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Original article

Synthesis and antibacterial activity of 4-benzoyl-1-methyl-5-phenyl-1*H*-pyrazole-3-carboxylic acid and derivatives

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Abstract

Some new 1*H*-pyrazole-3-carboxylic acid and pyridazinone derivatives were synthesized and evaluated for their antibacterial activities against *Bacillus cereus* ATCC 7064, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 4230 and *Pseudomonas putida* using tube dilution method. The minimal inhibitory concentrations (MICs) experiments revealed that all chemical compounds showed inhibitor effects on the growth of the test microorganisms. Moreover, the results of this research showed that the compound named as **5c** was the best compound in the series, exhibiting antibacterial activity against both Gram-positive and Gram-negative bacteria.

Keywords: Pyrazole; Pyridazine; Antibacterial activity

1. Introduction

In recent years, the number of life-threatening infectious caused by multi-drug resistant Gram-positive and Gram-negative pathogen bacteria have reached an alarming level in many countries around the world [1,2]. A number of clinical reports in the United States and worldwide have independently described the emergence of vancomycin resistance in methicillin-resistance *Staphylococcus aureus* (MRSA) isolates and other human pathogen Gram-negative isolates [3]. Infections caused by these microorganisms pose a serious challenge to the medical community and need for an effective therapy has led to a search for novel antibacterial agents. Literatures report that pyrazole compounds, among their numerous pharmacological properties, possess also antimicrobial activity [4,5]. Cyclic oxalyl compounds of type 1 have been used successfully as starting materials to synthesize various 1,4,5-trisubstitue pyrazol-3-carboxylic acids and their derivatives via reaction with various hydrazines or hydrazones for about two decades [6–8]. Here, we report the chemical behavior of furandione **1** toward a aliphatichydrazine compound, and present the reactions of cyclic oxalyl compounds with methylhydrazine to prepare new pyrazole and pyridazine derivatives, and theirs antibacterial activity.

2. Chemistry

The furandione **1** was reacted with methylhydrazine to give the 1*H*-pyrazole-3-carboxylic acid **2** together with a new pyridazinone derivative **3, 2** was converted into the corresponding amide **5a**, ester **5b** or urea **5c** derivatives, respectively, via reactions of its acid chloride **4** with alcohol or various *N*-nucleophiles. Nitrile derivative **6** was obtained by dehydration of **5a** in a mixture of $SOCl_2$ and DMF (Scheme 1).

3. Experimental

Solvents were dried by refluxing with the appropriate drying agents (metallic sodium for ether; $CaCl_2$, or Na_2SO_4 for benzene, toluene...) and distilled before use. Melting points

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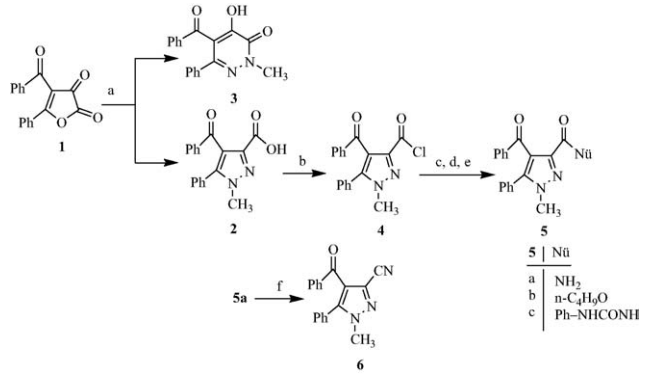

Scheme 1. Synthesis of compounds **2–6**. Reagents: (a) CH_3NHNH_2; (b) $SOCl_2$; (c) NH_3; (d) n-C_4-H_9OH; (e) Ph-$NHCONH_2$; (f) DMF-$SOCl_2$.

were determined on an Electrothermal Gallenkamp apparatus and are uncorrected. Microanalyses were performed on a Carlo Erba Elemental Analyzer Model 1108. The IR spectra were obtained in as potassium bromide pellets using a Mattson 1000 FTIR spectrometer. The ^{1}H and ^{13}C-NMR spectra were recorded on Varian XL-200 (200 MHz) and Varian XL-200 (50 MHz) spectrometers, respectively, using TMS as an internal standard. All experiments were followed by TLC using DC Alufolien Kieselgel 60 F 254 Merck and Camag TLC lamp (254/366 nm).

*3.1. 4-Benzoyl-1-methyl-5-phenyl-1H-pyrazole-3-carboxylic acid **2***

An equimolar mixture of furandione **1** (0.278 g, 1 mmole) and methylhydrazine (3.1 ml, 1 mmole) was mixture in room temperature in dry benzene (30 ml) for approximately 60 min. After the precipitate was filtered off and treated with dry ether to give a crude solid that was recrystallized from methyl alcohol. The yield 0.137 g (45%), mp 213 °C; IR: 3360–2800 cm^{-1} (b, OH, COOH), 1668 cm^{-1} (C=O, COOH), 1650 cm^{-1} (C=O, benzoyl); ^{1}H-NMR ($CDCl_3$): δ = 10.18–10.11 (b, 1H, OH), 7.67–7.13 (m, 10H, H_{arom}), 3.90 ppm (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ = 195.47 (C=O, benzoyl), 164.52 (C=O, COOH), 149.09 (C_3), 144.27 (C_5), 139.18, 134.98, 131.93, 131.79, 131.34, 130.70, 130.02, 129.49 (C-Ph), 122.63 (C_4), 40.40 ppm (N-CH_3). Anal. calcd. for $C_{18}H_{14}N_2O_3$: C, 70.58; H, 4.61; N, 9.15. Found: C, 70.65; H, 4.59; N, 9.14.

*3.2. 5-Benzoyl-4-hydroxsy-2-methyl-6-phenyl-2H-pyridazin-3-one **3***

After solvent of the filtrate of **2** was removed by evaporation, the oily residue treated with ether and the formed crude product was recrystallized from acetic acid to give 0.122 g (40%) of **3**, mp 218 °C; IR: 3117 cm^{-1} (OH), 3080 cm^{-1} (Ar-H), 1669 cm^{-1} (C=O, benzoyl), 1603 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$): δ = 9.41 (b, H, OH), δ = 7.8–7.2 (m, 10H, H_{arom}), 3.9 ppm (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ = 193.56 (C=O, benzoyl), 159.21 (C=O), 152.34 (C_4), 148.88 (C_5), 138.20, 137.11, 136.15, 131.52, 131.22, 130.81, 130.46, 130.27, 121.99, 42.56 ppm (N–CH_3). Anal. calcd. for $C_{18}H_{14}N_2O_3$: C, 70.58; H, 4.61; N, 9.15. Found: C, 70.57; H, 4.62; N, 9.16.

*3.3. 4-Benzoyl-1-methyl-5-phenyl-1H-pyrazole-3-carbonyl chloride **4***

Compound **2** (0.306 g, 1 mmole) and thionylchloride (1 ml, 13.8 mmoles) were refluxed on a steam bath for 5 h. After cooling, the crude precipitate was isolated by filtration and recrystallized from carbon tetrachloride, yield 0.216 g (68%), mp 160 °C; IR: 1750 (C=O, acyl), 1669 cm^{-1} (C=O, benzoyl); ^{1}H-NMR ($CDCl_3$): δ = 7.76–7.31 (m, 10H, H_{arom}), 3.96 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ = 192.13 (C=O, benzoyl), 163.27 (C=O, acyl), 147.69, (C_3), 144.67 (C_5), 139.25, 135.62, 132.11, 131.55, 131.31, 130.97, 130.54, 128.85 (C-Ph), 125.01 (C_4), 40.73 (N–CH_3). Anal. calcd. for $C_{18}H_{13}N_2O_2Cl$: C, 66.55; H, 4.03; N, 8.63. Found: C, 65.50; H, 4.05; N, 8.66.

*3.4. 4-Benzoyl-1-methyl-5-phenyl-1H-pyrazole-3-carboxamide **5a***

A moderate stream of gaseous ammonia was allowed to bubble through a solution of pyrazole-3-carboxylic acid chloride **4** (0.324 g, 1 mmole) in 10 ml of carbon tetrachloride during 30 min with ice-cooling. Then the crude precipitate was filtered off and recystallized from methanol to give 0.184 g (65%) of **5a**, mp 220 °C; IR: 3451–3320 cm^{-1} (NH), 1662 cm^{-1} (C=O, benzoyl), 1594 cm^{-1} (C=O, amide). ^{1}H-NMR($CDCl_3$): δ 7.69–7.20 (m, 10H, H_{arom}), 5.57–5.55 (bs, 2H, NH_2), 3.89 (s, 3H, N–CH_3). ^{13}C-NMR ($CDCl_3$): δ 194.35 (C=O, benzoyl), 164.48 (C=O, amide), 147.52 (C_3), 147.48 (C_5), 145.85, 140.19, 134.82, 132.09, 131.95, 131.77, 131.50, 131.42, 130.85, 130.67, 130.07, 129.97 (C-Ph), 122.65 (C_4), 39.99 (CH_3). Anal. calcd. for $C_{18}H_{15}N_3O_2$: C, 70.81; H, 4.91; N, 13.77. Found: C, 70.83; H, 4.88; N, 13.75.

*3.5. Butyl-4-benzoyl-1-methyl-5-phenyl-1H-pyrazole-3-carboxylate **5b***

The acid chloride **4** (0.324 g, 1 mmole) and excess of *n*-butyl alcohol (molar ratio 1:3) were refluxed together with catalytic amounts of pyridine for 2–3 h. After cooling the solution was acidified by adding diluted hydrochloric acid to give a crude solid, recrystallized from *n*-butyl alcohol. The yield was 0.159 g (44%), mp 99–100 °C; IR: 1703 (C=O, ester), 1664 cm^{-1} (C=O, benzoyl). ^{1}H-NMR ($CDCl_3$): δ = 7.78–7.26 (m, 10H, H_{arom}), 4.06–4.00 (t, 2H, OCH_2), 3.90 (s, 3H, N–CH_3), 1.33–1.02 (m, 4H, –CH_2–CH_2–), 0.79-0.72 (t, 3H, –CH_3), ^{13}C-NMR ($CDCl_3$): δ = 193.12 (C=O, benzoyl), 163.41 (C=O, ester), 146.36, (C_3), 143.01 (C_5), 140.23, 135.09, 131.64, 131.31, 130.76, 130.35, 129.67, (C-Ph), 124.09 (C_4), 67.05 (O–CH_2), 40.73 (N-CH_3), 32.19, 20.89 (–CH_2–), 15.58 (CH_3). Anal. calcd. for $C_{22}H_{22}N_2O_3$: C, 72.91; H, 6.12; N, 7.73. Found: C, 72.88; H, 6.08; N, 7.72.

3.6. N-[4-Benzoyl-1-methyl-5-phenyl-1H-pyrazole-3-carbonyl]-N′-phenyl urea **5c**

Compound **4** (0.324 g, 1 mmole) and phenyl urea (0.136 g, 1 mmole) after refluxing in xylene for 6 h and cooling to room temperature the precipitate was filtered off and recrystallized from xylene, yielding 0.212 g (50%), mp 227 °C; IR: 3244–3145 cm^{-1} (NH), 1711 cm^{-1} (C=O, urea), 1674 cm^{-1} (C=O), 1661 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$): δ = 10.24 (s, 1H, NH), 9.14 (s, 1H, NH), 7.81–7.04 (m, 15H, H_{arom}), 3.92 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$): δ = 193.19 (C=O), 163.14 (C=O), 152.23 (C=O, urea), 147.32 (C_3), 143.32 (C_5), 139.44, 139.22, 135.46, 131.88, 131.50, 130.90, 130.43, 129.26, 126.18, 123.90 (C-Ph), 122.35 (C_4), 40.30 (CH_3). Anal. calcd. for $C_{25}H_{20}N_4O_3$: C, 70.74 ,H, 4.75; N, 13.20. Found: C, 70.75; H, 4.73; N, 13.21.

3.7. 4-Benzoyl-1-methyl-5-phenyl-1H-pyrazole-3-carbonitrile **6**

A cold solution of the acid amide **5a** (0.287 g, 1 mmole) in a mixture of DMF (0.7 ml) and $SOCl_2$ (0.15 ml) was stirred at 0–5 °C for 2 h. After heating to room temperature, stirring was continued for overnight, then the reaction mixture poured over crushed ice and the separated solid filtered off, washed with water and recrystallized from methanol to give 0.186 g (65%) of **6**, mp: 169 °C; IR: 2237 cm^{-1} (CN), 1676 cm^{-1} (C=O, benzoyl). ^{13}C-NMR ($CDCl_3$-d_6): δ = 192.28 (C=O, benzoyl), 162.69 (C_3), 144.36 (C_5), 141.53 (C_4), 140.42, 139.22, 136.07, 133.85, 133.50, 133.20, 133.08, 132.74, 114.13 (C≡N), 41.72 ppm (CH_3). Anal. calcd. for $C_{18}H_{13}N_3O$: C, 75.26; H, 4.53; N, 14.63. Found: C, 75.33; H, 4.52; N, 14.64.

4. Antibacterial Activity

The chemical compounds were tested for antibacterial activity against human pathogenic Gram-negative (*E. coli* ATCC 4230, *Pseudomonas putida*) and Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 7064). The minimal inhibitory concentrations (MICs) of the chemical compounds assays were carried out as described by Clause [9] with minor modifications. Ampicillin was used as standard antibacterial agent. Solutions of the test compounds and ampicillin were dissolved in DMSO at concentration of 5000 μg ml^{-1}. The twofold dilution of the compounds and ampicillin were prepared (2500, 1250, 620, 310, 150, 70...) μg ml^{-1}. MIC tests were carried out in Muller-Hinton Broth (Difco) medium, pH 7.2, with an inoculum of (1–2) × 10^6 Colony Forming Unit ml^{-1} (CFU ml^{-1}) four microorganisms. The chemical compounds Muller-Hinton Broth serial tube dilutions inoculated with each bacterium were incubated on a rotary shaker at 37 °C for 18 h at 150 rpm. The minimum inhibitory concentrations of the chemical compounds were recorded as the lowest concentration of each chemical compounds in the tubes with no growth (i.e., no turbidity) of inoculated four bacteria. Muller-Hinton Broth medium containing DMSO inoculated microorganisms were used as a control. Experiments were repeated at least three to find out reproducibility of the assays.

5. Statistical analysis

Analysis of variance (ANOVA) was performed using a general linear model procedure. A randomized complete block design with repeated measurements was applied in this study. The significance of differences of the MIC values of three replicates were determined at the 95% confidence limit (P = 0.05). Differences among treatments were examined for levels of significance by the least significant differences (LSD) test (SAS Inst., Inc., Cary, NC).

6. Results and discussion

In this study, compounds **2, 3, 5a–c** and **6** have been evaluated for their antibacterial activity against Gram-negative (*Escherichia coli* ATCC 4230 and *Pseudomonas putida* ATCC) and Gram-positive (*Bacillus cereus* ATCC 7064 and *Staphylococcus aureus* ATCC 6538). The MICs (μg ml^{-1}) of tested compounds against bacteria are showed in Table 1.

The results revealed that all chemical compounds exhibited promising antibacterial activity against test bacteria. Compounds had relatively a good antibacterial activity against

Table 1
Minimal inhibitory concentrations (MICs) [a] of the compounds **2, 3, 5a–c** and **6** derivatives against Gram–positive and Gram–negative bacteria [b]

Compound	*Bacillus cereus* ATCC 7064	*Staphylococcus aureus* ATCC 6538	*Escherichia coli* ATCC 4230	*Pseudomonas putida*
2	1250	620	620	1250
3	1250	620	620	620
5a	620	310	620	620
5b	620	620	310	620
5c	620	310	310	310
6	620	620	620	310
Ampicillin	50	12	12	25

[a] MICs values were determined as μg ml^{-1} active compounds in medium.
[b] The MIC values (μg ml^{-1}) with different letters in the same column are significantly different (P < 0.05).

Gram-negative bacteria more than Gram-positive bacteria. Particularly, the compound named as **5c** exhibited more antibacterial potencies than other compounds against Gram-positive and Gram-negative bacteria (Table 1). In addition, did not found significantly differences between antibacterial activities of other compounds against test microorganisms.

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