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Research Articles

Substituted Heterocyclic Thioureas I

Antitubercular Activity

By ARTHUR C. GLASSER and RICHARD M. DOUGHTY

A series of substituted 2-pyridyl and 4-(1-phenyl-2,3-dimethyl)-5-pyrazolone thioureas have been synthesized and tested for their antitubercular activity against *Mycobacterium tuberculosis* in Dubos medium. The minimum inhibitory concentrations of the compounds studied ranged from 10 mg. per 100 ml. to 0.16 mg. per 100 ml.

THIOUREA and its simple derivatives have been shown to have a limited tuberculostatic activity (1). *p*-Aminophenyl alkyl ethers also have a definite inhibitory effect (2), and when the two classes of compounds are combined through N-substituted thioureas this activity is enhanced (3–7). The tuberculostatic activity of the thioureas has been reviewed by Schroeder (8). This study is concerned with the influence on tuberculostatic activity by the substitution of the thiourea molecule with heterocyclic rings such as 2-pyridyl- or 4-(1-phenyl-2, 3-dimethyl)-5-pyrazolone in conjunction with alkyl or *p*-alkoxyphenyl substitution. The 1-*p*-alkoxy-

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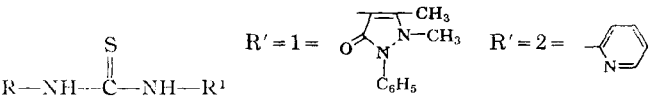
TABLE I.—SUBSTITUTED HETEROCYCLIC THIOUREAS



No.	R	R′	M.p.[a], °C.	R.S.[b]	Yield[c], %	Formula	N Analyses[d] Calcd.	N Analyses[d] Found	Min. Inhib. Concn., mg./100 ml.
1	C_6H_5	1	207–08[e]	E	90	$C_{18}H_{18}N_4OS$	16.5	16.3	2.5
2	C_6H_{11}	1	252–53d decompn.	C	82	$C_{18}H_{24}N_4OS$	16.3	16.8	5
3	4-$CH_3OC_6H_4$	1	200–01[f]	M	89	$C_{19}H_{20}N_4O_2S$	15.2	15.3	2.5
4	4-$C_2H_5OC_6H_4$	1	200–01.5[g]	M	87	$C_{20}H_{22}N_4O_2S$	14.7	14.7	2.5
5	4-*n*-$C_3H_7OC_6H_4$	1	198–200d	AM	69	$C_{21}H_{24}N_4O_2S$	14.1	14.6	2.5
6	4-*i*-$C_3H_7OC_6H_4$	1	202–03d	M	89	$C_{21}H_{24}N_4O_2S$	14.1	14.3	0.16
7	4-*n*-$C_4H_9OC_6H_4$	1	201–02.5	T	87	$C_{22}H_{26}N_4O_2S$	13.6	13.2	10
8	4-*sec*-$C_4H_9OC_6H_4$	1	195–96d	MI	70	$C_{22}H_{26}N_4O_2S$	13.6	13.6	5
9	4-*n*-$C_5H_{11}OC_6H_4$	1	173–75d	C	81	$C_{23}H_{28}N_4O_2S$	13.2	12.7	5
10	4-*sec*-$C_5H_{11}OC_6H_4$	1	180–81d	C	71	$C_{23}H_{28}N_4O_2S$	13.2	13.0	2.5
11	C_2H_5	2	122.5–23	A	65	$C_8H_{11}N_3S$	23.2	22.9	2.5
12	*n*-C_3H_7	2	87.5–88.5	A	68	$C_9H_{13}N_3S$	21.5	21.3	5
13	*i*-C_3H_7	2	129.5–30[h]	A	78	$C_9H_{13}N_3S$	21.5	21.0	2.5
14	C_3H_5	2	101–02[i]	A	90	$C_9H_{11}N_3S$	21.7	21.2	5
15	*n*-C_4H_9	2	83–84	A	84	$C_{10}H_{15}N_3S$	20.5	20.0	10
16	*n*-C_5H_{17}	2	80–82	A	75	$C_{14}H_{23}N_3S$	15.8	15.6	1.25
17	C_6H_5	2	177–78[j]	B	92	$C_{12}H_{11}N_3S$	18.3	18.1	1.25
18	C_6H_{11}	2	147–48	A	63	$C_{12}H_{17}N_3S$	17.8	17.5	2.5

[a] Fisher-Johns Block, uncorrected. [b] Recrystallization solvents: A-alcohol, B-acetone, C-Methanol, E-Ether, I-Isooctane, M-Methylcellosolve, T-Tolune. [c] Yields based on recrystallized product. [d] Weiler and Strauss, Oxford, England. [e] Reported, Takahasi, T., *Yakugaku Zasshi* **79,** 162(1959), 208°; through *Chem. Abstr.*, **53,** 13128e(1959). [f] Reported, *ibid.*, 199°. [g] Reported, *ibid.*, 198°. [h] Reported, Roy, A., and Guha, R. C., *J. Sc. Research (India)*, **9B,** 9262(1950), 129–130°. [i] Reported, *ibid*, 100–101°. [j] Reported, *ibid.*, 167°.

phenyl-3-(2-pyridyl) thioureas have been investigated (5). With our observation of the activity of the 1-*n*-octyl-3-(2-pyridyl) thiourea it was decided to investigate the other members of the 2-pyridyl series along with the 5-pyrazolone series.

The *in vitro* determination of the tuberculostatic activity was carried out using the virulent H37Rv strain of *M. tuberculosis* grown on Dubos medium with added Tween 80 and beef serum. A comparison of the biological activities as seen from Table I shows the inhibitory concentrations range in activity from a low value of 10 mg./100 ml. to an upper limit of 0.16 mg./100 ml. Maximum activity of the 2-pyridyl series rests in the phenyl and *n*-octyl derivatives, while maximum for the 5-pyrazolone series is shown by the isopropoxyphenyl derivative. In the *p*-alkoxyphenyl derivatives the increased activity of the lower alkyl chains is in keeping with observations made on the 4,4′-dialkoxythiocarbanilides (9).

Synthesis of the thioureas involved the reaction of 2-aminopyridine or 4-aminoantipyrine with the appropriate isothiocyanate in absolute alcohol or thiophene free benzene (10). The alkyl isothiocyanates were prepared by the general method of Moore (11). The aryl isothiocyanates were made with some modification following the process of Van der Kirk (12), or the action of acetic anhydride on the appropriate thiocarbanilide (13). The *p*-alkoxyaniline derivatives needed for the isothiocyanate syntheses were prepared by the method of Bucchi (14).

EXPERIMENTAL

Biological Test Method.—The culture used was the H37Rv strain of *Mycobacterium tuberculosis* var. *hominis* obtained from the American Type Culture Collection.[1] The organism to be used as inoculant was propagated in Dubos broth base.[2] These cultures were grown in 100 ml. of broth in aluminum foil capped Erlenmeyer flasks for 2 weeks before inoculating tubes in the dilution series. Incubation was at 37° for all cultures and dilution tubes.

In the dilution series 4.5 ml. of Dubos broth base, enriched with 10% Dubos Medium Serum,[2] was placed in all tubes except the first tube of the series which received initially 9.0 ml. of the enriched medium. Ten milligrams of the compounds per milliliter of a solvent consisting of equal parts of propylene glycol, Tween 80, ethanol and distilled water was diluted to 10 ml. with nonenriched Dubos broth base. Using a sterile Swinny hypodermic adaptor and a membrane filter, Millipore HA type,[3] 1.0 ml. was aseptically filtered into the first tube of a series. These were mixed by drawing up portions of the medium into a sterile syringe, then 4.5 ml. was withdrawn aseptically from the first tube and placed in the second. This was mixed as before and 4.5 ml. transferred to the next tube. This was continued through all tubes of a specific series; 4.5 ml. was discarded from the last tube in the series. This resulted in increasing dilutions of 2× magnitude

[1] American Type Culture Collection, 2112 M Street N. W., Washington 7, D. C.
[2] Difco Laboratories, Detroit 2, Mich.
[3] Millipore Filter Corp., Bedford, Mass.

from 10 mg. % in the first tube to 0.0195 mg. % in the last tube of a ten tube series.

One-half milliliter of the propagation culture was added to each tube of the diluted agent in Dubos medium in a series. Centrifugation in a Hopkins vaccine tube of the propagation cultures showed the concentration of organisms to average about 0.0007 ml. packed cells per 0.5 ml. of culture.

All tubes in the tests were read at the end of 10 days and again at the end of 2 weeks. There was little difference in the two readings. First, tubes which developed a characteristic pellicle were read as positive for growth and noted. Acid-fast slides were prepared from doubtful positive tubes and also from the most dilute tubes showing macroscopically no growth. These were read "positive" if there were acid-fast bacilli in all fields, and "doubtful" if only a few acid-fast organisms found on the whole smear. Frequently, the microscopic examination resulted in lowering the evaluation of an agent. The compound 4,4'-diaminodiphenylsulfone was used as a standard for the activity of the compounds.

p-Alkoxyaniline Intermediates.—*p*-Phenetidine and *p*-anisidine are commercial products. Following the directions given by Bucchi (14), with the exception of an increased hydrolysis period to 8 hours, the following *p*-alkoxyanilines were prepared: *p*-propoxy (15), *p*-isopropoxy (14), *p*-*n*-butoxy (15), *p*-*n*-amoxy (15). *p*-*sec*-Butoxyaniline was prepared by this method in 80% yield, b.p. 150–151° (23 mm.).

Anal.—Calcd. for $C_{10}H_{15}NO$: N 8.47. Found: N 8.40.

p-*sec*-Amoxyaniline was also prepared by this method in 71% yield, b.p. 161–163° (23 mm.).

Anal.—Calcd. for $C_{11}H_{17}NO$: N 7.86. Found: N 7.79.

Aryl Isothiocyanates.—Phenyl isothiocyanate was a commercial product. The following *p*-alkoxyphenyl isothiocyanates were prepared by the action of sodium chloroacetate on the corresponding dithiocarbamates followed by decomposition of the resulting salt with zinc chloride (12): *p*-methoxyphenyl (12), *p*-ethoxyphenyl (12), *p*-propoxyphenyl (5), *p*-isopropoxyphenyl (5), *p*-butoxyphenyl (5), and *p*-amoxyphenyl (5). Prepared in this same manner was *p*-*sec*-butoxyphenyl isothiocyanate, b.p. 176–178° (28 mm.).

Anal.—Calcd. for $C_{11}H_{13}NOS$: N 6.77. Found: N 6.91.

Also prepared in this fashion was *p*-*sec*-amoxyphenyl isothiocyanate b.p. 157–158° (3mm.).

Anal.—Calcd. for $C_{12}H_{15}NOS$: N 6.36. Found: N 6.27.

2-Pyridyl Thioureas.—A 10-Gm. (0.07 mole) quantity of cyclohexyl isothiocyanate (16) was refluxed with 6.7 Gm. (0.07 mole) of 2-aminopyridine in 15 ml. of thiophene free benzene for 1 hour. The reaction mixture was concentrated through removal of 10 ml. of benzene and allowed to stand overnight. The resulting solid mass was recrystallized from alcohol to yield 10.5 Gm. (63%) of 1-cyclohexyl-3-(2-pyridyl) thiourea, m.p. 147–148°.

Anal.—Calcd. for $C_{12}H_{17}N_3S$: N 17.8. Found: N 17.4.

4-(1-Phenyl-2,3-dimethyl)-5-pyrazolone Thioureas.—*p*-Isopropoxyphenyl isothiocyanate weighing 1.93 Gm. (0.01 mole) and 2.03 Gm. (0.01 mole) of 4-aminoantipyrine were refluxed for ½ hour in 50 ml. of absolute alcohol. The reaction mixture was chilled in ice and filtered to yield after drying in a vacuum desiccator 3.6 Gm. of the thiourea. Concentration of the mother liquor and chilling yielded another 0.3 Gm. for a total of 3.9 Gm. of crude product, m.p. 198–201°. The product recrystallized from methylcellosolve yielded 3.4 Gm. (89%) 1-*p*-isopropoxyphenyl-3-[4-(1-phenyl-2,3-dimethyl)-5-pyrazolone] thiourea, m.p. 202–203° decompn.

Anal.—Calcd. for $C_{21}H_{24}N_4O_2S$: N 14.1. Found: N 14.3.

The pyrazolone thioureas proved to be photosensitive, and upon exposure to light for various periods of time developed a yellow color.

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