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STUDY OF THE TUBERCULOSTATIC ACTIVITY OF CERTAIN DERIVATIVES OF UNSATURATED ALDEHYDES AND KETONES OF THE 5-NITROFURAN SERIES

The urgency of the problem of finding new antituberculosis agents which, unlike the majority of those now used—streptomycin, preparations of the isoniazid series, and others—possess a pronounced tuberculostatic action also against strains of *Mycobacterium tuberculosis* resistant to the named substances has led us to turn our attention to a class of compounds little studied in this respect: derivatives of the 5-nitrofuran series.

The first information on the tuberculostatic activity of nitrofuran derivatives is contained in the works of Mizuno (^1), which appeared in 1948. The antituberculosis properties of nitrofurans were also studied by a number of other investigators at a later time. These authors established that the highest activity is shown by the oximes of β-(5-nitrofuryl-2)-acrolein (I) and 5-(5′-nitrofuryl-2′)-pentadien-2,4-al-1 (II) (^2,5), which inhibit the growth of certain strains of *Mycobacterium tuberculosis* ($H_{37}Rv$, Ravenel, and others) at a dilution of 10^{-6}.

Recently, reports by Ivanov and Kozhukharov with co-workers (^6,7) have appeared, devoted to the study of N-(5-nitrofurfurylidene-2)-isonicotinoylhydrazone (III), first synthesized by S. A. Giller (^8) in 1953. Compound III, according to the data of the authors mentioned, proved to be a highly effective antituberculosis agent both in *vitro* and in *vivo*.

In the present work we investigated, in comparison with isoniazid and streptomycin, the tuberculostatic activity of derivatives of α,β-unsaturated and polyene aldehydes and ketones of the 5-nitrofuran series, synthesized by us for the first time, of general type IV (^8-14) (see Fig. 1)

$$O_2N - \langle\text{furan}\rangle - (CH = CR') - C = Y$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \Big| R''$$

(IV)

where $R' = H$, alkyl, alkoxymethyl, $R'' = H$, alkyl, and Y denotes various functional groups.

Taking into account the observation of Saikachi et al. (^15), who established that, when hydrogens in the vinylidene group of similar compounds are replaced by methyl–

Fig. 1. Tuberculostatic activity of compounds of the 5-nitrofuran series. Solid lines–sensitive strains (Akademia), broken lines–resistant strains (D).

$$R_1 = O_2N - \langle\text{furan}\rangle - C_5H_4N\text{-}4 = \text{pyridyl}$$

$$\begin{aligned}
R_2 &= R_1 - CH = CH- \\
R_3 &= R_1 - CH = C(CH_3)- \\
R_4 &= R_1 - CH = C(C_2H_5)- \\
R_5 &= R_1 - CH = C(\text{iso-}C_3H_7)- \\
R_6 &= R_1 - CH = C(C_5H_{11})- \\
R_7 &= R_1 - CH = C(CH_2OCH_3)- \\
R_8 &= R_1 - CH = C(CH_2OC_2H_5)- \\
R_9 &= R_1 - (CH = CH)_2-
\end{aligned}$$

$$-N - CH_2 \qquad\qquad -N - CH_2$$

$$X = CO \qquad CO \qquad Z = OC \qquad CH_2$$

$$NH \qquad\qquad O$$

Logarithms of dilution numbers
4.0 5.0 6.0 7.0
LD_{50}, mg/kg

Isoniazid
Streptomycin –172

Group	Substituent	Compound	LD_{50}, mg/kg
R_1	–CHO	(V)	43 (38-48)
R_1	–$CH(OCOCH_3)_2$	(VI)	70 (49-112)
R_1	–$CH(OC_2H_5)_2$	(VII)	300 (273-330)
R_1	–CH=NOH	(VIII)	28 (23-34)
R_1	–$CH{=}NNHCOCH_2CN$	(IX)	330 (282-375)

Group	Substituent	Compound	LD_{50}, mg/kg
R_1	–CH=NNHCOC$_5$H$_4$N	(III)	2350 (2000-2794)
R_2	–CHO	(X)	8.6 (6.1-12.0)
R_2	–CH(OCOCH$_3$)$_2$	(XI)	34 (26-43)
R_2	–CH(OC$_2$H$_5$)$_2$	(XII)	18.5 (15.6-21.3)
R_2	–CH=NOH	(I)	133 (123-148)
R_2	–CH=NNHCOCH$_2$CN	(XIII)	1150 (885-1495)
R_2	–CH=NNHCOC$_5$H$_4$N	(XIV)	940 (832-1062)
R_3	–CHO	(XV)	–
R_3	–CH(OCOCH$_3$)$_2$	(XVI)	–
R_3	–CH=NNHCONH$_2$	(XVII)	–
R_3	–CH=NNHCSNH$_2$	(XVIII)	–
R_3	–CH=NNHCOCH$_2$CN	(XIX)	–
R_3	–CH=NNHCOC$_5$H$_4$N	(XX)	–
R_3	–CH=N–X	(XXI)	–
R_3	–CH=N–Z	(XXII)	–
R_4	–CH(OCOCH$_3$)$_2$	(XXIII)	–
R_4	–CH=NNHCONH$_2$	(XXIV)	–
R_4	–CH=NNHCSNH$_2$	(XXV)	–
R_4	–CH=NNHCOCH$_2$CN	(XXVI)	–
R_4	–CH=NNHCOC$_5$H$_4$N	(XXVII)	–
R_4	–CH=N–X	(XXVIII)	–
R_4	–CH=N–Z	(XXIX)	–
R_5	–CH=NNHCONH$_2$	(XXX)	–
R_5	–CH=NNHCSNH$_2$	(XXXI)	–
R_5	–CH=NNHCOCH$_2$CN	(XXXII)	–
R_5	–CH=N–X	(XXXIII)	–

Group	Substituent	Compound	LD_{50}, mg/kg
R_6	–CH=NNHCOCH$_2$CN	(XXXIV)	–
R_7	–CHO	(XXXV)	–
R_7	–CH=NOH	(XXXVI)	–
R_7	–CH=NNHCSNH$_2$	(XXXVII)	–
R_7	–CH=NNHCOCH$_2$CN	(XXXVIII)	–
R_7	–CH=NNHCOC$_5$H$_4$N	(XXXIX)	1250 (1213-1398)
R_7	–CH=N–Z	(XL)	–
R_8	–CH=NNHCOCH$_2$CN	(XLI)	–
R_9	–CHO	(XLII)	16.5 (14-20)
R_9	–CH(OCOCH$_3$)$_2$	(XLIII)	49 (36-65)
R_9	–CH=NNHCONH$_2$	(XLIV)	9200 (6345-13340)
R_9	–CH=NNHCSNH$_2$	(XLV)	535 (457-626)
R_9	–CH=NOH (m.p. 194-5°)	(II)	180 (90-360)
R_9	–CH=NOH (m.p. 159-160°)	(XLVI)	72 (55-94)
R_9	–CH=NNHCOCH$_2$CN	(XLVII)	6250 (4664-8375)
R_9	–CH=NNHCOC$_5$H$_4$N	(XLVIII)	1850 (1360-2516)
R_9	–CH=N–X	(IL)	14000 (12390-15820)
R_9	–CH=N–Z	(L)	4600 (4107-5152)
R_2	–CO–CH$_3$	(LI)	174 (112-236)
R_2	–C(CH$_3$)=NOH	(LII)	170 (145-199)
R_2	–C(CH$_3$)=NNHCOCH$_2$	(LIII)	2750 (2318-3182)
R_2	–C(CH$_3$)=NNHCOC$_5$H$_4$N	(LIV)	1000 (671-1329)
R_2	–CO–C(CH$_3$)$_3$	(LV)	215 (176-262)

and ethyl, their antitubercular activity increases, we synthesized substances of

type IV containing the following radicals in the "side chain" : methyl, ethyl, isopropyl, amyl, methoxymethyl, and ethoxymethyl.

Experiments to determine tuberculostatic activity were carried out *in vitro* on *Mycobacterium tuberculosis* typus humanus, strains Academia, H_{37}Rv, and typus bovinus, strains Ravenel and B_8, which are sensitive to streptomycin and isoniazid, and also on typus bovinus strain "Vallée" and typus humanus strain D (isolated from clinical material), resistant to streptomycin and isoniazid.

The tuberculous mycobacteria were cultivated on modified Model semisolid media at pH 7.0 and a temperature of 37° for 14–30 days. For the inoculum, a suspension of tuberculous mycobacteria in physiological saline was used, calculated to contain 1 million bacteria per 1 ml (according to the optical standard). To check the tuberculostatic titer, the method of serial dilutions was used, adding a solution of the preparation in different concentrations, beginning with the saturated limit.

Most of the compounds studied proved to be poorly soluble in water; therefore they were first dissolved in dimethylformamide and, before setting up the experiment, diluted with such an amount of medium that the given concentration of dimethylformamide would not inhibit the growth of mycobacteria. The growth of mycobacteria was observed macroscopically. The conclusion regarding the tuberculostatic activity of the compounds studied was made on the basis of 616 experiments.

Determination of acute toxicity was carried out in experiments on white mice weighing from 15 to 25 g, with intraperitoneal administration of the preparations. In view of the poor solubility of the compounds studied, they were first wetted with a nonionic detergent, a 6% solution of TWEEN-80, and then their suspensions were prepared in isotonic sodium chloride solution. The doses of the preparations were calculated per 1 kg of mouse weight. Six to nine doses were tested for each compound; each dose was tested on 6 mice. The experimental animals were observed for 72–120 hours from the time of administration of the preparations, and the number of deaths was recorded. The experimental material obtained in these experiments was processed statistically by the method of graphical probit analysis according to Litchfield and Wilcoxon at $P = 0.05$, on the basis of which the values of LD_{50} and their confidence limits were calculated.

In Fig. 1, the tuberculostatic activity, on a logarithmic scale of the number of dilutions, of the investigated 5-nitrofuran derivatives is graphically shown both against sensitive (Academia) and streptomycin- and isoniazid-resistant (D) strains of tuberculous mycobacteria, and the acute toxicity for the most active compounds is indicated.

As can be seen from Fig. 1, compounds V, VI, VIII, X, XI, XII, XLII, XLIII, and others, which are aldehydes, diacetates, diethylacetals, or aldoximes of the 5-nitrofuran series, although distinguished by high activity, are of little suitability as chemotherapeutic agents for the treatment of tuberculosis because of their high toxicity, lying within the range $LD_{50} = 15$–71 mg/kg. An exception to this

rule is 5-nitrofurfural diethylacetal (VII), which inhibits the growth of mycobacteria at a dilution of $8 \cdot 10^{-6}$ and does not exhibit high toxicity (LD_{50} = 300 mg/kg).

On the other hand, compounds containing known "tuberculostatic" groups, such as, for example, the isonicotinoylhydrazone group, not only inhibit the growth of the tubercle bacillus at high dilutions, but are also comparatively low-toxic ($LD_{50} > 1000$ mg/kg). Thus, these substances, also distinguished by high effectiveness in inhibiting the growth of mycobacterial strains resistant to other drugs, undoubtedly represent an interesting group of new preparations, among which especially prominent are N-[α-ethyl-β-(5-nitrofuryl-2)-acrylidene]-isonicotinoylhydrazone (XXVII) and N-[α-methoxymethyl-β-(5-nitrofuryl-2)-acrylidene]-isonicotinoylhydrazone (XXXIX).

Also highly active against resistant strains and comparatively low-toxic as a tuberculostatic agent, not belonging to any of the types of antitubercular substances known up to now, proved to be 1-(5′-nitrofuryl-2′)-4,4-dimethylpenten-1-ol-3 (LV).

In conclusion, it should be pointed out that we have found certain regularities in the relationship between the structure of the compounds studied and their tuberculostatic activity. With an increase in the number of vinylidene groups in the side chain of the nitrofuran-derivative molecule, a certain increase in activity is observed. For example, the oximes of β-(5-nitrofuryl-2)-acrolein (I) and 5-(5′-nitrofuryl-2′)-pentadien-2,4-al-1 (II and XLVI) are more active than the oxime of 5-nitrofurfural (VIII). The activity of compounds XXVII and XXXIX, which contain a substituent in the α-position, is at the level of the tuberculostatics currently known to be the most active, while at the same time they have very low toxicity; this confirms the positive influence of α-alkyl and α-methoxymethyl substituents in vinylidene groups on increasing the tuberculostatic activity of substances of type IV as well.

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Note: Figure translations are in progress. See original paper for figures.