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Original article

Design and microwave-assisted synthesis of 5-trifluoromethyl-4,5-dihydro-1*H*-pyrazoles: Novel agents with analgesic and anti-inflammatory properties

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Abstract

In this work, we reported the synthesis and evaluation of the analgesic and anti-inflammatory properties of novel 3- or 4-substituted 5-trifluoromethyl-5-hydroxy-4,5-dihydro-1*H*-1-carboxyamidepyrazoles (where 3-/4-substituent = H/H, Me/H, Et/H, Pr/H, *i*-Pr/H, Bu/H, *t*-Bu/H, Ph/H, 4-Br-Ph/H and H/Me) designed in the exploration of the bioisosteric replacement of benzene present in salicylamide with a 5-trifluoromethyl-4,5-dihydro-1*H*-pyrazole scaffold. Target compounds were synthesized from the cyclocondensation of 4-alkoxy-1,1,1-trifluoromethyl-3-alken-2-ones with semicarbazide hydrochloride through a rapid one-pot reaction via microwave irradiation. In addition to spectroscopic data, the structure of the compounds was supported by X-ray diffraction. Subcutaneous administration of the 5-trifluoromethyl-4,5-dihydro-1*H*-pyrazoles decreased pain-related behavior during neurogenic and inflammatory phases of the formalin test in mice. Moreover, the more active analgesic compounds (3-/4- = Et/H and H/Me) significantly decreased carrageenan-induced paw edema in mice. The data obtained in this work suggest that the synthesized compounds could be promising candidates for the future development of novel analgesic and anti-inflammatory agents.

Keywords: Pyrazoles; Microwave-assisted synthesis; Analgesic; Anti-inflammatory; Pain

1. Introduction

Although pain is the most common complaint in the medical field, the arsenal of effective and safe analgesics is still relatively small. Thus, the identification of compounds that can effectively treat painful states without induction of side effects remains a major challenge in biomedical research [1].

Pyrazoles and their derivatives are widely known for their excellent effectiveness as analgesics and antipyretics, dipyrone being a well documented and commercially available example [1]. This compound is a nonsteroidal anti-inflammatory drug (NSAID) used with success in the treatment of fever and pain showing a relatively weak anti-inflammatory effect [1,2]. Other NSAIDs have been used in the treatment of chronic arthropathies, as well as for fever and pain. However, long-term use of these agents is limited by a high incidence of unpleasant effects in the gastrointestinal tract since these

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complications are a major cause of morbidity and mortality produced by NSAIDs [3]. Nonetheless, some data have shown that the ulcerogenic activity of dipyrone in rats and humans is substantially lower than the risk associated with other NSAIDs, such as acetylsalicylic acid and diclofenac, commonly used for pain relief [4,5]. These findings suggest better tolerability of dipyrone in relation to other NSAIDs.

In this context, our research group has reported the biological effect of novel pyrazole derivatives in animal models of inflammation, fever and pain [6–11]. The lead-like compound, 3-methyl-5-hydroxy-5-trichloromethyl-4,5-dihydro-1*H*-1-carboxyamidepyrazole (MPCA) possessed analgesic, but not anti-inflammatory properties against neurogenic, inflammatory and visceral pain in rodents [6]. This effect seemed to be related with the activation of spinal α_2-adrenoceptors and 5-HT receptors, but not with opioid receptor stimulation [7].

In accordance with the increasing interest in the medicinal chemistry community in technologies and concepts that facilitate a more rapid synthesis and, consequently, the screening of novel chemical substances [12], we have demonstrated the use of microwave irradiation in heterocyclic preparations [13,14]. This form of energy has been recently used to accelerate organic reactions due to its high heating efficiency, providing a remarkable rate of enhancement and a dramatic reduction in reaction times. Reactions that require hours or even days by conventional heating can often be accomplished in seconds or minutes by microwave heating [15].

In this study, our initial efforts were focused on the design of new pyrazole derivatives to be used as analgesics. For this purpose, we have explored the hypothesis that benzene, which is present in salicylamide, could be mimicked with an appropriate 3- or 4-substituted 5-trifluoromethyl-4,5-dihydro-1*H*-pyrazole scaffold (Fig. 1). Salicylamide is a salicylate derivative endowed with good analgesic and antipyretic activity [16,17]. In addition, the presence of a trifluoromethyl group within cyclic compounds, especially at a strategic position, has become an important aspect to be studied in pharmaceutical research, due to the unique physical and biological properties of fluorine [18]. In many systems, the substitution of a methyl group with a trifluoromethyl group, for example, has resulted in added lipophilicity, which may lead to easier absorption and transportation of molecules within biological systems and thereby improve the overall pharmacokinetic properties of drug candidates [18,19].

Thus, in an attempt to identify novel pyrazole derivatives endowed with analgesic activity and as a part of our search for basic information about the structural requirements for the analgesic properties of such compounds, we have synthesized of a series of 3-alkyl(aryl)-5-hydroxy-5-trifluoromethyl-4,5-dihydro-1*H*-1-carboxyamidepyrazoles using microwave induced techniques (Scheme 1). Furthermore, we investigated the analgesic and anti-inflammatory profile of the synthesized compounds in mice. The effects of bioactive pyrazoles on other behavioral measures, such as spontaneous and forced locomotion were also evaluated.

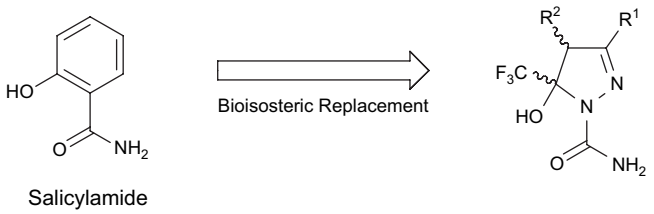


Fig. 1. Design of the new 5-trifluoromethyl-4,5-dihydro-1*H*-pyrazoles based on the bioisosteric replacement of the benzene scaffold of salicylamide.

2. Results and discussion

2.1. Chemistry

Over the last decade, our research group has reported the general synthesis of 1,1,1-trihalo-4-alkoxy-3-alken-2-ones, which are 1,3-dielectrophilic building blocks, and have demonstrated their use in the preparations of a wide range of heterocyclic compounds [20]. In this study, the 1,1,1-trifluoromethyl-4-alkoxy-3-alken-2-ones **1** were synthesized from the reaction of the respective enol ether or acetal with trifluoroacetic anhydride [21,22]. The 5-trifluoromethyl-4,5-dihydro-1*H*-pyrazoles **2** were synthesized by the cyclocondensation reaction of the enones **1** with semicarbazide hydrochloride (Scheme 1).

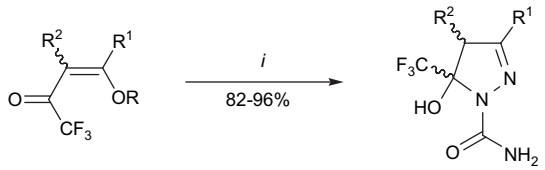
The mixture of enones **1** and semicarbazide hydrochloride reacted in the presence of pyridine and methanol/water solution (3:1 v/v) as solvent. The solution was submitted to microwave irradiation (100 W) for 4 min, at a temperature of 70 °C and at 2.2 bar of pressure, to produce 5-trifluoromethyl-4,5-dihydro-1*H*-pyrazoles **2** with yields of 82–96%. The new method for forming 5-trifluoromethyl-4,5-dihydro-1*H*-pyrazoles under microwave irradiation offers several advantages including faster reaction rates, fewer byproducts, and higher yields, while the conventional method entails moderate yields and a long process (ca. 24 h) [23].

^{1}H and ^{13}C NMR, mass spectral data and elemental analysis of all compounds are in full agreement with the proposed structure. In addition to these data, the structures of the two most active compounds (**2c** and **2j**) were supported by crystallographic studies (Figs. 2 and 3). On the basis of these experiments, it was observed that the presence of intramolecular hydrogen bonds between the carbonyl and the hydroxyl groups of tetrasubstituted pyrazole rings generated a six membered ring with O···H bond distances of 2.17 Å [O(5)–H(5)···O(11)] and 2.28 Å [O(5)–H(5)···O(11)] in structures of **2c** and **2j**, respectively [25].

2.2. Pharmacological studies

2.2.1. Formalin test

All compounds **2a–j** were screened for analgesic activity using the formalin test in mice. In Figs. 4 and 5 the effect of these novel pyrazole derivatives on formalin-induced neurogenic and inflammatory pain is shown. The synthesized compounds **2a**, **2c–e**, **2g**, **2i** and **2j** exhibited a significant analgesic effect on neurogenic and inflammatory phases of the formalin test. Compound **2b** was ineffective on neurogenic



1a-j 2a-j

Entry	R	R^1	R^2	Yield (%)
a	Et	H	H	90
b	Me	Me	H	96
c	Me	Et	H	91
d	Me	Pr	H	87
e	Me	*i*-Pr	H	90

Entry	R	R^1	R^2	Yield (%)
f	Me	Bu	H	89
g	Me	*t*-Bu	H	82
h	Me	Ph	H	85
i	Me	4-Br-Ph	H	91
j	Et	H	Me	94

Scheme 1. Reagents and conditions: (*i*) $NH_2NHCONH_2 \cdot HCl$, $MeOH/H_2O$, Py, MW, 100 W, 70 °C, 2.2 bar, 4 min.

pain, causing a significant analgesic effect only in the inflammatory phase of the test. On the other hand, compound **2f** showed a significant analgesic effect in the neurogenic phase but was ineffective in the inflammatory phase (data not shown in Fig. 5). Furthermore, the 5-trifluoromethyl-4,5-dihydro-1*H*-pyrazole **2h** proved to be ineffective against the pain response induced by formalin (data not shown in Fig. 5). Interestingly, compounds **2d** and **2e** presented an analgesic profile that was not linear with the dose. In the inflammatory phase, **2d** presented an effect at doses of 75 and 750 μmol/kg but not at 250 μmol/kg. On the other hand, **2e** had an analgesic effect at all doses tested in the inflammatory phase, but, surprisingly, the greatest effect was achieved at the smallest dose (25 μmol/kg). It would be possible to explain this dose-independent profile through future research on the mechanism(s) of action for the 5-trifluoromethyl-4,5-dihydro-1*H*-pyrazole derivatives.

Another interesting observation was the different profile of the action of compound **2b** in relation to its analogue 3-methyl-5-hydroxy-5-trichloromethyl-4,5-dihydro-1*H*-1-carboxyamidepyrazole (MPCA), which has been previously reported [6]. Compound **2b** was ineffective against formalin-induced neurogenic pain, while MPCA caused analgesia at a similar dose as that administered in the present study [6]. However, **2b** was more potent than MPCA to induce analgesia against inflammatory pain. Therefore, the replacement of the trichloromethyl group at position 5 of the pyrazole ring by a trifluoromethyl group seems to increase the analgesic potency of the novel pyrazoles. Compound **2h** is structurally similar to 3-phenyl-5-hydroxy-5-trichloromethyl-4,5-dihydro-1*H*-1-carboxyamidepyrazole (PPCA), a pyrazole derivative also previously described [7,8]. Different from PPCA, **2h**

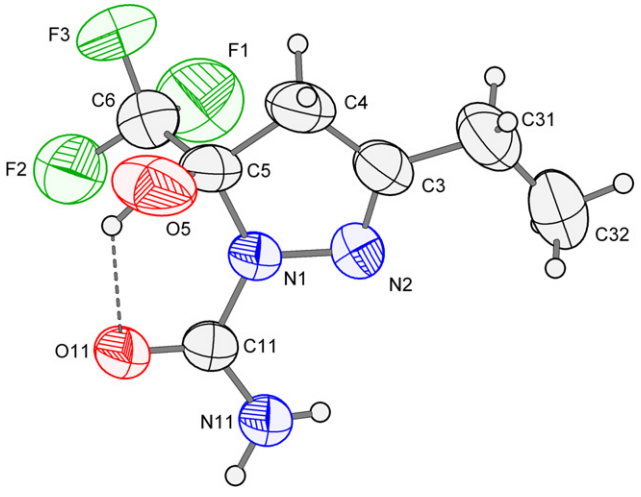


Fig. 2. ORTEP [24] obtained from crystal structure of 3-ethyl-5-hydroxy-5-trifluoromethyl-4,5-dihydro-1*H*-1-carboxyamidepyrazole (**2c**). Displacement ellipsoids are drawn at the 50% probability level. The intramolecular hydrogen bond is indicated with dashed lines.

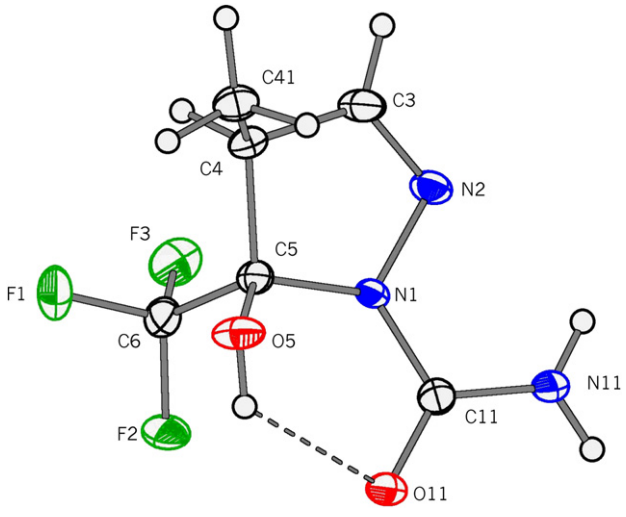


Fig. 3. ORTEP [24] obtained from crystal structure of 5-hydroxy-4-methyl-5-trifluoromethyl-4,5-dihydro-1*H*-1-carboxyamidepyrazole (**2j**). Displacement ellipsoids are drawn at the 50% probability level. The intramolecular hydrogen bond is indicated with dashed lines.

did not exhibit an analgesic effect in mice. This discrepancy may be explained by the different models of pain used to evaluate the analgesic effect of PPCA and **2h**. One significant change caused by the replacement of trichloromethyl with a trifluoromethyl group is related to their differences in size. While the van der Waals radius of chlorine has been shown to be 1.81 Å [26], the fluorine atom has shown a van der Waals radius of 1.47 Å [27]. This greater size of chlorine could cause steric effects at putative receptor sites, thus changing the pharmacological properties of the compounds.

In order to determine the most promising compounds for the next tests, we calculated the analgesic efficacy of the 4,5-dihydro-1*H*-pyrazoles in both the neurogenic and inflammatory phases. As shown in Table 1, compounds **2c** and **2j**, at a dose of 1000 μmol/kg, presented the best analgesic efficacy against neurogenic and inflammatory pain. Therefore, these compounds were selected for evaluation with hot-plate, carrageenan-induced paw edema, rotarod and spontaneous locomotor activity tests. Furthermore, the analgesic efficacy of **2c** and **2j** in the formalin test was compared with that of dipyrone and salicylamide (Fig. 6). Compounds **2c**, **2j** and dipyrone, but not salicylamide, were effective against formalin-induced neurogenic pain. Moreover, compounds **2c** and **2j** were equally effective against inflammatory pain, but more effective than salicylamide and dipyrone. These data suggest that the replacement of the benzene from salicylamide with a 5-trifluoromethyl-4,5-dihydro-1*H*-pyrazole scaffold was well tolerated leading to compounds with better analgesic efficacy than salicylamide. It is noteworthy that the crystallographic studies revealed that the 4,5-dihydro-1*H*-pyrazole rings, similar to the benzene ring, showed a planar structure where the sum of internal angles [N(1)–N(2)–C(3)–C(4)–C(5)] was found to be 539.65° and 539.8° for compounds **2c** and **2j**, respectively, deviating only slightly from the ideal value of 540°. This fact may be associated with the retention of analgesic activity of the pyrazole derivatives of salicylamide as the structural planarity often guides the molecular elements toward the correct binding pockets of the putative target.

The analgesic profile of compounds **2a–j** also suggests that the analgesic efficacy is greater when R^1 is an ethyl or when R^2 is a methyl group such as in compounds **2c** and **2j**, respectively. Furthermore, a change in the methyl group from R^2 to R^1 seemed to decrease the analgesic effect (**2j** versus **2b** compound).

Another interesting finding is that most of the pyrazole derivatives, similar to dipyrone and unlike most of the nonsteroidal anti-inflammatory drugs (NSAIDs), were effective in preventing the neurogenic pain response induced by formalin [28–30]. The analgesic effect of such pyrazole compounds during the neurogenic phase of the formalin test may suggest that they have a different mechanism of action than classical NSAIDs. Neurogenic pain generally responds poorly to conventional analgesics and its treatment can be difficult [31,32]. Therefore, to discover new drugs for treatment of this kind of pain is clinically relevant.

2.2.2. Hot-plate test

The subcutaneous administration of pyrazole compounds **2c** and **2j** (1000 μmol/kg) did not produce an analgesic effect according to the thermal behavior model of pain, the hot-plate test, maintained at 50 °C (Fig. 7). On the other hand, dipyrone (1500 μmol/kg), included in the experiment as an internal standard, increased the latency response after subcutaneous administration.

There is controversy in the literature about the usefulness of the thermal stimulus test for the evaluation of the analgesic efficacy of mild analgesics, such as NSAIDs. Some authors consider that NSAIDs, as well as dipyrone, are not effective in thermal stimulation tests [33,34]. In contrast, other groups have found that dipyrone is effective against the nociception produced by the thermal stimulus [35–38]. The discrepancies in the literature are probably due to differences in the intensity of the thermal stimulus applied [39–41]. When the intensity of the thermal stimulus is around 50 °C, the antinociceptive effect of mild analgesics has been detected [39,41]. In our work, high doses of **2c** and **2j** were not evaluated due to their low solubility in the vehicle used. The lack of analgesic effect may, therefore, be attributed to the low doses. In summary, our findings suggest that **2c** and **2j** have a different analgesic profile in relation to dipyrone, perhaps because they present a different mechanism of action or different analgesic potency.

2.2.3. Evaluation of anti-inflammatory action in carrageenan-induced paw edema test

The pyrazole compounds **2c** and **2j** (1000 μmol/kg) were evaluated for anti-inflammatory activity by the carrageenan-induced paw edema test, a suitable experimental animal model, widely used for determining acute inflammation [42]. The inflammation induced by carrageenan is believed to be biphasic [42]. The early phase (up to 1 h) involves the release of histamine, serotonin and bradykinin and the late phase (over 1 h) is due to the release of prostaglandin-like substances [43,44]. Based on this, a decrease in the second phase may be, at last in part, explained by inhibition of cyclooxygenase [44].

Fig. 8 shows that the intraplantar injection of carrageenan in mice produced an increase in paw volume, a signal of inflammation. However, carrageenan-induced paw edema was significantly prevented in a time-dependent manner by previous treatment with compounds **2c** and **2j** at a dose of 1000 μmol/kg. In fact, when the paw volume was evaluated 30 min after the carrageenan injection, **2c** and **2j** did not prevent the development of paw edema. In contrast, **2c** and **2j** presented an anti-edematogenic effect when evaluated 4 h after the induction of inflammation. This inhibition of paw edema, only in the late phase of inflammation, suggests that the 5-trifluoromethyl-4,5-dihydro-1*H*-pyrazoles **2c** and **2j** act on prostaglandin biosynthesis, which is involved in the late phase of carrageenan-induced inflammation.

It is important to consider that **2c** and **2j** inhibited carrageenan-induced inflammation almost equally ($43.7 \pm 8.1\%$ and $34.6 \pm 12.8\%$, respectively), suggesting that the R^1 or R^2

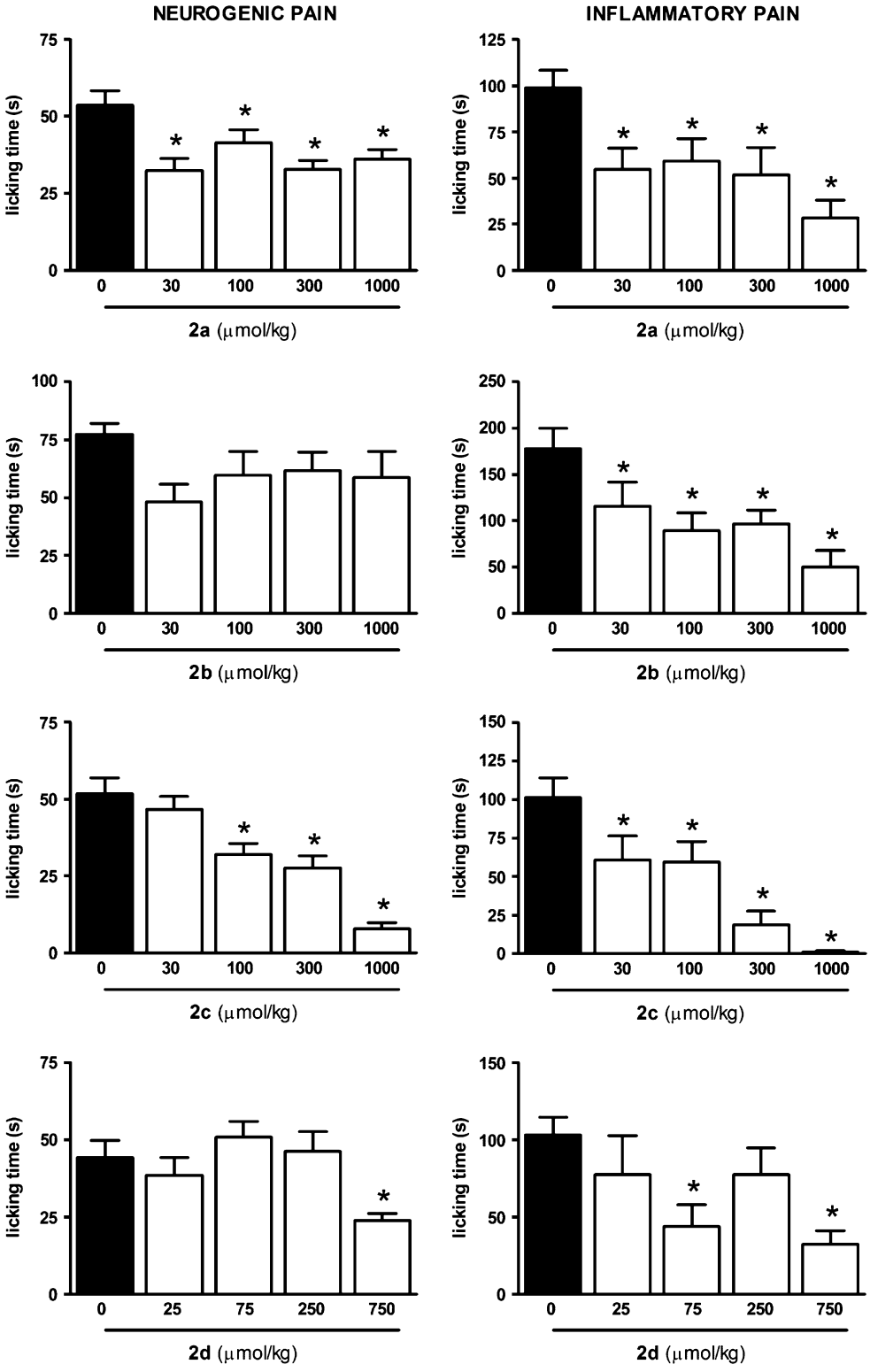


Fig. 4. Effect of novel pyrazole derivatives **2a–d** on nociception during neurogenic and inflammatory phases of the formalin test in mice. Data are reported as mean ± SEM; $*p < 0.05$ compared to vehicle (SNK test).

substituent did not significantly affect the anti-inflammatory effect.

In contrast with compounds **2c** and **2j**, the trichloromethylated analogue, MPCA, was ineffective against carrageenan-induced paw edema [6]. As the substitutions in R^1 and R^2 positions seem not to modify the anti-inflammatory efficacy of these compounds, it is possible that such an effect is due to the trifluoromethyl group attached at position 5 of

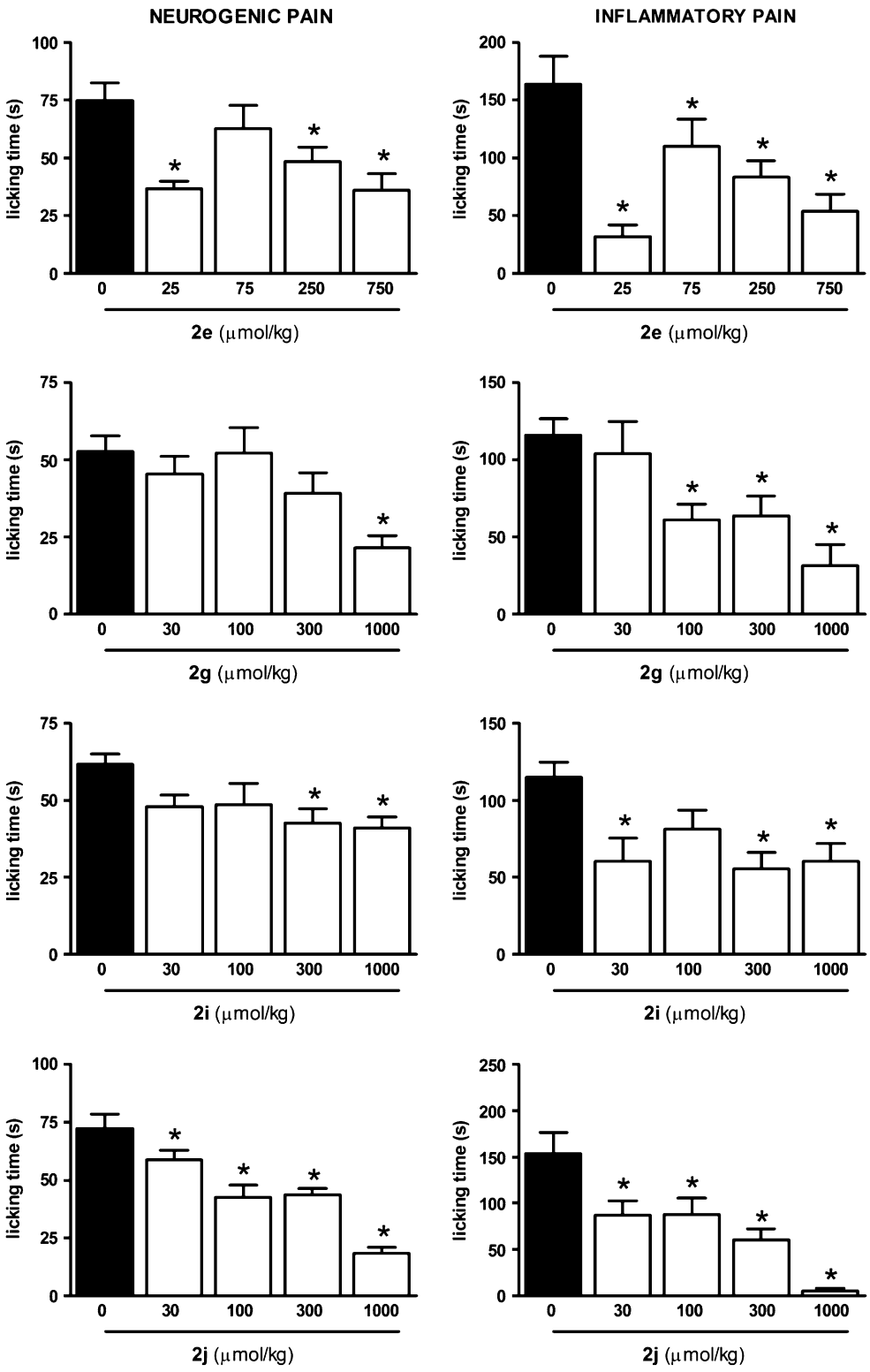


Fig. 5. Effect of novel pyrazole derivatives **2e**, **2g**, **2i** and **2j** on nociception during neurogenic and inflammatory phases of the formalin test in mice. Data are reported as mean ± SEM; *$p < 0.05$ compared to vehicle (SNK test).

4,5-dihydro-1*H*-pyrazole ring. One plausible hypothesis for this fact is that the presence of CF_3 group provides compounds metabolically more stable in comparison to their isostere CCl_3 group [27]. Such an alteration is based on the premise that fluorine forms a strong bond with carbon (bond energy C−F = 116 kcal/mol) [27], which has an increased oxidative and thermal stability compared with the carbon−chlorine bond (C−Cl = 87 kcal/mol) [45]. Therefore, this greater stability can lead to better bioavailability of the trifluoromethylated compounds in relation to their trichloromethylated

Table 1
Analgesic efficacy of 4,5-dihydro-1*H*-pyrazoles **2a–j** (1000 μmol/kg) on neurogenic and inflammatory phases of the formalin test in mice

Entry	Analgesia (%)		N	
	Neurogenic phase	Inflammatory phase	Vehicle	Treated
2a	32.8 ± 6.2*	70.8 ± 9.6*	17	15
2b	23.3 ± 11.9	55.5 ± 10.5*	9	8
2c	87.9 ± 3.6*	98.2 ± 1.3*	14	9
2d	48.2 ± 4.7*	70.1 ± 8.4*	16	12
2e	51.9 ± 9.5*	67.0 ± 8.8*	10	9
2f	46.0 ± 7.2*	−22.3 ± 15.1	6	8
2g	59.1 ± 7.6*	72.9 ± 11.9*	12	11
2h	10.0 ± 11.8	8.2 ± 17.1	8	8
2i	33.3 ± 5.9*	47.3 ± 9.8*	17	18
2j	74.6 ± 3.6*	97.2 ± 1.6*	10	10

Values are reported as means ± SEM; *N* is the number of animals in each group.
* Statistically different from vehicle ($p < 0.05$; SNK test).

analogues thus being able to demonstrate a significant effect in the late phase of carrageenan-induced inflammation. However, further investigation is necessary to clarify this point.

2.2.4. Rotarod test and spontaneous locomotor activity evaluation

Tables 2 and 3 show that **2c** and **2j** did not alter rotarod performance and spontaneous locomotor activity of mice, respectively. However, salicylamide increased spontaneous locomotor activity. These results indicate that the observed decrease in pain-related behavior in both the neurogenic and inflammatory phases of the formalin test, after administration of **2c** and **2j**, was not related to gross motor impairment since locomotion was not affected. Nevertheless, salicylamide increased the locomotor activity of mice, suggesting that it induces other effects besides analgesia. In addition, the compounds did not cause the death of the animals in a 72 h observation period, an indication of low toxicity, at least after acute administration.

3. Conclusion

In summary, we have demonstrated here, for the first time, that the 5-trifluoromethyl-4,5-dihydro-1*H*-pyrazole scaffold behaves like benzene bioisosteres, supplying novel analgesic and anti-inflammatory pyrazole derivatives. The synthesized compounds **2a–j** were obtained with the use of microwave-assisted synthesis in a significantly shorter time (4 min) and with high yields (82–96%). Our results suggest that 4,5-dihydro-1*H*-pyrazoles **2a**, **2c–g**, and **2i–j**, unlike most of the NSAIDs, have an additional and interesting analgesic action, in that these compounds were effective on neurogenic pain. However, further investigations are necessary to elucidate their mechanism of action and their effect on other models of pain, including chronic and neuropathic pain models. Finally, the results showed that 5-hydroxy-5-trifluoromethyl-4,5-dihydro-1*H*-1-carboxyamidepyrazoles are promising candidates for the future development of novel drugs for the treatment of pain and inflammatory diseases.

4. Experimental protocols

4.1. Chemistry

Unless otherwise indicated, all common reagents were used as obtained from commercial suppliers without further purification. The solvents were dried and purified according to recommended procedures [46]. All melting points were measured using a Reichert-Thermovar apparatus and are uncorrected. Yields listed are of isolated compounds. ^{1}H and ^{13}C NMR spectra were acquired on a Bruker DPX 200 or Bruker DPX 400 spectrometer (^{1}H at 200.13 MHz or 400.13 MHz and ^{13}C at 50.32 MHz or 100.63 MHz, respectively) at 300 K, 5 mm sample tubes, and with a digital resolution of ±0.01 ppm. $CDCl_3$ was used as solvent with TMS as internal standard. Mass spectra were registered in a HP 5973 MSD connected to a HP 6890 GC and interfaced by a Pentium PC. The GC was equipped with a split–splitless injector, autosampler cross-linked HP-5 capillary column (30 m, 0.32 mm of internal diameter), and helium was used as the carrier gas. The reactions were performed in a multimode microwave *ETHOS 1* (*Milestone Inc*) which possesses a twin magnetron with a maximum delivered power of 1300 W. The temperature and the pressure were measured throughout with an ATC-400 CE and APC-55 detector, respectively. The crystal data were recorded on a Bruker Kappa Apex

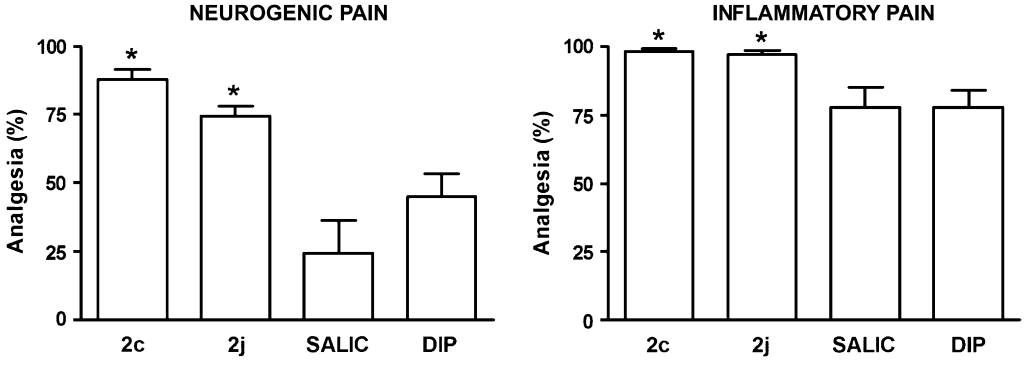


Fig. 6. Comparison between analgesic efficacy of **2c**, **2j** (1000 μmol/kg), salicylamide (1500 μmol/kg) (SALIC) and dipyrone (1000 μmol/kg) (DIP) in the formalin test. Data are reported as mean ± SEM for $n = 6–9$, * denotes a significant difference with regards to salicylamide and dipyrone ($p < 0.05$; SNK test).

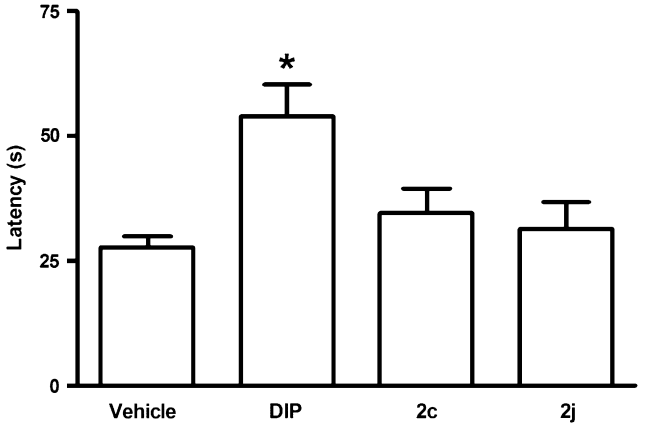


Fig. 7. Effect of pyrazole derivatives **2c** and **2j** (1000 μmol/kg), dipyrone (1500 μmol/kg) or vehicle on the latency to response from thermal stimulus on the hot-plate test. Data are reported as mean ± SEM for $n = 14–15$ animals per group; $^*p < 0.05$ compared to vehicle (SNK test).

II CCD area detector with graphite monochromatized Mo Kα radiation ($\lambda = 0.71073$ Å). The data were processed with SAINT and SADABS. The structure was solved by direct methods (SHELXS-97) and additional atoms were located in the Fourier map and refined on F2 (SHELXL-97) using the SHELXTL [47] and Wingx [48] packages. The CHN elemental analyses were performed on a Perkin-Elmer 2400 CHN elemental analyzer (Federal University of São Paulo, USP/Brazil).

*4.1.1. General procedure for the synthesis of 5-trifluoromethyl-4,5-dihydro-1H-pyrazoles **2a–j***

A solution of 4-alkoxy-1,1,1-trifluoro-3-alken-2-ones 1 (2 mmol) and semicarbazide hydrochloride (0.268 g, 2.4 mmol) in methanol/water 3:1 v/v (6 mL) and pyridine (2 mL) was stirred for a few minutes. The mixture was then irradiated in a microwave *ETHOS 1* at 100 W, 2.2 bar of pressure for 4 min. The temperature was set to 70 °C and the irradiation was automatically stopped at this temperature. After cooling to room temperature, the solution was extracted with chloroform (2 × 20 mL) and ethyl acetate (2 × 20 mL). The organic layers were washed with a solution of H_2O/HCl (10:1) (2 × 10 mL) and with distilled water (2 × 10 mL). Finally, the organic layers were combined and dried with magnesium sulfate, the solvent was removed by rotatory evaporation; the 5-hydroxy-5-trifluoromethyl-4,5-dihydro-1*H*-1-carboxyamidepyrazoles **2a–j** were isolated. When necessary, the products were recrystallized from hexane.

*4.1.1.1. 5-Hydroxy-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazole (**2a**).* Yield 1.8 mmol (90%); mp 125–127 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ (*J*, Hz) 3.18 (dd, 1H, $J_{H4a-H4b} = 19.6$, H4a), 3.35 (dd, 1H, $J_{H4b-H4a} = 19.6$, H4b), 6.89 (s, 1H, H3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (J_{C-F}, Hz) 45.2 (C4), 89.4 (q, $^2J = 33.9$, C5), 123.1 (q, $^1J = 286.1$, CF_3), 143.1 (C3), 156.3 (C=O); MS-EI (70 eV): *m/z* (%) = 197 (M^+, 1), 154 (9), 128 (4), 85 (100), 69 (14); Anal. Calcd. for $C_5H_6F_3N_3O_2$: C, 30.47%; H, 3.07%; N, 21.32%. Found: C, 30.69%; H, 2.86%; N, 21.12%.

*4.1.1.2. 5-Hydroxy-3-methyl-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazole (**2b**).* Yield 1.92 mmol (96%); mp 108–110 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ (*J*, Hz) 2.02 (s, 3H, Me), 3.11 (d, 1H, $J_{H4a-H4b} = 19.0$, H4a), 3.24 (d, 1H, $J_{H4b-H4a} = 19.1$, H4b), 5.6 (br, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (J_{C-F}, Hz) 15.3 (CH_3), 47.4 (C4), 90.6 (q, $^2J = 33.9$, C5), 123.1 (q, $^1J = 286.1$, CF_3), 152.9 (C3), 156.7 (C=O); MS-EI (70 eV): *m/z* (%) = 211 (M^+, 6), 168 (26), 99 (100), 69 (14); Anal. Calcd. for $C_6H_8F_3N_3O_2$: C, 34.13%; H, 3.82%; N, 19.90%. Found: C, 34.43%; H, 3.57%; N, 19.64%.

*4.1.1.3. 3-Ethyl-5-hydroxy-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazole (**2c**).* Yield 1.82 mmol (91%); mp 83–85 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (*J*, Hz) 1.15 (t, 3H, CH_3), 2.34 (q, 2H, CH_2), 3.11 (d, 1H, $J_{H4a-H4b} = 19.0$, H4a), 3.24 (d, 1H, $J_{H4a-H4b} = 19.0$, H4b), 5.09 (br, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (J_{C-F}, Hz) 9.7 (CH_3),

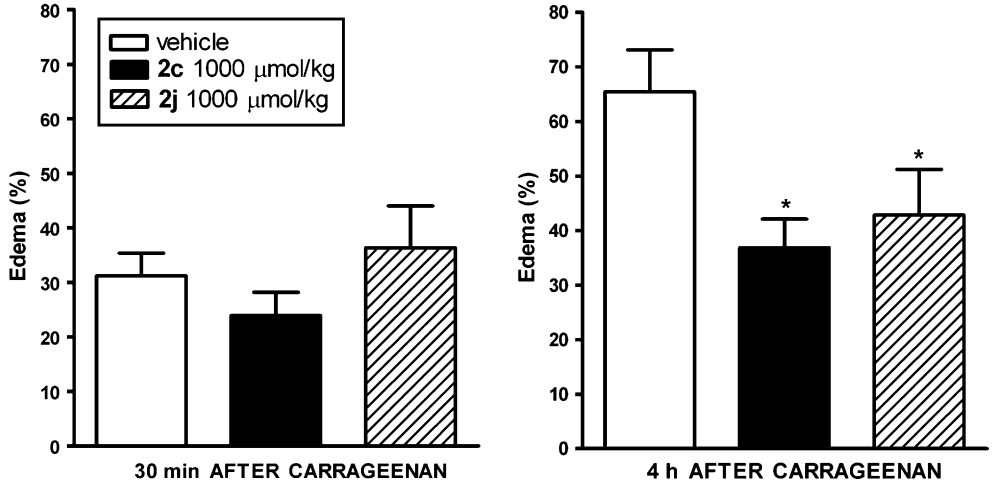


Fig. 8. Effect of pyrazole derivatives **2c** and **2j** (1000 μmol/kg) or vehicle on the paw edema induced by carrageenan (20 μl, 1.5%, ipl) 30 min and 4 h after paw edema-induction in mice. Data are reported as mean ± SEM for $n = 14–16$ animals per group; $^*p < 0.05$ compared to vehicle (SNK test).

Table 2
Effects of 4,5-dihydro-1*H*-pyrazoles **2c** and **2j** on rotarod performance

Group	Fall latency (s)	Number of falls
Vehicle	76.9 ± 17.0	5.4 ± 1.3
2c 1000 μmol/kg	112.5 ± 21.3	3.7 ± 2.1
2j 1000 μmol/kg	62.6 ± 17.6	5.1 ± 1.0

Data are means ± SEM for 14 to 15 animals per group.

22.7 (CH_2), 45.7 (C4), 90.3 (q, $^2J = 33.9$, C5), 123.1 (q, $^1J = 286.1$, CF_3), 156.8 (C=O), 157.3 (C3); MS-EI (70 eV): *m/z* (%) = 225 (M^+, 5), 182 (17), 113 (100), 85 (18), 69 (7); Anal. Calcd. for $C_7H_{10}F_3N_3O_2$: C, 37.34%; H, 4.48%; N, 18.66%. Found: C, 37.15%; H, 4.13%; N, 18.29%.

*4.1.1.4. 5-Hydroxy-3-propyl-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazole (**2d**).* Yield 1.74 mmol (87%); mp 68–70 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ (*J*, Hz) 0.97 (t, 3H, CH_3), 1.62 (sex, 2H, CH_2), 2.31 (t, 2H, CH_2), 3.09 (d, 1H, $J_{H4a-H4b} = 18.9$, H4a), 3.24 (d, 1H, $J_{H4a-H4b} = 18.9$, H4b), 5.66 (br, 2H, NH_2); ^{13}C NMR (50 MHz, $CDCl_3$) δ (J_{C-F}, Hz) 13.4 (CH_3), 19.4 (CH_2), 31.5 (CH_2), 46.0 (C4), 90.4 (q, $^2J = 33.9$, C5), 123.2 (q, $^1J = 286.1$, CF_3), 156.4 (C3), 156.8 (C=O); MS-EI (70 eV): *m/z* (%) = 239 (M^+, 6), 196 (18), 168 (8), 127 (100), 85 (29), 69 (8); Anal. Calcd. for $C_8H_{12}F_3N_3O_2$: C, 40.17%; H, 5.06%; N, 17.57%. Found: C, 40.40%; H, 4.85%; N, 17.28%.

*4.1.1.5. 5-Hydroxy-3-isopropyl-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazole (**2e**).* Yield 1.8 mmol (90%); mp 78–80 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ (*J*, Hz) 1.18 (d, 6H, C_2H_6), 2.63 (sep, 1H, CH), 3.10 (d, 1H, $J_{H4a-H4b} = 18.8$, H4a), 3.26 (d, 1H, $J_{H4a-H4b} = 18.8$, H4b), 5.35 (br, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (J_{C-F}, Hz) 19.5 (C_2H_6), 29.4 (CH), 44.1 (C4), 90.5 (q, $^2J = 33.9$, C5), 123.2 (q, $^1J = 286.1$, CF_3), 156.9 (C=O), 160.7 (C3); MS-EI (70 eV): *m/z* (%) = 239 (M^+, 19), 196 (57), 181 (29), 127 (100), 85 (98), 69 (23); Anal. Calcd. for $C_8H_{12}F_3N_3O_2$: C, 40.17%; H, 5.06%; N, 17.57%. Found: C, 40.30%; H, 4.90%; N, 17.52%.

*4.1.1.6. 3-Butyl-5-hydroxy-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazole (**2f**).* Yield 1.78 mmol (89%); mp 59–61 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ (*J*, Hz) 0.93 (t, 3H, CH_3), 1.36 (sex, 2H, CH_2), 1.55 (quin, 2H, CH_2), 2.33 (t, 2H, CH_2), 3.09 (d, 1H, $J_{H4a-H4b} = 18.8$, H4a), 3.23 (d, 1H, $J_{H4a-H4b} = 18.8$, H4b), 5.06 (br, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (J_{C-F}, Hz) 12.3 (CH_3), 21.9 (CH_2), 27.8 (CH_2), 29.0 (CH_2), 45.9 (C4), 90.0 (q, $^2J = 33.9$, C5), 123.1(q, $^1J = 286.1$, CF_3), 156.4 (C3), 156.8 (C=O); MS-EI (70 eV): *m/z* (%) = 253 (M^+, 9), 210 (25), 168 (71), 141 (100), 85 (43), 69 (16); Anal. Calcd. for $C_9H_{14}F_3N_3O_2$: C, 42.69%; H, 5.57%; N, 16.59%. Found: C, 42.83%; H, 5.19%; N, 16.21%.

Table 3
Effect of 4,5-dihydro-1*H*-pyrazoles **2c** and **2j** and salicylamide on spontaneous locomotor activity

Group	Number of crossing	*N*
Vehicle	146.1 ± 24.7	10
2c 1000 μmol/kg	165.6 ± 30.5	8
Vehicle	115.5 ± 10.0	14
2j 1000 μmol/kg	167.3 ± 31.2	9
Vehicle	45.6 ± 7.2	9
Salicylamide 1500 μmol/kg	102.1 ± 26.4*	7
Vehicle	114.3 ± 22.8	6
Dipyrone 1000 μmol/kg	87.7 ± 29.2	7

Data are means ± SEM; *N*, number of animals in each group. *$p < 0.05$ compared to vehicle (SNK test).

*4.1.1.7. 3-tert-Butyl-5-hydroxy-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazole (**2g**).* Yield 1.64 mmol (82%); mp 100–102 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ (*J*, Hz) 1.19 (s, 9H, C_3H_9), 3.13 (d, 1H, $J_{H4a-H4b} = 18.8$, H4a), 3.28 (d, 1H, $J_{H4a-H4b} = 18.8$, H4b), 4.99 (br, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (J_{C-F}, Hz) 27.7 (C_3H_9), 34.0 (*t*-bu), 43.0 (C4), 90.8 (q, $^2J = 33.2$, C5), 123.3 (q, $^1J = 286.8$, CF_3), 156.7 (C=O), 163.2 (C3); MS-EI (70 eV): *m/z* (%) = 253 (M^+, 7), 195 (63), 178 (7), 141 (100), 85 (53), 69 (22); Anal. Calcd. for $C_9H_{14}F_3N_3O_2$: C, 42.69%; H, 5.57%; N, 16.59%. Found: C, 43.90%; H, 5.69%; N, 16.80%.

*4.1.1.8. 5-Hydroxy-3-phenyl-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazole (**2h**).* Yield 1.7 mmol (85%); mp 129–131 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (*J*, Hz) 3.56 (d, 1H, $J_{H4a-H4b} = 18.8$, H4a), 3.70 (d, 1H, $J_{H4a-H4b} = 18.8$, H4b), 5.68 (br, 2H, NH_2), 7.43–7.69 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ (J_{C-F}, Hz) 43.9 (C4), 91.0 (q, $^2J = 33.9$ Hz, C5), 123.2 (q, $^1J = 286.8$, CF_3), 126.4, 128.8, 129.8, 130.9 (Ph), 151.7 (C3), 156.4 (C=O); MS-EI (70 eV): *m/z* (%) = 273 (M^+, 33), 230 (90), 161 (100), 118 (26), 103 (52), 77 (86); Anal. Calcd. for $C_{11}H_{10}F_3N_3O_2$: C, 48.36%; H, 3.69%; N, 15.38%. Found: C, 48.10%; H, 3.39%; N, 15.25%.

*4.1.1.9. 3-(4-Bromophenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazole (**2i**).* Yield 1.82 mmol (91%); mp 194–196 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ (*J*, Hz) 3.52 (d, 1H, $J_{H4a-H4b} = 18.8$, H4a), 3.64 (d, 1H, $J_{H4a-H4b} = 18.8$, H4b), 5.6 (br, 2H, NH_2), 7.52 (d, 2H, Ph), 7.57 (d, 2H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ (J_{C-F}, Hz) 49.1 (C4), 91.4 (q, $^2J = 33.9$, C5), 123.3 (q, $^1J = 286.8$, CF_3), 130.9, 133.3, 134.3, 137.6 (Ph), 156.2 (C=O), 161.4 (C3); MS-EI (70 eV): *m/z* (%) = 351 (M^+, 15), 308 (47), 239 (100), 160 (47), 102 (55), 75 (26); Anal. Calcd. for $C_{11}H_9BrF_3N_3O_2$: C, 37.52%; H, 2.58%; N, 11.93%. Found: C, 37.69%; H, 2.65%; N, 12.15%.

*4.1.1.10. 5-Hydroxy-4-methyl-5-trifluoromethyl-4,5-dihydro-1H-1-carboxyamidepyrazole (**2j**).* Yield 1.88 mmol (94%); mp 118–120 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (*J*, Hz) 1.25 (d, 3H, $J_{CH3-H4} = 7.5$, CH_3), 3.43 (qd, 1H, $J_{H4-CH3} = 7.5$, $J_{H4-H3} = 1.5$, H4), 5.64 (br, 2H, NH_2), 6.79 (d, 1H, $J_{H3-H4} = 1.5$ Hz, H3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (J_{C-F}, Hz) 10.0 (CH_3), 48.2 (C4), 85.1 (q, $^2J = 33.2$, C5),

123.5 (q, $^1J = 286.8$, CF_3), 148.5 (C3), 156.8 (C=O); MS-EI (70 eV): *m/z* (%) = 211 (M^+, 3), 168 (17), 142 (22), 99 (100), 69 (10); Anal. Calcd. for $C_6H_8F_3N_3O_2$: C, 34.13%; H, 3.82%; N, 19.90%. Found: C, 34.38%; H, 3.55%; N, 19.65%.

4.2. Pharmacological studies

4.2.1. Animals

Adult male albino mice (30–40 g) were housed in groups of 20 per cage, at a controlled temperature (22 ± 1 °C), on a 12 h light/12 h dark cycle and with standard lab chow and water ad libitum. The animals were acclimated to the experimental room for at least 2 h before the experiments. Each animal was used only once. All experiments were performed in accordance with the ethical guidelines established for investigations of experimental pain in conscious animals [49] and were approved by the Committee on the Use and Care of Laboratory Animals of our University (register number 23081.018371/2006-94).

4.2.2. Formalin test

The analgesic activity was evaluated by the formalin test carried out as previously described [50]. Animals were injected subcutaneously with 20 μl of 1.5% formalin (v/v) into the dorsal right hindpaw.

The time spent licking or biting the injected paw or leg was recorded for 30 min at 5 min intervals. Subcutaneous administration of formalin causes a typical biphasic response of licking and biting. During the first 5 min the animals lick or bite the injected paw because formalin probably causes direct stimulation of nerve terminals and this phase is termed ''neurogenic''. A second burst of licking or biting occurs between 15 and 30 min after the injection and seems to be related to the inflammatory response elicited by formalin and this phase is termed ''inflammatory'' [50].

Vehicle (saline plus 5% Tween 80) or the 4,5-dihydro-1*H*-pyrazoles **2a–j** (25–1000 μmol/kg) were injected subcutaneously into the dorsal region 20 min before formalin injection into the paw. Salicylamide and dipyrone were used as internal standards, at effective doses previously reported [16,17] or determined by pilot experiments. Immediately after formalin injection, the mice were placed in individual glass cages ($20 \times 20 \times 20$ cm^3), which served as the observation chamber, and were evaluated for 30 min.

Furthermore, the floor of the observation chamber was divided into 12 equal areas and the number of areas that a mouse crossed with all paws was recorded and was assessed simultaneously with the formalin-induced pain evaluation. The number of crossings was used as a spontaneous locomotor activity index.

Initially, the compounds were administered at the maximal possible dose in accordance with their solubility in vehicle (saline plus 5% Tween 80). Therefore, **2a–c**, **2g–j** were injected at a dose of 1000 μmol/kg, while **2d–f** were injected at a dose of 750 μmol/kg. When the compounds that produced analgesia were identified, a dose–response curve was constructed for each one.

4.2.3. Hot-plate test

The hot-plate test was carried out to determine whether the novel pyrazoles produced analgesia in a thermal model of pain. The test was performed according to the method described by Ferreira et al. [51]. In these experiments, the hot-plate (Ugo Basile, model-DS 37) was maintained at 50 ± 1 °C. Animals were placed in a glass cylinder of 24 cm diameter on the heated surface, and the time between placement and shaking, lifting or licking of the hind paws, or jumping, was recorded as the index of response latency. The cutoff latency was set to 90 s to prevent tissue damage. Twenty-four hours before the experiment, all mice were acclimated to the experimental procedure in order to minimize novelty-induced analgesia [52]. On the day of the experiment the animals were injected with vehicle or pyrazole derivatives (1000 μmol/kg, subcutaneously) and the latency time was determined 30 min thereafter. Dipyrone (1500 μmol/kg, subcutaneously) was used as an internal standard at effective doses previously determined by pilot experiments.

4.2.4. Carrageenan-induced paw edema

We employed the classical model of carrageenan-induced paw edema to assess the anti-inflammatory effect of the novel pyrazoles [42,53]. Briefly, carrageenan was diluted in physiological saline at a concentration of 15 mg/ml. This solution was boiled for 1–2 s and cooled at room temperature. The mice received 20 μl of this solution in the right hindpaw, subcutaneously, on the intraplantar side. Edema formation was quantified by changes in paw volume measured before, 30 min and 4 h after carrageenan injection. The paw edema evaluation was made by immersing the injected paw into a cuvette filled with a solution of 2.5% extran in water (v/v). The cuvette was fixed on the plate of an electronic scale (precision of 0.01 g), and the careful immersion of the paw into cuvette's solution was accompanied by an increase in the weight displayed. The weight in grams is related to the increase of the liquid column in the cuvette, but not to the mass of the paw. Since the cuvette's solution density was 1 mg/ml, the value displayed by the balance was promptly assumed as the paw volume. In order to evaluate the effect of the pyrazole compounds, the animals received subcutaneously vehicle or pyrazole derivatives (1000 μmol/kg) 30 min before intraplantar carrageenan injection and the paw edema was calculated as to the following equation: % edema = $[(V_{initial} - V_{final})/V_{initial}] \times 100$; where $V_{initial}$ is the volume of the paw before carrageenan injection and V_{final} is the volume of the paw 30 min or 4 h after carrageenan injection.

4.2.5. Rotarod performance test

The rotarod test was used to assess whether the pyrazole derivatives caused gross motor impairment in the animals. Twenty-four hours before the experiment, all mice were trained in the rotarod (3.7 cm in diameter, 8 rpm) until they could remain in the apparatus for 60 s without falling. On the day of the experiment, the animals were injected with vehicle or the pyrazole derivatives (1000 μmol/kg) and subjected to the rotarod 30 min thereafter. The latency to fall from the

apparatus was recorded with a stopwatch for up to 240 s, as was the number of falls in a 4 min session [54].

4.2.6. Statistical analysis

Data were analyzed by one-way ANOVA. *Post hoc* Student–Newman–Keuls test (SNK) was carried out when appropriate.

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