Anti-HIV Activity of New Substituted 1,3,4-Oxadiazole Derivatives and their Acyclic Nucleoside Analogues

Wael A. El-Sayed\textsuperscript{a}, Farag A. El-Essawy\textsuperscript{b}, Omar M. Ali\textsuperscript{b}, Barsis S. Nasr\textsuperscript{b}, Mohamed M. Abdalla\textsuperscript{c}, and Adel A.-H. Abdel-Rahman\textsuperscript{b,*}

\textsuperscript{a} Photochemistry Department, National Research Center, El Dokki, Cairo, Egypt
\textsuperscript{b} Faculty of Science, Department of Chemistry, Menoufia University, Shebin El-Koom, Egypt. E-mail: adelnassar63@hotmail.com
\textsuperscript{c} Research Unit, Univet Pharmaceutical Co., Cairo, Egypt

* Author for correspondence and reprint requests

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A number of new 5-\{(naphthalen-5-yloxy)methyl\}-1,3,4-oxadiazole derivatives, 2 – 5 and 8 – 11, were synthesized. The 2-5-\{(naphthalen-5-yloxy)methyl\}-1,3,4-oxadiazol-2-ylthio|acetohydrazones 6\textsubscript{a} and 6\textsubscript{b} were synthesized by the reaction of the hydrazide 4 with the corresponding monosaccharides. Cyclization of the sugar hydrazones 6\textsubscript{a} and 6\textsubscript{b} with acetic anhydride afforded the substituted oxadiazoline derivatives 7\textsubscript{a} and 7\textsubscript{b}. The synthesized compounds were evaluated for their antiviral activity against, the human immunodeficiency virus (HIV-1) and some of these compounds showed moderate to high antiviral activity.

Key words: Sugar Hydrazones, 1,3,4-Oxadiazoles, Acyclic Nucleosides, Antiviral Activity

Introduction

The synthesis and screening of compounds as important objectives in pharmaceutical chemistry to get new potent and active leads increases rapidly. Among the five-membered nitrogen heterocycles, 1,3,4-oxadiazoles are associated with a broad spectrum of biological activities (Zareen et al., 2004; El-Azzouny et al., 2003; Loetchutinat et al., 2003). Their derivatives possess antibacterial (Ates et al., 1997), antimicrobial (Rahman and Farghaly, 2004), insecticidal (Li et al., 2003), herbicidal, fungicidal (Zou et al., 2002), anti-inflammatory (Palaska et al., 2002), and hypoglycaemic (Mhasalkar et al., 1971) characteristics, and antiviral (El-Emam et al., 2004) and antitumour activities (Loszqkiewicz et al., 2003). On the other hand, the acyclic C-nucleoside analogues possess a wide range of biological properties, including antibiotic, antiviral, and antitumour activities (Holy, 1987; Remy and Secrist, 1985; Larson et al., 1983; El Ashry and El Kilany, 1996, 1997, 1998; Chu and Cutler, 1986; Markar and Keseru, 1997; Franchetti et al., 1997; Hammerschmidt et al., 1997). The most unique feature of C-nucleosides is that the sugar chain is connected to the pendant heterocyclic base by a C-C bond instead of the C-N bond of the natural nucleosides. As a result, they are resistant to chemical and enzymatic hydrolytic cleavage. Our interest in the attachment of various carbohydrate residues to newly synthesized 1,3,4-oxadiazoles enhances our attempt to modify leading compounds synthesized for antiviral screening (El-Essawy et al., 2008; Ali et al., 2007; El-Sayed et al., 2008). Owing to these facts, our aim in the present work is the synthesis of new 2,5-disubstituted 1,3,4-oxadiazole derivatives as well as the attachment of the synthesized derivatives to several carbohydrate moieties.

Experimental

General

Melting points were determined using a Büchi apparatus. IR spectra (KBr) were recorded with a Bruker-Vector22 instrument (Bruker, Bremen, Germany). \textsuperscript{1}H NMR spectra were recorded with a Varian Gemini spectrometer at 300 MHz and 200 MHz with TMS as internal standard. Chemical shifts are reported in \( \delta \) scale (ppm) relative to TMS as a standard, and the coupling constants (\( J \) values) are given in Hz. The progress of the reactions was monitored by TLC using aluminium silica gel plates 60 F245. EI mass spectra were recorded with a HP D5988 A 1000 MHz instrument (Hewlett-Packard, Palo Alto, CA, USA). Antiviral activity against hepatitis C virus (HCV) and human immunodeficiency virus (HIV) was...
HIV inhibitory activity and reverse transcriptase inhibition with therapeutic windows

Cells and viruses

The established human cells and laboratory-derived virus isolates used in these evaluations have previously been described (Buckheit et al., 1995; Byrnes et al., 1993). These cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Fresh human cells were obtained from the American Red Cross (Baltimore, MD, USA).

Antiviral and cross-resistance assays

The inhibitory activities of the compounds against HIV were evaluated by microtiter anti-HIV assays with CEM-SS cells or fresh human peripheral blood mononuclear cells (PBMCs); these assays quantify the ability of a compound to inhibit HIV-induced cell killing or HIV replication. Quantification was performed by the tetrazolium dye XTT assay (CEM-SS, 174 × CEM, MT2, and AA5 cell-based assays), in which a coloured formazan product is formed by viable cells, RT assay (U937- and PBMC-based assays), and/or p24 enzyme-linked immunosorbent assay (monocyte-macrophage assay). Antiviral and toxicity data are reported as the concentration of drug required to inhibit the virus-induced cell killing or virus production by 50% (EC₅₀) and the concentration of drug required to reduce the cell viability by 50% (IC₅₀). For comparison, atevirdine was used as standard drug.

In vitro assays of anti-HIV activity

Purified RT assays of each newly synthesized compound were conducted for determining the RT inhibitory activity against purified recombinant HIV-1 RT using the cell-free Quan-T-RT assay system (Amersham Corp., Arlington Heights, IL, USA), which utilizes the scintillation proximity assay (SPA) principle (Zarling et al., 1991; Bosworth and Towers, 1989). In this assay, a DNA/RNA template is bound to SPA beads via an iotin/streptavidin linkage. The primer DNA is a 16-mer oligo (T), which has been annealed to a poly (rA) template. The primer-template is bound to a streptavidin-coated SPA bead. [³H]TTP (thymidine 5’ triphosphate) is incorporated into the primer by reverse transcription. In brief, [³H]TTP, at a final content of 18500 Bq (0.5 μCi)/sample, was diluted in the RT assay buffer [49.5 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 8.0, 80 mM KCl, 10 mM MgCl₂, 10 mM dithiothreitol, 2.5 mM EGTA, 0.05% Nonidet P-40] and added to annealed DNA/RNA bound to SPA beads. The compound being tested was added to the reaction mixture at 0.001–100 μM. Addition of 10 μM of recombinant HIV RT and incubation at 37 °C for 1 h resulted in the extension of the primer by incorporation of [³H]TTP. The reaction was stopped by addition of 0.2 ml of 120 mM EDTA. The samples were counted in an open window using a Beckman LS 7600 instrument and IC₅₀[RT] values (concentration at which the compound inhibits recombinant RT by 50%) were calculated by comparing the measurements to that of an untreated sample.

Results and Discussion

Chemistry

The starting material 2-(naphthalen-5-yloxy)-acetohydrazide (1) was synthesized following a reported procedure (Palaska et al., 2002) by refluxing its corresponding ethyl ester and hydrazine hydrate in ethanol. When the acid hydrazide 1 reacted with CS₂ in alkaline medium it afforded 5-[(naphthalen-5-yloxy)methyl]-1,3,4-oxadiazole-2-thiol (2) in 78% yield. Reaction of the 1,3,4-oxadiazole derivative 2 with ethyl chloroacetate afforded the S-substituted ethyl ester derivative 3 in 75% yield. The 1H NMR spectrum of 3 showed the signals of the ethyl group as a triplet at δ 1.15 ppm and a quartet at δ 4.09 ppm, the two singlet peaks for the remaining CH 2 groups at δ 4.23 and 5.59 ppm in addition to signals for the aromatic protons at δ 7.14 – 8.19 ppm. Treatment of 3 with hydrazine hydrate gave the corresponding acid hydrazide 4 in 75% yield. Its 1H NMR spectrum showed the signals of the ethyl group as a triplet at δ 1.15 ppm and a quartet at δ 4.09 ppm, the two singlet peaks for the remaining CH 2 groups at δ 4.23 and 5.59 ppm in addition to signals for the aromatic protons at δ 7.14 – 8.19 ppm. Treatment of 3 with hydrazine hydrate gave the corresponding acid hydrazide 4 in 75% yield. Its structure was proved by means of IR, 1H NMR and mass spectra which all agreed with the assigned structure. Reaction of 4 with CS₂ in the presence of potassium hydroxide gave 5-[[naphthalen-5-yloxy]methyl]-1,3,4-oxadiazol-2-ylthio]methyl]-1,3,4-oxadiazole-2-thiol (5) in 72% yield. Its 1H NMR spectrum showed two singlet peaks at δ 4.86 and 5.33 ppm for the two CH 2 groups at δ
7.09 – 7.89 ppm and the NH group at δ 13.8 ppm (Fig. 1).

When the hydrazide 4 was allowed to react with a number of monosaccharides, the corresponding aldehyde sugar hydrazones were obtained. Thus, reaction of 4 with D-galactose and D-xylose in an aqueous ethanolic solution and catalytic amount of acetic acid gave the corresponding sugars 2-[5-[(naphthalen-5-yloxy)methyl]-1,3,4-oxadiazol-2-ylthio]acetoxyhydrzones 6a and 6b in 72 – 78% yields. The structures of these compounds were confirmed by analytical and spectral data. The IR spectra of 6a and 6b showed the presence of characteristic absorption bands in the region 3381 – 3450 cm⁻¹ corresponding to the hydroxy groups. The ¹H NMR spectra showed the signals of the sugar chain protons at δ 3.39 – 5.42 ppm, the C-1 methine proton as doublet in the range δ 7.15 – 7.55 ppm in addition to the aromatic protons in the region δ 7.50 – 8.16 ppm. The reaction
of the sugar aryldrazones with boiling acetic anhydride is well known to give the respective per-\(O,N\)-acetyl 1,3,4-oxadiazole derivatives 7a and 7b (Abdel-Aal et al., 2006, 2008; Somogyi, 1977, 1978). However, reaction of the sugar hydrazones 6a and 6b with acetic anhydride at reflux temperature afforded the substituted 1,3,4-oxadiazole derivatives 7a and 7b in 65–68% yields. Their structures were established on the basis of spectral and analytical data. The IR spectra of 7a and 7b showed characteristic absorption bands at 1653–1678 cm\(^{-1}\) and 1746–1775 cm\(^{-1}\) corresponding to the carbonyl amide and the carbonyl ester groups, respectively, indicating the presence of an \(N\)-acetyl group in addition to the \(O\)-acetyl group.

The \(^1\)H NMR spectra of 7a and 7b showed the signals of the \(O\)-acetyl-methyl protons as singlets in the range \(\delta 1.95–2.07\) ppm and the \(N\)-acetyl-methyl protons in the range \(\delta 2.19–2.21\) ppm. The rest of the sugar chain protons appeared in the range \(\delta 3.98–5.37\) ppm in addition to the aromatic protons, as multiplets, in the region \(\delta 7.20–8.17\) ppm (Fig. 1).

Alkylation of the oxadiazole thione 2 with methyl or ethyl iodide in alkaline medium afforded the 2-(alkylthio)-5-[(naphthalen-5-yloxy)methyl]-1,3,4-oxadiazoles 8a and 8b in 76–79% yields. Hydrazinolysis of 8a and 8b gave the required hydrazine derivative 1-{5-[(naphthalen-5-yloxy)methyl]-1,3,4-oxadiazol-2-yl]hydrazine (9) in 77% yield. The \(^1\)H NMR spectra of 8a and 8b showed the signals of the methyl group for 8a and the ethyl group as triplet and quartet for 8b which disappeared in the spectrum of 9 in which the NH\(_2\) signal appeared at \(\delta 6.07\) ppm (Fig. 2).

When the oxadiazole thione 2 reacted with acrylonitrile the corresponding \(N\)-substituted alkyl nitrile derivative 10 was obtained in 72% yield. Its IR spectrum showed a characteristic peak at 2225 cm\(^{-1}\) for the CN group, and its \(^1\)H NMR spectrum showed the signal for the two CH\(_2\) groups each as a triplet at \(\delta 3.09\) and 3.34 ppm. Treatment of 10 with hydrazine hydrate in ethanol at reflux temperature afforded 3-{5-[(2-naphthyl-oxy)methyl]-2-thioxo-1,3,4-oxadiazol-3(2\(H\))-yl]-propanimido-hydrazide (11) (70%). Its structure was proved by means of IR, \(^1\)H NMR and mass spectra which all agreed with the assigned structure (Fig. 2).

**Anti-HIV activity**

The newly synthesized compounds were evaluated for their HIV inhibitory activity as reverse transcriptase inhibitors by using microtiter anti-HIV assays with CEM-SS cells or fresh human peripheral blood mononuclear cells. The results of

![Fig. 2. Synthesis of the hydrazide and imidrazone of 1,3,4-oxadiazoles.](image-url)
the antiviral activity test (Table 1) revealed that compound 6b showed the highest activity with an IC_{50} value of 1.44 \mu M and a therapeutic index of 3.15 \cdot 10^7 followed by compounds 4 and 8a with IC_{50} values of 1.88 and 2.12 \mu M. Compounds 7b and 11 showed moderate activities while 6a and 7a showed the weakest activity among the series of tested compounds. Furthermore, the anti-HIV activity observed for the 1,3,4-oxadiazolylthio sugar hydrazone derivative 6b indicated the importance of the free hydroxy xylotetritolyl moiety as the activity was reduced when this group was protected as in the corresponding O-acetylated derivative 7b or the galactopentitolyl derivative 6a.

Conclusion

From the results of or antiviral activity test and the structure activity relationship, it can be concluded that the attachment of a free hydroxy sugar moiety increases the activity against HCV and HIV compared to the corresponding O-acetylated analogues. Furthermore, the free hydroxy galactopentitolyl moiety derived from the aldohexose D-galactose showed higher anti-HCV activity than the xylotetritolyl moiety derived from the aldopentose D-xylose, concerning the anti-HIV activity, the latter exhibited higher activity than the corresponding galactopentitolyl moiety.

Table I. HIV inhibition activities (reverse transcriptase inhibition) with therapeutic windows.

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC_{50} [\mu M]</th>
<th>IC_{50} [\mu M]</th>
<th>Therapeutic index</th>
</tr>
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<tbody>
<tr>
<td>4</td>
<td>3.24 \cdot 10^{-3}</td>
<td>1.88</td>
<td>2.88 \cdot 10^{7}</td>
</tr>
<tr>
<td>6a</td>
<td>1.1 \cdot 10^{-5}</td>
<td>12.89</td>
<td>6.24 \cdot 10^{8}</td>
</tr>
<tr>
<td>6b</td>
<td>5.26 \cdot 10^{-4}</td>
<td>1.44</td>
<td>3.15 \cdot 10^{7}</td>
</tr>
<tr>
<td>7a</td>
<td>5.23 \cdot 10^{-4}</td>
<td>12.44</td>
<td>5.78 \cdot 10^{6}</td>
</tr>
<tr>
<td>7b</td>
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<td>3.11</td>
<td>3.45 \cdot 10^{6}</td>
</tr>
<tr>
<td>8a</td>
<td>3.81 \cdot 10^{-3}</td>
<td>2.12</td>
<td>8.14 \cdot 10^{6}</td>
</tr>
<tr>
<td>11</td>
<td>2.72 \cdot 10^{-3}</td>
<td>2.9</td>
<td>5.12 \cdot 10^{6}</td>
</tr>
</tbody>
</table>

EC_{50} and IC_{50} values were estimated by logistic regression analysis. One-way ANOVA (P < 0.01) was used to test treatment differences in EC_{50} and IC_{50} values. After determination of the significant factor by ANOVA, individual group differences were analyzed using Holm-Sidak’s procedure (Guo and Romano, 2007) for multiple comparisons versus control.


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