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Synthesis and pharmacological evaluation of pyrazine *N*-acylhydrazone derivatives designed as novel analgesic and anti-inflammatory drug candidates

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ABSTRACT

In this paper, we report the synthesis and pharmacological evaluation of pyrazine *N*-acylhydrazone (NAH) derivatives (**2a**–**s**) designed as novel analgesic and anti-inflammatory drug candidates. This series was planned by molecular simplification of prototype **1** (LASSBio-1018), previously described as a non-selective cyclooxygenase inhibitor. Derivatives **2a**–**s** were evaluated in several animal models of pain and inflammation, standing-out compound **2o** (2-*N*′-[(*E*)-(3,4,5-trimethoxyphenyl) methylidene]-2-pyrazinecarbohydrazide; LASSBio-1181), that was also active in a murine model of chronic inflammation (i.e., adjuvant-induced arthritis test in rats) and can be considered a new analgesic and anti-inflammatory lead for drug development.

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1. Introduction

Lipid mediators play pivotal roles in immune regulation, in self-defense response and in the maintenance of homeostasis in living systems. They are produced by multi-step enzymatic pathways, which are initiated by the de-esterification of membrane phospholipids by phospholipase A_2 or sphingomyelinase, and include prostaglandins (PGs), leukotrienes (LTs) and platelet-activating factor (PAF). These mediators exert their biological effects by binding to cognate receptors, which are members of the G protein-coupled receptor (GPCR) superfamily and play a fundamental role in inflammatory and immune responses.[1]

Inflammation is an adaptive response that is triggered by noxious stimuli and conditions, including infection and tissue injury, being characterized by symptoms such as pain, redness, heat, and swelling. If the acute inflammatory response fails to eliminate the pathogen, the inflammatory process persists and acquires new characteristics. The neutrophil infiltrate is replaced by macrophages, and, in case of infection, also by T cells. If the combined effect of these cells is still insufficient, a chronic inflammatory state ensues, involving the formation of granulomas and lymphoid cells.[2]

The treatment of acute inflammatory state is commonly based on the use of non-steroidal anti-inflammatory drugs (NSAIDs), which are also effective as analgesics, being frequently prescribed to treat various forms of pain, particularly chronic musculoskeletal pain. Such drugs exert their anti-inflammatory and analgesic activities by inhibiting the cyclooxygenase (COX), an enzyme that catalyzes the incorporation of two oxygen atoms into arachidonic acid to form PG endoperoxides, which are converted into various types of PGs, depending on the cell or tissue, by terminal PG synthases. Two COX isoenzymes have been identified: a constitutive form, COX-1, and an inducible form, COX-2.[1–3]

Recently, the *N*-acylhydrazone moiety was considered as a single molecular framework and as a privileged structure employed to the design of several new lead-compounds with diverse pharmacological activities, including analgesic and anti-inflammatory effects.[4]

In this report, we describe the design, synthesis and pharmacological evaluation of pyrazine *N*-acylhydrazone (NAH) derivatives (**2a**–**s**) designed as novel analgesic and anti-inflammatory drug candidates, planned by molecular simplification of prototype **1** (LASSBio-1018), previously described as non-selective COX inhibitor (Fig. 1).[5]

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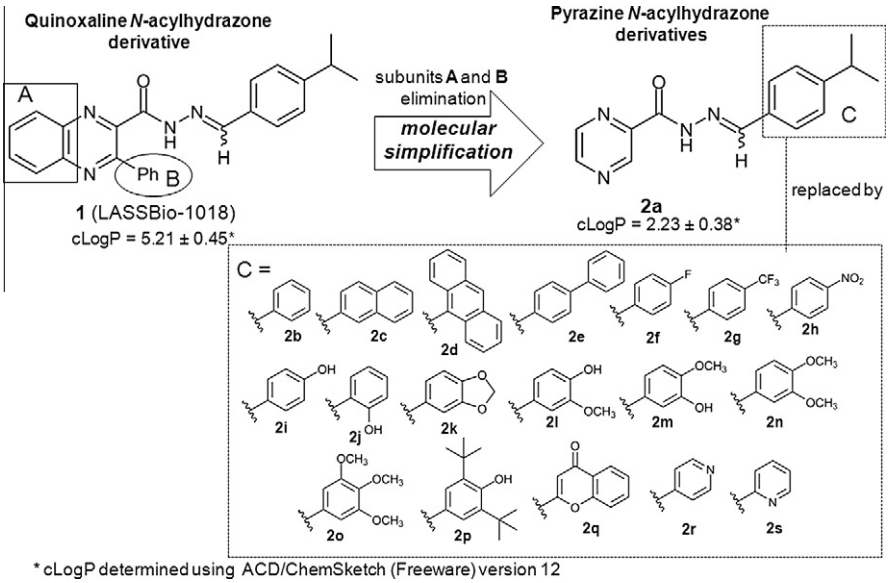


Figure 1. Design concept of pyrazine *N*-acylhydrazone derivatives **2a**–**s**.

The molecular simplification process was based on the exclusion of subunits **A** and **B** (Fig. 1) present in the lead-compound **1** (LASSBio-1018), aiming to reduce log *P* value and consequently improving aqueous solubility (Fig. 1). The resulting pyrazine pattern **2** was designed in order to keep the vicinal relationship of N1 with the carbonyl group of *N*-acylhydrazone moiety; preserving all other structural features of **1**. A congeneric series of analogues was designed by replacing the *para*-isopropylphenyl moiety of **2a** by four other substituents, as follows: Type 1: non-substituted aromatic rings [i.e., **2b**, **2c**, **2d** and **2e**]; type 2: phenyl substituted by electron-donors or electron-withdrawing groups [i.e., **2f**, **2g**, **2h**, **2i**, **2j** and **2p**]; type 3: polyoxygenated aromatic rings [i.e., **2k**, **2l**, **2m**, **2n** and **2o**]; and type 4: heteroaromatic rings [i.e., **2q**, **2r** and **2s**].

2. Chemistry

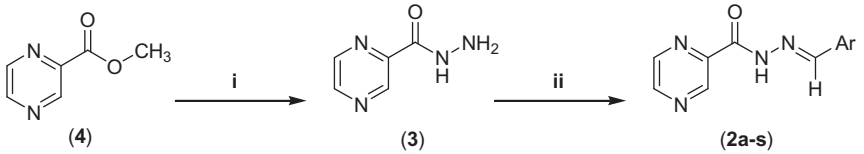
The obvious synthetic route planned to achieve the pyrazine *N*-acylhydrazone derivatives **2a**–**s** is depicted in Scheme 1. The key intermediate **3** was obtained in 87% yield by hydrazinolysis of methyl pyrazine-2-carboxylate (**4**), using hydrazine monohydrate 98% in ethanol. With the hydrazide intermediate **3** in hands, the pyrazine *N*-acylhydrazone derivatives **2a**–**s** were obtained, in good yields, by condensing the hydrazide intermediate **3**[5,16] with the corresponding aromatic aldehydes (**W**-ArCHO) in ethanol, using hydrochloric acid as catalyst.[6]

The careful analysis of the ^{1}H NMR spectra of compounds **2a**–**s** indicated the diastereoselectivity of the condensation step as depicted by the presence of only one imino-hydrogen (N=C*H*) signal for each *N*-acylhydrazone derivative (δ 8.32–8.82), which was attributed to the (*E*)-diastereomer.[7]

3. Results and discussion

All compounds (**2a**–**s**) were evaluated in the screening dose of 100 μmol/kg (po) employing chemical and thermal models of acute pain and inflammatory tests, using thalidomide (TNF-α inhibitor), indomethacin (COX-1 selective inhibitor) and celecoxib (COX-2 selective inhibitor) as drug standards. Morphine was used as standard in the hot-plate test. The lead-compound **1** (LASSBio-1018; 100 μmol/kg, po) was also used as standard in all assays, except CFA-induced arthritis. Celecoxib was administered concomitantly with thalidomide to evaluate an occasional synergistic effect.

The analgesic activity of pyrazine *N*-acylhydrazone derivatives **2a**–**s** was initially evaluated employing the acetic acid-induced abdominal writhing model in mice.[8] As shown in Table 1, all derivatives produced marked inhibition of acetic acid-induced writhing response. Compounds **2o** (97.1%) and **2q** (86.3%) proved to be significantly more active than standard celecoxib, indomethacin, thalidomide and **1**.



Ar = 4-isopropylphenyl (**2a**); phenyl (**2b**); 2-naphthyl (**2c**); 9-antracenyl (**2d**); 4-phenylbenzene (**2e**); 4-fluorophenyl (**2f**); 4-trifluoromethylphenyl (**2g**); 4-nitrophenyl (**2h**); 4-hydroxyphenyl (**2i**); 2-hydroxyphenyl (**2j**); 3,5-di(*tert*-butyl)-4-hydroxyphenyl (**2p**); 1,3-benzodioxole (**2k**); 4-hydroxy-3-methoxyphenyl (**2l**); 3-hydroxy-4-methoxyphenyl (**2m**); 3,4-dimethoxyphenyl (**2n**); 3,4,5-trimethoxyphenyl (**2o**); 4-oxo-4*H*-2-chromene (**2q**); 4-pyridinyl (**2r**) and 2-pyridinyl (**2s**).

Scheme 1. Reagents and conditions: (i) Hydrazine monohydrate 98%, EtOH, rt, 2 h, 87%; (ii) W-ArCHO, EtOH, HCl cat., rt, 2 h, 69–96%.

Table 1
Effect of pyrazine *N*-acylhydrazone derivatives **2a–s**, **1** (LASSBio-1018), thalidomide, celecoxib and indomethacin (100 μmol/kg, po) on the 0.6% acetic acid-induced abdominal constrictions in mice, for a period of 25 min

Substance	*N*	Writhing number mean ± S.E.M.	% of inhibition mean ± S.E.M.
Control	10	55.0 ± 2.3	—
Thalidomide	6	14.7 ± 1.1***	73.3 ± 5.8***
Celecoxib	6	23.7 ± 2.3***	56.9 ± 4.2***
T + C[a]	6	26.9 ± 2.6***	51.1 ± 8.4***
Indomethacin	6	14.8 ± 2.2***	73.1 ± 4.0***
1	6	12.5 ± 2.6***	77.3 ± 4.7***
2a	6	17.8 ± 2.9***	67.6 ± 5.3***
2b	6	28.4 ± 1.1***	42.4 ± 6.2***
2c	6	17.4 ± 1.5***	68.4 ± 2.7***
2d	6	36.5 ± 3.1***	33.6 ± 5.7***
2e	6	23.5 ± 2.0***	57.3 ± 3.7***
2f	6	14.0 ± 4.8***	74.5 ± 8.7***
2g	6	25.0 ± 2.7***	54.5 ± 4.9***
2h	6	32.8 ± 4.1***	40.4 ± 7.5***
2i	6	21.3 ± 1.1***	61.4 ± 2.0***
2j	6	14.8 ± 3.4***	73.1 ± 6.2***
2k	6	12.0 ± 1.0***	78.2 ± 1.9***
2l	6	29.2 ± 1.5***	47.0 ± 3.4***
2m	6	19.3 ± 2.8***	64.8 ± 5.0***
2n	6	14.5 ± 3.0***	73.6 ± 5.5 ***
2o	6	1.60 ± 0.5***	97.1 ± 0.9***
2p	6	15.6 ± 1.6***	71.6 ± 3.0***
2q	6	7.5 ± 2.3***	86.3 ± 4.2***
2r	6	32.8 ± 7.1***	40.4 ± 13.0***
2s	6	13.5 ± 3.5***	75.5 ± 6.4***

Data are expressed as mean ± S.E.M. Statistical differences between the treated and the control groups were evaluated by ANOVA and Dunnett tests and the asterisks denote the levels of significance in comparison with control groups.
*** P <0.05.
[a] Thalidomide (50 μmol/kg, po) administered concomitantly with celecoxib (50 μmol/kg, po).

Table 2
Effect of pyrazine *N*-acylhydrazone derivatives **2a–s**, **1** (LASSBio-1018), thalidomide, indomethacin and celecoxib (100 μmol/kg, po) on formalin (2.5%) test in mice

Substance	*n*	Phase 1 mean ± S.E.M.	Phase 2 mean ± S.E.M.	% of inhibition mean ± S.E.M.
Control	6	61.7 ± 6.2	192.4 ± 32.4	—
Thalidomide	6	44.6 ± 6.0	63.8 ± 10.5**	66.7 ± 5.5**
Celecoxib	6	47.4 ± 6.1	61.0 ± 7.3**	68.4 ± 3.7**
T + C[a]	6	46.1 ± 9.5	52.9 ± 10.4**	72.4 ± 5.4**
Indomethacin	6	59.0 ± 3.4	117.2 ± 10.6*	39.0 ± 5.5*
1	6	49.6 ± 5.0	64.3 ± 19.5**	66.5 ± 10.1**
2a	6	70.1 ± 5.5	64.4 ± 11.1**	66.5 ± 5.7**
2b	6	55.1 ± 11.5	84.9 ± 6.6**	60.8 ± 7.0**
2c	6	38.8 ± 5.0	61.4 ± 13.5	68.0 ± 7.0
2d	6	46.8 ± 7.9	104.2 ± 18.2*	45.75 ± 9.5
2e	6	45.9 ± 5.3	106.7 ± 4.5*	46.9 ± 3.2*
2f	6	49.0 ± 4.4	53.1 ± 10.4**	72.3 ± 5.4**
2g	6	65.8 ± 6.3	158.6 ± 30.3	27.7 ± 15.1
2h	6	64.1 ± 6.9	106.8 ± 24.1*	44.4 ± 12.5*
2i	6	65.8 ± 7.1	161.5 ± 16.9	16.7 ± 8.4
2j	6	11.9 ± 4.2***	161.2 ± 20.6	16.2 ± 8.3
2k	6	38.2 ± 5.6	102.2 ± 17.8*	46.7 ± 9.2*
2l	6	49.9 ± 5.2	131.0 ± 16.5	31.8 ± 8.6
2m	6	52.8 ± 6.0	169.3 ± 20.2	14.7 ± 9.7
2n	6	58.1 ± 10.8	139.6 ± 33.4	29.1 ± 16.3
2o	6	61.0 ± 5.2	26.8 ± 12.7**	86.0 ± 6.6**
2p	6	51.4 ± 5.5	140.5 ± 16.1	26.8 ± 8.4
2q	6	57.3 ± 5.9	90.8 ± 13.5**	52.7 ± 7.0**
2r	6	54.2 ± 6.5	79.9 ± 14.9**	58.4 ± 7.8**
2s	6	56.8 ± 3.9	79.3 ± 6.0**	56.4 ± 6.1**

Data are expressed as mean ± S.E.M. Statistical differences between the treated and the control groups were evaluated by ANOVA and Dunnett tests and the asterisks denote the levels of significance in comparison with control groups.
* P <0.05.
** P <0.01.
[a] Thalidomide (50 μmol/kg, po) administered concomitantly with celecoxib (50 μmol/kg, po).

In the formalin test,[9] which consists of two different phases—a neurogenic phase, generated by the activation of nociceptive neurons; and a second phase (called inflammatory phase), induced by activation of ventral horn neurons at the spinal cord level—10

Table 3
Time course effect of pyrazine *N*-acylhydrazone derivatives **2a–s** (100 μmol/kg, po), **1** (LASSBio-1018) (100 μmol/kg, po), morphine (15 μmol/kg, ip), thalidomide (100 μmol/kg, po) and celecoxib (100 μmol/kg, po) in the hot-plate test in mice

Substance	*n*	0 min mean ± S.E.M.	60 min mean ± S.E.M.	90 min mean ± S.E.M.	120 min mean ± S.E.M.	150 min mean ± S.E.M.
Control	6	6.1 ± 0.6	4.9 ± 0.5	5.9 ± 0.8	5.6 ± 0.5	5.1 ± 0.4
Morphine	6	6.9 ± 0.4*	12.8 ± 0.4*	10.3 ± 0.8*	9.7 ± 0.7*	9.7 ± 0.9*
Thalidomide	6	4.4 ± 0.5	48 ± 0.7	7.5 ± 1.1*	6.8 ± 0.9	5.9 ± 0.6
Celecoxib	6	4.0 ± 0.3	5.5 ± 0.6	5.7 ± 0.8	4.4 ± 0.7	3.8 ± 0.8
T + C[a]	6	3.1 ± 0.4	4.1 ± 0.3	6.9 ± 0.7	5.4 ± 1.3	2.9 ± 0.4
1	6	3.3 ± 1.0	4.6 ± 0.8	5.0 ± 1.1	5.4 ± 0.8	4.3 ± 0.4
2a	6	5.3 ± 0.8	3.3 ± 0.6	4.7 ± 0.8	5.2 ± 0.8	4.6 ± 1.2
2b	6	6.7 ± 1.4	4.4 ± 1.1	4.2 ± 1.3	4.0 ± 0.6	5.2 ± 0.7
2c	6	3.4 ± 0.7	2.8 ± 0.9	3.5 ± 0.8	4.4 ± 0.7	4.5 ± 1.1
2d	6	4.4 ± 0.8	3.7 ± 1.0	6.2 ± 0.8	5.6 ± 1.3	6.6 ± 1.8
2e	6	2.8 ± 0.5	2.7 ± 0.5	3.5 ± 0.8	4.0 ± 0.7	4.5 ± 1.1
2f	6	6.1 ± 0.7	4.6 ± 0.9	5.4 ± 0.8	4.8 ± 0.8	3.1 ± 0.7
2g	6	3.9 ± 0.6	3.1 ± 0.9	3.9 ± 0.5	2.4 ± 0.7	4.0 ± 0.5
2h	6	2.9 ± 0.8	3.1 ± 0.6	5.4 ± 1.1	4.9 ± 1.1	4.9 ± 1.2
2i	6	2.9 ± 0.6	3.1 ± 0.5	3.4 ± 0.7	5.3 ± 0.9	4.0 ± 0.7
2j	6	4.7 ± 0.6	4.0 ± 1.1	5.2 ± 0.4	6.4 ± 1.3	5.2 ± 1.2
2k	6	3.3 ± 0.5	2.4 ± 0.5	3.0 ± 0.6	4.4 ± 0.7	3.2 ± 0.7
2l	6	6.2 ± 0.8	2.6 ± 0.7	3.2 ± 0.7	3.4 ± 0.9	4.4 ± 0.7
2m	6	3.1 ± 0.4	2.0 ± 1.0	4.0 ± 1.2	1.9 ± 0.4	4.7 ± 0.6
2n	6	3.7 ± 0.8	4.8 ± 1.0	4.2 ± 0.9	6.3 ± 1.1	3.8 ± 0.8
2o	6	3.9 ± 0.6	4.0 ± 0.9	4.8 ± 0.9	4.3 ± 0.6	5.3 ± 1.0
2p	6	3.1 ± 0.5	2.7 ± 0.7	3.4 ± 0.9	3.4 ± 0.4	5.4 ± 1.5
2q	6	3.0 ± 0.3	2.5 ± 0.9	3.5 ± 0.6	6.2 ± 1.2	4.2 ± 1.2
2r	6	4.1 ± 0.3	3.5 ± 0.3	3.2 ± 0.6	5.4 ± 0.7	4.5 ± 0.8
2s	6	3.3 ± 0.7	2.4 ± 1.0	4.4 ± 1.1	4.5 ± 0.8	3.0 ± 0.6

Data are expressed as mean ± S.E.M. Statistical differences between the treated and the control groups were evaluated by ANOVA and Dunnett tests and the asterisks denote the levels of significance in comparison with control groups.
* P <0.05.
[a] Thalidomide (50 μmol/kg, po) administered concomitantly with celecoxib (50 μmol/kg, po).

compounds, that is, **2a**, **2b**, **2e**, **2f**, **2h**, **2k**, **2o**, **2q**, **2r** and **2s**, were active in the second phase, indicating their ability to inhibit nociception associated with inflammatory response (Table 2). Only compound **2j** was able to inhibit the first phase (i.e., neurogenic phase) of the formalin test (Table 2). Considering that peripherally-acting drugs, such as Aspirin® and glucocorticoids, inhibit the second phase of formalin test, without significant response in the neurogenic phase,[10] as observed for standards thalidomide, indomethacin and celecoxib (Table 2), these results suggest an anti-inflammatory profile for compounds **2a**, **2b**, **2e**, **2f**, **2h**, **2k**, **2o**, **2q**, **2r** and **2s**.

In order to investigate an occasional central antinociceptive activity for compounds **2a**–**s**, they were evaluated in the hot-plate test[11] using morphine (15 μmol/kg, ip), thalidomide (100 μmol/kg, po), celecoxib (100 μmol/kg, po) and **1** (100 μmol/kg, po) as standards. As shown in Table 3, the administration of pyrazine *N*-acylhydrazone derivatives **2a**–**s** did not increase significantly the reaction time to the nociceptive stimuli in the hot-plate test, while morphine induced a marked increase in the latency of the animals at 60 min (12.8 ± 0.4 s), 90 min (10.3 ± 0.8 s), 120 min (9.7 ± 0.7 s) and 150 min (9.7 ± 0.9 s), indicating that compounds **2a**–**s** probably do not show any central antinociceptive activity.

To confirm the anti-inflammatory profile of pyrazine *N*-acylhydrazone derivatives **2a**–**s**, as suggested by the results obtained in the formalin test, the zymosan-induced peritonitis assay was performed.[12]

As depicted in Table 4, the pyrazine *N*-acylhydrazone derivatives, with exception of compounds **2a**, **2d**, **2g**, **2h** and **2q**, were able to inhibit cell migration in zymosan-induced peritonitis model, especially compound **2k**, which presented 57.2% of inhibition.

Table 4
Effect of pyrazine *N*-acylhydrazone derivatives **2a**–**s**, **1** (LASSBio-1018), thalidomide, indomethacin and celecoxib (100 μmol/kg, po) on the zymosan-induced peritonitis in mice

Substance	*N*	Cell Number × 106/mL mean ± S.E.M.	% of inhibition mean ± S.E.M.
Control	6	25.5 ± 1.5	—
Saline	6	2.6 ± 0.6	—
Thalidomide	6	9.8 ± 1.3*	61.3 ± 6.4*
Celecoxib	6	19.9 ± 2.1	24.1 ± 6.7
T + C[a]	6	4.8 ± 1.8**	80.9 ± 6.9**
Indomethacin	6	5.3 ± 1.3**	79.4 ± 5.0**
1	6	29.3 ± 4.0	0.0 ± 0.0
2a	6	23.7 ± 4.8	19.4 ± 13.5
2b	6	18.9 ± 2.3*	21.7 ± 8.8*
2c	6	15.1 ± 2.5*	40.8 ± 9.3*
2d	6	27.3 ± 4.1	0.0 ± 0.0
2e	6	22.4 ± 1.6*	14.4 ± 5.2*
2f	6	20.0 ± 1.6*	24.5 ± 6.3*
2g	6	29.8 ± 1.3	0.0 ± 0.0
2h	6	28.6 ± 3.4	0.0 ± 0.0
2i	6	15.3 ± 3.9*	43.1 ± 12.9*
2j	6	12.4 ± 2.3*	51.2 ± 9.0*
2k	6	10.9 ± 1.0*	57.2 ± 4.0*
2l	6	16.7 ± 2.2*	34.7 ± 8.4*
2m	6	12.6 ± 2.0*	50.4 ± 7.8*
2n	6	12.4 ± 1.7*	51.3 ± 6.8*
2o	6	15.6 ± 2.2*	38.8 ± 8.7*
2p	6	14.2 ± 1.4*	44.4 ± 5.6*
2q	6	28.2 ± 5.9	0.0 ± 0.0
2r	6	15.1 ± 1.8*	12.7 ± 12.7*
2s	6	22.7 ± 4.4	42.9 ± 15.4

Data are expressed as mean ± S.E.M. Statistical differences between the treated and the control groups were evaluated by ANOVA and Dunnett tests and the asterisks denote the levels of significance in comparison with control groups.
* P <0.05.
** P <0.01.
[a] Thalidomide (50 μmol/kg, po) administered concomitantly with celecoxib (50 μmol/kg, po).

In order to analyze the effects of pyrazine *N*-acylhydrazone derivatives **2a**–**s** on other components of inflammatory response, these compounds were studied using capsaicin-induced ear edema in mice. In this model, topical application of capsaicin in the ear of mice produces neurogenic acute inflammatory responses, such as axon reflex vasodilatation, plasma leakage and erythema.[13,14]

As demonstrated in Table 5, compounds **2d**, **2g**, **2l**, **2n**, **2o** and **2q** were able to produce an antiedematogenic activity. However, the pyrazine *N*-acylhydrazone derivatives **2o** (LASSBio-1181) and

Table 5
Effect of pyrazine *N*-acylhydrazone derivatives **2a**–**s**, **1** (LASSBio-1018), thalidomide, indometacin and celecoxib (100 μmol/kg, po) in capsaicin-induced ear edema in mice

Substance	*n*	% of edema mean ± S.E.M.	% of inhibition mean ± S.E.M.
Control	10	58.5 ± 7.5	—
Thalidomide	6	46.6 ± 6.8	25.2 ± 8.8
Celecoxib	6	25.5 ± 3.5**	56.4 ± 5.9**
T + C[a]	6	24.9 ± 4.2**	57.4 ± 7.2**
Indomethacin	6	29.7 ± 6.8*	49.3 ± 11.6*
1	6	30.7 ± 5.0	47.5 ± 8.7
2a	6	31.2 ± 9.8	46.6 ± 16.8
2b	6	40.6 ± 7.2	30.5 ± 12.4
2c	6	37.8 ± 2.7	35.4 ± 4.7
2d	6	26.5 ± 6.5**	54.7 ± 11.1**
2e	6	32.9 ± 13.2	47.7 ± 20.0
2f	6	39.1 ± 7.6	33.1 ± 13.0
2g	6	30.2 ± 2.3*	48.3 ± 4.0*
2h	6	34.0 ± 6.4	41.9 ± 11.0
2i	6	33.7 ± 1.6	42.3 ± 2.7
2j	6	33.9 ± 9.9	42.0 ± 16.9
2k	6	32.3 ± 8.6	44.7 ± 11.3
2l	6	21.3 ± 6.7*	63.5 ± 11.5*
2m	6	42.8 ± 3.4	27.0 ± 5.7
2n	6	29.5 ± 5.9**	49.6 ± 10.1**
2o	6	17.6 ± 5.3**	70.0 ± 9.1**
2p	6	39.5 ± 6.3	32.3 ± 10.8
2q	6	26.1 ± 6.7**	55.3 ± 11.5**
2r	6	45.7 ± 6.9	32.3 ± 6.9
2s	6	32.2 ± 10.7	44.9 ± 18.2

Data are expressed as mean ± S.E.M. Statistical differences between the treated and the control groups were evaluated by ANOVA and Dunnett tests and the asterisks denote the levels of significance in comparison with control groups.
* P <0.05.
** P <0.01.
[a] Thalidomide (50 μmol/kg, po) administered concomitantly with celecoxib (50 μmol/kg, po).

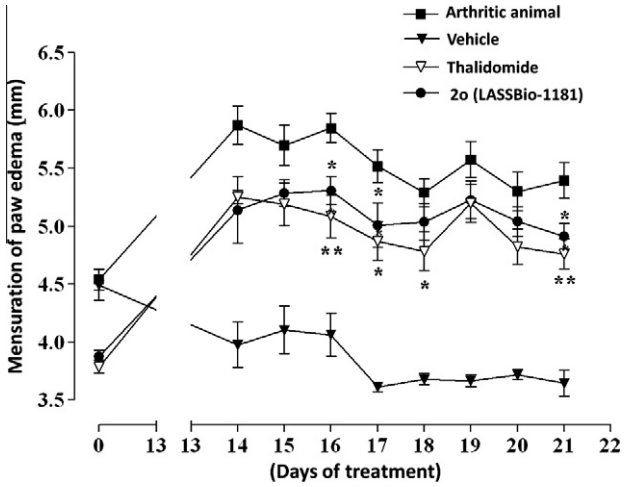


Figure 2. Effect of compound **2o** (LASSBio-1181) and thalidomide (100 μmol/kg, po) on the CFA-induced arthritis in rats. Each point represents the mean ± S.E.M. of six animals. Statistical differences between the treated and the control groups were evaluated by ANOVA and Dunnett tests and the asterisks denote the levels of significance in comparison with control groups.**P <0.01 and *P <0.05.

2l (LASSBio-1182) can be pointed as the most active derivatives when compared to standards thalidomide, celecoxib, indomethacin and **1**.

The set of results herein described (e.g., writhing test, formalin and hot-plate models, zymosan-induced peritonitis and capsaicin-induced ear edema) enabled **2o** to be selected as the most important compound with anti-inflammatory and analgesic profiles among the pyrazine *N*-acylhydrazone derivatives **2a**–**s**. Therefore, prototype **2o** (LASSBio-1181) was further evaluated in a chronic inflammatory model, using complete Freund's adjuvant (CFA)-induced arthritis test in rats.[15] As illustrated in Figure 2, in the CFA-induced arthritis model, the treatment with **2o** (100 μmol/Kg, po) and thalidomide (100 μmol/Kg, po) for seven days resulted in statistically significant inhibition of paw edema on the 16th, 17th and 21st days, indicating their ability to control a chronic inflammatory process.

4. Conclusion

We have designed and synthesized nineteen pyrazine *N*-acylhydrazone derivatives **2a**–**s** structurally planned by molecular simplification of the lead-compound **1** (LASSBio-1018). Derivatives **2a**–**s** were found to have antinociceptive and anti-inflammatory activities, especially **2o** (2-*N*′-[(*E*)-(3,4,5-trimethoxyphenyl)methylidene]-2-pyrazinecarbohydrazide; LASSBio-1181). This compound presented a better pharmacological profile than prototype **1** and was also active in a murine model of chronic inflammation (i.e., adjuvant-induced arthritis test in rats). Therefore, compound **2o** (LASSBio-1181) can be considered a new analgesic and anti-inflammatory lead for drug development.

5. Experimental

5.1. General

Reactions were routinely monitored by thin-layer chromatography (TLC) in silica gel (F245 Merck plates) and the products visualized with iodine or ultraviolet lamp (254 and 365 nm). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were determined in DMSO-d_6 or $CDCl_3$ solutions using a Bruker AC-200 or a Varian UNITY-300 spectrometer. Peak positions are given in parts per million (δ) using tetramethylsilane as internal standard, and coupling constant values (J) are given in Hertz. Signal multiplicities are represented by: s (singlet), d (doublet), t (triplet), q (quadruplet), qu (quintuplet), m (multiplet) and br (broad signal). Infrared (IR) spectra were obtained using an ABB FTLA2000-100 IR spectrometer. Samples were examined as potassium bromide (KBr) disks. Elemental microanalyses were obtained on an Elemental Analyzer (Flash EA 1112 Series, Thermo Scientific) from vacuum-dried samples. The analytical results for C, H, and N, were within ±0.4% of the theoretical values. Melting points were determined using a Quimis instrument and are uncorrected. All described products showed ^{1}H and ^{13}C NMR spectra according to the assigned structures. All organic solutions were dried over anhydrous sodium sulphate and all organic solvents were removed under reduced pressure using a rotatory evaporator.

5.1.1. Pyrazine-2-carbohydrazide (3)

Hydrazine monohydrate 98% (1.77 mL; 1.81 g; 36.2 mmol) was added to a 5 mL ethanolic solution of methyl 2-pyrazinecarboxylate (**4**) (0.5 g; 3.62 mmol), and the reaction mixture was stirred for 2 h, at room temperature, when TLC indicated the end of reaction. The solvent was removed under reduced pressure, followed by addition of a water/ice mixture (1:1) to the obtained residue, promoting extensive precipitation. The solid was filtered through a Buckner funnel and recovered as yellow needles in 87% yield, mp 158–159 °C. The melting point and ^{1}H NMR data for compound **3** are in agreement with previous reports.[16]

^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 4.56 (br, 2H, NH_2); 8.69 (dd, 1H, J = 1.4 and 2.4 Hz, H5); 8.82 (d, 1H, J = 2.4 Hz, H6); 9.12 (d, 1H, J = 1.4 Hz, H3); 10.11 (s, 1H, CON*H*). ^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 143.7 (C5); 144.0 (C3); 145.4 (C2); 147.8 (C6); 162.0 (*C*=O). IR (ν_{max}, KBr) ν (cm^{-1}): 3306, 3242, 3054, 1679, 1576, 1307, 858. Anal. Calcd for $C_5H_6N_4O$: C, 43.48; H, 4.38; N, 40.56. Found: C, 43.56; H, 4.36; N, 40.26.

5.1.2. General procedure for the preparation of pyrazine-2-*N*-acylhydrazones (2a–s)

The corresponding aromatic or heteroaromatic aldehyde (0.72 mmol) was added to a solution of pyrazine-2-carbohydrazide (**3**) (100 mg; 0.72 mmol) in absolute ethanol (6 mL) containing one drop of 37% hydrochloric acid. The mixture was stirred at room temperature for 2 h until extensive precipitation was visualized. Afterwards, the solvent was partially concentrated at reduced pressure and the resulting mixture was poured into cold water. After neutralization with 10% aqueous sodium bicarbonate solution, the precipitate formed was filtered out and dried under vacuum producing the desired *N*-acylhydrazone derivatives **2a**–**s**, as described below.

5.1.2.1. *N*′-[(1*E*)-(4-(1-Methylethyl)phenyl)methylidene]pyrazine-2-carbohydra-zide (2a; LASSBio-1244). The title compound was obtained as a white solid by the condensation of **3** with 4-(1-methylethyl)benzaldehyde in 77% yield, mp 232–235 °C. ^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ (ppm): 1.27 (d, 6H, J = 6.8 Hz, CH_3); 2.93 (m, 1H, ArCH(CH_3)$_2$); 7.29 (d, 2H, J = 7.9 Hz, H3′ and H5′); 7.75 (d, 2H, J = 7.9 Hz, H2′ and H6′); 8.32 (s, 1H, N=C*H*); 8.56 (dd, 1H, J = 1.4 and 2.4 Hz, H5); 8.82 (d, 1H, J = 2.4 Hz, H6); 9.52 (d, 1H, J = 1.4 Hz, H3); 10.67 (s, 1H, CON*H*). ^{13}C NMR (50 MHz, $CDCl_3$, TMS) δ (ppm): 23.8 (*C*H$_3$); 34.2 ArCH(CH$_3$)$_2$); 127.0 (C3′ and C5′); 128.1 (C2′ and C6′); 131.0 (C1′); 142.5 (C5); 144.0 (C3); 145.0 (C2); 147.8 (C6); 150.0 (N=*C*H); 152.3 (C4′); 158.8 (*C*=O). IR (ν_{max}, KBr) ν (cm^{-1}): 3298, 3080, 2963, 2871, 1679, 1268, 828. Anal. Calcd for $C_{15}H_{16}N_4O$: C, 67.15; H, 6.01; N, 20.88. Found: C, 67.28; H, 6.12; N, 20.77.

5.1.2.2. *N*′-[(1*E*)-Phenylmethylidene]pyrazine-2-carbohydrazide (2b; LASSBio-1243). The title compound was obtained as a white solid by the condensation of **3** with benzaldehyde in 87% yield, mp 227–230 °C. The melting point and ^{1}H NMR data are in agreement with those previously published.[16,18] ^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 7.47 (br, 3H, H3′, H4′ and H5′); 7.73 (br, 2H, H2′ and H6′); 8.64 (s, 1H, N=C*H*); 8.79 (dd, 1H, J = 1.1 and 2.1 Hz, H5); 8.92 (d, 1H, J = 2.1 Hz, H6); 9.26 (d, 1H, J = 1.1 Hz, H3); 12.28 (s, 1H, CON*H*). ^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 127.8 (C3′ and C5′); 129.4 (C2′ and C6′); 130.9 (C4′); 134.7 (C1′); 143.9 (C5); 144.6 (C3); 145.2 (C2); 148.4 (C6); 150.5 (N=*C*H); 160.1 (*C*=O). IR (ν_{max}, KBr) ν (cm^{-1}): 3296, 3035, 1679, 1271, 845, 759, 693. Anal. Calcd for $C_{12}H_{10}N_4O$: C, 63.71; H, 4.46; N, 24.76. Found: C, 63.69; H, 4.42; N, 25.10.

5.1.2.3. *N*′-[(1*E*)-Naphthalen-2-ylmethylidene]pyrazine-2-carbohydrazide (2c; LASSBio-1190). The title compound was obtained as yellow needles by the condensation of **3** with naphthalene-2-carbaldehyde in 82% yield, mp 190–193 °C. ^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 7.65 (m, 3H, H5′, H6′ and H7′); 8.02 (m, 3H, H3′, H4′ and H8′); 8.82 (br, 2H, H1′ and N=C*H*); 8.94 (dd, 1H, J = 1.0 and 2.0 Hz, H5); 9.31 (d, 1H, J = 2.0 Hz, H6); 9.38 (d, 1H, J = 1.0 Hz, H3); 12.36 (s, 1H, CON*H*). ^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 124.6 (C3′); 126.1 (C7′); 126.9 (C5′); 127.9 (C6′); 128.0 (C4′); 129.4 (C8′); 130.0

(C8a′); 131.0 (C2′); 131.4 (C1′); 134.1 (C4a′); 143.9 (C5); 144.7 (C3); 145.1 (C2); 148.4 (C6); 150.1 (N=*C*H); 160.0 (*C*=O). IR (ν_{max}, KBr) ν (cm^{-1}): 3302, 3044, 1701, 1268, 770. Anal. Calcd for $C_{16}H_{12}N_4O$: C, 69.55; H, 4.38; N, 20.28. Found: C, 69.46; H, 4.36; N, 20.26.

5.1.2.4. *N′*-[(1*E*)-Anthracen-9-ylmethylidene]pyrazine-2-carbohydrazide (2d; LASSBio-1192). The title compound was obtained as a yellow solid by the condensation of **3** with anthracene-9-carbaldehyde in 74% yield, mp 248–250 °C. ^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 7.63 (m, 4H, H2′, H3′, H6′ and H7′); 8.16 (d, 2H, *J* = 8.2 Hz, H4′ and H5′); 8.74 (br, 1H, H10′); 8.53 (m, 3H, H1′, H8′ and N=C*H*); 8.97 (dd, 1H, *J* = 1.4 and 2.4 Hz, H5); 9.34 (d, 1H, *J* = 2.4 Hz, H6); 9.88 (d, 1H, *J* = 1.4 Hz, H3); 12.60 (s, 1H, CON*H*). ^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 125.5 (C9′); 125.6 (C1′ and C8′); 126.1 (C3′ and C6′); 127.8 (C2′ and C7′); 129.6 (C4′ and C5′); 130.4 (C4a′ and C5a′); 130.5 (C10′); 131.5 (C1a′ and C8a′); 143.9 (C5); 144.7 (C3); 145.1 (C2); 148.5 (C6); 149.9 (N=CH); 160.1 (*C*=O). IR (ν_{max}, KBr) ν (cm^{-1}): 3201, 3051, 1670, 1282, 869, 729. Anal. Calcd for $C_{20}H_{14}N_4O$: C, 73.61; H, 4.32; N, 17.17. Found: C, 73.26; H, 4.29; N, 16.81.

5.1.2.5. *N′*-[(1*E*)-Biphenyl-4-ylmethylidene]pyrazine-2-carbohydrazide (2e; LASSBio-1191). The title compound was obtained as a white solid by the condensation of **3** with biphenyl-4-carbaldehyde in 88% yield, mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6, TMS) δ (ppm): 7.38 (t, 1H, *J* = 7.2 Hz, H4″); 7.48 (dd, 2H, *J* = 7.2 and 7.4 Hz, H3″ and H5″); 7.71 (d, 2H, *J* = 7.4 Hz, H2″ and H6″); 7.77 (d, 2H, *J* = 8.4 Hz, H2′ and H6′); 7.82 (d, 2H, *J* = 8.4 Hz, H3′ and H5′); 8.67 (s, 1H, N=C*H*); 8.78 (dd, 1H, *J* = 1.2 and 2.4 Hz, H5); 8.91 (d, 1H, *J* = 2.4 Hz, H6); 9.26 (d, 1H, *J* = 1.2 Hz, H3); 12.24 (s, 1H, CON*H*). IR (ν_{max}, KBr) ν (cm^{-1}): 3285, 3013, 1677, 1275, 835, 764, 699. Anal. Calcd for $C_{18}H_{14}N_4O$: C, 71.51; H, 4.67; N, 18.53. Found: C, 71.60; H, 4.61; N, 18.47.

5.1.2.6. *N′*-{(1*E*)-[4-Fluorophenyl]methylidene}pyrazine-2-carbohydrazide (2f; LASSBio-1270). The title compound was obtained as a beige solid by condensation of **3** with 4-fluorobenzaldehyde in 96% yield, mp 210–212 °C. The melting point and ^{1}H NMR data are in agreement with those previously published.[16,18] ^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 7.30 (dd, 2H, $^3J_{H,H}$ = 8.5 Hz, $^3J_{H,F}$ = 8.9 Hz, H3′ and H5′); 7.79 (dd, 2H, $^3J_{H,H}$ = 8.5 Hz, $4J_{H,F}$ = 5.4 Hz, H2′ and H6′); 8.64 (s, 1H, N=C*H*); 8.79 (dd, 1H, *J* = 1.4 and 2.4 Hz, H5); 8.92 (d, 1H, *J* = 2.4 Hz, H6); 9.26 (d, 1H, *J* = 1.4 Hz, H3); 12.29 (s, 1H, CON*H*). ^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 115.9 (d, $^2J_{C,F}$ = 22 Hz, C3′ and C5′); 129.4 (d, $^3J_{C,F}$ = 9 Hz, C2′ and C6′); 130.7 (C1′); 143.3 (C5); 144.0 (C3); 144.6 (C2); 147.8 (C6); 148.7 (N=*C*H); 159.5 (*C*=O); 163.3 (d, $^1J_{C,F}$ = 246 Hz, C4′). IR (ν_{max}, KBr) ν (cm^{-1}): 3297, 3022, 1678, 1295, 1146, 835. Anal. Calcd for $C_{12}H_9FN_4O$: C, 59.01; H, 3.71; N, 22.94. Found: C, 58.97; H, 3.49; N, 22.89.

5.1.2.7. *N′*-{(1*E*)-[4-(Trifluoromethyl)phenyl]methylidene}pyrazine-2-carbo-hydrazide (2g; LASSBio-1193). The title compound was obtained as a beige solid by the condensation of **3** with 4-(trifluoromethyl)benzaldehyde in 77% yield, mp 240–243 °C. ^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 7.82 (d, 2H, *J* = 7.8 Hz, H3′ and H5′); 7.94 (d, 2H, *J* = 7.8 Hz, H2′ and H6′); 8.72 (s, 1H, N=C*H*); 8.80 (dd, 1H, *J* = 1.4 and 2.4 Hz, H5); 8.94 (d, 1H, *J* = 2.4 Hz, H6); 9.28 (d, 1H, *J* = 1.4 Hz, H3); 12.49 (s, 1H, CON*H*). ^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 124.1 (q, $^1J_{C,F}$ = 276 Hz, CF_3); 125.7 (q, $^3J_{C,F}$ = 4 Hz, C3′ and C5′); 127.8 (C2′ and C6′); 130.3 (q, $^2J_{C,F}$ = 32 Hz, C4′); 138.1 (C1′); 143.3 (C5); 144.2 (C3); 144.4 (C2); 148.0 (C6); 148.1 (N=CH); 159.8 (*C*=O). IR (ν_{max}, KBr) ν (cm^{-1}): 3298, 3056, 1683, 1272, 1116, 840. Anal. Calcd for $C_{13}H_9F_3N_4O$: C, 53.07; H, 3.08; N, 19.04. Found: C, 53.18; H, 3.14; N, 19.19.

5.1.2.8. *N′*-[(1*E*)-(4-Nitrophenyl)methylidene]pyrazine-2-carbohydrazide (2h; LASSBio-1194). The title compound was obtained as a yellow solid by the condensation of **3** with 4-nitrobenzaldehyde in 93% yield, mp >250 °C. The melting point and ^{1}H NMR data are in agreement with those previously published.[16,18] ^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 7.99 (d, 2H, *J* = 8.9 Hz, H2′ and H6′); 8.32 (d, 2H, *J* = 8.9 Hz, H3′ and H5′); 8.76 (s, 1H, N=C*H*); 8.81 (dd, 1H, *J* = 1.4 and 2.4 Hz, H5); 8.95 (d, 1H, *J* = 2.4 Hz, H6); 9.29 (d, 1H, *J* = 1.4 Hz, H3); 12.60 (s, 1H, CON*H*). IR (ν_{max}, KBr) ν (cm^{-1}): 3303, 3029, 1686, 1527, 1368, 1270, 853. Anal. Calcd for $C_{12}H_9N_5O_3$: C, 53.14; H, 3.34; N, 25.82. Found: C, 53.48; H, 3.22; N, 25.72.

5.1.2.9. *N′*-[(1*E*)-(4-Hydroxyphenyl)methylidene]pyrazine-2-carbohydrazide (2i; LASSBio-1185). The title compound was obtained as a yellow solid by the condensation of **3** with 4-hydroxybenzaldehyde in 87% yield, mp >250 °C. The melting point and ^{1}H NMR data are in agreement with those previously published.[16,18] ^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 6.85 (d, 2H, *J* = 8.5 Hz, H3′ and H5′); 7.56 (d, 2H, *J* = 8.5 Hz, H2′ and H6′); 8.53 (s, 1H, N=C*H*); 8.76 (dd, 1H, *J* = 1.4 and 2.4 Hz, H5); 8.90 (d, 1H, *J* = 2.4 Hz, H6); 9.25 (d, 1H, *J* = 1.4 Hz, H3); 9.97 (s, 1H, O*H*); 12.06 (s, 1H, CON*H*). IR (ν_{max}, KBr) ν (cm^{-1}): 3300, 3274, 3002, 1678, 1268, 846. Anal. Calcd for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.16; N, 23.13. Found: C, 59.68; H, 4.24; N, 23.39.

5.1.2.10. *N′*-[(1*E*)-(2-Hydroxyphenyl)methylidene]pyrazine-2-carbohydrazide (2j; LASSBio-1186). The title compound was obtained as a beige solid by the condensation of **3** with 2-hydroxybenzaldehyde in 95% yield, mp 198–200 °C. The melting point and ^{1}H NMR data are in agreement with those previously published.[16,18] ^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 6.90 (d, 1H, *J* = 6.5 Hz, H3′); 6.95 (dd, 1H, *J* = 7.5 and 7.8 Hz, H5′); 7.31 (dd, 1H, *J* = 6.5 and 7.8 Hz, H4′); 7.51 (d, 1H, *J* = 7.5 Hz, H6′); 8.78 (dd, 1H, *J* = 1.4 and 2.4 Hz, H5); 8.81 (s, 1H, N=C*H*); 8.91 (d, 1H, *J* = 2.4 Hz, H6); 9.26 (d, 1H, *J* = 1.4 Hz, H3); 11.26 (s, 1H, O*H*); 12.59 (s, 1H, CON*H*). IR (ν_{max}, KBr) ν (cm^{-1}): 3416, 3291, 3052, 1670, 1266, 856, 761. Anal. Calcd for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.16; N, 23.13. Found: C, 59.58; H, 4.12; N, 23.40.

5.1.2.11. *N′*-[(1*E*)-1,3-Benzodioxol-5-ylmethylidene]pyrazine-2-carbohydrazide (2k; LASSBio-1180). The title compound was obtained as a yellow solid by the condensation of **3** with 1,3-benzodioxole-5-carbaldehyde in 84% yield, mp 227 °C. ^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 6.10 (s, 2H, CH_2); 6.99 (d, 1H, *J* = 7.8 Hz, H5′); 7.16 (dd, 1H, *J* = 1.4 and 7.8 Hz, H6′); 7.31 (d, 1H, *J* = 1.4 Hz, H2′); 8.54 (s, 1H, N=C*H*); 8.78 (dd, 1H, *J* = 1.4 and 2.4 Hz, H5); 8.92 (d, 1H, *J* = 2.4 Hz, H6); 9.25 (d, 1H, *J* = 1.4 Hz, H3); 12.21 (s, 1H, CON*H*). ^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 102.2 (*C*H_2); 105.7 (C2′); 109.1 (C5′); 124.3 (C6′); 129.1 (C1′); 143.9 (C5); 144.6 (C3); 145.3 (C2); 148.3 (C6); 148.6 (N=*C*H); 149.9 (C3′); 150.2 (C4′); 159.9 (*C*=O). IR (ν_{max}, KBr) ν (cm^{-1}): 3278, 3068, 1682, 1257, 866. Anal. Calcd for $C_{13}H_{10}N_4O_3$: C, 57.78; H, 3.73; N, 20.73. Found: C, 57.60; H, 3.67; N, 20.64.

5.1.2.12. *N′*-[(1*E*)-(4-Hydroxy-3-methoxyphenyl)methylidene]pyrazine-2-carbo-hydrazide (2l; LASSBio-1182). The title compound was obtained as a beige solid by the condensation of **3** with 4-hydroxy-3-methoxybenzaldehyde in 95% yield, mp >250 °C. ^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 3.81 (s, 3H, CH_3); 6.97 (d, 1H, *J* = 8.2 Hz, H5′); 7.05 (d, 1H, *J* = 8.2 Hz, H6′); 7.28 (s, 1H, H2′); 8.48 (s, 1H, N=C*H*); 8.78 (dd, 1H, *J* = 1.4 and 2.4 Hz, H5); 8.91 (d, 1H, *J* = 2.4 Hz, H6); 9.25 (d, 1H, *J* = 1.4 Hz, H3); 9.37(s, 1H, O*H*); 12.12 (s, 1H, CON*H*). ^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 55.6 (*C*H_3); 111.9 (C2′); 112.5 (C5′); 120.6 (C6′); 127.0 (C1′); 143.3 (C5); 144.0 (C3); 144.8 (C2); 146.9 (C6); 147.7

(N=CH); 150.0 (C3′); 150.1 (C4′); 159.3 (C=O). IR (ν_{max}, KBr) ν (cm^{-1}): 3368, 3265, 3095, 1673, 1274, 864. Anal. Calcd for $C_{13}H_{12}N_4O_3$: C, 57.35; H, 4.44; N, 20.58. Found: C, 57.28; H, 4.40; N, 20.77.

5.1.2.13. *N′*-[(1*E*)-(3-Hydroxy-4-methoxyphenyl)methylidene] pyrazine-2-carbohy-drazide (2m; LASSBio-1183). The title compound was obtained as a yellow solid by the condensation of **3** with 3-hydroxy-4-methoxybenzaldehyde in 95% yield, mp 221–223 °C. ^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 3.84 (s, 3H, C*H*$_3$); 6.85 (d, 1H, *J* = 8.2 Hz, H5′); 7.08 (d, 1H, *J* = 8.2 Hz, H6′); 7.32 (s, 1H, H2′); 8.51 (s, 1H, N=C*H*); 8.77 (dd, 1H, *J* = 1.4 and 2.4 Hz, H5); 8.90 (d, 1H, *J* = 2.4 Hz, H6); 9.25 (d, 1H, *J* = 1.4 Hz, H3); 9.60 (s, 1H, O*H*); 12.10 (s, 1H, CON*H*). ^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 55.6 (*C*H$_3$); 109.2 (C5′); 115.5 (C2′); 122.5 (C6′); 125.5 (C1′); 143.3 (C5); 144.0 (C3); 144.8 (C2); 147.7 (C6); 148.1 (N=*C*H); 149.3 (C3′); 150.4 (C4′); 159.2 (*C*=O). IR (ν_{max}, KBr) ν (cm^{-1}): 3439, 3264, 3086, 1673, 1286, 862. Anal. Calcd for $C_{13}H_{12}N_4O_3$: C, 57.35; H, 4.44; N, 20.58. Found: C, 57.29; H, 4.43; N, 20.81.

5.1.2.14. *N′*-[(1*E*)-(3,4-Dimethoxyphenyl)methylidene]pyrazine-2-carbohydrazide (2n; LASSBio-1184). The title compound was obtained as a beige solid by the condensation of **3** with 3,4-dimethoxybenzaldehyde in 71% yield, mp 187–189 °C. ^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 3.80 (s, 3H, C4′-OC*H*$_3$); 3.82 (s, 3H, C3′-OC*H*$_3$); 7.03 (d, 1H, *J* = 8.2 Hz, H5′); 7.20 (dd, 1H, *J* = 1.7 and 8.2 Hz, H6′); 7.39 (d, 1H, *J* = 1.7 Hz, H2′); 8.52 (s, 1H, N=C*H*); 8.76 (dd, 1H, *J* = 1.4 and 2.4 Hz, H5); 8.90 (d, 1H, *J* = 2.4 Hz, H6); 9.24 (d, 1H, *J* = 1.4 Hz, H3); 12.18 (s, 1H, CON*H*). ^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 56.0 (C3′–O*C*H$_3$); 56.1 (C4′–O*C*H$_3$); 109.0 (C5′); 112.0 (C2′); 122.9 (C6′); 127.3 (C1′); 143.9 (C5); 144.5 (C3); 145.2 (C2); 148.3 (C6); 149.6 (N=*C*H); 150.7 (C3′); 151.6 (C4′); 160.0 (*C*=O). IR (ν_{max}, KBr) ν (cm^{-1}): 3424, 3050, 1688, 1314, 867. Anal. Calcd for $C_{14}H_{14}N_4O_3$: C, 58.73; H, 4.93; N, 19.57. Found: C, 58.59; H, 4.96; N, 19.71.

5.1.2.15. *N′*-[(1*E*)-(3,4,5-Trimethoxyphenyl)methylidene]pyrazine-2-carbohydra-zide (2o; LASSBio-1181). The title compound was obtained as a yellow solid by the condensation of **3** with 3,4,5-trimethoxybenzaldehyde in 73% yield, mp 168–170 °C. ^{1}H NMR data are in agreement with those previously published.[17] ^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 3.71 (s, 3H, C4′-OC*H*$_3$); 3.84 (s, 6H, C3′-OC*H*$_3$ and C5′-OC*H*$_3$); 7.03 (s, 2H, H2′ and H6′); 8.55 (s, 1H, N=C*H*); 8.78 (dd, 1H, *J* = 1.1 and 2.3 Hz, H5); 8.92 (d, 1H, *J* = 2.3 Hz, H6); 9.25 (d, 1H, *J* = 1.1 Hz, H3); 12.24 (s, 1H, CON*H*). ^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 56.6 (C3′-O*C*H$_3$ and C5′-O*C*H$_3$); 60.7 (C4′-O*C*H$_3$); 105.2 (C2′ and C6′); 130.1 (C1′); 140.2 (C4′); 143.9 (C5); 144.6 (C3); 145.3 (C2); 148.3 (C6); 150.4 (N=*C*H); 153.8 (C3′ and C5′); 160.1(*C*=O). IR (ν_{max}, KBr) ν (cm^{-1}): 3192, 3015, 1690, 1295, 849. Anal. Calcd for $C_{15}H_{16}N_4O_4$: C, 56.96; H, 5.10; N, 17.71. Found: C, 56.68; H, 5.14; N, 17.69.

5.1.2.16. *N′*-[(1*E*)-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)methylidene]pyrazine-2-carbohydrazide (2p; LASSBio-1187). The title compound was obtained as a white solid by the condensation of **3** with 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde in 87% yield, mp >250 °C. ^{1}H NMR data are in agreement with those previously published.[19] ^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 1.41 (s, 18H, C*H*$_3$); 7.19 (s, 1H, O*H*); 7.48 (s, 2H, H2′ and H6′); 8.54 (s, 1H, N=C*H*); 8.78 (dd, 1H, *J* = 1.0 and 2.4 Hz, H5); 8.91 (d, 1H, *J* = 2.4 Hz, H6); 9.24 (d, 1H, *J* = 1.0 Hz, H3); 12.03 (s, 1H, CON*H*). IR (ν_{max}, KBr) ν (cm^{-1}): 3631, 3257, 3041, 2954, 1677, 1293, 859. Anal. Calcd for $C_{20}H_{26}N_4O_2$: C, 67.77; H, 7.39; N, 15.81. Found: C, 67.70; H, 7.38; N, 15.70.

5.1.2.17. *N′*-[(1*E*)-(4-Oxo-4*H*-chromen-3-yl)methylidene]pyrazine-2-carbohydra-zide (2q; LASSBio-1188). The title compound was obtained as a yellow solid by the condensation of **3** with 4-oxo-4*H*-chromene-3-carbaldehyde in 69% yield, mp 240–242 °C. ^{1}H NMR (300 MHz, DMSO-d_6, TMS) δ (ppm): 7.54 (dt, 1H, *J* = 1.2 and 8.5 Hz, H6′); 7.71 (dd, 1H, *J* = 1.2 and 8.5 Hz, H8′); 7.85 (dt, 1H, *J* = 1.7 and 8.5 Hz, H7′); 8.12 (dd, 1H, *J* = 1.7 and 8.5 Hz, H5′); 8.76 (s, 1H, N=C*H*); 8.77 (dd, 1H, *J* = 1.5 and 2.5 Hz, H5); 8.84 (s, 1H, H2′); 8.89 (d, 1H, *J* = 2.5 Hz, H6); 9.23 (d, 1H, *J* = 1.5 Hz, H3); 12.42 (s, 1H, CON*H*). IR (ν_{max}, KBr) ν (cm^{-1}): 3284, 3097, 1687, 1642, 1334, 849, 765. Anal. Calcd for $C_{15}H_{10}N_4O_3$: C, 61.22; H, 3.43; N, 19.04. Found: C, 61.01; H, 3.48; N, 18.88.

5.1.2.18. *N′*-[(1*E*)-Pyridin-4-ylmethylidene]pyrazine-2-carbohydrazide (2r; LASSBio-1189). The title compound was obtained as a yellow solid by the condensation of **3** with pyridine-4-carbaldehyde in 56% yield, mp >250 °C. ^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 7.66 (d, 2H, *J* = 5.6 Hz, H2′ and H6′); 8.63 (s, 1H, N=C*H*); 8.65 (d, 2H, *J* = 5.6 Hz, H3′ and H5′); 8.80 (dd, 1H, *J* = 1.4 and 2.1 Hz, H5); 8.94 (d, 1H, *J* = 2.1 Hz, H6); 9.28 (d, 1H, *J* = 1.4 Hz, H3); 12.56 (s, 1H, CON*H*). ^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 121.7 (C2′ and C6′); 141.8 (C1′); 143.9 (C5); 144.8 (C3); 144.9 (C2); 148.0 (C6); 148.6 (N=*C*H); 150.9 (C3′ and C5′); 160.5 (*C*=O). IR (ν_{max}, KBr) ν (cm^{-1}): 3380, 3054, 1696, 1272, 841. Anal. Calcd for $C_{11}H_9N_5O$: C, 58.14; H, 3.99; N, 30.82. Found: C, 58.22; H, 3.97; N, 30.43.

5.1.2.19. *N′*-[(1*E*)-Pyridin-2-ylmethylidene]pyrazine-2-carbohydrazide (2s; LASSBio-1269). The title compound was obtained as a yellow solid by the condensation of **3** with pyridine-2-carbaldehyde in 69% yield, mp 208–210 °C. ^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 7.43 (m, 1H, H4′); 7.86 (m, 1H, H5′); 8.01 (d, 1H, *J* = 7.9 Hz, H6′); 8.61 (d, 1H, *J* = 4.4 Hz, H3′); 8.67 (s, 1H, N=C*H*); 8.79 (dd, 1H, *J* = 1.4 and 2.4 Hz, H5); 8.92 (d, 1H, *J* = 2.4 Hz, H6); 9.27 (d, 1H, *J* = 1.4 Hz, H3); 12.52 (s, 1H, CON*H*). ^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 120.7 (C6′); 125.1 (C4′); 137.5 (C5′); 143.9 (C5); 144.8 (C3); 145.1 (C2); 148.4 (C6); 150.1 (N=*C*H); 150.5 (C3′); 153.7 (C1′); 160.6 (*C*=O).

IR (ν_{max}, KBr) ν (cm^{-1}): 3431, 3093, 1700, 1270, 861, 774. Anal. Calcd for $C_{11}H_9N_5O$: C, 58.14; H, 3.99; N, 30.82. Found: C, 58.17; H, 3.97; N, 30.51.

5.2. Biological assays

5.2.1. Reagents

Acetic acid (Merck), arabic gum (Sigma–Aldrich), morphine sulphate (Dimorf-Cristalia-BR), Zymosan A (Sigma–Aldrich), complete Freund's adjuvant (CFA) (Sigma–Aldrich), celecoxib (Merck Sharp & Dome), indomethacin (Merck Sharp & Dome) and thalidomide (Sigma–Aldrich) were obtained from commercial sources. A solution of 2.5% formalin was prepared with formaldehyde (Merck) in saline (NaCl 0.9%). In all experiments, Indometacin, celecoxib, thalidomide, LASSBio-1018 (**1**) and compounds **2a**–**s** were used as suspensions in arabic gum.

5.2.2. Animals

Experiments were conducted using Swiss mice obtained from the BIOCEN—UFAL breeding unit, weighing 20–30 g each, males or females, adult, with 6–8 weeks of age, distributed in groups up to 6–8 animals for treatment. Wistar rats (130–200 g), males or females, were used in the experiment of induction of arthritis. The animals were maintained with free access to food and water and kept at 25–28 °C under a controlled 12 h light/dark cycle. All animals were manipulated according to the norms established by the Ethics Commission—UFAL for handling animals (Protocol number: 006443/2005-78).

5.2.3. Writhing test

This test was performed as described by Collier and co-workers.[8] Acetic acid (0.6%, v/v) was administered ip in a volume of 0.1 mL/10 g. The number of writhes, a response consisting of contraction of an abdominal wall, pelvic rotation followed by hind limb extension, was counted during continuous observation for 20 min beginning 5 min after the acetic acid injection. The pyrazine *N*-acylhydrazone derivatives **2a**–**s**, indomethacin, celecoxib and thalidomide were administered at the dose of 100 μmol/kg (po), 40 min before the acetic acid injection. Control group received 10 mL/kg of vehicle (arabic gum) (po) and another group was treated concomitantly with 50 μmol/kg of thalidomide (po) and 50 μmol/kg of celecoxib (po). Antinociceptive activity was expressed as inhibition percentage of the usual number of writhing observed in control animals.

5.2.4. Formalin-induced pain in mice

The formalin test was performed as described by Hunskaar and Hole.[9] Animals received 20 μL of a 2.5% formalin solution (0.92% formaldehyde in saline) in the ventral surface of the right hind paw. They were observed from 0 to 5 min (neurogenic phase) and from 15 to 30 min (inflammatory phase) after injection and the time they spent licking the injected paw was recorded and considered as indicative of nociception. The pyrazine *N*-acylhydrazone derivatives **2a**–**s**, indomethacin, celecoxib and thalidomide were administered at the dose of 100 μmol/kg (po), 40 min before formalin injection. Control group received 10 mL/kg of vehicle (arabic gum) (po) and another group was treated concomitantly with 50 μmol/kg of thalidomide (po) and 50 μmol/kg of celecoxib (po).

5.2.5. Hot-plate test

Mice were treated according to the method described by Eddy and Leimbach.[11] Each mouse was placed on the hot-plate set at 54 ± 1.0 °C and the time of paw licking was recorded before and 30 min after oral administration of the tested compounds. The pyrazine *N*-acylhydrazone derivatives **2a**–**s**, celecoxib and thalidomide were administered at the dose of 100 μmol/kg (po). Control group received 10 mL/kg of vehicle (arabic gum) (po) and another group was treated concomitantly with 50 μmol/kg of thalidomide (po) and 50 μmol/kg of celecoxib (po). Morphine was also used as a drug standard at the dose of 15 μmol/kg (ip). Analgesia was defined as an increase in the latency of paw licking, and the latency times were compared with the values obtained for control. Sixty seconds were taken as the cut-off time to avoid mouse tissue damage.

5.2.6. Zymosan-induced peritonitis

Peritoneal inflammation was induced according to the method described by Doherty et al.[12] A solution of Zymosan A (Sigma–Aldrich) (2 mg/mL) was prepared in saline (NaCl 0.9%) and injected into the peritoneal cavity of mice (0.5 mL). Six hours after injection of Zymosan A, the animals were killed by cervical dislocation and the peritoneal cavity was washed with 3 mL of cold Hank's. The pyrazine *N*-acylhydrazone derivatives **2a**–**s**, indomethacin, celecoxib and thalidomide were administered at the dose of 100 μmol/kg (po), 40 min before Zymosan A injection. Control group received 10 mL/kg of vehicle (arabic gum) (po) and another group was treated concomitantly with 50 μmol/kg of thalidomide (po) and 50 μmol/kg of celecoxib (po). The number of cells was quantified by optical microscope, using the 100× lens.

5.2.7. Capsaicin-induced mouse ear edema

The capsaicin-induced ear edema model was performed as described by Mantione and Rodriguez.[13] The pyrazine *N*-acylhydrazone derivatives **2a**–**s**, indomethacin, celecoxib and thalidomide were administered at the dose of 100 μmol/kg (po), 40 min before capsaicin injection. Control group received 10 mL/kg of vehicle (arabic gum) (po) and another group was treated concomitantly with 50 μmol/kg of thalidomide (po) and 50 μmol/kg of celecoxib (po). Capsaicin (250 μg) was injected into the inner surface of the right ear of mice 40 min after oral administration of the tested compounds. The left ear received acetone, delivered in the same manner. Thirty minutes after capsaicin application, mice were killed and both ears were removed. Circular sections were taken using a 6 mm diameter cork borer and weighed. The weight gain caused by capsaicin was measured by subtracting the weight of untreated left ear sections from that of treated right ear sections. Ear edema was quantified as the difference in weight between the challenged and the unchallenged ear. Inhibition percentage was calculated using $(C - T)/C \times 100(\%)$, where C and T indicate non-treated (vehicle) edema and drug-treated edema, respectively.

5.2.8. Induction of arthritis by complete Freund's adjuvant (CFA)

The arthritic syndrome was induced in rats (130–170 g) according to the method proposed by Newbould, with some modifications.[15] Complete Freund's adjuvant (CFA) (0.1 mL) was injected into the right paw of rats. After 13 days, animals with pronounced arthritis were separated into groups of 6, so that each group had approximately similar mean arthritic scores. Compounds **2o** and thalidomide were administered at the dose of 100 μmol/kg (po) from the 14th to the 21st day after immunization. The control and arthritic groups were treated with 10 mL/kg of vehicle (arabic gum) (po) during the same period. The paw volumes were measured on the 1st, 14th, 15th, 16th, 17th, 18th, 19th, 20th and 21st days using a digital caliper (Mitutoyo). Mean paw volumes were obtained daily for treated groups and compared with paw volumes of the positive control group.

5.2.9. Statistical analysis

Data obtained from animal experiments are represented by mean ± standard error of the mean (mean ± S.E.M.). Statistical differences between the treated and the control groups were evaluated by test t of Student or ANOVA in the tutorial Prisma®. Values were considered significant if $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

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