Fluorometric Assay of Angiotensin Converting Enzyme. Materials. Porcine plasma converting enzyme was purchased from Miles Laboratories, IL. Hippurylhistidylleucine, histidylleucine, and *o*-phthaldehyde was obtained from Sigma Chemical Co., St, Louis, MO. Angiotensin I was supplied by Bachem Fine Chemicals, Marina Del Rey, CA. All chemicals used were reagent grade.

Enzyme Assay.[25] The enzyme activity was measured by the fluorometric determination of histidylleucine, a product of the enzyme reaction. The substrate used was either hippurylhistidylleucine or angiotensin I. The concentration ranges were 2.6×10^{-4} to 2.04×10^{-3} M and 1.1×10^{-4} to 1.4×10^{-3} M for hippurylhistidylleucine and angiotensin I, respectively. The assay was carried out by mixing 10 μL of phosphate buffer (0.1 M, pH 7.6, containing 0.3 M NaCl) containing the testing inhibitor with substrate dissolved in 10 μL of phosphate buffer. Then, 1 mg of porcine plasma converting enzyme in 50 μL of buffer was added. The mixture was incubated at 37 °C for 90 min with constant shaking, and the reaction was stopped by adding 50 μL of 10% TCA. The samples were then diluted with 0.7 mL of water, followed by 0.4 mL of 2 N NaOH. To the alkalized mixture, 0.1 mL of 1% (w/v) *o*-phthaldehyde in methanol was added. After exactly 4 min, 0.2 mL of 6 N HCl was added. The contents of all tubes were thoroughly mixed after each addition. The samples were then centrifuged at 10000*g* for 10 min and the fluorescence of the supernatant was measured with exitation at 365 nm and emission at 495 nm on an Aminco Bowman fluorometer. The fluorescent product of histidylleucine with *o*-phthaldehyde is not stable in alkaline solution but is stabilized upon acidification. The fluorescence of the acidified solution is stable up to 1 h, so all readings should be made within 1 h.[26] Each sample was run in duplicate and an average of the two readings obtained was calculated.

A standard curve of histidylleucine was always prepared with each assay by mixing various amounts of histidylleucine with 1 mg of enzyme in 70 μL of phosphate buffer. The tubes containing the standards were treated exactly as those containing the samples.

A reagent blank containing all reagents but no substrate was also run for each assay.

Test for the Effect of Inhibitors. For testing the effect of inhibitors on angiotensin converting enzyme, two assays were run in parallel. One contained the substrate (1–2 mM), enzyme (1 mg), and various concentrations (1 nM–10 mM) of an inhibitor; the other contained only the substrate and enzyme. The assay conditions were the same as described above. The product formed with an inhibitor relative to that without an inhibitor was calculated to give the percent of inhibition. By plotting the percent of inhibition vs. various concentrations of an inhibitor, the I_{50} was obtained.

For determination of the K_i of an inhibitor, the enzyme assay was carried out as described above using various concentrations of substrate with and without an inhibitor. When hippurylhistidylleucine was used as substrate, the concentrations were 2×10^{-4} to 2.1×10^{-3} M. When angiotensin I was used as substrate, the concentrations were 1.1×10^{-4} to 1.4×10^{-3} M. The K_i determinations for **1** and **20** were performed using Hip-His-Leu as substrate at two different inhibitor concentrations (0.52 and 0.39 μM for **1** and 0.14 and 0.08 μM for **20**). By using the Michaelis–Menten equation[27] and double-reciprocal plot,[28] the amount of product formed at each substrate concentration with and without the inhibitor can be graphed and the K_m and K_i can be calculated.

Guinea Pig Ileum Assay. Male guinea pigs weighing 200 g were sacrificed. The ileum was isolated from each animal and a 10-cm segment nearest to the caecum junction was discarded. A 2–3 cm segment was suspended in a tissue bath filled with a modified Krebs solution at 37 °C and bubbled with 95% O_2–5% CO_2. The contractions were monitored with a transducer (Grass FT 0.03) in the presence of a 1-g load. A polygraph (Grass Model 7D) was used to record the contractions.

After equilibration, uniform contractions were observed at 10-min intervals after adding angiotensin I (25 ng/mL). In order to test the inhibitory activity (IC_{50}) of a drug, the method described by Rubin[12,29] was used. Each drug was kept in the bath 2 min before adding angiotensin I. After each drug test, the ileum was washed three times with Krebs solution, allowed to rest 8 min, and then tested again.

The Krebs solution had the following composition, mM: NaCl, 118; KCl, 4.75; $NaHCO_3$, 25; KH_2PO_4, 1.19; $MgSO_4$, 1.20; $CaCl_2$, 2.54; glucose, 11.

Acknowledgment. We thank the Heart, Lung, and Blood division of the National Institutes of Health who (Grant HL 19538) provided the funds for this work. Our appreciation is also extended to Lewis Cary and Dr. David Thomas for NMR and mass spectral results, respectively. We thank Dr. Joseph DeGraw for helpful discussions on various aspects of this work.

(25) B. S. Tsai and M. J. Peach, *J. Med. Chem.*, 18, 1180 (1975).
(26) Y. Piquilloud, A. Reinharz, and M. Roth, *Biochim. Biophys. Acta*, **206**, 136 (1970).
(27) L. Michaelis and M. L. Menten, *Biochem. Z.*, **49**, 333 (1913).
(28) I. H. Segel, in "Biochemical Calculations", 2nd ed, Wiley, New York, 1976, p 250.
(29) B. Rubin, E. H. O'Keefe, D. G. Kotler, D. A. DeMaio, and D. W. Cushman, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **34**, 770 (1975).

Antihypertensive 5,6-Diarylpyridazin-3-ones[1]

Russell Buchman, James A. Scozzie, Zaven S. Ariyan, Richard D. Heilman, Donna J. Rippin, W. J. Pyne, Larry J. Powers,*

Diamond Shamrock Corporation, T. R. Evans Research Center, Painesville, Ohio 44077

and Richard J. Matthews

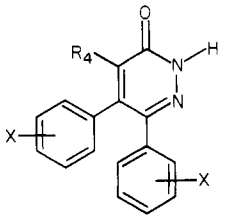
Pharmakon Laboratories, Scranton, Pennsylvania 18510. *Received December 17, 1979*

The synthesis of a series of 5,6-diarylpyridazinones is described. Some members of this series display an antihypertensive effect in both the spontaneously hypertensive rat (SHR) model and the deoxycorticosteroid (DOCA) model of hypertension. The most potent compounds in the series have halogen substituents on the 5,6-diphenyl rings, a β-substituted alkyl group at the 2 position of the ring, and an acetyl or cyano substituent at the 4 position.

There have been previous reports of antihypertensive agents which contain a pyridazinone ring system. These compounds can be classified into three groups. One series, hydrazino-substituted pyridazines, is probably related to

0022-2623/80/1823-1398$01.00/0

Table I. Pyridazinones



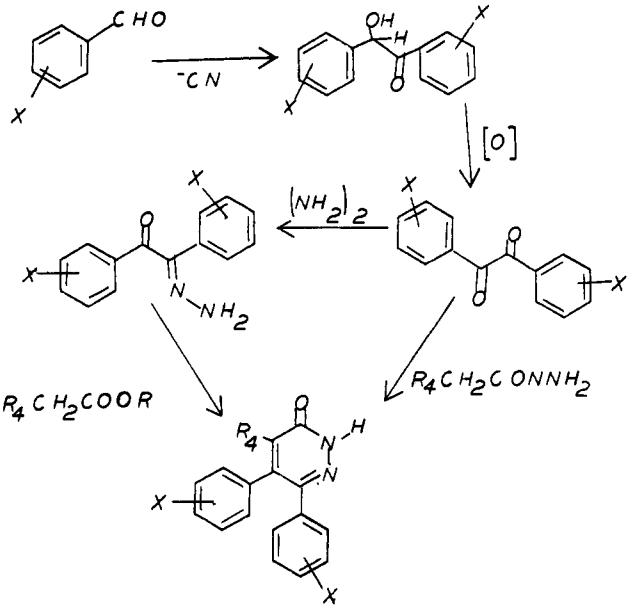
no.	R_4	X	mp, °C	solvent	% yield	method	formula
1	C_6H_5	H	290-292[a]	MeCN	30	A	$C_{22}H_{16}N_2O$
2	CN	4-CH_3	287-289	MeCN	40	A	$C_{19}H_{15}N_3O$
3	CN	3,4-Cl_2	236-237	C_6H_6	37	A	$C_{17}H_7Cl_4N_3O$
4	CN	4-Br	262-264	MeCN	49	A	$C_{17}H_9Br_2N_3O$
5	C_6H_5	4-CH_3	282-284	MeCN	48	A	$C_{24}H_{20}N_2O$
6	2-$CH_3OC_6H_4$	H	264-267	MeCN-MeOH	28	A	$C_{23}H_{18}N_2O_2$
7	CH_3CO	4-Cl	269-271	MeCN	20	A	$C_{18}H_{12}Cl_2N_2O_2$
8	4-$CH_3C_6H_4$	H	256-258	MeCN-MeOH	35	B	$C_{23}H_{18}N_2O$
9	4-ClC_6H_4	H	268-270	MeOH	25	A	$C_{22}H_{15}ClN_2O$
10	3-ClC_6H_4	H	228-230	MeCN	43	A	$C_{22}H_{15}ClN_2O$
11	4-BrC_6H_4	H	278-280	MeCN	36	A	$C_{22}H_{15}BrN_2O$
12	2,5-$(CH_3O)_2C_6H_3$	H	240-242	MeOH-EtOAc	35	A	$C_{24}H_{28}N_2O_3$
13	4-$CH_3OC_6H_4$	H	265-267	MeOH	31	A	$C_{23}H_{18}N_2O_2$
14	$C_6H_5SO_2$	H	254-256	EtOH	34	A	$C_{22}H_{16}N_2O_3S$
15	3,4,5-$(CH_3O)_3$-C_6H_2	4-Cl	248-251	MeCN	21	B	$C_{25}H_{20}Cl_2N_2O_4$
16	$(CH_3)_2CHCO$	4-Cl	241-242	EtOH	17	C	$C_{20}H_{16}Cl_2N_2O_2$
17	$CH_3(CH_2)_2CO$	4-Cl	209-211	MeCN	14	C	$C_{20}H_{16}Cl_2N_2O_2$
18	CH_3CO	4-CH_3O	239-242	MeCN	13	D	$C_{20}H_{18}N_2O_4$
19	CH_3CO	4-F	278-281	MeCN	34	D	$C_{18}H_{12}F_2N_2O_2$
20	CH_3CO	H	232-234[b]			A	$C_{18}H_{14}N_2O_2$
21	CN	2-Cl	263-264			A	$C_{17}H_9Cl_2N_3O$
22	CN	4-Cl	271-272	EtOH	50	A	$C_{17}H_9Cl_2N_3O$
23	CH_3CNNH_2	H	264-266	MeOH	30	*c*	$C_{18}H_{18}N_4O$
24	COOH	4-Cl	278-279[d]		50	c	$C_{17}H_{10}Cl_2N_2O_4$

[a] Lit.[8] mp 272-273 °C (C_6H_6). [b] Lit.[8] mp 232-233 °C (EtOH). [c] See Experimental Section. [d] Lit.[8] mp 274 °C dec (EtOH).

hydralazine in activity.[2a-c] A second group of antihypertensive pyridazinones, which have a 3-amino-2-hydroxypropyloxy substituent,[3] probably act as β-blocking agents. Finally, there have been reports of 4,5-dihydropyridazinones that have antihypertensive activity, although they have no classical hypotensive pharmacophore.[4a-c] In addition, Nannini[5] has recently reported a series of 5,6-diphenylpyridazinones with analgetic and antiinflammatory activity. We now report the synthesis of a series of 5,6-diarylpyridazinones which are active in several models of hypertension in the rat.

Our interest in this area was initiated by the observation that compound **88** lowers blood pressure of the spontaneously hypertensive rat (SHR). The objective of our project was to explore the structure-activity relationships of substituents at the N-2, the 5,6-diaryl, and the 4 positions.

Scheme I



Chemistry. The synthetic route used to generate the 5,6-diarylpyridazinones is essentially that reported by Schmidt and Druey.[6a,b] (Scheme I). The pyridazinone ring system is generated by either the condensation of a substituted acetic acid ester with a benzil monohydrazone or the condensation of a hydrazide with a benzil. The

(1) Presented in part before the Division of Medicinal Chemistry at the 180th National Meeting of the American Chemical Society. See "Abstracts of Papers", 180th National Meeting of the American Chemical Society, San Francisco, Calif., Aug 1980, American Chemical Society, Washington, D.C., 1980, Abstr MEDI 50.

(2) (a) C. DePonti, U. Bardi, and M. Marchetti, *Arzneim.-Forsch.*, **26**, 2089 (1976); (b) F. Parravicini, G. Scarpitta, L. Dorigotti, and G. Pifferi, *Farmaco, Ed. Sci.*, **34**, 299 (1979); (c) R. Pellegrini and G. Abbondati, *ibid.*, **32**, 19 (1977).

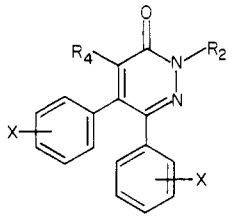
(3) SmithKline Corp., U.S. Patent 4 011 321 (1977).

(4) (a) W. V. Curran and A. Ross, *J. Med. Chem.*, **17**, 273 (1974); (b) F. J. McEvoy and G. R. Allen, Jr., *ibid.*, **17**, 281 (1974); (c) J. L. Grace, R. Z. Gussin, L. M. Luchuck, L. Ellenbogen, and P. S. Chan, *Pharmacologist*, 18, 187 (1976).

(5) G. Nannini, G. Biasoli, E. Perrone, A. Forgione, A. Buttinoni, and M. Ferrari, *Eur. J. Med. Chem.*, 14, 53 (1979).

(6) (a) P. Schmidt and J. Druey, *Helv. Chim. Acta*, **37**, 1467 (1954); (b) P. Schmidt and J. Druey, *Helv. Chim. Acta*, **37**, 134 (1954).

Table II. 2-Substituted Pyridazinones



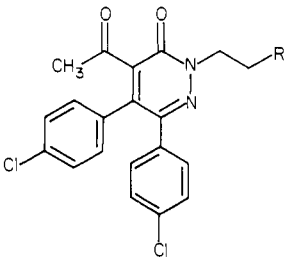
no.	R_2	R_4	X	mp, °C	solvent	% yield	synth method	formula
25	CH_3	CH_3CO	H	151-152[a]	MeOH	15	E	$C_{19}H_{16}N_2O_2$
26	$(CH_2)_2OH$	4-$CH_3CONHC_6H_4$	4-Cl	283-284	MeOH	47	E	$C_{26}H_{21}Cl_2N_3O_3$
27	$(CH_2)_2OH$	CH_3CO	4-Cl	191-193	MeOH	68	E	$C_{20}H_{16}Cl_2N_2O_3$
28	$(CH_2)_2OH$	C_6H_5	4-CH_3	192-194	EtOAc	91	E	$C_{26}H_{24}N_2O_2$
29	$(CH_2)_3COOC_2H_5$	CN	4-Cl	115-117	EtOAc	17	F	$C_{23}H_{19}Cl_2N_3O_3$
30	$(CH_2)_2OH$	C_6H_5CO	H	176-177	MeOH	50	E	$C_{24}H_{20}N_2O_3$
31	$(CH_2)_2OH$	2-$CH_3OC_6H_4$	H	197-199	EtOAc	66	E	$C_{25}H_{22}N_2O_3$
32	$(CH_2)_3COOH$	C_6H_5	H	171-173	$CHCl_3$	44	*b*	$C_{26}H_{22}N_2O_2$
33	$(CH_2)_3COOH$	CN	4-Cl	163-165	$CHCl_3$-CCl_4	29	*b*	$C_{21}H_{15}Cl_2N_3O_3$
34	$(CH_2)_2OH$	2-$C_{10}H_7$[c]	H	197-199	EtOAc	42	E	$C_{23}H_{22}N_2O_2$
35	$(CH_2)_2OH$	CN	4-F	212-215	EtOAc	62	E	$C_{19}H_{13}F_2N_3O_2$
36	$(CH_2)_2OH$	CN	3,4-Cl_2	198-202	$CHCl_3$-MeOH	26	E	$C_{19}H_{11}Cl_4N_3O_4$
37	$(CH_2)_2OH$	3-ClC_6H_4	H	153-155	C_6H_{12}-EtOAc	71	E	$C_{24}H_{19}ClN_2O_2$
38	$(CH_2)_2OH$	3-$CH_3OC_6H_4$	H	139-142	EtOAc	71	E	$C_{25}H_{22}N_2O_3$
39	$(CH_2)_2OH$	3,4-$(CH_3O)_2C_6H_3$	H	188-191	C_6H_6	45	E	$C_{26}H_{24}N_2O_4$
40	$(CH_2)_2OH$	2,5-$(CH_3O)_2C_6H_3$	H	211-214	C_6H_6	36	E	$C_{26}H_{24}N_2O_4$
41	$(CH_2)_2OH$	3,4-$(CH_3O)_2C_6H_3$	H	164-167	C_6H_{12}-EtOAc	31	E	$C_{28}H_{28}N_2O_6$
42	$(CH_2)_2OH$	CH_3CHOH	H	130-133	C_6H_{14}-EtOAc	60	*b*	$C_{20}H_{20}N_2O_3$
43	$(CH_2)_3COOC_2H_5$	CH_3CO	H	88-91	C_6H_{12}-EtOAc	71	F	$C_{24}H_{24}N_2O_4$
44	$(CH_2)_2OH$	$COOC_2H_5$	4-Cl	142-143	CCl_4-C_6H_{12}	48	E	$C_{21}H_{18}Cl_2N_2O_4$
45	$(CH_2)_2OH$	4-BrC_6H_4	H	184-187	EtOAc	72	E	$C_{24}H_{19}BrN_2O_2$
46	$(CH_2)_2OH$	4-$CH_3OC_6H_4$	H	150-153	C_6H_{12}-EtOAc	70	E	$C_{25}H_{22}N_2O_3$
47	$(CH_2)_3COOH$	CH_3CO	H	170-172	MeCN	53	*b*	$C_{22}H_{20}N_2O_4$
48	$(CH_2)_2OH$	$CH_3(CH_2)_2CO$	4-Cl	149-151	EtOH-H_2O	50	E	$C_{22}H_{20}Cl_2N_2O_3$
49	$(CH_2)_2OH$	$(CH_3)_2CHCO$	4-Cl	135-136	EtOH-H_2O	75	E	$C_{22}H_{20}Cl_2N_2O_3$
50	$CH_2CHOHCH_3$	CH_3CO	4-Cl	162-164	C_6H_6-C_6H_{12}	50	F	$C_{21}H_{18}Cl_2N_2O_3$
51	CH_2COCH_3	CN	4-Cl	160-162	*i*-PrOH	60	F	$C_{20}H_{13}Cl_2N_3O_2$
52	$(CH_2)_2Cl$	CN	4-Cl	172-175	C_6H_6	45	*b*	$C_{19}H_{12}Cl_3N_3O$
53	$CH(CH_3)CH_2OH$	CH_3CO	4-Cl	190-192	C_6H_{12}-C_6H_6	53	F	$C_{21}H_{18}Cl_2N_2O_3$
54	$(CH_2)_2OH$	H	4-Cl	177-178	C_6H_6	77	*b*	$C_{18}H_{14}Cl_2N_2O_2$
55	$(CH_2)_2OH$	COOH	4-Cl	245 dec	MeCN	83	*b*	$C_{19}H_{14}Cl_2N_2O_4$
56	$(CH_2)_2Cl$	CH_3CO	4-Cl	178-180	MeCN	58	*b*	$C_{10}H_{15}Cl_3N_2O_2$
57	$(CH_2)_2OH$	4-$(CH_3)_2NC_6H_4$	4-Cl	154-157 dec	EtOAc-EtOH	35	E	$C_{26}H_{23}Cl_2N_3O_2$·HCl
58	CH_3	CN	H	219-220[d]	C_6H_6	50	F	$C_{18}H_{13}N_3O$
59	CH_2COOH	CH_3CO	4-Cl	225-226		45	*b*	$C_{21}H_{14}Cl_2N_2O_4$
60	CH_2COOCH_3	CH_3CO	4-Cl	160-162	*i*-PrOH	41	F	$C_{21}H_{16}Cl_2N_2O_4$
61	CH_2COCH_3	CH_3CO	4-Cl	173-175	MeOH-H_2O	49	F	$C_{21}H_{16}Cl_2N_2O_3$
62	$(CH_2)_2NCH(CH_3)_2$	CN	4-Cl	212-213	EtOAc-MeOH	5	*b*	$C_{22}H_{21}Cl_2N_4O$·HCl
63	$CH_2CHOHCH_2NHCH(CH_3)_2$	CH_3CO	4-Cl	208-210	EtOAc	11	*b*	$C_{24}H_{25}Cl_2N_3O_3$·HCl
64	$CH_2CHOHCH_2Cl$	CH_3CO	4-Cl	157-158	C_6H_6-C_6H_{12}	10	*b*	$C_{21}H_{17}Cl_3N_2O_3$
65	CH_2CHOCH_2	CH_3CO	4-Cl	128-129	C_6H_6-C_6H_{12}	10	*b*	$C_{21}H_{16}Cl_2N_2O_3$
66	$(CH_2)_3N(CH_3)_2$	CH_3CO	4-Cl	153-156	EtOAc-C_6H_{12}	30	F	$C_{23}H_{23}Cl_2N_3O_2$
67	CH_2COOCH_3	CN	4-Cl	148-151	$CHCl_3$-C_6H_{12}	20	F	$C_{20}H_{13}Cl_2N_3O_3$
68	$(CH_2)_2$-*N*-PHTH	CH_3CO	4-Cl	185-186	MeCN	64	F[e,f]	$C_{28}H_{18}Cl_2N_3O_4$
69	$CH{=}CH_2$	CH_3CO	4-Cl	145-148	MeCN	36	*b*	$C_{20}H_{14}Cl_2N_2O_2$
70	$CH(CH_3)CH_2OH$	$CH_3(CH_2)_2CO$	4-Cl	128-131	CCl_4	25	F	$C_{23}H_{22}Cl_2N_2O_3$
71	CH_2CONH_2	CH_3CO	4-Cl	228-230	MeCN	91	F	$C_{20}H_{15}Cl_2N_3O_3$
72	$(CH_2)_2I$	CH_3CO	4-Cl	163-165	MeOH	49	*b*	$C_{20}H_{15}Cl_2IN_2O_2$
73	$(CH_2)_3COOC_2H_5$	CH_3CO	4-Cl	100-103	C_6H_6-pet. ether	55	F	$C_{24}H_{22}Cl_2N_2O_4$
74	$(CH_2)_2OH$	C_6H_5	4-Cl	179-180	MeCN-H_2O	77	E	$C_{29}H_{18}Cl_2N_2O_2$
75	$(CH_2)_2NC_5H_5^+Cl^-$	CH_3CO	4-HO	300	MeOH-EtOAc	52	*b*	$C_{25}H_{22}ClN_3O_4$
76	$(CH_2)_2OH$	CH_3CO	4-HO	235-237	EtOH-H_2O	26	*b*	$C_{20}H_{18}N_2O_5$
77	$(CH_2)_2OH$	CH_3CO	4-$C_6H_5CH_2O$	175-178	95% EtOH	85	E	$C_{34}H_{30}N_2O_5$
78	$(CH_2)_2OH$	CH_3CO	4-CH_3O	146-148	C_6H_{12}-EtOAc	86	E	$C_{22}H_{22}N_2O_5$
79	CH_2CH_3	CH_3CO	4-Cl	149-151	*i*-PrOH	77	F	$C_{20}H_{16}Cl_2N_2O_2$
80	$CH_2COOC_2H_5$	CH_3CO	4-CH_3O	99-103	C_6H_{14}-$CHCl_3$	43	F	$C_{24}H_{24}N_2O_6$
81	$(CH_2)_2OH$	CH_3CO	4-F	171-174	C_6H_{14}-$CHCl_3$	45	E	$C_{20}H_{16}F_2N_2O_3$
82	$(CH_2)_2OCH_3$	CH_3CO	4-Cl	127-128		26	F[e]	$C_{21}H_{18}Cl_2N_2O_5$
83	$(CH_2)_3OH$	CH_3CO	4-Cl	136-138	EtOH-H_2O	21	F	$C_{23}H_{22}Cl_2N_2O_3$
84	$(CH_2)_6$-*N*-PHTH	CH_3CO	4-Cl	192-193	95% EtOH	89	F[f]	$C_{32}H_{27}Cl_2N_3O_3$
85	$(CH_2)_2OH$	CH_3CH_2CO	4-Cl	157-158	MeOH-H_2O	67	E	$C_{21}H_{18}Cl_2N_2O_3$

Table II (*Continued*)

no.	R_2	R_4	X	mp, °C	solvent	% yield	synth method	formula
86	$(CH_2)_2OH$	C_6H_5	H	189 dec	MeOH	25	E	$C_{24}H_{20}N_2O_2$
87	$(CH_2)_2OH$	CN	4-CH_3O	193-194	EtOH	25	F	$C_{21}H_{20}N_2O_2$
88	$(CH_2)_2OH$	CN	4-Cl	122-123	C_6H_6-pet. ether	61	E	$C_{19}H_{13}Cl_2N_3O_2$
89	$(CH_2)_2OH$	CN	2-Cl	182-183	C_6H_6-pet. ether	81	E	$C_{19}H_{13}Cl_2N_2O_2$
90	$(CH_2)_2OH$	CH_3CO	H	126-128	C_6H_6-C_6H_{14}	69	E	$C_{20}H_{18}N_2O_3$

[a] Lit.[8] mp 158-159 °C (EtOH). [b] See Experimental Section. [c] 2-Naphthyl. [d] Lit.[8] mp 211-212 °C (EtOH). [e] Tosylate used as alkylating agent. [f] PHTH = phthalimido.

Table III. 2-[2-(Alkyl(acyl)amino)ethyl]pyridazinones



no.	R	mp, °C	solvent	% yield	synth method	formula
91	$N(CH_3)_2$	149-150	C_6H_{12}-$CHCl_3$	63	F	$C_{22}H_{21}Cl_2N_3O_2$
92	c-$N(CH_2CH_2)_2O$	194-198	EtOH-H_2O	27	F	$C_{24}H_{23}Cl_2N_3O_3$
93	$NH_2 \cdot HBr$	160-162	95% EtOH	30	*a*	$C_{20}H_{17}Cl_2N_3O_2$
94	c-NC_5H_{10}	155-157	*i*-PrOH	27	F	$C_{25}H_{25}Cl_2N_3O_2$
95	$N(CH_3)_2 \cdot HCl$	253-255	95% EtOH	40	*a*	$C_{21}H_{21}Cl_2N_3O_2$
96	c-NC_4H_8	184-186	95% EtOH	34	F	$C_{24}H_{23}Cl_2N_3O_2$
97	$N{=}C(CH_3)_2$	191-193	*i*-PrOH	9	*a*	$C_{23}H_{21}Cl_2N_3O_2$
98	$N^+(CH_3)_3I^-$	192-200	*n*-PrOH	57	*a*	$C_{23}H_{24}Cl_2IN_3O_2$
99	$NHCOCH_3$	154-157	EtOAc-pet. ether	43	*a*	$C_{22}H_{19}Cl_2N_3O_3$
100	$NHCH(CH_3)_2$	135-138	*i*-PrOH	33	*a*	$C_{23}H_{23}Cl_2N_3O_2$
101	$NHCO_2CH_2CH_3$	161-163	EtOAc		*a*	$C_{23}H_{21}Cl_2N_3O_4$
102	c-$N(CH_2CH_2)_2N$-$CO_2CH_2CH_3$	129-131	*n*-heptane		F	$C_{24}H_{28}Cl_2N_4O_4$

[a] See Experimental Section.

second route has the advantage of being one step shorter; however, it cannot be used to generate 4-acylpyridazinones. The synthesis and chemistry of pyridazinones have been the subject of two reviews.[7a,b]

The synthesis of the 5,6-bis(4-chlorophenyl)-pyridazinone, **7**, did not prove to be as straightforward as expected. Although the synthesis of **20**, the analogue with no substituents on the 5,6-phenyl rings, by method A (see Experimental Section) proceeds in good yield,[8] the condensation of ethyl acetoacetate and *p,p'*-dichlorobenzil monohydrazone by the same method gave a complex mixture of products.

Systematic modification of the reaction conditions used to generate **1** revealed that cleaner reactions resulted if alkoxide was not used as a catalyst. It was found that the reaction could be forced to completion by azeotropic removal of the water and alcohol formed during the reaction (method D). Further investigation revealed that **7** and related pyridazinones could be formed in acceptable yields by heating the monohydrazone and acetoacetic acid ester in refluxing Me_2SO (method C) without catalyst. This method proved not to be suitable for large-scale (kilogram) reactions, as increasing the concentration (from 0.7 to 2.6 M) of the monohydrazone decreased (from 63 to 35%) the yield of the product.

The pyridazinones could be alkylated in good yield with a variety of electrophilic reagents. The alkylations were conveniently carried out in DMF using potassium carbonate (method F) or potassium hydroxide (method E) as the catalyst. No significant amount of O-alkylated product was observed in these reactions.

In order to synthesize pyridazinones with phenolic substituents on the 5,6-diphenyl rings, the solvolysis of N-substituted pyridazinones with ether substituents on the 5,6-diaryl rings was investigated. Fusion of the *p*-methoxy analogue **78** with pyridine hydrochloride did cleave the methyl ethers but also led to the displacement of the 2-hydroxyl substituent to give the 2-pyridinium ethyl analogue **75**. An attempt to hydrolyze the methyl ethers of **78** with 48% hydrogen bromide gave a mixture of six components, which were not identified. The phenolic analogue **76** was successfully synthesized by hydrolysis of the 5,6-bis[(benzyloxy)phenyl]pyridazinone, **77**, with hydrogen bromide in acetic acid.[9]

The 2-(2-chloroethyl) derivatives can be obtained in good yield by reaction of the corresponding 2-hydroxyethyl analogues with either thionyl chloride or phosphorous oxychloride (Scheme II). An attempt to displace the chloride of **56** with potassium phthalimide gave only the *N*-vinyl compound **69**. The *N*-vinyl compound could also be obtained in good yield by reaction of the 2-chloroethyl analogue **56** with potassium carbonate in DMF. The phthalimide, **68**, was obtained (Scheme II) by reaction of

(7) (a) J. W. Mason and D. L. Aldous, *Chem. Heterocycl. Compd.*, **28**, 23 (1973); (b) M. Tisler and B. Stanovnik, *Adv. Heterocycl. Chem.*, **9**, 211 (1968).

(8) Ciba Pharmaceutical Products, U.S. Patent 2 839 532 (1958).

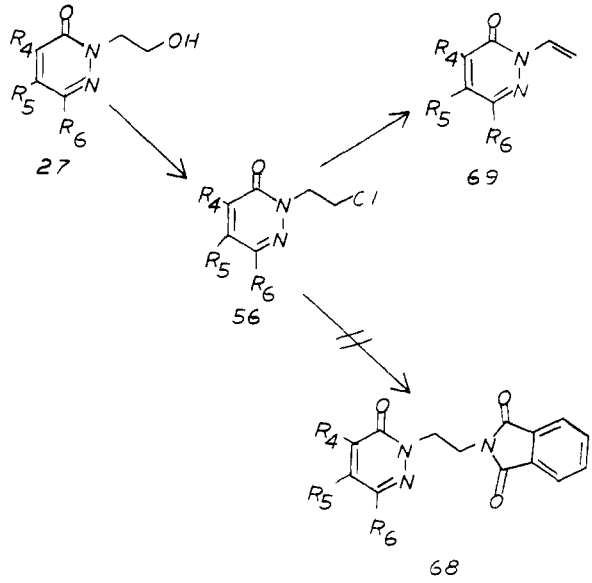
(9) *p,p'*-(Benzyloxy)benzil (mp 127–129 °C) was obtained by alkylation of *p,p'*-hydroxybenzil [H. Gilman and H. S. Broadbent, *J. Am. Chem. Soc.*, **70**, 2619 (1948) and N. J. Lenard, R. T. Rapola, H. L. Herzog, and E. R. Blout, *ibid.*, 71, 2997 (1949)] with benzyl bromide.

Table IV. Antihypertensive Activity. SHR Model

compd[a]	po dose, mg/kg	av blood pressure,[a] mmHg predose	4 h PD[b] 1	24 h PD[b] 1	4 h PD[b] 2	24 h PD[b] 2	heart rate[c]
22	100	205	171[d]	177[d]	173[d]	175[d]	0
27	100	218	173[d]	185	174[d]	193	+
	30	213	200	192	164[d]	206	+
	10	202	187	203	191	197	+
33	100	215	202	204	175[d]	180[d]	0
53	100	212	186	174[d]	191	201	0
57	100	223	184[d]	199	188[d]	208	+
62	100	220	218	206	209	173[d]	
73	100	196	192	174[d]	188	181	0
81	100	186	138[d]	160	166	180	0
82	100	186	142[d]	148[d]	144[d]	158	0
86	100	200	173[d]	187	176[d]	188	+
90	75	200	181	163[d]	164[d]	176	0
91	100	201	182	179	155[d]	144[d]	0
	30	199	182	164	167	193	0
92	100	239	230	201[d]	199[d]	222	0
	100	201	140[d]	161[d]	146[d]	177[d]	0
93	25	194	184	162[d]	177	183	0
94	100	192	180	163[d]	165	176	0
95	100	205	200	170[d]	160[d]	184[d]	+
96	100	192	155[d]	138[d]	142[d]	146[d]	0
	30	212	200	187[d]	191	199	0
	10	208	205	204	202	207	–
97	100	209	203	158[d]	134[d]	164[d]	0
	30	211	201	196	190[d]	198	0
	10	210	214	208	205	209	0
99	100	220	149[d]	146	136[d]	145[d]	
100	100	216	211	181	143[d]	176	0
102	100	200	186	182	151[d]	183	0
α-Me-Dopa	100	178	161[d]	174	155[d]	166	
guanethidine	50	187	154[d]	166[d]	138[d]	146[d]	

[a] Compounds 1–15, 17-19, 23–26, 28-32, 34-52, 54-56, 58-60, 63-72, 74-80, 83-85, 87-89, 98, and 101 were tested po at 100 mg/kg and gave no significant ($p = <0.05$) lowering of blood pressure; compound 20 was tested at 0.50 mg/kg po and compound 21 at 15 mg/kg. Neither of these compounds caused a significant lowering of blood pressure. Compounds 16 and 61 were not screened due to lack of sufficient sample. [b] PD = postdose. [c] + = increased heart rate, – = decreased heart rate, 0 = no significant change in heart rate. [d] Indicates that level of significance $p = <0.05$.

Scheme II



7 with *N*-(2-tosylethyl)phthalimide or *N*-(2-bromoethyl)-phthalimide. Hydrolysis of **68** with hydrogen bromide and acetic acid provided the 2-amino analogue **93**. The free base of **93** on trituration with acetone yielded the 2-isopropylidene analogue **97**, which was reduced to the 2-isopropylamine **100**.

Biological Activity. The effect of the compounds on the blood pressure of the spontaneously hypertensive rat (SHR) is shown in Table IV. Two of the more active compounds were evaluated in the deoxycortiosteroid acetate (DOCA) hypertensive model. As shown in Table V, these compounds lowered the blood pressure of the DOCA rat. Compound **27** was also evaluated in the normotensive rat. No hypotensive effect was observed. In the SHR model the hypotensive effect was usually accompanied by a significant increase in heart rate. An exception to this is the majority of the 2-(alkyl(acyl)amino)ethyl analogues (**91–97, 99, 100,** and **102**). With the exception of compound **95**, the compounds in this subseries either had no effect on heart rate or caused a decrease in heart rate. While some of these compounds (**96, 97,** and **99**) caused a greater drop in blood pressure at 100 mg/kg than **27**, this greater efficacy was not observed at lower doses.

The following observations can be made about the effects of structural variation at the ring positions. The R_2 position does not need to be substituted to have an active antihypertensive compound; however, the compounds which were most potent have a heteroatom two carbons removed from the pyridazinone ring. These active analogues and the substituent present in each are **27** and **53** (OH), **91** and analogues (amine), **82** (OCH_3), and **71** (amide). A separate group of active antihypertensive agents contained a 4-butyric acid residue at the R_2 position (**29, 33,** and **73**). It is interesting to note that compounds which have an acetamide residue at R_2 (**71**) or a substituent which contains both an alkyl and oxygen function β to the pyridazinone ring (**50** and **61**) have significantly increased acute toxicity.

At the R_4 position the acetyl group was of prime interest. Increasing the size of the acyl function (**30, 48, 49,** and **85**) decreases activity. A 4,5,6-triphenyl analogue (**86**) does

Table V. Antihypertensive Activity. DOCA (D) and Normotensive (N) Models

compd	po dose, mg/kg	model	av blood pressure, mmHg				
			predose	4 h PD 1	24 h PD 1	4 h PD 2	24 h PD 2
88	10	D	194	181	184	178	180
88	25	D	208	168[a]	178	157[a]	180
88	50	D	200	165[a]	171[a]	156[a]	168[a]
88	100	D	200	157[a]	158[a]	145[a]	152[a]
27	10	D	224	191	192	188	210
27	25	D	235	194[a]	191[a]	188[a]	208[a]
27	50	D	224	171[a]	184[a]	182[a]	213[a]
27	100	D	222	169[a]	178[a]	164[a]	195
27	100	N	125	123	125	127	130
guanethidine	25	D	194	163[a]	181	163[a]	183

[a] Indicates that level of significance $p = <0.05$.

have moderate activity, but evaluation of several analogues which have substituted phenyl groups on the 4 position failed to substantially increase activity.

The substitution of Cl or F at the 4′ position of the aryl rings at the 5 and 6 positions of the pyridazinone ring enhanced activity relative to the unsubstituted analogues. Analogues with alkyl groups on the 5,6-diaryl rings were usually active but not as active as the corresponding halo derivatives. Substitution of alkoxy analogues on these rings generally lowered antihypertensive activity.

Experimental Section

Elemental analyses were performed by the Central Analytical Department of Diamond Shamrock or by Galbraith Laboratories, Knoxville, Tenn. All analyses (C, H, and N) are within ±0.4% of the calculated values. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Structural assignments are supported by IR, NMR and, where necessary, by mass spectra. Homogeneity of all compounds was evaluated prior to submission of the compounds for biological evaluation by silica gel TLC. The yields listed in the table represent the first crop obtained from the recrystallization and, therefore, represent a low estimate of the overall yield.

Method A. EtOH, dried by distillation from Mg/I_2, was added to a dry flask (N_2 atmosphere) containing clean Na (1.1 equiv). After the Na reacted, the acetic acid ester (1.1 equiv) was added dropwise to the cold (0–5 °C) alkoxide solution. The benzil monohydrazone (1 equiv) was then added as a solid. After heating the reaction mixture at reflux for 3 h, it was cooled and poured into 1 N HCl. The resulting precipitate was separated by filtration and washed with water. The product was air-dried and recrystallized.

Method B. The benzil monohydrazone (1 equiv), acetic acid ester (1 equiv), and potassium *tert*-butoxide (1.1 equiv) were slurried in *i*-PrOH, and the solution was heated at reflux for 2 h. The reaction mixture was then cooled and poured into 1 N HCl. After filtration of the mixture, the precipitate was washed with H_2O, air-dried, and recrystallized.

Method C. Me_2SO (1.3 L/mol) was heated to reflux and the bis(*p*-chlorobenzil) monohydrazone (1 equiv) was added. A stream of N_2 was passed over the solution during the course of the reaction. The acylacetic acid ester (4 equiv) was added, and the reaction was maintained at 175 °C for 1.25 h. After the reaction was diluted by pouring onto ice (3× volume increase), toluene (2.6 L/mol) was added and the slurry stirred. The suspension was filtered and the residue was washed with MeOH, toluene, and petroleum ether.

Method D. The benzil monohydrazone (1 equiv) and xylene (600 mL/mol) were stirred while the mixture was heated rapidly to 120 °C. The acylacetic acid ester (1.35 equiv) was added rapidly, followed immediately by pyridine (1.1 equiv). The reaction mixture was heated at reflux for 2.5 h, during which time H_2O was collected by azeotrope in a Dean–Stark trap. The reaction was cooled, and the resulting precipitate was washed with xylene and air-dried.

Method E. The pyridazinone (1 equiv) was dissolved in DMF (4 mL/mmol) and ethylene carbonate (1.1 equiv) was added. KOH (0.01 equiv) was added and the flask was placed in an oil bath (110–120 °C) until CO_2 evolution ceased (ca. 3.5 h). The reaction mixture was cooled and diluted with 3 volumes of H_2O to give a precipitate, which was separated, washed with H_2O, and recrystallized.

Method F. The pyridazinone (1 equiv) and anhydrous K_2CO_3 (1 equiv) were slurried with DMF (4.0 mL/mmol). The alkyl halide (1.1 equiv) was added and the flask immersed in an oil bath (80–100 °C) for 3 h. The reaction mixture was cooled and diluted with 3 volumes of H_2O. The resulting precipitate was separated, washed with H_2O, and recrystallized after air-drying.

4-Acetyl-5,6-diphenyl-2*H*-pyridazin-3-one Hydrazone (23). Compound **20** (5.0 g, 0.017 mol) was dissolved in 100 mL of absolute EtOH. Eighty-five percent hydrazine hydrate (1.2 g, 0.02 mol) was added, and the reaction mixture was heated at reflux for 3 h. When the mixture cooled, a white precipitate formed which was separated by filtration and recrystallized from MeOH.

Carboxylic Acid Derivatives 24, 32, 33, 47, and 59. The carboxylic acid ester (1 equiv) and Na_2CO_3 (4 equiv) were heated at reflux in 75% EtOH (6 mL/mmol) for 8 h. The solvent was removed from the reaction mixture, and the residue was partitioned between H_2O and EtOAc. The aqueous layer was washed two times with EtOAc and then acidified with 37% HCl. The resulting precipitate was separated by filtration, washed with H_2O, air-dried, and recrystallized.

2-(2-Hydroxyethyl)-4-(1-hydroxyethyl)-5,6-diphenyl-2*H*-pyridazin-3-one (42). Compound **90** (10.65 g, 0.03 mol) was stirred in EtOH (200 mL), and $NaBH_4$ (0.28 g, 0.0075 mol) was added as a solid. The reaction was allowed to stir for 5 h at 20 °C and the solvent was removed. The residue was dissolved in EtOAc and washed with H_2O. After the organic layer was dried (Na_2SO_4), the solvent was removed in vacuo. The residual oil was crystallized by trituration with hexane and recrystallized from EtOAc/hexane.

2-(2-Chloroethyl)pyridazinones (52 and 56). The 2-(2-hydroxyethyl)pyridazinone (1 equiv; **88** or **27**) was dissolved in dioxane (60 mL) and $POCl_3$ (1.3 equiv) was added. Upon addition of pyridine (1.2 equiv), the reaction mixture became homogeneous. After 4 h at 20 °C, 1 N HCl (20 mL) was added to give a precipitate, which was separated, washed with 1 N HCl, and recrystallized from MeCN.

2-(2-Hydroxyethyl)-5,6-bis(4-chlorophenyl)-2*H*-pyridazin-3-one (54). Compound **55** (10 g, 0.025 mol) was added to refluxing decahydronaphthalene. After heating at reflux for 6 h, the reaction mixture was cooled and diluted with petroleum ether. The resulting precipitate was separated and recrystallized from C_6H_6.

2-(2-Hydroxyethyl)-5,6-bis(4-chlorophenyl)-3-oxopyridazin-4-ylcarboxylic Acid (55). Compound **44** was dissolved in absolute EtOH (6 mL/mmol). Following addition of KOH (1.1 equiv), the reaction mixture was placed on a steam bath. When the solvent had evaporated, the residue was dissolved in warm H_2O (6 mL/mmol) and filtered. The filtrate was acidified with 37% HCl, and the resulting precipitate was separated by filtration, air-dried, and recrystallized.

2-(Isopropylamino)-Substituted Analogues (62 and 63). The 2-alkylchloropyridazinone was heated at reflux for 18 h in isopropylamine. The excess amine was removed in vacuo and the residue dissolved in Et_2O. The resulting solution was washed with H_2O and dried (K_2CO_3). The addition of 17% HCl/Et_2O (w/w)

to the Et_2O solution gave a precipitate which was recrystallized from MeOH/EtOAc after decanting the Et_2O.

Reaction of 7 with Epichlorohydrin. Compound **7** (50 g, 0.14 mol), K_2CO_3 (20 g), and epichlorohydrin (12 mL) were allowed to react in DMF (200 mL) at 80 °C for 2 h. The cooled reaction mixture was poured into H_2O (700 mL) and the slurry was extracted with EtOAc. The EtOAc solution was washed with a saturated NaCl solution and dried (K_2CO_3). The solvent was removed to give a residual oil (55 g). An aliquot (12 g) of this oil was dissolved in 20% EtOAc/$CHCl_3$, and the solution was chromatographed on dry column silica gel (80-mm tubing × 1.2 m). The column was eluted with 2 L of 20% EtOAc/$CHCl_3$, and the UV-absorbing region was cut into 2-in. segments. The segments were extracted with MeCN and analyzed on silica gel plates ($CHCl_3$/EtOAc, 1:1 as developing solvent). Fractions 2–4 were combined and the solvent removed. The residue was recrystallized from hexane to give **65**. The solvent was removed from fraction 7 to give a residue which was recrystallized from cyclohexane/C_6H_6 to give **64**.

4-Acetyl-5,6-bis(4-chlorophenyl)-2-vinyl-2*H*-pyridazin-3-one (69). Compound **56** (8.86 g, 0.02 mol), K_2CO_3 (3.0 g, 0.022 mol), and potassium phthalimide (4.07 g, 0.022 mol) were stirred in DMF (60 mL) at 50 °C for 48 h. The reaction mixture was cooled and diluted with 1 N NaOH, and the resulting suspension was extracted with EtOAc. The solvent was evaporated from the organic layer in vacuo, and the residue was recrystallized from MeCN.

4-Acetyl-5,6-bis(4-chlorophenyl)-2-(2-iodoethyl)-2*H*-pyridazin-3-one (72). Compound **56** (15.0 g, 0.035 mol) and NaI (15.9 g, 0.1 mol) were heated at reflux in Me_2CO for 36 h. The reaction mixture was cooled and the solvent removed in vacuo to give a tan solid which was recrystallized.

1-[2-[4-Acetyl-5,6-bis(4-hydroxyphenyl)-3-oxo-2*H*-pyridazin-2-yl]ethyl]pyridinium Chloride (75). Compound **78** (14 g, 0.04 mol) and pyridine hydrochloride (42 g) were mixed in an argon atmosphere. The flask was placed in an oil bath (215 °C) for 0.5 h. After cooling, the reaction mixture was poured into 1 N HCl (250 mL). This gave initially a dark solution in which a precipitate formed. The precipitate was separated by filtration, washed with H_2O, and recrystallized.

4-Acetyl-5,6-bis(4-hydroxyphenyl)-2-(2-hydroxyethyl)-2*H*-pyridazin-3-one (76). Compound **77** (5.46 g, 0.010 mol), 48% HBr (25 mL), and glacial AcOH (25 mL) were heated on a steam bath for 25 min. The orange solution was evaporated in vacuo to a foam, which was applied to a dry column of silica gel and eluted with EtOAc/$CHCl_3$ (4:1). Fractions containing material having R_f 0.4 were eluted with EtOAc. The extract was evaporated in vacuo to a yellow foam, which was triturated with 95% EtOH/Et_2O/petroleum ether to give a tan crystalline solid (1.5 g). On recrystallization from EtOH/H_2O a tan crystalline material, which retained an indeterminant amount of solvent of recrystallization, was obtained. The product was dried under high vacuum at 150 °C for 3 days to give 0.96 g of product.

4-Acetyl-5,6-bis(4-chlorophenyl)-2-(2-aminoethyl)-2*H*-pyridazin-3-one Hydrobromide (93). Compound **68** (16.1 g, 0.03 mol), glacial AcOH (100 mL), and 48% HBr (50 mL) were combined and stirred under reflux for 16 h. Since TLC [silica gel; toluene/EtOAc/HCO_2H (15:10:1)] showed the presence of the phthalimide, additional 48% HBr (50 mL) was added and refluxing continued for 4 h. Then another portion of 48% HBr (50 mL) was added and refluxing continued for an additional 4 h. The reaction mixture was allowed to stand at 20 °C for 15 h and then evaporated in vacuo. The orange semisolid residue was triturated with anhydrous Me_2CO and filtered. The air-dried solid was recrystallized from 95% EtOH following treatment with charcoal. The solid obtained was recrystallized three times from 95% EtOH and air-dried to give 4.7 g of product.

4-Acetyl-5,6-bis(4-chlorophenyl)-2-[2-(dimethylamino)ethyl]-2*H*-pyridazin-3-one Hydrochloride (95). To a stirred, warm solution of **91** (7.0 g, 0.016 mol) and anhydrous Me_2CO (30 mL) was added 37% HCl (2 mL) in one portion. A white crystalline solid separated immediately. The mixture was cooled (0 °C) and filtered, and the solid obtained was washed with anhydrous Et_2O and air-dried. The product (6.6 g) was recrystallized twice from 95% EtOH and air-dried to give 3.1 g of **95**.

4-Acetyl-5,6-bis(4-chlorophenyl)-2-[2-(trimethylamino)ethyl]-2*H*-pyridazin-3-one Iodide (98). A mixture of **91** (16.0 g, 0.037 mol) and MeI (100 mL, 228.0 g, 1.61 mol) was stirred for 18 h at 20 °C. The resulting suspension was filtered and the solid was washed with anhydrous Et_2O (3×). The solid was then recrystallized from *n*-PrOH to give 13.2 g of **98**.

4-Acetyl-5,6-bis(4-chlorophenyl)-2-[2-(isopropylideneamino)ethyl]-2*H*-pyridazin-3-one (97). The crude free amine, 4-acetyl-5,6-bis(4-chlorophenyl)-2-(2-aminoethyl)-2*H*-pyridazin-3-one, was triturated with Me_2CO. The solid which separated was collected by filtration and air-dried. The solid obtained was recrystallized from MeCN to give an orange crystalline material, which was applied to a dry column of silica gel and eluted with $CHCl_3$/Me_2CO (1:1). Fractions containing product (R_f 0.4) were eluted with Me_2CO and evaporated in vacuo to a yellow semisolid. Trituration with Et_2O gave a solid which was separated by filtration and air-dried. This yellow powder was recrystallized from *i*-PrOH and air-dried to give a yellow crystalline solid.

4-Acetyl-5,6-bis(4-chlorophenyl)-2-[2-(isopropylamino)ethyl]-2*H*-pyridazin-3-one (100). To a stirred solution of **97** (16.0 g, 0.036 mol) and anhydrous MeOH (1 L) was added 1 N HCl (10 mL). The pH of the resulting solution was ca. 5. $NaBH_3CN$ (1.6 g, 0.025 mol) was added in one portion, and the resulting solution was stirred at 20 °C for 2 h. Since TLC [silica gel; EtOAc/MeOH (9:1)] indicated starting material was present, an additional amount of $NaBH_3CN$ (0.80 g, 0.0125 mol) was added. Stirring was continued for 2 h, and then another portion of $NaBH_3CN$ (0.80 g, 0.0125 mol) was added. After an additional 2 h of stirring at 20 °C, the solution was evaporated in vacuo. The residue was dissolved in $CHCl_3$, washed with H_2O (2×), dried ($CaSO_4$), and evaporated in vacuo. The residue was chromatographed on a dry silica gel column and eluted with EtOAc/MeOH (1:1). Fractions containing product were eluted with Me_2CO and evaporated in vacuo, and the residue was dissolved in $CHCl_3$. The solution was dried ($CaSO_4$) and concentrated in vacuo to a syrup, which began to crystallize on standing. The syrup was dissolved in hot *i*-PrOH and allowed to cool. The yellow crystalline material which separated was collected by filtration and air-dried (3.5 g). A second crop was obtained from the mother liquors (3.0 g). The two crops were combined and dissolved in *i*-PrOH, H_2O was added, and the solution was allowed to stand at 20 °C for 18 h. The supernatant was decanted from the small amount of yellow crystalline solid which separated and was evaporated in vacuo to a tan amorphous solid. The solid was dried under vacuum at 80 °C overnight to give 5.3 g of product.

Acylation of 4-Acetyl-5,6-bis(4-chlorophenyl)-2-(2-aminoethyl)-2*H*-pyridazin-3-one (99 and 101). Compound **93** was dissolved in warm H_2O, and the pH of the solution adjusted to ca. 10 with 1 N NaOH. The resulting mixture was extracted with $CHCl_3$. The combined $CHCl_3$ extracts were dried ($CaSO_4$) and evaporated in vacuo to a brown syrup. This crude free amine was used without further purification.

To a stirred (0 °C) solution of 0.05 mol of the crude amine and 150 mL of pyridine was added, dropwise, 0.05 mol of the required acylating agent. When the addition was complete, the cooling bath was removed and the mixture was allowed to stir at 20 °C for 18 h. The mixture was poured into H_2O and extracted with $CHCl_3$ (**99**) or EtOAc (**101**). The organic extract was dried (Na_2SO_4) and concentrated in vacuo. The resulting residue was either recrystallized (**99**) or chromatographed on a dry silica gel column with $CHCl_3$/EtOAc (2:1) and then recrystallized (**101**).

Antihypertensive SHR Rat Assay. Spontaneously hypertensive rats, 12 to 16 weeks of age, were used in this assay. Systolic blood pressure was determined by the tail cuff method, utilizing capacitance transducers for the detection of pressure, an aneroid manometer for measuring pressure, and an oscilloscope for visualizing the disappearance and/or appearance of the pressure pulse. Heart rate was measured by a biotachometer. Groups of five rats having systolic blood pressure of 170 mmHg or greater were chosen, and the test compound was administered at 100 mg/kg po as a solution or suspension in 0.25% methylcellulose (MC) at a volume of 5 mL/kg. One group served as the control and received the vehicle. Four and twenty-four hours after dosing, systolic blood pressure and heart rate were recorded. A second dose of compound was administered, and blood pressure and heart rate were determined at 4 and 24 h after the second dose.

The systolic blood pressures and heart rates were compared to predose "control" values statistically using the Student's t test. Compounds having a level of significance $p = <0.05$ are regarded as active.

Antihypertensive DOCA Rat Assay. Sprague–Dawley male rate weighing 90–100 g were used in this assay. Deoxycorticosterone acetate (DOCA) was administered subcutaneously at a dose of 100 mg/rat per day for 5 days a week for 3 weeks. One percent saline was provided ad libitum for the 3-week period. Tap water was substituted for the 1% saline at the end of the treatment period.

Systolic blood pressure were determined by the tail cuff method, utilizing capacitance transducers for the detection of pressure, an aneroid manometer for measuring pressure, and an oscilloscope for visualizing the disappearance and/or appearance of the pressure pulse. Groups of five rats having systolic blood pressure of 170 mmHg or greater were chosen, and the test compound was administered at 100 mg/kg po as a solution or suspension in 0.25% methylcellulose (MC) at a volume of 5 mL/kg. One group served as the control and received the vehicle. Systolic blood pressures were recorded prior to dosing and again 4 h after drug. If a significant hypotensive effect was obtained at 4 h posttreatment, the pressure was again measured at 24 h posttreatment.

The 0- and 4-h postdose systolic blood pressures were compared statistically using the Student's t test. Compounds having a level of significance $p = <0.05$ were regarded as active.

Synthesis and Antihypertensive Activity of 5-Amino-2-pyridinecarboxylic Acid Derivatives

Neville Finch,* Thomas R. Campbell, Charles W. Gemenden, and Henry J. Povalski

Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, New Jersey 07901.
Received June 12, 1980

The synthesis of various substituted 5-amino-2-pyridinecarboxylic acids and their derivatives is described by three general methods: (1) reductive alkylation of methyl 5-amino-2-pyridinecarboxylates (**2**) and subsequent hydrolysis; (2) alkylation of the urethane (**9**), followed by hydrolysis; and (3) selective $NaBH_4$ reduction of the appropriate amide of (**2**), followed by hydrolysis. A more specific process was used for the 5-(phenylamino) compound, i.e., nucleophilic displacement of nitrite from methyl 5-nitro-2-pyridinecarboxylate by sodioformanilide and subsequent hydrolysis. Many of these 2-pyridinecarboxylic acid derivatives were potent antihypertensive agents in the spontaneously hypertensive rat (SHR). Optimization of structural parameters for this activity yielded compounds **54**, **55**, **34**, **65**, and **22**, which were selected for further study in the renal hypertensive dog (RHD). Based on these studies, one compound, 5-[(4-fluorobenzyl)amino]-2-pyridinecarboxylic acid (**65**), was selected for preclinical toxicity evaluation. Based on the toxicological findings, it was decided not to pursue compound **65** clinically.

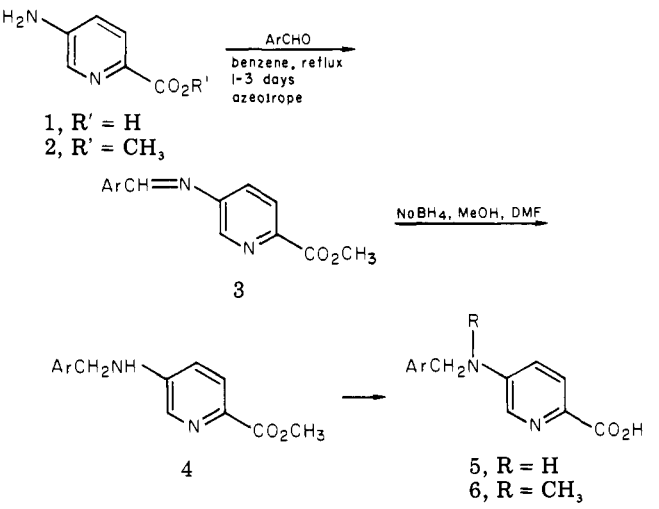
The reasons for our interest in vasodilators were described previously.[1] Fusaric acid, 5-butylpicolinic acid, is still actively studied as such, both preclinically[2] and clinically.[3] Synthesis of analogues in attempts to improve the profile continue.[4]

Our earlier efforts to improve on fusaric acid by studying 5-thio-2-pyridinecarboxylic acid derivatives were unsuccessful.[1] The more interesting compounds based on SHR (spontaneously hypertensive rat) data lacked sufficient efficacy in the RHD (renal hypertensive dog). We now describe compounds of the fusaric acid type, 5-amino-2-pyridinecarboxylic acids, which show good efficacy in both SHR and RHD models.

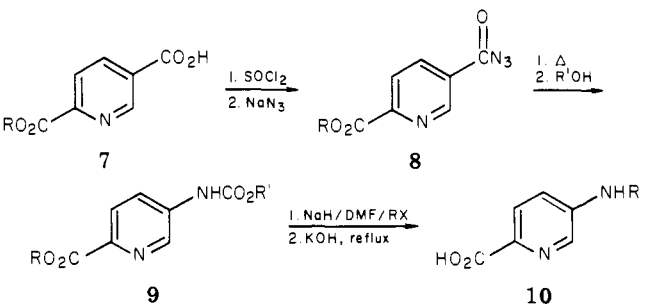
Chemistry. A very limited amount of work has been done with 5-amino-2-pyridinecarboxylic acids. Only the parent compound[5,6] and its 5-(dimethylamino)[5] and a few *N*-acyl derivatives have been described.[7]

We developed four methods to provide access to a broad range of substituted 5-amino-2-pyridinecarboxylic acids. For the most important structural types, i.e., substituted 5-(benzylamino)-2-pyridinecarboxylic acids, two of these methods were principally used. One is the reduction of the Schiff base from an aromatic aldehyde and methyl 5-amino-2-pyridinecarboxylate with sodium borohydride, followed by hydrolysis (Scheme I). The other method is the alkylation of the urethane derived from the Curtius reaction on ethyl 5-carboxy-2-pyridinecarboxylate, followed by hydrolysis (Scheme II). For other structural types,

Scheme I



Scheme II



(1) N. Finch, T. R. Campbell, C. W. Gemenden, M. J. Antonaccio, and H. J. Povalski, *J. Med. Chem.*, 21, 1269 (1978).
(2) L. Piesche, H. Hilse, and P. Oehme, *Pharmazie*, 34, 332 (1979).
(3) M. Miyahara, H. Miyazake, H. Takayama, and Y. Minatogawa, *Jpn. Circ. J.*, 41, 1161 (1977).
(4) H. Yamanaka, M. Mizugaki, M. Sagi, and K. Edo, *Heterocycles*, 12, 1323 (1979).
(5) L. W. Deady, R. A. Shanks, A. D. Campbell, and S. Y. Chooi, *Aust. J. Chem.*, 24, 385 (1971).
(6) J. Delarge, *Pharm. Acta Helv.*, 44, 637 (1969).
(7) G. H. Cooper and R. L. Rickard, *J. Chem. Soc. C*, 3257 (1971).

0022-2623/80/1823-1405$01.00/0