Journal of the Pharmaceutical Society of Korea
16, 162-175(1972)

Synthesis and Evaluation of Antimicrobial-antitumor Activities of Methylthiosemicarbazones and Thiocarbohydrazones

Shanghi Rhee*

(Received December 25, 1972)

Abstract—Fifty six compounds of 4-methylthiosemicarbazone and thiocarbohydrazone derivatives were prepared and subjected to biological tests. The following five compounds, 2-hydroxybenzaldehyde monothiocarbohydrazone (2), 4-methylbenzaldehyde monothiocarbohydrazone (8), 1-(2-hydroxybenzaldehyde)-5-(4-hydroxy-3-methoxybenzaldehyde) dithiocarbohydrazone (45), 1-(2-hydroxybenzaldehyde)-5-furfural dithiocarbohydrazone (46) and 1-benzaldehyde-5-cinnamaldehyde dithiocarbohydrazone (49) exhibited marked antimicrobial activity against *E. coli*, *St. aureus* and *P. chrysogenum*. In addition to these compounds, 3-methoxybenzaldehyde monothiocarbohydrazone (12) and 4-methylbenzaldehyde dithiocarbohydrazone (29) showed marked inhibition of HeLa cell growth at the concentration of 10μg/ml. It was generally observed that most compounds demonstrated significant antifungal activity against *P. chrysogenum* but only one compound, 3-hydroxy-4-methoxybenzaldehyde dithiocarbohydrazone (39), exerted antituberculosis activity against *M. tuberculosis* $H_{37}RV$ at the concentration of 10μg/ml.

Quite a number of research reports[1-12] in medicinal chemistry have shown that antimicrobial and antitumor activities are considerably related with chelating activity.

It is also well established that the transition and heavy metals are in vivo firmly bound to macromolecular structures and are essential components of many biologically active macromolecules.[13-15]

* Dept. of Pharmacodynamics, College of Pharmacy, Seoul National University, Seoul, Korea.

As a consequence of these consideration the ligand must be so structured that it can adequately contact the bound metal ion in its intracellular environment.

In addition, the conjugate N-N-S tridentate ligand system was reported to be a common feature of those compounds with carcinostatic potencies.[16]

The biological mechanism of these compounds is reported to be associated with its chelating activity. In connection with these reports, the N-N-S tridentate ligand which is involved in the compounds prepared by the author is one of the essential abstraction of these kinds.

Actually, the weak antileukemic activity of the compound, 2-formylpyridine thiosemicarbazone,[17] stood as a unique observation until the current study was lauched in 1963.

Since Brockman, *et al.*[17] first have observed the antileukemic effect of 2-formylpyridine thiosemicarbazone, various thiosemicarbazones for aromatic and heterocyclic aldehyde have been examined for antitumor activity[18] and the antimicrobial activities of many thiosemicarbazone derivatives have long been recognized.[19-23]

It has been suggested that the antimicrobial activity of thiosemicarbazones is due to their ability to form complexes with copper ions.[24]

By the report of Krishina,[25] the fact was presented that 1-formylisoquinoline thiosemicarbazone, a broad spectrum antitumor agent, caused marked inhibition of the synthesis of DNA by preventing the conversion of ribonucleotides to deoxyribonucleotide form.[26-28]

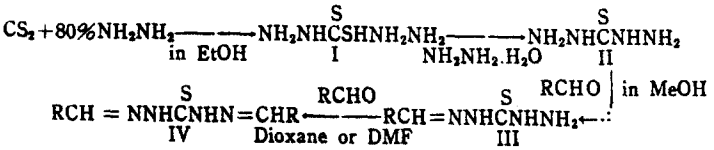
A similar mechanism of action of thiosemicarbazone derivatives of the pyridine ring system appears to be operative with relatively high therapeutic indices as antineoplastic agent.[29-33]

In view of such pharmacological properties of thiosemicarbazone derivatives, a number of modification have been made in the thiosemicarbazone side chain to ascertain the importance of this part of the molecule for antimicrobial activity.

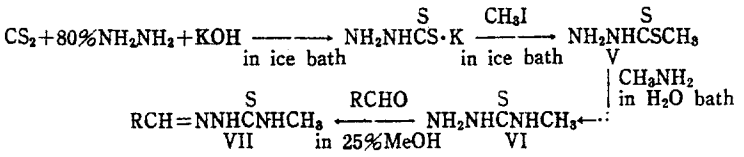
Author's attention was turned to the modification of 4-methyl-thiosemicarbazide and thiocarbohydrazide because of their pharmacological properties.

It is considerably noted that Schiff bases containing >C=N group are known to have slight antitumor activities[34] and the azomethine linkage can be regarded as an isostere of cytotoxic agent,[35] the azo compounds.

These studies mentioned so far prompted the author to synthesize the derivatives of 4-methylthiosemicarbazone and thiocarbohydrazone as shown in Scheme I and Scheme II.



Scheme I—Synthesis of Thiocarbohydrazones.



Scheme II—Synthesis of 4-Methylthiosemicarbazones.

The compounds were subjected to biological tests for antimicrobial activity against *E. coli*, *St. aureus*, *P. chrysogenum* and *M. tuberculosis* as well as antitumor activity in vitro against HeLa cell.

Chemistry—The preparation of N^4-substituted thiosemicarbazides was described by Kazakov, *et al.*,[36] Nardi, *et al.*,[37] and McElhinney.[38] The reaction of thiocarbohydrazone with carbonyl-containing compounds was reported by several authors.[39-41]

Generally, the condensation of an aldehyde or ketone with thiocarbohydrazide proceeds readily, but there is a question whether a mono or di-substituted compound is obtained in case of one step reaction.

Stephen and Wilson[42] recognized that the second hydrazine group reacts with ketones much less readily than the first one; to obtain di-substituted compound they used excess ketone or aldehyde. In preparing dithiocarbohydrazones, however, the ratio of carbonyl-compound to thiocarbohydrazide used was never less than 4 : 1 and frequently the former was used as the reaction medium.[43]

With consideration of the above mentioned reports, the author prepared di-substituted compounds from mono-substituted compounds with so called two step reaction.

Table I—Thiocarbohydrazones ($R{=}NNHC(=S)NHN{=}R'$).

Compd No.	R	R′	Formula	Recrystn Solvent	Yield (%)	m.p.	Analyses(%)	
							Calcd	Found
1	CHC_6H_4-3-OH	H_2	$C_8H_{10}N_4OS$	MeOH	87.3	190–193	C 45.71	45.49
							H 4.76	4.50
							N 26.70	26.99
2	CHC_6H_4-2-OH	H_2	$C_8H_{10}N_4OS$	MeOH	83.6	189–190	C 45.71	45.56
							H 4.76	4.85
							N 26.70	26.81
3	CHC_6H_4-4-Cl	H_2	$C_8H_9ClN_4S$	MeOH+DMF	94.7	201–203	C 42.10	42.06
							H 3.95	4.09
							N 24.56	24.04
4	CHC_6H_4-3-Cl	H_2	$C_8H_9ClN_4S$	Dioxane	91.5	200	C 42.10	42.45
							H 3.95	3.71
							N 24.56	24.71
5	CHC_6H_3-2, 4-Cl_2	H_2	$C_8H_8Cl_2N_4S$	MeOH	87.7	220	C 36.50	36.55
							H 3.04	2.86
							N 21.30	20.82

Table I—Continued

Compd No.	R	R′	Formula	Recrystn Solvent	Yield (%)	m.p.	Analyses(%)	
							Calcd	Found
6	CHC_6H_4-2-NO_2	H_2	$C_8H_9N_5O_2S$	Dioxone	90.3	205	C 40.20	40.14
							H 3.77	3.53
							N 29.30	29.86
7	CHC_6H_4-4-NO_2	H_2	$C_8H_9N_5O_2S$	DMF	90.7	203–204	C 40.20	39.92
							H 3.77	3.65
							N 29.30	29.20
8	CHC_6H_4-4-Me	H_2	$C_9H_{12}N_4S$	Dioxone	91.4	171–174	C 51.92	52.17
							H 5.77	5.76
							N 26.92	27.40
9	CHC_6H_4-3-Me	H_2	$C_9H_{12}N_4S$	MeOH +Benzene	86.2	215–218	C 51.92	52.25
							H 5.77	5.68
							N 26.92	27.00
10	CHC_6H_4-2-OMe	H_2	$C_9H_{12}N_4OS$	Dioxone	92.8	181–182	C 48.21	47.94
							H 5.36	5.30
							N 25.00	24.95
11	CHC_6H_4-4-OMe	H_2	$C_8H_{12}N_4OS$	DMF+EtOH	91.9	202–204	C 48.21	48.21
							H 5.36	5.37
							N 25.00	25.12
12	CHC_6H_4-3-OMe	H_2	$C_8H_{12}N_4OS$	DMF+EtOH	90.7	219–221	C 48.21	48.64
							H 5.36	5.43
							N 25.00	25.03
13		H_2	$C_7H_9N_5S$	DMF+EtOH	93.8	211–212	C 43.10	43.58
							H 4.62	4.71
							N 35.90	35.61
14		H_2	$C_7H_9N_5S$	DMF+EtOH	90.1	204–206	C 43.10	42.85
							H 4.62	4.70
							N 35.90	35.76
15	CHC_6H_4-3,4-$(OMe)_2$	H_2	$C_{10}H_{14}O_2S$	DMF+EtOH	92.9	217–219	C 47.24	46.99
							H 5.51	5.58
							N 22.01	21.87
16	$CHC_{10}H_6$-2-OH	H_2	$C_{12}H_{12}N_4OS$	DMF	93.3	210 (DEC)	C 55.38	55.35
							H 4.62	4.96
							N 21.53	21.96
17		H_2	$C_{10}H_{11}N_5S$	DMF+H_2O	89.8	217–220	C 51.50	51.17
							H 4.72	4.45
							N 30.04	29.61
18	$CCH_3C_3H_5$	H_2	$C_9H_{12}N_4S$	DMF+H_2O	89.2	183–186	C 51.92	51.64
							H 5.77	5.88
							N 26.92	27.29

Table I—Continued

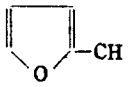
Compd No.	R	R′	Formula	Recrystn Solvent	Yield (%)	m.p.	Analyses(%)	
							Calcd	Found
19	$CMeC_6H_4$-4-NH_2	H_2	$C_9H_{13}N_5S$	DMF+H_2O	90.4	186–189	C 48.43	48.04
							H 5.83	5.84
							N 31.40	31.26
20	CHC_6H_4-4-NMe_2	H_2	$C_{10}H_{13}N_5S$	Dioxone	93.4	190	C 50.63	50.93
							H 6.33	6.32
							N 29.53	29.13
21	CHC_6H_3-4-OH-5-OMe	H_2	$C_9H_{12}N_4O_2S$	Dioxane	94.5	199–201	C 45.00	44.65
							H 5.00	5.10
							N 23.33	23.18
22	CHC_6H_3-4-OMe-5-OH	H_2	$C_9H_{12}N_4O_2S$	MeOH	88.2	187–188	C 45.00	44.73
							H 5.00	5.08
							N 23.33	23.65
23		H_2	$C_6H_8N_4OS$	Dioxane	90.4	192–195	C 39.13	39.28
							H 4.35	4.40
							N 30.43	30.55
24	$CHCH{=}CHC_6H_5$	H_2	$C_{10}H_{12}N_4S$	Dioxane	94.2	174–177	C 54.54	54.94
							H 5.45	5.50
							N 25.45	25.07
25	D-$CHCH_2OH(CHOH)_3$	H_2	$C_6H_{14}N_4O_4S$	DMF+H_2O	72.3	170–173 (DEC)	C 30.25	29.94
							H 5.88	6.09
							N 23.52	23.94
26	L-$CHCH_2OH(CHOH)_3$	H_2	$C_6H_{14}N_4O_4S$	DMF+H_2O	69.4	169–166	C 30.25	30.34
							H 5.88	5.86
							N 23.52	23.44
27	CHC_6H_3-2,4-Cl_2	CHC_6H_4-2,4-Cl_2	$C_{16}H_{10}Cl_4N_4S$	DMF+H_2O	32.8	210–213	C 42.85	43.16
							H 2.39	2.36
							N 13.33	13.63
28	CHC_6H_4-2-NO_2	CHC_6H_4-2-NO_2	$C_{15}H_{12}N_6O_4S$	Dioxane	30.9	182–185	C 48.40	47.99
							H 3.23	3.27
							N 22.60	22.47
29	CHC_6H_4-4-Me	CHC_6H_4-4-Me	$C_{17}H_{18}N_4S$	Dioxane	42.2	208–210	C 65.80	65.71
							H 5.81	5.79
							N 18.06	17.89
30	CHC_6H_4-3-Me	CHC_6H_4-3-Me	$C_{17}H_{18}N_4S$	Dioxane	48.1	190–193	C 65.80	66.01
							H 5.81	5.86
							N 18.06	18.03
31	CHC_6H_4-2-OMe	CHC_6H_4-2-OMe	$C_{17}H_{17}N_4O_2S$	Dioxane	43.8	206–209	C 59.64	59.70
							H 5.26	5.28
							N 16.37	16.37

Table I—Continued

Compd No.	R	R′	Formula	Recrystn Solvent	Yield (%)	m.p.	Analyses(%) Calcd	Found
32	CHC_6H_4-3-OMe	CHC_6H_4-3-OMe	$C_{17}H_{18}N_4O_2S$	DMF+EtOH	34.9	196–199	C 59.64	59.46
							H 5.24	5.25
							N 16.37	16.41
33			$C_{12}H_{12}N_6S$	DMF+EtOH	41.5	214–216	C 54.93	55.02
							H 4.23	4.29
							N 29.57	29.53
34			$C_{13}H_{12}N_6S$	DMF+EtOH	38.8	222–225	C 54.93	54.87
							H 4.23	4.25
							N 29.57	29.63
35			$C_{23}H_{18}N_4S$	Dioxane	49.6	213–215	C 72.25	71.98
							H 4.71	4.74
							N 14.66	14.65
36	$CHC_{10}H_6$-2-OH	$CHC_{10}H_6$-2-OH	$C_{23}H_{18}N_4O_2S$	DMF	40.7	294–296	C 66.67	66.53
							H 4.35	4.40
							N 13.52	13.54
37	CHC_6H_3-4-OH -5-OMe	CHC_6H_3-4-OH -5-OMe	$C_{17}H_{18}N_4O_4S$	Dioxane	43.9	220	C 54.55	54.39
							H 4.81	4.83
							N 14.97	14.92
38	CHC_6H_3-4-OH -6-OMe	CHC_6H_3-4-OH -6-OMe	$C_{17}H_{18}N_4O_4S$	Dioxane	43.2	204	C 54.55	54.32
							H 4.81	4.79
							N 14.97	15.02
39	CHC_6H_3-4-OMe -5-OH	CHC_6H_3-4-OMe -5-OH	$C_{17}H_{18}N_4O_4S$	Dioxane	46.7	215 (DEC)	C 54.55	54.53
							H 4.81	4.90
							N 14.97	14.93
40	CHC_6H_3-3-OH -4-OMe		$C_{18}H_{12}N_6O_3S$	DMF+H_2O	40.1	218–220	C 42.86	42.78
							H 3.29	3.26
							N 23.08	22.96
41	CHC_6H_3-4-OH -5-OMe		$C_{13}H_{12}N_6O_5S$	DMF+H_2O	30.8	245–247	C 42.86	42.85
							H 3.29	3.31
							N 23.08	23.14
42	CHC_6H_4-4-OH		$C_{12}H_{10}N_6O_4S$	MeOH	34.9	>300	C 43.11	42.97
							H 2.99	3.02
							N 25.15	25.18
43	CHC_6H_5		$C_{12}H_{10}N_6O_3S$	DMF+H_2O	30.1	206–209	C 45.28	45.31
							H 3.14	3.17
							N 26.42	26.36
44			$C_{10}H_8N_6O_4S$	DMF+MeOH +Aceton	31.3	214–217	C 38.96	38.95
							H 2.59	2.48
							N 27.27	27.09

Table I—Continued

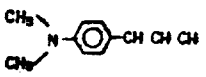
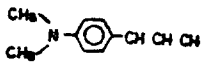
Compd No.	R	R′	Formula	Recrystn Solvent	Yield (%)	m.p.	Analyses(%) Calcd	Found
45	CHC_6H_4-2-OH	CHC_6H_3-4-OH -5-OMe	$C_{16}H_{16}N_4O_3S$	Dioxane	37.7	189–190	C 55.81	55.67
							H 4.65	4.81
							N 16.28	16.59
46	CHC_6H_4-2-OH		$C_{13}H_{12}N_4O_2S$	EtOH	30.3	189–190	C 54.17	53.85
							H 4.17	4.14
							N 19.44	19.01
47	CHC_6H_4-2-OH	$CH(CH)_2C_6H_5$	$C_{17}H_{18}N_4OS$	Dioxane	42.2	210–213	C 62.96	62.60
							H 4.93	4.97
							N 17.28	17.15
48	CHC_6H_4-4-Me	CHC_6H_4-2-Me	$C_{17}H_{18}N_4S$	MeOH	40.5	220–222	C 65.81	65.52
							H 5.80	5.64
							N 18.06	17.89
49	CHC_6H_5	$CH(CH)_2C_6H_5$	$C_{17}H_{16}N_4S$	Dioxane +H_2O	37.0	192–195	C 66.23	65.94
							H 5.19	5.21
							N 18.18	17.96
50	CHC_6H_3-4 -OH-5-OMe		$C_{20}H_{22}N_5O_2S$	DMF+H_2O	39.1	145(DEC)	C 60.45	60.35
							H 5.79	5.73
							N 17.63	17.68
51			$C_{17}H_{19}N_5OS$	EtOH	33.0	156–158	C 59.82	59.46
							H 5.57	5.62
							N 20.52	20.13

Table II—4-Methylthiosemicarbazones ($CH_3NHCSNHN{=}R$).

Compd No.	R	Formula	Recrystn Solvent	Yield (%)	m.p.	Analyses(%) Calcd	Found
52	CHC_6H_4-3-Me	$C_{10}H_{13}N_3S$	MeOH	79.2	134–136	C 57.97	57.94
						H 6.28	6.39
						N 20.29	20.37
53	CHC_6H_4-2-Me	$C_{10}H_{13}N_3S$	MeOH +Benzene	88.6	168–171	C 57.97	57.90
						H 6.28	6.33
						N 20.29	20.15
54	CHC_6H_4-2-OH	$C_9H_{11}N_3OS$	MeOH	86.0	206–208	C 51.67	51.77
						H 5.26	5.46
						N 20.10	20.41
55		$C_7H_9N_3OS$	EtOH	79.9	163–165	C 45.90	46.08
						H 4.92	5.12
						N 22.95	22.81
56	CHC_6H_4-4-OMe	$C_{10}H_{13}N_3OS$	EtOH	87.3	202–205	C 53.81	53.89
						H 5.83	5.83
						N 18.83	18.64

Table III—*In vitro* biological activity of thiocarbohydrazones and 4-methylthiosemicarbazones.

Compd[a] No.	*P. chrysogenum* (μg/disk)		*St. aureus* (μg/disk)		*E. coli* (μg/disk)		HeLa cell (μg/ml)	
	10	1	10	1	10	1	10	1
1	##[b]	+						
2	##	##	##	−	##	−		
3	##	+	##	−				
4			+	−				
8	##	##	##	−	+	−	75.8[c](58.7)[d]	122.8(4.5)
9	##	−						
10	##	+						
12							55.5(82.1)	110.9(18.2)
16			+	−				
17	##	##						
18	##	−						
19	##	−						
20	##	+					72.9(62.0)	112.0(17.0)
21	##	−						
22	##	−						
23	##	−						
24	##	##			##	−		
28							66.3(69.8)	118.2(9.8)
29							58.9(78.2)	92.7(39.2)
30	##	##	+	−				
35							68.4(67.3)	123.5(3.7)
37							65.4(70.7)	116.7(11.5)
38	##	##			##	−		
39	##	##						
43							71.5(63.7)	124.9(2.1)
44							78.6(55.5)	120.3(7.4)
45	##	##	##	−	##	−		
46	##	##	##	−	##	−		
47			##	−				
48	##	##						
49	##	##	+	−	##	−		
52	##	−			##	−		
53	##	−			+	−		
54			##	+	+	−		
55	##	+						

a. None of the compds except these compd No. showed any biological activity

b. Zone of inhibition ##: 8.0 mm, ##: 6.5—8.0mm, +: 6.5mm in dia, −: no inhibition

c. Protein conc. in μg. Cell control: 136.8 μg, Solvent control: 126.7μg, Initial protein content: 40.0μg.

d. Percent inhibition of control growth.

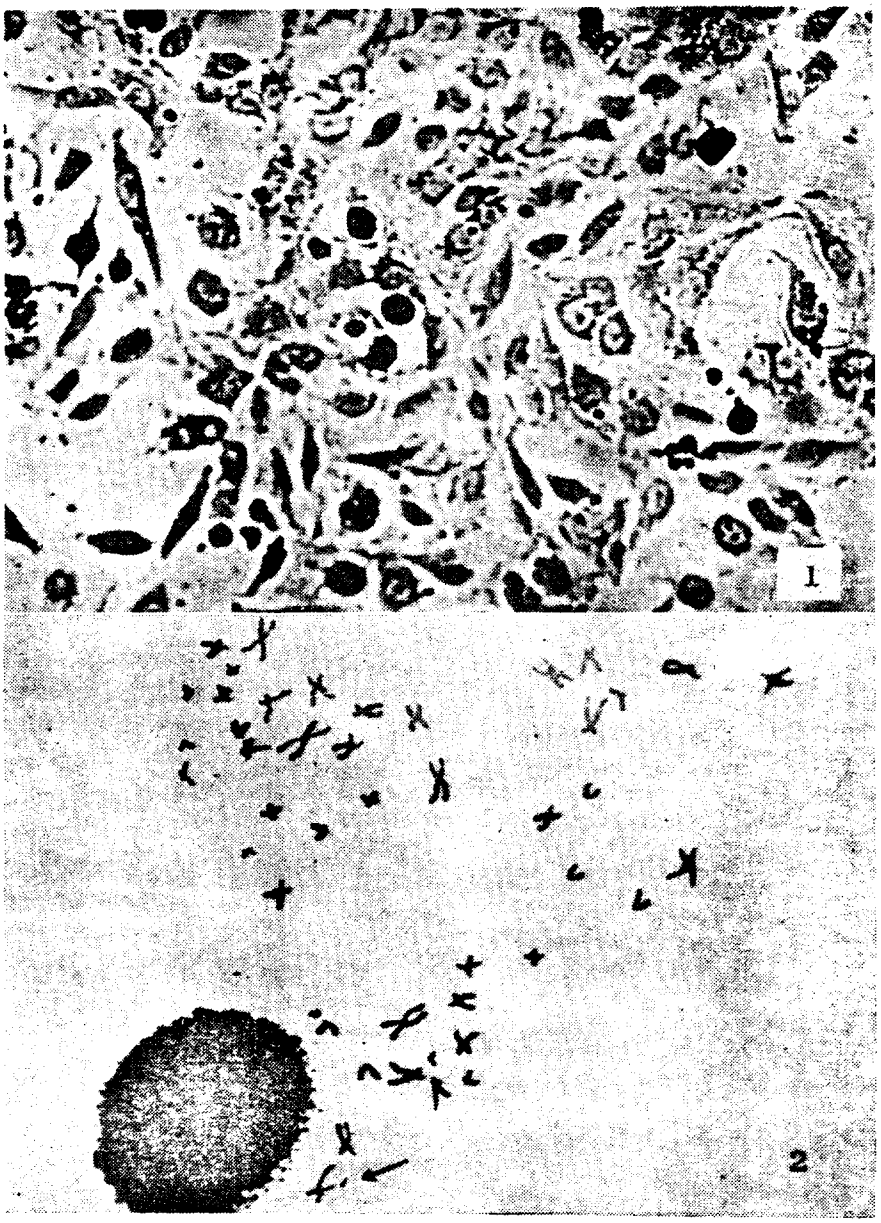


Fig. 1—Morphological changes of HeLa-S3 cells treated with compound 12(10μg/ml) for 72hr. The nuclei separated by cell disintegration appear as dark spherules of variable size, ×84.

Fig. 2—The chromosomal feature of HeLa-S3 cells treated with compound 12 (10μg/ml) for 72hr. Note arrow of chromosomal breakage appears in metaphase, stained by Giemsa method, ×1500.

Biological Activity.—As shown in table III, compounds 2, 8, 45, 46, 49 demonstrated common antimicrobial activity on *E. coli*, *St. aureus* and *P. chrysogenum*. Furthermore, 8 showed inhibitory effect on the growth of HeLa cell.

The data obtained from antimicrobial test by tube dilution method showed that *M. tuberculosis* was highly resistant to these compounds; only 15 compounds (11, 12, 13, 15, 20, 21, 22, 29, 32, 33, 37, 39, 40, 50, 56) of them exerted insufficient inhibitory effect producing more than 100 discrete colonies in case of compounds 15, 21, and 32, 20-100 colonies in case of compounds 12 and 20, and less than 20 colonies in case of the other ten compounds just listed previously even at the concentration of 100μg/ml level, whereas no compounds were active on this organism at 10μg/ml level except 39 producing confluent growth.

This would indicate that the antituberculosis activity appeared limited to 39 only with a minimal inhibitory concentration (MIC) of more than 10μg/ml (10 ppm).

One explanation of the result can be given by the fact that many of these compounds were so insoluble in vitro that they left small to large compound deposits in case of tube dilution method.

Turning attention to the results for antimicrobial activity against *P. chrysogenum*, it was interesting that the parent compound, thiocarbohydrazide, was significantly active at the concentration of 1μg/disk and most of thiocarbohydrazones have also antifungal activity.

In the preliminary test for cytotoxicity it was found that many a compound showed inhibition of growth to 50 percent of control growth at the concentration of 10μg/ml.

Especially, 12 and 29 exhibited significant inhibition of cell growth at the concentration of 10μg/ml, respectively.

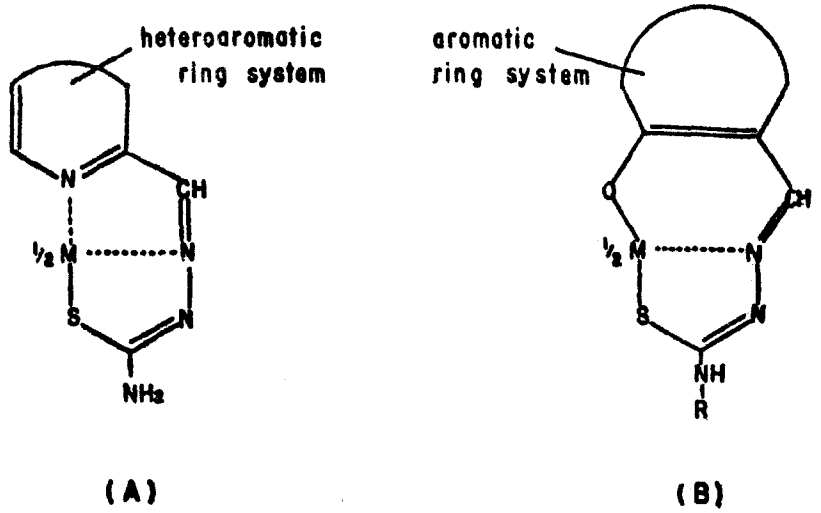


Fig. 3—The provisional postulation for chelation.

HeLa cell morphology by microscopic observation showed disintegrated cell fusion followed by chromosomal breakage. All these observed phenomena were approximately identical with the data of protein measurement.

Structure-Activity Relationship—All these biologically active properties are in agreement with the postulated involvement of a chelation mechanism which is related to a conjugate N-N-S tridentate ligand system[16] and the presence of an intact terminal NH_2 group on the side chain of the parent compound also appeared to be critical for maximum antifungal activity.

It is, too, of structural interest that, among the compounds synthesized, most of the compounds with orthohydroxyl-containing substituents demonstrated antimicrobial activity.

In view of the essential structural features of 1-formylisoquinoline thiosemicarbazone[44] postulated by French and Freedlander[45] (Fig. 3-A), it seems possible to postulate a provisional structural model of the mechanism related to structure-activity relationship of these compounds with orthohydroxyl-containing substituents (Fig. 3-B).

The author feels the available evidence points to the necessity of knowing more about the detailed structure for biological activity.

EXPERIMENTAL

All melting points were determined using a Fisher-Johns melting point appratus and were uncorrected. Microanalyses were perfomed at Yanagimoto Lab., Japan. Infrared spectra were measured at Lab. of Dong-A Pharm. Co., Ltd, Korea.

Hydrazinium dithiocarbazinate(I)—This intermediate was prepared by the procedure of Stolle.[46]

Thiocarbohydrazide(II)—This material was prepared by the method of Audrieth, *et al.*[47]

Methyldithiocarbazinate(V)—This interamediate was prepared by the method of Audrieth, *et al.*[47]

4-Methylthiosemicarbazide(VI)—This material was prepared by the method of R.S. McElhinney.[38]

Monothiocarbohydrazones(III)—0.01 Mole of thiocarbohydrazide was dissolved in 30 ml of hot 25% MeOH. To this solution 0.01 mole of aldehyde in 20 ml of MeOH was added. The mixture was refluxed on hot plate for 0.5-1 hr. The mixture was allowed to cool to room temp. and the reaction product was filtered, recrystallized from appropriate solvents.

Dithiocarbohydrazones(IV)—The following synthesis is typical of the procedure used for preparing this series of compounds. 0.01 Mole of V was dissolved in hot solvent (MeOH, dioxane or DMF). To this solution 0.01 mole of aldehydes in 20 ml of MeOH was added. The reaction mixture was refluxed on hot plate for 2-3 hr, a little concentrated and cooled to room temp. Some drops of water were added to the reaction mixture to

precipitate. The products were filtered off and recrystallized.

4-Methylthiosemicarbazone(VII)—0.01 Mole of aldehyde in MeOH was added, in one portion, to a solution of 4-methylthiosemicarbazide in hot 25% MeOH. The reaction mixture was refluxed on hot plate for 0.5-2 hr and allowed to cool to room temp. The formed precipitate was collected by filtration and recrystallized.

Microbial Sensitivity Test—The microbiological properties were studied against *St. aures* ATCC 6538P. *E. coli* NIHJ and *P. chrysogenum Q* 176 using paper disk method,[48-50] and against *M. tuberculosis* $H_{37}RV$ by tube dilution method.[48] *St. aureus* was controlled to 20% transmittance at 650*mm* and 0.5ml was mixed with 100ml of pepton caseine agar. In the case of *P. chrysogenum* the inoculum was made by adding 1ml of spore suspension having 30,000-50,000 spores per ml to the 100 ml of media. One loopful organisms per 500m*l* of media was used for the inoculum of *E. coli*. For *M. tuberculosis* $H_{37}RV$ suspension, a scrape of growth from LJ medium equal to about 4 or 5 colonies was placed in the bottle containing 1ml of saline. Each compounds was dissolved in 0.1 ml of DMF and diluted with 0.5% CMC solution and applied to prepare 200, 100, 10 and 1μg disks (Whatman No.1 6.5mm in diameter). Zone of inhibition was measured by Fisher-Lilly antibiotic zone reader.

Tissue Culture—Anticancer activities in vitro of the compounds were evaluated by the monolayer cell culture method.[51-54] HeLa-S3 strain obtained from NIH was used for the screening test. Stock culures were cultivated on basal medium (Medium-199) plus 10% calf serum. Cells were removed from glass tube by trypsinization. Dispersed cells were centrifuged, washed twice with Medium-199 minus 10% calf serum and diluted to 10 ml of suspension in the same medium plus 10% calf serum and incubated. An aliquot of the cell suspension was centrifuged, washed twice with Earle's salt (minus bicarbonate) solution, and protein content was determined according to the method of Oyama-Eagle[53,55] for initial protein content. The stock suspension was diluted to 40-50 μg of cell protein/ml with medium-199 plus 10% calf serum. 10mg of the compounds was dissolved in 0.1 ml of DMF and diluted in 0.5% CMC solution to give the concentrations of 1,000, 500, 100 and 10μg/ml, respectively.

0.1ml of these test solutions was added to the 16×122 mm screw capped culture tubes and next 0.9 ml of above medium was implanted in two tubes with each four concentration levels per compound. The controls were also run, which consist of four tubes, each containing the cells without compounds. After inoculation the tube rack was agitated gently to give a uniform distribution of cells and was incubated at 37° at 10° angle. After 72 hr incubation the culture tubes were replaced in a warm-air heater which maintained the cells at 37° during microscopic examination and washing of the tube.

After observing cell morphology growth rate of cells was calculated based on the protein content determined by methods of Oyama-Eagle[53] using bovine serum albumin as a reference.

ACKNOWLEDGEMENT

It is with gratitude that I express my indebtedness to Prof. Yun Sung Chough for his guidance and advice given throughout the course of this investigation, and to Mr. Yong Bae Kim, managing director of production of Dong-A Pharm. Co., Ltd for his heartful encouragement.

I also wish to thank Messrs. Yong Back Lee, Yong Jae Lee of Dong-A Pharm. Co., Ltd. and Mr. Sung Bok Back of NIH, Korea for their help in biological evaluation.

Appreciations are also in the assistance of my colleagues, Messrs. Geon Chi Kim, Gun Il Kang and Chung Ik Yang.

REFERENCES

1. R.G. Wilkinson, R.G. Shepherd, J.P. Thomas, and C. Baughn, *J. Am. Chem. Soc.*, **83**, 2212(1961)
2. A. Furst, ***Chemistry of Chelation in Cancer***, Thomas, (1963)
3. W.O. Foye, *J. Am. Pharm. Assoc.*, **44**, 415 (1955)
4. F. Bernheim, *J. Biol. Chem.*, **143**, 383 (1942)
5. J. Cymerman-Craig and S.D. Rubbo, *Nature*, **178**, 34 (1955)
6. Yun Sung Chough, *J. Pharm. Soc. Korea*, 10, 8 (1966)
7. E. Carl and P. Marquardt, *Z. Naturforschg.*, **4B**, 280 (1949)
8. K. Liebermeister, *Z. Naturforschg.*, **5 B**, 79 (1950)
9. G.J. Van Giessen and H.G. Petering, *J. Med. Chem.*, 11, 695 (1968)
10. H.G. Petering, H.H. Buskirk, and G.E. Underwood, *Cancer Res.*, **24**, 367 (1964)
11. J.A. Crim, H.H. Buskirk, and G.L. Underwood, *Cancer Res.*, 27, 1109 (1967)
12. H.G. Petering, H. H. Buskirk, and J.A. Crim, *Cancer Res.*, 27, 1115 (1967)
13. F.A. French and E.J. Blanz, Jr., *Japanese Cancer Association GANN Monograph*, **2**, 51 (1966)
14. W.E.C. Wacker and B.L. Vallee, *J. Biol. Chem.*, 234, 3257 (1959)
15. C.A. Tobias; *Acta Unio Intern. Contra Cancrum*, **7**, 874 (1952)
16. F.A. French and E.J. Blanz, Jr., *J. Med. Chem.*, **9**, 585 (1966)
17. R.W. Brockman, J.R. Thomson, M.J. Bell, and H.E. Skipper, *Cancer Res.*, **16**, 167 (1956)
18. M.A. Chirigos, *Cancer Res.*, **23**, 1646 (1963)
19. G. Domagk, R. Behnisch, F. Mietzsch, and H. Schmidt, *Naturwissenschaften*, **33**, 315 (1946)
20. R. Donovick, F. Pansy, G. Stryker, and J. Bernstein, *J. Bacteriol.*, **59**, 667 (1950)
21. H.C. Caldwell and W.L. Nobles, *J. Pharm. Sci.* **45**, 729 (1956)
22. B.G. Benns, B.A. Gingras, and C.H. Bayley, *Applied Microbiology*, **8**, 353 (1960)
23. B.A. Gingras, G. Colin, and C.H. Bayley, *J. Pharm. Sci.*, **54**, 1674 (1965)
24. E. Carl and and P. Marquardt, *Z. Naturforschg.*, **4B**, 280 (1949)
25. K.C. Agrawal and A. C. Sartorelli, *J. Med. Chem.*, **12**, 771 (1969)
26. A.C. Sartorelli, *Biochem. Biophys. Res. Commun.*, **27**, 26 (1967)
27. A.C. Sartorelli, *Pharmacologist*, **9**, 192 (1967)
28. A.C. Sartorelli, M.S. Zedeck, K.C. Agrawal, and E.C. Moore, *Fed. Proc.*, 27, 650 (1968)

29. A.C. Sartorelli and B.A. Booth, *Proc. Am. Assoc. Cancer Res.*, 9, 61 (1968)
30. A.C. Sartorelli, B.A. Booth, and E.C. Moore, *Proc. Am. Assoc. Cancer Res.*, 10, 299 (1969)
31. P.N. Harris, Japanese Cancer Association GANN Monograph, 3, 51 (1966)
32. F.A. French, *Cancer Res.*, 28, 2419 (1968)
33. F.A. French and E.J. Blanz, Jr., *Cancer Res.*, 26, 1638 (1966)
34. E.M. Hodnett and W. Willie, *Proc. Okla. Acad. Sci.*, 46, 107 (1966)
35. W.C. J. Ross, G.P. Warwick, and J.J. Roberts, *J. Chem. Soc.*, 3110 (1955)
36. V. Ya. Kazakov and I. Ya. Postovskii, *Chem. Abstr.*, 55, 6483b (1961)
37. D. Nardi, E. Massarani, A. Tajana, L. Degen, and M.J. Magistretti, *J. Med. Chem.*, 10, 530(1967)
38. R.S. McElhinney, *J. Chem. Soc.*, 950 (1966)
39. P.C. Guha and S.C. Dey, *Quart. J. Indian Chem. Soc.*, 2, 225 (1925)
40. J. Sandström, *Acta Chem. Scand.*, 14, 1037 (1960)
41. J. Sandström, *Acta Chem. Scand.*, 17, 1595 (1963)
42. H.W. Stephen and F.J. Wilson, *J. Chem. Soc.*, 2531 (1926)
43. D.W. Wiles and T. Suprunchuk, *J. Med. Chem.*, 13, 323 (1970)
44. F.A. French and E.J. Blanz, Jr., *Cancer Res.*, 25, 1454 (1965)
45. F.A. French and B.L. Freedlander, *Cancer Res.*, 18, 1290 (1958)
46. R. Stolle and P. E. Bowles, *Ber.*, 41, 1099 (1908)
47. L.F. Audrieth, E.S. Scott, and P.S. Kippur, *J. Org. Chem.*, 19, 733 (1954)
48. B.G. Benns, B.A. Gingras, C.H. Bayley, *Applied Microbiology*, 8, 353 (1960)
49. J.G. Vincent and H.W. Vincent, *Proc. Soc. Exptl. Biol. Med.* 55, 162 (1944)
50. L.R. Worthen and H.W. Bond, *J. Pharm. Sci.*, 59, 1185 (1970)
51. H. Eagle and G.E. Foley, *Cancer Res.*, 18, 1017 (1958)
52. C.G. Smith, W.L. Lummis, and J.E. Grady, *Cancer Res.*, 19, 843 (1959)
53. V.I. Oyama and H. Eagle, *Proc. Soc. Exptl. Biol. Med.*, 91, 305 (1956)
54. M. Dawson and W.F. Dryden, *J. Pharm. Sci.*, 56, 545 (1967)
55. O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, 193, 265 (1951)