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

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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 **6a** and **6b** were synthesized by the reaction of the hydrazide **4** with the corresponding monosaccharides. Cyclization of the sugar hydrazones **6a** and **6b** with acetic anhydride afforded the substituted oxadiazoline derivatives **7a** and **7b**. 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 (Liszkiewicz *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). ^{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 δ 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 aluminum 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

tested at Research Unit, Univet Pharmaceutical Co., Cairo, Egypt.

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% (EC_{50}) and the concentration of drug required to reduce the cell viability by 50% (IC_{50}). 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. [^{3}H]TTP (thymidine 5' triphosphate) is incorporated into the primer by reverse transcription. In brief, [^{3}H]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 $MgCl_2$, 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 [^{3}H]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 $IC_{50[RT]}$ 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 CS_2 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 ^{1}H 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 structure was proved by means of IR, ^{1}H NMR and mass spectra which all agreed with the assigned structure. Reaction of **4** with CS_2 in the presence of potassium hydroxide gave 5-{[5-[(naphthalen-5-yloxy)methyl]-1,3,4-oxadiazol-2-ylthio]methyl}-1,3,4-oxadiazole-2-thiol (**5**) in 72% yield. Its ^{1}H NMR spectrum showed two singlet peaks at δ 4.86 and 5.33 ppm for the two CH_2 groups in addition to the signals of the aromatic protons at δ

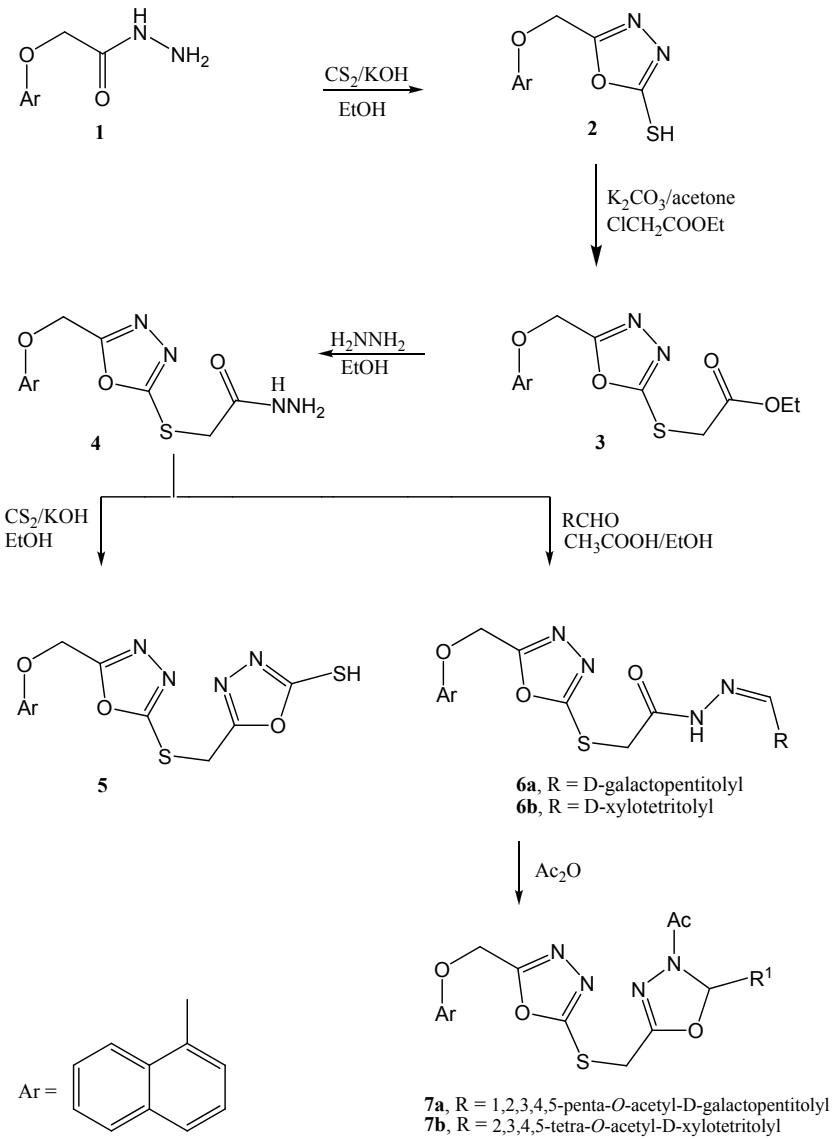

Fig. 1. Synthesis of disubstituted 1,3,4-oxadiazoles.

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 aldehydo 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}acetohydrazones **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 arylhydrazones 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 δ 1.95–2.07 ppm and the *N*-acetyl-methyl protons in the range δ 2.19–2.21 ppm. The rest of the sugar chain protons appeared in the range δ 3.98–5.37 ppm in addition to the aromatic protons, as multiplets, in the region δ 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 δ 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 δ 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

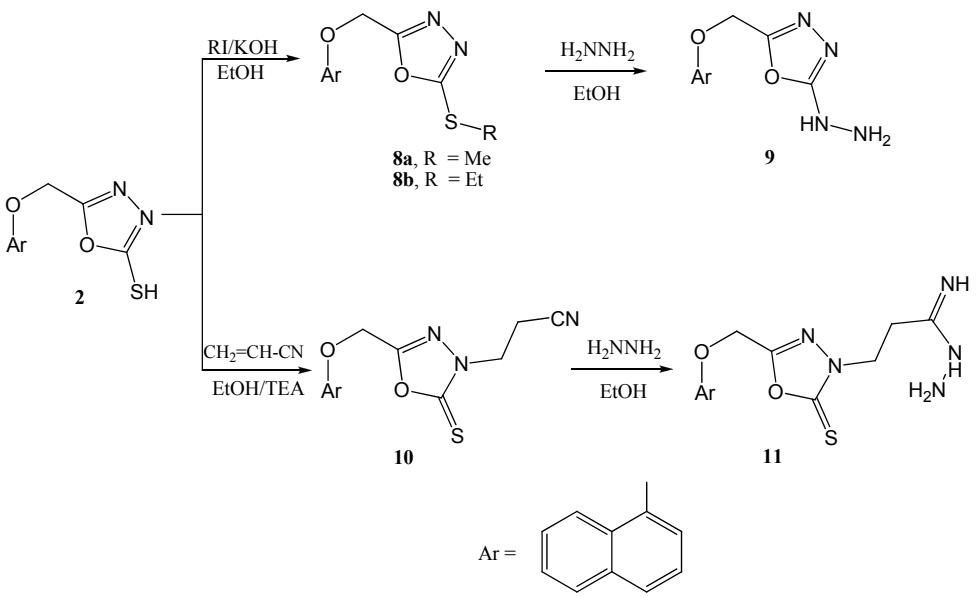

Fig. 2. Synthesis of the hydrazide and imidrazone of 1,3,4-oxadiazoles.

the antiviral activity test (Table I) revealed that compound **6b** showed the highest activity with an IC_{50} value of 1.44 μM 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 μM. 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.

Compound	EC_{50} [μM][a]	IC_{50} [μM][b]	Therapeutic index[c]
4	$3.24 \cdot 10^{-3}$	1.88	$2.88 \cdot 10^7$
6a	$1.1 \cdot 10^{-5}$	12.89	$6.24 \cdot 10^8$
6b	$5.26 \cdot 10^{-4}$	1.44	$3.15 \cdot 10^7$
7a	$5.23 \cdot 10^{-4}$	12.44	$5.78 \cdot 10^6$
7b	$1.56 \cdot 10^{-3}$	3.11	$3.45 \cdot 10^6$
8a	$3.81 \cdot 10^{-3}$	2.12	$8.14 \cdot 10^6$
11	$2.72 \cdot 10^{-3}$	2.9	$5.12 \cdot 10^6$

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.

[a] Compound concentration required to inhibit the virus-induced cell killing by 50%.

[b] Compound concentration required to achieve 50% inhibition of recombinant HIV-1 RT activity.

[c] The therapeutic index is the toxic dose of a drug for 50% of the population (TD_{50}) divided by the minimum effective dose for 50% of the population (ED_{50}).

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