Design, Synthesis, and Cytotoxic Evaluation of Etodolac-1,3,4-oxadiazole-1,2,3-triazole Molecules

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Synthesis, etodolac, oxadiazoles, triazoles, cytotoxicity

Abstract
A new series of etodolac-1,3,4-oxadiazole-1,2,3-triazole derivatives was designed and synthesized from commercially available starting materials by employing a simple synthetic sequence. The in vitro evaluation of the synthesized analogues displayed promising cytotoxic activity. Among the tested compounds 7c, 7l, and 7n exhibited highest cytotoxic activity against MCF-7 (breast), A549 (lung), and DU-145 (prostate) human cancer cell lines.

Key words
1,3,4-Oxadiazole scaffold containing pharmaceuticals

Figure 1
1,3,4-Oxadiazole scaffold containing pharmaceuticals

1. Esterification
2. NH2NH2
3. CS2

K2CO3
DMF

Etodolac
NSAID

15 Examples
cytotoxic evaluation

CuAc

Figure 2
1,2,3-Triazole scaffold containing pharmaceuticals

Carboxyamidotriazole (CAI)
tert-Butylidemethylsilylspiroaminooxathiole-dioxide (TSAO)

Etodolac (1) is a nonsteroidal anti-inflammatory drug used for the treatment of the symptoms of rheumatoid arthritis and osteoarthritis.7 It has also been found to exhibit potent antitumor activity against various human cancer cell lines.8 Recent studies revealed that etodolac is a selective COX-2 inhibitor, suppressing proliferation and inducing apoptosis in prostate cancer cells with no effect on normal prostate stromal cells.9 A literature review suggested that the derivatization of the carboxylate functional group of nonsteroidal anti-inflammatory drugs can result in reduced ulcerogenic potential with retained anti-inflammatory activity.10 During the last few decades, various etodolac congeners have been evaluated for their biological activities, including anticancer activity.11 Very recently, our research group has reported the synthesis of a novel series of 1,2,3-triazole–etodolac derivatives and evaluated their anticancer activity.12 Most of these compounds exhibited potent anticancer activity against A549 human lung cancer cell lines.

Similarly, the 1,2,3-triazole scaffold is found in pharmaceuticals including the non-nucleoside reverse transcriptase inhibitor tert-butyldimethylsilylspiroaminooxathiole-dioxide (TSAO) and the anticancer drug (carboxyamidotriazole, CAI) (Figure 2).4,5 These heterocyclic scaffolds possess specific properties such hydrogen bonding capability, moderate dipole character and molecular rigidity, and can be constructed through a ‘click’ chemistry approach.6

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Organic molecules containing 1,3,4-oxadiazole and 1,2,3-triazole nuclei exhibit a broad spectrum of biological properties such as antimicrobial, antituberular, antiviral, analgesic, and anticancer activities.1,2 Particularly, the 1,3,4-oxadiazole heterocyclic nucleus has been widely exploited for a spectrum of therapeutic applications such as antibacterial (Furamizole), antihypertensive (Nesapidil), HIV-integrase inhibition (Raltegravir), and anticancer (Zibotentan) treatments (Figure 1).3
In recent years, it has also been reported that the incorporation of the 1,2,3-triazole moiety with 1,3,4-oxadiazoles in a single molecule provides promising biological activities. Therefore, combining etodolac with 1,3,4-oxadiazole and substituted 1,2,3-triazole is predicted to give novel molecules with good cytotoxic activities. In this context, we herein report an efficient synthesis of a series of etodolac derivatives, linking as key fragments 1,3,4-oxadiazole and 1,2,3-triazole scaffolds (Figure 3). The cytotoxic activity of a series of etodolac-1,3,4-oxadiazole-1,2,3-triazoles was based on IC50 values obtained against MCF-7 (breast), A549 (lung), and DU-145 (prostate) human cancer cell lines.

The target etodolac-1,3,4-oxadiazole-1,2,3-triazole derivatives 7a–o were synthesized from commercially available etodolac (1) via a conventional five-step sequence as depicted in Scheme 1 and Table 1. The known intermediate etodolac-hydrazide 3 was prepared from etodolac (1) according to the previous reported procedure. Etodolac-hydrazide 3 was transformed into the corresponding 1,3,4-oxadiazole-2-thiol 4 by heating to reflux with carbon disulfide (CS2) in ethanolic sodium hydroxide solution. The structure of compound 4 was confirmed based on proton resonances appearing at δ = 13.97 (s, 1 H) ppm in the 1H NMR spectrum, indicating the presence of the -SH group. Additionally, the 13C NMR spectrum showed a signal at δ = 163.5 ppm, corresponding to one carbon of the oxadiazole ring. A molecular ion peak at m/z 344 [M+H]+ in the ESI mass spectrum further supported the structural assignment. Next, the key intermediate 5, with a terminal alkyne, was prepared through reaction of propargyl bromide with compound 4 in dimethylformamide (DMF) using K2CO3 as base at room temperature. The structure of 5 was confirmed by 1H NMR spectroscopic analysis, indicating the presence of a CH2 group at δ = 3.41 ppm and absence of a peak at δ = 13.97 (s, 1 H) ppm in compound 5. Additionally, the 13C NMR spectrum showed signals at δ = 24.02 ppm due to the CH2 carbon and terminal alkyne resonances at δ = 79.91 and 80.61 ppm. Further support was provided by the appearance of a molecular ion at m/z 382 [M+H]+ in the ESI mass spectrum.

Finally, the 1,2,3-triazol ring of target molecules 7a–o could be constructed by applying a ‘click’ reaction. 1,3-Dipolar cycloaddition of 5 with a series of substituted phenylazides 6a–o in the presence of CuSO4·5H2O and sodium L-ascorbate in DMF at room temperature provided the desired target molecules 7a–o in excellent isolated yields (Table 1). All synthesized compounds were characterized by 1H NMR, 13C NMR spectroscopy and mass spectrometry.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substituted aryl azide 6a–o</th>
<th>Etodolac-1,3,4-oxadiazole-1,2,3-triazole derivatives 7a–o</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6a</td>
<td>7a, 86%</td>
</tr>
</tbody>
</table>

Figure 3 Design of etodolac-1,3,4-oxadiazole-1,2,3-triazole derivatives

Scheme 1 Synthesis of etodolac-1,3,4-oxadiazole intermediate
Table 1 (continued)

<table>
<thead>
<tr>
<th></th>
<th>Structure</th>
<th>Yield (%)</th>
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<tbody>
<tr>
<td>2</td>
<td>6b</td>
<td>7b, 90%</td>
</tr>
<tr>
<td>3</td>
<td>6c</td>
<td>7c, 92%</td>
</tr>
<tr>
<td>4</td>
<td>6d</td>
<td>7d, 94%</td>
</tr>
<tr>
<td>5</td>
<td>6e</td>
<td>7e, 90%</td>
</tr>
<tr>
<td>6</td>
<td>6f</td>
<td>7f, 94%</td>
</tr>
<tr>
<td>7</td>
<td>6g</td>
<td>7g, 92%</td>
</tr>
<tr>
<td>8</td>
<td>6h</td>
<td>7h, 93%</td>
</tr>
<tr>
<td>9</td>
<td>6i</td>
<td>7i, 87%</td>
</tr>
<tr>
<td>10</td>
<td>6j</td>
<td>7j, 89%</td>
</tr>
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</table>
The novel etodolac-1,3,4-oxadiazole-1,2,3-triazole derivatives 7a–o were screened for their in vitro cytotoxicity on three human cancer cell lines, namely MCF-7 (breast), A549 (lung), and DU-145 (prostate), using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The IC50 values were determined from the plot of percent inhibition (from control) versus concentration; the results are illustrated in Table 2. Doxorubicin was used as a positive control to validate the MTT assay. Compounds 7c, 7l, and 7n showed promising cytotoxicity against all cancer cell lines, with IC50 values ranging from 1.07 to 3.5 μM, among which the derivatives 7c and 7n with a methoxy group at the para-position of the phenyl ring displayed similar activity against all cancer cell lines. The para-methoxy-ortho-nitro phenyl derivative 7c showed the highest potency against both MCF-7 (breast) and A549 (lung) cancer cell lines, with IC50 values of 1.07 μM and 1.4 μM, respectively. It was observed that, only compounds 7a, 7c, 7n, and 7o showed promising activity against the DU-145 (prostate) cancer cell line, among which derivative 7o, with an electron-withdrawing trifluoromethyl group at the meta-position of the phenyl ring, proved to be optimal with an IC50 value of 1.3 μM. The para-methoxy-ortho-nitro phenyl derivative 7c showed an IC50 value of 1.5 μM. Compounds 7d, 7g, 7k, and 7m, possessing electron-donating methyl groups on the phenyl ring, exhibited lower cytotoxic activities.

Table 2  IC50 (μM) Against Human Tumor Cell Lines for 7a–o

<table>
<thead>
<tr>
<th>Compound</th>
<th>MCF-7 (breast cancer)</th>
<th>A549 (lung cancer)</th>
<th>DU-145 (prostate cancer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>47.8</td>
<td>&gt;100</td>
<td>13.3</td>
</tr>
<tr>
<td>7b</td>
<td>&gt;100</td>
<td>50.1</td>
<td>17.1</td>
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<tr>
<td>7c</td>
<td>1.07</td>
<td>1.4</td>
<td>1.5</td>
</tr>
<tr>
<td>7d</td>
<td>83.1</td>
<td>64.5</td>
<td>63.0</td>
</tr>
<tr>
<td>7e</td>
<td>96.6</td>
<td>52.4</td>
<td>&gt;100</td>
</tr>
<tr>
<td>7f</td>
<td>15.1</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>7g</td>
<td>15.3</td>
<td>&gt;100</td>
<td>79.4</td>
</tr>
</tbody>
</table>
In summary, we have demonstrated the design, synthesis, and cytotoxic evaluation of a series of novel etodolac-1,3,4-oxadiazole-1,2,3-triazole derivatives against MCF-7 (breast), A549 (lung), and DU-145 (prostate) cancer cell lines. From the initial screening of the tested compounds, it was observed that some of the analogues were active against human cancer cell lines, with IC50 values of ≤15 μM. Cytotoxicity profiling indicated that compounds 7c, 7l, and 7n show the best cytotoxicity against all three tested cancer cell lines. Notably, the most active compound 7e showed a broad range of cytotoxicity in all three cancer cell lines with a remarkable IC50 value of 1.07 μM against the MCF-7 (breast cancer) cell line.

5-(1,8-Diethyl-1,3,4,9-tetrahydropyranol,3,4-b]indol-1-y)methyl)-1,3,4-oxadiazole-2-thiol (4)

Etodolac 1,4-oxadiazol-2-thione 4 was synthesized from 3 (3 g, 9.93 mmol) by heating to reflux with KOH (14.9 mmol) and C5H5N (14.9 mmol) in absolute EtOH (30 mL) for 8 h. After completion, the reaction mixture was cooled to r.t. and diluted with ice water (30 mL). Acidification with 1N HCl with stirring formed a precipitate, which was filtered and dried to give 4.

Yield: 2.9 g (80%); white solid; m.p. 149–153 °C.

FTIR: 3372 (indole), 1250 (C-O-C oxadiazoles stretch), 2549 (SH) cm⁻¹.

1H NMR (400 MHz, CDCl3): δ = 0.72 (t, J = 7.33 Hz, 3 H), 1.33 (t, J = 7.33 Hz, 3 H), 1.84–1.99 (m, 1 H), 2.05–2.20 (m, 1 H), 2.62–2.95 (m, 4 H), 3.28–3.44 (m, 2 H), 3.90–4.11 (m, 2 H), 6.91–7.04 (m, 2 H), 7.29 (d, J = 7.69 Hz, 1 H), 10.07 (s, 1 H), 13.97 (br s, 1 H).

13C NMR (100 MHz, CDCl3): δ = 7.11, 13.65, 21.47, 23.49, 30.49, 33.82, 60.20, 75.26, 108.03, 115.02, 118.60, 125.45, 126.33, 134.03, 134.35, 160.52, 171.84.

Yield: 2.7 g (81%); white solid; m.p. 103–105 °C.

Synthesis of Etodolac-1,3,4-oxadiazole-1,2,3-triazole Derivatives 7a–o; General Procedure

Terminal alkyne 5 was coupled with arylazide 6a–o using CuSO4·5H2O (0.049 g, 0.2 mmol) and sodium t-butoxide (0.039 g, 0.2 mmol) in DMF at r.t. for 20–30 min to afford the desired compounds 7a–o in good to excellent yields. The reaction was found to be complete within 20–30 min, although no ultrasound or microwave irradiation was used.

1-(5-[(1-(4-Bromophenyl)-1H-1,2,3-triazol-4-yl)methyl]thio)-1,3,4-oxadiazol-2-yl)methyl)-1,8-diethy-1,3,4,9-tetrahydropyranol,3,4-b]indole (7a)

Yield: 0.127 g (86%); white solid; m.p. 172 °C.

1H NMR (400 MHz, CDCl3): δ = 0.80 (t, J = 7.73 Hz, 3 H), 1.33 (t, J = 7.6 Hz, 3 H), 1.94–2.02 (m, 1 H), 2.08–2.17 (m, 1 H), 2.72–2.90 (m, 4 H), 3.40 (s, 2 H), 3.99–4.04 (m, 1 H), 4.07–4.13 (m, 1 H), 4.58 (s, 2 H), 7.01 (d, J = 7.62 Hz, 1 H), 7.07 (t, J = 7.62 Hz, 1 H), 7.36 (d, J = 7.62 Hz, 1 H), 7.57–7.61 (m, 2 H), 7.62–7.66 (m, 2 H), 8.12 (s, 1 H), 8.86 (br s, 1 H).

13C NMR (100 MHz, CDCl3): δ = 7.24, 13.76, 21.75, 23.70, 26.22, 30.61, 33.77, 60.40, 75.54, 108.14, 115.19, 118.81, 119.77, 120.80, 121.41, 125.63, 126.51, 128.71, 132.33, 134.52, 135.31, 143.50, 159.96, 163.19, 165.12.

ESI-MS: m/z = 581 [M+2H].

1,8-Diethyl-1-[(5-[(1-(3-Nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl]thio)-1,3,4-oxadiazol-2-yl)methyl]-1,3,4,9-tetrahydropyranol,3,4-b]indole (7b)

Yield: 0.127 g (90%); pale-yellow solid; m.p. 130–132 °C.

1H NMR (400 MHz, CDCl3): δ = 0.79 (t, J = 7.33 Hz, 3 H), 1.33 (t, J = 7.58 Hz, 3 H), 1.91–2.03 (m, 1 H), 2.07–2.18 (m, 1 H), 2.70–2.91 (m, 4 H), 3.42 (s, 2 H), 3.98–4.14 (m, 2 H), 4.60 (s, 2 H), 7.00 (d, J = 7.2 Hz, 1 H), 7.05 (t, J = 7.5 Hz, 1 H), 7.34 (d, J = 7.7 Hz, 1 H), 7.72 (t, J = 8.1 Hz, 1 H), 8.10–8.14 (m, 1 H), 8.27 (s, 1 H), 8.28–8.32 (m, 2 H), 8.57–8.61 (m, 1 H), 8.83 (br s, 1 H).

13C NMR (100 MHz, CDCl3): δ = 7.56, 13.75, 21.21, 24.12, 26.61, 31.11, 34.87, 60.90, 75.41, 109.35, 115.34, 116.02, 119.84, 120.70, 121.26, 123.33, 125.89, 126.12, 126.58, 130.95, 134.55, 134.70, 137.48, 144.48, 148.88, 164.01, 165.91.

ESI-MS: m/z = 546 [M+].

1,8-Diethyl-1-[(5-[(1-(4-Methoxy-2-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl]thio)-1,3,4-oxadiazol-2-yl)methyl]-1,3,4,9-tetrahydropyranol,3,4-b]indole (7c)

Yield: 0.137 g (92%); pale-yellow solid; m.p. 89–90 °C.
1H NMR (400 MHz, CDCl3): δ = 0.79 (t, J = 7.32 Hz, 3 H), 1.30 (t, J = 7.32 Hz, 3 H), 1.94–2.02 (m, 1 H), 2.08–2.17 (m, 1 H), 2.72–2.90 (m, 4 H), 3.38–3.44 (m, 2 H), 3.94 (s, 3 H), 3.99–4.06 (m, 1 H), 4.07–4.15 (m, 1 H), 4.59 (s, 2 H), 6.69 (d, J = 7.01 Hz, 1 H), 7.05 (d, J = 7.62 Hz, 1 H), 7.22 (dd, J = 8.85, 8.65 Hz, 1 H), 7.35 (d, J = 7.85 Hz, 1 H), 7.43–7.46 (m, 1 H), 7.55 (d, J = 7.74 Hz, 1 H), 7.94 (s, 1 H), 8.93 (br s, 1 H).

13C NMR (100 MHz, CDCl3): δ = 75.1, 131.7, 22.25, 24.11, 26.76, 31.02, 34.89, 56.38, 60.88, 75.37, 109.21, 110.55, 115.98, 119.33, 119.75, 120.59, 122.81, 125.35, 126.11, 126.66, 129.41, 134.69, 134.74, 143.13, 145.11, 160.92, 163.95, 165.95.

ESI-MS: m/z = 576 [M + H].

1-((5-(((1-(2,4-Dimethylphenyl)-1H,7,8-Diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indole (7d)

Yield: 0.129 g (94%); white solid; m.p. 130–131 ℃.

1H NMR (400 MHz, CDCl3): δ = 0.79 (t, J = 7.32 Hz, 3 H), 1.30 (t, J = 7.62, 3 H), 1.93–2.01 (m, 1 H), 2.09–2.16 (m, 1 H), 2.17 (s, 3 H), 2.72–2.78 (m, 1 H), 2.80–2.89 (m, 3 H), 3.41 (s, 2 H), 3.98–4.04 (m, 1 H), 4.06–4.12 (m, 1 H), 4.61 (s, 2 H), 6.99 (d, J = 7.01 Hz, 1 H), 7.05 (t, J = 7.62 Hz, 1 H), 7.28–7.43 (m, 5 H), 7.88 (s, 1 H), 9.01 (br s, 1 H).

13C NMR (100 MHz, CDCl3): δ = 7.50, 13.69, 17.77, 22.21, 24.10, 26.83, 31.94, 34.84, 60.86, 75.37, 109.15, 115.95, 119.73, 120.59, 125.85, 126.08, 126.64, 128.89, 129.93, 131.45, 133.48, 134.70, 134.76, 140.08, 142.45, 164.16, 165.88.

ESI-MS: m/z = 515 [M + H].

1,8-Diethyl-1-((5-(((1-(4-Chlorophenyl)-1H,7,8-Diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indole (7e)

Yield: 0.126 g (89%); pale-yellow solid; m.p. 112–113 ℃.

1H NMR (400 MHz, CDCl3): δ = 0.80 (t, J = 7.21 Hz, 3 H), 1.34 (t, J = 7.58 Hz, 3 H), 1.95–2.03 (m, 1 H), 2.09–2.17 (m, 1 H), 2.72–2.91 (m, 4 H), 3.40 (s, 2 H), 3.98–4.05 (m, 1 H), 4.06–4.13 (m, 1 H), 4.59 (s, 2 H), 7.01 (d, J = 7.08 Hz, 1 H), 7.06 (t, J = 7.58 Hz, 1 H), 7.35 (d, J = 7.73 Hz, 1 H), 7.47–7.51 (m, 2 H), 7.63–7.67 (m, 2 H), 8.11 (s, 1 H), 8.85 (br s, 1 H).

13C NMR (100 MHz, CDCl3): δ = 7.01, 13.58, 21.48, 24.32, 25.98, 30.36, 33.45, 60.11, 75.34, 107.77, 114.89, 118.49, 119.46, 120.68, 120.93, 125.38, 126.29, 129.09, 133.58, 134.22, 134.29, 134.60, 140.01, 162.76, 164.83.

ESI-MS: m/z = 535 [M + H].
Yield: 0.125 g (91%); white solid; m.p. 168–169 °C.

ESI-MS: m/z = 544 [M+H].

1-(5-(((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-1,3,4-oxadiazol-2-yl)methyl)-1,3,4,9-tetrahydropyran-3,4-bindole (7k)

Yield: 0.122 g (92%); white solid; m.p. 156–158 °C.

ESI-MS: m/z = 531 [M+H].

1,8-Diethyl-1-((5-(((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)thio)-1,3,4-oxadiazol-2-yl)methyl)-1,3,4,9-tetrahydropyran-3,4-bindole (7o)

Yield: 0.132 g (90%); white solid; m.p. 146–148 °C.

ESI-MS: m/z = 501 [M+H].

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Supporting Information

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References


