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Synthesis, Structure, and Antibacterial Activity of Novel 5-Arylpyrazole Derivatives

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A series of novel 1-(acetyl,carboxamide,carbothioamide)substituted-5-(substituted-phenyl)-3-methy-4,5-dihydropyrazole derivatives have been synthesized and characterized by ^{1}H NMR, IR, ESI-MS, and elemental analysis. Compounds **6h** and **6q** were further characterized by single crystal X-ray structural analysis. All of the compounds have been screened for their antibacterial potential in vitro against *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 35218, *Pseudomonas fluorescens* ATCC 13525, and *Staphylococcus aureus* ATCC 6538. Among the tested compounds, some of them display significant activity against the tested strains, and compounds **5ac** and **6h** show potent activity with a minimum inhibitory concentration value of 1.562 μg mL^{-1} against *B. subtilis* ATCC 6633, which is comparable to the positive control penicillin. Structure–effect relationships are also discussed based on the experimental data.

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Introduction

The identification of novel structures that can be potentially useful in the design of new, potent antibacterial agents is still a major challenge to medicinal chemistry researchers. Many pyrazole derivatives are acknowledged to possess a wide range of bioactivities that include antibacterial activity.[1–6] Much attention has been directed towards pyrazole derivatives as antimicrobial agents after the discovery of pyrazofurin, which demonstrated a broad spectrum of antimicrobial activity.[7] Hoffmann–La Roche's group[8,9] has developed a new lead DNA gyrase inhibitor (compound **1**, Fig. 1), which has strong inhibitory activity against DNA gyrase, and this inhibitory effect can cause bacterial cell death.[10] Recently, it was reported that compounds **2a**, **2b**, and **3** (Fig. 1) are selective inhibitors of DNA gyrase with moderate antibacterial activity.[11,12] Among the compounds mentioned above, it can be seen that those that contain a diaryl pyrazole template show weak antibacterial activity. A possible reason is that these compounds cannot penetrate the membrane of bacterial cells easily. Motivated by the aforementioned findings, our aim here is to find new, potent DNA gyrase inhibitors with high antibacterial activity.

Among the previous research, few reports have been dedicated to the synthesis and antibacterial activity evaluation of 1-(acetyl,carboxamide,carbothioamide)substituted-5-(substituted-phenyl)-3-methy-4,5-dihydropyrazole derivatives, therefore, we have designed and synthesized a series of novel 5-aryl pyrazole and diaryl pyrazole derivatives that have a similar structure to compound **3** (Fig. 1). The antibacterial activity of the prepared compounds have been tested against *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 35218, *Pseudomonas fluorescens* ATCC 13525, and *Staphylococcus aureus* ATCC 6538 using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric method. The results show that some compounds in this series exhibited potent antibacterial activity against *B. subtilis* ATCC 6633 and *P. fluorescens* ATCC 13525, and compounds **5ac** and **6h** display potent activity with a minimum inhibitory concentration (MIC) value of 1.562 μg mL^{-1} against *B. subtilis* ATCC 6633, which is comparable to the positive control penicillin.

Results and Discussion

Synthesis

In order to optimize the reaction conditions for preparation of compound **4**,[13] the synthesis of **4b** was carried out under different conditions. The effects of solvent system, base, reaction time, and temperature are summarized in Table 1. A yield as high as 75.8% can be achieved when a mixture of sodium hydroxide and 4-hydroxybenzaldehyde (2:1, mol ratio) is reacted in ethanol at 25°C for 20 h. Compound **5** was obtained by cyclization of the α,β-unsaturated ketones, in which different a solvent was selected, such as *n*-butanol[14] and *N*,*N*-dimethylformamide (DMF). Compound **5bd** was obtained on refluxing in DMF but not in *n*-butanol. The reaction conditions for the synthesis of compounds **5** are illustrated in Table 2.

Compounds **6g**–**p** were obtained in chloroform by refluxing for 6 h with pyridine as catalyst. No product was obtained when the temperature was below 40°C and the reaction time was less

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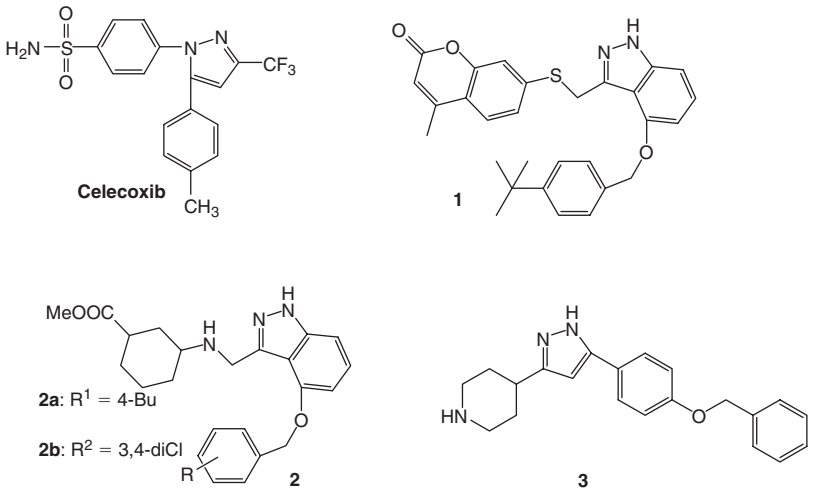


Fig. 1.

Table 1. Yields of 4b at different reaction conditions
—, no product

Entry	Solvent system	Base proportion[A]	Time [h]	Temp. [°C]	Yield[B] [%]
1	CH_3COCH_3-H_2O	1:1	20	25	—
2	CH_3COCH_3-H_2O	1:2	24	30	—
3	CH_3COCH_3-C_2H_5OH	1:1.5	10	20	35.8
4	CH_3COCH_3-C_2H_5OH	1:2	15	15	69.7
5	CH_3COCH_3-C_2H_5OH	1:2	24	28	73.2
6	CH_3COCH_3-C_2H_5OH	1:2	20	25	75.8

[A]Mol of 4-hydroxybenzaldehyde: mol of sodium hydroxide.
[B]Isolated yields after recrystallization, yields are based on 4-hydroxybenzaldehyde.

Table 2. The reaction conditions for the transformation to 5

Entry	Solvent	Reagent	Time [h]	Temp [°C]	Yield [%]
5ac	CH_3COOH (98%)	$N_2H_4{\cdot}H_2O$	3	Reflux	79.8
5bc	CH_3COOH (98%)	$N_2H_4{\cdot}H_2O$	3	Reflux	70.5
5bd	DMF	Semicarbazide	3	Reflux	31.2
5be	*n*-butanol	Thiosemicarbazide	6	Reflux	30.1
5bf	C_2H_5OH	$N_2H_4{\cdot}H_2O$	3	Reflux	50.2

than 3 h. The effect of the position of the hydroxy group of the 5-arylpyrazole on the yields of compounds **6g**–**p** was studied, and it can be concluded that *para*-substituted hydroxy groups afforded higher yields than *ortho*-substituted hydroxy groups. A possible reason may be steric hindrance effects.

To optimize the reaction conditions for the preparation of compound **6q** (**6r**), the synthesis was carried out with different bases, such as $NaHCO_3$, $KHCO_3$, Et_3N, NaOH, and pyridine. It was found that the yield was up to 62.1% when the reaction mixture was refluxed for 10 h in acetone catalyzed by NaOH. In addition, the synthesis conditions of **7s** (**7t**) is in analogy with **6q**, however, amide was obtained instead of benzoate under the similar conditions. The presence of the amide

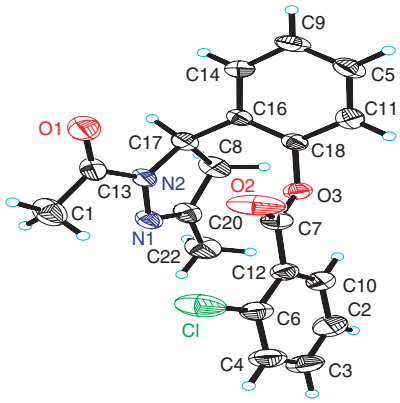


Fig. 2. ORTEP drawing of **6h**.

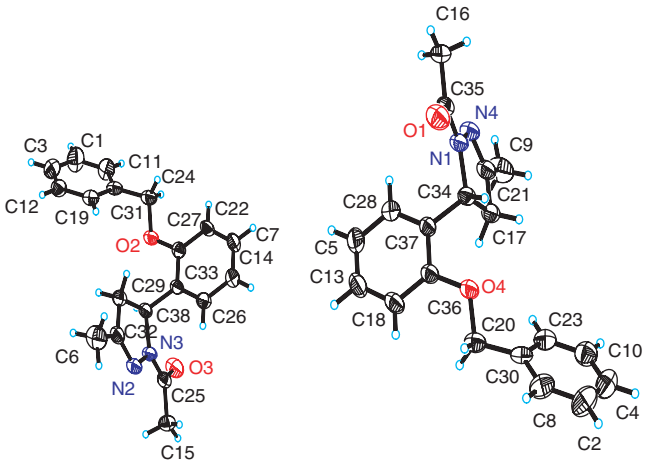


Fig. 3. ORTEP drawing of **6q**.

(–CONH–) and hydroxy (–OH) groups were confirmed by ^{1}H NMR spectroscopy, the signals of which were easily recognized at δ_H 1.61 (br s, 1H) and 5.17 (br s, 1H).

The single-crystal X-ray structure of compounds **6h** and **6q** are illustrated in Figs 2 and 3.

Table 3. Minimum inhibitory concentrations (MIC, $\mu g\,mL^{-1}$) of the synthesized compounds

Compounds	Microorganisms			
	Gram positive		Gram negative	
	B. subtilis ATCC 6633	*S. aureus* ATCC 6538	*P. aeruginosa* ATCC 13525	*E. coli* ATCC 35218
5ac	1.562	>50	3.125	>50
5bc	12.5	>50	12.5	12.5
5bd	12.5	>50	12.5	>50
5be	12.5	>50	12.5	>50
5bf	12.5	>50	12.5	>50
6g	6.25	>50	6.25	6.25
6h	1.562	>50	12.5	>50
6i	12.5	>50	3.125	6.25
6j	3.125	>50	12.5	>50
6k	12.5	>50	12.5	>50
6l	6.25	>50	6.25	>50
6m	12.5	>50	12.5	>50
6n	12.5	>50	12.5	>50
6o	12.5	>50	12.5	>50
6p	12.5	>50	12.5	>50
6q	3.125	>50	6.25	12.5
6r	12.5	>50	12.5	>50
7s	6.25	>50	12.5	>50
7t	6.25	>50	6.25	>50
Penicillin	1.562	1.562	6.25	6.25
Kanamycin	0.39	1.562	3.125	3.125
Novobiocin	0.78	3.125	1.562	3.125

In **6h**, the bond length of C(20)–N(1) (1.278(5) Å) is shorter than that of a typical C=N bond (1.34 Å), which is indicative of significant double bond character; the C(17)–N(2) (1.468(3) Å) distance is in line with a normal C–N (1.47 Å) distance, which is indicative of a single bond. The bond angles of C(17)–N(2)–N(1), C(8)–C(20)–N(1), and C(20)–N(1)–N(2) are 113.6(3)°, 114.5(7)°, and 107.6(3)°, respectively. The benzene ring I [C(2), C(3), C(4), C(6), C(10), C(12)] is fairly planar, and the deviation from the least-squares plane through the ring atoms is 0.0024°. While for the second benzene ring II [C(5), C(9), C(11), C(16), C(18), C(14)], the deviation from the least-squares plane through the ring atoms is 0.0034°. The dihedral angles between the plane of the pyrazole ring [N(1), N(2), C(8), C(17), C(20)] and the planes of benzene ring I and benzene ring II are 88.4(9)° and 86.6(8)°, respectively.

In **6q**, the bond length of C(32)–N(2) (1.281(4) Å) is shorter than that of typical a C=N bond (1.34 Å), which is indicative of significant double bond character; the C(38)–N(3) (1.471(3) Å) distance is in line with a normal C–N (1.47 Å) distance, which is indicative of a single bond. The bond angles of C(38)–N(3)–N(2) and N(3)–N(2)–C(32) are 113.1(2)° and 107.4(2)°, respectively. The benzene ring I [C(3), C(12), C(19), C(31), C(11), C(1)] is fairly planar, and the deviation from the least-squares plane through the ring atoms is 0.0039°. While for the second benzene ring II [C(27), C(22), C(7), C(14), C(26), C(33)], the deviation from the least-squares plane through the ring atoms is 0.0111°. The dihedral angles between the plane of the pyrazole ring [N(2), N(3), C(38), C(29), C(32)] and the planes of benzene ring I and benzene ring II are 129.8(5)° and 109.6(4)°, respectively.

In-Vitro Antibacterial Assay

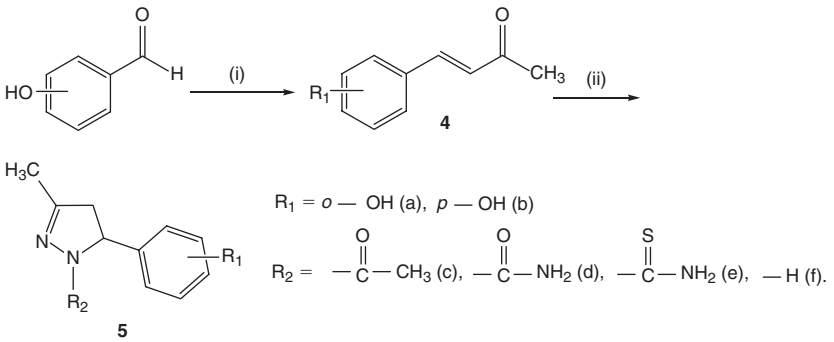
All the synthesized compounds were screened for antibacterial activity against two Gram-positive bacterial strains (*B. subtilis* ATCC 6633 and *S. aureus* ATCC 6538) and two Gram-negative bacterial strains (*E. coli* ATCC 35218 and *P. fluorescens* ATCC 13525) by the MTT method. The MICs of the compounds against the four strains are presented in Table 3. The antibacterial activity of reference compounds kanamycin, penicillin, and novobiocin are also included.

Compounds **5ac** and **6h** display potent activity with MIC values of $1.562\,\mu g\,mL^{-1}$ against *B. subtilis* ATCC 6633, which is comparable to the positive control penicillin. Compounds **6g**, **6j**, **6l**, **6q**, **7s**, and **7t** show moderate antibacterial activity against *B. subtilis* ATCC 6633 with MIC values of 3.125–$6.25\,\mu g\,mL^{-1}$, while the other compounds display mild antibacterial activity against *B. subtilis* ATCC 6633 with MIC values of $12.5\,\mu g\,mL^{-1}$.

Compounds **5ac** and **6i** also exhibit significant antibacterial activity, it being equal to that of kanamycin against *P. aureus* ATCC 13525 with MIC values of $3.125\,\mu g\,mL^{-1}$, while compounds **6g**, **6l**, **6q**, and **7t** show moderate antibacterial activity against *P. aureus* ATCC 13525 with MIC values of $6.25\,\mu g\,mL^{-1}$. Other compounds display mild antibacterial activity against *P. aureus* ATCC 13525 with MIC values of $12.5\,\mu g\,mL^{-1}$.

Among all the synthesized compounds, compounds **6g** and **6i** exhibit high antibacterial activity against *E. coli* ATCC 35218, while compounds **5bc** and **6q** display moderate antibacterial activity against *E. coli* ATCC 35218 with MIC values of $12.5\,\mu g\,mL^{-1}$. Other compounds show mild antibacterial activity against *E. coli* ATCC 35218 with MIC values of $50\,\mu g\,mL^{-1}$. However, the compounds prepared were relatively inactive against *S. aureus* ATCC 6538 with MIC values of more than $50\,\mu g\,mL^{-1}$ compared with the three tested strains above.

Based on the experimental data presented in Table 3, it can be concluded that *N*-acetyl arylpyrazole derivatives exhibit higher



Scheme 1. Reagent and conditions: (i) CH_3COCH_3, NaOH, EtOH, 25°C, 15 h; (ii): (**5ac**, **5bc**) $N_2H_4{\cdot}H_2O$, 98% CH_3COOH, reflux, 2 h. (**5bd**) semicarbazide, DMF, reflux, 4 h. (**5be**) thiosemicarbazide, *n*-butanol, reflux, 5 h. (**5bf**) $N_2H_4{\cdot}H_2O$, C_2H_5OH, reflux, 3 h.

antibacterial activity against selected strains than thiosulfate arylpyrazole and acetamide arylpyrazole derivatives. Furthermore, the fluorine and chlorine substituents play an important role on the antibacterial activity. In addition, the compounds that have phenolic hydroxy groups show a higher antibacterial activity against the tested strains than other compounds.

The biological activity of a particular substance depends on a complex sum of individual properties, which includes compound structure, affinity for the target site, survival in the medium of application, survival within the biological system, transport properties, and state of the target organism.[15] Structural analysis of these compounds indicates that the 1-acetyl-4,5-dihydro-3-methyl-5-(2-substitued-phenyl)-1*H*-pyrazole moiety may have potential antibacterial activity and deserves further investigation.

Conclusions

In this paper, a series of novel 1-(acetyl,carboxamide, carbothioamide)substituted-5-(substituted-phenyl)-3-methy-4,5-dihydropyrazole derivatives were synthesized and screened for their antibacterial potential in vitro against *B. subtilis* ATCC 6633, *E. coli* ATCC 35218, *P. fluorescens* ATCC 13525, and *S. aureus* ATCC 6538. Among the tested compounds, some of them displayed significant activity against the tested strains, and compounds **5ac** and **6h** displayed potent activity with MIC values of 1.562 $\mu g\,mL^{-1}$ against *B. subtilis* ATCC 6633, which was comparable to the positive control penicillin. Structural analysis of these compounds indicated that the 1-acetyl-4,5-dihydro-3-methyl-5-(2-substitued-phenyl)-1*H*-pyrazole moiety has potential antibacterial activity and deserves further investigation.

Experimental

General

Melting points were measured uncorrected. ^1H NMR spectra were recorded on a Varian INOVA300 (300 MHz) pulse Fourier-transform NMR spectrometer in $CDCl_3$ or (D_6)DMSO using tetramethylsilane as an internal standard. Elemental analyses were performed with a Vario-III CHN analyzer and were within ±0.4% of the theoretical values. Electrospray ionization (ESI) mass spectra were obtained on a Mariner System 5303 mass spectrometer. The single crystal structure was determined on a Bruker SMART 1000 CCD diffractometer. The reagents were all of analytical grade or chemically pure. The antibacterial activity of the reference compounds kanamycin, novobiocin (Nanjing Zhuyan Biotechnology Co. Ltd, Amresco 060D0504, Nanjing 210002, P. R. China), and penicillin (North China Pharmaceutical Co. Ltd, D0211107, Hebei 050015, P. R. China) are included.

Synthesis

4-(4-Hydroxyphenyl)but-3-en-2-one **4b**

Aqueous sodium ethanol solution (16.0 mmol) was added to a solution of salicylaldehyde (8.2 mmol) and acetone (82 mmol) at room temperature. The resulting yellow solution was then stirred at 25°C for 15 h. A brown mixture was obtained that was acidified by dropwise addition of aqueous HCl (12 mmol) at 25°C. The resulting solid was dried under vacuum to yield **4b** (1.01 g, 75.8%), mp 45–46°C. δ_H ($CDCl_3$) 2.38 (s, 3H), 5.96 (br s, 1H, OH), 6.58 (d, *J* 16.1, 1H), 6.87–7.46 (m, 4H, ArH), 7.51 (d, *J* 13.0, 1H). Compound **4a** was synthesized according to the same method.

4-(2-Hydroxyphenyl)but-3-en-2-one **4a**

Yield 1.07 g, 80.3%, mp 77–78°C. δ_H ($CDCl_3$) 2.29 (s, 3H), 6.08 (br s, 1H, –OH), 6.49 (d, *J* 16.1, 1H), 6.62–7.20 (m, 4H, ArH), 7.88 (d, *J* 13.5, 1H).

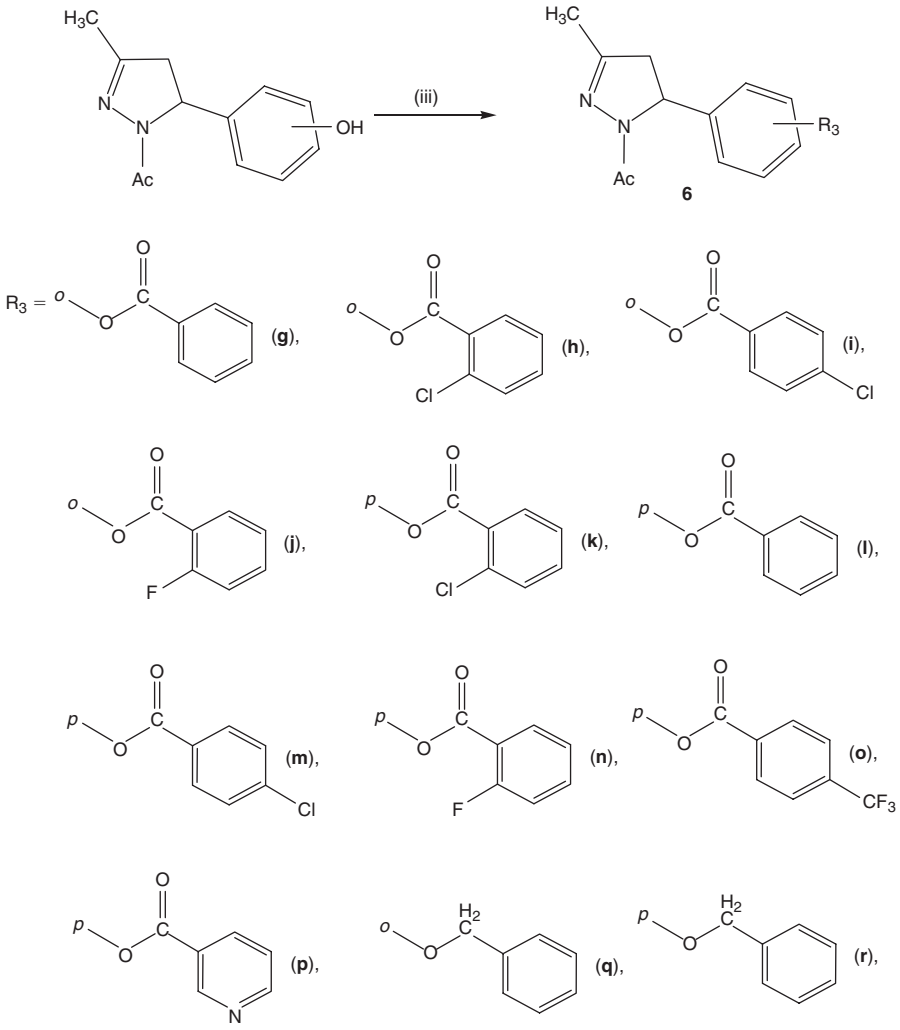
1-Acetyl-4,5-dihydro-3-methyl-5-(2-hydroxyphenyl)-1H-pyrazole **5ac**

To a solution of 4-(2-hydroxyphenyl)but-3-en-2-one (10 mmol) in acetic acid (20 mL) was added hydrazine monohydrate (40 mmol) and the reaction mixture was refluxed for 2 h. The mixture was cooled, poured into crush ice, and allowed to stand at room temperature overnight. The solid product was collected by filtration and recrystallized from acetone to give the title compound **5ac** as colourless crystals. Yield 58.7%, mp 175–176°C. δ_H ($CDCl_3$) 2.10 (s, 3H, Me), 2.22 (s, 3H, Me), 2.88 (dd, *J* 18.3 and 3.1, 1H, $4H_a$), 3.42 (dd, *J* 18.2 and 11.2, 1H, $4H_b$), 5.56 (dd, *J* 11.1 and 3.0, 1H, 5H), 6.69–7.10 (m, 4H, ArH), 9.19 (br s, 1H, OH). ν_{max} (KBr)/cm^{-1} 1648 (C=O), 1627 (C=N), 3430 (–OH). *m/z* (ESI) 218.0 ($C_{12}H_{14}N_2O_2$, $[M+H]^+$). (Found: C 66.12, H 6.21, N 12.59. Calc. for $C_{12}H_{14}N_2O_2$: C 66.04, H 6.47, N 12.84%.)

Compounds **5bc**–**5bf** (Scheme 1) were synthesized according to the same method. The reagents and solvents are shown in Table 2.

1-Acetyl-4,5-dihydro-3-methyl-5-(4-hydroxyphenyl)-1H-pyrazole **5bc**

The title compound was obtained as colourless crystals. Yield 61.3%, mp 210–211°C. δ_H ((D_6)DMSO) 1.98 (s, 3H, Me), 2.11



Scheme 2. Reagents and conditions: (iii): (a) acid chloride, pyridine, $CHCl_3$, reflux, 6 h. (b) 1-(chloromethyl)benzene, NaOH, CH_3COCH_3, reflux, 10 h.

(s, 3H, Me), 2.48 (dd, *J* 18.3 and 3.1, 1H, $4H_a$), 3.31 (dd, *J* 18.2 and 11.2, 1H, $4H_b$), 5.44 (dd, *J* 11.1 and 3.0, 1H, 5H), 6.64–6.67 (d, *J* 9.0, 2H), 6.90–6.92 (d, *J* 8.7, 2H), 9.28 (s, 1H, OH). ν_{max} (KBr)/cm^{-1} 1644 (C=O), 1620 (C=N), 3427 (OH). *m*/*z* (ESI) 218.2 ($C_{12}H_{14}N_2O_2$, $[M+H]^+$). (Found: C 65.82, H 6.42, N 12.66. Calc. for $C_{12}H_{14}N_2O_2$: C 66.04, H 6.47, N 12.84%.)

*1-Acetamide-4,5-dihydro-3-methyl-5-(4-hydroxyphenyl)-1*H*-pyrazole* ***5bd***

The title compound was obtained as colourless crystals. Yield 31.2%, mp 215–216°C. δ_H ((D_6)DMSO) 2.01 (s, 3H, Me), 2.80 (dd, *J* 18.2 and 3.2, 1H, $4H_a$), 3.76 (dd, *J* 18.3 and 11.7, 1H, $4H_b$), 5.58 (dd, *J* 3.2 and 11.3, 1H, 5H), 6.69 (d, *J* 9.0, 2H), 6.94 (d, *J* 8.8, 2H), 7.61–9.87 (br, 3H, OH, NH_2). ν_{max} (KBr)/cm^{-1} 3432 (–OH), 3340 and 3191 (NH_2), 1668 (C=O), 1624 (C=N). *m*/*z* (ESI) 219.4 ($C_{11}H_{13}N_3O_2$, $[M+H]^+$). (Found: C 60.44, H 5.68, N 18.83. Calc. for $C_{11}H_{13}N_3O_2$: C 60.26, H 5.98, N 19.17%.)

*1-Carbothioamide-4,5-dihydro-3-methyl-5-(4-hydroxyphenyl)-1*H*-pyrazole* ***5be***

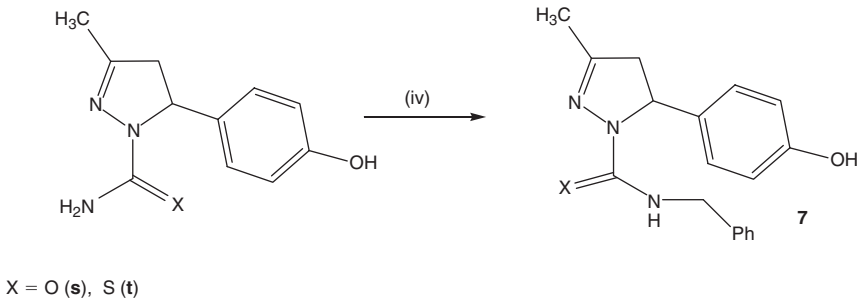
The title compound was obtained as colourless crystals. Yield 30.1%, mp 208–210°C. δ_H ((D_6)DMSO) 2.07 (s, 3H, Me), 2.77 (dd, *J* 18.4 and 3.3, 1H, $4H_a$), 3.80 (dd, *J* 18.4 and 11.4, 1H, $4H_b$), 5.44 (dd, *J* 3.3 and 11.4, 1H, 5H), 6.77 (d, *J* 9.0, 2H), 6.98 (d, *J* 8.7, 2H), 7.41–9.84 (br, 3H, OH, NH_2). ν_{max} (KBr)/cm^{-1} 3435 (–OH), 3347 and 3200 (NH_2), 1271 (C=S), 1627 (C=N). *m*/*z* (ESI) 235.0 ($C_{11}H_{13}N_3OS$, $[M+H]^+$). (Found: C 56.29, H 5.20, N 18.07. Calc. for $C_{11}H_{13}N_3OS$: C 56.15, H 5.57, N 17.86%.)

*1*H*-4,5-dihydro-3-methyl-5-(4-hydroxyphenyl)pyrazole* ***5bf***

The title compound was obtained as colourless crystals. Yield 40.2%, mp 155–156°C. δ_H ($CDCl_3$) 2.07 (s, 3H, Me), 2.89 (dd, J_1 10.9, J_2 16.1, 1H, $4H_a$), 3.97 (dd, J_1 10.9, J_2 16.1, 1H, $4H_b$), 5.37 (dd, J_1 3.6, J_2 10.8, 1H, 5H), 5.42 (br s, 1H, –OH), 6.44 (br s, 1H, NH), 6.72 (d, *J* 8.9, 2H), 6.98 (d, *J* 8.8, 2H). ν_{max} (KBr)/cm^{-1} 3443 (–OH), 3378 (NH), 1632 (C=N). *m*/*z* (ESI) 175.9 ($C_{10}H_{12}N_2O$, $[M+H]^+$). (Found: C 68.40, H 7.02, N 16.04. Calc. for $C_{10}H_{12}N_2O$: C 68.16, H 6.86, N 15.90%.)

General Procedure for the Preparation of ***6g–p***

To a solution of the 1-(5-(substituted-hydroxyphenyl)-3-methyl-4,5-dihydropyrazol-1-yl)ethanone (2 mmol), pyridine (0.010 mmol), and chloroform (30 mL), acid chloride (2.5 mmol)



Scheme 3. Reagent and conditions: (iv): 1-(cholormethyl)benzene, NaOH, CH_3COCH_3, reflux, 10 h.

was added dropwise at 25°C for 30 min. The reaction mixture was refluxed for 6 h and washed with 20 mL of H_2O and a 5% $NaHCO_3$ solution, respectively, and then dried over anhydrous $MgSO_4$. The product was collected by removing the solvent under vacuum, and the crude residue was purified with a silica gel column and was eluted with acetone/petroleum ether (4/1) to afford **6g**–**p** (Scheme 2).

*2-(1-Acetyl-3-methyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenyl Benzoate* ***6g***

The title compound was obtained as colourless crystals. Yield 46.2%, mp 135–136°C. δ_H ((D_6)DMSO) 1.81 (s, 3H, Me), 2.09 (s, 3H, Me), 2.63 (dd, J_1 5.4, J_2 18.3, 1H, $4H_a$), 3.38 (dd, J_1 12.0, J_2 18.2, 1H, $4H_b$), 5.40 (dd, J_1 5.4, J_2 12.0, 1H, 5H), 7.18–7.27 (m, 3H), 7.34–7.39 (m, 1H), 7.60–7.79 (m, 3H), 8.11 (d, J 7.2, 2H). ν_{max} (KBr)/cm^{-1} 1734 (COO), 1664 (C=O), 1600 (C=N). *m/z* (ESI) 322.9 ($C_{19}H_{18}N_2O_3$, $[M+H]^+$). (Found: C 71.01, H 5.42, N 8.77. Calc. for $C_{19}H_{18}N_2O_3$: C 70.79, H 5.63, N 8.69%.)

*2-(1-Acetyl-3-methyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenyl Chlorobenzoate* ***6h***

The title compound was obtained as colourless crystals. Yield 52.1%, mp 140–141°C. Crystal data of **6h**. $C_{19}H_{17}ClN_2O_3$, *M* 359.8, orthorhombic, space group *Pna*2(1), *a* 9.3230(19), *b* 15.291(3), *c* 12.440(3) Å, α 90°, β 90°, γ 90°, *V* 1773.4(6) nm^3, *T* 293(2) K, *Z* 4, D_c 1.336 g cm^{-3}, *F*(000) 744. Reflections collected/unique: 1503/1824, Fine, R_1 0.0390, $wR(F^2)$ 0.0948.

*2-(1-Acetyl-3-methyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenyl 4-Chlorobenzoate* ***6i***

The title compound was obtained as colourless crystals. Yield 56.1%, mp 158–159°C. δ_H ((D_6)DMSO) 2.00 (s, 3H, Me), 2.29 (s, 3H, Me), 2.75 (dd, J_1 4.4, J_2 18.1, 1H, $4H_a$), 3.39 (dd, J_1 11.5, J_2 18.0, 1H, $4H_b$), 5.48 (dd, J_1 4.6, J_2 11.7, 1H, 5H), 7.10–7.19 (m, 4H), 7.42 (d, J 8.4, 2H), 8.07 (d, J 8.4, 2H). ν_{max} (KBr)/cm^{-1} 1736 (COO), 1652 (C=O), 1610 (C=N). *m/z* (ESI) 356.9 ($C_{19}H_{17}ClN_2O_3$, $[M+H]^+$). (Found: C 63.74, H 5.07, N 7.71. Calc. for $C_{19}H_{17}ClN_2O_3$: C 63.96, H 4.80, N 7.85%.)

*2-(1-Acetyl-3-methyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenyl 2-Fluorobenzoate* ***6j***

The title compound was obtained as colourless crystals. Yield 45.2%, mp 151–152°C. δ_H ($CDCl_3$) 2.02 (s, 3H, Me), 2.41 (s, 3H, Me), 2.76 (dd, J_1 4.6, J_2 18.1, 1H, $4H_a$), 3.40 (dd, J_1 11.7, J_2 18.2, 1H, $4H_b$), 5.50 (dd, J_1 4.6, J_2 11.7, 1H, 5H), 7.08–7.20 (m, 6H), 7.47 (t, 1H), 8.10 (d, J 6.4, 1H). ν_{max} (KBr)/cm^{-1} 1742 (COO), 1647 (C=O), 1599 (C=N). *m/z* (ESI) 340.8 ($C_{19}H_{17}FN_2O_3$, $[M+H]^+$). (Found: C 66.89, H 4.82, N 8.01. Calc. for $C_{19}H_{17}FN_2O_3$: C 67.05, H 5.03, N 8.23%.)

*4-(1-Acetyl-3-methyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenyl 2-Chlorobenzoate* ***6k***

The title compound was obtained as colourless crystals. Yield 50.6%, mp 154–156°C. δ_H ($CDCl_3$) 2.07 (s, 3H, Me), 2.31 (s, 3H, Me), 2.74 (dd, J_1 4.4, J_2 18.1, 1H, $4H_a$), 3.37 (dd, J_1 11.7, J_2 18.1, 1H, $4H_b$), 5.47 (dd, J_1 4.4, J_2 11.7, 1H, 5H), 7.17–7.26 (m, 4H), 7.35–7.40 (m, 1H), 7.45–7.52 (m, 2H), 7.99 (d, J 6.4, 1H). ν_{max} (KBr)/cm^{-1} 1737 (COO), 1652 (C=O), 1592 (C=N). *m/z* (ESI) 356.2 ($C_{19}H_{17}ClN_2O_3$, $[M+H]^+$). (Found: C 64.12, H 5.04, N 7.61. Calc. for $C_{19}H_{17}ClN_2O_3$: C 63.96, H 4.80, N 7.85%.)

*4-(1-Acetyl-3-methyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenyl Benzoate* ***6l***

The title compound was obtained as colourless crystals. Yield 57.9%, mp 140–141°C. δ_H ($CDCl_3$) 2.04 (s, 3H, Me), 2.31 (s, 3H, Me), 2.74 (dd, J_1 4.6, J_2 18.1, 1H, $4H_a$), 3.37 (dd, J_1 11.7, J_2 18.1, 1H, $4H_b$), 5.48 (dd, J_1 4.6, J_2 11.5, 1H, 5H), 7.15 (d, J 8.6, 2H), 7.22 (d, J 8.8, 2H), 7.48–7.65 (m, 3H), 8.16 (d, J 7.1, 2H). ν_{max} (KBr)/cm^{-1} 1730 (COO), 1655 (C=O), 1600 (C=N). *m/z* (ESI) 322.0 ($C_{19}H_{18}N_2O_3$, $[M+H]^+$). (Found: C 71.1, H 5.8, N 9.0. Calc. for $C_{19}H_{18}N_2O_3$: C 70.8, H 5.6, N 8.7%.)

*4-(1-Acetyl-3-methyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenyl 4-Chlorobenzoate* ***6m***

The title compound was obtained as colourless crystals. Yield 61.2%, mp 174–175°C. δ_H ($CDCl_3$) 2.07 (s, 3H, Me), 2.31 (s, 3H, Me), 2.74 (dd, J_1 4.4, J_2 18.1, 1H, $4H_a$), 3.37 (dd, J_1 11.5, J_2 17.9, 1H, $4H_b$), 5.48 (dd, J_1 4.6, J_2 11.5, 1H, 5H), 7.16 (d, J 8.6, 2H), 7.26 (d, J 8.6, 2H), 7.49 (d, J 8.4, 2H), 8.12 (d, J 8.4, 2H). ν_{max} (KBr)/cm^{-1} 1731 (COO), 1662 (C=O), 1599 (C=N). *m/z* (ESI) 356.1 ($C_{19}H_{17}ClN_2O_3$, $[M+H]^+$). (Found: C 63.9, H 5.0, N 7.9. Calc. for $C_{19}H_{17}ClN_2O_3$: C 64.0, H 4.8, N 7.9%.)

*4-(1-Acetyl-3-methyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenyl 2-Fluorobenzoate* ***6n***

The title compound was obtained as colourless crystals. Yield 58.7%, mp 132–133°C. δ_H ((D_6)DMSO) 2.05 (s, 3H, Me), 2.32 (s, 3H, Me), 2.71 (dd, J_1 4.6, J_2 18.1, 1H, $4H_a$), 3.47 (dd, J_1 11.7, J_2 18.1, 1H, $4H_b$), 5.39 (dd, J_1 4.6, J_2 11.7, 1H, 5H), 7.12 (d, J 6.4, 2H, ArH), 7.16–7.44 (m, 5H, ArH), 8.14 (d, J 6.4, 1H). ν_{max} (KBr)/cm^{-1} 1743 (COO), 1658 (C=O), 1598 (C=N). *m/z* (ESI) 340.0 ($C_{19}H_{17}FN_2O_3$, $[M+H]^+$). (Found: C 67.0, H 5.2, N 8.4. Calc. for $C_{19}H_{17}FN_2O_3$: C 67.1, H 5.0, N 8.2%.)

4-(1-Acetyl-3-methyl-4,5-dihydro-1H-pyrazol-5-yl)phenyl 4-(Trifluoromethyl) Benzoate ***6o***

The title compound was obtained as colourless crystals. Yield 58.5%, mp 167–168°C. δ_H ($CDCl_3$) 2.00 (s, 3H, Me), 2.39 (s, 3H, Me), 2.71 (dd, J_1 4.4, J_2 18.1, 1H, $4H_a$), 3.52 (dd, J_1 11.7, J_2 18.1, 1H, $4H_b$), 5.44 (dd, J_1 4.4, J_2 11.7, 1H, 5H), 7.12 (d, J 8.6, 2H, ArH), 7.22 (d, J 8.6, 2H, ArH), 7.64 (d, J 8.4, 2H, CF_3-ArH), 8.10 (d, J 8.4, 2H, CF_3-ArH). ν_{max} (KBr)/cm^{-1} 1739 (COO), 1665 (C=O), 1621 (C=N). *m/z* (ESI) 390.8 ($C_{20}H_{17}F_3N_2O_3$, $[M+H]^+$). (Found: C 61.7, H 4.2, N 7.1. Calc. for $C_{20}H_{17}F_3N_2O_3$: C 61.5, H 4.4, N 7.2%.)

4-(1-Acetyl-3-methyl-4,5-dihydro-1H-pyrazol-5-yl)phenyl Nicotinate ***6p***

The title compound was obtained as colourless crystals. Yield 60.1%, mp 201–202°C. δ_H ($CDCl_3$), 2.03 (s, 3H, Me), 2.36 (s, 3H, Me), 2.77 (dd, J_1 4.6, J_2 18.0, 1H, $4H_a$), 3.49 (dd, J_1 11.7, J_2 18.0, 1H, $4H_b$), 5.47 (dd, J_1 4.6, J_2 11.7, 1H, 5H), 7.00 (d, J 8.6, 2H, ArH), 7.07 (d, J 8.6, 2H, ArH), 7.52 (q, 1H, PyH), 8.20 (d, J 6.8, 1H, PyH), 8.77 (d, J 6.8, 1H, PyH), 9.09 (s, 1H, PyH). ν_{max} (KBr)/cm^{-1} 1738 (COO), 1670 (C=O), 1621 (C=N). *m/z* (ESI) 322.8 ($C_{18}H_{17}N_3O_3$, $[M+H]^+$). (Found: C 67.0, H 5.1, N 13.4. Calc. for $C_{18}H_{17}N_3O_3$: C 66.9, H 5.3, N 13.0%.)

Procedure for the Preparation of ***6q***

1-(Chloromethyl)benzene (2.5 mmol) was added dropwise to a solution of **5a** (2 mmol), NaOH (0.010 mmol), and acetone (30 mL). The reaction mixture was refluxed for 10 h. The mixture was then cooled, acidified by dropwise addition of aqueous HCl (0.010 mmol), poured into crushed ice, and allowed to stand at room temperature overnight. The separated solid was washed by water, and recrystallized from ethyl acetate to afford compound **6q**. The structure was confirmed by X-ray crystallographic data. Compounds **6r**, **7s**, and **7t** were prepared following the same method.

1-(5-(2-(Benzyloxy)phenyl)-3-methyl-4,5-dihydropyrazol-1-yl)ethanone ***6q***

The title compound was obtained as colourless crystals. Yield 60.1%, mp 116–117°C. Crystal data for **6q**: $C_{19}H_{20}N_2O_2$, M 308.47, monoclinic, space group C, a 36.158(2), b 9.2767(11), c 21.5198(18) Å, α 90.00°, β 112.17(3)°, γ 90.00°, D_c 1.226 g cm^{-3}, Z 8, $F(000)$ 2624. Reflections collected/unique: 6537/2728, Fine, R_1 0.0675, wR_2 0.1531.

1-(5-(4-(Benzyloxy)phenyl)-3-methyl-4,5-dihydropyrazol-1-yl)ethanone ***6r***

The title compound was obtained as colourless crystals. Yield 65.2%, mp 90–91°C. δ_H ($CDCl_3$) 2.06 (s, 3H, Me), 2.29 (s, 3H, Me), 2.70 (dd, J_1 4.4, J_2 18.1, 1H, $4H_a$), 3.31 (dd, J_1 11.5, J_2 18.0, 1H, $4H_b$), 5.03 (s, 2H, $-CH_2-$), 5.38 (dd, J_1 4.4, J_2 11.5, 1H, 5H), 6.92 (d, J 8.4, 2H, ArH), 7.11 (d, J 8.4, 2H, ArH), 7.30–7.42 (m, 5H, ArH). ν_{max} (KBr)/cm^{-1} 1660 (C=O), 1612 (C=N), 1236 (–O–C–). *m/z* (ESI) 307.9 ($C_{19}H_{20}N_2O_2$, $[M+H]^+$). (Found: C 74.2, H 6.2, N 9.5. Calc. for $C_{19}H_{20}N_2O_2$: C 74.0, H 6.5, N 9.1%.)

N-Benzyl-5-(4-hydroxyphenyl)-3-methyl-4,5-dihydropyrazole-1-carboxamide ***7s*** *(Scheme 3)*

The title compound was obtained as colourless crystals. Yield 45.2%, mp 179–180°C. δ_H ($CDCl_3$) 1.61 (br s, 1H, NH), 2.04 (s, 3H, Me), 2.73 (dd, J_1 4.7, J_2 17.9, 1H, $4H_a$), 3.42 (dd, J_1 11.5, J_2 17.6, 1H, $4H_b$), 5.05 (s, 2H, CH_2), 5.17 (br s, 1H, OH), 5.34 (dd, J_1 5.1, J_2 11.5, 1H, 5H), 6.96 (d, 2H, J 8.6, ArH), 7.17 (d, 2H, J 8.6, ArH), 7.28–7.45 (m, 5H, ArH). ν_{max} (KBr)/cm^{-1} 3206 (NH), 1676 (C=O), 1629 (C=N). *m/z* (ESI) 309.0 ($C_{18}H_{19}N_3O_2$, $[M+H]^+$). (Found: C 69.8, H 6.0, N 13.2. Calc. for $C_{18}H_{19}N_3O_2$: C 69.9, H 6.2, N 13.6%.)

N-Benzyl-5-(4-hydroxyphenyl)-3-methyl-4,5-dihydropyrazole-1-carbothioamide ***7t***

The title compound was obtained as colourless crystals. Yield 48.2%, mp 185–186°C. δ_H ($CDCl_3$) 1.32 (br s, 1H, NH), 2.00 (s, 3H, Me), 2.78 (dd, J_1 4.6, J_2 17.9, 1H, $4H_a$), 3.49 (dd, J_1 11.5, J_2 17.6, 1H, $4H_b$), 5.04 (s, 2H, CH_2), 5.12 (br s, 1H, OH), 5.36 (dd, J_1 5.1, J_2 11.5, 1H, 5H), 7.01 (d, 2H, J 8.6, ArH), 7.14 (d, J 8.6, 2H, ArH), 7.28–7.47 (m, 5H, ArH). ν_{max} (KBr)/cm^{-1} 3154 (NH), 1286 (C=S), 1597 (C=N). *m/z* (ESI) 324.7 ($C_{18}H_{19}N_3OS$, $[M+H]^+$). (Found: C 66.3, H 6.1, N 12.9. Calc. for $C_{18}H_{19}N_3OS$: C 66.4, H 5.9, N 12.9%.)

Antibacterial Assay

The antibacterial activity of the synthesized compounds was tested against *B. subtilis* ATCC 6633, *E. coli* ATCC 35218, *P. fluorescens* ATCC 13525, and *S. aureus* ATCC 6538 using MH medium (Mueller–Hinton medium: casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract 1000 mL). The MICs of the test compounds were determined by a colorimetric method using the dye MTT.[16] A stock solution of the synthesized compound (100 μg mL^{-1}) in DMSO was prepared and graded quantities of the test compounds were incorporated in a specified quantity of sterilized liquid MH medium. A specified quantity of the medium that contained the compound was poured into microtitration plates. A suspension of the microorganisms was prepared to contain ~10^5 cfu mL^{-1} and applied to microtitration plates with serially diluted compounds in DMSO to be tested and incubated at 37°C for 24 h. After the MICs were visually determined on each of the microtitration plates, 50 μL of PBS (phosphate buffered saline 0.01 mol L^{-1}, pH 7.4, $Na_2HPO_4 \cdot 12H_2O$ 2.9 g, KH_2PO_4 0.2 g, NaCl 8.0 g, KCl 0.2 g, distilled water 1000 mL) that contained 2 mg of MTT per mL was added to each well. Incubation was continued at room temperature for 4–5 h. The content of each well was removed, and 100 μL of isopropyl alcohol that contained 5% 1 mol L^{-1} HCl was added to extract the dye. After 12 h of incubation at room temperature, the optical density was measured with a microplate reader at 570 nm.

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