Bioorganic & Medicinal Chemistry 17 (2009) 6517–6525

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and analgesic profile of conformationally constrained *N*-acylhydrazone analogues: Discovery of novel *N*-arylideneamino quinazolin-4(3*H*)-one compounds derived from natural safrole

Rodolfo C. Maia [a,b], Leandro L. Silva [a,c], Eduardo F. Mazzeu [a], Milla M. Fumian [a,c], Claudia M. de Rezende [b], Antonio C. Doriguetto [d], Rodrigo S. Corrêa [d,e], Ana Luisa P. Miranda [a,c], Eliezer J. Barreiro [a,b,c], Carlos Alberto Manssour Fraga [a,b,c,*]

[a] *LASSBio—Laboratório de Avaliação e Síntese de Substâncias Bioativas, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, PO Box 68023, ZIP 21941902 Rio de Janeiro, RJ, Brazil*
[b] *Programa de Pós-Graduação em Química, Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil*
[c] *Programa de Pós-Graduação em Farmacologia e Química Medicinal, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil*
[d] *Laboratório de Cristalografia, Departamento de Ciências Exatas, Universidade Federal de Alfenas (UNIFAL), ZIP 37130-000 Alfenas, MG, Brazil*
[e] *Instituto de Física de São Carlos, Universidade de São Paulo, 13560-970 São Carlos, SP, Brazil*

ARTICLE INFO

Article history:
Received 8 June 2009
Revised 3 August 2009
Accepted 5 August 2009
Available online 9 August 2009

Keywords:
N-Acylhydrazone
Quinazolin-4(3*H*)-one
Analgesic
Conformational restriction
Safrole
1,3-Benzodioxole

ABSTRACT

In this work we reported the synthesis and evaluation of the analgesic, anti-inflammatory, and platelet anti-aggregating properties of new 3-(arylideneamino)-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-one derivatives (**3a–j**), designed as conformationally constrained analogues of analgesic 1,3-benzodioxolyl-*N*-acylhydrazones (**1**) previously developed at LASSBio. Target compounds were synthesized in very good yields exploiting abundant Brazilian natural product safrole (**2**) as starting material. The pharmacological assays lead us to identify compounds LASSBio-1240 (**3b**) and LASSBio-1272 (**3d**) as new analgesic prototypes, presenting an antinociceptive profile more potent and effective than dipyrone and indomethacin used, respectively, as standards in AcOH-induced abdominal constrictions assay and in the formalin test. These results confirmed the success in the exploitation of conformation restriction strategy for identification of novel cyclic *N*-acylhydrazone analogues with optimized analgesic profile.

1. Introduction

The *N*-acylhydrazone (NAH) subunit has been described[1,2] as the pharmacophoric framework of several anti-inflammatory,[3] analgesic,[4–6] antiplatelet,[7–9] cardioactive,[10,11] and trypanocyde derivatives,[12] among others. Its importance and recurrent appearance in bioactive substances from different therapeutic classes has indicating the privileged status of the NAH moiety.[13]

The construction of NAH derivatives[4] (**1**) bearing the 1,3-benzodioxole ring derived from natural safrole[14] (**2**) lead us to found out novel orally active prototypes presenting remarkable analgesic profile when evaluated as inhibitor of AcOH-induced constrictions in mice, represented by phenyl derivative LASSBio-123 (**1a**) and *N*,*N*-dimethylaminophenyl derivative LASSBio-125 (**1b**), which showed ID_{50} of 6.9 and 95.5 μmol/kg, respectively. On the other hand, the thiophene-containing isoster LASSBio-294 (**1c**) and their corresponding imine-attached methyl analogue LASSBio-1029 (**1d**) presented also potent analgesic activity, that are, 8.1 and 5.9 μmol/kg, respectively, in the same pharmacological model (Fig. 1).

Trying to achieve the optimization of the analgesic profile presented by the *N*-acylhydrazone prototypes (**1a–d**), we planned the novel series of quinazolin-4(3*H*)-one derivatives (**3a–j**) using the strategy of conformational restriction[15] as tool for molecular modification and design (Fig. 1). We first promoted a cyclization between the position 6 of the 1,3-benzodioxole ring and the amidic nitrogen of the *NAH* function of (**1**), restricting the rotation of bonds A and B. On the other hand, we have also introduced a methyl group in the position 2 of the new quinazolin-4(3*H*)-one ring in order to restrict the free rotation of the single bond C, favoring the stabilization of the *s-cis* conformer (Fig. 1) by ca. 40 kcal/mol.[16] So, in addition to the quinazolin-4(3*H*)-one derivatives (**3a–d**), analogues to the corresponding *N*-acylhydrazones (**1a–d**), we have exploited the bioisosterism concept[17] for designing the structurally related carba-analogue derivative (**3e**) and the isosteric imine-attached heterocyclic derivatives (**3f–j**) (Fig. 1).

* Corresponding author. Tel.: +55 21 25626503; fax: +55 21 25626644.
E-mail address: cmfraga@pharma.ufrj.br (C.A.M. Fraga).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.08.009

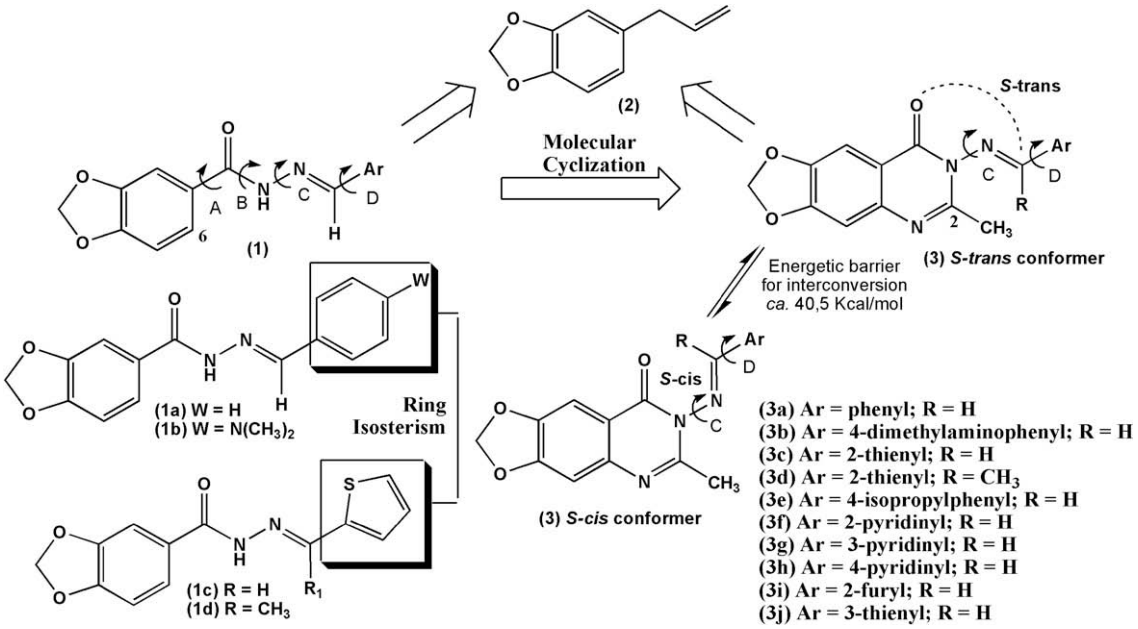


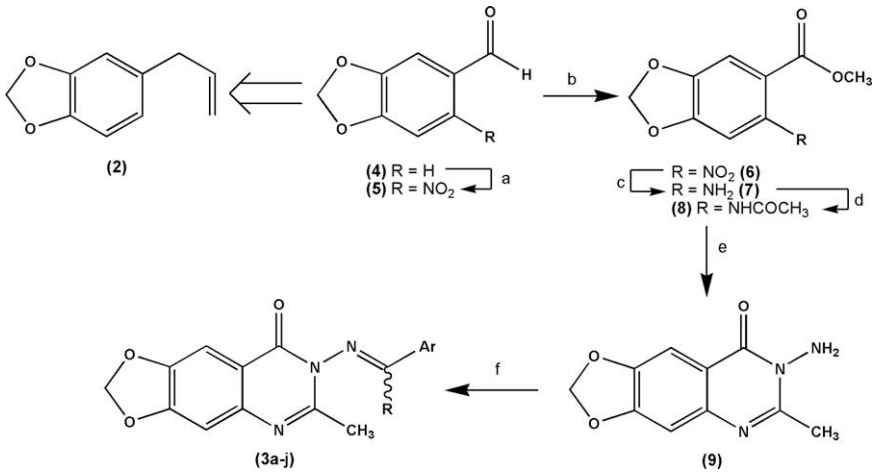
Figure 1. Design concept of new 3-(arylideneamino)-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-one derivatives (**3a**–**j**).

2. Results and discussion

2.1. Chemistry

The synthetic route used to construct the target compounds (**3a–j**) is depicted in Scheme 1. The starting material was piperonal (**4**) obtained in ca. 75% overall yield from isomerization and oxidative cleavage of the double bond of safrole (**2**),[18,19] an abundant Brazilian natural product.[14] Regioselective nitration of position 6 of the 1,3-bezondioxole ring of (**4**),[20] by using concentrated nitric acid, afforded the nitro-aldehyde derivative (**5**) in 95% yield, which was converted to the corresponding methyl ester (**6**) through the exploitation of oxidative Yamada's procedure.[21] Next, after the chemoselective reduction of nitro group of **(6)** by treatment with Fe^{o} and NH_4Cl in ethanol at reflux,[22] the obtained amino-ester derivative (**7**) was acylated by using acetic anhydride in ethanol under reflux,[23] yielding compound (**8**) in 85% yield.

The key quinazolin-4(3*H*)-one intermediate (**9**) was obtained in 90% yield by treatment of an ethanolic solution of the ester (**8**) with hydrazine hydrate at reflux.[23] The 'one-pot' formation of desired six-member heterocyclic ring of (**9**) was unambiguously confirmed by X-ray analysis (Fig. 2). The molecule is almost flat considering the non-H atoms (rms deviation = 0.0290 Å). The largest deviation from the least-squares plane through the all non-hydrogenous atoms occurs for atom N1 [displacement = 0.076(1) Å]. The positional parameters of the two H atoms connected to the amine N atom were not constrained during the refinements performed here permitting to determine the amine geometry. As expected for $-NH_2$ groups, our experimental data show that the amine H atoms are in an off-plane position. It was also shown that the position of the amine H atoms is a result of crystal packing forces or intermolecular bonding motifs. That means, intermolecular hydrogen bonds as described below. In the solid state, the compound (**9**) exhibits a strong hydrogen bond involving N1–H1b···O1, in which



Scheme 1. Synthesis of 3-(arylideneamino)-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-one derivatives (**3a**–**j**). Reagents and conditions: (a) concd HNO_3, 20–25 °C, 30 min, 95%; (b) I_2, KOH, MeOH, 0 °C, 1.5 h, 84%; (c) Fe, NH_4Cl, EtOH:H_2O, reflux, 2 h, 75%; (d) Ac_2O, EtOH, reflux, 1 h, 85%; (e) $NH_2NH_2{\cdot}H_2O$ 98%, EtOH, reflux, 1.5 h, 90%; (f) ArCHO EtOH, $HCl_{cat.}$, rt, 0.5–1 h or $ArCOCH_3$, EtOH, $HCl_{cat.}$, 50–60 °C, 96 h.

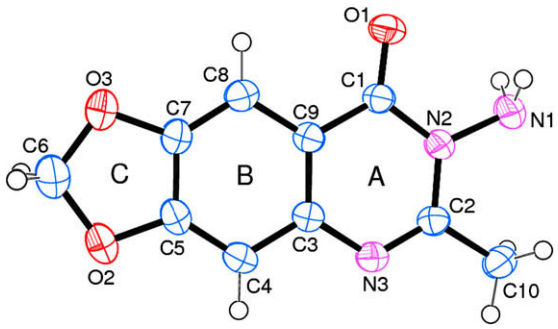


Figure 2. The structure of key 3-amino-6,7-methylenedioxy-quinazolin-4(3*H*)-one intermediate (**9**), showing the atom-numbering scheme. Displacement ellipsoids are drawn at the 50% probability level and the hydrogen atoms are represented as spheres of arbitrary radius.

forms a centrosymmetric dimer. Another classical hydrogen bond that also contributes for packing stabilization occurs between N1–H1a···N3. In this structure the non-classical hydrogen bonds [C8–H8···O2, C6–H6a···O1, and C6–H6b···O1] link the molecules of (**9**) to form sheets parallel to the (−3 0 4) plane. In addition, a π-stacking interaction plays an important role in the assembly. In this hydrophobic contact, the rings A of (**9**) are stacked along to the *c* axis, forming an infinite one-dimensional chain. All information concerning the intra- and intermolecular geometry is given in the Supplementary data.

Finally, the desired quinazolin-4(3*H*)-one compounds (**3a–c, 3e–j**) were obtained, in good yields (81–98%), by condensing intermediate **9** with the corresponding aromatic aldehydes (ArCHO) in ethanol at room temperature, using hydrochloric acid as catalyst.[4] On the other hand, compound **3d** was obtained through the condensation of **9** with 2-acetylthiophene under the same conditions except for the higher temperatures (50–60 °C) and longer time of reaction (96 h) needed to promote the full conversion of the starting material into the desired product (Table 1).

The next step of this work was to determine the relative configuration of the imine double bond in the novel quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivatives (**3a–j**), in order to assure the diastereomeric ratio, essential to the complete understanding of the biological results. The analysis of the ^{1}H NMR spectra of **3a–j**, allowed us to detect the presence of only one signal correspondent to the imine hydrogen (Table 1), demonstrating the diastereoselective profile of the last step of our synthetic route. The characterization of the relative configuration of target quinazolinon-4-one derivatives (**3**) exploited mass spectrometry as auxiliary tool, guided by the previous results from Pereira and co-workers[24] demonstrating that (*E*)-diastereomer of *N*-acylhydrazone derivatives presented a fragment in the mass spectrum referent to the McLafferty rearrangement[25] with high relative abundance, in contrast to the modest relative abundance (ca. 1%) observed for the corresponding (*Z*)-diastereomer, due to the unfavorable orientation between the carbonyl oxygen atom and the γ-hydrogen (Fig. 3). Considering that 3-arylideneamino quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivatives (**3**) have the *N*-acylhydrazone moiety internalized in the azaheterocyclic ring we assumed that they presented (*E*) configuration because of the high relative abundance evidenced for the peak with *m*/*z* 204.1, resultant from McLafferty rearrangement of representative isosteric derivatives (**3a**), (**3c**), (**3i**), and (**3j**) (Fig. 3).

2.2. Pharmacology

The evaluation of the analgesic profile of all quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivatives (**3a–j**) was performed using the classical acetic acid-induced mice abdominal constrictions assay,[26] in the screening concentration of 100 μmol/kg po and using dipyrone as standard drug. The results are displayed in Table 2.

Almost all 3-(arylideneamino)-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivatives (**3**) inhibited significantly the constrictions induced by acetic acid in a range from 16.9% to 45.3% (Table 2). Among them, the most active analgesic compounds were LASSBio-1240 (**3b**) and LASSBio-1272 (**3d**), respectively, with 45.3% and 43.0% of inhibition of induced constrictions.

The presence of a basic nitrogen in the aromatic ring attached to the imine subunit showed to be an important feature for the analgesic activity, as could be evidenced by comparison of the analgesic profile of 4-dimethylaminophenyl derivative (**3b**, 43% inhibition) with those displayed by its carbaisostere (**3e**, 32% inhibition) and additionally, corroborating this observation, phenyl derivative (**3a**) is less active than the 4-pyridinyl isostere (**3h**). In this context, observing the pharmacological behavior of derivatives (**3f**–**h**) from isomeric pyridinyl series and 4-dimethylaminophenyl derivative (**3b**), we can notice that the analgesic activity increases as the basic nitrogen is more distant from the quinazolin-4(3*H*)-one moiety, so the order of activity presented is, 4-dimethylaminophenyl (**3b**) **>** 4-pyridinyl (**3h**) > 3-pyridinyl (**3g**) > 2-pyridinyl (**3h**).

On the other hand, the change of the imine-attached hydrogen by a methyl group demonstrated to be very favorable for the analgesic activity, since thienylidene derivative (**3c**) inhibited in 10.7% the AcOH-induced abdominal constrictions while the corresponding methyl analogue (**3d**) presented remarkable antinociceptive profile, that is, 45.3% inhibition in the same bioassay.

Table 1
Yields and physical properties of the 3-(arylideneamino)-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivatives (**3a–j**)

Compound	Molecular formula[a]	Molecular weight	Yield[b] (%)	Melting point[c] (°C)	δ^d (ppm) N=CH
3a	$C_{17}H_{13}N_3O_3$	307.10	87	240–242	9.01
3b	$C_{19}H_{18}N_4O_3$	350.14	81	226–227	8.61
3c	$C_{15}H_{11}N_3O_3S$	313.05	92	238–239	9.23
3d	$C_{16}H_{13}N_3O_3S$	327.07	89	255–256	—
3e	$C_{20}H_{19}N_3O_3$	349.14	98	114–116	8.83
3f	$C_{16}H_{12}N_4O_3$	308.09	88	216–218	9.21
3g	$C_{16}H_{12}N_4O_3$	308.09	97	264–265	9.29
3h	$C_{16}H_{12}N_4O_3$	308.09	94	>270	9.46
3i	$C_{15}H_{11}N_3O_4$	297.07	99	225–226	8.90
3j	$C_{15}H_{11}N_3O_3S$	313.05	94	225–227	8.93

[a] The analytical results for C, H, N, S were within ±0.4% of calculated values.
[b] Isolated yield from the condensation of 3-amino-6,7-methylenedioxy-quinazolin-4(3*H*)-one intermediate (**9**) with the corresponding carbonyl derivative.
[c] Not corrected.
[d] Data obtained from ^{1}H NMR at 200 MHz, using $CDCl_3$ as solvent.

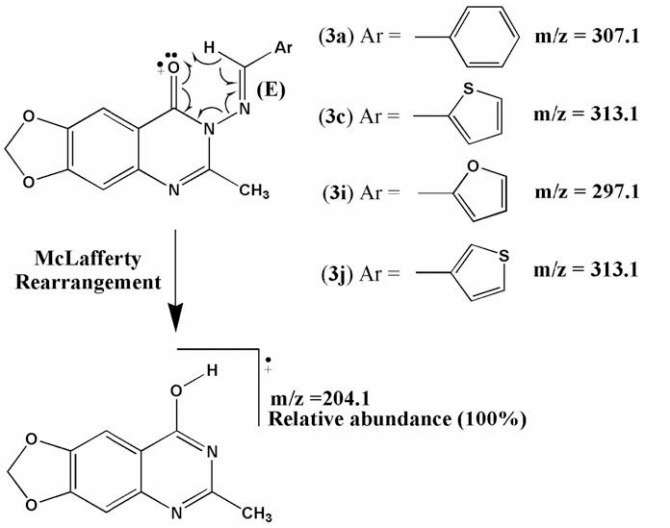


Figure 3. McLafferty rearrangement peak in the mass spectra of (*E*)-3-(arylideneamino)-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivatives (**3**).

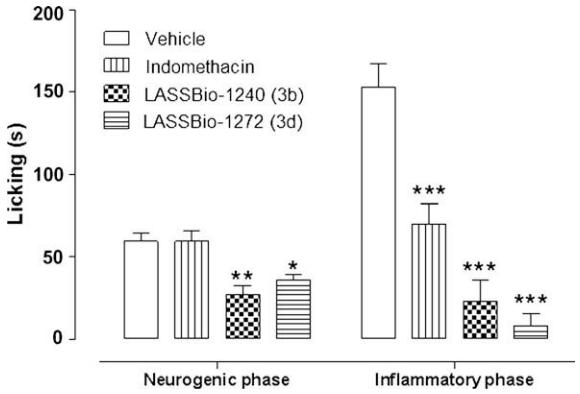


Figure 4. Effect of compounds LASSBio-1240 (**3b**) and LASSBio-1272 (**3d**) against formalin-induced pain in mice after ip administration of a dose of 100 μmol/kg. Each column represents the mean ± SEM. of 6–12 experimental values. **P* <0.05, ***P* <0.01, ****P* <0.001 Two-way ANOVA Bonferroni post-test.

For the two most active quinazolin-4(3*H*)-one compounds, that are, (**3b**) and (**3d**), was determined a dose–response curve to verify the potency and efficacy of these derivatives. LASSBio-1272 (**3d**) presented a maximum effect of 49.8 ± 2.16% inhibition of AcOH-induced constrictions while LASSBio-1240 (**3b**) showed a maximum effect of 41.2 ± 1.94%. Relative to the potency, LASSBio-1240 (**3b**) and LASSBio-1272 (**3d**) presented an ED_{50} = 5.4 and 3.5 μmol/kg, respectively, both higher than dipyrone (ED_{50} = 144.5 μmol/kg) used as standard analgesic drug (Table 2).

Compounds (**3b**) and (**3d**) also had their antinociceptive activity evaluated in the formalin test[27] (Fig. 4), being both able to be active in both phases of this pharmacological model after ip administration of a dose of 100 μmol/kg. However, they inhibited the nociceptive response more expressively in the inflammatory phase than in the neurogenic phase. Both derivatives showed to be more active than indomethacin, used as standard drug, in the two phases of this model.

The analgesic actions on neurogenic phase of formalin test lead us to investigate an eventual CNS-acting profile of compounds LASSBio-1240 (**3b**) and LASSBio-1272 (**3d**) in the hot-plate test (Fig. 5).[28] However, none of these two derivatives was able to increase the latency time of treated animals in this experimental protocol (Fig. 5).

On the other hand, when we performed a modified version of the classical hot-plate method,[29] suitable for measuring inflammatory nociception (hyperalgesia) through the previous treatment of the animals with carrageenan, the analgesic actions of quinazolin-

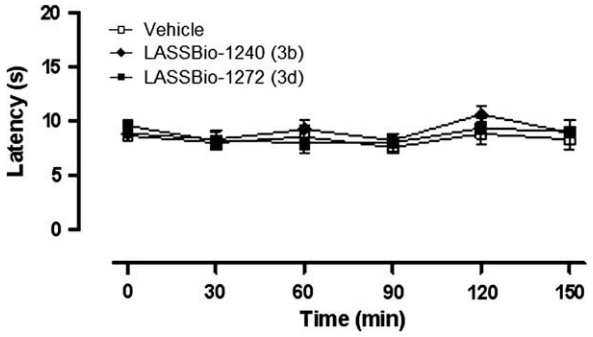


Figure 5. Effect of compounds LASSBio-1240 (**3b**) and LASSBio-1272 (**3d**) in the hot-plate test in mice, at 100 μmol/kg, ip. Each point represents the mean ± SEM of seven experimental values.

Table 2
Analgesic profile of 3-(arylideneamino)-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivatives (**3a–j**) and dipyrone in AcOH-induced abdominal constriction test in mice

Compound	Dose[a] (μmol/kg)	Number of constrictions[b]	*n*[c]	Inhibition[d] (%)	ED_{50} (μmol/kg)
Vehicle	—	64.40 ± 1.25	10	—	—
Dipyrone	100	41.22 ± 3.06	10	36.0*	144.5
3a	100	53.07 ± 1.05	10	17.6*	—
3b	100	36.67 ± 1.65	9	43.0*	5.4
3c	100	57.51 ± 2.07	8	10.7	—
3d	100	35.23 ± 1.81	9	45.3*	3.5
3e	100	43.22 ± 1.66	9	32.9*	—
3f	100	52.44 ± 2.04	9	18.5*	—
3g	100	41.70 ± 1.73	10	35.2*	—
3h	100	39.60 ± 1.98	8	38.5*	—
3i	100	53.52 ± 1.16	10	16.9*	—
3j	100	42.05 ± 1.57	9	34.7*	—

[a] All compounds were administered po.
[b] Results expressed in terms of mean ± SEM.
[c] *n* = number of animals.
[d] % of inhibition obtained by comparison with vehicle control group.
* *P* <0.01 One-way ANOVA (Dunnett's Multiple Comparison Test).

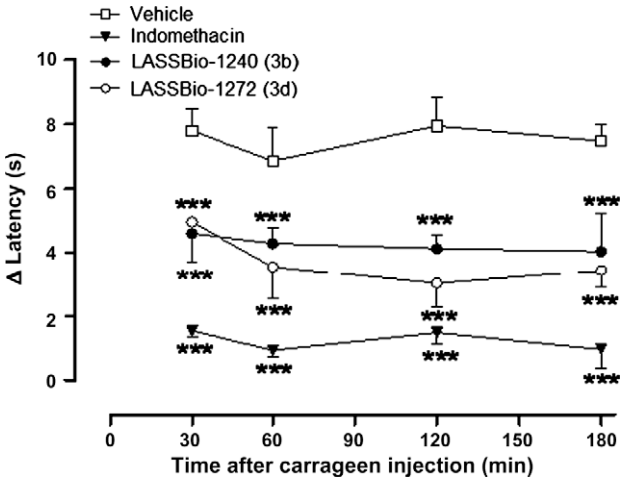


Figure 6. Effect of compounds LASSBio-1240 (**3b**) and LASSBio-1272 (**3d**) against carrageen-induced hyperalgesia in rats, at 100 μmol/kg, ip. Each point represents the mean ± SEM of 8–10 experimental values. ***P <0.001 Two-way ANOVA Bonferroni post-test.

4(3*H*)-onequinazolin-4(3*H*)-one derivatives LASSBio-1240 (**3b**) and LASSBio-1272 (**3d**) could be evidenced (Fig. 6).

In spite to be less effective than standard drug indomethacin, the compounds (**3b**) and (**3d**) showed to be able to reduce in ca. 50% the Δ latency time in comparison with vehicle group (Fig. 6), indicating their ability to control the hyperalgesic stimuli in inflammatory pain models, in agreement with the results obtained from formalin-induced murine hypernociception model.

We therefore turned our attention to the next step in the pharmacological evaluation of the derivatives LASSBio-1240 (**3b**) and LASSBio-1272 (**3d**) which consisted in the investigation of their anti-inflammatory profile by using classical carrageenan-induced rat paw edema (CIRPE) test (Fig. 7).[30] The results obtained from this model showed that the new quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one compounds did not possess a significant anti-inflammatory activity when compared with standard indomethacin, which was able to inhibit the edema formation in ca. 61% (Fig. 7)

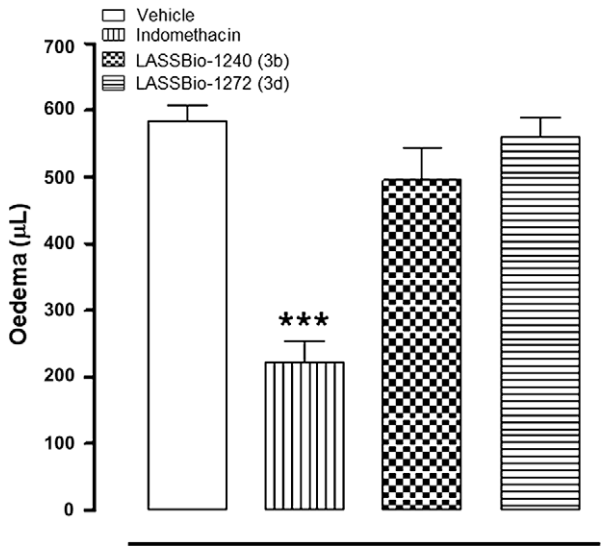


Figure 7. Effect of compounds LASSBio-1240 (**3b**) and LASSBio-1272 (**3d**) against carrageen-induced rat paw edema, at 100 μmol/kg, ip. Each point column the mean ± SEM of 8–10 experimental values. ***P <0.001 One-way ANOVA 'Dunnett's Multiple Comparison Test'.

at the same concentration utilized for (**3b**) and (**3d**), that is, 100 μmol/kg (ip).

Moreover, the new quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivatives (**3a–j**) were also screened in order to evaluate their effects on in vitro rabbit platelet aggregation[31] induced by arachidonic acid (AA, 100 μM). None of the compounds was able to inhibit significantly the aggregation induced by the arachidonic acid. These results indicated, in association with the absence of activity observed in the classical carrageenan-induced rat paw edema assay, that the compounds LASSBio-1240 (**3b**) and LASSBio-1272 (**3d**) do not exert their pharmacological effects through the inhibition of cyclooxigenase enzymes.

3. Conclusions

As concluding remarks, we can establish that the synthetic route used to access the new functionalized quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivatives (**3a–j**) described herein was efficient, furnishing the titled compounds in high overall yields (30–37%) and high diastereoselectivity, as they were obtained in diastereopure (*E*)-form.

The pharmacological assays lead us to identify compounds LASSBio-1240 (**3b**) and LASSBio-1272 (**3d**) as new analgesic prototypes, presenting an antinociceptive profile more potent and effective than the standards used, dipyrone, in AcOH-induced abdominal constrictions assay, and indomethacin, in the formalin test. These results confirmed the success in the exploitation of conformation restriction strategy for identification of novel cyclic *N*-acylhydrazone analogues with optimized analgesic profile. Complementary to this work, we are performing in the laboratory a deeper pharmacological study in order to elucidate the mechanism of action of prototypes LASSBio-1240 (**3b**) and LASSBio-1272 (**3d**), which will be described in due course.

4. Experimental protocols

4.1. Chemistry

Melting points were determined with a Quimis Q340M13 apparatus and are uncorrected. Proton magnetic resonance (^{1}H NMR), unless otherwise stated, was determined in deuterated chloroform containing ca. 1% tetramethylsilane as an internal standard with Bruker AC 200 spectrometer at 200 MHz. Splitting patterns are as follows: s, singlet; d, doublet; t, triplet; q, quartet; qt, quintet; dd, double doublet; br, broad; m, multiplet. Carbon magnetic resonance (^{13}C NMR) was determined in the same spectrometer described above at 50 MHz, using CF_3CO_2D as solvent. Infrared (IR) spectra were obtained with a Bomem FTLA2000 spectrophotometer by using potassium bromide plates. The mass spectra (MS) were obtained by electron impact (70 eV) with a GC/VG Micromass 12 spectrometer.

The progress of all reactions was monitored by thin-layer chromatography performed on aluminum sheets precoated with Silica Gel 60 (HF-254, Merck) to a thickness of 0.25 mm. The developed chromatograms were viewed under ultraviolet light at 254 nm.

4.1.1. 6-Nitro-1,3-benzodioxole-5-carbaldehyde (5)

A mixture of 0.7 g (4.7 mmol) of tritured piperonal (**4**) and 2.3 mL of concentrated nitric acid was kept at 20–25 °C, in a water bath, until the complete dissolution of starting material. After 30 min, a mixture of ice/water was added to the reaction media, promoting an intense precipitation of a yellow solid, which was collected by filtration, washed with 140 mL of distilled water and recrystallized with ethanol/water. Yield 0.85 g (93%); mp 91–92 °C (lit. 93–94°C[20]); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 6.33 (s,

2H, O–CH_2–O), 7.32 (s, 1H, H_4–Ar), 7.74 (s, 1H, H_7–Ar), 10.08 (s, 1H, –CHO); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 104.8 (O–CH_2–O), 105.4 (C_7–Ar), 107.4 (C_4–Ar), 127.9 (C_5–Ar), 146.1 (C_6–Ar), 151.7 (C_3–Ar), 152.3 (C_1–Ar), 188.6 (C=O); IR (ν_{max}, KBr): 1682, 1518, 1368, 1336, 1126, 1119, 1032, 1021 cm^{-1}.

4.1.2. Methyl 6-nitro-1,3-benzodioxole-5-carboxylate (6)

To a solution of nitro-aldehyde derivative (**5**) (1.2 g, 6.1 mmol) in absolute methanol (7.9 mL) cooled at 0 °C were successively added methanolic solutions (each 7.9 mL) of iodine (2.0 g, 18.3 mmol) and KOH (1.0 g, 18.3 mmol) at 0 °C. After stirring for 1.5 h at 0 °C, small amounts of saturated $NaHSO_3$ solution were added until the disappearance of the brown color. Next, the methanol was almost totally evaporated under reduced pressure. To the residue was added water and ice, and the desired nitro-ester (**6**) was obtained by filtration and recrystallized in ethanol/water as a light yellow solid. Yield 1.15 g (84%); mp 102–103 °C (lit. 102–103 °C[6]); ^{1}H NMR (200 MHz, $CDCl_3$,) δ: 3.88 (s, 3H, OCH_3), 6.17 (s, 2H, O–CH_2–O), 7.03 (s, 1H, H_4–Ar), 7.37 (s, 1H, H_7–Ar); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 53.4 (OCH_3), 103.7 (O–CH_2–O), 105.0 (C_7–Ar), 108.5 (C_4–Ar), 123.8 (C_5–Ar), 143.2 (C_6–Ar), 149.7 (C_3–Ar), 151.4 (C_1–Ar), 165.8 (C=O); IR (ν_{max}, KBr): 1717, 1547, 1509, 1488, 1362, 1272, 1108, 1038 cm^{-1}.

4.1.3. Methyl 6-amino-1,3-benzodioxole-5-carboxylate (7)

To a solution of nitro-ester (**6**) (1.0 g, 4.4 mmol) in 75 mL of an ethanol/water mixture (2:1), were successively added metallic iron (1.39 g, 24.9 mmol) and ammonium chloride (0.14 g, 2.7 mmol). Next, the reaction mixture was refluxed for 1 h, filtered under Celite® and the obtained solution was concentrated under reduced pressure. To the residue was added ice, and the resulting precipitate was filtered out furnishing the desired amino-ester derivative (**7**) as a light brown solid. Yield 0.66 g (75%); mp 175–176 °C (lit. 175–176 °C[3]); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.83 (s, 3H, OCH_3), 5.60 (br, 2H, NH_2), 5.88 (s, 2H, O–CH_2–O), 6.16 (s, 1H, H_4–Ar), 7.25 (s, 1H, H_7–Ar); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 51.3 (OCH_3), 96.7 (O–CH_2–O), 101.2 (C_7–Ar), 102.4 (C_4–Ar), 108.5 (C_5–Ar), 139.1 (C_6–Ar), 148.8 (C_3–Ar), 152.8 (C_1–Ar), 168.2 (C=O); IR (ν_{max}, KBr): 3449, 3350, 1671, 1616, 1479, 1401, 1235 cm^{-1}.

4.1.4. Methyl 6-acetylamino-1,3-benzodioxole-5-carboxylate (8)

A solution of amino-ester derivative (**7**) (1 g, 5.12 mmol) in absolute ethanol (25 mL) and acetic anhydride (10 mL, 12.7 mmol) was refluxed for 1 h and then concentrated under reduced pressure. To the obtained residue was added a water/ice mixture and the desired acetamide derivative (**8**) was collected by filtration as a white solid. Yield 1.03 g (85%); mp 182–184 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.19 (s, 3H, OCH_3), 3.86 (s, 3H, $NHCOCH_3$), 5.99 (s, 2H, O–CH_2–O), 7.38 (s, 1H, H_4–Ar), 8.29 (s, 1H, H_7–Ar); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 25.4 ($COCH_3$), 52.1 ($NHCOCH_3$), 101.4 (O–CH_2–O), 102.0 (C_4–Ar), 107.5 (C_7–Ar), 108.8 (C_5–Ar), 139.2 (C_6–Ar), 142.6 (C_3–Ar), 152.6 (C_1–Ar), 168.3 ($NHCOCH_3$), 168.9 (CO_2CH_3); IR (ν_{max}, KBr): 3273, 2960, 1698, 1674, 1630, 1536, 1495, 1446, 1374, 1248, 1181, 1129, 1039 cm^{-1}.

4.1.5. 3-Amino-2-methyl-6,7-methylenedioxyquinazolin-4(3*H*)-onequinazolin-4(3*H*)-one (9)

A solution of *ortho*-acetamide-ester (**8**) (1 g, 4 mmol) in 30 mL of ethanol containing 5.8 mL (30 equiv) of hydrazine monohydrate 98%, was maintained under reflux for 1.5 h. Then, this solution was concentrated under reduced pressure, followed by addition of a water/ice mixture (1:1) to the obtained residue, which promotes extensive precipitation. The solid was collected by filtration and recrystallized in ethanol/chloroform to furnish the desired derivative (**9**) as white needles. Yield 0.74 g (90%); mp 269–270 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.49 (s, 3H, CH_3), 6.15 (s, 2H, O–CH_2–O), 7.00 (s, 1H, H_5–Ar), 7.34 (s, 1H, H_8–Ar); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 19.3 (CH_3), 99.2 (O–CH_2–O), 103.8 (C_5–Ar), 104.1 (C_8–Ar), 113.4 (C_{4a}–Ar), 136.0 (C_{1a}–Ar), 148.9 (C_6–Ar), 155.0 (C_7–Ar), 158.5 (C_2–Ar), 159.5 (O=C_4–Ar); IR (ν_{max}, KBr): 3289, 3196, 1667, 1630, 1582, 1471, 1264, 1250, 1034 cm^{-1}.

4.1.6. General procedure for preparation of 3-(arylideneamino)-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivatives (3a–c) and (3e–j)

To a solution of 1 mmol of 3-amino-quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivative (**9**) in absolute ethanol (10 mL) containing two drops of 37% hydrochloric acid was added 1.1 mmol of corresponding aromatic aldehyde derivative. The mixture was stirred at room temperature for 1 h and then, it was poured into cold water, neutralized with 10% aqueous sodium bicarbonate solution, and the precipitate formed was filtered out and recrystallized from ethanol–water.

4.1.6.1. 3-(Benzylideneamino)-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivative (3a). The derivative (**3a**) was obtained, as a white solid, by condensation of (**9**) with benzaldehyde. Yield 87%; mp 240–242 °C. Elemental Anal. (CHNS) Calcd: C, 66.44; H, 4.26; N, 13.67. Found: C, 66.38; H, 4.21; N, 13.57; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.72 (s, 3H, CH_3), 6.14 (s, 2H, O–CH_2–O), 7.18 (m, 1H, $H_{4'}$–Ph), 7.27 (s, 1H, H_5–Ar), 7.56 (m, 2H, $H_{3'}$ and $H_{5'}$–Ph), 7.58 (s, 1H, H_8–Ar), 7.91 (d, 2H, J = 4.6 Hz, $H_{2'}$ and $H_{6'}$–Ph), 9.01 (s, 1H, N=CH–Ar); ^{13}C NMR (50 MHz, CF_3CO_2D) δ: 20.4 (CH_3), 100.2 (O–CH_2–O), 106.6 (C_5–Ar), 106.9 (C_8–Ar), 116.1 (C_{4a}–Ar), 131.6 ($C_{3'}$ and $C_{5'}$–Ph), 132.1 ($C_{2'}$ and $C_{6'}$–Ph), 133.3 ($C_{4'}$–Ph), 136.4 (C_{1a}–Ar), 137.5 ($C_{1'}$–Ar), 153.4 (N=CH–Ar), 159.3 (C_6–Ar), 159.7 (C_7–Ar), 160.0 (C_2–Ar), 177.9 (O=C_4–Ar); IR (ν_{max}, KBr): 3048, 2910, 1663, 1590, 1471, 1038 cm^{-1}; MS (70 eV) *m*/*z* (relative abundance): 307.1 (5.8%), 204.1 (100%), 163.1 (5.9%), 103.1 (34.5%).

4.1.6.2. 3-[(4-Dimethylaminobenzylidene)amino]-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivative (3b). The derivative (**3b**) was obtained, as a light yellow solid, by condensation of (**9**) with 4-dimethylaminobenzaldehyde. Yield 81%; mp 226–227 °C. Elemental Anal. (CHNS) Calcd: C, 65.13; H, 5.18; N, 15.99. Found: C, 65.18; H, 5.21; N, 16.07; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.63 (s, 3H, CH_3), 3.09 (s, 6H, N–$(CH_3)_2$), 6.11 (s, 2H, O–CH_2–O), 6.74 (d, 2H, J = 8.0 Hz, $H_{2'}$- and $H_{6'}$–Ph), 7.12 (s, 1H, H_5–Ar), 7.58 (s, 1H, H_8–Ar), 7.76 (d, 2H, J = 8.0 Hz, $H_{3'}$ and $H_{5'}$–Ph), 8.61 (s, 1H, N=CH–Ar); ^{13}C NMR (50 MHz, CF_3CO_2D) δ: 18.1 (CH_3), 47.1 ($N(CH_3)_2$), 97.9 (O–CH_2–O), 104.5 (C_5–Ar), 104.9 (C_8–Ar), 113.7 (C_{4a}–Ar), 121.1 ($C_{3'}$- and $C_{5'}$-Ph), 132.0 ($C_{2'}$- and $C_{6'}$-Ph), 133.0 ($C_{4'}$-Ph), 133.7 ($C_{1'}$-Ph), 145.7 (N=CH–Ar), 151.1 (C_{1a}–Ar), 156.2 (C_6–Ar), 157.3 (C_7–Ar), 158.0 (C_2–Ar), 171.2 (O=C_4–Ar); IR (ν_{max}, KBr): 3044, 2917, 1661, 1614, 1594, 1479, 1365, 1234, 1184, 1033 cm^{-1}.

4.1.6.3. 3-[(2-Thienylmethylidene)amino]-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivative (3c). The derivative (**3c**) was obtained, as a white solid, by condensation of (**9**) with 2-thiophenecarboxaldehyde. Yield 92%; mp 238–239 °C. Elemental Anal. (CHNS) Calcd: C, 57.50, H, 3.54; N, 13.41; S, 10.23. Found: C, 57.58; H, 3.51; N, 13.37; S, 10.17; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.65 (s, 3H, CH_3), 6.11 (s, 2H, O–CH_2–O), 7.08 (s, 1H, H_5–Ar), 7.17 (m, 1H, $H_{4'}$–Ar), 7.56 (m, 3H, H_8–Ar, $H_{3'}$-2-thienyl and $H_{5'}$-2-thienyl), 9.23 (s, 1H, N=CH–Ar); ^{13}C NMR (50 MHz, CF_3CO_2D) δ: 20.4 (CH_3), 100.2 (O–CH_2–O), 106.9 (C_5–Ar), 107.1 (C_8–Ar), 116.0 (C_{4a}–Ar), 131.1 ($C_{3'}$-2-thienyl), 131.5 ($C_{4'}$-2-thienyl), 136.1 (C_{1a}–Ar), 138.4 ($C_{5'}$-2-thienyl), 141.4 ($C_{2'}$-2-thienyl), 153.4 (N=CH–Ar), 159.5 (C_6–Ar), 159.7 (C_7–Ar), 160.2 (C_2–Ar), 170.4 (O=C_4–Ar); IR (ν_{max}, KBr): 3109, 3038, 2904, 1661,

1583, 1475, 1039 cm^{-1}. MS (70 eV) *m*/*z* (relative abundance): 313.1 (4.6%), 204.1 (100%), 163.1 (17%), 103.1 (34.5%).

4.1.6.4. 3-[(4-Isopropylbenzylidene)amino]-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivative (3e). The derivative (**3e**) was obtained, as a light yellow solid, by condensation of (**9**) with 4-isopropylbenzaldehyde. Yield 98%; mp 114–116 °C. Elemental Anal. (CHNS) Calcd: C, 68.75; H, 5.48; N, 12.03. Found: C, 68.85; H, 5.51; N, 12.97; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.15 (d, 6H, *J* = 9.0 Hz, $CH(CH_3)_2$), 2.57 (s, 3H, CH_3), 2.87 (m, 1H, $CH(CH_3)_2$), 6.03 (s, 2H, O–CH_2–O), 7.04 (s, 1H, H_5–Ar), 7.27 (d, 2H, *J* = 8.0 Hz, $H_{3'}$- and $H_{5'}$-Ph), 7.47 (s, 1H, H_8–Ar), 7.73 (d, 2H, *J* = 8.0 Hz, $H_{2'}$- and $H_{6'}$-Ph), 8.83 (s, 1H, N=CH–Ar); ^{13}C NMR (50 MHz, CF_3CO_2D) δ: 17.9 ($CH(CH_3)_2$), 21.9 (CH_3), 34.6 ($CH(CH_3)_2$), 97.7 (O–CH_2–O), 104.4 (C_5–Ar), 104.6 (C_8–Ar), 113.6 (C_{4a}–Ar), 127.3 ($C_{4'}$-Ph), 127.5 ($C_{3'}$- and $C_{5'}$-Ph), 130.0 ($C_{2'}$- and $C_{6'}$-Ph), 131.4 ($C_{1'}$-Ph), 134.0 (N=CH–Ar), 150.9 (C_{1a}–Ar), 157.2 (C_6–Ar), 157.5 (C_7–Ar), 158.5 (C_2–Ar), 175.7 (O=C_4–Ar); IR (ν_{max}, KBr): 3034, 2961, 2908, 1659, 1581, 1472, 1327, 1233, 1037 cm^{-1}.

4.1.6.5. 3-[(2-Pyridinylmethylidene)amino]-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivative (3f). The derivative (**3f**) was obtained as a white solid by condensation of (**9**) with 2-pyridinecarboxaldehyde. Yield 88%; mp 216–218 °C. Elemental Anal. (CHNS) Calcd: C, 62.33; H, 3.92; N, 18.17. Found: C, 62.45; H, 3.85; N, 18.03; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.63 (s, 3H, CH_3), 6.11 (s, 2H, O–CH_2–O), 7.02 (s, 1H, H_5–Ar), 7.43 (t, 1H, *J* = 5.0 Hz, H_5'-2-Py), 7.59 (s, 1H, H_8–Ar), 7.84 (t, 1H, *J* = 7.6 Hz, $H_{4'}$-2-Py), 8.17 (d, 1H, *J* = 7.6 Hz, $H_{6'}$-2-Py), 8.75 (d, 1H, *J* = 5.0 Hz, $H_{3'}$-2-Py), 9.21 (s, 1H, N=CH–Ar); ^{13}C NMR (50 MHz, CF_3CO_2D) δ: 21.1 (CH_3), 100.8 (O–CH_2–O), 107.2 (C_5–Ar), 108.0 (C_8–Ar), 116.5 (C_{4a}–Ar), 129.3 ($C_{4'}$ and $C_{5'}$-2-Py), 135.7 ($C_{6'}$-2-Py), 145.2 ($C_{1'}$-2-Py and C_{1a}-Ar), 152.0 ($C_{3'}$-2-Py), 153.9 (N=CH–Ar), 158.3 (C_2–Ar), 160.0 (C_6–Ar), 162.1 (C_7–Ar), 166.7 (O=C_4–Ar); IR (ν_{max}, KBr): 3135, 3006, 1665, 1625, 1472, 1401, 1041 cm^{-1}.

4.1.6.6. 3-[(3-Pyridinylmethylidene)amino]-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivative (3g). The derivative (**3g**) was obtained, as a white solid, by condensation of (**9**) with 3-pyridinecarboxaldehyde. Yield 97%; mp 264–265 °C. Elemental Anal. (CHNS) Calcd: C, 62.33; H, 3.92; N, 18.17. Found: C, 62.25; H, 4.00; N, 18.19; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.65 (s, 3H, CH_3), 6.12 (s, 2H, O–CH_2–O), 7.03 (s, 1H, H_5–Ar), 7.45 (dd, 1H, *J* = 4.0 and 8.0 Hz, $H_{5'}$-3-Py), 7.57 (s, 1H, H_8–Ar), 8.25 (d, 1H, *J* = 8.0 Hz, $H_{6'}$-3-Py), 8.75 (d, 1H, *J* = 4.0 Hz, $H_{4'}$-3-Py), 9.01 (s, 1H, $H_{2'}$-3-Py), 9.29 (s, 1H, N=CH–Ar); ^{13}C NMR (50 MHz, CF_3CO_2D) δ: 21.0 (CH_3), 100.7 (O–CH_2–O), 107.2 (C_5–Ar), 107.7 (C_8–Ar), 116.3 (C_{4a}–Ar), 131.0 ($C_{6'}$-3-Py), 135.1 ($C_{5'}$-3-Py), 135.9 (C_{1a}–Ar), 144.7 ($C_{1'}$-3-Py), 146.8 ($C_{4'}$-3-Py), 149.1 ($C_{2'}$-3-Py), 153.8 (N=CH–Ar), 158.3 (C_6–Ar), 160.0 (C_7–Ar), 161.5 (C_2–Ar), 167.1 (O=C_4–Ar); IR (ν_{max}, KBr): 3130, 3045, 1665, 1589, 1475, 1403, 1037 cm^{-1}.

4.1.6.7. 3-[(4-Pyridinylmethylidene)amino]-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-onequinazolin-4(3*H*)-one derivative (3h). The derivative (**3h**) was obtained, as a white solid, by condensation of (**9**) with 4-pyridinecarboxaldehyde. Yield 94%; mp >270 °C. Elemental Anal. (CHNS) Calcd: C, 62.33; H, 3.92; N, 18.17. Found: C, 62.05; H, 4.05; N, 18.37; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.68 (s, 3H, CH_3), 6.13 (s, 2H, O–CH_2–O), 7.05 (s, 1H, H_5–Ar), 7.57 (s, 1H, H_8–Ar), 7.77 (d, 2H, *J* = 6.0 Hz, $H_{2'}$- and $H_{6'}$-Ar), 8.80 (d, 2H, *J* = 6.0 Hz, $H_{3'}$- and $H_{5'}$-Ar), 9.46 (s, 1H, N=CH–Ar N); ^{13}C NMR (50 MHz, CF_3CO_2D) δ: 21.1 (CH_3), 100.8 (O–CH_2–O), 107.2 (C_5–Ar), 108.0 (C_8–Ar), 116.5 (C_{4a}–Ar), 129.3 ($C_{2'}$ and $C_{6'}$-4-Py), 135.7 ($C_{1'}$-4-Py), 145.2 ($C_{3'}$ and $C_{5'}$-4-Py), 152.0 (C_{1a}–Ar), 153.9 (N=CH–Ar), 158.3 (C_6–Ar), 160.0 (C_7–Ar), 162.1 (C_2–Ar), 166.7 (O=C_4–Ar); IR (ν_{max}, KBr): 3126, 3010, 1668, 1622, 1476, 1401, 1136 cm^{-1}.

4.1.6.8. 3-[(2-Furylidene)amino]-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-one derivative (3i). The derivative (**3i**) was obtained, as a light yellow solid, by condensation of (**9**) with 2-furfuraldehyde. Yield 99%; mp 225–226 °C. Elemental Anal. (CHNS) Calcd: C, 60.61; H, 3.73; N, 14.14. Found: C, 60.45; H, 3.80; N, 14.07; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.62 (s, 3H, CH_3), 6.11 (s, 2H, O–CH_2–O), 6.61 (dd, 1H, *J* = 1.7 and 3.5 Hz, $H_{4'}$-2-furyl), 7.02 (s, 1H, H_5–Ar), 7.05 (d, 1H, *J* = 3.5 Hz, $H_{5'}$-2-furyl), 7.56 (s, 1H, H_8–Ar), 7.68 (d, 1H, *J* = 1.7 Hz, $H_{3'}$-2-furyl), 8.90 (s, 1H, N=CH–Ar); ^{13}C NMR (50 MHz, CF_3CO_2D) δ: 20.5 (CH_3), 100.3 (O–CH_2–O), 106.9 (C_5–Ar), 107.2 (C_8–Ar), 115.9 ($C_{4'}$-2-furyl), 116.2 ($C_{3'}$-2-furyl), 129.1 (C_{4a}–Ar), 136.4 (C_{1a}–Ar), 148.0 ($C_{5'}$-2-furyl), 153.3 ($C_{2'}$-2-furyl), 153.5 (N=CH–Ar), 159.2 (C_6–Ar), 159.8 (C_7–Ar), 160.3 (C_2–Ar), 165.4 (O=C_4–Ar); IR (ν_{max}, KBr): 3142, 3042, 1664, 1620, 1588, 1475, 1405, 1307, 1233, 1036 cm^{-1}. MS (70 eV) *m*/*z* (relative abundance): 297.1 (7.7%), 204.1 (100%), 163.1 (10.5%), 120.1 (23.3%).

4.1.6.9. 3-[(3-Thienylidene)amino]-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-one derivative (3j). The derivative (**3j**) was obtained, as a white solid, by condensation of (**9**) with 3-thiophenecarboxaldehyde. Yield 94%; mp 225–227 °C. Elemental Anal. (CHNS) Calcd: C, 57.50; H, 3.54; N, 13.41; S, 10.23. Found: C, 57.37; H, 3.62; N, 13.47; S, 10.27; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.55 (s, 3H, CH_3), 6.04 (s, 2H, O–CH_2–O), 6.99 (s, 1H, H_5–Ar), 7.35 (dd, 1H, *J* = 2.0 and 4.0 Hz, $H_{4'}$-3-thienyl), 7.44 (s, 1H, H_8–Ar), 7.62 (d, 1H, *J* = 4.0 Hz, $H_{5'}$-3-thienyl), 7.79 (d, 1H, *J* = 2.0 Hz, $H_{2'}$-3-thienyl), 8.93 (s, 1H, N=CH–Ar); ^{13}C NMR (50 MHz, CF_3CO_2D) δ: 17.9 (CH_3), 97.8 (O–CH_2–O), 104.4 (C_5–Ar), 104.6 (C_8–Ar), 113.6 (C_{4a}–Ar), 124.2 ($C_{4'}$-3-thienyl), 128.2 ($C_{5'}$-3-thienyl), 133.2 ($C_{2'}$-3-thienyl), 134.0 ($C_{3'}$-3-thienyl), 137.8 (N=CH–Ar), 151.0 (C_{1a}–Ar), 156.9 (C_6–Ar), 157.3 (C_7–Ar), 157.5 (C_2–Ar), 169.2 (O=C_4–Ar); IR (ν_{max}, KBr): 3074, 3037, 2920, 1663, 1585, 1476, 1238, 1044 cm^{-1}. MS (70 eV) *m*/*z* (relative abundance): 313.1 (5.9%), 204.1 (100%), 163.1 (8.9%), 109.1 (21.6%).

4.1.7. Preparation of 3-[2-thienylethylidene)amino]-2-methyl-6,7-methylenedioxy-quinazolin-4(3*H*)-one derivatives (3d)

To a solution of 1 mmol of 3-amino-quinazolin-4(3*H*)-one derivative (**9**) in absolute ethanol (10 mL) containing two drops of 37% hydrochloric acid was added 1.1 mmol of 2-acetyl-thiophenecarboxaldehyde. The mixture was stirred at 50–60 °C for 96 h and then, it was poured into cold water, neutralized with 10% aqueous sodium bicarbonate solution, and the precipitate formed was filtered out and recrystallized from ethanol–water. Yield 89%; mp 255–256 °C. Elemental Anal. (CHNS) Calcd: C, 58.70; H, 4.00; N, 12.84; S, 9.80. Found: C, 58.55; H, 4.10; N, 12.77; S, 9.65; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.27 (s, 3H, CH_3–(Ar)C=N), 2.47 (s, 3H, CH_3), 6.11 (s, 2H, O–CH_2–O), 7.06 (s, 1H, H_5–Ar), 7.15 (t, 1H, *J* = 5.0 Hz, $H_{4'}$-2-thienyl), 7.55 (d, 1H, *J* = 5.0 Hz, $H_{3'}$-2-thienyl), 7.58 (s, 1H, H_8–Ar), 7.63 (d, 1H, *J* = 5.0 Hz, $H_{5'}$-2-thienyl); ^{13}C NMR (50 MHz, CF_3CO_2D) δ: 17.4 (N=C(Ar)–CH_3), 18.1 (Ar–CH_3), 97.9 (O–CH_2–O), 104.3 (C_5–Ar), 104.6 (C_8–Ar), 112.8 (C_{4a}–Ar), 128.9 ($C_{3'}$-2-thienyl), 134.4 ($C_{4'}$-2-thienyl), 136.7 (C_{1a}–Ar), 137.7 ($C_{5'}$-2-thienyl), 141.8 ($C_{2'}$-2-thienyl), 151.0 (N=CH–Ar), 151.6 (C_6–Ar), 157.4 (C_7–Ar), 157.8 (C_2–Ar), 159.9 (O=C_4–Ar); IR (ν_{max}, KBr): 2928, 1672, 1581, 1475, 1411, 1262, 1028, 705 cm^{-1}.

4.1.8. Single crystal X-ray diffraction

After the synthesis and purification procedures, a well-shaped single crystal of (**9**) was obtained by recrystallization from ethanol/chloroform (1:1 v/v) solution at room temperature. Intensity

data were measured with the crystal at room temperature (298 K) and with graphite monochromated Mo Kα radiation (λ = 0.71073 Å), using the Enraf-Nonius Kappa-CCD diffractometer. The cell refinements were performed using the software COLLECT[32] and SCALEPACK,[33] and the final cell parameters were obtained on all reflections. Data reduction was carried out using the software DENZO-SMN and SCALEPACK.[33] Since the absorption coefficient is insignificant for (**9**), no absorption correction was applied. The structure was solved using the software SHELXS-97,[34] where all the non-hydrogen atoms were readily found from the electronic density map constructed by Fourier synthesis. The model obtained was refined by full-matrix least-squares on F^2 using the software SHELXL-97.[34] The refinement was carried on with anisotropic thermal parameters for all the non-hydrogen atoms. With regard to the hydrogen atoms of (**9**), the C–H hydrogen atoms were positioned stereochemically and were refined with fixed individual displacement parameters [$U_{iso}(H) = 1.2U_{eq}$ (C^2_{sp}) or $1.5U_{eq}$ (C^3_{sp})] using a riding model with aromatic C–H bond length of 0.93 Å, methyl C–H one of 0.96 Å, and methylene C–H one of 0.97 Å. The hydrogen atoms linked to nitrogen of the amine group were located by difference Fourier synthesis and were set as isotropic. Crystal, collection and structure refinement data are summarized in Table 3. Tables were generated by WINGX[35] and the structure representations by ORTEP-3[36] and MERCURY.[37]

Atomic coordinates, bond lengths, angles and thermal parameters have been deposited at the Cambridge Crystallographic Data Center, deposition number CCDC 734003.

4.2. Pharmacology

4.2.1. Analgesic activity

4.2.1.1. Acetic acid-induced abdominal constrictions assay.[26]

The abdominal constrictions assay induced by acetic acid (0.6%; 0.1 mL/10 g) was performed using albino mice of both sexes (18–23 g). Compounds were administered orally (100 μmol/kg) as a suspension in 5% Arabic gum in saline (vehicle). Dipyrone (100 μmol/kg) was used as standard drug under same conditions. Acetic acid solution was administered intraperitoneally 1 h after administration of quinazolin-4(3*H*)-one compounds (**3a–j**). Ten minutes following intraperitoneally acetic acid injection the number of constrictions per animal was recorded for 20 min. Control animals received an equal volume of vehicle. Analgesic activity was expressed as percentage of inhibition of constrictions when compared with the vehicle control group (Table 2). The dose–response curve was constructed varying the molar concentration of compounds (**3b**) and (**3j**) from 0.3 to 100 μmol/kg. Results are expressed as the mean ± SEM of *n* animals per group. The data were statistically analyzed by the One-way ANOVA test for a significance level of $P < 0.01$.

Table 3
Crystal data and structure refinement for 3-amino-6,7-methylenedioxy-quinazolin-4(3*H*)-one intermediate (**9**)

Empirical formula	$C_{10}H_9N_3O_3$
Formula weight	219.20
Temperature (K)	298(2)
Wavelength (Å)	0.71073
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	
a (Å)	10.8231(3)
b (Å)	12.6064(4)
β (°)	103.983(2)
c (Å)	7.1213(2)
Volume (Å^3)	942.83(5)
Z	4
D_{calc} (Mg/m^3)	1.544
Absorption coefficient (mm^{-1})	0.117
F(0 0 0)	456
Crystal size (mm^3)	0.30 × 0.15 × 0.10
θ Range for data collection (°)	3.5–25.0
Index ranges	$-12 \leqslant h \leqslant 12$, $-14 \leqslant k \leqslant 14$, $-8 \leqslant l \leqslant 8$
Reflections collected	3221
Independent reflections [*R*(int)]	1651 [0.0110]
Completeness to $\theta = 25.0°$	99.2%
Max. and min. transmission	0.9931 and 0.9638
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	1651/0/154
Goodness-of-fit on F^2	1.070
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0399$, $wR_2 = 0.1076$
R indices (all data)	$R_1 = 0.0454$, $wR_2 = 0.1126$
Largest diff. peak and hole (e Å^{-3})	0.180 and −0.173

4.2.1.2. Formalin-induced pain in mice.

The formalin test was carried out as described by Hunskaar and Hole.[27] Albino swiss mices of both sexes weighting 18–25 g fasted for a period of 8 h were injected subplantarly with 20 μL of 2.5% formalin in one hind paw. The compounds were administrated intraperitoneally (100 μmol/kg in arabic gum 5% as vehicle) 60 min before formalin injection. The time the mice spent licking or biting the injected paw or leg was recorded. Two distinct periods of intensive licking activity were identified and scored separately unless otherwise stated. The first period (earlier or neurogenic phase) was recorded 0–5 min after formalin injection and the second period (later or inflammatory phase) was recorded 15–30 min after injection.

4.2.1.3. Hot-plate test.

The central analgesic activity was determined in vivo by the hot-plate test according to Kuraishi et al.[28] Swiss mice of both sexes were used, maintained with water ad libitum and fasted for a period of 8 h.

Animals were placed on heated plate at 55 ± 0.1 °C and their responses to thermal stimulation (licking or withdraw of hind paw) were timed. Two control measures were done (in the absence of test drugs) in intervals of 30 min to determine the control latency mean time and the cut-off time (maximum time of permanence of the animal in the plate), calculated as three times the control mean value. The response time for each mouse is registered at 30 min intervals after compounds administration for a total of 150 min. The quinazolin-4(3*H*)-one derivatives LASSBio-1240 (**3b**) and LASSBio-1272 (**3d**) were administrated intraperitoneally (100 μmol/kg, in tween 1% in saline as vehicle).

4.2.1.4. Modified hot-plate test (hyperalgesia).[29]

Wistar rats were placed individually on a hot plate with the temperature adjusted to 51 ± 0.1 °C. The latency of the withdrawal response of the left hind paw was determined at 0, 30, 60, 120, 180, and 240 min post-challenge. The left paw was stimulated with carrageenan (1 mg/paw) and the right with saline (0.9% NaCl), both in a final volume of 100 μL, injected intraplantarly (ipl). The time of maximum permanence permitted on the hot surface was 20 s. The quinazolin-4(3*H*)-one derivatives LASSBio-1240 (**3b**) and LASSBio-1272 (**3d**) were administrated intraperitoneally (100 μmol/kg, in 1% tween 80 in saline as vehicle) 60 min before carrageenan injection. Hyperalgesia to heat was defined as a decrease in withdrawal latency and calculated as follows: δ paw withdrawal latency (s) = (left paw withdrawal latency in time 0)—(left paw withdrawal latency in others times).

4.2.2. Anti-inflammatory activity

The anti-inflammatory activity was determined in vivo using the carrageenan-induced rat paw edema test according to Ferreira.[30] Albino rats of both sexes (150–200 g) were used in this protocol. Compounds were administered in the dose of choice as a suspension in 1% tween 80 in saline (vehicle). Control animals received equal volume of the vehicle. One hour after, the animals

were then injected with either 0.1 mL of 1% carrageenan solution in saline (0.1 mg/paw) or sterile saline (NaCl 0.9%), into the subplantar surface of one of the hind paws, respectively. The paw volumes were measured using a glass plethysmometer coupled to a peristaltic pump, 3 h after the subplantar injection. The edema was calculated as the volume difference between the carrageenan and saline-treated paw. Anti-inflammatory activity was expressed as percentage of inhibition of the edema when compared with vehicle control group.

4.2.3. Platelet anti-aggregating activity

4.2.3.1. Preparation of rabbit and human platelet rich plasma. Rabbit blood was collected from the central ear artery of rabbits weighing 2.5–3.0 kg. Blood samples were collected into 3.8% trisodium citrate (9:1 v/v). Platelet-rich plasma (PRP) was prepared by centrifugation at 1800 rpm for 10 min at room temperature. The platelet poor plasma (PPP) was prepared by centrifugation of the pellet at 3600 rpm for 10 min at room temperature. Platelet count was adjusted to $5 \times 108\ mL^{-1}$.

4.2.3.2. Platelet aggregation assay. Platelet aggregation was monitored by the turbidimetric method of Born and Cross[31] in a Chrono-Log aggregometer. PRP (400 μL) was incubated at 37 °C for 1 min with continuous stirring at 900 rpm. Aggregation of PRP was induced by arachidonic acid (200 μM). Test compounds and the vehicle (0.5% DMSO, 2 μL) were added to the PRP samples 5 min before addition of the aggregating agent. The DMSO used as vehicle did not have either pro- or antiplatelet aggregation activity. The platelet aggregation was expressed as percentage of aggregation for AA.

Acknowledgements

The authors thank Central Analítica (IQ-UFRJ) for technical facilities and CAPES (BR), CNPq (BR), FAPERJ (BR), PRONEX (BR) and INCT-INOFAR (BR, #573.564/2008-6) for financial support and fellowships.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.08.009.

References and notes

1. Fraga, C. A. M.; Barreiro, E. J. *Curr. Med. Chem.* **2006**, *13*, 167.
2. Rollas, S.; Küçükgüzel, S. G. *Molecules* **2007**, *12*, 1910.
3. Tributino, J. L. M.; Duarte, C. D.; Corrêa, R. S.; Dorigetto, A. C.; Ellena, J.; Romeiro, N. C.; Castro, N. G.; Miranda, A. L. P.; Barreiro, E. J.; Fraga, C. A. M. *Biorg. Med. Chem.* **2009**, *17*, 1125.
4. Lima, P. C.; Lima, L. M.; Silva, K. C. M.; Léda, P. H. O.; Miranda, A. L. P.; Fraga, C. A. M.; Barreiro, E. J. *Eur. J. Med. Chem.* **2000**, *35*, 187.
5. Bezerra-Neto, H. J. C.; Lacerda, D. I.; Miranda, A. L. P.; Alves, H. M.; Barreiro, E. J.; Fraga, C. A. M. *Bioorg. Med. Chem.* **2006**, *14*, 7924.
6. Duarte, C. D.; Tributino, J. L. M.; Lacerda, D. I.; Martins, M. V.; Alexandre-Moreira, M. S.; Dutra, F.; Bechara, E. J. H.; De-Paula, F. S.; Goulart, M. O. F.; Ferreira, J.; Calixto, J. B.; Nunes, M. P.; Bertho, A. L.; Miranda, A. L. P.; Barreiro, E. J.; Fraga, C. A. M. *Bioorg. Med. Chem.* **2007**, *15*, 2421.
7. Cunha, A. C.; Figueiredo, J. M.; Tributino, J. L. M.; Miranda, A. L. P.; Castro, H. C.; Zingali, R. B.; Fraga, C. A. M.; de Souza, M. C. B. V.; Ferreira, V. F.; Barreiro, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 2051.
8. Silva, G. A.; Costa, L. M. M.; Brito, F. C. F.; Miranda, A. L. P.; Barreiro, E. J.; Fraga, C. A. M. *Bioorg. Med. Chem.* **2004**, *12*, 3149.
9. Lima, L. M.; Frattani, F. S.; dos Santos, J. L.; Castro, H. C.; Fraga, C. A. M.; Zingali, R. B.; Barreiro, E. J. *Eur. J. Med. Chem.* **2008**, *43*, 348–356.
10. Gonzalez-Serratos, H.; Chang, R.; Pereira, E. F.; Castro, N. G.; Aracava, Y.; Melo, P. A.; Lima, P. C.; Fraga, C. A. M.; Barreiro, E. J.; Albuquerque, E. X. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 558.
11. Silva, A. G.; Zapata-Sudo, G.; Kummerle, A. E.; Fraga, C. A. M.; Barreiro, E. J.; Sudo, R. T. *Bioorg. Med. Chem.* **2005**, *13*, 3431.
12. Romeiro, N. C.; Aguirre, G.; Hernández, P.; González, M.; Cerecetto, H.; Aldana, I.; Pérez-Silanes, S.; Monge, A.; Barreiro, E. J.; Lima, L. M. *Bioorg. Med. Chem.* **2009**, *17*, 641.
13. Duarte, C. D.; Barreiro, E. J.; Fraga, C. A. M. *Mini-Rev. Med. Chem.* **2007**, *7*, 1108.
14. Barreiro, E. J.; Fraga, C. A. M. *Quím. Nova* **1999**, *22*, 744.
15. Wermuth, C. G. *The Practice of Medicinal Chemistry*, 2nd ed.; Academic Press: New York, 2003. Chapter 6.
16. The energetic barrier was determined using the semi-empirical method AM1 in the PC Spartan Pro program (Wavefunction Inc., Irvine, CA, 2000). It was calculated by studying the energetic demand involved in the rotation of the dihedral C–N–N=C in the novel quinazolin-4(3*H*)-one derivatives (**3**), when it was turned by 15 to 15 degrees.
17. Lima, L. M.; Barreiro, E. J. *Curr. Med. Chem.* **2005**, *12*, 23.
18. Barreiro, E. J.; Lima, M. E. F. *J. Pharm. Sci.* **1992**, *81*, 1219.
19. Barreiro, E. J.; Costa, P. R. R.; Coelho, F. A. S. *J. Chem. Res.* **1985**, 220.
20. Ekeley, J. B.; Klemme, M. *J. Am. Chem. Soc.* **1928**, *50*, 2711.
21. Yamada, S.; Morizono, D.; Yamamoto, K. *Tetrahedron Lett.* **1992**, *33*, 4329.
22. Lages, A. S.; Silva, K. C. M.; Miranda, A. L. P.; Fraga, C. A. M.; Barreiro, E. J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 183.
23. (a) Peet, N. P.; Sunder, S.; Cregge, R. J. *J. Org. Chem.* **1976**, *41*, 2733; (b) Dallacker, F. *Monasth. Chem.* **1959**, *90*, 846.
24. Pereira, A. S.; Violante, F. A.; Aquino Neto, F. R.; Cardoso, J. N.; Fraga, C. A. M.; Barreiro, E. J. *Anal. Lett.* **1998**, *31*, 719.
25. McLafferty, F. W. *Anal. Chem.* **1956**, *28*, 306.
26. Coolier, H. O. J.; Dinneen, L. C.; Johnson, C. A.; Schneider, C. *Br. J. Pharmacol. Chemother.* **1968**, *32*, 295.
27. Hunskaar, S.; Hole, K. *Pain* **1987**, *30*, 103.
28. Kuraishi, Y.; Harada, Y.; Aratani, S.; Satoh, M.; Takagi, H. *Brain Res.* **1983**, *273*, 245.
29. Lavich, T. R.; Cordeiro, R. S. B.; Silva, P. M. R.; Martins, M. A. *Braz. J. Med. Biol. Res.* **2005**, *38*, 445.
30. Ferreira, S. H. *J. Pharm. Pharmacol.* **1979**, *31*, 648.
31. Born, G. V. R.; Cross, M. J. *J. Physiol.* **1963**, *168*, 178.
32. Enraf-Nonius COLLECT. Nonius BV, Delft, The Netherlands, 1997–2000.
33. Otwinowski, Z.; Minor, W.. In *Methods in Enzymology*; Carter, C. W., Jr., Sweet, R. M., Eds.; Academic Press: New York, 1997; Vol. 276, pp 307–326.
34. Sheldrick, G. M. *Acta Crystallogr., Sect. A* **2007**, *64*, 112–122.
35. Farrugia, L. J. *J. Appl. Crystallogr.* **1999**, *32*, 837.
36. Farrugia, L. J. *J. Appl. Crystallogr.* **1997**, *30*, 565.
37. Bruno, I. J.; Cole, J. C.; Edgington, P. R.; Kessler, M. K.; Macrae, C. F.; McCabe, P.; Pearson, J.; Taylor, R. *Acta Crystallogr., Sect. B* **2002**, *58*, 389.