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Original article

Synthesis and analgesic activity of novel *N*-acylarylhydrazones and isosters, derived from natural safrole

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Abstract – A new series of antinociceptive compounds belonging to the *N*-acylarylhydrazone (NAH) class were synthesized from natural safrole (**7**). The most analgesic derivative represented by **10f**, [(4'-*N*,*N*-dimethylaminobenzylidene-3-(3',4'-methylenedioxyphenyl)-propionylhydrazine], was more potent than dipyrone and indomethacin, used as standards. The NAH compounds described herein were structurally planned by molecular hybridization and classical bioisosterism strategies on previously reported analgesic NAH in order to identify the pharmacophoric contribution of the *N*-acylarylhydrazone moiety and investigate the structure–activity relationship (SAR) in these series. © 2000 Éditions scientifiques et médicales Elsevier SAS

***N*-acylarylhydrazones and isosteric compounds / safrole in synthesis / analgesic activity**

1. Introduction

Pain is a fundamental event that is normally beneficial and works as a physiological advice for potentially tissue-damaging situations, e.g. the manifestation of inflammatory dysfunctions [1, 2]. However, the very significant emotional and subjective components of human pain and the therapy of chronically debilitating pain makes the search for new peripheral analgesic agents, potent, selective and with reduced toxicity very important.

The peripheral analgesic agents which modulate the inflammatory hyperalgesia can be divided into two main groups [2]: a) those which abolish the ongoing sensitization of pain receptors, e.g., dypirone, and b) those which prevent the sensitization of pain receptors, e.g. non-steroidal anti-inflammatory drugs (NSAID), such as indomethacin. The analgesic profile of classical NSAID [3] is a function of inhibition of the cyclooxygenase enzyme (COX), which reduces the level of prostaglandins, e.g. PGE_2, which sensitizes nociceptors at nerve fibre terminals [4]. Additionally, the 5-lipoxygenase (5-LO) product, such as leukotriene B_4 also contributes to the hyperalgesia seen during inflammation by decreasing the mechanical and thermal thresholds of C fibres [5]. For these reasons, compounds that achieve the dual inhibition of enzymes COX and 5-LO become an important therapeutic strategy in the combat of the pain in inflammatory diseases.

In the eighties, hydrazone-type containing compounds such as BW 755C (**1**) [6] and CBS 1108 (**2**) [7] were described as dual COX/5-LO inhibitors which present anti-inflammatory and analgesic activity. In fact, some recent evidences suggest that the hydrazone moiety present in phenylhydrazone derivative **3**, possess a pharmacophoric character for the inhibition of cyclooxygenase [8].

[#]This paper represents contribution # 36 of the LASSBio, UFRJ (Br.) (LASSBio, http://acd.ufrj.br/≈pharma/lassbio); For contribution # 35, see [24].

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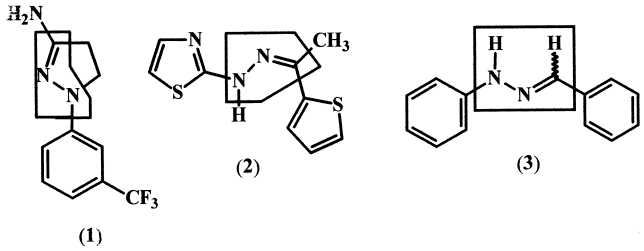


(1) (2) (3)

In the course of an ongoing research program aimed at the design, synthesis and pharmacological evaluation of new bioactive compounds acting at the arachidonic acid cascade enzyme level, we previously described the analgesic profile of four new series of heterocyclic *N*-acylarylhydrazones (NAH) belonging to *N*-phenylpyrazole 4-acylarylhydrazone derivatives **4** [9], *N*-phenyl-3-methyl-pyrazolo[3,4-b]pyridine 5-acylarylhydrazone derivatives **5** [10], functionalized imidazo[1,2-a]pyridine 3-acylarylhydrazone derivatives **6** [11] and safrole **7** derived isochromanyl compounds **8** [12] (*figure 1*).

These compounds have presented an important analgesic profile measured by the classical acetic acid-induced constrictions [13] which showed to be more influenced by the nature of phenyl ring substituent of the hydrazone sub-unit than the pattern of the heterocyclic ring of the *N*-acyl moiety. Additionally, these extensive studies allowed us to show that the more potent analgesic agents present the *para*-dimethylamino substituent at the phenyl ring of the hydrazone group, as represented by *N*-phenylpyrazole derivative **4**, selected as a new lead-compound for antinociceptive activity. In fact, derivative **4** showed a potency 11-fold greater than dipyrone as an ananalgesic agent p.o., at the same molar concentration [9].

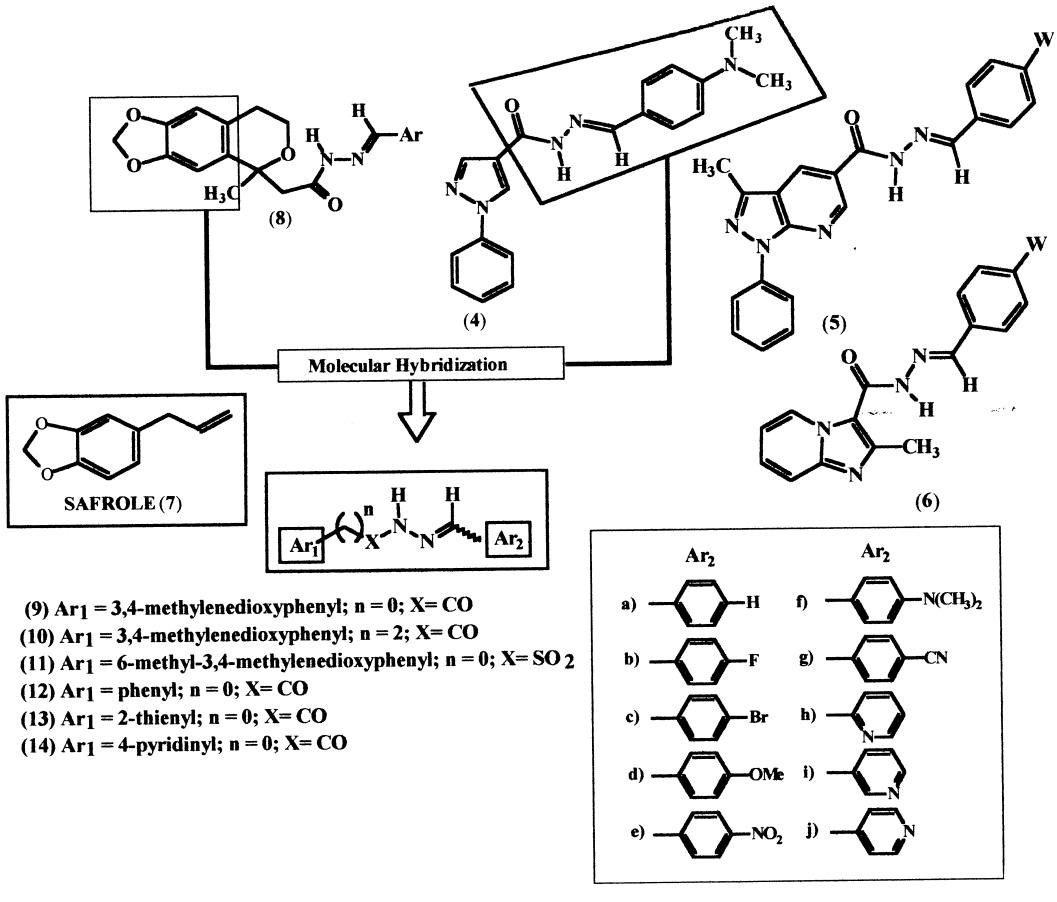


Figure 1. Rational concept to new *N*-acylarylhydrazone derivatives.

Continuing our studies on NAH derivatives that are attractive candidates as antinociceptive agents, we designed a new series of functionalized 3,4-methylenedioxybenzoylhydrazone compounds (**9a–j**) and bis-homologue derivatives (**10a–c**), exploring safrole **7**, an abundant Brazilian natural product obtained from Sassafras oil (*Ocotea pretiosa*), as starting material. The rational design of these new derivatives (**9** and **10**) was planned by molecular hybridization of previously described analgesic compounds **4** and **8**, keeping the 1,3-benzodioxole ring present in the non-addictive analgesic derivative **8** and introducing the NAH framework of the potent analgesic compound **4** (*figure 1*).

The nature of the *para*-substituent present in the phenyl group of the Ar_2 subunit (i.e. H, F, Br, OMe, NO_2, NMe_2 or CN) of the derivatives (**9a–g**), was defined in order to introduce, in this series of compounds, an important variation in σ_p-Hammett values (ranging from –0.83 (NMe_2) to +0.78 (NO_2)) which could be used to investigate any electronic contribution of this structural sub-unit on the analgesic activity. On the other hand, considering the importance of the presence of the *para*-dimethylaminophenyl group in the aza-arylidene moiety for the analgesic activity as evidenced in the previous series [9–12], we decided to investigate the contribution to the analgesic activity of the substitution of this pharmacophoric nitrogen-containing functional group by isosteric 2-, 3-, 4-pyridine rings in the Ar_2 subunit producing the derivatives (**9h–j**). Next, we decided to investigate the eventual importance of the distance between the two aromatic rings at both termini of the derivatives of series **9** by evaluating the analgesic activity of the more conformationally flexible analogues (**10a**, **c** and **f**), which presents a C-2 spacer unit mimicking the previously described class of active isochromanyl derivatives **8**.

Finally, we decided to promote a carbonyl-sulfone isosteric replacement in the NAH derivatives **9**, constructing the *N*-sulfonylarylhydrazone (NSH) series **11** in order to evaluate the eventual effect of the pKa increasing in the analgesic activity. In addition, in the series of derivatives **11**, the presence of the NSH unit introduced an additional hydrogen bond acceptor site (i.e., C=O (**9**) *vs.* O=S=O (**11**)) that could represent a new structural contribution for the antinociceptive profile of these compounds. The influence of a 1,3-benzodioxole ring, derived from the natural safrole **7** used as starting material, on the analgesic activity, was also studied by the evaluation of three isosteric aromatic NAH series, i.e. benzoyl derivatives **12**, 2-thienoyl derivatives **13** and isonicotinoyl derivatives **14** (*figure 1*).

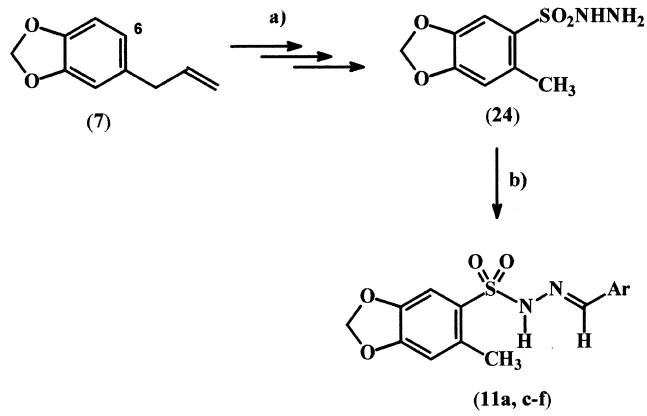


Figure 4. a) reference [23]; b) Ar–CHO, EtOH, HCl (cat.), 30 min, room temperature.

2. Chemistry

The obvious synthetic route planned to achieve the safrole derived NAH target compounds **9**, **10** and **11** are shown in *figures 2–4*, respectively. The derivatives of the series **9** (n = 0) were prepared from the functionalised aldehyde **15**, obtained in ca. 75% overall yield from the natural safrole **7**, using base catalysed isomerization of the double bond [14] followed by oxidative cleavage [15]. Employing the oxidative Yamada's procedure [16] compound **15** was 'one-pot' converted, in 90% yield, to the corresponding methyl ester **16** by treatment with 2.6 eq. of KOH and 1.3 eq. of iodine in methanol at 0 °C. Next, the key acylhydrazine intermediate **17** was obtained in 70% yield by treatment of an ethanolic solution of the ester **16** with hydrazine hydrate at reflux for 3.5 h [10] (*figure 2*).

The new NAH target compounds (**9a–j**) were obtained, in good yields, by condensing compound **17** with the corresponding aromatic aldehydes (ArCHO) in ethanol [10], using hydrochloric acid as catalyst (*figure 2*, *table I*).

The next step in this work was to determine the relative configuration of the imino double bond in *N*-acylarylhydrazone derivatives (**9a–j**), in order to assure the diastereomeric ratio, essential to the complete understanding of the biological results. The careful analysis of the ^{1}H-NMR spectra of (**9a–g** and **i–j**), allowed us to detect only the presence of one imino hydrogen signal (*table I*), which was attributed to the (*E*)-diastereomer, on the basis of the previous paper of Palla et al. [17] about the synthesis of the benzylidene benzoylhydrazone **12a**. Indeed, compound **12a** presents a singlet at δ 8.47 ppm in its ^{1}H-NMR spectrum, which by crystallographic analysis

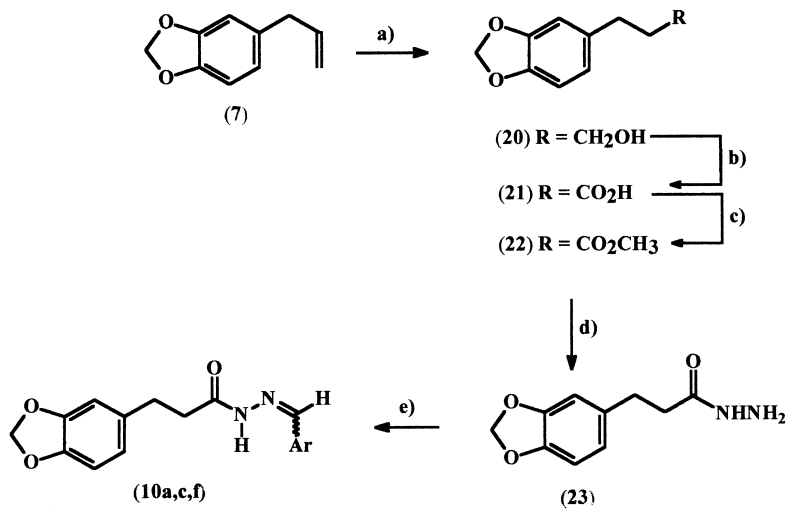


Figure 3. a) i– 1 M BH_3.THF, room temperature, 2 h, ii– H_2O_2 30%, 10% aq. NaOH, 60 °C, 4 h, 78%; b) H_2CrO_4, Me_2CO, 0–5 °C, 1 h, 92%; c) CH_3OH, H_2SO_4, reflux, 2 h, 90%; d) 80% aq. $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 3 h, 75%; e) ArCHO, EtOH, HCl (cat.), room temperature, 30 min.

indicated reference to the (*E*)-diastereomer [17] (*figure 5*). The same chemical shift could be observed for CH=N in the ^{1}H-NMR spectra of NAH compounds described herein (*tables I–III*), corresponding to the (*E*)-diastereomer. Moreover, we performed a brief study of the relative stability of both possible diastereomers by molecular modelling using the Hamiltonian AM1 in MOPAC 7.0 program as tools, that indicated a minor value for the heat formation of the diastereomer (*E*) in compound **9a**, indicating that the (*E*)-isomer could be preferentially formed (Guimarães C.W., Fraga C.A.M., Barreiro E.J., unpublished data).

Additionally, the relative configuration of compound **9h**, which also presents only one imino hydrogen (*table I*), was also characterized as (*E*), despite the possible intramolecular hydrogen bond interaction of N–H with the pyridine nitrogen contributing to stabilization of the (*Z*)-diastereomer (*figure 5*). In fact, this attribute was favoured by the previously published paper of Bell and Mortimore [18], describing the ^{1}H-NMR spectrum of

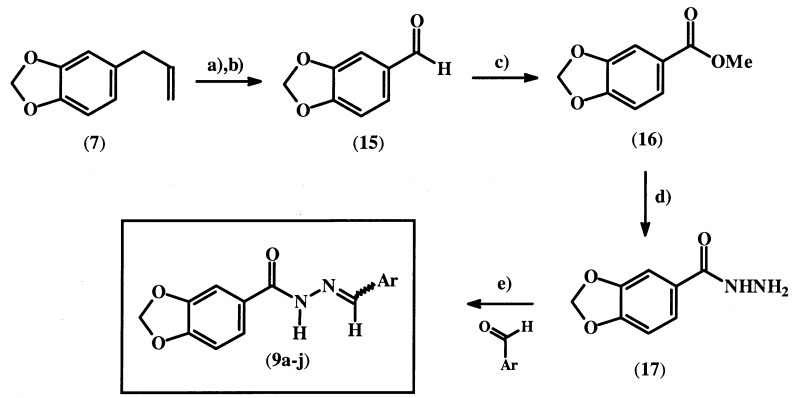


Figure 2. a) KOH aq. 3 N, n-BuOH, room temperature, 3 h, 98%; b) i– O_3 /O_2, AcOH, 0 °C, 1 h; ii– Zn°, AcOH, 93% (75%, 3 steps); c) I_2, KOH, MeOH, 0 °C, 1.5 h, 90%; d) $NH_2NH_2 \cdot H_2O$ 80%, EtOH, reflux, 3.5 h, 70%; e) Ar–CHO, EtOH, HCl (cat.), room temperature, 30 min.

Table I. 3,4-Methylenedioxybenzoylarylhydrazones **9a–j** and 3-(3',4'-methylenedioxyphenyl)-propionylarylhydrazone derivatives **10a**, **c** and **f**.

Compound	Molecular formula	Molecular weight	Yield (%)	M.p. (°C)	δ (ppm) N=CH	
					(E)	(Z)
9a	$C_{15}H_{12}N_2O_3$	268.26	67	180–181	8.42	–
9b	$C_{15}H_{11}FN_2O_3$	286.25	80	176–178	8.48	–
9c	$C_{15}H_{11}BrN_2O_3$	347.16	85	211–213	8.72	–
9d	$C_{16}H_{14}N_2O_4$	298.29	70	183–185	8.39	–
9e	$C_{15}H_{11}N_3O_5$	313.26	84	254–255	8.63	–
9f	$C_{17}H_{17}N_3O_3$	311.33	83	201–203	8.27	–
9g	$C_{16}H_{11}N_3O_3$	293.27	80	216–218	8.50	–
9h	$C_{14}H_{11}N_3O_3$	269.25	83	182–183	8.52	–
9i	$C_{14}H_{11}N_3O_3$	269.25	90	256–257	8.59	–
9j	$C_{14}H_{11}N_3O_3$	269.25	85	257–258	8.39	–
10a	$C_{17}H_{16}N_2O_3$	296.32	80	131–133	8.16	7.98
10c	$C_{17}H_{15}BrN_2O_3$	375.21	70	175–177	8.10	7.93
10f	$C_{19}H_{21}N_3O_3$	339.38	85	216–218	8.00	7.85

(*E*)-pyridine-2-carbaldehyde 2'-pyridylhydrazone **18**, where the signal referring to the imino hydrogen was displaced 0.73 ppm downfield in relation to that of the (*Z*)-diastereomer **19** (*figure 5*).

Next, the bis-homologous compounds (**10a**, **c** and **f**, n = 2, *table I*) were synthesized from natural safrole (**7**) by applying the route illustrated in *figure 3*.

Initially, the regioselective hydroboration of the terminal double bond of safrole **10** was performed, followed by an oxidative work-up to furnish the primary alcohol **20** in 78% yield [15]. The phenylpropionic ester **22** was prepared from **20** in 83% yield (2 steps), applying the classical sequence of Jones oxidation [19] followed by Fisher esterification of acid intermediate **21** [15] (*figure 3*).

The key intermediate phenylpropionylhydrazine (**23**) was obtained, in 75% yield, from ester **22** by applying the same experimental procedure described in the synthesis of the benzoylhydrazine derivative **17** [10]. Finally, the desired propionylarylhydrazone compounds (**10a**, **c** and **j**, *table I*) were prepared by condensation of propionylhydrazine derivative **23** with *para*-substituted benzaldehydes, selected on the basis of the results from the evaluation of analgesic activity of 3,4-methylenedioxybenzoylarylhydrazones (**9a–j**), described in *table IV*.

Intriguingly, the careful analysis of the ^{1}H-NMR spectra of these NAH derivatives (**10a**, **c** and **j**) indicated the presence of two singlet signals in almost the same relative proportion, referring to (*E*) and (*Z*)-imino hydrogens CH=N. The assignment of (*E*) and (*Z*) relative to the

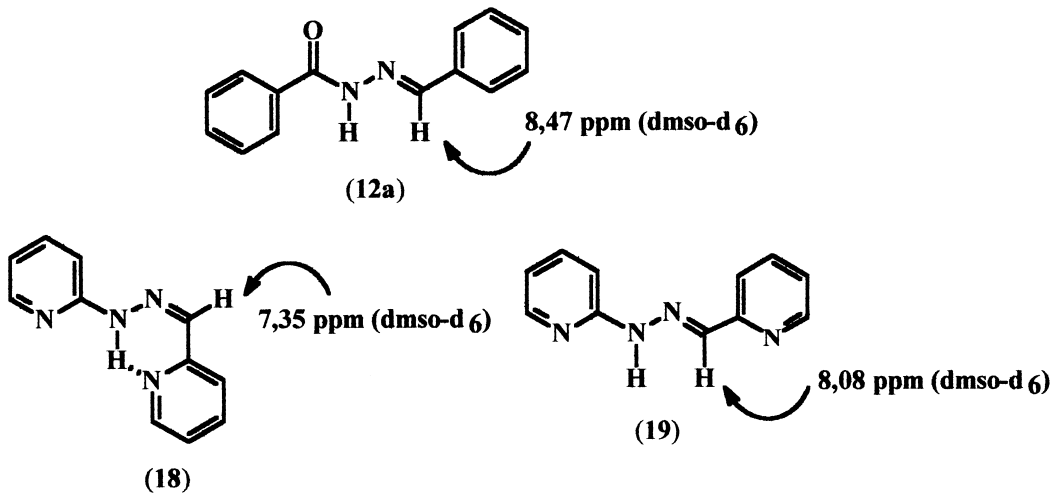


Figure 5.

Table II. 6-Methyl-3,4-methylenedioxyphenylsulfonylarylhydrazone derivatives **11a** and **c–f**.

Compound	Molecular formula	Molecular weight	Yield (%)	M.p. (°C)	δ (ppm) N=CH	
					(E)	(Z)
11a	$C_{15}H_{14}N_2O_4S$	318.34	92	165–166	7.97	–
11c	$C_{15}H_{13}BrN_2O_4S$	397.24	89	175–176	7.95	–
11d	$C_{16}H_{16}N_2O_5S$	348.37	93	164–165	7.91	–
11e	$C_{15}H_{13}N_3O_6S$	363.34	87	153–155	8.10	–
11f	$C_{17}H_{19}N_3O_4S$	361.41	89	173–175	7.82	–

configuration of **10** was made in agreement with previous results disclosed by Karabatsos et al. for the relative configuration of hydrazones and related compounds [20–22], which describes that the imino hydrogen of the (*E*)-diastereomer is downfielded by δ 0.2–0.3 ppm from the corresponding (*Z*)-diastereomer (*table I*). These results showed that the construction of an imino double bond of the phenylpropionylarylhydrazone derivatives **10** is not a diastereoselective process, in contrast with the previously described series **9**, probably due to minor steric interactions between the two phenyl rings in the (*Z*)-diastereomer caused by the presence of the C-2 ethylene spacer.

For the synthesis of the isosteric *N*-sulfonylarylhydrazone series **11** we explored the *N*-sulfonylhydrazine derivative **24** which was obtained, as described previously [23], in 31% overall yield from natural safrole **7** by applying a synthetic sequence that included the adequate cleavage of the allyl side chain, followed by mild regioselective sulfonation of the C-6 position of the methylenedioxyphenyl ring and the subsequent classical functional group interconversion: $SO_3K \rightarrow SO_2Cl \rightarrow SO_2NHNH_2$ (*figure 4*). Then, the target NSH derivatives **11** (*table II*) were obtained in good yields, from acid catalysed condensation of the compound **24** with *para*-substituted benzaldehydes (*figure 4*). The diastereocourse of this reaction follows that one described previously for 3,4-methylenedioxybenzoylarylhydrazones **9**, i.e. only one imino hydrogen signal was detected in the ^{1}H-NMR spectra, which was attributed to the (*E*)-diastereomer.

In order to conclude the synthetic work, the last series of NAH proposed, i.e. the isosteric benzoylhydrazone derivatives **12**, 2-thienoylhydrazone derivatives **13** and isonicotinoylhydrazone derivatives **14**, described in *table III*, were prepared in diastereopure form in a similar manner to the corresponding commercial acylhydrazines (purchased from Aldrich Co., USA) **25**, **26** and **27** (*figure 6*).

3. Results and discussion

The evaluation of the analgesic profile of all *N*-acylarylhydrazone derivatives (**9a–j**, **10a**, **c** and **f**, **11a** and **c–f**, **12f** and **h–j**, **13f** and **h–j** and **14f** and **h–j**) was performed using the classical acetic acid-induced mice abdominal constrictions test [14], p.o., with dipyrone and indomethacin as standards. The results are shown in *tables IV* and *V*.

Table III. Benzoyl-, thienoyl- and isonicotinoyl arylhydrazone derivatives **12f** and **h–j**, **13f** and **h–j**, and **14f** and **h–j**.

Compound	Molecular formula	Molecular weight	Yield (%)	M.p. (°C)	δ (ppm) N=CH	
					(E)	(Z)
12f	$C_{16}H_{17}N_3O$	267.32	90	175–176	8.32	–
12h	$C_{13}H_{11}N_3O$	225.24	85	166–168	8.48	–
12i	$C_{13}H_{11}N_3O$	225.24	84	184–185	8.53	–
12j	$C_{13}H_{11}N_3O$	225.24	80	162–163	8.47	–
13f	$C_{14}H_{15}N_3OS$	273.35	86	196–197	8.27	–
13h	$C_{11}H_9N_3OS$	231.27	87	192–193	8.43	–
13i	$C_{11}H_9N_3OS$	231.27	85	216–218	8.48	–
13j	$C_{11}H_9N_3OS$	231.27	83	192–194	8.40	–
14f	$C_{15}H_{16}N_4O$	268.31	85	195–196	8.33	–
14h	$C_{12}H_{10}N_4O$	226.23	85	145–147	8.39	–
14i	$C_{12}H_{10}N_4O$	226.23	83	232–233	8.51	–
14j	$C_{12}H_{10}N_4O$	226.23	90	224–225	8.44	–

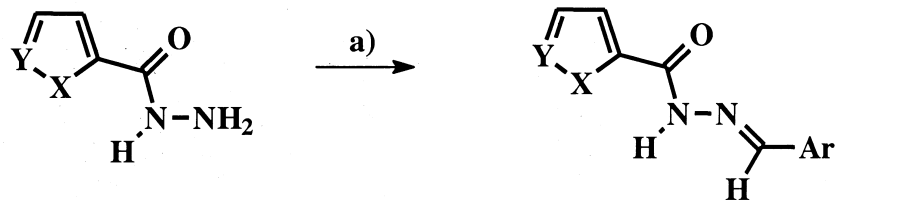
Table IV. Effect of safrole derived acylhydrazones **9a–j**, **10a**, **c** and **f**, and **11a** and **c–f**, dipyrone and indomethacin on the inhibition of abdominal constrictions induced by acetic acid (0.6 %, i.p.) in mice.

Compound	Dose[a] (μmol/kg)	*n*[b]	Constriction number	Inhibition (%)[c]
Control	–	32	93.0 ± 6.1	–
Vehicle control (arabic gum 5 %)	–	14	85.1 ± 2.6	8.5
Dipyrone	100	9	59.5 ± 4.4	35.9*
Indomethacin	100	10	41.9 ± 4.4	54.9*
9a	100	8	55.0 ± 3.9	40.9*
9b	100	9	60.0 ± 4.3	35.5*
9c	100	10	60.2 ± 4.9	35.3*
9d	100	9	65.9 ± 3.9	29.1*
9e	100	9	62.8 ± 6.3	32.5*
9f	100	9	45.4 ± 5.5	51.2*
9g	100	9	72.9 ± 8.7	21.6 ns[d]
9h	100	9	44.8 ± 6.5	51.8*
9i	100	7	42.1 ± 7.1	54.7*
9j	100	10	58.9 ± 4.6	36.7*
10a	100	11	68.9 ± 3.2	25.9*
10c	100	11	103.0 ± 5.6	–10.7 ns[d]
10f	100	10	30.6 ± 3.5	67.1*
11a	100	8	71.1 ± 4.8	23.5*
11c	100	9	85.3 ± 4.0	8.3 ns[d]
11d	100	10	69.2 ± 3.6	25.6*
11e	100	10	81.5 ± 6.3	12.4 ns[d]
11f	100	10	56.3 ± 4.4	39.5*

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

In the first series of derivatives, i.e. 3,4-methylenedioxybenzoylarylhydrazone compounds **9**, the most active derivatives (**9h** and **9i**) possess the pyridinyl ring at the aryl moiety of the acylhydrazone framework (**9i**, 54.7% of inhibition; **9h**, 51.8% of inhibition, *table V*) and presented the same activity of the indomethacin used as standard, at the same dose. The compound possessing the *para*-dimethylaminophenyl moiety (**9f**, 51.2%, *table IV*) was the third most active compound, presenting a relative index of analgesic activity related to dipyrone of ca. 1.5 (*table IV*).

The bis-homologation of compounds of series **9**, by introduction of an ethylene spacer, leading to more flexible phenylpropionylhydrazone derivatives **10**, produced a dissimilar influence in analgesic profile, i.e. significantly increased the potency of *para*-dimethyl-



(25) X = CH=CH; Y = CH
(26) X = S; Y = CH
(27) X = CH=CH; Y = N

(12f,h-j) X = CH=CH; Y = CH
(13f,h-j) X = S; Y = CH
(14f,h-j) X = CH=CH; Y = N

Figure 6. a) ArCHO, EtOH, HCl (cat.), room temperature, 30 min.

Table V. Effect of benzoylhydrazones **12f** and **h–j**, thienoylhydrazones **13f** and **h–j** and isonicotinoylhydrazones **14f** and **h–j**, dipyrone and indomethacin in the inhibition of abdominal constrictions induced by acetic acid (0.6 %, i.p.) in mice.

Compound	Dose[a] (μmol/kg)	*n*[b]	Constriction number	Inhibition (%)[c]
Control	–	32	93.0 ± 6.1	–
Vehicle control (arabic gum 5 %)	–	14	85.1 ± 2.6	8.5
Dipyrone	100	9	59.5 ± 4.4	35.9*
Indomethacin	100	10	41.9 ± 4.4	54.9*
12f	100	8	84.0 ± 6.3	9.8 ns[d]
12h	100	11	84.4 ± 4.6	9.3 ns[d]
12i	100	7	57.8 ± 9.5	37.8*
12j	100	7	57.6 ± 10.4	38.1*
13f	100	8	87.7 ± 6.1	5.7 ns[d]
13h	100	11	67.0 ± 5.8	27.9*
13i	100	9	63.0 ± 7.2	32.3*
13j	100	9	77.1 ± 6.6	17.1 ns[d]
14f	100	10	60.2 ± 2.0	35.3*
14h	100	10	65.7 ± 3.3	29.4*
14i	100	10	69.8 ± 4.5	24.9*
14j	100	10	67.6 ± 4.7	27.3*

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

aminophenylhydrazone derivative **10f** (67.1% (**10f**) vs. 51.2% (**9f**), *table IV*), which presents the most important dipyrone-relative index value of 1.86. In contrast, the C-2 spacer introduction produced a deleterious effect on the analgesic activity of NAH derivative **10a** (25.9% (**10a**) vs. 40.9% (**9a**), *table IV*), indicating that probably, in this more conformationally flexible series **10**, the pharmacophoric contribution to the bioreceptor recognition of the *para*-dimethylaminophenyl moiety is more important than conformational effects. In addition, in the isosteric sulfonylhydrazone derivatives (**11c–f**), the most active compound (**11f**, 39.5% inhibition, *table V*), presenting a dipyrone-relative index of ca. 1.0, also possess the *para*-dimethylaminophenyl unit at the hydrazone end. These results reinforce the pharmacophoric character for analgesic activity of this functionality, as observed with other series previously evaluated in our laboratory [9–12].

The contribution of the 3,4-methylenedioxy bridge in **9** to the analgesic activity was measured by comparison of the activities with derivatives **12**, where the 1,3-dioxolane ring was eliminated. The results, illustrated in *table V*, indicated that this molecular modification seems to be deleterious to the analgesic activity, except for the pyridinyl isoster **14f**, which presents again the *para*-dimethylaminophenyl substituent at the hydrazone unit. For instance, the corresponding benzoylhydrazone derivative **12f** and the isosteric thienoylhydrazone compound **13f** (*table V*) do not present any important analgesic activity, indicating that the presence of the natural methylenedioxy bridge seems to also be an important structural requirement for the analgesic activity measured by this bioassay. The important analgesic profile observed with the isosteric isonicotinoyl series (**14h–j**; 29.4%, 24.9% and 27.3% inhibition, respectively, *table V*) seems to be dependent on the 'symmetric' nature of these compounds, possessing a 'double' pyridine ring at both aromatic termini. The most active compound in this series (**14f**; 35.3% of inhibition, *table V*) also possess the *para*-dimethylaminophenyl substituent at the hydrazone moiety.

Finally, the analgesic activity of compounds of series **9** seem to be insignificantly influenced by the different electronic or lipophilic contributions of the *para*-substituent at the phenyl ring of the acylhydrazone framework. These compounds were unable to present any response in the hot-plate assay [24], using morphine as standard.

As concluding remarks, we can establish that the synthetic routes used to access the new active NAH/NSH derivatives (**9–14**) described herein, are useful and efficient, furnishing the titled compounds in high overall yield. The new *N*-acylarylhydrazone compounds **9**, **11**, **12**, **13** and **14** were obtained in diastereopure form, with the (*E*)-isomer being the major one. Only the derivatives of series **10** were obtained as a diastereomeric mixture, probably due to the presence of a more flexible chain that contributes to minimize the steric repulsions between the aromatic rings in the (*Z*)-diastereomer.

The pharmacological assays identified compound **10f** as the most analgesic agent, possessing the methylenedioxyphenyl moiety, originating from the natural product used as synthetic starting material, and presenting the *para*-dimethylaminophenyl unit at the hydrazone end. The absence of any detectable activity for this compound, as indicated by the hot-plate test using morphine as standard, confirms the antinociceptive non-central profile of this new derivative that was more active than indomethacin (1.22-fold) and dipyrone (1.86-fold) used as standards in the acetic acid induced constrictions test. In addition, the pharmacophoric contribution to the analgesic activity of the NAH functionality, the *para*-dimethylaminophenyl unit and the methylenedioxy bridge could be detected by comparison with the less active isosteric derivatives **11**, **12**, **13** and **14**.

Complementary to this work, we are performing in the laboratory a careful pharmacological study with the more analgesic derivative **10f** in order to elucidate the mechanism of action.

4. Experimental protocols

4.1. Chemistry

Melting points were determined with a Buchi 540 apparatus and are uncorrected. Proton magnetic resonance (^{1}H-NMR), unless otherwise stated, was determined in deuterated dimethylsulfoxide containing ca. 1% tetramethylsilane as an internal standard with Brucker AC 200, Brucker DRX 300 spectrometers at 200 MHz and 300 MHz, respectively. Splitting patterns are as follows: s, singlet; d, doublet; t, triplet; q, quartet; qt, quintet; dd, double doublet; br, broad; m, multiplet. Carbon magnetic resonance (^{13}C-NMR) was determined in the same spectrometers described above at 50 MHz and 75 MHz, respectively, using deuterated dimethylsulfoxide as internal standard. Infrared (IR) spectra were obtained with a Nicolet-550 Magna spectrophotometer by using potassium bromide plates. The mass spectra (MS) were obtained by electron impact (70 eV) with a GC/VG Micromass 12 spectrometer.

The progress of all reactions was monitored by TLC performed on 2.0 × 6.0 cm aluminium sheets precoated with silica gel 60 (HF-254, Merck) to a thickness of 0.25 mm. The developed chromatograms were viewed under ultraviolet light at 254 nm. For column chromatography Merck silica gel (70–230 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.

4.1.1. Methyl 3,4-methylenedioxybenzoate **16**

To a solution of piperonal **15** (0.45 g, 3.0 mmol) in absolute methanol (4 mL) cooled at 0 °C, were successively added methanolic solutions (each 3 mL) of iodine (1.00 g, 3.9 mmol) and KOH (0.44 g, 7.8 mmol) at 0 °C. After stirring for 1.5 h at 0 °C, small amounts of saturated $NaHSO_3$ solution were added until the disappearance of the brown colour. Next, the methanol was almost totally evaporated under reduced pressure. To the residue was added water, and the desired methyl 3,4-methylenedioxybenzoate **16** was obtained by filtration, in 90% yield, as a white solid, m.p. 53 °C. ^{1}H-NMR (200 MHz) $CDCl_3$: δ 7.63 (dd, 1H, H_6, *J*ax = 8.2 Hz, *J*bx = 1.7 Hz), 7.44 (d, 1H, H_2, *J*ax = 1.6 Hz), 6.82 (d, 1H, H_5, *J* = 8.2 Hz), 6.02 (s, 2H, O–C$\underline{H}_2$–O), 3.87 (s, 3H, OC$\underline{H}_3$) ppm; ^{13}C-NMR (50 MHz) $CDCl_3$: δ 166.0 (C=O), 151.4 (C_4), 147.5 (C_3), 125.1 (C_6), 124.0 (C_1), 109.3 (C_2), 107.7 (C_5), 101.6 (O–$\underline{C}H_2$–O), 51.9 (O$\underline{C}H_3$) ppm; MS (70 eV) m/z (relative abundance): 180 (50%), 149 (100%), 121 (20%), 91 (8%), 65 (18%); IR (KBr) cm^{-1}: 1 723 (ν C=O), 1 289 (ν C–O).

4.1.2. 3,4-Methylenedioxybenzoylhydrazine **17**

To a solution of 2.68 g (14.85 mmol) of **16** in 10 mL of ethanol, was added 15 mL of 80% hydrazine monohydrate. The reaction mixture was maintained under reflux for 3.5 h, when TLC indicated the end of the reaction. Then, the media was poured on ice and the resulting precipitate was filtered out affording the title compound in 70% yield, as a white solid, m.p. 170–171 °C. ^{1}H-NMR (200 MHz): δ 10.74 (s, 1H, CON$\underline{H}$-), 7.44 (dd, 1H, H_6, *J*ax = 8.2 Hz, *J*bx = 1.6 Hz), 7.36 (s, 1H, H_2), 7.17 (d, 1H, H_5, *J* = 8.2 Hz), 6.10 (s, 2H, O–CH_2–O), 4.44 (br, 2H, -NH_2) ppm, ^{13}C-NMR (50 MHz): δ 165.2 (C=O), 149.6 (C_4), 147.3 (C_3), 127.2 (C_1), 121.9 (C_6), 107.9 (C_2), 107.1 (C_5), 101.6 (O–CH_2–O) ppm; MS (70 eV) m/z (relative abundance): 180 (17%), 149 (100%), 121 (25%), 91 (8%), 65 (19%); IR (KBr) cm^{-1}: 3 303 (ν N–H), 3 220 (ν N–H), 1 605 (ν C=O), 1 262 (ν C–O).

4.1.3. General procedure for preparation of 3,4-methylenedioxybenzoyl-arylhydrazones **9a–j** (table I)

To a solution of 0.150 g (0.83 mmol) of **17** in absolute ethanol (7 mL) containing two drops of 37% hydrochloric acid, was added 0.87 mmol of corresponding aldehyde derivative. The mixture was stirred at room temperature for 30 min, then after extensive precipitation, was visualized. Next, the mixture was poured into cold water,

neutralized with 10% aqueous sodium bicarbonate solution and the precipitate formed was filtered out and dried.

4.1.3.1. Benzylidene 3,4-methylenedioxybenzoylhydrazine **9a**

The derivative **9a** was obtained as a white solid by condensation of **17** with benzaldehyde, (R_f acetone:toluene 30% = 0.50). ^{1}H-NMR (200 MHz): δ 11.70 (s, 1H, CONH-), 8.42 (s, 1H, -N=CH-), 7.70 (m, 2H, H_2 and H_4'), 7.51 (d, 1H, H_6, *J* = 8.1 Hz), 7.44 (m, 4H, H_2' and H_3'), 7.05 (d, 1H, H_5, *J* = 8.1 Hz), 6.17 (s, 2H, O–CH_2–O) ppm; ^{13}C-NMR (50 MHz): δ 162.3 (C=O); 150.2 (C_4), 148.1 (C_3), 147.5 (N=CH), 134.4 (C_1'), 130.0 (C_4'), 128.8 (C_2'), 127.2 (C_1), 127.0 (C_3'), 122.8 (C_6), 108.0 (C_2), 107.6 (C_5), 101.8 (O–CH_2–O) ppm; MS (70 eV) m/z (relative abundance): 268 (5%), 165 (28%), 149 (100%), 121 (13%), 65 (15%); IR (KBr) cm^{-1}: 3 448 (ν N–H), 1 636 (ν C=O), 1 601 (ν C=N), 1 300 and 1 261 (ν C–O). Anal. calcd. for $C_{15}H_{12}N_2O_3$: C, 67.16; H, 4.51; N, 10.44. Found: C, 67.25; H, 4.67; N, 10.28.

4.1.3.2. (4'-Fluorobenzylidene) 3,4-methylenedioxybenzoylhydrazine **9b**

The derivative **9b** was obtained as a white solid by condensation of **17** with 4-fluorobenzaldehyde (R_f acetone:toluene 30% = 0.52). ^{1}H-NMR (200 MHz): δ 11.79 (s, 1H, CONH-), 8.48 (s, 1H, -N=CH-), 7.83 (d, 2H, H_2', *J* = 7.5 Hz), 7.59 (d, 1H, H_6, *J* = 7.6 Hz), 7.52 (s, H_2), 7.34 (d, 2H, H_3', *J* = 7.5 Hz), 7.09 (d, 1H, H_5, *J* = 7.5 Hz), 6.18 (s, 2H, O–CH_2–O) ppm; ^{13}C-NMR (50 MHz): δ 162.2 (C=O), 160.6 (C_4'), 150.1 (C_4), 147.4 (C_3), 146.2 (-N=CH-), 131.0 (C_1'), 129.2 (C_2'), 127.0 (C_1), 122.8 (C_6), 115.6 (C_3'), 108.0 (C_2), 107.6 (C_5), 101.8 (O–CH_2–O) ppm; MS (70 eV) m/z (relative abundance): 286 (2%), 165 (24%), 149 (100%), 121 (18%), 91 (8%), 65 (2%); IR (KBr) cm^{-1}: 3 383 (ν N-H), 1 647 (ν C=O), 1 604 (ν C=N), 1 309 (ν C–O). Anal. calcd. for $C_{15}H_{11}FN_2O_3$: C, 62.94; H, 3.87; N, 9.79. Found: C, 63.03; H, 3.79; N, 9.81.

4.1.3.3. (4'-Bromobenzylidene) 3,4-methylenedioxybenzoylhydrazine **9c**

The derivative **9c** was obtained as a white solid, by condensation of **17** with 4-bromobenzaldehyde (R_f acetone:toluene 30% = 0.56). ^{1}H-NMR (200 MHz): δ 12.05 (s, 1H, CONH-), 8.72 (s, 1H, -N=CH-), 7.96 (m, 4H, H_2' and H_3'), 7.83 (d, 1H, H_6, *J* = 7.5 Hz), 7.75 (s, 1H, H_2), 7.38 (d, 1H, H_5, *J* = 7.5 Hz), 6.45 (s, 2H, O–CH_2–O) ppm; ^{13}C-NMR (50 MHz); δ 162.5 (C=O), 150.5 (C_4), 147.7 (C_3), 146.4 (-N=CH-), 134.0 (C_1'), 132.2 (C_2'), 129.1 (C_3'), 127.3 (C_1), 123.5 (C_4'), 123.2 (C_6), 108.3 (C_2), 107.9 (C_5), 102.1 (O–CH_2–O) ppm; MS (70 eV) m/z (relative abundance): 346 (2%), 165 (27%), 149 (100%), 121 (14%), 91 (8%), 65 (27%); IR (KBr) cm^{-1}: 3 244 (ν N–H), 1 648 (ν C=O), 1 598 (ν C = N), 1 290 and 1 260 (ν C–O). Anal. calcd. for $C_{15}H_{11}BrN_2O_3$: C, 51.89; H, 3.19; N, 8.07. Found: C, 51.83; H, 3.27; N, 8.15.

4.1.3.4. (4'-Methoxybenzylidene) 3,4-methylenedioxybenzoylhydrazine **9d**

The derivative **9d** was obtained as a white solid by condensation of **17** with 4-methoxybenzaldehyde (R_f acetone:toluene 30% = 0.46). ^{1}H-NMR (200 MHz): δ 11.39 (s, 1H, CONH-), 8.39 (s, 1H, -N=CH-), 7.66 (d, 1H, H_5, *J* = 8.6 Hz), 7.50 (d, 1H, H_6, *J* = 8.1 Hz), 7.44 (s, 1H, H_2), 7.05 (d, 2H, H_2', *J* = 6.5 Hz), 7.00 (d, 2H, H_3', *J* = 6.2 Hz), 6.15 (s, 2H, O–CH_2–O), 3.81 (s, 3H, OCH_3) ppm; ^{13}C-NMR (50 MHz): δ 162.1 (C=O), 160.8 (C_4'), 150.1 (C_4), 147.9 (C_3), 147.3 (-N=CH-), 128.6 (C_2'), 127.3 (C_1), 126.9 (C_1'), 122.7 (C_6), 114.3 (C_3'), 108.0 (C_2), 107.6 (C_5), 101.8 (O–CH_2–O), 55.2 (OCH_3) ppm; MS (70 eV) m/z (relative abundance): 298 (8%), 165 (30%), 149 (100%), 121 (15%), 91 (12%), 77 (8%), 65 (27%); IR (KBr) cm^{-1}: 3 262 (ν N–H), 1 647 (ν C=O), 1 605 (ν C=N), 1 254 and 1 288 (ν C–O), 1 437 (CH_3). Anal. calcd. for $C_{16}H_{14}N_2O_4$: C, 64.42; H, 4.73; N, 9.39. Found: C, 64.37; H, 4.79; N, 9.46.

4.1.3.5. (4'-Nitrobenzylidene) 3,4-methylenedioxybenzoylhydrazine **9e**

The derivative **9e** was obtained as a yellow solid by condensation of **17** with 4-nitrobenzaldehyde (R_f acetone:toluene 30% = 0.52). ^{1}H-NMR (300 MHz): δ 12.12 (s, 1H, CONH-), 8.63 (s, 1H, -N=CH-), 8.45 (d, 2H, H_3', *J* = 7.9 Hz), 8.10 (d, 2H, H_2', *J* = 8.2 Hz), 7.67 (d, 1H, H_5, *J* = 7.9 Hz), 7.60 (s, 1H, H_2), 7.20 (dd, 1H, H_6, *J*ax = 8.1 Hz, *J*bx = 1.2 Hz), 6.27 (s, 2H, O–CH_2–O) ppm; ^{13}C-NMR (75 MHz): δ 162.7 (C=O), 150.6 (C_4), 147.9 (C_4'), 147.6 (C_3), 145.0 (-N=CH-), 140.9 (C_1'), 128.1 (C_2'), 126.9 (C_1), 124.2 (C_3'), 123.4 (C_6), 108.3 (C_2), 107.4 (C_5), 102.1 (O–CH_2–O) ppm; MS (70 eV) m/z (relative abundance): 313 (6%), 165 (9%), 149 (100%), 121 (12%), 65 (11%); IR (KBr) cm^{-1}: 3 220 (ν N–H), 1 648 (ν C=O), 1 607 (ν C=N), 1 293 (ν C–O), 1 509 (ν NO_2). Anal. calcd. for $C_{15}H_{11}N_3O_5$: C, 57.51; H, 3.54; N, 13.41. Found: C, 57.45; H, 3.61; N, 13.37.

4.1.3.6. (4'-N,N-Dimethylaminobenzylidene) 3,4-methylenedioxybenzoylhydrazine **9f**

The derivative **9f** was obtained as an orange solid by condensation of **17** with 4-*N*,*N*-dimethylaminobenzaldehyde (R_f acetone:toluene 30% = 0.44). ^{1}H-NMR (200 MHz): δ 11.40 (s, 1H, CONH-), 8.27 (s, 1H, -N=CH-), 7.52 (d, 2H, H_2', *J* = 8.7 Hz), 7.49 (d, 1H, H_6, *J* = 8.2 Hz), 7.42 (s, 1H, H_2), 7.02 (d, 1H, H_5, *J* = 8.2 Hz), 6.74 (d, 2H,

H_3', *J* = 8.8 Hz), 6.12 (s, 2H, O–C$\underline{H}_2$–O), 2.98 (s, 6H, -N(C$\underline{H}_3$)$_2$) ppm; ^{13}C-NMR (50 MHz): δ 161.8 (C=O), 151.5 (C_4'), 149.9 (C_4), 148.2 (C_3), 148.4 (-N=$\underline{C}$H-), 147.4 (C_1'), 128.4 (C_2'), 127.6 (C_1), 122.6 (C_6), 111.8 (C_3'), 108.0 (C_2), 107.5 (C_5), 101.8 (O–$\underline{C}H_2$–O), 39.8 (-N($\underline{C}H_3$)$_2$) ppm; MS (70 eV) m/z (relative abundance): 311 (60%), 165 (12%), 149 (100%), 121 (17%), 91 (12%), 65 (19%); IR (KBr) cm^{-1}: 3 300 (ν N–H), 1 651 (ν C=O), 1 600 (ν C=N), 1 255 (ν C–O), 1 360 (ν N(CH_3)$_2$). Anal. calcd. for $C_{17}H_{17}N_3O_3$: C, 65.58; H, 5.50; N, 13.50. Found: C, 65.66; H, 5.61; N, 13.38.

4.1.3.7. (4'-Cyanobenzylidene) 3,4-methylenedioxybenzoylhydrazine **9g**

The derivative **9g** was obtained as a white solid by condensation of **17** with 4-cyanobenzaldehyde (R_f acetone:toluene 30% = 0.48). ^{1}H-NMR (200 MHz): δ 11.35 (s, 1H, -CON$\underline{H}$-), 8.50 (s, 1H, -N=C$\underline{H}$-), 7.93 (s, 4H, H_2' and H_3'), 7.58 (d, 1H, H_5, *J* = 7.5 Hz), 7.50 (s, 1H, H_2), 7.10 (d, 1H, H_6, *J* = 7.5 Hz), 6.27 (s, 2H, O–C$\underline{H}_2$–O) ppm; ^{13}C-NMR (50 MHz): δ 161.3 (C=O), 150.7 (C_4), 147.7 (C_3), 145.6 (-N=$\underline{C}$H-), 139.2 (C_1'), 133.0 (C_2'), 127.8 (C_3'), 127.1 (C_1), 123.4 (C_6), 118.9 (C_4'), 112.0 ($\underline{C}$≡N), 108.4 (C_2), 108.0 (C_5), 102.2 (O–$\underline{C}H_2$–O) ppm; MS (70 eV) m/z (relative abundance): 293 (4%), 165 (12%), 149 (100%), 121 (14%), 91 (8%), 65 (27%); IR (KBr) cm^{-1}: 3 420 (ν N–H), 2 227 (ν C≡N), 1 651 (ν C=O), 1 604 (ν C=N), 1 262 (ν C–O). Anal. calcd. for $C_{16}H_{11}N_3O_3$: C, 65.53; H, 3.78; N, 14.33. Found: C, 65.59; H, 3.65; N, 14.29.

4.1.3.8. (2'-Pyridinylidene) 3,4-methylenedioxybenzoylhydrazine **9h**

The derivative **9h** was obtained as a white solid by condensation of **17** with 2-pyridinecarboxaldehyde (R_f acetone:toluene 30% = 0.25). ^{1}H-NMR (200 MHz): δ 12.06 (s, 1H, -CON$\underline{H}$-), 8.63 (d, 1H, H_6', *J* = 4.5Hz), 8.52 (s, 1H, -N=C$\underline{H}$-), 7.95 (m, 2H, H_4' and H_5'), 7.57 (d, 1H, H_6, *J* = 8.2 Hz), 7.48 (m, 2H, H_2 and H_3'), 7.05 (d, 1H, H_5, *J* = 8.1 Hz), 6.13 (s, 2H, O–C$\underline{H}_2$–O) ppm; ^{13}C-NMR (50 MHz): δ 162.9 (C=O), 153.0 (C_4), 150.8 (C_2'), 149.0 (C_6'), 147.9 (C_4'), 147.8 (C_3), 146.8 (-N=$\underline{C}$H-), 127.2 (C_3'), 125.1 (C_5'), 123.7 (C_6), 123.5 (C_1), 108.6 (C_2), 108.3 (C_5), 102.3 (O–$\underline{C}H_2$–O) ppm; MS (70 eV) m/z (relative abundance): 269 (3%), 165 (11%), 149 (100%), 121 (12%), 91 (7%), 65 (20%); IR (KBr) cm^{-1}: 3 399 (ν N–H), 1 679 (ν C=O), 1 558 (ν C=N), 1 281 (ν C–O). Anal. calcd. for $C_{16}H_{11}N_3O_3$: C, 65.53; H, 3.78; N, 14.33. Found: C, 65.59; H, 3.65; N, 14.29.

4.1.3.9. (3'-Pyridinylidene) 3,4-methylenedioxybenzoylhydrazine **9i**

The derivative **9i** was obtained as a white solid by condensation of **17** with 3-pyridinecarboxaldehyde (R_f acetone:toluene 30% = 0.26). ^{1}H-NMR (200 MHz): δ 12.07 (s, 1H, -CONH-), 8.94 (s, 1H, H_2'), 8.86 (d, 1H, H_6', *J* = 4.9 Hz), 8.69 (d, 1H, H_4', *J* = 4.0Hz), 8.59 (s, 1H, -N=C$\underline{H}$-), 7.55 (dd, 1H, H_5', *J*ax = 7.4 Hz, *J*bx = 4.8 Hz), 7.52 (d, 1H, H_6, *J* = 8.2 Hz), 7.50 (s, 1H, H_2), 7.20 (d, 1H, H_5, *J* = 8.1 Hz), 6.10 (s, 2H, O–C$\underline{H}_2$–O) ppm; ^{13}C-NMR (50 MHz): δ 161.2 (C=O), 150.6 (C_4), 150.4 (C_2'), 148.3 (C_6'), 147.4 (C_3), 145.6 (-N=$\underline{C}$H-), 142.5 (C_3'), 132 4 (C_5'), 126.6 (C_4'), 125.9 (C_1), 123.2 (C_6), 108.1 (C_2), 107.9 (C_5), 101.9 (O–$\underline{C}H_2$–O) ppm; MS (70 eV) m/z (relative abundance): 269 (7%), 165 (10%), 149 (100%), 121 (10%), 91 (7%); IR (KBr) cm^{-1}: 3 446 (ν N–H), 1 659 (ν C=O), 1 589 (ν C=N), 1 281 (ν C-O). Anal. calcd. for $C_{16}H_{11}N_3O_3$: C, 65.53; H, 3.78; N, 14.33. Found: C, 65.50; H, 3.70; N, 14.18.

4.1.3.10. (4'-Pyridinylidene) 3,4-methylenedioxybenzoylhydrazine **9j**

The derivative **9j** was obtained as a white solid by condensation of **17** with 4-pyridinecarboxaldehyde (R_f acetone:toluene 30% = 0.29). ^{1}H-NMR (200 MHz): δ 11.98 (s, 1H, -CON$\underline{H}$-), 8.39 (s, 1H, -N=C$\underline{H}$-), 8.63 (d, 2H, H_2', *J* = 5.9 Hz), 7.64 (d, 2H, H_3', *J* = 5.6 Hz), 7.50 (d, 1H, H_6, *J* = 8.1 Hz), 7.32 (s, 1H, H_2), 7.05 (d, 1H, H_5, *J* = 8.1 Hz), 6.18 (s, 2H, O–C$\underline{H}_2$–O) ppm; ^{13}C-NMR (50 MHz): δ 161.2 (C=O), 150.4 (C_4), 150.2 (C_2'), 147.4 (C_3), 144.9 (-N=$\underline{C}$H-), 141.6 (C_4'), 126.8 (C_1), 123.4 (C_6), 120.9 (C_3'), 108.1 (C_2), 107.8 (C_5), 101.9 (O–$\underline{C}H_2$–O) ppm; MS (70 eV) m/z (relative abundance): 269 (10%), 165 (16%), 149 (100%), 121 (14%), 91 (5%), 65 (10%); IR (KBr) cm^{-1}: 3 487 (ν N–H), 1 655 (ν C=O), 1 600 (ν C=N), 1 261 (ν C–O). Anal. calcd. for $C_{16}H_{11}N_3O_3$: C, 65.53; H, 3.78; N, 14.33. Found: C, 65.69; H, 3.71; N, 14.41.

4.1.3.11. 3-(3',4'-Methylenedioxyphenyl) propan-1-ol **20** *[15]*

A solution of 0.92 g (5.7 mmol) of safrole **7** in 25 mL of dry THF, at room temperature, was treated with 6 mL (6 mmol) of a 1 M solution of BH_3.THF, under nitrogen atmosphere. The resulting solution was stirred at room temperature for 2 h. Then, methanol was added dropwise until no further gas was evolved, and 2.4 mL of a 10% aqueous NaOH solution (6 mmol) and 2.4 mL of 30% aqueous H_2O_2 solution were added at 0 °C. The suspension formed was maintained at 60 °C for 4 h. After cooling, the reaction mixture was partitioned between ethyl ether (20 mL) and water (10 mL), followed by

separation of the organic phase. The aqueous layer was further extracted with ethyl ether (3 × 10 mL) and the organic extracts were combined, treated with 10 mL of a 10% aqueous HCl solution and submitted to the usual work-up which furnished compound **20** as a yellow oil, 78% yield (R_f ethyl acetate: hexane 30% = 0.35). ^{1}H-NMR (200 MHz) $CDCl_3$: δ 6.67 (m, 3H, H_2, H_5 and H_6), 5.90 (s, 2H, O–C$\underline{H}_2$–O), 3.61 (t, 2H, C$\underline{H}_2$–OH, *J* = 8.3 Hz), 2.60 (t, 2H, Ar–CH_2, *J* = 10.0 Hz), 1.81 (qt, 2H, C$\underline{H}_2$–CH_2–CH_2, *J* = 9.9 Hz) ppm; ^{13}C-NMR (50 MHz) $CDCl_3$: δ 147.3 (C_4), 145.4 (C_3), 135.5 (C_1), 120.9 (C_6), 108.7 (C_5), 107.9 (C_2), 100.5 (O–$\underline{C}H_2$–O), 61.7 ($\underline{C}H_2$–OH), 34.2 (CH_2–$\underline{C}H_2$–CH_2), 31.6 (Ar–$\underline{C}H_2$) ppm; MS (70 eV) m/z (relative abundance): 180 (42%), 163 (4%), 149 (100%), 121 (16%); IR (KBr) cm^{-1}: 3 359 (ν O–H), 1 245 (ν C–O).

4.1.3.12. 3-(3',4'-Methylenedioxyphenyl) propanoic acid **21** *[15]*

An 8 N solution of Jones reagent [19] (ca. 10 mL) was added dropwise to a solution of 1.15 g (6.4 mmol) of alcohol **20** in 10 mL of acetone at 0 °C. After 1 h 10 mL of isopropanol was added and the reaction mixture was filtered. The filtrate was concentrated under reduced pressure to obtain a residue which was diluted with 50 mL of a mixture of diethylether/water (2:1). The organic layer was separated, dried and evaporated to give 1.14 g (92%) of the acid **21** as a white solid, m.p. 81–82 °C (R_f acetone:toluene 30% = 0.47). ^{1}H-NMR (200 MHz): δ 10.12 (s, 1H, -COO$\underline{H}$), 6.81 (d, 1H, H_5, *J* = 8.0 Hz), 6.78 (s, 1H, H_2), 6.67 (d, 1H, H_6, *J*ax = 7.9 Hz), 5.92 (s, 2H, O–CH_2–O), 2.74 (t, 2H, C$\underline{H}_2$COOH, *J* = 7.5 Hz), 2.48 (t, 2H, Ar–C$\underline{H}_2$, *J* = 7.5 Hz) ppm; ^{13}C-NMR (50 MHz): δ 179.1 (C=O), 147.5 (C_4), 145.9 (C_3), 133.8 (C_1), 120.9 (C_6), 108.6 (C_2), 108.2 (C_5), 100.7 (O–$\underline{C}H_2$–O), 35.8 ($\underline{C}H_2$–COOH), 30.2 (Ar–CH_2) ppm; IR (KBr) cm^{-1}: 3 000 (ν O–H), 1 700 (ν C=O), 1 035 (ν C–O).

4.1.3.13. Methyl 3-(3',4'-methylenedioxyphenyl) propanoate **22**

To a solution of 0.580 g (3 mmol) of the acid **21** in 10 mL of MeOH were added 0.3 mL of concentrated H_2SO_4 and the reaction mixture was refluxed for 2 h, when analysis by TLC indicated the end of the reaction. Then, the solvent was concentrated at reduced pressure and the residue obtained was neutralized with 10% aqueous $NaHCO_3$ solution. Extraction of the aqueous phase with ethyl acetate (3 × 10 mL), followed by the usual work-up of organic layers, furnished 0.62 g (90%) of the corresponding ester derivative **22** as a light yellow oil, (R_f acetone:toluene 30% = 0.81). ^{1}H-NMR (200 MHz) $CDCl_3$: δ 7.30 (s, 1H, H_2), 6.98 (d, 1H, H_5, *J* = 5.7 Hz), 6.70 (d, 1H, H_6, *J* = 5.6 Hz), 3.80 (s, 3H, OCH_3), 2.86 (t, 2H, -C$\underline{H}_2$–COOMe, *J* = 7.7 Hz), 2.58 (t, 2H, Ar–C$\underline{H}_2$-, *J* = 7.9 Hz) ppm; ^{13}C-NMR (50 MHz) $CDCl_3$: δ 173.1 (C=O), 147.5 (C_4), 145.8 (C_3), 134.2 (C_1), 120.9 (C_6), 108.6 (C_2), 106.4 (C_5), 100.7 (O–$\underline{C}H_2$–O), 51.5 (O$\underline{C}H_3$), 35.9 ($\underline{C}H_2$COOMe), 30.6 (Ar–CH_2) ppm; IR (KBr) cm^{-1}: 1 703 (ν C=O), 1 259 (ν C–O).

4.1.3.14. 3-(3',4'-Methylenedioxyphenyl) propionylhydrazine **23**

To a solution of 3.08 g (14.8 mmol) of ester **22** in 10 mL of ethanol, was added 15 mL of 80% hydrazine monohydrate. The reaction mixture was maintained under reflux for 3 h, when TLC indicated the end of the reaction. Then, the media was poured onto ice and the resulting precipitate was filtered out, affording 2.31 g (75%) of the propionylhydrazine derivative **23** as a white solid, m.p. 144–146 °C (R_f acetone: toluene 30% = 0.42). ^{1}H-NMR (200 MHz): δ 8.95 (s, 1H, -CON$\underline{H}$-), 6.82 (s, 1H, H_2), 6.78 (d, 1H, H_5, *J* = 7.7 Hz), 6.63 (d, 1H, H_6, *J* = 7.9 Hz), 5.95 (s, 2H, O–CH_2–O), 4.19 (br, 2H, CONHN$\underline{H}_2$), 2.74 (t, 2H, C$\underline{H}_2$–CONH, *J* = 7.7 Hz), 2.28 (t, 2H, Ar–CH_2, *J* = 7.7 Hz) ppm; ^{13}C-NMR (50 MHz): δ 170.7 (C=O), 147.1 (C_4), 145.3 (C_3), 135.0 (C_1), 120.9 (C_6), 108.7 (C_2), 108.0 (C_5), 100.6 (O–$\underline{C}H_2$–O), 35.3 ($\underline{C}H_2$–CONH), 30.7 (Ar–$\underline{C}H_2$) ppm; MS (70 eV) m/z (relative abundance): 208 (33%), 176 (18%), 148 (12%), 135 (100%), 91 (10%), 77 (12%), 65 (8%); IR (KBr) cm^{-1}: 3 311 and 3 287 (ν N–H), 1 632 (ν C=O), 1 245 (ν C-O).

4.1.4. General procedure for preparation of 3-(3',4'-methylenedioxyphenyl)propionylaryl-hydrazones **10a**, **c** *and* **f** *(table I)*

To a solution of 0.150 g (0.72 mmol) of propionyl-hydrazine **23** in absolute ethanol (7 mL) containing two drops of 37% hydrochloric acid, was added 0.76 mmol of the corresponding benzaldehyde derivative. The mixture was stirred at room temperature for 30 min, then, after extensive precipitation, was visualized. Next, the mixture was poured into cold water and the precipitate formed was filtered out and dried.

4.1.4.1. Benzylidene 3-(3',4'-methylenedioxyphenyl) propionylhydrazine **10a**

The derivative **10a** was obtained as a white solid by condensation of **23** with benzaldehyde (R_f acetone:toluene 30% = 0.52). ^{1}H-NMR (200 MHz): δ 11.29 and 11.25 (s, 1H, -CON$\underline{H}$-), 8.16 and 7.98 (s, 1H, -N=C$\underline{H}$- of (*E*) and (*Z*)-diastereomer, respectively), 7.68 (dd, 2H, H_2', *J*ax = 7.5 Hz, *J*bx = 1.5 Hz), 7.43 (m, 3H, H_3' and H_4'), 6.80 (m, 3H, H_2, H_5 and H_6), 5.95 and 5.97 (s, 2H,

-O–CH_2–O-), 2.50 (t, 2H, CH_2–CONH, *J* = 7.4 Hz), 2.85 (t, 2H, Ar–C<u>H</u>$_2$, *J* = 8.0 Hz) ppm; ^{13}C-NMR (50 MHz): δ 173.5 (C=O), 167.8 (C_4), 147.1 (C_3), 145.8 (-N=<u>C</u>H-), 145.3 (C_1'), 134.3 (C_4'), 128.7 (C_2'), 127.3 (C_1), 126.9 (C_3'), 119.8 (C_6), 108.8 (C_2), 108.01 (C_5), 100.6 (O–<u>C</u>H_2–O), 36.07 and 34.05 (-<u>C</u>H_2α), 30.4 and 29.76 (-<u>C</u>H_2β) ppm; MS (70 eV) m/z (relative abundance): 295 (24%), 148 (40%), 135 (100%), 121 (5%); IR (KBr) cm^{-1}: 3 311.3 and 3 287.4 (ν N–H), 1 632.8 (ν C=O), 1 245.2 (ν C-O). Anal. calcd. for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45. Found: C, 69.02; H, 5.60; N, 9.53.

4.1.4.2. (4'-Bromobenzylidene) 3-(3',4'-methylenedioxyphenyl) propionylhydrazine **10c**

The derivative **10c** was obtained as a white solid by condensation of **23** with 4-bromobenzaldehyde (R_f acetone:toluene 30% = 0.41). ^{1}H-NMR (200 MHz): δ 11.41 and 11.32 (s, 1H, -CONH-), 8.10 and 7.93 (s, 1H, -N=CH- of (*E*) and (*Z*)-diastereomer, respectively), 7.60 (m, 4H, H_2' and H_3'), 6.73 (s, 1H, H_2), 6.70 (m, 2H, H_5 and H_6), 5.95 and 5.94 (s, 2H, -O–CH_2–O), 2.84 (m, 4H, C<u>H</u>$_2$–CONH and Ar–CH_2) ppm; ^{13}C-NMR (50 MHz): δ 174.5 (C=O), 147.9 (C_4), 145.4 (C_3), 145.1 (-N=<u>C</u>H-), 135.6 (C_2'), 134.0 (C_1'), 129.2 (C_3'), 128.3 (C_1), 123.5 (C_4'), 121.5 (C_6), 109.3 and 109.2 (C_2), 108.5 (C_5), 101.0 (-O–<u>C</u>H_2–O), 36.5 and 34.5 (<u>C</u>H_2–CONH), 30.8 and 30.2 (Ar–<u>C</u>H_2); MS (70 eV) m/z (relative abundance): 375 (7%), 135 (100%), 148 (43%); IR (KBr) cm^{-1}: 3 100 (ν N–H), 1 679 (ν C=O), 1 394 (ν C–O). Anal. calcd. for $C_{17}H_{15}BrN_2O_3$: C, 54.42; H, 4.03; N, 7.47. Found: C, 54.52; H, 4.12; N, 7.56.

4.1.4.3. (4'-N,N-Dimethylaminobenzylidene)3-(3',4'-methylenedioxyphenyl) propionylhydrazine **10f**

The derivative **10f** was obtained as a light orange solid by condensation of **23** with 4-*N*,*N*-dimethylaminobenzaldehyde (R_f acetone:toluene 30% = 0.56). ^{1}H-NMR (200 MHz): δ 11.06 and 10.99 (s, 1H, -CONH-), 8.00 and 7.85 (s, 1H, -N=CH-, of (*E*) and (*Z*)-diastereomer, respectively), 7.46 (m, 4H, H_2' and H_3'), 6.79 (s, 1H, H_2), 6.72 (d, 1H, H_5, *J* = 6.6 Hz), 6.70 (d, 1H, H_6, *J* = 6.7 Hz), 5.96 and 5.95 (s, 2H, -O–CH_2–O-), 2.95 (s, 6H, N(CH_3)$_2$), 2.82 (t, 2H, C<u>H</u>$_2$–CONH), 2.42 (t, 2H, Ar–CH_2) ppm; ^{13}C-NMR (50 MHz): δ 174.5 (C=O), 151.5 (C_4), 147.4 (C_3), 144.2 (-N=<u>C</u>H-), 121.1 (C_6), 128.6 (C_2'), 111.7 (C_3'), 108.9 (C_2), 108.2 (C_5), 100.6 (O–<u>C</u>H_2–O), 36.8 (N(<u>C</u>H_3)$_2$), 34.9 (<u>C</u>H_2–CONH), 30.4 (Ar–<u>C</u>H_2) ppm; MS (70 eV) m/z (relative abundance): 339 (50%), 294 (17%), 208 (15%), 148 (60%), 135 (100%), 121 (6%); IR (KBr) cm^{-1}: 3 405 (ν N–H), 1 668 (ν C=O), 1 247 (ν C–O). Anal. calcd. for $C_{19}H_{21}N_3O_3$: C, 67.24; H, 6.24; N, 12.38. Found: C, 67.28; H, 6.22; N, 12.43.

4.1.5. General procedure to obtain 6-methyl-3,4-methylenedioxybenzenesulphonyl-hydrazones **11a** *and* **c–f** (table II)

To a solution of 0.19 g (0.83 mmol) of **24** in absolute ethanol (20 mL) containing two drops of 37% hydrochloric acid, was added 0.83 mmol of the corresponding benzaldehyde derivative. The end of the reaction was observed by TLC, after that the mixture was poured into cold water and the precipitate formed was filtered out and dried.

4.1.5.1. Benzylidene-6-methyl-3,4-methylene-dioxybenzenesulfonylhydrazine **11a**

The derivative **11a** was obtained as a white solid by condensation of **24** with benzaldehyde. ^{1}H-NMR (200 MHz): δ 11.45 (s, 1H, SO_2N<u>H</u>-), 7.97 (s, 1H, -N=C<u>H</u>-), 7.55 (m, 5H, H_2', H_3' and H_4'), 7.47 (s, 1H, H_2), 6.92 (s, 1H, H_5), 6.09 (s, 2H, O–C<u>H</u>$_2$–O), 2.59 (s, 3H, Ar–C<u>H</u>$_3$) ppm; ^{13}C NMR (50 MHz): δ 150.58 (C_4), 145.8 (<u>C</u>=NH), 145.23 (C_3), 133.59 (C_1), 133.05 (C_6), 129.95 (C_1'), 129.63 (C_4'), 128.46 (C_2'), 126.41 (C_3'), 111.54 (C_5), 108.79 (C_2), 102.01 (O–<u>C</u>H_2–O), 19.78 (Ar–<u>C</u>H_3) ppm; MS (70 eV) m/z (relative abundance): 318 (20%), 200 (9%), 135 (40%), 90 (100%), 77 (44%); IR (KBr) cm^{-1}: 3 227 (ν N–H), 1 612 (ν C=N), 1 326 and 1 122 (ν S–N). Anal. calcd. for $C_{15}H_{14}N_2O_4S$: C, 56.59; H, 4.43; N, 8.80. Found: C, 56.64; H, 4.55; N, 8.91.

4.1.5.2. (4'-Bromobenzylidene)-6-methyl-3,4-methylene-dioxybenzenesulfonyl hydrazine **11c**

The derivative **11c** was obtained as a white solid by condensation of **24** with 4-bromobenzaldehyde. ^{1}H-NMR (200 MHz): δ 11.67 (s, 1H, SO_2N<u>H</u>-), 7.95 (s, 1H, -N=C<u>H</u>-), 7.57 (d, 2H, H_3', *J* = 7.90 Hz), 7.37 (s, 1H, H_2), 6.94 (d, 2H, H_2', *J* = 8.00 Hz), 6.32 (s, 1H, H_5), 5.99 (s, 2H, O–C<u>H</u>$_2$–O), 2.57 (s, 3H, Ar–C<u>H</u>$_3$) ppm; ^{13}C-NMR (50 MHz): δ 150.82 (C_4), 145.38 (<u>C</u>=NH), 144.66 (C_3), 133.19 (C_1), 132.97 (C_6), 131.71 (C_1'), 129.86 (C_2'), 128.40 (C_3'), 123.09 (C_4'), 111.77 (C_5), 108.96 (C_2), 102.26 (O–<u>C</u>H_2–O), 19.99 (Ar–<u>C</u>H_3) ppm; MS (70 eV) m/z (relative abundance): 369 (2%), 200 (26%), 169 (31%), 135 (34%), 89 (100%), 77 (38%); IR (KBr) cm^{-1}: 3 201 (ν N–H), 1 611 (ν C=N), 1 301 and 1 122 (ν S–N). Anal. calcd. for $C_{15}H_{13}BrN_2O_4S$: C, 45.35; H, 3.30; N, 7.05. Found: C, 45.46; H, 3.22; N, 7.13.

4.1.5.3. (4'-Methoxybenzylidene)-6-methyl-3,4-methylene-dioxybenzenesulfonyl hydrazine **11d**

The derivative **11d** was obtained as a white solid by condensation of **24** with 4-methoxybenzaldehyde. ^{1}H-NMR (200 MHz): δ 11.22 (s, 1H, SO_2N<u>H</u>-), 7.91 (s, 1H, -N=C<u>H</u>-), 7.49 (d, 2H, H_3', *J* = 8.50 Hz), 7.35 (s, 1H, H_2), 6.96 (d, 2H, H_2', *J* = 8.50 Hz), 6.91 (s, 1H, H_5), 6.09 (s,

2H, O–C<u>H</u>$_2$–O), 3.76 (s, 3H, -OC<u>H</u>$_3$), 2.57 (s, 3H, Ar–C<u>H</u>$_3$) ppm; ^{13}C-NMR (50 MHz): δ 160.55 (C$_4$'), 150.51 (C$_4$), 145.9 (<u>C</u>=NH), 145.18 (C$_3$), 133.06 (C$_1$), 133.02 (C$_6$), 127.98 (C$_1$'), 126.23 (C$_2$'), 114.08 (C$_3$'), 111.51 (C$_5$), 108.77 (C$_2$), 101.98 (O–<u>C</u>H$_2$–O), 55.04 (-O<u>C</u>H$_3$), 19.80 (Ar–<u>C</u>H$_3$) ppm; MS (70 eV) m/z (relative abundance): 348 (15%), 200 (12%), 149 (47%), 135 (62%), 120 (100%), 77 (50%); IR (KBr) cm^{-1}: 3 217 (ν N–H), 1 607 (ν C=N), 1 441 (OCH$_3$), 1 321 and 1 118 (ν S–N). Anal. calcd. for $C_{16}H_{16}N_2O_5S$: C, 55.16; H, 4.63; N, 8.04. Found: C, 55.22; H, 4.72; N, 7.98.

4.1.5.4. (4'-Nitrobenzylidene)-6-methyl-3,4-methylene-dioxybenzenesulfonylhydrazine **11e**

The derivative **11e** was obtained as a white solid by condensation of **24** with 4-nitrobenzaldehyde. ^{1}H-NMR (200 MHz): δ 12.01 (s, 1H, SO$_2$N<u>H</u>-), 8.25 (d, 2H, H$_3$', *J* = 8.79 Hz), 8.10 (s, 1H, -N=C<u>H</u>-), 7.80 (d, 2H, H$_2$', *J* = 8.80 Hz), 7.40 (s, 1H, H$_2$), 6.97 (s, 1H, H$_5$), 6.14 (s, 2H, O–CH$_2$–O), 2.60 (s, 3H, Ar–C<u>H</u>$_3$) ppm; ^{13}C-NMR (50 MHz): δ 151.02 (C$_4$), 147.71 (C$_4$'), 145.48 (<u>C</u>=NH), 143.27 (C$_3$), 139.90 (C$_1$), 133.28 (C$_6$), 129.67 (C$_1$'), 127.52 (C$_2$'), 123.99 (C$_3$'), 111.88 (C$_5$), 109.00 (C$_2$), 102.38 (O–<u>C</u>H$_2$–O), 19.99 (Ar–<u>C</u>H$_3$) ppm; MS (70 eV) m/z (relative abundance): 200 (47%), 151 (52%), 135 (100%), 89 (94%), 77 (71%); IR (KBr) cm^{-1}: 3 221 (ν N–H), 1 595 (ν C=N), 1 326 and 1 158 (ν S–N). Anal. calcd. for $C_{15}H_{13}N_3O_6S$: C, 49.58; H, 3.61; N, 11.56. Found: C, 49.64; H, 3.70; N, 11.48.

4.1.5.5. (4'-N,N-Dimethylaminobenzylidene)-6-methyl-3,4-methylenedioxybenzene sulfonylhydrazine **11f**

The derivative **11f** was obtained as a white solid by condensation of **24** with 4-*N,N*-dimethylaminobenzaldehyde. ^{1}H-NMR (200 MHz): δ 11.02 (s, 1H, SO$_2$N<u>H</u>-), 7.82 (s, 1H, -N=C<u>H</u>-), 7.36 (d, 2H, H$_3$', *J* = 8.40 Hz), 7.32 (s, 1H, H$_2$), 6.92 (s, 1H, H$_5$), 6.65 (d, 2H, H$_2$', *J* = 8.37 Hz), 6.09 (s, 2H, O–C<u>H</u>$_2$–O), 2.92 (s, 6H, -N(C<u>H</u>$_3$)$_2$), 2.56 (s, 3H, Ar–CH$_3$) ppm; ^{13}C-NMR (50 MHz): δ 151.29 (C$_4$'), 150.49 (C$_4$), 147.14 (<u>C</u>=NH), 145.20 (C$_3$), 133.05 (C$_1$), 130.16 (C$_6$), 129.07 (C$_1$'), 127.81 (C$_2$'), 121.04 (C$_3$'), 111.59 (C$_5$), 108.87 (C$_2$), 102.05 (O–<u>C</u>H$_2$–O), 40.74 (-N(<u>C</u>H$_3$)$_2$), 19.97 (Ar–<u>C</u>H$_3$) ppm; MS (70 eV) m/z (relative abundance): 361 (17%), 200 (3%), 160 (100%), 135 (48%), 77 (21%); IR (KBr) cm^{-1}: 3 205 (ν N–H), 1 607 (ν C=N), 1 360 (N(CH$_3$)$_2$), 1 317 and 1 122 (ν S–N). Anal. calcd. for $C_{17}H_{19}N_3O_4S$: C, 56.49; H, 5.30; N, 11.63. Found: C, 56.53; H, 5.27; N, 11.69.

4.1.6. General procedure for preparation of isosteric benzoylhydrazone derivatives **12f** *and* **h–j**, *2-thienoylhydrazone derivatives* **13f** *and* **h–j** *and isonicotinoylhydrazone derivatives* **14f** *and* **h–j** (table III)

To a solution of 0.83 mmol of the commercial acylhydrazine **25**, **26** or **27** in absolute ethanol (7 mL) containing two drops of 37% hydrochloric acid, was added 0.87 mmol of the corresponding aromatic aldehyde derivative. The mixture was stirred at room temperature for 30 min, then, after extensive precipitation, was visualized. Next, the mixture was poured into cold water, neutralized with 10% aqueous sodium bicarbonate solution and the precipitate formed was filtered out and dried.

4.1.6.1. (4'-N,N-Dimethylaminobenzylidene) benzoylhydrazine **12f**

The derivative **12f** was obtained as an orange solid by condensation of **25** with 4-*N,N*-dimethylaminobenzaldehyde (R$_f$ acetone:toluene 30% = 0.44). ^{1}H-NMR (200 MHz): δ 11.55 (s, NH), 8.32 (s, -N=<u>C</u>H-), 7.90 (d, H$_2$', *J* = 8.5 Hz), 7.54 (m, H$_2$, H$_3$ and H$_4$), 6.76 (d, H$_3$', *J* = 8.9 Hz), 2.96 (s, 6H, N(C<u>H</u>$_3$)$_2$) ppm; ^{13}C-NMR (50 MHz): δ 162.7 (C=O), 151.5 (C$_4$'), 148.7 (N=<u>C</u>H), 133.8 (C$_1$), 131.4 (C$_4$), 128.4 (C$_2$), 128.3 (C$_3$), 127.4 (C$_2$'), 121.6 (C$_1$'), 111.5 (C$_3$'), 39.7 (N(<u>C</u>H$_3$)$_2$) ppm; MS (70 eV) m/z (relative abundance): 267 (1%), 205 (3%), 121 (13%), 105 (7%), 91 (105); IR (KBr) cm^{-1}: 3 234 (ν N–H), 1 657 (ν C=O), 1 287 (ν C–O). Anal. calcd. for $C_{16}H_{17}N_3O$: C, 71.89; H, 6.41; N, 15.72. Found: C, 71.99; H, 6.52; N, 15.84.

4.1.6.2. (2'-Pyridinylidene) benzoylhydrazine **12h**

The derivative **12h** was obtained as a white solid by condensation of **25** with 2-pyridinecarboxaldehyde, m.p. 166–168 °C (R$_f$ acetone:toluene 30% = 0.20). ^{1}H-NMR (200 MHz): δ 12.04 (s, -CON<u>H</u>-), 8.61 (d, H$_6$', *J* = 4.8 Hz), 8.48 (s, -N=C<u>H</u>-), 7.91 (m, 4H, H$_2$, H$_3$' and H$_5$'), 7.56 (m, 3H, H$_3$ and H$_4$'), 7.40 (t, H$_4$, *J* = 6.2 Hz) ppm; ^{13}C-NMR (50 MHz): δ 163.3 (C=O), 153.2 (C$_2$'), 149.5 (C$_4$' and C$_6$'), 148.0 (-N=<u>C</u>H-), 133.2 (C$_1$), 131.9 (C$_4$), 128.4 (C$_2$), 127.7 (C$_3$), 124.3 (C$_3$'), 119.9 (C$_5$') ppm; MS (70 eV) m/z (relative abundance): 225 (4%), 121 (12%), 105 (100%); IR (KBr) cm^{-1}: 3 420 (ν N–H), 1 643 (ν C=O), 1 583 (ν C=N), 1 289 (ν C–O). Anal. calcd. for $C_{13}H_{11}N_3O$: C, 69.32; H, 4.92; N, 18.66. Found: C, 69.24; H, 4.85; N, 18.70.

4.1.6.3. (3'-Pyridinylidene) benzoylhydrazine **12i**

The derivative **12i** was obtained as a white solid by condensation of **25** with 3-pyridinecarboxaldehyde (R$_f$ acetone:toluene 30% = 0.25). ^{1}H-NMR (200 MHz): δ 12.02 (s, -CON<u>H</u>-), 8.89 (s, H$_2$'), 8.63 (dd, H$_6$', *J*ax = 4.7 Hz, *J*bx = 1.6 Hz), 8.53 (s, -N=C<u>H</u>-), 8.17 (d, 2H, H$_2$, *J*

= 7.9 Hz), 7.95 (d, H_4', *J* = 6.6 Hz), 7.55 (m, 4H, H_3, H_4 and H_5'); ^{13}C-NMR (50 MHz): δ 163.4 (C=O), 150.7 (C_6'), 148.7 (-N=CH-), 145.1 (C_2'), 133.5 (C_5'), 133.2 (C_1), 131.9 (C_4), 130.3 (C_3'), 128.5 (C_2), 127.7 (C_3), 124.0 (C_4') ppm; MS (70 eV) m/z (relative abundance): 225 (3%), 121 (24%), 105 (100%), 77 (42%); IR (KBr) cm^{-1}: 3 467 (ν N–H), 1 656 (ν C=O), 1 571 (ν C=N), 1 296 (ν C–O). Anal. calcd. for $C_{13}H_{11}N_3O$: C, 69.32; H, 4.92; N, 18.66. Found: C, 69.31; H, 4.99; N, 18.57.

4.1.6.4. (4'-Pyridinylidene) benzoylhydrazine **12j**

The derivative **12j** was obtained as a white solid by condensation of **25** with 4-pyridinecarboxaldehyde (R_f acetone:toluene 30% = 0.30). ^{1}H-NMR (200 MHz): δ 12.18 (s, -CONH-), 8.68 (d, 2H, H_2', *J* = 6.5 Hz), 8.47 (s, -N=CH-), 7.95 (d, H_3', *J* = 6.8 Hz), 7.61 (m, 5H, Ph) ppm; ^{13}C-NMR (50 MHz): δ 163.5 (C=O), 150.3 (C_2'), 145.4 (-N=CH-), 141.5 (C_4'), 133.1 (C_1), 132.1 (C_4), 128.6 (C_2), 127.8 (C_3'), 121.1 (C_3) ppm; MS (70 eV) m/z (relative abundance): 225 (4%), 121 (27%), 105 (100%), 77 (44%); IR (KBr) cm^{-1}: 3 471 (ν N–H), 1 638 (ν C=O), 1 570 (ν C=N), 1 293 (ν C–O). Anal. calcd. for $C_{13}H_{11}N_3O$: C, 69.32; H, 4.92; N, 18.66. Found: C, 69.23; H, 5.01; N, 18.73.

4.1.6.5. (4'-N,N-Dimethylaminobenzylidene) 2-thienoylhydrazine **13f**

The derivative **13f** was obtained as an orange solid by condensation of **26** with 4-*N*,*N*-dimethylaminobenzaldehyde (R_f acetone:toluene 30% = 0.10). ^{1}H-NMR (200 MHz): δ 11.60 (s, CONH-), 8.27 (s, -N=CH-), 7.94 (m, 3H, H_3, H_4 and H_5), 7.57 (d, H_2', *J* = 7.8 Hz), 6.77 (d, H_3', *J* = 8.0 Hz), 2.99 (s, 6H, -N(CH_3)$_2$) ppm; ^{13}C-NMR (50 MHz): δ 160.8 (C=O), 151.5 (C_4'), 148.5 (-N=CH-), 134.6 (C_3), 134.4 (C_4), 133.4 (C_1'), 128.5 (C_2'), 126.5 (C_5), 121.4 (C_2), 111.9 (C_3'), 39.7 (-N(CH_3)$_2$) ppm; MS (70 eV) m/z (relative abundance): 273 (0%), 158 (7%), 111 (82%), 83 (50%), 55 (100%); IR (KBr) cm^{-1}: 3 380 (ν N–H), 1 651 (ν C=O), 1 630 (ν C=N), 1 260 (ν C–O), 1 350 (ν N(CH_3)$_2$). Anal. calcd. for $C_{14}H_{15}N_3OS$: C, 61.51; H, 5.53; N, 15.37. Found: C, 61.63; H, 5.48; N, 15.44.

4.1.6.6. (2'-Pyridinylidene) 2-thienoylhydrazine **13h**

The derivative **13h** was obtained as a white solid by condensation of **26** with 2-pyridinecarboxaldehyde (R_f acetone:toluene 30% = 0.10). ^{1}H-NMR (200 MHz): δ 12.01 (s, -CONH-), 8.61 (dd, H_3', *J*ax = 4.6 Hz, *J*bx = 1.6 Hz), 8.43 (s, -N=CH), 8.00 (m, 4H, H_3, H_4, H_5 and H_4'), 7.42 (t, H_5', *J* = 5.9 Hz), 7.22 (dd, H_6', *J*ax = 5.2 Hz, *J*bx = 1.5 Hz) ppm; ^{13}C-NMR (50 MHz): δ 162.0 (C=O), 153.0 (C_2'), 149.6 (C_6'), 147.7 (-N=CH-), 140.2 (C_4'), 136.9 (C_3), 134.4 (C_4), 129.3 (C_5'), 128.1 (C_3'), 124.3 (C_5), 120.0 (C_2) ppm; MS (70 eV) m/z (relative abundance): 231 (7%), 120 (100%), 111 (85%), 92 (56%), 65 (25%); IR (KBr) cm^{-1}: 3 420 (ν N–H), 1 643 (ν C=O), 1 583 (ν C=N), 1 350 (ν C–O). Anal. calcd. for $C_{11}H_9N_3OS$: C, 57.13; H, 3.92; N, 18.17. Found: C, 57.24; H, 3.88; N, 18.26.

4.1.6.7. (3'-Pyridinylidene) 2-thienoylhydrazine **13i**

The derivative **13i** was obtained as a white solid by condensation of **26** with 3-pyridinecarboxaldehyde (R_f acetone:toluene 30% = 0.12). ^{1}H-NMR (200 MHz): δ 12.01 (s, -CONH-), 8.91 (s, H_2'), 8.61 (dd, H_6', *J*ax = 4.8 Hz, *J*bx = 1.6 Hz), 8.48 (s, -N=CH), 8.04 (m, 3H, H_3, H_4 and H_5), 7.49 (dd, H_5', *J*ax = 7.7 Hz, *J*bx = 4.8 Hz), 7.22 (dd, H_4', *J*ax = 4.8 Hz, *J*bx = 3.8 Hz) ppm; ^{13}C-NMR (50 MHz): δ 161.4 (C=O), 150.6 (C_2'), 148.7 (C_6'), 144.8 (-N=CH-), 141.3 (C_3'), 135.0 (C_3), 133.6 (C_4), 132.6 (C_4'), 126.7 (C_5), 124.0 (C_5'), 121.2 (C_2) ppm; MS (70 eV) m/z (relative abundance): 231 (5%), 120 (2%), 111 (100%), 65 (25%); IR (KBr) cm^{-1}: 3 400 (ν N–H), 1 648 (ν C=O), 1 564 (ν C=N), 1 286 (ν C-O). Anal. calc. for $C_{11}H_9N_3OS$: C, 57.13; H, 3.92; N, 18.17. Found: C, 57.09; H, 3.99; N, 18.10.

4.1.6.8. (4'-Pyridinylidene) 2-thienoylhydrazine **13j**

The derivative **13j** was obtained as a white solid by condensation of **26** with 4-pyridinecarboxaldehyde (R_f acetone:toluene 30% = 0.23). ^{1}H-NMR (200 MHz): δ 12.01 (s, -CONH-), 8.62 (d, 2H, H_2', *J* = 5.8 Hz), 8.40 (s, -N=CH-), 8.01 (m, 2H, H_3 and H_5), 7.69 (d, H_3', *J* = 5.6 Hz), 7.23 (dd, H_4, *J*ax = 5.0 Hz, *J*bx = 3.7 Hz) ppm; ^{13}C-NMR (50 MHz): δ 161.6 (C=O), 150.3 (C_2'), 145.6 (-N=CH-), 141.3 (C_4'), 136.9 (C_3), 135.0 (C_4), 126.8 (C_5), 124.3 (C_3'), 121.0 (C_2) ppm; MS (70 eV) m/z (relative abundance): 231 (5%), 127 (40%), 111 (100%); IR (KBr) cm^{-1}: 3 400 (ν N–H), 1 644 (ν C=O), 1 595 (ν C=N), 1 390 (ν C–O). Anal. calcd. for $C_{11}H_9N_3OS$: C, 57.13; H, 3.92; N, 18.17. Found: C, 57.02; H, 3.84; N, 18.19.

4.1.6.9. (4'-N,N-Dimethylaminobenzylidene) isonicotinoylhydrazine **14f**

The derivative **14f** was obtained as an orange solid by condensation of **27** with 4-*N*,*N*-dimethylaminobenzaldehyde (R_f acetone:toluene 30% = 0.40) ^{1}H-NMR (200 MHz): δ 11.81 (s, CONH-), 8.76 (m, 4H, H_2 and H_3), 8.33 (s, -N=CH), 7.56 (d, 2H, H_2', *J* = 8.3 Hz), 6.73 (d, 2H, H_3', *J* = 8.4 Hz), 2.97 (s, 6H, -N(CH_3)$_2$) ppm; ^{13}C-NMR (50 MHz): δ 161.7 (C=O), 151.6 (C_2), 150.2 (C_4'), 149.8 (C_1'), 146.1 (-N=CH-), 140.1 (C_4), 128.5 (C_2'), 121.3 (C_3), 112.3 (C_3'), 40.3 (-N(CH_3)$_2$) ppm; MS (70 eV) m/z (relative abundance): 268 (68%), 162 (20%), 146 (100%), 118 (18%), 78 (25%); IR (KBr) cm^{-1}: 3 390

(ν N–H), 1 646 (ν C=O), 1 604 (ν C=N), 1 190 (ν C–O), 1 389 (ν $N(CH_3)_2$). Anal. calcd. for $C_{15}H_{16}N_4O$: C, 67.15; H, 6.01; N, 20.88. Found: C, 67.19; H, 5.95; N, 21.01.

4.1.6.10. (2'-Pyridinylidene) isonicotinoylhydrazine **14h**

The derivative **14h** was obtained as a white solid by condensation of **27** with 2-pyridinecarboxaldehyde (R_f acetone:toluene 30% = 0.11). ^{1}H-NMR (200 MHz): δ – (s, -CONH-), 8.81 (dd, 2H, H_2, *J*ax = 5.1 Hz, *J*bx = 1.4 Hz), 8.65 (d, H_6' *J* = 5.3 Hz), 8.39 (s, -N=CH-), 8.28 (dd, H_4', *J*ax = 7.8 Hz, *J*bx = 1.5Hz), 8.12 (d, H_3', *J* = 5.2 Hz), 8.07 (dd, 2H, H_3, *J*ax = 5.0 Hz, *J*bx = 1.6 Hz), 7.77 (dd, H_5', *J*ax = 6.1 Hz, *J*bx = 1.4 Hz) ppm; ^{13}C-NMR (50 MHz): δ 165.9 (C=O), 150.1 (C_2'), 149.2 (C_2), 148.2 (C_6'), 147.6 (C_4'), 146.2 (-N=CH-), 145.7 (C_4), 129.8 (C_5'), 128.0 (C_3'), 126.6 (C_3) ppm; MS (70 eV) m/z (relative abundance): 226 (6%), 122 (50%), 106 (100%), 78 (75%), 63 (14%); IR (KBr) cm^{-1}: 3 450 (ν N–H), 1 672 (ν C=O), 1 559 (ν C=N), 1 284 (ν C–O). Anal. calcd. for $C_{12}H_{10}N_4O$: C, 63.71; H, 4.46; N, 24.77. Found: C, 63.79; H, 4.54; N, 24.83.

4.1.6.11. (3'-Pyridinylidene) isonicotinoylhydrazine **14i**

The derivative **14i** was obtained as a white solid by condensation of **27** with 3-pyridinecarboxaldehyde (R_f acetone:toluene 30% = 0.13). ^{1}H-NMR (200 MHz): δ – (s, -CONH-), 8.87 (s, H_2'), 8.78 (dd, 2H, H_2, *J*ax = 4.5 Hz, *J*bx = 1.6 Hz), 8.62 (dd, H_6', *J*ax = 4.8 Hz, *J*bx = 1.7 Hz), 8.51 (s, -N=CH-), 8.16 (dd, H_4', *J*ax = 8.0 Hz, *J*bx = 1.9 Hz), 7.83 (dd, 2H, H_3, *J*ax = 4.5 Hz, *J*bx = 1.7 Hz), 7.49 (dd, H_5', *J*ax = 8.1 Hz, *J*bx = 4.8 Hz) ppm; ^{13}C-NMR (50 MHz): δ 162.6 (C=O), 150.7 (C_2'), 150.5 (C_2), 148.6 (C_6'), 146.3 (-N=CH-), 141.5 (C_3'), 133.6 (C_4'), 130.4 (C_4), 123.9 (C_3), 121.6 (C_5') ppm; MS (70 eV) m/z (relative abundance): 226 (4%), 122 (56%), 106 (100%), 78 (80%), 63 (16%); IR (KBr) cm^{-1}: 3 490 (ν N–H), 1 682 (ν C=O), 1 570 (ν C=N), 1 292 (ν C–O). Anal. calcd. for $C_{12}H_{10}N_4O$: C, 63.71; H, 4.46; N, 24.77. Found: C, 63.82; H, 4.40; N, 24.80.

4.1.6.12. (4'-Pyridinylidene) isonicotinoylhydrazine **14j**

The derivative **14j** was obtained as a white solid by condensation of **27** with 4-pyridinecarboxaldehyde (R_f acetone:toluene 30% = 0.17). ^{1}H-NMR (200 MHz): δ 12.31 (s, -CONH-), 8.78 (d, H_2, *J* = 5.9 Hz), 8.65 (d, 2H, H_2', *J* = 5.7 Hz), 8.44 (s, -N=CH-), 7.82 (d, 2H, H_3, *J* = 5.9 Hz), 7.67 (d, 2H, H_3', *J* = 5.7 Hz) ppm; ^{13}C-NMR (50 MHz): δ 162.0 (C=O), 150.4 (C_2), 150.3 (C_2'), 146.7 (-N=CH-), 141.2 (C_4), 140.1 (C_4'), 121.6 (C_3), 121.1 (C_3') ppm; MS (70 eV) m/z (relative abundance): 226 (5%), 122 (67%), 106 (100%), 78 (77%), 63 (13%); IR (KBr) cm^{-1}: 3 462 (ν N–H), 1 684 (ν C=O), 1 560 (ν C=N), 1 289 (ν C–O). Anal. calcd. for $C_{12}H_{10}N_4O$: C, 63.71; H, 4.46; N, 24.77. Found: C, 63.73; H, 4.38; N, 24.73.

4.2. Pharmacology

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 [14]. Albino mice of both sexes (18–23 g) were used. Compounds were administered orally (100 μmol/kg) as a suspension in 5% arabic gum in saline (vehicle). Indomethacin (100 μmol/kg) and dipyrone (100 μmol/kg) were used as standard drugs under the same conditions. Acetic acid solution was administered i.p. 1 h after administration of acylhydrazone compounds **9a–j**, **10a**, **c** and **f**, **11a** and **c–f**, **12f** and **h–j**, **13f** and **h–j**, and **14f** and **h–j**. Ten minutes following 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 percentage of inhibition of constrictions when compared with the vehicle control group (*tables IV* and *V*). Results are expressed as the mean ± SEM of *n* animals per group. The data were statistically analysed by the Student's *t*-test for a significance level of $P < 0.05$.

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