mixture was stirred for 16 h at room temperature with maintenance of the pH at 8.0. The solution was dialyzed three times for a total of 24 h at 0 °C against water (10 L each, containing 100 μL of 1 N NaN_3) and the product, maleimidobenzoyl-BSA (MBSA), was lyophilized. The Acm group of the peptides 1–4 was cleaved by treatment of the Acm-protected peptides 1–4 dissolved in 2 mL of 3 M NaOAc at pH 4.0, with the addition of 4.2 mg of $Hg(OAc)_2$ for 20 min at room temperature. The reaction mixture was diluted with 4 mL of water, and gaseous H_2S was bubbled through for 30 min. The black suspension was filtered through Celite, the reaction vial and the filter were rinsed with 4 mL of water, and the filtrate fractions were combined and degassed in vacuo for 10 min. This solution was directly used for the coupling of the hapten peptides to the carrier by adding these solutions to MBSA in 15 mL of 50 mM Tris·HCl at pH 7.5 and nitrogen bubbling for 3 h. The reaction mixtures were dialyzed three times against water, as described above, and then lyophilized. Eleven milligrams of 1 coupled to 105 mg of MBSA produced 99.0 mg of conjugate, 9.5 mg of 2 to 102 mg of MBSA produced 90.0 mg, 10 mg of 3 to 100 mg of MBSA produced 97 mg, and 8.3 mg of 4 to 100 mg of MBSA finally produced 59.5 mg of conjugate.

Immunization Procedures. Female Balb/c mice (Charles River, St. Constant, Quebec) received weekly injections (500 or 50 ng/mL) of immunogen for 3 consecutive weeks. An additional immunization was given 3 weeks later. On each occasion the animals received a single intraperitoneal (0.1 mL) plus two intradermal (0.05 mL) injections of the immunogen in the form of an emulsion in complete Freund's adjuvant. Ten days after the last immunization, the mice were bled and the individual sera tested in an enzyme-linked immunosorbant assay (ELISA).[18] Crossreactivity with similar immunogens and titre of the sera were assessed. In the present study, 24 mice were used; six mice were immunized with each of the four immunogens 1–MBSA, 2–MBSA, 3–MBSA, and 4–MBSA.

ELISA Protocol. The enzyme-linked immunosorbent assay (ELISA) is a procedure that allows relatively rapid estimation of the specificity of antisera. In this test, the natural antigen (SP, NKA, NKB) is absorbed to the plastic surface of a well in a microtiter plate and serum potentially containing antibodies specific for these antigen is added. If the antigen is recognized and bound by antibodies, an indirect anti-antibody–enzyme test is used to visualize these immobilized antibodies.

All steps were performed at room temperature in polyvinyl microtitre plates (Immunlon II, Dynatech Labs, Alexandria, VA). The various stages were carried out in 50-μL volumes for a period of 1 h, unless otherwise stated. After each step the wells were washed three times with phosphate-buffered saline (PBS, 0.9%, pH 7.4), and excessive liquid was removed by blotting the plates on absorbant paper towels. In addition to the immunogens described above, SP, NKA, and NKB (Peninsula, Belmont, CA) and BSA were used as additional antigens in the ELISA. All antigens were dissolved in PBS and added to separate wells on the plate. After the incubation period, the contents of the plate were discarded and the wells washed. Saturation of nonspecific binding sites in each well was achieved with a 1% ovalbumin solution in PBS. After the excess of ovalbumin was discarded, diluted serum (1:50; in PBS) was pipeted into each well; any specific antibodies in the serum should then bind to the antigen-coated wells. Rabbit anti mouse antibodies (Sigma M9637; in PBS with 0.2% Triton) were then added in excess to each plate to bind to the mouse sera–antigen complex, formed in the previous stage. Mouse anti-peroxidase antibody,[18] a third antibody, was then added to the wells and left for 45 min to form the final antigen–antibody–antibody–antibody bridge through the still free second binding site of the rabbit antibody. Horseradish peroxidase (Sigma Tyr^6, 5 μg/mL in PBS) was added to the wells, left for 30 min, and washed off three times in order to remove any unbound enzyme. Finally, hydrogen peroxide (0.01%) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, Sigma, 0.04%) in phosphate–citrate buffer (150 mM) at pH 4.0 were added, and the mixture was left for another 30 min. The subsequent coloration due to oxidized ABTS was measured at 414 nm in a Bio-Rad EIA reader (Model 2550), and values from antigen-coated wells were compared to those that were treated only with PBS.

Acknowledgment. We are gratefully indebted to C. Thêberge for secretarial help and M.-R. Lefebvre and R. Laprise for technical assistance. These studies were funded through grants from the Canadian Medical Research Council to Dr. E. Escher and Dr. A. C. Cuello. E.E. is a Chercheur-Boursier of the Fonds de la Recherche en Santé du Québec.

Registry No. 1, 115437-76-8; 2, 115437-77-9; 3, 115437-78-0; 4, 115437-79-1; H-Aca-Arg-Pro-Lys(NO_2Z)-Pro-Gln-Gln-Aca-Cys(Acm)-OH, 115437-80-4; BOC-Cys(Acm)-OCs, 79396-90-0; BOC-Aca-OH, 6404-29-1; *cyclo*[Aca-Arg-Pro-Lys(NO_2Z)-Pro-Gln-Gln-Aca-Cys(Acm)], 115462-18-5.

(18) Semenenko, F. M.; Bramwell, S.; Sidebottom, E.; Cuello, A. C. *Histochemistry* **1985**, *83*, 405.

Drug-Induced Modifications of the Immune Response. 12. 4,5-Dihydro-4-oxo-2-(substituted amino)-3-furancarboxylic Acids and Derivatives as Novel Antiallergic Agents

Robert A. Mack, Walter I. Zazulak, Lesley A. Radov, Jane E. Baer, Jeffrey D. Stewart, Philip H. Elzer, C. Richard Kinsolving, and Vassil St. Georgiev*

Departments of Organic Chemistry and Pharmacology, Pennwalt Corporation, Pharmaceutical Division, Rochester, New York 14623. *Received January 11, 1988*

The synthesis of a series of novel 4,5-dihydro-4-oxo-2-(substituted amino)-3-furancarboxylic acids, salts, esters, and amides is described. The title compounds when tested in the mediator-induced dermal vascular permeability and active anaphylaxis assays in rats demonstrated moderate to potent antiallergic activity. The [2-*trans*-(4-methylphenyl)cyclopropyl]amino analogue **53** emerged as the most active derivative. Thus, when administered intraperitoneally to rats at a dose of 100 mg/kg, it inhibited the action of the mediators serotonin, histamine, and bradykinin by 100%. In the active anaphylaxis assay in rats, compound **30** suppressed the edema by 81% at a dose of 100 mg/kg, following intraperitoneal administration.

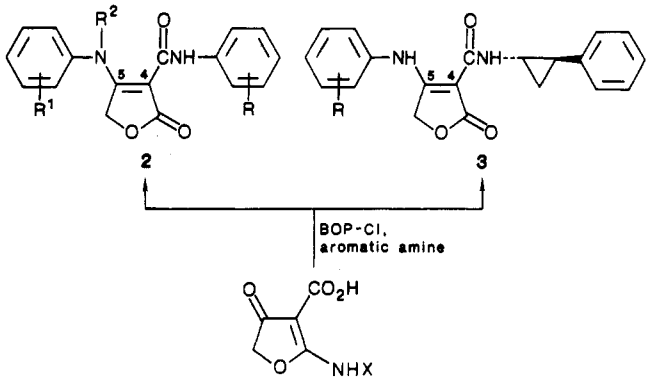
During the past few years our interest has been directed toward the synthesis of a new class of antiallergic agents, the 2(5*H*)-furanone derivatives **2** and **3**.[1,2] The γ-lactone amides **2** and **3** were obtained by a novel rearrangement of 2-(substituted amino)-4,5-dihydro-4-oxo-3-furancarboxylic acids (**1**) in the presence of 1 equiv of each

(1) Mack, R. A.; Georgiev, V. St.; DeCory, T. R.; Radov, L. A. *Eur. J. Med. Chem.* **1987**, *22*, 521.

(2) Georgiev, V. St.; Mack, R. A.; Walter, D. J.; Radov, L. A.; Baer, J. E. *Helv. Chim. Acta* **1987**, *70*, 1526.

0022-2623/88/1831-1910$01.50/0

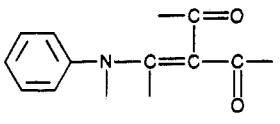
Scheme I



N,N-bis(2-oxo-3-oxazolidinyl)phosphorodiamidic chloride (BOP-Cl) and an appropriately substituted aromatic amine (Scheme I).[3-6]

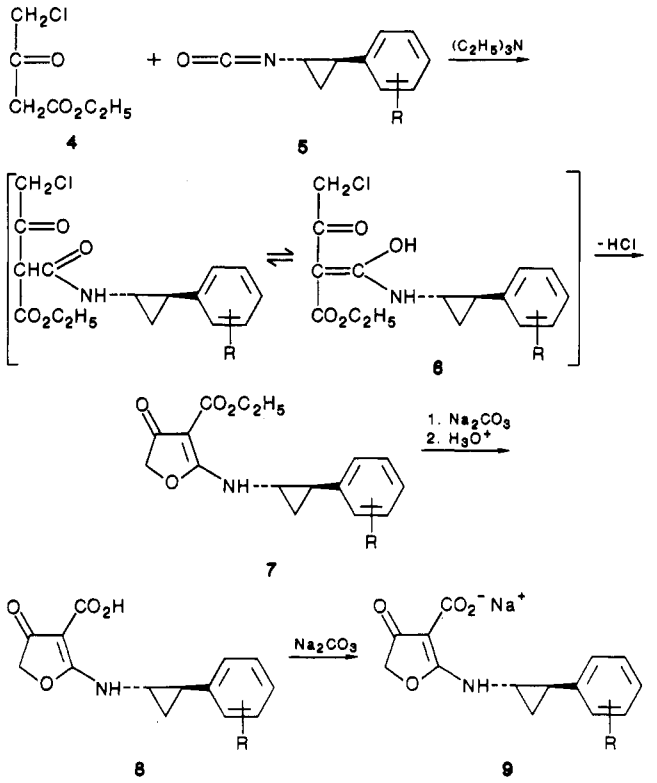
When tested for antiallergic activity in the rat dermal vascular permeability assay, a number of compounds **2** inhibited the action of mediators (serotonin, histamine, and bradykinin) on the small blood vessels.[1] The most active analogues of the series, **2a** (R = R^2 = H, R^1 = 4-Cl) and **2b** (R = R^1 = R^2 = H) inhibited the effect of bradykinin by 50 and 63%, respectively, when injected intraperitoneally at doses of 100 mg/kg. Similarly, the *N*-(2-*trans*-phenylcyclopropyl) amides **3** also displayed the same activity under comparable experimental conditions.[2] Thus, the inhibitory activity against bradykinin ranged between 67 and 100%, with compounds **3a** (R = 3-CH_3), **3b** [R = 3,5-$(CH_3O)_2$] and **3c** (R = 4-Cl) being the most active ones with 80, 88, and 100% inhibition, respectively. The potency against serotonin and histamine, although less pronounced, was still significant. For example, in the cases of compounds **3a–c**, the suppression of the serotonin and histamine action was by 43, 62, and 94% and by 72, 64, and 92%, respectively.[2] The obtained experimental results have provided clear evidence that replacing the C-4 *N*-aryl amide group of **2** with the *N*-(2-*trans*-phenylcyclopropyl) amide moiety of **3** markedly increased the antiallergic activity of the 2(5*H*)-furanones through the inhibition of the mediator-induced vascular permeability of the small blood vessels. In addition, derivatives **2** and **3** were also screened for antiallergic activity in the rat active anaphylaxis assay. At doses of 100 mg/kg (intraperitoneal administration), compounds **2a** and **3c** inhibited the edema by 39 and 70%, respectively; by comparison, the corresponding value for the standard drug theophylline was 66% when given orally at a dose of 90 mg/kg.[2]

One feature in the molecules of analogues **2** and **3** is the presence of unit A. A similar arrangement is also conspicuous in the molecule of the 4,5-dihydro-4-oxo-3-furancarboxylic acids **1**. Therefore, it became of interest



A

Scheme II



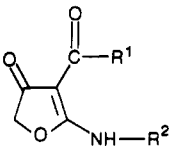
to investigate the antiallergic activity of compounds **1** and determine whether the structural difference between the 3(2*H*)- (**1**) and 2(5*H*)-furanones (**2** and **3**) would be sufficient to cause any change in the degree and/or nature of antiallergic activity. In addition to the free acids **1**, a number of their salts, esters, and amides were also investigated for activity. In the present communication we report the results of our studies.

Chemistry

A number of 4,5-dihydro-4-oxo-2-(substituted amino)-3-furancarboxylic acids and esters have previously been prepared as intermediates in the synthesis of various naturally occurring furoquinolines[7-12] or as products of an unusual heterocyclization reaction that involved a cyclic C–O insertion of an isocyanate synthon.[13] Recently,[14] we also reported the synthesis of a series of 4,5-dihydro-4-oxo-2-[(2-*trans*-phenylcyclopropyl)amino]-3-furancarboxylic acids and ethyl esters using the Capuano–Fischer approach.[13] The latter were prepared by a base-catalyzed cyclocondensation of ethyl 4-chloroacetoacetate (**4**) with an appropriately substituted 2-*trans*-phenylcyclopropyl isocyanate (**5**). The cyclocondensation most likely proceeded through the formation of a ketene *N*-acetal intermediate **6** and a C–O insertion into a furan ring to generate the desired ethyl 4,5-dihydro-4-oxo-2-[(2-*trans*-phenylcyclopropyl)amino]-3-furancarboxylate (**7**). Compound **7** was hydrolyzed under basic conditions to

(3) Mack, R. A.; Georgiev, V. St. *J. Org. Chem.* **1987**, *52*, 477.
(4) Mack, R. A.; Carroll, P. J.; Georgiev, V. St. *J. Heterocycl. Chem.* **1988**, *25*, 603.
(5) Carroll, P. J.; Mack, R. A.; Georgiev, V. St. *Acta Crystallogry, Sect. C: Cryst. Struct. Chem.* **1988**, *C44*, 294.
(6) Kuipers, W. J.; Mack, R. A.; Georgiev, V. St. *Magn. Reson. Chem.* **1988**, *26*, 89.
(7) Prabhakar, S.; Pai, B. R.; Ramachandran, V. N. *Indian J. Chem.* **1971**, *9*, 191.
(8) Govindachari, T. R.; Prabhakar, S.; Ramachandran, V. N.; Pai, B. R. *Indian J. Chem.* **1971**, *9*, 1031.
(9) Mester, I.; Ionescu, M. *Phytochemistry* **1971**, *10*, 2205.
(10) Ramachandran, V. N.; Pai, B. R.; Somasundaram, N.; Swaminathan, G. S. *Indian J. Chem.* **1973**, *11*, 1088.
(11) Gais, H. J.; Hafner, J. *Heterocycles* **1976**, *4*, 1921.
(12) Yazima, T.; Munakata, K. *Agric. Biol. Chem.* **1980**, *44*, 235.
(13) Capuano, L.; Fischer, W. *Chem. Ber.* **1976**, *109*, 212.
(14) Georgiev, V. St.; Mack, R. A.; Kinsolving, C. R. *Heterocycles* **1986**, *24*, 3195.

Table I. 4,5-Dihydro-4-oxo-2-(substituted amino)-3-furancarboxylic Acids, Salts, Esters, and Amides



26-56

compd	R^1	R^2	mp, °C	recrystn solvent	formula[a]
13			93–95	cyclohexane	$C_{14}H_{15}NO_4$
14			118–121	cyclohexane–ethyl acetate	$C_{12}H_{11}NO_4$
17			101–103	cyclohexane	$C_{15}H_{17}NO_4$
18			129–131	cyclohexane–ethyl acetate	$C_{13}H_{13}NO_4$
26	OC_2H_5	CH_3	179–181[b]	2-propanol	$C_6H_{11}NO_4$
27	OH	CH_3	170–172	ethanol	$C_6H_7NO_4$
28	OC_2H_5	$CH(CH_3)_2$	118–121[c]	ethyl acetate	$C_{10}H_{15}NO_4$
29	OC_2H_5	C_6H_5	115–117[d]	2-propanol	$C_{13}H_{13}NO_4$
30	OH	C_6H_5	159–161[e]	toluene	$C_{11}H_9NO_4$
31	OC_2H_5	$C_6H_4OCH_3$-2	174–176	ethyl acetate	$C_{14}H_{15}NO_5$
32	OH	$C_6H_4OCH_3$-2	161–164	2-propanol	$C_{12}H_{11}NO_5$
33	OC_2H_5	$C_6H_4CF_3$-3	167–170	2-propanol	$C_{14}H_{12}F_3NO_4$
34	OH	$C_6H_4CF_3$-3	170–173	toluene	$C_{12}H_8F_3NO_4$
35	OC_2H_5	$C_6H_4NO_2$-3	182–184	2-propanol	$C_{13}H_{12}N_2O_6$
36	OH	$C_6H_4NO_2$-3	215–218	tetrahydrofuran–water	$C_{11}H_8N_2O_6$
37	OC_2H_5	C_6H_4Cl-4	179–181[f]	2-propanol	$C_{13}H_{12}ClNO_4$
38	OC_2H_5	C_6H_4Br-4	172–175	2-propanol	$C_{13}H_{12}BrNO_4$
39	OH	C_6H_4Br-4	195–197	xylene	$C_{11}H_8BrNO_4$
40	OC_2H_5	$C_6H_4CO_2C_2H_5$-4	152–153	2-propanol	$C_{16}H_{17}NO_6$
41	OH	$C_6H_4CO_2H$-4	222 dec	aqueous ethanol	$C_{12}H_9NO_6$
42	OC_2H_5	C_6H_4CH-3	108–110	2-propanol	$C_{14}H_{15}NO_4$
43	OC_2H_5	1-naphthyl	139[g]	2-propanol	$C_{17}H_{15}NO_4$
44	OH	1-naphthyl	160–161	2-propanol	$C_{15}H_{11}NO_4$
45	ONa	C_6H_5	>220 dec	water	$C_{11}H_8NNaO_4$
46	NHC_6H_5	C_6H_5	182–184	ethanol	$C_{17}H_{14}N_2O_3$
47	NHC_6H_4Cl-4	C_6H_4Cl-4	241–243	dioxane	$C_{17}H_{12}Cl_2N_2O_3$
48	$NHC_6H_4OCH_3$-4	$C_6H_4OCH_3$-4	202–204	tetrahydrofuran	$C_{19}H_{18}N_2O_5$
49	$NHC_6H_4OCH_3$-2	$C_6H_4OCH_3$-2	172–174	ethanol	$C_{19}H_{18}N_2O_5$
50	$NHC_6H_4CH_3$-2	$C_6H_4CH_3$-2	188–190	95% ethanol	$C_{19}H_{18}N_2O_3$
51	OC_2H_5	2-*trans*-phenylcyclopropyl	140–142[h]	2-propanol	$C_{16}H_{17}NO_4$
52	OC_2H_5	2-*trans*-(4-chlorophenyl)cyclopropyl	146–148[h]	2-propanol	$C_{16}H_{16}ClNO_4$
53	OH	2-*trans*-(4-methylphenyl)cyclopropyl	155–157[h]	ethanol	$C_{15}H_{15}NO_4$
54	OH	2-*trans*-phenylcyclopropyl	155–157	ethanol	$C_{14}H_{13}NO_4$
55	OH	2-*trans*-(4-chlorophenyl)cyclopropyl	173–175	ethanol	$C_{14}H_{12}ClNO_4$
56	ONa	2-*trans*-phenylcyclopropyl	158–163 dec	water	$C_{14}H_{14}NNaO_4$

[a] See the Experimental Section for details. Elemental analyses were within 0.4% of theory. [b] Literature[13] mp 184 °C (ethyl acetate). [c] Literature[15] mp 123–124 °C (petroleum ether). [d] Literature[13] mp 119 °C (ethyl acetate). [e] Literature[13] mp 165 °C (benzene). [f] Literature[13] mp 171 °C (ethyl acetate). [g] Literature[13] mp 139 °C (ethyl acetate). [h] See ref 14.

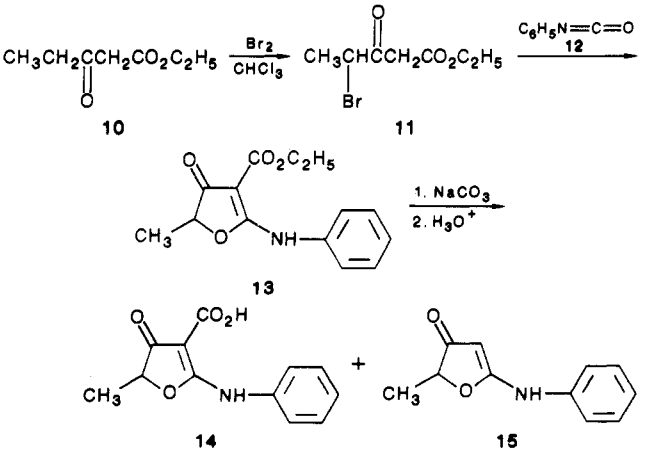
provide the corresponding free acid **8**. Exposure to aqueous sodium carbonate solution led to the preparation of sodium salt **9** (Scheme II).

When a substituted phenyl isocyanate was used in place of 2-*trans*-phenylcyclopropyl isocyanate, the corresponding 4,5-dihydro-4-oxo-2-[(substituted phenyl)amino]-3-furancarboxylic acids and esters were obtained (Table I). Replacement of the isocyanate precursor **5** with either methyl or isopropyl isocyanate furnished the *N*-methyl (**26**)[13] or *N*-isopropyl (**28**)[15] ethyl esters. The cyclocondensation reaction of 1-naphthyl isocyanate with ethyl 4-chloroacetoacetate (**4**) generated the *N*-(1-naphthyl) analogue **43**.[13]

The synthesis of ethyl 4,5-dihydro-5-methyl-4-oxo-2-(phenylamino)-3-furancarboxylate (**13**) was accomplished by treating ethyl 4-bromo-3-oxopentanoate (**11**) with phenyl isocyanate (**12**) (Scheme III). Compound **11** was prepared according to the procedure of Svendsen and Boll[16] by bromination of ethyl propionylacetate (**10**).

During the base-catalyzed hydrolysis of ester **13** to the free acid **14**, an unexpected decarboxylation took place

Scheme III



giving the 2-methyl-5-(phenylamino)-3(2*H*)-furanone **15**. While various decarboxylation reactions of furancarboxylic[17–21] and tetrahydrofurancarboxylic acids[22,23] have

(15) Mulholland, T. P. C.; Foster, R.; Haydock, D. B. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1225.
(16) Svendsen, A.; Boll, P. M. *Tetrahedron* **1973**, *29*, 4251.
(17) Nazarova, Z. N. *Zh. Org. Khim.* **1974**, *10*, 1341.
(18) Lyalin, V. V.; Grigorash, R. V.; Alekseeva, L. A.; Yagupol'skii, L. M. *Zh. Org. Khim.* **1975**, *11*, 1086.

Table II. Antiallergic Activity of 4,5-Dihydro-4-oxo-2-(substituted amino)-3-furancarboxylic Acids and Derivatives in the Mediator-Induced Dermal Vascular Permeability in Rats

compd	dose mg/kg	route of administration	no. of rats	% inhibition of mediator activity		
				serotonin	histamine	bradykinin
13	100	ip[a]	10	37.0[b]	40.0[b]	46.0[b]
26	100	ip[a]	10	38.0[b]	50.0[b]	25.0[b]
32	100	po[c]	10	30.0[b]	27.0[b]	38.0[b]
33	100	po[c]	10	9.7	4.1	40.0[b]
34	100	po[c]	10	40.0[b]	26.0[b]	54.0[b]
36	100	ip[a]	10	18.0[b]	16.0[b]	48.0[b]
37	100	po[c]	10	6.6	3.3	9.5
39	100	po[c]	10	39.0[b]	45.0[b]	68.0[b]
40	100	po[c]	10	18.0[b]	21.0[b]	21.0[b]
41	100	po[c]	10	63.0[b]	64.0[b]	64.0[b]
47	100	ip[a]	10	14.0	15.0	44.0[b]
48	100	ip[a]	10	13.0	8.0	39.0[b]
49	100	ip[a]	10	18.0[b]	1.0	39.0[b]
50	100	ip[a]	10	9.0	0.0	7.0
51	100	po[c]	10	57.0[b]	35.0[b]	59.0[b]
52	100	ip[a]	10	51.0[b]	60.0[b]	76.0[b]
53	100	ip[a]	10	100.0[b]	100.0[b]	100.0[b]
55	100	ip[a]	10	54.0[b]	56.0[b]	71.0[b]
cyproheptadine hydrochloride	1	ip[a]	10	93.0[b]	93.0[b]	23.0[b]

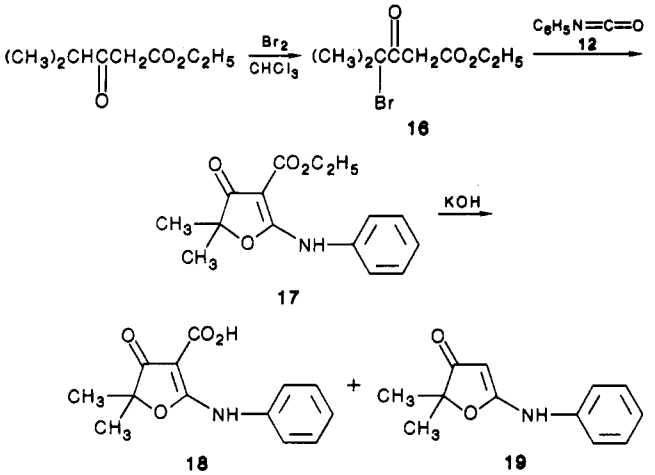
[a] ip = intraperitoneal administration 1 h prior to challenge. [b] Statistically significant ($p \leq 0.05$). [c] po = oral administration 1 h prior to challenge.

been reported, scant information regarding the decarboxylation of furanones exists, and this almost exclusively with γ-lactones.[24,25] To our knowledge, there are no reports dealing with the decarboxylation of 2*H*-furan-3-one-4-carboxylic acids, thus making the synthesis of compound **15** the first such example.

The synthesis of ethyl 4,5-dihydro-5,5-dimethyl-4-oxo-2-(phenylamino)-3-furancarboxylate (**17**) was accomplished in a similar fashion by reacting ethyl 4-bromo-4-methyl-3-oxopentanoate (**16**)[16] with phenyl isocyanate (**12**) (Scheme IV). However, when the cyclocondensation reaction was carried out in the usual manner by mixing together first the bromo ester **16** and the phenyl isocyanate (**12**) in petroleum ether–ethyl acetate and then adding the triethylamine, the yield of the resulting *gem*-dimethyl ester **17** was only 36%. In order to improve the yield of **17**, the reaction procedure was modified by dropwise addition of ethyl 4-bromo-4-methyl-3-oxopentanoate to a solution of phenyl isocyanate and triethylamine in methylene chloride. The change of solvent and the reverse addition of the reagents led to significantly higher yields of **17** (≈70%). Hydrolysis of ester **17** in aqueous sodium carbonate was unsatisfactory, providing the free acid **18** in only 6% yield. However, when the hydrolysis was carried out with potassium hydroxide in 50% ethanol at 75–80 °C, the yield of **18** improved to 58%. As with the case of the 5-methyl analogue **14**, in addition to the free acid **18**, the corresponding decarboxylated 3(2*H*)-furanone **19** was also formed.

In order to further diversify the present series of 4-oxo-3-furancarboxylic acids, we prepared a number of the

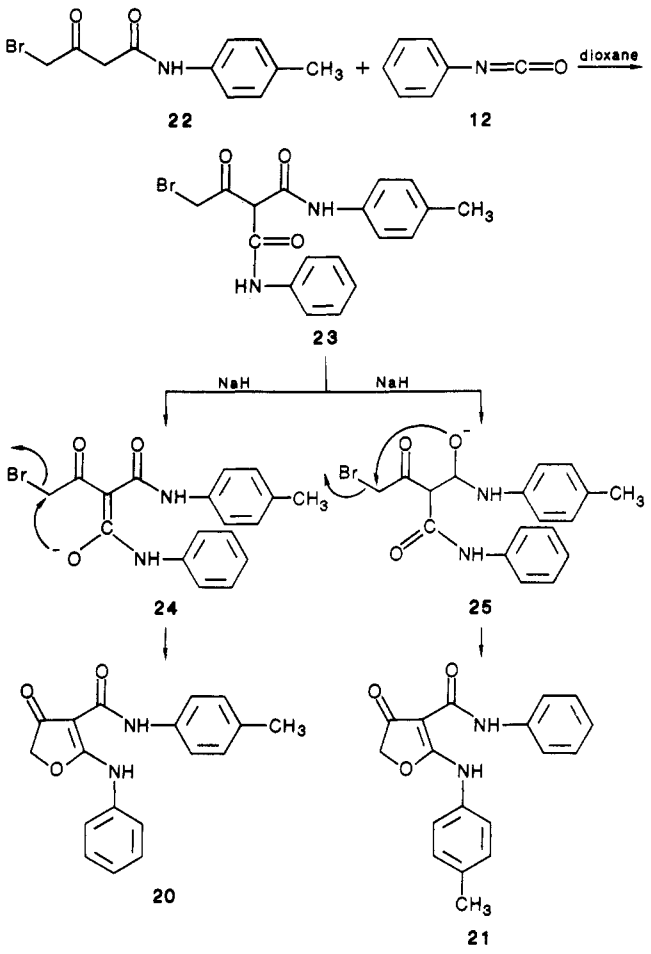
Scheme IV



corresponding amide derivatives by using the procedure of Ibrahim et al.[26] In 1985, the latter described the synthesis of *N*-(4-methylphenyl)-4,5-dihydro-4-oxo-2-(phenylamino)-3-furancarboxamide (**20**).[27] In our hands, the procedure of Ibrahim et al. provided a product that appeared as one spot on TLC[28] but had an unclear melting point and a questionable ^{1}H NMR spectrum. Whereas Ibrahim et al. reported NMR values (from a 90-MHz instrument) with no signal multiplicity noted, we observed the methyl protons as a doublet at δ 2.32 (on a 60-MHz instrument). The 200-MHz ^{1}H NMR spectrum of **20** revealed doublets for each of the NH protons, as well as for the furan methylene and the CH_3 protons. When put through an analytical HPLC, the product was found to consist of *two* components that were present in a ratio of

(19) Naoi, Y.; Nakano, M.; Sakai, K.; Fujii, K. Japan Patent 78 56,656; *Chem. Abstr.* **1979**, *90*, 6628*k*.
(20) Naoi, Y.; Nakano, M.; Sakai, K.; Fujii, K. Japan Patent 78 56,658; *Chem. Abstr.* **1979**, *90*, 6231*f*.
(21) Shiono Koryo Kaisha, Ltd. Japan Patent 59 05,159 [84 05,159]; *Chem. Abstr.* **1984**, *101*, 7022*w*.
(22) Miyoshi, M.; Matsumoto, K.; Iwasaki, T. Japan Patent 78 77,054; *Chem. Abstr.* **1978**, *89*, 179842*p*.
(23) Ireland, R. E.; Norbeck, D. W.; Mandel, G. S.; Mandel, N. S. *J. Am. Chem. Soc.* **1985**, *107*, 3285.
(24) Zalinyan, M. G.; Kazaryan, Sh. A.; Dangyan, M. T. *Arm. Khim. Zh.* **1972**, *25*, 941.
(25) Institute of Pharmacology (Peopl. R. China) *K'o Hsueh T'ung Pao* **1976**, *21*, 515, 509; *Chem. Abstr.* **1977**, *86*, 171690*e*.
(26) Ibrahim, N. S.; Sadek, K. U.; Aziz, S. I.; Elnagdi, M. H. *Z. Naturforsch. B: Anorg. Chem., Org. Chem.* **1985**, *40b*, 131.
(27) In the reaction sequence of Ibrahim et al.,[26] equimolecular amounts of 4-bromo-4′-methylacetoacetanilide (**22**) and phenyl isocyanate (**12**) were mixed in anhydrous dioxane and stirred at room temperature in the presence of sodium hydride (100%, 2 equiv) for 5 h. A yield of 85% of pure **20** was claimed, having a mp of 192 °C (ethanol).
(28) The TLC was developed using a 1:1 mixture of ethyl acetate–hexane on 5 × 10 cm silica gel plates by E. Merck 60 F254, 0.25 mm, thick layer.

Scheme V



approximately 1:1.[29] These observations lead us to conclude that the existing procedure of Ibrahim et al., in fact, results in a mixture of two regioisomers (**20** and **21**) arising from two alternate modes of cyclization (**24** → **20** and **25** → **21**) from a common precursor (**23**) (Scheme V).

In order to avoid the formation of regioisomeric mixtures, all other 4-oxo-3-furancarboxamides made during the present study (Table I) were synthesized from pairs of 4-bromoacetoacetanilides and phenyl isocyanates having the same substitution pattern on the phenyl rings, thus insuring the identical product from either mode of cyclization.[30] Either sodium hydride or triethylamine was effective as base catalyst.[32] All of the starting 4-bromoacetoacetanilides were made by modified procedures of existing methods.[34-36]

The 4,5-dihydro-4-oxo-2-(substituted amino)-3-furancarboxylic acids, salts, ethyl esters, and amides prepared during the present study are listed in Table I.

Table III. Antiallergic Activity of 4,5-Dihydro-4-oxo-2-(substituted amino)-3-furancarboxylic Acids and Derivatives in the Active Anaphylaxis Assay in Rats

compd	dose mg/kg	route of administration	no. of rats	edema mean values (Δ ± SD)	% inhibition
13	100	ip[a]	25	1.44 ± 1.76	61.0[b]
14	100	ip[a]	20	2.00 ± 1.89	60.0[b]
17	100	ip[a]	21	7.71 ± 4.71	16.0
18	100	ip[a]	20	1.95 ± 1.82	44.0[b]
30	100	ip[a]	15	0.47 ± 0.67	81.0[b]
32	100	ip[a]	20	3.45 ± 2.67	45.0[b]
34	100	ip[a]	19	3.35 ± 2.82	45.0[b]
36	100	ip[a]	30	7.29 ± 3.03	39.0[b,c]
39	100	ip[a]	29	3.24 ± 2.18	27.0[b]
41	100	ip[a]	28	2.00 ± 2.70	52.0[b]
44	100	ip[a]	15	1.20 ± 1.66	64.0[b]
45	100	ip[a]	8	2.75 ± 2.31	17.0
46	100	ip[a]	20	2.45 ± 1.57	46.0[b]
53	100	ip[a]	18	1.58 ± 0.99	45.0[b]
55	100	ip[a]	30	4.10 ± 1.95	6.0
56	100	ip[a]	25	3.72 ± 1.46	22.0[b]
theophylline	90	po[d]	20	3.75 ± 1.77	66.0[b]

[a] ip = intraperitoneal administration 1 h prior to challenge. [b] Statistically significant ($p \leq 0.05$). [c] Increase of edema. [d] po = oral administration 1 h prior to challenge.

Results and Discussion

The 4,5-dihydro-4-oxo-2-(substituted amino)-3-furancarboxylic acids and derivatives were tested for antiallergic activity in vivo in the mediator (serotonin, histamine, and bradykinin)-induced dermal vascular permeability and the active anaphylaxis assays in rats. The testing results are summarized in Tables II and III.

In the rat dermal vascular permeability test, the compounds were screened for their ability to inhibit the action of the mediators on the small blood vessels. The administration of the test compounds to rats was either by intraperitoneal or oral route at doses of 100 mg/kg (Table II). The screening results have provided some useful structure–activity correlations. The C-2 substitution proved to be an important factor for activity. Thus, the 2-(2-*trans*-phenylcyclopropyl)amino derivatives **51–55** showed the highest potency among all of the compounds tested. The 4-methylphenyl analogue **53** was the most active by inhibiting the action of all three mediators by 100%. Furthermore, substituting the methyl group with an electronegative function such as chlorine (**52** and **55**) significantly reduced the activity. Lack of substitution on the phenyl ring (as in ester **51**) did not change the potency (relative to that of the 4-chlorophenyl analogues **52** and **55**) but lowered the activity against both histamine and bradykinin. With the exception of the dicarboxylic acid **41**, the 2-phenylamino derivatives **29–42** were less potent as compared to their 2-(2-*trans*-phenylcyclopropyl)amino counterparts **51–55**. The weak activity of the 4-chlorophenyl ester **37** relative to the 2-(2-*trans*-phenylcyclo-

(29) HPLC conditions: a Perkin-Elmer Series 4B unit with LC-95 detector and Hewlett-Packard 3392A integration was used with a Du Pont Golden Series C-8, 3 μm, 6.2 mm × 8 cm column. The mobile phase was methanol–water (55:45) with each solvent containing 0.2% acetic acid and 0.2% triethylamine and a flow rate of 3.0 mL/min. The detector wavelength was 254 nm at 0.2 or 0.5 AUFS (absorbance units full scale). The retention times for the respective regioisomers was 24.22 and 25.46 min.

(30) Attempts to prepare the 4-oxo-3-furancarboxamides using alkyl or aromatic amines and 1,3-dicyclohexylcarbodiimide (DCC) in methylene dichloride solution under various reaction conditions proved to be unsuccessful. Furthermore, the use of *N,N*-bis(2-oxo-3-oxazolidinyl)phosphorodiamidic chloride, a recently reported[31] reagent for activation of carboxyl groups, in the presence of triethylamine and an aromatic amine, failed to generate the expected 4-oxo-3-furancarboxamides. Instead, a novel 3(2*H*)-furanone–2(5*H*)-furanone rearrangement[3-6] took place leading to the facile preparation of a new class of γ-lactone amides **2** and **3**.[1,2]

(31) Diago-Meseguer, J.; Palomo-Coll, A. L.; Fernández-Lizarbe, J. R.; Zugaza-Bilbao, A. *Synthesis* **1980**, 547.

(32) When analyzed by HPLC under similar experimental conditions[29] and 200-MHz ^{1}H NMR,[33] each of the 4-oxo-3-furancarboxamides **46–50** was confirmed to be one single compound.

(33) See the Experimental Section for details.

(34) Mallams, A. K.; Israelstam, S. S. *J. Org. Chem.* **1964**, *29*, 3554.

(35) Ali, M. I.; Abou-State, M. A.; Hassan, N. M. *J. Chem. Eng. Data* **1972**, *17*, 106.

(36) Chudgar, R. J.; Trivedi, K. N. *J. Indian Chem. Soc.* **1969**, *46*, 6.

propyl)amino analogues (especially **52** and **55**) leads to the conclusion that the introduction of a cyclopropyl group between the heterocyclic and phenyl rings is essential for an increased activity against the action of all three mediators. Overall, greater potency against bradykinin (as compared to serotonin and histamine) is consistently observed, as best examplified by compounds **33**, **36**, and **39** and the carboxamide derivatives **47–49**. Among the ethyl esters and the free acids, the latter seem to be the more active compounds. The 3-carboxamide analogues were the least potent in their inhibition of the mediator-induced dermal vascular permeability in rats.

When screened in the rat active anaphylaxis model (following intraperitoneal administration at doses of 100 mg/kg), most of the compounds tested showed moderate to potent inhibition of the edema (Table III). The highest activity was observed with the unsubstituted 2-phenylamino acid **30**. Substitution at the phenyl ring, in general, decreased the activity; in the case of the 3-nitrophenyl analogue **36**, the result was actually an increase in edema. The 2-(1-naphthyl)amino ester **43** and the C-5-methylated derivatives **13**, **14**, and **18** were also potent inhibitors of anaphylaxis. The latter three compounds, similar to **30**, had no substituents on the phenyl ring. As with the case in the mediator-induced vascular permeability assay, the free acids were found to be more potent than their ethyl esters. The observed decrease in activity of the sodium salt **45** as compared to the free acid **30** may be due to a difference in bioavailability, since both compounds were suspended rather than solubilized in 1% Methocel. That, in turn, may alter their absorption and metabolism sufficiently enough to cause the observed difference in activity. Overall, several of the tested compounds (**13**, **14**, **30**, and **44**) displayed activity that was equal to or better than that of the standard drug theophylline.

In addition to their antiallergic activity, the two 2-(2-*trans*-phenylcyclopropyl)amino analogues **51** and **54**, as previously reported,[14] exerted antimicrobial activity in vitro against a variety of Gram-positive and Gram-negative bacteria and antifungal activity against *Candida albicans* and two dermatophytes, *Trichophyton mentagrophytes* and *Microsporum audouinii*.

In summary, the present series of 4,5-dihydro-4-oxo-2-(substituted amino)-3-furancarboxylic acids and derivatives represent a novel class of antiallergic agents, as demonstrated by their ability to inhibit both the actions of mediators on the small blood vessels in the dermal vascular permeability assay in rats and the active anaphylaxis in rats. Furthermore, the obtained experimental results lend further support to the stipulation[1] of unit A as a pharmacophore for antiallergic activity. The first examples of a new decarboxylation reaction of 2*H*-furan-3-one-4-carboxylic acids have been discussed, as well as the lack of regiospecificity of a previously known method for synthesis of *N*-phenyl-4,5-dihydro-4-oxo-2-(phenylamino)-3-furancarboxamide derivatives.[40]

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The infrared (IR) spectra were obtained on a Nicolet MX-1 FT spectrometer as KBr disks. The proton nuclear magnetic resonance (^{1}H NMR) spectra were taken on a Varian EM-360A (60 MHz) spectrometer with tetramethylsilane as an internal standard; the 200-MHz ^{1}H NMR spectra were recorded on a Bruker-IBM 200 SY Fourier transform spectrometer with the same internal standard. All spectra were consistent with the assigned structures. Elemental analyses were within the acceptable limits of 0.4% of theory.

Ethyl 4-Bromo-3-oxopentanoate (11). Under a nitrogen atmosphere, a solution of bromine (9.07 mL, 0.177 mol) in 20 mL of chloroform was added dropwise over a period of 2 h to a solution of ethyl propionylacetate (25.52 g, 0.177 mol) in 155 mL of chloroform, at 0–5 °C (ice bath). The reaction mixture was stirred for 30 min at 0–10 °C and then allowed to stand at room temperature overnight. While stirring, a stream of air was bubbled through the solution for 1 h. After drying over anhydrous sodium sulfate, the solvent was evaporated under reduced pressure leaving compound **11**[16] (39.52 g, 100%) as a yellow oil.

Ethyl 4,5-Dihydro-5-methyl-4-oxo-2-(phenylamino)-3-furancarboxylate (13). Under a nitrogen atmosphere, triethylamine (33.9 mL, 0.24 mol) was added dropwise over a 2-h period to a solution of ethyl (2-bromopropionyl)acetate (**11**) (45.94 g, 0.2 mol) and phenyl isocyanate (**12**) (26.87 g, 0.24 mol) in a mixture of 400 mL of petroleum ether and 40 mL of ethyl acetate at 0–10 °C. The reaction mixture was stirred at 10–15 °C for 1 h and then allowed to stand at room temperature overnight. The crude solid precipitate was filtered off and sucked dry on the filter. After being stirred in 500 mL of 1 N hydrochloric acid for 1 h, the crude **13** was washed with water and recrystallized from cyclohexane to provide 44.56 g (83%) of pure product melting at 93–95 °C. IR (KBr): 3195, 2961, 1678, 1653, 1620, 1593, 1578, 1567, 1495, 1220, 1194, 1083, 790, and 755 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 10.45 (br s, 1 H), 7.74–7.22 (m, 5 H), 4.94 (q, 1 H, J = 7.0 Hz), 4.55 (q, 2 H, J = 7.0 Hz), 1.81 (d, 3 H, J = 7.0 Hz), 1.62 (t, 3 H, J = 7.0 Hz). Anal. ($C_{14}H_{15}NO_4$) C, H, N.

4,5-Dihydro-5-methyl-4-oxo-2-(phenylamino)-3-furancarboxylic Acid (14). A solution of 2-anilino-5-methyl-3-(ethoxycarbonyl)-4(5*H*)-oxofuran (**13**) (25.68 g, 98 mmol) and anhydrous sodium carbonate (20.83 g, 196 mmol) in 295 mL of water was refluxed for 2 h. After cooling, the insoluble solid precipitate (see compound **15** below) was filtered off, and the filtrate was acidified with concentrated hydrochloric acid (pH 1). Sodium chloride was added until complete precipitation was noted. The crude product was filtered off and washed thoroughly with water. Following repeated recrystallization from cyclohexane, a total of 8.90 g (39%) of pure **14** was obtained, mp 118–121 °C (cyclohexane–ethyl acetate). IR (KBr): 3285, 3190, 1737, 1660, 1635, 1600, 1555, 1442, 1340, 1280, 1170, 1042, and 748 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 10.52 (s, 1 H), 9.05 (br s, 1 H), 7.83–7.07 (m, 5 H), 5.04 (q, 1 H, J = 7.0 Hz), 1.62 (d, 3 H, J = 7.0 Hz). Anal. ($C_{12}H_{11}NO_4$) C, H, N.

2-Methyl-5-(phenylamino)-3(2*H*)-furanone (15). The insoluble solid precipitate, which was isolated at the end of the hydrolysis of ester **13** (see the preceding experiment) was purified by flash chromatography on silica gel with ethyl acetate–methanol (9:1) as eluent. Recrystallization from ethyl acetate provided pure **15** (1.23 g, 7%) as white crystals melting at 191–193 °C. IR (KBr): 3205, 3160, 3115, 3000, 2970, 2930, 2880, 2835, 2800, 1645, 1595, 1560–1480, 1443, 1360, 1288, 1257, 1163, 1105, 1068, 1042, 762, 745, 718, 686, and 667 cm^{-1}. ^{1}H NMR ($CDCl_3$–DMSO-d_6): δ 8.60 (br s, 1 H), 7.62–6.78 (m, 5 H), 4.78 (s, 1 H), 4.63 (q, 1 H, J = 7.0 Hz), 1.37 (d, 3 H, J = 7.0 Hz). Anal. ($C_{11}H_{11}NO_2$) C, H, N.

Ethyl 4-Bromo-4-methyl-3-oxopentanoate (16). Derivative **16** was prepared by a procedure similar to that described for the synthesis of **11** from ethyl isobutyrylacetate (5.26 g, 316 mmol) and bromine (1.62 mL, 316 mmol); yield 7.60 g (100%).[16]

Ethyl 4,5-Dihydro-5,5-dimethyl-4-oxo-2-(phenylamino)-3-furancarboxylate (17). Under a nitrogen atmosphere, a solution of ethyl 4-bromo-4-methyl-3-oxopentanoate (**16**) (38.08 g, 155.5 mmol) in 130 mL of methylene chloride was added dropwise over a 4-h period to a solution of phenyl isocyanate (**12**) (21 mL, 192.7 mmol) and triethylamine (26.4 mL, 189.6 mmol) in 335 mL of methylene chloride, at 0–5 °C (ice bath). The reaction mixture was stirred at 10–15 °C for 30 min and then at room temperature overnight. The resulting solution was washed sequentially with 250 mL each of 1 N hydrochloric acid, saturated sodium chloride solution, and saturated sodium bicarbonate solution and then dried over anhydrous sodium sulfate. Following evaporation of

(37) Clemens, R. J.; Hyatt, J. A. *J. Org. Chem.* **1985**, *50*, 2431.
(38) Yoshikawa, T.; Urabe, Y.; Kizota, R. *Yakugaku Zasshi* **1969**, *89*, 759.
(39) Blume, H.; Hesse, B.; Kochmann, W.; Mueller, S.; Pallas, M. Ger. (East) Patent 100,139; *Chem. Abstr.* **1974**, *80*, P 95562*y*.
(40) For Part 11 of this series see: Mullen, G. B.; Swift, P. A.; Georgiev, V. St. *J. Pharm. Sci.* **1987**, *76*, 930.

the solvent under reduced pressure, the resulting crude **17** was flash chromatographed over silica gel with ethyl acetate–hexane (3:2) as eluent. Recrystallization from cyclohexane yielded 30.13 g (70%) of pure product, mp 101–103 °C. IR (KBr): 3195, 2961, 1678, 1653, 1620, 1578, 1567, 1495, 1220, 1194, 1083, 790, and 755 cm^{-1}. ^1H NMR ($CDCl_3$): δ 10.45 (br s, 1 H), 7.74–7.22 (m, 5 H), 4.94 (q, 1 H, J = 7.0 Hz), 4.55 (q, 2 H, J = 7.0 Hz), 1.81 (d, 3 H, J = 7.0 Hz), 1.62 (t, 3 H, J = 7.0 Hz). Anal. ($C_{14}H_{15}NO_4$) C, H, N.

4,5-Dihydro-5,5-dimethyl-4-oxo-2-(phenylamino)-3-furancarboxylic Acid (18). To a solution of potassium hydroxide (87% pure; 16.79 g, 0.26 mol) in 570 mL of 50% aqueous ethanol was added solid ester **17** (17.90 g, 65.02 mmol) in one portion. The reaction mixture was heated at 75–80 °C for 6 h and then concentrated under reduced pressure, and the resulting insoluble material was filtered off. The filtrate was cooled to 0–5 °C (ice bath) and acidified with concentrated hydrochloric acid (pH 1) to precipitate a crude solid mixture comprised of the free acid **18** and its decarboxylated analogue, the 2,2-dimethyl-5-(phenylamino)-3(2*H*)-furanone (**19**). The mixture of **18** and **19** was partially dissolved in refluxing cyclohexane, and the insoluble material was filtered off. The resulting filtrate, after cooling, provided a white solid precipitate, which was recrystallized from cyclohexane–ethyl acetate to give 9.30 g (58%) of acid **18**, mp 129–131 °C. IR (KBr): 3300, 3190, 1729, 1655, 1633, 1596, 1550, 1445, 1339, 1270, 1165, 1057, and 759 cm^{-1}. ^1H NMR ($CDCl_3$): δ 9.13 (s, 1 H), 9.02 (br s, 1 H), 7.73–7.07 (m, 5 H), 1.61 (s, 6 H). Anal. ($C_{13}H_{13}NO_4$) C, H, N.

The cyclohexane-insoluble product was recrystallized from toluene to yield 2.40 g (18%) of the decarboxylated analogue **19**, mp 200–202 °C. IR (KBr): 3170, 3130, 2975, 2930, 2845, 2780, 1648, 1610, 1573, 1510, 1492, 1450, 1430, 1375, 1354, 1268, 1180, 1000, 813, 781, 765, 746, 700, and 678 cm^{-1}. ^1H NMR ($CDCl_3$–DMSO-d_6): δ 9.98 (br s, 1 H), 7.58–6.96 (m, 5 H), 4.80 (s, 1 H), 1.45 (s, 6 H). Anal. ($C_{12}H_{13}NO_2$) C, H, N.

Ethyl 4,5-Dihydro-4-oxo-2-[(4-(ethoxycarbonyl)phenyl)amino]-3-furancarboxylate (40). General Procedure. Triethylamine (9.87 mL) was added dropwise over a 1-h period to a cooled (0–5 °C) solution of ethyl 4-chloroacetoacetate (9.87 g, 60 mmol) and ethyl *p*-isocyanatobenzoate (14.34 g, 75 mmol) in 60 mL of petroleum ether–ethyl acetate (10:1) under a nitrogen atmosphere. The reaction mixture was stirred at 0–5 °C for 1 h. The resulting precipitate was filtered and washed sequentially with petroleum ether, 1 N hydrochloric acid, and water to furnish pure **40** (13.0 g), mp 152–153 °C (2-propanol). IR (KBr): 3160, 2985, 2930, 1717, 1654, 1630, 1601, 1574, 1430, 1385, 1282, 1118, 1078, and 850 cm^{-1}. ^1H NMR ($CDCl_3$): δ 10.50 (br s, 1 H), 7.33–8.30 (q, 4 H, J = 8.5 Hz), 4.70 (s, 2 H), 4.38 (q, 4 H, J = 7.5 Hz), 1.40 (t, 6 H, J = 7.5 Hz). Anal. ($C_{16}H_{17}NO_6$) C, H, N.

The following ether esters were obtained by procedures similar to that described for **40**: **ethyl 4,5-dihydro-4-oxo-2-(methylamino)-3-furancarboxylate (26),**[13] **ethyl 4,5-dihydro-4-oxo-2-(isopropylamino)-3-furancarboxylate (28),**[15] **ethyl 4,5-dihydro-4-oxo-2-(phenylamino)-3-furancarboxylate (29).**[13]

Ethyl 4,5-Dihydro-4-oxo-2-[(2-methoxyphenyl)amino]-3-furancarboxylate (31). Compound **31** was hydrolyzed to the corresponding free acid without further purification.

Ethyl 4,5-Dihydro-4-oxo-2-[3-(trifluoromethyl)phenyl]amino]-3-furancarboxylate (33). Anal. ($C_{14}H_{12}F_3NO_4$) C, H, F, N.

Ethyl 4,5-Dihydro-4-oxo-2-[(3-nitrophenyl)amino]-3-furancarboxylate (35). Compound **35** was hydrolyzed to the corresponding free acid without further purification.

Ethyl 4,5-Dihydro-4-oxo-2-[(4-chlorophenyl)amino]-3-furancarboxylate (37).[13]

Ethyl 4,5-Dihydro-4-oxo-2-[(4-bromophenyl)amino]-3-furancarboxylate (38). Compound **38** was hydrolyzed to the corresponding free acid without further purification.

Ethyl 4,5-Dihydro-4-oxo-2-[(3-methylphenyl)amino]-3-furancarboxylate (42). Anal. ($C_{14}H_{15}NO_4$) C, H, N.

Ethyl 4,5-Dihydro-4-oxo-2-[(1-naphthyl)amino]-3-furancarboxylate (43).[13]

4,5-Dihydro-4-oxo-2-[(4-carboxyphenyl)amino]-3-furancarboxylic Acid (41). General Procedure. Ethyl ester **40** (24.21 g, 0.759 mol) was suspended in 8% aqueous sodium carbonate (500 mL) and stirred. By use of a glass subsurface delivery tube, steam was passed through the mixture for 30 min, and then the resulting solution was cooled to ambient temperature and filtered, and the filtrate was acidified (pH 1) by gradual addition of concentrated HCl at 0–5 °C (ice bath). The precipitated free acid **41** was filtered off, rinsed with water, and dried on the filter. Recrystallization from aqueous ethanol furnished 13.32 g (67%) of pure **41**, mp >220 °C dec. IR (KBr): 3350–2300, 3280, 3055, 1762, 1663, 1640, 1554, 1440, 1178, and 790 cm^{-1}. ^1H NMR ($CDCl_3$–DMSO-d_6): δ 9.90 (br s, 1 H), 9.50 (br s, 2 H), 7.50–8.40 (m, 4 H), 4.70 (br s, 2 H). Anal. ($C_{12}H_9NO_6$) C, H, N.

The following 3-furancarboxylic acids were synthesized by procedures similar to the one used in the preparation of **41**. In addition to steam passage through the reaction mixture for a period ranging between 30 min and 1.5 h, some of the ethyl esters required an additional heating at reflux (1–3 h) in order to complete the hydrolysis. The obtained yields were usually 60–70% of theory.

4,5-Dihydro-4-oxo-2-(methylamino)-3-furancarboxylic Acid (27). Anal. ($C_6H_7NO_4$) C, H, N.

4,5-Dihydro-4-oxo-2-(phenylamino)-3-furancarboxylic Acid (30).[13]

4,5-Dihydro-4-oxo-2-[(2-methoxyphenyl)amino]-3-furancarboxylic Acid (32). Anal. ($C_{12}H_{11}NO_5$) C, H, N.

4,5-Dihydro-4-oxo-2-[[3-(trifluoromethyl)phenyl]amino]-3-furancarboxylic Acid (34). Anal. ($C_{12}H_8F_3NO_4$) C, H, F, N.

4,5-Dihydro-4-oxo-2-[(3-nitrophenyl)amino]-3-furancarboxylic Acid (36). Anal. ($C_{11}H_8N_2O_6$) C, H, N.

4,5-Dihydro-4-oxo-2-[(4-bromophenyl)amino]-3-furancarboxylic Acid (39). Anal. ($C_{11}H_8BrNO_4$) C, H, N.

4,5-Dihydro-4-oxo-2-[(1-naphthyl)amino]-3-furancarboxylic Acid (44). Anal. ($C_{15}H_{11}NO_4$), C, H, N.

The 4,5-dihydro-4-oxo-2-[(2-*trans*-phenylcyclopropyl)amino]-3-furancarboxylic acids **53–55**, the sodium salt **56**, and the ethyl esters **51** and **52** were prepared by previously reported procedures.[14]

4,5-Dihydro-4-oxo-2-(phenylamino)-3-furancarboxylic Acid Sodium Salt (45). 4,5-Dihydro-4-oxo-2-(phenylamino)-3-furancarboxylic acid ethyl ester (**29**) (10.13 g, 41 mmol) was added in one portion to a solution of sodium carbonate monohydrate (9.92 g, 80 mmol) in 100 mL of water. Steam was then passed through the mixture until the ester completely dissolved (1–2 h). The reaction mixture was filtered while still hot. Cooling of the filtrate (ice–water) provided the crystalline sodium salt. Recrystallization of the latter in a minimum amount of water gave 4.53 g of pure **45**, mp >220 °C. IR (KBr): 3270, 3210, 2935, 1690, 1635, 1577, 1540, 1465, 1438, 1313, 1245, 1060, 1041, 1024, 765, and 718 cm^{-1}. ^1H NMR ($CDCl_3$–DMSO-d_6): δ 10.51 (s, 1 H), 7.78–6.73 (m, 5 H), 4.20 (s, 2 H). Anal. ($C_{11}H_8NNaO_4$) C, H, N, Na.

***N*-Phenyl-4,5-dihydro-4-oxo-2-(phenylamino)-3-furancarboxamide (46).** Under a nitrogen atmosphere, a solution of bromine (1.16 mL, 22.59 mmol) in 17 mL of acetic acid containing a small crystal of iodine was added over a period of 2.5 h to a solution of acetoacetanilide (4.0 g, 22.57 mmol) in 12 mL of acetic acid at room temperature (cooling with cold water). The reaction mixture was stirred at room temperature for 3 h and then poured into 100 mL of ice-cold water, and the crude solid product was filtered off. Following crystallization from ethanol, 3.30 g of 4-bromoacetoacetanilide were obtained, mp 130–132 °C [lit.[34] mp 136–138 °C (benzene)].

Under a nitrogen atmosphere, sodium hydride (97%; 0.633 g, 25.61 mmol) was weighed into a dry reaction vessel and covered with 55 mL of anhydrous dioxane. A solution of phenyl isocyanate (1.39 mL, 12.81 mmol) in 5 mL of anhydrous dioxane was then added over a 5–10-min period at room temperature (cooling with cold water). Next, 4-bromoacetoacetanilide (3.28 g, 12.8 mmol) was added portionwise over a 20-min period. The reaction mixture was stirred at 20–25 °C for 5 h, filtered under nitrogen atmosphere, and poured into 300 mL of ice-cold water. After acidifying (pH 5–6) with concentrated hydrochloric acid, the crude product was filtered off and crystallized from ethanol to yield 2.8 g of amide **46**, mp 182–184 °C. IR (KBr): 3230, 3135, 1653, 1617, 1589, 1578, 1548, 1505, 1493, 1310, 1067, 772, and 754 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): 10.78 (br s, 1 H), 9.82 (br s, 1 H), 7.97–7.10 (m, 10 H), 4.81 (s, 2 H); (200 MHz, $CDCl_3$) 10.72 (s, 1 H, NH), 9.72

(s, 1 H, NH), 7.69–7.00 (m, 10 H, aromatic), 4.78 (s, 2 H, CH_2). Anal. ($C_{17}H_{14}N_2O_3$) C, H, N.

N-(4-Chlorophenyl)-4,5-dihydro-4-oxo-2-[(4-chlorophenyl)amino]-3-furancarboxamide (47). Under a nitrogen atmosphere, a solution of bromine (2.56 mL, 0.05 mol) in 20 mL of acetic acid was added over a period of 2 h to a solution of 4′-chloroacetoacetanilide (10.58 g, 0.05 mol) in 40 mL of acetic acid at room temperature (cooling with cold water). The reaction mixture was stirred for 18 h and then poured into 200 mL of ice-cold water to precipitate the crude 4-bromo-4′-chloroacetoacetanilide. The latter was recrystallized from chloroform to furnish 11.55 g (80%) of pure product melting at 116–118 °C [lit.[35] mp 125–126 °C (chloroform)].

The *N*-(4-chlorophenyl)-4,5-dihydro-4-oxo-2-[(4-chlorophenyl)amino]-3-furancarboxamide (47) was obtained by a procedure similar to the one described in the synthesis of **46**, starting from 4-bromo-4′-chloroacetoacetanilide (10.17 g, 35 mmol) and 4-chlorophenyl isocyanate (4.57 mL, 35 mmol); yield 8.30 g, mp 241–243 °C (dioxane). IR (KBr): 3210, 3160, 3105, 1655, 1615, 1585, 1570, 1539, 1500, 1483, 1400, 1302, 1242, 1203, 1090, 1067, 830, and 810 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$–TFA-*d*): δ, 7.45 (d, 8 H, *J* = 3.0 Hz), 5.10 (s, 2 H); (200 MHz, $CDCl_3$–TFA-*d*) 10.55 (s, 1 H, NH), 9.75 (s, 1 H, NH), 7.89–6.89 (m, 8 H, aromatic), 5.06 (s, 2 H, CH_2). Anal. ($C_{17}H_{12}Cl_2N_2O_3$) C, H, Cl, N.

N-(4-Methoxyphenyl)-4,5-dihydro-4-oxo-2-[(4-methoxyphenyl)amino]-3-furancarboxamide (48). 4-Bromo-4′-methoxyacetoacetanilide was prepared by a procedure similar to that described for the preparation of 4-bromo-4′-chloroacetoacetanilide (see the preceding experiment) from 4′-methoxyacetoacetanilide (10.57 g, 0.05 mmol) and bromine (2.56 mL, 0.05 mol); yield 6.80 g (48%), mp 131–134 °C (ethanol) [lit.[35] mp 135 °C (ethanol)].

Under a nitrogen atmosphere, a solution of triethylamine (6.02 mL, 43.2 mmol) in 30 mL of anhydrous methylene chloride was added over a period of 2 h to a solution of 4-bromo-4′-methoxyacetoacetanilide (6.18 g, 21.6 mmol) and 4-methoxyphenyl isocyanate (3.22 mL, 24.84 mmol) in 120 mL of methylene chloride at 5–10 °C. The reaction mixture was stirred at 5–10 °C for 1 h and at room temperature for 18 h. The solvent was evaporated under reduced pressure, leaving a residual solid, which was stirred in petroleum ether for 1 h, filtered off, and stirred in 1 N hydrochloric acid for 30 min. The crude **48** was recrystallized from tetrahydrofuran to afford 5.0 g of pure product, melting at 202–204 °C. IR (KBr): 3250, 3170, 2945, 2830, 1665, 1639, 1597, 1589, 1570, 1538, 1506, 1483, 1247, 1073, 1030, and 828 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 10.62 (br s, 1 H), 9.60 (br s, 1 H), 7.63–6.72 (m, 8 H), 4.77 (s, 2 H), 3.82 (s, 6 H); (200 MHz, $CDCl_3$) 10.57 (s, 1 H, NH), 9.59 (s, 1 H, NH), 7.76–6.73 (m, 8 H, aromatic), 4.76 (s, 2 H, CH_2), 3.62 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3). Anal. ($C_{19}H_{18}N_2O_5$) C, H, N.

N-(2-Methoxyphenyl)-4,5-dihydro-4-oxo-2-[(2-methoxyphenyl)amino]-3-furancarboxamide (49). The 4-bromo-2′-methoxyacetoacetanilide[39] was made by bromination of 2′-methoxyacetoacetanilide (10.57 g, 0.05 mol) with bromine (2.56 mL, 0.05 mol) in acetic acid as described in the preparation of 4-bromo-4′-chloroacetoacetanilide (see the synthesis of **47**); yield 4.70 g, mp 79–81 °C (95% ethanol). Anal. ($C_{11}H_{12}BrNO_3$) C, H, N.

Compound **49** was obtained from 4-bromo-2′-methoxyacetoacetanilide (6.73 g, 23.52 mmol) and 2-methoxyphenyl isocyanate (3.65 mL, 27.45 mmol) by a procedure similar to that described in the synthesis of **48**; yield 4.50 g, mp 172–174 °C (ethanol). IR (KBr): 3260, 3225, 2920, 1650, 1615, 1694, 1580, 1540, 1480, 1455, 1248, 1115, 1061, 1020, and 740 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 11.10 (br s, 1 H), 10.26 (br s, 1 H), 8.68–8.37 (m, 1 H), 7.92–7.69 (m, 1 H), 7.37–6.87 (m, 6 H), 4.80 (s, 2 H), 3.98 (s, 3 H), 3.96 (s, 3 H). Anal. ($C_{19}H_{18}N_2O_5$) C, H, N.

N-(2-Methylphenyl)-4,5-dihydro-4-oxo-2-[(2-methylphenyl)amino]-3-furancarboxamide (50). The 4-bromo-2′-methylacetoacetanilide was prepared in 70% yield (9.40 g) from 2′-methylacetoacetanilide (9.56 g, 0.05 mmol) and bromine (2.56 mL, 0.05 mol) by a procedure similar to that of 4-bromo-4′-chloroacetoacetanilide (see the synthesis of **47**); mp 85–86 °C (ethyl acetate–hexane) [lit.[36] mp 85 °C (ethanol)].

Derivative **50** was synthesized by a procedure similar to that of compound **48**, starting from 4-bromo-2′-methylacetoacetanilide (9.24 g, 34.2 mmol) and 2-methylphenyl isocyanate (4.88 mL, 39.36 mmol); yield 5.70 g, mp 188–190 °C (95% ethanol). IR (KBr): 3220, 3135, 3010, 2962, 1656, 1624, 1595, 1580, 1545, 1502, 1470, 1450, 1301, 1251, 1065, 780, and 748 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$–DMSO-d_6): δ 10.72 (br s, 1 H), 9.77 (br s, 1 H), 8.32–8.06 (m, 1 H), 7.77–7.02 (m, 3 H), 4.83 (s, 2 H), 2.43 (s, 3 H), 2.41 (s, 3 H); (200 MHz, DMSO-d_6) 10.60 (s, 1 H, NH), 9.80 (s, 1 H, NH), 8.28–8.02 (m, 1 H, aromatic), 7.73–6.73 (m, 7 H, aromatic), 4.86 (s, 2 H, CH_2), 2.39 (s, 3 H, CH_3), 2.35 (s, 3 H, CH_3). Anal. ($C_{19}H_{18}N_2O_3$) C, H, N.

4-Bromo-4′-methylacetoacetanilide (22). Under a nitrogen atmosphere, a solution of 2,2,6-trimethyl-4*H*-1,3-dioxen-4-one (95%; 7.48 g, 0.05 mol) and *p*-toluidine (5.36 g, 0.05 mol) in 10 mL of toluene was heated at 110–115 °C for 3.5 h.[37] Following the removal of the solvent under reduced pressure and recrystallization of the crude product from carbon tetrachloride, 3.10 g (32%) of pure *N*-(4-methylphenyl)-3-oxobutanamide was obtained, mp 92–94 °C (lit.[38] mp 93 °C).

By the method of Mallams and Israelstam,[34] *N*-(4-methylphenyl)-3-oxobutanamide (4.25 g, 22.22 mmol) was brominated with bromine (3.55 g, 22.24 mmol) to furnish 4.17 g (70%) of **22**, mp 146–147 °C (ethanol) [lit.[35] mp 147 °C (ethanol)].

Attempted Preparation of N-(4-Methylphenyl)-4,5-dihydro-4-oxo-2-(phenylamino)-3-furancarboxamide (20). The synthesis of **20** was attempted, via the procedure of Ibrahim et al.[26] as follows:

Under a nitrogen atmosphere, phenyl isocyanate (**12**) (0.22 mL, 2.036 mmol) was added in one portion to a suspension of 97% sodium hydride (0.1 g, 4.065 mmol) in 10 mL of anhydrous dioxane at 15–20 °C (cooling with cold water). After the mixture was stirred for 10 min, solid 4-bromo-4′-methylacetoacetanilide (**22**) (0.55 g, 2.036 mmol) was added portionwise over a period of 35 min, at 15–20 °C. The reaction mixture was stirred for 15 min at 15–20 °C and for 5 h at 20–25 °C and then filtered. The filtrate was poured into 25 mL of ice water and acidified with concentrated hydrochloric acid (pH 5). The resulting tan precipitate was filtered off, rinsed with water, and sucked dry on the filter. Recrystallization from ethanol provided a crystalline one-spot product on TLC (ethyl acetate–hexane, 1:1) (0.40 g, 63.7%). A sample of the product when put through an analytical HPLC was shown to be a 1:1 mixture of two regioisomers (**20** and **21**). ^1H NMR (200 MHz, $CDCl_3$): δ 10.77 (s, 0.5 H, NH), 10.63 (s, 0.5 H, NH), 9.73 (s, 0.5 H, NH), 9.65 (s, 0.5 H, NH), 7.70–7.06 (m, 9 H, aromatic), 4.80 (s, 1 H, CH_2), 4.79 (s, 1 H, CH_2), 2.37 (s, 1.5 H, CH_3), 2.34 (s, 1.5 H, CH_3).

Active Anaphylaxis in Rats. Sprague–Dawley rats were immunized with a single intraperitoneal injection of 500 μg of a bovine serum albumin(BSA)–alum complex. Fourteen days later, a local anaphylaxis was elicited by a subcutaneous injection (0.1 mL) of 100 μg of BSA in the intraplantar surface of the right hindpaw. Ninety minutes following this challenge, the paw volume was determined by using the mercury plethysmometer. The difference in mean paw volume between the saline and drug-treated groups of animals were compared. The drug at a dose of 100 mg/kg was administered to the rats intraperitoneally 1 h prior to the challenge. Theophylline (90 mg/kg) was the positive reference utilized in the assay. The difference in paw volume of the individual rats per group were averaged to give the value (mean ± standard deviation) at 0 and 90 min. The change in the volume at the 90-min time increment was recorded for each group. The percent of inhibition of edema was calculated as follows: [[Δ(control group volume) − Δ(test group volume)]/Δ(control group volume)] × 100 = percent inhibition of edema.

Dermal Vascular Permeability Assay in Rats. Sprague–Dawley rats were injected intravenously with 1.0 mL of a 0.5% solution of Evan's blue dye. Ten minutes later, the animals were intradermally injected (0.1 mL) on the back with 0.1 μg of serotonin, 1.0 μg of bradykinin, and 2.0 μg of histamine. Five minutes following the injection of the allergic mediators, the rats were sacrificed, the skin was reflected, and the mean diameter of each of the blue wheals was determined. The drug (100 mg/kg) was administered intraperitoneally 1 h prior to the administration of the dye. The difference in the mean wheal size between the saline and the drug-dosed groups of rats was then determined. Cyproheptadine (1 mg/kg) was included in each assay as a positive standard drug. The wheals were measured with vernier calipers. Any measurement under 5 mm in either direction was regarded

as no reaction, i.e. zero measurement. The mean of each wheal was calculated by multiplying two measurements (in two perpendicular directions for each wheal) together and taking the square root of the product.

Acknowledgment. We thank Brian W. Soper for the technical assistance. The help of William J. Kuipers in obtaining the 200-MHz ^1H NMR spectra is gratefully acknowledged.

Registry No. 4, 638-07-3; 10, 4949-44-4; 11, 36187-69-6; 12, 103-71-9; 13, 115320-88-2; 14, 115320-89-3; 15, 115320-90-6; 16, 51287-60-6; 17, 115320-91-7; 18, 115320-92-8; 19, 115320-93-9; 20, 96286-63-4; 21, 115320-94-0; 22, 23976-42-3; 26, 58337-19-2; 27, 115320-95-1; 28, 36717-55-2; 29, 58337-16-9; 30, 58337-23-8; 31, 115320-96-2; 32, 106212-55-9; 33, 115320-97-3; 34, 106212-56-0; 35, 115320-98-4; 36, 106212-54-8; 37, 58337-17-0; 38, 115320-99-5; 39, 106212-58-2; 40, 115321-00-1; 41, 115321-01-2; 42, 115321-02-3; 43, 58337-18-1; 44, 115321-03-4; 45, 115321-04-5; 46, 115321-05-6; 47, 115321-06-7; 48, 115321-07-8; 49, 115321-08-9; 50, 115321-09-0; 51, 103921-11-5; 52, 103921-12-6; 53, 103921-15-9; 54, 103921-14-8; 55, 103921-16-0; 56, 103921-17-1; MeNCO, 624-83-9; *i*-PrNCO, 1795-48-8; *o*-$OCNC_6H_4OMe$, 700-87-8; *m*-$F_3CC_6H_4NCO$, 329-01-1; *m*-$O_2NC_6H_4NCOp$, 3320-87-4; *p*-ClC_6G_4NCO, 104-12-1; *p*-BrC_6H_4NCO, 2493-02-9; *p*-$OCNC_6H_4CO_2Et$, 30806-83-8; M-MeC_6H_4NCO, 621-29-4; *p*-$MeOC_6H_4NCO$, 5416-93-3; *o*-MeC_6H_4NCO, 614-68-6; ethyl isobutyrylacetate, 7152-15-0; 1-naphthyl isocyanate, 86-84-0; acetoacetanilide, 102-01-2; 4-bromoacetoacetanilide, 1205-74-9; 4′-chloroacetoacetanilide, 101-92-8; 4-bromo-4′-chloroacetoacetanilide, 19359-22-9; 4′-methoxyacetoacetanilide, 5437-98-9; 4-bromo-4′-methoxyacetoacetanilide, 20335-27-7; 2′-methoxyacetoacetanilide, 52405-54-6; 4-bromo-2′-methoxyacetoacetanilide, 52405-54-6; 2′-methylacetoacetanilide, 93-68-5; 4-bromo-2′-methylacetoacetanilide, 23976-47-8; 2,2,6-trimethyl-4*H*-1,3-dioxen-4-one, 5394-63-8; *p*-toluidine, 106-49-0; *N*-(4-methylphenyl)-3-oxobutamide, 2415-85-2.

Renin Inhibitors Containing Hydrophilic Groups. Tetrapeptides with Enhanced Aqueous Solubility and Nanomolar Potency

Mark G. Bock,* Robert M. DiPardo, Ben E. Evans, Roger M. Freidinger, Kenneth E. Rittle, Linda S. Payne, Joshua Boger,† Willie L. Whitter, Bruce I. LaMont, Edgar H. Ulm, Edward H. Blaine, Terry W. Schorn, and Daniel F. Veber

Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486, and Rahway, New Jersey 07065.
Received February 16, 1988

Nineteen tetrapeptides containing statine (Sta) and 4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA) were prepared. Solubility measurements of these compounds were carried out in H_2O and in pH 7.4 phosphate buffer solution, and their partition coefficients were determined in a 1:1 1-octanol/sodium phosphate–citric acid buffer system. The tetrapeptides were tested in vitro for their ability to inhibit porcine, canine, and human plasma renins. Four compounds, **6**, **12**, **14**, and **20**, were potent inhibitors against all renins tested ($IC_{50} = 10^{-9}$ M). Compound **12** was administered orally to dogs and substantially inhibited plasma renin activity for up to 5 h. The addition of polar groups to the C-terminus of Sta- and ACHPA-containing tetrapeptides renders them soluble in aqueous milieu and provides a valuable tool with which to examine the role of the renin–angiotensin system in physiological and pathological circumstances.

A potential alternative to angiotensin converting enzyme (ACE) inhibitors in the treatment of various hypertensive states is an agent that blocks the preceding step in the enzymatic sequence, the renin reaction. Over the past decade, several different categories of renin inhibitors have appeared[1] with one of the most promising types, the renin substrate analogues,[2] achieving potency levels in the nanomolar range. In fact, one of these compounds has been tested in humans and found to lower blood pressure.[3] However, despite encouraging results regarding potency and specificity, important questions remain to be answered before medicinally useful renin inhibitors result that rival the therapeutic profile of ACE inhibitors.

In our analysis of these problems, we concluded that a key issue concerning the efficacy of renin inhibitors relates to their solubility in physiological media. The therapeutic potential of many peptidal renin inhibitors is limited because of their hydrophobic nature and low aqueous solubility. In the past, this shortcoming was remedied by the addition of hydrophilic amino acids and oligopeptides to either terminus of the peptide renin inhibitors.[4] Alternative solubilizing tactics such as employing various aqueous vehicles[5] or derivatization with polysaccharides[6] have also been explored. Nevertheless, even in the most favorable cases, only moderate success has been achieved.

† Rahway, NJ.

(1) (a) Renin antibodies: Romero, J. C.; Hoobler, S. W.; Kozak, T. J.; Warzynski, R. *J. Am. J. Physiol.* **1973**, *225*, 810. Dzau, V. J.; Kopelman, R. I.; Barger, A. C.; Haber, E. *Science (Washington, D.C.)* **1980**, *207*, 1091. (b) Phospholipid renin inhibitors: Sen, S.; Smeby, R. R.; Bumpus, F. M. *Biochemistry* **1967**, *6*, 1572. Miyazaki, M.; Yamamoto, K. *Proc. Soc. Exp. Biol. Med.* **1977**, *155*, 468. (c) Fermentation product pepstatin: Gross, F.; Lazar, J.; Ortho, H. *Science (Washington, D.C.)* **1972**, *175*, 656.

(2) (a) Burton, J.; Poulson, K.; Haber, E. *Biochemistry* **1975**, *14*, 3892. (b) Poulson, K.; Haber, E.; Burton, J. *Biochim. Biophys. Acta* **1976**, *452*, 533. (c) Szelke, M.; Leckie, B. J.; Tree, M.; Brown, A.; Grant, J.; Hallett, A.; Hughes, M.; Jones, D. M.; Lever, A. F. *Hypertension* **1982**, *4*(II), 59. (d) Szelke, M.; Leckie, B. J.; Hallet, A.; Jones, D. M.; Sueiras, J.; Atrash, B.; Lever, A. F. *Nature (London)* **1982**, *299*, 555. (e) Boger, J.; Lohr, N. S.; Ulm, E. H.; Poe, M.; Blaine, E. H.; Fanelli, G. M.; Lin, T.-Y.; Payne, L. S.; Schorn, T. W.; LaMont, B. I.; Vassil, T. C.; Stabilito, I. I.; Veber, D. F.; Rich, D. H.; Bopari, A. S. *Nature (London)* **1983**, *303*, 5912.

(3) (a) Zusman, R.; Christensen, D.; Burton, J.; Nussberger, J.; Dobbs, A.; Haber, E.; Szelke, M.; Donovan, B. *Clin. Res.* **1983**, *31*, 538A. (b) Webb, D. J.; Cumming, A. M. M.; Leckie, B. J.; Lever, A. F.; Morton, J. J.; Robertson, J. I. S. *Lancet* **1983**, 1486.

0022-2623/88/1831-1918$01.50/0