Department of Chemistry, University of New Mexico

Imidazo[4,5-d]pyridazines. IV. Synthesis of 4,7 Disubstituted Derivatives and 1-Benzyl-2-substituted Thio Derivatives

G. Anne Gerhardt (I), Duane L. Aldous (I) (2), and Raymond N. Castle (3)

Seventy-five new imidazo[4,5-d]pyridazines and three new imidazoles have been prepared for antitumor testing. Antitumor screening results are reported for thirty-five of these compounds. No significant activity was observed.

The antitumor activity shown by such compounds as 5-amino-7-(2,4-dichlorobenzylthio)-*v*-triazolo[d]-pyrimidine (4) and halobenzyl substituted 4-mercaptocinnolines (5) prompted the preparation of the following series of compounds.

The imidazo[4,5-d]pyridazine-4,7-dithiol, as well as the monomethyl and dimethyl derivatives had been reported previously by Castle and Seese (6). The imidazo[4,5-d]pyridazine-4,7-dithiol was allowed to react with various alkyl, benzyl and carboxyalkyl halides in a 1:1 or a 1:2 ratio to produce the expected mono- or disubstituted thio derivatives (Tables I and II). However, when the imidazo[4,5-d]pyridazine-4,7-dithiol was allowed to react with two moles of *o*-chlorobenzyl, *p*-chlorobenzyl or 2,4-dichlorobenzyl iodides in 2.5 *N* potassium hydroxide solution the 4(7)-substituted thio-7(4)-hydroxy-1-substituted imidazo[4,5-d]pyridazines were obtained (Table III). In a manner similar to the preparation of the 4,7-diaminoimidazo[4,5-d]pyridazine (6), various other substituted 4,7-diamino derivatives (Table VII) were obtained by allowing the 4,7-bismethylthioimidazo-[4,5-d]pyridazine to react with substituted amines in a stainless steel rocking autoclave for approximately 8 hours at 195°. In several instances unexpected products were obtained, for example, the treatment of the 4,7-bismethylthioimidazo[4,5-d]-pyridazine with 6-30 molar excess of diisopropyl amine or di-(3-methylbutyl) amine gave only the mono-substituted product, 4(7)-substituted amino-7(4)-methylthioimidazo[4,5-d]pyridazine (Table VIII). When a molar excess of di-*n*-propylamine, di-*n*-butylamine or diethylamine was allowed to react in the autoclave under similar conditions, only the 4(7)-hydroxy-7(4)-methylthioimidazo[4,5-d]pyridazine (Table VIII) was obtained.

The 1-benzylimidazo[4,5-d]pyridazine-4,7-diol-2-thiol was prepared from diethyl 1-benzyl-2-mercaptoimidazole-4,5-dicarboxylate which had been reported by Carbon (7). When the 1-benzylimidazo-[4,5-d]pyridazine-4,7-diol-2-thiol was allowed to react with various alkyl and benzyl halides the 1-benzyl-2-substituted thioimidazo[4,5-d]pyridazine-4,7-dioles were obtained as shown in Table IV. The same type of product was obtained when the mercapto diester was first treated with a given halide and then cyclized by reaction with hydrazine. It was found that when the diethyl 1-benzyl-2-mercaptoimidazole-4,5-dicarboxylate was allowed to react with the halide in all cases the diethyl 1-benzyl-2-substituted thioimidazole-4,5-dicarboxylates appeared as oils. These were not analyzed but were characterized by cyclization with hydrazine to the corresponding imidazo[4,5-d]pyridazines as shown in Table V. In several instances the side products of the reactions were isolated and identified as the 1-benzyl-2-substituted thioimidazole-4,5-dicarboxylic acids or the half-acid, half-ester of the 1-benzyl-2-mercaptoimidazole dicarboxylic acids (Table VI).

1-Benzylimidazo[4,5-d]pyridazine-4,7-diol-2-thiol when allowed to react with a six-fold molar excess of phosphorus pentasulfide in boiling pyridine solution for 7 hours, a good yield of 1-benzylimidazo[4,5-d]-pyridazine-2,4(7)-dithiol-7(4)-ol was obtained. However, when the same proportions of reactants were allowed to reflux for 24 hours, the 1-benzylimidazo-[4,5-d]pyridazine-2,4,7-trithiol was obtained in 85% yield.

The infrared and ultraviolet spectra have been determined for all of the compounds reported and are consistent with the structures proposed.

Thirty-five of the compounds have been screened (8) either against experimental rodent tumors or in cell culture or both. The activity of a compound is considered to be statistically significant if the T/C is 0.53 or less in SA-180 or CA-755. In the LE-1210 system, the activity is considered to be statistically significant if the T/C is 1.25 or more. In the cell culture system (KB cells), the activity of the compound is considered to be statistically significant if the ED_{50} is 4×10^0 μ g/ml. or less. The slope is simply the difference in response for a one-log difference in dose, the response being the ratio of the growth of the test sample to that of the untreated control. These data are recorded in Table IX (8).

EXPERIMENTAL (9)

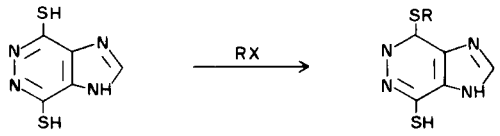
General Procedure for the Mercapto Substitution in Imidazo[4,5-d]-pyridazines.

Method A.

Equimolar amounts of the thiol compound and the appropriate halide dissolved in 1.25 *N* potassium hydroxide were heated with stirring on

TABLE I

4(7)Monosubstituted Thioimidazo[4, 5-d]pyridazine-7(4)-thiols

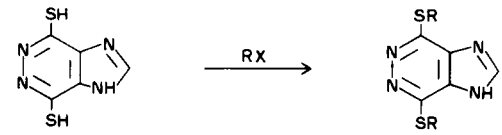


R	X	m.p. °(a)	Method of Prep.	Yield % (b)	Recrystallization Solvent	Formula	C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
n-Propyl	I	257-258	A		Absolute Ethanol	$C_8H_{10}N_4S_2$	42.46	42.84	4.45	4.14	24.76	24.92
Isopropyl	I	254-255.5	A		Glacial Acetic Acid	$C_8H_{10}N_4S_2$ (c)	42.46	42.62	4.45	4.84	24.76	24.95
n-Butyl	I	244-246	A	58	Benzene-Dioxane	$C_9H_{12}N_4S_2$ (d)	44.98	45.28	5.03	5.18	23.31	23.40
Allyl	Br	244.5-245	B	65	Absolute Ethanol	$C_8H_8N_4S_2$	42.85	42.86	3.60	3.19	24.98	25.15
3-Methylbutyl	I	239-241	A	44	Benzene-Dioxane	$C_{10}H_{14}N_4S_2$	47.21	47.18	5.55	5.66	22.03	22.30
β-Hydroxyethyl	Br	360	B	55	Aqueous Ethanol	$C_7H_8N_4OS_2$	36.83	36.82	3.53	3.36		
Carboxymethyl	Cl	248 dec.	C	66	Water	$C_7H_6N_4O_2S_2$	34.70	34.58	2.50	2.53		
β-Carboxyethyl	Cl	256 dec.	C	71	Water	$C_8H_8N_4O_2S_2$	37.49	36.92	3.15	3.11	21.86	22.01
Carboxamidomethyl	Cl	360	B	76	Pyridine	$C_7H_7N_5OS_2$	34.85	34.86	2.93	3.35		
Benzyl	Cl	283-285	A	82	Absolute Ethanol	$C_{12}H_{10}N_4S_2$	52.52	53.00	3.67	4.21		
o-Chlorobenzyl	Cl	244.5-246	A	58	Ethanol-Benzene	$C_{12}H_9ClN_4S_2$	46.68	46.43	2.94	3.56	18.14	17.55
2,4-Dichlorobenzyl	I	269-271	A	75	Dioxane	$C_{12}H_8Cl_2N_4S_2$	41.98	41.82	2.35	2.50		
3,4-Dichlorobenzyl	I	301-303	A	81	Dioxane	$C_{12}H_8Cl_2N_4S_2$	41.98	41.70	2.35	2.55		
o-Fluorobenzyl	Br	239-241	B	36	95% Ethanol	$C_{12}H_9FN_4S_2$	49.30	49.32	3.10	3.30	19.16	18.93
m-Fluorobenzyl	Br	270-272	B	43	95% Ethanol	$C_{12}H_9FN_4S_2$	49.30	49.32	3.10	3.30	19.16	18.94
p-Fluorobenzyl	Br	276-277	B	51	95% Ethanol	$C_{12}H_9FN_4S_2$	49.30	49.41	3.10	3.46	19.16	18.95
p-Nitrobenzyl	Cl	298-299	B	44	Aqueous Pyridine	$C_{12}H_9N_5O_2S_2$	45.11	45.06	2.84	2.85		
p-Iodobenzyl	I	288-289	B	18	Aqueous Pyridine	$C_{12}H_9IN_4S_2$	36.00	36.39	2.27	2.27		
p-Chlorophenacyl	Br	263-264	B	22	Aqueous Pyridine	$C_{13}H_9ClN_4OS_2$	46.44	46.54	2.57	2.40		
p-Bromophenacyl	Br	261-262	B	20	Aqueous Pyridine	$C_{13}H_9BrN_4OS_2$	40.96	41.15	2.38	2.44		

(a) M.p. given for analytical sample. (b) Percent yield is reported on the crude product. (c) S Calcd. 28.59; found 28.69. (d) S Calcd. 26.69; found 26.72.

TABLE II

4,7-Bis-substituted Thioimidazo[4, 5-d]pyridazines

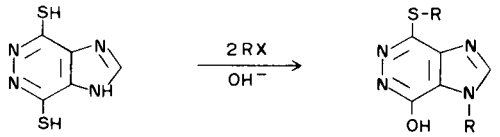


R	X	m.p. °(e)	Method of Prep.	Yield % (f)	Recrystallization Solvent	Formula	C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
n-Propyl	I	126-128	A (a)		Benzene-Cyclohexane	$C_{11}H_{16}N_4S_2$	49.21	48.70	6.01	5.79		
Isopropyl	I	127-129	A (a)	28	Benzene	$C_{11}H_{16}N_4S_2$	49.21	49.71	6.01	6.30		
Benzyl	Cl	125	A (a)		Aqueous Acetic Acid	$C_{19}H_{16}N_4S_2$ (b)	62.61	62.16	4.42	4.54	15.37	15.64
o-Nitrobenzyl	Br	97-98	B	22	Methanol	$C_{19}H_{14}N_6O_4S_2$	50.20	50.34	3.11	3.22		
3,4-Dichlorobenzyl	I	186-187.5	D	60	Aqueous Acetic Acid	$C_{19}H_{12}Cl_4N_4S_2$	45.42	45.46	2.41	2.72	11.15	10.91
p-Chlorobenzyl	I	147-149	D (c)	72	Glacial Acetic Acid	$C_{19}H_{14}Cl_2N_4S_2$	52.65	52.34	3.26	3.16		
p-Iodobenzyl	Br	315-317	B	41	Aqueous Pyridine	$C_{19}H_{14}I_2N_4S_2$	37.02	36.46	2.29	2.35	9.09	8.90
2,4-Dinitrophenyl	Cl	323-324	B	77	Glacial Acetic Acid	$C_{17}H_8N_8O_8S_2$	39.54	39.24	1.56	1.66		
p-Bromophenacyl	Br	216-217	B	88	Aqueous Pyridine	$C_{21}H_{14}Br_2N_4O_2S_2$	43.62	43.36	2.44	2.37		
p-Iodophenacyl	Br	212-214	B	43	Dioxane	$C_{21}H_{14}I_2N_4O_2S_2$ (d)	37.52	38.24	2.10	2.29	8.33	8.09

(a) 2 Molar equivalents of halide. (b) S Calcd. 17.60; found 17.80. (c) 1:1 Molar equivalents. (d) S Calcd. 9.54; found 9.66. (e) M.p. given for the analytical sample. (f) Percent yield is reported on the crude product.

TABLE III

4(7)Substituted Thio-7(4)hydroxy-1-substituted Imidazo[4,5-d]pyridazines

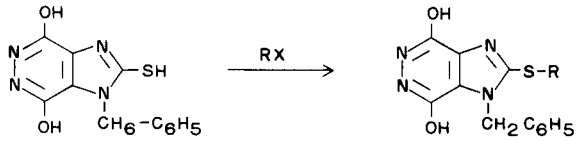


R	X	m.p. °(d)	Method of Prep.	Yield % (e)	Recrystallization Solvent	Formula	C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
o-Chlorobenzyl	I	268-270	D	62	Glacial Acetic Acid	$C_{19}H_{14}Cl_2N_4OS$ (a)	54.69	54.81	3.38	3.20	13.43	13.41
p-Chlorobenzyl	I	269-271	D	67	Benzene-Ethanol	$C_{19}H_{14}Cl_2N_4OS$ (b)	54.69	54.50	3.38	3.36	13.43	12.96
2,4-Dichlorobenzyl	I	275-277	D	31	Glacial Acetic Acid	$C_{19}H_{12}Cl_4N_4OS$ (c)	46.93	47.00	2.49	2.37		

(a) S Calcd. 7.69; found 8.21. (b) S Calcd. 7.69; found 8.42. (c) S Calcd. 6.60; found 6.80. (d) M.p. given for analytical sample. (e) Percent yield is reported on the crude product.

TABLE IV

1-Benzyl-2-substituted Thioimidazo[4,5-d]pyridazine-4,7-diols

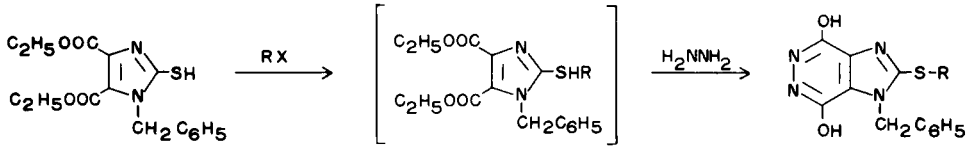


R	X	m.p. °(a)	Method of Prep.	Yield % (b)	Recrystallization Solvent	Formula	C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
2,4-Dichlorobenzyl	I	269	A	quant.	Acetic acid	$C_{19}H_{14}Cl_2N_4O_2S$	52.66	52.47	3.26	3.64	12.93	13.09
3,4-Dichlorobenzyl	I	299	A	quant.	Acetic acid	$C_{19}H_{14}Cl_2N_4O_2S$	52.66	52.66	3.26	2.93	12.93	12.52
2,6-Dichlorobenzyl	I	282-283	A	quant.	95% Ethanol	$C_{19}H_{14}Cl_2N_4O_2S$	52.66	52.27	3.26	3.03	12.93	12.85
p-Fluorobenzyl	Br	258	A	84	Acetic acid-water	$C_{19}H_{15}FN_4O_2S$	59.67	59.77	3.95	4.15	14.65	14.39
o-Fluorobenzyl	Br	236-238	A	86	Acetic acid	$C_{19}H_{15}FN_4O_2S$	59.67	59.71	3.95	4.20	14.65	14.19
m-Fluorobenzyl	Br	240-242	A	quant.	Acetic acid-water	$C_{19}H_{15}FN_4O_2S$	59.67	59.76	3.95	4.33	14.65	14.18
p-Bromobenzyl	Br	292-293	A	quant.	Acetic acid	$C_{19}H_{15}BrN_4O_2S$	51.47	51.51	3.41	3.27	12.64	12.28
o-Bromobenzyl	I	260-261	A	77	Acetic acid	$C_{19}H_{15}BrN_4O_2S$	51.47	51.19	3.41	3.79	12.64	12.99
o-Chlorobenzyl	Cl	263-264	A	93	Acetic acid	$C_{19}H_{15}ClN_4O_2S$	57.21	57.69	3.79	3.92	14.05	13.54
o-Iodobenzyl	I	262-263	A	96	Acetic acid	$C_{19}H_{15}IN_4O_2S$	46.54	46.87	3.08	3.27	11.43	11.06
Benzyl	Br	261	A	88	Acetic acid-water	$C_{19}H_{16}N_4O_2S$	62.62	62.86	4.43	4.73	15.37	14.95
p-Phenylethyl	Br	250	A	72	Acetic acid	$C_{20}H_{18}N_4O_2S$	63.47	63.96	4.79	5.16	14.81	14.58
Isopropyl	I	260-262	A	93	Acetic acid-water	$C_{15}H_{16}N_4O_2S$	56.94	57.00	5.10	5.14	17.71	17.70
Isobutyl	I	254-256	A	53	Acetic acid-water	$C_{16}H_{18}N_4O_2S$	58.16	58.09	5.49	5.41	16.96	17.10
n-Amyl	I	254-256	A		Acetic acid-water	$C_{17}H_{20}N_4O_2S$	59.28	59.56	5.85	6.11	16.27	15.86
3-Methylbutyl	I	240-241	A		Acetic acid	$C_{17}H_{20}N_4O_2S$	59.28	59.26	5.85	5.86	16.27	16.09
Cyclohexyl	I	262-262.5	A		Acetic acid-water	$C_{18}H_{20}N_4O_2S$	60.65	60.69	5.66	5.40	15.72	15.52
$HOOC-CH_2-$	Cl	277 dec.	C	quant.	Water-ethanol	$C_{14}H_{12}N_4O_4S$	50.59	50.76	3.64	3.84	16.86	16.53
$HOOC-CH_2-CH_2-$	Cl	210 dec.	C	50	Acetic acid	$C_{15}H_{14}N_4O_4S$	52.01	52.26	4.07	4.25	16.18	15.96
$H_2N-\overset{O}{\overset{\|}{C}}-CH_2-$	Cl	261-263	B		Acetic acid-ethanol	$C_{14}H_{13}N_5O_3S$	50.74	51.10	3.95	4.04	21.14	21.03

(a) M.p. given for analytical sample. (b) Percent yield is reported on the crude product.

TABLE V

1-Benzyl-2-substituted Thioimidazo[4,5-d]pyridazine-4,7-diols

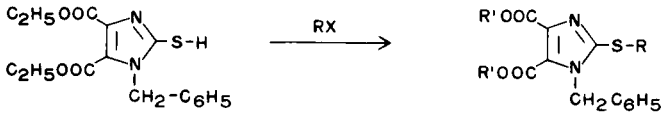


R	X	m.p. °(f)	Yield %	Recrystallization Solvent	Formula	Calcd.	Found	Calcd.	Found	Calcd.	Found
Methyl	I	312-315	90	Ethanol-water	$C_{13}H_{12}N_4O_2S$ (a)	54.15	54.33	4.20	4.07	19.43	19.13
Ethyl	I	258-260	60	Ethanol-water	$C_{14}H_{16}N_4O_3S$ (b)	52.48	52.15	5.04	5.34	17.49	17.21
n-Propyl	I	255-257	68	Ethanol-water	$C_{15}H_{16}N_4O_2S$ (c)	56.94	57.38	5.10	5.34	17.71	17.98
n-Butyl	I	250	24	Ethanol	$C_{16}H_{18}N_4O_2S$	58.16	58.50	5.49	5.56	16.96	17.17
sec-Butyl	Br	283	73	Ethanol-water	$C_{16}H_{18}N_4O_2S$	58.16	58.48	5.49	5.71	16.96	16.54
t-Amyl	I	256-258	79	Ethanol	$C_{17}H_{20}N_4O_2S$	59.28	59.04	5.85	5.53	16.27	16.74
Cyclopentyl	Br	262-265	76	Ethanol-water	$C_{17}H_{18}N_4O_2S$ (d)	59.63	59.77	5.30	5.47	16.36	16.17
p-Chlorobenzyl	Cl	265-268	96	Ethanol-water	$C_{19}H_{17}ClN_4O_3S$ (e)	54.74	55.00	4.11	3.98	13.44	13.85

(a) S Calcd., 11.12; found, 11.43. (b) Monohydrate; S Calcd., 10.01; found 9.93. (c) S Calcd., 10.13; found, 9.78. (d) S Calcd., 9.37; found 9.27. (e) Monohydrate; S Calcd., 7.69; found 8.01. (f) M.p. is given for the analytical sample.

TABLE VI

Thioimidazole-4,5-dicarboxylates

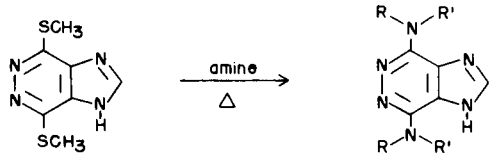


R'	R'	R	X	m.p. °(a)	Recrystallization Solvent	Formula	C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
H	H	Benzyl	Cl	190-191	Acetic acid-water	$C_{19}H_{16}N_2O_4S$	61.94	61.83	4.38	4.66	7.61	7.70
H	H	*sec*-Butyl	Br	155-157	Acetic acid-water	$C_{16}H_{18}N_2O_4S$	57.46	57.30	5.43	5.38	8.38	8.30
C_2H_5	H	H	Br or I	191-192	Ethanol-benzene	$C_{14}H_{14}N_2O_4S$ (b)	54.89	54.83	4.61	4.97	9.15	9.24

(a) M.p. is given for the analytical sample. (b) S Calcd. 10.43; found 10.57.

TABLE VII

Aminoimidazo[4,5-d]pyridazines

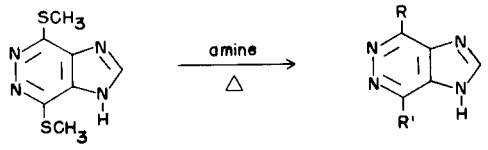


R	R'	m.p. °(a)	Recrystallization Solvent	Formula	C Calcd.	C Found	H Calcd.	H Found
H	Methyl	314-315	Ethanol-benzene	$C_7H_{10}N_6 - {}^1/_4\,H_2O$ (b)	46.03	46.39	5.79	5.69
H	Ethyl	247-249	Ethanol-benzene	$C_9H_{14}N_6 - {}^1/_2\,H_2O$	50.21	50.27	7.03	6.97
H	*n*-Butyl	110	Dioxane-ligroin	$C_{13}H_{22}N_6$	59.51	59.74	8.45	8.52
H	β-Hydroxyethyl	251-253	Water	$C_9H_{14}N_6O_2$	45.36	45.70	5.92	6.50
H	Dimethylaminopropyl	174-176	Benzene	$C_{15}H_{28}N_8$	56.23	56.35	8.81	8.93
H	Diethylaminopropyl	140-142	Benzene-cyclohexane	$C_{19}H_{36}N_8$	60.60	60.34	9.64	9.23
Methyl	Methyl	209-211	Benzene-ethanol	$C_8H_{14}N_6$	52.41	52.23	6.84	6.58

(a) M.p. is given for the pure sample. (b) N Calcd. 46.02; found 45.86; reaction mixture heated for 24 hours at 170-175°.

TABLE VIII

Substituted Imidazo[4,5-d]pyridazines

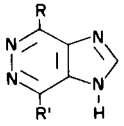


R	R'	m.p. °(a)	Recrystallization Solvent	Formula	Calcd.	Found	Calcd.	Found
Diisopropylamino	Methylthio	305-307	Benzene-ethanol	$C_{12}H_{19}N_5S$ (b)	54.30	54.12	7.21	7.40
Di-(3-methylbutyl)amino	Methylthio	319-321	Benzene-ethanol	$C_{16}H_{27}N_5S$	59.75	60.04	8.47	8.42
Hydroxy	Methylthio	322-323	Ethanol	$C_6H_6N_4OS$ (c)	39.55	39.86	3.32	3.62

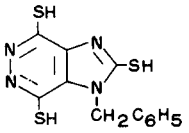
(a) M.p. is given for the pure sample. (b) Heated 13 hours at 195°. (c) Obtained when the amine used was diethylamine, di-*n*-propylamine, or di-*n*-butylamine.

TABLE IX (8)

Antitumor and Cell Culture Activity



R	R'	CCNSC Number	Antitumor activity (T/C) SA 180	CA 755	LE 1210	Cell Culture Slope	ED 50 in μ g./ml.
n-Propylthio	Thiol	40 8410				-0.54	1.4×10^1
Isopropylthio	Thiol	62 413	1.39	0.63	0.94		$>1.0 \times 10^2$
Allylthio	Thiol	64 809	0.74	1.00	0.98	-0.75	2.3×10^1
n-Butylthio	Thiol	62 407	0.81	0.78	1.02	-0.93	2.7×10^1
3-Methylbutylthio	Thiol	62 408	0.88	1.18	0.90	-0.95	2.6×10^1
β-Hydroxyethylthio	Thiol	64 813	1.01	0.80	1.00		$>1.0 \times 10^2$
Carboxymethylthio	Thiol	64 812	0.94	0.85	1.00		$>1.0 \times 10^2$
Carboxyethylthio	Thiol	64 811	0.92	0.97	1.00		$>1.0 \times 10^2$
Carboxamidomethylthio	Thiol	40 9364				-1.38	4.4×10^1
Benzylthio	Thiol	62 405	1.08	0.90	1.09	-0.33	2.6×10^1
o-Chlorobenzylthio	Thiol	62 406	1.34	0.71	1.05	-0.83	3.3×10^1
2,4-Dichlorobenzylthio	Thiol	64 806			0.89	-0.55	1.0×10^1
3,4-Dichlorobenzylthio	Thiol	64 805	0.76	1.12	1.05	-0.59	1.2×10^1
o-Fluorobenzylthio	Thiol	40 9357				-1.09	3.6×10^1
m-Fluorobenzylthio	Thiol	64 789			0.94		$>1.0 \times 10^2$
p-Fluorobenzylthio	Thiol	64 788			0.91		$>1.0 \times 10^2$
p-Iodobenzylthio	Thiol	64 790			0.89		$>1.0 \times 10^2$
p-Nitrobenzylthio	Thiol	64 810	1.04	1.06	1.02		$>1.0 \times 10^2$
p-Chlorophenacylthio	Thiol	64 793			0.97	-0.55	1.3×10^1
p-Bromophenacylthio	Thiol	64 792			0.89		$>1.0 \times 10^2$
n-Propylthio	*n*-Propylthio	64 804	1.00	1.04	1.02	-1.02	2.3×10^1
Benzylthio	Benzylthio	40 8409				-1.02	2.6×10^1
p-Chlorobenzylthio	*p*-Chlorobenzylthio	64 807	0.76	0.97	1.00	-1.02	2.3×10^1
3,4-Dichlorobenzylthio	3,4-Dichlorobenzylthio	64 808			1.00		$>1.0 \times 10^2$
2,4-Dinitrobenzylthio	2,4-Dinitrobenzylthio	40 9359				-0.99	2.6×10^1
p-Bromophenacylthio	*p*-Bromophenacylthio	64 791			0.86		$>1.0 \times 10^2$
Hydrazino	Hydrazino	64 803	0.60	1.03	1.22	-0.89	3.1×10^1
n-Butylamino	*n*-Butylamino	40 9363				-0.55	1.4×10^{-1}
						-0.48	1.3×10^{-1}
							$<1.0 \times 10^{-1}$
						-0.79	2.8×10^{-2}
Ethanolamino	Ethanolamino	64 801			0.96		$>1.0 \times 10^2$
Dimethylaminopropylamino	Dimethylaminopropylamino	64 802	0.97	1.16	1.01		$>1.0 \times 10^2$
			very toxic				
Diethylaminopropylamino	Diethylaminopropylamino	69 059				-0.37	1.4×10^2
Diisopropylamino	Methylthio	64 799			0.90	-1.02	2.3×10^1
Diisoamylamino	Methylthio	40 9361					$>1.0 \times 10^1$
Hydroxy	Methylthio	64 800	0.72	0.85	0.92	-0.98	2.2×10^1



		66 080	0.81	1.01	1.02		$>1.0 \times 10^2$
							$>1.0 \times 10^1$

the steam bath for 0.5-5 hours. Sometimes ethanol was added to increase the solubility of the halide. Upon cooling, the 1-benzyl-2-substituted thioimidazo[4,5-d]pyridazine-4,7-diols were precipitated whereas the substituted thioimidazo[4,5-d]pyridazines were precipitated only upon acidification to *p*H 6 with acetic acid.

The solid was collected, washed with ethanol-water or water, dried and purified.

Method B.

To the rapidly stirred solution of the thiol compound (0.05 mole) dissolved in 150 ml. of 28% ammonium hydroxide was added, over a 15 minute period, an equimolar amount of the appropriate halide dissolved in 25 ml. of hot dioxane. The mixture was warmed to 35-40° and then allowed to return to room temperature with continuous stirring for 2.5-4.5 hours. The 1-benzyl-2-substituted thioimidazo[4,5-d]-pyridazine-4,7-diol precipitated immediately; however, the substituted-thioimidazo[4,5-d]pyridazines were obtained on acidification with acetic acid to *p*H 6. The solid was air dried and purified.

Method C.

Similar to method A, equimolar amounts of the chloroalkanoic acid and the thiol dissolved in potassium hydroxide, were refluxed for 2-4.5 hours after which the solution was acidified with concentrated hydrochloric acid to *p*H 1-2. The 1-benzyl-2-substituted thioimidazo[4,5-d]-pyridazine-4,7-diols were heated on the steam bath for 2 hours in the acidic solution, cooled, collected and dried. The substituted thioimidazo[4,5-d]pyridazines were collected, dissolved in a saturated sodium bicarbonate solution for 2 hours, filtered and reprecipitated by acidification with concentrated hydrochloric acid. The solid was collected and purified.

Method D.

Two molar equivalents of the halide dissolved in 60 ml. of 95% ethanol was added to a stirred solution of 0.05 mole of the dithiol in 60 ml. of 2.5 *N* potassium hydroxide. The isolation procedure was the same as method A.

1-Benzyl-2-substituted Thioimidazo[4,5-d]pyridazine-4,7-diols from Diethyl 1-Benzyl-2-mercaptoimidazole-4,5-dicarboxylate.

To a solution containing 10 g. (0.03 mole) of diethyl 1-benzyl-2-mercaptoimidazole-4,5-dicarboxylate in 50 ml. of potassium hydroxide (1.25 *N*) was added 0.03 mole of the appropriate halide. The mixture was stirred at room temperature for 1 hour and then heated on the steam bath for an additional 0.5-1 hour. After cooling, the solution was extracted three times with ether. The ethereal layer was dried over anhydrous magnesium sulfate and reduced in volume, leaving the diethyl 1-benzyl-2-substituted thioimidazole-4,5-dicarboxylate as a crude oil. The crude product was dissolved in methanol and allowed to react with anhydrous hydrazine. After refluxing for 2 hours, the solution was cooled, filtered and the filtrate acidified with concentrated hydrochloric acid. The resulting mixture was warmed on the steam bath for 1-2 hours; the solid was collected, washed, and air dried giving the 1-benzyl-2-substituted thioimidazo[4,5-d]-pyridazine-4,7-diol.

In some instances, the water layer from the above extraction was acidified to yield two additional compounds. At *p*H 6-7, the 1-benzyl-2-substituted thioimidazole-4,5-dicarboxylic acid precipitated (Table VI). A yellow-green oil appeared at approximately *p*H 3 and was recovered by extracting the acidic solution with ether. The ethereal layer was dried and the ether removed. The half-ester, half-acid of the starting material (Table VI) was recovered and recrystallized from ethanol-benzene.

General Procedure for the Reaction of an Amine with Bismethylthioimidazo[4,5-d]pyridazine (Table VII, Table VIII).

A mixture of 4,7-bismethylthioimidazo[4,5-d]pyridazine and the appropriate amine was dissolved in 200 ml. of absolute ethanol and heated in a stainless steel rocking autoclave at 195° for 8-9 hours. The solution was filtered and the filtrate reduced to dryness under reduced pressure. The resulting solid was recrystallized as indicated in the corresponding table.

4,7-Bishydrazinoimidazo[4,5-d]pyridazine.

To 4.24 g. (0.02 mole) of bismethylthioimidazo[4,5-d]pyridazine was added 29 ml. (29.32 g.) of hydrazine. After the solution was refluxed for 1.5 hours, the excess hydrazine was removed under reduced pressure and the resulting solid washed repeatedly with ethanol, yield, 2.65 g. (74%), m.p. > 360°. A sulfur test was negative.

Anal. Calcd. for $C_5H_8N_8$: C, 33.34; H, 4.48; N, 62.20. Found: C, 33.03; H, 4.40; N, 61.32.

1-Benzylimidazo[4,5-d]pyridazine-4,7-diol-2-thiol.

Diethyl 1-benzyl-2-mercaptoimidazole-4,5-dicarboxylate (15 g., 0.045 mole) was dissolved in 36 ml. of hot methanol. After the addition of 4.5 g. (0.14 mole) of hydrazine, the solution was refluxed with stirring for 2 hours. The resulting precipitate was collected and suspended in water. The solution was made acidic to congo-red paper with concentrated hydrochloric acid and warmed for 20 minutes on the steam bath, yielding 6 g. (50%), m.p. 333-334° dec.

The analytical sample was prepared by recrystallization from acetic acid (norite), m.p. 336° dec.

U. V. λ max (95% C_2H_5OH): 206 (ε, 22,300); 234 (sh) (ε, 15,000); 270 mμ (ε, 23,000).

Infrared cm^{-1}: 3000 (s), 2900 (s), 1675 (s), 1600 (s), 1475 (s), 1425 (s), 1330 (m), 1275 (m), 1220 (s), 1150 (w), 1135 (w), 1120 (w), 1075 (w), 1045 (w), 1030 (w), 970 (w), 960 (w), 830 (m), 790 (w), 740 (w), 705 (s), 675 (w), 665 (w), 600 (w), 575 (w), 555 (w), 530 (m), 460 (w).

Anal. Calcd. for $C_{12}H_{10}N_4O_2S$: C, 52.54; H, 3.68; N, 20.43. Found: C, 52.41; H, 3.66; N, 20.62.

1-Benzylimidazo[4,5-d]pyridazine-2,4(7)-dithiol-7(4)-ol.

1-Benzylimidazo[4,5-d]pyridazine-4,7-diol-2-thiol (8 g., 0.029 mole) was dissolved in 255 ml. of warm dry pyridine. After cooling, 40 g. of phosphorus pentasulfide was added and the mixture refluxed for 7 hours. The excess pyridine was removed under reduced pressure and water added. The solution was warmed on the steam bath for 3 hours. After cooling, the solid was collected, dissolved in 20% sodium hydroxide (norite), filtered and acidified with concentrated hydrochloric acid, yielding 7.5 g. (89%), m.p. 271-272° dec.

An analytical sample was prepared by taking a portion of the above solid and suspending it in ethyl acetate. The remaining residue was dissolved in sodium hydroxide and acidified with concentrated hydrochloric acid, m.p. 315° dec.

U. V. λ max (0.5 *N* NaOH): 221 (ε, 22,100); 268 mμ (ε, 24,000).

Infrared cm^{-1}: 3075 (s), 3025 (s), 2950 (s), 1675 (m), 1580 (m), 1530 (s), 1500 (s), 1435 (s), 1270 (s), 1205 (s), 1150 (m), 1095 (s), 1075 (m), 1025 (w), 990 (w), 925 (w), 760 (w), 730 (m), 700 (s), 595 (w), 550 (w), 455 (m).

Anal. Calcd. for $C_{12}H_{10}N_4OS_2$: C, 49.63; H, 3.47; N, 19.30. Found: C, 49.93; H, 3.76; N, 19.02.

1-Benzylimidazo[4,5-d]pyridazine-2,4,7-trithiol.

A mixture of 10 g. (0.0365 mole) of 1-benzylimidazo[4,5-d]pyridazine-4,7-diol-2-thiol, 285 ml. of dry pyridine and 50 g. of phosphorus pentasulfide was refluxed for 24 hours. After removal of the excess pyridine, water was added slowly and the resulting mixture heated on the steam bath for 6 hours. The mixture was cooled and the solid collected, washed with ethanol and dried; yield 9.5 g. (85%), m.p. 235-240°. A portion of the solid was recrystallized from ethyl acetate and then dissolved in base, filtered and acidified with concentrated hydrochloric acid to give the analytically pure sample, m.p. 253-255° dec.

U. V. λ max (0.5 *N* NaOH): 230 (ε, 15,000); 282 (ε, 30,900); 310 mμ (sh) (ε, 17,800).

Infrared cm^{-1}: 3050 (s), 2975 (s), 1675 (m), 1575 (m), 1500 (s), 1460 (s), 1425 (s), 1390 (s), 1200 (s), 1115 (w), 1075 (w), 1035 (m), 1000 (w), 985 (w), 930 (m), 905 (w), 890 (w), 725 (m), 695 (s), 660 (m), 590 (w), 560 (w), 510 (w), 440 (m).

Anal. Calcd. for $C_{12}H_{10}N_4S_3$: C, 47.03; H, 3.29; N, 18.28. Found: C, 47.35; H, 3.72; N, 18.35.

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(1) In partial fulfillment of the requirements for the Ph.D. degree in Chemistry at the University of New Mexico.

(2) Present address: E. I. du Pont de Nemours and Company, Kinston, North Carolina.

(3) Communications concerning this paper should be directed to Professor Raymond N. Castle.

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(8) The screening data were kindly supplied by Dr. J. E. Leiter, CCNSC, National Institutes of Health, Bethesda, Maryland.

(9) All melting points were determined in a Thomas Hoover capillary melting point apparatus or in a copper block and are uncorrected. The elemental analyses were determined by the following institutions: The University of New Mexico; New Mexico Highlands University; Tanabe Seiyaku Co., Ltd. Tokyo, Japan; and Weiler and Strauss, Oxford, England.

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