mp 140–142° dec. Recrystallization from EtOH gave an analytical sample, mp 142–144° dec. *Anal.* ($C_{17}H_{18}N_2O_4S_2$) C, H, N.

3-Piperonylrhodanine (VIII).—A solution of 4.7 g (0.05 mole) of $ClCH_2CO_2H$ in 5 ml of H_2O was treated with 1 g (0.025 mole) of NaOH and sufficient Na_2CO_3 to bring the pH to 7.5. To this stirred solution was added, during 30 min, 12.2 g (0.05 mole) of VII. During the addition, 15 ml of H_2O and 40 ml of DMF were added to facilitate the solution of the carbamate salt. The mixture was stirred for 36 hr and the resulting solid, mp 144–145° dec, was collected by filtration and washed (H_2O, EtOH). It was slurried in 50 ml of H_2O and acidified with 6 *N* HCl to pH 2. The mixture was heated at 80° for 15 min and cooled. The yellow solid was collected by filtration, washed (H_2O), and dried *in vacuo* to give 6.5 g (52% yield) of product, mp 117–118°. Recrystallization from aq EtOH gave analytically pure VIII, mp 117–118°. *Anal.* ($C_{11}H_9NO_3S_2$) C, H, N.

3-(3,4-Methylenedioxyphenyl)alanine (IX).—A mixture of 7 g (0.03 mole) of IIa and 66 g (0.21 mole) of $Ba(OH)_2 \cdot 8H_2O$ in 350 ml of H_2O was refluxed with stirring for 3 days until evolution of NH_3 had ceased. The reaction mixture was cooled to 20° and acidified with concd H_2SO_4 to *p*H 1. The pptd $BaSO_4$ was filtered and washed with 200 ml of H_2O. The filtrate and washings were combined and evaporated to one-third of the original volume. It was then neutralized with NH_4OH. The resulting precipitate was collected by filtration and recrystallized from H_2O to give 1.4 g (22%) of IX, mp 253–254° dec (lit.[6] mp 250–255°). *Anal.* ($C_{10}H_{11}NO_4$) C, H, N.

3-(3,4-Methylenedioxyphenyl)-2-thiopyruvic Acid (X).—A mixture of 12 g (0.045 mole) of IIId and 100 ml of 4 *N* NaOH was refluxed with stirring for 30 min. The clear solution was cooled rapidly to 10° and acidified with 100 ml of cold 4 *N* HCl. After 15 min the pale yellow precipitate was collected by filtration, washed with H_2O, and dried, mp 198–200° dec. Two recrystallizations from MeOH gave 4 g (40%) of X, mp 201–203° (lit.[19] mp 208–210° dec.). *Anal.* ($C_{10}H_8O_4S$) C, H, S.

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Studies on the *in Vivo* Antiviral Effects of Benzothiazole Derivatives against Various Influenza A2 Strains

Stig Åkerfeldt

Virology Department, Research Department of the KABI Group, S-104 25 Stockholm 30, Sweden

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During an investigation of the effect of various heterocyclic ring systems on mice infected with various influenza virus strains, it was found that 2-aminobenzothiazole, when administered intraperitoneally to mice, gave a protection to the animals quite comparable to that of aminoadamantane. A structure–activity study was then undertaken to investigate whether certain structural changes could improve the antiviral effect of the compound. A remarkable sensitivity to variations in structure was found. Indeed, out of 17 benzothiazoles only the 2-amino- and 2-amino-4-chloro derivatives showed significant protective effect at the dose levels tested.

All 17 compounds were also tested in *in vitro* systems using human amnionic cells infected with rhino virus 33342, adeno virus 3, and herpes simplex virus. No protective effects could be demonstrated with any compound in the concentration range of 1–50 μg/ml.

Hungarian workers[1,2] have reported the effectiveness of some 2-(pyridyl)benzothiazole compounds against certain strains of influenza virus when tested in chick embryo chorioallantoic membrane cultures. However, the investigated substances were completely ineffective *in vivo* in mice.[2] Recently Paget, *et al.*,[3] have demonstrated the *in vivo* effect of certain benzothiazoleureas against Coxsackie A 21 virus in mice.

Table I describes the results of the *in vivo* tests.

Table I

Effect of Benzothiazole Derivatives in Mice Infected with Influenza A2/Stockholm/63

Substituents	Dose, mg/animal	% survivors treated group	% survivors control group
None	0.5 ip	10	33
	1.0 ip	10	20
2-NH_2	0.4 intranasal	10	7
	0.5 po	20	20
	0.1–1.5 ip	See Table II	
2-NH_2-4-Cl·HBr	0.1–0.5	See Table II	
2-NH_2-6-Cl	0.5 ip	0	27
	0.5 ip	25	20
2-NH_2-4,6-Cl_2	0.5 ip	Toxic dose	
	0.1 ip	Toxic dose	
2-NH_2-5,6-Cl_2	0.5 ip	Toxic dose	
	0.1 ip	0	20
2-NH_2-6-Br	0.5 ip	30	46
2-NH_2-6-Me	0.5 ip	0	27
2-NH_2-5,6-Me_2	0.5 ip	10	20
2-NH_2-6-EtO	0.5 ip	30	20
2-NH_2-6-Me	0.5 ip	40	24
2-NH_2-6-SO_3H	0.5 ip	0	0
2-NH_2-5-Me-7-SO_3H	0.5 ip	10	0
5-NH_2-2-Me	1.0 ip	0	20
2-(4-$H_2NC_6H_4$)-6-Me	0.5 ip	40	33
6-OH-2-SO_2NH_2	4.0 ip	10	7
6-EtO-2-SO_2NH_2	4.0 ip	0	7

The substances were administered either intraperitoneally (ip) or intranasally to male mice (10–12 g, 2–3 weeks old, NMRI-strain) 15 min before intranasal infection with virus of the Et_2O-anesthetized animals. For each experiment 10 mice were used. The number of surviving animals was recorded daily for 15 days.

Benzothiazole derivatives are often rather toxic compounds, producing tremors in the animals at near-toxic dose levels. The acute LD_{50} values for 2-aminobenzothiazole and for 2-amino-4-chlorobenzothiazole were estimated at 180 mg/kg in the mouse strain used. The toxic properties of the compounds studied may explain the lower number of surviving animals in certain treated groups as compared to the corresponding control groups.

Table II shows experiments where 2-aminobenzothiazole, 2-amino-4-chlorobenzothizaole, and aminoadamantane·HCl were tested against various mouse-adapted influenza strains. A single dose of substance was administered ip 15 min before intranasal infection of Et_2O-anesthetized animals. From the results with

(1) L. Vaczi, G. Hadhazy, K. Hideg, L. Gergely, O. H. Hankovszky, and F. D. Toth, *Acta Virol.*, **12**, 371 (1968).

(2) L. Gergely, F. D. Toth, and G. Hadhazy, *Acta Microbiol. Acad. Sci. Hung.*, **15**, 145 (1968).

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TABLE II
EFFECT OF TWO BENZOTHIAZOLE DERIVATIVES ON MICE INFECTED WITH VARIOUS INFLUENZA STRAINS

	2-Aminobenzothiazole			2-Amino-4-chlorobenzothiazole·HBr			Aminoadamantane·HCl		
Influenza strain	Dose mg/animal (ip)	% survivors treated group	% survivors control group	Dose mg/animal (ip)	% survivors treated group	% survivors control group	Dose mg/animal (ip)	% survivors treated group	% survivors control group
A2/Stockholm/10/63	0.1	30	7	0.1	20	20	1.0	70	8
A2/Stockholm/10/63	0.3	0	7	0.3	50	20			
A2/Stockholm/10/63	0.5	70	7	0.5	90	20	1.0	30	20
A2/Stockholm/10/63	0.5	60	7	0.5	100	20			
A2/Stockholm/10/63	0.5	50	20						
A2/Stockholm/10/63	1.5	60	7	0.5	60	8			
A2/Stockholm/10/63	1.5	80	47	0.5	25	20			
A2/Japan/57	0.5	10	13	0.5	30	13	1.0	50	13
A2/England/64	0.5	60	87	0.5	100	87	1.0	90	87
A2/Singapore/57	0.5	40	60	0.5	60	60	1.0	60	60
A2/Taiwan/64	0.5	10	33	0.5	0	33	1.0	70	33
A2/Hongkong/1/68	0.5	90	17	0.5	90	17	1.0	70	8

2-aminobenzothiazole it would appear that the antiviral effect is not improved by increasing the dose level above 0.5 mg per animal.

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Cycloserine Derivatives[1]

CHARLES H. STAMMER,[2] C. CHANDRASEKHARAN KARTHA, NISHITH C. CHATURVEDI, AND JAMES D. MCKINNEY

Department of Chemistry, University of Georgia, Athens, Georgia 30601

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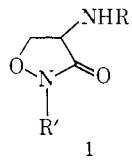
The known[3] biological activity of D-cycloserine (**1a**), a broad spectrum antibiotic, led use to examine derivatives of this compound for antimalarial activity.

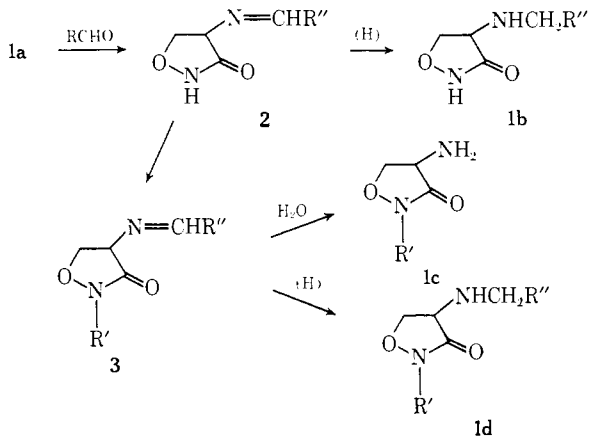


1

a, R = R′ = H
b, R = aralkyl; R′ = H
c, R = H; R′ = aralkyl
d, R = R′ = aralkyl

We have previously[4] reported the synthesis of several derivatives of cycloserine having general structures **1b** (R = 2-hydroxy-5-chlorobenzyl) and **1c** (R′ = Ph_3C). The pathway by which this was done is shown in Scheme I. In this report, several new Schiff bases (**2**) are described which can be reduced with $NaBH_4$ to

SCHEME I



type **1b** or alkylated giving **3** which is further hydrolyzed or reduced to **1c** or **1d**.

Of greatest chemical interest is the considerable variation in the ease of racemization of Schiff bases **2** as a function of the aldehyde used. Earlier work[4b] showed that when 5-chlorosalicylaldehyde was employed, the Schiff base was optically quite stable[4a] in solution but was completely racemized during conversion into its 2-trityl derivative **3**, R′ = Ph_3C, or its 2-Me derivative[5] **3**, R′ = Me. These 2-alkylations were carried out in the presence of K_2CO_3 which apparently catalyzed the racemization of the products by abstraction of a proton from the asymmetric center.[4b] Polarimetric measurements on a solution of the Schiff base Na salt in dimethoxyethane showed that the rate of racemization more than doubled after the addition of the alkyl halide. Hydrolysis of both of these Schiff bases gave the racemic 2-alkylated cycloserines in good yield. $NaBH_4$ reduction of 5-chlorosalicylidene-D-cycloserine, however, gave[4b] an optically active derivative **1b**, R = 5-chloro-2-hydroxybenzyl. The 5-nitrosalicylidene Schiff base **2**, R = 5-nitrosalicyl, had the same optical properties, *i.e.*, alkylation gave an optically inactive 2-derivative while reduction gave an active *N*-5-nitro-2-hydroxybenzyl-D-cycloserine.

In an attempt to synthesize an optically active 2-tritylcycloserine, the Schiff base **2**, R″ = 2-hydroxy-

(1) Supported in part by Contract DA-49-193-MD-2993 from the U. S. Army Medical Research and Development Command.
(2) To whom inquiries should be addressed.
(3) Francis C. Neuhaus, in "Antibiotics," Vol. I, D. Gottlieb and P. L. Shaw, Ed., Springer-Verlag, Heidelburg, 1967, Chapter 2.
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(5) Ph.D. Thesis of James D. McKinney, University of Georgia, Athens, Georgia, 1968.