Antinociceptive efficacy of the μ-opioid/nociceptin peptide-based hybrid KGNOP1 in inflammatory pain without rewarding effects in mice: An experimental assessment and molecular docking

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Table S1. Antinociceptive effect of KGNOP1 and morphine after s.c. administration in the formalin test in mice

Treatment **Dose**	**Phase I** **%Antinociceptive effect**	**Phase II** **%Antinociceptive effect**
KGNOP1		
0.12 µmol/kg	33.9 ± 3.8	2.3 ± 11.1
0.49 µmol/kg	33.7 ± 9.3	51.8 ± 9.7
1.22 µmol/kg	55.6 ± 7.3	71.4 ± 6.8
ED_{50} (µmol/kg) (95% CL)	1.17 (0.41-3.35)	0.55 (0.22-1.33)
Morphine		
3.11 µmol/kg	19.4 ± 6.7	24.8 ± 11.6
7.77 µmol/kg	32.6 ± 5.7	57.5 ± 8.3
15.5 µmol/kg	38.4 ± 10.9	79.1 ± 7.5
ED_{50} (µmol/kg) (95% CL)	-[a]	6.44 (3.20-12.7)

Data are shown as percent (%) antinociceptive effect calculated for the acute nociceptive pain (Phase I, 0-5 min) and inflammatory pain (Phase II, 15-60 min) as: $100 \times [(C - T)/C]$, where C is the mean time in control (saline) group and T is the time in drug-treated group. The antinociceptive effective dose (ED_{50}) and 95% confidence limit (CL) values were calculated using linear regression. -[a] not calculable. Values represent the mean ± SEM (n = 6-8 mice per group).

Table S2. Antinociceptive effect of KGNOP1 and morphine after s.c. administration to mice with CFA-induced inflammatory pain

Treatment **Dose**	**30 min**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**	**6 h**	**7 h**	**8 h**	**24 h**
Thermal sensitivity (Hargreaves test)										
KGNOP1										
0.49 µmol/kg	23.5	38.1	59.8	40.2	25.4	15.7	9.3	-1.46	6.12	-3.79
	±12.0	±12.5	±16.2	±7.7	±7.3	±8.0	±6.0	±4.4	±3.5	±4.5
1.22 µmol/kg	31.9	54.4	98.6	78.6	58.2	26.0	26.7	10.2	9.15	6.72
	±11.0	±12.3	±16.2	±11.5	±11.2	±7.2	±9.4	±5.2	±5.4	±3.7
Morphine	68.6	36.1	19.6	13.8	-1.96	0.43	-0.40			0.00
15.5 µmol/kg	±2.8	±3.6	±2.8	±0.5	±2.3	±1.5	±2.6	-	-	±0.6
Mechanical sensitivity (von Frey test)										
KGNOP1	-6.71	65.4	19.1	-8.73	21.1	-0.50	-12.9	-8.30	-2.42	-8.66
1.22 µmol/kg	±23.2	±21.2	±24.1	±31.8	±40.6	±25.8	±26.6	±7.3	±26.5	±18.4
Morphine	63.4	50.6	18.9	6.62	1.57	11.7	-2.29	-[a]	-[a]	1.68
15.5 µmol/kg	±10.2	±16.7	±7.5	±9.6	±3.3	±6.5	±6.0			±9.0

Data are shown as percentages (%) reversal of thermal/mechanical sensitivity calculated as: $100 \times [(T_1 - T_0)/(T_{BL} - T_0)]$, where T_0 is the nociceptive value at 72 hours post-inoculation with CFA (defined as 0 hours), T_1 is the value obtained following drug administration and T_{BL} is the basal value before CFA inoculation. -[a] not determined. Values represent the mean ± SEM (n = 6-8 mice per group).

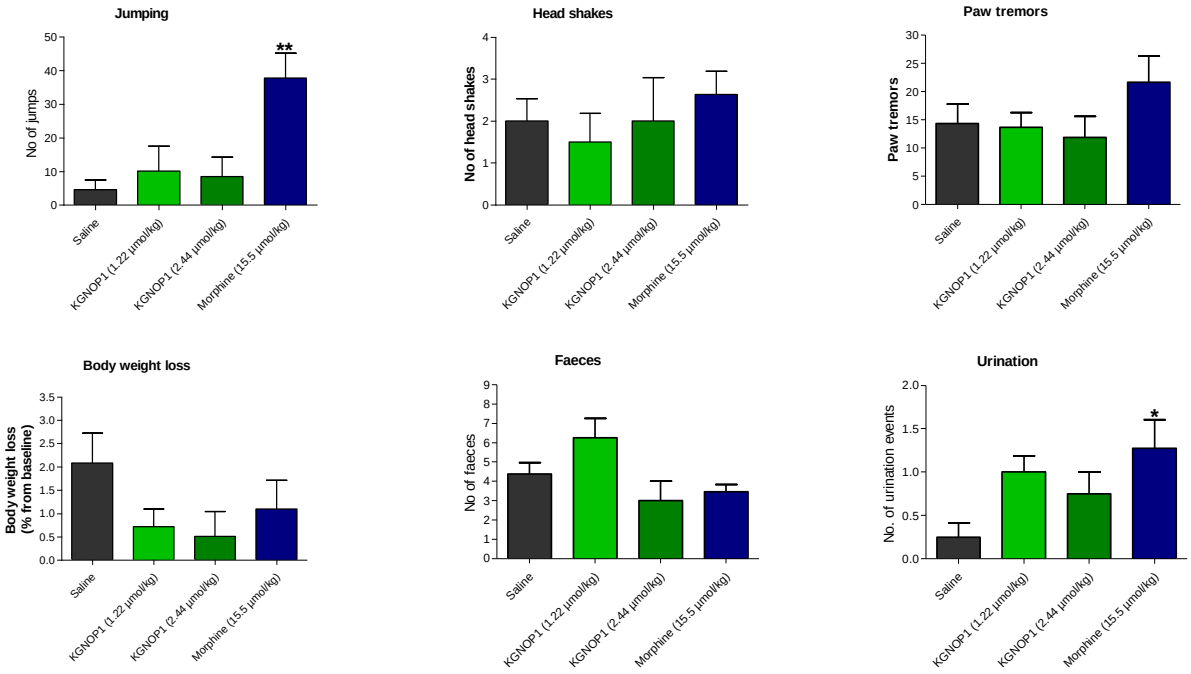

Figure S1. Effect of KGNOP1 and morphine on naloxone-precipitated withdrawal syndrome in mice after s.c. administration. Groups of mice received saline (control), KGNOP1or morphine, daily for 5 days. Naloxone (1 mg/kg, s.c.) was injected two hours after last drug treatment to precipitate withdrawal signs. Number of occurring events were recorded for 15 min after naloxone administration. Data are presented as the mean ± SEM (n = 8 mice per group). **P* < 0.05 and ***P* < 0.01 vs. saline group, one-way ANOVA followed by Dunnett's *post-hoc* test.