

OPTIMIZATION REACTION OF SOME 1, 4-DISUBSTITUTED THIOSEMICARBAZIDES WITH TUBERCULOSTATIC ACTIVITY

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Six new 1,4-disubstituted thiosemicarbazides were synthesized and their obtaining reactions were optimized in a 3^2 factorial experiment. The structural features of the analyzed compounds were established by spectral means. The tuberculostatic activity against Mycobacterium Tuberculosis has been tested for different concentrations of the studied thiosemicarbazides in DMSO + phosphate buffer in volumetric ratios 1:4. The highest tuberculostatic activity has been registered for compound IV.

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1. Introduction

The 1,4-disubstituted thiosemicarbazides is a group of substances with therapeutic interest.

The tuberculostatic activity of various thiosemicarbazides [1-6] was noted by many authors, as well as stimulating action of enzymes like lipase or amylase in gastric secretions [2-4]. Other authors notify that the compounds with this structure show antiviral [2-5, 7-10], cytostatic [11,12], antibacterial [13-15], anticonvulsant [16], tuberculostatic activity [17, 18] antifungal activity [14,19, 20].

Some authors of the above mentioned works consider that the N₁-N₄-disubstituted thiosemicarbazides molecules consist from a toxophore group, which is an active group and a haptophore portion that allows close attachment to the cellular constituents. According to this description, the thiourea group (– NH – CS – NH –) represents toxophore group and the radicals attached to the two nitrogen atoms are haptophore portions.

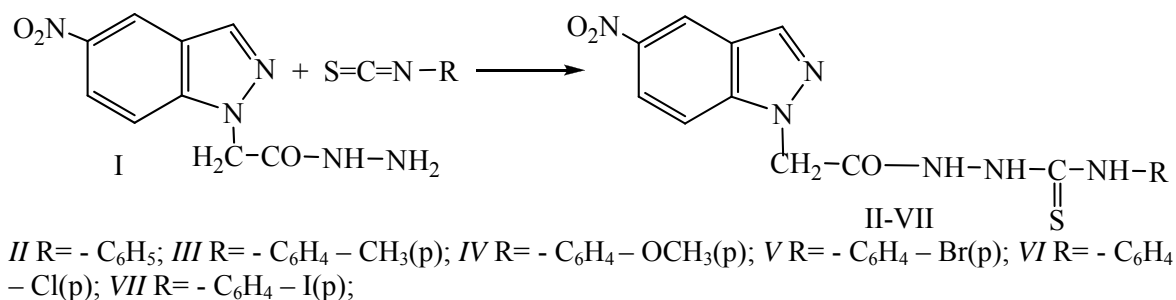
The conclusions of authors cited above are the thiosemicarbazides activity depends largely on the structure haptophore portions, so the entirely molecule structure.

Based on this findings we decided to synthesize the new series of 4-substituted thiosemicarbazides based on 5-nitroindazole.

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2. Experimental

In this paper, the 5-nitroindazole-1-yl-acetylhydrazide (*I*) [21] was treated, according to known procedures [6, 22-24], with phenyl-, p-tolyl-, p-methoxyphenyl-, p-bromophenyl-, p-chlorophenyl-, p-iodophenyl- isothiocyanate in anhydrous methanol at reflux for two hours, leading to new 1-acyl- 4-aryl-thiosemicarbazides (*II-VII*). (Scheme 1) [24].



Scheme 1. Obtaining of 1-acyl- 4-aryl-thiosemicarbazides (*II-VII*)

The chemical structures of the synthesized compounds were elucidated by means of elemental and spectral analysis (FT-IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, SM).

FT-IR spectra of the studied thiosemicarbazide derivatives present an intense absorption band at $2918\text{-}3123\text{ cm}^{-1}$, specific for NH group. The absorption band from $1619\text{-}1621\text{ cm}^{-1}$ is specific for CO group, and the C=S group is revealed by an absorption band at $1137\text{-}1250\text{ cm}^{-1}$. The NO_2 group is identified by $\nu\text{ NO}_2$ symmetric at $1338\text{-}1343\text{ cm}^{-1}$ and by $\nu\text{ NO}_2$ asymmetric at $1525\text{-}1535\text{ cm}^{-1}$. The compounds (*V-VII*) show FT-IR absorption bands at $625\text{-}721\text{ cm}^{-1}$. FT-IR spectra of compound *IV* is given in Fig.1

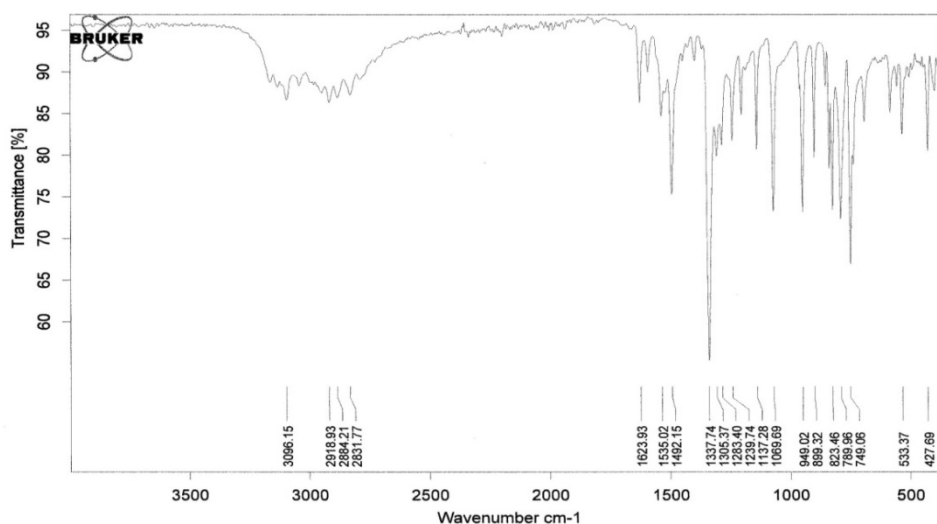


Fig. 1. The IR spectrum of 1-(5'-nitroindazol-1'-yl-acetyl)-4-(p-methoxyphenyl) thiosemicarbazide *IV*

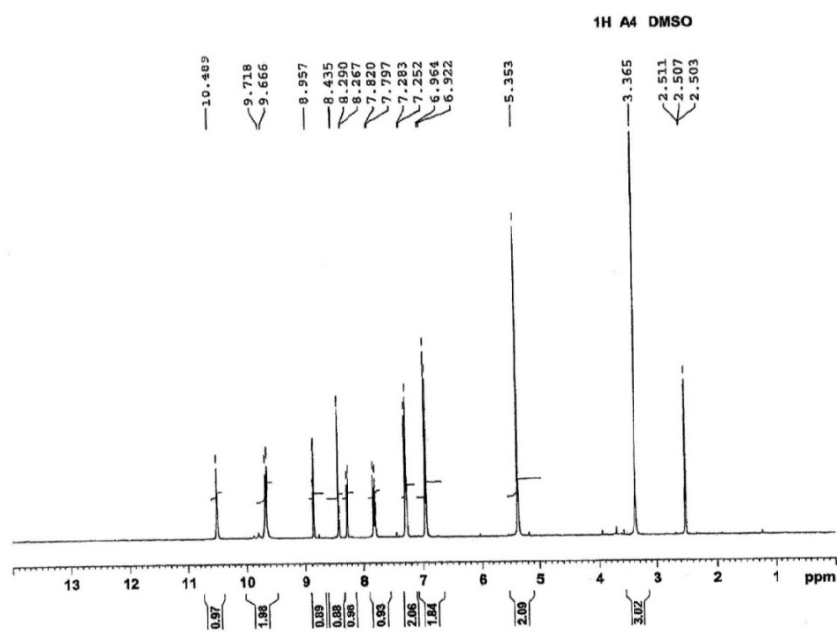


Fig. 2. The ^1H -RMN spectrum of 1-(5'-nitroindazol-1'-yl-acetyl)-4-(p-methoxyphenyl) thiosemicarbazide **IV**

The ^1H -NMR spectra confirm the presence of the protons bound at nitrogen showing signals at 9.66-11.02 ppm and at 7.17-8.95 ppm, respectively aromatic protons. The protons from metin group show signals at 2.30 ppm (s, 1H) and at 3.36 ppm (s, 2H).

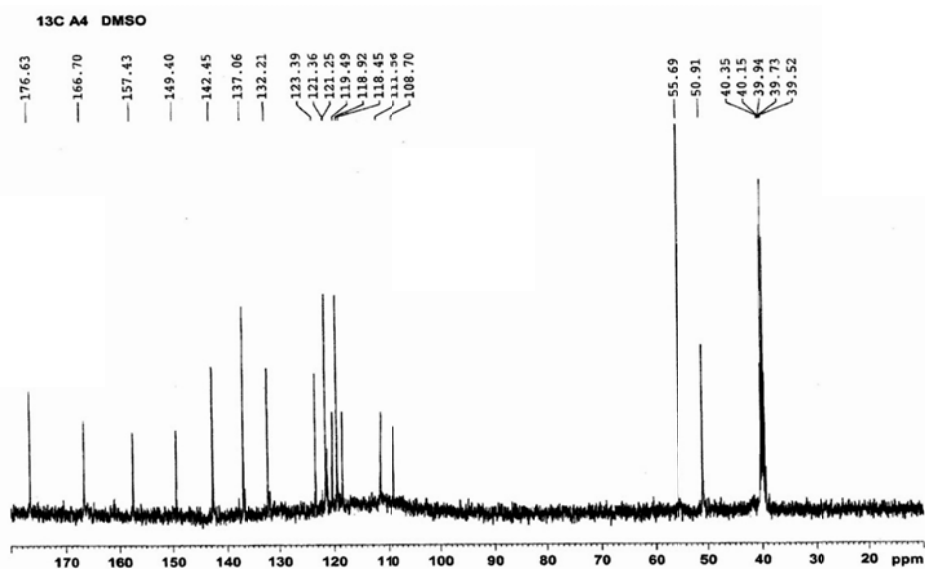


Fig. 3. The ^{13}C -RMN spectrum of 1-(5'-nitroindazol-1'-yl-acetyl)-4-(p-methoxyphenyl) thiosemicarbazide **IV**

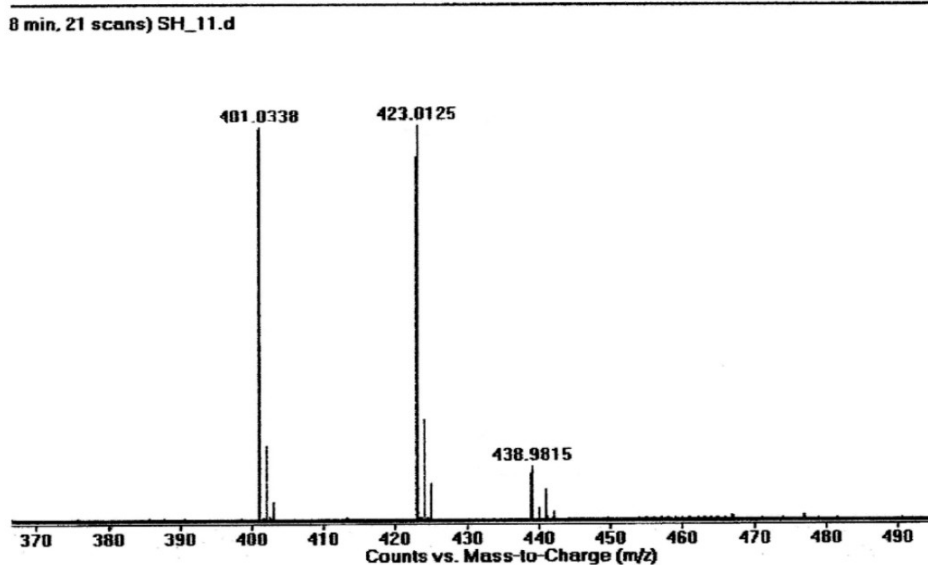


Fig. 4. The mass spectrum of 1-(5'-nitroindazol-1'-yl-acetyl)-4-(p-methoxyphenil) thiosemicarbazide IV

In the ^{13}C -NMR spectra, C=S resonates at 176.63-187.16 ppm and the signals corresponding to C=O appear at 165.58-168.89 ppm.

The mass spectrum confirms the supposed chemical structures. So, all thiosemicarbazides appear the signals of sodium adducts ($\text{M} + \text{Na}$), for values m/z 393, 408, 423, 472, 833, 519. In the same spectra were also identified protonated ionic species [$\text{M} + \text{H}$].

3. Optimization process

Considering the connection between the chemical structure and the potential biological activity of thiosemicarbazides (*II-VII*), we thought of notable importance to establish the optimal conditions for the additive reaction of hydrazide (*I*) to the six aromatic isothiocyanates.

Our preliminary researches revealed that the reaction time and temperature are factors with major influence on the reaction yield when the compounds *II-VII* are prepared.

In these conditions the reaction yield η was considered as being optimization indicator, while the reaction time $X_1 = t$ (expressed in hours) and temperature $X_2 = T$ (expressed in $^{\circ}\text{C}$) were considered as relevant variables.

Three values of each system variable were considered in a 3^2 factorial experiment [25-30] organized in order to determine the best conditions in which the studied reactions take place. In the title of the factorial experiment 3 is the number of the variation levels for each relevant variable of the system and 2 represents the number of the relevant variables taken into consideration in this study.

Let us consider the influence of the system variables and of their conjugate effects on the reaction field as being described by the polynomial (1).

The factorial programs permit to avoid a long and laborious series of experiments for determination the model coefficients a_0 , a_i ($i=1,2$) and a_{ij} ($i, j=1,2$), usually named regression coefficients, from relation (1). The relation (1) must be applied to each studied thiosemicarbazide for the experimental data.

$$\eta = a_0 + a_1x_1 + a_2x_2 + a_{11}x_1^2 + a_{22}x_2^2 + a_{12}x_1x_2 \quad (1)$$

In relation (1), x_1 and x_2 are a-dimensional variables for the reaction time ($i=1$) and for reaction temperature ($i=2$). They are defined by;

$$x_i = \frac{X_i - \bar{X}_i}{\Delta X_i} \quad i = 1,2 \quad (2)$$

The a-dimensional variables can be converted in real variables by using relation:

$$X_i = \bar{X}_i + x_i \Delta X_i \quad i = 1,2 \quad (3)$$

In relations (2) and (3), the following notations were made:

$$\bar{X}_i = \frac{X_{i \max} + X_{i \min}}{2} \quad (4)$$

for the middle of the variable variation domain and

$$2\Delta X_i = X_{i \max} - X_{i \min} \quad (5)$$

for the length of the variation domain.

A-dimensional variables x_1 and x_2 are usually defined in order to facilitate simulation. For real variables of the system, $X_1 = t$ and $X_2 = T$, which vary in the ranges:

$$X_1 \in [X_{1 \min}, X_{1 \max}] \text{ and } X_2 \in [X_{2 \min}, X_{2 \max}], \quad (6)$$

the a-dimensional variables x_1 and x_2 have the corresponding unitary limit values.:

$$x_{i \min} = \frac{X_{i \min} - \bar{X}_i}{\Delta X_i}; \quad x_{i \min} = -1; \quad i = 1,2 \quad (7)$$

$$x_{i \max} = \frac{X_{i \max} - \bar{X}_i}{\Delta X_i}; \quad x_{i \max} = 1; \quad i = 1,2 \quad (8)$$

The real and the a-dimensional variables of the reactions considered here are contained in Table 1 which also contains the mass of the substances II-VII obtained in the 3^2 factorial experiment made for each compound II-VII. The substances were arranged in Table 1 after the common reaction temperature. The a-dimensional variables are given between parentheses in Table 1.

Table 1 Experimental quantities obtained in reactions for determining the reaction yields of compounds II, III, V, VI.

Nr.	$X_1 [h](x_1)$	$X_2 [^{\circ}C](x_2)$	$m[g](II)$ $m_{calc.} = 1.85$	$m[g](III)$ $m_{calc.} = 1.92$	$m[g](V)$ $m_{calc.} = 2.24$	$m[g](VI)$ $m_{calc.} = 2.02$
1	2.5 (-1)	55 (-1)	1.43	1.60	1.73	1.57
2	2.5 (-1)	60 (0)	1.45	1.65	1.76	1.60
3	2.5 (-1)	65 (1)	1.44	1.61	1.73	1.56
4	3 (0)	55 (-1)	1.47	1.62	1.78	1.58
5	3 (0)	60 (0)	1.51	1.67	1.81	1.61
6	3 (0)	65 (1)	1.49	1.62	1.78	1.58
7	3.5 (1)	55 (-1)	1.45	1.61	1.75	1.56
8	3.5 (1)	60 (0)	1.48	1.66	1.77	1.60
9	3.5 (1)	65 (1)	1.45	1.63	1.74	1.56

From the mass experimentally determined, $m_{exp.}$, the reaction yield can be approximated by using relation (9).

$$\eta = \frac{m_{exp.}}{m_{calc.}} \quad (9)$$

In (9) $m_{calc.}$ is the computed mass of the corresponding compound for the same quantity of the initial precursor as in the experiment.

Table 1 continued Experimental quantities obtained in reactions for determining the reaction yields of compounds IV and VII.

Nr.	$X_1 [h](x_1)$	$X_2 [^{\circ}C](x_2)$	$m[g](IV)$ $m_{calc.} = 2.00$	$m[g](VII)$ $m_{calc.} = 2.48$
1	1.5 h ($x_1 = -1$)	50 ⁰ C ($x_1 = -1$)	1.51	1.91
2	1.5 h ($x_1 = -1$)	55 ⁰ C ($x_1 = 0$)	1.54	1.96
3	1.5 h ($x_1 = -1$)	60 ⁰ C ($x_1 = 1$)	1.51	1.92
4	2.0 h ($x_1 = 0$)	50 ⁰ C ($x_1 = -1$)	1.53	1.94
5	2.0 h ($x_1 = 0$)	55 ⁰ C ($x_1 = 0$)	1.59	1.99
6	2.0 h ($x_1 = 0$)	60 ⁰ C ($x_1 = 1$)	1.52	1.94
7	2.5 h ($x_1 = 1$)	50 ⁰ C ($x_1 = -1$)	1.51	1.91
8	2.5 h ($x_1 = 1$)	55 ⁰ C ($x_1 = 0$)	1.55	1.96
9	2.5 h ($x_1 = 1$)	60 ⁰ C ($x_1 = 1$)	1.50	1.92

The regression coefficients a_0 , a_i ($i=1,2$) and a_{ij} ($i, j=1,2$) can be evaluated by the least (smallest) square method [24,25,28] using the data from Tables 1. They are given in Table 2. The values of the regression coefficients can indicate the degree of influence of the corresponding parameter and, their sign indicates an increase (+) or a decrease (-) in the reaction yield determined by the corresponding parameter (or by the conjugate influence of two parameters).

Table 2 Regression coefficients from (1) with the data of Table 1.

Compound	a_0	a_1	a_2	a_{12}	a_{11}	a_{22}
<i>II</i>	81.44	0.562	0.232	-0.273	-2.318	-1.188
<i>III</i>	87.07	0.33	0.453	0.24	-0.96	-2.11
<i>IV</i>	78.96	0.067	-0.132	-0.11	-1.297	-2.312
<i>V</i>	80.55	0.285	-0.187	-0.175	-1.765	-1.3
<i>VI</i>	79.81	0.017	-0.075	0.087	-0.723	-1.698
<i>VII</i>	80.08	0.007	0.11	0	-0.887	-1.867

Let us make t-student test in order to establish the relevance of the regression coefficients from Table 2. In this purpose, three supplemental measurements were made in the center of the variation domain of the relevant variables. The average value of the reaction yield in the center of the variation domain was computed with formula (10) and it is given in Table 3.

Table 3 Reaction yield in the center of the variation domain, average yield; square average deviation and precision.

Compound	η_1	η_2	η_3	$\bar{\eta}$	S_η	P
<i>II</i>	81.16	81.75	81.50	81.47	0.0026	0.017
<i>III</i>	86.90	87.05	87.20	87.05	0.0225	0.050
<i>IV</i>	79.01	79.50	79.20	79.24	0.0025	0.017
<i>V</i>	80.50	80.69	80.92	80.70	0.0623	0.083
<i>VI</i>	79.56	79.80	79.98	79.78	0.0444	0.070
<i>VII</i>	80.10	80.15	80.80	80.35	0.1325	0.121

$$\bar{\eta} = \frac{\eta_1 + \eta_2 + \eta_3}{3} \quad (10)$$

The square average deviation for the supplemental experiments was estimated by using equation (11).

$$S_\eta = \frac{\sum_{i=1}^3 (\bar{\eta} - \eta_i)^2}{2} \quad (11)$$

The precision of measurements can be estimated by the formula (12).

$$P = \sqrt{\frac{S_\eta}{N}} \quad (12)$$

The total number of the experimental data in a 3^2 factorial experiment is $N = 9$.

If one supposes that all regression coefficients from relation (1) were determined with the same precision, P , (given by relation (12)), the t-student test for each regression coefficient can be calculated with relation (13)

$$t_j = \frac{|a_j|}{P}; j = 1, 2, \dots, p \quad (13)$$

The values of the t-student coefficients are given in Table 4. Let us suppose that the maximal value of the t-student test indicating the dependence of the reaction yield on the corresponding parameter is equal to unity.

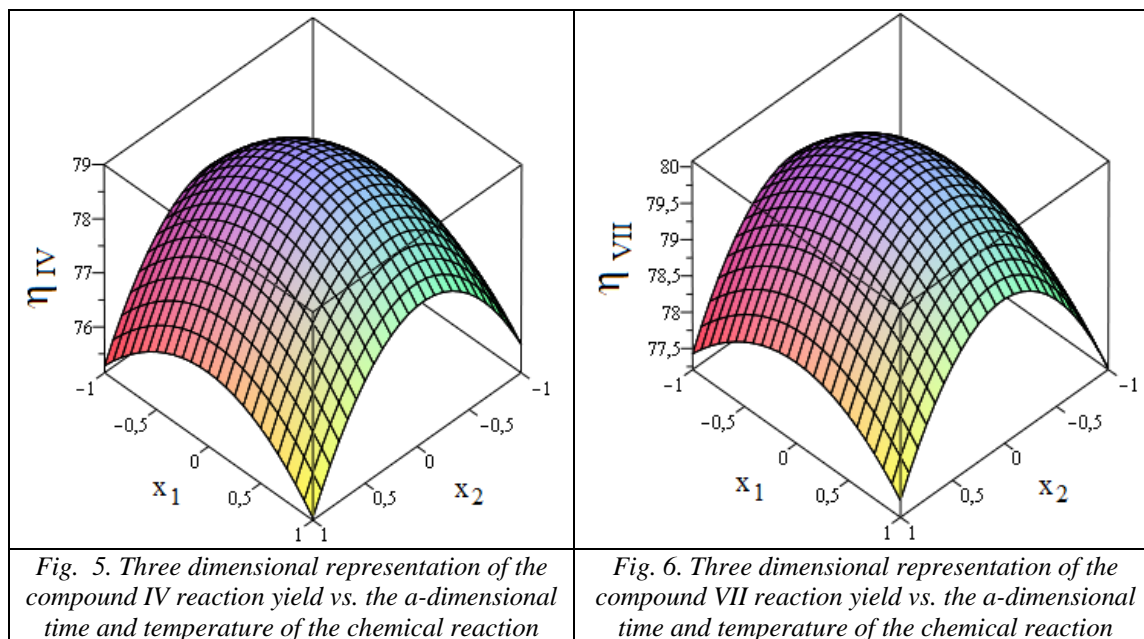


Table 4 t_{ij} -student test for regression coefficients from relation (1) with the data of Table 1.

Compound	a_0	a_1	a_2	a_{12}	a_{11}	a_{22}
II	4790.6	33.1	13.7	16.10	136.4	69.88
III	1741.4	6.6	9.1	4.8	19.2	42.2
IV	4644.7	3.94	7.76	6.47	76.29	136
V	970	3.43	2.25	2.10	21.26	15.66
VI	1140.1	0.24	1.07	1.24	10.34	24.25
VII	661.8	0.057	0.11	0.91	7.33	15.43

If one supposes that the highest value of t_{ij} coefficients for the significant parameters is the unity, the final formulae obtained in optimization of the synthesis reactions for the studied compounds II-VII, after applying t-student test are written below:

$$\eta_{II} = 81.44 + 0.562x_1 + 0.232x_2 - 0.273x_1x_2 - 2.318x_1^2 - 1.188x_2^2 \quad (14)$$

$$\eta_{III} = 87.07 + 0.33x_1 + 0.453x_2 + 0.240x_1x_2 - 0.960x_1^2 - 2.11x_2^2 \quad (15)$$

$$\eta_{IV} = 78.96 + 0.067x_1 - 0.130x_2 - 0.110x_1x_2 - 1.297x_1^2 - 2.318x_2^2 \quad (16)$$

$$\eta_V = 80.55 + 0.285x_1 - 0.187x_2 - 0.175x_1x_2 - 1.765x_1^2 - 1.300x_2^2 \quad (17)$$

$$\eta_{VI} = 79.81 - 0.750x_2 + 0.087x_1x_2 - 0.723x_1^2 - 1.698x_2^2 \quad (18)$$

$$\eta_{VII} = 80.08 - 0.887x_1^2 - 1.867x_2^2 \quad (19)$$

The maximal yield values of the studied reactions are given in Table 5.

Table 5 Extreme values resulting from optimization.

Compound	x_{1extr}	x_{2extr}	η_{extr}
II	0.116	0.084	81.482
III	0.186	0.117	87.127
IV	0.027	-0.029	78.962
V	0.084	-0.077	80.569
VI	0.010	-0.021	79.810
VII	0.003	0.029	80.081

The highest value for the reaction yield was obtained for compound *IV*, the most active from the tuberculostatic action point of view.

4. Tuberculostatic activity

We assessed the tuberculostatic activity in order to evaluate the relationship between the chemical structure and the pharmacodynamic activity.

The compounds *II* - *VII* were tested against *Mycobacterium tuberculosis*, and their activity compared with that of the isonicotinic acid hydrazide (INH), showing that they do have tuberculostatic activity.

The serial dilution technique was applied using the Youmans medium in bovine serum. H₃₇R_v microbial strain hominic variety was used, as inoculums in a 10⁻² mg/5ml concentrated culture medium [31].

The compounds were tested in different concentrations of 5, 10, 20, 30 and 40 µg /ml.

Due to solubility difficulties of these compounds in saline, we had to dissolve them in nonpolar organic solvents (DMSO or DMF) prior to testing. We used 100 µg substance in 1 ml solvent made of DMSO and phosphate buffer, in a ratio of 1:4 (V/V).

The readings were done at 6 and 15 days after inoculations.

We found that the most active thiosemicarbazide is compound *IV* : 1-(5'-nitroindazole-1' -yl-acetyl)-4-(p-methoxyphenyl)-thiosemicarbazide and this shows a minimum inhibitory concentration of 10 µg/ml similar to that of the reference tuberculostatic. The next most active is compound *III*: 1-(5' - nitroindazole - 1' -yl-acetyl) - 4 - (p-tolyl)-thiosemicarbazide (CMI = 25 µg/ml).

Table 6. Tuberculostatic activity of compounds *II* - *V* against *Mycobacterium tuberculosis*, in vitro, compared to that of the isonicotinic acid hydrazide (INH).

Compound	Thiosemicarbazides concentration in culture medium (µg/mL)										CMI (µg/mL)
	5		10		20		30		40		
	6days	15days	6days	15days	6days	15days	6days	15days	6days	15days	
<i>II</i>	++	++	++	++	+	+	+	-	-	-	35
<i>III</i>	++	+++	+++	+++	++	++	++	++	-	-	45
<i>IV</i>	++	++	+	+	-	-	-	-	-	-	25
<i>V</i>	++	++	-	-	-	-	-	-	-	-	10
INH	-	-	-	-	-	-	-	-	-	-	10

- No growth; + reduced growth; ++ moderate growth; +++ intense growth.

Analysis of the structure and the in vitro antituberculostatic activity of the investigated compounds shows:

- substitution of nitrogen bound in position 4 in the phenyl ring of the thiosemicarbazides leads to changes in the antibacterial activity. Substitution with methoxy group in the para position of the benzene ring of the compound *IV* leads to an increase in activity, while the presence of a methyl group in the same position makes compound *III* less active.

- none of the compounds have any tuberculostatic activity below 10 µg/ml.

- presence of bromine, like in compound *II* of the thiosemicarbazides, leads to a decrease in the antibacterial activity.

5. Conclusions

By using a 3² factorial experiment the trials for obtaining the most efficient reaction conditions for obtaining the compounds *II-VII* studied in this paper were minimized. The highest

value for the reaction yield was obtained for compound *IV* which it the most active against *Mycobacterium tuberculosis*. They are promising precursors for antituberculose drugs.

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