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Original article

Synthesis and pharmacological evaluation of *N*-phenyl-acetamide sulfonamides designed as novel non-hepatotoxic analgesic candidates

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ABSTRACT

In this paper we report the design, synthesis and pharmacological evaluation of a series of *N*-phenyl-acetamide sulfonamide derivatives (**5a**–**g**), planned by structural modification on the prototype paracetamol (**1**). In this series (**5a**–**g**), compound LASSBio-1300 (**5e**; $ID_{50} = 5.81$ μmol/kg) stands out as a new non-hepatotoxic analgesic drug candidate. The increase of area, volume and eletrostatic potential of paracetamol's analogues seems to be beneficial to the analgesic activity. Unlike paracetamol (**1**) and the other analogues (**5a**, **5d**–**g**), compounds **5b** and **5c** presented an important anti-hypernociceptive activity associated to inflammatory pain.

1. Introduction

Paracetamol (acetaminophen, 4-hydroxyacetanilide, **1**) was synthesized in 1878 and the first clinical use was reported in 1893, but only after it was identified as the active metabolite of phenacetin (**2**) and acetanilide (**3**) that it was marketed as a drug [1].

Introduced worldwide in the 1950s as an antipyretic and analgesic drug, paracetamol (**1**) is still one of the most popular over-the-counter drugs, which is frequently used on a prescription basis for the relief of acute and chronic pain. Within the therapeutic range, it is, usually, well tolerated, with few side effects. However, when taken in over dosage, paracetamol (**1**) may cause severe and sometimes fatal hepatic necrosis [2,3].

The mechanism of paracetamol's hepatotoxicity is well known and is directly associated to its hepatic metabolism. It is primarily metabolized by the liver, being 90% eliminated as glucoronide and sulphate metabolites, 5% excreted unchanged and 5% oxidized, by cytochrome P4502E1 (CYP2E1), to *N*-acetyl-*p*-benzoquinone imine (NAPQI) (Scheme 1) [4].

Hepatotoxicity arises only through NAPQI (**4**), a highly reactive compound that normally conjugates with glutathione, being eliminated as an inactive metabolite. However, in conditions where the production of NAPQI exceeds that of glutathione, this reactive metabolite binds covalently to liver proteins and causes dose-related liver injury by centrilobular necrosis [5,6].

In a continuing effort to develop new analgesic drug candidates, we report in this paper the design, synthesis and pharmacological evaluation of *N*-phenyl-acetamide sulfonamide derivatives (**5a**–**g**), planned by structural modification on the prototype paracetamol (**1**).

The design concept considered the need to carry out structural modifications in the toxicophoric unit of paracetamol (**1**), avoiding its biotransformation to the reactive metabolite NAPQI (**4**).

The structural design of this new series was accomplished first by applying a non-classical bioisosterism [7], represented by the

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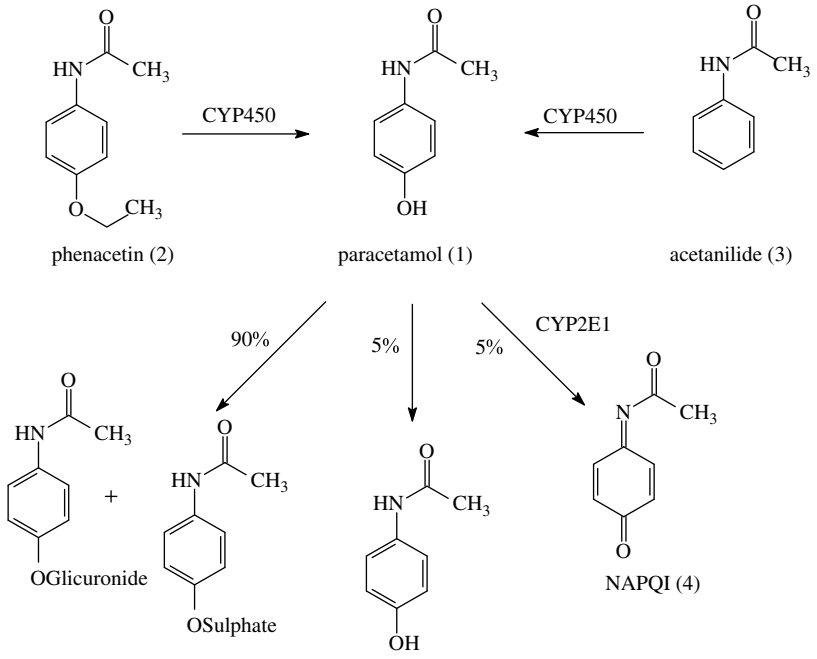
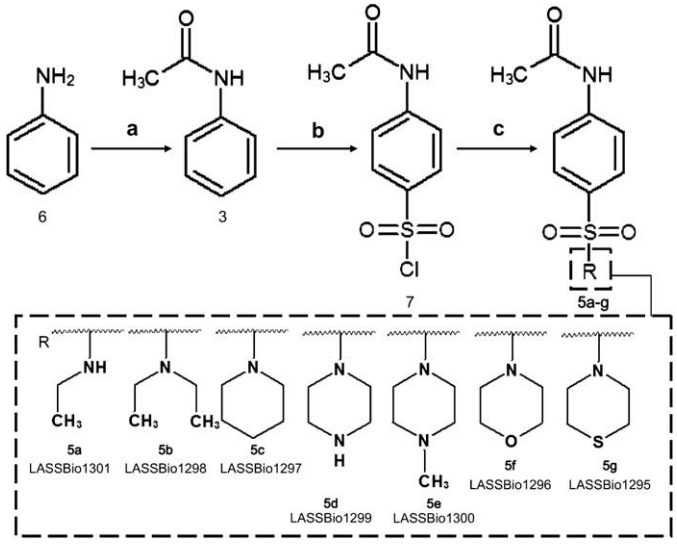


Scheme 1.

replacement of the phenolic hydroxyl group (subunit B) by a non-oxidizable one, comprised by the sulfonamide subunit (Chart 1). Then, the introduction of an ethyl side chain was performed, since it is also present in the structure of the analgesic phenacetin (**2**), linked to the nitrogen of the sulfonamide moiety, in a classical example of the homologation strategy (Chart 1) [7]. Moreover, in order to investigate the importance of stereoelectronic and conformational parameters to the analgesic activity, the nature of the sulfonamide moiety (subunit C) was modified by the introduction of diethyl (**5c**), piperidine (**5d**), piperazine (**5d**), *N*-methyl-piperazine (**5e**), morpholine (**5f**) and thiomorpholine (**5g**) units (Chart 1).



Scheme 2. Reagents and conditions: a) acetic anhydride, glacial acetic acid, CH_3CO_2Na anhydrous, r.t., 30 min, 90%; b) HSO_3Cl, 60 °C, 30 min, 85%; c) functionalized amines, CH_2Cl_2, r.t., 30 min, 50–70%.

2. Results and discussion

2.1. Chemistry

The synthesis of these new series was undertaken using a classical methodology, based on several functional group interconversions, employing the sulfonyl chloride derivative (**7**) as the key intermediate. This compound could be easily obtained, in high yield, by the synthetic sequence depicted in Scheme 2. The acetylation of aniline (**6**) with acetic anhydride, furnished the acetanilide (**3**) in 90% yield [8]. The second step in the synthesis of **7** was based on a regioselective electrophilic aromatic substitution employing chlorosulfonic acid, obtaining **7** in 85% yield [9,10]. With this key intermediate in hands, the target compounds could be prepared by condensation of the 4-(acetylamine)benzenesulfonyl chloride (**7**) with functionalized amines to give the desired sulfonamide derivatives (**5a**–**g**) in 50–70% yield, as shown in Scheme 2 [11].

2.2. Analgesic and hypothermic activities

The analgesic activity of *N*-phenyl-acetamide sulfonamide derivatives (**5a**–**g**) was initially performed employing the acetic

Table 1
Effect of derivatives **5a**–**g** (100 μmol/kg, p.o.), and paracetamol (100 μmol/kg, p.o.) on acetic acid-induced writhing in mice.

Substance	*n*	Writhing number mean ± S.E.M.[a]	% of inhibition
Control	8	43.2 ± 1.7	–
1 (Paracetamol)	8	28.5 ± 3.2*	34.0%
5a (LASSBio-1301)	8	22.2 ± 4.1**	48.6%
5b (LASSBio-1298)	8	23.4 ± 4.5**	45.8%
5c (LASSBio-1297)	8	30.4 ± 2.5*	29.6%
5d (LASSBio-1299)	8	16.8 ± 3.1**	61.1%
5e (LASSBio-1300)	8	10.8 ± 1.7**	75.0%
5f (LASSBio-1296)	8	24.5 ± 3.7**	43.2%
5g (LASSBio-1295)	8	24.2 ± 3.5**	44.0%

[a] The asterisks denote the significance levels in comparison with control groups, *$P < 0.05$ and **$P < 0.01$.

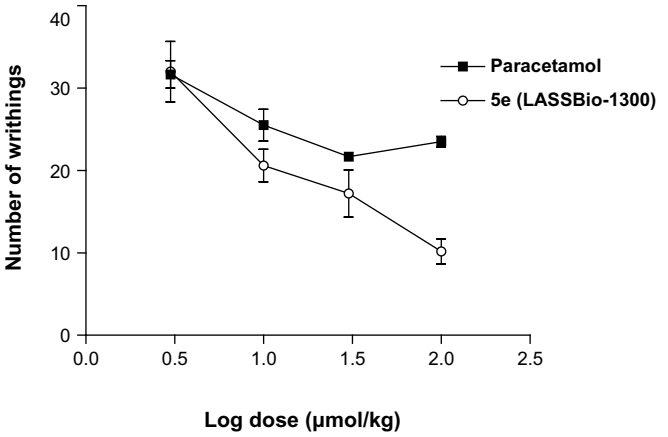


Fig. 1. Dose–response curves of the antinociceptive effect of paracetamol (■) (100, 30, 10, and 3 μmol/kg) and **5e** (LASSBio-1300) (○) (100, 30, 10, and 3 μmol/kg) on the 0.6% acetic acid-induced abdominal constrictions observed in a period of 25 min in mice. Data are expressed as the total number writhings calculated for 8 animals. $^{*}P < 0.05$, ANOVA followed by Dunnett's test.

acid-induced abdominal writhing model in mice [12–14], using paracetamol (**1**) as standard compound.

In this model the *N*-phenyl-acetamide sulfonamide derivatives (**5a**–**g**) and paracetamol (**1**) were evaluated at the dose of 100 μmol/kg, by oral administration. The results, illustrated in Table 1, demonstrated that all derivatives (**5a**–**g**) produced marked inhibition of acetic acid-induced writhing response, compounds **5d** (LASSBio-1299; 61.1%) and **5e** (LASSBio-1300; 75.0%) being significantly more active than the standard paracetamol (34.0%) in the screening dose of 100 μmol/kg, p.o. Thus, LASSBio-1300 (**5e**) was selected in order to study its dose–response curve. As depicted in Fig. 1, paracetamol (dose = 100.0, 30.0, 10.0, and 3.0 μmol/kg) and LASSBio-1300 (dose = 100.0, 30.0, 10.0, and 3.0 μmol/kg) produced a dose-related inhibition of acetic acid-induced abdominal constrictions in mice, with ID_{50} values of 19.27 μmol/kg and 5.81 μmol/kg, respectively.

In order to determine an eventual central antinociceptive activity, compounds **5a**–**g** (100 μmol/kg, p.o.) were also evaluated in the hot-plate test [15], using morphine (15 μmol/kg, i.p.) and paracetamol (100 μmol/kg, p.o.) as standards. In this model, the heat induces a cutaneous thermonociceptive effect and the stimulus integration occurs due to the stimulation of non-mielinized C fibers of slow conduction [16]. Although the hot-plate test is commonly used to assess narcotic analgesics, other centrally acting drugs, including sedatives and muscle relaxants or psychotomimetics, have shown activity in this test while indomethacin and other NSAIDs (non-steroidal anti-inflammatory drugs) have no effect [17–19].

As shown in Table 2, the oral administration of compounds **5g** (LASSBio-1295) and **5e** (LASSBio-1300) and paracetamol increased significantly the reaction time to the nociceptive response in the hot-plate test, while morphine induced a marked increase in the latency of the animals at (9.0 ± 1.4 s) 60 min and (7.5 ± 0.5 s) 90 min. No significant activity was found for compounds **5a**–**f** (Table 2). The observations that **5g** (LASSBio-1295), **5e** (LASSBio-1300) and paracetamol (**1**) increased the baseline of animals in the hot-plate model suggest that these compounds present supraspinal analgesic activity.

The anti-hyperalgesic activity of *N*-phenyl-acetamide sulfonamide derivatives (**5a**–**g**) and paracetamol (**1**) was determined using the formalin test [20,21]. Formalin is known to produce biphasic pain behaviors. The first transient phase is ascribed to the direct effect of formalin on sensory C fibers, and the second prolonged phase is associated to the development of an inflammatory response and the release of algesic mediators [20–23]. The results obtained, depicted in Fig. 2, show a significant antinociceptive response observed in the first phase (i.e. central phase) of the formalin-induced pain after oral administration (100 μmol/kg) of paracetamol (**1**), **5f** (LASSBio-1296) and **5d** (LASSBio-1299). On the other hand, the analysis of the second phase of the formalin test (i.e. inflammatory phase) indicated an anti-hyperalgesic activity only for compounds **5c** (LASSBio-1297) and **5b** (LASSBio-1298).

Considering the hypothermic action, in mice, of paracetamol (**1**) and its derivatives [24], which seems to be mediated by inhibition of prostaglandin endoperoxide synthase 1 gene-derived protein (i.e. COX-3) [25,26], we investigated the ability of this prototype (**1**) and its sulfonamide analogues **5a**–**g** to interfere on the body temperature, using the test behavioral measures of rectal temperature in mice [27]. In this model, the temperature was measured with room temperature of 25 °C. After 1 h of acclimatization, the baseline temperature was determined. The same was measured before and after administration of test compounds (**5a**–**g**) and paracetamol (**1**), at the screening dose of 100 μmol/kg, p.o. As shown in Table 3, LASSBio-1298 (**5b**) and LASSBio-1300 (**5e**) were the most active compounds, leading to a decrease in temperature by 1.8 °C. The compounds LASSBio-1296 (**5f**), LASSBio-1297 (**5c**) and paracetamol (**1**), in the same conditions, also induced a significant decrease of body temperature.

Aiming to verify the comparative hepatotoxicity of paracetamol (**1**) and its *N*-phenyl-acetamide sulfonamide analogues (**5a**–**g**), we investigated the effect of paracetamol (**1**) and LASSBio-1300 (**5e**) on liver functions and on the morphology of liver parenchyma of animals treated for five days with **1** and **5e** (100 μmol/kg, p.o.).

Table 2
Time course effect of derivatives **5a**–**g** (100 μmol/kg, p.o.), morphine (15 μmol/kg, i.p.) and paracetamol (100 μmol/kg, p.o.) in the hot-plate test in mice.

Substance	*n*	Latency before the treatment[a] (s)	Time after the treatment[a,b]			
		0 min	30 min	60 min	90 min	120 min
Control	8	1.8 ± 0.3	3.4 ± 0.8 (47.1%)	2.1 ± 0.5 (14.3%)	3.9 ± 0.8 (53.8%)	3.4 ± 0.7 (47.1%)
Morphine	8	1.9 ± 0.2	9.0 ± 1.4 (78.9%)**	7.5 ± 0.5 (74.7%)**	4.5 ± 0.5 (57.8%)	2.8 ± 0.2 (32.1%)
1 Paracetamol	8	3.9 ± 0.4	8.1 ± 0.3 (51.8%)	9.9 ± 2.4* (60.6%)	8.6 ± 1.7 (54.6%)	8.6 ± 1.1 (54.6%)
5a (LASSBio-1301)	6	4.7 ± 0.6	6.1 ± 0.8 (23.0%)	5.0 ± 0.8 (6.0%)	7.0 ± 1.0 (32.8%)	6.2 ± 1.1 (24.2%)
5b (LASSBio-1298)	6	4.0 ± 0.9	4.1 ± 1.0 (2.4%)	2.8 ± 0.5 (0%)	4.0 ± 0.5 (0%)	3.4 ± 0.6 (0%)
5c (LASSBio-1297)	6	3.6 ± 0.4	4.9 ± 0.9 (26.5%)	4.2 ± 0.8 (14.3%)	4.4 ± 0.4 (18.2%)	5.2 ± 0.7 (30.7%)
5d (LASSBio-1299)	6	3.9 ± 0.6	5.1 ± 1.3 (23.5%)	4.2 ± 0.6 (7.2%)	6.2 ± 0.6 (37.1%)	3.7 ± 0.5 (0%)
5e (LASSBio-1300)	6	3.7 ± 0.6	8.7 ± 1.1** (57.5%)	7.1 ± 1.0* (47.9%)	4.6 ± 0.9 (19.6%)	4.7 ± 1.0 (21.3%)
5f (LASSBio-1296)	6	4.6 ± 0.2	5.9 ± 0.6 (22.0%)	6.0 ± 0.8 (23.3%)	5.8 ± 0.5 (20.7%)	5.0 ± 1.0 (8.0%)
5g (LASSBio-1295)	6	3.0 ± 0.2	4.0 ± 0.6 (25.0%)	4.2 ± 0.9 (28.6%)	5.8 ± 0.6 (48.3%)	8.6 ± 1.4** (65.1%)

[a] The readings represent the mean ± S.E.M.
[b] The asterisks denote the significance levels in comparison with control groups, $^{**}P < 0.01$.

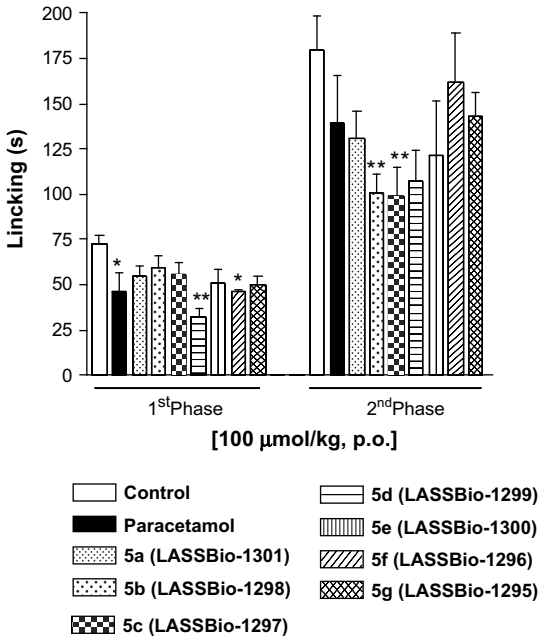


Fig. 2. Effects of paracetamol (**1**), **5a** (LASSBio-1301), **5b** (LASSBio-1298), **5c** (LASSBio-1297), **5d** (LASSBio-1299), **5e** (LASSBio-1300), **5f** (LASSBio-1296), **5g** (LASSBio-1295) on the formalin-induced nociception in mice. Animals were treated with paracetamol and compounds **5a**–**g** (dose = 100 μmol/kg, p.o.) or the same volume of vehicle (arabic gum) (the control) 40 min before 20 μL of 2% formalin in 0.9% saline was injected s.c. into the dorsal surface of the right hind paw. The time spent licking and biting the injected paw was measured with a hand-stop watch from 0 to 5 min (the first phase—A) and from 15 to 30 min (the second phase—B) after formalin injection. Data were presented as the mean ± S.E.M. ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control.

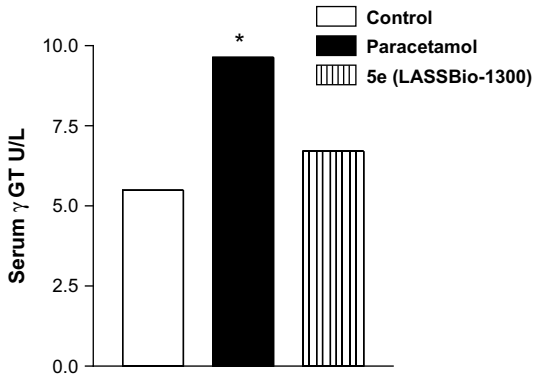


Fig. 3. Effect of LASSBio-1300 (**5e**) and paracetamol (**1**) treatment on serum γGT levels measured at 5 days of administration daily (100 μmol/kg, p.o.). Values are expressed in U/L ($n = 6$). Treatment vs. control group: $^{*}P < 0.01$.

For the liver function the serum gamma-Glutamyl Transferase (γGT) activity was determined. The γGT is an enzyme embedded in the hepatocyte plasma membrane and considered to be one of the best indicators of liver damage [28]. As illustrated in Fig. 3, mice treated with paracetamol (**1**) showed a significant increase in serum γGT, when compared to control, while no significant effect was observed for LASSBio-1300 (**5e**).

The microscopic liver analysis of animals treated with saline (control group) and with LASSBio-1300 (**5e**) demonstrated a regular morphology of the liver parenchyma, with well-designed and evident hepatic cells and sinusoids (Fig. 4A and B). On the other hand, the liver of animals treated with paracetamol (**1**) showed histological changes in the micro architecture of the liver lobule and degenerated hepatocytes, showing perinuclear vacuolization in most areas (Fig. 4C).

Taken together, these results seem to demonstrate hepatic cell damage caused by paracetamol (**1**), which has not been observed in the treatment with LASSBio-1300 (**5e**) in the same conditions.

In attempts to correlate the biological results with the molecular conformational and stereoelectronic properties, the conformer distribution of paracetamol (**1**) and its analogues (**5a**–**g**) was carried out using the semi empirical AM1 Hamiltonian within Spartan'08 for Windows Software [29,30]. In this study were found 3, 20, 50, 11, 13, 44, 14 and 11 conformers for compounds **1**, **5a**, **5b**, **5c**, **5d**, **5e**, **5f** and **5g**, respectively. The difference in energy between the conformer of maximum and minimum energy for all the studied compounds varied between 6.59 kcal/mol and 11.79 kcal/mol. The alignment of all the conformers of each compound was performed based on a selection of a set of atoms (CO, NH, C2, C3, C5, C6 and SO_2) and the results are depicted in Fig. 5. Considering that a drug's bioactive conformation often does not correspond to the absolute minimum of energy, for each set of conformers the molecular electrostatic potential surface was calculated (Fig. 5). Others molecular properties, including area, volume, dipole moment, polar surface area and partition coefficient were calculated, as available in the Spartan package, and the results obtained for the conformer of minimum and maximum energy are depicted in Table 4.

As seen in Fig. 5, two main differences can be drawn comparing the set of conformers of paracetamol (**1**) and its analogues. These differences are based on the increase in area and volume, introduced by the replacement of the phenolic hydroxyl group by a sulfonamide unit, and the introduction of a new negative potential region, localized at the terminal moiety of piperazine, *N*-methyl-piperazine, morpholine and thiomorpholine rings, present in the structures of compounds **5d**, **5e**, **5f** and **5g**, respectively. Considering the better peripheral analgesic activity for paracetamol analogues, with exception of compound **5c**, (Table 1) we can speculate that the improvement on the electron-rich regions, represented by the eletrostatic

Table 3
Activity of derivatives **5a**–**g** (100 μmol/kg, p.o.) and paracetamol (100 μmol/kg, p.o.) on the body temperature of mice.

Substance	*n*	Temperature (°C) (mean ± S.E.M) (before treatment)[a]	Temperature (°C) (mean ± S.E.M) (after treatment)[a]	Δ Temperature[b] (°C)
1 Paracetamol	8	38.0 ± 0.2	36.9 ± 0.3	−1.1**
5a (LASSBio-1301)	8	36.4 ± 0.3	36.6 ± 0.2	0.2
5b (LASSBio-1298)	8	37.8 ± 0.2	36.0 ± 0.3	−1.8**
5c (LASSBio-1297)	8	37.3 ± 0.2	36.5 ± 0.2	−0.8*
5d (LASSBio-1299)	8	36.6 ± 0.4	36.8 ± 0.3	0.2
5e (LASSBio-1300)	8	37.3 ± 0.4	35.5 ± 0.2	−1.8**
5f (LASSBio-1296)	8	37.8 ± 0.1	36.4 ± 0.2	−1.4**
5g (LASSBio-1295)	8	37.5 ± 0.2	36.8 ± 0.2	−0.7

[a] The readings represent the mean ± S.E.M. of 8 animals.
[b] The asterisks denote the significance levels in comparison with control groups, $^{**}P < 0.01$.

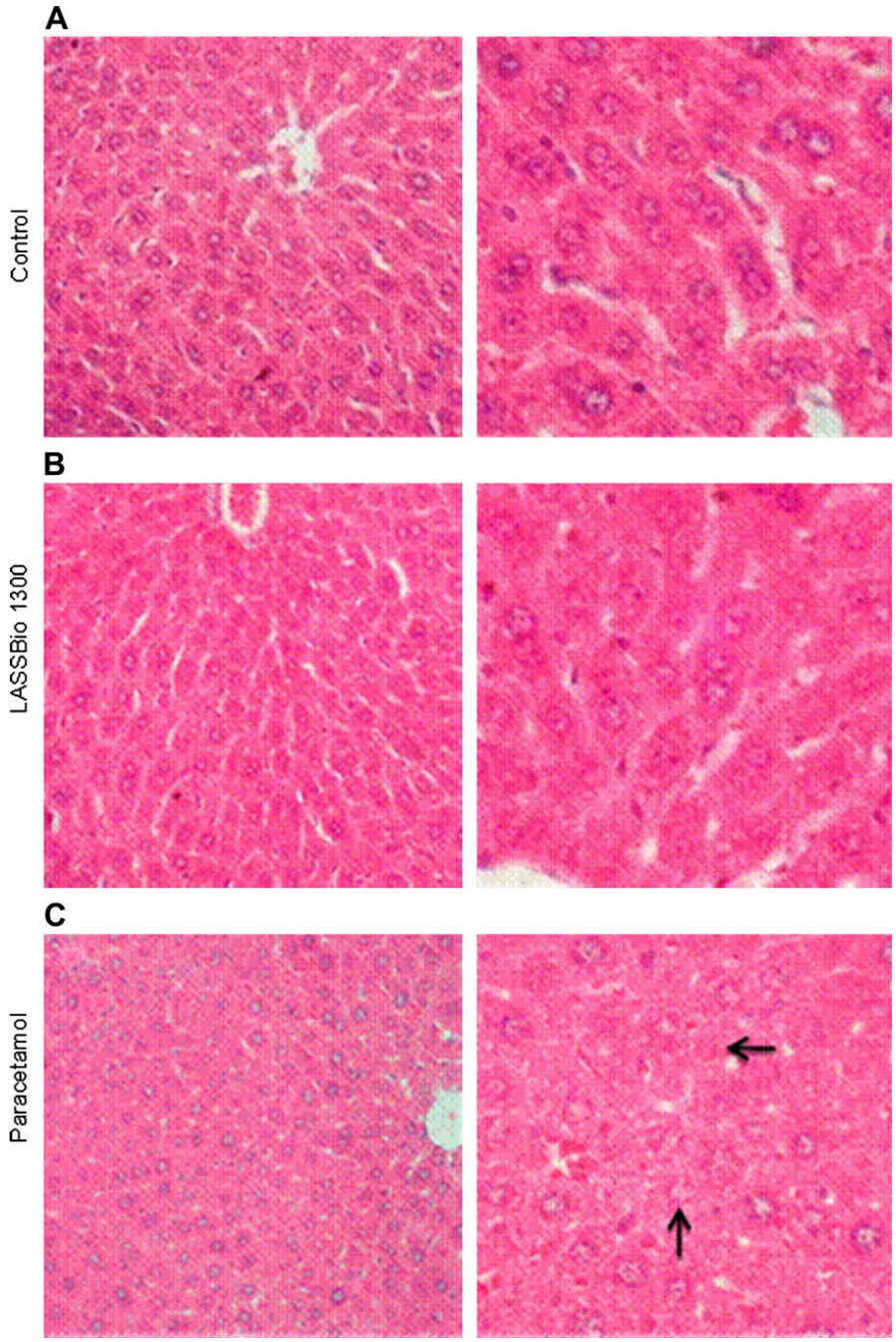


Fig. 4. Histological observation of the liver. Mice were treated with LASSBio-1300 (**5e**, 100 μmol/kg, p.o.) or paracetamol (100 μmol/kg, p.o.) for 5 days. Control group was administered only with vehicle. Hepatic tissue was collected 24 h after the last treatment and subjected to H&E staining. Left slides are 100× magnification and right slides are 400× magnification. The arrow in the figure indicates the hepatocyte necrosis or hepatocyte degeneration.

potential surfaces illustrated in Fig. 5, was beneficial to the analgesic profile of compounds **5a** and **5b** and **5d–g**. Although, the more active compound (**5e**, LASSBio-1300) being the one with higher volume, area and dipole moment, none direct correlation between the calculated stereoelectronic properties and the biological results can be drawn. This fact can be explained considering the limitation of try to establish a structure–activity relationship when the target/mechanism of action is unknown, and also when the pharmacological profile was determined in vivo, the results of which are, consequently, influenced by pharmacokinetic parameters.

Aiming to establish the theoretical potential to qualify compounds **5a–g** as drug candidates, we calculated the overall drug-score values, using a web-based system named Osiris Property Explorer (http://www.organic-chemistry.org/prog/peo/) which combines drug-likeness, solubility, c log *P*, molecular weight, and toxicity risks in one handy value that may be used to judge the compound's overall potential to qualify for a drug. Values = 1.0 mean no risk and values ≤0.6 mean high risk [31]. As illustrated in Table 4, the *N*-phenyl-acetamide sulfonamide derivatives (**5a–g**) presented a higher drug-score than prototype paracetamol, with the exception of compound **5b**.

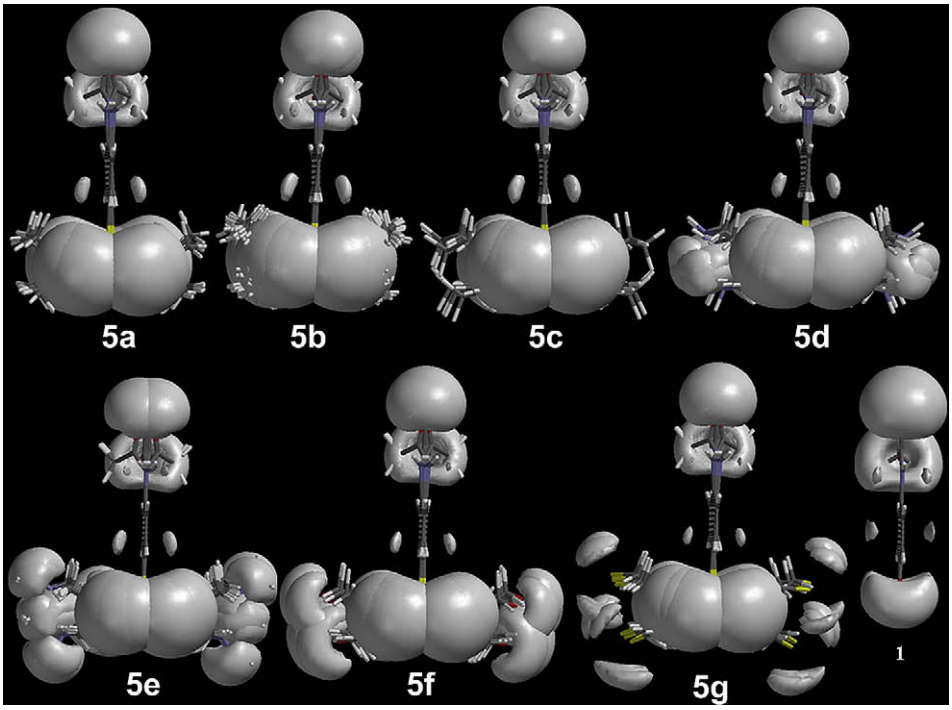


Fig. 5. Three-dimensional isopotential surface of all the conformers of paracetamol (**1**) and its analogues **5a**–**g**. The solid contours represent a constant potential of −83.68 kJ/mol showing the position and orientation of the lone pair electrons at approximately 1.4 Å away from the van der Waals surface of the molecule.

3. Conclusions

A new series of analgesic drug candidates were identified. In this series (**5a**–**g**), planned by molecular modifications on the prototype paracetamol (**1**), compound LASSBio-1300 (**5e**; $ID_{50} = 5.81$ μmol/kg, p.o.) stands out as a new non-hepatotoxic drug candidate, more potent than the standard **1** ($ID_{50} = 19.27$ μmol/kg, p.o.) and presenting a better hypothermic profile in a screening dose of 100 μmol/kg. The increase of area, volume and eletrostatic potential of paracetamol's analogues seems to be beneficial for the analgesic activity. Unlike paracetamol (**1**) and the other analogues (**5a**, **5d**–**g**), compounds **5b** and **5c** presented an important anti-hypernociceptive activity associated to inflammatory pain.

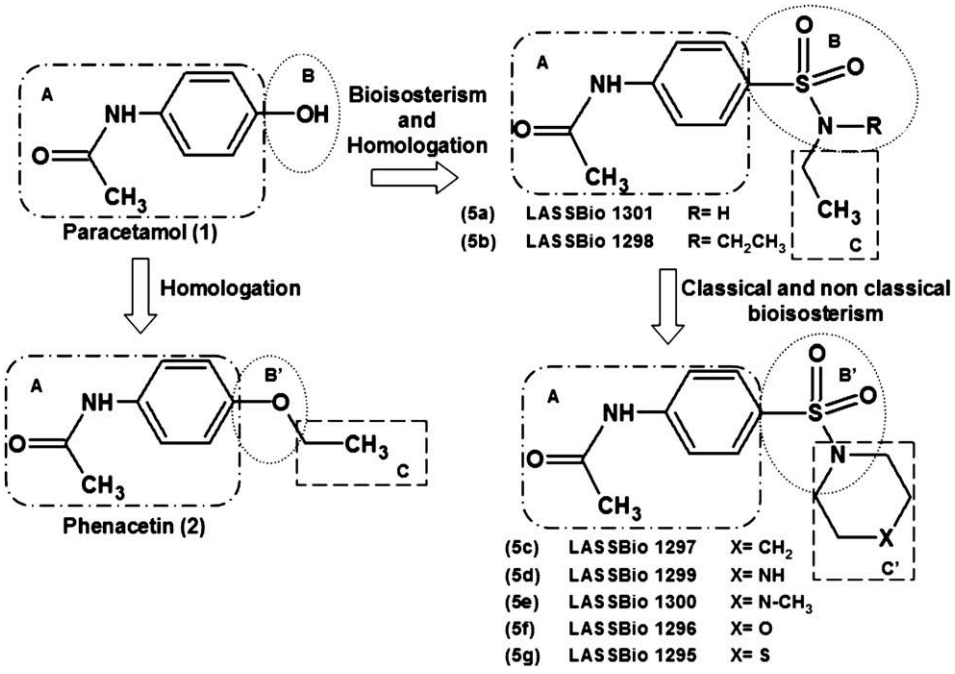


Chart 1.

Table 4
Selected molecular properties: heat of formation (ΔH_f), surface area, volume of a space-filling model, dipole, polar surface area (PSA), calculated partition coefficient (*c* log *P*) and Drug-score values.

Compound	ΔH_f[a] (kcal/mol)	Area[a] (Å^2)	Volume[a] (Å^3)	Dipole[a] (Debye)	PSA[a] (Å^2)	*c* log *P*[b]	Drug-score[c]
1	−247.30	180.99	158.47	2.61	42.86	0.494	0.20
	−237.35	178.96	157.85	2.48	45.07		
5a	−371.22	265.72	232.41	7.04	67.60	0.791	0.95
	−363.91	263.11	231.59	4.31	69.86		
5b	−369.13	300.66	270.91	7.0	55.32	1.874	0.56
	−359.88	299.71	270.40	4.54	57.71		
5c	−379.37	301.60	275.89	6.80	55.19	2.009	0.91
	−372.78	299.63	275.21	4.89	58.03		
5d	−300.00	297.84	270.45	5.97	67.73	0.748	0.95
	−291.78	295.96	269.77	4.66	58.03		
5e	−288.19	316.71	289.83	7.58	57.65	1.323	0.95
	−276.40	315.97	289.23	4.79	60.61		
5f	−497.15	293.93	267.35	6.70	63.05	0.762	0.95
	−488.37	292.28	266.68	3.53	65.82		
5g	−313.88	301.65	276.01	6.63	55.17	1.497	0.93
	−306.33	299.53	275.28	3.39	57.78		

[a] Calculated using Spartan'08 for Windows.
[b] Calculated using ChemOffice 2004 (Chem3D Ultra 8.0) Software.
[c] Calculated using Osiris program (http://www.organic-chemistry.org/prog/peo/).

4. Experimental section

4.1. Chemistry

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 solutions using a Bruker AC-200 spectrometer. Peak positions are given in parts per million (δ) from tetramethylsilane as internal standard, and coupling constant values (J) are given in Hz. Signal multiplicities are represented by: s (singlet), d (doublet), t (triplet), q (quadruplet), qu (quintuplet) and m (multiplet). 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 in rotatory evaporator.

4.1.1. N-*Phenyl-acetamide* (**3**)

A solution of 2.1 g (25.6 mmol) of sodium acetate anhydrous in 8.4 g (8.0 mL, 139.9 mmol) of glacial acetic acid was prepared. 8.18 g of aniline (8.0 mL, 87.8 mmol) was slowly added, and then 9.2 g (8.5 mL, 90.1 mmol) of acetic anhydride was also added. The reaction mixture was stirred for about 30 min, at room temperature, when the end of reaction was observed by TLC. After cooling, the *N*-phenyl-acetamide (**3**) was filtered through a Buckner funnel, washed twice with 200 mL of water and recovered as a white shiny powder in 90% yield, mp 116 °C.

^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 2.03 (s, 3H, COC$\underline{H}_3$); 7.01 (t, 1H, J = 7.5 Hz, H4); 7.28 (t, 2H, J = 8.0, 7.5 Hz, H3 and H5); 7.57 (d, 2H, J = 8.0 Hz, H2 and H6); 9.93 (s, 1H, CONH).

^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 24.6 (CO$\underline{C}$H$_3$); 119.5 (C-3 and C-5); 123.5 (C-4); 129.2 (C-2 and C-6); 139.9 ($\underline{C}$-1); 168.8 (C=O).

IR (ν_{max}, KBr) ν (cm^{-1}): 3299, 1666, 755, 698.

Anal. Calcd. for C_8H_9NO: C, 71.09; H, 6.71; N, 10.36. Found: C, 71.02; H, 6.78; N, 10.38.

4.1.2. 4-(Acetylamine)-benzenesulfonyl chloride (7)

9.12 g (5.2 mL, 78.27 mmol) of chlorosulfonic acid was slowly added to 2.0 g (14.80 mmol) of *N*-phenyl-acetamide (**3**). The resulting mixture was stirred and heated at 60 °C for 30 min. After cooling, the 4-(acetylamine)-benzenesulfonyl chloride (**7**) was filtered through a Buckner funnel, washed twice with 100 mL of water and recovered as a white hygroscopic powder in 85% yield, mp 143–148 °C. The melting point and IR data for compound (**7**) are in agreement with previews reports [8–10].

IR (ν_{max}, KBr) ν (cm^{-1}): 3310, 1683, 1374, 1168, 839.

4.1.3. General procedures for the preparation of sulfonamides ***5a–g***

The functionalized amines (5.0 mmol) were added to a solution of sulfonyl chloride derivative (**7**) (0.5 g; 2.14 mmol) in 50 mL of methylene chloride. The reaction mixture was stirred for about 30 min, at room temperature, when the end of the reaction was observed by TLC. The sulfonamide derivatives **5a–g** were isolated by addition of 50 mL of methylene chloride and extraction with 10% aq HCl and brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated at reduced pressure to give the sulfonamide derivatives **5a–g** in good yields.

4.1.3.1. N-*[4-(Ethylamino-4-ylsulfonyl)phenyl]acetamide (**5a**; LASSBio-1301).* The title compound was obtained as a white crystal, in 50% yield, by condensing **7** with ethylamine, mp 155–157 °C.

^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 0.94 (t, 3H, J = 7.2 Hz, RNHCH$_2$C$\underline{H}_3$); 2.08 (s, 3H, COC$\underline{H}_3$); 2.74 (qu, 2H, J = 7.2 Hz and 5.7 Hz, RNHC$\underline{H}_2$CH$_3$); 7.42 (t, 1H, J = 5.7 Hz, RN$\underline{H}$CH$_2$CH$_3$); 7.69 (d, 2H, J = 8.9 Hz, H2 and H6); 7.77 (d, 2H, J = 8.9 Hz, H3 and H5); 10.41 (s, 1H, CONH).

^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 14.7 (RNHCH$_2$$\underline{C}H_3$); 24.3 (CO$\underline{C}H_3$); 37.5 (RNH$\underline{C}H_2CH_3$); 118.7 (C-2 and C-6); 127.6 (C-3 and C-5); 134.2 (C-4); 142.7 (C-1); 169.1 (C=O).

IR (ν_{max}, KBr) ν (cm^{-1}): 3330, 3149, 1679, 1318, 1154, 834.

Anal. Calcd. for $C_{10}H_{14}N_2O_3S$: C, 49.57; H, 5.82; N, 11.56. Found: C, 49.29; H, 5.66; N, 11.45.

4.1.3.2. N-*[4-(Diethylamino-4-ylsulfonyl)phenyl]acetamide (**5b**; LASSBio-1298).* The title compound was obtained as an orange solid, in 59% yield, by condensing **7** with diethylamine, mp 71–73 °C.

^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 1.01 (t, 6H, J = 7.2 Hz, RN(CH$_2$C$\underline{H}_3$)$_2$); 2.08 (s, 3H, COC$\underline{H}_3$); 3.12 (q, 4H, J = 7.2 Hz,

RN(CH_2CH_3)$_2$); 7.70 (d, 2H, J = 9.0 Hz, H2 and H6); 7.77 (d, 2H, J = 9.0 Hz, H3 and H5); 10.34 (s, 1H, CONH).

^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 14.1 (RN(CH_2CH_3)$_2$); 24.2 ($COCH_3$); 41.8 (RN(CH_2CH_3)$_2$); 118.8 (C-2 and C-6); 127.9 (C-3 and C-5); 133.4 (C-4); 143.0 (C-1);169.1 (C=O).

IR (ν_{max}, KBr) ν (cm^{-1}): 3235, 1681, 1330, 1149, 935, 848.

Anal. Calcd. for $C_{12}H_{18}N_2O_3S$: C, 53.31; H, 6.71; N, 10.36. Found: C, 53.22; H, 6.78; N, 10.28.

4.1.3.3. N-*[4-(Piperidin-4-ylsulfonyl)phenyl]acetamide (**5c**; LASSBio-1297).* The title compound was obtained as a yellow crystal, in 70% yield, by condensing **7** with piperidine, mp 118–120 °C.

^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 1.34 (m, 2H, H4′); 1.52 (m, 4H, H3′ and H5′); 2.09 (s, 3H, $COCH_3$); 2.83 (t, 4H, J = 5.3 Hz, H2′ and H6′); 7.64 (d, 2H, J = 8.9 Hz, H2 and H6); 7.80 (d, 2H, J = 8.9 Hz, H3 and H5); 10.38 (s, 1H, CONH).

^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 23.5 ($COCH_3$); 24.7 (C-4′); 25.2 (C-3′ and C-5′); 47.2 (C-2′ and C-6′); 119.2 (C-2 and C-6); 129.2 (C-3 and C-5); 129.5 (C-4); 143.9 (C-1);169.7 (C=O).

IR (ν_{max}, KBr) ν (cm^{-1}): 3261, 1675, 1336, 1162, 850.

Anal. Calcd. for $C_{13}H_{18}N_2O_3S$: C, 55.30; H, 6.43; N, 9.92. Found: C, 55.24; H, 6.47; N, 9.72.

4.1.3.4. N-*[4-(Piperazin-4-ylsulfonyl)phenyl]acetamide (**5d**; LASSBio-1299).* The title compound was obtained as a beige powder, in 50% yield, by condensing **7** with piperazine, mp 150–152 °C.

^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 2.09 (s, 3H, $COCH_3$); 2.39 (br, 1H, NH); 2.73 (s, 8H, H2′-H6′); 7.64 (d, 2H, J = 8.9 Hz, H2 and H6); 7.82 (d, 2H, J = 8.9 Hz, H3 and H5); 10.41 (s, 1H, CONH).

^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 24.7 ($COCH_3$); 45.1 (C-3′ and C-5′); 47.1 (C-2′ and C-6′); 119.2 (C-2 and C-6); 128.8 (C-4); 129.4 (C-3 and C-5); 144.0 (C-1); 169.7 (C=O).

IR (ν_{max}, KBr) ν (cm^{-1}): 3410, 3294, 1687, 1342, 1172, 840.

Anal. Calcd. for $C_{12}H_{17}N_3O_3S$: C, 50.87; H, 6.05; N, 14.83. Found: C, 50.77; H, 6.08; N, 14.87.

4.1.3.5. N-*[4-[(4-Methyl-1-piperazinyl)sulfonyl]phenyl]acetamide (**5e**; LASSBio-1300).* The title compound was obtained as a yellow crystal, in 67% yield, by condensing **7** with *N*-methyl-piperazine, mp 175 °C.

^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 2.09 (s, 3H, $COCH_3$); 2.12 (s, 3H, $RNCH_3$); 2.33 (t, 4H, J = 4.8 Hz, H3′ and H5′); 2.84 (t, 4H, J = 4.8 Hz, H2′ and H-6′); 7.65 (d, 2H, J = 8.5 Hz, H2 and H6); 7.82 (d, 2H, J = 8.5 Hz, H3 and H5); 10.41 (s, 1H, CONH).

^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 24.2 ($COCH_3$); 45.3 ($RNCH_3$); 45.8 (C-3′ and C-5′); 53.5 (C-2′ and C-6′); 118.7 (C-2 and C-6); 128.3 (C-4); 128.8 (C-3 and C-5); 143.5 (C-1); 169.2 (C=O).

IR (ν_{max}, KBr) ν (cm^{-1}): 3345, 1704, 1343, 1162, 839.

Anal. Calcd. for $C_{13}H_{19}N_3O_3S$: C, 52.51; H, 6.44; N, 14.13. Found: C, 52.34; H, 6.59; N, 14.03.

4.1.3.6. N-*[4-(Morpholin-4-ylsulfonyl)phenyl]acetamide (**5f**; LASSBio-1296).* The title compound was obtained as a yellow crystal, in 55% yield, by condensing **7** with morpholine, mp 174–176 °C.

^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 2.09 (s, 3H, $COCH_3$); 2.81 (t, 4H, J = 4.8 Hz, 4.3 Hz, H2′ and H6′); 3.61 (t, 4H, J = 4.8 Hz, 4.3 Hz, H3′ and H5′); 7.65 (d, 2H, J = 8.9 Hz, H2 and H6); 7.83 (d, 2H, J = 8.9 Hz, H3 and H5); 10.41 (s, 1H, CONH).

^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 24.2 ($COCH_3$); 45.9 (C-2′ and C-6′); 65.3(C-3′ and C-5′); 118.7 (C-2 and C-6); 127.8 (C-4); 129.0 (C-3 and C-5); 143.7 (C-1); 169.2 (C=O).

IR (ν_{max}, KBr) ν (cm^{-1}): 3305, 1679, 1344, 830.

Anal. Calcd. for $C_{12}H_{16}N_2O_4S$: C, 50.69; H, 5.67; N, 9.85. Found: C, 50.67; H, 5.67; N, 9.76.

4.1.3.7. N-*[4-(Thiomorpholin-4-ylsulfonyl)phenyl]acetamide (**5g**; LASSBio-1295).* The title compound was obtained as a yellow powder, in 65% yield, by condensing **7** with thiomorpholine, mp 207–209 °C.

^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ (ppm): 2.09 (s, 3H, $COCH_3$); 2.65 (t, 4H, J = 5.0 Hz, H2′ and H6′); 3.16 (t, 4H, J = 5.0 Hz, H3′ and H5′); 7.67 (d, 2H, J = 8.9 Hz, H2 and H6); 7.82 (d, 2H, J = 8.9 Hz, H3 and H5); 10.40 (s, 1H, CONH).

^{13}C NMR (50 MHz, DMSO-d_6, TMS) δ (ppm): 24.2 ($COCH_3$); 26.4 (C-3′ and C-5′); 47.8 (C-2′ and C-6′); 118.8 (C-2 and C-6); 128.5 (C-3 and C-3); 129.5 (C-4); 143.5 (C-1); 169.2 (C=O).

IR (ν_{max}, KBr) ν (cm^{-1}): 3352, 1702, 1321, 1153, 835.

Anal. Calcd. for $C_{12}H_{16}N_2O_3S_2$: C, 47.98; H, 5.37; N, 9.33. Found: C, 47.84; H, 5.33; N, 9.24.

4.2. Biological assays

4.2.1. Animals

Swiss mice weighing 20–30 g (from the BIOCEN-UFAL) were housed in group cages and maintained on a 12 h light/12 h dark cycle. Animals had free access to food and water at all times. Experiments were carried out according to a protocol approved by the Animal Welfare Committee of Federal University of Alagoas (UFAL) and in according with the ethical guidelines for investigation of experimental pain in conscious animals [33].

4.2.2. Reagents

Acetic acid (Merck), arabic gum (Sigma Aldrich), morphine sulphate (Dimorf-Cristalia-BR), and paracetamol (Sigma Aldrich) were obtained from commercial sources. A solution of formalin 2.5% was prepared with formaldehyde (Merck) in saline (NaCl 0.9%). Paracetamol (**1**) and its analogues **5a**–**g** were used as suspension in arabic gum in all the experiments and oral administrations.

4.2.3. Acetic acid-induced writhing

This test was performed as described by Collier et al. [12], Koster et al. [13] and Fontenele et al. [14]. Acetic acid (0.6%, v/v) was administered i.p. 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 from 5 min after the acetic acid injection. Paracetamol (**1**) and the *N*-phenyl-acetamide sulfonamide derivatives (**5a**–**g**) (all 100 μmol/kg, oral administration) were administered 60 min before the acetic acid injection. Antinociceptive activity was expressed as inhibition percent of the usual number of writhing observed in control animals. Dose–response curves were obtained for paracetamol (**1**) and LASSBio-1300 (**5e**) (100, 30, 10 and 3 μmol/kg) using groups of 8 animals. Control animals received the vehicle. The ID_{50} values (i.e. dose which reduces response by 50% relative to the control values), to paracetamol and LSSBio-1300 (**5e**) were determined by linear regression from individual experiments with linear regression Graph Pad Prisma software®.

4.2.4. Hot-plate test

Mice were treated according to the method described by Franzotti et al. [15]. The animals (n = 6) were placed on a hot-plate set at 55 ± 1 °C. Reaction time was recorded when the animals licked their fore and hind-paws and jumped at 30, 60, 90 and 120 min after oral administration of 100 μmol/kg of paracetamol (**1**) or the *N*-phenyl-acetamide sulfonamide derivatives (**5a**–**g**) or reference drug (morphine, 15 μmol/kg. i.p.). Baseline was considered as the mean of reaction time obtained at 30 and 60 min before administration of

5a–**g** or **1** or morphine and was defined as normal reaction of animal to the temperature.

4.2.5. Formalin-induced nociception

The procedure used was essentially the same as that described previously [20,21]. Animals received 20 μL of 2.5% formalin solution (0.92% formaldehyde in saline) in the ventral surface of the right hind paw. Animals were observed from 0 to 5 min (neurogenic phase) and from 15 to 30 min (inflammatory phase) and the time that they spent licking the injected paw was recorded and considered as indicative of nociception. Animals received paracetamol (**1**) or the *N*-phenyl-acetamide sulfonamide derivatives (**5a**–**g**) (100 μmol/kg, oral administration) 40 min beforehand. Control animals received vehicle (arabic gum).

4.2.6. Rectal temperature

The rectal temperature of the mice was determined with a digital thermometer at a distance of 2.5 mm from the anus. The measurement was performed at room temperature of 25 °C. Mice with normal rectal temperature 37.0–38.0 °C were selected and used in the experiment. The baseline rectal temperature was measured after 1 h acclimatization. Shortly after, animals received paracetamol or the *N*-phenyl-acetamide sulfonamide derivatives (**5a**–**g**) (100 μmol/kg, oral administration). After 1 h of administration the rectal temperature was measured [27].

4.2.7. Serum enzyme assessment

Animals received vehicle ($n = 5$), paracetamol (100 μmol/kg, p.o., $n = 6$) and LASSBio-1300 (**5e**) (100 μmol/kg, p.o., $n = 6$) for five days. 24 h after the last dose of vehicle, paracetamol and LASSBio-1300 (**5e**), animals in all groups were anesthetized with ethyl ether and the blood was collected. The serum was separated by centrifugation at 3000 × *g* for 15 min and stored at 4 °C for the assay of γGT using γ Glutamyl Transferase kinetic method kit (LABORLAB, Brazil) [32].

4.2.8. Histology

For light microscopic investigations, following the blood collection, the animals were euthanised by means of CO_2 and for each animal, ten representative samples of the liver (two each from the left, right, left middle, right middle and caudate lobes) were placed in 10% neutral buffered formalin and routinely in an automatic tissue processor, embedded in paraffin, sectioned at 4–6 μm and stained with haematoxylin and eosin (H&E). The prepared samples were histologically examined and the changes observed in each individual liver lobe were observed.

4.2.9. Statistical analysis

Data obtained from animal experiments were expressed as the mean standard error (Mean ± S.E.M.). Statistical differences between the treated and the control groups were evaluated by ANOVA and Dunnett hoc tests. $P < 0.05$ was considered to be significant ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

5. Molecular modeling

The conformer distribution of *N*-phenyl-acetamide sulfonamide derivatives (**5a**–**g**) and paracetamol (**1**) was carried out using the semi empirical AM1 Hamiltonian [29] within Spartan'08 Windows program on a Pentium IV 1.5 GHz. The stereoelectronic properties were calculated on all the conformers of the molecules, as available in the Spartan package [30].

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