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Synthesis and pharmacological evaluation of novel antinociceptive N-substituted-phenylimidazolyl-4-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 (*NAH*), belonging to the N-substituted-phenylimidazolyl-4-acylhydrazone class (**3a**–**o**). These compounds were planned by applying the molecular hybridization strategy to propose the structural modifications on the previously described functionalized 2-methyl-imidazolyl-3-acylhydrazone class (**2**), which presented an important analgesic profile. This new series (**3**) was synthesized in order to investigate the possible pharmacophoric contribution of the N-heteroaromatic ring and N-acylhydrazone moieties to the analgesic activity. Compounds **3g** and **3n** are the most potent analgesic agents from this series, at the screening dose of 100 mg/kg *p.o.* and compounds **3e**, **3j** and **3o** presented the best antiinflammatory properties at the same screening concentration.

Keywords: N-substituted-phenylimidazolyl-4-acylhydrazone derivatives; Antiinflammatory activity; Analgesic activity

1. Introduction

In the course of an ongoing research program aiming at the design, synthesis and pharmacological evaluation of new bioactive *N*-acylhydrazone (*NAH*) derivatives [1–3], we described previously the analgesic and antiinflammatory profile of functionalized imidazo[1,2-*a*]pyridine 3-acylhydrazone derivatives (**1**) [1]. Next, we designed a second class of azaheterocyclic *NAH* derivatives, now belonging to 2-methyl-imidazolyl-3-acylhydrazone class (**2**) [4]. Series (**2**) was structurally planned by molecular simplification of **1** (Fig. 1) and presented the most important analgesic *NAH* derivatives obtained in the laboratory [5,6] when orally administered to mice in doses of 100 μM. These results were preliminary rationalized on the basis of the contribution of the less hydrophobic imidazole ring, compared with the previous series of *NAH* derivatives.

We wish describe in this paper a new class of imidazolyl *NAH* derivatives **3** following our studies on new azaheterocyclic *N*-acylhydrazone (*NAH*) derivatives as antinociceptive agents. These compounds were structurally planned by applying the molecular hybridization strategy on the previous class of 2-methyl-imidazolyl-3-acylarylhydrazone (**1**) derivatives and aminopyrine (**4**), a known pyrazolone with analgesic properties [7] (Fig. 1).

The rational basis of the molecular hybridization employed in the structural design of the new series **3** maintains the NAH moiety (sub-unit **A**, Fig. 1) and the imidazole ring presents in the most active compound of the previous series (**2**) [4]. In addition, we thought that it could be interesting to retain a similar substitution pattern around the imidazole nucleus and namely the NAH chain, with an *ortho*-monovalent isosteric substituent, i.e. C-5 methyl ($\sigma_{ortho} = 0.84$; MR = 5.65) in (**2**) and C-5 chloro ($\sigma_{ortho} = 0.76$; MR = 6.03) [8] in (**3**), such that the heterocyclic ring would retain a similar overall shape compared to the previously analgesic series **2**. Finally, we introduced the sub-unit **C** (Fig. 1) from

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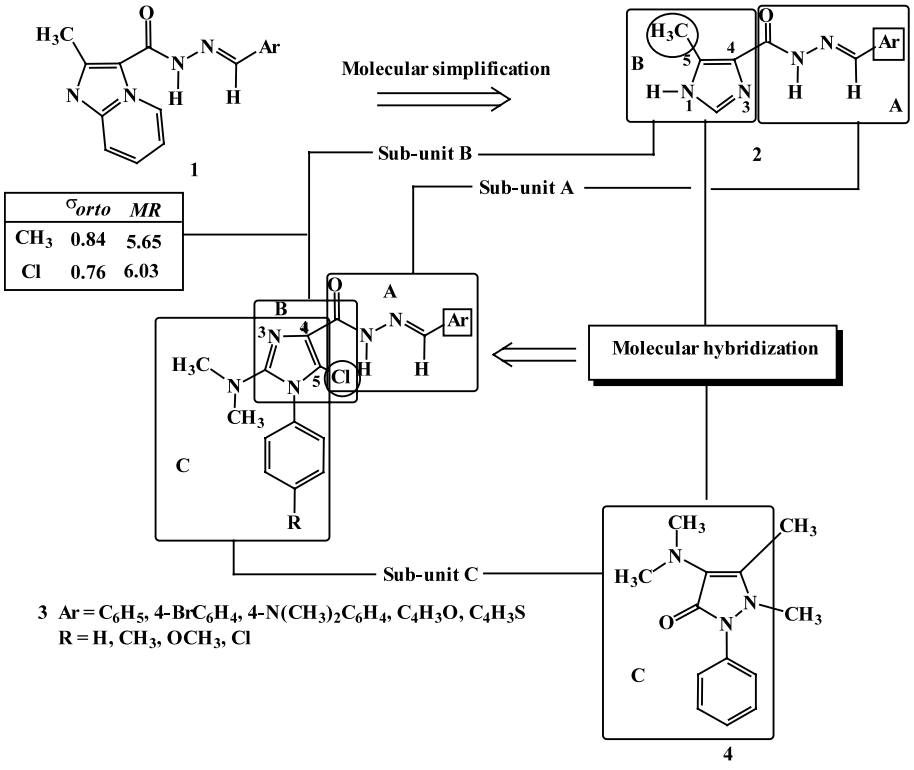
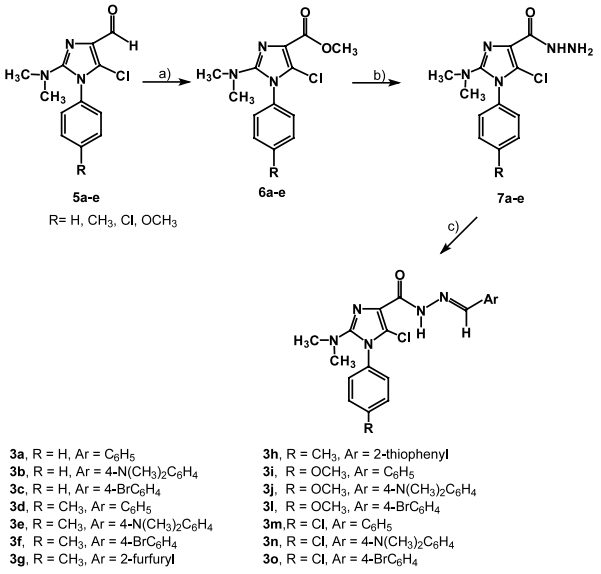


Fig. 1. Design of N-substituted-phenylimidazolyl-4-acylhydrazone derivatives **3a**–**o**.

pyrazolone (**4**), keeping the *N*-phenyl and *C*-dimethylamine substituent arriving to the structure of series **3**. The selection of Ar group at end of imine double bond in this series was based on the previously described one as well as the nature of the **R** substituent at the *N*-phenyl ring [4–6].

2. Results and discussion

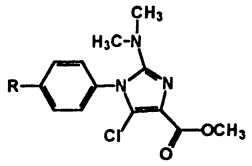
The synthesis of the new substituted NAH derivatives **3a**–**o** is depicted in Scheme 1. The known *N*-substituted 5-chloro-2-(dimethylamino)imidazole-4-carboxaldehydes **5a**–**e**, possessing different substituents at the *N*-phenyl moiety (R = H, Me, Cl and OMe), were prepared according to the procedure developed for construction of nitrogen heterocycles, under Vilsmeier conditions [9]. The oxidation of **5a**–**e** with manganese dioxide and sodium cyanide in MeOH, afforded the desired methyl esters **6a**–**e** [10], which were next converted to the corresponding hydrazides intermediates **7a**–**e** by treatment with hydrazine hydrate (Table 1). Finally, compounds **7a**–**e** were obtained by condensing with functionalized aromatic aldehydes, i.e. benzaldehyde, 4-dimethylaminobenzaldehyde, 4-bromobenzaldehyde, furan-2-carboxaldehyde and thiophene 2-carboxaldehyde, at reflux in methanol, to produce the desired *N*-substituted-phenylimidazolyl-4-acylhydrazones (**3a**–**o**) in good yields, as illustrated in Table 2.



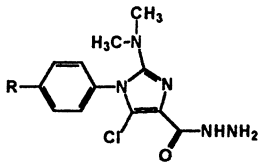
Scheme 1. (a) NaCN, MnO_2, MeOH, rt, 12h, 84–92%. (b) NH_2NH_2. H_2O 80%, EtOH, reflux, 8–10h, 70–93%. (c) Ar-CHO, EtOH, HCl (cat.), reflux, 2h, 85–96%.

The structure of these stable and crystalline compounds were fully characterized by the usual methods. (IR, ^1H, ^{13}C NMR). IR spectra of these new NAH derivatives presented the N–H absorption at 3304–3489 cm^{-1}, the C=O at 1655–1748 cm^{-1} and C=N at 1570–1645 cm^{-1} stretching bands (Table 2). The *E*-configuration of the C=N double bond in the compounds **3a**–**o**

Table 1
Yields, IR and ^{1}H NMR spectral data of compounds **6a–d** and **7a–d**



6a-d

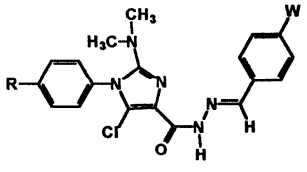


7a-d

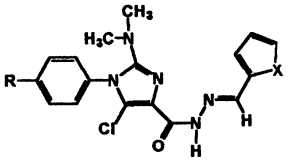
Compound	R	Yield (%)	M.p. (°C)	IR [b] (cm^{-1}) C=O	IR (cm^{-1}) (N–H)	^{1}H NMR and δ (ppm)
6a	H	92	75–76 [a]	1709	–	7.58–7.50 (m, 3H, H3 and H4′), 7.36–7.30 (m, 2H, H2′), (s, 3H, OCH_3) 2.64 (s, 6H, $N(CH_3)_2$)
6b	CH_3	90	135–136 [a]	1709	–	7.21 (d, 2H, $J = 8.3$ Hz, H3′), 7.12 (d, 2H, $J = 8.3$ Hz, H2′), 3.83 (s, 3H, OCH_3), 2.57 (s, 6H, $N(CH_3)_2$), 2.36 (s, 3H, CH_3)
6c	OCH_3	84	145–150 [a]	1677	–	7.50 (d, 2H, $J = 8.8$ Hz, H2′), 7.36–7.26 (m, 2H, H3′), 3.91 (s, 3H OCH_3), 3.91 (s, 3H, $O(CH_3$ aromatic), 2.64 (s, 6H, $N(CH_3)_2$)
6d	Cl	85	188–189 [a]	1720	–	7.64 (d, 2H, $J = 8.8$ Hz, H3′), 7.47 (d, 2H, $J = 8.8$ Hz, H2′), 8.70 (br, 1H, $NHNH_2$), 4.33 (br, 2H, $NHNH_2$), 2.55 (s, 6H, $N(CH_3)_2$)
7a	H	74	195–196	1653	3301; 3206	7.56–7.43 (m, 5H, aromatic), 3.35 (br, 3H, $NHNH_2$), 2.53 (s, 6H, $N(CH_3)_2$)
7b	CH_3	93	204–205	1653	3301; 3206	7.34 (d, 2H, $J = 7.3$ Hz, H3′), 7.28 (d, 2H, $J = 7.3$ Hz, H2′), 8.78 (br, 1H, $NHNH_2$), 4.50 (br, 2H, $NHNH_2$), 2.50 (s, 6H, $N(CH_3)_2$), 2.39 (s, 3H, CH_3)
7c	OCH_3	70	194–196	1654	3430; 3299	7.32 (d, 2H, $J = 9.0$ Hz, H2′), 7.10 (d, 2H, $J = 9.0$ Hz, H3′), 8.32 (br, 1H, $NHNH_2$), 4.37 (br, 2H, $NHNH_2$), 3.80 (s, 3H, OCH_3), 2.53 (s, 6H, $N(CH_3)_2$)
7d	Cl	77	224–226	1656	3297; 3204	7.64 (d, 2H, $J = 8.8$ Hz, H3′), 7.47 (d, 2H, $J = 8.8$ Hz, H2′), 8.70 (br, 1H, $NHNH_2$), 4.33 (br, 2H, $NHNH_2$), 2.55 (s, 6H, $N(CH_3)_2$)

[a] Purified by silica gel colunm chromatography.
[b] Obtained from KBr plates.

Table 2
N-Substituted-phenylimidazolyl-4-acylarylhydrazone derivatives **3a–o**



3a-f and 3i-o

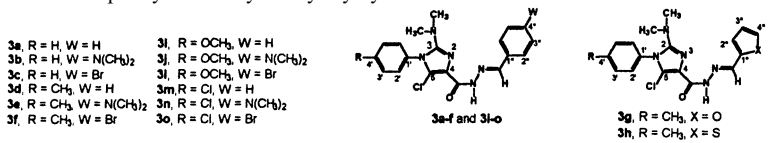


3g-h

Compound	R	W/X	Yield (%)	M.p. (°C)	Molecular formula [a]	Molecular weight	R (cm^{-1})
3a	H	H	91	185	$C_{19}H_{18}ClN_5O$	367.84	3303 (N–H), 1655 (C=O), 1574 (C=N)
3b	H	$N(CH_3)_2$	95	176–179	$C_{21}H_{23}ClN_6O$	410.90	3344 (N–H), 1661 (C=O), 1600 (C=N)
3c	H	Br	91	171–174	$C_{19}H_{17}BrClN_5O$	446.73	3304 (N–H), 1674 (C=O), 1590 (C=N)
3d	CH_3	H	85	184–185	$C_{20}H_{20}ClN_5O$	381.86	3314 (N–H), 1672 (C=O), 1571 (C=N)
3e	CH_3	$N(CH_3)_2$	96	190–191	$C_{22}H_{25}ClN_6O$	424.93	3304 (N–H), 1684 (C=O), 1599 (C=N)
3f	CH_3	Br	89	169–170	$C_{20}H_{19}BrClN_5O$	460.76	3281 (N–H), 1673 (C=O), 1590 (C=N)
3g	CH_3	O	90	200–201	$C_{18}H_{18}ClN_5O_2$	371.82	3442 (N–H), 1669 (C=O), 1572 (C=N)
3h	CH_3	S	84	250–252	$C_{18}H_{18}ClN_5OS$	387.89	3424 (N–H), 1672 (C=O), 1573 (C=N)
3i	OCH_3	H	90	184–185	$C_{19}H_{18}ClN_5O$	402.28	3210 (N–H), 1678 (C=O), 1556 (C=N)
3j	OCH_3	$N(CH_3)_2$	95	199–200	$C_{21}H_{22}Cl_2N_6O$	445.35	3218 (N–H), 1747 (C=O), 1557 (C=N)
3i	OCH_3	Br	70	183–185	$C_{19}H_{16}BrCl_2N_5O$	481.18	3303 (N–H), 1748 (C=O), 1589 (C=N)
3m	Cl	H	63	193–194	$C_{20}H_{20}ClN_5O_2$	397.86	3231 (N–H), 1655 (C=O), 1570 (C=N)
3n	Cl	$N(CH_3)_2$	90	177–178	$C_{22}H_{25}ClN_6O_2$	440.93	3271 (N–H), 1746 (C=O), 1645 (C=N)
3o	Cl	Br	95	175–176	$C_{20}H_{19}BrClN_5O_2$	476.76	3297 (N–H), 1747 (C=O), 1661 (C=N)

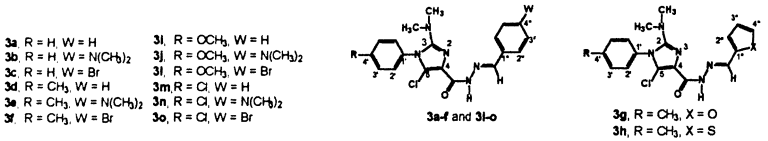
[a] The analytical results for C, H, N were within $\pm 0.4\%$ of calculated values.

Table 3
^{1}H NMR spectral data at 200 MHz (DMSO-d_6) for N-substituted-phenylimidazolyl-4-acylarylhydrazone derivatives **3a**–**o**



Compound	δ (ppm)								
	H-2′	H-3′	H-4′	N=CH	H-2″	H-3″	H-4″	N–H	Other
3a	7.53–7.44 (m)	7.53–7.44 (m)	7.53–7.44 (m)	8.57 (s)	7.62–7.56 (m)	7.73–7.69 (m)	7.73–7.69 (m)	11.16 (s)	2.60 (s) $N(CH_3)_2$ (ring)
3b	7.58–7.48 (m)	7.53–7.48 (m)	7.53–7.48 (m)	8.38 (s)	7.53 (d) $J = 8.3$ Hz	6.76 (d) $J = 8.3$ Hz	–	10.84 (s)	2.59 (s) $N(CH_3)_2$ (ring), 2.96 (s) $N(CH_3)_2$
3c	7.62–7.46 (m)	7.62–7.46 (m)	7.62–7.46 (m)	8.55 (s)	7.65 (s)	7.65 (s)	–	11.30 (s)	2.61 (s) $N(CH_3)_2$
3d	7.30 (d) $J = 8.9$ Hz	7.39 (d) $J = 8.9$ Hz	–	8.55 (s)	7.73–7.68 (m)	7.48–7.39 (m)	7.48–7.39 (m)	11.00 (s)	2.40 (s) CH_3, 2.63 (s) $N(CH_3)_2$
3e	7.30 (d) $J = 8.4$ Hz	7.39 (d) $J = 8.4$ Hz	–	8.35 (s)	7.52 (d) $J = 9.0$ Hz	6.75 (d) $J = 9.0$ Hz	–	10.66 (s)	2.40 (s) CH_3, 2.60 (s) $N(CH_3)_2$ (ring), 2.97 (s) $N(CH_3)_2$
3f	7.31 (d) $J = 8.5$ Hz	7.39 (d) $J = 8.5$ Hz	–	8.52 (s)	7.64 (s)	7.64 (s)	–	11.06 (s)	2.40 (s) CH_3, 2.60 (s) $N(CH_3)_2$
3g	7.30 (d) $J = 8.2$ Hz	7.39 (d) $J = 8.2$ Hz	–	8.46 (s)	6.85 (d) $J = 3.2$ Hz	6.65 (d) $J = 3.2$ and 1.2 Hz	7.82 (d) $J = 1.2$ Hz	11.00 (s)	2.39 (s) CH_3, 2.59 (s) $N(CH_3)_2$
3h	7.27 (d) $J = 8.2$ Hz	7.38 (d) $J = 8.2$ Hz	–	8.62 (s)	7.52 (d) $J = 5.1$ Hz	7.08 (dd) $J = 5.1$ and 3.6 Hz	7.26 (d) $J = 3.6$ Hz	11.05 (s)	2.38 (s) CH_3, 2.56 (s) $N(CH_3)_2$
3i	7.36 (d) $J = 8.9$ Hz	7.11 (d) $J = 8.9$ Hz	–	8.46 (s)	7.72–7.68 (m)	7.45–7.40 (m)	7.45–7.40 (m)	11.20 (s)	2.60 (s) $N(CH_3)_2$, 3.82 (s) OCH_3
3j	7.35 (d) $J = 8.9$ Hz	7.10 (d) $J = 8.9$ Hz	–	8.35 (s)	7.51 (d) $J = 8.9$ Hz	6.74 (d) $J = 8.9$ Hz	–	10.71 (s)	2.61 (s) $N(CH_3)_2$ (ring), 2.96 (s) $N(CH_3)_2$
3i	7.36 (d) $J = 9.0$ Hz	7.11 (d) $J = 9.0$ Hz	–	8.52 (s)	7.64 (s)	7.64 (s)	–	11.11 (s)	2.62 (s) $N(CH_3)_2$, 3.83 (s) OCH_3
3m	7.54 (d) $J = 8.5$ Hz	7.68 (d) $J = 8.5$ Hz	–	8.60 (s)	7.70–7.66 (m)	7.56–7.44 (m)	7.56–7.44 (m)	11.20 (s)	2.60 (s) $N(CH_3)_2$
3n	7.54 (d) $J = 8.5$ Hz	7.68 (d) $J = 8.5$ Hz	–	8.34 (s)	7.52 (d) $J = 8.9$ Hz	6.76 (d) $J = 8.9$ Hz	–	10.84 (s)	2.60 (s) $N(CH_3)_2$, 2.97 (s) $N(CH_3)_2$
3o	7.54 (d) $J = 8.9$ Hz	7.69 (d) $J = 8.9$ Hz	–	8.35 (s)	7.65 (s)	7.65 (s)	–	11.30 (s)	2.61 (s) $N(CH_3)_2$

Table 4
^{13}C NMR spectral data at 50 MHz (DMSO-d_6) for *N*-substituted-phenylimidazolyl-4-acylarylhydrazone derivatives **3a**–**o**



Compound	δ (ppm)													
	C-2	C-4	C-5	C-1′	C-2′	C-3′	C-4′	C-1″	C-2″	C-3″	C-4″	C=N	C=NO	Other
3a	151.7	125.5	118.6	135.0	127.6	130.0	129.3	134.9	128.6	130.3	130.2	147.8	157.7	40.8 $N(CH_3)_2$
3b	151.6	125.7	118.0	135.0	128.6	130.3	130.1	122.3	128.9	112.3	151.9	148.6	157.3	39.6 $N(CH_3)_2$, 40.8 $N(CH_3)_2$
3c	151.5	125.6	118.8	134.8	128.6	130.2	129.3	134.3	128.7	132.3	123.5	146.6	157.6	40.4 $N(CH_3)_2$
3d	151.0	124.9	117.6	139.1	127.6	129.9	131.8	134.4	126.7	129.5	128.5	147.1	157.1	20.5 CH_3, 40.5 $N(CH_3)_{2\ ring}$
3e	151.0	125.4	117.4	139.1	127.6	130.3	131.9	121.8	128.2	111.7	151.4	148.0	156.8	20.5 CH_3, 39.6 $N(CH_3)_2$ 40.6 $N(CH_3)_{2\ ring}$
3f	151.0	124.8	117.9	139.1	127.6	129.9	131.7	133.7	128.6	131.5	122.8	145.8	157.1	20.5 CH_3, 40.5 $N(CH_3)_2$
3g	151.7	125.6	118.6	139.8	128.4	130.7	132.4	150.3	113.3	112.7	137.6	145.4	158.1	21.3 CH_3, 40.8 $N(CH_3)_2$
3h	151.2	124.9	118.0	139.3	127.8	130.2	131.9	139.4	130.3	128.5	127.5	142.4	159.6	20.7 CH_3, 40.7 $N(CH_3)_2$
3i	151.2	124.7	118.3	126.8	129.2	114.7	159.6	134.1	126.8	129.7	128.6	147.1	157.2	40.6 OCH_3, 40.6 $N(CH_3)_2$
3j	151.3	124.8	118.2	126.9	129.3	114.7	159.6	121.8	128.6	111.7	154.2	147.9	156.7	39.6 $N(CH_3)_2$, 40.4 $N(CH_3)_{2\ ring}$
3l	151.2	124.6	118.4	126.9	129.3	114.7	159.6	133.8	128.7	131.7	122.9	145.9	157.0	55.9 OCH_3, 40.6 $N(CH_3)_2$
3m	151.5	125.4	118.6	133.8	130.0	129.3	134.7	135.0	127.5	130.6	130.3	147.9	157.6	40.8 $N(CH_3)_2$
3n	151.4	125.4	118.3	133.8	129.9	129.7	134.2	121.7	128.3	111.8	154.3	148.6	156.7	39.7 $N(CH_3)_2$, 40.7 $N(CH_3)_{2\ ring}$
3o	151.2	125.1	118.2	133.8	130.0	129.8	134.3	133.3	128.9	131.8	123.1	146.2	157.2	40.8 $N(CH_3)_2$

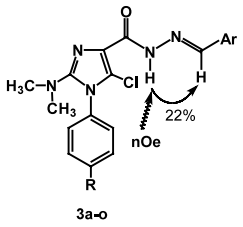


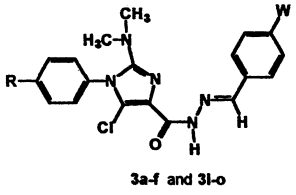
Fig. 2. (*E*)-configuration of derivatives **3a**–**o**.

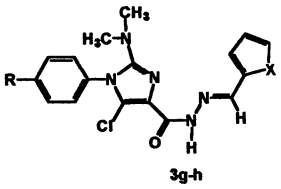
was determined by carefully ^{1}H, ^{13}C NMR analysis (Tables 3 and 4) [11,12]. In fact, the (*E*)-diastereomers of NAH presented the N=CH signal at 7.83–8.33 ppm. By applying the NOE differential experiments we were able to detect the steric vicinity of the N–H and N=CH hydrogens. For instance, the irradition of the N–H signal (10.66 ppm) expressively enhanced (22%) the N=CH hydrogen signal, occurring at 8.35 ppm (Fig. 2).

The analgesic and anti-inflammatory activities of the new N-substituted-phenylimidazolyl-4-acylhydrazone derivatives (**3a**–**o**) were evaluated using the acetic acid-induced mice abdominal constrictions test [13] and the carrageenan-induced rat paw edema test [14], respectively. The results are disclosed in Tables 5 and 6, respectively. All compounds were studied with a concentration of 100 μmol/kg.

The most potent analgesic compound **3g** (R = CH_3 and R = 2-furyl) (Table 5) showed 45.8% of inhibition of the induced contortions, higher than dipyrone used as standard (35.9%) at the same dose, followed by compound **3n** (R = Cl and W = $N(CH_3)_2$), which presented 35.2% of inhibition. Interestingly, the corresponding 5-metylimidazolyl-4-acylhydrazone derivative (**2**), described in the previous series [4] presented 72% of inhibition, suggesting that the presence of 2-*N*-dimethylamine unit and the *N*-phenyl at the imidazole ring was detrimental to the analgesic activity of this series. In addition, the *nor*-chloro congener (**3b**) of **3n**, presented only 18.3% of inhibition, and the corresponding *para*-methyl isostere (**3e**) was practically inactive. These results seem to indicate that the analgesic activity identified for derivatives possessing the *N*-dimethylphenyl unit at the imine terminus (i.e. **3n**, **3b**, **3j**, **3e**) is related to a combination of π_{arom} and σ parameters of the *para*-substituent of the *N*-phenyl ring. In contrast, the analgesic activity of other imidazole derivatives (**3a**, **3c**–**d**, **3f**–**i**, **3l**–**m**, **3o**) seems to be related to a balance of different structural contributions.

Table 5
Effect of *N*-substituted-phenylimidazolyl-4-acylarylhydrazone derivatives **3a–o** and dipyrone on the inhibition of abdominal constrictions induced by acetic acid (0.6%, *i.p.*) in mice

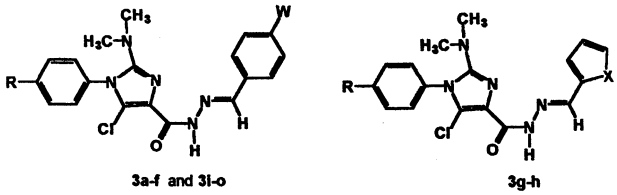




Compound [a]	R	W/X	N [b]	Constrictions count	Inhibition (%) [c]	Relative activity [d]
Vehicle control (arabic gum 5%)	–	–	10	79.8 ± 4.3	–	–
Dipyrone	–	–	10	38.7 ± 4.9	35.9	1.00
3a	H	H	11	56.6 ± 5.5	28.3	0.79
3b	H	$N(CH_3)_2$	12	64.5 ± 4.9	18.3	0.51
3c	H	Br	10	72.0 ± 4.3	8.9 [e]	0.25
3d	CH_3	H	8	66.6 ± 3.7	15.7 [e]	0.44
3e	CH_3	$N(CH_3)_2$	9	72.8 ± 3.4	8.4 [e]	0.23
3f	CH_3	Br	7	71.0 ± 4.2	10.1 [e]	0.28
3g	CH_3	O	9	42.8 ± 5.0	45.8 [f]	1.28
3h	CH_3	S	10	75.8 ± 6.2	1.3 [e]	0.04
3i	OCH_3	H	8	69.5 ± 3.9	12.1 [e]	0.34
3j	OCH_3	$N(CH_3)_2$	10	64.5 ± 5.5	18.3 [e]	0.51
3l	OCH_3	Br	9	58.3 ± 6.7	26.2 [f]	0.73
3m	Cl	H	10	87.2 ± 4.1	−10.3 [e]	−0.29
3n	Cl	$N(CH_3)_2$	13	51.2 ± 4.9	35.2 [f]	0.98
3o	Cl	Br	14	61.43 ± 5.9	22.2 [f]	0.62

[a] All test compounds were administered at a dose of 100 μmol/kg p.o.
[b] N = number of animals.
[c] % inhibition obtained by comparison with vehicle control group.
[d] Analgesic activity relative to dipyrone.
[e] Not significant.
[f] $p < 0.05$ (Student's t-test). Results are expressed as mean ± SEM.

Table 6
Effect of *N*-substituted-phenylimidazolyl-4-acylarylhydrazone derivatives **3a–o** and indomethacin in the inhibition of carrageenan-induced rat paw edema



Compound [a]	R	W/X	N [b]	Volume variation (μL) [c]	Inhibition (%) [d]
Vehicle control (arabic gum 5%)	–	–	10	478.0±30.0	–
Indomethacin	–	–	9	228.1±31.9	52.3
3a	H	H	10	341.3±43.5	28.6 [f]
3b	H	$N(CH_3)_2$	11	435.1±36.7	90.0 [e]
3c	H	Br	10	452.5±25.5	5.3 [e]
3d	CH_3	H	8	404.3±38.5	15.5 [e]
3e	CH_3	$N(CH_3)_2$	6	299.3±26.2	37.5 [f]
3f	CH_3	Br	7	431.7±35.0	9.7 [e]
3g	CH_3	O	6	343.5±41.2	28.2 [f]
3h	CH_3	S	6	399.2±56.2	16.5 [e]
3i	OCH_3	H	6	390.2±31.7	18.4 [e]
3j	OCH_3	$N(CH_3)_2$	6	334.0±40.0	30.1 [f]
3l	OCH_3	Br	6	341.2±44.9	28.6 [f]
3m	Cl	H	7	341.7±47.4	28.6 [f]
3n	Cl	$N(CH_3)_2$	7	345.6±36.7	27.7 [f]
3o	Cl	Br	6	325.3±49.4	32.0 [f]

[a] All test compounds were administered at a dose of 100 μmol/kg p.o.
[b] N = number of animals.
[c] Volume variation is the difference between the volumes of carrageenan- and saline-treated paws at the 3 h after carrageenan injection paw.
[d] Percentage of inhibition obtained by comparison with the vehicle control group.
[e] Not significant.
[f] $p < 0.05$ (Student's t-test). Results are expressed as mean ± SEM.

The results of the anti-inflammatory bioassay for series **3**, also measured with a concentration of 100 μmol/kg (Table 6) indicated that derivatives possessing the *para*-dimethylaminophenyl unit at the imine end were, with exception of **3b**, was the most active ones, inhibiting the edema formation for 3 h after the induction in 37.5%. For instance, the *N-para*-methoxyphenyl analogue **3j** presented 30.1% of edema inhibition followed by the corresponding *N-para*-chlorophenyl isoster (**3n**) that presenting 27.7% of inhibition. In addition, the bis-halogenated derivative **3o** presented 32.0% of edema inhibition. The data disclosed in Table 6 indicate that all compounds having the *N-para*-chlorophenyl moiety were active in this essay. For instance, compounds **3m** and **3n** presented an edema inhibition of 28% The anti-inflammatory profile of the most analgesic derivative of this series, compound **3g** was measured as 28.2% and was similar to the previously described compound of series **2**, which presented 33% of edema inhibition at the same concentration [4].

Furthermore, the effect of the most anti-inflammatory derivatives (**3a**, **3e**, **3g**, **3l**–**m** and **3o**) in the gastric mucosa was also investigated, indicating a very poor ulcerative behavior when essayed at the concentration of 300 μmol/kg (data not shown).

3. Conclusions

These results identified the 2-furyl compound (**3g**) as the most attractive compound, with the best antinociceptive profile, and present an attracting anti-inflammatory behavior for this new series of imidazole NAH derivatives (**3**).

4. Experimental

4.1. Chemistry

M.p.s were determined with a Thomas Hoover apparatus and are uncorrected. ^{1}H NMR spectra, unless otherwise stated, were determined in deuterated Me_2SO containing ca. 1% Me_4Si as an internal standard using a Bruker AC 200 spectrometer at 200 MHz. Splitting

patterns are as follows: s, singlet; d, doublet; dd, double doublet; br, broad; m, multiplet. ^{13}C NMR was determined using the same spectrometer described above at 50 MHz, using deuterated Me_2SO as internal standard. IR spectra were obtained using a Bruker IFS66 spectrophotometer by using KBr plates. Microanalysis data was obtained using a Perkin–Elmer 240 analyzer, using a Perkin–Elmer AD-4 balance.

The progress of all reactions was monitored by TLC performed on 2.0×6.0 cm^2 aluminum sheets precoated with silica gel 60 (HF-254, E. Merck) to a thickness of 0.25 mm. The developed chromatograms were viewed under ultraviolet light at 254 nm. For column chromatography E. Merck silica gel (60–200 mesh) was used. The N-substituted 5-chloro-2-(dimethylamino)imidazole-4-carboxaldehydes **5a**–**e** were prepared by known procedure in the literature [9].

4.1.1. General procedure for the preparation of methyl N-substituted 5-chloro-2-(dimethylamino)imidazole-4-carboxylate (*6a*–*e*)

To a mixture of 1.45 mmol of N-substituted 5-chloro-2-(dimethylamino)imidazole-4-carboxaldehydes **5a**–**e** in methanol (18 ml), and 0.35 g (7.14 mmol) of NaCN, activated MnO_2 1.60g (18.4 mmol) was added. The reaction was stirred at room temperature for 12 h and then the suspension was filtered through Celite® and treated with CH_2Cl_2 (3×20 ml). The organic layers were joined, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to furnish crude methyl esters **6a**–**e**, which were purified by flash column chromatography using 80% *n*-C_6H_{14}–EtOAc as eluent (see Table 1).

4.1.2. General procedure for preparation of the N-substituted-phenylimidazolyl-4-carbohydrazide derivatives (*7a*–*e*)

A solution the appropriate methyl ester derivates **6a**–**e** (3.41 mmol) and 3.2 ml of 80% hydrazine monohydrate in 16 ml of EtOH, was stirred at reflux for 8–10 h. After this time, the reaction mixture was concentrated under reduced pressure and the colored solid formed was collected by filtration, washed with cold water and dried under vacuum to give the desired imidazolyl hydrazides **7a**–**e**, as showed in Table 1.

4.1.3. General procedure for the preparation of the N-substituted-phenylimidazolyl-4-acylhydrazone derivatives (*3a*–*o*)

To a solution of hydrazide derivatives **7a**–**e** (0.50 mmol) in 15 ml of EtOH, was added an equimolar amount of the appropriates aromatic aldehydes in the presence catalytic amount of 37% aqueous HCl. The reaction was stirred for 2 h, at reflux. Then, solvent was evaporated and the colored precipitate was collected by filtration, washed with cold water and dried under vacuum. The imidazolylhydrazone derivates **3a**–**o** were purified in column flash chromatography using 50% *n*-C_6H_{14}–EtOAc as eluent (Tables 3 and 4).

4.2. Pharmacology

4.2.1. Analgesic activity

The analgesic activity was determined in vivo by using abdominal constriction test induced by acetic acid 0.6% (0.1 ml/10g) in mice [11]. Albino mice of both sexes (18–23 g) were used. Compounds were administered orally (100 μmol/kg; 0.1 ml/20 g) as a suspension in 5% arabic gum in saline (vehicle). Dipyrone (100 μmol/kg) was used as the standard drug under same conditions. Acetic acid solution was administered *i.p*. One hour after administration of the NAH compounds **3a**–**o**. Ten minutes after *i.p.* injection of the acetic acid solution, the number of constrictions *per* animal was recorded for 20 min. Control animals received on equal volume of vehicle. Analgesic activity was expressed as percentage of inhibition of constrictions when compared with the vehicle control group. Results are expressed as the mean ± SEM of *n* animals per group. The data were statistically analyzed by Student's *t*-test for significance level of $^*\rho < 0.05$.

4.2.2. Antiinflammatory activity

The antiinflammatory activity was determined in vivo using the carrageenan induced rat paw edema test according to Pereira and coworkers [12]. Albino rats of both sexes (150–200 g) were used. Compounds were administered orally 100 μmol/kg (0.1 ml/20g) as a suspension in 5% arabic gum in saline (vehicle). Control animals received on equal volume of the vehicle. One hour after, the animals were then injected with either 0.1 ml of 1% carrageenan solution in saline (0.1 mg/paw) and sterile saline (NaCl 0.9%), into the subplantar surface of one of the hind paws, respectively. The paw volumes were measured using a glass plethysmometer coupled to a peristaltic pump, 3 h after the subplantar injection. The edema was calculated as the volume variation between the carrageenan and saline-treated paw. Indomethacin (100 μmol/kg) and was used as standard in the same conditions. Antiinflammatory activity was expressed as percentage of inhibition of the edema when compared with vehicle control group.

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

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