crystallize upon addition of a few drops of petroleum ether (b.p. 30–60°). The crystals were collected, washed with a few drops of ethyl acetate, and recrystallized from the same solvent after clarification with charcoal; m.p. 124–130°; yield 50 mg. (30%); ultraviolet absorption spectra: in 0.1 *N* NaOH, λ_{max} 219 mμ (log ε 4.42), 275 mμ (log ε 4.11); in 0.1 *N* hydrochloric acid, λ_{max} 277 mμ (log ε 3.90).

Anal. Calcd. for $C_{13}H_{14}Cl_2N_2O_5$: C, 44.72; H, 4.04; Cl, 20.31. Found: C, 44.38; H, 3.86; Cl, 19.89.

When chromatogrammed on Whatman 3 MM paper and developed with 0.1 *M* acetate buffer of pH 4.4, ascending flow, 3,5-dichloro-4-methylaminobenzoylglutamic acid showed R_f 0.83.

Discussion

Although derived from methotrexate, DCM differs from it in several aspects. In comparison with methotrexate, DCM is superior as a therapeutic agent in mouse leukemia L1210 and less toxic toward the host animals.[18] Another distinct feature revealed by our work is that DCM is rapidly oxidized to the 7-hydroxy derivative both *in vivo* and *in vitro* while methotrexate apparently is not.[7,19]

The metabolic fate of pteroylglutamic acid and derivatives in man and other animals has not been extensively investigated previously.[20] Because of its occurrence in human urine, xanthopterin has been suggested[21] as a final catabolic product of pteroylglutamic acid in man. It was postulated that initially pteroylglutamic acid was cleaved between position 9 and N^{10}, followed by oxidation of the pteridine part, finally resulting in 7-hydroxylation. Xanthine oxidase was implicated in the last step even though the enzyme is inert toward intact pteroylglutamic acid.[22] There was no evidence that pteroylglutamic acid was oxidized at position 7 as in our studies with DCM although pteroylglutamic acid probably indirectly gave rise to simple pteridines such as xanthopterin.[15]

Incubation of pteroylglutamic acid with either chicken liver extracts[23a] or rat liver slices (but not homogenate)[23b] afforded a diazotizable amine, most likely *p*-aminobenzoylglutamic acid. The fate of the pteridine moiety was not defined. In any event, 7-hydroxylation of pteroylglutamic acid was not encountered during incubation.

Simple pteridines, on the other hand, are subjected to 7-hydroxylation when incubated with either liver preparations or xanthine oxidase. For example, 2-amino-4-hydroxypteridine is oxidized to isoxanthopterin by chicken liver extract[24a] as well as by xanthine oxidase from milk[24b]; likewise, xanthopterin is oxidized to leucopterin.[24b–e] In all cases, an oxygen atom is introduced at position 7, parallel to DCM. Yet interestingly, DCM is not oxidized by milk or calf liver xanthine oxidase.[7] Although methotrexate and aminopterin were not oxidized by rat liver homogenate,[7] such an oxidation was recently reported by the incubation of these drugs with an enzyme system isolated from rabbit liver.[19c]

(18) (a) A. Goldin, S. M. Humphreys, J. M. Venditti, and N. Mantel, *J. Natl. Cancer Inst.*, **22**, 811 (1959); (b) J. M. Venditti, S. R. Humphreys, N. Mantel, I. Kline, and A. Goldin, *Cancer Res.*, **20**, 698 (1960).

(19) (a) E. S. Henderson and C. Denham, *Proc. Am. Assoc. Cancer Res.*, **4**, 27 (1963); see, however: (b) D. G. Johns, J. W. Hollingsworth, A. R. Cashmore, I. H. Plenderleith, and J. R. Bertino, *J. Clin. Invest.*, **43**, 621 (1964); (c) D. G. Johns, A. T. Iannotti, A. C. Sartorelli, B. A. Booth, and J. R. Bertino, *Life Sci.*, **3**, 1383 (1964).

(20) B. Anderson, E. H. Belcher, I. Chanarin, and D. L. Mollin, *Brit. J. Haematol.*, **6**, 439 (1960).

(21) J. A. Blair, *Biochem. J.*, **68**, 385 (1958).

(22) J. A. Blair, *ibid.*, **65**, 209 (1957).

(23) (a) S. Futterman and M. Silverman, *J. Biol. Chem.*, **224**, 31 (1957); (b) J. S. Dinning, J. T. Sime, and P. L. Day, *Federation Proc.*, **15**, 549 (1956).

(24) (a) T. Taira, *Nature*, **189**, 231 (1961); (b) F. Bergmann and H. Kwietny, *Biochim. Biophys. Acta*, **28**, 613 (1958); (c) H. Wieland and R. Leibig, *Ann.*, **555**, 146 (1944); (d) H. G. Petering and J. A. Schmitt, *J. Am. Chem. Soc.*, **72**, 2995 (1950); (e) F. Korte and H. Bannuscher, *Ann.*, **62**,2 126 (1959).

Synthesis and Pharmacological Action of 3-Amino-2,1-benzisothiazoles

ROBERT F. MEYER, BETTY L. CUMMINGS, PAUL BASS,[1a] AND H. O. J. COLLIER[1b]

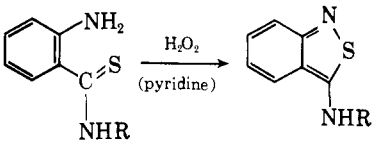
Research Division, Parke, Davis and Company, Ann Arbor, Michigan, and Hounslow, England

Received January 7, 1965

A series of 21 novel 3-amino-2,1-benzisothiazoles was prepared and evaluated for certain pharmacological effects. Some members show potent gastric antisecretory activity in the rat, antinociceptive action in the mouse, and antibradykinin activity in the guinea pig. The structure–activity relationships indicate that these three biological actions are not interrelated to a specific chemical moiety.

Continuous effort is exerted to develop novel heterocyclic systems that possess interesting pharmacological activities. This had led us to the synthesis and testing of a series of 3-amino-2,1-benzisothiazoles. Pharmacological observations indicated that certain independent members of the series possess gastric antisecretory, antibradykinin, antinociceptive, and mild antierythema activity.

Chemistry.—Although 2,1-benzisothiazole (thioanthranil) itself has long been known[2] and was prepared both by reductive cyclization[2] from *o*-nitro-α-toluenethiol and recently by oxidative cyclization[3] from *o*-amino-α-toluenethiol, no 3-amino- or 3-substituted amino-2,1-benzisothiazoles are reported in the literature. Goerdeler and co-workers oxidized some β-aminothiocrotonamides to give 5-aminoisothiazoles.[4] We prepared 3-amino- and 3-monosubstituted amino-



(1) (a) Department of Pharmacology. (b) Department of Pharmacology, Hounslow, England.

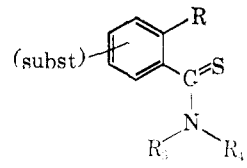
(2) S. Gabriel and T. Posner, *Ber.*, **28**, 1027 (1895); S. Gabriel and R. Stelzner, *ibid.*, **29**, 161 (1896).

(3) J. Goerdeler and J. Kandler, *ibid.*, **92**, 1679 (1959).

(4) J. Goerdeler and H. W. Pohland, *Ber.*, **94**, 2950 (1961); **96**, 526 (1963).

Table I

Thiobenzamides

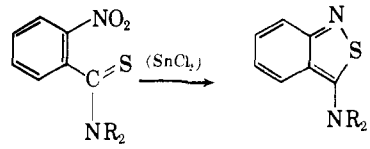


Starting material for 2,1-benzisothiazole	R	M.p., °C.	Recrystn. solvent[a]	Yield, %	Formula	% calcd. C	% calcd. H	% found C	% found H
1	NH_2	121–122	E or W	71	$C_7H_8N_2S$[b]				
2	NH_2	107–108[c]	B	65	$C_8H_{10}N_2S$	57.80	6.06	57.91	5.94
3	NH_2	67–68[c]	B	81	$C_9H_{12}N_2S$	59.97	6.71	60.06	6.84
4	NH_2	94–95[c]	B	65	$C_{10}H_{14}N_2S$	61.82	7.26	61.66	7.40
5	NH_2	99–100	B	68	$C_{10}H_{14}N_2S$	61.82	7.26	62.10	7.32
6	NH_2	94–95	CT	53	$C_{10}H_{16}N_2S$	63.42	7.74	63.12	7.47
7	NH_2	88–89[c]	CT	46	$C_{11}H_{16}N_2S$	63.42	7.74	62.98	7.73
8	NH_2				$C_{14}H_{10}N_2S$[d]				
9	NH_2	122–123	P	86	$C_{13}H_{12}N_2S$	68.39	5.30	68.52	5.42
10	NO_2	160–161[c]	B	77	$C_9H_{10}N_2SO_2$	51.41	4.79	51.58	4.84
11	NO_2	129–130[c]	P	91	$C_{11}H_{12}N_2O_2S$	55.92	5.12	55.93	5.21
12	NH_2	114–115	P	32	$C_8H_9BrN_2S$	39.20	3.70	39.44	3.81
13	NO_2	159–160	P	68	$C_9H_9ClN_2O_2S$	44.17	3.71	44.43	3.57
14	NH_2	150–151	P	69	$C_7H_7ClN_2S$[e]				
15	NH_2	99–100	CT	59	$C_8H_9ClN_2S$	47.87	4.52	47.56	4.33
16	NO_2	126–127	P	69	$C_9H_9ClN_2O_2S$	44.17	3.71	44.49	3.48
17	NO_2	182–184	P	58	$C_{11}H_{11}ClN_2O_4S$	46.08	3.87	46.23	3.93
18	NH_2	102–103[c]	P	71	$C_{10}H_{14}N_2S$	61.78	7.26	61.74	7.25
19	NH_2	92–93	P	45	$C_{10}H_{14}N_2OS$	57.10	6.71	57.13	6.38
20	NH_2	114–115	P	66	$C_{11}H_{16}N_2O_2S$	54.97	6.71	55.10	6.78
21	NH_2	131–132	P	70	$C_{11}H_{16}N_2O_2S$	54.97	6.71	55.25	6.93

[a] B = benzene, E = ethanol, T = tetrahydrofuran, W = water, P = 2-propanol, A = acetonitrile, Et = ether, CT = carbon tetrachloride. [b] A. Reissert and F. Grube [*Ber.*, **42**, 3710 (1909)] report m.p. 121.5°. [c] This melting point was taken on a Fisher-Johns block and was not corrected. In order to obtain the corrected melting point, 6° has to be subtracted from the given uncorrected melting point. [d] Low-melting, crude thioamide was used without purification for the next step. [e] Ch. Grundmann and H. Ulrich [*J. Org. Chem.*, **24**, 272 (1959)] report m.p. 150–151°.

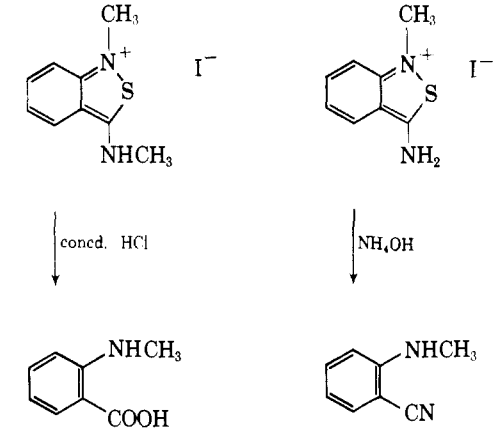
2,1-benzisothiazoles by oxidative cyclization of the corresponding *o*-aminothiobenzamides.

The 3-disubstituted amino-2,1-benzisothiazoles were conveniently obtained by reductive cyclization of the corresponding *o*-nitrothiobenzamides.



The N-mono- and N,N-disubstituted thioamides were obtained readily from the corresponding amides by reaction with phosphorus pentasulfide in pyridine followed by hydrolysis of the phosphorus compound by heating it in water preferably in presence of an organic solvent.

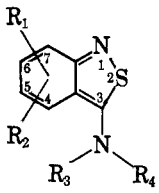
The 2,1-benzisothiazoles were yellow crystalline compounds which formed stable salts with mineral acids and could be alkylated with reactive haloalkyls. In three cases the alkylation was carried out with methyl iodide. Methylation in two of the three cases was proved to occur at the ring nitrogen by hydrolysis to a known derivative of anthranilic acid. 1-Methyl-3-methylamino-2,1-benzisothiazolium iodide on hydrolysis with refluxing concentrated HCl gave N-methylanthranilic acid; on the other hand, 1-methyl-3-amino-2,1-benzisothiazolium iodide on treatment with aqueous ammonia gave *o*-methylaminobenzonitrile.



All 2,1-benzisothiazoles prepared showed characteristic absorption in the ultraviolet at λ_{max} *ca.* 380 and *ca.* 230 mμ. Furthermore, the blue-green color reaction of all 2,1-benzisothiazoles prepared in the presence of alcoholic ferric chloride proved very useful for identification purposes. This blue-green color faded rapidly on addition of a few drops of water which suggested that a metal complex (but no chelate) was formed which breaks up in water. Absorption of the metal complex in the ultraviolet occurred at the same wave length as the 2,1-benzisothiazoles. Metal complexing was observed with other metal salts too, such as cobaltous chloride. Recrystallization from absolute etha-

TABLE II

3-AMINO-2,1-BENZISOTHIAZOLES



No.	R_1, R_2	R_3, R_4	M.p., °C.	Recrystn. solvent[a]	Yield, %	Formula	% calcd. C	% calcd. H	% calcd. N	% found C	% found H	% found N
1	H, H	H, H	178–179[b]	B	62	$C_7H_6N_2S$[c]	55.97	4.04	18.65	56.28	4.18	18.48
1a[d]	H, H	H, H	>200 dec.	E	88	$C_7H_6N_2S \cdot CH_3I$	32.89	3.11		32.84	3.00	
1b[d]	H, H	H, H	112–113	T + W	76	$C_7H_6N_2S \cdot HCl$	45.04	3.78	19.01	45.17	3.86	19.09
2	H, H	H, CH_3	206–207[b]	P	46	$C_8H_8N_2S$	58.51	4.91	17.06	58.46	4.64	17.22
2a[d]	H, H	H, CH_3	210 dec.[b]	A	66	$C_8H_8N_2S \cdot CH_3I$	35.30	3.62		35.41	3.72	
2b[d]	H, H	H, CH_3	>200 dec.	P	75	$C_8H_8N_2S \cdot HCl$[e]	47.87	4.52		48.13	4.51	
3	H, H	H, C_2H_5	195–196[b]	E	52	$C_9H_{10}N_2S$	60.64	5.60	15.72	60.70	5.59	15.77
4	H, H	H, $(CH_2)_2CH_3$	167–168[b]	P	54	$C_{10}H_{12}N_2S$	62.46	6.29		62.71	6.27	
5	H, H	H, $CH(CH_3)_2$	188–189	B	45	$C_{10}H_{12}N_2S$	62.46	6.29		62.66	6.24	
6	H, H	H, $CH_2CH(CH_3)_2$	179–181	P	69	$C_{11}H_{14}N_2S$	64.04	6.84		63.98	6.60	
7	H, H	H, $CH(CH_3)C_2H_5$	206–207[b]	E	58	$C_{11}H_{14}N_2S$	64.04	6.84		64.06	6.80	
8	H, H	H, $CH_2C_6H_5$	181–182	E	59	$C_{14}H_{12}N_2S$	69.97	5.03		70.24	5.06	
9	H, H	H, C_6H_5	>240 dec.	E	50	$C_{13}H_{10}N_2S$	69.00	4.46		68.99	4.20	
10	H, H	CH_3, CH_3	120–121[b]	Et or W	24	$C_9H_{10}N_2S$	60.64	5.66	15.72	60.68	5.74	15.58
11	H, H	$(CH_2)_4$	144–145[b]	P	22	$C_{11}H_{12}N_2S$	64.67	5.92	13.71	64.65	5.83	13.66
11b[d]	H, H	$(CH_2)_4$	210–215[b]	P	72	$C_{11}H_{12}N_2S \cdot HCl$[f]	54.88	5.45	11.64	55.03	5.22	11.73
12	H, 5-Br	H, CH_3	244–249	E	30	$C_8H_7BrN_2S$	39.52	2.90		39.74	2.87	
13	H, 5-Cl	CH_3, CH_3	140–141[b]	P	29	$C_9H_9ClN_2S$	50.92	4.27	13.17	50.83	4.04	13.05
14	H, 6-Cl	H, H	191–192	B	87	$C_7H_5ClN_2S$	45.53	2.73	15.17	45.79	2.49	15.21
15	H, 6-Cl	H, CH_3	250–251	P	33	$C_8H_7ClN_2S$	48.37	3.55	14.10	48.60	3.61	13.92
16	H, 6-Cl	CH_3, CH_3	134–135[b]	P	57	$C_9H_9ClN_2S$	50.92	4.27	13.17	50.82	4.47	13.38
16a[d]	H, 6-Cl	CH_3, CH_3	254–260	A	92	$C_9H_9ClN_2S \cdot CH_3I$	33.87	3.41		33.99	3.34	
17	H, 6-Cl	$(CH_2CH_2)_2O$	125–126	P	33	$C_{11}H_{11}ClN_2OS$	51.86	4.35	11.00	51.60	4.12	11.12
18	H, 6-CH_3	H, C_2H_5	225–226[b]	E	60	$C_{10}H_{12}N_2S$	62.46	6.28		62.50	6.21	
19	H, 6-OCH_3	H, C_2H_5	181–182	B	43	$C_{10}H_{12}N_2OS$	57.65	5.82		57.69	5.72	
20	5-OCH_3, 6-OCH_3	H, C_2H_5	187–188	E	62	$C_{11}H_{14}N_2O_2S$	55.44	5.92		55.49	5.97	
20a	5-OCH_3, 6-OCH_3	H, C_2H_5	240–242	P	78	$C_{11}H_{14}N_2O_2S \cdot HCl$	48.08	5.50		48.08	5.50	
21	5-OCH_3, 7-OCH_3	H, C_2H_5	184–185	E	58	$C_{11}H_{14}N_2O_2S$	55.44	5.92	11.75	55.65	5.89	11.80

[a] Footnote *a*, Table I. [b] This melting point was taken on a Fisher-Johns block and was not corrected. In order to obtain the corrected melting point, 6° has to be subtracted from the given uncorrected melting point. [c] S: calcd., 21.35; found, 21.30. [d] a = methiodide, b = hydrochloride. [e] Cl^-: calcd., 17.67; found, 17.25. [f] Cl^-: calcd., 14.73; found, 14.30.

nol gave a 2:1 complex of the 3-dimethylaminobenzisothiazole with cobaltous chloride.

Titration in 50% MeOH indicated that 3-amino-2,1-benzisothiazoles are basic (comparable in strength with *m*- or *p*-chloroaniline), 3-amino-2,1-benzisothiazole having $pK_a' = 3.8$, 3-methylamino-2,1-benzisothiazole $pK_a' = 3.9$, and 3-dimethylamino-2,1-benzisothiazole $pK_a' = 3.5$.

Experimental[5]

3-Alkylamino-2,1-benzisothiazoles. *o*-Amino-N-ethylthiobenzamide.—A mixture of 82 g. (0.5 mole) of *o*-amino-N-ethylbenzamide[6] and 111 g. (0.5 mole) of phosphorus pentasulfide in 200 ml. of pyridine was refluxed for 1.5 hr., cooled, and poured into 2 l. of ice-water. The yellow solid was removed by filtration and washed with water, then heated at reflux with a mixture of 1 l. of benzene and 500 ml. of water until two clear phases were obtained (5–6 hr.). The organic layer was separated, washed with water, concentrated, and cooled. The thioamide was obtained as a yellow crystalline solid (see Table I).

3-Ethylamino-2,1-benzisothiazole (3).—A solution of 36 ml. (0.315 mole) of 30% H_2O_2 was added dropwise with stirring to a solution of 54 g. (0.3 mole) of *o*-amino-N-ethylthiobenzamide in 100 ml. of pyridine at 35°. The reaction mixture was allowed to stand for 16 hr.; the yellow crystalline solid was removed by filtration, washed with water, and recrystallized from ethanol (see Table II): ultraviolet in absolute methanol, λ_{max} 378 230 mμ (E_{max} 478, 1948).

3-Dialkylamino-2,1-benzisothiazoles. *o*-Nitro-N,N-dimethylthiobenzamide.—A mixture of 194 g. of *o*-nitro-N,N-dimethylbenzamide, 222 g. of phosphorus pentasulfide, and 800 ml. of pyridine was refluxed for 1.5 hr. The reaction mixture was poured into 3 l. of ice-water, and the yellow solid was filtered and washed with water. The solid was suspended in a mixture of 2 l. of benzene and 500 ml. of water and refluxed until a clear two-phase system was obtained (about 4 hr.). The organic layer was washed with water and concentrated to small volume.

3-Dimethylamino-2,1-benzisothiazole (10).—A solution of 248 g. (1.1 moles) of stannous chloride dihydrate in 250 ml. of concentrated HCl was added dropwise to a well-stirred suspension of 105 g. (0.5 mole) of finely powdered *o*-nitro-N,N-dimethylthiobenzamide in 1 l. of concentrated HCl at 25°. The mixture was stirred an additional 3 hr. at 25°, then cooled in ice. The solid tin complex was removed by filtration and washed with benzene. The solid was suspended in a mixture of 1 l. of benzene and 500 ml. of water and, below 20°, an excess of 50% aqueous NaOH was added.

The benzene layer was separated, washed with water, and concentrated to small volume to give, on cooling, a yellow crystalline solid which was recrystallized readily from ether or water; ultraviolet in absolute methanol, λ_{max} 378, 232 mμ (E_{max} 499, 2200).

The **hydrochlorides** were prepared by adding slightly more than 1 equiv. of concentrated HCl to the alcoholic solution of the benzisothiazole. The **methiodides** were obtained by refluxing the benzisothiazole with an excess of CH_3I in acetonitrile for 3 hr.

Cobaltous Chloride Complex of 3-Dimethylamino-2,1-benzisothiazole.—A solution of 2 g. of 3-dimethylamino-2,1-benziso

(5) Melting points were determined on a Thomas–Hoover capillary melting point apparatus and are corrected unless stated otherwise. Some intermediates were prepared by reference methods: 4-methyl-2-nitrobenzoic acid [A. Claus and J. Joachim, *Ann.*, **266**, 210 (1892)] and 4-methoxy-2-nitrobenzoic acid were prepared by the method of L. Katz, L. S. Karger, W. Schroeder, and M. S. Cohen, *J. Org. Chem.*, **18**, 1399 (1953), and 4,5-dimethoxy-2-nitrobenzoic acid [R. Pschorr and C. Sumuleanu, *Ber.*, **32**, 3412 (1899)] and 2-amino-3,5-dimethoxy-N-ethylbenzamide according to P. Friedländer, "Fortschritte der Teerfarbenfabrikation," Vol. 17, J. Springer, Berlin, 1932, p. 473.

(6) R. H. Clark and E. D. Wagner, *J. Org. Chem.* **9**, 55 (1944).

thiazole in 25 ml. of alcohol was added with stirring to a solution of 6.7 g. of cobaltous chloride hexahydrate in 100 ml. of alcohol. Immediately a grass green precipitate was obtained. It was filtered, washed with alcohol, and dried at 80°, yielding 2.3 g. of product, m.p. 300–310° dec. Recrystallization from alcohol gave a dark green crystalline solid, m.p. 310–320° dec.; ultraviolet in absolute methanol, λ_{max} 379, 232 mμ (E_{max} 382, 1640).

Anal. Calcd. for $(C_9H_{16}N_2S)_2 \cdot CoCl_2$: C, 44.45; H, 4.15; Cl, 14.58; N, 11.52. Found: C, 44.79; H, 4.37; Cl, 14.53; N, 11.65.

Degradation of Methiodides. *o*-Methylaminobenzonitrile from 1-Methyl-3-amino-2,1-benzisothiazolium Iodide (1a).—A solution of 7 g. of **1a** in 150 ml. of boiling water was made alkaline with 10 ml. of concentrated NH_4OH solution. A tan oil separated which crystallized on cooling. A sample was recrystallized from 2-propanol yielding sulfur. Another sample recrystallized from CS_2 gave *o*-methylaminobenzonitrile, m.p. 70–71°, identical in every respect with a reference sample.

N-Methylanthranilic Acid from 1-Methyl-3-methylamino-2,1-benzisothiazolium Iodide (2a).—A solution of 2 g. of **2a** in 50 ml. of concentrated HCl was refluxed for 15 hr. The pale yellow solution was evaporated to dryness, and the solid was taken up in water and neutralized to give on cooling a crystalline precipitate of N-methylanthranilic acid, m.p. 176–178°, identical in every respect with a reference sample.

Pharmacology

Method.—A modified pylorus ligation technique[7] was used for studying the gastric antisecretory properties of the compounds. Forty-eight hours prior to testing, Sprague–Dawley male rats (100–120 g.) were placed in individual cages. They were deprived of their regular rations but had access to two cubes of sugar and water *ad libitum*. This "fasting" period was sufficient to empty the stomach and eliminate the necessity for gastric lavage. Following pylorus ligation, wound clips were used to close the incision. At this time, the compound or carrier vehicle was administered subcutaneously. Four hours after ligation, the animals were rendered unconscious with CO_2. The stomach secretions were centrifuged and volume, less sediment, was recorded.

In the preliminary studies each compound was administered at a dose level of 25 mg./kg. to six rats. The compounds were dissolved in 0.9% saline or suspended in 1% Methocel. A carrier vehicle control group was included in each test. The volume of secretion was calculated/100 g. of body weight. The mean value of the control group represented 100% secretion; the mean value of each group receiving a compound was compared to the control. Compounds at doses of 25 mg./kg. which did not reduce volume of secretion by 25% were considered inactive.

Compounds that reduced secretion by 25% or more were tested further at log interval doses. Four or six animals per group were used in this portion of the study. Calculations were performed as above, and the per cent reduction of secretion *vs.* mg./kg. dose was plotted on semilogarithmic paper. A line was interpolated between the three or four points of the graph and the value that would produce a 50% reduction of secretion (ED_{50}) was read from the graph. An ED_{50} of less than 10 mg./kg. was confirmed and the average value reported.

Five compounds of the series were investigated for cholinergic, adrenergic, and ganglionic blocking properties in an anesthetized dog preparation. Each compound was tested for antagonization of the blood pressure effects of acetylcholine (15 γ/kg.), epinephrine (2 γ/kg.), and 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP, 15 γ/kg.). Each of the five benzisothiazole derivatives were administered every 20 min. in increasing log intervals (log 2) starting with 0.5 mg./kg. to an accumulative dose of 64 mg./kg. Each injection of compound was followed by an agonist substance at 5-min. intervals. Both agonist and antagonist were administered intravenously. Water-insoluble compounds were dissolved in 50% propylene glycol.

The test of antinociceptive activity was performed in mice in two parts: (1) responsiveness to an artery clip placed on the tail[8]; and (2) ability to stay on the surface of a slowly rotating drum.[9] Drugs were administered subcutaneously in solution in 0.9% NaCl or in suspension in 20% gum acacia in water. An artery clip of 200–300-g. opening tension was placed for 10 sec. on the base of each mouse's tail. The median effective dose (ED_{50}) of a drug was that at which 50% of animals failed to turn and bite the clip. Immediately after this part of the test was completed, mice were placed on the wire mesh covered surface of a cylindrical drum, 12-in. diameter, rotating once/min. The median discoordinating dose (FD_{50}) was that at which 50% of animals fell from the drum. On the basis of experiments with known analgesics,[10] a value of 1.5 or more for the ratio FD_{50}/ED_{50} was taken to indicate valid antinociceptive action.

Antibradykinin activity was tested against bradykinin-induced bronchoconstriction in the guinea pig.[11] Animals were anesthetized with urethan (1.25 g./kg. i.p.) and prepared for recording changes in the resistance of the lungs to inflation during intermittent ventilation at constant positive pressure. Bradykinin, histamine, and test drug were administered intravenously on a 5-min. dosage cycle. The minimal effective dose (MED) was the least dose of drug that depressed by >50% the bronchoconstrictor response to a dose of bradykinin that was double the dose before drug, without depressing the response to histamine. When the response to histamine was also depressed, the activity is recorded as nonspecific. Compounds 1 and 15 had such a nonspecific response.

Results

The most active gastric secretory inhibitor was **2** (Table III). Generally, with increasing substitution at the 3-amino function, activity was decreased (**6–8** and **11**). Whereas ring substitution in the lower 3-monoalkylamino series reduced activity (for example, **12** and **15**), most of the antisecretory activity in the 3-dimethylamino series was retained after nuclear substitution, especially when the substituent was chlorine (**13** and **16**).

A moderate degree of specific antibradykinin activity is present in many members of the series, **2–5**, **10**, **13**, **14**, **16–18**, and **20** having activity approximately equal to or greater than that of aminopyrine.[11] In general, substitution in the ring did not greatly affect activity, but lengthening of the alkyl chain lessened it (**6–9** and **11**).

Antinociceptive activity, when present, was also of the same order as that of aminopyrine in this test.[10] Antinociceptive activity was rarer than antibradykinin, but with the exception of **19**, all compounds showing antinociceptive also showed antibradykinin activity. In the antinociceptive test, also, higher homologs were less potent. Methoxy substitution in position 6 or 5,6 had little effect, but substitution with chlorine markedly attenuated the compounds' action (**14** and **16**).

Compounds **2**, **10**, **16**, **10**, and **21** were tested for certain autonomic nervous system effects. They were devoid of anticholinergic, ganglionic, or adrenergic blocking properties at accumulative doses as high as 64 mg./kg. Compounds **2** and **16** demonstrated a prolonged hypotensive effect at the 16- and 32-mg./kg. dose.

Discussion

The question arises whether the pharmacological activities shown by compounds of this series are correlated. Spearman's coefficient of rank correla-

(7) H. Shay, D. C. H. Sun, and M. Gruenstein, *Gastroenterology*, **26**, 906 (1954).

(8) C. Bianchi and J. Franceschini, *Brit. J. Pharmacol.*, **9**, 280 (1954).

(9) H. O. J. Collier, R. A. Hall, and E. C. Fieller, *Analyst*, **74**, 592 (1949).

(10) H. O. J. Collier, "Pharmacometrics," Vol. 1, D. R. Laurence and A. L. Bacharach, Ed., Academic Press Inc., New York, N. Y., 1964, p. 183.

(11) H. O. J. Collier and P. C. Shorley, *Brit. J. Pharmacol.*, **15**, 601 (1960).

TABLE III

PHARMACOLOGICAL ACTIVITIES[a]

No.[b]	Gastric antisecretory, s.c. in rat, ED_{50}	Antibradykinin, i.v. in guinea pig, MED	Antinociceptive, s.c. in mouse, ED_{50}	Antinociceptive, s.c. in mouse, FD_{50}
1	58	2[c]	87	70
1a	>25	16	39	78
2	1.5	8	152	>400
2a	2.4	32	34	59
2b	1.8	8	56	54
3	3.0	1	37	255
4	>25	8	128	239
5	3.6	8	66	360
6	23	>32	>200	>200
7	19	16	>400	>400
8	>50	>16	>200	>200
9	>25	>16	>200	>200
10	4.8	8	79	115
11	>50	16	203	>400
12	>25	>8	>200	>200
13	2.8	4	>400	>400
14	11	8	>200	>200
15	54	16[c]	>100	>100
16	3.1	8	>200	>200
16a	50	>4	>25	>25
17	6	2	>200	>200
18	15	4	250	>800
19	4.2	>32	63	136
20	7.4	4	56	136
20b	11	1	77	114
21	>25	>32	>200	>200
Aminopyrine	82	8	99	160

[a] All values are in mg./kg. [b] a = methiodide, b = hydrochloride. [c] Nonspecific response.

tions[12] were determined between pairs of properties where the potencies had been tested. The coefficients (r_s) were not significant at a 0.5 level between the compounds' antisecretory–antibradykin, antisecretory–antinociceptive, or antibradykinin–antinociceptive potencies. This would indicate no relationship of pharmacological properties among compounds in this series based on their potencies in the individual tests.

(12) R. G. D. Steel and J. H. Torrie, "Principles and Procedures of Statistics" McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p. 409.

Some of these compounds were tested for their ability to delay the onset of erythema upon exposure to ultraviolet irradiation.[13] Previous experience with established nonsteroidal antiinflammatory compounds had shown some correlation between antiultraviolet erythema and antibradykinin activity.[10] A few members of the benzisothiazole series effectively delayed skin erythema, **16** being the most active and showing potency of the order of phenylbutazone. However, **16** was not the most active antibradykinin compound. Generally, within this series, the relationships between activity against ultraviolet erythema and against bradykinin were poor. Nevertheless, there is evidence of the existence of both properties in this series, but to test this relationship more closely would require more precise experimental values.

Nonsteroid antiinflammatory drugs are active in the antibradykinin test,[11] but, except for aminopyrine and antipyrine (phenazole), they are inactive in the antinociceptive test used.[10] On the contrary, narcotic analgesics are inactive in the antibradykinin and active in the antinociceptive test. Some of the benzisothiazoles resemble aminopyrine and antipyrine in being active in both tests. Aminopyrine also resembles these benzisothiazoles in showing antisecretory activity.

Highest potency of the gastric antisecretory property was obtained with lower 3-monoalkylamino-2,1-benzisothiazoles. The site of action of these compounds has not been elucidated. It apparently is not related to any anticholinergic or ganglionic-blocking properties since the series is devoid of such autonomic activity.

Acknowledgments.—We would like to thank Misses M. A. Patterson and R. A. Purdon for their assistance with the gastric antisecretory tests, Dr. D. A. McCarthy for the autonomic nervous system experiments, and Mr. C. Schneider, Mrs. P. J. Piper, Mrs. R. J. Skerry, Misses S. Horwood-Barrett, G. C. Clarke, and P. Shorley for the antibradykinin and antinociceptive tests. Appreciation is also extended to Dr. E. F. Elslager for encouragement, to Mr. C. Childs and associates for the microanalytical data, to Dr. J. M. Vandenbelt and associates for spectral data, Miss C. Eady for tabulating pharmacological results, and Mr. W. M. Pearlman for catalytic hydrogenations.

(13) C. V. Winder, J. Wax, V. Burr, M. Been, and C. E. Rosiere, *Arch. Intern. Pharmacodyn.*, **116**, 261 (1958).