Antiallergic and Cytoprotective Activity of New *N*-Phenylbenzamido Acid Derivatives

Francesco Makovec,* Walter Peris, Laura Revel, Roberto Giovanetti, Daniele Redaelli, and Lucio C. Rovati

Rotta Research Laboratorium, 20052 Monza (Milan), Italy

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A series of new *N*-phenylbenzamido acid derivatives was synthesized and evaluated for their ability to inhibit the IgE-mediated passive cutaneous anaphylaxis in the rat (PCA), as well as for their capacity to inhibit gastric mucosal damage induced by the oral administration of absolute alcohol in the rat. Some of these new derivatives exhibit potent antiallergic and cytoprotective activity, 20–80 times higher than that of the reference, disodium cromoglycate (DSCG). Structure–activity relationships are discussed. The antiallergic activity of one of the more potent compounds of this series, i.e. 4-(1*H*-tetrazol-5-yl)-*N*-[4-(1*H*-tetrazol-5-yl)phenyl]benzamide (compound **44**, CR 2039) was further evaluated in vivo. This compound antagonizes the bronchoconstriction induced by aerosolized ovalbumin in both anesthetized and conscious IgE sensitized guinea pigs with ID_{50} of 3.7 mg/animal (tracheal insufflation) and 20 mg/kg (im). Further cytoprotective effects were evaluated in gastric ulcer models induced by the acute oral administration of hypertonic sodium chloride solution or by acetic acid and by the subchronic administration of glucose in fasted animals. In the models used experimentally CR 2039 is effective, whereas DSCG seems to be devoid of any protective activity. Such a potent antiallergic and mucosal protectant could provide a new potential agent in the therapy of atopic allergic diseases.

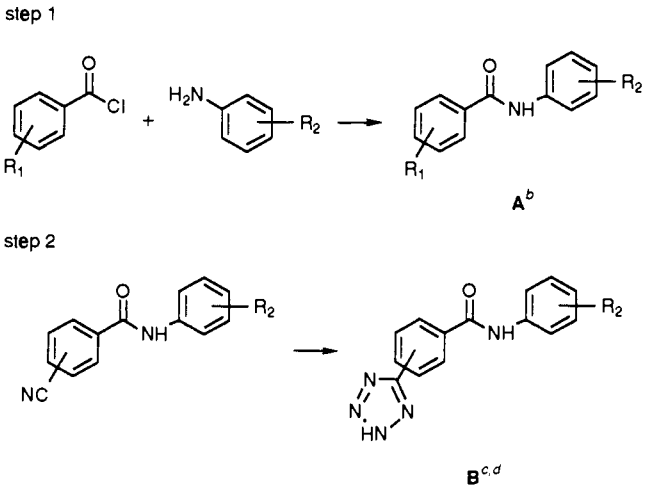
The introduction onto the market of disodium cromoglycate (DSCG), proposed as a therapeutic agent for the prevention and treatment of asthma, rhinitis, and other allergic disorders, has stimulated research toward the discovery of new antiallergic drugs with increased potency and/or more favorable pharmacokinetic patterns.[1,2] In fact DSCG is poorly absorbed by the gastrointestinal tract, and therefore the drug is only effective when administered by inhalation.[3]

DSCG and many other antiallergic compounds that have previously been reported are dicarboxylic aromatic acids or their esters.[4,5] As for example, the DSCG molecule is typically characterized by the presence of the two carboxylic groups placed on two hydrophobic moieties linked together by a 2-hydroxytrimethylenedioxy chain. The aim of our investigation was to explore the possibility that the introduction of appropriate substituents in a simple structure like the *N*-phenylbenzamide moiety carrying acidic groups (compounds **A** and **B** of Scheme I and shown in Table I) could lead to obtaining new molecular entities exhibiting potent antiallergic properties.

Chemistry

The new *N*-phenylbenzamido acid derivatives were synthesized by a two-step method illustrated in Scheme I. Therefore the *N*-phenylbenzamides of formula **A** were prepared by reacting the appropriate aroyl chloride of formula $R_1ArCOCl$ with equivalent quantities of substituted anilines of formula NH_2ArR_2 under Schotten–Baumann conditions. The preparation of 1*H*-tetrazol-5-yl-*N*-phenylbenzamido derivates **B** was achieved by heating at 100 °C the derivates **A** of Scheme I (step 2) with a slight excess of NaN_3 and NH_4Cl in DMF.

Scheme I. Synthesis of Compounds 1–47 of Table I



[a] Reagents: step 1 (1) base, H_2O/THF; (2) acid. Step 2 (1) NaN_3/NH_4Cl, DMF; (2) acid. [b] The physical properties of compounds **A** are reported in Table I and Table IV (compounds **49–58**). [c] Compounds **B** are the 1*H*-tetrazol-5-yl-*N*-phenylbenzamido derivatives **21**, **27**, **35**, **36**, **38**, **41–47**. Their physical properties are reported in Table I. [d] **37** is obtained by dehydratation of **38** with P_2O_5 in pyridine.

The physicochemical characteristics of the new *N*-phenylbenzamido derivatives (compounds **A** and **B** of Scheme I) are given in Tables I and IV.

Results and Discussion

The results obtained are presented in Table II. Biological activity was assessed by studying the antianaphylactic reaction on an IgE-mediated PCA model in rats and by the evaluation of cytoprotective activity on the ex-

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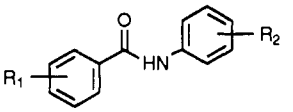
Table I. Physical Properties of Acidic *N*-Phenylbenzamides (1–48) Prepared by Scheme I



compd	R_1	R_2	mp[a] (°C)	recryst solvent	formula
1	H	3,5-COOH	339	DMF/H_2O 1:1	$C_{15}H_{11}NO_5$
2	4-Me	3,5-COOH	340	DMF/H_2O 2:1	$C_{16}H_{13}NO_5$
3	4-Pr	3,5-COOH	333	DMF/H_2O 2:1	$C_{16}H_{17}NO_5$
4	4-Bu	3,5-COOH	311[b]	DMF/H_2O 1:1	$C_{19}H_{19}NO_5$[c]
5	4-OH	3,5-COOH	333	DMF/H_2O 2:1	$C_{15}H_{11}NO_6$
6	3,4-OH	3,5-COOH	322	DMF/H_2O 1:1	$C_{15}H_{11}NO_7$
7	3,5-OH	3,5-COOH	342	EtOH/H_2O 1:1	$C_{15}H_{11}NO_7$
8	4-OMe	3,5-COOH	329	DMF/H_2O 1:1	$C_{16}H_{13}NO_6$
9	3,4-OMe	3,5-COOH	333	DMF/H_2O 1:1	$C_{17}H_{15}NO_7$
10	3,5-OMe	3,5-COOH	335	DMF/H_2O 1:1	$C_{17}H_{15}NO_7$
11	3,4,5-OMe	3,5-COOH	340	DMF/H_2O 1:1	$C_{18}H_{17}NO_8$
12	4-OPr	3,5-COOH	324	DMF/H_2O 1:1	$C_{16}H_{17}NO_6$
13	3-Cl	3,5-COOH	330	DMF/H_2O 1:1	$C_{15}H_{10}ClNO_5$
14	4-Cl	3,5-COOH	347	MeOH/H_2O 9:1	$C_{15}H_{10}ClNO_5$
15	2,4-Cl	3,5-COOH	329	DMF/H_2O 1:1	$C_{15}H_9Cl_2NO_5$
16	4-CF_3	3,5-COOH	315	DMF/H_2O 1:1	$C_{16}H_{10}F_3NO_5$
17	3-CN	3,5-COOH	331	DMF/H_2O 3:1	$C_{16}H_{10}N_2O_5$
18	4-CN	3,5-COOH	324	DMF/H_2O 2:1	$C_{16}H_{10}N_2O_5$
19	4-NO_2	3,5-COOH	325	MeOH/H_2O 9:1	$C_{15}H_{10}N_2O_7$
20	4-COOH	3,5-COOH	332	DMF/H_2O 1:1	$C_{16}H_{11}NO_7$[d]
21	4-Trz[e]	3,5-COOH	303	DMF/H_2O 4:1	$C_{16}H_{11}N_5O_5$
22	4-CH	3,4-COOH	305	DMF/H_2O 4:1	$C_{16}H_{10}N_2O_5$
23	4-NO_2	3,4-COOH	243	DMF/H_2O 2.5:1	$C_{15}H_{10}N_2O_7$
24	4-CN	2,4-COOH	321	DMF/H_2O 5:1	$C_{16}H_{10}N_2O_5$
25	4-CN	2,3-COOH	248	DMF/H_2O 5:1	$C_{16}H_{10}N_2O_5$
26	4-CN	2,5-COOH	330	DMF	$C_{16}H_{10}N_2O_5$
27	4-Trz[e]	2,5-COOH	319	DMF/H_2O 2:1	$C_{16}H_{11}N_5O_5$
28	4-NO_2	4-COOH	323	DMF/H_2O 2:1	$C_{14}H_{10}N_2O_5$
29	4-CN	4-COOH	320	DMF/H_2O 2:1	$C_{15}H_{10}N_2O_3$
30	4-CN	3-COOH	261	DMF/H_2O 2:1	$C_{15}H_{10}N_2O_3$
31	4-CN	4-Trz[e]	284	DMF/H_2O 3:1	$C_{15}H_{10}N_6O$
32	4-CN	3-Trz[e]	273	DMF/H_2O 1:1	$C_{15}H_{10}N_6O$
33	4-CN	3-COOH-5-CH_2OH	286	DMF/H_2O 1:1	$C_{16}H_{12}N_2O_4$
34	4-CN	3-COOH-5-$CONH_2$	341	DMF/H_2O 2.5:1	$C_{16}H_{11}N_3O_4$
35	3-Trz[e]	H	250	DMF/H_2O 2:1	$C_{14}H_{11}N_5O$
36	4-Trz[e]	H	282	DMF/H_2O 2:1	$C_{14}H_{11}N_5O$
37	4-Trz[e]	4-CN	258	DMF/H_2O 3:1	$C_{15}H_{10}N_6O$
38	4-Trz[e]	4-$CONH_2$	302	DMF	$C_{15}H_{12}N_6O_2$
39	4-COOH	4-CN	288	DMF/H_2O 1:1	$C_{15}H_{10}N_2O_3$
40	4-COOH	4-COOH	360	DMF	$C_{15}H_{11}NO_5$
41	4-Trz[e]	4-COOH	321	DMF/H_2O 2:1	$C_{15}H_{11}N_5O_3$
42	4-Trz[e]	2-Trz[e]	256	DMF/H_2O 2:1	$C_{15}H_{11}N_9O$
43	4-Trz[e]	3-Trz[e]	283	DMF/H_2O 3:1	$C_{15}H_{11}N_9O$
44	4-Trz[e]	4-Trz[e]	301	DMF/MeOH 1:1	$C_{15}H_{11}N_9O$
45	3-Trz[e]	2-Trz[e]	227	DMF/H_2O 2:1	$C_{15}H_{11}N_9O$
46	3-Trz[e]	3-Trz[e]	290	DMF/H_2O 2:1	$C_{15}H_{11}N_9O$
47	3-Trz[e]	4-Trz[e]	287	DMF/H_2O 3:1	$C_{15}H_{11}N_9O$
48[f]	4-Trz[e]	4-Trz[e]	153	DMF/H_2O 1:1	$C_{16}H_{13}N_9O$[g]

[a] Values obtained from DSC for the compounds with melting point higher than 270 °C. [b] Glass transition. [c] H: calcd, 5.61; found, 5.68. [d] N: calcd, 4.25; found, 4.32. [e] Trz: 1*H*-Tetrazol-5-yl. [f] *N*-Methyl. [g] H: calcd, 3.77; found, 3.83.

perimental ulcer induced by alcohol in rats since it was demonstrated that DSCG is effective in preventing gastric mucosal necrosis induced by alcohol.[6] Initially we synthesized a homologous series of *N*-[3,5-$(COOH)_2$-phenyl]-benzamide derivates, carrying two carboxylic groups in 3,5-R_2 positions. This was done, as stated above, by taking into account that DSCG as well as many other putative antiallergic compounds described thus far are strongly acidic. The R_1 unsubstituted parent compound (compound 1) as well as the 4-Me derivative (compound 2) are devoid of any activity up to 10 mg/kg on the PCA test, whereas the introduction of bulkier alkyl groups, such as 4-propyl or 4-butyl starts to produce compounds endowed with little though significant antianaphylactic activity (compounds 3 and 4, ID_{50} 6.7 and 12.3 mg/kg, respectively). The introduction in R_1 of electron-donor groups such as OH, $(OH)_2$, OMe, $(OMe)_2$, $(OMe)_3$, *O*-propyl (compounds 5–12), as well as the introduction of halogen substituents (compounds 13–16) instead of the alkyl bulky groups, produces a complete loss of activity. On the contrary, the introduction of electron withdrawing groups such as NO_2 and CN (compounds 17–19) induces, in some cases, a strong increase in activity. The best result is obtained when R_1 is 4-CN (compound 18, ID_{50} 1.7 mg/kg), which has about the same activity as DSCG (ID_{50} 1.5 mg/kg) in the PCA test.

The introduction in R_1 of another acidic group also gives good results, especially when R_1 is the 4-(1*H*-tetrazol-5-yl) group (compound 21), that is the most effective

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Table II. Antiallergic (PCA) and Cytoprotective Activities of *N*-Phenylbenzamido Acid Derivatives in the Rat

compd	ID_{50}, mg/kg		compd	ID_{50}, mg/kg	
	PCA[a]	cytoprotective[b] activity		PCA	cytoprotective activity
1	inactive[c]	inactive[d]	**25**	15.8 (7.4–33.7)	135 (79.6–230)
2	inactive	inactive	**26**	1.5 (0.5–4.7)	26.8 (19.5–36.8)
3	6.7 (4.3–10.4)	94.2 (34.8–255)	**27**	1.2 (0.5–2.8)	31.1 (12.2–79.4)
4	12.3 (5.5–27.5)	91.9 (36.8–230)	**28**	inactive	inactive
5	inactive	inactive	**29**	inactive	inactive
6	inactive	inactive	**30**	inactive	inactive
7	inactive	90.2 (31.8–256)	**31**	2.4 (0.8–7.0)	54.4 (18.8–158)
8	inactive	inactive	**32**	inactive	21.4 (7.7–59.7)
9	inactive	116 (35–388)	**33**	inactive	inactive
10	inactive	108 (47.5–246)	**34**	5.7 (1.1–30.3)	87.2 (58.3–131)
11	inactive	inactive	**35**	4.5 (2.3–8.8)	38.1 (19.6–74.4)
12	inactive	78.0 (36.1–169)	**36**	8.4 (0.7–97.6)	40.0 (26.5–60.3)
13	inactive	inactive	**37**	1.2 (0.6–2.4)	19.7 (10.5–37)
14	inactive	97.3 (37.4–253)	**38**	2.0 (0.7–5.2)	33.0 (7.1–57.2)
15	inactive	103 (70.1–151)	**39**	inactive	inactive
16	inactive	inactive	**40**	25.4 (16.4–39.5)	54.3 (32.2–195)
17	5.0 (1.5–16.7)	140 (61.5–318)	**41**	2.3 (0.4–12.7)	3.5 (2.0–6.2)
18	1.7 (0.4–7.4)	32.1 (23.6–43.8)	**42**	1.5 (0.4–6.1)	6.1 (3.6–10.4)
19	9.8 (3.7–26.3)	61.1 (48.5–76.8)	**43**	0.03 (0.008–0.12)	1.1 (0.4–2.7)
20	2.1 (1.2–3.8)	32.2 (14.2–73.2)	**44**	0.09 (0.04–0.23)	2.5 (1.1–5.5)
21	1.2 (0.7–1.9)	8.2 (5.3–12.7)	**45**	1.4 (0.6–2.9)	9.7 (6.2–15.2)
22	10.1 (5.0–20.3)	inactive	**46**	1.6 (0.9–3)	15.8 (6.9–36.3)
23	13.2 (7.6–22.8)	inactive	**47**	0.05 (0.02–0.16)	0.6 (0.2–2.1)
24	19.5 (8.5–44.5)	66.3 (54.2–80.9)	**48**	3.8 (1.9–7.6)	60.0 (24.4–150)
DSCG	1.5 (1.0–2.2)	50.8 (20.3–127.2)	tranilast	3.6 (1.6–8.1)	inactive
nedocromil	1.3 (0.8–2.2)	inactive			

[a] ID_{50}: compound dose in mg/kg and $p = 0.05$ fiducial limits required to inhibit by 50% the PCA reaction induced by 25 mg/kg of ovalbumin given iv (bolus). [b] ID_{50}: compound dose in mg/kg and $p = 0.05$ fiducial limits required to inhibit by 50% the ulcerogenic effect induced by oral administration of 1.5 mL/animal of absolute alcohol. [c] Inactive: the protective effect at 10 mg/kg (im) is less than 20%. [d] Inactive: the protective effect at 100 mg/kg (iv) is less than 20%.

Table III. Further Cytoprotective Evaluation of Compound 44 (CR 2039) in Comparison to Reference Drugs

gastric ulcer model	route of admin	ID_{50}[a] mg/kg (fiducial limits 95%)	
		44	comparison drugs
ethanol	iv	2.5[b] (1–5)	DSCG: 50.8[b] (20–127) PGE: 0.08 (0.03–0.2) cimetidine: IN[c]
sodium chloride	iv	18.2 (7–44)	DSCG: IN[c] PGE: 0.05 (0.02–0.12) cimetidine: 48 (19–120)
acetic acid	im	9.4 (4–20)	DSCG: IN[c] cimetidine: IN[c]
glucose	sc	23.9 (8–69)	DSCG: IN[c]

[a] ID_{50}: compound dose in mg/kg and $p = 0.05$ fiducial limits required to inhibit by 50% the gastric ulcer induced by challenge in conscious rats. For protocol, see Experimental Section under Biological Methods. [b] Values drawn from Table II. [c] No significant activity at 100 mg/kg.

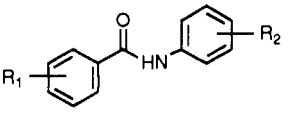
compound (ID_{50} 1.2 mg/kg) of this series of *N*-[3,5-$(COOH)_2$-phenyl]benzamide derivates.

Because of these encouraging results, another series of *N*-phenylbenzamides was synthesized in which R_2 was again the dicarboxylic groups $(COOH)_2$ but placed in different positions from those discussed above [i.e. the 3,5-$(COOH)_2$] and in which R_1 was chosen among 4-CN, 4-NO_2, or 4-(1*H*-tetrazol-5-yl) groups. The derivatives having the two carboxylic groups (R_2) in position 3,4; 2,4; and 2,3 (compounds **22–25**) are all barely effective in comparison to the corresponding *N*-[3,5-$(COOH)_2$-phenyl]-benzamides described above, whereas compounds **26** and **27**, carrying the two carboxylic groups in the 2,5-R_2 position, exhibit the same potency as the corresponding 3,5-$(COOH)_2$ derivatives (i.e., the compounds **18** and **21**).

Later, a series of derivatives carrying only one acidic substituent in R_1 or in R_2 was synthesized. The compounds in which the acidic group is placed on the *N*-phenyl ring (compounds **28–32**) are all ineffective, apart from compound **31**, in which R_2 is the 4-(1*H*-tetrazol-5-yl) substituent. This compound has about the same activity of compounds **18** and **26**, so one could speculate that in this series the introduction of the 4-(1*H*-tetrazol-5-yl) group in R_2 is equivalent to the introduction in the same moiety of the 3,5-$(COOH)_2$ or 2,5-$(COOH)_2$ groups.

It is worthy to note that the substitution of one R_2-carboxylic group of compound **18** with the CH_2OH group or with the carbamoyl group is not worthwhile, producing compounds which are poorly active or completely ineffective (compounds **33** and **34**). In the case in which the only acidic group is placed in R_1, i.e. in the benzamide moiety (compounds **35–39**), the best substitution is achieved if the acidic group is the 4-(1*H*-tetrazol-5-yl) substituent and R_2 is the 4-CN group (compound **37**) which exhibits an ID_{50} of 1.2 mg/kg.

Table IV. Physical Properties of *N*-Phenylbenzamides (**49–58**) Prepared by Scheme I



compd	R_1	R_2	mp (°C)	recryst solvent	formula
49	3-CN	H	173	EtOH 96%	$C_{14}H_{10}N_2O$
50	4-CN	H	180	DMF/H_2O 1:1	$C_{14}H_{10}N_2O$
51	4-CN	4-$CONH_2$	328[a]	DMF	$C_{15}H_{11}N_3O_2$
52	4-CN	2-CN	194	DMF/H_2O 3:1	$C_{15}H_9N_3O$
53	4-CN	3-CN	216	DMF/H_2O 2:1	$C_{15}H_9N_3O$
54	4-CN	4-CN	267	THF/H_2O 2:1	$C_{15}H_9N_3O$
55	3-CN	2-CN	188	DMF/H_2O 3:1	$C_{15}H_9N_3O$
56	3-CN	3-CN	162	DMF/H_2O 3:1	$C_{15}H_9N_3O$
57	3-CN	4-CN	220	DMF/H_2O 2:1	$C_{15}H_9N_3O$
58[b]	4-CN	4-CN	163	DMF/H_2O 1:1	$C_{16}H_{11}N_3O$

[a] From DSC analysis. [b] *N*-Methyl.

Nevertheless, the best antiallergic activity of the series as a whole is obtained when an acidic group is introduced at the same time in both benzene rings of the *N*-phenylbenzamide structure (compounds **40–47**).

The most active compounds, i.e. compounds **43**, **44**, and **47** are obtained when R_1 and R_2 are both the 1*H*-tetrazol-5-yl group in position 3 or 4 of the two benzene rings. For instance, compound **43** (ID_{50} 0.03 mg/kg) is about 50 times more effective than the reference compound (DSCG) on this experimental model, whereas less effective are the compounds in which in R_2 the 1*H*-tetrazol-5-yl group is in the ortho position (compounds **42** and **45**) or when both the 1*H*-tetrazol-5-yl groups are in the meta position (compound **46**). It is also to be noted that if the hydrogen atom of the secondary benzamide group is substituted by the methyl group, the antiallergic activity is strongly reduced (compound **48**, ID_{50} 3.8 mg/kg) when compared with the activity of the parent compound (compound **44**, ID_{50} 0.09 mg/kg).

The calculated distance between the aromatic carbon atoms carrying the two tetrazolyl groups of compounds **43**, **44**, and **47** is about 8.5–9.5 Å. When this distance is reduced and it is included in the range 6.8–8 Å (compounds **42**, **45**, and **46**), then the activity on PCA is reduced by 40–50 times and becomes the same as that exhibited by DSCG.

To better understand the structure–activity relationships between these acidic benzanilides and other potent acidic antiallergic compounds previously described, a computer-assisted conformational analysis was carried out. The low-energy conformation was determined for both new benzanilide derivatives and some paradigmatic biacidic antiallergic representatives (e.g. lodoxamide, a dioxamic acid derivative,[7] nedocromil, a 2,8-dicarboxylic pyranoquinoline,[8] and sudexamox, a xanthone derivative[9]).

The structure of compound **47** was fitted to the low-energy structure of the other reference antiallergics. The rigid 3-(1*H*-tetrazol-5-yl)benzamido group was taken as an anchor group and this allowed the other moiety of the molecule to orient independently within the space that the computer graphics gave approximately as the best superposition of acidic groups of the different structures. However, for all molecules the minimized structure energy, that is, 70.14 kcal/mol for compound **47**, 43.3 kcal/mol for lodoxamide, 44.8 kcal/mol for nedocromil, and 40.02 kcal/mol for sudexamox, was maintained.

As shown in Figure 1, in each case compound **47** exhibits an extensive overlap with the structure of the antiallergics. The main difference between the molecular structure of compound **47** and the other examined antiallergics (with the exception of its higher flexibility) is that the two acidic groups are about 9 Å apart, whereas in all three reference compounds this distance is about 7–7.5 Å. This also occurs, as stated above, for the less effective ditetrazolylbenzamides of this series, i.e. compounds **42**, **45**, and **46**.

As far as the cytoprotective activity of these *N*-phenylbenzamido derivates is concerned, the results are generally in accordance with those obtained by the PCA test, with some exceptions. In the series of *N*-[3,5-$(COOH)_2$-phenyl]benzamide derivatives (compounds **1–21**), the derivatives in which R_1 consists of alkyl groups (compounds **2–4**), electron donor groups (compounds **5–12**), or halogen groups (compounds **13–16**) are all barely effective or ineffective at all. In fact, among these derivatives the most effective is compound **12**, a 4-OPr benzamido derivative, that exhibits an ID_{50} of only 78 mg/kg. Cytoprotective activity increases when R_1 is an electron withdrawing group (such as NO_2 or CN) or a carboxylic group is placed in the para position (compounds **18–20**) and especially when R_1 is the 4-(1*H*-tetrazol-5-yl) substituent (compound **21**, ID_{50} 8.2 mg/kg). The derivatives having the two carboxylic groups (R_2) in positions 3,4; 2,4; and 2,3 (compounds **22–25**) are less effective than the corresponding 3,5-$(COOH)_2$-R_2 derivatives (compounds **18**, **19**) whereas the 2,5-$(COOH)_2$-R_2 derivative (compound **26**) has about the same activity as the corresponding 3,5-$(COOH)_2$-R_2 compound **18**. On the contrary, the cytoprotective activity of compound **27**, in which R_1 is 4-(1*H*-tetrazol-5-yl) and R_2 is 2,5-$(COOH)_2$, is about 4 times less effective than corresponding compound **21**. All the derivatives carrying only one carboxylic group in R_2 (compounds **28–30**) are also in this case ineffective, whereas

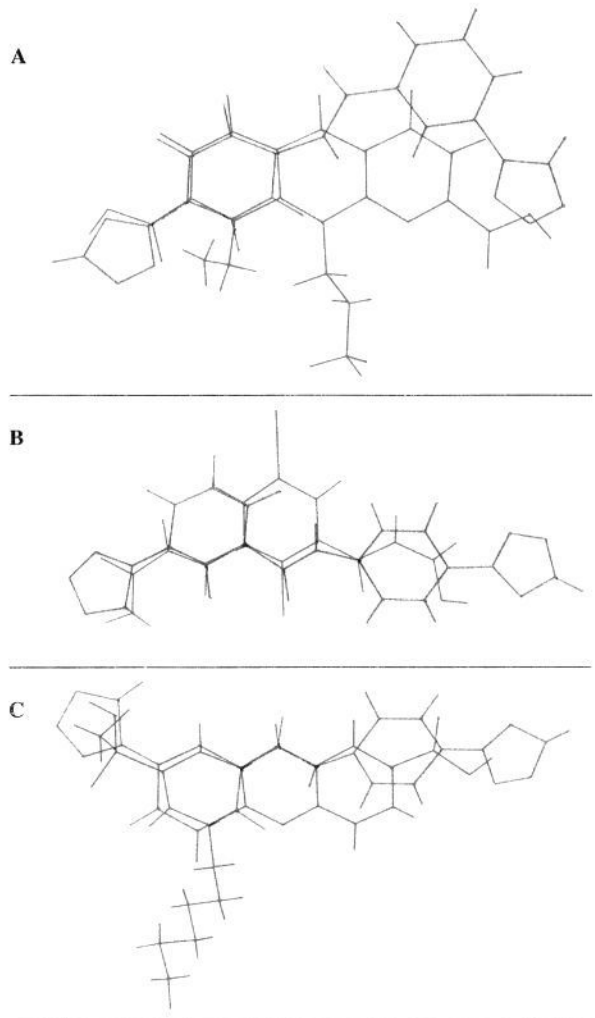


Figure 1. Computer superposition of **47** on nedocromil (A), lodoxamide (B) and sudexamox (C), showing acidic groups matching. Blue, compound **47**; red, other antiallergics.

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if the acidic group is the 1*H*-tetrazol-5-yl substituent, some slight activity is achieved, especially with compound **32** (ID_{50} = 21.4 mg/kg), which, on the contrary, is inactive in the PCA test.

In the case in which the unique acidic group is the 4-(1*H*-tetrazol-5-yl) group placed in R_1, corresponding compounds **35–38** exhibit mild cytoprotective activity, and among such compounds the best cytoprotection is obtained when R_2 is 4-CN (compound **37**, ID_{50} = 19.7 mg/kg). The corresponding compound in which the 4-(tetrazol-5-yl) group is replaced by the carboxylic group (compound **39**) is completely inactive. In the case of cytoprotective activity, too, the best results are obtained when R_1 and R_2 are both the 1*H*-tetrazol-5-yl group placed alternately in position 3 or 4 of the two aromatic rings (compounds **43** and **47**) as well as in the para-para positions (compound **44**). As for example, compound **47** is the most potent derivative of the whole series, showing an ID_{50} of 0.6 mg/kg and, therefore, with regard to the cytoprotective activity it is about 80 times more potent than the reference compound. In fact, under our experimental conditions DSCG seems to be poorly effective, exhibiting an ID_{50} of 50.8 mg/kg, as well as the other antiallergics tested, i.e. nedocromil and tranilast.[10] The introduction of the two tetrazol-5-yl groups in other positions of the two benzene moieties is, also in this case, less favorable (compounds **42**, **45**, and **46**), whereas the substitution of the tetrazol-5-yl group in R_2 of compound **44** by the carboxylic group in the same position (compound **41**) allows high activity to be maintained (ID_{50} = 3.5 mg/kg). This result is different with regard to the activity exhibited by the two compounds **41** and **44** on the PCA test, in which compound **44** was about 25 times more potent than compound **41**.

When both of the two 4-(1*H*-tetrazol-5-yl) groups of compound **44** are substituted by the carboxylic group, the activity is strongly reduced (compound **40**, ID_{50} 54.3 mg/kg). We can therefore conclude that a necessary condition to obtain (in this series of *N*-phenylbenzamides) compounds having at least a fair amount of activity, i.e. compounds exhibiting an ID_{50} lower than 10 mg/kg im on the PCA model and an ID_{50} lower than 30 mg/kg iv on the alcohol ulcer model, is that the R_2 substitution consists of 2,5- or 3,5-dicarboxylic groups and R_1 is the 4-CN or the 4-(tetrazol-5-yl) substituent (compounds **18**, **21**, **26**, and **27**). High activity is obtained when R_1 substitution consists of the 4-(tetrazol-5-yl) group joined with a substitution in the *N*-phenyl ring in which R_2 consists of the carboxylic group (compound **41**) or groups such as 4-CN and 4-$CONH_2$ that can in vivo change into the carboxylic group (compounds **37** and **38**).

Better results are however obtained when both the acidic groups are the 1*H*-tetrazol-5-yl group, the potency depending on the position of the substituent in the two aromatic rings. In both pharmacological models used, the para-para and meta-para substitution gave the best results. On the contrary, the introduction of three acidic groups is less favorable (compounds **21** and **27**), and the activity is strongly reduced as well when the secondary amido group is methylated (compound **48**) as already stated for the PCA model.

Compound **44** (coded CR 2039, Figure 2), one of the more potent antianaphylactic and cytoprotective agents

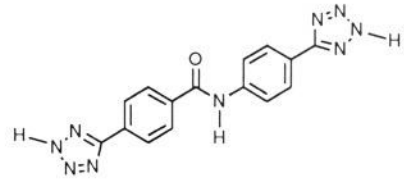

Figure 2. Compound **44** (CR 2039).

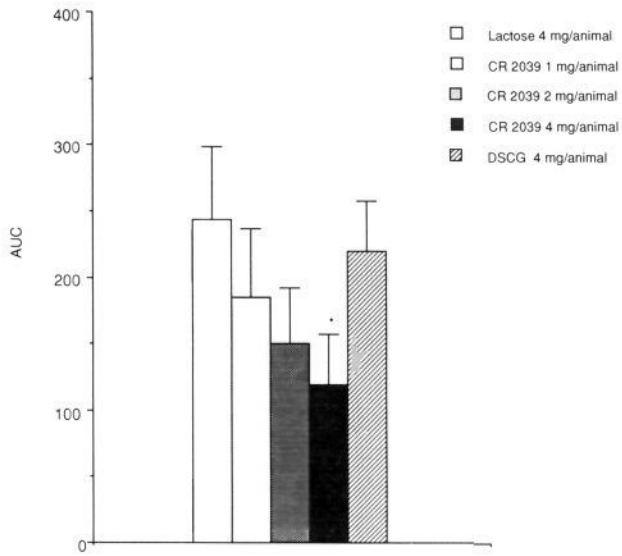

Figure 3. Effects of **44** (CR 2039) in comparison with DSCG in antagonizing bronchial anaphylaxis in anesthetized sensitized (IgE) guinea pig induced by aerosolized ovalbumin (0.5% w/v) given per 1 min. In abscissa given doses of drugs administered as disodium salt by dry powder insufflation 15 min before challenge. Data are mean ±SD of bronchoconstriction area (AUC) (n = 6); *$p < 0.05$ vs lactose (Anova, polynomial analysis). The calculated ID_{50} of CR 2039, i.e. the dose in mg/kg and p = 0.05 fiducial limits required to reduce by 50% the AUC induced by ovalbumin is 3.7 (2.3–6.1) mg/animal.

of this series, was chosen in order to further evaluate the feature of its activity on other experimental models. Thus, the antianaphylactic activity of compound **44** was investigated in a bronchial anaphylaxis model in an actively IgE-mediated sensitized guinea pig, in which the anesthetized animals were challenged with ovalbumin 0.15 mg/kg iv, and compound **44** as well as DSCG, used as reference, were administered by dry powder insufflation 15 min before challenge. Compound **44** dose-dependently (in the range 1–4 mg/animal) antagonizes bronchoconstriction induced by an aerosol administration of ovalbumin with an ID_{50} of 3.7 mg/animal, whereas DSCG in the dose of 4 mg/animal exhibits a nonsignificant activity, giving only about 10% protection (Figure 3). In conscious sensitized (IgE) guinea pigs in which bronchial anaphylaxis was induced by aerosol antigen given 15 min after im drug administration, and bronchospasm was recorded as latency time to first bronchoconstriction symptoms, compound **44** increases latency time in the range of 10–100 mg/kg im with an ID_{50} of 20 mg/kg. In this model DSCG is practically ineffective up to 100 mg/kg. On the other hand, compound **44** does not antagonize the bronchoconstriction induced by histamine on the same experimental model, demonstrating the lack of any direct antihistaminic activity. The results obtained are shown in Figure 4.

In order to confirm the hypothesis that the protective effect of compound **44** on stomach gastric mucosa vs ethanol, as well as its protective effect on the airway

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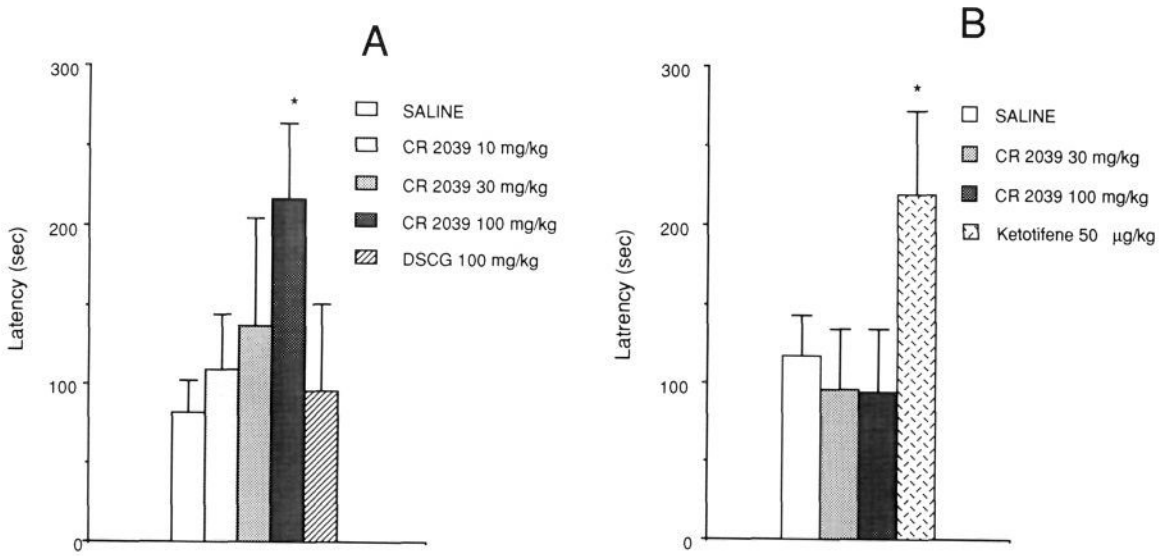

Figure 4. Effects of **44** (CR 2039) in comparison with DSCG and ketotifene in antagonizing bronchoconstriction (BR) latency (sec) in different models: (A) BR induced by aerosolized ovalbumin (0.5% w/v) in conscious sensitized guinea pigs; (B) BR induced by aerosolized histamine (0.03% w/v) in conscious naive guinea pigs. In abscissa given doses of **44** or comparison drugs by im, 15 min before challenge. Each point represents the mean of at least five different experiments. The calculated ID_{50} of CR 2039 in experiment A, i.e. the dose in mg/kg and $p = 0.05$ fiducial limits required to increase by 50% the BR latency induced by ovalbumin, is 17 (4–74). *$p < 0.05$ vs saline with Anova, polynomial analysis.

epithelium shown on the allergic reactions of models described above, could be a general feature of its pharmacological action, the cytoprotective activity of the compound was investigated also on other different models with experimentally induced gastric damage. Indeed, compound **44**, administered parenterally, exhibits a marked protective effect in gastric ulcers acutely induced in the rat by different agents, such as sodium chloride and acetic acid, as well as in a subchronic model, the glucose-induced ulcer. In fact, in gastric ulcers induced by a 25% NaCl in water solution given orally in the rat, compound **44** dose-dependently antagonizes the gastric damage with an ID_{50} of 18.2 mg/kg iv. In the acetic acid ulcer model the compound (given three times during the 24 h required for the test), exhibits the same pattern of activity with an ID_{50} of 9.4 mg/kg im. In glucose-induced ulcers, a model in which gastric damage is induced by feeding the animals solely with a liquid meal made up of an aqueous glucose solution, compound **44** administered sc twice daily for 9 days has a protective ID_{50} of about 24 mg/kg. In comparison, DSCG and the reference antiulcer cimetidine, given under the same conditions up to 100 mg/kg, do not show any protective effect in any of the cited models, apart from the NaCl ulcer test, in which cimetidine shows a weak activity. On the contrary, the prostaglandin E_2 (PGE) exhibits a potent cytoprotective effect in both ethanol and sodium chloride ulcer models with an ID_{50} of 0.08 and 0.05 mg/kg, respectively. These results are shown in Table III.

Compound **44**, as well as other ditetrazolylbenzanilides of this series, are poorly absorbed by the oral route. The oral bioavailability of this compound in rat and guinea pig is about 1%; however, the bronchial absorption is of the same order of magnitude as after im administration, i.e. about 70%. The structural requirements for activity and for good oral absorption have often proven to be mutually exclusive in the field of antiallergic compounds. Also in this series, many compounds having less acidic properties (e.g. compound **37** which exhibits about the same activity as DSCG) show excellent oral absorption. Nevertheless, their activity is about 30–40 times lower than that shown by the most potent ditetrazolylbenzanilides. This feature does not necessarily mean a therapeutic disadvantage, because inhaled drugs for the treatment of asthma are now widely used, and it is shared by other antiallergic compounds such as DSCG and nedocromil, which are first line agents in the treatment of mild to moderate asthma.

Conclusions

We have described the synthesis and some pharmacological properties of a new class of acidic *N*-phenylbenzamides. Among these derivatives, compound **44** (CR 2039) and other bis(1*H*-tetrazol-5-yl)-*N*-phenylbenzamides exhibit potent antiallergic and cytoprotective activities. Compound **44** seems to have the same pattern of activity as DSCG, the standard reference, i.e. the prevention of the release of histamine and other autacoids from sensitized cells responsible for allergic reactions. But this derivative, as well as other compounds of the series, is much more potent than the standard agent in conventional tests, and moreover it seems to possess additional pharmacological action. In fact, it also exhibits potent cytoprotective effects on different experimental models, and this strong capacity to prevent the breakdown of the membrane epithelium may be useful in the clinical management of allergic diseases.

Experimental Section

The following procedures were adopted: ^1H NMR spectra were recorded at 60 MHz on a Varian EM360L or at 300 MHz on a Bruker CXP-300 instrument; infrared spectra were recorded on a Perkin-Elmer 1420 ratio recording IR spectrophotometer with a 3700 data station. Melting points were determined on a Buchi 535 apparatus and are uncorrected. Melting points higher than 270 °C were determined by DSC method on a Perkin-Elmer DSC7 PC series apparatus. Elemental analyses were performed by Redox (Cologno Monzese, MI), and the analytical results were within ±0.4% of the theoretical values unless otherwise noted. TLC was carried out using Merck silica gel GF_{254} plates. Aniline, 2-aminobenzonitrile, 3-aminobenzonitrile, 4-aminobenzonitrile, 4-aminobenzoic acid, 3-aminobenzoic acid, 5-aminoisophthalic acid, 3-aminophthalic acid, 4-aminobenzamide, 4-nitrophthalic

acid, benzoyl chloride, terephthaloyl chloride, 4-cyanobenzoyl chloride, 4-propylbenzoyl chloride, 4-methylbenzoyl chloride, 4-methoxybenzoyl chloride, 3,4-dimethoxybenzoyl chloride, 3,5-dimethoxybenzoyl chloride, 3,4,5-trimethoxybenzoyl chloride, 3-chlorobenzoyl chloride, 4-chlorobenzoyl chloride, 2,4-dichlorobenzoyl chloride, 4-(trifluoromethyl)benzoyl chloride, 4-nitrobenzoyl chloride, 4-butylbenzoic acid, 3-cyanobenzoic acid, and 4-propoxybenzoic acid were purchased from commercial suppliers and were used without further purification. Some of the acyl chlorides were synthesized from the corresponding commercially available acids by refluxing with thionyl chloride and were purified by conventional methods. 4-Aminophthalic acid,[11] 4-aminoisophthalic acid,[12] 3-aminoterephthalic acid,[13] 3-carboxy-5-carbamoylaniline,[14] 3-carboxy-5-hydroxymethylaniline,[15] 4-(tetrazol-5-yl)aniline,[16] 3-(tetrazol-5-yl)aniline,[16] terephthaloyl monochloride,[17] 4-hydroxybenzoyl chloride,[18] and *N*-methyl-4-cyanoaniline,[19] were prepared by cited literature methods. 3,4- and 3,5-dihydroxybenzoyl chloride were synthesized according to the procedure used for 4-hydroxybenzoyl chloride.

4-Cyano-*N*-(4-cyanophenyl)benzamide (54). To a mechanically stirred solution of 17.8 g (0.151 mol) of 4-aminobenzonitrile and 23.2 mL (0.166 mol) of triethylamine dissolved in 50 mL of THF at 10 °C was added a solution of 25 g (0.151 mol) of 4-cyanobenzoyl chloride in 30 mL of THF dropwise. Stirring was continued at 10 °C for 1 h and at room temperature for 3 h. The precipitate was filtered and then the crude solid was collected and washed with 100 mL of 1 N HCl. The solid was filtered, washed on the filter with water to neutral pH, and dried to give 35 g (94%) of **54**. An analytical sample was obtained by recrystallization from THF/water 2:1: mp 267 °C; TLC benzene/AcOH/MeOH 45:8:8 (R_f 0.78); IR (KBr) 3348, 2225, 1680, 1596, 1519, 1405, 1317, 1252, 841 cm^{-1}; ^{1}H-NMR 60 MHz (DMSO-d_6) 8.00 (8 H, m, aromatics), 10.8 (1 H, s, NHCO) ppm. Anal. ($C_{15}H_9N_3O$) C, H, N. With this procedure, compounds A of Scheme I, shown in Tables I and IV, were synthesized.

4-(1*H*-Tetrazol-5-yl)-*N*-[4-(1*H*-tetrazol-5-yl)phenyl]benzamide (44) (CR 2039). To a mechanically stirred solution of 30 g (0.121 mol) of **54** in 200 mL of DMF at 50 °C were added 31.6 g (0.485 mol) of sodium azide and 26 g (0.485 mol) of ammonium chloride. The obtained suspension was stirred at 100 °C for 24 h, cooled at room temperature, diluted with ice, and acidified with concentrated HCl under nitrogen flow in a well-ventilated hood (Warning: in this phase toxic fumes of HN_3 were generated). The solid was filtered and washed to neutral pH with warm water to afford 40 g. The crude product was crystallized from DMF/methanol 1:1 to give 29 g of 44 (72% yield): mp 301 °C; TLC methyl ethyl ketone/AcOH/MeOH 8:0.3:1 (R_f 0.44); IR (KBr) 3303, 1651, 1601, 1572, 1542, 1508, 1436, 1334, 847 cm^{-1}; ^{1}H-NMR 300 MHz (DMSO-d_6) 8.10 (4 H, m, aromatics), 8.27 (4 H, m, aromatics), 10.75 (1 H, s, CONH) ppm. Anal. ($C_{15}H_{11}N_9O$) C, H, N. With this procedure, compounds **B** of Scheme I and shown in Table I were synthesized.

4-(1*H*-Tetrazol-5-yl)-*N*-[4-(1*H*-tetrazol-5-yl)phenyl]benzamide, Disodium Salt. The disodium salt of the compound 44 (CR2039) was prepared by conventional methods: mp 390 °C; IR (KBr) 3440–3330, 1674, 1613, 1594, 1539, 1518, 1446, 1420, 1318, 1004, 843, 738; ^{1}H-NMR 300 MHz (CD_3OD) 7.86 ppm (4 H, m, aromatics), 8.07 ppm (4 H, m, aromatics), 8.20 ppm (2 H, m, aromatics). Anal. ($C_{15}H_9N_9Na_2O$) C, H, N, Na.

Computer-Aided Design. The software used for superimposition of lodoxamide, nedocromil, and sudexamox on 47 was MAD (Molecular Advanced Design, available from Aquitaine Systemes, Tour ELF, La Defense, Paris, France) and run on an IBM 6150 with the graphics station IBM 5085. The optimization of low-energy conformers was conducted by Monte Carlo–Metropolis algorithm, using a dynamic weighting of the randomly chosen rotation axes to be modified. The starting data set is minimized using a function that randomly selects the rotation axes. During the first part of the calculation, the "heaviest" rotation axes are favored, followed by the "lightest" ones (second part), and finally the "heaviest" ones again (third part). Fifty iterations maximum or dE less than 0.1 kcal/mol were performed for optimization, according to Newton-Raphson.

Biological Tests. Male Sprague-Dawley rats (150–250 g) and male Hartley guinea pigs (350–500 g) were used. DSCG, ketotifene, nedocromil, PGE, cimetidine, and ovalbumin (grade V) were purchased from Sigma, Bordetella Pertussis from Difco (Milano, Italy), aluminum hydroxide and Evans blue from Fluka (Buchs, Switzerland), sodium thiopental from Abbott, absolute alcohol, sodium chloride, acetic acid, and glucose from Carlo Erba (Milano, Italy).

The compounds under investigation were dissolved as the sodium salt in saline. Estimated doses at 50% effect (ID_{50}) and their $p = 0.05$ fiducial limits were calculated from the regression line of the percentage of maximum effect, discarding the 6% tails, on the logarithm of the dose.

(1) Antiallergic Activity. Rat Passive Cutaneous Anaphylaxis (PCA). PCA was performed using standard techniques as previously described.[20] Briefly, IgE antiserum was prepared in male Sprague-Dawley rats immunized by im administration of ovalbumin (25 mg) in 0.5 mL of Bordetella Pertussis suspension. Animals were bled 14 days after immunization and their sera were pooled and diluted to produce, after intradermal administration of 0.05 mL, a 10-mm diameter skin reaction. A volume of 0.05 mL of this IgE antiserum was injected intradermally at two different sites on the shaved back of naive young rats. After a 24-h sensitization period, the animals were challenged intravenously with 5 mL/kg of saline solution containing 25 mg of egg albumin and 25 mg of Evans blue. Thirty minutes after challenge the animals were sacrificed, the dorsal skin was removed, and the long and short axis of each wheal were measured. Test compounds were administered by im bolus 2 mL/kg, 15 min before challenge in at least three different doses ($n \geq 15$).

Antigen-Induced Bronchial Anaphylaxis in the Sensitized Guinea Pig. Male guinea pigs were sensitized by an sc injection of 0.5 mL of saline containing 10 μg of ovalbumin dispersed in 1 mg of $Al(OH)_3$. The injection was repeated after 14 days, and 1 week later the animals were anesthetized with sodium thiopental 50 mg/kg ip; the bronchial response was recorded according to the Konzett and Rossler method.[21] The trachea was cannulated and the lung ventilated mechanically by a Palmer constant volume respiration pump (53 strokes/min, 6 mL/stroke). Pulmonary inflation pressure was recorded from a T-shaped cannula inserted into the trachea connected to a Statham pressure transducer (Model P 23 DB) and to a polygraph (Battaglia Rangoni). Systemic arterial blood pressure and heart rate were also continuously recorded from the cannulated carotid artery. After a 10-min basal recording, the animals were pretreated with test compounds by dry powder insufflation[22] 15 min before challenge. The ovalbumin challenge was performed

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by aerosol, with an ultrasonic nebulizer (2.75-μm median particle diameter, 4 mL/min aerosol output) which was connected to the ventilator circuit, and the animals were ventilated for 1 min with air containing aerosolized ovalbumin (0.3 mg/mL). Bronchoconstriction was recorded for 10 min after antigen challenge and expressed as percent increase versus basal tone. Area under bronchoconstriction curve (AUC) was calculated in the period 0–10 min.

In experiments where bronchoconstriction was studied in conscious guinea pigs, the actively sensitized animal was placed in a Plexiglas box and challenged 15 min later with aerosolized ovalbumin (0.5% w/vol in saline). Test compounds were administered by im bolus 2 mL/kg, 15 min before challenge in at least three different doses and in triplicate ($n \geq 15$). The latency time to the first sign of bronchoconstriction (abdominal contraction) was measured and the animal was drawn out from the box immediately after.

(2) Cytoprotective Activity. Ethanol-Induced Gastric Lesions. Male conscious rats of 140–160 g body weight and fasted 24 h prior to the experiment were used. Water was allowed ad libitum. Gastric lesions were induced by oral administration of 1.5 mL of absolute ethanol.[23] The tested drugs were administered intraveneously (iv) (5 mL/kg) 15 min before challenge. One hour after the ethanol administration, the animals were sacrificed by cervical dislocation. The stomachs were taken out and dissected along the greater curvature, and the mucosa was examined by an investigator, unaware of the treatment given. The grading of necrotic lesions was quantified according to an arbitrary scoring that takes into account the length of the necrotic area (<2 mm, 2–4 mm, >4 mm) and the number of ulcers in each length category (lesion index). For each treatment group, the mean of lesion index as well as the effect percent vs the control group was calculated. The substances under investigation were administered in at least three dose levels to groups of five animals ($n \geq 15$).

Sodium Chloride-Induced Gastric Lesions. Male conscious rats of 140–160 g body weight and fasted 24 h prior the experiment were used. Water was allowed ad libitum. Gastric lesions were induced by oral administration of 1.5 mL/animal of 25% (w/v) NaCl hypertonic solution.[23] The tested drugs were administered iv (5 mL/kg) 15 min before NaCl administration. One hour after the challenge administration, the animals were sacrificed by cervical dislocation. The stomachs were taken out and dissected along the greater curvature, and the mucosa was examined by an investigator, unaware of the treatment given. The grading of necrotic lesions was quantified according to the method described above for the ethanol ulcer. For each treatment group the mean of lesion index as well as the percent of effect vs the control group was calculated. Compound 44 and PGE were administered at five dose levels to groups of five animals ($n = 25$), whereas DSCG and cimetidine were tested in duplicate at 30 and 100 mg/kg ($n = 20$).

Acetic Acid-Induced Gastric Lesions. Male conscious rats of 140–160 g body weight and fasted 24 h prior to the experiment were used. Water was allowed ad libitum. Gastric lesions were induced by local administration of a 30% water solution of acetic acid.[24] Under light ether anesthesia, the rats were laparomized, their stomachs were exposed, and 50 μL of acetic acid solution was administered into the submucosa of the greater curve. The abdominal wall was sutured, and the rats were placed in fasting cages. Twenty-four hours after acetic acid administration, the animals were sacrificed by excess of ether. The stomachs were taken out and dissected along the greater curvature, and the mucosa examined by an investigator, unaware of the treatment given. The grading of necrotic lesions was quantified according to the method described above for the ethanol ulcer. The tested drugs were administered im three times at −0.5, 4, and 21 h from challenge. For each treatment group, the mean of lesion index as well as the effect percent vs the control group was calculated. Compound 44 was administered at five dose levels to groups of five animals ($n = 25$), whereas DSCG and cimetidine were tested in duplicate at 100 mg/kg ($n = 10$).

Glucose-Induced Gastric Lesions. Male conscious rats of 180–200 g body weight and fasted 24 h prior to the experiment were used. Water was allowed ad libitum. Gastric lesions were induced by feeding the animals for 9 days solely with a liquid meal constituted by an aqueous 20% (w/v) solution of glucose.[24] On the 9th day after the outset, the animals were sacrificed by cervical dislocation. The stomachs were taken out and dissected along the greater curvature, and the mucosa was examined by an investigator, unaware of the treatment given. The grading of necrotic lesions was quantified according to an arbitrary scoring that takes into account the number of necrotic lesions as well as the extension of the area of the rumen that was impaired. The tested drugs were administered sc twice daily for 9 days. For each treatment group, the mean of lesion index as well as the effect percent vs control group was calculated. Compound 44 was administered at four dose levels to groups of five animals in duplicate ($n = 40$), whereas DSCG was tested in duplicate at 100 mg/kg ($n = 10$).

Supplementary Material Available: Tables containing the atomic coordinates, bond distances, bond angles, and torsion angles for computer-aided design for compound 47, lodoxamide, nedocromil, and sudexamox, and elemental analysis data for 1–58 (36 pages). Ordering information is given on any current masthead page.

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