Synthesis and Antimicrobial Activity of New Substituted 1,2,4-Triazoles and their Acyclic *C*-Nucleoside Analogues

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A number of new substituted 1,2,4-triazole {[(1,2,4-triazolyl)ethyl]tetrazolyl} derivatives, their sugar hydrazones, and their acyclic *C*-nucleoside analogues were synthesized and tested for their antimicrobial activity against *Bacillus subtilis* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), and *Streptomyces* species (Actinomycetes). The synthesized compounds displayed different degrees of antimicrobial activities or inhibitory actions.

Key words: 1,2,4-Triazoles, Sugar Hydrazones, Acyclic Nucleosides

Introduction

The synthesis and screening of compound libraries increased rapidly and became an important major objective in pharmaceutical chemistry. Among the five-membered nitrogen heterocycles, 1,2,4-triazoles are associated with a broad spectrum of biological activities. Many 1,2,4-triazoles have been reported to possess antibacterial, antifungal, antiviral, anti-inflammatory, anticonvulsant, antidepressant, antitubercular, antihypertensive, analgesic, hypoglycemic, herbicidal, and sedative properties (Palaska *et al.*, 2002; Amir and Shikha, 2004; Demirbas *et al.*, 2004; Colanceska-Ragenovic *et al.*, 2001; Labanauskas *et al.*, 2004; Al-Soud *et al.*, 2004, Jones *et al.*, 1965; Unangst *et al.*, 1992; Mullican *et al.*, 1993; Shams El-Dine and Hazzaa, 1974; Stillings *et al.*, 1986; Kane *et al.*, 1988; George *et al.*, 1971; Gall *et al.*, 1978). On the other hand, the acyclic *C*-nucleosides possess a wide range of biological properties, including antibiotic, antiviral, and antitumour activities (Holy, 1987; Remy and Secrist, 1985; Larson *et al.*, 1983; El Ashry and El Kilany, 1996, 1997, 1998; Chu and Cutler, 1986; Markar and Keseru, 1997; Franchetti *et al.*, 1997; Hammerschmidt *et al.*, 1997). The most unique feature of *C*-nucleosides is that the sugar chain is connected to the pendant heterocyclic base by a C–C bond instead of the C–N bond of the natural nucleoside. As a result, they are resistant to chemical and enzymatic hydrolytic cleavage. Our interest in the attachment of various carbohydrate residues to newly synthesized five-membered heterocycles (El-Sayed *et al.*, 2008; 2009a, b; Ali *et al.*, 2007) prompted us to study the modification of leading compounds synthesized for antimicrobial screening. Owing to these facts, our aim in the present work was the synthesis and antimicrobial evaluation of new 2,5-disubstituted 1,2,4-triazoles as well as the attachment of the synthesized derivatives to carbohydrate moieties to get the corresponding sugar hydrazones and acyclic *C*-nucleoside analogues.

Experimental

General

Melting points were determined using a Büchi apparatus. IR spectra (KBr) were recorded with a Bruker-Vector22 instrument (Bruker, Bremen, Germany). ^{1}H NMR spectra were recorded with a Varian Gemini spectrometer at 300 MHz and 200 MHz with TMS as internal standard. Chemical shifts are reported in δ scale (ppm) relative to TMS as a standard, and the coupling constants (J values) are given in Hz. The progress of the reactions was monitored by TLC using aluminum silica gel plates 60 F_{245}. EI-mass spectra were recorded with a HP D5988 A 1000 MHz instrument (Hewlett-Packard, Palo Alto, CA, USA).

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Sample preparation

Each of the test compounds and standards was 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 were supplied by Botany Department, Faculty of Science, Menoufia University, Shebin El-Koam, Egypt, namely *Bacillus subtilis* (ATCC 6633) (Gram-positive), *Pseudomonas aeruginosa* (ATCC 27853) (Gram-negative), and *Streptomyces* species (Actinomycetes). The bacterial strains were maintained on MHA (Mueller-Hinton agar, 17.5 g casein hydrolysate, 1.5 g soluble starch, 1000 mL beef extract) 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 a 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 a cork-borer, cups of about 10 mm diameter were produced (Jorgensen *et al.*, 1999).

Agar diffusion technique

The antibacterial activities of the synthesized compounds were tested against *Bacillus subtilis* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), and *Streptomyces* species (Actinomycetes) using MH medium. A stock solution of each synthesized compound (500 μg/mL) in DMSO was prepared, and graded quantities of the test compounds were incorporated in a specified quantity of sterilized liquid MH medium. Different concentrations of the test compounds in DMF were placed separately in cups in the agar medium. All plates were incubated at 37 ºC overnight. The inhibition zones were measured after 24 h. The minimum inhibitory concentration (MIC) was defined as the intercept of the graph of logarithmic concentrations versus diameters of the inhibition zones (Janssen *et al.*, 1987; Greenwood, 2000).

Results and Discussion

Chemistry

The starting compound 2[-(naphthalen-1-yloxy)-acetyl]-*N*-phenylhydrazinecarbothioamide (**1**) was synthesized from the corresponding acid hydrazide following a reported procedure (Abdel-Aal *et al.*, 2003). Heating **1** in 2 M NaOH solution at reflux temperature afforded the 1,2,4-triazole derivative **2** in 81% yield (Fig. 1). The ^{1}H NMR spectrum of **2** showed the CH_2 signal as a singlet at δ 5.21 ppm, the signals of the aromatic protons at δ 6.78–8.14 ppm in addition to the NH signal at δ 12.14 ppm.

Alkylation of **2** with methyl or ethyl iodide in alkaline medium afforded the corresponding *S*-methyl or *S*-ethyl derivatives **3a** and **3b** in 76% and 79% yields, respectively. Hydrazinolysis of **3a** and **3b** gave 3-hydrazinyl-5-[(naphthalen-1-yloxy)methyl]-4-phenyl-4*H*-1,2,4-triazole (**4**) in 80% yield. The ^{1}H NMR spectra of **3a** and **3b** showed the signals of the methyl group and the ethyl group as triplet and quartet which disappeared in the ^{1}H NMR spectrum of **4** in which the NH_2 signal appeared at δ 5.88 ppm.

Reaction of **2** with ethyl chloroacetate afforded the *S*-substituted ethyl ester derivative **5** in 75% yield. The ^{1}H NMR spectrum of **5** showed the signals of the ethyl group as a triplet at δ 1.18 ppm and a quartet at δ 4.19 ppm. The two singlet peaks for the remaining CH_2 groups appeared at δ 4.28 ppm and 5.29 ppm in addition to signals for the aromatic protons at δ 6.194–8.18 ppm.

Treatment of **5** with hydrazine hydrate gave the corresponding acid hydrazide **6** in 81% yield. Its structure was proved by means of IR, ^{1}H NMR and mass spectra which all agreed with the assigned structure.

When the hydrazide **6** was allowed to react with D-galactose or D-arabinose in an aqueous ethanolic solution with a catalytic amount of acetic acid, the corresponding sugar 2-{5-[(naphthalen-1-yloxy)methyl]-4-phenyl-4*H*-1,2,4-triazol-3-ylthio}acetohydrazones **7a** and **7b** were obtained in 72 and 78% yields. The structures of these compounds were confirmed by analytical and spectral data. The IR spectra of **7a** and **7b** showed the presence of characteristic absorption bands corresponding to the hydroxy groups in the region 3389–3466 cm^{-1}. The ^{1}H NMR spectra showed signals of the sugar chain protons at δ 3.37–5.44 ppm, the C-1 methine proton as a doublet in the range δ 7.08–7.58 ppm in addition to the aromatic protons in the region δ 6.98–8.18 ppm.

The reaction of the sugar arylhydrazones with boiling acetic anhydride is well known to give either the corresponding per-*O,N*-acetyl derivatives

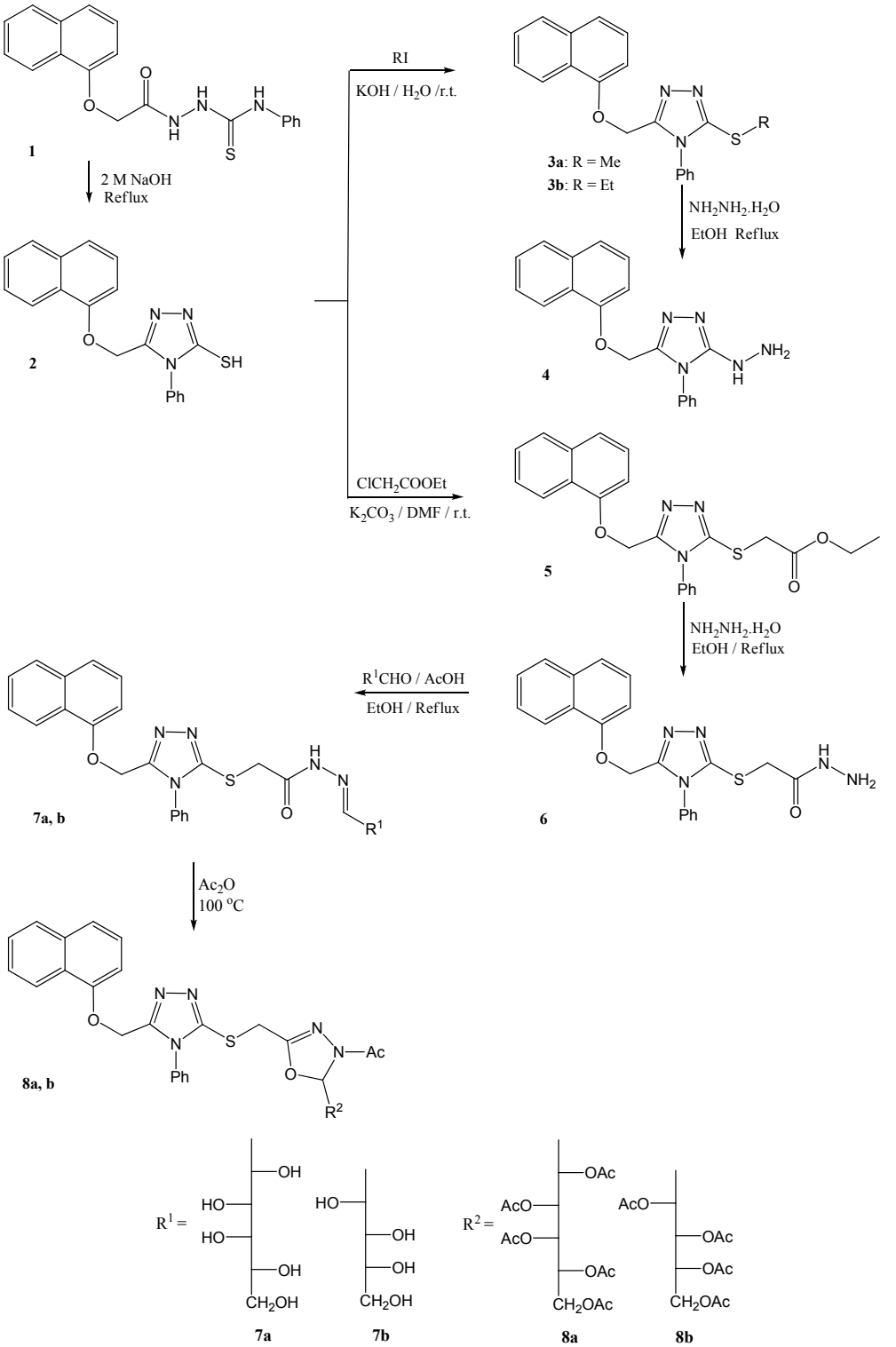

Fig. 1. Synthesis of 1,2,4-triazole and 1,3,4-oxadiazoline sugar derivatives.

or the respective per-*O,N*-acetyl-1,3,4-oxadiazolin derivatives (Abdel-Aal *et al.*, 2006, 2008; Somogyi, 1977, 1978). However, reaction of the sugar hydrazones **7a** and **7b** with acetic anhydride at 100 °C gave the sugar-substituted 1,3,4-oxadiazoline derivatives **8a** and **8b** in 62 and 69% yields. The IR spectra of **8a** and **8b** showed characteristic absorption bands at 1660–1670 cm^{-1} and 1742–1748 cm^{-1} corresponding to the carbonyl amide and the carbonyl ester groups, respectively, indicating the presence of an *N*-acetyl group in addition to the *O*-acetyl groups. The ^{1}H NMR spectra of **8a** and **8b** showed signals of the *O*-acetyl-methyl protons as singlets in the range δ 1.89–2.05 ppm and the *N*-acetyl-methyl protons in the range δ 2.20–2.24 ppm. The rest of the sugar chain protons appeared in the range δ 3.92–5.48 ppm in addition to multiplets of the aromatic protons in the region δ 7.12–8.18 ppm (Fig. 1).

On the other hand, the *N*-substituted 1,2,4-triazole derivative **9** was used as a key starting material for a number of tetrazole derivatives. Thus, reaction of **9** with sodium azide in DMF in the presence of ammonium chloride at 100 °C gave the tetrazole derivative **10**. Its ^{1}H NMR spectrum showed signals of two CH_2 groups each as a triplet at δ 4.45 and 4.72 ppm in addition to the NH signal at δ 12.41 ppm.

Reaction of **10** with ethyl chloroacetate afforded the *N*-substituted ethyl ester derivative **11** in 75% yield. The ^{1}H NMR spectrum of **11** showed signals of the ethyl group as a triplet at δ 1.21 ppm and a quartet at δ 4.22 ppm, two triplet signals for the remaining CH_2 groups at δ 4.48 and 4.78 ppm in addition to a singlet at δ 5.48 ppm for the *N*-CH_2CO group.

Treatment of the *N*-substituted ethyl ester **11** with hydrazine hydrate gave the corresponding acid hydrazide **12** in 80% yield. Its structure was proved by means of IR, ^{1}H NMR and mass spectra which all agreed with the assigned structure.

When the hydrazide **12** was allowed to react with D-galactose or D-arabinose the corresponding sugar (*E*)-2-{5-[2-(4*H*-1,2,4-triazol-4-yl)ethyl]-2*H*-tetrazol-2-yl}-*N*'-acetohydrazones **13a** and **13b** were obtained in 78 and 80% yields. The IR spectra of **13a** and **13b** showed absorption bands corresponding to the hydroxy groups in the region 3442–3469 cm^{-1}. The ^{1}H NMR spectra showed signals corresponding to the sugar chain protons in addition to those of aromatic protons.

Reaction of **13a** and **13b** with acetic anhydride at 100 °C gave the sugar-substituted 1,3,4-oxadiazoline derivatives **14a** and **14b** in 66 and 71% yields. The IR spectra of **14a** and **14b** showed absorption bands at 1658–1672 cm^{-1} and 1744–1748 cm^{-1} corresponding to the carbonyl amide and the carbonyl ester groups, respectively. The ^{1}H NMR spectra showed signals of the *O*-acetyl-methyl protons as singlets in the range δ 1.92–2.08 ppm and the *N*-acetyl-methyl protons in the range δ 2.18–2.25 ppm in addition to the other sugar protons at δ 3.89–5.45 ppm and the aromatic protons in the region δ 7.08–8.18 ppm (Fig. 2).

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 are presented in Table I. The results of the antimicrobial activity test revealed that **8b**, **10**, and

Table I. 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	100	500	125
3a	250	–[a]	250
3b	125	125	500
4	125	500	125
5	–	250	100
6	–	75	75
7a	125	125	250
7b	125	–	500
8a	100	125	–
8b	75	125	100
10	75	100	75
11	250	125	–
12	75	–	125
13a	125	500	250
13b	100	100	250
14a	250	–	125
14b	100	250	75
Penicillin	31	46	33

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

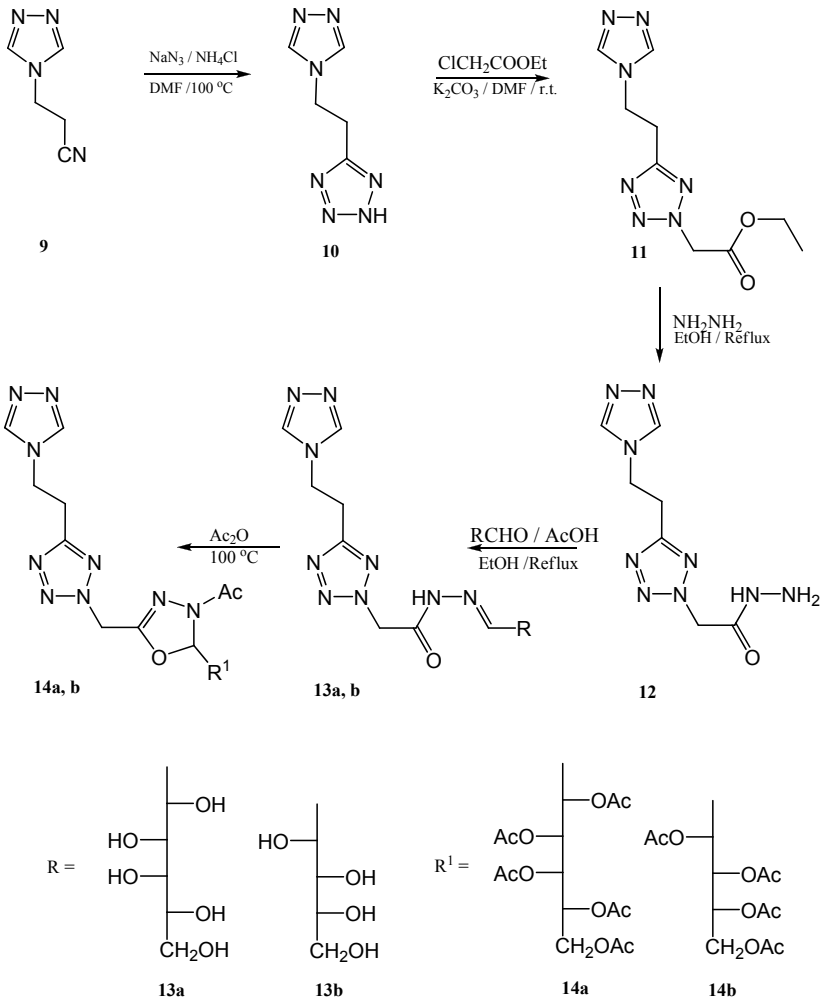

Fig. 2. Synthesis of {[(1,2,4-triazolyl)ethyl]tetrazolyl} sugar derivatives.

12 showed the highest activity against *B. subtilis* with MIC values of 75 μg/mL followed by compounds **2**, **8a**, **13b**, and **14b**. Compound **6** showed the highest inhibitory activity against *P. aeruginosa*, whereas **6**, **10**, and **14b** were the most active among the series of tested compounds against *Streptomyces* species with MIC values of 75 μg/mL. The results also revealed that some compounds showed little or no activity against the microorganisms (Table I).

The structure-activity relationship indicated that the acyclic nucleoside analogue **8b** in which the 1,2,4-triazole is substituted with an 1,3,4-oxadiazoline moiety attached to an acyclic sugar revealed high antimicrobial activity against *B. subtilis*, and **14b** exhibited the highest activity against *Streptomyces* species. In addition, acyclic nucleosides with the five-carbon-sugar arabinose showed higher activity than the corresponding galactose derivatives. Furthermore, 1,2,4-triazoles carrying a tetrazole moiety with a free NH group in the tetrazolyl ring revealed higher activity against *Bacillus subtilis* and *Streptomyces* species. Furthermore, the 1,2,4-triazole acid hydrazide derivatives exhibited higher activity than the corresponding hydrazine analogues or ester derivatives.

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