cyloyl carbonate (Nujol) 1795 (OCOO), 1740 cm^{-1} ($COOC_6H_5$); nmr ($CDCl_3$) δ 7.0–7.9 (m, 18, C_6H_4, C_6H_5); mp 130°; mass spectrum *m/e* (rel intensity) 362 (59), 361 (83), 242 (59), 241 (100), 197 (34), 196 (83), 121 (51), 120 (63), 92 (57). *Anal.* ($C_{27}H_{18}O_7$) C, H.

The antiinflammatory tests were carried out as follows. Male albino rats (6–8 per group) weighing 150–160 g were used. The polymers were made up as suspensions in Tragacanth. The control vehicle was 1 drop of DMF diluted by the same factor. This itself does not cause any effect. Edema in one leg was obtained by an injection of carrageenin in the usual method. After 1 hr, when it was ensured that all animals had a well-developed inflammation, the compounds were injected subcutaneously into the nekscruff in 1 ml/kg doses. The control group had carrageenin alone with 1 ml/kg sc of the vehicle, while a positive control was considered to be a relatively high dose of aspirin (200 mg/kg). All compounds were given as 200 mg/kg doses, after this was found to be well tolerated in all compounds. After injection of the compounds, differences in the paws were measured every hour for 4 hr and then again at 6, 8, 10, and 20 hr.

Acknowledgment. We wish to thank Dr. Harold Fenton of Pharmatox for carrying out the pharmacological tests.

References

(1) B. Z. Weiner, M. Tahan, and A. Zilkha, *J. Med. Chem.*, 15, 410 (1972).
(2) B. Z. Weiner and A. Zilkha, *J. Med. Chem.*, 16, 573 (1973).
(3) R. J. Cornell and L. G. Donaruma, *J. Polym. Sci., Part A*, 3, 827 (1965).
(4) R. J. Cornell and L. G. Donaruma, *J. Med. Chem.*, 8, 388 (1965).
(5) M. Tahan, N. Lender, and A. Zilkha, *Isr. J. Chem.*, 10, 835 (1972).
(6) L. G. Donaruma and J. Razzano, *J. Med. Chem.*, 9, 258 (1966).
(7) L. G. Donaruma and J. Razzano, *J. Med. Chem.*, 14, 244 (1971).
(8) H. Jatzkwitz, *Z. Physiol. Chem.*, 297, 149 (1954).
(9) H. Jatzkwitz, *Z. Naturforsch. B*, 10, 27 (1955).
(10) S. N. Ushakov and T. A. Kononova, *Dokl. Akad. Nauk SSSR*, 129, 1309 (1959).
(11) S. N. Ushakov and T. A. Kononova, U.S.S.R. Patent 136551; *Zh. Khim.*, 2L284 (1962).
(12) H. Jatzkewitz, German Patent 1,041,052 (1959); *Chem. Abstr.*, 54, 24435e (1960).
(13) K. Kratzl and E. Kaufmann, *Monatsh. Chem.*, 92, 371 (1961).
(14) K. Kratzl, E. Kaufmann, O. Kraupp, and H. Stormann, *Monatsh. Chem.*, 92, 379 (1961).
(15) V. A. Kropachev, E. M. Lavrent'eva, K. S. Podgorskaya, and T. E. Semenova, *Vysokomol. Soedin., Ser. B*, 11, 857 (1969).
(16) M. Covello, G. Ciampa, and A. Vittoria, *Ann. Chim. (Rome)*, 57, 644 (1967).
(17) G. Ciampa, C. Gaieco, F. Manna, C. Silipo, and A. Vittoria, *Chim. Ind., (Milan)*, 51, 595 (1969).
(18) S. N. Ushakov, L. B. Trukhmanova, E. V. Drozodova, and T. M. Markelova, *Dokl. Akad. Nauk SSSR*, 141, 1117 (1961).
(19) H. S. Yanai and W. H. Watanabe, U. S. Patent 3,489,739 (1970).
(20) B. Z. Weiner and A. Zilkha, *Isr. J. Chem.*, 11, 567 (1973).

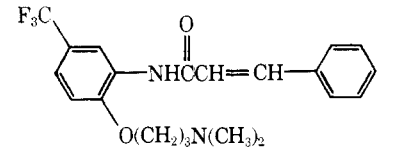
Immunosuppressive and Antiinflammatory Activities of Cinanserin and Its Analogs[1]

Robert C. Millonig,* Blanche J. Amrein, Joel Kirschbaum, and Sidney M. Hess

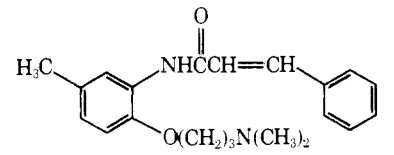
The Squibb Institute for Medical Research, Princeton, New Jersey 08540. *Received December 21, 1973*

Cinanserin, 2′-(3-dimethylaminopropylthio)cinnamanilide, has been shown to be a potent antagonist of serotonin (Krapcho, *et al.*,[2] Rubin, *et al.*,[3] Furgiuele, *et al.*[4]) that also exhibits analgesic activity (Rubin, *et al.*[5]). Preliminary studies in our laboratory showed that it also possessed immunosuppressive activity. Subsequent studies showed cinanserin to be more active than azathioprine in suppressing the uptake of ^{14}C-labeled leucine and thymidine by phytohemagglutinin (PHA)-stimulated human lymphocytes, in prolonging the survival time of skin grafts between congenic strains of mice differing at the H-2 locus (Schwartz, *et al.*[6]), and in protecting rats against paralysis in experimental allergic encephalomyelitis (Babington and Wedeking[7]). About 100 analogs of cinanserin have been synthesized and evaluated for immunosuppressive and antiserotonin activities. Although most compounds of these series showed both immunosuppressive and antiserotonin activities, and of similar degree, several members exhibited a marked separation of these activities (Krapcho, *et al.*[8]). The immunosuppressive activity of cinanserin was further evaluated and the antiinflammatory activities of several analogs of cinanserin were compared with those of cinanserin, azathioprine, and indomethacin.

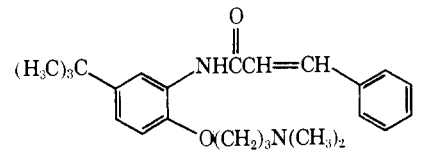
Chart I. Chemical Structure of Cinanserin, 2′-(3-Dimethylaminopropoxy)-5′-trifluoromethylcinnamanilide (I), 2′-(3-Dimethylaminopropoxy)-5′-methylcinnamanilide (II), 5′-*tert*-Butyl-2′-(3-dimethylaminopropoxy)cinnamanilide (III), and 2-Chloro-2′-(3-dimethylaminopropoxy)cinnamanilide (IV)[a]



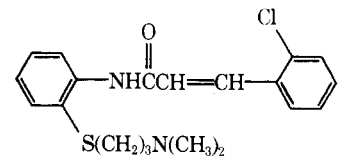
I



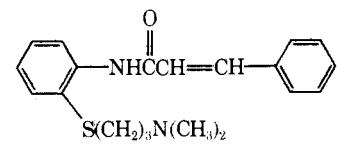
II



III



IV



cinanserin

[a] With the exception of III, the preparation of the above compounds was reported in ref 8. Compound III (mp 199–201°, crystallized from isopropyl alcohol) was synthesized by C. F. Turk of these laboratories from 4-*tert*-butyl-2-nitrophenol according to the procedure used for the preparation of II.[8]

Experimental Section

Immunosuppressive Activity of Cinanserin. Primary Immune Response. Hemagglutination. The effect of cinanserin on

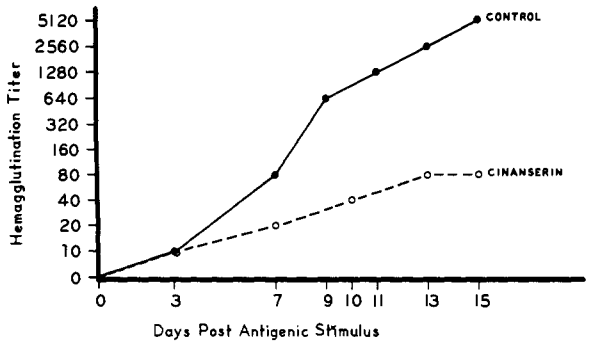


Figure 1. Effect of cinanserin on the production of sheep red blood cell agglutinins in the mouse. Each point on the graph represents the hemagglutination titer of pooled sera from ten mice. The S.E. limits for control (n = 7) and cinanserin-treated mice (n = 7) at day 15 comprise a ± twofold dilution.

the primary immune response of mice injected with sheep red blood cells was measured by the procedure described by Nathan, *et al.*,[9] for the detection of agents that interfere with the immune response. Male Swiss mice (Harpaul Farms, Franklin Park, N. J.), 19–21 g, were allotted to two groups of 70 animals each. One group was treated daily with cinanserin, 25 mg/kg, administered subcutaneously for 4 successive days, starting on the day of antigenic stimulation. The second group, sensitized controls, received no treatment. The antigenic stimulus for each mouse consisted of 0.25 ml of a 30% suspension of tanned sheep red blood cells, given intravenously. Ten mice from each group were sacrificed by decapitation on days 0, 3, 7, 9, 11, 13, and 15 after the antigenic stimulation. The blood was collected and pooled, and separated serum was assayed for the presence of agglutinins to sheep red blood cells.

Dosage Regimen Studies. The effect of dosage regimen on the immunosuppressive activity of cinanserin was determined by use of the mouse–sheep red blood cell hemagglutination system described above. Five regimens were evaluated (see Figure 2 for details) with ten mice per experimental group.

Biological Activities of Cinanserin and Related Compounds. The biological activities of three immunosuppressive analogs, 2′-(3-dimethylaminopropoxy)-5′-trifluoromethylcinnamanilide (**I**), 2′-(3-dimethylaminopropoxy)-5′-methylcinnamanilide (II), 5′-*tert*-butyl-2′-(3-dimethylaminopropoxy)cinnamanilide (III), and one antiserotonin analog, 2-chloro-2′-(3-dimethylaminopropoxy)-cinnamanilide (IV) have been compared with those of cinanserin, azathioprine, and indomethacin. The structures of cinanserin and its analogs are shown in Chart I. The immunosuppressive activity of these compounds was measured in the mouse–sheep red blood cell hemagglutinin system and in mouse–spleen cell culture. The antinflammatory activities of these compounds were measured in carrageenin-induced edema, erythrocyte membrane stabilization, allergic encephalomyelitis, and adjuvant-induced arthritis. Details of these procedures have been described elsewhere.[7,10,11]

Results and Discussion

Cinanserin suppressed the production in mice of humoral antibody to sheep red blood cells (see Figure 1). Subsequent to synthesis, early appearance of antibody was not delayed by treatment with this compound. The antibody levels in mice treated with cinanserin rose slowly and reached a maximal titer by day 13. In contrast, antibody levels in the sera of control mice approached a maximum at day 15, at which time these mice had a 32-fold to 64-fold greater hemagglutinin titer than did mice treated with cinanserin. Ultracentrifugal analyses of the test and control sera collected on day 15 showed that mice immunized with sheep red cells produced an antibody of high molecular weight (18S). The amount of 18S antibody formed was reduced 43% by cinanserin, when this agent had been administered at the time of antigenic stimulation. This decrease in the amount of circulating antibody might have reflected suppression of antibody synthesis, cleavage of the 18S disulfide bonds *in vivo*, or both. We attempted, therefore, to demonstrate the effects of the test compound on the 18S component and on the hemagglutination titer *in vitro*. Cinanserin, at a concentration of 6 mg/ml, had no effect *in vitro* on the concentration of the 18S component in normal or antigen-treated mouse serum. It also had no effect on the concentration of the 18S component of purified human γ globulin.

The effect of dosage regimens on the immunosuppressive activity of cinanserin in the mouse–sheep red blood cell hemagglutinin system is shown in Figure 2. The maximum immunosuppressive activity observed (expressed as the ratio of the hemagglutination titer of control mice to that of treated mice × 100) was 128 ± twofold dilution and was achieved by each of several dosage regimens. When cinanserin had been administered only prior to antigenic stimulus, no immunosuppressive activity was observed. When dosage with cinanserin had been started be-

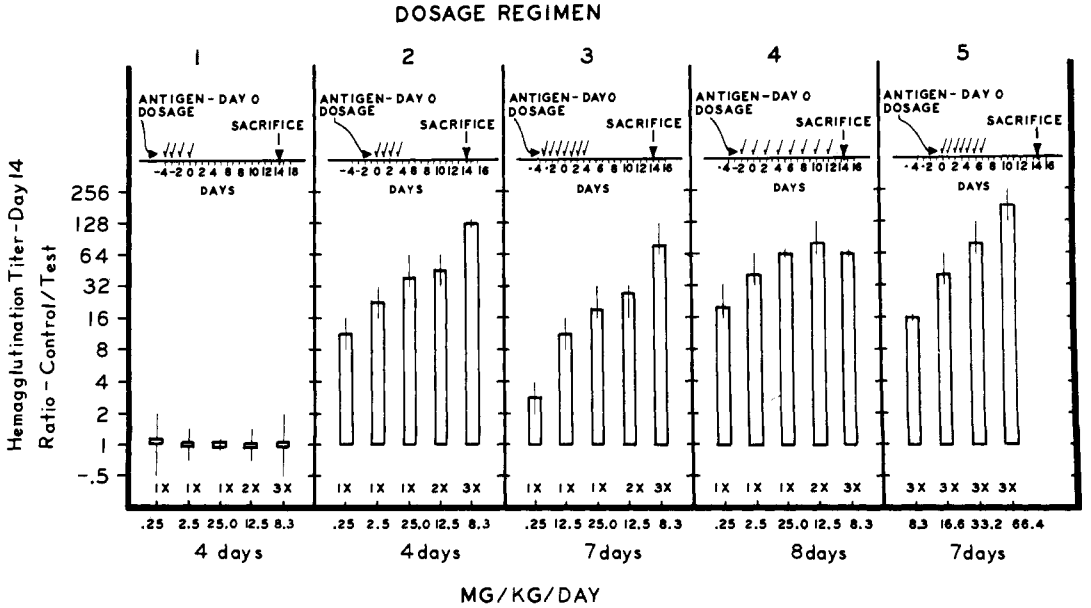


Figure 2. Effect of cinanserin on the production of sheep red cell agglutinins in the mouse. Dosage-regimen studies. Each bar on the graph represents the mean (n = 10) ratio of the hemagglutination titer of the sera of the control mice on day 14 to those of cinanserin-treated mice on day 14.

Table I. Comparison of the Biological Activities of 2′-(3-Dimethylaminopropoxy)-5′-trifluoromethylcinnamanilide (I), 2′-(3-Dimethylaminopropoxy)-5′-methylcinnamanilide (II), 5′-*tert*-Butyl-2′-(3-dimethylaminopropoxy)cinnamanilide (III), and 2-Chloro-2′-(3-dimethylaminopropoxy)cinnamanilide (IV) with Those of Cinanserin, Imuran, and Indomethacin

Test	Dose and route of administration[a]	Measurement	I	II	III	IV	Cinanserin	Azathioprine	Indomethacin
Antiserotonin		0.25–1 × BAS = 1 1–4 × BAS = 2 4–16 × BAS = 3 16–64 × BAS = 4 >64 × BAS = 5	1	1	1	4	5	ND	ND
Hemagglutinin synthesis	25 sc	Ratio, control/test	32	64	64	2	64	64	64
DNA synthesis		I_{50}, μg/ml	>2<10	5	2	5	10	10	>100
Carrageenin-induced edema	po	ID_{50}, mg/kg	>250	400	250	450	300	>150[b]	7 ± 2.9[c]
Membrane stabilization		I_{50}, μg/ml	3	16	3	20	372	>450	36
Experimental allergic encephalomyelitis	0.75	% protection							46
	1.5								54
	3.0								61
	3.0 po								44
	15						28		
	18.75							10	
	30		17		41				
	37.5							59	
	60		22	37	60	31	50	73	
	60 po							55	
	75							66[d]	
	90		66	30	*d*				
	120			78					
	120 po			48					
Adjuvant-induced arthritis	0.1	% inhibition, local/systemic, % survival							20/19, 100
	0.5								47/48, 100
	2.5								66/53, 100
	10.0				0/0, 100				
	20.0				12/21, 90				
	30.0			8/50, 100					
	40.0				26/26, 90				
	50.0						4/10, 100		
	60.0		14/30, 100	17/36, 94	43/57, 84	28/54, 100		73/98, 100	
	60.0 po		4/23, 90						
	120.0 po		5/21, 70	30/55, 60	56/86, 80				

[a] The compounds were administered ip on a mg/kg basis, except where indicated. [b] 7% inhibition of edema formation at 150 mg/kg. [c] ID_{50} ± standard deviation. [d] Toxicity (deaths in experimental groups) observed.

fore or on the day of antigenic stimulation and continued for 4 or 7 days after antigenic stimulation, immunosuppressive activity was also observed. When cinanserin had been administered to mice for 7 days in three daily doses up to 66.6 mg/kg each, starting on the day of antigenic stimulation, optimum immunosuppressive activity occurred.

The immunosuppressive and antiinflammatory activities of cinanserin and its analogs were compared with those of azathioprine and indomethacin (Table I). Cinanserin, a potent inhibitor of serotonin, possessed immunosuppressive activity comparable to that of azathioprine. The immunosuppressive and antiserotonin activities of cinanserin have been separated.[7] Three cinanserin analogs, I, II, and III, possessed excellent immunosuppressive activity but little or no antiserotonin activity. IV possessed excellent antiserotonin activity but little immunosuppressive activity. Cinanserin and azathioprine were ineffective as stabilizers of the red cell membrane, whereas the cinanserin analogs and indomethacin were quite effective. Cinanserin and its analogs, as well as azathioprine, effectively suppressed DNA synthesis by mouse spleen cells in culture, protected rats against paralysis in experimental allergic encephalomyelitis, and possessed only minimal

activity in the inhibition of carrageenin-induced edema in the rat paw. II, III, IV, azathioprine, and indomethacin were effective in suppression of both local and systemic lesions in adjuvant-induced arthritis. II, III, IV, and azathioprine were more effective in suppressing systemic lesions than in suppressing local lesions; whereas indomethacin was equally effective in suppressing local and systemic lesions.

References

(1) Presented in part at the 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, Abstract MEDI 034.
(2) J. Krapcho, B. Rubin, A. Drungis, E. Spitzmiller, C. Turk, J. Williams, B. Craver, and J. Fried, *J. Med. Chem.*, **6**, 219 (1963).
(3) B. Rubin, J. J. Piala, J. C. Burke, and B. N. Craver, *Arch. Int. Pharmacodyn. Ther.*, **152**, 132 (1964).
(4) A. R. Furgiuele, J. P. High, and Z. P. Horovitz, *Arch. Int. Pharmacodyn. Ther.*, **155**, 225 (1965).
(5) B. Rubin, J. Krapcho, and J. High, *Life Sci.*, **5**, 845 (1966).
(6) G. H. Schwartz, E. Ambinder, R. R. Riggio, K. H. Stenzel, and A. L. Rubin, *Clin. Res.*, **16**, 323 (1968).
(7) R. G. Babington and P. W. Wedeking, *J. Pharmacol. Exp. Ther.*, **177**, 454 (1971).
(8) J. Krapcho, R. C. Millonig, C. F. Turk, and B. J. Amrein, *J. Med. Chem.*, **12**, 164 (1969).
(9) H. C. Nathan, S. Bieber, G. Elion, and G. H. Hitchings, *Proc. Soc. Exp. Biol. Med.*, **107**, 796 (1961).
(10) S. M. Hess and R. C. Millonig in "Inflammation Mechanisms and Control," Academic Press, New York and London, 1972, p 1.
(11) R. C. Millonig, M. B. Goldlust, W. E. Maguire, B. Rubin, E. Schulze, R. J. Wojnar, A. R. Turkheimer, W. F. Schreiber, and R. J. Brittain, *J. Med. Chem.*, **16**, 780 (1973).

Antiparasitic Thiocyanatobenzothiazoles

Robert J. Alaimo,* Stanford S. Pelosi,

Chemistry Division

Christopher J. Hatton,

Veterinary Research Division

and Joseph E. Gray

Medical Microbiology Division, Norwich Pharmacal Company, Division of Morton-Norwich Products, Inc., Norwich, New York 13815.

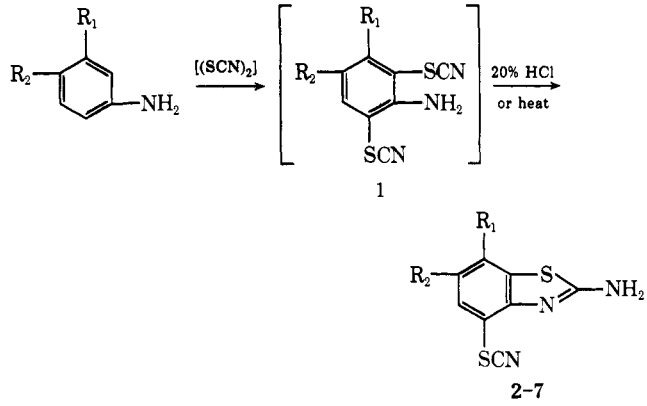
Received January 24, 1974

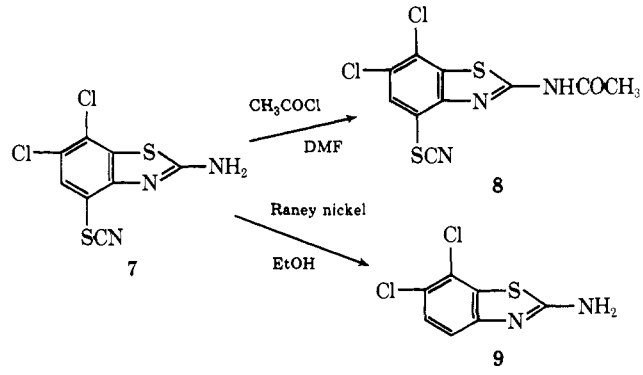
As part of a continuing program directed toward the development of novel antiparasitic agents,[1,2] a series of thiocyanatobenzothiazoles was synthesized.

The general method for the preparation of the benzothiazoles **2–7** involves the reaction of a suitably substituted aniline with excess thiocyanogen in anhydrous methanol solution.[3,4] The thiocyanogen was generated *in situ* by the action of bromine on sodium thiocyanate in methanol. The intermediate dithiocyanatoanilines 1 were occasionally isolable in this procedure but were generally smoothly cyclized in high yields in 20% HCl or by heating.[4] Slight modifications of the work-up procedure were necessary depending on the aniline substitution and are described in the Experimental Section. The synthesis of 2–7 is shown in Scheme I, and the structures are shown in Table I.

Since the cyclization of 1 can proceed to give either the 5,6- or 6,7-disubstituted benzothiazole derivatives, it became necessary to assign the proper structure. The chlorine atoms in **7** were found to be in the 6 and 7 positions as shown after removal of the thiocyanato group by boiling with Raney nickel in alcohol. The resulting product was 2-amino-6,7-dichlorobenzothiazole (**9**), which had been previously characterized.[3] The structures of the other disubstituted analogs (**5**, **6**) were assigned on the basis of the chemical shift in the nmr spectrum of the 5-proton which was in agreement with that of **7**. Details of thiocyanate removal and the evidence for structural assignment are included in the Experimental Section.

Scheme I





Acylation of **7** with acetyl chloride in DMF provided the 2-acetamido compound **8** in moderate yield. The position of acylation in 2-aminobenzothiazoles has been previously demonstrated to be on the 2-amino group.[5]

The thiocyanatobenzothiazoles **2–8** were tested for anthelmintic and antifungal activity against several test organisms, and the results are shown in Table I. The activities of the reference drugs, *dl*-tetramisole (**10**), bunamidine (**11**), and nystatin (**12**), are included for comparison.

Anthelmintic Testing. The anthelmintic activity of the compounds **2–8** was determined against *Ascaris suum* and *Hymenolepis nana* in the mouse, and the results of the testing are shown in Table I. The biologic method and the reference standards have been previously reported.[1,2]

The activity data of **2–8** against these two helminths failed to establish any structure–activity relationships. Only compound **3** had comparable activity to the reference drugs 10 and 11.

Antifungal Testing. The antifungal activity of **2–8** was determined in Sabouraud's liquid medium BBL and the MIC values against a number of yeast species are shown in Table I.[6] The most active compounds tested were **5** and **7**, and, in addition, both significantly inhibited the growth of *Candida albicans* and *Microsporum canis* in the agar diffusion–cylinder cup test.[6]

Experimental Section

Melting points were determined in open capillary tubes using a Mel-Temp melting point apparatus and are uncorrected. The