# CHEMOTHERAPY OF TUBERCULOSIS, I. IN VITRO ACTIVITY OF THIOSEMICARBAZONES AND RELATED COMPOUNDS*

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In 1946, the antituberculous activity *in vitro* of several thiosemicarbazones of aromatic aldehydes and ketones was first reported by DOMAGK, BEHNISCH, MIETZSCH and SCHMIDT[1]. Subsequently, in 1948, qualitative differences in activity among various thiosemicarbazones were indicated[2]. The clinical investigations with these compounds were undertaken by MONCORPS, KALKOFF, KUHLMANN, KLEE, HEILMEYER, MALLUCHE and others. Excellent reviews of investigations about these thiosemicarbazones, particularly, *p*-acetylaminobenzaldehyde thiosemicarbazone (TB 1/698), developed in Germany were published in 1950 by the German workers[3)4)5)],

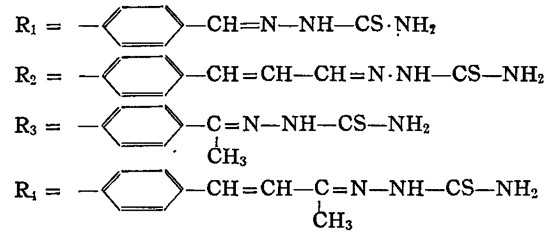
HOGGARTH, MARTIN, STOREY and YOUNG[6] published in 1949 their excellent quantitative *in vivo* evaluation of a considerable number of thiosemicarbazones and related compounds. Furthermore, extensive synthesis[9] of thiosemicarbazones and compounds of related structure amounted to nearly 100 and their activities *in vitro*[7] against the BCG strain of *Mycobacterium tuberculosis* and in the mouse[8] were reported in 1950~1951.

Since TB1 is a drug with apparent degree of toxicity, development of thiosemicarbazones more active as well as less toxic, would be an important addition to the currently available antituberculous agents. It is well known in the field of local anesthetics[10)11)] and antibacterial substance[12)13)] that a compound and its vinylog are similar in biological action. With thiosemicarbazone series, the relation between benzaldehyde or acetophenone thiosemicarbazones and their vinylogs in biological activity has not yet fully established; it is merely known[7] that *p*-ethoxy-, and *p*-dimethylaminobenzaldehyde thiosemicarbazones are superior to their respective vinylogs and furaldehyde thiosemicarbazone is inferior to its vinylog in antituberculous activity. A program was therefore inaugurated in this laboratory to clarify this point, expecting less toxic agents among cinnamaldehyde thiosemicarbazones.

During the course of a study on acyl derivatives of *p*-amino-cinnamaldehyde thiosemicarbazone, the *p*-dimethylacrylyl derivative was found to have markedly low toxicity[14] when tested by subcutaneous injection of its oily suspension. This was presumed to be attributed to the presence of $\alpha$, $\beta$-double bond in the dimethylacrylyl radical, since caproyl radical having almost the same nu-

Table 1. Comparison among four thiosemicarbazones having same substituents.

| No. | Thiosemicarbazone | Formula | Minimum tuberculostatic concentration (γ/ml) |
|---|---|---|---|
| 1 | *p*-Nitrobenzaldehyde | $NO_2 \cdot R_1$ | 1.56 |
| 2 | *p*-Nitrocinnamaldehyde | $NO_2 \cdot R_2$ | 3.12 |
| 3 | *p*-Nitroacetophenone | $NO_2 \cdot R_3$ | 3.12 |
| 4 | *p*-Nitrobenzalacetone | $NO_2 \cdot R_4$ | 3.12 |
| 5 | *p*-Aminobenzaldehyde | $NH_2 \cdot R_1$ | 12.5 |
| 6 | *p*-Aminocinnamaldehyde | $NH_2 \cdot R_2$ | 12.5 |
| 7 | *p*-Aminoacetophenone | $NH_2 \cdot R_3$ | 12·5 |
| 8 | *p*-Aminobenzalacetone | $NH_2 \cdot R_4$ | 25.0 |
| 9 | *p*-Acetamidobenzaldehyde | $CH_3CONH \cdot R_1$ | 0.78 |
| 10 | *p*-Acetamidocinnamaldehyde | $CH_3CONH \cdot R_2$ | 3.12 |
| 11 | *p*-Acetamidoacetophenone | $CH_3CONH \cdot R_3$ | 6.25 |
| 12 | *p*-Acetamidobenzalacetone | $CH_3CONH \cdot R_4$ | 12.5 |



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mber of carbon atoms had not such an effect. It was decided therefore to prepare crotonyl and methacrylyl derivatives in attempting to enhance the antibacterial effectiveness.

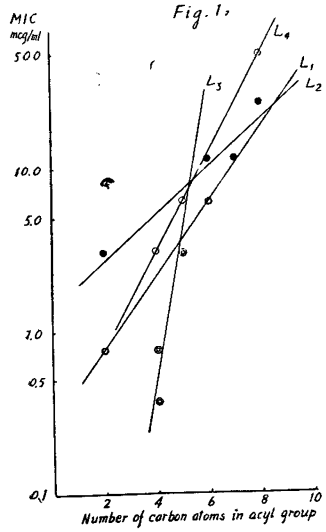
Although thiosemicarbazones have been claimed to have specific activity against tubercle bacilli, α-bromo-*p*-nitrocinnamaldehyde thiosemicarbazone was found to show apparent antibacterial action against *Staphylococcus aureus* and *Escherichia coli* which led to preparing various derivatives of α-bromo-*p*-nitrocinnamaldehyde and the corresponding ketones.

## Materials and Methods

*Chemical* : All compounds tested, consisting of 27 thiosemicarbazones, 2 aldehydes, 2 ketones, 4 semicarbazones, 4 guanylhydrazone hydrochlorides, and 3 nitromethane derivatives were prepared in this laboratory and details of synthesis except for several compounds have been published elsewhere[15) 16) 17)]. Dihydrostreptomycin and isonicotinic acid hydrazide were included as references.

*Bcteriological* : The *in vitro* test using *Mycobacterium tnberculosis* var. *hominis*, strain H 37 Rv in YOUMANS medium, described previously[18)] was used. The bacteriostatic studies were also performed on *Staphylococcus aureus*, strain 209 P and *Escherichia coli*, KAUFFMANN's standard strain No. 6, in a bouillon or in a casamino acid semi-synthetic medium (casamino acid, 10% ; glucose, 1% ; $Na_2HPO_4$, 0.14% ; $KH_2PO_4$, 0.1% ; NaCl, 0.2% ; $MgSO_4$, 0.01% ; nicotinamide, $10^{-4}$M. The pH was adjusted to 6.8 ~7.0 with 10% NaOH), deivsed by us. Casamino acid was prepared by the method of MUELLER[19)]. The chemicals were dissolved with a minimum of heating in ethyleneglycol to make 0.5% solutions. Subsequent dilutions were made into distilled water and then into media.

Fig. 1.




## Results and Discussion

1. Against *M. tuberculosis*

In Table 1 are listed 12 thiosemicarbazones of *p*-substituted benzaldehydes, cinnamaldehydes, acetophenones, and benzalacetones which will be referred to hereafter as $R_1$, $R_2$, $R_3$, and $R_4$, respectively. The magnitude of the activity is seen to be in the following order ; $R_1>R_2=R_3=R_4$ for *p*-nitroderivatives, $R_1=R_2=R_3>R_4$ for *p*-amino derivatives, $R_1>R_2>R_3>R_4$ for *p*-acetylamino derivatives. Examination of Table 2 which lists *p*-acetylamino derivatives of benzaldehyde and cinnamaldehyde thiosemicarbazones, obviously shows that the relation, $R_1>R_2$ is always hold with the exception of *p*-HOOC–$CH_2$–$CH_2$–CONH– derivatives (No. 20 and No. 21). It is clearly obse-

Table 2. Acyl derivatives of *p*-aminobenzaldehyde and *p*-aminocinnamaldehyde thiosemicarbazones.

| No. | Thiosemicarbazone | Formula | Minimum tuberculostatic concentration (γ/ml) |
|---|---|---|---|
| 13 | *p*-Crotonylamino-benzaldehyde | $CH_3CH=CH-CONH-R_1$ | 0.39 |
| 14 | *p*-Crotonylamino-cinnamaldehyde | $CH_3CH=CH-CONH-R_2$ | 3.12 |
| 15 | *p*-Methacrylylamino-benzaldehyde | $CH_2=C(CH_3)-CONH-R_1$ | 0.78 |
| 16 | *p*-(β,β-dimethylacrylylamino)-benzaldehyde | $(CH_3)_2C=CH-CONH-R_1$ | 3.12 |
| 17 | *p*-(β,β-dimethylacrylylamino)-cinnamaldehyde | $(CH_3)_2C=CH-CONH-R_2$ | 6.25 |
| 18 | *p*-Caproylamino-benzaldehyde | $CH_3[CH_2]_4CONH-R_1$ | 6.25 |
| 19 | *p*-Caproylamino-cinnamaldehyde | $CH_3[CH_2]_4CONH-R_2$ | 12.5 |
| 20 | *p*-(β-carboxypropionylamino)-benzaldehyde | $HOOC\cdot CH_2\cdot CH_2\cdot CONH-R_1$ | 25.0 |
| 21 | *p*-(β-carboxypropionylamino)-cinnamaldehyde | $HOOC\cdot CH_2\cdot CH_2\cdot CONH-R_2$ | 12.5 |
| 22 | *p*-Benzoylamino-cinnamaldehyde | $C_6H_5-CONH\cdot R_2$ | 12.5 |
| 23 | *p*-Phenylacetylamino-cinnamaldehyde | $C_6H_5-CH_2CONH\cdot R_2$ | 25.0 |
| 24 | *p*-Cinnamoylamino-cinnamaldehyde | $C_6H_5-CH=CH-CONH\cdot R_2$ | 50.0 |
| 25 | *p*-(*p*-Tolylsulfonylamino)-cinnamaldehyde | $CH_3-C_6H_4-SO_2-NH\cdot R_2$ | 25.0 |

rved that the substitution of the strongly hydrophilic group, -NHCO-$CH_2CH_2COOH$ results in marked loss of activity in $R_1$ series in accordance with BERNSTEIN's observation[7] regarding $SO_3$ group, but not so much in $R_2$ series. The order of activity of various substituting groups, is seen to be $CH_3CO-NH \geqq NO_2 > NH_2$ in the aldehyde series ($R_1$ and $R_2$) and $NO_2 > CH_3CONH > NH_2$ in the ketone series ($R_3$ and $R_4$).

It is evident from Fig. 1 that the increase in numbers of carbon stoms in acy group causes the decrease of antitubercu-lous activity. Therefore, the linear regressions between the numbers of carbon atoms in acyl group and logarithm of the minimum inhibitory concentration were calculated withthe following result :

Table 3 $\alpha$, $\beta$-Unsaturated aldehydes and ketones and their derivatives.

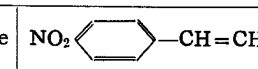
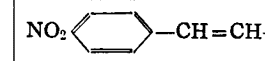
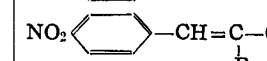
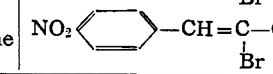
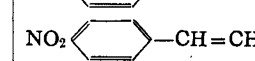
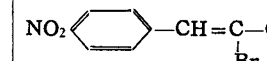
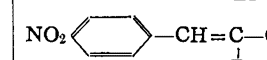
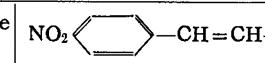
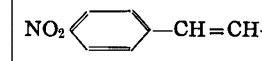
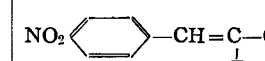
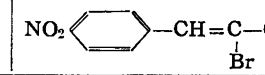
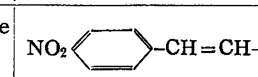
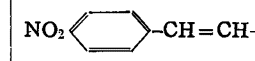
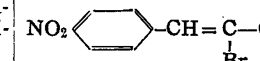
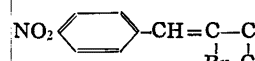
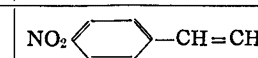
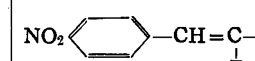
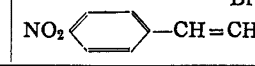
| No. | Compound | Formula | Minimum tuberculostatic concentration ($\gamma$/ml) |
|---|---|---|---|
| 26 | *p*-Nitrocinnamaldehyde |  | 25 |
| 27 | *p*-Nitrobenzalacetone |  | 50 |
| 28 | $\alpha$-Bromo-*p*-nitro-cinnamaldehyde |  | 10 |
| 29 | 1-(*p*-Nitrophenyl)-2-bromo-1-buten-3-one |  | 25 |
| 2 | *p*-Nitrocinnamaldehyde thiosemicarbazone |  | 3.12 |
| 4 | *p*-Nitrobenzalacetone thiosemicarbazone |  | 3.12 |
| 30 | $\alpha$-Bromo-*p*-nitro-cinnamaldehyde thiosemicarbazone |  | 12.5 |
| 31 | 1-(*p*-Nitrophenyl)-2-bromo-1-buten-3-one thiosemicarbazone |  | 25 |
| 32 | *p*-Nitrocinnamaldehyde semicarbazone |  | >40 |
| 33 | *p*-Nitrobenzalacetone semicarbazone |  | >20 |
| 34 | $\alpha$-Bromo-*p*-nitro-cinnamaldehyde semicarbazone |  | >10 |
| 35 | 1-(*p*-Nitrophenyl)-2-bromo-1-buten-3-one semicarbazone |  | >10 |
| 36 | *p*-Nitrocinnamaldehyde guanylhydrazone hydrochloride |  | 50 |
| 37 | *p*-Nitrobenzalacetone guanylhydrazone hydrochloride |  | 50 |
| 38 | $\alpha$-Bromo-*p*-nitro-cinnamaldehyde guanylhydrazone hydrochloride |  | 50 |
| 39 | 1-(*p*-Nitrophenyl)-2-bromo-1-buten-3-one guanylhydrazone hydrochloride |  | 50 |
| 40 | 1-(*p*-Nitrophenyl)-4-nitro-1, 3-butadiene |  | 25> >10 |
| 41 | 1-(*p*-Nitrophenyl)-2-bromo-4-nitro 1, 3-butadiene |  | 50> >20 |
| 42 | $\omega$-Nitro-*p*-nitrostyrene |  | 50 |
| Control | Dihydrostreptomycin | | 0.78 |
| | Isonicotinic acid hydrazide | | 0.06 |

Table 4. Active compounds against *Staphylococcus aureus* and *Escherichia coli*.

| No. | Minimum bacteriostatic concentration (γ/ml) | | | |
|---|---|---|---|---|
| | Bouillon | | Casamino acid semi-synthetic medium | |
| | Against *Staph. aureus* | Against *E. coli* | Against *Staph. aureus* | Against *E. coli* |
| 30 | 50 | 100 | 12.5 | 50 |
| 38 | 50 | 100 | 6.25 | 12.5 |
| 39 | 25 | 100 | 6.25 | 12.5 |

$L_1$ ; $y=2.226x-0.559$ for saturated acyl group attached to $R_1$

$L_2$ ; $y=0.141x+0.208$* for saturated acyl group attached to $R_2$

$L_3$ ; $y=0.753x+3.27$ for unsaturated acyl group attached to $R_1$

$L_4$ ; $y=0.237x-0.423$* for unsaturated acyl group attached to $R_2$

where x=numbers of carbon atoms in acyl group,

y=minimum inhibitory concentration transformed into log unit.

Since the difference between two regression coefficients for $R_2$ series ($L_2$ and $L_4$) is significant at 5% level, it may be said that the antituberculous activity of the $R_2$ series with unsaturated acyl group ($L_4$) increases more rapidly with decreasing numbers of carbon atoms than that of the same series with saturated acyl group ($L_2$), and that the α, β-unsaturated acyl derivatives are more active than the corresponding saturated acyl derivatives, provided that the numbers of carbon atoms involved in acyl group do not exceed 5.

*p*-Crotonylaminobenzaldehyde thiosemicarbazone (No. 13), the best of compounds examined has been found inferior to TB 1 when tested on tuberculous mice[20]. The antituberculous activities of several aldehydes, and ketones, and their thiosemicarbazones, semicarbazones, guanylhydrazones, and nitromethane derivatives are examined in order to make clear the effect of the introduction of Br into the aldehyde or ketone moiety. The results are summarized in Table 3. Respective comparison of No. 2 and No. 4 with No. 30 and No. 31 shows that the substitution of Br at the α-position of α, β-unsaturated carbonyl thiosemicarbazone results in the reduction of the activity. It has been reported that the bromo-substitution at the aromatic nucleus of α, β-unsaturated ketones effects the reduction in the activity against tubercle bacillus and the increase in the action against the other bacteria[21]. As will be described later, Br on the side chain also provides the thiosemicarbazones with the antibacterial property against *Staph. aureus* and *E. coli*. It is, however difficult to draw a conclusion as to the general effect of Br, because of the antituberculous action of the aldehyde or ketone itself favored by the presence of Br on the α-position.

α, β-Unsaturated aldehydes (No. 26 and No. 28) are more active than the corresponding ketones (No. 27 and No. 29). Their antibacterial actions which may be due to the presence of –CO–CH=CH– groupings in the molecules,[22][23] are enhanced (No. 2 and No. 4) or not changed (No. 30 and No. 31) by condensations with thiosemicarbazide and reduced by formations of semicarbazones and guanylhydrazones. Condensation with nitromethane gives no appreciable change in the activity with No. 26 but results in some drop with No. 28. ω-Nitro-*p*-nitrostyrene (No. 42) is inferior to its vinylog (No. 40). T. URBANSKI[24] reported that ω-nitrostyrene inhibits growth of saprophitic micobacteria at concentrations of 50–150 γ/ml in YOUMANS medium.

2. Against *Staph. aureus* and *E. coli*

Of 25 thiosemicarbazones reported herein, only α-bromo-*p*-nitrocinnamaldehyde thiosemicarbazone (No. 30) showed appreciable activity. Guanylhydrazones bearing Br also have the antimicrobial activity. Three effective drugs are listed in Table 4. ω-Nitro-*p*-nitrostyrene has been found bacteriostatic against *Staph. aureus* at a concentration of 7.2 γ/ml by SCHALES[25], α-bromo-*p*-nitrocinnamaldehyde against the same organism at a concentration of 0.4 γ/ml by AFFONSO ;[26] the corresponding values in our hands were 100 γ/ml and 50 γ/ml, respectively.

* Statistically significant at 5% level.

## Summary

1. Minimum inhibitory concentrations against *Mycobacterium tuberculosis*, $H_{37}Rv$, *Staphylococcus aureus*, 209 P, and *Escherichia coli* were determined of twenty-seven thiosemicarbazones, two aldehydes, two ketones, four semicarbazones, four guanylhydrazones, and three nitromethane derivatives. *p*-Crotonylaminobenzaldehyde thiosemicarbazone gave the best value against *M. tuberculosis*.

2. The relationship between chemical structure and tuberculostatic activity *in vitro* was discussed.

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