TABLE I

4,6-DIAMINO-1-ALKYL-1,2-DIHYDRO-2,2-DISUBSTITUTED-*s*-TRIAZINE HYDROCHLORIDES (Ib)

	R_1	R_2	R_3	M.p., °C.[a]	Yield, %	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Ib-1	$C_6H_5CH_2CH_2$	CH_3	CH_3	195–198	20	55.11	55.34	7.15	7.01	24.86	24.83
Ib-2	$C_6H_5CH_2CH_2$	$CH_3(CH_2)_3$	H	240–241	7	56.95	56.89	7.51	7.62	23.72	23.46
Ib-3	$C_6H_5CH_2CH_2$	3,4-$(CH_2O_2)C_6H_3$	H	208–212	21	57.82	57.97	5.39	5.62	18.71	18.97
Ib-4	$C_6H_5CH_2CH_2$	C_6H_{11}	H	249–250	13	60.78	60.66	7.50	7.67	20.85	20.56
Ib-5	$C_6H_5CH_2CH_2$	$-(CH_2)_2-CH(CH_3)-(CH_2)_2-$	—	223–224	19	60.79	61.02	7.80	7.76	20.85	21.25
Ib-6	$CH_3(CH_2)_4$	*p*-FC_6H_4	H	201–203	20	53.26	53.18	6.75	6.69	22.32	22.10
Ib-7	$(CH_3)_2-CH-(CH_2)_2$	*p*-FC_6H_4	H	199–200[b]	51	17.41	17.47	6.22	5.97	21.69	25.01
Ib-8	$CH_3-(CH_2)_7$	*p*-ClC_6H_4	H	196 dec.[b]	20	51.21	51.11	6.84	6.72	21.07	21.12
Ib-9	$C_6H_5CH_2CH_2$	*p*-FC_6H_4	H	214–216	33	58.77	59.09	5.22	5.60	20.23	19.82
Ib-10	$C_6H_5CH_2CH_2$	*p*-$CH_3SC_6H_4$	H	229–230	20	57.51	57.61	5.60	5.92	18.63	18.61

[a] All compounds were recrystallized from ethanol–ether. [b] Nitrate salts. Prepared by substituting nitric acid for hydrochloric acid as catalyst (see Experimental section).

section for 4,6-diamino-1-amyl-1,2-dihydro-2-(*p*-fluorophenyl)-*s*-triazine hydrochloride. Table I summarizes some representative compounds while Table II lists infrared and ultraviolet data.

Yields in reactions with aliphatic aldehydes were very low (*e.g.*, Ib-2) while certain other carbonyl compounds (*i.e.*, pyridine-4-carboxaldehyde, *p*-dimethylaminobenzaldehyde and cycloheptanone) gave unpurifiable mixtures. An examination of Table II reveals that all infrared spectra of Ib exhibit strong peaks near 6.4 and 6.6 μ, supporting the assignment of Degraw[12] of absorptions due to the triazine ring near 6.40 and 6.60 μ.

TABLE II

SPECTRAL DATA OF Ib

Compound	Ultraviolet (Ethanol) λ_{max}	ϵ	Infrared (KBr)[a] μ			
Ib-1	246	10650	5.95	6.15	6.35	6.70
Ib-2	245	7290	5.95	6.10	6.35	6.68
Ib-3	248	12200	5.96	6.10	6.40	6.70
Ib-4	246	7200	5.96	6.01	6.35	6.71
Ib-5	247	8460	5.96	6.10	6.50	6.75
Ib-6	250	6780	5.96	6.10	6.40	6.70
Ib-7	251	6340	5.96	6.09	6.35	6.62
Ib-8	251	7070	5.95	6.05	6.30	6.66
Ib-9	249	8680	5.97	6.10	6.40	6.71
Ib-10	264	20590	5.95	6.10	6.40	6.72

[a] Strong intensity bands in all cases.

Compounds of type Ib were administered subcutaneously to guinea pigs at 20, 40, or 50 mg./kg. and blood glucose levels measured. Blood glucose was determined on diluted whole blood samples using a micro-adaptation of the method of Hoffman[13] on an Auto-Analyzer. Most derivatives of Ib had little effect on blood glucose levels, however. Table III summarizes those compounds having a mild effect. Increasing doses did not increase this effect. For comparison, data on phenethylbiguanide hydrochloride are included in Table III. They show that compounds of type Ib do not approach the potency of phenethylbiguanide as hypoglycemic agents. Apparently, any similarities in structure to the cyclic form of II are either too subtle or are offset by the possible change in pharmacodynamic properties due to the difference in basic strength of diamino dihydrotriazines[3a] as compared to biguanides (*e.g.*, pK_a of 9–10 *vs.* pK_a *ca.* 12 for the latter).

Possible antitumor activity is presently under study while no antiviral activity could be detected in representative structures Ib.

(12) J. J. Degraw, L. O. Ross, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 1933 (1961).

(13) W. S. Hoffman, *J. Biol. Chem.*, **120**, 51 (1937).

TABLE III

ADMINISTRATION OF Ib TO GUINEA PIGS[a]

Compound	R_1	R_2	R_3	Dose, mg./kg.	% Fall in blood glucose[b]
Ib-1	$C_6H_5CH_2CH_2$	CH_3	CH_3	20	25
				50	20
Ib-2	$C_6H_5CH_2CH_2$	n-C_3H_7	H	20	25
Ib-9	$C_6H_5CH_2CH_2$	*p*-FC_6H_4	H	20	22
Ib-10	$C_6H_5CH_2CH_2$	*p*-$CH_3SC_6H_4$	H	40	18
PhenethylbiguanideHCl				15	40[c]

[a] Subcutaneous administration. [b] Pooled values for 6 animals at 3 hr. post-administration. Percentage fall from normal blood glucose level. [c] Value for 6 animals at 4 hr. post-administration.

Experimental

4,6-Diamino-1-amyl-1,2-dihydro-2-(*p*-fluorophenyl)-*s*-triazine Hydrochloride (Ib-6).—In a 250 ml., 3-necked flask under a Soxhlet extractor containing calcium sulfate was placed 2.08 g. (0.010 mole) of N^1-(*n*-amyl)-biguanide hydrochloride, 1.36 g. (0.011 mole) of *p*-fluorobenzaldehyde, 0.10 ml. (0.0012 mole) 12 *N* hydrochloric acid and 75 ml. of ethanol. The solution was placed under a nitrogen atmosphere and refluxed for 24 hr. Tests on reaction samples with copper ammonium sulfate[14] solution showed all biguanide to be gone after approximately 20 hr. of reflux. Concentration to 25 ml. under vacuum and cooling yielded 0.620 g. (20%) of white solid in two crops, m.p. 201–203°. Certain derivatives of Ib required the addition of ether and cooling in order to induce crystallization. An analytical sample was prepared by recrystallizing from ethanol–ether. Table I summarizes physical properties of Ib.

Acknowledgment.—The author is indebted to Mr. C. F. Gerber for the blood glucose determinations and to Mr. Nelson Treadway, Jr., for his technical assistance.

(14) Ref. 3a, footnote 35.

Synthesis of Substituted 2-Phenyl-1,4-benzothiazin-3(4H)-ones and their Activity as Inhibitors of 1,4-Dipyrrolidino-2-butyne

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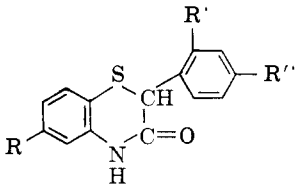
Received August 15, 1962

During the course of pharmacological evaluation of a variety of compounds, 4-(2-diethylaminoethyl)-2-

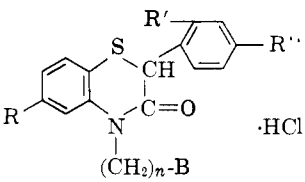
TABLE I



	R	R'	R''	M.p., °C.[a]	Yields, %	Formula	Nitrogen analysis, % Calcd.	Found
A	Cl	H	H	217–219	36	$C_{14}H_{10}ClNOS$	5.08	5.31
B	CF_3	H	H	216–217	65	$C_{15}H_{10}F_3NOS$	4.53	4.62
C	H	Cl	H	199–200	89	$C_{14}H_{10}ClNOS$	5.08	5.38
D	H	H	Cl	197–199	71	$C_{14}H_{10}ClNOS$	5.08	5.18
E	H	H	OCH_3	192–193	55	$C_{15}H_{13}NO_2S$	5.16	5.04

[a] All compounds were purified by crystallization from acetonitrile.

TABLE II



No.	R	R'	R''	n	B	M.p., °C.[a]	Formula	Analyses, % Chlorine Calcd.	Chlorine Found	Nitrogen Calcd.	Nitrogen Found
I	H	H	H	2	$N(C_2H_5)_2$	173–175[b]	$C_{20}H_{25}ClN_2OS$	9.41	9.68	7.43	7.72
II	H	H	H	3	$N(CH_3)_2$	210–212[b]	$C_{19}H_{23}ClN_2OS$	9.77	9.75	7.72	7.46
III	H	H	H	2		225–227[b]	$C_{21}H_{23}ClN_2OS$	9.11	9.22	7.20	7.00
IV	H	H	H	2	$N[CH(CH_3)_2]_2$	238–240	$C_{22}H_{29}ClN_2OS$	8.75	8.67	6.92	7.01
V	H	H	H	2		237–239[b]	$C_{20}H_{23}ClN_2O_2S$	9.07	8.96	7.17	7.22
VI	H	H	H	2		269–270	$C_{21}H_{27}Cl_2N_3OS$[c]	16.11	15.94	9.54	9.60
VII[d]	Cl	H	H	2	$N(C_2H_5)_2$	208–210	$C_{20}H_{24}Cl_2N_2OS$	17.13	17.32	6.81	6.51
VIII	CF_3	H	H	2	$N(C_2H_5)_2$	166–168	$C_{21}H_{24}ClF_3N_2OS$	7.97	8.13	6.30	6.22
IX	H	Cl	H	2	$N(C_2H_5)_2$	170–172	$C_{20}H_{24}Cl_2N_2OS$	17.13	16.97	6.81	6.66
X	H	H	Cl	2	$N(CH_3)_2$	220–222	$C_{18}H_{20}Cl_2N_2OS$	18.50	18.38	7.31	7.01
XI	H	H	Cl	2	$N(C_2H_5)_2$	155–157	$C_{20}H_{24}Cl_2N_2OS$	8.62[e]	8.74	6.81	6.74
XII	H	H	OCH_3	2	$N(C_2H_5)_2$	144–146	$C_{21}H_{27}ClN_2O_2S$	8.71	8.74	6.89	6.60

[a] These salts were crystallized from ethanol, except I and II (acetonitrile), IX (isopropyl alcohol) and X (methanol). [b] A. Funke, G. Funke and B. Millet, *Bull. Soc. Chim. France*, **28**, 1524 (1961), prepared these compounds using different experimental conditions and reported these melting points: I, 158–160°; II, 175°; III, 202–204°; and V, 198–200°. [c] Dihydrochloride salt. [d] This material and compound A of Table I were prepared by E. Spitzmiller. [e] Ionic chloride.

phenyl-1,4-benzothiazin-3(4H)-one hydrochloride (compound I, Table II) was found to be active in inhibiting the tremors produced by 1,4-dipyrrolidino-2-butyne.[1] The ability of a compound to antagonize the effects of 1,4-dipyrrolidino-2-butyne has been made the basis of a method for the selection of compounds for evaluation in Parkinsonism.[2] In order to establish a structure–activity relationship for this class of compounds, the modified structures listed in Table II were prepared. These products were obtained, usually in 60–85% yields, by heating a suspension of 2-phenyl-1,4-benzothiazin-3(4H)-one[3] (or a substituted analog listed in Table I) with one equivalent of sodamide in toluene, followed by the appropriate basically-substituted alkyl halide and then refluxing the mixture for three hours. The infrared data were in agreement with the assigned structures. New benzothiazin-3-ones (Table I) were obtained from the corresponding derivatives of 2-aminobenzenethiol and α-haloarylacetic acids and esters.[4,5]

The compounds were administered subcutaneously or orally to mice 50 min. before intraperitoneal injection of 20 mg./kg. of 1,4-dipyrrolidino-2-butyne dihydrochloride. The ED_{50} of I required to inhibit *all* tremors after a 2 hr. period was 32 mg./kg. by the subcutaneous route. The estimated LD_{50} of I was 625 mg./kg. (s.c.). With the exception of II, which had the same activity as I, modifications of the basic side-chain (III–VI) gave compounds which were less than one-third as active as I. Of the compounds containing substituents in either the nucleus or the phenyl ring in the 2-position, only the 4-chlorophenyl analog, XI, was more potent than I. The ED_{50} of XI was 21 mg./kg. (s.c.) and 53 mg./kg. (oral) and the LD_{50} was estimated at 705 mg./kg. (s.c.). The ED_{50} of benztropine (3-diphenylmethoxy-

(1) Tremorine®.

(2) M. E. Farquharson and R. G. Johnston, *Brit. J. Pharmacol.*, **14**, 559 (1959), and references cited therein.

(3) O. Unger and G. Graff, *Ber.*, **30**, 2389 (1897). An improved procedure for the preparation of this material is given in the Experimental part.

(4) J. M. Bruce and F. K. Sutcliffe, *J. Chem. Soc.*, 4793 (1957).

(5) F. G. Baddar, *J. Am. Chem. Soc.*, **76**, 1161 (1954).

tropane methanesulfonate) in the same test was 4.3 mg./kg. (s.c.). The reported LD_{50} of benztropine is 55 mg./kg. (s.c.).[7] Compound XI, although less potent than benztropine, has a better therapeutic ratio in this test.

Experimental

2-Phenyl-1,4-benzothiazin-3(4H)-one.—A well-stirred solution of 325 g. (2.6 moles) of 2-aminobenzenethiol in 600 ml. of xylene was treated in one portion with a solution of 281 g. (1.3 moles) of α-bromophenylacetic acid[4] in 600 ml. of xylene. Cooling in a water bath was necessary to maintain the reaction temperature below 80°. After the exothermic reaction subsided, the mixture was heated to (and maintained at) 100° for 30 min. while 21 ml. of water distilled azeotropically from the reaction mixture. The latter was then heated at 137–142° for 1 hr. (during which time an additional 2 ml. of water was collected), cooled to room temperature and the precipitate filtered and washed with xylene. The air-dried solid (554 g.) was triturated with 1 l. of water to remove 2-aminobenzenethiol hydrobromide. It was suspended in 1 l. of 5% sodium bicarbonate and filtered to give 290 g. of product, m.p. 198–204°. After trituration with 1 l. of cold acetonitrile, the colorless product weighed 285 g. (91%) and melted at 202–204°. Crystallization from ethanol raised the m.p. to 205–206° (reported[3] m.p. is 204°), λ_{max}^{Nujol} 3.15, 5.95 μ.

4-(2-Diethylaminoethyl)-2-phenyl-1,4-benzothiazin-3(4H)-one Hydrochloride (I).—A slurry of 50.0 g. (1.28 moles) of sodamide in 3 l. of toluene was treated with 300 g. (1.24 moles) of the above material (m.p. 202–204°) in one portion; the mixture was stirred and refluxed for 1 hr., cooled and treated with 200 g. (1.48 moles) of 2-diethylaminoethyl chloride [b.p. 50–55° (30 mm.)]. The resulting solution was stirred at room temperature for 30 min., refluxed for 3 hr., cooled and treated with 300 ml. of water. The layers were separated and the organic phase washed with 100 ml. of water; the aqueous layer was discarded and the organic phase then extracted with 900 ml. of 2 *N* hydrochloric acid. The acidic aqueous phase was cooled and treated with 400 ml. of 20% NaOH solution to liberate the base. The mixture was extracted 5 times with 600 ml. portions of ether and the combined ether phase dried over magnesium sulfate. After evaporation of the solvent, the residue was distilled to give 374 g. of a yellow-orange liquid; b.p. 200–210° (0.5 mm.). A solution of this material in 200 ml. of ethanol was treated with the equivalent quantity of hydrogen chloride in 200 ml. of ethanol and the resulting solution diluted to 2 l. with ether to give a crystalline product weighing 304 g., m.p. 158–175°. This material was triturated with 800 ml. of acetonitrile and filtered to give 380 g. (81%) of a colorless product; m.p. 173–175°, λ_{max}^{Nujol} 3.78, 5.95 μ. Recrystallization of this material from acetonitrile did not change the melting point.

Acknowledgment.—The authors are indebted to Dr. Bernard Rubin and Mr. Jack High for the pharmacological data, to Miss Barbara Keeler for interpretation of the infrared spectra, and to Mr. Joseph Alicino and his associates for the analyses reported herein.

Antiviral Activity of 2,2-Dichloro-4′-formylacetanilide Thiosemicarbazone

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Received February 27, 1962

Hamre and her associates[2] first reported the antivaccinial activity of 4′-formylacetanilide thiosemicarbazone (I), and this activity was confirmed by Thompson and co-workers.[3] Because substitution of a dichloroacetyl group on an amino group of certain drugs leads to an enhancement of their activity, it was decided to prepare and test a sample of the title compound (II), for comparison of its properties with those of I.

By deacetylation of 4′-formylacetanilide (III), *p*-aminobenzaldehyde (IV) was obtained. This preparation is given in some detail because, although IV (which polymerizes with ease) has been described repeatedly in the literature, no satisfactory method for its preparation and preservation could be found therein. Compound IV then was acylated with dichloroacetyl chloride to give 2,2-dichloro-4′-formylacetanilide (V), which was condensed with thiosemicarbazide, affording II in quantitative yield.

In tests for antiviral activity, compound II was found to be about as active as I *versus* vaccinia (see Table I). Both compounds were inactive *versus* four other viruses in mice.

Experimental

***p*-Aminobenzaldehyde (IV).**—To a refluxing solution of 81.6 g. (0.5 mole) of III in 100 ml. of absolute ethanol and 200 ml. of water was added slowly a solution of 22 g. (0.55 mole) of sodium hydroxide in 55 ml. of water, and the solution was boiled for 20 min. Nuchar (0.5 g.) was added and the suspension was boiled in an open flask and fluted-filtered, giving a pale-orange filtrate which deposited some red-orange oil. (a) The supernatant liquor was decanted, boiled until the odor of ethanol had disappeared, fluted-filtered, cooled in ice, and nucleated, to give crop 1 (12 g.) of IV. (b) The oil was kept overnight in the refrigerator, giving a mass of orange crystals which was extracted with three 200-ml. portions of boiling water; the extracts were combined and treated as before, to give crop 2 (24 g.) of IV. Crops 1 and 2 were combined (36 g.) and recrystallized from 400 ml. of boiling water, giving 23.5 g. of IV as yellow platelets. The mother liquor was combined with those of crops 1 and 2; this solution was extracted with neutral 1,2-dichloroethane[4] and the extract was dried (anhydrous sodium sulfate) and evaporated to give crop 3 (22 g.) of yellow crystals; total yield, 45.5 g. (75%); m.p. 71°; lit. m.p.[5] 71°. Solutions of IV in neutral 1,2-dichloroethane may be kept in the refrigerator for several days without formation of the polymer[6] (which is insoluble in this solvent).

2,2-Dichloro-4′-formylacetanilide (V).—To a solution of 22 g. of IV in 100 ml. of anhydrous, neutral 1,2-dichloroethane[4] at 0° was added 17.5 ml. of dry pyridine. With exclusion of moisture, a solution of 22 ml. of dichloroacetyl chloride in 25 ml. of 1,2-dichloroethane[4] was added dropwise, with magnetic stirring, during 1.75 hr. at 0°. Water (1 + 1 + 3 ml.) now was added at 5-min. intervals, with stirring at 0°; 100 ml. of water was added and the product was isolated in the usual way, to give 26.4 g. of a yellow-orange, crystalline mass. A portion (20 g.) was recrystallized from 95% ethyl alcohol (2 vols.) and the crystals were washed with 20 ml. of this solvent, to give 12.3 g. of very pale-yellow crystals; m.p. 128–129°. Its infrared absorption spectrum showed bands that were absent from the spectrum of compound II: at 1701, 1511, 1422, 1325, 1255, 1220, 1020, 778, and 715 cm.$^{-1}$.

Anal. Calcd. for $C_9H_7Cl_2NO_2$: C, 46.58; H, 3.04; Cl, 30.56; N, 6.04. Found: C, 46.50; H, 3.16; Cl, 30.58; N, 6.26.

2,2-Dichloro-4′-formylacetanilide Thiosemicarbazone (II).—To a refluxing solution of 5.8 g. (0.025 mole) of V in 75 ml. of absolute ethanol plus 100 ml. of water was added a hot solution of

(1) Division of Physical Chemistry, National Bureau of Standards, Washington 25, D. C.

(2) D. Hamre, J. Bernstein, and R. Donovick, *Proc. Soc. Exptl. Biol. Med.*, **73**, 275 (1950); K. A. Brownlee and D. Hamre, *J. Bacteriol.*, **61**, 127 (1951); D. Hamre, K. A. Brownlee, and R. Donovick, *J. Immunol.*, **67**, 305 (1951).

(3) R. L. Thompson, M. L. Price, and S. A. Minton, Jr., *Proc. Soc. Exptl. Biol. Med.*, **78**, 11 (1951); S. A. Minton, Jr., J. E. Officer, and R. L. Thompson, *J. Immunol.*, **70**, 222 (1953); R. L. Thompson, S. A. Minton, Jr., J. E. Officer, and G. H. Hitchings, *ibid.*, **70**, 229 (1953); R. L. Thompson, J. Davis, P. B. Russell, and G. H. Hitchings, *Proc. Soc. Exptl. Biol. Med.*, **84**, 496 (1953).

(4) Commercial 1,2-dichloroethane is acidic; it was prewashed with aqueous sodium bicarbonate solution and water.

(5) L. C. Janse, *Rec. trav. chim.*, **40**, 285 (1921).

(6) Polymer may be dissolved from glassware by means of concentrated nitric acid.