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Short communication

Synthesis and evaluation of analgesic, antiinflammatory and antiplatelet properties of new 2-pyridylarylhydrazone derivatives†

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Abstract – This work describes recent results from our research program aiming at the synthesis and pharmacological evaluation of new compounds acting as antiinflammatory, analgesic and platelet antiaggregatory. In this paper the synthesis and the pharmacological profile as analgesic, antiinflammatory and anti-platelet of new functionalized 2-pyridylarylhydrazone derivatives **5a–r** are discussed. This class of *N*-heterocyclic derivatives represents a new series of prototype candidates with analgesic and antiinflammatory properties possessing also an important anti-aggregating activity. The pharmacological results herein disclosed suggest that the anti-inflammatory and analgesic activities of these new pyridynehydrazone derivatives observed in the carrageenan pleurisy model and acetic acid writhing test, respectively, is probably due to an interference on the arachidonic acid (AA) metabolism. The most important antiinflammatory derivative 2-(2-formylfurane)pyridylhydrazone **5p** presented a 79% inhibition of pleurisy at a dose of 80.1 μmol/kg. We also described the results concerning the mechanism of action of this series of *N*-heterocyclic derivatives in platelet aggregation which suggest a Ca^{2+} participation, probably by a complexation scavenger mechanism. Compound 2-(2-formylfurane)pyridylhydrazone **5p** was able to complex Ca^{2+} in in vitro experiments at 100 μM concentration, indicating that this series of compounds can act as Ca^{2+} scavenger depending on the nature of the aryl moiety present at the imine subunit. © Elsevier, Paris

2-pyridylarylhydrazone derivative / analgesic activity / antiinflammatory activity / 2-pyridylarylhydrazone synthesis / platelet aggregation inhibitors

1. Introduction

The therapeutic benefits of inhibitors of the arachidonic acid cascade (AAC) enzymes have been well established for a number of pathological conditions that involve inflammation, bronchial asthma, allergy and thrombotic or thromboembolic diseases [1]. These therapeutical effects are based on the important role played by the AAC products on the pathogenesis of these disorders. In fact, while a number of mechanisms have been proposed to explain the pharmacological effects of non-steroidal antiinflammatory drugs (NSAID's), it is believed that the inhibition of eicosanoid biosynthesis at the prostaglandin endoperoxide synthase or cyclooxigenase (PGHS or COX, EC 1.14.99.1) level is the one of greatest importance [2]. The recent discovery of an isoform of the PGHS, named PGHS-2 or COX-2, as a mitogen-inducible enzyme, especially involved in the physiopathological states, such as inflammation, opens a new perspective to PGHS inhibitors used in therapeutics [3]. The involvement of eicosanoids and other agents, as mediators of pain after noxious stimuli can explain the analgesic properties of AAC enzymes inhibitors in relieving moderate pain.

The constrictory and platelet-aggregatory activity of thromboxane A_2 (TXA_2), an unstable metabolite of AAC, have stimulated efforts to develop inhibitors of thromboxane synthase (TXS), antagonists of thromboxane receptor (TPant) and dual compounds having both activities as possible antithrombotic agents [4]. Those agents, in contrast to today's established drugs (i.e. aspirin, heparin and warfarin), will provide more specific and mechanism-based therapies, for the treatment of cardiovascular, renal and pulmonary diseases [5].

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In a previous work from our laboratory we disclosed the synthesis and the pharmacological profile of N-phenylpyrazole arylhydrazones derivatives **1** [6] and 4-(1-phenyl)-pyrazolo[3,4-*b*]pyridyl arylhydrazones derivatives **2** [7], designed as structurally hybrid compounds from the hydrazone derivatives BW-755c **3** [8] and CBS-1108 **4** [9] (*a* + *b*, *figure 1*). Both series of derivatives **1** and **2** were active as analgesic, antiinflammatory and anti-platelet agents possibly acting at the AAC level (A.L.P. Miranda and E.J. Barreiro, unpublished results). The most analgesic compound **1a** (*figure 2*) belonging to the N-phenylpyrazole arylhydrazones series presents a N-dimethylphenyl unit at the arylhydrazone framework. These findings prompt us to synthesize new structurally simple isosteres, as the 2-pyridylarylhydrazone derivatives **5** (*figure 1*), described in this paper, in order to investigate the possible pharmacophoric contribution of both the functionalized arylhydrazone units and the N-heterocyclic moiety in these activities. Previous results disclosed by Mansuy et al. [10, 11] indicated an inhibitory activity of phenylhydrazone derivatives on CitP450 dependent enzymes **6** (*figure 2*). Considering that some enzymes

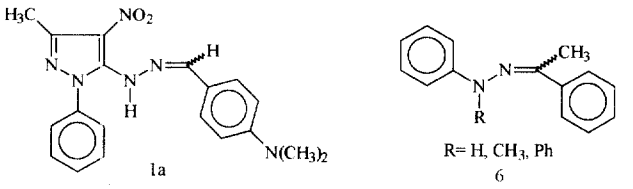


Figure 2. Structure of compounds **1a** and **6**.

of AAC are CitP450-dependent, due to their oxidase character, we became more interested to undertake the studies described herein.

The structure of the new 2-pyridylarylhydrazone derivatives **5** was planned by applying the classical bioisosterism concept of heteroaromatic ring replacement [12] on the previously described **1** and **2** derivatives, considered as lead-compound for this new series (*figure 1*). The series **5** represents a structural simplification with respect to **1** and **2**, where the *ortho*-

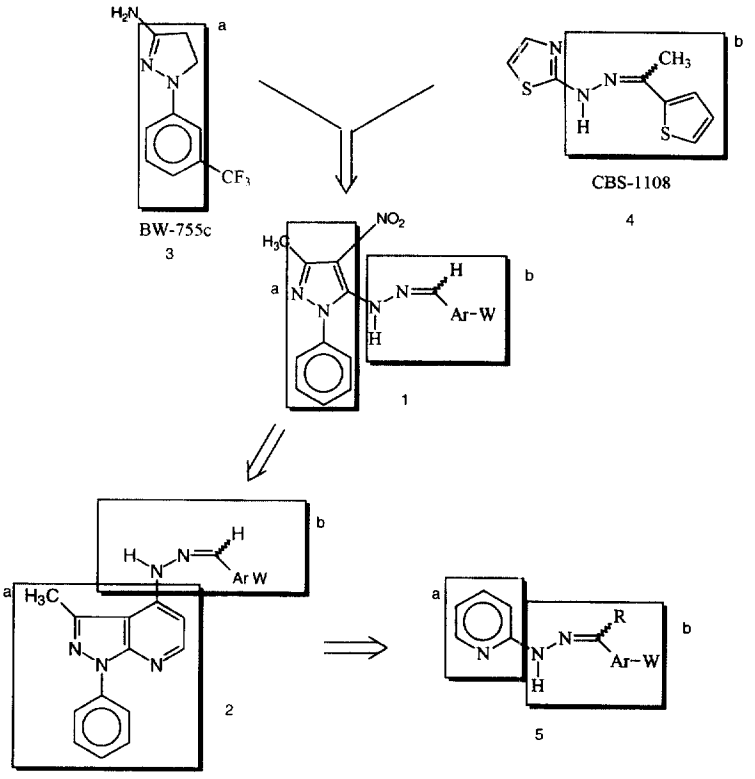


Figure 1. Genesis of 2-pyridylarylhydrazone derivatives **5**.

dinitrogen relationship present in **1** was maintained between the hydrazonic side chain and the N-heterocyclic atom (*figure 1*). We also considered for derivatives **5** that the –NH– unit of the pyridylhydrazone moiety (i.e., 2-Py–NH–N=CH–) could work as a surrogate for the bis-allylic methylene group present in the (*Z*,*Z*)-1,4-pentadiene (i.e., –CH=CH–CH_2–CH=CH–) framework of AA placed at C-5/C-9, one of the recognition sites for 5-lipoxigenase (5-LO) action on AA (*figure 3*). With this rational basis we hope to introduce a mimic structural unit which could favor the 5-LO recognition giving to this new class of derivatives **5** some dual inhibitory character between PGHS and 5-LO enzymes.

Otherwise, in order to investigate the eventual role of the aryl and the *para*-phenyl substituent of the hydrazone subunit on the biological activity, we decided to vary the heteroaryl rings (e.g. 2-furyl, 2-thiophenyl and 2-pyridyl) and also place different *para*-W-substituent in the phenyl ring (W = H, *p*-OMe, *p*-OH, *p*-NMe_2, *p*-CN, *p*-NO_2) of the hydrazone unit. These structural modifications would add different electronic and lipophilyc properties to these new derivatives with a useful contribution to understanding the structure–activity relationships (*figure 4*).

2. Chemistry

The series of 2-pyridylarylhydrazone derivatives **5a–r** were synthesized using classical synthetic methods as illustrated in *figure 5*, were the 2-hydrazinepyridine **9** was considered as key intermediate. This compound was obtained in 54% overall yield from 2-aminopyridine **7** which was transformed in the corresponding 2-bromo derivative **8** by treatment with bromine and bromidric acid as described by Allen and Thirtle [13]. The 2-bromo compound **8** was next treated in S_NAr conditions with hydrazine hydrate to furnish the desired 2-hydrazinepyridyne **9** derivative [14]. Finally, the title compounds were prepared by condensation at room temperature of an acidic ethanolic solution of **9** with the appropriated aryl- or heteroarylaldehydes or acetophenones, producing the desired compounds **5a–r** in very good yields.

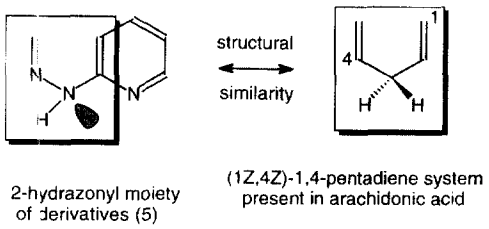


Figure 3. Structural relationship between the (1Z,4Z)-1,4-pentadiene system of arachidonic acid and the 2-pyridylarylhydrazone derivatives **5**.

The complete structural assignment of these new derivatives was performed by usual spectroscopic methods. A careful ^1H and ^{13}C nuclear magnetic resonance spectra analysis (*tables I* and *II*, respectively) was performed in order to investigate the presence of diastereoisomers (*E*) and (*Z*) at C=N bond level (*figure 6*), possibly formed during the acidic conden-

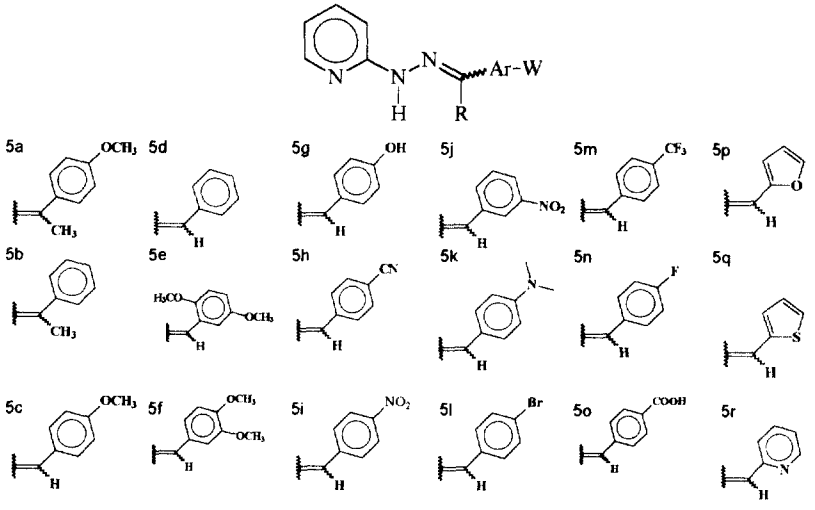


Figure 4. New 2-pyridylarylhydrazone derivatives **5a–r**.

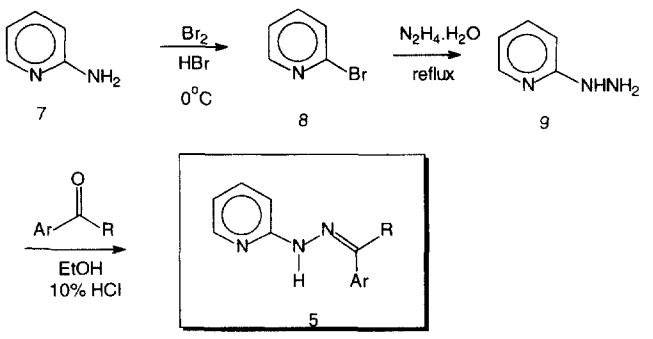


Figure 5. Synthesis of 2-pyridylarylhydrazone derivatives.

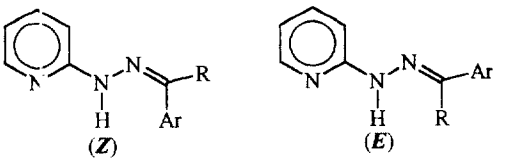
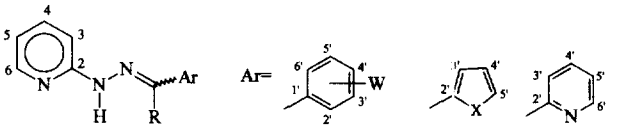


Figure 6. (*E*) and (*Z*) diastereomers for derivatives **5**.

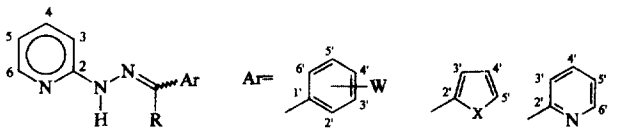
sation step to construct the hydrazone moiety of **5** [15, 16]. From this spectroscopic analysis we are able to detect that the (*Z*)-isomer was the major diastereoisomer for compounds **5c** (W = 4-OCH_3), **5e** (W = 2,5-OCH_3), **5k** (W = 4-$N(CH_3)_2$) and **5r** (Ar = 2-pyridyl).

Table I. ^{1}H-NMR spectral data of the 2-pyridylarylhydrazone derivatives. DMSO-d_6 was used as solvent and TMS was used as internal standard.



No.	R	Ar-W	H-3	H-4	H-5	H-6	N=C-*H*		H-2'	H-3'	H-4'	H-5'	H-6'	NH	Others
							E	*Z*							
5a	CH_3	4-OCH_3-C_6H_4	7.56 (d 9.0)	8.10 (t 6.8)	7.06 (t 6.8)	8.07 (t 6.8)			8.02 (d 8.0)	6.98 (d 8.0)				11.80 (s)	1.30 3.79
5b	CH_3	C_6H_5	7.78 (d 10.0)	8.10 (t 7.0)	7.50 (t 8.1)	8.10 (d 7.0)			8.20 (d 7.0)	7.48 (m)	7.48 (m)			12.20 (s)	1.35
5c	H	4-OCH_3-C_6H_4	7.29 (d 9.0)	8.0 (t 9.0)	7.02 (m)	8.07 (d 9.0)	8.6 (s)	8.3 (s)	7.78 (d 8.0)/ 7.89 (d 8.0)	7.02 (d)				13.10 (s)	3.80
5d	H	C_6H_5	7.18 (m)	8.01 (t 7.8)	7.02 (t 7.8)	7.83 (s 2.0)	8.22 (s)		7.83 (m)	7.40 (m)	7.40 (m)			13.00 (s)	
5e	H	2.5-$(OCH_3)_2$-C_6H_3	6.90 (d 3.0)/ 6.85 (d 3.0)	7.60 (t 8.0/ 2.0)	6.71 (d 6.0)	8.05 (d 6.0)	8.33 (s)			6.95 (s)	7.20 (d 8.0)		7.39 (d 3.0)		3.78 3.80
5f	H	3.4-$(OCH_3)_2$-C_6H_3	7.22 (d)	8.06 (t)	7.02 (t 6.0)	8.05 (d 8.0)	8.60 (s)		7.80 (d)			7.00 (d)	7.00 (d)	13.20 (s)	3.80
5g	H	4-OH-C_6H_4	7.30 (d 9.0)	8.00 (t 9.0)	6.90 (t 9.0)	8.05 (d 9.0)	8.30 (s)		7.80 (d 8.0)	6.87 (d 8.0)		6.87 (d 8.0)	7.80 (d 8.0)	13.30 (s)	9.70
5h	H	4-CN-C_6H_4	7.30 (d 6.5)	8.10 (m)	7.10 (t 6.5)	8.15 (m)	8.40 (s)		8.15 (m)	7.80 (d 8.0)		7.80 (d 8.0)	8.15 (m)	13.70	
5i	H	4-NO_2-C_6H_4	7.42 (d 8.7)	7.95 (t 7.4)	7.00 (t 6.3)	7.79 (d 2.2)	8.43 (s)		8.09 (d 7.4)	8.19 (d 8.6)		8.19 (s)	8.09 (d 7.4)	13.72 (s)	
5j	H	3-NO_2-C_6H_4	7.36 (d 8.6)	8.10 (m)	7.13 (t 6.2)	8.16 (d 6.20)	8.50 (s)		8.90		8.50 (s)	7.75 (t 7.7)	8.24 (d 8.1)	13.60 (s)	
5k	H	4-$N(CH_3)_2$-C_6H_4	6.70 (d 8.2)	7.13 (t 8.2)	6.27 (t)	7.62 (d)	8.00 (s)	7.45 (s)	7.00 (d 8.3)	6.27 (d)		6.27 (d)	7.00 (d 8.3)	10.07 (s)	2.53
5l	H	4-Br-C_6H_4	7.30 (d 10.0)	8.12 (t 6.0)	7.12 (t 6.1)	8.06 (dd 6.0)	8.34 (s)		7.92 (d 8.0)	7.63 (d 8.0)		7.63 (d 8.0)	7.92 (d 8.0)	13.61 (s)	
5m	H	4-CF_3-C_6H_4	7.69 (d)	8.40 (m)	7.29 (t)	8.42 (d)	9.00 (s)		8.40 (m)	7.85 (d)		7.85 (d)	8.40 (m)	13.60 (s)	
5n	H	4-F-C_6H_4	7.28 (d)	8.11 (t 6.0)	7.07 (t 6.6)	8.07 (d)	8.36 (s)		7.30 (dd)	8.05 (dd)		8.05 (dd)	7.30 (dd)	13.30 (s)	
5o	H	4-COOH-C_6H_4	7.44 (d 8.8)	8.12 (m)	7.13 (t 6.9)	8.20 (d 6.2)	8.50 (s)		8.14 (d 8.14)	8.00 (d 8.4)		8.00 (d 8.4)	8.14 (d 8.4)	13.50 (s)	10.10
5p	H	2-furyl	7.10 (d 8.3)	7.60 (t 7.4)	6.72 (t 6.5)	8.07 (d 4)	7.90 (s)			6.69 (d 3.0)	6.56 (s)	7.72 (s)	7.90 (s)	10.63 (s)	
5q	H	2-thiophene	7.75 (s)	8.05 (d)	7.16 (t)	8.05 (d 8.6)	8.64 (s)	8.56 (s)		7.26 (d 8.6)	7.03 (s)	7.16 (s)		13.25 (s)	
5r	H	2-pyridyl	7.45 (d 8.4)	8.23 (m)	7.22 (t 5.6)	8.46 (d 5.5)	8.46 (s)			8.23 (d 8.4)	8.37 (t 5.5)	7.82 (t 5.5)	8.46 (d)	11.00 (s)	

Table II. ^{13}C-NMR spectral data of the 2-pyridylarylhydrazone derivatives. DMSO-d_6 was used as solvent and TMS was used as internal standard.



No.	R	Ar-W	C-1	C-2	C-3	C-4	C-5	C=N	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'	Others
5a	CH_3	4-OCH_3–C_6H_4	150.2	112.9	136.8	114.5	143.9	153.8	129.2	128.9	113.7	160.8	113.7	128.9	15.5 55.4
5b	CH_3	C_6H_5	154.2	113.2	139.1	115.3	144.4	151.4	139.1	138.4	127.9	130.2	127.9	138.4	16.0
5c	H	4-OCH_3–C_6H_4	149.4	112.0	136.7	114.4	144.1	147.9 161.5	126.1	129.6 130.0	114.4	161.5	114.4	129.6 130.0	55.6
5d	H	C_6H_5	150.0	112.1	137.9	115.9	145.8	149.5	134.0	129.5	128.2	131.8	128.2	129.5	
5e	H	2.5-OCH_3–C_6H_3	151.2	115.4	137.9	114.9	147.76	132.1	124.2	157.0	113.1	106.4	153.3	109.0	56.1; 55.4
5f	H	3.4-OCH_3–C_6H_3	146.0	114.2	139.9	110.8	140.5	142.9	118.2	110.0	149.0	150.0	108.2	107.8	52.3
5g	H	4-OH–C_6H_4	149.1	112.1	136.5	114.0	143.8	148.3	124.4	129.7	115.8	160.3	115.8	129.7	
5h	H	4-CN–C_6H_4	149.4	112.2	137.9	115.3	144.9	145.4	128.1	128.5	132.4	132.4	132.4	128.5	
5i	H	4-NO_2–C_6H_4	148.6	110.35	138.22	113.8	143.6	146.62	142.9	121.9	136.5	142.5	136.5	121.9	
5j	H	3-NO_2–C_6H_4	149.6	112.2	137.4	115.2	145.0	145.1	135.4	124.7	148.3	130.0	130.1	134.0	
5k	H	4-$N(CH_3)_2$–C_6H_4	150.2	105.6	137.3	113.7	147.4	139.5	124.8	126.7	111.6	156.9	111.6	126.7	39.5
5l	H	4-Br–C_6H_4	149.6	114.8	137.4	111.9	144.2	146.2	132.8	131.7	129.6	123.9	129.6	131.7	
5m	H	4-CF_3–C_6H_4	161.1	112.1	137.2	114.7	144.4	146.7	149.6	115.7	116.2	166.1	116.2	115.7	
5n	H	4-F–C_6H_4	149.5	112.0	137.1	114.6	144.3	146.5	130.2	116.0	130.3	165.9	130.3	116.0	
5o	H	4-COOH–C_6H_4	149.6	111.7	137.3	114.7	143.9	146.0	131.9	127.5	129.5		129.5	127.5	166.5
5p	H	2-furyl	156.9	106.2	138.1	115.4	147.8	129.5		152.0	110.2	112.0	143.6		
5q	H	2-thiophene	119.5	114.7	137.5	112.0	142.9	128.3		144.5	137.8	132.3	130.5		
5r	H	2-pyridyl	149.9	112.5	140.4	117.0	145.1	143.5		148.5	124.9	138.3	126.7	145.6	

The assignment of the (*Z*)-configuration to the N=CH hydrazone double bond was performed considering literature data [17]. In fact, the chemical shift due the –CH= hydrogen atom in the (*Z*)-isomer is usually upfield with respect to the (*E*)-isomer, appearing in these new pyridine hydrazone derivatives **5** at the range δ 7.45–8.30 ppm in the ^{1}H NMR spectrum [17] (*table I*). Otherwise, the hydrazonic carbon atom of these derivatives occurs upfield in the ^{13}C NMR spectra for the (*Z*)-isomer (*table II*), at the range δ 132–147 ppm in aggreement with the results of Bunnel and Fuchs [18]. In addition, in the ^{1}H NMR spectra of these derivatives we are able to identify a signal occurring at δ 10.07–13.3 ppm which was attributed to the –NH– of the hydrazone moiety. This signal has a chemical shift displacement in aggreement with very recent results described by Easmon [19, 20] for heteroarylhydrazones belonging to the thiazolyl class, where the authors determine the nitrogen-double bond configuration by the relative chemical shift of –NH–, occurring in the (*Z*)-isomer upfield (δ 9.0–12.0 ppm) with respect to the (*E*)-isomer (δ 11.0–14.0 ppm). In the ^{1}H NMR spectra of these derivatives the aromatic hydrogens could be depicted between δ 7.0–8.5 ppm. In the ^{1}H NMR spectra of the hydrazonic methylated derivatives **5a** and **5b** we are able to observe a singlet signal at δ 2.23 ppm, attributed to the methyl hydrogens of (*Z*)-isomer [21].

The correct attribution of carbon atom chemical shifts in the ^{13}C NMR spectra was performed by employing DEPT (Distortionless Enhancement by Polarization Transfer), APT (Attached Proton Test) and homonuclear 2D spectra ($^1Hx^{13}C$–COSY^1–J_{CH}) long distance [$^1Hx^{13}C$–COSY–$^nJ_{CH}$ (n = 2 and 3, COLOC = COrrelation spectroscopy LOng-range Coupling)] (*table II*) [22].

Interestingly, we were able to observe an important variation in the diastereomeric ratio of (*Z*)- and (*E*)-isomers for different compounds. For instance, compounds **5c** (W = 4-OCH_3), **5e** (W = 2,5-OCH_3), **5k** (W = 4-$N(CH_3)_2$) and **5r** (Ar = 2-pyridyl) showed a signal at δ 161.0 ppm in the ^{13}C NMR spectra, corresponding to the CH=N hydrazonic carbon atom of the minor (*E*)-isomer. On the other hand, it was not possible to detect the presence of any important amount of the (*E*)-isomer in compounds **5c** (W = H), **5f** (W = 3,4-OCH_3), **5g** (W = 4-OH), **5h** (W = 4-CN), **5i** (W = 4-NO_2), **5j** (W = 3-NO_2), **5l** (W = 4-Br), **5m** (W = 4-CF_3), **5n** (W = 4-F), **5o** (W = 4-COOH), **5p** (Ar = 2-furyl) and **5q** (Ar = 2-thiophenyl). This data indicated that in this series of hydrazone derivatives **5** the possible azo-form tautomer is not present and it must be improbable that it could be involved as a transient intermediate in an eventual isomerization (*E*) ⇔ (*Z*) process, as suggested by Karabatsos [21] for structurally related derivatives.

These spectroscopic results indicated that the acidic condensation step used to construct the hydrazone function of these derivatives represents a diastereoselective process, favouring the (*Z*)-diastereomer for the great majority of derivatives **5** prepared in this work.

Table III. Analgesic activity of 2-pyridylarylhydrazone derivatives **5a–r** (100 μmol/kg p.o.) and indomethacin (100 μmol/kg p.o.) by the acetic acid constriction test.

Compound	N[a]	Writhes (no.)	Inhibition (%)
Control	26	100.3 ± 4.0	–
Arabic gum	26	93.8 ± 4.6	6.5
Indomethacin	9	26.3 ± 6.9	73.7[b]
5a	7	68.1 ± 5.7	32.1[b]
5b	12	104.4 ± 7.1	–4.1
5c	8	75.8 ± 3.7	24.4[b]
5d	10	66.4 ± 5.7	33.8[b]
5e	8	78.6 ± 8.2	21.6
5f	7	97.9 ± 7.3	2.4
5g	8	49.0 ± 5.0	51.2[b]
5h	7	58.3 ± 7.3	41.8[b]
5i	9	62.5 ± 4.4	37.6[b]
5j	10	45.1 ± 4.4	55.0[b]
5k	8	45.0 ± 6.7	55.5[b]
5l	9	76.2 ± 8.6	24.0[b]
5m	9	88.3 ± 7.8	11.9[b]
5n	11	91.0 ± 6.1	9.3
5o	10	64.2 ± 14.4	36.0[b]
5p	7	54.7 ± 2.8	44.9[b]
5q	7	76.3 ± 11.1	24.0[b]
5r	8	95.9 ± 7.6	4.8
9	5	96.8 ± 2.8	3.5

[a]*N*: number of experiments; [b]$p < 0.05$ compared to the appropriate control (Student's *t*-test).

3. Pharmacology

The pharmacological profile of these new derivatives **5a–r** was initially investigated by testing their analgesic activity employing the acetic acid-induced mouse abdominal constriction test [23]. The results obtained are illustrated in *table III*. Between the most active derivatives, three of them, **5k** (*para*-dimethylamino, 55.5% of inhibition), **5g** (*para*-hydroxyl, 51.2% of inhibition) and **5h** (*para*-cyano, 41.8% of inhibition) present a *para*-substituent pattern in the phenyl ring of the hydrazone moiety. These findings were in accordance with previous results obtained with structurally related series synthesized in our laboratory [24, 25] where the most active derivatives possess a *para*-dimethylamino group as substituent of the phenyl ring of the hydrazone moiety. Curiously, the meta-nitro **5j** (55.0 %) was more powerful than the *para*-isomer **5i** (37.6%) in the analgesic assay. Among the heterocycle-substituted hydrazones the 2-furyl derivative **5p** (44.9%) presents a more important analgesic activity than the isostere 2-thiophene **5q** (24.0%) and the 2-pyridine **5r** (4.8%) derivative not presented any activity of importance.

The antiinflammatory properties of compounds **5b** (W = H), **5d** (W = H), **5k** (W = 4-$N(CH_3)_2$) and **5p** (Ar = 2-furyl), were evaluated in vivo on the carrageenan-induced rat paw edema (100 μmol/kg, p.o.) [26], using indomethacin (100 μmol /kg, p.o.) as standard (*table IV*). Compounds **5b** (W = H; R = CH_3) and **5d** (W = H; R = H) were tested in order to verify an eventual CH_3 group influence in the activity, especially due to possible steric restrictions. Since compounds **5k** (W = 4-$N(CH_3)_2$) and **5p** (Ar = 2-furyl) were among the most active in respectively the analgesic and anti-platelet aggregation assays, they were selected to verify their antiinflammatory profile. These compounds were not capable of inhibiting the inflammatory response induced by carrageenan in the rat

Table IV. Antiinflammatory activity of 2-pyridylarylhydrazone derivatives **5b,d,k,p** (100 μmol/kg p.o.) and indomethacin (100 μmol/kg p.o.) on carrageenan-induced rat paw edema test.

Compound	N[a]	Volume (μL)	Inhibition (%)
Control	24	508.4 ± 18.6	–
Indomethacin	18	252.1 ± 33.6	50.41%[b]
5b	5	558.6 ± 40.6	–9.87%
5d	9	535.3 ± 42.4	–5.28%
5k	5	650.1 ± 77.5	–27.88%[b]
5p	7	648.0 ± 19.4	–27.46%[b]

[a]*N*: number of experiments; [b]$p < 0.05$ compared to the appropriate control (Student's *t*-test).

paw edema. Curiously, the derivatives **5k** (W = 4-$N(CH_3)_2$) and **5p** (Ar = 2-furyl) induced an increase in the rat paw edema formation when given p.o. 1 h before the carrageenan administration (*table IV*).

It is well known that dual 5-LO/COX inhibitors as phenidone or BW-755c are able to prevent the exudation and the cellular infiltration in the pleurisy assay [27]. Thus, in order to verify the existence of any 5-LO/COX-related activity for 2-pyridylarylhydrazone derivatives, **5c** (W = 4-OCH_3), **5k** (W = 4-$N(CH_3)_2$) and **5p** (Ar = 2-furyl) were assayed in carrageenan-induced pleurisy [28]. The results obtained are illustrated in *table V*. The furyl derivative **5p** (80 μmol/kg, p.o.) was the most active compound showing a potent antiinflammatory activity comparable to that of indomethacin or phenydone, employed as standard. It was able to inhibit significantly both PMN migration and exudation associated to the carrageenan-induced pleurisy. Compound **5k** (W = $N(CH_3)_2$) was only able to inhibit the cellular migration, without having any important activity over the exudation. On the other hand compound **5c** (W = 4-OCH_3) inhibited only the exudation. These results seem to indicate that the bioactivity of these compounds in pleurisy depends on the nature of the aryl substituent at the hydrazone unit.

In previous work we verified the platelet antiaggregating activity of 2-pyridylarylhydrazone derivatives [29]. The anti-platelet activity was measured in vitro on the responses induced by ADP (5 μM), collagen (5 μg/mL) and arachidonic acid (AA) (200 μM) on citrated platelet-rich rabbit and human plasma (PRP). None of the compounds **5a–r** tested showed a statistically significant inhibitory activity on the ADP-induced rabbit platelet aggregation test, even at final concentration of 100 μM. Compound **5p** (Ar = 2-furyl) was the most active in the series, it fully inhibited collagen-induced aggregation, showing a potent inhibitory activity on the arachidonic acid-induced aggregation, with an IC_{50} of 0.35 μM. Additionally, this derivative was able to inhibit the secondary ADP-induced aggregation of human citrated platelet-rich plasma [29], a response mainly mediated by arachidonic acid metabolites [30]. These results corroborate our suggestion that this compound possibly was acting on the AA metabolism.

In order to verify the possible mechanism of action of compound **5p** (Ar = 2-furyl) in the aggregation process, we decide to investigate the platelet aggregation induced by thrombin. This process is Ca^{2+}-dependent and it does not have an important participation of the AAC [31]. Then, the next step in this study was to verify the antiplatelet activity of the isosteric related derivatives **5d** (W = H) and **5p** (Ar = 2-furyl) on this system. These derivatives were used at 100 μM concentration in platelet aggregation response induced

Table V. Antiinflammatory activity of 2-pyridylarylhydrazone derivatives **5c,k,p**, indomethacin and phenidone in the carrageenan-induced pleurisy.

Compound[a]	Dose (μmol/kg) sc.	N[b]	PNM x 10^6	(%) PMN migration inhibition	Exudation (mL)	% exudation inhibition (mL)
Carrageenan		15	21.7 ± 2.2	–	1.04 ± 0.2	–
Indomethacin	29.0	10	5.44 ± 1.8	74.9[c]	0.52 ± 0.1	50.0[c]
Phenidone	617.0	10	4.12 ± 2.1	81.0[c]	0.42 ± 0.2	60.0[c]
5c	66.0	10	17.40 ± 1.7	19.6	0.60 ± 0.2	42.3[c]
5k	62.5	10	8.24 ± 1.3	62.0[c]	0.76 ± 0.3	26.9
5p	80.1	10	4.55 ± 1.2	79.0[c]	0.39 ± 0.2	62.5[c]

[a]The compounds were diluted in propyleneglycol; [b]*N*: number of experiments; [c]$p < 0.005$ compared to the appropriate control (Student's *t*-test).

by thrombin in the presence of different Ca^{2+} concentrations. Washed rabbit PRP was used as described by Cazenave and coworkers [32]. Induction of platelet aggregation by thrombin at different Ca^{2+} concentrations (e.g., 0.5 μM and 2 μM) is illustrated in *table VI*. Compound **5p** (Ar = 2-furyl) inhibited the thrombin-induced platelet aggregation in 40% at the lowest Ca^{2+} concentration used. However, this activity was abolished at higher Ca^{2+} concentration. These results indicated that compound **5p** (Ar = 2-furyl) interfered with the platelet's Ca^{2+}, probably by Ca^{2+} scavenging (*figure 7*). In contrast, compound **5d** (W = H), a classical isoster of **5p** (Ar = 2-furyl) inhibited platelet aggregation induced by thrombin in 50%, independently of the Ca^{2+} concentration, probably due the absence of chelating properties at the aromatic substituent (*figure 7*). These results seem indicate that the 2-furyl ring present in **5p** is an important structural requirement for the Ca^{2+}-dependent activity. Conversely, both derivatives **5p** (Ar = 2-furyl) and **5d** (W = H) were more active than ASA (27% at 500 μM) (*table VI*). The Ca^{2+} chelating properties of compound **5p** (Ar = 2-furyl) were measured by UV in the presence of a stechiometric quantity of Ca^{2+}, showing a hyperchromic displacement of the standard curves as illustrated in *figure 8*, confirming the chelating behaviour of the 2-furyl moiety in this compound.

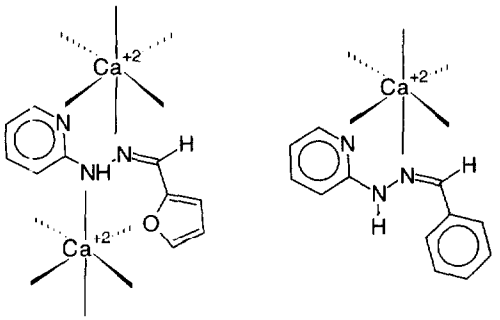


Figure 7. Representation of chelating Ca^{2+} for derivatives **5d** (W = H) and **5p** (Ar = 2-furyl).

4. Conclusions

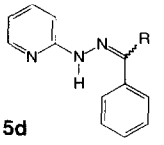
Based on the data shown here, we can conclude that the introduction of some minor structural modifications on the aromatic framework of the hydrazono unit of derivatives **5** interfere significantly on the observed bioactivities. The next step of this work will be the determination of AA metabolites in order to clarify where these compounds could be acting at AAC. The results described here are very important for guiding future structure–activity relationship (SAR) studies that are in progress in our laboratory with new series of hydrazone compounds.

5. Experimental protocols

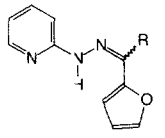
5.1. Chemistry

Thin-layer chromatography on precoated silica-gel plates (Merk 60 F254) was used to control the course of reactions and the purity of the products. The detection of components was made by UV light exposition and by treatment with iodine vapors. Melting points were determined in a capillary melting point apparatus with Buchi 510 and are uncorrected. The structures of all compounds were confirmed by their infrared spectra using a Perkin-Elmer spectrometer. The ^1H and ^{13}C NMR

Table VI. Effect of 2-pyridylhydrazone derivatives **5d**,**p** and ASA on the in vitro thrombim-induced platelet aggregation of rabbit-citrated platelet-rich plasma.



5d



5p

Compounds	Concentration (μM)	N^a	Aggregation		Inhibition (%)	
			0.5 mM Ca^{2+}	2 mM Ca^{2+}	0.5 mM Ca^{2+}	2 mM Ca^{2+}
Control	–	3	41.3 ± 1.2	51.4 ± 1.8	–	–
ASA	500	3	46.4 ± 9.0	36.9 ± 4.1	−12.5	27.9[b]
5d	100	3	19.7 ± 6.7	30.8 ± 5.2	52.4[b]	40.14[b]
5p	100	3	25.3 ± 4.9	48.4 ± 1.8	39.4[b]	5.7

[a]*N*: number of experiments; [b]$p < 0.05$ compared to appopriate control (Student's *t*-test).

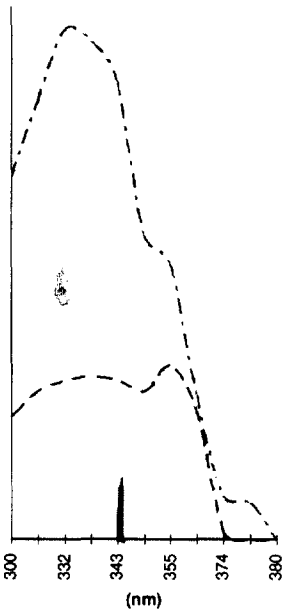


Figure 8. UV absorption: Ca^{2+} (100 μM) (—); compound **5p** (100 μM) (– – –); Ca^{2+} + **5p** (– • – • –).

spectra were recorded using a Bruker AC-200 or a Varian XI-200 spectrometer. Chemical shift values are reported in δ units (ppm) relative to tetramethylsilane used as an internal standard.

5.1.1. 2-Bromopyridine ***8***

A 7.9 mL (0.16 mol) volume of a 47% hydrobromic acid solution was placed in a three-necked flask fitted with a mechanical stirrer, dropping funnel and a thermometer for low temperatures. The reaction vessel was cooled to 10–20 °C in an ice-salt bath, and 1.5 g (0.16 mol) of 2-aminopyridine was added dropwise over a period of 10 min. A volume of 2.4 mL (0.049 mol) of bromine was added dropwise to a reaction flask while keeping the temperature at 0 °C, and maintaining the flask under magnetical stirring. Subsequently, an aqueous solution of 2.75 g (0.040 mol) of sodium cyanide in 40.0 mL was carefully added, over a period of 2 h, in a temperature maintained at 0 °C. After an additional 30 min stirring period, an aqueous solution of 60.0 g (1.5 mol) of sodium hydroxide in 6.0 mL of water was added at a rate that kept the temperature of the reaction vessel at 20–25 °C. Then, the nearly colorless reaction mixture was extracted with ethyl ether (3 x 30 mL) and dried over solid potassium hydroxide/magnesium sulfate powder. After solvent elimination at reduced pressure, the oily product was distilled at 74–75 °C/13 mm Hg to furnish 60% yield of 2-bromopyridine [13].

5.1.2. 2-Hydrazinepyridine ***9***

A solution of 2-bromopyridine **9** (0.009 mol) and hydrazine hydrate (0.02 mol) was placed in a three-necked flask fitted with a mechanical stirrer, a dropping funnel and thermometer. The reaction mixture was stirred at reflux for 4 h under an inert atmosphere of N_2. The reaction mixture was next extracted at room temperature with ethyl ether (3 x 50 mL). The organic layer was evaporated under reduced pressure to provide, in 54% yield, the 2-hydrazinepyridyne as a white oil; mp: 46 °C [33] IR (cm^{-1}): 3326 (NH), 1628 (C–N), 1493 (C–C).

5.1.3. General procedure for the preparation of arylhydrazones

An ethanolic solution of 2-hydrazinepyridyne **9** (0.0014 mol) in 20 mL was added to the suitable arylaldehyde or arylketones in ethanol at room temperature. The reaction mixture was stirred at reflux during 2 h. The mixture was then cooled to room temperature and concentrated at reduced pressure to furnish a solid preparation that was recrystallized from ethanol/water to furnish the desired hydrazone derivatives as colored solid in 90% yield.

4'-Methoxyketophenone-2-pyridylhydrazone ***5a:*** (79%); mp: 100–103 °C; IR (cm^{-1}): 3400 (NH), 1695 (C=N), 1615 (C=C), 1298 (C–O), 728 (C–H).

Ketofenone-2-pyridylhydrazone ***5b:*** (76%); mp: 138–139 °C; IR (cm^{-1}): 3414 (NH), 1650 (C=N), 1606 (C=C), 756 (C–H).

4-Methoxybenzaldehyde-2-pyridylhydrazone ***5c:*** (96%); mp: 115–118 °C; IR (cm^{-1}): 3385 (NH), 1648(C=N), 1607 (C=C), 768 (C–H).

Benzaldehyde-2-pyridylhydrazone ***5d:*** (85%); mp: 130–132 °C; IR (cm^{-1}): 3320 (NH), 1650 (C=N), 1610 (C=C), 1244 (C–O), 770 (C–H).

2,5-Dimethoyibenzaldehyde-2-pyridylhydrazone ***5e:*** (96%); mp: 100 °C; IR (cm^{-1}): 3388 (NH), 1701 (C=N), 1648 (C=C), 1234 (C–O), 776 (C–H).

3,4-Dimethoxybenzaldhyde-2-pyridylhydrazone ***5f:*** (95%); mp: 130–135 °C; IR (cm^{-1}): 3297 (NH), 1664 (C=N), 1613 (C=C), 1262 (C–O).

4-Hydroxybenzaldehyde-2-pyridylhydrazone ***5g:*** (95%); mp: 130–134 °C; IR (cm^{-1}): 1645 (C=N), 1590 (C=C), 1162 (C–O), 775 (C–H).

4-Cyanobenzaldehyde-2-pyridylhydrazone ***5h:*** (96%); mp: 122–124 °C; IR (cm^{-1}): 2217 (C N), 1637 (C=N), 1592 (C=C), 757 (C–H).

4-Nitrobenzaldehyde-2-pyridylhydrazone ***5i:*** (90%); mp: 185–188 °C; IR (cm^{-1}): 3375 (NH), 1600 (C=C), 1525 (N–O), 1330 (N–O), 780 (C–H).

3-Nitrobenzaldehyde-2-pyridylhydrazone ***5j:*** (82%); mp: 190 °C; IR (cm^{-1}): 2682 (NH), 1649 (C=N), 1623 (C=C), 1520 (N–O), 1359 (N–O), 761 (C–H).

4-Dimethylaminobenzaldehyde-2-pyridylhydrazone ***5k:*** (95%); mp: 123–125 °C; IR (cm^{-1}): 1650 (C=N), 1600 (C=C), 780 (C–H).

2-Bromobenzaldehyde-2-pyridylhydrazone ***5l:*** (70%); mp: 180–183 °C; IR (cm^{-1}): 1653 (C=N), 1600 (C=C), 766 (C–H), 508 (C–Br).

4-Trifluoromethylbenzaldehyde-2-pyridylhydrazone ***5m:*** (90%); mp: 255–257 °C; IR (cm^{-1}): 1649 (C=N), 1609 (C=C), 1111 (C–F), 767 (C–H).

4-Fluorobenzaldehyde-2-pyridylhydrazone ***5n:*** (65%); mp: 228–230 °C; IR (cm^{-1}): 3416 (NH), 1635 (C=N), 1609 (C=C), 1229 (C–F), 764 (C–H).

4'-Carboxybenzaldehyde-2-pyridylhydrazone ***5o:*** (80%); mp: 290–291 °C; IR (cm^{-1}): 3346 (NH), 1688 (C=O), 1648 (C=N), 1603 (C=C), 766 (C–H).

2-(2-Formylfurane)pyridylhydrazone-2-pyridylhydrazone ***5p:*** (81%); mp: 58–62 °C; IR (cm^{-1}): 3419 (NH), 3095 (C–H), 1659 (C=N), 1608 (C=C), 755 (C–H).

2-(2-Formylthiophene)pyridylhydrazona-2-pyridylhydrazone ***5q:*** (83%); mp: 143 °C; IR (cm^{-1}): 3136 (NH), 1608 (C=N), 1601(C=C), 1422 (C–S), 768 (C–H).

2-(2-Formylpyridyl)pyridilidrazona-2-pyridylhydrazone ***5r:*** (65%); mp: 270–272 °C; IR (cm^{-1}): 3455 (NH), 3084 (C–H), 1671 (C=N), 1605 (C=C), 761 (C–H).

5.2. Pharmacology

5.2.1. Analgesic activity

An acetic acid writhing test [23] was used on mice of both sexes, weighing 18–25 g. The test compounds (100 μmol/kg) were administered p.o. Arabic gum was used as vehicle (0.1 mL/20 g of animal weight). After 1 h of treatment, the animals were injected intraperitoneally with 0.10 mL/10 g of animal weight of 0.6% acetic acid solution. The number of abdominal constrictions of injected mice was recorded during 30 min after i.p. injection. The values obtained were compared with those obtained on untreated control mice and the activity expressed as percent of inhibition. Mean values were analyzed statistically by the Student's *t*-test for a p^* value of < 0.05.

5.2.2. Antiinflammatory activity

The paw edema inhibition test [26] was used on rats of both sexes (body weight 150–200 g). The test compounds (100 μmol/kg) were administered p.o. Arabic gum was used as vehicle (0.1 mL/20 g of animal weight). After 1 h, 0.1 mL of a 1% carrageenan suspension, in 0.9% NaCl solution, was injected subcutaneously into the plantar aponeurosis of one hind paw, the other one received the same volume of 0.9% NaCl solution. The paw volume was measured 3 h later by a water plethysmometer. The mean variation of paw volume was compared with that of a control group (rats that were treated with the vehicle). The values of the percentage of inhibition were calculated.

5.2.3. Pleurisy

Wistar rats (200 ± 50 g) were anaesthesized with ether and 500 μL of a 1% carrageenan solution, in 0.9% NaCl, was injected into the pleural cavity [28]. The exudate was collected by pleural lavage 4 h after the injection of the irritant. Cell numbers were counted in a Neubauer chamber. The compounds **5c** (66 μmol/kg), **5k** (62.5 μmol/kg), **5p** (80.1 μmol/kg) were diluted in propyleneglycol (0.1 mL/20 g of animal weight). Indomethacin (29.0 μmol/kg) and phenidone (617.0 μmol/kg) were used as reference compounds. All compounds were administered subcutaneously (s.c.).

5.2.4. Anti-platelet activity [34]

Rabbit blood was collected by heart puncture from rabbits weighing 2.5–3.0 kg. Human blood was obtained by vein puncture of the median cubital vein from healthy volunteers who had not received any medication for 1 week before the study. Blood samples were collected into 3.8% trisodium citrate (9:1 v/v).

Platelet-rich plasma (PRP) was prepared by centrifugation at 500 *g* for 10 min at room temperature. The platelet-poor plasma (PPP) was prepared by centrifugation of the pellet at 2000 *g* for 10 min at room temperature. Platelets were counted and diluted to 5 x 10^8 platelets/mL with PPP. Platelet aggregation was monitored by the turbidimetric method of Born and Cross [34] in a Chrono-Log aggregometer. PRP (400 μL) was incubated at 37 °C for 1 min with continuous stirring at 900 rpm. Aggregation was induced by the addition of 5 μL of an ADP solution (final concentration of 5 μM, in distilled water), 4 μL of a collagen suspension (5 μg/mL in saline) or 2 μL of a arachidonic acid solution (200 μM in ethanol). Test compounds and the solvent used in dissolution of the 2-pyridylarylhydrazone compounds (0.5% DMSO, 2 μL in volume) were added to the PRP samples 5 min before addition of the aggregating agent. Indomethacin (10 μM), a classical cyclooxygenase inhibitor, was used as standard. The DMSO used as vehicle did not have either pro- or anti-platelet aggregation activity.

The platelet aggregation was expressed as percentage of aggregation for ADP and AA and as the maximum rate of aggregation (slope) for collagen.

Data were analyzed statistically by analysis of variance for a p^* value of < 0.05 (one-way, Scheffé test) and were expressed as mean ± s.e.m. for *N* experiments in triplicate.

5.2.5. Preparation of washed platelets [32]

Rabbit blood, obtained as described above, was collected into EDTA 0.2 M (1.0 mL) and 0.95% NaCl solution (1.5 mL). PRP, prepared as described above, was centrifugated at 500 *g* for 10 min at room temperature. The supernatant was discarded and the platelet pellet was resuspended in an equal volume of a modified Tyrode buffer (0.21 g/L $MgCl_2$, 8.00 g/L NaCl, 0.20 g/L KCl, 2.76 g/L $NaHCO_3$, 57.00 mg/L $NaH_2PO{\cdot}H_2O$, 1.00 g/L glucose, 3.50 g/L gelatin), pH 6.5, which contained 1 M $MgCl_2$ (1mL/mL) and 0.1 M EGTA (2mL/mL). This procedure was repeated to eliminate residual contamination. The platelets were recentrifuged at 2.000 *g* for 10 min at room temperature and were finally resuspended in Tyrode buffer pH 7.35, to a final platelet count of 5 x 10^8 platelets/mL. 2 mM $CaCO_2$ was added 1 min before the addition of the aggregation inducer agent.

5.3. Statistics

Results were expressed as the mean ± s.e. for *N* experiments. Statistical analysis was determined by the Student's unpaired '*t*' test and analysis of variance (Scheffé test) for a p^* value of < 0.05 considered significant.

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