6th International Electronic Conference on Medicinal Chemistry

1-30 November 2020

sciforum.net/conference/ECMC2020

sponsored by pharmaceuticals

From LP2 to 2S-LP2: discovery of a biased dual-target mu/delta opioid receptor agonist for pain management

Rita Turnaturi[1*], Carmela Parenti[2], Girolamo Calò[3], Santina Chiechio[2], Agostino Marrazzo[1] and Lorella Pasquinucci[1]

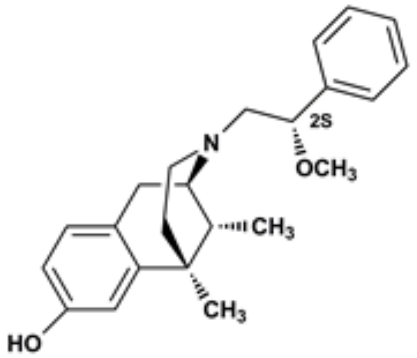
[1] Department of Drug Sciences, Medicinal Chemistry Section, University of Catania, Catania, Italy

[2] Department of Drug Sciences, Pharmacology and Toxicology Section, University of Catania, Catania, Italy

[3] Department of Medical Sciences, Pharmacology section, University of Ferrara, Ferrara, Italy

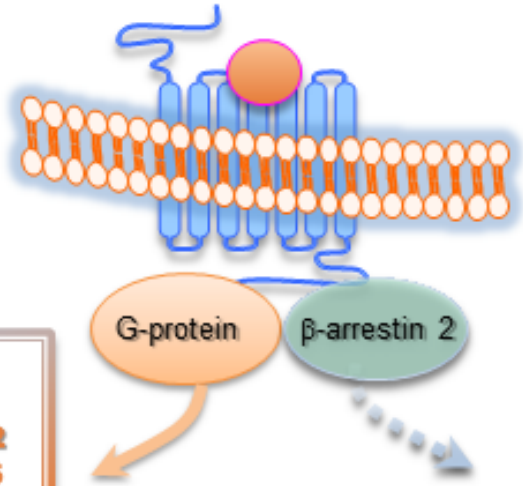
* Corresponding author: rita.turnaturi@unict.it

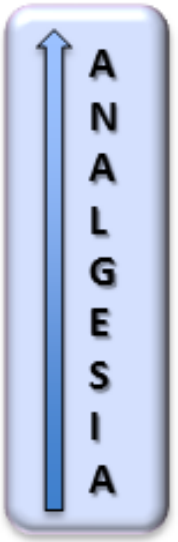
From LP2 to 2S-LP2: discovery of a biased dual-target mu/delta opioid receptor agonist for pain management



2S-LP2

MOR/DOR biased ligand





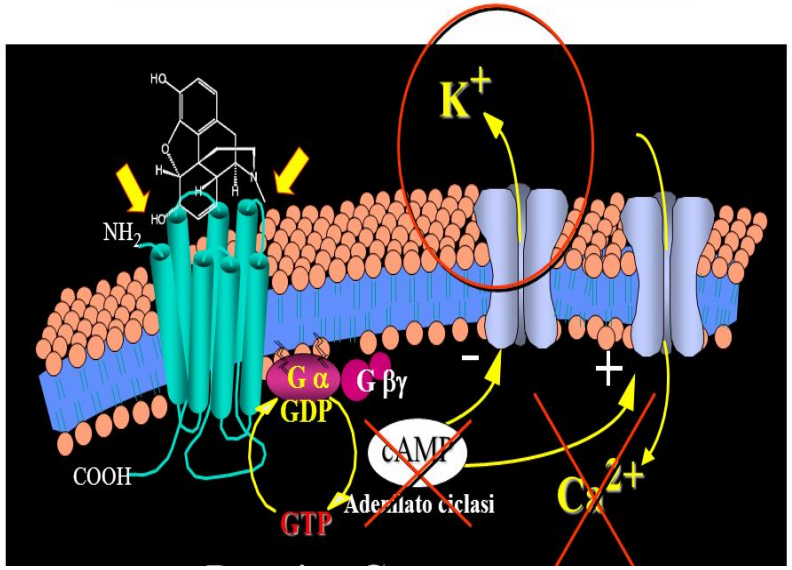
K_i^{MOR} = 0.50 nM
K_i^{DOR} = 2.59 nM
ED_{50} = 0.6 mg/kg

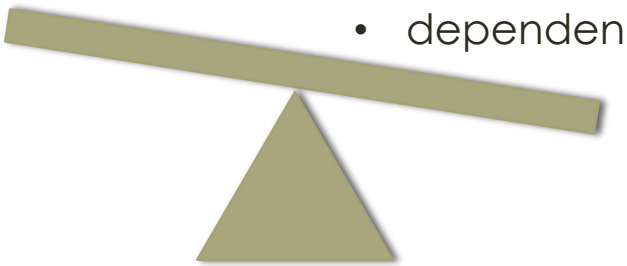
$pEC_{50}^{MOR/G\ protein}$ = 8.33
$pEC_{50}^{DOR/G\ protein}$ = 7.46
$pEC_{50}^{MOR/\beta\text{-}arrestin\ 2}$ = 6.82
$pEC_{50}^{DOR/\beta\text{-}arrestin\ 2}$ = 5.86

Abstract: Opioid analgesics, such as morphine, elicit analgesia primarily through mu opioid receptor (MOR), whose activation determines also side effects. Although indispensable for the management of acute severe pain, classical analgesics are unsuccessful for persistent pain treatment. MOR/delta opioid receptor (DOR) agonists represent a strategy to overcome the default in chronic pain treatment. In this context, we identified the dual-target ligand LP2 with high MOR (K_i= 1.08 nM) and DOR (K_i= 6.6 nM) affinity coupled to an agonist profile *versus* these receptors (IC_{50}^{MOR}= 21.5 nM and IC_{50}^{DOR}= 4.4 nM). In tail flick test, LP2 produced a long-lasting antinociception naloxone-reversed (ED_{50} of 0.9 mg/kg i.p.). Here, our efforts were focused on demonstrating whether the LP2 dual-target profile could be useful for persistent pain states. Thus, LP2 was evaluated in an animal model of inflammatory and neuropathic pain. Moreover, both 2*R*- and 2*S*-diastereoisomers of LP2 were synthesized and their pharmacological profile was compared each other and with LP2.
Specifically, 2*S*-LP2 showed an increased antinociceptive effect than LP2 consistent with the *in vitro* functional profile. Moreover, 2*S*-LP2 resulted a biased MOR/DOR agonist with functional selectivity for G-protein signaling and reduced β-arrestin 2 recruitment, an effectiveness profile in chronic pain conditions management.

Keywords: Pain; benzomorphan; dual-target; Mu opioid receptor; delta opioid receptor.

Opioids are the gold standard for the pain management



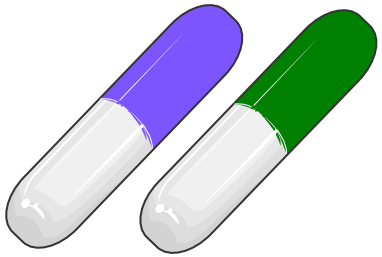


Multitarget opioid ligands

may be potential drugs for the pain management on the basis of their low propensity to induce side effects.

Turnaturi R. et al., Eur J Med Chem, 2016

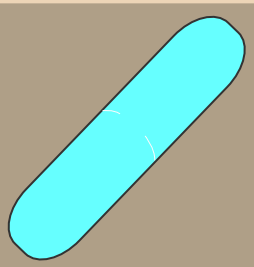
Multitarget therapy



drug cocktail

poor patient compliance

complex pharmacokinetic/pharmacodynamic relationships leading to unpredictable variability between patients



multitarget ligand

lower risk of drug-drug interaction

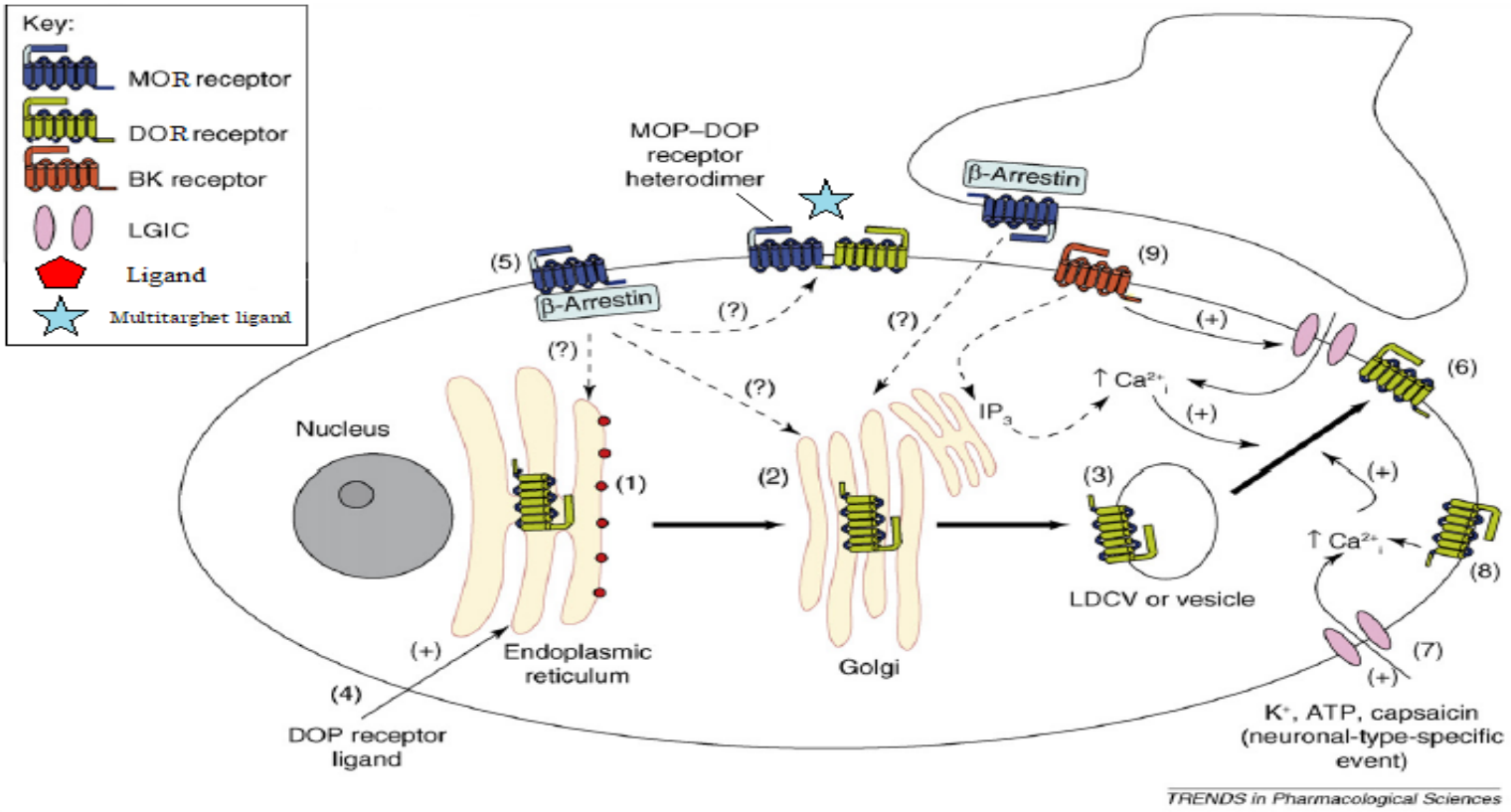
development of agents that modulate multiple targets simultaneously, with the aim of enhancing efficacy or improving safety

Dietis N, et al. British Journal of Anaesthesia, 2009
Turnaturi R et al. European Jounral of Medicinal Chemistry, 2016
Turnaturi R et al. Current Medicinal Chemistry, 2016.

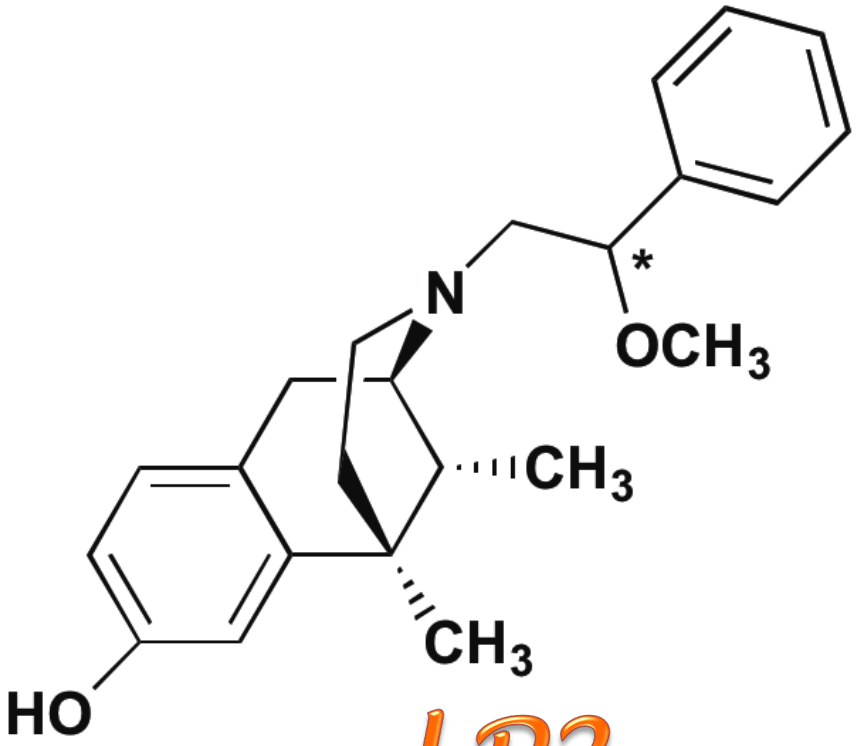
MOR agonist + DOR agonist

increased analgesia with an improved side-effect profile

Balboni G,et al. ACS Chem Neurosci. 2010.
Purington LC, et al. ACS Chem Biol 2011.
Daniels D.J., et al. Procl Natl Acad Sci USA 2005.



Benzomorphan-based compound



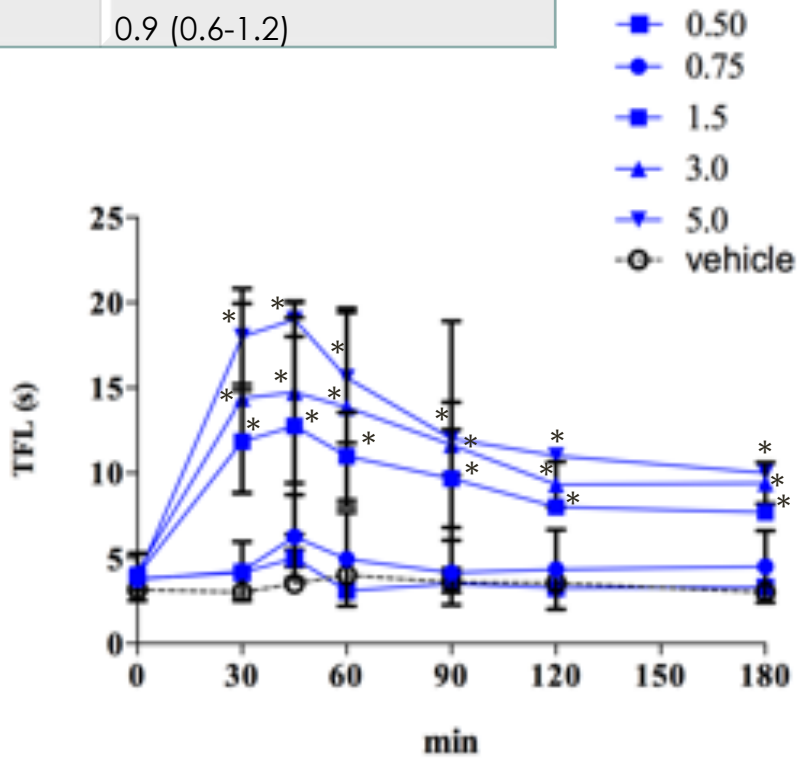
LP2

- affinity vs MOR K_i= 1.08 nM and DOR K_i= 6.6 nM
- agonist functional activity at MOR IC_{50}= 21.5 nM in GPI and DOR IC_{50}= 4.4 nM in MVD

Pasquinucci L, et al. Bioorg Med Chem, 2017

LP2 antinociceptive effect

Compound	ED_{50} mg/kg ip (C.L.)
LP2	0.9 (0.6-1.2)



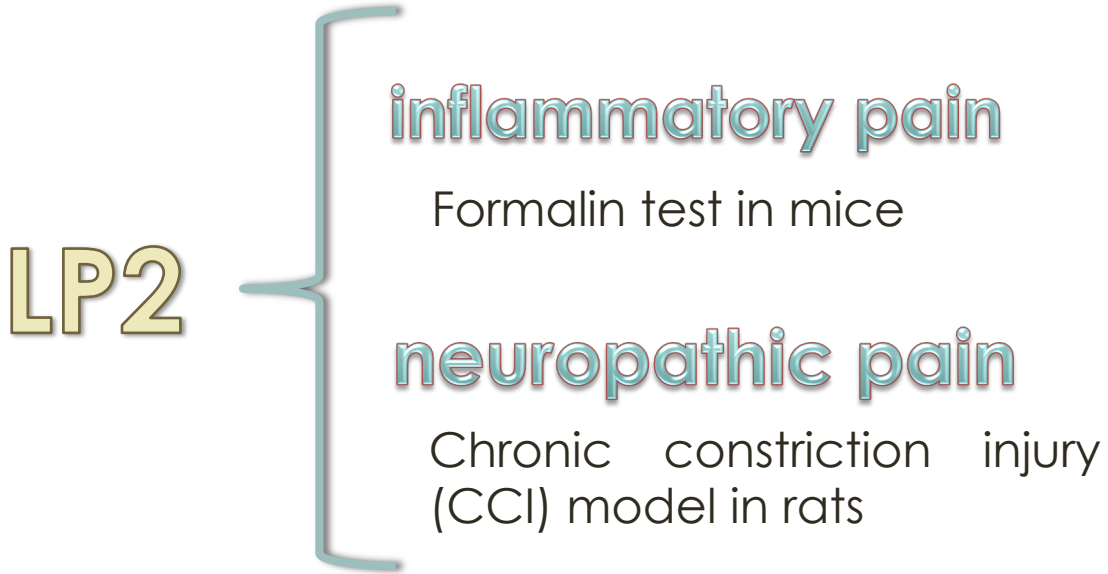
*P<0.05 vs saline treated mice

LP2 elicits a long-lasting antinociceptive effect analgesic effect naloxone-reversed.

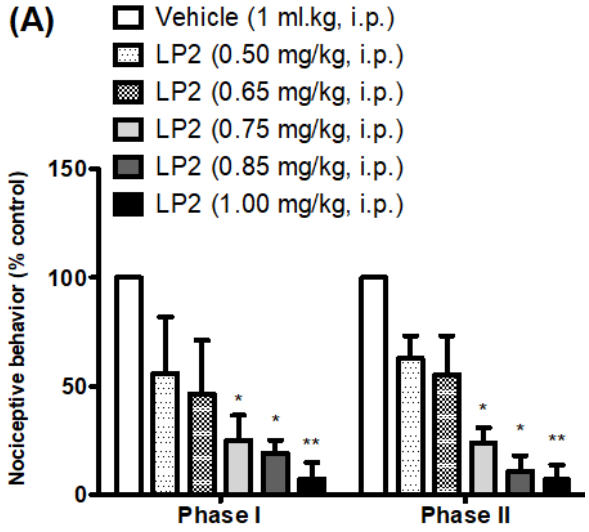
Pasquinucci L, et al. Bioorg Med Chem, 2017

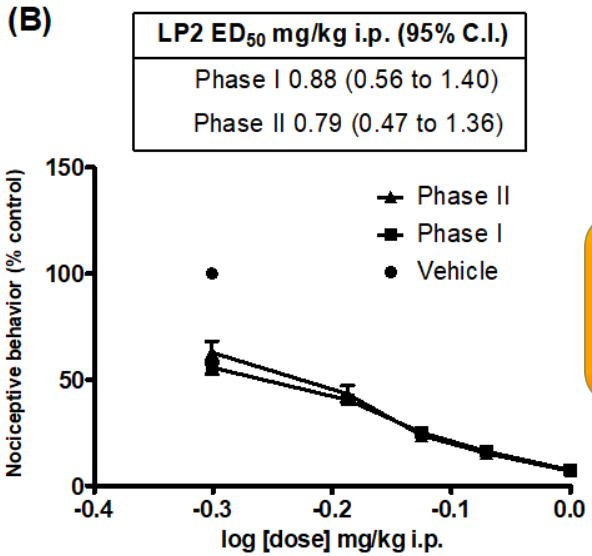
- LP2 elicits a significant long-lasting antinociceptive effect opioid receptor-mediated
- LP2 acts as a dual-target opioid ligand combining a potent MOR–DOR agonist activity.

These results emphasize its potential application for **persistent pain treatment**.



Inflammatory pain in mice



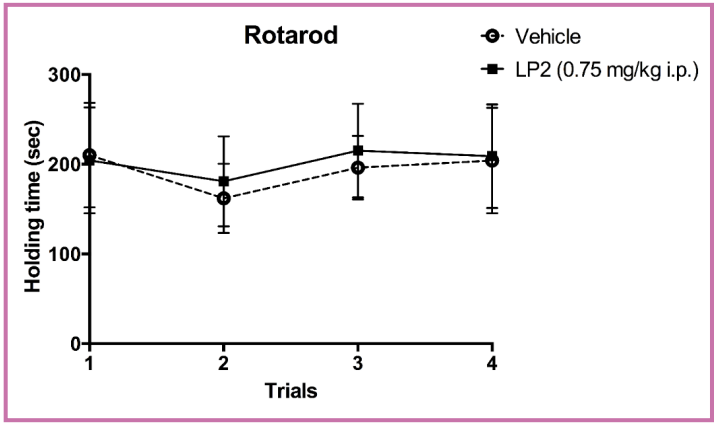


Formalin test in mice

Formalin induces a biphasic response

LP2 dose-dependently reduced both phases of the formalin test.

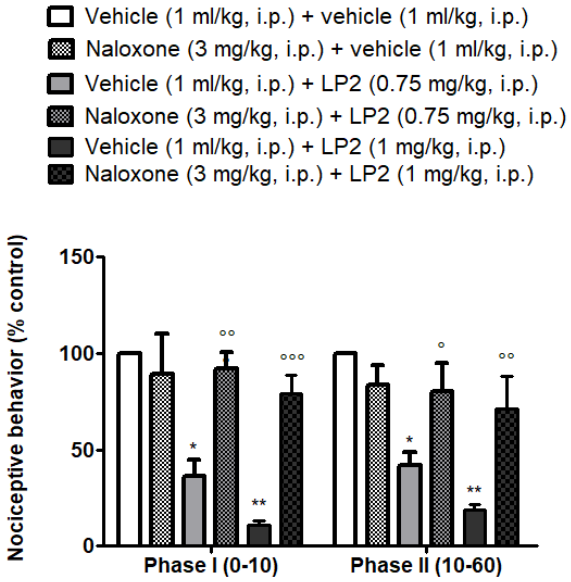
LP2. at the doses of 0.75 mg/kg i.p did not affect motor performance

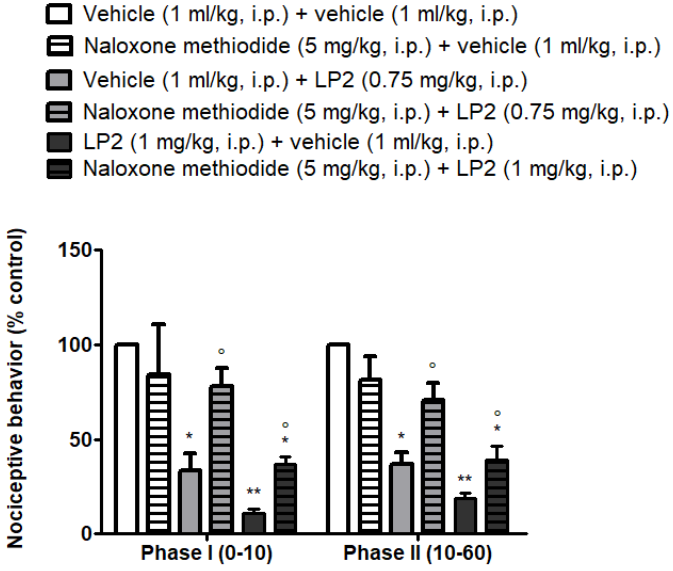


Pasquinucci L, et al. Eur.J. Pharm: 2019

Inflammatory pain in mice

to evaluate whether LP2 analgesic effect is mediated in the central or peripheral nervous system mice were pretreated with naloxone or naloxone methiodide

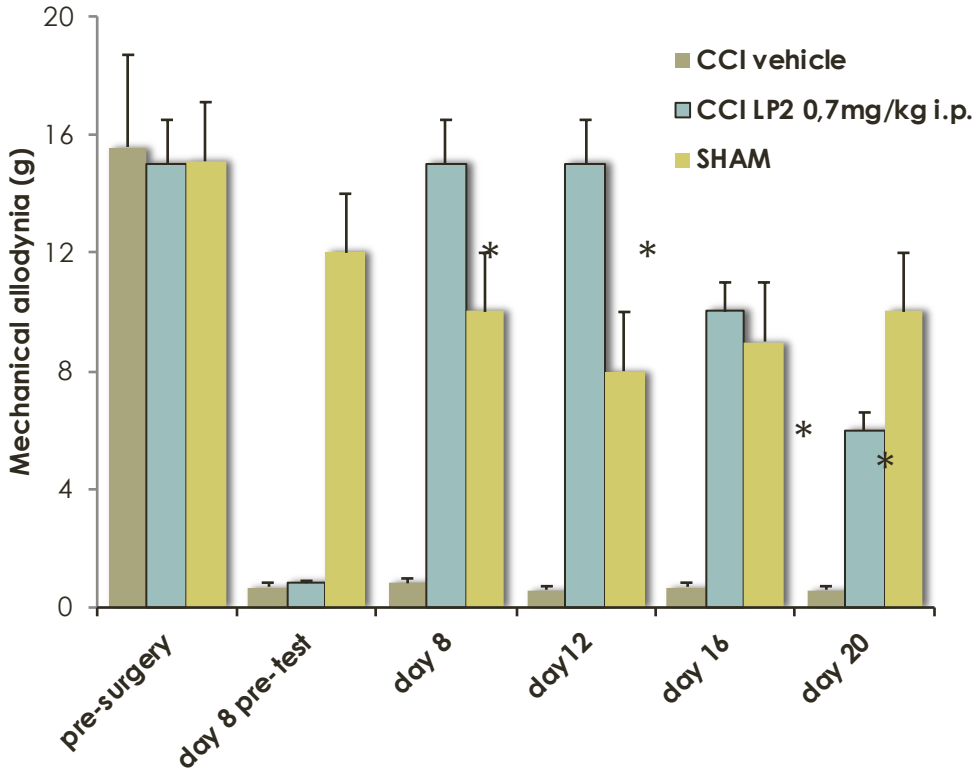




naloxone significantly antagonized the antinociceptive effects of LP2 in both phases.

naloxone methiodide significantly antagonized the analgesic effect of 0.75 mg/kg but not 1.0 mg/kg of LP2 in both phases

Neuropathic pain in CCI rats



Injured rats received vehicle or LP2 (0.7 mg/kg i.p.) for 12 days (from day 8 to day 20 post surgery).

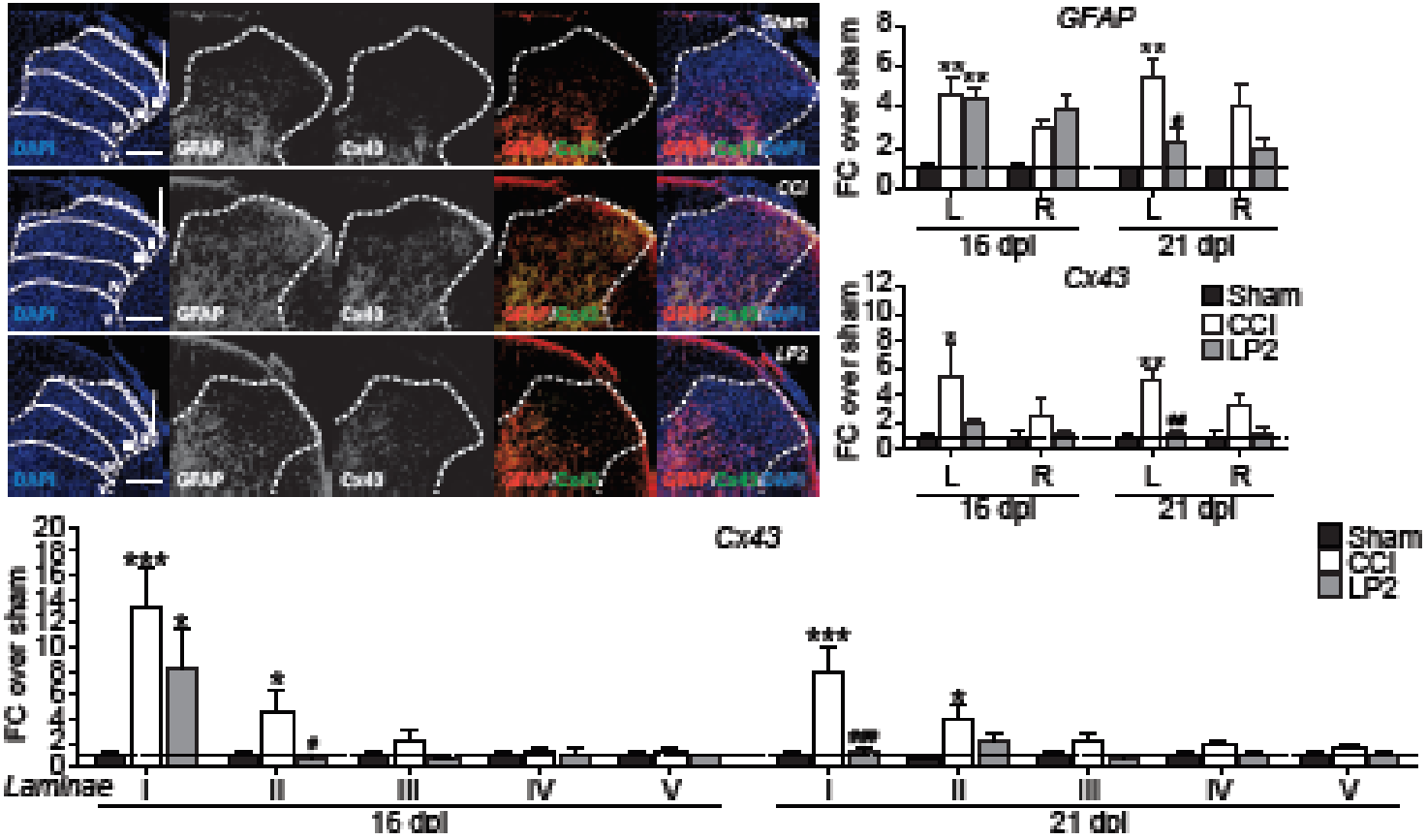
The effect of LP2 was assessed on tactile allodynia 45 min post i.p. injection

LP2 mediated significant anti-allodinic effects

Vicarioi N et al. Mol Neurobiol. 2019

Vicarioi N et al. Mol Neurobiol. 2019

Neuropathic pain in CCI rats

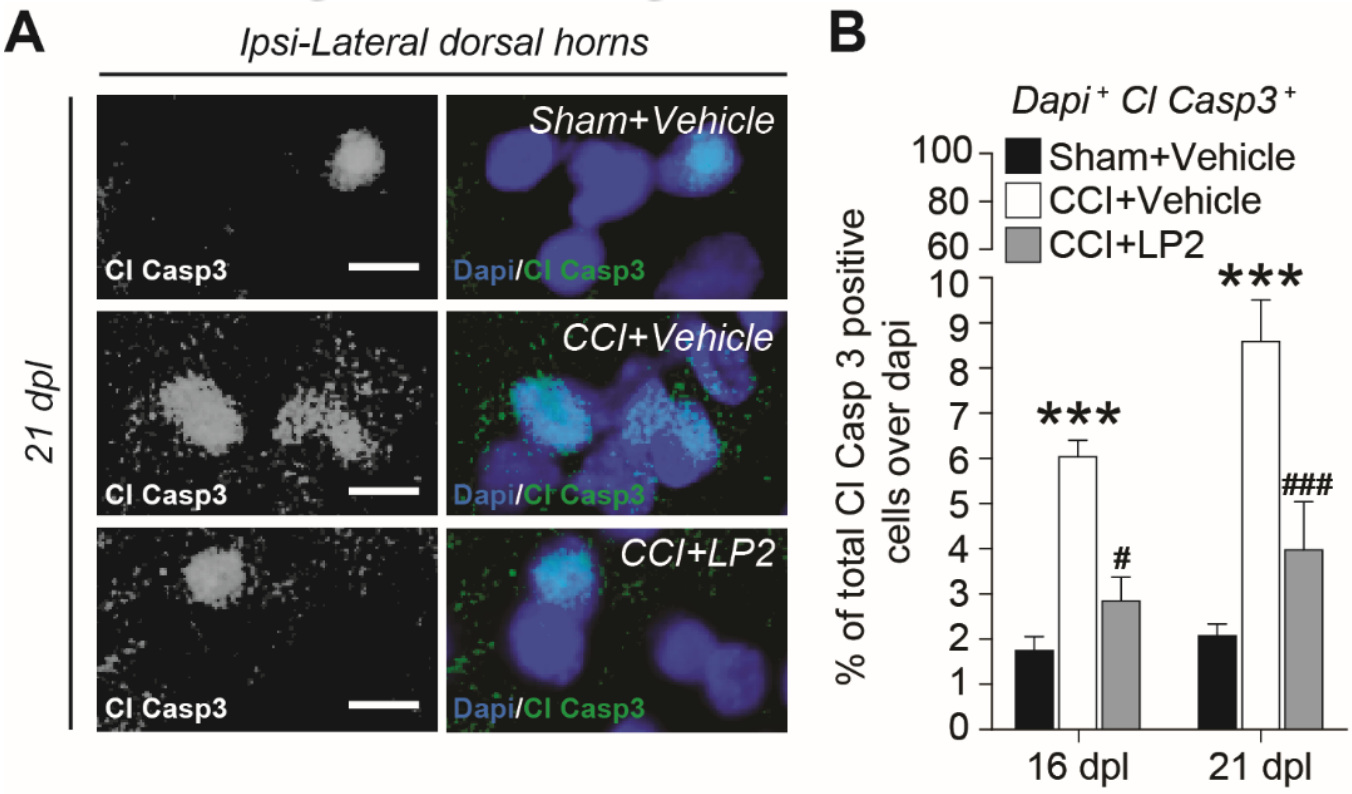


in CCI injured rats MOR/DOR agonist LP2 mediates a significant reduction of GFAP and Cx43 levels in laminae I and II

Vicarioi N et al. Mol Neurobiol. 2019

Vicarioi N et al. Mol Neurobiol. 2019

Neuropathic pain in CCI rats



MOR/DOR agonist LP2 induced a significant reduction of cleaved caspase 3 positive cells in ipsilateral dorsal horns of CCI rats

Vicarioi N et al. Mol Neurobiol. 2019

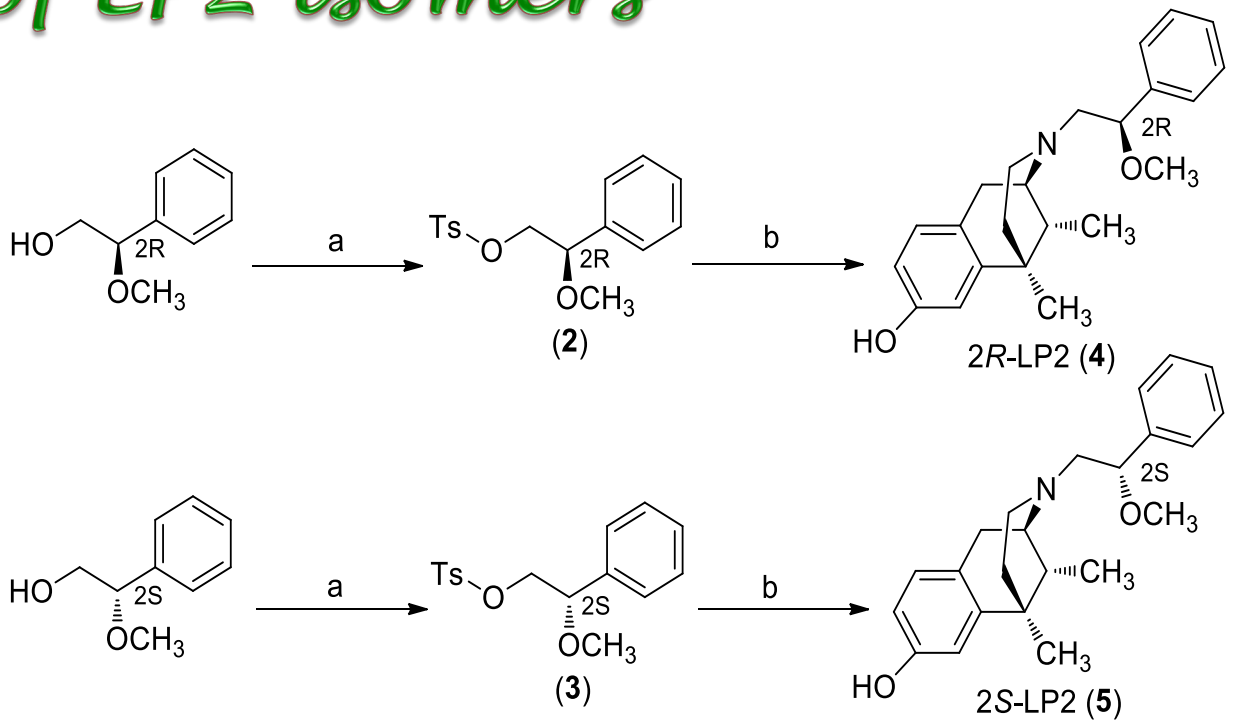
Vicarioi N et al. Mol Neurobiol. 2019

Stereoselective target interaction of LP2 isomers

Stereocenter role

Stereoselective drug-target interaction

Asymmetric synthesis



Reagents and conditions: a) TsCl, TEA, Bu_2SnO, CH_2Cl_2, rt, 3 h; b) (-)-*cis*-*N*-normetazocine, $NaHCO_3$, KI, DMF, 65 °C, 24 h.

Pasquinucci L, et al. Eur.J. Med Chem: 2019

In vitro assay: competition binding at MOR, DOR and KOR

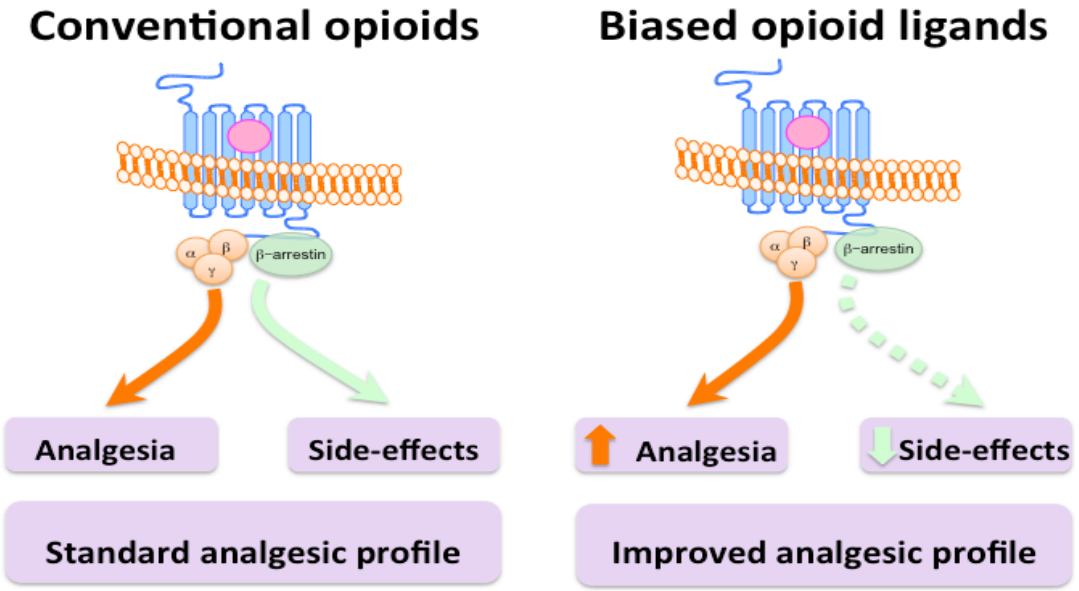
Compd	K_i (nM) ± SEM [a,b]			K_i ratio	
	MOR	DOR	KOR	DOR/MOR	KOR/MOR
LP2 (1)	1.08 ± 0.10	6.60 ± 0.60	15.22 ± 0.80	6.11	14.10
2R-LP2 (4)	12.30 ± 0.42	151.10 ± 0.60	236.00 ± 0.83	12.28	19.20
2S-LP2 (5)	**0.50 ± 0.03**	**2.59 ± 0.05**	**26.50 ± 0.44**	**5.18**	**53.00**

[a] Values are means ± SEM of three separate experiments, each carried out in duplicate. [b] K_i values were obtained as [^{3}H]DAMGO displacement for MOR, [^{3}H]DPDPE displacement for DOR, and [^{3}H]U69,593 displacement for KOR, using nonlinear regression analysis (GraphPad Prism).

2S-LP2 showed an improved MOR and DOR affinity profile in comparison to its 2R-antipode and LP2

Pasquinucci L, et al. Eur.J. Med Chem: 2019

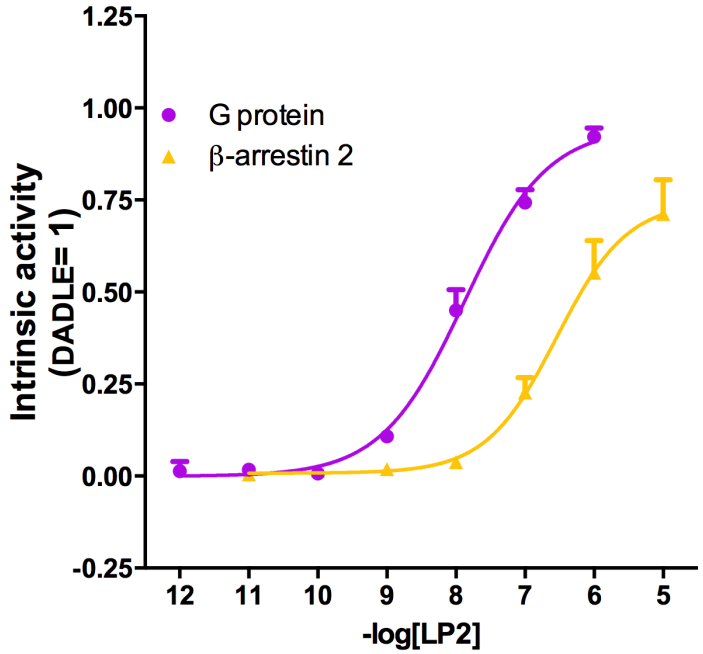
Functional activity at MOR and DOR by BRET assay



It has been demonstrated the biased opioid ligand potential to be more effective antinociceptive drugs with fewer side-effects.

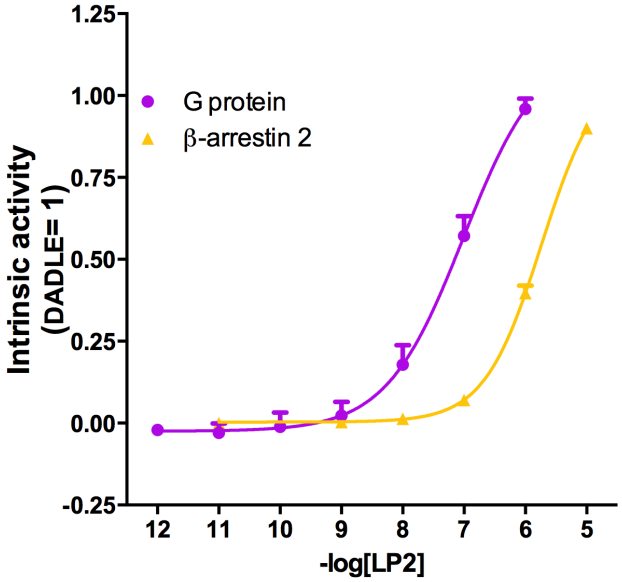
Violin, J.D. *et al. Trends Pharmacol. Sci. 2014*
Turnaturi R et al. Eur J Med Chem, 2019

Functional activity at MOR by BRET assay



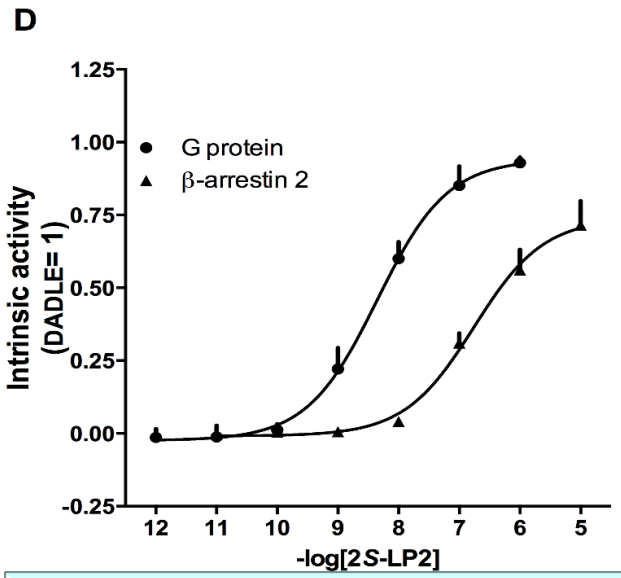
	MOR/G protein		MOR/ β-arrestin 2		
	pEC_{50} ($CL_{95\%}$)	α ± SEM	pEC_{50} ($CL_{95\%}$)	α ± SEM	Bias factor (CL95%)
LP2	7.90 (7.51-8.30)	0.92 ± 0.02	6.56 (6.22-6.90)	0.71 ± 0.09	0.57 (0.03-1.16)
DADLE	6.89 (6.56-7.22)	1.00	5.86 (5.71-6.01)	1.00	0

Functional activity at DOR by BRET assay



	DOR/G protein		DOR/ β-arrestin 2		
	pEC_{50} ($CL_{95\%}$)	α ± SEM	pEC_{50} ($CL_{95\%}$)	α ± SEM	Bias Factor (CL95%)
LP2	7.02 (6.83-7.21)	0.96 ± 0.03	5.65 (5.48-5.82)	0.90 ± 0.01	2.03 (1.57-2.49)
DADLE	6.89 (6.56-7.22)	1.00	5.86 (5.71-6.01)	1.00	0

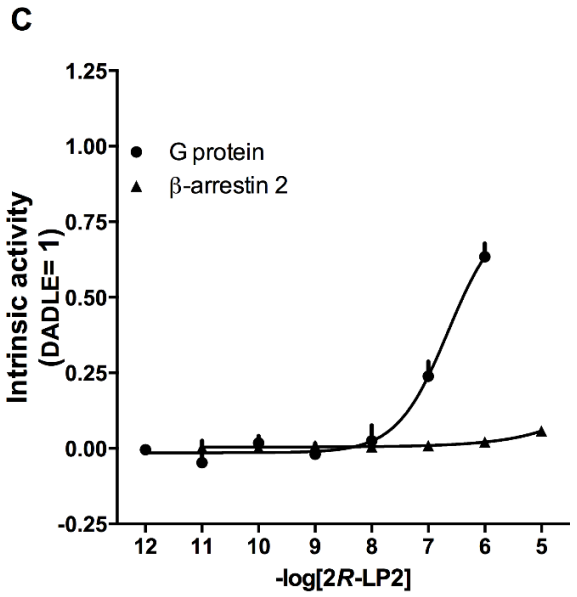
Functional activity at MOR by BRET assay



2*S*-LP2 promoted MOR/G-protein interaction with pEC_{50}= 8.33

A weak MOR/β-arrestin 2 interaction pEC_{50}= 6.82 was recorded for LP2

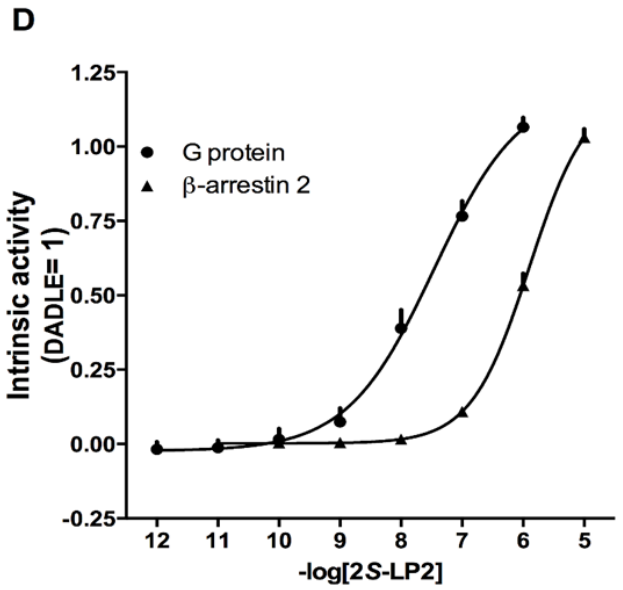
BIAS FACTOR 0.82



2*R*-LP2 was able to elicit a weak stimulatory response only at the highest concentration tested

Pasquinucci L, et al. Eur.J. Med Chem: 2019

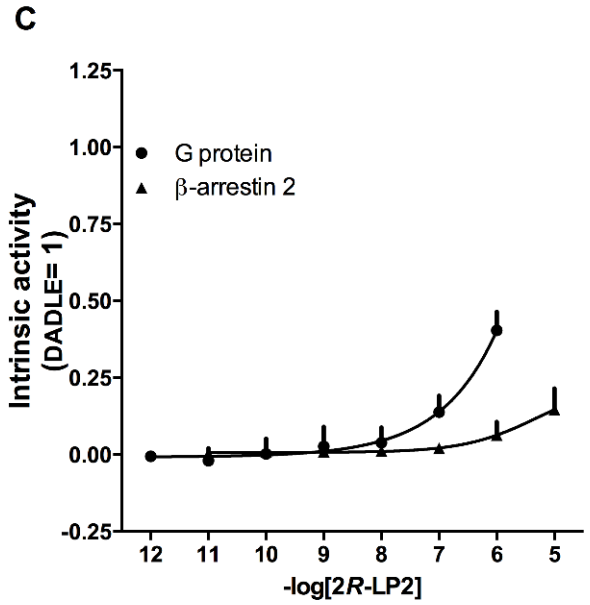
Functional activity at DOR by BRET assay



2*S*-LP2 promoted DOR/G-protein interaction with pEC_{50}= 7,49

A weak MOR/β-arrestin 2 interaction pEC_{50}= 5,73 was recorded for LP2

BIAS FACTOR 2,31

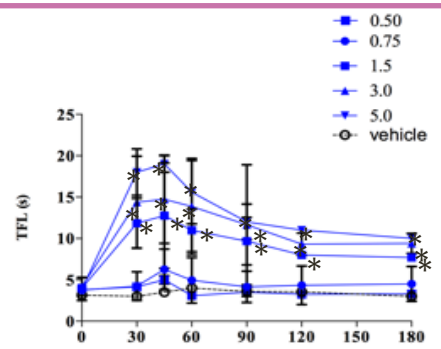


2*R*-LP2 was able to elicit a weak stimulatory response only at the highest concentration tested

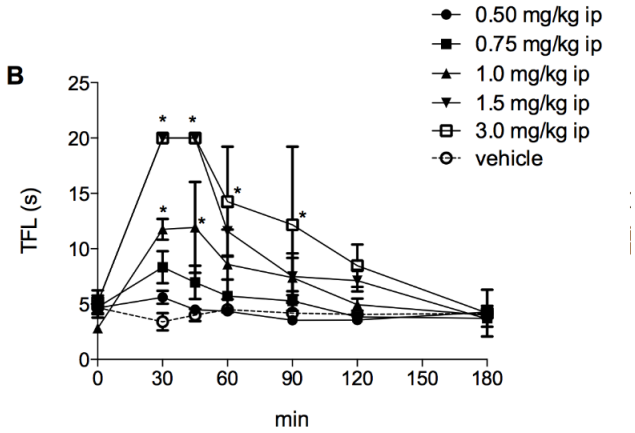
Pasquinucci L, et al. Eur J Med Chem: 2019

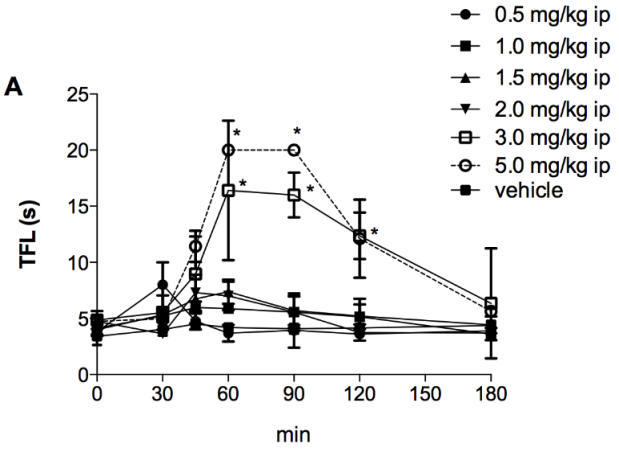
Pasquinucci L, et al. Eur.J. Med Chem: 2019

2R- and 2S-LP2 antinociceptive effect



LP2
ED_{50} of 0.9 mg/kg ip
(0.6-1.2, $CL_{95\%}$)





2S-LP2 ED_{50} of 0.6 mg/kg i.p. (0.4-0.8, $CL_{95\%}$)

2R-LP2 ED_{50} of 1.8 mg/kg ip (1.2-2.8, $CL_{95\%}$)

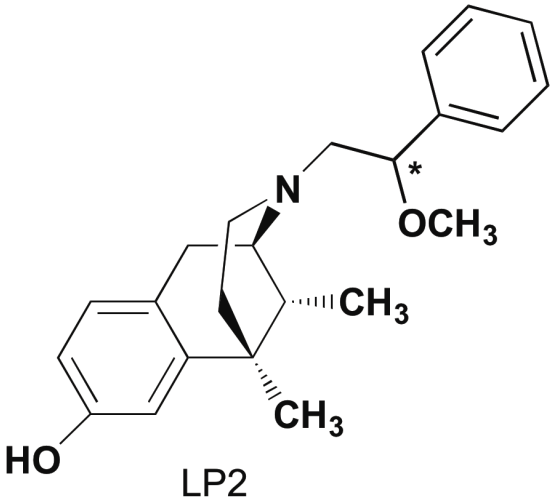
2S-LP2 resulted 3 times 1,5 times more potent than 2R-LP2 and LP2, respectively.

Pasquinucci L, et al. Eur.J. Med Chem: 2019

conclusions

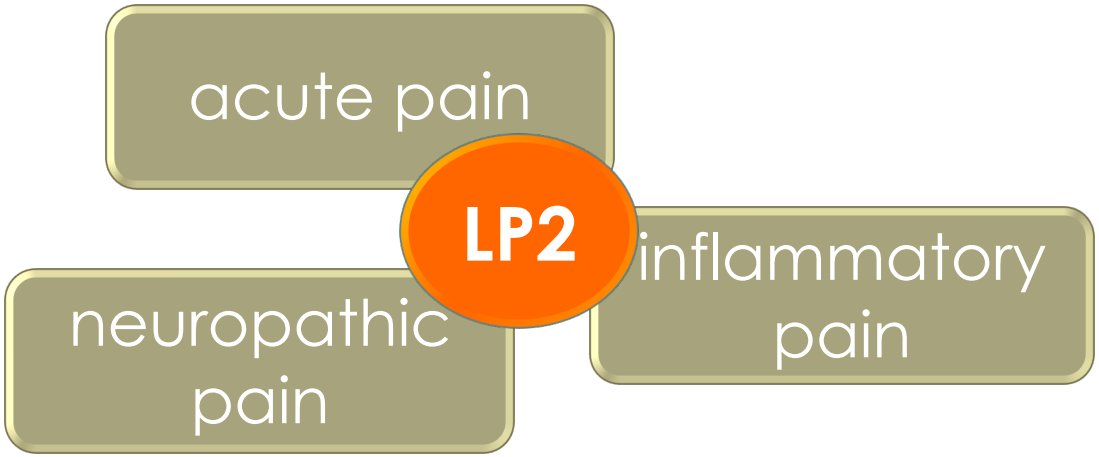
LP2

Dual-target MOR-DOR agonist



LP2

significantly reduced pain behaviors in neuropathic and in inflammatory pain



conclusions

2S-LP2

Dual-target biased MOR-DOR agonist

Improved MOR and DOR affinity profile

Improved antinociceptive profile

conclusions

LP2 and 2*S*-LP2 effects could be a consequence of

simultaneously targeting of MOR and DOR

Cahill et al., 2007; Wang et al., 2010; Zhang et al., 2006
Zhang and Pan, 2010

functional selectivity (biased agonist)

Violin JD et al. 2014,

pronounced DOR agonist profile

Acknowledgements:

Prof. Santina Chiechio

Prof. Carmela Parenti

Prof. Lorella Pasquinucci

Prof. Girolamo Calò

Dr. Nunzio Vicario

....and you for your kind attention!