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Design, synthesis, and preliminary evaluation as antimicrobial activity of novel *spiro*-1, 3-thiazolidine C-acyclic nucleoside analogs

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Synthesis of a new class of 1, 3-thiazolidine nucleoside analogs is described. Reaction of 2-amino-2-deoxy-*D*-glucopyranose hydrochloride **2** with carbon disulfide yielded 5-hydroxy-4-(*D*-arabino-1, 2, 3, 4-tetrahydroxybutyl)-thiazolidin-2-thione **3,** which on acetylation yielded 5-acetoxy-4-(*D*-arabino-1, 2, 3, 4-tetraacetoxy-butyl)-thiazolidin-2-thione **4**. The acetylated sugar **4** reacted with hydrazonoyl chlorides **1a–f,** affording the 5-acetoxy-4-(*D*-arabino-1, 2, 3, 4-tetraacetoxybutyl)-*spiro*-[1,3]thiazolidine-2,2′-[1,3,4]thiadiazole derivatives **8a–f**. The antibacterial activity of the novel 1, 3-thiazolidine-2,2′-*spiro*-[1,3,4]thiadiazole nucleoside analogs is highlighted. All compounds with free NH group in the thiazolidine series **8a–f** showed significant biological activity against all the standard strains.

Keywords: cycloaddition reaction; 1-aza-2-azoniaallene; *spiro*-1, 3-thiazolidine C-acyclic nucleoside analogs; antimicrobial activity

1. Introduction

The chemical modification of nucleic acid fragments offers a continuous challenge for the organic chemists in search of compounds with anticancer (*1*) and antibacterial activity (*2*). Accordingly, considerable efforts have been made to develop new nucleoside analogs likely to exhibit improved activity or decreased toxicity with respect to troglitazone (*1*). In this context, the design of novel 1, 3-thiazolidine rings has resulted in the discovery of effective biologically active agents; in particular, promising results have been obtained from a new generation of nucleoside analogs where the chroman moiety has been replaced by the 5-acestoxy-4-(*D*-arabino-1, 2, 3, 4-tetraacetoxybutyl)-thiazolidin-2-thione (*1*).

1, 3-Thiazolidines are a new class of antimicrobial agents with activity against broad spectrum of Gram-positive pathogens, such as *Staphylococci*, *Streptococci*, and *Enterococci* (*3*). These compounds inhibit the protein synthesis in actively growing bacteria. A mechanism of action supports 1, 3-thiazolidine moiety being binded to the bacterial 50 S ribosomal subunits and

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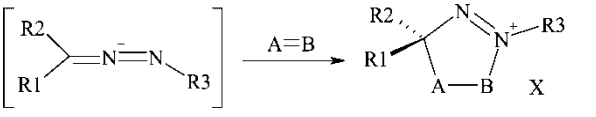
inhibition of formation of the 70 S ribosomal initiation complex (*4*), inhibiting the bacterial protein synthesis. The 1, 3-thiazolidine structure is relatively simple and allows for diverse synthetic modifications.

Much attention has been given to the synthesis of a new class of *spiro*-1, 3-thiazolidine nucleoside analogs using 1, 3-dipolar cycloaddition reaction procedure (*5*), largely because of their biological activities. Nitrilimines represent an important class of highly reactive 1, 3-dipoles used intensively for cycloaddition reactions with numerous unsaturated functional groups (*6–8*). El-Gazzar *et al.* (*9–11*) reported the reactions of nitrilimines with isothiocyanates to afford 1, 2, 4-thiadiazoles. On the other hand, regiospecific dipolar cycloaddition reactions of nitrilimine derivatives with thiobenzophenone and 2-thiooxoadamantanone have been reported (*12, 13*). The 1, 3, 4-thiadiazole ring containing structures exhibited biological activities, such as leishmanicidal (*14*), anticonvulsant (*15*), and antituberculosis (*16*). In the previous papers, we described the chemistry of nitrilimines toward isothiocyanates (*9–11*) and the compounds containing thioxo group (*5*). Here, we investigate the reaction of 5-acetoxy-4-(*D*-arabino-1, 2, 3, 4-tetraacetoxybutyl)-thiazolidin-2-thione with a variety of nitrilimines and evaluate the biological activity of the new products.

While 1, 3-dipolar cycloadditions of neutral 1, 3-dipoles are widely used in preparative organic chemistry (*17*), reports on cycloadditions of cationic four-electron-three-center components to multiple bonds are scarce. Interesting inorganic examples of such "1, 3-dipolar cycloadditions with reverse electron demand" have been reported for certain sulfur–nitrogen compounds (*18*). For instance, the ion $S{=}N^{+}{=}N$ behaves as a 1, 3-dipole undergoing cycloadditions to alkynes, alkene, and nitriles. In contrast, the economically important nitronium ion $O{=}N^{+}{=}O$ acts as a strong electrophile effecting, for example, aromatic nitration.

Recently, we reported preparations of azoniaallene salts as reactive intermediates, among others of 1-aza-2-azoniaallene salts **1**. This salt react as four-electron-three-center components in cycloaddition with many types of multiple bonds (Scheme 1) (*19*).



1
1-aza-2-azoniaallene salt

A=B carbodiimides, nitriles, isocyanates, alkene, alkynes

Scheme 1.

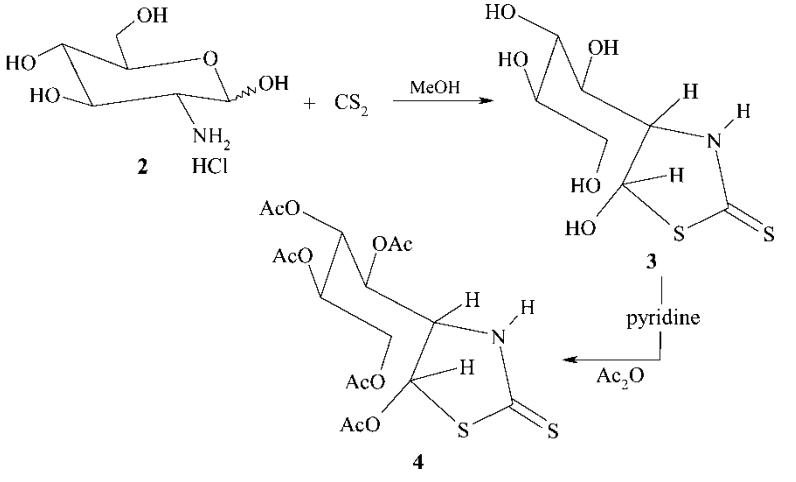
Cation **1** undergo cycloaddition to electron-rich alkenes with complete retention of configuration of the alkene (*20*). This led us to assume that the addition of cation **1** to alkenes and alkynes are concerted reactions, a view that is supported by semi-empirical AM1 calculations. However, cycloadditions of cations **1** to the triple bond of nitriles are most likely two-step processes with nitrilium ions as intermediates (*21*). Likewise, according to AM1 calculations, cycloadditions of heteroalllenes **1** to carbodiimides and isocyanates (*22*) should proceed in two steps.

Recently, we also reported the synthesis of 2, 5- and 2, 3-dihydro-2-(glucosylimino)-1, 3, 4-thiadiazoles formed by the reaction of a glucosyl isothiocyanate with certain salts **1** (*23*). Furthermore, 2, 3-dihydro-2-(iminoalkyl)-1, 3, 4-thiadiazolium salts were produced by cycloadditions of 1-aza-2-azoniaallene salt derived from camphor with isothio-cyanates (*24*). However, it soon turned out that reactions of heteroallenes **1** with isothiocyanates could lead to different products, depending on the substitution pattern of cation **1**. Here, we report the results of a more systematic investigation of reaction of cations **1** with thioxo group in 1, 3-thiazolidine.

2. Results and discussion

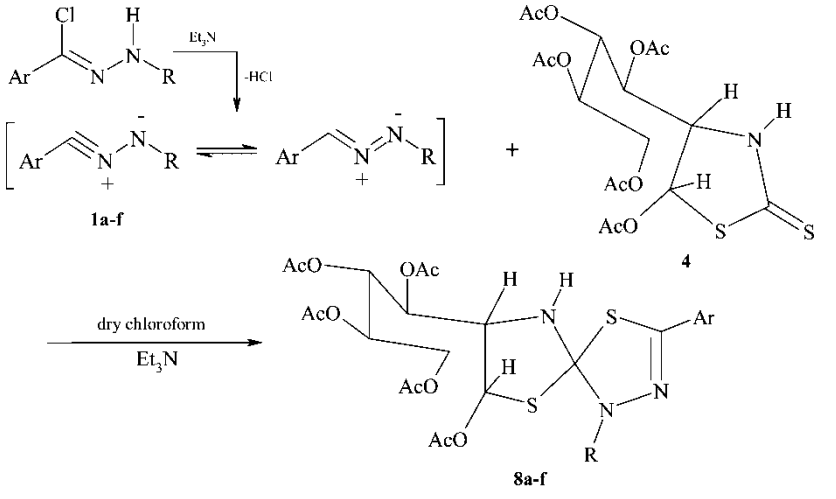
2.1. *Chemistry*

Our synthetic strategy was based on the reaction of carbondisulfide with glucosamine (**2**), which is the most abundant naturally occurring chemical element. 5-Hydroxy-4-(*D*-arabino-1, 2, 3, 4-tetrahydroxybutyl)-thiazolidin-2-thione **3** was obtained and acylated at room temperature in acetic anhydride-pyridine to afford the 5-acetoxy-4-(*D*-arabino-1, 2, 3, 4-tetraacetoxybutyl)-thiazolidin-2-thione **4** (Scheme 2). The structure of the product **4** was established by elemental analyses and spectral data (IR, ^{1}H and ^{13}C-NMR, MS). The IR spectrum of **4** displayed five carbonyl absorption bands around 1732–1751 and 3260 cm^{-1} for NH group. ^{1}H-NMR ($CDCl_3$) of **4** showed the corresponding signals for five acetyl protons at δ 1.61, 1.70, 1.81, 1.91, and 2.00. Moreover, the ^{13}C-NMR showed signals for five acetyl groups around 20.54–20.84.



Scheme 2.

In continuation of work (*9–11*) on heteroallenes, we report here an investigation into their 1, 3-dipolar cycloaddition reaction with 1, 3-thiazolidin-2-thione. Thus, stirring 5-acetoxy-4-(*D*-arabino-1, 2, 3, 4-tetraacetoxybutyl)-thiazolidin-2-thione **4** with *C*-phenyl-*N*-phenyl hydrazonyl chloride **1a** in refluxing dry chloroform containing triethylamine for 5 h afforded 5-acetoxy-4-(*D*-arabino-1, 2, −3, 4-tetraacetoxybutyl)-3′, 5′-diphenyl-*spiro*-[1, 3]thiazolidine-2, 2′-[1,3,4]thiadiazole **8a** as only one product, as indicated by Thin Layer Chromatography (TLC) (Scheme 3). The formula of the product **8a** was established by elemental analyses and spectral data (IR, ^{1}H and ^{13}C-NMR, MS). IR spectrum showed five absorption bands characterized of acetyl groups around 1730–1754 cm^{-1} and a broad band for NH group at 3225 cm^{-1}. Its ^{1}H NMR spectrum gave data with supports assigned structure and showed that the signals around δ 1.86–2.17 supported for five acetyl group, 4.03 (d, 1H, J = 6.5 Hz thiazolidine proton), 4.26 [dd, 2H (H-4′, H-4″), J = 9.9 Hz, J = 8.6 Hz, CH_2O-], 5.09 (br, 1H, H-1′), 5.31 (d, J = 8.7 Hz, H-3′), 5.46 (d, J = 7.6 Hz, H-2′), 6.34 (br, 1H, thiazolidine), 6.83–6.88 (m, 2H, phenyl), 7.23–7.48 (m, 5H, phenyl), 7.86–7.97 (m, 3H, phenyl), and 9.87 (brs, 1H, NH, D_2O exchangeable). Also, its ^{13}C-NMR revealed the absence of the signal characterized of the thione group and showed five signals characterized of 5CH_3 of acetyl groups at δ 20.38, 20.53, 20.66, 20.76, and 20.93 ppm; five signals characterized of C=O of acetyl groups at 169.5, 169.5, 169.6,



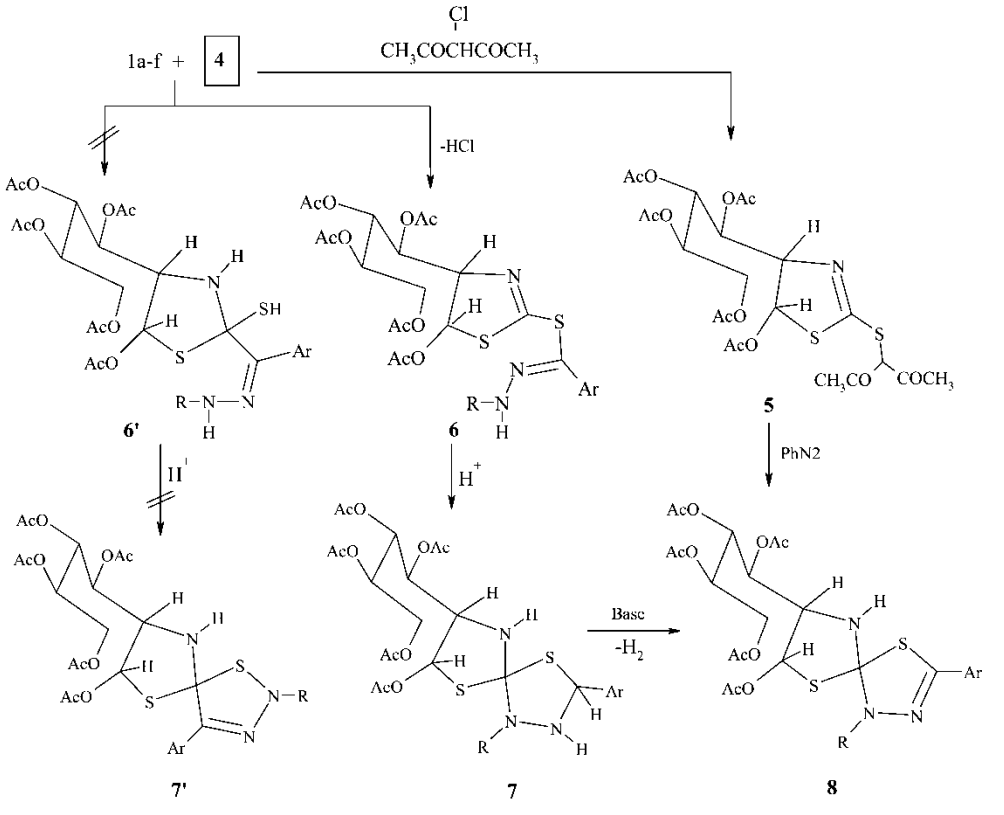
Scheme 3.

169.7, and 170.2 ppm; also the signals of the remaining carbons of the sugar moiety and thiazolidine; and the signal characterized of C-2 at 98.70 ppm, which are in agreement with that of Jochims *et al.* (*25*).

On the other hand, the reaction of compound **4** with 2-oxo-*N*-(4-sub-phenyl)-propane hydrazonoyl chloride **1b–e** under the same reaction condition afforded 5-acetoxy-4-(*D*-arabino-1, 2, 3, 4-tetraacetoxy-butyl)-3′-acetyl-5′-phenyl/(sub-phenyl)-*spiro*-[1, 3]-thiazolidine-2,-2′-[1,3,4]thiadiazole **8b–e**. The structures of **8b–e** were deduced on the basis of analytical and spectral data. Thus, the IR spectra for **8b–e** revealed the presence of CO group at 1750 cm^{-1}, and ^{13}C-NMR showed signals corresponding to *N*-acetyl groups around 25 ppm for methyl group and 185 ppm for carbonyl group. Also, the reaction of compound **4** with chloro-(4-tolylhydrazono)-ethylacetate **1f** afforded 5-acetoxy-4-(*D*-arabino-1, 2, 3, 4-tetraacetoxybutyl)-3′-ethylcarboxylate-5′-(4-tollyl)-*spiro*-[1, 3]thiazolidine-2,2′-[1,3,4]thiadiazole **8f**. The IR spectrum of **8f** revealed the presence of C=O (ester) at 1705 cm^{-1}. ^{1}H-NMR showed signals at 1.25–1.31 ppm (t, 3H, CH_3, J=6.1 Hz) and 4.28–4.31 ppm (q, 2H, CH_2, J = 6.9 Hz), which support the assigned structure. Also, the appearance of signal at δ 97.92 for (C-2) in the ^{13}C-NMR spectrum of **8f** supports the assigned structure. The mass spectra of **8f** showed the prominent ion peak at m/z 668 (M^+, 100) and m/z 577 (M^+-91, 88).

Our objective was to identify the possible intermediate(s) involved in the reaction of 1, 3-thiazolidin-2-thione with benzonitrilium *N*-phenylimide derivatives **1a–f**, which were recently reported from our laboratory (*11*). The required reactant **5** was prepared from **4** with 3-chloro-pentane-2, 4-dione in ethanolic potassium hydroxide solution, as outlined in Scheme 4. The latter compound **5** was treated with benzenediazonium chloride in ethanol in the presence of sodium acetate, which underwent a Japp–Klingemann reaction (*26*) and directly yielded the corresponding 5-acetoxy-4-(*D*-arabino-1, 2, 3, 4-tetraacetoxybutyl)-3′-acetyl-5′-phenyl-*spiro*-[1,3]thiazolidine-2,2′-[1,-3,4]thiadiazole (**8b**). Their structure was supported by elemental and spectral (^{1}H, ^{13}C-NMR, IR, and mass) analyses, which are identical to the data of the structure that is produced from the reaction of 1, 3-thiazolidin-2-thione **3** with benzonitrilium *N*-phenylimide **1b** (Scheme 4).

In the light of the foregoing results, the mechanism outlined in Scheme 4 seems to be the most plausible pathway for the formation of **8a–f** from the reaction of **4** with **1a–f**. The reaction involves an initial formation of the thiohydrazonate derivative **6** not **6′** (*27*), which undergoes



a; R = Ar = C_6H_5

b; R = $COCH_3$, Ar = C_6H_5

c; R = $COCH_3$, Ar = *p*-C_6H_4-CH_3

d; R = $COCH_3$, Ar = *p*-C_6H_4-Cl

e; R = $COCH_3$, Ar = *p*-C_6H_4-NO_2

f; R = $COOC_2H_5$, Ar = *p*-C_6H_4-CH_3

Scheme 4.

intramolecular cyclization once it is formed to yield the *spiro*-thiadiazole derivative **7**. The latter finally exhibits a base-catalyzed ring oxidation to afford the end products **8a–f**.

Table 1. Preliminary antimicrobial activity test for tested compounds (micro-organism inhibition zone in millimeter diameter).

Compound numbers	Gram-negative bacteria			Gram-positive bacteria	Fungi	
	E. coli	*Klebsiella pneumonia*	*Pseudomonas aerugniosa*	*Staphylococcus aureus*	*C. albicans*	*C. glabrata*
8a	19	18	23	18	–	–
8b	20	21	23	22	10	10
8c	22	21	22	22	19	14
8d	22	19	23	20	18	17
8e	22	23	23	22	–	–
8f	23	23	24	20	–	–
Nystatin	–	–	–	–	20	19
Sterptomycin	25	25	25	25	–	–

Inhibition zone = 6 – 10 mm slight activity, 11–15 mm moderate activity, more than 15 mm high activity.

Table 2. MIC of compounds **8a–f** (μg/mL).

Compound numbers	*E. coli*	*K. pneumonia*	*P. aerugniosa*	*S. aureus*	*C. albicans*
8a	30	30	30	30	–
8b	30	30	30	30	–
8c	20	20	20	20	50
8d	20	20	20	20	50
8e	30	30	30	30	–
8f	30	20	20	20	–

2.2. *Antimicrobial activity*

From the data obtained in (Table 1), it is clear that all the compounds under test were found to be active against Gram-negative and Gram-positive bacteria compared with the reference drug streptomycin. On the other hand, compounds **8d** and **8c** possess high activity toward the fungi as compared with the reference drug nystatin, whereas the rest of the compounds showed no activity toward the fungi. The minimum inhibitory concentration (MIC) of the most active compounds toward the micro-organisms ranged between 20–30 µg/disk (Table 2).

3. Experimental

3.1. *Synthesis*

All melting points were uncorrected and measured using an Electro-thermal IA 9100 apparatus (Shimadzu, Japan). Microanalytical data were performed by Vario, Elementar apparatus (Shimadzu) (Table 3). IR spectra (KBr) were recorded on a Perkin-Elmer 1650 spectrometer (USA). ^{1}H-NMR spectra were determined on a JEOL EX-270 run for HNMR at 270 MHz and for CNMR at 67.5 MHz, or on a JEOL ECA-500 run for HNMR at 500 MHz and for CNMR at 125 MHz, and chemical shifts were expressed in parts per minute relative to $SiMe_4$ as internal standards. Mass spectra were recorded on 70 eV EI Ms-QP 1000 EX (Shimadzu, Japan). The starting materials, 5-hydroxy-4-(*D*-arabino-1, 2, 3, 4-tetrahydroxybutyl)-thiazolidin-2-thione **3**

Table 3. Physical and chemical properties of synthesized compounds.

Compound numbers	Yield (%)	M.p. (°C)	Molecular formula (M_r)	Found/calcd. (%) C	H	N
3	82	144–146	$C_7H_{13}NO_5S_2$ (255.3)	32.93	5.13	5.49
				32.87	5.10	5.45
4	76	214–216	$C_{17}H_{23}NO_{10}S_2$ (465.5)	43.86	4.98	3.01
				43.79	5.01	3.03
8a	64	243–245	$C_{30}H_{33}N_3O_{10}S_2$ (660.2)	54.65	5.05	6.36
				54.63	5.02	6.38
8b	71	227–229	$C_{26}H_{31}N_3O_{11}S_2$ (625.6)	49.91	4.99	6.71
				50.01	4.95	6.74
8c	67	235–237	$C_{27}H_{33}N_3O_{11}S_2$ (639.6)	50.69	5.20	6.57
				50.72	5.18	6.54
8d	69	207–210	$C_{26}H_{30}ClN_3O_{11}S_2$ (660.1)	47.30	4.58	6.36
				47.26	4.56	6.40
8e	73	230–233	$C_{26}H_{30}N_4O_{13}S_2$ (670.6)	46.56	4.51	8.35
				46.52	4.48	8.38
8f	71	202–205	$C_{28}H_{34}N_3O_{12}S_2$ (668.7)	50.29	5.12	6.28
				50.31	5.10	6.31

and 5-acetoxy-4-(*D*-arabino-1, 2, 3, 4-tetraacetoxybutyl)-thiazolidin-2-thione **4,** were prepared according to Jochims *et al.* (*25*). The hydrazonoyl chloride **1a–f** was prepared as the same in the literature (*28, 29*). The biological evaluations of the products were carried out at the Fermentation Biotechnology and Applied Microbiology (Ferm-BAM) Center at Al-Azhar University, Cairo, Egypt.

3.1.1. *Synthesis of 5-hydroxy-4-(D-arabino-1,2,3,4-tetrahydroxybutyl)-thiazolidin-2-thione (**3**)*

A mixture of compound **1** (0.01 mol) and carbon disulphide (excess 10 mL) was heated under reflux in methanolic potassium hydroxide solution (12%) for 14 h. The reaction mixture was allowed to cool to 0°C for 3 h, the precipitate was filtered off, washed by water (100 mL), dried and crystallized from absolute ethanol; IR (KBr) cm^{-1}: 3500 (brs, OH), 3240 (brs, NH), 2943 (CH alkyl);^{1}H-NMR ($CDCl_3$) ppm: δ 3.31 (m, 5OH, D_2O exchangeable), 3.76–3.86 (3d, 3H, $J = 9.6, 3.8, 3.7$ Hz), 4.70–4.73 (d, 1H, $J = 8.2$ Hz), 4.97–5.02 (2d, 2H, $J = 6.2, 5.5$ Hz), 5.91 (br, 1H), and 8.35 (brs, 1H, NH, D_2O exchangeable); MS: m/z (%); 255 (M^+, 100).

3.1.2. *Synthesis of 5-acetoxy-4-(D-arabino-1,2,3,4-tetraacetoxybutyl)-thiazolidin- 2-thione (**4**)*

A mixture of compound **2** (0.01 mol) and acetic anhydride (excess 10 mL) was stirred in 20 mL pyridine at room temperature for 8 h (under TLC control). The reaction mixture was allowed to cool to 0°C for 24 h, the precipitate was filtered off, washed by 10% water/HCl (100 mL), dried, and crystallized from absolute ethanol; IR (KBr) cm^{-1}: 3260 (brs, NH), 2923 (CH alkyl), 1751–1732 (5 C=O, acetyl);^{1}H-NMR (DMSO-d_6)ppm: δ 1.61, 1.70, 1.81, 1.91. 2.00 (5s, 5 $COCH_3$), 3.77–3.83 (t, H-4′, H-4″ of $J = 7.1, 7.6$ Hz, CH_2O-), 3.86–3.90 (m, H-1′, CH), 4.71–4.74 (dd, H-3′, CH), 4.96–4.98 (dd, H-2′, CH), 5.15 (d, 1H, thiazolidine, $J = 6.1$ Hz), 5.93 (d, 1H, thiazolidine, $J = 5.6$ Hz), and 8.42 for NH, D_2O exchangeable; ^{13}C-NMR ($CDCl_3$) ppm: δ 20. 54, 20.60, 20.68, 20.70, 20.84 (5 CH_3), 61.72 (CH_2), 68.02, 68.10, 68.13 (3CH), 68.89, 81.43 (2CH-thiazolidine), 170.2, 169.9, 169.7, 169.6, 169.5 (5 C=O), 196.4 (C=S); MS: m/z (%); 465 (M^+, 100).

3.1.3. *Synthesis of 5-acetoxy-4-(D-arabino-1,2,3,4-tetraacetoxybutyl)-spiro-[1,3]-thiazolidine-2,2′ -[1,3,4]thiadiazole derivatives (**8a–f**) – general procedure*

A mixture from compound **3** (0.01 mol) and the appropriate hydrazonoyl chlorides **4a–f** (0.01 mol) was stirred under reflux in dry chloroform (30 mL) and four drops of triethylamine for 5 h. The solvent was evaporated under reduced pressure. The solid produced was washed three times with 30 mL absolute ethanol and crystallized from an appropriate solvent to produce **8a–f** in high yields.

3.1.4. *Synthesis of 5-acetoxy-4-(D-arabino-1,2,3,4-tetraacetoxybutyl)-3′,5′-diphenyl-spiro-[1,3]thiazolidine-2,2′-[1,3,4]thiadiazole (**8a**)*

The compound was obtained from **4** (0.01 mol) and *N*-phenylbenzene-carbo-hydrazonoyl chloride **1a** (0.01 mol), as white powder and crystallized from dimethyl-formamide; IR (KBr) cm^{-1}: 3265 (brs, NH), 2931 (CH alkyl), 1754–1730 (5 C=O, acetyl);^{1}H-NMR (DMSO-d_6) ppm: δ 1.86, 1.96, 2.03, 2.11. 2.17 (5s, 5 $COCH_3$), 4.00–4.06 (d, 1H, $J = 6.1$ Hz, thiazolidine), 4.20–4.32 (dd, 2H, $J = 9.9$ Hz, $J = 8.6$ Hz), 5.09 (br, 1H), 5.29–5.32 (d, 1H, $J = 8.7$ Hz), 5.45–5.48 (d, 1H, $J = 7.6$ Hz), 6.34 (br, 1H, thiazolidine), 6.83–6.88 (m, 2H, phenyl), 7.23–7.48 (m, 5H, phenyl),

7.86–7.97 (m, 3H, phenyl), and 9.87 (brs, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) ppm: δ 20. 38, 20.53, 20.66, 20.76, 20.93 (5 CH_3), 61.72 (CH_2), 67.54, 67.89, 68.11 (3CH), 68.79, 81.38 (2CH-thiazolidine), 98.70 (C-2), 120.1, 120.5, 122.3, 126.0, 128.8, 129.2, 134.2, 144.2 (10C-SP^2 with four asymmetric carbons), 169.5, 169.5, 169.6, 169.7, 170.2 (5 C=O); MS: m/z (%); 660 (M^+, 100), 583 (M^+-77, 56).

3.1.5. *Synthesis of 5-acetoxy-4-(D-arabino-1,2,3,4-tetraacetoxybutyl)-3′-acetyl-5′-phenyl-spiro-[1,3]thiazolidine-2,2′-[1,3,4]thiadiazole (**8b**)*

The compound was obtained from **4** (0.01 mol) and 2-oxo-*N*-phenyl-propane hydrazonoyl chloride **1b** (0.01 mol) as yellow powder and crystallized from dimethylformamide; IR (KBr) cm^{-1}: 3255 (brs, NH), 2919 (CH alkyl), 1754–1733 (6 C=O, acetyl);^{1}H-NMR (DMSO-d_6) ppm: δ 1.96, 1.98, 2.00, 2.08, 2.13, 2.20 (6s, 6 $COCH_3$), 4.06–4.23 (d, 1H, $J = 7.6$ Hz thiazolidine), 4.32–4.36 (dd, 2H, CH_2O-), 5.31 (br, 1H), 5.32–5.33 (d, 1H, $J = 8.1$ Hz), 5.48–5.50 (d, 1H, $J = 7.4$ Hz), 6.39 (s, 1H, thiazolidine), 7.40–7.46 (m, 5H, phenyl), and 10.77 (brs, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) ppm: δ 20.30, 20.34, 20.40, 20.48, 20.61, 25.34 (6 CH_3), 61.48 (CH_2), 61.75, 67.72, 67.95 (3CH), 68.03, 81.32 (2CH-thiazolidine), 97.84 (C-2), 123.5, 126.4, 128.7, 128.9, 129.1, 141.5 (6C-SP^2, phenyl), 169.5, 169.6, 169.7, 169.9, 170.1, 188.2 (6 C=O acetyl); MS: m/z (%); 625 (M^+, 100), 582 (M^+-43, 64), 548 (M^+-77, 52).

3.1.6. *Synthesis of 5-acetoxy-4-(D-arabino-1,2,3,4-tetraacetoxybutyl)-3′-acetyl-5′-(4-tollyl)-spiro-[1,3]thiazolidine-2,2′-[1,3,4]thiadiazole (**8c**)*

The compound was obtained from **4** (0.01 mol) and 2-oxo-*N*-(4-tollyl)-propane hydrazonoyl chloride **1c** (0.01 mol), as white powder and crystallized from absolute ethanol; IR (KBr) cm^{-1}: 3250 (brs, NH), 2926 (CH alkyl), 1756–1730 (6 C=O, acetyl);^{1}H-NMR (DMSO-d_6) ppm: δ 1.30, 1.98, 1.99, 2.07, 2.11, 2.13, 2.14 (7s, CH_3, 6 $COCH_3$), 4.27–4.29 (d, 1H, thiazolidine), 4.30–4.33 (dd, 2H, $J = 8.0$, 8.2 Hz, CH_2O-), 5.10–5.11 (br, 1H), 5.32–5.37 (d, 1H, $J = 8.2$ Hz), 5.39–5.48 (d, 1H, $J = 7.3$ Hz), 6.38–6.41 (s, 1H, thiazolidine), 7.14–7.20 (d, 2H, phenyl, $J = 8.3$ Hz), 7.22–7.26 (d, 2H, phenyl, $J = 8.4$ Hz), and 10.46 (brs, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) ppm: δ 14.13, 20.34, 20.35, 20.43, 20.49, 20.61, 20.78 (7 CH_3), 61.65 (CH_2), 62.09, 64.12, 67.95 (3CH), 68.05, 81.35 (2CH-thiazolidine), 98.05 (C-2), 114.6, 129.7, 131.4, 140.3 (6C-SP^2 with two asymmetric carbons), 169.3, 169.4, 169.5, 169.7, 170.3, 184.5 (6 C=O acetyl); MS: m/z (%); 639 (M^+, 100), 548 (M^+-91, 43).

3.1.7. *Synthesis of 5-acetoxy-4-(D-arabino-1,2,3,4-tetraacetoxybutyl)-3′-acetyl-5′-(4-chlorophenyl)-spiro-[1,3]thiazolidine-2,2′-[1,3,4]thiadiazole* (***8d***)

The compound was obtained from **4** (0.01 mol) and 2-oxo-*N*-(4-chloro-phenyl)-propane hydrazonoyl chloride **1d** (0.01 mol) as yellow powder and crystallized from absolute ethanol; IR (KBr) cm^{-1}: 3240 (brs, NH), 2921 (CH alkyl), 1754–1736 (6 C=O, acetyl);^{1}H-NMR (DMSO-d_6) ppm: δ 1.99, 2.00, 2.08, 2.10, 2.14, 2.19 (6s, 6 $COCH_3$), 3.92–4.19 (d, 1H, $J = 8.9$ Hz, thiazolidine), 4.24 – 4.4.33 (dd, 2H, CH_2O-), 4.34 – 4.48 (d, 1H, $J = 7.8$ Hz), 5.26–5.30 (d, 1H, $J = 8.4$ Hz), 5.31–5.36 (d, 1H, $J = 7.2$ Hz), 6.40 (br, 1H, thiazolidine), 7.36–7.38 (d, 2H, phenyl, $J = 8.2$ Hz), 7.45–7.47 (d, 2H, phenyl, $J = 8.3$ Hz), and 10.78 (brs, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) ppm: δ 20.40, 20.42, 20.44, 20.50, 20.64, 25.34 (6 CH_3), 61.70 (CH_2), 62.15, 64.23, 67.50 (3CH), 68.00, 81.31 (2CH-thiazolidine), 96.90 (C-2), 122.8, 122.9,

129.3, 142.6 (6C-SP^2 with two asymmetric carbons), 169.2, 169.4, 169.6, 169.8, 170.2, 183.7 (6 C=O acetyl); MS: m/z (%); 660 (M^+, 100), 661 (M^++1, 38), 617 (M^+−43, 53).

3.1.8. *Synthesis of 5-acetoxy-4-(D-arabino-1,2,3,4-tetraacetoxybutyl)-3′-acetyl-5′-(4-nitro-phenyl)-spiro-[1,3]thiazolidine-2,2′-[1,3,4]thiadiazole (**8e**)*

The compound was obtained from **4** (0.01 mol) and 2-oxo-*N*-(4-nitrophenyl)-propane hydrazonoyl chloride **1e** (0.01 mol) as yellow powder and crystallized from absolute ethanol; IR (KBr) cm^{-1}: 3245 (brs, NH), 2918 (CH alkyl), 1756–1728 (6 C=O, acetyl); ^{1}H-NMR (DMSO-d_6) ppm: δ 1.97, 1.99, 2.04, 2.09, 2.15, 2.24 (6s, 6 $COCH_3$), 3.97–4.04 (d, 1H, J = 6.0 Hz, thiazolidine), 4.19–4.24 (dd, 2H, J = 13.9 Hz, CH_2O-), 5.08 (br, 1H), 5.26–5.30 (d, 1H, J = 8.5 Hz), 5.44–5.47 (d, 1H, J = 7.3 Hz), 6.38 (br, 1H, thiazolidine), 7.12–7.15 (d, 2H, phenyl, J = 8.3 Hz), 7.23–7.28 (d, 2H, phenyl, J = 8.2 Hz), and 10.50 (brs, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) ppm: δ 20.33, 20.36, 20.40, 20.49, 20.60, 20.77 (6 CH_3), 61.56 (CH_2), 62.17, 64.22, 67.54 (3CH), 68.04, 81.34 (2CH-thiazolidine), 99.00 (C-2), 114.7, 125.7, 126.4, 141.3 (6C-SP^2 with two asymmetric carbons), 169.4, 169.5, 169.6, 169.7, 170.0, 182.9 (6 C=O acetyl); MS: m/z (%); 670 (M^+, 33), 548 (M^+−122, 42).

3.1.9. *Synthesis of 5-acetoxy-4-(D-arabino-1,2,3,4-tetraacetoxybutyl)-3′-ethylcarboxylate-5′-(4-tollyl)-spiro-[1,3]thiazolidine-2,2′-[1,3,4]thiadiazole (**8f**)*

The compound was obtained from **4** (0.01 mol) and chloro-(4-tolyl-hydrazono)-ethylacetate **1f** (0.01 mol) as yellow powder and crystallized from absolute ethanol/dioxane; IR (KBr) cm^{-1}: 3235 (brs, NH), 2919 (CH alkyl), 1752–1730 (5 C=O acetyl), 1705 (C=O ester);^{1}H-NMR (DMSO-d_6) ppm: δ 1.25–1.31 (t, 3H, CH_3), 1.62, 1.71, 1.80, 1.90.2.00 (5s, 5 $COCH_3$), 2.22 (s, 3H, CH_3), 3.99–4.06 (q, 2H, CH_2), 4.24–4.30 (t, H-4′, H-4″ with J = 7.0, 7.5 Hz, CH_2O-), 5.08–5.27 (m, H-1′, CH), 5.29–5.32 (dd, H-3′, CH), 5.44–5.47 (dd, H-2′, CH), 4.26 (m, 1H, thiazolidine), 6.35 (s, 1H, thiazolidine), 7.10–7.13 (d, 2H, phenyl, J = 8.2 Hz), 7.22–7.25 (d, 2H, phenyl, J = 8.3 Hz), and 10.45 (brs, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) ppm: δ 14.23, 20.33, 20.41, 20.55, 20.69, 20.86 (6 CH_3), 61.68, 62.12 ($2CH_2$), 62.19, 64.20, 67.83 (3CH), 68.06, 81.33 (2CH-thiazolidine), 97.92 (C-2), 112.9, 129.7, 131.4, 140.4 (6C-SP^2 with two asymmetric carbons), 159.4, 169.4, 169.5, 169.6, 169.7, 170.1 (6 C=O); MS: m/z (%); 668 (M^+, 100), 577 (M^+− 91, 88).

3.2. *Antimicrobial test*

The bacterial isolates representing Gram-negative and Gram-positive bacteria were recovered on nutrient and macconky agar. The two fungal isolates, *Candida albicans* and *C. glabrate*, were isolated on Sabourauds dextrose agar (Oxoid). They are isolated from clinical samples and identified to the species level according to different API systems (Biomerilux). The selected compounds were tested *in vitro* using the agar disc diffusion method (*30, 31*) using sterptomycin and nystatin as reference drugs for bacteria and fungi, respectively. The antimicrobial potentialities of the tested compounds were estimated by placing presterilized filter paper disks (5 mm in diameter) impregnated with 50 μg/disk using dimethylsulfoxide (DMSO) as a solvent, which showed no inhibition zones. The inhibition zones of the tested compounds were measured after 24–28 h incubation at 37°C for bacteria and at 28°C after 5 days for fungi (Table 1).

The MIC determined method of the biologically active compounds (Table 2) was applied using different concentrations per disk against Gram-negative, Gram-positive, and fungi. Reference

nystatin and sterptomycin disks were supplied from Pasteur laboratory in Egypt at a concentration of 100 units and 30 μg, respectively. The activity of the selected compounds is tested at concentration of 50 μg/disk.

References

(1) Chen, H.; Fan, Y-H.; Natarajan, A.; Guo, Y.; Iyasere, J.; Harbinski, F.; Luus, L.; Christ, W.; Aktas, H.; Halperin, J.A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5401–5405.
(2) Mitsuya, H.; Broader, S. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 1911–1915.
(3) Nasr, M.N.; Gineinah, M.M.; El-Bendary, E.R. *Arch. Pharm.* **2003**, *336*, 560–566.
(4) Belleau, B.; Brasilli, L.; Chan, L.; Zacheri, B. *Cameron J. Bioorg. Med. Chem. Lett*. **1993**, *3*, 1723–1928.
(5) Padwa, A. In *Comprehensive Organic Chemistry;* Trost, B.M., Flemin, I., Eds., Pergmon: Oxford, 1991; Vol. 4, p 1069.
(6) Hegab, M.I.; El-Gazzar, A.B.A.; Gad, F.A.; Ellithey, M. *J. Sulf. Chem.* **2007**, *28*, 101–108.
(7) Molteni, G.; Ponti, A. *Tetrahedron* **2003**, *59*, 5225–5229.
(8) Molteni, G.; Proggini, G.; Pilati, T. *Tetrahedron: Asymmetry* **2002**, *13*, 2499–2495.
(9) El-Gazzar, A.B.A.; Scholten, K.; Guo, Y.; Weibenbach, K.; Hitzler, M.G.; Roth, G.; Fischer, H.; Jochims, J.C. *J. Chem. Soc. Perkin Trans.* **1999**, *1*, 1999–2010.
(10) El-Gazzar, A.B.A.; Hegab, M.I.; Hassan, N.A. *Sulf. Lett*. **2002**, *25* (2), 45–62.
(11) El-Gazzar, A.B.A.; Hegab, M.I.; Hassan, N.A. *Sulf. Lett.***2002**, *25* (4), 161–171.
(12) Dunstan, J.B.F.; Elsey, G.M.; Russel, R.A.; Savage, G.P.; Simpson, G.W.; Tiekink, E.R.T. *Aus. J. Chem*. **1998**, *51*, 499–509.
(13) Katada, T.; Eguchi, S.; Sasaki, T. *J. Chem. Soc., Perkin Trans.* **1984**, *1*, 2649–2653.
(14) Foroumadi, A.; Pournourmohammadi, S.; Soltani, F.; Asgharian-Rezaee, M.; Dabiri, S.; Kharazmi, A.; Shafiee, A. *Bioorg. Med. Chem. Lett*. **2005**, *15*, 1983–1985.
(15) Dogan, N.H.; Duran, A.; Rollas, S.; Sener, G.; Uysal, M.K.; Gulen, D. *Bioorg. Med. Chem.* **2002**, *10*, 2893–2898.
(16) Foroumadi, A.; Kiani, Z.; Soltani, F. *Farmaco* **2003**, *58*, 1073–1076.
(17) Padwa, A., 1,3 Dipolar Cycloadditions: Volume 1, 2: John Wiley: New York, 1984.
(18) Parsons, S.; Passmore, J.; Schriver, M.J.; Sun, X. *Inorg. Chem.* **1991**, *30*, 3342–3348.
(19) Liblong, S.W.; Oakley, R.T.; Cordes, A.W.; Noble, M.C. *Can. J. Chem.* **1983**, *61*, 2062–2067.
(20) Wirschun, W.; Winkler, M.; Lutz, K.; Jochims, J.C. *J. Chem. Soc., Perkin Trans.* **1998**, *1*, 1755–1762.
(21) Wang, Q.; Amer, A.; Mohr, S.; Ertel, E.; Jochims, J.C. *Tetrahedron* **1993**, *49*, 9973–9986.
(22) Wang, Q.; Mohr, S.; Jochims, J.C. *Chem. Ber*. **1994**, *127*, 947–953.
(23) Al-Masoudi, N.; Hassan, N.A.; Al-Soud, Y.A.; Schmidt, P.; Gaafar, A.M.; Wang, M.; Marino, S.; Amer, A.; Jochims, J.C. *J. Chem. Soc., Perkin Trans.* **1998**, *1*, 947–954.
(24) Hassan, N.A.; Mohamed, T.K.; Abdel-Hafez, O.M.; Lutz, M.; Karl, C.C.; Wirschun, W.; Al-Soud, Y.A.; Jochims, J.C. *J. Pract. Chem.* **1998**, *340*, 151–159.
(25) Jochims, J.C.; Seeliger, A.; Taigel, G. *Chem. Ber*. **1967**, *100*, 845–854.
(26) Philips, R.R. *Org. React. (N.Y.)* **1969**, *10*, 143.
(27) Hassneen, H.M.; Abdallah, T.A. *Molecules* **2003**, *8*, 333–341.
(28) Wolkoff, P.; Nemeth, S.T.; Gibson, M.S. *Can. J. Chem.*, **1975**, *53*, 3211–3215.
(29) Hegarty, A.F.; Cashoman, M.; Aylward, J.B.; Scott, F.L. *J. Chem. Soc. B*. **1971**, *57*, 1879–1883.
(30) Weinstein, M.J.; Wagman, G.H. ANTIBIOTICS. Amsterdam, Oxford, New York: Elsevier Scientific Publishing Company; 1978, Vol. 3, pp 231–272.
(31) Barry, A.L.; Thornsbery, C. in Susceptibility testing; diffusion tests procedures. In Manual of Clinical Microbiology, Lennette, J.P. Ed. American Society for microbiology. Washington, D.C., p 561, 1981.