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Design and Synthesis of Novel Potent Antinociceptive Agents: Methyl-imidazolyl *N*-Acylhydrazone Derivatives†

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Abstract—This paper describes recent results of design, synthesis and pharmacological evaluation of new *N*-heterocyclic functionalized *N*-acylhydrazone compounds, belonging to the 2-methyl-imidazolyl-3-acylhydrazone class (**4a**–**e**). These compounds were planned by applying the molecular simplification strategy to propose the structural modifications on the previously described functionalized imidazo[1,2-*a*]pyridine 3-acylhydrazone series (**2**), which presented an important analgesic profile. This new series (**4**) was synthesized in order to investigate the possible pharmacophoric contribution of the *N*-heteroaromatic ring and *N*-acylhydrazone moieties to the analgesic activity. Compounds **4a**–**b** are the most potent antinociceptive agents from this series.

Introduction

In the course of an ongoing research program aimed at the design, synthesis and pharmacological evaluation of new bioactive compounds acting at arachidonic acid cascade enzymes level, we described previously the analgesic and platelet anti-aggregating profile of *N*-phenylpyrazole 4-acylhydrazone (NAH) derivatives (**1**),[1] functionalized imidazo[1,2-*a*]pyridine 3-acylhydrazone derivatives (**2**)[2] and *N*-phenyl-3-methyl-pyrazolo[3,4-*b*]pyridine 5-acylhydrazone derivatives (**3**)[3] (Fig. 1). Compounds from series **2**, particularly the *para*-bromophenyl derivative (**2b**), presented an important analgesic profile when orally administered to mice in doses of 100 μM.[2]

As our studies progressed with new heterocyclic *N*-acylhydrazone (NAH) derivatives as candidates to antinociceptive agents, we designed a new class of azaheterocyclic NAH derivatives (**4a**–**e**). This series was structurally planned by employing the molecular simplification strategy to introduce further structural modifications on the previously active series (**2**) (path a, Fig. 1). Thus, we assume that the imidazole ring introduced in **4**, which represents an isosteric replacement, could imitate the aromatic system of the lead series (**2**) and give rise to a different hydrophobic character to the *N*-heterocyclic framework linked to the *para*-substituted aryl-*N*-acylhydrazone moiety, considered as the principal pharmacophoric unit involved in the analgesic activity.[3]

In the new imidazolic series of NAH derivatives (**4**), we elected only the groups associated with the most active derivatives in the previous lead series (e.g. **4a**–**b**, NMe_2, Br)[1–3] as the *para*-substituents of the phenyl ring at the hydrazone motif. In addition, we decided to introduce the 3,5-di-*tert*-butylphenol substituent (**4e**) due to the known redox properties of this framework,[4] which is present in other anti-inflammatory derivatives.[5,6] We also decided to synthesize the unsubstituted phenyl ring compound (**4c**) to verify the contribution of the *para*-substituents present in **4a**–**b**, as well as the isosteric 2-furyl derivative (**4d**).[7–9]

The rational design of compounds from series **4** may provide evidence to the contribution of the *N*-heterocyclic moiety in the anti-inflammatory and analgesic activities, considering that the imidazolic system in the new series (**4**) presents a lower hydrophobic character

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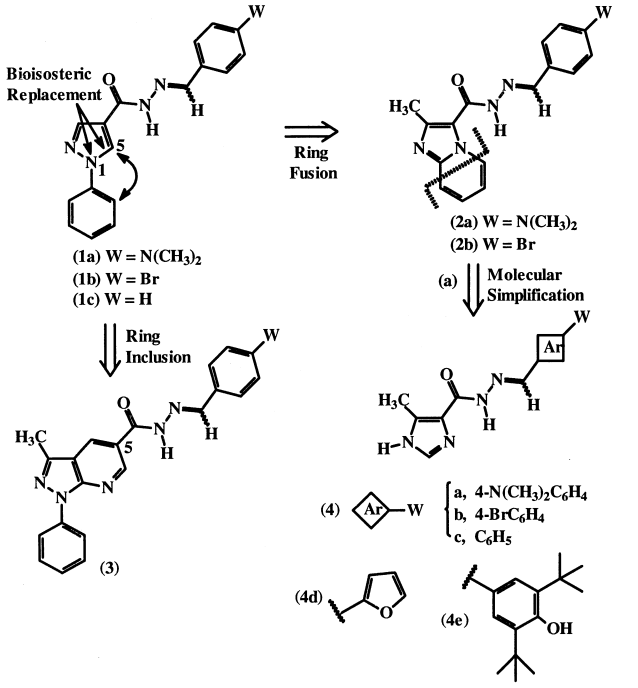
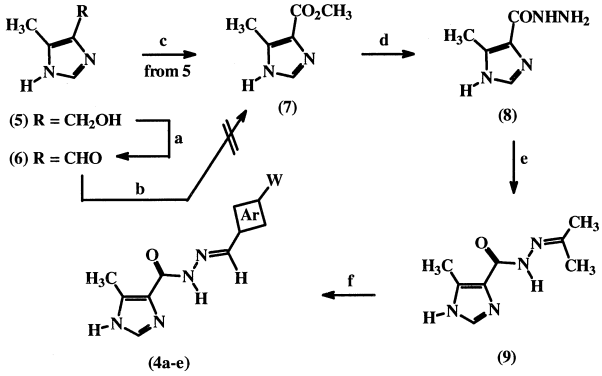


Figure 1. Design concept of the new imidazolyl NAH derivatives (**4**).



Scheme 1. Synthetic route for the preparation of imidazolyl NAH derivatives (**4**). (a) MnO_2, THF, reflux, 3 h, 85%; (b) I_2, KOH, MeOH, rt, 24 h; (c) NaCN, MnO_2, MeOH, rt, 20 h, 70%; (d) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 1.5 h; (e) $(H_3C)_2C{=}O$, EtOH, $HCl_{(cat.)}$, reflux, 12 h, 75% (2 steps); (f) Ar-CHO, MeOH, $HCl_{(cat.)}$, reflux, 20 h.

than the previous lead series (**2**). Furthermore, the presence of the methyl group in the imidazole ring of **4** ensures the same *ortho*-relationship with the supposed pharmacophoric unit as in the lead series (**2**) and could prevent the typical tautomerism of the imidazolic system, as observed in other therapeutically useful imidazolic compounds.[10]

Results and Discussion

The most convenient method previously developed for the preparation of the desired imidazolic NAH compounds (**4a–e**) was the condensation of imidazolyl hydrazide (**8**) with elected aromatic aldehydes, which were performed in the precedent series.[1–3] The key intermediate hydrazide (**8**) can be obtained from the oxidation of 5(4)-methyl-4(5)-hydroxymethyl imidazole (**5**)[11] to the corresponding methyl ester derivative (**7**) followed by treatment with hydrazine hydrate (Scheme 1). Therefore, we initially decided to use the Yamada method[12] for the conversion of the aldehyde (**6**) into methyl 5-methylimidazole-4-carboxylate (**7**). The oxidation of alcohol **5**[11] using MnO_2 in THF,[13] in reflux, gave a yield of 85% of the corresponding aldehyde (**6**), as a pale-yellow solid.[14] Unfortunately, under Yamada conditions (i.e., I_2 in methanolic KOH), we were not able to obtain the desired methyl ester derivative (**7**) from the aldehyde (**6**). In order to circumvent this unexpected result, we proceeded with the investigation of the oxidation method described by Lai and Anderson[15] using MnO_2/NaCN in methanol, directly over alcohol (**5**), to obtain methyl ester (**7**). Fortunately, this process gave a yield of 70% of methyl ester (**7**).[16]

The N-4 tautomer was found to be the major compound in the ester (**7**) and its identity was revealed through the analysis of ^1H and ^{13}C NMR spectra, considering the chemical shift of the imidazolic hydrogen and C-2, respectively. Thus, Montgomery and co-workers[17] indicated that in structurally related compounds possessing an electron-withdrawing group (EWG) at C-4 and a methyl substituent at C-5 from the imidazole ring, the N-4 tautomer is favored and presents H-2 as a single signal in the ^1H NMR spectra upfield shifted at δ 2.53. Due to the C-2 from the imidazole ring, the signal in the ^{13}C NMR spectra occurs at δ 134.2. These data are consistent with the pattern observed for the ester (**7**) (see Experimental). In general, the population of the imidazole tautomer can be estimated from the electronic influence of the ring substituents. In fact, the presence of the electron-releasing group (ERG) favored the N-5 tautomer while the EWG favored the N-4 tautomer.[18]

Afterwards, as it was initially established by the synthetic route, the hydrazide derivative (**8**) was prepared by treating **7** with an 80% hydrazine hydrate solution. Unfortunately, although the thin-layer chromatography (TLC) indicated the formation of a new polar compound, which was initially assumed as the desired hydrazide (**8**), all attempts to obtain this key intermediate from the reaction mixture were unsuccessful. In order to circumvent this experimental limitation, we decided to trap this intermediate in situ, by carefully adding a carbonyl compound. None of our attempts to use aromatic aldehydes were successful. Fortunately, by adding an excess of acetone at the final stage of the reaction, as indicated by TLC analysis, the yield of the corresponding acetone–hydrazone obtained **9** was almost 80%. The structure of this stable and crystalline compound was fully spectroscopically characterized by the usual methods. The success of this procedure over the aromatic aldehyde addition was probably due to the distinct solubility properties exhibited by the two different classes of carbonyl compounds in the reaction medium. This procedure is particularly advantageous because the hydrazone adduct (**9**) can be stocked for several weeks at room temperature and can be easily prepared in a molar scale.

After the key intermediate acetone–hydrazone adduct (**9**) was prepared using an efficient method, a '*trans-hydrazonation*' reaction was performed by treating **9**

with an excess of the appropriate aromatic aldehyde in acidic conditions (Scheme 1). As a matter of fact, using this procedure, the new desired imidazolic NAH compounds (**4a**–**e**) were obtained in adequate yields, as illustrated in Table 1.

The (*E*)–(*Z*) diastereomeric ratio of these new NAH compounds (**4a**–**e**) was next investigated by spectroscopic methods. The careful analysis of ^{1}H and ^{13}C NMR spectra indicated the presence of the (*Z*)-isomer formed in the '*trans-hydrazonation*' process as the major one.[19] The double-bond configuration was identified by the **H**C=N chemical shift in the ^{1}H NMR spectra, occurring in all derivatives, at the range of δ 8.35–8.50 ppm in accordance with previous observations from the precedent series (**1–2**)[1–3,19] (Table 1).

Molecular modeling studies using AM1 Hamiltonian[20] were next carried out with the unsubstituted derivative (**4c**) in order to investigate the conformational behavior of the NAH motif around the C-4 from the imidazole nucleus. From these studies we were able to observe that the *S-cis*-like conformation **A** of the N-4 tautomer from compound **4c** was ca. 6.0 kcal/mol more stable than the corresponding *S-trans* **A′** conformation (Fig. 2). Besides, this conformation (**A**) (Fig. 2) could be favored by a five-member-like intramolecular hydrogen bond involving the hydrogen atom of the *N*-acylhydrazone moiety and the nitrogen atom N-3 of the imidazole ring. Hydrogen bonding was also observed in the N-5 tautomer (conformation B′; Figure 2). However, this one is 3 kcal/mol less stable.

The analgesic and anti-inflammatory activities of the new imidazolyl hydrazone derivatives (**4a**–**e**) were evaluated using the acetic acid-induced mice abdominal constrictions test[21] and the carrageenan-induced rat paw edema test,[22] respectively. The results for the analgesic activity are shown in Table 2. All compounds studied, initially with a concentration of 100 μmol/kg, were able to inhibit ca. 70% of abdominal induced constrictions, with a higher potency than indomethacin (55.4%) and dipyrone (42.1%), in the same dose. Thus, due to this high analgesic activity, we decided to investigate these compounds with half of the dose, i.e., with a concentration of 50 μmol/kg. In this concentration, all *N*-acylhydrazone derivatives (**4a**–**e**) were more active than dipyrone in the concentration of 100 μmol/kg. The most potent analgesic compound (**4b**) showed 61.7% of inhibition of the induced contortions, followed by the *para*-dimethylaminophenyl compound (**4a**), which presented 58% of inhibition. Both compounds (**4a**–**b**) were more active than indomethacin twice as much as its dose.

The results of the anti-inflammatory bioassay for series **4** are shown in Table 3. Compounds **4a**–**e** inhibited significantly 35–45% of the edema formation for 3 h after the induction. In this bioassay, **4b** was the most active

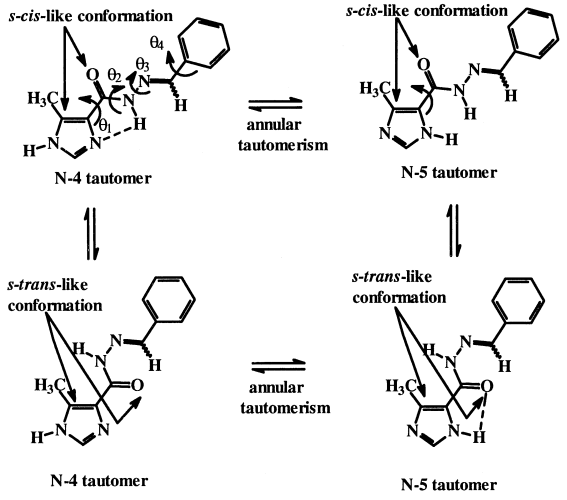


Figure 2. Ring isomerism and conformers of the new class of NAH derivatives (**4**).

Table 2. Effects of 5-methyl-imidazolyl-4-acylhydrazone derivatives (**4a–e**), dipyrone and indomethacin in the inhibition of abdominal constrictions induced by acetic acid (0.6%, ip) in mice

Compound	Dose[a] (μmol/kg)	*n*[b]	Constrictions number	Inhibition (%)[c]
Vehicle control (Arabic gum 5%)	—	10	93.8±4.9	—
Dipyrone	100	9	54.3±5.5	42.1*
Indomethacin	100	14	41.9±4.4	55.3*
4a	100	10	31.3±5.5	66.6*
	50	8	37.5±3.4	58.0*
4b	100	9	26.8±3.4	71.4*
	50	8	34.1±2.8	61.7*
4c	100	10	22.5±4.9	76.0*
	50	7	43.0±5.3	51.7*
4d	100	9	26.2±3.0	72.0*
	50	9	40.2±3.3	54.8*
4e	100	10	21.9±3.8	76.6*
	50	9	43.3±3.8	51.3*

[a]All compounds were administered po.
[b]*n* = number of animals.
[c]% of inhibition obtained by comparison with vehicle control group. *$P < 0.05$ (Student's *t*-test). Results are expressed as mean ± SEM.

Table 1. Physical properties and yields of 5-methyl-imidazolyl 4-acylhydrazone derivatives (**4a–e**)

Compound	Molecular formula	Molecular weight	Yield (%)	mp (°C)	δ (ppm) N=C<u>H</u>
4a	$C_{14}H_{17}N_5O$	271	69	247–250 (dec.)	8.35
4b	$C_{12}H_{11}N_4OBr$	307	76	> 250 (dec.)	8.50
4c	$C_{12}H_{12}N_4O$	228	73	> 250 (dec.)	8.48
4d	$C_{10}H_{10}N_4O_2$	218	93	158–160	8.47
4e	$C_{20}H_{28}N_4O_2$	356	60	176–179	8.37

Table 3. Effects of 5-methyl-imidazolyl-4-acylhydrazone derivatives (**4a**–**e**) and indomethacin in the inhibition of carrageenan-induced rat paw edema

Compound	Time (h)[a]	n[b]	Dose[c] (μmol/kg)	Volume variation (μL)[d]	Inhibition (%)[e]
	1		—	267.2±47.2	—
Vehicle control	2	9	—	564.8±52.1	—
(Arabic gum 5%)	3		—	773.9±36.2	—
	1			104.9±13.5	60.7*
Indomethacin	2	7	100	140.3±13.8	75.1*
	3			153.2±11.9	80.2*
	1			169.0±19.2	36.7*
4a	2	12	100	358.1±31.7	36.6*
	3			485.2±34.8	37.3*
	1			116.6±10.8	56.4*
4b	2	11	100	375.4±41.9	33.5*
	3			509.4±49.4	34.2*
	1			153.4±22.8	42.6*
4c	2	12	100	317.3±24.1	43.8*
	3			444.5±44.8	42.6*
	1			191.8±25.8	28.2*
4d	2	9	100	411.1±23.6	27.2*
	3			513.8±31.8	33.6*
	1			157.3±22.9	41.1*
4e	2	8	100	326.4±35.1	42.2*
	3			479.5±60.1	38.0*

[a]Time after carrageenan injection (0.1 mg/paw).
[b]n = number of animals.
[c]All compounds were administered po.
[d]Volume variation is the difference between the volumes of carrageenan treated paw and saline treated paw.
[e]% of inhibition obtained by comparison with vehicle control group.
* $P < 0.05$ (Student's t-test). Results are expressed as mean ± SEM.

compound presenting 56.4% of inhibition at the first hour with a concentration of 100 μmol/kg.

The anti-inflammatory profile of this series of NAH (**4**) was clearly distinct from that previously described for compounds of series **2**.[1,2,23] In fact, the 2-furyl derivative from the *N*-phenylpyrazolic series (**2**) was not able to present any important effect in the carrageenan-induced rat paw edema test.[23,24] Curiously, the corresponding *para*-bromophenyl derivative belonging to this series (**2**) was not able to present any anti-inflammatory activity in identical concentrations in the same pharmacological assay.[2] Furthermore, the effect of these compounds (**4**) in the gastric mucosa was also investigated, indicating a weak ulcerative behavior for compound **4c** when used in the concentration of 300 μmol/kg.

These results suggest that this new series of imidazolic NAH derivatives (**4**) presents a very important analgesic activity profile in comparison with the most hydrophobic series (**2**) previously described. Therefore, the *N*-heteroaromatic ring of derivatives (**4**) is probably responsible for the improvement of the analgesic activity due to the less hydrophobic character of the imidazolic ring and a nitrogen atom is sterically less hindered in this series.

Conclusions

Our results indicate that the isosteric replacement of the imidazo[1,2-*a*]pyridine system from the original lead compound (**2**) by the imidazole ring in the new series of imidazolyl *N*-acylhydrazone derivatives (**4**) represents an optimization of the analgesic activity in this class of derivatives. Moreover, compounds **4a** and **4b** were identified as the most potent analgesic agents, suggesting that the imidazole ring plays an important role in the analgesic activity observed.

Experimental

Chemistry

Infrared (IR) spectra were obtained with a Perkin–Elmer 1600 spectrometer by using potassium bromide plates. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were determined in dimethylsulfoxide (DMSO-d_6) containing ca. 1% TMS as an internal standard with AC 200 Bruker and VXR 300 Varian and Gemini 300 Varian spectrophotometers. Splitting patterns are as follows: s, singlet; d, doublet; m, multiplet; b, broad. The signals due to the hydrogen attached to the imidazole ring are indicated as Im-H. The mass spectra were obtained with a GC/VG Micromass 12 at 70 eV. Microanalysis data was obtained using a Perkin–Elmer 240a analyzer. Melting points were determined with a Quimis 340 apparatus and are uncorrected.

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. Melting points were determined through a Quimis 120 capillary melting point apparatus and are uncorrected. The solvents employed in the reactions were previously distilled.

Methyl 5-methyl-imidazole-4-carboxylate (7).[15] To a mixture of 2.24 g (20 mmol) of 4(5)-methyl-5(4)-hydroxymethyl-imidazole (**5**) and 1.63 g of sodium cyanide (33.2 mmol) in 200 mL of methanol, was added 8.7 g activated manganese dioxide[15] (100 mmol) over 5 min. The mixture was stirred for 20 h at room temperature and the suspension was filtered through Celite, and washed with dichloromethane (3×20 mL). The organic fractions were combined, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure, to give 2.24 g (80%) of the methyl ester (**7**) as a white solid, mp 171–173 °C; MS (70 eV) relative abundance: 140 (M^+, 100%), 125 (21%), 109 (95%); IR (KBr) cm^{-1}: 1708 (ν C=O); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.62 (s, 1H, Im H-2), 3.95 (s, 3H, OCH_3), 2.59 (s, 3H, Im-CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 163.5 (C=O), 137.5 (C-5), 135.0 (C-2), 132.0 (C-4), 52.5 (OCH_3), 14.0 (Im-CH_3).

(Isopropylidene)-5-methyl-imidazole-4-carbohydrazide (9). One gram of 80% aqueous hydrazine monohydrate (20 mmol) was added to a solution of 1 g of methyl ester (**7**) (7.14 mmol) in 2.5 mL of absolute ethanol and the resulting mixture was refluxed for 4 h. The solvent was evaporated under reduced pressure and to the crude resulting oil was added 15 mL of *n*-hexane, 5 mL of

ethanol, 5 mL of acetone, one drop of hydrochloridric acid and the mixture was refluxed for 2 h. The evaporation under reduced pressure and the filtration of the resulting solid in Buchner furnished 0.91 g (71%) of the acetone–hydrazone derivative (**9**) as a yellow solid, mp 245–250 °C (dec.); MS (70 eV) 180 (M^+, 5%); 165 (5%), 109 (100%); IR (KBr) cm^{-1}: 3385, 3252 and 3151 (ν N–H), 1668 (ν C=O); ^1H NMR (200 MHz, DMSO-d_6) δ 9.85 (br, 1H, CONH), 7.60 (s, 1H, Im H-2), 3.50 (br, 2H, NH_2), 2.45 (s, 3H, Im-CH_3), 2.00 (s, 3H, N=CCH_3), 1.90 (s, 3H, N=CCH_3); ^{13}C NMR (50 MHz, DMSO-d_6) δ 160.0 (C=O), 154.5 (Me_2C=N), 134.0 (C-2), 133.0 (C-5), 128.5 (C-4), 25.5 (CH_3), 17.0 (CH_3), 14.0 (Im-CH_3).

General procedure for the preparation of *N*-acylhydrazone derivatives (4a–e). Equimolar amounts of the appropriate aromatic aldehydes and two drops of hydrochloridric acid, acting as a catalyst, were added to a solution of acetone–hydrazone derivative (**9**) in 20 mL of ethanol. At the end of the reaction, which was monitored by TLC analysis, compounds **4a**–**e** were isolated by concentrating the reaction mixture under reduced pressure, followed by the addition of dichloromethane (ca. 25 mL), cooling and filtration under vacuum.

(4-Dimethylaminebenzylidene)-5-methyl-imidazole-4-carbohydrazide (4a). Derivative **4a** was obtained with a yield of 69% by trans-hydrazonation of **9** with 4-dimethylaminobenzaldehyde as a yellow solid, mp 247–250 °C (dec.); MS (70 eV) relative abundance: 271 (M^+, 74%), 163 (52%), 146 (100%), 125 (7%), 109 (43%); IR (KBr) cm^1: 3468, 3432, 3386 and 3236 (ν N–H), 1664 (ν C=O), 1599 (ν C=C); ^1H NMR (200 MHz, DMSO-d_6) δ 12.70 (br, 1H, Im-NH), 11.00 (br, 1H, CONH), 8.35 (s, 1H, CH=N), 7.63 (s, 1H, Im H-2), 7.50 (d, 2H, C-4′, J= 15 Hz), 6.75 (d, 2H, C-3′, J= 15 Hz), 2.96 ($N(CH_3)_2$), 2.45 (s, 3H, Im-CH_3); ^{13}C NMR (50 MHz, DMSO-d_6) δ 159.8 (C=O), 151.6 (C-4′), 147.7 (CH=N), 133.7 (Im C-2), 132.5 (Im C-5), 128.6 (C-3′ and C-5′), 128.0 (Im C-4), 122.0 (C-1′), 112.0 (C-2′ and C-6′), 40.0 ($N(CH_3)_2$) and 11.0 (Im-CH_3). Anal. calcd for $C_{14}H_{17}N_5O$: C, 61.96; H, 6.32; N, 25.82. Found: C, 62.07; H, 6.34; N, 25.90.

(4-Bromobenzylidene)-5-methyl-imidazole-4-carbohydrazide (4b). Derivative **4b** was obtained with a yield of 76% by trans-hydrazonation of **9** with 4-bromobenzaldehyde, as a yellow solid, mp > 250 °C (dec.); MS (70 eV) relative abundance: 306 (M^+, 5%), 308 (5%), 198 (5%), 200 (5%), 125 (33%), 109 (100%); IR (KBr) cm^{-1}: 3471, 3322, 3386 and 3186 (ν N–H), 1668 (ν C=O), 1591 (ν C=C); ^1H NMR (200 MHz, DMSO-d_6) δ 12.60 (br, 1H, Im-NH), 11.40 (br, 1H, CONH), 8.50 (s, 1H, CH=N), 7.68 (s, 1H, Im H-2), 7.65 (m, 2H, C-2′ and C-6′), 7.45 (m, 2H, C-3′ and C-5′), 2.50 (s, 3H, Im-CH_3); ^{13}C NMR (50 MHz, DMSO-d_6) δ 159.8 (C=O), 146.4 (CH=N), 134.8 (Im C-2), 133.7 (C-1′), 129.6 (C-4′), 129.2 (Im C-5), 128.8 (C-3′ and C-5′), 127.9 (Im C-4), 126.9 (C-2′ and C-6′), 10.7 (Im-CH_3). Anal. calcd for $C_{12}H_{11}BrN_4O$: C, 47.06; H, 3.62; N, 18.30. Found: C, 46.98; H, 3.69; N, 18.38.

(Benzylidene)-5-methyl-imidazole-5-carbohydrazide (4c). Derivative **4c** was obtained with a yield of 73% by trans-hydrazonation of **9** with benzaldehyde as a yellow solid, mp > 250 °C; MS (70 eV) relative abundance: 228 (M^+, 7%), 125 (30%), 109 (100%); IR (KBr) cm^{-1}: 3314, 3207 and 3174 (ν N–H), 1668 (ν C=O), 1593 (ν C=C); ^1H NMR (200 MHz, DMSO-d_6) δ 12.50 (br, 1H, Im-NH), 11.42 (s, 1H, CONH), 8.48 (s, 1H, N=CH), 7.70 (s, 1H, Im H-2), 7.60 (m, 5H, Ph), 2.55 (s, 3H, Im-CH_3); ^{13}C NMR (50 MHz, DMSO-d_6) δ 159.8 (C=O), 145.0 (CH=N), 134.1 (C-1′), 133.7 (Im C-2), 132.8 (Im C-5), 131.7 (C-3′ and C-5′), 128.7 (C-2′ and C-6′), 128.0 (Im C-4), 122.7 (C-4′), 10.6 (Im-CH_3). Anal. calcd for $C_{12}H_{12}N_4O$: C, 63.13; H, 5.30; N, 24.56. Found: C, 63.18; H, 5.39; N, 24.48.

(2′-Furylidene)-5-methyl-imidazole-4-carbohydrazide (4d). Derivative **4d** was obtained with a yield of 93% by trans-hydrazonation of **9** with furfuraldehyde as a yellow solid, mp 158–160 °C; MS (70 eV) relative abundance: 218 (M^+, 18%), 125 (16%), 109 (100%); IR (KBr) cm^{-1}: 3468, 3332 and 3214 (ν N–H), 1662 (ν C=O) and 1592 (ν C=C); ^1H NMR (200 MHz, DMSO-d_6) δ 11.75 (br, 1H, Im-NH), 8.47 (s, 1H, N=CH), 8.04 (br, 1H, CONH), 7.82 (s, 1H, C-5′), 6.89 (s, 1H, C-3′), 6.78 (s, 1H, Im H-2), 6.6 (s, 1H, C-4′), 2.6 (s, 3H, Im-CH_3); ^{13}C NMR (50 MHz, DMSO-d_6) δ 158.7 (C=O), 149.6 (C5), 144.8 (CH=N), 136.8 (C-2′), 133.6 (Im C-2), 133.3 (Im C-5), 126.6 (Im C-4), 112.9 (C-4′), 112.1 (C-3′) and 10.6 (Im-CH_3). Anal. calcd for $C_{10}H_{10}N_4O_2$: C, 55.03; H, 4.62; N, 25.68. Found: C, 55.11; H, 4.69; N, 55.08.

(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-5-methyl-imidazole-4-carbohydrazide (4e). Derivative **4e** was obtained with a yield of 60% by trans-hydrazonation of **9** with 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde as a yellow solid, mp 176–179 °C; MS (70 eV) relative abundance: 356 (M^+, 25%), 341 (3%), 248 (3%), 233 (17%), 125 (27%), 109 (100%); IR (KBr) cm^{-1}: 3438 (ν O–H), 3318, 3376 and 3167 (ν N–H), 1651 (ν C=O), 1605 (ν C=C); ^1H NMR (200 MHz, DMSO-d_6) δ 12.34 (br, 1H, Im-NH), 10.92 (s, 1H, CONH), 8.37 (s, 1H, N=CH), 7.60 (s, 1H, Im H-2), 7.45 (s, 2H, H6-H2), 7.24 (s, 1H, OH), 2.48 (s, 3H, Im-CH_3), 1.41 (s, 18H, C(CH_3)$_3$); ^{13}C NMR (50 MHz, DMSO-d_6) δ 159.5 (C=O), 155.7 (C-4′), 147.7 (N=CH), 139.1 (C-3′ and C-5′), 133.3 (Im C-4), 132.1 (Im C-2), 129.0 (C-1), 126.0 (Im C-5), 123.6 (C-2′ and C-6′), 34.4 ($C(CH_3)_3$), 30.1 (C(CH_3)$_3$), 10.4 (Im-CH_3). Anal. calcd for $C_{20}H_{28}N_4O_2$: C, 67.37; H, 7.92; N, 15.72. Found: C, 67.42; H, 7.89; N, 15.78.

Molecular modeling

All modeling procedures were performed on a Silicon Graphics workstation IRIX 6.3 (CPU R5000). The molecular models were built and pre-optimized on the Builder module of InsightII (Molecular Simulations Inc., San Diego, CA) and conformational analysis was done using the CVFF forcefield within Search-Compare module in InsightII environment (Molecular Simulations Inc., San Diego, CA).

Conformational analysis by molecular mechanics was done by filtering the conformations in a range of 5 kcal/mol. The starting conformation was, in each case, the

minimized structure after building structures for **4c**. The dihedral angles were defined as follows: $\theta_1 = 0$–$180°$, $\theta_2 = 180°$, $\theta_3 = 0$–$360°$ with increments of 60° and $\theta_4 = 0$–$180°$, with increments of 60° (see Figure 2 for torsion angle definition).

The conformations obtained from molecular mechanics were then reoptimized by AM1, using the keywords AM1, MMOK, EF, HESS = 1, GRAD and PRECISE.[20]

Pharmacology

Analgesic activity. The analgesic activity was determined in vivo by the abdominal constriction test induced by 0.6% (0.1 mL/10 g) acetic acid in mice.[21] Albino mice of both sexes (18–23 g) were used. Compounds **4a**–**e** were administered orally (100 μmol/kg; 0.1 mL/20 g) as a suspension in 5% Arabic gum in saline (vehicle). Indomethacin (100 μmol/kg) and dipyrone (100 μmol/kg) were used as standard drugs in the same conditions. Acetic acid solution was administered ip 1 h later. Ten minutes following ip 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 control group.

Anti-inflammatory activity. The anti-inflammatory activity was determined in vivo using the carrageenan-induced rat paw edema test described by Ferreira.[22] Fasted albino rats of both sexes (150–200 g) were used. Compounds **4a**–**e** were administered orally and intraperitoneally (100 μmol/kg; 0.1 mL/20 g) as a suspension in 5% Arabic gum in saline (vehicle). Control animals received an equal volume of vehicle. One hour later, the animals were injected with either 0.1 mL of 1% carrageenan solution in saline (0.1 mg/paw) or sterile saline (NaCl 0.9%), into the subplantar surface of one of the hind paws respectively. The paw volume was measured using a glass plethysmometer coupled to a peristaltic pump, at each hour, up to 4 h after the subplantar injection. The edema was calculated as the volume variation between the carrageenan and saline treated paw. Indomethacin (100 μmol/kg) was used as the standard drug in the same conditions. Anti-inflammatory activity was expressed as % of inhibition of the edema when compared with the control group.

Statistics. Results are expressed as the mean ±SEM of '*n*' animals per group. The data were statistically analyzed by the Student's *t*-test for a significance level of $*P < 0.05$.

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