Synthesis of novel trifluoromethyl-substituted spiro-[chromeno[4,3-*d*]pyrimidine-5,1'-cycloalkanes], and evaluation of their analgesic effects in a mouse pain model

Helio G. Bonacorso, Wilian C. Rosa, Sara M. Oliveira, Indiara Brusco, Evelyne S. Brum, Melissa B. Rodrigues, Clarissa P. Frizzo, Nilo Zanatta

PII: S0960-894X(17)30173-7
DOI: http://dx.doi.org/10.1016/j.bmcl.2017.02.036
Reference: BMCL 24708

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 29 December 2016
Revised Date: 14 February 2017
Accepted Date: 16 February 2017

Please cite this article as: Bonacorso, H.G., Rosa, W.C., Oliveira, S.M., Brusco, I., Brum, E.S., Rodrigues, M.B., Frizzo, C.P., Zanatta, N., Synthesis of novel trifluoromethyl-substituted spiro-[chromeno[4,3-*d*]pyrimidine-5,1'-cycloalkanes], and evaluation of their analgesic effects in a mouse pain model, *Bioorganic & Medicinal Chemistry Letters* (2017), doi: http://dx.doi.org/10.1016/j.bmcl.2017.02.036

Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

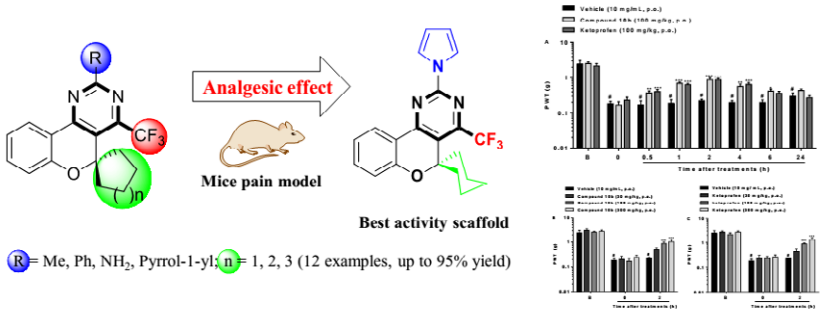
Synthesis of novel trifluoromethyl-substituted spiro-[chromeno[4,3-*d*]pyrimidine-5,1'-cycloalkanes], and evaluation of their analgesic effects in a mouse pain model

Leave this area blank for abstract info.

Helio G. Bonacorso[*a], Wilian C. Rosa[a], Sara M. Oliveira[b], Indiara Brusco[b], Evelyne S. Brum[b], Melissa B. Rodrigues[a], Clarissa P. Frizzo[a], and Nilo Zanatta[a]

[a] *Núcleo de Química de Heterociclos (NUQUIMHE), Departamento de Química, Universidade Federal de Santa Maria, 97.105-900, Santa Maria, RS, Brazil.*

[b] *Laboratório de Neurotoxicidade e Psicofarmacologia, Departamento de Bioquímica Toxicológica, Universidade Federal de Santa Maria, 97.105-900, Santa Maria, RS, Brazil.*



Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

Synthesis of novel trifluoromethyl-substituted spiro-[chromeno[4,3-*d*]pyrimidine-5,1'-cycloalkanes], and evaluation of their analgesic effects in a mouse pain model

Helio G. Bonacorso[a*], Wilian C. Rosa[a], Sara M. Oliveira[b], Indiara Brusco[b], Evelyne S. Brum[b], Melissa B. Rodrigues[a], Clarissa P. Frizzo[a], and Nilo Zanatta[a]

[a] *Núcleo de Química de Heterociclos (NUQUIMHE), Departamento de Química, Universidade Federal de Santa Maria, 97.105-900, Santa Maria, RS, Brazil.*
[b] *Laboratório de Neurotoxicidade e Psicofarmacologia, Departamento de Bioquímica Toxicológica, Universidade Federal de Santa Maria, 97.105-900, Santa Maria, RS, Brazil.*

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Chromeno[4,3-*d*]pyrimidines
Trifluoromethyl
Spiro compounds
Arthritic pain
Analgesic effects

ABSTRACT

Herein we report the synthesis of twelve 2,5-substituted 4-(trifluoromethyl)-spirochromeno[4,3-*d*]pyrimidines (**7**–**10**), as well as an evaluation of their analgesic effect in a mouse pain model. The nine new chromeno[4,3-*d*]pyrimidines (**7–9**) were synthesized from the cyclocondensation reactions of three 2,2,2-trifluoro-1-(4-methoxyspiro[chromene-2,1'-cycloalkane]-3-yl)ethanones (**3**) containing 5-, 6- and 7-membered spiro-cycloalkanes, with some well-known amidine salts (**4–6**) [$NH_2CR(=NH)$] — in which R = Me, Ph, and NH_2 — at yields of 60–95 %. Subsequently, three new 2-(pyrrol-1-yl)-4-(trifluoromethyl)-chromeno[4,3-*d*]pyrimidines (**10**) were obtained through a Clauson-Kaas reaction between the respective 2-(amino)-4-(trifluoromethyl)-chromeno[4,3-*d*]pyrimidines (**9**) and 2,5-dimethoxy-tetrahydrofuran. The analgesic evaluation showed that these 4-(trifluoromethyl)chromeno[4,3-*d*]pyrimidines (100 mg/kg, p.o.) and Ketoprofen (100 mg/kg, p.o.) significantly reduced capsaicin-induced spontaneous nociception. Moreover, the 2-pyrrolyl-spirocyclohexane derivative **10b** (100 and 300 mg/kg, p.o.) had an anti-allodynic effect comparable to Ketoprofen (100 and 300 mg/kg, p.o.) in the arthritic pain model, without causing locomotor alterations in the mice. These results suggest that the compound **10b** is a promising molecule for new analgesic drugs in the treatment of pathological pain, such as in arthritis.

Benzopyrano[4,3-*d*]pyrimidines are important pharmacophores that have shown anti-inflammatory, antiplatelet, and antithrombotic activity.[1] Between 2001 and 2006, Bruno *et al.* developed a large number of benzopyrano[4,3-*d*]pyrimidines from 3-formylchromone and amidines, via cyclocondensation reactions endowed with interesting pharmacological properties such as anti-inflammatory, analgesic, and, in particular, *in vitro* anti-aggregating activity. Moreover, these compounds showed gastrointestinal tolerability and did not induce gastrolesivity.[2–4]

In 2006, Hegab *et al.* synthesized some spirocyclohexane-substituted aldazino-, pyrazolo-, thieno-, and thiooxopyrimidino-chromenes via reaction of the corresponding *β*-chlorocarboxaldehyde with hydrazine hydrate, mercaptoacetic acid, and thiourea, respectively. Some of these compounds showed good anti-inflammatory, analgesic, anticonvulsant, and antiparkinsonian activity in comparison with indomethacin, diclofenace, carbamazepine, and benztropine. In particular, the spiro[chromeno[4,3-*d*]pyrimidine-5,1'-cyclohexane]-2(1*H*)-thione showed higher anti-inflammatory activity than indomethacin and diclofenace. However none of the molecules were trifluoromethyl-substituted, and, furthermore, the spiro-carbocyclic moiety was limited to a six-membered ring.[5]

Relatively few papers have reported on the formation of benzopyrano[4,3-*d*]pyrimidines with a limited number of substituents, starting from 3-formylchromone or its equivalents, via [3+3] cyclocondensation reaction. Thus, in 2010, Li *et al.* reported on an efficient combinatorial synthesis of substituted benzopyrano[4,3-*d*]pyrimidines, via a three-component *one-pot* reaction employing iodochromone, alkynes, and amidines, together with sequential Sonogashira coupling, condensation, and cycloaddition reaction. This protocol enabled the heterocyclic system to be constructed efficiently, with good to excellent yields.[6]

In the context of spiro compounds, heterocycles, in particular, are present in a large number of natural compounds, including plants and animals. It is also known that spiro heterocycles have a wide variety of biological activities; for example, spiro-oxindole derivatives have antitumor, antibiotic, and antimicrobial (anti-HIV and antimalarial) activity.[7,8] Due to being tachykinin

antagonists, aza-spiro compounds have been used to treat depression, anxiety, pain, and migraine.[6] Some spiro heterocycles derived from benzopyrans are aldose reductase inhibitors, and some possess antidiabetic activity.[8]

Due to the capacity for fluorine atom changes in the physical and chemical properties of the molecules to which these atoms are bonded, researchers have shown great interest in organofluorine pharmaceuticals, which have at least one fluorine atom in their structure and account for about 20–30 % of approved drugs and about 40% of the new compounds entering phase III trials.[9–11] Among the properties that can be changed, the most notable are the pKa value, the electrostatic interactions between receptor and ligand, the increase in lipophilicity, and increases in molecular stability within the body.[9] Over the last decade, our research group has worked on the synthesis of new trifluoromethyl-substituted heterocycles — such as 5,6-dihydrobenzo[*h*]quinazolines[12,13] and pyrazoles[14,15] and their associated derivatives — which have shown promising pharmacological properties such as analgesic and anti-inflammatory activity.

Building on our general interest in the synthesis of trifluoromethyl-substituted aza-heterocycles with promising biological applications,[16] we observed a dearth of studies involving benzopyrano[4,3-*d*]pyrimidines. Thus, given that these scaffolds exhibit most of the characteristic properties previously described, we considered them to be valuable and possible drug candidates for the treatment of arthritic pain.

Pathological states of pain are difficult to treat, and pain is one of the most prevalent conditions that limits productivity and reduces the quality of a patient's life,[17] which is why it is the main motivation for patients seeking medical care.[18] In arthritis, the joint nerves become sensitized, producing acute and chronic pain.[19] The current therapies for alleviating joint pain, although widely used, have limited effectiveness, and some drugs lead to adverse effects, which makes their clinical use problematic and precludes their long-term use.[20,21] In this scenario, the discovery and development of safer and more effective therapies for treating arthritic pain is required. The Complete Freund's Adjuvant (CFA) has been used as an experimental model to induce chronic inflammatory pain in rodents, similar to how *M. tuberculosis* (CFA's component) produces arthritis in humans.[22,23]

In our previous work, we were pleased to disclose the excellent reactivity of 2,2,2-trifluoro-1-[4-methoxy-spiro(2*H*-chromen-2,10-cycloalkane)-3-yl]ethanones — which had been prepared from spiro[chroman-2,10-cycloalkan]-4-ones — in the regioselective synthesis of 1(2)-methyl(phenyl)-3-(trifluoromethyl)-1,4(2,4)-dihydro-spiro(chromen[4,3-*c*]pyrazole-4,n'-cycloalkanes).[24] Also synthesized from spiro[chroman-2,10-cycloalkan]-4-ones were novel 7-amine-spiro[chromeno[4,3-b]quinoline-6,1'-cycloalkanes] (see Figure 1 – **I**) such as new tacrine hybrids, which were subjected to AChE and cytotoxicity activity and molecular docking studies, and gave promising results.[25]

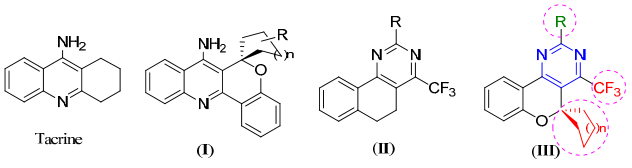
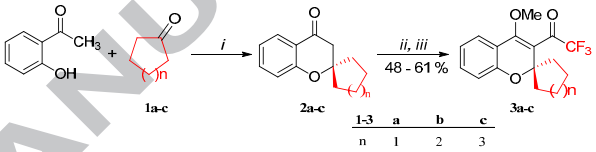


Figure 1. Tacrines, quinazolines and pyrimidines of pharmacological interest.

In order to keep exploring the synthetic potential of polyfunctional 2,2,2-trifluoro-1-[4-methoxy-spiro(2*H*-chromen-2,10-cycloalkane)-3-yl]ethanones for synthetizing scaffolds more complex than 5,6-dihydrobenzo[*h*]quinazolines (Figure 1 - II), we wish to disclose our efforts toward studying the synthesis and antinociceptive activity, in an arthritic pain model induced by CFA in mice, of a series of novel 4-(trifluoromethyl)-spiro[chromeno[4,3-*d*]pyrimidine-5,1'- cycloalkanes] (Figure 1 – III).

*General synthesis of 4-(trifluoromethyl)spiro[chromeno[4,3-d]pyrimidine-5,1'-cycloalkanes] (**7a–c**, **8a–c**, and **9a–c**):* Firstly, the starting materials involved in this study were synthesized according to the reaction pathways illustrated in Scheme 1. As depicted in Scheme 1, and according to the methodology reported by us previously,[24] the a series of 2,2,2-trifluoro-1-[4-methoxy-spiro(2*H*-chromen-2,1'-cycloalkan)-3-yl]ethanones **3a–c** was synthesized from the Kabbe adducts, namely, spiro[chroman-2,1'-cycloalkan]-4-ones (**2a–c**). Yields of 48–61 % were obtained when trifluoroacetylation reactions of mixtures of enolethers and acetals derived from a series of three spiro ketones (Kabbe adducts)[26] were performed at a reaction temperature of 60 °C for 36 h, employing anhydrous chloroform as the solvent.



Reactional conditions[24]: (*i*) Pyrrolidine, Toluene, reflux, 2 h; (*ii*) $CH(OMe)_3$, *p*-TsOH, MeOH, reflux, 48 h; (*iii*) $(CF_3CO)_2O$, Py, $CHCl_3$, 60 °C, 36 h.

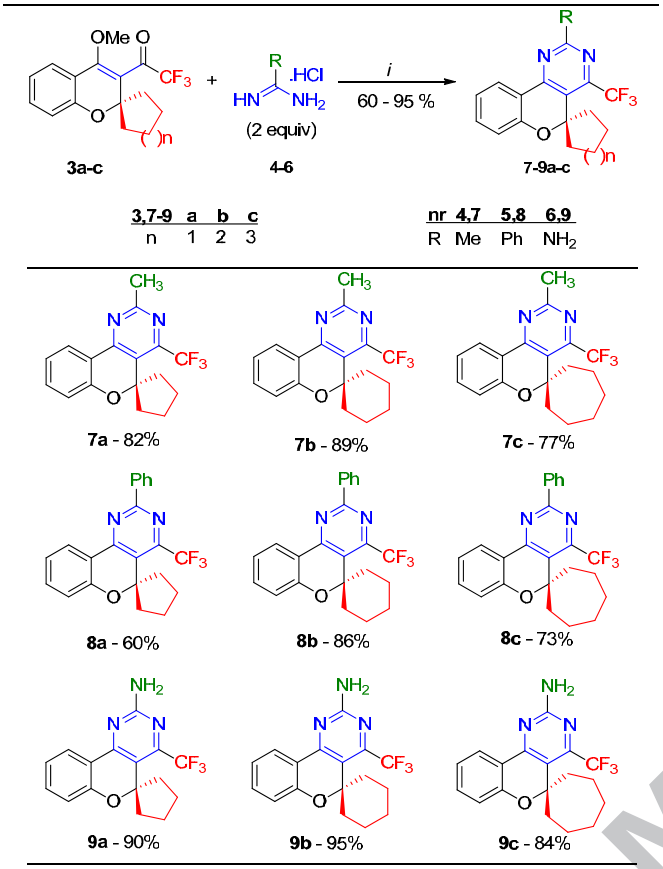
Scheme 1. Synthetic route to the chromanones **2a-c** and the tricyclic ethanones **3a-c**

Prior to use for the next reaction step, compounds **3a–c** were fully characterized by the NMR and GC-MS techniques, as well as by comparison with data from the literature.[24]

In order to obtain a novel series of 4-(trifluoromethyl)spiro[chromeno[4,3-*d*]pyrimidines-5,1'-cycloalkanes] (**7a–c**, **8a–c**, and **9a–c**), we firstly tried to find the best reaction conditions. We initially selected the reaction involving the guanidine hydrochloride (**6**) and 2,2,2-trifluoro-1-[4-methoxy-spiro(2*H*-chromen-2,1'-cyclohexane)-3-yl]ethanone (**3b**), in order to obtain the spirotetracyclic pyrimidine **9b** — see Table 1. The reaction showed poor results when using green solvents such as methanol and ethanol (entries 1 and 2). Unfortunately, in water the formation of the product was not observed (entry 3), but when acetonitrile was used as solvent, a better result was achieved, which led to product **9b** at a 48 % yield (entry 4). It was also determined that the presence of basic medium for neutralizing the hydrochloric acid and releasing the guanidine is very important for this reaction, because in the absence of a base, the formation of product **9b** was not observed (entry 5).

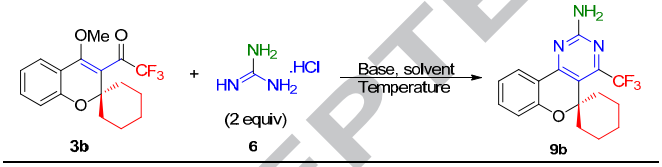
Among the organic and inorganic bases tested, we found that sodium hydroxide was the best alkaline medium (entries 6–9). Likewise, variations of the molar ratio, from 1:1:1 to 1:2.5:2.5, were tested for the ethanone **3b,** guanidine hydrochloride (**6**), and NaOH, respectively, and we concluded that the best molar ratio was 1:2:2 of (entries 10–12). Thus, it was found that the best reaction condition to obtain the desired product **9b** employed **3b**:**6**:NaOH at a molar ratio of 1:2:2, in refluxing MeCN (82 °C) for 24 h (Table 1, entry 11, 95 % yield).

Table 1. Optimization of the reaction conditions[a] for the synthesis of compound **9b**.



[a] Reactional conditions: (*i*) Ketones **3a-c** (1 mmol), amidine salt **4-6** (2 mmol), NaOH (2 mmol), MeCN, 82 °C, 24 h. [b] Isolated yields.

Scheme 2. Synthetic route to 4-(trifluoromethyl)spiro[chromeno[4,3-*d*]pyrimidine-5,1'-cycloalkanes] (**7-9a–c**).



Entry	Solvent (15 mL)	Base	Temp (°C)	Yield (%) (**9b**)[f]
1	MeOH	KOH	64	28
2	EtOH	KOH	78	34
3	H_2O	KOH	100	-[e]
4	MeCN	KOH	82	48
5	MeCN	-	82	-[e]
6	MeCN	NaOH	82	56
7	MeCN	Na_2CO_3	82	-[e]
8	MeCN	K_2CO_3	82	-[e]
9	MeCN	Et_3N	82	-[e]
10[b]	MeCN	NaOH	82	70
11[c]	MeCN	NaOH	82	95
12[d]	MeCN	NaOH	82	93

[a] Standard reaction condition: **3b** (1.0 mmol), **6** (1.0 mmol), base (1 mmol), for 24 h.
[b] Reaction condition: **3b** (1.0 mmol), amidine **6** (1.5 mmol), NaOH (1.5 mmol).
[c] Reaction condition: **3b** (1.0 mmol), amidine **6** (2 mmol), NaOH (2 mmol).
[d] Reaction condition: **3b** (1.0 mmol), amidine **6** (2.5 mmol), NaOH (2.5 mmol).
[e] Recovery of starting materials.
[f] Yield of isolated product.

Subsequently, in an attempt to include alkyl, aryl, amino, thiomethyl, etc. at the 2-position of the pyrimidine ring; and five-, six-, and seven-membered spirocarbocyclic moieties at the 5-position, we tried to expand the reaction scope, by applying the optimal reaction condition determined for the synthesis of **9b** to the reaction involving ethanones **3a–c** and some other nitrogenated 1,3-dinucleophiles.

Thus, the novel series of 4-(trifluoromethyl)spiro[chromeno[4,3-*d*]pyrimidine-5,1'-cycloalkanes] (**7a–c**, **8a–c**, and **9a–c**) was successfully synthesized from the [3+3] cyclocondensation reactions of 2,2,2-trifluoro-1-[4-methoxy-spiro(2*H*-chromen-2,1'-cycloalkan)-3-yl]ethanones **3a–c** with three substituted amidine chlorides — acetamidine (**4**), benzamidine (**5**), and guanidine (**6**) — as shown in Scheme 2. When the reactions were performed in refluxing acetonitrile at 82 °C for 24 h — in accordance with the optimal reaction condition developed for **9b** and with the assistance of TLC monitoring — yields in the range of 60–95 % were obtained. The best results were also obtained when the solutions of **3a–c** diluted in acetonitrile were added dropwise to the mixtures of amidines **4–6** and sodium hydroxide (powder) — the latter was dissolved beforehand in acetonitrile at room temperature.

Curiously, when similar reaction conditions were applied to other amidines, such as formamidine, 2-methyl-2-thiopseudourea, aminoguanidine, nitroguanidine, urea, and thiourea, no reactions took place. The negative results for the reactions using urea and thiourea were expected because these dinucleophiles are highly electronically deactivated. However, up until now we have not been able to explain the unsuccessful results for the reactions involving aminoguanidine and formamidine with a view to isolating the 4-(trifluoromethyl)spiro[chromeno[4,3-*d*]pyrimidine-5,1'-cycloalkanes].

The structures of the novel 4-(trifluoromethyl)spiro[chromeno[4,3-*d*]pyrimidine-5,1'-cycloalkanes] (**7a–c**, **8a–c**, and **9a–c**) were deduced from: ^{1}H- and ^{13}C-NMR experiments (solvent $CDCl_3$), by comparison with the NMR data of other previously synthesized chromeno[4,3-*d*]pyrimidines,[1–6] and from the mass spectrometry analysis (GC-MS and HRMS). Their purity was confirmed by CHN elemental analysis. Complementarily, 2-amino-4-(trifluoromethyl)-spiro[chromeno[4,3-*d*]pyrimidine-5,1'-cycloheptane] (**9c**) had its structure confirmed by single crystal X-ray diffraction, as shown in Figure 2. The ORTEP clearly shows a tetracyclic structure with the presence of an amino group, a trifluoromethyl substituent, and a spirocycloheptane moiety at the 2-, 4- and 5-position, respectively.

*General synthesis of 2-(1H-pyrrol-1-yl)-4-(trifluoromethyl)spiro[chromeno[4,3-d]pyrimidine-5,1'-cycloalkanes] (**10a–c**):* Five-membered heterocycles, such as pyrroles, are part of the structural unit of many natural compounds,[28] essential drugs,[29] and pharmacologically valuable synthetic substances with antibacterial,[30] antifungal,[31] antioxidant,[32] antitumor, and anti-HIV activity,[33] and they are widely used in the pharmaceutical industry[34] and in functional materials.[35]

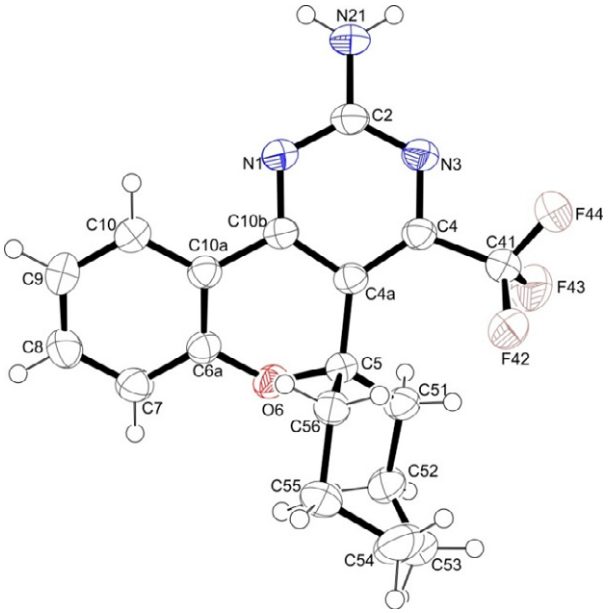


Figure 2. ORTEP of the crystal structure of 2-amino-4-(trifluoromethyl)-spiro[chromeno[4,3-*d*]pyrimidine-5,1'-cycloheptane] (**9c**) (CCDC 1420775).[27] Displacement ellipsoids are drawn at the 50 % probability level.

Thus, we sought to promote further functionalization of the 2-amino-4-(trifluoromethyl)-spiro[chromeno[4,3-*d*]pyrimidine-5,1'-cycloalkanes] **9a–c** by a Clauson-Kaas reaction with 2,5-dimethoxytetrahydrofuran in an acid medium.[36] Notwithstanding the achievement of the desired 2-(1*H*-pyrrol-1-yl)-4-(trifluoromethyl)spiro[chromeno[4,3-*d*]pyrimidine-5,1'-cycloalkanes] (**10a–c**), we were pleased to observe that the one-pot procedure provided a better yield than the two-step protocol for compounds **10b** and **10c**, in addition to not requiring a purification step (Scheme 3).

Effect of treatments on capsaicin-induced nociception:

The capsaicin is a lipophillic vanilloid compound that renders "hot" chili peppers pungent. It binds to specific vanilloid receptors on the peripheral terminals of nociceptive neurons, causing pain.[41] The capsaicin test represents an appropriate and simple nociception model, because the application of capsaicin in humans and in experimental animals evokes intense pain.[37] In the present research, the intraplantar administering of capsaicin led to paw licking, which was characterized as spontaneous nociception. The treatment with the new 4-(trifluoromethyl)spiro[chromeno[4,3-*d*]pyrimidine-5,1'-cycloalkanes] (**8a–c**, **9a–c**, and **10a–c**; 100 mg/kg, p.o.) or Ketoprofen (100 mg/kg, p.o.) was able to prevent capsaicin-induced nociception at 1 h after their administering, when compared to animals treated with the vehicle (Figure 3). The Ketoprofen had a maximum antinociceptive effect of 80±3%; while compound **10b**, which had a maximum inhibition of 83±5%, was more effective in preventing spontaneous nociception than the other compounds; therefore, it was chosen for the execution of subsequent tests. These results support the peripheral antinociceptive action of the compounds tested. Unfortunately, due to the low solubility in the vehicle used, compounds **7a–c** could not be tested.

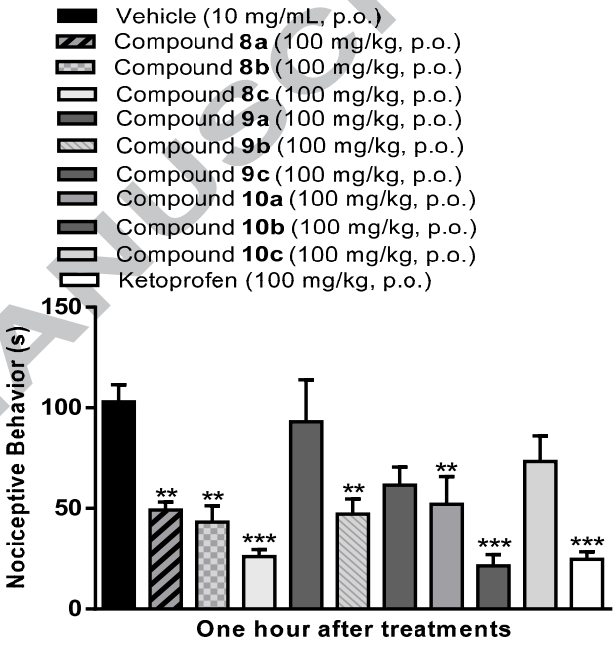


Figure 3. Antinociceptive effect of pyrimidine compounds or Ketoprofen on the spontaneous nociception induced by intraplantar capsaicin administering (1 nmol/paw, i.pl.). The antinociceptive effect was evaluated at 1 h after treatment with: the vehicle (10 mL/kg, p.o.), pyrimidine compounds (100 mg/kg, p.o.), or Ketoprofen (100 mg/kg, p.o.). **P<0.01; ***P<0.001 when compared to the vehicle group; one-way ANOVA followed by the Bonferroni post hoc test. Data are expressed as the mean + SEM (n = 5–8 per group).

Effects of treatments on an arthritic pain model:

Sensory alterations are the most debilitating effects in inflammatory processes; for example, pain during movement or stimulation, which has been one of the main motivations in patients seeking medical care.[18] In order to investigate the antinociceptive effect of compound **10b** and Ketoprofen in a clinically relevant pain model, the animals were subjected to intraplantar CFA injections, in an arthritic pain model with some similarities to forms of arthritis in humans.[38] Since the TRPV1 receptor is involved in arthritic pain and the compound **10b** was effective in reducing nociception capsaicin-induced, a TRPV1 agonist, we tested its effect on a CFA-induced arthritic pain model.[42] The animals that received intraplantar CFA injections showed a decrease in paw withdrawal threshold at 48 h after the administering (baseline 2; Time 0) in comparison with the baseline values (baseline 1, B — before CFA injection; Figure

4A), which were considered to be mechanical allodynia. The treatment of animals with compound **10b** (100 mg/kg, p.o.) and Ketoprofen (100 mg/kg, p.o.) led to reductions in the CFA-induced mechanical allodynia of up to 68±5% and 65±5%, respectively, at 2 h after administering, when compared to animals treated with the vehicle. Ketoprofen reduced the mechanical allodynia between 0.5 and 4 h after being administered, while compound **10b** reduced the mechanical allodynia between 0.5 and 6 h after being administered (Figure 4A).

Given that the best nociception inhibition was observed at 2 h, this time was chosen for the analysis of the dose-response curve (30–300 mg/kg, p.o.) in the CFA-induced arthritic pain model. The anti-allodynic effect of post-treatment with compound **10b** and Ketoprofen occurred at doses of 100 and 300 mg/kg, respectively. The calculated inhibitory dose (ID_{50}) value for compound **10b** was 422.3 (113–1577) mg/kg, with maximum inhibition of 66±8% (300 mg/kg); while for the Ketoprofen it was 169.2 (95–301) mg/kg, with maximum inhibition of 75±11% (300 mg/kg), at 2 h after the treatments (Fig. 4B and 4C). Previous studies have shown that in rats and mice, non-steroidal anti-inflammatory drugs (NSAID), such as Ketoprofen, reduce the development of allodynia induced by the injection of CFA.[39,40] In the present study, compound **10b** seemed to be as effective as the Ketoprofen against CFA-induced arthritic pain in mice.

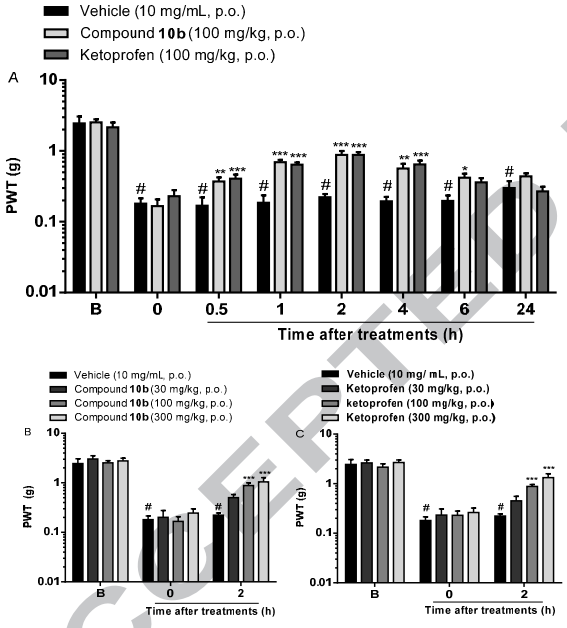


Figure 4. A) Time-response curve for the antinociceptive effect of the vehicle (10 mL/kg, p.o.), compound **10b** (100 mg/kg, p.o.), and Ketoprofen (100 mg/kg, p.o.) on CFA-induced mechanical allodynia, in mice evaluated between 0.5 and 24 h after treatments; and (B) and (C): Dose-response curves for the antinociceptive effect of the vehicle (10 mL/kg, p.o.), compound **10b** (30–300 mg/kg, p.o.), and Ketoprofen (30–300 mg/kg, p.o.) on CFA-induced mechanical allodynia, at 2 h after treatment. Point B represents the mechanical allodynia measured before the CFA injection; while point 0 represents the mechanical allodynia measured immediately before drug treatment and 48 h after CFA injection. $^{\#}P < 0.001$ when compared to the baseline threshold (baseline 1; B); one-way ANOVA followed by the Bonferroni post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ when compared with the vehicle group; two-way ANOVA followed by the Bonferroni post hoc test. Data represent the mean + SEM (n = 6–7 per group).

In conclusion, we successfully developed an attractive strategy for the direct assembly of the new 4-(trifluoromethyl)spiro[chromeno[4,3-*d*]pyrimidines-5,1'-cycloalkane] derivatives **7a–c**, **8a–c**, and **9a–c**, via [3+3] cyclocondensation reactions between 2,2,2-trifluoro-1-(4-methoxyspiro[chromene-2,1'-cycloalkane]-3-yl)ethanones (**3a–c**) and different amidine chlorides (**4–6**), which furnished yields of 60–95 %. Furthermore, we demonstrated the use of the 2-amino-substituted 4-(trifluoromethyl)spiro[chromeno[4,3-*d*]pyrimidines-5,1'-cycloalkanes] (**9**) in *N*-derivatization reactions for the synthesis of the 2-pyrrolyl derivatives (**10**), by employing 2,5-dimethoxytetrahydrofuran in acetic acid medium (Clauson-Kaas reaction) and two different methods — the first method involved two steps and furnished overall yields of 38–49 %, while the second one-pot method had a yield of 40–65 % and was the most advantageous considering the experimental features.

We also showed that most of the tested compounds (**8a–c**, **9a–c**, and **10a–c**, but not **7a–c**) and Ketoprofen had an antinociceptive effect in the capsaicin test, most notably compound **10b**. Moreover, compound **10b** has an anti-allodynic effect similar to Ketoprofen in an arthritic pain model, and it does not cause any changes in the locomotor activity (data not shown). Although the efficacy of compound **10b** is not greater than the efficacy of Ketoprofen, it could be used for the treatment of pathological pain such as is arthritis.

Acknowledgments

The authors thank: the Coordination for Improvement of Higher Education Personnel (CAPES) for the fellowships, and the National Council for Scientific and Technological Development (CNPq) for the financial support (Process numbers 306.883/2015-5 and 471.269/2013-1-CNPq/Universal).

Supplementary Material

Supplementary material for the review process was prepared and provided as a separate electronic file.

References and notes

1. Bruno, O.; Brullo, C.; Schenone, S.; Ranise, A.; Bondavalli, F.; Barocelli, E.; Tognolini, M.; Magnanini, F.; Ballabeni, V. *Il Farmaco* **2002**, *57*, 753.
2. Bruno, O.; Brullo, C.; Schenone, S.; Bondavalli, F.; Raise, A.; Tognolini, M.; Ballabeni, V.; Barocelli, E. *Bioorg. Med. Chem.* **2004**, *12*, 553.
3. Bruno, O.; Schenone, S.; Ranise, A.; Bondavalli, F.; Barocelli, E.; Ballabeni, V.; Chiavarini, M.; Bertoni, S.; Tognolini, M.; Impicciatore, M. *Bioorg. Med. Chem.* **2001**, *9*, 629.
4. Bruno, O.; Brullo, C.; Bondavalli, F.; Ranise, A.; Schenone, S.; Tognolini, M.; Ballabeni, V.; Barocelli, E. *Med. Chem.* **2007**, *3*, 127.
5. Li, D.; Duan, S.; Hu, Y. *J. Comb. Chem.* **2010,** *12,* 895.
6. Hegab, M. I.; Abdulla, M. M. *Arch. Pharm. Chem. Life Sci.* **2006**, *339*, 41.
7. Pradhan, R.; Patra, M.; Behera, A. K.; Mishra, B. K.; Behera,R. K. *Tetrahedron*, **2006**, *62*, 779.
8. Singh, S. G.; Desta, Y. Z. *Chem. Rev.* **2012**, *112*, 6104.
9. Kaur, K.; Kumar, V.; Gupta, G. K. *J. Fluorine Chem.* **2015**, *178,* 306.

10. Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. *Chem. Soc. Rev.* **2008**, *37*, 320.
11. Zhou, Y.; Wang, J.; Gu, Z.; Wang, S.; Zhu, W.; Acenã, J. L; Soloshonok, V. A; Izawa, K.; Liu, H. *Chem. Rev.* **2016**, *116*, 422.
12. Bonacorso, H. G; Rosa, W. C.; Oliveira, S. M.; Brusco, I.; Dalla Pozza, C. C.; Nogara, P. A.; Wiethan, C. W.; Rodrigues, M. B.; Frizzo, C. P.; Zanatta, N. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 4808.
13. Bonacorso, H. G.; Rosa, W. C.; Oliveira, S. M.; Brusco, I.; Pozza, C. C. D.; Frizzo, C. P.; Zanatta, N. BR Patent Deposited on National Institute for Industrial Property INPI (BR 10 2015 020925 8) in Agreement to Federal University of Santa Maria, 2015.
14. Lobo, M. M.; Oliveira, S. M.; Brusco, I.; Machado, P.; Timmers, L. S. M.; Souza, O. N.; Martins, M. A. P.; Bonacorso, H. G.; Santos, J. M.; Canova, B.; Silva, T. V. F.; Zanatta, N. *Eur. J. Med. Chem.* **2015**, *102*, 143.
15. Sauzem, P. D.; Machado, P.; Rubin, M. R.; Sant'Anna, G. S.; Faber, H. B.; Souza, A. H.; Mello, C. F.; Beck, P.; Burrow, R. A.; Bonacorso, H. G.; Zanatta, N.; Martins, M. A. P. *Eur. J. Med. Chem.* **2008**, *43*, 1237.
16. Lobo, M. M.; Viau, C. M.; dos Santos, J. M.; Bonacorso, H. G.; Martins, M. A. P.; Amaral, S. S.; Saffi, J.; Zanatta, N. *Eur. J. Med. Chem.* **2015**, *101*, 836.
17. Woller, S. A.; Hook, M. A. *Exp. Neurol.* **2013**, *247*, 328.
18. Simons, L.; Elman, I.; Borsook, D. *Neurosci. Biobehav. Rev.* **2014**, *39*, 61.
19. McDougall, J. J. *Arthritis Res. Ther.* **2006**, *8*, 220.
20. Cazacu, I.; Mogosan, C.; Loghin, F. *Clujul Med.* **2015**, *88*, 128.
21. Oliveira, S. M.; Silva, C. R.; Wentz, A. P.; Paim, G. R.; Correa, M. S.; Bonacorso, H. G.; Prudente, A. S.; Otuki, M. F.; Ferreira, J. *Pharmacol. Biochem. Behav.* **2014**, *124*, 396.
22. Hogan, Q. *Reg. Anesth. Pain. Med.* **2002**, *27*, 385.
23. Nagakura, Y.; Okada, M.; Kohara, A.; Kiso, T.; Toya, T.; Iwai, A. *J. Pharmacol. Exp. Ther.* **2003**, *306*, 490.
24. Bonacorso, H. G.; Garcia, F. D.; Belo, C. B.; Tier, A. Z.; Frizzo, C. P.; Martins, M. A. P.; Zanatta, N. *J. Fluorine Chem.* **2014**, *166*, 44.
25. Bonacorso, H. G.; Silva, L. B.; Rocha, J. B. T.; Nogara, P. A.; Waczuk, E. P.; D'A. Silva, F.; Bueno, D. C.; Kader, Y. N. A. M.; Martins M. A. P.; Zanatta, N. *Tetrahedron Lett.* **2015**, *56*, 7024.
26. Kabbe, H. J. *Synthesis* **1978**, 886.
27. The crystallographic data for the structure of **9c** have been deposited with the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 1420775. Copies of the data can be obtained free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax:+441223336033 or deposit@ccdc.com.ac.uk).
28. Young, I. S.; Thornton, P. D.; Thompson, A. *Nat. Prod. Rep.* **2010**, *27*, 1801.
29. Chen, G.; Wang, H. F.; Pei, Y. H. J. *Asian Nat. Prod. Res.* **2013**, *16*, 105.
30. Al-Mourabit, A.; Zancanella, M. A.; Tilvi, S.; Romo, D. *Nat. Prod. Rep.* **2011**, *28*, 1229.
31. Wang, M. Z.; Xu, H.; Liu, T. W.; Feng, Q.; Yu, S. J.; Wang, S. H.; Li, Z. M. *Eur. J. Med. Chem.* **2011**, *46*, 1463.
32. Wang, D.; Hu, X.; Zhao, G. *Int. J. Food Sci. Technol.* **2008**, *43*, 1880.
33. Boonlarppradab, C.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Org. Lett.* **2008**, *10*, 5505.
34. Walsh, C. T.; Garneau-Tsodikova, S.; Howard- Jones, A. *Nat. Prod. Rep.* **2006**, *23*, 517.
35. Langford, R. M. *Clin. Rheumatol.* **2006**, *25*, S2.
36. Clauson-Kaas, N.; Zdenˇk, T. *Acta Chem. Scand.* **1952**, *6*, 667.
37. Diniz, D. M.; Souza, A. H.; Pereira, E. M. R.; Silva, J. F.; Rigo, F. K.; Romano-Silva, M. A.; Binda, N.; Castro, C. J.; Cordeiro, M. N.; Ferreira, J.; Gomez, M. V. *Pharmacol. Biochem. Behav.* **2014**, *126*, 97.
38. Billiau, A.; Matthys. P. *Cytokine Growth Factor Rev.* **2011**, *22*, 339.
39. Lee, J. Y.; Kim, J. H.; Kang, S. S.; Bae, C. S.; Choi, S. H. *Am. J. Chin. Med.* **2004**, *32*, 521.
40. Solankar, A. K.; Jagtap, A. G.; *Indian J. Exp. Biol.* **2005**, *43*, 46.
41. Lowin, T.; Apitz, M.; Anders, S.; Straub, R. H.; *Arthritis Res. Ther.* **2015**, *17*, 321.
42. Caterina, M. J.; Leffler, A.; Malmberg, A. B.; Martin, W. J.; Trafton, J.; Petersen-Zeitz, K. R.; Koltzenburg, M.; Basbaum, A. I.; Julius, D. *Science* **2000,** *288*, 306.