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Design and synthesis of 3,4-methylenedioxy-6-nitrophenoxyacetylhydrazone derivatives obtained from natural safrole: New lead-agents with analgesic and antipyretic properties

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Abstract—In this work, we reported the synthesis and evaluation of the analgesic, anti-inflammatory, and antipyretic properties of new 2-(6-nitro-benzo[1,3]dioxol-5-yloxy)-acetylhydrazone derivatives (**3**), designed exploring molecular hybridization and isosteric replacement approaches between nimesulide (**1**) and carbanalogue NAH series (**2**) developed at LASSBio. Target compounds were synthesized in very good yields exploiting abundant Brazilian natural product safrole (**4**) as starting material. The evaluation of the antinociceptive properties of this series led us to discover a new potent prototype of analgesic and antipyretic agent, that is, NAH derivative **3c**, named LASSBio-891, which showed to be more potent than dipyrone used as standard.

1. Introduction

Pain is an unpleasant and subjective sensation resulting from a harmful sensorial stimulation that alerts the body about a current or potential damage to its tissues and organs.[1] It is estimated that more than 75 million people refer to health services annually, presenting some form of recurrent or persistent pain.[2] In spite of painful sensation can be solved most efficiently by removal of the underlying cause, the pain-causing stimulus cannot always be either easily defined or quickly removed. Therefore, the health professionals are usually faced with the necessity to manage the symptomatology of the pain.[1]

One of the most important pain-inducer factors is the inflammation process that frequently occurs in response to a tissue damage, resulting in a series of characteristic aspects correlated with the evolution of the disease, that is, fever, lethargy, anorexy, and generalized pain in muscles and joints as well as an arisen of the hypersensibility to pain near the damaged place.[3]

Among other mediators, the prostaglandins produced in the initial period of the inflammatory injury are responsible for the local vasodilatation and potentialization of the edema, leading to the development of redness and heat in the inflamed tissue.[3] In special, prostaglandins E_2 (PGE_2) and I_2 (PGI_2) contribute to the development of the hyperalgesia associated with the inflammatory process.[4]

The ability of non-steroidal anti-inflammatory drugs (NSAIDs) to modulate the pain, inflammation, and fever made them one of the most used therapeutical classes in the world.[5] The discovery in 1992 of a second

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isoform of the cyclooxygenase (COX-2), related to the bioformation of prostaglandins in inflamed sites, contributed to the sprouting of a new class of NSAIDs, which act as selective COX-2 inhibitors, with lower side effects.[6]

Among the most potent drugs that appeared in the initial phase of the development of selective COX-2 inhibitors are the sulides, such as nimesulide (**1**), which was launched in 1985, before the COX-2 discovery, as an important non-steroidal anti-inflammatory (NSAI) agent without gastric effects.[7] At that moment, the therapeutical profile of (**1**) was attributed to its radical scavenger behavior, which may be explained by the presence of the *para*-nitrosulfonylaminophenyl unit in its structure, pharmacophoric for its redox properties.[8] Later, this compound was analyzed in relation to its action on COX isoforms, having been shown to be 1400-fold more selective for COX-2.[9]

Considering this panorama, our laboratory had synthesized structurally diverse *N*-acylarylhydrazone (NAH) derivatives presenting important analgesic, anti-inflammatory, and anti-thrombotic profiles.[10,11] In this context, the analgesic activity of 1,3-benzodioxolyl NAH series (**2**) was characterized.[12] indicating to us the biophoric characteristics of these new prototypes of powerful antinociceptive drug candidates. For instance, the most active compound of this family, that is, *para*-dimethylamino derivative (**2a**), was able to inhibit in 67% the acetic acid-induced abdominal constrictions in mice, having presented a superior inhibitory profile when compared with that displayed by the classical standards dipyrone and indomethacin at the same dose, that is, 36% and 56%, respectively.[12] A proposal for the molecular mechanism of COX inhibition by hydrazone derivatives was described by Mahy et al.,[13] which have identified an isosteric relationship between benzo-bis-azaallyl moiety of the arylhydrazones and the bis-allyl methylene subunit of the arachidonic acid. This structural similarity seems to be the primary reason for molecular recognition of the corresponding frameworks by the target enzyme, in both structures.

In the course of an ongoing research program aimed at the design of new bioactive compounds acting at the arachidonic acid cascade enzyme's level, we described in this manuscript the design, the synthesis, and the pharmacological evaluation of a new series of functionalized 2-(6-nitro-benzo[1,3]dioxol-5-yloxy)-acetylhydrazone (NBNAH) derivatives (**3a–j**),[14] exploring safrole (**4**), an abundant Brazilian natural product,[15] as starting material (Fig. 1).

The title compounds (**3**) were structurally designed through the isosteric exchange[16] of the benzylic

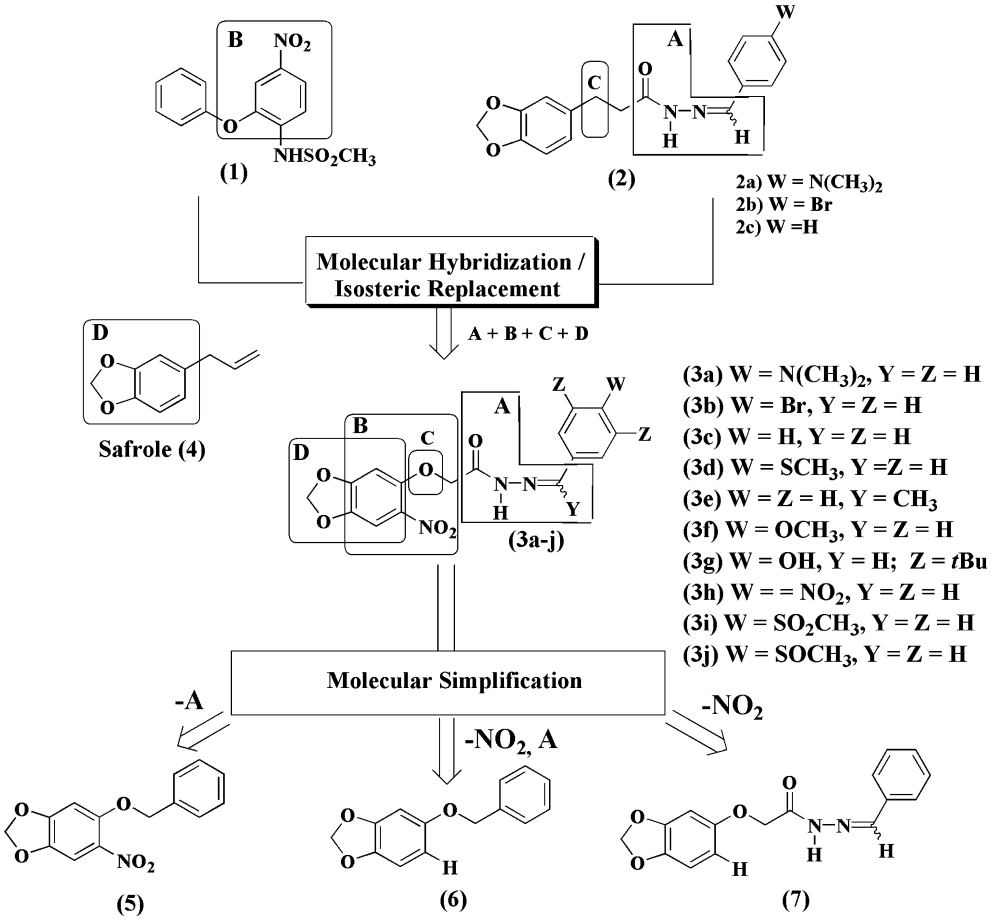


Figure 1. Design concept of new 2-(6-nitro-benzo[1,3]dioxol-5-yloxy)-acetylhydrazone (NBNAH) derivatives (**3a–j**).

methylene group (C, Fig. 1) of *N*-acylhydrazone series (**2**) by an oxygen atom, in order to evaluate any eventual influence of the conformational restriction in the pharmacophoric NAH side chain, promoted by a possible intramolecular H-bond formation, in the antinociceptive profile. Moreover, the nitro group was introduced at C-6 position of the 1,3-benzodioxole ring in order to investigate its internal 'catalytic' effect in the formation of free radicals on the *N*-acylhydrazone moiety, intending to improve the radical scavenger profile in these new compounds,[8] aiming the optimization of its pharmacological profile through the selective modulation of its redox properties,[13,17] The substituents of the terminal phenyl moiety, attached at the imine function of NAH derivatives (**3**), were rationally elected in order to acquire information about the influence of electronic and physical–chemical parameters on the pharmacological activity.

Additionally, in order to study and validate the pharmacophoric character of the *N*-acylhydrazone and nitro moieties related to anti-inflammatory and analgesic activities of the new compounds (NBNAH, **3**), we also elect as target the 5-(benzyloxy)-6-nitrobenzo[1,3]dioxole derivative (NBB, **5**), 2-(benzo[1,3]dioxol-5-yloxy)-acetylhydrazone derivative (BNAH, **7**), and 5-(benzyloxy)-benzo[1,3]dioxole derivative (BB, **6**), designed by molecular simplification of series (**3**) by removing, respectively, the *N*-acylhydrazone moiety (A), the nitro group, and both functionalities (Fig. 1).

2. Chemistry

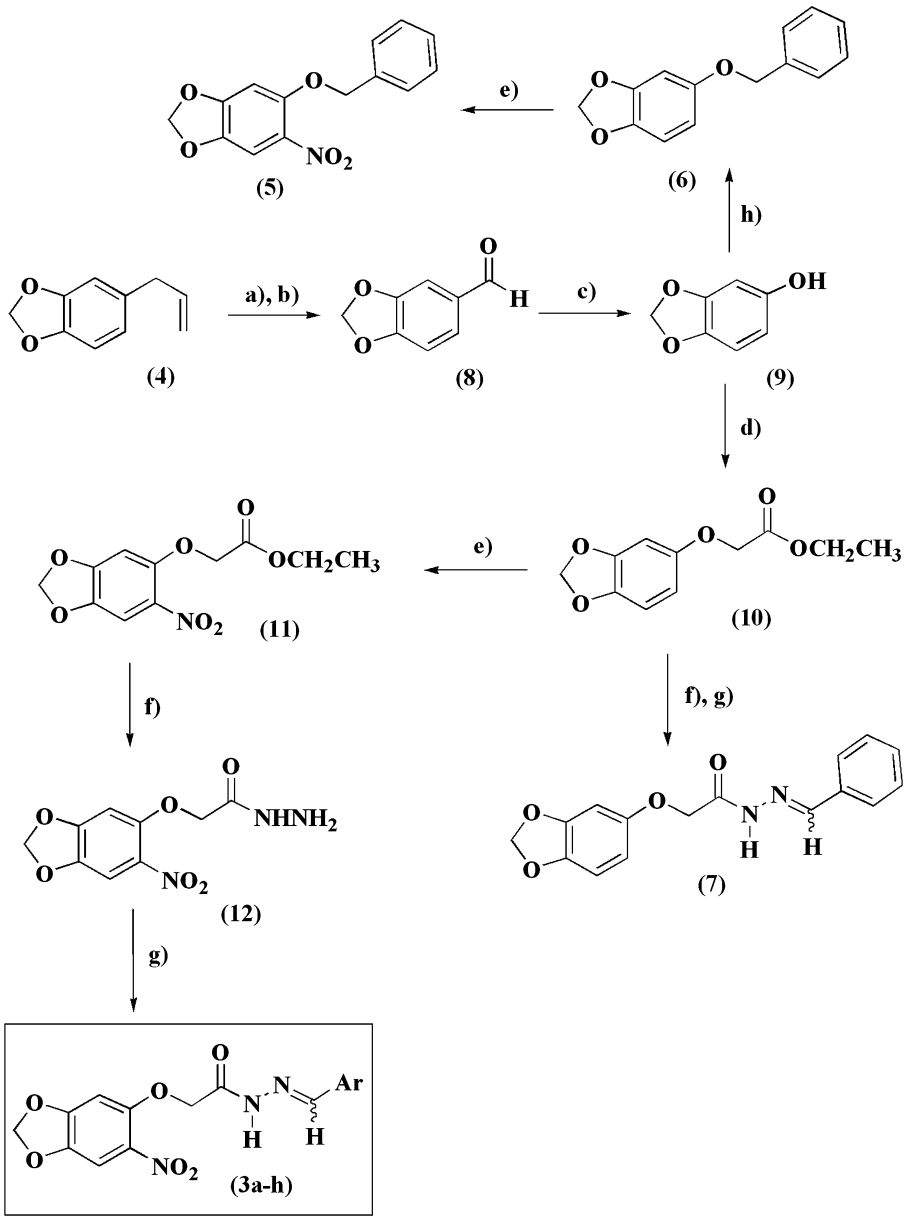
The obvious synthetic route planned to obtain the desired NBNAH derivatives (**3a**–**j**) is shown in Scheme 1. The new NAH derivatives of series (**3**) were prepared from piperonal (**8**), obtained in ca. 75% overall yield from the natural safrole (**4**),[18] using base-catalyzed isomerization of the terminal double bond followed by oxidative cleavage.[19] Performing a Bayer–Villiger oxidation of the aromatic aldehyde (**8**) through its treatment with performic acid generated 'in situ,' we were able to obtain the corresponding phenolic derivative sesamol (**9**) in 90% yield.[20] Next, the aryl-ether derivative (**10**) was prepared, in 85% yield, exploring the O-alkylation of (**9**), by treating the phenolate intermediate formed in basic conditions with ethyl 2-bromoacetate.[21] Next, regioselective nitration at C-6 of 1,3-benzodioxole ring of the derivative (**10**) with nitric acid in chloroform[22] furnished the nitro-ester derivative (**11**) in high yield, which after treatment with hydrazine hydrate in ethanol[12,23–26] resulted in the formation of the corresponding hydrazide (**12**), the key-intermediate of this synthesis route, in 85% yield.

Finally, the new safrole-derived NBNAH derivatives (**3a**–**h**) were obtained, in good yields, by condensing hydrazide derivative (**12**) with different aromatic aldehydes (ArCHO) in ethanol, using hydrochloric acid as catalyst.[12,23,25,26]

The methylsulfone (**3i**) and methylsulfoxide (**3j**) NAH derivatives were, respectively, prepared by chemoselective oxidation of the corresponding thiomethyl NAH derivative **3d** exploiting Oxone® ($2KHSO_5{\cdot}KHSO_4{\cdot}K_2SO_4$) supported in alumina[27] or 3-chloroperoxybenzoic acid (MCPBA) in dichloromethane[28] (Scheme 2).

The next step of this work was to determine the relative configuration of the imine double bond of *N*-acylarylhydrazone derivatives (**3a**–**j**), in order to assure the diastereomeric ratio essential to the complete understanding of the biological effects. The careful analysis of the ^{1}H NMR spectra of (**3a**–**j**) allowed us to detect the presence of two singlet signals related to the imine hydrogen, which were attributed to the respective (*E*) and (*Z*)-diastereomers. The presence of both diastereomers in this series of NAH derivatives (**3**) is in agreement with previous results from our laboratory that indicated a similar behavior in other NAH series presenting a C_2 spacer unit between 1,3-benzodioxole ring and the acyl moiety.[10,12,29,30] Studies using NOESY and COSY techniques of bidimensional ^{1}H NMR[31] did not achieve success in the identification of the relative configuration of the diastereomers, probably due to the conformational flexibility around amide bond. The assignment of the relative configuration of the diastereomers of NAH derivatives (**3a**–**j**) was made in agreement with previous results obtained by Karabatsos et al.,[32–34] which describes that imine-attached hydrogen signal of (*E*)-diastereomer is downfielded by 0.2–0.3 ppm from the corresponding hydrogen atom signal in (*Z*)-diastereomer. Therefore, after careful analysis of the ^{1}H NMR spectra of the mixture of diastereomers of NAH derivatives (**3a**–**j**), we were able to evidence that the major one presents (*E*) configuration similar to that we have found for the *N*-acylhydrazones of the previous series (**2**).[12] In order to obtain additional evidences about the relative configuration of the imine double bond of nitro-acylarylhydrazone derivatives (**3a**–**j**), we performed a brief molecular modeling study employing the semi-empirical AM1 method[35] in the Spartan Pro 1.5.0 program[36] using the non-substituted derivative (**3c**) as model (Fig. 2). Systematic conformational analysis was carried out to determine the heat formation value of the more stable diastereomeric form. For derivative (**3c**), the obtained value for the more stable conformer of the (*E*)-diastereomer was −33.75 kcal/mol, while the energy found for the corresponding (*Z*)-diastereomer was −31.27 kcal/mol (Fig. 2). Although the calculated difference in heat formation seems to be insufficient to let us to identify unambiguously, what diastereomeric form of (**3c**) predominates, these studies indicated that (*E*)-diastereomer, presenting minor heat formation, could be preferentially formed.

The ratio between (*E*)/(*Z*) diastereomers could be defined from the relative integration of imine-attached hydrogen in the corresponding ^{1}H NMR spectra (Table 1) and was confirmed by reversed-phase high performance liquid chromatography (RP-HPLC), also employing the acylhydrazone derivative (**3c**) as model compound (Fig. 3). The RP-HPLC analysis has demonstrated a ratio between (*E*)/(*Z*)-diastereomers of 2.8:1, in agreement with the relationship that we have obtained by ^{1}H NMR (see Table 1). In order to explain the polarity difference between the (*E*) and (*Z*)-diastereomers of



Scheme 1. Reagents and conditions: (a) KOH aq 3 N, *n*-BuOH, room temperature, 3 h, 98%; (b) i—O_3/O_2, AcOH, 0 °C, 1 h; ii—Zn^0, AcOH, 93% (75%, three steps); (c) i—30% H_2O_2, HCO_2H, CH_2Cl_2, reflux, 18 h; ii—NaOH 1,5 N, room temperature, 15 min, 77%; (d) K_2CO_3, DMF, then 2-bromoethyl acetate, room temperature, 20 h, 85%; (e) HNO_3, $CHCl_3$, 0 °C, 3 h, 90% (**11**) and 75% (**5**); (f) $NH_2NH_2{\cdot}H_2O$ 80%, EtOH, room temperature, 5 min, 85%; (g) Ar-CHO, EtOH, 37% aq HCl (cat.), room temperature, 30 min; (h) K_2CO_3, DMF, then benzyl bromide, 20 h, 95%.

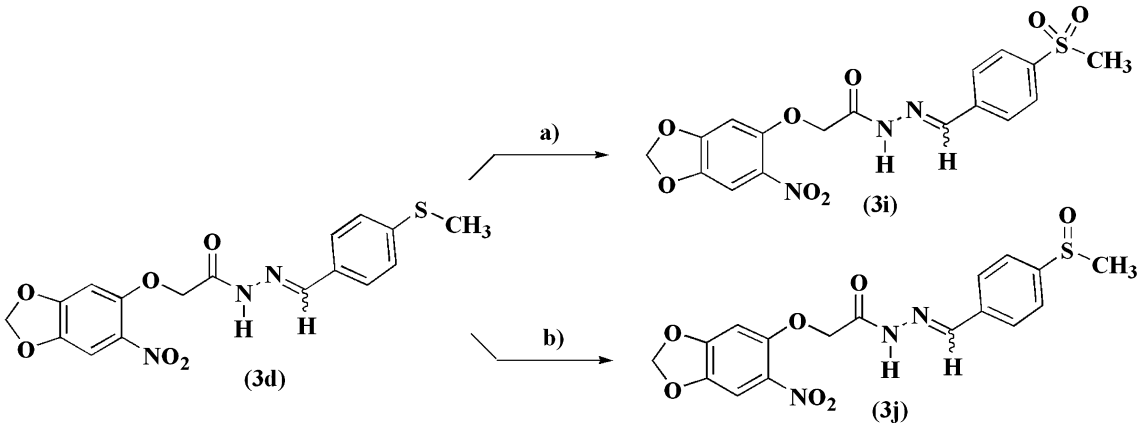
3c in the HPLC (Fig. 3), the dipole moment (μ) of the minimum energy conformers of each diastereomer was calculated using the Hartree–Fock ab initio method, in the program Spartan 1.5.0 Pro.[36] The correlation between the obtained μ values, that is, 6.91 for (*E*)-diastereomer and 7.22 for (*Z*)-diastereomer, with the elution order evidenced by HPLC, corroborated that the major diastereomer of the mixture should present the relative (*E*)-configuration.

Next, the target compounds **5**, **6**, and **7** were prepared from natural safrole (**4**), by applying the synthetic route illustrated in Scheme 1. Alkylation of sesamol (**9**) with benzyl bromide furnished the expected arylbenzylether (**6**) in 95% yield, using the same experimental protocol described previously.[21] Next, derivative (**6**) was regioselectively nitrated with nitric acid (67%) in chloroform at 0 °C, resulting in the formation of the corresponding nitroarylether derivative (**5**) in 75% yield.[22]

Thereafter, treatment of previously obtained aryl-ether derivative (**10**) with hydrazine hydrate gave the corresponding hydrazide intermediate, which after acid-catalyzed condensation with benzaldehyde furnished the desired BNAH derivative (**7**), in 59% overall yield (two steps)[12,23–26] (Scheme 1).

3. Pharmacological results

The analgesic and anti-inflammatory profiles of new NBNAH derivatives (**3a**–**j**) and its structurally simplified



Scheme 2. Reagents and conditions: (a) Oxone®, alumina, MeOH, reflux, 8 h, 85%; (b) MCPBA, CH_2Cl_2, 0 °C, 3 h, 80%.

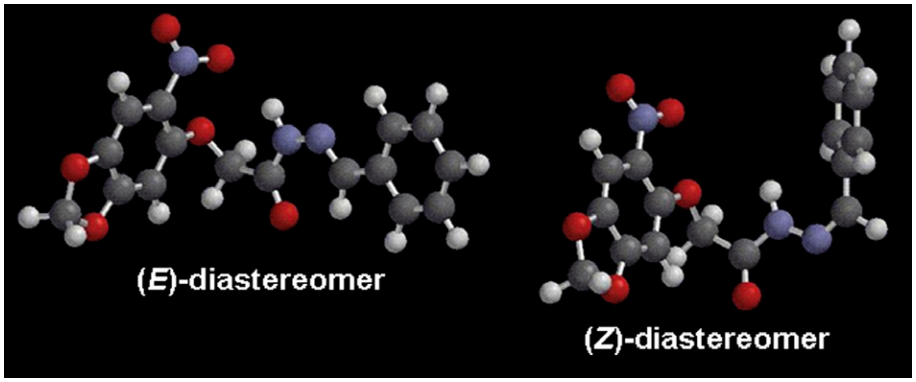


Figure 2. Minimum energy conformers of the diastereomers of NAH derivative (**3c**) obtained by applying semiempirical AM1 method.

Table 1. Physical and spectral properties of the NBNAH derivatives (**3a**–**j**)

Compound	Molecular formula[a]	M_W	Yield (%)	Mp (°C)	δ (ppm)[c] N=CH		Ratio (*E*)/(*Z*)[d]
					(*E*)	(*Z*)	
3a	$C_{18}H_{18}N_4O_6$	386.36	98	222–224	8.09	7.86	1.8/1
3b	$C_{16}H_{12}BrN_3O_6$	422.19	95	238–240	8.23	7.97	1.2/1
3c	$C_{16}H_{13}N_3O_6$	343.29	98	220–222	8.25	8.00	2.8 /1
3d	$C_{17}H_{15}N_3O_6S$	389.07	92	210–212	8.19	7.95	2.1/1
3e	$C_{17}H_{15}N_3O_6$	357.32	62	198–200	—		1.8/1[e]
3f	$C_{17}H_{15}N_3O_7$	373.32	98	238–240	8.18	7.94	2.3/1
3g	$C_{24}H_{29}N_3O_7$	471.50	98	248–250	8.16	7.90	1.7/1
3i	$C_{17}H_{15}N_3O_8S$	421.38	85[b]	184–186	7.95–7.61 (m)		2.1/1[e]
3j	$C_{17}H_{15}N_3O_7S$	405.38	80[b]	230–232	8.31–7.70 (m)		2.1/1[e]

[a] The analytical results for C, H, N, and S were within ±0.4% of calculated values.
[b] Obtained by chemoselective oxidation of the corresponding methylsulfide derivative (**3d**) as described in Scheme 2.
[c] Data obtained at 200 MHz, using DMSO-d_6 as solvent.
[d] Data obtained by relative integration of the corresponding imine-attached hydrogen of the (*E*)- and (*Z*)-diastereomers.
[e] Data obtained by relative integration of methyl group-attached hydrogens of the corresponding (*E*)- and (*Z*)-diastereomers.

analogues NBB (**5**), BB (**6**), and BNAH (**7**) (Fig. 1) were evaluated using, respectively, the classical tests of acetic acid-induced abdominal constrictions in mice[37] and carrageenan-induced rat paw edema (CIRPE),[38] employing dipyrone and nimesulide as standards. The obtained results are illustrated in Figures 4 and 5, respectively.

The analysis of these results allowed us to evidence that the most active analgesic compounds were the 4-dimethylamino derivative (**3a**) and its corresponding non-substituted analogue (**3c**), that were able to reduce remarkably the AcOH-induced constrictions, presenting 59.8% and 57.8% of inhibition, respectively. This profile is in agreement with that observed for the non-nitrated carbanalog (**2a**) (67.1% of inhibition) described previously,[12] indicating, in comparison with compound (**3a**), that no significative difference in the antinociceptive activity could be evidenced. However, a great difference in the analgesic profiles of compound (**3b**) and its non-nitrated carbanalog (**2b**) (non-significative

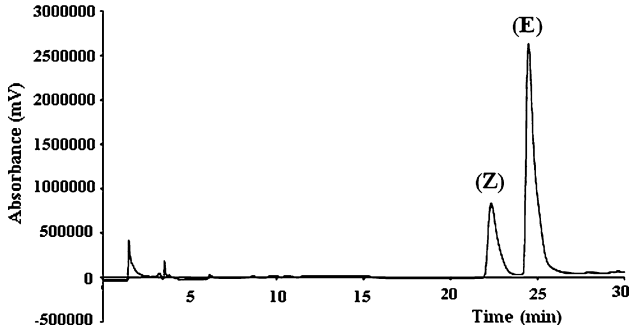


Figure 3. HPLC chromatogram of (*E*)/(*Z*)-diastereomeric mixture of NBNAH derivative **3c**. The retention time found for (*Z*)-diastereomer was 22.5 min, while the major (*E*)-diastereomer elutes after 25.0 min.

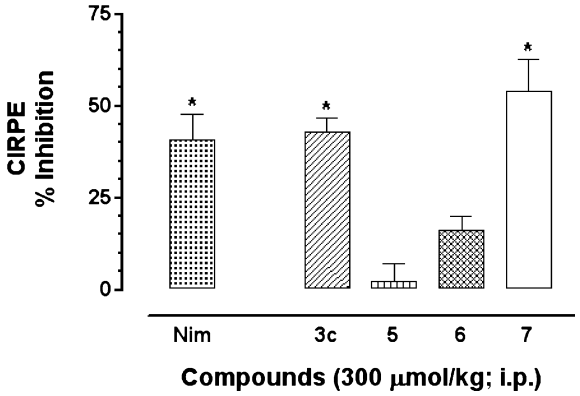


Figure 5. Anti-inflammatory effects of NBNAH derivative (**3c**), NBB derivative (**5**), BB derivative (**6**), BNAH derivative (**7**), and nimesulide (Nim). All compounds were administered i.p. at a dose of 300 μmol/kg. Results are expressed as means ± SEM for $n = 10$ animals. % of inhibition was obtained by comparison with vehicle control group (data not shown). *$p < 0.05$ (Student's *t*-test).

inhibition), as well as between compound (**3c**) and its non-nitrated carbanalog (**2c**) (25.9% of inhibition), was observed (Fig. 4). These characteristics allow us to state that the introduction of the nitro moiety and the isosteric replacement in the spacer subunit of NAH derivatives of series (**2**) generating the new NAH series (**3**) produced strong beneficial effect over its analgesic profile. In spite of that, we did not have any structural elements to infer which modification was responsible for the general increase in the antinociceptive activity of the NBNAH derivatives (**3**). In order to clarify this point, the analgesic profile of compound (**7**), non-nitrated analogue of (**3c**), was also assayed in the same pharmacological protocol presenting only 12.9% of inhibition at the same dose (Fig. 4). This expressive reduction of the analgesic activity has indicated to us the pharmacophoric character of the *ortho*-nitrophenyl framework for the antinociceptive profile of the new NBNAH derivatives of series (**3**).

Additionally, the suppression of the *N*-acylhydrazone subunit in the structure of the derivatives does not eliminate the analgesic activity in total but promotes its significative reduction as evidenced by the direct comparison of the antinociceptive profile of BB derivative (**5**) (35.6%) with that of the NAH-containing structural analogue (**3c**) (57.8%).

In addition, upon removal of both nitro and acylhydrazone moieties from the structure of (**3**), the analgesic activity of compound (**6**) (13.2%) was abolished, confirming the importance of these two subunits for the antinociceptive profile of the NBNAH derivatives (**3**). Furthermore, the unsubstituted NAH derivative (**3c**) was able to inhibit dose-dependently (5, 10, 50, 100, and 300 μmol/kg) the acetic acid-induced abdominal constrictions with an ID_{50} value of 14.48 μmol/kg which is ten times more potent than the standard analgesic drug dipyrone (ID_{50} = 144.5 μmol/kg), in the same pharmacological protocol.

The anti-inflammatory profile of NBNAH derivatives (**3a**–**j**), NBB (**5**), BB (**6**), and BNAH derivative (**7**) was evaluated in the CIRPE test at a dose of 300 μmol/kg[38] (Fig. 5).

The NAH derivatives of series (**3**) presented a poor anti-inflammatory profile (data not shown), excepting for the unsubstituted derivative (**3c**) (42.8%) identified as the most active one, suggesting that the elected *para*-substituents introduced at terminal phenyl ring do not play in

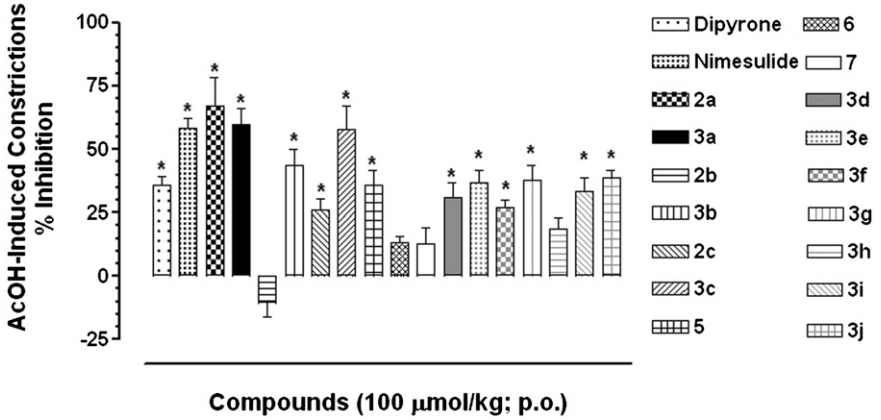


Figure 4. Analgesic effects of NBNAH derivatives (**3a**–**j**), NBB (**5**), BB (**6**), BNAH (**7**), dipyrone, and nimesulide on the acetic acid-induced (0.6%, ip) abdominal constrictions in mice. All compounds were administered p.o. at a dose of 100 μmol/kg. Results are expressed as means ± SEM for $n = 10$ animals. % of inhibition was obtained by comparison with vehicle control group (data not shown). *$p < 0.05$ (Student's *t*-test).

favor of the anti-inflammatory activity observed through this pharmacological protocol.

On the other hand, the comparison between the anti-edematogenic activity of compound (**3c**) with that of the NAH-suppressed analogue (**5**) (2.26% of inhibition) could demonstrate unambiguously the pharmacophoric character of the acylhydrazone moiety for the anti-inflammatory profile, as previously described for other NAH series developed in our laboratory.[11]

Additionally, in order to investigate the relevance of the nitrophenyl group for the anti-inflammatory profile of nitro-acylhydrazone derivative (**3c**), BNAH compound (**7**) was comparatively assayed in the CIRPE test. This compound was able to inhibit by 54.0% the edema indicating that, in spite of *ortho*-nitrophenyl moiety has a pharmacophoric character for the analgesic profile of the NBNAH derivatives (**3**), its presence does not seem to interfere with the anti-inflammatory profile of these new NAH derivatives.

Comparing the anti-edematogenic profile obtained with the simplified analogue (**6**), we can infer, once again, the great importance of the NAH framework for the anti-inflammatory profile.

Due to the fact that the NAH derivative (**3c**) only presented anti-inflammatory activity at a high dose, that is, 300 μmol/kg, we decided to investigate its anti-inflammatory profile through the administration by the alternative oral route. The obtained results are shown in Figure 6. The compound (**3c**) (20.9% of inhibition) had its anti-inflammatory activity reduced to half when orally administered. This result indicates that the nitro-acylhydrazone derivative (**3c**) and eventually its structural analogues are suffering a pharmacokinetic effect, which may contribute to reduce their bioavailability, when administered by oral route. The stomachs of the animals used in the CIRPE assay were qualitatively examined and no gastric irritation was observed for all compounds even at the dose of 300 μmol/kg.[39]

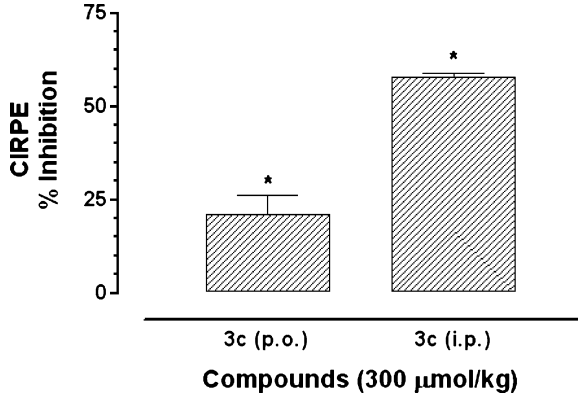


Figure 6. Comparison of the anti-inflammatory profile of the NBNAH derivative (**3c**) administered by oral versus intra-peritoneal routes. The test compound was administered at a dose of 300 μmol/kg. Results are expressed as means ± SEM for $n = 10$ animals. % of inhibition was obtained by comparison with vehicle control group (data not shown). *$p < 0.05$ (Student's *t*-test).

Moreover, considering the well-known antipyretic effect of analgesic drugs like dipyrone, we decided also to investigate the behavior of the two more potent nitro-acylhydrazone derivatives (**3a** and **3c**) in lipopolysaccharide-induced fever model,[40] as depicted in Figure 7. The results permitted us to evidence the potent and long-lasting antipyretic effect of the non-substituted acylhydrazone derivative (**3c**), which displayed a similar profile to the standard drug dipyrone in reversing the LPS-induced fever at the fourth and fifth hour of the assay. However, in spite of dipyrone not being more able to reduce the rectal temperature of the animals treated with LPS at the sixth hour of the experiment, compound **3c** remains to present an expressive antipyretic effect p.o. at the same molar concentration.

Among the new NAH derivatives (**3**), designed by hybridization strategy and isosteric replacement of the initial elected prototype (**2**), was discovered benzylidene-2-(6-nitro-benzo[1,3]dioxol-5-yloxy)-acetylhydrazine (**3c**), named LASSBio-891, which showed to be ten times more potent than dipyrone as analgesic agent. This compound, synthesized in 43% overall yield from Brazilian abundant natural product safrole (**4**) by applying classical methodology, represents a new lead-compound for an antinociceptive and antipyretic drug.

The investigation on further SAR and the complete mechanism of action of LASSBio-891 is now in progress. The present results highlighted the importance of NAH-moiety as an easily prepared biophore representing a very useful privileged structure.

4. Experimental protocols

4.1. Chemistry

Melting points were determined with a Quimis 340 apparatus and are uncorrected. ^{1}H NMR spectra were determined in deuterated chloroform or dimethylsulfoxide containing ca. 1% tetramethylsilane as an internal

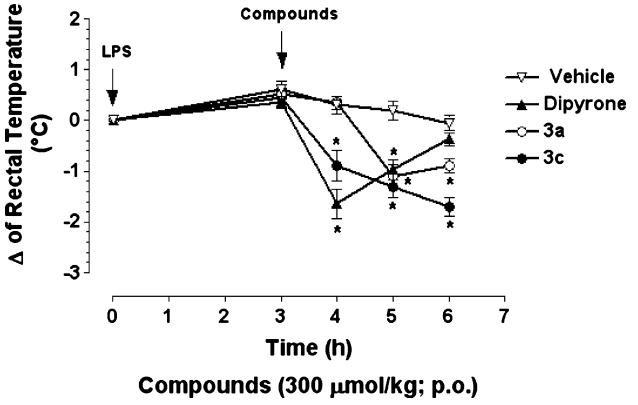


Figure 7. Antipyretic effects of the NBNAH derivatives (**3a**), (**3c**) and dipyrone on LPS-induced fever. All compounds were administered p.o. at a dose of 300 μmol/kg. Results are expressed as means ± SEM of the differences (Δ) of rectal temperature for $n = 8$ animals. *$p < 0.05$ when compared with the (Δ) rectal temperature at the third hour (Student's *t*-test and ANOVA).

standard, with Brucker DPX-200 and Varian Gemini XI-200 spectrometers, operating at 200 MHz. ^{13}C NMR spectra were determined in the same spectrometers described above at 50 MHz, employing the same solvents. Microanalyses were obtained with Perkin Elmer 240 analyzer, using Perkin Elmer AD-4 balance.

IR spectra were obtained with BOMEM FTLA 2000-100 spectrophotometer by using potassium bromide pellets. UV spectra were determined in methanol (TEDIA) solution on a Shimadzu UV 1601 spectrophotometer. HPLC analyses were performed on a Shimadzu LC10 AD apparatus, with photodiode detector (SPD-M10A) at 270 and 250 nm, equipped with a Shimadzu RP-C18 (250 × 4 mm) column. Analysis was done in gradient mode, using a mixture of acetonitrile/water as mobile phase at flow rate of 1 mL/min.

The progress of all reactions was monitored by TLC, which was performed on 2.0 × 6.0 cm 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 (254–265 nm) and treated with iodine vapor. For column chromatography Merck silica gel (70–230 mesh) was used. Reagents and solvents were purchased from commercial suppliers and used as received. The usual work-up means that the organic extracts, prior to concentration under reduced pressure, were treated with a saturated aqueous sodium chloride solution, referred as brine, dried over anhydrous sodium or magnesium sulfate, and filtered.

4.1.1. Benzo[1,3]dioxol-5-ol (sesamol) (9). To a magnetically stirred solution of piperonal (**8**) (10 g, 66.6 mmol) in 330 mL of dichloromethane were added 18 mL of 30% aq H_2O_2 (166.5 mmol, 2.5 equiv) and 14 mL of formic acid (4.0 equiv). The reaction mixture was maintained under reflux for 18 h. After cooling, 350 mL of 1.5 N NaOH solution (7.9 equiv) was added and the mixture was stirred for additional 15 min. The aqueous layer was additionally extracted with dichloromethane (4× 200 mL) to remove neutral compounds. Next, the organic layer was discarded and the remaining aqueous phase had its pH adjusted to 1–2 with concentrated HCl and extracted with dichloromethane (5× 200 mL). The organic layer was separated, dried over anhydrous magnesium sulfate, filtered, and vacuum concentrated, furnishing 8.64 g of a crude pink residue. Microdistillation of this residue yielded 7.08 g (77%) of pure sesamol (**9**) as a yellowish crystalline solid. Mp 64–66 °C [lit.[41] mp 67 °C].

4.1.2. Ethyl (benzo[1,3]dioxol-5-yloxy)acetate (10). A suspension of sesamol (**9**) (3.34 g, 24.2 mmol) in 40 mL of freshly distilled DMF containing anhydrous potassium carbonate (10.0 g, 3.0 equiv) was stirred for 30 min at room temperature, followed by addition of ethyl 2-bromoacetate (3.25 mL, 1.0 equiv). The reaction mixture was stirred for 20 h, when the TLC analysis permitted us to evidence the total consumption of **9**. Next, the reaction mixture was poured into a 1:1 mixture of crushed ice and water (50 mL) and the resulting precipitate was filtered under reduced pressure, to furnish 4.6 g (85%) of the desired ester derivative **10**, as a beige crystalline solid. Mp 60–62 °C. ^1H NMR ($CDCl_3$) δ: 6.70 (d, Ar-H_7, ^3J = 8.4 Hz), 6.54 (d, Ar-H_4, ^4J = 2.5 Hz), 6.32 (dd, Ar-H_6, ^3J = 8.4 Hz, ^4J = 2.5 Hz), 5.92 (s, O–CH_2–O), 4.54 (s, O–CH_2C=O), 4.27 (q, O–CH_2CH$_3$, J = 6.8 Hz), 1.30 (t, O–CH$_2$CH_3, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ: 169.1 (*C*=O), 153.5 (C_5), 148.5 (C_1), 142.6 (C_3), 108.0 (C_7), 106.2 (C_6), 101.4 (O*C*H$_2$O), 98.7 (C_4), 66.7 (O–*C*H$_2$C=O), 61.4 (O–*C*H$_2$CH$_3$), 14.3 (O–CH$_2$*C*H$_3$); IR (KBr) cm^{-1}: 1754 (νC=O), 1194 (νC–O).

4.1.3. Ethyl [(6-nitro-benzo[1,3]dioxol-5-yl)oxy]acetate (11). To a solution of ethyl ester derivative **10** (0.5 g, 2.23 mmol) in 30 mL of chloroform, cooled at 0 °C, was added dropwise 2.85 mL of concentrated HNO_3 (d = 1.41 g/mL) (20 equiv). After stirring for 3 h at room temperature, the end of the reaction was detected by TLC analysis and then, a sufficient amount of a saturated Na_2CO_3 solution (ca. 15 mL) was slowly added to neutralize the media. The organic phase was separated, concentrated at reduced pressure, and the obtained residue was washed with ice-cold water. The resulting precipitate was collected by filtration to give 0.54 g (90%) of desired ester derivative **11**, as a yellow solid, mp 85–87 °C. ^1H NMR ($CDCl_3$) δ: 7.42 (s, Ar-H_7), 6.60 (s, Ar-H_4), 6.08 (s, O–CH_2–O), 4.72 (s, O–CH_2C=O), 4.26 (q, O–CH_2CH$_3$, J = 7.0 Hz), 1.29 (t, O–CH$_2$CH_3, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ: 167.6 (*C*=O), 152.4 (*C*5), 149.5 (*C*1), 141.8 (*C*3), 133.3 (*C*6), 102.8 (O*C*H$_2$O), 105.2 (*C*7), 97.7 (*C*4), 67.6 (O–*C*H$_2$C=O), 61.34 (O–*C*H$_2$CH$_3$), 13.73 (O–CH$_2$*C*H$_3$); IR (KBr) cm^{-1}: 1726 (νC=O), 1622 (ν_{ass} N–O), 1193 (ν C–O), 858 (νC–N).

4.1.4. 2-[(6-Nitro-benzo[1,3]dioxol-5-yl)oxy]acetylhydrazide (12). A solution of 0.2 g of the ethyl ester derivative **11** (0.74 mmol) and 2.96 mL of 80% aq hydrazine monohydrate (7.4 mmol) in 20 mL of ethanol was stirred at room temperature for 5 min. The reaction mixture was immediately poured into a mixture of crushed ice-water giving a crude precipitate, which was collected by filtration and recrystallized in an ethanol/water to give 0.16 g (85%) of hydrazide derivative **12**, as a light yellow amorphous solid, mp 198–200 °C. ^1H NMR (DMSO-d_6) δ: 9.06 (s, CON*H*NH$_2$), 7.55 (s, Ar-H_7), 7.01 (s, Ar-H_4), 6.17 (s, O–CH_2–O), 4.66 (s, O–CH_2C=O), 4.38 (s, CONHNH_2); ^{13}C NMR (DMSO-d_6) δ: 166.3 (*C*=O), 153.2 (C_5), 150.2 (C_1), 141.6 (C_3), 141.6 (C_6), 105.4 (C_7), 103.7 (O*C*H$_2$O), 97.8 (C_4), 68.5 (O–*C*H$_2$C=O); IR (KBr) cm^{-1}: 3415 (ν_{ass} N–H), 3383 (ν_s N–H), 3340 (ν_{as} O=CN–H), 3126 (ν_s O=CN–H), 1667 (ν C=O), 1625 (ν_{ass} N–O), 1195 (ν C–O).

4.1.5. General procedure for preparation of the 2-[(6-nitro-1,3-benzodioxol-5-yl)oxy]acetylhydrazones (3a–h). To a solution of 0.3 g (1.18 mmol) of hydrazide **12** in absolute ethanol (35 mL) containing two drops of 37% hydrochloric acid (d = 1.19 g/mL) was added ca. 1.24 mmol of the desired aromatic aldehyde, previously dissolved in ca. 5 mL of ethanol. The reaction mixture was stirred at room temperature for ca. 30 min, furnishing an extensive precipitation. Next, the solvent was

concentrated at reduced pressure and the resulting residue poured into ice-cold water. After neutralization with 10% aq sodium bicarbonate solution, the precipitate formed was filtered out and dried under vacuum to give desired *N*-acylhydrazone derivatives (**3a**–**h**), generally as a colored solid.

4.1.5.1. (4′-Dimethylaminobenzylidene)-2-(6-nitro-benzo[1,3]dioxol-5-yloxy)-acetylhydrazine (3a). The derivative **3a** was obtained in 98% yield, by condensation of **12** with 4-dimethylaminobenzaldehyde, as a light orange solid, mp 222–224 °C. ^{1}H NMR (DMSO-d_6) δ: 11.37/11.14 (s, CON*H*–), 8.09/7.86 (s, –N=C*H*), 6.70–7.59 (m, Ar-H_7), 6.70–7.59 (m, Ar-$H_{2'}$), 6.70–7.59 (m, Ar-$H_{3'}$), 7.07/7.04 (s, Ar-H_4), 6.19/6.16 (s, O–CH_2–O), 5.30/4.80 (s, O–CH_2C=O), 2.96 (s, Ar–NCH_3); ^{13}C NMR (DMSO-d_6) δ: 167.6/162.5 (C=O), 152.7/152.4 (C_5), 151.6/151.4 (C_1), 150.4/149.8 (C_3), 148.7 (*C*=N), 141.2/140.7 (C_6), 121.2/121.0 ($C_{1'}$), 132.1 ($C_{4'}$), 128.5/128.2 ($C_{2'}$), 111.7 ($C_{3'}$) 104.9/104.7 (C_7), 103.3/103.0 (O*C*H$_2$O), 97.4/97.2 (C_4), 68.26/66.7 (O–*C*H$_2$C=O), 39.7 (–N*C*H$_3$); IR (KBr) cm^{-1}: 3331 (ν_{ass} –NH–), 3146 (ν_s –NH–), 1684 (ν C=O), 1507 and 1324 (ν_{ass} N–O), 1266 and 1033 (ν C–O).

4.1.5.2. (4-Bromobenzylidene)-2-(6-nitro-benzo[1,3]dioxol-5-yloxy)-acetylhydrazine (3b). The derivative **3b** was obtained in 95% yield, by condensation of **12** with 4-bromobenzaldehyde, as a light yellow solid, mp 238–240 °C. ^{1}H NMR (DMSO-d_6) δ: 11.71/11.54 (s, CON*H*–), 8.23/7.97 (s, –N=C*H*), 7.54–7.70 (m, Ar-H_7, Ar-$H_{2'}$ and Ar-$H_{3'}$), 7.09 (s, Ar-H_4), 6.19/6.16 (s, O–CH_2–O), 5.35/4.85 (s, O–CH_2C=O); ^{13}C NMR (DMSO-d_6) δ: 168.3/163.4 (*C*=O), 152.4 (C_5), 150.2/149.6 (C_1), 146.7 (*C*=N), 142.7 (C_3), 141.2/140.7 (C_6), 133.2 ($C_{1'}$), 123.5 /123.1 ($C_{4'}$), 129.0/128.0 ($C_{2'}$), 131.8/131.7 ($C_{3'}$), 104.9/104.7 (C_7, O*C*H$_2$O), 97.5/97.3 (C_4), 68.1/66.7 (O–*C*H$_2$C=O); IR (KBr) cm^{-1}: 3318 (ν_{ass} –NH–), 3186 (ν_s –NH–), 1682 (ν C=O), 1523 and 1329 (ν_{ass} N–O), 1259 and 1036 (ν C–O).

4.1.5.3. Benzylidene-2-(6-nitro-benzo[1,3]dioxol-5-yloxy)-acetylhydrazine (3c). The derivative **3c** was obtained in 98% yield, by condensation of **12** with benzaldehyde, as a light yellow solid, mp 220–222 °C. ^{1}H NMR (DMSO-d_6) δ: 11.63/11.47 (s, CON*H*–), 8.25/8.00 (s, –N=C*H*), 7.21–7.89 (m, Ar-H_7, Ar-$H_{2'}$, Ar-$H_{3'}$ and Ar-$H_{4'}$), 7.04 (s, Ar-H_4), 6.18/6.15 (s, O–CH_2–O), 5.34/4.84 (s, O–CH_2C=O); ^{13}C NMR (DMSO-d_6) δ: 168.8 (*C*=O), 153.0 (C_5), 150.8 (C_1), 144.6 (*C*=N), 141.4 (C_6), 134.5 ($C_{1'}$), 130.6 ($C_{4'}$), 129.3 ($C_{2'}$), 127.8 ($C_{3'}$), 105.3 (C_7), 103.6 (O*C*H$_2$O), 97.9 (C_4), 67.4 (O–*C*H$_2$C=O), 39.7 (–N*C*H$_3$); IR (KBr) cm^{-1}: 3449 (ν_{ass} –NH–), 3186 (ν_s –NH-), 1684 (ν C=O), 1519 and 1328 (ν_{ass} N–O), 1143 and 1035 (ν C–O).

4.1.5.4. (4′-Methylthiobenzylidene)-2-(6-nitro-benzo-[1,3]dioxol-5-yloxy)-acetylhydrazine (3d). The derivative **3d** was obtained in 92% yield, by condensation of **12** with 4-(methylthio)benzaldehyde, as a dark yellow solid, mp 210–212 °C. ^{1}H NMR (DMSO-d_6) δ: 11.59/11.40 (s, CON*H*–), 8.19/7.95 (s, –N=C*H*), 7.27–7.65 (m, Ar-H_7, Ar-$H_{2'}$ and Ar-$H_{3'}$), 7.06 (s, Ar-H_4), 6.18/6.15 (s, O–CH_2–O), 5.33/4.83 (s, O–CH_2C=O), 2.5 (s, -SCH_3); ^{13}C NMR (DMSO-d_6) δ: 168.7/163.8 (*C*=O), 153.3/153.0 (C_5), 150.9/150.3 (C_1), 148.2 (C_3), 144.2 (*C*=N), 141.9/141.4 (C_6), 130.9 ($C_{4'}$), 126.1 ($C_{1'}$), 128.2/128.0 ($C_{2'}$), 131.8/131.7 ($C_{3'}$), 105.5/105.3 (C_7), 103.9/103.6 (O*C*H$_2$O), 98.1/97.9 (C_4), 68.8/67.4 (O–*C*H$_2$C=O), 14.8 (–S*C*H$_3$); IR (KBr) cm^{-1}: 3301 (ν_{ass} –NH–), 3123 (ν_s –NH–), 1716 (ν C=O), 1498 and 1324 (ν_{ass} N–O), 1263 and 1028 (ν C–O).

4.1.5.5. (1′-Phenylethylidene)-2-(6-nitro-benzo[1,3]dioxol-5-yloxy)-acetylhydrazine (3e). The derivative **3e** was obtained in 62% yield, by condensation of **12** with acetophenone, as a light yellow solid, mp 198–200 °C. ^{1}H NMR (DMSO-d_6) δ: 10.89/10.35 (s, CON*H*–), 7.21–7.89 (m, Ar-H_7, Ar-$H_{2'}$, Ar-$H_{3'}$ and Ar-$H_{4'}$), 7.13/7.04 (s, Ar-H_4), 6.19/6.15 (s, O–CH_2–O), 5.37/4.92 (s, O–CH_2C=O), 2.33/2.25 (s, –CH_3); ^{13}C NMR (DMSO-d_6) δ: 169.6/163.8 (*C*=O), 153.6/153.5 (*C*=N), 152.9 (C_5), 150.8/150.3 (C_1), 141.7/141.2 (C_6), 133.2 ($C_{1'}$), 132.7/132.1 ($C_{4'}$), 128.8 ($C_{2'}$), 129.9/129.6 ($C_{3'}$), 105.5/105.2 (C_7), 103.8/103.5 (O*C*H$_2$O), 97.7/97.6 (C_4), 68.5/67.7 (O–*C*H$_2$C=O), 14.0 (–*C*H$_3$); IR (KBr) cm^{-1}: 3354 (ν_{ass} –NH–), 3125 (ν_s –NH–), 1709 (ν C=O), 1510 and 1325 (ν_{ass} N–O), 1266 and 1024 (ν C–O).

4.1.5.6. (4′-Methoxybenzylidene)-2-(6-nitro-benzo[1,3]dioxol-5-yloxy)-acetylhydrazine (3f). The derivative **3f** was obtained in 98% yield, by condensation of **12** with 4-methoxybenzaldehyde, as a light yellow solid, mp 238–240 °C. ^{1}H NMR (DMSO-d_6) δ: 11.46 (s, CON*H*–), 8.18/7.94 (s, –N=C*H*), 6.96–7.66 (m, Ar-H_7, Ar-$H_{2'}$, Ar-$H_{3'}$ and Ar-H_4), 6.17/6.14 (s, O–CH_2–O), 5.30/4.81 (s, O–CH_2C=O), 3.79 (s, –OCH_3); ^{13}C NMR (DMSO-d_6) δ: 168.4/163.5 (*C*=O), 161.5/161.2 ($C_{4'}$), 153.2/152.9 (C_5), 150.8 (C_1), 150.2 (C_3), 144.4 (*C*=N), 141.2 (C_6), 129.3/129.0 ($C_{1'}$), 127.0/126.9 ($C_{2'}$), 105.4/105.2 (C_7), 103.5 (O*C*H$_2$O), 97.8 (C_4), 67.2 (O–*C*H$_2$C=O), 55.7 (–O*C*H$_3$); IR (KBr) cm^{-1}: 3444 (ν_{ass} –NH–), 3188 (ν_s –NH–), 1682 (ν C=O), 1505 and 1329 (ν_{ass} N–O), 1255 and 1034 (ν C–O).

4.1.5.7. (3′,5′-Di-*tert*-butyl-4′-hydroxybenzylidene)-2-(6-nitro-benzo[1,3]dioxol-5-yloxy)-acetylhydrazine (3g). The derivative **3g** was obtained in 98% yield, by condensation of **12** with 3′,5′-di-*tert*-butyl-4′-hydroxybenzaldehyde, as a light yellow solid, mp 248–250 °C. ^{1}H NMR (DMSO-d_6) δ: 11.47/11.19 (s, CON*H*–), 8.16/7.90 (s, –N=C*H*), 7.43–7.59 (m, Ar-H_7 and Ar-$H_{2'}$), 7.08/7.02 (Ar-H_4), 6.18/6.15 (s, O–CH_2–O), 5.30/4.82 (s, O–CH_2C=O), 1.39 (s, –C(CH_3)$_3$); ^{13}C NMR (DMSO-d_6) δ: 168.3/163.3 (*C*=O), 156.8/156.4 ($C_{4'}$), 153.2/152.9 (C_5), 150.8 (C_1), 150.2/149.8 (C_3), 145.6 (*C*=N), 141.7/141.2 (C_6), 132.6 ($C_{1'}$), 125.7/125.6 ($C_{2'}$), 105.4/105.2 (C_7), 103.7/103.5 (O*C*H$_2$O), 98.0/97.6 (C_4), 68.7/68.2 (O–*C*H$_2$C=O), 34.9 (–*C*(CH$_3$)$_3$); IR (KBr) cm^{-1}: 3355 (ν_{ass} –NH–), 3129 (ν_s –NH–), 1705 (ν C=O), 1515 and 1322 (ν_{ass} N–O), 1269 and 1037 (ν C–O).

4.1.5.8. (4′-Nitrobenzylidene)-2-(6-nitro-benzo[1,3]dioxol-5-yloxy)-acetylhydrazine (3h). The derivative **3h** was obtained in 75% yield, by condensation of **12** with

4-nitrobenzaldehyde, as a dark yellow solid, mp 242–244 °C. ^{1}H NMR (DMSO-d_6) δ: 11.91/11.60 (s, CONH–), 8.36/8.09 (s, –N=CH), 8.26 (d, Ar-$H_{2'}$, ^4J = 8.2 Hz), 7.97 (d, Ar-$H_{3'}$, ^4J = 8.2 Hz), 7.58/7.52 (s, Ar-H_7), 7.10–7.08 (s, Ar-H_4), 6.18/6.15 (s, O–CH_2–O), 5.39/4.88 (s, O–CH_2C=O); ^{13}C NMR (DMSO-d_6) δ: 169.2 (C=O), 152.9 (C_5), 150.6 (C_1), 148.2 (C=N), 142.1 (C_3), 141.3 ($C_{4'}$), 140.7 ($C_{1'}$), 132.8 (C_6), 128.6/128.4 ($C_{2'}$), 105.2 (C_7), 103.6 (OCH$_2$O), 97.8 (C_4), 67.3 (O–CH$_2$C=O); IR (KBr) cm^{-1}: 3298 (ν_{ass} –NH–), 1705 (ν C=O), 1508 and 1342 (ν_{ass} N–O), 1265 and 1035 (ν C–O).

4.1.5.9. (4′-Methylsulfonylbenzylidene)-2-(6-nitrobenzo[1,3]dioxol-5-yloxy)-acetylhydrazine (3i). To a solution of 0.3 g (0.77 mmol) of the nitro-acylhydrazone derivative **3d** in 30 mL of methanol were added 0.84 g neutral alumina (Merk, Germany) and 1.66 g (2.69 mmol) of Oxone® (Aldrich Co, USA). The resulting suspension was refluxed for 8 h, when TLC analysis indicated the total consumption of the **3d**. Then, reaction mixture was concentrated at reduced pressure and the obtained crude residue was submitted to silica gel flash column chromatography eluted with dichloromethane to give **3i** in 85% yield, mp 184–186 °C. ^{1}H NMR (DMSO-d_6) δ: 11.68 (s, CONH–), 7.61–7.95 (m, –N=CH, Ar-$H_{2'}$ and Ar-H$_{3'}$), 7.57/7.51 (s, Ar-H_7), 7.20/7.07 (s, Ar-H_4), 6.18/6.15 (s, O–CH_2–O), 5.35/4.85 (s, O–CH_2C=O), 3.23/3.21 (s, –SO$_2$CH_3); ^{13}C NMR (DMSO-d_6) δ: 173.8/165.0 (C=O), 153.3 (C_5), 149.3 (C_1), 141.9 (C=N), 141.5 (C_3), 141.0 ($C_{4'}$), 139.1 ($C_{1'}$), 132.8 (C_6), 128.4/128.1 ($C_{2'}$), 125.2/124.6 ($C_{3'}$), 106.4 (C_7), 104.8 (OCH$_2$O), 103.5 (C_4), 61.4/60.7 (O–CH$_2$C=O), 43.9 (–SO$_2$CH$_3$); IR (KBr) cm^{-1}: 3299 (ν_{ass} –NH–), 1676 (ν C=O), 1509 and 1323 (ν_{ass} N–O), 1311 and 1147(ν SO$_2$), 1266 and 1028 (ν C–O).

4.1.5.10. (4′-Methylsulfinylbenzylidene)-2-(6-nitrobenzo[1,3]dioxol-5-yloxy)-acetylhydrazine (3j). To a solution of 0.3 g (0.77 mmol) of the nitroacylhydrazone derivative **3d** in 8 mL of dichloromethane was carefully added 0.96 mmol of 3-chloroperoxybenzoic acid and the resulting mixture was stirred at 0 °C, for 4 h. Thereafter, the reaction mixture was neutralized with a saturated sodium bicarbonate solution and extracted with dichloromethane (3× 50 mL). The collected organic layers were dried over anhydrous sodium sulfate and concentrated at reduced pressure. The resulting crude residue was submitted to silica gel flash column chromatography eluted with dichloromethane to furnish the desired oxidized derivative **3j** in 80% yield, as a yellow solid, mp 230–232 °C. ^{1}H NMR (DMSO-d_6) δ: 11.84/11.74 (s, CONH–), 7.70–8.31 (m, –N=CH, Ar-$H_{2'}$ and Ar-$H_{3'}$), 7.57/7.52 (s, Ar-H_7), 7.08 (s, Ar-H_4), 6.18/6.15 (s, O–CH_2–O), 5.36/4.86 (s, O–CH_2C=O), 2.76 (s, –SOCH_3); ^{13}C NMR (DMSO-d_6) δ: 169.2/169.0 (C=O), 153.0 (C4′), 150.8 (C5), 148.3 (C1), 141.9/141.4 (C=N), 139.3 (C3), 136.8 (C1′), 132.8 (C6), 128.4/128.2 (C2′), 124.6 (C3′), 105.5/105.3 (C7), 103.9/103.6 (OCH$_2$O), 98.2/97.9 (C4), 68.9/67.4 (O–CH$_2$C=O), 44.0/43.0 (–SOCH$_3$); IR (KBr) cm^{-1}: 3299 (ν_{ass} –NH–), 1708 (ν C=O), 1509 and 1323 (ν_{ass} N–O), 1268 and 1032 (ν C–O).

4.1.6. 5-(Benzyloxy)-1,3-benzodioxole (6). A suspension of sesamol (**9**) (3.34 g, 24.2 mmol) in 40 mL of freshly distilled DMF containing anhydrous potassium carbonate (10.0 g, 3.0 equiv) was stirred for 30 min at room temperature, followed by addition of benzyl bromide (2.9 mL, 1 equiv). The reaction mixture was then stirred for 20 h, when the TLC analysis permitted us to evidence the total consumption of **9**. Next, the reaction mixture was poured into a 1:1 mixture of crushed ice and water (50 mL). The resulting precipitate was filtered under reduced pressure to furnish 4.6 g (95%) of the desired ester derivative **6**, as a beige crystalline solid. Mp 52–54 °C. ^{1}H NMR (CDCl$_3$) δ: 7.30–7.45 (m, Ar-$H_{2'}$, Ar-$H_{3'}$ and Ar-$H_{4'}$), 6.69 (d, Ar-H_7, ^3J = 8.4 Hz), 6.56 (d, Ar-H_4, ^4J = 2.5 Hz), 6.39 (dd, Ar-H_6, ^3J = 8.4 Hz, ^4J = 2.5 Hz), 5.89 (s, O–CH_2–O), 4.98 (s, O–CH_2Ph); ^{13}C NMR (CDCl$_3$) δ: 154.1 (C_5), 148.1 (C_1), 141.6 (C_3), 127.7 ($C_{4'}$), 136.9 ($C_{1'}$), 127.2 ($C_{2'}$), 128.3 ($C_{3'}$), 107.7 (C_7), 106.0 (C_6), 101.9 (OCH$_2$O), 98.3 (C_4), 70.8 (O–CH$_2$Ph); IR (KBr) cm^{-1}: 1183 (ν C–O).

4.1.7. 5-(Benzyloxy)-6-nitro-1,3-benzodioxole (5). To a solution of benzyl ether derivative **6** (0.5 g, 2.23 mmol) in 30 mL of chloroform, cooled at 0 °C, was added dropwise 2.85 mL of concentrated HNO_3 (d = 1.41 g/mL) (20 equiv). After stirring for 3 h, the end of the reaction was detected by TLC analysis and a sufficient amount of a saturated Na_2CO_3 solution (ca. 15 mL) was added. The organic phase was separated, concentrated at reduced pressure, and the obtained residue was washed with ice-cold water. The resulting precipitate was collected by filtration to give 0.45 g (75%) of desired nitroether derivative **5**, as a beige solid, mp 108–110 °C. ^{1}H NMR (DMSO-d_6) δ: 7.54 (s, Ar-H_7), 7.30–7.48 (m, Ar-$H_{2'}$, Ar-H_3 and Ar-$H_{4'}$), 7.18 (s, Ar-H_4), 6.15 (s, O–CH_2–O), 4.98 (s, O–CH_2Ph); ^{13}C NMR (DMSO-d_6) δ: 167.6 (C=O), 153.2 (C_5), 150.1 (C_1), 141.2 (C_3), 136.5 ($C_{1'}$), 133.3 (C_6), 128.9 ($C_{3'}$), 128.5 ($C_{4'}$), 127.8 ($C_{2'}$), 105.4 (C_7), 103.6 (OCH$_2$O), 97.8 (C_4), 71.8 (O–CH$_2$Ph); IR (KBr) cm^{-1}: 1507 and 1331 (ν_{as} N–O), 1194 (ν C–O).

4.1.8. 2-(1,3-Benzodioxol-5-yloxy)acetylhydrazide (13). A solution of 0.2 g of the ethyl ester derivative **10** (0.74 mmol) and 2.96 mL of 80% aq hydrazine monohydrate (7.4 mmol) in 20 mL of ethanol was stirred at room temperature for 5 min. The reaction mixture was then poured into a mixture of crushed ice-water giving a crude precipitate, which was collected by filtration and recrystallized in an ethanol/water mixture, resulting in 0.12 g (80%) of hydrazide derivative **13**, as a white solid, mp 198–200 °C. ^{1}H NMR (DMSO-d_6) δ: 9.28 (s, CONHNH$_2$), 6.80 (d, Ar-H_7, ^3J = 8.4 Hz), 6.66 (d, Ar-H_4, ^4J = 2.4 Hz), 6.38 (dd, Ar-H_6, ^3J = 8.4 Hz and ^4J = 2.4 Hz), 5.95 (s, O–CH_2–O), 4.39 (s, O–CH_2C=O), 4.32 (s, CONHNH$_2$); ^{13}C NMR (DMSO-d_6) δ: 167.1 (C=O), 153.6 (C_5), 148.3 (C_1), 142.0 (C_3), 108.4 (C_6), 106.5 (C_7), 101.5 (OCH$_2$O), 98.6 (C_4), 67.6 (O–CH$_2$Ph); IR (KBr) cm^{-1}: 3320 (ν_{ass} N–H), 3240 (ν_s N–H), 3212 (ν_{as} O=CN–H), 3062 (ν_s O=CN–H), 1678 (ν C=O), 1195 (ν C–O).

4.1.9. Benzylidene 2-(benzo[1,3]dioxol-5-yloxy)-acetylhydrazine (7). To a solution of 0.3 g (1.43 mmol) of hydrazide **13** in absolute ethanol (35 mL) containing two drops of 37% hydrochloric acid (d = 1.19 g/mL) was added 1.50 mmol of benzaldehyde, previously dissolved in ca. 5 mL of ethanol. The reaction mixture was stirred at room temperature for 30 min. Next, the solvent was concentrated at reduced pressure and the resulting residue was poured into ice-cold water and neutralized with 10% aq sodium bicarbonate solution, the precipitate formed was filtered out and dried under vacuum to furnish 0.29 g (70% yield) of the desired acylhydrazone derivative (**7**), as a light beige solid, mp 173–174 °C. ^{1}H NMR (DMSO-d_6) δ: 11.52/11.32 (s, CON*H*–), 8.36/8.01 (s, –N=C*H*), 7.67–7.71 (m, Ar-$H_{2'}$), 7.42–7.43 (m, Ar-$H_{3'}$ and Ar-$H_{4'}$), 6.77–6.85 (m, Ar-H_7), 6.68 (d, Ar-H_6, ^4J = 2.3 Hz and ^3J = 11.1 Hz), 6.39 (d, Ar-H_4, ^4J = 2.3 Hz), 5.97/5.95 (s, O–CH_2–O), 5.05/4.59 (s, O–CH_2C=O); ^{13}C NMR (DMSO-d_6) δ: 169.6/164.8 (*C*=O), 154.1/153.6 (C_5), 148.5 (*C*=N), 148.4 (C_1), 148.3 (C_3), 142.2/141.8 ($C_{1'}$), 134.6/134.4 ($C_{4'}$), 129.2 ($C_{2'}$), 127.6/127.4 ($C_{3'}$), 108.5/108.4 (C_7), 106.5/106.3 (C_6), 101.6/101.5 (O*C*H$_2$O), 98.6/98.5 (C_4), 67.9/66.0 (O–*C*H$_2$C=O); IR (KBr) cm^{-1}: 3093 (ν_s -NH-), 1681 (ν C=O), 1141 and 1035 (ν C–O).

4.2. Pharmacology

4.2.1. Analgesic activity. The analgesic activity was determined in vivo by the acetic acid-induced (0.6%, 0.1 mL/10 g) abdominal constriction test in mice.[37] Albino mice of both sexes (18–23 g) were used. Compounds were administered orally (100 μmol/kg) as a suspension in 5% Arabic gum in saline (vehicle). Dipyrone (100 μmol/kg) and nimesulide (**1**) (100 μmol/kg) were used as standard drugs under the same conditions. Acetic acid solution was administered i.p. 1 h after administration of the compounds. Ten minutes after the i.p. 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 % of inhibition of constrictions when compared with the vehicle control group. Results are expressed as means ± SEM of n animals per group. The data were statistically analyzed by the Student's t-test for a significance level of $^*p < 0.05$.

When appropriated, the ID_{50} values were determined by non-linear regression using GraphPad Prism software (Version 3.00, 1999).

4.2.2. Anti-inflammatory activity. The anti-inflammatory activity was determined in vivo by the carrageenan-induced rat paw edema test according to Ferreira.[38] Fasted albino rats of both sexes (150–200 g) were used. Test compounds and the standard anti-inflammatory drug nimesulide (**1**) were administered orally and intraperitoneally (300 μmol/kg) as a suspension in 5% Arabic gum in saline (vehicle). Control animals received an equal volume of vehicle. On hour later, 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 paw, respectively. The paw volumes were measured using a glass plethysmograph coupled to a peristaltic pump, 3 h after the subplantar administration of carrageenan. The edema was calculated as the volume variation between the carrageenan-injected paw and the saline-treated paw. The anti-inflammatory activity was expressed as % of inhibition of the edema when compared with vehicle control group. Results are expressed as means ± SEM of n animals per group. The data were statistically analyzed by the Student's t-test for a significance level of $^*p < 0.05$.

At the end of the anti-inflammatory experiments, ulcerogenic effects in rats were investigated as described earlier.[39] Briefly, animals were euthanized and the stomachs excised along its greater curvature for visualization of gastric lesions with a stereomicroscope.

4.2.3. Antipyretic activity. The antipyretic effect was determined in vivo by using lipopolysaccharide (LPS)-induced fever (50 μg/kg, i.p.) in mice.[40] Albino mice of both sexes (28–32 g) were used. The rectal temperature was measured by inserting a lubricated digital thermometer, with a 0.1 °C precision, 3 cm into the rectum of the animal. Two control measurements were carried out at 1 h interval before administration of LPS. The second recorded temperature, taken immediately before LPS injection, was defined as the basal rectal temperature. Three hours after LPS, the rectal temperatures were recorded again immediately before the oral administration of the compounds (300 μmol/kg) as a suspension of 5% Arabic gum in saline (vehicle). The rectal temperatures were recorded during the following 3 h. Dipyrone (300 μmol/kg) was used as standard under the same conditions. Results are expressed as means ± SEM of the differences (Δ) between the rectal temperature at each time and the basal one, for n animals per group. Data were statistically analyzed by the Student's t-test and ANOVA (one-way) for a significance level of $^*p < 0.05$ when compared with the (Δ) rectal temperature at the third hour.

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