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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

The antinociceptive evaluation of 2,3-substituted-1,3-thiazolidin-4-ones through thermal stimulation in mice

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Abstract The present study assessed the 2,3-substituted-1,3-thiazolidin-4-ones antinociceptive potential looking at the acute nociception model induced by thermal stimulation in mice. This was done to contribute to the development of new analgesic drugs, in addition to the fact that 4-thiazolidinones are an important scaffold associated with many pharmacological activities. The synthesized compounds were characterized by GC-MS and NMR of ^{1}H and ^{13}C and administered at a dose of 100 mg/kg hydrochloride salt (ip). Sodium dipyrone (250 and 500 mg/Kg; ip) and tramadol hydrochloride (25 and 50 mg/Kg; ip) were used as positive controls. The hot plate test was done at a temperature of 50 ± 0.1 °C and animals assessed at 30, 60, and 90 min after the drugs were administered. Among the fourteen compounds tested, nine (**5Aa**, **5Ab**, **5Ac**, **5Ad**, **5Ba**, **5Bb**, **5Bd**, **5Ea**, and **5Fa**) showed significant increases in latency time when compared to saline (negative control) and compared to sodium dipyrone (500 mg/Kg; ip) in a 30-min assessment. The highest latency times were obtained for 3-(2-piperidin-1-yl)ethyl)thiazolidin-4-one derivatives (**5Ab**, **5Ac**, and **5Ad**). This highlights three findings about the chemical structure that improve activity: (i) an ethylenic link; (ii) a six-membered piperidine; (iii) an aliphatic substituent at the 2-position of thiazolidinone ring.

Keywords Antinociception · 4-Thiazolidinones · Analgesic · Acute pain · Hot plate test

Introduction

In 1979, the International Association for the Study of Pain defined pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage,” which is still valid today (Kopf and Patel 2010). For pain treatment, non-pharmacological, pharmacological, and invasive methods are used (Costa et al. 2007; Teixeira et al. 2013; Nossaman et al. 2010). Thus three classes of analgesic drugs may be described, opioid analgesic, non-steroidal anti-inflammatory (NSAIDs), and adjuvant analgesics, such as antidepressant, anticonvulsant, and anxiolytic medications. The combination of these analgesic drugs is very useful for chronic pain therapy (Costa et al. 2007; Liu et al. 2010; Shinde et al. 2015).

The most commonly used method for the relief of acute pain is systemic analgesics (Camu and Vanlersberghe 2002). However, independently of the type of pain, acute or

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chronic, the pharmacological therapy based on opioids and NSAIDS has several side effects, such as abuse, overdose, tolerance, dependence, sedation, gastrointestinal dysfunction, among others. The patients sometimes require other medication for controlling and managing these side effects (Bjarnason et al. 1993; Nelson and Camilleri 2015), thereby decreasing the adherence to pharmacological treatment (Kurita and Pimenta 2003). Thus, researchers shall develop new drugs for pain treatment rather safer and more effective, with side effect reduction that limits medication use, with greater therapeutic success for the user (Verçoza et al. 2009).

In this sense, the 1,3-thiazolidin-4-ones ring has become very important within the scientific community due to its multiple biological actions (Jain et al. 2012; Tripathi et al. 2014). Accordingly, researchers have established actions opposing germs and other infectious agents (virus, bacteria, fungi and parasites), against chronic diseases such as diabetes, hyperlipidemia and convulsions, as well as the presence of an anti-inflammatory and analgesic effect, among others (Tripathi et al. 2014; Cunico et al. 2008). Moreover, 1,3-thiazolidin-4-one derivatives have a low cost and large synthesis versatility, and also large structural variety depending on the adopted synthesis method. There are usually substituents at the 2, 3, and 5-position of the ring that promote changes in chemical, physical, and biological parameters (Jain et al. 2012; Cunico et al. 2008).

Therefore, taking advantage of the experience of our group in the synthesis of thiazolidinones and seeking to contribute to the development of new drugs for pain, the present study aimed at performing the synthesis of 2,3-substituted-1,3-thiazolidin-4-ones and antinociceptive activity through the acute nociception model, induced by thermal stimulation (hot plate test) in mice.

Material and methods

Chemical part

All common reagents and solvents were used and obtained from commercial suppliers without further purification. Reactions progress were monitored by a thin-layer chromatography (hexane:ethyl acetate 3:1) and/or by a Shimadzu Gas Chromatograph GC-2010, HP-1 column (cross linked methyl siloxane, 30 m × 0.32 mm × 0.25 µm): Column head pressure, 14 psi, program: $T0 = 60$ °C; $t0 = 2.0$ min; rate 10.0 °C min^{-1}; Tf = 280 °C; tf = 13.0 min; Inj. = 250 °C; Det. = 280 °C. The melting points were determined using open capillaries on a Fisatom model 430 apparatus and are uncorrected. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DRX 400 spectrometer (^{1}H at 400 MHz and ^{13}C at 100 MHz), on a Bruker Avance 500 spectrometer (^{1}H at 500 MHz and ^{13}C at 125 MHz) or on a Bruker AC-200F spectrometer (^{1}H at 200 MHz and ^{13}C at 50 MHz) in $CDCl_3$ or D_2O containing tetramethylsilane as an internal standard. The mass spectra were obtained on a Shimadzu GC-MS-QP2010SE with a split-splitless injector and equipped with a RDX-SMS capillary column (30 m × 0.25 mm × 0.25 µm); helium was used as the carrier gas (56 kPa).

General procedure for the synthesis of thiazolidinones ***4Aa–f, 4Ba–d, 4Ca–Fa***

The compounds **4Aa**, **4Ac**, **4Ae,** and **4Af** were obtained according Kunzler et al. (2013). The compounds **4Ea** and **4Fa** were obtained according Gouvea et al. (2012). For novel compounds, at first, the reaction occurs through a solution of 1 mmol of amine **1A–F** and 1 mmol of aldehyde or ketone **2a–f** in reflux of toluene (30 mL) for 2 h using a Dean-Stark trap. After, 3 mmol of mercaptoacetic acid **3** was added and the mixture was heated for more 3 h. The organic layer was washed with saturated $NaHCO_3$ (3 × 30 mL), dried with $MgSO_4$ and concentrated to give the products that were purified by column chromatography using hexane:ethyl acetate (7:3) as eluent. The atom numbering for NMR analyzes identification of compounds **4** and **5** are given in Fig. 1.

2-Butyl-3-(2-(piperidin-1-yl)ethyl)thiazolidin-4-one (**4Ab**) It was obtained as an oil; ^{1}H NMR δ ($CDCl_3$, 400 MHz, ppm, J_{H-H} = Hz); 4.76 (td, 1H, $^3J = 8.5$, $^4J = 2.3$, H-2); 3.74 (ddd, 1H, $^2J = 13.7$, $^3J = 7.7$, $^3J = 6.0$, H-6a); 3.50 (dd, 1H, $^2J = 15.5$, $^4J = 1.5$, H-5a); 3.41 (d, 1H, $^2J = 15.5$, H-5b); 3.04 (dt, 1H, $^2J = 13.9$, $^3J = 6.9$, H-6b); 2.47 (ddd, 1H, $^2J = 13.4$, $^3J = 7.3$, $^3J = 6.5$, H-7a); 2.35 (ddd, 1H, $^2J = 12.8$, $^3J = 7.1$, $^3J = 5.8$, H-7b); 2.33–2.39 (m, 4 H); 1.81–1.89 (m, 1H); 1.56–1.63 (m, 1H); 1.46–1.52 (m, 4H); 1.33–1.38 (m, 2H); 1.24–1.32 (m, 4H); 0.82–0.87 (m, 3H). ^{13}C NMR δ ($CDCl_3$, 100 MHz, ppm); 171.0 (C-4); 62.0 (C-2); 56.1 (C-7); 54.6 (2 C); 39.8 (C-6); 35.2; 32.0 (C-5); 26.3; 25.8 (2C); 24.1; 22.3; 14.0. MS (70 eV): *m/z* (%) = 270 (M^+, 1); 111 (3); 102 (1); 98 (100); 84 (2).

2-(4-Fluorophenyl)-3-(2-(pyrrolidin-1-yl)ethyl)thiazolidin-4-one (**4Ba**) It was obtained as a solid m.p.: 56–58 °C; ^{1}H

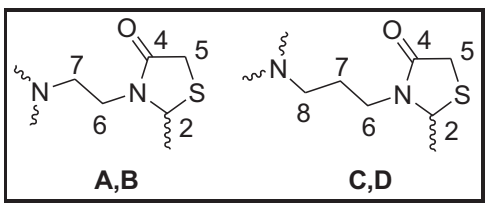


Fig. 1 The atom numbering for compounds **4** and **5**

NMR δ ($CDCl_3$, 200 MHz, ppm, J_{H-H} = Hz); 7.26–7.34 (m, 2H, Ar); 7.02–7.12 (m, 2H, Ar); 5.86 (s, 1H, H-2); 3.75–3.88 (m, 2H, H-5a, H-6a); 3.71 (d, 1H, $^2J = 15.5$, H-5b); 2.65–2.88 (m, 2H, H-6b, H-7a); 2.46–2.60 (m, 5H, H-7b, H-8); 1.74–1.80 (m, 4H). ^{13}C NMR δ ($CDCl_3$, 50 MHz, ppm, J_{C-F} = Hz); 171.3 (C-4); 162.9 (d, $^1J = 247.7$, Ar),135.3 (d, $^4J = 3.2$, Ar), 129.1 (d, 2C, $^3J = 8.5$, Ar), 116.0 (d, 2C, $^2J = 21.8$, Ar); 63.2 (C-2); 54.0 (2C, C-8); 52.9 (C-7); 41.2 (C-6); 32.9 (C-5); 23.4 (2C). MS (70 eV): *m/z* (%) = 294 (M^+, 2); 292 (M − 4, 0.5); 224 (0.5); 153 (1); 139 (2); 97 (6); 84 (100); 69 (3).

2-Butyl-3-(2-(pyrrolidin-1-yl)ethyl)thiazolidin-4-one (**4Bb**) It was obtained as an oil; ^{1}H NMR δ ($CDCl_3$, 400 MHz, ppm, J_{H-H} = Hz); 4.70 (td, 1H, $^3J = 8.5$, $^4J = 2.3$, H-2); 3.77 (ddd, 1H, $^2J = 14.2$, $^3J = 8.2$, $^3J = 6.0$, H-6a); 3.51 (dd, 1H, $^2J = 15.5$, $^4J = 1.5$, H-5a); 3.42 (d, 1H, $^2J = 15.5$, H-5b); 3.10 (ddd, 1H, $^2J = 13.9$, $^3J = 7.9$, $^3J = 6.0$, H-6b); 2.69 (ddd, 1H, $^2J = 12.0$, $^3J = 8.4$, $^3J = 5.9$, H-7a); 2.50–2.56 (m, 5H); 1.82–1.90 (m, 1H); 1.72 (br, 4H); 1.55–1.65 (m, 1H); 1.25–1.31 (m, 4H); 0.84–0.87 (m, 3H). ^{13}C NMR δ ($CDCl_3$, 100 MHz, ppm); 171.1 (C-4); 61.9 (C-2); 54.1 (2C); 53.0 (C-7); 41.4 (C-6); 35.3; 32.0 (C-5); 26.2; 23.4 (2C); 22.3; 14.0; MS (70 eV): *m/z* (%) = 256 (M^+, 1); 252 (M − 4, 2); 102 (1); 97 (9); 84 (100); 69 (3).

2-Phenyl-3-(2-(pyrrolidin-1-yl)ethyl)thiazolidin-4-one (**4Bc**) It was obtained as an oil; ^{1}H NMR δ ($CDCl_3$, 400 MHz, ppm, J_{H-H} = Hz); 7.27–7.33 (m, 3H, Ar); 7.20–7.24 (m, 2H, Ar); 5.78 (d, 1H, $^4J = 1.6$, H-2); 3.74 (dt, 1H, $^2J = 13.7$, $^3J = 6.7$, H-6a); 3.72 (dd, 1H, $^2J = 15.5$, $^4J = 1.9$, H-5a); 3.64 (d, 1H, $^2J = 15.4$, H-5b); 2.72–2.79 (m, 2H, H-6b, H-7a); 2.61 (dt, 1H, $^2J = 13.5$, $^3J = 6.8$, H-7b); 2.37–2.40 (m, 4H); 1.65–1.69 (m, 4H). ^{13}C NMR δ ($CDCl_3$, 100 MHz, ppm); 171.3 (C-4); 139.6 (Ar); 129.1 (Ar); 129.0 (2C, Ar); 127.0 (2C, Ar); 64.0 (C-2); 54.1 (2C); 53.1 (C-7); 41.6 (C-6); 32.9 (C-5); 23.4 (2C); MS (70 eV): *m/z* (%) = 276 (M^+, 1); 272 (M-4, 2); 178 (0.5); 135 (2); 121 (3); 97 (10); 84 (100); 70 (3).

3-(3-(Diethylamino)propyl)-2-(4-fluorophenyl)thiazolidin-4-one (**4Ca**) It was obtained as an oil; ^{1}H NMR δ ($CDCl_3$), 600 MHz, ppm, J_{H-H} = Hz); 7.35 (dd, 2H, $^3J = 8.6$, $^4J = 5.2$, Ar); 7.08 (t, 2H, $^3J = 8.5$, Ar); 5.75 (s, 1H, H-2); 3.79 (dd, 1H, $^2J = 15.6$, $^4J = 1.5$, H-5a); 3.72 (d, 1H, $^2J = 15.6$, H-5b); 3.62 (dt, 1H, $^2J = 14.2$, $^3J = 7.6$, H-6a); 2.78 (q, 4H, $^3J = 7.2$); 2.74–2.76 (m, 1H, H-6b); 2.65–2.70 (m, 1H, H-8a); 2.59–2.63 (m, 1H, H-8b); 1.75–1.82 (m, 2H, H-7); 1.12 (t, 6H, $^3J = 7.2$). ^{13}C NMR δ ($CDCl_3$, 150 MHz, ppm, J_{C-F} = Hz); 171.5 (C-4); 163.0 (d, $^1J = 248.8$, Ar); 134.9 (d, $^4J = 3.1$, Ar); 129.2 (d, 2C, $^3J = 8.4$, Ar); 116.1 (d, 2C, $^2J = 21.9$, Ar); 62.7 (C-2); 49.1 (C-8); 45.6 (2C); 40.6 (C-6); 32.9 (C-5); 22.7 (C-7); 9.3 (2C). MS (70 eV): *m/z* (%) = 310 (M^+, 0.5); 281 (2); 182 (1); 109 (10); 86 (100); 72 (10).

2-(4-Fluorophenyl)-3-(3-(piperidin-1-yl)propyl)thiazolidin-4-one (**4Da**) It was obtained as an oil; ^{1}H NMR δ ($CDCl_3$, 500 MHz, ppm, J_{H-H} = Hz); 7.23 (dd, 2H, $^3J = 8.6$, $^4J = 5.1$, Ar); 7.00 (t, 2H, $^3J = 8.5$, Ar); 5.64 (d, 1H, $^4J = 1.7$, H-2); 3.72 (dd, 1H, $^2J = 15.6$, $^4J = 1.8$, H-5a); 3.62 (d, 1 H, $^2J = 15.6$, H-5b); 3.57 (ddd, 1H, $^2J = 14.1$, $^3J = 8.0$, $^3J = 6.6$, H-6a); 2.63 (ddd, 1H, $^2J = 13.9$, $^3J = 8.0$, $^3J = 5.9$, H-6b); 2.18–2.23 (m, 5H, H-8a); 2.10–2.16 (m, 1H, H-8b); 1.60–1.66 (m, 1H, H-7a); 1.52–1.57 (m, 1H, H-7b); 1.45–1.49 (m, 4H); 1.34 (sl, 2H). ^{13}C NMR δ ($CDCl_3$, 125 MHz, ppm, J_{C-F} = Hz); 171.0 (C-4); 162.9 (d, $^1J = 248.0$, Ar); 135.3 (d, $^4J = 6.4$, Ar); 128.9 (d, 2C, $^3J = 8.2$, Ar); 115.9 (d, 2C, $^2J = 21.8$, Ar); 62.9 (C-2); 56.0 (C-8); 54.3 (2C); 41.2 (C-6); 32.8 (C-5); 25.7 (2C); 24.2; 23.9 (C-7). MS (70 eV): *m/z* (%) = 322 (M^+, 2); 238 (1); 182 (1.5); 127 (4); 112 (4.5); 98 (100); 84 (9).

*General procedure for the synthesis of hydrochloride salt **5Aa–f, 5Ba–d**, and **5Ca–Ga***

In a solution of thiazolidinone **4** in dichloromethane (20 ml) was flowing the hydrochloric acid ($HCl_{(g)}$) (generate for the reaction of H_2SO_4 concentrated with NaCl) in an open vessel at room temperature for 30 min. The hydrochloride salt was filtered off under vacuum. When necessary, the salt was extracted with distilled water from the organic layer.

4-(2-(Piperidin-1-yl)ethyl)-1-thia-4-azaspiro[4.5]decan-3-one chlorohydrate (**5Ad**) It was obtained as a brown solid; ^{1}H NMR δ (D_2O, 400 MHz, ppm, J_{H-H} = Hz); 3.59 (t, 2H, $^3J = 6.8$, H-6); 3.48–3.52 (m, 2H); 3.48 (s, 2H, H-5); 3.12 (t, 2H, $^3J = 6.8$, H-7); 2.83 (dt, 2H, $^2J = 12.4$, $^4J = 2.3$); 1.74–1.83 (m, 4H); 1.63–1.70 (m, 5H); 1.28–1.60 (m, 6H); 0.93–1.04 (m, 1H); ^{13}C NMR δ (D_2O, 100 MHz, ppm); 175.1 (C-4); 75.2 (C-2); 55.8 (C-7); 53.9 (2C); 37.1 (2C); 36.4 (C-6); 30.6 (C-5); 23.7; 23.0 (2C); 22.9 (2C); 21.0. MS (70 eV) (free base **4Ad**): *m/z* (%) = 171 (M^+-111, 0.5); 128 (2); 111 (6); 98 (100); 84 (2).

4-(2-(Pyrrolidin-1-yl)ethyl)-1-thia-4-azaspiro[4.5]decan-3-one chlorhydrate (**5Bd**) It was obtained as a dark brown solid; ^{1}H NMR δ (D_2O, 400 MHz, ppm, J_{H-H} = Hz); 3.60–3.66 (m, 2H); 3.58 (t, 2H, $^3J = 6.8$, H-6); 3.49 (s, 2H, H-5); 3.25 (t, 2H, $^3J = 6.7$, H-7); 2.96–3.03 (m, 2H); 1.94–2.06 (m, 2H); 1.83–1.91 (m, 2H); 1.75–1.82 (m, 2H); 1.64–1.71 (m, 4H); 1.49 (d, 1H, $^2J = 13.0$); 1.32–1.44 (m, 2H); 0.93–1.05 (m, 1H). ^{13}C NMR δ (D_2O, 100 MHz, ppm); 175.0 (C-4); 75.2 (C-2); 54.8 (2C, C-8); 54.0 (C-7); 37.8 (C-6); 37.0 (2C); 30.6 (C-5); 23.7; 23.1 (2C); 22.6

(2C). MS (70 eV) (free base **4Bd**): *m/z* (%) = 264 (M^+-4, 3); 171 (1); 128 (1); 97 (15); 84 (100); 69 (3).

Biological part

Animals

Experiments were performed using 60–90 day-old adult male (Swiss) mice. Animals were maintained in controlled environmental conditions (22 ± 1 °C, 12/12 h light/dark cycle, relative humidity (45–55%) and free access to food and water (Luszczki 2010). All experiments were based on precepts and ethical considerations, to investigate experimental pain in animals (Zimmermann 1983). The project was approved by the University Ethics Committee, registered under the number (CEEA 2231).

Standard drugs and negative control

Sodium dipyrone (Novalgina®, Aventis Pharma Ltd) was diluted in water at two concentration 250 and 500 mg/Kg. Tramadol hydrochloride (Tramal®, Pfizer Ltd) was diluted in water at two concentration 25 and 50 mg/Kg. Saline solution (sodium chloride 0.9%, Equiplex Ltd) was used as a negative control. Vehicle and drugs were administered intraperitoneally in volume of 0.1 ml/10 g body weight.

Hot plate test

The hot plate test is a standard model test used to determine antinociceptive efficacy of drugs with central activity through acute thermal stimulation (Siegfried et al. 1987). The apparatus used (Hot Plate, model EFF361, Insigth®) consisted of an aluminum plate that is evenly heated, and it is surrounded by a transparent acrylic rectangular cage, which keeps animals confined, where access is done only by a higher opening that allows researchers to remove and include animals.

Animals were randomized into groups of eight subjects and were habituated in a room for at least 30 min before the experiments (Luszczki 2010). Twenty-four hour before each experiment, animals were weighed and accustomed to procedure, to avoid the occurrence of new-induced analgesia which could provide false results (Siegfried et al. 1987). In habituation, the mice were exposed to the same conditions that would be subject on the day of the experiment, except to the derivatives treatment, such as the researcher manipulation, the injection (saline) and the mice insertion in the equipment. Lastly, the animals were observed, for a minute, for their pain responses and then they were removed from the equipment. If positive for pain responses, the animals were disposed from the next stage (test).

In the day of the test, the mice were placed separately on the apparatus, and the latency time (in seconds) for the nociceptive response (jumping or hind paw licking) was measured with a manual chronometer in times of 30, 60, and 90 min after the saline injection (NaCl 0.9%, 10 ml/Kg, ip), 2,3-substituted-1,3-thiazolidin-4-ones derivatives (100 mg/Kg, ip), dipyrone (250 and 500 mg/Kg, ip) or tramadol (25 and 250 mg/Kg, ip); it was settled the upper cut-off time of 50 s, to avoid possible tissue damage in animals. The temperature established in the experiment was set to 50 ± 0.1 °C.

Finally, the animals were submitted to the hot plate test for three different moments after treatment administration, in 30, 60, and 90 min, and the latency time data were registered for further analysis (Vigorita et al. 2001).

Statistical analysis

Data were analyzed by using variance analysis (ANOVA) followed by Duncan's test in the SPSS 11.0.1 software. Furthermore, all data were expressed as mean ± standard error and the level of significance was set as $P < 0.05$.

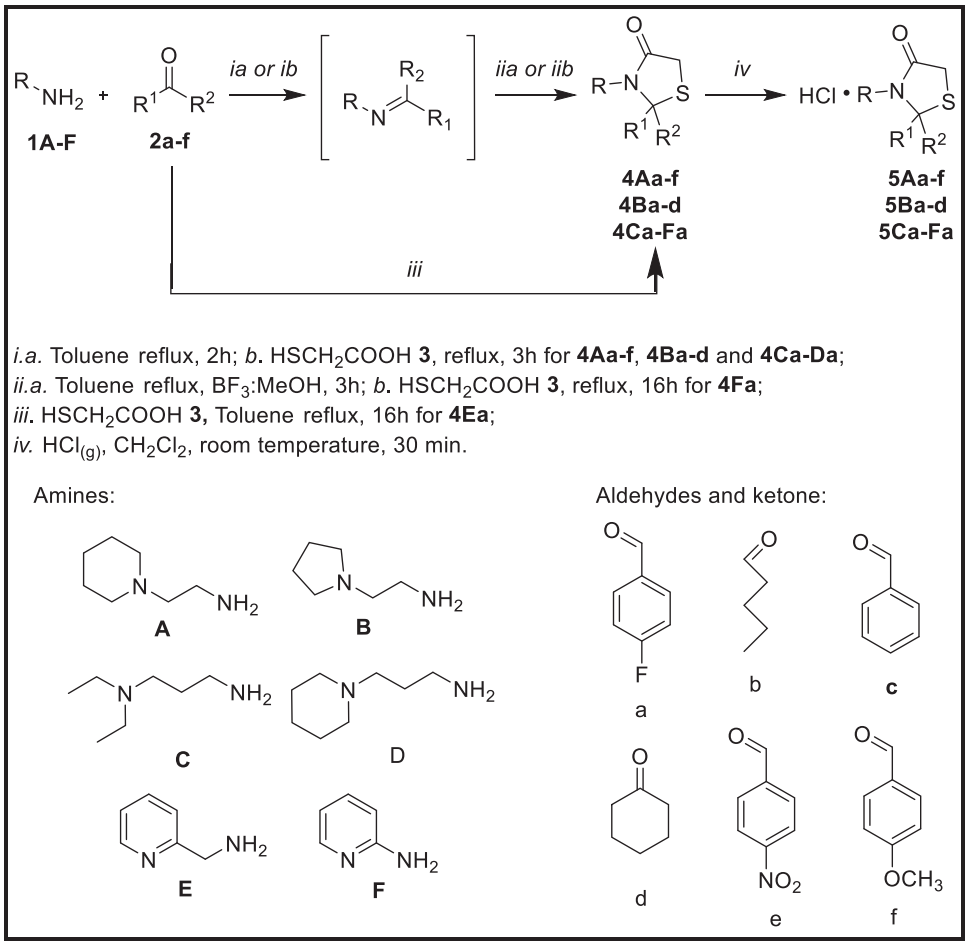
Results and discussion

Chemistry

Fourteen 1,3-thiazolidin-4-ones, eight of them unpublished in the literature, were synthesized for biological study. The new ones and the compounds **4Aa** (Kunzler et al. 2013), **4Ac** (Sun and Kyle 2003), **4Ae** (Kunzler et al. 2013), **4Af** (Kunzler et al. 2013), **4Ea** (Gouvêa et al. 2012), and **4Fa** (Gouvêa et al. 2012) were synthesized from reactions between amines **1A–F** with aldehydes or ketone **2a–f** in toluene reflux. Scheme 1 shows the conditions to the synthesis of desired compounds.

According to many studies in literature, the antinociceptive activity of some 1,3-thiazolidin-4-ones might be associated with their capacity to inhibit cyclooxygenase (COX) enzymes including COX-2 selective inhibition, (Geronikaki et al. 2008; Taranalli et al. 2008; Unsal-Tan et al. 2012; Vigorita et al. 2003; Zarghi et al. 2007). Enzyme inhibition shows anti-inflammatory, analgesic and antipyretic activities due to the reduction of prostaglandin synthesis (Davis and Brogden 1994). In this way, a preview study showed the analgesic and anti-inflammatory activities of 2-(aryl)-3-(4-sulfonamidebenzene)-1,3-thiazolidin-4-ones. The compound with 4-fluorophenyl as an aryl group showed good results compared to NSAID nimesulide (Prasit et al. 1999). In this sense, this study began

Scheme 1 Synthesis of thiazolidinones **4** and their hydrochloride salts **5**



researching 2-(4-fluorophenyl)-thiazolidinones (**4Aa–Ga**) with different amine cores (**A–F**) linked at 3-position at the thiazolidinone ring (Scheme 1).

Thiazolidinones **4Ab–f** and **4Bb–d** were synthesized according to biological results. For this, the amines **A** (2-(piperidin-1-yl)ethan-1-amine) and **B** (2-(pyrrolidin-1-yl) ethan-1-amine) were maintained and the 4-fluorobenzaldehyde (**a**) was modified in the synthetic process, obtaining compounds based on other carbonyl group (aldehydes and cyclohexanone, **b–f**).

In sequence, all thiazolidinones underwent a reaction to form a hydrochloride salt (**5**) in the basic nitrogen that all amines have in their structures. Due to salt formation, no surfactant agent was needed to apply it in an animal model.

Efficient methodologies were studied at aiming to obtain all all the proposed products in moderate to good yields (Table 1). The structure of known thiazolidinones (**4Aa, 4Ac, 4Ae, 4Af, 4Ea**, and **4Fa**) was confirmed by mass spectrometry. The new compounds were fully identified and characterized by gas chromatography-mass spectrometry and ^{1}H and ^{13}C NMR. The NMR of compounds **4Ab**, **4Ba**, **4Bb**, **4Bc**, **4Ca**, and **4Da** was performed for the free base and compounds **5Ad** and **5Bd** for the hydrochloride salt.

The 1,3-thiazolidin-4-ones were identified by classical H-2 and H-5 signals at ^{1}H NMR. Due to the presence of a stereogenic center at 2-position, the H-5 hydrogens are diastereotopic and present two signals: one at ~3.5 ppm for H-5a (doublet of doublet with $^{2}J = 15.5$ Hz and $^{4}J = 1.5$ Hz); and other at ~3.4 ppm for H-5b (doublet with $^{2}J =$ 15.5 Hz). The H-2 was assigned as a simplet or doublet at ~5.7 ppm, with the exception of thiazolidinones from valeraldehyde (**4Ab** and **4Bb**) those H-2 appear as triplet of doublet at ~4.7 ppm. For thiazolidinone salt from cyclohexanone (**5Ad** and **5Bd**), the both H-5 appear as simplet at ~3.4 ppm since these compounds have no stereogenic center at 2-position.

At ^{13}C NMR, the mainly signals for thiazolidinones **4** are: C-4 at ~170 ppm; C-2 at ~62 ppm; and C-5 at ~32 ppm. Compounds **5** have a deshield C-2 at ~75 ppm and a shielded C-5 at ~30 ppm.

Antinociceptive study

The hydrochloride salts **5** were tested in an animal model. In the hot plate test, pain response is described as latency time (in seconds), thus the high latency times indicate good

Table 1 Chemical parameters of 2,3-substituted-1,3-thiazolidin-4-ones

	Yield (%)	MF	MW (g/mol)	Log *P**	Hydrochloride salt (5)	
					MW (g/mol)	Dose (μmol/kg)**
4Aa	74	$C_{16}H_{21}FN_2OS$	308.41	2.72	344.87	289.9
4Ba	89	$C_{15}H_{19}FN_2OS$	294.39	2.30	330.85	302.2
4Ca	22	$C_{16}H_{23}FN_2OS$	310.43	2.77	346.89	288.2
4Da	52	$C_{17}H_{23}FN_2OS$	322.44	2.83	358.90	278.6
4Ea	44	$C_{15}H_{13}FN_2OS$	288.34	2.81	324.80	307.8
4Fa	59	$C_{14}H_{11}FN_2OS$	274.31	3.03	310.77	321.7
4Ab	64	$C_{14}H_{26}N_2OS$	270.43	2.31	306.89	325.8
4Ac	68	$C_{16}H_{22}N_2OS$	290.42	2.56	326.88	305.9
4Ad	48	$C_{15}H_{26}N_2OS$	282.44	2.33	318.90	313.5
4Ae	33	$C_{16}H_{21}FN_3O_3S$	335.42	2.52	371.88	268.9
4Af	71	$C_{17}H_{24}FN_2O_2S$	320.45	2.44	356.91	280.2
4Bb	62	$C_{13}H_{24}N_2OS$	256.41	1.90	292.87	341.4
4Bc	66	$C_{15}H_{20}N_2OS$	276.40	2.14	312.86	319.6
4Bd	15	$C_{14}H_{24}N_2OS$	268.42	1.91	304.88	328.0

FM molecular formula, *MW* molecular weight

*Log P calculated by software Chendraw® Ultra, version 8.0.3

Dose in μmol/Kg corresponding to 100 mg/Kg of hydrochloride salt (5**)

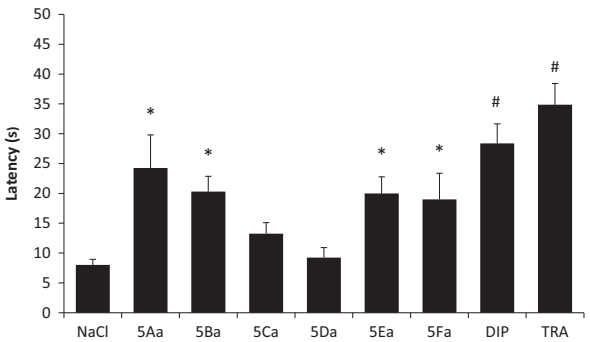


Fig. 2 Latency time of 3-amino-2-(4-fluorophenyl)-1,3-thiazolidin-4-ones (**5Aa–Ga**) in hot plate test evaluation in 30 min (100 mg/Kg, ip). *DIP* Sodium dipyrone (250 mg/Kg); *TRA* Tramadol hydrochloride (25 mg/Kg). *$P < 0.05$ X saline; # $P < 0.05$ X negative control and compounds. ANOVA/DUNCAN

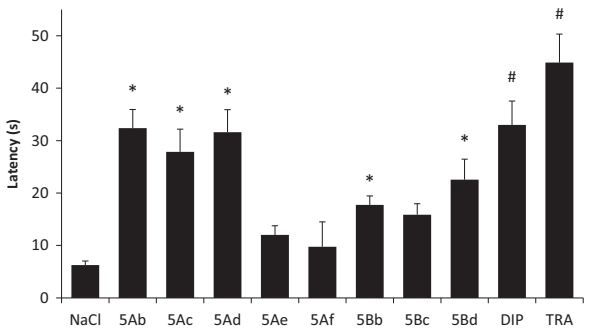


Fig. 3 Latency time of 3-(piperidin-1-yl)-ethyl-2-substituted-1,3-thiazolidin-4-ones (**5Ab–f**) and 3-((pyrrolidin-1-yl)-ethyl)-2-substituted-1,3-thiazolidin-4-ones (**5Bb–d**) in hot plate test evaluation in 30 min (100 mg/Kg, ip). *DIP* Sodium dipyrone (500 mg/Kg); *TRA* Tramadol hydrochloride (50 mg/Kg). *$P < 0.05$ X saline; # $P < 0.05$ X negative control and compounds. ANOVA/DUNCAN

inhibition of nociception. The latency times of thiazolidin-4-ones in the hot plate test are shown in Figs. 2, 3. Generally, the findings agree with others studies in the field, evidencing, once again, that 1,3-thiazolidin-4-ones are an important scaffold associated with many pharmacological activities (Tripathi et al. 2014). Under these circumstances, from fourteen compounds tested, nine showed significant increases in latency time when compared to saline (negative control).

Figure 2 exhibits the effect of compounds **5Aa–Ga** on latency time 30 min after injection. Four (**5Aa**, **5Ba**, **5Ea**, and **5Fa**) revealed an increase in latency to nociceptive response when compared to saline ($P < 0.05$) by ANOVA/DUNCAN, demonstrating that these compounds are capable of promoting antinociceptive activity. However, none was better than dipyrone or tramadol, two well-known analgesic drugs. These standard drugs were selected as positive controls, due to their strong analgesic effect, low cost and greater current therapeutic use (Teixeira et al. 2013; Silva and Moraes 2010, Edwards et al. 2010; Tulunay et al. 2004). Although morphine is the standard drug to evaluate analgesic medication, tramadol hydrochloride was chosen due to its greater use to relieve acute or chronical pain of moderate or severe intensity (Silva and Moraes 2010; Teixeira et al. 2013; Giraldes et al. 2016). Nevertheless, to maintain the same analgesic effect, morphine (mg) was converted to tramadol (mg), using tables of equianalgesic opioid doses in parenteral administration

(1 mg morphine = 10 mg of tramadol) (Kopf and Patel 2010).

The difference in increases of latency time among **5Aa**, **5Ba**, **5Ea**, **5Fa**, and dipyrone possibly occurred due to the dose used. Sodium dipyrone (M.W. 333.3 g/mol; 250 mg/kg, 750 µmol/Kg; ip) had a molar mass similar to 3-(amino)-2-(4-fluorophenyl)-1,3-thiazolidin-4-ones **5Aa–Ga** (M.W. 310.81–358.94 g/mol; 100 mg/kg, 278.6–323.7 µmol/Kg; ip). The 100 mg/kg dose was based on previous works (Taranalli et al. 2008; Vigorita et al. 2001).

In the 60 and 90 min evaluation (see supplementary information), there were decreases in latency times, which were expected except for compound **5Ea** that still maintained higher levels of antinociceptive activity than saline over these times. This suggests that the compounds tested have a short effect and fast metabolization, but further studies are necessary to confirm this hypothesis. Yet a previous study investigated the anti-inflammatory and analgesic activity of 3,3′-(1,2-ethanediyl)-bis[2-aryl-4-thiazolidinone], obtaining good results and latency times in a hot plate test as phenylbutazone and indomethacin did, within up to 180 min after treatment. These compounds have two 1,3-thiazolidin-4-one nuclei connected by an ethyl bridge (position 3) and two substituted phenyl at 2-position (Vigorita et al. 2001).

Based on the results shown in Fig. 2, a 30-min assessment was chosen in the sequence of the study in order to plan a new structural modification in the two compounds with the highest latency times: 2-(4-fluorophenyl)-3-(piperidin-1-ylethyl)-1,3-thiazolidin-4-one (**5Aa**; 24.3 ± 5.6 s); and 2-(4-fluorophenyl)-3-(pyrrolidin-1-ylethyl)-1,3-thiazolidin-4-one (**5Ba**; 20.3 ± 2.6 s). Similarly to Vigorita et al. (2001), our work showed good results for compounds with ethyl as a bridge (**A** and **B**). In this way, this distance between the atoms of nitrogen may seem important for the antinociceptive activity since the increase of chain showed no statistical activity (propyl link, **5Ca** and **5Da**). Compounds with shorter distances, methyl (**5Ea**) or none (**5Fa**), also showed good results in 30 min (Fig. 2). Therefore, comparing compounds with different links between nitrogens, the order of activity was: ethyl > methyl or none » propyl.

Further, analogs from **A** (**5Ab–f**) and **B** (**5Bb–d**) were studied. Compounds **5Ab**, **5Ac**, **5Ad**, **5Bb**, and **5Bd** (five of eight) revealed increased latency times when compared to saline in the 30-min evaluation after treatment (Fig. 3). The highest latency times were obtained by 2-butyl-3-(2-piperidin-1-yl)ethyl)thiazolidin-4-one (**5Ab**; 32.4 ± 3.5 s), 2-phenyl-3-(2-piperidin-1-yl)ethyl)thiazolidin-4-one (**5Ac**; 27.8 ± 4.3 s) and 4-(2-piperidin-1-yl)ethyl)-1-thia-4-azaspiro[4.5]decan-3-one (**5Ad**; 31.6 ± 4.3 s). These compounds have better antinociceptive activity than **5Aa** (24.3 ± 5.6 s, 4-fluorophenyl substituent) and showed a nociceptive response compared to dipyrone (33.0 ± 4.6 s). When evaluating these compounds in 60 and 90 min, none showed analgesic effect (see Supplementary material). Moreover, literature findings demonstrated that 1,3-thiazolidin-4-one derivatives have a similar anti-inflammatory and analgesic efficacy to NSAIDs (Geronikaki et al. 2008; Vigorita et al. 2001).

It is highlighted that all compounds with six-membered piperidine (**5A**) showed better results than compounds with five-membered pyrrolidine (**5B**) comparing the same 2-position substituent (**5Aa–d** compared to **5Ba–d**, Figs. 2, 3). These results suggested that, in addition to an ethylenic link, a six-member ring is also important to activity.

Another important finding is the influence of substituents R^1 and R^2 on antinociceptive activity. Comparing the compounds with aromatic substituents, phenyl (**c**) has better antinociceptive activity than aryl, no matter what the nature of the aryl substituent, since both electron-withdrawing fluoro (**a**) or nitro (**e**) and electron-releasing methoxy (**f**) decrease the activity. The best results were found for compounds bearing an aliphatic substituent, from valeraldehyde (**5Ab**) and from ciclohexanone (**5Ad**). These results suggested that an aliphatic group is also important for this activity. Comparing compounds with different groups at 2-position of thiazolidinone ring, the order of activity was: aliphatic > phenyl > aryl.

Conclusion

In conclusion, this work described the antinociceptive effect of 1,3-thiazolidin-4-ones through thermal stimulation in mice. The 1,3-thiazolidin-4-ones were synthesized from one-pot or multicomponent procedures using different amines **1A–F** and aldehydes or ketone **2a–f**. Fourteen compounds were obtained in moderate to good yields, identified and characterized. Nine thiazolidinones showed a significant increase in latency time in the hot plate test. Three of them (**5Ab**, **5Ac**, and **5Ad**) showed the best antinociceptive activity after 30 min at 100 mg/Kg. It can highlight three findings about chemical structure: (i) the distance between nitrogens is important to activity and the best result was found with an ethylenic link; (ii) the presence of six-membered heterocycle piperidine is more efficient; (iii) an aliphatic substituent at 2-position of thiazolidinone improves the activity. In addition there are ongoing anti-inflammatory and anti-pyretic studies as well as the evaluation of the antinociceptive mechanism.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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