Novel Bronchodilators: Synthesis, Transamination Reactions, and Pharmacology of a Series of Pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxides

Nuria Campillo, Concepción García, Pilar Goya, Ibon Alkorta, and Juan A. Páez*

Instituto de Química Médica CSIC, Juan de la Cierva 3, 28006 Madrid, Spain

Received April 26, 2000

The synthesis, pharmacological evaluation, and structure−activity relationships of a new class of bronchodilator agents, derivatives of pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxides are described. The compounds were prepared by reaction of 3,4,5-triamino-1,2,6-thiadiazine 1,1-dioxide with suitable 1,2-dicarbonyl compounds or α-hydroxyiminoketones and subsequent N-alkylation. A transamination procedure for synthesizing derivatives with different substituents at the 4-amino group is reported for the first time. The pyrazino[2,3-*c*][1,2,6]thiadiazine derivatives were screened for tracheal relaxing activity in vitro, and the active compounds were evaluated in vivo in guinea pigs as bronchodilator agents in comparison to theophylline. Among the compounds studied, the most interesting properties were displayed by the 4-amino-1-ethyl-6-methyl derivative (**21**). The toxicological evaluation of this derivative is also reported.

Asthma and chronic obstructive pulmonary disease (COPD) are characterized by chronic obstruction of the flow of air through the airways. COPD involves chronic bronchitis or emphysema and its irreversible nature contrasts with the reversible condition of asthma.[1−5] Asthma is an extremely common disorder, responsible for more pediatric hospital admissions than any other single illness, and COPD represents now the fourth to fifth most common cause of death.

Although different,[1,2] the pharmacological treatment of both diseases is similar because the inflammatory/bronchospastic component is the amenable aspect to therapy, and thus β_2-adrenoreceptor agonists, anticholinergics, and theophylline, among other drugs, can be used with different degrees of efficacy.[6−11] Corticosteroids are the mainstay of nonbronchodilator therapy for asthma; however, their utility in COPD is less clear. At present, therapies for COPD are not very effective, and all these medications have well-established unwanted secondary effects, and so new therapeutic approaches for the treatment of these lung diseases still represent an important challenge in medicinal chemistry.[1,2,12−13]

Within this context, we wish to report a novel class of bronchodilator agents not related structurally to any of the bronchodilators known, which shows interesting tracheal relaxing properties (comparable to theophylline) and which are derived from the pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide system.[14,15] This heterocycle first reported by our group,[16] has been the subject of our research in relation to its particular structural features,[17−20] and we have also described the diuretic properties of some particular derivatives[21] and the ability to inhibit platelet aggregation of some aryl substituted compounds.[22,23]

Chemistry

The general synthetic route for the formation of substituted pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxides may comprise three steps involving ring formation between the 3,4,5-triamino-1,2,6-thiadiazine 1,1-dioxide **1**[24] and suitable 1,2-dicarbonyl compounds or α-hydroxyiminoketones, subsequent introduction of the substituent at the N-1 position, and transamination of the amino group at the 4-position.

The new pyrazino[2,3-*c*][1,2,6]thiadiazine derivatives prepared for this work are gathered in Table 1. In the first step, formation of the heterocyclic system, different methods can be used depending on the kind of substitution desired at the 6- and 7-positions. In the case of 6,7 symmetrically disubstituted derivatives, the synthesis can be achieved from *o*-diaminothiadiazines and 1,2-dicarbonyl compounds.[16]

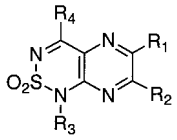
When the dicarbonyl compound used to build the pyrazino moiety has different substituents, the two possible isomers at positions 6 and 7 can be formed. The selectivity is specially high when one of the substituents of the dicarbonyl compound is a methyl group, and in this case, 6-methyl derivatives are obtained.[21] However, from α-hydroxyiminoketones it is possible to selectively obtain the 6- or 7-substituted derivatives depending on whether the substituent is linked to the carbonyl or the hydroxyimino function, respectively.

Thus, the synthesis of N(1)H-pyrazinothiadiazines **2**−**4** was carried out starting from **1**,[24] and dicarbonyl compounds such as 1,2-cycloheptanedione,[25] 4-methyl-2,3-pentanedione, or 2,3-heptanedione (Scheme 1). In the reaction of **1** with 2-oxobutanaldehyde,[26] the selectivity is high and only 7-ethyl derivative **5** is obtained. The 7-unsubstituted pyrazinothiadiazines were synthesized from α-hydroxyiminoketones, and thus, 6-methyl, 6-ethyl, 6-propyl, 6-isopropyl, and 6-*tert*-butyl derivatives **6**−**10** were prepared by reaction of **1** and the corresponding α-hydroxyiminoketones obtained from the β-oxoesters using the Freon method.[27] Compounds **11**−**16** were synthesized following previously published procedures[16,17,23] and were used as starting compounds to prepare other N-substituted derivatives in this work.

* To whom correspondence should be addressed. Fax: 91 5644853. E-mail: juan@suricata.iqm.csic.es.

10.1021/jm000970x CCC: $19.00
Published on Web 09/19/2000

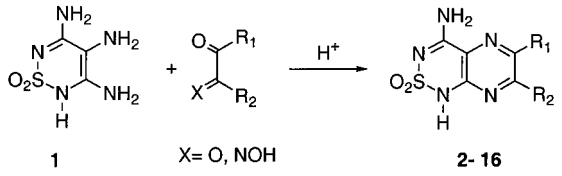
Table 1. Physicochemical Data for 4-aminopyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxides **2**−**10**, **17**−**32**, and **38**−**48**



	R_1	R_2	R_3	R_4	mp (°C)	recryst solv	formula	anal.[a]
2	$CH_2(CH)_3CH_2$		H	NH_2	280	-[b]	$C_{10}H_{13}N_5O_2S$	C, H, N, S
3	Me	*i*-Pr	H	NH_2	257−259	EtOH/water	$C_9H_{13}N_5O_2S$	C, H, N, S
4	Me	Bu	H	NH_2	211−213	water/MeOH	$C_{10}H_{15}N_5O_2S$	C, H, N, S
5	H	Et	H	NH_2	190−192	acetic ac/water	$C_7H_9N_5O_2S$	C, H, N, S
6	Me	H	H	NH_2	282−284	water	$C_6H_7N_5O_2S$	C, H, N, S
7	Et	H	H	NH_2	169−170	water	$C_7H_9N_5O_2S$	C, H, N, S
8	Pr	H	H	NH_2	184−186	water	$C_8H_{11}N_5O_2S$	C, H, N, S
9	*i*-Pr	H	H	NH_2	212−214	water	$C_8H_{11}N_5O_2S$	C, H, N, S
10	*t*-Bu	H	H	NH_2	285−287	water/MeOH	$C_9H_{13}N_5O_2S$	C, H, N, S
17	$CH_2(CH)_3CH_2$		Me	NH_2	267−269	water/MeOH	$C_{11}H_{15}N_5O_2S$	C, H, N, S
18	Me	*i*-Pr	Et	NH_2	164−166	water/MeOH	$C_{11}H_{17}N_5O_2S$	C, H, N, S
19	Me	Bu	Me	NH_2	149−151	water/MeOH	$C_{11}H_{17}N_5O_2S$	C, H, N, S
20	H	Et	Et	NH_2	168−170	water/EtOH	$C_9H_{13}N_5O_2S$	C, H, N, S
21	Me	H	Et	NH_2	171−173	water/EtOH	$C_8H_{11}N_5O_2S$	C, H, N, S
22	Et	H	Et	NH_2	172−174	water/MeOH	$C_9H_{13}N_5O_2S$	C, H, N, S
23	Pr	H	Et	NH_2	114−116	MeOH/water	$C_{10}H_{15}N_5O_2S$	C, H, N, S
24	*i*-Pr	H	Et	NH_2	150−152	MeOH/water	$C_{10}H_{15}N_5O_2S$	C, H, N, S
25	*t*-Bu	H	Et	NH_2	171−73	water/MeOH	$C_{11}H_{17}N_5O_2S$	C, H, N, S
26	Me	H	Me	NH_2	214	water/EtOH	$C_7H_9N_5O_2S$	C, H, N, S
27	H	Ph	Me	NH_2	295	EtOH/water	$C_{12}H_{11}N_5O_2S$	C, H, N, S
28	Ph	Me	Me	NH_2	246−247	EtOH/water	$C_{13}H_{13}N_5O_2S$	C, H, N, S
29	H	H	Et	NH_2	207−209	water	$C_7H_9N_5O_2S$	C, H, N, S
30	Me	H	CH_2COOEt	NH_2	208−210	EtOH/water	$C_{10}H_{13}N_5O_4S$	C, H, N, S
31	Me	Me	CH_2CH_2OH	NH_2	171−172	water	$C_9H_{13}N_5O_3S$	C, H, N, S
32	Me	Me	CH_2COOH	NH_2	292	−	$C_9H_{11}N_5O_4S$	C, H, N, S
38	Ph	H	Et	NHEt	227−229	EtOH/water	$C_{15}H_{17}N_5O_2S$	C, H, N, S
39	H	Ph	Me	$NH(CH_2)_2NH_2$	172−174	EtOH/water	$C_{14}H_{16}N_6O_2S$	C, H, N
40	H	Ph	Me	$NHNH_2$	278	EtOH	$C_{12}H_{12}N_6O_2S$	C, H, N
41	H	Ph	Et	$NHCH_2Ph$	155	EtOH/water	$C_{20}H_{19}N_5O_2S$	C, H, N, S
42	Me	H	Et	$NHCH_2Ph$	193−194	EtOH	$C_{15}H_{17}N_5O_2S$	C, H, N, S
43	Me	H	Et	NHEt	166−168	EtOH/water	$C_{10}H_{15}N_5O_2S$	C, H, N, S
44	Me	Me	Me	$NHCH_2$-2py	214−216	water/EtOH	$C_{14}H_{16}N_6O_2S$	C, H, N
45	Me	Et	Et	$NHCH_2CH_2OH$	181−182	water/MeOH	$C_{12}H_{19}N_5O_3S$	C, H, N, S
46	Me	Et	Et	$NHNH_2$	170−172	water/MeOH	$C_{10}H_6N_6O_2S$	C, H, N, S
47	Me	Et	Et	$NH(CH_2)_2NMe_2$	127	EtOH/water	$C_{14}H_{24}N_6O_2S$	C, H, N, S
48	Ph	Me	Me	*N*-pyrrolidinyl	245−247	EtOH	$C_{17}H_{19}N_5O_2S$	C, H, N, S

[a] Elemental analyses were within ±0.4 of the calculated values for the formulas given. [b] Purified by chromatography on silica gel using hexane/acetone/acetic acid (12/4/1) as eluent.

Scheme 1



	R_1	R_2
2	CH_2-$(CH_2)_3$-CH_2	
3	Me	i-Pr
4	Me	Bu
5	H	Et
6	Me	H
7	Et	H
8	Pr	H
9	i-Pr	H
10	*t*-Bu	H

	R_1	R_2
11	H	H
12	H	Ph
13	Me	Me
14	Me	Et
15	Ph	H
16	Ph	Me

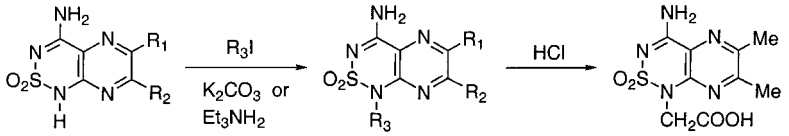
The N(1)-substituted compounds **17**−**32** described in this work are gathered in Table 1 and Scheme 2. Substitution at position 1 from the corresponding N(1)H-pyrazinothiadiazines 2,2-dioxides can be performed with alkyl halides in acetone using either potassium carbonate or triethylamine as base. Thus, reaction of N(1)H-pyrazino[2,3-*c*][1,2,6]thiadiazine derivatives **2**−**10** in acetone and triethylamine as base, with methyl or ethyl iodide, afforded the corresponding methyl or ethyl derivatives **17**−**25**, respectively.

Derivatives **26**−**28** were obtained by reaction of compounds **6, 12,**[17] and **16**[22] with methyl iodide in acetone, and potassium carbonate. Treatment of compounds **11**[16] and **6** with ethyl iodide or ethyl bromoacetate in the presence of triethylamine, afforded the corresponding derivatives **29** and **30**, respectively.

On the other hand, the preparation of compound **31** was carried out by reaction of **13**[16] with 2-bromoethanol (Scheme 2). The carboxymethyl derivative **32** was obtained by hydrolysis of 1-ethoxycarbonylmethyl derivative **33**.[21] Finally, compounds **34**−**37** were prepared from **12**−**15** according to previously reported procedures[21] (Scheme 2).

For the modification of the 4-position of the heterocyclic system, in principle alkylation at the exocyclic amino group with alkyl halides in basic medium is possible.[28] Following this method, the 4-ethylamino derivative **38** was prepared from the reaction of 4-amino-6-phenyl compound **37** with ethyl iodide and potassium

Scheme 2

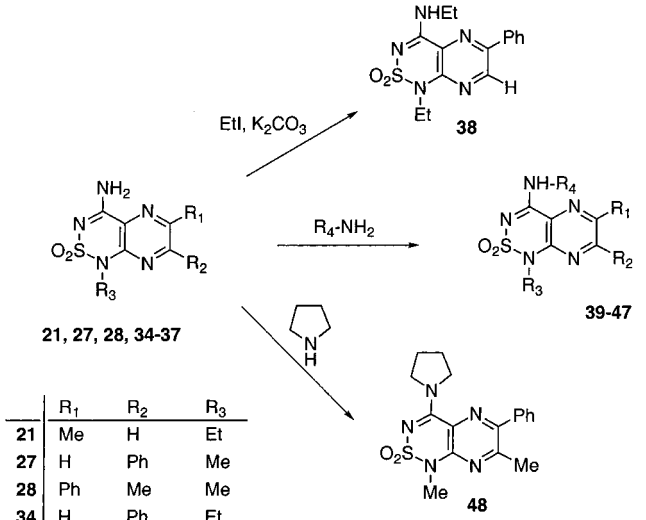


2- 16 **17- 31, 33-37** **32**

	R_1	R_2	R_3
17	CH_2-$(CH_2)_3$-CH_2		Me
18	Me	i-Pr	Et
19	Me	Bu	Me
20	H	Et	Et
21	Me	H	Et
22	Et	H	Et
23	Pr	H	Et
24	i-Pr	H	Et
25	*t*-Bu	H	Et
26	Me	H	Me

	R_1	R_2	R_3
27	H	Ph	Me
28	Ph	Me	Me
29	H	H	Et
30	Me	H	CH_2COOEt
31	Me	Me	CH_2CH_2OH
33	Me	Me	CH_2COOEt
34	H	Ph	Et
35	Me	Me	Me
36	Me	Et	Et
37	Ph	H	Et

Scheme 3



38 **21, 27, 28, 34-37** **39-47** **48**

	R_1	R_2	R_3
21	Me	H	Et
27	H	Ph	Me
28	Ph	Me	Me
34	H	Ph	Et
35	Me	Me	Me
36	Me	Et	Et
37	Ph	H	Et

	R_1	R_2	R_3	R_4
39	H	Ph	Me	$(CH_2)_2NH_2$
40	H	Ph	Me	NH_2
41	H	Ph	Et	CH_2Ph
42	Me	H	Et	CH_2Ph
43	Me	H	Et	Et
44	Me	Me	Me	CH_2-2py
45	Me	Et	Et	CH_2CH_2OH
46	Me	Et	Et	NH_2
47	Me	Et	Et	$(CH_2)_2NMe_2$

carbonate (Scheme 3). However, in this procedure it is very difficult, in some cases, to prevent the formation of mixtures of mono and dialkyl products.

Therefore, a different approach to the preparation of 4-amino substituted pyrazino[2,3-*c*][1,2,6]thiadiazines has been developed and consists of a transamination reaction, described here for the first time. Thus, reaction of the N1-substituted 4-aminopyrazinothiadiazines with an excess of the corresponding amine in alcoholic medium yielded the 4-amino-substituted derivatives.

The alkylamino derivatives of the pyrazinothiadiazines synthesized are shown in Scheme 3. Thus, different 4-substituted derivatives **39**−**47** were obtained from **21**, **27**, and **34**−**36** by reaction with primary amines such as benzylamine, ethylenediamine, ethanolamine, *N*,*N*-dimethylethylenediamine, 2-picolylamine, and hydrazine in methanol. This method can also be used with secondary amines, like pyrrolidine, and so it was possible to obtain the pyrrolidinyl derivative **48** from compound **28**.

The structures of all the new compounds synthesized have been established on the basis of their analytical and spectroscopic data. The ^{13}C NMR chemical shifts are gathered in Table 2. The positions of the 6- and 7-substituents were established according to the long-range coupling constants and chemical shifts of the C-7 and C-6 carbons.

Pharmacological Results

The N1-substituted compounds synthesized in this work **17**−**26, 29**−**32,** and **38**−**48**, together with derivatives **33**−**37** and **49**−**68**[21−23] previously available, were screened for tracheal relaxing activity in vitro[29] on guinea pig trachea, and the results obtained are gathered in Table 3.

On the basis of the corresponding tracheal relaxing screening results, selected compounds were further evaluated as bronchodilator agents in vivo[30] in comparison to theophylline. Finally, the most promising compound was subjected to toxicity studies.

The results obtained for the tracheal relaxing activity of pyrazino[2,3-*c*][1,2,6]thiadiazine compounds and theophylline are shown in Table 3. Derivatives with a percentage of tracheal tone inhibition more than 50% at concentrations of 30 μg/mL, similar to theophylline, were considered with pharmacological activity significance.

In the tracheal relax assay (Table 3), the activity of the 4-NH_2 derivatives was similar to the corresponding 4-amino substituted compounds when the group at the 4-position is hydrazino or alkylamino except in the case of the 4-(ethylamino)-6-phenyl derivative **38**, which

Table 2. ^{13}C NMR Spectral Data (δ, DMSO-d_6) for Compounds **2**−**1** **17**−**32**, and **38**−**48**

	C-4	C-7	C-8a	C-6	C-4a	other signals
2	158.8	163.7	146.8	151.4	118.2	37.3, 36.3, 31.2, 26.0, 25.6
3	158.8	165.5	147.3	144.5	118.8	20.5, 31.4, 20.9
4	158.8	161.3	147.1	145.4	118.7	20.5, 33.9, 29.1 21.9, 13.5
5	158.7	163.6	148.7	137.2	120.2	28.1, 12.7
6	158.8	148.7	149.5	144.0	120.3	20.2
7	158.7	148.2	147.4	151.5	121.1	27.0, 13.0
8	158.6	148.4	147.4	150.2	121.3	35.6, 21.8, 13.4
9	158.7	147.3	147.4	154.9	121.0	32.5, 21.9
10	158.7	145.8	147.0	156.9	120.0	35.9, 29.4
17	158.7	163.3	146.8	150.2	119.2	27.6, 37.5, 35.9, 31.1, 25.8, 25.5
18	158.5	164.8	146.6	143.5	119.6	37.5, 31.3, 20.8, 20.1, 13.7
19	158.7	160.8	147.1	144.5	119.6	33.9, 28.5, 27.8, 21.8, 20.2, 13.7
20	158.6	163.3	148.0	136.2	120.9	37.6, 28.2, 13.8, 12.4
21	158.7	148.5	146.9	145.9	122.2	37.7, 20.2, 13.9
22	158.6	147.8	146.9	150.4	122.2	37.6, 26.8, 13.8, 13.0
23	158.8	148.4	147.2	149.7	122.5	38.0, 35.7, 22.1, 14.1, 13.8
24	158.7	147.0	147.0	154.0	122.1	37.4, 32.4, 21.9, 13.9
25	158.6	145.5	155.9	146.5	121.5	37.6, 35.8, 29.4, 13.8
26	158.5	148.4	147.5	145.9	122.3	28.1, 20.1
27	157.9	154.2	148.3	133.3	121.6	134.3, 131.0, 128.7, 127.4, 27.6
28	158.6	156.7	147.1	145.6	120.4	136.8, 129.3, 128.7, 128.2, 23.67, 28.0
29	158.4	148.7	148.6	136.7	123.6	37.7, 12.8
30	158.4	148.3	146.8	146.5	122.2	167.3, 61.0, 43.0, 20.2, 13.9
31	159.0	158.3	146.9	145.3	119.8	58.4, 43.6, 22.6, 20.8
32	158.7	158.2	146.4	145.8	119.7	168.9, 22.5, 20.8, 42.7
38	156.4	145.4	147.1	143.6	122.5	134.5, 129.7, 128.8, 126.6, 37.9, 35.9, 13.9, 13.7
39	154.2	155.6	148.2	133.6	122.4	134.5, 131.0, 129.2, 127.7, 44.1, 40.0, 28.0
40	151.2	153.3	148.0	133.5	122.7	134.6, 131.2, 129.1, 127.5, 28.1
41	154.1	155.5	148.0	134.4	121.8	133.5, 128.9, 128.2, 127.5, 131.2, 128.9, 127.4, 43.9, 37.4, 13.5
42	156.0	148.3	146.6	145.8	122.3	43.9, 37.7, 20.0, 13.7
43	155.6	148.1	146.5	145.7	122.4	37.6, 35.8, 22.0, 13.6, 13.7
44	156.3	158.0	146.8	144.9	119.9	156.1, 148.8, 136.6, 122.3, 121.3, 45.5, 27.8, 22.5, 20.5
45	156.3	161.5	146.3	144.5	119.6	58.4, 43.4, 37.6, 27.5, 20.3, 13.9, 10.7
46	152.5	160.5	145.9	144.1	119.8	37.4, 27.3, 20.1, 10.7, 13.8
47	156.1	161.5	146.3	144.5	119.5	56.8, 45.1, 38.6, 37.6, 27.5, 20.3, 13.9, 10.7
48	154.4	154.8	148.5	144.9	123.9	137.5, 128.8, 128.7, 128.5, 51.9, 51.0, 28.7, 27.0, 23.5, 23.4

shows a lower MIC (10 μg/mL) than the corresponding unsubstituted 4-amino derivative **37**.

Regarding substituents at 1-position, the tracheal relaxant activity increases by the presence of a methyl group (**35**, **52**; MIC = 3 μg/mL), except for 1-ethyl-6-methyl derivative **21**, which shows activity at 30 μg/mL while the corresponding methyl compound **26** is devoid of activity (Table 3). This 1-ethyl compound **21**, as will be shown later, is the most interesting derivative concerning its bronchodilator properties in vivo.

The influence of the substitution at 6- and 7-positions depends on the group attached to the other position without being possible to establish general structure−activity relationships. Thus, in the 7H- and 7-methyl series, the 6-ethyl group shows a higher activity, while in the 7-ethyl and 7-phenyl series it is the 6H group, and in the 7-phenyl series the 6H or 6-Me groups.

The compounds which showed a similar or higher activity in comparison to theophylline were further evaluated in guinea pigs to determine their bronchodilator activity in vivo.

The bronchoconstriction in guinea pigs was determined by the Konzett and Rossler test[30] using histamine as spasmogen. The activity of pyrazino[2,3-*c*][1,2,6]-thiadiazine derivatives in relation to theophylline was studied at 25 and 100 mg/kg (the evaluation method is described in the Experimental Section). In Figure 1 are gathered those derivatives which had a significant activity at 25 mg/kg or 100 mg/kg ($p < 0.05$). All derivatives showed interesting bronchodilator properties although the 1-ethyl-6-methylpyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-dioxide **21** is clearly the most interesting compound, with an activity 30% higher than theophylline at 100 mg/kg.

Finally, toxicological studies have also been carried out, and thus, LD_{50} values have been determined in mouse and rat and are shown in Figure 2. The values obtained for the LD_{50} in these animal species indicate that **21** is, in both cases, less toxic than theophylline, being the LD_{50} of compound **21** 3.6 times higher in mouse and 7.7 times higher in rat. (Figure 2). This may represent an advantage over theophylline since it can eventually result in a better therapeutic index.

Conclusions

From a synthetic point of view, new derivatives of pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide have been prepared, and the transamination procedure to obtain 4-aminosubstituted derivatives within this series is reported here for the first time.

Regarding their activity, several compounds have shown interesting results in tracheal relaxation screening in vitro, and bronchodilator properties, comparable to theophylline, in guinea pigs in vivo.

From these, 4-amino-1-ethyl-6-methylpyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-dioxide (**21**) emerges as a promising candidate. The inhibition of the bronchoconstriction induced by other spasmogens (histamine, methacholline, arachidonic acid, or PAF) in vivo and the ability of compound **21** to relax human bronchus and to inhibit human bronchial phosphodiesterase activity have been published elsewhere.[31] All these findings, together with its LD_{50} values here determined suggest that pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide **21** can be regarded as a new bronchodilator agent useful for lung diseases.

Experimental Section

Chemistry. **General.** Melting points were determined with a Reichert-Jung Thermovar micro melting point apparatus and are uncorrected. ^1H NMR spectra (200 or 300 MHz) and ^{13}C NMR spectra (75 MHz) were recorded on a Gemini or Varian XL-300 spectrometer and are reported in ppm on the δ scale. The signal of the solvent was used as reference. 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 1,2-cycloheptanedione[25] and the ethylglyoxal[26] were prepared from cycloheptanone and butanaldehyde with selenium dioxide, respectively, following described procedures. In the case of the α-hydroxyiminoketones, the syntesized compounds were obtained from β-oxoesters by means of Freon's method.[27]

4-Amino-1,6,7,8,9,10-hexahydrocyclohepta[*b*]pyrazino-[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (2). To a suspension of 1 (1.30 g, 7.3 mmol) in acetic acid (60 mL, 80 °C) and concentrated hydrochloric acid (0.2 mL) was added 1,2-cycloheptanedione (0.95 g, 7.3 mmol). After heating the mixture at 80 °C for 5 h, the precipitate was filtered, washed

Table 3. Relaxant Tracheal Activity in Vitro of Pyrazino[2,3-*c*]-1,2,6-thiadiazine 2,2-Dioxides **17**−**26** and **29**−**68**[a]

	R_1	R_2	R_3	R_4	tracheal %inhibition[b]	relaxation MIC[c]
17	CH_2-$(CH_2)_3CH_2$		Me	NH_2	88	10
18	Me	*i*-Pr	Et	NH_2	55	30
19	Me	Bu	Me	NH_2	64	10
20	H	Et	Et	NH_2	80	30
21	Me	H	Et	NH_2	67	30
22	Et	H	Et	NH_2	72	10
23	Pr	H	Et	NH_2	90	10
24	*i*-Pr	H	Et	NH_2	57	30
25	*t*-Bu	H	Et	NH_2	86	30
26	Me	H	Me	NH_2	45	*d*
29	H	H	Et	NH_2	13	*d*
30	Me	H	CH_2COOEt	NH_2	20	*d*
31	Me	Me	CH_2CH_2OH	NH_2	59	30
32	Me	Me	CH_2COOH	NH_2	13	*d*
33	Me	Me	CH_2COOEt	NH_2	15	*d*
34	H	Ph	Et	NH_2	95	3
35	Me	Me	Me	NH_2	92	3
36	Me	Et	Et	NH_2	0	*d*
37	Ph	H	Et	NH_2	20	*d*
38	Ph	H	Et	NHEt	74	10
39	H	Ph	Me	$NH(CH_2)_2NH_2$	6	*d*
40	H	Ph	Me	$NHNH_2$	81	10
41	H	Ph	Et	$NHCH_2Ph$	20	*d*
42	Me	H	Et	$NHCH_2Ph$	27	*d*
43	Me	H	Et	NHEt	63	30
44	Me	Me	Me	$NHCH_2$−2-Py	31	*d*
45	Me	Et	Et	$NHCH_2CH_2OH$	41	*d*
46	Me	Et	Et	$NHNH_2$	72	30
47	Me	Et	Et	$NH(CH_2)_2NMe_2$	34	*d*
48	Ph	Me	Me	*N*-pyrrolidinyl	10	*d*
49	H	H	Me	NH_2	22	*d*
50	H	Me	Et	NH_2	42	*d*
51	H	Ph	CH_2COOEt	NH_2	72	10
52	Me	Me	Et	NH_2	33	*d*
53	Me	Me	Pr	NH_2	58	30
54	Me	Et	Me	NH_2	95	3
55	Me	Et	Et	NHMe	75	30
56	Me	Et	Pr	NH_2	51	30
57	Me	Pr	Et	NH_2	34	*d*
58	Me	Ph	Et	NH_2	100	3
59	Et	Me	Et	NH_2	95	10
60	Et	Et	Et	NH_2	0	*d*
61	*i*-Pr	*i*-Pr	Et	NH_2	0	*d*
62	$(CH_2)_2$-	$(CH_2)_2$-	Et	NH_2	82	10
63	Ph	H	Me	NH_2	56	30
64	Ph	Me	Et	NH_2	93	30
65	Ph	Ph	Et	NH_2	24	*d*
66	Cl	Ph	Me	NH_2	86	30
67	Br	Me	Et	NH_2	88	10
68	Br	Ph	Et	NH_2	63	30
theophylline					60	30

[a] The evaluation method is described in the Experimental Section. Test compound inhibition by more than 50% was considered of pharmacological significance. The compounds were assayed in duplicate for each concentration. [b] Percentage of inhibition at 30 μg/mL of test compound. [c] Results expressed as the lowest concentration (μg/mL) required to obtain a inhibitory response by more than 50%. [d] Compounds devoid of tracheal relaxing activity at 30 μg/mL.

with water, and recrystallized from the appropriate solvent (see Table 1) to give **2** (1.12 g, 57%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 11.94 (br s, 1H, NH), 8.57 (br s, 1H, NH_2), 8.32 (br s, 1H, NH_2), 3.00 (t, 4H, $2CH_2$), 1.85 (m, 2H, CH_2), 1.67 (m, 4H, $2CH_2$).

4-Amino-7-isopropyl-6-methyl-1*H*-pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxide (3). To a suspension of **1** (2.00 g, 11.3 mmol) in acetic acid (60 mL, 80 °C) was added 4-methyl-2,3-pentanedione (1.59 g, 12.4 mmol). After heating the mixture at 80 °C for 6 h, the precipitate was filtered, washed with water, and recrystallized from the appropriate solvent (see Table 1) to give **3** (1.53 g, 53%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 11.91 (br s, 1H, NH), 8.50 (br s, 1H, NH_2), 8.35 (br s, 1H, NH_2), 3.28 (m, 1H, CH), 2.54 (s, 2H, CH_3), 1.07 (d, 6H, $2CH_3$).

4-Amino-7-butyl-6-methyl-1*H*-pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxide (4). To a suspension of **1** (1.50 g, 8.5 mmol) in acetic acid (50 mL, 80 °C) was added 2,3-heptanedione (1.19 g, 9.3 mmol). After heating the mixture at 80 °C for 6 h, the precipitate was filtered, washed with water, and recrystallized from the appropriate solvent (see Table 1) to give **4** (1.64 g, 73%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 11.87 (br s, 1H, NH), 8.44 (br s, 1H, NH_2), 8.29 (br s, 1H, NH_2), 2.79 (t, 2H, (C-6)-CH_2), 2.51 (s, 3H, (C-7)-CH_3), 1.64 (m, 2H, CH_2), 1.37 (m, 2H, CH_2), 0.91 (t, 3H, CH_3).

4-Amino-7-ethyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (5). To a suspension of **1** (5.00 g, 28.2 mmol) in water (100 mL), ethanol (66 mL), and concentrated hydrochloric acid (0.5 mL) was added ethylglyoxal (3.00 g, 35.0 mmol). The reaction mixture was stirred at room temperature for 4 days and then evaporated to dryness. The residue was purified by chromatography on silica gel using dichloromethane/methanol (15/1) as eluent and recrystallized from the appropriate solvent (see Table 1) to yield **5** (0.98 g, 16%). ^{1}H NMR

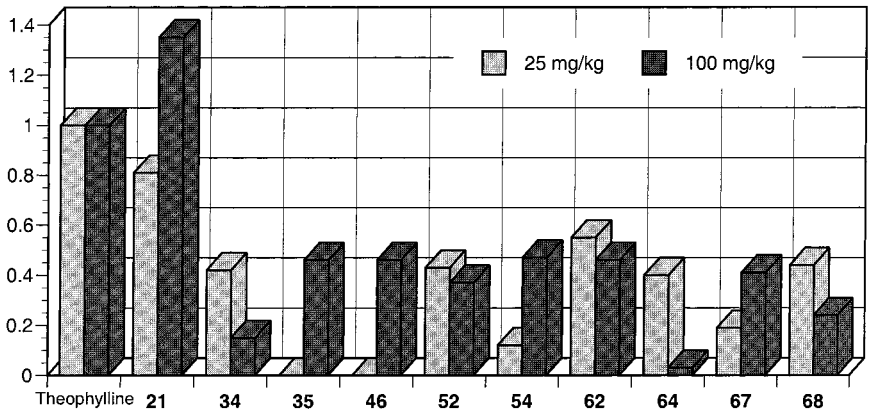


Figure 1. Comparison of bronchodilatory effect of selected compounds versus theophylline in the model of bronchospasm induced by histamine in guinea pig. The activity is expressed as the percentage of the inhibitory response vs theophylline (see Experimental Section). The relation values are means of 5−6 experiments ($p < 0.05$).

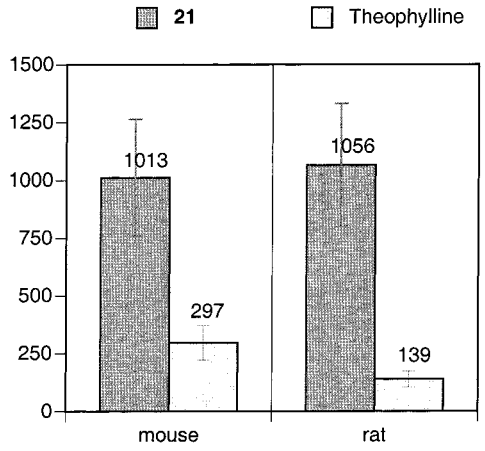


Figure 2. LD_{50} values (mg/kg) of **21** in mouse or rat in comparison to theophylline.

(200 MHz, DMSO-d_6): δ 12.11 (br s, 1H, NH), 8.53 (br s, 2H, NH_2), 8.31 (s, 1H, CH), 2.82 (q, 2H, CH_2), 1.23 (t, 3H, CH_3).

General Procedure for the Synthesis of Compounds 6−10 from 3,4,5-Triamino-2*H*-1,2,6-thiadiazine 1,1-Dioxide. To a suspension of **1** in methanol and concentrated hydrochloric acid was added the corresponding α-hydroxyimino compound, and the mixture heated at reflux. The reaction mixture was evaporated to dryness, and water was added to the residue. The precipitate was filtered, washed with water, and recrystallized from the appropriate solvent (see Table 1).

4-Amino-6-methyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (6). From **1** (20.00 g, 112.8 mmol), methanol (800 mL), concentrated hydrochloric acid (2 mL), and pyruvaldoxime (18.00 g, 206.7 mmol). Reaction time 24 h. Yield **6** (21.70 g, 91%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.05 (br s, 1H, NH), 8.58 (br s, 1H, NH_2), 8.51 (s, 1H, 7-H), 8.44 (br s, 1H, NH_2), 2.49 (s, 3H, CH_3).

4-Amino-6-ethyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (7). From **1** (1.50 g, 8.4 mmol), methanol (60 mL), concentrated hydrochloric acid (0.2 mL), and 1-hydroxyimino-2-butanone (1.75 g, 16.6 mmol). Reaction time 5 h. Yield **7** (0.79 g, 42%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.06 (br s, 1H, NH), 8.61 (br s, 1H, NH_2), 8.56 (s, 1H, 7-H), 8.55 (br s, 1H, NH_2), 2.80 (q, 2H, CH_2), 1.25 (t, 3H, CH_3).

4-Amino-6-propyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (8). From **1** (1.00 g, 5.6 mmol), methanol (40 mL), concentrated hydrochloric acid (0.15 mL), and 1-hydroxyimino-2-pentanone (1.31 g, 11.3 mmol). Reaction time 5 h. Yield **8** (1.08 g, 80%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.10 (br s, 1H, NH), 8.62 (br s, 1H, NH_2), 8.52 (s, 1H, 7-H), 8.42 (br s, 1H, NH_2), 2.73 (t, 2H, CH_2), 1.74 (m, 2H, CH_2), 0.87 (t, 3H, CH_3).

4-Amino-6-isopropyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (9). From **1** (1.00 g, 5.6 mmol), methanol (40 mL), concentrated hydrochloric acid (0.15 mL), and 1-hydroxyimino-3-methyl-2-butanone (1.31 g, 11.3 mmol). Reaction time 5 h. Yield **9** (0.96 g, 71%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.08 (br s, 1H, NH), 8.61 (br s, 1H, NH_2), 8.56 (s, 1H, 7-H), 8.38 (br s, 1H, NH_2), 3.10 (m, 1H, CH), 1.25 (d, 6H, $2CH_3$).

4-Amino-6-*tert*-butyl-1*H*-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (10). From **1** (1.00 g, 5.6 mmol), methanol (40 mL), concentrated hydrochloric acid (0.15 mL), and 1-hydroxyimino-3-dimethyl-2-butanone (1.53 g, 11.3 mmol). Reaction time 5 h. Yield **10** (1.07 g, 75%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.10 (br s, 1H, NH), 8.72 (s, 1H, 7-H), 8.63 (br s, 1H, NH_2), 8.35 (br s, 1H, NH_2), 1.35 (s, 9H, $3CH_3$).

General Procedure for the Synthesis of *N*-1-Alkylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxides (17−31). 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 stirred at room temperature or heated at reflux and then evaporated to dryness, and diluted hydrochloric acid was added to the residue. The precipitate was filtered and recrystallized from the appropriate solvent (Table 1).

4-Amino-1-methyl-6,7,8,9-tetrahydro-10*H*-cyclohepta-[*b*]pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (17). From **2** (0.60 g, 2.2 mmol), acetone (25 mL), triethylamine (0.3 mL, 2.3 mmol), and methyl iodide (0.5 mL, 8.0 mmol). Reaction time 7 days at room temperature. Yield **17** (0.40 g, 63%). ^{1}H NMR (200 MHz, DMSO-d_6) δ: 8.73 (br s, 1H, NH_2), 8.59 (br s, 1H, NH_2), 3.33 (s, 3H, CH_3), 3.04−3.00 (m, 4H, $2CH_2$), 1.82 (m, 2H, CH_2), 1.66 (m, 4H, $2CH_2$).

4-Amino-1-ethyl-7-isopropyl-6-methylpyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-Dioxide (18). From **3** (1.00 g, 3.9 mmol), acetone (30 mL), triethylamine (0.5 mL, 6.2 mmol), and ethyl iodide (0.4 mL, 4.7 mmol). Reaction time 4 days (heated at reflux). Yield **18** (0.79 g, 71%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.65 (br s, 1H, NH_2), 8.49 (br s, 1H, NH_2), 4.01 (q, 2H, N-CH_2), 3.28 (m, 1H, CH), 2.55 (s, 3H, CH_3), 1.32−1.19 (m, 9H, $3CH_3$).

4-Amino-7-butyl-1,6-dimethylpyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxide (19). From **4** (1.00 g, 3.7 mmol), acetone (30 mL), triethylamine (0.6 mL, 4,3 mmol), and methyl iodide (0.7 mL, 11.2 mmol). Reaction time 24 h at room temperature. Yield **19** (0.65 g, 62%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.72 (br s, 1H, NH_2), 8.58 (br s, 1H, NH_2), 3.35 (s, 3H, N−CH_3), 2.85 (t, 2H, C7−CH_2), 2.52 (s, 3H, C6-CH_3), 1.70 (q, 2H, CH_2), 1.38 (m, 2H, CH_2), 0.92 (t, 3H, CH_3).

4-Amino-1,7-diethylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (20). From **5** (1.00 g, 4.4 mmol), acetone (25 mL), triethylamine (0.6 mL, 4.6 mmol), and ethyl iodide (0.7 mL, 11.2 mmol). Reaction time 6 days at room temperature. Yield

20 (0.58 g, 56%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.79 (br s, 1H, NH_2), 8.66 (s, 1H, CH), 8.61 (br s, 1H, NH_2), 4.02 (q, 2H, CH_2), 2.87 (q, 2H, CH_2), 1.28 (t, 6H, $2CH_3$).

4-Amino-1-ethyl-6-methylpyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxide (21). From **6** (5.20 g, 24.4 mmol), acetone (725 mL), potassium carbonate (1.68 g, 12.2 mmol), and ethyl iodide, (3.0 mL, 37.5 mmol). Reaction time 20 days at room temperature. Yield **21** (4.30 g, 73%).

From **6** (0.50 g, 2.3 mmol), acetone (60 mL), triethylamine (0.4 mL, 2.9 mmol), and ethyl iodide (0.4 mL, 5.0 mmol). Reaction time 8 h (heated at reflux). Yield (0.31 g, 54%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.70 (br s, 2H, NH_2), 8.63 (s, 1H, 7-H), 4.02 (q, 2H, CH_2), 2.53 (s, 3H, CH_3), 1.27 (t, 3H, CH_3).

4-Amino-1,6-diethylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (22). From **7** (0.79 g, 3.4 mmol), acetone (30 mL), triethylamine (0.5 mL, 3.6 mmol), and ethyl iodide (0.4 mL, 5.0 mmol). Reaction time 15 days at room temperature. Yield **22** (0.57 g, 60%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.80 (br s, 1H, NH_2), 8.66 (s, 1H, 7-H), 8.62 (br s, 1H, NH_2), 4.03 (q, 2H, CH_2), 2.83 (q, 2H, CH_2), 1.28 (t, 6H, $2CH_3$).

4-Amino-1-ethyl-6-propylpyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxide (23). From **8** (0.60 g, 2.4 mmol), acetone (20 mL), triethylamine (0.4 mL, 2.9 mmol), and ethyl iodide (0.3 mL, 3.7 mmol). Reaction time 15 days at 4 °C. Yield **23** (0.28 g, 46%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.79 (br s, 1H, NH_2), 8.64 (s, 1H, 7-H), 8.61 (br s, 1H, NH_2), 4.03 (q, 2H, N-CH_2), 2.78 (t, 2H, CH_2), 1.75 (m, 2H, CH_2), 1.27 (t, 3H, CH_3), 0.91 (t, 3H, CH_3).

4-Amino-1-ethyl-6-isopropylpyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxide (24). From **9** (1.50 g, 6.2 mmol), acetone (100 mL), triethylamine (0.9 mL, 6.4 mmol), and ethyl iodide (0.7 mL, 8.7 mmol). Reaction time 6 days at room temperature. Yield **24** (0.93 g, 56%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.82 (br s, 1H, NH_2), 8.67 (s, 1H, 7-H), 8.59 (br s, 1H, NH_2), 4.02 (q, 2H, CH_2), 3.13 (sep, 1H, CH), 1.29−1.27 (m, 9H, $3CH_3$).

4-Amino-6-*tert*-butyl-1-ethylpyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxide (25). From **10** (0.82 g, 3.2 mmol), acetone (35 mL), triethylamine (0.5 mL, 3.6 mmol), and ethyl iodide (0.6 mL, 7.5 mmol). Reaction time 5 days at room temperature. Yield **25** (0.56 g, 61%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.83 (br s, 2H, NH_2, 7-H, 8.56 (br s, 1H, NH_2), 4.03 (q, 2H, CH_2), 1.37 (s, 9H, $3CH_3$), 1.28 (t, 3H, CH_3).

4-Amino-1,6-dimethylpyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxide (26). From **6** (1.50 g, 7.0 mmol), acetone (50 mL), potassium carbonate (0.50 g, 3.6 mmol), and methyl iodide (2.0 mL, 32.1 mmol). Reaction time 10 h at room temperature. Yield **26** (0.65 g, 41%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.84 (br s, 1H, NH_2), 8.71 (br s, 1H, NH_2), 8.63 (s, 1H, CH), 3.34 (s, 3H, CH_3), 2.52 (s, 3H, CH_3).

4-Amino-1-methyl-7-phenylpyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxide (27). From **12**, (3.00 g, 11.0 mmol), acetone (180 mL), potassium carbonate (0.75 g, 5.5 mmol), and methyl iodide (4.0 mL, 64.2 mmol). Reaction time 5 days at room temperature. Yield **27** (2.26 g, 76%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.03 (s, 1H, CH), 8.90 (br s, 1H, NH_2), 8.88 (br s, 1H, NH_2), 8.32−8.27 (m, 2H, Ph), 7.60−7.57 (m, 3H, Ph), 3.48 (s, 3H, CH_3).

4-Amino-1,7-dimethyl-6-phenylpyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxide (28). From **16** (2.00 g, 6.9 mmol), acetone (80 mL), potassium bicarbonate (0.75 g, 7.6 mmol), and methyl iodide (3.0 mL, 48.2 mmol). Reaction time 24 h at room temperature. Yield **28** (1.71 g, 82%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.77 (br s, 1H, NH_2), 8.59 (br s, 1H, NH_2), 7.74−7.49 (m, 5H, Ph), 4.07 (s, 3H, CH_3), 2.63 (s, 3H, CH_3).

4-Amino-1-ethylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (29). From **11** (1.00 g, 5.0 mmol), acetone (55 mL), triethylamine (0.7 mL, 5.0 mmol), and ethyl iodide (0.7 mL, 8.7 mmol). Reaction time 5 days at room temperature. To the reaction mixture was added dichloromethane, and the organic layer was separated, evaporated to dryness, and recrystallized from water to yield **29** (0.57 g, 50%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.83 (br s, 2H, NH_2), 8.72 (d, 1H, CH), 8.42 (d, 1H, CH), 4.04 (q, 2H, CH_2), 1.28 (t, 3H, CH_3).

4-Amino-1-[(ethoxycarbonyl)methyl]-6-methylpyrazino-[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (30). From **6** (2.50 g, 11.7 mmol), acetone (100 mL), triethylamine (1.7 mL, 12,2 mmol), and ethyl bromoacetate (1.4 mL, 12.8 mmol). Reaction time 2 days at room temperature. Yield **30** (1.25 g, 36%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.95 (br s, 1H, NH_2), 8.82 (br s, 1H, NH_2), 8.61 (s, 1H, CH), 4.69 (s, 2H, N-CH_2), 4.10 (q, 2H, O-CH_2), 2.55 (s, 3H, CH_3), 1.61 (t, 3H, CH_3).

4-Amino-1-(2-hydroxyethyl)-6,7-dimethylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (31). From **13**, (2.00 g, 8.8 mmol), acetone (200 mL), potassium carbonate (0.60 g, 4.4 mmol), tetrabutylammonium iodide (1.65 g, 4.0 mmol), and 2-bromoethanol (0.8 mL, 11.2 mmol). Reaction time 7 days at room temperature. Yield **31** (0.74 g, 31%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.67 (br s, 1H, NH_2), 8.52 (br s, 1H, NH_2), 4.88 (t, 1H, OH), 4.04 (s, 2H, CH_2), 3.62 (q, 2H, CH_2), 3.31 (s, 3H, CH_3), 2.55 (s, 3H, CH_3).

4-Amino-1-carboxymethyl-6,7-dimethylpyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-Dioxide (32). A solution of **37** (0.80 g, 2.5 mmol) in 2 N hydrochloric acid (70 mL) was heated 70 °C for 5 h. Then, the precipitate was filtered to give **32** (0.42 g, 58%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.77 (br s, 1H, NH_2), 8.63 (br s, 1H, NH_2), 4.58 (s, 2H, CH_2), 2.52 (s, 3H, CH_3), 2.51 (s, 3H, CH_3).

1-Ethyl-4-(ethylamino)-6-phenylpyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxide (38). From **37** (1.00 g, 3.6 mmol), potassium carbonate (0.25 g, 1.8 mmol), acetone (50 mL), and ethyl iodide (0.6 mL, 7.5 mmol). Reaction time 5 days (heated at reflux). Yield **38** (0.72 g, 60%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.46 (t, 1H, NH), 9.39 (s, 1H, CH), 8.34−7.49 (m, 5H, Ph), 4.09 (q, 2H, CH_2), 3.48 (q, 2H, CH_2), 1.34 (t, 3H, CH_3), 1.22 (t, 3H, CH_3).

Transamination. General Procedure for the Synthesis of Compounds 39−48. To a solution of the N-1-substituted pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide in methanol, the correspondig amine was added. The reaction mixture was stirred at room temperature or heated at reflux and evaporated to dryness, and water was added to the residue. The precipitate was filtered and recrystallized from the appropriate solvent (see Table 1).

4-[(2-Aminoethyl)amino]-1-methyl-7-phenylpyrazino-[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (39). From **27** (1.00 g, 3.4 mmol), methanol (45 mL), and ethylendiamine (0.6 mL, 34.4 mmol). Reaction time 6 h (heated at reflux). Yield **39** (0.67 g, 56%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.04 (s, 1H, CH), 8.32−7.58 (m, 5H, Ph), 3.49 (s, 3H, CH_3), 3.41 (m, 2H, CH_2), 2.78 (t, 2H, CH_2).

4-Hydrazino-1-methyl-7-phenylpyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxide (40). From **27** (1.00 g, 3.4 mmol), methanol (90 mL), and hydrazine hydrate (1.5 mL, 30.7 mmol). Reaction time 20 h at room temperature. Yield **40** (0.51 g, 48%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.95 (s, 1H, CH), 8.26−7.53 (m, 5H, Ph), 3.44 (s, 3H, N-CH_3).

4-Benzylamino-1-ethyl-7-phenylpyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxide (41). From **34** (0.60 g, 1.98 mmol), methanol (35 mL), and benzylamine (2.0 mL, 18.3 mmol). Reaction time 5 days (heated at reflux). Yield **41** (0.53 g, 68%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.96 (br s, 1H, NH), 9.05 (s, 1H, CH), 8.30−8.26 (m, 2H, Ph), 7.62−7.59 (m, 3H, Ph), 7.36−7.25 (m, 5H, Ph), 4.62 (s, 2H, CH_2), 4.15 (q, 2H, CH_2), 1.38 (t, 3H, CH_3).

4-Benzylamino-1-ethyl-6-methylpyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxide (42). From **21** (0.80 g, 3.3 mmol), methanol (30 mL), and benzylamine (3.0 mL, 27.5 mmol). Reaction time 62 h (heated at reflux). Yield **42** (0.44 g, 40%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.77 (t, 1H, NH), 8.65 (s, 1H, CH), 7.35−7.25 (m, 5H, Ph), 4.60 (d, 2H, NH-CH_2), 4.02 (q, 2H, CH_2), 2.55 (s, 3H, C6-CH_3), 1.27 (t, 3H, CH_3).

1-Ethyl-4-(ethylamino)-6-methylpyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxide (43). From **21** (0.90 g, 3.7 mmol), methanol (40 mL), and ethylamine (2 mL, 35.3 mmol). Reaction time 48 h (heated at reflux). Yield **43** (0.82 g, 81%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.25 (t, 1H, NH), 8.62 (s, 1H,

CH), 4.01 (q, 2H, CH_2), 3.41 (q, 2H, CH_2), 2.54 (s, 3H, C6−CH_3), 1.27 (t, 3H, CH_3), 1.16 (t, 3H, CH_3).

1,6,7-Trimethyl-4-(2-picolylamino)pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxide (44). From **35** (1.00 g, 4.1 mmol), ethanol (50 mL), and 2-picolylamine (3.0 mL, 29.1 mmol) Reaction time 62 h (heated at reflux). Yield **44** (0.87 g, 65%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.63 (br s, 1H, NH), 8.53 (dd, 1H, py), 7.77 (m, 1H, py), 7.27−7.35 (m, 2H, py), 4.72 (s, 2H, CH_2), 3.36 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 2.55 (s, 3H, CH_3).

1,7-Diethyl-4-[(2-hydroxy)ethylamino]-6-methylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (45). From **36** (1.50 g, 5.6 mmol), methanol (50 mL), and ethanolamine (3.0 mL, 49.7 mmol). Reaction time 28 h (heated at reflux). Yield **45** (1.70 g, 97%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.88 (br s, 1H, NH), 4.90 (t, 1H, OH), 4.04 (q, 2H, CH_2), 3.59 (q, 2H, CH_2), 3.45 (t, 2H, CH_2), 2.90 (t, 2H, CH_2), 2.54 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.26 (t, 3H, CH_3).

1,7-Diethyl-4-hydrazino-6-methylpyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxide (46). From **36** (1.50 g, 5.6 mmol), methanol (50 mL), and hydrazine monohydrate (2.0 mL, 41.0 mmol). Reaction time 4 h at room temperature. Yield **46** (1.41 g, 88%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.01 (q, 2H, CH_2), 2.87 (q, 2H, CH_2), 2.50 (s, 3H, CH_3), 1.29 (t, 3H, CH_3), 1.25 (t, 3H, CH_3).

1,7-Diethyl-6-methyl-4-[(*N*,*N*-dimethylaminoethyl)amino]pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxide (47). From **36** (1.00 g, 3.7 mmol), methanol (40 mL), *N*,*N*-dimethylethylenediamine (3.0 mL, 27.6 mmol). Reaction time 20 h (heated at reflux). Yield **47** (1.06 g, 84%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.82 (br s, 1H, NH), 4.03 (q, 2H, N-CH_2), 3.47 (t, 2H, NH-CH_2), 2.90 (q, 2H, CH_2), 2.54 (s, 3H, CH_3), 2.47 (t, 2H, CH_2-N), 2.17 (s, 6H, 2CH_3), 1.30 (t, 3H, CH_3), 1.26 (t, 3H, CH_3)

1,7-Dimethyl-6-phenyl-4-pyrrolidinylpyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-Dioxide (48). From **28** (0.80 g, 2.7 mmol), methanol (30 mL), and pyrrolidine (1.0 mL, 11.9 mmol). Reaction time 90 h at room temperature. Yield **48** (0.48 g, 50%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.51−7.24 (m, 5H, Ph), 4.16 (t, 2H, N-CH_2), 3.81 (t, 2H, N-CH_2), 3.55 (s, 3H, N-CH_3), 2.64 (s, 3H, CH_3), 2.03−1.59 (m, 4H, 2CH_2).

Pharmacological Assays. In Vitro Tracheal Muscle Relaxation. Relaxation of the spontaneous tone in isolated tracheal muscle was determined.[29] The isolated zigzag cut guinea pig trachea is permitted to develop contraction when placed in physiological salt solution at 37 °C under 0.5 g resting tension. Test substance (μg/mL) inhibition of tracheal tone by more than 50%, relative to maximal relaxation induced by 0.3 μg/mL epinephrine, was considered significant activity.

Bronchodilatory Activity Studies in Vivo. The brochoconstriction in male Dunkin−Hartley guinea pigs (n = 5−6, 400−450 mg) was determined using the Konzett and Rossler technique.[30] Guinea pigs were anaesthetized with urethane (1.5 g/kg i.p.), and the trachea, jugular vein, and duodenum were cannulated. Animals were ventilated using a pump at 5 strokes/min with a stroke volume of 10 mL/kg body weight. After stabilization of the animals, changes in airway resistance were induced by i.v. injection of histamine (7.5 μg/kg). Ten minutes after two similar responses to the spasmogen had been obtained, a single dose of compound or theophylline was given intraduodenally. Bronchoconstrictor challenges were repeated at 5, 15, 30, and 60 min after administration of the test compound. Test compound activity was calculated by comparing the increase in pulmonary inflation presure in response to each bronchoconstriction before and after compound administration and expresing the difference as percentage change. (Test Duncan−Kramer $p < 0.05$). Activity is expressed as the percentage of the inhibitory response versus theophylline (value = 1 at 25 mg/kg and 100 mg/kg).

Acute Lethal Toxicity. LD_{50} value was determined according to the Litchfield and Wilcoxon method[32] from the mortality in male swiss mice (n = 10, 20−25 g) and male CFY/SD rat (n = 10, 150−200 g), after oral administration of compound **21** and theophylline.

Acknowledgment. We are grateful to E. Carrasco and M. Grau from Prodesfarma S. A., Barcelona, Spain, for biological assays.

Supporting Information Available: Elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Magnussen, H.; Richter, K.; Taube, C. Are chronic obstructive pulmonary disease (COPD) and asthma different diseases? *Clin. Exp. Allergy* **1998**, *28*, 187−94.
(2) Hay D. W. P.; Barnette M. S. Current and potential new therapies for chronic obstructive pulmonary disease. *Annu. Rep. Med. Chem.* **1999**, *34*, 111−120.
(3) Chung, K. F. Role of inflammation in the hyperreactivity of the airways in asthma. *Thorax* **1986**, *41*, 657−662.
(4) Farley, J. M. Inhaled Toxicants and Airway Hiperresponsiveness. *Annu. Rev. Pharmacol. Toxicol.* **1992**, *32*, 67−88.
(5) Duplantier, A. J.; Cheng, J. B. Emerging Opportunities in the Treatment of Asthma and Allergy. *Annu. Rep. Med. Chem.* **1994**, *29*, 73−81.
(6) Frew, A. J.; Holgate, S. T. Clinical Pharmacology of Asthma. Implications for treatment. *Drugs* **1993**, *46*, 847−862.
(7) Soong, S. C. Bronchodilators. Part I: Adrenergic Drugs. *Drugs Today* **1984**, *20*, 439−464.
(8) Nasser, S. S. M.; Rees, P. J. Theophylline. Current thoughts on the Risk and Benefits of its use in Asthma. *Drug Safety* **1993**, *8*, 12−18.
(9) Wettergel, R. Theophylline past present and future. *Arzneimit. Forsch* **1998**, *48*, 535−539.
(10) Rabe, K. F.; Dent, G. Theophylline and airway inflammation. *Clin. Exp. Allergy* **1998**, *28*, 35−41.
(11) Jacobs, R. T.; Veale, C. A.; Wolanin, D. J. Pulmonary and Anti-Allergy Agents. *Annu. Rep. Med. Chem.* **1992**, *27*, 109−118.
(12) Schudt, C.; Tenor, H.; Hatzelmann, A. PDE isoenzymes as targets for anti-asthma drugs. *Eur. Respir. J.* **1995**, *8*, 1179−1183.
(13) Rogers, D. F.; Laurent, G. J. New ideas on the pathophysiology and treatment of lung disease. *Thorax* **1998**, *53*, 200−203.
(14) Carrasco, E.; Goya, P.; Grau, M.; Paez, J. A. Preparation of 1N substituted pyrazino[2,3-*c*]-1,2,6-thiadiazine 2,2-dioxides and their use in pharmaceuticals. (Prodesfarma, S. A.; C.S.I.C., Spain). Eur. Pat. Appl. EP 580916, 1994.
(15) Carrasco, E.; Goya, P.; Grau, M.; Paez, J. A. 1N substituted pyrazino[2,3-*c*]-1,2,6-thiadiazine 2,2-dioxides. (Prodesfarma, S. A.; C.S.I.C., Spain). U.S. Patent 5,580,869, 1996.
(16) Goya, P.; Páez, J. A.; Pfleiderer, W. Pyrazino[2,3-*c*][1,2,6]-thiadiazine 2,2-Dioxides. Sulfur Dioxide Analogues of Pteridines. *J. Heterocycl. Chem.* **1984**, *21*, 861−864.
(17) Alkorta, I.; Goya, P.; Páez, J. A.; Pfleiderer, W. Synthesis and Physico-Chemical Properties of 6- and 7-Monosubstituted Pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxides. *Pteridines* **1990,** *2*, 3−7.
(18) Goya, P., Herrero, A.; Jimeno M. L.; Ochoa C.; and Páez, J. A. Tautomerism in Pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-Dioxides. *Heterocycles* **1988**, *27*, 2201−2209.
(19) Alkorta, I.; García-Gómez, C.; Páez, J. A.; Goya, P. Theoretical and Experimental Analysis of Properties in Heterocycles Containing the Aminosulphonylamino Moiety. *J. Phys. Org. Chem.* **1996**, *9*, 203−211.
(20) Campillo N.; Alkorta, I.; Páez J. A.; Goya, P. Solvent Effect on the Tautomerism of 4-Aminopyrazino[2,3-*c*]-1,2,6-Thiadiazine 2,2-Dioxide. *J. Chem. Soc., Perkin Trans. 2*, **1998**, 1889−92.
(21) Goya, P.; Páez, J. A.; Alkorta, I.; Carrasco, E.; Grau, M.; Antón, F.; Julia, S.; Martínez-Ripoll, M. N-Substituted Pyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-Dioxides. A New Class of Diuretics. *J. Med. Chem.* **1992**, *35*, 3977−3983.
(22) Campillo, N.; García, C.; Goya, P.; Páez, J. A.; Carrasco, E.; Grau, M. Novel arylpyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxides as inhibitors of platelet aggregation: 1. Synthesis and Pharmacological Evaluation. *J. Med. Chem.* **1999**, *42*, 1698−1704.
(23) Campillo, N.; Goya, P.; Páez, J. A. Novel Arylpyrazino[2,3-*c*]-[1,2,6]thiadiazine 2,2-dioxides as platelet aggregation inhibitors. 2. Optimization by Quantitative Structure−Activity Relationships. *J. Med. Chem.* **1999**, *42*, 3279−3288.
(24) García-Muñoz, G.; Madroñero, R.; Ochoa, C.; Stud, M. Synthesis of Purina-Like Ring System Derived From 1,2,6-Thiadiazine 1,1-Dioxide. *J. Heterocycl. Chem.* **1976**, *13*, 793−796.
(25) Van der Haar, R. W.; Voter, R. C.; Banks, C. V. The Synthesis of 1,2-Cycloheptane-dione Dioxime. *J. Org. Chem.* **1949**, *14*, 836−838.

(26) Riley, H. L.; Morley, J. F.; Child-Friend, N. A. Selenium Dioxide, a New Oxidising Agent. Part I. Its Reaction with Aldehydes and Ketones. *J. Chem. Soc* **1932**, *Part I*, 1875−1883.
(27) Freon, P. Essais de préparation du méthylglyoxal et de son acetal. *Ann. Chim.* **1939**, *11*, 460.
(28) Goya, P.; Páez, J. A.; Pfleiderer, W. Reactivity of 4-amino-6,7-diphenyl-8H-pyrazino[2,3-*c*][1,2,6]thiadiazine 2,2-dioxide towards methylating agents. *J. Heterocycl. Chem.* **1990**, *27*, 785−6.
(29) Luduena, F. P.; Euler, V.; Tullar, B. F.; Lands, A. M. Effect of the optical isomers of some sympathomimetic amines on the guinea pig bronchioles. *Arch. Int. Pharmacod.* **1957**, *111*, 392−400.
(30) Konzett, H.; Rossler, R. Experimental research on brochomuscle. (Versuchsanordnung zu untersuchunger an der bronchialmuskulatur). *Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmakol.* **1940**, *195*, 71−74.
(31) Cortijo, J.; Martí-Cabrera, M.; Berto, L.; Antón, F.; Carrasco, E.; Grau, M.; Morcillo, E. J. Pharmacological activity of PF-904 in guinea pig in vivo, and on human bronchus and neutrophils in vitro. *Eur. J. Pharmacol.* **1997**, *333*, 69−78.
(32) Litchfield, T.; Wilcoxon, F. A Simplified Method of Evaluating Dose−Effect experiments. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99−113.

JM000970X