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Inhibition of Clinically Significant Bacterial Organisms In Vitro by 2-Acetylpyridine Thiosemicarbazones

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Antibacterial activity of 65 2-acetylpyridine thiosemicarbazones and related compounds was determined by using clinical isolates of nine bacterial genera. Minimal inhibitory concentrations (MICs) of 0.002 to 0.062 μg/ml were obtained with 23% of the compounds for *Neisseria gonorrhoeae* and 0.016 to 0.062 μg/ml with 17% of the compounds for *N. meningitidis*. *Staphylococcus aureus* was inhibited in the MIC range of 0.125 to 0.5 μg/ml by 18% of the thiosemicarbazones, whereas 26% inhibited group D enterococcus with an MIC of 0.25 to 2.0 μg/ml. Poor antibacterial activity was shown toward the gram-negative bacilli, i.e., *Pseudomonas*, *Klebsiella-Enterobacter*, *Shigella*, *Escherichia coli*, and *Proteus*.

2-Acetylpyridine thiosemicarbazones have been found by Klayman et al. (4, 5) to exhibit antimalarial activity in mice infected with *Plasmodium berghei* (Fig. 1). In these studies, it was noted that such activity was limited to those compounds in which the alkylidene group is attached to the 2-position, rather than the 3- or 4-position, of the pyridine ring, and also to those in which a thiocarbonyl, rather than a carbonyl group, is present. The incorporation of N^4 of the thiosemicarbazone moiety into a six- or seven-membered ring appears to be important for optimization of antimalarial properties.

Other classes of thiosemicarbazones are reported to be inhibitory to *Mycobacterium tuberculosis* (1, 9, 10), *M. leprae* (9), *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and enterococcus (6). Accordingly, an investigation was initiated to examine the potential antibacterial activity of a representative group of 2-acetylpyridine thiosemicarbazones as well as several closely related compounds. The potential advantage of such a group of compounds is that they are not found in nature and, therefore, the existing bacterial population would not have had an opportunity to develop resistance by prior exposure.

(This work was presented in part at the 79th Annual Meeting of the American Society for Microbiology, 4-8 May 1979, Los Angeles, Calif.)

MATERIALS AND METHODS

Organisms. Five clinical isolates of each of the following bacterial genera were obtained from the Clinical Microbiology Laboratory, Walter Reed Army Medical Center: *S. aureus*, group D enterococcus, *Pseudomonas* spp. (*P. aeruginosa*, *P. fluorescens*), *Klebsiella-Enterobacter* spp. (*K. pneumoniae*, *E. aerogenes*), and *Proteus mirabilis*. S. B. Formal, Department of Bacterial Diseases, Walter Reed Army Institute of Research, kindly provided cultures of *Shigella dysenteriae* 1 (smooth), *S. flexneri* 2a, a *Shigella* hybrid X16 (2), *S. dysenteriae* 60R (rough), and an invasive *Escherichia coli* strain, 10673/76. Five *Neisseria meningitidis* isolates and five *N. gonorrhoeae* isolates were obtained from the collection of the Department of Bacterial Diseases. An additional 30 *N. gonorrhoeae* isolates, which included 15 β-lactamase producers, were kindly provided by C. Thornsberry, Communicable Disease Center, Atlanta, Ga.

Test compounds. The 2-acetylpyridine thiosemicarbazones were prepared by the Organic Synthesis Section of the Division of Experimental Therapeutics. They include 26 N^4-monosubstituted (4) and 24 N^4,N^4-disubstituted (5) derivatives, 9 thiosemicarbazone derivatives of other 2-acylpyridines, and 6 related compounds. In addition, penicillin G and ampicillin were used as antibiotic comparisons to determine minimal inhibitory concentrations (MICs) for *N. gonorrhoeae* and *N. meningitidis*, respectively.

To determine structure-activity relationships, the thiosemicarbazones are divided as follows: type A includes those in which N^4 is monosubstituted (Table 1); type B includes those which are disubstituted at N^4 by alkyl or cycloalkyl groups (Table 2); type C includes those in which the N^4 atom is incorporated in a heterocyclic ring system (Table 3); type D includes 2-acyl (other than acetyl) pyridine thiosemicarbazones (Table 4); and type E includes miscellaneous com-

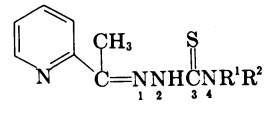
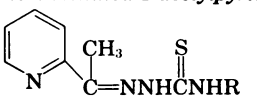


FIG. 1. *Chemical structure of 2-acetylpyridine thiosemicarbazones.*

TABLE 1. *MICs of N^4-monosubstituted 2-acetylpyridine thiosemicarbazones*

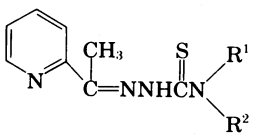


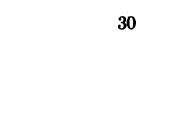
Compound	R	MIC (μg/ml) for:								
		S. aureus	Group D enterococcus	*Pseudomonas*	*Klebsiella-Enterobacter*	*Shigella*	*E. coli*	*Proteus*	*N. meningitidis*	*N. gonorrhoeae*
1	H	>16	>16	>512	128–512	64–256	256	256–512	>1	0.25–0.5 (0.5)[a]
2	CH_3	>16	>16	256–>512 (>512)	128–512	32–128	32	256–512 (512)	0.5–1 (0.5)	0.5
3	C_2H_5	>16	>16	>512	256–>512	32–128	>512	>512	0.25–0.5 (0.25)	0.5
4	C_3H_7	2–4	>16	>512	>512	64–256 (64)	>512	>512	0.25–0.5	0.5–1 (0.5)
5	$CH_2CH{=}CH_2$	2–8	8	>512	>512	32–256	>512	>512	0.5–1	0.062–0.25 (0.125)
6	$CH_2C{\equiv}CH$	>16	>16	>512	>512	64–>512	>512	>512	0.25–0.5	0.25–0.5 (0.5)
7	C_4H_9	1–4	>16	>512	>512	>512	>512	>512	0.125–0.25	0.062–0.25 (0.125)
8	C_5H_{11}	1	4–8 (4)	>512	>512	512–>512	>512	>512	0.25–0.5 (0.25)	0.031–0.125 (0.062)
9	C_6H_{13}	2–4 (2)	2	>512	>512	>512	>512	>512	0.25	0.062–0.5 (0.125)
10	C_7H_{15}	1–4	2	>512	>512	>512	>512	>512	0.25–1 (0.25)	0.125–0.25 (0.125)
11	C_8H_{17}	4–>16 (>16)	8–16	>512	>512	>512	>512	>512	0.125–0.5	0.25–0.5 (0.5)
12	1,1,4,4-Tetramethylbutyl	4–8	4	128–>512 (>512)	>512	>512	>512	>512	0.5–1 (1)	0.25–1 (0.5)
13	$C_{10}H_{21}$	>16	>16	>512	>512	>512	>512	>512	0.25–0.5 (0.25)	1–>1 (>1)
14	$C_6H_5CH_2$	2–4 (2)	>16	>512	>512	>512	>512	>512	0.5	0.062–0.25 (0.125)
15	$2\text{-}CH_3C_6H_5CH_2$	1–2 (2)	16–>16 (16)	>512	>512	256–>512 (>512)	>512	256–>512 (>512)	0.125–0.25	0.062–0.5 (0.125)
16	$C_6H_5CH_2CH_2$	1	4–8 (8)	>512	>512	512–>512 (>512)	>512	>512	0.125–0.25	0.125–0.25 (0.25)
17	$C_6H_5CH_2CH_2CH_2$	2	1–4	>512	>512	>512	>512	>512	0.125–0.25	0.031–0.125
18	C_6H_5	8	8	>512	>512	512–>512 (>512)	>512	>512	0.25–>1 (>1)	0.031–0.25 (0.125)

19	3-FC_6H_4	8–16 (16)	16	>512	>512	>512	>512	>512	0.25	0.125–0.5 (0.125)
20	4-ClC_6H_4	>16	16–>16 (>16)	>512	256–>512 (>512)	64–512	512	64–512 (512)	0.25–1	0.004–0.062 (0.031)
21	4-MeC_6H_4	16–>16 (16)	>16	>512	128–>512	128–256 (128)	128	128–256 (128)	0.125–1	0.031–0.25 (0.062)
22	4-$F_3CC_6H_4$	>16	>16	>512	>512	>512	>512	>512	0.5–>1	0.125–0.5 (0.25)
23	2-Pyridyl	>16	>16	512–>512	256–>512	128–>512 (512)	>512	128–512	>1	0.125–0.25 (0.25)
24	2-Picolyl	>16	>16	>512	>512	256–>512	>512	>512	1–>1 (>1)	0.25–0.5 (0.25)
25	Cyclohexyl	0.5–1 (0.5)	2–>8 (8)	>512	>512	>512	>512	>512	0.25	0.062–0.25 (0.125)
26	1-Adamantyl	1–4 (1)	1–2 (2)	>512	256–>512 (>512)	>512	>512	256–>512	0.25–1 (0.5)	0.125–1 (0.5)

[a] Numbers in parentheses are the predominant values in the MIC range.

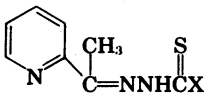
TABLE 2. *MICs of N^4,N^4-disubstituted (noncyclic) 2-acetylpyridine thiosemicarbazones*

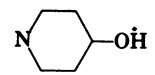
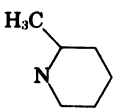
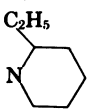
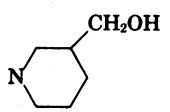
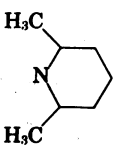
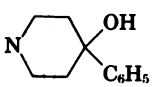
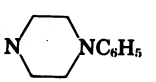


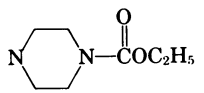
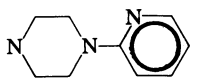
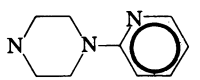
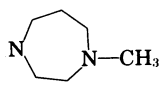
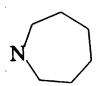
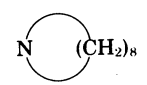
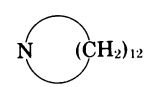
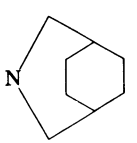
Compound	R^1	R^2	MIC (μg/ml) for:								
			S. aureus	Group D enterococcus	*Pseudomonas*	*Klebsiella-Enterobacter*	*Shigella*	*E. coli*	*Proteus*	*N. meningitidis*	*N. gonorrhoeae*
27	CH_3	CH_3	>16	>16	256–512 (256)[a]	128–256 (128)	64–128	64	64–128 (64)	0.062–0.125 (0.062)	0.002–0.008 (0.004)
28	C_2H_5	C_2H_5	2	8–>16 (>16)	128	128–512	32–64	128	128	0.031–0.062	0.016–0.062 (0.031)
29	Isobutyl	Isobutyl	0.5–1	1–4 (2)	64–128	64–512 (64)	64–256	64	64–128	0.062–0.5 (0.062)	0.25–0.5 (0.5)
30	CH_3		>16	>16	256	256–>512	128–256	512	256	>1	>1
31	CH_3	Cyclohexyl	1	1	64	128–256	64	64	64–128	0.031–0.062	0.031–0.125 (0.062)
32	CH_3	Cyclooctyl	0.25–0.5	0.5–1 (0.5)	64–128	32–>512	64–512	64	64	0.062–0.125	0.062–0.25 (0.125)

[a] See Table 1.

TABLE 3. *MICs of N^4,N^4-disubstituted (azacyclic) 2-acetylpyridine thiosemicarbazones*

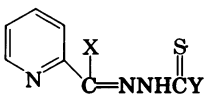


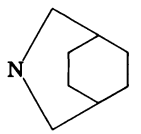
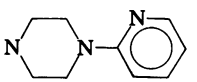
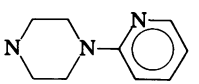
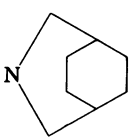
Compound	X	*S. aureus*	Group D enterococcus	*Pseudomonas*	*Klebsiella-Enterobacter*	*Shigella*	*E. coli*	*Proteus*	*N. meningitidis*	*N. gonorrhoeae*
33		0.5	>16	128	64–256	32–128	128	64–128	0.016–0.031 (0.031)[a]	0.008–0.062
34		0.5	1–2 (1)	256–>512 (256)	>512	64–128 (128)	128	128–512	0.031–0.062 (0.031)	0.016–0.062 (0.031)
35		>16	>16	256	128–512	64–128	128	128	>1	0.031–0.125
36		1	4–>16	128–256	128–256 (256)	128	128	128	0.031–0.125 (0.062)	0.016–0.062 (0.031)
37		0.5	1	128	128–256	64–128 (128)	128	128	0.062–0.125 (0.062)	0.5
38		>16	>16	128–256 (256)	128–256	32–64 (64)	64	128	0.5–>1 (>1)	0.031–0.125 (0.062)
39		>16	>16	256	256–>512	256–>512 (>512)	>512	512	>1	0.25–0.5 (0.5)
40		2	4	64	128–256	64–128 (128)	128	128–256 (128)	0.062–0.125	0.031–0.062 (0.062)
41		16–>16	>16	>512	>512	>512	>512	>512	0.031–0.125	0.004–0.031 (0.016)

42		>16	>16	512->512 (>512)	256->512 (>512)	128->512 (>512)	>512	256->512	0.062–0.5	0.031–0.062 (0.031)
43		2–4 (4)	>16	64->512 (128)	256->512 (>512)	>512	>512	>512	0.062–0.25 (0.125)	0.004–0.031 (0.016)
44	 ·2HCl[b]	4–8 (8)	>16	128->512 (128)	128–256 (256)	128–256 (128)	256	256–512 (256)	0.25–0.5 (0.5)	0.031–0.125 (0.031)
45		2	4	128->512 (128)	128–256 (256)	64–256 (128)	128	128	0.031–0.125	0.016–0.062 (0.031)
46		16	>16	128–256	128–256	64–128 (64)	128	128	>1	0.062–0.25 (0.125)
47		0.5	0.5–1	64–128	64->512 (>512)	64–256	64	32	0.062–0.125 (0.062)	0.016–0.125 (0.062)
48		0.5	0.5	64–128	128	32–64 (64)	64	64	0.062–0.125	0.062–0.125 (0.125)
49		0.5–2	0.5	512->512	256->512	64	128	128–256	0.031–0.125	0.5–1 (0.5)
50		0.25	2	128	64–128 (64)	64	64	64–256 (128)	0.031–0.125	0.008–0.031 (0.016)

[a] See Table 1.
[b] Water soluble.

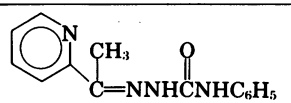
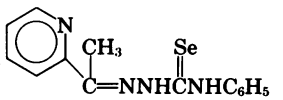
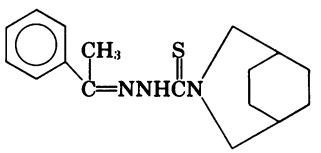
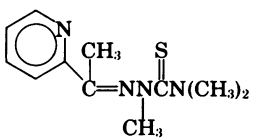
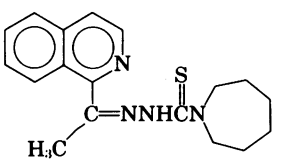
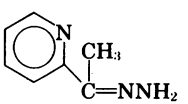
TABLE 4. *MICs of derivatives of other 2-acylpyridine thiosemicarbazones*



Compound	X	Y	MIC (μg/ml) for:								
			S. aureus	Group D enterococcus	*Pseudomonas*	*Klebsiella-Enterobacter*	*Shigella*	*E. coli*	*Proteus*	*N. meningitidis*	*N. gonorrhoeae*
51	C_2H_5	$NHCH_2CH{=}CH_2$	2–4	>16	>512	>512	64–>512	>512	>512	0.125–0.25	0.25–0.5
52	C_2H_5	$N(CH_3)_2$	0.5–1 (1)[a]	8–16 (16)	128–256 (256)	128–256	32–128	128	64–128	0.031	0.008–0.062 (0.016)
53	C_2H_5		0.25–0.5	0.5	128	64–256	32–64	64	64	0.031–0.062	0.031–0.125 (0.062)
54	C_2H_5		0.125	0.25	64	64–512	>512	>512	32–64	0.016–0.031 (0.016)	0.062–0.125 (0.062)
55	C_2H_5		0.5–2	1	64–128	128–256	64	64	64	0.016–0.062	0.031–0.062 (0.062)
56	$CH(CH_3)_2$		0.5	0.5	64	64–>512	64	64	64–128 (64)	0.062	0.031–0.125 (0.062)
57	$CH(CH_3)_2$		0.5–1 (0.5)	16	256	512–>512 (>512)	64–256	128	512	0.016–0.062	0.008–0.016 (0.016)
58	$CH(CH_3)_2$		0.5	0.5	64–128	256–512	64	64	256	0.031–0.062 (0.062)	0.031–0.125
59	$CH(CH_3)_2$		0.125–0.25	0.5–1	>512	64–>512	64	64	512	0.062–0.125	0.125–0.25 (0.125)

[a] See Table 1.

TABLE 5. *MICs of miscellaneous compounds related to 2-acylpyridine thiosemicarbazones*

Compound	Structure	MIC (μg/ml) for:								
		S. aureus	Group D enterococcus	*Pseudomonas*	*Klebsiella-Enterobacter*	*Shigella*	*E. coli*	*Proteus*	*N. meningitidis*	*N. gonorrhoeae*
60		>16	>16	>512	>512	512->512 (>512)[a]	>512	>512	>1	>1
61		4–8 (4)	16->16 (>16)	>512	>512	>512	>512	>512	1->1 (1)	1->1 (1)
62		>16	>16	>512	>512	64->512 (>512)	>512	>512	>1	>1
63		>16	>16	>512	>512	>512	>512	>512	>1	>1
64		4–8 (8)	8–16 (8)	64–128	256	64	64	128–256	0.25–1 (1)	0.062–0.5 (0.25)
65		>16	>16	128–512	512	128->512	512	512	>1	>1

[a] See Table 1.

pounds related to 2-acetylpyridine thiosemicarbazones (Table 5).

Testing procedure. The standard macro-broth dilution method described by Washington and Barry (11) was used to determine MICs for all microorganisms tested except the *N. gonorrhoeae* cultures, for which the agar plate dilution method (11) was used. Mueller-Hinton broth (Difco Laboratories) with 1% of a modified defined supplement (12) was used for the agar plate dilution procedure. This supplement consisted of 81 ml of aqueous solution A (containing 40.5 g of dextrose and 0.002 g of cocarboxylase) which was mixed with 19 ml of aqueous solution B [83 mg of $Fe(NO_3)_3 \cdot 9H_2O$ and 1 g of L-glutamine]. It was then filter sterilized and kept frozen until used.

Because of the poor water solubility of the test compounds, it was necessary, with only one exception (compound 44), to dissolve them initially in dimethyl sulfoxide (DMSO). The control tubes or plates also contained the highest concentration of DMSO present in each particular dilution series. Further dilutions were done by using Mueller-Hinton broth or, in the case of *N. gonorrhoeae* testing, GC broth (3) with defined supplement.

The concentrations of the test compounds were twofold dilutions from 16 to 0.25 μg/ml for the gram-positive bacteria, 512 to 32 μg/ml for the gram-negative enteric bacteria, and 1 to 0.002 μg/ml for the two *Neisseria* species.

In the *N. gonorrhoeae* testing, the 0.5 MacFarland standard was used to standardize each inoculum in GC broth from an overnight agar plate culture. This inoculum was diluted 20-fold (11) and applied to the thiosemicarbazone-containing plates and the controls, using a Lidwell apparatus (7).

Cultures were incubated under appropriate atmospheric conditions for 24 h at 37°C and then read.

RESULTS

The antibacterial data have been organized according to the five chemical structure types mentioned previously (Tables 1–5) for each organism.

S. aureus. An MIC range of 0.125 to 0.5 μg/ml was selected to ascertain those compounds with the most promising activity. None of the type A compounds were inhibitory; 1 of 6 type B, 6 of 18 type C, 5 type D, and no type E compounds were in the selected range.

Group D enterococcus. With the criterion of 0.25 to 2 μg/ml as the MIC range, there were three compounds of type A, two of type B, six of type C, six of type D, and no type E compounds in this category.

In contrast to the MIC values for the gram-positive microorganisms mentioned above, those compounds that were considered the most promising for the gram-negative bacillus genera had at least 250- to 2,000-fold greater MICs, i.e., 32 to 256 μg/ml at best. There were no significant differences in MICs for the different species within each particular genus tested.

***Pseudomonas* spp.** None of the type A compounds had this MIC range, i.e., 32 to 256 μg/ml, whereas four type B compounds, six type C, five type D, and one type E were so designated.

***Klebsiella-Enterobacter* spp.** No type A or type E compounds were within the same MIC range, but two type B, ten type C, and three type D compounds were found to have this designation.

***Shigella* spp.** Within the MIC range of 32 to 64 μg/ml, none of the compounds were type A, but two were type B, four were type C, five were type D, and one was type E.

E. coli. The invasive isolate tested had an MIC profile involving one type A compound, four type B compounds, four type C compounds, five type D compounds, and one type E compound, all of which were no greater than 64 μg/ml.

***Proteus* sp.** None of the type A or E compounds, but one type B compound, two type C compounds, and three type D compounds, had MIC values no greater than 64 μg/ml.

The *Neisseria* species were by far the most susceptible of all the isolates tested. A majority of the 2-acylpyridine thiosemicarbazones had an MIC profile of ≤0.25 μg/ml for these cultures.

N. meningitidis. The MIC values of 0.016 to 0.062 μg/ml were selected as the range for the most promising inhibitory activity. No type A or type E compounds had these values, but two type B compounds, two type C compounds, and seven type D compounds did. These cultures had an ampicillin MIC range of 0.008 to 0.016 μg/ml.

N. gonorrhoeae. For these isolates, the MIC profile of 0.002 to 0.062 μg/ml was selected for the compounds tested. Of these, one type A, two type B, nine type C, three type D, and no type E compounds were in this range. The penicillin-susceptible and -resistant strains were inhibited to the same extent by the test compounds. The penicillin-susceptible cultures had an MIC range of 0.031 to 1 μg/ml for penicillin G, whereas the penicillin-resistant isolates had MIC values of 2 to 4 μg/ml.

There was no inhibition of the bacterial cultures in any of the DMSO-containing control tubes and plates.

DISCUSSION

To evaluate the 2-acetylpyridine thiosemicarbazones and related compounds, the term "prime" was applied to describe the most active inhibitors for a particular organism group. These compounds were chosen for MIC levels which include no more than 25% of the test agents. A survey of the activity of the prime thiosemicar-

TABLE 6. *Compilation of prime[a] 2-acetylpyridine thiosemicarbazones and related compounds*

Organism	A[b]			B				C										D							E	MIC (μg/ml)[c]
	2[d]	9	10	20	26	27	28	29	31	32	33	34	36	37	38	40	41	42	43	44	45	46	47	48	49	50	52	53	54	55	56	57	58	59	65	
S. aureus										x	x	x		x									x	x		x		x		x		x	x		0.5
Group D			x	x		x			x	x		x		x									x	x	x	x	x	x	x	x		x	x		2
Enterococcus																																				
Pseudomonas								x	x	x	x	x			x		x						x	x		x		x	x	x		x		x	128
Klebsiella-						x			x		x		x	x	x	x				x	x	x		x			x		x		x				256
Enterobacter																																				
Shigella							x		x						x									x	x		x		x	x		x	x	x	64
E. coli		x				x		x	x	x					x								x	x		x		x	x		x	x	x	64
Proteus										x													x	x			x	x	x						64
N. meningitidis							x		x		x	x															x	x	x	x	x		x		0.062
N. gonorrhoeae				x		x	x				x	x	x			x	x	x	x		x					x	x		x		x				0.062

[a] Prime compounds for a particular organism are those which are most inhibitory and are selected by starting with the lowest MIC and proceeding stepwise to higher values until no more than 25% of the test compounds are identified.

[b] Compound type.

[c] Maximum MIC determinant for prime compounds.

[d] Compound number.

bazones (Table 6) revealed that the N,N-disubstituted compounds had considerably higher activity than those which were N-monosubstituted. Of the 65 compounds that we examined, 7 had a broad antibacterial spectrum; i.e., they exhibited good inhibition of at least five of the organism groups. These compounds, 31, 33, 48, 50, 53, 55, and 56, have in common N,N-disubstitution and, except for compound 31, have N^4 included in a ring system of five to nine atoms.

It is of interest that a one- or two-carbon extension of the alkylidene chain attached to the pyridine ring (specifically 45 → 53 and 56; 43 → 53) served in two instances to broaden the activity of the parent compound. This trend, however, was not observed with compound 50, where extension of the 2-pyridylethylidene chain (as in 54 and 59) produced the opposite effect. Preliminary toxicity data obtained in mice (M. Grenan, Walter Reed Army Institute of Research, personal communication) indicate that extension of the alkylidene chain by one methylene group has a beneficial effect in that it raises the 50% lethal dose when the compound is administered by the intraperitoneal, subcutaneous, or oral route.

S. aureus was inhibited by the prime compounds in the MIC range of 0.25 to 0.5 μg/ml. Virtually all of these compounds were types C and D, in which the N^4 atom of the thiosemicarbazone moiety is incorporated into an N-containing ring.

The prime compounds, in the case of group D enterococcus, were effective in the range of 0.25 to 2 μg/ml. Activity seems to be distributed through all chemical types; however, N^4,N^4-disubstitution is favored over N^4-monosubstitution.

Inhibition of the gram-negative bacilli, namely, *Pseudomonas*, *Klebsiella-Enterobacter*, *Shigella*, *E. coli*, and *Proteus*, by the compounds under investigation was at such high concentrations as to reduce their promise as therapeutic agents.

It is against *N. menigitidis* and *N. gonorrhoeae*, however, that the 2-acetylpyridine thiosemicarbazones are most active. Inhibition of the former species by a majority of the compounds tested was achieved with an MIC of ≤0.125 μg/ml. Furthermore, the prime compounds, which include virtually all of chemical type D, were capable of inhibiting *N. meningitidis* in the range of 0.016 to 0.062 μg/ml. *N. gonorrhoeae* organisms were even more susceptible, some 41% of which have an MIC of ≤0.125 μg/ml. The prime compounds, most of which come from chemical type C, were active in the range of 0.008 to 0.062 μg/ml. Compound 27, the most

inhibitory, has an MIC range of 0.002 to 0.008 μg/ml. That the penicillin-susceptible and -resistant (i.e., β-lactamase producing) strains were inhibited to the same extent by the thiosemicarbazones is not unexpected in view of the absence of a β-lactam ring in their structure. Of the two groups of *Neisseria*-inhibiting prime compounds, there are only six in common, i.e., compounds 28, 33, 34, 52, 55, and 57.

Increasing the water solubility of the 2-acetylpyridine thiosemicarbazones by modification of their structure to include a sugar moiety (compound 30) or by preparation of the dihydrochloride salt (see compound 44 versus 43) did not serve to impart greater antibacterial activity but, in fact, resulted in compounds with reduced activity.

A comparison of thiosemicarbazone 18 with the corresponding semicarbazone 60 and selenosemicarbazone 61 suggests that such modifications of the thiocarbonyl group do not improve antibacterial activity. The importance of the pyridine ring is demonstrated by a comparison between the 2-acetyl pyridine analog 50 and its acetophenone analog 62. The replacement of the nitrogen atom in the aromatic ring by a carbon atom converts a broad-spectrum prime compound into one which is inactive. Compound 27, where N^4 is substituted by two methyl groups, is particularly inhibitory toward *N. gonorrhoeae*. However, placement of an additional methyl group on the N^2 of the thiosemicarbazone moiety (compound 63) eliminated activity. Exchange of 2-acetylpyridine by 1-acetylisoquinoline (which may be viewed as a 2-acetylbenzo[c]pyridine) as in comparable compounds 45 and 64, respectively, causes diminution of antibacterial activity. Finally, 2-acetylpyridine hydrazone, 65, which is part of the 2-acetylpyridine thiosemicarbazone molecule, through its demonstrated lack of activity illustrates the importance of the thiocarbamoyl [$R^1R^2NC(=S)$] moiety.

From these data, it appears that there is potential clinical application for several 2-acetylpyridine thiosemicarbazones, especially those which are N^4,N^4-disubstituted, against infections involving *N. gonorrhoeae*, *N. meningitidis*, *S. aureus*, and group D enterococci. There appears to be less clinical applicability for these compounds against the gram-negative bacilli tested.

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