Bulgarian Chemical Communications, Volume 51, Special Issue A (pp. 179 -185) 2019

Synthesis and preliminary antioxidant activity evaluation of new pyrrole based aryl hydrazones

D. G. Tzankova[1], S. P. Vladimirova[2], L. P. Peikova[1], M. B. Georgieva[1*]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy - Medical University – Sofia, 2 Dunav str., 1000 Sofia, Bulgaria*

[2]*Department of Organic Synthesis and Fuels, University of Chemical Technology and Metallurgy, 8 Kl. Ohridski Blvd, 1756 Sofia, Bulgaria*

Received February 15, 2019; Revised March 16, 2019

Six new hydrazones of ethyl 5-(4-bromophenyl)-1-(1-hydrazinyl-4-methyl-1-oxopentan-2-yl)-2-methyl-1H-pyrrole-3-carboxylate were prepared in a micro synthesis conditions with 62 – 84 % yields. The applied synthetic methodology assured low harmful emissions and reagent economy. The performed IR, ^{1}H, ^{13}C NMR, MS spectral analysis and microanalysis were consistent with the assigned evaluated structures. The substances purity was confirmed by TLC characteristics and corresponding melting points. All new compounds were subjected for evaluation of their free radical scavenging activity; using classical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) tests. Compounds **5** and **5d** were determined to express good DPPH activity, and three of the new structures (**5**, **5b** and **5d**) expressed good ABTS activity, when compared to butylated hydroxytoluene (BHT), used as a control.

Key words: pyrrole hydrazones, synthesis, radical scavenging activity

INTRODUCTION

Pyrrole is a five-member heterocyclic organic compound with C_4H_4NH general formula, and a subject of a host of investigations [1].

Pyrrole and its derivatives form a significant class of organic compounds due to their active role in many diseases. The development of novel pyrrolyl hydrazide-hydrazones containing the combination of different pharmacophores in a pyrrole ring system led to the formation of more active compounds [2, 3].

Pyrroles contain an active hydrogen atom (N-H) and possess antioxidant activity [4] thus pyrrole and its derivatives might act as a natural antioxidants against oxidative stress [5, 6]. On the other hand pyrrole-containing molecules provide a possible *in vivo* protection against free radical damage as pointed out in some literary data [4, 7-10]. However, so far, there is still lack of information on the relationship between the structure and the antioxidative activity of most organic compounds, especially pyrrole derivatives.

Free radicals, especially those derived from oxygen, can react with various biological molecules because of their high reactivity. These radicals play a role in most major health problem of the industrialized world, such as cardiovascular diseases, cancer, neurological diseases and aging [11, 12]. To date, a large number of antioxidants has been either synthesized or separated from naturally occurring resource such as fruit, vegetables, plant and marine animals, and many of which exhibit a good antioxidative activity against DPPH, ABTS, hydroxyl radical and so on [13]. Particularly, many natural compounds containing a pyrrole-ring moiety are of great interests because of their biological activities, which have been widely used in medicine and agriculture [14].

This study is focused on the synthesis of new N-pyrrolyl hydrazide-arylhydrazones and establishing their antioxidant activity.

EXPERIMENTAL PART

All experiments were monitored by thin layer chromatography (TLC), performed on aluminium sheets Silicagel 60 F_{254} (Merck, Darmstadt, Germany), using $CHCl_3/CH_3CH_2OH$ as a mobile phase. Yields were calculated for purified products. Melting points were measured in open capillary tubes with a Digital Melting Point Apparatus IA 9200 ELECTROTHERMAL (Southend-on-Sea, England) and are uncorrected. All names were generated by using structure –to – name algorithm of ChemBioDrawUltra software, Version 11.0, CambridgeSoft. The IR spectra 400 – 4000cm^{-1} were recorded on a Nicolet iS10 FT-IR Spectrometer using ATR technique with Smart iTR adapter. ^{1}H- and ^{13}C-NMR measurements were performed using a Bruker Spectrospin WM 600 and 250 spectrometer (Faenlanden, Switzerland) operating at 600 and 250 MHz respectively, as δ (ppm) relative to TMS as internal standard and the coupling constants (*J*) are expressed in Hertz (Hz). All NH protons were D_2O exchangeable. Mass spectra were recorded on 6410 Agilent LCMS triple quadrupole mass spectrometer (LCMS) with an

* To whom all correspondence should be sent:
E-mail: maya.bg77@gmail.com

electrospray ionization (ESI) interface. Elemental analyses were performed by the microanalytical laboratory of Faculty of Pharmacy (Medical University-Sofia) on Euro EA 3000-Single, EUROVECTOR SpA analyser.

Antioxidant assays were carried out using a Shimadzu UV-1203 spectrophotometer (Japan).

All the commercially available reagents were purchased from Merck (Darmstadt, Germany).

Synthesis of the N-pyrrolylcarboxylic acid (3)

The target compound (3) was synthesized through Paal-Knorr cyclization according to literary method [15].

2-(5-(4-bromophenyl)-3-(ethoxycarbonyl)-2-methyl-1H-pyrrol-1-yl)-4-methyl pentanoic acid (3): Yield 97%, mp 131,9-133,8°C, Rf = 0.62(10 : 0.6). IR (cm^{-1}): 2960 (νOH), 2865 (νCOOH), 1739, 1635 (νCO), 826 (p-disubstituted C_6H_4); ^{1}H-NMR (δ, 600 MHz, $CDCl_3$): 0.54, 0.55 [d, J = 6.31, 3H, CH$\underline{CH_3}$], 0.69, 0.70 [d, J = 6.6, 3H, CH$\underline{CH_3}$], 1.08 - 1.14 [m, 1H, $CH_2\underline{CH}$], 1.91 [m, 2H, $\underline{CH_2}CH$], 1.34 [t, 3H, $CH_2\underline{CH_3}$], 2.58 [s, 3H, CH_3(2)], 4.26 – 4.30 [m, 2H, $\underline{CH_2}CH_3$], 4.92 – 4.95 [q, J = 5.86, 1H, $\underline{CH}CH_2$], 6.57 [s, 1H, H(4)], 7,25, 7.26 [d, J = 8.37, 2H, H(3'), H(5')], 7.26 [s, 1H, OH], 7.53,7.54 [d, J=6.6, 2H, H(2'), H(6')]; ^{13}C-NMR (δ, 600 MHz, $CDCl_3$): 176.0, 165.5, 136.9, 131.9, 131.8, 131.5, 131.3, 122.4, 113.5, 110.5, 59.7, 56.4, 39.6, 25.5, 22.8, 21.4, 14.5, 13.0; MS (FTMS + pESI, m/z): 422.09. Anal. Calc. for $C_{20}H_{24}BrNO_4$:C 56.88, H 5.73, Br 18.92, N 3.32, O 15.15%; Found: C 57.27, H 6.03, Br 18.67, N 2.92, O 15.11%.

Synthesis of intermediate ethyl ester of the N-pyrrolylcarboxylic acid (4)

$SOCl_2$ (0.04mol) was added drop wise at 0°C to dry ethanol (50 mL) under intensive stirring. Thereafter, the obtained N-pyrrolylcarboxylic acid (**3**) (0.01 mol) was added immediately and the mixture was refluxed for 2−3 h to complete the reaction (TLC control). The solvent was removed under reduced pressure and the resulted oil was dissolved in chloroform and washed successively with 5% solution of Na_2CO_3 and water. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue consisted of the relevant ethyl ester, which was used in the next step without isolation and additional purification [16].

Synthesis of the N-pyrrolylcarbohydrazide 5

The target carbohydrazide (**5**) was synthesized by selective hydrazinolysis of the relevant intermediate ethyl ester (**4**) according to the literary method [16] presented on **Scheme 2.**

Ethyl 5-(4-bromophenyl)-1-(1-hydrazinyl-4-methyl-1-oxopentan-2-yl)-2-methyl-1H-pyrrole-3-carboxylate (5): Yield: 84%, mp 140-142°C, Rf = 0.64 (10 : 0.4). IR (cm^{-1}): 3344, 3270 (NH_2), 3230 (NH), 2863 - 2958(CH_3 and CH_2), 1684 with shoulder at 1629 ($COOC_2H_5$, CO - Amide I), 1570 (Amide II), 1247 (C-O), 836 (p-disubstituted C_6H_4); ^{1}H-NMR (δ, 600 MHz, DMSO): 0.53, 0.54 [d, J = 6.16, 3H, CH$\underline{CH_3}$], 0.70, 0.71 [d, J = 6.61, 3H, CH$\underline{CH_3}$], 1.07 -1.14 [m, 1H, $CH_2\underline{CH}$], 1.35 [t, 3H, $CH_2\underline{CH_3}$], 1.81 [brs, 1H, $\underline{CH_2}CH$], 2.07 [brs, 1H, $\underline{CH_2}CH$], 2.53 [s, 3H, CH_3(2)], 3.74 [brs, 1H, NH$\underline{NH_2}$], 4.26 – 4.30 [q, J = 7.04, 2H, $\underline{CH_2}CH_3$], 4.80 – 4.82 [q, J = 4.98, J = 4.99, 1H, $\underline{CH}CH_2$], 6.60 [s, 1H, H(4)], 7.09 [brs, 1H, NH$\underline{NH_2}$], 7,14, 7.15 [d, J = 8.37, 2H, H(3'), H(5')],7.26 [s, 1H, CONH], 7.53,7.54 [d, J=8.21, 2H, H(2'), H(6')]; ^{13}C-NMR (δ, 600 MHz, DMSO): 171.3, 165.0, 136.6, 133.8, 132.0, 131.1, 130.9, 122.5, 114.5, 111.3, 59.8, 57.2, 39.1, 24.6, 22.9, 21.4, 14.5, 13.2; MS (FTMS + pESI, m/z): 438.12. Anal. Calc. for $C_{20}H_{26}BrN_3O_3$:C 55.05, H 6.01, Br 18.31, N 9.63, O 11.00%; Found: C 55.45, H 5.82, Br 18.56, N 9.33, O 10.84%

General procedure for the synthesis of the targeted hydrazones:

Carbohydrazide (**5)** (1.8 mmol) and any of the carbonyl partners **a, b, c, d, f or g** (1.8 mmol) were dissolved in glacial acetic acid (1.5mL) in a reaction vial of 5 mL and stirred at 100 °C for 40-50 min. to complete the reaction under TLC-control. The products were isolated after adding water and recrystallized from ethanol.

(E)-ethyl 5-(4-bromophenyl)-1-(1-(2-(2-hydroxybenzylidene) hydrazinyl)-4-methyl-1 oxopentan-2-yl)-2-methyl-1H-pyrrole-3-carboxylate(5a): Yield: 72%, mp. 115,5-120,9°C, Rf = 0.64 (10 : 0.4). IR (cm^{-1}): 3230 (OH), 3047 (NH), 2867 – 2958 (CH_3 and CH_2), 1667 ($COOC_2H_5$), 1621 (Amide I), 1570 (Amide II), 1243 (C-O), 818 (p-disubstituted C_6H_4), 751 (o-disubstituted C_6H_4); ^{1}H-NMR (δ, 250 MHz, $CDCl_3$): 0.55, 0.58 [d, J = 6.23, 3H, CH$\underline{CH_3}$], 0.69, 0.72 [d, J = 6.48, 3H, CH$\underline{CH_3}$], 1.08 -1.16 [m, 1H, $CH_2\underline{CH}$], 1.32 [t, 3H, $CH_2\underline{CH_3}$], 1.71 – 1.82 [m, 1H, $\underline{CH_2}CH$],], 2.09 – 2.25 [m, 1H, $\underline{CH_2}CH$], 2.56 [s, 3H, CH_3(2)], 4.19 – 4.27 [q, J = 6.35, J = 6.85, 2H, $\underline{CH_2}CH_3$], 4.91 – 4.97 [m, 1H, $\underline{CH}CH_2$], 6.62 [s, 1H, H(4)], 6.90 [t, 1H, OH], 7.00, 7.03 [d, J = 8.56, 1H, H(3")], 7.16, 7.19 [d, J = 7.71, 2H, H(3'), H(5')], 7.16, 7.19 [d, J = 7.71, 1H, H(5")], 7.26 [s, 1H, H(4")], 7.32 [t, 1H, H(6")], 7.54,7.57 [d, J=7.95, 2H, H(2'), H(6')], 8.38 [s, 1H, CONH],8.91 [s, 1H, CH=N], ^{13}C-NMR (δ, 250 MHz, $CDCl_3$):166.4, 165.1, 158.7, 152.5, 136.8, 133.7,132.4, 132.2, 131.9, 131.2, 130.9, 122.7, 119.5, 117.0, 117.4, 114.9, 111.8, 59.9, 57.6, 39.4,

24.8, 22.8, 21.5, 14.4, 13.2; MS (FTMS + pESI, m/z):542.15. Anal. Calc. for $C_{27}H_{30}BrN_3O_4$:C 60.00, H 5.59, Br 14.78, N 7.77, O 11.86%; Found: C 59.60, H 5.90, Br 14.93, N 7.55, O 12.02%

(E)-ethyl 5-(4-bromophenyl)-1-(1-(2-(4-(dimethylamino) benzylidene) hydrazinyl)-4-methyl-1 oxopentan-2-yl)-2-methyl-1H-pyrrole-3-carboxylate (5b): Yield: 62%, mp 115,8-119,9°C, Rf = 0.37(10 : 0.4). IR (cm^{-1}): 3213 (NH), 2802 – 2955 (CH_3 and CH_2), 1687 ($COOC_2H_5$), 1673 (Amide I), 1556 (Amide II), 1242 (C-O), 814 (p-disubstituted C_6H_4); ^{1}H-NMR (δ, 250 MHz, $CDCl_3$): 0.55, 0.57 [d, J = 5.99, 3H, CH$\underline{CH_3}$], 0.66 - 0.75 [m, 3H, CH$\underline{CH_3}$], 1.13 -1.19 [m, 1H, $CH_2\underline{CH}$], 1.31 – 1.39 [m, 3H, $CH_2\underline{CH_3}$], 1.83 – 1.93 [m, 1H, $\underline{CH_2}CH$],], 2.01 – 2.14 [m, 1H, $\underline{CH_2}CH$], 2.60 [s, 3H, CH_3(2)], 3.02 [s, 6H, $NCH_3(CH_3)$], 4.23 – 4.34 [m, 2H, $\underline{CH_2}CH_3$], 4.90 – 4.96 [m, 1H, $\underline{CH}CH_2$], 6.57 [s, 1H, H(4)], 6.66 – 6.69 [m, 2H, H(3”), H(5”)], 7,18 - 7.21 [d, J = 7.95, 2H, H(3’), H(5’)],7.26 [t, 1H, CONH], 7.40 – 7.44 [d, J = 8.31, 2H, H(2”), H(6”)], 7.52 - 7.61 [m, 2H, H(2’), H(6’)], 7.67 [s, 1H, CH=N]; ^{13}C-NMR (δ, 250 MHz, $CDCl_3$):166.2, 165.6, 150.5, 145.6, 136.9, 133.8, 132.4, 131.6, 131.4, 130.9, 128.8, 122.6, 121.8, 113.1, 112.2, 111.9, 110.6, 59.3, 56.1, 40.4, 40.3, 39.3, 24.7, 22.8, 21.2, 14.6, 13.6; MS (FTMS + pESI, m/z): 569.19. Anal. Calc. for $C_{29}H_{35}BrN_4O_3$:C 61.37, H 6.22, Br 14.08, N 9.87, O 8.46%; Found: C 61.55, H 6.17, Br14.46, N 9.65, O 8.17%.

(E)-ethyl 5-(4-bromophenyl)-1-(1-(2-(4-methoxybenzylidene) hydrazinyl)-4-methyl-1-oxopentan-2-yl)-2-methyl-1H-pyrrole-3-carboxylate (5c): Yield: 74%,mp 165,8-168,9°C, Rf = 0.56 (10 : 0.4).IR (cm^{-1}): 3181 (NH), 2839 – 2956 (CH_3 and CH_2), 1693 ($COOC_2H_5$), 1675 (Amide I), 1569 (Amide II), 1245 (C-O), 830 (p-disubstituted C_6H_4); ^{1}H-NMR (δ, 250 MHz, $CDCl_3$):0.55 - 0.63 [m, 3H, CH$\underline{CH_3}$], 0.65 - 0.76[m, 3H, CH$\underline{CH_3}$], 1.31 – 1.39 [m, 3H, $CH_2\underline{CH_3}$] 1.78 - 1.90 [m, 1H, $CH_2\underline{CH}$], , 1.96 – 2.16 [m, 2H, $\underline{CH_2}CH$],], 2.60 [s, 3H, CH_3(2)], 3.84 [s, 3H, O$\underline{CH_3}$], 4.23 – 4.31 [q, J = 5.87, J = 6.85, 2H, $\underline{CH_2}CH_3$], 4.92 – 4.96 [m, 1H, $\underline{CH}CH_2$], 6.57 [s, 1H, H(4)], 6.87 – 6.93 [m, 1H, CH=N], 7,18 - 7.26 [m,2H, H(3’), H(5’)], 7.30 – 7.33 [d, J = 8.56, 2H, H(3”), H(5”)], 7.40 – 7.43 [d, J = 8.07, 2H, H(2”), H(6”)], 7.52 - 7.55 [m, 2H, H(2’), H(6’)], 7.66 – 7.73 [m, 1H, CONH]; ^{13}C-NMR (δ, 250 MHz, $CDCl_3$):166.7, 165.5, 162.2, 150.3, 144.9, 136.8, 133.7, 132.3, 131.6, 131.5, 129.9, 125.7, 122.6, 114.4, 114.3, 113.2, 110.6, 59.4, 56.0, 55.4, 39.4, 24.7, 22.8, 21.2, 14.5, 13.5; MS (FTMS + pESI, m/z):554.16. Anal. Calc. for $C_{28}H_{32}BrN_3O_4$:C 60.65, H 5.82, Br 14.41, N 7.58, O 11.54%; Found: C 60.33, H 5.90, Br 14.17, N 7.59, O 12.01%.

(E)-ethyl 5-(4-bromophenyl)-1-(1-(2-(4-hydroxy-3-methoxybenzylidene) hydrazine-yl)-4-methyl-1-oxopentan-2-yl)-2-methyl-1H-pyrrole-3-carboxylate (5d): Yield: 76%, mp 190,5-194,2°C, Rf = 0.27 (10 : 0.4). IR (cm^{-1}): 3366 (OH), 3266 (NH), 2869 – 2958 (CH_3 and CH_2), 1667 ($COOC_2H_5$), 1600 (Amide I), 1569 (Amide II), 1244(C-O), 815 (p-disubstituted C_6H_4); ^{1}H-NMR (δ, 250 MHz, $CDCl_3$): 0.54 - 0.62 [m, 3H, CH$\underline{CH_3}$], 0.68 - 0.74 [m, 3H, CH$\underline{CH_3}$], 1.14 -1.27 [m, 1H, $CH_2\underline{CH}$], 1.31– 1.39 [m, 3H, $CH_2\underline{CH_3}$], 1.79 – 1.83 [m, 1H, $\underline{CH_2}CH$],], 1.95 – 2.20 [m, 1H, $\underline{CH_2}CH$], 2.60 [s, 3H, CH_3(2)], 3.64 [s, 3H, O$\underline{CH_3}$], 4.23 – 4.34 [m, 2H, $\underline{CH_2}CH_3$], 4.92 – 4.95 [m, 1H, $\underline{CH}CH_2$], 5.77 – 5.83 [m, 1H, OH], 6.58 [s, 1H, H(4)], 6.90 [s, 1H, H(5”)], 7.00 - 7.06 [m, 1H, H(6”)], 7,17, 7.20 [d, J = 7.70, 2H, H(3’), H(5’)],7.26 – 7.30 [d, J = 8.68, 1H, CONH], 7.39 – 7.47 [m, 1H, H(2”)], 7.50 - 7.57 [m, 2H, H(2’), H(6’)], 7.70 [s, 1H, CH=N]; ^{13}C-NMR (δ, 250 MHz, $CDCl_3$):166.6, 165.5, 150.2, 148.4, 147.1, 136.8, 133.5, 132.1, 131.6, 131.5, 130.9, 123.5, 122.6, 114.3, 110.5, 107.9, 106.9, 59.4, 56.2, 56.2, 39.5, 24.5, 22.8, 21.1, 14.5, 13.5; MS (FTMS + pESI, m/z): 572.16. Anal. Calc. for $C_{28}H_{32}BrN_3O_5$:C 58.95, H 5.65, Br 14.01, N 7.37, O 14.02%; Found: C 59.21, H 5.67, Br 14.31, N 7.31, O 13.50%.

(E)-ethyl 5-(4-bromophenyl)-1-(1-(2-(furan-2-ylmethylene) hydrazinyl)-4-methyl-1 oxopentan-2-yl)-2-methyl-1H-pyrrole-3-carboxylate (5f): Yield: 68%, mp 180,8-182,6°C, Rf = 0.78 (10 : 0.4). IR (cm^{-1}): 3243 (NH), 2878 – 2962 (CH_3 and CH_2), 1690 ($COOC_2H_5$), 1670 (Amide I), 1567 (Amide II), 1238 (C-O), 826 (p-disubstituted C_6H_4); ^{1}H-NMR (δ, 250 MHz, $CDCl_3$):0.58[s, 3H, CH$\underline{CH_3}$], 0.66 - 0.75[m, 3H, CH$\underline{CH_3}$], 1.26 - 1.39 [m, 3H, $CH_2\underline{CH_3}$], 1.90 -1.96 [m, 1H, $CH_2\underline{CH}$], 2.13 – 2.23 [m, 1H, $\underline{CH_2}CH$],], 2.72 [s, 1H, $\underline{CH_2}CH$], 2.59 [s, 3H, CH_3(2)], 4.23 – 4.35 [m, 2H, $\underline{CH_2}CH_3$], 4.95 – 5.00 [m,1H, $\underline{CH}CH_2$], 6.46 [s, 1H, H(4)], 6.52 – 6.55 [m, 1H, H(3”)], 6.60 – 6.64 [m, 1H, CONH], 6.71 [t, 1H, H(2”)], 7,14, 7.17 [d, J = 8.07, 2H, H(3’), H(5’)], 7.26, 7.29 [d, J = 8.32, 1H, H(4”)], 7.43 - 7.53 [m,2H, H(2’), H(6’)], 7.63 (s, 1H, CH=N]; ^{13}C-NMR (δ, 250 MHz, $CDCl_3$):167.3, 165.1, 148.5, 144.6, 136.5, 133.7, 131.8, 132.1, 131.6, 131.4, 122.7, 116.8, 114.4, 112.8, 110.6, 59.8, 58.3, 39.2, 22.9, 22.6, 21.3, 14.5, 13.4; MS (FTMS + pESI, m/z): 514.13. Anal. Calc. for $C_{25}H_{28}BrN_3O_4$:C 58.37, H 5.49, Br 15.53, N 8.17, O 12.44%; Found: C 58.20, H 5.78, Br 15.64, N 7.82, O 12.56%.

(E)-ethyl 5-(4-bromophenyl)-2-methyl-1-(4-methyl-1-oxo-1-(2-(2-oxoindolin-3-ylidene) hydrazinyl) pentan-2-yl)-1H-pyrrole-3-carboxylate (5g): Yield: 71%, mp 283,5-285,4°C, Rf = 0.33 (10 : 0.4). IR (cm^{-1}): 3227 (NH), 2875 – 2957 (CH_3 and CH_2), 1702 ($COOC_2H_5$), 1687 (Amide I), 1572 (Amide II), 1246 (C-O), 822 (p-disubstituted C_6H_4), 747 (o-disubstituted C_6H_4); ^{1}H-NMR (δ, 250 MHz, DMSO):0.56, 0.58[d, J = 4.77, 3H, CH$\underline{CH_3}$], 0.67, 0.69[d, J = 4.76, 3H, CH$\underline{CH_3}$], 1.04 -1.17 [m, 1H, $CH_2\underline{CH}$], 1.27 [t, 3H, $CH_2\underline{CH_3}$], 1.72 – 1.86 [m, 1H, $\underline{CH_2}$CH],], 1.89 - 2.01 [m, 1H, $\underline{CH_2}$CH], 2.61 – 2.68 [m, 3H, CH_3(2)], 4.14 – 4.23 [q, J = 7.09, J = 7.21, 2H, $\underline{CH_2}CH_3$], 5.04 – 5.14 [m, 1H, $\underline{CH}CH_2$], 6.50 [s, 1H, H(4)], 6.92 - 6.96 [d, J = 7.94, 1H, H(3'), H(5')],7.04 - 7.11 [m, 1H, H(3'), H(5')], 7.29, 7.32 [d, J = 8.31, 1H, H(6")], 7.29, 7.32 [d, J = 8.31, 1H, H(7")], 7.35 – 7.44 [m, 1H, H(8")], 7.53 - 7.67 [m,2H, H(2'), H(6')], 7.53 – 7.67 [m, 1H, H(5")], 11.18 – 11.25 [m, 1H, Izatin NH], 13.38 – 13.40 [m, 1H, CONH]; ^{13}C-NMR (δ, 250 MHz, DMSO):164.2, 162.7, 142.6, 133.3, 132.8, 132.2, 132.1, 131.9, 131.6, 122.7, 121.5, 119.5, 112.7, 111.3, 59.0, 53.3, 39.5, 24.3, 22.4, 21.2, 14.3, 9.7; MS (FTMS + pESI, m/z):567.14. Anal. Calc. for $C_{28}H_{29}BrN_4O_4$:C 59.47, H 5.17, Br 14.13, N 9.91, O 11.32%; Found: C 59.29, H 5.57, Br 14.36, N 9.81, O 10.97%.

Antioxidant activity

DPPH radical scavenging activity

Free radical scavenging activity was measured by using the DPPH method [17]. Different concentrations (0.125-1 mmol/L) (1 mL) of compounds in methanol were added to the 1 mL methanol solution of DPPH (1 mmol/L). After 30 min incubation at room temperature, their absorptions were read at 517 nm against a control sample containing the methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Percentage of the DPPH radicals scavenged by the studied concentration was calculated according to equation:

$$DPPH_{radicalscavengingactivity}\% = \frac{Abs_{control}-Abs_{sample}}{Abs_{control}} x100,\% \quad (1)$$

where $Abs_{control}$ is the absorbance of DPPH radical in methanol, Abs_{sample} is the absorbance of DPPH radical solution mixed with sample.

The IC_{50} value of the sample (concentration of sample where the absorbance of DPPH decreases 50% with respect to the absorbance of blank) was determined. Butylated hydroxytoluene (BHT) was used as positive control. All determinations were performed in triplicate (n = 3).

ABTS-radical scavenging assay

For ABTS assay, the procedure followed the method of Arnao et al. [18] with some modifications [17]. The stock solutions included 7 mmol/L solution of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2.4 mmol/L solution of potassium persulphate. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 14 hours at room temperature in dark. The solution was then diluted by mixing 2 mL ABTS solution with50 mL methanol to obtain an absorbance of 0.305 ± 0.01 units at 517 nm using a spectrophotometer. A fresh ABTS solution was prepared for each assay. Different concentrations (1 mL) of compounds were allowed to react with 1 mL of the ABTS solution and the absorbance was taken at 517 nm after 7 min. The capability to scavenge the ABTS radical was compared with that of BHT and was calculated using the following equation:

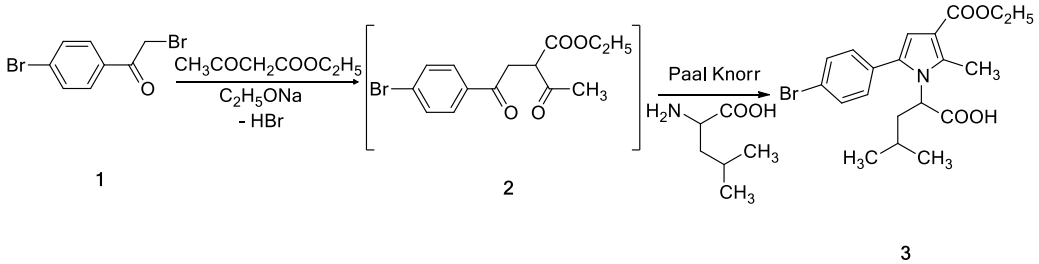
$$ABTS_{radicalscavengingactivity}\% = \frac{Abs_{control}-Abs_{sample}}{Abs_{control}} x100,\% \quad (2)$$

where $Abs_{control}$ is the absorbance of ABTS radical in methanol; Abs_{sample} is the absorbance of an ABTS radical solution mixed with sample.

The IC_{50} value of the sample (concentration of sample where the absorbance of ABTS decreases 50% with respect to the absorbance of blank) was determined. BHT was used as positive control. All determinations were performed in triplicate (n = 3).

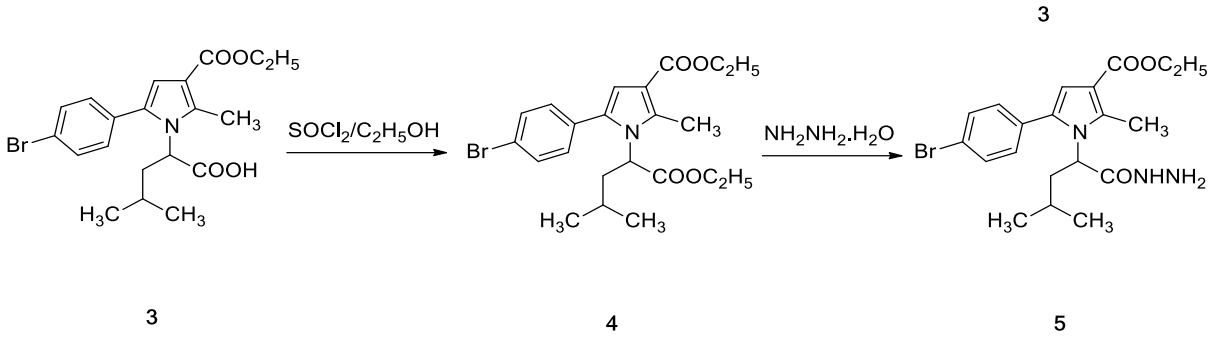
RESULTS

The necessary N-pyrrolylcarboxylic acid (**3**) was prepared through a classical Paal-Knorr cyclization according to the procedure presented on **Scheme 1.**



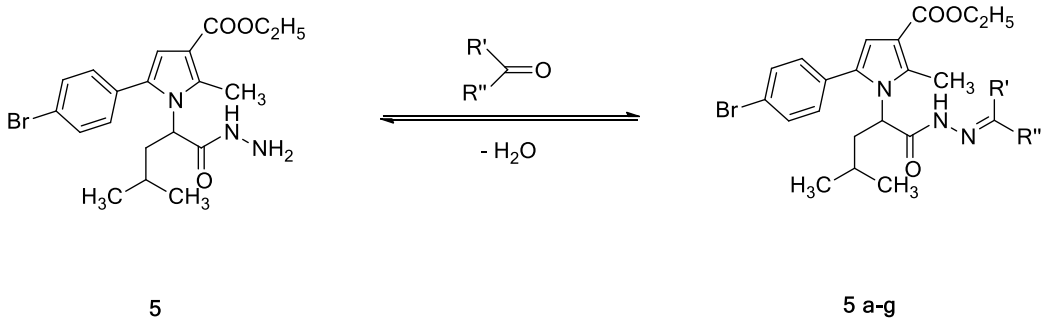
Scheme 1. Synthesis of the initial N-pyrrolyl carboxylic acid.

The target carbohydrazide (**5**) have been obtained by selective hydrazinolysis of the relevant intermediate ethyl ester (**4**) shown in **Scheme 2.** Synthesis of hydrazones were based on a method defined on **Scheme 3** through condensation of the corresponding N-pyrrolyl hydrazide (**5**) with any of the carbonyl compounds **a – g** presented on **Fig. 1.**



3 4 5

Scheme 2. Synthesis of the new N-pyrrolyl hydrazide (5).



5 5 a-g

Scheme 3. General synthesis of the target compounds.

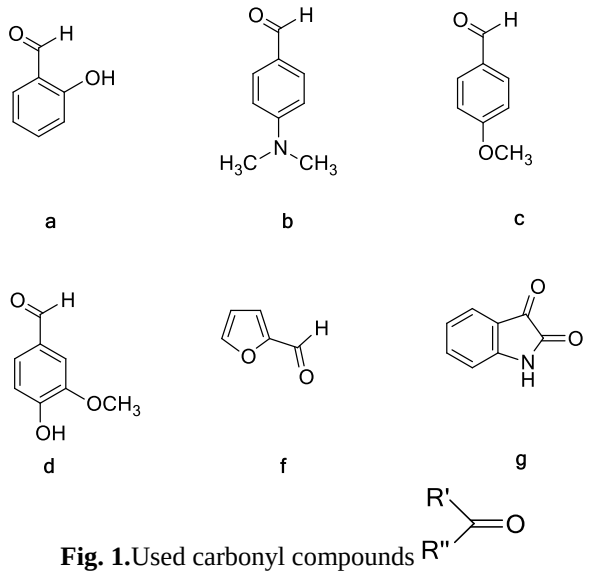


Fig. 1. Used carbonyl compounds

The final hydrazones were synthesized in a micro synthesis apparatus, assuring about 62 – 84% yields, low harmful emissions and reagent economy thus following the requirements of green chemistry.

The purity of the new compounds was confirmed by TLC characteristics and their structure was elucidated, with IR, ^{1}H and ^{13}C NMR spectral data followed by MS spectral and elemental analysis. The spectral results correspond with the assigned structures.

Antioxidant assays

Two common methods [17, 18] for determination of free radicals scavenging activity were applied in this paper in an attempt to establish the possible antioxidant effects, demonstrated from the newly synthesized N-pyrrolyl hydrazide hydrazones. The described methods were chosen for their user-friendly mechanisms for antioxidant activity determination since they require a simple

machine like a spectrophotometer, which is commonly available in most laboratories.

Firstly as method for measurement of the free radical scavenging activity was applied the general DPPH method with slight modifications [19], using the basic property of the stable organic free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and its absorption band at 517 nm. The method is based on loss of this absorption when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow [20].

All 7 newly synthesized compounds were tested for their interaction with the stable free radical DPPH and this interaction, in turn, indicates their radical scavenging activity. At all the concentrations tested, the DPPH radical scavenging activity of the initial hydrazide was higher than that of BHT, the reference antioxidant. All hydrazone derivatives perform radical scavenging activity close to the one of the reference as seen from the results presented on **Fig. 2**.

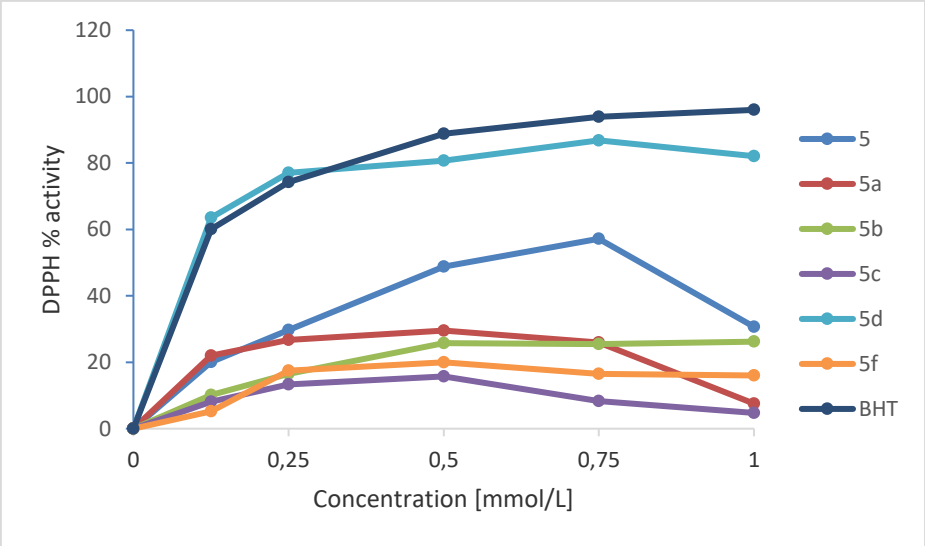


Fig. 2. DPPH radical scavenging activity of studied compounds **5** and **5a-f** and BHT.

Additionally the effect on 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was also evaluated. Based on the applied methodology, the inhibitory effects of different concentrations of the tested compounds (0.125-1 mmol/L) on ABTS (**Fig.3**) were determined by recording the absorbance of the reaction mixture at 517 nm. The corresponding graphical dependency was observed for the change in the measured absorption as presented on **Fig. 3.**

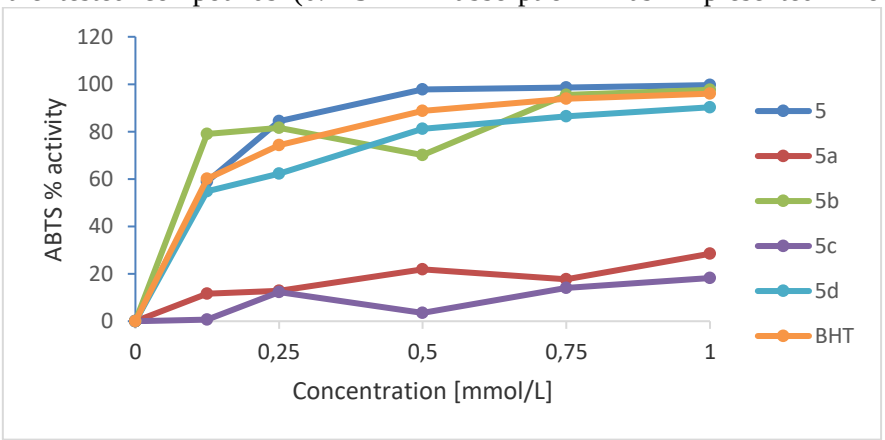


Fig.3. ABTS-radical scavenging activity of studied compounds **5** and **5a-d** and BHT.

For this method three of the examined structures performed antioxidant effect comparable with the one of the used butylated hydroxytoluene (BHT), applied as a reference – **5**, **5b** and **5d**.

The corresponding radical scavenging activities expressed as IC_{50} mmol/L of inhibition against DPPH and ABTS of the compounds were compared with those of BHT, used as positive control. All determinations are performed in triplicate (n = 3), and the results are presented in **Table 1**.

Among the analyzed structures, DPPH radical scavenging activity was shown by compounds **5** (IC_{50} 0.971 mmol/L) and **5d** (IC_{50} 0.184 mmol/L),

with the highest antioxidant activity demonstrated by the hydrazide **5**.

Table 1. DPPH and ABTS-radical scavenging activities of new compounds.

Sample	DPPH IC_{50} [mmol/L]	ABTS, IC_{50} [mmol/L]
5	0.971	0.144
5a	-	-
5b	-	0.126
5c	-	-
5d	0.184	0.266
5f	-	-
5g	-	-
BHT	0.189	0.189

For the other tested method three of the tested compounds expressed ABTS-radical scavenging activity decreasing in order: **5d** (IC_{50}0.266 mmol/L) >**BHT** (IC_{50}0.189 mmol/L) >**5** (IC_{50}0.144 mmol/L) >**5b** (IC_{50}0.126 mmol/L).

The expressed high radical scavenging activity of the initial hydrazide **5** may be due to the presence of free NH-NH_2 being able to donate much easier H-atom from the amino group. The expressed activity of **5d** is also expected, due to the presence of additional phenolyc OH group. The results reveal that introducing aryl groups in the side chain, leads to expected decrease of the antioxidative activity against the two radicals as demonstrated also in [14].

The performed preliminary tests show that ABTS methodology is more suitable for advanced evaluation of radical scavenging activity of molecules, based on N-pyrrolyl hydazide-hydrazone moiety in compare to the DPPH test. This suggestion complies with some literary data demonstrating the low efficiency of the popular DPPH radical [4] when evaluating the radical scavenging activity of poly substituted pyrrole based chemical structures.

We think that this might be due to the proposed antioxidant mechanism of substituted pyrroles which is based on the consideration that the synthesized pyrrole derivatives are expected to act as hydrogen atom donors from the un-substituted 4^{th} C-H group to provide antioxidant activity. This and the fact that the popular **DPPH•** radical for monitoring HAT activities of phenols gives anomalous kinetic solvent effects makes this methodology unreliable for determination of HAT activities in protic solvents, as in our case.

CONCLUSIONS

One new N-pyrrolylcarbohydrazide and six new hydrazones were synthesized. The structures of the new compounds were elucidated by IR, ^{1}H and ^{13}C NMR spectral data followed by MS data. The purity of the obtained compounds was proven by the corresponding melting points, TLC characteristics and elemental analyses. The obtained IC_{50} values from the performed preliminary *in vitro* evaluation of the radical scavenging activity underline the initial N-pyrrolylcarbohydrazide as the strongest antioxidant. The applied ABTS methodology may be considered as more suitable for preliminary evaluation of radical scavenging activity of molecules based on N-pyrrolyl hydazide-hydrazone moiety when compared with the used DPPH method.

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