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Synthesis and Antibacterial Activity of some 5,5′-(1,4-phenylene)-bis-1,3,4-Oxadiazole and bis-1,2,4-Triazole Derivatives as Precursors of New *S*-Nucleosides

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ABSTRACT

Five compounds, namely 5,5′-benzene-1,4-diylbis(1,3,4-oxadiazole-2-thiol)6 and 5,5′-benzene-1,4-diylbis(1*H*-1,2,4-triazole-3-thiol) 7a and its derivatives 7b–d were synthesized. Two related *S*-nucleosides 9 and 10 have been prepared from 6 and 7a. Some of these synthesized compounds were tested *in vitro* by spotting on Mueller Hinton Agar medium against some Gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis* and three Gram-negative bacteria *Escherichia coli, Pseudomonasaeruginosa, Pseudomonas fluorescens* and compared with the known antibiotics cephalosporin (cefotaxim) and gentamycin. Compound 6 showed significant inhibitory activity against Gram-positive *E. faecalis* and Gram-negative *E. coli* bacteria while the others have shown variable inhibition activity.

KEYWORDS

Terephthalic acid, 1,3,4-oxadiazole, 1*H*-1,2,4-triazole, *S*-nucleosides, antimicrobial activity.

1. Introduction

The emergence of bacterial resistance to β-lactam antibiotics, macrolides, quinolones and vancomycin is becoming a major worldwide health problem, with antibiotic resistance among Gram-positive bacteria becoming increasingly serious.[1,2] These facts inspired us to synthesize new compounds with potential anti-bacterial and anti-viral inhibitory effect such as 1,3,4-oxadiazoles, 1,2,4-triazoles and nucleosides related to them. Nucleoside analogues constitute an important class of therapeutic agents in the treatment of cancers and viral infections. The mode of action of these derivatives is based upon their intracellular conversion to their phosphorylated forms (nucleotides), which can be used in cellular biosynthesis.[3,4] The literature shows intensive work describing various kinds of nucleoside analogues concerning chemical modification of nucleobases[5–7] and/or the sugar moieties,[8–10] and more complicated analogs like dinuclear,[11] single or multiple-headed nucleosides.[12–13] Some related single *S*-nucleosides in two instances were found to have a wide range of biological activity, such as enzyme inhibitory, anti-bacterial,[14,15] anti HIV-1 inhibitory,[16] anti-tumor,[17] anti-inflammatory,[18] anti-fungal[19] and anti-cancer action.[20] Although a tremendous amount of work has been done in the synthesis of new series of nucleoside analogues, none regarding synthesis and biological activity of multiple *S*-nucleosides were reported. This prompted us to choose synthesizing the intermediates 5,5′-benzene-1,4-diylbis(1,3,4-oxadiazole-2-thiol) **6** and 5,5′-benzene-1,4-diylbis(1*H*-1,2,4-triazole-3-thiol) **7a**, starting from terephthalic acid (TPA), as precursors for building multiple *S*-nucleosides **9** and **10**. TPA has been considered to be non-genotoxic chemical compound and it is a mild respiratory tract and eye irritant, but it was found to be toxic at high doses.[21] Studies have shown that variation of the carboxylate functions of TPA resulted in a reduced ulcerogenic effect, while retaining the anti-inflammatory and analgesic activities.[22–25] Furthermore, substituted 1,3,4-oxadiazole and 1,2,4-triazole derivatives have been reported to show a broad spectrum of biological activities including anticancer,[26,27] antibacterial,[28–30] anti-inflammatory and analgesic effects.[22] We anticipated that the oxadiazole and triazole derivatives of terephthalic acid may show significant biological activity. Thus all the synthesized derivatives from TPA represented by compounds **4–7c** were synthesized for evaluation of their preliminary *in vitro* antibacterial activity against some Gram-positive and Gram-negative bacterial strains.

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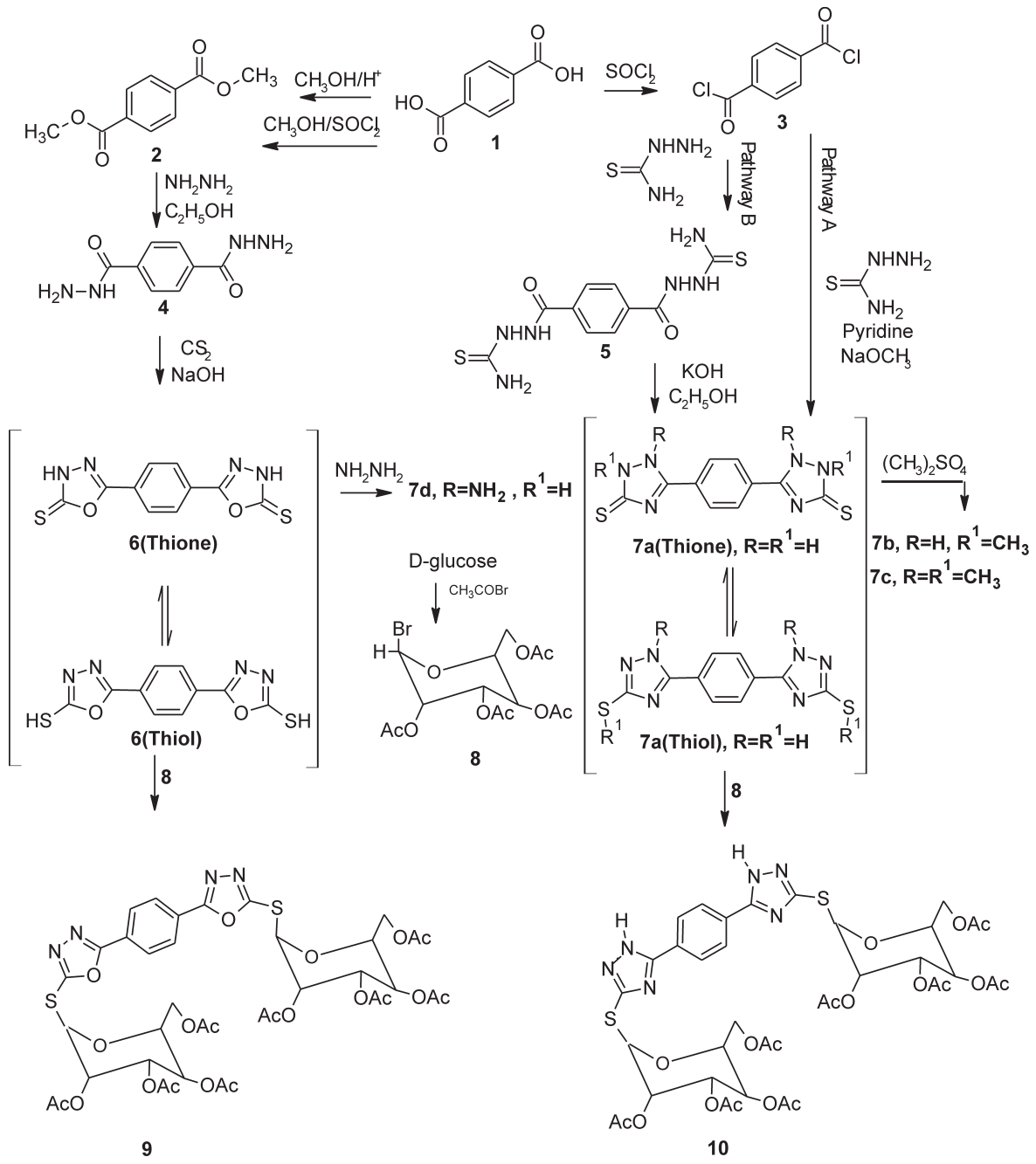
2. Results and Discussion

2.1. Synthesis

The bisoxadiazole **6** was synthesized according to Scheme 1. The ester **2** has been prepared by two methods, either by refluxing the acid **1** in methanol with catalytic amount of sulphuric acid or by treating TPA with thionyl chloride in methanol under reflux. In both cases the yield exceeded 90 %.

The hydrazide **4** was isolated in 85 % yield when the ester was refluxed with 64 % hydrazine. The yield was reduced appreciably when the ester was treated with 90 % hydrazine. The bisoxadiazole **6** was obtained after refluxing the hydrazide **4** with CS_2 in methanol in presence of sodium hydroxide to give **6** as a high melting yellow crystallinein 80 % yield.[31–33] The bisoxadiazole **6** exists largely in the form of thione with a trace of thiol form as shown by strong absorption in IR at 1421 cm^{-1} for C=S and a weak peak appeared at 2360 cm^{-1} for SH stretching band. This was confirmed by ^{1}H-NMR and ^{13}C-NMR as exhibited by C=S signal at 206 ppm.[39] The mass spectrum showed molecular ion at *m*/*z* 277.9933.



Scheme 1
Synthetic pathway for the preparation of compounds **6**, **7a–d**, **8**, **9** and **10**.

The synthesis of bistriazole **7a**[34–38] was achieved *via* formation of terephthalolyl chloride **3** which was prepared from TPA and thionyl chloride in an excellent yield. The yields varied between 95 % and 98 % depending upon the concentration of thionyl chloride in the reaction medium. Using excess of thionyl chloride without any other solvent gave higher percentage of **3** and shortened the reaction time from 24 h to 5 h. Two pathways **A** and **B** were followed to reach **7a** from **3**. Pathway **A**, the direct method, consisted of treating **3** with thiosemicarbazide in pyridine solution, the adduct without isolation was then treated with methanolic solution of freshly prepared sodium methoxide to give **7a** in good yield (72 %). IR spectrum of the product **7a** suggested the thione form predominated, as exhibited by the characteristic band for C=S at 1230 cm^{-1}.[39]

The second pathway **B**, the indirect method, used to the synthesize **7a**, included the preparation and isolation of **5** by means of treating **3** with thiosemicarbazide. The structure **5** was established based on IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. Its IR spectrum had the characteristic bands for NH, CON and CSNH at 3275, 1669 and 1267 cm^{-1}, respectively. ^{1}H-NMR confirmed the presence of NH by the signal at 10.49 ppm. ^{13}C-NMR had signals at 127.6 ppm (for aromatic carbons), 165.0 ppm (CON) and 181.9 ppm (for the thiourea carbon $CSNH_2$).[39] Base-catalyzed cyclization of **5** in the presence of KOH gave **7a** in an excellent yield of 96 %, identical to that obtained by the direct method. The product **7a** was poorly soluble in $CHCl_3$ and DMSO which made it difficult to perform ^{1}H-NMR and ^{13}C-NMR spectroscopic measurements. Monomethyl **7b** and dimethyl **7c** derivatives were prepared to overcome this problem. The monomethyl triazole **7b** was prepared in 75 % yield by treating **7a** with one molar equivalent of dimethylsulfate. ^{1}H-NMR spectrum of **7b** in DMSO exhibited a singlet at 3.2 ppm

integrated for 6H assigned for the two S-CH_3 groups. ^{13}C-NMR spectrum of the same compound exhibited the characteristic carbons at δ 15.1 (S-CH_3), 127.6 (aromatic), 131.6 (aromatic), 159.7 (C-3 heterocyclic) and 160.6 ppm (C-5 heterocyclic).[37] Its mass spectrum displayed MH^+ at *m/z* 305.0651 which is in accord with the molecular formula $C_{12}H_{13}N_6S_2$. The dimethyl triazole **7c** was obtained in 72 % yield by treating **7a** with a six-fold excess of dimethylsulfate. Compound **7c** had a lower m.p. of 193–197 °C, and gave an ^{1}H-NMR spectrum which exhibited two singlets at 3.90 and 2.95 ppm, each integrated for H. The first was attributed to the two N-CH_3 groups and the other to the two S-CH_3 groups. ^{13}CNMR for had heteroaromatic C signals at 161.3, 153.6, 131.4, 126.2, 35.3 and 15.8 ppm.[40] The mass spectrum of compound **7c** displayed MH^+at *m/z* 333.0951 which is in agreement with the molecular formula $C_{14}H_{17}N_6S_2$.

5,5′-Benzene-1,4-diylbis(1-amino-1*H*-1,2,4-triazole-3-thiol) **7d**[41–43] was prepared by treating bisoxadiazole **6** with hydrazine. IR spectroscopy showed an absorption band at 2360 cm^{-1} for SH and 1423 cm^{-1} for C=S, while ^{1}H-NMR exhibited a signal at 5.80 ppm for NH_2. The mass spectrum of **7d** showed at *m/z* 306.0475 which is in agreement with its molecular formula $C_{10}H_{10}N_8S_2$.

The primary attempt to form the *S*-nucleosides **9** and **10** was done by linking oxadiazole **6** and triazole **7a** with glucopyranosyl bromide **8** by an aid of triethylamine at room temperature for 24 hours. TLC, IR and ^{1}H-NMR for **9** and **10** showed glycosidation of the SH of **6** and **7a** by means of disappearance of both the SH band in the IR spectrum and SH signal in the ^{1}H-NMR spectrum at δ 2.51 ppm, with the appearance of sugar protons (see experimental section). The incorporation of sugar moieties was also supported by mass spectra of **9** which showed MH^+at *m/z* 939.1917 and of **10**, having MH^+ at *m/z* 937.2238.

3. Antibacterial Evaluations

Since TPA is known to be a non-genotoxic compound and anti-bacterial inhibitor,[21] the inhibitory effect of its derivatives **4–7a–c** in DMSO (10 % v:v)were tested upon *in vitro* against Gram-positive, *Staphylococcus aureus* ATCC25923 and *Enterococcus faecalis* ATTC 29212 and three Gram-negative bacteria *Escherichia coli* ATCC 25924, *Pseudomonas aeruginosa* ATCC 10145, *Pseudomonas fluorescens.* DMSO, which is known as bacterial static in the above-mentioned concentration, was used as negative control and standard disks (Mast Diagnostics, UK), saturated with known antibiotic Cefotaxim and Gentamycin as positive control were applied. After incubation at 37 °C for 24 h, the zone of inhibition of growth around each disk was measured in millimetres and zone diameters were interpreted in accordance with CLSI and NCCLS (for *Campylobacter* spp.) guidelines (CLSI, 2006; NCCLS, 2003, 2005).[44–46] The experiments were performed in duplicates and the average results are summarized in Table 1.

Furthermore, the minimum inhibition concentrations were determined in triplicates against the test bacteria and the average results shown in Table 2. Cefotaxim's MIC interpretive breakpoints were used as standard as follows: ≤1 μg mL^{-1}, susceptible; ≥1–4 μg mL^{-1}, intermediate; ≥4 μg mL^{-1}, resistant.[50,51] Hydrazide **4** and thiosemicarbazide **5** showed no effect on any examined bacteria at concentration higher than 5.0 μg mL^{-1}. Compound **6** showed an intermediate effect on *E. faecalis* and *E. coli* at concentration (1.25 μg mL^{-1}), triazole **7a** exhibited asimilar effect on *P. aeruginosa* while methyl triazole **7b** showed a similar effect on *P. aeruginosa* and *E. coli* at the same concentration. The highest effect was observed by dimethyl triazole **7c** upon *E. coli* at lowest concentration (0.36 μg mL^{-1}).

4. Experimental

4.1. General

All reactions were monitored by TLC, silica gel F_{254}, made by Merck, Germany. The melting points were measured with a BÜCHI 540 melting point apparatus and are uncorrected. The IR spectra were recorded using KBr discs in a GENESIS II FTIR spectrophotometer, in units of cm^{-1}. The ^{1}H and ^{13}C NMR spectra in **1d** were recorded on a Bruker AC 250 M spectrometer in either DMSO-d_6 or $CDCl_3$ and referenced to TMS. Coupling constants, *J,* are given in Hz, and chemical shifts in ppm. The mass spectra were recorded on a Micro Tof-Q 98 mass spectrometer at Faculté de chimie des matériaux, Université de Luxembourg, Luxembourg', using glycerol as matrix, and reported in units of *m/z.* Microorganisms in this study were supplied by the university hospital of Oran and identified by the laboratory of applied microbiology, University of Oran EsSenia. The Mueller Hinton medium was supplied by (Difco).

4.2. *Dimethyl Terephthalate* **2**

Method A: terephthalic acid **1** (18.33 g, 0.05 mol), methanol (200 mL) and H_2SO_4 (5 mL) were heated under reflux for 4 h. Solid $NaHCO_3$ was added to neutralize the acid to pH 7 and filtered off. The filtrate was evaporated to dryness under vacuum to give white solid, recrystallized from ether-petroleum ether 40–60 °C to give colourless crystalline dimethyl terephthalate **2** (19.25 g, 93 %). M.p. 141–142 °C (lit. 141–142 °C).[52] δ_H(DMSO-d_6, 250 MHz) 8.1 (4H, s, H-aromatic), 3.9(6H,s, OCH_3); δ_C(DMSO-d_6, 62.5 MHz) 51.6, 135.0, 128.0 and 165.0.

Method B: TPA**1** (18.33 g, 0.05 mol), methanol (200 mL) and

Table 1 Antibacterial screening of compounds **4–7a–c*** by a disk diffusion method in Mueller Hinton medium incubated at 37 °C, measured in mm.

Compound	Gram-positive		Gram-negative		
	S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*	*P. fluorescens*
4	–	–	–	–	–
5	10(R)†	–	–	–	–
6	9(R)	16(S)	23(S)	–	9(R)
7a	–	–	–	10(R)	–
7b	10(R)	10(R)	9(R)	12(R)	10(R)
7c	–	9(R)	9(R)	12(R)	9(R)
DMSO (10 % v:v)	–	–	–	–	–
Gentamycin	29(S)	29(S)	25(S)	12(R)	11(R)
Cefotaxim	33(S)	32(S)	29(S)	20(I)	11(R)

Key to the inhibition zones activities. * Concentration 10 μg mL^{-1}. †Abbreviations: S, susceptible; I, intermediate ; R, resistant. For gentamycin: S ≥15 mm; I = 13–14 mm; R ≤12 mm, and for cefotaxim: S ≥32 mm; I = 15–22 mm; R ≤14 mm.[50]

Table 2 Inhibition of microorganisms by compounds **4–7a–c** at minimum concentrations after 24 h.

Compound	Gram-positive		Gram-negative		
	S. aureus /μg mL^{-1}	*E. faecalis* /μg mL^{-1}	*E. coli* /μg mL^{-1}	*P. aeruginosa* /μg mL^{-1}	*P. fluorescens* /μg mL^{-1}
4	10.00	10.00	10.00	10.00	10.00
5	5.00	10.00	10.00	10.00	10.00
6	5.00	1.25	1.25	10.00	5.00
7a	10.00	10.00	10.00	1.25	10.00
7b	5.00	5.00	1.25	1.25	5.00
7c	10.00	5.00	0.63	5.00	5.00

thionyl chloride, (20 mL) were heated under reflux for 1 h. Working up as above, gave similar results.

4.3. *Terephthalolyl Dichloride* **3**

Method A: TPA (0.50 g, 0.01 mol) and thionyl chloride (15 mL) were heated under reflux for 5 h until the acid was dissolved completely. Excess reagent was removed under vacuum to give the bis-acyl chloride terephthalate **3** as yellowish solid (0.60 g, 98 %), m.p. 82–84.5 °C (lit. 79–81 °C); δ_H(DMSO-d_6, 250 MHz) 8.21 (4H, s, H-aromatic); δ_C(DMSO-d_6, 62.5 MHz) 130.1, 138.0 and 166.3.

Method B. TPA (8.30 g, 0.50 mol), chloroform (100 mL) and thionyl chloride (16 mL), added to it DMF (5 mL) dropwise while stirring at room temperature. The mixture was refluxed for 24 h at 80 °C. The solvents were removed under vacuum to give a yellowish crystalline **3** (9.65 g, 95 %), m.p. 82–84.5 °C.

4.4. *Terephthalicdihydrazide* **4**

Dimethyl terephthalate **2** (6.00 g, 0.03 mol), absolute ethanol (60 mL) and 64 % hydrazine hydrate (20 mL) were heated under reflux at 110 °C for 4 h. The dihydrazide (4) precipitated and was filtered off. The filtrate was evaporated to dryness to give an additional solid which was combined with the previous batch and recrystallized from water to give a yellowish-white solid **4** (5.10 g, 85 %), m.p. > 350 °C; ν_{max}: 3323 (NH), 1696 (CONH), 1650 (CONH) and 1622 (C=C aromatic); δ_H(DMSO-d_6, 250 MHz) 10.57 (2H, s, 2 × CONH), 7.91(4H,s, H-aromatic), and 3.90 (4H, d, 2 × NH_2); δ_C(DMSO-d_6, 62.5 MHz) 165.0, 135.0 and 128.0.

4.5. *2,2′-(Benzene-1,4-diyldicarbonyl)dihydrazinecarbothioamide* **5**

Terephthalolyl dichloride **3** (10.00 g, 0.05 mol), THF (150 mL) and thiosemicarbazide (17.90 g, 0.21 mol) was added over a period of 15 min with an aid of stirring at room temperature. Stirring continued at room temperature for 4 h when the colour of the solution changed to green. The aqueous solution of sodium bicarbonate 5 % (100 mL) was added dropwise while stirring to give a solid precipitate. Filtered to give white crystalline material, recrystallized from methanol to give **5** (12.60 g. 83 %), m.p. 248–250 °C; ν_{max}: 3275 (NH), 1669 (CO), 1618 (CN) and 1267 (CS); δ_H(DMSO-d_6, 250 MHz) 10.49 (4H, s, 2 × NH-NH), 9.36 (4H, s, 2 × NH_2), and 8.07 (4H, s, aromatic); δ_C(DMSO-d_6, 62.5 MHz) 127.6, 135.2, 165.0, and 181.9 ; HRMS (ESI) calculated for $C_{10}H_{12}N_6O_2S_2$, 312.0462, found 312.0471 (M^+).

4.6. *5,5′-Benzene-1,4-diylbis(1,3,4-oxadiazole-2-thiol)* **6**

The hydrazide **4** (5.00 g, 0.02 mol), ethanol (60 mL), a solution of NaOH (0.16 g, 0.04 mol) in ethanol (20 mL) was added and the reaction mixture was stirred magnetically at room temperature. Carbon disulphide (15 mL) was added dropwise and the reaction mixture was refluxed at 80 °C for 3 h then allowed to cool down to room temperature. The volatile solvent was removed partially at room temperature, acidification with HCl (10 %) to pH 5–6 to give a solid precipitate which was filtered under vacuum and washed with an iced cold ethyl acetate. The filtrate was extracted twice with ethyl acetate, then evaporated to dryness to give a yellow solid which recrystallized from ethanol/petroleum ether 40–60 to give a yellow solid **6** (5.08 g, 80 %), m.p. > 350 °C; ν_{max}: 3369(NH), 2360 (SH), 1583 (C=N), 1421 (CS) and 1152 (C-O); δ_H(DMSO-d_6, 250 MHz) 10.32 (2H, s, 2 × NH), 7.81 (4H, s, aromatic), and 2.51 (2H, s, 2 × S-H); δ_C(DMSO-d_6, 62.5 MHz) 206.0, 180.0, 164.0, 162.0; HRMS (ESI) calculated for $C_{10}H_6N_4O_2S_2$277.9932, found 277.9933 (M^+).

4.7. *5,5′-Benzene-1,4-diylbis(1*H*-1,2,4-triazole-3-thiol)* **7a**

Method A: compound **3** (6.90 g, 0.03 mol) was dissolved in pyridine (60 mL), thiosemicarbazide (18.71 g, 0.06 mol) was added gradually while stirring at 0 °C then continued for 24 h at room temperature. Pyridine was removed under vacuum to give a brownish solid. The latter was dissolved in methanol (50 mL), freshly prepared sodium methoxide in methanol (Na, 3.00 g, 0.055 mol, methanol 15 mL) was added to it and refluxed for 20 h. The solvent was removed under vacuum, water (50 mL) was added cautiously and the mixture was acidified with orthophosphoric acid. The pink precipitate formed filtered off to give a solid which recrystallized from methanol/ethyl acetate to yield white crystalline **7a** (7.5 g, 72 %), m.p. > 350 °C; ν_{max}: 3324(NH), 1584 (C=N) and 1421 (CS). The compound **7a** was poorly soluble in most organic solvents which made it difficult to perform ^{1}H-NMR and ^{13}C-NMR.

Method B: compound **5** (3.14 g, 0.01 mol) was dissolved in ethanol (200 mL). Ethanolic solution of KOH (KOH, 1.71 g, in ethanol 20 mL) was added and the mixture was refluxed for 15 h. The bulk of the volatiles were removed under vacuum. The precipitate formed was diluted with water (100 mL) and filtered. The filtrate was acidified with orthophosphoric acid to give a white solid which recrystallized to white crystalline **7a** (2.6 g, 96 %), m.p. > 350 °C; ν_{max}: identical as above.

4.8. *5,5′-Benzene-1,4-diylbis[3-(methylsulfanyl)-1*H*-1,2,4-triazole]* **7b**

Compound **7a** (0.50 g, 0.01 mol) was dissolved in aqueous sodium hydroxide solution (NaOH, 0.14 g, H_2O 10 mL). Dimethylsulfate (0.12 g, 0.01 mol) was added and the mixture was refluxed for 1 h. Solvent removed under vacuum to give a white solid, recrystallized from methanol to give white crystalline **7b** (0.40 g, 75 %), m.p. >350 °C; ν_{max}: 3324 (NH) and 1618 (C=N); δ_H($CDCl_3$, 250 MHz) 9.71(1H, s, NH), 9.45(s.1H, NH), 7.96 (4H, s, H-aromatic), 3.60 (3H, s, S–CH_3) and 3.21 (3H, s, S–CH_3); δ_C($CDCl_3$, 62.5 MHz) 159.7, 151.2, 131.6, 127.6 and 15.12 ; HRMS (ESI) calculated for $C_{12}H_{13}N_6S_2$ 305.0643, found 305.0651 (MH^+).

4.9. *5,5′-Benzene-1,4-diylbis[1-methyl-3-(methylsulfanyl)-1H-1,2,4-triazole]* **7c**

Compound **7a** (1.40 g, 0.01 mol) was dissolved in aqueous potassium hydroxide solution (KOH, 1.05 g, H_2O 25 mL). Dimethylsulfate (5.00 g, 0.03 mol) was added and the mixture was stirred at room temperature for ½ h. Solvent removed under vacuum to give a white solid, recrystallized from methanol to give white crystalline **7c** (1.20 g, 75 %), m.p.193–197 °C. ν_{max}: 1618 (C=N); δ_H($CDCl_3$, 250 MHz) 8.21 (4H, s, aromatic), 3.90 (6H, s, 2 × N-CH_3), and 2.95 (6H, s. 2 × S-CH_3); δ_C($CDCl_3$, 62.5 MHz) 161.3, 153.6, 131.4, 126.2, 35.3 and 15.8 ; HRMS (APCI) calculated for $C_{14}H_{17}N_6S_2$333.0956, found 333.0951 (MH^+).

4.10. *5,5′-Benzene-1,4-diylbis(1-amino-1H-1,2,4-triazole-3-thiol)* **7d**

Oxadiazole **6** (5.00 g, 0.02 mol), ethanol (20 mL) and hydrazine hydrate 64 % (10 mL) were refluxed at 110 °C for 9 h. Ethanol was removed under vacuum; the crude **7d** was precipitated and recrystallized from methanol to give colourless crystalline **7d** (4.00 g, 71 %). M.p. > 350 °C; ν_{max}: 3433 (NH and NH_2), 2360 (SH), 1636 (C=N) and 1423 (C=S); δ_H(DMSO-d_6, 250 MHz) 9.80 (2H, s, 2 × NH), 7.85 (4H, s, aromatic), 5.80 (4H, s, 2 × NH_2) and 3.35 (2H, s, 2 × SH); δ_C($CDCl_3$, 62.5 MHz) 127.2, 131.3 and 151.8; HRMS (ESI) calculated for $C_{10}H_{10}N_8S_2$ 306.0470, found 306.0475 (M^+).

4.11. *2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl Bromide* **8**

D-Glucose (12.50 g) was dissolved in acetic anhydride (50 mL), perchloric acid ($HClO_4$) (3.0 mL) was added dropwise to a stirred solution. Temperature should not exceed 50 °C. The reaction mixture was maintained at 30–40 °C for ½ h.The reaction mixture was cooled to 20 °C, acetyl bromide (20 mL) was added dropwise followed by water (5 mL) and stirring was continued for 2 h. Chloroform (50 mL) was added followed by water (500 mL). The organic layer was washed with dilute solution of sodium bicarbonate to neutrality and dried over anhydrous $MgSO_4$. Filtration and evaporation gave a solid substance which was recrystallized from ether-petroleum ether 40–60, (v:v, 1:3) to give a colourless crystalline solid **8** (25.00 g, 57.5 %), m.p. 83–87 °C (lit. 88–89 °C);[53] ν_{max}: 1744 (CO); δ_H(DMSO-d_6, 250 MHz) 6.32 (1H, d,*J* 3.6, 1-H), 5.22 (3H, m, 2-H, 3-H, 4-H), 5.12 (1H, dd, *J* 3.27 and 3.6, 6-H), 4.81 (1H, dd, *J* 4.03 and 4.05, 6-H), 4.35 (1H, ddd, *J* 1.9, 4.05 and7.5, 5-H), 2.09, 2.07, 2.03 and 2.01 (12H, 4s, CH_3CO-4 C2″, C3″, C4″, C6″) ; δ_C(DMSO-d_6, 62.5 MHz) 20.1, 21.9, 63.4, 68.0, 70.9, 79.1, 88.9, 159.3, 171.2; HRMS (ESI) calculated for $C_{14}H_{19}BrO_9$ 410.01212, found 410.0122 (M^+).

4.12. *4-Bis[5′-S-(2″,3″,4″,6″-tetra-O-acetate-1″-S-glucosidyl)-1′,3′,4′,oxadiazo-2′-yl]phenelyne* **9**

Bisoxadiazole **6** (0.28 g, 0.01 mol) was dissolved in dimethylformamide (10 mL), triethylamine (0.4 mL) was added. The bromide **8** (1.0 g, 0.01 mol) was added and the reaction mixture was stirred at room temperature for 24 h during which the reaction mixture changed into brown. The solvents were removed under vacuum, water (50 mL) was added and extracted with CH_2Cl_2 (3 × 50 mL). The organic layer was washed three times with water (50 mL), dried over anhydrous $MgSO_4$, filtered and evaporated under vacuum to dryness at room temperature to give **9** as a yellowish syrup which solidified on standing for a week (0.75 g, 80 %); ν_{max}: 1635 and 1585 (C=C and C=N), 1152 (C-O) and 1742 (C=O); δ_H(DMSO-d_6, 250 MHz) 7.90 (4H, s, aromatic-H), 5.81, 5.75 (1H, dd, *J* 3.65 and 3.65, 1″-H), 5.10–5.20 (3H, m, 2″-H, 3″-H, 4″H), 5.00, 4.70 (1H, dd, *J* 3.67 and 7.97, 6″H), 4.13 (1H, ddd, J 3.8 and4.05, 5″-H), 2.90, 2.80, 2.71 and 2.45 (12H, 4s, 4 × CH_3CO- C″,C″,C4″,C6″) ; δ_C(DMSO-d_6, 62.5 MHz) 20.1, 21.9, 31.1, 63.8, 68.0, 70.1, 73.0, 77.9, 79.1, 87.3, 88.9, 126.9, 129.1, 159.4, 164.0, 171.3; HRMS (ESI) calculated for $C_{38}H_{43}N_4O_{20}S_2$ 939.1912, found 939.1917 (MH^+).

4.13. *1,4-Bis[5′-S-(2″,3″,4″,6″-tetra-O-acetate-1″-S-glucosidyl)-1′H-1′,2′,4′-triazo-3′-yl]phenelyne* **10**

Method A: compound **7a** (1.10 g, 0.01 mol) was dissolved in chloroform (75 mL), aqueous solution of NaOH (0.40 g, NaOH in H_2O, 25 mL) was added. Benzyl triethyl ammonium chloride (4.63 g and bromoglucoside tetra-acetate **8** (4.00 g were added to the chloroform solution and the mixture was heated under reflux for 48 h with vigorous stirring. The two layers formed were separated. The aqueous phase was extracted with chloroform (20 mL) and the extract was added to the organic phase. The latter was dried over anhydrous $MgSO_4$, filtered and evaporated under vacuum on a water bath to give brownish syrup solidified on standing **10** (3.77 g, 43 %); ν_{max} 3330 (NH), 1725 (broad C=O) and 1620 (CN); δ_H(DMSO-d_6, 250 MHz) 10.31 (2H, s, 2NH), 8.10, 7.97 (4H, s, 4H- phenylene), 6.20 (1H, d, *J* 3.75, 1″-H), 5.30 (3H, m, 2″-H, 3″-H, 4″-H), 5.00, 4.50 (2H, dddd, *J* 3.61,3.75,3.0 and 3.37, 2–6″-H), 4.35 (1H, ddd, *J* 3.37,3.75 and 4.55, 5″-H), 2.90, 2.70, 2.51 and 2.22 (12H, 4s, 4 × CH_3CO- C2″, C3″, C4″, C6″); δ_C (DMSO-d_6, 62.5 MHz) 20.4, 22.2, 32.8, 65.1, 69.0, 70.2, 72.0, 77.1, 78.7, 87.0, 89.3, 128.7, 159.9, 171.3; HRMS (ESI) calculated for $C_{38}H_{45}N_6O_{18}S_2$ 937.2232, found 937.2238 (MH^+).

Method B: compound **7a** (1.38 g, 0.05 mol), was dissolved in DMF (15 mL), triethylamine (TEA) (1.0 mL, 0.01 mol) and added to it bromoglucosidetetraacetate **8** (4.93 g, 0.01 mol). The mixture was stirred at room temperature for 24 h. Volatile solvents were removed under vacuum at low temperature. The residual syrup was dissolved in dichloromethane (50 mL) and washed twice with H_2O (20 mL). The organic layer dried over anhydrous $MgSO_4$, filtered and evaporated to dryness at low temperature to give a brownish syrup solidified on standing **10** (4.70 g, 60 %). IR and ^{1}H-NMR were similar to that found in method A.

5. Antimicrobial Susceptibility Testing[45]

5.1. *Antibacterial Tests*

A disk diffusion assay according to the standard protocols (NCCLS, 2003, 2005; CLSI, 2006) was used[42–44] to determine the susceptibility of two Gram-positive bacteria *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATTC 29212 and three Gram-negative bacteria *Escherichia coli* ATCC 25924, *Pseudomonas aeruginosa* ATCC 10145, *Pseudomonas fluorescens* using Gentamycin and Cefotaxim as references.The bacterial suspension (in 0.9 % NaCl) turbidity were adjusted to 0.5 McFarland, then the suspensions were spread with a sterile cotton swab confluently over the entire surface of Mueller Hinton agar (Merck, Germany).

The filter paper disk method was performed in duplicate using fresh Mueller Hinton agar medium. This agar medium was inoculated with 0.5 mL of cultures containing about 10^6 CFU/mL. Filter paper disks (5 mm diameter) saturated with dimethylsulphoxide (10 % DMSO v:v) solutions of each compound was placed on the indicated agar medium. The incubation time was 24 h at 37 °C. The blank test disk with DMSO was used. Inhibitory activity was evaluated by measuring the diameter of clear zone observed around the disk in mm.

5.2. *The Minimum Inhibition Concentration (MIC) Tests*

Each 1 mL of the original concentration (10 μg mL^{-1}) in DMSO of the compounds **4, 5, 6** and **7a–c** were diluted with DMSO five times in test tubes to 5.0, 2.5, 1.25, 0.625, 0.312 μg mL^{-1} and optical density at 600 nm was measured at 24 h.

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