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Synthesis of Pyrano, Pyrido, Oxazino, and Spiro Pyrazole Derivatives and Their Antimicrobial Activity

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Abstract—Condensation of 5-methyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one with 4-hydroxybenzaldhyde in alcoholic sodium hydroxide yielded 4-(4-hydroxybenzylidene)-5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one, and treatment of the latter with acetylacetone, hydrazine hydrate, ethyl cyanoacetate, and ethyl acetoacetate gave pyranopyrazole, pyrazolopyrazole, and pyrazolopyridine derivatives. 5-Methyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one was reacted with 2,4-dichlorobenzoyl isothiocyanate, thiosemicarbazide, and hydrazine hydrate to afford pyrazolooxazine, pyrazolopyrazole, and spiro[pyrazole-3,3′-pyrazolo[3,4-*c*]pyrazole] derivatives. Some of the newly synthesized compounds showed high antimicrobial activity against three microbial strains (*S. aureus*, *E. coli*, *C. albicans*).

Keywords: pyranopyrazole, pyrazolopyrazole, pyrazolopyridine, pyrazolooxazine, antimicrobial activity

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INTRODUCTION

In recent years, much attention has been focused on the synthesis of nitrogen heterocycles because of their biological and medicinal importance. Pyrazolones and their derivatives constitute a significant class of heterocyclic compounds which can be found in many natural and synthetic products and medicinally active molecules [1, 2]. They are interesting because of their substantial pharmacological and biological activities such as antioxidant, antiplatelet, anti-inflammatory, antitumor, enzyme inhibitory, analgesic, antifungal, and antibacterial [3–10]. Therefore, the synthesis of functionalized pyrazolone structures is a hot topic in synthetic chemistry, and a great deal of work has been reported on this subject [11–14].

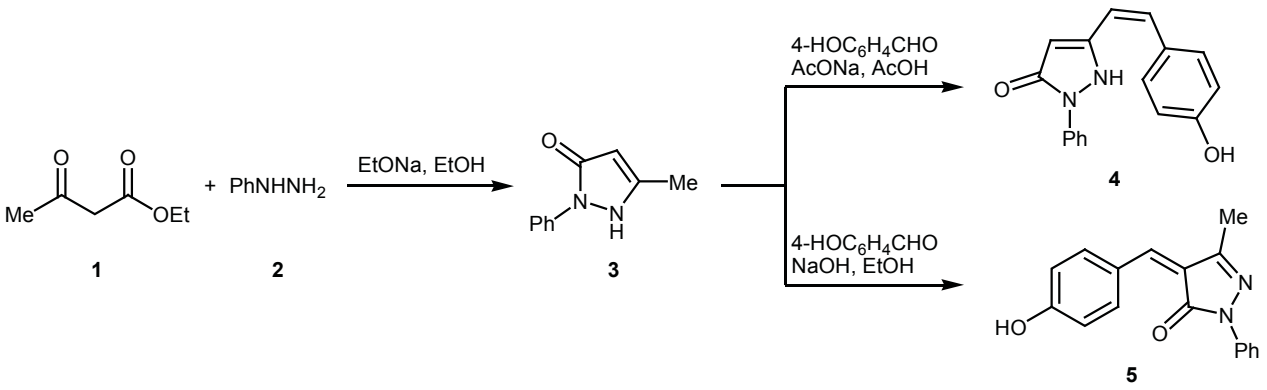
Increasing antibiotic resistance in microbial populations requires search for alternate cellular targets for new antimicrobial agents. It is well known that small modifications in the structure of targets change their biological character and physiochemical properties. A survey of literature on antimicrobial activity of various types of compounds revealed that the presence of certain pharmacophore such as pyrazole in any molecule plays an important role in improving the activity [15–17].

Herein we report the synthesis and antimicrobial activity of some new fused pyrazole derivatives such as pyranopyrazole, pyrazolopyrazole, pyrazolopyridine, and pyrazolooxazine.

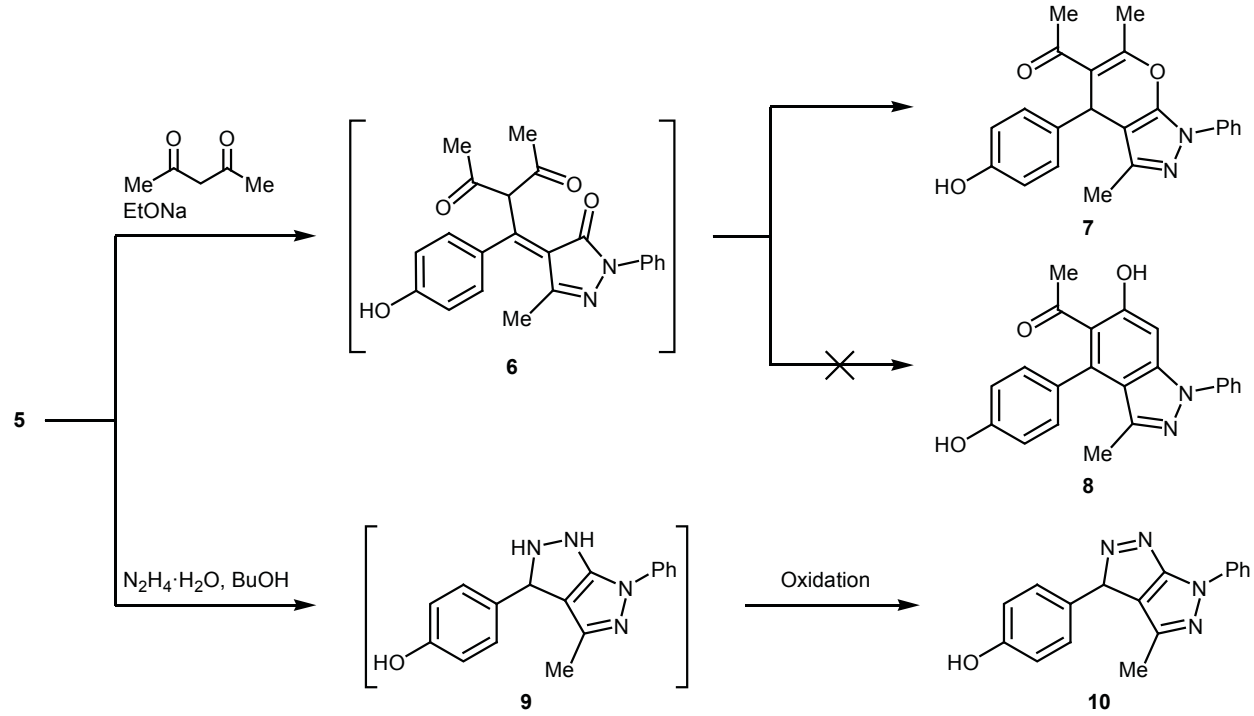
RESULTS AND DISCUSSION

5-Methyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one (**3**) was synthesized by the condensation of ethyl acetoacetate (**1**) with phenylhydrazine (**2**). The reaction of **3** with 4-hydroxybenzaldehyde in acetic acid in the presence of sodium acetate afforded pyrazolone **4** due to the activation of the 5-CH_3 group via protonation of the endocyclic nitrogen atom, whereas pyrazolone **5** was obtained under basic conditions [18, 19] (Scheme 1). The IR spectrum of **4** contained bands at 3178 and 1654 cm^{-1} for N–H and C=O stretching vibrations, respectively, The ^1H NMR spectrum of **4** showed singlets at δ 10.83 and 10.59 ppm for OH and NH protons and two doublets at δ 6.89–7.08 ppm with J = 8 Hz, which were assigned to *cis*-oriented protons at the exocyclic C=C double bond. In the ^{13}C NMR spectrum of **4**, the carbonyl carbon atom resonated at δ_C 163.06 ppm. The mass spectrum of **4** showed the molecular ion peak at m/z 278.32 and the base peak at m/z 77 $[Ph]^+$. In the IR spectrum of **5** we observed OH and C=O peaks at 3411 and 1650 cm^{-1}, respectively. Its

Scheme 1.



Scheme 2.

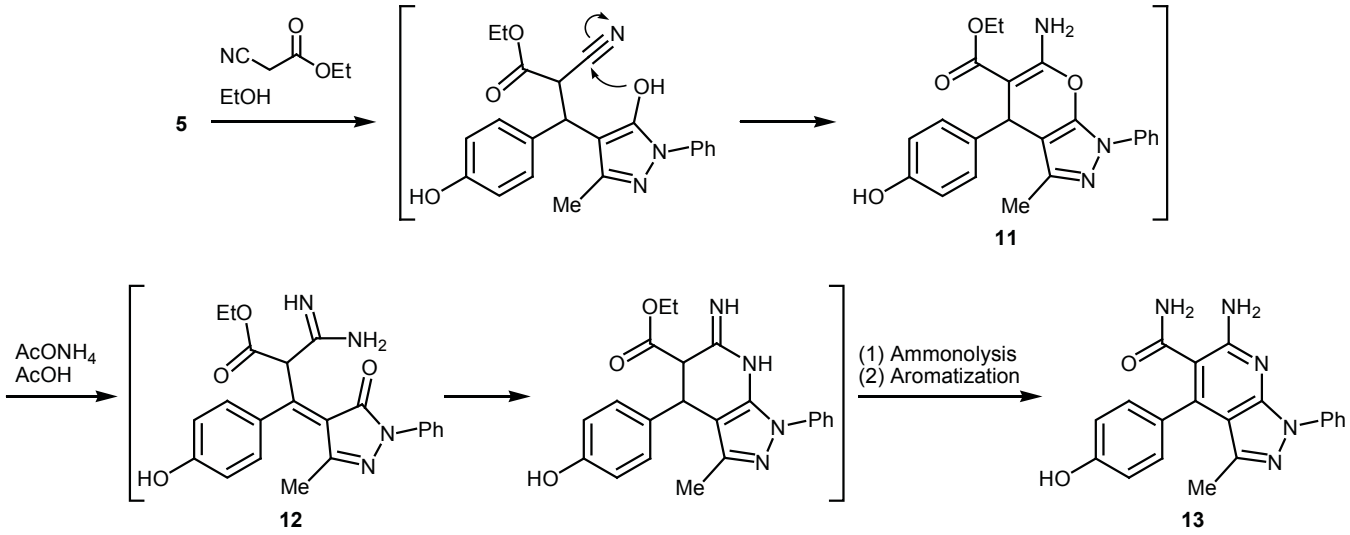


^{1}HNMR spectrum showed singlets at δ 10.84 (OH) and 2.49 ppm (CH_3), and the ^{13}C NMR spectrum contained signals at δ_C 163.06 (C=O) and 13.16 ppm (CH_3).

The reaction of benzylidenepyrazole **5** with acetylacetone in basic medium resulted in cyclization to pyranopyrazole derivative **7**, whereas no indazole **8** was formed. Obviously, compound **7** is formed via intramolecular cyclodehydration of intermediate Michael adduct **6** which could not be isolated (Scheme 2). Compound **7** displayed IR peaks at 3402 and 1654 cm^{-1} due to O–H and C=O stretchings, respectively. Its ^{1}H NMR spectrum showed a downfield D_2O exchangeable singlet at δ 9.19 ppm for the OH proton, a singlet at δ 4.83 ppm from the CH proton of the pyran ring, and three methyl proton singlets at δ 2.70, 2.26, and 2.03 ppm. The ^{13}C NMR spectrum of **7** contained signals at δ_C 162.32 ppm (C=O) and at δ_C 35.79, 32.34, 30.77, and 11.62 ppm for sp^3-carbons (CH, CH_3). The mass spectrum of **7** showed the molecular ion peak at *m*/*z* 360 $[M]^+$.

Intermolecular cyclization via conjugate addition of hydrazine hydrate to compound **5**, followed by oxidation of intermediate **9**, afforded pyrazolopyrazole derivative **10** (Scheme 2). The IR spectrum of **10** lacked C=O absorption, but OH and C=N peaks were observed at 3452 and 1654 cm^{-1}, respectively. The OH proton of **10** resonated in the ^{1}H NMR spectrum at δ 10.33 ppm as a D_2O-exchangeable singlet, and the

Scheme 3.



CH and methyl proton signals were located at δ 4.80 and 2.26 ppm, respectively. The ^{13}C NMR spectrum of **10** showed C=N carbon signals at δ_C 163.05 ppm. The mass spectrum contained the molecular ion peak at *m*/*z* 290 $[M]^+$.

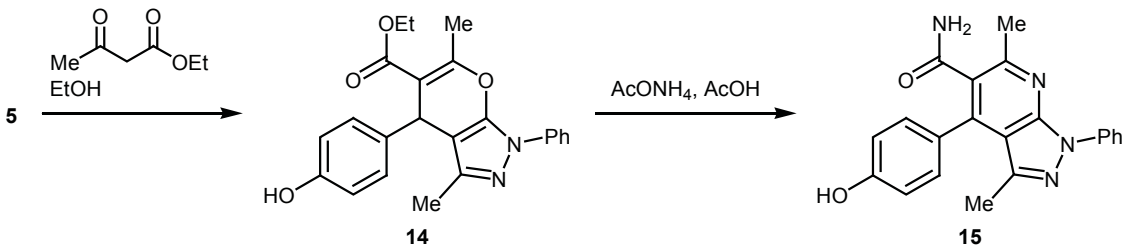
Treatment of **5** with ethyl cyanoacetate in presence of ammonium acetate in acetic acid afforded pyrazolopyridine **13** as final product. The reaction is likely to involve initial formation of pyranopyrazole **11** and its recyclization to pyrazolopyridine **13** through intermediate **12**, followed by imine–enamine tautomerization, ammonolysis, and aromatization [20] (Scheme 3). The IR spectrum of **13** showed bands at 3066, 1640, and 1504 cm^{-1} for NH_2, C=O, and C=N groups. Its ^{1}H NMR spectrum revealed the presence of singlets at δ 9.17, 4.82, and 3.91 ppm assignable for OH and two NH_2 protons. In the ^{13}C NMR spectrum of **13**, the carbonyl carbon resonated at δ_C 162.32 ppm. Its mass spectrum contained the molecular ion peak at *m*/*z* 359.

Likewise, the cyclocondensation of **5** with ethyl acetoacetate in the presence of ammonium acetate in acetic acid gave pyrazolopyridine **15** (Scheme 4). The mechanism of formation of **15** is likely to be similar to that proposed for compound **13**. In the IR spectrum of **15** we observed absorption peaks at 3417 (OH), and 1699 cm^{-1} (C=O), and signals at δ 12.3 (OH) and 4.82 ppm (NH_2) were present in its ^{1}H NMR.

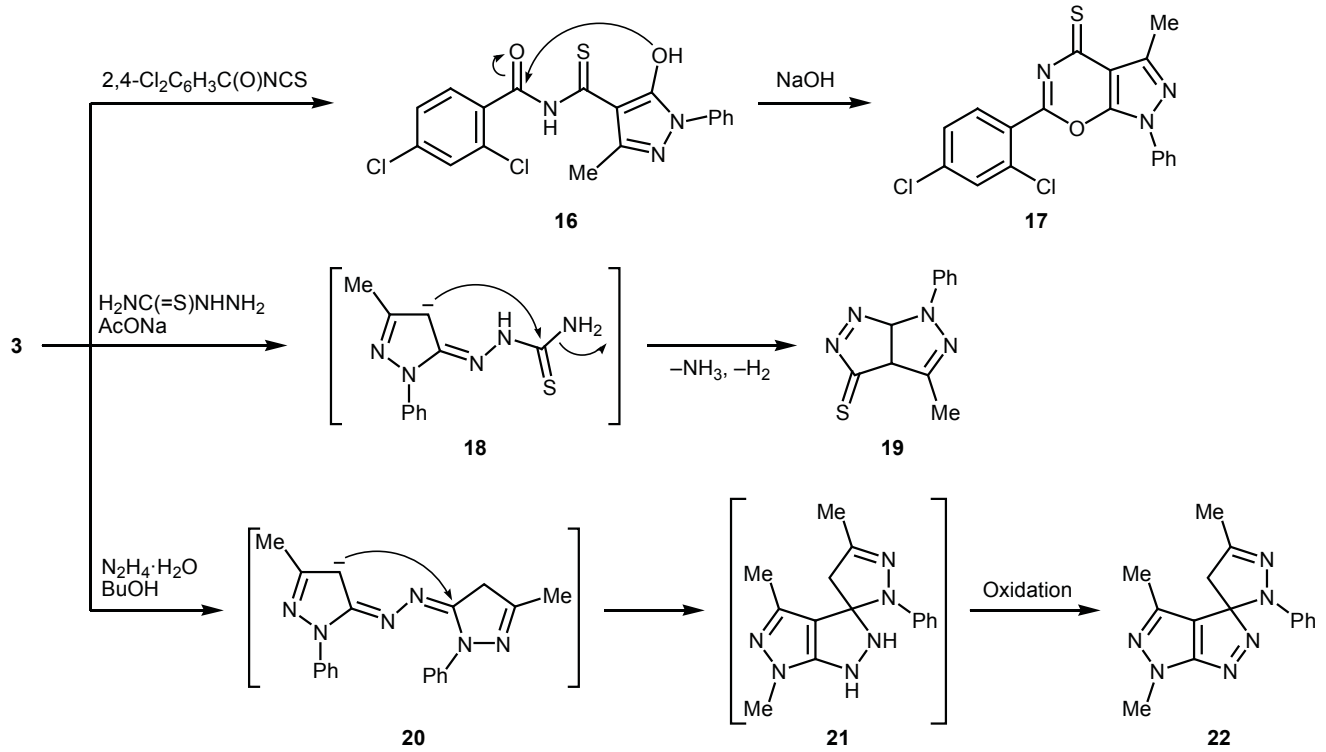
Michael addition of the activated enamino carbon atom of pyrazolone **3** to the electrophilic carbon atom of 2,4-dichlorobenzoyl isothiocyanate yielded thioamide derivative **16** (Scheme 5). It should be noted that thioamide derivative **16** exist in solution as a mixture of thione and thiol tautomers (Scheme 6) as indicated by its IR and ^{1}H NMR spectra. The IR spectrum of **16** revealed peaks at 3375, 2245, 1647, 1620, and 1256 cm^{-1} assignable to OH, SH, C=O, and C=S groups. The ^{1}H NMR spectrum of **16** showed downfield D_2O exchangeable singlets at δ 11.98, 11.65, and 9.58 ppm for SH, OH, and NH protons.

Base-catalyzed cyclization of **16** afforded pyrazolooxazine **17** (Scheme 5) which displayed IR absorption bands at 1651 (C=N) and 1242 cm^{-1} (C=S). Aromatic protons of **17** resonated as a multiplet signal at δ 7.92–7.42 ppm in the ^{1}H NMR spectrum. In the ^{13}C NMR

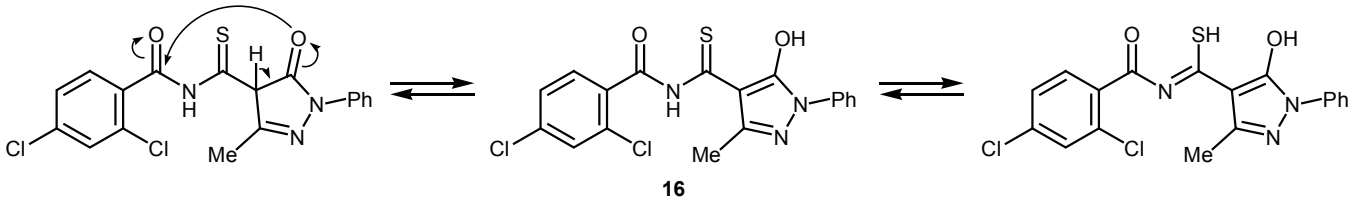
Scheme 4.



Scheme 5.



Scheme 6.



spectrum of **17**, signals at δ_C 167.30 (C=S) and 135.9 ppm (C=N) were present. The mass spectrum of **17** showed a peak at *m*/*z* 390.29 corresponding to its molecular ion.

The reaction of **3** with thiosemicarbazide in acetic acid in the presence of sodium acetate yielded pyrazolopyrazole **19**. Compound **19** is likely to be formed as a result of intramolecular cyclization of intermediate thiosemicarbazone **18** via attack of the enamino carbon atom on the thioxo group with extrusion of ammonia molecule and subsequent oxidation (Scheme 5). Pyrazolopyrazole **19** showed IR peaks for C=N and C=S groups at 1616 and 1292 cm^{-1}, respectively. Its ^1H NMR spectrum (DMSO-d_6) revealed multiplet signals at δ 7.94–7.26 ppm for aromatic protons and a singlet at δ 2.49 ppm for CH_3 group. Treatment of **3** with hydrazine hydrate afforded spiro pyrazole derivative **22**. Presumably, initially formed bis-hydrazone **20** underwent intramolecular cyclization via addition of enamino carbon atom of one pyrazole fragment to the hydrazone carbon atom of the other pyrazole fragment, followed by oxidation (Scheme 5). In the IR spectrum of **22**, an absorption band at 1616 cm^{-1} (C=N) was observed. Its ^1H NMR spectrum showed a multiplet at δ 7.75–7.17 ppm for aromatic protons and two doublets at δ 2.71–2.30 ppm for methylene protons.

Antimicrobial activity. The synthesized compounds were evaluated for their in vitro antimicrobial activity against three microbial strains (*S. aureus*, *E. coli*, and *C. albicans*) by using agar diffusion assay, and the results for each tested compound were represented as inhibition zone diameters in mm (Table 1). Compounds **16** and **17** containing a 2,4-dichlorophenyl moiety showed high antibacterial activity against *S. aureus* and *E. coli*, whereas compounds **4**, **5**, **7**, **10**, **13**, **15**, **19** and **22** were weakly active against the same

Table 1. Inhibition zone diameters[a] (*d*) and activity indices (*I*) for the newly synthesized compounds against some bacterial and fungal strains

Compd. no.	*E. coli*		*S. aureus*		*C. albicans*	
	d, mm	*I*, %	*d*, mm	*I*, %	*d*, mm	*I*, %
4	3	11	4	17	7	26
5	8	31	10	42	15	56
7	9	35	13	55	18	67
10	3	12	7	30	9	34
13	11	43	18	76	22	82
15	12	46	16	67	13	48
16	20	80	19	79	12	45
17	22	82	21	88	3	12
19	10	38	14	58	15	55
22	7	26	9	37	17	63
Ampicillin	26	100	24	100	NA	–
Clotrimazole	NA	–	NA	–	27	100

[a] NA stands for no activity.

bacterial strains. Compound **13** showed the highest antifungal activity against *C. albicans*; the other compounds exhibited weak activity against *C. albicans*.

EXPERIMENTAL

The melting points were measured on a Gallenkamp apparatus and are uncorrected. The IR spectra were recorded in KBr on a Perkin Elmer FT/IR-400 spectrophotometer. The ^{1}H and ^{13}C NMR were recorded on a Bruker spectrometer at 400 and 100 MHz, respectively, using DMSO-d_6 as solvent. The mass spectra (electron impact, 70 eV) were run on an MS-S988 instrument. Elemental analyses and antimicrobial study were carried out at the Faculty of Science, Mansoura University (Egypt).

5-Methyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one (3) [18]**.** A solution of ethyl acetoacetate (**1**, 13 mL, 0.1 mol) and phenylhydrazine (**2**, 10 mL, 0.1 mol) in ethanolic sodium ethoxide (0.015 mol in 3 mL of ethanol) was refluxed for 2 h. The mixture was concentrated, and the precipitate was filtered off and recrystallized from ethanol. Yield 85%, pale yellow solid, mp 118–120°C; published data [20]: mp 126–127°C. IR spectrum, ν, cm^{-1}: 3414 (N–H), 3055 (C–H_{arom}), 2924 (C–H_{aliph}), 1604 (C=O), 1519 (C=C). ^{1}H NMR spectrum, δ, ppm: 2.09 s (3H, CH_3), 5.36 s (1H, =CH), 7.14–7.81 m (5H, H_{arom}), 11.45 s (1H, NH).

5-[(*Z*)-2-(4-Hydroxyphenyl)ethenyl]-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one (4). A mixture of compound **3** (1.7 g, 0.01 mol), 4-hydroxybenzaldehyde (1.2 g, 0.01 mol), and sodium acetate (0.82 g, 0.01 mol) in acetic acid (10 mL) was refluxed for 3 h. The mixture was cooled to room temperature and poured into ice water, and the precipitate was filtered off, dried, and recrystallized from ethanol. Yield 80%, orange crystals, mp 230–233°C. IR spectrum, ν, cm^{-1}: 3406 (O–H), 3178 (N–H), 3070 (C–H_{arom}), 1654 (C=O), 1593 (C=N), 1543 (C=C). ^{1}H NMR spectrum, δ, ppm: 4.95 s (1H, 4-H), 6.90 d (1H, J = 8 Hz, CH=CH), 6.98 d (2H, J = 8 Hz, H_{arom}), 7.14–7.68 m (3H, H_{arom}), 7.74 d (1H, J = 8 Hz, CH=CH), 7.84 d (2H, J = 8 Hz, H_{arom}), 8.56–8.60 m (2H, H_{arom}), 9.77 s (1H, NH, D_2O exchangeable),10.83 s (1H, OH, D_2O exchangeable). ^{13}C NMR spectrum, δ_C, ppm: 73.1, 115.85, 118.2, 122.6, 124.3, 124.9, 128.8, 137.4, 138.4, 148.5, 151.8, 161.9, 163.06. Mass spectrum, *m*/*z* (I_{rel}, %): 278 (45) $[M]^+$, 185 (15), 145 (40), 127 (22), 115 (40), 91 (35), 77 (100), 51 (28). Found, %: C 73.35; H 5.05; N 10.05. $C_{17}H_{14}N_2O_2$. Calculated, %: C 73.37; H 5.07; N 10.07. *M* 278.

(*Z*)-4-(4-Hydroxybenzylidene)-5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (5). A mixture of compound **3** (1.7 g, 0.01 mol) and 4-hydroxylbenzaldhyde (1.2 g, 0.01 mol) in a solution of sodium hydroxide (0.4 g, 0.01 mol) in ethanol (20 mL) was refluxed for 6 h. The mixture was cooled to room temperature and poured into ice water, and the precipitate was filtered off, dried, and recrystallized from ethanol. Yield 70%, orange crystals, mp 235–237°C; published data [18, 19]: mp 230°C. IR spectrum, ν, cm^{-1}: 3414 (O–H), 3170 (C–H_{arom}), 2800 (C–H_{aliph}), 1581 (C=O),

1500 (C=N). ^{1}H NMR spectrum, δ, ppm: 2.31 s (3H, CH_3), 6.61–6.64 m (3H, H_{arom}), 6.80 d (2H, J = 8 Hz, H_{arom}), 7.00 d (2H, J = 8 Hz, H_{arom}), 7.16-8.57 m (3H, H_{arom}), 7.73 s (1H, =CH), 10.84 s (1H, OH, D_2O exchangeable). ^{13}CNMR spectrum, δ_C, ppm: 13.16 115.8, 118.2, 122.6, 124.3, 124.9, 128.8, 137.4, 138.4, 148.5, 151.8, 161.9, 163.06. Mass spectrum, m/z (I_{rel}, %): 278 (10) $[M]^+$, 264 (25), 186 (50), 145 (50), 127 (75), 116 (60), 77 (68), 51 (100). Found, %: C 73.35; H 5.05; N 10.05. $C_{17}H_{14}N_2O_2$. Calculated, %: C 73.37; H 5.07; N 10.07. M 278.

1-[4-(4-Hydroxyphenyl)-3,6-dimethyl-1-phenyl-1,6-dihydropyrano[2,3-*c*]pyrazol-5-yl]ethanone (7). A mixture of compound **5** (2 g, 0.01 mol), acetylacetone (1.1 mL, 0.01 mol), and sodium ethoxide (0.01 mol) in ethanol (20 mL) was refluxed for 4 h. The mixture was cooled to room temperature and poured into ice water, and the precipitate was filtered off, dried, and recrystallized from ethanol. Yield 50%, dark brown crystals, mp 200–202°C. IR spectrum, ν, cm^{-1}: 3402 (O–H), 3066 (C–H_{arom}), 1597 (C=O), 1504 (C=N), 1249 (C–O). ^{1}H NMR spectrum, δ, ppm: 2.28 s (3H, CH_3), 2.31 s (3H, CH_3), 2.33 s (3H, CH_3), 4.83 s (1H, 4-H), 6.53–6.65 m (3H, Ph), 6.98 d (2H, J = 8 Hz, H_{arom}), 7.02 d (2H, J = 8 Hz, H_{arom}), 7.23–7.94 m (2H, Ph), 9.19 s (1H, OH, D_2O exchangeable). ^{13}C NMR spectrum, δ_C, ppm: 11.62, 30.77, 32.34, 35.79, 114.85, 120.49, 125.51, 128.10, 128.93, 132.27, 146.18, 155.49, 162.32. Mass spectrum, m/z (I_{rel}, %): 360 (10) $[M]^+$, 302 (35), 185 (28), 174 (75), 115 (48), 76 (74), 44 (35). Found, %: C 73.30; H 5.56; N 7.75. $C_{22}H_{20}N_2O_3$. Calculated, %: C 73.32; H 5.59; N 7.77. M 360.

4-(4-Methyl-6-phenyl-3,6-dihydropyrazolo-[3,4-*c*]pyrazol-3-yl)phenol (10). A mixture of compound **5** (2 g, 0.01 mol) and hydrazine hydrate (0.5 mL, 0.01 mol) in butan-1-ol (20 mL) was refluxed for 4 h. The mixture was cooled to room temperature and poured into ice water, and the crystals were collected by filtration and recrystallized from ethanol. Yield 55%, light orange crystals, mp 210–212°C. IR spectrum, ν, cm^{-1}: 3421 (O–H), 3066 (C–H_{arom}), 2920 (C–H_{aliph}), 1654 (C=N), 1597 (C=C). ^{1}H NMR spectrum, δ, ppm: 2.30 s (3H, CH_3), 4.80 s (1H, 3-H), 6.63 d (2H, J = 8 Hz, H_{arom}), 6.92–7.90 m (5H, Ph), 8.62 d (2H, J = 8 Hz, H_{arom}), 10.83 s (1H, OH, D_2O exchangeable). ^{13}C NMR spectrum, δ_C, ppm: 13.14, 69.5, 114.8, 115.8, 118.2, 120.4, 122.6, 124.3, 124.8, 128.7, 128.8, 137.42, 138.44, 148.53, 151.82, 161.97, 163.06. Mass spectrum, m/z (I_{rel}, %): 290 (12) $[M]^+$, 246 (20), 190 (15), 177 (22), 115 (30), 77 (94), 42 (100). Found, %: C 70.30; H 4.82; N 19.28. $C_{17}H_{14}N_4O$. Calculated, %: C 70.33; H 4.86; N 19.30. M 290.

6-Amino-4-(4-hydroxyphenyl)-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (13). A mixture of compound **5** (2 g, 0.01 mol), ethyl cyanoacetate (1 mL, 0.01 mol), and ammonium acetate (11 g) in acetic acid (20 mL) was refluxed for 2 h. The mixture was cooled to room temperature and poured into ice water, and the crystals were collected by filtration and recrystallized from ethanol. Yield 40%, dark brown crystals, mp 180–182°C. IR spectrum, ν, cm^{-1}: 3417 (O–H), 3136 (NH_2), 3066 (C–H_{arom}), 2920 (C–H_{aliph}), 1654 (C=O), 1597 (C=N). ^{1}H NMR spectrum, δ, ppm: 2.29 s (3H, CH_3), 3.91 br.s (2H, NH_2), 4.87 s, 2H, NH_2), 6.66 d (2H, J = 8 Hz, H_{arom}), 7.04 d (2H, J = 8 Hz, H_{arom}), 7.21–7.94 m (5H, Ph), 9.17 s (1H, OH, D_2O exchangeable). ^{13}C NMR spectrum, δ_C, ppm: 11.65, 114.89, 115.8, 118.3, 120.5, 125.5, 128.8, 128.9, 132.3, 137.44, 146.2, 155.54, 162.32. 359 (20) $[M]^+$, 248 (30), 143 (25), 115 (100), 78 (35), 63 (43), 50 (32). Found, %: C 66.82; H 4.75; N 19.47. $C_{20}H_{17}N_5O_2$. Calculated, %: C 66.84; H 4.77; N 19.49. M 359.

4-(4-Hydroxyphenyl)-3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15). A mixture of compound **5** (2 g, 0.01 mol), ethyl acetooacetate (1.3 mL, 0.01 mol), and ammonium acetate (11 g) in acetic acid (20 mL) was refluxed for 2 h. The mixture was cooled to room temperature and poured into ice water, and the crystals were collected by filtration and recrystallized from ethanol. Yield 45%, dark brown crystals mp 100–102°C. IR spectrum, ν, cm^{-1}: 3417 (O–H), 3197 (NH_2), 3066 (C–H_{arom}), 2904 (C–H_{aliph}), 1701 (C=O), 1597 (C=N). ^{1}H NMR spectrum, δ, ppm: 1.64 s (3H, CH_3), 2.09 s (3H, CH_3), 4.83 s (2H, NH_2), 6.66 d (2H, J = 8 Hz, H_{arom}), 7.04 d (2H, J = 8 Hz, H_{arom}), 7.21–7.94 m (5H, Ph), 11.05 s (1H, OH, D_2O exchangeable). Mass spectrum, m/z (I_{rel}, %): 358 (10) $[M]^+$, 260 (20), 226 (22), 248 (30), 143 (25), 115 (100), 78 (35), 63 (43), 50 (32). Found, %: C 70.36; H 5.04; N 15.60. $C_{21}H_{18}N_4O_2$. Calculated, %: C 70.38; H 5.06; N 15.63. M 358.

2,4-Dichloro-*N*-(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazole-4-carbothioyl)benzamide (16). A mixture of compound **3** (1.7 g, 0.01 mol) and 2,4-dichlorobenzoyl isothiocyanate (0.01 mol) in dioxane (20 mL) was refluxed for 1 h. The mixture was allowed to cool to room temperature and poured into an ice-cooled mixture of water and acetic acid, and the precipitate was filtered off, dried, and recrystallized from water and DMF. Yield 60%, brown crystals, mp 98–

100°C. IR spectrum, ν, cm^{-1}: 3375 (O–H), 3186 (C–H_{arom}), 3062 (C–H_{aliph}), 1647 (C=O), 1620 (C=O), 1589 (C=N), 1554 (C=C), 1256 (C=S). ^1H NMR spectrum, δ, ppm: 2.35 s (3H, CH_3), 7.15–7.45 m (5H, Ph), 7.65–8.25 m (3H, H_{arom}), 9.58 s (1H, NH, D_2O exchangeable), 11.65 s (1H, OH, D_2O exchangeable), 11.98 s (1H, SH, D_2O exchangeable). Found, %: C 53.19; H 3.20; N 10.32; S 7.87. $C_{18}H_{13}C_{l2}N_3O_2$. Calculated, %: C 53.21; H 3.23; N 10.34; S 7.89.

6-(2,4-Dichlorophenyl)-3-methyl-1-phenyl-1,7a-dihydropyrazolo[4,3-*e*][1,3]oxazine-4(3a*H*)-thione (17). A mixture of compound **16** (0.01 mol) and sodium hydroxide (0.01 mol) in ethanol (20 mL) was refluxed for 2 h. The mixture was allowed to cool to room temperature and poured into ice water, and the precipitate was filtered off, dried, and recrystallized from ethanol. Yield 55%, rose crystals, mp 190–192°C. IR spectrum, ν, cm^{-1}: 3186 (C–H_{arom}), 2904 (C–H_{aliph}), 1651 (C=N), 1620 (C=C), 1242 (C=S). ^1H NMR spectrum, δ, ppm: 2.49 s (3H, CH_3), 7.42–7.48 m (5H, Ph), 7.61–7.92 m (3H, H_{arom}). ^{13}CNMR spectrum, δ_C, ppm: 13.14, 119.25, 120.45, 122.23, 125.22, 126.21, 127.01, 127.26, 129.15, 130.44, 130.91, 134.20, 135.9, 167.3. Mass spectrum, *m*/*z* (I_{rel}, %): 388 (100) $[M]^+$, 390 (50) $[M + 2]^+$, 392 (10) $[M + 4]^+$, 353 (25), 338 (32), 109 (22), 80 (45), 67 (48), 57 (32), 44 (62). Found, %: C 55.66; H 2.84; N 10.79; S 8.24. $C_{18}H_{11}C_{l2}N_3OS$. Calculated, %: C 55.68; H 2.86; N 10.82; S 8.26. *M* 388.

4-Methyl-6-phenylpyrazolo[3,4-*c*]pyrazole-3(6*H*)-thione (19). A mixture of compound **3** (1.7 g, 0.01 mol), thiosemicarbazide (0.91 g, 0.01 mol), and sodium acetate (0.8 g, 0.01 mol) in acetic acid (10 mL) was refluxed for 6 h. The mixture was cooled to room temperature and poured into ice water, and the precipitate was filtered off, dried, and recrystallized from ethanol. Yield 40%, yellow crystals, mp 195–196°C. IR spectrum, ν, cm^{-1}: 3059 (C–H_{arom}), 2908 (C–H_{aliph}), 1616 (C=N), 1496 (C=C), 1292 (C=S). ^1H NMR spectrum, δ, ppm: 2.30 s (3H, CH_3), 7.26–7.94 m (5H, Ph). Mass spectrum, *m*/*z* (I_{rel}, %): 230 (100) $[M]^+$, 246 (20), 190 (15), 177 (22), 115 (30), 77 (2). Found, %: C 57.35; H 4.35; N 24.30; S 13.90. $C_{11}H_{10}N_4S$. Calculated, %: C 57.37; H 4.38; N 24.33; S 13.92. *M* 230.

4′,5-Dimethyl-2,6′-diphenyl-2,4-dihydro-6′*H*-spiro[pyrazole-3,3′-pyrazolo[3,4-*c*]pyrazole] (22). A mixture of compound **3** (1.7 g, 0.01 mol) and hydrazine hydrate (0.5 mL, 0.01 mol) in butan-1-ol (20 mL) was refluxed for 6 h. The mixture was cooled to room temperature and poured into ice water, and the precipitate was filtered off, dried, and recrystallized from ethanol. Yield 45%, dark brown crystals, mp 280–282°C. IR spectrum, ν, cm^{-1}: 3066 (C–H_{arom}), 2688 (C–H_{aliph}), 1597 (C=N), 1558 (C=C). ^1H NMR spectrum, δ, ppm: 0.85 s (3H, CH_3), 1.22 s (3H, CH_3), 2.50 d.d (2H, CH_2), 7.75–7.17 m (10H, Ph). Mass spectrum, *m*/*z* (I_{rel}, %): 342 (12) $[M]^+$, 327 (20), 131 (100), 105 (25), 77 (39), 77 (100). Found, %: C 70.14; H 5.28; N 24.50. $C_{20}H_{18}N_6$. Calculated, %: C 70.16; H 5.30; N 24.54. *M* 342.

Antimicrobial evaluation. The synthesized compounds were evaluated against gram positive (*S. aureus*) and gram negative bacteria (*E. coli*), as well as fungi (*C. albicans*). Each compound was dissolved in DMSO to a concentration of 1 mg/mL. Whatman filter paper discs with a standard size (5 cm) were prepared and sterilized in an autoclave. The paper discs were soaked in a solution of a compound to be tested with a required concentration and were placed aseptically in Petri dishes containing nutrient agar media (agar 20 g + beef extract 3g + peptone 5 g) seeded with *S. aureus*, *E. coli*, or *C. albicans*. The Petri dishes were incubated at 36°C, and the inhibition zones were measured after incubation for 24 h. Each treatment was replicated three times. Ampicillin and clotrimazole were used as reference drugs [21]. The activity index was calculated as the percent ratio of the inhibition zone diameters for a test compound and standard drug.

CONFLICT OF INTEREST

The authors declared the absence of conflict of interest.

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