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SYNTHESIS AND COMPARATIVE STUDY OF TUBERCULOSTATIC ACTIVITY OF PYRROLE-BASED HYDRAZONES RELATED TO STRUCTURAL VARIATIONS

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Summary. As continuance of some previous investigations, a series of twenty five new hydrazones representing derivatives of pyrrole were synthesized and structurally elucidated. New variations were introduced in the nature of the halogen substituent and the length of the „spacer" $(-CH_2-)_n$ in the acyl chain. The products were evaluated *in vitro* against *Mycobacterium tuberculosis* strain H37Rv as potential tuberculostatics. The corresponding activity expressed as % Growth Inhibition was registered. In a comparative structure-activity analysis of the total set of compounds, the effect of the halogen upon the tuberculostatic activity was found to dominate over the length of the acyl chain. The most favorable combination of both structural characteristics comprises Cl-substituted hydrazones with a spacer $(-CH_2-)_2$.

Key words: antituberculars, carbohydrazides, hydrazones, pyrrole, synthesis

СИНТЕЗ НА ПИРОЛ-СЪДЪРЖАЩИ ХИДРАЗОНИ И СРАВНИТЕЛЕН АНАЛИЗ НА ЗАВИСИМОСТТА НА ТУБЕРКУЛОСТАТИЧНАТА ИМ АКТИВНОСТ ОТ СТРУКТУРНИ ПРОМЕНИ

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Резюме. Като продължение на предишни разработки бяха синтезирани и анализирани серии от нови двадесет и пет хидразона, представляващи производни на пирола. Нововъведенията се характеризират с промяна в природата на халогенния заместител и дължината на „спейсъра" $(-CH_2-)_n$ в ацилната верига. Продуктите бяха изследвани за вероятна противотуберкулозна активност *in vitro* спрямо *Mycobacterium tuberculosis* щам H37Rv. Регистрирана е съответната активност, изразена като % Growth Inhibition. От извършения сравнителен структурен анализ на групата съединения бе установено, че влиянието на халогена върху противотуберкулозната активност е доминиращо в сравнение с това на дължината на ацилната верига. Най-добрата комбинация от двете структурни характеристики включва Cl-заместени хидразони със спейсър $(-CH_2-)_2$.

Ключови думи: антитуберкулозни препарати, карбохидразиди, хидразони, пирол, синтез

Introduction

Tuberculosis (TB), disproportionally affecting the world's poorest populations, remains one of the biggest public health problems in the 21st century. The spread of multidrug-resistant TB (MDR-TB) and the appearance of extensively drug-resistant TB (XDR-TB) pose new challenges for the prevention, treatment and control of this deadly disease. The control of TB is complicated by the fact that about a third of the world's population has latent TB. About 10% of those latently infected eventually develop active form of disease during their lifetime [6]. The disease particularly affects persons from countries where TB incidence is high, persons who have HIV infection, and persons who have recently been exposed to someone with TB. Drug resistant TB, caused by inconsistent or partial treatment, is also a growing concern [10].

One of the most active agents used for treatment of TB is Isoniazid, discovered by Domagk in 1952 [6]. In the last few years, a generation of Isoniazid hydrazones was presented as a part of the search for new more active antitubercular agents [9, 12-16]

Recently series of pyrrole derivatives comprising a hydrazone moiety was reported by us, whose *in vitro* evaluation, performed by the American organization TAACF [19], acting as a contractor, appointed this class of compounds as promising and defined their participation in the search for new tuberculostatics [1-5].

Comparing some of the previously synthesized and reported by us analogous compounds with general formulae, given in Figure 1, we observed some contradictory influence of the length of the „spacer" $(-CH_2-)_n$ in the molecule of the initial carbohydrazide [5].

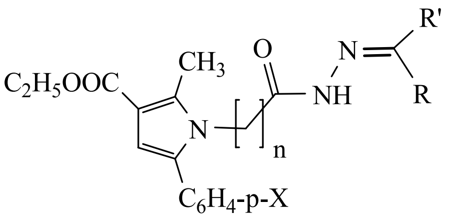


Fig. 1. *General formulae of the previously synthesized and evaluated hydrazide-hydrazones*

In order to clarify this, we designed, synthesized and evaluated the antimycobacterial activity of a new series of pyrrole based hydrazones. The introduced differences consisted mainly in:

a) prolongation of the "spacer" between the nitrogen atom in the central pyrrole ring and the hydrazide part of the molecule by additional methylene group;

b) the nature of the halogen atom at 4th position in the aromatic ring in the carbohydrazide part of the molecule.

Thus obtained hydrazide, containing also a Br atom as halogen substituent, was used as a condensing partner in the followed hydrazone synthesis. The antitubercular activity of the target hydrazones was evaluated in vitro by the partner organization NIAID, using SRI International as a new contracting agent.

Materials and methods

Chemistry

Apparatuses and devices

For determination of the melting points, a capillary Digital Melting Point Apparatus IA 9200 ELECTROTHERMAL (Southend-on-Sea, England) was used and the obtained values were not corrected. The used chromatographic system for TLC control and purity elucidation is based on an aluminium sheets Silica gel 60 F_{254} (Merck, Darmstadt, Germany), using $CHCl_3/CH_3CH_2OH$ as a mobile phase. Detection at UV 254 nm. Yields were calculated for purified products. No efforts were made to optimize yields.The IR spectra 400 – 4000 cm^{-1} were recorded on a Nicolet iS10 FT-IR Spectrometer in KBr. The ^{1}H-NMR spectra were registered at 250 MHz on spectrometer Bruker-Spectrospin WM250MHz (Faenlanden, Switzerland) as δ (ppm) relative to TMS as internal standard and the coupling constants (*J*) are expressed in Hertz (Hz). All OH and NH protons were D_2O exchangeable. All names were generated by using ACD/Name Version 2.51 [7].

The starting materials were of commercially available research – grade chemicals, Merck (Darmstadt, Germany).

General procedure for the synthesis of the targeted hydrazones:

The targeted products were synthesized in micro-synthetic apparatus (ALDRICH Ace Micro-Lab Kit, St. Louis, USA) by condensation of 0.05 mol of parental carbohydrazides **1, 2, 11** and **13** and 0.05 mol of any of the above mentioned carbonyl partners. The reaction was performed in Reaction vial of 5 ml wherein 1.0 ml glacial acetic acid was added. The reaction was stirred at water boiling bath for 45 min. For multifunctional carbonyl partners the temperature was maintained at up to 60°C, for avoiding concurrent reactions. For these conditions the reaction time was prolonged to 1-1.5 hours. The reaction was controlled by TLC and stopped after exhaustion of the starting compounds.

The obtained products were isolated in water and purified by re-crystallization from ethanol, where necessary. The structure of the new compounds was confirmed by IR and ^{1}H-NMR spectral analyses, and their purity was elucidated by TLC, melting points and elemental analyses (C, H, N), whereat the obtained values were in acceptable error range (within ± 0.4%).

Synthesis of carbohydrazide 13

Since carbohydrazide **13** was not found to be reported in the literature, it was prepared by selective hydrazinolysis of the relevant intermediate ethyl ester according to the published general procedure for synthesis of N-pyrrolylcarbohydrazides [1] and given in Figure 2.

The IUPAC names of the newly synthesized hydrazones together with the interpretation of their IR and ^{1}H-NMR spectra are presented below; the chemical structures, the relevant melting points, the TLC characteristics (R_f at the relevant ratio

$CHCl_3/CH_3CH_2OH$ used as a mobile phase) and the individual yields are presented in Table 1.

Ethyl 5-(4-bromophenyl)-1-(3-hydrazinyl-3-oxopropyl)-2-methyl-1H-pyrrole-3-carboxylate (**13**): IR (KBr): ν 3321, 2974, 1684, 1524, 1250, 839; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.34 (t, 3H, CH_2CH_3), 2.23 (t, 2H, CH_2CH_2CO), 2.61 [s, 3H, $CH_3(2)$], 4.23-4.31 [m, 4H, 2 x CH_2], 4.71 [brs, 2H, $NHNH_2$], 6.56 [s, 1H, H(4)], 7.20, 7.24 [d, 2H, H(3'), H(5'), J = 8.68], 7.26 (s, 1H, CONH), 7.53, 7.56 [d, 2H, H(2'), H(6'), J = 8.68].

Ethyl 1-(3-(2-benzylidenehydrazinyl)-3-oxopropyl)-5-(4-bromophenyl)-2-methyl-1H-pyrrole-3-carboxylate (**13a**): IR (KBr): ν 3081, 2975, 1680, 1669, 1522, 1250, 774, 689, 756; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.32 (t, 3H, CH_2CH_3), 2.69 [s, 3H, $CH_3(2)$], 2.96 (t, 2H, CH_2CH_2CO), 4.24,4.26 [d, 2H, CH_2CH_3, J = 7.09], 4.33 (t, 2H, CH_2N), 6.55 [s, 1H, H(4)], 7.23,7.25 [d, 2H, H(2'), H(6'), J = 8.43], 7.40, 7.43 [m, 3H, H(3"), H(4"), H(5")], 7.45, 7.50 [d, 2H, H(3'), H(5'), J = 8.56], 7.50-7.57 [m, 2H, H(2"), H(6")], 7.65 (s, 1H, CH=N), 9.30 (s, 1H, CONH).

Ethyl 5-(4-bromophenyl)-1-(3-(2-(2-hydroxybenzylidene)hydrazinyl)-3-oxopropyl)-2-methyl-^{1}H-pyrrole-3-carboxylate (**13b**): IR (KBr): ν 3435, 3069, 2359-2976, 1700, 1665, 1524, 1250, 833, 759; ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.33 (t, 3H, CH_2CH_3), 2.66 [s, 3H, $CH_3(2)$], 2.79 (t, 2H, CH_2CH_2CO), 4.21, 4.29 [q, 2H, CH_2CH_3, J = 7.1, J = 7.09], 4.35 (t, 2H, CH_2N), 6.55 [s, 1H, H(4)], 6.92-7.00 [m, 2H, H(3"), H(5")], 7.20-7.25 [m, 3H, H(3'), H(5'), H(6")], 7.26 (s, 1H, CH=N), 7.31-7.37 [m, 1H, H(4")], 7.46-7.50 [d, 2H, H(2'), H(6'), J = 8.64], 7.79 (s, 1H, CONH), 9.74 (s, 1H, OH).

Ethyl 5-(4-bromophenyl)-1-(3-(2-(4-methoxybenzylidene)hydrazinyl)-3-oxopropyl)-2-methyl-1H-pyrrole-3-carboxylate (**13c**): IR (KBr): ν 3860-3920, 3216, 1678, 1660, 1609, 1254, 823; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.36 (t, 3H, CH_2CH_3), 2.72 [s, 3H, $CH_3(2)$], 2.98 (t, 2H, CH_2CH_2CO), 3.69 (s, 3H, OCH_3), 4.24-4.31 [q, 2H, CH_2CH_3, J = 7.1, J = 7.09], 4.35 (m, 2H, CH_2N), 6.59 [s, 1H, H(4)], 6.98,6.95 [d, 2H, H(3"), H(5"), J = 8.81], 7.26 [s, 1H, H(2")], 7.29, 7.30 [d, 2H, H(3'), H(5'), J = 1.72], 7.50,7.51 [d, 2H, H(2'), H(6'), J = 2.08], 7.53,7.54 [d, 1H, H(6"), J = 2.52], 7.63 (s, 1H, CH=N), 9.21 (s, 1H, CONH).

(E)-ethyl 5-(4-bromophenyl)-2-methyl-1-(3-(2-((5-nitrofuran-2-yl)methylene)hydrazinyl)-3-oxopropyl)-1H-pyrrole-3-carboxylate (**13d**): IR (KBr): ν 3300, 2860-3000, 1675, 1691, 1565, 1249, 810; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.22 (t, 3H, CH_2CH_3), 2.62 [s, 3H, $CH_3(2)$], 2.79 (t, 2H, CH_2CH_2CO), 4.07-4.15 [q, 2H, CH_2CH_3, J = 7.09, J = 7.21], 4.27 (t, 2H, CH_2N), 6.35 [s, 1H, H(4)], 7.09,7.11 [d, 1H, FurH(2"), J = 3.91], 7.31, 7.35 [d, 2H, H(3'), H(5'), J = 8.44], 7.54,7.58 [d, 2H, H(2'), H(6'), J = 8.56], 7.75,7.76 [d, 1H, FurH(3"), J = 3.91], 7.78 (s, 1H, CH = N), 11.64 (s, 1H, CONH).

Ethyl 5-(4-bromophenyl)-1-(3-(2-(4-ethoxy-4-oxobutan-2-ylidene)hydrazinyl)-3-oxopropyl)-2-methyl-1H-pyrrole-3-carboxylate (**13e**): IR (KBr): ν 3200-3100, 3000-2900, 1737, 1679, 1521, 1252, 819; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.29-1.39 (m, 6H, 2 x CH_2CH_3), 1.60 (s, 3H, CCH_3), 1.88 (s, 2H, CCH_2), 2.62 [s, 1H, $CH_3(2)$], 2.66 [s, 2H, $CH_3(2)$], 2.83 (t, 1H, CH_2CH_2CO), 3.24 (t, 1H, CH_2CH_2CO), 4.16-4.34 [m, 6H, 3 x CH_2], 6.56 [s, 1H, H(4)], 7.31, 7.24,7.28 [d, 2H, H(2'), H(6'), J = 8.44], 7.29 (s, 1H, CONH), 7.53,7.56 [d, 2H, H(3'), H(5'), J = 8.44].

Diethyl 5-((2-(3-(5-(4-bromophenyl)-3-(ethoxycarbonyl)-2-methyl-1H-pyrrol-1-yl)propanoyl)hydrazono)methyl)-3-methyl-1H-pyrrole-2,4-dicarboxylate (**13g**): IR (KBr): ν 3446-2980, 2359, 1700, 1699, 1558, 1250, 835; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.28-1.43 (m, 9H, 3 x CH_2CH_3), 2.58 [s, 3H, $CH_3(4")$], 2.67 [s, 3H, $CH_3(2)$], 2.89 (t, 2H, CH_2CH_2CO), 4.19-4.45 [m, 8H, 4 x CH_2], 6.56 [s, 1H, H(4)], 7.22 (s, 1H, CH=N), 7.26 [s, 2H, H(3'), H(5')], 7.45,7.50 [d, 2H, H(2'), H(6'), J = 8.44], 8.32 (s, 1H, CONH), 9.22 (s, 1H, PyrNH).

Ethyl 5-(4-bromophenyl)-1-(3-(2-((4-(ethoxycarbonyl)-3,5-dimethyl-1H-pyrrol-2-yl)methylene) hydrazinyl)-3-oxopropyl)-2-methyl-1H-pyrrole-3-carboxylate (**13h**): IR (KBr): ν 3130, 2872-3015, 1699, 1674, 1558, 1252, 833; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.31-1.39 (m, 6H, 2 x CH_2CH_3), 2.26,2.29 [d, 3H, CH3(3"), J = 7.33], 2.46 [s, 1H, $CH_3(2)$], 2.55 [s, 2H, $CH_3(2)$], 2.60 [s, 1H, $CH_3(5")$], 2.66 [s, 2H, $CH_3(5")$], 2.83 (t, 2H, CH_2CH_2CO), 4.22-4.34 [m, 6H, 3 x CH_2], 6.56 [s, 1H, H(4)], 7.18-7.24 [m, 2H, H(3'), H(5')], 7.26 (s, 1H, CH=N), 7.46-7.56 [m, 2H, H(2'), H(6')], 7.56 (s, 1H, CONH), 9.05 (s, 1H, PyrNH).

Ethyl 1-(3-(2-((4-acetyl-3,5-dimethyl-1H-pyrrol-2-yl)methylene)hydrazinyl)-3-oxopropyl)-5-(4-bromophenyl)-2-methyl-1H-pyrrole-3-carboxylate (**13i**): IR (KBr): ν 3505-3121, 3015-2863, 1675, 1616, 1558, 1249, 817; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.30-1.34 (m, 3H, CH_2CH_3), 1.71 [brs, 3H, $CH_3(2)$], 2.08,2.14

[d, 3H, CH_3(2"), J = 14.55], 2.23-2.49 [m, 3H, $COCH_3$], 2.63, 2.66 [d, 3H, CH_3(4"), J = 9.66], 2.86 (brs, 2H, CH_2CH_2CO), 4.28 [brs, 4H, 2 x CH_2], 6.57 [s, 1H, H(4)], 7.22 (s, 1H, CH=N), 7.26 [s, 2H, H(3'), H(5')], 7.39-7.55 [m, 2H, H(2'), H(6')], 8.24-8.55 (m, 1H, CONH), 8.52-8.94 (s, 1H, PyrNH).

Ethyl 1-(3-(2-((1-acetyl-1H-indol-3-yl)methylene)hydrazinyl)-3-oxopropyl)-5-(4-bromophenyl)-2-methyl-1H-pyrrole-3-carboxylate (**13l**): IR (KBr): ν 3019, 2814-2359, 1724, 1660, 1558, 1252, 831, 755; ^{1}H NMR (250 MHz, DMSO-d6) δ 1.21-1.28 (m, 3H, CH_2CH_3), 2.50 (t, 5H, CH_2CH_2CO, $NCOCH_3$), 2.62 [s, 3H, CH_3(2)], 4.11,4.18 [q, 2H, CH_2CH_3, J = 7.09, J = 5.87], 4.21-4.35 (m, 2H, CH_2N), 6.41 [s, 1H, H(4)], 7.28-7.44 [m, 4H, H(3'), H(5'), IndolH(6), IndolH(7)], 7.47-7.50 [d, 1H, H(2'), J = 8.43], 7.60-7.63 [d, 1H, H(6'), J = 8.44], 7.86, 7.89 [d, 1H, IndolH(2), J = 7.46], 8.06 (s, 1H, CH=N), 8.25-8.38 [m, 2H, IndolH(5), IndolH(8)], 11.28 (s, 1H, CONH).

Ethyl 4-((2-(3-(5-(4-bromophenyl)-3-(ethoxycarbonyl)-2-methyl-1H-pyrrol-1-yl)propanoyl)hyd-razono)methyl)-3,5-dimethyl-1H-pyrrole-2-carboxylate (**13m**): IR (KBr): ν 3220, 2839-3035, 1684, 1653, 1558, 1250, 815; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.21-1.32 (m, 6H, 2 x CH2CH3), 2.20 [s, 2H, CH3(4")], 2.26 [s, 2H, CH3(2")], 2.32 [s, 1H, CH3(4")], 2.37 [s, 1H, CH3(2")], 2.59 [s, 3H, CH_3(2)], 2.72 (t, 2H, CH_2CH_2CO), 4.12-4.21 [m, 4H, 2 x CH_2], 4.24-4.38 (m, 2H, CH_2=N), 6.40 [s, 1H, H(4)], 7.32-7.40 [m, 2H, H(3'), H(5')], 7.52-7.63 [m, 2H, H(2'), H(6')], 7.68 (s, 1H, CH=N), 10.76 (s, 1H, CONH), 11.60 (s, 1H, PyrNH).

Ethyl 1-(3-(2-((1H-pyrrol-2-yl)methylene)hydrazinyl)-3-oxopropyl)-5-(4-bromophenyl)-2-methyl-1H-pyrrole-3-carboxylate (**13n**): IR (KBr): ν 3340, 2977, 1683, 1653, 1558, 1250, 816; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.33 (t, 3H, CH_2CH_3), 2.68 [s, 3H, CH_3(2)], 2.85 (t, 2H, CH_2CH_2CO), 4.24-4.27 (m, 2H, CH_2=N), 4.28-4.31 (m, 2H, CH_2CH_3), 6.22-6.27 [m, 1H, PyrH(3")], 6.44-6.47 [m, 1H, PyrH(4")], 6.56 [s, 1H, H(4)], 6.92-6.94 [m, 1H, PyrH(5")], 7.22 (s, 1H, CH=N), 7.26 [s, 2H, H(3'), H(5')], 7.48 [s, 1H, H(2')], 7.52 [s, 1H, H(6')], 7.53 (s, 1H, CONH), 9.11 (s, 1H, PyrNH).

Ethyl 5-(4-bromophenyl)-2-methyl-1-(3-oxo-3-(2-(2-oxoindolin-3-ylidene)hydrazinyl)propyl)-1H-pyrrole-3-carboxylate (**13o**): IR (KBr): ν 3329, 2839-3031, 1695, 1653, 1558, 1248, 833, 775; ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.23 (t, 3H, CH_2CH_3), 2.60 [s, 3H, CH3(2)], 2.85 (brs, 2H, CH2CH2CO), 4.09,4.18 [m, 2H, CH_2CH_3], 4.31 (m, 2H, CH_2N), 6.40 [s, 1H, H(4)], 6.86-6.89 [d, 1H, H(5"), J = 7.7], 7.00 [t, 1H, H(6")], 7.34-7.39 [m, 4H, H(3'), H(5'), H(7"), H(8")], 7.54-7.57 [m, 2H, H(2'), H(6'),], 7.95 (brs, 1H, CONH), 10.75 (s, 1H, IzatinNH).

Ethyl 5-(4-bromophenyl)-2-methyl-1-(3-oxo-3-(2-(pyridin-3-ylmethylene)hydrazinyl)propyl)-1H-pyrrole-3-carboxylate (**13p**): IR (KBr): ν 3305, 2843-3015, 1699, 1669, 1558, 1249, 809; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.31 (t, 3H, CH_2CH_3), 2.69 [s, 3H, CH_3(2)], 2.94 (t, 2H, CH_2CH_2CO), 4.19-4.28 (q, 2H, CH_2CH_3, J = 7.09, J = 7.1), 4.36 (t, 2H, CH_2=N), 6.56 [s, 1H, H(4)], 7.22,7.25 [d, 2H, H(3'), H(5'), J = 8.43], 7.26 (s, 1H, CH=N), 7.45,7.49 [d, 2H, H(2'), H(6'), J = 8.56], 7.67 [s, 1H, H(5")], 7.87,7.96 [d, 2H, H(4"), J = 7.95], 8.66-8.72 [brs, 2H, H(2"), H(6")], 9.65 (s, 1H, CONH).

Ethyl 5-(4-bromophenyl)-1-(3-(2-(2-hydroxy-5-nitrobenzylidene)hydrazinyl)-3-oxopropyl)-2-methyl-1H-pyrrole-3-carboxylate (**13x**): IR (KBr): ν 3286, 3133, 2863-3006, 1695, 1662, 1521, 1248, 835, 819 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.25 (t, 3H, CH_2CH_3), 2.62 [s, 3H, CH_3(2)], 2.83 (t, 2H, CH_2CH_2CO), 4.14-4.21 [q, 2H, CH_2CH_3, J = 7.09, J = 7.09], 4.28 (brs, 2H, CH_2N), 6.36 [s, 1H, H(4)], 7.07 [s, 1H, H(4")], 7.51-7.54 [d, 2H, H(2'), H(6'), J = 8.55], 7.59-7.62 [d, 2H, H(3'), H(5'), J = 8.44], 8.13-8.17 [m, 2H, H(3"), H(6")], 8.35 (s, 1H, CH=N), 8.50,8.51 (d, 1H, ArOH, J = 2.82), 11.39 (s, 1H, CONH).

Ethyl 5-(4-chlorophenyl)-1-(2-(2-(4-ethoxy-4-oxobutan-2-ylidene)hydrazinyl)-2-oxoethyl)-2-me-thyl-1H-pyrrole-3-carboxylate (**1e**): IR (KBr): ν 3160-3200, 1720, 1680, 1540, 1240, 850 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.28 (t, 3H, CH_2CH_3), 1.36 (t, 3H, CH_2CH_3), 1.95 [s, 3H, CH_3(2)], 2.54 (s, 3H, CCH_3), 3.30 (s, 2H, $COOCH_2$), 4.15-4.24 [q, 2H, CH_2CH_3, J = 6.22, J = 7.21], 4.27-4.35 [q, 2H, CH_2CH_3, J = 7.09, J = 7.09], 4.91 (s, 2H, CH_2N), 6.64 [s, 1H, H(4)], 7.29-7.31 [d, 2H, H(3), H(5), J = 2.08], 7.35-7.38 [d, 2H, H(2), H(6), J = 7.09], 8.76 (s, 1H, CONH).

Ethyl 5-(4-chlorophenyl)-1-(2-(2-((4-(ethoxycarbonyl)-3,5-dimethyl-1H-pyrrol-2-yl)methylene) hydrazinyl)-2-oxoethyl)-2-methyl-1H-pyrrole-3-carboxylate (**1h**): IR (KBr): ν 3220, 2840-3000, 1710, 1660, 1560, 1200, 830 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.26 (t, 6H, 2 x CH_2CH_3), 2.21 [s, 3H, CH_3(3")], 2.40 [s, 3H, CH_3(5")], 2.43 [s, 3H, CH_3(2)], 4.11-4.24 (m, 4H, 2 x CH_2CH_3), 5.02 (s, 2H, CH_2N), 6.49 [s, 1H, H(4)], 7.30,7.33 [d, 2H, H(3’), H(5’), J = 11.74], 7.45, 7.49 [d, 2H, H(2’), H(6’), J = 8.56], 7.90 (s, 1H, CH=N), 11.27 (s, 1H, CONH), 11.41 (s, 1H, PyrNH).

Ethyl 1-(2-(2-((1-acetyl-1H-indol-3-yl)methylene)hydrazinyl)-2-oxoethyl)-5-(4-chlorophenyl)-2-methyl-1H-pyrrole-3-carboxylate (**1l**): IR (KBr): ν 3286, 3133, 2863-3006, 1695, 1662, 1521, 1248, 835 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.30 (t, 3H, CH_2CH_3), 2.55 [s, 3H, $CH_3(2)$], 2.78 (s, 3H, $NCOCH_3$), 4.25, 4.32 [q, 2H, CH_2CH_3, J = 7.11, J = 10.88], 4.54 (s, 2H, CH_2N), 6.62 [s, 1H, H(4)], 7.24 [s, 1H, IndolH(2)], 7.28-7.29 [d, 3H, H(3’), H(5’), IndolH(6), J = 11.49], 7.37-7.40 [d, 3H, H(2’), H(6’), IndolH(7), J = 8.44], 7.45-7.53 [m, 2H, IndolH(5), IndolH(8)], 8.11 (s, 1H, CH=N), 10.17 (s, 1H, CONH).

Ethyl 5-(4-chlorophenyl)-1-(2-(2-(2-hydroxy-5-nitrobenzylidene)hydrazinyl)-2-oxoethyl)-2-methyl-1H-pyrrole-3-carboxylate (**1x**): IR (KBr): ν 3240, 3123, 2875-3010, 1675, 1649, 1516, 1235, 830 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6) δ 1.27 (t, 3H, CH_2CH_3), 2.49 [s, 3H, $CH_3(2)$], 4.15-4.23 [q, 2H, CH_2CH_3, J = 7.21, J = 7.09], 4.68 (s, 1H, CH_2N), 5.11 (s, 1H, CH_2N), 6.48 [s, 1H, H(4)], 7.07 [s, 1H, H(3”)], 7.29-7.38 [m, 2H, H(3’), H(5’)], 7.43-7.51 [m, 2H, H(2’), H(6’)], 8.10-8.18 [m, 1H, H(4”)], 8.30 [s, 1H, H(6”)], 8.44 (s, 1H, CH=N), 8.56 (s, 1H, ArOH), 11.62 (s, 1H, CONH).

Ethyl 5-(4-chlorophenyl)-1-(3-(2-((4-(ethoxycarbonyl)-3,5-dimethyl-1H-pyrrol-2-yl)methylene) hydrazinyl)-3-oxopropyl)-2-methyl-1H-pyrrole-3-carboxylate (**2h**): IR (in KBr): ν 3300, 2900-3006, 1670, 1652, 1518, 1230, 825 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.33-1.42 (m, 6H, 2 x CH_2CH_3), 2.28 [s, 1H, $CH_3(3”)$], 2.31 [s, 2H, $CH_3(3”)$], 2.48 [s, 1H, $CH_3(5”)$], 2.58 [s, 2H, $CH_3(5”)$], 2.63 [s, 1H, $CH_3(2)$], 2.68 [s, 2H, $CH_3(2)$], 2.83-2.89 (m, 2H, CH_2CH_2CO), 4.23-4.28 (m, 2H, CH_2N), 4.29-4.31 [m, 4H, 2 x CH_2CH_3], 6.58 [s, 1H, H(4)], 7.26-7.31 [m, 2H, H(3’), H(5’)], 7.32-7.35 [d, 2H, H(2’), H(6’), J = 4.28], 7.37-7.41 (m, 1H, CH=N), 7.62 (s, 1H, CONH), 9.23 (s, 1H, PyrNH).

Ethyl 4-((2-(3-(5-(4-chlorophenyl)-3-(ethoxycarbonyl)-2-methyl-1H-pyrrol-1-yl)propanoyl) hydrazono)methyl)-3,5-dimethyl-1H-pyrrole-2-carboxylate (**2m**): IR (KBr): ν 3300-3400, 3000-3100, 2800-3000, 1680, 1650, 1220, 790 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6) δ 1.28-1.32 (m, 6H, 2 x CH_2CH_3), 2.46 [s, 3H, $CH_3(4”)$], 2.49 [s, 3H, $CH_3(2”)$], 2.60 [s, 3H, $CH_3(2)$], 3.12-3.21 [m, 4H, 2 x CH_2], 4.21-4.29 (m, 2H, CH_2=N), 6.40 [s, 1H, H(4)], 7.42-7.46 [m, 2H, H(3’), H(5’)], 7.88-7.90 [m, 2H, H(2’), H(6’)], 8.56 (s, 1H, CH=N), 9.90 (s, 1H, CONH), 12.04 (s, 1H, PyrNH).

Ethyl 5-(4-chlorophenyl)-1-(3-(2-(2-hydroxy-5-nitrobenzylidene)hydrazinyl)-3-oxopropyl)-2-me-thyl-1H-pyrrole-3-carboxylate (**2x**): IR (KBr): ν 3270, 3100, 2880-3000, 1685, 1660, 1521, 1240, 830 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.26-1.40 (m, 3H, CH_2CH_3), 2.44-2.49 (m, 1H, CH_2CH_2CO), 2.71 [s, 3H, $CH_3(2)$], 2.78-2.83 (m, 1H, CH_2CH_2CO), 4.21-4.30 [q, 2H, CH_2CH_3, J = 7.09, J = 6.97], 4.37-4.45 (m, 2H, CH_2N), 6.57 [s, 1H, H(4)], 7.09 [s, 1H, H(3”)], 7.29 [s, 2H, H(3’), H(5’)], 7.32-7.33 [d, 2H, H(2’), H(6’), J = 2.69], 7.89 [s, 1H, H(4”)], 8.23 [s, 2H, CH=N, H(6”)], 9.82 (s, 1H, ArOH), 10.54 (s, 1H, CONH).

Ethyl 5-(4-bromophenyl)-1-(2-(2-(4-ethoxy-4-oxobutan-2-ylidene)hydrazinyl)-2-oxoethyl)-2-methyl-1H-pyrrole-3-carboxylate (**11e**): IR (in KBr): ν 3170, 2860-2950, 1720, 1680, 1535, 1240, 835 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 1.26 (t, 3H, CH_2CH_3), 1.34 (t, 3H, CH_2CH_3), 1.93 (s, 3H, CH_3C=N), 2.51 [s, 3H, $CH_3(2)$], 3.29 (s, 2H, CH_2C=N), 4.17-4.19 [m, 2H, CH_2CH_3], 4.27-4.29 [m, 2H, CH_2CH_3], 4.89 (s, 2H, CH_2N), 6.62 [s, 1H, H(4)], 7.19-7.20 [d, 2H, H(3’), H(5’), J = 7.48], 7.49-7.50 [d, 2H, H(2’), H(6’), J = 7.48], 8.70 (s, 1H, CONH).

Diethyl 5-((2-(2-(5-(4-bromophenyl)-3-(ethoxycarbonyl)-2-methyl-1H-pyrrol-1-yl)acetyl) hydrazono)methyl)-3-methyl-1H-pyrrole-2,4-dicarboxylate (**11g**): IR (in KBr): ν 3190, 2880-3000, 1700, 1670, 1570, 1270, 820 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6) δ 1.26-1.31 (m, 9H, CH_2CH_3), 2.44 [s, 3H, $CH_3(4”)$], 2.47 [s, 3H, $CH_3(2)$], 4.18-4.28 [m, 6H, CH_2CH_3], 5.17 (s, 2H, CH_2N), 6.50 [s, 1H, H(4)], 7.24-7.28 [d, 2H, H(3’), H(5’), J = 8.44], 7.58-7.61 [d, 2H, H(2’), H(6’), J = 8.44], 8.47 (s, 1H, CH=N), 11.95 (s, 1H, CONH), 12.00 (s, 1H, Pyr-NH).

Ethyl 1-(2-(2-((4-acetyl-3,5-dimethyl-1H-pyrrol-2-yl)methylene)hydrazinyl)-2-oxoethyl)-5-(4-bromophenyl)-2-methyl-1H-pyrrole-3-carboxylate (**11i**): IR (in KBr): ν 3800-3960, 3200, 1690, 1650, 1580, 1220, 810 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.24 (t, 3H, CH_2CH_3), 2.00-2.46 [m, 12H, $CH_3(2)$, $CH_3(2”)$, $COCH_3$, $CH_3(5”)$], 4.13-4.18 [q, 2H, CH_2CH_3, J = 7.09, J = 7.08], 4.83-4.90 (m, 2H, CH_2N), 6.52 [s, 1H, H(4)], 7.10-7.20 [q, 2H, H(3’), H(5’), J = 8.31, J = 8.19], 7.27 (s, 1H, CH=N), 7.36-7.39 [d, 2H, H(2’), H(6’), J = 5.75], 7.78 (s, 1H, CONH), 10.82 (s, 1H, Pyr-NH).

Ethyl 5-(4-bromophenyl)-1-(2-(2-(2-hydroxy-5-nitrobenzylidene)hydrazinyl)-2-oxoethyl)-2-me-thyl-1H-pyrrole-3-carboxylate (**11x**): IR (in KBr): ν 3410, 3190, 2860-3000, 1690, 1630, 1560, 1240, 850, 830 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.34 (t, 3H, CH_2CH_3), 2.54 [s, 3H, CH_3(2)], 4.23-4.32 [q, 2H, CH_2CH_3, J = 7.1, J = 7.21], 4.64 (s, 1H, CH_2N), 4.93 (s, 1H, CH_2N), 6.61 [s, 1H, H(4)], 7.24 [t, 2H, H(3'), H(5')], 7.36(s, 1H, CH=N), 7.48-7.51 [d, 2H, H(2'), H(6'), J = 8.31], 8.13-8.17 [m, 2H, H(3"), H(6")], 8.24 (s, 1H, ArOH), 7.01, 7.05 [d, 1H, H(4"), J = 9.17], 8.33 (brs, 1H, CONH).

Screening for tuberculostatic activity

The tuberculostatic activities of the new hydrazones were tested *in vitro* at the SRI International, a contractor for NIAID, USA [17].

The evaluation was performed according to the SRI International Screening Program [18].

Single point concentration procedure: All compounds are initially screened against *Mycobacterium tuberculosis* strain H37Rv in the Single point concentration procedure [8]. This assay is the primary screen. If compounds are active at 10 µg/mL level, they are further tested in an MIC assay at 8 concentrations in a dose range between 10 and 0.078 µg/mL.

The procedure is performed as follows: 20 µl of the 3.2 mg/ml test compound is added to a 96-well microtiter plate. Two fold dilutions are made by the addition of 20 µl of diluent. Each dilution is further diluted 1:10 in sterile water (10 µl of dilution to 90 µl of sterile water). 6.25 µl of each dilution is transferred to duplicate 96-well test plates. 93.75 µl of the cell suspension (~ 10^4 bacteria) in 7H9 medium is added to the test plates. Positive (include rifampicin and isoniazid), negative (include cell culture with solvent and water; and cell culture only), sterility (include media only; and media with solvent and water) and resazurin (includes one plate containing the diluted compound with resazurin only) controls are tested. The 96-well test plates are incubated at ambient 37°C incubator for 6 days. After the 6 day incubation, 5 µl of a 0.05% sterile resazurin solution is added to each well of the 96-well plate. The plates are placed in an ambient 37°C incubator for 2 days. After the 2 day incubation, a visual evaluation and fluorimetric read-out is performed. The results are given as % Growth inhibition.

Results and Discussion

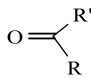
Synthesis of the new structural analogues

Compound IDs **1-12** have already been assigned to carbohydrazides used in our previous researches [1-5]. The intermediate carbohydrazide **13** of the corresponding pyrrolyl carboxylic acid is a result of a multistaged synthetic process whereat the classical Paal-Knorr pyrrole synthesis was used as key reaction to provide the central pyrrole cycle. The synthesis of the parental carbohydrazide is presented on Fig. 2.

Synthesis of pyrrole-based hydrazones

The new hydrazones were synthesized according to Fig. 3 by condensation between the relevantly substituted carbohydrazides **1, 2, 11** and **13** and any of the carbonyl compounds a-x presented in Fig. 4.

where X = p-Cl or p-Br, n = 1 or 2, and



could be any of the carbonyl compounds **a-x** presented in Fig. 4.

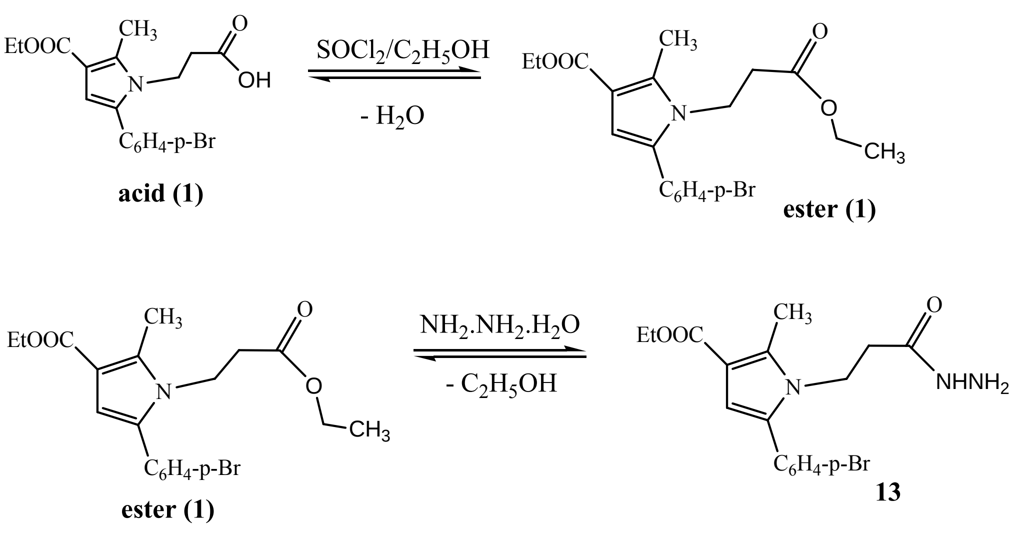
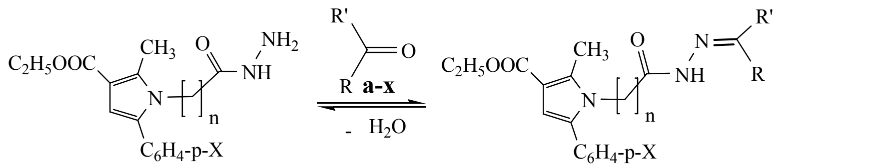


Fig. 2. *Synthesis of carbohydrazide 13 from the relevant pyrrolyl carboxylic acid*



1, 2, 11 or13 **1, 2, 11 or 13(a,b,c,d,e,g,h,i,l,m,n,o,p,x) n=1 or 2**

Fig. 3. *General reaction scheme for the hydrazone syntheses*

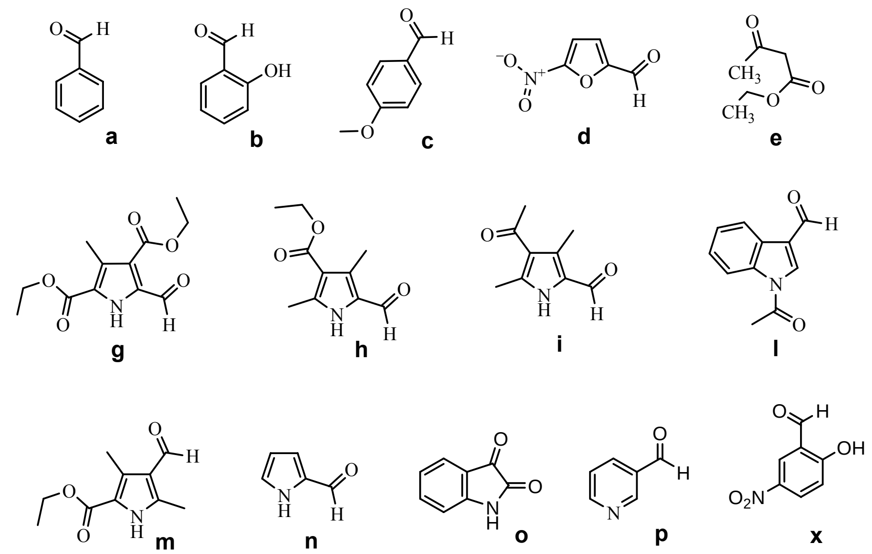


Fig. 4. *Set of carbonyl compounds, used as reaction partners in the hydrazone syntheses*

The carbonyl partners have been selected to allow comparison with the antitubercular and antibacterial agents evaluated previously.

The pyrrole carbonyl compounds **g, h, i, m** and compound **l** were synthesized in our laboratory using previously published method [11].

The IDs of the obtained hydrazones contain information about the both reaction partners, whereas the digit represents the used carbohydrazide **1, 2, 11, 13** and the letter, corresponds to one of the applied carbonyl partners **a – x**.

As a result from the performed synthesis carbohydrazide **13** and its fourteen hydrazones along with additional eleven hydrazones of hydrazides **1, 2** and **11** were obtained with yields in the range of 45-90%.

Screening results

The tuberculostatic activity of the new series of hydrazones against Mycobacterium tuberculosis strain H37Rv was evaluated by SRI International [17] as "% Growth Inhibition" (registered in the range of -8.99 to 2.37%), unlike the previously used by TAACF format of "% Inhibition" (in the range of 0-100%) or IC50 [1-5]. In the search for possible structure-activities trends, an attempt was made to transform the total available screening results in a common general format to make possible an approximate comparison of data related to analogous compounds, but expressed in different scales. Just for this purpose and with the help of simplifying considerations, some of the current results were recalculated from "% Growth Inhibition" to "% inhibitory activity" related to that of Isoniazide (accepted to be a standard with 100%). The following approximations were applied:

In case the registered value is in format "% growth inhibition":

Recalculated % inhibitory activity = % Growth Inhibition + 29.44

where 29.44 is the absolute value of the poorest registered (with negative sign) activity.

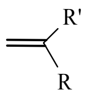
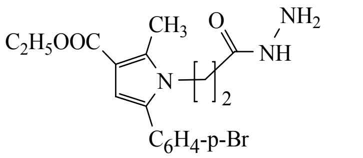
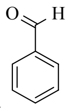
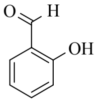
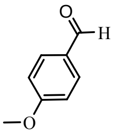
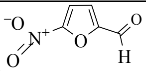
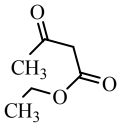
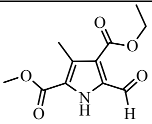
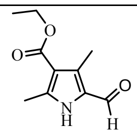
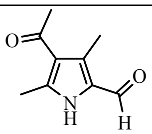
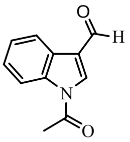
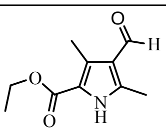
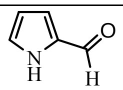
In case the registered value is in format IC50:

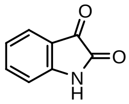
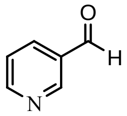
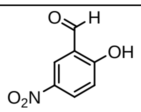
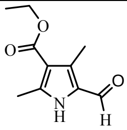
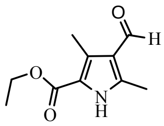
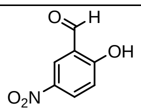
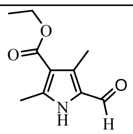
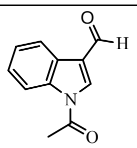
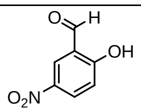
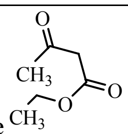
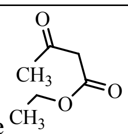
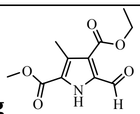
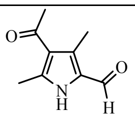
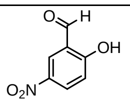
Recalculated % inhibitory activity = 0.06/IC50*100

where 0.06 is the IC50 value of the selected standard Izoniazide used as reference.

The primary screening results together with some structural characteristics related to the new compounds are presented in Table 1.

Table 1. *Inhibitory activities versus relevant structural characteristics of the new compounds*

ID	Structural characteristics			Inhibitory activity		M.p.(°C)	TLC: Rf ($CHCl_3$:EtOH)	Yield (%)
	X	n		% Growth inhibition/IC50	% Inhibition: *measured **recalculated			
13				-8.99	20.45**	167-169	0.38/ (10:0.25)	76
13a	Br	2	 a	2.37	27.36**	150-152	0.41/ (10:0.25)	78
13b	Br	2	 b	-2.75	26.69**	210-211	0.43/ (10:0.25)	68
13c	Br	2	 c	-2.61	26.83**	184-188	0.46/ (10:0.25)	86
13d	Br	2	d 	-5.81	23.63**	193-195	0.60/ (10:0.5)	51
13e	Br	2	 e	-4.04	25.40**	129-130	0.57/ (10:0.25)	58
13g	Br	2	 g	0.50	29.94**	136-138	0.36/ (10:0.25)	78
13h	Br	2	 h	-4.68	24.76**	118-120	0.76/ (10:0.5)	46
13i	Br	2	 i	-5.87	23.57**	170-175	0.57/ (10:0.25)	69
13l	Br	2	 l	-2.52	26.92**	233-234	0.60/ (10:0.5)	72
13m	Br	2	 m	-1.84	27.60**	270-272	0.60/ (10:0.5)	88
13n	Br	2	 n	-4.97	24.47**	154-155	0.59/ (10:0.5)	84

13o	Br	2	 **o**	-2.23	27.21**	221-222	0.31/ (10:0.25)	78
13p	Br	2	 **p**	-3.48	25.96**	127-129	0.53/ (10:0.5)	72
13x	Br	2	 **x**	-6.10	23.34**	231-233	0.70/ (10:0.5)	85
2h	Cl	2	 **h**	-5.37	24.07**	264-265	0.49/ (10:0.5)	65
2m	Cl	2	 **m**	-3.14	26.30**	184-185	0.48/ (5:1)	57
2x	Cl	2	 **x**	-7.24	22.20**	225-226	0.68/ (10:0.5)	82
1h	Cl	1	 **h**	8.48	37.92**	244-245	0.78/ (10:1)	85
1l	Cl	1	 **l**	-6.53	22.91**	220-222	0.84/ (10:0.5)	49
1x	Cl	1	 **x**	-9.15	20.29**	227-229	0.57/ (10:1)	63
1e	Cl	1	 **e**		89*	209-211	0.64/ (10:0.25)	78
11e	Br	1	 **e**	/ > 100	6.00**	220-221	0.54/ (10:0.25)	57
11g	Br	1	 **g**	/ > 100	6.00**	265-267	0.47/ (10:0.5)	69
11i	Br	1	 **i**	/ > 100	6.00**	212-213	0.46/ (10:0.5)	89
11x	Br	1	 **x**	/ > 100	6.00**	229-232	0.35/ (10:1)	75
Isoniazide					100			

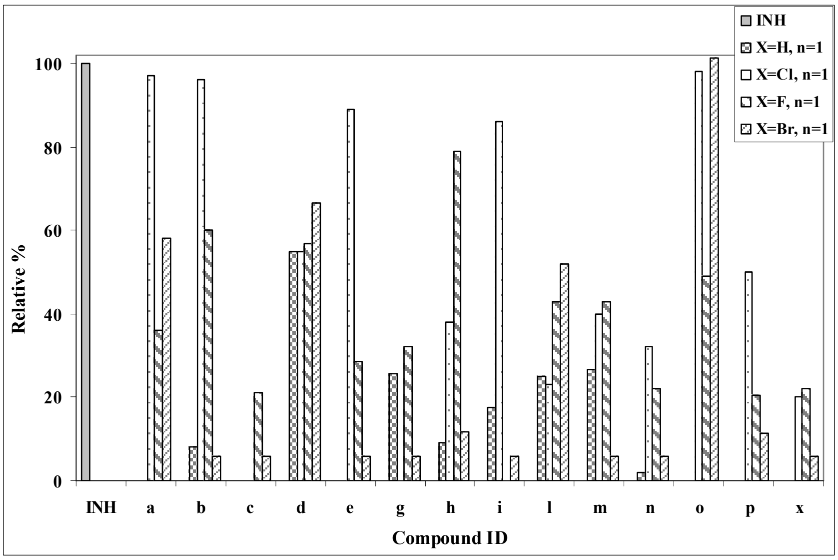


Fig. 5. *Influence of the nature of halogen substituent for analogues Series with "spacer" n =1 upon the tuberculostatic activity*

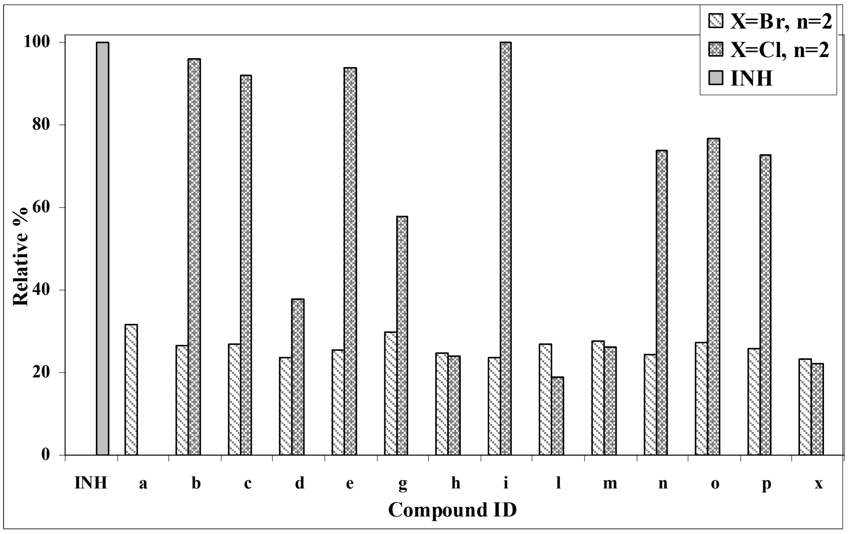


Fig. 6. *Parallel comparison of the effects of substituents Cl and Br in* ***Series 2*** *and* ***Series 13*** *(having a spacer n = 2) upon the tuberculostatic activity*

Comparative analysis

For the needs of comparative analysis on the influence of the halogen substituent X and the length of the "spacer" $(-CH_2-)_n$ (according to Fig. 1), the set of data related to the newly synthesized compounds was completed with the relevant characteristics of the structural analogs, previously investigated in our laboratory [1-5].

The analyzed compounds are summarized in the following Series, according to the mentioned attributes. In the following graphs, the letters **a–x** account for the respective carbonyl compounds used as synthetic partners and given on Fig. 4: **Series 01:** X = H, n = 1 [1]; **Series 1:** X = Cl, n = 1 [1-3]; **Series 2:** X = Cl, n = 2 [2,3]; **Series 4:** X = F, n = 1 [3,4]; **Series 11:** X = Br, n = 1 [5]; **Series 13:** X = Br, n = 2 – newly synthesized compounds.

Thus Figure 5 expresses the influence of the halogen substituent in compounds having the same "spacer" n = 1 within the range of available data.

Within the compared four series the highest activity was observed predominantly in the hydrazones of **Series 1** (X = Cl) followed by the activity of **Series 4** (X = F) before that of **Series 11** (X = Br).

The influence of Cl is even more emphatically favorable than the effect of Br in the next compared **Series 2** and **Series 13** (both with a spacer n = 2), whereat the average activity within the Cl-substituted series is about 4 times higher compared to that of the Br-analogs. (Figure 6).

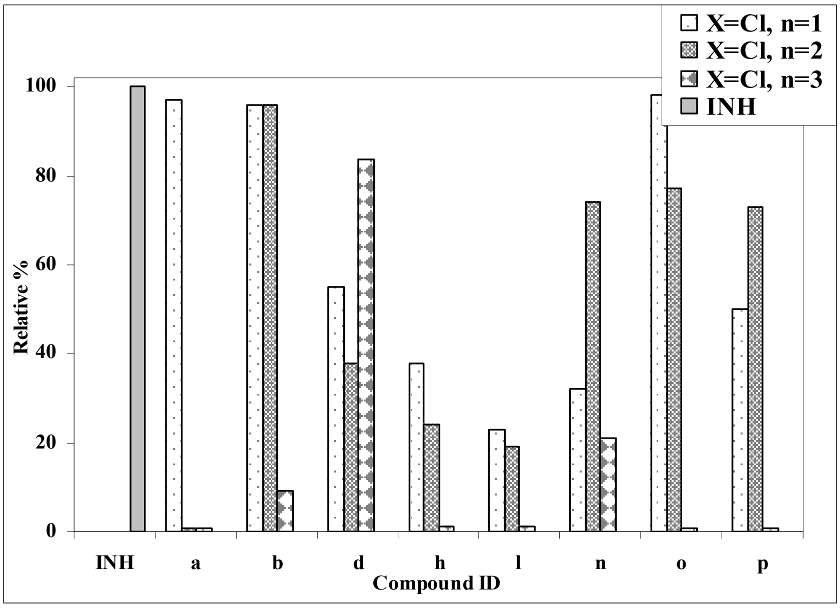


***Fig. 7.** Influence of the "spacer" (-CH_2-)$_n$ on the activity*

An attempt was made to trace the influence of the "spacer" (-CH_2-)$_n$: n = 1, 2 or 3 at a fixed (and found to be most favorable) halogen X = Cl .

A graphical presentation of this analysis is shown on Figure 7.

The graph indicates that the influence of spacer may not be interpreted separately. Nevertheless the compounds with the longest spacer (n = 3) are evidently found to be less active (with one exception: d).

Conclusion

New pyrrole based hydrazones, designed and synthesized as potential tuberculostatics, completed the series of recently published structural analogs. In a comparative structure-activity analysis of the total set of compounds, the effects of halogen and the length of the acyl chain were studied. The effect of the halogen upon the tuberculostatic activity was found to dominate. The most favorable combination of both structural characteristics comprises **Cl**-substituted hydrazones with a spacer (-CH_2-)$_2$ and could be helpful in future design and optimizations in the search for new antituberculosis agents.

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