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Synthesis and antimicrobial activity of some new N-glycosides of 2-thioxo-4-thiazolidinone derivatives

Nadia Hanafy Metwally *, Magda Ahmed Abdalla, Mosselhi Abdel Nabi Mosselhi, Ebrahim Adel El-Desoky

Department of Chemistry, Faculty of Science, Cairo University, Giza, Egypt

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ABSTRACT

5-Arylidene-2-thioxo-4-thiazolidinones **3a**–**f** react with each of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl and α-D-galactopyranosyl bromides **4a**,**b** in acetone in the presence of aqueous potassium hydroxide at room temperature to afford *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) or *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) 2-thioxo-4-thiazolidinone derivatives **5a**–**f**. Similarly, the reaction of 5-cycloalkylidene-2-thioxo-4-thiazolidinones **7a**,**b** with **4a** gave the corresponding N-glucosides **8a**,**b**. Also, 5-pyrazolidene rhodanines **10a**–**e** react with **4a** to afford the new N-glucosides **11a**–**e**. Treatment of compounds **15** and **16** with **4a** in the presence of few drops of triethylamine or in KOH solution accomplished the mono- and bis-nucleosides **17** and **18**, respectively. Some selected products were tested for their antimicrobial activities.

1. Introduction

2-Thioxo-4-thiazolidinone (rhodanine) and its derivatives have broad spectrum of biological activities as antibacterial,[1–3] antifungal,[4] antidiabetic,[5] antitubercular,[6,7] anti-HIV,[8,9] antiparasitic,[10] hypnotic,[11] and anthelmintic agents.[12] Rhodanines also act as analogs of purine bases in nucleic acid synthesis.[13] During the last decade thiazole nucleoside analogs have been recognized as potent and increasingly important antimetabolite agents.[14–16]

In continuation of our previous work[17–23] in the development of an efficient procedure for the synthesis of some new 4-thiazolidinone derivatives, we report herein some new *N*-glucopyranosyl rhodanine derivatives to search for more effective biologically active agents. 2-Thioxo-4-thiazolidinone (**1**) seems to be a good candidate to fulfill our objective via its condensation with different aldehydes and ketones to afford the new 5-aralkylidene rhodanines, which are the key intermediates for the preparation of *N*-glucopyranosyl rhodanine derivatives.

2. Results and discussion

Compound **1** reacts with aromatic aldehydes **2a**–**f** in glacial acetic acid at reflux in the presence of anhydrous sodium acetate to give 5-arylidene-2-thioxo-4-thiazolidinones **3a**–**f**. Compounds **3** can then be treated with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl and α-D-galactopyranosyl bromides **4a**,**b** in acetone in the presence of aqueous potassium hydroxide at room temperature to afford one isolable product, **5** (Scheme 1). The structures of the products **5** were established and confirmed on the basis of their elemental analyses and spectral data (MS, IR, and ^{1}H NMR). For example, considering the IR spectrum of product **5b** as a typical example, the product was characterized by the presence of acetoxy carbonyl groups at ν = 1747 cm^{-1}. ^{1}H NMR spectroscopy was also used to confirm the product. Thus, the ^{1}H NMR spectrum of **5b** showed the anomeric proton as a doublet at δ = 6.34 ppm with a $J_{1',2'}$ = 9.3 Hz, which corresponds to the diaxial orientation of the H-1′ and H-2′ protons indicating the β-configuration. The other six protons of the glucopyranosyl ring resonate between δ = 3.89–6.13 ppm region, in addition to the other expected signals for aromatic and olefinic protons (see Section 4).

Similarly, we prepared another series of rhodanine derivatives **7** using cycloalkanones instead of aromatic aldehydes. Thus, the fusion of **1** with cycloalkanones **6a**,**b** in the presence of ammonium acetate led to the formation of the corresponding 5-cycloalkylidene-2-thioxo-4-thiazolidinones **7a**,**b**. The reaction of compounds **7** with **4a** in acetone in the presence of aqueous potassium hydroxide gave the corresponding N-glucosides **8a**,**b** (Scheme 2). The structures of the isolated products were confirmed based on the elemental analyses and the spectral data (see Section 4).

Our study was extended to introduce a pyrazole moiety into thiazole derivatives, which has wide applications in different biological and medicinal fields besides their application in organic chemistry.[24] Thus, the reaction of **1** with pyrazole-4-carboxaldehyde derivatives **9a**–**e** under reflux in glacial acetic acid in the

* Corresponding author.
E-mail address: nhmmohamed@yahoo.com (N.H. Metwally).

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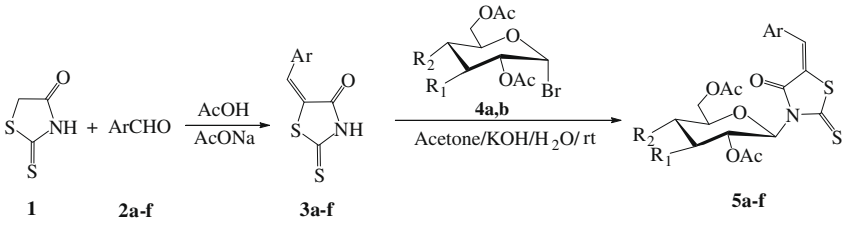
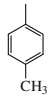
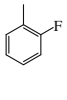
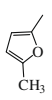
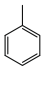
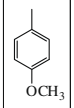
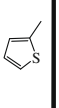
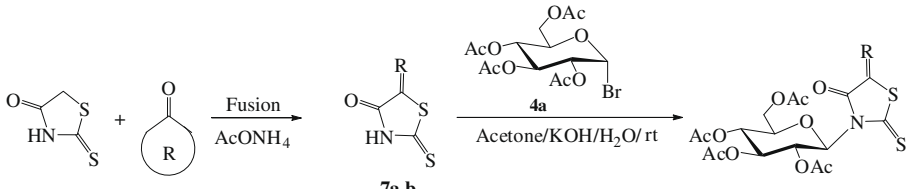
presence of anhydrous sodium acetate afforded the corresponding 5-pyrazolidene rhodanines **10a–e** (Scheme 3). The structures of the isolated products were confirmed on the basis of their elemental and spectral data (MS, IR, and ^{1}H NMR). The IR spectra of compounds **10** showed absorption bands characteristic for N–H and C=O groups. Also, as an example, the ^{1}H NMR spectrum of **10a**



1 **2a-f** **3a-f** **5a-f**

2,3,5	a	b	c	d	e	f
Ar						
R_1	H	H	H	OAc	OAc	OAc
R_2	OAc	OAc	OAc	H	H	H

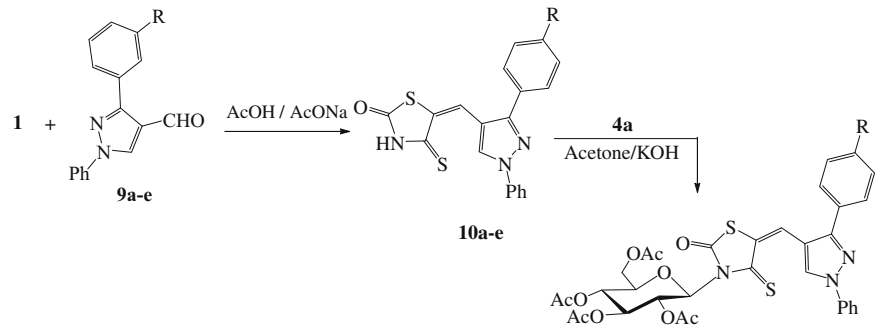
Scheme 1.



1 **6a,b** **7a,b** **8a,b**

6,7,8	a	b
R		

Scheme 2.



9a-e **10a-e** **11a-e**

9,10,11	a	b	c	d	e
R	H	CH_3	NO_2	Cl	Br

Scheme 3.

showed three singlet signals at δ = 7.27, 7.99, and 9.07 ppm, due to the olefinic proton, N–H, and pyrazole protons. The results of mass spectra together with elemental analyses are in good agreement with structures **10**.

Compounds **10a**–**e** react with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**4a**) in acetone in the presence of potassium hydroxide at room temperature to afford the new N-glucosides **11a**–**e** (Scheme 3). The structures of the isolated products were confirmed based on elemental analyses and spectral data (MS, IR, and ^{1}H NMR and ^{13}C NMR). IR spectra of the products revealed absorption bands for acetoxy carbonyl groups at $\nu \sim 1746$ cm^{-1}. The ^{1}H NMR spectrum of product **11a**, as typical example, showed the anomeric proton as doublet at δ = 6.35 ppm with $J_{1',2'}$ = 9.6 Hz corresponding to a diaxial orientation of the H-1′ and H-2′ protons. The other six protons of the glucopyranosyl ring resonated at δ = 3.89–6.12 ppm region, besides the other expected signals for pyrazolyl and olefinic protons. Also the ^{13}C NMR spectrum was characterized by a signal of 81.9 ppm corresponding to the C-1′ atom of the β-D-glucopyranose ring, the four signals appearing at δ = 169.2–170.5 ppm are due to the four acetoxy carbonyl carbon atoms, while the six signals at δ = 20.3–20.7 ppm are attributed to the acetate methyl carbon atoms. Other signals at δ = 61.6, 67.5, 67.9, 73.2, and 74.8 ppm were assigned to C-6′,-4′, -2′,-3′, and -5′, respectively (see Section 4).

Recently, it has been reported that bis-heterocyclic compounds exhibit much higher biological activity as antifungicidal and antibacterial activities than mono-heterocyclic compounds.[25–28] Based on these findings, we decided to synthesize some bis-N-glucosides starting with bis-thiazolidinone derivative **12**.[29] The reaction of bis-thiazolidinone **12** with two moles of compound **4a** in dimethylformamide in the presence of few drops of triethylamine at room temperature afforded the bis-N-glucoside derivative **13** (Scheme 4). The assigned structure for product **13** was confirmed by elemental and spectral data. The IR spectrum for product **13** showed characteristic bands at ν = 1746 cm^{-1} due to acetoxy carbonyl groups. The ^{1}H NMR spectrum showed the anomeric protons as doublet at δ = 6.46 ppm with $J_{1',2'}$ = 9.3 Hz indicating the β-configuration. The other protons of glucopyranosyl rings resonated at δ = 3.88–5.85 ppm region. The remaining acetoxy groups appear in their expected region (see Section 4).

Similarly, the reaction of dialdehydic compound **14**[30] with one mole of 2-thioxo-4-thiazolidinone **1** in glacial acetic acid at reflux in the presence of anhydrous sodium acetate afforded the corresponding 5-ylidene-2-thioxo-4-thiazolidinone **15**. On the other hand, reaction of **14** with two moles of **1** under the same reaction conditions led to formation of the bis-ylidene-2-thioxo-4-thiazolidinone **16**. Treatment of compounds **15** and **16** with **4a** in the presence of few drops of triethylamine or in KOH solution accomplished the mono- and bis-nucleosides **17** and **18**, respectively (Scheme 5). All the synthesized products **15**–**18** were confirmed based on elemental and spectral data (IR, ^{1}H NMR and MS).

3. Antimicrobial activity

The compounds **5a**–**c**, **11a**,**b**,**e** were tested for their antimicrobial activities using two fungal species, namely *Aspergillus flavus* **AF** and *Candida albicans* **CA** as well as two bacteria species, *Escherichia coli* **EC** (Gram negative) and *Staphylococcus aureus* **SA** (Gram positive). A solution of each compound at a concentration of 20 mg/mL was prepared and the inhibition zone diameter in centimeter (IZD) was used as the criterion for antimicrobial activity. The fungicide amphotericin B and the bactericide tetracycline were used as references to evaluate the potency of the tested compounds under the same conditions. The results are depicted in Table 1. The results revealed that all compounds exhibited considerable inhibition action against **EC** and **SA** but not antifungal activity.

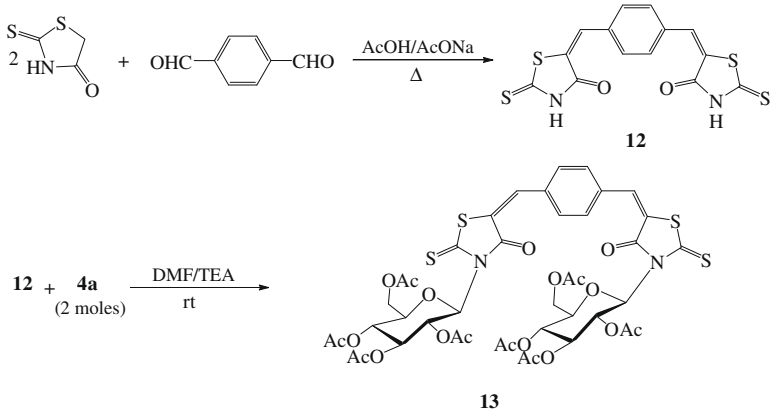
Compound **11e** exhibits moderate activity against *E. coli* **EC**; therefore, we determined its MIC using the agar dilution method. The results indicate that the MIC of compound **11e** is 143 μg/mL, whereas that of the tetracycline standard is 39 μg/mL.

4. Experimental

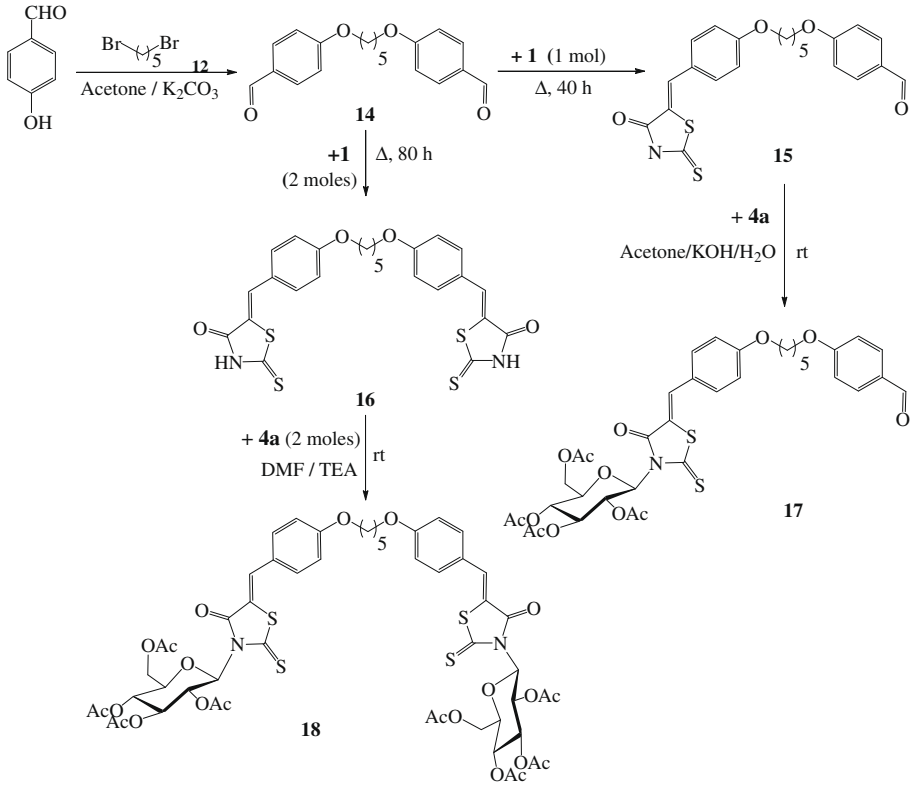
All evaporations were carried out under reduced pressure at 60 °C. TLC was carried out on aluminum sheet Silica Gel 60 (Fluka) and detected by short UV light. All melting points were measured on an electrothermal melting point apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded in deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO-d_6) at 300 MHz on a Varian Mercury VXR-300 NMR spectrometer. Chemical shifts were referenced to that of the solvent. Infrared spectra were recorded in potassium bromide disks on a Pye-Unicam, SP300 and Shimadzu, FT IR 8101 PC infrared spectrophotometers. Biological activity determinations were carried out at the Regional Center for Mycology and Biotechnology at Al-azhar University, Cairo, Egypt. Mass spectra were recorded on a Shimadzu GC MS-QP 1000 EX mass spectrometer at 70 eV. Elemental analyses were carried out at the Microanalytical Center of Cairo University, Giza, Egypt. The 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**4a**) was prepared according to the literature.[31,32]

4.1. 5-[(1-Phenyl-3-aryl-1*H*-pyrazol-4-yl)methylene]-2-thioxo-4-thiazolidinones 10a–e; general procedure

To a suspension of rhodanine **1** (0.66 g, 5 mmol) in acetic acid (5 mL) were added the aldehydes **9a**–**e** (5 mmol), followed by the



Scheme 4.



Scheme 5.

Table 1
Antimicrobial activity of compounds **5a–c**, **11a**,**b**,**e**

Sample		Inhibition zone diameter (mm/mg sample)			
		Escherichia coli (G^-)	*Staphylococcus aureus* (G^+)	*Aspergillus flavus* (fungus)	*Candida albicans* (fungus)
Control: DMSO		0.0	0.0	0.0	0.0
Standard	Tetracycline Antibacterial agent	33	31	—	—
	Amphotericin B Antifungal agent	—	—	16	19
	5a	10	11	0.0	0.0
	5b	12	12	0.0	0.0
	5c	11	11	0.0	0.0
	11a	10	10	0.0	0.0
	11b	12	11	0.0	0.0
	11e	12	11	0.0	0.0

addition of anhydrous sodium acetate (5 mmol). The mixture was heated until judged complete by TLC (0.5–2 h). The solid product formed upon cooling was filtered off, washed with water, dried, and finally recrystallized from the proper solvent.

4.2. 5-[(1,3-Diphenyl-1*H*-pyrazol-4-yl)methylene]-2-thioxo-4-thiazolidinone 10a

Yield 50%; yellow crystals, mp 305–307 °C; (ethanol–DMF); IR (KBr) ν 3130 (NH), 1692 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.27 (s, 1H, =CH), 7.38–7.56 (m, 8H, Ar-H), 7.84 (m, 2H, Ar-H), 7.99 (s, 1H, NH), 9.07 (s, 1H, pyrazolyl-H); MS: m/z (%) = 364 (M^++1, 4), 363 (M^+, 52), 275 (70), 77 (100). Anal. Calcd for $C_{19}H_{13}N_3OS_2$ (363): C, 62.79; H, 3.61; N, 11.56; S, 17.64. Found: C, 62.85; H, 3.56; N, 11.62; S, 17.59.

4.3. 5-[(3-(4-Methylphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene]-2-thioxo-4-thiazolidinone 10b

Yield 66%; yellow crystals, mp 286 °C; (ethanol–DMF); IR (KBr) ν 3134 (NH), 1691 (C=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 2.40 (s, 3H, CH_3), 7.41, 7.54, 8.02 (3m, 11H, Ar-H + NH + =CH), 8.71 (s, 1H, pyrazolyl-H); MS: m/z (%) = 378 (M^++1, 27), 377 (M^+, 61), 290 (96), 77 (100). Anal. Calcd for $C_{20}H_{15}N_3OS_2$ (377): C, 63.64; H, 4.01; N, 11.13; S, 16.99. Found: C, 63.58; H, 3.97; N, 11.20; S, 17.02.

4.4. 5-[(3-(4-Nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene]-2-thioxo-4-thiazolidinone 10c

Yield 59%; yellow crystals, mp 302–304 °C; (ethanol–DMF); IR (KBr) ν 3160 (NH), 1694 (C=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 7.41, 7.58, 7.96, 8.41 (4m, 11H, Ar-H + NH + =CH), 8.76 (s, 1H, pyrazolyl-H); MS: m/z (%) = 409 (M^++1, 16), 408 (M^+, 57), 321 (58), 275 (28), 77 (100). Anal. Calcd for $C_{19}H_{12}N_4O_3S_2$ (408): C, 55.87; H, 2.96; N, 13.72; S, 15.70. Found: C, 55.79; H, 3.01; N, 13.68; S, 15.78.

4.5. 5-[(3-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene]-2-thioxo-4-thiazolidinone 10d

Yield 78%; yellow crystals, mp 245–247 °C; (ethanol–DMF); IR (KBr) ν 3122 (NH), 1688 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.45, 7.84 (2m, 11H, Ar-H + NH + =CH), 8.52 (s, 1H, pyrazolyl-H); MS: m/z (%) = 398 (M^++1, 7.5), 397 (M^+, 43), 310 (56), 275 (23), 77 (100). Anal. Calcd for $C_{19}H_{12}ClN_3OS_2$ (397): C, 57.35; H, 3.04; Cl, 8.91; N, 10.56; S, 16.12. Found: C, 57.43; H, 2.96; Cl, 8.87; N, 10.49; S, 16.19.

4.6. 5-[(3-(4-Bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene]-2-thioxo-4-thiazolidinone 10e

Yield 65%; yellow crystals, mp 290 °C; (ethanol); IR (KBr) *ν* 3130 (NH), 1690 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 7.40, 7.54, 7.83 (3m, 11H, Ar-H + NH + =CH), 8.72 (s, 1H, pyrazolyl-H); MS: *m*/*z* (%) = 443 (M^++1, 30), 442 (M^+, 27), 356 (41), 275 (57), 77 (100). Anal. Calcd for $C_{19}H_{12}BrN_3OS_2$ (442): C, 51.59; H, 2.73; Br, 18.06; N, 9.50; S, 14.50. Found: C, 51.70; H, 2.65; Br, 17.95; N, 9.58; S, 14.61.

4.7. 4-(5-(4-((4-Oxo-2-thioxothiazolidin-5-ylidene)methyl)phenoxy)pentyloxy)-benzaldehyde 15

A solution of 2-thioxo-4-thiazolidinone **1** (1.33 g, 0.01 mmol) and 1,5-bis(4-formylphenoxy)pentane **14** (3.12 g, 0.01 mmol) was dissolved in glacial acetic acid (50 mL) containing anhydrous sodium acetate (0.01 mol). The mixture was heated at reflux for 40 h, and then allowed to cool to room temperature. The resulting solid was collected by filtration and crystallized from DMF (yield = 72%), mp 220 °C.

4.8. 5,5′-(4,4′-(Pentane-1,5-diylbis(oxy))bis(4,1-phenylene))bis(methan-1-yl-1-ylidene)bis(2-thioxothiazolidin-4-one) 16

A solution of 2-thioxo-4-thiazolidinone **1** (2.66 g, 0.02 mmol) and 1,5-bis(4-formylphenoxy)pentane **14** (3.12 g, 0.01 mmol) was dissolved in glacial acetic acid (50 mL) containing anhydrous sodium acetate (0.01 mol). The mixture was heated at reflux for 80 h, and then allowed to cool to room temperature. The resulting solid was collected by filtration and crystallized from DMF (yield = 69%), mp 264–266 °C.

4.9. Glycosidation of compounds 3a–f, 7a,b, 10a–e, and 15; General procedure

A solution of the appropriate 5-arylidene or 5-cycloalkylidene-2-thioxo-4-thiazolidinone **3a**–**f**, **7a**,**b**, **10a**–**e**, or **15** (1 mmol) and potassium hydroxide (0.056 g, 1 mmol) in water (1 mL) was treated with a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl or D-galactopyranosyl bromide **4a**,**b** (1.1 mmol) in acetone (30 mL). The mixture was stirred at room temperature until judged complete by TLC (1–4 days), filtered and the solution was concentrated under reduced pressure. The obtained residue was triturated with MeOH, the solid product formed was filtered off, washed with water then ethanol, dried, and finally recrystallized from the proper solvent to afford the respective *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl or galactopyranosyl)-5-arylidene or 5-cycloalkylidene-2-thioxo-4-thiazolidinone derivative **5a**–**f**, **8a**,**b**, **11a**–**e**, or **17**, respectively.

4.10. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-(4-methylphenylmethyl-ene)-2-thioxo-4-thiazolidinone 5a

Yield 40%; yellow crystals, mp 214 °C; (ethanol); IR (KBr) *ν* 1749 (CH_3CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 2.42 (s, 3H, CH_3), 1.95, 2.04, 2.07, 2.10 (4s, 12H, $4CH_3CO$), 3.88 (m, 1H, H-5′), 4.25 (m, 2H, H-6a′, H-6b′), 5.28 (t, *J* = 9.6 Hz, 1H, H-4′), 5.39 (t, *J* = 9 Hz, 1H, H-3′), 6.16 (t, *J* = 9 Hz, 1H, H-2′), 6.35 (d, *J* = 9.3 Hz, 1H, H-1′), 7.29 (d, *J* = 8.4, 2H, Ar-H), 7.39 (d, *J* = 7.8, 2H, Ar-H), 7.71 (s, 1H, =CH). Anal. Calcd for $C_{25}H_{27}NO_{10}S_2$ (565): C, 53.09; H, 4.81; N, 2.48; S, 11.34. Found: C, 53.30; H, 4.93; N, 2.60; S, 11.29.

4.11. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-(2-fluorophenylmethylene)-2-thioxo-4-thiazolidinone 5b

Yield 52%; yellow crystals, mp 179 °C; (ethanol); IR (KBr) *ν* 1747 (CH_3CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 1.95, 2.05, 2.07, 2.11 (4s, 12H, $4CH_3CO$), 3.89 (m, 1H, H-5′), 4.25 (m, 2H, H-6a′, H-6b′), 5.29 (t, *J* = 9.9 Hz, 1H, H-4′), 5.38 (t, *J* = 9 Hz, 1H, H-3′), 6.13 (t, *J* = 9 Hz, 1H, H-2′), 6.34 (d, *J* = 9.3 Hz, 1H, H-1′), 7.20, 7.44 (2m, 4H, Ar-H), 7.95 (s, 1H, =CH). Anal. Calcd for $C_{24}H_{24}FNO_{10}S_2$ (569): C, 50.61; H, 4.25; N, 2.46; F, 3.34; S, 11.26. Found: C, 50.81; H, 4.18; N, 2.52; S, 11.32.

4.12. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-[(5-methylfuran-2-yl)methyl-ene]-2-thioxo-4-thiazolidinone 5c

Yield 90%; brown crystals, mp 137 °C; (ethanol); IR (KBr) *ν* 1752 (CH_3CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 1.93, 2.03, 2.06, 2.09 (4s, 12H, $4CH_3CO$), 2.42 (s, 3H, CH_3), 3.86 (m, 1H, H-5′), 4.23 (m, 2H, H-6a′, H-6b′), 5.25 (t, *J* = 9.6 Hz, 1H, H-4′), 5.38 (t, *J* = 9.3 Hz, 1H, H-3′), 6.13 (t, *J* = 9.3 Hz, 1H, H-2′), 6.22 (d, *J* = 4.2 Hz, 1H, furanyl H-4), 6.34 (d, *J* = 9.3 Hz, 1H, H-1′), 6.78 (d, *J* = 3.3 Hz, 1H, furanyl H-3), 7.38 (s, 1H, =CH). Anal. Calcd for $C_{23}H_{25}NO_{11}S_2$ (555): C, 49.72; H, 4.54; N, 2.52; S, 11.54. Found: C, 49.88; H, 4.43; N, 2.46; S, 11.63.

4.13. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-5-benzylidene-2-thioxo-4-thiazolidinone 5d

Yield 61%; yellow crystals, mp 130 °C; (ethanol); IR (KBr) *ν* 1755 (CH_3CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 1.91, 1.95, 1.98, 2.08 (4s, 12H, $4CH_3CO$), 4.0 (m, 3H, H-5′, H-6a′, H-6b′), 4.55 (t, *J* = 9.1 Hz, 1H, H-4′), 5.27 (t, *J* = 9 Hz, 1H, H-3′), 5.95 (t, *J* = 9 Hz, 1H, H-2′), 6.46 (d, *J* = 8.98 Hz, 1H, H-1′), 7.86 (m, 6H, =CH + Ar-H). MS: *m*/*z* = 551. Anal. Calcd for $C_{24}H_{25}NO_{10}S_2$ (551): C, 52.26; H, 4.57; N, 2.54; S, 11.63. Found: C, 52.38; H, 4.60; N, 2.71; S, 11.55.

4.14. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-5-(4-methoxyphenylmethyl-ene)-2-thioxo-4-thiazolidinone 5e

Yield 62%; yellow crystals, mp 145 °C; (ethanol); IR (KBr) *ν* 1756 (CH_3CO) cm^{-1}; MS: *m*/*z* = 581. Anal. Calcd for $C_{25}H_{27}NO_{11}S_2$ (581): C, 51.63; H, 4.68; N, 2.41; S, 11.03. Found: C, 51.88; H, 4.70; N, 2.41; S, 11.12.

4.15. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-5-[(thiophen-2-yl)methylene]-2-thioxo-4-thiazolidinone 5f

Yield 61%; yellow crystals, mp 114 °C; (ethanol); IR (KBr) *ν* 1748 (CH_3CO) cm^{-1}; MS: *m*/*z* = 557. Anal. Calcd for $C_{22}H_{23}NO_{10}S_3$ (557): C, 47.39; H, 4.16; N, 2.51; S, 17.25. Found: C, 47.51; H, 4.25; N, 2.66; S, 17.15.

4.16. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-cyclopentylidene-2-thioxo-4-thiazolidinone 8a

Yield 30%; brown crystals, mp 170–172 °C; (ethanol); IR (KBr) 1749 (CH_3CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 1.95, 2.03, 2.05, 2.09 (4s, 12H, $4CH_3CO$), 1.84, 2.37, 2.99 (3m, 8H, cyclopentylidene-H), 3.86 (m, 1H, H-5′), 4.23 (m, 2H, H-6a′, H-6b′), 5.25 (t, *J* = 9.9 Hz, 1H, H-4′), 5.37 (t, *J* = 9 Hz, 1H, H-3′), 6.11 (t, *J* = 9.3 Hz, 1H, H-2′), 6.33 (d, *J* = 9.6 Hz, 1H, H-1′). Anal. Calcd for $C_{22}H_{27}NO_{10}S_2$ (529): C, 49.90; H, 5.14; N, 2.64; S, 12.11. Found: C, 50.02; H, 5.00; N, 2.55; S, 12.19.

4.17. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-cyclohexylidene-2-thioxo-4-thiazolidinone 8b

Yield 62%; orange crystals, mp 152–155 °C; (water–ethanol); IR (KBr) *ν* 1751 (CH_3CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 1.95, 2.03, 2.06, 2.10 (4s, 12H, $4CH_3CO$), 1.77, 2.22 (2m, 10H, cyclohexylidene-H), 3.85 (m, 1H, H-5′), 4.24 (m, 2H, H-6a′, H-6b′), 5.21 (t, *J* = 9.3 Hz,

1H, H-4′), 5.36 (t, *J* = 9 Hz, 1H, H-3′), 6.10 (t, *J* = 9 Hz, 1H, H-2′), 6.34 (d, *J* = 9.6 Hz, 1H, H-1′). Anal. Calcd for $C_{23}H_{29}NO_{10}S_2$ (543): C, 50.82; H, 5.38; N, 2.58; S, 11.80. Found: C, 50.76; H, 5.49; N, 2.45; S, 11.87.

4.18. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-[(1,3-diphenyl-1*H*-pyrazol-4-yl)methylene]-2-thioxo-4-thiazolidinone 11a

Yield 69%; yellow crystals, mp 194 °C; (ethanol); IR (KBr) *ν* 1746 (CH_3CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 1.95, 2.04, 2.06, 2.10 (4s, 12H, $4CH_3CO$), 3.89 (m, 1H, H-5′), 4.25 (m, 2H, H-6a′, H-6b′), 5.26 (t, *J* = 9.9 Hz, 1H, H-4′), 5.39 (t, *J* = 9.6 Hz, 1H, H-3′), 6.12 (t, *J* = 9 Hz, 1H, H-2′), 6.35 (d, *J* = 9.6 Hz, 1H, H-1′), 7.38, 7.55, 7.68, 7.82 (4m, 11H, Ar-H + =CH), 8.16 (s, 1H, pyrazolyl). ^{13}C NMR (300 MHz, $CDCl_3$) *δ* 20.3, 20.50, 20.53, 20.7 ($4CH_3CO$), 61.6 (C-2′), 67.5 (C-6′), 67.9 (C-4′), 73.2 (C-3′), 74.8 (C-5′), 81.9 (C-1′), 116.4 (C=C for rhod.), 118.6, 119.5, 124.9, 127.5, 127.8, 128.9, 129.1, 129.6, 131.1, 139.0, 155.2 (Ar-C), 165.3 (C=O), 169.2, 169.5, 170.0, 170.5 (CH_3CO), 192.6 (C=S); MS: *m*/*z* (%) = 693 (M^+, 4), 692 (8), 364 (16), 276 (31), 169 (100), 109 (99), 77 (31). Anal. Calcd for $C_{33}H_{31}N_3O_{10}S_2$ (693): C, 57.13; H, 4.50; N, 6.06; S, 9.24. Found: C, 57.26; H, 4.40; N, 5.89; S, 9.35.

4.19. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-[(3-(4-methylphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene]-2-thioxo-4-thiazolidinone 11b

Yield 81%; yellow crystals, mp 200–202 °C; (ethanol–dioxane); IR (KBr) *ν* 1740 (CH_3CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 1.95, 2.04, 2.06, 2.10 (4s, 12H, $4CH_3CO$), 2.45 (s, 3H, CH_3), 3.90 (m, 1H, H-5′), 4.22 (m, 2H, H-6a′, H-6b′), 5.26 (t, *J* = 9.9 Hz, 1H, H-4′), 5.41 (t, *J* = 9.6 Hz, 1H, H-3′), 6.17 (t, *J* = 9 Hz, 1H, H-2′), 6.33 (d, *J* = 9.6 Hz, 1H, H-1′), 7.32–7.78, 7.81 (2m, 10H, Ar-H + =CH), 8.14 (s, 1H, pyrazolyl H). Anal. Calcd for $C_{34}H_{33}N_3O_{10}S_2$ (707): C, 57.70; H, 4.70; N, 5.94; S, 9.06. Found: C, 57.53; H, 4.76; N, 5.88; S, 9.01.

4.20. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-[(3-(4-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene]-2-thioxo-4-thiazolidinone 11c

Yield 25%; yellow crystals, mp 142–144 °C; (ethanol–dioxane); IR (KBr) *ν* 1743 (CH_3CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 1.95, 2.01, 2.06, 2.09 (4s, 12H, $4CH_3CO$), 3.88 (m, 1H, H-5′), 4.23 (m, 2H, H-6a′, H-6b′), 5.26 (t, *J* = 9.9 Hz, 1H, H-4′), 5.4 (t, *J* = 9.3 Hz, 1H, H-3′), 6.04 (t, *J* = 9.3 Hz, 1H, H-2′), 6.31 (d, *J* = 9 Hz, 1H, H-1′), 7.41–7.89, 8.21 (2m, 8H, Ar-H + =CH), 8.38 (m, 3H, Ar-H + pyrazol-yl-H). Anal. Calcd for $C_{33}H_{30}N_4O_{12}S_2$ (738): C, 53.65; H, 4.09; N, 7.58; S, 8.68. Found: C, 53.81; H, 4.20; N, 7.45; S, 8.61.

4.21. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-[(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene]-2-thioxo-4-thiazolidinone 11d

Yield 40%; yellow crystals, mp 234–236 °C; (ethanol–dioxane); IR (KBr) *ν* 1740 (CH_3CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 1.95, 2.04, 2.06, 2.10 (4s, 12H, $4CH_3CO$), 3.91 (m, 1H, H-5′), 4.23 (m, 2H, H-6a′, H-6b′), 5.26 (t, *J* = 9.9 Hz, 1H, H-4′), 5.39 (t, *J* = 9.3 Hz, 1H, H-3′), 6.09 (t, *J* = 9.3 Hz, 1H, H-2′), 6.32 (d, *J* = 9 Hz, 1H, H-1′), 7.41–7.72, 7.85 (2m, 10H, Ar-H + =CH), 8.15 (s, 1H, pyrazolyl-H). ^{13}C NMR (300 MHz, $CDCl_3$) *δ* 20.3–20.7 ($4CH_3CO$), 61.6 (C-2′), 67.8 (C-6′), 68.0 (C-4′), 73.2 (C-3′), 75.0 (C-5′), 82.0 (C-1′), 116.4 (*C*=C for rhod.), 118.8, 119.6, 124.2, 127.6, 128.0, 129.2, 129.7, 130.1, 135.4, 139.0 (Ar-C), 163.6 (C=O), 169.2, 169.5, 170.1, 170.5 (CH_3CO), 192.5 (C=S). Anal. Calcd for $C_{33}H_{30}ClN_3O_{10}S_2$ (727.5): C, 54.43; H, 4.15; N, 5.77; S, 8.81. Found: C, 54.55; H, 4.05; N, 5.74; S, 8.76.

4.22. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-[(3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene]-2-thioxo-4-thiazolidinone 11e

Yield 45%; yellow crystals, mp 247–249 °C; (ethanol–dioxane); IR (KBr) *ν* 1747 (CH_3CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 1.87, 1.96, 2.01, 2.02 (4s, 12H, $4CH_3CO$), 3.82 (m, 1H, H-5′), 4.17 (m, 2H, H-6a′, H-6b′), 5.20 (t, *J* = 9.9 Hz, 1H, H-4′), 5.30 (t, *J* = 9.3 Hz, 1H, H-3′), 6.02 (t, *J* = 9.3 Hz, 1H, H-2′), 6.26 (d, *J* = 9 Hz, 1H, H-1′), 7.34–7.72 (m, 10H, Ar-H + =CH), 8.06 (s, 1H, pyrazolyl-H). Anal. Calcd for $C_{33}H_{30}BrN_3O_{10}S_2$ (773): C, 51.30; H, 3.91; N, 5.44; S, 8.30. Found: C, 51.39: H, 4.00; N, 5.53; S, 8.39.

4.23. 1,4-Bis[*N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-thioxo-4-thiazolidinon-5-ylidenemethyl]benzene 13

Compound **13** was prepared by the method described above for the synthesis of compounds **5a–f**, **8a**,**b**, **11a–e**, and **17**. However, DMF in the presence of Et_3N was used as the basic medium instead of potassium hydroxide. Yield 30%; red crystals, mp 242–244 °C; (ethanol–dioxane); IR (KBr) *ν* 1746 (CH_3CO) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) *δ* 1.85, 1.93, 1.98, 2.00 (4s, 24H, $8CH_3CO$), 3.88 (m, 2H, H-5′, H-5″), 4.29 (m, 4H, H-6a′, H-6b′, H-6a″, H-6b″), 5.01 (t, *J* = 9.6 Hz, 2H, H-4′, H-4″), 5.52 (t, *J* = 9.3 Hz, 2H, H-3′, H-3″), 5.85 (t, *J* = 9.3 Hz, 2H, H-2′, H-2″), 6.46 (d, *J* = 9.3 Hz, 2H, H-1′, H-1″), 7.51 (s, 2H, 2 =CH), 7.70 (m, 4H, Ar-H). Anal. Calcd for $C_{42}H_{44}N_2O_{20}S_4$ (1024): C, 49.21; H, 4.33; N, 2.73; S, 15.51. Found: C, 49.11; H, 4.25; N, 2.63; S, 15.62.

4.24. 4-[5-(4-((*N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenoxy)pentyloxy]benzaldehyde 17

Yield 25%; yellow crystals, mp 106–108 °C; (ethanol); IR (KBr) *ν* 1752 (CH_3CO) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) *δ* 1.79–1.83 (m, 10H, pentyl-H), 1.88, 1.96, 2.00, 2.02 (4s, 12H, $4CH_3CO$), 4.08 (m, 1H, H-5′), 4.27 (m, 2H, H-6a′, H-6b′), 5.01 (t, *J* = 9.6 Hz, 1H, H-4′), 5.59 (t, *J* = 9.3 Hz, 1H, H-3′), 5.92 (t, *J* = 9.3 Hz, 1H, H-2′), 6.53 (d, *J* = 9.3 Hz, 1H, H-1′), 7.10, 7.22, 7.61, 7.85 (4d, 8H, Ar-H), 7.77 (s, 1H, =CH), 9.85 (s, 1H, CHO). Anal. Calcd for $C_{36}H_{39}NO_{13}S_2$ (757): C, 57.06; H, 5.19; N, 1.85; S, 8.46. Found: C, 57.16; H, 5.08; N, 1.73; S, 8.42.

4.25. 5,5′-[4,4′-(Pentane-1,5-diylbis(oxy))bis(4,1-phenylene)]bis(methan-1-yl-1-ylidene)bis[*N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-thioxo-4-thiazolidinone] 18

Compound **18** was prepared by the same method described above for the synthesis of compounds **5a–f**, **8a**,**b**, **11a–e**, and **17**. However, DMF in the presence of Et_3N was used as the basic medium instead of potassium hydroxide. Yield 35%; yellow crystals, mp 231–233 °C; (ethanol–dioxane); IR (KBr) *ν* 1751 (CH_3CO) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) *δ* 1.79–1.83 (m, 10H, pentyl-H), 1.88, 1.95, 1.98, 2.02 (4s, 24H, $8CH_3CO$), 4.11 (m, 2H, H-5′, H-5″), 4.28 (m, 4H, H-6a′, H-6b″, H-6a″, H-6b″), 5.01 (t, *J* = 9.6 Hz, 2H, H-4′, H-4″), 5.58 (t, *J* = 9.3 Hz, 2H, H-3′, H-3″), 5.91 (t, *J* = 9.3 Hz, 2H, H-2′, H-2″), 6.52 (d, *J* = 9.3 Hz, 2H, H-1′, H-1″), 7.12, 7.61 (2d, 8H, Ar-H), 7.77 (s, 2H, 2 =CH). Anal. Calcd for $C_{53}H_{58}N_2O_{22}S_4$ (1203): C, 52.90; H, 4.86; N, 2.33; S, 10.66. Found: C, 52.86; H, 4.90; N, 2.29; S, 10.70.

5. Antimicrobial assay

The antimicrobial activity of the tested samples was determined using a modified Kirby–Bauer disk diffusion method.[33]

Briefly, 100 μL of the test bacteria/fungi was grown in 10 mL of fresh media until a count of approximately 108 cells/mL for bacteria or 105 cells/mL for fungi was obtained.[34] Then, 100 μL of microbial suspension was spread onto agar plates corresponding to the broth from which they were maintained.

The inoculated plates were then treated with *A. flavus*, *S. aureus*, *Bacillus subtilis* or *E. coli* at 25 °C for 48 h. In the case of *Pseudomonas aeruginosa* plates were incubated at 35–37 °C for 24–48 h and for *C. albicans* the plates were incubated at 30 °C for 24–48 h. After incubation, the diameters of the inhibition zones were measured in millimeters.[33] Standard disks of tetracycline (antibacterial agent), amphotericin B (antifungal agent) served as positive controls for antimicrobial activity and filter disks impregnated with 10 μL of solvent (distilled water, chloroform, DMSO) were used as a negative control.

The agar used was Mueller–Hinton agar, which was rigorously tested for composition and pH. Furthermore, the depth of the agar in the plate is a factor to be considered in the disk diffusion method. This method is well documented and standard zones of inhibition have been determined for susceptible and resistant values. The zone diameters were measured with slipping calipers of the National Committee for Clinical Laboratory Standards (NCCLS, 1993). Agar-based methods such as Etest and disk diffusion can be good alternatives because they are simpler and faster than broth-based methods.[35,36]

6. MIC determination

Stationary-phase cultures of each bacterium were prepared at 37 °C and used to inoculate fresh 5.0 mL culture to an OD_{600} of 0.05. The 5.0 mL cultures were then incubated at 37 °C until an OD_{600} of 0.10 was achieved from which standardized bacterial suspensions were prepared to a final cell density of 6×10^5 CFU/mL. Serial dilutions from the treatments (0–320 μg/mL) were prepared and mixed with 5.0 mL of the standardized bacteria suspension then added to the plates and incubated for 24 h at 37 °C. The colony forming units (CFUs) were counted for each dilution.[37]

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