ChemInform Abstract: New Orally Effective 3-(2-Nitro)phenylpropanamide Analgesic Derivatives: Synthesis and Antinociceptive Evaluation.

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · SEPTEMBER 2013

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2013.08.041 · Source: PubMed

European Journal of Medicinal Chemistry 69 (2013) 728–734

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

New orally effective 3-(2-nitro)phenylpropanamide analgesic derivatives: Synthesis and antinociceptive evaluation

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ARTICLE INFO

Article history:
Received 23 April 2013
Received in revised form
19 August 2013
Accepted 25 August 2013
Available online 13 September 2013

Keywords:
6-Nitrophenylpropanamide
TDAE
Analgesic
Peripheral antinociceptive activity
Writhing test
Hot-plate test

ABSTRACT

A series of substituted 6-nitrophenylpropanamide derivatives (**1**–**20**) were synthesized using either the TDAE strategy or classical organic reactions. All these compounds were characterized by fusion point, ^{1}H NMR, ^{13}C NMR, elemental analysis or mass spectrometry data. Because of their structural analogy with recently published compounds possessing antinociceptive properties, our derivatives were screened for peripheral analgesic activities on acetic acid-induced writhing in mice. Compound **13** showed the best result at 100 μmol/kg *ip* (50% inhibition *vs* 59% for aspirin). This antinociceptive activity was maintained after oral administration (40% inhibition *vs* 31.6% for aspirin). Both hot-plate and actimetry-based tests were non-significant suggesting the analgesic activity of **13** linked to a peripheral mechanism.

1. Introduction

Pain is an unpleasant and a subjective sensation resulting from a harmful sensorial stimulation that alerts the body about current or potential damage to its tissues and organs [1]. Prescribing analgesics is the first response of health professionals, before removal of the underlying causes. The World Health Organization classifies analgesic drugs into three categories, by analgesic strength [2]. The first category includes paracetamol, aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs). The second and the third are composed of opioid drugs such as codeine and morphine acting against moderate and severe pain respectively.

Among the non-steroidal anti-inflammatory agents, arylalkanoic acids are the most widely investigated compounds. A typical molecule offer valuable features like a carboxyl group separated by one or more carbon atoms from an aromatic nucleus. The lead compound for aryl acetic acids is ibuprofen.

However, although these drugs are very effective and abundantly prescribed, they have revealed some gastric or intestinal adverse effects leading to numerous hospitalizations or death [3].

As a solution, many research groups transformed the carboxylic acid moiety of commercially available NSAIDs into carboxamide or ester forms in order to increase their cyclooxygenase-2 selectivity and their oral absorption, and to hide the irritant carboxylic acid group. Derivatizations studies of this kind were performed on indometacine [4] and mefenamic acid [5,6], profens [7], and phenylacetic acids [8].

In parallel, three research groups developed alternative arylalkanoic scaffolds bearing a carboxamide moiety which showed promising analgesic and anti-inflammatory activity combined with a safe ulcerogenic profile (Fig. 1, formula A, B and C) [9–14]. Kwak

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http://dx.doi.org/10.1016/j.ejmech.2013.08.041

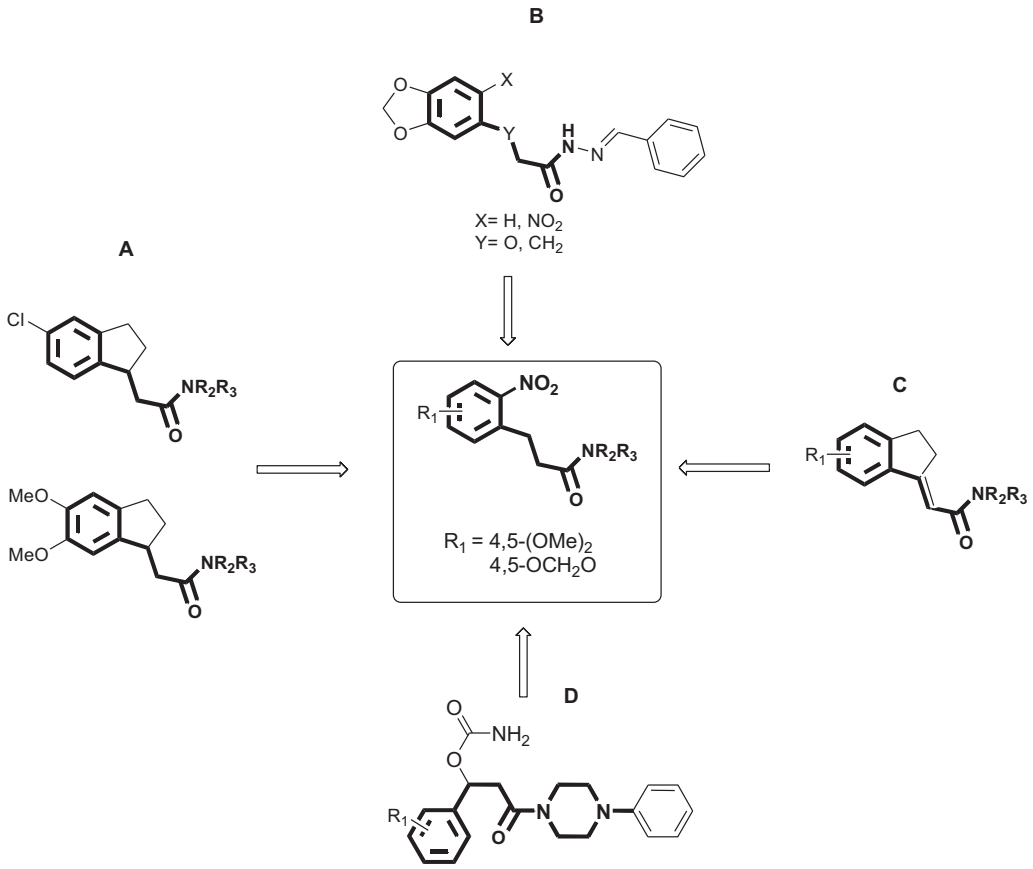


Fig. 1. Design of 3-(2-nitrophenyl)propanamides.

et al. developed carbamic acid derivatives bearing similar scaffolds and phenylpiperazinyl moiety (Fig. 1 formula D). These derivatives not only showed peripheral antinociceptive property but also anti-anxiety and antidepressant activities on mice [15].

Combining these promising structures enabled us to reveal the N-substituted 3-(2-nitrophenyl)propanamide skeleton as a new potentially analgesic scaffold (Fig. 1).

Recently, we developed a new synthesis methodology using tetrakis(dimethylamino)ethylene (TDAE) to prepare *N*,*N*-disubstituted 3-(6-nitrobenzo[*d*][1,3]dioxol-5-yl)propanamide and 3-(4,5-dimethoxy-2-nitrophenyl)propanamide derivatives [16]. This and previous studies [17,18] demonstrated the nitro group requirement in *ortho*- or in *para*- position from chloride to performed the carbon-halide reduction *via* the TDAE strategy. Consequently, we focus our interest on the synthesis of nitrated compounds. In continuation of our work directed towards the development of original synthetic methods in medicinal chemistry [19–25] and the preparation of new potentially analgesic active compounds [26,27], we report herein the synthesis of new 3-(2-nitrophenyl)propanamide derivatives and their analgesic properties.

2. Results–discussion

2.1. Chemistry

Preparation of the 3-(2-nitrophenyl)propanamides and esters (**1**–**20**) was realized *via* two different pathways (Scheme 1).

First, we used the S_N2 initiated by TDAE. This methodology led us to previously synthesize compounds **1**–**6** [16].

The second pathway used phenylpropanoic acids as starting materials using classical organic reactions. This yielded more synthesis alternatives such as un-nitrated derivatives or 2-substituted amides, *via* a simple, quick and cost-effective strategy. The 3-(benzo[*d*][1,3]dioxol-5-yl)propanoic acid was first reacted with nitric acid in dichloromethane, leading to nitrated derivatives in quantitative yield. These, or the starting propanoic acid, were reacted with thionyl chloride to obtain acyl chlorinated intermediates. After evaporation of the solvent, the crude was reacted with the corresponding amine reagent, leading to the expected products **7**–**17** in moderate to good yields (34–95%) (Table 1).

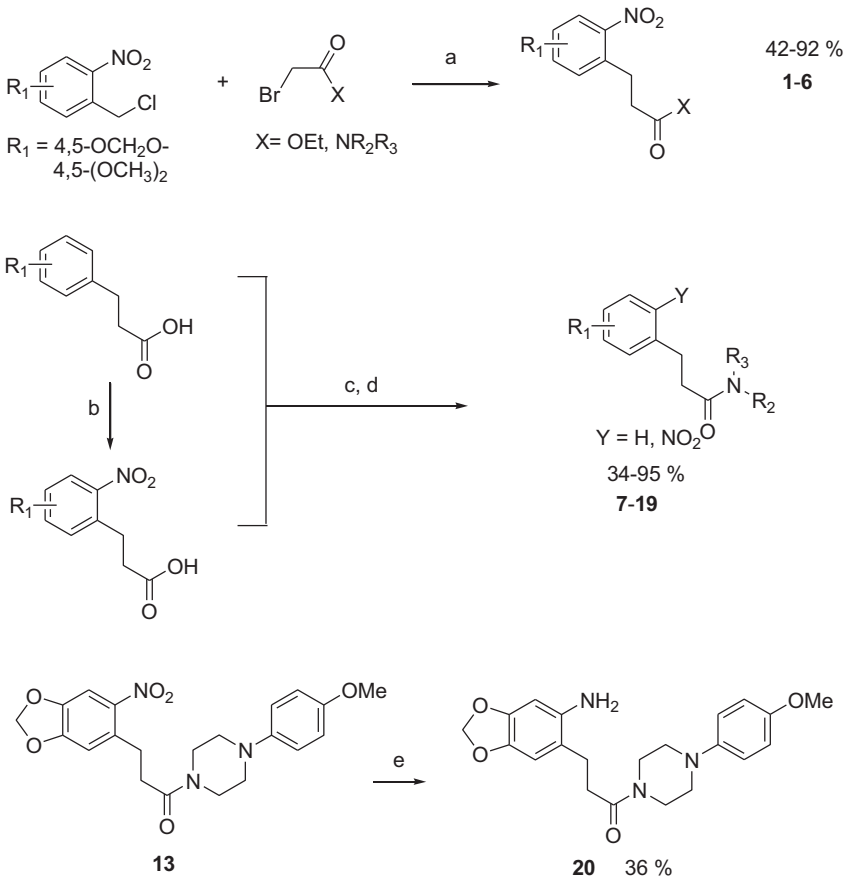
To complete the pharmacomodulation of compound **13**, reduction of its nitro group appeared an attractive option and was performed using molecular hydrogen in dioxane leading to compound **20** in 36% yield.

2.2. Analgesic activity

2.2.1. Antinociceptive screening

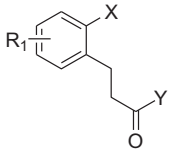
Antinociceptive activity of synthesized compounds was first assessed through the acetic acid-induced writhing test. A screening protocol was used to select structures possessing analgesic activity after 3 mg/kg intra-peritoneal (*ip*) administration in mice (Table 2). No toxic effect was observed during the protocol.

In 6-nitrobenzo[*d*][1,3]dioxol-5-yl series (**1**–**4**, **8**–**20**), non-significant results were observed with the non-substituted carboxamide derivative **8** or with compounds bearing an aromatic ring (**9**, **10**). However, substitution by aliphatic groups such as *n*-hexyl (**11**), piperidinyl (**1**), morpholinyl (**2**), piperazinyl (**12**, **13**) led to better results. Focusing on compound **13**, analogous derivatives with periodic structural changes were synthesized and screened (**3**, **14**–**17**, **20**). These compounds did not show improved antinociceptive activity. We can observe that derivatives **17** and **20**, the



Scheme 1. Reagents and conditions: a) TDAE (1 equiv.), DMF, −20 °C 1 h then 50 °C 1 h; b) HNO_3, CH_2Cl_2, 1.5 h; c) $SOCl_2$, N_2, CH_2Cl_2, 1 h; d) Amine derivatives, TEA, CH_2Cl_2; e) H_2/Ni Raney, dioxane, 40 °C.

Table 1
Formula and yield of compounds **1**–**20**.



Compound	R_1	X	Y	Yield (%)[a]
1[b]	4,5-OCH_2O–	NO_2	Piperidin-1-yl	42
2[b]	4,5-OCH_2O–	NO_2	Morpholin-1-yl	68
3[b]	4,5-OCH_2O–	NO_2	4-(3,4-Di-Cl-Ph)-piperazin-1-yl	62
4[b]	4,5-OCH_2O–	NO_2	OEt	85
5[b]	4,5-$(OCH_3)_2$–	NO_2	Piperidin-1-yl	65
6[b]	4,5-$(OCH_3)_2$–	NO_2	Morpholin-1-yl	62
7[c]	4,5-$(OCH_3)_2$–	H	Piperidin-1-yl	82
8	4,5-OCH_2O–	NO_2	NH_2	81
9	4,5-OCH_2O–	NO_2	NH-Ph	81
10	4,5-OCH_2O–	NO_2	NH-Pyridin-2-yl	76
11	4,5-OCH_2O–	NO_2	NH-*n*-hexyl	95
12	4,5-OCH_2O–	NO_2	(2-Hydroxyethyl)piperazin-1-yl	73
13	4,5-OCH_2O–	NO_2	4-(4-MeOPh)piperazin-1-yl	72
14	4,5-OCH_2O–	NO_2	4-(4-F-Ph)piperazin-1-yl	34
15	4,5-OCH_2O–	NO_2	4-(2-MeOPh)piperazin-1-yl	75
16	4,5-OCH_2O–	NO_2	4-Ph-piperazin-1-yl	68
17	4,5-OCH_2O–	H	4-(4-MeOPh)piperazin-1-yl	62
18[c]	4,5-OCH_2O–	H	Piperidin-1-yl	74
19	4,5-OCH_2O–	H	2-Me-piperidin-1-yl	70
20	4,5-OCH_2O–	NH_2	4-(4-MeOPh)piperazin-1-yl	36

[a] % All yields refer to the chromatographically isolated pure products.
[b] Synthesis and analytical description previously performed by our team [16].
[c] Compounds previously described [28,29].

Table 2
Acetic acid-induced writhing assay after ip administration at 3 mg/kg of compound **1**–**20**.

Compound	Dose (mg/kg)	Writhing ± SEM	% Inhibition[a]
Control	–	21.0 ± 5.2	
Aspirin	15	7.9 ± 0.63	62.3***
1	3	15.7 ± 1.2	25.2**
2	3	14.4 ± 1.7	31.4***
3	3	16.4 ± 1.5	22.0*
4	3	12.5 ± 1.6	40.5***
5	3	14.0 ± 1.7	33***
6	3	18.2 ± 0.6	15.3
7	3	22.0 ± 4.0	<0
8	3	18.1 ± 1.2	14.8
9	3	18.3 ± 2.1	12.9
10	3	18.9 ± 1.6	10
11	3	12.9 ± 1.2	38.6***
12	3	14.1 ± 2.3	32.9***
13	3	13.2 ± 2.5	37.2***
14	3	18.0 ± 1.3	14.3
15	3	20.2 ± 1.3	4.8
16	3	16.7 ± 1.8	21.5*
17	3	20.5 ± 2.6	2.4
18	3	9.9 ± 2.5	53.0***
19	3	15.1 ± 2.2	20.1*
20	3	16.5 ± 2.0	21.5*

[a] Statistical significance between control and treated groups (ANOVA + PLSD of Fischer: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). $n = 50$ mice for control and aspirin groups, $n = 10$ for other treatment groups.

Table 3
Acetic acid-induced writhing assay after *ip* administration at 100 μmol/kg of compounds **1**, **5**, **11**, **13**.

Molecule	Dose (mg/kg)	Writhings ± SEM	% Inhibition[a]
Control	–	21.2 ± 1.0	
Aspirin	100 μmol/kg	8.7 ± 1.1	59.0***
1	100 μmol/kg	11.7 ± 1.1	44.8***
5	100 μmol/kg	12.9 ± 1.4	39.1***
11	100 μmol/kg	15.7 ± 1.2	26.0**
13	100 μmol/kg	10.6 ± 1.3	50.0***

[a] Statistical significance between control and treated groups (ANOVA + PLSD of Fischer: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$). $n = 50$ mice for control and aspirin groups, $n = 10$ for other treatment groups.

un-nitrated and the aniline analog compound of **13**, were not or less active than **13**.

In 4,5-dimethoxyphenyl series, only compound **18** showed effective biological action.

These promising results led us to investigate compounds **1**, **5**, **11** and **13** more closely, all at the same higher dose (100 μmol/kg; at least 10 times higher than the screening dose) to compare their analgesic activity and determine to what extent dose explained the observed the antinociceptive effect (Table 3).

Surprisingly, derivative **11** did not show a significant activity at a higher dose than during screening, while the analgesic activity of compounds **1**, **5** and **13** was slightly better. Compound **13**, which showed the best analgesic activity (in term of percentage of writhing inhibition) at 100 μmol/kg, was then further investigated.

2.2.2. *Investigation of compound **13***

First, in order to distinguish any locomotor effect in the inhibition of writhing by derivatives **13**, an actimetry-based test was performed. No effect was observed at 33 and 100 μmol/kg (data not shown). Inhibition of writhing was then not related to any locomotor inhibition but rather to an analgesic effect. Second, compound **13** was tested for inhibition of acetic acid-induced writhing test at gradual increased oral dosages (10, 33.3, 100 μmol/kg) (Fig. 2). Positive control was aspirin at the highest dose (100 μmol/kg).

Compound **13** showed a significant antinociceptive activity at the three doses, respectively with 28.6, 35.7, 40.3% inhibition of constrictions. This result does not reveal a clear dose-dependent effect, since almost maximum analgesic action was obtained from the lowest dose tested. However, results obtained at 100 μmol/kg were slightly better than for aspirin at the same dose (31.2%).

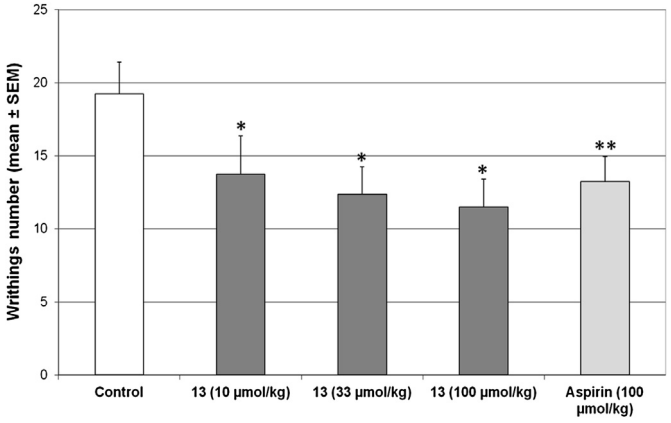


Fig. 2. Acetic acid-induced writhing assay after oral administration: analgesic activity of compound 13. $n = 8$ mice per treatment groups. $^{*}p < 0.05$, $^{**}p < 0.01$ versus control group (ANOVA and PLSD of Fischer).

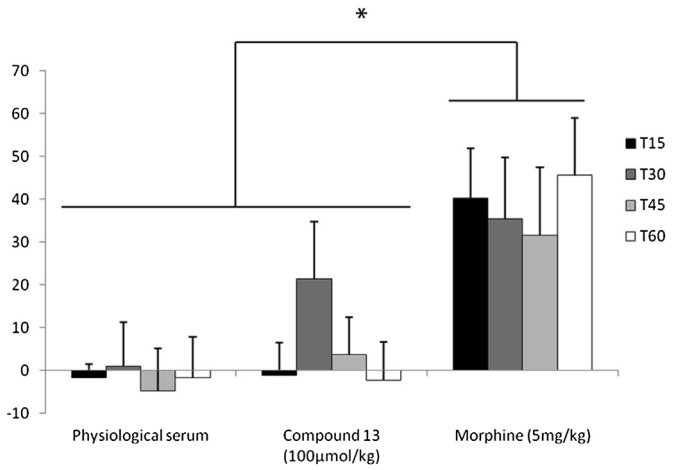


Fig. 3. The analgesic activity results of the hot plate experiment for compound 13 at 100 μmol/kg, physiological serum and morphine at 5 mg/kg. $^{*}p < 0.05$ vs vehicle control group data expressed as mean ± SEM, one-way ANOVA followed by a Fisher PLSD test.

Interestingly, while a decrease was observed in the percentage of inhibition after oral administration of aspirin compared to the same 100 μmol/kg dose administrated intraperitonally (59% after *ip* administration versus 31.2% after oral administration) the decrease is not as great under the same conditions for compound **13** (50% versus 40.3%) suggesting that compound 13 offers slightly better oral biodisponibility.

In addition, to assess the pharmacological mechanism (peripheral and/or central) involved in the analgesic effect of compound **13**, a hot plate test using a 100 μmol/kg intraperitoneal injection was performed. Compound **13** did not produce any significant increase in hot-plate latency compared to control group (Fig. 3). This result suggested that the compound **13** antinociceptive activity may be related to a peripheral mechanism.

3. Conclusion

20 new 3-(2-nitrophenyl)propanamide derivatives were synthesized using either classical methodology or an original TDAE strategy. When screened, 8 of them bearing aliphatic substituent on the carboxamide group showed a significant peripheral analgesic activity at a very low dose (3 mg/kg). One of the 8, compound **13** was more thoroughly investigated. Its analgesic activity was demonstrated and was clearly maintained through oral administration contrary to aspirin. Although the mechanism was not elucidated, it appeared to be mediated in a peripheral way. The structural analogy with other new derivatives showing anti-inflammatory activity makes the hypothesis of a peripheral mechanism plausible. 3-(2-nitrophenyl)propanamide compounds require further investigation to confirm them as new potent analgesic therapeutics agent and demonstrated their anti-inflammatory activity.

4. Experimental section

4.1. Chemistry

Melting points were determined on a Büchi capillary melting point apparatus and are uncorrected. Element analyses were performed by the centre de Microanalyse of the Aix-Marseille University. Both ^{1}H and ^{13}C NMR spectra were determined on a Bruker AC 200 spectrometer. The ^{1}H the ^{13}C chemical shifts are reported from $CDCl_3$ peaks: ^{1}H (7.26 ppm) and ^{13}C (76.9 ppm). Absorptions

are reported with the following notations: s, singlet; d, doublet; t, triplet; q, quartet; m, a more complex multiplet or overlapping multiplets. The following adsorbents were used for column chromatography: silica gel 60 (Merck, particle size 0.063–0.200 mm, 70–230 mesh ASTM). TLC was performed on 5 cm × 10 cm aluminum plates coated with silica gel 60 F254 (Merck) in an appropriate solvent.

4.1.1. Procedure for nitration 3-(benzo[d][1,3]dioxol-5-yl)propanoic acid and 3-(3,4-dimethoxyphenyl)propanoic acid

3-(Benzo[*d*] [1,3]dioxol-5-yl)propanoic acid and 3-(3,4-dimethoxyphenyl)propanoic acid was nitrated in 6 position of the phenyl moiety according to the procedure described by Bezerra-Netto [13].

4.1.2. Procedure for carboxamide synthesis

A solution of 1 mmol of the corresponding propanoic acid mixed with one drop of pyridine in 10 mL of dichloromethane (DCM), cooled at 0 °C, was added dropwise to 1.2 mmol of thionyl chloride. After stirring for 1 h at rt, the crude was evaporated at reduced pressure. The crude was dissolved in DCM at 0 °C and mixed with 1.2 mmol of the corresponding amine reagent and with 0.8 mmol of triethylamine. After stirring for 3 h, the crude was diluted with 40 mL of DCM and washed with water (3 × 100 mL). The organic phase was separated, dried with Na_2SO_4 and concentrated at reduced pressure. Purification was performed by silica gel chromatography.

*4.1.2.1. 3-(6-Nitrobenzo[d][1,3]dioxol-5-yl)propanamide (**8**).* Yellow solid, mp 182 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.36 (t, J = 7.8 Hz, 2H, CH_2); 2.96 (t, J = 7.8 Hz, 2H, CH_2); 6.17 (s, 2H, CH_2); 6.77 (s, 1H, NH); 7.00 (s, 1H, CH); 7.27 (s, 1H, NH); 7.54 (s, 1H, CH). ^{13}C NMR (50 MHz, DMSO-d_6) δ 28.4 (CH_2); 35.6 (CH_2); 103.2 (CH_2); 105.1 (CH); 110.5 (CH); 133.3 (C); 142.6 (C); 146.3 (C); 151.5 (C); 173.0 (C). Anal. Calcd for $C_{10}H_{10}N_2O_5$: C, 50.42; H, 4.23; N, 11.76. Found: C, 50.88; H, 4.07; N, 11.32.

*4.1.2.2. 3-(6-Nitrobenzo[d][1,3]dioxol-5-yl)-N-phenylpropanamide (**9**).* Yellow solid, mp 148 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.71 (t, J = 7.5 Hz, 2H, CH_2); 3.26 (t, J = 7.5 Hz, 2H, CH_2); 6.07 (s, 2H, CH_2); 6.85 (s, 2H, CH_2); 7.09 (t, J = 7.3 Hz, 1H, CH); 7.26–7.33 (m, 2H, 2× CH); 7.47–7.50 (m, 3H, 3× CH). Amidic proton did not appear in theses experimental conditions. ^{13}C NMR (50 MHz, $CDCl_3$) δ 30.0 (CH_2); 38.2 (CH_2); 102.9 (CH_2); 105.7 (CH); 111.2 (CH); 119.9 (2× CH); 124.3(CH); 128.9 (2× CH); 133.4 (C); 137.7 (C); 142.6 (C); 146.8 (C); 152.0 (C); 169.9 (CO). Anal. Calcd for $C_{16}H_{14}N_2O_5$: C, 61.14; H, 4.49; N, 8.91. Found: C, 61.39; H, 4.48; N, 8.77.

*4.1.2.3. 3-(6-Nitrobenzo[d][1,3]dioxol-5-yl)-N-(pyridine-2-yl)propanamide (**10**).* Yellow solid, mp 177 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.79 (t, J = 7.3 Hz, 2H, CH_2); 3.26 (t, J = 7.3 Hz, 2H, CH_2); 6.07 (s, 2H, CH_2); 6.84 (s, 1H, CH); 7.04–7.07 (m, 1H, CH); 7.50 (s, 1H, CH); 7.67–7.76 (m, 1H, CH); 8.19–8.24 (m, 2H, CH); 8.75 (s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 29.4 (CH_2); 38.0 (CH_2); 102.9 (CH_2); 105.8 (CH); 111.0 (CH); 114.3 (CH); 119.8 (CH); 133.0 (C); 138.8 (CH); 142.8 (C); 146.8 (C); 147.1 (C); 151.8 (C); 152.2 (C); 170.4 (CO). Anal. Calcd for $C_{15}H_{13}N_3O_5$: C, 57.14; H, 4.16; N, 13.33. Found: C, 56.52; H, 4.15; N, 12.77.

*4.1.2.4. N-Hexyl-3-(6-nitrobenzo[d][1,3]dioxol-5-yl)propanamide (**11**).* Yellow solid, mp 105 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.80 (t, J = 6.7 Hz, 3H, CH_3); 1.19 (m, 6H, 3× CH_2); 1.38 (m, 2H, CH_2); 2.46 (t, J = 7.3 Hz, 2H, CH_2); 3.10 (t, J = 7.3 Hz, 2H, CH_2); 3.11–3.18 (m, 2H, CH_2); 6.02 (s, 2H, CH_2); 6.03 (s, 1H, NH); 6.76 (s, 1H, CH); 7.40 (s, 1H, CH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.8 (CH_3); 22.3 (CH_2); 26.3 (CH_2); 29.3 (CH_2); 29.9 (CH_2); 31.3 (CH_2); 36.9 (CH_2); 39.4 (CH_2); 102.7 (CH_2); 105.4 (CH); 110.9 (CH); 133.5 (C); 142.3 (C); 146.4 (C); 151.7 (C); 171.3 (CO). Anal. Calcd for $C_{16}H_{22}N_2O_5$: C, 59.61; H, 6.88; N, 8.69. Found: C, 59.64; H, 7.13; N, 8.59.

*4.1.2.5. 1-(4-(2-Hydroxyethyl)piperazin-1-yl)-3-(6-nitrobenzo[d][1,3]dioxol-5-yl)propan-1-one (**12**).* Yellow solid, mp 103 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.45 (m, 5H, OH, 2× CH_2); 2.53 (t, J = 5.3 Hz, 2H, CH_2); 2.67 (t, J = 7.2 Hz, 2H, CH_2); 3.15 (t, J = 7.2 Hz, 2H, CH_2); 3.46 (t, J = 5.3 Hz, 2H, CH_2); 3.62 (t, J = 5.2 Hz, 4H, 2× CH_2); 6.06 (s, 2H, CH_2); 6.84 (s, 1H, CH); 7.47 (s, 1H, CH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 29.9 (CH_2); 33.9 (CH_2); 41.6 (CH_2); 45.4 (CH_2); 52.5 (CH_2); 53.0 (CH_2); 57.8 (CH_2); 59.3 (CH_2); 102.8 (CH_2); 105.6 (CH); 111.2 (CH); 133.7 (C); 142.7 (C); 146.6 (C); 151.8 (C); 170.1 (CO). Anal. Calcd for $C_{16}H_{21}N_3O_6$: C, 54.69; H, 6.02; N, 11.96. Found: C, 54.77; H, 6.31; N, 11.58.

*4.1.2.6. 1-(4-(4-Methoxyphenyl)piperazin-1-yl)-3-(6-nitrobenzo[d][1,3]dioxol-5-yl)propan-1-one (**13**).* Yellow solid, mp 143 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.75 (t, J = 7.3 Hz, 2H CH_2); 2.96–3.05 (m, 4H, 2× CH_2); 3.21 (t, J = 7.3 Hz, 2H CH_2); 3.63 (m, 2H, CH_2); 3.77 (s, 3H, CH_3); 3.78 (m, 2H, CH_2); 6.08 (s, 2H, CH_2); 6.82–6.94 (m, 5H, 5× CH); 7.50 (s, 1H, CH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 30.0 (CH_2); 34.0 (CH_2); 41.7 (CH_2); 45.5 (CH_2); 51.1 (CH_2); 51.4 (CH_2); 55.6 (CH_3); 102.9 (CH_2); 105.7 (CH); 111.4 (CH); 114.6 (2× CH); 119.0 (2× CH); 133.8 (C); 142.8 (C); 144.8 (C); 146.7 (C); 151.9 (C); 154.5 (C); 170.1 (CO). Anal. Calcd for $C_{21}H_{23}N_3O_6$: C, 61.01; H, 5.61; N, 10.16. Found: C, 61.00; H, 5.77; N, 10.10.

*4.1.2.7. 1-(4-(4-Fluorophenyl)piperazin-1-yl)-3-(6-nitrobenzo[d][1,3]dioxol-5-yl)propan-1-one (**14**).* Yellow solid, mp 159 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.74 (t, J = 7.2 Hz, 2H CH_2); 2.99–3.08 (m, 4H, 2× CH_2); 3.20 (t, J = 7.2 Hz, 2H CH_2); 3.63 (t, J = 4.6 Hz, 2H CH_2); 3.70 (t, J = 4.6 Hz, 2H CH_2); 6.06 (s, 2H, CH_2); 6.87–7.02 (m, 5H, 5× CH); 7.50 (s, 1H, CH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 30.0 (CH_2); 33.9 (CH_2); 41.4 (CH_2); 45.3 (CH_2); 50.7 (CH_2); 51.0 (CH_2); 102.8 (CH_2); 105.6 (CH); 111.3 (CH); 115.8 (d, J = 22.3 Hz, 2× CH); 118.8 (d, J = 8.0 Hz, 2× CH); 133.6 (C); 142.7 (C); 147.0 (C); 151.9 (C); 155.6 (C); 157.4 (d, J = 202.7 Hz, C); 170.1 (CO). Anal. Calcd for $C_{20}H_{20}FN_3O_6$: C, 59.85; H, 5.02; N, 10.47. Found: C, 59.77; H, 5.16; N, 10.47.

*4.1.2.8. 1-(4-(2-Methoxyphenyl)piperazin-1-yl)-3-(6-nitrobenzo[d][1,3]dioxol-5-yl)propan-1-one (**15**).* Yellow solid, mp 143 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.74 (t, J = 7.3 Hz, 2H CH_2); 2.94–3.02 (m, 4H, 2× CH_2); 3.20 (t, J = 7.3 Hz, 2H CH_2); 3.63 (t, J = 4.7 Hz, 2H, CH_2); 3.79 (t, J = 4.7 Hz, 2H, CH_2); 3.86 (m, 3H, CH_3); 6.05 (s, 2H, CH_2); 6.84–6.90 (m, 4H, 4× CH); 6.98–7.02 (m, 1H, CH); 7.48 (s, 1H, CH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 29.9 (CH_2); 33.8 (CH_2); 41.8 (CH_2); 45.7 (CH_2); 50.5 (CH_2); 50.9 (CH_2); 55.3 (CH_3); 102.8 (CH_2); 105.5 (CH); 111.3 (2× CH); 118.3 (CH); 120.9 (CH); 123.5 (CH); 133.7 (C); 140.4 (C); 142.7 (C); 146.6 (C); 151.7 (C); 152.2 (C); 170.1 (CO). Anal. Calcd for $C_{21}H_{23}N_3O_6$: C, 61.01; H, 5.61; N, 10.16. Found: C, 61.16; H, 5.60; N, 10.24.

*4.1.2.9. 3-(6-Nitrobenzo[d][1,3]dioxol-5-yl)-1-(4-phenylpiperazin-1-yl)propan-1-one (**16**).* Yellow solid, mp 186 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.75 (t, J = 7.2 Hz, 2H CH_2); 3.07–3.14 (m, 4H, 2× CH_2); 3.19 (t, J = 7.2 Hz, 2H CH_2); 3.62 (m, 2H, CH_2); 3.78 (m, 2H, CH_2); 6.05 (s, 2H, CH_2); 6.87–6.93 (m, 3H, 3× CH); 7.24–7.32 (m, 3H, 3× CH); 7.51 (s, 1H, CH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 30.0 (CH_2); 34.0 (CH_2); 41.7 (CH_2); 45.5 (CH_2); 49.4 (CH_2); 49.6 (CH_2); 102.8 (CH_2); 105.7 (CH); 111.4 (CH); 116.6 (2× CH); 120.5 (CH); 129.2 (2× CH); 133.7 (C); 142.8 (C); 146.7 (C); 150.9 (C); 151.9 (C); 170.2 (CO). Anal. Calcd for $C_{20}H_{21}N_3O_5$: C, 62.62; H, 5.52; N, 10.96. Found: C, 62.72; H, 5.61; N, 10.76.

*4.1.2.10. 3-(Benzo[d][1,3]dioxol-5-yl)-1-(4-(4-methoxyphenyl)piperazin-1-yl)propan-1-one (**17**).* Yellow solid, mp 143 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.59 (t, J = 7.1 Hz, 2H CH_2); 2.86–2.99 (m, 6H, 3× CH_2); 3.50(t, J = 4.9 Hz, 2H CH_2); 3.73–3.74 (m, 5H, CH_3, CH_2); 5.85 (s, 2H, CH_2); 6.83–6.73 (m, 3H, 3× CH); 6.78–6.88 (s, 4H, 4× CH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 31.0 (CH_2); 34.9 (CH_2); 41.4 (CH_2); 45.4 (CH_2); 50.6 (CH_2); 50.8 (CH_2); 55.3 (CH_3); 100.6 (CH_2); 108.1 (CH); 108.8 (CH); 114.2 (2× CH); 118.6 (2× CH); 121.0 (CH); 134.7 (C); 145.5 (C); 145.7 (C); 147.4 (C); 154.1 (C); 171.3 (CO). Anal. Calcd for $C_{21}H_{24}N_2O_4$: C, 68.46; H, 6.47; N, 7.60. Found: C, 68.21; H, 6.54; N, 7.34.

*4.1.2.11. 3-(Benzo[d][1,3]dioxol-5-yl)-1-(2-methylpiperidin-1-yl)propan-1-one (**19**).* Yellow oil. Mixture of 2 rotamers. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.15 (m, 6H, CH_3); 1.53–1.62 (m, 12H, CH_2); 2.53 (t, J = 7.7 Hz, 2H, CH_2); 2.64 (m, 1H, CH_2); 2.88 (t, J = 7.7 Hz, 2H, CH_2); 3.03 (m, 1H, CH_2); 3.03 (m, 1H, CH_2); 3.57 (d, J = 12.9 Hz, 1H, CH_2); 4.09 (m, 1H, CH_2); 4.52 (d, J = 12.9 Hz, 1H, CH_2); 4.93 (m, 1H, CH_2); 5.91 (s, 4H, CH_2); 6.64–6.75 (m, 6H, CH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 15.4 (CH_3); 16.5 (CH_3); 18.6 (2× CH_2); 25.4 (CH_2); 26.1 (CH_2); 29.7 (CH_2); 30.6 (CH_2); 31.3 (2× CH_2); 35.3 (CH_2); 35.7 (CH_2); 36.2 (CH_2); 40.6 (CH_2); 43.6 (CH); 48.1 (CH); 100.7 (CH_2); 108.1 (CH); 108.8 (CH); 121.1 (CH); 135.2 (C); 145.7 (C); 147.5 (C); 170.4 (CO). HRMS (EI): calcd for $C_{16}H_{21}NO_3$: $[M + H]^+$: 276.1594, found: 276.1593.

*4.1.2.12. 3-(6-Aminobenzo[d][1,3]dioxol-5-yl)-1-(4-(4-methoxyphenyl)piperazin-1-yl)propan-1-one (**20**).* Brown solid, mp 102 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.63 (t, J = 7.1 Hz, 2H CH_2); 2.78–2.89 (m, 4H, 2× CH_2); 2.96 (t, J = 4.9 Hz, 2H CH_2); 3.52 (t, J = 4.9 Hz, 2H CH_2); 3.72–3.75 (m, 7H, NH_2, CH_2, CH_3); 5.78 (s, 2H, CH_2); 6.26 (s, 1H, CH); 6.54 (s, 1H, CH); 6.84 (m, 4H, 4× CH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 26.5 (CH_2); 32.8 (CH_2); 41.7 (CH_2); 45.6 (CH_2); 50.9 (2× CH_2); 55.4 (CH_3); 98.2 (CH_2); 100.4 (CH); 109.5 (CH); 114.4 (2× CH); 117.6 (C); 118.8 (2xCH); 139.0 (C); 140.3 (C); 145.1 (C); 146.5 (C); 154.3 (C); 171.1 (CO). HRMS (EI): calcd for $C_{21}H_{25}N_3O_4$: $[M + H]^+$: 384.1918, found: 384.1923.

4.2. In vivo biology

4.2.1. Writhing test

The test employed [30] was essentially that described by Hendershot and Forsaith [31], however, acetic acid [32], rather than phenylquinone was used to elicit stretching. Groups of 10 mice (20–24 g) were injected i.p. with 10 ml/kg of 0.5% aqueous acetic acid. The mice were placed in an observation beaker, and the number of stretches per animal was counted during a 10 min period starting 10 min after acetic acid treatment. A stretch was defined as a sequence of arching of the back, pelvic rotation and hind limb extension. Tested and reference compounds were administered 15 min before acetic acid solution.

4.2.2. Hot plate test

The method employed for measuring central analgesic effect was first described by Woolfe and McDonald [33]. Briefly, every mouse was individually placed on a plate heated to 55 °C and the time until forepaw licking occurs was recorded by stop-watch. We measured the reaction times of groups of 10 mice twice before injections (mice must react between 4 and 12 s). Compound **13** was tested at 100 μmol/kg ip and reaction times were determined at 15, 30, 45 and 60 min after injection. If an animal did not respond by 30 s (cutoff time), it was removed from the plate to avoid tissue damage. Morphine used as reference at 5 mg/kg induced abolition of avoidance behavior (mean reaction times, 30 s, 26 s, 25 s, 25 s at 15, 30, 45 and 60 min, respectively; $p < 0.0001$ *vs* control at the four times, PLSD of Fisher). Analgesic activity was quantified for each mouse by calculating the percentage of the maximum possible effect (MPE%, where MPE% = [(test latency − control latency)/(cut-off point − control latency) × 100]).

4.2.3. Locomotor activity

Motor activity was measured in Plexiglas cages (1911 14 cm) placed in frames mounted with computer-monitored photocell beams (IMETRONIC). Horizontal locomotion was measured by the number of cage crossings. Behavioral data were collected by an Imetronic interface connected to a PC. Mice were injected intraperitoneally with drug or saline and immediately placed back into the chamber for 1 h. The device (PC) allows *a posteriori* analysis of potential modifications in of locomotion occurring during a period 25 min–35 min post-treatment (i.e. the same period post-treatment as that used to the number of constrictions in the writhing test). Methylphenidate (10 mg/kg) and haloperidol (0.5 mg/kg) used as stimulating and depressive references, respectively, produce an increase (280% increase, $p < 0.001$, PLSD of Fischer) and a decrease (80%, $p < 0.001$, PLSD of Fischer) in horizontal locomotion.

4.2.4. Statistical analyses

All quantitative data were expressed as mean ± SEM and were analyzed using analysis of variance (ANOVA) followed, when significant effects were found by post-hoc multiple comparison tests (PLSD of Fisher). p-values less than 0.05 were considered to be significant. Statistical analysis was performed with STATVIEW® software.

Acknowledgments

This work was supported by the CNRS and Aix-Marseille University. The authors thank V. Remusat for ^{1}H and ^{13}C spectra recording.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.08.041.

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