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The Reaction of Cyanoacetic Acid Hydrazone with 2-Acetylfuran: Synthesis of Coumarin, Pyridine, Thiophene and Thiazole Derivatives with Potential Antimicrobial Activities

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Abstract

The hydrazone-hydrazone derivative **1** was formed through the reaction of cyanoacetic acid hydrazone with 2-acetylfuran. Compound **1** underwent a series of hetrocyclization reactions through its reaction with different chemical reagents to produce arylidene, coumarin, aryl hydrazone, pyridine, thiophene and thiazole derivatives **2–10**. The MIC values for the newly synthesized products were tested against *E. coli*, *B. cereus*, *B. subtilis* and *C. albicans* compared with ampicilline and cycloheximide as reference drugs.

Keywords

Pyridine • Thiophene • Thiazole • Antimicrobial

Introduction

It is well known that the hydrazone group plays an important role for the antimicrobial activity. Furthermore, a number of hydrazone-hydrazone claimed to possess interesting antibacterial-antifungal [1–3] anticonvulsant [4, 5] anti-inflammatory [6, 7] antimalarial [8] and antituberculosis activities [9–15]. We report here the synthesis of a series of

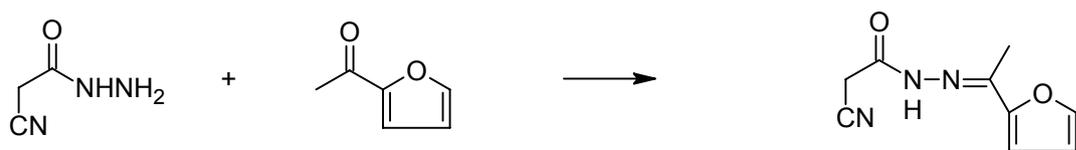
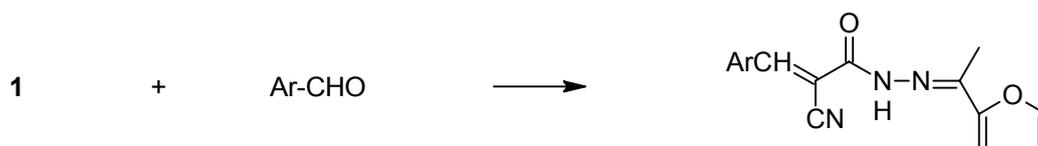
hydrazide-hydrzones via the reaction of cyanoacetylhydrazine with 2-acetylfuran with the aim of obtaining new hydrazide-hydrazones with wide spectrum of pharmaceutical applications,.

Results and Discussions

Cyanoacetylhydrazine reacts with 2-acetylfuran to give the hydrazide-hydrazone derivative **1**. The structure of compound **1** was established on the basis of analytical and spectral data. Thus ^1H NMR spectrum showed the presence of a singlet at δ 1.01 ppm corresponding to CH_3 group, a singlet at δ 3.31 ppm for CH_2 group, a multiplet at δ 6.42–7.29 ppm for the furan protons and a singlet at δ 7.90 ppm for an NH group. Moreover, the ^{13}C NMR data showed the presence of δ 18.7 (CH_3), 27.9 (CH_2), 110.3, 129.6, 137.2, 143.9 (furan C), 156.9 (C=N), 168.4 (C=O). Further elucidation for the structure of **1** was obtained through studying its chemical reactivity through some chemical reagents. Thus, the reaction of **1** with aromatic aldehydes either benzaldehyde, 4-chlorobenzaldehyde or 4-methoxybenzaldehyde gave the benzalidene derivatives **2a–c**, respectively. Analytical and spectral data are in agreement with the proposed structures. On the other hand, the reaction of **1** with salicylaldehyde gave the coumarin derivative **3**. The reaction goes in analogy with the reported literature [16, 17]. The reaction of **1** with either benzenediazonium chloride, 4-chlorobenzenediazonium chloride and 4-methylbenzenediazonium chloride gave the aryl hydrazone derivatives **4a–c**, respectively.

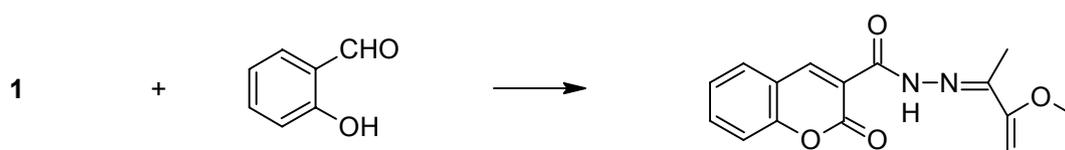
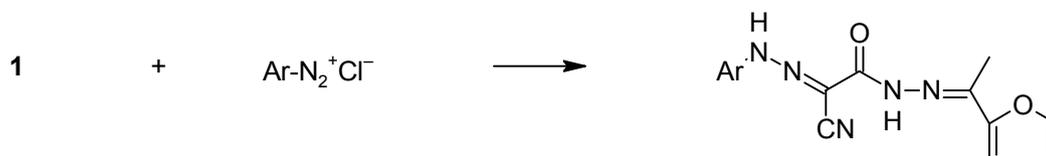
The reaction of compound **1** with cinnamionitrile derivatives was studied. Thus, the reaction of **1** with either α -cyanocinnamionitrile or ethyl α -cyanocinnamate gave the pyridine derivatives **6a** and **6b**, respectively. The reaction took place through the intermediate formation of **5a,b**. Structures of **6a** and **6b** were based on analytical and spectral. Thus, the ^1H NMR spectrum of **6a** showed the presence of a singlet at δ 1.05 corresponding to the CH_3 group, a singlet at δ 4.87 for an NH_2 group, a multiplet at δ 7.28–7.41 phenyl and furan protons. Further confirmation for the structures of **6a** and **6b** were obtained through their synthesis via another reaction root. Thus, the reaction of compound **2a** with either malononitrile or ethyl cyanoacetate gave the same pyridine derivatives **6a** and **6b**, respectively (m.p., mixed m.p. and fingerprint IR). The reaction of **1** with either malononitrile or ethyl cyanoacetate gave the pyridin-2-one derivatives **7a** and **7b**, respectively. Analytical and spectral data are consistent with the proposed structures.

The reaction of **1** with either malononitrile or ethyl cyanoacetate and elemental sulfur in the presence of triethyl amine gave the thiophene derivatives **8a** and **8b**, respectively. The reaction goes in parallel to the reported Gewald's thiophene synthesis [18]. Similarly, the reaction of **1** with cyclohexanone and elemental sulfur gave the 4,5,6,7-tetrahydro-benzothiophene derivative **9**. Formation of **9** took place according the similar reported reactions of cyclohexanone with methylene reagents and elemental sulfur [19]. On the other hand the reaction of **1** with elemental sulfur and phenylisothiocyanate gave the thiazole derivative **10**. Formation of the latter product took place in parallel to the reported Hanzesch reported reaction [20]. Structures of compounds **9** and **10** were based on analytical and spectral data.

**1**

Ar = Ph
 Ar = 4-Cl-Ph
 Ar = 4-MeO-Ph

2a: Ar = Ph
2b: Ar = 4-Cl-Ph
2c: Ar = 4-MeO-Ph

**3**

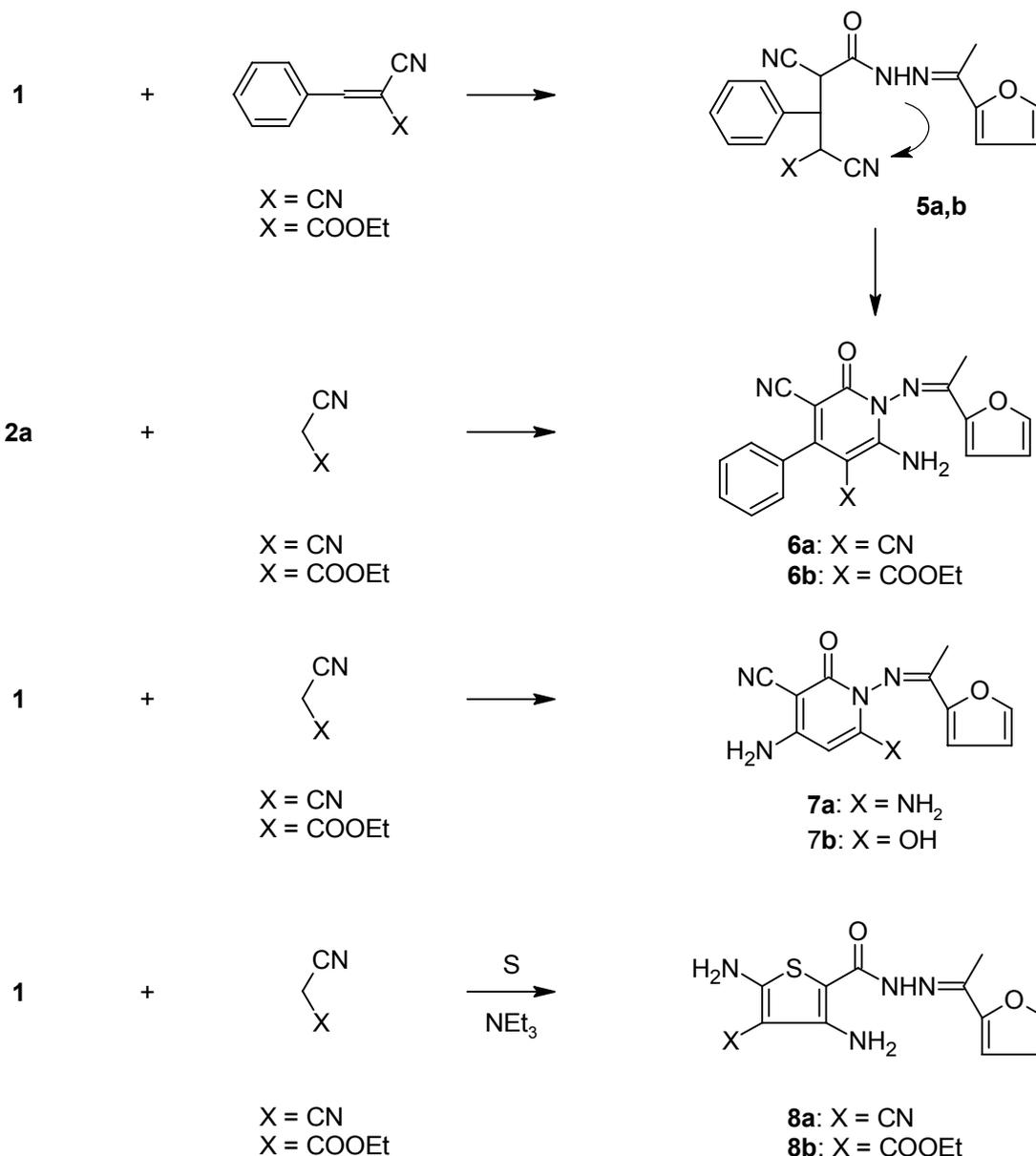
Ar = Ph
 Ar = 4-Cl-Ph
 Ar = 4-MeO-Ph

4a: Ar = Ph
4b: Ar = 4-Cl-Ph
4c: Ar = 4-MeO-Ph

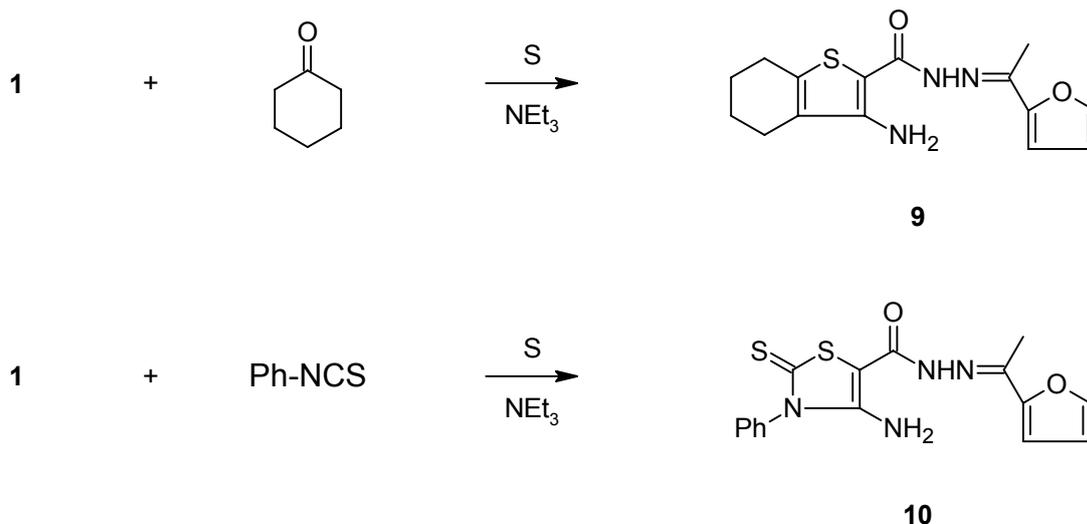
Sch. 1.

According to the results shown in Table 1, only two compounds **1** and **4b** are moderately active against *E. coli* in the concentrations tested (average MIC $12.5 \mu\text{g mL}^{-1}$), the cyanoacetyl and 4-chlorophenyl derivatives of furan moieties being responsible for such activity. However, compound **1** was the higher active compound. Compounds **2a**, **6b** and **7b** are highly active compounds against *B. Cereus*. Also compound **6b** showed the highest activity towards *B. subtilis* with respect to ampicilline. The compounds **2c**, **4a**, **6a** and **8b** shows the highest activity against *C. albicans*. Comparing the SAR of compounds **6a,b** compound **6b** with the Ethylester group showed the highest activity towards *B. subtilis*. On the other hand considering the hydrazone derivatives **4a–c**, the phenylhydrazone

compound **4a** showed the highest activity towards *C. albicans*. Substitution with 4-chloro group as in **4b** showed no activity towards *C. albicans*. Compounds **6a,b** and **7a,b** (the substituted hydrazone derivatives) one can notice that the activity against *B. cereus* was increased due to the existence of the pyridine ring, but compounds **6b** and **7b** are higher active than the corresponding **6a** and **7a** due to the presence of the ethyl pyridine-3-carboxylate moiety and hydroxy group respectively. Finally compound **6b** is the only highest active against *B. subtilis* due to the presence of the ethyl pyridine-3-carboxylate moiety.



Sch. 2.

**Sch. 3.****Experimental****Synthetic methods, analytical and spectral data**

Melting points were determined on an electrothermal apparatus (Buchi 535, Switzerland) in an open capillary tube and are uncorrected. Elemental analyses were determined on a Yanaco CHN Corder elemental analyzer (Japan). IR spectra expressed in ($\nu \text{ cm}^{-1}$) were recorded in KBr pellets on a PA-9721 IR spectrophotometer (Shimadzu, Japan). ^1H NMR & ^{13}C NMR spectra were obtained on a Jeol 300 MHz (Japan) spectrometer in $\text{DMSO-}d_6$ as solvent, using TMS as internal reference and chemical shifts (δ) are expressed in ppm. Mass spectra were recorded on Kratos (75e-v) Ms Equipment (Germany). Synthetic pathways are presented in Schemes 1, 2 and 3. Physicochemical and spectral data for the synthesized compounds are given. The antimicrobial activities of the tested compounds are given in the table. All compounds produced in this work are novel.

2-Cyano-*N'*-(1-furan-2-ylethylidene)acetohydrazide (1)

To a solution of 2-cyanoacetohydrazide (1.0 g, 0.01 mol) in 1,4-dioxane (30 mL) 2-acetylfuran (1.7 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 1 h then left to cool. The solid product formed upon pouring onto ice/water was collected by filtration.

1. Colourless crystals of mp 155 °C (ethanol). MS (m/z , %): 191 (M^+). IR (KBr), vcm^{-1} : 3466, 3326 (NH), 3020 (furan CH), 2970, 2893 (CH_3 , CH_2), 2222 (CN), 1693 (CO), 1640 (C=N), 1632 (C=C). ^1H NMR (300 MHz, $\text{DMSO-}d_6$, TMS): δ 1.01 (s, 3H, CH_3), 3.31 (s, 2H, CH_2), 6.42-7.29 (m, 3H, furan CH), 7.90 (s, 1H, NH). ^{13}C NMR (300 MHz, $\text{DMSO-}d_6$, TMS): δ 18.7 (CH_3), 27.9 (CH_2), 110.3, 129.6, 137.2, 143.9 (furan C), 156.9 (C=N), 168.4 (C=O). Anal. Calcd. for $\text{C}_9\text{H}_9\text{N}_3\text{O}_2$: C, 56.54; H, 4.74; N, 21.98. Found: C, 56.44; H, 4.61; N, 22.05.

**2-Cyano-*N'*-(1-furan-2-ylethylidene)-3-phenylprop-2-enehydrazide (2a),
3-(4-chlorophenyl)-2-cyano-*N'*-(1-furan-2-ylethylidene)prop-2-enehydrazide (2b) and**

**2-cyano-N'-(1-furan-2-ylethylidene)-3-(4-methoxyphenyl)prop-2-enehydrazide (2c)
N'-(1-furan-2-ylethylidene)-2-oxo-2H-chromene-3-carbohydrazide (3)**

General procedure: Equimolecular mixture of **1** (3.21 g, 0.01 mol) and either benzaldehyde (1.08 g, 0.01 mol), *p*-chlorobenzaldehyde (1.38 g, 0.01 mol), 4-methoxybenzaldehyde (1.34 g, 0.01 mol) or salicylaldehyde (1.22 g, 0.01 mol) in 1,4-dioxane (20 mL) containing piperidine (0.50 mL) was heated under reflux for 3 h. The reaction mixture was left to cool then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product, formed in each case, was collected by filtration.

2a. Colorless crystals of mp 233–225 °C (ethanol). MS (*m/z*, %): 279 (M^+). IR (KBr), vcm^{-1} : 3452, 3336 (NH), 3059 (aromatic CH), 2983 (CH_3), 2220 (CN), 1687 (CO), 1643 (C=N), 1636 (C=C). ^1H NMR (300 MHz, $\text{DMSO-}d_6$, TMS): 1.06 (s, 3H, CH_3), 5.99 (s, 1H, CH), 6.40–7.47 (m, 8H, C_6H_5 , furan CH), 7.89 (s, 1H, NH). ^{13}C NMR (300 MHz, $\text{DMSO-}d_6$, TMS): δ 18.7 (CH_3), 110.3, 115.6, 119.6, 125.6, 126.3, 128.8, 136.8, 143.9 (furan C, Benzene C), 111.0, 148.6 (CH=C), 156.6 (C=N), 168.3 (C=O). Anal. Calcd. for $\text{C}_{16}\text{H}_{13}\text{N}_3\text{O}_2$: C, 68.81; H, 4.69; N, 15.05. Found C, 68.77; H, 4.49; N, 14.72.

2b. Colorless crystals of mp 199–201 °C (ethanol). MS (*m/z*, %): 313 (M^+). IR (KBr), vcm^{-1} : 3448, 3342 (NH), 3061 (aromatic CH), 2980, 2883 (CH_3), 2222 (CN), 1690 (CO), 1646 (C=N), 1632 (C=C). ^1H NMR (300 MHz, $\text{DMSO-}d_6$, TMS): 1.02 (s, 3H, CH_3), 6.01 (s, 1H, CH), 6.36–7.44 (m, 7H, C_6H_4 , furan CH), 7.87 (s, 1H, NH). ^{13}C NMR (300 MHz, $\text{DMSO-}d_6$, TMS): 18.7 (CH_3), 112.4, 114.2, 118.9, 125.6, 126.3, 127.9, 136.8, 144.6 (furan C, Benzene C), 111.0, 148.6 (CH=C), 153.6 (C=N), 168.9 (C=O). Anal. Calcd. for $\text{C}_{16}\text{H}_{12}\text{ClN}_3\text{O}_2$: C, 61.25; H, 3.86; N, 13.39. Found: C, 61.11; H, 4.09; N, 13.49.

2c. Colorless crystals of mp >300 °C (ethanol). MS (*m/z*, %): 309 (M^+). IR (KBr), vcm^{-1} : 3446, 3340 (NH), 3058 (aromatic CH), 2977 (CH_3), 2220 (CN), 1668 (CO), 1644 (C=N), 1636 (C=C). ^1H NMR (300 MHz, $\text{DMSO-}d_6$, TMS): 1.03 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 6.04 (s, 1H, CH), 6.38–7.40 (m, 7H, C_6H_4 , furan CH), 7.89 (s, 1H, NH). ^{13}C NMR (300 MHz, $\text{DMSO-}d_6$, TMS): 18.8, 24.3 (2 CH_3), 110.1, 115.8, 118.8, 125.6, 125.8, 128.8, 135.8, 143.7 (furan C, Benzene C), 110.9, 148.3 (CH=C), 156.5 (C=N), 168.0 (C=O). Anal. Calcd. for $\text{C}_{17}\text{H}_{15}\text{N}_3\text{O}_3$: C, 66.01; H, 4.89; N, 13.58. Found: C, 65.99; H, 4.66; N, 13.43.

3. Colorless crystals of mp 229–231 °C (ethanol). MS (*m/z*, %): 296 (M^+). IR (KBr), vcm^{-1} : 3452, 3330 (NH), 3059 (aromatic CH), 2978 (CH_3), 1688–1685 (2CO), 1640 (C=N), 1638 (C=C). ^1H NMR (300 MHz, $\text{DMSO-}d_6$, TMS): 1.21 (s, 3H, CH_3), 6.89 (s, 1H, coumarin H-4), 6.92–7.38 (m, 7H, C_6H_4 , furan CH), 8.22 (s, 1H, NH). ^{13}C NMR (300 MHz, $\text{DMSO-}d_6$, TMS): 18.8 (CH_3), 110.3, 119.6, 120.8, 121.3, 121.8, 122.6, 124.7, 128.3, 136.8, 143.9 (furan C, coumarin C), 158.3 (C=N), 166.5, 168.8 (2C=O). Anal. Calcd. for $\text{C}_{16}\text{H}_{12}\text{N}_2\text{O}_4$: C, 64.86; H, 4.08; N, 9.40. Found: C, 64.85; H, 4.33; N, 9.27.

**2-Cyano-N'-(1-furan-2-ylethylidene)-2-(phenylhydrazono)acetohydrazide (4a),
2-cyano-N'-(1-furan-2-ylethylidene)-2-[(4-methylphenyl)hydrazono]acetohydrazide (4b) and
2-cyano-N'-(1-furan-2-ylethylidene)-2-[(4-methoxyphenyl)hydrazono]acetohydrazide (4c)**

To a solution of compound **1** (3.21 g, 0.01 mol) in ethanol (40 mL) containing sodium acetate (10.0 g) either benzenediazonium chloride (1.40 g, 0.01 mol), 4-chlorobenzene-

diazonium chloride (1.75 g, 0.01 mol) or 4-methylbenzenediazonium chloride (1.55 g, 0.01 mol) with continuous stirring at 0 °C. The solid product, formed in each case, was collected by filtration.

4a. Colorless crystals of mp 198-201 °C (ethanol). MS (*m/z*, %): 295 (M^+). IR (KBr), vcm^{-1} : 3466, 3328 (2NH), 3062 (aromatic CH), 2982, 2893(CH_3), 2222 (CN) 1690 (CO), 1648 (C=N), 1635 (C=C). ^1H NMR (300 MHz, $\text{DMSO-}d_6$, TMS): 1.19 (s, 3H, CH_3), 6.87-7.39 (m, 8H, C_6H_5 , furan CH), 7.99, 8.20 (2s, 2H, 2NH). ^{13}C NMR (300 MHz, $\text{DMSO-}d_6$, TMS): 18.6 (CH_3), 116.7 (CN), 110.3, 119.6, 120.4, 120.8, 121.3, 123.0, 128.0, 134.6, 144.2 (furan C, benzene C), 158.6 (C=N), 165.8, 168.9 (2C=O). Anal. Calcd. for $\text{C}_{15}\text{H}_{13}\text{N}_5\text{O}_2$: C, 61.01; H, 4.44; N, 23.72. Found: C, 60.88; H, 4.09; N, 23.3.

4b. Colorless crystals of mp >300 °C (ethanol). MS (*m/z*, %): 329 (M^+). IR (KBr), vcm^{-1} : 3451, 3322 (2NH), 3060 (aromatic CH), 2980 (CH_3), 2221 (CN) 1690 (CO), 1646 (C=N), 1632 (C=C). ^1H NMR (300 MHz, $\text{DMSO-}d_6$, TMS): 1.16 (s, 3H, CH_3), 7.03 -7.37 (m, 7H, C_6H_4 , furan CH), 7.97, 8.22 (2s, 2H, 2NH). ^{13}C NMR (300 MHz, $\text{DMSO-}d_6$, TMS): 18.7 (CH_3), 116.9 (CN), 111.8, 119.2, 120.6, 121.8, 123.6, 128.5, 133.9, 140.5, 144.2 (furan C, benzene C), 158.6 (C=N), 165.8, 168.9 (2C=O). Anal. Calcd. for $\text{C}_{15}\text{H}_{12}\text{ClN}_5\text{O}_2$: C, 54.42; H, 3.66; N, 21.24. Found: C, 54.64; H, 3.67; N, 21.24.

4c. Colorless crystals of mp 215-217 °C (ethanol). MS (*m/z*, %): 309 (M^+). IR (KBr), vcm^{-1} : 3463, 3347 (2NH), 3058 (aromatic CH), 2982 (CH_3), 2222 (CN), 1693 (CO), 1642 (C=N), 1635 (C=C). ^1H NMR (300 MHz, $\text{DMSO-}d_6$, TMS): 1.18 (s, 3H, CH_3), 2.88 (s, 3H, CH_3), 7.19 -7.39 (m, 7H, C_6H_4 , furan CH), 7.83, 8.25 (2s, 2H, 2NH). ^{13}C NMR (300 MHz, $\text{DMSO-}d_6$, TMS): 18.8, 120.7 (2 CH_3), 116.7 (CN), 110.6, 119.4, 120.6, 121.0, 121.3, 123.2, 128.2, 134.8, 144.0 (furan C, benzene C), 158.9 (C=N), 166.2, 168.7 (2C=O). Anal. Calcd. for $\text{C}_{16}\text{H}_{15}\text{N}_5\text{O}_2$: C, 62.13; H, 4.89; N, 22.64. Found: C, 62.09; H, 4.89; N, 22.74.

6-Amino-1-[(1-furan-2-ylethylidene)amino]-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (6a) and ethyl 2-amino-5-cyano-1-[(1-furan-2-ylethylidene)amino]-6-oxo-4-phenyl-1,6-dihydropyridine-3-carboxylate (6b)

Method (A): To a solution of compound **1** (3.21 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (1.0 mL), either benzalmalononitrile (1.54 g, 0.01 mol) or ethyl benzalacetate (2.01 g, 0.01 mol) was added. The reaction mixture, in each case was heated under reflux for 3 h then poured onto ice/water and the formed solid product was collected by filtration.

Method (B): To a solution of compound **2a** (3.21 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (1.0 mL) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 6 h the poured onto ice/water and the formed solid product was collected by filtration.

6a. Colorless crystals of mp 188-190 °C (1,4 dioxane). MS (*m/z*, %): 343 (M^+). IR (KBr), vcm^{-1} : 3460, 3387 (NH_2), 3054 (aromatic CH), 2989 (CH_3), 2225, 2221 (2CN), 1690 (CO), 1640 (C=N), 1637 (C=C). ^1H NMR (300 MHz, $\text{DMSO-}d_6$, TMS): 1.05 (s, 3H, CH_3), 4.87 (s, 2H, NH_2), 7.28 -7.41 (m, 8H, C_6H_5 , furan CH). ^{13}C NMR (300 MHz, $\text{DMSO-}d_6$, TMS): 18.8 (CH_3), 116.9, 117.3 (2CN), 111.6, 119.3, 120.4, 121.4, 122.7, 123.0, 126.2, 134.7, 145.6

(furan C, benzene C), 158.7 (C=N), 165.2 (C=O). Anal. Calcd. for C₁₉H₁₃N₅O₂: C, 66.47; H, 3.82; N, 20.40. Found: C, 66.61; H, 3.49; N, 20.69.

6b. Colorless crystals of mp >300 °C (ethanol). MS (*m/z*, %): 390 (M⁺). IR (KBr), vcm⁻¹: 3452, 3376 (NH₂), 3056 (aromatic CH), 2985, 2888 (CH₃, CH₂), 2227, (CN), 1689, 1686 (2CO), 1638 (C=N), 1632 (C=C). ¹H NMR (300 MHz, DMSO-*d*₆, TMS): 1.09 (s, 3H, CH₃), 1.16 (t, 3H, J = 6.79 Hz, CH₃), 4.24 (q, 2H, J = 6.79 Hz, CH₂), 4.79 (s, 2H, NH₂), 7.30–7.45 (m, 8H, C₆H₅, furan CH). ¹³C NMR (300 MHz, DMSO-*d*₆, TMS): 18.8, 20.8 (2CH₃), 62.6 (CH₂), 116.6 (CN), 110.9, 119.0, 120.8, 121.6, 122.9, 123.7, 126.0, 134.9, 144.3 (furan C, benzene C), 158.8 (C=N), 165.0 (C=O). Anal. Calcd. for C₂₁H₁₈N₄O₄: C, 64.08; H, 4.62; N, 14.16. Found: C, 64.61; H, 4.65; N, 14.35.

4,6-Diamino-1-[(1-furan-2-ylethylidene)amino]-2-oxo-1,2-dihydropyridine-3-carbonitrile (7a) and ethyl 4-amino-5-cyano-1-[(1-furan-2-ylethylidene)amino]-6-oxo-1,6-dihydropyridine-2-carboxylate (7b)

Equimolecular amounts of compound **1** (0.01 mol) and either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) in 1,4-dioxane (30 mL) containing triethylamine (1.5 mL) was heated under reflux for 5 h. The reaction mixture was left to cool and evaporated under vacuum. The remaining product was triturated with ethanol and the formed solid product was collected by filtration.

7a. Colorless crystals of mp 202–204 °C (ethanol). MS (*m/z*, %): 257 (M⁺). IR (KBr), vcm⁻¹: 3473, 3351 (2NH₂), 3058 (aromatic CH), 2987 (CH₃), 2223, (CN), 1687 (CO), 1641 (C=N), 1634 (C=C). ¹H NMR (300 MHz, DMSO-*d*₆, TMS): 1.11 (s, 3H, CH₃), 4.81, 4.93 (2s, 4H, 2NH₂), 6.61 (s, 1H, pyridine H-3), 6.82–6.91 (m, 3H, furan CH). ¹³C NMR (300 MHz, DMSO-*d*₆, TMS): 18.6 (CH₃), 116.9 (CN), 112.6, 118.8, 120.3, 121.9, 122.6, 130.2, 134.8, 140.6 (furan C, pyridine C), 159.4 (C=N), 165.8 (C=O). Anal. Calcd. for C₁₂H₁₁N₅O₂: C, 56.03; H, 4.31; N, 27.22. Found: C, 55.84; H, 4.33; N, 26.81.

7b. Colorless crystals of mp 176–177 °C (ethanol). MS (*m/z*, %): 258 (M⁺). IR (KBr), vcm⁻¹: 3530, 3380 (OH, NH₂), 3053 (aromatic CH), 2989 (CH₃) 2221, (CN), 1686 (CO), 1640 (C=N), 1631 (C=C). ¹H NMR (300 MHz, DMSO-*d*₆, TMS): 1.13 (s, 3H, CH₃), 4.83 (s, 2H, NH₂), 6.48 (s, 1H, pyridine H-3), 6.80–6.93 (m, 4H, furan CH), 10.25 (s, 1H, OH). ¹³C NMR (300 MHz, DMSO-*d*₆, TMS): 18.2 (CH₃), 116.5 (CN), 112.9, 119.1, 120.6, 122.3, 122.9, 130.7, 140.3, 144.8 (furan C, pyridine C), 159.6 (C=N), 165.3 (C=O). Anal. Calcd. for C₁₂H₁₀N₄O₃: C, 55.81; H, 3.90; N, 21.70. Found: C, 55.63; H, 4.07; N, 21.82.

3,5-Diamino-4-cyano-N'-(1-furan-2-ylethylidene)thiophene-2-carbohydrazide (8a), ethyl 2,4-diamino-5-[[2-(1-furan-2-ylethylidene)hydrazino]carbonyl]thiophene-3-carboxylate (8b) and 3-amino-N'-(1-furan-2-ylethylidene)-4,5,6,7-tetrahydro-1-benzothiophene-2-carbohydrazide (9)

General procedure: To a solution of compound **1** (3.21 g, 0.01 mol) in absolute ethanol (50 mL) containing triethylamine (1.0 mL) either malononitrile (0.66 g, 0.01 mol), ethyl cyanoacetate (1.13 g, 0.01 mol) or cyclohexanone (0.98 g, 0.01 mol) together with elemental sulfur (0.32 g, 0.01 mol) were added. The whole reaction mixture was heated

under reflux for 1 h then poured onto ice/water mixture and the formed solid product, in each case, was collected by filtration.

8a. Colorless crystals of mp >300 °C (ethanol). MS (m/z , %): 289 (M^+). IR (KBr), $\nu_{cm^{-1}}$: 3462, 3326 (2NH₂, NH), 3056 (aromatic CH), 2978 (CH₃) 2226 (CN), 1683 (CO), 1644 (C=N), 1635 (C=C). ¹H NMR (300 MHz, DMSO-*d*₆, TMS): 1.08 (s, 3H, CH₃), 4.80, 4.93 (2s, 4H₂NH₂), 6.78-6.90 (m, 4H, furan CH), 8.1 (s, 1H, NH). ¹³C NMR (300 MHz, DMSO-*d*₆, TMS): 18.7 (CH₃), 115.9 (CN), 110.2, 112.5, 116.9, 120.5, 124.4, 132.7, 134.9, 140.4 (furan, thiophene C), 156.9 (C=N), 165.6 (C=O). Anal. Calcd. for C₁₂H₁₁N₅O₂S: C, 49.82; H, 3.83; N, 24.21; S, 11.08. Found: C, 49.67; H, 3.59; N, 24.07; S, 10.83.

8b. Colorless crystals of mp 209–211 °C (ethanol). MS (m/z , %): 336 (M^+). IR (KBr), $\nu_{cm^{-1}}$: 3462, 3326 (2NH₂, NH), 3056 (aromatic CH), 2978 (CH₃) 2880 (CH₂), 2226 (CN), 1683 (CO), 1644 (C=N), 1635 (C=C). ¹H NMR (300 MHz, DMSO-*d*₆, TMS): 1.07 (s, 3H, CH₃), 1.14 (t, 3H, J = 7.36 Hz, CH₃), 4.22 (q, 2H, J = 7.36 Hz, CH₂), 4.79, 4.87 (2s, 4H, 2NH₂), 6.63-6.85 (m, 3H, furan CH), 8.22 (s, 1H, NH). ¹³C NMR (300 MHz, DMSO-*d*₆, TMS): 18.3, 24.8 (2CH₃), 62.6 (CH₂), 110.8, 112.2, 116.6, 120.4, 124.4, 132.9, 134.7, 140.4 (furan, thiophene C), 156.6 (C=N), 165.6, 166.0 (2C=O). Anal. Calcd. for C₁₄H₁₆N₄O₄S: C, 49.99; H, 4.79; N, 16.66; S, 9.53. Found: C, 49.85; H, 4.33; N, 16.27; S, 9.71.

9. Colorless crystals of mp 190–191 °C (ethanol). MS (m/z , %): 303 (M^+). IR (KBr), $\nu_{cm^{-1}}$: 3459, 3421 (NH₂, NH), 3049 (CH aromatic), 2988, 2876 (CH₃, CH₂), 1686 (CO), 1644 (C=N), 1632 (C=C). ¹H NMR (300 MHz, DMSO-*d*₆, TMS): 1.11 (CH₃), 2.21-2.26 (m, 4H, 2CH₂), 2.30-2.36 (m, 4H, 2CH₂), 4.78 (s, 2H, NH₂), 6.48-6.82 (m, 3H, furan CH), 8.01 (s, 1H, NH). ¹³C NMR (300 MHz, DMSO-*d*₆, TMS): 18.6 (CH₃), 20.3, 123.5, 124.2 (4CH₂), 120.5, 122.3, 124.6, 126.8, 134.2, 140.6 (thiophene, furan C), 156.3 (C=N), 165.9 (C=O). Anal. Calcd. for C₁₅H₁₇N₃O₂S: C, 59.38; H, 5.65; N, 13.85; S, 10.57. Found: C, 59.26; H, 5.52; N, 13.55; S, 10.33.

4-amino-N'-(1-furan-2-ylethylidene)-3-phenyl-2-thioxo-2,3-dihydro-1,3-thiazole-5-carbohydrazide (10)

To a solution of compound **1** (3.21 g, 0.01 mol) in absolute ethanol (30 mL) containing triethylamine (1.0 mL) and elemental sulfur (0.32 g, 0.01 mol), phenylisothiocyanate (1.30 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then left to cool. The solid product formed upon pouring onto ice/water was collected by filtration.

10. Colorless crystals of mp 160 °C (acetic acid). MS (m/z , %): 358 (M^+). IR (KBr), $\nu_{cm^{-1}}$: 3466, 3329 (NH₂, NH), 3020 (CH aromatic), 2986 (CH₃), 1644 (C=N), 1629 (C=C), 1205-1196 (C=S). ¹H NMR (300 MHz, DMSO-*d*₆, TMS): 1.13 (CH₃), 4.70 (s, 2H, NH₂), 6.49-6.77 (m, 3H, furan CH), 7.29-7.35 (m, 5H, C₆H₅), 8.11 (s, 1H, NH). ¹³C NMR (300 MHz, DMSO-*d*₆, TMS): 18.5 (CH₃), 73.9 (thiazole C-5), 115.6, 118.3, 120.9, 122.4, 126.8, 128.4, 130.5, 133.5, 140.1 (furan C, thiazole C-6, benzene C), 156.7 (C=N), 166.1 (C=O), 188.0 (C=S). Anal. Calcd. for C₁₆H₁₄N₄O₂S₂: C, 53.61; H, 3.94; N, 15.63; S, 17.89. Found: C, 53.28; H, 3.55; N, 15.73; S, 17.57.

Antimicrobial Activity

The *in vitro* antimicrobial activity of the newly heterocyclic derivatives against two strains of Gram positive bacteria (*Bacillus subtilis* CECT 498 and *Bacillus cereus* CECT 148), one

strain of Gram negative bacteria (*Escherichia coli* ECT 101) and *Candida albicans* CECT 1394 as a representative species of fungi was investigated. The newly synthesized products were dissolved in aqueous ethanol to give a logarithmic series of concentrations from 2 to 256 mg/L upon tenfold dilution with the growth medium and spore suspension of the test fungi. The toxicity of compounds was determined via a pipette additions into the wells of multi-well slides, followed with 25 μL of the culture medium. The incubated slides were then incubated at 25 °C until short germ tubes appeared; approximately 50 μm in length (at 0 h) was measured. Five μL volumes of the prepared compound test solutions were added to the incubated wells, one control well on each slide being treated with solvent only. The slides were then returned to the incubator until germ tubes 400 μm (\pm 50 μm) long were visible in the control wells. Further growth was arrested by the addition of lactophenol aniline blue to each of the wells. The minimal inhibitory concentration (MIC in $\mu\text{g mL}^{-1}$) was determined using an adaptation of agar streak dilution method based on radial diffusion. Under the same conditions ampicillin (antibacterial) and cycloheximide (antifungal) were used as standards. The MIC was considered to be the lowest concentration of the tested compound (in dimethylformamide) which inhibits growth of bacteria or fungi on the plate (Table 1). The diameters of the inhibition zones corresponding to the MICs are also presented in Table 1.

Tab. 1. Antimicrobial activities of the tested compounds

Comp. No.	MIC ($\mu\text{g mL}^{-1}$) (zone of inhibition, mm)			
	<i>E. coli</i>	<i>B. cereus</i>	<i>B. subtilis</i>	<i>C. albicans</i>
1	12.50 (6)	22.52 (8)	20.55 (4)	8.65 (4)
2a	–	6.05 (15)	21.01 (8)	33.23 (6)
2b	–	11.32 (3)	18.22 (8)	12.40 (4)
2c	–	12.30 (4)	3.72 (6)	16.58 (12)
3	–	22.38 (8)	22.83 (6)	24.18 (3)
4a	–	19.15 (4)	20.46 (9)	0.40 (10)
4b	16.45(6)	18.23 (5)	22.45 (8)	40.18 (6)
4c	–	18.12 (5)	12.25 (2)	23.64 (6)
6a	–	10.05 (6)	16.42 (2)	4.55 (10)
6b	–	0.03 (9)	4.13 (10)	0.61 (6)
7a	–	18.24 (7)	6.18 (4)	0.40 (5)
7b	–	6.25 (15)	22.01 (8)	30.23 (6)
8a	–	16.22 (3)	18.32 (8)	14.40 (4)
8b	–	10.38 (4)	6.22 (6)	12.55 (12)
9	–	25.36 (8)	23.63 (6)	26.12 (3)
10	–	21.15 (4)	23.16 (9)	38.15 (4)
Ampicillin	6.25 (10)	3.13 (15)	12.50 (10)	–
Cycloheximide	–	–	–	12.50 (15)

Authors' Statement

Competing Interests

The authors declare no conflict of interest.

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