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Synthesis, Structure and Antiradical Activity of Functionally Substituted Hydrazides of Isonicotinic Acid

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Abstract

The purpose of this work is the synthesis of new isonicotinic acid hydrazones, the study of their structure, reactivity and biological screening of some synthesized compounds. The reactions leading to new N-arylidene(alkylidene) hydrazones via the condensation of isonicotinic acid hydrazide with various derivatives of aromatic aldehydes were studied. The structure of the new functionally substituted isonicotinic acid hydrazones was established by FTIR, ^{1}H and ^{13}C NMR, two-dimensional COSY (^{1}H-^{1}H) NMR spectroscopy and HMQC (^{1}H-^{13}C). The antiradical activity of the synthesized derivatives was studied using diphenylpycrylhydrazide radical (DPPH) assay. It was shown that N-(3-ethoxy-4-hydroxybenzylidene)isonicotinohydrazide possesses antiradical activity (IC_{50} (DPPH) 103.0 μM). The antiradical properties of compounds of phenolic nature are in agreement with the energies of homolytic O-H bond dissociation calculated with the use of density functional theory.

1. Introduction

The synthesis of compounds with desired properties, including the modification of molecules of known drugs, is one of the areas of search for new biologically active substances.

Various derivatives of isonicotinic acid hydrazide (INH) can be considered promising compounds. Many of them are widely used as effective drugs in medical practice (ftivazide, methazide, phenazide, etc.) [1–2]. To date, numerous derivatives with a wide variation of anti-tuberculosis activity and toxicity have been synthesized based on INH [3–8]. Thus, aldazon with the structure of 1-isonicotinoyl-2-glucosyl-hydrazone has biological activity comparable to isoniazid, while possessing fifteen-fold reduced toxicity and prolonged action [9]. In some cases, the so-called «hybrid» structures were obtained which combine moieties of different biologically active compounds, such as acylhydrazone based on isoniazid and vitamin B_6 [9].

Recently, there have been many reports that isoniazid and its derivatives used to prevent and treat tuberculosis are not free from side effects [10]. As pharmacological agents, they can cause changes in the metabolic and structural-functional state of the organs and systems of the living organism. It is important to note that the toxicity of isoniazid is associated mainly with its metabolites [11, 12]. Therefore, further search and synthesis of isoniazid analogues, possibly with another type of metabolism and decreased toxicity, is an interesting and promising task.

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2. Experimental part

The FTIR spectrum of clathrate samples was obtained using a Cary 600 series IR Fourier spectrometer manufactured by Agilent Technologies (USA) in the range of 4000–400 cm^{-1}. The NMR ^{1}H and ^{13}C spectra of compounds 1–15 were recorded on a JNM-ECA Jeol 400 spectrometer (frequency 399.78 and 100.53 MHz, respectively) using DMSO-d_6 as a solvent. Residual protons or carbon atoms of DMSO-d_6 were used as a reference. IR spectra were recorded on a Cary 600 Series FTIR IR spectrophotometer (Agilent Technologies, USA) using a diamond single-reflection module Gladiatr (PIKE, USA). The IR measurements were carried out at a resolution of 4.0 cm^{-1} with 40 scans. Melting points were determined on an OptiMelt device. TLC analysis was performed using Silufol UV-254 plates with iodine vapor visualization.

2.1 Quantum-chemical calculations

Quantum-chemical calculations were performed using the Gaussian 09w program (Revision D.01) using the method of density functional theory (DFT). The B3LYP hybrid functional and 6-31+G(d,p) basis set were applied. The solvent effect of methanol was taken into account within the polarized continuum model (PCM).

2.2 Synthetic procedures

General method of obtaining compounds 1-15.

To a mixture of 0.007 mol of isonicotinic acid hydrazide in 10 ml ethanol, 0.007 mol of aldehyde dissolved in 10 ml ethanol was added on stirring at room temperature. The reaction mixture was refluxed with stirring for 1–4 h at 50 °C. Completion of the reaction was controlled by TLC. The solution was cooled, the precipitate was filtered out. The yields and melting temperatures of compounds 1-15 are given in Table 1.

N-(4-Diethylaminobenzylidene)isonicotinohydrazide (1) IR (KBr, ν_{max}/cm^{-1}): 3214 (N-H), 1697 ($C{=}O_{amid}$), 1601 (C=N). ^{1}H NMR, δ, ppm (*J*, Hz): 1.06 t (6H, $H^{20,20,20,22,22,22}$, ^{3}J 6.9), 3.30-3.35 m (4H, $H^{19,19,21,21}$), 6.66 d (2H, $H^{14,16}$, ^{3}J 10.0), 7.48 d (2H, $H^{13,17}$, ^{3}J 10.0), 7.77 dd (2H, $H^{3,5}$, ^{3}J 6.9, ^{4}J 2.3), 8.72 dd (2H, $H^{2,6}$, ^{3}J 6.0, ^{4}J 2.3), 8.25 s (1H, H^{11}), 11.72 s (1H, H^{9}). ^{13}C NMR, δ_C, ppm: 12.96 ($C^{20,22}$), 44.26 ($C^{19,21}$), 111.56 ($C^{14,16}$), 120.76 (C^{12}), 122.00 ($C^{3,5}$), 129.56 ($C^{13,17}$), 141.37 ($C^{4,11}$), 149.57 ($C^{2,6}$), 150.41 (C^{15}), 161.48 (C^{7}) (Figs. S1 – S4). Found, %: C 69.06; H 7.01; N 18.99. $C_{17}H_{20}N_4O$. Calculated, %: C 68.89; H 6.80; N 18.90.

N-(Diethylamino-2-hydroxybenzylidene)isonicotinohydrazide (2). IR (KBr, ν_{max}/cm^{-1}): 3228 (N-H), 1692 ($C{=}O_{amid}$), 1605 (C=N). ^{1}H NMR, δ, ppm (*J*, Hz): 1.05 t (6H, $H^{20,20,20,22,22,22}$, ^{3}J 6.9), 3.29-3.35 m (4H, $H^{19,19,21,21}$), 6.08 s (1H, H^{16}), 6.23 d (1H, H^{14}, ^{3}J 8.2), 7.19 d (1H, H^{13}, ^{3}J 8.7), 7.78 d (2H, $H^{3,5}$, ^{3}J 2.7), 8.40 s (1H, H^{11}), 8.73 d (2H, $H^{2,6}$, ^{3}J 2.7), 11.26 s (1H, H^{24}), 11.99 s (1H, H^{9}). ^{13}C NMR, δ_C, ppm: 13.04 ($C^{20,22}$), 44.34 ($C^{19,21}$), 97.89 (C^{16}), 104.27 (C^{14}), 106.77 (C^{12}), 121.94 ($C^{3,5}$), 132.15 (C^{13}), 140.77 (C^{4}), 150.84 ($C^{2,6}$), 150.90 (C^{15}), 151.32 (C^{11}), 160.32 (C^{17}), 161.10 (C^{7}) (Figs. S5 – S8). Found, %: C 65.82; H 6.93; N 18.17. $C_{17}H_{20}N_4O_2$. Calculated, %: C 65.37; H 6.45; N 17.94.

N-(4-Dimethylaminobenzylidene)isonicotinohydrazide (3). IR (KBr, ν_{max}/cm^{-1}): 3232 (N-H), 1691 ($C{=}O_{amid}$), 1608 (C=N). ^{1}H NMR, δ, ppm (*J*, Hz): 2.92 s (6H, $H^{19,19,19,20,20,20}$), 6.71 d (2H, $H^{14,16}$, ^{3}J 8.8), 7.52 d (2H, $H^{13,17}$, ^{3}J 8.8), 7.78 d (2H, $H^{3,5}$, ^{3}J 6.0), 8.28 s (1H, H^{11}), 8.73 d (2H, $H^{2,6}$, ^{3}J 4.0), 11.78 br. s (1H, H^{9}). ^{13}C NMR, δ_C, ppm: 40.12 ($C^{19,20}$), 112.27 ($C^{14,16}$), 121.54 (C^{12}), 122.02 ($C^{3,5}$), 129.20 ($C1^{3,17}$), 141.33 (C^{4}), 150.35 (C^{11}), 150.79 ($C^{2,6}$), 152.19 (C^{15}), 161.59 (C^{7}) (Figs. S9 – S12). Found, %: C 67.49; H 6.33; N 21.15. $C_{15}H_{16}N_4O$. Calculated, %: C 67.15; H 6.01; N 20.88.

N-(Pyridine-4-ylmethylene)isonicotinohydrazide (4). IR (KBr, ν_{max}/cm^{-1}): 3240 (N-H), 1698 ($C{=}O_{amid}$), 1610 (C=N). ^{1}H NMR, δ, ppm (*J*, Hz): 7.65 d (2H, $H^{13,17}$, ^{3}J 5.6), 7.79 d (2H, $H^{3,5}$, ^{3}J 5.6), 8.42 s (1H, H^{11}), 8.63 d (2H, $H^{14,16}$, ^{3}J 5.2), 8.76 d (2H, $H^{2,6}$, ^{3}J 5.6), 12.28 br. s (1H, H^{9}). The ^{13}C NMR, δ_C, ppm: 121.65 ($C^{13,17}$), 122.08 ($C^{3,5}$), 140.69 (C^{4}), 141.73 (C^{12}), 147.21 (C^{11}), 150.85 ($C^{14,16}$), 150.91 ($C^{2,6}$), 162.56 (C^{7}) (Figs. S13 – S16). Found, %: C 64.09; H 4.80; N 25.09. $C_{12}H_{10}N_4O$. Calculated, %: C 63.71; H 4.46; N 24.76.

N-(2-Hydroxy-5-nitrobenzylidene)isonicotinohydrazide (5). IR (KBr, ν_{max}/cm^{-1}): 3240 (N-H), 1694 ($C{=}O_{amid}$), 1605 (C=N). ^{1}H NMR, δ, ppm (*J*, Hz): 7.05 d (1H, H^{14}, ^{3}J 9.2 Hz), 7.80 d (2H, $H^{3,5}$, ^{3}J 5.5), 8.11 d (1H, H^{15}, ^{3}J 8.6), 8.54 s (1H, H^{11}), 8.69 s (1H, H^{17}), 8.75 d (2H, $H^{2,6}$, ^{3}J 5.5), 12.15 br. s (1H, H^{18}), 12.37 br. s (1H, H^{9}). ^{13}C NMR, δ_C, ppm: 117.60 (C^{14}), 120.51 (C^{12}), 127.35 (C^{15}), 140.34 (C^{16}), 145.48 (C^{17}), 162.12 (C^{13}), 122.05 ($C^{3,5}$),

150.89 ($C^{2,6}$), 140.45 (C^{4}), 123.91 (C^{11}), 163.09 (C^{7}) (Figs. S17 – S20). Found, %: C 54.89; H 3.83; N 19.91. $C_{13}H_{10}N_4O_4$. Calculated, %: C 54.55; H 3.52; N 19.57.

N-(5-Nitrofuran-2-il-methylene)isonicotinohydrazide (6). IR (KBr, ν_{max}/cm^{-1}): 3245 (N-H), 1696 (C=O_{amid}), 1610 (C=N). ^{1}H NMR, δ, ppm (*J*, Hz): 7.29 d (1H, H^{13}, ^3J 4.3), 7.76-7.79 m (3H, $H^{3,5,14}$), 8.36 s (1H, H^{11}), 8.77 d (2H, $H^{2,6}$, ^3J 5.5), 12.41 br. s (1H, H^{9}). ^{13}C NMR, δ_C, ppm: 115.08 (C^{14}), 116.64 (C^{13}), 122.07 ($C^{3,5}$), 137.20 (C^{11}), 140.35 (C^{4}), 150.99 ($C^{2,6}$), 151.83 (C^{12}), 152.59 (C^{15}), 162.53 (C^{7}) (Figs. S21 – S24). Found, %: C 51.12; H 3.47 N 21.87. $C_{11}H_8N_4O_4$. Calculated, %: C 50.77; H 3.10; N 21.53.

N-(2-Bromo-3-phenylallylidene)isonicotinohydrazide (7). IR (KBr, ν_{max}/cm^{-1}): 3240 (N-H), 1696 (C=O_{amid}), 1611 (C=N). ^{1}H NMR, δ, ppm (*J*, Hz): 7.38-7.43 m (3H, $CH^{15,17,19}$), 7.67 s (1H, H^{13}), 7.77 d (2H, $H^{3,5}$, ^3J 5.6), 7.84 d (2H, $H^{16,18}$, 3J 6.4), 8.34 s (1H, H^{11}), 8.75 d (2H, $H^{2,6}$, ^3J 6.0), 12.19 s (1H, H^{9}). ^{13}C NMR, δ_C, ppm: 119.50 (C^{12}), 122.07 ($C^{3,5}$), 128.99 ($C^{15,19}$), 130.01 (C^{17}), 130.34 ($C^{16,18}$), 135.04 (C^{14}), 140.85 (C^{4}), 149.53 (C^{11}), 150.90 ($C^{2,6}$), 162.27 (C^{7}) (Figs. S25 – S28). Found, %: C 54.90; H 3.79; N 12.96. $C_{15}H_{12}BrN_3O$. Calculated, %: C 54.56; H 3.66; N 12.73.

N-(5-Bromo-2-hydroxybenzylidene)isonicotinohydrazide (8). IR (KBr, ν_{max}/cm^{-1}): 3250 (N-H), 1688 (C=O_{amid}), 1612 (C=N). ^{1}H NMR, δ, ppm (*J*, Hz): 6.87 d (1H, H^{14}, 3J 8.7), 7.40 d (1H, H^{15}, ^3J 8.7, 4J 2.7), 7.79 d (1H, H^{17}, ^4J 2.3 Hz), 7.81 d (2H, $H^{3,5}$, ^3J 6.0), 8.61 s (1H, H^{11}), 8.76 d (2H, $H^{2,6}$, ^3J 6.0), 11.10 br. s (1H, H^{18}), 12.33 br. s (1H, H^{9}). ^{13}C NMR, δ_C, ppm: 111.09 (C^{14}), 119.22 (C^{16}), 121.82 (C^{12}), 122.06 ($C^{3,5}$), 130.63 (C^{17}), 134.42 (C^{15}), 140.41 (C^{4}), 146.84 (C^{11}), 150.91 ($C^{2,6}$), 156.99 (C^{13}), 162.01 (C^{7}) (Figs. S29 – S32). Found, %: C 49.06; H 3.34; N 13.45. $C_{13}H_{10}BrN_3O_2$. Calculated, %: C 48.77; H 3.15; N 13.13.

N-(3-Bromo-4-fluorobenzylidene)isonicotinohydrazide (9). IR (KBr, ν_{max}/cm^{-1}): 3245 (N-H), 1692 (C=O_{amid}), 1605 (C=N). ^{1}H NMR, δ, ppm (*J*, Hz): 7.45 t (1H, H^{16}, ^3J 8.5), 7.78 d (3H, $H^{3,5,17}$, ^3J 4.9), 8.05 d (1H, $H^{2,6}$, ^3J 6.7), 8.39 s (1H, H^{11}), 8.75 d (2H, $H^{2,6}$, ^3J 4.3), 12.20 s (1H, H^{9}). ^{13}C NMR, δ_C, ppm: 109.22 (C^{14}), 109.44 (C^{14}), 117.79 (C^{16}), 122.09 ($C^{3,5}$), 129.09 (C^{17}), 132.39 (C^{13}), 132.90 (C^{12}), 140.78 (C^{4}), 146.81 (C^{11}), 150.89 ($C^{2,6}$), 158.53 (C^{15}), 161.00 (C^{15}), 162.30 (C^{7}) (Figs. S33 – S36). Found, %: C 48.76; H 3.02; N 13.34. $C_{13}H_9BrFN_3O$. Calculated, %: C 48.47; H 2.82; N 13.04.

N-(3-Fluorobenzylidene)isonicotinohydrazide (10). IR (KBr, ν_{max}/cm^{-1}): 3237 (N-H), 1693 (C=O_{amid}), 1605 (C=N). ^{1}H NMR, δ, ppm (*J*, Hz): 7.22-7.26 m (1H, H^{15}), 7.44-7.56 m (3H, $H^{13,14,17}$), 7.80 d (2H, $H^{3,5}$, ^3J 6.1), 8.44 s (1H, H^{11}), 8.75 d (2H, $H^{2,6}$, ^3J 4.6), 12.15 br. s (1H, H^{9}). ^{13}C NMR, δ_C, ppm: 113.63 (C^{17}), 113.85 (C^{17}), 117.52 (C^{15}), 117.73 (C^{15}), 122.07 ($C^{3,5}$), 124.15 (C^{13}), 131.42 (C^{14}), 131.49 (C^{14}), 137.09 (C^{12}), 137.17 (C^{12}), 140.84 (C^{4}), 148.15 (C^{11}), 150.87 ($C^{2,6}$), 161.73 (C^{16}), 162.30 (C^{7}), 164.15 (C^{16}) (Figs. S37 – S40). Found, %: C 64.43; H 4.29; N 17.62. $C_{13}H_{10}FN_3O$. Calculated, %: C 64.19; H 4.14; N 17.28.

N-(4-Fluorobenzylidene)isonicotinohydrazide (11). IR (KBr, ν_{max}/cm^{-1}): 3241 (N-H), 1687 (C=O_{amid}), 1603 (C=N). ^{1}H NMR, δ, ppm (*J*, Hz): 7.24-7.29 m (2H, $H^{14,16}$), 7.76-7.79 m (4H, $H^{3,5,13,17}$), 8.43 s (1H, H^{11}), 8.5 d (2H, $H^{2,6}$, ^3J 6.1), 12.05 br. s (1H, H^{9}). ^{13}C NMR, δ_C, ppm: 116.38 ($C^{14,16}$), 116.60 ($C^{14,16}$), 122.05 ($C^{3,5}$), 129.97 ($C^{13,17}$), 120.05 ($C^{13,17}$), 131.15 (C^{12}), 140.96 (C^{4}), 148.44 (C^{11}), 150.86 ($C^{2,6}$), 162.18 (C^{7}), 162.59 (C^{15}), 165.08 (C^{15}) (Figs. S41 – S44). Found, %: C 64.55; H 4.32; N 17.73. $C_{13}H_{10}FN_3O$. Calculated, %: C 64.19; H 4.14; N 17.28.

N-(2,3,4-Trimethoxybenzylidene)isonicotinohydrazide (12). IR (KBr, ν_{max}/cm^{-1}): 3239 (N-H), 1690 (C=O_{amid}), 1605 (C=N). ^{1}H NMR, δ, ppm (*J*, Hz): 3.74 c (3H, $H^{21,21,21}$), 3.82 s (6H, $H^{19,19,19,23,23,23}$), 6.91 d (1H, H^{16}, ^3J 8.6), 7.61 d (1H, H^{17}, ^3J 8.5), 7.80 d (2H, $H^{3,5}$, ^3J 4.3), 8.74 d (2H, $H^{2,6}$, ^3J 4.3), 8.62 s (1H, H^{11}), 11.97 s (1H, H^{9}). ^{13}C NMR, δ_C, ppm: 56.49 (C^{23}), 61.00 (C^{21}), 62.39 (C^{19}), 109.26 (C^{16}), 120.59 (C^{12}), 121.20 (C^{17}), 142.04 (C^{14}), 153.32 (C^{13}), 155.95 (C^{15}), 122.03 ($C^{3,5}$), 150.82 ($C^{2,6}$), 141.05 (C^{4}), 145.08 (C^{11}), 161.81 (C^{7}) (Figs. S45 – S49). Found, %: C 61.25; H 5.78; N 13.71. $C_{16}H_{17}N_3O_4$. Calculated, %: C 60.94; H 5.43; N 13.33.

N-(3-Ethoxy-4-hydroxybenzylidene)isonicotinohydrazide (13). IR (KBr, ν_{max}/cm^{-1}): 3241 (N-H), 1690 (C=O_{amid}), 1602 (C=N). ^{1}H NMR, δ, ppm (*J*, Hz): 1.32 t (3H, $H^{20,20,20}$, ^3J 7.6), 4.03 q (2H, $H^{19,19}$, ^3J 7.6), 6.84 d (1H, H^{16}, ^3J 9.2), 7.08 d (1H, H^{17}, ^3J 6.2), 7.28 s (1H, H^{13}), 7.78 d (2H, $H^{3,5}$, ^3J 6.1), 8.31 s (1H, H^{11}), 8.74 d (2H, $H^{2,6}$, ^3J 6.1), 9.50 br. s (1H, H^{21}), 11.85 br. s (1H, H^{9}). ^{13}C NMR, δ_C, ppm: 15.23 (C^{20}), 64.43 (C^{19}), 110.95 (C^{13}), 116.10 (C^{16}), 122.02 ($C^{3,5}$), 122.90 (C^{17}), 125.91 (C^{12}),

141.22 (C^4), 147.76 (C^{14}), 150.09 (C^{15}), 150.15 (C^{11}), 150.81 ($C^{2,6}$), 161.88 (C^7) (Figs. S49 – S52). Found, %: C 63.43; H 5.69; N 14.98. $C_{15}H_{15}N_3O_3$. Calculated, %: C 63.15; H 5.30; N 14.73.

N-(2-Octyl-3-phenylallylidene)isonicotinohydrazide (14). IR (KBr, ν_{max}/cm^{-1}): 3251 (N-H), 1690 (C=O_{amid}), 1605 (C=N). ^{1}H NMR, δ, ppm (*J*, Hz): 0.73-0.79 m (3H, $H^{25,25,25}$), 1.05-1.09 m (2H, $H^{23,23}$), 1.21-1.29 m (4H, $H^{22,22,24,24}$), 1.51 m (2H, $H^{21,21}$), 2.53-2.58 m (2H, $H^{20,20}$), 6.79 br. s (1H, H^{13}), 7.28-7.39 m (4H, $H^{16,18,15,19}$), 7.55 br. s (1H, CH^{11}), 7.76 d (2H, $H^{3,5}$, ^3J 6.0), 8.13 br. s (1H, H^{17}), 8.73 d (2H, $H^{2,6}$, ^3J 6.0), 11.86 s (1H, H^9). ^{13}C NMR, δ_C, ppm: 14.46 (C^{25}), 22.59 (C^{24}), 26.22 (C^{20}), 28.53 (C^{21}), 29.45 (C^{22}), 31.30 (C^{23}), 122.04 ($C^{3,5}$), 128.43 ($C^{16,18}$), 128.96 ($C^{15,19}$), 136.58 (C^{14}), 137.15 (C^{13}), 137.99 (C^{12}), 141.18 (C^4), 149.83 (C^{11}), 150.82 ($C^{2,6}$), 154.22 (C^{17}), 161.93 (C^7) (Figs. S53 – S56). Found, %: C 75.42; H 7.23; N 12.59. $C_{21}H_{25}N_3O$. Calculated, %: C 75.19; H 7.51; N 12.53.

N-(4-Styrylbenzylidene)isonicotinohydrazide (15). IR (KBr, ν_{max}/cm^{-1}): 3252 (N-H), 1690 (C=O_{amid}), 1598 (C=N). ^{1}H NMR, δ, ppm (*J*, Hz): 7.21-7.27 m (3H, $H^{23,18,19}$), 7.33-7.37 m (4H, $H^{14,16,21,25}$), 7.57-7.71 m (4H, $H^{13,17,22,24}$), 7.74-7.79 m (2H, $H^{3,5}$), 8.46 s (1H, H^{11}), 8.75 d (2H, $H^{2,6}$, ^3J 4.0), 11.86 s (1H, H^9). ^{13}C NMR, δ_C, ppm: 122.07 ($C^{3,5}$), 127.20 ($C^{14,16}$), 127.46 ($C^{21,25}$), 128.21 ($C^{13,17}$), 128.49 ($C^{18,23}$), 129.30 ($C^{22,24}$), 130.27 (C^{19}), 133.66 (C^{12}), 137.36 (C^{20}), 139.61 (C^4), 140.99 (C^{15}), 144.92 (C^{11}), 149.11 ($C^{2,6}$), 162.10 (C^7) (Figs. S57 – S59). Found, %: C 77.29; H 5.38; N 12.59. $C_{21}H_{17}N_3O$. Calculated, %: C 77.04; H 5.23; N 12.84.

2.3 Antiradical activity

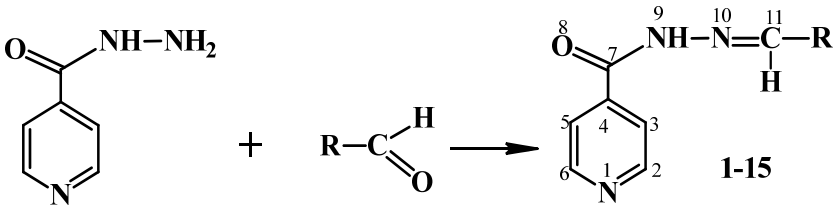
To assess the antiradical activity of the studied compounds in the DPPH assay, a methanol solution of DPPH (100 μM) was used. For the selection of substances with pronounced antiradical activity, 2 ml of 100 μM DPPH methanol solution was mixed with 20 μl of the studied compound dissolved in methanol at a concentration of 5 μM. The final concentration of the tested substance in the reaction mixture was 50 μM. 10 minutes after the addition of the tested compound to the DPPH radical solution, the absorbance decrease was measured at 515 nm. For substances capable of decreasing the absorbance by more than 30%, tests for interaction with the DPPH radical in the final concentrations 100, 75, 50, 25, 20, 10 and 5 μM of the studied compounds in methanol were performed. Afterward, the concentration of each tested substance capable of reducing the absorbance by 50% – IC_{50} (DPPH) was determined (Table 2) [13].

3. Results and discussion

Continuing research on the modification of isonicotinic acid hydrazide, we found it interesting to obtain new INH-based hydrazones which contain various pharmacophoric groups. In this work, N-arylidene(alkylidene)hydrazones 1–15 were synthesized by condensation of isonicotinic acid hydrazide with a series of aromatic and heteroaromatic aldehydes. The condensation reaction was carried out by heating equimolar amounts of aldehyde and INH in ethyl alcohol at 60–70 °C for 3–5 h. The products 1–15 are easily crystallizable substances from white to yellow color, soluble in many organic solvents; compound yields were 63–98% (Table 1).

The composition and structure of compounds 1–15 were confirmed by the methods of IR, ^{1}H and ^{13}C NMR spectroscopy, as well as by two-dimensional COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C) spectra.

In the IR spectra of compounds, there are bands of intense absorption in the region of 3214–3252 cm^{-1}, which correspond to the stretching fluctuations of NH, and the band 1690–1697 cm^{-1}, corresponding to the stretching fluctuations of the C-O_{amid} groups. The absorption in the range of 1870–1955 cm^{-1} is explained by the overtone of pyridine rings. The bands corresponding to the stretching fluctuations C= N appear at 1598–1612 cm^{-1} and OH groups appear in the region at 3390–



3450 cm^{-1}, respectively. In the ^{1}H-NMR spectrum of compound 1, signals of the diethylamino fragment are observed as a triplet ($H^{20,20,20,22,22,22}$) with ^{3}J 6.9 Hz at 1.06 ppm and a multiplet ($H^{19,19,21,21}$) at 3.30–3.35 ppm. Doublets at 6.66 ppm with ^{3}J 10.0 Hz and 7.48 ppm with ^{3}J 10.0 Hz were assigned to the benzylidene protons $H^{14,16}$ and $H^{13,17}$, respectively. Protons of the pyridine ring $H^{3,5}$ and $H^{2,6}$ are observed as doublets of doublets at 7.77 ppm with J 6.9 and 2.3 Hz and 8.72 ppm with J 6.0 and 2.3 Hz, respectively. A signal of CH=N benzylidene proton H^{11} appears as a singlet at 8.25 ppm. A signal of hydrazide proton H^9 is observed downfield at 11.72 ppm.

In the ^{13}C NMR spectrum of compound 1, the signals of diethylamino group are observed at 12.96 ($C^{20,22}$) and 44.26 ppm ($C^{19,21}$). Carbon atoms of the benzylidene fragment give signals at 111.56 ($C^{14,16}$), 120.76 (C^{12}), 129.56 ($C^{13,17}$), 141.37 (C^{11}) and 150.41 ppm (C^{15}). Signals of the carbon atoms in the pyridine ring appear at 122.00 ($C^{3,5}$), 141.37 (C^4) and 149.57 ($C^{2,6}$). In the downfield region at 161.48 ppm appears the signal of the carbonyl C^7 atom.

The structure of compound 1 was also confirmed by two-dimensional NMR spectroscopy COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C) with the aim to establish spin-spin interactions of homo- and heteronuclear nature. The observed correlations in molecule 1 are presented in Figs. 1 and 2. In the ^{1}H-^{1}H COSY spectrum, spin-spin correlations through three bonds of the neighboring aliphatic protons were observed for the diethylamino group $H^{20,22}$-$H^{19,21}$ (1.03, 3.33 and 3.32, 1.05), benzylidene fragment $H^{14,16}$-$H^{13,17}$ (6.66, 7.48 and 7.48, 6.66), and the pyridine ring $H^{3,5}$-$H^{2,6}$ (7.76, 8.72 and 8.71, 7.77) (Fig. 1). Heteronuclear interactions of protons with carbon atoms through a single bond were

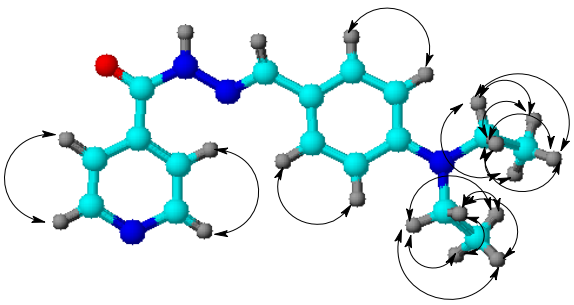


Fig. 1. Correlation scheme of COSY (^{1}H-^{1}H) of compound 1.

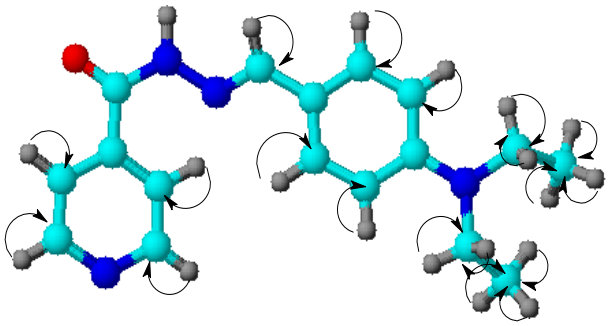


Fig. 2. Correlation scheme of HMQC (^{1}H-^{13}C) of compound 1.

established using ^{1}H-^{13}C HMQC spectroscopy for the pairs $H^{20,22}$-$C^{20,22}$ (1.03, 12.93), $H^{14,16}$-$C^{14,16}$ (6.63, 111.53), $H^{13,17}$-$C^{13,17}$ (7.45, 129.54), $H^{3,5}$-$C^{3,5}$ (7.76, 121.92), and $H^{2,6}$-$C^{2,6}$ (8.70, 150.73) (Fig. 2).

We have studied antiradical activity of the synthesized compounds 1–5, using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay. In our experiments, 100 μM methanol solution of DPPH was used. For a preliminary selection of substances with pronounced antiradical activity, the synthesized compounds were studied at a concentration of 50 μM. For a compound capable of reducing the optical density by more than 30%, further experiments were conducted with concentrations of the studied hydrazides from 5 to 100 μM. Finally, a concentration of a tested compound reducing the optical density by 50% – IC_{50} (DPPH) was determined (Table 2) [13].

Table 2 shows that N-(3-ethoxy-4-hydroxybenzylidene) isonicotinohydrazide 13 reduced the absorbance of the initial DPPH radical solution by 30.2%, being of interest for further investigation.

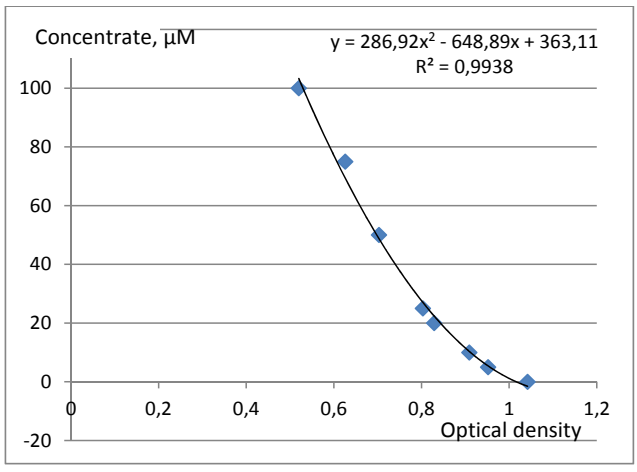


Fig. 3. Plot of absorbance at 515 nm of the DPPH radical solution vs concentration of compound 13.

Table 1
Yields and melting points of compounds 1–15.

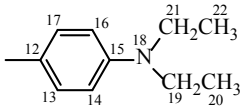
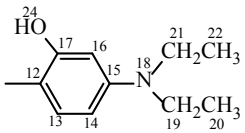
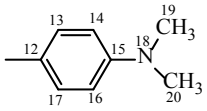
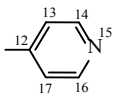
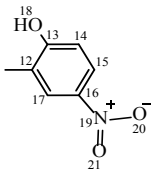
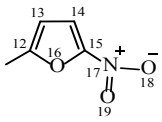
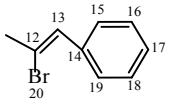
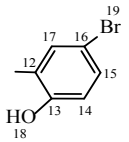
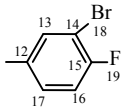
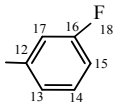
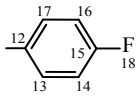
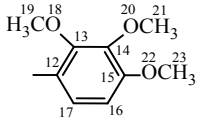
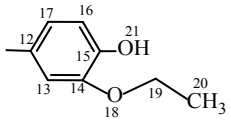
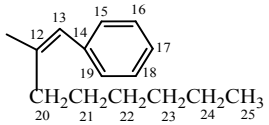
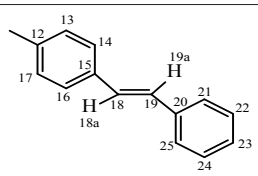
Compd. No.	R	Yeld, %	Melting point, °C
1		96	192-193
2		82	224-225
3		73.9	162-165
4		95	219-220
5		90	285-287
6		93	220-222
7		63	211-213
8		91	320-322
9		90	212-213
10		89	202-203
11		88	185-186
12		63	170-172
13		67.4	245-247
14		98	110-112
15		87.8	255-256

Table 2
Absorbance at 515 nm of a 100 μM solution of DPPH radical in methanol after 10 min of incubation with the tested compound in a final concentration of 50 μM.

Compd. No.	Compound	Absorbance	A decrease in absorbance of the initial DPPH radical solution, % of the control
1	N-(4-diethylaminobenzylidene)-isonicotinohydrazide	0.954	2.4
2	N-(diethylamino-2-hydroxy-benzylidene)isonicotinohydrazide	0.841	13.9
3	N-(4-dimethylaminobenzylidene) isonicotinohydrazide	0.841	13.9
4	N-(pyridine-4-ylmethylene)-isonicotinohydrazide	0.976	0.1
5	N-(2-hydroxy-5-nitrobenzylidene)-isonicotinohydrazide	0.972	0.5
6	N-(5-nitrofuran-2-yl-methylene) isonicotinohydrazide	0.974	0.3
7	N-(2-bromo-3-phenylallylidene)-isonicotinohydrazide	0.965	1.2
8	N-(5-bromo-2-hydroxyben-zylidene)isonicotinohydrazide	0.975	0.2
9	N-(3-bromine-4-fluoroben-zylidene)isonicotinohydrazide	0.975	0.2
10	N-(3-fluorobenzylidene)-isonicotinohydrazide	0.974	0.3
11	N-(4-fluorobenzylidene)-isonicotinohydrazide	0.974	0.3
12	N-(2,3,4-trimethoxybenzylidene)-isonicotinohydrazide	0.957	2.0
13	N-(3-ethoxy-4-hydroxyben-zylidene)isonicotinohydrazide	0.682	30.2
14	N-(2-octyl-3-phenylallylidene)-isonicotinohydrazide	0.977	0.2
15	N-(4-styrylbenzylidene)-isonicotinohydrazide	0.976	0.1
	Control (DPPH solution without test sample)	0.977	-

Table 3
Absorbance at 515 nm of 100 μM DPPH radical solution in methanol after 10 min of incubation with compound 13 in the final concentrations in the reaction mixture of 100, 75, 50, 25, 20, 10, and 5 μM.

#	Final concentration of the compound 13 in the reaction mixture, μM	Absorbance
1	100	0.520
2	75	0.626
3	50	0.703
4	25	0.803
5	20	0.829
6	10	0.909
7	5	0.952
	Control (DPPH solution without test sample)	1.042

Other compounds did not show antiradical activity in the conditions of DPPH assay. Hence, in the second series of experiments we have studied the ability of compound 13 in different concentrations (from 5.0 to 100 μM) to interact with the DPPH radical (Table 3).

From the derived curve (Fig. 3), we have determined the concentration of N-(3-ethoxy-4-hydroxybenzylidene) isonicotinohydrazide 13, capable of reducing by 50% the absorbance of the 100 μM DPPH radical solution. For compound 13, the IC_{50} (DPPH) value is equal to 103.0 μM.

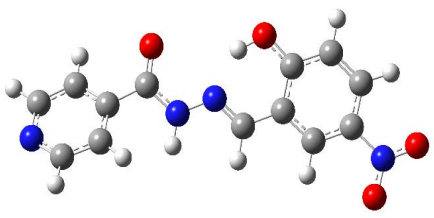
It is known [14] that the interaction of a phenolic compound with the DPPH radical occurs through the homolytic cleavage of the O–H bond leading to a formation of phenoxyl radical:

$$DPPH^{\bullet} + ArOH \rightarrow DPPH\text{-}H + ArO^{\bullet}$$

Since this stage is rate-limiting [15], the reactivity of a potential antioxidant ArOH with respect to the DPPH radical should be largely determined by the energy of homolytic dissociation of O–H bond:

$$ArOH \rightarrow ArO^{\bullet} + H^{\bullet}, \quad \Delta H_d$$

(a)



(b)

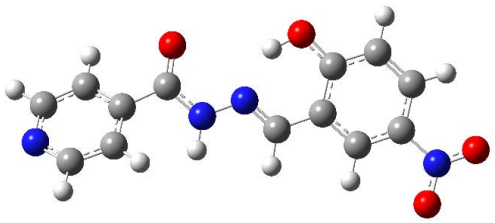


Fig. 4. Structures of compounds 5 (a) and 13 (b) in methanol solution according to the results of DFT geometry optimization.

We have calculated the enthalpy ΔH_d as a measure of O–H bond dissociation energy in hydroxyl-containing compounds 2, 5, 8, 13 using the quantum-chemical density functional theory (DFT) at B3LYP/6-31+G(d,p) level. Geometry optimizations were performed both for molecules 2, 5, 8, 13 and for the corresponding phenoxyl radicals. The solvent (methanol) was accounted for within the PCM solvation model. It should be noted that in the found energy minima, the hydroxyl-containing molecules are stabilized by intramolecular hydrogen bonds OH···N or OH···O since the calculated distances between the phenolic hydrogen atom and the heteroatom are 1.76, 1.73, 1.76, and 2.11 Å for compounds 2, 5, 8 and 13, respectively (see examples in Fig. 4). According to our DFT data, the pyridine ring is rotated about C–CO bond adopting the out-of-plane torsion angle of 29–32° relative to the hydrazide fragment. The DFT calculation shows that the geometrical structures of the $ArO^{\bullet}$ radicals differ only slightly from the structures of the corresponding initial molecules.

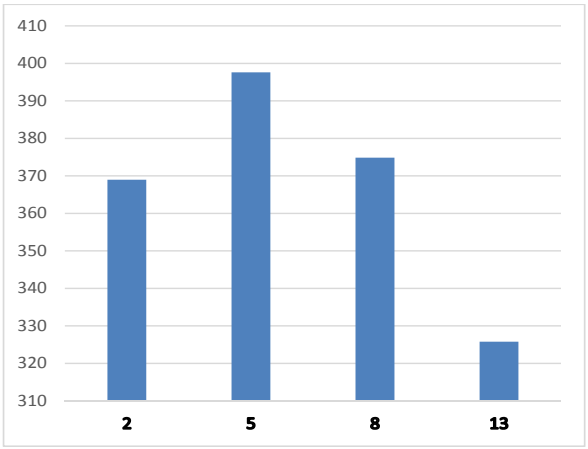


Fig. 5. Heats of phenoxyl radical formation (ΔHd, kJ/mol) from compounds 2, 5, 8, and 13 according to the results of DFT calculations.

The O–H bond dissociation energies calculated as enthalpies ΔH_d for the studied compounds were equal to 369.0 (2), 397.6 (5), 374.9 (8), 325.8 kJ/mol (13) (Fig. 5).

The phenoxyl radical is most easily formed from compound 13 (Fig. 5). This result is consistent with the observed antiradical activity. Previously, for chalcone derivatives with pronounced antiradical properties, we also obtained ΔHd values of about 320–330 kJ/mol [16]. For inactive hydrazides 5 and 8, the energy of homolytic dissociation of the O-H bond is 50–70 kJ/mol higher than that of molecule 13. Nitro-substituted derivative 5 has the highest ΔH_d value – obviously due to the strong acceptor character which destabilizes the corresponding phenoxy radical. Isonicotinohydrazide 2, although possessing an increased heat of radical formation in comparison with compound 13, still has a slight antiradical activity. In this regard, the presence of approximately the same activity value for dimethylamino derivative 3 that does not contain phenolic hydroxyl is not entirely clear. The mechanism of interaction of molecule 3 with the DPPH radical requires further investigation, since other similar compounds, for which the formation of phenoxyl radicals is impossible, do not reduce optical density in experiments with DPPH.

Thus, an increased tendency to homolytic dissociation of the O–H bond may be one of the main reasons for the observed antiradical activity of compound 13.

4. Conclusion

Reactions leading to the formation of new N-arylidene (alkylidene)hydrazones by condensation of isonicotinic acid hydrazide with various derivatives of aromatic aldehydes have been studied. The results of FTIR, ^{1}H and ^{13}C NMR, COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C) two-dimensional NMR spectroscopy confirmed the structure of new functionally substituted isonicotinic acid hydrazones. A high antiradical activity of N-(3-methoxy-4-hydroxybenzylidene)-isonicotinohydrazide was established, and the IC_{50} of this compound was 103.0 microns. Therefore, this compound is promising for further pharmacological studies.

Acknowledgements

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at DOI: https://doi.org/10.18321/ectj1502.

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