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Synthesis, antibacterial and antioxidant evaluation of 4-substituted 1-(4-methoxyphenyl)pyrrolidin-2-ones

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4-Substituted 1-(4-methoxyphenyl)pyrrolidin-2-ones containing azole, diazole, oxadiazole, thiadiazole, and triazole fragments have been synthesized, and the characterization of the obtained products is presented. The study compounds have been analyzed for antioxidant and antibacterial activities. Two types of hetero systems – 5-thioxo-4,5-dihydro-1,3,4-oxadiazole and [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine – showed a moderate activity against *Rhizobium radiobacter*, *Xanthomonas campestris*, and *Escherichia coli* microorganisms. Some compounds were tested for their antioxidant activity.

Keywords: 1,4-disubstituted pyrrolidin-2-ones, azoles, antibacterial, antioxidant activities.

Introduction

Among azoles, many biologically active compounds are known. Synthetic azole derivatives possess antituberculous [1], antifungal [2–4], and antimicrobial [5–7] activity. They also may be used as crop protectors [8, 9]. The 1,3,4-oxadiazole compounds often display antifungal, herbicidal [10], and insecticidal [11, 12] effects. 1,2,4-Triazoles have attracted particular attention due to the wide range of their biological properties such as antibacterial and antifungal [13–16], anticancer–antitumour [17], antidepressant [18]. Strains of the *Rhizobium* species (formerly *Agrobacterium*, which was reclassified based on 16S rDNA analyses) are aerobic, motile, oxidase-positive, and non–spore-forming gram-negative bacilli. Among the species of *Rhizobium* (i. e. *R. radiobacter, R. rhizogenes, R. rubi, R. undicola,* and *R. vitis), R. radiobacter* is the species that most commonly causes disease in humans. Since the first case of human infection with *R. radiobacter* in a patient with prosthetic aortic valve endocarditis was reported in 1980, *R. radiobacter* has been recognized as an opportunistic human pathogen. Most of patients with *R. radiobacter* infection have debilitating underlying diseases [19]. Bacteria belonging to the genus *Xanthomonas* are one of the most omnipresent groups of Gram-negative plant pathogenic bacteria and cause a variety of diseases in multiple plants [20]. *Xanthomonas campestris* pv. *campestris* (Xcc), the cause of black rot of crucifers, is a seed-borne bacterium which occurs worldwide [21]. Antioxidants are extensively studied for their capacity to protect an organism and cell from damage induced by oxidative stress. Scientists in many different disciplines have become more interested in new compounds, either synthesized or obtained from natural sources that could provide active components to prevent or reduce the impact of oxidative stress on a cell [22].

As a continuation of our interest in the synthesis and application of new azole derivatives [23–25] the title compounds with O, S, and N heteroatoms were synthesized.

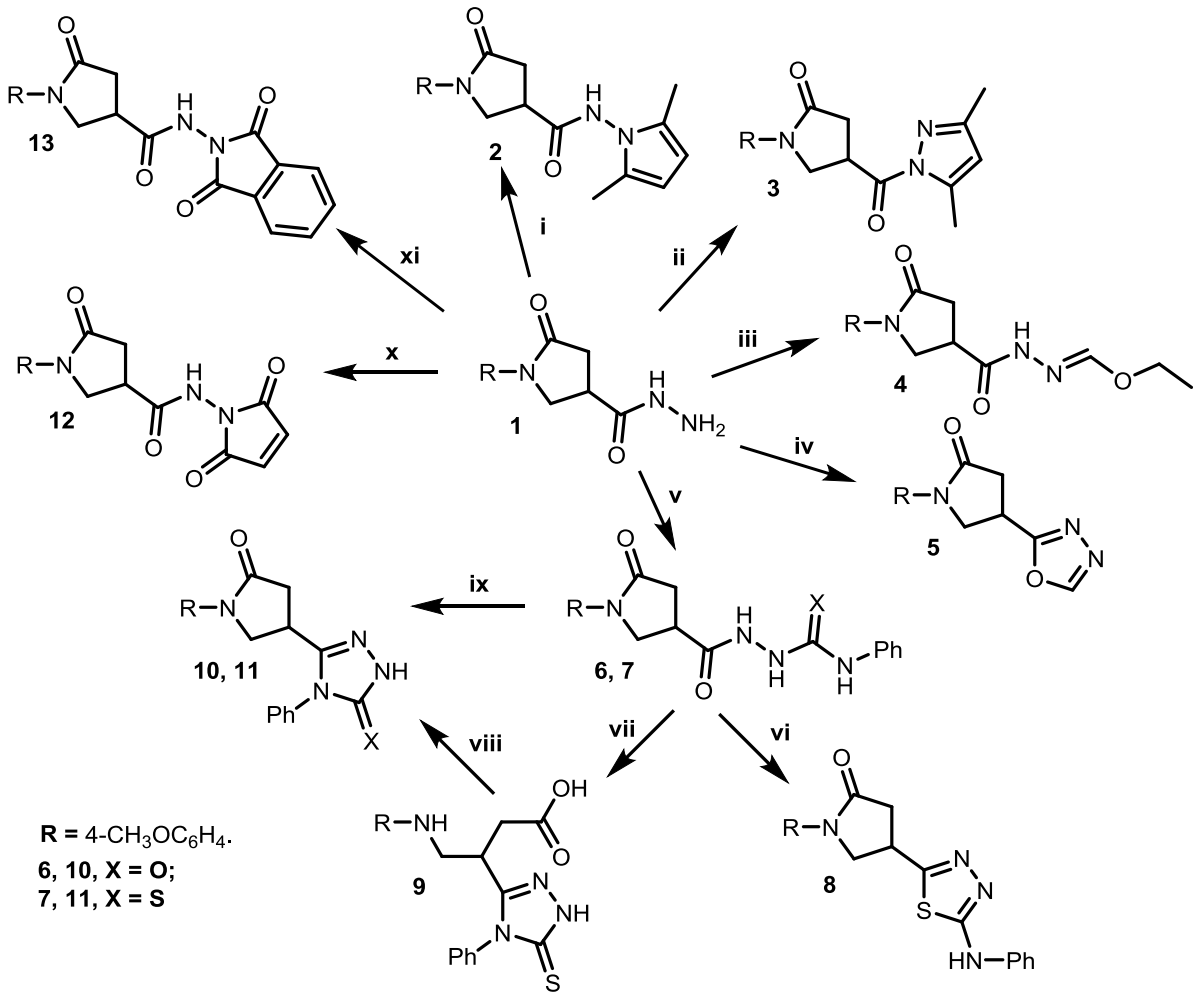
Results and discussion

Chemistry

The pathway of synthesis of the new 1,4-disubstituted pyrrolidinones is shown in Scheme 1 (**2–13**) and Scheme 2 (**14–23**).

In this work, functionalized pyrrole and pyrazole derivatives **2, 3** were prepared by refluxing a mixture of the carbohydrazide **1** with diketones – 2,5-hexanedione or 2,4-pentanedione in 2-propanol in the presence of the catalytic amount of hydrochloric or acetic acids. The obtained products were identified from the characteristic proton resonances of two CH_3 groups of the pyrrole ring at 2.00 ppm and signal at 5.66 ppm, attributed to the CH groups of compound **2**, whereas, the ones at 2.19 ppm, 2.48 ppm (CH_3 groups), and 6.11 ppm (CH group) in the ^{1}H NMR spectrum of compound **3** confirmed the formation of a pyrazole ring. Refluxing of hydrazide **1** in the excess of triethyl orthoformate, in the presence of *p*-toluenesulfonic acid for 20 hours, gave 2-substituted oxadiazole **5**, and the shortening of the reaction time to about 1 minute allowed us to isolate the hydrazone-type intermediate **4.** The NMR spectra of compound **4** exhibit four sets of resonances; therefore, four different spatial states may exist in the DMSO-d_6 solution. *N*-Phenylhydrazinecarboxamide **6** and *N*-phenylhydrazinecarbothioamide **7** were synthesized by

the interaction of the hydrazide **1** with phenyliso- and phenylisothiocianates in methanol. These compounds were used for the synthesis of heterocycles **8–11.** It was found that intramolecular heterocyclization of thiosemicarbazide **7** in acidic conditions led to the formation of 1,3,4-thiadiazole **8**, while under the basic conditions 1,2,4-triazoles **10** and **11** were obtained from compounds **6, 7.** The conversion of semicarbazides **5, 6** was carried out by refluxing them in a 2 % aqueous NaOH solution with the subsequent acidification of the reaction mixture with acetic acid. The singlets at 11.87 ppm and 13.86 ppm have been ascribed to protons of the NH groups of 1,2,4-triazoles **10, 11** in the ^{1}H NMR spectra. The resonances at 147.3 ppm and 152.8 ppm have been assigned to C=N, and the ones at 155.9 ppm and 170.5 ppm have been attributed to C=O and C=S carbon atoms, respectively, of the 5-membered heterocycle moiety of compounds **10, 11** in ^{13}C NMR spectra.



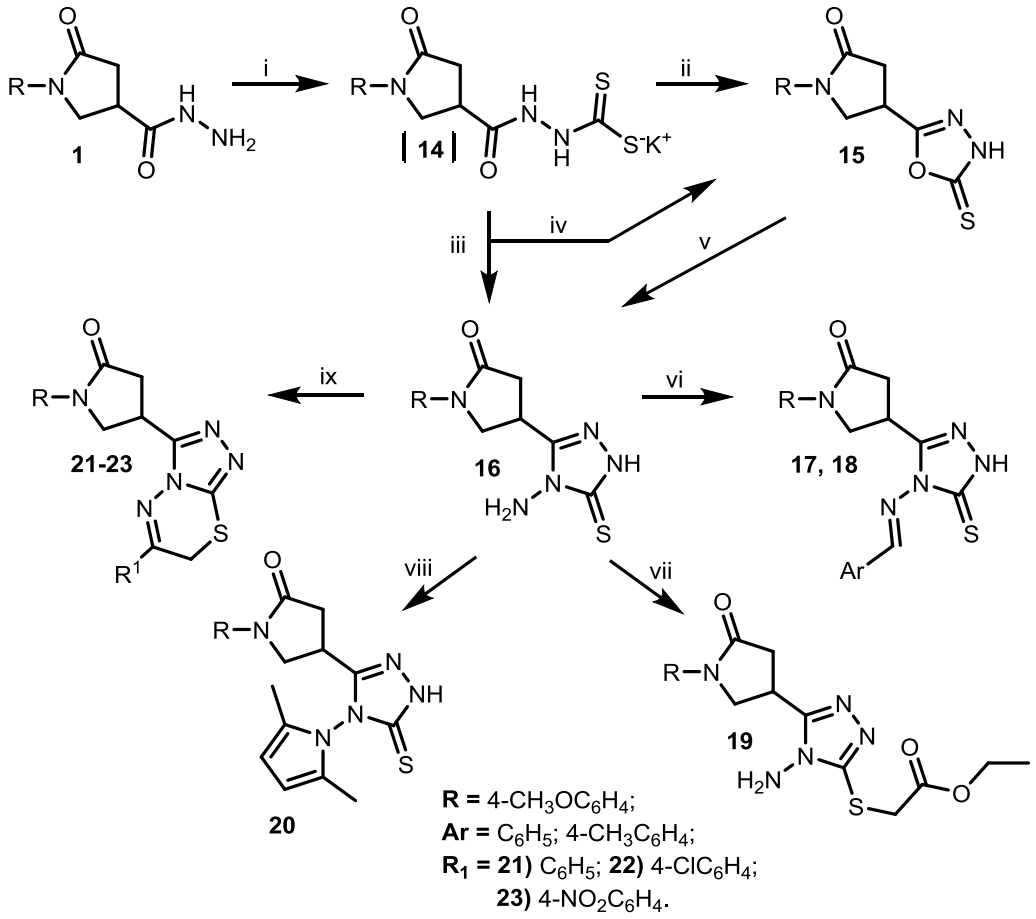
Reagents and conditions: **i:** 2,5-Hexanedione, CH_3COOH, 2-PrOH, Δ, 3 h; **ii:** 2,4-Pentanedione, HCl, 2-PrOH, Δ, 2 h; **iii:** Ethyl orthoformate, heated to boiling; **iv:** Ethyl orthoformate, *p*-toluenesulfonic acid, Δ, 20 h; **v:** PhNCO, MeOH, r.t., 2 h; PhNCS, MeOH, Δ, 1 h; **vi:** H_2SO_4, r.t., stirred 1 h, 2 % Na_2CO_3, pH 10; **vii:** 20 % NaOH, Δ, 3 h, CH_3COOH, pH 7; **viii:** 10 % HCl, heated to boiling; **ix:** 2 % NaOH, Δ, 3 h, 10 % HCl, pH 2; **x:** Maleic anhydride, 1,4-dioxane, Δ, 3 h; **xi:** Phthalic anhydride, CH_3COOH, Δ, 24 h, H_2O

Scheme 1. Synthesis of azoles **2-13**

4-[(4-Methoxyphenyl)amino]-3-(4-phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)butanoic acid (**9**) was synthesized from thiosemicarbazide **7** by cleaving the pyrrolidinone ring with a 20 % NaOH solution under reflux. The same triazole cycle formation took place. Heating this compound in a 10 % HCI solution lead to the formation of triazole **11.** The proton resonance of the NH group at 5.48 ppm in ^{1}H NMR spectrum confirmed the existence of an open chain in compound **9**. Compounds **12, 13** with dioxodihydropyrrolylic fragments were synthesized from hydrazide **1** and maleic or phthalic anhydride respectively. The reactions were carried out at reflux in 1,4-dioxane or acetic acid.

One of the ways to obtain oxadiazole and triazole heterosystems is their synthesis from dithiocarbazates. For the synthesis of oxadiazole and triazole derivatives, hydrazide **1** was heated with carbon disulfide in methanol in the presence of potassium hydroxide. Upon refluxing, the formed potassium dithiocarbazate **14** was dissolved in water, and subsequent acidification of the reaction

mixture with diluted hydrochloric acid to pH 1 gave 1-(4-methoxyphenyl)-4-(4,5-dihydro-5-thioxo-1,3,4-oxadiazol-2-yl)pyrrolidin-2-one (**15**). The formation of the oxadiazolethione ring in compound **15** has been proven by the signals at 163.9 ppm (O-C=N) and 177.9 ppm (C=S) in ^{13}C NMR spectrum and by the broad singlet at 14.78 ppm (NH) in ^{1}H NMR spectrum. A characteristic absorption band of the NH group of compound **15** was observed at 3056 cm^{-1} in the IR spectrum. The absorption bands at 1658 cm^{-1} and 1248 cm^{-1} have been ascribed to the C=O group of the pyrrolidinone ring and the C=S group of the oxadiazole cycle, respectively.



Reagents and conditions: **i:** KOH, CS_2, MeOH, Δ, 24 h; **ii:** 10 % HCl, pH 1; **iii:** $N_2H_4 \cdot H_2O$, Δ, 8 h, CH_3COOH, pH 7; **iv:** 10 % HCl, pH 1; **v:** $N_2H_4 \cdot H_2O$, H_2O, Δ, 24 h, CH_3COOH, pH 6; **vi:** ArCHO 2-PrOH, HCl, Δ, 8 h; **vii:** Ethyl chloroacetate, triethylamine, 2-PrOH, Δ, 7 h; **viii:** 2,5-Hexanedione, HCl, EtOH, Δ, 7 h; **ix:** α-Haloketone, triethylamine, MeOH, Δ, 4 h

Scheme 2. Synthesis of the 1,4-disubstituted pyrrolidinone derivatives **14**–**23**

Aminotriazole **16** was obtained by heating potassium dithiocarbazate **14** with hydrazine hydrate or heating the corresponding 1,3,4-oxadiazole **15** with hydrazine hydrate in water. The resonances at 152.6 ppm (C=N) and at 171.1 ppm (C=S) in ^{13}C NMR spectrum as well as the ones at 5.56 ppm (NH_2) and 13.61 ppm (NH/SH) in ^{1}H NMR spectrum revealed the formation of the 5-membered triazole **16**. The condensation of aminotriazole **16** with aromatic aldehydes was carried out providing the corresponding Schiff bases **17, 18**. Due to the above-mentioned condensation reaction, the resonances of protons of the NH_2 group (5.56 ppm) disappeared in ^{1}H NMR spectra of aminotriazole **16**. Treatment of the 4-(4-amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-1-(4-methoxyphenyl)-pyrrolidin-2-one (**16**) with 1.2 equivalents of ethyl chloroacetate in the presence of triethylamine resulted in the formation of the only *S*-alkylated product **19**. During reaction of 4-amino-1,2,4-triazole **16** with 2,5-hexanedione, performed in the refluxing 2-propanol in the presence of a catalytic amount of hydrochloric acid, the *N*-substituted pyrrole derivative **20** was synthesized. The formation of a 2,5-dimethylpyrrole ring, included into the **20** composition, has been proven by the singlets at 1.98 ppm (CH_3), 5.95 ppm (=CH), and 14.27 ppm (NH) in ^{1}H NMR spectrum. In the final stage of this synthetic work, the 4-(6-(4-substituted phenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-1-(4-methoxyphenyl)pyrrolidin-2-ones **21**–**23** were synthesized from aminotriazole **16** and the corresponding α-bromoketone in methanol in the presence of triethylamine.

Biology

Results of DPPH scavenging activity screening. The antioxidative activity of the synthesized compounds was evaluated by the 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl (DPPH) radical scavenging method. The effect of antioxidants on DPPH radical scavenging has been thought to be due to their hydrogen donating ability. The decrease in absorbance of the DPPH radical was caused by antioxidants because of the reaction between antioxidant molecules and the radical.

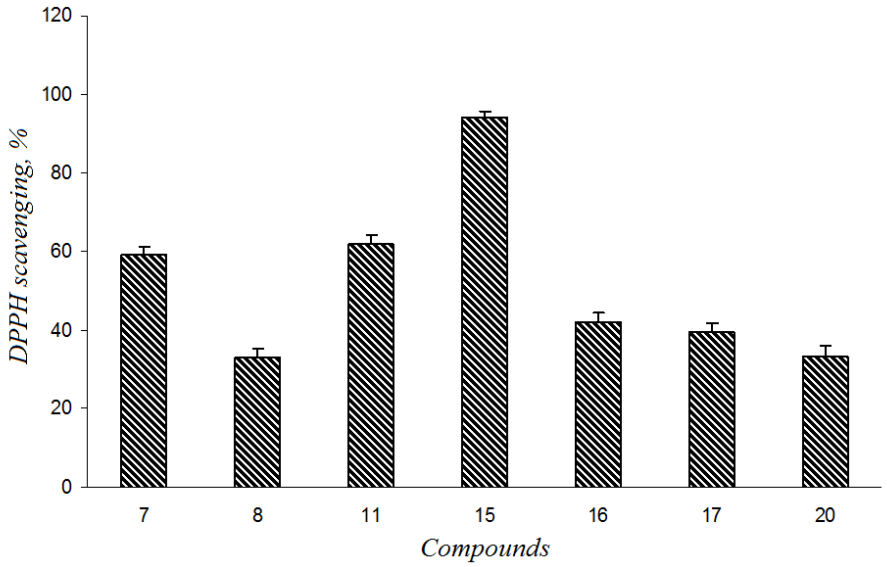


Fig. 1. DPPH scavenging activity

As seen from the results presented in Fig. 1, compound **15** showed a very high DPPH scavenging ability (92 %). Compounds **7** and **11** also showed an approx. 61 % scavenging action. Antioxidative activity of compounds **8, 16, 17,** and **20** was moderate.

Results of antibacterial activity screening. The synthesized compounds **2–5, 9, 11–13,** and **15–23** were evaluated for their antibacterial activity against *R. radiobacter*, *Xanthomonas campestris*, *Escherichia coli* strains by using the diffusion technique. The activity of the tested compounds were compared with that of the known antibacterial agent ampicillin. *R. radiobacter* was sensitive to compounds **21–23** (1000 μg/ml), however it was the most sensitive to compound **15** (concentrations 300–1000 μg/ml). *E. coli* was sensitive to compounds **21** and **22** (1000 μg/ml) and *X. campestris* – to compound **22** (1000 μg/ml). Both microbial strains were most sensitive to compound **15** (concentrations 300–1000 μg/ml).

The research presented herein has demonstrated that among all the compounds tested, compounds with 5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl- and 6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl substituents possess the highest antibacterial activity. The highest antibacterial activity was shown by 1-(4-methoxyphenyl)-4-(4,5-dihydro-5-thioxo-1,3,4-oxadiazol-2-yl)pyrrolidin-2-one **(15).** Based on these experiments, the antibacterial activity of the investigated compounds may be arranged in the following order:

15>22>23>21

Increasing antibacterial activity against *Rhizobium radiobacter*

As seen from the comparison of the antibacterial data for **21–23** series compounds, the introduction of substituents in the benzene ring has increased the antibacterial effect.

Experimental section

Chemistry

The starting materials and solvents were obtained from Sigma-Aldrich Chemie GmbH (Germany) and Fluka (Switzerland) and were used without further purification. The methods used to follow the reactions were TLC and NMR. The NMR spectra were recorded on a Varian Unity Inova (300 MHz) spectrometer (Varian, Inc., USA)**.** Chemical shifts are expressed as δ, ppm relative to TMS. IR spectra (ν, cm^{-1}) were recorded on a Perkin–Elmer BX FT–IR spectrometer (Perkin–Elmer Inc., USA) using KBr tablets. Mass spectra were obtained on a Waters ZQ 2000 spectrometer (Waters, Germany) using the atmospheric pressure chemical ionization (APCI) mode and operating at 25 V. Elemental analyses were performed on a CE-440 elemental analyzer (Exeter Analytical Inc., USA). Melting points were determined on a B-540 Melting Point Analyzer (Büchi Corporation, USA) and are uncorrected. TLC was performed using Merck, Silica gel 60 F_{254} (Kieselgel 60 F_{254}) silica gel plates.

***N*-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-1-(4-methoxyphenyl)-5-oxopyrrolidine-3-carboxamide (2).** A mixture of the hydrazide **1** (2.50 g, 10 mmol), 2,5-hexanedione (2.28 g, 20 mmol), glacial acetic acid (1 ml) and 2-propanol (15 ml) was refluxed for 3 h, the solvent was separated under reduced pressure, the residue was diluted with water (50 ml), and the solution was heated to a gentle boil. The precipitate formed upon cooling the reaction mixture was filtered off, washed with water, dried and recrystallized from 2-propanol to give **2** (3.10 g, 95 %) as a brownish solid. M.p. 147–148 ºC (2-propanol).

IR (KBr, cm^{-1}): 3259 (NH), 1682, 1664 (2C=O).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 2.00 (6H, s, $2CH_3$), 2.67–2.91 (2H, m, CH_2CO), 3.43–3.51 (1H, m, CH), 3.74 (3H, s, OCH_3), 3.29–4.13 (2H, m, NCH_2), 5.66 (2H, s, 2CH), 7.57 (2H, d, *J* = 9.0 Hz, $H_{ar\text{-}3,5}$), 7.96 (2H, d, *J* = 9.0 Hz, $H_{ar\text{-}2,6}$), 10.92 (1H, s, NH).

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 10.9 (CH_3), 34.0, 35.3, 50.6 ($C_{pyrrolid}$), 55.1 (OCH_3), 103.0 (CH), 113.8, 121.3, 132.2, 155.9 (C_{ar}), 126.7 (C-N), 171.0, 171.8 (2C=O).

MS (APCI) m/z: 328 $[M+H]^+$ (10 %), 350 $[M+Na]^+$ (100 %), 351 $[M+Na+H]^+$ (30 %).

Calculated, %: C 66.04, N 12.84, H 6.47. $C_{18}H_{21}N_3O_3$.

Found, %: C 66.21, N 12.92, H 6.44.

4-[(3,5-Dimethyl-1*H*-pyrazol-1-yl)carbonyl]-1-(4-methoxyphenyl)pyrrolidin-2-one (3). A mixture of hydrazide **1** (2.50 g, 10 mmol), 2,4-pentanedione (2.00 g, 20 mmol), 2-propanol (15 ml) and conc. hydrochloric acid (0.5 ml) was heated under reflux for 2 h, the solvent was separated under reduced pressure, the residue was diluted with water (50 ml), and the solution was heated to a gentle boil. Upon cooling the reaction mixture, the precipitate was filtered off, washed with water, dried and crystallized from a mixture of 2-propanol and water (2:1) to give **3** (2.32 g, 74 %) as a white solid. M.p. 94–95 ºC (2-propanol and water).

IR (KBr, cm^{-1}): 1727, 1695 (2C=O), 1611 (C=N).

^{1}H NMR (300 MHz, $CO(CD_3)_2$): δ_H 2.19 (3H, s, N=C-CH_3), 2.48 (3H, s, N-C-CH_3), 2.67–2.78 (2H, m, CH_2CO), 3.74 (3H, s, OCH_3), 4.01–4.25 (2H, m, NCH_2), 4.48–4.58 (1H, s, CH_2CH), 6.11 (1H, s, CH), 6.89 (2H, d, *J* = 9.2 Hz, $H_{ar\text{-}3,5}$), 7.57 (2H, d, *J* = 9.2 Hz, $H_{ar\text{-}2,6}$).

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 14.0, 14.5 ($2CH_3$), 35.9, 37.0, 51.6 ($C_{pyrrolid}$), 55.8 (OCH_3), 112.3 (C*C*H), 114.8, 122.4, 134.0, 157.5 (C_{ar}), 145.2 (N-*C*-CH_3), 153.4 (N=*C*-CH_3), 171.9, 173.8 (2C=O).

MS (APCI) m/z: 314 $[M+H]^+$ 315 (80 %) $[M+1+H]^+$ (30 %).

Calculated, %: C 65.16, N 13.41, H 6.11. $C_{17}H_{19}N_3O_3$.

Found, %: C 65.42, N 13.20, H 6.34.

Ethyl *N*-{[1-(4-methoxyphenyl)-5-oxopyrrolidin-3-yl]carbonyl}hydrazonoformate (4). A mixture of the hydrazide **1** (1.25 g, 5 mmol), and ethyl orthoformate (10 ml) was heated to boiling and then cooled down. The formed residue was filtered off, washed with ether, dried, and crystallized from toluene to give **4** (0.73 g, 48 %) as a white solid. M.p. 163–164 ºC (toluene).

IR (KBr, cm^{-1}): 3233 (NH), 1670, 1615 (2C=O), 1514 (C=N).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.22–1.31 (3H, m, CH_3), 2.61–2.72 (2H, m, CH_2CO), 3.73 (3H, s, OCH_3), 3.77–4.21 (5H, m, OCH_2CH_3 and CH and CH_2N), 6.85 (1H(0.2 *Z*), s, CH), 6.90 (1H(0.5 *Z*), s, CH), 6.93 (2H, d, *J* = 9.0 Hz, $H_{ar\text{-}3,5}$), 7.54 (2H, d, *J* = 9.0 Hz, $H_{ar\text{-}2,6}$), 7.94 (1H(0.1 *E*), s, CH), 8.22 (1H(0.2 *E*), s, CH), 10.05 (1H(0.2 *Z*), s NH), 10.54 (1H(0.5 *Z*), s, NH), 10.76 (1H(0.1 *E*), s, NH), 10.79 (1H(0.2 *E*), s, NH).

^{1}H NMR (300 MHz,$CDCl_3$) δ_H 1.35 (3H, t, *J* = 6.0 Hz, CH_3), 2.75–3.02 (2H, m, CH_2CO), 3.77 (3H, s, OCH_3), 3.90–4.17 (5H, m, OCH_2CH_3 and CH and CH_2N), 6.44 (1H(0.8 *Z*), s, CH), 6.65 (1H(0.2 *E*), s, CH), 6.87 (2H, d, *J* = 9.0 Hz, $H_{ar\text{-}3,5}$), 7.47 (2H, d, *J* = 9.0 Hz, $H_{ar\text{-}2,6}$), 8.77 (1H(0.8 *Z*), s, NH), 9.06 (1H(0.2 *E*), s, NH).

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 14.1, 15.3, 15.5 (OCH_2CH_3), 32.5, 34.2, 34.7, 35.7, 50.4, 51.1 ($C_{pyrrolid}$), 55.2 (OCH_3), 62.5, 67.2 (OCH_2CH_3), 113.8, 121.2, 132.4, 155.4 (C_{ar}), 143.2, 145.4, 155.8, (CH), 168.0, 168.6, 171.4, 171,5 (CONH).

^{13}C NMR (75 MHz, $CDCl_3$) δ_C 15.4 (OCH_2CH_3), 33.5, 35.2, 50.9 ($C_{pyrrolid}$), 55.5 (OCH_3), 68.1 (OCH_2CH_3), 114.1, 114.2, 122.3, 132.2, 156.9 (C_{ar}), 142.0, 145.3 (2CH), 172.0, 172.7 (2C=O).

MS (APCI) m/z: 306 $[M+H]^+$ (10 %), 328 $[M+Na]^+$ (100 %), 329 $[M+Na+H]^+$ (30 %).

Calculated, %: C 59.01, N 13.76, H 6.27. $C_{15}H_{19}N_3O_4$.

Found, %: C 58.86, N 13.95, H 6.05.

1-(4-Methoxyphenyl)-4-(1,3,4-oxadiazol-2-yl)pyrrolidin-2-one (5). A mixture of the hydrazide **1** (2.50 g, 10 mmol), ethyl orthoformate (11.86 g, 80 mmol) and *p*-toluenesulfonic acid (0.1 g, 0.6 mmol) was heated under reflux for 20 h and then cooled down. The formed residue was filtered off, washed with water, dried, and crystallized from ethanol to give **5** (2.20 g, 85 %) as a white solid. M.p. 135–136 ºC (from ethanol) in the literature [26] m.p. 133–134 ºC (from 2-propanol).

2-{[1-(4-Methoxyphenyl)-5-oxopyrrolidin-3-yl]carbonyl}-*N*-phenylhydrazinecarboxamide (6). A mixture of hydrazide **1** (3.74 g, 15 mmol), phenyl isocyanate (2.02 g, 17 mmol) and methanol (20 ml) was stirred for 2 h at room temperature. The precipitate was filtered off, washed with methanol and water, crystallized from 1,4-dioxane to give **6** (4.42 g, 80 %) as a white solid. M.p. 157 ºC (from 1,4-dioxane decomp.).

IR (KBr, cm^{-1}): 3312, 3279, 3197 (3NH), 1673, 1637, 1610 (3C=O).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 2.62–2.82 (2H, m, $COCH_2$), 3.73 (3H, s, OCH_3), 3.82–4.05 (3H, m, CH, NCH_2), 6.91–7.55 (9H, m, H_{ar}), 8.10 (1H(0.8 *Z*), s, CON*H*Ph), 8.43 (1H(0.2 *E*), s, CON*H*Ph), 8.80 (1H(0.8 *Z*), s, N*H*CONH), 9.00 (1H(0.2 *E*), s, N*H*CONH), 9.27 (1H(0.2 *Z*), s, N*H*$COCH_{pyrrolid}$), 9.94 (1H(0.8 *E*), s, *NH*$COCH_{pyrrolid}$).

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 33.6, 35.1, 50.6 ($C_{pyrrolid}$), 55.0 (OCH_3), 113.7, 118.3, 121.1, 121.6, 128.5,

132.1, 132.0, 139.3, 155.3, 155.9 (C_{ar}), 171.0, 171.1, 172.4 (3C=O).

MS (APCI) m/z: 369 $[M+H]^+$ (100 %), 370 $[M+1+H]^+$ (50 %).

Calculated, %: C 61.95, N 15.21, H 5.47. $C_{19}H_{20}N_4O_4$.

Found, %: 61.70, N 15.34; H 5.67.

2-{[1-(4-Methoxyphenyl)-5-oxopyrrolidin-3-yl]carbonyl}-*N*-phenylhydrazinecarbothioamide (7). A mixture of the hydrazide **1** (4.98 g, 20 mmol), phenyl isothiocyanate (2.70 g, 20 mmol) and methanol (40 ml) was refluxed for 1 h and then cooled down. The precipitate was filtered off, washed with methanol, crystallized from 1,4-dioxane to give **7** (5.80 g, 75 %) as a white solid. M.p. 144–145 ºC (1,4-dioxane).

IR (KBr, cm^{-1}): 3314, 3267, 3180 (3NH), 1714, 1672 (2C=O), 1247 (C=S).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 2.65–2.82 (2H, m, CH_2CO), 3.33–3.34 (1H, m, CH), 3.74 (1H, s, OCH_3), 3.89–4.05 (2H, m, NCH_2), 6.93–7.55 (9H, m, H_{ar}), 9.57, 9.69, 10.21 (3H, 3s, NH).

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 34.1, 35.2, 50.6 ($C_{pyrrolid}$), 55.2 (OCH_3), 113.8, 121.3, 126.4, 128.1, 128.4, 132.3, 139.0, 155.8 (C_{ar}), 170.9, 171.4 (2C=O).

MS (APCI) m/z: 385 $[M+H]^+$ (30 %), 407 $[M+Na]^+$ (100 %), 408 $[M+H+Na]^+$ (40 %).

Calculated, %: C 59.36, N 14.57, H 5.24. $C_{19}H_{20}N_4O_3S$.

Found, %: C 59.14, N 14.61, H 5.36.

1-(4-Methoxyphenyl)-4-[5-(phenylamino)-1,3,4-thiadiazol-2-yl]pyrrolidin-2-one (8). A mixture of thiosemicarbazide **7** (0.54 g, 1.4 mmol) and sulphuric acid (1 ml) was stirred at room temperature for 1 h. Then 2 % aqueous Na_2CO_3 solution was added to the reaction mixture to pH 10. The formed residue was filtered off, washed with water and dried, crystallized from 1,4-dioxane to give **8** (0.46 g, 90 %) a white solid.

IR (KBr, cm^{-1}): 3288 (NH), 1644 (C=O).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 2.76–3.05 (2H, m, CH_2CO), 3.74 (1H, s, OCH_3), 3.97–4.13 (2H, m, NCH_2), 4.22–4.28 (1H, m, CH), 6.93–7.61 (9H, m, H_{ar}), 10.36 (1H, s, NH).

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 32.2, 37.9, 52.9 ($C_{pyrrolid}$), 54.9 (OCH_3), 113.5, 117.0, 121.1, 121.5, 128.7, 131.9, 140.3, 155.6 (C_{ar}), 160.1, 164.2 (2C=N), 170.7 (C=O).

MS (APCI) m/z: 367 $[M+H]^+$ (100 %), 368 $[M+1+H]^+$ (40 %).

Calculated, %: C 62.28, N 15.29, H 4.95. $C_{19}H_{18}N_4O_2S$.

Found, %: C 62.55, N 15.02, H 4.88.

4-[(4-Methoxyphenyl)amino]-3-(4-phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)butanoic acid (9). A mixture of thiosemicarbazide **7** (1.00 g, 2.6 mmol) and 20 % sodium hydroxide solution (25 ml) was heated under reflux for 3 h and then cooled down. Then the reaction mixture was acidified with acetic acid to pH 7, the formed residue was filtered off, washed with water and dried to give **9** (0.30 g, 30 %) as a white solid. M.p. 139–140 ºC (2-propanol).

IR (KBr, cm^{-1}): 3600 (OH), 3370, 3136 (2NH), 1689 (C=O), 1250 (C=S).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 2.26–2.79 (2H, m, CH_2CO), 2.90–2.99 (1H, m, CH), 3.07–3.18 (2H, m, CH_2N), 3.60 (3H, s, OCH_3), 5.48 (1H, br. s, NH), 6.06 (2H, d, J = 8.9 Hz, $H_{ar\text{-}3,5}$), 6.53 (2H, d, J = 8.9 Hz, $H_{ar\text{-}2,6}$), 7.37–7.63 5H, (m, $H_{ar'}$), 13.86 (1H, br. s, NNH).

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 32.0, 34.9, 46.3 ($C_{pyrrolid}$), 55.3 (OCH_3), 112.7, 114.6, 128.8, 129.5, 129.8, 133.5, 141.5, 153.8 (C-4), 150.7 (C=N), 167.6 (C=S), 173.1 (C=O).

MS (APCI) m/z: 453 $[H+3Na]^+$ (70 %).

Calculated, %: C 59.36, N 14.57, H 5.24. $C_{19}H_{20}N_4O_3S$.

Found, %: C 59.57, N 14.33, H 4.49.

5-[1-(4-Methoxyphenyl)-5-oxopyrrolidin-3-yl]-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (10). A mixture of semicarbazide **6** (1.00 g, 2.7 mol) and 25 ml of 2 % aqueous NaOH solution was heated under reflux for 3 h, cooled and acidified with 10 % hydrochloric acid to pH 2. The formed residue was filtered off, washed with water and dried, crystallized from 1,4-dioxane to give **10** (0.58 g, 61 %) as a white solid. M.p. 174–175 ºC (1,4-dioxane).

IR (KBr, cm^{-1}): 3184 (NH), 1704, 1692 (2C=O).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 2.53–2.73 (2H, m, CH_2CO), 3.57–3.65 (1H, m, CH), 3.72 (1H, s, OCH_3), 3.75–3.97 (2H, m, NCH_2), 6.92 (2H, d, J = 9.0 Hz, $H_{ar\text{-}3,5}$), 7.44 (2H, d, J = 9.0 Hz, $H_{ar\text{-}2,6}$), 7.45–7.59 (5H, m, $H_{ar'}$), 11.87 (1H, s, NH).

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 27.8, 35.3, 50.5 ($C_{pyrrolid}$), 55.1 (OCH_3), 113.7, 121.4, 127.6, 127.7, 128.9, 129.5, 132.0, 132.6, 155.9 (C_{ar}), 147.3 (C=N), 154.6, 170.7 (2C=O).

MS (APCI) m/z: 373 $[M+Na]^+$ (100 %).

Calculated, %: C 65.13, N 15.99, H 5.18. $C_{19}H_{18}N_4O_3$.

Found, %: C 65.32, N 15.75, H 5.04.

1-(4-Methoxyphenyl)-4-(4-phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)pyrrolidin-2-one (11). Method A. A mixture of thiosemicarbazide **7** (1.00 g, 2.6 mol) and 25 ml of 2 % aqueous NaOH solution was heated under reflux for 3 h, cooled and acidified with 10 % HCl to pH 2. The formed residue was filtered off, washed with water and dried, crystallized from methanol to give **11** (0.80 g, 84 %) as a white solid. M.p. 267–268 ºC (methanol).

Method B. A mixture of the compound **9** (0.38 g, 1 mmol), 10 % HCl (7 ml) was heated to boiling and then cooled down. The formed residue was filtered off, washed with water and dried, crystallized from methanol to give **11** (0.33 g, 90 %) as a white solid.

IR (KBr, cm^{-1}): 3047 (NH), 1701 (C=O), 1249 (C=S).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 2.53–2.76 (2H, m, CH_2CO), 2.51–2.58 (1H, m, CH), 3.72 (3H, s, OCH_3), 3.75–4.02 (2H, m, NCH_2), 6.92 (2H, d, J = 9.0 Hz, $H_{ar\text{-}}$

$_{3,5}$), 7.43 (2H, d, J = 9.0 Hz, $H_{ar-2,6}$), 7.49–7.63 (5H, m, $H_{ar'}$), 13.89 (1H, s, NH).

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 27.8, 35.7, 50.7 ($C_{pyrrolid}$), 55.1 (OCH_3), 113.7, 121.5, 128.5, 129.5, 129.7, 131.9, 133.4, 156.0 (C_{ar}), 152.8 (C=N), 168.3 (C=S), 170.5 (C=O).

MS (APCI) m/z: 389 $[M+Na]^+$ (100 %).

Calculated, %: C 62.28, N 15.29, H 4.95. $C_{19}H_{18}N_4O_2S$.

Found, %: C 62.54, N 15.21, H 4.96.

***N*-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-1-(4-methoxyphenyl)-5-oxopyrrolidine-3-carboxamide (12).** A mixture of the hydrazide **1** (1.0 g, 4 mmol), maleic anhydride (0.40 g, 4 mmol) and 1,4-dioxane (20 ml) was heated under reflux for 3 h, then cooled down, and the formed residue was filtered off, washed with 1,4-dioxane, crystallized from 1,4-dioxane to give **12** (1.03 g, 78 %) as a white solid.

IR (KBr, cm^{-1}): 3221, 3048 (2NH), 1723, 1675, 1636, 1620 (4C=O).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 2.57–2.81 (2H, m, $COCH_2$), 3.34–3.37 (1H, m, CH), 3.74 (3H, s, OCH_3), 3.81–4.05 (2H, m, NCH_2), 6.30, 6.36 (4H, 2d, J = 12,0 Hz, $H_{pyrrole}$), 6.94 (2H, d, J = 9.0 Hz, $H_{ar-3,5}$), 7.54 (2H, d, J = 9.0 Hz, $H_{ar-2,6}$), 10.44 (1H, s, NH).

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 33.8, 35.3, 50.8 ($C_{pyrrolid}$), 55.2 (OCH_3), 113.8, 121.3, 127.3, 155.8 (C_{ar}), 132.2, 132.4 ($C_{pyrrole}$), 162.6, 167.0, 171.0, 171.2 (4C=O).

MS (APCI) m/z: 330 $[M+H]^+$ (100 %).

Calculated, %: C 58.36, N 12.76, H 4.59. $C_{16}H_{15}N_3O_5$.

Found, %: C 58.18, N 12.53, H 4.90.

***N*-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-1-(4-methoxyphenyl)-5-oxopyrrolidine-3-carboxamide (13).** A mixture of the hydrazide **1** (1.0 g, 4 mmol), phthalic anhydride (0.59 g, 4 mmol), and glacial acetic acid (5 ml) was heated under reflux for 24 h. The mixture was filtered off, the filtrate was diluted with water (5 ml), and the formed residue was filtered off, washed with water, crystallized from 2-propanol to give **13** (0.65 g, 43 %) as a white solid. M.p. 181–182 ºC (2-propanol).

IR (KBr, cm^{-1}): 3526, 3433, 3309 (3NH), 1743, 1722, 1690, 1677 (4C=O).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 2.57–2.90 (2H, m, $COCH_2$), 3.45–3.53 (1H, m, CH), 3.69 (3H, s, OCH_3), 3.83–4.12 (2H, m, NCH_2), 6.91 (2H, d, J = 9.0 Hz, $H_{ar-3,5}$), 7.50 (2H, d, J = 9.0 Hz, $H_{ar-2,6}$), 7.87–7,94 (4H, m, $H_{ar'}$), 10.98 (1H, s, NH).

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 33.4, 34.9, 50.2 ($C_{pyrrolid}$), 54.8 (OCH_3), 113.4, 121.0, 123.4, 129.0, 131.7, 134.9, 155.5 (C_{ar}), 164.6, 170.5, 171.9 (4C=O).

MS (APCI) m/z: 380 $[M+H]^+$ (100 %), 381 $[M+1+H]^+$ (40 %).

Calculated, %: C 63.32, N 11.08, H 4.52. $C_{20}H_{17}N_3O_5$.

Found, %: C 63.48, N 10.87, H 4.76.

1-(4-Methoxyphenyl)-4-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)pyrrolidin-2-one (15). A mixture of the hydrazide **1** (7.48 g 30 mmol), potassium hydroxide (5.22 g, 93 mmol), carbon disulphide (4.26 g, 56 mmol) and methanol (65 ml) was refluxed for 24 h, cooled down to room temperature, acidified with hydrochloric acid 10 % to pH 1. The precipitate was filtered off, washed with water, dried and crystallized from ethanol to give **15** (5.56 g, 64 %) as a white solid.

IR (KBr, cm^{-1}): 3056 (NH), 1658 (C=O), 1248 (C=S).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 2.76–2.99 (2H, m, CH_2CO), 3.74 (3H, s, OCH_3), 3.89–3.96 (1H, m, CH), 4.01–4.20 (2H, m, NCH_2), 6.95 (2H, d, J = 9.0 Hz, $H_{ar-3,5}$), 7.53 (2H, d, J = 9.0 Hz, $H_{ar-2,6}$), 14.78 (1H, pl. s, NH).

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 27.9, 34.8, 50.1 ($C_{pyrrolid}$), 55.2 (OCH_3), 113.8, 121.5, 131.9, 156.0 (C_{ar}), 163.9 (C=N), 170.3 (C=O), 177.9 (C=S).

MS (APCI) m/z: 292 $[M+H]^+$ (100 %).

Calculated, %: C 53.60, N 14.42, H 4.50. $C_{13}H_{13}N_3O_3S$.

Found, %: C 53.78, N 14.38, H 4.37.

4-(4-Amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-1-(4-methoxyphenyl)pyrrolidin-2-one (16). Method A. A mixture of the hydrazide **1** (7.48 g 30 mmol), potassium hydroxide (5.23 g, 93 mmol), carbon disulphide (4.29 g, 56 mmol), and methanol (65 ml) was refluxed for 24 h, cooled down to room temperature, and diethyl ether (45 ml) was poured into the reaction mixture. The precipitate was filtered off, washed with diethyl ether (3×50 ml) and dried. A mixture of the obtained dry solid, hydrazine hydrate (4.50 g, 90 mmol) and water (10 ml) was refluxed for 8 h. Then the mixture was cooled down to room temperature, neutralized with acetic acid to pH 7, the formed precipitate of the residue was filtered off, washed with water, dried, and crystallized from ethanol to give **16** (4.60 g, 50 %). Then the filtrate was acidified with 10 % hydrochloric acid to pH 1 to give compound **15** (2.43 g, 28 %) (Specification **iv** in Scheme 2).

Method B. A mixture of the compound **15** (3.00 g, 14 mmol), hydrazine hydrate (2.05 g, 40 mmol) and water (10 ml) was refluxed for 24 h, then cooled down, acidified with acetic acid to pH 6. The precipitate was filtered off, washed with water, dried, and crystallized from ethanol to give **16** (3.00 g, 70 %). M.p. 191–192 ºC (ethanol).

IR (KBr, cm^{-1}): 3056 (NH), 1658 (C=O), 1248 (C=S).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 2.79–2.98 (2H, m, CH_2CO), 3.73 (3H, s, OCH_3), 3.81–3.90 (1H, m, CH), 4.01–4.21 (2H, m, NCH_2), 5.56 (2H, s, NH_2), 6.94 (2H, d, J = 9.0 Hz, $H_{ar-3,5}$), 7.52 (2H, d, J = 9.0 Hz, $H_{ar-2,6}$), 13.61 (1H, s, NH).

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 27.3, 35.0, 50.7 ($C_{pyrrolid}$), 55.2 (OCH_3), 113.8, 121.4, 132.2, 155.9 (C_{ar}), 152.6 (C=N), 167.1 (C=O), 171.1 (C=S).

MS (APCI) m/z: 306 $[M+H]^+$ (90 %), 307 $[M+1+H]^+$ (20 %).

Calculated, %: C 51.13, N 22.94, H 4.95. $C_{13}H_{15}N_5O_2S$.

Found, %: C 51.36, N 23.02, H 5.23.

General synthetic procedure of 4-{4-[aryl substituted amino]-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl}-1-(4-methoxyphenyl)pyrrolidin-2-ones (17, 18).

A mixture of aminotriazole (0.50 g, 1.6 mmol), the corresponding benzaldehyde (1.76 mmol), 2-propanol (10 ml) and hydrochloric acid (2 drops) was refluxed for 8 h and cooled down. The precipitate was filtered off, washed with water, dried, and crystallized from 1,4-dioxane to give **17** or **18**.

4-{4-[Benzylideneamino]-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl}-1-(4-methoxyphenyl)pyrrolidin-2-one (17) was obtained according to the general procedure from benzaldehyde to give a white solid (0.25 g, 40 %). M.p. 209–210 ºC (1,4-dioxane).

IR (KBr, cm^{-1}): 3052 (NH), 1667 (C=O), 1610 (C=N), 1250 (C=S).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 2.84–2.98 (2H, m, CH_2CO), 3.73 (3H, s, OCH_3), 3.99–4.09 (2H, m, NCH_2), 4.17–4.22 (1H, m, CH), 6.93 (2H, d, *J* = 9.0 Hz, $H_{ar\text{-}3,5}$), 7.90 (2H, d, *J* = 9.0 Hz, $H_{ar\text{-}2,6}$), 7.51–7.64 (5H, m, $H_{ar'}$), 10.12 (1H, s, N=CH), 13.95 (1H, s, NH).

MS (APCI) m/z: 394 $[M+H]^+$ (100 %), 395 $[M+1+H]^+$ (30 %).

Calculated, %: 61.05, N 17.80, H 4.87. $C_{20}H_{19}N_5O_2S$.
Found, %: C 61.29, N 17. 57, H 4.81.

4-{4-[(4-Methoxybenzylidene)amino]-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl}-1-(4-methoxyphenyl)pyrrolidin-2-one (18) was obtained according to the general procedure from 4-methoxybenzaldehyde to give a white solid (0.53 g, 78 %). M.p. 206–208 ºC (1,4-dioxane). **IR** (KBr, cm^{-1}): 3283 (NH), 1691 (C=O), 1602 (C=N), 1242 (C=S).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 2.80–2.96 (2H, m, CH_2CO), 3.73, 3.84 (6H, 2s, $2OCH_3$), 3.69–4.07 (2H, m, NCH_2), 4.15–4.21 (1H, m, CH), 6.93, 7.08, 7.52, 7.85 (8H, 4d, *J* = 9.0 Hz, H_{ar}), 9.88 (1H, s, N=CH), 13.91 (1H, s, NH).

MS (APCI) m/z: 424 $[M+H]^+$ (100 %), 425 $[M+H+1]^+$ (30 %).

Calculated, %: 59.56, N 16.54, H 5.00. $C_{21}H_{21}N_5O_3S$.
Found, %: C 59.54, N 16.66, H 5.17.

Ethyl ({4-amino-5-[1-(4-methoxyphenyl)-5-oxopyrrolidin-3-yl]-4*H*-1,2,4-triazol-3-yl}sulfanyl)acetate (19). A mixture of the aminotriazole **16** (1.53 g, 5.0 mmol), ethyl chloroacetate (0.74 g, 6 mmol), triethylamine (0.71 g, 7 mmol) and 2-propanol (15 ml) was refluxed for 7 h and cooled down. The precipitate was filtered off, washed with water, dried, and crystallized from methanol to give **19** (1.31 g, 67 %) as a yellow solid. M.p. 203–204 ºC (methanol).

IR (KBr, cm^{-1}): 3345 (NH_2), 1732 (C=O), 1674, 1642 (2C=N).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.18 (3H, t, *J* = 8.0 Hz, CH_3), 2.81–2.98 (2H, m, NCH_2), 3.74 (3H, s, OCH_3), 3.86–3.96 (1H, m, CH), 4.01–4.21 (2H, m, CH_2CO), 4.04 (2H, s, CH_2), 4.10 (2H, q, *J* = 8.0 Hz, CH_2CH_3), 5.84 (2H, s, NH_2), 6.95 (2H, d, *J* = 8.0 Hz, $H_{ar\text{-}3,5}$), 7.53 (2H, d, *J* = 8.0 Hz, $H_{ar\text{-}2,6}$).

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 14.1 (CH_3), 26.8 (SCH_2), 33.0, 36.1, 51.5 ($C_{pyrrolid}$), 55.3 (OCH_3), 61.2 (CH_2CH_3), 113.9, 121.4, 132.4, 155.9 (C_{ar}), 151.8 (C=N), 156.7 (C-S), 168.5, 171.4 (2C=O).

MS (APCI) m/z: 392 $[M+H]^+$ (100 %).

Calculated, %: 52.16, N 17.89, H 5.41. $C_{17}H_{21}N_5O_4S$.
Found, %: C 52.39, N 17.62, H 5.61.

4-[4-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]-1-(4-methoxyphenyl)pyrrolidin-2-one (20). A mixture of the aminotriazole **16** (1.00 g, 3.3 mmol), 2,5-hexanedione (0.57 g, 5 mmol), ethanol (10 ml), and hydrochloric acid (2 drops) was refluxed for 7 h and cooled down. The precipitate was filtered off, washed with water, dried, and crystallized from 1,4-dioxane to give **20** (1.06 g, 84 %) as a white solid. M.p. 201–202 ºC (1,4-dioxane).

IR (KBr, cm^{-1}): 3051 (NH), 1700 (C=O), 1630 (C=N), 1253 (C=S).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.98 (6H, s, CH_3), 2.45–2.80 (2H, m, CH_2CO), 3.48–3.56 (1H, m, CH), 3.73 (3H, s, OCH_3), 3.80–4.07 (2H, m, NCH_2), 5.95 (2H, s, CH-CH), 6.93 (2H, d, *J* = 9.0 Hz, $H_{ar\text{-}3,5}$), 7.45 (2H, d, *J* = 9.0 Hz, $H_{ar\text{-}2,6}$), 14.27 (1H, s, NH).

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 11.0 ($2CH_3$), 27.6, 35.2, 50.2 ($C_{pyrrolid}$), 55.3 (OCH_3), 105.8 (CH-CH), 113.9, 121.6, 131.8, 156.1 (C_{ar}), 127.4 (CH_3C), 152.2 (C=N), 167.7 (C=S), 170.3 (C=O).

MS (APCI) m/z: 384 $[M+H]^+$ (100 %).

Calculated, %: 59.51, N 18.26, H 5.52. $C_{19}H_{21}N_5O_2S$.
Found, %: C 59.69, N 18.01, H 5.62.

4-(6-(4-Substituted phenyl)-7-bromoaceto-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)-1-(4-methoxyphenyl)pyrrolidin-2-ones (21–23)

A mixture of the aminotriazole **16** (0.31 g, 1 mmol), the corresponding α-haloketone (1.2 mmol), triethylamine (0.12 g, 1.2 mmol), and methanol (7 ml) was refluxed for 4 h and cooled down. The precipitates were filtered off, washed with water, dried, and crystallized from methanol to give **21–23**.

1-(4-Methoxyphenyl)-4-(6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)pyrrolidin-2-one (21) was obtained according to the general procedure from 2-bromoacetophenone to give a white solid (0.23 g, 57 %). M.p. 86–87 ºC (methanol).

IR (KBr, cm^{-1}): 1699 (C=O), 1513, 1464, 1466 (3C=N).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 2.92–3.08 (2H, m, CH_2CO), 3.74 (3H, s, OCH_3), 4.10–4.18 (2H, m, NCH_2), 4.28 (t, 1H, *J* = 8.4 Hz, CH), 4.43 (2H, s, SCH_2), 6.94 (2H, d, *J* = 9.1 Hz, $H_{ar\text{-}3,5}$), 6.90–8.04 (9H, m, H_{ar}).

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 23.0 (SCH_2), 27.0, 35.8, 51.1 ($C_{pyrrolid}$), 55.2 (OCH_3), 113.8, 121.4, 127.5, 129.0, 132.0, 132.3, 133.4, 153.1 (C_{ar}), 141.3 (CH_2-*C*=N), 155.1, 155.9 (2C=N), 171.2 (C=O).

Calculated, %: C 62.21, N 17.27, H 4.72. $C_{21}H_{19}N_5O_2S$.
Found, %: C 62.45, N 17.42, H 4.97.

4-[6-(4-Chlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]-1-(4-methoxyphenyl)pyrrolidin-2-one (22) was as obtained according to the general procedure from 4-chloro-2′-bromoacetophenone to give a white solid (0.33 g, 75 %). M.p. 209–210 ºC (methanol).

IR (KBr, cm^{-1}): 1676 (C=O), 1510, 1496, 1467 (3C=N).

^{1}H NMR (300 MHz, DMSO-d_6): δ 2.90–3.07 (2H, m, CH_2CO), 3.74 (3H, s, OCH_3), 4.10–4.18 (2H, m, NCH_2), 4.24–4.30 (1H, m, CH), 4.42 (2H, s, SCH_2), 6.94 (2H, d, *J* = 9.1 Hz, $H_{ar\text{-}3,5}$), 7.56 (2H, d, *J* = 9.1 Hz, $H_{ar\text{-}2,6}$), 7.62 (2H, d, *J* = 8.7 Hz, $H_{ar\text{-}3',5'}$), 8.03 (2H, d, *J* = 8.7 Hz, $H_{ar\text{-}2',6'}$).

^{13}C NMR (75 MHz, DMSO-d_6): δ 22.9 (SCH_2), 27.0, 35.8, 51.1 ($C_{pyrrolid}$), 55.2 (OCH_3), 113.8, 121.4, 129.1, 129.3, 132.2, 132.3, 136.8, 153.9 (C_{ar}), 141.2 (CH_2-*C*=N), 154.0, 155.9 (2C=N), 171.2 (C=O).

Calculated, %: C 57.34, N 15.92, H 4.12. $C_{21}H_{18}ClN_5O_2S$.

Found, %: C 57.53, N 16.05, H 4.37.

1-(4-Methoxyphenyl)-4-[6-(4-nitrophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]pyrrolidin-2-one (23) was as obtained according to the general procedure from 4-nitro-2′-bromoacetophenone to give a brown solid (0.35 g, 78 %). M.p. 212–213 ºC (methanol).

IR (KBr, cm^{-1}): 1684 (C=O), 1512, 1498, 1467 (3C=N).

^{1}H NMR (300 MHz, DMSO-d_6): δ_H 2.88–3.12 (2H, m, CH_2CO), 3.73 (3H, s, OCH_3), 4.11–4.21 (2H, m, NCH_2), 4.24–4.33 1H, (m, CH), 4.49 (2H, s, SCH_2), 6.94 (2H, d, *J* = 9.0 Hz, $H_{ar\text{-}3,5}$), 7.56 (2H, d, *J* = 9.0 Hz, $H_{ar\text{-}2,6}$), 8.25 (2H, d, *J* = 8.9 Hz, $H_{ar\text{-}2',6'}$), 8.35 (2H, d, *J* = 8.9 Hz, $H_{ar\text{-}3',5'}$).

^{13}C NMR (75 MHz, DMSO-d_6): δ_C 23.1 (SCH_2), 27.0, 35.7, 51.1 ($C_{pyrrolid}$), 55.2 (OCH_3), 113.8, 121.4, 123.9, 128.9, 132.3, 139.3, 149.1, 153.3 (C_{ar}), 141.1 (CH_2-*C*=N), 154.1, 155.9 (2C=N), 171.1 (C=O).

Calculated, %: C 55.99, N 18.66, H 4.03. $C_{21}H_{18}N_6O_4S$.

Found, %: C 56.15, N 18.82, H 4.17.

Biology

Method for determination of antioxidant activity. The free radical scavenging activity of the compounds was measured by the widely used DPPH method [27]. Briefly, 1 cm^3 of a 1 mM solution of DPPH in ethanol was added to the solutions of tested compounds (1 mg cm^{-3} in DMSO). The mixture was shaken vigorously and allowed to stand at room temperature for 20 min. Afterwards, the absorbance was measured at 517 nm in a spectrophotometer (UV-200-RS). The lower absorbance of the reaction mixture indicated a higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated according to the following equation: DPPH scavenging effect % = $(A_0–A_1/A_0)\times100$, where A_0 is the absorbance of the control reaction, and A_1 is the absorbance in the presence of the samples.

Microbiology. The antibacterial activity was tested by using the disk diffusion technique. The microbial strains *R. radiobacter*, *X. campestris*, and *E.coli* were commercially available from the German Collection of Microorganisms and Cell Cultures (DSMZ). The zone of inhibition of bacterial growth was investigated. The main solutions (1 mg/cm^3) of the synthesized compounds were prepared in DMSO and diluted to different concentrations (250, 300, 400, 450, 500, 750, 1,000 μg/cm^3) with DMSO. Cultures of *R. radiobacter*, *X. campestris*, and *E. coli* were cultivated in Petri dishes for 24 h at 37 °C on the Luria–Bertani (LB) agar medium. A bacterial suspension was prepared from cultivated bacterial cultures, and 50 mm^3 of the inoculum containing bacterial cells (10^8 CFU/cm^3) was spread over the LB agar medium. Filter paper disks were prepared by adding 25 mm^3 of each compound solution and then placed on the LB agar medium. Ampicillin was used as the positive control.

Conclusions

A variety of 4-substituted 1-(4-metoxyphenyl)pyrrolidin-2-ones containing azole, diazole, oxadiazole, thiadiazole, and triazole fragments were synthesized from 1-(metoxyphenyl)-5-oxopyrrolidine-3-carbohydrazide by condensation reactions or by the modification of the obtained compounds. All structures of the new compounds described here have been confirmed by the synthetic, analytical, and spectroscopic data. All compounds were screened for antibacterial activity and only two types of hetero systems – 5-thioxo-4,5-dihydro-1,3,4-oxadiazole and [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine – showed a moderate activity against *R. radiobacter*, *X. campestris*, and *E. coli* microorganisms. Some compounds were tested for their antioxidant activity. The best activity was shown by 1-(4-methoxyphenyl)-4-(4,5-dihydro-5-thioxo-1,3,4-oxadiazol-2-yl)pyrrolidin-2-one, whereas the displacement of the oxygen atom by the nitrogen one in the 1,3,4-oxadiazole ring decreased the antioxidant activity.

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4-PAKEISTŲJŲ 1-(4-METOKSIFENIL)PIROLIDIN-2-ONŲ SINTEZĖ IR ANTIBAKTERINIS BEI ANTIOKSIDACINIS AKTYVUMAS

S a n t r a u k a

Pasinaudojus 1-(4-metoksifenil)-5-oksopirolidin-3-karbohidrazido kondensacijos reakcijomis arba modifikuojant gautuosius produktus susintetinta daug 4-pakeistųjų 1-(4-metoksifenil)pirolidin-2-onų, turinčių azolo, diazolo, oksadiazolo, tiadiazolo, triazolo fragmentus. Šių struktūrų susidarymas patvirtintas remiantis analitinių ir spektroskopinių tyrimų duomenimis. Ištirtas naujų junginių antibakterinis aktyvumas prieš *Rhizobium radiobacter*, *Xanthomonas campestris* ir *Escherichia coli* bakterijų padermes. Tik dvi heterociklinės sistemos – 5-tiokso-4,5-dihidro-1,3,4-oksadiazolas ir [1,2,4]triazol[3,4-*b*][1,3,4]tiadiazinas – pasižymėjo vidutiniu poveikiu prieš minėtus mikroorganizmus. Ištyrus keleto susintetintų junginių antioksidacines savybes, nustatyta, kad labiausiai oksidacinius procesus slopino 1-(4-metoksifenil)-4-(4,5-dihidro-5-tiokso-1,3,4-oksadiazol-2-il)pirolidin-2-onas, deguonies atomą 1,3,4-oksadiazolo žiede pakeitus azoto atomu, junginio antioksidacinis poveikis sumažėjo.