Heterocyclic Thioureas II

Antitubercular Activity of Diheterocyclic Thioureas

By ARTHUR C. GLASSER and RICHARD M. DOUGHTY

The preparation and *in vitro* examination for antitubercular activity of a series of heterocyclic substituted (1-phenyl-2,3-dimethyl-5-pyrazolon-4-yl) thioureas are described. The second heterocyclic rings substituted on the molecules are examples of *N*- and *O*-heteroparaffinics, *N*-π deficient and *N*-π excessive rings. The compounds studied ranged in minimum inhibitory concentrations from 0.63 mg. per cent to a lower level of activity of 10 mg. per cent.

IT IS WELL KNOWN that certain 1,3-di(4-substituted phenyl)-thioureas have the ability to inhibit the growth of *M. tuberculosis* (1). Extensive series of related molecules of this general type have been investigated for this activity (2, 3). A previous communication of this series has reported on the activity of a series of 1-*p*-alkoxyphenyl-3-(5-pyrazolon-4-yl)- and (2-pyridyl)-thioureas (4). It was determined in the first study that one of the compounds, 1-*p*-isopropoxyphenyl-3-[(1-phenyl-2,3-dimethyl)-5-pyrazolon-4-yl] -2-thiourea, showed *in vitro* activity at the 0.16 mg. % level. This observation led to the synthesis of a second series of related thioureas in which both substituents of the molecule were of a heterocyclic nature, one of which was the 5-pyrazolone moiety of the before-mentioned compound. This study reports the effects upon the tuberculostatic activity of the second substituent being (*a*) *N* and *O* heteroparaffinic molecules, such as alkyl substituted piperidine, piperazine, and morpholine, (*b*) π-deficient *N*-heteroaromatic rings such as alkyl and dialkyl substituted pyridine, and (*c*) π-excessive *N*-heterocyclic ring such as substituted 5-pyrazolone.

As a group the *N*- and *O*-heteroparaffinics and the π deficient *N*-heterocyclic compounds showed about the same general level of activity as the *p*-alkoxyphenyl compounds reported previously. The most active compound of the present series at 0.63 mg. % was compound *17*. The rings involved here are of the π excessive type, and this type of electron abundance coupled with a thion–thiol enolization type reaction could possibly be used for a comparison to the presence of excessive electrons in the 4-position of such molecules as *p*-isopropoxyphenyl substituted pyrazolone thioureas reported previously. An attempt to place a center of electron density in the 4-position of some of the π-deficient molecules is now underway in this laboratory as a means of further investigating this observation.

The preparative methods used for the thioureas were essentially the same as reported previously in which the isothiocyanate, prepared by the action of thiophosgene on the appropriate amine in methylene chloride, was allowed to react with an amine in absolute alcohol or thiophene free benzene (5). The aminoalkylpyridines, morpholine, piperidine, and methylpiperazine used were commercial products purified by recrystallization or distillation just prior to use. The cyclic secondary amines, 4-ethyl, 4-*n*-propyl, and 4-isopropylpiperidine, were prepared by the reduction of the corresponding 4-alkylpyridine derivative using sodium and *n*-butanol reduction followed by catalytic hydrogenation in the presence of palladium-on-carbon in procedures similar to the method of Wawzonek (6).

The *in vitro* determination of the tuberculostatic activity was carried out generally as previously reported using the H37Rv strain of *M. tuberculosis* var. *hominis*[1] grown on Dubos media with added polysorbate 80[2] and beef serum (4). The insoluble nature of compounds *12* and *13* required the incorporation of methylcellosolve into the solvent used for the preparation of the original dilution for testing. This combined solvent showed inhibiting properties of significant nature, and thus detracted from the validity of the readings compared to those of the regular solvent which had been previously shown to be noninhibiting. However, it was felt that as members of the series it was desirable to have some sort of evaluation of these compounds, so they are included as qualified. A summary of structures, properties, and testing results are shown in Table I.

EXPERIMENTAL

1-Phenyl-2,3-dimethyl-5-pyrazolon-4-isothiocyanate.—A solution of 5.8 Gm. (0.05 mole) of thio-

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[1] American Type Culture Collection, 2112 M Street, N.W. Washington, D. C., 20037.

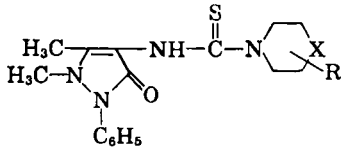
[2] Polyoxyethylene 20 sorbitan mono-oleate. Marketed as Tween 80 by the Atlas Powder Co., Wilmington, Del.

phosgene in 20 ml. of methylene chloride was added dropwise to a stirred solution of 10.2 Gm. (0.05 mole) of 4-aminoantipyrine in 30 ml. of methylene chloride contained in a 250-ml. flask surrounded by an ice bath. The reaction mixture was allowed to warm to room temperature and stand overnight. The resulting solid was filtered off and recrystallized from benzene-ether to give 6.4 Gm. (54%) of needles, m.p. 146 to 147°.

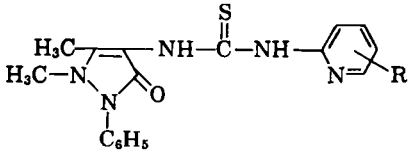
Anal.—Calcd. for $C_{12}H_{11}N_3OS$: C, 58.75; H, 4.52; N, 17.13. Found: C, 58.92; H, 4.50; N, 16.89.

4-Isopropylpiperidine.—A solution of 40.5 Gm. (0.3 mole) of 4-isopropylpyridine in 500 ml. of *n*-butyl alcohol was placed in a three-necked 1000-ml. flask equipped with an efficient condenser and stirrer. The solution was preheated to 50–60° and 70 Gm. of sodium added at such a rate that reflux was maintained. An additional 150 ml. of alcohol was added in portions to maintain the contents of the flask in a fluid condition. After the addition of the sodium was completed, the mixture was heated at reflux for 25 minutes to effect solution of all the sodium. The solution was cooled to about 75° and 200 ml. of ice water slowly added. After cooling to room temperature, the aqueous layer was separated and the alcoholic solution made acid to litmus with 6 *N* hydrochloric acid. The acidic solution was steam distilled to remove all the butyl alcohol, made alkaline to litmus with 10 *N* sodium hydroxide solution, and steam distilled until the distillate was no longer basic to litmus. The basic distillate was saturated with potassium carbonate and the amine extracted with ether. The ethereal solution of the amine was dried over anhydrous sodium sulfate, the ether removed by evaporation at reduced pressure, and the residue fractionated through a glass helices column to yield 8.5 Gm. of 4-isopropyl-1,2,5,6-

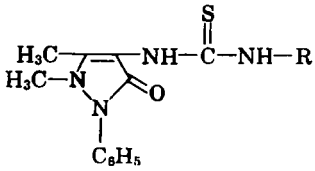
TABLE I.—DIHETEROCYCLIC THIOUREAS



No.	R	X	Yield, %	RS[c]	M.p., °C.[a]	Formula	*N*-Analyses[b] Calcd.	Found	Min. Inhib. mg. %
1	H	CH_2	62	Ac	188–189[e]	$C_{17}H_{22}N_4OS$	16.95	17.15	2.5
2	2-Methyl	CH_2	91	A	170–171	$C_{18}H_{24}N_4OS$	16.16	14.90	2.5
3	3-Methyl	CH_2	90	A	175–176	$C_{18}H_{24}N_4OS$	16.26	15.91	5.0
4	4-Methyl	CH_2	93	A	184–185	$C_{18}H_{24}N_4OS$	16.26	16.01	2.5
5	2,6-Dimethyl	CH_2	95	A	174–175	$C_{19}H_{26}N_4OS$	15.63	15.49	1.25
6	2-Ethyl	CH_2	85	A	149–150	$C_{19}H_{26}N_4OS$	15.63	15.74	5.0
7	4-Ethyl	CH_2	91	A	168 to 168.5	$C_{19}H_{26}N_4OS$	15.63	15.41	5.0
8	4-*n*-Propyl	CH_2	85	A	147 to 147.5	$C_{20}H_{28}N_4OS$	15.02	15.16	5.0
9	4-*i*-Propyl	CH_2	90	A	188–189	$C_{20}H_{28}N_4OS$	15.02	14.80	10.0
10	H	O	79	B	211–212[d]	$C_{16}H_{20}N_4OS$	16.81	16.46	5.0
11	H	$=N-CH_3$	70	C	168–169	$C_{17}H_{23}N_5OS$	20.27	20.59	2.5



12	H		65	A	248–249	$C_{17}H_{17}N_5OS$	20.63	20.41	0.63
13	4-Methyl		87	A	222–223	$C_{18}H_{19}N_5OS$	19.81	19.75	2.5
14	5-Methyl		73	A	240 to 241.5	$C_{18}H_{19}N_5OS$	19.81	19.47	2.5
15	6-Methyl		76	A	213 to 214.5	$C_{18}H_{19}N_5OS$	19.81	19.63	5.0
16	4,6-Dimethyl		86	A	223–224	$C_{19}H_{21}N_5OS$	19.06	19.17	2.5



17	4-Antipyryl		81	D	245–247 dec.	$C_{23}H_{24}N_6O_2S$	18.72	18.51	0.63

[a] Melting points determined on Fisher-Johns block and uncorrected. [b] Microanalyses by Weiler and Straus, Oxford, England and Galbraith Laboratories, Knoxville, Tenn. [c] Reported, Takahaski, T., and Kanematsa, K., *Yakugaku Zasshi*, **79** 162(1959), 190°. [d] Reported, *ibid.*, 213°, through *Chem. Abstr.*, **53**, 13138e(1959). [e] Recrystallization solvents: Ac—acetone, A—ethyl alcohol, B—benzene, C—chloroform, and D—methyl alcohol.

tetrahydropyridine, b.p. 176 to 177.5° at 738 mm., taken as a heart cut for a yield of 20%. Picrate 170–171°, (Lit. picrate 170 to 170.5°) (7).

Anal.—Calcd. for $C_8H_{15}N$: C, 76.74; H, 12.07; N, 11.19. Found: C, 76.83; H, 12.01; N, 10.93.

A solution of 8 Gm. of 4-isopropyl-1,2,5,6-tetrahydropyridine in 30 ml. of glacial acetic acid was subject to hydrogenation at 45 lb./in.^{2} in the presence of 2.5 Gm. of 10% palladium-on-charcoal until hydrogen was no longer taken up. After filtering off the catalyst, the solution was made alkaline to litmus with 10 *N* sodium hydroxide solution, and the alkaline solution steam distilled until the distillate was neutral to litmus. The distillate was saturated with potassium carbonate, and the amine separated and dried over potassium hydroxide pellets. Careful fractionation through a glass helices column resulted in 5.6 Gm. (69%) of 4-isopropylpiperidine, b.p. 171–172° at 733 mm., n_D^{20} 1.4577, chloroplatinate 172–173°. (Lit. b.p. 168–171°, chloroplatinate 172°) (8).

Anal.—Calcd. for $C_8H_{17}N$: C, 75.52; H, 13.47; N, 11.01. Found: C, 75.38; H, 13.42; N, 11.22.

1,1-(3-Isopropylpentamethylene)-3-(1-phenyl-2,3-dimethyl-5-pyrazolon-4-yl)-2-thiourea.—A solution of 0.57 Gm. (0.05 mole) of 4-isopropylpiperidine and 1.0 Gm. (0.04 mole) of 1-phenyl-2,3-dimethyl-5-pyrazolon-4-isothiocyanate in 10 ml. of absolute alcohol was refluxed on a steam bath for 2 hours. After evaporation of the reaction mixture to one-third its original volume, crystals formed on cooling. Filtration and recrystalization from alcohol gave 1.35 Gm. (90%) of the thiourea, m.p. 188–189°.

Anal.—Calcd. for $C_{20}H_{28}N_4OS$: C, 64.48; H, 7.57; N, 15.02. Found: C, 64.67; H, 7.61; N, 14.80.

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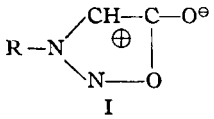
Prediction of Stability in Pharmaceutical Preparations XII

Solvolysis of Various Alkyl Sydnones

By EDWARD R. GARRETT

The kinetics of solvolysis of various alkyl sydnones—furfuryl, methyl, propyl, phenethyl, isopropyl, *s*-butyl, *t*-butyl, and 1,1,3,3 tetramethylbutyl, have been studied spectrophotometrically as a function of pH and temperature; rate-pH profiles have been determined. The order of reactivity on alkaline catalyzed solvolysis generally decreased in the order cited. Except for the furfuryl sydnone, the order of reactivity reversed on acid catalyzed solvolysis. The heats of activation for a specific type solvolysis were generally similar, and the greatest differences were in the entropies of activation. Only in the specific cases of 1,1,3,3-tetramethylbutyl, *t*-butyl, and furfuryl sydnones was a solvent or pH-independent solvolysis observed. In the alkaline pH range, the spectra shifts to that of the *N*-nitroso alkylamino acetic acid, whereas in acid solution absorbance is lost with time.

VARIOUS *meso*-IONIC 3-alkyl sydnones (1, 2) have been synthesized in these laboratories



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by Kier and associates (3, 4) as potential therapeutic agents. They are pharmacologically active as central nervous system stimulants with a particularly stimulating effect on respiration. Several also possess antitumor activity (5).

It has been well known that the sydnones are hydrolyzed by hot aqueous alkali with regeneration of the original *N*-nitroso acid from which they are prepared and by hot acid to give a R-hydrazine, a carboxylic acid, and carbon dioxide (2, 6, 7).

It has also been stated that the stability of the sydnone ring, I, does not appear to depend to a significant extent on the nature of R (7). However, our preliminary kinetic studies had shown that this was not the case—that even

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