Novel dihydropyrazole Derivatives Linked with 4H-Chromene: Microwave-Promoted Synthesis and Antibacterial Activity

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Abstract: Seven novel 6-(1-acetyl-3-methyl-4,5-dihydro-1H-pyrazol-5-yl)-2-amino-4-substituted-phenyl-4H-chromene-3-carbonitrile derivatives were synthesized and characterized by ESI-MS, ^{1}H NMR and ^{13}C NMR. All of the compounds have been screened for their antibacterial activity. The results show that compounds **7e** and **7f** displayed significant activity with MIC of 1.562 μg/mL against *B. subtilis* ATCC 6633.

Keywords: Microwave-promoted, synthesis, characterize, dihydropyrazole, 4H-chromene, antibacterial activity.

INTRODUCTION

Now, multidrug-resistant Gram-positive bacteria have started posing serious issues in medical science to deal with. To overcome the limitations of the known inhibitors, it has become imperative to identify new class of compound. Many pyrazole derivatives are well acknowledged to possess a wide range of antibacterial bioactivities [1-3]. Much attention was paid to pyrazole as a potential antimicrobial agent after the discovery of the natural pyrazole C-glycoside. Hoffmann-La Roche's group [4, 5] has developed a new lead DNA gyrase inhibitor (compound **1**, Fig. **1**). Recently, antidepressant, antihypertensive, anti-tubulin, antiviral, antioxidative [10].

Motivated by the aforementioned findings, we anticipate that the presence of 4H-chromene in the 4,5-dihydropyrazole moiety may play an important role in the antimicrobial activities. However, to date, few reports have been dedicated to the synthesis and antimicrobial activity evaluation of 4H-chromene -4,5-dihydropyrazole derivatives. Herein, in continuation to extend our research on antibacterial compounds containing 4,5-dihydropyrazole moiety, we designed a series of novel 4,5-dihydropyrazole derivatives

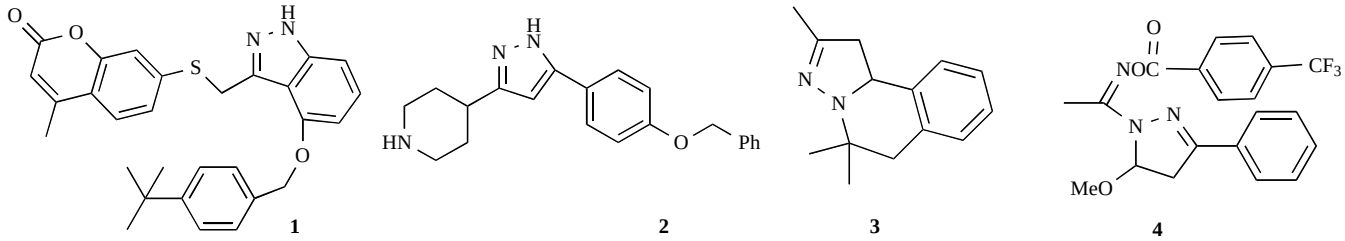

Fig. (1). Recently disclosed pyrazole as antibacterial inhibitors.

Tanitame, A. *et al.* [6] have found compound **2** (Fig. **1**) as potent and selective inhibitors of DNA gyrase. In our previous work, we found that compound **3** (Fig. **1**) possessed potent antibacterial activity. We also reported that some 4,5-dihydropyrazole derivatives and some 4,5-dihydropyrazole ethanone oxime ester derivatives compound **4** (Fig. **1**) showed antibacterial bioactivities [7-9].

4H-Chromenes are important classes of oxygenated heterocyclic compounds. More recently, there has been increased interest in the synthesis of 4H-chromene since bioactive natural product containing 4H-chromene ring system possesses wide spectrum of activities including as linked with 4H-chromene. The structures of novel compounds were confirmed by spectral analysis. The antibacterial activity of the synthesized compounds was tested against *B. subtilis* ATCC 6633, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 13525 and *S. aureus* ATCC 6538.

RESULTS AND DISCUSSION

The synthetic route to target compound was shown in Scheme **1**. Compounds **5, 6** (Scheme **1**) were synthesized according to the previously published report [11]. Phenol with malononitrile and substituted aldehyde proceeding in ethanolic piperidine to form chromenes was a classical procedure. This procedure employs piperidine as a hazardous organic base, refluxing for long hours and gives low yields of products. In order to optimize the reaction conditions and to greenify the classical method to a clean, economical

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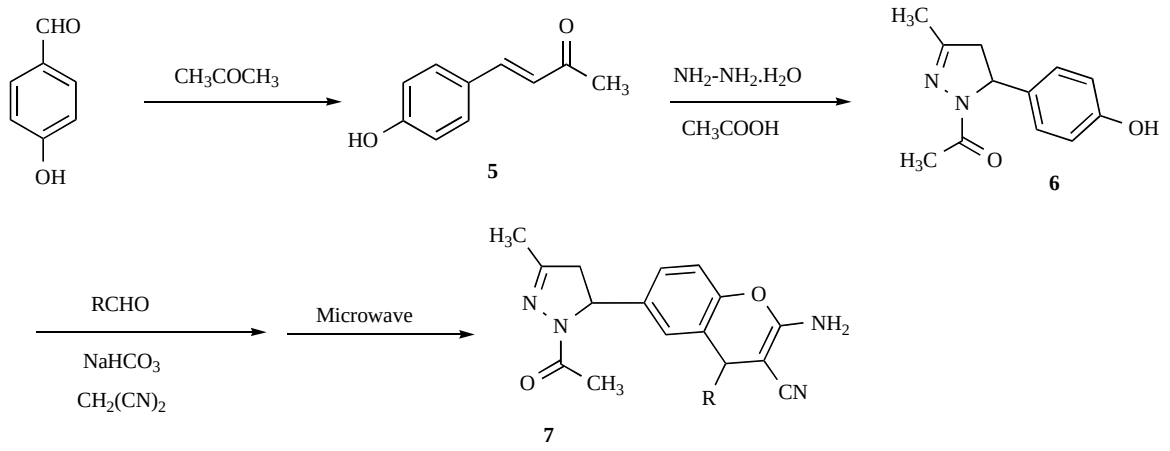

Scheme 1. Synthesis of dihydropyrazole derivatives Linked with 4H-Chromene.

7a: R= benzenyl; **7b**: R= 4-methoxy-benzenyl; **7c**: R= 2,4-dichloro-benzenyl; **7d**: R= 4-trifluoromethyl -benzenyl; **7e**: R= 2-furanyl; **7f**: R= 3-pyridinyl; **7g**: R= 2-naphthalenyl.

method for preparation of chromene, the syntheses were carried out in presence of various bases, for example: $NaHCO_3$, $KHCO_3$, Na_2CO_3, $NaCH_3CO_2$, pyridine. It was found that a yield up to 72% when the reaction mixture was refluxed for 20 min in ethanol under microwave irradiation catalyzed by $NaHCO_3$. Using the above optimal condition, compounds **7a-7g** were prepared.

The activities of synthesized compounds were tested against *B. subtilis* ATCC 6633, *E. coli* ATCC 35218, *P. fluorescens* ATCC 13525 and *S. aureus* ATCC 6538 which may be the cause agents of some serious infections in humans using MH medium (Mueller-Hinton medium: casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract 1000 mL). The MICs of the compounds against four bacteria are presented in Table **1**. Also included are the activities of reference compounds kanamycin, penicillin and novobiocin (Nanjing Zhuyan Biotechnology Co. Ltd, Amresco 060D0504, Nanjing 210002, China). The compounds **7e** and **7f** showed antibacterial activities against *B. subtilis* ATCC 6633 with the MIC of 1.562 μg/mL, comparable to that of positive control penicillin. The compounds **7a, 7c, 7e** and **7f** showed moderate antibacterial activities against *S. aureus* ATCC 6538 with MIC of 6.25 μg/mL. All the compounds showed poor antibacterial activities against *P. fluorescens* ATCC 13525 and *E. coli* ATCC 35218.

EXPERIMENTAL

Analysis and Instruments

Melting points were measured and not corrected. The ^1H NMR spectra were recorded on a Varian INOVA300 (300 MHz) pulse Fourier-transform NMR spectrometer in $CDCl_3$. ESI mass spectra were obtained on a Mariner System 5303 mass spectrometer. The studies were carried out in a microwave reactor (Discover, CEM-SP1245). Analytical TLC (E.Merck, Type 5554 plates) was performed on silica gel GF254. Column chromatographic purification was carried out using silica gel. All reagents were of analytical grade or chemically pure.

Table 1. Minimum Inhibitory Concentrations (MIC-μg/mL) of the Title Compounds

Compounds	Microorganisms			
	Gram Positive		Gram Negative	
	Bacillus Subtilis	*Staphylococs aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*
7a	6.25	6.25	50	>50
7b	6.25	25.0	50	50
7c	12.5	6.25	50	>50
7d	25.0	25.0	50	50
7e	1.562	6.25	>50	50
7f	1.562	6.25	>50	50
7g	50	25	50	25
Novobiocin[a]	0.78	3.125	6.25	6.25
Penicillin[a]	1.562	1.562	6.25	6.25
Kanamycin[a]	0.78	1.562	3.125	3.125

[a]Used as a positive control.
Negative control DMSO, no activity.

Syntheses: general synthetic procedure for 6-(1-acetyl-3-methyl-4,5-dihydro-1H-pyrazol-5-yl)-2-amino-4-substituted-phenyl-4H-chromene-3-carbonitrile derivatives (7)

To a solution of 1-(5-(4-hydroxyphenyl)-3-methyl-4,5-dihydropyrazol-1-yl)ethanone **6** (2 mmoL) and aqueous Na_2HCO_3 (2 mmoL) in C_2H_5OH (30 mL) at 20 °C for 30 min. Then the respective aldehyde (2 mmol) and malononitrile (2 mmol) were added, the reaction mixture was microwave irradiation for 20 min at 60 °C. Upon completion, the reaction mixture was allowed to cool to room temperature and then maintained at 0~5°C for 10 h. The product was collected by filtration, and the crude residue was purified by recrystallization (acetone/petroleum, *V:V* = 1:2) to give **7** as colorless solids. Their spectra are provided in the supporting information.

7a: Colorless crystal, yield 70%. mp 201~203 °C, ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.12 (s, 3H, Me), 2.36 (s, 3H, Me-amide), 3.01 (dd, *J*=18.2 and 3.0 Hz, 1H, pyrazole, 4-H_a), 3.49 (dd, *J*=18.2 and 11.1 Hz, pyrazole, 1H, 4-H_b), 4.82 (s, 1H, 4H-chromene), 5.73 (dd, *J*=11.1 and 3.0 Hz, 1H, pyrazole, 5-H), 6.30 (s, 2H, NH_2), 6.68-7.27 (m, 8H, ArH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 17.8, 22.7, 31.3, 42.9, 54.5, 62.3, 118.8, 119.2, 120.6, 126.2, 127.1, 128.3, 128.9, 130.7, 136.8, 143.1, 150.7, 157.5, 170.1, 179.2; ESI-MS: 371.5 ($C_{22}H_{20}N_4O_2$, $[M+H]^+$).

7b: Colorless crystal, yield 52%. mp 216~217 °C, ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.10 (s, 3H, Me), 2.31 (s, 3H, Me-amide), 3.00 (dd, *J*=18.2 and 3.0 Hz, 1H, pyrazole, 4-H_a), 3.55 (dd, *J*=18.2 and 11.1 Hz, pyrazole, 1H, 4-H_b), 3.78 (s, 3H, OMe), 4.87 (s, 1H, 4H-chromene), 5.69 (dd, *J*=11.1 and 3.0 Hz, 1H, pyrazole, 5-H), 6.37 (s, 2H, NH_2), 6.68-7.02 (m, 7H, ArH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 17.2, 22.2, 31.0, 43.3, 54.2, 61.8, 62.0, 115.4, 116.6, 116.9, 121.1, 125.1, 129.3, 130.5, 131.3, 140.0, 152.0, 157.8, 159.3, 169.4, 179.0; ESI-MS: 401.7 ($C_{23}H_{22}N_4O_3$, $[M+H]^+$).

7c: Colorless crystal, yield 72%. mp 225~227 °C, ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.19 (s, 3H, Me), 2.36 (s, 3H, Me-amide), 3.03 (dd, *J*=18.2 and 3.0 Hz, 1H, pyrazole, 4-H_a), 3.50 (dd, *J*=18.2 and 11.1 Hz, pyrazole, 1H, 4-H_b), 4.82 (s, 1H, 4H-chromene), 5.76 (dd, *J*=11.1 and 3.0 Hz, 1H, pyrazole, 5-H), 6.48 (s, 2H, NH_2), 6.71-7.26 (m, 6H, ArH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 17.2, 20.2, 22.7, 43.8, 54.3, 62.1, 117.0, 117.5, 122.7, 125.0, 128.3, 128.9, 132.4, 133.0, 133.5, 136.1, 140.1, 149.7, 151.0, 157.9, 169.0, 178.2; ESI-MS: 440.9 ($C_{22}H_{18}Cl_2N_4O_2$, $[M+H]^+$).

7d: Colorless crystal, yield 51%. mp 208~209 °C, ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.17 (s, 3H, Me), 2.39 (s, 3H, Me-amide), 3.01 (dd, *J*=18.2 and 3.0 Hz, 1H, pyrazole, 4-H_a), 3.37 (dd, *J*=18.2 and 11.1 Hz, pyrazole, 1H, 4-H_b), 4.89 (s, 1H, 4H-chromene), 5.62 (dd, *J*=11.1 and 3.0 Hz, 1H, pyrazole, 5-H), 6.48 (s, 2H, NH_2), 6.69-7.44 (m, 7H, ArH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 17.9, 22.8, 30.8, 43.5, 54.7, 61.1, 118.2, 118.4, 121.5, 125.2, 126.4, 127.0, 128.7, 128.9, 130.1, 139.6, 145.0, 152.4, 159.1, 169.3, 178.7; ESI-MS: 439.7 ($C_{23}H_{19}F_3N_4O_2$, $[M+H]^+$).

7e: Colorless crystal, yield 59%. mp 208~209 °C, ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.12 (s, 3H, Me), 2.33 (s, 3H, Me-amide), 3.04 (dd, *J*=18.2 and 3.0 Hz, 1H, pyrazole, 4-H_a), 3.31 (dd, *J*=18.2 and 11.1 Hz, pyrazole, 1H, 4-H_b), 5.05 (s, 1H, 4H-chromene), 5.68 (dd, *J*=11.1 and 3.0 Hz, 1H, pyrazole, 5-H), 5.93 (d, 1H, *J*=3.2 Hz, furan-H), 6.22 (dd, 1H, *J*=3.2 and 1.7 Hz, furan-H), 6.41 (s, 2H, NH_2), 6.67-7.29 (m, 4H, ArH and furan-1H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 17.0, 22.3, 31.5, 43.9, 53.8, 59.9, 107.2, 110.5, 115.8, 117.2, 117.8, 123.5, 128.0, 138.5, 142.0, 151.0, 152.2, 158.3, 169.9, 178.0; ESI-MS: 363.0 ($C_{20}H_{18}N_4O_3$, $[M+H]^+$).

7f: Colorless crystal, yield 62%. mp 219~221 °C, ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.18 (s, 3H, Me), 2.37 (s, 3H, Me-amide), 3.00 (dd, *J*=18.2 and 3.0 Hz, 1H, pyrazole, 4-H_a), 3.36 (dd, *J*=18.2 and 11.1 Hz, pyrazole, 1H, 4-H_b), 4.86 (s, 1H, 4H-chromene), 5.69 (dd, *J*=11.1 and 3.0 Hz, 1H, pyrazole, 5-H), 6.49 (s, 2H, NH_2), 6.73-8.69 (m, 7H, ArH and pyridine-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.8, 21.4, 30.9, 43.5, 53.8, 59.9, 118.0, 118.5, 122.1, 124.0, 130.9, 132.0, 136.2, 137.5, 147.0, 149.4, 151.7, 153.3, 158.0, 169.2, 178.4; ESI-MS: 374.0 ($C_{21}H_{19}N_5O_2$, $[M+H]^+$).

7g: Colorless crystal, yield 67%. mp 226~227 °C, ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.15 (s, 3H, Me), 2.35 (s, 3H, Me-amide), 2.98 (dd, *J*=18.2 and 3.0 Hz, 1H, pyrazole, 4-H_a), 3.39 (dd, *J*=18.2 and 11.1 Hz, pyrazole, 1H, 4-H_b), 4.81 (s, 1H, 4H-chromene), 5.69 (dd, *J*=11.1 and 3.0 Hz, 1H, pyrazole, 5-H), 6.41 (s, 2H, NH_2), 6.66-7.86 (m, 10H, ArH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 17.3, 21.9, 31.2, 43.9, 54.6, 60.1, 117.8, 118.0, 121.3, 125.1, 125.3, 127.2, 128.1, 128.4, 128.6, 128.7, 129.4, 131.0, 132.3, 134.5, 134.9, 137.6, 151.0, 153.5, 169.0, 178.0; ESI-MS: 422.1 ($C_{26}H_{22}N_4O_2$, $[M+H]^+$).

Bioassay Conditions: *In Vitro* Antibacterial Activity

The antibacterial activities of the synthesized compounds were tested against *B. subtilis*, *E. coli*, *P. fluorescens* and *S. aureus* using MH medium (casein hydrolysate 17.5g, 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 [12]. A stock solution of the synthesized compound (100 μg/mL) in DMSO was prepared and graded quantities of the test compounds were incorporated in specified quantity of sterilized liquid MH medium. A specified quantity of the medium containing the compound was poured into microtitration plates. Suspension of the microorganism was prepared to contain approximately 10^5 cfu/mL (colony forming units) and applied to microtitration plates with serially diluted compounds in DMSO for testing and incubation 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, pH 7.4: $Na_2HPO_4.12H_2O$ 2.9 g, KH_2PO_4 0.2 g, NaCl 8.0 g, KCl 0.2 g, distilled water 1000 mL) containing 2 mg of MTT/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 isopropanol containing 5% 1 mol/L 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 550 nm. The MICs were observed.

CONCLUSION

A series of novel 6-(1-acetyl-3-methyl-4,5-dihydro-1H-pyrazol-5-yl)-2-amino-4-substituted-phenyl-4H-chromene-3-carbonitrile derivatives **7** were synthesized. The compounds

were evaluated and assayed for their antibacterial (*B. subtilis* ATCC 6633, *E. coli* ATCC 35218, *P. fluorescens* ATCC 13525 and *S. aureus* ATCC 6538) activity by MTT method. The results showed that compounds **7e** and **7f** possess potent antibacterial activity.

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