Serpil Demirci*

Synthesis and biological activity studies of some new hybrid compounds derived from antipyrine

DOI 10.1515/hc-2016-0002
Received January 10, 2016; accepted February 17, 2016

Abstract: *N*-Benzyl-*N'*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)urea **(1)** was obtained from antipyrine. The reaction of **1** with ethyl bromoacetate produced the 1,3-oxazole derivative **2**. Compounds **5a–c** were obtained from antipyrine by three steps via intermediary of the ester **3** and hydrazide **4**. The microwave supported cyclocondensation of **5a–c** with 4-chlorophenacyl bromide and ethyl bromoacetate afforded the corresponding 1,3-thiazoles **6** or 1,3-thia(oxa)zolidines **7**. The intramolecular cyclization of **5a–c** in the presence of NaOH produced the corresponding triazoles **8a–c**. The synthesis of the hybrid compound **9** containing a penicillin skeleton was carried out by the treatment of **8a** with (+)-6-aminopenicillanic acid (6-apa) in the presence of formaldehyde. The structural assignments of new compounds were based on their elemental analysis and spectral (IR, ^{1}H-NMR, ^{13}C-NMR and LC-MS) data. All compounds except **1** and **7b** show moderate antimicrobial activity.

Keywords: antimicrobial activity; antipyrine; Mannich reaction; microwave.

Introduction

The development of drug resistance is becoming a worldwide concern with rapid increases in multidrug resistant bacteria [1, 2]. Literature survey has revealed that more than one-third of the world populations are infected by bacterial pathogens and nearly two million people per year die due to these infections [3]. Therefore, the design and synthesis of new and potent antibacterial agents without cross resistance with the present antibacterial agents is a crucial task for the effective treatment of bacterial infections. A molecule that includes more than one pharmacophore, each with a different mode of action, could be beneficial for the treatment of microbial infectious [4–6].

Antipyrine (AP) was first synthesized by Knorr [7] in 1883 and there has been a continuous interest in the studies of antipyrine derivatives (APDs). Broad bioactivities of APDs have been investigated including antitumor [8], antimicrobial [9], antiviral [10], analgesic, and anti-inflammatory effects [11]. APDs have been accepted as important model compounds in the biological systems [12].

A triazole scaffold is a lead structure for the synthesis of antimicrobial agents. It has been reported that the primary structural requirement for the antimicrobial azole class is a weakly basic imidazole or triazole ring bonded by a nitrogen-carbon linkage to the rest of the structure [13]. Selected antifungal agents of this type are given in Figure 1 [14].

This report deals with the synthesis of novel thiourea and 1,2,4-triazol derivatives that incorporate antipyrine moiety, The newly synthesized compounds were evaluated as potential antimicrobial agents against Gram positive and Gram negative bacteria and fungi [15, 16].

Results and discussion

The synthetic chemistry is outlined in Scheme 1. The treatment of antipyrine with benzyl isocyanate produced the urea derivative **1**. The synthesis of compound **2** was achieved by condensation of compound **1** and ethyl bromoacetate. Product **2** is a hybrid molecule containing antipyrine and a 1,3-oxazole ring. The synthesis of compound **3** was performed by the reaction of antipyrine with ethyl bromoacetate in tetrahydrofuran in the presence of triethylamine at room temperature. Then, compound **3** was heated under reflux with hydrazine hydrate in ethanol to give the hydrazide **4**. The treatment of hydrazide **4** with several isocyanates and isothiocyanates produced the key intermediate products **5a–c**. Compounds **5a–c** exhibit spectral and elemental analysis data consistent with the proposed structures.

With the aim to obtain hybrid compounds containing antipyrine and 1,3-thiazole moieties together, **6a,b**, the carbo(thio)amides **5a, 5c** were heated under reflux in the presence 4-chlorophenacyl bromide and anhydrous

***Corresponding author: Serpil Demirci,** Giresun University, Kadir Karabas School of Applied Sciences, Department of Crop Production and Technologies, 28000, Bulancak, Giresun, Turkey, e-mail: demirciserpil17@gmail.com

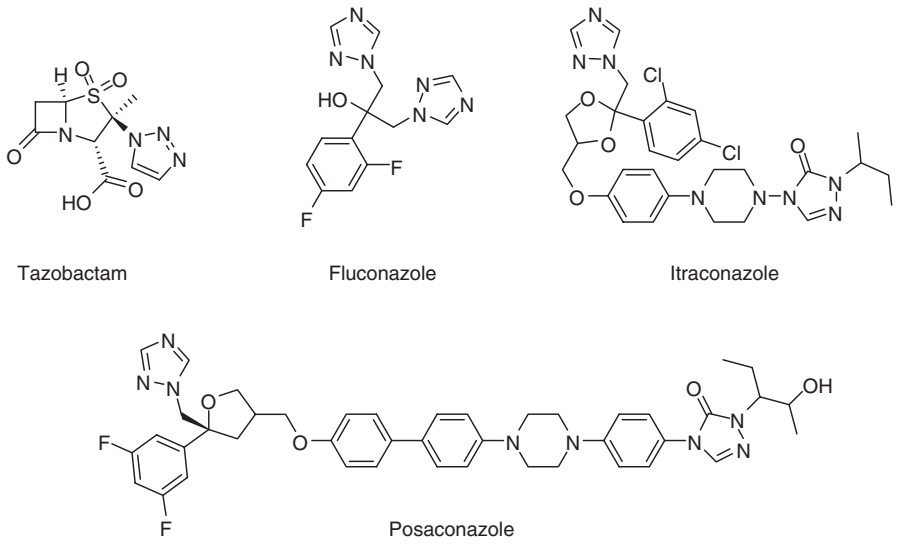


Figure 1 Selected antifungal imidazoles and triazoles.

sodium acetate. Other hybrid compounds **7a–c** incorporating antipyrine and 1,3-oxa(thia)zolidine moieties in the molecular framework were obtained by the reaction of compounds **5a–c** with ethyl bromoacetate in ethanolic solution in the presence of sodium acetate. The treatment of intermediate products **5a–c** with sodium hydroxide afforded derivatives **8a–c**. The idea was to merge two bioactive nuclei namely antipyrine and triazole in a single molecule. Elemental analysis and FT-IR, ^{1}H-NMR, ^{13}C NMR, LC-MS spectral data were obtained for all these products. In particular, in the ^{13}C NMR spectra of compounds **8a–c**, the chemical shifts of C-3 and C-5 carbon atoms belonging to 1,2,4-triazole nucleus are consistent with the literature values for related compounds [17–19]. It is known that compounds **8a** and **8c** can exist as thioxo-mercapto tautomeric forms, while type **8b** compounds are generally ketones [20].

The synthesis of compound **9** was performed by Mannich reaction between compound **8a** and (+)-6-aminopenicillanic acid (6-apa) at room temperature. The aim was to join the antipyrine nucleus with a penicillin skeleton which belongs to β-lactam class antibiotics. β-Lactam derivatives constitute a class of important antibacterial agents in the current clinical regimen. Besides their applications as antibacterials, β-lactams are increasingly used as inhibitors of other medicinally important targets [21]. Compound **9** display spectral data and elemental analysis results consistent with the assigned structure.

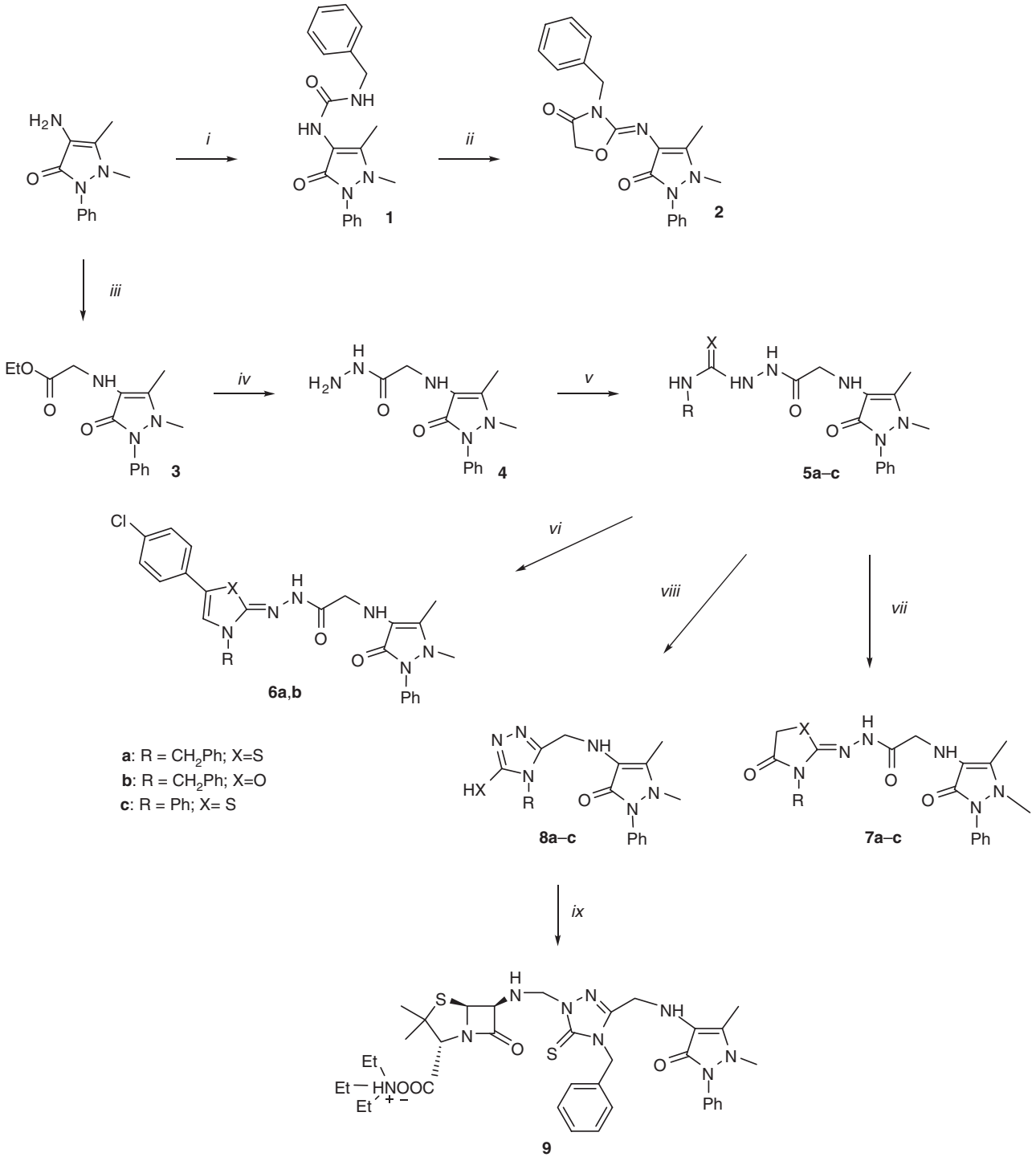
The newly synthesized compounds were evaluated *in vitro* for their antimicrobial activities and the results are shown in Table 1 (only positive results are presented). None of the tested compounds, except **9**, display activity against Gram negative bacteria *Escherichia coli* (Ec), *Yersinia pseudotuberculosis* (Yp) and *Pseudomonas aeruginosa* (Pa). Compounds **5c, 6a, 7c**, **8a**, **8c** and **9** display moderate antimicrobial activity against Gram positive bacteria *Staphylococcus aureus* (Sa), *Enterococcus faecalis* (Ef), *Bacillus cereus* (Bc), *Micobacterium smegmatis* (Ms), an atypical tuberculosis factor, and *Candida albicans* (Ca) and *Saccaromyces cerevisiae* (Sc), yeast-like fungi.

Conclusion

This study reports the synthesis of some new antipyrine derivatives incorporating several other heterocyclic moieties having importance for biological activity in a single structure. The structures of new compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis techniques. The synthesized compounds were screened for antimicrobial activities. Compounds, **5c, 6a, 7c, 8a, 8c** and **9** exhibit moderate activities against some of the test microorganisms.

Experimental

All chemicals were purchased from Fluka Chemie AG Buchs (Switzerland) and used without further purification. Melting points were determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on silica-gel 60 F254 aluminum sheets. The mobile phase was ethanol/ethyl ether, 1:1, and detection was made using UV light. FT-IR spectra were



Scheme 1 Synthesis of compounds **1–9**.
i: $PhCH_2NCO$, 13 h, reflux, *ii*: $BrCH_2CO_2Et$, CH_3COONa, $CHCl_3$, 18 h, reflux. *iii*: $BrCH_2CO_2Et$, TEA, in THF, 24 h, rt., *iv*: H_2NNH_2, in EtOH, 15 h, reflux, *v*: RNCX, in EtOH, reflux (5 h for **5a**) (16 h for **5b** and **5c**), *vi*: $BrCH_2COC_6H_4Cl$, CH_3COONa, in EtOH, MW (15 min., 150 W for **6a**) (15 min., 200 W for **6b**), *vii*: $BrCH_2COOEt$, CH_3COONa, MW (20 min., 150 W for **7a** and **7b**) (5 min., 200 W for **7c**), *viii*: NaOH, EtOH-water, 3 h, reflux, *ix*: 6-apa, HCOH, DMF, 4 h, rt.,

recorded using a Perkin Elmer 1600 series FT-IR spectrometer using KBr pellets. ^{1}H-NMR and ^{13}C-NMR spectra were registered in DMSO-d_6 on a Bruker Avene II 400 NMR spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). Microwave-assisted syntheses were carried out using mono-mode CEM-Discover microwave apparatus. The elemental analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer. The mass spectra were obtained on a Quattro LC-MS (70 eV) instrument.

Table 1 Antimicrobial activity of selected compounds. See the text for definitions of microorganisms.

No	Microorganisms and inhibition zone (mm)								
	Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca	Sc
5c	–	–	–	12	6	6	16	10	15
6a	–	–	–	10	–	6	–	7	12
7c	–	–	–	6	6	6	–	6	6
8a	–	–	–	6	–	–	–	6	6
8c	–	–	–	9	–	–	8	10	20
9	15	10	–	15	10	10	13	6	8
Amp.	10	18	18	35	10	15	–	–	–
Strep.	–	–	–	–	–	–	35	–	–
Flu.	–	–	–	–	–	–	–	25	>25

Ec, *Escherichia coli* ATCC 25922; Yp, *Yersinia pseudotuberculosis* ATCC 911; Pa, *Pseudomonas aeruginosa* ATCC 43288; Sa, *Staphylococcus aureus* ATCC 25923; Ef, *Enterococcus faecalis* ATCC 29212; Bc, *Bacillus cereus* 702 Roma; Ms, *Mycobacterium smegmatis* ATCC607; Ca, *Candida albicans* ATCC 60193; Sc, *Saccharomyces cerevisiae* RSKK 251; Amp.: ampicillin, Strep.: streptomycin, Flu.: fluconazole; –, no activity of test concentrations.

N-Benzyl-*N′*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)urea (1)

A solution of antipyrine (10 mmol) and benzyl isocyanate (10 mmol) in absolute ethanol (10 mL) was heated under reflux for 13 h. On cooling the mixture to room temperature, a solid was formed. This crude product was collected by filtration and crystallized from ethyl acetate/*n*-hexane (2:1) to give the target product: yield 92%; mp 188–189°C; FT-IR: 3274, 3077, 1765 cm^{-1}; ^1H NMR: δ 2.14 (s, 3H), 3.00 (s, 3H), 4.23 (bs, 2H, *J* = 5.8 Hz), 6.75 (s, 2H, NH, D_2O exchangeable), 7.24 (bs, 2H), 7.29 (d, 2H, *J* = 5.0 Hz), 7.36 (bs, 2H), 7.46 (d, 4H, *J* = 7.0 Hz); ^{13}C NMR: δ 14.1, 35.2, 61.3, 116.2, 125.8, 127.5, 129.5, 130.0, 131.2, 134.3, 140.3, 152.6; LC-MS *m/z* (%): 355.21 ($[M+1+H_2O]^+$ 45), 321.11 (100). Anal. Calcd for $C_{19}H_{20}N_4O_2$: C, 67.84; H, 5.99; N, 16.66. Found: C, 67.79; H, 5.98; N, 16.62.

3-Benzyl-2-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)imino]-1,3-oxazolidin-4-one (2)

A mixture of compound **1** (10 mmol) and ethyl bromoacetate in dry chloroform was heated under reflux in the presence of dried sodium acetate (50 mmol) for 18 h. Then, the mixture was cooled to room temperature and the precipitated salt was separated by filtration. The filtrate was concentrated under reduced pressure and the resultant solid was crystallized from ethyl acetate to give the target product: yield 27%; mp 177–178°C; IR: 3076, 1770, 1628 cm^{-1}; ^1H NMR: 2.14 (s, 3H), 3.00 (s, 3H), 3.34 (s, 2H), 4.25 (d, 2H, *J* = 5.8 Hz), 6.75 (t, 1H, *J* = 5.6 Hz), 7.25–7.32 (m, 6H), 7.37 (s, 1H), 7.48 (t, 2H, *J* = 7.8 Hz); ^{13}C NMR: 14.1, 35.4, 56.3, 61.3, 118.2, 125.9, 127.2, 129.8, 131.0, 131.8, 132.3, 134.6, 140.3, 148.9, 152.6, 167.0; LC-MS *m/z* (%): 393.14 ($[M+1]^+$ 50), 385.22 (100). Anal. Calcd for $C_{19}H_{20}N_4O_2S$: C, 64.27; H, 5.14; N, 14.28. Found: C, 64.29; H, 5.18; N, 14.22.

Ethyl *N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)glycinate (3)

To the mixture of antipyrine (10 mmol) and triethylamine (10 mmol) in dry tetrahydrofuran, ethyl bromoacetate (10 mmol) was added dropwise at 0–5°C. Then, the mixture was allowed to reach room temperature and stirred for 12 h (the progress of the reaction was monitored by TLC). The precipitated triethylammonium salt was removed by filtration and the solution was concentrated under reduced pressure. The obtained yellow solid was crystallized from ethyl acetate/*n*-hexane (2:1) to give the desired product: yield 92%; mp 72–73°C; IR: 3251, 3062, 1729, 1117 cm^{-1}; ^1H NMR: δ 1.13 (t, 3H, *J* = 7.0 Hz), 2.13 (s, 3H,), 2.76 (s, 3H), 3.85 (d, 2H, *J* = 5.4 Hz), 4.03 (q, 2H, *J* = 7.0 Hz), 7.22 (bs, 1H, NH D_2O exchangeable), 7.39–7.46 (m, 5H); ^{13}C NMR: δ 10.8, 14.8, 19.2, 47.0, 60.7, 120.5, 122.9, 125.8, 126.1, 129.5, 136.0, 162.0, 172.7; LC-MS *m/z* (%): 328.41 ($[M+K]^+$ 10), 312.43 ($[M+Na]^+$ 13), 304.44 (100), 289.48 ($[M]^+$ 10). Anal. Calcd for $C_{15}H_{19}N_3O_3$: C, 62.27; H, 6.62; N, 14.52. Found: C, 62.26; H, 6.67; N, 14.51.

2-[(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)aminoaceto hydrazide (4)

Hydrazine hydrate (25 mmol) was added to the solution of compound **3** (10 mmol) in ethanol and the mixture was heated under reflux for 15 h and then cooled. The resultant white crystals were filtered off and crystallized from ethyl acetate: yield 82%; mp 52–53°C; IR: 3371, 3047, 1704 cm^{-1}; ^1H NMR: δ 2.13 (s, 3H), 2.77 (s, 3H), 3.59 (s, 2H), 3.98 (bs, 2H, NH_2, D_2O exchangeable), 7.23–7.26 (m, 2H, 2NH, D_2O exchangeable), 7.379–7.47 (m, 5H); ^{13}C NMR: δ 10.7, 37.6, 48.8, 119.7, 123.1, 123.6, 126.5, 129.8, 135.5, 162.6, 171.2; LC-MS *m/z* (%): 275.41 ($[M]^+$ 10), 257.43 ($[M-H_2O]^+$ 13), 165.10 (100). Anal. Calcd for $C_{13}H_{17}N_5O_2$: C, 56.71; H, 6.62; N, 25.44. Found: C, 56.80; H, 6.64; N, 25.41.

Synthesis of compounds 5a–c

The mixture of compound **4** (10 mmol) and iso(thio)cyanate (10 mmol) in absolute ethanol was heated under reflux for 5 h (for **5a**), 15 h (for **5b**) and 16 h (for **5c**) with the progress of the reaction monitored by TLC. Upon cooling the resultant white solid was filtered and crystallized from ethanol to afford the desired compound.

***N*-Benzyl-2-{[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)amino]acetylhydrazine carbothioamide (5a)** Yield 66%, mp 290°C; IR: 3353, 3328, 3293, 3241, 3061, 1650, 1284 cm^{-1}; ^1H NMR: δ 2.14 (s, 3H), 2.80 (s, 3H), 3.63 (s, 2H), 4.60 (bs, 2H), 7.20 (s, 2H), 7.27 (bs, 6H), 7.37 (d, 2H, *J* = 7.0 Hz), 8.65 (s, 2H, NH, D_2O exchangeable), 9.31 (s, 1H, NH, D_2O exchangeable), 9.98 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 10.6, 37.9, 41.5, 46.3, 118.9, 126.3, 127.8, 127.9, 129.1, 129.3, 129.7, 135.9, 136.6, 145.2, 151.6, 162.7, 168.2; LC-MS *m/z* (%): 424.62 ($[M]^+$ 18), 251.48 (100). Anal. Calcd for $C_{21}H_{24}N_6O_2S$: C, 59.41; H, 5.70; N, 19.80. Found: C, 59.39; H, 5.64; N, 19.81.

***N*-Benzyl-2-{2-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)amino]acetyl}hydrazine carboxamide (5b)** Yield 32%, mp 241–242°C; IR: 3325, 3267, 3052, 1702, 1649 cm^{-1}; ^1H NMR: δ 2.12 (s, 3H), 3.00 (s, 3H), 3.71 (bs, 2H), 4.22 (d, 2H, *J* = 5.2 Hz), 7.19–7.34

(m, 8H), 7.47 (m, 2H); ^{13}C NMR: δ 12.0, 35.2, 61.2, 63.0, 106.2, 125.9, 127.5, 129.0, 130.0, 134.3, 140.4, 152.7; LC-MS *m/z* (%): 429.24 ([M+2+Na]$^+$, 50), 399.23 (100). Anal. Calcd for $C_{21}H_{24}N_6O_3$: C, 61.75; H, 5.92; N, 20.58. Found: C, 61.77; H, 5.94; N, 20.51.

2-{[(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl) amino]acetyl}-*N*-phenylhydrazine carbothioamide (5c) Yield 42%; mp 168–169°C; IR: 3264, 3014, 1657, 1288 cm^{-1}; ^{1}H NMR: δ 1.94 (s, 3H), 2.77 (s, 3H), 4.08 (s, 2H), 7.28–7.48 (m, 10H), 9.71 (s, 1H, NH, D_2O exch.), 9.98 (s, 1H, NH, D_2O exchangeable), 10.71 (s, 1H, NH, D_2O exchangeable), 13.89 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 7.5, 9.7, 54.0, 116.0, 116.7, 120.9, 122.4, 125.5, 128.0, 128.3, 133.4, 144.0, 151.0, 155.5, 161.6; LC-MS *m/z* (%): 443.27 (100), 449.21 ([M+K]$^+$ 10), 429.26 ([M+1+H_2O]$^+$, 45). Anal. Calcd for $C_{20}H_{22}N_6O_2S$: C, 58.52; H, 5.40; N, 20.47. Found: C, 58.57; H, 5.44; N, 20.50.

Synthesis of compounds 6a,b

A mixture of compound **5** (10 mmol) and dried sodium acetate (20 mmol) in absolute ethanol was stirred at room temperature for 15 min. Then, 4-chlorophenacyl bromide (15 mmol) was added and the mixture irradiated in a closed vessel with the pressure control at 124°C for 10 min (hold time) at 150 W (for **6a**) and 160°C for 15 min (hold time) at 200 W (for **6b**) maximum power. The solution was poured into ice water and the resultant white solid was collected by filtration and crystallized from butylacetate/diethylether (1:2) to afford the target compound.

***N'*-[3-Benzyl-5-(4-chlorophenyl)-1,3-thiazol-2(3*H*)-ylidene]-2-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl) amino]acetohydrazide (6a)** Yield 45%; mp 180°C; IR: 3381, 3227, 3056, 1721, 1588 cm^{-1};. ^{1}H NMR: δ 2.00 (s, 3H), 2.80 (s, 3H), 4.10 (s, 2H), 4.31 (bs, 1H), 5.38 (s, 2H), 7.31–7.45 (m, 14H); ^{13}C NMR: δ 10.5, 37.6, 46.3, 58.2, 104.5, 118.4, 123.5, 126.6, 127.8, 128.4, 129.0, 129.3, 129.7, 130.8, 135.7, 136.4, 139.5, 145.7, 151.6, 162.8, 169.5; LC-MS *m/z* (%): 559.55 ([M]$^+$ 45), 541.06 ([M-H_2O]$^+$, 25), 321.41 (100). Anal. Calcd for $C_{29}H_{27}ClN_6O_2S$: C, 62.30; H, 4.87; N, 15.03. Found: C, 62.37; H, 5.81; N, 15.01.

***N'*-[5-(4-Chlorophenyl)-3-phenyl-1,3-thiazol-2(3*H*)-ylidene]-2-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl) amino]acetohydrazide (6b)** Yield 70%; mp 140–141°C; IR: 3279, 3227, 3056, 1702, 1589 cm^{-1}; ^{1}H NMR: δ 1.76 (s, 3H), 1.88 (s, 3H), 3.35 (s, 2H), 6.56 (s, 1H), 7.11–7.36 (m, 14H); ^{13}C NMR: δ 18.9, 25.0, 44.2, 109.3, 120.0, 128.1, 129.0, 129.4, 130.3, 130.6, 133.5, 138.5, 159.9, 166.5; LC-MS *m/z* (%): 568.05 ([M+1+Na]$^+$, 100), 545.06 ([M]$^+$, 25). Anal. Calcd for $C_{28}H_{25}ClN_6O_2S$: C, 61.70; H, 4.62; N, 15.42. Found: C, 61.77; H, 4.64; N, 15.41.

Synthesis of compounds 7a–c

A mixture of compound **5** (15 mmol) and dried sodium acetate (20 mmol) in absolute ethanol was stirred at room temperature for 15 min. Then, ethyl bromoacetate (15 mmol) was added and the mixture was irradiated in a closed vessel with the pressure control at 125°C for 20 min (hold time) at 150 W (for **7a** and **7b**) and 160°C for 5 min (hold time) at 200 W (for **7c**) maximum power. The solution poured into ice water and the resultant white solid was collected by filtration and crystallized from ethyl acetate to give the target compound.

***N'*-[3-Benzyl-4-oxo-1,3-thiazolidin-2-ylidene]-2-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)amino]acetohydrazide (7a)** Yield 75%; mp 140°C; IR: 3289, 3287, 3058, 1702, 1689, 1599 cm^{-1}; ^{1}H NMR: δ 1.89 (s, 3H), 2.87 (s, 3H), 3.37 (s, 2H), 4.01 (s, 2H), 4.77 (s, 2H), 7.32 (m, 10H); ^{13}C NMR: δ 10.0, 24.6, 32.6, 45.6, 51.4, 120.1, 123.0, 127.9, 128.6, 129.3, 134.4, 136.0, 149.9, 155.7, 160.0; LC-MS *m/z* (%): 487.55 ([M+Na]$^+$, 100), 464.06 ([M]$^+$, 25). Anal. Calcd for $C_{23}H_{24}N_6O_3S$: C, 59.47; H, 5.21; N, 18.09. Found: C, 59.37; H, 5.21; N, 18.01.

***N'*-[3-Benzyl-4-oxo-1,3-oxazolidin-2-ylidene]-2-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)amino]acetohydrazide (7b)** Yield 77%; mp 177–178°C; IR: 3158, 3021, 1713, 1702, 1593 cm^{-1}; ^{1}H NMR: δ 2.14 (s, 3H), 2.99 (s, 3H), 3.46 (s, 4H), 4.24 (s, 2H, *J* = 5.0 Hz), 6.74 (bs, 2H), 7.30–7.34 (m, 10H); ^{13}C NMR: δ 10.0, 24.6, 32.6, 45.6, 51.4, 120.1, 123.0, 127.9, 128.6, 129.3, 134.4, 136.0, 149.9, 155.7, 160.0; LC-MS *m/z* (%): 487.55 ([M+Na]$^+$, 100), 464.06 ([M]$^+$, 25). Anal. Calcd for $C_{23}H_{24}N_6O_3S$: C, 59.47; H, 5.21; N, 18.09. Found: C, 59.37; H, 5.21; N, 18.01.

2-[(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl) amino]-*N'*-(4-oxo-3-phenylthiazolidin-2-ylidene)acetohydrazide (7c) Yield 71%; mp 118–119°C; IR: 3235, 3278, 3093, 1727, 1722, 1589 cm^{-1}; ^{1}H NMR: δ 1.91 (s, 3H), 2.77 (s, 3H), 4.01–4.16 (m, 4H), 6.92 (t, 1H, *J* = 6.0 Hz), 7.24 (s, 2H), 7.30 (d, 2H, *J* = 7.8 Hz), 7.44 (s, 1H), 7.53 (m, 4H), 9.86 (s, 2H, NH, D_2O exchangeable); ^{13}C NMR: δ 14.5, 34.6, 37.2, 62.2, 105.0, 117.5, 127.6, 129.0, 129.7, 130.0, 130.6, 130.8, 133.2, 135.5, 141.6, 156.5, 163.2, 168.8; LC-MS *m/z* (%): 450.00 ([M]$^+$, 10), 321.11 (100). Anal. Calcd for $C_{22}H_{22}N_6O_3S$: C, 58.65; H, 4.92; N, 18.65. Found: C, 58.67; H, 4.94; N, 18.61.

Synthesis of compounds 8a–c

A solution of compound **5** (10 mmol) and NaOH (0.4 g, 10 mmol) in ethanol-water (10 mL, 1:1), was heated under reflux for 3 h, then cooled to room temperature and acidified to pH 4 with 37% HCl. The precipitate formed was filtered off, washed with water, and crystallized from ethanol to give the target product.

4-{[(4-Benzyl-5-mercapto-4*H*-1,2,4-triazol-3-yl)methyl]amino}-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one (8a) Yield 73%; mp 139–140°C; IR: 3325, 3033, 2767, 1649 cm^{-1}; ^{1}H NMR: δ 2.00 (s, 3H), 2.80 (s, 3H), 4.81 (s, 2H), 4.11 (s, 2H), 5.37 (s, 1H), 7.32 (m, 8H), 7.43 (d, 2H, *J* = 6.6 Hz), 13.07 (s, 1H); ^{13}C NMR: δ 10.5, 37.5, 46.3, 54.2, 105.6, 123.5, 126.6, 127.5, 127.8, 128.4, 129.0, 129.3, 129.7, 130.1, 135.6, 136.4, 145.8, 151.6, 162.8; LC-MS *m/z* (%): 443.21 ([M-2+K]$^+$ 100), 429.03 ([M+Na]$^+$, 61), 424.13 ([M+H_2O]$^+$, 25), 406.37 ([M]$^+$, 21). Anal. Calcd for $C_{21}H_{24}N_6OS$: C, 62.05; H, 5.45; N, 20.67. Found: C, 62.39; H, 5.54; N, 20.81.

4-{[(4-Benzyl-5-hydroxy-4*H*-1,2,4-triazol-3-yl)methyl]amino}-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one (8b) Yield 95%; mp 256–257°C; IR: 3521, 3287, 3098, 1712 cm^{-1}; ^{1}H NMR: δ 2.48 (bs, 3H), 3.56 (bs, 3H), 4.21 (d, 4H, *J* = 5.6 Hz), 7.01 (s, 1H, NH, D_2O exchangeable), 7.25 (bs, 10H), 7.81 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 14.5, 34.5, 43.3, 44.2, 108.5, 123.5, 125.6, 127.6, 127.6, 128.6, 129.0, 131.6, 135.7, 137.9, 146.6, 156.6, 162.8; LC-MS *m/z* (%): 390.21 ([M]$^+$, 70), 372.26 ([M-H_2O]$^+$, 45), 241 (100). Anal. Calcd for $C_{21}H_{22}N_6O_2$: C, 64.60; H, 5.68; N, 21.52. Found: C, 64.57; H, 5.69; N, 21.50.

4-{[(5-Mercapto-4-phenyl-4*H*-1,2,4-triazol-3-yl)methyl]amino}-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one (8c) Yield 57%; mp 239–240°C; IR: 3267, 3056, 2651, 1721 cm^{-1}; ^1H NMR: δ 1.94 (s, 3H), 2.77 (s, 3H), 4.07 (s, 2H), 7.33–7.48 (m, 10H), 13.87 (s, 1H); ^{13}C NMR: δ 10.4, 37.4, 41.6, 118.1, 123.5, 125.6, 128.7, 129.7, 130.0, 134.0, 135.5, 145.5, 154.1, 162.5; LC-MS *m/z* (%): 415.07 ([M+Na]$^+$, 25), 393.09 ([M+1]$^+$, 100). Anal. Calcd for $C_{20}H_{20}N_6OS$: C, 61.20; H, 5.14; N, 21.41. Found: C, 61.27; H, 5.14; N, 21.41.

N,N-Diethylethanaminium-6-{[(4-benzyl-3-{[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)amino]methyl}-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)methyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (9)

6-Apa (10 mmol) was added to a solution of compound **8a** (10 mmol) in DMF and the mixture was stirred at room temperature in the presence of formaldehyde (37%, 7.4 mL) for 4 h. Then, water was added and the mixture kept overnight at 0°C. The solid separated was collected by filtration and crystallized from ethyl acetate/petroleum ether (1:2) to give the target product: yield 70%; mp 129–130°C; IR: 3203, 3060, 1763, 1728, 1215 cm^{-1}; ^1H NMR: δ 1.12 (t, 9H, *J* = 7.8 Hz), 1.45 (s, 3H), 1.53 (s, 3H), 2.24 (s, 3H), 2.51 (s, 2H), 2.91 (q, 6H, *J* = 7.2 Hz), 3.06 (s, 3H), 4.06 (s, 1H), 4.47 (s, 2H), 4.52 (s, 1H), 4.67 (d, 2H, *J* = 5.8 Hz), 5.32 (d, 1H, *J* = 3.4 Hz), 7.19–7.28 (m, 5H), 7.33 (m, 2H), 7.47 (t, 3H, *J* = 7.8 Hz), 9.52 (s, 2H, 2NH, D_2O exchangeable); ^{13}C NMR: δ 9.2, 14.2, 27.4, 30.2, 34.2, 46.3, 47.9, 50.3, 51.3, 59.3, 62.3, 64.3, 75.5, 107.3, 124.9, 127.6, 128.8, 130.0, 131.8, 133.0, 134.6, 140.1, 148.9, 163.6, 165.3, 167.0, 171.9; LC-MS *m/z* (%): 734.14 ([M-1]$^+$, 70), 585.22 (100). Anal. Calcd for $C_{36}H_{49}N_9O_4S_2$: C, 58.75; H, 6.71; N, 17.13. Found: C, 58.69; H, 6.78; N, 17.12.

Antimicrobial Activity Assessment by agar-well diffusion method

All tested microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey): *E. coli* ATCC35218, *E. aerogenes* ATCC13048, *Y. Pseudotuberculosis* ATCC911, *P. aeruginosa* ATCC43288, *S. aureus* ATCC25923, *E. faecalis* ATCC29212, *B. cereus* 709 Roma, *M. smegmatis* ATCC607, *C. Albicans* ATCC60193, *C. tropicalis* ATCC 13803, *A. niger* RSKK 4017 and *S. cerevisiae* RSKK 251. The compounds were weighed and dissolved in dimethyl sulfoxide to prepare a stock solution of 5000 mg/mL. Agar-well diffusion method screening test using agar-well diffusion method [22] as adapted earlier [23] was used. Each microorganism was suspended in Mueller Hinton (MH) (Difco Detroit, MI) broth and diluted approximately to 10^6 colony forming unit (cfu)/mL. They were 'flood-inoculated' onto the surface of MH agar and Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) and then dried. For *C. albicans* and *C. tropicalis*, SDA was used. Five millimeter diameter wells were cut from the agar using a sterile cork borer, and 50 mL of the extract substances was delivered into the wells. The plates were incubated for 18 h at 35°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 mg), streptomycin (10 mg) and fluconazole (5 mg) were standard drugs. Dimethyl sulfoxide and ethanol were used as solvent controls. The antimicrobial activity results are summarized in Table 1.

Acknowledgments: This work was supported by Karadeniz Technical University, BAP, Turkey (Ref. No. 8663 and 8623).

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