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Novel *N*-substituted benzomorphan-based compounds: from MOR-agonist/DOR-antagonist to biased/unbiased MOR agonists.

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ABSTRACT: Modifications at the basic nitrogen of the benzomorphan scaffold allowed the development of compounds able to segregate physiological responses downstream of the receptor signaling, opening new possibilities in opioid drug development. Alkylation of the phenyl ring in the *N*-substituent of the MOR-agonist/DOR-antagonist LP1, resulted in retention of MOR affinity. Moreover, derivatives **7a**, **7c** and **7d** were biased MOR agonists towards ERK1,2 activity stimulation, whereas derivative **7e** was a low potency MOR agonist on adenylate cyclase inhibition. They were further screened in the mouse tail flick test and PGE2-induced hyperalgesia and drug-induced gastrointestinal transit.

During the last decade efforts were made to develop effective multitarget opioid ligands as an alternative strategy to overcome the typical side effects associated to opioid selective agonists.[1-3] For instance, valid analgesic effect with lower propensity to produce tolerance and physical dependence was reported for both dual MOR/DOR agonist[4-6] and MOR agonist/DOR antagonist ligands given in persistent pain models.[7-9]

Recently, the concept of biased agonists,[10] able to differentially activate GPCR downstream pathways, became a new approach in the design of novel drug candidates. It was reported that opioid compounds promoting G-protein signaling produce analgesia, while β-arrestin recruitment is responsible for opioid side effects such as constipation.[11-13]

Benzomorphan nucleus represents a versatile template[14,15] for the development of a specific functional profile by modifying *N*-substituent or 8-OH group. In this context, the introduction of a tertiary *N*-Methyl-*N*-phenylethylamino group as *N*-substituent conferred a MOR agonist profile *in vitro* and *in vivo* (**1**, Figure 1).[16] The replacement of the *N*-ethylamino spacer with the *N*-acetamido one was detrimental for MOR, DOR and KOR recognition,[17] while an *N*-propanamido spacer improved the opioid binding profile. In particular, LP1 (**2**, Figure 1), with an *N*-phenylpropanamido substituent, resulted *in vitro* and *in vivo* a potent MOR agonist/DOR antagonist[18] able to counteract nociceptive pain and behavioral signs of persistent pain with low tolerance-inducing capability.[19,20] The phenyl replacement with the bulkier *N*-naphthyl ring (**3**, Figure 1), switched the MOR efficacy profile from agonism to antagonism.[21] Analogously, the increased steric hindrance of the aromatic moiety with indoline, tetrahydroquinoline or diphenylamine group affected the shift from MOR agonism to antagonism.[22] More recently, a dual MOR/DOR agonist, endowed of a significant long-lasting antinociceptive effect,[23-25] was developed through the introduction of the short and flexible 2*R*/*S*-methoxy ethyl spacer as *N*-substituent (LP2 **4**, Figure 1). Moreover, the 2*S* diastereoisomer of LP2 was found a potent G-protein biased MOR/DOR agonist with a 3-times lower ED_{50} value.[26]

Since minor structural modifications often result in significant changes in the pharmacological profile of opioid ligands, we expanded our SAR studies by the synthesis of LP1 derivatives **7a-e**, variously alkylated at the phenyl ring of the *N*-propanamido substituent, and **11a-e**, featured also by a tertiary *N*-propanamido substituent. Finally, derivatives **14a-c**, bearing a secondary or tertiary *N*-ethylamino spacer, were synthesized (Figure 1).

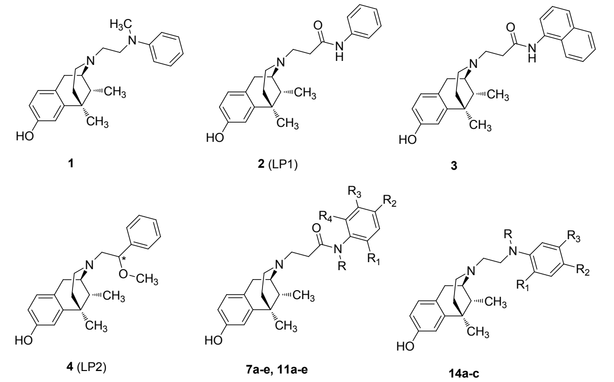
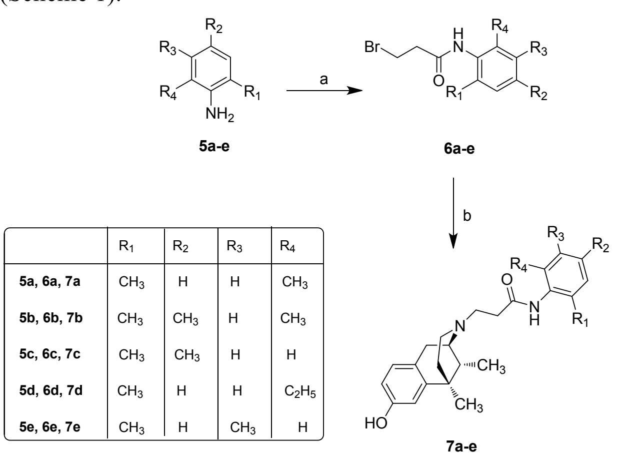


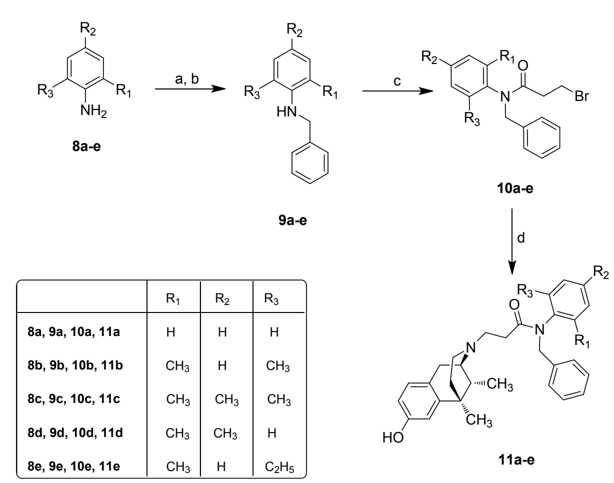
Figure 1. Benzomorphan-based compounds structures.

According to the previously reported method,[17,28] we prepared derivatives **7a-e**, **11a-e** and **14a-c** as reported in Schemes 1-3. After *cis*-(±)-*N*-normetazocine resolution,[27] the target compounds **7a-e** were obtained by alkylation of *cis*-(−)-(1*R*,5*R*,9*R*)-*N*-normetazocine with the respective amides **6a-e** (Scheme 1).



	R_1	R_2	R_3	R_4
5a, 6a, 7a	CH_3	H	H	CH_3
5b, 6b, 7b	CH_3	CH_3	H	CH_3
5c, 6c, 7c	CH_3	CH_3	H	H
5d, 6d, 7d	CH_3	H	H	C_2H_5
5e, 6e, 7e	CH_3	H	CH_3	H

Scheme 1. Synthesis of *N*-substituted normetazocine derivatives **7a-e**. Reagents and conditions: a) 3-bromopropionyl chloride (1.5 eq), 4-(dimethylamino)pyridine (DMAP) (0.47 eq), dry THF, rt, 3h; b) (–)-*cis*-(1*R*,5*R*,9*R*)-*N*-normetazocine (1 eq), $NaHCO_3$ (1.5 eq), KI, DMF, 65 °C, 20 h.

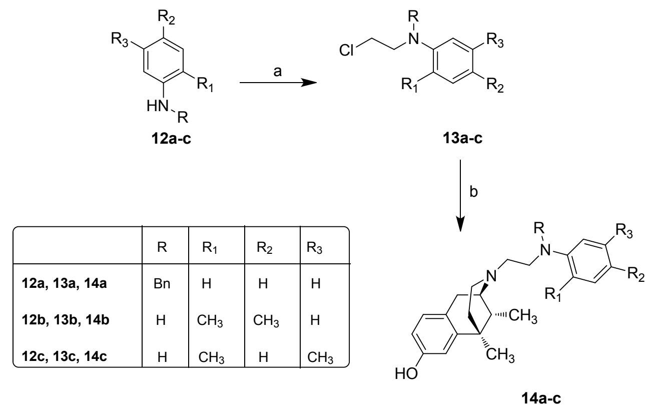


	R_1	R_2	R_3
8a, 9a, 10a, 11a	H	H	H
8b, 9b, 10b, 11b	CH_3	H	CH_3
8c, 9c, 10c, 11c	CH_3	CH_3	CH_3
8d, 9d, 10d, 11d	CH_3	CH_3	H
8e, 9e, 10e, 11e	CH_3	H	C_2H_5

Scheme 2. Synthesis of *N*-substituted normetazocine derivatives **11a-e**. Reagents and conditions: a) benzaldehyde (1 eq), MeOH, reflux, 3 h; b) $NaBH_4$ (0.5 M solution in EtOH) reflux, 6 h; c) 3-bromopropionyl chloride (1.5 eq), 4-(dimethylamino)pyridine (DMAP) (0.47 eq), dry THF, rt, 3h; d) (–)-*cis*-(1*R*,5*R*,9*R*)-*N*-normetazocine (1 eq), $NaHCO_3$ (1.5 eq), KI, DMF, 65 °C, 20 h.

N-benzyl anilines **9a-e**, obtained by reductive amination with $NaBH_4$, were acylated with 3-bromopropionyl chloride to obtain the respective amides **10a-e**. Derivatives **11a-e** were prepared according to the synthetic route shown in Scheme 2.

The *N*-(2-chloroethyl)anilines **13a-c** were obtained by alkylation with 1-bromo-2-chloroethane.[29] Then, the next step to get target derivatives **14a-c** was carried out as reported in Scheme 3. All newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis.



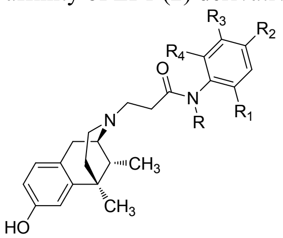
	R	R_1	R_2	R_3
12a, 13a, 14a	Bn	H	H	H
12b, 13b, 14b	H	CH_3	CH_3	H
12c, 13c, 14c	H	CH_3	H	CH_3

Scheme 3. Synthesis of *N*-substituted normetazocine derivatives **14a-c**. Reagents and conditions: a) 1-bromo-2-chloroethane (0.3 eq), CH_3CN, 110 °C in sealed tube, 10 min; b) (–)-*cis*-(1*R*,5*R*,9*R*)-*N*-normetazocine (1 eq), $NaHCO_3$ (1.5 eq), KI, DMF, 50 °C, 12 h.

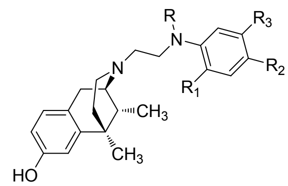
To investigate the SAR of synthesized novel derivatives, their binding and efficacy profile at MOR, DOR and KOR was explored. Binding at MOR, DOR and KOR was evaluated by competitive displacement of [^{3}H]DAMGO, [^{3}H]DPDPE and [^{3}H]U69,593, respectively.[30] K_i values of derivatives **7a-e**, **11a-e** and **14a-c**, calculated using nonlinear regression analysis (GraphPad Prism), are listed in Table 1.

The synthesized derivatives showed a broad range of binding affinity for MOR (K_i= 7.4-1,540 nM) and low or no affinity for DOR and KOR. Derivatives **7a** and **7e**, having methyl groups in position 2',6' and 2',5' respectively, possessed the highest MOR affinity, followed by derivatives **7b**, **7d** and **7c** having slight less affinity for this receptor. A third methyl group in position 4' (**7b**), as well as an ethyl group in position 6' (**7d**), reduced MOR affinity by 6- and 2-times compared to **7a** and **7e**. The dimethyl alkylation in position 2' and 4' (**7c**) resulted in a worse MOR binding profile. Thus, methylation in *orto* and *meta* is well tolerated while the *para*-methylation was unfavorable. In MOR-ligand interaction the negative influence of *para* substitution, with both electron-withdrawing or electron-donor groups, was outlined.[22] A worse DOR and KOR binding profile was recorded for derivatives **7a-e**. Indeed, in comparison to LP1 their DOR and KOR affinity were from 7- to 69-times and from 7- to 61-times lower, respectively. The introduction of a benzyl pendant at the amidic nitrogen (**11a**) and the simultaneous phenyl ring methylation (**11b-e**) resulted detrimental for opioid binding affinity, mainly at DOR and KOR (Table 1). Such modifications hindered the ligands to adopt a compatible ligand-receptor conformation. Derivatives **14a-c**, featured by an *N*-ethylamino spacer, showed MOR affinity higher than derivatives **11a-e** and lower than derivatives **7a-e**. The steric hindrance at the amine nitrogen in derivative **14a** resulted in a dramatically loss of opioid receptor affinity, mainly at MOR, respect to compound 1 (K_i^{MOR} = 6.1 nM), featured by a tertiary *N*-Methyl-*N*-phenylethylamino group.

Table 1. Opioid receptor binding affinity of LP1 (**2**) derivatives **7a-e**, **11a-e** and **14a-c**.



7 and **11**



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Cmp						Ki (nM) ± SEM [a,b]		
	R	R_1	R_2	R_3	R_4	MOR	DOR	KOR
7a	H	CH_3	H	H	CH_3	7.4 ± 0.75	277 ± 12	252 ± 10
7b	H	CH_3	CH_3	H	CH_3	43.7 ± 2	> 2,000	313 ± 15
7c	H	CH_3	CH_3	H	H	20.8 ± 1	474 ± 20	340 ± 13
7d	H	CH_3	H	H	C_2H_5	14.9 ± 0.92	198 ± 8	320 ± 11
7e	H	CH_3	H	CH_3	H	7.9 ± 0.65	478 ± 22	480 ± 22
11a	Bn	H	H	H	H	606 ± 34	> 5,000	> 5,000
11b	Bn	CH_3	H	H	CH_3	400 ± 24	> 5,000	> 3,000
11c	Bn	CH_3	CH_3	H	CH_3	504 ± 27	> 5,000	444 ± 24
11d	Bn	CH_3	CH_3	H	H	165 ± 7	> 5,000	> 2,000
11e	Bn	CH_3	H	H	C_2H_5	1,540 ± 59	> 5,000	151 ± 6
14a	Bn	H	H	H	H	244 ± 12	> 1,000	> 3,000
14b	H	CH_3	CH_3	H	H	129 ± 10	> 5,000	> 1,000
14c	H	CH_3	H	CH_3	H	182 ± 13	> 5,000	> 2,000
1[c]						6.1 ± 0.50	147 ± 5.700	31 ± 1.300
2 (LP1)[d]						0.83 ± 0.05	29 ± 1.00	110 ± 6.00
DAMGO						0.90 ± 0.04	-	-
Naltrindole						-	0.83 ± 0.04	-
U50,488						-	-	0.27±0.03

[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. [c] Ref. [16]. [d] Ref. [17].

To examine the functional significance of derivatives **7a-e**, displaying the best MOR binding profile, we tested their ability to affect agonist-mediated AC inhibition. Opioid receptors signal through Gi/Go proteins to inhibit AC,[31-34] which is known to be one of their major pathway to induce analgesia.[12] For that reason, HEK293 cells stably expressing the MOR were treated with increasing concentrations of derivatives **7a-e**, and the levels of forskolin-stimulated AC activity were tested. Derivatives **7a-7d** were unable to inhibit AC even at high concentrations up to 10^{-5} M (data not shown). Treatment of HEK293 cells with compound **7e** resulted with 50±3 % inhibition of cAMP accumulation at a concentration of 10 μM (Figure 2). However, this effect was much lower than that detected with LP1 (Figure 2). These results suggest that derivative **7e** could be considered as an effective MOR agonist with low potency on AC inhibition. To further identify whether derivatives **7a**, **7c**, **7d** and **7e** behave as MOR agonists we measured alterations of ERK1,2 phosphorylation mediated by these derivatives upon MOR activation. It was previously demonstrated that opioid receptors stimulate ERK1,2 via pertussis toxin-sensitive Gi/o-protein signaling mechanism[32-34] and regulate additional effectors by interacting with other scaffolding proteins.[35] In addition, it is well known that signaling of opioid receptors through ß-arrestin pathway leads to ERK1,2 activation.[36] Serum-starved HEK293 cells expressing stably the MOR were challenged with derivatives **7a**, **7c**, **7d** and **7e** with different time intervals ranging from 5-15 min. As shown in Figure 3, western blotting with a specific phospho-ERK1,2 antibody revealed an increase in ERK1,2 phosphorylation reaching a peak within 5 min administration for tested derivatives, which decreased after 15 min compound exposure. The same pattern of increased ERK1/2 phosphorylation was also shown for LP1. However, the levels of ERK1,2 phosphorylation mediated by derivative **7e** retain even after 15 min of receptor stimulation. These results suggest that **7a**, **7c**, **7d** and **7e** act as potent MOR agonists with derivatives **7a**, **7c**, **7d** exerting biased agonist properties towards ERK1,2 activity stimulation.

To determine if *in vitro* biased and unbiased G-protein profile could reflect what is happening in animal pain models, derivatives **7a** and **7e** were further screened in the mouse-tail flick test. **7a** and **7e**, in a dose range from 2.5 up to 7.5 mg/kg i.p., did not significantly modify TFLs, during the entire time of observation (90 min, Figure 4 panel A and B, respectively) compared to the group of mice treated with saline ($p > 0.05$ vs saline-treated mice).

Considering that PGE2-induced hyperalgesia is well known to be triggered by cAMP accumulation and the consequent protein kinase A activation,[37] we selected this assay as a suitable index of the behavioral effects of the derivatives **7a**, **7c**, **7d** and **7e** through cAMP inhibition. The administration of PGE2 induced a marked decrease in the withdrawal latency of the injected paw to heat stimulation, in comparison to saline-injected controls, denoting the development of thermal hyperalgesia. There were no statistically significant differences between the values obtained in the paw contralateral to PGE2 or saline (data not shown). Both morphine (1-3 mg/kg, s.c.) and LP1 (1-4 mg/kg, s.c.) induced a dose-dependent increase in paw withdrawal latency in PGE2-treated mice, reaching values similar to control animals (i.e. a full antihyperalgesic effect) (Figure 5) at the highest doses tested. In contradiction, the administration of **7a**, **7c**, **7d** or **7e** (8-16 mg/kg, s.c.) did not induce any significant antihyperalgesic effect (Figure 5). These results are in agreement with the inhibition of cAMP accumulation by morphine and LP1 and the absence of any effect detected by **7a**, **7c**, **7d** or **7e** in the same experiments.

As constipation is a known opioid-induced side effect dependent on the activation of ß-arrestin pathway,[12,13] we also tested the effects of derivatives **7a**, **7c**, **7d** and **7e** on gastrointestinal transit. Immediately after the evaluation of the behavioral responses to heat stimulus, mice received intragastrically an activated charcoal solution. The charcoal meal travelled about 30 cm of the small intestine in either mouse treated i.pl. with saline or PGE2, indicating that the administration of PGE2 do not influence gastrointestinal transit (Figure 6).

Morphine already induced significant gastrointestinal transit inhibition at a dose devoid of antihyperalgesic effect (1 mg/kg), and this effect dose-dependently increased reaching values of distance travelled by the charcoal meal as low as 10.5 cm at the highest tested dose of the opioid (3 mg/kg) (compare Figures 5 and 6). Instead, LP1 inhibited gastrointestinal transit only at the highest dose tested (4 mg/kg), which induced a maximal antihyperalgesic effect (compare Figures 5 and 6). These results indicate that the MOR agonist/DOR antagonist LP1 has a more favorable safety profile than morphine.

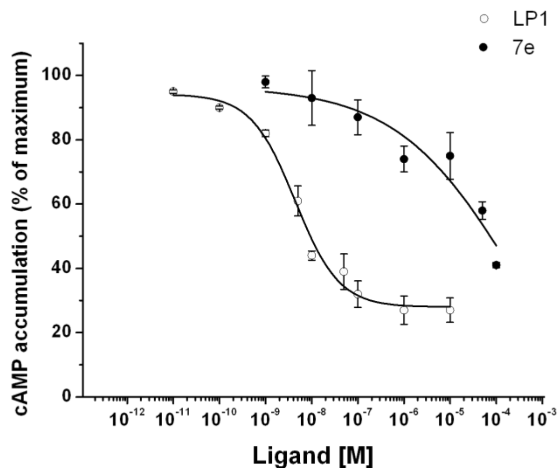

Figure 2. Effect of derivative **7e** and LP1 on MOR-mediated cAMP accumulation. The inhibition of cAMP accumulation was measured as described in Material and methods in HEK293 cells stably expressing the MOR in the presence of various concentrations of LP1 and **7e**, in response to treatment with 50 μM forskolin. The IC_{50} values of LP1 and **7e** are 4.8 x10-9 ± 0.5 M and 2.4 x10-4 ± 0.83 M respectively. Data represent as cAMP accumulation (% of maximum) and are the average of ± SEM of triplicate determinations from three independent experiments.

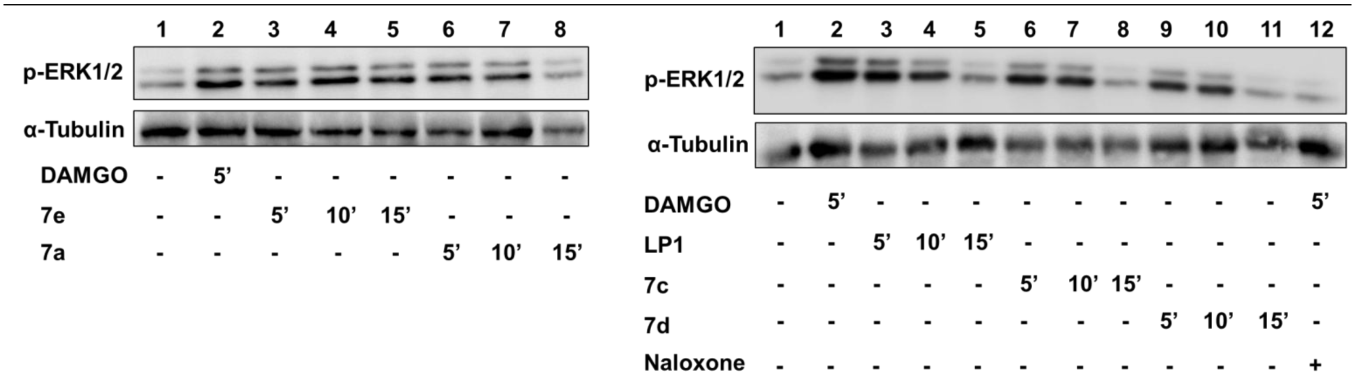

Figure 3. Effect of derivatives **7a**, **7c**, **7d** and **7e** on ERK1,2 phosphorylation mediated upon MOR activation. Stably transformed HEK293 cells expressing the MOR were challenged with 1 μM of derivatives **7a**, **7c**, **7d** and **7e** for 5, 10 and 15 min and cell lysates were resolved in SDS-PAGE (10%). The ERK1,2 phosphorylation mediated by DAMGO and LP1 (1 μM) after 5 min exposure was used as positive controls. Phosphorylation of ERK1,2 was abolished upon pretreatment of the cells with naloxone (10 μM, 30 min), prior to 5 min DAMGO administration (negative control). The phosphorylated-ERK1,2 was visualized by immunoblotting with a phosphor-ERK1,2 (upper panel). Equal loading was verified by stripping and reprobing the PVDF membrane with a specific α-tubulin antibody (lower panel). Results are representative of three independent experiments.

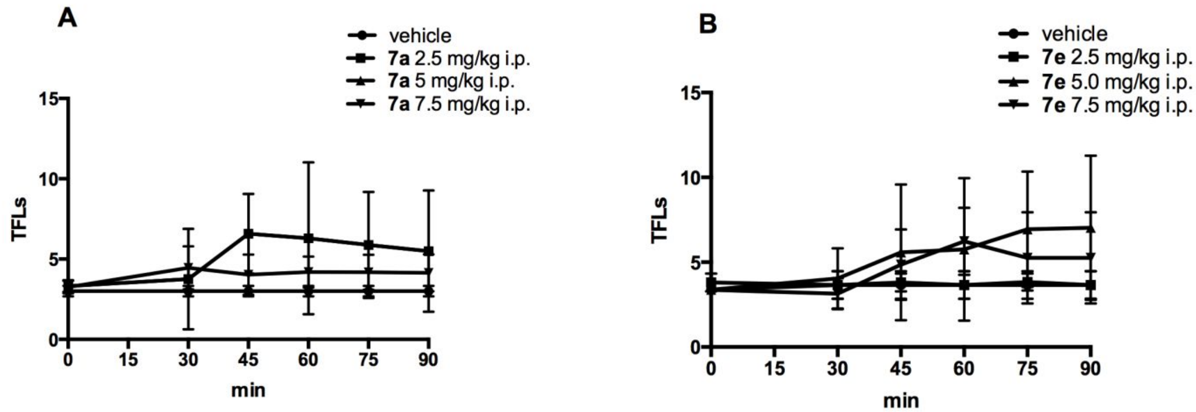

Figure 4. Time-course (min) of derivatives **7a** and **7e**-induced antinociceptive effect measured by tail flick test (panel A and B, respectively). Results are expressed in seconds (s). Data are means ± SEM from 6 to 8 mice. *P < 0.05 vs saline-treated mice.

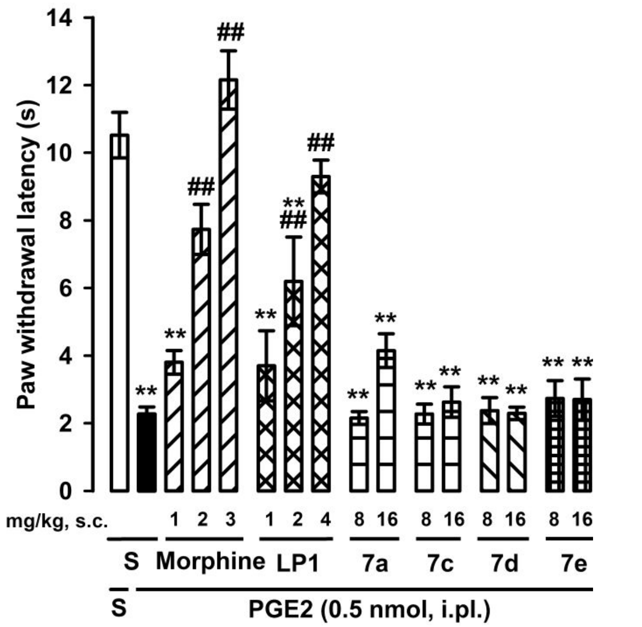

Figure 5. Effects of morphine, LP1 and derivatives **7a**, **7c**, **7d** and **7e** on PGE2-induced heat hyperalgesia. The results represent the latency to hindpaw withdrawal in response to radiant heat in mice treated intraplantarly (i.pl.) with PGE2 or saline (S). Mice were tested (in the paw injected with PGE2 or its solvent) 10 min after the intraplantar injection. Morphine, LP1, **7a**, **7c**, **7d**, **7e** or their solvent (S) were administered subcutaneously (s.c.) 20 min before the i.pl. injection. Statistically significant differences between the values obtained in mice i.pl. injected with saline and PGE2: *p<0.05, **p < 0.01, and between the values obtained in mice treated with PGE2 alone or associated with morphine or LP1: ##p < 0.01 (one-way ANOVA followed by Bonferroni test).

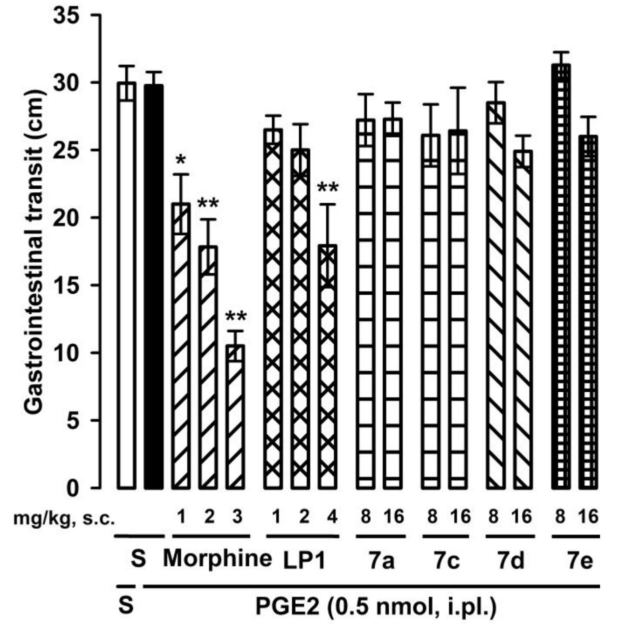

Figure 6. Effects of morphine, LP1 and derivatives **7a**, **7c**, **7d** and **7e** on gastrointestinal transit. Immediately after the evaluation of PGE2-induced hyperalgesia [i.e. 30 minutes after the subcutaneous (s.c.) administration of morphine, LP1, **7a**, **7c**, **7d** or **7e** or saline (S)], mice were given a 0.5% charcoal suspension intragastrically. Transit of the charcoal was measured 30 min after its ingestion. Each bar and vertical line represents the mean ± SEM of values obtained in 6-7 mice. Statistically significant differences between the values obtained in saline-treated group and mice treated with morphine or LP1: *p<0.05, **p < 0.01 (one-way ANOVA followed by Bonferroni test).

However, the administration of **7a**, **7c**, **7d** or **7e** (8-16 mg/kg, s.c.) did not alter gastrointestinal transit distances, as the charcoal meal travelled approximately 30 cm of the small intestine in all cases (Figure 6). Although these derivatives were able to act in vitro as opioid agonists mediating ERK1,2 phosphorylation, someone could assume that they may activate the ß-arrestin pathway and thus are unable to decrease gastrointestinal transit. Animals administered with these derivatives did not show either a Straub tail response (data not shown), which is a known centrally-induced opioid effect.[38]

In summary, we have repurposed the *N*-modified benzomorphan scaffold to develop novel LP1 derivatives to further understanding the requirements for MOR interaction. A secondary amido *N*-substituent, as well as an *orto*- and/or *orto*/*meta*-methyl introduction to the phenyl ring, resulted in retention of MOR agonism. Derivatives **7a**, **7e**, **7c** and **7d** resulted MOR agonists with a peculiar functional profile, being **7e** a biased MOR agonist, able to stimulate G-protein pathway, and **7a**, **7c** and **7d** unbiased MOR agonists, able to stimulate ERK1,2 activity.

ERKs activation can be facilitated by distinct pathways mediated by G-proteins or β-arrestins dependent pathways. Fast activation of ERKs (2 min) is usually mediated by G-proteins resulted in the nuclear translocation of phosphorylated ERKs, whereas a slower activation of ERKs (10 min), the time sets that was used in our studies, is mediated by β-arrestins and resulted in the cytosolic retention of the phosphorylated ERKs. Different MOR agonists activate ERKs via β-arrestins dependent or independent pathways, thus resulting in differential subcellular localization of activated ERKs and altering their effect on gene transcription driven by the agonist [36]. In addition to opioid receptor-mediated activation of ERKs via β-arrestins, β2AR stimulation resulted in ERKs activation via a β-arrestin dependent pathway.[39]

Compounds provided with functional selectivity could open new possibilities in opioid drug development. Indeed, biased MOR agonists toward G-protein are analgesics with low side effects incidence while biased MOR agonist toward β-arrestin could be useful to treat hypermotility disorders.

Besides the notable antinociceptive and antihyperalgesic effect, the dual-target profile of LP1 conferred a safer profile resulting in a less gastrointestinal transit inhibition than morphine. In accordance with in vitro data, synthesized derivatives did not elicit any significant antinociceptive and antihyperalgesic effect. Differences of pharmacokinetic could explain the low correlation between the in vivo inability to decrease gastrointestinal transit and the in vitro evidence. In conclusion, we found hits able to segregate physiological responses downstream of the receptor signaling that could be optimized.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures for the synthesis and characterization of the compounds, radioligand binding, adenylyl cyclase inhibition, ERK1,2 activations, tail-flick, PGE2-induced hyperalgesia, drug-induced gastrointestinal transit inhibition assays. This material is available free of charge via the Internet.

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Author contributions

L.P., R.T. and C.P. designed all paper experiments, analyzed and discussed results and wrote the paper. L.P., R.T. and E.A. designed and synthesized new compounds. C.P. performed in vivo experiment. O.P. and E.Ar. performed and analyzed radioligand binding experiments. A.M., L.S. and M.D. participated to the statistical analysis and characterized compounds. Z.G. and P.P. performed and analyzed in vitro functional experiments. E.J.C. and M.C.R.-C. performed and analyzed in vivo experiments. All authors have participated in the writing refinement and given approval to the final version of the manuscript.

Note

The authors declare no conflict of interest.

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ABBREVIATIONS

MOR, mu opioid receptor; DOR, delta opioid receptor; GPCR, G-protein coupled receptor; KOR, kappa opioid receptor; K_i, inhibition constant; AC, adenylyl cyclase; HEK293, human embryonic kidney 293; ERK1,2, extracellular regulated kinase 1 and 2; TFLs, tail flick latencies; PGE2, Prostaglandin E2; β-arrestins, β2AR, β-adrenergic receptor; s.c., subcutaneous; i.p., intraperitoneal; i.pl. intraplantar.

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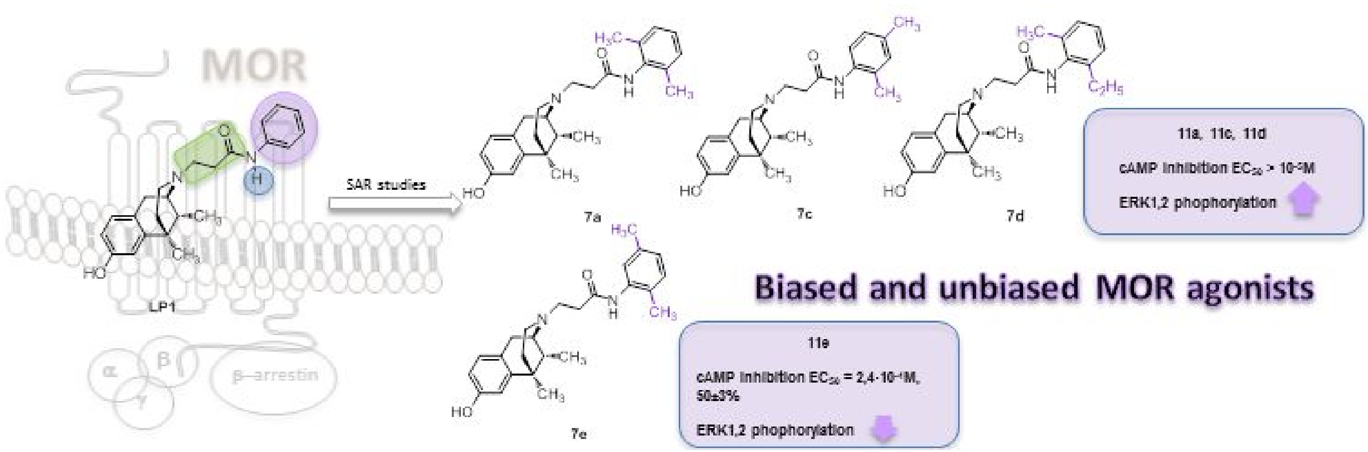

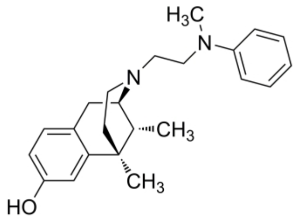

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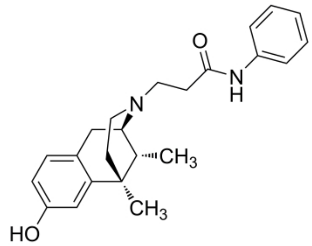

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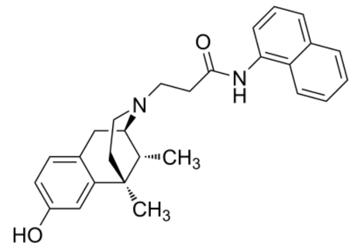

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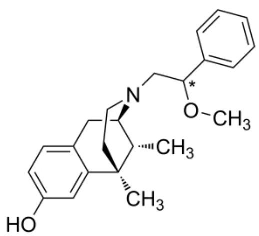

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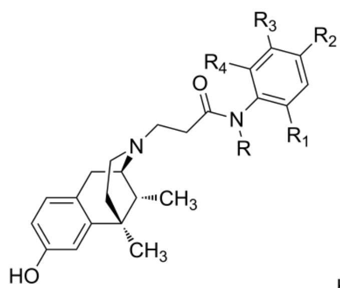

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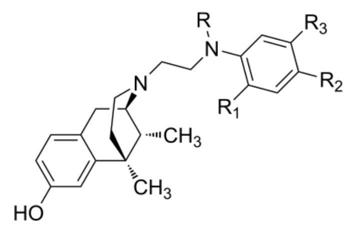

14a-c

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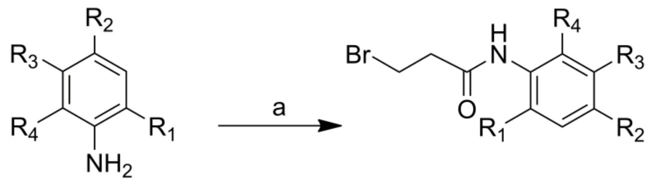
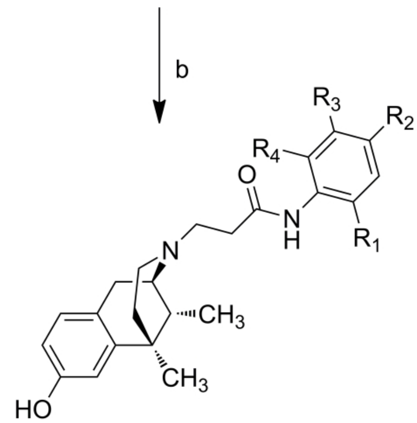


5a-e **6a-e**



7a-e

	R_1	R_2	R_3	R_4
5a, 6a, 7a	CH_3	H	H	CH_3
5b, 6b, 7b	CH_3	CH_3	H	CH_3
5c, 6c, 7c	CH_3	CH_3	H	H
5d, 6d, 7d	CH_3	H	H	C_2H_5
5e, 6e, 7e	CH_3	H	CH_3	H

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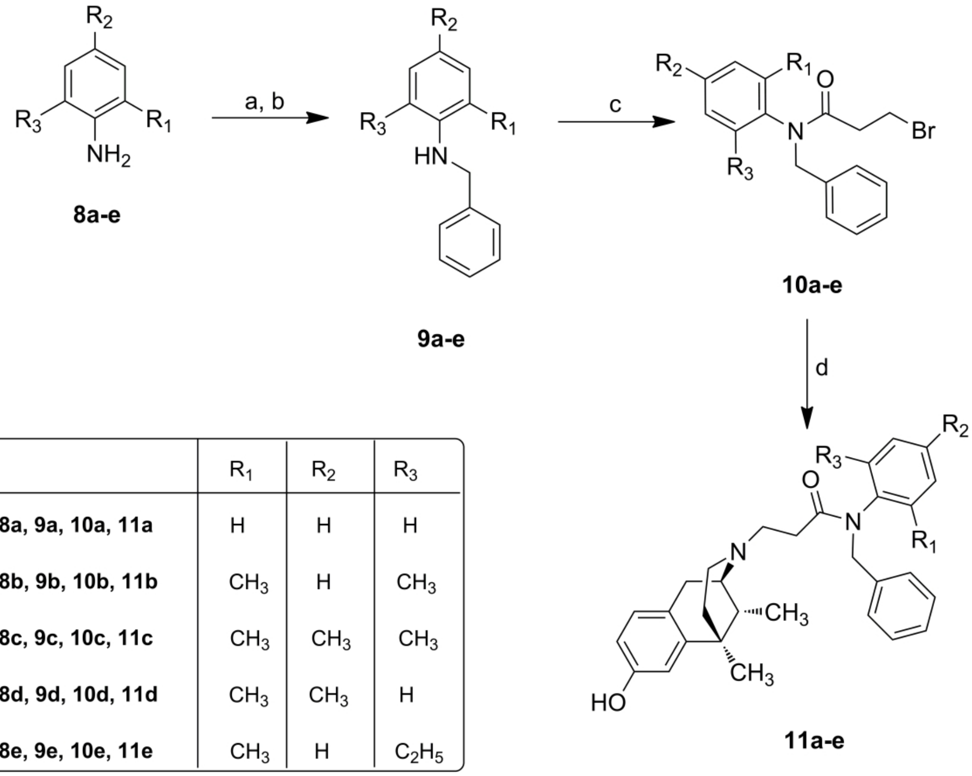
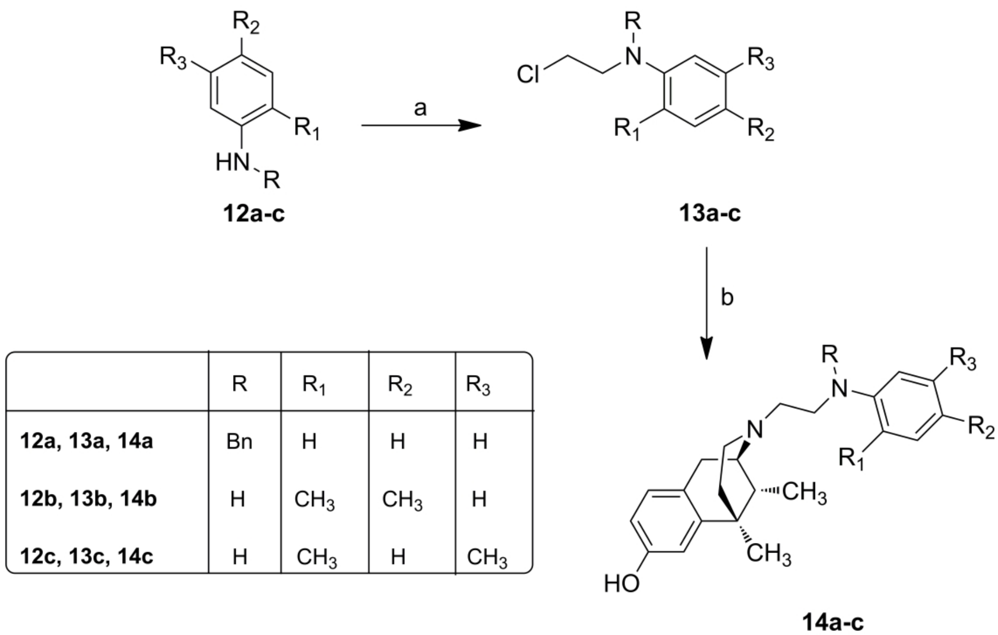


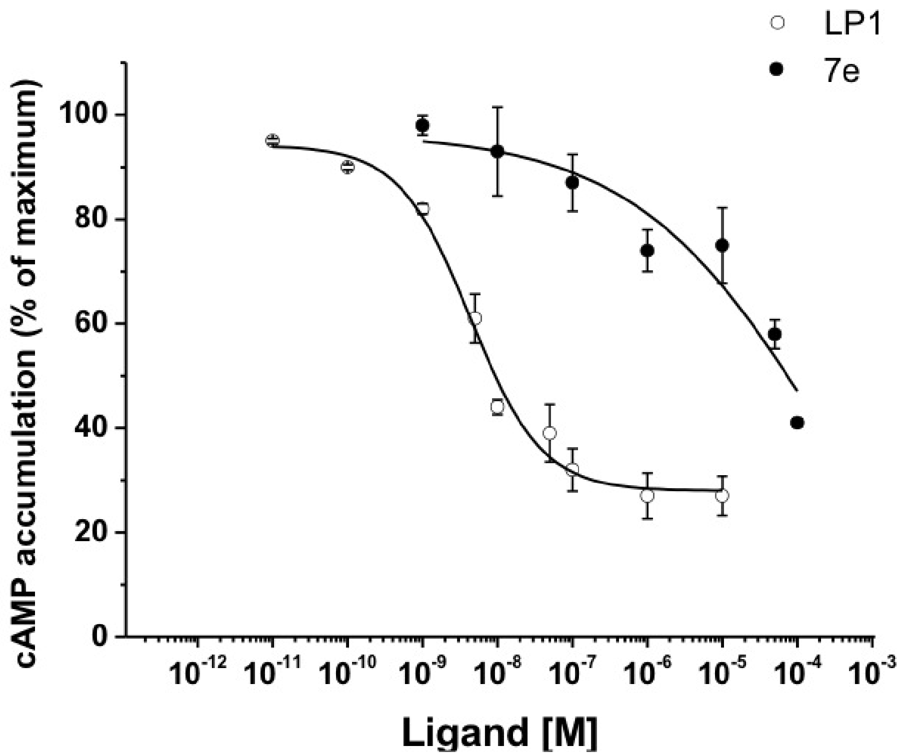
	R_1	R_2	R_3
8a, 9a, 10a, 11a	H	H	H
8b, 9b, 10b, 11b	CH_3	H	CH_3
8c, 9c, 10c, 11c	CH_3	CH_3	CH_3
8d, 9d, 10d, 11d	CH_3	CH_3	H
8e, 9e, 10e, 11e	CH_3	H	C_2H_5

146x113mm (1000 x 1000 DPI)



	R	R_1	R_2	R_3
12a, 13a, 14a	Bn	H	H	H
12b, 13b, 14b	H	CH_3	CH_3	H
12c, 13c, 14c	H	CH_3	H	CH_3

142x91mm (1000 x 1000 DPI)



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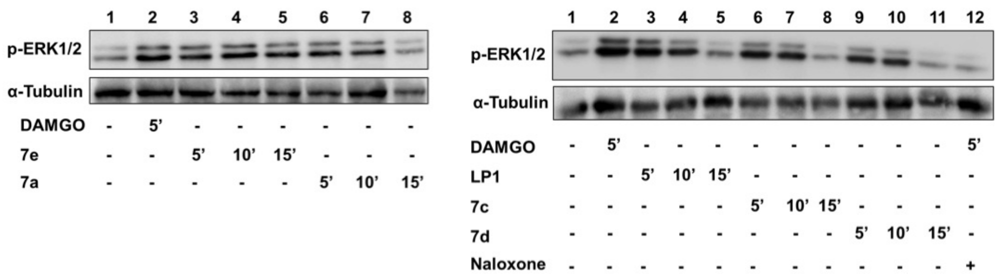

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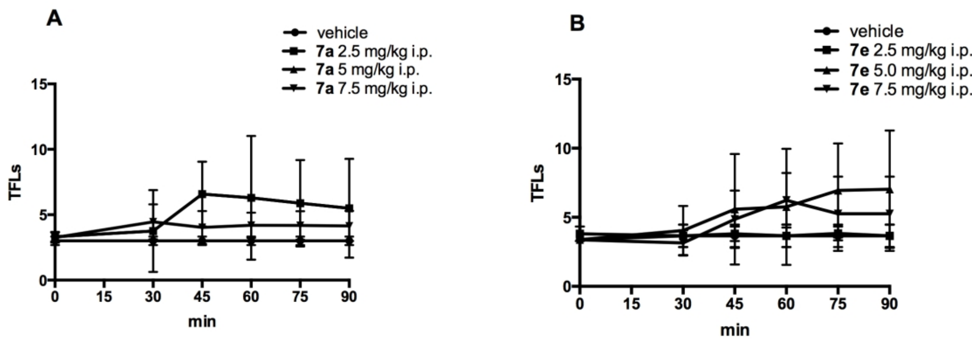

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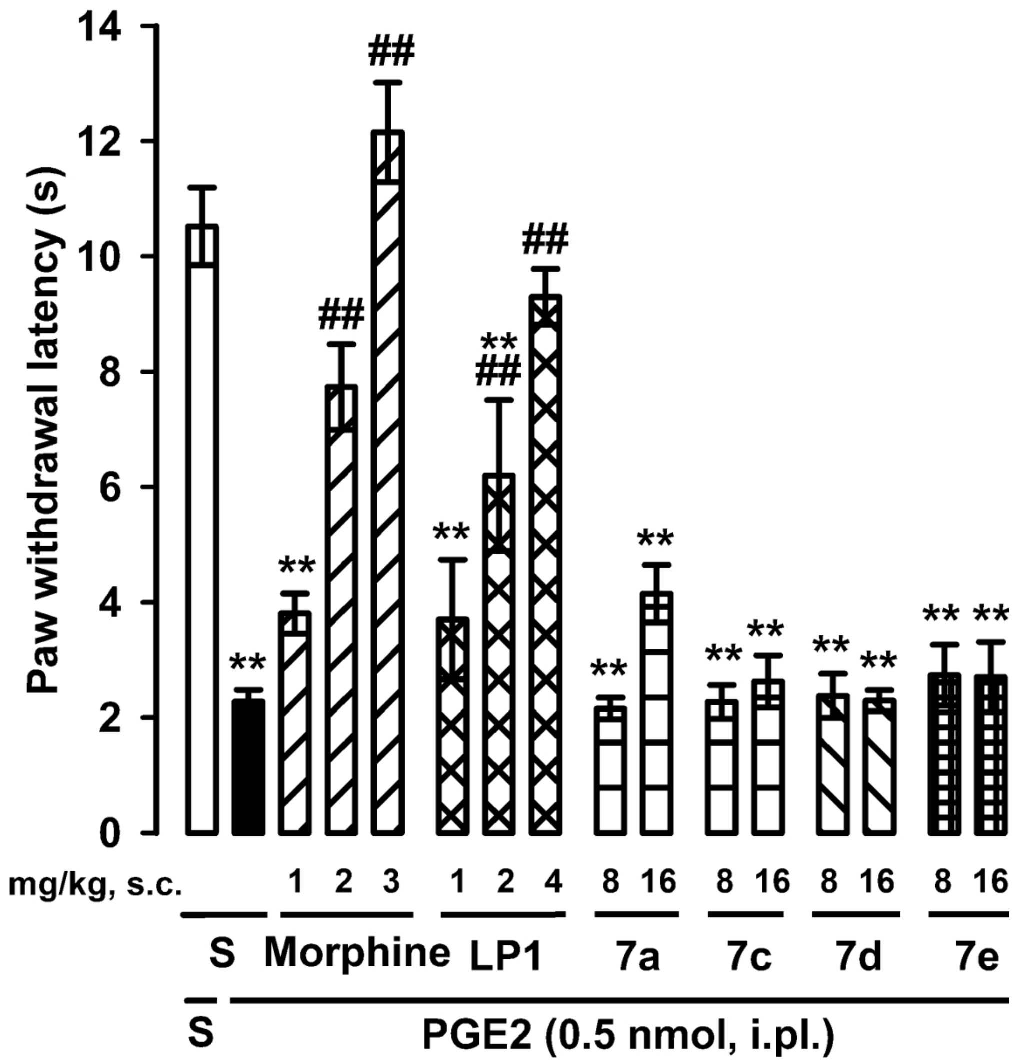
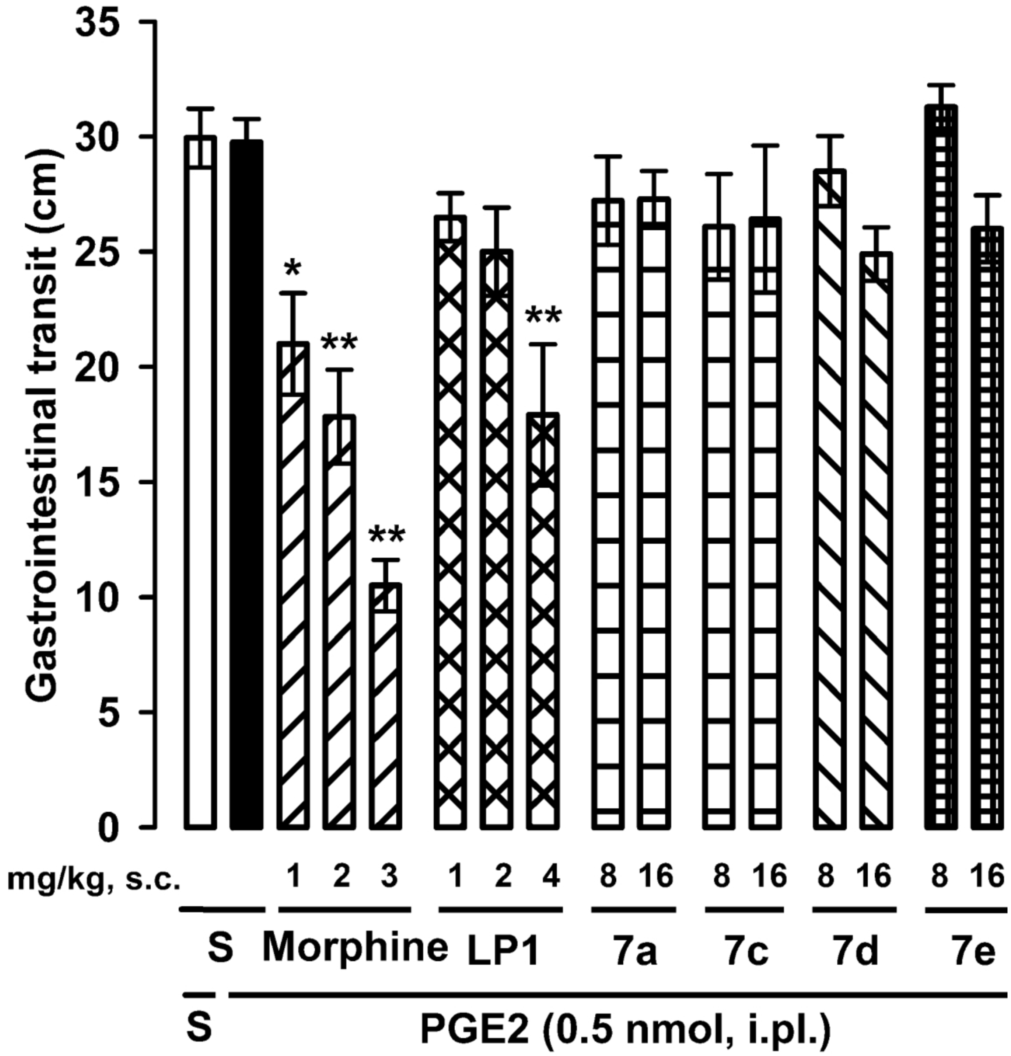
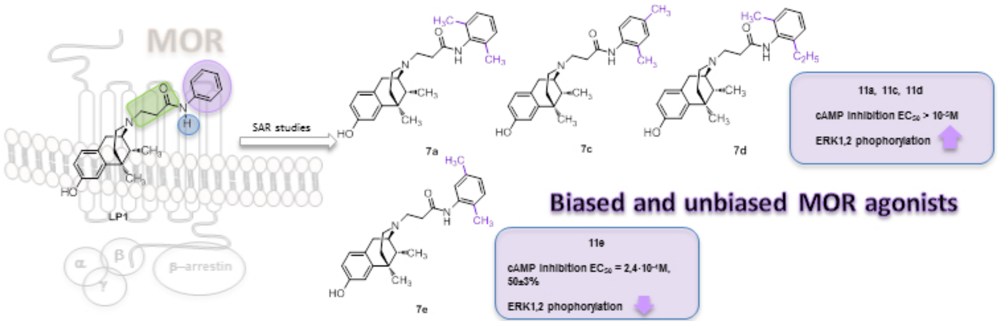

Paw withdrawal latency (s)
14
12
10
8
6
4
2
0
##
##
**
##
##
**
**
**
**
**
**
**
**
**
**
**
mg/kg, s.c.
1 2 3
1 2 4
8 16
8 16
8 16
8 16
S
Morphine
LP1
7a
7c
7d
7e
S
PGE2 (0.5 nmol, i.pl.)



Gastrointestinal transit (cm)
35
30
25
20
15
10
5
0
*
**
**
**
mg/kg, s.c.
1 2 3
1 2 4
8 16
8 16
8 16
8 16
S
Morphine
LP1
7a
7c
7d
7e
S
PGE2 (0.5 nmol, i.pl.)



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