Full Paper

Synthesis and Antiviral Evaluation of Novel 5-(*N*-Aryl-aminomethyl-1,3,4-oxadiazol-2-yl)hydrazines and Their Sugars, 1,2,4-Triazoles, Tetrazoles and Pyrazolyl Derivatives

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A number of new *N*-arylaminomethyl-1,3,4-oxadiazole derivatives **2**, **3a**,**b**, and **9**–**12a**,**b** were prepared. Sugar (5-*N*-arylaminomethyl-1,3,4-oxadiazol-2-yl) hydrazones **4**–**6a**,**b** were synthesized by the reaction of the hydrazino derivatives **3a**,**b** with the corresponding monosaccharides. The novel acyclo-*C*-nucleosides **7**, **8a**,**b** were prepared by heterocyclization of the sugar hydrazones **4**, **5a**,**b** with acetic anhydride. A number of the synthesized compounds were tested for their antiviral activity against herpes simplex virus type-1 (HSV-1) and hepatitis-A virus (HAV, MBBcell culture-adapted strain). The results revealed that the sugar hydrazones **6a**,**b** showed higher antiviral activity compared to the other hydrazones and their acetylated derivatives.

Keywords: Acyclo-nucleosides / Antiviral activity / 1,3,4-Oxadiazoles / Sugar hydrazones / Triazolo-oxadiazoles

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Introduction

1,3,4-Oxadiazoles represent an important class of heterocyclic compounds. Their derivatives possess a broad spectrum of biological activity in both agrochemicals and pharmaceuticals such as antibacterial [1], antimicrobial [2], insecticidal [3], herbicidal, fungicidal [4], anti-inflammatory [5], hypoglycemic [6], hypotension characteristics [7], antiviral [8], and antitumor activities [9]. The nucleosides as well as their acyclic and *C*-nucleoside analogues possess a wide range of medicinal properties, including antibiotic, antiviral, and antitumor activities [10–19]. We have been interested in the attachment of carbohydrate moieties to heterocycles, in the synthesis of acyclonucleoside analogues as well as heterocycles from carbohydrate precursors [20–25]. The aim of the present work was to attach carbohydrate residues to oxadiazoles in order to find new biologically active leads with good solubility in biological systems. Thus, synthesis of novel 1,3,4-oxadiazol-2-yl-hydrazines and their sugar derivatives has been achieved. Moreover, the cyclization of sugar hydrazones to novel acyclonucleoside analogues, fused oxadiazolo-triazoles, and tetrazoles as well as oxadioazolyl-triazoles has been carried out. The antiviral activity of selected members has been evaluated.

Results and discussion

Chemistry

Alkylation of the oxadiazole thiones **1a**,**b** with ethyl iodide in alkaline medium afforded 5-*N*-arylaminomethyl-2-ethylmercapto-1,3,4-oxadiazoles **2a**,**b**. Hydrazinolysis of which gave the required hydrazine derivatives 5-*N*-arylaminomethyl-2-hydrazino-1,3,4-oxadiazoles **3a**,**b** in good yields. The ^{1}H-NMR spectra of **2a**,**b** showed the signals of the ethyl group as triplet and quartet which disappeared in the spectra of **3a**,**b**, whereas the NH_2 signal appeared at δ 5.60 and 5.65 ppm for **3a** and **3b**, respectively in addition to the aromatic protons in the range δ 6.15–6.95 ppm. The ^{13}C-NMR spectrum of **3b** showed the

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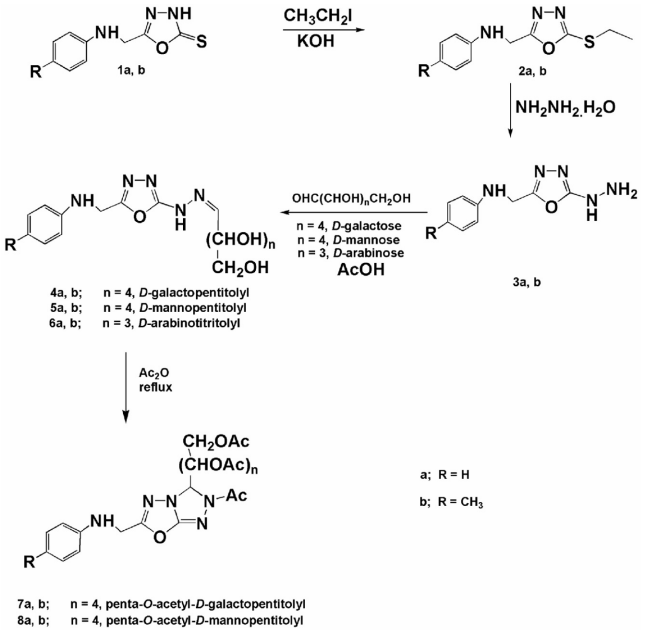
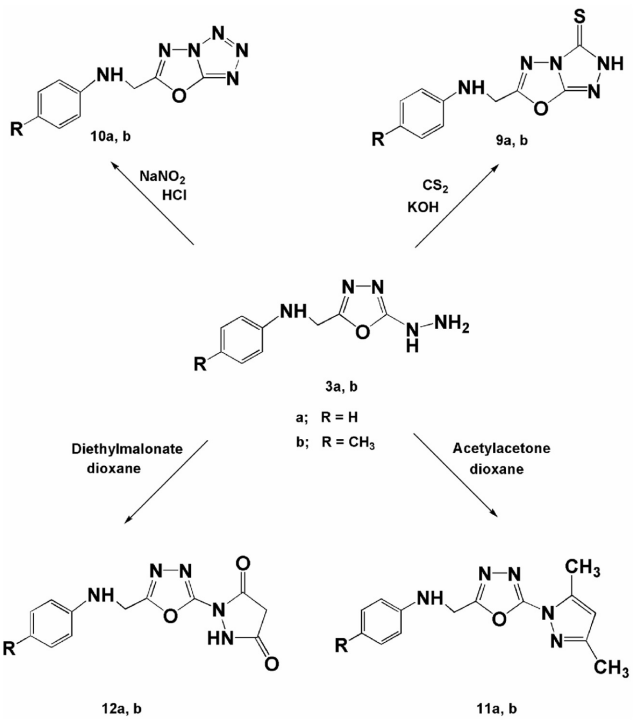


Scheme 1. Synthesis route of presented compounds **1**–**8**.



Scheme 2. Synthesis route of presented compounds **9**–**12**.

signals of C=N group at 152.77 ppm and the aromatic carbons at δ 112.44–144.87 ppm.

When compounds **3a**,**b** were allowed to react with a number of monosaccharides, the corresponding aldehydo sugar hydrazones were obtained (Scheme 1). Thus, reaction of the hydrazine derivatives **3a**,**b** with D-galactose, D-mannose and D-arabinose in an aqueous ethanolic solution and catalytic amount of acetic acid gave the corresponding sugar (5-*N*-arylaminomethyl-1,3,4-oxadiazol-2-yl)hydrazones **4**–**6a**,**b**. The structures of these compounds were confirmed by the analytical and spectral data. The IR spectra of **4**–**6a**,**b** showed the presence of characteristic absorption bands corresponding to the hydroxyl groups in the region 3200–3500 cm^{-1}. The ^{1}H-NMR spectra showed the signals of the sugar chain protons at δ 3.30–5.78 ppm, the C-1 methine proton as doublet in the range δ 6.38–7.65 ppm in addition to the aromatic protons in the region δ 6.50–7.80 ppm.

The reaction of sugar arylhydrazones with boiling acetic anhydride is well known to give the respective per-*O*,*N*-acetyl derivatives [26–29]. However, treatment of the sugar hydrazones **4**–**5a**,**b** with boiling acetic anhydride did not afford the expected per-*O*,*N*-acetyl or their per-*O*-acetyl derivatives, but gave compounds **7**–**8a**,**b**. The later structures were deduced from their IR, ^{1}H-NMR, and ^{13}C-NMR spectra. The IR spectra of **7**–**8a**,**b** showed characteristic absorption bands in the carbonyl frequency region 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 *N*-acetyl group in addition to the *O*-acetyl groups. The ^{1}H-NMR spectra of **7**–**8a**, **b** showed the signals of the *O*-acetyl-methyl protons as singlets in the range δ 1.83–2.20 ppm and the *N*-acetyl-methyl protons in the range δ 2.24–2.30 ppm. The rest of the alditolyl chain protons appeared in the range δ 3.77–5.75 ppm in addition to the aromatic protons as multiplets in the region δ 6.59–7.05 ppm. The ^{13}C-NMR spectrum of **7a** showed the resonances of the acetyl-methyl carbons at δ 20.35–29.76 ppm. The value of the chemical shift of the C-1 of the sugar proton appeared at δ 92.08 ppm whose value indicated its *N*,*N*-acetal nature rather than being a C=N; the later should appear at a lower field. The signals at δ 168.95–178.24 ppm corresponded to the carbonyl groups. The elemental analyses were in agreement with the assigned structures (Scheme 1). Consequently, the reactions of **4**, **5a**,**b** with boiling acetic anhydride caused a novel cyclization accompanying the acetylation to give 2-acetyl-3-(1′,2′,3′,4′,5′-penta-*O*-acetylsugar)-6-*N*-arylaminomethyl-2,3-dihydro[1,2,4]triazole[3,4-*b*]-[1,3,4-oxadiazoles] **7**–**8a**,**b**.

The literature indicated the broad utility of heterocyclic hydrazines for the synthesis of several condensed systems containing triazole and tetrazole ring systems [30, 31]. Thus, 5-*N*-arylaminomethyl-2-hydrazino-1,3,4-oxadiazoles **3a**,**b** were utilized in preparing a series of condensed heterocyclic compounds (Scheme 2). The reaction of compounds **3a**,**b** with carbon disulphide in the

presence of alcoholic potassium hydroxide and subsequent acidification gave 6-*N*-arylamino-methyl[1,2,4]triazolo[3,4-*b*][1,3,4]oxadiazole-(2*H*)-3-thiones **9a**,**b** in 77 – 79% yield. The IR spectra of **9a**,**b** showed the presence of characteristic absorption bands corresponding to the C=N and C=S groups. The ^{1}H-NMR spectra showed the presence of a singlet at δ 13.75 or 14.05 ppm corresponding to the NH group of **9a** and **9b**, respectively, in addition to the aromatic protons at δ 7.22 – 7.65 ppm.

When compounds **3a**,**b** were reacted with sodium nitrite and hydrochloric acid (Scheme 2), the corresponding 5-*N*-arylamino-methyl-1,3,4-oxadiazolo[1,5-*d*][1,2,3,4]-tetrazoles **10a**,**b** were obtained. Their IR spectra showed the absence of the NH_2 absorption band and the presence of characteristic absorption band corresponding to the C=N group. Their ^{1}H-NMR spectra showed the CH_2 group as a doublet at δ 3.39 and 4.25 ppm for **10a** and **10b**, respectively and the aromatic protons as multiplets at δ 6.63 – 7.66 ppm.

Condensation of **3a**,**b** with 1,3-dicarbonyl compounds gave the respective pyrazole and pyrazolidinone derivatives. Thus, reaction of **3a**,**b** with acetylacetone in dioxane gave 2-(3,5-dimethylpyrazol-1-yl)-5-*N*-arylamino-methyl-1,3,4-oxadiazoles **11a**,**b**, via a known mechanism [32] that involved initial reaction of the NH_2 group of **3a**,**b** with one carbonyl group of the diketone, followed by cyclization of the remaining carbonyl to form the pyrazole ring (Scheme 2).

Cyclocondensation of **3a**,**b** with diethylmalonate (Scheme 2) gave the 2-[3,5-dioxo-(2*H*)-pyrazol-1-yl]-5-*N*-5-arylaminomethyl-1,3,4-oxadiazoles **12a**,**b**. The IR spectra of compounds **12a**,**b** showed the presence of the expected characteristic absorption band in the carbonyl frequency region. The ^{1}H-NMR spectrum of compounds **11a** showed the signals at δ 2.20 and 2.23 ppm as singlets corresponding to the methyl groups and the aromatic protons appeared in the range δ 6.80 – 7.10 ppm.

Antiviral activity

A plaque infectivity assay was carried out testing a number of selected compounds for their antiviral activity. The test was performed to include three possibilities for antiviral activity: virucidal effect, virus adsorption, and effect on virus replication for both HAV and HSV-1. The antiviral activity against herpes simplex virus (HSV) revealed that compounds **6a** and **6b** showed the highest activity at concentration 20 μg/10^5 and 10 μg/10^5, respectively (Fig. 1). Compounds **5b** and **7a** showed little activity at both concentrations.

For the activity against hepatitis-A virus (HAV), the results showed that compound **6a** showed the highest activity at both concentration 10 and 20 μg/10^5 (Fig. 2). In conclusion, the sugar hydrazones **6a**,**b** showed higher antiviral activity compared to the other hydrazones and their acetylated derivatives.

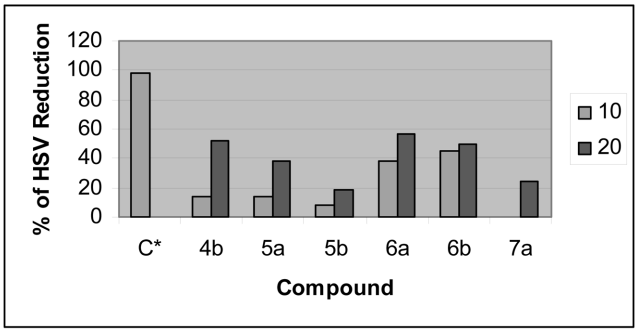


Figure 1. Effect of some novel compounds on Herpes simplex virus-1 reduction in comparison with acyclovir (C*) as a control.

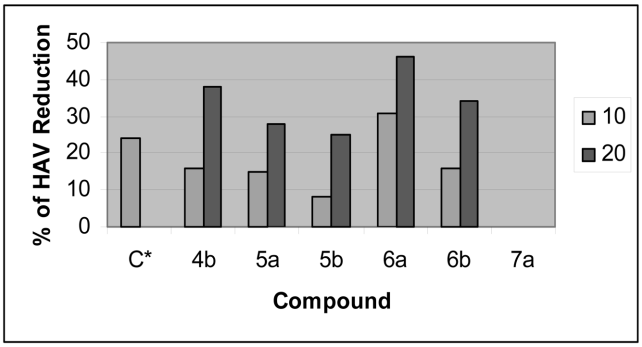


Figure 2. Effect of some novel compounds on Hepatitis A virus reduction in comparison with amantadine (C*) as a control.

Physical properties and spectral data of the newly synthesized compounds are listed in Tables 1 and 2.

The authors have declared no conflict of interest.

Experimental

General

Melting points were determined with a Kofler block apparatus (C. Reichert, Vienna, Austria) and are uncorrected. The IR spectra were recorded on a Perkin-Elmer model 1720 FTIR spectrometer for KBr disc (Perkin-Elmer, Norwalk, CT, USA). NMR spectra were recorded on a Varian Gemini 200 NMR Spectrometer at 300 MHz for ^{1}H (Varian Inc., Palo Alto, CA, USA) and 75 MHz for ^{13}C or on a Bruker Ac-250 FT spectrometer at 250 MHz for ^{1}H and at 62.9 MHz for ^{13}C with TMS as a standard (Bruker Bioscience, Billerica, MA, USA). The progress of the reactions was monitored by TLC using aluminum silica gel plates 60 F 245. Elemental analyses were performed at the Microanalytical Data Centre at Faculty of Science, Cairo University, Egypt. Viral screening against HAV and HSV was conducted at the Environmental Virology Laboratory, Department of Water Pollution Research, National Research Centre, Cairo, Egypt.

Table 1. Physicochemical properties of the newly synthesized compounds.

Compound	Mp. (°C) from ethanol	Mol. Formula	Yield (%)	Analysis % (Calcd./Found)			IR Spectra (cm^{-1})
				C	H	N	
2a	158–159	$C_{11}H_{13}N_3OS$	77	56.15	5.57	17.86	1610 (C=N)
		(235.31)		55.77	5.52	17.50	3415 (NH)
2b	162–163	$C_{12}H_{15}N_3OS$	79	57.81	6.06	16.85	1605 (C=N)
		(349.33)		57.44	5.72	16.48	3423 (NH)
3a	239–231	$C_9H_{11}N_5O$	78	52.67	5.40	34.13	1615 (C=N)
		(205.22)		52.71	5.39	34.05	3291 (NH)
							3393 (NH_2)
3b	225–227	$C_{10}H_{13}N_5O$	79	54.78	5.98	31.94	1614 (C=N)
		(219.24.36)		55.14	5.62	31.59	3310 (NH)
							3351 (NH_2)
4a	188–190	$C_{15}H_{21}N_5O_6$	83	49.04	5.76	19.06	1605 (C=N)
		(367.38)		48.82	5.51	18.82	3130 (NH)
							3391–4440 (OH)
4b	187–189	$C_{16}H_{23}N_5O_6$	82.5	50.39	6.08	18.36	1610 (C=N)
		(381.38)		50.66	5.91	18.12	3310 (NH)
							3350–3490 (OH)
5a	187–189	$C_{15}H_{21}N_5O_6$	82	49.04	5.76	19.06	1605 (C=N)
		(367.38)		48.69	5.48	18.84	3120 (NH)
							3398–3450 (OH)
5b	184–185	$C_{16}H_{23}N_5O_6$	78.5	50.39	6.08	18.36	1590 (C=N)
		(381.38)		50.69	5.89	18.15	3285 (NH)
							3373–3420 (OH)
6a	188–189	$C_{14}H_{19}N_5O_5$	79	49.85	5.68	20.76	1615 (C=N)
		(337.33)		50.05	5.78	20.51	3150 (NH)
							3320–3400 (OH)
6b	184–185	$C_{15}H_{21}N_5O_5$	81.5	51.28	6.02	19.93	1605 (C=N)
		(351.39)		51.41	6.19	19.71	3160 (NH)
							3477–3500 (OH)
7a	145–147	$C_{27}H_{33}N_5O_{12}$	68.5	52.34	5.37	11.30	1658 (OCN)
		(619.58)		52.12	5.24	11.41	1751 (OAc)
							3472 (NH)
7b	151–152	$C_{28}H_{35}N_5O_{12}$	73.5	53.08	5.57	11.05	1653 (OCN)
		(633.60)		53.42	5.53	11.02	1751 (OAc)
							3481 (NH)
8a	149–150	$C_{27}H_{33}N_5O_{12}$	71.5	52.34	5.37	11.32	1657 (OCN)
		(619.58)		52.22	5.18	11.71	1746 (OAc)
							3466 (NH)
8b	151–152	$C_{28}H_{35}N_5O_{12}$	72	53.08	5.57	11.05	1678 (OCN)
		(633.60)		52.82	5.83	10.82	1775 (OAc)
							3371(NH)
9a	205–207	$C_{10}H_9N_5OS$	77	48.57	3.67	28.32	1605 (C=N)
		(247.29)		48.45	3.55	28.15	3368 (NH)
9b	208–207	$C_{11}H_{11}N_5OS$	79	50.56	4.24	26.80	1615 (C=N)
		(261.30)		50.38	4.11	26.65	3334 (NH)
10a	225–226	$C_9H_8N_6O$	77	50.00	3.73	38.87	1610 (C=N)
		(216.20)		49.82	3.52	38.77	3105 (NH)
10b	223–225	$C_{10}H_{10}N_6O$	79	52.17	4.38	36.50	1620 (C=N)
		(230.27)		51.95	4.58	36.28	3350 (NH)
11a	217–218	$C_{14}H_{15}N_5O$	85	62.44	5.61	26.01	1615 (C=N)
		(269.30)		62.62	5.62	25.82	3180 (NH)
11b	219-221	$C_{15}H_{17}N_5O$	87	63.59	6.05	24.72	1615 (C=N)
		(238.33)		63.25	5.83	24.43	3250 (NH)
12a	209–210	$C_{12}H_{11}N_5O_3$	75	52.75	4.06	25.63	1604 (C=N)
		(273.25)		52.37	3.84	25.21	1677 (C=O)
							3371 (NH)
12b	210–212	$C_{13}H_{13}N_5O_3$	76	54.35	4.56	24.38	1610 (C=N)
		(287.27)		54.12	4.31	24.13	1662 (C=O)
							3434 (NH)

Table 2. ^{1}H- and ^{13}C-NMR spectra of the newly synthesized compounds.

H^1 – NMR/^{13}C-NMR (d, ppm)	Compound
1.19 (t, 3H, J = 6.2 Hz, CH_3), 3.84 (d, 2H, J = 5.4 Hz, CH_2), 4.09 (q, 2H, J = 6.2 Hz, CH_2), 5.70 (t, 1H, J = 5.4 Hz, NH), 6.47 (m, 2H, J = 8.5 Hz, Ar-2H), 6.89 (m, 3H, J = 8.5 Hz, Ar-3H).	**2a**
1.19 (t, 3H, J = 6.2 Hz, CH_3), 2.14 (s, 3H, CH_3), 3.84 (d, 2H, J = 5.4 Hz, CH_2), 4.09 (q, 2H, J = 6.2 Hz, CH_2), 5.70 (t, 1H, J = 5.4 Hz, NH), 6.47 (d, 2H, J = 8.5 Hz, Ar-2H), 6.89 (d, 2H, J = 8.5 Hz, Ar-2H).	**2b**
4.30 (d, 2H, J = 5.4 Hz, CH_2), 5.60 (s, 2H, NH_2), 6.05 (s, 1H, NH), 6.65 (m, 3H, Ar-3H), 7.15 (m, 2H, Ar-2H), 13.15 (s, 1H, NH).	**3a**
2.12 (s, 3H, CH_3), 4.12 (d, 2H, J = 5.4 Hz, CH_2), 5.65 (s, 2H, NH_2), 5.80 (s, 1H, NH), 6.15 (d, 2H, J = 8.5 Hz, Ar-2H), 6.95 (d, 2H, J = 8.5 Hz, Ar-2H), 13.16 (s, 1H, NH)/19.98 (CH_3), 37.20 (CH_2), 112.62 (ArC-3,5), 124.69 (ArC-4), 129.09 (ArC-2,6), 145.79 (ArC-1), 152.77 (C=N), 162.81 (C=N).	**3b**
3.30-3.42 (m, 2H, H-6', H-6''), 3.61 (m, 1H, H-5'), 3.66 (m, 1H, H-4'), 3.71 (dd, 1H, J = 2.8 Hz, J = 5.8 Hz, H-3'), 4.29 (t, 1H, J = 5.8 Hz, H-2'), 4.33 (d, 2H, J = 5.4 Hz, CH_2), 4.53 (m, 1H, OH), 4.94 (d, 1H, J = 6.3 Hz, OH), 5.25 (m, 1H, OH), 5.75 (t, 1H, J = 4.5 Hz, OH), 5.85 (t, 1H, J = 4.5 Hz, OH), 5.96 (t, 1 H, J = 5.4 Hz, NH), 6.6 (m, 3H, Ar-3H), 7.4 (d, 1H, J = 5.8 Hz, H-1'), 7.6 (m, 2 H, Ar-2H), 11.2 (s, 1H, NH).	**4a**
2.14 (s, 3H, CH_3), 3.39 (m, 2H, H-6', H-6''), 3.54 (m, 1H, H-5'), 3.66 (m, 1H, H-4'), 4.02 (dd, 1H, J = 5.6 Hz, J = 2.6 Hz, H-3'), 4.11 (dd, 1H, J = 2.6 Hz, J = 5.8 Hz, H-2'), 4.31 (d, 2H, J = 5.4 Hz, CH_2), 4.45 (d, 1H, J = 2.8 Hz, OH), 4.85 (d, 1H, J = 3.5 Hz, OH), 5.19 (m, 1 H, OH), 5.30 (t, 1H, J = 5.8 Hz, OH), 5.55 (d, 1H, J = 3.5 Hz, OH), 5.75 (t, 1H, J = 5.4 Hz, NH), 6.51 (d, 2H, J = 8.5 Hz, Ar-2H), 6.91 (d, 2H, J = 8.5 Hz, Ar-2H), 7.45 (d, 1H, J = 5.8 Hz, H-1'), 11.01 (s, 1H, NH).	**4b**
3.36 (m, 1H, H-6`), 3.39 (dd, 1H, J = 10.5 Hz, 3.6 Hz, H-6''), 3.53 (dd, 1H, J = 3.6 Hz, 6.2 Hz, H-5'), 3.61 (m, 1H, H-4'), 3.79 (dd, 1H, 2.8 Hz, 6.2 Hz, H-3'), 4.17 (d, 2H, J = 5.4 Hz, CH_2), 4.18 (dd, 1H, J = 2.8 Hz, J = 5.8 Hz, H-2'), 4.34 (m, 1H, J = 6.2 Hz, OH), 4.9 (d, 1H, J = 2.8 Hz, OH), 4.99 (d, 1H, J = 3.6 Hz, OH), 5.03 (t, 1H, J = 4.5 Hz, OH), 5.18 (m, 1H, OH), 6.02 (t, 1H, J = 5.4 Hz, NH), 6.65 (m, 3H, Ar-3H), 7.09 (m, 2H, Ar-2H), 7.32 (m, 2H, Ar-2H), 6.38 (d, 1H, J = 5.8 Hz, H-1'), 11.21 (s, 1H, NH).	**5a**
3.04 (m, 1H, H-5'), 3.22 (dd, 1H, J = 9.8 Hz, J = 2.8 Hz, H-5''), 3.49 (m, 1H, H-4'), 3.75 (t, 1H, J = 2.2 Hz, H-3'), 3.90 (dd, 1H, J = 5.8 Hz, J = 2.2 Hz, H-2'), 4.22 (d, 2H, J = 5.4 Hz, CH_2), 4.45 (m, 1H, OH), 5.17 (d, 1H, J = 2.8 Hz, J = 6.3 Hz, OH), 5.57 (t, 1H, J = 2.2 Hz, OH), 5.79 (d, 1H, J = 4.5 Hz, OH), 5.90 (t, 1H, J = 5.4 Hz, NH), 7.54 (m, 2H, Ar-2H), 7.80 (m, 3H, Ar-3H), 7.65 (d, 1H, J = 5.8 Hz, H-1'), 11.48 (s, 1H, NH).	**6a**
2.14 (s, 3H, CH_3), 3.42 (m, 2H, H-5', H-5''), 3.68 (m, 1H, H-4'), 3.72 (t, 1H, J = 2.8 Hz, H-3'), 4.16 (d, 2H, J = 5.4 Hz, CH_2), 4.28 (dd, 1H, 2.8 Hz, J = 5.8 Hz, H-2'), 4.43 (d, 1H, J = 2.8 Hz, OH), 5.15 (d, 1H, J = 4.5 Hz, OH), 5.25 (m, 1H, OH), 5.60 (t, 1H, J = 6.2 Hz, OH), 5.78 (t, 1H, J = 5.4 Hz, NH), 6.5 (m, 2H, Ar-2H), 6.9 (d, 2H, J = 8.5 Hz, Ar-2H), 7.40 (d, 1H, J = 5.8 Hz, H-1'), 11.11 (s, 1H, NH).	**6b**
1.83, 1.98, 2.04, 2.11, 2.20, 2.30 (6s, 18H, 6CH_3), 4.15 (dd, 1H, J = 11.2 Hz, J = 2.4 Hz, H-5''), 4.20 (dd, 1H, J = 10.6 Hz, J = 2.4 Hz, H-5''), 4.53 (d, 2H, J = 5.4 Hz, CH_2), 5.15 (dd, 1H, J = 2.8 Hz, J = 6.5 Hz, H-4'), 5.25 (dd, 1H, J = 3.2 Hz, J = 6.5 Hz, H-3'), 5.50 (dd, 1H, J = 3.2 Hz, J = 6.2 Hz, H-2'), 5.70 (t, 1H, J = 6.2 Hz, H-1'), 5.73 (t, 1H, J = 5.4 Hz, NH), 5.78 (d, 1H, J = 6.2 Hz, triazole-H), 6.61 (m, 3H, Ar-2H), 7.01 (m, 3H, Ar-2H)/20.35, 20.50, 20.58, 20.67, 20.76, 29.67 (6CH_3), 39.57 (CH_2), 62.82 (C-5'), 65.38 (C-4'), 68.36 (C-3'), 69.7 (C-2'), 71.04 (C-1'), 92.08 (C-N), 113.05 (ArC-2,6), 128.57 (ArC-4), 129.96 (ArC-3,5), 143.32 (C-1), 158.45 (C=N), 160.35 (C=N), 168.95 (C=O), 169.64 (C=O), 169.75 (C=O), 169.93 (C=O), 170.05 (C=O), 178.24 (C=O).	**7a**
1.98, 2.01, 2.05, 2.09, 2.13, 2.24, 2.36 (7s, 21H, 7CH_3), 3.77 (dd, 1H, J = 2.6 Hz, J = 11.8 Hz, H-5'), 4.03 (dd, 1H, J = 3.5 Hz, J = 11.8 Hz, H-5''), 4.26 (dd, 1H, J = 3.5 Hz, J = 5.8 Hz, H-4'), 4.55 (d, 2H, J = 5.4 Hz, CH_2), 5.13-5.16 (m, 2H, H-2', H-3'), 5.37 (dd, 1H, J = 3.2 Hz, J = 6.2 Hz, H-1'), 5.45 (d, 1H, J = 6.2 Hz, triazole-H), 5.72 (t, 1H, J = 5.4 Hz, NH), 6.62 (d, 2H, J = 8.5 Hz, Ar-2H), 7.03 (m, 2H, Ar-2H).	**7b**
2.03, 2.06, 2.08, 2.11, 2.17, 2.24 (6s, 18H, 6CH_3), 3.95 (dd, 1H, J = 18.8 Hz, J = 2.5 Hz, H-5''), 4.15 (dd, 1H, J = 11.3 Hz, J = 2.5 Hz, H-5''), 4.38 (m, 1H, H-4'), 4.55 (d, 2H, J = 5.4 Hz, CH_2), 5.15 (dd. 1H, J = 4.8 Hz, J = 2.2 Hz, H-3'), 5.20 (t, 1H, J = 3.2 Hz, H-2'), 5.45 (dd, 1H, J = 3.2 Hz, J = 6.2 Hz, H-1'), 5.71 (d, 1H, J = 6.2 Hz, triazole-H), 5.81 (t, 1H, J = 5.4 Hz, NH), 6.59 (m, 2H, Ar-3H), 7.03 (m, 3H, Ar-2H).	**8a**
5.05 (d, 2H, J = 5.4 Hz, CH_2), 5.70 (t, 1H, J = 5.4 Hz, NH), 7.40 (m, 3H, Ar-3H), 7.55 (m, 2H, Ar-2H), 14.05 (s, 1H, NH).	**9a**
2.20 (s, 3H, CH_3), 5.05 (d, 2H, J = 5.4 Hz, CH_2), 5.75 (t, 1H, J = 5.4 Hz, NH), 7.22 (d, 2H, J = 8.5 Hz, Ar-2H), 7.65 (d, 2H, J = 8.5 Hz, Ar-2H), 13.75 (s, 1H, NH).	**9b**
3.39 (d, 2H, J = 5.4 Hz, CH_2), 6.22 (t, 1H, J = 5.4 Hz, NH), 6.63 (m, 3H, Ar-3H), 7.15 (m, 2H, Ar-2H).	**10a**
2.25 (s, 3H, CH_3), 4.25 (d, 2H, J = 5.4 Hz, CH_2), 5.78 (t, 1H, J = 5.4 Hz, NH), 7.15 (d, 2H, J = 8.5 Hz, Ar-2H), 7.66 (d, 2H, J = 8.5 Hz, Ar-2H).	**10b**
2.20 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 4.40 (d, 2H, J = 5.4 Hz, CH_2), 6.03 (t, 1H, J = 5.4 Hz, NH), 6.15 (s, 1H, CH), 6.59 (m, 1H, Ar-1H), 6.75 (m, 2H, Ar-2H), 7.01 (m, 2H, Ar-2H).	**11a**
3.67 (s, 2H, CH_2), 4.23 (d, 2H, J = 5.4 Hz, CH_2), 5.78 (t, 1H, J = 5.4 Hz, NH), 6.56 (m, 3H, Ar-3H), 7.07 (m, 2H, Ar-2H), 9.04 (s, 1H, NH).	**12a**
2.15 (s, 3H, CH_3), 3.61 (s, 2H, CH_2), 4.42 (d, 2H, J = 5.4 Hz, CH_2), 5.65 (t, 1H, J = 5.4 Hz, NH), 6.45 (d, 2H, J = 8.5 Hz, Ar-2H), 6.88 (d, 2H, J = 8.5 Hz, Ar-2H), 9.05 (s, 1H, NH).	**12b**

Chemistry

5-N-Arylaminomethyl-2-ethylmercapto-1,3,4-oxadiazoles ***2a,b***

To a solution of *N*-arylaminomethyl-1,3,4-oxadiazole-2-thione **1a**,**b** (0.01 mol) and potassium hydroxide (0.01 mol) in water (25 mL), was added ethyl iodide (0.01 mol). The solution was stirred at room temperature for 2 h. The resulting precipitate was filtered off and crystallized from ethanol.

5-N-Arylaminomethyl-2-hydrazino-1,3,4-oxadiazoles ***3a,b***

A solution of *N*-arylaminomethyl-2-ethylmercapto-1,3,4-oxadiazole **2a**,**b** (0.01 mol) and hydrazine hydrate (0.03 mol) in ethanol was heated under reflux for 6 h. The solution was cooled and the resulting precipitate was filtered and crystallized from ethanol.

Sugar (5-N-arylaminomethyl-1,3,4-oxadiazol-2-yl-hydrazones ***4–6a–c***

General procedure: To a well-stirred solution of the respective monosaccharide (0.01 mol) in water (2 mL), and glacial acetic acid (0.2 mL) was added the appropriate 5-*N*-arylaminomethyl-2-hydrazino-1,3,4-oxadiazole **3a**,**b** (0.01 mol) in ethanol (10 mL). The mixture was heated under reflux for 3 h, the resulting solution was concentrated and left to cool. The precipitate formed was filtered off, washed with water and ethanol, then dried and crystallized from ethanol.

2-Acetyl-3-(1′,2′,3′,4′,5′-penta-O-acetyl-alditol-1-yl)-6-N-arylamino-methyl-2,3-dihydro[1,2,4]triazolo[3,4-b][1,3,4]-oxadiazoles ***7–8a,b***

General procedure: A solution of sugar (5-*N*-arylaminomethyl-1,3,4-oxadiazol-2-yl)-hydrazones **4–5a**,**b** (1 mmol) in acetic anhydride (5 mL) was boiled under reflux for 1 h. The resulting solution was poured onto crushed ice, and the product that separated out was filtered off, washed with sodium hydrogen carbonate and water, and was then dried.

6-N-Arylaminomethyl[1,2,4]triazolo[3,4-b][1,3,4]-oxadiazole-(2H)-3-thiones ***9a,b***

To a solution of *N*-arylaminomethyl-2-hydrazino-1,3,4-oxadiazole **3a, b** (0.02 mol) in ethanol (50 mL) was added a solution of potassium hydroxide (0.02 mol) dissolved in water (2 mL) and carbon disulphide (5 mL). The solution was heated under reflux for 15 h. The solvent was evaporated and the residue was dissolved in water, filtered off, and acidified with dilute hydrochloric acid. The formed precipitate was filtered off, washed with water, and crystallized from ethanol.

5-N-Arylaminomethyl-1,3,4-oxadiazolo[1,5-d][1,2,3,4]-tetrazoles ***10a,b***

A solution of sodium nitrite (0.01 mol) in water (3 mL) was added to a cooled and stirred solution of 5-*N*-arylaminomethyl-2-hydrazino-1,3,4-oxadiazole **3a**,**b** (0.01 mol) in 50% aqueous HCl (10 mL) over the time span of 1 h. The resulting precipitate was filtered off and recrystallized from ethanol.

2-(3,5-Dimethylpyrazol-1-yl)-5-N-arylaminomethyl-1,3,4-oxadiazoles ***11a,b*** *and 2-[3,5-Dioxo-(2H)-pyrazolidin-1-yl]-5-N-arylaminomethyl-1,3,4-oxadiazoles* ***12a,b***

General procedure: Acetylacetone or diethylmalonate (0.01 mol) was added to a solution of 5-*N*-arylaminomethyle-2-hydrazino-1,3,4-oxadiazole **3a**,**b** (0.01 mol) in dioxane (20 mL) as well as a few drops of triethylamine. The mixture was refluxed for 4 h. The reaction mixture was then concentrated, cooled to room temperature, and the formed precipitate was filtered off, and crystallized from ethanol to give compounds **11a**,**b** or **12a**,**b**.

Antiviral screening

Preparation of the compounds for bioassay

The test compounds (100 mg) were dissolved each in 1 mL of 10% DMSO (in water). The final concentration was 100 μg/μL (stock solution). The stock solutions were decontaminated by addition of 50 μg/mL antibiotic antimycotic mixture (10 000 U penicillin G sodium, 10 000 μg streptomycin sulfate, and 250 μg amphotericin B; PAA Laboratories GmbH, Austria).

Cell culture

African green monkey kidney-derived cells (Vero) and human hepatoma cell line (HepG2) were used. Cells were propagated in Dulbeccos' Minimal Essential Medium (DMEM) supplemented with 10% fetal bovine serum, 1% antibiotic-antimycotic mixture. The pH was adjusted at 7.2–7.4 by 7.5% sodium bicarbonate solution. The mixture was sterilized by filtration through 0.2 μm-pore size nitrocellulose membrane.

Viruses

Herpes simplex virus type-1 (HSV-1) and Hepatitis-A virus (HAV, MBB-cell culture adapted strain) were obtained from Environmental Virology Lab., Department of Water Pollution Research, National Research Centre, Cairo, Egypt.

Cytotoxicity assay

Cytotoxicity was assayed for both dimethyl sulfoxide (DMSO) and the tested compounds. Serial dilutions were prepared and inoculated on Vero cells grown in 96-well tissue culture plates. The maximum tolerated concentration (MTC) for each compound was determined both by cell morphology and cell viability by staining with trypan blue dye.

Plaque reduction infectivity assay

A 6-well plate was cultivated with cell culture (10^5 cell/mL) and incubated for 2 days at 37°C. HSV-1 and HAV were diluted to give 10^4 PFU/mL as final concentration for each virus and mixed with the tested compound at the previous concentration and incubated overnight at 4°C. Growth medium was removed from the multiwell plate and the virus-compound mixture was inoculated (100 μL/well). After 1 h contact time, the inoculum was aspirated and 3 mL of MEM with 1% agarose was overlaid the cell sheets. The plates were left to solidify and incubated at 37°C until the development of virus plaques. Cell sheets were fixed in 10% formaline solution for 2 h and stained with crystal violet stain. Control virus and cells were treated identically without the chemical compounds. Virus plaques were counted and the percentage of reduction was calculated [33].

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