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SYNTHESIS AND BIOLOGICAL ACTIVITY OF MONO- AND DISUBSTITUTED 1,2,4-TRIAZOLE DERIVATIVES

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Abstract – Novel mono- and disubstituted triazole derivatives were synthesized by condensation reactions of 1-aryl-3-hydrazinocarbonyl-5-oxopyrrolidinones with organic and inorganic cyanates, their structures were investigated and antimicrobial activity was evaluated.

INTRODUCTION

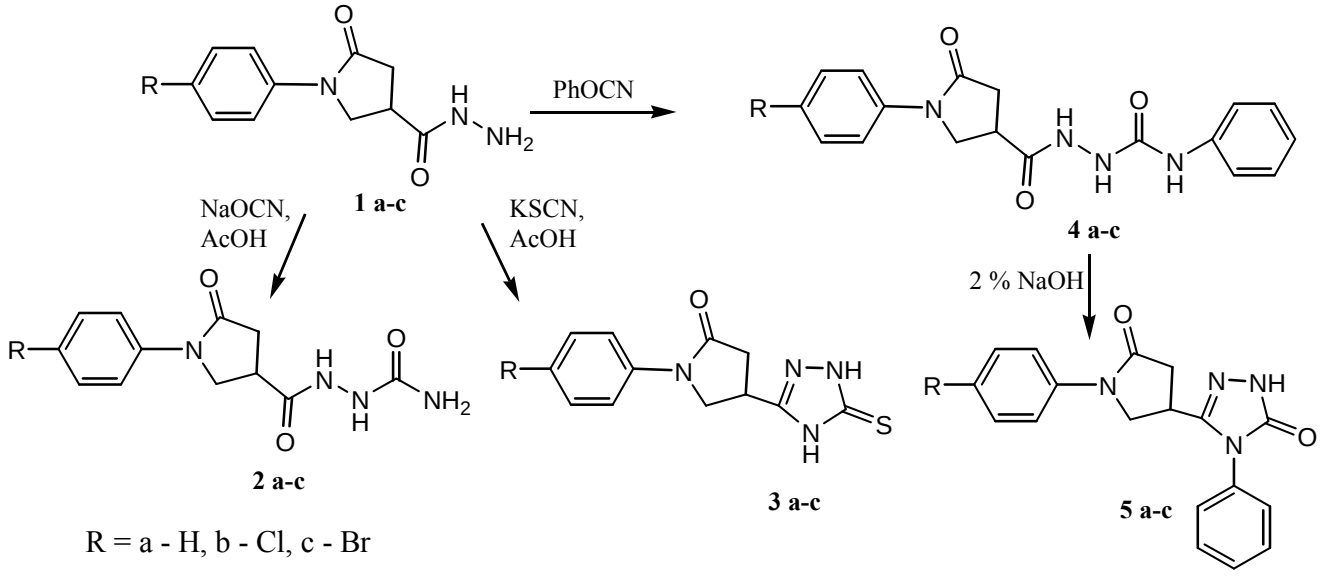
Nitrogen-containing five-membered heterocyclic compounds, azoles, diazoles, triazoles, and their derivatives, are especially important and widespread natural products which can also be successfully synthesized in the laboratory. Synthetic compounds of this class participate in biochemical processes and often have a wide range of biological activity. 1,2,4-Triazoles are associated with diverse pharmacological activities such as anti-inflammatory,[1,2] antiviral,[3,4] anticancer,[5,6] antitubercular,[7,8] antifungal and antimicrobial.[9–15]

Cyclocondensation of carboxylic acid hydrazides is a widely known method for the synthesis of azoles. The goal of this work was investigation of the reactions of 1-aryl-3-hydrazinocarbonyl-5-oxopyrrolidines **1a–c** with organic and inorganic cyanates and thiocyanates aiming to synthesize variously substituted triazole systems, and to determine the structures of the synthesized compounds by spectroscopic methods.

The results of the assessment of the antimicrobial activity of the newly synthesized compounds are reported.

RESULTS AND DISCUSSION

In this work, 2-[(5-oxo-1-phenylpyrrolidin-3-yl)carbonyl]hydrazinecarboxamide (**2a**), 2-{[1-(4-chlorophenyl)-5-oxopyrrolidin-3-yl]carbonyl}hydrazinecarboxamide (**2b**), and 2-{[1-(4-bromophenyl)-5-oxopyrrolidin-3-yl]carbonyl}hydrazinecarboxamide (**2c**) were prepared by heating under reflux 1-aryl-3-hydrazinocarbonyl-5-oxopyrrolidines **1a–c** with sodium cyanate in dilute acetic acid (Scheme). The structures of the synthesized compounds were confirmed by the IR, ^{1}H, ^{13}C NMR, mass spectra and elemental analysis data. ^{1}H NMR spectra of **2a–c** have disclosed that these compounds exist as rotamers in DMSO-d_6 solution. For example, in the spectrum for **2a** the NH protons in $NHCONH_2$ fragment are observed as two sets of resonances due to the restricted rotation around the CO-NH bond indicating the existence of *E*/*Z* izomers with the ratio 0.90:0.10. Since the spectral line corresponding to the *Z* isomer is more shielded and, thus, is always observed at higher magnetic field, the conclusion can be drawn that the *Z* isomers predominate in this case. The protons of $NHCOCH_{pyrrolid}$ give a double set of spectral lines as well, but in the opposite order of intensities with the ratio of 0.15:0.85. Therefore, rotation of these two fragments around the amide bond is stabilized differently by hydrogen bonding (between molecules of the compound and solvent).



Scheme

The analogous reaction of **1a–c** with potassium thiocyanate instead of sodium cyanate resulted in the formation of different structure compounds which precipitated from the reaction mixture already while

heated. The analysis of the ^{1}H and ^{13}C NMR spectra of these compounds revealed that the cyclic compounds, 1-aryl-4-(4,5-dihydro-5-thioxo-1*H*-1,2,4-triazol-3-yl)pyrrolidin-2-ones **3a–c**, were obtained. Their ^{1}H NMR spectra indicate the NH/SH tautomerism. For example, in the spectrum for **3b** the intensity ratio of spectral lines attributed to NH proton (13.37 ppm) and SH proton (1.94 ppm) is 0.57: 0.43.

The reactions of **1a–c** with phenyl isocyanate were carried out in methanol under reflux. The structures of the obtained 2-(1-aryl-3-carbonyl-5-oxopyrrolidinyl)-*N*-phenylhydrazinocarboxamides **4a–c** were identified by the analysis of their ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectrum for **4a** shows that the influence of benzene ring on the shielding of NH group proton is weaker than the one of -NHCO- fragment, therefore, the NH proton in CONHPh fragment is more shielded than the other NH proton at the same carbonyl group (NHCONH). In the ^{13}C NMR spectrum for this compound, the characteristic signals assigned to pyrrolidinone carbons and four resonances (C-2, C-3 and C-2", C-3", double intensity) for each benzene ring are observed. Three carbonyl carbon atoms resonate at 155 ppm (-NHCONH), 171.88 ppm (pyrrolidinone ring), and 172.51 ppm (CONH). The NMR spectra for **4a–c**, have revealed that in the DMSO-d_6 solutions these compounds, the same as **2a–c,** exist as a mixture of rotamers due to the restricted rotation around the amide bonds.

As known from the literature, semicarbazides can undergo cyclization to diazole and triazole derivatives. In this work, cyclization of phenylhydrazinocarboxamides **4a–c** in 2% NaOH solution provided the corresponding 1,2,4-triazol-3-ones **5a–c**. The formation of cyclic compounds was confirmed by their NMR spectra which display resonances characteristic to this five-membered heterocycle. For example, in the ^{1}H NMR spectrum for **5b** the signal of NH group proton is observed at 13.86 ppm, whereas, in the ^{13}C NMR spectrum C=N group carbon resonates at 152.71 ppm, and the C=O group carbon gives a signal at 168.31 ppm.

ANTIMICROBIAL ACTIVITY

Antimicrobial activity of the synthesized compounds **2a-c**, **3a-c**, **4a-c**, and **5c** against *Escherichia coli, Staphylococcus aureus, Mycobacterium luteum*, *Candida tenuis* and *Aspergillus niger* strains was evaluated by diffusion method in agar (data of inhibition zone of microorganisms) and by "serial dilution" method (determination of minimal bacteriostatic (MBSC) and minimal bactericidic (MBCC) concentrations, minimal fungistatic (MFSC) and minimal fungicidic (MFCC) concentrations).[16,17]

Data obtained by the first method revealed that **2a**, **3a**, and **3c** showed antibacterial and antifungal activity at the indicated concentrations (Table 1).

Table 1. Antibacterial and fungicidal activities of the synthesized compounds determined by diffusion method in agar

Compound	Concentration, %	Inhibition diameter of microorganism growth, mm				
		Antibacterial activity			Fungicidal activity	
		E.coli	*St.aureus*	*Myc.luteum*	*C. tenuis*	*Asp. niger*
2a	0.5	8.0	0	0	0	0
3a	0.5	0	0	11.0	12.3	0
	1.0	0	0	7.0	9.0	0
3c	0.5	0	0	7.0	0	0

As seen from the data in Table 2, substances with antibacterial and fungicidal activity in low concentrations are identified among the synthesized compounds by "serial dilution" method. Compounds **3a** and **3c** had good antibacterial activity against *St. aureus*; **3a–c**, and **5c** were active against *Myc. luteum*. Activity of the other compounds in the investigated concentrations was not detected.

Table 2. Antibacterial and fungicidal activity of the synthesized compounds determined by "serial dilution" method

Compound	Bacteria cultures						Fungi cultures			
	E.coli		*St.aureus*		*Myc.luteum*		*C. tenuis*		*Asp. niger*	
	MBCS, μg cm^{-3}	MBCC, μg cm^{-3}	MBCS, μg cm^{-3}	MBCC, μg cm^{-3}	MBCS, μg cm^{-3}	MBCC, μg cm^{-3}	MFSC, μg cm^{-3}	MFCC, μg cm^{-3}	MFSC, μg cm^{-3}	MFCC, μg cm^{-3}
2a	+	+	+	+	+	+	250.0	*	+	+
2b	+	+	+	+	500.0	*	+	+	+	+
2c	+	+	+	+	250.0	*	500.0	*	+	+
3a	+	+	31.2	250.0	15.6	62.5	7.8	15.6	+	+
3b	+	+	+	+	125.0	500.0	62.5	250.0	+	+
3c	500.0	*	125.0	500.0	31.2	62.5	31.2	62.5	500.0	*
4a	+	+	+	+	+	+	+	+	+	+
4b	+	+	+	+	+	+	125.0	*	+	+

4c	+	+	+	+	+	+	500.0	*	+	+
5c	+	+	+	+	250.0	500.0	500.0	*	+	+

+ – growth of microorganisms;
* – in the investigated concentrations the indexes of biocidic effect were not determined.

Investigation of fungicidal activity of the synthesized compounds showed that biocidic effect against *Asp. niger* practically was not determined in concentrations 500 μg cm^{-3} and lower. Yeast culture of fungus *C. tenuis* appeared sensitive to majority of the tested compounds. Among them, **3a–c** had effective indexes of fungistatic activity in concentrations 7.8–62.5 μg cm^{-3} and showed fungicidal activity in concentrations 15.6–250 μg cm^{-3}.

CONCLUSIONS

The convenient synthesis route of 1,2,4-triazole derivatives containing aromatic and 2-pyrrolidinone substituents from the corresponding carbohydrazides is reported. The carried out investigations have revealed that 1-aryl-4-(4,5-dihydro-5-thioxo-1*H*-1,2,4-triazol-3-yl)pyrrolidin-2-ones **3a–c** show good antibacterial and fungicidal activities at low concentrations in relation to the cultures *Staphylococcus aureus*, *Mycobacterium luteum*, and *Candida tenuis*.

EXPERIMENTAL

Melting points were determined on an Auto probe analyzer APA 1 and are uncorrected. NMR spectra were recorded on a Varian Unity Inova (300 MHz) spectrometer using DMSO-d_6 as a solvent. Chemical shifts (δ) are reported in parts per million (ppm) relative to internal standard TMS (0 ppm) for ^{1}H NMR, and DMSO-d_6 (39.5 ppm) for ^{13}C NMR. The IR spectra were measured as potassium bromide pellets on a Perkin–Elmer Spectrum BX FT–IR spectrometer. Elemental analyses (C, H, N) were performed on an Elemental Analyzer CE-440. Silica gel plates (Silufol UV-254) were used for analytical purposes.

General procedure for the preparation of 2-(1-Aryl-3-carbonyl-5-oxopyrrolidinyl)-hydrazinocarboxamides 2a-c. A mixture of **1a-c** (0.01 mol), sodium cyanate (0.03 mol), water (20 mL) and glacial acetic acid (5 mL) was heated under reflux for 1 h and cooled down. The crystalline precipitate **2a–c** was filtered, washed with water, and recrystallized from the appropriate solvent.

2-[(5-Oxo-1-phenylpyrrolidin-3-yl)carbonyl]hydrazinecarboxamide (**2a**). Yield 2.00 g (76%), mp 183–184 °C (from water). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.60–2.80 (m, 2H, CH_2CO), 3.20–3.30 (m, 1H, CH), 3.85–4.05 (m, 2H, NCH_2), 6.02 (s, 2H (0.90), (*Z*) NH_2), 6,25 (s, 2H (0.10), (*E*) NH_2), 7.11–7.65

(m, 5H, H_{ar}), 7.83 (s, 1H (0.90), (*Z*) NHCONH$_2$), 8.20 (s, 1H (0.10), (*E*) NHCONH$_2$), 9.12 (s, 1H (0.15), (*Z*) NHCOCH$_{pyrrolid}$), 9.78 (s, 1H (0.85), (*E*) NHCOCH$_{pyrrolid}$). ^{13}C NMR (75 MHz, DMSO-*d*$_6$): δ 33.72, 35.45, 50.38, 119.29, 123.96, 128.62, 139.08, 158.72, 172.17. IR (KBr): ν 3568, 3437, 3224 (2NH, NH$_2$), 1736, 1690, 1648 (3C=O) cm^{-1}. MS (APCI, 20V): *m/z* 263 (M+H)$^+$ (30%), 264 (M+1+H)$^+$ (10%). *Anal*. Calcd for $C_{12}H_{14}N_4O_3$: C, 54.96; H, 5.38; N, 21.36. Found: C, 55.08; H, 5.25; N, 21.55.

2-{[1-(4-Chlorophenyl)-5-oxopyrrolidin-3-yl]carbonyl}hydrazinecarboxamide (**2b**). Yield 1.78 g (60%), mp 193–194 °C (from MeOH). ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 2.61–2.82 (m, 2H, COCH$_2$), 3.20–3.30 (m, 1H (0.87), CH), 3.44–3.53 (m, 1H (0.13), CH), 3.84–4.04 (m, 2H, NCH$_2$), 6.02 (br.s, 2H (0.89), (*Z*) NH$_2$), 6.25 (br.s, 2H (0.11), (*E*) NH$_2$), 7.40–7.71 (m, 4H, H_{ar}), 7.82 (br.s, 1H (0.87), (*Z*) NHCONH$_2$), 8.13 (br.s, 1H (0.13), (*E*) NHCONH$_2$), 9.14 (br.s, 1H (0.13), (*Z*) CHCONH), 9.77 (br.s, 1H (0.87), (*E*) CHCONH). ^{13}C NMR (75 MHz, DMSO-*d*$_6$): δ 33.54, 35.35, 50.15, 115.72, 121.01, 132.01, 135.24, 138.59, 159.61, 172.14. IR (KBr): ν 3481 (NH), 3320–3150 (NH+NH$_2$), 1716, 1668, 1590 (C=O) cm^{-1}. MS (APCI, 20V): *m/z* 297 (M+H)$^+$ (40%), 299 (M+2+H)$^+$ (20%). *Anal*. Calcd for $C_{12}H_{13}ClN_4O_3$: C, 48.58; H, 4.42; N, 18.88. Found: C, 48.39; H, 4.60; N, 18.73.

2-{[1-(4-Bromophenyl)-5-oxopyrrolidin-3-yl]carbonyl}hydrazinecarboxamide (**2c**). Yield 2.10 g (62%), mp 188–189 °C (from water). ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 2.60–2.81 (m, 2H, CH$_2$CO), 3.22–3.31 (m, 1H, CH), 3.83–4.03 (m, 2H, NCH$_2$), 6.00 (s, 2H (0,81), (*Z*) NH$_2$), 6.23 (s, 2H (0.19), (*E*) NH$_2$), 7.53–7.64 (m, 4H, H_{ar}), 7.80 (s, 1H (0.89), (*Z*) NHCONH$_2$), 8.08 (s, 1H (0.11), (*E*) NHCONH$_2$), 9.13 (br.s, 1H (0.14), (*Z*) NHCOCH$_{pyrrolid}$), 9.75 (br.s, 1H (0.86), (*E*) NHCOCH$_{pyrrolid}$). ^{13}C NMR (75 MHz, DMSO-*d*$_6$): δ 33.56, 35.44, 50.25, 115.78, 121.08, 131.41, 134.44, 138.37, 158.71, 172.16. IR (KBr): ν 3447, 3293, 3196, 3069 (NH, NH$_2$), 1713, 1682, 1618 (3C=O) cm^{-1}. MS (APCI, 20V): *m/z* 341 (M+H)$^+$ (20%), 343 (M+2+H)$^+$ (20%), 363 (M+Na)$^+$ (100%), 365 (M+Na+1+H)$^+$ (95%). *Anal*. Calcd for $C_{12}H_{13}BrN_4O_3$: C, 42.25; H, 3.84; N, 16.42. Found: C, 42.45; H, 3.74; N, 16.25.

General procedure for the preparation of 1-Aryl-4-(4,5-dihydro-5-thioxo-1*H*-1,2,4-triazol-3-yl)pyrrolidin-2-ones 3a–c. A mixture of **1a–c** (5 mmol), potassium thiocyanate (15 mmol) and glacial acetic acid (5 mL) was heated under reflux for 20 h, diluted with water (10 mL), and cooled down. Precipitate **3a–c** was filtered, washed with water, and recrystallized from 50% acetic acid.

1-Phenyl-4-(4,5-dihydro-5-thioxo-1*H*-1,2,4-triazol-3-yl)pyrrolidin-2-one (**3a**). Yield 0.54 g (41%), mp 267–268 °C. ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 1.86 (s, 0.2H, SH), 2.60–2.85 (m, 2H, CH$_2$CO), 3.27–3.35 (m, 1H, CH), 3.85–4.10 (m, 2H, NCH$_2$), 7.11–7.65 (m, 5H, H_{arom}), 10.2 (s, 0.8H, NH). ^{13}C NMR (75 MHz, DMSO-*d*$_6$): δ 33.41 (C-4‘), 35.21 (C-3‘), 50.10 (C-5‘), 119.02 (C-2), 123.72 (C-4), 128.34 (C-3), 138.73 (C-1), 154.31 (C_a), 165.55 (C_d), 171.38 (C-2‘). IR (KBr): ν 3213, 3063 (2NH), 1680 (C=O), 1599 (C=N), 1235 (C=S) cm^{-1}. MS (APCI, 20V): *m/z* 261(M+H)$^+$ (20%), 262 (M+1+H)$^+$ (30%).

Anal. Calcd for $C_{12}H_{12}N_4OS$: C, 55.37; H, 4.65; N, 21.52. Found: C, 55.18; H, 4.70; N, 21.42.

1-(4-Chlorophenyl)-4-(4,5-dihydro-5-thioxo-1*H*-1,2,4-triazol-3-yl)pyrrolidin-2-one (**3b**). Yield 0.66 g (45%), mp 246–247 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.94 (s, 0.43H, SH), 2.74–2.96 (m, 2H, CH_2CO), 3.70–3.81 (m, 1H, CH), 3.97–4.17 (m, 2H, NCH_2), 7.43, 7.67 (2d, 4H, J = 9.1 Hz, H_{ar}), 13.37, 13.40 (2s, 0.57H + 1H, NH-C=S + NH). ^{13}C NMR (75 MHz, DMSO–d_6): δ 27.95 (C-4‘), 36.13 (C-3‘), 50.76 (C-5‘), 121.03 (C-2), 127.95 (C-4), 128.59 (C-3), 137.89 (C-1), 152.51 (C_a), 166.74 (C_d), 171.58 (C-2‘). IR (KBr): ν 3101, 2930 (2NH), 1664 (C=O), 1586 (C=N), 1319 (C=S) cm^{-1}. MS (APCI, 20V): *m/z* 295 $(M+H)^+$ (100%), 297 $(M+2+H)^+$ (40%). *Anal*. Calcd for $C_{12}H_{11}ClN_4OS$: C, 48.90; H, 3.76; N, 19.01. Found: C, 48.72; H, 3.94; N, 19.20.

1-(4-Bromophenyl)-4-(4,5-dihydro-5-thioxo-1*H*-1,2,4-triazol-3-yl)pyrrolidin-2-one (**3c**). Yield 0.76 g (45%), mp 229–230 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.88 (s, 0.2H, SH), 2.72–2.94 (m, 2H, CH_2CO), 3.71–3.76 (m, 1H, CH), 3.94–4.15 (m, 2H, NCH_2), 7.50-7.61 (m, 4H, H_{ar}), 13.51 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 27.14, 35.36, 49.90, 115.26, 120.56, 130.69, 137.48, 151.70, 165.92, 170.81. IR (KBr): ν 2938, 2867 (2NH), 1660 (C=O), 1492 (C=N), 1320 (C=S) cm^{-1}. MS (APCI, 20V): *m/z* 339 $(M+H)^+$ (25%), 341 $(M+2+H)^+$ (20%), 362 $(M+Na)^+$ (40%), 363 $(M+Na+H)^+$ (70%). *Anal*. Calcd for $C_{12}H_{11}BrN_4OS$: C, 42.48; H, 3.24; N, 15.52. Found: C, 43.30; H, 3.32; N, 15.33.

General procedure for the preparation of 2-(1-Aryl-3-carbonyl-5-oxopyrrolidinyl)-*N*-phenylhydrazinocarboxamides 4a–c. The mixture of **1a–c** (0.01 mol), phenyl isocyanate (0.02 mol) and MeOH (15 mL) was heated under reflux for 2 h and cooled down. The precipitated **4a–c** was filtrated, washed with MeOH, and recrystallized from 1,4-dioxane.

2-(1-Phenyl-3-carbonyl-5-oxopyrrolidinyl)-*N*-phenylhydrazinocarboxamide (**4a**). Yield 2.46 g (73%), mp 210–211 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.63–2.86 (m, 2H, CH_2CO), 3.26–3.37 (m, 1H, CH), 3.88–4.10 (m, 2H, NCH_2), 6.97–7.67 (m, 10H, H_{ar}), 8.11, 8.44 (2s, 2H (0.89:0.11), CONHPh), 8.80, 9.02 (2s, 1H (0.89:0.11), NHCONH), 9.26, 9.95 (2s, 1H (0.15:0.85), $NHCOCH_{pyrrolid}$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.76 (C-4’), 35.54 (C-3’), 50.43 (C-5’), 118.49 (C-2”), 119.38 (C-2), 121.94 (C-4”), 124.06 (C-4), 128.70 (C-3 and C-3”), 139.14, 139.53 (C-1 and C-1”), 155.23 (NHCONH), 171.88 (C-2’), 172.51 (CONH). IR (KBr): ν 3325, 3200, 3061 (3NH), 1679, 1611, 1594 (3C=O) cm^{-1}. MS (APCI, 20V): *m/z* 339 $(M+H)^+$ (40%), 340 $(M+1+H)^+$ (20%). *Anal*. Calcd for $C_{18}H_{18}N_4O_3$: C, 63.89; H, 5.36; N, 16.56. Found: C, 63.79; H, 5.38; N, 16.45.

2-(1-(4-Chlorophenyl)-3-carbonyl-5-oxopyrrolidinyl)-*N*-phenylhydrazinocarboxamide (**4b**). Yield 1.90 g (51%), mp 225–226 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.63–2.87 (m, 2H, CH_2CO), 3.27–3.37 (m, 1H, CH), 3.87–4.10 (m, 2H, NCH_2), 6.92–7.73 (m, 9H, H_{ar}), 8.10, 8.42 (2s, 2H (0.88:0.12), CONHPh), 8.79, 9.01 (2s, 1H (0.88:0.12), NHCONH), 9.26, 9.95 (2s, 1H (0.13:0.87), $NHCOCH_{pyrrolid}$).

^{13}C NMR (75 MHz, DMSO-d_6): δ 33.65 (C-4'), 35.49 (C-3'), 50.34 (C-5'), 118.47 (C-2"), 120.82 (C-2), 121.92 (C-4"), 127.76 (C-4), 128.57, 128.63 (C-3 and C-3"), 138.01 (C-1), 139.51 (C-1"), 155.20 (NHCONH), 172.10, 172.41 (CONHNH or C-2'). IR (KBr): ν 3327, 3198, 3113 (3NH), 1680, 1614, 1594 (3C=O) cm^{-1}. MS (APCI, 20V): *m/z* 373 $(M+H)^+$ (70%), 375 $(M+2+H)^+$ (30%). *Anal*. Calcd for $C_{18}H_{17}ClN_4O_3$: C, 57.99; H, 4.60; N, 15.03. Found: C, 57.83; H, 4.60; N, 14.85.

2-(1-(4-Bromophenyl)-3-carbonyl-5-oxopyrrolidinyl)-*N*-phenylhydrazinocarboxamide (**4c**). Yield 3.17 g (76%), mp 218–219 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.73–2.97 (m, 2H, CH_2CO), 3.39–3.42 (m, 1H, CH), 3.96–4.19 (m, 2H, NCH_2), 7.03–7.76 (m, 9H, H_{ar}), 8.20 (s, 1H, CONHPh), 8.89 (s, 1H, NHCONH), 10.06 (s, 1H, $NHCOCH_{pyrrolid}$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.58, 35.45, 50.22, 115.81, 118.40, 121.09, 121.86, 128.57, 131.42, 138.36, 139.44, 155.14, 172.073, 172.34. IR (KBr): ν 3333, 3259, 3230 (3NH), 1702, 1616, 1597 (3C=O) cm^{-1}. MS (APCI, 20V): *m/z* 417$(M+H)^+$ (20%), 419 $(M+2+H)^+$ (10%), 439 $(M+Na)^+$ (95%), 441 $(M+Na+1+H)^+$ (100%). *Anal*. Calcd for $C_{18}H_{17}BrN_4O_3$: C, 51.81; H, 4.11; N, 13.43. Found: C, 52.00; H, 4.22; N, 13.49.

General procedure for the preparation of 5-(1-Aryl-5-oxopyrrolidin-3-yl)-4-phenyl-2,4-dihydro-1,2,4-triazol-3-ones 5a–c. The mixture of **4a–c** (1 mmol) and 2% aqueous NaOH solution (10 mL) was heated under reflux for 3 h, cooled down, and acidified with dilute HCl (1:1) to pH 2. The precipitated **5a–c** was filtered, washed with water, dried, and recrystallized from 1,4-dioxane.

5-(1-Phenyl-5-oxopyrrolidin-3-yl)-4-phenyl-2,4-dihydro-1,2,4-triazol-3-one (**5a**). Yield 0.14 g (44%), mp 88–89 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.54–2.78 (m, 2H, CH_2CO), 3.57–3.66 (m, 1H, CH), 3.82–4.8 (m, 2H, NCH_2), 7.10–7.66 (m, 10H, H_{ar}), 11.85 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 27.47 (C-4'), 35.26 (C-3'), 49.87 (C-5'), 119.21 (C-2), 127.49 (C-4), 128.30 (C-3"), 128.60 (C-3), 129.23 (C-2"), 130.08 (C-4), 132.31 (C-1"), 138.59 (C-1), 154.26 (C_a), 167.47 (C=O), 171.96 (C-2'). IR (KBr): ν 3105 (NH), 1699, 1685 (2C=O), 1598 (C=N) cm^{-1}. MS (APCI, 20V): *m/z* 321 $(M+H)^+$ (100%), 322 $(M+1+H)^+$ (30%), 323 $(M+2+H)^+$ (10%). *Anal*. Calcd for $C_{18}H_{16}N_4O_2$: C, 67.49; H, 5.03; N, 17.49. Found: C, 67.31; H, 5.23; N, 17.60.

5-[1-(4-Chlorophenyl)-5-oxopyrrolidin-3-yl]-4-phenyl-2,4-dihydro-[1,2,4]triazol-3-one (**5b**). Yield 0.2 g (56%), mp 173–174 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.56–2.80 (m, 2H, CH_2CO), 3.50–3.63 (m, 1H, CH), 3.82–4.09 (m, 2H, NCH_2), 7.39–7.63 (m, 9H, H_{ar}), 13.86 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 27.60 (C-4'), 35.90 (C-3'), 50.34 (C-5'), 120.99 (C-2), 127.86 (C-4), 128.46 (C-3"), 128.51 (C-3), 129.51 (C-2"), 129.66 (C-4), 133.40 (C-1"), 137.71 (C-1), 152.71 (C_a), 168.31 (C=O), 171.30 (C-2'). IR (KBr): ν 3197 (NH), 1698, 1688 (2C=O), 1495 (C=N) cm^{-1}. MS (APCI, 20V): *m/z* 355 $(M+H)^+$ (100%), 357 $(M+2+H)^+$ (40%). *Anal*. Calcd for $C_{18}H_{15}ClN_4O_2$: C, 60.94; H, 4.26; N, 15.79. Found: C, 60.87; H, 4.39; N, 15.84.

5-[1-(4-Bromophenyl)-5-oxopyrrolidin-3-yl]-4-phenyl-2,4-dihydro-[1,2,4]triazol-3-one (**5c**). Yield 0.28 g (70%), mp 186–187 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.55–2.75 (m, 2H, CH_2CO), 3.59–3.62 (m, 1H, CH), 3.80–4.0 (m, 2H, NCH_2), 7.46–7.58 (m, 9H, H_{ar}), 11.83 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 27.62, 35.92, 51.34, 119.31, 127.76, 128.48, 128.62, 129.45, 130.02, 133.42, 137.86, 153.40, 168.51, 171.86. IR (KBr): ν 3045 (NH), 1709, 1695 (2C=O), 1495 (C=N) cm^{-1}. MS (APCI, 20V): *m/z* 399 $(M+H)^+$ (40%), 401 $(M+2+H)^+$ (40%), 421 $(M+Na)^+$ (95%), 423 $(M+Na+1+H)^+$ (95%). *Anal*. Calcd for $C_{18}H_{15}BrN_4O_2$: C, 51.55; H, 3.79; N, 12.21. Found: C, 51.58; H, 3.81; N, 12.03.

Evaluation of antimicrobial activity. 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 cm^{-3}. The duration of incubation for bacteria was 24 h at 35 °C and 48–72 h at 28–30 °C for fungi.

The determination of minimal bacteriostatic and minimal bactericidic concentrations (MBSC and MBCC), minimal fungicidic and minimal fungistatic concentrations (MFCC and MFSC) of the synthesized compounds by "serial dilution" was carried out. The certain volume of solution of the compound in DMSO was brought in nutrient medium (nutrient meat-extract for bacteria, wort for fungi). The inoculum of bacteria and fungi was inoculated in nutrient medium. The duration of incubation was 24–72 h at 37 °C for bacteria and at 30 °C for fungi. The results were estimated by the presence or absence of growth of the microorganisms.

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