Synthesis and Antimicrobial Activity of New Tetrazoles Incorporating Isoindole-1,3-dione Moiety and Their Sugar Derivatives

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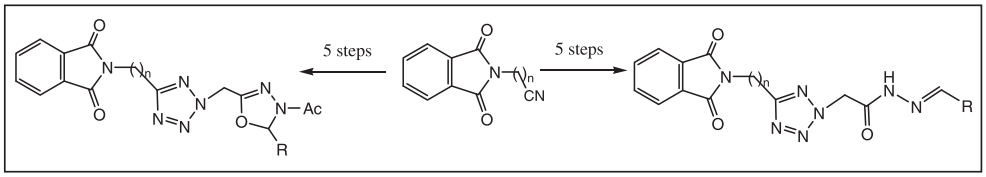

New tetrazole derivatives linked to isoindole-1,3-dione were prepared starting from the corresponding nitrile derivatives. The substituted hydrazides, their corresponding sugar hydrazones, and the derived acyclic *C*-nucleoside analogs were also synthesized. The antimicrobial results indicated that most of the tested compounds exhibited moderate to high activity, whereas few compounds were found to exhibit little or no activity against the tested microorganisms.

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INTRODUCTION

The synthesis of nitrogen-containing heterocyclic compounds has increasingly been a subject of great interest because of their importance. Heterocyclic compounds containing isoindole moiety are of interest because they show some pharmacological and biological activities [1–4]. Isoindole derivatives have been shown to possess antineoplastic and antiviral [5], antimalarial [6], antimycobacterium tuberculosis activity [7], antitumor [8], and antimicrobial activities [9]. Tetrazole derivatives found wide applications as carboxylic surrogates, bioisosteres of carboxylic acids [10,11] and lipophilic spacers in pharmaceuticals resulting in compounds with antihypertensive, antiallergic, and antibiotic activities [12]. Many tetrazole derivatives endowed with a pronounced antimicrobial activity and *N*-substituted tetrazoles showed interesting antinociceptive activity [13]. On the other hand, the nucleosides as well as their acyclic and *C*-nucleoside analogs possess a wide range of medicinal properties, including antibiotic, antiviral, and antitumor activities [14–19]. In view of the reported diverse activities for isoindole and tetrazole structural moieties, especially antimicrobial activity, and as continuation of our effort [20–24], to identify new candidates that may be of value in designing new, potent, selective, and less toxic antimicrobial agent, we report herein the synthesis and antimicrobial activity of new tetrazoles incorporating isoindole-1,3-dione moiety.

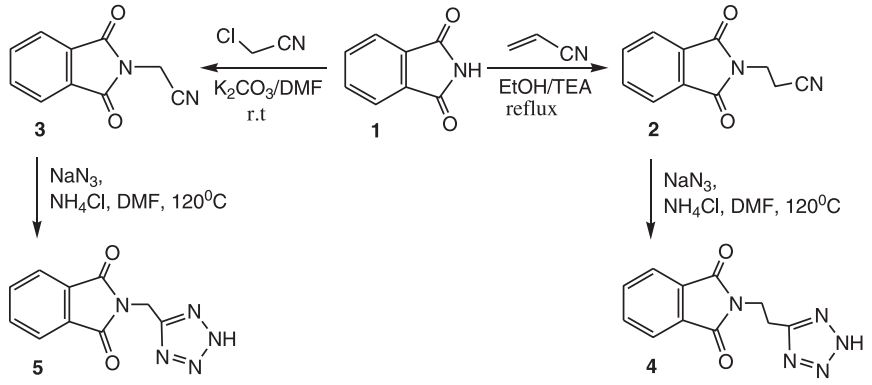
RESULTS AND DISCUSSION

The key tetrazole compounds **4** and **5** were synthesized by refluxing phthalamide with acrylonitrile in ethanol or stirring with chloroacetonitrile in DMF at room temperature, respectively, followed by reaction with sodium azide and ammonium chloride in DMF. The IR spectra revealed the disappearance of the CN absorption band present in the nitrile derivatives **2** and **3** and presence of absorption bands to the NH group in the region 3446–3470 cm^{-1} (Scheme 1).

When tetrazole derivatives **4** and **5** were stirred with ethyl chloroacetate in DMF in the presence of potassium carbonate, they afforded the ethyl ester derivatives **6** and **7**. The IR spectrum of the latter ester derivatives showed the presence of characteristic absorption bands corresponding to the carbonyl groups in the region 1739 and 1746 cm^{-1} and (C═N) in 1618–1620 cm^{-1}. Acid hydraziedes **8** and **9** were obtained by refluxing the corresponding ester precursors with hydrazine hydrate in ethanol. The IR spectra of the produced hydrazides showed the presence of characteristic absorption bands corresponding to the NH and NH_2 groups in addition to the carbonyl and C═N groups. The ^1H NMR spectra showed the signals of the NH_2 and NH protons and revealed the absence of the ethyl group signals present in their corresponding esters (see Experimental Section).

When hydrazide derivatives **8** and **9** were allowed to react with D-galactose and D-xylose in an aqueous ethanolic solution and a catalytic amount of glacial acetic acid, the corresponding sugar hydrazones **10–13** were obtained in 71–73% yields. The reaction of sugar hydrazones with acetic anhydride is well known to give products depending on the applied reaction conditions. The expected products **14–17** in which per-*O*-acetylation of the sugar chain hydroxyls were obtained, in 74.5–86.7% yields, by carrying out the reaction sugar hydrazones **10–13** with acetic anhydride in pyridine at room

Scheme 1



temperature. The IR spectra of the acetylated hydrazones **14–17** showed characteristic absorption bands corresponding to the carbonyl ester and carbonyl amide groups in addition to the NH absorption bands. On the other hand, when the reaction with acetic anhydride was carried out using acetic anhydride only at 100°C, the oxadiazoline acyclic *C*-nucleoside analogs **18–21** were obtained [25–28]. The structures of these derivatives were established by analytical and spectral data that agreed with the assigned structures. The ^{1}H NMR spectra of the oxadiazoline derivatives **18–21** showed the signals of the *O*-acetyl-methyl protons as singlets in the range 1.95–2.19 ppm and the *N*-acetyl-methyl protons in the range 2.27–2.29 ppm. The rest of the sugar chain protons appeared in the range 3.94–5.89 ppm in addition to aromatic proton signals as multiplet at 7.71–8.04 ppm. The disappearance of the chemical shift higher than 7.0 ppm for the H-1 sugar proton (originally present in their sugar hydrazone precursors) and instead the appearance of the doublet signal at δ 5.85–5.89 ppm indicated the nature of the C-1 of the original sugar moiety as -O-*CH*-N-Ac (C-1 in the original sugar chain moiety and C-2 in the oxadiazoline ring). These spectral characteristics indicated the *N,N*-acetal nature of the this carbon rather than being a C═N, which indicated that cyclization to the oxadiazoline ring has taken place and also in agreement with the reporting literature. This relatively higher chemical shift values (higher than 7.0 ppm) appeared in their acyclic acetylated hydrazone derivatives (**14–17**) [Scheme 2].

Antimicrobial activity. The synthesized compounds were evaluated for their antimicrobial activity 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 1. The MIC values of the most active compounds were in accordance with the results obtained in the primary screening.

The result revealed that compounds showed varying degrees of inhibition against the tested microorganisms. In general, compounds **4**, **10**, **13**, and **21** displayed the highest activity with MIC value of 75 μg/mL at least against two of the microorganisms under test. Compound **11** displayed the highest inhibition activity against *Sreptomyces* species also with MIC value of 75 μg/mL. Some of the other compounds revealed moderate activity, whereas others revealed little or no activity.

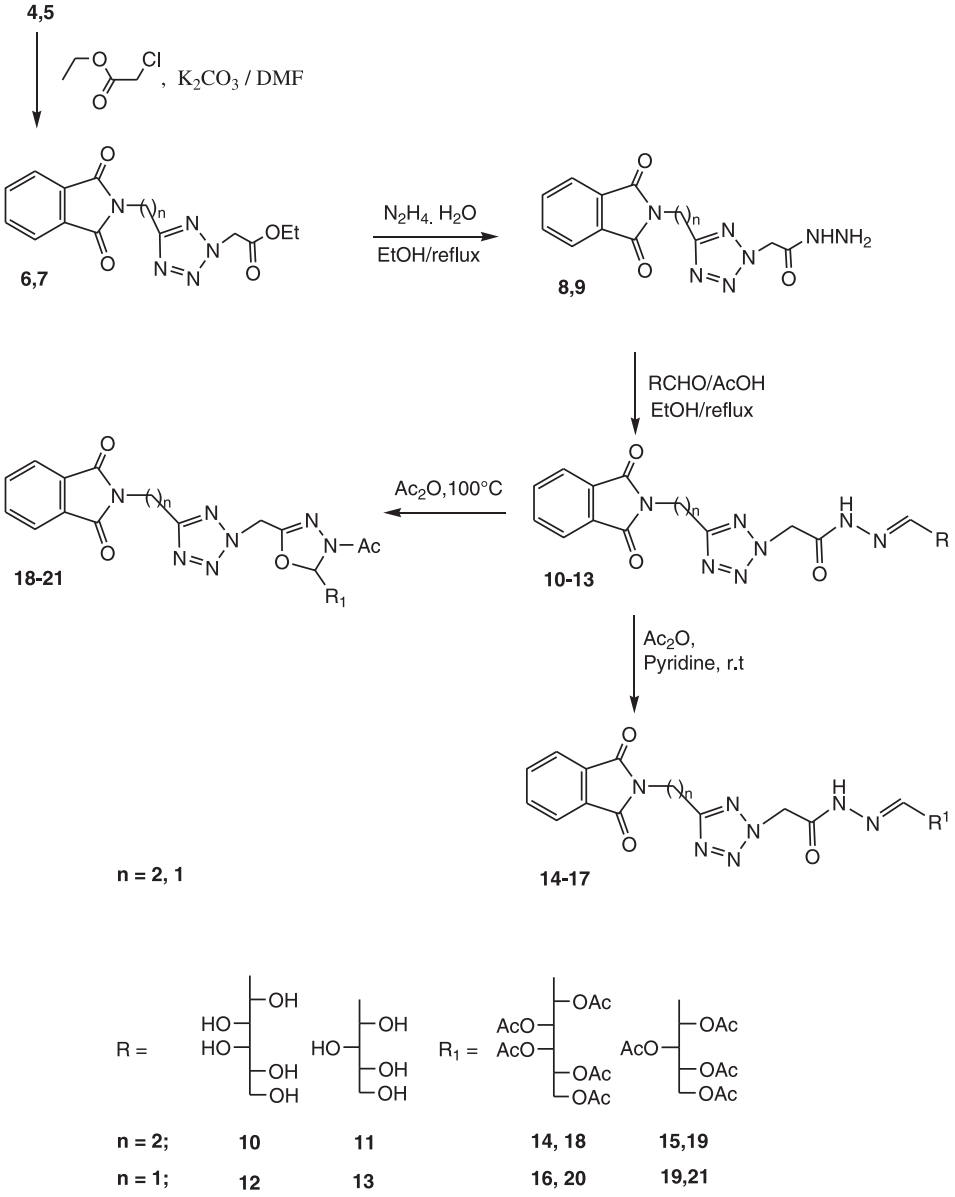
The antimicrobial activity results and structure activity relationship indicated that the attachment of acyclic sugar moieties to tetrazole ring system linked through alkyl linkage to isoindole resulted in increase of antimicrobial activity. Furthermore, the hydrazones incorporating free hydroxyl sugar chains showed higher activity than the corresponding acetylated analogs. In addition, the acyclic *C*-nucleoside analog with the five carbon sugar xylose attached to the oxadiazoline base showed high inhibition activity against *B. subtilis* and *P. aeruginosa*. Furthermore, the length of the alkyl chain, consisting of one or two carbon atoms, connecting the isoindole and tetrazole moieties revealed no effect on inhibition activities of tested compounds as higher activities were not observed for only one compound type.

In conclusion new tetrazole derivatives incorporating isoindole-1,3-dione moiety and their sugar hydrazones as well as acyclic *C*-nucleoside analogs were prepared and studied for their antimicrobial activity. The attachment of acyclic free hydroxyl sugar moieties resulted in higher antimicrobial activities.

EXPERIMENTAL

Melting points were determined with a *kofler* block apparatus (VEB Wägetechnik Rapido, Germany) and are uncorrected. The IR spectra were recorded on a perkin-Elmer model 1720 FTIR spectrometer (Perkin-Elmer, USA) for KBr disk. NMR spectra

Scheme 2



were recorded on a Varian Gemini 200 NMR Spectrometer (Varian, Palo Alto, CA) at 300 MHz for ^{1}H NMR with TMS as a standard. Mass spectra were measured on a Kratos 50 TC spectrometers (Kratos Analyti-cal Instruments, Ramsey, NJ). The progress of the reactions was monitored by TLC using aluminum silica gel plates 60 F 245. Elemental analyses were performed at the Microanalytical Data Center at Faculty of Science, Cairo University, Egypt.

3-(1,3-Dioxoisoindolin-2-yl)propanenitrile (2). To a solution of phthalimide (**1**) (4.41 g, 0.03 mol) in ethanol (50 mL), triethyl amine (1.01 g, 0.01 mol) and acrilonitrile (1.59 g, 0.03 mol) were added. The reaction mixture was reflux for 5 h. The precipitate that separated on cooling was filtered off as white crystals. Yield: 4.01 g (66.8%); mp 150–151°C; IR (KBr) υ: 2252 (C≡N), 1672 (C═O). ^{1}H NMR ($CDCl_3$) δ: 3.05 (t, 2H, J = 5.6 Hz, CH_2), 4.16 (t, 2H, J = 5.6 Hz, CH_2), 7.76–8.05 (m, 4H, Ar-H). *Anal*. calcd. for $C_{11}H_8N_2O_2$: C, 66.00; H, 4.03; N, 13.99. Found: C, 65.86; H, 3.96; N, 13.79.

2-(1,3-Dioxoisoindolin-2-yl)acetonitrile (3). To a solution of phthalimide (1.47 g, 0.01 mol) and potassium carbonate (1.40 g, 0.01 mol) in *N,N*-dimethylformamide (30 mL), monochloroacetonitrile (0.76 g, 0.01 mol) was added. The reaction mixture was stirred overnight at room temperature. The mixture was poured onto cold water, and the precipitate was filtered off as white crystals. Yield: 1.5 g (87.2%); mp 130–131°C; IR (KBr) υ: 2246 (C≡N), 1680 (C═O); ^{1}H NMR ($CDCl_3$) δ: 4.56 (s, 2H, CH_2), 7.76–7.95 (m, 4H, Ar-H); MS (*m/z*): 187 (M^+ + 1); *Anal*. calcd. for $C_{10}H_6N_2O_2$: C, 64.52; H, 3.25; N, 15.05. Found: C, 64.41; H, 3.17; N, 14.86.

Table 1

Minimum inhibitory concentrations (MIC-μg/mL) of the title compounds. Negative control DMSO, no activity.

Compound	*Bacillus subtilis* (Gram positive)	*Pseudomonas aeruginosa* (Gram negative)	*Streptomyces* species (actinomycetes)
2	250	125	250
3	250	250	[a]—
4	75	100	75
5	100	100	125
6	250	500	500
7	250	500	—
8	125	125	100
9	125	250	100
10	75	75	100
11	100	100	75
12	125	125	100
13	75	100	75
14	500	—	125
15	250	250	125
16	500	250	—
17	125	250	250
18	125	250	125
19	100	100	100
20	125	100	250
21	75	75	100
Penicillin	31	45	34

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

General procedure for the preparation of tetrazole derivatives 4 and 5. A solution of nitrile derivative **2** or **3** (0.01 mol), sodium azide (1.95 g, 0.03 mol), and ammonium chloride (0.53 g, 0.01 mol) in *N,N*-dimethyformamide (10 mL) was heated at 120°C for 3 h. The solvent was removed under reduced pressure; the residue was dissolved in water (100 mL) and carefully acidified with conc. hydrochloric acid at pH = 2 in ice bath. The precipitated tetrazole that separated was filtered off, dried, and recrystallized.

2-[2-(2H-Tetrazol-5-yl)ethyl]isoindoline-1,3-dione (4). Yield: 2.17 g (89.3%) as pale yellow powder; mp 196–197°C; IR(KBr) υ: 3446 (NH), 1709 (C═O), 1625 (C═N). ^{1}H NMR ($CDCl_3$) δ: 3.01 (t, 2H, *J* = 5.5 Hz, CH_2), 4.19 (t, 2H, *J* = 5.5 Hz, CH_2), 7.68–8.01 (m, 4H, Ar-H), 9.95 (bs, 1H, NH). *Anal*. calcd. for $C_{11}H_9N_5O_2$: C, 54.32; H, 3.73; N, 28.79. Found: C, 54.18; H, 3.66; N, 28.59.

2-[(2H-Tetrazol-5-yl)methyl]isoindoline-1,3-dione (5). Yield: 2.07 g (90.4%) as white powder; mp 226–227°C; IR (KBr) cm^{-1}: υ 3470 (NH), 1707 (C═O), 1624 (C═N). ^{1}H NMR ($CDCl_3$) δ: 4.35 (s, 2H, CH_2), 7.66–7.98 (m, 4H, Ar-H), 10.03 (bs, 1H, NH); *Anal*. calcd. for $C_{10}H_7N_5O_2$: C, 52.40; H, 3.08; N, 30.56. Found: C, 52.24; H, 3.02; N, 30.40.

General procedure for the preparation of ester derivatives 6 and 7. A solution of tetrazole derivative **4** or **5** (0.01 mol) and potassium carbonate (1.38 g, 0.01 mol) in *N,N*-dimethylformamide (30 mL) was stirred for 2 h. Ethyl chloroacetate (1.24 g, 0.01 mol) was added, and the mixture was stirred overnight. The mixture was poured on crushed ice. The precipitate was filtered off as white powder.

Ethyl 2-{5-[2-(1,3-dioxoisoindolin-2-yl)ethyl]-2H-tetrazol-2-yl}acetate (6). Yield: 2.2 g (66.9%); mp 83–84°C; IR (KBr) υ: 1746 (C═O), 1680 (C═O), 1618 (C═N); ^{1}H NMR ($CDCl_3$) δ: 1.25 (t, 3H, *J* = 4.8 Hz, CH_3), 3.01 (t, 2H, *J* = 5.5 Hz, CH_2), 4.09 (t, 2H, *J* = 5.5 Hz, CH_2), 4.22 (q, 2H, *J* = 4.8 Hz, CH_2), 5.16 (s, 2H, CH_2), 7.70–7.96 (m, 4H, Ar-H). MS (*m/z*): 330 ($M^+ + 1$); *Anal*. calcd. for $C_{15}H_{15}N_5O_4$: C, 54.71; H, 4.59; N, 21.27. Found: C, 54.49; H, 4.50; N, 21.19.

Ethyl 2-{5-[(1,3-dioxoisoindolin-2-yl)methyl]-2H-tetrazol-2-yl}acetate (7). Yield: 2.4 g (76%); mp 114–115°C; IR (KBr) υ: 1739 (C═O), 1675 (C═O), 1620 (C═N); ^{1}H NMR ($CDCl_3$) δ: 1.30 (t, 3H, *J* = 4.6 Hz, CH_3), 4.24 (q, 2H, *J* = 4.6 Hz, CH_2), 4.34 (s, 2H, CH_2), 5.16 (s, 2H, CH_2), 7.74–7.86 (m, 4H, Ar-H); MS (*m/z*): 316 ($M^+ + 1$); *Anal*. calcd. for $C_{14}H_{13}N_5O_4$: C, 53.33; H, 4.16; N, 22.21. Found: C, 53.20; H, 4.09; N, 22.11.

General procedure for the preparation of hydrazide derivatives 8 and 9. To a solution of the respective ester **6** or **7** (0.01 mol) in absolute ethanol (40 mL), hydrazine hydrate (1.5 g, 0.03 mol) was added. The reaction mixture was heated under reflux for 3 h. The excess of ethanol was removed under reduced pressure, and the resulting precipitate was filtered off as gray powder and crystallized from ethanol.

2-{5-[2-(1,3-Dioxoisoindolin-2-yl)ethyl]-2H-tetrazol-2-yl} acetohydrazide (8). Yield: 3.99 g (94.9%); mp burns at 234–235°C; IR (KBr) υ: 3441, 3276 (NH_2 and NH), 1690 (C═O), 1658 (C═O), 1615 (C═N); ^{1}H NMR (DMSO-d_6) δ: 3.04 (t, 2H, *J* = 5.6 Hz, CH_2), 4.15 (t, 2H, *J* = 5.6 Hz, CH_2), 5.32 (s, 2H, CH_2), 5.63 (bs, 2H, NH_2), 7.68–7.99 (m, 4H, Ar-H), 9.18 (bs 1H, NH); MS (*m/z*): 316 ($M^+ + 1$); *Anal*. calcd. for $C_{13}H_{13}N_7O_3$: C, 49.52; H, 4.16; N, 31.10. Found: C, 49.31; H, 4.05; N, 30.88.

2-{5-[(1,3-Dioxoisoindolin-2-yl)methyl]-2H-tetrazol-2-yl} acetohydrazide (9). Yield: 2.7 g (89.7%); mp burns at 219–220°C; IR (KBr) υ: 3435, 3271 (NH_2 and NH), 1691 (C═O), 1653 (C═O), 1608 (C═N); ^{1}H NMR (DMSO-d_6) δ: 4.35 (s, 2H, CH_2), 5.36 (s, 2H, CH_2), 5.66 (bs, 2H, NH_2), 7.71–8.01 (m, 5H, Ar-H), 9.25 (bs, 1H, NH); MS (*m/z*): 302 ($M^+ + 1$); *Anal*. calcd. For $C_{12}H_{11}N_7O_3$: C, 47.84; H, 3.68; N, 32.55. Found: C, 47.68; H, 3.60; N, 32.42.

General procedure for the synthesis of sugar hydrazones 10–13. A solution of the monosaccharide (0.015 mol) in least amount of water (1 mL) was treated with a solution of hydrazide (0.01 mol) in ethanol (60 mL) and few drops of glacial acetic acid. The mixture was heated under reflux for 2 h. The excess of ethanol was removed under reduced pressure, and the residue was triturated with (10 mL) diethyl ether to produce compounds **10–13** as brown viscous material.

D-Galactose-2-{5-[2-(3,1-dioxoisoindolin-2-yl)ethyl]-2H-tetrazol-2-yl}acetohydrazone (10). Yield: 3.5 g (73.4%). 3442–3292 (OH and NH), 1688 (C═O), 1661 (C═O), 1615 (C═N); ^{1}H NMR (DMSO-d_6) δ 3.09 (t, 2H, *J* = 5.5 Hz, CH_2), 3.25–3.44 (m, 3H, H-5, H-6, H-6′), 3.94–4.18 (m, 2H, H-3, H-4), 4.29 (m, 1H, H-2), 4.35 (t, 2H, *J* = 5.5 Hz, CH_2), 4.42 (bs, 1H, OH), 4.60 (m, 2H, 2xOH), 5.05 (m, 2H, 2xOH), 5.37 (s, 2H, CH_2), 7.44 (d, 1H, *J* = 2.5 Hz, H-1), 7.73–7.97 (m, 4H, Ar-H), 11.60 (bs, 1H, NH); MS (*m/z*): 478 ($M^+ + 1$); *Anal*. calcd. for $C_{19}H_{23}N_7O_8$: C, 47.80; H, 4.86; N, 20.54. Found: C, 47.66; H, 4.81; N, 20.38.

D-Xylose2-{5-[2-(3,1-dioxoisoindolin-2-yl)ethyl]-2H-tetrazol-2-yl}acetohydrazon (11). Yield: 3.3 g (71.6%). 3448–3297 (OH and NH), 1685 (C═O), 1660 (C═O), 1618 (C═N); ^{1}H NMR (DMSO-d_6) δ 3.07 (t, 2H, *J* = 5.8 Hz, CH_2), 3.25–3.51 (m, 3H, H-4, H-5, H-5′), 4.14 (m, 1H, H-3), 4.32 (m, 1H, H-2), 4.39 (t, 2H, *J* = 5.4 Hz, CH_2), 4.50 (bs, 2H, 2xOH), 5.20 (m, 2H, 2xOH), 5.42 (s, 2H, CH_2), 7.53 (d, 1H, *J* = 2.5 Hz, H-1), 7.73–7.97 (m, 4H, Ar-H), 11.50 (bs, 1H, NH); MS (*m/z*): 448 ($M^+ + 1$); *Anal*. calcd. For $C_{18}H_{21}N_7O_7$: C,

48.32; H, 4.73; N, 21.91. Found: C, 48.19; H, 4.64; N, 21.79.

D-Galactose-2-{5-[(1,3-dioxoisoindolin-2-yl)methyl]-2H-tetrazol-2-yl}acetohydrazone (12). Yield: 3.5 g (75.5%); IR (KBr) υ: 3393 (OH), 3209 (NH), 1665 (C═O), 1608 (C═N); %). ^{1}H NMR (DMSO-d_6) δ 3.25–3.92 (m, 3H, H-5, H-6, H-6′), 4.11–4.15 (m, 1H, H-3,4), 4.32 (s, 2H, CH_2), 4.42 (m, 1H, H-2), 4.48 (bs, 1H, OH), 4.62 (m, 2H, 2xOH), 5.03 (m, 2H, 2xOH), 5.34 (s, 2H, CH_2), 7.45 (d, 1H, J = 2.5 Hz, H-1), 7.70–7.98 (m, 4H, Ar-H), 11.58 (bs, 1H, NH); MS (*m/z*): 464 (M^+ + 1); *Anal*. calcd. for $C_{18}H_{21}N_7O_8$: C, 46.65; H, 4.57; N, 21.16. Found: C, 46.51; H, 4.51; N, 21.02.

D-Xylose-2-{5-[(1,3-dioxoisoindolin-2-yl)methyl]-2H-tetrazol-2-yl} acetohydrazone (13). Yield: 3 g (69%); IR (KBr) υ: 3398 (OH), 3200 (NH), 1661 (C═O), 1612 (C═N); %). ^{1}H NMR (DMSO-d_6) δ 3.20–3.74 (m, 3H, H-4, H-5, H-5′), 4.12 (m, 1H, H-3), 4.35 (s, 2H, CH_2), 4.45 (m, 1H, H-2), 4.65 (m, 2H, 2xOH), 5.15 (m, 2H, 2xOH), 5.35 (s, 2H, CH_2), 7.457 (d, 1H, J = 2.5 Hz, H-1), 7.75–7.99 (m, 4H, Ar-H), 11.18 (bs, 1H, NH); MS (*m/z*): 434 (M^+ + 1); *Anal*. calcd. for $C_{17}H_{19}N_7O_7$: C, 47.11; H, 4.42; N, 22.62. Found: C, 46.92; H, 4.38; N, 22.50.

General procedure for the synthesis of *O*-acetylated sugar hydrazones 14–17. To a solution of sugar hydrazones **10–13** (0.01 mol) in pyridine (15 mL), acetic anhydride (3.06 g, 0.03 mol) was added. The reaction mixture was stirred for 5 h. The mixture was cooled and poured on crushed ice; add hydrochloric acid with stirring until the odor of pyridine was removed and extracted by chloroform. The product was separated in pure form as brown viscous material.

2,3,4,5,6-Penta-O-acetyl-D-(+)-galactose-{2-[(1,3-dioxoisoindolin-2-yl)ethyl]-2H-tetrazol-2-yl}acetylhydrazone (14). Yield: 5.1 g (74.2%); IR (KBr) υ: 3423 (NH), 1737 (C═O), 1660 (C═N); ^{1}H NMR (DMSO-d_6) δ 1.95, 2.02, 2.10, 2.13, 2.16 (5s, 15H, 5xCH_3CO), 3.04 (t, 2H, J = 4.8 Hz, CH_2), 4.09 (m, 2H, H-6,6′), 4.20 (t, 2H, J = 4.8 Hz, CH_2), 4.50 (m, 1H, H-5), 4.71 (m, 1H, H-4), 5.18 (dd, 1H, J = 6.8 Hz, J = 9.4 Hz, H-3), 5.40 (s, 2H, CH_2), 5.48 (t, 1H, J = 9.8 Hz, H-2), 7.46 (d, 1H, J = 9.8 Hz, H-1), 7.74–8.00 (m, 4H, Ar-H), 11.30 (bs, 1H, NH); MS (*m/z*): 688 (M^+ + 1); *Anal*. calcd. for $C_{29}H_{33}N_7O_{13}$: C, 50.66; H, 4.84; N, 14.26. Found: C, 50.49; H, 4.79; N, 14.14.

2,3,4,5-Tetra-O-acetyl-D-(+)-xylose-{2-[(1,3-dioxoisoindolin-2-yl)ethyl]-2H-tetrazol-2-yl}acetylhydrazone (15). Yield: 4.3 g (71.5%); IR (KBr) ν 1525 cm^{-1} (C═N), 1750 cm^{-1} (C═O), 3439 cm^{-1} (NH); ^{1}H NMR (DMSO-d6) δ: 1.95, 2.03, 2.11, 2.15 (4s, 12H, 4xCH_3CO), 3.06 (t, 2H, J = 4.0 Hz, CH_2), 4.17 (m, 2H, H-5,5′), 4.29 (t, 2H, J = 4.1 Hz, CH_2), 4.39 (m, 1H, H-4), 5.38 (s, 2H, CH_2), 5.74 (m, 1H, H-3), 5.88 (dd, 1H, J = 6.8 Hz, J = 9.2 Hz, H-2), 7.42 (d, 1H, J = 9.2 Hz, H-1), 7.72–8.02 (m, 4H, Ar-H), 10.69 (bs, 1H, NH).; MS (*m/z*): 616 (M^+ + 1); *Anal*. calcd. for $C_{26}H_{29}N_7O_{11}$: C, 50.73; H, 4.75; N, 15.93. Found: C, 50.50; H, 4.70; N, 15.76.

2,3,4,5,6-Penta-O-acetyl-D-(+)-galactose-{2-[(1,3-dioxoisoindolin-2-yl)methyl]-2H-tetrazol-2-yl}acetylhydrazone (16). Yield: 4.75 g (70.5%). IR (KBr) υ: 3411 (NH), 1722 (C═O), 1662 (C═O), 1627 (C═N); ^{1}H NMR (DMSO-d_6) δ 1.96, 2.04, 2.11, 2.14, 2.17 (5s, 15H, 5xCH_3CO), 4.10 (m, 2H, H-6,6′), 4.37 (s, 2H, CH_2), 4.51 (m, 1H, H-5), 5.42 (s, 2H, CH_2), 4.69 (m, 1H, H-4), 5.17 (m, 1H, H-3), 5.45 (dd, J = 6.4 Hz, J = 8.8 Hz, 1H, H-2), 7.42 (d, 1H, J = 9.2 Hz, H-1), 7.72-8.01 (m, 4H, Ar-H), 11.27 (bs, 1H, NH); MS (*m/z*): 674 (M^+ + 1), *Anal*. calcd. for $C_{28}H_{31}N_7O_{13}$: C, 49.93; H, 4.64; N, 14.56. Found: C, 49.71; H, 4.58; N, 14.38.

2,3,4,5-Tetra-O-acetyl-D-(+)-xylose-{2-[(1,3-dioxoisoindolin-2-yl)methyl]-2H-tetrazol-2-yl}acetylhydrazone (17). Yield: 4.55 g (75%). IR (KBr) υ: 3411 (NH), 1732 (C═O), 1664 (C═O), 1626 (C═N); ^{1}H NMR (DMSO-d_6) δ 1.97, 2.04, 2.12, 2.15 (5s, 15H, 5xCH_3CO), 4.12 (m, 2H, H-6,6′), 4.36 (s, 2H, CH_2), 4.52 (m, 1H, H-5), 5.44 (s, 2H, CH_2), 4.72 (m, 1H, H-4), 5.21 (m, 1H, H-3), 5.47 (dd, J = 6.4 Hz, J = 8.8 Hz, 1H, H-2), 7.45 (d, 1H, J = 9.2 Hz, H-1), 7.75–7.99 (m, 4H, Ar-H), 11.25 (bs, 1H, NH); MS (*m/z*): 602 (M^+ + 1); *Anal*. calcd. for $C_{25}H_{27}N_7O_{11}$: C, 49.92; H, 4.52; N, 16.30. Found: C, 49.77; H, 4.37; N, 16.19.

General procedure for the synthesis oxadiazolin derivatives 18–21. A solution of sugar hydrazone **10–13** (0.01 mol) in acetic anhydride (30 mL) was heated at 100°C for 3 h. The mixture was cooled and poured on crushed ice and separated from chloroform extract in pure form as brown viscous material.

4-Acetyl-5-(1,2,3,4,5-penta-O-acetyl-D-galactopentitolyl)-5-{2-[(1,3-dioxoisoindolin-2-yl)ethyl)-2H-tetrazol-2-yl)methyl]-2,3-dihydro}-1,3,4-oxadiazoline (18). Yield: 6.3 g (86.4%); IR (KBr): ν 1730 (C = O), 1683 (C = O), 1620 (C = N); ^{1}H NMR ($CDCl_3$): δ 1.95, 1.96, 2.01, 2.10, 2.19, 2.28 (6s, 18H, 6xCH_3CO), 3.02 (t, 2H, J = 4.4 Hz, CH_2), 3.98 (m, H, H-5), 4.05 (dd, H, J = 2.8 Hz, J = 11.2 Hz, H-5′), 4.21 (t, 2H, J = 4.5 Hz, CH_2), 4.86 (m, 1H, H-4), 5.16 (m, 1H, H-3), 5.20 (t, 1H, J = 7.4 Hz, H-2), 5.30 (s, 2H, CH_2), 5.37 (dd, 1H, J = 8.8, 7.4 Hz, H-1′), 5.89 (d, 1H, J = 8.8 Hz, oxadiazoline H-5), 7.70-8.01 (m, 4H, Ar-H); MS (*m/z*): 730 (M^+ + 1); *Anal*. calcd. for $C_{31}H_{35}N_7O_{14}$: C, 51.03; H, 4.83; N, 13.44. Found: C, 50.80; H, 4.76; N, 13.32.

4-Acetyl-5-(1,2,3,4-tetra-O-acetyl-D-xylotetritolyl)-5-{2-[(1,3-dioxoisoindolin-2-yl)ethyl)-2H-tetrazol-2-yl)methyl]-2,3-dihydro}-1,3,4-oxadiazoline (19). Yield: 4.9 g (74.5%); IR (KBr): ν 1733 (C═O), 1680 (C═O), 1618 (C═N); ^{1}H NMR ($CDCl_3$): δ 1.97, 1.99, 2.03, 2.17, 2.29 (5s, 15H, 5xCH_3CO), 3.05 (t, 2H, J = 4.4 Hz, CH_2), 3.97 (m, 1H, H-4), 4.03 (dd, H, J = 2.8 Hz, J = 11.2 Hz, H-4′), 4.24 (t, 2H, J = 4.5 Hz, CH_2), 5.15 (m, 1H, H-3), 5.18 (t, 1H, J = 7.4 Hz, H-2), 5.32 (s, 2H, CH_2), 5.39 (dd, 1H, J = 8.8, 7.4 Hz, H-1), 5.87 (d, 1H, J = 8.8 Hz, oxadiazoline H-5), 7.73–7.99 (m, 4H, Ar-H); MS (*m/z*): 658 (M^+ + 1); *Anal*. calcd. for $C_{28}H_{31}N_7O_{12}$: C, 51.14; H, 4.75; N, 14.91. Found: C, 50.92; H, 4.68; N, 14.81.

4-Acetyl-5-(1,2,3,4,5-penta-O-acetyl-D-galactopentitolyl)-5-{2-[(1,3-dioxoisoindolin-2-yl)methyl)-2H-tetrazol-2-yl)methyl]-2,3-dihydro}-1,3,4-oxadiazoline (20). Yield: 6.5 g (78.3%); IR (KBr): ν 1734 (C═O), 1683 (C═O), 1621 (C═N); ^{1}H NMR ($CDCl_3$): δ 1.96, 1.97, 2.06, 2.12, 2.18, 2.27 (6s, 18H, 6xCH_3CO), 3.98 (m, 1H, H-5), 4.07 (dd, H, J = 2.8 Hz, J = 11.8 Hz, H-5′), 4.33 (s, 2H, CH_2), 4.93 (m, 1H, H-4), 5.09 (m, 1H, H-3), 5.14 (t, 1H, J = 7.6 Hz, H-2), 5.32 (s, 2H, CH_2), 5.37 (dd, 1H, J = 7.4 Hz, 8.6 Hz, H-1), 5.86 (d, 1H, J = 8.6 Hz, oxadiazoline H-5), 7.69–8.03 (m, 4H, Ar-H); MS (*m/z*): 716 (M^+ + 1); *Anal*. calcd. for $C_{30}H_{33}N_7O_{14}$: C, 50.35; H, 4.65; N, 13.70. Found: C, 50.01; H, 4.57 N, 13.49.

4-Acetyl-5-(1,2,3,4-tetra-O-acetyl-D-xylotetritolyl)-5-{2-[(1,3-dioxoisoindolin-2-yl)methyl)-2H-tetrazol-2-yl)methyl]-2,3-dihydro}-1,3,4-oxadiazoline (21).Yield: 5.2 g (80.8%); IR (KBr): ν 1739 (C═O), 1672 (C═O), 1618 (C═N); ^{1}H NMR ($CDCl_3$): δ 1.95, 1.96, 2.11, 2.15, 2.28 (5s, 15H, 5xCH_3CO), 3.94 (m, 1H, H-4), 4.07 (dd, H, J = 2.8 Hz, J = 11.6 Hz, H-4′), 4.39 (s, 2H, CH_2), 5.02 (m, 1H, H-3), 5.12 (t, 1H, J = 7.6 Hz, H-2), 5.39 (s, 2H, CH_2), 5.35 (dd, 1H, J = 7.4 Hz, 8.6 Hz, H-1), 5.85 (d, 1H, J = 8.6 Hz, oxadiazoline H-5), 7.71–8.04 (m, 4H, Ar-H); MS (*m/z*): 644 (M^+ + 1); *Anal*. calcd. for $C_{27}H_{29}N_7O_{12}$: C, 50.39; H, 4.54; N, 15.24. Found: C, 50.22; H, 4.41; N, 15.08.

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

Sample preparation. Each of the test compounds and standards was dissolved in 12.5% DMSO at concentrations of 500 μg/mL. Further dilutions of the compounds and standards were made in test medium.

Culture of microorganisms. Bacterial strains were supplied from the Botany Department, Faculty of Science, Menoufia University, Egypt, namely *B. subtilis* (ATCC 6633) (gram positive), *P. aeruginosa* (ATCC 27853) (gram negative) and *Streptomyces* species (actinomycetes). The bacterial strains were maintained on MHA (Mueller–Hinton agar) medium (Oxoid, Chemical Co.) for 24 h at 37°C. The medium was molten on a water bath, incubated with 0.5 mL of the culture of the specific microorganism, and poured into sterile Petri dishes to form a layer of about 3–4 mm. The layer was allowed to cool and harden. With the aid of cork-borer, cups of about 10-mm diameter were produced [29].

Agar diffusion technique. Antibacterial activities were tested using MH medium (17.5 g case in hydrolysate, 1.5 g soluble starch, 1000 mL beef extract). A stock solution of each synthesized compound (500 μg/mL) in DMSO was prepared and incorporated in 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°C overnight. The inhibition zones were measured after 24 h. The minimum inhibitory concentration (MIC) was defined as the intercept of the grave of logarithm concentrations versus diameter of the inhibition zones [30].

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