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Antiplatelet Properties of Novel *N*-Substituted-phenyl-1,2,3-triazole-4-acylhydrazone Derivatives

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Abstract—This paper describes the design, synthesis and pharmacological evaluation of new *N*-acylhydrazone (NAH) compounds, belonging to the *N*-substituted-phenyl-1,2,3-triazole-4-acylhydrazone class (**2a**–**p**). Classical heteroaromatic ring bioisosterism strategies were applied to the previously reported *N*-phenylpyrazolyl-4-acylhydrazone derivative **1**, elected as lead-compound due to its important anti-aggregating profile on arachidonic acid induced platelet aggregation ($IC_{50} = 24 \pm 0.5\,\mu M$), from which emerge this new series **2**. These new compounds **2a**–**p** were readily synthesized, characterized and tested on platelet aggregation assays induced by collagen (5 μg/mL), ADP (5 μM) and arachidonic acid (100 μM) in rabbit citrated platelet-rich plasma. Compounds **2b**, **2d**, and **2h** were found to be the most potent, exhibiting a significant antiplatelet activity on arachidonic acid- and collagen-induced platelet aggregation. In addition, these new antiplatelet agents are free of gastric ulcerogenic effect and presented discrete anti-inflammatory and analgesic properties. The *N*-*para*-chlorophenyltriazolyl-4-acylhydrazone compound **2h** produced the highest inhibitory effect on collagen ($IC_{50} = 21.6 \pm 0.4\,\mu M$) and arachidonic acid-induced platelet aggregation ($IC_{50} = 2.2 \pm 0.06\,\mu M$), suggesting that the nature of the substituent on the phenyl ring of the *N*-heteroaromatic system of NAH moiety may be an important structural requirement for the improvement of antiplatelet activity, in comparison with lead-series **1**.
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Introduction

Thromboxane A_2 (TXA_2) and its metabolic precursor, prostaglandin endoperoxide H_2 (PGH_2), are potent inducers of platelet aggregation and vasoconstriction and are also involved in several pathological processes including thromboembolic diseases.[1,2] Several classes of compounds such as nonsteroidal anti-inflammatory drugs (NSAIDS) have been used in the treatment of various circulatory disorders.[3,4] Acetylsalicylic acid (ASA), the most popular antiplatelet drug and COX-1 inhibitor, inhibits biosynthesis of PGH_2 and consequently reduces TXA_2 level in platelets, resulting in an effective way to prevent thrombotic events.[5] However, long-term treatment using ASA causes adverse gastrointestinal side effects and reduces prostaglandin I_2 levels, an important vasodilator and antiplatelet eicosanoid.[1,4,5] Other antiplatelet agents acting by different mechanisms of action have been developed and described, but the demand still remains for innovative antithrombotic drugs, which are safer than those currently adopted for clinical use.[6–8]

In our laboratory various *N*-acylheteroarylhydrazones (NAH) have been synthesized and were found to be very effective in antiplatelet activity on arachidonic acid induced platelet aggregation in rabbit citrated platelet-rich plasma.[9–12] The *N*-phenylpyrazolyl-4-acylhydrazone derivative (**1**) was the most active in this series ($IC_{50} = 24 \pm 0.5\,\mu M$) (Fig. 1).[12,13] In an effort to optimize

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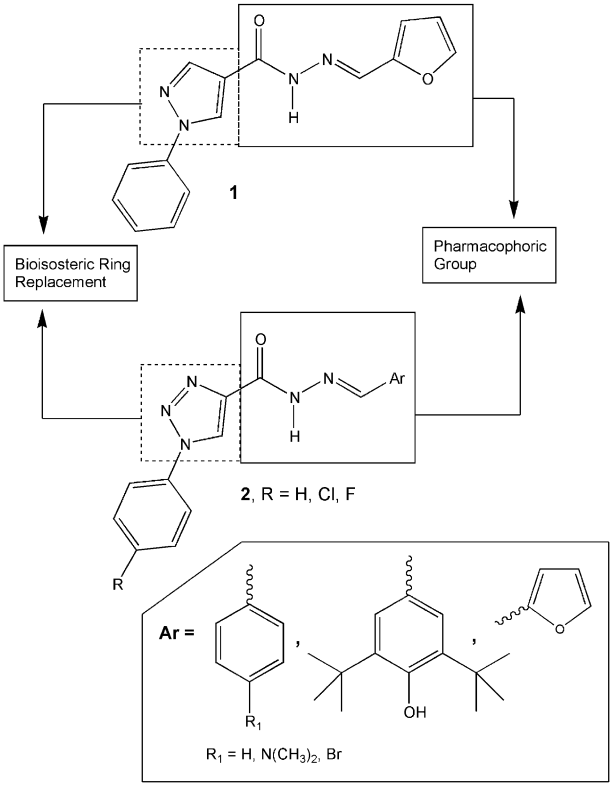


Figure 1. Design concept of *N*-substituted-phenyl-1,2,3-triazole-4-acylhydrazone derivatives.

the antiplatelet activity of structurally simple azaheterocyclic *N*-acylhydrazone derivatives, we describe in this paper a new class of *N*-substituted-phenyl-1,2,3-triazole-4-acylhydrazone derivatives **2a–p**, structurally designed by applying classical heteroaromatic ring bioisosterism strategy to the previously described antiplatelet class **1**.[13]

Considering that the *N*-phenylpyrazolyl moiety in series (**1**) which has the NAH chain with identical *ortho*-substituents, represented by C-3 and C-5 hydrogens of the pyrazolyl ring, we decided to investigate the eventual effect of '*desymmetrization*' around this pharmacophoric chain constructing the isosteric *N*-substituted-phenyl-1,2,3-triazole system of compounds **2a–p**.

This isosteric ring replacement maintained a similar lipophilic character of the *N*-heteroaromatic ring, represented by the presence of 6-π electrons, connected to an additional phenyl ring in both series (Fig. 1).

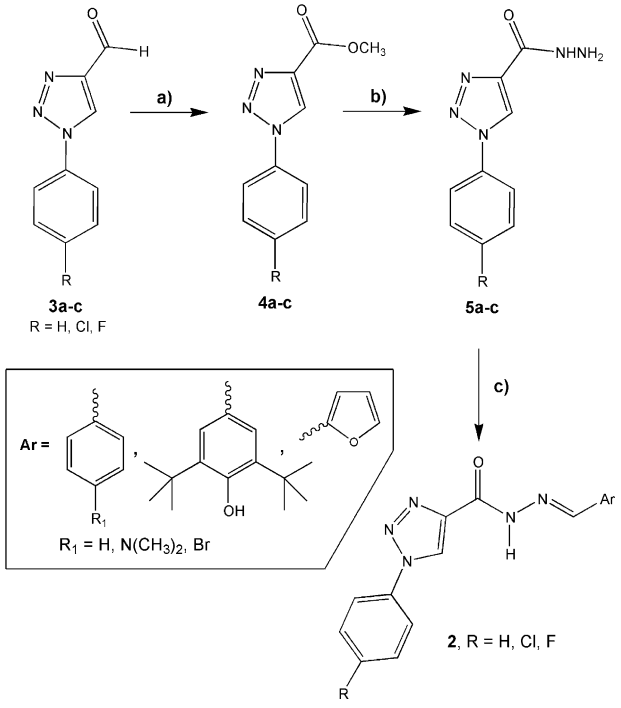
In addition, we also decided to investigate the influence of the aryl substituent of the imine function on antiplatelet activity by introducing different aromatic rings such as 2-furyl (**2e**, **2j**, and **2p**), *para*-phenyl substituted (**2a–c**, **2f–h** and **2l–n**) and 3,5-di*tert*-butyl-4-hydroxyphenyl rings (**2d**, **2f**, and **2e**) (Fig. 1). Furthermore, we also modified the electronic and lipophilic contributions of the *N*-phenyl ring by introducing two different halogen atoms as *para*-substituents (Fig. 1).

Chemistry

The synthesis of the new *N*-substituted-phenyl-1,2,3-triazole-4-acylhydrazone derivatives **2a–p** (Scheme 1) was initiated from the *N*-substituted triazole-4-carboxaldehydes **3a–c** prepared in good yields, employing the reaction of diazomalonaldehydes with appropriate amines in acetic acid, according to the procedure described by Arnold and coworkers.[14] Converting the aldehyde function of **3a–c** to the corresponding methyl ester **4a–c** by oxidation with manganese dioxide in the presence of sodium cyanide in methanol,[15] we were able to obtain the corresponding acylhydrazides intermediates **5a–c** by treatment with hydrazine hydrate in ethanol at reflux. Finally, the new *NAH* compounds **2a–p** were prepared in good yields by condensing compounds **5a–c** with the suitable aromatic aldehydes in ethanol using hydrochloric acid as catalyst (Scheme 1).

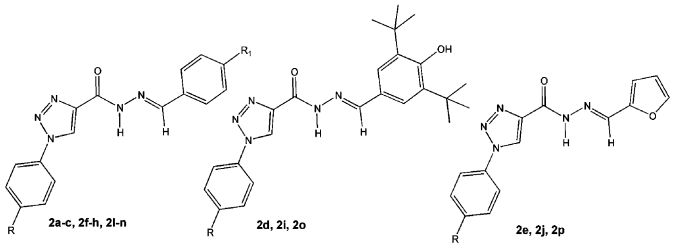
All new acylhydrazone derivatives (**2a–p**) were obtained as stable solid as illustrated in Table 1.

In order to assure the diastereomeric ratio of the *N*-acylarylhydrazone derivatives (**2a–p**), essential to the complete understanding of the biological results, the next step in this work was to determine the relative configuration of the imine double bond in this series. A detailed analysis of the ^{1}H NMR spectra of these new derivatives (**2a–p**) allowed us to detect only the presence of one hydrogen signal, which was attributed to the (*E*)-diastereomer. The assignment of (*E*)-configuration to the hydrazone double bond was performed by the chemical shift comparison with previous data obtained in our laboratory[9–11,16] and is in agreement with data from Karabatsos[22] and Fuchs.[23] In fact the –CH=N hydrazonic



Scheme 1. (a) NaCN, MnO_2, MeOH, rt, 5–6 h (71–85%); (b) $NH_2NH_2{\cdot}H_2O$ 80%, EtOH, reflux, 5–6 h (68–84%); (c) ArCHO, EtOH, HCl (cat.), reflux (32–98%).

Table 1. *N*-substituted-phenyl-1,2,3-triazole-4-acylhydrazone derivatives **2a–p**



Compd	R	R_1	Yield (%)	Mp (°C)	Molecular formula[a]	Molecular weight	R (cm^{-1})
2a	H	H	42	>250	$C_{16}H_{13}N_5O$	291.31	3400 (N–H), 1673(C=O)
2b	H	$N(CH_3)_2$	95	>250	$C_{18}H_{18}N_6O$	334.38	3428 (N–H), 1666 (C=O)
2c	H	Br	93	>250	$C_{16}H_{12}BrN_5O$	370.21	3400(N–H), 1680 (C=O)
2d	H	—	78	>250	$C_{24}H_{29}N_5O_2$	419.52	3424 (N–H), 1671 (C=O)
2e	H	—	58	211–212	$C_{14}H_{11}N_5O_2$	281.27	3400 (N–H), 1672 (C=O)
2f	Cl	H	88	>250	$C_{16}H_{12}ClN_5O$	325.76	3453 (N–H), 1682 (C=O)
2g	Cl	$N(CH_3)_2$	60	>250	$C_{18}H_{17}ClN_6O$	368.82	3446 (N–H), 1671 (C=O)
2h	Cl	Br	84	>250	$C_{16}H_{11}BrClN_5O$	404.65	3453 (N–H), 1682 (C=O)
2i	Cl	—	76	>250	$C_{24}H_{28}ClN_5O_2$	453.97	3445 (N–H), 1658 (C=O)
2j	Cl	—	76	>250	$C_{14}H_{10}ClN_5O_2$	315.72	3449 (N–H), 1667 (C=O)
2l	F	H	98	>250	$C_{16}H_{12}FN_5O$	309.30	3457 (N–H), 1672 (C=O)
2m	F	$N(CH_3)_2$	56	250–251	$C_{18}H_{17}FN_6O$	352.37	3446 (N–H), 1671 (C=O)
2n	F	Br	71	>250	$C_{16}H_{11}BrFN_5O$	388.20	3448 (N–H), 1677 (C=O)
2o	F	—	32	248–249	$C_{24}H_{28}FN_5O_2$	437.51	3448 (N–H), 1650 (C=O)
2p	F	—	92	>250	$C_{14}H_{10}FN_5O_2$	299.26	3445 (N–H), 1665 (C=O)

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

proton in the (*E*)-isomer occurs at 8.25–8.71 ppm and the iminic carbon atom appears at 145.2–150.7 ppm in the ^{13}C NMR spectra suggesting the (*E*)-isomer.[23,24]

Results and Discussion

These new derivatives **2a–p** were next screened in order to evaluate their effects on in vitro rabbit platelet aggregation induced by arachidonic acid (AA) (100 μM), collagen (5 μg/mL) or adenosine 5-diphosphate (ADP) (5 μM) (Table 2).[17] The initial screening at 100 μM concentration revealed the compounds **2b** (R = H and $R_1 = N(CH_3)_2$), **2d** (R = H) and **2h** (R = Cl and R_1 = Br) as those with the most important antiplatelet profiles on the arachidonic acid induced assay (89, 82 and 100%, respectively). It is interesting to observe that the introduction of a *para*-halogen substituent in the *N*-phenyl ring of **2b** (cf. **2g** and **2m**) and **2d** (cf. **2i** and **2o**) was deleterious to antiplatelet activity in the AA-induced assay. In contrast, the presence of the *para*-chloro instead of the *para*-fluoro substituent in the *N*-phenyl ring of the *para*-bromophenyl NAH derivatives [i.e., **2c** (13%), **2h** (100%) and **2n** (13%)] furnished

Table 2. Effect of *N*-substituted-phenyl-1,2,3-triazole-4-acylhydrazone derivatives **2a–p** (100 μM) and indomethacin (10 μM) on in vitro platelet aggregation of rabbit citrated platelet-rich plasma induced by arachidonic acid, collagen and ADP

Compd	R	R_1	Arachidonic acid (200 μM)			Collagen (5 μg/mL)			ADP (5 μM)		
			n	Aggregation (%)	Inhibition (%)	*n*	Aggregation (slope)	Inhibition (%)	*n*	Aggregation (%)	Inhibition (%)
Control	—	—	5	100±3	—	5	7.5±0.8	—	5	100±2	—
DMSO	—	—	5	94±3	6	5	6.9±1.0	8	5	97±2	3
Indomethacin	—	—	4	0±0	100*	4	0.3±0.1	96*	4	96±2	4
2a	H	H	3	92±5	7	3	7.3±1.0	0	3	76±7	24
2b	H	$N(CH_3)_2$	3	11±2	89*	4	4.9±0.8	59*	6	91±5	9
2c	H	Br	3	87±5	13	3	7.4±1.1	7	3	100±3	0
2d	H	—	4	18±6	82*	3	2.5±0.5	69*	3	86±5	14
2e	H	—	3	84±3	16	3	6.0±1.1	35*	3	72±8	28*
2f	Cl	H	4	80±5	20	3	7.5±0.6	0	3	100±2	0
2g	Cl	$N(CH_3)_2$	4	93±2	7	3	7.4±0.7	2	3	50±5	50*
2h	Cl	Br	4	0±0	100*	3	1.6±0.2	80*	6	96±8	2
2l	Cl	—	3	92±5	8	6	6.2±1.2	40*	3	88±9	12
2j	Cl	—	3	89±3	11	3	7.2±0.5	1	3	55±7	45*
2l	F	H	3	83±7	17	3	7.3±0.4	2	3	85±3	25
2m	F	$N(CH_3)_2$	3	91±5	9	3	7.5±0.5	0	3	65±3	35*
2n	F	Br	4	87±4	13	3	7.4±0.7	1	4	92±5	8
2o	F	—	3	94±6	6	3	5.8±1.5	23	3	100±1	0
2p	F	—	4	98±5	2	5	7.5±0.4	0	3	98±3	2

n, number of independent experiments in triplicate. Results are expressed as mean±SEM. *$p < 0.05$ (Student's *t*-test).

the most potent compound in this assay. The 2-furyl NAH derivatives **2e**, **2j** and **2p** presented a poor activity in the AA-induced assay (16, 11 and 14%, respectively), while **2j** was active at the screening concentration in the ADP-induced test (45%), contrasting with the original series represented by compound **1** (Table 2).

Compounds **2b**, **2d**, and **2h** also showed an important antiplatelet profile in collagen-induced platelet aggregation (59, 69 and 80%, respectively) (Table 2). These active compounds (**2b**, **2d**, and **2h**) were not able to inhibit neither the ADP-induced platelet aggregation (Table 2) nor the aggregatory activity of U-46619 (not shown). This last evidence indicated that the antiplatelet activity was not due to an effect at TXA_2 receptor level.

The antiplatelet potency of compounds **2b**, **2d**, and **2h** was confirmed by determination of IC_{50} values obtained from concentration–response curves (Table 3). The *N*-*para*-chlorophenyl isoster **2h** was the most potent antiplatelet compound in the AA- and collagen-induced tests ($IC_{50} = 2.2 \pm 0.06$ and $21.6 \pm 0.1\,\mu M$; respectively) (Table 3). From data illustrated in Table 3, we can conclude that compound **2h** was much more potent (27-fold) than the *N*-nor-*para*-chlorophenyl analogue **2b**, which presented an $IC_{50} = 96 \pm 8\,\mu M$ on the AA-induced assay. Compound **2h** was also more potent as an antiplatelet agent than the lead-derivative **1** ($IC_{50} = 24 \pm 0.5\,\mu M$), representing an optimization of this initial series. Interestingly, the NAH derivative **2d** presented an IC_{50} value ($21 \pm 2\,\mu M$) similar to compound **1** IC_{50} value (Table 3).

The pharmacological investigation of the anti-inflammatory properties of compounds **2b**, **2d**, and **2h** were next assayed in the carrageenan induced rat paw edema ($100\,\mu mol/kg$, po), using indomethacin as standard. In addition, the acetic acid-induced mice abdominal constrictions test was used to investigate the analgesic profile of these NAH derivatives. The results obtained indicated that only compound **2h** exhibited a discrete antiinflammatory/analgesic activity, inhibiting 30.5% of the edema formation and 23.1% of the induced abdominal constrictions. Moreover, a weak analgesic effect was also observed for compound **2b** (18.9%). Finally, the gastric mucosa was investigated, indicating an absence of any ulcerative behavior for these three NAH derivatives, based on the lack of any kind of lesions on the animals stomachs (Table 4).[18,19]

The constitutive isoform of COX-1 present in the platelets is blocked by acetylsalicylic acid (ASA) and indomethacin, preventing the formation of TXA_2, thereby inhibiting arachidonic acid and collagen but not ADP-induced rabbit platelet aggregation.[19–21] Compounds **2b**, **2d**, and **2h** exhibited a similar ASA profile, suggesting that they also modulate TXA_2 formation.[19] For this reason, we decided to investigate the effect of the most potent antiplatelet derivative **2h** on the TXA_2 production. The observed results for thromboxane production by platelets in response to collagen induction measuring the bioformation of TXB_2, a stable metabolite of TXA_2, in the presence or absence of compound **2h**, confirmed that the production of TXA_2 is effectively suppressed by this compound (Fig. 2).

The analysis of the structure–activity relationships suggests that the triazole ring in the new series of *N*-substituted-phenyl-1,2,3-triazole-4-acylhydrazone (NAH) class (**2a–p**) is not directly involved in the establishment of the antiplatelet activity showed by compound **2h**. This hypothesis is brought about due to the results of compound **2e** on platelet aggregation assay. Derivative **2e**, which has only the isosteric replacement of the pyrazole system from the lead-derivative **1** by the triazole ring, did not present any significant activity on arachidonic induced platelet aggregation at $100\,\mu M$, when compared to the lead-derivative **1** ($16 \times 100\%$, respectively). Therefore, it seems reasonable to conclude that, within this series, the nature of the substituents introduced in *N*-phenyl ring and arylhydrazone moiety could be crucial to determine the potential of the biological activity. Hence, the most significant antiplatelet profile of the isoster compound **2h** ($R = Cl$ and $R_1 = Br$) could be correlated with the lipophilic influence and inductive electron attracting properties of halogenated substituents on the biological activity.

Table 3. IC_{50} (μM) of *N*-substituted-phenyl-1,2,3-triazole-4-acylhydrazone derivatives **2b**, **2d**, and **2h** (0.1–100 μM) on arachidonic acid (AA) and collagen-induced platelet aggregation

Compd	R	R_1	IC_{50} (μM)	
			AA	Collagen
2b	H	$N(CH_3)_2$	96 ± 10	32 ± 3
2d	H	—	21 ± 2	73 ± 8
2h	Cl	Br	2.2 ± 0.06	21.6 ± 0.1
Indomethacin	—	—	0.6 ± 0.02	3.1 ± 0.9

IC_{50} values are determined by nonlinear regression (% of inhibition × log concentration) using the Microcal Origin program (Version 4.0).

Table 4. Effects of *N*-substituted-phenyl-1,2,3-triazole-4-acylhydrazone derivatives **2b**, **2d**, and **2h** ($100\,\mu mol/kg$) and indomethacin ($100\,\mu mol/kg$) on carrageenan-induced rat paw edema assay and abdominal constrictions induced by acetic acid (0.6%, ip) in mice

Compd	R	R_1	Carrageenan-induced rat paw edema			Abdominal constrictions induced by acetic acid			Ulcerogenic effects
			n	Volume variation (μL)	Inhibition (%)	*n*	Constrictions count	Inhibition (%)	
Vehicle control	—	—	10	491.4 ± 38.6	—	10	79.0 ± 4.9	—	
2b	H	$N(CH_3)_2$	10	579.0 ± 18.5	n.s.	14	60.7 ± 4.2	19.0	—
2d	H	—	7	482.3 ± 29.5	n.s.	13	69.8 ± 4.7	11.7	—
2h	Cl	Br	10	341.3 ± 43.5	30.5*	12	64.0 ± 4.1	23.1	—
Indomethacin	—	—	9	228.1 ± 31.9	53.5*	10	38.7 ± 4.9	51.0*	+ + + + +

Compounds are administered po. Results are expressed as mean ± SEM. *n*, number of animals; $*p < 0.05$ (Student's *t*-test); n.s., non significant. Percentage of inhibition obtained by comparison with vehicle control group.

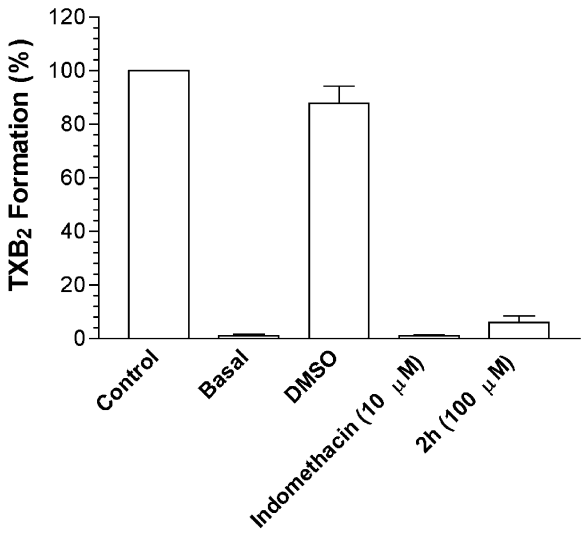


Figure 2. Effects of NAH derivative **2h** (100 μM) and indomethacin (10 μM) on platelets TXB_2 formation induced by collagen. Results are expressed as mean ± SEM for $n = 3$ independent experiments. Basal = unstimulated platelets. Control = platelets stimulated by collagen 5 mg/mL. Control was employed as 100% of TXB_2 formation. $^*p < 0.05$ (Student's *t*-test).

Conclusions

In summary, from the present study it is evident that the most potent antiplatelet compound is **2h**, which presents a similar profile to ASA without the undesired gastric ulceration. Therefore, this series of NAH derivatives represents a novel family of antiplatelet agents.

Experimental

Chemistry

Melting points were determined with a Quimis 340 apparatus and are uncorrected. ^{1}H NMR spectra, unless otherwise stated, were obtained 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 spectra were obtained using the same spectrometer described above at 50 MHz, using deuterated dimethylsulfoxide as internal standard. IR spectra were obtained using a Bruker IFS66 spectrophotometer employing potassium bromide plates. Microanalysis data were obtained using a Perkin–Elmer 240 analyzer, using a Perkin–Elmer AD-4 balance. The progress of all reactions was monitored by TLC performed on 2.0 × 6.0 cm 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. E. Merck silica gel (60–200 mesh) was used for column chromatography. The diazomalonaldehyde used in synthesis was prepared by a known procedure described in the literature.[14]

General procedure for the preparation of 4-formyltriazoles 3b–c

A solution of diazomalonaldehyde (0.482 g) in methanol (1.0 mL) and acetic acid (0.7 mL) was added dropwise to a stirring solution of *p*-chloro or *p*-fluoro aniline (368 mmol) in methanol (9 mL). The stirring was continued for 1 h at room temperature and a colored solid was formed which was collected by filtration and washed with cold water and dried under vacuum. The triazole was suspended in acetyl acetate (20 mL), and heated in a boiling water-bath and then an aqueous solution of HCl (0.1 N, 10 mL) was added. After cooling the solution, the organic phase was separated and washed with an aqueous solution of sodium bicarbonate (15 mL). After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure to furnish crude triazole, which were purified by short column flash chromatography using 80% *n*-hexane–ethyl acetate as eluent. The following triazoles **3b–c** were thus prepared.

(*p*-Chlorophenyl)-4-formyl-1,2,3-triazole (3b). Derivative **3b** was obtained as a yellow solid in 76% yield mp 155–156 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 10.2 (s, 1H, C**H**O), 7.79 (d, 2H, H2′ and H6′, $J = 8.4$ Hz), 7.64 (d, 2H, H3′ and H5′, $J = 8.4$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 186.0 (C=O), 146.0 (C4), 137.6 (C4′), 133.9 (C1′), 130.7 (C3′ and C5′), 126.2 (C5), 122.5 (C2′ and C6′). IR (KBr) cm^{-1} 1705 (ν C=O).

1-(*p*-Fluorophenyl)-4-formyl-1,2,3-triazole (3c). Derivative **3c** was obtained as a yellow solid in 78% yield, mp 150–152 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 10.2 (s, 1H, C**H**O), 7.78 (dd, 2H, H2′ and H6′, $J = 4.5$ and 9.0 Hz), 7.32 (dd, 2H, H3′ and H5′, $J = 7.8$ and 9.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 185.2 (C=O), 146.4 (C4), 165.3 (d, C4′, $J = 255$ Hz), 131.7 (C1′), 125.7 (C5), 123.3 (d, C2′ and C6′, $J = 15$ Hz), 117.3 (d, C3′ and C5′, $J = 30$ Hz). IR (KBr) cm^{-1} 1699 (ν C=O).

General procedure for the preparation of methyl *N*-substituted-phenyl-4-carbethoxy-1,2,3-triazoles (4a–c)

To a mixture of 1.00 mmol of 4-formyltriazoles **3a–c** in methanol (30 mL) was added 0.08 g (1.67 mmol) of sodium cyanide and 0.44 g of activated manganese dioxide (7.91 mmol). The reaction was stirred at room temperature for 6 h and the suspension was filtered through Celite® and treated with dichloromethane (3 × 20 mL). The organic layers were joined, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to furnish crude methyl esters **4a–c**, which were purified by column flash chromatography using 80% *n*-hexane–ethyl acetate as eluent.

1-Phenyl-4-carbethoxy-1,2,3-triazole (4a). Derivative **4a** was obtained as yellow solid in 71% yield, mp 115 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.54 (s, 1H, H5), 8.03–7.97 (m, 2H, H2′ and H6′), 7.68–7.51 (m, 3H, H3′-H5′), 3.92 (s, 3H, OC**H**$_3$); ^{13}C NMR (50 MHz, DMSO-d_6) δ 160.5 (C=O), 136.0 (C1′), 139.5 (C4), 129.4 (C3′ and C5′), 129.3 (C4′), 127.2 (C5), 120.5 (C2′ and C6′), 51.9 (O**C**H$_3$). IR (KBr) cm^{-1} 1734 (ν C=O).

1-(*p*-Chlorophenyl)-4-carbethoxy-1,2,3-triazole (4b). Derivative **4b** was obtained as yellow solid in 85% yield, mp 174–178 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.54 (s, 1H, H5), 8.0 (d, 2H, H2′ and H6′, J = 8.8 Hz), 7.67 (d, 2H, H3′ and H5′, J = 8.8 Hz), 3.88 (s, 3H, OCH_3); ^{13}C NMR (50 MHz, DMSO-d_6) δ 160.7 (C=O), 140.6 (C4), 139.6 (C4), 135.3 (C1′), 134.6 (C4′), 130.0 (C3′ and C5′), 125.3 (C5), 121.8 (C2′ and C6′), 52.3 ($O\mathbf{C}H_3$). IR (KBr) cm^{-1} 1707 (ν C=O).

1-(*p*-Fluorophenyl)-4-carbethoxy-1,2,3-triazole (4c). Derivative **4c** was obtained as yellow solid in 79% yield, mp 186 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.50 (s, 1H, H5), 8.02 (dd, 2H, H2′ and H6′, J = 8.8 and 4.8 Hz), 7.46 (dd, 2H, H3′ and H5′, J = 8.8 and 8.7 Hz), 3.90 (s, 3H, $OC\mathbf{H}_3$); ^{13}C NMR (50 MHz, DMSO-d_6) δ 162.5 (d, C4′, J = 245.5 Hz), 160.9 (C=O), 140.0 (C4), 133.1 (C1′), 127.9 (C5), 123.4 (d, C2′ and C6′, J = 8.9 Hz), 117.2 (d, C3′ and C5′, J = 23.2 Hz), 52.5 ($O\mathbf{C}H_3$). IR (KBr) cm^{-1} 1707 (ν C=O).

General procedure for preparation of the *N*-substituted-phenyl-4-carbohydrazide-1,2,3-triazole 5a–c

A solution of the appropriate methyl ester derivatives **4a–c** (3.70 mmol) and 3.7 mL of 80% hydrazine monohydrate in 10 mL of ethanol, was stirred at reflux for 5–6 h. The reaction mixture was then concentrated under reduced pressure and the colored solid which formed was collected by filtration, washed with cold water and dried under vacuum to give the desired hydrazides **5a–c**.

1-Phenyl-1*H*-1,2,3-triazole-4-carbohydrazide (5a). Derivative **5a** was obtained as a yellow solid in 77% yield by reaction of methyl ester **4a** with hydrazine monohydrate, mp 186 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.90 (br, 1H, N–H), 9.28 (s, 1H, H5), 7.99–7.95 (m, 2H, H2′ and H6′), 7.67–7.49 (m, 2H, H3′ and H5′) 4.58 (br, 2H, NH_2); ^{13}C NMR (50 MHz, DMSO-d_6) δ 158.9 (C=O), 142.7 (C4), 136.1 (C1′), 129.9 (C3′ and C5′), 129.1 (C4′), 124.2 (C5),120.4 (C2′ and C6′). IR (KBr) cm^{-1} 3318 and 3151 (ν N–H), 1671 (ν C=O).

1-(4-Chlorophenyl)-1*H*-1,2,3-triazole-4-carbohydrazide (5b). Derivative **5b** was obtained in 68% yield by reaction of methyl ester **4b** with hydrazine monohydrate, as a yellow solid, mp 230–233 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.72 (1H, s, N–H), 9.22 (s, 1H, H5), 7.99 (d, 2H, H2′ and H2′, J = 8.9 Hz), 7.64 (d, 2H, H3′ and H5′, J = 8.9 Hz) 4.52 (br, 2H, $N\mathbf{H}_2$); ^{13}C NMR (50 MHz, DMSO-d_6) δ 158.9 (C=O), 142.9 (C4), 135.0 (C1′), 133.3 (C4′), 129.7 (C3′ and C5′), 124.3 (C5), 122.0 (C2′ and C6′). IR (KBr) cm^{-1} 3285 and 3087 (ν N–H), 1661 (ν C=O).

1-(4-Fluorophenyl)-1*H*-1,2,3-triazole-4-carbohydrazide (5c). Derivative **5c** was obtained in 84% yield by reaction of methyl ester **4c** with hydrazine monohydrate, as a yellow solid, mp 236–240 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.74 (br, 1H, N–**H**), 9.22 (s, 1H, H5), 8.20–7.93 (m, 2H, H2′ and H6′), 7.44 (dd, 2H, H3′ and H5′, J = 8.9 and 8.9 Hz), 4.52 (br, 2H, $N\mathbf{H}_2$); ^{13}C NMR (50 MHz, DMSO-d_6) δ 161.7 (d, C4′, J = 244.8 Hz), 158.7 (C=O), 142.6 (C4), 132.7 (C1′), 124.3 (C5), 122.7 (d, C2′ and C6′, J = 8.7 Hz), 116.5 (d, C3′ and C5′, J = 23.3 Hz). IR (KBr) cm^{-1} 3327 and 3117 (ν N–H), 1661 (ν C=O).

General procedure for the preparation of the *N*-substituted-phenyl-1,2,3-triazole-4-acylhydrazone derivatives 2a–p

An equimolar amount of appropriates aromatic aldehydes was added to a solution of hydrazide derivatives **5a–c** (0.50 mmol) in 15 mL of ethanol, in the presence of a catalytic amount of hydrochloric acid. The reaction was stirred for 4–6 h, at reflux and the solvent was evaporated and the colored precipitate was collected by filtration, washed with cold water, ethyl ether and dried under vacuum to give the desired *N*-acylhydrazone derivatives **2a–p**.

Benzylidene-1*H*-1-(phenyl)-1,2,3-triazole-4-carbohydrazide (2a). Derivative **2a** was obtained as a white solid by condensation of **5a** with benzaldehyde. ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.22 (s, 1H, N–**H**), 9.47 (s, 1H, H5), 8.59 (s, 1H, N=C**H**), 8.0 (d, 2H, H2′ and H6′, J = 7.7 Hz), 7.45–7.74 (m, 6H, H3′, H4′, H5′, H2″, H4″ and H6″); ^{13}C NMR (50 MHz, DMSO-d_6) δ 156.6 (C=O), 149.3 (N=**C**H), 143.4 (C4), 136.9 (C1′), 130.9 (C3″ and C5″), 130.6 (C4″), 129.9 (C3′ and C5′), 129.5 C4′), 126.5 (C5), 127.9 (C2″ and C6″), 121.2 (C2′ and C6′).

(4′-*N*,*N*-Dimethylaminobenzylidene)-1*H*-1-(phenyl)-1,2,3-triazole-4-carbohydrazide (2b). Derivative **2b** was obtained as a yellow solid by condensation of **5a** with 4-*N*,*N*-dimethylaminobenzaldehyde. ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.89 (br, 1H, N–**H**), 9.43 (s, 1H, H5), 8.43 (s, 1H, N=C**H**), 7.67–7.53 (m, 3H, H3′, H4′ and H5′), 8.03–7.98 (m, 2H, H2′ and H6′), 7.54 (d, 2H, H2″ and H6″, J = 8.9 Hz), 6.77 (d, 2H, H3″ and H5″, J = 8.9 Hz), 2.99 (s, 6H, $N(C\mathbf{H}_3)_2$); ^{13}C NMR (50 MHz, DMSO-d_6) δ 155.3 (C=O), 151.5 (C4″), 149.3 (N=**C**H), 142.9 (C4), 136.2 (C1′), 129.7 (C3′ and C5′), 129.0 (C4′), 128.4 (C2″ and C6″), 125.1 (C5), 121.4 (C1″), 120.4 (C2′ and C6′), 111.6 (C3″ and C5″), 40.0 ($N(\mathbf{C}H_3)_2$).

(4′-Bromobenzylidene)-1*H*-1-(phenyl)-1,2,3-triazole-4-carbohydrazide (2c). Derivative **2c** was obtained as a yellow solid by condensation of **5a** with 4-bromobenzaldehyde. ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.3 (s, 1H, N–**H**), 9.49 (s, 1H, H5), 8.56 (s, 1H, N=C**H**), 8.00 (d, 2H, H2′ and H6′, J = 7.6 Hz), 7.69 (d, 2H, H2″ and H6″, J = 7.9 Hz) 7.65 (d, 2H, H3′ and H5′, J = 7.9 Hz); ^{13}C NMR (50 MHz, DMSO-d_6) δ 156.6 (C=O), 147.9 (N=**C**H), 143.3 (C4), 136.8 (C1′), 134.1 (C1″), 132.3 (C3″ and C5″), 129.9 (C3′ and C5′), 129.6 (C2″ and C6″), 129.0 (C4′), 126.4 (C5), 124.0 (C4″), 121.1 (C2′ and C6′).

(3′-5′-Di-*tert*-butyl-4′-hydroxybenzylidene)-1*H*-1-(phenyl)-1,2,3-triazole-4-carbohydrazide (2d). Derivative **2d** was obtained as a yellow solid by condensation of **5a** with 3′-5′-di-*tert*-butyl-4′-hidroxybenzylideno. ^{1}H NMR

(200 MHz, DMSO-d_6) δ 11.94 (s, 1H, N–**H**), 9.44 (s, 1H,**H**5), 8.49 (s, 1H, N=C**H**), 8.00 (d, 2H, H2′ and H6′, J = 7.9 Hz), 7.66–7.44 (m, 2H, H3′, H4′ and H5′), 7.47 (s, 2H, H2″ and H6″), 5.56 (s, 1H, O**H**), 1.41 (s,18H, 6C**H**$_3$) ppm; ^{13}C NMR (50 MHz, DMSO-d_6) δ 156.8 (C=O), 156.1 (C4″), 150.6 (N=**C**H), 143.2 (C4), 139.7 (C3″ and C5″), 134.1 (C1′), 133.3 (C1″), 130.4 (C3′ and C5′), 129.2 (C4′), 126.2 (C5), 124.5 (C2″ and C6″), 121.0 (C2′ and C6′), 35.0 (**C**(CH$_3$)$_3$), 30.7 (6 **C**H$_3$).

(2′-Furylidene)-1*H*-1-(phenyl)-1,2,3-triazole-4-carbohydrazide (2e). Derivative **2e** was obtained as a white solid by condensation of **5a** with furfural. ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.26 (s,1H, N–**H**), 9.47 (s,1H H5), 8.50 (s, 1H, N=C**H**), 7.99 (d, 2H, H2′ and H6′, J = 7.6 Hz), 7.85 (d, 1H, H4″, J = 1.8 Hz), 7.66–7.49 (m, 3H, H3′, H4′ and H5′), 6.90 (d, 1H, H2″, J = 3.4 Hz), 6.64 (dd, 1H, H3″, J = 3.4 and 1.8 Hz); ^{13}C NMR (50 MHz, DMSO-d_6) δ 155.8 (C=O), 145.2 (N=**C**H), 149.9 (C1″), 145.2 (N=**C**H), 142.6 (C4), 138.2 (C4=), 136.2 (C1′), 129.9 (C3′ and C5′), 129.2 (C4′), 125.8 (C5), 120.5 (C2′ and C6′), 113.5 (C2″), 112.2 (C3″).

(Benzylidene)-1*H*-1-(*p*-chlorophenyl)-1,2,3-triazole-4-carbohydrazide (2f). Derivative **2f** was obtained as a white solid by condensation of **5b** with benzaldehyde. ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.24 (s, 1H, N–**H**), 9.50 (s, 1H,**H**5), 8.59 (s, 1H, N=C**H**), 8.05 (d, 2H, H2′ and H6′, J = 8.8 Hz), 7.72 (d, 2H, H3′ and H5′, J = 8.8 Hz), 7.73–7.69 (m, 2H, H2″ and H6″), 7.48–7.46 (m, 3H, H3″, H4″ and H5″); ^{13}C NMR (50 MHz, DMSO-d_6) δ 156.3 (C=O), 149.3 (N=**C**H), 143.3 (C4), 135.5 (C4′), 134.7 or 134.1 (C1′ or C1″), 130.7 (C3″ and C5″), 130.4 (C4″), 129.3 (C3′ and C5′), 127.9 (C2″ and C6″), 126.5 (C5), 122.7 (C2′ and C6′).

(4′-*N*,*N*-Dimethylaminobenzylidene)-1*H*-1-(*p*-chlorophenyl)-1,2,3-triazole-4-carbohydrazide (2g). Derivative **2g** was obtained as a white solid by condensation of **5b** with 4-*N*,*N*-dimethylaminobenzaldehyde. ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.06 (s, 1H, N–**H**), 9.60 (s, 1H, **H**5), 8.54 (s, 1H, N=C**H**), 8.17 (d, 2H, H2′ and H6′, J = 5.8 Hz), 7.83 (d, 2H, H3′ and H5′, J = 8.7 Hz, m), 7.66 (d, 2H, H2″ and H6″, J = 8.7 Hz), 6.89 (d, 2H, H3″ and H5″, J = 8.7 Hz), 3.10 (s, 6H, N(C**H**$_3$)$_2$); ^{13}C NMR (50 MHz, DMSO-d_6) δ 155.9 (C=O), 152.2 (C4″), 150.0 (N=**C**H), 143.6 (C4), 135.4 (C4′), 134.2 (C1′), 130.5 (C3′ and C5′), 129.2 (C2″ and C6″), 126.2 (C5), 121.9 (C1″), 122.8 (C2′ and C6′), 112.3 (C3″ and C5″), 40.4 (N(**C**H$_3$)$_2$).

(4-Bromobenzylidene)-1*H*-1-(*p*-chlorophenyl)-1,2,3-triazole-4-carbohydrazide (2h). Derivative **2h** was obtained as a white solid by condensation of **5b** with 4-bromobenzaldehyde. ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.34 (s, 1H, N–**H**), 9.52 (s, 1H, H5), 8.56 (s, 1H, N=C**H**), 8.05 (d, 2H, H2′ and H6′, J = 8.8 Hz), 7.71 (d, 2H, H3′ and H5′, J = 8.8 Hz), 7.68 (d, H2″ and H6″, J = 7.9 Hz), 7.65 (d, H3″ and H5″, J = 7.9 Hz); ^{13}C NMR (50 MHz, DMSO-d_6) δ 156.5 (C=O), 147.9 (N=**C**H), 143.0 (C4), 135.6 (C4′), 134.2 or 134.1 (C1′ or C1″), 132.5 (C3″ and C5″), 130.5 (C3′ and C5′), 126.1 (C5), 124.0 (C4″), 122.9 (C2′ and C6′).

(3′-5′-Di-*tert*-butyl-4′-hydroxybenzylidene)-1*H*-1-(*p*-chlorophenyl)-1,2,3-triazole-4-carbohydrazide (2i). Derivative **2i** was obtained as a white solid by condensation of **5b** with 3′-5′-di-*tert*-butyl-4′-hydroxybenzaldehyde. ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.94 (s, 1H, N–**H**), 9.44 (s, 1H, H5), 8.48 (s, 1H, N=C**H**), 8.04 (d, 2H, H2′ and H6′, J = 8.8 Hz), 7.69 (d, 2H, H3′ and H5′, J = 8.8 Hz), 7.47 (s, 2H, H2″ and H6″), 5.56 (s, 1H, O**H**), 1.48 (s, 18H, 6C**H**$_3$); ^{13}C NMR (50 MHz, DMSO-d_6) 156.8 (C=O), 156.1 (C4″), 150.7 (N=**C**H), 143.5 (C4), 139.7 (C3″ and C5″), 134.1 (C1′), 133.3 (C1″), 130.4 (C3′ and C5′), 126.2 (C5), 124.5 (C2″ and C6″), 122.7 (C2′ and C6′), 35.0 (**C**(CH$_3$)$_3$), 30.7 (6 **C**H$_3$).

(2′-Furylidene)-1*H*-1-(*p*-chlorophenyl)-1,2,3-triazole-4-carbohydrazide (2j). Derivative **2j** was obtained as a brown solid by condensation of **5b** with furfural. ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.25 (s, 1H, N–**H**), 9.51 (s, 1H, H5), 8.47 (s, 1H, N=C**H**), 8.05 (d, 2H, H2′ and H6′, J = 8.9 Hz), 7.87 (d, 1H, H4″, J = 1.7 Hz), 7.71 (d, 2H, H3′ and H5′, J = 8.9 Hz), 6.95 (d, 1H, H2″, J = 3.3 Hz), 6.65 (1H, dd, H3″, J = 3.3 and 1.7 Hz); ^{13}C NMR (50 MHz, DMSO-d_6) δ 156.3 (C=O), 149.9 (C1″), 145.7 (N=**C**H), 143.3 (C4), 138.8 (C4″), 134.1 (C1′), 130.4 (C3′ and C5′), 126.5 (C5), 122.7 (C2′ and C6′), 114.1 (C2″), 112.7 (C3″).

(Benzylidene)-1*H*-1-(*p*-fluorophenyl)-1,2,3-triazole-4-carbohydrazide (2l). Derivative **2l** was obtained as a white solid by condensation of **5c** with benzaldehyde. ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.4 (s,1H, N–**H**), 9.6 (s, 1H, **H**5), 8.71 (s, 1H, N=C**H**), 8.18 (dd, 2H, H2′ and H6′, J = 8.9 and 4.5 Hz), 7.87–7.84 (m, 2H, H3′ and H5′), 7.65–7.58 (m, 5H, H2″-H6″); ^{13}C NMR (50 MHz, DMSO-d_6) δ 162.5 (d, J = 246 Hz, C4′), 156.5 (C=O), 149.2 (N=**C**H), 143.2 (C4), 134.6 (C1″), 133.2 (C1′), 130.7 (C3″ and C5″), 129.4 (C4″), 127.7 (C2″ and C6″), 126.6 (C5), 123.5 (d, C2′ and C6′, J = 8.9 Hz), 117.3 (d, C3′ and C5′, J = 23.2 Hz).

(4′-*N*,*N*-Dimethylaminebenzylidene)-1*H*-1-(*p*-fluorophenyl)-1,2,3-triazole-4-carbohydrazide (2m). Derivative **2m** was obtained as a white solid by condensation of **5c** with 4-*N*,*N*-dimethylaminobenzaldehyde. ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.89 (s, 1H, N–**H**), 9.40 (s, 1H, H5), 8.43 (s, 1H, N=C**H**), 8.06 (d, 2H, H2′ and H6′, J = 8.4 and 4.5 Hz), 7.51 (d, 2H, H3′ and H5′, J = 8.7 and 8.1 Hz), 7.47 (d, 2H, H2″ and H6″, J = 8.5 Hz), 6.77 (d, 2H, H3″ and H5″, J = 8.5 Hz), 2.98 (s, 6H, N(C**H**$_3$)$_2$); ^{13}C NMR (50 MHz, DMSO-d_6) δ 162,6 (d, C4′, J = 245 Hz), 155,4 (C=O), 151,5 (C4″), 149,3 (N=**C**H), 142,9 (C4), 132.8 (C1′), 128.5 (C2″ and C6″), 125.6 (C5), 122.9 (d, C2′ and C6′, J = 8.9 Hz), 121.4 (C1″), 116.7 (d, C3′ and C5′, J = 23.3 Hz), 111.7 (C3″ and C5″), 40.4 (N(**C**H$_3$)$_2$).

(4-Bromobenzylidene)-1*H*-1-(*p*-fluorophenyl)-1,2,3-triazole-4-carbohydrazide (2n). Derivative **2n** was obtained as a white solid by condensation of **5c** with 4-bromobenzaldehyde. ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.01 (s, 1H, N–**H**), 9.32 (s, 1H, **H**5), 8.55 (s, 1H, N=C**H**), 8.06–7.99 (m, 2H, H2′ and H6′), 7.46 (dd, 2H, H3′ and H5′, J = 8.8 and 8.8 Hz), 7.69 (d, 2H, H2″ and H6″, J = 7.9 Hz), 7.69 (d, 2H, H3″ and H5″, J = 7.9 Hz); ^{13}C

NMR (50 MHz, DMSO-d_6) δ 156.8 (C=O), 143.1 (C4), 162.6 (d, C4′, J = 245 Hz), 133.4 or 133.3 (C1′ or C1″), 132.3 (C3″ and C5″), 129.5 (C2″ and C6″), 126.6 (C5), 123.9 (C4″), 123.7 (d, C2′ and C6′, J = 8.9 Hz), 117.2 (d, C3′ and C5′, J = 23.2 Hz).

(3′-5′-Di-*tert*-butyl-4′-hidroxybenzylidene)-1*H*-1-(*p*-fluorophenyl)-1,2,3-triazole-4-carbohydrazide (2o). Derivative **2o** was obtained as a brown solid by condensation of **5c** with 3′-5′-di-*tert*-butyl-4′-hidroxybenzaldehyde. ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.2 (s, 1H, N–**H**), 9.42 (1H, s, H5), 8,49 (s, 1H, N=C**H**), 8.03 (dd, 2H, H2′ and H6′, J = 6.9 and 4.6 Hz), 7.53–7.36 (m, 2H, H3′ and H5′), 7.47 (s, 2H, H2″ and H6″), 1.48 (s, 18H, 6C**H**$_3$), 5.60 (s, 1H, s, O**H**); ^{13}C NMR (50 MHz, DMSO-d_6) 156.8 (C=O), 156.1 (C4″), 150.6 (N=**C**H), 143.4 (C4), 139.7 (C3″ and C5″), 133.3 or 133.2 (C1′), 133.3 or 133.2 (C1″), 124.5 (C2″ and C6″), 126.3 (C5), 123.5 (d, C2′ and C6′, J = 8.9 Hz), 117.3 (d, C3′ and C5′, J = 23.1 Hz), 35.0 (**C**(CH$_3$)$_3$), 30.7 (6 **C**H$_3$).

(2′-Furylidene)-1*H*-1-(*p*-fluorophenyl)-1,2,3-triazole-4-carbohydrazide (2p). Derivative **2p** was obtained as a brown solid by condensation of **5c** with furfural. ^{1}H NMR (200 MHz, DMSO-d_6) δ 12,4 (s, 1H, N–**H**), 9.60 (s, 1H, H5), 8.60 (s, 1H, N=C**H**), 8.17 (d, 2H, H2′ and H6′, J = 9.0 and 4.5 Hz), 7.99 (d, 1H, H4″, J = 1.8 Hz), 7.61 (d, 2H, H3′ and H5′, J = 9.9 and 9.0 Hz), 7.06 (d, 1H, H2″, J = 3.3 Hz), 6.77 (dd, 1H, H3″, J = 3.3 and 1.8 Hz); ^{13}C NMR (50 MHz, DMSO-d_6) δ 162.4 (d, C4′, J = 246 Hz), 156.3 (C=O), 149.9 (C1″), 145.8 (N=**C**H), 143.2 (C4), 138.8 (C4″), 133.2 (C1′), 126.5 (C5), 123.5 (d, C2′ and C6′, J = 8.9 Hz), 117.3 (d, C3′ and C5′, J = 23.2 Hz), 114.1 (C2″), 112.8 (C3″).

Pharmacology

Antiplatelet activity. Blood was withdrawn from rabbit ear central artery and mixed with 3.8% trisodium citrate (9:1 v/v). Platelet-rich plasma (PRP) was prepared by centrifugation at 500g × 10 min at room temperature. The platelet-poor plasma (PPP) was prepared by centrifugation of the pellet at 2000g × 10 min at room temperature. Platelet aggregation was monitored by the turbidimetric method of Born and Cross[17] in a Chrono-Log aggregometer. PRP (300 μL) was incubated at 37 °C for 1 min with continuous stirring at 900 rpm. Platelet aggregation was induced by ADP (5 μM), collagen suspension (5 μg/mL) or AA (100 μM). Compounds or vehicle DMSO (0.5% v/v) were incubated with the PRP samples 5 min before addition of the aggregating agent. DMSO presented neither pro- nor antiplatelet aggregation activity. Indomethacin (10 μM) was used as standard. The platelet aggregation was expressed as percentage of aggregation for ADP and AA and as the maximum rate of aggregation (slope) for collagen. All concentrations values were expressed as final concentration in PRP.

Platelet TXB_2 formation. Washed platelets were incubated with test compounds (100 μM) for 5 min and treated with collagen (5 μg/mL) for 5 min. EDTA (2 mM) and indomethacin (50 μM) were added to halt TXB_2 formation. Finally, platelets were centrifuged at 13,000g × 5 min and the supernatant was assayed for TXB_2 using EIA kits (Biotrak, Amershan).

Anti-inflammatory activity. The anti-inflammatory activity was determined in vivo using the carrageenan induced rat paw edema test according to Ferreira.[18] Albino rats of both sexes (150–200 g) were used. Compounds were orally administered (100 μmol/kg, 0.1 mL/20 g animal weight) as a suspension in 5% arabic gum (Sigma) in saline (vehicle). Control animals received equal volume of the vehicle. One hour after, the animals were then injected with either 0.1 mL of 1% carrageenan solution in saline (0.1 mg/paw) or sterile saline (NaCl 0.9%), into the subplantar surface of one of the hind paws, respectively. The paw volumes were measured using a glass plethysmometer coupled to a peristaltic pump, 3 h after the subplantar injection. The edema was calculated as the volume difference between the carrageenan and saline-treated paw. Indomethacin (100 μmol/kg) was used as standard in the same conditions. Antiinflammatory activity was expressed as percentage of inhibition of the edema when compared with vehicle control group. The stomachs of the animals were also verified to estimate ulcerogenic effects. After the assays, the stomachs are opened across the great curvature, rinsed with saline and observed in a stereomicroscope for ulcers. A score of 2 and 5 were attributed for punctual and haemorragic lesions, respectively.

Analgesic activity. The analgesic activity was determined in vivo by using the abdominal constriction test induced by acetic acid 0.6% (0.1 mL/10 g) in mice.[19] Albino swiss 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). Indomethacin (100 μmol/kg) was used as the standard drug under the same conditions. Acetic acid solution was administered ip 1 h after administration of the NAH compounds **2a–p**. Ten minutes after ip injection of the acetic acid solution, 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.

Statistical analysis. All results are expressed as mean ± SEM for n animals or experiments per group. Data were statistically analyzed by Student's t-test for a significance level of $^*p < 0.05$.

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