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Synthesis and analgesic profile of novel *N*-containing heterocycle derivatives: arylidene 3-phenyl-1,2,4-oxadiazole-5-carbohydrazide☆

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Abstract

This paper describes recent results of a research program aimed at the synthesis and pharmacological evaluation of new heterocyclic *N*-acylhydrazone (NAH) compounds, belonging to the arylidene (3-phenyl)-1,2,4-oxadiazolyl-5-carboxyhydrazide (**8a**–**p**) series. These compounds were structurally planned by applying the molecular hybridization strategy on previously described arylidene 1-phenylpyrazole-4-carbohydrazide (**5**) derivatives, considered as lead-compounds, which present potent analgesic properties. The analgesic profile of the title compounds **8a**–**p**, evaluated in the model of abdominal constrictions induced by acetic acid, showed that the 4-methoxybenzylidene derivatives **8c** and **8k** were the most active ones, exhibiting a relative analgesic activity comparable with that of dipyrone **1** used as standard. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: 3-Phenyl-1,2,4-oxadiazole derivatives; *N*-Acylhydrazone derivatives; Analgesic properties

1. Introduction

In the course of an ongoing research program aimed at designing, synthesizing and pharmacologically evaluating new bioactive compounds acting at arachidonic acid cascade enzyme levels, we have previously described the pharmacological profile of the furylidene 1-phenyl-3-methyl-4-nitropyrazole-5-hydrazine (**2**) derivative [1], developed as a hybrid isoster of both BW-755c **3** [2] and CBS-1108 **4** [3], which have dual inhibitory properties at COX and 5-LO levels (Fig. 1). In a following study we discovered the analgesic and platelet anti-aggregation profiles of the arylidene 1-phenylpyrazole-4-carbohydrazide (**5**) series, planned by applying a molecular simplification approach on series **2**. Compounds of formulae **2**, **3** and **5** are related in the same way to the well known drug dipyrone **1**. In fact, compound **5b** displayed 11.0- and 1.19-fold greater potency than that of dipyrone **1** as an analgesic agent, in a model of abdominal constrictions induced by acetylcholine [4] and acetic acid [5], respectively. More recently, we showed the analgesic activity of a series of *N*-acylhydrazones derived from pyrazolo[3,4-*b*]pyridine (**6**) and imidazo[1,2-*a*]pyridine (**7**) rings [6,7], structurally planned from **5** (Fig. 1), where the most active derivatives were **6b** and **7b**, respectively, presenting a *para*-dimethylamino phenyl ring at hydrazone moiety.

These results led us to design new structurally related derivatives, keeping the NAH framework and modifying the nature of the *N*-heterocyclic moiety present in **5** by using bioisosterism as our strategy [8]. Thus, using this rational basis, we describe in this paper the synthesis, structural properties and pharmacological evaluation of a new series of arylidene 3-phenyl-1,2,4-oxadiazole-5-carbohydrazide derivatives **8**, planned by bioisosteric heterocyclic replacement of the *N*-phenylpyrazole ring present in lead series **5**, with

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the 3-phenyl-1,2,4-oxadiazole ring (Fig. 1) [9]. This structural modification introduces a new electronic environment at the acyl function of the hydrazone motif with possible distinct conformational preferences, which could play an interesting role in the biological profile of this NAH series. For instance, in the lead-se-

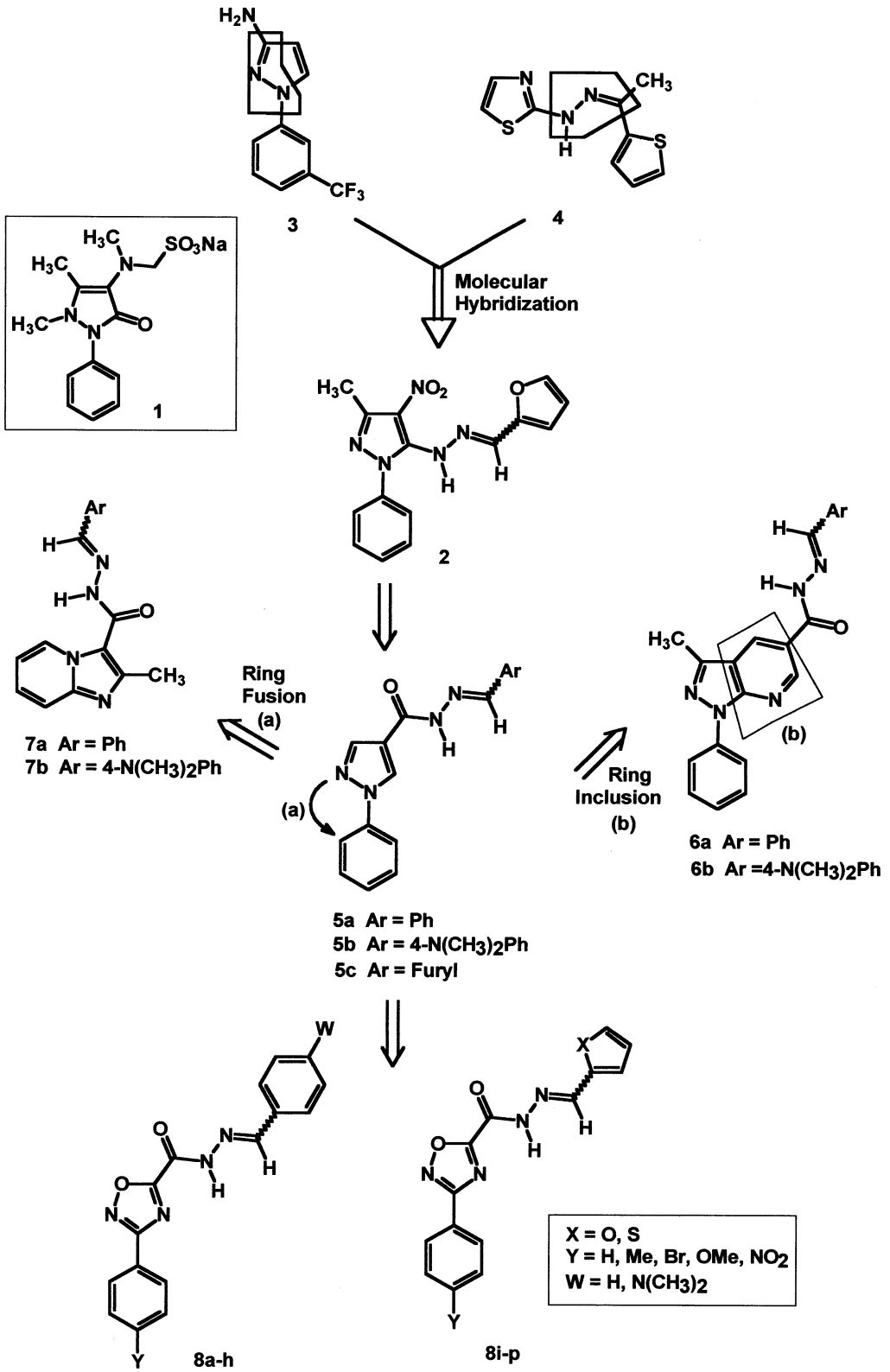
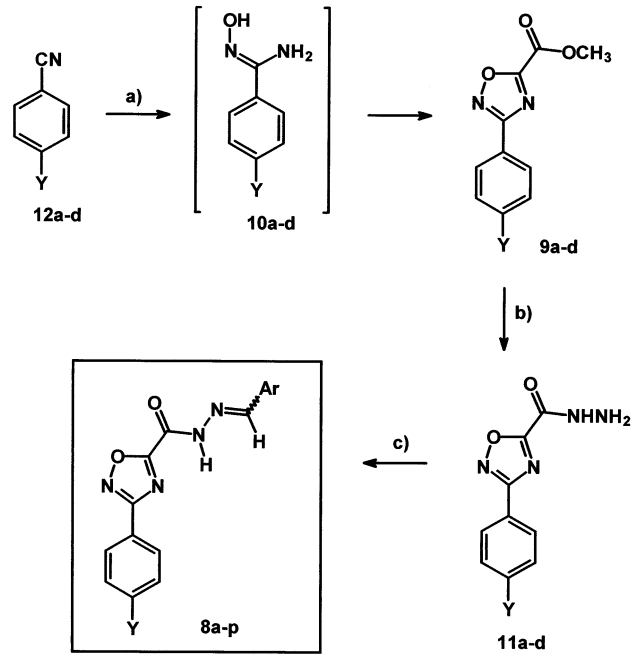


Fig. 1. Design concept of new *N*-acyl-arylhydrazone derivatives **8a**–**p**.



Scheme 1. (a) i- $NH_2OH \cdot HCl$, Na_2CO_3, methanol, water, reflux, 4 h, 70–80%; ii- $ClCOCOOCH_3$, THF, reflux, 2.5 h, 42–52%. (b) 80% aq. $NH_2NH_2 \cdot H_2O$, ethanol, r.t., 1 h, 80–90%. (c) Benzaldehyde or 4-*N*,*N*-dimethylaminobenzaldehyde or furfuraldehyde or thiophene 2-carboxaldehyde, $H_2SO_{4\ (cat.)}$, ethanol, water, reflux, 2.5 h.

ries **5** the acyl functions have two aromatic CH at the *ortho* position, whereas this new series **8** possess two distinct aromatic heteroatoms, e.g. O and N (Fig. 1). Due to this distinct neighborhood around the acyl function, we decided to investigate by molecular mechanics, using the AM1 method, the relative stability of the two possible *S-cis* versus *S-trans* conformations (vide conformers **A**, **C** versus **B**, **D** (Table 5)) in this series of derivatives. This conformational study was also undertaken in order to identify any possible 'pseudo-*ortho*-effect' caused by the distinct heteroatoms in the heterocyclic ring favoring one of the possible conformations. For instance, we can anticipate that the masked amide function present in the NAH functionality of **8** could be involved in an intramolecular H-bonding, due to its –NH-donor character. This structural feature could be responsible for an eventual favored stability between the two mentioned acyl conformations (Table 5).

The nature of the *para*-substituent Y present in the phenyl group at C-3 of 1,2,4-oxadiazole ring (i.e. H, CH_3, OCH_3, NO_2) in the derivatives **8a**–**p**, was defined in order to introduce in this new series of compounds a variation in σ_p-Hammett values [ranging from -0.21 (OCH_3) to $+0.78$ (NO_2)] which could be used to investigate any electronic contribution by this structural sub-unit on the analgesic activity. Additionally, considering the SAR of previously related acylhydrazone series **2**, **5**, **6** and **7**, the new envisaged derivatives should present at the arylhydrazone unit, phenyl (W = H, **8a**–**d**), 4-dimethylaminophenyl (W = $N(CH_3)_2$, **8e**–**h**) and furyl (X = O, **8i**–**l**) residues. Bioisosteric thienyl series (X = S, **8m**–**p**) were also designed in order to investigate the role of lipophilicity increase in the bio-evaluation of these new derivatives, considering the important lipophilic contribution of the thienyl ring [10] (Fig. 1).

2. Chemistry

The new substituted arylidene 3-phenyl-1,2,4-oxadiazole-5-carbohydrazide (**8a**–**p**) derivatives were synthesized using the route illustrated in Scheme 1.

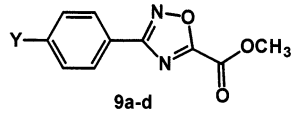
The key synthetic intermediates for the target compounds **8a**–**p** were the corresponding hydrazides **11a**–**d**, which by condensation with the appropriate aromatic aldehydes, i.e. benzaldehyde, 4-dimethylaminobenzaldehyde, furfural or thiophene 2-carboxaldehyde, at reflux in ethanol, produced the new desired arylidene 3-phenyl-1,2,4,-oxadiazole-5-carbohydrazide (**8a**–**p**) derivatives [6,7].

The initial synthetic step, illustrated in Scheme 1, was the construction of the 1,2,4-oxadiazole system present in the methyl 3-aryl-1,2,4-oxadiazole-5-carboxylate derivatives **9a**–**d**, chosen as a precursor for the hydrazides **11a**–**d**. This was performed by exploring the classical methodology to access the 1,2,4-oxadiazole system [11], by treatment of the appropriate benzonitrile **12a**–**d** with hydroxylamine in ethanol at reflux [12]. This procedure produced the substituted benzamidoxime precursor derivatives **10a**–**d**, which were next treated, in situ, with methyl oxalylchloride in tetrahydrofuran, at reflux, to furnish, in yields varying between 42 and 52%, the desired 1,2,4-oxadiazole-5-carboxylate (**9a**–**d**) derivatives (Table 1). These derivatives were then treated with hydrazine hydrate in ethanol [6,7] to give the hydrazides **11a**–**d** in 80–90% yield (Table 1).

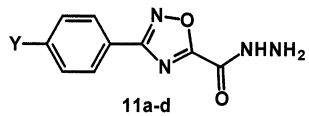
After purification by recrystallization from an ethanol/water mixture, the target compounds **8a**–**p** were obtained in good yields, as crystalline derivatives (Tables 2 and 3). The ^1H NMR spectra in DMSO-d_6 of **8a**–**p** (Table 4) showed a broad singlet varying between δ 12.50 to 13.00 ppm similar to N–*H* hydrogen, exchangeable with D_2O, indicating that the hydrazone form (*H*) is preferred over the possible tautomeric diazo form (*D*) [13,14] (Scheme 2).

The careful analysis of the ^1H NMR spectra of 1,2,4-oxadiazole carbohydrazone derivatives, **8b** and **8c**, showed the presence of two components in each. These were assigned as (*E*)-**8** and (*Z*)-**8** diastereomers and downfield shifts of C*H*=N hydrogen indicated that the (*E*)-isomer was the major one in the mixture [15–17] (Table 4). For all remaining compounds only the (*E*)-diastereomer was detected.

Table 1
Yields, physical and spectroscopic data of compounds **9a–d** and **11a–d**



9a-d



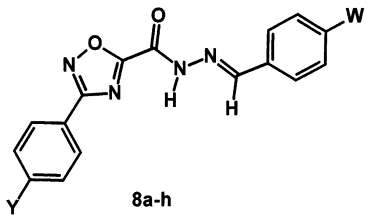
11a-d

Comp.	Y	Molecular formula [a]	Yield (%)	M.p. (°C)	IR [d] (cm^{-1}) (C=O)	IR [d] (cm^{-1}) (N–H); (NH_2)	^{1}H NMR [e] δ (ppm)
9a	H	$C_{10}H_8N_2O_3$	42	98–100 [b]	1748		8.28–8.10 (m, 2H, H-2′), 7.59–7.40 (m, 3H, H-3′ and H-4′), 4.15 (s, 3H, COOCH_3)
9b	CH_3	$C_{11}H_{10}N_2O_3$	52	89–90 [b]	1744		8.05 (d, 2H, $J = 8.1$ Hz, H-2′), 7.42 (d, 2H, $J = 8.1$ Hz, H-3′), 4.10 (s, 3H, COOCH_3), 2.40 (s, 3H, ArCH_3)
9c	OCH_3	$C_{11}H_{10}N_2O_4$	42	136–138 [b]	1753		8.02 (d, 2H, $J = 8.7$ Hz, H-2′), 7.15 (d, 2H, $J = 8.7$ Hz, H-3′), 4.10 (s, 3H, COOCH_3), 3.80 (s, 3H, ArOCH_3)
9d	NO_2	$C_{10}H_7N_3O_5$	52	142–143 [b]	1757		8.44 (d, 2H, $J = 9.0$ Hz, H-3′), 8.31 (d, 2H, $J = 9.0$ Hz, H-2′), 4.20 (s, 3H, COOCH_3)
11a	H	$C_9H_8N_4O_2$	90	219–220 [c]	1686	3319; 3247	10.11 (br, 1H, CONHNH_2), 8.15–8.00 (m, 2H, H-2′), 7.60–7.75 (m, 3H, H-3′ and H-4′), 5.10 (br, 3H, CONHNH_2)
11b	CH_3	$C_{10}H_{10}N_4O_2$	90	187–188 [b]	1687	3319; 3241	10.02 (br, 1H, CONHNH_2), 8.07 (d, 2H, $J = 8.1$ Hz, H-2′), 7.42 (d, 2H, $J = 8.1$ Hz, H-3′), 4.47 (br, 2H, CONHNH_2), 2.40 (s, 3H, ArCH_3)
11c	OCH_3	$C_{10}H_{10}N_4O_3$	80	166–167 [b]	1694	3331; 3153	9.98 (br, 1H, CONHNH_2), 8.03 (d, 2H, $J = 8.7$ Hz, H-2′), 7.15 (d, 2H, $J = 8.7$ Hz, H-3′), 4.45 (br, 2H, CONHNH_2), 3.80 (s, 3H, ArOCH_3)
11d	NO_2	$C_9H_7N_5O_4$	90	219–220 [c]	1702	3357; 3280	10.61 (br, 1H, CONHNH_2), 8.46 (d, 2H, $J = 9.0$ Hz, H-3′), 8.30 (d, 2H, $J = 9.0$ Hz, H-2′), 4.57 (br, 2H, CONHNH_2)

[a] The analytical results for C,H,N were within $\pm 0.4\%$ of calculated values.
[b] Recrystallized from ethanol.
[c] Recrystallized from ethanol/water.
[d] Obtained from KBr plates.
[e] Recorded at 200 MHz, using DMSO-d_6 as solvent.

The molecular modeling studies of the conformational preference of the acyl function were carried out using the AM1 Hamiltonian [18]. After geometry optimizations, we were not able to find any suggestive differences in the heat formation by comparing conformers presenting the same relative configuration, i.e. *S-cis*/*S-cis* (**A**) or *S-cis*/*S-trans* (**C**) versus *S-trans*/*S-cis* (**B**) or *S-trans*/*S-trans* (**D**), respectively (Table 5). How-

Table 2
Substituted arylidene 3-phenyl-1,2,4-oxadiazole-5-carbohydrazide derivatives **8a–h**

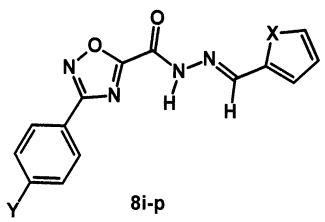


8a-h

Comp.	Y	W	Molecular formula [a]	Yield (%)	Molecular weight	M.p. (°C) [b]	δ (ppm) N=C*H* [c]		Diastereomeric (*E*):(*Z*) ratio
							(*E*)	(*Z*)	
8a	H	H	$C_{16}H_{12}N_4O_2$	80	292	186–187	8.61	[d]	
8b	CH_3	H	$C_{17}H_{14}N_4O_2$	70	306	214–215	8.63	8.71	3:1
8c	OCH_3	H	$C_{17}H_{14}N_4O_3$	75	322	205–206	8.63	8.70	1.8:1
8d	NO_2	H	$C_{16}H_{11}N_5O_4$	80	337	266–267	8.63	[d]	
8e	H	$N(CH_3)_2$	$C_{18}H_{17}N_5O_2$	70	335	202–204	8.45	[d]	
8f	CH_3	$N(CH_3)_2$	$C_{19}H_{19}N_5O_2$	85	349	234–236	8.45	[d]	
8g	OCH_3	$N(CH_3)_2$	$C_{19}H_{19}N_5O_3$	80	365	218–219	8.50	[d]	
8h	NO_2	$N(CH_3)_2$	$C_{18}H_{16}N_6O_4$	80	380	272–274	8.45	[d]	

[a] The analytical results for C, H, N were within $\pm 0.4\%$ of calculated values.
[b] Recrystallized from ethanol/water.
[c] Recorded at 200 MHz, using DMSO-d_6 as solvent.
[d] Only the signal of the (*E*)-diastereomer was detected.

Table 3
Substituted arylidene 3-phenyl-1,2,4-oxadiazole-5-carbohydrazide derivatives **8i–p**

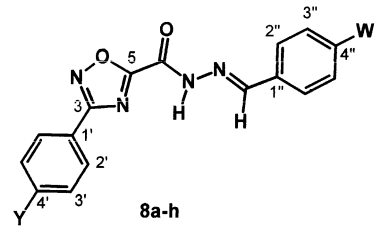


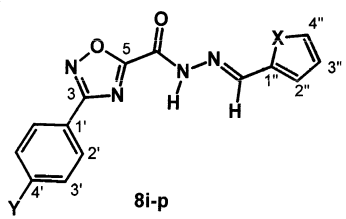
8i-p

Comp.	Y	X	Molecular formula [a]	Yield (%)	Molecular weight	M.p. (°C) [b]	δ (ppm) N=C*H* [c]	
							(*E*)	(*Z*)
8I	H	O	$C_{14}H_{10}N_4O_3$	70	282	165–166	8.51	[d]
8j	CH_3	O	$C_{15}H_{12}N_4O_3$	70	296	205–206	8.51	[d]
8k	OCH_3	O	$C_{15}H_{12}N_4O_4$	70	312	225–227	8.51	[d]
8l	NO_2	O	$C_{14}H_{19}N_5O_5$	80	327	256–257	8.51	[d]
8m	H	S	$C_{14}H_{10}N_4O_2S$	60	298	201	8.82	[d]
8n	CH_3	S	$C_{15}H_{12}N_4O_2S$	55	312	202–204	8.82	[d]
8o	OCH_3	S	$C_{15}H_{12}N_4O_3S$	60	328	214–215	8.80	[d]
8p	NO_2	S	$C_{14}H_9N_5O_4S$	60	343	254–255	8.81	[d]

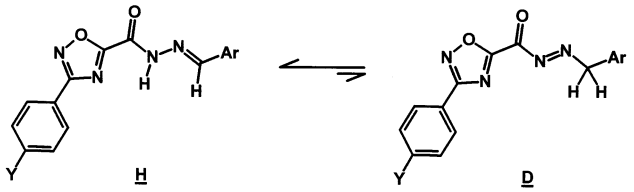
[a] The analytical results for C, H, N were within $\pm 0.4\%$ of calculated values.
[b] Recrystallized from ethanol/water.
[c] Recorded at 200 MHz, using DMSO-d_6 as solvent.
[d] Only the signal of the (*E*)-diastereomer was detected.

Table 4
^{1}H NMR data at 200 MHz (DMSO-d_6) for compounds **8a**–**p**



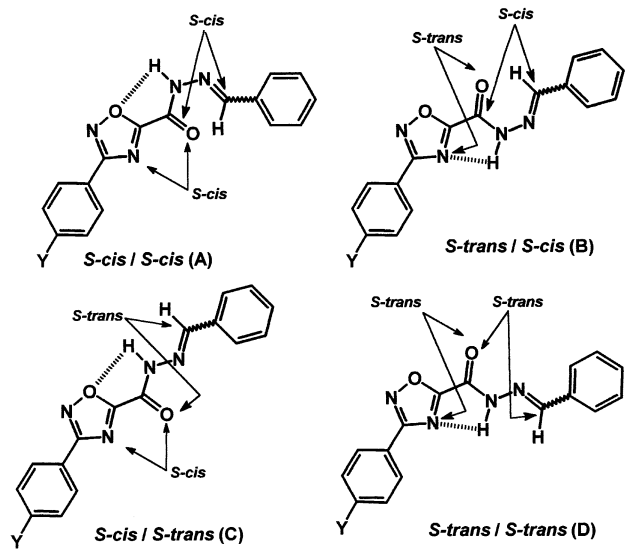


Comp.	δ (ppm)							
	H-2′	H-3′	H-4′	H-2″	H-3″	H-4″	N–H	Other
8a	8.13–8.03 (m)		7.66–7.57 (m)	7–80–7.70 (m)		7.52–7.43 (m)	12.80 (s)	
8b	8.00 (d) $J = 8.1$ Hz	7.40 (d) $J = 8.1$ Hz		7.78–7.70 (m)		7.55–7.45 (m)	12.80 (s)	2.40 (s) CH_3
8c	8.03 (d) $J = 8.7$ Hz	7.15 (d) $J = 8.7$ Hz		7.90–7.83 (m)	7.80–7.70 (m)	7.55–7.40 (m)	12.80 (s)	3.80 (s) OCH_3
8d	8.30 (d) $J = 9.0$ Hz	8.46 (d) $J = 9.0$ Hz		7.80–7.70 (m)		7.55–7.42 (m)	12.90 (s)	
8e	8.10 (m)	7.70–7.60 (m)		7.55 (d) $J = 7.7$ Hz	6.75 (d) $J = 7.7$ Hz		12.50 (s)	3.00 (s) $N(CH_3)_2$
8f	8.00 (d) $J = 8.1$ Hz	7.55 (d) $J = 8.1$ Hz		7.40 (d) $J = 8.7$ Hz	6.75 (d) $J = 8.7$ Hz		12.50 (s)	3.00 (s) $N(CH_3)_2$ 2.40 (s) CH_3
8g	8.10 (d) $J = 8.7$ Hz	7.60 (s) $J = 8.7$ Hz		7.15 (d) $J = 8.7$ Hz	6.75 (d) $J = 8.7$ Hz		12.50 (s)	3.00 (s) $N(CH_3)_2$ 3.90 (s) OCH_3
8h	8.35 (d) $J = 8.9$ Hz	8.50 (d) $J = 8.9$ Hz		7.60 (d) $J = 8.9$ Hz	6.75 (d) $J = 8.9$ Hz		12.50 (s)	3.00 (s) $N(CH_3)_2$
8i	8.11 (d) $J = 7.7$ Hz	7.68–7.61 (m)		7.06 (d) $J = 3.3$ Hz	6.70 (dd) $J = 2.2/2.2$ Hz	7.93 (d) $J = 1.1$Hz	12.80 (br)	
8j	8.01 (d) $J = 8.2$ Hz	7.44 (d) $J = 8.2$ Hz		7.06 (d) $J = 3.2$ Hz	6.70 (dd) $J = 1.5/1.5$ Hz	7.93 (d) $J = 1.4$ Hz	12.80 (br)	2.51 (s) CH_3
8k	8.04 (d) $J = 8.1$ Hz	7.18 (d) $J = 8.1$ Hz		7.06 (d) $J = 3.3$ Hz	6.70 (dd) $J = 1.9/1.9$ Hz	7.93 (d) $J = 1.6$ Hz	12.80 (br)	3.86 (s) OCH_3
8l	8.36 (d) $J = 8.4$ Hz	8.48 (d) $J = 8.4$ Hz		7.06 (d) $J = 3.4$ Hz	6.69 (dd) $J = 1.9/1.9$ Hz	7.93 (d) $J = 1.9$ Hz	13.00 (br)	
8m	8.10 (d) $J = 6.0$ Hz	7.70–7.60 (m)		7.57 (d) $J = 3.3$ Hz	7.19 (d) $J = 3.3$ Hz	7.77 (d) $J = 4.2$ Hz	12.80 (s)	
8n	8.01 (d) $J = 7.1$ Hz	7.44 (d) $J = 7.1$ Hz		7.57 (d) $J = 2.8$ Hz	7.19 (d) $J = 2.8$ Hz	7.77 (d) $J = 3.6$ Hz	12.90 (s)	2.41 (s) CH_3
8o	8.02 (d) $J = 8.2$ Hz	7.14 (d) $J = 8.2$ Hz		7.54 (d) $J = 3.5$ Hz	7.16 (dd) $J = 4.7/4.7$ Hz	7.76 (d) $J = 4.7$ Hz	12.90 (s)	3.85 (s) OCH_3
8p	8.35 (d) $J = 7.4$ Hz	8.48 (d) $J = 7.4$ Hz		7.57 (d) $J = 3.0$ Hz	7.20 (dd) $J = 3.0/3.0$ Hz	7.77 (d) $J = 4.5$ Hz	12.90 (s)	



Scheme 2.

Table 5
Heat formation values for conformers **A**–**D** of the diastereomers (*E*) and (*Z*) of compounds **8a**–**d**, calculated using the semi-empirical AM1 method



Diastereomer/conformer	Comp.			
	8a Y = H	**8b** Y = CH_3	**8c** Y = OCH_3	**8d** Y = NO_2
	ΔH_f (kcal/mol)			
(*Z*)-**A**	122.39	114.06	66.26	126.67
(*Z*)-**B**	121.65	113.52	66.63	132.51
(*Z*)-**C**	120.08	112.20	64.68	125.35
(*Z*)-**D**	119.80	111.96	64.46	125.11
(*E*)-**A**	118.29	110.36	79.79	123.54
(*E*)-**B**	117.91	110.00	79.60	123.16
(*E*)-**C**	123.38	115.52	67.95	128.74
(*E*)-**D**	123.15	115.27	91.78	128.47

ever, we were able to show that the *S-cis* arrangement of the *N*-acylhydrazone chain in the conformers (**A**) and (**B**), presented a major stability in the range ca. 5 kcal/mol, when compared with the corresponding extended conformations (**C**) and (**D**) (Table 5). In addition, these studies also indicated that the (*E*)-diastereomer of the derivatives **8a**–**d**, are ca. 3 kcal/mol more stable than the corresponding (*Z*)-diastereomer (Table 5), in agreement with the experimental data obtained from the ^{1}H NMR spectra (Tables 2 and 3).

In Table 6 the ^{13}C NMR chemical shift assignments of the carbon atoms in this series of new derivatives **8a**–**p** are described. The attributions of carbon chemical shifts in series **8** were proposed by applying special NMR techniques such as APT, DEPT and HETERO-COSY. Curiously we were not able to detect any important signal in the spectra of compounds **8b** and **8c**, due the minor diastereomeric imine double bond carbon atom which occurs in the major (*E*)-isomer of **8a**–**p** in the range δ 140.9–152.3 ppm.

3. Experimental

3.1. Synthesis

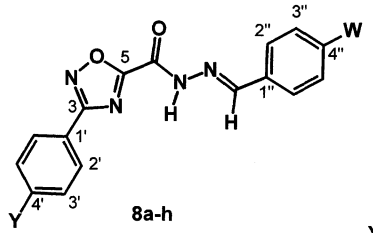
Melting points were determined with a Thomas Hoover apparatus and are uncorrected. ^{1}H NMR, unless otherwise stated, was determined in deuterated dimethylsulfoxide containing ca. 1% tetramethylsilane as an internal standard using a Bruker AC 200 spectrometer at 200 MHz. Splitting patterns are as follows: s, singlet; d, doublet; dd, double doublet; br, broad; m, multiplet. ^{13}C NMR was determined using the same spectrometers described above at 50 MHz, using deuterated dimethylsulfoxide as internal standard. IR spectra were obtained using a Bruker IFS66 spectrophotometer by using potassium bromide plates. Microanalysis data was obtained using a Perkin–Elmer 240 analyzer, using a Perkin–Elmer AD-4 balance.

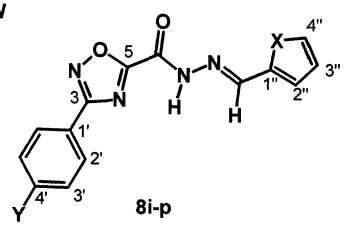
The progress of all reactions was monitored by TLC performed on 2.0 cm × 6.0 cm aluminum sheets precoated with silica gel 60 (HF-254, E. Merck) to a thickness of 0.25 mm. The developed chromatograms were viewed under ultraviolet light at 254 nm. For column chromatography E. Merck silica gel (60–200 mesh) was used. The usual work-up means that the organic extracts prior to concentration, under reduced pressure, were treated with a saturated aqueous sodium chloride solution, referred to as brine, dried over anhydrous sodium sulfate and filtered.

3.2. General procedure for the preparation of substituted methyl 3-phenyl-1,2,4-oxadiazole-5-carboxylates (**9a**–**d**) [11,12]

To a solution of 5 g (48 mmol) of hydroxylamine hydrochloride and 2.57 g (24 mmol) of sodium carbonate in 20 ml of water, 48 mmol of corresponding benzonitrile derivative **12** were added. Then, 100 ml of methanol were added and the mixture was stirred under reflux for ca. 4 h, when TLC analysis indicated the end of the reaction. Next, the mixture was filtered and the resulting precipitate chromatographed in a silica gel column, using chloroform as eluent, to furnish the corresponding benzamidoxime derivatives **10a**–**d**, which were immediately submitted to the next step.

Table 6
^{13}C NMR data at 50 MHz (DMSO-d_6) for compounds **8a**–**p**





Comp.	δ (ppm)												
	C-3	C-5	C-1′	C-2′	C-3′	C-4′	C-1″	C-2″	C-3″	C-4″	C=N	C=O	Other
8a	149.5	168.2	125.5	127.3	129.4	132.1	133.6	127.6	129.0	130.9	151.6	168.7	
8b	149.5	168.1	122.7	127.2	129.9	142.1	133.6	127.6	129.0	130.9	151.6	168.5	21.1 [CH_3]
8c	149.6	167.9	117.7	129.0	114.8	161.5	133.6	127.6	128.9	130.9	151.6	168.4	55.0 [OCH_3]
8d	149.5	166.9	133.6	129.0	124.7	149.4	131.3	127.6	128.5	131.0	151.8	169.2	
8e	149.0	168.1	129.5	127.3	129.4	132.0	120.6	129.1	111.7	152.0	152.3	169.0	39.9 [$N(CH_3)_2$]
8f	149.0	168.1	122.7	129.1	129.9	142.1	120.6	127.2	111.7	152.0	152.2	168.8	39.9 [$N(CH_3)_2$] 21.1 [CH_3]
8g	149.0	167.8	120.6	129.1	114.8	162.0	117.7	129.0	111.7	152.0	152.2	168.7	55.4 [OCH_3] 39.9 [$N(CH_3)_2$]
8h	149.5	167.7	131.4	129.1	124.7	152.4	120.6	128.7	111.8	152.0	152.3	169.4	39.9 [$N(CH_3)_2$]
8i	149.5	168.3	125.6	127.4	129.6	132.3	149.0	115.7	112.7	146.3	140.9	168.8	
8j	149.7	168.3	122.7	127.4	130.1	142.3	149.0	115.6	112.7	146.3	140.9	168.7	21.3 [CH_3]
8k	149.7	168.0	117.6	129.2	115.0	162.2	149.0	115.6	112.7	146.3	140.9	168.5	55.6 [OCH_3]
8l	149.5	167.0	131.5	128.9	124.7	146.3	149.0	115.7	112.7	146.3	141.0	168.0	
8m	149.1	167.9	125.2	127.0	129.1	131.8	138.0	132.2	127.8	130.0	146.1	168.4	
8n	149.1	167.9	122.5	126.9	129.7	138.0	141.9	132.2	127.8	132.0	146.1	168.3	20.9 [CH_3]
8o	148.3	167.8	117.6	128.9	114.8	162.0	138.2	132.4	128.0	130.2	146.2	168.4	55.4 [OCH_3]
8p	149.6	167.0	131.2	128.9	124.8	149.3	138.4	132.8	128.3	130.5	146.6	169.3	

To a solution of 36.8 mmol of the benzamidoxime derivatives **10a**–**d** in 30 ml of dry THF 4.5 ml (48.92 mmol) of methyl oxalylchloride were added and the mixture was maintained under reflux for 2.5 h. The solvent was then completely removed under reduced pressure and ca. 30 ml of cold water were added to the formed residue. The methyl esters **9a**–**d** were obtained after filtration, as described in Table 1.

*3.3. General procedure for the preparation of substituted 3-phenyl-1,2,4-oxadiazole-5-carbohydrazide (**11a**–**d**) derivatives [6]*

To a solution of 9.8 mmol of methyl ester derivatives **9a**–**d** in 30 ml of ethanol, was added 1.8 ml (30 mmol) of 80% hydrazine monohydrate. This mixture was stirred at room temperature for ca. 1 h, when TLC analysis indicated the end of the reaction. Then, the media were poured on ice and the resulting precipitate was filtered out, affording the corresponding carbohydrazide derivatives **11a**–**d**, as described in Table 1.

*3.4. General procedure for the preparation of substituted benzylidene 3-aryl-1,2,4-oxadiazole-5-carbohydrazide (**8a**–**p**) [6,7]*

A solution of 1.47 mmol of hydrazide derivatives **11a**–**d** in 10 ml of water, containing two drops of concentrated sulfuric acid, was refluxed for ca. 30 min, until complete solubilization. Then, a solution of 1.50 mmol of aromatic aldehyde derivative in 3 ml of ethanol was added and the mixture was additionally refluxed for 2.5 h. Next, the mixture was poured into cold water, neutralized with 10% aqueous sodium bicarbonate solution and the precipitate formed was filtered out and dried, furnishing the title compounds **8a**–**p**, as described in the Tables 2 and 3.

3.5. Molecular modeling

Geometry optimizations were performed at SCF level using ther AM1 Hamiltonian [18], within the MOPAC version 6.0 package [19] on an IBM RISC system/6000 workstation under the IBM AIX version 3.0 operational system and on a Pentium 100 MHz running under the FreeBSD Unix system. The potential energy surface slices were pointwise calculated for the torsional angles, which were varied independently between 0 and 180° with a 30° increment. Minimum energy structures were then reoptimized adopting keywords GNORM = 0.01 and PRECISE, and unequivocally characterized by Hessian Matrix analysis (no negative eigenvalues were found).

3.6. Pharmacological assays

The analgesic activity was determined in vivo by the abdominal constriction test induced by acetic acid 0.6% (0.1 ml/10 g) in mice [20]. 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 **1** (100 μmol/kg) was used as the standard drug under the same conditions. Acetic acid solution was administered i.p. 1 h after administration of the acylhydrazone compounds **8a**–**p**. Ten minutes after i.p. acetic acid injection, the number of constrictions per animal was recorded for 20 min. Control animals received an equal volume of vehicle. Analgesic activity was expressed as a percentage of inhibition of constrictions when compared with the vehicle control group (Table 7). 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 $P < 0.05$.

4. Results and discussion

The evaluation of the analgesic profile of all *N*-acylhydrazone derivatives **8a**–**p** was performed using the classical acetic acid-induced mice abdominal constrictions test [20], p.o., with dipyrone **1** as standard. The results are disclosed in Table 7.

In the series of substituted benzylidene 3-phenyl-1,2,4-oxadiazole-5-carbohydrazide derivatives **8a**–**h**, the most active derivatives (**8c** and **8g**) possess an electron donating group, i.e. $Y = OCH_3$, at the *para* position of oxadiazole 3-phenyl ring (**8c**, 52.3% of inhibition, **8g**, 49.2% of inhibition, Table 4) and presented a similar relative activity index (rai) to dipyrone **1** of 0.97 and 0.92, respectively. These results seem to indicate that the *para*-*N*,*N*-dimethylaminophenyl unit has a minor contribution to the analgesic activity. In fact, the lead-compound **5b** presented, in the same test, a 1.19-fold rai, remaining the most active compound. Also, when we compare the potency of the derivatives **8a** and **8e**, the presence of the *para*-substituent at the phenyl ring of the hydrazone moiety seems to be less important to the analgesic activity than the *para*-substituent at the *N*-phenyl ring of the heterocycle unit. For instance, the introduction of the *N*-*para*-nitrophenyl unit, furnishing derivatives **8d** and **8h**, gives a similar analgesic profile to both compounds (35.9 and 37.2%, respectively), indicating that the presence of an electron-attracting group at the *N*-*para*-phenyl substituent is probably deleterious to the activity. In contrast, the most analgesic compounds in this oxadiazole series (**8d** and **8k**) posses an electron-donating substituent

Table 7
Effect of substituted benzylidene 3-aryl-1,2,4-oxadiazole-5-carbohydrazide derivatives **8a–p**, dipyrone **1** and 4′-*N*,*N*-dimethylaminobenzylidene 1-phenylpyrazole-4-carbohydrazide (**5b**) in the inhibition of abdominal constrictions induced by acetic acid (0.6%, i.p.) in mice

Comp.	Y	W/X	*n* [a,b]	Constrictions count	Inhibition (%) [c]	Relative activity [d]
Vehicle control (arabic gum 5%)			20	89.0 ± 2.9		
Dipyrone **1**			10	41.3 ± 3.1	53.6 *	1.00
8a	H	H	10	84.7 ± 6.4	4.8 ns [e]	0.09
8b	CH_3	H	6	70.8 ± 3.7	20.4 ns [e]	0.38
8c	OCH_3	H	8	42.4 ± 5.6	52.3 *	0.97
8d	NO_2	H	8	57.0 ± 5.1	35.9 *	0.67
8e	H	$N(CH_3)_2$	8	70.5 ± 2.4	20.8 ns [e]	0.39
8f	CH_3	$N(CH_3)_2$	10	70.6 ± 6.7	20.7 ns [e]	0.39
8g	OCH_3	$N(CH_3)_2$	8	45.2 ± 2.3	49.2 *	0.92
8h	NO_2	$N(CH_3)_2$	8	55.9 ± 3.2	37.2 *	0.69
8i	H	O	9	61.9 ± 5.5	30.4 *	0.57
8k	OCH_3	O	11	42.7 ± 3.3	52.0 *	0.97
8l	NO_2	O	9	52.6 ± 4.4	40.9 *	0.76
8m	H	S	8	44.4 ± 4.7	50.1	0.93
8o	OCH_3	S	9	51.4 ± 4.0	42.2 *	0.79
8p	NO_2	S	8	45.0 ± 3.3	49.4 *	0.92
5b			12	32.1 ± 2.8	63.9 *	1.19

[a] All test compounds were administered at a concentration of 100 μM/kg p.o.
[b] n = number of animals.
[c] % inhibition obtained by comparison with vehicle control group.
[d] Analgesic activity relative to dipyrone **1**.
[e] ns = not significant.
* $P < 0.05$ (Student's t-test). Results are expressed as mean ± SEM.

(i.e. OMe) at this position. This rationalization became contradictory when we observed the activity of compound **8p**, which presented a *para*-nitrophenyl substituent and a 2-thienyl ring instead of the *para*-dimethylaminophenyl unit at the hydrazone end. In fact, this major activity could result from a balance of different structural contributions, as a major lipophilic nature of the 2-thienyl ring compensating for the presence of the nitro group.

Finally, we can not discard that the observed analgesic activity described herein to this new 1,2,4-oxadiazole series of NAH derivatives, results from modulation of different mechanisms.

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