viscous oil was obtained; it crystallized on trituration with petroleum ether (bp 90–120°); dinitrophenylhydrazone, from EtOH, mp 187–189°. *Anal.* ($C_{18}H_{18}Cl_2N_5O_4$) N.

Dimethyl 4-methylbenzenephosphonate [V, R = PO(OCH$_3$)$_2$] was obtained according to Kossolapoff,[5a] bp 143–146° (2–3 mm), n^{20}D 1.5060, yield 81%. A side product was also obtained, bp 180° (2–3 mm), n^{20}D 1.5470, yield 16%, that remains as a residue during distillation of the reaction mixture and which is assumed (after analysis of the nitrated product) to be tetramethyl methylbenzene-2,4-diphosphonate.

V [R = PO(OCH$_3$)$_2$] (2 g, 0.01 mole) in 20 ml of HCl (*d* 1.19) was refluxed for 7 hr and gave after 24 hr of standing at room temperature 1.1 g (65%) of 4-methylbenzenephosphonic acid, mp 189–190° (H_2O).[10]

Dimethyl 3-Nitro-4-methylbenzenephosphonate [VI, R = PO(OCH$_3$)$_2$].—A mixture of 5.2 ml of HNO_3 (*d* 1.42) and 6 ml of H_2SO_4 (*d* 1.83) was added slowly to a cooled and stirred solution (0–5°) of 16 g (0.08 mole) of V [R = PO(OCH$_3$)$_2$] in 60 ml of H_2SO_4 (*d* 1.83). After complete addition, the mixture was stirred for an additional 0.5 hr and poured over 300 g of crushed ice. The resulting oil was extracted (C_6H_6), washed (H_2O), dried (Na_2SO_4), and evaporated to dryness, giving 16.5 g (84%) of VI as an oil, bp 170° (2–3 mm), n^{20}D 1.5330. *Anal.* ($C_9H_{12}NO_5P$) N.

The following compounds were obtained similarly: **methyl 3-nitro-4-methylbenzenesulfonate,** mp 52–53° (C_6H_6–petroleum ether, 1:1), yield 98% [*Anal.* ($C_7H_7O_5S$) N]; **3-nitro-4-methylbenzenesulfonyl morpholide,** mp 119–120° [*Anal.* ($C_{11}H_{14}N_2O_5S$)N].

VI (2 g, 0.008 mole) in 20 ml of HCl (*d* 1.19) was refluxed for 7 hr and gave, after 2 days of standing at room temperature, 0.9 g (53%) of 3-nitro-4-methylbenzenephosphonic acid: mp 189° (EtOAc);[10] uv spectrum, λ_{max} 256 mμ (ε 4265).

Dimethyl 3-Amino-4-methylbenzenephosphonate [VII, R = PO(OCH$_3$)$_2$].—VI [R = PO(OCH$_3$)$_2$] (10 g, 0.04 mole) in 200 ml of anhydrous MeOH was hydrogenated at atmospheric pressure and room temperature with 6 g of 5% Pd–$CaCO_3$ catalyst. The theoretical amount of H_2 was absorbed in 0.3 hr. After catalyst removal and solvent evaporation under reduced pressure, the oily residue thus obtained was taken up in dry C_6H_6, treated with charcoal, and evaporated to dryness giving 6.5 g (74%) of VII [R = PO(OCH$_3$)$_2$] as an oil: n^{20}D 1.5490; uv spectrum, λ_{max} 305 mμ (ε 2340).

Similarly prepared were **3-amino-4-methylbenzenesulfonyl morpholide** (VIIm, Table II) and **methyl 3-amino-4-methylbenzenesulfonate** (VIIl, Table II).

Hydroxyethylation was carried out according to the previously described method.[2] The following compounds were obtained (melting point, yield, N analysis): 3-[*N*,*N*-bis(2-hydroxyethyl)amino]-4-methylbenzenesulfonyl morpholide (oil, 86%, ($C_{15}H_{24}N_2O_5S$) N); 3-[*N*,*N*-bis(2-hydroxyethyl)amino]-4-methylbenzenesulfonic acid (150–153°, 90%, ($C_{11}H_{17}NO_5S$) N), dimethyl 3-[*N*,*N*-bis(2-hydroxyethyl)amino]-4-methylbenzenephosphonate (oil (n^{20}D 1.5244), 93%, ($C_{13}H_{22}NO_5P$) N), and 3-[*N*,*N*-bis(2-hydroxyethyl)amino]-4-methylnitrobenzene (112–113° (toluene), 80%, ($C_{11}H_{16}N_2O_4$) N). Methyl 3-[*N*,*N*-bis(2-hydroxyethyl)amino]-4-methylbenzenesulfonate was not isolated, being directly subjected to chlorination.

Chlorination was accomplished as previously reported.[2] During chlorination of sulfonic derivatives methyl ester hydrolysis occured, giving directly II (R = SO_3H).

3-[*N*,*N*-Bis(2-chloroethyl)amino]-4-methylbenzenephosphonic Acid (In).—To 5 g (0.016 mole) of the corresponding bis(hydroxyethyl) derivative in 75 ml of dry C_6H_6 was added 8 ml of freshly distilled $SOCl_2$, and the mixture was refluxed for 2 hr. After solvent and excess $SOCl_2$ removal under vacuum, 30 ml of HCl (*d* 1.19) was added, and the solution was heated to boiling for 0.5 hr, treated with charcoal, and filtered to remove the resins. To the filtrate, 25 ml of HCl (*d* 1.19) was added, and the solution was refluxed for 10 hr and allowed to stand 2 days at room temperature. HCl removal under vacuum gave 4 g of a yellow oily residue consisting of a mixture of In and monomethyl ester of In.

Separation of the two compounds was done by dissolving the residue in H_2O, filtering the insoluble ester, and concentrating the solution after adding few drops of HCl; or, the residue was dissolved in the minimum amount of H_2O–EtOH (1:1) and passed over Merck 2 (weak basic) ion-exchange resin, which retained only the free acid In. Its elution was carried out with H_2O–EtOH–HCl (*d* 1.19), 9:9:1. Analysis of the solid (no melting point till 350°) was in agreement with the calculated data for In; uv spectrum, λ_{max} 257 mμ (ε 3454).

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Immunosuppressive Activity of 2′-(3-Dimethylaminopropylthio)cinnamanilide (Cinanserin) and Related Compounds. IV[1]

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Several test procedures have previously shown cinanserin, a potent inhibitor of serotonin, to be more active than azathioprine as an immunosuppressive agent. Seventy-two compounds related to cinanserin were tested for immunosuppressive activity using the mouse–sheep red blood cell procedure and compared with their antiserotonin activity. The syntheses and physical properties of the new analogs of cinanserin are also reported. Although most compounds of this series showed a similar degree of immunosuppressive and antiserotonin activities, several members exhibited a marked separation of these responses. Five compounds showing high-immunosuppressive low-antiserotonin activities are presently undergoing further biological evaluation.

Cinanserin,[2] a potent serotonin inhibitor, suppresses the primary immune response of mice to sheep red blood cells.[3] Subsequent studies showed cinanserin to be more active than azathioprine in suppressing the uptake of C^{14}-labeled leucine and thymidine by human lymphocytes stimulated by phytohemagglutinin and in prolonging the time of survival of skin grafts between congenic strains of mice differing at the H-2 locus.[4] Cinanserin also suppresses the secondary immune response of mice to sheep red blood cells and the develop-

(1) Previous paper: J. Krapcho and C. F. Turk, *J. Med. Chem.*, **9**, 809 (1966).

(2) Cinanserin is the approved generic name for 2′-(3-dimethylaminopropylthio)cinnamanilide.

(3) R. C. Millonig, B. J. Amrein, J. Kirschbaum, and A. Borman, *Proc. Soc. Exptl. Biol. Med.*, in press.

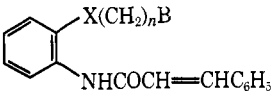
(4) G. H. Schwartz, E. Amblinder, R. R. Riggio, K. H. Stenzel, and A. L. Rubin, *Clin. Res.*, **16**, 323 (1968).

ment of allergic aspermatogenesis in the guinea pig,[5] causes no bone marrow toxicity,[3] and may be useful in the treatment of autoimmune diseases and in the prevention of graft rejections.

In order to determine a structure–activity relationship in this series, 72 compounds related to cinanserin (**1**) were screened for immunosuppressive activity using a slight modification[3] of the mouse–sheep red blood cell test[6] and compared with the antiserotonin effect, as measured on the isolated rat uterus[7] (Tables I–III).

TABLE I

MODIFICATION OF BASICALLY SUBSTITUTED SIDE CHAIN



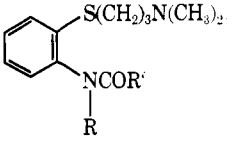
No.	X	n	B	Immunosuppressive[a] act.	Antiserotonin[b] act.
1	S	3	$N(CH_3)_2$	5	5
2	S	3	$N(C_2H_5)_2$	1	5
3	S	3	$N[CH(CH_3)_2]_2$	3	2
4	S	3	$N(CH_3)CH_2CH_2OH$	3	2
5	S	3	$N(CH_2CH_2OH)_2$	4	3
6	S	3	NC_4H_8O[c]	3	3
7	S	3	$NC_4H_8NCH_3$[c]	1	3
8	S	3	$NC_4H_8NCH_2CH_2OH$[c]	2	3
9	S	3	$NC_4H_8NCH_2CH_2C_6H_5$[c]	1	5
10	S	3	$NC_4H_8NC_6H_5$[c]	3	4
11	S	3	NC_4H_8N-2-pyridyl[c]	1	5
12	S	3	$NHC(NH_2)=NH$	3	5
13	S	d	$N(CH_3)_2$	4	3
14	S	2	$N(CH_3)_2$	4	3
15	S	2	$NHCH_3$	4	3
16	S	2	NH_2	1	2
17	S	2	$N(CH_3)CH_2C_6H_5$	2	3
18	S	2	NC_5H_{10}[c]	1	1
19	S	2	NC_4H_8O[c]	3	4
20	S	2	NC_4H_8N-2-$(CH_3O)C_6H_4$[c]	0	2
21	S	2	$NHC(NH_2)=NH$	4	5
22	S	2	$N(CH_3)C(NH_2)=NH$	1	4
23	S	2	$NHC(NHCH_3)=NH$	3	2
24	S	2	$N(CH_3)C[N(CH_3)_2]=NH$	1	1
25	O	4	$N(CH_3)_2$	2	2
26	O	3	$N(CH_3)_2$	4	5
27	O	2	$N(CH_3)_2$	3	3
28	O	3	NC_4H_8N-2-$(CH_3O)C_6H_4$[c]	4	3
29		3	$N(CH_3)_2$	4	1
30		2	$N(CH_3)_2$	3	3
31		1	$N(CH_3)_2$	0	2
32	SO_2	3	$N(CH_3)_2$	3	1
33	CO	3	$N(CH_3)_2$	1	5
34	CO	2	$N(CH_3)_2$	2	1
35	CONH	2	$N(CH_3)_2$	0	1
36	$CON(CH_3)$	2	$N(CH_3)_2$	1	2
37	COO	2	$N(CH_3)_2$	3	2
38	NHCO	2	$N(CH_3)_2$	3	1
39	$N(CH_3)$	2	$N(CH_3)_2$	0	1

[a] Activity in the mouse–sheep red blood cell test.[3] Antibody index = ratio of the concentration of the antibodies in the sera of the control to that in the sera of the drug-treated mice (25 mg/kg). In the case of azathioprine or cinanserin, it was necessary to make a 64-fold dilution of the control sera to match the hemagglutination titer of the sera from the drug-treated mice. Activity ratings: dilution of 64 = 5, 32 = 4, 16 = 3, 8 = 2, 2–4 = 1 and 1 = 0. [b] Activity measured on the isolated rat uterus:[7] BAS = 1; >64× = 5, 16–64× = 4, 4–16× = 3, 1–4× = 2, 0.25–1× = 1, and <0.25× = 0. [c] NC_4H_8O = morpholino, NC_4H_8N = piperazino, NC_5H_{10} = piperidino, C_4H_3S = 2-thienyl, C_6H_{11} = cyclohexyl, and C_7H_9 = 2-norbornen-5-yl. [d] $(CH_2)_n$ = $CH_2CH(CH_3)CH_2$.

The synthetic routes used to prepare the new members of this series of compounds are summarized in the Experimental Section and the physical properties of these materials are reported in Table IV.

TABLE II

MODIFICATIONS OF ACYLAMIDO GROUP



No.	R	R′	Immunosuppressive act.[a]	Antiserotonin act.[b]
40	H	$CH_2C_6H_5$	0	1
41	H	$(CH_3)_2C_6H_5$	2	1
42	H	C_6H_5	1	2
43	H	$CH=CHCH_3$	3	3
44	H	$(CH=CH)_2CH_3$	0	1
45	H	$C\equiv CC_6H_5$	2	2
46	H	$C(CH_3)=CHC_6H_5$	2	1
47	H	$C(C_2H_5)=CHC_6H_5$	4	1
48	H	$C(C_3H_7)=CHC_6H_5$	4	3
49	H	$C(C_4H_9)=CHC_6H_5$	4	2
50	H	$CH=C(CH_3)C_6H_5$	3	3
51	H	$CH=CHC_4H_3S$[c]	4	4
52	H	$CH=CHC_6H_{11}$[c]	5	2
53	H	$CH=CHC_7H_9$[c]	1	1
54	CH_3	$CH=CHC_6H_5$	1	1
55	$CH_2C_6H_5$	$CH=CHC_6H_5$	1	2
56	$COCH_3$	$CH=CHC_6H_5$	2	3
57	H	CH=CH-2-(Cl)C_6H_4	0	4
58	H	CH=CH-3-(Cl)C_6H_4	3	2
59	H	CH=CH-4-(Cl)C_6H_4	3	4
60	H	CH=CH-4-(F)C_6H_4	3	5
61	H	CH=CH-4-$(CF_3)C_6H_4$	3	4
62	H	CH=CH-4-$(CH_3)C_6H_4$	4	4
63	H	CH=CH-3,4,5-$(OCH_3)_3C_6H_2$	2	2

[a–c] See corresponding footnotes in Table I.

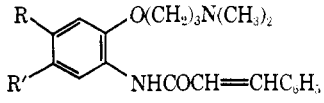
A study of the immunosuppressive and the antiserotonin results indicates that most of the compounds of this series showed comparable activity; however, several exceptions are apparent. Compounds **29**, **47**, **52**, **65**, and **67** showed a high immunosuppressive response with low antiserotonin activity, whereas **2**, **9**, **11**, **33**, and **57** exhibited weak immunosuppressive but strong antiserotonin activity. The most active immunosuppressive compounds contain the 3-dimethylaminopropyl side chain. A comparison of a series of guanidine analogs (**21–24**) showed that methylation of the latter group resulted in less active products. The removal (**29**) or replacement of the thio linkage of cinanserin (**1**) by oxa (**26**) slightly decreased activity; however, the substitution by SO_2, CO, CONH, $CON(CH_3)$, COO, NHCO, and $N(CH_3)$ linkages (**32–39**) resulted in compounds with low to moderate immunosuppressive activity. The *meta* and *para* isomers (**69** and **70**) were also less active than cinanserin. Introduction of substituents in either of the phenyl rings usually decreased the immunosuppressive and antiserotonin response; however, the analogs containing a CH_3 or a CF_3 group in the anilide ring (**65** and **67**) exhibited high immunosuppressive activity. Substitution of H of the amido group by Me, $PhCH_2$, or Ac (**54-56**), or the replacement of the cinnamoyl group by crotonyl, sorboyl, benzoyl, phenacetyl, phenylpropionyl, and phenylpropioloyl (**40–45**), gave products with less immunosuppressive activity. Alkylation of the α carbon of the cinnamoyl portion with Et, Pr, and Bu groups (**47–49**), or replacement of the phenyl group by α-thienyl (**51**) and cyclohexyl (**52**), did not alter immunosuppressive activity. The *cis* isomer (**72**) was less active than cinanserin (*trans* form)

(5) R. C. Millonig, manuscript in preparation.

(6) H. C. Nathan, S. Bieber, G. B. Elion, and G. H. Hitchings, *Proc. Soc. Exptl. Biol. Med.*, **107**, 796 (1961).

(7) B. Rubin, J. J. Piala, J. C. Burke, and B. N. Craver, *Arch. Intern. Pharmacodyn.*, **152**, 132 (1964)

TABLE III
NUCLEAR-SUBSTITUTED AND RELATED COMPOUNDS



No.	R	R'	Immunosuppressive activity[a]	Antiserotonin activity[b]
64	OCH_3	H	2	1
65	H	CH_3	5	1
66	H	Cl	1	1
67	H	CF_3	4	1
68	H	NO_2	1	1
69	3′-(3-Dimethylaminopropoxy)cinnamanilide		1	0
70	4′-(3-Dimethylaminopropylthio)cinnamanilide		2	0
71	3-Cinnamamido-2-(3-dimethylaminopropoxy)pyridine		1	1
72	*cis*-Cinanserin		2	2
73	2-(3-Dimethylaminopropylthio)aniline		0	0
	Azathioprine[d]		5	*c*
	Cyproheptadine[d]		2	5

[a,b] See corresponding footnotes in Table I. [c] Not tested. [d] Reference compounds.

TABLE IV
NEW COMPOUNDS

No.	Mp, °C[a]	Yield, %	Formula[b]	Analyses[c]
3	66–68	7	$C_{24}H_{32}N_2OS \cdot C_6H_8O_7 \cdot 2H_2O$	C, H, N, S
4	107–109	31	$C_{21}H_{26}N_2O_2S \cdot HCl$	Cl, S
6	178–180	36	$C_{22}H_{26}N_2O_2S \cdot HCl$	Cl, S
12	138–140	10	$C_{19}H_{22}N_4OS \cdot 0.5H_2SO_4 \cdot 0.5H_2O$	C, H, N, S
13	149–151	79	$C_{21}H_{26}N_2OS \cdot HCl$	N, S
18	189–191	69	$C_{22}H_{26}N_2OS \cdot HCl$	Cl, N
21	210–212	38	$C_{18}H_{20}N_4OS \cdot 0.5H_2SO_4$	N, S
22	174–176	55	$C_{19}H_{22}N_4OS \cdot HCl$	Cl, N
23	85–87	8	$C_{18}H_{22}N_4OS \cdot C_6H_8O_7$	C, H, N, S
24	148–150	35	$C_{21}H_{26}N_4OS \cdot HCl$	Cl, N
32	164–166	19	$C_{20}H_{24}N_2O_3S \cdot C_6H_{13}NO_3S$	N, S
46	136–138	68	$C_{21}H_{26}N_2OS \cdot C_6H_8O_7$[d]	C, H, N, S
47	123–125	51	$C_{22}H_{28}N_2OS \cdot C_6H_8O_7$[d]	C, H, N, S
49	80–90	50	$C_{24}H_{32}N_2OS \cdot C_6H_8O_7 \cdot 0.5H_2O$[d]	C, H, N, S
50	144–146	54	$C_{21}H_{26}N_2OS \cdot HCl$	Cl, S
51	121–123	53	$C_{18}H_{22}N_2OS_2 \cdot HCl$	Cl, S
52	135–137	66	$C_{20}H_{30}N_2OS \cdot HCl$	Cl, S
53	123–125	25	$C_{21}H_{28}N_2OS \cdot C_6H_{13}NO_3S$[d]	N, S
54	166–168	50	$C_{21}H_{26}N_2OS \cdot C_2H_2O_4$	C, H, N, S
56	152–154	44	$C_{22}H_{26}N_2O_2S \cdot HCl$	Cl, N
58	172–174	79	$C_{20}H_{23}ClN_2OS \cdot HCl$	Cl, S
60	118–120	77	$C_{20}H_{23}FN_2OS \cdot HCl$	Cl, S
61	162–164	60	$C_{21}H_{23}F_3N_2OS \cdot HCl$	Cl, S
62	175–177	82	$C_{21}H_{26}N_2OS \cdot HCl$	Cl, S
65	172–174	72	$C_{21}H_{26}N_2O_2 \cdot HCl$	Cl, N
72	88–93	22	$C_{20}H_{24}N_2OS \cdot C_2H_2O_4$	N, S

[a] Crystallization solvents: MeCN, **6, 13, 18, 24, 32, 47, 49, 50, 52, 53, 60, 61, 72**; EtOH, **46, 54, 58, 62, 65**; *i*-PrOH, **22**; acetone, **51**; butanone, **56**; MeOH–Et_2O, **3, 21**; MeCN–MeOH, **4**; $CHCl_3$–Et_2O, **12**; and EtOH–Et_2O, **23**. [b] Salts: $C_6H_8O_7$ = citric acid, $C_6H_{13}NO_3S$ = cyclohexanesulfamic acid, and $C_2H_2O_4$ = oxalic acid. [c] Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. [d] Previously purified as oxalic acid salts: **46**, mp 141–143° (EtOH); **47**, mp 124–126° (EtOH); **49**, mp 118–120° (EtOH); **53**, mp 111–113° (MeCN).

as an immunosuppressive agent. Five compounds of this series (**29**, **47**, **52**, **65**, and **67**) are presently undergoing further evaluation as potential immunosuppressive agents.

Experimental Section

Melting points were taken in a Thomas–Hoover capillary melting point apparatus and are corrected. The ir spectra of all compounds were in agreement with the assigned structures.

Most of the compounds listed in Table IV were obtained by the interaction of 2′-(3-dimethylaminopropylthio)aniline with the appropriate substituted-acryloyl chloride in the usual manner.[8] In the preparation of **65**, 4-methyl-2-nitrophenol was converted to the product according to the method used to obtain **26**.[8]

2′-(3-Chloropropylthio)cinnamanilide was treated with NaI and then with the appropriate amine in the usual manner[1] to give **3**, **4**, and **6**.

The synthesis of 2′-(3-aminopropylthio)cinnamanilide [from N-(3-bromopropyl)phthalimide] was carried out in the same manner as that of 2′-(2-aminoethylthio)cinnamanilide (**A**).[8] These amines were then refluxed for 2 hr with 2-methyl-2-thiopseudourea sulfate in DMF to give **12** and **21**. When **A** was treated with N,S-dimethylthiopseudourea hydriodide in the same manner, **23** was obtained. By interaction of 2′-[2-(methylamino)ethylthio]cinnamanilide hydrochloride[8] with dimethylcyanamide in DMF for 20 hr and with excess cyanamide in EtOH for 2 hr, **22** and **24** were obtained.

Treatment of 2,3-dihydro-2-phenyl-1,5-benzothiazepin-4(5H)-one with 1-(2-chloroethyl)piperidine in the usual manner[9] gave **18**.

A solution of **1** in AcOH was treated with excess H_2O_2 at 40° and then refluxed for 1 hr. The product thus obtained (**32**) was purified as the oxalate, mp 194–196° (MeOH). We have found that our previous preparation,[8] in which the reaction mixture was not heated above 40°, contained some of the corresponding sulfoxide.

Addition of the base of **1** to liquid NH_3 containing 1 equiv of KNH_2, followed by a slight excess of MeI, gave a mixture of the base of **54** and unchanged starting material. Extraction of the residue with hexane, followed by evaporation, gave the base of **54**.

A mixture of **1** and excess Ac_2O was refluxed for 1 hr, partially concentrated, and then diluted (Et_2O) to give **56**.

The *cis* form of cinanserin (**72**) was obtained by photolysis of a 10% solution of **1** in EtOH for 2 hr using a 450-W photochemical lamp. The solvent was removed under reduced pressure and the residue was triturated with Me_2CO. The solid (unchanged **1**) was filtered off, the solvent was evaporated, and the residue was converted to the base. The latter was triturated with warm hexane and cooled to remove a further quantity of the hexane-insoluble *trans* isomer. Evaporation of the solvent gave the *cis* base (>80% pure, nmr data) which was dissolved in MeCN and treated with an equivalent quantity of oxalic acid to give **72** as a nearly colorless crystalline product (>70% pure, nmr data).

Acknowledgments.—We are indebted to Dr. B. Rubin and Mrs. E. O. Grigas for the pharmacological data, to Dr. A. I. Cohen for the nmr data, and to Mr. J. Alicino and his staff for the analyses reported herein.

(8) J. Krapcho, E. R. Spitzmiller, C. F. Turk, and J. Fried, *J. Med. Chem.*, **7**, 376 (1964).
(9) J. Krapcho, E. R. Spitzmiller, and C. F. Turk, *ibid.*, **6**, 514 (1963).