Novel Arylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxides as Platelet Aggregation Inhibitors. 2. Optimization by Quantitative Structure−Activity Relationships‡

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In the previous paper (Part 1), we described the synthesis and antiplatelet activity of a series of phenyl- and heteroarylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxides. In this paper, we report the optimization of the platelet aggregation inhibitory activity by an iterative sequence of quantitative structure−activity relationship studies which encompassed synthesis and evaluation of the effects of structure variations at the 1-, 6-, and 7-positions of the heterocyclic system. A model has been established that correctly correlates antiplatelet activity in this series with the partial atomic charges calculated by a local density functional ab initio method. As a result of this study, the experimental platelet aggregation inhibitory activity of the lead compound was improved 300-fold.

Introduction

In a previous paper,[1] we described the synthesis and pharmacological evaluation of a new family of platelet aggregation inhibitors derived from 6- or 7-phenylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide. The most promising compounds of the series were N-1-substituted 6,7-diphenyl- and 6-phenylpyrazino[2,3-*c*][1,2,6]thiadiazine derivatives which showed significant inhibition of platelet aggregation. The fact that pyrazinothiadiazines are in general deprived of significant toxicity, together with the preliminary results obtained ex vivo of their inhibition of platelet aggregation induced by different agonists, prompted us to optimize this series and to design new active compounds by means of a QSAR study.

The general methodology for this kind of work has been followed establishing a mathematical model which correlates the pharmacological activity of the compounds with some parameter that describes the structural features.[2,3] For the measurement of the pharmacological activity, a quick simple method, i.e., determination of the EC_{100}, has been used, and as structural descriptors the partial atomic charges obtained by quantum mechanical calculations were used. The initial model was then used to design compounds with a predicted increased activity which were synthesized and their experimental antiplatelet properties determined and confirmed. These results were used to establish the final model which was validated from a statistical point of view.

QSAR Study

Training Set. Compound **1**, 1-ethyl-6,7-diphenylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide, with an EC_{100} = 0.3 μg/mL was chosen as the lead compound. One possible way to optimize a lead when no structural information about the target is available is to modify the structure by variations of the substituents at particular sites. Thus, in a first approach, three different positions of the pyrazinothiadiazine system were considered responsible for modulating the biological activity and were subject to modification.

In position 1 different groups such as methyl, ethyl, *tert*-butoxy- and methoxycarbonylmethyl, carbamoylmethyl, and benzyl were chosen since from our previous experience the corresponding *N*(1)*H*-pyrazino[2,3-*c*]-[1,2,6]thiadiazine derivatives are devoid of activity. For positions 6 and 7, a variety of alkyl, aryl, halogen, alkoxy, and hydrogen were considered with the restriction that at least one of them should be an aromatic group which is necessary, according to our preliminary results for antiplatelet activity.

Taking into account all these facts, we selected among the different pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxides previously synthesized by us a first training set of 17 derivatives (**1**−**17**) which are gathered in Table 1.

Pharmacological Activity. As pharmacological response we have used the effective concentration (EC_{100}, μg/mL) which produced a 100% of inhibition of platelet aggregation (rabbit platelet-rich plasma) induced by arachidonic acid.[4] This relatively simple method for determining the platelet aggregation inhibitory activity is sufficient for our mathematical model, and as already mentioned, we have previously observed that compounds giving positive results in this assay also show interesting properties ex vivo.

Structural Parameters. The standard Hansch parameters were not suitable for these molecules,[2] so we decided to use data derived from quantum mechanical ab initio calculations to correctly describe the structure. For each of the 17 selected compounds, the Hirshfeld partial charges of each atom of the heterocycle were calculated by a local density functional (LDF) ab initio method[5−7] using the DMol program.[8] The feasability of this method for calculating molecules incorporating the N−SO_2−N rest had already been proved

‡ This paper is dedicated to Professor José Elquero on his 65th birthday.

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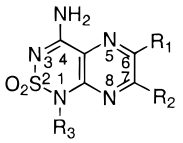
Table 1. Experimental and Calculated EC_{100} and Residual of Compounds **1**−**45** from Eq 1 and Final Model Eq 2



					eq 1			eq 2		
compd	R_1	R_2	R_3	EC_{100}[a]	resid	fitted	pred	resid	fitted	pred
1[b,c]	Ph	Ph	Et	0.3	−0.428	0.728	•	−0.842	1.142	•
2[b,c]	Ph	Ph	CH_2Ph	3.0	0.829	2.171	•	0.205	2.795	•
3[b,c]	Ph	Ph	CH_2CO_2Et	0.1	−1.035	1.135	•	−1.012	1.112	•
4[b,c]	Ph	Ph	$CH_2CO_2{}^tBu$	1.0	−0.262	1.262	•	−0.302	1.302	•
5[b,c]	Ph	Ph	CH_2CONH_2	3.0	1.650	1.350	•	1.562	1.438	•
6[b,c]	Ph	H	Me	0.3	−0.673	0.973	•	−0.723	1.023	•
7[b,c]	Ph	H	Et	1.0	0.589	0.411	•	0.550	0.450	•
8[b,c]	Ph	Me	Et	1.0	−0.747	1.747	•	−1.090	2.090	•
9[b,c]	Me	Ph	Et	1.0	−0.376	1.376	•	−0.803	1.803	•
10[b,c]	Br	Ph	CH_2CO_2Et	10	−0.688	10.688	•	−0.345	10.345	•
11[b,c]	Cl	Ph	Et	10	0.651	9.349	•	1.039	8.961	•
12[b,c]	2-pyridyl	2-pyridyl	Me	3.0	0.414	2.586	•	0.674	2.327	•
13[b,c]	2-thienyl	2-thienyl	Et	3.0	0.076	2.924	•	−0.621	3.621	•
14[b]	H	Ph	Et	>10	•	•	18.436	•	•	17.616
15[b]	H	Ph	CH_2CO_2Et	>10	•	•	16.949	•	•	15.947
16[b]	2-furyl	2-furyl	Me	1.0	•	•	2.895	•	•	3.611
17[b]	Br	Ph	Et	1.0	•	•	13.247	•	•	12.479
18[c]	4-MeOPh	4-MeOPh	Me	0.001	•	•	−1.538	0.569	−0.568	•
19[c]	2-FPh	H	Me	0.03	•	•	−0.233	0.292	−0.262	•
20[c]	Ph	OMe	Me	0.1	•	•	−1.459	0.150	−0.050	•
21[c]	4-FPh	4-FPh	Me	0.3	•	•	0.263	−0.350	0.650	•
22[c]	4-ClPh	4-ClPh	Et	3.0	•	•	2.180	0.616	2.384	•
23[c]	4-BrPh	4-BrPh	Me	1.0	•	•	0.303	0.424	0.576	•
24[c]	Ph	OEt	Me	0.30	•	•	−1.419	0.230	0.070	•
25[c]	4-ClPh	OEt	Et	10	•	•	12.423	−0.223	10.223	•
26	H	4-MePh	Me	>10	•	•	17.037	•	•	16.658
27	3-MeOPh	3-MeOPh	Me	>10	•	•	−2.512	•	•	−1.773
28[d]	4-ClPh	4-ClPh	Me	NDe	•	•	2.426	•	•	2.494
29[d]	2-ClPh	2-ClPh	Me	ND	•	•	6.828	•	•	6.438
30[d]	Ph	4-ClPh	Me	ND	•	•	1.104	•	•	1.424
31[d]	4-ClPh	Ph	Me	ND	•	•	1.402	•	•	1.706
32[d]	Ph	4-NO_2Ph	Me	ND	•	•	1.452	•	•	1.240
33[d]	4-NO_2Ph	Ph	Me	ND	•	•	2.999	•	•	2.909
34[d]	4-ClPh	4-MePh	Me	ND	•	•	1.075	•	•	1.564
35[d]	4-MePh	4-ClPh	Me	ND	•	•	0.978	•	•	1.368
36[d]	Ph	4-HOPh	Me	ND	•	•	0.360	•	•	1.100
37[d]	H	4-ClPh	Me	ND	•	•	17.016	•	•	16.417
38[d]	H	4-NO_2Ph	Me	ND	•	•	17.651	•	•	16.270
39[d]	H	3-NO_2Ph	Me	ND	•	•	17.584	•	•	16.434
40[d]	Cl	4-ClPh	Et	ND	•	•	9.232	•	•	8.896
41[d]	4-MePh	H	Me	ND	•	•	1.125	•	•	1.268
42[d]	4-MeOPh	H	Me	ND	•	•	1.740	•	•	1.953
43[d]	4-ClPh	H	Et	ND	•	•	2.705	•	•	2.606
44[d]	4-NO_2Ph	H	Et	ND	•	•	3.549	•	•	3.039
45[d]	3-NO_2Ph	H	Me	ND	•	•	3.475	•	•	3.118

[a] Activity expressed as the EC_{100} (μg/mL), effective concentration which produced 100% inhibitory response. ASA, EC_{100} = 2.5 μg/mL. [b] Compounds included in ref 1. [c] Compounds included in the model 2. [d] Compounds not synthesized. [e] Not determined.

by us, in a previous study in which different quantum mechanical calculations were tested on related substances.[9,10]

QSAR Analysis

The structures and experimental platelet aggregation inhibitory activities of the training set compounds (**1**−**17**) are gathered in Table 1, and the values of the calculated charges are in Table 2.

Different models involving combinations of all the partial atomic charges were tested according to standard deviation (s) and correlation coefficient (R^2) criteria. The initial models tested were those including the variables which showed a higher standard deviation and no significant collinearity. In the best model found, the platelet aggregation inhibitory activity can be expressed as a function of the atomic charges of N5, C7, C4a, N1, and C6 atoms by the following eq 1 (model 1):

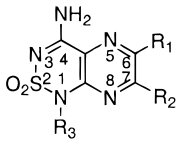
$$EC_{100} = -18.04 + 1719.91X_1 + 4482.12X_2 + 40658.12X_3 - 79373.68X_4 \quad (1)$$

$$n = 13\ (\mathbf{1}-\mathbf{13})\ R^2 = 0.95\ R_a{}^2 = 0.92\ s = 0.96\ F_{4,8} = 35.39\ p < 0.0001$$

$$X_1 = (\mathrm{N5})^2\ X_2 = (\mathrm{N5})^2\mathrm{C7}\ X_3 = (\mathrm{N5})^2\mathrm{C4a}\ X4 = (\mathrm{N1})^2\mathrm{C6}$$

It should be noted that, although traditional QSAR is

Table 2. Hirshfeld Partial Charges (au) of Candidate Compounds **1**−**45**[a]



compd	R_1	R_2	R_3	C-4a	N-5	C-6	C-7	N-1
1	Ph	Ph	Et	0.01510	−0.1058	0.0399	0.0620	−0.0575
2	Ph	Ph	CH_2Ph	0.01250	−0.1087	0.0401	0.0656	−0.0549
3	Ph	Ph	CH_2CO_2Et	0.01900	−0.1041	0.0429	0.0648	−0.0568
4	Ph	Ph	$CH_2CO_2{}^tBu$	0.01840	−0.1054	0.0422	0.0648	−0.0582
5	Ph	Ph	CH_2CONH_2	0.01780	−0.1051	0.0427	0.0649	−0.0565
6	Ph	H	Me	0.01980	−0.1038	0.0368	0.0193	−0.0559
7	Ph	H	Et	0.02030	−0.1033	0.0367	0.0185	−0.0574
8	Ph	Me	Et	0.01490	−0.1061	0.0362	0.0701	−0.0588
9	Me	Ph	Et	0.01620	−0.1147	0.0471	0.0574	−0.0639
10	Br	Ph	CH_2CO_2Et	0.01780	−0.1182	0.0300	0.0550	−0.0610
11	Cl	Ph	Et	0.01930	−0.1222	0.0461	0.0558	−0.0613
12	2-pyridyl	2-pyridyl	Me	0.02050	−0.1010	0.0323	0.0547	−0.0556
13	2-thienyl	2-thienyl	Et	0.01180	−0.1080	0.0286	0.0444	−0.0556
14	H	Ph	Et	0.01650	−0.1132	−0.0082	0.0582	−0.0619
15	H	Ph	CH_2CO_2Et	0.01870	−0.1112	−0.0032	0.0622	−0.0585
16	2-furyl	2-furyl	Me	0.01200	−0.1099	0.0293	0.0477	−0.0598
17	Br	Ph	Et	0.02000	−0.1206	0.0311	0.0561	−0.0611
18	4-MeOPh	4-MeOPh	Me	0.01120	−0.1062	0.0387	0.0598	−0.0600
19	2-FPh	H	Me	0.02130	−0.1000	0.0366	0.0149	−0.0548
20	Ph	OMe	Me	0.00300	−0.1019	0.0325	0.1247	−0.0569
21	4-FPh	4-FPh	Me	0.01570	−0.1055	0.0388	0.0607	−0.0597
22	4-ClPh	4-ClPh	Et	0.01640	−0.1066	0.0383	0.0604	−0.0573
23	4-BrPh	4-BrPh	Me	0.01630	−0.1023	0.0386	0.0613	−0.0556
24	Ph	OEt	Me	0.00230	−0.1024	0.0321	0.1245	−0.0569
25	4-ClPh	OEt	Et	0.03200	−0.1027	0.0306	0.1246	−0.0548
26	H	4-MePh	Me	0.01350	−0.1135	−0.0090	0.0579	−0.0592
27	3-MeOPh	3-MeOPh	Me	0.01340	−0.1030	0.0423	0.0645	−0.0587
28	4-ClPh	4-ClPh	Me	0.01770	−0.1078	0.0400	0.0622	−0.0592
29	2-ClPh	2-ClPh	Me	0.01970	−0.1101	0.0314	0.0538	−0.0588
30	Ph	4-ClPh	Me	0.01600	−0.1068	0.0396	0.0610	−0.0592
31	4-ClPh	Ph	Me	0.01590	−0.1067	0.0390	0.0620	−0.0587
32	Ph	4-NO_2Ph	Me	0.02090	−0.1040	0.0418	0.0605	−0.0582
33	4-NO_2Ph	Ph	Me	0.01850	−0.1052	0.0376	0.0639	−0.0564
34	4-ClPh	4-MePh	Me	0.01440	−0.1073	0.0384	0.0616	−0.0590
35	4-MePh	4-ClPh	Me	0.01550	−0.1074	0.0398	0.0605	−0.0596
36	Ph	4-HOPh	Me	0.01270	−0.1085	0.0387	0.0607	−0.0602
37	H	4-ClPh	Me	0.01530	−0.1124	−0.0086	0.0572	−0.0571
38	H	4-NO_2Ph	Me	0.02180	−0.1095	−0.0052	0.0578	−0.0568
39	H	3-NO_2Ph	Me	0.01970	−0.1100	−0.0082	0.0574	−0.0555
40	Cl	4-ClPh	Et	0.01900	−0.1220	0.0452	0.0541	−0.0612
41	4-MePh	H	Me	0.01900	−0.1057	0.0374	0.0185	−0.0569
42	4-MeOPh	H	Me	0.01820	−0.1077	0.0365	0.0171	−0.0577
43	4-ClPh	H	Et	0.02020	−0.1047	0.0356	0.0198	−0.0535
44	4-NO_2Ph	H	Et	0.02360	−0.1013	0.0350	0.0231	−0.0501
45	3-NO_2Ph	H	Me	0.02220	−0.1042	0.0356	0.0225	−0.0534

[a] Only the values of the charges used in eqs 1 and 2 are given.

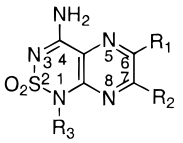
usually carried out using a logarithmic form of the biological activity, in our case, we were not able to find a correlation between the charges and log EC_{100}. However, since, as is later dealt with, it was possible with this initial model to predict the EC_{100} within this experimental domain, we decided to use it for our optimization purposes.

From a chemical point of view it is reasonable to assume that the charges at the atoms of the heterocyclic system bearing the different substituents and the contiguous atoms should be more influenced, and so it is not surprising that the model involves these particular atoms and not others.

In this first approach, we could see (Table 1) that it was possible to reproduce the pharmacological activity of the training set compounds (compare exptl EC_{100} with calcd EC_{100} derivatives **1**−**13**), except in compounds **16** and **17**. These data points were omitted from eq 1 since they showed a residual about or higher than 1.5 the standard deviation (*s*). However from this initial model, although not statistically validated, it was possible to predict those compounds devoid of activity (see the calcd EC_{100} values of derivatives **14** and **15** which show an exptl $EC_{100} > 10$, Table 1). Therefore, we decided to use this eq to identify new derivatives with an increased activity.

If we now think of structural variations at positions 1, 6, and 7 and include for these last two differently substituted aryl rests, the number of possible compounds to be synthesized is very large. This is usually the case in a QSAR study as this, and therefore, it is necessary to use some criterion in order to somehow restrict the experimental domain. In principle, we included as candidates only those compounds which could be accessible from a chemical point of view, and also we limited it to compounds bearing methyl or ethyl

Table 3. Physicochemical Data for Pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxides



compd	R_1	R_2	R_3	mp (°C)	recryst solvents	formula	M_r	anal.[a]
18	4-MeOPh	4-MeOPh	Me	255−6	MeOH/H_2O	$C_{20}H_{19}N_5O_4S$	425.47	C,H,N,S
19	2-FPh	H	Me	245−7	EOH	$C_{12}H_{10}FN_5O_4S$	307.31	C,H,N,S
20	Ph	OMe	Me	208	EtOH/H_2O	$C_{13}H_{13}N_5O_2S$	319.34	C,H,N,S
21	4-FPh	4-FPh	Me	224−6	EtOH/H_2O	$C_{18}H_{13}F_2N_5O_2S$	401.39	C,H,N,S
22	4-ClPh	4-ClPh	Et	294−6	EtOH	$C_{19}H_{15}Cl_2N_5O_2S$	448.33	C,H,Cl,N,S
23	4-BrPh	4-BrPh	Me	268−70	EtOH/H_2O	$C_{18}H_{13}Br_2N_5O_2S$	527.23	C,H,N,S
24	Ph	OEt	Me	249−50	EtOH/acetone	$C_{14}H_{15}N_5O_3S$	343.19	C,H,N,S
25	4-ClPh	OEt	Et	212	EtOH/H_2O	$C_{15}H_{16}ClN_5O_3S$	381.84	C,H,N,S
26	H	4-MePh	Me	282−4	EtOH/H_2O	$C_{13}H_{13}N_5O_2S$	303.34	C,H,N,S
27	3-MeOPh	3-MeOPh	Me	224−6	MeOH	$C_{20}H_{19}N_5O_4S$	425.47	C,H,N,S
48	4-MeOPh	4-MeOPh	H	277−8	EtOH/H_2O	$C_{19}H_{17}N_5O_4S$	411.43	C,H,N,S
49	3-MeOPh	3-MeOPh	H	251−3	MeOH	$C_{19}H_{17}N_5O_4S$	411.43	C,H,N,S
50	4-ClPh	4-ClPh	H	>350	EtOH/H_2O	$C_{17}H_{11}Cl_2N_5O_2S$	420.27	C,H,Cl,N,S
51	2-ClPh	2-ClPh	H	310	EtOH/H_2O	$C_{17}H_{11}Cl_2N_5O_2S$	420.27	C,H,Cl,N,S
52	4-FPh	4-FPh	H	>350	H_2O/EtOH	$C_{17}H_{11}F_2N_5O_2S$	387.36	C,H,N,S
53	4-BrPh	4-BrPh	H	>350	AcOH/EtOH	$C_{17}H_{11}Br_2N_5O_2S$	509.19	C,H,Br,N,S
54	Ph	4-ClPh	H	>350	EtOH/H_2O	$C_{17}H_{12}ClN_5O_2S$	385.83	C,H,Cl,N,S
55	4-ClPh	Ph	H	>350	EtOH/H_2O	$C_{17}H_{12}ClN_5O_2S$	385.83	C,H,Cl,N,S
56	Ph	4-NO_2Ph	H	338−40	MeOH/H_2O	$C_{17}H_{12}N_6O_4S$	396.38	C,H,N,S
57	4-NO_2Ph	Ph	H	>350		$C_{17}H_{12}N_6O_4S$	396.38	C,H,N,S
58	4-ClPh	4-MePh	H	331−3		$C_{18}H_{14}ClN_5O_2S$	399.50	C,H,N,S
59	4-MePh	4-ClPh	H	>350	MeOH/H_2O	$C_{18}H_{14}ClN_5O_2S$	399.50	C,H,Cl,N,S
60	Ph	4-HOPh	H	>350	EtOH/H_2O	$C_{17}H_{13}N_5O_3S$	367.38	C,H,N,S
61	H	4-MePh	H	345	EtOH/H_2O	$C_{12}H_{11}N_5O_2S$	289.32	C,H,N,S
62	H	4-ClPh	H	>350	EtOH/H_2O	$C_{11}H_8ClN_5O_2S$	309.73	C,H,Cl,N,S
63	H	4-NO_2Ph	H	>350	EtOH/H_2O	$C_{11}H_8N_6O_4S$	320.28	C,H,N,S
64	H	3-NO_2Ph	H	280−2	EtOH/H_2O	$C_{11}H_8N_6O_4S$	320.28	C,H,N,S
65	Cl	4-ClPh	H	>350	MeOH/H_2O	$C_{11}H_8Cl_2N_5O_2S$	344.17	C,H,Cl,N,S
66	4-MePh	H	H	>350	MeOH/H_2O	$C_{12}H_{11}N_5O_2S$	289.32	C,H,N,S
67	4-MeOPh	H	H	318−20	EtOH/H_2O	$C_{12}H_{11}N_5O_3S$	305.31	C,H,N,S
68	4-ClPh	H	H	>350	H_2O/EtOH	$C_{11}H_8ClN_5O_2S$	309.73	C,H,Cl,N,S
69	4-NO_2Ph	H	H	>350	EtOH/AcOH	$C_{11}H_8N_6O_4S$	320.28	C,H,N,S
70	3-NO_2Ph	H	H	328−30	H_2O/EtOH	$C_{11}H_8N_6O_4S$	320.28	C,H,Cl,N,S
71	2-FPh	H	H	330−2	EtOH/H_2O	$C_{11}H_8FN_5O_2S$	293.27	C,H,N,S

[a] Elemental analyses were within ±0.4 of the calculated values for the formulas given.

at N-1, since none of the more complicated substituents at these positions significantly modified the activity as was shown in the first part of this study.[1] For positions 6 and 7 differently substituted aromatic rings (with activating and deactivating substituents at various positions), 6-bromo, 6-chloro, 7-alkoxy, etc., were in principle plausible.

However, the problem was still the very large number of possible compounds to be synthesized, and in these cases, experimental design schemes are of great help in focusing on the most informative experiments; i.e., only those derivatives that are more representative of the experimental domain should be synthesized.[11,12]

The proposed candidate molecules **1**−**45** (45 compounds) are gathered in Table 1. The experimental domain was designed including only N-1-substituted derivatives of parent *N*(1)*H*-pyrazinothiadiazines synthesized (Table 3). Having calculated the partial atomic charges, by the ab initio LDF method used before, and taking as mathematical model eq 1, it was possible to apply the experimental design methodology[12] for selecting the compounds. Thus, derivatives **24**−**26** were chosen according to the criteria of minimization of the variance function and the efficiency *G*.[13] The solution of 16 candidate molecules showed a variance function of 0.86 and maximum efficiency (36%) in comparison to the initial solution of 13 molecules (variance function 7.45 and $G = 5\%$). Besides, derivatives **18**−**20** and **27** were also selected since they were predicted to have an improved activity according to eq 1 (Table 1). The fact that some compounds have predicted negative activities deserves comment. Although these values have no physical meaning, they lie within the range of the standard deviation. In any case, experiences with negative values are indicative of compounds with low EC_{100}, therefore high activity and thus potential candidates to be synthesized.

All the selected derivatives were synthesized (see Chemistry, Table 3), and their platelet aggregation inhibitory activity was determined. In addition, compounds **21**−**23** were also evaluated. Table 1 shows their experimental EC_{100} values together with those calculated for synthesized compounds **18**−**27** and the predicted values for the rest of the compounds **28**−**45** using eq 1.

As can be seen, experimental EC_{100} values for derivatives **18**−**26** are in good agreement with those predicted by equation 1. The values calculated or predicted from the final model failed for compounds **16**, **17**, and **27**. The elimination of compounds **16** and **17** from the final model (EC_{100} for **27** > 10) has to be done on a statistical basis since, although compound **16** is relatively unstable

Table 4. Equation Corresponding to Final Model (Eq 2) and Statistical Data

$$EC_{100} - \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 \quad (2)$$

$n = 21$ (**1**−**13**, **18**−**25**) $R_2 = 0.95$ $R_a^2 = 0.94$[a] $s = 0.80$

$F_{4,16} = 83.88$ $p < 0.0001$

X_i	variable[b]	coefficient	SE	t value	p value
	const	−17.272	1.662	−10.39	<0.0001
X_1	$(N5)^2$	1835.312	188.951	9.71	<0.0001
X_2	$(N5)^2C7$	3885.427	605.719	6.41	<0.0001
X_3	$(N5)^2C4a$	29092.447	2841.672	10.23	<0.0001
X_4	$(N1)^2C6$	−73862.781	8840.759	−8.35	<0.0001

[a] R_a^2: adjusted correlation coefficient (correlation coefficient adjusted with the degrees of freedom). [b] N5, C7, C4a, N1, C6: Hirshfeld partial charges calculated from DMol program.

in solution, there is not sufficient evidence to assume a different chemical behavior in the pharmacological assays.

Thus, by means of this methodology, it was possible to predict compounds of this series with a considerably improved activity, and indeed compounds **18** and **19** once synthesized did show experimental EC_{100} of 0.001 and 0.03, respectively. In fact, compound **18** represents a 300-fold improvement of the starting lead compound **1**. To refine our initial model these values were introduced providing a final model from 21 compounds (equation 2) which correctly correlates antiplatelet activity of this series with the atomic charges of certain atoms (Table 4).

Statistical Analysis and Validation of the Model. The multiple regression eq provides information on the statistical quality of our model which should be evaluated not only by how well it fits the data but also by how well it predicts the data.

The final model 2 is statistically significant with a degree of confidence of 95% according to the F statistic and p value. The F value represents the statistical significance of the regression model and is calculated as the ratio between regression and residual variances, and p is the probability that the correlation may be due to chance (Table 4).

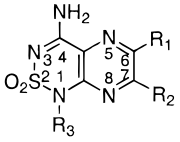
To know the predictive ability of the final model, to be sure that it is not misinterpreted, and to prevent the phenomenon of chance correlation in QSAR studies, use of validation methods is recommended. In this respect, the first procedure used to check the predictive ability of the final model was the leave-one-out method.[14] The statistical results summarized in Table 5 together with the value of R^2_{cv} (0.95) show that the final model has a high predictive ability.

The second method used was the so-called randomization of the responses into an array of reordered variables.[15] In this test, the values in the response column are shuffled randomly while the predictor columns are kept constant and a new model is established. This process is repeated a number of times, and the estimates of R_a^2 and R^2_{cv} are calculated. The best results obtained of 10 runs of the randomization test are given in Table 6. These values provide an estimate of the relevance of the real QSAR model. Looking at Table 6 we can see that the R_a^2 and R^2_{cv} values of the random models (a−e) are smaller and the PRESS (predictive residual sum of squares) values increase dramatically. This result shows that the final model 2 is very reliable for predicting data and does not imply chance correlation.

Chemistry

The N-substituted compounds synthesized for the QSAR study are gathered in Scheme 1. The substitution at position 1 can be performed by different procedures such as alkyl sulfates in water or alkyl halides in

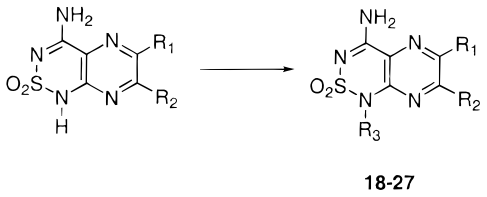
Table 5. Summary of Statistical Results of Leave-One-Out Test



compd	R_1	R_2	R_3	EC_{100} exptl	EC_{100} calcd (eq 2)	predicted leave-one-out	PRESS[a]	residual
1	Ph	Ph	Et	0.3	1.142	1.194	9.463	−0.894
2	Ph	Ph	CH_2Ph	3.0	2.795	2.774	10.169	0.226
3	Ph	Ph	CH_2CO_2Et	0.1	1.112	1.243	9.059	−1.143
4	Ph	Ph	$CH_2CO_2{}^tBu$	1.0	1.302	1.340	10.113	−0.340
5	Ph	Ph	CH_2CONH_2	3.0	1.438	1.299	7.559	1.701
6	Ph	H	Me	0.3	1.023	1.192	9.570	−0.892
7	Ph	H	Et	1.0	0.450	0.320	9.841	0.680
8	Ph	Me	Et	1.0	2.090	2.150	8.962	−1.150
9	Me	Ph	Et	1.0	1.803	2.376	9.111	−1.376
10	Br	Ph	CH_2CO_2Et	10	10.345	10.697	9.975	−0.967
11	Cl	Ph	Et	10	8.961	7.976	8.113	2.024
12	2-pyridyl	2-pyridyl	Me	3.0	2.327	2.218	9.688	0.782
13	2-thienyl	2-thienyl	Et	3.0	3.621	3.963	9.618	−0.963
18	4-MeOPh	4-MeOPh	Me	0.001	−0.568	−0.643	9.849	−0.106
19	2-FPh	H	Me	0.03	−0.262	−0.363	10.100	0.393
20	Ph	OMe	Me	0.1	−0.050	−0.140	10.179	0.240
21	4-FPh	4-FPh	Me	0.3	0.650	0.680	10.082	−0.380
22	4-ClPh	4-ClPh	Et	3.0	2.384	2.353	9.816	0.647
23	4-BrPh	4-BrPh	Me	1.0	0.576	0.542	10.021	0.458
24	Ph	OEt	Me	0.30	0.070	−0.083	10.127	0.383
25	4-ClPh	OEt	Et	10	10.223	11.517	9.877	−1.517

[a] PRESS: predictive sum of squares of final model (eq 2) with the ith compound excluded from the regression.

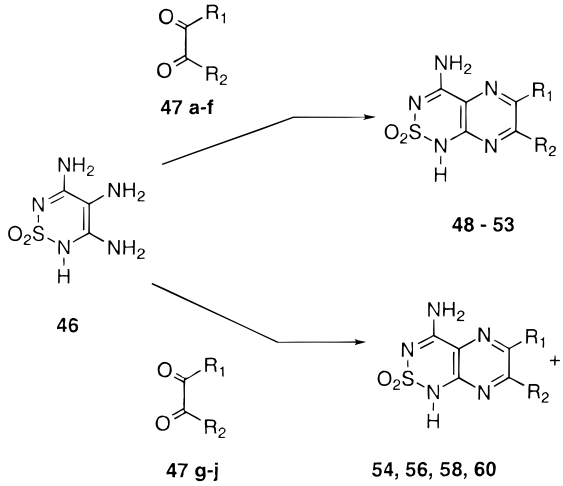
Scheme 1



18-27

	R_1	R_2	R_3
18	4-MeOPh	4-MeOPh	Me
19	2-FPh	H	Me
20	Ph	OMe	Me
21	4-FPh	4-FPh	Me
22	4-ClPh	4-ClPh	Et
23	4-BrPh	4-BrPh	Me
24	Ph	OEt	Me
25	4-ClPh	OEt	Et
26	H	4-MePh	Me
27	3-MeOPh	3-MeOPh	Me

Scheme 2



47 a-f

48 - 53

46

47 g-j

54, 56, 58, 60

55, 57, 59

47	$R_1 = R_2$	
a	4-MeOPh	**48**
b	3-MeOPh	**49**
c	4-ClPh	**50**
d	2-ClPh	**51**
e	4-FPh	**52**
f	4-BrPh	**53**

	R_1	R_2	
47g, 54	Ph	4-ClPh	**55**
47h, 56	Ph	4-NO_2Ph	**57**
47i, 58	4-ClPh	4-MePh	**59**
47j, 60	Ph	4-OHPh	

acetone.[1] Compounds **18**−**27** were obtained from the corresponding *N*(1)*H*-pyrazinothiadiazines with alkyl halides in acetone since the *N*(1)*H*-aryl derivatives are very insoluble in water.

In Table 3 are shown the novel *N*-alkyl-substituted pyrazino[2,3-*c*][1,2,6]thiadiazines **18**−**27** and the *N*(1)*H*-derivatives **48**−**71** synthesized for the QSAR study.

Reaction of **49** and **52** with methyl iodide in the presence of potassium carbonate afforded compounds **21** and **27**, respectively. The 1-methyl (**18**−**20**, **23**, **24**, **26**) and 1-ethyl (**22**, **25**) derivatives were prepared from the corresponding *N*(1)*H*-compounds with methyl iodide and ethyl iodide, respectively, using triethylamine to avoid the precipitation of the corresponding potassium salt in acetone of the arylpyrazinothiadiazine derivative.

The synthesis of *N*(1)*H*-pyrazinothiadiazines was carried out from **46**[16] with 1,2-dicarbonyl compounds, α-keto aldehydes, and α-hydroxyimino ketones (Table 3). Reaction of **46** with 4,4′-dimethoxybenzil, 3,3′-dimethoxybenzil, 4,4′-dichlorobenzil, 2,2′-dichlorobenzil, 4,4′-difluorobenzil, and 4,4′-dibromobenzil (**47a**−**f**) afforded the corresponding 6,7-bisarylpyrazinothiadiazines **48**−**53** (Scheme 2).

When the dicarbonyl compounds are not symmetric, the two different isomers in positions 6 and 7 can be obtained, and reaction of **46** with 1-(4-chlorophenyl)-2-phenyl-1,2-ethanedione, 1-(4-nitrophenyl)-2-phenyl-1,2-ethanedione, and 1-(4-chlorophenyl)-2-(4-methylphenyl)-1,2-ethanedione (**47g**−**i**) afforded a mixture of the corresponding pyrazinothiadiazines derivatives **54**−**55**, **56**−**57**, and **58**−**59**, respectively. However, when the reaction was carried out with 1-(4-hydroxyphenyl)-2-phenyl-1,2-ethanedione (**47j**) the regioselectivity is high and only one compound **60** was isolated (Scheme 2).

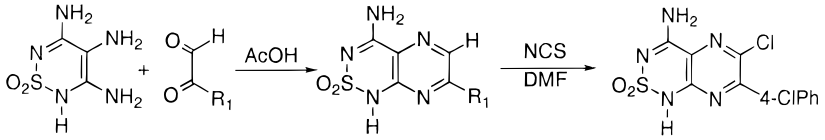
To obtain 6-unsubstituted arylpyrazinothiadiazines, reaction of **46** with α-keto aldehydes was carried out. Thus, compounds **61**−**64** were prepared from 4-methylphenylglyoxal, 4-chlorophenylglyoxal, 4-nitrophenylgly-

Table 6. Summary of Results of the Randomization Test

model	R_a^2 [a]	PRESS[b]	R^2_{cv}[c]
2	0.94	10.21	0.95
a	0.69	37.81	0.83
b	0.55	60.28	0.73
c	0.53	47.17	0.79
d	0.51	138.44	0.60
e	0.43	161.28	0.54

[a] R_a^2: adjusted correlation coefficient (correlation coefficient adjusted with the degrees of freedom). [b] PRESS: predictive residual sum of squares. [c] R^2_{cv}: cross-validated correlation coefficient R^2 (a R^2_{cv} value of 1.0 corresponds to perfect prediction).

Scheme 3



46 **47 k- n** **61-64** **65**

47	R_1	
k	4-MePh	**61**
l	4-ClPh	**62**
m	4-NO_2Ph	**63**
n	3-NO_2Ph	**64**

Table 7. ^{13}C NMR Data for Compounds **18**−**27** and **48**−**71**

compd	C-4	C-7	C-8a	C-6	C-4a	other signals
18	158.6	154.6	146.7	144.2	120.2	160.6; 159.6; 131.5; 131.1; 129.6; 113.9; 113.7; 55.3; 55.4; 28.1
19	158.3	148.2	147.0	140.4	122.6	161.6; 131.9; 131.4; 125.1; 116.3; 124.8; 28.5;
20	158.4	159.5	147.5	134.4	114.6	134.1; 129.1; 128.9; 128.1; 55.1; 28.2
21	158.3	154.2	146.9	143.3	120.6	165.0; 160.0; 133.6; 133.3; 132.2; 132.0; 131.7; 115.5; 115.1; 114.8; 29.1
22	158.3	154.1	146.5	143.0	120.9	136.0; 135.7; 134.8; 133.6; 131.6; 128.5; 128.2; 37.9; 13.9
23	157.9	153.9	147.0	142.9	120.9	136.1; 135.9; 131.5; 131.1; 130.9; 123.4; 122.7; 27.9
24	158.6	159.3	147.8	134.8	114.6	134.4; 129.4; 129.2; 128.4; 64.5; 14.2; 28.5
25	158.5	159.2	146.9	135.0	114.8	133.1; 132.9; 130.7; 128.1; 64.2; 38.0;14.2
26	157.9	154.2	148.3	133.1	121.2	141.3; 131.6; 129.4; 127.3; 20.5; 27.5
27	158.3	155.0	147.0	144.2	120.8	158.9; 158.8; 138.5; 138.3; 129.3; 129.0; 121.9; 115.2; 114.2; 28.1; 55.0
48	158.8	155.3	147.9	145.5	119.0	160.2; 159.3; 131.2; 130.9; 130.0; 113.6; 113.5; 55.2
49	158.5	155.8	147.0	145.4	120.0	158.7; 159.0; 138.5; 138.4; 129.0; 129.2; 122.0; 121.9; 115.1; 115.0; 55.0
50	158.3	154.8	147.1	144.2	120.2	153.8; 133.7; 133.1; 132.9; 127.2; 127.0; 117.4
51	158.0	155.5	147.6	144.5	120.7	135.3; 135.2; 132.5; 132.1; 131.6; 131.3; 130.9; 130.6; 129.3; 129.2; 126.6
52	158.4	155.0	147.9	144.5	119.9	164.9; 160.0; 133.6; 132.0; 131; 9; 115.1; 115.0
53	158.3	154.9	147.1	144.2	120.2	136.3; 136.2; 131.8; 131.7; 131.2; 131.0; 123.4; 122.2
54	158.6	154.2	147.2	145.6	120.3	137.1; 136.1; 134.5; 131.6; 129.8; 129.7; 128.2
55	158.5	156.2	147.1	144.4	120.1	137.0; 136.2; 133.5; 131.5; 129.7; 128.7; 128.1
56	158.3	156.5	147.1	143.8	120.8	147.7; 136.7; 131.0; 129.8; 128.7; 128.1
57	158.3	153.9	147.5	145.6	120.1	147.0; 143.8; 136.5; 129.7; 128.3
58	158.3	154.5	146.7	145.4	120.6	129.3; 136.2; 134.0; 133.2; 131.2; 129.4; 128.5; 127.2
59	158.3	155.9	147.0	144.0	119.6	138.0; 134.2; 134.0; 131.3; 129.4; 128.6; 128.1
60	158.9	156.3	147.2	145.7	119.3	159.1; 138.0; 131.8; 129.8; 128.7; 127.9; 115.3
61	158.4	154.9	148.6	134.8	120.5	141.5; 131.7; 129.7; 127.5; 20.9
62	158.3	153.6	148.6	136.3	121.1	134.3; 133.3; 129.3; 129.2
63	158.1	152.6	148.6	135.7	122.2	148.8; 140.5; 128.9; 124.0; 122.2
64	158.1	152.4	148.5	135.2	121.9	148.3; 136.2; 133.7; 130.8; 125.6; 122.1
65	157.2	155.3	147.5	137.1	120.1	135.3; 133.9; 133.4; 128.3
66	158.6	145.7	147.6	144.8	121.0	139.3; 131.9; 129.4; 126.4; 20.8
67	158.8	145.6	147.4	144.8	129.0	160.5; 128.1; 127.3; 114.3; 55.3
68	158.5	145.9	148.2	143.3	121.1	134.5; 133.7; 128.8; 128.3
69	158.2	146.8	148.5	142.0	121.5	140.0; 147.8; 127.6; 123.9
70	158.3	146.5	148.4	142.3	121.3	148.6; 136.6; 132.8; 130.3; 123.9; 121.0
71	158.4	148.5	147.6	141.2	121.9	159.8; 131.6; 131.1; 122.8; 124.9; 116.2

oxal, and 3-nitrophenylglyoxal (**47k**−**n**). The 6-chloro-7-(4-chlorophenyl) derivative **65** was obtained by chlorination of compound **62** with *N*-chlorosuccinimide in DMF (Scheme 3).

Another procedure to prepare compounds with different substituents at 6- and 7-positions involves the use of α-hydroxyimino ketones as starting materials. Thus, reaction of **46** with hydroxyiminoacetophenones **47o**−**t** afforded the corresponding 6-arylpyrazinothiadiazines **66**−**71** (Scheme 4).

The structures of all the new compounds synthesized have been established on the basis of their analytical and spectroscopic data. The ^{13}C and ^{1}H NMR chemical shifts are gathered in Tables 7 and 8. The positions of the 6- and 7-substituents were established on the basis of the long-range coupling constants and chemical shifts of the C-7 and C-6 carbons. In the case of compound **60**, the structure was assigned using an HMQC experiment.

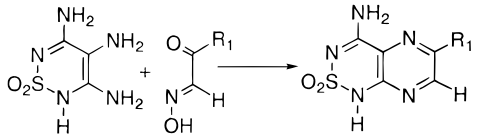
Conclusions

In a series of novel arylpyrazino[2,3-*c*][1,2,6]thiadiazines, a statistically significant correlation between the platelet aggregation inhibition (EC_{100}) and partial Hirshfeld charges has been established. Although this is not a conventional QSAR analysis in the sense that no correlation could be found when using logarithmic values of the pharmacological activity and that two outliers had to be removed, it has been possible to use the model for predictive purposes. Thus, new compounds were designed and synthesized, and among them, 4-amino-1-methyl-6,7-bis(4-methoxyphenyl)pyrazino-[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (**18**), when evaluated as a platelet aggregation inhibitor, showed high activity

Table 8. ^{1}H NMR (DMSO-d_6) Data for Compounds **18**−**27** and **48**−**71**

compd	NH_2	R_1, R_2	R_3
18	8.90; 8.77	7.51 (d, 4H, Ar); 6.96 (m, 4H, Ar); 3.80 (s, 3H, Me); 3.79 (s, 3H, Me)	3.47 (s, 3H, Me)
19	9.11; 8.98	9.11 (s, 1H, H-7); 8.31 (s, 1H, Ar); 7.45−7.35 (m, 3H, Ar)	3.44 (s, 3H, Me)
20	8.66; 8.56	8.16−7.44 (m, 5H, Ar); 4.11 (s, 3H, Me)	3.45 (s, 3H, Me)
21	8.95; 8.86	7.17−5.58 (m, 8H, Ar)	3.45 (s, 3H, Me)
22	8.91; 8.83	7.58−7.41 (m, 8H, Ar)	4.15 (q, 2H, CH_2); 1.35 (t, 3H, Me)
23	8.97; 8.88	7.57−7.49 (m, 8H, Ar)	3.46 (s, 3H, Me)
24	8.70; 8.57	8.11−8.08 (m, 2H, Ar); 7.51−7.25 (m, 8H, Ar); 3.95 (s, 3H, Me)	5.17 (s, 2H, CH_2)
25	8.63; 8.57	8.25 (d, 2H, Ar); 7.55 (d, 2H, Ar); 4.05 (q, 2H, CH2); 1.37 (t, 3H, Me)	4.59 (q, 2H,CH_2); 1.45 (t, 3H, Me)
26	8.95; 8.61	8.17 (d, 2H, Ar); 7.40 (d, 2H, Ar); 2.42 (s, 3H, Me)	3.52 (s, 3H, Me)
27	8.87	6.91−7.31 (m, 8H, Ar); 3.68 (s, 3H, Me)	3.64 (s, 3H, Me)
48	8.62; 8.32	7.46 (d, 2H, Ar); 7.40 (d, 2H, Ar); 6.94 (d, 2H, Ar) 6.90 (d, 2H, Ar); 3.78 (s, 3H, Me); 3.77 (s, 3H, Me)	12.55 (br s, 1H, NH)
49	8.65; 8.45	7.31−6.87 (m, 8H, Ar); 3.67 (s, 3H, OMe); 3.63 (s, 3H, OMe)	12.40 (br s, 1H, NH)
50	8.71; 8.63	7.54 (d, 4H, Ar); 7.42 (d, 4H, Ar)	12.49 (br s, 1H, NH)
51	8.73; 8.55	7.48−7.26 (m, 8H, Ar)	12.62 (br s, 1H, NH)
52	8.69; 8.60	8.56−5.14 (m, 8H, Ar)	12.41 (br s, 1H, NH)
53	8.73; 8.71	7.65−7.36 (m, 8H, Ar)	12.52 (br s, 1H, NH)
54	8.70; 8.61	7.53−7.32 (m, 9H, Ar)	12.41 (br s, 1H, NH)
55	7.58	7.53−7.32 (m, 9H, Ar)	12.41 (br s, 1H, NH)
56	8.78; 8.72	8.16−7.26 (m, 9H, Ar)	12.40 (br s, 1H, NH)
57	8.78; 8.72	8.16−7.26 (m, 9H, Ar)	12.40 (br s, 1H, NH)
58	8.69; 8.55	7.60−7.13 (m, 8H, Ar); 2.31 (s, 3H, Me)	12.37 (br s, 1H, NH)
59	8.66; 8.52	7.60−7.13 (m, 8H, Ar); 2.30 (s, 3H, Me)	12.37 (br s, 1H, NH)
60	8.63; 8.49	9.95 (br s, 1H, OH); 7.52 (m, 2H, Ar); 7.49−7.35 (m, 3H, Ar); 7.27 (d, 2H, Ar); 6.71 (d, 2H, Ar)	12.29 (br s, 1H, NH)
61	8.12	8.96 (s, 1H, H-6); 8.12 (d, 2H, Ar); 7.40 (d, 2H, Ar); 2.40 (s, 3H, Me)	12.21 (br s, 1H, NH)
62	8.63	9.01 (s, 1H, H-6); 8.24 (d, 2H, Ar); 7.67 (d, 2H, Ar)	12.31 (br s, 1H, NH)
63	8.70	9.10 (s, 1H, H-6); 8.49−8.44 (m, 5H, Ar)	12.46 (br s, 1H, NH)
64	8.76	9.18 (s, 1H, H-6); 9.00 (s, 1H, Ar); 8.71 (d, 1H, Ar); 8.46 (dd, 1H, Ar); 7.92 (t, 1H, Ar)	12.52 (br s, 1H, NH)
65	8.74. 8.65	7.80 (d, 2H, Ar); 7.63 (d, 2H, Ar)	12.63 (br s, 1H, NH)
66	8.74; 8.71	9.21 (s, 1H, H-7); 8.21 (d, 2H, Ar); 7.31 (d, 2H, Ar); 2.36 (s, 3H, Me)	12.28 (br s, 1H, NH)
67	8.44; 8.68	9.19 (s, 1H, H-7); 8.27 (d, 2H, Ar); 7.05 (d, 2H, Ar); 3.76 (s, 3H, OMe)	12.45 (br s, 1H, NH)
68	8.81; 8.71	9.26 (s, 1H, H-7); 8.36 (d, 2H, Ar); 7.57 (d, 2H, Ar)	12.40 (br s, 1H, NH)
69	8.93; 8.80	9.38 (s, 1H, H-7); 8.80 (d, 2H, Ar); 8.62 (d, 2H, Ar)	
70	8.98; 8.75	9.39 (s, 1H, H-7); 9.08 (m, 1H, Ar); 8.72 (dd, 2H, Ar); 7.30 (dd, 1H, Ar); 7.81 (t, 1H, Ar)	
71	8.58; 8.42	8.99 (d, 1H, H-7); 8.29 (dt, 1H, Ar); 7.56−7.52 (m, 1H, Ar); 7.43 (m, 2H, Ar)	12.44 (br s, 1H, NH)

Scheme 4



46 **47 o-t** **66-71**

47	R_1	
o	4-MePh	**66**
p	4-MeOPh	**67**
q	4-ClPh	**68**
r	4-NO_2Ph	**69**
s	3-NO_2Ph	**70**
t	2-FPh	**71**

(EC_{100} = 0.001 μg/mL). This value represents a 300-fold improvement over the starting lead compound and a 2500-fold improvement over aspirin in the same experimental conditions. On the basis of these results, further research in order to study the mechanism of action and to evaluate potential use as antiplatelet agents of this series is in progress.

Experimental Section

Chemistry. Melting points were determined with a Reichert-Jung Thermovar micromelting point apparatus and are uncorrected. ^{1}H NMR spectra (300 MHz) and ^{13}C NMR spectra (75 MHz) were recorded on a Varian XL-300 spectrometer and are reported in ppm on the δ scale. The signal of the solvent was used as reference. Mass spectra (electron impact, 70 eV) were obtained on a VG 12-250 (VG Masslab). Elemental analyses were performed on a Heraeus CHN-O-Rapid analyzer. Column chromatography was carried out on silica gel (Merck, particle size 70−230 mesh).

The α-keto aldehydes were prepared from the procedures reported by Cava[17] (**47k**,**i**) and Steinbach[18] (**47m**,**n**). In the case of α-hydroxyimino ketones the compounds **47o**−**t** were synthesized following the procedure of Slater.[19]

Theoretical Calculations. The studied compounds were built with standard bond lengths and angles by using the molecular modeling package Insight II.[20] All the structures were fully optimized without any symmetry restrictions in the gas phase. The LDF calculations were carried out using the DMol program[8] distributed by MSI. A double-ζ numerical basis set with polarization functions in all the atoms and the Janak−Moruzzi−Williams (JMW) exchange correlation potential[21] were used. The geometry of the molecules was optimized until the gradient was smaller than 0.001 au. Then, the partial Hirshfeld charges were calculated.[7]

Statistical Analysis. Multiple regression analysis were carried out with the StatView, SPSS, and NEMROD programs.[13] The collinearity of the variables in the experimental domain is not significant with a correlation coefficient between −0.04 and 0.40 which indicates that our descriptors are not intercorrelated. The experimental design methodology was performed using an exchange algorithm included in the DOPTDC program.[13]

In Vitro Platelet Aggregation Studies. The arylpyrazinothiadiazine derivatives were screened for their platelet aggregation inhibitory activity in vitro on citrated rabbit platelet-rich plasma in which aggregation was induced with arachidonic acid.[1,4]

General Procedure for the Preparation of *N*(1)-Alkylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxides 18−27. To the corresponding 4-amino-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide derivative in acetone, and either potassium carbonate or triethylamine, was added the alkyl halide. The reaction mixture was refluxed and evaporated to dryness, and water was added to the residue. The precipitate was filtered and recrystallized from the appropriate solvent.

4-Amino-6,7-bis(4-methoxyphenyl)-1-methylpyrazino-[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (18): from **48** (2.90 g, 4.02 mmol), triethylamine (0.5 mL, 4.0 mmol), methyl iodide (0.8 mL, 12.1 mmol), and acetone (200 mL); reaction time 72 h; yield (2.10 g, 73%).

4-Amino-6-(2-fluorophenyl)-1-methylpyrazino[2,3-c]-[1,2,6]thiadiazine 2,2-dioxide (19): from **71** (0.90 g, 3.1 mmol), triethylamine (0.4 mL, 3.1 mmol), methyl iodide (0.6 mL, 9.3 mmol), and acetone (80 mL); reaction time 72 h; yield (0.47 g, 54%).

4-Amino-7-methoxy-1-methyl-6-phenylpyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-dioxide (20): from 4-amino-7-methoxy-6-phenylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide[22] (1.70 g, 5.7 mmol), triethylamine (0.8 mL, 5.7 mmol), and methyl iodide (0.45 mL, 6.8 mmol); reaction time 24 h; yield (1.30 g, 74%).

4-Amino-6,7-bis(4-fluorophenyl)-1-methylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (21): from **52** (1.50 g, 3.8 mmol), potassium carbonate (0.30 g, 1.9 mmol), methyl iodide (1.2 mL, 19.8 mmol), and acetone (150 mL); reaction time 24 h; yield (1.14 g, 73%).

4-Amino-6,7-bis(4-chlorophenyl)-1-ethylpyrazino[2,3-c][1,2,6]thiadiazine 2,2-dioxide (22): From **50** (1.10 g, 6.7 mmol), triethylamine (0.3 mL, 2.6 mmol), ethyl iodide (0.4 mL, 5.4 mmol), and acetone (50 mL); reaction time 200 h; yield (0.69 g, 62%).

4-Amino-6,7-bis(4-bromophenyl)-1-methylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (23): from **53** (1.30 g, 2.5 mmol), triethylamine (0.32 mL, 2.5 mmol), methyl iodide (excess), and acetone (60 mL); reaction time 72 h; yield (0.80 g, 60%).

4-Amino-7-ethoxy-1-methyl-6-phenylpyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-dioxide (24): from 4-amino-7-ethoxy-6-phenyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide[23] (1.50 g, 4.7 mmol), triethylamine (0.7 mL, 4.7 mmol), methyl iodide (0.6 mL, 9.4 mmol), and acetone (100 mL); reaction time 90 h; yield (1.11 g, 69%).

4-Amino-6-(4-chlorophenyl)-7-ethoxy-1-ethylpyrazino-[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (25): from 4-amino-6-(4-chlorophenyl)-7-ethoxy-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide[23] (3.60 g, 4.2 mmol), triethylamine (0.6 mL, 4.2 mmol), ethyl iodide (0.6 mL, 9.4 mmol), and acetone (300 mL); reaction time 90 h; yield (2.12 g, 60%).

4-Amino-1-methyl-7-(4-tolyl)pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-dioxide (26): from **61** (1.60 g, 5.5 mmol), triethylamine (0.7 mL, 5.5 mmol), methyl iodide (0.5 mL, 8.3 mmol), and acetone (120 mL); reaction time 120 h; yield (0.77 g, 57%).

4-Amino-6,7-bis(3-methoxyphenyl)-1-methylpyrazino-[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (27): from **49** (1.50 g, 3.6 mmol), potassium carbonate (0.25 g, 1.8 mmol), methyl iodide (5.0 mL, 70 mmol), and acetone (150 mL); reaction time 6 h; yield (1.04 g, 66%).

General Procedure for the Synthesis of Compounds 48−52 and 54−64 from Dicarbonyl Compounds. To a suspension of **46** (1.0 mmol) in methanol or ethanol and either concentrated hydrochloric acid or acetic acid was added the corresponding carbonyl compound (1.1 mmol), and the mixture refluxed. The reaction mixture was evaporated to dryness, and water was added to the residue. The precipitate was filtered, washed with dichloromethane, and recrystallized from the appropriate solvent.

4-Amino-6,7-bis(4-methoxyphenyl)-1*H*-pyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-dioxide (48): from **46** (4.0 g, 22.6 mmol), methanol (300 mL), concentrated hydrochloric acid (1 mL), and 4,4′-dimethoxybenzil (**47a**) (7.1 g, 25.0 mmol); reaction time 24 h; yield (1.60 g, 61%); previously reported from compound **46** and 4-methoxybenzaldehyde.[24]

4-Amino-6,7-bis(3-methoxyphenyl)-1*H*-pyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-dioxide (49): from **46** (2.00 g, 11.3 mmol), methanol (60 mL), concentrated hydrochloric acid (1.0 mL), and 3,3′-dimethoxybenzil (**47b**) (3.50 g, 12.3 mmol); reaction time 72 h; yield (1.91 g, 41%).

4-Amino-6,7-bis(4-chlorophenyl)-1*H*-pyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-dioxide (50): from **46** (2.50 g, 14.1 mmol), ethanol (120 mL), concentrated hydrochloric acid (1.0 mL), and 4,4′-dichlorobenzil (**47c**) (4.30 g, 15.5 mmol); reaction time 72 h; yield (1.85 g, 40%); previously reported from compound **46** and 4-chlorobenzaldehyde.[24]

4-Amino-6,7-bis(2-chlorophenyl)-1*H*-pyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-dioxide (51): from **46** (2.50 g, 14.1 mmol), ethanol (120 mL), concentrated hydrochloric acid (1.0 mL), and 2,2′-dichlorobenzil (**47d**) (4.30 g, 15.5 mmol); reaction time 72 h; yield (1.85 g, 40%).

4-Amino-6,7-bis(4-fluorophenyl)-1*H*-pyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-dioxide (52): from **46** (1.50 g, 6.2 mmol), methanol (50 mL), concentrated hydrochloric acid (0.6 mL), and 4,4′-difluorobenzil (**47e**) (1.50 g, 6.2 mmol); reaction time 72 h; yield (2.0 g, 36%).

4-Amino-6,7-bis(4-bromophenyl)-1*H*-pyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-Dioxide (53). To a solution of 4,4′-dibromobenzil (**47f**) (7.40 g, 20.1 mmol) in dimethyl sulfoxide (200 mL) at 80 °C was added compound **46** (3.00 g, 16.8 mmol) and the mixture stirred for 24 h at 80 °C. Then, the solvent was evaporated to dryness, and water was added to the residue. The precipitate was filtered and recrystallized from acetic acid/ethanol to yield **53** (2.30 g, 25%).

4-Amino-7-(4-chlorophenyl)-6-phenyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (54) and 4-amino-6-(4-chlorophenyl)-7-phenyl-1*H*-pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-dioxide (55): from **46** (1.00 g, 5.6 mmol), 1-(4-chlorophenyl)-2-phenyl-1,2-ethanedione (**47g**) (1.50 g, 6.2 mmol), methanol (40 mL), and concentrated hydrochloric acid (0.6 mL); reaction time 24 h; yield (0.90 g, 43%); separated by chromatography on silica gel using $CHCl_3$/AcOH (100/3) as eluent.

4-Amino-7-(4-nitrophenyl)-6-phenyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (56) and 4-amino-6-(4-nitrophenyl)-7-phenyl-1*H*-pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-dioxide (57): from **46** (1.00 g, 5.6 mmol), 1-(4-nitrophenyl)-2-phenyl-1,2-ethanedione (**47h**) (1.50 g, 6.2 mmol), methanol (40 mL), and concentrated hydrochloric acid (0.6 mL); reaction time 48 h; yield (0.89 g, 41%); separated by chromatography on silica gel using CH_2Cl_2/MeOH (40/1) as eluent.

4-Amino-7-(4-chlorophenyl)-6-(4-tolyl)-1*H*-pyrazino-[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (58) and 4-amino-6-(4-chlorophenyl)-7-(4-tolyl)-1*H*-pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-dioxide (59): from **46** (1.00 g, 5.6 mmol), 1-(4-chlorophenyl)-2-(4-methylphenyl)-1,2-ethanedione (**47i**) (1.60 g, 6.2 mmol), methanol (80 mL), and concentrated hydrochloric acid (0.6 mL); reaction time 72 h; yield (1.30 g, 57%); separated by chromatography on silica gel using CH_2Cl_2/MeOH (40/1) as eluent.

4-Amino-7-(4-hydroxyphenyl)-6-phenyl-1*H*-pyrazino-[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (60): from **46** (0.68 g, 3.8 mmol), 1-(4-hydroxyphenyl)-2-phenyl-1,2-ethanedione (**47j**) (1.00 g, 4.2 mmol), methanol (30 mL), and concentrated hydrochloric acid (0.15 mL); reaction time 48 h; yield (1.40 g, 68%); EM *m/e* 367 (M^+).

4-Amino-7-(4-tolyl)-1*H*-pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-dioxide (61): from **46** (8.30 g, 46.5 mmol), 4-methylglyoxal (**47k**) (9.90 g, 67.0 mmol), and acetic acid (170 mL); reaction time 48 h; yield (7.30 g, 55%).

4-Amino-7-(4-chlorophenyl)-1*H*-pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-dioxide (62): from **46** (2.60 g, 14.5 mmol), 4-chlorophenylglyoxal (**47l**) (3.30 g, 18.2 mmol), and acetic acid (50 mL); reaction time 48 h yield (1.18 g, 46%).

4-Amino-7-(4-nitrophenyl)-1*H*-pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-dioxide (63): from **46** (0.70 g, 4.4 mmol),

4-nitrophenylglyoxal (**47m**) (0.90 g, 5.2 mmol), and acetic acid (40 mL); reaction time 72 h; yield (0.33 g, 23%).

4-Amino-7-(3-nitrophenyl)-1*H*-pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-dioxide (64): from **46** (5.00 g, 28.0 mmol), 3-nitrophenylglyoxal (**47n**) (6.00 g, 33.5 mmol), and acetic acid (100 mL); reaction time 48 h; yield (3.05 g, 37%).

General Procedure for the Synthesis of Compounds 66−71 from a-Hydroxyimino Ketones. To a suspension of **46** (1.0 mmol) in methanol and water was added the corresponding α-hydroxyimino ketone (1.2 mmol), and the mixture was stirred. The reaction mixture was evaporated to dryness, and water was added to the residue. The precipitate was filtered, washed with dichloromethane, and recrystallized from the appropriate solvent.

4-Amino-6-(4-tolyl)-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide (66): from **46** (9.30 g, 52.5 mmol), 4-methyl-2-(hydroxyimino)acetophenone (**47o**) (12.00 g, 78.0 mmol), methanol (60 mL), and water (60 mL); reaction time 48 h; yield (5.92 g, 48%).

4-Amino-6-(4-methoxyphenyl)-1*H*-pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-dioxide (67): from **46** (4.20 g, 23.6 mmol), 4-methoxy-2-(hydroxyimino)acetophenone (**47p**) (5.70 g, 34.3 mmol), methanol (90 mL), and water (90 mL); reaction time 120 h; yield (3.40 g, 47%).

4-Amino-6-(4-chlorophenyl)-1*H*-pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-dioxide (68): from **46** (8.30 g, 47.0 mmol), 4-chloro-2-(hydroxyimino)acetophenone (**47q**) (11.30 g, 61.5 mmol), methanol (70 mL), and water (70 mL); reaction time 120 h; yield (6.96 g, 46%).

4-Amino-6-(4-nitrophenyl)-1*H*-pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-dioxide (69): from **46** (12.00 g, 0.07 mol), 4-nitro-2-(hydroxyimino)acetophenone (**47r**) (14.70 g, 0.09 mol), methanol (100 mL), and water (100 mL); reaction time 48 h; yield (5.42 g, 25%).

4-Amino-6-(3-nitrophenyl)-1*H*-pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-dioxide (70): from **46** (3.00 g, 16.8 mmol), 3-nitro-2-(hydroxyimino)acetophenone (**47s**) (3.70 g, 18.9 mmol), methanol (60 mL), and water (60 mL); reaction time 48 h; yield (0.98 g, 30%).

4-Amino-6-(2-fluorophenyl)-1*H*-pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-dioxide (71): from **46** (1.00 g, 5.6 mmol), 2-fluoro-2-(hydroxyimino)acetophenone (**47t**) (1.00 g, 7.0 mmol), methanol (30 mL), and water (30 mL); reaction time 48 h; yield (1.06 g, 64%).

4-Amino-6-chloro-7-(4-chlorophenyl)-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (65). To a solution of **62** (1.50 g, 5.0 mmol) in DMF (30 mL) was added *N*-chlorosuccinimide (1.76 g, 10.1 mmol). The reaction mixture was stirred to room temperature for 24 h, and then the solvent was evaporated to dryness and water was added to the residue. The precipitate was filtered and recrystallized from MeOH/H_2O to give **65** (0.80 g, 47%): EM *m/e* 343 (M^+).

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