N-(4-Isoxazolylthiazol-2-yl)oxamic Acid Derivatives as Potent Orally Active Antianaphylactic Agents

Dario Chiarino, Giancarlo Grancini, Viviana Frigeni,* Ivano Biasini, and Angelo Carenzi

Zambon Group Spa, Via Lillo Del Duca, 10-20091 Bresso, Milano, Italy. *Received April 11, 1990*

A series of N-(4-isoxazolylthiazol-2-yl)oxamic acid derivatives was synthesized and tested on the passive cutaneous anaphylaxis (PCA) model in rats to verify its potential antianaphylactic activity. These compounds were prepared by reaction of an appropriate bromoacetylisoxazole with thiourea to give the corresponding aminothiazole and subsequent condensation with an oxalic acid monoester chloride to yield, following the usual process, the oxamic acid derivatives. Most of the new compounds exhibited, by intraperitoneal route in rats, a very potent antianaphylactic activity on PCA response, higher than that of the reference compound disodium cromoglycate (DSCG). The new derivatives, in contrast with DSCG, were effective on PCA even by oral route. The most interesting derivative of the new series was N-[4-(3-methyl-5-isoxazolyl)-2-thiazolyl]oxamic acid 2-ethoxyethyl ester (**49**), which was also active and more potent than DSCG in experimental models involving either IgE- or IgG-mediated anaphylactic responses at bronchopulmonary level.

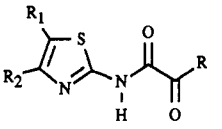
Current prophylaxis of allergic asthma includes treatment with disodium cromoglycate (DSCG). Although DSCG is known to be effective only by inhaled administration and not when given by oral route, it is almost completely devoid of side effects in comparison with other agents. Hence, there has been an increasing interest in the synthesis of new antianaphylactic compounds with a chemical structure other than that of DSCG but with the same mechanism of action, i.e. inhibition of asthmogenic mediator release from mast cells and eosinophils, in order to develop orally active drugs.[1-3] In fact, some oxamic acid derivatives were already reported in the literature[4-8] which seem to answer this purpose.

In our laboratories a series of 4-substituted isoxazolyl-2-thiazolyloxamate derivatives was synthesized and studied on an IgE-mediated PCA model in rats to verify its potential antianaphylactic activity. The most interesting compound of this series was then selected for a further in vivo and in vitro pharmacological evaluation as antianaphylactic agent at bronchopulmonary level.

The new N-(4-isoxazolyl-thiazol-2-yl)oxamic acid derivatives are represented by the general formulas **1a-c** wherein the meaning of X, Y, and R are described for all compounds in Tables III-VI.

Chemistry

The new oxamic acid derivatives of general formulas **1a-c** were readily synthetized as shown in Scheme I. The preparation of a number of starting isoxazole ketones **2** was previously described.[9-15] The new ketones were synthesized by 1,3-dipolar cyclization between a nitrile oxide generated in situ and an appropriate alkynol, followed by oxidation of the resulting alcohol with chromic anhydride in acetic acid as compounds **4-6** (Scheme II). Otherwise, sodium monoenolate of 2,4-pentanedione and 3-methoxy-1-buten-3-one were used as dipolarophiles to give directly the ketones as for **7** and **8**.



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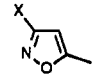
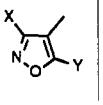
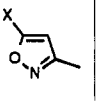
	a	b	c
R_1	H, C_2H_5	H	H
R_2			

The bromination of ketones affords the corresponding α-bromo ketones (**9-24**, Table I) often together with α,α-dibromo ketones as minor products.

The 2-aminothiazole intermediates **25-33** and **35-41**, listed in Table II, were prepared by refluxing the α-bromo ketones with thiourea in ethanol. 2-Amino-4-(3-hydroxymethyl-5-isoxazolyl)thiazole (**34**) (Table II) was obtained by reduction of the corresponding 3-carbethoxy derivative **33** with $NaBH_4$ in methanol and dimethylformamide.

Oxamic acid esters **42-61**, listed in Tables III and IV, were prepared by reaction of 2-aminothiazole with the appropriate oxalic acid monoester chloride in pyridine or in THF with triethylamine as hydrochloric acid acceptor.

The oxamic acids were obtained from the corresponding esters by mild alkaline hydrolysis followed by acidification and their salts of ethanolamine and lysine were prepared by the usual methods (compounds **62-76**, Tables V and VI).

Biological Results and Discussion

The new oxamic acid derivatives were studied by oral and intraperitoneal route as potential antianaphylactic agents on the PCA model in rats. The results we obtained

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(15) Caramella, P.; Cereda, E. *Synthesis* **1971**, 433.

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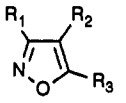
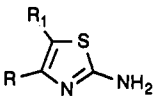
Table I. α-Bromo Ketones



no.	R_1	R_2	R_3	mp, °C, or bp, °C (mmHg)	recrystn solvent	% yield
9	Cl	H	$BrCH_2CO$	97–99 (2.0)		96
10	Br	H	$BrCH_2CO$	53–54	*i*-PrOH	81
11	Me	H	$BrCH_2CO$	68–70	*i*-PrOH	91
12	$PhCH_2O$	H	$BrCH_2CO$	80–81	*i*-PrOH	83
13	Me	H	$BrCH_2CO$	44–46	*i*-PrOH	87
14	$MeOCH_2$	H	$BrCH_2CO$	oil[a]		91
15	Ph	H	$BrCH_2CO$	116–118	EtOH	89
16	2-Cl-6-F-C_6H_3	H	$BrCH_2CO$	146–150 (0.2)		85
17	EtOCO	H	$BrCH_2CO$	74–75	*i*-PrOH	83
18	Br	H	BrCH(Et)CO	53–54	hexane	98
19	Me	$BrCH_2CO$	H	101–103	CCl_4	80
20	Ph	$BrCH_2CO$	Me	46–48	*i*-PrOH	87
21	2-Cl-6-F-C_6H_3	$BrCH_2CO$	Me	74–75	*i*-PrOH	86
22	$BrCH_2CO$	H	Me	65–66	ligroin	76
23	$BrCH_2CO$	H	$HOCH_2$	160 (0.3)		94
24	$BrCH_2CO$	H	Ph	128–130	*i*-PrOH	82

[a] Controlled by ^1H NMR and used crude.

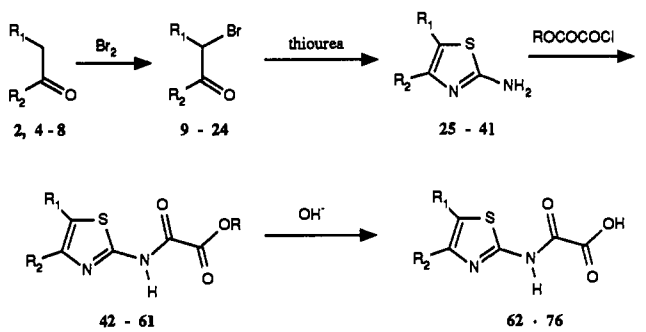
Table II. 2-Aminothiazoles



no.	R	R_1	mp, °C	recrystn solvent	% yield
25	3-chloro-5-isoxazolyl	H	168–170	MeCN	77
26	3-bromo-5-isoxazolyl	H	160–162	MeOH	97
27	3-methoxy-5-isoxazolyl	H	215–217	MeOH	84
28	3-benzyloxy-5-isoxazolyl	H	129–131	MeCN	76
29	3-methyl-5-isoxazolyl	H	208–210	MeCN	57
30	3-(methoxymethyl)-5-isoxazolyl	H	137–138	MeCN	49
31	3-phenyl-5-isoxazolyl	H	191–192	MeCN	66
32	3-(2-chloro-6-fluorophenyl)-5-isoxazolyl	H	168–169	MeCN	57
33	3-(etoxycarbonyl)-5-isoxazolyl	H	156–157	MeCN	87
34	3-(hydroxymethyl)-5-isoxazolyl	H	183–185	MeCN	97
35	3-bromo-5-isoxazolyl	Et	151–152	MeCN	71
36	3-methyl-4-isoxazolyl	H	178–179	MeCN	50
37	5-methyl-3-phenyl-4-isoxazolyl	H	223–224	MeOH	70
38	3-(2-chloro-6-fluorophenyl)-5-methyl-4-isoxazolyl	H	148–149	*i*-PrOH	82
39	5-methyl-3-isoxazolyl	H	242 dec[a]	95% EtOH	76
40	5-(hydroxymethyl)-3-isoxazolyl	H	185–187	MeOH	63
41	5-phenyl-3-isoxazolyl	H	215–216	MeOH	75

[a] Characterized as hydrobromide.

Scheme I

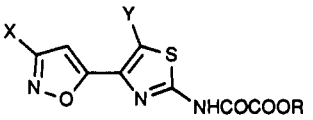


are reported in Tables III–VI.

The isoxazole ring coupled to the thiazolyloxamic moiety leads to derivatives endowed with antianaphylactic activity when administered either by oral or by intraperitoneal route (Tables III–VI). However, some specific structural conditions are required in order to obtain compounds endowed with a potent antianaphylactic activity. For instance, the position of the bond between the thiazolyloxamic acid moiety and the isoxazole ring plays an important role. In fact, the highest antianaphylactic activity was obtained when the thiazolyloxamic acid moiety is linked with the position 3 or 5 of the isoxazole ring, while the link with position 4 of the isoxazole ring was generally followed by a decrease in the antianaphylactic activity (see **49** and **59** vs **56**). Furthermore, within the series of *N*-[4-(5-isoxazolyl)-2-thiazolyl]oxamic acid ester derivatives (Table III) the nature of the X substituent is the main requirement to obtain compounds provided with a potent antianaphylactic activity by oral route: the most effective compounds were obtained when X was an alkyl, a substituted alkyl, or a carbethoxy group (Table III).

Within the new series of *N*-[4-(5-isoxazolyl)-2-thiazolyl]oxamic acid and salt derivatives, besides the alkyl or the substituted alkyl, the phenyl group on the isoxazole ring also accounts for the potent antianaphylactic properties of the new compounds (Table V). With regard to the variation in the antianaphylactic activities of the different salt forms (e.g. compounds **63–65**), it should be

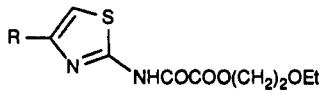
Table III. *N*-[4-(3-Substituted-5-isoxazolyl)-2-thiazolyl]oxamic Acid Esters



no.	X	Y	R	mp, °C, from MeCN	% yield	rat PCA ED_{50}, mg/kg po	rat PCA ED_{50}, mg/kg ip
42	Cl	H	$EtOCH_2CH_2$	154–156	81	>30	6 (3–10)
43	Br	H	$EtOCH_2CH_2$	162–164	70	>30	11 (11–13)
44	Br	H	$MeOCH_2CH_2$	175–177	61	>100	7 (5–6)
45	Br	H	Et	196–198	79	>100	12 (8–16)
46	Br	Et	$EtOCH_2CH_2$	128–129	92	9 (6–14)	3 (2–4)
47	MeO	H	$EtOCH_2CH_2$	142–143	78	11 (10–13)	3 (2–5)
48	$PhCH_2O$	H	$EtOCH_2CH_2$	142–144	82	>100	3.7 (2.9–4.8)
49	Me	H	$EtOCH_2CH_2$	155–156	91	1.3 (0.8–2.3)	0.04 (0.01–0.11)
50	Me	H	Et	180–181	81	0.7 (0.2–2.5)	0.2 (0.07–0.5)
51	$MeOCH_2$	H	$EtOCH_2CH_2$	136–137[a]	86	0.7 (0.08–5.8)	2.4 (1–5.8)
52	Ph	H	t-Bu	212–213 dec	64	7– (2–30)	9 (3–25)
53	2-Cl-6-F-C_6H_3	H	$EtOCH_2CH_2$	140–141	87	>30	1.1 (0.2–6.5)
54	2-Cl-6-F-C_6H_3	H	$PhCH_2$	199–200	67	>30	>30
55	EtOCO	H	$EtOCH_2CH_2$	145–146	75	1.9 (1.5–2.5)	0.08 (0.004–1.4)
DSCG						NA[b]	12 (9–16)

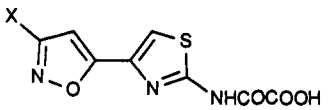
[a] Crystallized from EtOH. [b] Not active.

Table IV. *N*-[4-[3-(or 4)-Isoxazolyl]-2-thiazolyl]oxamic Acid Esters



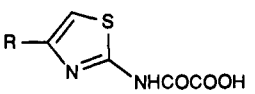
no.	R	mp, °C	recrystn solvent	% yield	rat PCA ED_{50}, mg/kg po	rat PCA ED_{50}, mg/kg ip
56	3-Me-4-isoxazolyl	150–151	MeCN	82	>100	>100
57	5-Me-3-Ph-4-isoxazolyl	113–115	EtOH	80	>100	>10
58	3-(2-Cl-6-F-C_6H_3)-5-Me-4-isoxazolyl	47–48	MeCN	85	>30	>10
59	5-Me-3-isoxazolyl	115–116	CCl_4	80	2 (0.2–2.7)	1.3 (0.8–2.3)
60	5-Ph-3-isoxazolyl	146–147	MeCN	82	13 (10–17)	0.2 (0.1–0.4)
61	5-$HOCH_2$-3-isoxazolyl	159–161	MeCN	58	14 (11–18)	1.8 (1.3–2.5)

Table V. *N*-[4-(3-Substituted-5-isoxazolyl)-2-thiazolyl]oxamic Acids and Salts



no.	X	salt	mp, °C	recrystn solvent	% yield	rat PCA ED_{50}, mg/kg po	rat PCA ED_{50}, mg/kg ip
62	Cl	2-aminoethanol	211–212	70% EtOH	71	>30	>30
63	Br		217–218		85	>30	3
64	Br	2-aminoethanol	190–193	70% EtOH	59	>30	7 (5–10)
65	Br	L-lysine	196–197	75% EtOH	71	8 (5–15)	0.5 (0.1–2.3)
66	MeO	2-aminoethanol	214–215	70% EtOH	69	8 (5–11)	1.2 (0.5–1.6)
67	Me	L-lysine	208–209	80% MeOH	55	6 (3–12)	0.3 (0.29–0.31)
68	$HOCH_2$	2-aminoethanol	196–197	75% MeOH	69	0.7 (0.4–1)	0.007 (0.001–0.045)
69	Ph	2-aminoethanol	207–208	85% EtOH	73	0.6 (0.4–0.8)	0.24 (0.19–0.30)
70	Ph	L-lysine	227–228	40% MeOH	73	2.4 (0.8–7.6)	0.24 (0.15–0.40)
71	2-Cl-6-F-C_6H_3	2-aminoethanol	230–231	70% EtOH	56	>30	2.4 (1.8–3.2)

Table VI. *N*-[4-(3-(or 4)-Isoxazolyl]-2-thiazolyl]oxamic Acids and Salts



no.	R	salt	mp, °C	recrystn solvent	% yield	rat PCA ED_{50}, mg/kg po	rat PCA ED_{50}, mg/kg ip
72	3-Ph-4-isoxazolyl	L-lysine	187–188	*i*-PrOH	62	>100	>100
73	3-(2-Cl-6-F-C_6H_3)-4-isoxazolyl	2-aminoethanol	164–166	*i*-PrOH	60	>100	>30
74	5-Me-3-isoxazolyl	L-lysine	213–214	70% EtOH	81	17 (6–48)	1.7 (0.8–3.4)
75	5-Ph-3-isoxazolyl	2-aminoethanol	210–211	75% EtOH	69	2.6 (2.0–3.3)	0.23 (0.14–0.29)
76	5-$HOCH_2$-3-isoxazolyl	2-aminoethanol	185–187	MeOH	61	>30	>100

Scheme II

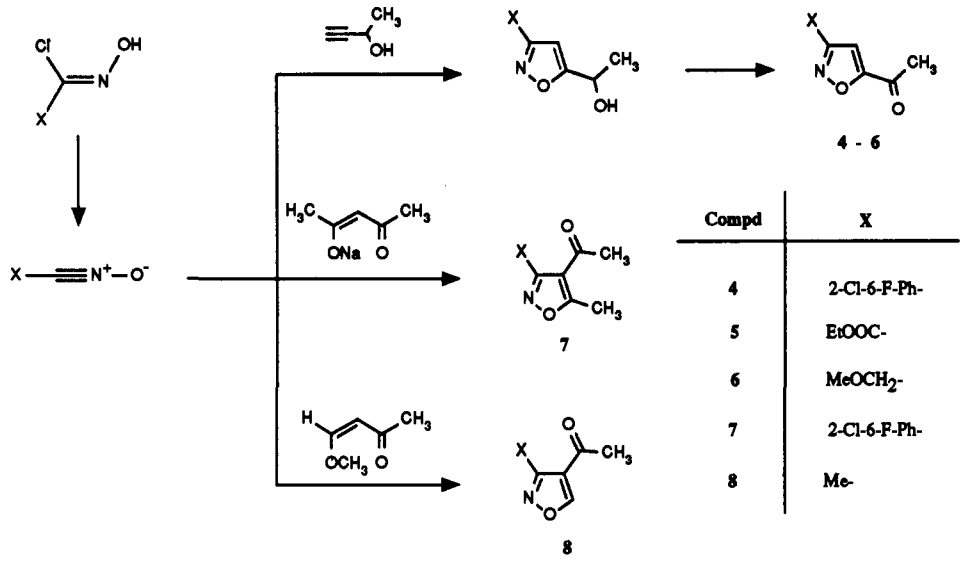


Table VII. Antianaphylactic Activity (ED_{50}) of Compound **49** at the Bronchopulmonary Level

experimental model	species	route	ED_{50} (CL 95%), mg/kg	
			49	DSCG
		po	4.7 (0.9–24.3)	>100
IgE-mediated PPA	rat	iv	0.09 (0.05–0.17)	0.82 (0.33–2.06)
IgG-mediated APA	guinea pig	ip	185 (91–376)	>1000

pointed out that it could be ascribed to a different bioavailability of these salt forms since they all were tested by keeping the pretreatment time constant (Table V).

Quite a number of the new compounds when tested by intraperitoneal injection into rats on PCA were about 20–50 times more effective than DSCG and moreover, unlike DSCG, they were also fully effective when given by oral route. In order to further investigate the pharmacological profile of the new derivatives, compound **49** was selected since, at first, its antianaphylactic activity by oral route fell within the range of activity displayed by the other very effective derivatives of the series; in addition, compound **49** also has the advantage of its better physicochemical stability besides its easier manufacturing procedures when compared to those of other members of the series (e.g. compound **68**). Thus, the antianaphylactic effectiveness of **49** was investigated in experimental models involving either IgE-mediated responses in rats, i.e. passive pulmonary anaphylaxis (PPA), or IgG-mediated responses in guinea pigs, i.e. active pulmonary anaphylaxis (APA). DSCG was used as reference compound. As clearly shown in Table VII, compound **49** displayed a clear-cut activity by oral route in inhibiting IgE-mediated PPA (ED_{50} = 4.7 mg/kg po) in contrast with the reference compound DSCG, which did not display any significant activity up to 100 mg/kg po. Compound **49** was also more effective than DSCG on PPA model when both derivatives were administered by intravenous route (compound **49** ED_{50} = 0.09 mg/kg iv vs DSCG ED_{50} = 0.82 mg/kg iv). Furthermore, as can be seen in the same table, also in the IgG-mediated APA response compound **49** administered by intraperitoneal route was more effective than DSCG (compound **49** ED_{50} = 185 mg/kg ip vs DSCG ED_{50} > 1000 mg/kg ip).

With regard to the in vitro studies, compound **49** showed a potent inhibitor effect on antigen-induced slow reacting substance of anaphylaxis (SRS-A-like material) release from sensitized guinea pig lung fragments (Table VIII). These results confirmed, at first, the antianaphylactic activity of compound **49** already observed in vivo and, then,

Table VIII. Inhibitory Effect of Compound **49** and DSCG on Antigen-Induced SRS-A-like Material Release from Sensitized Guinea Pig Lung Fragments

compd	concn, M	% inhibn of SRS-A-like material release[a]	compd	concn, M	% inhibn of SRS-A-like material release
49	10^{-8}	18 ± 5	DSCG	10^{-6}	20 ± 5
	10^{-7}	43 ± 5		10^{-5}	38 ± 3
	10^{-6}	48 ± 6*		10^{-4}	46 ± 5
	10^{-5}	52 ± 5*			

[a] An asterisk denotes $p < 0.05$ vs DSCG corresponding concentrations (Student's unpaired two-tailed t test).

suggested a direct effect of compound **49** on cells primarily involved in the release of chemical mediators responsible for the asthmogenic response. In addition, further in vitro studies demonstrated that compound **49**'s antianaphylactic activity could not be assigned to its antagonistic activity on chemical receptors such as acetylcholine, histamine, or serotonin receptors because it did not antagonize the constrictive effects of these mediators on isolated guinea pig ileum preparation (data not shown).

In conclusion, we have described the synthesis and the antianaphylactic activity of a new series of (isoxazolylthiazolyl)oxamic acid derivatives. Most of these compounds possess a significant antianaphylactic effectiveness on PCA in rats, higher than that of DSCG and, more interestingly, in contrast with DSCG they are fully effective even when given by oral route. Compound **49**, which stands out among the derivatives of the new series for its very potent antianaphylactic activity on PCA in rats, is also very effective in inhibiting both IgE- and IgG-mediated anaphylaxis at the bronchopulmonary level. Compound **49** seems to act through a similar mechanism of action to DSCG, i.e. a direct inhibition of asthmogenic mediator release, without any antagonistic activity on chemical receptors. Hence, compound **49** could be of great interest as an antianaphylactic agent in the management of asthma.

Experimental Section

Melting points were determined with a Büchi SMP 20 apparatus and were uncorrected. NMR spectra were obtained with EM-360L and Gemini 200-MHz Varian spectrometers using trimethylsilane as internal standard. The purity of all new derivatives (**25–76**) was verified by means of elemental analysis for C, H, N, S (±0.4% of the calculated values).

1-[3-(2-Chloro-6-fluorophenyl)-5-isoxazolyl]ethanol (3). To a solution of 28.4 g (136 mmol) of α,2-dichloro-6-fluorobenzaldoxime[16] and 19.14 g (273 mmol) of 3-butyn-2-ol in 250 mL of benzene was added 27.6 g of triethylamine dropwise at 8–10 °C. After the addition, the mixture was stirred at 60 °C for 1 h and then was cooled, washed with 10% HCl and water, and evaporated. The oily residue was purified by distillation to give 25 g (75%) of **3**: bp 140–145 °C (0.3 mmHg); NMR ($CDCl_3$) ppm 7.31 (m, 3 H), 6.40 (s, 1 H), 5.11 (q, 1 H), 1.63 (t, 3 H).

3-(2-Chloro-6-fluorophenyl)-5-acetylisoxazole (4). To a stirred solution of 20 g (83 mmol) of **3**, previously prepared in 125 mL of acetic acid cooled at 5 °C, was added dropwise a solution of 6 g (60.5 mmol) of CrO_3 in 6.5 mL of water and 87 mL of acetic acid. The mixture was stirred overnight at room temperature. The acetic acid was evaporated, the residue was dissolved in water, and the resulting solution was neutralized with $NaHCO_3$ and extracted with ether. The organic extract was evaporated and the oily residue was purified by distillation to give 15.8 g (80%) of product, bp 120–122 °C (0.4 mmHg), as colorless oil that spontaneously turned into a crystalline solid: mp 46–47 °C; NMR (Me_2SO-d_6) ppm 7.60 (m, 4 H), 2.81 (s, 3 H).

The following compounds were prepared as above.

3-Carbethoxy-5-acetylisoxazole (5) was obtained from 1-(3-carbethoxy-5-isoxazolyl)ethanol:[17] yield 80%; mp 67–68 °C (from diisopropyl ether); NMR ($CDCl_3$) ppm 7.3 (s, 1 H), 4.52 (q, 2 H), 2.69 (s, 3 H), 1.52 (t, 3 H).

3-(Methoxymethyl)-5-acetylisoxazole (6) was obtained from 1-(3-methoxymethyl-5-isoxazolyl)ethanol:[17] yield 58.5%; bp 72–74 °C (0.4 mmHg); NMR ($CDCl_3$) ppm 6.99 (s, 1 H), 4.60 (s, 2 H), 3.41 (s, 3 H), 2.58 (s, 3 H).

3-(2-Chloro-6-fluorophenyl)-5-methyl-4-acetylisoxazole (7). To a solution of 2.3 g (100 mmol) of clean sodium in 220 mL of anhydrous ethanol was added 10 g (100 mmol) of acetylacetone. The mixture was cooled and stirred at 0 °C and a solution of 17 g (82 mmol) of α,2-dichloro-6-fluorobenzaldoxime[16] in 65 mL of anhydrous EtOH was added dropwise while the temperature was kept below 5 °C. After stirring overnight at room temperature, the mixture was neutralized with 10% HCl, and EtOH was evaporated. The residue was taken up with 100 mL of water and 150 mL of ether. The organic layer was separated and evaporated and the oily residue was purified by distillation to give 14.5 g (70%) of colorless oil: bp 129–131 °C (0.5 mmHg); NMR ($CDCl_3$) ppm 7.60 (m, 3 H), 2.81 (s, 3 H), 2.29 (s, 3 H).

3-Methyl-4-acetylisoxazole (8). To a solution of 23.6 g (400 mmol) of acetaldoxime in 95 mL of 5% HCl cooled at −5 °C, was added 29.8 g (20.3 mL, 420 mmol) of chlorine in 2 h. After 1 h at −10 °C the solution was extracted four times with 90 mL of CH_2Cl_2. The organic extracts were washed with water, and 20 g (200 mmol) of 1-methoxy-1-buten-3-one was added. Then, 44.5 g (440 mmol) of triethylamine was added dropwise under stirring at −5 °C in 2 h. After stirring overnight, the reaction mixture was washed with 5% HCl and water. The solvent was evaporated and the oily residue (24.8 g) was purified by fractionated distillation to give 16 g (64%) of **8** as a colorless oil, bp 75–77 °C (3 mmHg), that crystallized from 2-propanol: mp 46–48 °C; NMR ($CDCl_3$) ppm 8.83 (s, 1 H), 2.46 (s, 3 H), 2.44 (s, 3 H).

3-Carbethoxy-5-(bromoacetyl)isoxazole (17). To a solution of 38.3 g (209 mmol) of **6** in 300 mL of $CHCl_3$, containing 6 mL of acetic acid and warmed at 48–50 °C, a solution of 35.1 g (220 mmol) of bromine in 90 mL of $CHCl_3$ was added dropwise in 10 min. The colorless solution was poured into 400 mL of an ice-water mixture. The organic layer was separated, washed with water, and dried on Na_2SO_4. The solvent was evaporated to give 54.8 g of crude **17**, which is contaminated by α,α-dibromo ketone [NMR ($CDCl_3$) ppm 6.77 (s, 1 H, $CHBr_2$)] but can be purified by crystallization from diisopropyl ether: mp 74–75 °C; NMR ($CDCl_3$) ppm 7.53 (s, 1 H), 4.58 (s, 2 H), 4.52 (q, 2 H), 1.47 (t, 3 H).

All α-bromo ketones listed in Table I were prepared as above. Compound **14** was obtained and utilized as crude product.

2-Amino-4-(3-chloro-5-isoxazolyl)thiazole (25). A mixture of 11.2 g (50 mmol) of **9** and 7.6 g (100 mmol) of thiourea in 165 mL of EtOH was refluxed for 90 min and then cooled in an ice bath for 1 h. The hydrobromide precipitate was separated by filtration and taken up with a mixture of 25 mL of 10% NaOH and 100 mL of ethyl acetate. The organic layer was separated, washed with water, and evaporated to give 7.7 g (77%) of **25**, which was purified by recrystallization from MeCN, mp 169–170 °C; NMR (Me_2SO-d_6) ppm 7.4 (s, 1 H), 6.9 (s, 1 H).

2-Aminothiazoles **25–33** and **35–41** listed in Table II were synthesized as described above.

2-Amino-4-[3-(hydroxymethyl)-5-isoxazolyl]thiazole (34). To a stirred solution of 13.9 g (58 mmol) of **33**, in 40 mL of DMF and 80 mL of MeOH warmed at 65 °C, was added 4.4 g (116 mmol) of $NaBH_4$ portionwise. After 90 min the mixture was cautiously acidified with 60 mL of 10% HCl and evaporated. The residue was dissolved in water, and K_2CO_3 was added until a definite alkaline reaction occurred. The precipitate was separated by filtration and washed with water to give 11.1 g (97%) of crude **34**, which was crystallized from MeCN: mp 184–185 °C; NMR (Me_2SO-d_6) ppm 7.41 (s, 1 H), 6.59 (s, 1 H), 5.52 (t, 1 H), 4.61 (d, 2 H).

2-Ethoxyethyl *N*-[4-(3-Methyl-5-isoxazolyl)-2-thiazolyl]oxamate (49). To a stirred mixture of 9.7 g (53.5 mmol) of **29** in 107 mL of pyridine, cooled at 5 °C, was added 11.1 g (61.5 mmol) of 2-ethoxyethyloxalyl chloride dropwise. After stirring overnight, the reaction mixture was poured into 200 g of crushed ice, acidified with 20% HCl, and extracted with CH_2Cl_2. The organic extracts were washed with water, dried, and evaporated. The residue was crystallized from MeCN to give 15.9 g (91%) of **41**: MP 157.5–159 °C; NMR (Me_2SO-d_6) ppm 13.35 (s, 1 H), 7.90 (s, 1 H), 6.90 (s, 1 H), 4.37 (m, 2 H), 3.66 (m, 2 H), 3.46 (t, 2 H, J = 7 Hz), 2.27 (s, 3 H), 1.09 (t, 3 H, J = 7 Hz).

The same procedure was utilized to prepare oxamic acid esters **42–51** and **53–61**, listed in Tables III and IV.

***tert*-Butyl *N*-[4-(3-Phenyl-5-isoxazolyl)-2-thiazolyl]oxamate (52).** A solution of 5.84 g (46 mmol) of oxalyl chloride in 70 mL of THF was stirred under nitrogen at 0 °C and 3.4 g (46 mmol) of *t*-BuOH was added at once. After 1 h, a solution of 9.72 g (40 mmol) of **31** and 9.30 g (92 mmol) of triethylamine was added in 60 mL of THF and the mixture was stirred at room temperature for 48 h. The precipitate was collected by filtration and washed with THF. Both the combined filtrate and the washings were evaporated, and the residue was dissolved in $CHCl_3$. The solution was washed several times with 10% HCl and water, dried, and evaporated to give 12 g of crude product **52**, which was crystallized from MeCN, giving 9.5 g (64%) of pure **52**: mp 212–213 °C dec; NMR (Me_2SO-d_6) ppm 8.3–7.4 (m, 5 H), 8.13 (s, 1 H), 7.38 (s, 1 H), 1.60 (s, 9 H).

***N*-[4-(3-Bromo-5-isoxazolyl)-2-thiazolyl]oxamic Acid (63) and Its 2-Aminoethanol Salt (64) and L-Lysine Salt (65).** A suspension of 12.6 g (36.4 mmol) of **45** in 500 mL of 0.1 N NaOH was stirred at 40 °C for 45 min. The reaction mixture was cooled, washed twice with 150 mL of ethyl ether, treated with charcoal, and filtered. The filtrate was acidified with 60 mL of 1 N HCl and the precipitate was collected by filtration and washed with water on the filter, giving 9.80 g (84.5%) of **63**: mp 217–218.5 °C dec; NMR (Me_2SO-d_6) ppm 8.13 (s, 1 H), 7.16 (s, 1 H).

A suspension of 2.85 g (8.95 mmol) of **63** in 25 mL of EtOH was gently refluxed and 0.59 g (9.66 mmol) of 2-aminoethanol in 10 mL of EtOH and 20 mL of water was quickly added. The reaction mixture was cooled at 40 °C overnight and the precipitate was collected by filtration and recrystallized from 65 mL of a EtOH-water (2:1) mixture to give 2 g (59%) of **64**: mp 190–193 °C dec; NMR (Me_2SO-d_6) ppm 8.01 (s, 1 H), 7.05 (s, 1 H), 3.71 (m, 2 H), 3.00 (m, 2 H).

To a solution of 1.25 g (8.6 mmol) of L-lysine in 140 mL of 75% methanol, kept at gentle reflux, was added 2.6 g (8.2 mmol) of **63** all at once. The mixture was cooled at 0 °C for 1 h and the

(16) Cannata, V. Ger. Patent, 2,323,809, 1973; *Chem. Abstr.* **1974**, *80*, 479839*q*.

(17) Theobald, H.; Wuerzer, B.; Kiehs, K. Eur. Pat. 28, 355; 1981; *Chem. Abstr.* **1981**, *95*, 115522*a*.

precipitate was collected by filtration, giving 2.7 g (17%) of **65**: mp 196–197 °C dec; NMR (D_2O) ppm 7.51 (s, 1 H), 6.5 (s, 1 H), 3.88 (t, 1 H), 3.15 (m, 2 H), 2.2–1.38 (m, 6 H).

Oxamic acids and their salts **62–67** and **69–75**, listed in Tables V and VI, were prepared in a similar way.

***N*-[4-[5-(Hydroxymethyl)-3-isoxazolyl]-2-thiazolyl]oxamic Acid 2-Aminoethanol Salt (76).** To a stirred solution of 14.8 g (75 mmol **of 40** and 16 g (158 mmol) of triethylamine in 150 mL of THF, under nitrogen and cooled at 0 °C, was added 27 g (150 mmol) of 2-ethoxyethyloxalyl chloride dropwise. After stirring overnight, the solvent was distilled off and to the residue was added 75 mL of 1 N NaOH. The mixture was stirred at 40 °C to obtain a complete solution. After 40 min the solution was cooled and acidified with 2 N HCl. The precipitate was collected by filtration and dried to give 15 g (75%) of acid. This was dissolved in 175 mL of hot MeOH and 3.4 g (56 mmol) of 2-aminoethanol was added dropwise. The solution was cooled and the precipitate was filtered and dried to give 15 g (61%) of **77**: mp 185–187 °C; NMR (Me_2SO-d_6) ppm 7.91 (s, 1 H), 6.83 (s, 1 H), 4.60 (s, 2 H), 3.72 (m, 2 H), 3.15 (m, 2 H).

Compound **68** was prepared in the same manner, starting from **34**.

Biological Methods. Antiserum Production (IgE Antibody Antiovoalbumin). The production of homocytotropic serum necessary for accomplishment of the experiment was obtained according to the method described by Mota.[18]

Female Sprague–Dawley rats (supplied by Charles River, Calco, Italy), weighing about 200 g, were used. Animals were sensitized by intramuscolar administration of ovoalbumin in liquid petrolatum (1 mg/mL per rat) and by intraperitoneal administration of a 1 mL suspension of *Bordetella pertussis* at a concentration of 4×10^9 killed cells/mL. Sixteen days later the rats were exsanguinated; the blood was collected, pooled, and stored at −20 °C. Before experiments, different dilutions of serum were tested in order to optimize the anaphylactic response in naive rats (see below).

Passive Cutaneous Anaphylaxis (PCA) in Rats. PCA-induced reaction was performed according to the method described by Goose and Blair.[19] Diluted antiserum (IgE antibody antiovoalbumin) was injected intradermally (0.1 mL) at four different sites on the shaved back of naive rats. Forty-eight hours later rats were intravenously injected into the caudal vein with a saline solution containing 5 mg of ovoalbumin (challenge) and 2.5 mg of Evans Blue (1 mL/rat in 2 s). Drugs, suspended in 2% gum arabic, were given by oral (10 mL/kg) or by intraperitoneal route (5 mL/kg) 10 or 5 min before challenge, respectively. Thirty minutes after challenge, rats were sacrificed and the diameter of the blue spots at each site of serum application was measured. The antianaphylactic activity was evaluated in terms of reduction in spot area with respect to control rats. Control rats were passively sensitized rats treated with 2% gum arabic (10 mL/kg po or 5 mL/kg ip) before the injection containing both challenge and Evans Blue. Drug ED_{50} values were calculated by linear-regression analysis on at least three different dose levels for each compound.

Passive Pulmonary Anaphylaxis (PPA) in Anesthetized Rats. The production of homocytotropic serum (IgE antibody antiovoalbumin) necessary for accomplishment of the experiment was obtained as described above. PPA was induced according to the method described by Leibowitz et al.[20] and bronchial resistance was measured by the Konzett and Rössler technique described by Collier et al.[21] Rats were passively sensitized by intravenous injection of serum antiovoalbumin (1 mL/rat). Forty-eight hours later rats were anesthetized by urethane (1 g/kg ip) and nembutal (30 mg/kg ip) and prepared for bronchospasm measurement. To this purpose the trachea was cannulated with a T-shaped cannula and connected, at one side, with a respirator (Ugo Basile) operating at 70 breaths/min and, at the other side, with a flow transducer (Bronchospasm Transducer 7020, Ugo Basile) connected with an integrator (Brush) and with a polygraph (Brush Clevite) to measure and record the air overflow following bronchospasm. A catheter was inserted into the jugular vein for drug administration. The anaphylactic reaction was induced by intravenous injection of ovoalbumin 4 mg/kg per mL of saline in 2 s (challenge). The subsequent bronchoconstriction was measured in terms of air overflow. Drugs were administered either orally (10 mL of 2% gum arabic/kg) or intravenously (1 mL of 10% dimethyl sulfoxide/kg in 8 s) 30 or 15 min before ovoalbumin, respectively. Drug effect was observed as reduction of antigen-induced air overflow with respect to antigen-induced air overflow observed in control rats (10 mL of 2% gum arabic/kg po or 1 mL of 10% dimethyl sulfoxide/kg iv). Drug ED_{50} values were calculated by linear-regression analysis.

Active Pulmonary Anaphylaxis (APA) in Guinea Pigs. Experiments were performed according to the method described by Herxheimer and Stresemann.[22] Guinea pigs of either sex (supplied by Alserio Allevamenti S.r.l., Cremona, Italy), weighing 300–400 g, were used. Guinea pigs were sensitized by intraperitoneal injection of a single dose of ovoalbumin in liquid petrolatum (20 mg/mL per guinea pig). After 3 weeks, each guinea pig was placed in a Plexiglas chamber and exposed to challenge (2% ovoalbumin aqueous solution) dispensed by a nebulizer at constant air pressure until the guinea pig exhibited sever dyspnea. The time elapsing from the beginning of the challenge procedure and the appearance of severe dyspnea was measured: generally, severe dyspnea appeared in about 80 s (baseline condition) in control sensitized guinea pigs (5 mL of 2% gum arabic/kg ip plus challenge). Treated guinea pigs not showing signs of bronchospasm within 3 min were considered protected and withdrawn from the aerosol exposure (cutoff time). Drugs, suspended in 2% gum arabic were given intraperitoneally (5 mL/kg) 30 min before ovoalbumin challenge. Drug activity was estimated as capacity to delay the appearance of dyspnea, measured in seconds. Drug ED_{50} values were calculated by linear-regression analysis.

Antigen-Induced Slow Reacting Substance of Anaphylaxis (SRS-A) Release from Sensitized Guinea Pig Lung Fragments. Experiments were performed by using the method described by Augstein et al.[23] Guinea pigs of either sex (supplied by Alserio Allevamenti S.r.l., Cremona, Italy), weighing 300–400 g were used. Guinea pigs were sensitized by a single dose of ovoalbumin in liquid petrolatum (20 mg/mL per guinea pig ip) and sacrificed by decapitation three weeks later. Lungs were withdrawn, washed, triturated, and suspended in Krebs' solution (0.1 g of tissue/mL). This suspension was distributed in tubes and incubated for 15 min in a shaking water bath at 37 °C. Then, drug was added and, after 5 min, each tissue suspension was exposed to the antigen (1 μg of ovoalbumin) for 15 additional min at 37 °C. The reaction was stopped by filtration and the filtered fluid containing the released material was stored at −70 °C until determinations were performed. SRS-A-like activity of the fluids was determined by measuring the spasmogenic action of fluids on isolated guinea pig ileum preparation. Mepyramine (10^{-8} M) and atropine (10^{-6} M) were added to the bath to avoid undesirable histamine- and acetylcholine-mediated effects. The antagonizing action of compound FPL 55712 (10^{-7} M) on the spasmogenic activity of the fluids was used to confirm the SRS-A nature of this contraction.

Drug activity was expressed as inhibition of SRS-A-like material release assuming as 100% the SRS-A-like material released from control challenged lung fragments. In control samples saline was added instead of drugs. The comparison between compound **49** and DSCG was evaluated by Student's unpaired two-tailed *t* test.

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