M aqueous ammonium acetate (NH_4OAc) (pH 6.9) and CH_3CN and appropriate gradients. A flow rate of 7.0–8.0 mL/min was used. Elution of the peptides was monitored at 214 and/or 280 nm. Collected fractions were readily screened by analytical HPLC and pooled accordingly. The peptides thus obtained were subjected to rotary evaporation, in vacuo, to remove CH_3CN and then lyophilized twice. Purified peptides were analyzed for homogeneity by analytical HPLC on a μBondapak C_{18} (0.39 × 15 cm, 10-μm particle size) column using appropriate linear gradients of 0.01% aqueous TFA (pH 2.9) and 0.01% TFA–CH_3CN and of 0.01 M ammonium acetate (pH 6.9) and CH_3CN. Their amino acid composition and peptide content were assessed by quantitative amino acid analysis after acidic hydrolysis in vacuo (6 N HCl, 110 °C, 18 h), as we previously described.[19] Individual amino acid recovery ranged from 0.83 to 1.08/residue, except for Cys and Trp. The molecular mass of peptides **6**, **13**, **19**, **29**, and **30** and therefore Cys and Trp integrity were assessed by FAB-MS on a Kratos MS-50 TATC instrument.

HSV-1 Ribonucleotide Reductase Assay. The inhibitory effect of the synthetic peptides, on HSV-1 RR activity, was determined as previously described[19] using HSV-1 RR partially purified from quiescent BHK-21/C13 cells infected with strain F at 20 plaque-forming units per cell. The specific activity of the viral reductase preparation was 37 units/mg protein, one unit of RR being defined as the amount of enzyme generating 1 nmol of deoxycytidine 5′-diphosphate per hour under the assay conditions.

Acknowledgment. We gratefully acknowledge Claire Guilbaut for preparing the RR extract and Marie-Françoise Marchal for secretarial assistance. This research was supported by the Medical Research Council of Canada and Bio-Méga Inc. Pierrette Gaudreau is the recipient of a scholarship from "Fonds de la Recherche en Santé du Québec".

Bis Basic Substituted Diaminobenzobisthiazoles as Potential Antiarthritic Agents

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A series of benzobisthiazoles were screened for antiinflammatory activity in the carrageenan paw edema and adjuvant arthritis tests. Compound **26**, 2,6-bis(*N*,*N*-diethylamino)benzo[1,2-*d*:5,4-*d*′]bisthiazole, was found to inhibit the swelling of the uninjected paw in the prophylactic adjuvant arthritis model with an ED_{50} of 2.3 mg/kg orally. As with most compounds of this series, **26** was inactive in acute model of inflammation, such as paw edema; like steroids, it showed activity in the granuloma pouch assay but did not inhibit cyclooxygenase, indicating a mode of action different from the classical nonsteroidal antiinflammatory drugs (NSAID's). At doses higher than those producing antiinflammatory activity, **26** had some immunoregulating properties.

Ever since it became clear that the classical nonsteroidal antiinflammatory drugs (NSAID's) produced gastrointestinal side effects by virtue of cyclooxygenase (CO) inhibition, research in medicinal laboratories has been directed at finding compounds which interfere with the underlying cause of the arthritic diseases. The name given to these elusive agents, "disease modifying agents" (DMA's), indicates the vagueness of the concept. One avenue of research was to concentrate on the aberrant autoimmune reaction believed to be the result of an inflammatory stimulus of unknown origin.[1]

At the onset of the present work, very few chemicals were known to possess a selective mode of action on either the humoral or the cellular arms of the immune system.[2] One such experimental agent was tilorone (**90a**). Originally found to be an interferon inducer,[3] it was later shown to suppress cell-mediated responses and, in contrast, to enhance antibody production in animals models.[4] The compound was also reported to suppress adjuvant induced arthritis in rats[4–6] and experimental allergic encephalomyelitis,[4,6] two cell-mediated, delayed type reactions.

Tilorone has since generated an active search for bioisosteric analogues with an improved biological profile (for review see ref 7). In our laboratories, we have examined, as a potential source of antiarthritic drugs, the benzobisthiazole systems having as basic side chains (alkylamino)acetamido or related functions in lieu of the alkylamino ether moiety of tilorone. The compounds were screened in the acute paw edema and the chronic adjuvant arthritis assays. In the latter test, high activity in suppressing the secondary inflammation in the uninjected paw was considered to be the result of interference with the immune response.[8]

Chemistry

Basic [(alkylamino)acyl]amino side chains were introduced into the diaminobenzobisthiazoles I, II, III, IV, and V (Y_1 = Y_2 = NH_2) (Tables I and II). Two general methods were used: (1) chloroacylation with excess chloroacyl chloride (or anhydride) followed by treatment of the resulting chloroacetylamines with appropriate al-

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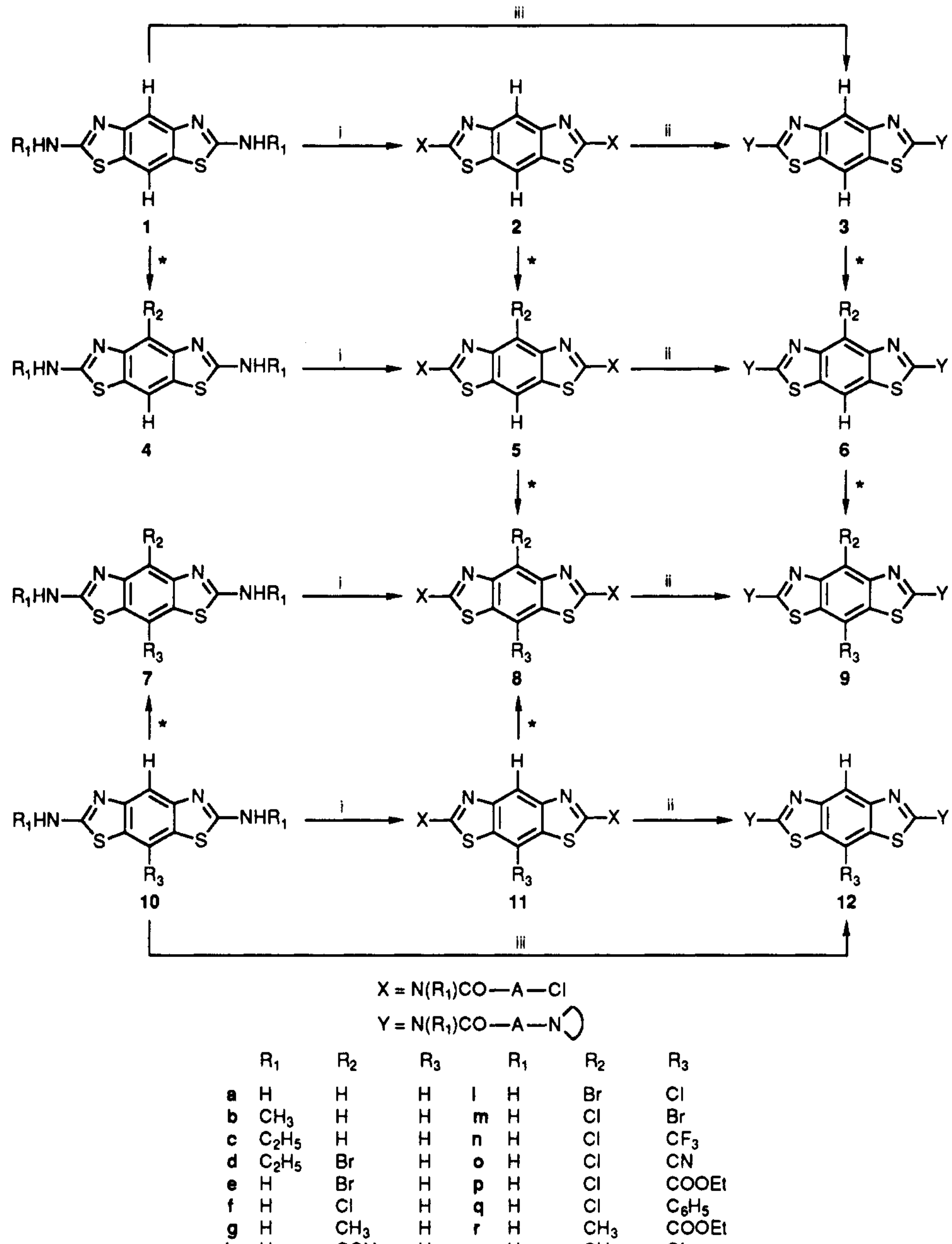
Scheme I[a]



X = N(R1)CO—A—Cl

Y = N(R1)CO—A—N⟩

	R_1	R_2	R_3		R_1	R_2	R_3
a	H	H	H	l	H	Br	Cl
b	CH_3	H	H	m	H	Cl	Br
c	C_2H_5	H	H	n	H	Cl	CF_3
d	C_2H_5	Br	H	o	H	Cl	CN
e	H	Br	H	p	H	Cl	COOEt
f	H	Cl	H	q	H	Cl	C_6H_5
g	H	CH_3	H	r	H	CH_3	COOEt
h	H	OCH_3	H	s	H	CH_3	Cl
i	H	NO_2	H	t	H	CH_3	COONa
j	H	NH_2	H	v	H	H	Cl
k	H	Cl	Cl	w	H	H	COOEt

[a] (i) ClCO-A-Cl; (ii) H-N⟩ (method A); (iii) EtONa + EtOOC-A-N⟩ (method B). (*) See Table II reaction sequences. (A) = CH_2, CH_2CH_2, $CH(CH_3)$. (-N⟩) = aliphatic, heterocyclic, and carbocyclic amines. Schemes I and II illustrate routes for compounds of types I and V (Tables I and II). Types II, III, and IV (Table I) were prepared by similar routes from appropriate starting materials (Table III).

kylamines, heteroalkylamines, or heterocyclic amines (method A) and (2) aminolysis of ethyl aminoalkyl carboxylates with the diaminobenzobisthiazoles I–IV ($Y_1 = Y_2 = NH_2$) in the presence of sodium ethoxide[9] (method B) (Schemes I and II). The later method was especially indicated when the competing transacylation reaction ii (method A) was favored over the desired alkylation of the amine, or when no reaction occurred. In several cases we could isolate the monoaminoacylamines which originated either from incomplete reaction (method B) or from cleavage of one of the chloroacetylamine bonds in method A. This cleavage was to some extent dependent on the solvent used. In most cases ethanol and DMF afforded a mixture of products, while dioxane reduced the cleavage to a minimum. These monoaminoacetamides could be used to prepare a few unsymmetrical diaminoacylamides. Compound **89**, for example, was prepared from 2-amino-6-[[(diethylamino)acetyl]amino]benzo[1,2-*d*:5,4-*d*′]bisthiazole (**87**) by method A with piperidine. Direct monoacylation was never observed. Even when a large excess of diaminobenzobisthiazole was used, only mixtures of mono- and diacylated products resulted. Nuclear substituted products (Table II) were prepared through acylation, by method A or B, of their corresponding diaminobenzobisthiazoles (Table III), or through introduction of substituents in the proper preacylated compounds by standard methods (see reaction sequences in Table II). Starting materials listed in Table III were prepared by standard procedures[10,11] from the appropriate phenylene-

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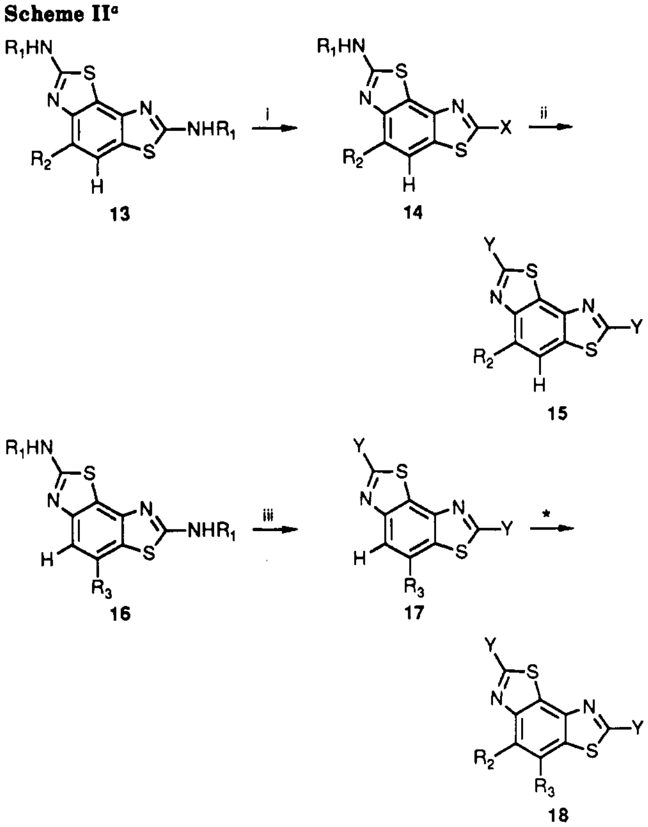
Scheme II[a]



X = N(R_1)CO—A—Cl
Y = N(R_1)CO—A—N⊃

	R_1	R_2	R_3
X	H	Cl	H
Y	H	H	OCH_3
Z	H	Cl	OCH_3

[a] (i) ClCO-A-Cl; (ii) H-N⊃ (method A); (iii) EtONa + EtOOC-A-N⊃ (method B). (*) See Table II reaction sequences. (A) = CH_2, CH_2CH_2, $CH(CH_3)$. (-N⊃) = aliphatic, heterocyclic, and carbocyclic amines. Schemes I and II illustrate routes for compounds of types I and V (Tables I and II). Types II, III, and IV (Table I) were prepared by similar routes from appropriate starting materials (Table III).

diamines. Where necessary, the linear versus angular nature of the products was determined by UV[11] or NMR[12] analysis. The bis[[(alkylamino)alkyl]amines] **85** and **86** were prepared via the corresponding thioureas **21a** and **21b** (Scheme III). In the same fashion the bisacetic acid **83** was obtained from ester **22c** via **21c**. These derivatives, however, proved to be unsuitable precursors for the synthesis of the carbamoylmethyl derivative **84**, in which carbonyl and methylene were reversed as compared with the amide **26**. Instead **84** was prepared by the reaction of 2,6-dichlorobenzo[1,2-*d*:5,4-*d*′]bisthiazole **23** and glycine diethylamide[14] (Scheme IV). The product could also be obtained in small yield through oxidative cyclization of 1,3-bis[*N*′-(*N*,*N*-diethylacetamido)thioureido]benzene (**21c**). Attempts to obtain **85** directly by reduction of **26** were unsuccessful.

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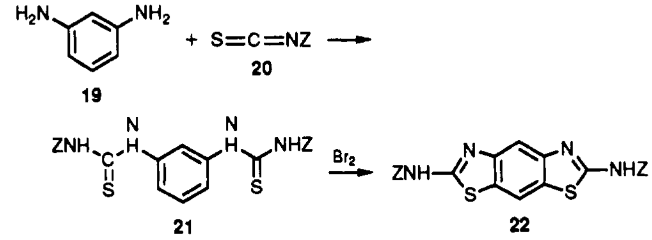
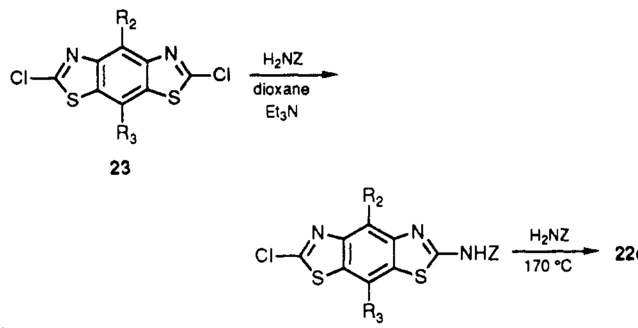
Scheme III[a]



	Z	
a	$CH_2CH_2N(CH_2CH_3)_2$	85*
b	$CH(CH_3)CH_2CH_2CH_2N(CH_2CH_3)_2$	86*
c	$CH_2COOCH_2CH_3$	
d	CH_2COOH	83*
e	$CH_2CON(CH_2CH_3)_2$	84*

[a] (*) Compound number from Table I.

Scheme IV



24
Z = CH_2CONEt_2

Biological Results and Discussions

Tables I–III show the activities in the acute carrageenin paw edema (CPE) and in the chronic rat adjuvant arthritis (AA) tests. A prophylactic treatment protocol, as described in the Experimental Section, was used in the latter case. The percent change in body weight, in the AA test, as compared to the weight of the untreated animals, was considered a preliminary indication of the compound's toxicity.

Few compounds showed significant activity in the CPE assay even at the high screening dose of 200 mg/kg and, for these, there was no correlation with the inhibition of edema observed in the injected paw in the D–AA screen. It is well accepted that in D–AA the injected paw swelling is related to the acute phase of inflammation. Moreover when retested at 50 mg/kg, these compounds, except **40**, showed no activity (compounds of Table II were not retested). The high activity of the bisamines **111** and **131** in CPE may be attributed to the stimulation of the adrenal cortex. Indeed **111** was found negative in adrenalectomized animals. In the AA screen, for the straight-chain alkylamino derivatives, high activity resulted with the methyl (**25**) and the ethyl (**26**, **34**) while the higher homologues (**27**, **28**, **29**, **35**) were inactive.

Elongation (**30**), branching (**31**), or shortening (**79**) of the acylamino linkage resulted in loss of activity. It was established also that the nitrogen of the amido group could not bear groups larger than methyl without compromising the activity: see **26**, **32** versus **33** (Table I) and **94** versus **107** (Table II).

Bis(monoalkylamino) derivatives would present greater activity than their corresponding dialkyl analogues. See **34** versus **26**; **37** and **40** versus **43** and **44**, respectively. However, the monoethyl **34**, at the screening dose of 50 mg/kg, caused the death of all animals at day 4 of the experiment. This is in sharp contrast with the monocycloalkylamino derivatives which induced, at the same dose, a body weight gain in the animals.

In the heterocyclic amine series significant activity was observed for quite a few piperidino (51–63) and piperazino (64–77) derivatives. With respect to ring substitution only the monomethyl- or monoethylpiperidino compounds presented activity. Rings having two methyl groups or larger, bulkier substituents had no activity with the exception of some *N*-phenyl analogues, e.g. **73**.

Substitution at the tricyclic nucleus in the 4-position (Table II) in general had a negative influence; either the activity was retained but with a corresponding increase in the toxicity of the compound, as for the methyl (**91**), 4-chloro (**93**), and 4-bromo (**94**) derivatives, or the activity was completely abolished, as for the 4-methoxy (**92**), 4-nitro (**95**), and 4-amino (**96**) derivatives. No clear SAR was recognized for substitution in the 8-position nor in the 4- plus 8-positions.

Linear and angular anellation isomers of benzo[1,2-*d*:5,4-*d*′]bisthiazole (I) with the basic [(diethylamino)acetyl]amino side chains of the prototype **26** had no activity in AA; see **80**, **81**, **82** (Table I) and **108** (Table II). The borderline inhibition observed with **108** was probably a reflection of the severe loss in body weight induced by the compound.

The mono[[(dialkylamino)acyl]amide] **87** showed only weak activity in comparison with **26**. This seems surprising in view of the precursor activity, discussed below.

The precursor **111** had been reported to be active in some inflammatory tests,[11] and we also observed potent activity in AA (Table III). However, it seems unlikely that the antiarthritic activities of the bis[[(alkylamino)acyl]amines] are due to in vivo cleavage to the diamine **111** for the following reasons: (a) It would be unusual that some (aminoacyl)amides are metabolized to the diamine and others are not. (b) The diamine **127**, isomeric to **111**, is highly active while the bis[(aminoacyl)amide] **80**, isomeric to the prototype **26** is not. (c) Conversely, the 2,6-diamino ring-substituted analogues **113**, **114**, **115** (Table III) were all found inactive as such but were quite active after introduction of the [(diethylamino)acetyl]amino side chains **94**, **99**, and **100** (Table II). Lastly, it would appear that the presence of a carbonyl function in the side chains is a requisite for activity. Thus, the reduced analogue **85** turned out to be completely inactive. Shifting the carbonyl function as in the carbamyl derivative **84** reduced the activity considerably as compared with **26**. A few selected members of the series were further evaluated in a secondary screening program comprising established adjuvant arthritis (E–AA) (with a therapeutic dosing regiment), granuloma pouch, and analgesic assays.

Interestingly, all compounds found active in the E–AA assay significantly restored the body weight of the arthritic animals. In particular, with compound **26** the increase was greater than in the polyarthritic controls. The oral ED_{50} for this product was found to be 41 mg/kg for the injected paw and 5 mg/kg for the uninjected paw.

We could not detect any significant analgesic activity for **26** by the acetic acid writhing test. The granuloma pouch assay primarily detects steroidal compounds and as a rule gives negative results for the NSAID's. In that assay **26** significantly inhibited the exudate volume (ED_{50} 35.7 mg/kg) and the granuloma weight (ED_{50} 46.7 mg/kg).

Unlike typical NSAID's **26** did not induce ulceration in rats up to 200 mg/kg, the highest dose tested. As expected, the compound did not inhibit human platelet cyclooxygenase in vitro at concentration from 0.1 to 30 μM.[15]

This product inhibited the T-cell-dependent antibody production in the Jerne plaque forming cell (PFC) assay (Table IV). However, this was observed at doses at least 10 times in excess of those necessary for anti-AA activity. It is also to be noted that, in our hands, tilorone failed to enhance, in the same assay, antibody production.

Preliminary acute toxicity studies in mice, rats, rabbits, and guinea pigs established oral LD_{50}'s of 2000 mg/kg. Further studies to determine the pharmacological and immunological profile will be published elsewhere.

Conclusion

Bis basic side chains were introduced in each one of the isosteric structures of diaminobenzobisthiazoles I–V, and the compounds were evaluated for antiinflammatory activity. Only the linear structure I was found active. This activity was restricted to the bis(lower alkylamino) (e.g. **26**), the bis(cycloalkylamino) (e.g. **37**), and some of the bis(heterocyclic amino) derivatives (e.g. **53**). Furthermore an acetamido linkage to the nucleus was a requisite for activity. These products had no significant activity in a model of acute inflammation but were orally active in model of chronic inflammation. In particular compound **26** had an oral ED_{50} of 2.3 mg/kg in D–AA and 5.0 mg/kg in E–AA for the uninjected paw. Unlike NSAID's **26** showed activity in granuloma pouch assay, which suggested some analogy with steroids. The product had no effect on CO activity in vitro and as expected induced no ulceration in vivo in rats. Immunosuppression could be involved although inhibitory effect in Jerne plaque cells assay was seen at doses 10× the dose required for AI activity.

Experimental Section

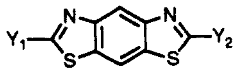
Infrared spectra were recorded with a Perkin-Elmer Model 237B or 267 spectrophotometer using KBr disks. ^1H NMR spectra were taken in the indicated solvent with a Varian Model T-60 NMR spectrometer. UV spectra were recorded in 0.1 N HCl with a Perkin-Elmer Model 320 instrument. Melting points were determined with an electrothermal melting point apparatus and are uncorrected. Elemental analyses were performed by Micro Tech Laboratories Inc., Skokie, IL and were within ±0.4% of the calculated values except as indicated.

2,6-Bis[[(*N*,*N*-diethylamino)acetyl]amino]benzo[1,2-*d*:5,4-*d*′]bisthiazole (26) and 2-Amino-6-[[(*N*,*N*-diethylamino)acetyl]amino]benzo[1,2-*d*:4,5-*d*′]bisthiazole (87). Method A. A stirred mixture of 2,6-bis[(chloroacetyl)amino]benzo[1,2-*d*:4,5-*d*′]bisthiazole (**132**) (15.2 g, 0.0405 mol) and diethylamine (16 g, 0.22 mol) in dioxane (120 mL) was heated at 100 °C overnight in a pressure vessel. After cooling, the solvent was evaporated in vacuo, the residue was taken up in chloroform (500 mL), and the solution was washed with water and brine and dried over $MgSO_4$. Evaporation of this solution left 15 g of a crude solid, which was applied to a 450-g dry silica gel column and eluted with chloroform/methanol, 97/3. Fractions were pooled according to TLC. Compound **26** (9.0 g, 49.5%) eluted first. It crystallized from ethanol: mp 225–227 °C; IR 3400, 1700, 1660 sh, 1640 sh, 1615, 1550 cm^{-1}; UV λ_{max} (log ε) 214 (4.46), 277 (4.68), 319 (sh) (4.36), 332 nm (4.46); NMR ($CDCl_3$) δ 1.1 (t, 12 H), 2.7 (q, 8 H), 3.3 (s, 4 H), 8.15 (s, 2 H), 10.5 (s, br, 2 H ex). Anal. ($C_{20}H_{28}N_6O_2S_2$) C, H, N, S. Following **26**, the monoacylated product **87** was collected. It melted at 203–205 °C after one crystallization from ethanol/DMF (0.30 g, 2.2%): IR 3300 sh, 3190, 3060, 1710, 1680, 1670, 1650, 1575 sh, 1555 cm^{-1}; NMR (Me_2SO-d_6) δ 1.0 (t, 6 H), 2.6 (q, 4 H), 3.4 (s, 2 H), 7.45 (s, br, 2 H ex), 7.7 (s, 1 H), 8.2 (s, 1 H), 8–10 (1 H, ex). Anal. ($C_{14}H_{17}N_5OS_2$) C, H, N, S.

2,6-Bis[(chloroacetyl)amino]benzo[1,2-*d*:5,4-*d*′]bisthiazole (132). Chloroacetyl chloride (20 mL, 0.294 mol) was added portionwise, at 5–10 °C, to a vigorously stirred suspension of 2,6-diaminobenzo[1,2-*d*:5,4-*d*′]bisthiazole[10] (**111**) (18.5 g, 0.0832 mol) in DMF (130 mL). The mixture was then heated on a water

(15) Farina, P.; Graham, A. Personal communication.

Table I. Chemical and Biological Screening Data for *N*-Aminoacyl (25–82, 87–89), *N*-Carboxy and *N*-Carbamoylalkyl (83–84), and *N*-Aminoalkyl (85–86) Derivatives of Diaminobenzobisthiazoles[a] of Structures I, II, III, and IV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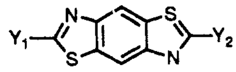
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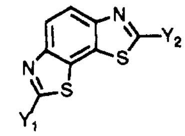
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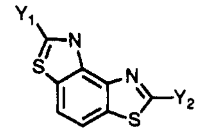

IV

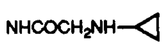
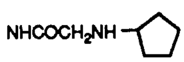
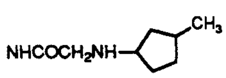
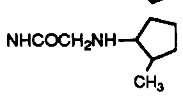
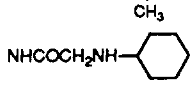
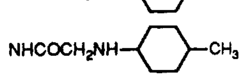
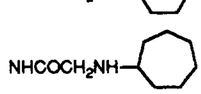
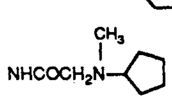
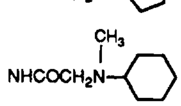
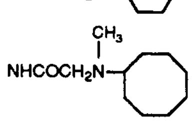
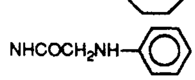
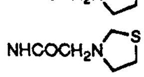
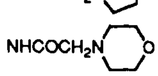
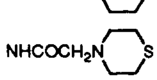
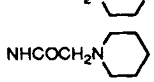
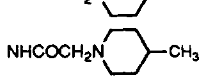
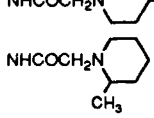
compd	type	$Y_1 = Y_2$	method[b]	mp, °C[c]	crystn[d] solv	formula[e]	CPE[f,g] (mg/kg) % inhibn	D-adjuvant arthritis[f,g] injd/uninjd paw (mg/kg) % inhibn	D-adjuvant arthritis[f,g] % change body wt
25	I	$NHCOCH_2N(CH_3)_2$	B	288–290	I	$C_{16}H_{20}N_6O_2S_2$	8 (200)	1/81 (50)*	−37
26	I	$NHCOCH_2N(C_2H_5)_2$	A, B	225–227	II	$C_{20}H_{28}N_6O_2S_2$	9 (200)	76/98 (50)*[h]	−22
27	I	$NHCOCH_2N(C_3H_7)_2$	A	≥190 dec	III	$C_{24}H_{36}N_6O_2S_2 \cdot 2HCl$	46 (200)* 1 (50)	29/47 (50)	−11
28	I	$NHCOCH_2N(i\text{-}C_3H_7)_2$	B	208–210	II	$C_{24}H_{36}N_6O_2S_2$	0 (50)	0/0 (50)	−5
29	I	$NHCOCH_2N(CH_2CH_2OC_2H_5)_2$	A	82–84	II	$C_{28}H_{44}N_6O_6S_2$	0 (50)	16/21 (50)	0
30	I	$NHCOCH_2CH_2N(C_2H_5)_2$	B	>300 dec	I	$C_{22}H_{32}N_6O_2S_2 \cdot 0.1H_2O$	16 (50)	29/48 (50)	−5
31	I	$NHCOC(CH_3)HN(C_2H_5)_2$	A	230–235 dec	IV	$C_{22}H_{32}N_6O_2S_2 \cdot 2HCl$	0 (50)	0/0 (50)	−19
32	I	$N(CH_3)COCH_2N(C_2H_5)_2$	A	244–246 dec	V	$C_{22}H_{32}N_6O_2S_2 \cdot 2HCl$	16 (200)	0/16 (50)	−16
33	I	$N(C_2H_5)COCH_2N(C_2H_5)_2$	A	133–135	II	$C_{24}H_{36}N_6O_2S_2$	18 (200)	14/0 (50)[i]	−4
34	I	$NHCOCH_2NHC_2H_5$	B	230–250 dec	VI	$C_{16}H_{20}N_6O_2S_2 \cdot 2HCl$	21 (50)	79/100 (25)*[j]	−28
35	I	$NHCOCH_2NHCH(CH_3)_2$	B	255–257	VII	$C_{18}H_{24}N_6O_2S_2 \cdot 0.5H_2O$[k1]	28 (50)	9/11 (50)	+7
36	I		B	203–205	VII	$C_{18}H_{20}N_6O_2S_2 \cdot H_2O$	18 (200)	0/16 (50)	+14
37	I		B	258–260	VII	$C_{22}H_{28}N_6O_2S_2 \cdot 0.75H_2O$	13 (200) 9 (50)	43/70 (50)*	+10
38	I		B	220–222	VII	$C_{24}H_{32}N_6O_2S_2$[k2]	11 (200)	11/30 (50)	−7
39	I		B	238–240	VII	$C_{24}H_{32}N_6O_2S_2$	36 (200)* 18 (50)	0/0 (50)	−22
40	I		B	257–259 dec	VII	$C_{24}H_{32}N_6O_2S_2$	20 (200) 15 (50)	3/59 (50)*	+8
41	I		B	250–252 dec	VII	$C_{26}H_{36}N_6O_2S_2$[k3]	3 (50)	21/54 (50)	−4
42	I		B	240–242	VII	$C_{26}H_{36}N_6O_2S_2$[k4]	0 (50)	6/59 (50)*	+1
43	I		A	189–191	II	$C_{24}H_{32}N_6O_2S_2$	6 (50)	21/3 (50)	−18
44	I		A	235–237 dec	V	$C_{26}H_{34}N_6O_2S_2 \cdot 2HCl \cdot 1.5H_2O$	6 (50)	32/27 (100)	−4
45	I		A	137–139	II	$C_{30}H_{44}N_6O_2S_2$	0 (50)	22/29 (50)	−3
46	I		B	295–297 dec	VIII	$C_{24}H_{20}N_6O_2S_2$[k5]	0 (50)	0/0 (50)	−11
47	I		A	290–292 dec	VII	$C_{20}H_{24}N_6O_2S_2$	17 (50)	18/21 (50)	−21
48	I		A	263–265	VII	$C_{18}H_{20}N_6O_2S_4 \cdot 0.5H_2O$	11 (50)	26/27 (50)	+11
49	I		A	280–295 dec	VI	$C_{20}H_{24}N_6O_2S_2 \cdot 2HCl$	17 (50)	18/21 (50)	−21
50	I		A	303–305 dec	VII	$C_{20}H_{24}N_6O_2S_4$	7 (50)	0/43 (200)	+6
51	I		A	293–295 dec	VI	$C_{22}H_{28}N_6O_2S_2 \cdot 2HCl \cdot 0.5H_2O$	0.8 (100)	56/79 (200)*	−6
52	I		A	245–247	VIII	$C_{24}H_{32}N_6O_2S_2$	20 (200)	70/100 (50)*	−26
53	I		A	235 dec	II	$C_{24}H_{32}N_6O_2S_2$	0 (50)	41/82 (50)*	+6

Table I (Continued)

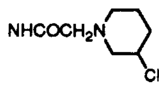
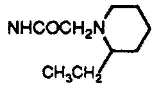
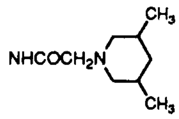
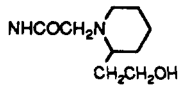
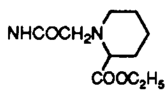
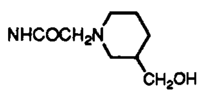
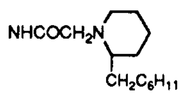
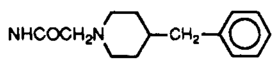
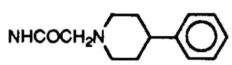
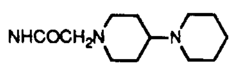
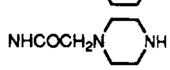
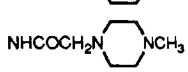
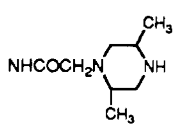
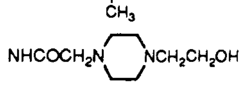
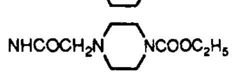
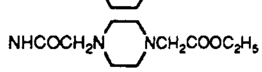
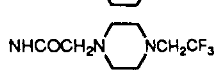
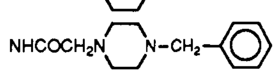
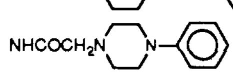
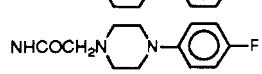
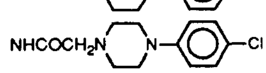
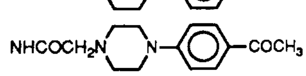
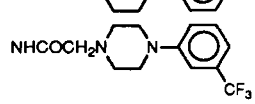
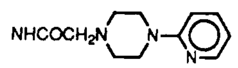
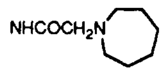
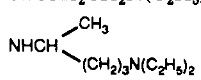
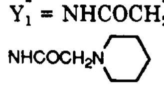
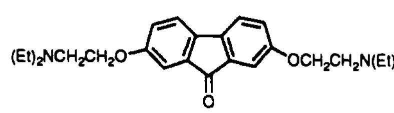
compd	type	$Y_1 = Y_2$	method[b]	mp, °C[c]	crystn[d] solv	formula[e]	CPE[f,g] (mg/kg) % inhibn	D-adjuvant arthritis[f,g] injd/uninjd paw (mg/kg) % inhibn	D-adjuvant arthritis[f,g] % change body wt
54	I		A	240–242	I	$C_{24}H_{32}N_6O_2S_2$	19 (200)	34/63 (200)*	+6
55	I		A	201–203	II	$C_{26}H_{36}N_6O_2S_2$	12 (200)	34/65 (200)*	−1
56	I		A	262–265	VII	$C_{26}H_{36}N_6O_2S_2$	15 (200)	29/11 (50)	−3
57	I		A	185–187	II	$C_{26}H_{36}N_6O_4S_2 \cdot 0.5H_2O$	30 (200)* 6 (50)	4/5 (50)	−9
58	I		A	138–140	II	$C_{28}H_{36}N_6O_6S_2$	20 (200)	6/0 (50)	−16
59	I		A	226–228	VII	$C_{24}H_{32}N_6O_4S_2$	16 (200)	25/47 (50)	−4
60	I		A	110	IV	$C_{36}H_{52}N_6O_2S_2 \cdot 0.75H_2O$	0 (200)	0/0 (50)	+1
61	I		A	233–235	VII	$C_{36}H_{40}N_6O_2S_2$	9 (200)	15/13 (50)	−16
62	I		A	288–290 dec	VII	$C_{34}H_{36}N_6O_2S_2$	1 (50)	40/12 (50)	−12
63	I		A	272–274	VII	$C_{32}N_{46}N_8O_2S_2 \cdot 0.33H_2O$	0 (200)	46/38 (50)	−3
64	I		A	230–250 dec	VII	$C_{20}H_{26}N_8O_2S_2 \cdot H_2O$	19 (50)	31/27 (50)	−9
65	I		A	256–258	II	$C_{22}H_{30}N_8O_2S_2 \cdot 0.33H_2O$	28 (50)	0/0 (50)	−25
66	I		B	260 dec	II	$C_{24}H_{34}N_8O_2S_2 \cdot 4HCl$	0 (200)	0/0 (50)	−6
67	I		A	238–240	VII	$C_{24}H_{34}N_8O_4S_2 \cdot H_2O$	24 (200)	16/72 (50)*	0
68	I		A	225 dec	X	$C_{26}H_{34}N_8O_6S_2 \cdot 2HCl$	17 (200)	26/60 (50)*	−17
69	I		A	158–160	II	$C_{28}H_{38}N_8O_6S_2 \cdot H_2O$	14 (200)	0/0 (50)	−11
70	I		B	270–275 dec	I	$C_{24}H_{28}F_6N_8O_2S_2$	25 (200)	0/10 (50)	−13
71	I		A	247–249	VII	$C_{34}H_{38}N_8O_2S_2 \cdot H_2O$	26 (200) 4 (50)	5/6 (50)	−6
72	I		A	293–295	VII	$C_{32}H_{34}N_8O_2S_2 \cdot 0.33H_2O$	0 (200)	40/56 (50)[l]	+1
73	I		A	268–270	VII	$C_{32}H_{32}F_2N_8O_2S_2$	11 (200)	41/79 (50)*	+12
74	I		A	225–227	VII	$C_{32}H_{32}Cl_2N_8O_2S_2 \cdot H_2O$	12 (200)	21/41 (50)	−2
75	I		A	>330	VII	$C_{36}H_{38}N_8O_4S_2 \cdot 2H_2O$	40 (200)* 1 (50)	0/0 (50)	−7
76	I		A	232–234	I	$C_{34}H_{32}F_6N_8O_2S_2$	0 (200)	39/73 (50)*	+4

Table I (Continued)

								D-adjuvant arthritis[f,g]	
compd	type	$Y_1 = Y_2$	method[b]	mp, °C[c]	crystn[d] solv	formula[e]	CPE[f,g] (mg/kg) % inhibn	injd/uninjd paw (mg/kg) % inhibn	% change body wt
77	I		A	285 dec	VII	$C_{30}H_{32}N_{10}O_2S_2{\cdot}H_2O$	9 (200)	0/16 (50)	−2
78	I		A	245–247	II	$C_{24}H_{32}N_6O_2S_2$	0 (50)	26/27 (50)	
79	I	$NHCONHC_2H_5$	E	>330 dec	VII	$C_{14}H_{16}N_6O_2S_2$	6 (200)	0/15 (50)	−1
80	II	$NHCOCH_2N(C_2H_5)_2$	A	280–315 dec	V	$C_{20}H_{28}N_6O_2S_2{\cdot}2HCl$	9 (50)	11/0 (5)[m]	−33
81	III	$NHCOCH_2N(C_2H_5)_2$	A	283–283.5	X	$C_{20}H_{28}N_6O_2S_2{\cdot}2HCl{\cdot}0.5H_2O$	0 (50)	2/36 (50)	−20
82	IV	$N(CH_3)COCH_2N(C_2H_5)_2$	A	165–167	II	$C_{22}H_{32}N_6O_2S_2{\cdot}0.25H_2O$[k6]	21 (200)	0/34 (50)	+4
83	I	$NHCH_2COOH$	C	310 dec	VII	$C_{12}H_{10}N_4O_4S_2$[k7]	2 (200)	0/2 (200)	−25
84	I	$NHCH_2CON(C_2H_5)_2$	D	235–237	VII	$C_{20}H_{28}N_6O_2S_2$	5 (200)	58/55 (50)*[,n]	−18
85	I	$NHCH_2CH_2N(C_2H_5)_2$	C	oil	IX	$C_{20}H_{32}N_6S_2{\cdot}0.5H_2O$	14 (100)	33/0 (100)	+3
86	I		C	145–150 d	XII	$C_{26}H_{44}N_6S_2{\cdot}H_2O$	0 (200)	0/0 (50)	−17
		Y_1, Y_2							
87	I	$Y_1 = NH_2$; $Y_2 = NHCOCH_2N(C_2H_5)_2$	A	203–205	I	$C_{14}H_{17}N_5OS_2$[k8]	13 (50)	34/61 (50)*	−20
88	I	$Y_1 = NHCOCH_2Cl$; $Y_2 = NHCOCH_2N(C_2H_5)_2$	A	280–282	II	$C_{16}H_{18}ClN_5O_2S_2$	25 (200) 14 (50)	38/0 (50)	−16
89	I	$Y_1 = NHCOCH_2N(C_2H_5)_2$ 	A	221–223	XI	$C_{21}H_{28}N_6O_2S_2$	20 (50)	50/58 (50)*	−14
90a	tilorone						*o*	59/45 (100)*[,p] 53/0 (50)	+10 −8
90b	cyclophosphamide						18 (100)[q]	86/100 (25)*[,g]	−19
90c	indomethacine						63 (10)* 34 (3)*	71/83 (2.5)* 61/69 (1.0)*	+18 +28

[a] [ ]: Type I, [1,2-*d*:5,4-*d*′]; type II, [1,2-*d*:4,5-*d*′]; type III, [1,2-*d*:4,3-*d*′]; type IV, [2,1-*d*:3,4-*d*′]; type V (see Table II), [1,2-*d*:3,4-*d*′]. [b] A and B, Schemes I and II; C, Scheme III; D, Scheme IV; E, see Experimental Section. [c] Uncorrected. [d] I, EtOH–DMF; II, EtOH; III, ethanol–ether; IV, free base crystallized from ethanol and treated with ethereal HCl; V, the free base as eluted from a SiO_2 column was treated with HCl; VI, EtOH–H_2O; VII, DMF; VIII, EtOH–$CHCl_3$; IX, CH_3OH; X, H_2O; XI, CH_3CN; XII, see Experimental Section. [e] All analyses were within ±0.4% of theoretical value except where indicated. [f] Animals were dosed orally. See Experimental Section for description of screening tests. [g] Statistically significant results with two-tail student's tests ($p < 0.05$) are marked with an asterisk (*). [h] The oral ED_{50} was established for the uninjected paw at 2.3 (0.8–6.9) mg/kg, $p = 0.05$. [i] 2/6 animals died. [j] At 50 mg/kg, all animals died. [k1] H: calcd, 5.86; found, 5.43. [k2] H: calcd, 6.44; found, 6.03. [k3] S: calcd, 12.13; found, 12.55. [k4] S: calcd, 12.13; found, 11.70. [k5] N: calcd, 17.20; found, 16.72. [k6] S: calcd, 13.32; found, 13.77. [k7] S: calcd, 18.95; found, 18.53. [k8] N: calcd, 20.88; found, 21.35. [l] 1/6 animals died. [m] Compound was screened at $^1/_5$ the LD_{50}. [n] At 200 mg/kg, all rats died between day 8 and day 12. [o] Mengel et al., see ref 4b, reported the ED_{70} at 82 mg/kg, the compound being administered 24 and 1 h prior to the carrageenan challenge. [p] At 100 mg/kg p.o., significant inhibition in the injected paw and complete inhibition in the noninjected paw was reported by Mengel et al., see refs 4a and 7b. [q] Doses were administered subcutaneously.

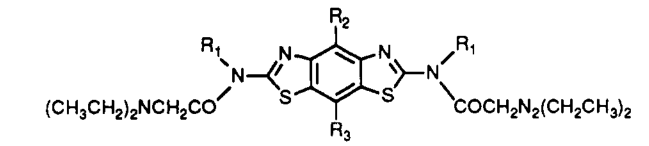
bath for 1 h. A solid crystallized and was collected after cooling. It was treated with sodium bicarbonate solution, washed well with water, and dried. The product (23 g, 73.6%) was sufficiently pure (by TLC) to be used as such. It crystallized from DMF/H_2O: mp 320 °C; IR 3220, 1685, 1620, 1560 cm^{-1}; NMR (Me_2SO-d_6) δ 4.4 (s, 4 H), 8.0 (s, 1 H), 8.4 (s, 1 H), 12.9 (s, br, 2 H ex). Anal. ($C_{12}H_8Cl_2N_4O_2S_2$) C, H, Cl, N, S.

4-Bromo-8-chloro-2,6-bis[[(*N*,*N*-diethylamino)acetyl]amino]benzo[1,2-*d*:5,4-*d*′]bisthiazole (104). Method B. A mixture of crude 2,6-diamino-4-bromo-8-chlorobenzo[1,2-*d*:5,4-*d*′]bisthiazole (**124**) (2.6 g, 0.01 mol), 30 mL of 1 M sodium ethoxide solution in ethanol (0.03 mol), and ethyl *N*,*N*-diethylglycinate (4.5 g, 0.028 mol) was stirred at room temperature overnight. The residue obtained on evaporation of the resulting solution was dissolved in water (200 mL), neutralized with 2 N HCl, and extracted with chloroform. The extract was washed, dried, and taken to near dryness in vacuo. The concentrated solution was then chromatographed on a 60-g dry silica gel column, using chloroform as eluant. The first eluates (TLC) contained the title product (2.1 g, 37%). It crystallized from DMF/chloroform: mp 265–267 °C; IR 3430, 3270, 1700, 1670 sh, 1600, 1580 cm^{-1}; NMR ($CDCl_3$) δ 1.2 (t, 6 H), 2.7 (q, 4 H), 10.3 (s, br, 2 H ex); UV λ_{max} (log ε) 220 (4.34), 278 (sh) (4.60), 286 (4.72), 323 (sh) (4.28), 338 nm (4.20). Anal. ($C_{20}H_{16}BrClN_6O_2S_2$) C, H, S, Br.

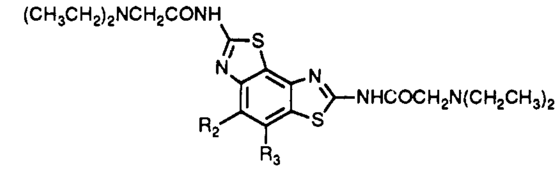
4,8-Dichloro-2,6-bis[[(*N*,*N*-diethylamino)acetyl]amino]benzo[1,2-*d*:5,4-*d*′]bisthiazole (98). Chlorine (6.0 g, 0.085 mol) was absorbed into trimethyl phosphate (TMP) (50 mL) at −10 °C, and the solution was added to a stirred suspension of 2,6-bis[[(*N*,*N*-diethylamino)acetyl]amino]benzo[1,2-*d*:5,4-*d*′]bisthiazole (**26**) (7.0 g, 0.0156 mol) in TMP (50 mL) at −10 °C. The resulting solution was kept at 0–5 °C for 8 h, poured into ice/water (150 mL), and neutralized with ammonium hydroxide. The pale off-white solid was collected, washed with water, dried, dissolved in methylene chloride, and treated with charcoal. The colorless solution was evaporated to dryness, and the residue was crystallized twice from DMF to yield 3.5 g (44%) of compound **98**: mp 278–280 °C; IR 3450, 3220, 1710 sh, 1700, 1610, 1550 cm^{-1}. NMR ($CDCl_3$) δ 1.1 (t, 12 H), 2.7 (q, 8 H), 3.4 (s, 4 H), 10.4 (s, br, 2 H ex). Anal. ($C_{20}H_{26}Cl_2N_6O_2S_2$) C, H, Cl, N, S.

2,6-Bis[(chloroacetyl)amino]-4-nitrobenzo[1,2-*d*:5,4-*d*′]bisthiazole (137). Fuming nitric acid (10 mL) was added dropwise to a stirred suspension of 2,6-bis[(chloroacetyl)amino]benzo[1,2-*d*:5,4-*d*′]bisthiazole (**132**) (1.8 g, 4.8 mmol) in acetic acid (50 mL) with heating on a water bath. The reaction mixture was then heated to reflux for 0.5 h, cooled, and diluted with ice water. The precipitated solid was collected and crystallized twice from DMF to give **137**: 1.2 g (60%); mp 300 °C; IR 3310, 3210, 1700, 1515 cm^{-1}; NMR (Me_2SO-d_6) δ 4.5 (s, 4 H),

Table II. Chemical and Biological Screening Data for Substituted 2,6-[[(Diethylamino)acetyl]amino]benzo[1,2-*d*:5,4-*d*′]bisthiazoles (**90–107**) and 2,7-[[(Diethylamino)acetyl]amino]benzo[1,2-*d*:3,4-*d*′]bisthiazoles (**108–110**)



I



V

compd	type	R_1	R_2	R_3	method (Scheme I) reaction sequences[a]	mp, °C[b]	crystn[c] solvent	formula[d]	CPE[e,f] (200 mg/kg) % inhibn	D-adjuvant arthritis[e,f] injd/uninjd paw (mg/kg) % inhibn	D-adjuvant arthritis[e,f] % change body wt
91	I	H	CH_3	H	**4g** $\xrightarrow{i}$ **5g** $\xrightarrow{ii}$ **6g**	225 dec	V	$C_{21}H_{30}N_6O_2S_2{\cdot}2HCl{\cdot}H_2O$	8	59/100 (50)*	−37
92	I	H	OCH_3	H	**4h** $\xrightarrow{iii}$ **6h**	175–177	II	$C_{21}H_{30}N_6O_3S_2{\cdot}H_2O$	17	19/0 (50)	+10
93	I	H	Cl	H	**4f** $\xrightarrow{iii}$ **6f**	225–226	II	$C_{20}H_{27}ClN_6O_2S_2$	8	94/100 (50)*[g]	−46
94	I	H	Br	H	**1a** $\xrightarrow{iv}$ **4e** $\xrightarrow{iii}$ **6e** $\xleftarrow{v}$ **3a**	196–198	II	$C_{20}H_{27}BrN_6O_2S_2$	0	80/100 (50)*	−45
95	I	H	NO_2	H	**1a** $\xrightarrow{i}$ **2a** $\xrightarrow{vi}$ **5i** $\xrightarrow{ii}$ **6i**	276–278	I	$C_{20}H_{27}N_7O_4S_2$	23	11/12 (50)[h]	−16
96	I	H	NH_2	H	**4j** $\xrightarrow{iii}$ **6j**	160–162	II	$C_{20}H_{29}N_7O_2S_2$	53*	26/38 (50)	−35
97	I	H	H	COOEt	**10w** $\xrightarrow{i}$ **11w** $\xrightarrow{ii}$ **12w**	215–217	II	$C_{23}H_{32}N_6O_4S_2$	32*	7/0 (50)	−9
98	I	H	Cl	Cl	**1a** $\xrightarrow{iii}$ **3a** $\xrightarrow{vii}$ **9k**	278–280	VII	$C_{20}H_{26}Cl_2N_6O_2S_2$	19	30/71 (10)*[i]	−33
99	I	H	Cl	CF_3	**7n** $\xrightarrow{i}$ **8n** $\xrightarrow{ii}$ **9n**	240–245	IV	$C_{21}H_{26}ClF_3N_6O_2S_2{\cdot}2HCl{\cdot}0.2H_2O$	18	57/100 (25)*[j]	−22
100	I	H	Cl	CN	**7o** $\xrightarrow{i}$ **8o** $\xrightarrow{ii}$ **9o**	260–265	IV	$C_{21}H_{26}ClN_7O_2S_2{\cdot}2HCl$	19	80/100 (50)*	−35
101	I	H	Cl	C_6H_5	**7q** $\xrightarrow{iii}$ **9q**	247–249	VIII	$C_{26}H_{31}ClN_6O_2S_2$	35*	13/7 (50)	−6
102	I	H	Cl	COOEt	**10w** $\xrightarrow{i}$ **11w** $\xrightarrow{vi}$ **8p** $\xrightarrow{ii}$ **9p**	260–262	VIII	$C_{23}H_{31}ClN_6O_4S_2$	5	0/0 (50)	−16
103	I	H	Cl	Br	**4f** $\xrightarrow{i}$ **5f** $\xrightarrow{v}$ **8m** $\xrightarrow{ii}$ **9m**	278–280	VII	$C_{20}H_{26}BrClN_6O_2S_2{\cdot}0.5H_2O$	24	55/72 (50)*	−33
104	I	H	Br	Cl	**10v** $\xrightarrow{iv}$ **7l** $\xrightarrow{iii}$ **9l**	265–267	XII	$C_{20}H_{26}BrClN_6O_2S_2$	8	68/100 (50)*	−49
105	I	H	CH_3	Cl	**4g** $\xrightarrow{i}$ **5g** $\xrightarrow{ii}$ **6g** $\xrightarrow{vii}$ **9s**	245–247	VII	$C_{21}H_{29}ClN_6O_2S_2{\cdot}0.75H_2O$	18	42/16 (50)	−37
106	I	H	CH_3	COOEt	**7r** $\xrightarrow{iii}$ **9r**	256–257	VII	$C_{24}H_{34}N_6O_4S_2$	35*	15/50 (50)*	−12
107	I	C_2H_5	Br	H	**1c** $\xrightarrow{iv}$ **4d** $\xrightarrow{i}$ **5d** $\xrightarrow{ii}$ **6d**	158–160	II	$C_{24}H_{35}BrN_6O_2S_2$	16	0/0 (50)	−16
					(Scheme II)						
108	V	H	Cl	H	**13x** $\xrightarrow{i}$ **14x** $\xrightarrow{ii}$ **15x**	220–225	V	$C_{20}H_{27}ClN_6O_2S_2$	35*	0/62 (100)*	−58
109	V	H	H	OCH_3	**16y** $\xrightarrow{iii}$ **17y**	176–178	II	$C_{21}H_{30}N_6O_3S_2$	32*	47/51 (50)*	+7
110	V	H	Cl	OCH_3	**17y** $\xrightarrow{vii}$ **18z**	172–174	II	$C_{21}H_{29}ClN_6O_3S_2{\cdot}0.25H_2O$	17	38/8 (50)	−11

[a] Numbers are those assigned to structures in Scheme I and II; (i) $ClCOCH_2Cl$; (ii) $HNEt_2$ (method A); (iii) NaOEt + $EtOOCCH_2NEt_2$ (method B); (iv) in situ bromination of the starting material (e.g. **1a**, **1c**, or **71**) formed by oxidative cyclization of the appropriate 1,3-phenylenebisthiourea with >3 Br_2 equivalents. See Experimental Section (method E) **115**, **124**, and **126**; (v) Br_2; (vi) HNO_3; (vii) Cl_2. [b] Uncorrected. [c] See Table I, footnote *d*. [d] All compounds had elemental analyses within ±0.4% of theoretical value. [e] Animals were dosed orally. See Experimental Section for tests description. [f] Statistically significant results with two-tail student's tests ($p < 0.05$) results are marked with an asterisk (*). [g] 3/6 animals died. [h] 2/6 animals died. [i] At 50 mg/kg, 3/6 animals died. [j] At 50 mg/kg 2/6 animals died.

8.9 (s, 1 H), 13.3 (s, 2 H ex). Anal. ($C_{12}H_7Cl_2N_5O_4S_2$) C, H, Cl.

8-Bromo-4-chloro-2,6-bis[(chloroacetyl)amino]benzo[1,2-*d*:5,4-*d*′]bisthiazole (139). Bromine (2.85 mL, 0.056 mol) was added portionwise to a stirred suspension of 4-chloro-2,6-bis-[(chloroacetyl)amino]benzo[1,2-*d*:5,4-*d*′]bisthiazole (**138**) (7.0 g, 0.0171 mol) in TMP (80 mL), and the mixture was stirred at room temperature overnight. The solution was then poured on ice/water, and the resulting solid was collected, washed, and crystallized three times from DMF to yield 5.0 g (77%) of pure **139**: mp 320 °C; IR 3280, 1700, 1610, 1550 cm^{-1}. Anal. ($C_{12}H_6BrCl_3N_4O_2S_2$) H, N; C: calcd, 29.49; found, 29.93.

2,6-Diamino-4-chloro-8-(trifluoromethyl)benzo[1,2-*d*:5,4-*d*′]bisthiazole (113). Method F. A bromine (3 mL, 0.058 mol) solution in acetic acid (30 mL) was added over a period of 20 min to an ice-cold stirred mixture of 2-chloro-5-(trifluoromethyl)-1,3-phenylenediamine[16] (5.3 g, 0.025 mol) and potassium thiocyanate (19.4 g, 0.20 mol) in acetic acid (150 mL) containing methanol (20 mL). The reaction mixture was stirred at room temperature for 1 h, poured into water (200 mL), and basified with ammonium hydroxide to precipitate the free base. It crystallized from DMF: 6.0 g (73%); mp 320 °C; IR 3495, 3280, 1640, 1530 cm^{-1}. Anal. ($C_9H_4ClF_3N_4S_2$) C, H, Cl, F, N, S.

2,6-Diamino-4-chloro-8-cyanobenzo[1,2-*d*:5,4-*d*′]bisthiazole (114). The title product was prepared from 2-chloro-5-cyano-1,3-phenylenediamine[16] by method F—see above—in 66% yield. It crystallized from methanol: mp 350 °C; IR 3400, 3275, 3070, 2225, 1630, 1540 cm^{-1}. Anal. ($C_9H_4ClN_5S_2{\cdot}{}^1/_4DMF$) C, H, N, Cl.

2,6-Diamino-8-carboxy-4-methylbenzo[1,2-*d*:5,4-*d*′]bisthiazole Sodium Salt (116). The title product was prepared from 3,5-diamino-4-methylbenzoic acid[17] in 46% yield by method F, see above. The crude product was crystallized from 1 N sodium hydroxide solution to yield the sodium salt: mp 350 °C; IR 3600–2700, 1670, 1635, 1615, 1540, 1375 cm^{-1}; NMR (Me_2SO-d_6) δ 2.4 (s, 3 H), 7.0 (s, 4 H ex). Anal. ($C_{10}H_7N_4O_2S_2Na{\cdot}H_2O$) C, H, N, S.

2,6-Diamino-8-carbethoxy-4-methylbenzo[1,2-*d*:5,4-*d*′]-bisthiazole (121). The title product was prepared from ethyl 3,5-diamino-4-methylbenzoate[18] in 80% yield (crude) by method F, see above. The product crystallized from DMF: mp 350 °C; IR 3430, 3300, 3150, 1660, 1640 sh, 1630, 1530 cm^{-1}; NMR (Me_2SO-d_6) δ 1.4 (t, 3 H), 2.7 (s, 3 H), 4.2 (q, 2 H), 7.5 (s, br, 4 H ex). Anal. ($C_{12}H_{12}N_4O_2S_2$) C, H, N, S.

2,4,6-Triaminobenzo[1,2-*d*:5,4-*d*′]bisthiazole (122). The title product was prepared from 2-amino-1,3-phenylenediamine[19] by method F, see above. The crude product (47%) obtained after treatment with ammonia was collected, washed, and dried: mp 350 °C; IR 3410, 3320, 3275, 3140, 2250 w, 1620 s, 1530 cm^{-1}. The weak band at 2250 cm^{-1} would indicate some uncyclized product in this sample. The product was used as such to prepare **96**.

2,6-Diamino-4-chloro-8-phenylbenzo[1,2-*d*:5,4-*d*′]bisthiazole (123). The title product was prepared from 3,5-diamino-4-chlorobiphenyl[20] in 63% yield by method F, see above. It crystallized from DMF: mp 350 °C; IR 3400, 3350, 3100, 1615, 1515 cm^{-1}; NMR (Me_2SO-d_6) δ 7.6 (m, 9 H, 4 ex). Anal. ($C_{14}H_9ClN_4S_2{\cdot}{}^1/_3H_2O$) C, H, S.

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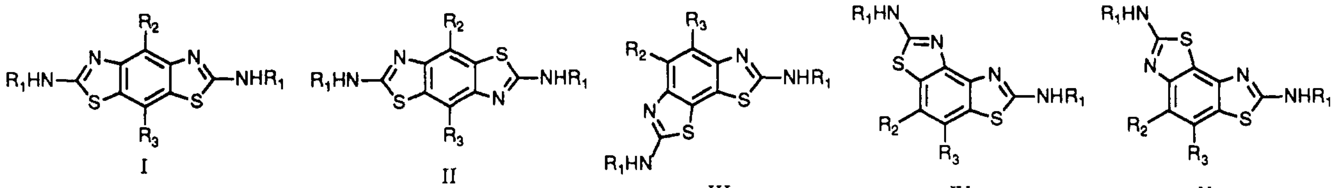
(20) Wright, J. B. Ger. Offen. 2,525,249, 1976; *Chem. Abstr.* **1976**, *84*, 135315m.

Table III. Chemical and Biological Screening Data for Diaminobenzobisthiazoles,[a] Types I–V, Used as Starting Materials in Tables I and II



I II III IV V

compd (Schemes I and II)	type	R_1	R_2	R_3	method[b] (lit.)	crystn[c] solvent	mp, °C[d]	formula[e]	CPE[f,g] (mg/kg) % inhibn	D-adjuvant arthritis[f,g] injd/uninjd paw (mg/kg) % inhibn	D-adjuvant arthritis[f,g] % change body wt
111 (**1a**)	I	H	H	H	(10), (11)	XIII	>400	$C_8H_6N_4S_2$	60 (200)*[,h]	79/92 (100)*	−20
112 (**4b**)	I	CH_3	H	H	(13)	VII	318–319	$C_8H_{10}N_4S_2·2HCl$	24 (200)	71/100 (25)*[,i]	−30
113 (**7n**)	I	H	Cl	CF_3	F	VII	>320	$C_9H_4ClF_3N_4S_2$	13 (200)	0/12 (200)	+3
114 (**7o**)	I	H	Cl	CN	F	XIV	>320	$C_9H_4ClN_5S_2·0.25H_2O$	7 (200)	47/37 (200)	+3
115 (**4e**)	I	H	Br	H	G	XIII	>320	$C_8H_5BrN_4S_2$	33 (200)*	47/37 (200)	+10
116 (**7t**)	I	H	CH_3	COONa	F	XV	>320	$C_{10}H_7N_4O_2S_2Na·H_2O$	22 (200)	7/0 (50)	−15
117 (**7w**)	I	H	H	COOEt	H	II	>320	$C_{11}H_{10}N_4O_2S_2·1.25H_2O$	2 (200)	23/0 (50)	−14
118 (**4g**)	I	H	CH_3	H	(13)	VII	>320	$C_9H_8N_4S_2$			
119 (**4h**)	I	H	OCH_3	H	(13)	VII	305 dec	$C_9H_8N_4OS_2$			
120 (**4k**)	I	H	Cl	H	(13)	XIII	>350	$C_8H_5ClN_4S_2$			
121 (**7p**)	I	H	CH_3	COOEt	F	VII	>320	$C_{12}H_{12}N_4O_2S_2$			
122 (**4j**)	I	H	NH_2	H	F	XVI	>320	$C_8H_7N_5S_2$[j]			
123 (**7g**)	I	H	Cl	C_6H_5	F	VII	>320	$C_{14}H_9ClN_4S_2·0.33H_2O$			
124 (**7l**)	I	H	Br	Cl	G	XVI	>320	$C_8H_4BrClN_4S_2$[j]			
125 (**1c**)	I	C_2H_5	H	H	(11)	VII	306–308	$C_{12}H_{14}N_4S_2$			
126 (**4d**)	I	C_2H_5	Br	H	G	VII	273–275	$C_{12}H_{13}BrN_4S_2$[j]			
127	II	H	H	H	(11)	XIV	350	$C_8H_6N_4S_2$	4 (100)	88/100 (10)*[,k]	−30
128[l]	III	H	H	H	(11)	XVII	320	$C_8H_6N_4S_2$			
129	IV	CH_3	H	H	(11)	XIII	315–316	$C_{10}H_{10}N_4S_2$			
130 (**13x**)	V	H	Cl	H	(11)	XIII	360	$C_8H_5ClN_4S_2$			
131 (**16y**)	V	H	H	OCH_3	H	II	320	$C_9H_8N_4OS_2·{}^1/_4H_2O$	43 (200)*	34/35 (50)[m]	−29

[a] See Table I, note *a*. [b] As described in literature (lit.) or in the Experimental Section, methods F–H. [c] II, ethanol; VII, DMF; XIII, pyridine; XIV, DMF–H_2O; XV, H_2O; XVI, product was not purified and used as such; XVII, product was purified via the hydrochloride and then neutralized with ammonia. [d] Uncorrected. [e] The elemental analysis of new compounds within ±0.4% of the theoretical value. [f] Animals were dosed orally. For description of screening tests see Experimental Section. [g] Statistically significant results with two-tail student's tests ($p < 0.05$) are marked with an asterisk (*). [h] Product showed no activity when tested with adrenalectomized animals. [i] 50 mg/kg for 4 days and 25 mg/kg for 10 days; 2/6 animals died. At 10 mg/kg, compound was inactive. [j] Product was not analyzed, see Experimental Section. [k] At 50 mg/kg, all animals died by day 7. [l] Prepared from 1,4-phenylenediamine; product contained 25% of the corresponding linear isomer, 127, by NMR analysis. [m] 2/6 animals died on days 2 and 3.

Table IV. Effect of **26** in Plaque-Forming Cell (PFC) in AKR Mice[a]

compd	dose,[b] mg/kg po	N[c]	total PFC/spleen % suppression[d]
26	10	6	11
	50	6	67*
	100	6	82*
	200	6	83*
90a	125	6	28

[a] See experimental. [b] Day 1–3. [c] Number of animals. [d] Results with $p < 0.05$ are marked with an asterisk (*).

2,6-Diamino-8-carbethoxybenzo[1,2-*d*:5,4-*d*′]bisthiazole (117). Method H. A solution of bromine (2.7 g, 17 mmol) in chloroform (10 mL) was added dropwise to 5-carbethoxy-1,3-phenylenebisthiourea (**150**) (2.4 g, 8.05 mmol) in chloroform (100 mL). When the addition was completed, the mixture was refluxed for 2 h, and the solid was collected, resuspended in water, and neutralized with ammonium hydroxide. The base was collected, washed, dried, and crystallized from ethanol, yielding **117** (0.90 g, 38%): mp 350 °C; IR 3320, 3180, 1650 cm^{-1}; NMR (Me_2SO-d_6) δ 1.2 (t, 3 H), 4.4 (q, 2 H), 7.4 (4 H ex), 7.55 (s, 1 H); UV λ_{max} (log ϵ) 215 (3.79), 238 (4.15), 254 (sh) (3.96), 346 nm (3.61). Anal. ($C_{11}H_{10}N_4O_2S_2·1.25H_2O$) C, H, N, S.

2,7-Diamino-4-methoxybenzo[1,2-*d*:3,4-*d*′]bisthiazole (131). The title compound was prepared in 48% yield from 5-methoxy-1,3-phenylenebisthiourea by method H, see above: mp 255 °C; IR 3340, 3200, 1640 cm^{-1}; NMR (Me_2SO-d_6) δ 3.85 (s, 3 H), 6.8 (s, 1 H), 7.68 (s, br, 4 H ex); UV λ_{max} (log ϵ) 210 (4.30), 221 (sh) (4.28), 252 (4.30), 306 nm (sh) (3.63). Anal. ($C_9H_8N_4OS_2·{}^3/_4H_2O$) C, H, N, S.

2,6-Diamino-4-bromobenzo[1,2-*d*:5,4-*d*′]bisthiazole (115). Method G. Bromine (9 mL, 0.175 mol) was added dropwise to a solution of 1,3-phenylenebisthiourea[10] (8.5 g, 0.0377 mol) in chloroform (150 mL). The mixture was stirred at room temperature overnight and then refluxed for 2 h. After cooling, a solid precipitated, which was collected, suspended in water, and treated with ammonium hydroxide. The free base was collected, washed with water, and crystallized from pyridine to yield 2.8 g (27.5%) of a light pink crystalline solid: mp 320 °C; IR 3450, 3280, 1630, 1535 cm^{-1}; NMR (Me_2SO-d_6) δ 7.6 (s, br, 4 H ex), 7.8 (s, 1 H); UV λ_{max} (log ϵ) 210 (3.98), 253 (4.21), 304 (sh) (3.72), 315 nm (3.87). Anal. ($C_8H_5BrN_4S_2$) C, H, N.

2,6-Diamino-4-bromo-8-chlorobenzo[1,2-*d*:5,4-*d*′]bisthiazole (124). The title product was prepared from 5-chloro-1,3-phenylenebisthiourea and excess bromine according to method G, see above. The crude product was treated with boiling DMF (25 mL/g), the insoluble material was removed by filtration, and the filtrate was rotoevaporated to dryness. The residue (mp 300 °C; IR 3470, 3270, 3100, 1630, 1540 cm^{-1}) was used as such for the preparation of **104**.

4-Bromo-2,6-bis(ethylamino)benzo[1,2-*d*:5,4-*d*′]bisthiazole (126). The title product was prepared from *N*′,*N*′-diethyl-1,3-phenylenebisthiourea[11] and excess bromine in 49% yield according to method G, see above. The product crystallized from DMF: mp 273–275 °C; IR 3200, 2975, 1675 sh, 1625, 1570 cm^{-1}; NMR (Me_2SO-d_6) δ 1.2 (t, 6 H), 3.3 (m, 4 H), 7.8 (s, 1 H), 8.1 (t, 2 H ex). The product was used as such for the preparation of **107**.

5-Carbethoxy-1,3-phenylenebisthiourea (149). A mixture of ethyl 3,5-diaminobenzoate[21] (10 g, 0.0395 mol) and ammonium

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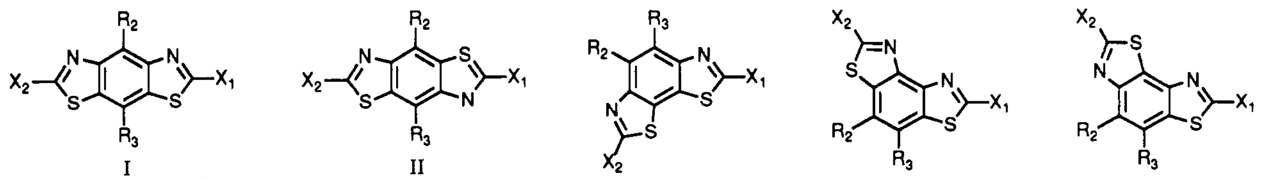
Table V. Intermediate Acyl Chlorides of Diaminobenzobisthiazoles[a]



I II III IV V

compd	type	$X_1 = X_2$	R_2	R_3	mp, °C[b]	formula[c]
132	I	$NHCOCH_2Cl$	H	H	>300	$C_{12}H_8Cl_2N_4O_2S_2$
133	I	$NH(CH_3)COCH_2Cl$	H	H	>250 dec	$C_{14}H_{12}Cl_2N_4O_2S_2$
134	I	$NH(C_2H_5)COCH_2Cl$	H	H	>220 dec	$C_{16}H_{16}Cl_2N_4O_2S_2$
135	I	$NHCOCH(CH_3)Cl$	H	H	265–270	$C_{14}H_{12}Cl_2N_4O_2S_2 \cdot 1.5DMF$
136	I	$NHCOCH_2Cl$	CH_3	H	>300	$C_{13}H_{10}Cl_2N_4O_2S_2$
137	I	$NHCOCH_2Cl$	NO_2	H	>300	$C_{12}H_7Cl_2N_5O_4S_2$
138	I	$NHCOCH_2Cl$	Cl	H	>300[d]	$C_{12}H_7Cl_3N_4O_2S_2$
139	I	$NHCOCH_2Cl$	Cl	Br	>300	$C_{12}H_6BrCl_3N_4O_2S_2$[e]
140	I	$NHCOCH_2Cl$	Cl	CF_3	>300	$C_{13}H_6Cl_3F_3N_4O_2S_2$
141	I	$NHCOCH_2Cl$	Cl	CN	>300	$C_{13}H_6Cl_3N_5O_2S_2 \cdot 0.33H_2O$
142	I	$NHCOCH_2Cl$	H	COOEt	>300 dec	$C_{15}H_{12}Cl_2N_4O_4S_2 \cdot 0.5H_2O$
143	I	$NH(C_2H_5)COCH_2Cl$	Br	H	>300 dec	$C_{16}H_{15}BrCl_2N_4O_2S_2$
144	II	$NHCOCH_2Cl$	H	H	>300 dec	$C_{12}H_8Cl_2N_4O_2S_2$
145	III	$NHCOCH_2Cl$	H	H	>300[f,g]	$C_{12}H_8Cl_2N_4O_2S_2 \cdot 0.33H_2O$
146	IV	$N(CH_3)COCH_2Cl$	H	H	280–285	$C_{14}H_{12}Cl_2N_4O_2S_2$
147	V	$NHCOCH_2Cl$	Cl	H	>300	$C_{12}H_7Cl_3N_4O_2S_2 \cdot 0.67DMF$
		X_1, X_2				
148	I	$X_1 = NHCOCH_2N(C_2H_5)_2$; $X_2 = NHCOCH_2Cl$	H	H	280–282	$C_{16}H_{18}ClN_5O_2S_2$

[a] See Table I, note *a*. [b] Products were crystallized from DMF except as indicated. [c] Analyses were within ±0.4% of the theoretical value except where indicated. [d] Product could not be crystallized. It was used crude and not analyzed. [e] C: calcd, 29.50; found, 29.93. [f] DMF–CH_3COOH. [g] Product contained 25% of isomer **144** (NMR).

thiocyanate (6.5 g, 0.0854 mol) in water (30 mL) was heated to reflux for 3 h. The reaction product was cooled, the solid was collected and crystallized from water and a trace amount of DMF to yield 2.4 g (20.4%) of the title product: mp 210–212 °C; NMR (Me_2SO-d_6) δ 1.2 (t, 3 H), 4.4 (q, 2 H), 7.58 (s, 4 H ex), 7.8 (s, 2 H), 7.9 (s, 1 H), 10.0 (s, 2 H ex). Anal. ($C_{11}H_{14}N_4O_2S_2 \cdot {}^1/_4H_2O$) C, H; N: calcd, 18.50; found, 18.09.

5-Chloro-1,3-phenylenebisthiourea (150). 5-Chloro-1,3-phenylenediamine[22] (15.5 g, 0.109 mol), carbon disulfide (53 mL, 0.109 mol), and triethylamine (53 g, 0.524 mol) were mixed together in acetone (450 mL) and stirred at room temperature for 2 days. A solid was collected which was added to methanol (100 mL) and treated with iodomethane (25 g, 0.173 mol) for 2 h at room temperature. Dimethyl 5-chloro-1,3-phenylenebisdithiocarbamate precipitated. It was collected and dissolved in methanol (200 mL), and the solution was treated with concentrated ammonia (75 mL). The mixture was heated to reflux for 2 h, cooled to room temperature, and the resulting suspension was filtered. The solid was washed with methanol and dried. Another crop was obtained by evaporation of the mother liquor and treatment of the residue with ethanol: yield 11.4 g (38%); mp 185–187 °C; NMR (Me_2SO-d_6) δ 7.3 (s, 2 H), 7.5 (s, 1 H), 7.5 (s, br, 4 H ex), 9.7 (s, 2 H ex). The product was used as such.

5-Methoxy-1,3-phenylenebisthiourea (151). This product was obtained from 5-methoxy-1,3-phenylenediamine[23] in three steps as described above for 5-chloro-1,3-phenylenebisthiourea: yield 53%; mp 193–195 °C; IR 3380, 3250, 3150, 1615 cm^{-1}; NMR (Me_2SO-d_6) δ 3.7 (s, 3 H), 6.8 (d, 2 H), 7.0 (t, 1 H), 7.5 (s, 4 H ex), 9.5 (s, 2 H ex). The product was used as such.

2,6-Bis[(carboxymethyl)amino]benzo[1,2-*d*:5,4-*d′*]bisthiazole (83). 2,6-Bis[(carbethoxymethyl)amino]benzo[1,2-*d*:5,4-*d′*]bisthiazole (**22c**) (18 g, 0.0545 mol) in a mixture of ethanol (300 mL) and 1 N NaOH (150 mL) was heated in a water bath for 30 min. Water (300 mL) was added, and the resulting solution was concentrated in vacuo to about 300 mL and then neutralized with 2 N HCl. The solid was collected, washed with water, dried, and crystallized from DMF to yield 13 g (84%) of the title product melting at 310 °C: IR 3500–2600 with max. at 2900, 1640, 1620 sh, 1565, 1420, 1300 cm^{-1}; NMR (TFA-*d*) δ 4.6 (s, 4 H), 8.0 (s, 1 H), 8.3 (s, 1 H). Anal. ($C_{12}H_{10}N_4O_4S_2$) C, H, N; S: calcd, 18.95; found, 18.53.

2,6-Bis[(carbethoxymethyl)amino]benzo[1,2-*d*:5,4-*d′*]bisthiazole (22c). Bromine (26 g, 0.163 mol) in chloroform (100 mL) was added dropwise at room temperature to a stirred solution of 1,3-bis[*N′*-(carbethoxymethyl)thioureido]benzene (**21c**) (31.5 g, 0.079 mol) in chloroform (500 mL). The resulting mixture was heated to reflux for 1 h. A solid was collected, suspended in water, treated with ammonia, and again collected by filtration. It was washed well with water and then crystallized from ethanol/DMF, 2/1, to yield 20 g (65%) of the title compound: mp 215–217 °C; IR 3340, 3300, 1735 sh, 1725, 1600, 1550 cm^{-1}; NMR (Me_2SO-*d*) δ 1.2 (t, 6 H), 4.2 (m, 8 H), 7.4 (s, 1 H), 8.0 (s, 1 H), 8.4 (t, br, 2 H ex). Anal. ($C_{16}H_{18}N_4O_4S_2$) C, H, N, S.

1,3-Bis[*N′*-(carbethoxymethyl)thioureido]benzene (21c). A stirred mixture of 1,3-phenylenediamine (2.5 g, 0.023 mol) and ethyl isothiocyanoacetate[24] (8.0 g, 0.055 mol) in dioxane (30 mL) was heated on a water bath for 1 h. The resulting solution was evaporated, and the residue was crystallized from ethanol to give 8.0 g (87%) of the title product: mp 122–124 °C; IR 3340, 1740, 1620 m, 1550, 1530 cm^{-1}; NMR (Me_2SO-d_6) δ 1.2 (t, 6 H), 4.15 (m, 8 H), 7.2 (s, br, 3 H), 7.4 (s, br, 1 H), 8.0 (t, 2 H ex), 9.6 (s, br, 2 H ex). Anal. ($C_{16}H_{22}N_4O_4S_2$) C, H, N, S.

2,6-Bis[[(*N*,*N*-diethylcarbamoyl)methyl]amino]benzo[1,2-*d*:5,4-*d′*]bisthiazole (84). Method D. A stirred mixture of 2-chloro-6-[[(*N*,*N*-diethylcarbamoyl)methyl]amino]benzo[1,2-*d*:5,4-*d′*]bisthiazole (**24**) (4.0 g, 0.0113 mol) and glycine–diethylamide[14] (1.7 g, 0.0135 mol) was heated neat at 170 °C for 30 min, allowed to cool to room temperature, and treated with ethanol. The resulting solid was collected and crystallized from DMF: yield 2.3 g (45%); mp 235–237 °C; IR 3250, 1640, 1600 w, 1540 cm^{-1}; NMR ($CDCl_3$) δ 1.2 (m, 12 H), 3.4 (m, 8 H), 4.3 (s, br, 4 H), 6.6 (s, br, 2 H ex), 7.8 (s, 1 H), 7.9 (s, 1 H). Anal. ($C_{20}H_{28}N_6O_2S_2$) C, H, N, S.

2-Chloro-6-[[(*N*,*N*-diethylcarbamoyl)methyl]amino]benzo[1,2-*d*:5,4-*d′*]bisthiazole (24). A stirred mixture of 2,6-dichlorobenzo[1,2-*d*:5,4-*d′*]bisthiazole[10] (9.4 g, 0.036 mol), gly-

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cine–diethylamide[14] (9.4 g, 0.072 mol), and triethylamine (8.0 g, 0.079 mol) in dioxane (50 mL) was heated to reflux for 1 h. The reaction mixture was evaporated, the residue was dissolved in chloroform, and the solution was washed with H_2O and dried. It was then concentrated to a few milliliters and applied to a 200-g silica gel column. The reaction products were eluted with chloroform/methanol, 95/5. The first fraction (8.5 g, 66%) crystallized from ethanol/DMF and corresponded to the title product: mp 178–180 °C; IR 3220, 1640 sh, 1630, 1600, 1560 cm^{-1}; NMR ($CDCl_3$) δ 1.1 (m, 6 H), 3.4 (m, 4 H), 4.25 (d, 2 H), 6.9 (s, br, 1 H ex), 7.8 (s, 1 H), 8.0 (s, 1 H). Anal. ($C_{14}H_{15}ClN_4OS_2$) C, H, Cl, N. A second fraction yielded 2.0 g of the bis-adduct (identical IR to **84** above), which was contaminated with an impurity of near identical R_f, and which could not be removed by crystallization.

2,6-Bis[[2-(*N*,*N*-diethylamino)ethyl]amino]benzo[1,2-*d*:5,4-*d'*]bisthiazole Tetrahydrochloride (85). Method C. 2-(Diethylamino)ethyl isothiocyanate[25] (8.0 g, 0.051 mol) was added to a solution of 1,3-phenylenediamine (2.7 g, 0.025 mol) in dioxane (20 mL), and the mixture was heated to reflux for 3 h. The product, 1,3-bis[N^1-[(*N*,*N*-diethylamino)ethyl]thioureido]benzene (**21a**) [11 g, 99%; NMR ($CDCl_3$) δ 0.9 (m, 12 H), 2.5 (q, 12 H), 3.6 (q, br, 6 H, 2 H ex), 7.2 (m, 6 H, 2 H ex)], obtained on evaporation, was used as such for the oxidative cyclization of the next step. The compound (7.0 g, 0.0165 mol) was dissolved in chloroform (100 mL), and excess HCl gas was bubbled through the solution. Bromine (4.5 g, 0.028 mol) in chloroform (70 mL) was then added dropwise, at room temperature, and under strong stirring to the resulting suspension of the hydrochloride. The mixture was then heated to reflux for 1 h and cooled, and the resulting solid was collected. It was dissolved in water, and the solution was treated with ammonia and extracted with chloroform (3 × 100 mL). The combined extracts were washed with water and brine and dried over sodium sulfate. The residue obtained on evaporation (6.2 g) was passed through a column of silica gel using chloroform/methanol, 9/1, as eluant. The title product (2.5 g, 36%) was obtained as thick oil: IR 3360, 2970, 2830, 1620, 1550 cm^{-1}; NMR ($CDCl_3$) δ 1.0 (t, 12 H), 2.5 (m, 12 H), 3.4 (t, 4 H), 6.2 (s, br, 2 H ex), 7.6 (s, 1 H), 7.65 (s, 1 H). Anal. ($C_{20}H_{32}N_6S_2 \cdot 0.5H_2O$) C, H, N, S. The oil was dissolved in chloroform, and the hydrochloride was precipitated from etheral HCl. It was recrystallized from methanol, mp 195–200 °C. Anal. ($C_{20}H_{32}N_6S_2 \cdot 4HCl \cdot 2CH_3OH$) C, H, N.

2,6-Bis[[5-(diethylamino)-2-pentyl]amino]benzo[1,2-*d*:5,4-*d'*]bisthiazole Tetrahydrochloride (86). The title product was prepared as described above for compound **85** from 1,3-phenylenediamine and 1-(diethylamino)-4-isothiocyanatopentane. The latter product was prepared according to Schmidt et al.[26] It had a bp of 68–72 °C (0.1 mmHg): IR 2950, 2800, 2100, 1475, 1450 cm^{-1}. The hydrochloride was obtained from an ethanolic solution of the free base with excess ethereal HCl. It became a crystalline solid after treatment with acetone, ether, and ethyl acetate: yield 57%; mp 145–150 °C dec; NMR (Me_2SO-d_6) δ 1.2 (t, 21 H), 1.8 (m, br, 8 H), 3.1 (m, br, 14 H), 4.3 (m, br, 2 H), 8.0 (s, 1 H), 8.4 (s, 1 H), 10.7–11.0 (m, br, 7 H ex). Anal. ($C_{26}H_{44}N_6S_2 \cdot 4HCl \cdot EtOH$) C, Cl, S; H: calcd, 7.80; found, 7.32; N: calcd, 12.07; found, 12.54. Neutralization of an aqueous solution of this salt with 2 N NaOH afforded the pure free base as a solid, mp 85–86 °C. Anal. ($C_{26}H_{44}N_6S_2 \cdot H_2O$) C, H, N, S.

2,6-Bis(*N*-ethylureido)benzo[1,2-*d*:5,4-*d'*]bisthiazole (79). Method E. A stirred mixture of 2,6-diaminobenzo[1,2-*d*:5,4-*d'*]bisthiazole[10] (4.45 g, 0.020 mol) and ethyl isocyanate (3.0 g, 0.042 mol) in dioxane (100 mL) was heated to reflux for 2 h. The resulting suspension was left at room temperature overnight. A beige solid (5.6 g, 77%) was collected, washed, dried, and crystallized from DMF: mp 330 °C; IR 3400, 3260, 3200 sh, 3080, 1680, 1660, 1615 br cm^{-1}; NMR (CF_3COOD) δ 1.3 (t, 6 H), 3.5 (q, 4 H), 8.4 (s, 1 H), 8.5 (s, 1 H), 12 (s, br, 4 H). Anal. ($C_{14}H_{16}N_6O_2S_2$) C, H, N.

Biological Methods. Carrageenan Paw Edema. Edema was produced in the right hind paw of male Sprague–Dawley rats (150 ± 10 g) by the subplantar injection of 0.1 mL of a 1% carrageenan suspension in saline.[27] Test compounds or vehicle was administered orally in 0.5% methylcellulose 1 h prior to carrageenan injection. Five rats were used in each group. The paw volume was determined by measuring the amount of mercury displaced after immersing the paw to the level of the lateral malleolus. Paw volumes were measured just prior to test compound administration and again 3 h after carrageenan injection and the difference was designated as edema volume. Statistics were performed using Analysis of Variance (ANOVA) and Dunnett's *t* test.

Ulcerogenicity Test. Ulcerogenic activity was evaluated by the method described by Wong et al.[30]

Acetic Acid Writhing. The analgesic activity of **26** was determined by using the procedure of Hendershot and Forsaith,[31] but with acetic acid[32] in place of phenylquinone.

Plaque Forming Cell (PFC) Test. The PFC assay was performed according to the method of Jerne et al.[33] For each test three groups consisting of six AKR mice were set up. One control group was neither immunized nor treated and was used to establish the number on nonspecific PFC/spleen. Mice in another control group were immunized on day 0 with 0.2 mL of a 15% suspension of sheep erythrocytes and the test groups were treated with test drug starting 1 day after immunization and continued until day 3. All groups were killed on day 4, and the spleens were removed. The spleen cells were teased out into a culture medium, washed, counted, and diluted to a suitable concentration. Aliquots were then mixed with sheep erythrocytes and agarose and plated in triplicate on Petri dishes. These were incubated for 1 h at 37 °C, and then fresh guinea pig serum was added to provide complement. After incubation for a further 30 min, the plates were stained with benzidine, and the number of plaques were counted. The total PFC/spleen in the test group was expressed as a percentage of those in the control group. The data were analyzed using the Student's *t* test or Analysis of Variance.

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Registry No. **19**, 108-45-2; **20a**, 32813-52-8; **20b**, 104093-88-1; **20c**, 24066-82-8; **20d**, 4385-41-5; **20e**, 137697-50-8; **21a**, 137697-51-9; **21b**, 137697-52-0; **21c**, 137697-55-3; **21d**, 137697-53-1; **21e**, 137697-54-2; **22c**, 137697-56-4; **24**, 137697-58-6; **25**, 70175-42-7; **26**, 70175-10-9; **27**, 74071-27-5; **27**·2HCl, 70175-45-0; **28**, 70175-69-8; **29**, 70175-70-1; **30**, 70175-73-4; **31**, 137698-01-2; **31**·2HCl, 137697-59-7; **32**, 70175-14-3; **32**·2HCl, 137697-60-0; **33**, 137698-02-3; **33**·2HCl, 74071-07-1; **34**, 70175-44-9; **35**, 74071-42-4; **36**, 74071-28-6; **37**, 70175-51-8; **38**, 74071-29-7; **39**, 74071-30-0; **40**, 70175-50-7; **41**, 70175-48-3; **42**, 70175-49-4; **43**, 70175-24-5; **44**, 70175-21-2; **44**·2HCl, 70175-22-3; **45**, 70175-23-4; **46**, 70175-71-2; **47**, 70175-17-6; **48**, 70175-18-7; **49**, 74083-79-7; **49**·2HCl, 137697-61-1; **50**, 70175-19-8; **51**, 74071-25-3; **52**, 70175-64-3; **53**, 70175-66-5; **54**, 70175-57-4; **55**, 70175-59-6; **56**, 74071-09-3; **57**, 74071-35-5; **58**, 74071-11-7; **59**, 137697-62-2; **60**, 74071-36-6; **61**, 74071-10-6; **62**, 70175-20-1; **63**, 137697-63-3; **64**, 70175-68-7; **65**, 70175-67-6; **66**, 137698-03-4; **66**·4HCl, 74071-38-8; **67**, 70175-55-2; **68**, 70175-52-9; **68**·2HCl, 74071-31-1; **69**, 74071-32-2; **70**, 74071-33-3; **71**, 74071-41-3; **72**, 74083-81-1; **73**, 70175-61-0; **74**, 70175-63-2; **75**, 137697-64-4; **76**, 74071-37-7; **77**, 74071-40-2; **78**, 70175-47-2; **79**, 137697-65-5; **80**, 137698-04-5; **80**·2HCl, 137697-66-6; **81**, 137698-07-8; **81**·2HCl,

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New 8-(Trifluoromethyl)-Substituted Quinolones. The Benefits of the 8-Fluoro Group with Reduced Phototoxic Risk

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A series of 8-(trifluoromethyl)-substituted quinolones has been prepared and evaluated for in vitro and in vivo antibacterial activity, and phototolerance in a mouse phototolerance assay. These analogues were compared to the corresponding series of 6,8-difluoro- and 6-fluoro-8*H*-quinolones (ciprofloxacin type). Although their in vitro antibacterial activities are less than the 6,8-difluoro analogues, the 8-(trifluoromethyl)quinolones are generally equivalent to their 8*H* analogues. In vivo, they are comparable to the 6,8-difluoro series and show up to 10-fold improvement in efficacy when compared to their ciprofloxacin counterparts vs *Streptococcus pyogenes* and *Streptococcus pneumonia*. In the phototolerance model, the 8-(trifluoromethyl)quinolones are comparable to the 8*H*-quinolones. Both of these series display much higher no effect doses (greater tolerance) than the corresponding 6,8-difluoroquinolones.

The quinolone antibacterials have emerged as an area of intense interest because of their broad spectrum of activity in vitro and their in vivo chemotherapeutic efficacy.[1] Several quinolones are already being marketed, e.g., norfloxacin **1**,[2] ofloxacin **2**,[3] enoxacin (Flumark) **3**,[4] and ciprofloxacin **4**.[5] The success of these compounds has caused an increase in efforts to produce even more efficacious agents, leading to the current list of compounds with exciting clinical potential. These candidates include lomefloxacin (**5**),[6] tosufloxacin (**6**),[7] sparfloxacin (AT-4140/CI-978) (**7**),[8] WIN 57273 (**8**),[9] and PD 127391 (**9**)[10]

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