2-Acetylpyridine Thiosemicarbazones and *Mycobacterium leprae*[1]

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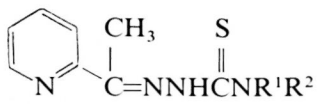
Thiacetazone (amithiozone, TB1, 4'-formylacetanilide thiosemicarbazone) was introduced in the treatment of leprosy by Lowe ([7]). Many patients relapsed, however, after being treated with thiacetazone (as single-drug therapy) for several years. Because of its limited activity and because of the incidence of side effects, use of the drug in leprosy largely stopped, and its chief use is now as a secondary drug in the treatment of tuberculosis in certain countries. The most extensive experimental studies of thiacetazone in mice infected with *Mycobacterium leprae* have been carried out by Colston, *et al.* ([2]), who have also reviewed the earlier work. They found that the usual minimal effective dosage was 0.03% in the diet, corresponding to a minimal inhibitory concentration of 0.2 μg/ml in the serum.

Recently, a number of newly synthesized 2-acetylpyridine thiosemicarbazones (see figure below)



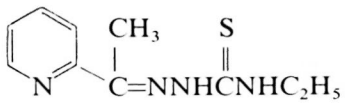
were reported to have considerable antimalarial ([4, 5]) and antimicrobial ([3]) activity. Studies with mycobacteria showed that compounds with appropriate lipophilicities had minimal inhibitory concentrations of less than 1 μg/ml for certain cultures ([1, 8]). Preliminary results with *M. leprae* in mice indicated distinct activity which for the most active compounds exceeded that of thiacetazone ([8]).

Using the kinetic method for studies of drugs against *M. leprae*, we have now retested four of the more promising compounds together with thiacetazone itself.

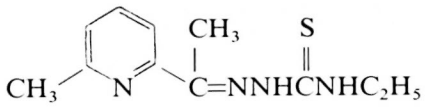
MATERIALS AND METHODS

Details of the animal methods have already been published ([9, 10]). In brief, CFW female mice were inoculated in the foot pad with 5000 *M. leprae* in mouse passage, and the bacterial growth curve was monitored by counts of acid-fast bacteria in the infected foot pads of four individual mice from the control group (untreated) at 28-day intervals beginning on day 70. The drugs were administered in a dosage of 0.05% in the diet from day 70 through day 126 (The Figure). On day 126 and again on day 154, counts were performed on four individual mice from all of the groups. The significance of the differences between groups was estimated by the two-sample rank test ([10]).

The new drugs tested were 2-acetylpyridine 4-ethylthiosemicarbazone (**A**), 2-acetyl-6-methylpyridine 4-ethylthiosemicarbazone (**B**), 2-acetylpyridine 4-allylthiosemicarbazone (**C**), and 2-acetyl-6-methylpyridine 4-allylthiosemicarbazone (**D**) ([6]). All of the compounds were ground in a mortar and passed through a



A



B

C

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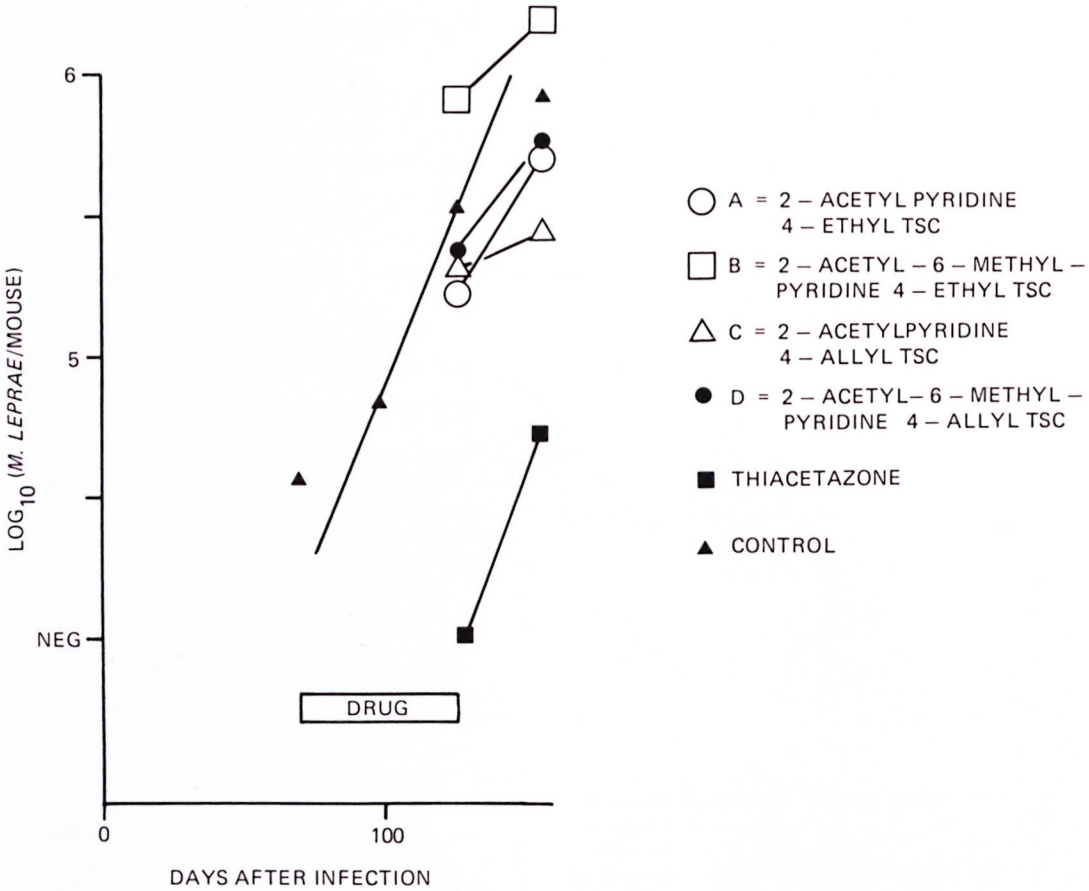
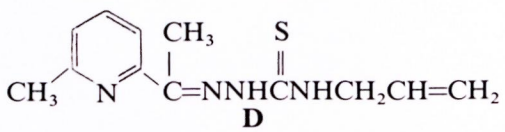


THE FIGURE. Activity of thiosemicarbazones for *M. leprae*. Drugs were administered in a dosage of 0.05% for the period shown. Line drawn for the control is for a generation time of 12.5 days through the average near the end of the logarithmic phase. Other lines connect the averages for each drug. Values for individual mice are not shown, but were used to calculate the probability values presented in The Table.



D

200 mesh sieve in order to ensure uniform particle size.

RESULTS

Three of the new drugs had minimal activity, at most, and all four were significantly less active than thiacetazone (The Figure, The Table). The results for thiacetazone are in agreement with those previously obtained by the kinetic method by Colston, *et al.* ([2]) with a dosage of 0.03% in the diet for 60 days. A dosage of 0.1% is needed to produce growth delay that is distinctly in excess of the period of drug administration.

DISCUSSION

We are unable to account for the apparent discrepancy between the present results and

THE TABLE. *Activity of thiosemicarbazones for* M. leprae.

Thiosemicarbazones[a]	Growth delay (days)	Probability values vs	
		Control	TBl
Compound A	14	<0.04	<0.0002
Compound B	0	NS[b]	0.0002
Compound C	34	<0.03	<0.0008
Compound D	16	NS	<0.0004
Thiacetazone (TB1)	>62	<0.0002	—

[a] 0.05% in the diet, day 70–126.
[b] NS = not statistically significant.

those obtained previously by another method ([8]). In that work, the drug was administered from day 30 through day 180, and the *M. leprae* in the foot pads were then counted. Nine 2-acetylpyridine compounds were tested and those we have designated A and C were the most active; both gave better results than did thiacetazone. Because the foot pad tissues were pooled for each group, statistical analysis was not possible. The correlation observed between the lipophilicity of the 2-acetylpyridine thiosemicarbazones and the counts of *M. leprae,* however, suggested reasonable consistency.

SUMMARY

Four 2-acetylpyridine thiosemicarbazones were tested in mice against *Mycobacterium leprae* by the kinetic method and found to be nearly inactive in a dosage of 0.05% in the diet. At the same dosage, thiacetazone, as a positive control, exhibited its expected activity.

RESUMEN

Usando el método cinético se probó el efecto de cuatro 2-acetilpiridina-tiosemicarbozonas contra el *Mycobacterium leprae* inoculado en ratón y se encontró que estas drogas fueron practicamente inactivas a dosis de 0.05% en la dieta. La misma dosis de tiacetazona, un control positivo, mostró su actividad esperada.

RÉSUMÉ

Au moyen d'une méthode cynétique, on a étudié chez des souris l'activité contre *Mycobacterium leprae* de quatre 2-acetylpyridine thiosémicarbazone; on a observé qu'elles étaient à peu près inactives au dosage de 0.05% dans l'alimentation. Au même dosage, la thiacetazone, utilisée comme témoin positif, a démontré l'activité à laquelle on s'attendait.

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