8.49 (m, 1 H, pyridine H-6), 8.81 (s, 1 H, exchangeable with D_2O, NH); high-resolution MS calcd for $C_7H_{10}N_4S$ (M^+) 182.0626, found 182.0617 ± 0.002.

Methyl 3-[1-(3-Pyridazinyl)ethyl]hydrazinecarbodithioate (25). A stirred suspension of 12[13] (7.00 g, 31 mmol) in ethanol (100 mL) was treated portionwise with sodium borohydride (2.34 g, 62 mmol) over a period of 30 min. The solution was then stirred overnight at room temperature, diluted with water (100 mL), and cautiously neutralized with ~10 mL of glacial acetic acid. After evaporation of ethanol, the product separated from the remaining solution on cooling. Subsequent filtration and drying afforded 3.74 g (53%) of colorless needles, mp 159–160 °C. An analytically pure sample was obtained upon recrystallization from ethanol: IR (KBr) 3210, 3120, 3050, 2920, 2860, 1580 cm^{-1}; MS m/z 228 (M^+, 8), 181 (10), 122 (100), 108 (96), 107 (49); ^1H NMR (DMSO-d_6) δ 1.34 (d, J = 6.7 Hz, 3 H, CCH_3), 2.36 (s, 3 H, SCH_3), 4.51 (m, 1 H, q with J = 6.7 Hz after addition of D_2O, NCH), 6.18 (d, J = 4.0 Hz, 1 H, exchangeable with D_2O, CHN*H*), 7.58–7.74 (m, $J_{4,5}$ = 8.6 Hz, $J_{5,6}$ = 4.6 Hz, 1 H, pyridazine H-5), 7.77–7.91 (m, $J_{4,5}$ = 8.6 Hz, $J_{4,6}$ = 1.9 Hz, 1 H, pyridazine H-4), 9.08–9.17 (m, $J_{4,6}$ = 1.9 Hz, $J_{5,6}$ = 4.6 Hz, 1 H, pyridazine H-6), 10.90 (s, 1 H, exchangeable with D_2O, NHCS). Anal. ($C_8H_{12}N_4S_2$) C, H, N.

General Procedure for the Preparation of Thiosemicarbazides 26a–d. A mixture of compound 25 (1.50 g, 6.6 mmol) and 1 equiv of the appropriate cyclic amine in methanol (15 mL) was reacted and worked up as described for the preparation of thiosemicarbazones 18–24, method A. For yields, recrystallization solvents, and physical data, see Table III.

Biological Methods. Assays for Inhibition of Cell Proliferation and of Virus-Induced Cytopathic Effects. The assay procedure described by Pauwels et al.[29] for measuring inhibition of HIV was used with minor modifications. Briefly, the HTLV-I-transformed T4-cell line MT4, which was previously shown to be highly permissive for HIV-infection, was used as the target cell. Inhibition of HIV-1 (strain HTLV-IIIB) induced cytopathic effect was determined by measuring the viability of both HIV and mock-infected cells. Viability was assessed spectrophotometrically (at 540 nm) via in situ reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Virus-infected and uninfected cultures without compound were included as controls as were uninfected cells treated with compound. The cell concentration was chosen so that the number of cells/mL increased by a factor of 10 during the 4 days of incubation in the mock-infected cultures. Virus inoculum was adjusted to cause cell death in 90% of the target cells after 4 days of incubation. The virus was adsorbed to a 10-fold concentrated cell suspension at 37 °C for 1 h. Then, the infected cells were diluted 1:10 and added to microtiter plates containing the test compounds. Thus, compounds were added postadsorption.

The procedure was adopted for determination of herpes simplex virus type I (HSV-1) induced cytopathic effects in HUT78 cells, a T4-cell line derived from cutaneous T cell lymphoma. HSV-1 was found to cause death of these cells after 4 days of incubation. Inhibition of cell proliferation was measured as described above for MT4 cells by comparing cell numbers of drug treated and untreated uninfected control cells after 4 days by viability staining.

Acknowledgment. We thank Mrs. E. Mlynar and Mrs. M. Figl for careful technical assistance. Moreover, J.E. gratefully acknowledges a grant from the Republic of Austria (Nord-Süd Dialog Programm).

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4-Methyl-3-(arylsulfonyl)furoxans: A New Class of Potent Inhibitors of Platelet Aggregation

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A series of 4-methyl-3-(arylthio)furoxans were synthesized by oxidation of 1-(arylthio)-2-methylglyoxymes with dinitrogen tetroxide. Reduction with trimethyl phosphite of the furoxan derivatives afforded the corresponding furazans, while oxidation with an equimolar amount of 30% hydrogen peroxide in acetic acid or with an excess of 81% hydrogen peroxide in trifluoroacetic acid afforded the corresponding arylsulfinyl and arylsulfonyl analogues, respectively. All the furoxan and furazan derivatives showed activity as inhibitors of platelet aggregation. 4-Methyl-3-(arylsulfonyl)furoxans were the most potent derivatives of the series. 4-Methyl-3-(phenylsulfonyl)furoxan (**10a**), one of the most active derivatives, inhibits the AA-induced increase of cytosolic free Ca^{2+} and production of malondialdehyde. A primary action of the compound on cyclooxygenase is excluded, as a stable epoxymethano analogue of prostaglandin H_2 does not reverse the inhibitory effect of **10a**. This compound produces a significant increase in cGMP which is likely to cause inhibition at an early stage of the platelet activation pathway.

Several events are involved in the stimulus–response coupling in platelets. The major metabolic responses are phosphoinositide metabolism, increase of cytosolic calcium, protein phosphorylation, arachidonic acid release, and prostanoid synthesis.[1,2] These responses are not a linear progression of events, but probably there is a high degree of cooperativity and feed-back among them.[3] The cyclic nucleotides cAMP and cGMP play important regulatory roles in platelets. Increases in either cAMP and cGMP inhibit platelet activation through inhibition of agonist-induced Ca^{2+} elevation.[4]

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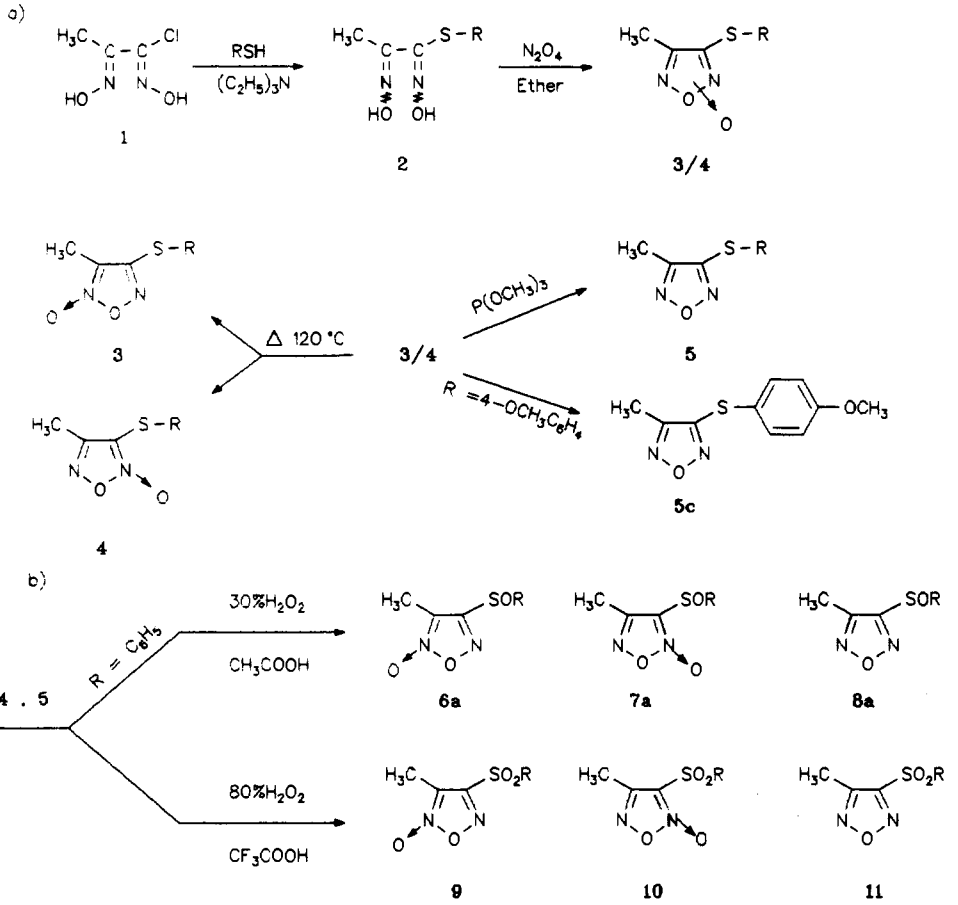
Scheme I



Today there is a great interest in searching for new structures able to inhibit platelet aggregation, since they could be potentially used in the prevention or in the treatment of thrombotic diseases.

During our study of the pharmacochemistry of furoxan ring we designed, as potential analgesic and antiinflammatory agents, a few furoxan compounds having as substituents $S(O)_n$ (n = 0, 1, 2) functions, likewise to what reported in literature for some [aryl-$S(O)_n$]imidazoles (n = 0, 1, 2).[5] Since in the early stage of this work we noticed that some of the derivatives we designed were potent inhibitors of platelet aggregation, we decided to look deeper into this aspect. In this paper we report the synthesis of a series of (arylsulfonyl)furoxans and -furazans and studies on their ability to prevent arachidonic acid (AA) induced platelet aggregation.

Chemistry

The arylthio intermediates **3–5** used to prepare the final arylsulfonyl derivatives **9–11** were synthesized according to Scheme Ia.

The starting materials used to obtain compounds **3–5** were 1-(arylthio)-2-methylglyoximes (**2**). These latter compounds were synthesized starting from *anti*-1-chloro-2-methylglyoxime (**1**) and the appropriate thiols, in ether solution, in the presence of triethylamine. By oxidation of **2** with dinitrogen tetroxide a mixture of furoxans **3/4** was obtained. This mixture, when reduced with trimethyl phosphite, gave furazans **5** or, when resolved by flash chromatography, afforded furoxan isomers **3** and **4**. Before the chromatographic resolution the original mixture of **3/4** was heated at 120 °C and allowed to reach thermodynamic equilibrium composition. 3-Methyl-4-[(4-methoxyphenyl)thio]furazan (**5c**) was prepared by reducing the mixture **3c/4c** with stannous chloride.

Oxidation of arylthio derivatives **3–5** (R = C_6H_5) with equimolar amounts of 30% hydrogen peroxide in acetic acid solution afforded the corresponding phenylsulfinyl analogues **6a**, **7a**, and **8a** in fair yields. By contrast, an excess of 81% hydrogen peroxide in trifluoroacetic acid solution afforded the arylsulfonyl derivatives **9–11** in high yield (Scheme Ib).

Analytical characterization of compounds **2–11** is reported in Table I. ^{13}C NMR data of the phenyl derivatives **3a–11a** are shown in Table II.

Biological Results and Discussion

Sulfonyl derivatives and phenylthio and phenylsulfinyl analogues described in the present work were examined for their inhibition of arachidonic acid (AA) induced human platelet aggregation.

A typical antiaggregation experiment is shown in Figure 1. The results expressed as IC_{50} are collected in Table III. Table III shows that 4-methyl-3-(phenylsulfonyl)furoxan (**10a**) is a potent inhibitor of platelet aggregation induced by AA, triggering its action at a concentration close to those of dazoxiben (inhibitor of thromboxane synthase[9]),

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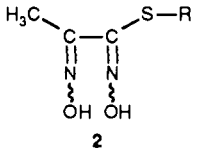
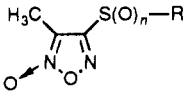
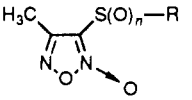
Table I. Analytical Characterization of Compounds 2-11



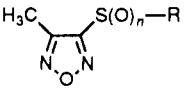
2



3, 6, 9



4, 7, 10



5, 8, 11

compds	n	R	mp, °C	recrystn solvent	formula	M+	yields, %	analyses[e]
2a		C_6H_5	161-162	EtOAc/PE[a]	$C_9H_{10}N_2O_2S$	210	92	C,H,N
2b		p-$CH_3C_6H_4$	192-193	EtOAc/PE	$C_{10}H_{12}N_2O_2S$	224	93	C,H,N
2c		p-$CH_3OC_6H_4$	170-171	EtOAc/PE	$C_{10}H_{12}N_2O_3S$	240	89	C,H,N
2d		p-ClC_6H_4	185-186	EtOAc/PE	$C_9H_9N_2O_2SCl$	244	95	C,H,N
2e		p-FC_6H_4	173-174	EtOAc/PE	$C_9H_9N_2O_2SF$	228	90	C,H,N
3a	0	C_6H_5	87[b]	$CHCl_3$/PE	$C_9H_8N_2O_2S$	208	40	
3b	0	p-$CH_3C_6H_4$	52-53[c]	MeOH/H_2O	$C_{10}H_{10}N_2O_2S$	222	49	
3c	0	p-$CH_3OC_6H_4$	73-74	$CHCl_3$/PE	$C_{10}H_{10}N_2O_3S$	238	50	C,H,N
3d	0	p-ClC_6H_4	49-50[c]	MeOH/H_2O	$C_9H_7N_2O_2SCl$	242	45	
3e	0	p-FC_6H_4	58-60[c]	MeOH/H_2O	$C_9H_7N_2O_2SF$	226	42	
4a	0	C_6H_5	oil		$C_9H_8N_2O_2S$	208	31	C,H,N
4b	0	p-$CH_3C_6H_4$	oil		$C_{10}H_{10}N_2O_2S$	222	32	C,H,N
4c	0	p-$CH_3OC_6H_4$	oil		$C_{10}H_{10}N_2O_3S$	238	20	C,H,N
4d	0	p-ClC_6H_4	44-45	MeOH/H_2O	$C_9H_7N_2O_2SCl$	242	39	C,H,N
4e	0	p-FC_6H_4	oil		$C_9H_7N_2O_2SF$	226	30	C,H,N
5a	0	C_6H_5	27-28[b]	MeOH/H_2O	$C_9H_8N_2OS$	192	90	
5b	0	p-$CH_3C_6H_4$	19-20[c]	MeOH/H_2O	$C_{10}H_{10}N_2OS$	206	90	
5c	0	p-$CH_3OC_6H_4$	44-45	PE	$C_{10}H_{10}N_2O_2S$	222	45	C,H,N
5d	0	p-ClC_6H_4	47-48[c]	MeOH/H_2O	$C_9H_7N_2OSCl$	226	90	
5e	0	p-FC_6H_4	oil		$C_9H_7N_2OSF$	210	90	C,H,N
6a	1	C_6H_5	54-55	MeOH/H_2O	$C_9H_8N_2O_3S$	224	27	C,H,N
7a	1	C_6H_5	56-57	MeOH/H_2O	$C_9H_8N_2O_3S$	224	35	C,H,N
8a	1	C_6H_5	oil		$C_9H_8N_2O_2S$	192	35	C,H,N
9a	2	C_6H_5	89-90[b]	MeOH/H_2O	$C_9H_8N_2O_4S$	240	87	
9b	2	p-$CH_3C_6H_4$	117-118[c]	MeOH/H_2O	$C_{10}H_{10}N_2O_4S$	254	70	
9c	2	p-$CH_3OC_6H_4$	88-89	MeOH/H_2O	$C_{10}H_{10}N_2O_5S$	270	85	C,H,N
9d	2	p-ClC_6H_4	119-120[c]	MeOH/H_2O	$C_9H_7N_2O_4SCl$	274	90	
9e	2	p-FC_6H_4	100-101[c]	MeOH/H_2O	$C_9H_7N_2O_4SF$	258	87	
9f	2	C_2H_5	38-40[d]					
10a	2	C_6H_5	98-99[b]	MeOH/H_2O	$C_9H_8N_2O_4S$	240	87	
10b	2	p-$CH_3C_6H_4$	118-119[c]	MeOH/H_2O	$C_{10}H_{10}N_2O_4S$	254	75	
10c	2	p-$CH_3OC_6H_4$	101-102	MeOH/H_2O	$C_{10}H_{10}N_2O_5S$	270	87	C,H,N
10d	2	p-ClC_6H_4	130-132[c]	MeOH/H_2O	$C_9H_7N_2O_4SCl$	274	90	
10e	2	p-FC_6H_4	99-100[c]	MeOH/H_2O	$C_9H_7N_2O_4SF$	258	85	
10f	2	C_2H_5	oil[d]		$C_5H_8N_2O_4S$	192		C,H,N
11a	2	C_6H_5	30-31[b]	MeOH/H_2O	$C_9H_8N_2O_3S$	224	95	
11b	2	p-$CH_3C_6H_4$	77-78[c]	MeOH/H_2O	$C_{10}H_{10}N_2O_3S$	238	90	
11c	2	p-$CH_3OC_6H_4$	67-68	MeOH/H_2O	$C_{10}H_{10}N_2O_4S$	254	90	C,H,N
11d	2	p-ClC_6H_4	100-101[c]	MeOH/H_2O	$C_9H_7N_2O_3SCl$	258	93	
11e	2	p-FC_6H_4	67-68[c]	MeOH/H_2O	$C_9H_7N_2O_3SF$	242	90	
11f	2	C_2H_5	28[d]					

[a] PE = petroleum ether, 40-60 °C. [b] Compounds obtained in ref 7 by a different synthetic pathway. The melting points and the spectral data agree. [c] Compounds obtained in ref 6 by a different synthetic pathway. The melting points and the spectral data agree. [d] Compounds prepared according to ref 7. **10f** was isolated by column chromatography (eluent petroleum ether–methylene chloride, 70:30). [e] Elemental analyses agree with calculated values within ±0.4%.

aspirin (inhibitor of cyclooxygenase[10]), and sodium nitroprusside (SNP), a potent antiaggregatory agent, whose action is mediated by a cGMP level increase.[11] The corresponding 3-methyl isomer **9a** and the furazan analogue **11a** are about 10 times less effective.

Lowering the oxidation level of the sulfur atom reduces the activity of **10a**. In fact the sulfoxide **7a** and the sulfide **4a** show similar antiaggregation action, 10 times lower than that shown by the lead **10a**. The corresponding 3-methyl isomers **6a** and **3a** and the analogue furazans **8a** and **5a** show the same activity level.

Substitution of the phenyl moiety in **10a** with an ethyl group to give **10f** does not substantially modify the activity. This same situation occurs moving from **9a** to **9f**, while **11f** appears to be less active than its analogue **11a**.

Modification of the phenyl substitution pattern in **9a**, **10a**, and **11a** does not influence activity. Therefore the low precision of the biological assay prevented us from finding in each series of sulfonyl derivatives any clear correlation between IC_{50} and physicochemical properties of the substituents.

The SAR picture discussed above shows that the antiaggregatory action is maximized by the presence of the substructure I.

Data reported here indicate that 3-(arylsulfonyl)-4-methylfuroxans represent a new effective class of antiaggregatory derivatives.

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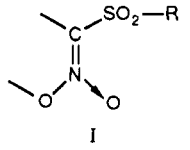

I

Compound **10a**, one of the most active derivatives of the series, increases the intracellular cGMP levels in a dose-dependent manner (Table IV) and promotes a dose-dependent inhibition of AA-induced $[Ca^{2+}]_i$ increase (Table V) similar to SNP and related vasodilators.[11]

We carried out on **10a** an extensive biochemical characterization whose details are reported in ref 12.

Among the results obtained, it is interesting to point out that the effect on the platelet aggregation of this compound is also evident with other agonists like collagen, ADP, or thrombin. Measurements of MDA production in 1 mM AA-stimulated PRP (0.41 ± 0.08 μmol MDA per 5 min per 10^{11} cells) showed a total inhibition of AA-induced MDA formation. In order to investigate on the possible involvement of cyclooxygenase in the inhibition mechanism, U-46619 (the stable epoxymethane analogue of cyclic endoperoxide PGH_2 which stimulates $[Ca^{2+}]$ influx and aggregation in aspirin-treated platelets[1]) was used. **10a**-dependent inhibition of AA-induced aggregation was not reversed by repetitive addition of U-46619. This finding strongly suggests that **10a** effect is independent of the inhibition of cyclooxygenase, although it does not exclude thromboxane synthase involvement. However, when 1 μM U-46619 was used to stimulate platelet-rich plasma (PRP), **10a** inhibited the U-446619-induced aggregation.

These findings suggest that the inhibitory effect of **10a** may involve an early step in the pathway of platelet activation. The increased level of intracellular cGMP may be a possible candidate for this effect, as cGMP appears to act at an early point in the cell activation cascade.[13]

At the moment we have no evidence as to whether these compounds increase cGMP by NO release[14] or by a direct interaction with guanylate cyclase. Studies are in progress to investigate this point.

Experimental Section

Chemistry. Melting points were taken on a capillary melting point apparatus (Büchi 530) and are uncorrected. All compounds were routinely checked by IR spectrophotometry (Perkin-Elmer Model 781), ^1H and ^{13}C NMR (Bruker AC-200) spectroscopy, and mass spectrometry (Varian CH7 MAT). The spectra are in keeping with the proposed structures. Silica gel (Merck Kieselgel 100), 70–230 mesh ASTM, was employed for column chromatography. TLC was carried out on 5 × 20 plates precoated with Merck silica gel 60 F_{254}, layer thickness 0.25 mm. Anhydrous magnesium sulfate was used as drying agent. Petroleum ether, bp 40–60 °C, was used for chromatographic purifications and crystallizations. Derivative 1 was synthesized according to ref 15. Dinitrogen tetroxide was distilled from phosphorus pentoxide.

General Method for the Preparation of Dioxime Derivatives 2a–e. To a stirred solution of 1 (0.27 g, 2.00 mmol) and of the appropriate 4-substituted thiophenol in ether (18 mL) was added triethylamine (0.54 mL, 4.00 mmol) dissolved in 1.5 mL of ether.

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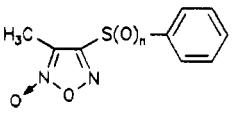
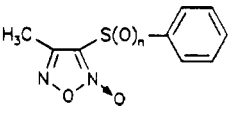
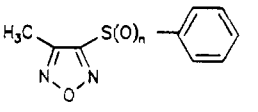
Table II. ^{13}C Chemical Shifts of the Derivatives **3a–11a** (δ, ppm from TMS)[a]



3a,6a,9a



4a,7a,10a



5a,8a,11a

compd	*n*	CH_3	CCH_3	CS(O)*n*	C-1	C-2/6	C-3/5	C-4
3a	0	7.6	113.2	154.3	127.3	132.5*	129.7*	129.3
4a	0	11.4	156.5	111.4	129.3	131.0*	129.6*	128.8
5a	0	8.1	151.4*	151.2*	128.6	132.1*	129.5*	128.9
6a	1	7.4	110.3	161.6	139.3	124.2	129.8	132.2
7a	1	11.5	152.1	118.9	139.3	123.8	129.7	132.5
8a	1	8.3	149.8	157.6	140.0	124.1	129.6	131.9
9a	2	7.9	109.8	158.9	137.0	128.8*	129.7*	135.6
10a	2	12.2	151.7	117.6	137.1	128.4*	129.5*	135.6
11a	2	8.5	149.1	156.6	137.8	128.6*	129.6*	135.2

*Tentatively assigned. [a] The assignments of the furoxan carbons were done according to criteria reported in ref 8.

Table III. Effects on Platelet Aggregation of (Phenylthio) (**3a–5a**), (Phenylsulfinyl)- (**6a–8a**), and (Arylsulfonyl) Furoxan (**9a–11f**) and Furazan Derivatives

compd	IC_{50},[a] μM	compd	IC_{50},[a] μM
3a	42 (±19)	**9c**	27 (±10)
4a	45 (±21)	**10c**	3.7 (±2.2)
5a	34 (±18)	**11c**	24 (±6)
6a	38 (±12)	**9d**	34 (±18)
7a	37 (±12)	**10d**	1.4 (±0.9)
8a	32 (±16)	**11d**	41 (±18)
9a	44 (±19)	**9e**	41 (±18)
10a	3.6 (±1.8)	**10e**	2.6 (±2.1)
11a	35 (±18)	**11e**	67 (±14)
9b	23 (±7)	**9f**	21 (±6)
10b	1.2 (±0.6)	**10f**	1.8 (±0.75)
11b	53 (±24)	**11f**	100 (±20)
SNP[b]	1.0 (±0.5)		

[a] Data represent means ± SD of four or five experiments. [b] Sodium nitroprusside.

The reaction mixture was stirred for 1 h at room temperature and then was washed with 0.5 N sulfuric acid, sodium bicarbonate solution, and finally with water. The ether layer, dried over magnesium sulfate, was evaporated in vacuo. The residue recrystallized gave the title products. Yields and characterization of the products are reported in Table I.

General Method for the Preparation of 4-(Arylthio)-3-methylfuroxans (3a–e) and of 3-(Arylthio)-4-methylfuroxans (4a–e). An ice–water cooled, stirred solution of the appropriate dioxime (3.93 mmol) in dry ether (2.5 mL) was treated with dinitrogen tetroxide (0.30 mL, 4.72 mmol). The reaction solution was stirred for 1 h at 0–5 °C and was then left at room temperature for 16 h. The ether was removed in vacuo and the residue was treated with water and extracted with methylene chloride. The combined organic layers were distilled in vacuo to give a mixture of the two isomeric furoxans **3**/4 in the approximate ratio of 3/1 by NMR detection. (A ratio of 7/1 was observed for the mixture **3c**/4c.) This mixture was heated at 120 °C for 2 h in order to reach a thermodynamic equilibrium composition.

Flash chromatography (eluting with petroleum ether–methylene chloride, 70:30, 4-methyl isomers first eluted) gave the title compounds.

Yields and characterization of the products are reported in Table I.

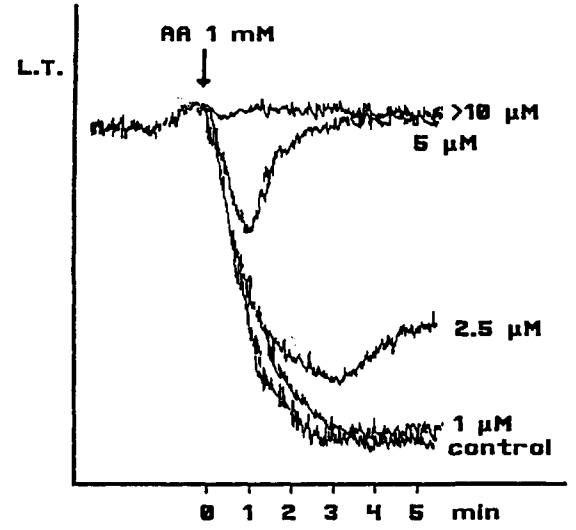


Figure 1. Aggregation traces of human PRP challenged with 1 mM arachidonic acid (AA). The PRP was preincubated for 5 min with **10a** and then stimulated with AA. The IC_{50} for all compounds tested was calculated from a similar set of data. L.T. = light transmission.

Table IV. Effect of **10a** on the Intracellular cGMP Level in Human Platelets

10a, μM	0	1	10	100
cGMP pmol/10^9 cells[a]	0.24	0.53	1.1	3.1

[a] Data are means from two separate experiments performed in triplicate.

Table V. Effect of **10a** on the $[Ca^{2+}]_i$ in Human Platelets during AA-Induced Aggregation[a]

10a, μM	0	5	10	20
$[Ca^{2+}]_i$, μM	0.68 ± 0.12	0.42 ± 0.07	0.23 ± 0.06	0.12

[a] Basal value before stimulation is 0.12 ± 0.01 μM. In the table are reported the maximal values reached 1 min after AA (10 μM) addition. Data represent means ± SD of four experiments.

General Method for the Preparation of 3-(Arylthio)-4-methylfurazans (5a,b,d,e). The mixture of the appropriate parent furoxans was reduced using trimethyl phosphite according to the procedure reported in refs 6 and 7. Yields and characterization of products are reported in Table I.

3-[(4-Methoxyphenyl)thio]-4-methylfurazan (5c). A mixture of the two isomer furoxans **3c/4c** (2.00 g, 8.40 mmol) was added to a solution of stannous chloride dihydrate (22.75 g, 0.101 mol) in 37% hydrochloric acid (12 mL) and acetic acid (50 mL). The reaction mixture was kept at 95 °C for 48 h, cooled, and poured into water. The resulting mixture was extracted with ether. The combined ether layers were washed with water, dried, and evaporated in vacuo. The residue was purified by flash chromatography (eluting with petroleum ether–chloroform, 70:30). Yields and characterization of the products are reported in Table I.

General Method for the Preparation of 3-Methyl-4-(phenylsulfinyl)furoxan (6a), 4-methyl-3-(phenylsulfinyl)furoxan (7a), and 3-methyl-4-(phenylsulfinyl)furazan (8a). Hydrogen peroxide (30%, 0.50 mL, 4.9 mmol) was added to a solution of the appropriate sulfide (4.80 mmol) in acetic acid (10 mL). The reaction flask was stoppered and kept at room temperature for 48 h. The reaction solution was treated with an ice–water mixture and then extracted with ether. The organic layers were washed with sodium bicarbonate solution, dried, and evaporated in vacuo. The sulfoxide was separate from the large amount of unreacted sulfide and from minor amount of sulfone by flash chromatography (eluting with petroleum ether–chloroform, 65:35). Yields and characterization of the products are reported in Table I.

General Method for the Preparation of 3-(Arylsulfonyl)-4-methylfurazans (11a–e), 4-(Arylsulfonyl)-3-methylfuroxans (9a–e) and 3-(Arylsulfonyl)-4-methylfuroxans (10a–e). Hydrogen peroxide (81%, 0.3 mL, 9.6 mmol) was added to a stirred solution of the appropriate sulfide (2.40 mmol) dissolved in trifluoroacetic acid (5.0 mL). After 1 h, the reaction solution was poured into water–ice mixture. The resulting precipitate was collected by filtration. Yields and characterization of the products are reported in Table I.

Platelet Aggregation. Human blood from healthy volunteers who had not taken any aspirin-like drug during the previous two weeks, was collected in one-tenth volume of 3.8% trisodium citrate in plastic tubes. Platelet-rich plasma (PRP, pH 7.6) was prepared by centrifugation at room temperature for 15 min at 200*g*. Platelet-poor plasma (PPP) was prepared by subsequent centrifugation at 1500*g* for 10 min. Aggregation studies in PRP were performed according to the light transmission method of Born in a dual channel aggregometer (Elvi 840, Elvi Logos, Milan).

Arachidonic acid (AA) (1 mM) was used as inducer. All the drugs tested were dissolved in DMSO–ethanol and added to PRP 5 min prior to agonist. Control samples received in parallel the same volume addition of DMSO–ethanol. Identical platelet count in corresponding samples was adjusted. All aggregation studies were repeated at least four times with platelets obtained from different donors.

Measurements of Cytoplasmic Free Calcium $[Ca^{2+}]_i$. Washed platelets were prepared from fresh human blood anticoagulated with 0.15 volumes of ACD (85 mM trisodium citrate, 111 mM dextrose, 71 mM citric acid). PRP (pH 6.8) was obtained by centrifugation at 200*g* for 15 min at room temperature. The platelets were loaded with quin2 by incubating the PRP at 37 °C for 30 min with 15 μM quin2 AM (acetoxymethyl ester)[16] and pelleted by centrifugation at 800*g* at room temperature for 15 min. The supernatant was discarded, and the cells were gently resuspended in a physiological saline containing 145 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 10 mM Na-Hepes, 10 mM glucose, pH 7.4 at 37 °C. The platelet suspension was adjusted to a density of approximately 2×10^{10} cells/mL and kept at room temperature in a plastic tube until use. The experiments with quin2-loaded platelets were carried out within 40 min after final resuspension, as platelet responsiveness was constant during that period. $CaCl_2$ (1 mM) was added, and the cells were equilibrated at 37 °C for 3 min before addition of agonists. The drugs tested were added to platelet suspensions 5 min prior to agonist; control samples received in parallel the same volume addition of DMSO–ethanol, and the final solvent concentration never exceeded 0.1%. Measurements of $[Ca^{2+}]_i$ from the fluorescence of intracellular quin2 was performed as described[16] in a Perkin-Elmer LS-5 spectrofluorimeter (Perkin-Elmer Corp., Norwalk, CT); the fluorimetric cuvette holder was thermostated (37 °C); standard monocromator settings were 339-nm excitation (5-nm slit) and 500-nm emission (10-nm slit). Calibration of the fluorescence signal in terms of $[Ca^{2+}]_i$ was performed as described.[16] F_{max} was obtained by adding a large concentration of calcium ionophore (ionomycin) in the presence of 1 mM external Ca^{2+}; to obtain F_{min}, the dye fluorescence was quenched by addition of 2 mM free Mn^{2+}, which gave just the autofluorescence of the cells. Quin2 release into the medium during incubation with the inhibitors tested was negligible, and autofluorescence of unloaded cells was only slightly affected.

cGMP Measurement. For the determination of platelet cGMP content, citrated PRP was incubated (37 °C) with the drug tested at different concentrations. After incubation, ice-cold ACD was added to PRP, platelets were quickly pelleted, the supernatant was aspirated, and 0.5 mL of ice-cold 6% trichloroacetic acid was added to the pellet. After freeze–thawing, the cellular extracts were centrifugated at 2000*g* for 10 min at 4 °C. The trichloroacetic acid was removed from the supernatant fraction by extracting the samples six times with 2 mL of water–saturated ether, and the samples were kept frozen (–20 °C) until analysis. The amount of intracellular cGMP was measured with the Amersham cGMP radioimmunoassay system, and the results were expressed as picomoles of cGMP per 10^9 cells.

(16) Hallam, T. J.; Sanchez, A.; Rink, T. J. Stimulus-response coupling in human platelets. Changes evoked by platelet-activating factor in cytoplasmic free calcium monitored with the fluorescent calcium indicator quin2. *Biochem. J.* 1984, *218*, 819–827.