Synthesis and Antiallergy Activity of [1,3,4]Thiadiazolo[3,2-*a*]-1,2,3-triazolo[4,5-*d*]pyrimidin-9(3*H*)-one Derivatives. I

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A series of 6-substituted [1,3,4]thiadiazolo[3,2-*a*]-1,2,3-triazolo[4,5-*d*]pyrimidin-9(3*H*)-one derivatives 4a—z were synthesized from 5-substituted 1,3,4-thiadiazol-2-amines 5 by the following consecutive reactions: pyrimidine ring closure with bis(2,4,6-trichlorophenyl) malonate, nitration, chlorination, amination, hydrogenation and diazotization. The structure of 4 was confirmed by an alternate synthesis of 4, involving reaction of 5-substituted 2-azido-1,3,4-thiadiazole 13 with ethyl cyanoacetate, followed by the Dimroth rearrangement and ring closure. The antiallergic activities (anti-passive peritoneal anaphylaxis, anti-passive cutaneous anaphylaxis and anti-slow reacting substance of anaphylaxis activities) of the products were evaluated.

Keywords [1,3,4]thiadiazolo[3,2-*a*]-1,2,3-triazolo[4,5-*d*]pyrimidin-9(3*H*)-one; 1,3,4-thiadiazol-5-amine; Dimroth rearrangement; antiallergy; anaphylaxis; SRS-A

Disodium cromoglycate (DSCG) **1** is a well known inhibitor of chemical mediator release from sensitized mast cells, and inhalation of this drug is widely used for the treatment of bronchial asthma. Since its discovery, a great many studies have been reported from numerous laboratories aiming to find additional DSCG-like antiallergic agents, especially orally effective ones.[1] Most of the compounds examined have similar structural features to **1**: they each have a carbonyl group and an acidic group attached to the same ring or adjacent rings.

In our search for a new orally active antiallergic drug, we focused our attention on the effects of various 4*H*-pyrimidin-4-ones fused at the 2- and 3-positions with a heteroaromatic compound instead of the 4*H*-1-benzopyran-4-one of **1**, because of their good oral absorbability. We have reported previously the syntheses and antiallergic activities of 2-substituted 5-oxo-5*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidine-6-carboxylic acids **2** and 2-substituted 6-(5-tetrazolyl)-5*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-ones **3**; the substituent at the 2-position has a major influence on the oral activity against passive cutaneous anaphylaxis (PCA) and an aryl derivative is, in general, superior to an alkyl one.[2]

On the other hand a 4,5-ring fused 1*H*-1,2,3-triazole moiety shows an acidic character and is considered as one of the bioisosteres of the carboxyl group. Our interest, as a result, was next focused on the 6-substituted [1,3,4]thiadiazolo[3,2-*a*]-1,2,3-triazolo[4,5-*d*]pyrimidin-9(3*H*)-ones **4**. This paper deals with the syntheses and antiallergic activities of these new ring system compounds.

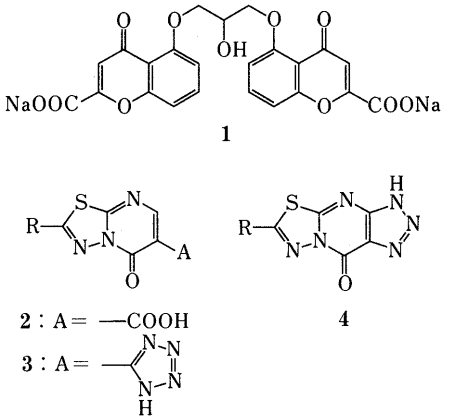
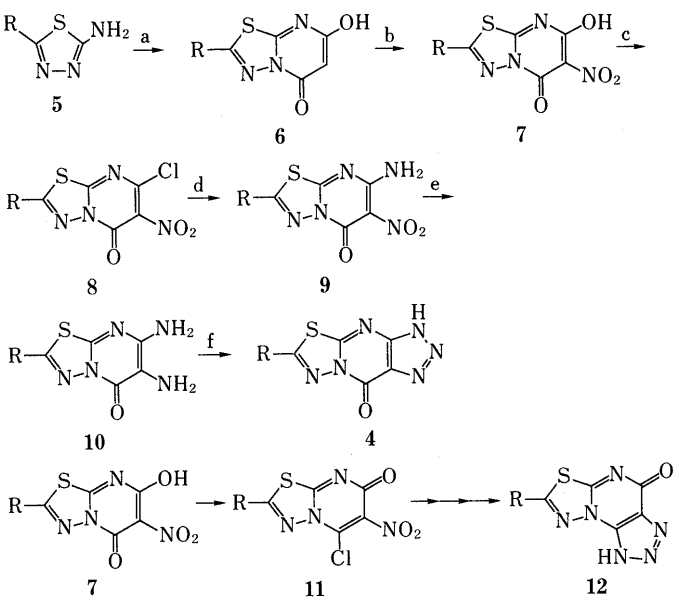


Chart 1

Chemistry Kummer *et al.*[3] and Temple[4] reported the syntheses of compounds having 1,2,3-triazolo[4,5-*d*]pyrimidin-7(1*H*)-one fused at positions 5 and 6 with a heterocycle, namely pyrido[1,2-*a*]-1,2,3-triazolo[4,5-*d*]pyrimidin-10(1*H*)-ones and 6,7-dihydro-3*H*-imidazo[1,2-*a*]-1,2,3-triazolo[4,5-*d*]pyrimidin-9(4*H*)-ones. In addition, Knowles *et al.*[5] obtained [1,2,3]triazolo[4′,5′:4,5]pyrimido[2,1-*a*]isoquinolin-8(9*H*)-ones starting from pyrimido-[2,1-*a*]isoquinoline-2,4(3*H*)-dione by the following consecutive reactions: chlorination, amination, nitrosation, hydrogenation and diazotization.

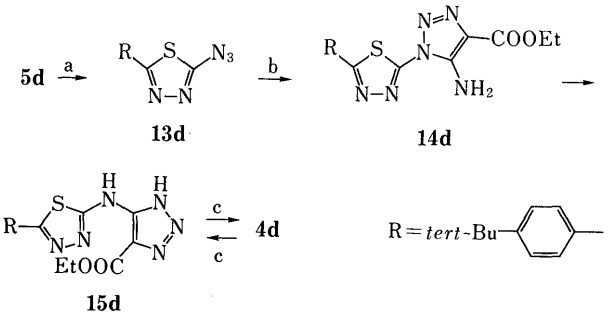
Since we wished to vary the substituent at the 6-position of **4**, we modified Knowles's stepwise synthetic method.



reagents: a) $CH_2(COO\text{-}2,4,6\text{-}Cl_3C_6H_2)_2$; b) fuming or concentrated HNO_3–AcOH; c) $POCl_3$–$PhNEt_2$ or Pr_3N; d) NH_4OH; e) tin powder–HCl; f) $NaNO_2$–HCl

Chart 2

Our series of reactions, nitration of the hydroxy-oxopyrimidine derivatives followed by the chlorination, amination, hydrogenation and diazotization (method A, Chart 2) is simple and not laborious. The starting materials, 5-substituted-1,3,4-thiadiazol-2-amines **5**, were prepared from corresponding nitriles (method C) or aldehydes (method D) according to the method of Heindl *et al.*[6] or Kubota *et al.*,[7] respectively. In the case of method C, the nitrile derivative was allowed to react with thiosemicarbazide not in aqueous trifluoroacetic acid (TFA)[6] but under nonaqueous conditions. Pyrimidine ring closure was achieved mostly by heating the amines **5** with bis(2,4,6-trichlorophenyl) malonate[8] in Dowtherm A.[9] Nitration of **6** with fuming or concentrated HNO_3 in AcOH followed by chlorination with $POCl_3$ and diethylaniline or tripropylamine afforded single chloro–nitro compounds, to which we tentatively assigned the structure **8**.[10] If chlorination occurred at the 5-position, the final product might be an angular type compound **12** from the chloro–nitro compound **11** (Chart 2). Treatment of a suspension of **8** in dioxane or EtOH with concentrated NH_4OH provided **9**. After hydrogenation of **9** with tin powder in a mixture of concentrated HCl and dioxane, diazotization of the crude **10**·HCl with $NaNO_2$ in 6 N HCl gave the triazole derivative **4**; the infrared (IR) spectra showed the presence of the carbonyl group but the position of the hydrogen of the triazole ring is undecided. During these processes almost every intermediate could be used without further purification.



reagents: a) TsN_3–$PhCH_2(Me)_3N^+Cl^-$–40% NaOH; b) $NCCH_2$-COOEt–NaOEt; c) NaOEt

Chart 3

In order to determine the structure of the final product, not **12** but **4**, 2-azido-5-(4-*tert*-butylphenyl)-1,3,4-thiadiazole (**13d**), prepared from the amine **5d** and *p*-toluenesulfonyl azide[11] according to the procedure of Anselme and Fisher,[12] was allowed to react with ethyl cyanoacetate in the presence of NaOEt in EtOH (Chart 3). When the mixture was stirred at room temperature, a yellow precipitate (compound **A**) separated out within 20 min and another product (compound **B**), whose *Rf* value is smaller than that of compound **A**, was obtained from the mother liquor by means of preparative thin layer chromatography (TLC). The ^1H-nuclear magnetic resonance (NMR) spectra of **A** and **B** in $CDCl_3$ were very similar to each other, both having signals due to 4-*tert*-butylphenyl and ester ethyl protons, except that **A** showed amino proton signals at 7.1 ppm. The IR spectra of **A** and **B** both exhibited carbonyl bands at 1680 cm^{-1} and the bands at 3470 and 3350 cm^{-1} in the spectrum of compound **A** suggested the presence of an amino group. Heating **A** in EtOH at refluxing temperature for 10 min gave **B**, but the reverse reaction did not

TABLE I. Data for 6-Substituted [1,3,4]Thiadiazolo[3,2-*a*]-1,2,3-triazolo[4,5-*d*]pyrimidin-9(3*H*)-ones **4**

Compd. No.	R	Method[a] (Yield, %)	mp (°C) (Recrystn. solvent)[b]	Formula	Analysis (%) Calcd (Found)			Antiallergy activity		
					C	H	N	PPA test SRS-A[c]	PPA test His[d,e,g]	PCA test[d-f]
4a	C_6H_5–	A (57.3)	Over 300 ($CHCl_3$–EtOH)	$C_{11}H_6N_6OS$	48.88 (48.79)	2.24 2.55	31.10 30.79)	++	82[e]	38[e]
4b	4-MeC_6H_4–	B (60.9)	Over 300 —	$C_{12}H_8N_6OS$	50.70 (50.60)	2.84 3.16	29.56 29.43)	+++	91[e]	31[e]
4c	4-iso-PrC_6H_4–	A (67.2[h])	269—271 (EtOH)	$C_{14}H_{12}N_6OS$	53.83 (54.27	3.87 4.08	26.91 26.73)	+++	85[e]	18[f]
4d	4-*tert*-BuC_6H_4–	A (85.4) B (35.5)	293—296 (dec.) ($CHCl_3$–EtOH)	$C_{15}H_{14}N_6OS$	55.20 (55.39	4.32 4.54	25.75 25.82)	+++	83[e]	46[e]
4e	3,4-$(CH_2)_4C_6H_3$–	B (34.9)	284—287 —	$C_{15}H_{12}N_6OS$	55.54 (55.70	3.73 3.94	25.91 25.50)	++	77[e]	44[e]
4f	4-$MeOC_6H_4$–	A (64.0)	285—287 (DMF)	$C_{12}H_8N_6O_2S$ ·$DMFH_2O$	45.69 (45.40	3.83 3.56	25.66 25.59)	++	93[e]	26[f]
4g	2-Furyl-	A (64.0[i]) B (23.6)	Over 300 ($CHCl_3$–EtOH)	$C_9H_4N_6O_2S$	41.54 (41.69	1.55 1.92	32.30 32.05)	++	82[e]	50[e]
4h	5-*tert*-Bu-2-thienyl-	A (51.7)	253—256 ($CHCl_3$–IPA–Et_2O)	$C_{13}H_{12}N_6OS_2$	46.97 (46.85	3.64 3.64	25.29 24.96)	+++	91[e]	34[e]
4i	4,5-$(Me)_2$-2-thienyl-	A (64.5)	Over 300 (DMF)	$C_{11}H_8N_6OS_2$ ·$1/2H_2O$	42.16 (42.28	2.90 2.88	26.82 26.82)	++	73[e]	22[f]
4j	$C_6H_5CH_2$–	A (60.7)	Over 300 ($CHCl_3$–EtOH)	$C_{12}H_8N_6OS$	50.69 (50.59	2.84 2.93	29.56 29.57)	++	99[e]	48[e]
4k	$C_6H_5(CH_2)_2$–	A (66.7)	295—296 ($CHCl_3$–EtOH)	$C_{13}H_{10}N_6OS$	52.34 (52.50	3.38 3.56	28.17 28.20)	++	80[e]	52[e]
BW-755C[21]								++	18[g]	ND
Proxicromil[22]								++	49[e]	22[e]

a) See text. *b*) —: not recrystallized. *c*) +++: over 50% inhibition at 1 mg/kg, ++: 60—50% inhibition at 5 mg/kg. $N=5$. *d*) Inhibition % at 1 mg/kg. *e*) $p<0.01$, *f*) $p<0.05$ significantly different from vehicle control. $N=5$. ND: not done. *g*) Tested at 5 mg/kg. *h*) Yield from **8c**. *i*) Yield from **8g**. DMF: dimethylformamide. IPA: isopropyl alcohol.

TABLE II. Data for 6-Substituted [1,3,4]Thiadiazolo[3,2-*a*]-1,2,3-triazolo[4,5-*d*]pyrimidin-9(3*H*)-ones **4**

Compd. No.	R	Method[a] (Yield, %)	mp (°C) (Recrystn. solvent)	Formula	Analysis (%) Calcd (Found) C	H	N	Antiallergy activity Anti-SRS-A[b]	PCA test[c-f]	Toxicity[g]
4k	$C_6H_5(CH_2)_2-$							1.00	80[d]	0/10
4l	$C_6H_5(CH_2)_3-$	A (18.8)	265—267 ($CHCl_3$–MeOH)	$C_{14}H_{12}N_6OS$	53.83 (53.58)	3.87 (3.93)	26.91 (26.48)	0.18	21[e]	ND
4m	$C_6H_5(CH_2)_4-$	A (35.5)	279—281 ($CHCl_3$–EtOH)	$C_{15}H_{14}N_6OS$	55.20 (54.91)	4.32 (4.27)	25.75 (25.36)	0.13	—	ND
4n	$C_6H_5OCH_2-$	A (37.1[h])	Over 300 (DMF)	$C_{12}H_8N_6O_2S$ 1/4DMF	48.07 (47.77)	3.09 (2.93)	27.48 (27.33)	0.01	33[d]	ND
4o	$C_6H_5SCH_2-$	A (63.6)	249—251 ($CHCl_3$–EtOH)	$C_{12}H_8N_6OS_2$	45.30 (45.63)	2.55 (2.60)	26.57 (26.41)	0.29	22	ND
4p	$2\text{-}MeC_6H_4(CH_2)_2-$	A (75.7)	277—279 (DMF–EtOH)	$C_{14}H_{12}N_6OS$	53.83 (53.62)	3.87 (3.99)	26.91 (26.43)	4.6	35[d]	0/5
4q	$3\text{-}MeC_6H_4(CH_2)_2-$	A (69.5)	276—278 ($CHCl_3$–EtOH)	$C_{14}H_{12}N_6OS$	53.83 (53.86)	3.87 (4.03)	26.91 (26.88)	1.13	26[d]	0/5
4r	$4\text{-}MeC_6H_4(CH_2)_2-$	A (64.5)	284—285 (AcOH)	$C_{14}H_{12}N_6OS$	53.83 (53.76)	3.87 (4.18)	26.91 (26.57)	0.39	36[d]	0/5
4s	$3\text{-}MeOC_6H_4(CH_2)_2-$	A (54.7)	285—287 (DMF)	$C_{14}H_{12}N_6O_2S$	51.21 (51.19)	3.68 (3.92)	25.59 (25.31)	0.56	—	ND
4t	$4\text{-}MeOC_6H_4(CH_2)_2-$	A (63.7)	270—272 (DMF)	$C_{14}H_{12}N_6O_2S$	51.21 (51.25)	3.68 (3.76)	25.59 (25.48)	0.06	23[d]	ND
4u	$3\text{-}C_6H_{13}OC_6H_4(CH_2)_2-$	A (66.8)	212—214 ($CHCl_3$–EtOH)	$C_{19}H_{22}N_6O_2S$	57.27 (57.16)	5.56 (5.57)	21.09 (21.11)	2.98	15[f]	ND
4v	$2\text{-}ClC_6H_4(CH_2)_2-$	A (62.0)	273—276 (dec.) ($CHCl_3$–EtOH)	$C_{13}H_9ClN_6OS$	46.92 (47.15)	2.72 (3.04)	25.26 (24.98)	3.7	30[d]	ND
4w	$3\text{-}ClC_6H_4(CH_2)_2-$	A (71.3)	277—279 ($CHCl_3$–EtOH)	$C_{13}H_9ClN_6OS$	46.92 (46.93)	2.72 (2.87)	25.26 (25.30)	6.0	16	0/5
4x	$4\text{-}FC_6H_4(CH_2)_2-$	A (63.1)	284—286 ($CHCl_3$–EtOH)	$C_{13}H_9FN_6OS$	49.36 (49.30)	2.87 (3.06)	26.57 (26.63)	1.67	44[d]	0/5
4y	$4\text{-}ClC_6H_4(CH_2)_2-$	A (53.1)	290—292 (DMF)	$C_{13}H_9ClN_6OS$	46.92 (46.68)	2.73 (2.99)	25.26 (24.80)	1.47	34[d]	1/5
4z	$2\text{-}CF_3C_6H_4(CH_2)_2-$	A (58.0)	280 (dec.) ($CHCl_3$–EtOH)	$C_{14}H_9F_3N_6OS$	45.90 (45.60)	2.48 (2.65)	22.94 (22.78)	7.04	66[d,f]	0/5
Proxicromil[22]								ND	22[d]	ND
FPL-55712[23]								54.0	—	ND

a) See text. *b*) Relative activity (**4k** = 1.0). $N = 5$. ND: not done. *c*) Inhibition %, —: no effect. *d*) $p < 0.01$, *e*) $p < 0.05$, significantly different from the vehicle control. $N = 5$. *f*) Tested at 10 mg/kg. *g*) Number of dead mice/number of tested mice, ND: not done. *h*) Yield from **8n**.

occur. Refluxing the solution of **B** in EtOH with NaOEt afforded a mixture of **B** and **4d**, which was identified by comparing its spectral data with those of **4d** prepared by the above-mentioned stepwise method (method A). Further, the treatment of **4d** under the same conditions also afforded a mixture of **4d** and **B**. From these facts it is concluded that the reaction of **13d** and ethyl cyanoacetate affords first compound **A**, **14d**, which is then converted to compound **B**, **15d**, by means of the Dimroth rearrangement.[13] The structure of **4d**, which is synthesized by method A, is unquestionably the linear **4d**, not the angular one **12d** (Chart 2). Four kinds of **4** were synthesized *via* azide derivatives (method B). Among the starting azides, **13e** existed in a thiadiazolotetrazole form, because its IR spectra lacked the azide band in the region of about 2100 to 2250 cm^{-1}. The scope and limitations of this reaction will be reported in a separate paper. Tables I and II show the physicochemical properties of the synthesized compounds **4**.

Pharmacological Evaluation and Discussion The 6-aryl (**4a**—**i**), 6-benzyl (**4j**) and 6-phenethyl (**4k**) derivatives were tested for their ability to inhibit the passive peritoneal anaphylaxis (PPA) and the PCA reactions in rats as described in the experimental section, and their activities are listed in Table I. The 6-phenethyl derivatives (**4k**—**z**) were assayed for antagonistic activity against slow reacting substance of anaphylaxis (SRS-A), anti-PCA activity and acute toxicity, and these results are shown in Table II.

Every compound in Table I has marked activity with regard to histamine (His) release inhibition in PPA. On the other hand, the activity against PCA varies from compound to compound, because it relates not only to the intrinsic histamine release–inhibitory activity but also to absorption, distribution, metabolism and excretion. As regards SRS-A, compounds having a monoalkylaryl group at the 6-position (**4b**—**d**, **h**) generally show potent inhibition of SRS-A release and antagonistic activity against SRS-A. Aiming to find a compound which has both antagonistic activity to SRS-A and anti PCA activity, we further synthesized compounds containing phenylalkyl substituents. Antagonizing activity to SRS-A reached maximum when the number of the methylenes between the phenyl and the tricyclic ring is 2 (**4k**, **l**, **m**). Replacement of this methylene bridge by oxymethyl (**4n**) or thiomethyl (**4o**) decreased the activity. A methoxy substituent on the phenyl ring (**4s**, **t**) also decreased the activity.

These novel ring system compounds thus proved to be orally available antiallergy agents of a new type, characteristically having dual antiallergy, anti PCA and anti SRS-A, activities.

Experimental

All melting points were determined on a Yanagimoto MP-1 melting point apparatus and are uncorrected. IR spectra were obtained with a Hitachi 260-30 or 270-30 spectrophotometer. ^{1}H-NMR spectra were measured on a Hitachi R-40 spectrometer using tetramethylsilane as an internal standard.

Method A. 7-Hydroxy-2-(2-phenylethyl)-5*H*-1,3,4-thiadiazolo[3,2-*a*]-pyrimidin-5-one (6k) A mixture of **5k** (10.30 g, 50.0 mmol) and bis-(2,4,6-trichlorophenyl) malonate (25.00 g, 55.0 mmol) in Dowtherm A (50 ml) was heated at 120—130 °C for 1 h with stirring. After the mixture had been cooled to room temperature, EtOH was added, and the resulting precipitate was collected and washed thoroughly with ether to give **6k** (9.90 g, 72.3%), mp 255—258 °C. *Anal.* Calcd for $C_{13}H_{11}N_3O_2S$: C, 57.13; H, 4.06; N, 15.37. Found: C, 57.20; H, 4.13; N, 15.38.

Compound **6a**, **6c**, **6d**, **6f**—**j** and **6l**—**z** were prepared by an analogous procedure, and data are listed in Table III.

7-Hydroxy-6-nitro-2-(2-phenylethyl)-5*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-one (7k) Concentrated HNO_3 (4.0 ml, 53 mmol) was added dropwise to a stirred suspension of **6k** (9.50 g, 34.8 mmol) in AcOH (70 ml) at room temperature within 20 min, and stirring was continued for a further 2 h at the same temperature. The precipitate was collected and washed successively with ice water and a mixture of iso-PrOH and ether to give **7k** (10.40 g, 93.7%), mp 188—192 °C. *Anal.* Calcd for $C_{13}H_{10}N_4O_4S$: C, 49.05; H, 3.17; N, 17.60. Found: C, 49.21; H, 3.30; N, 17.81.

Compounds **7a**, **7c**, **7d**, **7f**—**j** and **7l**—**z** were prepared by an analogous procedure, and data are listed in Table IV.

7-Chloro-6-nitro-2-(2-phenylethyl)-5*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-one (8k) A suspension of **7k** (5.00 g, 15.7 mmol) in $POCl_3$ (30 ml, 322 mmol) was heated to 70—90 °C with stirring, $PhNEt_2$ (2 ml, 12.5 mmol) was added within 3 min and the mixture was heated for 3 h at the same temperature. After being cooled to room temperature, the reaction mixture was poured into ice water and stirred for 30 min. The resulting precipitate was collected, washed with water, and recrystallized from $CHCl_3$–EtOH to give **8k** (4.55 g, 86.2%), mp 197—199 °C. *Anal.* Calcd for $C_{13}H_9ClN_4O_3S$: C, 46.36; H, 2.69; N, 16.64. Found: C, 46.13; H, 2.65; N, 16.76.

TABLE III. 2-Substituted 7-Hydroxy-5*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-ones **6**

Compd. No.	mp (°C) (Yield, %)	Formula	Analysis (%) Calcd (Found)		
			C	H	N
6a	273—275 (91.1)	$C_{11}H_7N_3O_2S$	53.87 (53.87	2.88 2.73	17.13 17.42)
6c	248—255 (91.1)	$C_{14}H_{13}N_3O_2S$	58.52 (58.33	4.56 4.55	14.46 14.46)
6f	284—286 (76.4)	$c_{12}H_9N_3O_3S$	52.36 (52.17	3.30 3.10	15.27 15.24)
6g	Over 300 (95.8)	$C_9H_5N_3O_3S$	45.95 (45.44	2.14 2.49	17.86 17.97)
6h	255—259 (79.3)	$C_{13}H_{13}N_3O_2S_2$	50.79 (50.54	4.26 3.89	13.67 13.75)
6i[a]	278—282 (78.1)	$C_{11}H_9N_3O_2S_2$	47.30 (47.19	3.25 3.37	15.04 15.15)
6j	256—257 (71.4)	$C_{12}H_9N_3O_2S$	55.59 (55.79	3.50 3.50	16.21 16.60)
6k	255—258 (72.3)	$C_{13}H_{11}N_3O_2S$	57.13 (57.20	4.06 4.13	15.37 15.38)
6l	202—204 (84.2)	$C_{14}H_{13}N_3O_2S$	58.52 (58.37	4.56 4.57	14.62 14.71)
6m	215—218 (73.4)	$C_{15}H_{15}N_3O_2S$	59.78 (59.78	5.02 5.02	13.95 14.23)
6n	251—254 (44.0)	$C_{12}H_9N_3O_3S$	52.36 (51.78	3.30 3.12	15.26 15.71)
6o	191—193 (73.4)	$C_{12}H_9N_3O_2S_2$	49.47 (49.37	3.11 2.89	14.42 14.62)
6p	243—247 (87.2)	$C_{14}H_{13}N_3O_2S$	58.52 (58.34	4.56 4.63	14.62 14.52)
6q	252—254 (87.5)	$C_{14}H_{13}N_3O_2S$	58.52 (58.42	4.56 4.54	14.62 14.65)
6r	258—261 (91.7)	$C_{14}H_{13}N_3O_2S$	58.52 (58.44	4.56 4.61	14.62 14.53)
6s	232—233 (90.0)	$C_{14}H_{13}N_3O_3S$	55.43 (55.40	4.32 4.43	13.85 13.83)
6t	250—252 (84.4)	$C_{14}H_{13}N_3O_3S$	55.43 (55.87	4.32 4.37	13.85 13.83)
6v	236—237 (50.9)	$C_{13}H_{10}ClN_3O_2S$	50.73 (50.74	3.28 3.24	13.65 13.80)
6w	254—255 (68.7)	$C_{13}H_{10}ClN_3O_2S$	50.73 (50.96	3.28 3.15	13.65 14.09)
6y	273—275 (73.5)	$C_{13}H_{10}ClN_3O_2S$	50.73 (51.03	3.28 3.16	13.65 13.96)
6z	224—226 (76.0)	$C_{14}H_{10}F_3N_3O_2S$	49.27 (49.40	2.95 3.16	12.31 12.44)

a) Recrystallized from DMF.

TABLE IV. 2-Substituted 7-Hydroxy-6-nitro-5*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-ones **7**

Compd. No.	mp (°C) (Yield, %)	Formula	Analysis (%) Calcd (Found)		
			C	H	N
7a	253—255 (85.6)	$C_{11}H_6N_4O_4S$	45.52 (45.55	2.08 1.95	19.30 19.00)
7c	255—257 (81.2)	$C_{14}H_{12}N_4O_4S$	50.59 (50.70	3.64 3.60	16.86 16.92)
7d	245—246 (dec.) (59.6[a])	$C_{15}H_{14}N_4O_4S$	52.01 (52.08	4.07 4.43	16.18 16.18)
7f	259—261 (dec.) (55.8)	$C_{12}H_8N_4O_5S$	45.00 (44.93	2.52 2.72	17.49 17.38)
7g	(Unclear) (87.1)	$C_9H_4N_4O_5S$	38.57 (38.25	1.44 1.75	20.00 19.90)
7h[b]	198—203 (95.2)	$C_{13}H_{12}N_4O_4S_2 \cdot 1/2H_2O$	43.20 (43.00	3.63 3.42	15.50 15.48)
7i[b]	Over 300 (91.4)	$C_{11}H_8N_4O_4S_2$	40.74 (40.80	2.49 2.59	17.27 17.37)
7j	167—169 (84.6)	$C_{12}H_8N_4O_4S$	47.37 (47.41	2.65 2.72	18.41 18.55)
7k	188—192 (93.7)	$C_{13}H_{10}N_4O_4S$	49.05 (49.21	3.17 3.30	17.60 17.81)
7l	143—144 (91.4)	$C_{14}H_{12}N_4O_4S$	50.60 (50.47	3.64 3.70	16.86 16.91)
7m	158—159 (91.5)	$C_{15}H_{14}N_4O_4S$	52.02 (52.08	4.07 4.18	16.18 16.07)
7n	208—210 (dec.) (95.1)	$c_{12}H_8N_4O_5S$	45.00 (45.15	2.52 2.27	17.49 17.78)
7o	190—192 (89.5)	$C_{12}H_8N_4O_4S_2$	42.85 (42.94	2.40 2.01	16.66 16.57)
7p	194—196 (86.9)	$C_{14}H_{12}N_4O_4S$	50.60 (50.94	3.64 3.71	16.86 16.73)
7q	152—153 (95.9)	$C_{14}H_{12}N_4O_4S$	50.60 (50.89	3.64 3.66	16.86 16.87)
7r	169—172 (96.0)	$C_{14}H_{12}N_4O_4S$	50.60 (50.37	3.64 3.73	16.86 16.67)
7s	187—189 (95.7)	$C_{14}H_{12}N_4O_5S$	48.27 (48.60	3.47 3.62	16.08 15.80)
7t	178—179 (99.6)	$C_{14}H_{12}N_4O_5S$	48.27 (48.34	3.47 3.53	16.08 15.90)
7u[c]	71—72 (75.4[d])	$C_{19}H_{22}N_4O_5S \cdot 1/4H_2O$	53.95 (53.93	5.36 5.34	13.25 13.48)
7v	194—195 (96.6)	$C_{13}H_9ClN_3O_4S$	44.26 (44.52	2.57 2.71	15.88 15.99)
7w	178—180 (82.4)	$C_{13}H_9ClN_3O_4S$	44.26 (44.37	2.57 2.27	15.88 15.77)
7x	227—228 (55.5[e])	$C_{13}H_9FN_4O_4S$	46.43 (46.44	2.70 2.36	16.66 16.56)
7y	219—221 (92.2)	$C_{13}H_9ClN_3O_4S$	44.26 (44.59	2.57 2.73	15.88 15.65)
7z	159—161 (81.0)	$C_{14}H_9F_3N_4O_4S$	43.53 (43.68	2.35 2.48	14.50 14.63)

a) Yield from **5d**. *b*) Recrystallized from DMF. *c*) Recrystallized from aqueous MeOH. *d*) Yield from **5u**. *e*) Yield from **5x**.

TABLE V. 2-Substituted 7-Chloro-6-nitro-5*H*-1,3,4-thiadiazolo[3,2-*a*]-pyrimidin-5-ones **8**

Compd. No.	Recrystn. solvent[a] (Yield, %)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N
8a	— (59.2)	259—262	$C_{11}H_5ClN_4O_3S$	42.80 (42.42)	1.63 1.76	18.15 18.02)
8c	$CHCl_3$–IPA–Et_2O (91.1)	195—197	$C_{14}H_{11}ClN_4O_3S$	47.93 (48.08)	3.16 3.09	15.97 15.99)
8d	— (98.3)	245—248	$C_{15}H_{13}ClN_4O_3S$	49.38 (49.02)	3.32 3.27	15.36 15.48)
8f	— (86.8)	265—270 (dec.)	$C_{12}H_7ClN_4O_4S$	42.55 (42.62)	2.08 2.24	16.54 16.52)
8g	$CHCl_3$–EtOH (59.8)	257—259	$C_9H_3ClN_4O_4S$	36.19 (35.95)	1.04 1.22	18.76 18.72)
8h	$CHCl_3$–IPA–Et_2O (88.1)	139—141	$C_{13}H_{11}ClN_4O_3S_2$	42.10 (42.25)	2.99 2.99	15.11 15.12)
8i	AcOH (76.9)	279—282	$C_{11}H_7ClN_4O_3S_2$	38.54 (38.57)	2.06 2.13	16.34 16.41)
8j	$CHCl_3$–EtOH (87.8)	174—176	$C_{12}H_7ClN_4O_3S$	44.71 (44.81)	2.12 2.42	17.36 17.42)
8k	$CHCl_3$–EtOH (86.2)	197—199	$C_{13}H_9ClN_4O_3S$	46.36 (46.13)	2.69 2.65	16.64 16.76)
8l	$CHCl_3$–EtOH (82.5)	168—170	$C_{14}H_{11}ClN_4O_3S$	47.94 (48.03)	3.16 3.17	15.97 16.10)
8m	$CHCl_3$–EtOH (75.5)	127—129	$C_{15}H_{13}ClN_4O_3S$	49.39 (49.64)	3.59 3.66	15.36 15.35)
8n	$CHCl_3$–EtOH (98.9)	235—245 (Unclear)	$C_{12}H_7ClN_4O_4S$	42.55 (42.19	2.08 1.75	16.54 16.28)
8o	$CHCl_3$–EtOH (85.3)	151—152	$C_{12}H_7ClN_4O_3S_2$	40.62 (40.58	1.99 1.56	15.79 15.63)
8p	— (90.1)	168—175	$C_{14}H_{11}ClN_4O_3S$	47.94 (47.98	3.16 3.24	15.97 15.78)
8q	— (96.5)	200—204	$C_{14}H_{11}ClN_4O_3S$	47.94 (47.79	3.16 3.12	15.97 15.85)
8r	— (81.4)	220—221.5	$C_{14}H_{11}ClN_4O_3S$	47.94 (47.79	3.16 3.12	15.97 15.85)
8s	— (54.7)	183—185	$C_{14}H_{11}ClN_4O_4S$	45.85 (45.83	3.02 2.78	15.28 15.15)
8t	— (64.4)	203—205	$C_{14}H_{11}ClN_4O_4S$	45.85 (45.56	3.02 2.90	15.28 15.37)
8u	Ether (76.9)	59—60	$C_{19}H_{21}ClN_4O_4S$	52.23 (52.20	4.84 4.84	12.82 12.85)
8v	— (62.3)	166—167	$C_{13}H_8Cl_2N_4O_3S$	42.06 (41.61	2.17 2.34	15.09 14.63)
8w	— (82.5)	200—202	$C_{13}H_8Cl_2N_4O_3S$	42.06 (41.70	2.17 1.89	15.09 14.99)
8x	$CHCl_3$–EtOH (62.3)	196—198	$C_{13}H_8ClFN_4O_3S$	44.01 (43.95	2.27 1.80	15.79 15.64)
8y	$CHCl_3$–EtOH (94.9)	223—225	$C_{13}H_8Cl_2N_4O_3S$	42.06 (42.08	2.17 1.77	15.09 15.01)
8z	— (87.7)	167—168.5	$C_{14}H_8ClF_3N_4O_3S$	41.54 (41.31	1.99 2.14	13.84 13.92)

a) —: Not recrystallized.

TABLE VI. 2-Substituted 7-Amino-6-nitro-5*H*-1,3,4-thiadiazolo[3,2-*a*]-pyrimidin-5-ones **9**

Compd. No.	Recrystn. solvent[a] (Yield, %)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N
9a	— (94.4)	Over 300	$C_{11}H_7N_5O_3S$	45.67 (45.79	2.44 2.26	24.21 24.27)
9d	— (72.2)	Over 300	$C_{15}H_{15}N_5O_3S$	52.16 (51.23	4.38 4.62	20.28 20.37)
9f	DMF (60.0)	Over 300	$C_{12}H_9N_5O_4S$	45.14 (45.01	2.84 3.20	21.94 21.85)
9h	— (82.0)	272—275	$C_{13}H_{13}N_5O_3S_2$	44.43 (44.26	3.73 3.55	19.93 19.80)
9i	DMF (80.7)	Over 300	$C_{11}H_9N_5O_3S_2$	40.86 (41.25	2.81 3.13	21.66 21.70)
9j	$CHCl_3$–EtOH (59.6)	286—289	$C_{12}H_9N_5O_3S$	47.52 (47.61	2.99 2.63	23.09 22.84)
9k	$CHCl_3$–EtOH (90.2)	268—270	$C_{13}H_{11}N_5O_3S$	49.21 (49.39	3.49 3.18	22.07 21.95)
9l	— (88.8)	252—255	$C_{14}H_{13}N_5O_3S$	50.75 (50.85	3.95 3.79	21.14 21.17)
9m	— (86.0)	249—252	$C_{15}H_{15}N_5O_3S$	52.16 (52.13	4.38 4.32	20.28 20.13)
9o	— (28.9)	150—155	$C_{12}H_9N_5O_3S_2$	42.98 (42.91	2.71 2.55	20.88 20.82)
9p	— (73.6)	264—268	$C_{14}H_{13}N_5O_3S$	50.75 (50.73	3.95 4.03	21.14 21.04)
9q	— (75.7)	262—264	$C_{14}H_{13}N_5O_3S$	50.75 (50.47	3.95 4.06	21.14 20.73)
9r	$CHCl_3$–EtOH (76.2)	273—274	$C_{14}H_{13}N_5O_3S$	50.75 (50.61	3.95 4.06	21.14 20.96)
9s	— (88.2)	264—267	$C_{14}H_{13}N_5O_4S$	48.41 (48.19	3.77 4.04	20.16 19.90)
9t	— (82.5)	254—257	$C_{14}H_{13}N_5O_4S$	48.41 (48.27	3.77 4.00	20.16 19.89)
9u	— (75.0)	180—187	$C_{19}H_{23}N_5O_4S$	54.66 (54.82	5.55 5.59	16.78 16.81)
9v	— (84.5)	235—243	$C_{13}H_{10}ClN_5O_3S$	44.39 (44.39	2.87 3.02	19.91 19.46)
9w	— (80.5)	248—249	$C_{13}H_{10}ClN_5O_3S$	44.38 (44.54	2.87 2.44	19.91 19.82)
9x	— (87.6)	257—258	$C_{13}H_{10}FN_5O_3S$	46.56 (46.59	3.01 2.68	20.89 20.55)
9y	— (88.1)	248—250	$C_{13}H_{10}ClN_5O_3S$	44.38 (44.69	2.87 2.83	19.91 19.84)
9z	$CHCl_3$–EtOH (81.0)	265—269 (dec.)	$C_{14}H_{10}F_3N_5O_3S$	43.64 (43.77	2.62 2.79	18.18 17.88)

a) —: Not recrystallized.

Compound **8a**, **8c**, **8d**, **8f**—**j** and **8l**—**z** were prepared by an analogous procedure, and data are listed in Table V.

7-Amino-6-nitro-2-(2-phenylethyl)-5*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-one (9k) Concentrated NH_4OH (3.0 ml, 44 mmol) was added to a suspension of **8k** (4.00 g, 11.9 mmol) in dioxane (30 ml) while maintaining the reaction temperature below 40 °C. The mixture was stirred for a further 15 min at 60 °C, and then cooled, and the precipitate formed was collected and recrystallized from $CHCl_3$–EtOH to obtain **9k** (3.40 g, 90.2%), mp 268—270 °C. *Anal.* Calcd for $C_{13}H_{11}N_5O_3S$: C, 49.21; H, 3.49; N, 22.07. Found: C, 19.53; H, 3.86; N, 22.44.

Compounds **9a**, **9c**, **9d**, **9f**—**j** and **9l**—**z** were prepared by an analogous procedure, and some data are listed in Table VI.

6-(2-Phenylethyl)-[1,3,4]thiadiazolo[3,2-*a*]-1,2,3-triazolo[4,5-*d*]pyrimidin-9-(3*H*)-one (4k) *via* 6,7-Diamino-2-(2-phenylethyl)-5*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-one (10k) A suspension of **9k** (3.40 g, 10.7 mmol) and tin powder (6.00 g, 50.6 mg·atom) in 80% dioxane (60 ml) was heated to reflux, and concentrated HCl (10 ml) was added followed by heating at reflux until **9k** was no longer detectable on TLC. Any insoluble material was filtered off and the filtrate was concentrated *in vacuo*. The filtrate was cooled to room temperature, then the precipitate was collected and suspended in a mixture of concentrated HCl (40 ml) and water (40 ml). The suspension was cooled to below 5 °C and a solution of $NaNO_2$ (2.00 g, 29.0 mmol) in water (5 ml) was added dropwise while maintaining the same temperature. The mixture was stirred for a further 1 h at the same temperature and diluted with water. The resulting precipitate was collected and recrystallized from $CHCl_3$–EtOH to obtain **4k** (2.13 g, 66.7%), mp 295—296 °C. *Anal.* Calcd for $C_{13}H_{10}N_6OS$: C, 52.34; H, 3.38; N, 28.17. Found: C, 52.50; H, 3.56; N, 28.20. IR (KBr): 3140, 3080, 1700, 1565, 1520, 1470, 1265, 1000, 970, 770 cm^{-1}. ^{1}H-NMR ($CDCl_3$–TFA) δ: 3.0—3.68 (4H, m, $C\underline{H}_2C\underline{H}_2$), 7.06—7.52 (5H, m, $C_6\underline{H}_5$).

Compounds **4a**, **4c**, **4d**, **4f**—**j** and **4l**—**z** were prepared by an analogous procedure, and data are listed in Tables I and II.

Method B. 6-(4-*tert*-Butylphenyl)-[1,3,4]thiadiazolo[3,2-*a*]-1,2,3-triazolo[4,5-*d*]pyrimidin-9(3*H*)-one (4d) from 2-Azido-5-(4-*tert*-butylphenyl)-1,3,4-thiadiazole (13d) A solution of NaOEt prepared from Na (0.29 g, 12.6 mg·atom), ethyl cyanoacetate (1.43 g, 12.6 mmol) and **13d** (1.64 g, 6.3 mmol) in EtOH (5 ml) was stirred at room temperature for 30 min followed by heating to reflux for 2.5 h. The mixture was poured into dil. HCl. The resulting precipitate was collected and washed successively with water and cold MeOH to give **4d** (0.73 g, 35.5%), which was identical with **4d** prepared from **9d**.

Compounds **4b**, **4e** and **4g** were prepared by an analogous procedure, and data are listed in Table I.

Reaction of 13d and Ethyl Cyanoacetate; Ethyl 5-Amino-1-[5-(4-*tert*-butylphenyl)-1,3,4-thiadiazol-2-yl]-1*H*-1,2,3-triazole-4-carboxylate (14d) and Ethyl 5-[5-(4-*tert*-Butylphenyl)-1,3,4-thiadiazol-2-yl]amino-1*H*-1,2,3-triazole-4-carboxylate (15d) A solution of NaOEt prepared from 50%

NaH (6 mg, 0.125 mmol), ethyl cyanoacetate (1 drop) and **13d** (30 mg, 0.12 mmol) in EtOH (0.5 ml) was stirred at room temperature for 20 min. The separated yellow precipitate was collected and washed with EtOH to obtain **14d**, mp 197 °C (dec.). IR (KBr): 3470, 3350, 2960, 1680, 1625, 1540, 1480, 1450, 1330, 1280, 1115, 1105, 980, 960, 840, 780 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (9H, s, $3\times CH_3$), 1.44 (3H, t, J=7.2 Hz, $COOCH_2CH_3$), 4.45 (2H, q, J=7.2 Hz, $COOCH_2CH_3$), 7.53 (2H, d, J=8.5 Hz, $C_{3'}$ and $C_{5'}$–H), 7.88 (2H, d, J=8.5 Hz, $C_{2'}$ and $C_{6'}$-H), 7.1 (2H, br m, NH_2). The mother liquor was concentrated *in vacuo* and TLC of the residue afforded **15d**. *Anal.* Calcd for $C_{17}H_{20}N_6O_2S$: C, 54.82; H, 5.41; N, 22.56. Found: C, 54.74; H, 5.54; N, 22.41. IR (KBr): 3220, 2950, 1680, 1600, 1580, 1450, 1100 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.35 (9H, s, $3\times CH_3$), 1.46 (3H, t, J=7.0 Hz, $COOCH_2CH_3$), 4.52 (2H, q, J=7.0 Hz, $COOCH_2CH_3$), 7.50 (2H, d, J=8.5 Hz, $C_{3'}$ and $C_{5'}$-H), 7.76 (2H, d, J=8.5 Hz, $C_{2'}$ and $C_{6'}$-H). Heating of a solution of **14d** in EtOH to reflux resulted in the formation of **15d** (detected by TLC). No change was found on heating a solution of **15d** in EtOH to reflux (TLC).

6-(4-*tert*-Butylphenyl)-[1,3,4]thiadiazolo[3,2-*a*]-1,2,3-triazolo[4,5-*d*]pyrimidin-9(3*H*)-one (4d) from 15d A solution of **15d** (372 mg, 1.0 mmol) and EtONa (70.0 mg, 1.0 mmol) in EtOH (2 ml) was heated to reflux for 2 h. The solvent was removed *in vacuo* and the cooled residue was treated with concentrated HCl. The resulting precipitate was collected and washed successively with water and cold MeOH to give **4d** (220 mg, 67.4%), which was identical with **4d** derived from **9d**. TLC showed the presence of **15d** in the mother liquor.

Reaction of 4d and NaOEt A solution of **4d** (163 mg, 0.5 mmol) and NaOEt (35 mg, 0.5 mmol) in EtOH (1 ml) was heated to reflux for 3 h. TLC showed that the solution contained a mixture of **4d** and **15d** (*ca.* 2 : 1).

Method C. 2-Amino-5-phenyl-1,3,4-thiadiazole (5a) A stirred mixture of benzonitrile (51.5 g, 0.50 mol) and thiosemicarbazide (50.0 g, 0.55 mol) in TFA (150 ml) was heated at 60—80 °C for 6 h. After being cooled to room temperature, the reaction mixture was poured into dilute NH_4OH. The resulting precipitate was collected and dissolved in hot EtOH. The solution was made alkaline by addition of NH_4OH and concentrated. The precipitate was collected, washed with H_2O and dried to give **5a** (67.8 g, 76.6%), mp 231—232 °C, which was identical with **5a** prepared by the reported procedure.[14] Data of the new compounds prepared by an analogous procedure are listed in Table VII.

Method D. 2-Amino-5-(2-furyl)-1,3,4-thiadiazole (5g) Furfural (52.0 g, 0.54 mol) was treated according to the method of Kubota *et al.*[7] to afford **5g** (33.8 g, 37.7%), mp 250—255 °C, which was identical with **5g** prepared by the reported procedure.[15] **5h**: 22.2% yield, mp 203—205 °C.

13d A solution of **5d** (1.17 g, 5.0 mmol), *p*-toluenesulfonyl azide (1.97 g, 10.0 mmol) and benzyl trimethylammonium chloride (0.05 g) in a mixture of 40% NaOH (10 ml) and CH_2Cl_2 (20 ml) was stirred at room temperature for 18 h. After the addition of iced water, the organic layer was separated, washed with water and dried. The solvent was removed *in vacuo*, and the residue was recrystallized from MeOH to afford **13d** (0.82 g, 63.1%), mp 137 °C (dec.). *Anal.* Calcd for $C_{12}H_{13}N_5S$: C, 55.58; H, 5.05; N, 27.01. Found: C, 55.79; H, 5.12; N, 26.93. IR (KBr): 2950, 2125, 1440, 1270, 1110, 1080, 835, 585 cm^{-1}.

Compounds **13b**, **13e** and **13g** were prepared by an analogous procedure, and data are listed in Table VIII.

Biological Assay Male Sprague-Dawley rats (Charles River Japan, Inc.) weighing 300—350 g (for preparation of rat antiserum), 170—270 g (PCA assay) or 250—400 g (PPA assay), and male Std : ddY mice weighing 15—25 g (acute toxicity assay) were used.

Rat Antiserum Containing Homocytotropic Antibody[16] The rats were immunized intramuscularly with 1.0 mg of egg albumin dissolved in 0.2 ml of saline and intraperitoneally (i.p.) with 2×10^{10} *Bordetella pertussis* organisms in 1.0 ml of saline. Fourteen days later the rats were bled and the serum containing homocytotropic antibody was pooled. The 48-h PCA titer of this serum was found to be 64—128.

PCA The antiserum was diluted with saline so as to form a blue spot having a diameter of about 10 mm in the following control group. The diluted antiserum (0.05 ml) was injected into the shaved dorsal skin of rats. After a 48 h latent period, 1.0 ml of saline containing 5.0 mg each of albumin and Evans blue dye was injected intravenously and the rats were killed 30 min later. The diameter in millimeters of the blue spot at each antiserum injection site was measured. The test compounds (20 mg/kg in 0.5% carboxymethyl cellulose (CMC)) were given orally 30 min before the antigen challenge. Percent inhibition was calculated by using the formula, $\% = 100(1 - a/b)$, where a is the sum of the reaction diameters in the treated animals and b is the sum of the reaction diameters in the control animals.

TABLE VII. 5-Substituted 1,3,4-Thiadiazol-2-amines **5**

Compd. No.	Method[a]	mp (°C) (Yield, %)	Formula	Analysis (%) Calcd (Found)		
				C	H	N
5c	C	215—217 (55.6)	$C_{11}H_{13}N_3S$	60.24 (60.25	5.97 5.94	19.16 19.19)
5h	D	203—205 (22.2)	$C_{10}H_{13}N_3S_2$	50.18 (49.97	5.47 5.20	17.56 17.95)
5i	C	237—238 (67.9)	$C_8H_9N_3S_2$	45.47 (45.47	4.29 4.38	19.89 19.81)
5n	C	206—209 (77.1)	$C_9H_9N_3OS$	52.16 (51.47	4.38 4.25	20.27 19.88)
5o	C	173—175 (59.9)	$C_9H_9N_3S_2$	48.40 (48.16	4.06 3.78	18.82 18.77)
5p	C	193—194 (64.5)	$C_{11}H_{13}N_3S$	60.24 (60.41	5.98 5.93	19.16 19.20)
5q	C	173.5—174.5 (69.8)	$C_{11}H_{13}N_3S$	60.24 (60.47	5.98 5.98	19.16 19.03)
5r	C	214 (60.6)	$C_{11}H_{13}N_3S$	60.24 (60.59	5.98 6.12	19.16 19.23)
5s	C	167—168 (68.0)	$C_{11}H_{13}N_3OS$	56.15 (56.32	5.57 5.57	17.86 17.82)
5t	C	212—213 (57.0)	$C_{11}H_{13}N_3OS$	56.15 (56.28	5.57 5.56	17.86 17.79)
5u	C	164—165 (29.5)	$C_{16}H_{23}N_3OS$	62.92 (62.94	7.59 7.53	13.76 13.64)
5v	C	196—198 (72.0)	$C_{10}H_{10}ClN_3S$	50.10 (49.69	4.20 4.17	17.53 17.50)
5w	C	170—172 (38.0)	$C_{10}H_{10}ClN_3S$	50.10 (50.14	4.20 4.15	17.53 17.82)
5x	C	217—218 (71.7)	$C_{10}H_{10}FN_3S$	53.79 (54.08	4.51 4.70	18.82 18.72)
5y	C	219—221 (76.9)	$C_{10}H_{10}ClN_3S$	50.10 (50.08	4.20 4.12	17.53 17.73)
5z[b]	C	205.5—206.5 (66.4)	$C_{11}H_{10}F_3N_3S$	48.35 (48.23	3.69 3.62	15.38 15.52)

a) See text. *b*) Recrystallized from EtOH.

TABLE VIII. 5-Substituted 2-Azido-1,3,4-thiadiazoles **13**

Compd. No.	Form[a]	Recrystn. solvent (Yield, %)	mp (°C)	Formula	Analysis (%) Calcd (Found)			IR (KBr, cm^{-1})
					C	H	N	
13b	A	MeOH (72.4)	129—131 (dec.)	$C_9H_7N_5S$	49.76 (49.96	3.25 3.33	32.24 32.51)	2130, 1600, 1440, 1270, 1255, 1240, 1210, 950, 815
13d	A	MeOH (63.1)	137 (dec.)	$C_{12}H_{13}N_5S$	55.58 (55.79	5.05 5.12	27.01 26.93)	2950, 2125, 1440, 1270, 1110, 1080, 835, 585
13e	B	MeOH (89.4[b])	113—114	$C_{12}H_{11}N_5S$	56.01 (56.17	4.31 4.38	27.22 26.89)	2920, 1600, 1470, 1430, 1420, 1220, 1200, 940, 820, 800
13g	A	MeOH (56.4)	107—108	$C_6H_{13}N_5OS$	37.30 (37.71	1.57 2.06	36.25 35.99)	2130, 1580, 1490, 1440, 1260, 1225, 750

a) A: azide form, B: thiadiazolotetrazole form. *b*) Yield from 4,5,6,7-tetrahydro-2-naphthonitrile.

The statistical significance of differences was determined by using Dunnett's multiple comparison technique.

PPA[17] The rats were passively sensitized by i.p. injection of 5.0 ml of 5-fold-diluted antiserum, and 2 h later they were challenged i.p. with 5.0 ml of phosphate-buffered saline containing 2 mg of egg albumin and 0.9 mM $CaCl_2$. After 5 min the rats were killed, and the peritoneal fluid was harvested and centrifuged at $300 \times g$ at 4 °C for 10 min. The amounts of SRS-A and histamine in the supernatant were determined. The test compounds [1 or 5 mg/kg/5 ml phosphate-buffered saline containing 0.9 mM $CaCl_2$, NaOH (equimolar to the test compound) and 2% dimethyl sulfoxide (DMSO) (or 2% EtOH)] were administered i.p. 30 s before the challenge with albumin. The amount of histamine in the supernatant was measured by the fluorometric method of Shore *et al.*[18] and SRS-A was bioassayed according to Stechschulte *et al.*[19] using a guinea pig ileum specimen in the presence of 10^{-6} M mepyramine and 5×10^{-7} M atropine. Percent inhibition was calculated with the formula, $\% = 100(a-b)/(a-c)$, where a is the mean value of released amount in the control group, b is the mean value of released amount in the treated group and c is the spontaneously released amount. The statistical significance was determined by using Student's t test.

Antagonistic Activity Against SRS-A The *in vitro* screening system employed was the guinea pig ileum bioassay method of Orange and Austen.[20] SRS-A was obtained from the lung fragments of guinea pigs actively sensitized with albumin, and the SRS-A induced contractions were recorded isotonically. The dose of SRS-A that gave 50% of the maximal contraction was used for assay. Test compounds were added to the organ bath 3 min prior to the challenge with SRS-A. The range of concentration giving 50% inhibition with compound **4k** was from 6.5×10^{-5} to 4.3×10^{-6} M.

Acute Toxicity The test compounds were suspended in 0.5% CMC and administered orally at a dose of 2.0 g/kg, and after 24 h the mortality was evaluated.

References and Notes

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