principally of the steroid cholesterol, the solubility of the steroids examined is not of a magnitude that would lead one to propose a "like dissolves like" situation. From the solubilizing capacities which are comparable to those of other polyoxyethylene-type surfactants and from the spectral evidence, discussed in the following section, it seems likely that solubilization of the steroids takes place by association with the polyoxyethylene exterior of the surfactant micelles.

Spectral Studies—The wavelengths of maximum absorbance ($\lambda_{max.}$) of the three steroids in a few selected solvents and in various aqueous solutions of both unpurified and partially purified ethoxylated cholesterol are presented in Table II.

It may be seen that with an increase in the surfactant concentration up to 2% (w/v) there is a progressive shift toward lower wavelengths. This is in good agreement with the results of a previous study dealing with testosterone and polysorbates (3). At surfactant concentrations above 2% (w/v) the $\lambda_{max.}$ becomes essentially constant. These constant values of $\lambda_{max.}$ compare well with the $\lambda_{max.}$'s of the three steroids in polyethylene glycols 200, 300, and 400. The fact that the surfactant solutions were aqueous might account for the difference of 1 mμ. From these results it would thus appear that the polarity of the environment offered by aqueous ethoxylated cholesterol solutions to the Δ^4-3-keto chromophore of the three steroids is similar to that offered by polyethylene glycols. This observation further substantiates the previous proposal that solubilization of steroids by nonionic ethoxylated surfactants involves association of the steroid with the polyoxyethylene portion of the surfactant.

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ACKNOWLEDGMENTS AND ADDRESSES

Received December 23, 1968, from the *Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46206*

Accepted for publication February 2, 1969.

Presented to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Montreal meeting, May 1969.

* Present address: College of Pharmacy, University of Washington, Seattle, WA 98105

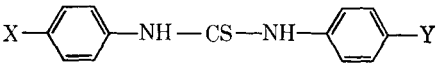
Chemotherapy of Tuberculosis, Part IX: Synthesis and Screening of New Thiazolyl Thiocarbanilides

B. S. KULKARNI, B. S. FERNANDEZ, M. R. PATEL, R. A. BELLARE, and C. V. DELIWALA*

Abstract □ Nearly eighty substituted thiocarbanilides—*viz.*, *p*-(2-thiazolyl)-, *p*-(4-thiazolyl)- and *p*-(5-thiazolyl)-*p*-alkoxy-thiocarbanilides, and *p*-*p'*-bis(4-thiazolyl)-thiocarbanilides, along with a few thiazolyl thiocarbanilides having halogens on the phenyl ring containing the *p'*-alkoxy group, have been synthesized and studied for *in vitro* antitubercular activity. Also described are over 40 new substituted thiazoles prepared as intermediates. *p*-(4-Thiazolyl)-*p'*-alkoxy-thiocarbanilides in general showed the maximum *in vitro* tuberculostatic activity in the present study. *p*-(2,5-Dimethyl-4-thiazolyl)-*p'*-*n*-propoxythiocarbanilide had the same *in vitro* tuberculostatic activity as isonicotinic acid hydrazide (INH) (0.04 mcg./ml.) but did not produce tuberculostatic serum concentration at the same oral dose level as INH.

Keyphrases □ Tuberculosis chromotherapeutic agents □ Thiazolyl thiocarbanilides—synthesis □ Antitubercular activity—thiazolyl thiocarbanilides

Since the discovery of antimycobacterial activity of thioureas (1) numerous publications have appeared showing the pronounced activity of thiocarbanilides both *in vitro* as well as in experimental animals coupled with only a low rate of development of resistance (2–5). Among the most potent compounds of this class are the thiocarbanilides bearing alkoxy groups in *para* positions (6–8). Some of these—*viz.*, 4,4'-diisoamyloxythiocarbanilide (9–11) (I), 4,4'-diethoxythiocarbanilide (12–15) (II) and 4-butoxy-4'-dimethylaminothiocarbanilide (16, 17) (III), have been used clinically for the treatment of tuberculosis and leprosy.



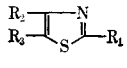
I, X = Y = isoamyloxy
II, X = Y = ethoxy
III, X = butoxy and Y = $N(CH_3)_2$
IV, X = 2-pyridyl and Y = isobutoxy

Doub *et al.* (18) have extended the series of alkoxythiocarbanilides by incorporating a heterocyclic ring—*viz.*, pyridyl as the substituent, and observed potentiation of antimycobacterial activity. One of the compounds from their series called thiocarbanidine (IV) showed high degree of antitubercular action in mice and guinea pigs (19). However, in clinical trials it was not effective, possibly due to poor absorption (20).

METHODS

Since thiazole and pyridine are isosteric and several thiazole derivatives are potent antibacterial agents, it seemed worthwhile to synthesize and study thiocarbanilides having thiazoles as substituents. Accordingly, the synthesis of *p*-(2-thiazolyl)-, *p*-(4-thiazolyl)-, and *p*-(5-thiazolyl)-, *p'*-alkoxythiocarbanilides was undertaken. A few thiazolyl thiocarbanilides having halogens on the phenyl ring

Table I—Substituted Thiazoles



Sr. No.	R_1	R_2	R_3	M.p., °C.	Mol. Formula	Nitrogen %, Found	Nitrogen %, Reqd.
1	p-CH_3O-C_6H_4	C_6H_5	H	98–99	$C_{16}H_{13}NOS$	5.19	5.24
2	3,4-$(CH_3O)_2$-C_6H_3	C_6H_5	H	186–187	$C_{17}H_{15}NO_2S$	4.42	4.71
3	3-Pyridyl	C_6H_5	H	223–224	$C_{14}H_{10}N_2S \cdot HBr$	8.65	8.77
4	p-$CH_3C_6H_4$	C_6H_5	CH_3	79–80	$C_{17}H_{15}NS$	5.20	5.28
5	p-CH_3O-C_6H_4	C_6H_5	CH_3	125–127	$C_{17}H_{15}NOS \cdot HBr$[a]	3.67	3.86
6	3,4-$(CH_3O)_2$-C_6H_3	C_6H_5	CH_3	201–202	$C_{18}H_{17}NO_2S$	4.29	4.50
7	CH_3	C_6H_5	CH_3	217–218	$C_{11}H_{11}NS \cdot HBr$	5.28	5.42
8	p-Cl-C_6H_4-CH_2	C_6H_5	CH_3	80–81	$C_{17}H_{14}ClNS$	4.46	4.67
9	3-Pyridyl	C_6H_5	CH_3	75–76	$C_{15}H_{12}N_2S$	10.93	11.11
10	H	p-NO_2-C_6H_4	H	183–184 (31)[b]	$C_9H_6N_2O_2S$		
11	C_6H_5	p-NO_2-C_6H_4	H	130–131	$C_{15}H_{10}N_2O_2S$[c]	9.71	9.93
12	p-CH_3-C_6H_4	p-NO_2-C_6H_4	H	150–151	$C_{16}H_{12}N_2O_2S$	9.46	9.46
13	p-CH_3O-C_6H_4	p-NO_2-C_6H_4	H	182–183	$C_{16}H_{12}N_2O_3S$	8.73	8.97
14	3,4-$(CH_3O)_2$-C_6H_3	p-NO_2-C_6H_4	H	204–205	$C_{17}H_{14}N_2O_4S$	7.88	8.18
15	CH_3	p-NO_2-C_6H_4	H	145–146 (32)	$C_{10}H_8N_2O_2S$		
16	$C_6H_5CH_2$	p-NO_2-C_6H_4	H	123–124	$C_{16}H_{12}N_2O_2S$	9.42	9.46
17	p-Cl-$C_6H_4CH_2$	p-NO_2-C_6H_4	H	115–116	$C_{16}H_{11}ClN_2O_2S$	8.45	8.47
18	C_6H_5-OCH_2	p-NO_2-C_6H_4	H	136–137	$C_{16}H_{12}N_2O_3S$	8.81	8.97
19	p-$CH_3C_6H_4OCH_2$	p-NO_2-C_6H_4	H	138–139	$C_{17}H_{14}N_2O_3S$	8.50	8.58
20	3-Pyridyl	p-NO_2-C_6H_4	H	168–169	$C_{14}H_9N_3O_2S$	14.72	14.84
21	H	p-NO_2-C_6H_4	CH_3	91–92	$C_{10}H_8N_2O_2S$	12.69	12.72
22	CH_3	p-NO_2-C_6H_4	CH_3	100–101	$C_{11}H_{10}N_2O_2S$	11.74	11.96
23	H	p-NH_2-C_6H_4	H	99–100 (22)	$C_9H_8N_2S$	17.14	17.25
24	C_6H_5	p-NH_2-C_6H_4	H	145–146	$C_{15}H_{12}N_2S$	10.97	11.11
25	p-CH_3-C_6H_4	p-NH_2-C_6H_4	H	138–140	$C_{16}H_{14}N_2S$	10.31	10.52
26	p-CH_3O-C_6H_4	p-NH_2-C_6H_4	H	179–180 (33)	$C_{16}H_{14}N_2OS$	9.82	9.93
27	3,4-$(CH_3O)_2$-C_6H_3	p-NH_2-C_6H_4	H	168–169 (33)	$C_{17}H_{16}N_2O_2S$	8.8	9.0
28	CH_3	p-NH_2-C_6H_4	H	145–146	$C_{10}H_{10}N_2S$[d]	14.51	14.73
29	$C_6H_5CH_2$	p-NH_2-C_6H_4	H	116–118	$C_{16}H_{14}N_2S$	10.41	10.52
30	p-Cl-C_6H_4-CH_2	p-NH_2-C_6H_4	H	150–151	$C_{16}H_{13}ClN_2S$	9.36	9.31
31	C_6H_5-OCH_2	p-NH_2-C_6H_4	H	104–105	$C_{16}H_{14}N_2OS$	9.72	9.93
32	p-CH_3-$C_6H_4OCH_2$	p-NH_2-C_6H_4	H	128–130	$C_{17}H_{16}N_2OS$	9.33	9.46
33	3-Pyridyl	p-NH_2-C_6H_4	H	177–178 (33)	$C_{14}H_{11}N_3S$	16.30	16.60
34	SH	p-NH_2-C_6H_4	H	245–247	$C_9H_8N_2S_2$[e]	13.39	13.43
35	H	p-NH_2-C_6H_4	CH_3	80–81	$C_{10}H_{10}N_2S$	14.68	14.73
36	C_6H_5	p-NH_2-C_6H_4	CH_3	243–244	$C_{16}H_{14}N_2S$	10.61	10.52
37	p-CH_3-C_6H_4	p-NH_2-C_6H_4	CH_3	128–129	$C_{17}H_{16}N_2S$	9.86	10.00
38	p-CH_3O-C_6H_4	p-NH_2-C_6H_4	CH_3	197–198	$C_{17}H_{16}N_2OS$	9.58	9.46
39	3,4$(CH_3O)_2$-C_6H_3	p-NH_2-C_6H_4	CH_3	189–190	$C_{18}H_{18}N_2O_2S$	8.49	8.58
40	CH_3	p-NH_2-C_6H_4	CH_3	106–107	$C_{11}H_{12}N_2S$	13.69	13.72
41	H	CH_3	C_6H_5	198–200	$C_{10}H_9NS \cdot HBr$	5.72	5.42
42	CH_3	CH_3	C_6H_5	206–207	$C_{11}H_{11}NS \cdot HBr$	4.99	5.18
43	H	CH_3	p-NO_2-C_6H_4	99–100	$C_{10}H_8N_2O_2S$	12.78	12.73
44	CH_3	CH_3	p-NO_2-C_6H_4	128–129	$C_{11}H_{10}N_2O_2S$	12.02	11.96
45	H	CH_3	p-NH_2-C_6H_4	143–144	$C_{10}H_{10}N_2S$	14.36	14.73
46	CH_3	CH_3	p-NH_2-C_6H_4	134–135	$C_{11}H_{12}N_2S$	13.30	13.23
47	p-NH_2-C_6H_4	H	H	123–124 (23)	$C_9H_8N_2S$		
48	p-NH_2-C_6H_4	CH_3	H	112–114 (23)	$C_{10}H_{10}N_2S$		
49	p-NH_2-C_6H_4	CH_3	CH_3	130–132 (23)	$C_{11}H_{12}N_2S$		

[a] *Anal.*—Found: C, 56.19; H, 4.26; requires C, 56.35; H, 4.14%. [b] Numbers in parentheses refer to references. [c] *Anal.*—Found: C, 67.89; H, 4.12; requires C, 68.05; H, 4.25%. [d] *Anal.*—Found: C, 54.77; H, 4.89; requires C, 54.88; H, 4.98%. [e] *Anal.*—Found: C, 71.21; H, 4.59; requires C, 71.42; H, 4.76%.

bearing the alkoxy group were synthesized since chloro analogs of (I) and (II) have shown enhanced antitubercular activity (21). *p-p'*-Bis(4-thiazolyl)thiocarbanilides were also synthesized.

The synthesis of *p*-thiazolyl-*p'*-alkoxythiocarbanilides (Tables II, III, and IV) reported in the present work was effected by the interaction of various 2-, 4-, and 5- (*p*-aminophenyl)thiazoles with appropriate *p*-alkoxyisothiocyanates in boiling benzene or acetone.

p-(Aminophenyl)thiazoles (Table I) required for this purpose were obtained by three different methods. ω-Chloro-*p*-aminoacetophenone or α-chloro-*p*-aminopropiophenone were prepared and condensed with appropriate thioamides; however, the yields were poor. In an alternative route ω-bromo-4-nitroacetophenone was condensed with the thioamides and the resultant 4-(*p*-nitrophenyl)-thiazoles were reduced to the corresponding amino derivatives. This method gave excellent yields. The 4-(*p*-nitrophenyl)thiazoles were also obtained, along with the 2- and 5-(*p*-nitrophenyl)thiazoles, by the nitration of the corresponding phenylthiazoles (except in thiazoles having a second phenyl ring) with satisfactory yields and good purity when the nitration was carried out below 5°. However higher temperatures resulted in the simultaneous formation of 2,4-di-nitro derivatives. The 4-(*p*-nitrophenyl)thiazoles prepared by either of these two methods were identical in all respects.

2-Phenylthiazoles were prepared by reacting α-haloketones with thiobenzamide in alcohol (22, 23) while 4-phenylthiazoles were obtained by reacting various thioamides with α-bromoacetophenone or α-bromopropiophenone and 5-phenylthiazoles were prepared by the interaction of α-bromo-α-phenylacetone with different thioamides.

The *p*-alkoxyphenylisothiocyanates were prepared by the decomposition of *p-p'*-dialkoxythiocarbanilides with acetic anhydride, the requisite *p,p'*-alkoxythiocarbanilides being obtained by the condensation of *p*-alkoxyanilines with carbon disulfide in the presence of potassium hydroxide (24–26).

The *p-p'*-bis(4-thiazolyl)thiocarbanilides (Table V) were similarly obtained from the 4-(*p*-aminophenyl)thiazoles and carbon disulfide.

ANTITUBERCULAR TESTING

The *in vitro* tuberculostatic activity was determined on cultures of *Mycobacterium tuberculosis* var. *hominis* (strain $H_{37}Rv$) cultivated in Youmans medium containing 10% horse serum by a modified method of Doub and Youmans (27) previously described by the authors (28). The results are presented in the last columns of Tables II–V. A majority of the compounds showed antitubercular activity.

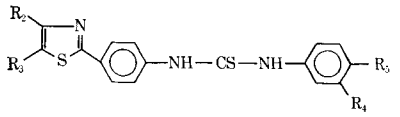


Table II—*p*-(2-Thiazolyl)-3′,4′-Substituted Thiocarbanilides

Sr. no.	R_2	R_3	R_5	R_4	M.p. °C. (uncorr.)	Yield, %	Mol. Formula	Nitrogen % Found	Nitrogen % Reqd.	Min. Inhib. Concn. *in vitro* Antitubercular Action meg./ml.
1	H	H	$-OCH_3$	H	165	78	$C_{17}H_{15}N_3OS_2$[a]	12.05	12.31	200
2	H	H	$-OC_2H_5$	H	154	68	$C_{18}H_{17}N_3OS_2$	11.47	11.83	200
3	H	H	*n*-OC_3H_7	H	165	60	$C_{19}H_{19}N_3OS_2$	11.34	11.39	Nil
4	H	H	iso-OC_3H_7	H	143–145	72	$C_{19}H_{19}N_3OS_2$	11.63	11.39	Nil
5	H	H	*n*-OC_4H_9	H	160	79	$C_{20}H_{21}N_3OS_2$	10.85	10.96	40
6	CH_3	H	$-OCH_3$	H	171	78	$C_{18}H_{17}N_3OS_2$[b]	11.43	11.83	20
7	CH_3	H	$-OC_2H_5$	H	159	72	$C_{19}H_{19}N_3OS_2$	11.78	11.39	20
8	CH_3	H	*n*-OC_3H_7	H	156	65	$C_{20}H_{21}N_3OS_2$	11.39	10.96	40
9	CH_3	H	iso-OC_3H_7	H	139	67	$C_{20}H_{21}N_3OS_2$	10.92	10.96	20
10	CH_3	H	*n*-OC_4H_9	H	150	60	$C_{21}H_{23}N_3OS_2$	10.39	10.58	40
11	CH_3	CH_3	$-OCH_3$	H	156	91	$C_{19}H_{19}N_3OS_2$	11.79	11.39	100
12	CH_3	CH_3	OC_2H_5	H	166	65	$C_{20}H_{21}N_3OS_2$	11.10	10.96	40
13	CH_3	CH_3	*n*-OC_3H_7	H	158	72	$C_{21}H_{23}N_3OS_2$	10.97	10.58	100
14	CH_3	CH_3	iso-OC_3H_7	H	134	68	$C_{21}H_{23}N_3OS_2$	9.96	10.59	40
15	CH_3	CH_3	*n*-OC_4H_9	H	160	81	$C_{22}H_{25}N_3OS_2$[d]	10.31	10.22	200
16	H	H	$-OCH_3$	Br	179–180	75	$C_{17}H_{14}BrN_3OS_2$	9.55	10.00	40
17	H	H	$-OC_2H_5$	Br	157	63	$C_{18}H_{16}BrN_3OS_2$[e]	9.98	9.68	20
18	H	H	Cl	Cl	174	52	$C_{16}H_{11}Cl_2N_3S_2$[f]	11.30	11.05	20
19	CH_3	H	$-OCH_3$	Br	172	50	$C_{18}H_{16}BrN_3OS_2$	9.65	9.68	40
20	CH_3	H	$-OC_2H_5$	Br	152	65	$C_{19}H_{18}BrN_3OS_2$	8.98	9.36	40
21	CH_3	H	Cl	Cl	162	71	$C_{17}H_{13}Cl_2N_3S_2$	11.23	10.67	100
22	CH_3	CH_3	$-OCH_3$	Br	171	73	$C_{19}H_{18}BrN_3OS_2$[g]	9.41	9.36	Nil
23	CH_3	CH_3	$-OC_2H_5$	Br	167	56	$C_{20}H_{20}BrN_3OS_2$	9.54	9.09	Nil
24	CH_3	CH_3	Cl	Cl	165	59	$C_{18}H_{15}Cl_2N_3S_2$	10.47	10.29	Nil

[a] *Anal.*—Found: C, 60.21; H, 4.60; requires C, 59.8; H, 4.40%. [b] *Anal.*—Found: C, 61.25; H, 4.91; requires C, 60.80; H, 4.79%. [c] *Anal.*—Found: C, 64.61; H, 5.83; requires C, 64.24; H, 6.08%. [d] *Anal.*—Found: C, 49.42; H, 3.38; requires C, 49.77; H, 3.68%. [e] *Anal.*—Found: C, 50.98; H, 3.10; requires C, 50.53; H, 2.89%. [f] *Anal.*—Found: C, 51.32; H, 4.16; requires C, 50.89; H. 4.02%.

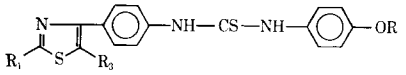


Table III—*p*-(4-Thiazolyl)-*p*′-Alkoxy Thiocarbanilides

Sr. no.	R	R_1	R_3	M.p. °C. (uncorr.)	Mol. Formula	Nitrogen % Found	Nitrogen % Reqd.	Min. Inhib. Concn. *in vitro* Antitubercular Action mcg./ml.
25	CH_3	H	H	167–169	$C_{17}H_{15}N_3OS_2$	12.52	12.31	200
26	C_2H_5	H	H	183	$C_{18}H_{17}N_3OS_2$	12.13	12.83	10
27	*n*-C_3H_7	H	H	180–181	$C_{19}H_{19}N_3OS_2$	11.44	11.38	10
28	iso-C_3H_7	H	H	157	$C_{19}H_{19}N_3OS_2$	11.71	11.38	10
29	*n*-C_4H_9	H	H	172–173	$C_{20}H_{21}N_3OS_2$	11.36	10.97	100
30	iso-C_4H_9	H	H	178	$C_{20}H_{21}N_3OS_2$	10.64	10.97	10
31	*n*-C_5H_{11}	H	H	172–173	$C_{21}H_{23}N_3OS_2$	11.27	10.58	20
32	CH_3	CH_3	H	159	$C_{18}H_{17}N_3OS_2$	12.03	11.83	10
33	C_2H_5	CH_3	H	172	$C_{19}H_{19}N_3OS_2$	11.40	11.38	10
34	*n*-C_3H_7	CH_3	H	168	$C_{20}H_{21}N_3OS_2$	11.12	10.97	20
35	iso-C_3H_7	CH_3	H	145–147	$C_{20}H_{21}N_3OS_2$	10.95	10.99	10
36	*n*-C_4H_9	CH_3	H	169	$C_{21}H_{23}N_3OS_2$	10.96	10.58	10
37	iso-C_4H_9	CH_3	H	172–173	$C_{21}H_{23}N_3OS_2$	10.54	10.58	20
38	*n*-C_5H_{11}	CH_3	H	140–141	$C_{22}H_{25}N_3OS_2$	10.21	10.22	10
39	CH_3	H	CH_3	165	$C_{18}H_{17}N_3OS_2$[a]	11.75	11.83	1
40	C_2H_5	H	CH_3	155–157	$C_{19}H_{19}N_3OS_2$	11.50	11.38	1
41	*n*-C_3H_7	H	CH_3	151–152	$C_{20}H_{21}N_3OS_2$	11.48	10.97	1
42	iso-C_3H_7	H	CH_3	193–195	$C_{20}H_{21}N_3OS_2$	11.63	10.97	1
43	*n*-C_4H_9	H	CH_3	168–169	$C_{21}H_{23}N_3OS_2$	10.88	10.58	1
44	iso-C_4H_9	H	CH_3	147	$C_{21}H_{23}N_3OS_2$	11.09	10.58	1
45	*n*-C_5H_{11}	H	CH_3	152–154	$C_{22}H_{25}N_3OS_2$	10.22	10.22	1
46	CH_3	CH_3	CH_3	170	$C_{19}H_{19}N_3OS_2$	12.05	11.38	10
47	C_2H_5	CH_3	CH_3	142–143	$C_{20}H_{21}M_3OS_2$	10.68	10.97	10
48	*n*-C_3H_7	CH_3	CH_3	145	$C_{21}H_{23}N_3OS_2$	10.14	10.58	0.04[b]
49	iso-C_3H_7	CH_3	CH_3	162	$C_{21}H_{23}N_3OS_2$	11.11	10.58	0.1
50	*n*-C_4H_9	CH_3	CH_3	161–163	$C_{22}H_{25}N_3OS_2$	10.10	10.22	0.1
51	iso-C_4H_9	CH_3	CH_3	155–157	$C_{22}H_{25}N_3OS_2$	10.17	10.22	2
52	*n*-C_5H_{11}	CH_3	CH_3	152	$C_{23}H_{27}N_3OS_2$	10.07	9.88	4

[a] *Anal.*—Found: C, 60.60; H, 4.82; requires C, 60.84; H, 4.79%. [b] This compound was also tested against Altere strain of *M. tuberculosis* which is resistant to streptomycin up to 1,000 mcg./ml. and was found active up to 0.1 mcg./ml.

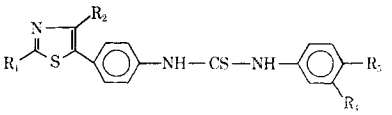


Table IV—*p*-(5-Thiazolyl)-3′,4′-Substituted Thiocarbanilides

Sr. no.	R_1	R_2	R_5	R_4	M.p. °C. (uncorr.)	Yield, %	Mol. Formula	Nitrogen % Found	Nitrogen % Reqd.	Min. Inhib. Concn. *in vitro* Antitubercular Action mcg./ml.
53	CH_3	CH_3	$-OCH_3$	H	121–122	74	$C_{19}H_{19}N_3OS_2$[a]	11.77	11.39	20
54	CH_3	CH_3	$-OC_2H_5$	H	137	82	$C_{20}H_{21}N_3OS_2$	10.69	10.96	20
55	CH_3	CH_3	*n*-OC_3H_7	H	164	79	$C_{21}H_{23}N_3OS_2$[b]	11.11	10.58	20
56	CH_3	CH_3	iso-OC_3H_7	H	195	61	$C_{21}H_{23}N_3OS_2$	10.45	10.58	20
57	CH_3	CH_3	*n*-OC_4H_9	H	156	67	$C_{22}H_{25}N_3OS_2$	9.62	10.22	20
58	H	CH_3	$-OCH_3$	Br	105	65	$C_{18}H_{16}BrN_3OS_2$[c]	9.42	9.68	100
59	H	CH_3	OC_2H_5	Br	132	72	$C_{19}H_{18}BrN_3OS_2$	9.72	9.36	40
60	H	CH_3	Cl	Cl	141	53	$C_{17}H_{13}Cl_2N_2S_2$	10.89	10.67	20
61	CH_3	CH_3	$-OCH_3$	Br	126	50	$C_{19}H_{18}BrN_3OS_2$[d]	9.41	9.36	20
62	CH_3	CH_3	$-OC_2H_5$	Br	161	68	$C_{20}H_{20}BrN_3OS_2$	8.80	9.09	20
63	CH_3	CH_3	Cl	Cl	158	70	$C_{18}H_{15}Cl_2N_3S_2$	10.36	10.29	20

[a] *Anal.*—Found: C, 62.3; H, 5.13; requires C, 61.8; H, 5.15%. [b] *Anal.*—Found: C, 63.78; H, 5.32; requires C, 63.48; H, 5.79. [c] *Anal.*—Found: C, 50.08; H, 3.96; requires C, 49.77; H, 3.69%. [d] *Anal.*—Found: C, 50.39; H, 4.23; requires C, 50.89; H, 4.02%.

p-(4-Thiazolyl)-*p*′-alkoxythiocarbanilides were the most active compounds in the present work. One compound from these series—*viz.*, *p*-(2,5-dimethyl-4-thiazolyl)-*p*′-*n*-propoxythiocarbanilide, 48, showed *in vitro* activity comparable to isonicotinic acid hydrazide (INH) (0.04 mcg./ml.). This compound was therefore, tested further against Altere strain of *M. tuberculosis*, which is a streptomycin-resistant strain withstanding a concentration of streptomycin up to 1,000 mcg./ml. and was found to be active up to 0.1 mcg./ml.

In order to determine whether adequate serum antitubercular activity of this compound could be maintained by oral administration, it was mixed with the stock diet to give final concentrations of 0.1 and 0.3% and the drugged diet so prepared was administered *ad libitum* to two groups of five guinea pigs, respectively. Additional control groups were also kept, one without any drug and another receiving diet mixed with 0.1% isonicotinic acid hydrazide (INH). On the 6th, 12th, 15th, and 20th day during the course of this drugged diet administration, blood samples were drawn (cardiac puncture) from each animal, samples of each group pooled and allowed to clot. The separated serum (0.5 ml.) from each group, after seitz filtration was added aseptically to 4.4 ml. of Youmans basal medium and seeded with 0.1 ml. of the inoculum of *M. tuberculosis* ($H_{37}Rv$) and incubated for 21 days. Under these conditions the serums of the group of animals receiving INH-drugged diet were found to be completely tuberculostatic at all the different intervals of sampling, while serums from the group receiving 0.1% of Cmpd. 48 showed no inhibition with 6-, 12-, and 15-days feeding and only partial inhibition with 20-days feeding. Even 0.3% concentration of Cmpd. 48 produced only partial inhibition in 6- and 12-day blood samples and complete tuberculostatic effect could only be seen after 15 and 20 days of feeding. Evidently the oral administration of Cmpd. 48 does not produce the same tuberculostatic serum concentration as INH although both have the same *in vitro* tuberculostatic activity.

Structure Activity Relationship—*p*-(4-Thiazolyl)-*p*′-alkoxythiocarbanilides were in general the most active compounds in the present work. Even in this series, compounds containing no substitution in the thiazole ring were less active. With the introduction of a methyl group in the ring the activity increased, the 5-methyl deriva-

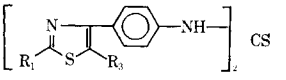


Table V—Bis(4-Thiazolyl)Thiocarbanilides

Sr. no.	R_1	R_3	M.p. °C. (uncorr.)	Mol. Formula	Nitrogen % Found	Nitrogen % Reqd.	Min. Inhib. Concn. *in vitro* Antitubercular Action mcg./ml.
64	H	H	205–207	$C_{19}H_{14}N_4S_3$	14.19	14.21	200
65	C_6H_5	H	214	$C_{31}H_{22}N_4S_3$	10.08	10.25	200
66	4-$CH_3C_6H_4$	H	164	$C_{33}H_{26}N_{43}$[a]	9.65	9.75	100
67	4-$OCH_3C_6H_4$	H	200–201	$C_{33}H_{26}N_4O_2S_3$	9.13	9.24	100
68	3,4(OCH_3)$_2C_6H_3$	H	113	$C_{35}H_{30}N_4O_4S_3$	8.23	8.40	100
69	CH_3	H	197	$C_{21}H_{18}N_4S_3$	13.17	13.26	200
70	$C_6H_5CH_2$	H	186–188	$C_{33}H_{26}N_4S_3$	9.55	9.75	100
71	4-$ClC_6H_4CH_2$	H	190	$C_{33}H_{24}Cl_2N_4S_3$	8.59	8.71	100
72	$C_6H_5OCH_2$	H	202–203	$C_{33}H_{26}N_4O_2S_3$	9.23	9.24	200
73	4-$CH_3C_6H_4OCH_2$	H	210	$C_{35}H_{30}N_4O_2S_3$	9.64	8.83	200
74	3-Pyridyl	H	214–215	$C_{29}H_{20}N_6S_3$	15.29	15.33	200
75	SH	H	231	$C_{19}H_{14}N_4S_5$	12.01	12.22	200
76	H	CH_3	204–205	$C_{21}H_{18}N_4S_3$	13.16	13.26	200
77	C_6H_5	CH_3	197–198	$C_{33}H_{26}N_4S_3$	9.66	9.75	100
78	4-$CH_3C_6H_4$	CH_3	200–202	$C_{35}H_{30}N_4S_3$	9.19	9.30	100
79	4-$CH_3OC_6H_4$	CH_3	222	$C_{35}H_{30}N_4O_2S_3$	8.70	8.83	200
80	3,4(CH_3O)$_2C_6H_3$	CH_3	267	$C_{37}H_{34}N_4O_4S_3$[b]	7.98	8.06	100
81	CH_3	CH_3	165–166	$C_{23}H_{22}N_4S_3$	12.29	12.44	200

[a] *Anal.*—Found: C, 68.81; H, 4.64; requires C, 68.99; H, 4.53%. [b] *Anal.*—Found: C, 63.71; H, 4.99; requires C, 63.98; H, 4.89%.

tives as a class being more active than the corresponding 2-methyl. But the length of the alkoxy group in 4′-position did not have significant influence on the antitubercular activity. With the introduction of a second methyl group in the thiazole ring to give 2,5-dimethylthiazole derivatives the activity increased still further but it was found that the activity of such thiocarbanilides was dependent on the nature of the 4′-alkoxy group.

Among the *p*-(2-thiazolyl)-3′,4′-substituted thiocarbanilides, the activity was found to be dependent on the substitution in the thiazole ring. Thus the unsubstituted thiazole derivatives gave very poor or no activity. Introduction of a methyl group in Position 4 of the thiazole ring improved the activity but an additional methyl group introduced in Position 5 reduced the activity. The activity was not much affected by the length of the alkoxy chain. In the *p*-(5-thiazolyl)-3′,4′-substituted thiocarbanilides however, the substituents either in the thiazole ring or the benzene ring did not significantly alter the activity. Bis-(4-thiazolyl)thiocarbanilides were the least active compounds in the present work.

EXPERIMENTAL

4-Phenyl-2,5-dimethylthiazole—To a solution of thioacetamide (2.47 g., 0.033 mole) in ethanol (20 ml.) was added α-bromopropiophenone (6.39 g., 0.033 mole). The mixture was refluxed on a steam bath for 2.5 hr. and partially concentrated. On cooling and diluting with ether, the hydrobromide of the required thiazole precipitated. It was collected by filtration and crystallized from ethanol, m.p. 217–218°; weight, 5.8 g.

4-(*p*-Nitrophenyl)-2,5-dimethylthiazole—To chilled concentrated sulfuric acid (20 ml.) was added 4-phenyl-2,5-dimethylthiazole hydrobromide (3.6 g., 0.013 mole) with stirring. The mixture was cooled to −5° and a nitrating mixture consisting of concentrated sulfuric acid (6 ml.) and fuming nitric acid (4 ml., sp. gr. 1.5) was slowly added maintaining the reaction temperature below 5°. After further stirring for a few minutes, the mixture was poured over 20% cold sodium hydroxide solution (about 150 ml.). The resulting precipitate was filtered, washed well with water, and crystallized from ethanol to give 2.5 g. (78%) of the desired compound m.p. 100–101°. (Found: N, 11.74; $C_{11}H_{10}N_2O_2S$ requires N, 11.96%).

4-(*p*-Aminophenyl)-2,5-dimethylthiazole—A mixture of 4-(*p*-nitrophenyl)-2,5-dimethylthiazole (5.8 g., 0.025 mole), iron powder (10 g.), and reducing solution (70 ml.) [made by adding formic acid (1 ml.) to acetic acid (7 ml.) and diluting the mixture to 100 ml. with 75% alcohol] was heated under reflux on a steam bath for 1 hr. and filtered while hot. The filtrate was treated with excess of solid sodium carbonate and again filtered. The sodium carbonate cake was repeatedly washed with hot ethanol; the combined filtrate and washings were concentrated on a steam bath and the residue was crystallized from alcohol to give 3.46 g. (68%) of the amine, m.p. 106–107°. (Found: C, 54.77, H, 4.89; N, 13.69; $C_{11}N_{12}N_2S$ requires C, 54.88; H, 4.98; N, 13.72%).

α-Chloro-*p*-aminopropiophenone—To a well-stirred suspension of powdered acetanilide (27 g., 0.2 mole) and anhydrous aluminum chloride (80 g.) in carbondisulfide (200 ml.) was added 2-chloropropionylchloride (46 g., 0.36 mole) at such a rate that a gentle refluxing was maintained. After the addition, the reaction mixture was refluxed on a steam bath for 1 hr. and then allowed to stand for about 20 min. The upper layer was removed by decantation and discarded. The residual slurry was decomposed by pouring over crushed ice containing a little hydrochloric acid. The *p*-acetamino-α-chloropropiophenone so obtained was collected by filtration, washed well, and dried. It was crystallized from benzene as shining yellow flakes; yield 39.5 g. (80%) m.p. 118–119°. (Found: N, 5.95; $C_{11}H_{12}ClNO_2$ requires N, 6.21%).

The above acetyl compound (24.6 g.) was added to 100 ml. of dilute hydrochloric acid (1:1) and the mixture boiled for about 10 min. The clear red solution was chilled by adding some crushed ice, decolorized with carbon, and filtered. Neutralization with dilute ammonium hydroxide solution gave α-chloro-*p*-aminopropiophenone. This was collected by filtration, washed, and pressed well. The moist solid was crystallized from benzene, yield 13.4 g. (65%) m.p. 98°.

4-(*p*-Aminophenyl)-2,5-dimethylthiazole—(Alternate Method)—A mixture of α-chloro-*p*-aminopropiophenone (9.17 g., 0.05 mole) and thioacetamide (4.13 g., 0.055 mole) in 25 ml. of alcohol was refluxed for 1 hr. and the solvent removed by evaporation. The residue was dissolved in water, treated with charcoal, and basified with ammonium hydroxide. The desired amine which separated was crystallized from hexane, m.p. 106–107°; mixed m.p. with a sample prepared by the earlier method was not depressed; yield 7.0 g. (68%).

All the other *p*-(aminophenyl)thiazoles were similarly prepared using the appropriate intermediates and are listed in Table I. The various intermediates required for the synthesis of these thiazoles were prepared according to the literature methods.

3,3′-Dibromo-4,4′-diethoxythiocarbanilide—A mixture of 3-bromo-4-ethoxyaniline (29) (21.6 g., 0.1 mole), carbon disulfide (12.7 ml., 0.02 mole) and alcohol (200 ml.) containing potassium hydroxide (0.05 g.) was refluxed on a water bath for 24 hr. The reaction mixture was cooled, the solid filtered, and crystallized from ethanol to give 35.6 g. (75%) of the desired thiocarbanilide m.p. 166–167°. (Found: C, 43.52; H, 3.81; N, 5.49; $C_{17}H_{18}Br_2N_2O_2S$ requires C, 43.03; H, 3.79; N, 5.91%).

3,3′-Dibromo-4,4′-dimethoxythiocarbanilide was similarly obtained in 65% yield, m.p. 180–181°. (Found: C, 39.82; H, 3.15; N, 6.4; $C_{15}H_{14}Br_2N_2O_2S$ requires C, 40.36; H, 3.14; N, 6.7%).

3-Bromo-4-ethoxyphenylisothiocyanate—A mixture of 3,3′-dibromo-4,4′-diethoxythiocarbanilide (23.7 g., 0.05 mole) and acetic anhydride (20.4 g., 0.2 mole) was refluxed for 10 min. and the mixture cooled, diluted with water, and neutralized with sodium carbonate. The solid thus separated was extracted with hexane and the extract concentrated to get 10.6 g. (60%) of the desired isothiocyanate. A small portion crystallized from dilute acetone m.p. 35°. (Found: N, 5.60; C_9H_8BrNOS requires N, 5.42%).

3-Bromo-4-methoxyphenyl isothiocyanate was similarly obtained in 50% yield m.p. 66–67°. (Found: N, 6.10; C_8H_6BrNOS requires N, 5.73%)

Other alkoxyisothiocyanates were similarly prepared: *p*-methoxy-(26), *p*-ethoxy-(26), *p*-*n*-propoxy-(26), *p*-isopropoxy-(18), *p*-*n*-butoxy-(26), *p*-isobutoxy-(18), *p*-*n*-amyloxy-(18), *p*-iso-amyloxy-(18), 3,4-dichlorophenylisothiocyanate-(30).

***p*-(2,5-Dimethyl-4-thiazolyl)-*p*′-*n*-propoxythiocarbanilide**—A solution of 4-(*p*-aminophenyl)-2,5-dimethylthiazole (4.08 g.; 0.02 mole) and *p*-*n*-propoxyphenylisothiocyanate (26) (3.54 g., 0.02 mole) in benzene (20 ml.) was refluxed for 1 hr. The solvent was removed and the syrupy residue was left overnight in contact with hexane. A granular solid thus obtained was filtered, washed with hexane, and crystallized from benzene. Yield, 5.0 g. (65%); m.p. 145°.

Other compounds were similarly prepared and are listed in Tables II–IV.

***p*,*p*′-Bis(2,5-dimethylthiazolyl)thiocarbanilide**—To a solution of 2,5-dimethyl-4-(*p*-aminophenyl)thiazole (6.1 g., 0.03 mole) in ethyl alcohol (60 ml.) containing potassium hydroxide (0.015 g.) was added carbon disulfide (4.6 g.). The mixture was refluxed for about 30 hr. and then cooled. The solid formed was removed by filtration and washed with a little ethanol to get 2.7 g. (40% yield) of the required thiourea, crystallized from ethanol m.p. 165–166°.

Other compounds were similarly prepared in yields ranging from 35 to 70%. These compounds were generally crystallized from ethanol or benzene and are listed in Table V.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 8, 1968 from the *Department of Chemotherapy, Haffkine Institute, Bombay-12 India.*

Accepted for publication March 25, 1969.

* To whom communications regarding this paper should be addressed.

The authors are indebted to Dr. J. Bretey (Pasteur Institute, Paris) for the Altere strain of *M. tuberculosis* and to Mr. M. T. Jaokar and his colleagues for the microanalyses; also to Dr. N. K. Dutta, Director, Haffkine Institute, Bombay for providing facilities to carry out the work.

Structure-Activity Relations in Organophosphorus-Inhibited Acetylcholinesterase Reactivators II: Methiodides of Hydroxyimino Derivatives of 1-Pyridyl-2-phenylethanes

PALMARISA FRANCHETTI*, MARIO GRIFANTINI, and SANTE MARTELLI

Abstract □ A series of methiodides from *syn*-2-hydroxyimino-1-pyridyl-2-phenylethanes, *syn*-1-hydroxyimino-1-pyridyl-2-phenylethan-2-ones, *syn*- and *anti*-2-hydroxyimino-1-pyridyl-2-phenylethan-1-ones, and *syn*-1,2-di(hydroxyimino)-1-pyridyl-2-phenylethanes was prepared. The influence of the structure of these new compounds on the *in vitro* reactivation of acetylcholinesterase inhibited by TEPP or DFP was investigated. The results obtained confirmed that quaternary pyridine derivatives containing a hydroxyimino group in the β position of the side chain generally keep a reactivating ability, in whatever position the connection with the ring may be; in some cases this activity is higher than that of the isomer containing the same group in the α position.

Keyphrases □ Acetylcholinesterase reactivators—structure-activity relationships □ Organophosphorus-inhibited cholinesterase—reactivators □ 1-Pyridyl-2-phenylethanes, hydroxyimino methiodides—synthesis □ UV spectrophotometry—identity, structure

It is well known that some quaternary pyridyloximes are used to reactivate the enzymatic activity of organophosphorus-inhibited acetylcholinesterase (1, 2). The most active are those in which the hydroxyimino group is placed on side chains in the 2 or 4 position of the ring (1, 3). Concerning the structure-activity relations of these drugs, Wilson (4) presented the hypothesis that reactivation principally depends on three factors: (*a*) nucleophilic reactivity; (*b*) stereochemistry; (*c*) complementarity of the molecule of the reactivator to enzymatic active sites. The *anti*-configurations in 2- and 4-formyl-*N*-methylpyridinium oxime iodides (2-PAM, 4-PAM) satisfy the stereochemical requirements so that the displacement of the organophosphorus group takes place. This assumption proved to be partly wrong when Poziomek *et al.* (5) succeeded in synthesizing the second isomer of 4-PAM, and were able to ascribe, unmistakably, the steric structure to the two geometric isomers by comparing their NMR spectra. Reactivation measurements showed the *syn*-isomer to be 2.5 times more effective.

In order to obtain more knowledge of structure-activity relations, the authors have recently undertaken a study on the reactivating properties of quaternary derivatives of pyridine containing either two hydroxyimino groups close to each other in the side chain or of one such group in the β position. In a preceding note (6) the results obtained on a series of methiodides of isomeric mono- and bis-hydroxyimino-β-pyridyl-propioanilides were reported; they pointed out the effectiveness of the hydroxyimino group situated one carbon atom distant from the ring.

In order to confirm these results, a series of methiodides of hydroxyimino derivatives of 1-pyridyl-2-phenylethanes, whose geometric configurations would be identical to those of the products reported previously