Synthesis of novel seco-acyclo-N-diazolyl-thione nucleosides analogous derived from acetic acid: characterization, complex formation with ions Pb(II), Hg(II) and antibacterial activity

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Abstract
Three diazoles namely 5-methyl-1,3,4-oxadiazole-2(3H)-thione, 5-methyl-1,3,4-thiadiazol-2(3H)-thione and 4-amino-5-methyl-2H-1,2,4-triazole-3-thiol were synthesized from acetic acid or ethyl acetate. Seco-acyclo-N-nucleoside analogous was synthesized by condensation of 1,3-benzylidine-glyceryl-2-tosylate with the three diazoles. Structural proof was based upon IR, 1H-NMR, 13C-NMR spectroscopy and MS measurements. The tendency to form complex between 1,3,4-oxadiazole and 1,3,4-thiadiazoles and Pb(II) and Hg(II) ions was achieved, and their structures were assigned by observing some changes in physical properties such as, MP, coloration, Rf (TLC), IR and UV spectroscopy. Most compounds were tested in vitro against Gram-positive and Gram-negative bacteria and showed variable activity. Hg2+ complexes of oxadiazole and thiadiazole derivatives exhibited appreciable antibacterial effect at lower MIC, compatible to the reference vancomycin. Similarly, oxadiazole-nucleoside exhibited high effect on Gram-positive bacteria.

Graphic abstract

Keywords 1,3,4-oxadiazole · 1,3,4-thiadiazol · 1,2,4-triazole · Acetic acid · Metallic complexes · Antibacterial activity

Introduction
Interest in acyclic nucleosides started since discovery of acyclovir (A) as a potent anti-herpes drug [1–3]. The unprecedented selectivity of acyclovir as an anti-viral drug and the subsequent clarification of its behavior toward virally coded enzymes instated massive work on acyclovir and its analogues [4–6]. The literature revealed a great number of works published in synthesis

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of seco-acyclo nucleosides and glycosides as analogues to acyclovir and its derivatives [7–9].

Several variations to acyclovir basic structure have been reported such as ganciclovir and its derivatives (B) in order to increase their bioavailability, since, like acyclovir, both are poorly absorbed from the alimentary tract and only give rise to low plasma level when they are given by mouth [10–12]. Penciclovir (C) and 2,6-diamino nucleosides similar to DAPD (D) which considered as prodrugs of their corresponding guanine derivatives to which they are converted by enzymic oxidation [13].

Many modifications, included replacing of purine with triazole-thione as in (E) [14] and changing the acyclic chains by sugar moiety like glucose in (F), have been exemplified and reviewed [12, 15, 16].

Some N-acyclo non-glycosides are expected to possess notable pharmacological applications like in ritonavir (G) which is using for treating HIV/hepatitis coinfection and as treatment options for severe acute respiratory syndrome coronavirus 2(SARS-CoV-2), commonly known as COVID-19 [17].

This paper deals with the synthesis of analogues to penciclovir (C) and DAPD (D) by modifying the sugar residue and replacing the guanine heterocyclic by biologically active 5-methyl-1,3,4-oxadiazole-2(3H)-thione, 5-methyl-1,3,4-thiadiazole-2(3H)-thione/thiol and 4-amino-5-methyl-2H-1,2,4-triazole-3-thiol. Moreover, preparation of Pb(II) and Hg(II) complexes with the prepared compounds and testing their bioactivity.

Results and Discussion

Synthesis of 5-methyl-1,3,4-oxadiazole-2(3H)-thione (4), 5-methyl-1,3,4-thiadiazole-2(3H)-thione/thiol (6) and 4-amino-5-methyl-2H-1,2,4-triazole-3-thiol (7)

Nucleobases 4, 6 and 7 were synthesized by a common route as summarized in Scheme 1.

Acetic acid (1) and ethyl acetate (2) are both readily available laboratory chemicals, when treated sepa-

rately with hydrazine hydrate 80%, both resulted acetyl hydrazide (3) in corresponding yields 64% and 93%. Oxadiazole (4) was obtained in good yield after heating (3) with CS₂ in alcoholic KOH at 80 °C for 27 h., followed by addition of HCl. Thiadiazole (6) and amino-triazole (7) were obtained after treatment hydrazide (3) with CS₂ and alcoholic KOH at 0 °C, then stirred for 2 h at room temperature to yield a solid potassium thionate salt (5). The latter without further purification, when treated with H₂SO₄ the result was the thiadiazole (6), while by treating the salt with hydrazine hydrate followed by adding HCl, it furnished amino-triazole (7). Structural elucidation of 4, 6 and 7 was proved by IR, ¹H-NMR and ¹³C-NMR (see experimental section).
Synthesis of Pb(II) and Hg(II) complexes with oxadiazole 4 and thiadiazole 6

Since the diazole-thiones similar to 4 and 6 showed tendency to form complexes with Fe(II), Hg(II) and Pd(II) ions [18–21]. Thus, when alcoholic solution of 4 and 6 was treated with Pb(OAc)$_2$ and HgCl$_2$, (see Scheme 2), gave precipitates with various colors and physical properties as summarized in Scheme 2, Tables 1 and 2.

Formation of complexes can be observed by the differences between starting compounds and resulted complexes in: melting points, coloration of mp, Rf’s, of TLC, characteristic IR bands of C=S group and UV absorptions, as shown in Tables 1 and 2.

Unfortunately, $^1$H- and $^{13}$C-NMR gave no useful information about the structure of complexes due to the absence of C-H bonds within the areas of complexation.

**Synthesis of N-nucleosides**

**Preparation of PTS-glycerol (as sugar analogue)**

Glycerol (12) as triol resembles the simple acyclic sugar analogues, when treated with benzaldehyde to protect 1, 3-diol groups to give inseparable mixture of stereo isomers of 1,3-O-benzyldenedes (13) and other traces of 1,2-O-benzyldene isomers [22]. No efforts were applied to separating and identifying that traces since eventually the benzyldene

Scheme 1 Synthetic route of nucleobases 4, 6 and 7

Scheme 2 Synthesis of complexes 8–11

Table 1 Physical properties of ligand 4 and its complexes 8 and 9

| Compound | M.P °C | Color  | $R_f$ Acetone / cyclohexane (v/v) | IR: $\nu$ (cm$^{-1}$) C=S | UV: $\lambda_{max}$ nm (log $\varepsilon$) |
|----------|-------|--------|------------------|------------------|------------------|
| 4        | 68    | Brown  | 0.82 (3/2)       | 1321             | 205(0.50), 260(1.01) |
| 8        | 78    | Yellow | 0.6 (3/2)        | 1457.92          | 265(1.35)         |
| 9        | 185   | White  | 0.47 (3/2)       | 1401.03          | 250(0.31)         |

Table 2 Physical properties of ligand 6 and its complexes 10 and 11

| Compound | M.P °C | Color  | $R_f$ Acetone / cyclohexane (v/v) | IR: $\nu$ (cm$^{-1}$) C=S | UV: $\lambda_{max}$ nm (log $\varepsilon$) |
|----------|-------|--------|------------------|------------------|------------------|
| 6        | 153   | White  | 0.63 (3/2)       | 1179.26          | 265(0.56), 310(2.11) |
| 10       | > 300 | White  | 0.59 (3/2)       | 1196.61          | 265(1.54)         |
| 11       | 245   | White  | 0.56 (3/2)       | 1198.54          | 260(1.18)         |
ring will be removed by hydrolysis and end up with symmetrical moiety, 2-O-glyceryl residue (Scheme 3).

**Synthesis of seco-acyclo N-Nucleosides 15, 16 and 17**

The diazoles 4, 6 and 7 were coupled smoothly by SN2 reaction with PTS-glycerol 14 to produce seco-acyclo N-Nucleosides 15, 16 and 17. Structural proof was assigned by IR, 1H-NMR, 13C-NMR and GC–MS measurements (see experimental section) (Scheme 4).

N1-H exhibited higher nucleophilicity to take part in SN2 substitution reaction as indicated by IR and C13 NMR spectra of the products 15–17 by means of existent of C=S bands (IR) and C=S signals (C13 NMR), respectively (see experimental section).

**Antibacterial evaluation**

All synthetic compounds were assayed in vitro using the paper disk diffusion method for their antibacterial and antifungal activities against Gram-positive bacteria, *Staphylococcus aureus*, *Enterococcus faecalis* and Gram-negative bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*. In primary screening, 10 mg mL−1 concentration of the synthesized compounds was taken and then, each compound showed an activity was diluted in DMSO for obtaining concentrations: 10/2, 10/4, 10/8, 10/16 mg mL−1, respectively. Vancomycin and colistin were used as positive standards and DMSO as negative standard. The results are tabulated in Table 3.

Compounds 1–3 showed no activity against microorganisms under consideration in concentration 10 mg mL−1.

Heterocyclic compounds 4, 6, 7 exhibited various scales of effect upon microorganisms under consideration. Compounds 4 and 7 showed effect of Gram-positive bacteria *S. aureus* and *E. faecalis* only, while 6 showed a negligible effect under experimental conditions.

Hg-metal complexes 9 and 11 showed appreciable effects on Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli*, more than standards vancomycin and colistin.

Among nucleosides, only 15, which possess oxadiazole ring, showed appreciable effect on Gram-positive bacteria *S. aureus* and *E. faecalis*.

**Experimental**

**General information**

All reactions were monitored by TLC, silica gel F254, made by Merck, Germany; iodine was used for visualization. Melting points were determined with a Reichert Thermowar hot plate apparatus and are uncorrected (Universidad de Alicante). IR spectra recorded with a FT-IR 4100LE (JASCO) (PIKE MIRacle ATR) (University of Alicante, Spain) and using KBr disks in a spectrophotometer JASCO FTIR (University of Oran, Es-Senia, Algeria), wavenumbers (υ) are given in cm−1.

UV spectra were recorded by Optizen View 4.2 Spectrometer. 1H NMR and 13C NMR 300 MHz (University of Oran, Es-Senia, Algeria), 1H NMR 300, 400 or 500 MHz and 13C NMR 75 MHz (University of Alicante, Spain), spectra were obtained with a Bruker AC-300 by using TMS as the internal standard. Mass spectra were obtained using a GC–MS Agilent 5973 spectrometer (University of Alicante, Spain), fragment ions in m/z in parentheses. The antibacterial tests were carried out in the university hospital of Oran (Algeria). The Mueller Hinton medium was supplied by (Difco).

**Synthesis**

**Acetohydrazide (3) Method 1**: Ethyl acetate (8.98 g, 102 mmol), hydrazine hydrate 98% (6.76 g, 135 mmol) was added dropwise, and the mixture was refluxed for 6 h. The precipitate was filtered off, washed with hexane and recrystallized from ethanol to give white hygroscopic crystalline 3 (7.01 g, 93%); mp: 58 °C; IR υ (cm−1): 3290.93 (NH, NH2); 3055.66 (-CH3); 1662.34 (O=C-N).

**Method 2**: Acetic acid (4 g, 60 mmol) was refluxed with hydrazine hydrate 80% (3.33 g, 60 mmol) in 10 ml of absolute ethanol for 5 h. The solid obtained was filtered off, washed with cold water, recrystallized from ethanol to give white crystals 3 (3.15 g, 64%); mp. 60 °C.
5-methyl-1,3,4-oxadiazole -2(3H)-thione (4) Acetohy-drazide (3, 0.32 g, 4.3 mmol) dissolved in ethanol (8 ml) was added to an ethanolic solution of KOH (0.32 g, in ethanol 15 ml). Carbon disulfide (4 ml) was dropped gradually, and the mixture was refluxed for 27 h. The reaction mixture was cooled to room temperature, acidified with concentrated HCl to pH 5–6, filtered off. The solution was extracted several times with ethyl acetate. The combine organic extracts were evaporated and dried. The resulting brown powder was recrystallized from methanol (0.4 g, 80%); mp: 68 °C; IR υ (cm⁻¹): 3436.53 (N–H), 2932.23(C-H), 2506.04 (C-SH), 1629.55 (C=N), 1322.93 (C=S); 1078.01 (C–O–C); 1H NMR (acetone-d₆, σ H ppm): 12.51 (s, 1H, NH), 1.69 (m, 3H,  CH₃); 13C NMR (acetone-d₆, σ c ppm): 180.68 (C, C=S), 163.22(C, O=C=N), 12.30 (C, CH₃).

5-methyl-1,3,4-thiadiazol-2(3H)-thione/thiol (6) Acetohydrazide (2.66 g, 35 mmol) was added to ethanolic solution of KOH (2.29 g, 40 mmol), and the reaction mixture was cooled in an ice bath. Then,  CS₂ (3 g) was added dropwise. After addition, the reaction mixture was stirred for 2 h. The solid obtained was filtered, washed with chilled acetone, dried, and used without further purification for the subsequent reaction.

The above solid was added slowly to concentrated H₂SO₄ (15 ml) with stirring at 0 °C. After addition, the mixture was stirred for further 3 h. The reaction mixture was added to ice cold water, and the solid obtained was filtered and washed with an excess of water till the filtrate became neutral. Then, it was dried and recrystallized from acetone/ water to give (1.66 g, 35%); mp: 153 °C; IR υ (cm⁻¹): 3423.03 (N–H), 2578.36(C-SH), 1632 (C=N), 1179.26(C=S); 1H NMR (DMSO-d₆, σ H ppm): 12.96 (s, 1H, NH), 9.20 (1H, SH), 1.36 (m, 3H,  CH₃); 13C NMR (DMSO, σ c ppm): 216.03 (C, C=S), 171.82 (C, N=C-S), 20.90 (C, CH₃).

4-amino-5-methyl-2H-1,2,4-triazole-3-thiol (7) A solution of acetohydradize (2.59 g, 35 mmol) in methanol was treated with a solution of potassium hydroxide (3 g, 53 mmol) in methanol (15 ml) at 0–5 °C with stirring. CS₂ (4 ml) was added slowly, and then, the reaction mixture was stirred overnight at room temperature. The solid product was filtered, washed with diethyl ether, and dried. It was directly used for the next step without further purification.

Hydrazine hydrate (5 ml) was added dropwise to the above solid in water (20 ml) and refluxed for 20 h. The reaction mixture was cooled to room temperature, diluted with water, and acidified with concentrated HCl. The resulting

| Compounds | Gram positive Staphylococcus aureus | Inhibition zone | | Enterococcus faecalis | Inhibition zone | | Escherichia coli | Inhibition zone | | Pseudomonas aeruginosa | Inhibition zone |
|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | d | e | a | b | c | d | e | a | b | c | d | e | a | b | c | d | e |
| 1 | – | – | – | – | – | – | – | – | – | – | 7 | – | – | – | – | – | – | – | – | – |
| 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 34 | 26 | 12 | – | – | 12 | 8 | – | – | – | – | – | – | 7 | – | – | – | – | – | – |
| 6 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 32 | 18 | – | – | – | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 21 | 18 | 14 | 13 | – | – | – | – | – | – | 24 | 23 | 19 | 18 | 14 | 9 | 8 | 8 | – | – |
| 10 | 7 | – | – | – | – | – | – | – | – | – | 9 | – | – | – | – | – | – | – | – | – |
| 11 | 20 | 18 | 16 | 14 | 11 | 9 | 8 | – | – | – | 21 | 19 | 18 | 14 | 13 | – | – | – | – | – |
| 15 | 21 | 14 | 16 | 12 | 8 | 19 | 12 | 10 | – | – | 7 | – | – | – | – | – | – | – | – | – |
| 16 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 17 | – | – | – | – | – | 17 | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| * | 18 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| ** | – | – | – | – | – | 16 | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| DMSO | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

Table 3: Antibacterial activity of acetic acid 1 and its synthesized derivatives 2–7, complexes 8–11 and nucleosides 15–17 in mm

a,b,c,d,e: concentrations: (10, 10/2, 10/4, 10/8, 10/16) mg/mL respectively

Key to the inhibition zones activities: (−) for no inhibition or undone, Highly active=Inhibition zone > 15 mm; Moderately active=Inhibition zone 10–15 mm; Slightly active=Inhibition zone 6–8 mm; Inactive=Inhibition zone < 6 mm

*Vancomycin, **Colistin
white precipitate was filtered, washed with cold water, and recrystallized from ethanol/water (2.5 g, 54%), mp: 115 °C; IR ν (cm⁻¹): 3268.19–3098.6 (NH, NH₂), 2933.55 (SH), 1610.28 (C=N); 1H NMR (DMSO-d₆, σH ppm): 7.69–7.27 (m, 4H, Ar–H), 5.81–5.30 (s, 4H, PhOCH), 4.84–4.15 (m, 2H, 2xCH₂), 3.94–3.40 (m, 1H, CHOCH), 2.19 (s, 1H, OH); ¹³C NMR (CDCl₃, σc ppm): 130.97–127.99 (C, CH ar), 105.63–102.71 (C, PhOCH), 68.51–64.84 (C, CH₂), 64.58–49.48 (C, CHOCH); GC–MS m/z: 179.1 (M⁺+H⁺) for C₁₀H₁₁O₃S as in structure 13.

2-phenyl-1,3-dioxan-5-yl 4-methylbenzenesulfonate (14) 2-Phenyl-[1,3]dioxan-5-ol 13 (2 g, 1.1 mmol) in ethanol, tosyl chloride (2 g, 10 mol) and some drops of pyridine were mixed and heated under reflux for 6 h, cooled to room temperature and neutralized by aqueous NaHCO₃. The product was extracted with dichloromethane, dried over MgSO₄, filtered, distilled to dryness to give white syrup 14 (1.42 g, 44.8%); IR ν (cm⁻¹): 1597.81 (C=C), 1351.55 (SO₂), 1094.46(C–O–C); ¹H NMR (DMSO-d₆, σH ppm): 7.78 (m, 4H, Ar–H α to CSO₂O), 7.47 (m, 2H, Ar–H β to methyl), 7.12 (d, 5H, Ar–H), 5.40–5.83 (s, 4H, PhOCH), 4.05 (m, 1H, CHOS), 3.64–3.64 (m, 4H, 2xCH₂ 1,3-dioxan ring), 2.41 (s, 3H, CH₃); ¹³C NMR (DMSO-d₆, σc ppm): 145.90–145.29 (C, C–CH₃), 138.22 (C, C¹ phenyl ring), 135.05–133.08 (C, CSO₂O), 130.63–129.61 (2C, CH₂ β to methyl), 127.95–125.36 (C, CH₂ Phenyl ring), 100.41–104.36 (C, PhOCH), 72.93 (C, C¹ 1,3-dioxan ring), 63.48–64.76 (2C, CH₂ 1,3-dioxan ring), 21.81 (C, CH₃); GC–MS m/z: 200–fragment of (M-199 for C₇H₁₂O₃S in structure 14).

5-Methyl-3-(2-phenyl-[1,3]-dioxan-5-yl)-3H-1,3,4-oxadiazole-2-thione (15) A solution of 9 (0.21 g, 0.6 mmol) in DMF (2 ml) was added to a solution of 4 (0.22 g, 1.8 mmol) in DMF (3 ml) and drops of DEA. The mixture was heated under reflux for 48 h. The reaction mixture was cooled, neutralized by water and dried with dichloromethane. Excess of solvent was evaporated to dryness to give a brown syrup 15 (0.3 g, 43%); IR ν (cm⁻¹): 1634.33(C=N), 1032(C–O–C); ¹H NMR (CDCl₃, σH ppm): 8.02–7.18 (m, 5H, Ar–H), 3.31(m, 2 × CH₂ 1,3-dioxan ring), 2.70 (m, 1H, HCN), 1.31 (m, 3H, CH₃); ¹³C NMR (CDCl₃, σc ppm): 162.63(C, C=), 140.46 (C, N=O oxadiazole ring), 140.29 (C, C¹ phenyl ring), 128.72 (2C, C¹, C² phenyl ring), 125.71 (3C, C², C³, C⁶ phenyl ring), 42.39 (C, 2xCH₂ 1,3-dioxan ring), 36.35 (C, N–C=H), 21.40(CH₂), GC–MS m/z: 279 (M⁺), for C₁₃H₁₄N₂O₅S as in structure 15.

(E)- and (Z)-2-phenyl-[1,3]dioxan-5-ol (13) Glycerol (9 g, 3 mol), benzaldehyde (7 g, 2 mol) in chloroform (25 ml) and ATPS (1.5 g) were mixed and heated under reflux for 6 h, cooled and neutralized by aqueous NaHCO₃ to pH=7. The product was extracted two times with dichloromethane, dried over MgSO₄, filtered, distinys to dryness to give colorless syrup. (8.5 g, 50%); IR ν (cm⁻¹): 3420.41(OH), 2859.38(CH ar), 1598.71(C=C); ¹H NMR (CDCl₃, σH ppm): 7.69–7.27 (m, 4H, Ar–H), 5.81–5.30 (s, 4H, PhOCH), 2.23 (s, 3H, CH₃); ¹³C NMR (DMSO-d₆, σc ppm): 145.90–145.29 (C, C–CH₃), 138.22 (C, C¹ phenyl ring), 135.05–133.08 (C, CSO₂O), 130.63–129.61 (2C, CH₂ β to methyl), 127.95–125.36 (C, CH₂ Phenyl ring), 100.41–104.36 (C, PhOCH), 72.93 (C, C¹ 1,3-dioxan ring), 63.48–64.76 (2C, CH₂ 1,3-dioxan ring), 21.81 (C, CH₃); GC–MS m/z: 200–fragment of (M-199 for C₇H₁₂O₃S in structure 14).

2-Methyl-5-(2-phenyl-[1,3]-dioxan-5-yl) sulfanyl- (1,3,4) thiadiazole (16) The compound 6 (0.25 g, 1.9 mmol) was dissolved in mixture of DMF and 1,4-dioxane (10 ml), and a solution of (14) (0.25 g, 0.74 mmol) in DMF (5 ml) was added. Diethylamine (1 ml) was added, and the reaction mixture was refluxed at for 72 h. The reaction mixture was neutralized with water and extracted with CH₂Cl₂ (3 × 10 ml). The organic layer was evaporated to dryness to give a brownish syrup 16 (0.44 g, 69%); IR ν (cm⁻¹): 1664.27(C=N), 1178.29(C=S); ¹H NMR (CDCl₃, σH ppm): 7.65 (m, 5H, Ar–
H), 5.40 (s, 1H, PhOCH), 3.23 (m, 2 × CH2 1,3-dioxan ring), 2.89 (m, 1H, HCN), 2.69 (s, 2H, NH2), 1.33 (m, 3H, CH3); 13CNMR (DMSO, σc ppm): 207.14 (C, C=S), 162.24 (C, N=C-S thiadiazole ring), 154.39 (C, C=S), 170.44 (C, C=S thiadiazole ring), 139.43 (C, C1 phenyl ring), 128.75 (2C, C3, C5 phenyl ring), 130.74 (3C, C2, C4, C6 phenyl ring), 133.46 (Ar–H), 5.29 (s, 1H, PhOCH), 3.15 (m, 2 × CH2 1,3-dioxan ring), 46.58 (C, 2xCH2 1,3-dioxan ring), 25.92 (C, N–C-H), 15.75 (C, CH3); GC–MS m/z: 292 (M+), for C13H16O2N4S as in structure 17.

4-Amino-5-methyl-2-[(2-phenyl-(1,3)-dioxan-5-yl)-2,4-dihydro-1,2,4-triazol-3-thione (17) Amino triazol 7 (0.3 g, 2.27 mmol) was dissolved in DMF (10 ml), and a compound 14 (0.6 g, 1.79 mmol) in DMF and DEA (1 ml) was mixed at room temperature for 24 h. The reaction mixture was neutralized by water and extracted with dichloromethane. Excess of solvent was evaporated to dryness to give a brown syrup 17 (0.93 g, 78%); IR u (cm−1): 3107.72 (NH2), 1663.34 (C=O); 1H NMR (DMSO-d6, σppm): 7.48 (m, 5H, Ar–H), 5.29 (s, 1H, PhOCH), 3.15 (m, 2 × CH2 1,3-dioxan ring), 2.89 (m, 1H, HCN), 2.69 (s, 2H, NH2), 1.33 (m, 3H, CH3); 13CNMR (DMSO, σc ppm): 170.44 (C, C=S), 154.39 (C, N=C-S thiadiazole ring), 170.44 (C, C=S), 139.43 (C, C1 phenyl ring), 128.75 (2C, C3, C5 phenyl ring), 130.74 (3C, C2, C4, C6 phenyl ring), 133.46 (Ar–H), 5.29 (s, 1H, PhOCH), 3.15 (m, 2 × CH2 1,3-dioxan ring), 46.58 (C, 2xCH2 1,3-dioxan ring), 25.92 (C, N–C-H), 15.75 (C, CH3); GC–MS m/z: 292 (M+), for C13H16O2N4S as in structure 17.

Antibacterial test

The respective different strain was spread separately on the Mueller–Hinton for antibiotic activity (CLSI 2004; CLSI 2012). Then, the test organism suspension was added and incubated at 37 °C for 24 h for bacteria studies. The drugs colistin and vancomycin were taken as standard drug to compare the results, and dimethylsulfoxide (DMSO) was taken as blank [24]. The respective different strain was spread separately on the Mueller–Hinton for antibacterial activity (CLSI 2004; CLSI 2012). Then, the test organism suspension was added and incubated at 37 °C for 24 h for bacteria studies. The drugs colistin and vancomycin were taken as standard drug to compare the results, and dimethylsulfoxide (DMSO) was taken as blank [24].

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