Preparation of New Oxadiazole Acyclic Nucleoside and Thioglycoside Analogs Containing Chromene Moeity with Antimicrobial Evaluation

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New sugar hydrazones linked to chroman ring system and their derived oxadiazole acyclic nucleoside analogs were synthesized from the substituted ethyl ester oxime derivative. The 2-substituted 1,3,4-oxadiazole-5-thione prepared from acid hydrazide was glycosylated to afford the corresponding thioglycosides, not the N-linked glycosides. The novel compounds were evaluated for their antimicrobial activity and showed different degrees of activities.

Keywords. Chromone; Oxadiazole; Acyclic Nucleoside; Antimicrobial

Chromenes and fused chromenes are biologically interesting compounds with antimicrobial activities [1–3], inhibitors of influenza virus sialidases [4,5], DNA strand breaking activity, and mutagenicity [6]. It is also known that many chromene containing compounds exhibit a wide spectrum of pharmacological activities [7,8], anti-HIV agents [9,10], antibacterials [11,12], and antifungals [13]. In general, a number of biologically active chromenes and chroman derivatives have been isolated from various natural sources. These substances have been identified as apoptosis-inducing [14]. Among the υe-membered nitrogen heterocycles, the 1,3,4-oxadiazoles are known to be associated with a broad spectrum of biological activities [15–17]. Their derivatives have been known to possess antibacterial [18], herbicidal, fungicidal [19], anti-inflammatories [20], hypoglycaemic [21], and hypotension characteristics [22], as well as antivirus [23] and antitumour activities [24]. Otherwise, the glycosylthio heterocycles [25–27] and the acyclic nucleoside (analogs with modification of both the glycon part and the heterocyclic base) have encouraged comprehensive research as biological inhibitors [28–30]. Several reports have documented the biological activity of Nucleosides and their analogs including antibiotic, antiviral, and antitumour activity [31–35]. Cyclization of diacylhydrazines is one of the preparation procedures of 1,3,4-Oxadiazole heterocycles [25-27] and the acyclic nucleoside (analogs with modification of both the glycon part and the heterocyclic base) have encouraged comprehensive research as biological inhibitors [28–30]. Several reports have documented the biological activity of Nucleosides and their analogs including antibiotic, antiviral, and antitumour activity [31–35]. Cyclization of diacylhydrazines is one of the preparation procedures of 1,3,4-Oxadiazole heterocycles [25-27].

General methods

All reagents were used in analytical grades. Solvents were distilled and recrystallized. Reactions were monitored by TLC (silica gel 60F254, Merck, Darmstadt, Germany). Melting points were determined on a melting point apparatus (Stuart Scientific, Stone, Staffordshire, UK) and were uncorrected. The IR spectra were recorded on a perkin-Elmer 1720 FTIR spectrometer (cm⁻¹), using KBr disks. NMR spectra were carried out with a 300 MHz at Cairo University. Chemical shifts (δ) are reported in parts per million (ppm) relative to the respective solvent or tetramethylsilane (TMS) as internal standard and standard abbreviations were used (s = apparent; b = broad; s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet). Elemental analyses were performed at the Micro analytical data centre at Faculty of Science, Cairo University, Egypt. Antimicrobial screening was conducted at the botany department, faculty of science, Menoufia University.

Ethyl 2-((2,2-dimethylchroman-4-ylideneamino)-oxy)acetate (3)

To a solution of compound 2 (1.91 g, 10 mmol) and dry potassium carbonate (1.40 g, 10 mmol) in 30 mL acetone, ethyl chloroacetate (1.20 g, 10 mmol) was added. The reaction mixture was allowed to stir at room temperature for 5 h and then poured onto ice water. The solid separated out was filtered off, washed with water and recrystallized from ethanol to give compound 3 as white crystals, Yield 1.98 g (72%), mp 129-130 °C; IR (KBr) ν: 1740 (C=O), 1120 (C-O) cm⁻¹; 1H NMR (CDCl₃, 300 MHz) δ: 1.19 (t, 3H, J = 3.3 Hz CH₂CH₃), 1.27 (s, 6H, 2CH₃), 2.90 (q, 2H, J = 5.2 Hz, CH₂CH₃), 503 (s, 2H, C=O). 

Experimental part

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All reagents were used in analytical grades. Solvents were distilled and recrystallized. Reactions were monitored by TLC (silica gel 60F254, Merck, Darmstadt, Germany). Melting points were determined on a melting point apparatus (Stuart Scientific, Stone, Staffordshire, UK) and were uncorrected. The IR spectra were recorded on a perkin-Elmer 1720 FTIR spectrometer (cm⁻¹), using KBr disks. NMR spectra were carried out with a 300 MHz at Cairo University. Chemical shifts (δ) are reported in parts per million (ppm) relative to the respective solvent or tetramethylsilane (TMS) as internal standard and standard abbreviations were used (s = apparent; b = broad; s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet). Elemental analyses were performed at the Micro analytical data centre at Faculty of Science, Cairo University, Egypt. Antimicrobial screening was conducted at the botany department, faculty of science, Menoufia University.

Ethyl 2-((2,2-dimethylchroman-4-ylideneamino)-oxy)acetate (3)

To a solution of compound 2 (1.91 g, 10 mmol) and dry potassium carbonate (1.40 g, 10 mmol) in 15 mL acetone, ethyl chloroacetate (1.20 g, 10 mmol) was added. The reaction mixture was allowed to stir at room temperature for 5 h and then poured onto ice water. The solid separated out was filtered off, washed with water and recrystallized from ethanol to give compound 3 as white crystals, Yield 1.98 g (72%), mp 129-130 °C; IR (KBr) ν: 1740 (C=O), 1120 (C-O) cm⁻¹; 1H NMR (CDCl₃, 300 MHz) δ: 1.19 (t, 3H, J = 3.3 Hz CH₂CH₃), 1.27 (s, 6H, 2CH₃), 2.90 (q, 2H, J = 5.2 Hz, CH₂CH₃), 503 (s, 2H, C=O). 

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(bs, 2H, NH), 10.12 (s, 1H, NH), 7.29-7.33 (m, 4H, ArH); \(^{13}C\) NMR (CDCl\(_3\), 75 MHz) \(\delta\): 19.23 (2CH\(_2\)), 35.33 (CH\(_3\)), 68.24 (CH\(_2\)), 72.64 (C(CH\(_3\))), 114.23, 119.65, 122.22, 129.77, 139.52, 148.88, 167.11 (C=C), 169.24 (C=O). Anal. Calcld. for C\(_19\)H\(_{27}\)N\(_3\)O\(_8\): C, 53.64; H, 5.80; N, 6.20. Found: C, 54.73; H, 5.80; N, 6.21.

D-Arabinose-\([2-(2,2-Dimethylchroman-4-ylideneaminoxy)]acetohydrazone (7)

Yield 1.41 g (75%), mp 197-198 °C; IR (KBr): \(\nu\): 3412 (OH), 3310 (NH), 1614 (C=N) cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 300 MHz): \(\delta\): 1.02 (s, 6H, 2 CH\(_3\)), 2.65 (2H, CH\(_2\)), 3.38-3.41 (m, 2H, H-6,6\(_2\)), 3.73 (m, 1H, H-5), 4.14-4.25 (m, 2H, H-1), 7.50 (1H, J = 7.5 Hz, H-1), 7.75-7.96 (m, 4H, ArH). 10.12 (s, 1H, NH). Anal. Calcld. for C\(_{19}\)H\(_{27}\)N\(_3\)O\(_8\): C, 54.73; H, 5.80; N, 6.20. Found: C, 54.73; H, 5.80; N, 6.21.

O-Acyethylsugar-\([2-(2,2-Dimethylchroman-4-ylideneaminoxy)methyl]-2,3-dihydro-1,3,4-oxadiazol-2-yl)butane-1,2,3,4-pentayl pentaacetate (10)

Yield 2.01 g (61%), mp 139-140 °C; IR (KBr): \(\nu\): 3150-3412 (OH), 3310 (NH), 1614 (C=O) cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 300 MHz): \(\delta\): 1.03 (s, 6H, 2 CH\(_3\)), 1.85, 197, 2.04, 2.14, 2.29 (5s, 15H, 5CH\(_3\)), 2.57 (2H, CH\(_2\)), 3.08 (2H, CH\(_2\)), 4.18 (dd, 1H, J = 11.4 Hz, 2H, H-4), 4.25 (dd, 1H, J = 11.4 Hz, H-2), 5.28 (dd, 1H, J = 6.5 Hz, H-2), 6.22 (1H, J = 7.4 Hz, H-2), 5.54 (dd, 1H, J = 7.4 Hz, H-2), 7.57 (2H, J = 7.2 Hz, H-2). 10.12 (s, 1H, NH). Anal. Calcld. for C\(_{31}\)H\(_{39}\)N\(_3\)O\(_{14}\): C, 54.73; H, 5.80; N, 6.21. Found: C, 54.73; H, 5.80; N, 6.21.
(s, 2H, CH₂), 3.22 (s, 2H, CH₂), 7.13-7.20 (m, 4H, ArH). 13C NMR (CDCl₃, 75 MHz) δ: 19.42, 20.44, 20.65, (6s, 18H, 4 CH₃CO and 2 CH₃), 2.50 (s, 2H, CH₂), 4.09 (s, 2H, CH₂), 4.16 (dd, 1H, J₅,₅₂ = 10.6 Hz, J₆,₆₂ = 2.8 Hz, H-6₂), 4.22 (dd, 1H, J₂,₃ = 10.6 Hz, J₃,₄ = 3.2 Hz, H-4), 5.21 (dd, 1H, J₁,₉ = 9.6 Hz, J₉,₁₀ = 9.4 Hz, H-3), 5.29 (t, 1H, J₂,₃ = 9.6 Hz, H-2), 5.78 (d, 1H, J₁,₉ = 10.4 Hz, H-1), 7.55-7.75 (m, 4H, ArH). Anal. Calcd. for C₂₆H₂₇N₃O₉S (563.16): C, 53.28; H, 5.12; N, 5.65.

2,2-dimethylchroman-4-one O-(5-(3,4,5-trihydroxy-6-hydroxymethyl)tetrahydro-2H-pyran-2-yl)-methyl oxime (14a)

Pale yellow powder Yield 1.73 g (74%), mp 197-198°C; IR (KBr) ν max 2980 (CH), 3483-3415 (OH); ¹H NMR (CDCl₃-d₆, 300 MHz) δ: 1.03 (s, 6H, 2CH₃), 2.89 (s, 2H, CH₂), 3.45 (m, 2H, H-6, H-6₂), 3.53 (m, 1H, H-5), 4.06 (s, 2H, CH₂), 4.14 (m, 2H, H-3, H-4), 4.38 (m, 1H, J₁,₉ = 9.2 Hz, H-1), 4.75 (t, 1H, J₂,₃ = 9.2 Hz, H-2), 4.75 (t, 1H, J₃,₄ = 9.2 Hz, H-3), 7.78-8.28 (m, 8H, ArH). Anal. calcd. for C₂₇H₂₉N₃O₁₀S (583.16): C, 53.28; H, 5.12; N, 5.65. Found: C, 52.18; H, 5.25; N, 5.65. 8.71 %.

Results and discussions

Chemistry

2,2-dimethylchroman-4-one oxime (2) was synthesized according to reported method [41]. Reaction of the 2,2-dimethylchroman-4-one 2 with ethyl chloroacetate in presence of potassium carbonate gave the corresponding ethyl O-substituted acetyl ester 3. The reaction of the latter ester compound with hydrazine hydrate afforded the corresponding acid hydrazide derivative 4. The structure of compound 4 was confirmed by the presence of new signal corresponding to the new ethoxy fragment in the ¹H NMR spectrum, which replaced...
The condensation of hydrazide 4 with D-galactose, D-mannose or D-arabinose in an aqueous ethanolic solution in the presence of a catalytic amount of acetic acid provided the corresponding sugar hydrazones 5-7 respectively. The structure of these derivatives was confirmed by the appearance of new typical signals of the sugar chain protons at δ 3.36-5.65 ppm and the C-1 methine proton as doublet in the range δ 7.46-7.51 ppm for the alditolyl sugar protons. Traditionally, the reaction of sugar arylhydrazones with acetic anhydride provides the corresponding per-O-acetyl derivatives. Alternatively, when the same reaction was proceeded in boiling acetic anhydride, cyclization usually takes place in addition to per-O-acetylation to give acyclic C-nucleoside analogs [29,42,43]. Thus, when the hydrazones 5-7 were heated in acetic anhydride at 100°C they gave the 1,3,4-oxadiazoline acyclic nucleoside analogs 8-10, respectively. IR spectra of compounds 8-10 showed absorption bands at 1672-1679 cm⁻¹ and 1736-1739 cm⁻¹ due to stretching vibration of the hydroxyl group and the disappearance of the acetyl carbonyl bands. 1H NMR spectra showed signals attributable to the new (NHNH₂) for hydrazide compounds and 13C NMR spectra showed signals attributable to hydroxyl protons in addition to the rest of the acetylated carbonyl groups, respectively. Moreover, 1H NMR indicated the appearance of new singlet signals corresponding to the (OCOCH₃) protons and (NCOCH₃) protons and also signals corresponding to the rest of the alditolyl chain protons. The 13C NMR spectrum of 8 and 10 indicated the presence of (OCOCH₃) carbons at δ 20.32-26.14 ppm and also the presence of signals at δ 91.24 and 91.30 ppm for (C-N-COH₂-C-1 in the original sugar chain moiety and C-2 in the oxadiazoline ring) which indicated its N,N-acetal nature rather than being a C=N. The signals at δ 161.9-172.20 ppm correspond to the carbonyl groups (scheme 1).

Refluxing of the acid hydrazide 4 with carbon disulfide in pyridine at 90°C yielded the 5-mercapto-1,3,4-oxadiazole derivative 11 in 77% yield, which in turn was reacted with 2,3,4,6-tetra-O-acetyl-α-D-galacto-2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide 12-c in DMF in the presence of triethylamine to give the corresponding substituted thioglycoside derivatives 13a-c in good yields. The 1H NMR of compounds 13a-c confirmed the synthesis of these compounds. In which, the spectrum showed signals attributable to the new anomeric proton of the sugar moiety at δ 5.78-5.79 ppm, combined with a coupling constant equal to 10.4 Hz and 9.8 Hz providing the β-orientation of the thioglycosidic bond. It was reported that, the anomeric proton of β-N-glycosides having an adjacent C=S appear at higher chemical shift (δ 6.9-7.2 ppm) due to the anisotropic deshielding effect of the C=S in the 1H NMR spectrum [44-47]. In addition, the absence of a signal corresponding to the C=S in the 13C NMR spectrum confirmed that the attachment of the sugar moiety has been taken place at the sulfur atom rather than to the nitrogen atom which has also been supported by the low chemical shift of the anomeric proton. Deacetylation of compounds 13a-c resulted into free thioglycosides 14a-c which were confirmed by the spectral and analytical data. The IR spectrum showed absorption band due to stretching vibration of the hydroxyl group and the disappearance of the acetyl carbonyl bands. 13C NMR spectra showed signals attributable to the new proton at δ 3.95-5.84 ppm (scheme 2).

**Antimicrobial activity**

The antimicrobial activities of the novel-synthesized compounds were determined by the agar diffusion method [48]. In which the zone of inhibition for each derivative measured by ruler to determine its size (table 1) and compared with that produced by the standard drug (tetracycline). The compounds were evaluated for antibacterial activity against bacteria, Gram-positive bacteria; Bacillus subtilis and Gram-negative bacteria; Escherichia coli. Moreover, the evaluation of these compounds against fungi (Aspergillus flavus and Candida albicans) were done. The results indicated generally that showed moderate activity against Gram-positive and Gram-negative strains, while some compounds revealed high activity against fungi. Compounds 6, 7, 14b and 14c were the most active against Escherichia coli with inhibition zone values in the range of 31-34 mm. While 5-7, 10, 14b and 14c revealed the highest activity against Bacillus subtilis with inhibition zone values in the range of 30-35 mm. On the other hand Compounds 4 and 6 showed high activity against the fungus microorganism Aspergillus flavus while 7 and 14b were the most active among the series of tested compounds against Candida albicans.
The antimicrobial activity and structure activity relationship proved that compounds with acyclic sugar moieties attached to the substituted acetyl hydrazinyl group, for example compounds 5, 6, and 7 exerted important effects on antibacterial activities. Alternatively, the acetylated sugar hydrazone derivatives 8, 9, and 10 showed lower activity than the free hydroxyl analogs. Furthermore, the attachment of free hydroxyl glycosyl moieties for example compounds 14a, 14b, and 14c resulted in relatively improves activities against Bacillus subtilis and Escherichia coli.

Conclusions

In conclusion, a series of novel sugar hydrazones linked to chromene ring system and their derived oxadiazole acyclic nucleoside analogs were designed and synthesized. All the new compounds were established by IR, NMR spectra and elemental analysis. The antimicrobial activities of these novel compounds were evaluated against Gram-positive and Gram-negative bacteria and also against fungi. Glycosylation of the substituted 1,3,4-oxadiazole derivatives resulted in the formation of the corresponding thioglycosides. Moreover SAR proved that 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 activities.

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References
3. EL-AGRODY, A. M., EL-HAKIM, M. H., ABD LATIF, M. S., FAKERY, A. H., EL-SAYED, E. S. M., EL-GHAREAB, K. A., Acta Pharm. 50, 2000, p. 111.