

Synthesis, Structure, and Antibacterial Evaluation of New *N*-Substituted-3-Amino-5-oxo-4-phenyl-2,5-dihydro-1*H*-pyrazole-1-carbothioamides

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ABSTRACT: Novel *N*-substituted-3-amino-5-oxo-4-phenyl-2,5-dihydro-1*H*-pyrazole-1-carbothioamide derivatives were synthesized by means of two methods. First is the cyclization reaction of 1-(cyanophenyl)acetyl-4-substituted thiosemicarbazide, and the second one is reaction of cyanophenyl acetic acid hydrazide with isothiocyanate. Structures of new compounds were confirmed by elemental analysis, ¹H NMR, and X-ray diffraction analysis. Biological evaluation showed that some of them possess promising antibacterial activities. © 2010 Wiley Periodicals, Inc. *Heteroatom Chem* 21:215–221, 2010; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.20598

INTRODUCTION

Nitrogen heterocycles are of synthesis interest because they constitute an important class of natural

and nonnatural products. The cyclization reaction of suitable linear compounds is one of the most common and popular methods for preparing these derivatives. Some asymmetrical ureas have been cyclized to produce several heterocycles, such as 1,3,4-thiadiazoles, 1,2,4-triazoles, and 1,3,5-triazines. The cyclization of 2,4-disubstituted thiosemicarbazides has been shown to be an excellent strategy for the synthesis of pyrazoles [1,2]. Much attention has been focused on these compounds as antiviral and anticancer agents after the discovery of the pyrazole C-glycoside pyrazofurin [3]. In addition, pyrazoles have a broad spectrum of biological activities, such as inflammatory [4], antipyretic [5], antidepressant [6], anticonvulsant [7], antifilarial agents [8], and antibacterial [9,10]. Various substituted pyrazoles, like 4-acyl-5-hydroxy-3-methyl-1*H*-pyrazole (or its tautomers), are used as the chelating and extracting reagents for many metal ions [11] and as starting materials for the synthesis of biologically active compounds as well as for the construction of condensed heterocyclic systems.

In view of these observations and in continuation of our research program on the synthesis of

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five-membered heterocyclic compounds [12–14], we present herein the synthesis, characterization, and the antibacterial evaluation of representative compounds of *N*-substituted-3-amino-5-oxo-4-phenyl-2,5-dihydro-1*H*-pyrazole-1-carbothioamides.

RESULTS AND DISCUSSION

Chemistry

The synthesis of 1-(cyanophenyl)acetyl-4-substituted thiosemicarbazide and *N*-substituted-3-amino-5-oxo-4-phenyl-2,5-dihydro-1*H*-pyrazole-1-carbothioamide derivatives is outlined in Schemes 1 and 2. Compound **1** was prepared by means of a known procedure in the reaction of corresponding ethyl cyanophenyl acetate with 80% hydrazine hydrate, at the room temperature, giving cyanophenyl acetic acid hydrazide **1** [15]. It was used as a key intermediate for the synthesis of final compounds. The treatment of compound **1** with ethyl and 4-methoxyphenyl isothiocyanate gave corresponding 1-(cyanophenyl)acetyl-4-substituted thiosemicarbazide **2a**, **2b**. The reaction was carried out by heating substrates in an oil bath. The conditions of the reactions were established experimentally. The cyclization of these compounds in alkaline **2a** and acid medium **2b** gave a new group of *N*-substituted-3-amino-5-oxo-4-phenyl-2,5-dihydro-1*H*-pyrazole-1-carbothioamide derivatives **3a**, **3b** (Scheme 1).

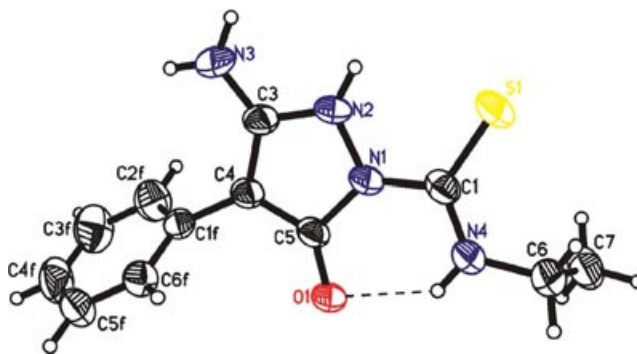
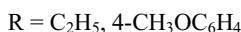
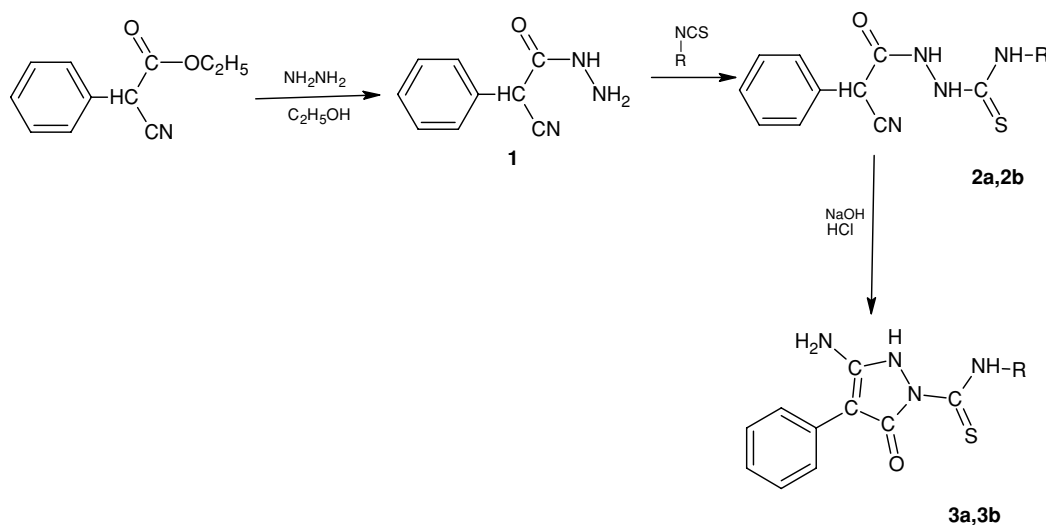


FIGURE 1 The molecular structure of compound **3a** with the atom-labeling scheme. The displacement ellipsoids are drawn at the 50% probability level.

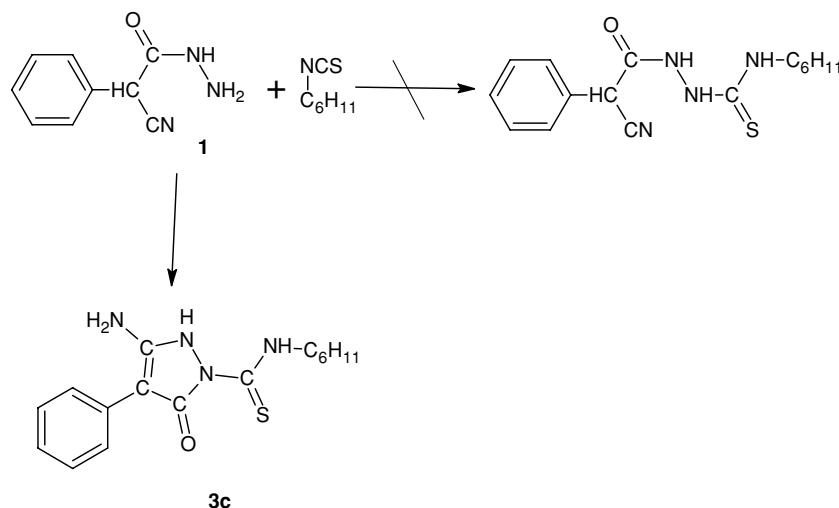
In the case of cyclohexyl isothiocyanate, we also expected to obtain thiosemicarbazide but the reaction went quite different. During this reaction, *N*-cyclohexyl-3-amino-5-oxo-4-phenyl-2,5-dihydro-1*H*-pyrazole-1-carbothioamide **3c** was obtained. The reaction led to cyclic compounds without the separation of mediate product (Scheme 2). The mechanism of the reaction will be further investigated.

Structure

Figure 1 shows the molecular structure of compound **3a**, together with the atomic numbering scheme



SCHEME 1



SCHEME 2

used. Selected bond distances and torsion angles are given in Table 1.

The structural research indicates that the compound **3a** adopts the keto-thione form. The main structural parameters of the molecule are similar to those found in closely related 5-oxo-1*H*-pyrazole-1-carbothioamide derivatives [16–19] in particular; the S atom of the carbothioamide group is *cis* oriented with respect to the protonated endocyclic N2 atom. This arrangement enables the formation of short intramolecular N4–H1n4...O1 hydrogen bond, which additionally stabilizes the conformation of the molecule. The central pyrazolone fragment in **3a** is essentially planar, with maximum mean plane deviation of 0.035(4) Å for atom N2, and it is coplanar with the amine N3 atom and the carbothioamide group, with an angle of 1.4(3)° between carbothioamide and pyrazole ring planes. Moreover, the flat thioamide group is conjugated with pyrazole ring, as evidenced by shortening of the N1–C5 and N1–C1 bonds in comparison with average values from

Cambridge Structural Database (CSD) [20]. However, the whole molecule is not planar; the pyrazole part forms dihedral angle of 61.3(2)° with the phenyl C1f–C6f ring plane, whereas the ethyl C7 atom is *-sc* oriented with respect to the thioamide C1 atom, as indicated by the torsion angle C1–N4–C6–C7 (Table 1). In the crystal structure, molecules of **3a** are linked by short intermolecular N2–H2/N3–H3a...O1 and C7–H7c...S1 hydrogen bonding interactions (Table 2) into one-dimensional extended chains along the *a* axis (Fig. 2).

Centrosymmetrically related chains are further stabilized by weak C6–H6a...O1 hydrogen bonds and aromatic π – π stacking interactions occurring between adjacent phenyl rings. The details of the crystal data, X-ray data collection, and refinement are given in Table 3.

Biological Activity

The newly synthesized compounds were screened for their antimicrobial activity against 10 reference strains of aerobic bacteria: 6 Gram-positive (*Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 6538, *S. epidermidis* ATCC 12 228, *Bacillus subtilis* ATCC

TABLE 1 Selected Bond Lengths (Å) and Torsion Angles (°)

Bond lengths (Å)			
N1–N2	1.387 (2)	C5–O1	1.244 (2)
N2–C3	1.344 (3)	N1–C1	1.381 (2)
C3–C4	1.387 (2)	C1–S1	1.667 (2)
C4–C5	1.406 (3)	C1–N4	1.321 (2)
N1–C5	1.412 (2)	N4–C6	1.452 (3)
N3–C3	1.333 (3)	C6–C7	1.500 (3)
Torsion angles (°)			
N2–N1–C1–N4	–179.3 (2)	S1–C1–N4–C6	–2.3 (3)
N2–N1–C1–S1	1.8 (2)	C1–N4–C6–C7	–87.1 (2)
N1–C1–N4–C6	178.9 (2)		

TABLE 2 Hydrogen-Bonding Geometry (Å, °)

	D–H	H...A	D...A	<DHA
N4–H1n4...O1	0.84	2.06	2.734 (3)	137
N2–H2...O1 ⁱ	0.86	2.28	2.928 (3)	133
N3–H3a...O1 ⁱ	0.86	2.46	3.119 (3)	134
C7–H7c...S1 ⁱⁱ	0.96	2.83	3.578 (3)	136
C6–H6a...O1 ⁱⁱⁱ	0.92	2.67	3.475 (3)	141

Symmetry codes: (i) $x - 1, y, z$; (ii) $x + 1, y, -z$; (iii) $2 - x, -y, 2 - z$

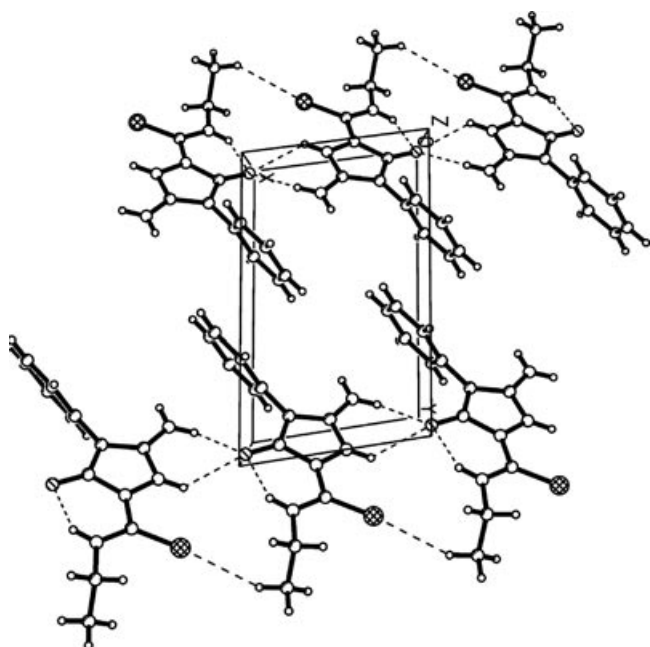


FIGURE 2 The packing arrangement of molecules, viewed along the *c* axis. Dashed lines indicate hydrogen bonds.

TABLE 3 Crystal Data and Structure Refinement for **3a**

Empirical formula	C ₁₂ H ₁₄ N ₄ OS
CCDC number	719663
Formula weight	262.33
Wavelength	0.71073
Crystal system	Triclinic
Space group	<i>P</i> -1
Unit cell dimensions	6.276 (3)
<i>a</i> , <i>b</i> , <i>c</i> (Å)	10.204 (4)
	10.513 (6)
α , β , γ (°)	79.01 (5)
	78.48 (5)
	79.96 (4)
<i>V</i> (Å ³)	641.1 (5)
<i>Z</i>	2
Density (calculated)	1.359
(g cm ⁻³)	
Crystal size (mm)	0.58 × 0.38 × 0.18
Absorption coefficient	0.246
(mm ⁻¹)	
<i>F</i> (000)	276
Index ranges	−8 ≤ <i>h</i> ≤ 8, −14 ≤ <i>k</i> ≤ 14, 0 ≤ <i>l</i> ≤ 14
Independent reflections	3761 [<i>R</i> (int) = 0.024]
Reflections with <i>I</i> > 2σ(<i>I</i>)	2236
Data/restraints/parameters	3761/0/167
GOOF	1.002
completeness to $\theta_{\max}$ =	0.998
30.07°	
Final <i>R</i> indices [<i>I</i> > 2σ(<i>I</i>)]	<i>R</i> ₁ = 0.0477, <i>wR</i> ₂ = 0.1203
<i>R</i> indices (all data)	<i>R</i> ₂ = 0.0976, <i>wR</i> ₂ = 0.1423
Largest diff. peak and hole	−0.37; 0.35
(e Å ⁻³)	

TABLE 4 Antibacterial activity of compounds **3a** and **2b** assessed by agar dilution method (adm) and broth microdilution method (bmm)

Bacterial strains	MIC (mg L ⁻¹)			
	3a		2b	
	adm	bmm	adm	bmm
<i>Staphylococcus aureus</i> ATCC 25923	7.82	15.63	62.5	62.5
<i>S. aureus</i> ATCC 6538	7.82	31.25	125	62.5
<i>S. epidermidis</i> ATCC 12228	7.82	7.82	62.5	125
<i>Bacillus subtilis</i> ATCC 6633	7.82	3.91	62.5	62.5
<i>B. cereus</i> ATCC 10876	7.82	31.25	62.5	62.5
<i>Micrococcus luteus</i> ATCC 10240	7.82	15.63	62.5	31.25
<i>Escherichia coli</i> ATCC 25922	250	62.5	>1000	>1000
<i>Klebsiella pneumoniae</i> ATCC 13883	250	250	>1000	>1000
<i>Proteus mirabilis</i> ATCC 12453	250	250	>1000	>1000
<i>Pseudomonas aeruginosa</i> ATCC 9027	1000	1000	>1000	>1000

6633, *B. cereus* ATCC 10876, *Micrococcus luteus* ATCC 10240) and 4 Gram-negative (*Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 12453, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 9027). The *in vitro* antimicrobial activities of substances at concentrations ranging from 3.91 to 1000 mg L⁻¹ were preliminary tested. Using the agar dilution method, it was found that compound **3a** possessed good activity against all tested Gram-positive bacteria with minimal inhibitory concentration (MIC) = 7.82 mg L⁻¹; the growth of Gram-negative bacteria was inhibited by much higher concentration with MIC = 250 mg L⁻¹ to 1000 mg L⁻¹ (Table 4).

Compound **2b** also showed good activity against all Gram-positive bacteria but with MIC values higher than those noted in the case of **3a** compound; no inhibitory effect of this compound was found against the tested Gram-negative bacteria (MIC > 1000 mg L⁻¹). Compound **3c** (data not shown) inhibited growth of Gram-positive bacteria with MIC = 500–1000 mg L⁻¹, being without the effect on growth of Gram-negative bacteria (MIC > 1000 mg L⁻¹). In our experiments, MICs of available antibiotics such as ampicillin, cefuroxime, and gentamicin, which have been extensively used to treat bacterial infections, were also estimated. The range of MICs of ampicillin was 0.03–0.06 mg L⁻¹.

TABLE 5 Antibacterial activity of compound **3a** against clinical isolates of *Staphylococcus aureus* assessed by broth microdilution method

Parameter	MIC (mg L ⁻¹)
Range of MIC (number of isolates)	15.63 (2) 31.25 (6) 62.50 (2)
Mode of MIC	31.25

for *Staphylococcus* species, 0.03–62.5 mg L⁻¹ for the other Gram-positive bacteria, and 7.81–125 mg L⁻¹ for Gram-negative *Enterobacteriaceae* rods; the range of MICs of cefuroxime was 0.049–1.95 mg L⁻¹ for all tested Gram-positive reference strains and 0.049–7.82 mg L⁻¹ for *Enterobacteriaceae* species; the range of MICs of gentamicin for *Staphylococci* was 0.015–0.06 mg L⁻¹, 0.015–0.49 mg L⁻¹, for other Gram-positive species, and 0.24–0.98 mg L⁻¹ for reference Gram-negative rods. The good antibacterial activities of **3a** and **2b** compounds were confirmed by using broth microdilution method. The difference among the MIC values determined by the two kinds of methods (the agar dilution procedure and broth microdilution test) is in accordance with the results communicated by other authors [21–23]. Moreover, it was found that compound **3a** inhibited the growth of 10 clinical isolates of *S. aureus* with MIC ranging from 15.63 to 62.5 mg L⁻¹; the mode MIC value was 31.25 mg L⁻¹ (Table 5).

The compound **3a** appears to be a promising precursor of agents with antibacterial activity against Gram-positive bacteria, both pathogenic (including *Staphylococcus* species) as well as opportunistic (e.g., *Micrococcus luteus*) [24]. Although this bacterial species is nonpathogenic and usually regarded as a contaminant, it should be considered as an emerging nosocomial pathogen in immunocompromised individuals causing recurrent bacterium, septic arthritis, and pulmonary diseases. The investigations on that field will be continued.

SUMMARY

The synthesis of *N*-substituted-3-amino-5-oxo-4-phenyl-2,5-dihydro-1*H*-pyrazole-1-carbothioamide derivatives **3a–3c** has been described in this paper. These compounds were obtained from appropriate thiosemicarbazides and 1-(cyanophenyl)acetic acid hydrazide. The final compound **3a** was confirmed by X-ray crystallographic studies. Selected compounds were screened for their *in vitro* antibacterial activity. These results showed that some of them possess promising antibacterial activities.

EXPERIMENTAL

All chemicals were purchased from Merck Co. or Lancaster (Gdańsk, Poland) and used without further purification. Melting points (°C) were determined in a Fisher–Johns block and were not corrected. Elemental analysis was made on Perkin–Elmer 2400 CHN analyzer, and the data were within ±0.4% of the theoretical values. ¹H and ¹³C NMR spectra were recorded on a Bruker AC 200F instrument (300 MHz) in DMSO-*d*₆ with TMS as internal standard. IR spectra were recorded in KBr disk using a Specord IR-75 spectrophotometer. The mass spectra were obtained with an AMD-604 mass spectrometer a 70-eV electron beam. The purity of obtained compounds was checked by TLC on aluminum oxide 60 F₂₅₄ plates (Merck) in a CHCl₃/C₂H₅OH (10:1 and 10:2) solvent system with UV or iodine visualization.

Synthesis of 1-(Cyanophenyl)acetyl-4-substituted Thiosemicarbazide (**2**)

Cyanophenyl acetic acid hydrazide **1** 1.7 g (10 mmol) and appropriate isothiocyanate (10 mmol) were heated in an oil bath at 110°C for 20 h. The formed product was washed with diethyl ether to remove the unreacted isothiocyanate, dried, and crystallized from ethanol.

1-(Cyanophenyl)acetyl-4-ethylthiosemicarbazide, 2a. Yield: 73%, mp 304–305°C. ¹H NMR (DMSO-*d*₆) δ: 1.19 (t, 3H, *J* = 7.0 Hz, CH₃), 3.62 (q, 3H, *J* = 6.8 CH₂), 5.92 (s, 1H, CH), 7.02–7.70 (m, 4H, CH_{arom}), 9.16, 10.49, 11.02 (3s, 3H, 3NH) ppm. ¹³C NMR (DMSO-*d*₆) δ: 13.01 (CH₃), 85.87 (CH₂), 122.45 (CH), 124.68, 126.74 (CH_{arom}), 127.66 (C_{arom}), 132.26 (CN), 153.51 (C=O), 166.84 (C=S) ppm. IR (KBr) ν: 3486, 3253, 3149 (NH), 2258 (CN), 1647 (C=O), 1181 (C=S) cm⁻¹. MS (EI) *m/z* (%): 262 (87) [M⁺], 175 (100).

1-(Cyanophenyl)acetyl-4-(4-methoxyphenyl)thiosemicarbazide, 2b. Yield: 71%, mp 184–185°C. ¹H NMR (DMSO-*d*₆) δ: 3.32 (s, 3H, CH₃), 5.00 (s, 1H, CH), 6.80–7.50 (m, 9H, CH_{arom}), 8.73, 9.49, 10.48 (3s, 3H, 3NH) ppm. ¹³C NMR (DMSO-*d*₆) δ: 53.75 (CH₃), 85.87 (CH), 122.44, 124.70, 126.71 (CH_{arom}), 132.23 (C_{arom}), 139.20 (CN), 153.60 (C=O), 166.70 (C=S) ppm. IR (KBr) ν: 3485, 3305, 3146 (NH), 2111 (CN), 1647 (C=O), 1153 (C=S) cm⁻¹. MS (EI) *m/z* (%): 324 (6) [M⁺], 91 (100).

Synthesis of *N*-Ethyl-3-amino-5-oxo-4-phenyl-2,5-dihydro-1*H*-pyrazole-1-carbothioamide **3a**

The thiosemicarbazide derivative **2a** (10 mmol) was dissolved in 2% NaOH (15 mL) and refluxed for 2 h.

After cooling, the solution was neutralized with 3 M HCl. The solid formed was filtered, dried, and crystallized from ethanol. Yield: 68%, mp 210–211°C. ^1H NMR (DMSO- d_6) δ : 1.19 (t, 3H, $J = 7.0$, CH_3), 3.60 (q, 2H, $J = 5.0$, CH_2), 6.74 (s, 2H, NH_2), 7.11–7.55 (m, 5H, CH_{arom}), 10.48 (s, 1H, 1H NH), 11.02 (t, 1H, $J = 5.0$, NH) ppm. ^{13}C NMR (DMSO- d_6) δ : 12.36 (CH_3), 84.90 (CH_2), 85.95 ($\text{C}=\text{C}$), 123.51, 124.82, 126.66, 127.32 (CH_{arom}), 130.08 (C_{arom}), 131.91 (CN), 152.74 ($\text{C}=\text{O}$), 160.58 ($\text{C}=\text{S}$), 173.02 ($\text{C}-\text{NH}_2$) ppm. IR (KBr) ν : 3479, 3311, 3153 (NH), 2122 (CN), 1647 ($\text{C}=\text{O}$), 1153 ($\text{C}=\text{S}$) cm^{-1} . MS (EI) m/z (%): 262 (40) [M^+], 175 (100).

Synthesis of N-(4-Methoxyphenyl)-3-amino-5-oxo-4-phenyl-2,5-dihydro-1H-pyrazole-1-carbothioamide 3b

The thiosemicarbazide derivative **2b** (10 mmol) was dissolved in 3 N HCl (15 mL) and refluxed for 2 h. After cooling, the formed precipitate was filtered off and then crystallized from ethanol. Yield: 70%, mp 217–218°C. ^1H NMR (DMSO- d_6) δ : 2.51 (s, 3H, CH_3), 6.80 (s, 2H, NH_2), 6.95–7.70 (m, 9H, CH_{arom}), 10.55 (s, 1H, NH), 11.42 (t, 1H, $J = 6.0$, NH) ppm. ^{13}C NMR (DMSO- d_6) δ : 14.86 (CH_3), 91.40 (C_4), 91.40 ($\text{C}=\text{C}$), 118.41, 129.05, 130.65 (CH_{arom}), 153.72 (C_{arom}), 156.42 ($\text{C}=\text{O}$), 163.44 ($\text{C}=\text{S}$), 168.57 ($\text{C}-\text{NH}_2$) ppm. IR (KBr) ν : 3485, 3321, 3149 (NH), 1653 ($\text{C}=\text{O}$), 1151 ($\text{C}=\text{S}$) cm^{-1} . MS (EI) m/z (%): 337 (25) [M^+], 175 (100).

Synthesis of N-Cyclohexyl-3-amino-5-oxo-4-phenyl-2,5-dihydro-1H-pyrazole-1-carbothioamide 3c. Cyanophenyl acetic acid hydrazide **1** 1.7 g (10 mmol) and cyclohexyl isothiocyanate (10 mmol) in 10 mL of anhydrous diethyl ether was kept at room temperature for 24 h. Then the formed compound was filtered off, washed with diethyl ether, and crystallized from ethanol. Yield: 65%, mp 223–225°C. ^1H NMR (DMSO- d_6) δ : 1.03–1.79 (m, 10H, $5 \times \text{CH}_2$), 4.07 (s, 1H, CH), 5.91 (s, 2H, NH_2), 7.69–7.61 (m, 5H, CH_{arom}), 9.06 (s, 1H, NH), 9.16 (s, 1H, NH) ppm. ^{13}C NMR (DMSO- d_6) δ : 23.22, 23.74, 30.77, 31.02 ($5 \times \text{CH}_2$), 51.03 (CH), 75.38 (C_4), 121.83, 122.33, 123.82, 126.67, 127.53 (CH_{arom}), 132.35 (C_{arom}), 153.53 ($\text{C}=\text{O}$), 166.80 ($\text{C}=\text{S}$), 78.83 ($\text{C}-\text{NH}_2$) ppm. IR (KBr) ν : 3484, 3239, 2927 (NH), 1644 ($\text{C}=\text{O}$), 1177 ($\text{C}=\text{S}$) cm^{-1} . MS (EI) m/z (%): 314 (5), 175 (100).

Crystallographic Measurement

Single-crystal diffraction data were measured at room temperature in the $\omega/2\theta$ mode on the Oxford Diffraction Xcalibur diffractometer using the graphite-monochromated Mo K_α radiation. The

stability of intensities was monitored by measurements of three standards every 100 reflections. Crystal structure was solved by direct methods using SHELXS97 [25] and refined by the full-matrix least squares on F^2 using the SHELXL97 [26]. All non-hydrogen atoms were refined with anisotropic displacement parameters. The hydrogen atom bonded to N4 was located from a difference Fourier map and was refined isotropically. The remaining hydrogen atoms were positioned geometrically and allowed to ride on their parent atoms, with $U_{\text{iso}}(\text{H}) = 1.2 U_{\text{eq}}(\text{C}, \text{N})$.

Supplementary Material

The CCDC-719663 for **3a** contains the supplementary crystallographic data for this paper. The data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0) 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk.

Antimicrobial Screening

Fresh bacterial suspensions with an optical density of 0.5 McFarland standard [150×10^6 CFU (colony forming units)/mL], were prepared in sterile saline (0.85% NaCl) and then were diluted 1:100 in the Mueller–Hinton broth. All stock solutions of the assayed compounds were prepared in dimethyl sulfoxide (DMSO) mixed with distilled water (1:1). It was found that DMSO at the final concentration in the medium had no influence on growth of the tested microorganisms. Ampicillin, cefuroxim, and gentamicin at the 0.0075–500 mg L^{-1} concentration were used as control antibacterial agents. The agar dilution procedure was performed using the Mueller–Hinton agar as the base medium to which serial dilutions of the tested compounds at concentrations from 1.96 to 1000 mg L^{-1} were added. The plates were poured on the day of testing. 10 μL of each bacterial suspension was put onto the Mueller–Hinton agar, containing the tested compounds; medium without the compounds was used as a control. The plates were incubated at 37°C for 18 h. The MIC was defined as the lowest concentration of the tested compounds that completely inhibited visible growth of bacteria. Antibacterial activity of the compounds was also determined spectrophotometrically by microdilution method with the Mueller–Hinton broth, containing from 0.49 to 1000 mg L^{-1} of the tested compounds. Two-fold dilutions of the tested compounds in the medium in the wells of the microdilution plates were prepared, and then each well

was inoculated with bacterial suspension with an optical density of 0.5 McFarland standard diluted 1:100 in the Mueller–Hinton broth (200 μ L of total volume per well). Twenty species of bacteria—the described above 10 reference strains of Gram-positive or Gram-negative microorganisms and 10 clinical isolates of *S. aureus*, were used in this period of our experiments. After incubation (37°C for 18 h), the optical density (OD₆₀₀) measurements were determined for bacterial culture in broth medium, and the MIC value were determined by comparison with the growth of control (compound-free) medium.

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