CHEMIJA. 2013. Vol. 24. No. 1. P. 74–80

Synthesis and antimicrobial activity of 1,3-disubstituted pyrrolidinones with hydrazone and naphthoquinone moieties

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1-(4-Aminophenyl)-5-oxo-3-pyrrolidinecarbohydrazide was converted into a series of new hydrazone derivatives and compounds, containing pyrazole, pyrrole, and 4-substituted benzene moieties. Reaction of 2,3-dichloro-1,4-naphthoquinone with the obtained compounds as well as with 5-oxo-1-(4-aminophenyl)pyrrolidine-3-carboxylic acid and its ester provided derivatives of 3-chloro-1,4-naphthoquinone, some of which exhibited good antifungal activities at low concentrations against *Candida tenuis* and *Aspergillus niger*.

Key words: hydrazone derivatives, 3-chloro-1,4-naphthoquinone derivatives, pyrazole, pyrrole, antifungal activity

INTRODUCTION

The incidence of bacterial and fungal infections is an important and challenging problem due to the emerging new infectious diseases and increasing multi-drug resistance of microbial pathogens [1]. For critically ill people with a compromised immune system, including AIDS patients, burn victims, individuals undergoing chemotherapy, and organ transplant recipients taking immunosuppressive drugs, fungal infections are a serious concern [2]. The widespread use of antibiotics has contributed to the growing infection rate as well since fungal infections occur after antibiotic therapy, which has the effect of killing the beneficial bacteria that normally suppress fungi. The development of new effective antifungal and antibacterial agents is strongly needed.

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Quinone and naphthoquinone fragments are often encountered in natural biologically active compounds. Natural naphthoquinone derivatives found in plants, such as juglone, lawsone, plumbagine, and lapachol, have antibacterial effect on several species of aerobic and anaerobic organisms [3–6]. Natural enantiomeric naphthoquinones alkannin and shikonin and their derivatives have been shown to be active against Gram-positive bacteria and fungi [7–9]. A series of 1,4-naphthoquinones containing a free or substituted amino group, including 2,3-diamino-1,4-naphthoquinone, has been found to act as antibacterial agents against *Staphylococcus aureus* with MIC values ranging from 30 to 125 μg/ml [10]. The recent studies have shown that the incorporation of chlorine atom in 1,4-naphthoquinone derivatives is essential for antifungal activity; thus, synthetic amino-1,4-naphthoquinone derivatives containing a chlorine atom have been reported to possess, among others, antibacterial and antifungal activities [11, 12].

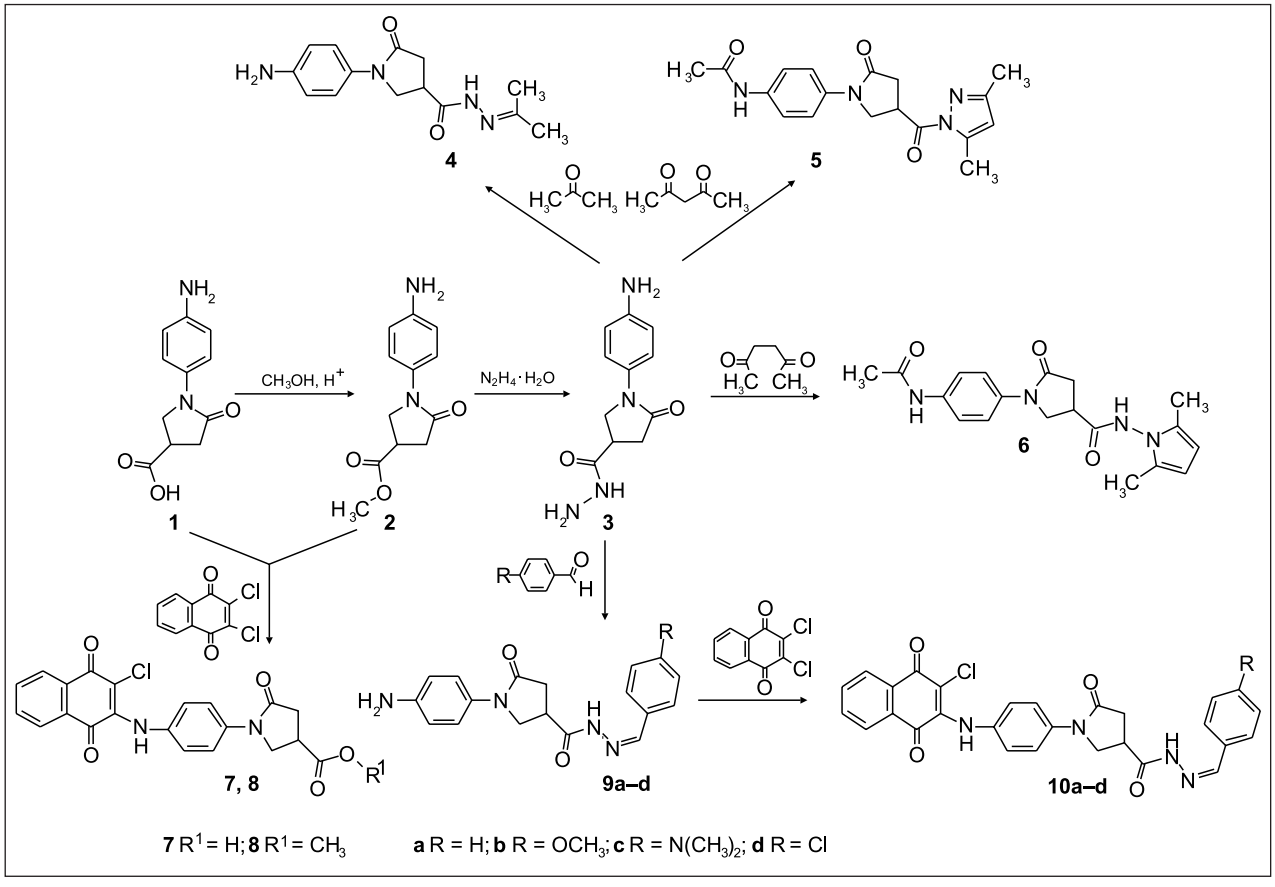
Hydrazone is another considerable pharmacophore group for antimicrobial activity [13]. Recently, hydrazone derivatives have attracted much attention as potent agents in development of novel antimicrobial agents [14–17].

As part of our research program on the synthesis of biologically active 1,4-naphthoquinone derivatives possessing antibacterial and antifungal activities [18–20], we report herein the synthesis of 1,3-disubstituted pyrrolidinones with hydrazone and naphthoquinone moieties.

RESULTS AND DISCUSSION

Chemistry

The synthetic strategies employed to obtain the target compounds **2–10** are presented in the Scheme. The starting compound, 1-(4-aminophenyl)-5-oxo-3-pyrrolidinecarboxylic acid (**1**), was converted into its ester under treatment with methanol in the presence of catalytic amount of sulfuric acid. Afterwards, methyl ester **2** was heated under reflux with hydrazine hydrate in propan-2-ol to give 1-(4-aminophenyl)-5-oxo-3-pyrrolidinecarbohydrazide (**3**). Condensation of acid hydrazide **3** with aromatic aldehydes, acetone, and diketones was investigated. It has been determined that hydrazine moiety only participated in the reaction of **3** with aromatic aldehydes and acetone, and thus respective hydrazones **4** and **9a–d** were obtained [21]. Whereas, pyrrole and pyrazole scaffold-containing compounds **5** and **6** were formed in the reaction with diketones, 2,4-pentanedione and 2,5-hexanedione. At the same time, acylation of the amine group in the aromatic ring took place. Reaction of compounds, containing a free amino group in the aromatic ring, **1**, **2** and **9** with 2,3-dichloro-1,4-naphthoquinone in ethanol at reflux temperature took place, as expected, via nucleophilic replacement of one chlorine atom in 2,3-dichloronaphthoquinone by an amine group. Thus, 1-{4-[(3-chloro-1,4-dioxo-1,4-dihydro-2-naphthalenyl)amino]phenyl}-5-oxo-3-pyrrolidinecarboxylic



Scheme. Synthesis of 1,3-disubstituted pyrrolidinones with hydrazone and naphthoquinone moieties

acid (**7**) and its ester **8**, as well as 1-{4-[(3-chloro-1,4-dioxo-1,4-dihydro-2-naphthalenyl)amino]phenyl}-5-oxo-*N*'-(phenylmethylidene)-3-pyrrolidinecarbohydrazides **10a–d** were synthesized.

The structures of all synthesized compounds have been confirmed by the NMR, IR, mass spectra, and elemental analyses data, which are provided in the Experimental section. The formation of heterocyclic systems in **5** and **6** molecules has been proven by the characteristic ^{1}H NMR resonances at 6.24 ppm and 5.67 ppm attributed to the CH group proton in the dimethylpyrazole moiety and two protons of CH groups in the dimethylpyrrole fragment, respectively.

An important feature of the NMR spectra for **4**, **9**, and **10** is the presence of double sets of some proton and carbon resonances, attributed to NH and CH= group atoms. Most probably, this is due to the restricted rotation around the CO–NH bond characteristic of amides and existence of s-*cis* and s-*trans* rotamers in DMSO-d_6 solution as well as the presence of unsaturated C=N bond which can condition the formation of positional isomers [21]. Positional isomers do not form in the case of **4** since two identical terminal methyl substituents are positioned at the double bond; therefore, only a mixture of s-*cis* and s-*trans* rotamers is observed in the ^{1}H NMR spectrum. For the same reason, protons of two methyl groups in **4** resonated as a set of four spectral lines. Bulky aromatic substituents are positioned at the double C=N bond in **10a–d** what allowed to admit that less restricted *E* diastereomer was formed in the reaction and the double set of resonances was recorded due to the existence of rotamers [22–25]. The predominant conformation in amides is a s-*cis* one, therefore the more intensive NH group proton resonance can be ascribed to a s-*cis* rotamer. The ratio of s-*cis* : s-*trans* rotamers was determined to be 40 : 60 for **4** and 60 : 40 for hydrazones **10a–d**. ^{1}H NMR spectra of **9b–d** display three or four singlets attributed to NH group proton, what has led to the conclusion that in DMSO-d_6 solutions these compounds form both positional isomers and rotamers due to the interaction with polar solvent. In case of **9a**, existence of the rotamers only has been observed.

Antimicrobial activity

The synthesized compounds **4**, **7**, **9a–d**, and **10a–d** were evaluated for their antibacterial and antifungal activity against strains of *Escherichia coli B-906, Staphylococcus aureus 209-P, Mycobacterium luteum B-917* (as nonpathogenic test bacteria culture representative of genus *Mycobacterium*), *Candida tenuis VKM Y-70*, and *Aspergillus niger VKM F-1119* by diffusion technique [26] and serial dilution technique (determination of minimal inhibition concentrations MIC) [27]. Their activities were compared with the known antibacterial agent vancomicine and antifungal agent nistatine.

However, test-cultures *E. coli* and *S. aureus* appeared not to be sensitive to the tested compounds investigated by diffusion technique at concentrations of 0.1 and 0.5%. Only *M. luteum* was slightly sensitive to **7** (diameter of inhibition zone at a concentration of 0.5% was 10.7 mm and it was 8.0 mm at 0.1%). When serial dilution technique was employed, **7** showed minimal inhibition action against *S. aureus* at 250 μg/ml, whereas **7** and **10c** suppressed growth of *M. luteum* at concentrations of 31.2 μg/ml and 125 μg/ml, respectively. For other compounds growth of bacteria strains was observed at the investigated concentrations.

The evaluation of antifungal activity of the synthesized compounds against strains of *C. tenuis* and *A. niger* gave much more promising results (Table). Diffusion technique has identified compound **7** with high antifungal activity at concentrations of 0.1 and 0.5% (21.7–26.0 mm and 20.0–24.4 mm, respectively) in comparison with nistatine. At 0.5% concentration, *C. tenuis* and *A. niger* were moderately sensitive to **10a**, **10c**, and **10d**. Diameters of inhibition zones for

Table. **Antifungal activity of 4, 7, 9b–d, and 10a–d determined by diffusion technique and serial dilution technique**

Compound	Inhibition diameter of microorganism growth, mm			MIC, μg/ml	
	Concentration	*C. tenuis*	*A. niger*	*C. tenuis*	*A. niger*
4				62.5	+
7	0.5	26.0	24.4	1.9	1.9
	0.1	21.7	20.0		
9b	0.5		7.0	+	+
9c				15.6	500.0
9d	0.5		6.0	31.2	+
10a	0.5	14.7	13.4	0.9	250.0
	0.1	8.0	6.0		
10b				+	500.0
10c	0.5	12.0	7.0	1.9	3.9
	0.1	7.0			
10d	0.5	15.4	10.7	0.9	125.0
	0.1	12.0			
C*	0.1	19.0	20.0		

* Nistatine was used as a control in the tests of antifungal activity of the synthesized compounds.

+ – growth of microorganisms.

strain *C. tenuis* were 14.7, 12.0, and 15.4 mm, and for fungi *A. niger* they were 13.4, 7.0, and 10.7 mm, respectively.

Compounds **7**, **10a**, **10c**, and **10d** showed MIC at concentrations of 0.9–1.9 μg/ml against *C. tenuis* and 1.9–500 μg/ml against *A. niger*. Compounds **9c** and **9d** showed MIC at a concentration of 15.6–31.2 μg/ml against *C. tenuis* and **9c** was active against *A. niger* at 500 μg/ml. Compound **4** possessed antifungal activity against *C. tenuis* at a concentration of 62.5 μg/ml.

Therefore, compounds with antifungal activity against fungi *C. tenuis* and *A. niger* were identified among the synthesized compounds at low concentrations, **7** being the most promising for which antifungal activity was observed at 1.9 μg/ml (*C. tenuis* and *A. niger*).

EXPERIMENTAL

Chemistry

Melting points were determined with an automatic APA1 melting point apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a *Varian Unity Inova* (300 MHz, 75 MHz) spectrometer operating in the Fourier transform mode. Chemical shifts (δ) are reported in parts per million (ppm) calibrated from TMS (0 ppm) as an internal standard for ^{1}H NMR and DMSO-d_6 (39.50 ppm) for ^{13}C NMR. IR spectra were recorded on a Perkin Elmer Spectrum BX FT-IR spectrometer using KBr tablets. Mass spectra were obtained on a Waters (Micromas) ZQ 2000 Spectrometer, using ESI mode. Elemental analyses (C, H, N) were performed with an Elemental Analyzer CE-440. Monitoring of the reaction course and purity of the synthesized compounds was carried out using TLC on Merck, Silica Gel 60 F_{254} (Kieselgel 60 F_{254}) silica gel plates.

Methyl 1-(4-aminophenyl)-5-oxo-3-pyrrolidinecarboxylate (2). A mixture of carboxylic acid **1** (11.0 g, 0.05 mol), methanol (25 ml, 0.62 mol), and sulfuric acid (1.5 ml) was heated at reflux temperature for 16 h. The excess of methanol was removed under reduced pressure. 10% Na_2CO_3 solution (40 ml) was poured onto the residue. The precipitate formed was filtered, washed with water, and recrystallized from ethanol. Yield 8.3 g (71%). M. p. 74–75.5 °C. ^{1}H NMR (DMSO-d_6) δ: 2.60–2.79 (m, 2H, CH_2CO); 3.37–3.38 (m, 1H, CH); 3.69 (s, 3H, OCH_3); 3.85–4.00 (m, 2H, CH_2N); 5.06 (s, 2H, NH_2); 6.56, 7.23 (2d, 4H, J = 8.7 Hz, ArH). ^{13}C NMR (DMSO-d_6) δ: 34.66 (CH); 34.94 (CH_2); 50.27 (NCH_2); 52.08 (CH_3); 113.55, 121.63, 128.00, 145.80 (ArC); 170.35, 173.27 (2CO). IR ν (cm^{-1}): 3 413, 3 346, 3 248 (NH_2, NH); 1 720, 1 684 (2C=O). MS (ESI, 20 V), *m/z* (%): 257 $[M+Na]^+$ (100); 235 $[M+H]^+$ (20). Anal. calcd. for $C_{12}H_{14}N_2O_3$, %: C, 61.53; H, 6.02; N, 11.96. Found, %: C, 61.28; H, 5.96; N, 11.78.

1-(4-Aminophenyl)-5-oxo-3-pyrrolidinecarbohydrazide (3). A mixture of methyl ester **2** (4.68 g, 0.02 mol), hydrazine hydrate (3 ml, 0.06 mol), and propan-2-ol (10 ml) was heated at reflux temperature for 30 min. Precipitate formed after cooling the reaction mixture to room temperature was filtered, washed with ethanol and diethyl ether, and recrystallized from ethanol. Yield 4.23 g (90%). M. p. 179–180.5 °C. ^{1}H NMR (DMSO-d_6) δ: 2.54–2.68 (m, 2H, CH_2CO); 3.08–3.20 (m, 1H, CH); 3.72–3.96 (m, 2H, CH_2N); 4.31 (s, 2H, $NH\underline{NH}_2$); 5.02 (s, 2H, NH_2); 6.56, 7.23 (2d, 4H, J = 9.0 Hz, ArH); 9.27 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 34.15 (CH); 35.38 (CH_2); 51.18 (NCH_2); 113.60, 121.54, 128.21, 145.67 (ArC); 170.86, 171.67 (2CO). IR ν (cm^{-1}): 3 310, 3 279, 3 147, 3 053 (NH_2, NH); 1 670, 1 647 (2C=O). Anal. calcd. for $C_{11}H_{14}N_4O_2$, %: C, 56.40; H, 6.02; N, 23.92. Found, %: C, 56.63; H, 6.15; N, 23.94%.

1-(4-Aminophenyl)-*N*'-(1-methylethylidene)-5-oxo-3-pyrrolidinecarbohydrazide (4). A mixture of hydrazide **3** (1.17 g, 5 mmol) and acetone (30 ml) was heated at reflux temperature for 4 h. Precipitate formed after cooling the reaction mixture to room temperature was filtered, washed with acetone, and recrystallized from 1,4-dioxane. Yield 0.97 g (71%). M. p. 91–92 °C. ^{1}H NMR (DMSO-d_6) δ: 1.88, 1.89, 1.94, 1.95 (4s, 6H, E-*cis*, Z-*cis*, E-*trans*, Z-*trans*, $N=C(CH_3)_2$); 2.52–2.72 (m, 2H, CH_2CO); 3.34–3.49 (m, 1H, CH); 3.75–4.04 (m, 2H CH_2N); 5.41 (s, 2H, NH_2); 6.58–7.30 (m, 4H, ArH); 10.23, 10.29 (2s, 1H (0.4/0.6), NH). IR ν (cm^{-1}): 3 420, 3 372, 3 288, 3 209 (NH_2, NH); 1 679, 1 643 (2C=O). MS (ESI, 20 V), *m/z* (%): 275 $[M+H]^+$ (80). Anal. calcd. for $C_{14}H_{18}N_4O_2$, %: C, 61.30; H, 6.61; N, 20.42. Found, %: C, 61.32; H, 6.56; N, 20.35.

***N*-(4-{4-[(3,5-Dimethyl-1*H*-pyrazol-1-yl)carbonyl]-2-oxo-1-pyrrolidinyl}phenyl)acetamide (5)**. A mixture of hydrazide **3** (0.70 g, 3 mmol), 2,4-pentanedione (1.2 g, 12 mmol), propan-2-ol (15 ml), and HCl (2 drops) was heated at reflux temperature for 5 h. The precipitate formed after cooling the reaction mixture to room temperature was filtered, washed with propan-2-ol, and recrystallized from propan-2-ol. Yield 0.82 g (80%). M. p. 170–171.5 °C. ^{1}H NMR (DMSO-d_6) δ: 2.05 (s, 3H, $COCH_3$); 2.23 (s, 3H, 5-CH_3); 2.44 (s, 3H, 3-CH); 2.73–2.96 (m, 2H, CH_2CO); 3.98–4.24 (m, 2H, CH_2N); 4.41–4.55 (m, 1H, CH); 6.24 (s, 1H, =CH); 7.58 (s, 4H, ArH); 9.96 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 13.49 (CH_3); 13.96 (CH_3); 23.8 (CH_3); 34.93 (CH); 35.92 (CH_2); 50.14 (NCH_2); 111.50 ($\underline{HC}$=CCH_3); 119.06, 119.98, 134.11, 135.60 (ArC); 143.80 ($\underline{HC}$=CCH_3); 152.05 ($CHCCH_3$); 168.00, 171.10, 172.56 (3CO). IR ν (cm^{-1}): 3 311, 3 262 (NH); 1 736, 1 695, 1 665 (3C=O). Anal. calcd. for $C_{18}H_{20}N_4O_3$, %: C, 63.52; H, 5.92; N, 16.46. Found, %: C, 63.33; H, 6.24; N, 16.32.

1-[4-(Acetylamino)phenyl]-N-(2,5-dimethyl-1H-pyrrol-1-yl)-5-oxo-3-pyrrolidinecarboxamide (6). A mixture of hydrazide **3** (1.17 g, 5 mmol), 2,5-hexanedione (1.14 g, 10 mmol), propan-2-ol (20 ml), and conc. acetic acid (10 ml) was heated under reflux for 4 h. The precipitate formed after cooling the reaction mixture to room temperature was filtered, washed with propan-2-ol and diethyl ether, and recrystallized from ethanol. Yield 1.1 g (62%). M. p. 224–225 °C. ^{1}H NMR

(DMSO-d_6) δ: 2.01 (s, 6H, $2CH_3$); 2.05 (s, 3H, $COCH_3$); 2.69–2.89 (m, 2H, CH_2CO); 3.41–3.52 (m, 1H, CH); 3.92–4.17 (m, 2H, CH_2N); 5.67 (s, 2H, 2CH); 7.60 (s, 4H, ArH); 9.96 (s, 1H, NH); 10.92 (s, 1H, NH-N). ^{13}C NMR (DMSO-d_6) δ: 10.87 ($2CH_3$); 23.83 (CH_3); 33.69 (CH); 35.44 (CH_2); 50.35 (NCH_2); 102.98 ($2CH_3C$=CH); 119.20, 119.90 (ArC); 126.63 ($2CH_3C$=CH); 134.15, 135.58 (ArC); 168.00, 171.19, 171.80 (3CO). IR ν (cm^{-1}): 3 305, 3 266, 3 200 (NH); 1 703, 1 683, 1 668 (3C=O). MS (ESI, 20 V), *m/z* (%): 355 $[M+H]^+$ (100). Anal. calcd. for $C_{19}H_{22}N_4O_3$, %: C, 64.39; H, 6.26; N, 15.81. Found, %: C, 64.50; H, 6.14; N, 15.72.

1-{4-[(3-Chloro-1,4-dioxo-1,4-dihydro-2-naphthalenyl) amino]phenyl}-5-oxo-3-pyrrolidinecarboxylic acid (7). A mixture of carboxylic acid **1** (1.10 g, 5 mmol), 2,3-dichloro-1,4-naphthoquinone (1.14 g, 5 mmol), and ethanol (40 ml) was heated at reflux temperature for 3 h. Afterwards, it was kept at room temperature for 20 h. The precipitate formed was filtered, dissolved in 10% Na_2CO_3 solution, solution was filtered and acidified with 30% acetic acid to pH 6. The precipitate formed was filtered, washed with water and ethanol, and recrystallized from ethanol. Yield 1.63 g (79%). M. p. 241–242.5 °C. ^{1}H NMR (DMSO-d_6) δ: 2.70–2.84 (m, 2H, CH_2CO); 3.28–3.43 (m, 1H, CH); 3.91–4.10 (m, 2H, CH_2N); 7.14–8.10 (m, 8H, ArH); 9.32 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 35.26 (CH); 35.36 (CH_2); 50.25 (NCH_2); 113.92, 119.13, 124.48, 126.26, 126.72, 130.30, 132.08, 133.38, 134.97, 135.04, 143.27 (ArC+2C=); 172.02, 174.50, 176.83, 180.23 (4CO). IR ν (cm^{-1}): 3 614 (COOH); 3 217 (NH); 1 731, 1 710, 1 639, 1 681 (4C=O). MS (ESI, 20 V), *m/z* (%): 434 $[M+Na]^+$ (100); 413 $[M+2H]^+$ (25). Anal. calcd. for $C_{21}H_{15}N_2O_5Cl$, %: C, 61.40; H, 3.68; N, 6.82. Found, %: C, 61.18; H, 3.94; N, 6.82.

Methyl 1-{4-[(3-chloro-1,4-dioxo-1,4-dihydro-2-naphthalenyl)amino]phenyl}-5-oxo-3-pyrrolidinecarboxylate (8). A mixture of methyl ester **2** (1.17 g, 5 mmol), 2,3-dichloro-1,4-naphthoquinone (1.14 g, 5 mmol), and ethanol (40 ml) was heated at reflux temperature for 4 h. Afterwards, it was kept at room temperature for 18 h. The precipitate formed was filtered and recrystallized from ethanol. Yield 1.55 g (73%). M. p. 81–82.5 °C. ^{1}H NMR (DMSO-d_6) δ: 2.69–2.89 (m, 2H, CH_2CO); 3.43–4.14 (m, 1H, CH); 3.71 (s, 3H, CH_3); 3.90–4.16 (m, 2H, CH_2N); 7.16, 7,62 (2d, 4H, J = 9.0 Hz, ArH); 7.85–8.10 (m, 4H, ArH); 9.38 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 34.81 (CH); 34.94 (CH_2); 49.70 (NCH_2); 52.11 (CH_3); 113.68, 118.78, 124.23, 125.89, 126.44, 130.16, 131.92, 133.06, 134.72, 134.75, 135.56, 143.06 (ArC); 171.34, 173.06, 176.52, 180.03 (4CO). IR ν (cm^{-1}): 3 311, 2 950 (NH); 1 743, 1 696, 1 674, 1 641 (4C=O). MS (ESI, 20 V), *m/z* (%): 425 $[M]^+$ (100); 427 $[M+2H]^+$ (30). Anal. calcd. for $C_{22}H_{17}N_2O_5Cl$, %: C, 62.20; H, 4.03; N, 6.59. Found, %: C, 61.98; H, 3.94; N, 6.51.

General procedure for the synthesis of hydrazones 9a–d. A mixture of hydrazide **3** (1.17 g, 5 mmol), corresponding aromatic aldehyde (5 mmol), and ethanol (40 ml) was heated at reflux temperature for 3 h. The precipitate formed after cooling the reaction mixture to room temperature was filtered, washed with propan-2-ol, and recrystallyzed from an appropriate solvent.

1-(4-Aminophenyl)-5-oxo-*N*'-(phenylmethylidene)-3-pyrrolidinecarbohydrazide (9a). Yield 1.08 g (67%). M. p. 204–205 °C (from 1,4-dioxane). ^{1}H NMR (DMSO-d_6) δ: 2.66–2.80 (m, 2H, CH_2CO); 3.27–3.32 (m, 3H, CH); 3.77–4.41 (m, 2H, CH_2N); 5.04 (s, 2H, NH_2); 6.50–7.76 (m, 10H, ArH + CH=N); 11.58, 11.65 (2s, 1H (0.6/0.4), NH). IR ν (cm^{-1}): 3 436, 3 343, 3 179 (NH_2, NH); 1 696, 1 664 (2C=O). MS (ESI, 20 V), *m/z* (%): 323 $[M+H]^+$ (100). Anal. calcd. for $C_{18}H_{18}N_4O_2$, %: C, 67.07; H, 5.63; N, 17.38. Found, %: C, 67.22; H, 5.68; N, 17.43.

1-(4-Aminophenyl)-*N*'-[(4-methoxyphenyl)methylidene]-5-oxo-3-pyrrolidinecarbohydrazide (9b). Yield 1.43 g (81%). M. p. 193–194.5 °C (from 1,4-dioxane). ^{1}H NMR (DMSO-d_6) δ: 2.69–2.86 (m, 2H, CH_2CO); 3.26–3.36 (m, 1H, CH); 3.80, 3.81 (2s, 3H, OCH_3); 3.87–4.10 (m, 2H, CH_2N); 6.57–8.50 (m, 9H, ArH + CH=N); 11.45, 11.50, 11.51, 11.58 (4s, 1H (0.5/0.10/0.25/0.15), NH). IR ν (cm^{-1}): 3 405, 3 335, 3 328, 3 238 (NH_2, NH); 1 681, 1 660 (2C=O). MS (ESI, 20 V), *m/z* (%): 353 $[M+H]^+$ (100). Anal. calcd. for $C_{19}H_{20}N_4O_3$, %: C, 64.76; H, 5.72; N, 15.90. Found, %: C, 64.88; H, 5.89; N, 15.98.

1-(4-Aminophenyl)-*N*'-[4-(dimethylamino)phenyl) methylidene]-5-oxo-3-pyrrolidinecarbohydrazide (9c). Yield 1.73 g (95%). M. p. 125–127 °C (from ethanol). ^{1}H NMR (DMSO-d_6) δ: 2.76–2.86 (m, 2H, CH_2CO); 2.96, 2.98, 3.02, 3.05 (4s, 6H, $N(CH_3)_2$); 3.82–4.19 (m, 3H, CH + CH_2N); 5.12 (br. s, 2H, NH_2); 6.54–8.46 (m, 9H, ArH + N=CH); 11.33, 11.45, 11.37 (3s, 1H (0.5/0.4/0.1), NH). IR ν (cm^{-1}): 3 447, 3 358, 3 215 (NH_2, NH); 1 685, 1 667, (2C=O). MS (ESI, 20 V), *m/z* (%): 366 $[M+H]^+$ (100). Anal. calcd. for $C_{19}H_{20}N_4O_3$, %: C, 65.73; H, 6.34; N, 19.16. Found, %: C, 65.56; H, 6.30; N, 19.18.

1-(4-Aminophenyl)-*N*'-[(4-chlorophenyl)methylidene]-5-oxo-3-pyrrolidinecarbohydrazide (9d). Yield 1.32 g (74%). M. p. 222–223 °C (from 1,4-dioxane). ^{1}H NMR (DMSO-d_6) δ: 2.65–2.89 (m, 2H, CH_2CO); 3.26–4.22 (m, 3H, CH + CH_2N); 5.37 (br. s, 2H, NH_2); 6.58–8.24 (m, 9H, ArH + CH=N); 11.64, 11.69, 11.74, 11.76 (4s, 1H (0.4/0.25/0.25/0.1), NH). IR ν (cm^{-1}): 3 432, 3 352, 3 092 (NH_2, NH); 1 679, 1 663 (2C=O). MS (ESI, 20 V), *m/z* (%): 357 $[M]^+$ (100); 359 $[M+2H]^+$ (30). Anal. calcd. for $C_{18}H_{17}N_4O_2Cl$, %: C, 60.59; H, 4.80; N, 15.70. Found, %: C, 60.82; H, 4.93; N, 15.81.

General procedure for the synthesis of hydrazones 10a–d. A mixture of the corresponding compound **9a–d** (5 mmol), 2,3-dichloro-1,4-naphthoquinone (1.14 g, 5 mmol), and ethanol (50 ml) was heated at reflux temperature for 6 h. The precipitate formed after cooling the reaction mixture to ambient temperature was filtered, washed with propan-2-ol, and recrystallyzed from the appropriate solvent.

1-{4-[(3-Chloro-1,4-dioxo-1,4-dihydro-2-naphthalenyl) amino]phenyl}-5-oxo-*N*'-(phenylmethylidene)-3-pyrrolidinecarbohydrazide (10a). Yield 1.48 g (58%). M. p. 182–184 °C (ethanol / chloroform 1/5). ^{1}H NMR (DMSO-d_6) δ: 2.30–2.92 (m, 2H, CH_2CO); 3.32–3.43 (m, 1H, CH); 3.91–4.21 (m, 2H, NCH_2); 7.12–8.13 (m, 14, ArH + CH=N); 9.36 (s, 1H, NH); 11.62, 11.69 (2s, 1H (0.6/0.4), CONH). IR ν (cm^{-1}): 3 336, 3 243, 3 065 (NH); 1 708, 1 676, 1 659, 1 638 (4C=O). MS (ESI, 20 V), *m/z* (%): 513 $[M]^+$ (100); 515 $[M+2H]^+$ (33). Anal. calcd. for $C_{28}H_{21}N_4O_4Cl$, %: C, 65.56; H, 4.13; N, 10.92. Found, %: C, 65.41; H, 4.09; N, 10.84.

1-{4-[(3-Chloro-1,4-dioxo-1,4-dihydro-2-naphthalenyl) amino]phenyl}-*N*'-[(4-methoxyphenyl)methylidene]-5-oxo-3-pyrrolidinecarbohydrazide (10b). Yield 1.63 g (60%). M. p. 229–230 °C (from 1,4-dioxane). ^{1}H NMR (DMSO-d_6) δ: 2.71–2.88 (m, 2H, CH_2CO); 3.81, 3.82 (2s, 3H, CH_3); 3.95–4.26 (m, 3H, CH + CH_2N); 6.94–8.20 (m, 13H, ArH + CH=N); 9.35 (s, 1H, NH); 11.47, 11.55 (2s, 1H (0.6/0.4), NHCO). IR ν (cm^{-1}): 3 337, 3 304, 3 240 (NH); 1 705, 1 674, 1 655, 1 639 (4C=O). MS (ESI, 20 V), *m/z* (%): 543 $[M]^+$ (100); 545 $[M+2H]^+$ (35). Anal. calcd. for $C_{29}H_{23}N_4O_5Cl$, %: C, 64.15; H, 4.27; N, 10.32. Found, %: C, 63.90; H, 4.42; N, 10.49.

1-{4-[(3-Chloro-1,4-dioxo-1,4-dihydro-2-naphthalenyl)amino]phenyl}-*N*'-{[4-(dimethylamino)phenyl] methylidene}-5-oxo-3-pyrrolidinecarbohydrazide (10c). Yield 2.18 g (78%). M. p. 156–157 °C (from methanol). ^{1}H NMR (DMSO-d_6) δ: 2.69–2.90 (m, 2H, CH_2CO); 2.97, 2.98 (2s, 6H, $2CH_3$); 3.95–4.28 (m, 3H, CH + CH_2N); 6.65–8.10 (m, 13H, ArH + CH=N); 9.37 (s, 1H, NH); 11.33, 11.45 (2s, 1H (0.6/0.4), CONH). IR ν (cm^{-1}): 3 320, 3 210, 3 064 (NH); 1 700, 1 677, 1 685, 1 654 (4C=O). MS (ESI, 20 V), *m/z* (%): 556 $[M]^+$ (100); 558 $[M+2H]^+$ (35). Anal. calcd. for $C_{30}H_{26}N_5O_4Cl$, %: C, 64.80; H, 4.71; N, 12.60. Found, %: C, 64.55; H, 4.63; N, 12.57.

1-{4-[(3-Chloro-1,4-dioxo-1,4-dihydro-2-naphthalenyl) amino]phenyl}-*N*'-[(4-chlorophenyl)methylidene]-5-oxo-3-pyrrolidinecarbohydrazide (10d). Yield 2.03 g (74%). M. p. 233–234 °C (from ethanol / chloroform). ^{1}H NMR (DMSO-d_6) δ: 2.68–2.93 (m, 2H, CH_2CO); 3.95–4.30 (m, 3H, CH + CH_2N); 7.16–8.30 (m, 13H, ArH + CH=N); 9.36 (s, 1H, NH); 11.66, 11.78 (2s, 1H (0.6/0.4), CONH). IR ν (cm^{-1}): 3 337, 3 304, 3 239 (NH); 1 725, 1 704, 1 675, 1 657 (4C=O). MS (ESI, 20 V), *m/z* (%): 547 $[M]^+$ (100); 549 $[M+2H]^+$ (70); 550 $[M+3H]^+$ (25). Anal. calcd. for $C_{28}H_{20}N_4O_4Cl_2$, %: C, 61.44; H, 3.68; N, 10.24. Found, %: C, 61.05; H, 3.63; N, 10.38.

Biology

Diffusion technique. Antimicrobial activity of the compounds was evaluated by diffusion in peptone on solid nutrient medium (nutrient agar – for bacteria, wort agar – for fungi). The microbial loading was 10^9 cells / 1 ml. The duration of incubation for bacteria was 24 h at 35 °C and for fungi it was 48–72 h at 28–30 °C. The results were recorded by measuring the zones surrounding the disk. Control disk contained vancomicine (for bacteria) or nistatine (for fungi) as a standard substance.

Serial dilution technique. Compounds were tested according to standard microbroth dilution for determination of minimal bacteriostatic and minimal bactericidic concentrations (MBSC and MBCC), minimal fungicidic and minimal fungistatic concentrations (MFCC and MFSC). The certain volume of solution of compound in DMSO was brought in nutrient medium (nutrient meat-extract – for bacteria, wort – for fungi). The tested compounds were dissolved in DMSO and the concentration range was 500–0.9 μg/ml. The inoculum of bacteria and fungi was inoculated in nutrient medium. The duration of incubation of bacteria was 24–72 h at 37 °C for bacteria and 30 °C for fungi. The results were estimated according to the presence or absence of growth of microorganisms.

CONCLUSIONS

New 1,3-disubstituted 5-oxopyrrolidine derivatives were synthesized and their antimicrobial activity against strains of bacteria *E. coli, S. aureus*, and *M. luteum*, as well as fungi *C. tenuis* and *A. niger* was tested.

The tested compounds did not exhibit a significant antibacterial activity. The highest antifungal activity was determined for 7 containing 2-chloro-3-aminophenyl-1,4-naphthoquinone moiety at the 1-position of 3-carboxy-5-oxopyrrolidine ring. Transformation of the carboxylic group to the hydrazone fragment led to the decrease in antifungal activities of the obtained compounds (**10a**, **10c**, and **10d**). Hydrazones **4** and **9a–d** inhibited growth of microorganisms even less than their structural analogues containing 2-chloro-1,4-naphthoquinone moiety.

Received 29 October 2012
Accepted 10 December 2012

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1,3-DIPAKEISTŲ PIROLIDINONŲ, TURINČIŲ HIDRAZONO IR NAFTOCHINONO FRAGMENTŲ, SINTEZĖ IR ANTIMIKROBINIS AKTYVUMAS

S a n t r a u k a

Iš 1-(4-aminofenil)-5-okso-3-pirolidinkarbohidrazido susintetinti nauji hidrazonai – junginiai, savo struktūroje turintys pirazolo, pirolo ir 4-pakeisto benzeno fragmentus. 2,3-Dichlor-1,4-naftochinonui reaguojant su gautaisiais produktais, o taip pat su 5-okso-1-(4-aminofenil)pirolidin-3-karboksirūgštimi bei jos esteriu susintetinti 2-pakeisti 3-chlor-1,4-naftochinono dariniai. Atlikus dalies susintetintų junginių biologinius tyrimus, nustatyta, kad du junginiai (**7**, **10c**) slopina *S. aureus* ir *M. luteum* bakterijų augimą, o vienas jų – junginys **7** – pasižymi ir didžiausiu fungicidiniu poveikiu prieš *C. tenuis* ir *A. niger*. Šio junginio karboksigrupės modifikavimas iki hidrazoninio fragmento (**4**, **10**) mažino tokių junginių fungicidinį veikimą.