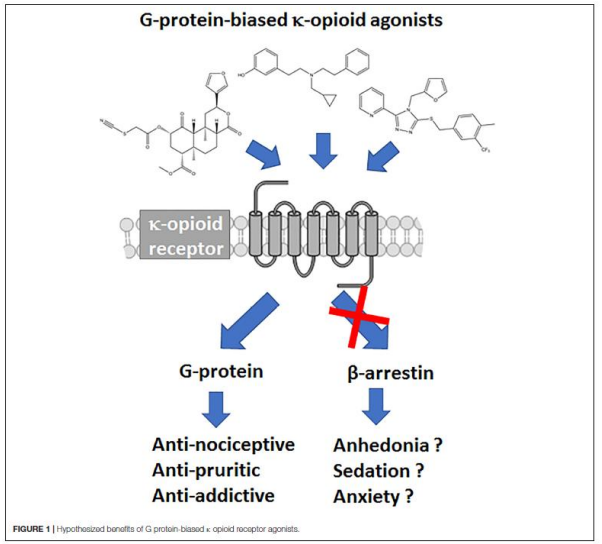
ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

KAPPA OPIOID RECEPTOR (KOR)

A promising pharmacological target for pain and neuropsychiatric disorders

- **Kappa opioid receptor (KOR)** is implicated in various physiological responses including nociception, stress, mood, feeding, gut motility.
- **KOR agonists** are being explored as alternatives to MOR analgesics for their low abuse potential and minimal gastrointestinal and respiratory side effects

CLINICAL RELEVANCE OF KOR AGONIST IS LIMITED BY SEVERE SIDE EFFECTS AS DYSPHORIA



[Mores et al., 2019]

- Chronic or repeated exposure to **stress** or drugs potentiates KOR function ultimately contributing to a hypodopaminergic state => endogenous KOR agonist, dynorphin, is released in distinct brain regions following the development of addiction, thus contributing to craving for substances of abuse.
- **Prolonged KOR activation in response to chronic stress** may lead to persistent symptoms typical of depressive disorders in human; **KOR agonists** promotes pro-depressant effects in rodents.
- **KOR** is expressed on pre-synaptic axons in nigrostriatal and mesolimbic neurons, thus modulating dopamine release: chronic KOR activation increases dopamine release => **KOR activation** may contribute to positive symptoms of schizophrenia.

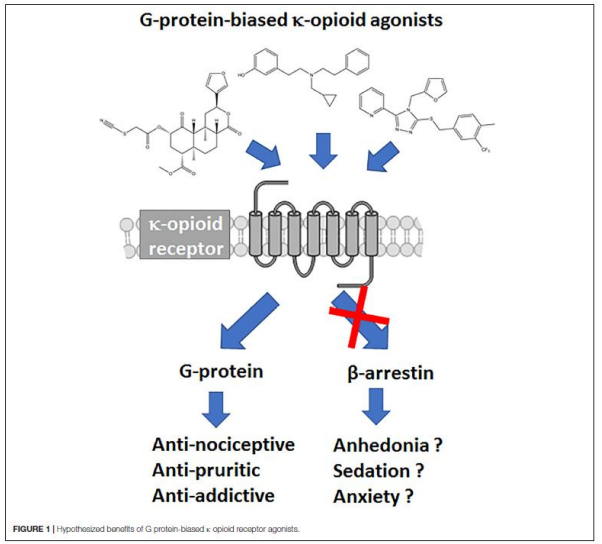
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

KAPPA OPIOID RECEPTOR (KOR)

A promising pharmacological target for pain and neuropsychiatric disorders

- **Kappa opioid receptor (KOR)** is implicated in various physiological responses including nociception, stress, mood, feeding, gut motility.
- **KOR agonists** are being explored as alternatives to MOR analgesics for their low abuse potential and minimal gastrointestinal and respiratory side effects



[Mores et al., 2019]

GROWING INTEREST IN KOR AGONISTS WITH LIMITED ACTIVATION OF ARRESTIN 3/p38MAPK SIGNALING
=> BETTER AND SAFER ANALGESICS?

GROWING INTEREST IN KOR ANTAGONISTS
AS NEW POTENTIAL THERAPIES FOR ADDICTION, DEPRESSIVE DISORDERS, SCHIZOPHRENIA

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

ATICAPRANT: A KOR ANTAGONIST FOR MDD

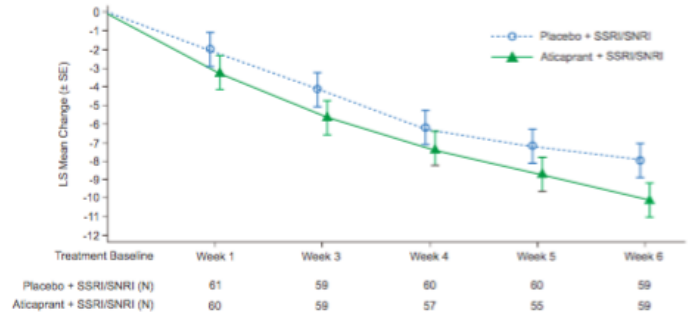
ARTICLE OPEN

Efficacy and safety of aticaprant, a kappa receptor antagonist, adjunctive to oral SSRI/SNRI antidepressant in major depressive disorder: results of a phase 2 randomized, double-blind, placebo-controlled study

Mark E. Schmidt[1]✉, Iva Kezic[1], Vanina Popova[1], Rama Melkote[2], Peter Van Der Ark[1], Darrel J. Pemberton[1], Guy Mareels[1], Carla M. Canuso[2], Maurizio Fava[3] and Wayne C. Drevets[4]

Received: 31 December 2023
Revised: 18 March 2024
Accepted: 4 April 2024
Published online: 22 April 2024

a. eITT Analysis Dataset



b. fITT Analysis Dataset

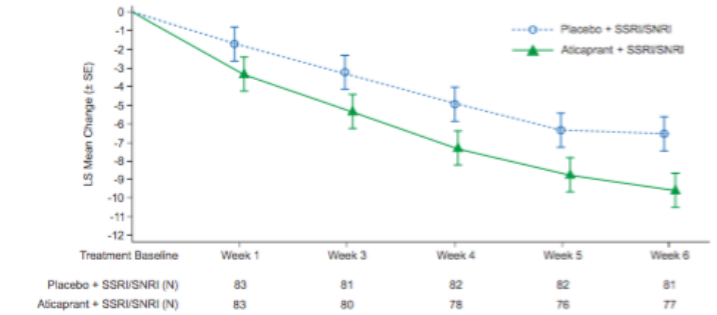
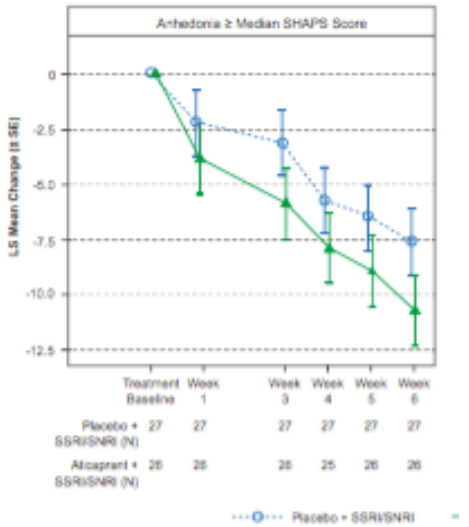
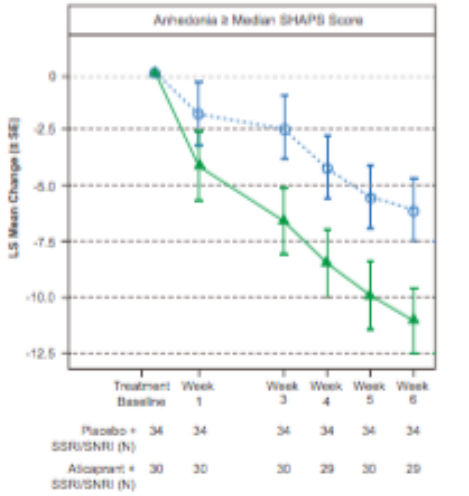


Fig. 1 MADRS total score: LS mean change from baseline (±SE) over 6 weeks. eITT enriched intent-to treat, fITT full intent-to-treat, LS least squares, MADRS Montgomery–Åsberg Depression Rating Scale, SE standard error, SNRI serotonin-norepinephrine reuptake inhibitor, SSRI selective serotonin reuptake inhibitor. Note: MADRS total score ranges from 0 to 60; a higher score indicates a more severe condition. Negative change in score indicates improvement. Negative difference favors aticaprant.

a. eITT Analysis Dataset



b. fITT Analysis Dataset

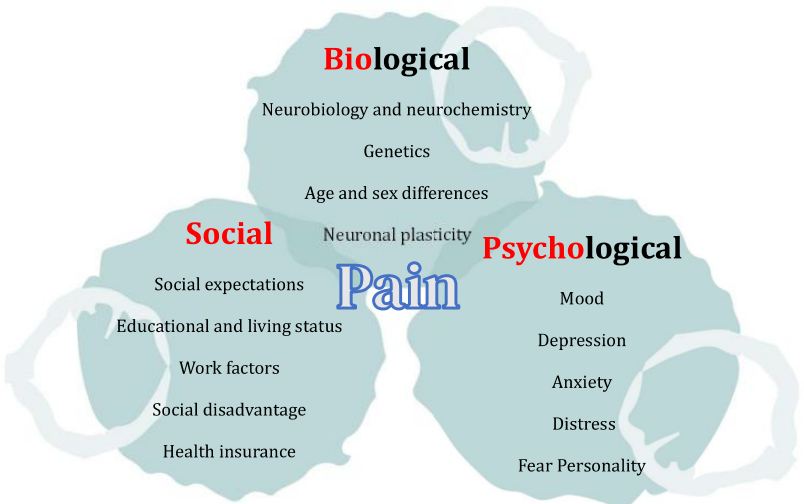


This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

PAIN AND COMORBID ANXIETY/DEPRESSION

A promising target for innovative KOR ligands?



- Chronic pain has several **psychosocial** and **functional consequences** which, in turn, **affect experience and reporting of pain** and related symptoms.
- **Depression, anxiety, and emotional distress**, along with a cluster of negative emotions, thoughts, and behaviours referred to as **negative affect**, strongly contribute to important **long-term consequences of chronic pain**.
- **Pre-morbid psychological dysfunction**, as well as **emotional distress**, represent a **risk factor** for the subsequent development of various **chronic pain** conditions

KOR activation within pain pathways => Abuse liability-free analgesia

Counteracting KOR dysregulated activation by endogenous dynorphin in the brain => antidepressive

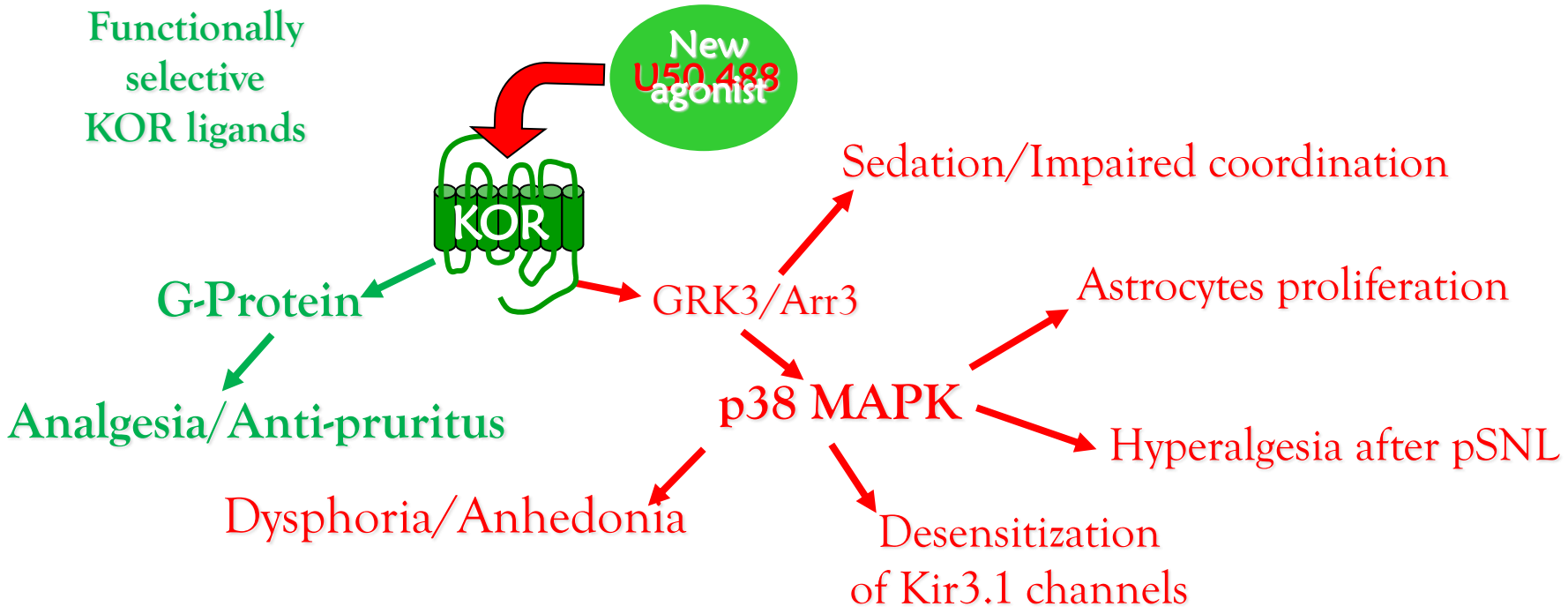
KOR innovative ligands with specific activity profiles => promising innovative therapeutics

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

FUNCTIONAL SELECTIVITY AT KOR

Pharmacological relevance and potential impact



Dunn AD, Reed B, Guariglia C, Dunn AM, Hillman JM, Kreek MJ. (2018)
Bruchas MR, Roth BL Trends Pharmacol Sci. (2016)
Morgenweck J, Frankowski KJ, Prisinzano TE, Aubé J, Bohn LM. Neuropharmacology (2015)
White KL, Robinson JE, Zhu H, DiBerto JF, Polepally PR, Zjawiony JK, Nichols DE, Malanga CJ, Roth BL. JPET (2015)
Pradhan AA, Smith ML, Kieffer BL, Evans CJ. British Journal of Pharmacology (2012)
Bruchas MR and Chavkin C. Psychopharmacology (Berl.) (2010)
Clayton CC, Xu M, Chavkin C. J Biol Chem (2009)
Xu M, Bruchas MR, Ippolito DL, Gendron L, Chavkin C. J Neurosci (2007)
Bruchas MR, Land BB, Aita M, Xu M, Barot SK, Li S, Chavkin C. J Neurosci (2007)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

INNOVATIVE KOR LIGANDS TO TREAT PAIN

TO DETAIL FUNCTIONAL SELECTIVITY AND SUBSEQUENT PHARMACOLOGICAL EFFECTS

CELL MODELS

HEK-293 cells stably expressing human KOR (HEK-293/hKOR)

U87-MG human astrocytoma cells endogenously expressing KOR

Normal Human Astrocytes (NHA) endogenously expressing KOR

- Inhibition of adenylyl cyclase
- Arrestin 3 recruitment at KOR
- ERK1/2 vs p38 MAPK phosphorylation
- Cell proliferation (in astrocytic models)

ANIMAL MODELS

Male CD1 mice (25-30 g)

- Warm-water tail-withdrawal test
- Acetic acid-induced visceral pain (writhing test)
- Oxaliplatin-induced neuropathic pain (cold plate)

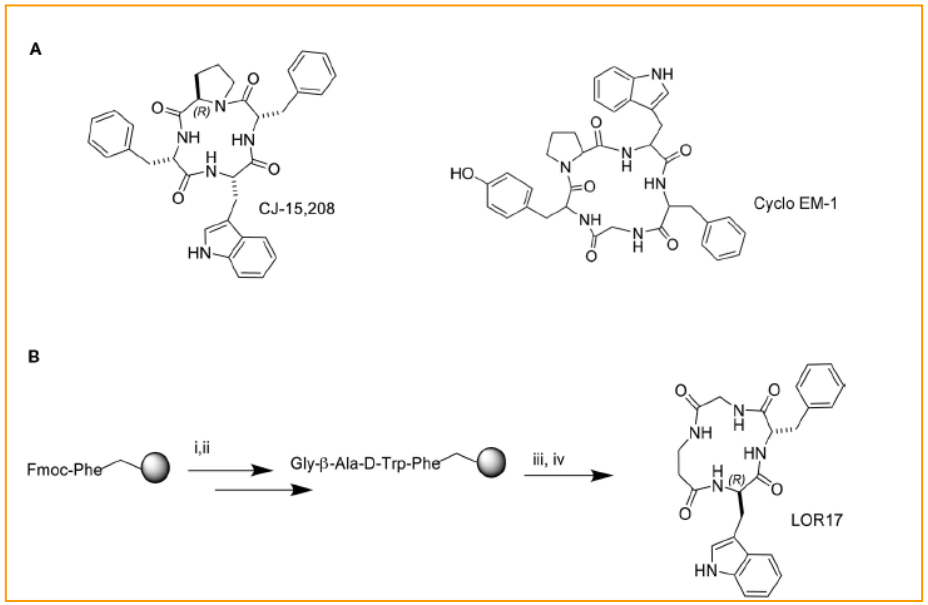
- Rotarod test (motor coordination)
- Hole-board test (locomotor and exploratory activities)
- Forced swim test (anhedonia-related behaviours)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

FUNCTIONAL SELECTIVITY AT KOR

G protein-mediated vs arrestin-dependent signalling: LOR17 vs U50,488

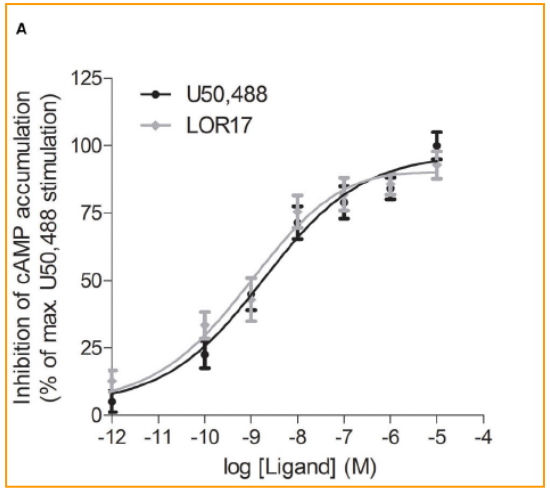


Prof. Luca Gentilucci
Dept. Chemistry
University of Bologna
Italy

TABLE 1 | *In vitro* affinity of LOR17 and reference compounds to opioid receptors.

Compound	Sequence	K_i MOR (nM)	K_i DOR (nM)	K_i KOR (nM)
DAMGO	H-Tyr-D-Ala-Gly-NMePhe-Glyol	1.5 ± 0.1		
DPDPE	H-Tyr-c[D-Pen-Gly-Phe-D-Pen]-OH		3.30 ± 0.05	
U50,488	not peptide			2.90 ± 0.04
LOR17	c[Phe-Gly-(β-Ala)-D-Trp]	>10^5	>10^5	1.19 ± 0.28

Mean of 6 determinations ± SD.



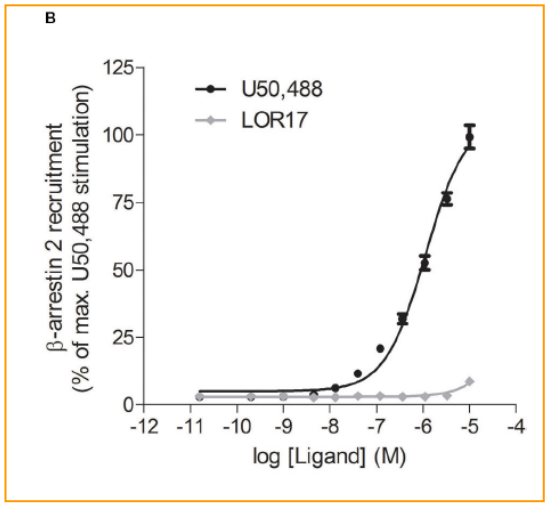


TABLE 3 | Inhibitory effects of LOR17 and U50,488 on forskolin-induced cAMP accumulation in different cell models.

COMPOUND	IC_{50} HEK-293/KOR (nM)	E_{max}HEK-293/KOR (%)	IC_{50} U87-MG (nM)	E_{max}U87-MG (%)	IC_{50} NHA (nM)	E_{max}NHA(%)
U50,488	1.6 ± 0.5	90 ± 2	1.2 ± 0.2	88 ± 3	2.2 ± 0.4	87 ± 3
LOR17	2.8 ± 0.6	85 ± 5	3.1 ± 0.8	87 ± 4	3.0 ± 0.2	88 ± 6

Mean ± SD of 6 independent experiments performed in triplicate.

Bedini A et al., Frontiers in Pharmacologyy2020

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

FUNCTIONAL SELECTIVITY AT KOR

Intrinsic efficacy, potency and efficacy of LOR17

Log (τ) ⟶ Intrinsic efficacy

E_{max} % ⟶ Maximal response

EC_{50} (nM) ⟶ Potency

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

FUNCTIONAL SELECTIVITY AT KOR

Intrinsic efficacy, potency and efficacy of LOR17

Log (τ)	cAMP HEK-293/KOR	cAMP U-87 MG	cAMP NHA
LOR17	0.7382 ± 0.026	0.4373 ± 0.017	0.5951 ± 0.028

E_{max} %	cAMP HEK-293/KOR	cAMP U-87 MG	cAMP NHA
U50,488	86.5 ± 5.1	83 ± 2	87.2 ± 4.4
LOR17	87.62 ± 2.64	75 ± 5	79 ± 9

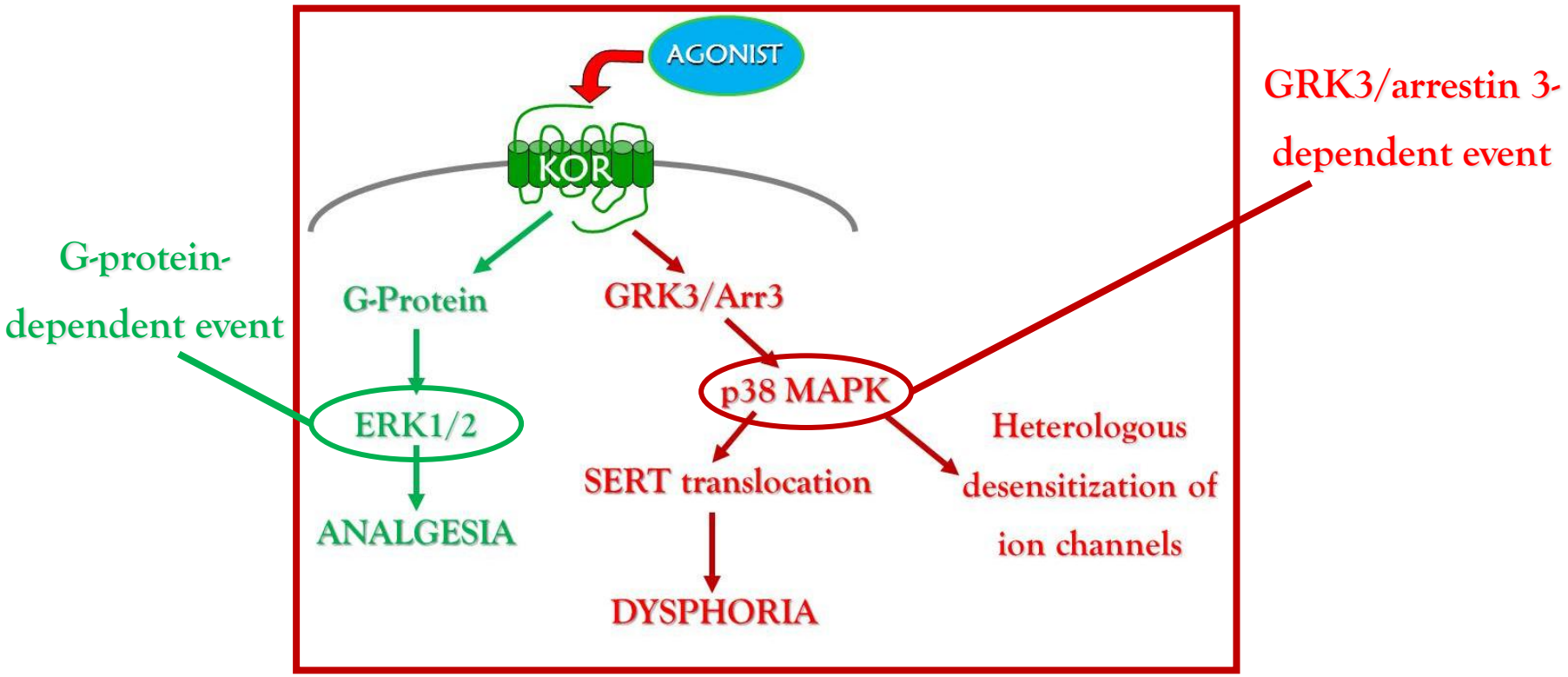
EC_{50} (nM)	cAMP HEK-293/KOR	cAMP U-87 MG	cAMP NHA
U50,488	1.3 ± 0.2	1.44 ± 0.08	1.68 ± 0.21
LOR17	2.59 ± 0.16	1.37 ± 0.24	2.24 ± 0.26

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

FUNCTIONAL SELECTIVITY AT KOR

Activation of ERK1/2 and p38MAPK: LOR17 vs U50,488



HEK-293/KOR
U87-MG
NHA

→ Serum starvation (16 h) → LOR17 or U50,488 (1 μM; 0-120 min)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

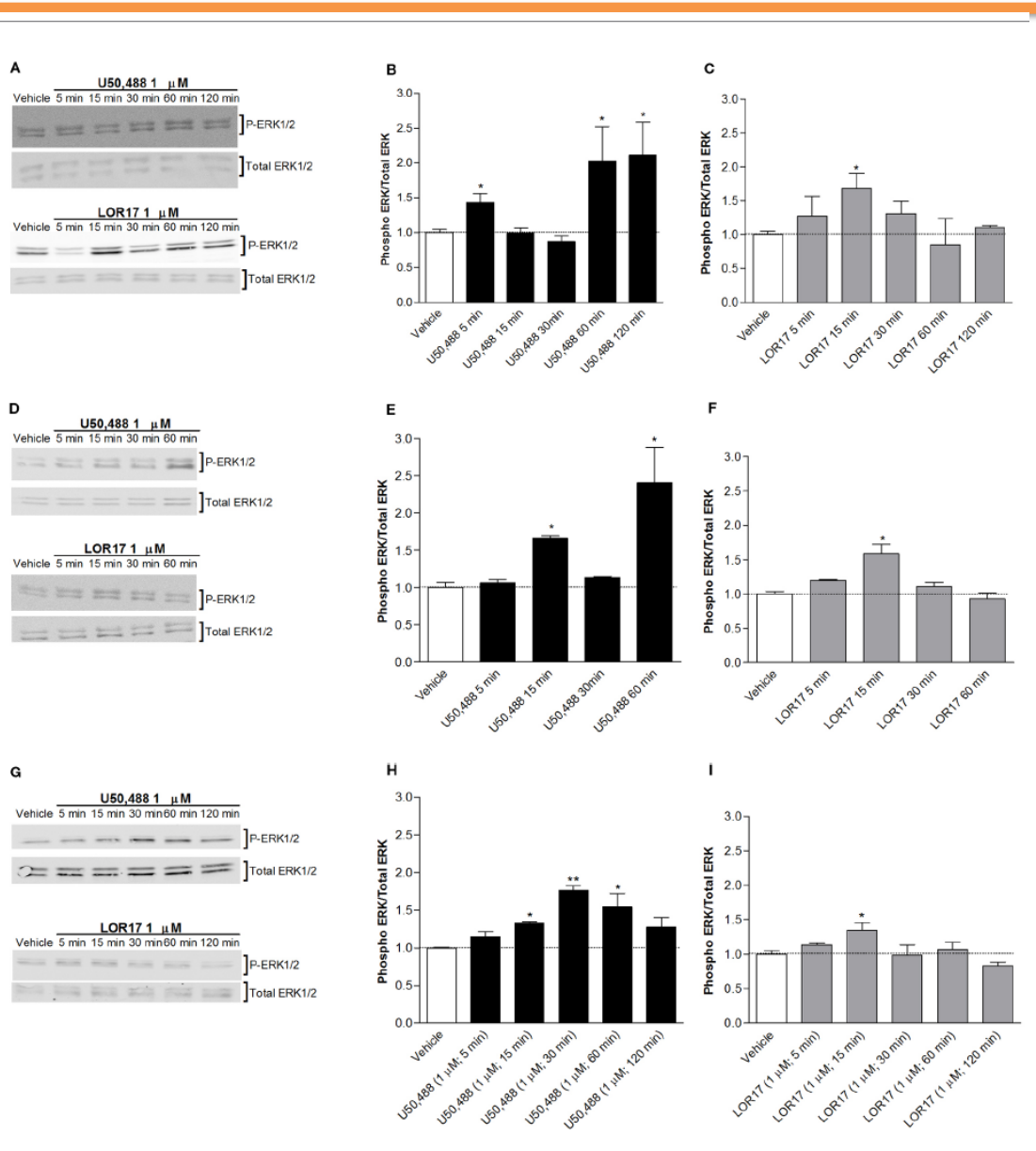
FUNCTIONAL SELECTIVITY AT KOR

Activation of ERK1/2 and p38MAPK: LOR17 vs U50,488

HEK-293/KOR

U87-MG

NHA



* p<0.05 vs Vehicle;

** p<0.01 vs Vehicle;

n = 6

[Bedini et al., 2020]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

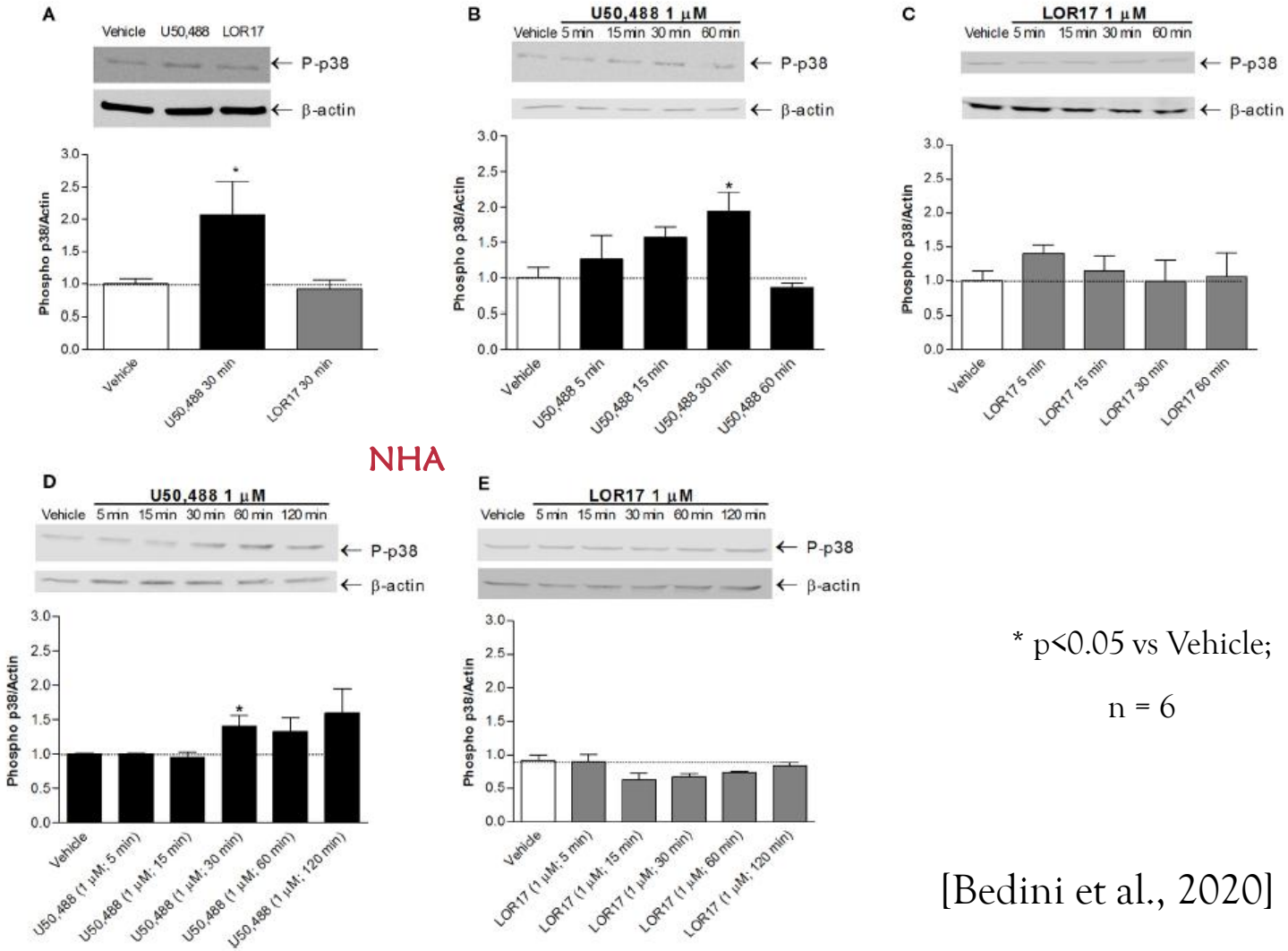
FUNCTIONAL SELECTIVITY AT KOR

Activation of ERK1/2 and p38MAPK: LOR17 vs U50,488

HEK-293/KOR

U87-MG

NHA



* $p<0.05$ vs Vehicle;

n = 6

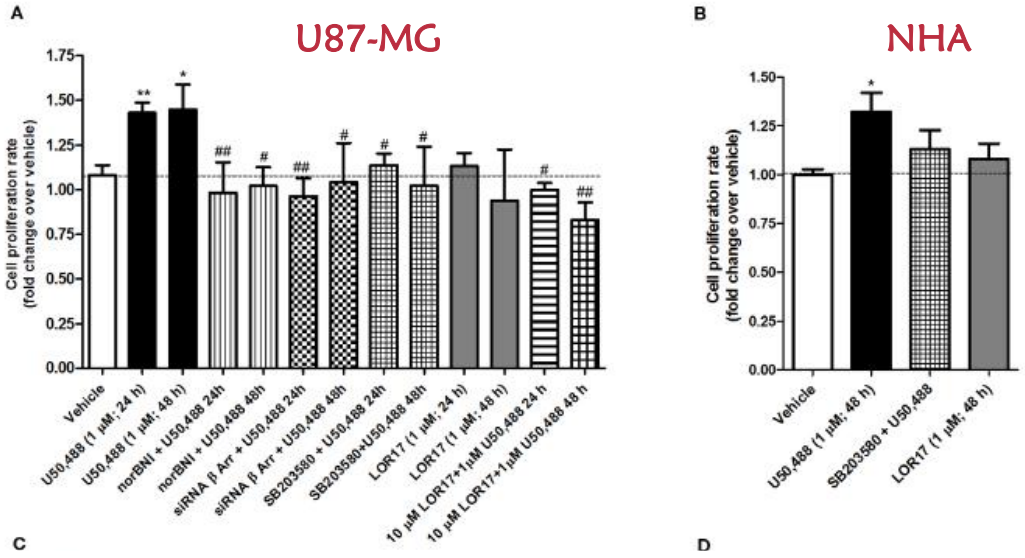
[Bedini et al., 2020]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

FUNCTIONAL SELECTIVITY AT KOR

Astrocyte cell proliferation: LOR17 vs U50,488



* p<0.05 vs Vehicle and LOR17 (1 μM; 48 h);
** p<0.01 vs Vehicle and LOR17 (1 μM; 24 h);
# p<0.05 vs U50,488 (1 μM; 48 h);
p<0.01 vs U50,488 (1 μM; 24 h)
§ p<0.05 vs U50,488 (1 μM; 24 h);
n = 8

[Bedini et al., 2020]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

FUNCTIONAL SELECTIVITY AT KOR

Antinociceptive effects of LOR17 in the warm water tail withdrawal test

Adult male CD-1 mice (25-30 g)

Water temperature = 55±2 °C

Cut-off = 10 sec

Latency to tail withdrawal

Methods described in: Bedini et al., 2010

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

FUNCTIONAL SELECTIVITY AT KOR

Antinociceptive effects of LOR17 in the warm water tail withdrawal test

Adult male CD-1 mice (25-30 g)

Water temperature = 55±2 °C

Cut-off = 10 sec

Vehicle (1:1 propylene glycol-saline; 0.1 ml/10 g i.p.)

LOR17 (5-20 mg/kg, i.p.)

U50,488 (5-20 mg/kg, i.p.)

norBNI (10 mg/kg, i.p.)

5, 15, 30, 45, 60, 90, 120 min

Latency to tail withdrawal

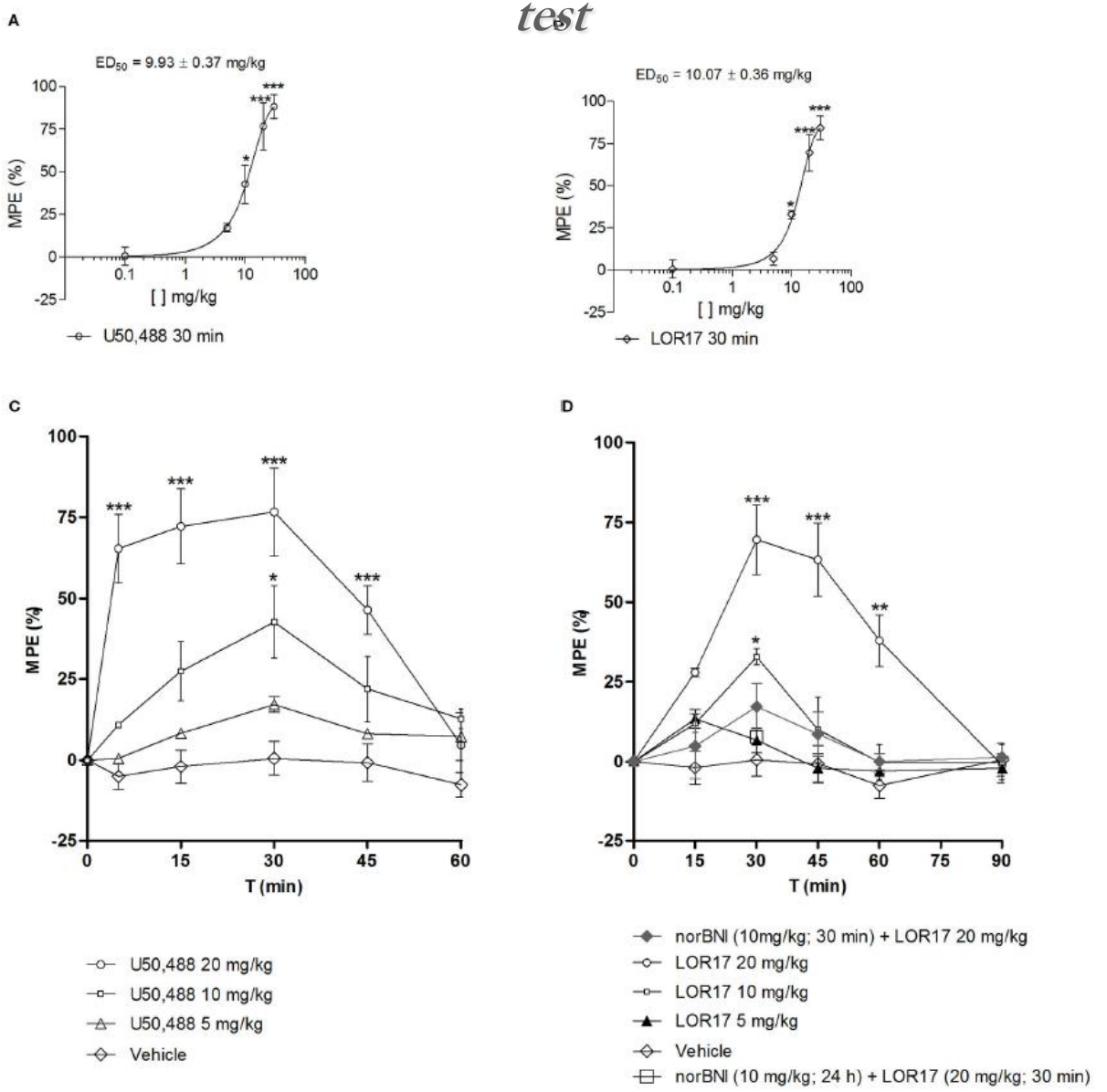
Methods described in: Bedini et al., 2010

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

FUNCTIONAL SELECTIVITY AT KOR

Antinociceptive effects of LOR17 in the warm water tail withdrawal test



*** p<0.001 vs Vehicle, 5 mg/kg and norBNI 10 mg/kg+LOR17 20 mg/kg;
** p<0.01 vs Vehicle and 5 mg/kg;
* p<0.05 vs Vehicle and 5 mg/kg;
n = 8-10

[Bedini et al., 2020]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

FUNCTIONAL SELECTIVITY AT KOR

Effects in a mouse model of chemotherapy-induced neuropathic pain

Prof. L. Di Cesare Mannelli
Dept. NEUROFARBA
University of Florence
Italy

Adult male CD-1 mice (25-30 g)

Oxaliplatin (2.4 mg/kg, i.p.)
5 consecutive days per week, for 2 weeks
Vehicle (5% glucose solution)

Vehicle (1:1 propylen glicole-saline, s.c.)
LOR17 (1-20 mg/kg, s.c.)
U50,488 (10-20 mg/kg, s.c.)

Methods described in:
Cavaletti et al., 2001
Brindisi et al., 2016

Bedini A et al., Frontiers in Pharmacology 2020

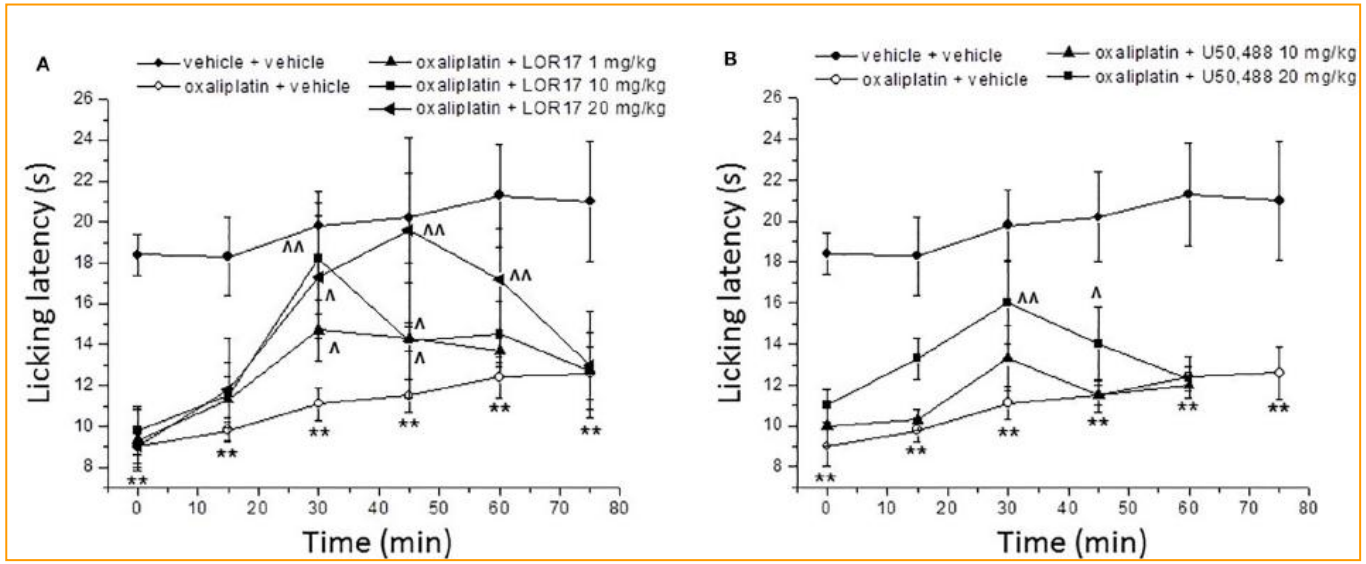
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

FUNCTIONAL SELECTIVITY AT KOR

Effects in a mouse model of chemotherapy-induced neuropathic pain

Prof. L. Di Cesare Mannelli
Dept. NEUROFARBA
University of Florence
Italy



** p<0.05 vs vehicle + vehicle;
^^ p<0.01 vs oxaliplatin + vehicle;
^ p<0.05 vs oxaliplatin + vehicle;
n = 9

TABLE 5 | Maximal effects elicited by LOR17 and U50,488 in counteracting oxaliplatin induced thermal hypersensitivity, as compared to vehicle.

Treatment	Licking latency (s)
oxaliplatin/vehicle	11.4 ± 1.2
oxaliplatin/U50,488 (10 mg/kg; 30 min)	12.6 ± 1.7
oxaliplatin/LOR17 (10 mg/kg; 30 min)	18.2 ± 1.4***
oxaliplatin/U50,488 (20 mg/kg; 30 min)	15.8 ± 1.6
oxaliplatin/LOR17 (20 mg/kg; 45 min)	19.6 ± 1.7***

*** p<0.001 vs oxaliplatin/vehicle, oxaliplatin/U50,488 (10 and 20 mg/kg; 30min); n = 10

TABLE 6 | Effect of LOR17 on motor coordination (rotarod test[a]), locomotor, and exploratory activitivities (hole-board test[b]) and pro-depressant like behaviour (forced swimming test[c]).

Treatment	Dose *mg kg⁻¹ s.c.*	Number of falls[a]				Hole[b]		Board[b]		Mobility time (s)[c]
		0 min	15 min	30 min	45 min	0 min	30 min	0 min	30 min	
vehicle		3.0 ± 0.6	2.3 ± 0.3	1.8 ± 0.6	1.3 ± 0.3	42.4 ± 4.6	48.2 ± 4.1	81.6 ± 13.1	106.2 ± 8.0	80.5 ± 9.6
LOR17	10	2.8 ± 0.7	1.6 ± 0.4	1.2 ± 0.2	0.6 ± 0.2	37.2 ± 3.9	39.0 ± 4.6	89.2 ± 7.3	97.2 ± 16.9	91.6 ± 12.3
U50,488	10	2.9 ± 0.4	3.2 ± 0.5$	2.6 ± 0.3$$	2.1 ± 0.4$$	/	/	/	/	21.0 ± 7.7 ***

$ p<0.05 vs vehicle and LOR17; $$ p<0.01 vs vehicle and LOR17;
***p<0.001 vs vehicle and LOR17; n = 12

Bedini A et al., Frontiers in Pharmacology 2020

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

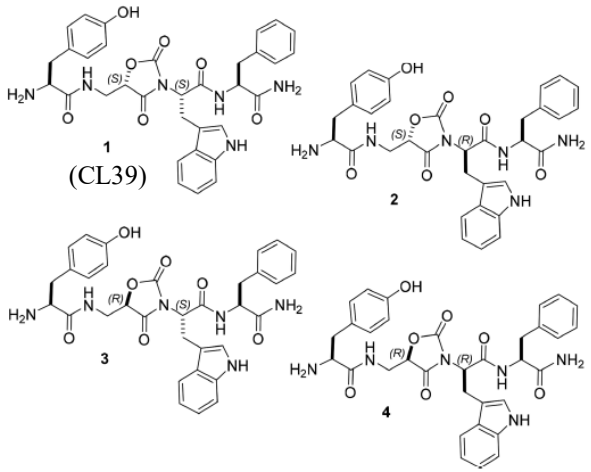
PARTIAL AGONISM AT KOR

G protein-mediated vs arrestin-dependent signalling: CL39 vs U50,488

ENDOMORPHIN-1

H-Tyr-Pro-Trp-Phe-NH_2

- Endogenous, MOR-selective agonist
- Antinociceptive upon icv and it administration
- Poor metabolic stability and BBB penetration



H-Tyr-(S/R)-Amo-(S/R)-Trp-PheNH_2

Amo => 5-(aminomethyl)oxazolidine-2,4-dione

De Marco R, Bedini A et al. J Med Chem. (2018)

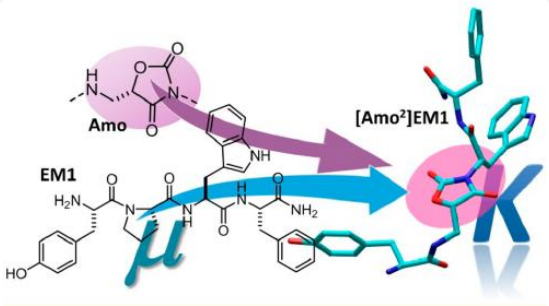


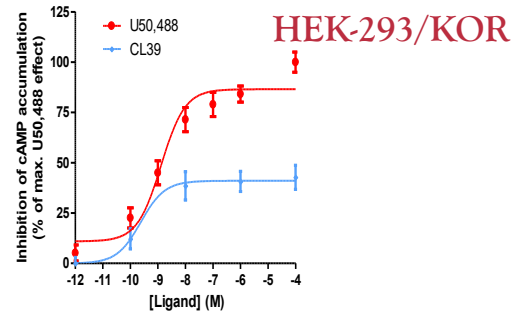
Table 1. In Vitro OR Affinities of the [Amo²]EMs and Reference Compounds for hORs

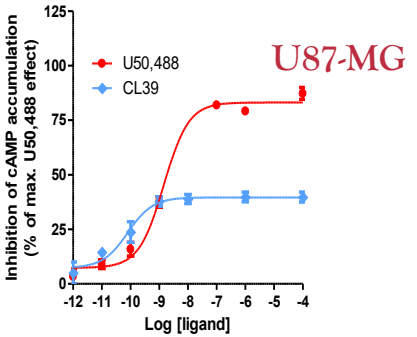
compd	purity (%)[a]	K_i (nM)[b] MOR	DOR	KOR
DAMGO		1.5 ± 0.1		
DPDPE			3.30 ± 0.05	
U50,488				2.90 ± 0.04
1 (CL39)	97	$>10^5$	$>10^5$	9.8 ± 4.1
2	95	$>10^5$	$>10^5$	$>10^5$
3	96	$>10^5$	$>10^5$	$>10^5$
4	98	240 ± 50	$>10^5$	$>10^5$

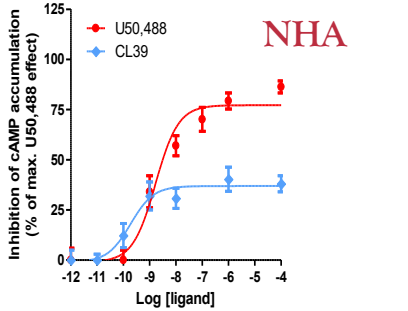
[a]Determined by RP-HPLC (General Methods). [b]Mean of 4–6 determinations ± SE.

COMPOUND	IC_{50} (nM)	E_{max} (%)
EM-1 in CHO[a]	1.0 ± 0.2	53
U50,488	1.2 ± 0.2	90
CL39	0.22 ± 0.02	40
4	0.016 ± 0.004	50

[a]Zadina JE et al., Nature (1997)







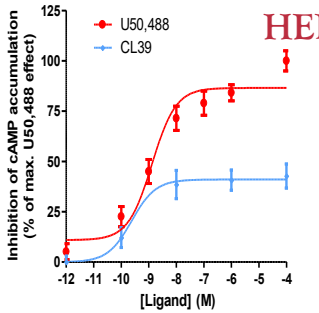
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

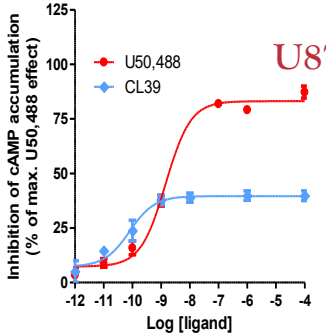
PARTIAL AGONISM AT KOR

G protein-mediated vs arrestin-dependent signalling: CL39 vs U50,488

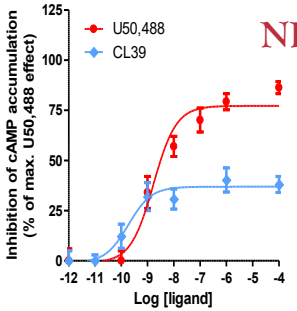
HEK-293/KOR

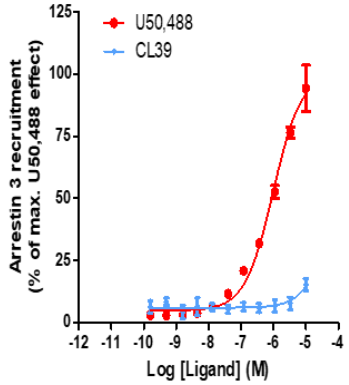


U87-MG

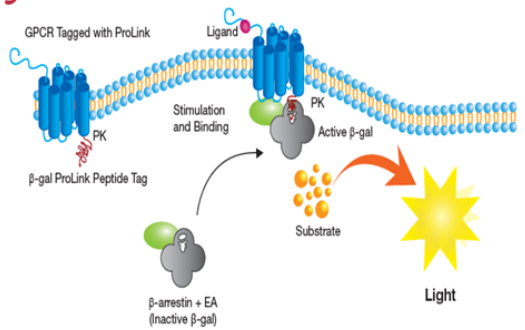


NHA



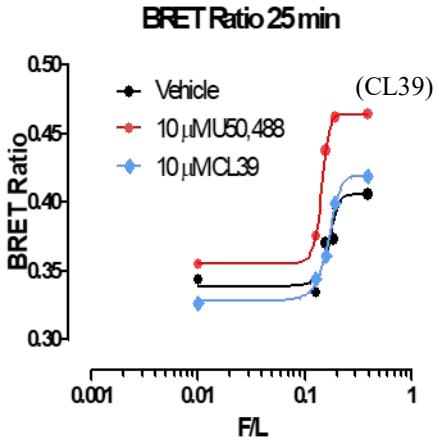


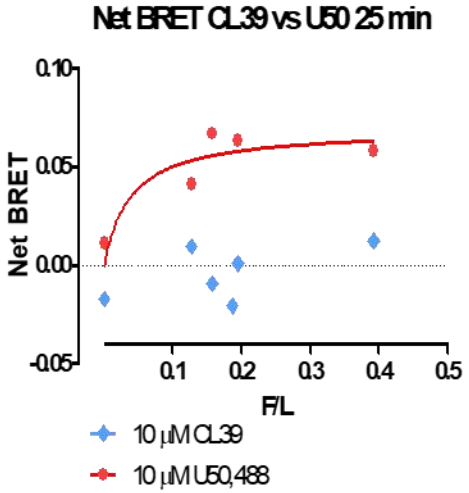
U2-OS

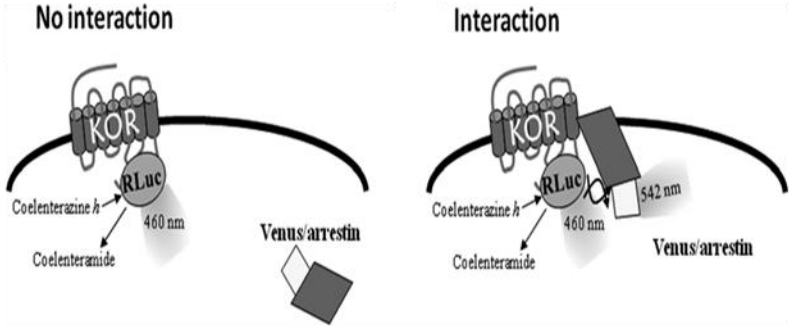


PathHunter β-Arrestin GPCR Assay

HEK-293







Bedini A. Methods Mol Biol. (2015)
Gimenez LE, Kook S, Vishnivetskiy SA, Ahmed MR, Gurevich EV, Gurevich VV. J Biol Chem. (2012)

Baiula et al., in preparation

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

PARTIAL AGONISM AT KOR

Intrinsic efficacy, potency and efficacy of CL39

Log (τ) ⟶ Intrinsic efficacy

E_{max} % ⟶ Maximal response

EC_{50} (nM) ⟶ Potency

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

A MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

PARTIAL AGONISM AT KOR

Intrinsic efficacy, potency and efficacy of CL39

Log (τ)	cAMP HEK-293/KOR	cAMP U-87 MG	cAMP NHA
CL39	- 0.1575 ± 0.072	-0.2017 ± 0.032	-0.2316 ± 0.022

E_{max} %	cAMP HEK-293/KOR	cAMP U-87 MG	cAMP NHA
U50,488	86.5 ± 5.1	83 ± 2	87.2 ± 4.4
CL39	41.12 ± 0.9	39.6 ± 1.5	55 ± 13

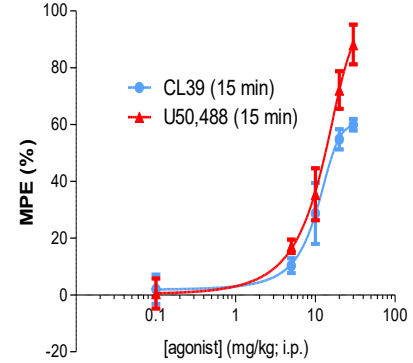
EC_{50} (nM)	cAMP HEK-293/KOR	cAMP U-87 MG	cAMP NHA
U50,488	1.3 ± 0.2	1.44 ± 0.08	1.68 ± 0.21
CL39	0.23 ± 0.01	0.10 ± 0.02	0.19 ± 0.02

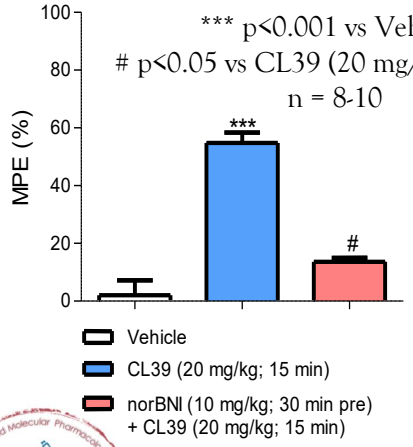
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

PARTIAL AGONISM AT KOR

In vivo pharmacological characterization of CL39

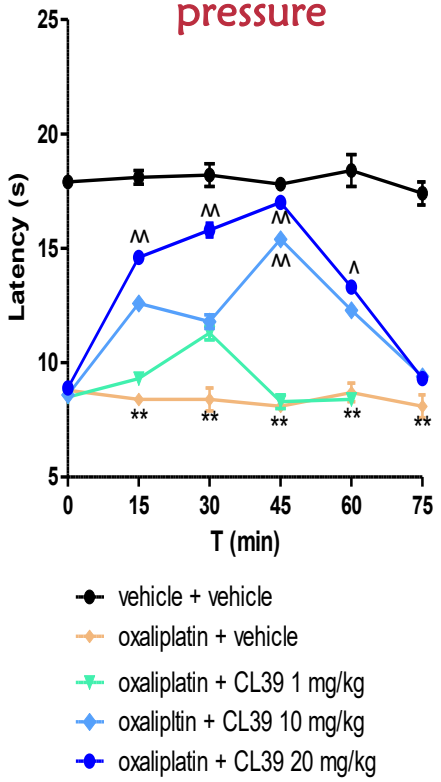
COMPOUND	ED_{50} (mg/kg)	E_{max} (%)
U50,488	9.93 ± 0.37	88.2 ± 14.0
CL39	10.10 ± 0.11	59.9 ± 3.49



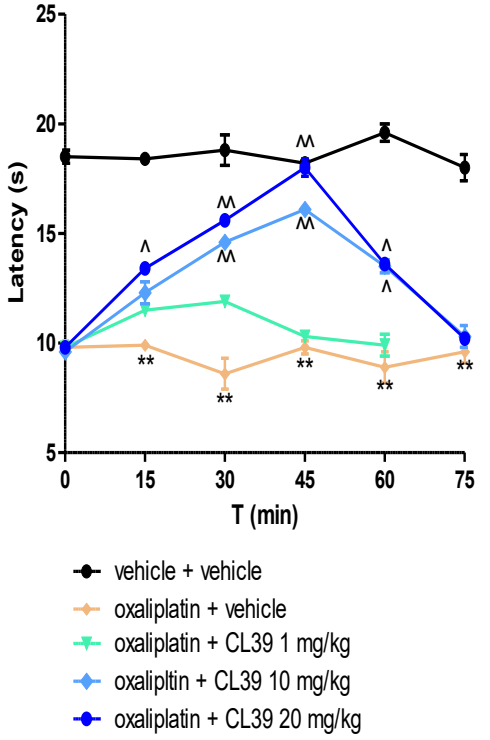


*** p<0.001 vs Vehicle;
# p<0.05 vs CL39 (20 mg/kg; 15 min)
n = 8-10

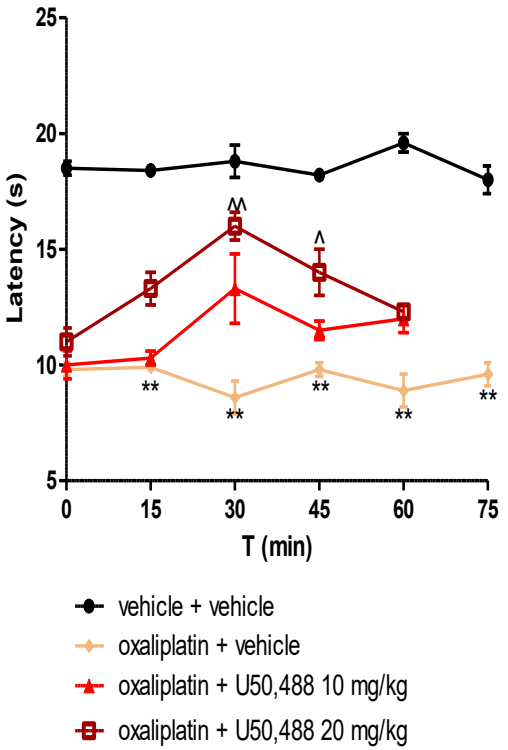
Paw pressure



Cold plate



Cold plate



** p<0.05 vs vehicle + vehicle;
^^ p<0.01 vs oxaliplatin + vehicle;
^ p<0.05 vs oxaliplatin + vehicle;
n = 10

Baiula et al., in preparation

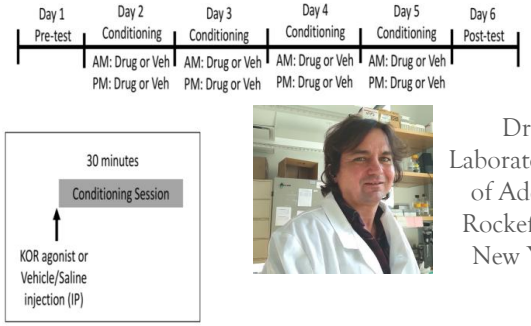
Prof. L. Di Cesare Mannelli
Dept. NEUROFARBA
University of Florence
Italy

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

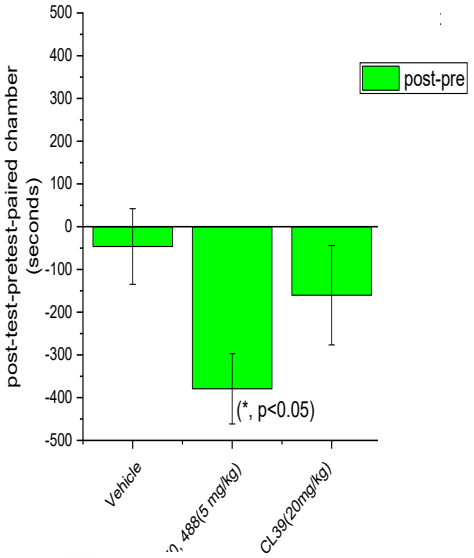
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

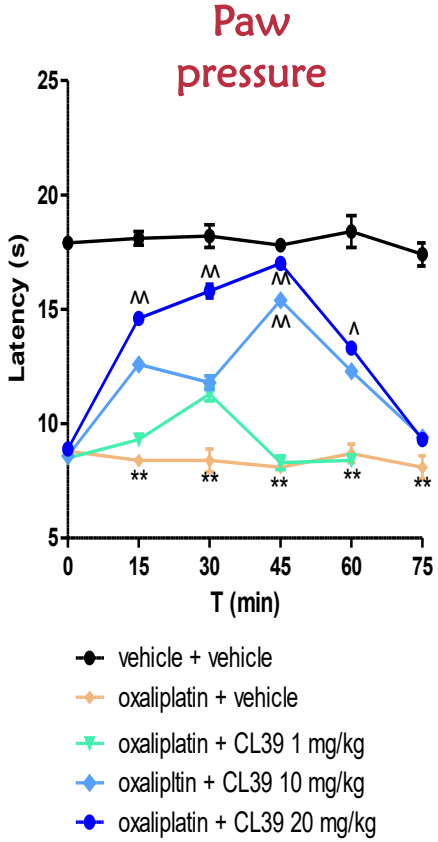
PARTIAL AGONISM AT KOR

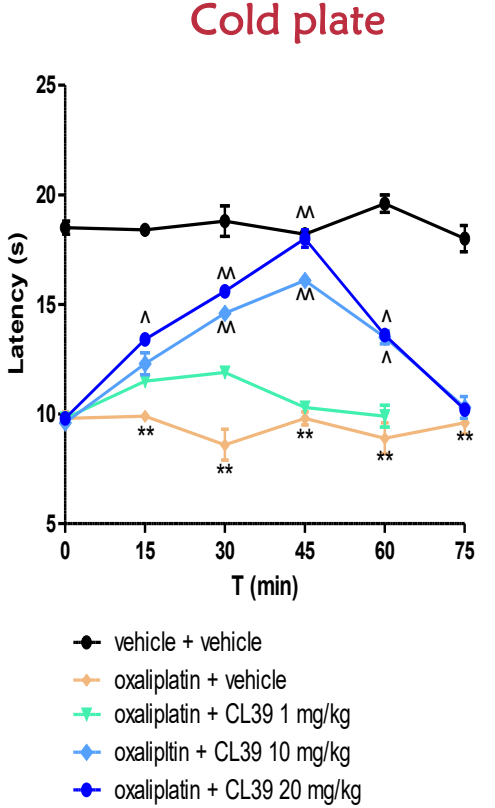
In vivo pharmacological characterization of CL39

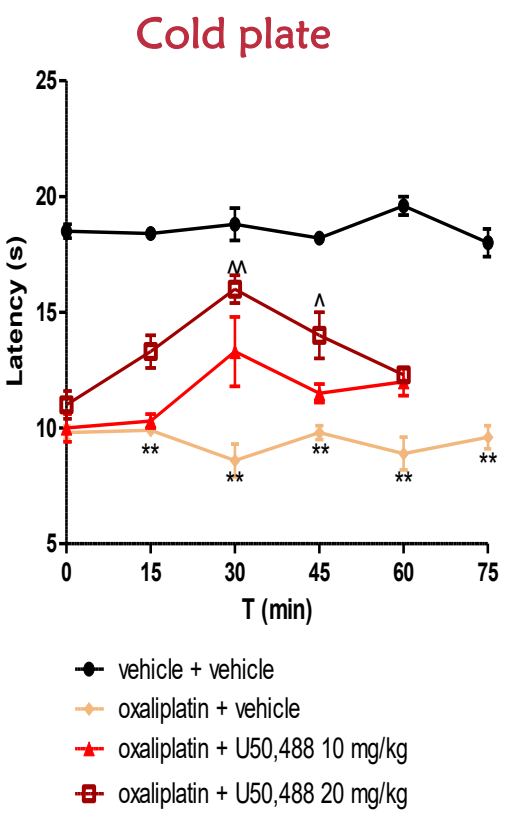


Dr. Brian Reed
Laboratory of the Biology
of Addictive Diseases
Rockefeller University,
New York, NJ (USA)









Baiula et al., in preparation

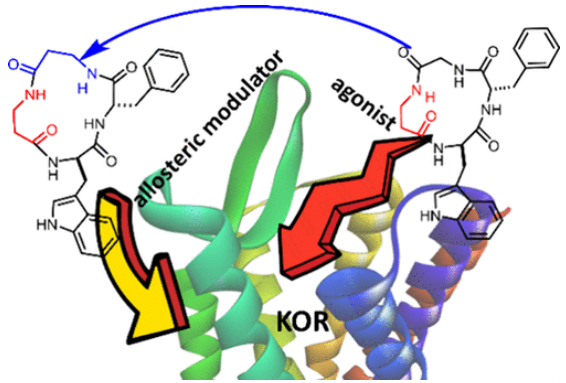
** p<0.05 vs vehicle + vehicle;
^^ p<0.01 vs oxaliplatin + vehicle;
^ p<0.05 vs oxaliplatin + vehicle;
n = 10

Prof. L. Di Cesare Mannelli
Dept. NEUROFARBA
University of Florence
Italy

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

RDM1127: THE FIRST KOR-SELECTIVE NAM



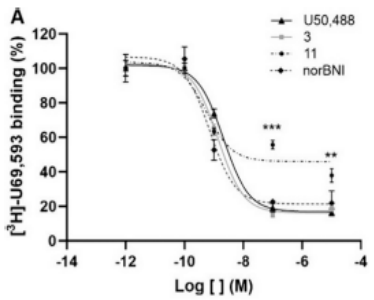
c[D-Trp-Phe-Gly-(β-Ala)]
KOR-selective,
G protein biased agonist
[Bedini et al., 2020]

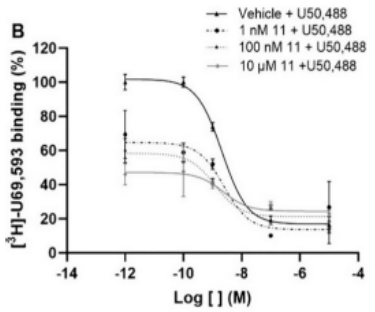
c[D-Trp-Phe-(β-Ala)-(β-Ala)]
KOR-selective,
negative allosteric modulator
[Zhao et al., 2024]

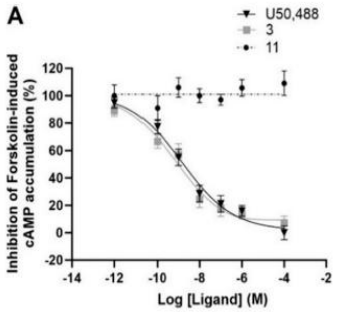
Table 1. K_i Affinity Values for h-ORs as Determined by Displacement Binding Assays for Cyclopeptides 3, 5–13, and the Reference Compounds[a]

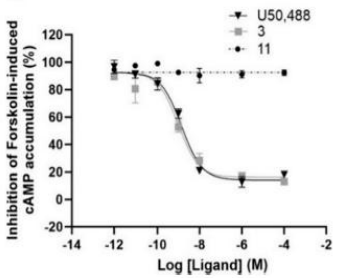
compd	ring size	sequence	K_i (nM) MOR	DOR	KOR
DAMGO		H-Tyr-D-Ala-Gly-NMePhe-Glyol	1.5 ± 0.1		
DPDPE		H-Tyr-c[D-Pen-Gly-Phe-D-Pen]OH		3.30 ± 0.05	
U50,488		non peptide			2.90 ± 0.04
CJ-15,208	12	c[Phe-D-Pro-Phe-Trp]	127 ± 13		32 ± 4
5	12	c[D-Trp-Phe-Gly-Gly]	>10^5	>10^5	>10^5
6	12	c[D-Trp-Phe-Gly-Ala]	111.2	>10^5	>10^5
7	12	c[D-Trp-Phe-Gly-D-Ala]	0.24 ± 0.04[b]	>10^5	>10^5
3[c]	13	c[D-Trp-Phe-Gly-β-Ala]	>10^5	>10^5	1.19 ± 0.28[c]
8	13	c[D-Trp-Phe-β-Ala-Gly]	>10^5	>10^5	>10^5
9	13	c[D-Trp-Phe-Aha]	>10^5	>10^5	>10^5
11	14	c[D-Trp-Phe-β-Ala-β-Ala]	>10^5	>10^5	0.55 ± 0.04[b]
13	14	c[D-Trp-Phe-Gly-GABA]	35.2	>10^5	>10^5

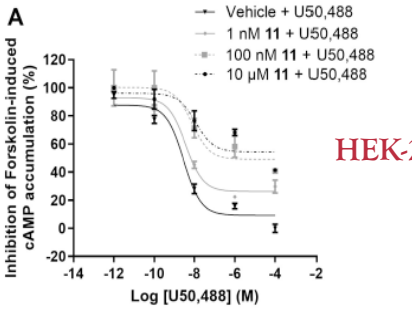
[a]Average of 4–6 determinations ± SE. [b]≪50% Radioligand displacement. [c]Reference 24.



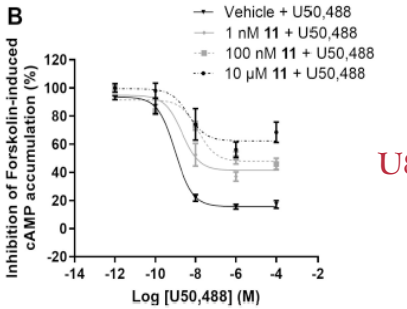




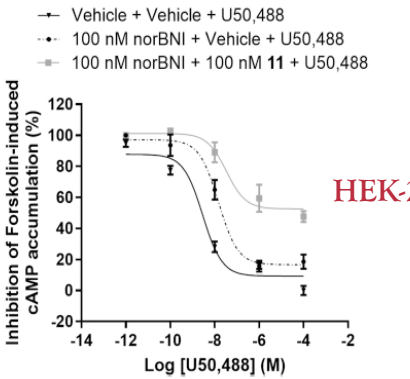




HEK-293/KOR



U87-MG



HEK-293/KOR

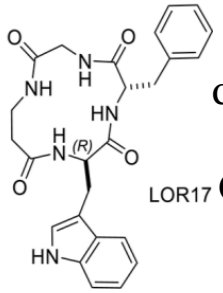
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

INNOVATIVE KOR LIGANDS FOR PAIN AND DEPRESSION

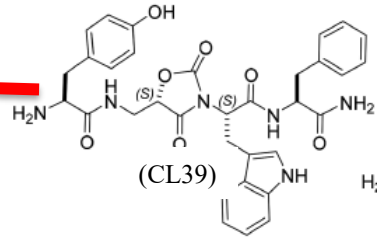
Two birds with one stone?

Chronic pain



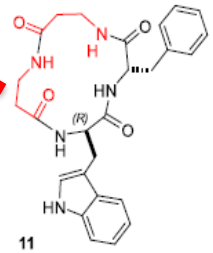
LOR17

LOR17
c[D-Trp-Phe-Gly-(β-Ala)]
KOR-selective,
G protein biased agonist



(CL39)

CL39
H-Tyr-Pro-Trp-Phe-NH2
KOR-selective
partial agonist,



11

RDM1127
c[D-Trp-Phe-(β-Ala)-(β-Ala)]
KOR-selective,
negative allosteric modulator

Depression

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

DEVELOPMENT OF BETTER AND SAFER THERAPEUTICS

The greater complexity of opioid receptor pharmacology

- **Multiple G proteins** (e.g., G_{i1}, G_{i2}, G_{i3}, G_z), as well as **arrestin isoforms** (i.e., arrestin 2 and arrestin 3), may interact with opioid receptors (also in a tissue-specific and time-specific fashion). *[Olsen et al., 2020].*
- Opioid-mediated modulation of the **same classes of intracellular effectors** (e.g., ERK1/2 or JNK) may occur through **both G protein- and/or arrestin-mediated** processes *[Kuhar, Bedini et al., 2015].*
- **Biphasic modulation of MAPK** activation by opioids may contribute to the **fine-tuning** of other physiologically **relevant second messengers and mediators** [*Schattauer, Bedini et al., 2019*].
- Opioid receptors (as many GPCRs) are **not isolated monads** but may interact to form **homodimers and heterodimers** *[Ferrè et al., 2014].*
- **Physiological conditions** (e.g., gender) and different **signalling mechanisms in vivo** may also deeply impact the effects elicited by agonists at opioid receptors *[Abraham et al., 2018].*

PAIN ITSELF MAY PROMOTE RELEVANT ALTERATIONS AT THE SYSTEMS LEVEL IN TERMS OF RECEPTOR AND EFFECTOR EXPRESSION AND FUNCTION

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

DEVELOPMENT OF BETTER AND SAFER THERAPEUTICS

The greater complexity of opioid receptor pharmacology

NEED FOR QUANTITATIVE UNDERSTANDING OF CHRONIC PAIN AND THE MECHANISMS OF DRUG ACTION IN THE BRAIN THAT RELIEVE PAIN IN A SYSTEMATIC AND/OR MECHANISM-BASED MANNER

NEED FOR MORE COMPREHENSIVE, INTEGRATED, NETWORK-CENTRIC APPROACHES TO FULLY DISSECT THE MULTIFACETED NETWORK OF SIGNALING EVENTS AND MOLECULAR PROCESSES UNDERLYING THERAPEUTIC AND ADVERSE EFFECTS

QUANTITATIVE SYSTEMS PHARMACOLOGY

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

BUILDING THE QSPainRelief PLATFORM

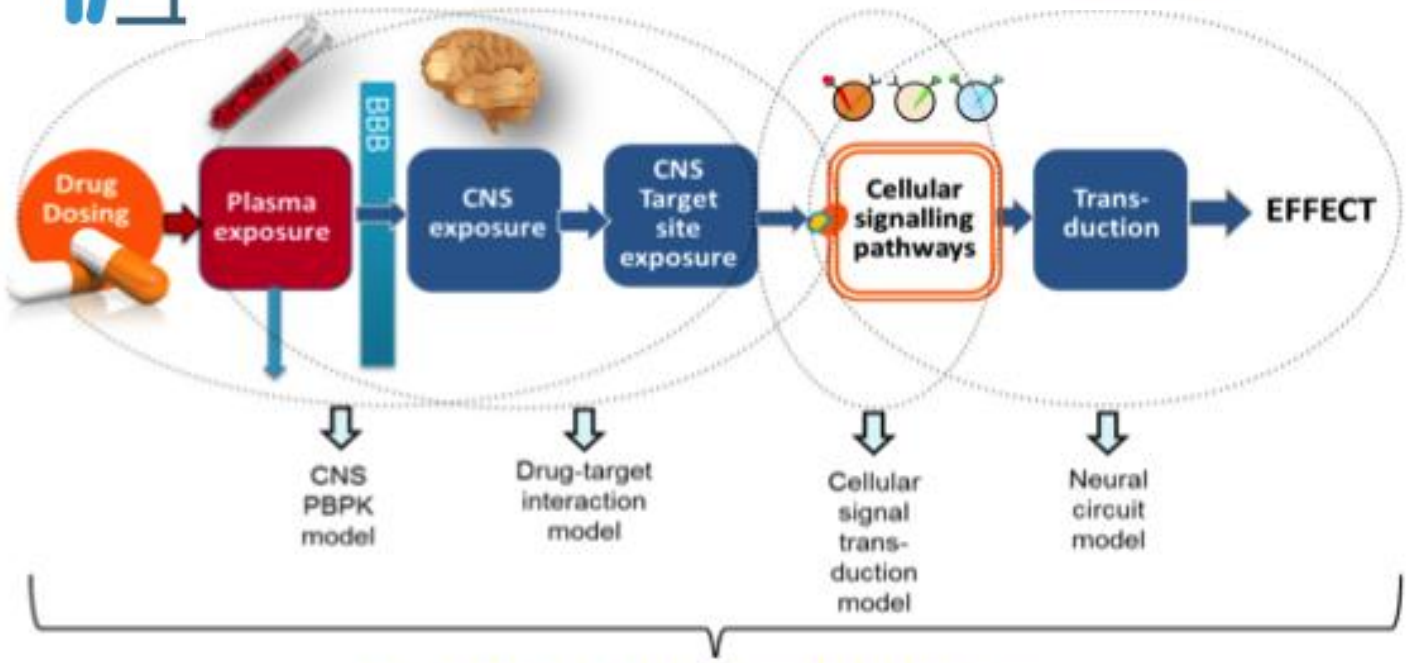
ASSEMBLING EXISTING AND NEW COMPUTATIONAL MODELS INTO A NOVEL PLATFORM EMPLOYING EXISTING AND NEWLY PRODUCED EXPERIMENTAL DATA AND PARAMETERS

Drug parameters (physio-chemical, PK, binding kinetic)
Systems parameters (species-specific)

Experimental **in vitro data** on pain/analgesia-related receptors/effectors **expression and activation**

Experimental **in vivo** data on analgesic vs adverse effects in an operant model of **neuropathic pain in mice**

Clinical data in **healthy volunteers** and in real-world **pain patients**



The QSPainRelief model platform

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

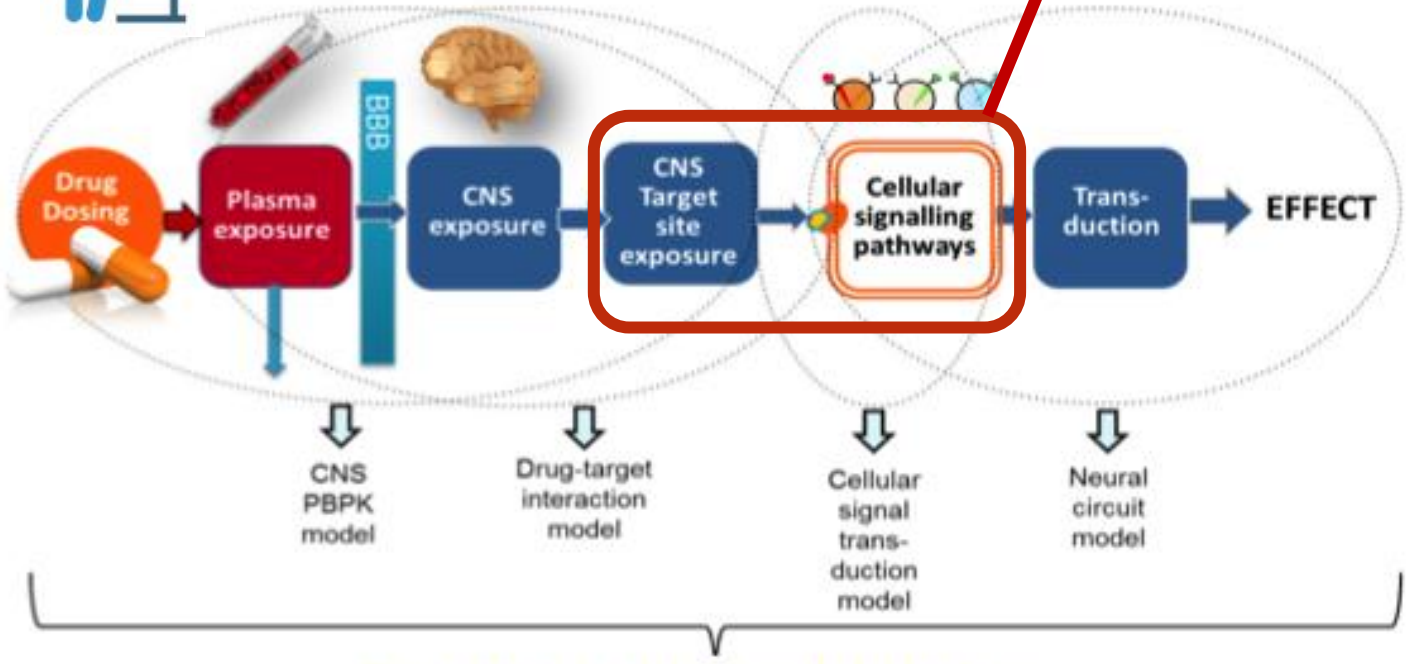
BUILDING THE QSPainRelief PLATFORM

ASSEMBLING EXISTING AND NEW COMPUTATIONAL MODELS INTO A NOVEL PLATFORM EMPLOYING EXISTING AND NEWLY PRODUCED EXPERIMENTAL DATA AND PARAMETERS

Drug parameters (physio-chemical, PK, binding kinetic)
Systems parameters (species-specific)

Experimental **in vitro data** on pain/analgesia-related receptors/effectors **expression and activation**

MOLECULAR PATHWAY ANALYSIS IN DIFFERENT IN VITRO NEURONAL CELL MODELS



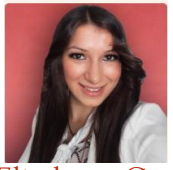
The QSPainRelief model platform

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

DRUG-MEDIATED EFFECTS ON MOR-CB1 INTERACTION AND MOR ACTIVATION

QSPainRelief

Ms. Berfin Gülave
LACDR
University of Leiden
The Netherland



Ms. Elisabetta Cuna

Prof. Monica Baiula

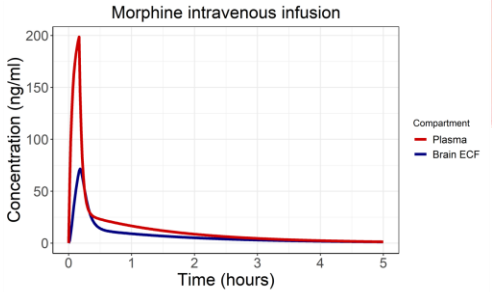


Figure 1. Simulated pharmacokinetic profile of morphine at plasma and brain extracellular fluid in humans, following i.v. infusion of 10 mg morphine over 10 minutes.

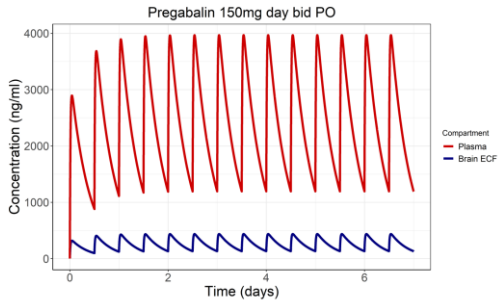
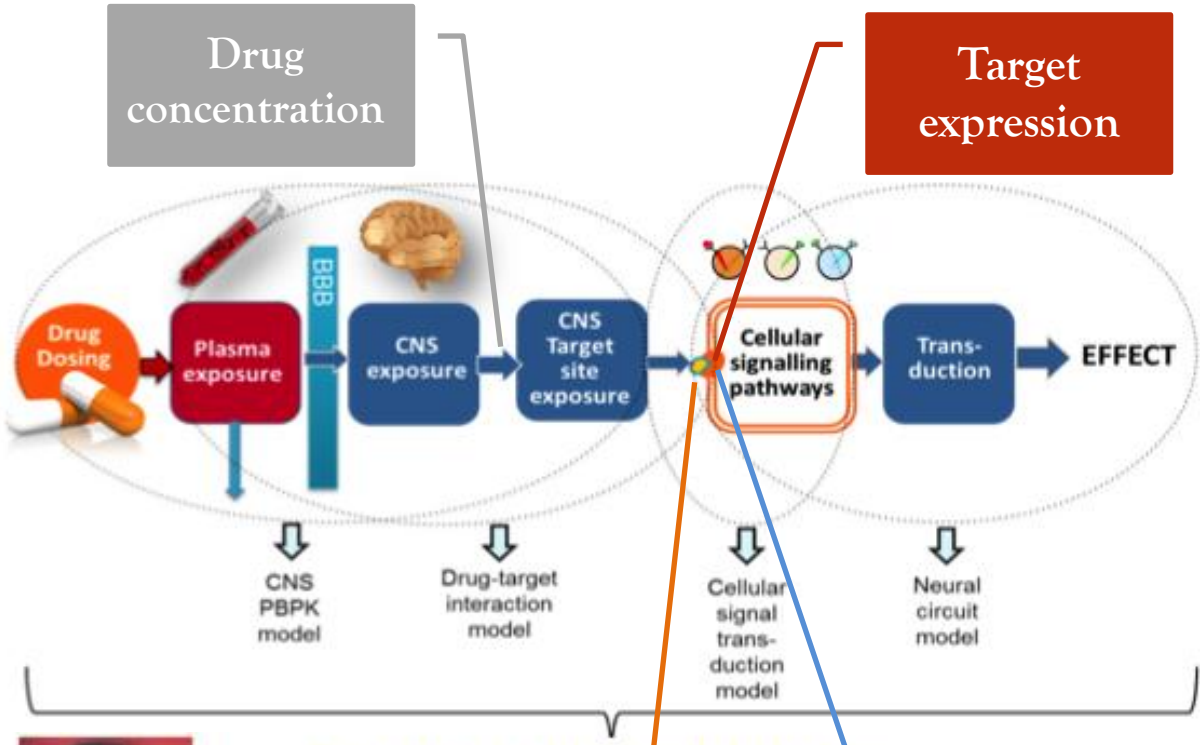
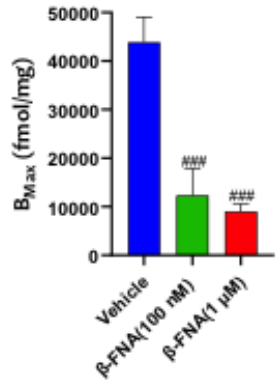
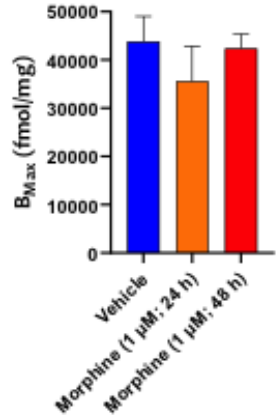


Figure 2. Simulated pharmacokinetic profile of pregabalin at plasma and brain extracellular fluid in humans, following oral administration of 150 mg pregabalin twice a day.



MOR => Mu opioid receptor, main target of opioid analgesics;
KOR => Kappa opioid receptor, involved in analgesic responses, secondary binding site for morphine (lower affinity as compared to MOR);
DOR => Delta opioid receptor, involved in analgesic and anxiolytic/antidepressant effects;
CB1 => Cannabinoid receptor type-1, involved in analgesic responses, possible interactions with opioid receptors (e.g.: signaling cross-talk, heterodimers);
D2R => Dopamine D2 receptor, involved in dopamine-mediated modulation of multiple processes including reward;
KCC2 => Potassium-chloride symporter, specifically expressed in neurons (CNS); expression and function altered in different chronic pain states;
α2δ => α2δ subunit of voltage-gated calcium channels, expressed in neurons (CNS), pharmacological target of pregabalin.





Institute of Neuroscience
Universidad Autonoma
de Barcelona
Barcelona (Spain)

Prof. Jesús Giraldo

Dr. Pedro Renault

The QSPainRelief model platform

Cooperativity/ cross-talk

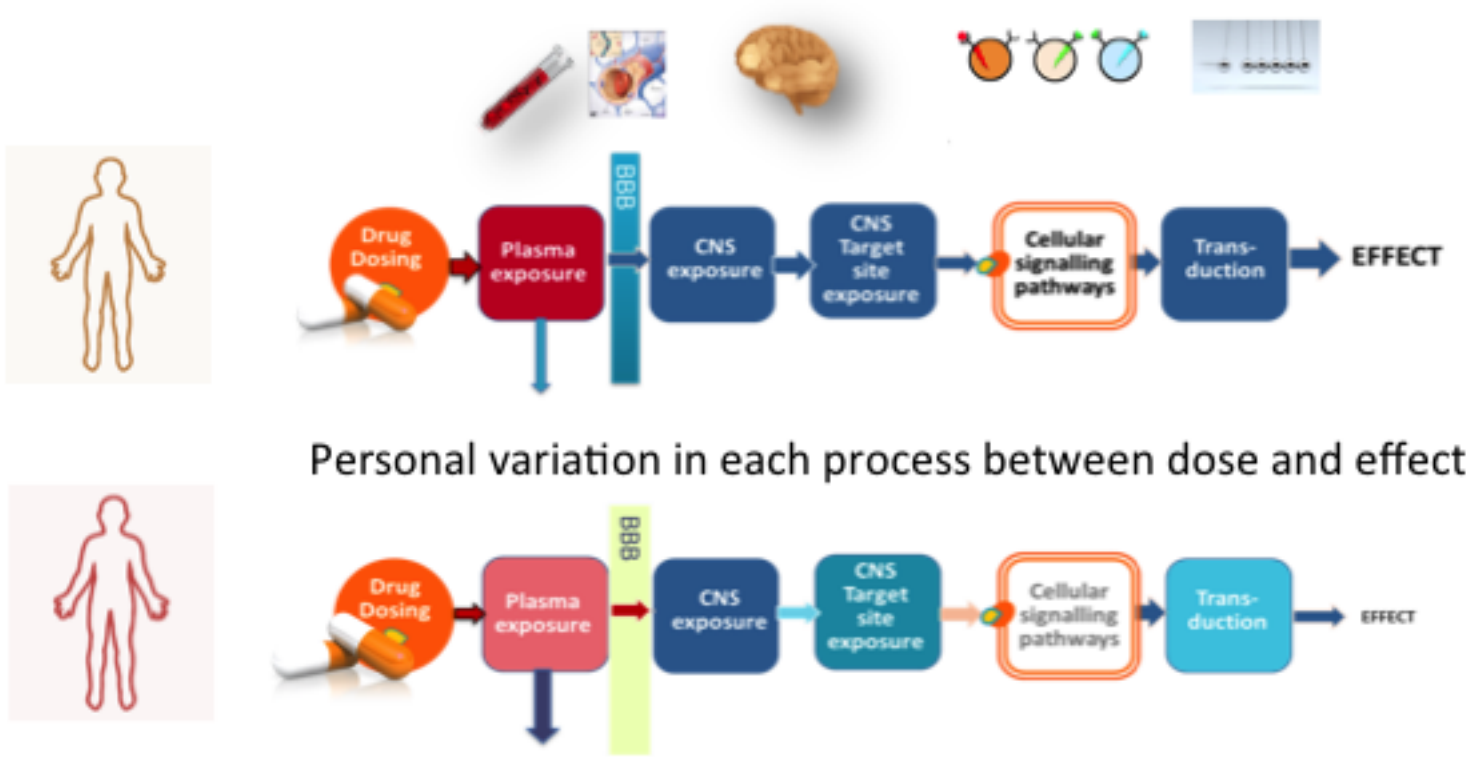
Receptor «activability»

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

QUANTITATIVE SYSTEMS PHARMACOLOGY

An intriguing approach to go beyond the complexity of opioid receptor pharmacology



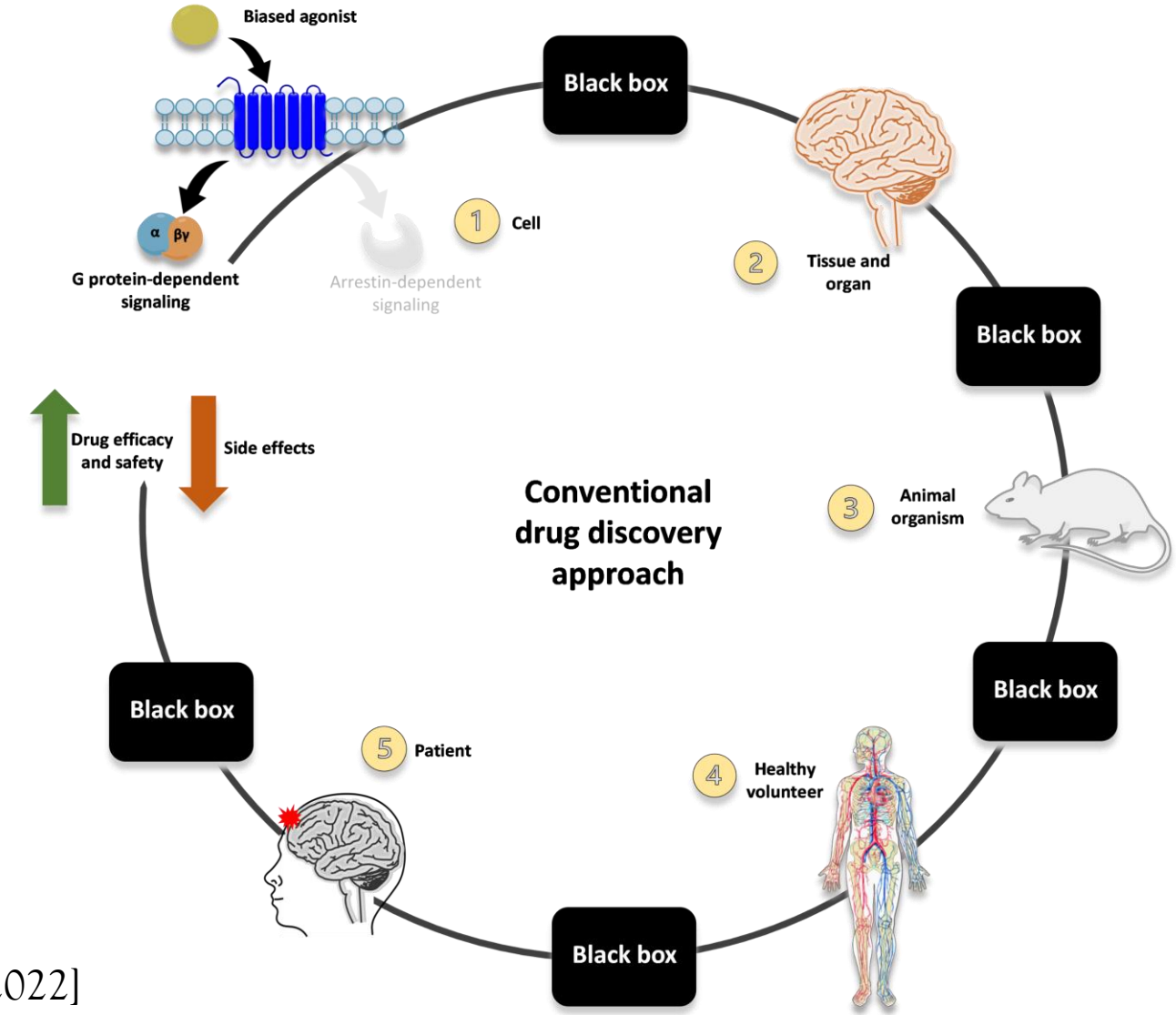
QSP platforms integrating at the systems level all the multiple determinants contributing to the multifaceted net-work of events
(including the multidimensional complexity of opioid receptors)
involved in chronic pain on one hand, and in analgesic and adverse effects of innovative drugs on the other

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

QUANTITATIVE SYSTEMS PHARMACOLOGY

An intriguing approach to go beyond the limitations of G protein biased analgesics



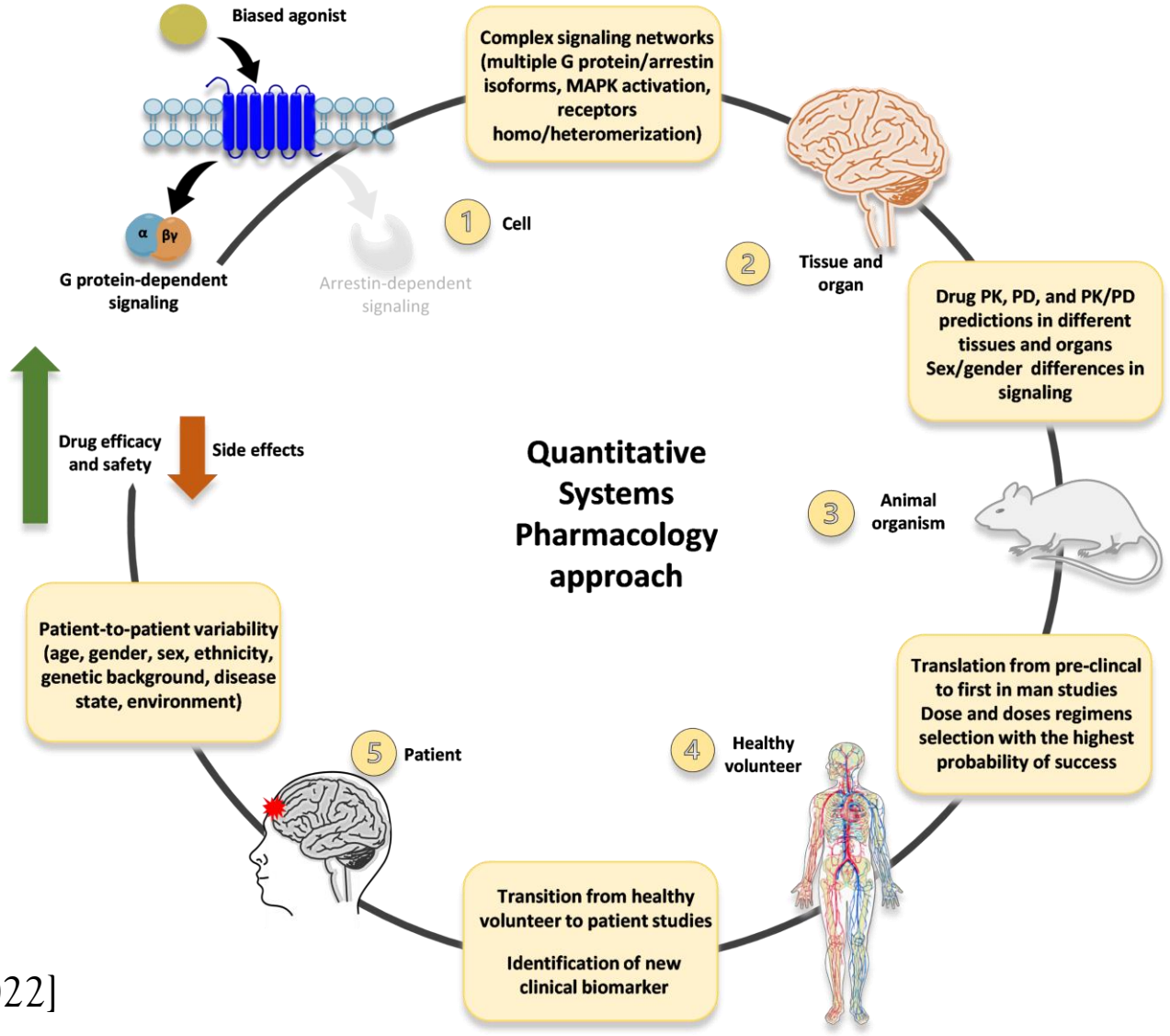
[Bedini et al., 2022]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

QUANTITATIVE SYSTEMS PHARMACOLOGY

An intriguing approach to go beyond the limitations of G protein biased analgesics



[Bedini et al., 2022]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

CONCLUDING REMARKS

- **Biased agonists** at MOR were believed to be **the key to improved analgesic**; now this is **highly debated**. We should learn from the paradigmatic story of TRV130.
- **Innovative KOR ligands** are emerging as promising candidates to **treat chronic pain and comorbid psychiatric disorders**; research efforts should be undertaken carefully and working hypothesis should be thoroughly validated.
- The **failure to translate** the intense endeavour of the last decades **into improved therapeutics** is indeed due to the complex multidimensional pharmacology of opioid receptors: the **multifaceted network** of signalling events and molecular processes underlying **therapeutic and adverse effects** induced by opioids requires more comprehensive, integrated, network-centric approaches to be fully dissected.
- **Quantitative Systems Pharmacology** is emerging as an intriguing approach to go beyond the greater complexity of opioid receptor pharmacology => **strong potential to significantly advance the quest for novel therapeutics with more favourable pharmacological profiles, due to the integration at the systems level of all the multiple determinants contributing to the multifaceted network of events involved in diseases onset and maintenance on one hand, and in therapeutic and adverse effects of innovative drugs on the other.**

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

CONCLUDING REMARKS

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

CONCLUDING REMARKS

Source: Shutterstock.com (royalty-free)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

IN MEMORIAM

Member of UNIBO team within QSPainRelief
Leader of Cellular and Molecular Pharmacology Unit
Director of Department of Pharmacy and Biotechnology
Career-long mentor

Prof. Santi Spampinato
(Catania, 7th May 1954 – Bologna, 20th November 2023

Strong and contagious passion for research.
Relentless dedication to teaching/mentoring generations of young researchers.
The deepest respect for colleagues and institutions.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

ACKNOWLEDGEMENTS

Cellular and Molecular Pharmacology Unit

Prof. Monica Baiula
Dr. Gabriele Campana
Ms. Elisabetta Cuna
Mr. Andrea Maurizio
Ms. Chiara Cimetti

Department of Chemistry
"G. Ciamician"
Selmi 2 - 40126 Bologna - Italy
Prof. Luca Gentilucci
Dr. Junwei Zhao
Mr. Marco Franescato

Department NEUROFARBA
University of Florence –
Florence, Italy
Prof. Carla Ghelardini
Prof. Lorenzo Di Cesare Mannelli
Dr. Laura Micheli

Universiteit Leiden
The Netherlands

Research Division of Systems Biomedicine and Pharmacology LACDR, Leiden University
Prof. Liesbeth de Lange
Ms. Berfin Gulave

UAB Universitat Autònoma de Barcelona

Institut de Neurociències and Unitat de Bioestadística
Prof. Jesus Giraldo
Dr. Pedro Renault

MOLLOY UNIVERSITY

Department of Biology, Chemistry, Earth & Environmental Science
Molloy University,
Rockville Center, NY (USA)
Dr. Brian Reed

Fondazione Umberto Veronesi
PER IL PROGRESSO DELLE SCIENZE

Horizon 2020 Programme

MIUR
MINISTERO DELL'ISTRUZIONE DELL'UNIVERSITA' E DELLA RICERCA

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 848068.

Thank you for your attention!!!