Synthesis and in Vitro Cytotoxic Activity of Novel Pyrazole-3,4-dicarboxylates

Hanan Sekkak, El Mostapha Rakib*, Medaghri-Alaoui Abdelouahid, Abderrafia Hafid, Abdelghani El Malki

Laboratoire de Chimie Organique et Analytique, Faculté des Sciences et Techniques, Université Sultan Moulay Slimane, Béni-Mellal, Morocco

Email: *elmostapha1@ymail.com

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ABSTRACT

N-Aryl-C-ethoxycarbonylnitrile imines (3a-g) react with ethyl cyanoacetate 1 in 1,3-dipolar cycloaddition to yield novel pyrazole-3,4-dicarboxylates (4a-g) in moderate yields. The reaction of pyrazole-3,4-dicarboxylates (4a, d) with hydrazine afforded pyrazolo[4,3-d]pyridazine-4,7-diones (5a, d) in good yields. All compounds were fully characterized by spectroscopic methods. Some of the newly synthesized compounds were evaluated for their cytotoxic activity against murine P815 mastocytoma cell line.

Keywords: Nitrile Imines; Cycloaddition; Pyrazoles; Cytotoxic Activity

1. Introduction

Pyrazole and its derivatives, occupy an important position in medicinal chemistry with a wide range of bioactivities. They possess anti-obesity [1], receptor antagonists [2], HIV reverse transcriptase inhibitors [3], and anti-hyperglycemic activities [4]. They are also used as anti-inflammatory [5,6], antipyretic [7], antiarrhythmic [8], antitumor [9,10], monoamine oxidase inhibiting [11] and antibacterial agents [12]. Considering the important bio- logical properties of pyrazole compounds, numerous methods toward the synthesis of pyrazoles have been developed over the past decades [13-17]. Despite numerous diverse approaches toward the synthesis of polysubstituted pyrazoles developed so far, it is still challenging to prepare polysubstituted pyrazoles with various substituents from readily available building blocks. Herein we report a facile approach to provide polysubstituted pyrazoles via 1,3-dipolar cycloaddition of ethyl cyanoacetate with N-Aryl-C-ethoxycarbonylnitrile imines. Evaluation of their cytotoxic activity toward cell line P815 is reported.

2. Results and Discussion

2.1. Chemistry

The ethyl hydrazono-α-bromoglyoxylates (2a-g) (Figure 1) selected to generate the corresponding N-aryl-C-ethoxycarbonylnitrile imines were prepared from the reaction of the adequate diazonium salts with ethyl acetoacetic esters [18]. The nitrile imines were generated in situ by basic treatment of the hydrazono-α-bromoglyoxylates [19,20].

We performed the cycloaddition reaction of ethyl cyanoacetate with nitrile imines obtained initially in situ from the treatment of hydrazonyl bromides with sodium ethoxide in dry ethanol at room temperature. The progress of the reaction was followed by TLC until all nitrilimines has been consumed. The overall reaction yielded a polysubstituted pyrazoles in moderate yields 40% - 56% (Scheme 1).

The structures of compounds (4a-g) were deduced from their IR, 1H NMR, 13C NMR and MS spectra. For example, the 1H NMR spectrum of (4a) exhibited two signals at 1.33, 1.39 for the methyl of ethoxy group and a broad signal at 5.40 ppm for NH2 group, along with multiplets for the aromatic protons at N-1 position of the pyrazolic cycle. In the IR spectrum of (4a), two absorption bands at 1725 and 1696 cm⁻¹, which are related to
two C=O stretching frequencies, clearly indicated the most significant functional groups of the product.

Pyrazoles (4a-g) are suitable intermediates for the preparation of new pyrazolopyridazinedione derivatives. In fact, the reaction between hydrazine hydrate and pyrazoles (4a, d) afforded pyrazolo[4,3-d]pyridazine-4,7-diones (5a, d) in good yields (Scheme 2).

The structures of the synthesized compounds (5a, d) were established on the basis of IR, 1H NMR, and 13C NMR spectral data. In the IR spectra of compound (5a) the absence of the absorption band at 1725 cm\(^{-1}\) and 1696 cm\(^{-1}\), for C=O ester confirms the formation of pyridazinedione cycle. The 1H NMR spectra of 5a exhibited two broad signals at \(\delta = 10.05\) and 12.11 ppm, respectively, due to the NH proton.

2.2. Cytotoxic Activity

The preliminary cytotoxic activities of some compounds against the murine P815 mastocytoma cell line were evaluated in vitro as shown in Table 1. The \(\text{IC}_{50}\) represents the drug concentration (\(\mu\)g/mL) required to inhibit cell growth by 50%. The polysubstituted pyrazoles (4a, d, g) have shown slight cytotoxic activity against cell line P815. Compound (4a) (R1 = Cl) showed significant activity against cell line P815 (\(\text{IC}_{50} = 32 \, \mu\text{g/mL}\)). It should be noted that the substituent at the position of benzene in compounds (4a-d), (4f), (4g) may also play an important role in determining relative activities.

### Table 1. In vitro cytotoxicity of some new substituted pyrazoles against the murine mastocytoma cell line P815

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁, R₂, R₃, R₄</th>
<th>IC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>R₁ = Cl, R₂ = R₃ = R₄ = H</td>
<td>32</td>
</tr>
<tr>
<td>4b</td>
<td>R₁ = NO₂, R₂ = R₃ = R₄ = H</td>
<td>105</td>
</tr>
<tr>
<td>4c</td>
<td>R₁ = CH₃, R₂ = R₄ = H, R₃ = Br</td>
<td>75</td>
</tr>
<tr>
<td>4d</td>
<td>R₁ = F, R₂ = R₃ = R₄ = H, R₃ = Br</td>
<td>98</td>
</tr>
<tr>
<td>4f</td>
<td>R₁ = R₂ = H, R₃ = CH₃, R₄ = NO₂</td>
<td>110</td>
</tr>
<tr>
<td>4g</td>
<td>R₁ = R₄ = H, R₂ = CH₃, R₃ = NO₂</td>
<td>107</td>
</tr>
</tbody>
</table>

3. Conclusion

In summary, with a simple approach, a new series of pyrazole-3,4-dicarboxylates (4a-g) was synthesized by 1,3-dipolar cycloaddition of N-aryl-C-ethoxycarbonyl nitrile imines with ethyl cyanoacetate. Novel pyrazolo [4,3-d]pyridazine-4,7-diones were also prepared by condensation reaction of hydrazine with pyrazole-3,4-dicarboxylates. In vitro cytotoxicity of some compounds were assessed and showed a lower cytotoxicity on murine mastocytoma cell line P815. Pyrazole (4a) with chlorine atom substitution on pyrazole-3,4-carboxylate at para position of benzene group, showed significant activity against cell line P815 (\(\text{IC}_{50} = 32 \, \mu\text{g/mL}\)). Compound (4a) can be useful as a lead for the development of novel anticancer agents.

4. Experimental Section

Melting points were determined using a Büchi-Tottoli apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 577 spectrometer using KBr disks; only noteworthy IR absorptions are listed (cm\(^{-1}\)). 1H and 13C NMR spectra were recorded in CDCl₃, DMSO-d₆ and...
solution (unless otherwise specified) with TMS as an internal reference using a Bruker AC 300 (1H) or 75MHz (13C) instruments. Chemical shifts are given in δ parts per million (ppm) downfield from TMS. Multiplicities of 13C NMR resources were assigned by distortionless enhancement by polarization transfer (DEPT) experiments. Low-resolution mass spectra (MS) were recorded on a Perkin-Elmer Sciei API 3000 spectrometer. Column chromatography was carried out on SiO2 (silica gel 60, F 254 Merck 0.063 - 0.200 mm). Thin-layer chromatography (TLC) was carried out on SiO2 (silica gel 60, F 254 Merck 0.063 - 0.200 mm), and the spots were located with UV light. Commercial reagents were used without further purification unless stated.

**Synthesis of polysubstituted pyrazoles (4a-g).** To a solution of ethyl cyanoacetate 1 (0.56 g, 0.005 mol) and hydrazonyl bromides 2a-g (0.005 mol) in dry ethanol, ethoxide sodium (0.68 g, 0.01 mol) was added. The mixture was stirred at room temperature for 12 h, during which the bromide dissolved and the crude pyrazole precipitated. The precipitate was filtered, washed with cold water, dried and purified by column chromatography (EtOAc/hexane 2/8).

**Diethyl 5-amino-1-(4-chlorophenyl)-1H-pyrazole-3,4-dicarboxylate (4a).** Yield: 56%, mp: 95°C - 97°C; IR (KBr, cm−1): 3405, 3328 (NH2), 1725 (CO), 1696 (CO), 1622 (C=NN); 1H NMR (CDCl3): δ 1.33 (t, 3H, CH3, J = 7.2 Hz), 1.39 (t, 3H, CH3, J = 7.2 Hz), 4.29 (q, 2H, OCH2, J = 7.2 Hz), 4.39 (q, 2H, OCH2, J = 7.2 Hz), 5.20 (s, 2H, NH2), 7.33 (d, 1H, J = 7.8 Hz), 7.46 (m, 2H); 13C NMR (CDCl3): δ 14.2 (CH3), 14.3 (CH3), 60.3 (CH2O), 61.8 (CH2O), 125.6 (2CH), 129.0 (C), 129.2 (2CH), 134.6 (C), 135.3 (C), 144.6 (C), 150.0 (C), 162.8 (CO), 163.7 (CO); MS m/z = 338 [M + 1], 340 [M + 3].

**Diethyl 5-amino-1-(4-nitrophenyl)-1H-pyrazole-3,4-dicarboxylate (4b).** Yield: 46%, mp: 138°C - 140°C; IR (KBr, cm−1): 3397, 3317 (NH2), 1728 (CO), 1690 (CO), 1622 (C=NN); 1H NMR (CDCl3): δ 1.34 (t, 3H, CH3, J = 7.2 Hz), 1.38 (t, 3H, CH3, J = 7.2 Hz), 4.30 (q, 2H, OCH2, J = 7.2 Hz), 4.40 (q, 2H, OCH2, J = 7.2 Hz), 5.61 (s, 2H, NH2), 7.81 (d, 2H, J = 8.0 Hz), 8.36 (d, 2H, J = 8.0 Hz); 13C NMR (CDCl3): δ 14.2 (CH3), 14.3 (CH3), 60.5 (CH2O), 62.1 (CH2O), 119.3 (C), 123.8 (2CH), 124.7 (C), 125.4 (2CH), 142.3 (C), 146.7 (C), 150.2 (C), 162.6 (CO), 163.5 (CO); MS m/z = 349 [M + 1].

**Diethyl 5-amino-1-(2-bromo-4-methylphenyl)-1H-pyrazole-3,4-dicarboxylate (4c).** Yield: 45%, mp: 93°C - 95°C; IR (KBr, cm−1): 3445, 3335 (NH2), 1730 (CO), 1690 (CO), 1616 (C=N); 1H NMR (CDCl3): δ 1.34 (t, 3H, CH3, J = 7.2 Hz), 1.39 (t, 3H, CH3, J = 7.2 Hz), 2.40 (s, 3H, CH3), 4.30 (q, 2H, OCH2, J = 7.2 Hz), 4.39 (q, 2H, OCH2, J = 7.2 Hz), 5.01 (s, 2H, NH2), 7.28 (m, 2H), 7.55 (d, 1H, J = 1.8 Hz); 13C NMR (CDCl3): δ 14.2 (CH3), 14.4 (CH3), 60.2 (CH2O), 61.7 (CH2O), 93.9 (C), 121.8 (C), 129.6 (CH), 132.7 (C), 134.2 (CH), 142.7 (C), 144.3 (C), 151.0 (C), 162.6 (CO), 163.7 (CO); MS m/z = 397 [M + 1], 399 [M + 3].

**Diethyl 5-amino-1-(2-bromo-4-fluorophenyl)-1H-pyrazole-3,4-dicarboxylate (4d).** Yield: 51%, mp: 105°C - 107°C; IR (KBr, cm−1): 3430, 3354 (NH2), 1718 (CO), 1697 (CO), 1621 (C=N); 1H NMR (CDCl3): δ 1.35 (t, 3H, CH3, J = 7.2 Hz), 1.39 (t, 3H, CH3, J = 7.2 Hz), 4.30 (q, 2H, OCH2, J = 7.2 Hz), 4.39 (q, 2H, OCH2, J = 7.2 Hz), 5.18 (s, 2H, NH2), 7.19 (m, 1H), 7.46 (m, 2H); 13C NMR (CDCl3): δ 14.2 (CH3), 14.3 (CH3), 60.3 (CH2O), 61.8 (CH2O), 94.0 (C), 116.3 (CH), 121.4 (CH), 123.2 (C), 131.7 (CH), 144.6 (C), 151.1 (C), 161.1 (C), 162.2 (C), 163.6 (CO), 164.7 (CO); MS m/z = 401 [M + 1], 403 [M + 3].

**Diethyl 5-amino-1-(2-bromo-4-fluorophenyl)-1H-pyrazole-3,4-dicarboxylate (4f).** Yield: 49%, mp: 96°C - 98°C; IR (KBr, cm−1): 3443, 3331 (NH2), 1731 (CO), 1691 (CO), 1617 (C=N); 1H NMR (CDCl3): δ 1.35 (t, 3H, CH3, J = 7.2 Hz), 1.39 (t, 3H, CH3, J = 7.2 Hz), 2.31 (s, 3H, CH3), 4.32 (q, 2H, OCH2, J = 7.2 Hz), 4.40 (q, 2H, OCH2, J = 7.2 Hz), 5.20 (s, 2H, NH2), 7.55 (d, 1H, J = 7.1 Hz), 8.26 (m, 2H); 13C NMR (CDCl3): δ 14.2 (CH3), 14.3 (CH3), 18.2 (CH3), 60.4 (CH2O), 61.9 (CH2O), 94.2

**Scheme 2. The reaction between hydrazine hydrate and pyrazoles.**
(C), 120.5 (C), 123.5 (CH), 125.1 (CH), 132.6 (CH), 135.6 (C), 144.7 (C), 146.8 (C), 150.8 (C), 162.4 (CO), 163.6 (CO); MS m/z = 363 [M + 1].

**Diethyl 5-amino-1-(2-methyl-3-nitrophenyl)-1H-pyrazole-3,4-dicarboxylate (4g).** Yield: 52%, mp: 120°C - 122°C; IR (KBr, cm⁻¹): 3381, 3317 (NH₂), 1695 (CO), 1693 (CO); ¹H NMR (CDCl₃): δ 1.35 (t, 3H, CH₃, J = 7.2 Hz), 1.39 (t, 3H, CH₃, J = 7.2 Hz), 1.87 (s, 3H, CH₃), 4.32 (q, 2H, OCH₂, J = 7.2 Hz), 4.40 (q, 2H, OCH₂, J = 7.2 Hz), 5.19 (s, 2H, NH₂), 7.51 (t, 1H, J = 7.5), 7.62 (d, 1H, J = 7.5 Hz), 8.02 (d, 1H, J = 7.5); ¹³C NMR (CDCl₃): δ 14.2 (CH₃), 14.3 (CH₃), 18.3 (CH₃), 60.4 (CH₂O), 61.8 (CH₂O), 94.1 (C), 126.2 (2CH), 127.7 (C), 132.4 (C), 132.7 (CH), 136.9 (C), 144.8 (C), 150.8 (C), 153.0 (C), 154.3 (C), 160.3 (C), 162.5 (C), 164.6 (C); MS m/z = 341 [M + 1], 343 [M + 3].

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