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ORIGINAL PAPER

Synthesis and antiviral evaluation of new 2,5-disubstituted 1,3,4-oxadiazole derivatives and their acyclic nucleoside analogues

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Abstract A number of new 5-[(naphthalen-1-yloxy)-methyl]-1,3,4-oxadiazole derivatives were synthesized. Sugar 2-[5-[(naphthalen-1-yloxy)methyl]-1,3,4-oxadiazol-2-ylthio]acetohydrazones were prepared by condensation of the hydrazide with the corresponding monosaccharides. Cyclization of the sugar hydrazones with acetic anhydride afforded the substituted oxadiazoline derivatives. The synthesized compounds displayed different degrees of antiviral activities or inhibitory actions against HCV and HIV viruses.

Keywords Sugar hydrazones · 1,3,4-Oxadiazoles · Acyclic nucleosides · Antiviral agents

Introduction

The synthesis and screening of compound libraries have increased rapidly and become important major objectives in pharmaceutical chemistry, leading to new potent leads. Among the five-membered nitrogen heterocycles, the 1,3,4-oxadiazoles are known to be associated with a broad spectrum of biological activities [1–3]. Their derivatives have been known to possess antibacterial [4], antimicrobial [5], insecticidal [6], herbicidal, fungicidal [7], anti-inflammatory [8], hypoglycaemic [9], and hypotension [10] characteristics, as well as antiviral [11] and anti-tumour activities [12]. On the other hand, the acyclic *C*-nucleoside analogues possess a wide range of biological properties, including antibiotic, antiviral, and anti-tumour activities [13–22]. 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 prompted us towards the modification of some lead compounds as antiviral agents [23–25]. Thus, the aim of this work is the synthesis of novel 2,5-disubstituted 1,3,4-oxadiazole derivatives that incorporate a variety of substituents with different electronic environments in addition to some selected carbohydrate moieties to be evaluated for their antiviral activities.

Results and discussion

The starting material 2-(naphthalen-1-yloxy)acetohydrazide (**1**) was synthesized following a previously reported procedure [8]. Reaction of the acid hydrazide **1** with CS_2 in alkaline medium afforded the corresponding 1,3,4-oxadiazole-2-thiol derivative **2** [26], which upon treatment with ethyl chloroacetate gave rise to the ethyl acetate analogue **3** in good yield. The ^{1}H NMR spectrum of **3** showed the signals of the ethyl group as triplet at $\delta = 1.15$ and quartet at $\delta = 4.09$ ppm, two singlets for the two CH_2 groups at $\delta = 4.23$ and 5.59 ppm in addition to signals for the aromatic protons at $\delta = 7.14$–8.19 ppm. Reflux of the ethyl

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acetate derivative **3** with $N_2H_4{\cdot}H_2O$ yielded the proposed acid hydrazide **4**. Reaction of the hydrazide **4** with CS_2 in the presence of KOH gave rise to the respective 1,3,4-oxadiazole-2-thiol **5**. Its ^{1}H NMR spectrum showed two singlet peaks at $\delta = 4.86$ and 5.33 ppm for the two CH_2 in addition to the signals of the aromatic protons at $\delta = 7.09$–7.89 ppm and the NH group at $\delta = 13.80$ ppm. Reaction of **4** with D-galactose and D-xylose in aqueous ethanolic solution containing a catalytic amount of acetic acid gave rise to the corresponding sugar acetohydrazones **6a** and **6b**. The structures of these compounds were confirmed by analytical and spectral data. The IR spectra of **6a** and **6b** showed the presence of absorption bands characteristic for the hydroxyl groups in the 3,381–3,450 cm^{-1} region. The ^{1}H NMR spectra revealed the signals of the sugar chain protons at $\delta = 3.39$–5.42 ppm, the C-1 methine proton as a doublet in the range $\delta = 7.15$–7.55 ppm in addition to the aromatic protons in the region $\delta = 7.50$–8.16 ppm. Reaction of sugar hydrazones with boiling acetic anhydride is known to give the respective per-*O,N*-acetyl derivatives [27–30]. However, unexpectedly, when the sugar hydrazones **6a** and **6b** were refluxed with acetic anhydride, the substituted 1,3,4-oxadiazoline derivatives **7a** and **7b** were obtained instead. The latter structures were established on the basis of their spectral and analytical data. The IR spectra of **7a** and **7b** showed characteristic absorption bands at 1,653–1,678 and 1,746–1,775 cm^{-1}, corresponding to the amide and ester carbonyl groups, respectively, indicating the presence of an *N*-acetyl group in addition to the *O*-acetyl groups. The ^{1}H NMR spectra of **7a** and **7b** showed the signals of the *O*-acetyl protons as singlets in the range $\delta = 1.95$–2.07 ppm and the *N*-acetyl 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 (Scheme 1).

Alkylation of the oxadiazole thiones **2** with methyl or ethyl iodide in alkaline medium afforded the respective alkylthio analogues **8a** and **8b** in reasonable yields. Treatment of **8a** and **8b** with hydrazine hydrate gave rise to the target hydrazine derivative **9**. The ^{1}H NMR spectra of **8a**, **8b** showed the signals of the methyl group for **8a** and the ethyl group as a triplet and quartet for **8b**, which disappeared in the spectrum of **9** in which the NH_2 signal appeared at $\delta = 6.07$ ppm. Reaction of the oxadiazole thione **2** with acrylonitrile in presence of triethylamine resulted in the formation of alkylnitrile derivative **10** in good yield. The IR spectrum of this compound showed a characteristic peak at 2,225 cm^{-1} for the CN group, while its ^{1}H NMR spectrum showed the signals characteristic for 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 the corresponding propanimido hydrazide **11** in reasonable yield. Its structure was confirmed by means of IR, ^{1}H NMR, and mass spectra, which were in agreement with the assigned structure (Scheme 2).

Antiviral activity

Anti-hepatitis C virus activity

The newly synthesized compounds were tested for their antiviral activity against hepatitis C virus (HCV) by using HCV replicon cells. The anti-HCV results (Table 1) showed that compound **6a** exhibited the highest activity with a minimum inhibitory concentration (MIC) of 1.33 μg/cm^3 followed by compounds **11** and **7a**. Compounds **6b** and **7b** showed moderate inhibition activities with MIC values of 5.12 and 5.16 μg/cm^3, while compounds **4** and **8a** were the least active against HCV in the series of tested compounds. Additionally, the structural activity relationship revealed that the antiviral activity observed for the substituted sugar hydrazone **6a** indicated the importance of the free hydroxyl galactopentitolyl moiety as the activity was reduced when this group was *O*-acetylated (**7a**) or replaced by the xylotetritolyl moiety (**6b**, Table 1).

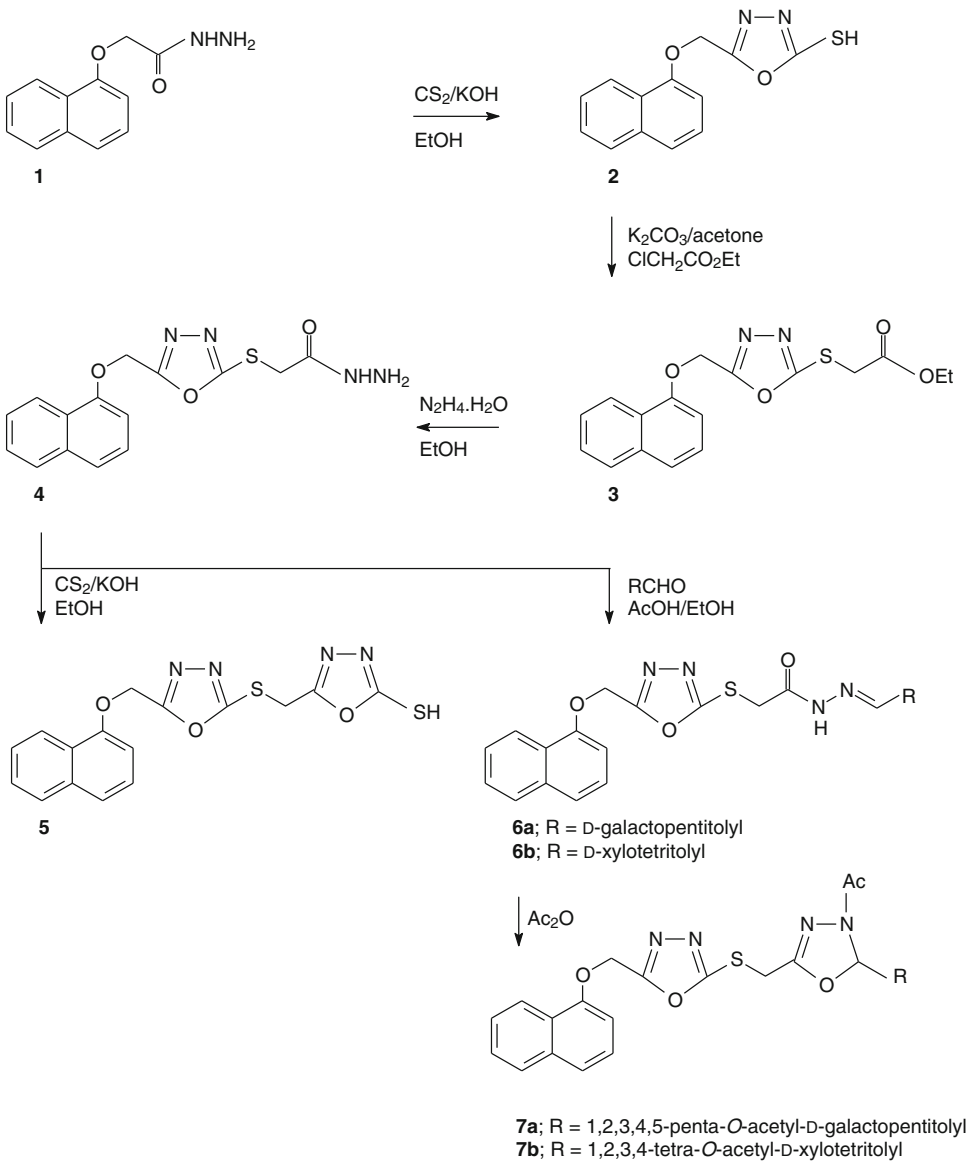
Anti-HIV activity

The newly synthesized compounds were evaluated for their HIV inhibition activity as reverse transcriptase inhibitors by using microtiter anti-HIV assays with CEM-SS cells or fresh human peripheral blood mononuclear cells (PBMCs). The results of antiviral activity (Table 2) revealed that compound **6b** showed the highest activity with an IC_{50} value of 1.44 μM and therapeutic index of 3.15×10^7 followed by compounds **4** and **8a** with $IC_{50} = 1.88$ and 2.12 μM. Compounds **7b** and **11** showed moderate activities, while compounds **6a** and **7a** showed the least 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 hydroxyl xylopentitolyl moiety as the activity was reduced when this group was replaced by its corresponding *O*-acetylated derivative (**7b**) or the galactopentitolyl moiety (**6a**, Table 2).

Conclusion

From the results of the antiviral activity and structural activity relationship, it could be concluded that the attachment of a free hydroxyl sugar moiety increases the activity against HCV and HIV viruses compared to its corresponding *O*-acetylated analogs. Furthermore, the free hydroxyl galactopentitolyl moiety derived from the aldohexose D-galactose showed higher anti-HCV activity

Scheme 1



than a xylotetritolyl moiety derived from the aldopentose D-xylose, while for anti-HIV activity the latter exhibited higher activity than the corresponding galactopentitolyl moiety.

Experimental

Melting points were determined using a Kofler block instrument. TLC was performed on plastic plates (Silica Gel 60 F_{254}; E. Merck, layer thickness 0.2 mm). NMR spectra were recorded on a Bruker AC 300 FT NMR spectrometer at 300 MHz for ^{1}H NMR and at 75 MHz for ^{13}C NMR with TMS as an internal standard. ES mass spectra were run on an Esquire 3000plus iontrap mass spectrometer from Bruker Daltonics. The microanalyses were performed at the microanalytical unit, Cairo University, Egypt, and were found to agree favorably with the calculated values. Antiviral activity of the synthesized compounds was conducted at the Research Unit, Univet Pharmaceutical Co., Egypt. 2-(Naphthalen-1-yloxy)acetohydrazide (**1**) and 5-[(naphthalen-1-yloxy)methyl]-1,3,4-oxadiazole-2-thiol (**2**) were prepared according to reported procedures [8] [26].

Ethyl 2-[5-[(naphthalen-1-yloxy)methyl]-1,3,4-oxadiazol-2-ylthio]acetate (**3**, $C_{17}H_{16}N_2O_4S$)

To a solution of 2.58 g **2** (10 mmol) and 1.38 g anhydrous K_2CO_3 (10 mmol) in 25 cm^3 DMF, 1.22 g ethyl chloroacetate (10 mmol) was added. The solution was stirred at room temperature for 6 h and then poured onto ice-cold H_2O. The resulting precipitate was filtered off and recrystallized from EtOH to give 2.64 g **3** (77%) as a yellow

Scheme 2

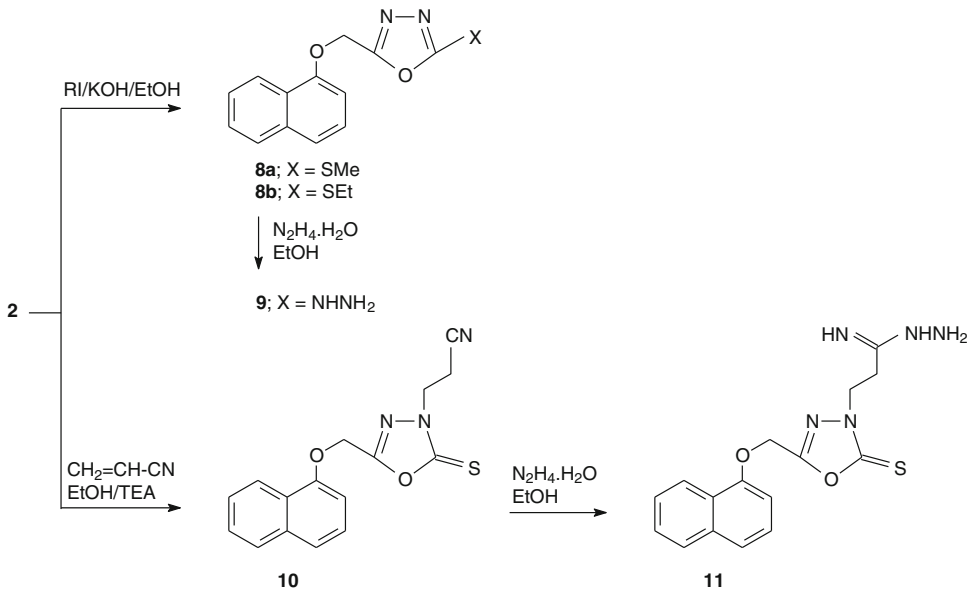


Table 1 Anti-hepatitis C virus (HCV) activity: minimum inhibition concentration (MIC)

Compound	MIC (μg/cm^3)	
	HCV	Subacute sclerosing anencephalitis (SSPE)
4	8.39	–
6a	1.33	4.24
6b	5.12	–
7a	2.56	5.8
7b	5.16	5.17
8a	8.24	–
11	2.32	–

Table 2 HIV inhibition activities (reverse transcriptase inhibition) with therapeutic windows

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

powder. M.p.: 112–113 °C; IR (KBr): $\bar{\nu} = 1{,}731$ (C=O), 1,643 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta = 1.15$ (t, $J = 5.6$ Hz, CH_3), 4.09 (q, $J = 5.6$ Hz, CH_2), 4.23 (s, CH_2), 5.59 (s, CH_2), 7.14 (m, Ar–H), 7.44 (d, $J = 8.2$ Hz, Ar–H), 7.47 (m, Ar–H), 7.52 (m, Ar–H), 7.55 (d, $J = 8.2$ Hz, Ar–H), 7.97 (d, $J = 8.5$ Hz, Ar–H), 8.19 (d, $J = 8.2$ Hz, Ar–H) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 8.10$ (CH_3), 41.84 (SCH_2), 60.60, 68.91 (2 OCH_2), 107.10, 120.52, 123.87, 125.99, 126.69, 128.99, 134.69, 155.96 (Ar–C), 158.77 (C-5, oxadiazole), 167.97 (C-2, oxadiazole), 175.90 (C=O) ppm; MS (ESI): $m/z = 383$ [M$^+$+K].

2-[5-[(Naphthalen-1-yloxy)methyl]-1,3,4-oxadiazol-2-ylthio]acetic acid hydrazide (**4**, $C_{15}H_{14}N_4O_3S$)

A solution of 1.72 g **3** (5 mmol) and 0.26 cm^3 $N_2H_4{\cdot}H_2O$ (10 mmol) in 25 cm^3 EtOH was heated under reflux for 6 h. The solution was cooled, and the resulting precipitate was filtered and recrystallized from EtOH to afford 1.23 g **4** (75%) as a white solid. M.p.: 204–205 °C; IR (KBr): $\bar{\nu} = 3{,}493$ (NH_2), 3,410 (NH), 1,687 (C=O), 1,643 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta = 3.85$ (s, CH_2), 5.37 (s, CH_2), 6.11 (s, NH_2), 7.22 (m, Ar–H), 7.44 (d, $J = 8.5$ Hz, Ar–H), 7.46 (m, Ar–H), 7.49 (m, Ar–H), 7.83 (d, $J = 8.2$ Hz, Ar–H), 7.86 (d, $J = 8.2$ Hz, Ar–H), 8.21 (d, $J = 8.5$ Hz, Ar–H), 9.39 (s, NH) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 40.34$ (SCH_2), 60.20 (OCH_2), 107.30, 120.72, 123.55, 125.45, 127.60, 134.61, 157.91 (Ar–C), 163.27 (C-5, oxadiazole), 169.27 (C-2, oxadiazole), 170.30 (C=O) ppm; MS (ESI): $m/z = 329$ [M$^+$−1].

5-[[5-[(Naphthalen-1-yloxy)methyl]-1,3,4-oxadiazol-2-ylthio]methyl]-1,3,4-oxadiazole-2-thiol

(**5**, $C_{16}H_{12}N_4O_3S_2$)

To a solution of 0.33 g **4** (1 mmol) in 15 cm^3 EtOH, a solution of 0.056 g KOH (1 mmol) in 2 cm^3 H_2O and 0.23 cm^3 CS_2 (3 mmol) was added. The solution was heated under reflux for 15 h. The solvent was evaporated, and the residue was dissolved in H_2O, filtered, and acidified with diluted HCl. The precipitate was filtered off, washed

with H_2O, and recrystallized from EtOH to afford 0.26 g **5** (72%) as a yellow solid. M.p.: 150–152 °C; IR (KBr): $\bar{\nu}$ = 3,415 (NH), 1,624 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ = 4.86 (s, CH_2), 5.33 (s, CH_2), 7.09 (m, Ar–H), 7.13 (d, J = 8.5 Hz, Ar–H), 7.24 (m, Ar–H), 7.53 (m, Ar–H), 7.86 (d, J = 8.2 Hz, Ar–H), 7.88 (d, J = 8.5 Hz, Ar–H), 7.89 (d, J = 8.2 Hz, Ar–H), 13.80 (s, SH) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 36.64 (SCH_2), 66.25 (OCH_2), 107.37, 120.83, 123.44, 125.73, 127.82, 134.69, 157.41 (Ar–C), 160.90, 163.17 (2 C-5, oxadiazole), 168.28, 168.59 (2 C-2, oxadiazole) ppm; MS (ESI): m/z = 395 [M^++Na].

*General procedure for the preparation of sugar acetohydrazones **6a**, **6b***

To a well-stirred solution of the monosaccharide (10 mmol) in 2 cm^3 H_2O and 0.3 cm^3 glacial AcOH in 10 cm^3 EtOH, 1.65 g **4** (10 mmol) was added. The mixture was heated under reflux for 3 h, and the resulting solution was concentrated and left to cool. The formed precipitate was filtered off, washed with H_2O, EtOH, and then dried and recrystallized from EtOH.

2-[5-[(Naphthalen-1-yloxy)methyl]-1,3,4-oxadiazol-2-ylthio]acetic acid 2-(D-galactopyranosylidene)hydrazide (**6a**, $C_{21}H_{24}N_4O_8S$)
Yield 78%; m.p.: 188-189 °C; IR (KBr): $\bar{\nu}$ = 3, 450 (OH), 3,388 (NH), 1,687 (C=O), 1,625 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ = 3.49 (m, H-6′, H-6″), 3.51 (m, H-5′), 4.18 (m, H-4′), 4.36 (dd, J = 2.8 Hz, 5.8 Hz, H-3′), 4.46 (t, J = 5.8 Hz, H-2′), 4.98 (s, CH_2), 4.61 (m, OH), 4.72 (d, J = 6.3 Hz, OH), 4.90 (m, OH), 4.97 (t, J = 4.5 Hz, OH), 5.12 (s, CH_2), 5.36 (t, J = 4.5 Hz, OH), 7.20 (m, Ar–H), 7.24 (d, J = 8.4 Hz, Ar–H), 7.44 (d, J = 8.4 Hz, Ar–H), 7.48 (m, Ar–H), 7.86 (m, Ar-2H), 8.16 (d, J = 8.2 Hz, Ar–H), 11.21 (s, NH) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 40.91 (SCH_2), 60.80 (C-2), 64.23 (OCH_2), 64.90 (C-6), 70.61 (C-4), 71.90 (C-3), 72.40 (C-5), 107.45, 120.60, 123.43, 125.25, 127.40, 134.33 (Ar–C), 153.40 (C-1), 163.26 (Ar–C), 166.41 (C-5, oxadiazole), 168.21 (C-2, oxadiazole), 171.10 (C=O) ppm; MS (ESI): m/z = 491 [M^+−1].

2-[5-[(Naphthalen-1-yloxy)methyl]-1,3,4-oxadiazol-2-ylthio]acetic acid 2-(D-xylopyranosylidene)hydrazide (**6b**, $C_{20}H_{22}N_4O_7S$)
Yield 72%; m.p.: 183–185 °C; IR (KBr): $\bar{\nu}$ = 3,381 OH), 3,295 (NH), 1,660 (C=O), 1,614 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ = 3.39 (m, H-5′), 3.44 (dd, J = 9.8 Hz, 2.8 Hz, H-5″), 3.62 (m, H-4′), 4.25 (t, J = 2.2 Hz, H-3′), 4.35 (dd, J = 5.8 Hz, 2.2 Hz, H-2′), 4.57 (d, J = 5.4 Hz, CH_2), 4.59 (m, OH), 4.86 (dd, J = 2.8 Hz, 6.3 Hz, OH), 4.88 (t, J = 2.2 Hz, OH), 5.11 (s, CH_2), 5.42 (d, J = 4.5 Hz, OH), 7.15 (d, J = 8.4 Hz, Ar–H), 7.22 (m, Ar–H), 7.41 (d, J = 8.5 Hz, Ar–H), 7.59 (m, Ar–H), 7.88 (m, Ar–H), 8.14 (d, J = 8.5 Hz, Ar–H), 11.09 (s, NH) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 40.88 (SCH_2), 60.85 (C-2), 64.20 (OCH_2), 65.33 (C-5), 70.44 (C-3), 71.40 (C-2), 72.43 (C-4), 107.41, 120.42, 123.33, 125.20, 127.31, 134.30 (Ar–C), 153.48 (C-1), 160.00 (Ar–C), 166.00 (C-5, oxadiazole), 168.00 (C-2, oxadiazole), 171.22 (C=O) ppm; MS (ESI): m/z = 485 [M^++Na].

*General procedure for the preparation of 1-(1,3,4-oxadiazol-3(2H)-yl)ethanones **7a**, **7b***

A solution of sugar hydrazones **6a**, **6b** (5 mmol) in 4 cm^3 Ac_2O was heated under reflux for 1.5 h. The resulting solution was poured onto crushed ice, and the separated product was filtered off, washed with 50 cm^3 $NaHCO_3$ solution, followed by 50 cm^3 H_2O, and then dried. The products were recrystallized from EtOH to give **7a**, **7b**.

1-[5-[[5-[(Naphthalen-1-yloxy)methyl]-1,3,4-oxadiazol-2-ylthio]methyl]-2-(1,2,3,4,5-penta-O-acetyl-D-galactopentitolyl)-1,3,4-oxadiazol-3(2H)-yl]ethanone (**7a**, $C_{33}H_{36}N_4O_{14}S$)
Yield 68%; m.p.: 123–124 °C; IR (KBr): $\bar{\nu}$ = 1,728 (C=O), 1,620 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ = 1.95, 1.96, 2.01, 2.07, 2.19, 2.21 (6 s, 6 CH_3), 2.85 (s, SCH_2), 3.98–4.05 (m, H-4′), 4.59 (s, OCH_2), 5.16 (dd, J = 2.8 Hz, 6.5 Hz, H-3′), 5.20 (dd, J = 3.2 Hz, 6.5 Hz, H-2′), 5.37 (dd, J = 3.2 Hz, 6.2 Hz, H-1′), 5.95 (d, J = 6.2 Hz, oxadiazoline-H), 7.20 (m, Ar–H), 7.22 (d, J = 8.5 Hz, Ar–H), 7.47 (m, Ar–H), 7.50 (m, Ar–H), 7.68 (d, J = 8.5 Hz, Ar–H), 7.88 (d, J = 8.2 Hz, Ar–H), 8.17 (d, J = 8.5 Hz, Ar–H) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 20.70, 20.82, 21.00, 21.80, 21.99, 23.24 (6 CH_3), 40.80 (SCH_2), 61.60 (C-5), 62.20 (C-2), 64.23 (OCH_2), 67.70 (C-3), 68.75 (C-4), 75.90 (C-1), 76.00 (C-2, oxadiazoline), 107.30, 120.40, 123.53, 125.45, 127.60, 134.63, 156.96 (Ar–C), 158.20 (C-5, oxadiazoline), 163.41 (C-5, oxadiazole), 168.14 (C-2, oxadiazole), 168.50 (C=O), 170.21 (5 C=O) ppm; MS (ESI): m/z = 767 [M^++Na].

1-[5-[[5-[(Naphthalen-1-yloxy)methyl]-1,3,4-oxadiazol-2-ylthio]methyl]-2-(1,2,3,4-tetra-O-acetyl-D-xylopentitolyl)-1,3,4-oxadiazol-3(2H)-yl]ethanone (**7b**, $C_{30}H_{32}N_4O_{12}S$)
Yield 65%; m.p.: 125–127 °C; IR (KBr): $\bar{\nu}$ = 1,728 (C=O), 1,624 cm^{-1} (C=N) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ = 1.95, 1.96, 2.01, 2.07, 2.19 (5 s, 5 CH_3), 2.82 (s, SCH_2), 3.98 (dd, J = 2.4 Hz, 11.2 Hz, H-4′), 4.05 (dd, J = 2.4 Hz, 10.6 Hz, H-4″), 4.14 (s, OCH_2), 5.16 (dd, J = 2.8 Hz, 6.5 Hz, H-3′), 5.20 (dd, J = 3.2 Hz,

6.5 Hz, H-2′), 5.37 (dd, $J = 3.2$ Hz, 6.2 Hz, H-1′), 5.95 (d, $J = 6.2$ Hz, oxadiazoline-H), 7.20 (m, Ar–H), 7.22 (d, $J = 8.5$ Hz, Ar–H), 7.47 (m, Ar–H), 7.50 (m, Ar–H), 7.68 (d, $J = 8.5$ Hz, Ar–H), 7.88 (d, $J = 8.2$ Hz, Ar–H), 8.17 (d, $J = 8.5$ Hz, Ar–H) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 20.68$, 20.80, 21.80, 21.98, 23.34 (5 CH_3), 40.70 (SCH_2), 61.63 (C-4), 62.15 (C-2), 65.55 (OCH_2), 68.70 (C-3), 75.83 (C-1), 76.12 (C-2, oxadiazoline), 107.33, 120.47, 123.59, 125.45, 127.62, 134.63, 156.90 (Ar–C), 158.25 (C-5, oxadiazoline), 163.32 (C-5, oxadiazole), 168.43 (C-2, oxadiazole), 168.55 (C=O), 170.33 (4 C=O) ppm; MS (ESI): $m/z = 658$ [$M^+ - CH_3$].

General procedure for the preparation of 2-(alkylthio)-5-[(naphthalen-1-yloxy)methyl]-1,3,4-oxadiazoles ***8a, 8b***

To a solution of 2.58 g **2** (10 mmol) and 0.56 g KOH (10 mmol) in a mixture of 25 cm^3 H_2O and 10 cm^3 EtOH, the alkyl iodide (10 mmol) was added. The solution was stirred at room temperature for 4 h. The resulting precipitate was filtered off and recrystallized from EtOH.

2-(Methylthio)-5-[(naphthalen-1-yloxy)methyl]-1,3,4-oxadiazole (**8a**, $C_{14}H_{12}N_2O_2S$)
Yield 79%; m.p.: 134–135 °C; IR (KBr): $\bar{\nu} = 1{,}643$ (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta = 2.33$ (s, CH_3), 4.25 (s, CH_2), 7.05 (m, Ar–H), 7.18 (d, $J = 8.5$ Hz, Ar–H), 7.43 (m, Ar–H), 7.52 (m, Ar–H), 7.60 (d, $J = 8.2$ Hz, Ar–H), 7.85 (d, $J = 8.2$ Hz, Ar–H), 8.14 (d, $J = 8.5$ Hz, Ar–H) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 14.50$ (SCH_3), 65.91 (OCH_2), 107.31, 120.42, 123.57, 125.45, 126.60, 134.58, 156.96 (Ar–C), 163.23 (C-5, oxadiazole), 168.43 (C-2, oxadiazole) ppm; MS (ESI): $m/z = 273$ [$M^+ + 1$].

2-(Ethylthio)-5-[(naphthalen-1-yloxy)methyl]-1,3,4-oxadiazole (**8b**, $C_{15}H_{14}N_2O_2S$)
Yield 76%; m.p.: 137–139 °C; IR (KBr): $\bar{\nu} = 1{,}640$ (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta = 1.36$ (t, $J = 5.8$ Hz, CH_3), 3.24 (q, $J = 5.8$ Hz, CH_2), 3.31 (s, CH_2), 3.60 (s, CH_2), 7.12 (m, Ar–H), 7.16 (d, $J = 8.5$ Hz, Ar–H), 7.44 (m, Ar–H), 7.51 (m, Ar–H), 7.54 (d, $J = 8.2$ Hz, Ar–H), 7.88 (d, $J = 8.2$ Hz, Ar–H), 8.19 (d, $J = 8.5$ Hz, Ar–H) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 14.30$ (CH_3), 33.00 (SCH_2), 65.87 (OCH_2), 107.34, 120.49, 123.55, 125.48, 126.55, 134.57, 156.90 (Ar–C), 163.27 (C-5, oxadiazole), 168.50 (C-2, oxadiazole) ppm; MS (ESI): $m/z = 286$ [M^+].

[5-[(Naphthalen-1-yloxy)methyl]-1,3,4-oxadiazol-2-yl]hydrazine (**9**, $C_{13}H_{12}N_4O_2$)
A solution of **8a** or **8b** (10 mmol) and 0.52 cm^3 $N_2H_4{\cdot}H_2O$ (20 mmol) in 5 cm^3 EtOH was heated under reflux for 6 h. The solution was cooled, and the resulting precipitate was filtered and recrystallized from EtOH to afford **9** as white crystals in 77% yield. M.p.: 161–162 °C; IR (KBr): $\bar{\nu} = 3{,}432$ (NH_2), 3,293 (NH), 1,650 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta = 5.38$ (s, CH_2), 6.07 (s, NH_2), 6.86 (m, Ar–H), 6.89 (d, $J = 8.2$ Hz, Ar–H), 7.29 (m, Ar–H), 7.49 (m, Ar–H), 7.88 (d, $J = 8.2$ Hz, Ar–H), 8.21(d, $J = 8.5$ Hz, Ar–H), 8.39 (d, $J = 7.8$ Hz, Ar–H), 9.49 (s, NH) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 68.80$ (OCH_2), 107.38, 120.52, 123.55, 125.56, 126.59, 134.59, 156.94 (Ar–C), 163.25 (C-5, oxadiazole), 169.35 (C-2, oxadiazole) ppm; MS (ESI): $m/z = 279$ [M^++Na].

3-[5-[(Naphthalen-1-yloxy)methyl]-2-thioxo-1,3,4-oxadiazol-3(2H)-yl]propanenitrile (**10**, $C_{16}H_{13}N_3O_2S$)
A mixture of 0.52 g **2** (20 mmol), 1.06 g acrylonitrile (20 mmol), and 1 g Et_3N (10 mmol) was dissolved in 30 cm^3 absolute EtOH. The reaction mixture was heated under reflux for 3 h and then cooled to room temperature. The obtained precipitate was filtered off, dried, and recrystallized from EtOH to give 0.45 g **10** (72%) as white crystals. M.p.: 123–125 °C; IR (KBr): $\bar{\nu} = 3{,}423$ (NH), 1,658 (C = N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta = 3.09$ (t, $J = 6.2$ Hz, CH_2), 4.34 (t, $J = 6.2$ Hz, CH_2), 5.52 (s, CH_2), 7.15 (m, Ar–H), 7.17 (d, $J = 8.5$ Hz, Ar–H), 7.44 (m, Ar–H), 7.56 (m, Ar–H), 7.58 (d, $J = 8.2$ Hz, Ar–H), 8.14 (d, $J = 8.2$ Hz, Ar–H), 8.17 (d, $J = 8.5$ Hz, Ar–H) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 14.23$ (CH_2), 47.53 (CH_2), 68.80 (OCH_2), 107.35 (Ar–C), 119.00 (CN), 120.50, 123.50, 125.50, 126.52, 134.50, 156.90 (Ar–C), 158.22 (C-5, oxadiazole), 177.11 (C=S) ppm; MS (ESI): $m/z = 334$ [M^++Na].

3-[5-[(Naphthalen-1-yloxy)methyl]-2-thioxo-1,3,4-oxadiazol-3(2H)-yl]propanimidic acid hydrazide (**11**, $C_{16}H_{17}N_5O_2S$)
A mixture of 0.62 g **10** (5 mmol), 8.5 cm^3 EtOH, and 0.26 cm^3 $N_2H_4{\cdot}H_2O$ (10 mmol) was refluxed for 4 h, and the solvent was removed under reduced pressure. The remaining precipitate was collected, dried, and recrystallized from EtOH to afford 0.48 g **11** in 70% yield as a yellow solid. M.p.: 138–140 °C; IR (KBr): $\bar{\nu} = 3{,}455$ (NH_2), 3,321 (NH), 1,625 (C = N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta = 3.09$ (t, $J = 6.2$ Hz, CH_2), 4.39 (t, $J = 6.2$ Hz, CH_2), 5.36 (s, CH_2), 5.86 (s, NH_2), 7.16 (d, $J = 8.5$ Hz, Ar–H), 7.44 (m, Ar–H), 7.48 (m, Ar–H), 7.51 (m, Ar–H), 7.56 (d, $J = 8.2$ Hz, Ar–H), 7.90 (d, $J = 8.2$ Hz, Ar–H), 8.20 (d, $J = 8.5$ Hz, Ar–H), 9.12 (s, NH), 10.21 (s, NH) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 23.20$ (CH_2), 43.33 (CH_2), 68.87 (OCH_2), 107.34, 120.52, 123.52, 125.47, 126.50, 134.50, 156.70 (Ar–C), 156.89 (C=NH), 158.29 (C-5, oxadiazole), 177.18 (C=S) ppm; MS (ESI): $m/z = 366$ [M^++Na].

Antiviral activity

Anti-hepatitis C virus activity

Screening of compounds and determination of their minimum inhibitory concentration (MIC) in HCV replicon cells were performed as follows:

Briefly, 1×10^4 replicon cells per well were placed in 96-well plates. On the following day, replicon cells were incubated at 37 °C for the indicated period of time with antiviral agents serially diluted in DMEM plus 2% FBS and 0.5% dimethyl sulfoxide. Total cellular RNA was extracted using an RNeasy-96 kit (QIAGEN, Valencia, CA), and the copy number of HCV RNA was determined using a quantitative RTPCR (QRT-PCR) assay. Each datum point represents the average of five replicates in cell culture. The cytotoxicity of the tested compound was measured under the same experimental settings using a tetrazolium (MTS)-based cell viability assay (Promega, Madison, WI). For the cytotoxicity assay with human hepatocyte cell lines, 1×10^4 parental Huh-7 cells per well or 4×10^4 HepG2 cells per well were used.

MIC of tested compounds and their combination in hamster brains for antiviral chemotherapy for subacute sclerosing panencephalitis (SSPE)

Under ether anesthesia, 50 cm^3 of either tested compound (or the newly synthesized compound) or their different combination solutions at dosages of 5, 10, and 20 mg/(kg day) was injected for 10 days intracranially to a depth of 2 mm by using a 27-gauge needle and was placed within the subarachnoid space. At 1, 2, 3, 5, 7, 10, 12, 15, and 20 days after the initial injection, four hamsters from each group were killed. The brains were aseptically removed, washed twice with phosphate-buffered saline (PBS), homogenized, and suspended in PBS. The suspension was centrifuged at 1,600*g* for 10 min. The supernatant was collected, ethanol was added to remove proteins, and the mixture was heated at 90 °C to evaporate the ethanol. The protein-free samples were used to evaluate the MIC in brain tissue by HPLC and bioassay.

Anti-HIV activity

Cells and viruses

The established human cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, penicillin (100 U/cm^3), and streptomycin (100 μg/cm^3). Fresh human cells were obtained from the American Red Cross (Baltimore, MD).

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), which is metabolized to a colored formazan product by viable cells, RT assay (U937- and PBMC-based assays), and/or p24 enzyme-linked immunosorbent assay (monocyte-macrophage assays). Antiviral and toxicity data are reported as the quantity of drug required to inhibit virus-induced cell killing or virus production by 50% (EC_{50}).

In vitro assays of anti-HIV activity

Each of the newly synthesized compounds was tested for RT inhibitory activity against purified recombinant HIV-1 RT using the cell-free Quan-T-RT assay system (Amersham Corp., Arlington Heights, IL), which utilizes the scintillation proximity assay (SPA) principle. In the 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 concentration of 0.5 μCi/sample, was diluted in RT assay buffer (49.5 mM 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 concentrations. 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 cm^3 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 untreated sample.

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