206 (1.8, B + 44), 190 (11.7, B + 28), 164 (64.2, BH_2^+), 163 (24.1, BH^+), 134 (31.7, $BH^+ - CH_3N$), almost identical to isomer **1a**, very similar to carbocyclic puromycin analogues described previously. Anal. ($C_{23}H_{31}N_7O_4 \cdot 2HCl \cdot H_2O$) C, H, N, Cl.

In the same way, starting with pure **4a**, a sample of **1a** was prepared and characterized as its dihydrochloride, giving white granules (50%) after crystallization from *i*-PrOH: mp 220–225 °C dec; $[\alpha]^{23}_{589}$ +31.4°, $[\alpha]^{23}_{436}$ +74.1° (c 0.11, H_2O): UV λ_{max} in nm ($\epsilon \times 10^{-3}$) 269 (20.4) in 0.1 N HCl, 275 (20.7) in H_2O, 276 (21.7) in 0.1 N NaOH; IR (KBr) and mass spectra almost identical to those of 1. Anal. ($C_{23}H_{31}N_7O_4 \cdot 2HCl$) C, H, N, Cl.

It was found most efficient to carry out the near quantitative deblocking on 1:1 mixtures of **4** and **4a**. When the resulting mixture of **1** and **1a** was dissolved in 95% EtOH along with 3 equiv of HCl (as described above) and seeded with a crystal of the dihydrochloride of **1**, the dihydrochloride formed slowly ([α] and mp same as analytical sample) in 33% yield (from the mixture of **1** and **1a**).

Acknowledgment. This investigation was supported by Grants CA13592 and CA23263 from the National Cancer Institute, DHHS.

3-(1*H*-Tetrazol-5-yl)-4(3*H*)-quinazolinone Sodium Salt (MDL 427): A New Antiallergic Agent

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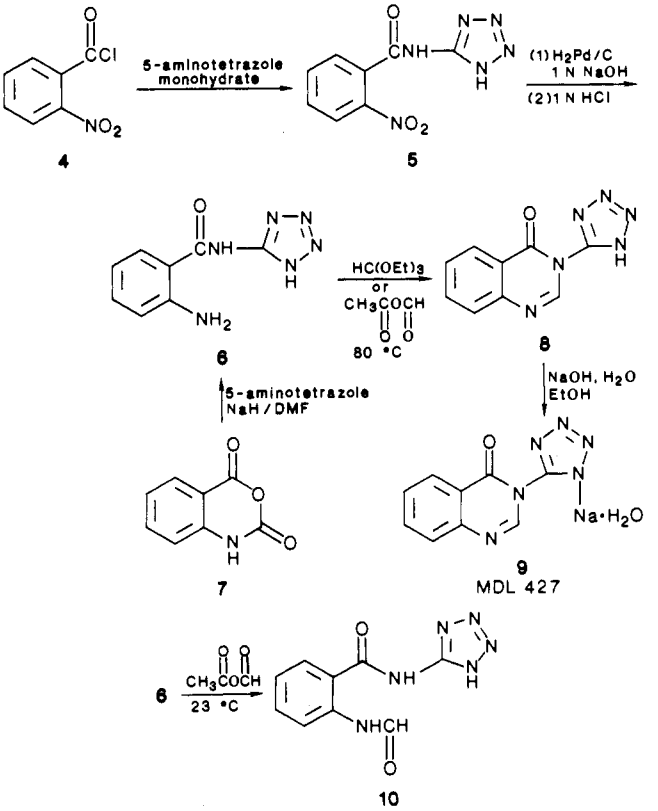
Syntheses for 3-(1*H*-tetrazol-5-yl)-4(3*H*)-quinazolinone sodium salt monohydrate (**9**; MDL 427) and the related formamido compound, 2-(formylamino)-*N*-1*H*-tetrazol-5-ylbenzamide (**10**), are described. Both compounds are active in the rat passive cutaneous anaphylaxis and passive peritoneal anaphylaxis tests. A 94:6 equilibrium mixture of **9** and ionized **10**, respectively, forms in aqueous buffer systems at a pH-dependent rate. In addition, analogues of 3-(1*H*-tetrazol-5-yl)-4(3*H*)-quinazolinone (**8**) bearing substituents on the benzene ring, substituents at the 2-position, and heteroaryl groups at the 3-position other than tetrazole were prepared. These analogue sets demonstrated that an accessible electrophilic center and an acidic functionality were requirements for good antiallergic activity.

The search for a clinically effective, orally active inhibitor of mediator release has been an active[1] and appropriate area of research since the discovery of disodium cromoglycate (DSCG).[2] Some of the antiallergic agents resulting from this effort have displayed activity in clinical settings,[3-8] a finding which provides encouragement for further work in this area.

We recently described a group of 6-oxo-6*H*-imidazo[4,5,1-*ij*]quinolin-4- and -5-carboxylic acid esters and related quinolines, some of which were active as mediator release inhibitors in our animal model.[9] We found that while imidazoquinoline **1** was active by both intraperitoneal and oral routes, the related ester **2** bearing the carboalkoxy group at the 4-position rather than the 5-position was

Scheme I



inactive by either route. This observation was a factor in our design of a series of 3-tetrazolylquinazolinones (general

(1) Schwender, C. F. *Drugs Future* **1983**, *8*, 699.
(2) Cox, J. S. C. G.; Beach, J. E.; Blair, A. M. J. N.; Clarke, A. J.; King, J.; Lee, T. B.; Loveday, D. E. E.; Moss, G. F.; Orr, T. S. C.; Ritchie, J. T.; Sheard, P. *Adv. Drug Res.* **1970**, *5*, 115.
(3) Clinically active candidates include ketotifen,[4] lodoxamide ethyl,[5] AH-7725,[6] BRL-10833,[7] and proxicromil.[8]
(4) (a) Haahtela, T. *Ann. Allergy* **1978**, *41*, 345. (b) Pauwels, R.; Lamont, H.; van der Straeten, M. *Clin. Allergy* **1978**, *8*, 289. (c) Gobel, P. *J. Int. Med. Res.* **1978**, *6*, 79. (d) *Br. J. Clin. Pharmacol.* **1979**, *8*, 65.
(5) Katcher, M. L.; Reed, C. E. *J. Allergy Clin. Immunol.* **1980**, *66*, 223.
(6) Church, M. K. *Med. Actual., Drugs Today (Barcelona)* **1978**, *14*, 281.
(7) (a) Lenney, W.; Milner, A. D.; Tyler, R. M. *Br. J. Dis. Chest* **1978**, *72*, 225. (b) Lumb, E. M.; McHardy, G. J. R.; Kay, A. B. *Br. J. Clin. Pharmacol.* **1979**, *8*, 65.
(8) Norman, P. S. *J. Allergy Clin. Immunol.* **1980**, *65*, 87.
(9) Peet, N. P.; Baugh, L. E.; Sunder, S.; Lewis, J. E. *J. Med. Chem.* **1985**, *28*, 298.

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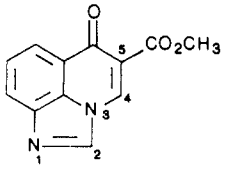
structure 3) as potential mediator release inhibitors.[10] The



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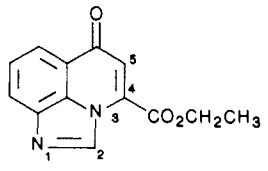
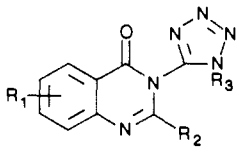


2



3

parent compound of this series, namely 3-(1*H*-tetrazol-5-yl)-4(3*H*)-quinazolinone sodium salt monohydrate (**9**; MDL 427), is a potent, orally active mediator release inhibitor of good duration in several animal models. Quinazolinone **9** is presently undergoing evaluation in several clinical trials.

Chemistry.[11] The synthesis of **9** is shown in Scheme I. Treatment of *o*-nitrobenzoyl chloride (**4**) with 5-aminotetrazole monohydrate provided a 79% yield of tetrazolamide **5**. Reduction of **5** to the corresponding amino compound **6** (74% yield) was effected by catalytic hydrogenation. It was most convenient to take advantage of the acidic nature of the tetrazolyl group and perform this reduction in 1 N sodium hydroxide. Cyclization of **6** with triethyl orthoformate gave an 86% yield of 3-(1*H*-tetrazol-5-yl)-4(3*H*)-quinazolinone (**8**), which was converted to its corresponding sodium salt monohydrate **9** with ethanolic sodium hydroxide.

Tetrazolamide **6** could alternatively be prepared by treating a dimethylformamide solution of 5-aminotetrazole sodium salt with isatoic anhydride (**7**), at elevated temperature. Although this route to **6** was shorter, the yield for this transformation was only 20%.

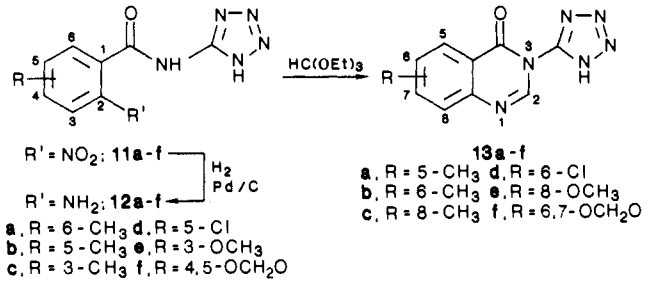
When tetrazolamide **6** was treated with acetic formic anhydride at ambient temperature, a 54% yield of 2-(formylamino)-*N*-1*H*-tetrazol-5-ylbenzamide (**10**)[12] resulted. However, when **6** was treated with the same formylating agent at 80 °C for 40 min, formylation was succeeded by cyclization to give quinazolinone **8** in 67% yield.

Scheme II shows the preparation of benzo-substituted derivatives of **8**. Compounds **13a–f** were prepared in the same manner as was **8**, through intermediate nitro (**11a–f**) and amino (**12a–f**) compounds. The substituted 2-nitrobenzoic acids used as starting materials were either commercially available or were synthesized. For instance, 4,5-(methylenedioxy)-2-nitrobenzoic acid, the starting material for the preparation of **13f**, was prepared by potassium permanganate oxidation of 6-nitropiperonal.

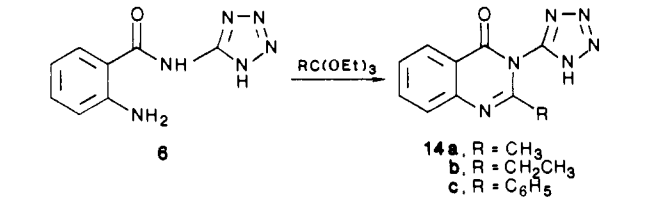
2-Substituted derivatives of **8** were prepared by treating tetrazolamide **6** with additional ortho esters. Scheme III shows the preparation of the 2-methyl, 2-ethyl, and 2-phenyl derivatives of **8**, i.e., compounds **14a**, **14b**, and **14c**, respectively.

The preparation of compounds bearing heterocyclic substituents other than the tetrazolyl moiety at the 3-

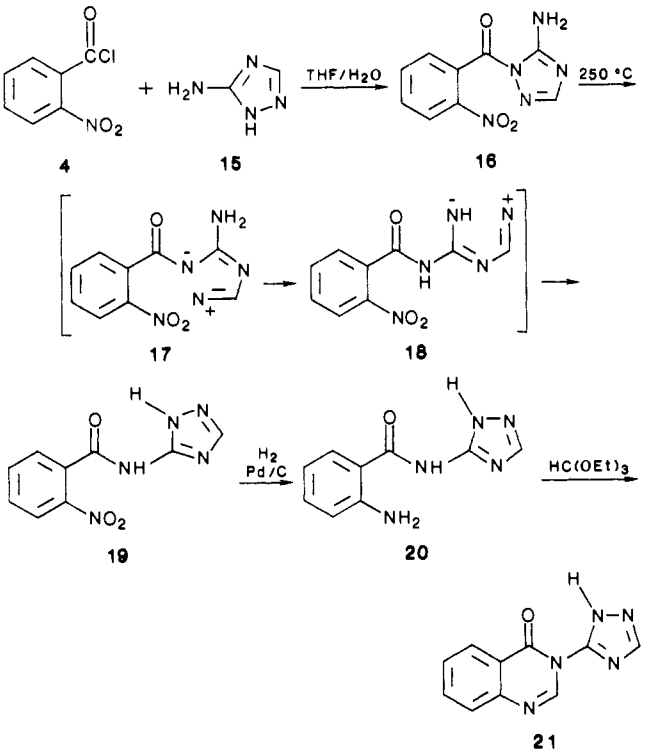
Scheme II



Scheme III



Scheme IV



position is shown in Schemes IV and V. Triazole **21**, pyrazole **25**, and triazole **29**, which are all structurally similar to **8**, were selected for study on the basis of their systematic decrease in acidity with respect to **8**.

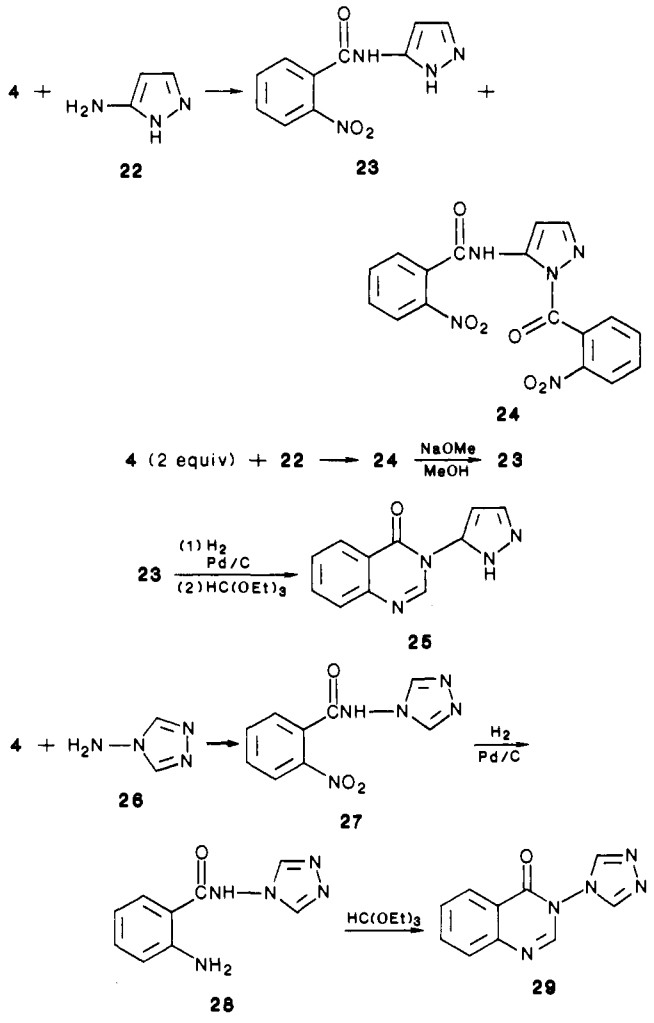
Acylation of 3-amino-1,2,4-triazole (**15**) with 2-nitrobenzoyl chloride (**4**) yielded a ring-acylated product, namely **16**, rather than the amino-acylated product which we required. However, we found that benzoyltriazole **16** could be thermally isomerized to (benzoylamino)triazole **19**. We feel that this rearrangement proceeds via zwitterion **17**, which results from rupture of the 1,2-nitrogen–nitrogen bond of **16**. Prototropic rearrangement of **17**, followed by rotation about the 3,4-carbon–nitrogen bond with developing single-bond character would give zwitterion **18**, which could then close to **19**. Thus, **16** is the kinetic product from the acylation of **15** with **4**, while **19** is thermodynamically more stable. Catalytic reduction of **19** gave amino compound **20**, which was cyclized to 3-(2*H*-1,2,4-triazol-3-yl)-4(3*H*)-quinazolinone (**21**). Precedent

(10) Peet, N. P.; Sunder, S. *3-(1H-Tetrazol-5-yl)-4(3H)-quinazolinones*; U.S. Patent 4 419 357, Dec 6, 1983.

(11) All compounds in Scheme I gave satisfactory elemental analyses. The IR, NMR, and mass spectra of these compounds were consistent with structure.

(12) Peet, N. P.; Sunder, S. *2-(Formylamino)-N-1H-tetrazol-5-ylbenzamide and Use as Antiallergic Agent*; U.S. Patent 4 567 193, Jan 28, 1986.

Scheme V



for the rearrangement of **16** to **19** is the reaction of 3-amino-1,2,4-triazole with phenyl isothiocyanate.[13] While a mixture of 5-amino-1-[(phenylamino)(thiocarbonyl)]-1,2,4-triazole and *N*-1,2,4-triazol-3-yl-*N'*-phenylthiourea resulted, the former could be thermally rearranged to the latter. Likewise, we[14] and others[15] have shown isocyanates will react with 5-aminotetrazole at the 4-position and undergo subsequent thermal rearrangement to 5-(carbamoylamino)tetrazoles.

The preparation of 3-(2*H*-pyrazol-3-yl)-4(3*H*)-quinazolinone (**25**) is shown in Scheme V. In this case, acylation of 3-aminopyrazole with **4** gave *N*-2*H*-pyrazol-3-yl-2-nitrobenzamide (**23**), as well as diacylated product **24**. We found that treatment of **24** with 1 equiv of sodium methoxide efficiently converted it to **23**. In fact, in later preparations it was most convenient to prepare **23** from **24**; treatment of **22** with 2 equiv of **4** gave only **24**, which avoided a separation problem. Catalytic hydrogenation of **23** gave the corresponding amino compound, which was treated directly with triethyl orthoformate to give pyrazole **25**.

The bottom of Scheme V shows the preparation of 3-(1,2,4-triazol-4-yl)-4(3*H*)-quinazolinone (**29**). Acylation of 4-amino-1,2,4-triazole with **4** cleanly gave nitro compound **27**, which was catalytically hydrogenated to the corresponding amino compound **28** and cyclized with triethyl orthoformate to give **29**.

(13) Kubota, S; Horie, K.; Misra, H. K.; Toyooka, K.; Uda, M.; Shibuya, M.; Terada, H. *Chem. Pharm. Bull.* **1985**, *33*, 662.
(14) Peet, N. P., submitted for publication in *J. Heterocycl. Chem.*
(15) Denny, G. H.; Cragoe, E. J.; Rooney, C. S. *J. Org. Chem.* **1980**, *45*, 1662.

Table I. Biological Activity of Quinazolinone **9** and Related Compounds

compd	rat PCA test[a] % inhibn ± SE ip[d]	rat PCA test[a] % inhibn ± SE po[e]	rat PCA test[a] ED_{50},[b] mg/kg, iv[f]	rat PPA test IC_{50},[c] μg/kg, ip[f]
6[g]	59 ± 10	7 ± 7[h]	10.5 (8.3–13.3)	10.5 (7.8–16.0)
8	100 ± 0	89 ± 5		
9	93 ± 7	97 ± 2	0.2 (0.1–0.3)	0.3 (0.2–0.7)
10[g]	89 ± 2	91 ± 6		
DSCG	100 ± 2	0 ± 0[h]	0.7 (0.6–0.9)	1.6 (0.8–3.0)

[a] All activities were significant at the $p < 0.001$ level unless otherwise stated. [b] ED_{50} is the dose required to limit the reaction to 50% of control values. [c] IC_{50} is the concentration that reduced the amount of histamine released to 50% of control values. [d] Animals dosed at 60 mg/kg. [e] Animals dosed at 100 mg/kg. [f] Parenthetical numbers represent 95% confidence limits. [g] This compound was tested as the extemporaneously prepared sodium salt. [h] Not significant.

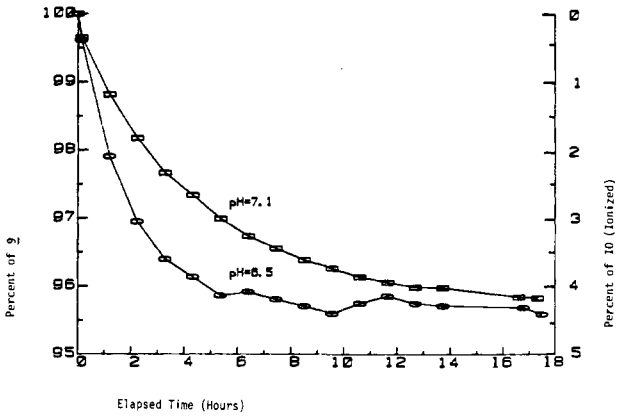


Figure 1. Equilibration of **9** in aqueous buffers.

Pharmacological Results and Discussion

The preclinical evaluation of quinazolinone **9** and the evaluation of related compounds included assessment of their activity in IgE-mediated immune reactions. The results are listed in Table I. Compound **9** inhibits passive cutaneous anaphylaxis (PCA) in the rat when administered by parenteral and oral routes. It has an intravenous ED_{50} in this assay of 0.2 mg/kg compared to an intravenous ED_{50} of 0.7 mg/kg for disodium cromoglycate. The compound has an oral ED_{50} of 1.5 mg/kg in the same model while DSCG has no appreciable oral activity. Peak activity was observed within 5 min of oral administration, but appreciable activity persisted for several hours. In contrast, the activity of tetrazole **8** diminished to a negligible level by 1 h. A possible explanation for this discrepancy is that when **9** is converted to **8** in the acidic medium of the stomach, it is deposited as a microprecipitate and is thus more bioavailable than the crystalline quinazolinone **8**.

To verify that the observed inhibition of the PCA was due to the inhibition of mediator release, the compounds were also tested for their ability to inhibit histamine release from the mixed population of cells in the peritoneal cavity of rats passively sensitized with IgE antibodies to ovalbumin (passive peritoneal anaphylaxis (PPA)). In this system, compound **9** has an IC_{50} of 0.3 μg/mL while DSCG has an IC_{50} of 1.6 μg/mL.

When compound **9** was placed in solution, an equilibrium between **9** and the water addition product **10** was achieved. The equilibrium mixture in the pH 6–9 range consists of 96% **9** and 4% **10** (ionized). Figure 1 shows

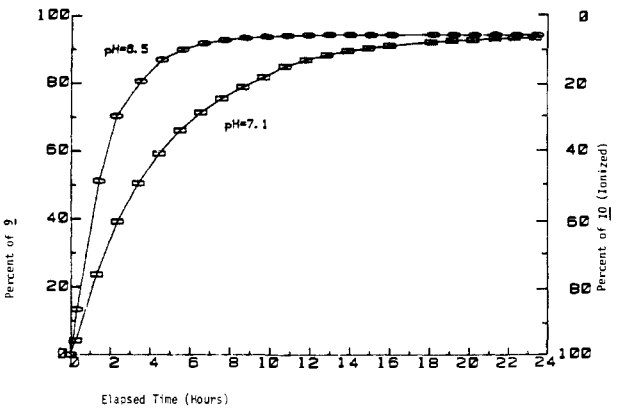
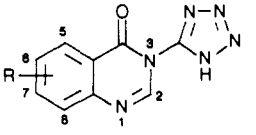


Figure 2. Equilibration of **10** in aqueous buffers.

Table II. Biological Activity of Benzo-Substituted Quinazolinones



compd	R	ip rat PCA test,[a] % inhibn ± SE[b]
8	H	100 ± 0
13a	5-CH_3	80 ± 12
13b	6-CH_3	100 ± 0
13c	8-CH_3	64 ± 11
13d	6-Cl	89 ± 11
13e	8-OCH_3	44 ± 8
13f	6,7-OCH_2O	90 ± 10

[a] Animals dosed at 60 mg/kg. [b] All activities were significant at the $p < 0.01$ level.

this developing equilibrium, as approached from pure **9**, at pH values of 6.5 (similar to that of urine) and 7.1 (similar to that of plasma). The equilibrium could also be approached from pure **10** (ionized) as well, as shown in Figure 2 at the same two pH values. Percentages of **9** and **10** in Figures 1 and 2 were determined by HPLC. A pH-dependent equilibrium similar to that displayed by **9** and **10** has been demonstrated for 5-aza-2′-deoxycytidine, a nucleoside antimetabolite, and the related formamide resulting from hydration.[16]

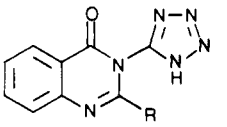
The biological data (Table I) did not allow us to distinguish between the activities of **9** and **10**. This may partly be due to their propensity to equilibrate, although the speed of this equilibration would suggest that equilibrium had not been attained during the course of the experiment. Amine **6** displayed negligible activity. However, since we determined that **6** was a decomposition product of both **9** and **10** at low pH values, we examined its biological activity as a potential metabolite.

Table II shows the ip rat PCA data for a limited number of benzo-substituted analogues of **8**. The activity of the 6-methyl compound (**13b**) is indistinguishable from **8** but better than the 5-methyl (**13a**) or 8-methyl (**13c**) compounds. The 8-substituted compounds (**13c** and **13e**) are the least active, which may indicate an unfavorable steric interaction at the site of activity.

The effect of substitution at the 2-position is seen from the compounds of Table III. All of the 2-substituted compounds are decidedly less active than **8**, which suggests the need for an accessible electrophilic center for interaction at the site of activity.

(16) Lin, K.; Momparler, R. L.; Rivard, G. E. *J. Pharm. Sci.* **1981**, *70*, 1228.

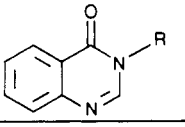
Table III. Biological Activity of 2-Substituted Quinazolinones



compd	R	ip rat PCA test,[a] % inhibn ± SE[b]
8	H	100 ± 0
14a	CH_3	22 ± 8
14b	CH_3CH_2	41 ± 12
14c	C_6H_5	27 ± 11

[a] Animals dosed at 60 mg/kg. [b] All activities were significant at the $p < 0.01$ level.

Table IV. Biological Activity of 3-Heteroaryl Quinazolinones



compd	R	ip rat PCA test,[a] % inhibn ± SE
8		100 ± 0[b]
21		75 ± 15[b]
25		5 ± 5[c]
29		0 ± 0[c]

[a] Animals dosed at 60 mg/kg. [b] Activity was significant at the $p < 0.01$ level. [c] Not significant.

Table IV contains a series of 3-heteroarylquinazolinones arranged in order of decreasing acidity. It is apparent that an acidic moiety is necessary for activity and that activity decreases dramatically with decreasing acidity.

In summary, we have described the chemistry and antiallergic pharmacology of our novel mediator release inhibitor MDL 427 (**9**). In addition, we have developed structure–activity relationships for tetrazole **8** and its analogues. Requirements for good antiallergic activity in this series include an accessible electrophilic center and acidic functionality.

Experimental Section

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer Model 727B spectrophotometer, NMR spectra with Varian T-60 and EM-360A spectrometers and a Perkin-Elmer R-32 (90 MHz) spectrometer, and MS at 70 eV with a Finnigan GC/MS Model 4023 (electron impact and chemical ionization) mass spectrometer. Combustion analyses fell within ±0.4% of the calculated values.

***N*-1*H*-Tetrazol-5-yl-2-nitrobenzamide (5).** To a solution of 5-aminotetrazole monohydrate (10.3 g, 0.100 mol) in tetrahydrofuran (300 mL) and water (15 mL) was added 2-nitrobenzoyl chloride (9.30 g, 50.0 mmol). After 30 min the solution was diluted with water (200 mL) and stored in the refrigerator. After 72 h the white solid was collected, washed with water, and dried to give 9.20 g (79%) of **5**: mp 273–274 °C dec (2-methoxyethanol); IR (Nujol) 3300–2400 (NH), 1680 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 13.8 (br s, 1, CONH), 8.38–8.20 (m, 1, C6-H), 8.05–7.75 (7, 3, remaining aromatic); MS (EI), *m/e* 234 (M^+). Anal. ($C_8H_6N_6O_3$) C, H, N.

***N*-1*H*-Tetrazol-5-yl-2-aminobenzamide (6). A. From 5.** A solution of benzamide **5** (19.0 g, 0.100 mol) in 1 N NaOH (100 mL) and EtOH (100 mL) was hydrogenated (5% Pd/C) in a Parr

apparatus. After hydrogen uptake had ceased, the catalyst was removed by filtration and the clear filtrate was acidified with 1 N HCl (110 mL). The resulting white solid was collected and dried to give 15.2 g (74%) of **6**: mp 253–254 °C; IR (Nujol) 3490 and 3380 (NH_2), 3250 (CONH) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.97–7.80 (m, 1 C6-H), 7.40–7.18 (m, 1 , C4-H), 6.90–6.76 (m, 1, C3-H), 6.70–6.48 (m, 1, C5-H); MS (EI), *m/e* 204 (M^+). Anal. ($C_8H_8N_6O$) C, H, N.

B. From Isatoic Anhydride (7). To a solution of 5-aminotetrazole monohydrate (11.3 g, 0.110 mol) in dimethylformamide (100 mL) was added dry sodium hydride (2.64 g, 0.110 mol). After 15 min of stirring, **7** (16.3 g, 0.100 mol) was added and heating was initiated. Gas evolution was noted at 60–80 °C and again at 120 °C. After 5 min at reflux, the mixture was cooled and diluted with water. A small amount of gelatinous solid was removed by filtration, and the filtrate was acidified with 1 N HCl (110 mL). The resulting precipitate was removed by filtration and discarded. The filtrate, upon standing for 2 days, deposited a pale-yellow solid, which was collected and dried to give 4.03 g (20%) of **6**, whose IR spectrum was identical to that of **6** made in part A. When this reaction was repeated using anhydrous 5-aminotetrazole (8.51 g, 0.100 mol), the yield of **6** was 4.15 g (20%).

3-(1*H*-Tetrazol-5-yl)-4(3*H*)-quinazolinone (8). A. From 6 and Triethyl Orthoformate. A mixture of **6** (5.00 g, 24.4 mmol) and triethyl orthoformate (50 mL) was heated at reflux for 15 h.[17] The mixture was cooled, and the precipitate was collected and dried to give 2.70 g (86%) of **8**: mp 260–261 °C (EtOH); IR (Nujol) 1700 (C=O), 1610 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.10 (**br** s, 1, NH), 8.91 (s, 1, C2-H), 8.45–8.15 (m, 1, C5-H), 8.10–7.45 (m, 3, C6-H, C7-H and C8-H); MS (EI), *m/e* 214 (M^+). Anal. ($C_9H_6N_6O$) C, H, N.

B. From 6 and Acetic Formic Anhydride. A solution of **6** (4.70 g, 23.0 mmol) and acetic formic anhydride[18] (4.80 g, 54.5 mmol) in dimethylformamide (40 mL) was heated at 80 °C for 40 min. After 3 h of stirring at ambient temperature, the solution was concentrated and the residue was triturated with water. The resulting solid was collected and dried to give 3.30 g (67%) of **8**, whose IR spectrum was identical to that of **8** made in part A.

3-(1*H*-Tetrazol-5-yl)-4(3*H*)-quinazolinone Sodium Salt Monohydrate (9). A solution of **8** (10.7 g, 50.0 mmol) was slurried in water (20 mL), and 5 N NaOH was added until solution resulted at 65–70 °C. Ethanol (75 mL) was added and the solution was cooled. The resulting white powder was collected and recrystallized ($EtOH$–H_2O)[20] to give 5.89 g (46%) of **9**: mp >300 °C; IR (KBr) 3650–3000 (H_2O), 1685 (C=O), 1660, 1610 cm^{-1}; ^1H NMR (D_2O) δ 8.25 (s, 1, C2-H), 8.10–7.83 (m, 1, C5-H), 7.83–7.30 (m, 3, C6-H, C7-H, C8-H). Anal. ($C_9H_5N_6O_2Na{\cdot}H_2O$) C, H, N.

2-(Formylamino)-*N*-1*H*-tetrazol-5-ylbenzamide (10). To a solution of **6** (4.70 g, 23.0 mmol) in dimethylformamide (30 mL) under a nitrogen atmosphere was added acetic formic anhydride[18] (4.70 g, 53.4 mmol). The precipitate that appeared within a few minutes was collected and washed with ether to give 2.86 g (54%) of **10**: mp 247–248 °C; IR (KBr) 3400–2200, with spikes at 3390 and 3240 (NH), 1725 (C=O), 1650 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 13.38 (br s, 1, NH), 11.20 (**br** s, 1, NH), 8.35 (br s, 1, CHO), 8.20–8.00 (m, 1, C6-H), 7.95–7.70 (m, 1, C3-H), 7.70–7.45 (m, 1, C4-H), 7.40–7.15 (m, 1, C5-H); MS (neg. CI), *m/e* 232 (M^-). Anal. ($C_9H_8N_6O_2$) C, H, N.

6-Methyl-2-nitro-*N*-1*H*-tetrazol-5-ylbenzamide (11a). A mixture of 2-methyl-6-nitrobenzoic acid (25.0 g, 0.138 mol), PCl_5 (28.7 g, 0.138 mol), and cyclohexane (300 mL) was heated at reflux for 1 h. The resulting solution was concentrated, and the concentrate was twice treated with $CHCl_3$ and concentrated to leave 26.7 g (97%) of 2-methyl-6-nitrobenzoyl chloride, which was dissolved in THF (50 mL) and added to a warm solution of 5-aminotetrazole monohydrate (28.0 g, 0.272 mol) in THF (300 mL) and water (15 mL). The solution was concentrated, and the residue was triturated with water to give a white solid, which was collected and dried to yield 32.0 g (96%) of **11a**: mp 230–232 °C; IR (Nujol) 3700–2200 (NH), 1685 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 15.6 (**br** s, 1, NH), 13.6 (**br** s, 1, NH), 8.24–8.07 (m, 1, C6-H), 7.94–7.58 (m, 2, remaining aromatic), 2.40 (s, 3, CH_3); MS (CI), *m/e* 249 ($M^+ + 1$). Anal. ($C_9H_8N_6O_3$) C, H, N.

In the same manner, 2-nitro-*N*-(tetrazolyl)benzamides **11b–f** were prepared.

5-Methyl-2-nitro-*N*-1*H*-tetrazol-5-ylbenzamide (11b): mp 278–279 °C; yield 89%; IR (Nujol) 3300–2300 (NH), 1670 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.6 (**br** s, 1, NH), 9.14 (d, 1, C3-H), 7.67 (s, 1, C6-H), 7.62 (d, 1, C4-H), 2.49 (s, 3, CH_3); MS (CI), *m/e* 249 ($M^+ + 1$). Anal. ($C_9H_8N_6O_3$) C, H, N.

3-Methyl-2-nitro-*N*-1*H*-tetrazol-5-ylbenzamide (11c): mp 262–263 °C dec (DMF–water); yield 97%; IR (Nujol) 3300–2300 (br NH), 1675 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 13.8 (**br** s, 1, NH), 8.65 (br s, 1, NH), 8.00–7.64 (m, 3, aromatic), 2.39 (s, 3, CH_3); MS (CI), *m/e* 249 ($M^+ + 1$). Anal. ($C_9H_8N_6O_3$) C, H, N.

5-Chloro-2-nitro-*N*-1*H*-tetrazol-5-ylbenzamide (11d): mp 268–269 °C (DMF); yield 87%; IR (Nujol) 3350–2300 (NH), 1685 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.8 (br s, 1, NH), 8.26 (d, 1, C3-H), 8.04 (d, 1, C6-H), 8.00–7.93 (m, 1, C4-H); MS (CI), *m/e* 269 ($M^+ + 1$). Anal. ($C_8H_5ClN_6O_3$) C, H, N.

3-Methoxy-2-nitro-*N*-1*H*-tetrazol-5-ylbenzamide (11e): mp 271 °C (DMF); NMR (Me_2SO-d_6) δ 15.9 (very **br** s, 1, NH), 12.9 (very **br** s, 1, NH), 7.90–7.50 (m, 3, aromatic), 3.97 (s, 3, CH_3); MS (CI), *m/e* 265 ($M^+ + 1$). Anal. ($C_9H_8N_6O_4$) C, H, N.

6-Nitro-*N*-1*H*-tetrazol-5-yl-1,3-benzodioxole-5-carboxamide (11f):[21] mp 279 °C dec (DMF–water); yield 81%; IR (Nujol) 3400–2250 (NH), 1690 (C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 13.7 (s, 1, NH), 8.07 (s, 1, aromatic), 7.68 (s, 1, aromatic), 6.63 (s, 2, CH_2); MS (CI), *m/e* 279 ($M^+ + 1$). Anal. ($C_9H_6N_6O_5$) C, H, N.

2-Amino-6-methyl-*N*-1*H*-tetrazol-5-ylbenzamide (12a). A solution of **11a** (7.50 g, 30.2 mmol) in 1 N NaOH (100 mL) containing 5% Pd/C (0.5 g) was treated with hydrogen at 50 psi in a Parr apparatus for 75 min. The catalyst was removed by filtration, and the filtrate was treated with 1 N HCl (100 mL). The resulting white solid was collected and dried to give 1.34 g (20%) of **12a**: mp 253–254 °C dec (DMF–water); IR (Nujol) 3500–2300, with spikes at 3490 and 3400 (NH), 1675 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.06 (t, 1, C4-H), 6.61 (d, 1, aromatic), 6.47 (d, 1, aromatic), 2.19 (s, 1, CH_3); MS (CI), *m/e* 219 ($M^+ + 1$). Anal. ($C_9H_{10}N_6O$) C, H, N.

In the same manner, 2-amino-*N*-(1*H*-tetrazol-5-yl)benzamides **12b–f** were prepared.

2-Amino-5-methyl-*N*-1*H*-tetrazol-5-ylbenzamide (12b): mp 245–246 °C; yield 88%; IR (Nujol) 3500–2800, with spikes at 3470 and 3360, 1660 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.80–7.68 (m, 1, C6-H), 7.23–7.05 (m, 1, C4-H), 6.75 (d, 1, C3-H); MS (EI), *m/e* 218 (M^+). Anal. ($C_9H_{10}N_6O$) C, H, N.

2-Amino-3-methyl-*N*-1*H*-tetrazol-5-ylbenzamide (12c): mp 262–263 °C dec (DMF–water); yield 73%; IR (Nujol) 3500–2200, with spikes at 3470, 3375, and 3250, 1655 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.76 (d, 1, C6-H), 7.23 (d, 1, C4-H), 6.57 (t, 1, C5-H), 2.14 (s, 3, CH_3); MS (CI), *m/e* 219 ($M^+ + 1$). Anal. ($C_9H_{10}N_6O$) C, H, N.

2-Amino-5-chloro-*N*-1*H*-tetrazol-5-ylbenzamide (12d): mp 255° (DMF); yield 73%; IR (Nujol) 3500–2300, with spikes at 3475 and 3375 (NH), 1660 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.96 (d, 1, C6-H), 7.40–7.20 (m, 1, C4-H), 6.85 (d, 1, C3-H); MS (CI), *m/e* 239 ($M^+ + 1$). Anal. ($C_8H_7ClN_6O$) C, H, N.

2-Amino-3-methoxy-*N*-1*H*-tetrazol-5-ylbenzamide (12e): mp 268–269 °C (DMF–water); yield 73%; IR (Nujol) 3450–2800, with spikes at 3410 and 3320, 1660 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.55 (d, 1, C6-H), 7.03 (d, 1, C4-H), 6.60 (t, 1, C5-H),

(17) In larger scale preparations, ethylation of the tetrazole ring was observed when triethyl orthoformate was used neat. To circumvent this side reaction, we used 3 equiv of triethyl orthoformate in a solvent such as 2-methoxyethanol or 1-methoxy-2-propanol.

(18) This reagent was prepared using the procedure of Krimin.[19] The ^1H NMR ($CDCl_3$) spectrum of the reagent, diagnostic of pure material, was as follows: δ 9.07 (s, 1, CH), 2.26 (s, 3, CH_3).

(19) Krimin, L. I. *Org. Synth.* **1970**, *50*, 1.

(20) Recrystallization from 2-propanol–water followed by air-drying consistently affords a 2.5 hydrate.

(21) 6-Nitro-1,3-benzodioxole-5-carboxylic acid, mp 160–163 °C [lit.[22] mp 172 °C], was prepared by oxidation of 6-nitro-1,3-benzodioxole-5-carboxaldehyde with aqueous potassium permanganate in 41% yield.

(22) Jobst; Hesse *Ann.* **1879**, *199*, 70.

3.85 (s, 3, CH_3); MS (CI), m/e 235 ($M^+ + 1$). Anal. ($C_9H_{10}N_6O_2$) C, H, N.

5-Methyl-3-(1*H*-tetrazol-5-yl)-4(3*H*)-quinazolinone (13a). A mixture of **12a** (1.30 g, 5.95 mmol) and triethyl orthoformate (10 mL) was heated at reflux for 1 h. The mixture was cooled, and the solid was collected, washed with ethanol, and dried to give 1.21 g (89%) of **13a**: mp 280–281 °C dec; IR (Nujol) 1695 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.61 (s, 1, C2-H), 7.95–7.35 (m, 4, aromatic and NH), 2.82 (s, 3, CH_3); MS (CI), m/e 229 ($M^+ + 1$). Anal. ($C_{10}H_8N_6O$) C, H, N.

In the same manner, 3-(1*H*-tetrazol-5-yl)-4(3*H*)-quinazolinones **13b–d** were prepared.

6-Methyl-3-(1*H*-tetrazol-5-yl)-4(3*H*)-quinazolinone (13b): mp 285–286 °C (Me_2SO–water); yield 90%; IR (Nujol) 3100–2000 (NH), 1690 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 11.1 (br s, 1, NH), 8.63 (s, 1, C2-H), 8.14–8.03 (m, 1, C7-H), 7.82–7.70 (m, 2, C5-H and C8-H). Anal. ($C_{10}H_8N_6O$) C, H, N.

8-Methyl-3-(1*H*-tetrazol-5-yl)-4(3*H*)-quinazolinone (13c): mp 268 °C dec (DMF); yield 81%; IR (Nujol) 3250 (NH), 1665 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.9 (br s, 1, NH), 8.71 (s, 1, C2-H), 8.20–8.04 (m, 1, C5-H), 7.90–7.73 (m, 1, C7-H), 7.53 (t, 1, C6-H), 2.59 (s, 3, CH_3); MS (CI), m/e 229 ($M^+ + 1$). Anal. ($C_{10}H_8N_6O$) C, H, N.

6-Chloro-3-(1*H*-tetrazol-5-yl)-4(3*H*)-quinazolinone (13d): mp 241 °C dec; yield 63%; ^1H NMR (Me_2SO-d_6) δ 14.8 (br s, 1, NH), 8.70 (s, 1, C2-H), 8.19 (d, 1, C5-H), 8.10–7.75 (m, 2, C7-H and C8-H); MS (CI), m/e 249 ($M^+ + 1$). Anal. ($C_9H_5ClN_6O$) C, H, N.

8-Methoxy-3-(1*H*-tetrazol-5-yl)-4(3*H*)-quinazolinone (13e): mp 260–261 °C dec (DMF); yield 91%; IR (Nujol) 3350–2500 (NH), 1660 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.63 (s, 1, C2-H), 7.90–7.40 (m, 3, aromatic), 3.98 (s, 3, CH_3); MS (CI), m/e 245 ($M^+ + 1$). Anal. ($C_{10}H_8N_6O_2$) C, H, N.

7-(1*H*-Tetrazol-5-yl)-1,3-dioxolo[4,5-*g*]quinazolin-8-(7*H*)-one (13f): mp 280 °C dec (DMF–MeOH); yield 60%; IR (Nujol) 3300–2500 (NH), 1675 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.56 (s, 1, C6-H), 7.53 (s, 1, C9-H), 7.24 (s, 1, C4-H). Anal. ($C_{10}H_6N_6O_3$) C, H, N.

2-Methyl-3-(1*H*-tetrazol-5-yl)-4(3*H*)-quinazolinone (14a). A mixture of **6** (3.80 g, 18.6 mmol), triethyl orthoacetate (3.30 g, 20.3 mmol), and ethanol (50 mL) was heated at reflux. After 18 h the solution was concentrated to a small volume and the crystals that formed were collected to give 3.20 g (75%) of **14a**: mp 225–226 °C; IR (Nujol) 1705 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 10.5 (br s, 1, NH), 8.17 (d, 1, C5-H), 7.95 (t, 1, C7-H), 7.74 (d, 1, C8-H), 7.61 (t, 1, C6-H); MS (CI), m/e 229 ($M^+ + 1$). Anal. ($C_{10}H_8N_6O$) C, H, N.

In the same manner, 3-(1*H*-tetrazol-5-yl)-4(3*H*)-quinazolinones **14b** and **14c** were prepared.

2-Ethyl-3-(1*H*-tetrazol-5-yl)-4(3*H*)-quinazolinone (14b): mp 210–212 °C; yield 69%; IR (Nujol) 1705 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 10.9 (br s, 1, NH), 8.18 (d, 1, C5-H), 7.95 (t, 1, C7-H), 7.77 (d, 1, C8-H), 7.60 (t, 1, C6-H); MS (EI), m/e 242 (M^+). Anal. ($C_{11}H_{10}N_6O$) C, H, N.

2-Phenyl-3-(1*H*-tetrazol-5-yl)-4(3*H*)-quinazolinone (14c): mp 252–253 °C (EtOH); yield 76%; IR (Nujol) 1705 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 11.9 (br s, 1, NH), 8.25 (d, 1, C5-H), 8.05–7.25 (m, 8, remaining aromatic); MS (EI), m/e 290 (M^+). Anal. ($C_{15}H_{10}N_6O$) C, H, N.

3-Amino-2-(2-nitrobenzoyl)-2*H*-1,2,4-triazole (16). To a mixture of **15** (16.8 g, 0.200 mol) and pyridine (100 mL) was added **4** (37.1 g, 0.200 mol) dropwise. The resulting solution was stirred at room temperature for 1 h. The mixture was diluted with water (1 L), and the solid was collected, washed with water, and dried to give 30.9 g (66%) of **16**: mp 234–236 °C (DMF–water); IR (Nujol) 3440, 3290, 3210 and 3120 (NH), 1715 (C=O), 1645 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.35–8.20 (m, 1, H ortho to C=O), 8.00–7.65 (m, 3, remaining benzoyl), 7.47 (s, 1, C5-H); MS (CI), m/e 234 ($M^+ + 1$). Anal. ($C_9H_7N_5O_3$) C, H, N.

2-Nitro-*N*-2*H*-1,2,4-triazol-3-ylbenzamide (19). Aminotriazole **16** (5.30 g, 22.7 mmol) was heated at 250 °C under nitrogen for 5 min. The solid was collected and washed with ethanol to leave 5.10 g (96%) of **19**: mp 275–276 °C dec (DMF–water); IR (Nujol) 3300–2400, with spike at 3230 (NH), 1660 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 13.3 (br s, 1, NH), 8.25–8.05 (m, 1, C6-H), 7.98 (br s, 1, triazole CH), 7.80 (m, 4, remaining aromatic); MS (CI), m/e 234 ($M^+ + 1$). Anal. ($C_9H_7N_5O_3$) C, H, N.

2-Amino-*N*-2*H*-1,2,4-triazol-3-ylbenzamide (20). A mixture of **19** (5.10 g, 21.9 mmol) and MeOH (225 mL) was hydrogenated (5% Pd/C) in a Parr apparatus for 1 h. The solid and catalyst were removed by filtration and extracted with boiling DMF. The extracts were filtered, and the filtrate deposited 2.00 g (45%) of **20** as a yellow solid: mp 294–296 °C (EtOH); IR (Nujol) 3500–2500 (NH), with NH_2 d at 3470 and 3360 and CONH at 3240, 1655 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 13.2 (br s, 1, NH), 7.89 (s, 1, triazole CH), 7.79 (d, 1, C6-H), 7.24 (t, 1, C4-H), 6.78 (d, 1, C3-H), 6.57 (t, 1, C5-H); MS (CI), m/e 204 ($M^+ + 1$). Anal. ($C_9H_9N_5O$) C, H, N.

3-(2*H*-1,2,4-Triazol-3-yl)-4(3*H*)-quinazolinone (21). A mixture of **20** (1.75 g, 8.61 mmol) and triethyl orthoformate (40 mL) was heated at reflux for 3.5 h. The solid was collected, washed with EtOH, and dried to leave 1.76 g (96%) of **21**: mp 284–285 °C (DMF–water); IR (Nujol) 3500–2400 (NH), 1685 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.74 (s, 1, C2-H), 8.42 (s, 1, triazole CH), 8.23 (d, 1, C5-H), 8.10–7.50 (m, 4, remaining aromatic and NH); MS (EI), m/e 213 (M^+). Anal. ($C_{10}H_7N_5O$) C, H, N.

Treatment of 22 with 1 equiv of 4. To a cold solution of **22** (9.97 g, 0.120 mol) in pyridine (100 mL) was added **4** (22.3 g, 0.120 g) dropwise. After stirring at room temperature for 1 h, the solution was diluted with water (800 mL) and the resulting solid was collected and dried to give 18.2 g of solid. The solid was treated with toluene at reflux, and the insoluble material was collected to afford 5.58 g (20%) of 2-nitro-*N*-(1*H*-pyrazol-3-yl)-benzamide (**23**): mp 207–208 °C (EtOH); IR (Nujol) 3460 (NH), 1675 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.3 (br s, 1, pyrazole NH), 11.05 (s, 1, CONH), 8.20–8.00 (m, 1, C6-H), 7.90–7.60 (m, 4, aromatic), 7.59 (d, 1, pyrazole CH); MS (EI), m/e 232 (M^+). Anal. ($C_{10}H_8N_4O_3$) C, H, N.

The filtrate from above deposited 5.72 g (12%) of 2-nitro-*N*-[1-(2-nitrobenzoyl)-1*H*-pyrazol-3-yl]benzamide (**24**): mp 179–180.5 °C; IR (Nujol) 3360 (NH), 1720 (C=O), 1690 (C=O), 1665 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 11.5 (s, 1, NH), 8.61 (d, 1, pyrazole CH), 8.40–7.50 (m, 8, aromatic), 7.14 (d, 1, pyrazole CH); MS (EI), m/e 381 (M^+). Anal. ($C_{17}H_{11}N_5O_6$) C, H, N.

Treatment of 22 with 2 equiv of 4. Treatment of **22** with 2 equiv of **4** as above gave a 69% yield of **24**. Subsequent treatment of **24** with 1 equiv of sodium methoxide for 3 h afforded a 76% yield of **23**.

3-(2*H*-Pyrazol-3-yl)-4(3*H*)-quinazolinone (25). A solution of **23** (5.00 g, 21.5 mmol) in MeOH (200 mL) was hydrogenated (10% Pd/C) in a Parr apparatus for 2 h. The catalyst was removed by filtration, and to the filtrate was added triethyl orthoformate (10 mL). After 18 h at reflux, the solution was concentrated to a small volume and the white solid that formed was collected to give 3.38 g (74%) of **25**: mp 192 °C; IR (Nujol) 1690 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 11.5 (br s, 1, NH), 8.57 (s, 1, C2-H), 8.20 (d, 1, C7-H), 7.87 (t, 1, pyrazole CH), 7.84–7.40 (m, 3, C6,7,8-H), 6.67 (t, 1, pyrazole CH); MS (EI), m/e 212 (M^+). Anal. ($C_{11}H_8N_4O$) C, H, N.

2-Nitro-*N*-4*H*-1,2,4-triazol-4-ylbenzamide (27). To a warm solution of **26** (16.8 g, 0.200 mol) in THF (300 mL) and water (5 mL) was added **4** (18.5 g, 0.100 mol) in THF (50 mL). After 3 h of warming, the solution was concentrated. The residue was triturated with water, and the resulting solid was collected to give 15.8 g (68%) of **27**: mp 202 °C (EtOH); IR (Nujol) 3500–2400 (NH), 1700 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.4 (br s, 1, NH), 8.77 (s, 2, triazole CH), 8.24 (d, 1, C6-H), 8.00–7.80 (m, 3, remaining aromatic); MS (CI), m/e 233 ($M^+ + 1$). Anal. ($C_9H_7N_5O_3$) C, H, N.

2-Amino-*N*-4*H*-1,2,4-triazol-4-ylbenzamide (28). A solution of **27** (15.4 g, 66.0 mmol) in EtOH (100 mL) and 1 N NaOH (75 mL) was hydrogenated in a Parr apparatus. The catalyst was removed by filtration, and the filtrate was neutralized with 1 N HCl (75 mL). The solution was extracted with CH_2Cl_2, and the combined extracts were dried (Na_2SO_4) and concentrated to a small volume. The white solid that formed was collected to give 4.00 g (30%) of **28**: mp 210–211 °C (EtOH); ^1H NMR (Me_2SO-d_6) δ 8.79 (s, 2, triazole CH), 8.27 (br s, 3, NH and NH_2), 7.66 (d, 1, C6-H), 7.29 (t, 1, C4-H), 6.81 (d, 1, C3-H), 7.62 (t, 1, C5-H). Anal. ($C_9H_9N_5O$) C, H, N.

3-(4*H*-1,2,4-Triazol-4-yl)-4(3*H*)-quinazolinone (29). A solution of **28** (4.00 g, 19.7 mmol) in triethyl orthoformate (25 mL)

was heated at reflux for 2.5 h. The mixture was cooled, and the solid was collected to give 3.10 g (74%) of **29**: mp 242-244 °C; IR (Nujol) 1700 (C=O), 1605 (C=N) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.87 (s, 2, triazole CH), 8.46 (s, 1, C2-H), 8.46 (d, 1, C5-H), 8.04-7.45 (m, 3, remaining aromatic). Anal. ($C_{10}H_7N_5O$) C, H, N.

Biological Test Procedures. The PCA test used in these studies is similar to that described by Goose and Blair.[23] The backs of anesthetized (sodium pentobarbital) male Sprague-Dawley rats were shaven prior to receiving 100-μL injections of two dilutions of an homologous antiserum rich in IgE antiovalbumin antibodies. The two dilutions were prepared to yield average reaction diameters of 7 and 14 mm in control animals. Forty-eight to seventy-two hours later, the rats received test compound by intraperitoneal (ip) injection (60 mg/kg, prepared in 50:50 polyglycol E-200-water, v:v) or oral (po) gavage (100 mg/kg, prepared in 20:80 ethanol-water, v:v). The rats were challenged intravenously with 0.1 mg of ovalbumin and 2.5 mg of Evan's Blue dye contained in 0.5 mL of saline 5 min after ip or 30 min after po compound administration. Thirty minutes after challenge, the rats were sacrificed, the dorsal skin was reflected, and the mean reaction diameters were determined from measurements of two perpendicular axes. The sum of the two mean diameters determined the score for each animal. A minimum of four animals was used for both treatment and vehicle control groups, but results from repeated trails were pooled, causing both treatment and control n values to range from 4 to 12. Percent inhibition was calculated on the basis of difference in scores between control and treated animals and reported as mean percent inhibition plus or minus the standard error.

The PPA test used in these studies is a modificaton of a test described by Spicer et al.[24] Rats were passively sensitized with 2 mL of a dilution of mouse ascites fluid rich in IgE-anti-DNP_3OV antibodies. (A 100-μL intradermal injection of the dilution would produce a PCA reaction approximately 14 mm in diameter.) Two hours later the animals were given an ip injection of either saline or test drug. Thirty seconds later the animals were challenged by an ip injection of 2 mg of ovalbumin in 5 mL of Burn's modified Tyrode's solution containing 25 units of heparin. Five minutes after challenge the animals were sacrificed and the peritoneal shock fluid was collected. Cells were removed by centrifugation (4000g/min). Protein was removed from each sample by precipitation with an equal volume of 0.8 N $HClO_4$ and recentrifugation (434000g/min). Histamine analysis was performed by use of a continuous-flow adaptation of the double extraction procedure described by von Redlich and Glick.[25]

Means of data from the PCA experiments were compared by use of the two-tailed Student's t test, with $p < 0.05$ chosen as the level of statistical significance.

Analytical Procedures. The HPLC conditions employed for the percent determinations of **9** and **10** were as follows: column, 10 μm Alltech C18, 250 mm × 6.4 mm o.d.× 4.6 mm i.d.; mobile phase, 0.005 M tetraethylammonium perchlorate in a mixture of 15:85 methanol-0.02 M McIlvaine[26] buffer; flow rate, 1.3 mL/min; injection volume, 20 μL; detection, UV absorbance at 254 nm.

Registry No. **4**, 610-14-0; **5**, 87693-28-5; **6**, 87693-21-8; **7**, 118-48-9; **8**, 87693-08-1; **9**, 87693-14-9; **10**, 95923-25-4; **11a**, 87693-22-9; **11a** (acid), 13506-76-8; **11a** (acid chloride), 66232-57-3; **11b**, 87693-23-0; **11b** (acid chloride), 38818-49-4; **11c**, 87693-24-1; **11c** (acid chloride), 50424-93-6; **11d**, 87693-25-2; **11d** (acid chloride), 4194-44-9; **11e**, 87693-26-3; **11e** (acid chloride), 15865-57-3; **11f**, 87693-27-4; **11f** (acid chloride), 50425-29-1; **12a**, 87693-15-0; **12b**, 87693-16-1; **12c**, 87693-17-2; **12d**, 87693-18-3; **12e**, 87693-19-4; **12f**, 87693-20-7; **13a**, 87693-02-5; **13b**, 87693-03-6; **13c**, 87693-04-7; **13d**, 87693-05-8; **13e**, 87693-06-9; **13f**, 87693-07-0; **14a**, 87693-09-2; **14b**, 87693-10-5; **14c**, 87693-11-6; **15**, 63598-72-1; **16**, 104035-16-7; **19**, 104035-17-8; **20**, 104035-18-9; **21**, 104035-19-0; **22**, 104035-20-3; **23**, 103060-67-9; **24**, 104035-22-5; **25**, 104035-23-6; **26**, 584-13-4; **27**, 104035-24-7; **28**, 104035-25-8; **29**, 104035-26-9; $H_3CC(OEt)_3$, 78-39-7; $EtC(OEt)_3$, 115-80-0; $C_6H_5C(OEt)_3$, 1663-61-2; 6-nitro-1,3-benzodioxole-5-carboxaldehyde, 712-97-0; 5-amino-tetrazole, 4418-61-5.

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(24) Spicer, B. A.; Ross, J. W.; Sharpe, T. J.; Smith, H. *Int. Arch. Allergy* **1978**, *56*, 493.
(25) von Redlich, D.; Glick, G. *Biochemistry* **1965**, *10*, 459.
(26) The McIlvaine buffer is prepared by mixing 81 mL of 0.2 M disodium phosphate and 19 mL of 0.1 M citric acid and diluting to 1 L. The solution is then adjusted to pH 7.0 with either the 0.2 M disodium phosphate or the 0.1 M citric acid.

Structure and Solution Conformation of the Cytostatic Cyclic Tetrapeptide WF-3161, *cyclo*[L-Leucyl-L-pipecolyl-L-(2-amino-8-oxo-9,10-epoxydecanoyl)-D-phenylalanyl]

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The sequence and configuration of amino acids in the cytostatic cyclic tetrapeptide WF-3161 are established as *cyclo*(L-Leu-L-Pip-L-Aoe-D-Phe) where Pip = pipecolic acid and Aoe = 2-amino-8-oxo-9,10-epoxydecanoic acid. In chloroform, WF-3161 adopts a conformation with a possible γ-turn between Leu NH and Aoe C=O and a cis amide bond between Leu and Pip. The torsion angles for this conformation are L-Aoe, ϕ, -95°, ψ, +85°, ω, -155°; D-Phe, ϕ, +120°, ψ, -80°, ω, -175°; L-Leu, ϕ, -145°, ψ, +35°, ω, -10°; L-Pip, ϕ, +20°, ψ, -135°, ω, -170°. The cis,trans,trans,trans amide bond sequence is related to the dimethyl sulfoxide conformation of chlamydocin, another cytostatic cyclic tetrapeptide.

WF-3161, a cyclic tetrapeptide isolated from culture filtrates of *Petriella guttulata*, is an inhibitor of cell growth in mouse P-388 leukemia cells (LD_{50} = 200 mg/kg).[2] Umehara et al. showed that WF-3161 contains the unusual amino acid 2-amino-8-oxo-9,10-epoxydecanoic acid (Aoe)[3] within the cyclic tetrapeptide *cyclo*(Leu-Pip-Aoe-Phe),[2] where Pip = pipecolic acid. However, the amino acid configurations were not assigned beyond the observation that L-Phe-L-Leu is present in partially hydrolyzed samples of WF-3161.

While characterizing the solution conformation of WF-3161 by nuclear magnetic resonance (NMR) spec-

(1) Abbreviations used herein: Pip, pipecolic acid (2-carboxypiperidine); Aoe, 2-amino-8-oxo-9,10-epoxydecanoic acid.
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