Acta Poloniae Pharmaceutica – Drug Research, Vol. 71 No. 5 pp. 771–780, 2014 ISSN 0001-6837
Polish Pharmaceutical Society

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF NEW NORBORNYL SYSTEM BASED OXADIAZOLE THIOGLYCOSIDES AND ACYCLIC NUCLEOSIDE ANALOGS

ASHRAF M. MOHAMED[1,2]*, WAEL A. EL-SAYED[3], HUSAM R.M. AL-QALAWI[1] and MOUSA O. GERMOUSH[4]

[1]Chemistry Department, College of Science, Aljouf University, Sakaka, Aljouf, Kingdom of Saudia Arabia
[2]Applied Organic Chemistry Department, National Research Centre, Dokki, Cairo, Egypt
[3]Photochemistry Department, National Research Centre, El-Dokki, Cairo, Egypt
[4]Biology Department, College of Science, Aljouf University, Sakaka, Aljouf, Kingdom of Saudia Arabia

Abstract: New sugar hydrazones linked to norbornyl ring system, their oxadiazole acyclic nucleoside analogs and the corresponding thioglycosides were synthesized. The synthesized compounds were tested for their antimicrobial activity and displayed different degrees of activities or inhibitory actions. Their oxadiazole acyclic nucleoside analogs and thioglycosides showed higher activities.

Keywords: norbornyl, oxadiazole, glycosides, antibacterial, antifungal

The fast and widespread evolution of antimicrobial resistance poses a grave threat to therapy of many microbial infections and necessitates imperative and scrupulous efforts to develop next generation of antibacterial and antifungal agents. Norbornyl ring system is the basic core in many naturally occurring compounds such as monoterpenoids. Camphor, one of the most important compounds within this system, has been widely used as a fragrance in cosmetics, as an active ingredient in some drugs (1). In addition, the monoterpenoid is present in a number of over-the-counter medications, mainly for external application, and is readily available in drugstores (2). Compounds exhibiting norbornyl ring system possess a broad range of biological properties such as insect repellent (3), a bacteriostatic and fungistatic agent (1) and an antitussive (4). Furthermore, compounds incorporating norbornyl ring system showed interesting biological activities with application in the pharmaceutical field such as antispasmodic (5) and as inhibitors of norepinephrine secretion (6). Among the five-membered nitrogen heterocycles, the 1,3,4-oxadiazoles have been associated with a broad spectrum of biological activities (7–10). Their derivatives have been reported to possess antimicrobial (11–14), insecticidal (15), herbicidal, fungicidal (16), anti-inflammatory (17, 18) as well as antiviral (19) and antitumor activities. A number of substituted 1,3,4-oxadiazoles linked to polycyclic alkyl unit **I** (19) and **II** (20) and others attached to sugar moieties **III** (21) and **IV** (22) showed high antimicrobial activities. On the other hand, the glycosylthio heterocycles (23–25) and the acyclic nucleoside analogs with modification of both the glycon part and the heterocyclic base have stimulated extensive research as biological inhibitors (26–28). Nucleosides and their analogs possess a wide range of medicinal properties, including antibiotic, antiviral, and antitumor activity (29–32). We have been interested in the synthesis of new nucleoside analogs by attachment of sugar moieties to newly synthesized heterocycles (22, 33–35) in an ongoing search for new compounds with potential biological activity. Consequently, we have considered the synthesis of norbornyl system based new oxadiazole thioglycosides and their acyclic analogs in addition to the evaluation of their antibacterial and antifungal activity.

* Corresponding author: e-mail: ammewas1@gmail.com

EXPERIMENTAL

Chemistry

Melting points were determined with a Kofler block apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer model 1720 FTIR Spectrometer for KBr discs. NMR spectra were recorded on a Varian Gemini 200 NMR Spectrometer at 300 MHz for ^{1}H 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. The progress of the reactions was monitored by

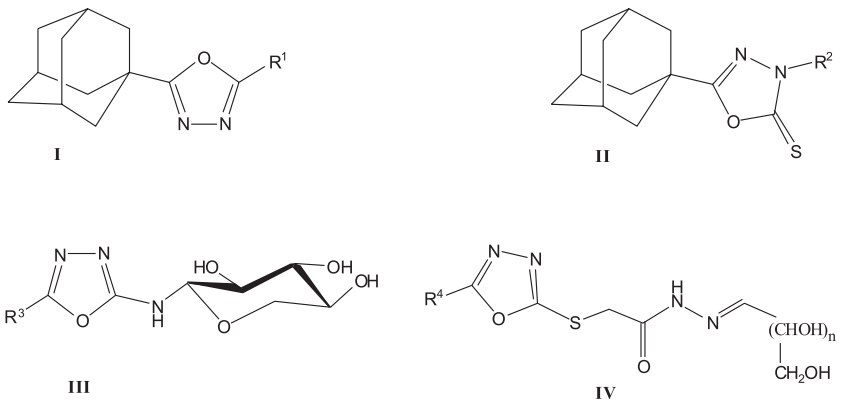
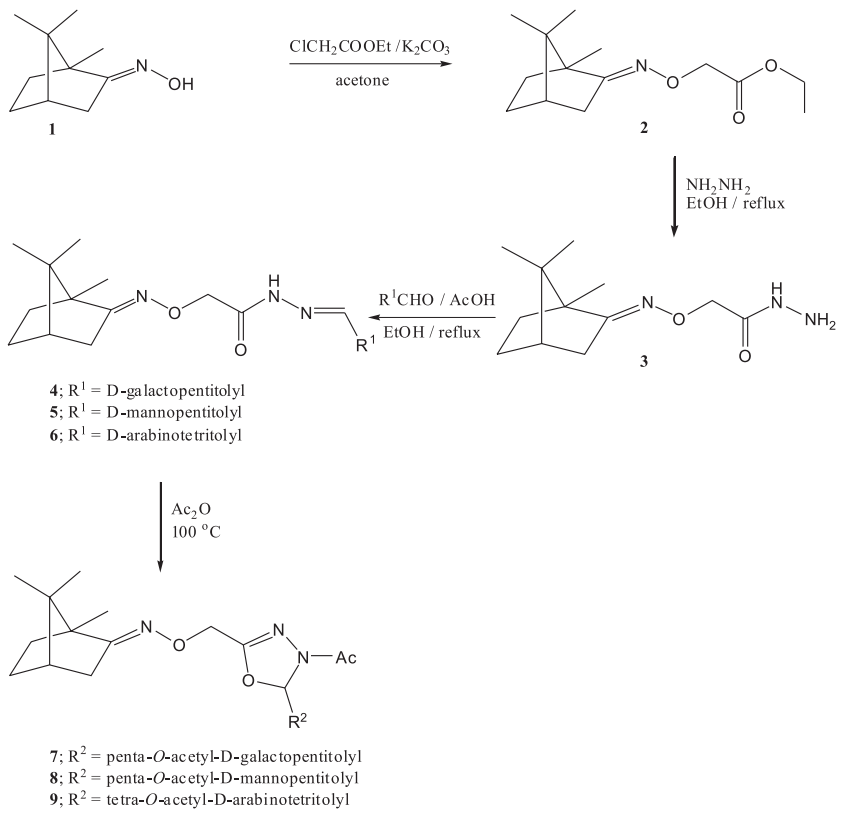


Figure 1. Antimicrobial 1,3,4-oxadiazole derivatives



Scheme 1. Synthesis of oxadiazoline acyclic sugar derivatives

TLC using aluminum silica gel plates 60 F 245. EI-mass spectra were measured on HP D5988 A 1000 MHz spectrometer (Hewlett-Packard, Palo Alto, CA, USA). Elemental analyses were performed at the Micro Analytical Data Center at Faculty of Science, Cairo University, Egypt. Antimicrobial activity was determined at the Biology Department, College of Science, Aljouf University.

Ethyl 2-(1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)aminooxy)acetate (2)

Ethyl chloroacetate (1.22 g, 10 mmoles) was added to a well stirred solution of compound **1** (1.67 g, 10 mmoles) and dry potassium carbonate (1.38 g, 10 mmoles) in acetone (15 mL). The reaction mixture was stirred at room temperature for 5 h and then poured on ice-cold water. The precipitated solid was filtered, washed with water and recrystallized from ethanol to give compound **2** as white crystals, 2.03 g.

2-[(1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ylidene)aminooxy]acetohydrazide (3)

Hydrazine hydrate (0.5 g, 10 mmoles) was added to a solution of compound **2** (2.54 g, 10 mmoles) in ethanol (25 mL) and the reaction mixture was heated under reflux for 3 h. After cooling, the precipitated solid was filtered, washed with ethanol and recrystallized from ethanol to afford compound **3** as white crystals, 2.03 g.

Sugar-[(1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)aminooxy]acetohydrazone (4–6)

General procedure: The acetylhydrazone **3** (1.20 g, 5 mmoles) dissolved in ethanol (15 mL) was

Table 1. Physical and analytical data for the synthesized compounds.

Comp. No.	M.p. (°C)	Yield (%)	Mol. formula (m.w.)	Analysis (%) calc. / found.		
				C	**H**	**N**
2	131–132	80	$C_{14}H_{23}NO_3$ (253.34)	66.37 66.02	9.15 9.11	5.53 5.27
3	189–190	85	$C_{12}H_{21}N_3O_2$ (239.31)	60.23 60.39	8.84 8.52	17.56 17.24
4	198–200	78	$C_{18}H_{31}N_3O_7$ (401.45)	53.85 53.49	7.78 8.11	10.47 10.24
5	196–198	80	$C_{18}H_{31}N_3O_7$ (401.45)	53.85 53.51	7.78 7.48	10.47 10.19
6	197–198	80	$C_{17}H_{29}N_3O_6$ (371.43)	54.97 54.62	7.87 7.42	11.31 11.12
7	130–132	74	$C_{30}H_{43}N_3O_{13}$ (653.67)	55.12 54.92	6.63 6.35	6.43 6.20
8	134-135	75	$C_{30}H_{43}N_3O_{13}$ (653.67)	55.12 54.95	6.63 6.38	6.43 6.29
9	137–138	70	$C_{27}H_{39}N_3O_{11}$ (581.61)	55.76 55.58	6.76 6.46	7.22 7.05
10	152–153	72	$C_{13}H_{19}N_3O_2S$ (281.37)	55.49 55.12	6.81 6.50	14.93 14.61
12a	139–141	73	$C_{27}H_{37}N_3O_{11}S$ (611.66)	53.02 52.85	6.10 5.92	6.87 6.59
12b	138–140	71	$C_{27}H_{37}N_3O_{11}S$ (611.66)	53.02 52.89	6.10 5.89	6.87 7.05
12c	141–142	70	$C_{24}H_{33}N_3O_9S$ (539.60)	53.42 53.15	6.16 6.38	7.79 7.45
13a	197–198	75	$C_{19}H_{29}N_3O_7S$ (443.51)	51.45 51.21	6.59 6.28	9.47 9.21
13b	194–195	75	$C_{19}H_{29}N_3O_7S$ (443.51)	51.45 51.19	6.59 6.35	9.47 9.24
13c	192–193	73	$C_{18}H_{27}N_3O_6S$ (413.49)	52.29 52.02	6.58 6.30	10.16 9.96

added to a solution of the respective monosaccharide (5 mmoles) in water (1 mL) and glacial acetic acid (1 mL). The mixture was heated under reflux for 4 h and the resulting solution was concentrated and left to cool. The formed precipitate was filtered off, washed with water and ethanol, then dried and recrystallized from ethanol to afford **4–6**.

O-Acetylsugar-5-[[(1,7,7-trimethylbicyclo[2.2.1]-heptan-2-ylidene)aminooxy]-methyl]-1,3,4-oxadiazol-3(2H)-yl}ethanone (7–9)

General procedure: A solution of sugar hydrazone **4–6** (5 mmol), in acetic anhydride (4 mL), was heated at 100°C for 1.0–1.5 h. The resulting solution was poured onto crushed ice, and the product that separated out was filtered off, washed with sodium hydrogen carbonate solution (50 mL), followed by water (50 mL) and then dried. The products were recrystallized from ethanol-water (2 : 1 v/v) to give oxadiazolines **7–9**.

5-{[-(1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ylidene)aminooxy]methyl}-1,3,4-oxadiazole-2(3H)-thione (10)

Carbon disulfide (3 mL) was added dropwise to a solution of hydrazide **3** (10 mmol, 2.40 g) in pyridine (30 mL). The solution was heated at 100°C for 12 h. The solvent was evaporated to half of its amount under reduced pressure and the residue was poured into ice-cold water containing acetic acid (2 mL). The obtained precipitate was filtered off, washed with water and recrystallized from ethanol to afford the oxadiazole **10** as yellow powder.

1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one-O-[5-glycopyranosylthio)-1,3,4-oxadiazol-2-yl]methyl oxime (12a–c)

General procedure: The bromosugar **11a–c** (6 mmol) was added to a well stirred solution of compound **10** (1.21 g, 5 mmol) in *N*,*N*-dimethylformamide (7 mL) containing Et_3N (0.85 mL, 6 mmol). The reaction mixture was stirred at room temperature until reaction was judged complete by TLC using chloroform/methanol 99.7 : 0.3 v/v). The mixture was concentrated under reduced pressure, diluted with CH_2Cl_2 (40 mL), and washed with water (3 × 30 mL). The organic layer was dried (Na_2SO_4), filtered, evaporated under reduced pressure, and the residue was triturated with petroleum ether (b.p. 40–60°C) (45 mL). The solid product was filtered, dried and recrystallized from ethanol.

1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one-O-[5-(D-glycopyranosylthio)-1,3,4-oxadiazol-2-yl]methyl oxime (13a–c)

General procedure: Dry gaseous ammonia was passed through a solution of the acetylated thioglycosides **12a–c** (5 mmol) in dry methanol (20 mL) at 0°C for 1 h, and then the mixture was stirred at room temperature for 5 h. The solvent was evaporated under reduced pressure at 40°C to give a solid residue, which was recrystallized from ethanol to give the corresponding free glycosides **13a–c**.

Antimicrobial screening

The synthesized compounds were tested for their antimicrobial activity against three microorganisms and the minimal inhibitory concentrations (MICs) of the tested compounds were determined by the dilution method.

Sample preparation

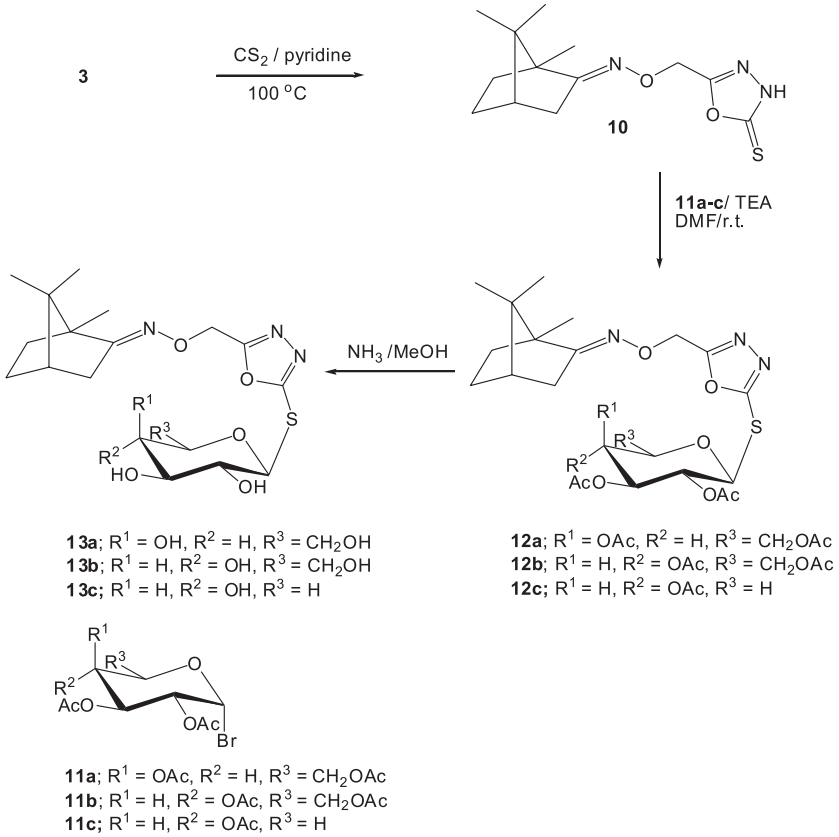
Each of the test compounds and standards were dissolved in 12.5% DMSO, at concentrations of 500 µg/mL. Further dilutions of the compounds and standards in the test medium were prepared at the required quantities.

Culture of microorganisms

Bacteria strains, namely: *Bacillus subtilis* (ATCC 6633) (Gram positive), *Pseudomonas aeruginosa* (ATCC 27853) (Gram negative) and *Streptomyces* species (Actinomycetes) were used in this investigation. The bacterial strains were maintained on MHA (Mueller-Hinton agar) medium (Oxoid, Chemical Co., UK) for 24 h at 37°C. The medium was molten on a water bath, inoculated with 0.5 mL of the culture of the specific microorganism and poured into sterile Petri dishes to form a layer of about 3–4 mm thickness. The layer was allowed to cool and harden. With the aid of corkborer, cups of about 10 mm diameter were produced (36).

Agar diffusion technique

The antibacterial activities of the synthesized compounds were tested against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Streptomyces* species using MH medium (17.5 g casein hydrolysate, 1.5 g soluble starch, 1000 mL beef extract). A stock solution of each synthesized compound (500 µg/mL) in DMSO was prepared and graded quantities of the test compounds were incorporated in specified quantity of sterilized liquid MH medium. Different concentrations of the test compounds in DMSO were placed separately in cups in the agar medium. All plates were incubated at 37°C overnight. The inhibition zones were measured after 24 h. The minimum inhibitory concentration (MIC) was defined



Scheme 2. Synthesis of 1,3,4-oxadiazole thioglycosides

as the intercept of the grave of logarithm concentrations *versus* diameter of the inhibition zones (37).

RESULTS AND DISCUSSION

Chemistry

Reaction of the bicycloheptanone oxime derivative **1** (38) with ethyl chloroacetate in the presence of potassium carbonate gave the ethyl *O*-substituted acetyl ester **2**. Hydrazinolysis of the latter ester with hydrazine hydrate afforded the corresponding acid hydrazide derivative **3** in 85% yield. The ^{1}H NMR spectrum of **2** showed signals corresponding to the ethyl group where the C*H*$_2$ appeared as quartet and disappeared in the ^{1}H NMR spectrum of the derived hydrazide, which revealed, instead, signals corresponding to NH and NH_2 groups. When the hydrazide **3** was allowed to react with D-galactose, D-mannose or D-arabinose in the presence of catalytic amount of acetic acid, the corresponding sugar hydrazones **4–6** were obtained, respectively. Their ^{1}H NMR spectra showed the signals of the sugar chain protons at δ 3.35–5.69 ppm for the alditolyl sugar protons and the hydroxyl protons in addition to the C-1 methine proton as doublet in the range of δ 7.46–7.51 ppm. It is well known that the reaction of sugar aroylhydrazones with acetic anhydride gives the respective per-*O*-acetyl derivatives. However, it has been reported (40–42) that when the reaction was carried out at high temperature in boiling acetic anhydride, cyclization usually takes place in addition to per-*O*-acetylation to afford acyclic *C*-nucleoside analogs. We reported previously (40, 41) the synthesis of 1,2,4-triazolo[1,3,4]oxadiazole and *N*-acetyl-1,3,4-oxadiazoline acyclic nucleoside analogs by the reaction of hydrazinyl sugars with boiling acetic anhydride. When the hydrazones **4–6** were heated in acetic anhydride at 100°C they gave the 1,3,4-oxadiazoline acyclic nucleoside analogs **7–9**, respectively. The structures of the oxadiazoline acetylated sugar derivatives were confirmed by their spectral and analytical data. Their IR spectra showed absorption bands in the carbonyl frequency region not only for the carbonyl ester but also corresponding to the carbonyl amide groups indicating the presence of *N*-acetyl group in addition to the *O*-

Table 2. Spectral data for the synthesized compounds.

Comp. No.	Spectrum	IR [KBr; ν cm^{-1}] , ^{1}H NMR [(DMSO) δ, ppm], ^{13}C NMR [(DMSO) δ, ppm], MS [(m/z), %]
2	IR	1736 (C=O), 1602 (C=N).
	^{1}H NMR	1.02 (s, 6H, 2 CH_3), 1.25 (s, 3H, CH_3), 1.52–1.77 (m, 7H, CH_3 and 2 CH_2), 2.50–2.55 (m, 3H, CH and CH_2), 3.90 (q, *J* = 5.2 Hz, 2H, CH_2), 4.89 (s, 2H, CH_2).
	^{13}C NMR	11.44, 15.20, 20.35 (4 CH_3), 28.30, 32.05, 33.39, 42.90, 46.28, 53.15 (norbornyl carbons), 62.15, 71.10 (2CH_2), 165.58 (C=N), 169.18 (C=O).
	MS	253 [$(M)^+$, 21].
3	IR	3431–3375 (NH_2 and NH), 1604 (C=N).
	^{1}H NMR	1.04 (s, 6H, 2 CH_3), 1.26 (s, 3H, CH_3), 1.55–1.80 (m, 4H, 2 CH_2), 2.50–2.56 (m, 3H, CH and CH_2), 4.95 (s, 2H, CH_2), 5.03 (bs, 2H, NH_2), 10.12 (bs, IH, NH).
	^{13}C NMR	11.48, 15.24 (3 CH_3), 28.41, 32.24, 33.90, 43.95, 47.02, 54.28 (norbornyl carbons), 72.27 (CH_2), 166.91 (C=N), 169.24 (C=O).
	MS	240 [$(M + H)^+$, 14].
4	IR	3540–3424 (OH), 3311 (NH), 1611 (C=N).
	^{1}H NMR	1.04 (s, 6H, 2 CH_3), 1.26 (s, 3H, CH_3), 1.55–1.80 (m, 4H, 2 CH_2), 2.50–2.59 (m, 3H, CH and CH_2), 3.36–3.39 (m, 2H, H-6,6'), 3.71–3.77 (m, 1H, H-5), 4.25–4.31 (m, 2H, H-4,3), 4.38–4.44 (m, 1H, H-2), 4.49–4.55 (m, 1H, OH), 4.61 (d, *J* = 6.4 Hz, 1H, OH), 4.95–4.08 (m, 3H, CH_2 and OH), 5.19–5.25 (m, 1H, OH), 5.63 (t, *J* = 4.6 Hz, 1H, OH), 7.46 (d, *J* = 7.5 Hz, 1H, H-1), 10.14 (s, IH, NH).
	^{13}C NMR	11.47, 15.22 (3 CH_3), 28.50, 32.29, 33.95, 44.11, 47.42, 54.30 (norbornyl carbons), 62.10 (C-6), 63.05 (C-5), 69.21 (C-4), 74.27 (C-3), 74.94 (CH_2), 75.88 (C-2), 152.16 (C-1), 166.08 (C=N), 169.15 (C=O).
5	IR	3563–3418 (OH), 3305 (NH), 1614 (C=N).
	^{1}H NMR	1.02 (s, 6H, 2 CH_3), 1.25 (s, 3H, CH_3), 1.54–1.78 (m, 4H, 2 CH_2), 2.52–2.58 (m, 3H, CH and CH_2), 3.38–3.41 (m, 2H, H-6,6'), 3.73–3.80 (m, 1H, H-5), 4.25–4.34 (m, 2H, H-4,3), 4.37–4.44 (m, 1H, H-2), 4.49–4.55 (m, 1H, OH), 4.62–4.70 (m, 1H, OH), 4.97–5.08 (m, 3H, CH_2 and OH), 5.20–5.26 (m, 1H, OH), 5.64–5.69 (m, 1H, OH), 7.50 (d, *J* = 7.5 Hz, 1H, H-1), 10.12 (s, IH, NH).
6	IR	3510–3412 (OH), 3310 (NH), 1614 (C=N).
	^{1}H NMR	1.03 (s, 6H, 2 CH_3), 1.24 (s, 3H, CH_3), 1.55–1.77 (m, 4H, 2 CH_2), 2.50–2.58 (m, 3H, CH and CH_2), 3.35–3.42 (m, 2H, H-5,5'), 3.70 (m, 1H, H-4), 4.17–4.26 (m, 2H, H-3), 4.39–4.45 (m, 1H, H-2), 4.48–4.55 (m, 1H, OH), 4.55 (d, *J* = 6.4 Hz, 1H, OH), 4.97–5.08 (m, 2H, CH_2), 5.18–5.24 (m, 1H, OH), 5.65–5.68 (m, 1H, OH), 7.51 (d, *J* = 7.5 Hz, 1H, H-1), 10.14 (s, IH, NH).
	^{13}C NMR	11.21, 15.22 (3 CH_3), 28.50, 32.31, 33.96, 44.12, 47.43, 54.31 (norbornyl carbons), 62.12 (C-5), 69.11 (C-4), 74.27 (C-3), 74.95 (CH_2), 75.73 (C-2), 152.18 (C-1), 166.05 (C=N), 169.20 (C=O).
7	IR	1738 (C=O), 1679 (C=O), 1612 (C=N).
	^{1}H NMR	1.05 (s, 6H, 2 CH_3), 1.27 (s, 3H, CH_3), 1.55–1.80 (m, 4H, 2 CH_2), 1.86, 1.98, 2.03, 2.11, 2.18, 2.28 (6s, 18H, 6CH_3), 2.55–2.59 (m, 3H, CH and CH_2), 4.17 (dd, *J* = 11.4 Hz, *J* = 2.8 Hz, 1H, H-5), 4.22 (dd, *J* = 11.4 Hz, *J* = 3.2 Hz, 1H, H-5'), 4.90–4.95 (m, 1H, H-4), 5.02 (s, 2H, CH_2), 5.27 (dd, *J* = 6.5 Hz, *J* = 7.4 Hz, 1H, H-3), 5.53 (dd, *J* = 7.4 Hz, *J* = 7.2 Hz, 1H, H-2), 5.72 (t, *J* = 7.2 Hz, 1H, H-1), 5.77 (d, *J* = 7.6 Hz, 1H, oxadiazoline-H).
	^{13}C NMR	11.18, 15.20, 20.32, 20.52, 20.64, 20.78, 21.05, 26.12 (9 CH_3), 28.82, 32.30, 33.91, 44.05, 47.40, 54.27 (norbornyl carbons), 72.95 (CH_2), 62.92 (C-5), 64.91 (C-4), 65.38 (C-3), 68.41 (C-2), 71.18 (C-1), 91.24 (*C*-N-Ac), 158.22 (oxadiazoline C-5), 163.02 (C=N), 169.15, 169.82, 170.24, 170.75, 171.10, 172.18 (6CO).
	MS	654 [$(M + H)^+$, 11].
8	IR	1736 (C=O), 1672 (C=O), 1614 (C=N).
	^{1}H NMR	1.04 (s, 6H, 2 CH_3), 1.25 (s, 3H, CH_3), 1.55–1.79 (m, 4H, 2 CH_2), 1.85, 1.97, 2.03, 2.10, 2.18, 2.27 (6s, 18H, 6CH_3), 2.56–2.60 (m, 3H, CH and CH_2), 4.18 (dd, *J* = 11.4 Hz, *J* = 2.8 Hz, 1H, H-5), 4.22 (dd, *J* = 11.4 Hz, *J* = 3.2 Hz, 1H, H-5'), 4.89–4.94 (m, 1H, H-4), 4.99 (s, 2H, CH_2), 5.27 (dd, *J* = 6.5 Hz, *J* = 7.4 Hz, 1H, H-3), 5.57 (dd, *J* = 7.4 Hz, *J* = 7.2 Hz, 1H, H-2), 5.75 (t, *J* = 7.2 Hz, 1H, H-1), 5.79 (d, *J* = 7.8 Hz, 1H, oxadiazoline-H).
	MS	654 [$(M + H)^+$, 9].

Table 2. Cont.

Comp. No.	Spectrum	IR [KBr; ν cm^{-1}], ^{1}H NMR [(DMSO) δ, ppm], ^{13}C NMR [(DMSO) δ, ppm], MS [(m/z), %]
9	IR	1739 (C=O), 1675 (C=O), 1615 (C=N).
	^{1}H NMR	1.03 (s, 6H, 2 CH_3), 1.28 (s, 3H, CH_3), 1.57–1.81 (m, 4H, 2 CH_2), 1.85, 1.97, 2.04, 2.14, 2.29 (5s, 15H, 5CH_3), 2.52-2.57 (m, 3H, CH and CH_2), 4.18 (dd, J = 11.4 Hz, J = 2.8 Hz, 1H, H-4), 4.25 (dd, J = 11.4 Hz, J = 3.2 Hz, 1H, H-4'), 5.11 (s, 2H, CH_2), 5.28 (dd, J = 6.5 Hz, J = 7.4 Hz, 1H, H-3), 5.54 (dd, J = 7.4 Hz, J = 7.2 Hz, 1H, H-2), 5.72 (t, J = 7.2 Hz, 1H, H-1), 5.77 (d, J = 7.6 Hz, 1H, oxadiazoline-H).
	^{13}C NMR	11.16, 15.21, 20.31, 20.50, 20.60, 21.05, 26.14 (8 CH_3), 28.83, 32.30, 33.90, 44.10, 47.40, 54.28 (norbornyl carbons), 74.90 (CH_2), 62.95 (C-4), 65.40 (C-3), 68.40 (C-2), 71.18 (C-1), 91.30 (*C*-N-Ac), 158.25 (oxadiazoline C-5), 162.95 (C=N), 169.10, 170.25, 170.75, 171.15, 172.20 (5CO).
10	IR	2986 (CH), 1615 (C=N).
	^{1}H NMR	1.03 (s, 6H, 2 CH_3), 1.28 (s, 3H, CH_3), 1.59–1.82 (m, 4H, 2 CH_2), 2.52–2.57 (m, 3H, CH and CH_2), 4.92 (s, 2H, CH_2), 12.31 (bs, 1H, NH).
	^{13}C NMR	11.17, 15.29 (3 CH_3), 28.74, 32.38, 33.76, 44.12, 47.46, 54.32 (norbornyl carbons), 72.65 (CH_2), 159.84 (oxadiazoline C-5), 163.95 (C=N), 179.95 (C=S).
	MS	281 [(M)$^+$, 18].
12a	IR	1612 (C=N), 1744 (C=O).
	^{1}H NMR	1.04, 1.25 (2s, 9H, 3 CH_3), 1.54–1.82 (m, 4H, 2 CH_2), 1.89, 1.92, 1.98, 2.03 (4s, 12H, 4CH_3CO), 2.49–2.55 (m, 3H, CH and CH_2), 4.05–4.10 (m, 1H, H-5), 4.14 (dd, $J_{6,6'}$ = 11.4 Hz, $J_{5,6}$ = 2.8 Hz, 1H, H-6), 4.22 (dd, $J_{6,6'}$ = 11.4 Hz, $J_{5,6'}$ = 3.2 Hz, 1H, H-6'), 4.94 (t, $J_{3,4}$ = 9.6 Hz, 1H, H-4), 5.14 (s, 2H, CH_2), 5.20 (dd, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 9.6 Hz, 1H, H-3), 5.28 (t, $J_{2,3}$ = 9.8 Hz, 1H, H-2), 5.79 (d, $J_{1,2}$ = 10.4 Hz, 1H, H-1).
	^{13}C NMR	11.17, 15.29, 19.27, 19.23, 20.44, 20.65 (4CH_3CO and 3 CH_3), 28.74, 32.38, 33.76, 44.12, 47.46, 54.32 (norbornyl carbons), 62.22 (C-6), 66.14 (C-4), 68.85 (C-3), 70.19 (C-2), 71.85 (CH_2), 72.28 (C-5), 91.02 (C-1), 159.05 (oxadiazole C-2), 160.34 (oxadiazole C-5), 163.75 (C=N), 169.41, 170.52, 171.11, 171.80 (4C=O).
	MS	612 [(M + H)$^+$, 8].
12b	IR	1614 (C=N), 1748 (C=O).
	^{1}H NMR	1.05, 1.25 (2s, 9H, 3 CH_3), 1.55–1.80 (m, 4H, 2 CH_2), 1.88, 1.92, 1.97, 2.03 (4s, 12H, 4CH_3CO), 2.48–2.54 (m, 3H, CH and CH_2), 4.09 (m, 1H, H-5), 4.15 (dd, $J_{6,6'}$ = 11.2 Hz, $J_{5,6}$ = 2.8 Hz, 1H, H-6), 4.20 (dd, $J_{6,6'}$ = 11.2 Hz, $J_{5,6'}$ = 3.4 Hz, 1H, H-6'), 5.05 (t, $J_{3,4}$ = 9.4 Hz, 1H, H-4), 5.15 (s, 2H, CH_2), 5.21 (dd, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 9.4 Hz, 1H, H-3), 5.29 (t, $J_{2,3}$ = 9.6 Hz, 1H, H-2), 5.78 (d, $J_{1,2}$ = 10.4 Hz, 1H, H-1).
	MS	612 [(M + H)$^+$, 19].
12c	IR	1611 (C=N), 1741 (C=O).
	^{1}H NMR	1.03, 1.25 (2s, 9H, 3 CH_3), 1.57–1.81 (m, 4H, 2 CH_2), 1.89, 1.95, 2.03 (3s, 9H, 3CH_3CO), 2.50–2.57 (m, 3H, CH and CH_2), 4.16 (dd, $J_{5,5'}$ = 10.6 Hz, $J_{4,5}$ = 2.8 Hz, 1H, H-5), 4.22 (dd, $J_{5,5'}$ = 10.6 Hz, $J_{4,5'}$ = 3.2 Hz, 1H, H-5'), 5.02 (t, $J_{3,4}$ = 9.2 Hz, 1H, H-4), 5.12 (s, 2H, CH_2), 5.24 (dd, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 9.2 Hz, 1H, H-3), 5.29 (t, $J_{2,3}$ = 9.6 Hz, 1H, H-2), 5.79 (d, $J_{1,2}$ = 9.8 Hz, 1H, H-1).
	^{13}C NMR	11.17, 15.29, 19.42, 20.44, 20.65 (3CH_3CO and 3 CH_3), 27.81, 32.30, 33.19, 44.35, 47.71, 55.02 (norbornyl carbons), 63.05 (C-5), 65.35 (C-4), 70.61 (C-3), 71.90 (CH_2), 72.97 (C-2), 91.27 (C-1), 158.68 (oxadiazole C-2), 160.21 (oxadiazole C-5), 161.94 (C=N), 169.82, 170.61, 171.91 (3C=O).
	MS	540 [(M + H)$^+$, 18].
13a	IR	1615 (C=N), 3488–3415 (OH).
	^{1}H NMR	1.06 (s, 6H, 2CH_3), 1.26 (s, 3H, CH_3), 1.57–1.85 (m, 4H, 2 CH_2), 2.52–2.64 (m, 3H, CH and CH_2), 3.47–3.56 (m, 2H, H-6,6'), 3.61–3.66 (m, 1H, H-5), 4.14–4.24 (m, 2H, H-3,4), 4.37–4.40 (m, 1H, H-2), 4.76–4.79 (m, 1H, OH), 4.85–4.90 (m, 1H, OH), 5.24–5.31 (m, 1H, OH), 5.38–5.45 (m, 1H, OH), 5.14 (s, 2H, CH_2), 5.80 (d, J = 10.4, 1H, H-1).
	^{13}C NMR	15.11, 23.11 (3CH_3), 27.88, 32.35, 33.21, 44.35, 47.73, 55.42 (norbornyl carbons), 63.60 (C-6), 66.44 (C-4), 68.69 (C-3), 71.29 (C-2), 71.98 (CH_2), 72.88 (C-5), 92.14 (C-1), 158.95 (oxadiazole C-2), 160.05 (oxadiazole C-5), 161.88 (C=N).
13b	IR	1612 (C=N), 3486–3410 (OH).
	^{1}H NMR	1.03 (s, 6H, 2CH_3), 1.25, (s, 3H, CH_3), 1.58–1.84 (m, 4H, 2 CH_2), 2.52–2.68 (m, 3H, CH and CH_2), 3.45–3.54 (m, 2H, H-6,6'), 3.53–3.59 (m, 1H, H-5), 4.14–4.24 (m, 2H, H-3,4), 4.36–4.39 (m, 1H, H-2), 4.75 (m, 1H, OH), 4.85–4.90 (m, 1H, OH), 5.22–5.26 (m, 1H, OH), 5.38–5.43 (m, 1H, OH), 5.11 (s, 2H, CH_2), 5.81 (d, J = 10.2, 1H, H-1).
13c	IR	1615 (C=N), 3481–3449 (OH).
	^{1}H NMR	1.04 (s, 6H, 2CH_3), 1.27 (s, 3H, CH_3), 1.59–1.86 (m, 4H, 2 CH_2), 2.52–2.67 (m, 3H, CH and CH_2), 3.39–3.48 (m, 2H, H-5,5'), 4.22–4.32 (m, 2H, H-3,4), 4.38–4.44 (m, 1H, H-2), 4.77–4.82 (m, 1H, OH), 5.24–5.30 (m, 1H, OH), 5.38–5.42 (m, 1H, OH), 5.08 (s, 2H, CH_2), 5.82 (d, J = 9.8, 1H, H-1).

acetyl groups. The ^{1}H NMR spectra showed signals corresponding to the *O*-acetyl-methyl protons in addition to the *N*-acetyl-methyl protons each as singlet and signals corresponding to the rest of the alditolyl chain protons. The cyclization of the sugar hydrazones was also confirmed by the ^{13}C NMR spectra of the resulting oxadiazoline derivatives. The value of the chemical shift of the *C*-N-Ac (C-1 in the original sugar chain moiety and C-2 in the oxadiazoline ring) appeared at δ 91.24 and 91.30 ppm, which indicated its *N,N*-acetal nature rather than being a C=N. In addition, the resonances of the acetyl-methyl carbons appeared at δ 20.31–26.14 ppm (Scheme 1).

When the acid hydrazide **3** was reacted with CS_2 in pyridine at 100°C it afforded the oxadiazole derivative **10** in 72% yield for which the IR and NMR spectra agreed with the assigned structure. When compound **10** was reacted with 2,3,4,6-tetra-*O*-acetyl-α-D-galacto-, 2,3,4,6-tetra-*O*-acetyl-α-D-gluco- or 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide **11a–c**, the corresponding substituted thioglycoside derivatives **12a–c** were afforded in 70–73% yields. The IR spectra of the resulting glycosides showed absorption bands in the range 1741–1748 cm^{-1} for the acetyl carbonyl groups. The ^{1}H NMR spectra showed signals corresponding to protons of the sugar moiety and carbonyl methyl protons in addition to the bicyclic ring protons. The anomeric proton chemical shift and its coupling constant values in the ^{1}H NMR spectra indicated the β-orientation of the thioglycosidic bond. The absence of a signal corresponding to the C=S in the ^{13}C NMR spectra confirmed that the attachment of the sugar moiety has been taken place at the sulfur atom rather than the nitrogen atom. Also, the anomeric proton of β-*N*-glycosides having an adjacent C=S was reported (43–45) to appear at higher chemical shift (δ 6.9–7.2 ppm) due to the anisotropic deshielding effect of the C=S (44, 46). Deacetylation of the thioglycoside derivatives **12a–c** afforded the free thioglycosides **13a–c** in 73–75% yields. The IR spectra of the deacetylated products showed absorption bands at 3410–3488 cm^{-1} for the hydroxyl groups and also revealed the absence of the acetyl carbonyl bands. Their ^{1}H NMR spectra showed signals corresponding to the hydroxyl protons at δ 4.75–5.45 ppm (Scheme 2).

Table 1. Minimum inhibitory concentration (MIC in μg/mL) of the title compounds. The negative control DMSO showed no activity.

Compound	*Bacillus subtilis* (Gram positive)	*Pseudomonas aeruginosa* (Gram negative)	*Streptomyces species* (Actinomycetes)
2	250	500	–[a]
3	250	250	125
4	100	125	100
5	100	100	75
6	75	75	100
7	125	250	500
8	125	125	125
9	100	125	100
10	125	250	250
12a	500	125	250
12b	250	250	125
12c	250	125	125
13a	125	125	100
13b	100	100	75
13c	75	75	100
Penicillin	31	46	33

[a] Totally inactive (MIC > 500 μg/mL).

Antimicrobial activity

The antimicrobial activity of the synthesized compounds was evaluated against three microorganisms; *Bacillus subtilis* (ATCC 6633) (Gram positive), *Pseudomonas aeruginosa* (ATCC 27853) (Gram negative), and *Streptomyces species* (Actinomycetes). The values of minimal inhibitory concentrations (MICs) of the tested compounds in addition to penicillin (46) as a reference drug are presented in Table 3. Compounds **6**, **13b** and **13c** were the most active against *Bacillus subtilis* whereas **6** and **13c** revealed the highest activity against *Pseudomonas aeruginosa*. Compounds **5** and **13b** showed high activity against the *Streptomyces species* with MIC values of 75 μg/mL.

The antimicrobial activity and structure activity relationship correlation indicated that the attachment of acyclic sugar moieties to the substituted acetyl hydrazinyl group resulted in higher inhibition activities. This is clear as the activity was lost in their preparation precursors. Furthermore, the 1,3,4-oxadiazole ring system linked to cyclic sugar moieties through a thioglycosidic linkage revealed higher activities. Moreover, the attachment of free hydroxyl glycosyl moieties resulted in distinct improvement in activities against *Bacillus subtilis* and *Pseudomonas aeruginosa*. Additionally, the sugar hydrazones with free hydroxyl acyclic sugar moieties showed higher activity values than the corresponding acetylated analogs.

The obtained results also indicated that the acyclic nucleoside analog with the acetylated arabinotetritolyl moiety attached to the oxadiazoline base exhibited relatively higher activity than the corresponding galactopentitolyl or mannopentitolyl residues against *Bacillus subtilis* and *Streptomyces species,* indicating the influence of the number and orientation of the free hydroxyl groups in sugar ring. On the other hand, the effect of attachment of thioglycosyl moiety to 1,3,4-oxadiazole ring was observed especially in the results of the prepared thioglycoside **13c** for *Bacillus subtilis* and *Pseudomonas aeruginosa*.

CONCLUSIONS

New sugar hydrazones linked to norbornyl ring system and their derived oxadiazole acyclic nucleoside analogs were prepared. The produced thioglycosides by glycosylation of the substituted 1,3,4-oxadiazoles showed higher activities. The attachment of acyclic sugar moieties to the substituted acetyl hydrazinyl derivatives as well as attachment of free hydroxyl glycosyl moieties to oxadiazole ring system resulted in relatively improved antimicrobial activities.

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Received: *26. 10. 2013*