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Synthesis of new 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thiourea and urea derivatives with anti-nociceptive activity

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ABSTRACT

Chalcones or 1,3-diaryl-2-propen-1-ones are known to be useful for treating pain, inflammation, and certain diseases although their uses have not been scientifically verified. Due to the limitations of opioid and NSAID therapy, there is a continuing search for new analgesics. A series of novel new 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thiourea and urea derivatives were synthesized and evaluated against writhing test in mice, following the aromatic substitution pattern proposed by Topliss. The results of the preliminary bioassays indicate that compound **3** presents promising anti-nociceptive activity in acetic acid-, formalin-, and glutamate-induced pain in mice, compared with some well-known non-steroidal anti-inflammatory and analgesic drugs.

1. Introduction

Chalcones have been considered the main biological precursors for the biosynthesis of flavonoids, which are common components of the human diet. Recent studies on the biological evaluation of chalcones have revealed many compounds, with a wide range of biological effects, including anti-cancer,[1] anti-inflammatory,[2,3] anti-mitotic,[4] anti-microbial,[5] anti-leishmanial,[6,7] anti-malarial,[8,9] anti-tubercular,[10] cardiovascular,[11] anti-lipidemic,[12] and anti-hyperglycemic agents.[13] Previous studies carried out in our laboratories have demonstrated that some chalcones,[14] or those compounds derived from xanthoxyline, have promising analgesic action in mice.[15–17]

Reactions of chalcones with isocyanates or isothiocyanates and amines give ureas and thioureas which are of considerable industrial importance, and are linked to a series of biological activities including herbicidal activity,[18] inhibition of nitric oxide,[19] anti-microbial,[20] anti-HIV,[21] anti-viral,[22] HDL-elevating,[11] and analgesic properties.[23,24] Some 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]-phenyl}-urea derivatives exhibited anti-inflammatory[3,25] and anti-malarial activity.[8,9] In view of the need to discover new analgesic agents, and our previous positive results on chalcones, we have synthesized new 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thiourea and urea derivatives, and evaluated the anti-nociceptive activity against different models of pain tested in mice. The most active compound was evaluated in more detail and compared with some reference drugs.

2. Results and discussion

2.1. Synthesis and chemical characterization

In order to determine the possible anti-nociceptive action of 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thiourea and urea derivatives, we initially prepared: 1-(4-acetylphenyl)-3-(4-chlorophenyl)thiourea **1** and 1-(4-acetylphenyl)-3-(4-chlorophenyl)urea **2**, which are obtained according to Scheme 1. A procedure based on a Claisen–Schmidt condensation was developed for the syntheses of five 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-ureas and five new 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thioureas (Scheme 1) following the aromatic substitution pattern proposed by Topliss.[26,27] The yields and melting points of the compounds **3**–**12** are listed in Table 1.

The chemical identification data (^{1}H NMR, ^{13}C NMR, IR, and elemental analysis) confirmed the structures of compounds **3**–**12**. In the infrared spectra of compounds **3**–**12**, it was possible to observe the absorptions between 3296 and 3344 cm^{-1} relating to NH stretch, absorptions in 1638–1660 cm^{-1} from α,β-unsaturated carbonyl moiety stretch and absorptions in 1708–1713 cm^{-1} from urea carbonyl moiety stretching (Table 1). Thiocarbonyl is less polar than the carbonyl group, and the link C=S is considerably

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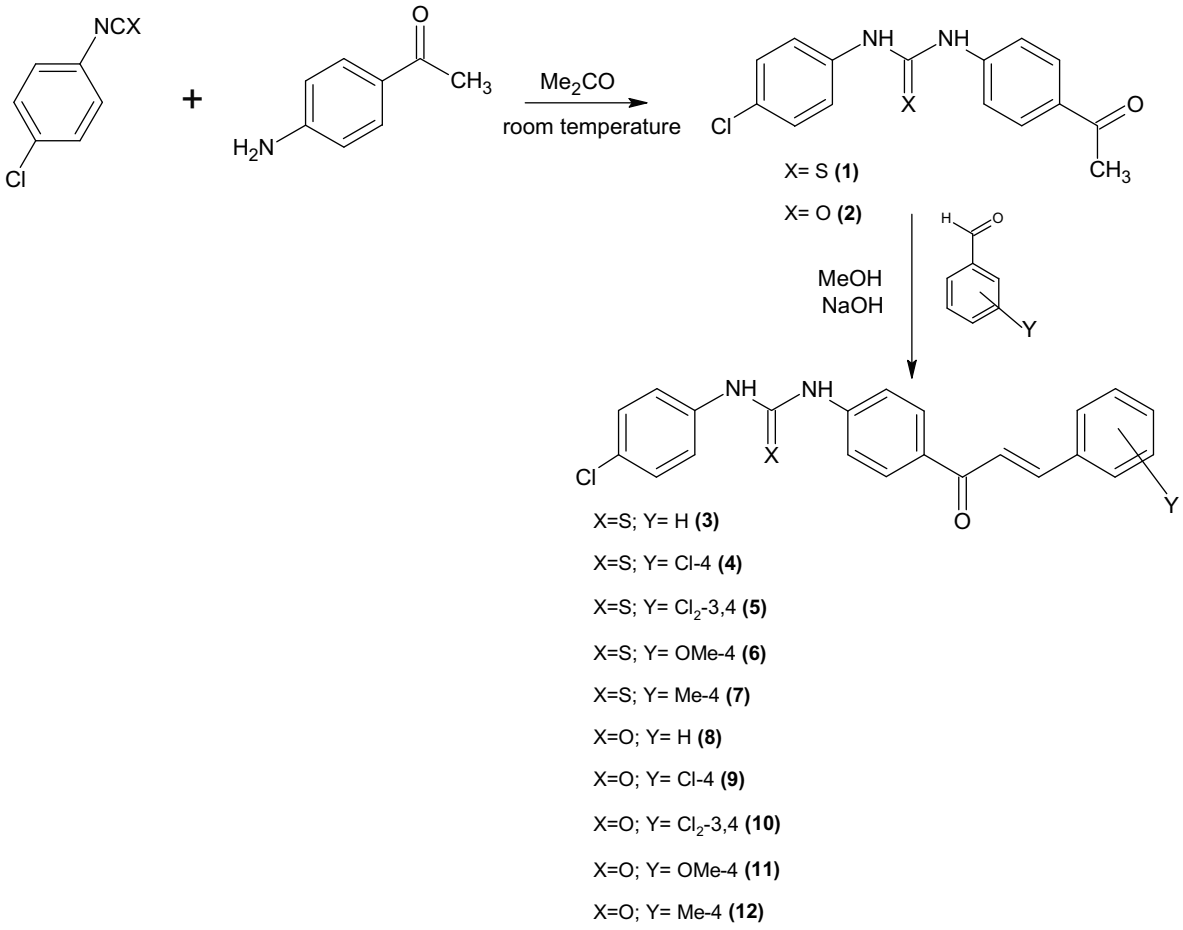
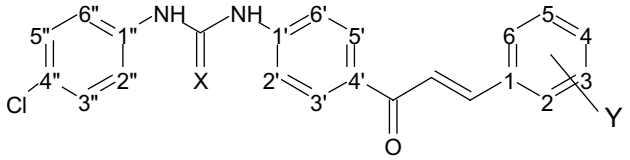


Scheme 1.

weaker, consequently, the band is not intense and it is located at lower frequencies than carbonyl, and is therefore more susceptible to the effects of coupling, their identification often being difficult and uncertain.[28]

Table 1
Structure and physical data of 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thiourea and urea derivatives



Compound	X	Y	Mp[a] (°C)		Yield (%)
			Found	Lit.	
3	S	–H	178–180	Unknown	50
4	S	4-Cl	187–190	Unknown	90
5	S	3,4-Cl_2	172–174	Unknown	80
6	S	4-OCH_3	195–197	Unknown	70
7	S	4-CH_3	208–210	Unknown	37
8	O	–H	202–204	223–224[b]	76
9	O	4-Cl	249–251	260–264[b]	87
10	O	3,4-Cl_2	198–200	Unknown	87
11	O	4-OCH_3	208–210	218–219[b]	88
12	O	4-CH_3	217–219	232–233[b]	93

[a] Melting points were uncorrected.
[b] Dominguez et al., 2005.[9]

The ^{1}H NMR spectra for all the synthesized compounds show signals at 9.5 ppm relating to hydrogens attached to the nitrogen. The signals for aromatic hydrogens are between 7.0 and 8.3 ppm. In this same region are the vinylic protons of *trans* olefinic protons which have larger coupling constants than those of their *cis* isomers. All the structures were geometrically pure and *E* configured (JHα–Hβ = 14–17 Hz). Some studies on chalcone derivatives[29,30] have shown this configuration exclusively.

Through the ^{13}C NMR data, a sign can be seen at 187 ppm, relating to chalcone carbonyl. This is followed by the sign for thiourea carbonyl, at 179 ppm, and the sign for urea carbonyl at 152 ppm. The quaternary and tertiary carbons are distributed in the 115–141 ppm region, as well as can be observed, in this band of chemical displacement, signals relating to olefinic carbons.

2.2. Evaluation of anti-nociceptive activity

This work aims to identify the effectiveness of 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thiourea and urea derivatives in a number of animal models of pain. In order to select the more active compounds, all the synthesized compounds were evaluated using the writhing test in mice, as a preliminary trial. A significant proportion of the work will be carried out using cells and tissues isolated from humans or from humanely killed animals, that is, in vitro. However, pain is a highly complex process requiring an input from many parts of the nervous system throughout the body, and, for this reason, in vitro testing alone

is not enough to determine if new medicines will be effective analgesics.[31,32]

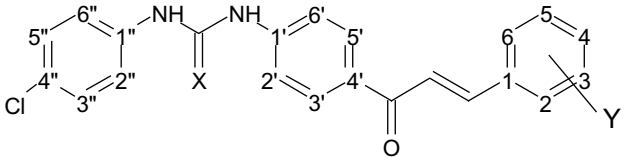
In a preliminary trial, as shown in Figure 1, all the compounds tested with the exception of compound **10** significantly inhibited acetic acid-induced abdominal constrictions when administered intraperitoneally at 10 mg/kg, compared with dipyrone, acetylsalicylic acid, and acetaminophen, which were used as reference drugs.

Due to the excellent results obtained in this test (Fig. 1), all the compounds were evaluated in more detail in the same model. The results shown in Table 2 indicate that nine of the compounds evaluated, five 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thioureas (**3**–**7**) and four 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-ureas (**8**, **9**, **11**, and **12**), which were tested in the writhing test, caused dose-dependent anti-nociceptive effects when given by the intraperitoneal route, inhibiting acetic acid-induced writhing responses in mice, with the exception of compound **10**, which was inactive at the dose of 60 mg/kg. 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thioureas presented a calculated ID_{50} value (95% confidence limit) of 3.03–13.14 μmol/kg, with maximum inhibition of 100 %, while the 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-ureas were less active with ID_{50} values ranging from 7.25 to 45.38 μmol/kg; with maximum inhibition of 84.4%. Some of the compounds tested were several times more active than the analgesic drugs used as reference: acetic salicylic acid (ASA), dipyrone, and acetaminophen (ACE), which presented ID_{50} values of 138.7 (73–243) μmol/kg, 162.2 (88–296) μmol/kg, and 125.0 (104–150) μmol/kg, respectively, in the same experimental model.

The results reported in the present investigation demonstrated for the first time that 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]-phenyl}-thioureas exert anti-nociceptive effects in mice. It is possible to observe in Table 2 that the percentage of maximal inhibition of 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thioureas showed higher values than that of 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-ureas, besides the lowest values of ID_{50}. In both series, the compounds without substituent in the aromatic ring (from aldehyde) were more active. Of these, compound **3** produced the most potent and dose-dependent action in writhing test model in mice, being approximately 47 times more active than the reference drugs in the intraperitoneal treatment.

Administered orally, compound **3** presents maximal inhibition of 55.0% at a dose of 200 mg/kg with ID_{50} value of 377.5 (344.63–413.48) μmol/kg (Fig. 2), being about two times more potent than the standard drugs (ASA and ACE) used as reference, which presented ID_{50} values of 605 (516–705) μmol/kg and ID_{50} = 1145 (708–1846) μmol/kg, respectively, in the same experi-

Table 2
Comparison of the anti-nociceptive effects of 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thiourea and urea derivatives with non-steroidal analgesic drugs (ASA, dipyrone, and acetaminophen) given intraperitoneally in mice, in the writhing test



Compound	X	Y	ID_{50} (μmol/kg, ip)[a]	MI[b]
3	S	–H	3.03 (1.99–4.58)	94.5 ± 1.1**
4	S	4-Cl	7.59 (6.26–9.25)	100.0 ± 0.0**
5	S	3,4-Cl_2	6.15 (4.91–7.69)	97.5 ± 0.6**
6	S	4-OCH_3	10.37 (8.21–13.07)	92.0 ± 1.4**
7	S	4-CH_3	13.14 (11.0–15.72)	75.3 ± 4.5**
8	O	–H	7.25 (6.11–8.63)	74.6 ± 4.1**
9	O	4-Cl	45.38 (37.71–54.63)	51.0 ± 2.9**
10	O	3,4-Cl_2	Not dose-dependent	21.6 ± 3.8**
11	O	4-OCH_3	31.05 (11.60–14.85)	84.4 ± 2.0**
12	O	4-CH_3	39.70 (35.12–44.87)	42.8 ± 4.2**
ASA	—	—	133.0 (73.0–243.0)	35.0 ± 2.0**
Dip.	—	—	162.0 (88.0–296.0)	33.0 ± 3.5**
ACE	—	—	125.0 (104.0–150.0)	38.0 ± 1.0**

[a] 95% confidence limit. Each group represents the mean of six to eight animals. Compounds, acetyl salicylic (ASA), dipyrone (Dip.) and acetaminophen (ACE) were given intraperitoneally at 10 mg/kg.
[b] Maximal inhibition.
** $p < 0.01$ compared with the corresponding control value.

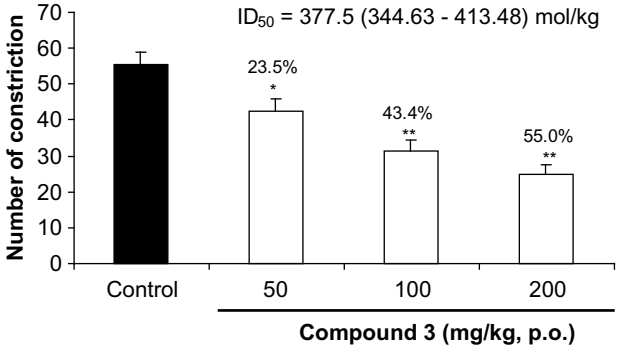


Figure 2. Effect of compound 1-(4-chlorophenyl)-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}thiourea **3**, administered orally, against acetic acid-induced abdominal constrictions in mice. Each column represents the mean ± s.e.m. of six experimental values. ** $p < 0.01$.

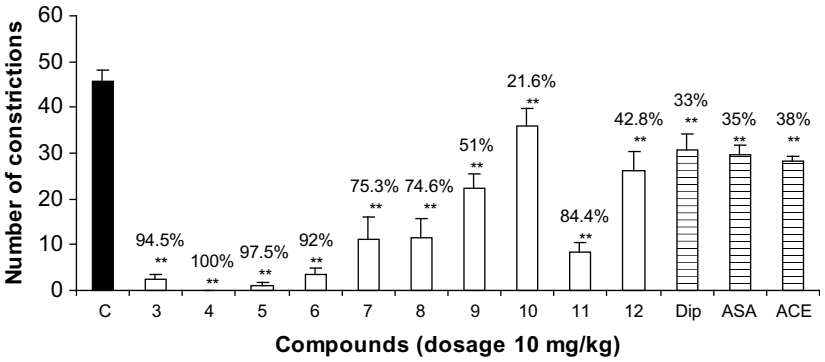


Figure 1. Comparison of the anti-nociceptive effects of 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thiourea and urea derivatives, non-steroidal anti-inflammatory and analgesic drugs (dipyrone, ASA, and ACE) with control (C) group, at a concentration of 10 mg/kg, against acetic acid-induced pain in mice. Each group represents the mean of six experiments and the vertical bars indicate the s.e.m. ** $p < 0.01$, compared with the corresponding control value.

mental model. These results seen in Figure 2 suggest that it is well absorbed by the gastrointestinal tract.

Analyzing some molecular properties and structural factors, according to the method proposed by Lipinski,[33,34] which evaluates in silico oral bioavailability, it can be observed that compound **3** exhibited five of the proposed parameters, unlike the other compounds of the series (**4**–**12**), which have infringed values of Log*P* proposed by Lipinski's' rule (Table 3).[33,34]

Although compound **3** proved to be the most promising candidate for a future drug, it is important to point out that one violation of the parameters proposed by Lipinski does not invalidate the biological potential of the molecules,[34,35] since this method, in principle, indicates a probable oral bioavailability only by passive transport. The excellent results for anti-nociceptive activity (intraperitoneally) suggest that compounds **4**–**9**, **11**, and **12** are not using any other transport to the active site. However, further biological assessments need to be conducted in order to identify possible mechanisms that enable us to definitively state which type of transport is being used by these compounds.

Acetic acid-induced pain in mice has the advantage that it allows evidence to be obtained for effects produced by weak analgesics. Indeed, this test works not only for all major and minor analgesics, but also for numerous other substances, including some that have no analgesic action, for example, adrenergic blockers,[36] anti-histamines,[37] muscle relaxants,[38] monoamine oxidase inhibitors[39] and neuroleptics.[40] Nevertheless, because all analgesics inhibit abdominal cramps, this method is useful for sifting molecules whose pharmacodynamic properties are unknown.[41,42] Compound **3** was therefore selected for more detailed studies in other models of pain, in order to correlate, at least partially, the mechanism of action of this compound.

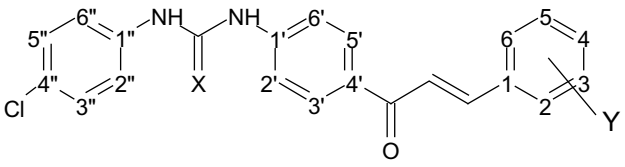
The evaluation in the formalin test presents a distinctive biphasic nociception response termed early and late phases. Drugs that act primarily on the central nervous system inhibit both phases equally, while peripherally acting drugs inhibit the late phase.[43,44] The early phase is probably a direct result of the stimulation of nociceptors in the paw, and reflects centrally mediated pain, while the late phase is due to inflammation with a release of serotonin, histamine, bradykinin, and prostaglandins[45] and at least to some degree, to the sensitization of central nociceptive neurons.[44–46]

In this model, compound **3** significantly inhibited both the first and second phases by systemic route, in a dose-dependent way. The calculated ID_{50} values were 69.32 (55.9–85.9) μmol/kg and 26.85 (23.0–31.3) μmol/kg, with maximum inhibitions of 53.9 and 78.7% at 30 mg/kg, for the first and second phases, respectively (Fig. 3). The ASA, an analgesic and anti-inflammatory drug which is frequently used in therapeutic treatments, was inactive in preventing the first phase of the formalin-induced (neurogenic) pain, and in the second phase it presented an ID_{50} value of 123.0 (77.0–209.0) μmol/kg, compound **3** being about five times more potent. In relation to dipyrone, which was active in both phases of this test, compound **3** was much more active, being about two times more active in the first phase, and 10 times more active in the late phase than dipyrone itself, presenting ID_{50} values of 154.5 (99.0–238.8) μmol/kg and 263.7 (234.0–296.9) μmol/kg, respectively.[47] Another interesting drug for comparison is the diclofenac, which presents a better response in the formalin-induced pain test, and was less active than compound **3**. This drug presented, in this test, values of >94 μmol/kg for the first phase and 34.5 (25.0–47.0) μmol/kg for the second phase[48].

The capsaicin test in mice has been used to access the anti-nociceptive effect of the tachykinin neurokinin-1 receptor antagonist, the glutamate receptor antagonist, the nitric oxide (NO) synthase inhibitor, and morphine.[49] Compound **3**, tested intraperitoneally in mice at 10 mg/kg, was ineffective in this model (Fig. 4). This result suggests that it does not involve the participation of the vanilloid receptors, owing to the lack of analgesic effects in the capsaicin model. The results support the hypothesis that the anti-nociceptive mechanism differs largely regarding their action on pain transmission in response to intraplantar injection of formalin or capsaicin although both formalin and capsaicin are neurogenic.

It is important to mention that some known non-steroidal anti-inflammatory drugs, including aspirin and acetaminophen, are

Table 3
Theoretical studies of solubility and permeability of 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thiourea and urea derivatives by Lipinski's rule of five



Compound	X	Y	No. of atom	Log*P*[a]	MW	No. of ON[b]	No. of OHNH[c]	No. of rotb.[d]	PSA[e]	No. of viol.[f]
3	S	–H	27	4.96	392.9	3	2	7	41.12	0
4	S	4-Cl	28	5.64	427.3	3	2	7	41.12	1
5	S	3,4-Cl_2	29	6.24	461.8	3	2	7	41.12	1
6	S	4-OCH_3	29	5.02	422.9	4	2	8	50.36	1
7	S	4-CH_3	28	5.41	406.9	3	2	7	41.12	1
8	O	–H	27	5.69	376.8	4	2	5	58.20	1
9	O	4-Cl	28	6.37	411.3	4	2	5	58.20	1
10	O	3,4-Cl_2	29	6.98	445.7	4	2	5	58.20	1
11	O	4-OCH_3	29	5.75	406.9	5	2	6	67.43	1
12	O	4-CH_3	28	6.14	390.9	4	2	5	58.20	1
ASA	—	—	13	1.43	180.2	4	1	3	63.60	0
ACE	—	—	11	0.68	151.2	3	2	1	49.33	0
Dip.	—	—	20	−2.62	296.3	7	1	4	98.23	0

[a] Method for Log*P* prediction developed at Molinspiration (miLog*P*2.2—November, 2005) is based on group contributions.
[b] Sum of N and O H-bond acceptors.
[c] Sum of NH and OH H-bond donors.
[d] Number of rotatable bond.
[e] Polar surface area.
[f] Number of violations.

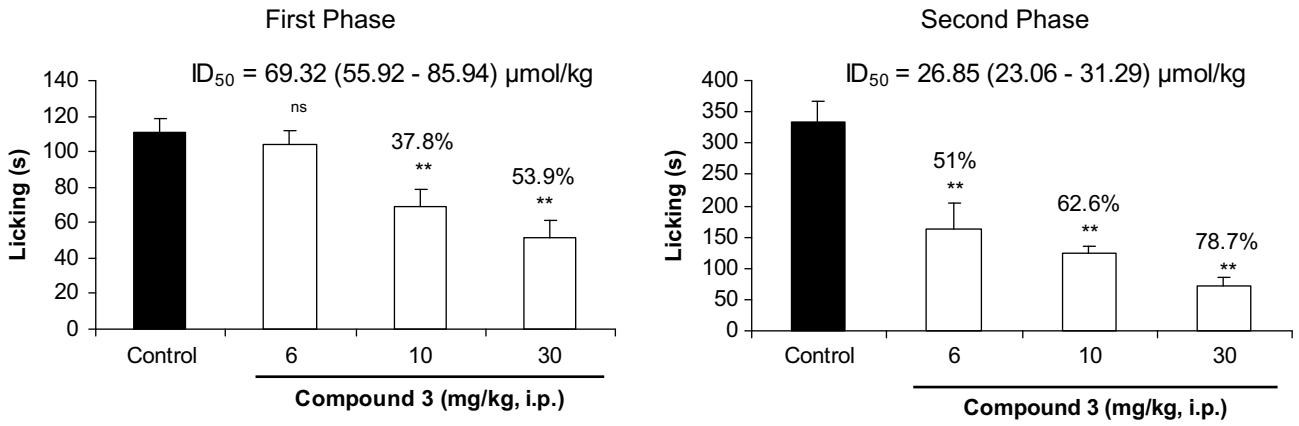


Figure 3. Effect of compound 1-(4-chlorophenyl)-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}thiourea (**3**), against formalin-induced pain in mice. Each column represents the mean ± s.e.m. of six experimental values. [ns]Not significant. [**]$p < 0.01$.

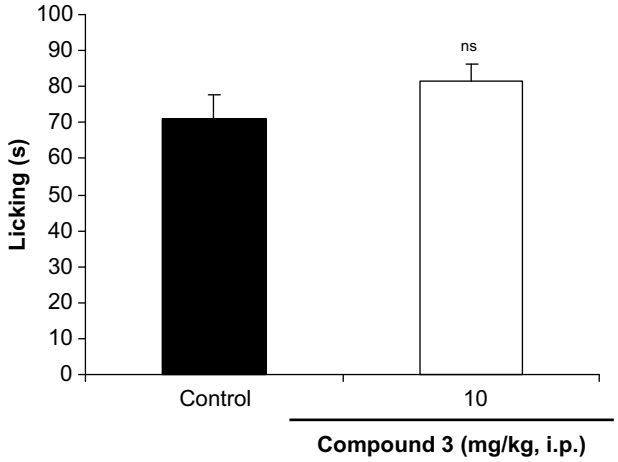


Figure 4. Effect of compound 1-(4-chlorophenyl)-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}thiourea (**3**), against capsaicin-induced pain in mice, at 10 mg/kg, ip. Each column represents the mean ± s.e.m. of six experimental values. [ns]Not significant.

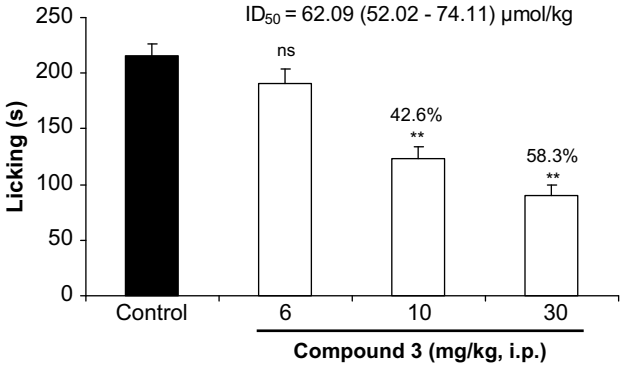


Figure 5. Effect of compound 1-(4-chlorophenyl)-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}thiourea (**3**), against glutamate-induced pain in mice. Each column represents the mean ± s.e.m. of six experimental values. [ns]Not significant. [**]$p < 0.01$.

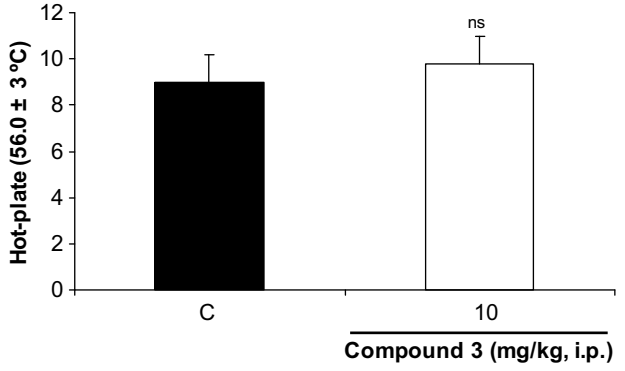


Figure 6. Effect of compound 1-(4-chlorophenyl)-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}thiourea (**3**), in the hot-plate test in mice, at 10 mg/kg, ip. Each column represents the mean ± s.e.m. of six experimental values. [ns]Not significant.

ineffective or exhibit weak activity in the first phase of the formalin test and in the capsaicin model, although they significantly inhibit the second phase of formalin-induced licking, compound **3** being the most powerful.

Compound **3** caused dose-related inhibition of the nociception elicited by intraplantar glutamate (20 μmol/paw), with a calculated ID_{50} value of 62.1 (52.0–74.1) μmol/kg and maximal inhibition of 58.3% at a dosage of 30 mg/kg (Fig. 5). These results suggest that this compound acts inhibiting the release of neuropeptides from the sensory fibers, namely, NKs and kinins.[50] The current results corroborate this evidence, indicating a relevant peripheral role in controlling the nociceptive processes.

When analyzed in the hot-plate method, compound **3** was not capable of increasing the latency period of pain induced by heating the plate at 10 mg/kg when administered intraperitoneally (Fig. 6). This method has been designed to perform rapid and precise screening of the central analgesic drugs.[51] Morphine (26.6 μmol/kg, sc), used as a reference drug in this test, caused a significant and marked analgesic effect.[51] Our results suggest that the mechanism by which compound **3** exerts analgesic activity does not involve the participation of the opioid system, owing to the lack of analgesic effects in the hot-plate test.

The manual procedure suggested by Topliss[27] is based on the assumption that the biological activity depends on the logic of the hydrophobic (π) and electronic (α) effects of the substituents in the aromatic ring, and on a combination of both. The projected order of potency of these five compounds for various parameter dependencies is listed in Table 4. Comparison with the actual experimentally determined potency order allows a possible deduction to be made concerning the probable operative parameters which in turn provides the basis for a new substituent selection. Preliminary evaluation assessed with compounds

Table 4
Potency order for various parameter dependencies[27]

Substituents	Parameters								
	π	$2\pi - \pi^2$	σ	$\pi + \sigma$	$2\pi - \sigma$	$\pi - \sigma$	$\pi - 2\sigma$	$\pi - 3\sigma$	E_s[a]
3,4-Cl_2	1	1–2	1	1	1	1–2	3–4	5	2–5
4-Cl	2	1–2	2	2	2–3	3	3–4	3–4	2–5
4-CH_3	3	3	4	3	2–3	1–2	1	1	2–5
4-OCH_3	4–5	4–5	5	5	4	4	2	2	2–5
H	4–5	4–5	3	4	5	5	5	3–4	1

[a] Unfavorable steric effect from 4 substitution.

3–**12** suggests that there is an unfavorable steric effect from substituent groups in the position 4; this suggestion could be seen through the analysis of Table 4 as suggested by Topliss.[27] Thus, the two most potent members of these series, the 4-unsubstituted compounds **3** and **8**, have the lowest steric requirements. However, it is useful to suggest molecular modeling in order to corroborate the influence of the steric effects of the substituent groups in the position 4.

3. Conclusions

In summary, the synthetic method permitted the preparation of 10 chalcone derivatives with good to reasonable yields (45–90%). Nine of these molecules (**3**–**9**, **11**, and **12**) presented anti-nociceptive activity, with lower ID_{50} values than those obtained for the positive control drugs (acetylsalicylic acid, acetaminophen, and dipyrone). The structure–activity analysis shows that 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thiourea derivatives were more potent than 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-urea derivatives, and there was an unfavorable steric effect from four substituents. The compounds not substituted in ring B, such as compounds **3** and **8**, presented greater anti-nociceptive activity. Compound **3** was a promising candidate for a future drug, because it was the most potent in the series tested, being approximately 47 times more active in the intraperitoneal treatment and two times when treated orally, compared with the reference drugs. In the formalin test, compound **3** significantly inhibited both the first and second phases, by systemic route, in a dose-dependent way, being more potent than diclofenac, a powerful anti-inflammatory drug used in therapeutic treatments. It also presents activity in the glutamate model. The mechanism by which this compound exerts analgesic activity still remains undetermined, but our results suggest that it does not involve the participation of the opioid system, or the vanilloid receptors, owing to the lack of analgesic effects in the hot-plate test and capsaicin model, respectively. The procedure manual method suggested by Topliss suggests that these compounds have an unfavorable steric effect from substitutions at position 4. It is important to mention that no violation of the Lipinski's parameters was observed, characterizing compound **3** as drug-like compound.

4. Experimental

4.1. Synthesis

A procedure based on Claisen–Schmidt condensation was developed for the syntheses of all the 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thiourea and urea derivatives. The syntheses of acetophenone derivatives (**1**–**2**) were obtained by reaction of *p*-amino acetophenone with the corresponding 4-chlorophenylisothiocyanate or 4-chlorophenyl-isocyanate derivatives. The subsequent treatment of these derivatives with solid sodium hydroxide, in methanol, at room temperature, and the corresponding substituted aromatic aldehydes by the use of Claisen–Schmidt condensation[52] yielded five thioureas (**3**–**7**) and five ureas (**8**–**12**) (Scheme 1).

4.2. General procedure for preparation of compounds

4.2.1. 1-(4-acetylphenyl)-3-(4-chlorophenyl)thiourea (1) and 1-(4-acetylphenyl)-3-(4-chlorophenyl)urea (2)[9]

A mixture of the corresponding 4-aminoacetophenone (1.5 mmol) and 4-chlorophenylisothiocyanate or 4-chlorophenyl-isocyanate (1.5 mmol) was dissolved in dry acetone (10 mL). The mixture was stirred at room temperature for 3–6 h. The resulting solids were collected on a filter and washed with dry acetone. The resulting solid was crystallized from ethanol to yield 1-(4-acetylphenyl)-3-(4-chlorophenyl)thiourea (**1**) and 1-(4-acetylphenyl)-3-(4-chlorophenyl)urea (**2**) in pure form.

4.2.2. 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thiourea and urea derivatives (3–12)[9]

Title compounds were prepared by reacting equivalent amounts of 1-(4-acetylphenyl)-3-(4-chlorophenyl)thiourea (**1**) and 1-(4-acetylphenyl)-3-(4-chlorophenyl)urea (**2**) with the corresponding substituted aldehydes, in the presence of excess sodium hydroxide (2.5 mmol) in dry methanol (5 mL). The mixture was stirred at room temperature, and the resulting solids were collected in a filter and washed three times with cold methanol. In most cases, off-white to bright-yellow solids were formed within 2–20 h. The product was recrystallized from the appropriate solvents (methanol) where necessary.

4.3. Physicochemical data of the synthesized compounds

The melting points were determined with a Microquimica AP-300 apparatus and are uncorrected. The IR spectra were recorded with an Abb Bomen FTLA 2000 or Perkin-Elmer 720 spectrometer on KBr disks. The NMR (^{1}H and ^{13}C NMR) spectra were recorded on a Brucker Ac-200 F (300 MHz) or Varian Oxford AS-400 (400 MHz) instrument, using tetramethylsilane as an internal standard. Elementary analysis was carried out using a Perkin-Elmer 2400. The percentages of elements determined (C, H, and N) were in agreement with the product formula (within ±0.4% of theoretical values for C). The solvents and reagents were purified in the usual manner where necessary.

4.3.1. 1-(4-Acetylphenyl)-3-(4-chlorophenyl)thiourea (1)

Yield 45%; mp 168–169 °C (from ethanol); IR (KBr) ν_{max} 3478 (NH), 1656 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.53 (s, 3H, COMe), 7.38 (d, 2H, H3″-5″, J = 8.80 Hz), 7.52 (d, 2H, H2″-6″, J = 8.80 Hz), 7.68 (d, 2H, H2′-6′, J = 8.80 Hz), 7.92 (d, 2H, H3′-5′, J = 8.80 Hz), 10.11 (br s, 1H, NH), 10.19 (br s, 1H, NH); ^{13}C NMR (300 MHz, DMSO-d_6) δ 26.47 (COMe), 121.77 (C2′-6′), 125.23 (C2″-6″), 128.35 (C3′-5′), 128.52 (C4″), 128.90 (C3″-5″), 132.22 (C4′), 138.14 (C1″), 143.92 (C1′), 179.40 ($CS(NH)_2$), 196.58 (CO).

4.3.2. 1-(4-Acetylphenyl)-3-(4-chlorophenyl)urea (2)

Yield 45%; mp 223–225 °C (from ethanol); IR (KBr) ν_{max} 3478 (NH), 1712 (CS(NH)$_2$), 1654 (CO) cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.53 (s, 3H, COMe), 7.33 (d, 2H, H3″-5″, J = 8.80 Hz), 7.50 (d, 2H, H2″-6″, J = 8.80 Hz), 7.58 (d, 2H, H2′-6′, J = 8.80 Hz), 7.90 (d, 2H, H3′-5′, J = 8.80 Hz), 8.93 (br s, 1H, NH), 9.12 (br s, 1H, NH); ^{13}C NMR (300 MHz, DMSO-d_6) δ 26.32 (COMe), 117.25 (C2′-6′), 119.97 (C2″-6″), 125.77 (C4″), 128.66 (C3′-5′), 129.62 (C3″-5″), 130.57 (C4′), 138.31 (C1″), 144.18 (C1′), 152.09 (CO(NH)$_2$), 196.29 (CO).

4.3.3. 1-(4-Chlorophenyl)-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}thiourea (3)

Yield 50%; mp 178–180 °C (from methanol); IR (KBr) ν_{max} 3453 (NH), 1640 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.40 (d, 2H, H3-5, J = 8.55 Hz), 7.53 (d, 2H, H3″-5″, J = 8.55 Hz), 7.68 (d, 1H, Hα, J = 15.87 Hz), 7.74 (m, 4H, H2-6, H2″-6″), 7.87 (dd, 1H, H4, J = 8.55 Hz), 8.06 (d, 1H, Hβ, J = 15.87 Hz), 8.18 (d, 2H, H2′-6′, J = 8.86 Hz), 8.29 (d, 2H, H3′-5′, J = 8.86 Hz), 10.15 (br s, 1H, NH), 10.24 (br s, 1H, NH); ^{13}C NMR (300 MHz, DMSO-d_6) δ 121.76 (C2′-6′), 124.04 (Cα), 125.25 (C2″-6″), 128.40 (C2-6), 129.10 (C4), 129.50 (C3-5), 130.05 (C4″), 130.94 (C3″-5″), 131.81 (C3′-5′), 132.56 (C4′), 135.68 (C1), 138.17 (C1″), 140.39 (C1′), 144.29 (Cβ), 179.36 (CS(NH)$_2$), 187.36 (CO). $C_{22}H_{17}ClN_2OS$ requires: C (67.25%) H (4.36%) N (7.13%). Found: C (67.15%) H (4.16%) N (7.00%).

4.3.4. 1-(4-Chlorophenyl)-3-{4-[(2*E*)-3-(4-chlorophenyl)prop-2-enoyl]-phenyl}thiourea (4)

Yield 90%; mp 187–190 °C (from methanol); IR (KBr) ν_{max} 3453 (NH) 1654 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.16 (d, 2H, H3″-5″, J = 8.65 Hz), 7.24 (d, 2H, H3-5, J = 8.65 Hz), 7.50 (d, 4H, H2″-6″ and H2-6, J = 8.65 Hz), 7.64 (d, 1H, Hα, J = 15.38 Hz), 7.91 (d, 2H, H2′-6′, J = 8.65 Hz), 7.95 (d, 1H, Hβ, J = 15.38 Hz), 8.00 (d, 2H, H3′-5′, J = 8.65 Hz), 9.96 (br s, 2H, NH); ^{13}C NMR (300 MHz, DMSO-d_6) δ 122.83 (Cα), 123.23 (C2′-6′), 127.45 (C2″-6″), 127.74 (C2-6), 128.43 (C4″), 128.87 (C3-5), 129.33 (C3″-5″), 129.99 (C4′), 130.28 (C3′-5′), 131.15 (C4), 134.06 (C1), 134.53 (C1″), 140.51 (C1′ and Cβ), 179.50 (CS(NH)$_2$), 187.73 (CO). $C_{22}H_{16}Cl_2N_2OS$ requires: C (61.83%) H (3.77%) N (6.56%). Found: C (61.90%) H (3.80%) N (6.50%).

4.3.5. 1-(4-Chlorophenyl)-3-{4-[(2*E*)-3-(3,4-dichlorophenyl)prop-2-enoyl]-phenyl}thiourea (5)

Yield 80%; mp 172–174 °C (from methanol); IR (KBr) ν_{max} 3451 (NH) 1642 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.40 (d, 2H, H3″-5″, J = 8.25 Hz), 7.55 (d, 1H, H6, J = 8.25 Hz), 7.68 (d, 1H, Hα, J = 15.6 Hz), 7.72 (d, 2H, H2″-6″, J = 8.25 Hz), 7.76 (d, 2H, H2′-6′, J = 8.25 Hz), 7.85 (d, 1H, H5, J = 8.25 Hz), 8.04 (d, 1H, Hβ, J = 15.6 Hz), 8.17 (d, 2H, H3′-5′, J = 8.25 Hz), 8.26 (s, 1H, H2), 10.16 (br s, 1H, NH), 10.25 (br s, 1H, NH); ^{13}C NMR (300 MHz, DMSO-d_6) δ 121.82 (C2′-6′), 124.07 (Cα), 125.28 (C2″-6″), 128.35 (C6), 128.43 (C3″-5″), 129.13 (C2), 129.53 (C3′-5′), 130.08 (C4″), 130.97 (C5) 131.32 (C4′), 131.84 (C4), 132.59 (C3), 135.68 (C1), 138.20 (C1″), 140.62 (Cβ), 144.32 (C1′), 179.39 (CS(NH)$_2$), 187.39 (CO). $C_{22}H_{15}Cl_3N_2OS$ requires: C (57.22%) H (3.27%) N (6.07%). Found: C (57.30%) H (3.50%) N (6.10%).

4.3.6. 1-(4-Chlorophenyl)-3-{4-[(2*E*)-3-(4-methoxyphenyl)prop-2-enoyl]-phenyl}thiourea (6)

Yield 70%; mp 195–197 °C (from methanol); IR (KBr) ν_{max} 3453 (NH) 1638 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.82 (s, 3H, OMe), 7.02 (d, 2H, H3-5, J = 8.86 Hz), 7.40 (d, 2H, H3″-5″, J = 8.86 Hz), 7.54 (d, 2H, H2-6), 7.70 (d, 1H, Hα, J = 15.75 Hz), 7.73 (d, 2H, H2″-6″, J = 8.86 Hz), 7.82 (d, 1H, Hβ, J = 15.75 Hz), 7.86 (d, 2H, H2′-6′, J = 8.86 Hz), 8.13 (d, 2H, H3′-5′, J = 8.86 Hz), 10.18 (br s, 1H, NH), 10.27 (br s, 1H, NH); ^{13}C NMR (300 MHz, DMSO-d_6) δ 55.35 (OMe), 114.39 (C3-5), 119.42 (Cα), 121.85 (C2′-6′), 125.25 (C2″-6″), 127.39 (C4″), 128.37 (C2-6), 128.45 (C4′), 129.18 (C3″-5″), 130.68 (C3′-5′), 133.14 (C1), 138.22 (C1″), 143.45 (Cβ), 143.83 (C1′), 161.28 (C4), 179.36 (CS(NH)$_2$), 187.51 (CO). $C_{23}H_{19}ClN_2O_2S$ requires: C (65.32%) H (4.53%) N (6.62%). Found: C (65,00%) H (4,55%) N (6,68%).

4.3.7. 1-(4-Chlorophenyl)-3-{4-[(2*E*)-3-(4-methylphenyl)prop-2-enoyl]-phenyl}thiourea (7)

Yield 37%; mp 208–210 °C (from methanol); IR (KBr) ν_{max} 3454 (NH) 1640 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.35 (s, 3H, Me), 7.21 (d, 2H, H3-5, J = 8.36 Hz), 7.25 (d, 2H, H3″-5″, J = 8.36 Hz), 7.27 (d, 2H, H2-6, J = 8.36 Hz), 7.65 (d, 1H, Hα, J = 15.38 Hz), 7.73 (d, 2H, H2″-6″, J = 8.36 Hz), 7.76 (d, 2H, H2′-6′, J = 8.36 Hz), 7.89 (d, 1H, Hβ, J = 15.38 Hz), 8.01 (d, 2H, H3′-5′, J = 8.36 Hz), 9.45 (br s, 2H, NH); ^{13}C NMR (300 MHz, DMSO-d_6) δ 21.06 (Me), 120.89 (Cα), 121.70 (C2′-6′), 123.61 (C2″-6″), 124.71 (C4″), 128.49 (C2-6), 128.81 (C3″-5″), 129.21 (C3-5), 129.50 (C3′-5′), 132.04 (C4′), 132.79 (C1), 139.18 (C4), 140.51 (C1″), 143.45 (C1′), 144.15 (Cβ), 179.27 (CS(NH)$_2$), 187.56 (CO). $C_{23}H_{19}ClN_2OS$ requires: C (67.89%) H (4.71%) N (6.88%). Found: C (66.98%) H (4.50%) N (6.92%).

4.3.8. 1-(4-Chlorophenyl)-3-{4-[(2*E*)-3-phenylprop-2-enoyl]-phenyl}urea (8)

Yield 76%; mp 202–204 °C (from methanol); IR (KBr) ν_{max} 3323 (NH) 1712 (CO urea) 1652 (COα,β-unsaturated) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.31 (d, 2H, H3,5, J = 8.95 Hz), 7.45 (m, 3H, H3″-5″, H4), 7.53 (d, 2H, H2-6, J = 8.95), 7.67 (d, 2H, H2″-6″, J = 8.95), 7.71 (d, 1H, Hα, J = 15.79 Hz), 7.87 (dd, 2H, H2′-6′, J = 8.95 Hz), 7.93 (d, 1H, Hβ, J = 15.79 Hz), 8.12 (d, 2H, H3′-5′, J = 8.95 Hz), 9.52 (br s, 2H, NH); ^{13}C NMR (300 MHz, DMSO-d_6) δ 117.40 (C2′-6′), 119.97 (C2″-6″), 122.02 (Cα), 125.60 (C4), 128.58 (C2-6), 128.72 (C3-5), 128.87 (C3″-5″), 130.02 (C3′-5′), 130.37 (C4″), 130.94 (C4′), 134.82 (C1), 138.51 (C1″), 142.99 (C1′), 144.61 (Cβ), 152.29 (CO(NH)$_2$), 187.28 (CO). $C_{22}H_{17}ClN_2O_2$ requires: C (70.12%) H (4.55%) N (7.43%). Found: C (69,80%) H (4,49%) N (7.22%).

4.3.9. 1-(4-Chlorophenyl)-3-{4-[(2*E*)-3-(4-chlorophenyl)prop-2-enoyl]-phenyl}urea (9)

Yield 87%; mp 249–251 °C (from methanol); IR (KBr) ν_{max} 3334 (NH) 1710 (CO urea) 1640 (COα,β-unsaturated) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.34 (d, 2 H, H3-5, J = 8.80 Hz), 7.51 (d, 2H, H3″-5″, J = 8.80 Hz), 7.53 (d, 2H, H2-6), 7.63 (d, 2H, H2″-6″), 7.70 (d, 1H, Hα, J = 15.85 Hz), 7.92 (d, 2H, H2′-6′, J = 8.80 Hz), 8.02 (d, 1H, Hβ, J = 15,85 Hz), 8.14 (d, 2H, H3′-5′, J = 8.80 Hz), 9.03 (br s, 1H, NH), 9.25 (br s, 1H, NH); ^{13}C NMR (300 MHz, DMSO-d_6) δ 117.37 (C2′-6′), 120 (Cα), 122.77 (C2″-6″), 125.77 (C2-6), 128.63 (C4), 128.89 (C3-5), 130.14 (C4″), 130.45 (C3″-5″), 130.97 (C3′-5′), 130.80 (C4′), 134.84 (C1), 138.28 (C1″), 141.58 (C1′), 144.41 (Cβ), 152.09 (CO(NH)$_2$), 187.19 (CO). $C_{22}H_{16}Cl_2N_2O_2$ requires: C (64.25%) H (3.92%) N (6.81%). Found: C (64.15%) H (3.50%) N (6.78%).

4.3.10. 1-(4-Chlorophenyl)-3-{4-[(2*E*)-3-(3,4-dichlorophenyl)prop-2-enoyl]-phenyl}urea (10)

Yield 87%; mp 198-200 °C (from methanol); IR (KBr) ν_{max} 3343 (NH) 1713 (CO urea) 1641 (COα,β-unsaturated) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.35 (d, 2H, H3″-5″, J = 8.83 Hz), 7.51 (d, 2H, H2″-6″, J = 8.83 Hz), 7.63 (d, 2H, H2′-6′), 7.67 (d, 1H, Hα, J = 15.44 Hz), 7.70 (d, 1H, H6, J = 8.83 Hz), 7.86 (d, 1H, H5, J = 8.83 Hz), 8.04 (d, 1H, Hβ, J = 15.44 Hz), 8.16 (d, 2H, H3′-5′, J = 8.83 Hz), 8.26 (s, 1H, H2), 9.12 (br s, 2H, NH); ^{13}C NMR (300 MHz, DMSO-d_6) δ 117.34 (C2′-6′), 120.00 (C2″-6″), 124.10 (Cα), 125.75 (C6), 128.63 (C3″-5″), 129.01 (C2), 130.02 (C4″), 130.22 (C3′-5′), 130.83 (C5), 130.92 (C4′), 131.78 (C4), 132.48

(C3), 135.74 (C1), 138.28 (C1″), 140.22 (Cβ), 144.55 (C1′), 152.09 ($CO(NH)_2$), 187.02 (CO). $C_{22}H_{15}Cl_3N_2O_2$ requires: C (59.28%) H (3.39%) N (6.28%). Found: C (59.30%) H (3.40%) N (6.18%).

4.3.11. 1-(4-Chlorophenyl)-3-{4-[(2*E*)-3-(4-methoxyphenyl)prop-2-enoyl]-phenyl}urea (11)

Yield 88%; mp 208–210 °C (from methanol); IR (KBr) ν_{max} 3343 (NH) 1708 (CO urea) 1641 (COα,β-unsaturated) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 3.81 (s, 3H, OMe), 7.00 (d, 2H, H3-5, J = 8.95 Hz), 7.32 (d, 2H, H3″-5″, J = 8.95 Hz), 7.52 (d, 2H, H2-6, J = 8.95), 7.64 (d, 2H, H2″-6″, J = 8.95 Hz), 7.68 (d, 1H, Hα, J = 15.26 Hz), 7.78 (d, 1H, Hβ, J = 15.26 Hz), 7.83 (d, 2H, H2′-6′, J = 8.42 Hz), 8.11 (d, 2H, H3′-5′, J = 8.42 Hz), 9.29 (br s, 2H, NH); ^{13}C NMR (300 MHz, DMSO-d_6) δ 55.32 (OMe), 114.36 (C3-5), 117.34 (C2′-6′), 119.48 (Cα), 119.97 (C2″-6″), 125.66 (C4″), 127.45 (C4′), 128.61 (C2-6), 129.88 (C3″-5″), 130.60 (C3′-5′), 131.29 (C1), 138.43 (C1″), 143.02 (Cβ), 144.23 (C1′), 152.18 (C4), 161.19 ($CO(NH)_2$), 187.19 (CO). $C_{23}H_{19}ClN_2O_3$ requires: C (67.90%) H (4.71%) N (6.89%). Found: C (67.20%) H (4.22%) N (6.92%).

4.3.12. 1-(4-Chlorophenyl)-3-{4-[(2E)-3-(4-methylphenyl)prop-2-enoyl]-phenyl}urea (12)

Yield 93%; mp 217–219 °C (from methanol); IR (KBr) ν_{max} 3343 (NH) 1709 (CO urea) 1643 (COα,β-unsaturated) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 2.34 (s, 3H, Me), 7.26 (d, 2H, H3-5, J = 8.83 Hz), 7.31 (d, 2H, H2-6, J = 8.83 Hz), 7.51 (d, 2H, H3″-5″), 7.66 (d, 2H, H2″-6″, J = 8.83 Hz), 7.68 (d, 1H, Hα, J = 16.18 Hz), 7.75 (d, 2H, H2′-6′, J = 8.83 Hz), 7.88 (d, 1H, Hβ, J = 16.18 Hz), 8.10 (d, 2H, H3′-5′, J = 8.83 Hz), 9.65 (br s, 2H, NH); ^{13}C NMR (300 MHz, DMSO-d_6) δ 21.03 (Me), 117.37 (C2′-6′), 119.97 (C2″-6″), 120.95 (Cα), 125.54 (C4″), 128.55 (C2-6), 128.75 (C3″-5″), 129.50 (C3-5), 129.93 (C3′-5′), 131.03 (C4′), 132.10 (C1), 138.60 (C4), 140.39 (C1″), 143.05 (C1′), 144.55 (Cβ), 152.32 ($CO(NH)_2$), 187.25 (CO). $C_{23}H_{19}ClN_2O_2$ requires: C (70.68%) H (4.90%) N (7.17%). Found: C (70,20%) H (4,70%) N (7.00%).

4.4. Biological assay

4.4.1. Animals

Swiss mice (25–35 g) were obtained from the Animal House of the University of Vale do Itajaí (Itajaí, Brazil). They were housed in automatically controlled temperature conditions (23 ± 2 °C and 12 h light–dark cycles). The animals were given access to water and Nuvital chow ad libitum, unless otherwise indicated. They remained in the appropriate laboratory at UNIVALI until several hours before the experiments. The allocation of animals into the different groups was randomized, and the experiments were carried out in blind conditions. Since some suffering might result from experiments, the IASP Committee for Research and Ethical Issues Guidelines[53] were followed.

4.4.2. Acetic acid-induced writhing

The abdominal constriction response caused by intraperitoneal injection of diluted acetic acid (0.6%), was carried out according to the procedures described previously[54,55] with minor modifications. The animals were pretreated with 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thiourea derivatives (**3**–**7**) or 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-urea derivatives (**8**–**12**) (1–60 mg/kg, ip) or standard drugs 30 min before acetic acid infection. The control animals received a similar volume of 0.9% NaCl (10 ml/kg, ip). All the experiments were carried out at 20–22 °C. After the challenge, each animal was placed in a separate glass funnel, and the number of abdominal contractions of the abdominal muscles together with stretching of the hind limbs was cumulatively counted over a period of 20 min. Anti-nociceptive activity control animals were pretreated with compounds 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]phenyl}-thiourea and urea derivatives or standard drugs. For compound **3**, the abdominal constriction response was analyzed, following pretreatment via oral route, of a dosage of 50–200 mg/kg.

4.4.3. Formalin test

The observation chamber was a glass cylinder of 20 cm in diameter, with a mirror at a 45° angle to allow clear observation of the animal's paws. The mice were treated with 0.9% saline solution (ip) or compound **3** (6–30 mg/kg, ip), 30 min before formalin injection. Each animal was placed in the chamber for 5 min before treatment, in order to acclimatize to the new environment. The formalin test was carried out as described by Hunskaar and Hole,[56] with minor modifications.[55,57] Twenty microliters of a 2.5% formalin solution (0.92% formaldehyde) in 0.9% saline solution were injected intraplantarly into the right hind paw. The animal was then returned to the chamber, and the amount of time spent licking the injected paw was considered as indicative of pain. Two distinct phases of intensive licking activity were identified: an early acute phase and a late or tonic phase (0–5 and 15–30 min after formalin injection, respectively).

4.4.4. Capsaicin-induced nociception

The procedure used was similar to that described previously.[49] After the adaptation period, 20 ml of capsaicin (1.6 μg/paw) was injected intraplantarly into the right hindpaw. The animals were observed individually for 5 min following capsaicin injection. The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of nociception. The animals were treated with compound **3** via ip (10 mg/kg) 30 min prior to capsaicin injection. The control animals received a similar volume of saline solution, intraperitoneally.

4.4.5. Hot-plate test

The hot-plate test was used to measure response latencies, according to the method described by Eddy and Leimback.[58] The mice were treated with saline solution, morphine (10 mg/kg, sc) or compound **3** (10 mg/kg, ip), and placed individually on a hot-plate maintained at 56 ± 1 °C. The time between placing the animal on the hot-plate and the occurrence of either the licking of the hind paws, shaking the paw or jumping off the surface was recorded as response latency. Mice with baseline latencies of more than 20 s were eliminated from the study, and the cut-off time for the hot-plate latencies was set at 30 s. The animals were treated 30 min before the assay.

4.4.6. Glutamate-induced nociception

The animals were treated with compound **3** via ip (6–30 mg/kg) 30 min before the glutamate injection. A volume of 20 μl of glutamate solution (30 μmol/paw), made up in phosphate-buffered saline (PBS), was injected intraplantarly under the surface of the right hind paw, as described previously by Beirith.[48] After injection with glutamate, the animals were individually placed into glass cylinders of 20 cm in diameter and observed from 0 to 15 min. The time spent licking or biting the injected paw was timed with a chronometer and considered as indicative of pain.

4.4.7. Statistical analysis

The results are presented as means ± SEM, except for the mean ID_{50} values (i.e. the dose of drugs or compounds reducing the anti-nociceptive responses by 50% relative to the control value) which are reported as geometric means accompanied by their respective 95% confidence limits. The statistical significance between the groups was determined by analysis of variance followed by Dunnett's multiple comparison test. *P*-values of less than 0.05 were

considered indicative of significance. The ID_{50} values were determined by graphical interpolation from the individual experiments.

4.4.8. Drugs

The following drugs were used: ASA, acetaminophen, dipyrone (all from Sigma Chemical) and acetic acid (E. Merck). All the compounds were dissolved in Tween 80 (E. Merck), plus 0.9% of NaCl solution. The final concentration of Tween 80 did not exceed 5% and did not cause any effect per se.

4.5. Solubility and permeability estimate: Lipinski's 'Rule of five'

Computational approaches were used to estimate the solubility and permeability of the synthesized compounds, using the 'rule of 5' proposed by Lipinski[33] and its extensions.[35] This rule predicts that poor absorption or permeation is more likely when there are more than 5 H-bond donors and, more than 10 H-bond acceptors, when the molecular weight (MWT) is greater than 500, the calculated CLog*P* is greater than 5 (or Mlog*P* > 4.15), and its extension parameters polar surface area (PSA) more than 140 $Å^2$ or the sum of the H-bond donors and acceptors is more than 12 and rotatable bond more than 10. These physicochemical parameters are associated with acceptable aqueous solubility and intestinal permeability, and comprise the first steps in oral bioavailability.[34]

The values for MWT, Log*P*, number of H-bond acceptors and donors, PSA and rotatable bond were obtained from the on-line program free molinspiration, by JME Editor, courtesy of Peter Ertl of Novartis, available on the website: http://www.molinspiration.com/cgi-bin/properties.

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