HETEROCYCLIC SEMICARBAZONES AND THIOSEMICARBAZONES. XXVI. STRUCTURE AND ANTITUBERCULOSIS ACTIVITY OF THIOSEMICARBAZONES OF HETEROCYCLIC DIKETONES

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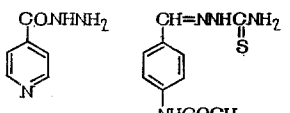
In the modern chemotherapy of tuberculosis, together with the antibiotic streptomycin, synthetic preparations are also used. The most important of them are derivatives of isonicotinic acid, derived from its hydrazide – tubazid (I), as well as derivatives of benzaldehyde thiosemicarbazone, the most important of which has been named thibone (II).



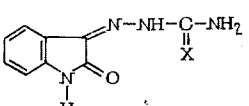
I

II

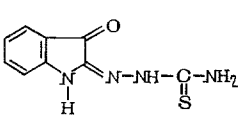
Streptomycin and I surpass II in chemotherapeutic index [1] and have found wider use. However, as these substances are used, strains of mycobacteria resistant to them are becoming increasingly widespread.

An advantage of II is its activity with respect to resistant tuberculosis pathogens [in our control experiments, it entirely suppressed the growth of sensitive mycobacteria (of the $H_{37}R_V$ strain) in vitro in a concentration of 0.3 μg, and that of resistant mycobacteria in a concentration of 0.5 μg].

In view of this, a further search for antituberculosis preparations, especially those active against resistant pathogens, in the series of thiosemicarbazones merits attention. A cycle of investigations along this line have been conducted by M. N. Shchukina, G. N. Pershin, et al. [2, 3].



III: X=S
IV: X=O



V

The main objects of our investigation were isatin thiosemicarbazone and a number of compounds related to it. The antituberculosis activity of isatin-β-thiosemicarbazone (III) was first noted in the patent [4], but in this case no quantitative evaluation of its action was given, as a result of which it is impossible to compare it with other preparations in this respect.* We have established that compound III manifests

*We should note that the melting point of III, cited in the patent [4], differs from the newer data [5, 6] by 50°, which is evidence of insufficient homogeneity of the preparation obtained by the authors of this work. Therefore the possibility remains that the antituberculosis activity of this preparation was due to the presence of impurities.

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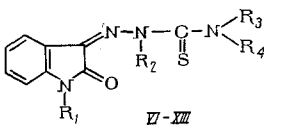
TABLE 1. Derivatives of Isatin-β-Thiosemicarbazone

Compound	R_1	R_2	R_3	R_4	Melting point (in degrees)	Method of synthesis†	Activity against tuberculosis mycobacteria: sensitive (culture $H_{37}R_V$)	resistant to I	resistant to streptomycin
III*	—	—	—	—	252	[5,6]	++	+	+
VI*	CH_3	H	H	H	243	[5,6]	±	—	—
VII	H	CH_3	H	H	195	[6]	—	—	—
VIII	CH_3	CH_3	H	H	172,5	[6]	—	—	—
IX	H	H	CH_3	CH_3	230	[6,7]	++	+++	++
X	CH_3	H	CH_3	CH_3	196	[6,7]	+++	+++	+++
XI*	H	H	n-C_4H_9	n-C_4H_9	152—153	[8]	+	±	±
XII	CH_3	H	n-C_4H_9	n-C_4H_9	120,5	[8]	+	+	+
XIII	H	H	NH_2	H	279	[9,10]	±	—	—

*The method of production is cited in the experimental section.
†Here and in Tables 2-4, the literature source is indicated.
Notation here and in Tables 2 and 3: —) preparation inactive in a concentration of 100 μg; ±) preparation suppresses growth of mycobacteria in a concentration of 100 μg; +) the same in a concentration of 50 μg; ++) in a concentration of 25 μg; +++) in a concentration of 10 μg; ++++) in a concentration of 5 μg; +++++) in a concentration of 2 μg; ++++++) in a concentration of 0.3 μg.

only weak activity. In experiments in vitro it suppresses the growth of sensitive mycobacteria at a concentration of 25 μg, and that of resistant mycobacteria at a concentration of 50 μg. Under the same conditions, isatin-α-thiosemicarbazone (V), isomeric to compound III, proved entirely inactive. The loss of activity is also detected when the sulfur atom in the side chain is replaced by an oxygen atom. The semicarbazone IV and its 1-methyl analog are inactive. Inactivation is also produced by the introduction of substituents in the 6-position of the benzene ring into the thiosemicarbazone III. Thus, 6-nitro- and 6-amino-derivatives of the thiosemicarbazone III are inactive. A substantial influence on the activity is exerted by replacement by alkyl radicals of the hydrogen atoms at the nitrogen atoms, the position of which plays the deciding role in this case. Table 1 presents data for the corresponding derivatives of III with the general formula:



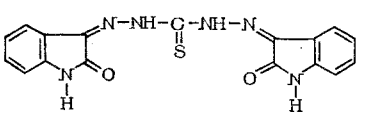
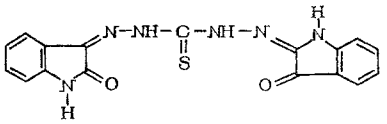
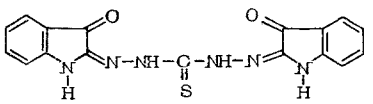
IV-XIII

As can be seen from Table 1, when the hydrogen atom in the 1-position is replaced by a methyl radical, the activity decreases, while in the case of replacement in the 2'-position of the thiosemicarbazone chain it disappears entirely. The introduction of methyl groups into the 4'-position leads to an increase in the activity against resistant mycobacteria, especially against cultures resistant to I. A certain inhibition of the growth of all three investigated types of mycobacteria by isatin-β-4',4'-dimethylthiosemicarbazone (IX) is already detected at a dose of 0.5 μg. Its 1-methyl derivative (X) proved even more active, entirely suppressing the growth both of sensitive and of resistant forms in a concentration of 10 μg. When the hydrogen atoms in the 4'-position of compound III are replaced by butyl radicals, however, the activity decreases somewhat.

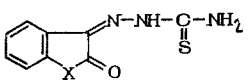
The derivative of III in which the hydrogen atom in the 4'-position is replaced by an amino group – isatin-β-thiocarbohydrazone (XIII) – is characterized by low activity. For comparison with it we investigated more complex derivatives – isatin dithiocarbohydrazones (Table 2).

As can be seen from Table 2, the highest activity of all the preparations that we tested is possessed by isatin α,β'-dithiocarbohydrazone (XV), which suppresses the growth of sensitive mycobacteria in a concentration of 0.3 μg. In this respect compound XV proved equal in activity to II. The growth of cultures resistant to I and streptomycin is entirely suppressed by XV in a concentration of 5 μg; however, a certain inhibition of growth already appears at 1 μg.

TABLE 2. Isatin Dithiocarbohydrazones

Compound	Structural formula	Melting point (in degrees)	Method of synthesis	Activity against tuberculosis mycobacteria: sensitive (culture $H_{37}R_V$)	resistant to I	resistant to streptomycin
XIV		283	[10]	—	—	—
XV		255	—	++++++	++++	++++
XVI		215	—	±	—	—

Together with isatin derivatives, we decided to investigate its analogs, containing a sulfur or oxygen atom in the five-membered ring instead of the nitrogen atom – thionaphthenequinone-β-thiosemicarbazone (XVII) and cumaranedione-β-thiosemicarbazone (XVIII).*



XVII: X = S

XVIII: X = O

The synthesis of compounds that were produced by us for the first time is described in our previous articles, with the exception of thionaphthenequinone 4',4'-dimethylthiosemicarbazone (XIX), the method of production of which is cited in the experimental section of this article.

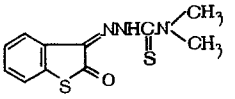
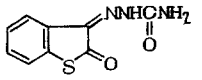
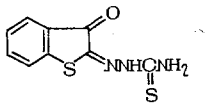
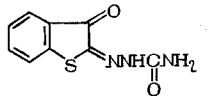
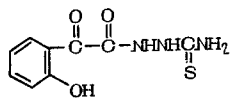
As can be seen from Table 3, XVII is more active than the isatin analog against sensitive mycobacteria, but is entirely inactive against forms resistant to I and streptomycin. Just as in the series of isatin derivatives, the replacement of the sulfur atom in the side chain by an oxygen atom leads to a loss of activity. However, the series of isatin thioanalogs exhibit their own peculiarities. Thus, replacement of hydrogen atoms in the 4'-position of the side chain of compound XVII, in contrast to isatin derivatives, leads to a loss of activity. Moreover, in this series of compounds the α-isomers show greater activity than the β-isomers. Of all the investigated thionaphthenequinone derivatives, the most active proved to be the α-thiosemicarbazone (XXI), which suppresses the growth of mycobacteria sensitive and resistant to I in a concentration of 10 μg, and the growth of those resistant to streptomycin in a concentration of 25 μg.

Compound (XVIII) – an oxygen analog of compound III – proved entirely inactive. In contrast to it, the product of the interaction of cumarenedione with thiosemicarbazide, formed with closing of the ring – the thiosemicarbazide of o-hydroxyphenylglyoxylic acid (XXIII) – shows weak activity against sensitive mycobacteria.

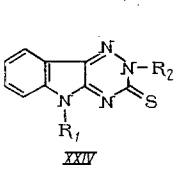
The compounds considered above are capable of undergoing conversions in different directions. Since such conversions also are not excluded in vivo, an investigation of the conversion products of these substances merits attention. One of the directions of these conversions is cyclization with the formation

*The compound XVII was mentioned in the patent [4] among thiosemicarbazones possessing antituberculosis activity in vitro, but it was evidently tested only against sensitive forms, and, moreover, this patent does not cite a quantitative comparison of its activity with the activity of other compounds.

TABLE 3. Products of the Interaction of Thionaphthenequinone and Cumaranedione with Semicarbazide and Thiosemicarbazide

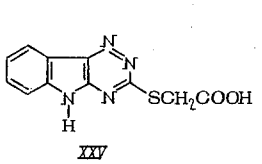
Compound	Structural formula	Melting point (in degrees C)	Method of synthesis	Activity against tuberculosis mycobacteria		
				sensitive (culture $H_{37}R_V$)	resistant to I	resistant to streptomycin
XVII	—	203	[11]	+++	—	—
XVIII	—	223	[9, 10]	—	—	—
XIX	XIX 	194	—	—	—	—
XX	XX 	207	[11]	—	—	—
XXI	XXI 	220	[12]	+++	+++	++
XXII	XXII 	257	[12]	+	±	±
XXIII	XXIII 	181	[10]	+	—	—

of derivatives of 1,3,4-triazacarbazole [13] or 1,2,4-triazacarbazole, respectively [14]. It was found that when the triazine ring is closed, the activity is lost. Of the compounds XXIV-XXVI, only the 1,2,4-derivative (XXVIb) and its thioanalog (XXVIc) exhibit weak activity (100 μg) against sensitive mycobacteria and against those resistant to streptomycin (XXVIb) and to I (XXVIc). In another direction, the compounds structurally related to III can be converted with cleavage of the five-membered ring to derivatives of phenylglyoxylic acid (XXVII) and products of its cyclization – phenyltriazines (XXVIII) [10, 11, 15, 16, 17).

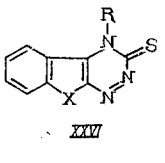


XXIV

a) $R_1=R_2=H$;
b) $R_1=CH_3$; $R_2=H$;
c) $R_1=H$; $R_2=CH_3$

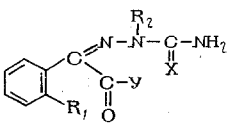


XXV



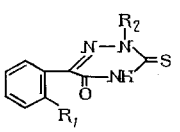
XXVI

a) X=NH, R=H
b) X=NH, R=NH_2
c) X=S, R=H



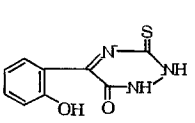
XXVII

R_1 = a H; b,c,i) NO_2; d) $NHCOCH_3$; e,f,g) OH; h) SH
R_2 = a,b,e-i) H; c,d) CH_3
X = a-g) S; h,i) O;
Y = a-f,h,i) OH; g) NHC_6H_5



XXVIII

R_1 = a) NH_2; b) Br; c) OH; d) SH;
R_2 = a,c,d) H; b) CH_3



XXIX

Of all the compounds XXVII-XXVIII, only the 2-methylthiosemicarbazone of acetylisatinic acid (XXVIId) had not been described; the method of its production is cited in the experimental section. All the derivatives of phenylglyoxylic acid (XXVII) are entirely inactive or possess only low activity (at a concentration no less than 100 μg). Thus, opening of the five-membered ring usually leads to a loss of activity. The only exception to this rule proved to be the semicarbazone of o-mercaptophenylglyoxylic acid (XXVIIh), which shows greater activity than thionaphthenequinone-β-semicarbazone (XX), from which it was produced.

The cyclization of thiosemicarbazones of o-substituted phenylglyoxylic acids (XXVII) to XXVIII does not lead to any increase in activity. Of the compounds XXVIII, weak activity (100 μg) is exhibited by XXVIIIa, and only against sensitive mycobacteria.

It is characteristic that tuberculosis mycobacteria are extremely sensitive even to negligible changes in the chemical structure of thiosemicarbazones and their derivatives. Thus, the syn-thiosemicarbazone of o-hydroxyphenylglyoxylic acid (XXVIIe) is more active than its anti-isomer (XXVIIf), while compound XXIX is more active than the substance XXVIIIc isomeric to it, which differs from it only in the sequence of NH and C=S groups in the triazine ring.

The data that we obtained permit us to conclude that of isatin derivatives, the greatest activity in vitro is possessed by compounds IX and X, which have a thiosemicarbazone chain in the β-position, which is substituted by alkyl radicals in the 4'-position. Also noteworthy is the high activity of XV. Among the derivatives of thionaphthenequinone, the α-thiosemicarbazone (XXI) is most interesting.

EXPERIMENTAL

Thionaphthenequinone-4',4'-dimethyl-β-thiosemicarbazone (XIX). Dimethylthiocarbaminoylthioglycolic acid was produced from 0.6 mole of dimethylamine according to the method of [18]. Carbon disulfide was added at 4-5°C over a period of 1 h. During mixing with sodium chloroacetate, the temperature rises to 30°C. To isolate the thioglycolic acid mentioned, 50 ml of concentrated hydrochloric acid was added, and the mixture treated with additional amount of the product was precipitated from the filtrate by the addition of 100 ml of concentrated hydrochloric acid. Yield 69%, colorless crystals, mp 145.5°C [18]. This substance was used without purification for the production of the 4,4-dimethylthiosemicarbazide. The latter is formed according to the method of [18] only with a low yield, together with a substantial amount of the thiocarbohydrazide. It is obtained in substantially more homogeneous form and with a higher yield according to the method of [19]. The product was isolated by cooling in ice and washed with ice water. Yield 72%, shining colorless crystals, mp 154°C [18] (from water). The absence of the thiocarbohydrazide in this sample was demonstrated by the fact that the product of its condensation with isatin does not contain isatin-β-thiocarbohydrazone according to the data of thin-layer chromatography.

A 0.771 g portion of the 4,4-dimethylthiosemicarbazide in 11 ml of water and 1.069 g of 2,3-thionaphthenequinone in 22 ml of alcohol were mixed, and heating was continued for 7 min. After 1 min an abundant precipitate formed. It was left for 1 h, filtered, and washed with aqueous alcohol (twice with 2.5 ml portions). Yield 82%, shining orange crystals, mp 194°C (from butanol). Found, %: N 16.08, 16.15; S 24.56, 24.13. $C_{11}H_{11}N_3OS_2$. Calculated, %: N 15.84, S 24.17.

2-Methylthiosemicarbazone of N-Acetylisatinic Acid (XXVIId). Produced by heating equimolar amounts of N-acetylisatinic acid and 2-methylthiosemicarbazide in aqueous alcohol. Yield 4.44%, mp 180°C. (from anhydrous alcohol). Found, %: N19.00, 19.16; S 11.19, 11.01. $C_{12}H_{14}N_4O_3S$. Calculated, %: N 19.04, S 10.90.

Isatin-β-thiosemicarbazone (III) (compare [5, 6]). A solution of 60 g isatin in 4 liters of water and a solution of 44 g thiosemicarbazide in 120 ml of water were mixed with boiling; heating was continued with mixing for 5-10 min, and the mixture filtered hot. The precipitate was washed with boiling water and dried at 105°C. Yield 82%, golden yellow flocs, mp 252°C (from 80% alcohol).

N-Methylisatin-β-thiosemicarbazone (VI) (compare [5, 6]).* The methylation of isatin with dimethyl sulfate according to the well-known procedures [20, 21] leads to the production of a substance that remains inhomogeneous according to the data of thin-layer chromatography even after repeated crystallization from benzene. Substantially purer N-methylisatin is produced according to a refined procedure [22]. A solution of 31 g sodium in 775 ml anhydrous alcohol was added to 232 g of finely pulverized isatin in 1160 ml an-

*With the participation of E. A. Rusakov.

hydrous alcohol and shaken for 1 h without access to moisture. The mixture was filtered, the precipitate triturated with ~500 ml of anhydrous alcohol, again filtered, washed with anhydrous alcohol, and dried in a vacuum desiccator over sodium hydroxide. Yield of the sodium salt 87.9%, dark violet crystals. Methyl iodide (410 ml) was added to the salt obtained and the mixture heated for 1 h at 100°C in a rotating autoclave. After cooling, the mixture was diluted with boiling water, the excess methyl iodide distilled off, and the product crystallized from ~10 liters of water with an addition of animal charcoal. N-methylisatin was obtained in the form of red needles. Yield 37%, mp 131.5-132°C.

A 9.1-g portion of thiosemicarbazide was added with boiling to a solution of 16.1 g N-methylisatin in 230 ml of 50% aqueous alcohol and the heating continued. After all of the substance passed into solution, 2 ml of 0.05 N hydrochloric acid was added. An abundant yellow precipitate immediately formed. The precipitate was left overnight, filtered, and washed several times with boiling water (total of ~500 ml). Yield 98.8%, mp 243°C (after two crystallizations from butanol).

Isatin-β-4',4'-dibutylthiosemicarbazone (XI). In the patent [7] it is indicated only that this substance is produced analogously to 4,4-dimethylthiosemicarbazone. According to our data, the reaction with carbon disulfide occurs just as in the production of 4,4-dimethylthiosemicarbazide, but instead of 102 ml of a 32% aqueous solution of dimethylamine, 112.5 ml of dibutylamine is added, and after the addition of carbon disulfide, the mixture is mixed in ice for another 1 h. To isolate the corresponding acid, 160 ml of concentrated hydrochloric acid was added immediately, and the mixture exposed overnight in a refrigerator. At first an oil formed, which was soon converted to colorless crystals. The yield of dibutylthiocarbaminoylthioglycolic acid was 88%, mp 69°C. This acid is sparingly soluble in water with heating, readily soluble in alcohol. A 102 g portion of the acid obtained was dissolved in 128 ml of hydrazine hydrate, the solution mixed for 30 min at 60°C, left for 2 h, 340 ml of water added, and extracted with ether (four times with 210 ml portions). The ether extracts were combined, washed with water (four times with 150 ml portions), and dried with anhydrous sodium sulfate. After the ether was distilled off, the viscous syrupy oil was dried in a vacuum desiccator over concentrated sulfuric acid, and 39.2 ml of concentrated sulfuric acid was added. Colorless crystals of 4,4-dibutylthiosemicarbazide hydrochloride were formed. After drying over phosphoric anhydride, the yield was 31.4%, mp 82°C (melts indistinctly). Since this substance is substantially more difficult to purify than the thiosemicarbazones formed from it, it proved advisable to use the sample obtained without preliminary purification.

Solutions of 3 g isatin in 68 ml of alcohol and 4.9 g 4,4-dibutylthiosemicarbazide hydrochloride in 10.5 ml alcohol were mixed with boiling, and heating was continued for 4 h. After cooling, the mixture was filtered to remove the negligible amount of impurity, the filtrate mixed with ether, and the solvent evaporated. The orange crystals obtained were triturated three times with ether and filtered off. Yield of XI 86.5%, shining orange crystals, mp 152-153°C (from alcohol).

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