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Regioselective Glycosylation: Synthesis, Characterization and Biological Evaluation of New Acyclo C-nucleosides Bearing 5-(substituted)-1,3,4-oxadiazole-2-thione, 5-(substituted)-4-amino-1,2,4-triazole-3-thiol and 5-(substituted)-1,2,4-triazole-3-thiones Moieties

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ABSTRACT

A several carboxylic acid derivatives 1a-b were used as starting materials to prepare an open chain C-nucleosides analogue, which exhibit distinguished antitumor and antiviral activities. Consequently, various analogues of C-nucleosides analogues have been synthesized to improve its chemotherapeutic index, including modification of the sugar ring as well as modification of the heterocyclic ring. In this study, subsequently after esterification, the compounds 2a-b were converted to acid hydrazides 3a-b by treatment with hydrazine hydrate. Moreover, the reaction of hydrazides with carbon disulfide in the presence of KOH afforded the 5-(5'-hydroxymethyl-2,2,2',2'-tetramethyl-[4,4']bi[[1,3]dioxolanyl]-5-yl) -3H-[1,3,4] oxadia zole -2-thione 4a-b. In parallel, the treatment of same hydrazides with ammonium thiocyanate in dry benzene under reflux produced [5'- (3-mercapto-1H-1,2,4-triazol-5-yl)-2,2,2',2'-tetra methyl-4,4'-bi-1,3-dioxol-5-yl]methanol 9a-b. The intermediates and the final products were fully characterized with IR, MS, ^{1}H-NMR and elemental analysis all synthesized compounds were found to have appreciable effects in antibacterial activity against some pathogenic bacteria that causes infectious diseases.

Key words: Triazolo-C-nucleosides, oxadiazolo-C-nucleosides, thiosemicarbazide, thiol-thione form, antibacterial, antifungal activities

INTRODUCTION

Triazoles and their derivatives have been proven to be effective bactericides, pesticides and fungicides. Further, some findings that the 1,2,3-triazole nucleus is associated with diverse pharmacological activities such as analgesic, antiasthematic, diuretic, antihypertensive and antiinflammatory properties have made them important chemotherapeutic agents (Hirota *et al*., 1991).

Derivatives of 1,3,4-oxadiazole are also known to have a broad spectrum of biological activities (Boschelli *et al*., 1993). Acyclic hydrazide have been in general use as the starting materials in some 1,2,4-triazole and 1,3,4-oxadiazole syntheses (Rostom *et al*., 2003). In addition there are some studies on electronic structures and thiol-thione tautomeric equilibrium of heterocyclic thione derivatives (Aydogan *et al*., 2002). Hydrazides and related compounds have been described as

useful building blocks for assembly of various heterocyclic rings. Thus, different carbohydrazides were found to be useful as medicines.

The relatively simple 1,2,4-triazoles display biological activities such as inhibition of cholinesterase, interference with mitosis and reversible denaturation of serum proteins. The 1,2,4-triazole thiones afford some protection of mice against irradiation with X-rays and have anti-inflammatory properties. They have appreciable biochemical effects when replacing histidine derivatives in nucleic acids. Also, compounds with a thiourea function NH-(CS) -NH have a strong potential for manufacturing drugs since the SH group can easily converted to their S-substituted derivatives (Cansiz *et al*., 2001).

As a continuation of our investigation of antimicrobial and antifungal activities of 1,3,4-oxadiazole-thione and 1,2,4-triazole-thiole derivatives. In the present study, we attempted to synthesize oxadiazole ring (similar development for triazole ring) having an amino function attached at C-5 from carboxylic acid hydrazide 3a-b with O-protected gluconic and glucaric acid having a terminal carboxyl function free 1a-b.This paper therefore describes the synthesis of some 1,3,4-oxadiazoles 4a-b, 4-amino triazole 5a-b, 1-amino-triazole 7a-b,and 1,2,4-triazole 9a-b having an protected glycosyl moiety attached to C-5 of the heterocyclic ring leading to acyclo-C-nucleosides.

MATERIALS AND METHODS

This study was conducted in the Biomolecular Engineering Laboratory of the Department of Industrial Chemistry at University of Sciences and Technologies in Oran-Algeria, in the period of 2006-2009.

General procedure: Melting points were determined on a BÜCHI 540 melting point apparatus and were uncorrected. The ^{1}H NMR spectra was recorded on a Varian-Mercury 250 MHz spectrometer, in $CDCl_3$ + DMSO-d_6 with TMS as an internal standard and the Mass spectra were recorded on a MAT312 mass spectrometer using glycerol as matrix. The IR spectra were measured as potassium bromide pellets using a Perkin-Elmer 1600 series FTIR spectrometer. Elemental analysis was performed on a Carlo Erba 1106 elemental analyzer. All the chemicals were obtained from Fluka Chemie AG Buchs (Switzerland).

General method for the synthesis of methyl carboxylic acid ester (2a-b): To a mixture of carboxylic acid 1a-b (0.15 moles) in methanol (90 mL), H_2SO_4 conc. (16 mL) was added dropwise with stirring and was refluxed on a oil bath at 80°C for 5 h. TLC eluted with Benzene/Ethanol; 5: 6 showed Rf: 0.55 for the acid 1a and Rf: 0.50 for the acid 1b. The aqueous mixture was extracted two times with n-hexane (25 mL).

The combined organic layers were washed with 5% aqueous $NaHCO_3$ (150 mL) until the pH reached 7 and then washed with 50 mL of water. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated to dryness to give a colourless oil; methyl carboxylate 2a-b. This product was recrystallized from ethanol/water (1:1) to afford the desired compound.

Methyl 2,3:4,5-di-O-isopropylidene-D-gluconate (2a): Crude brownish oil (18.23 g, 87%); (mp of crystallized product: 97-99°C); TLC (Benzene/Ethanol; 5:6, Rf: 0.78); IR (ν, cm^{-1}): 2850 (O-CH_3), 1685 (C = O); ^{1}H NMR (250 MHz, DMSO-d_6) δ ppm: 4.84 (d, 1H, H-2), 4.72 (dd, 1H, $J_{3,2}$ 5.4 Hz, $J_{3,4}$ 7.4 Hz, H-3), 4.20 (t, 1H, H-4), 4.09 (m, 1H, H-5), 3.83 (m, 1H, H-6'), 3.70 (ddd, 1H,

$J_{6,5}$ 4.4 Hz, $J_{6,6'}$ 12.1 Hz, H-6), 3.68 (s, 3H, s, CO_2CH_3), 3.65 (dd, 1H, $J_{OH,6}$ 7.9 Hz, $J_{OH,6'}$ 4.9 Hz, OH), 1.39, 1.41, 1.41, 1.45 (4s,12H, 2C $(CH_3)_2$); MS m/z: 291.14 (M+1), 292.14 (14.6%), 293.14 (2.4%); HRMS calcd /found for $C_{13}H_{22}O_7$, [M+H] = 291,137; 291,177; Anal. Calcd for $C_{13}H_{22}O_7$: C, 53.78; H, 7.64; Found: C, 53.38; H, 7.24.

Dimethyl 2,3:4,5-di-O-isopropylidene-D-glucarate (2b): Dense brownish oil (16.74 g, 95.37%); mp of crystallized product: 194°C; IR (ν, cm^{-1}): 2863 (O-CH_3), 1695 (C = O); ^{1}H NMR (250 MHz, DMSO-d_6) δ ppm: 4.89 (d,1H, H-2), 4.78 (dd,1H, $J_{3,2}$ 5.4 Hz, $J_{3,4}$ 7.4 Hz, H-3), 4.23 (t,1H, H-4), 4.13 (m, 1H, H-5), 3.70 (2s, 6H, $2CO_2CH_3$), 1.39, 1.41, 1.41, 1.45 (4s, 12H, 2C $(CH_3)_2$); MS m/z: 319.13 (M+1),320.13 (15.6%), 21.14 (2.8%); HRMS calcd/found for $C_{14}H_{22}O_8$, [M+H] = 319,131; 319,171;Anal. Calcd for $C_{13}H_{22}O_7$: C, 52.82; H, 6.97; Found: C, 52.42; H, 6.57.

General method for the synthesis of carboxylic hydrazide (3a-b): A mixture of corresponding compound 2a-b (0.032 moles) and an equivalent amount of ethanol (20 mL) and hydrazine hydrate 64% (6 mL) were mixed together and heated under reflux at 110°C for 6 h. After evaporating the reaction content under reduced pressure, a solid product was obtained. This was recrystallized from H_2O/MeOH to give carboxylic acid hydrazide 3a-b.

2,3:4,5-di-O-isopropylidene-D-gluconic acid Hydrazide (3a): Dense brownish oil (7.50 g, 79.09%); mp of crystallized product: 218-220°C; TLC (Benzène-Ethanol; 5:6, Rf: 0.71); IR (ν, cm^{-1}): 3125, 3320 (NH,NH_2), 1631 (C = O); ^{1}H NMR (250 MHz, DMSO-d_6) δ ppm: 9.43 (s, 1H, NH),4.84 (d, 1H, H-2), 4.72 (dd, 1H, $J_{3,2}$ 5.4 Hz, $J_{3,4}$ 7.4 Hz, H-3), 4.45 (s, 2H, NH_2),4.20 (t, 1H, H-4), 4.09 (m, 1H, H-5), 3.83 (m, 1H, H-6'), 3.70 (ddd, 1H, $J_{6,5}$ 4.4 Hz, $J_{6,6'}$ 12.1 Hz, H-6), 3.65 (dd, 1H, $J_{OH,6}$ 7.9 Hz, $J_{OH,6'}$ 4.9 Hz, OH), 1.45, 1.41, 1.41, 1.39 (4s,12H, 2C $(CH_3)_2$); MS m/z: 291.15 (M+1), 292.15 (13.5), 293.15 (2.1%); HRMS calcd /found for $C_{12}H_{22}N_2O_6$, [M+H] = 291,148; [M+1] = 291,188; Anal. Calcd for $C_{12}H_{22}N_2O_6$: C, 49.65; H, 7.64; N,9.65. Found: C, 49.25 H, 7.32; N, 9.33.

2,3:4,5-di-O-isopropylidene-D-glucaric acid Hydrazide (3b): Dense brownish oil (1.42 g, 91.42%) ; m.p of crystallized product: 62-64°C; TLC (Benzène-Ethanol; 5:6, Rf: 0.75); IR (ν, cm^{-1}): 3128, 3322 (NH,NH_2), 1635 (C = O); ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 9.49 (s, 2H, 2NH), 4.89 (d, 1H, H-2), 4.78 (dd,1H, $J_{3,2}$ 5.4 Hz, $J_{3,4}$ 7.4 Hz, H-3), 4.45 (s, 4H, $2NH_2$),4.13 (m, 1H, H-5),4.23 (t, 1H, H-4), 1.45, 1.41, 1.41, 1.39 (4s, 12H, 2C $(CH_3)_2$); MS m/z: 319.15 (M+1), 320.16 (13.5%), 321.16 (2.1%); HRMS calcd/found for $C_{12}H_{22}N_4O_6$, [M+H] = 319.154; [M+1] = 319.194; Anal. Calcd for $C_{12}H_{22}N_4O_6$: C, 45.28; H, 6.97; N, 17.60. Found: C, 44.85; H, 6.52; N, 17.03.

General method for the synthesis of 5-(substituted) -1,3,4-oxadiazole-2-thione (4a-b): These compounds were prepared according to the procedure reported in the literature[19b]. To a solution of appropriate carboxylic acid hydrazide 3a-b (10 mmol) in ethanol (150-200 mL), carbon disulfide (15 mmol) was added. This was followed by the addition of potassium hydroxide (0.84 g, 15 mmol) dissolved in 25 mL of water.

The reaction mixture was stirred and subjected to reflux at 110°C for 9 h. After reaction completion, excess ethanol was distilled off. The crude solid obtained was dissolved in excess water and acidified with 4N HCl to pH 2-3. The separated product was filtered, washed with water and recrystallized from $CHCl_3$/EtOH to afford compounds 4a-b.

5- ((4S,4'S,5'R) -5'- (hydroxymethyl) -2,2,2',2'-tetramethyl-4,4'bi (1,3-dioxolan) -5-yl) -1,3,4-oxadiazole-2 (3H) -thione (4a): Pink crystals (0.942g, 82.27%) ; m.p: 198-203°C; TLC (Benzène-Ethanol; 5:6, Rf: 0.65); IR (ν, cm^{-1}): 3125 (NH), 1592 (C = N), 1259 (C = S),1070 (C-O-C); ^1H NMR (250 MHz, DMSO-d_6) δ ppm: 7.01 (s, 1H, NH), 4.84 (d, 1H, H-2), 4.72 (dd, 1H, $J_{3,2}$ 5.4 Hz, $J_{3,4}$ 7.4 Hz, H-3), 4.20 (t, 1H, H-4), 4.09 (m, 1H, H-5), 3.83 (m, 1H, H-6'), 3.70 (ddd, 1H, $J_{6,5}$ 4.4 Hz, $J_{6,6'}$ 12.1 Hz, H-6), 3.65 (dd, 1H, $J_{OH,6}$ 7.9 Hz, $J_{OH,6'}$ 4.9 Hz, OH), 1.45, 1.41, 1.41, 1.39 (4s, 12H, 2C $(CH_3)_2$); MS m/z: 332.10 (M+1), 333.15 (15.5%), 334.10 (4.5%); HRMS calcd /found for $C_{13}H_{20}N_2O_6S$, [M+H] = 332,10; [M+1] = 332,12; Anal. Calcd for $C_{13}H_{20}N_2O_6S$: C, 46.98; H, 6.07; N,8.43; S, 9.65. Found: C, 46.95; H, 6.02; N, 8.39; S, 9.6.

5,5'- ((4S,4'R) -2,2,2',2'-tetramethyl-4,4'-bi (1,3-dioxolane) -5,5'-diyl) bis (1,3,4-oxa-diazole-2 (3H) -thione (4b): Crystalline solid (0.942 g, 82.27%); m.p:193-195°C; TLC (Benzène-Ethanol; 5:6, Rf: 0.58); IR (ν, cm^{-1}): 3125 (NH), 1593 (C = N), 1262 (C = S), 1075 (C-O-C); ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 14.60 (s, 2H, 2NH), 4.89 (d, 1H, H-2), 4.78 (dd, 1H, $J_{3,2}$ 5.4 Hz, $J_{3,4}$ 7.4 Hz, H-3), 4.13 (m, 1H, H-5), 4.23 (t, 1H, H-4), 1.45, 1.41, 1.41, 1.39 (4s, 12H, 2C $(CH_3)_2$); MS m/z: 403.067 (M+1), 404.06 (9.9%), 405.09 (4.5%); HRMS calcd/ found for $C_{14}H_{18}N_4O_6S_2$, [M+H] = 403,067; 403,107; Anal. Calcd for $C_{14}H_{18}N_4O_6S_2$: C, 41.78; H, 4.51; N,13.98; S, 15.94. Found: C, 41.73; H, 4.47; N, 13.92; S, 15.89.

General method for the synthesis of 5-(substituted) -4-amino-1,2,4-triazole-3-thiol (5a-b): Corresponding compound 4a-b (5 mmol) was dissolved in 80 mL ethanol and hydrazine monohydrate 64% (10 mL) was added and the reaction mixture was heated under reflux on an oil bath at 110°C for 8 h.

After evaporating it to dryness under reduced pressure, a solid was obtained. This was dissolved in 300 mL of H_2O and acidified with conc.HCl to pH 1.00. The precipitate was filtered, washed with H_2O and recrystallized from an appropriate solvent chloroform/ ethanol (2/1) to afford the desired compound 5a-b.

5'- (4-amino-5-mercapto-4H-1,2,4-triazol-3-yl) -2,2,2',2'-tetramethyl-4,4'-bi (1,3-dioxolan) -5-yl) methanol (5a): Yellow-brownish solid, (0.352 g, 84.41%) ; m.p:117-120°C; TLC (Benzène-Ethanol; 5:6, Rf: 0.63); IR (ν, cm^{-1}): 3330 (OH), 3326 (NH_2), 2620 (SH), 1599 (C = N); ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 13.79 (s, 2H, NH_2), 8.76 (s, H, SH), 5.17 (d, 1H, H-2), 4.59 (dd, 1H, $J_{3,2}$ 5.4 Hz, $J_{3,4}$ 7.4 Hz, H-3), 4.20 (t, 1H, H-4), 3.97 (m, 1H, H-5), 3.79 (m, 1H, H-6'), 3.65 (dd, 1H, $J_{OH,6}$ 7.9 Hz, $J_{OH,6'}$ 4.9 Hz, OH), 3.54 (ddd, 1H, $J_{6,5}$ 4.4 Hz, $J_{6,6'}$ 12.1 Hz, H-6), 1.45, 1.41, 1.41, 1.39 (4s,12H, 2C $(CH_3)_2$); MS m/z: 347.13 (M+1), 348.13 (16.2%), 348.12 (5.8%); HRMS calcd /found for $C_{13}H_{22}N_4O_5S$, [M+H] = 347.130; 348,120;Anal. Calcd for $C_{13}H_{22}N_4O_5S$: C, 45.07; H, 6.40; N,16.17; S, 9.26. Found: C, 45.03; H, 6.36; N, 16.13; S, 9.22.

5,5'- (2,2,2',2'-tetramethyl-4,4'-bi (1,3-dioxolane) -5,5'-diyl) bis (4-amino-4H-1,2,4-triazole-3-thiol) (5b): Yellow solid, (0.525 g, 78.16%) ; m.p: 224-228°C; TLC (Benzène-Ethanol; 5:6, Rf: 0.60); IR (ν,cm^{-1}): 3325 (OH), 3320 (NH_2), 2625 (SH), 1579 (C = N); ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 13.79 (s, 4H, $2NH_2$), 8.76 (s, 2H, 2SH), 5.17 (d, 1H, H-2), 5.17 (m, 1H, H-5), 4.59 (dd, 1H, $J_{3,2}$ 5.4 Hz, $J_{3,4}$ 7.4 Hz, H-3), 4.59 (t, 1H, H-4), 1.45, 1.41, 1.41, 1.39 (4s,12H, 2C $(CH_3)_2$); MS m/z: 431.12 (M+1), 432.10 (8.7%), 433.13 (4.5%); HRMS calcd/found for $C_{14}H_{22}N_8O_4S_2$, [M+H] = 431,099; 431,139; Anal. Calcd for $C_{14}H_{22}N_8O_4S_2$: C, 39.06; H, 5.15; N,26.03; S, 14.90. Found: C, 39.01; H, 5.11; N, 25.99; S, 14.86.

General method for the synthesis of thiocarbohydrazide (6a-b): The acid hydrazide 3a-b (0.01 mol) was added to absolute alcohol ethanol (250 mL), containing KOH (8.4 g, 0.15 mol) at room temperature. Carbon disulphide (9 mL, 0.015 mol) was added dropwise and heated under reflux on oil bath at 110°C for 10 h.

The ethanol was partially evaporated to 100 mL. After cooling it to room temperature, ether (200 mL) was added and a brownish precipitate appeared. This was recrystallized from chloroform/ethanol (1/1) to afford the desired product 6a-b.

Potassium2- (5'- (hydroxymethyl) -2,2,2',2'-tetramethyl-4,4'-bi (1,3-dioxolane-5-yl) carbonyl) hydrazinecarbothioate (6a): Yellow salt (needles) (0.530 g, 79.22%); m.p: 196°C; TLC (Benzène-Ethanol; 5:6, Rf: 0.70); IR (ν, cm^{-1}): 3120 (NH), 1667 (C = O), 1225 (C = S); ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 10.04 (s, 1H, NHNHCSO), 9.70 (s, 1H, NHNHCSO), 4.83 (d, 1H, H-2), 4.43 (dd, 1H, $J_{3,2}$ 5.4 Hz, $J_{3,4}$ 7.4 Hz, H-3), 4.20 (t, 1H, H-4), 3.97 (m, 1H, H-5), 3.79 (m, 1H, H-6'), 3.65 (dd, 1H, $J_{OH,6}$ 7.9 Hz, $J_{OH,6'}$ 4.9 Hz, OH), 3.54 (dd, 1H, $J_{6,5}$ 4.4 Hz, $J_{6,6'}$ 12.1 Hz, H-6), 1.45, 1.41, 1.41, 1.39 (4s, 12H, 2C $(CH_3)_2$); MS *m/z*: 389.071 (M+1), 390.07 (15.9%), 391.071 (13.4%).392.07 (1.8%); HRMS calcd /found for $C_{13}H_{21}KN_2O_7S$, [M+H] = 389,071; 389,111; Anal. Calcd for $C_{13}H_{21}KN_2O_7S$: C, 40.19; H, 5.45; N,7.21; S, 8.25. Found: C, 40.14; H, 5.41; N, 7.17; S, 8.20.

Dipotassium2,2'-[(2,2,2',2'-tetramethyl-4,4'-bi-1,3-dioxolane-5,5'-diyl) dicarbonyl] di-hydrazinecarbothioate (6b): Yellow salt (needles) (0.639 g, 79.08%); m.p 97-99°C; TLC (Benzène-Ethanol; 5:6, Rf: 0.70); IR (ν, cm^{-1}): 3120 (NH), 1660 (C = O): 1227 (C = S); ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 10.10 (s, 2H, 2NHNHCSO), 9.76 (s, 2H, 2NHNHCSO), 4.83 (d, 1H, H-2), 4.83 (m, 1H, H-5), 4.43 (dd, 1H, $J_{3,2}$ 5.4 Hz, $J_{3,4}$ 7.4 Hz, H-3), 4.43 (t, 1H, H-4), 1.45, 1.41, 1.41, 1.39 (4s,12H, 2C $(CH_3)_2$); MS *m/z*: 515.00 (M+1), 516.00 (9.9%), 517.00 (4.5%); HRMS calcd / found for $C_{14}H_{20}K_2N_4O_8S_2$, [M+H] = 515,000; 515,040; Anal. Calcd for $C_{14}H_{20}K_2N_4O_8S_2$: C, 32.67; H, 3.92; N,10.89; S, 12.46. Found: C, 32.63; H, 3.87; N, 10.84; S, 12.41.

General method for the synthesis of 5-(substituted) -2-amino-1,2,4-triazole-3-thiol (7a-b): Corresponding compound 6a-b (0.004 mole) dissolved in water (8 mL) and hydrazine hydrate 64% (4 mL) were heated under reflux on an oil bath at 110°C for 6 h. After evaporating it to dryness under reduced pressure, a solid was obtained. This was dissolved in 300 mL of H_2O and acidified with conc.HCl. to pH 1.0.

The precipitate was filtered, washed with H_2O and recrystallized from an appropriate solvent chloroform/ethanol (2/1) to afford the desired compound 7a-b.

[5'-(1-amino-5-mercapto-1H-1,2,4-triazol-3-yl) -2,2,2',2'-tetramethyl-4,4'-bi- (1,3-dioxolanyl) -5-yl] methanol (7a): Yellow crystals, (0.512 g, 85.51%); m.p:119-122°C; TLC (Benzène-Ethanol; 5:6, Rf: 0.63); IR (ν, cm^{-1}): 3330 (OH), 3175 (NH_2), 2580 (SH), 1620 (C = N); ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 13.15 (s, 2H, NH_2), 10.05 (s, 1H,-SH), 4.83 (d, 1H, H-2), 4.43 (dd, 1H, $J_{3,2}$ 5.4 Hz, $J_{3,4}$ 7.4 Hz, H-3), 4.20 (t, 1H, H-4), 3.97 (m, 1H, H-5), 3.79 (m, 1H, H-6'), 3.65 (dd, 1H, $J_{OH,6}$ 7.9 Hz, $J_{OH,6'}$ 4.9 Hz, OH), 3.54 (dd, 1H, $J_{6,5}$ 4.4 Hz, $J_{6,6'}$ 12.1 Hz, H-6), 1.45, 1.41, 1.41, 1.39 (4s, 12H, 2C $(CH_3)_2$); MS *m/z*: 347.13 (M+1), 348.13 (16.2%), 349.13 (5.8%); HRMS calcd / found for $C_{13}H_{22}N_4O_5S$, [M+H] = 347.129; 347.150; Anal. Calcd for $C_{13}H_{22}N_4O_5S$: C, 45.07; H, 6.40; N,16.17; S, 9.26. Found: C, 45.02; H, 6.35; N, 16.11; S, 9.21.

3,3'- (2,2,2',2'-tetramethyl-4,4'-bi-1,3-dioxolane-5,5'-diyl) bis (1-amino-1H-1,2,4-triazole -5-thiol) (7b): Yellow white crystals. (0.425 g, 79.16%) ; m.p: 274-277°C; TLC (Benzène-Ethanol; 5:6, Rf: 0.60); IR (ν, cm^{-1}): 3170 (NH_2), 2590 (SH),1620 (C = N); ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 13.25 (s, 4H, $2NH_2$), 10.05 (s, 2H, 2SH), 4.83 (d, 1H, H-2), 4.83 (m, 1H, H-5), 4.43 (dd, 1H, $J_{3,2}$ 5.4 Hz, $J_{3,4}$ 7.4 Hz, H-3), 4.43 (t, 1H, H-4), 1.45, 1.41, 1.41, 1.39 (4s, 12H, 2C $(CH_3)_2$); MS *m/z*: 431.12 (M+1), 432.12 (8.7%), 433.13 (4.5%); HRMS calcd /found for $C_{14}H_{22}N_8O_4S_2$, [M+H] = 431.121; 431,139; Anal. Calcd for $C_{14}H_{22}N_8O_4S_2$: C, 39.06; H, 5.15; N,26.03; S, 14.90. Found: C, 39.01; H, 5.11; N, 26.01; S, 14.86.

General method for the synthesis of thioamide-carbohydrazide (8a-b): The solution of corresponding compound 3a-b (0.066 moles) was dissolved in ethanol with stirring. Ammonium thiocyanate (1.6 g, 0.021 mole) and HCl (26 mL, 31%) were added and the reaction mixture was heated under reflux on an oil bath for 11 h. TLC eluted with ethanol/benzene 1:2 showed the development of a new spot, Rf = 0.55. After cooling the reaction mixture to room temperature, a mixture consisting of conc. HCl (3 mL) and water was added. The resulting crystalline solid was filtered, washed with water and recrystallized from toluene/petroleum-ether 60-80 to give different thiosemicarbazide carboxyl acid 8a-b.

2-{[5'- (hydroxymethyl) -2,2,2',2'-tetramethyl-4,4'-bi-1,3-dioxol-5-yl]carbonyl} Hydrazine-carbo-thioamide (8a): Solid pale yellow fibre (1.026 g, 85.22%); TLC (Benzène-Ethanol;5:6,Rf:0.67); IR (ν, cm^{-1}): 3300 (OH), 3170 (NH), 1660 (C = O), 1241 (C = S); ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 10.25 (s, 1H, CONHNHCS) 9.76 (s, 1H, CONHNHCS), 4.83 (d, 1H, H-2), 4.43 (dd, 1H, $J_{3,2}$ 5.4 Hz, $J_{3,4}$ 7.4 Hz, H-3), 4.20 (t, 1H, H-4), 3.97 (m, 1H, H-5), 3.79 (m, 1H, H-6'), 3.75 (s, 2H, NH_2), 3.65 (dd, 1H, $J_{OH,6}$ 7.9 Hz, $J_{OH,6'}$ 4.9 Hz, OH), 3.54 (dd, 1H, $J_{6,5}$ 4.4 Hz, $J_{6,6'}$ 12.1 Hz, H-6), 1.45, 1.41, 1.41, 1.39 (4s, 12H, 2C $(CH_3)_2$); MS m/z: 350.13 (M+1), 351.13 (16.2%), 352.13 (4.8%); HRMS calcd /found for $C_{13}H_{23}N_3O_6S$, [M+H] = 350,131; 350,171; Anal. Calcd for $C_{13}H_{23}N_3O_6S$: C, 44.69; H, 6.63; N,12.03; S, 9.18. Found: C, 44.63; H, 6.59; N, 11.98; S, 9.11.

2,2,2',2'-tetramethyl-4,4'-bi-1,3-dioxolane-5,5'-dihydrazinecarbothioamide (8b): White crystalline solid (1,67 g, 85.12%) ; TLC (Benzène-Ethanol; 5:6, Rf: 0.60); IR (ν, cm^{-1}): 3175 (NH),1665 (C = O),1250 (C = S); ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 10.23 (s, 2H, 2CONHNHCS), 9.76 (s, 2H, 2CONHNHCS), 4.83 (d, 1H, H-2).4.83 (m, 1H, H-5), 4.43 (dd, 1H, $J_{3,2}$ 5.4 Hz, $J_{3,4}$ 7.4 Hz, H-3), 4.43 (t, 1H, H-4), 3.95 (s, 4H, $2NH_2$), 1.45, 1.41, 1.41, 1.39 (4s, 12H, 2C $(CH_3)_2$),; MS m/z: 437.12 (M+1), 438.15 (8.7%), 439.12 (4.5%); HRMS calcd / found for $C_{14}H_{24}N_6O_6S_2$, [M+H] = 437,120; 437,160; Anal. Calcd for $C_{14}H_{24}N_6O_6S_2$: C, 38.52; H, 5.54; N,19.25; S, 14.69. Found: C, 38.48; H, 5.50; N, 19.20; S, 14.61.

General method for the synthesis of 5- (substituted) -1,2,4-triazole-3-thiol (9a-b): The corresponding thiosemicarbazide 8a-b (10 mmol) was dissolved in ethanol (200 mL). An aqueous potassium hydroxide (0.85 g, 20 mL, 15 mmol) was added and refluxed at 110°C for 4 h, to give one TLC spot (ethanol/benzene 2:4) at Rf_{8a} = 0.11, Rf_{8b} = 0.25 After evaporation at 45-60°C under reduced pressure, a solid appeared.

The precipitate was collected and crystallized from petroleum-ether/CH_2Cl_2 (1/4) to afford the desired compound as unless colour fibres 9a-b.

[5'- (5-mercapto-2H-1,2,4-triazol-3-yl) -2,2,2',2'-tetramethyl-4,4'-bi- (1,3-dioxolanyl) -5-yl] methanol (9a): White Crystalline fibres. (0.352 g, 84.41%) ; m.p: 119-122°C; TLC (Benzène-Ethanol; 5:6, Rf: 0.63); IR (ν, cm^{-1}): 3330 (OH), 3200 (NH), 2820 (SH), 1671 (C = N); ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 13.29 (s, H, NH), 10.25 (s, H, SH), 4.83 (d, 1H, H-2), 4.43 (dd, 1H, $J_{3,2}$ 5.4 Hz, $J_{3,4}$ 7.4 Hz, H-3), 4.20 (t, 1H, H-4), 3.97 (m, 1H, H-5), 3.79 (m, 1H, H-6'), 3.65 (dd, 1H, $J_{OH,6}$ 7.9 Hz, $J_{OH,6'}$ 4.9 Hz, OH), 3.54 (dd, 1H, $J_{6,5}$ 4.4 Hz, $J_{6,6'}$ 12.1 Hz, H-6), 1.45, 1.41, 1.41, 1.39 (4s, 12H, 2C $(CH_3)_2$); MS *m/z*: 332.12 (M+1), 33.12 (16.2%), 334.12 (5.8%); HRMS calcd/found for $C_{13}H_{21}N_3O_5S$, [M+H] = 332,120; 332,160; Anal. Calcd for $C_{13}H_{21}N_3O_5S$: C, 47.13; H, 6.39; N,12.69; S, 9.68. Found: C, 47.09; H, 6.33; N, 12.64; S, 9.63.

5,5'- (2,2,2',2'-tetramethyl-4,4'-bi-1,3-dioxolane-5,5'-diyl) bis (1H-1,2,4-triazole-3-thiol) (9 b): White Crystalline fibres (0.510 g, 84.16%) ; m.p: 274-277°C; TLC (Benzène-Ethanol; 5:6, Rf: 0.60); IR (ν, cm^{-1}): 3200 (NH), 2845 (SH), 1690 (C = N); ^1H NMR (250 MHz, DMSO-d_6) δ (ppm): 13.59 (s, 2H, 2NH),10.39 (s, 2H, 2SH), 4.83 (d, 1H, H-2), 4.83 (m, 1H, H-5), 4.43 (dd, 1H, $J_{3,2}$ 5.4 Hz, $J_{3,4}$ 7.4 Hz, H-3), 4.43 (t, 1H, H-4), 1.45, 1.41, 1.41, 1.39 (4s, 12H, 2C $(CH_3)_2$); MS *m/z*: 401.09 (M+1), 402.10 (8.7%), 403.13 (4.5%); HRMS calcd/found for $C_{14}H_{20}N_6O_4S_2$, [M+H] = 401,099; 401,139; Anal. Calcd for $C_{14}H_{20}N_6O_4S_2$: C, 41.99; H, 5.03; N,20.99; S, 16.01. Found: C, 41.95; H, 4.99; N, 21.08; S, 15.95.

Biological assay: The following bacteria were used for antibacterial study: *Staphylococcus aureus* ATCC 25923, *B. subtilis* ATCC 6633, *P. aeruginosa* ATCC 27833 and *E. coli* 25882. The following yeast-fungi were used for antifungal study: *C. albicans* ATCC 64550 and *C. krusei* ATCC14243 (Kuhler *et al*., 2002).

Inoculation suspensions: The microorganisms suspensions used for inoculation were prepared at 106 cfu mL^{-1} concentrations by diluting fresh cultures at MacFarland 0.5 density (108 cfu mL^{-1}). It was known that there were 5×104 cfu mL^{-1} microorganisms in each well after inoculation.

Medium: Mueller-Hinton Broth (Oxoid) was used for the dilution of microorganism suspensions and 2-fold dilutions of the compounds. Sabouraud liquid medium (Oxoid) was used for yeast-like fungi for the same purpose.

Equipment: FalconR microplates, which have 96 wells, were used for the microdilution method. A Brinkmann transfer pipette was used for 2-fold dilution of compounds in the wells.

Method: Microdilution was employed for antibacterial activity tests (Gokce and Bercin, 1996). The synthesized compounds and the stock solution of the standards were dissolved in dimethylsulfoxide for ketoconazole, in water for fluconazole and in phosphate buffer saline for ampicillin at 1000 μg mL^{-1} final concentration. The solutions of each compound at 500... 3.9 μg mL^{-1} concentrations were prepared in the wells by diluting with the media.

Suspensions of the microorganisms at 106 cfu mL^{-1} concentrations were inoculated to the two Fold-diluted solutions of the compounds; consequently the microorganism suspension in each well was approximately 5×105 cfu mL^{-1}. The solutions of DMSO-microorganism mixture, the pure microorganisms and pure media were used as control wells.

The microplates were then covered and incubated at 36°C for 24-48 h. Wet cotton-wool was placed in the incubation chamber in order to keep it sufficiently humid to avoid evaporation. After a certain period, the wells were evaluated. The concentrations of the wells where no growth was observed were evaluated as the MIC of the respective compounds.

RESULTS AND DISCUSSION

Synthesis: This global scheme shows the synthesis of open chain C-nucleosides possessing 1,3,4-oxadiazolo-2-thione ring 4a-b and 1,2,4-triazolo-3-thiol ring 5, 7, 9a-b derived from different carboxylic acids.

This route initially required a selective protection of the adjacent OH groups of many carboxylic acids 1a-b in order to leave the terminal OH group for further modifications (Belkadi and Ali-Othmane, 2006) (Fig. 1).

Compounds 2a-b were obtained from the reaction of compounds 1a-c with acetone in sulfuric acid media (90% yields) and their structures were confirmed using IR, ^{1}H NMR and ^{13}C NMR spectral. The IR spectrum of compounds (2a-b) showed absorption at 1685 cm^{-1} for the CO-ester group which is in accordance with the literature (Chavanne *et al.*, 1991). Compounds (3a-b) were prepared by the reaction of carboxylic acid ester derivatives (2a-b) with hydrazine monohydrate (64%) in ethanol media (Fig. 2).

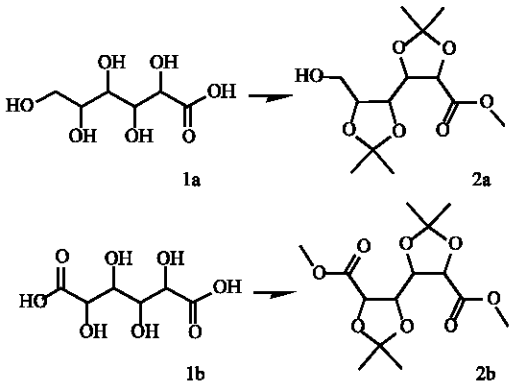


Fig. 1: Synthesis of (2.3:4.5) bisacetonide protected methyl-D-gluconate 2a and dimethyl bisacetonide protected-D-glucarate 2b

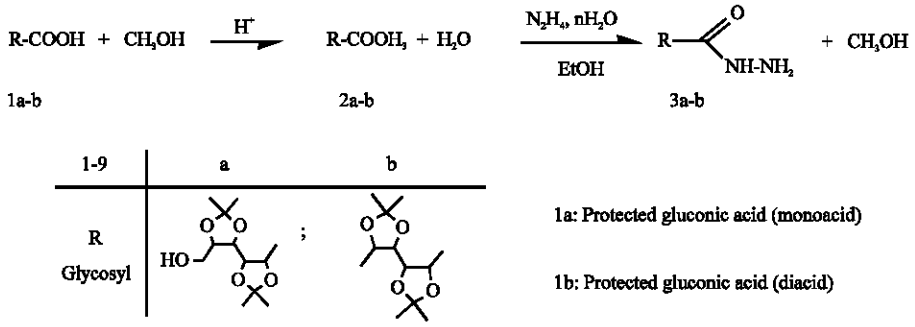


Fig. 2: Synthesis of hydrazides 3a-b from derivative carboxylic acid

These hydrazides 3a-b are used as the starting material for the common synthesis (three ways) described on the next and was obtained in 90% yield. The product exhibited characteristic IR bands 3125 cm^{-1} for NH and 1631 cm^{-1} for CO-N stretching. The ^1H NMR spectra of compounds 3a-b showed 2 different NH_2 signals (exch. with D_2O). The NH_2 signals disappeared when compounds 3a-b were converted to compounds 4a-b; instead a new signal originating from oxadiazole-NH was observed.

In the first way (Fig. 3), the hydrazide 3a-b was refluxed with CS_2 in absolute ethanol and KOH (0.015 moles) followed by acidification with HCl reveled into 5- ((4S,4'S,5'R) -5'-(hydroxymethyl) -2,2,2',2'-tetramethyl-4,4'bi (1,3-dioxolan) -5-yl) -1,3,4-oxadiazole-2 (3H) -thione 4a-b.

The mass spectrum showed a molecular ion at 331.15 (M^+) and the elemental analysis corresponded with structure 4. The characteristic bands in IR of this compound showed at regions 1259 cm^{-1} (C = S) and 1592 cm^{-1} (C = N). The position of the C = N band suggested that the oxadiazole existed as the thione tautomer 4a-b rather than the ene-thiol form 4 which normally exhibited a band at lower region (in about 1638 cm^{-1}) due to maximum conjugation (Aydogan *et al.*, 2002).

Further support for the thione form came from ^1H-NMR which exhibited only one proton as a singlet at lower field 7.01 ppm for N-H and no signal around 13 to 14 ppm where the S-H is normally shown at 3.5-6.5 ppm (Zamani *et al.*, 2003). The 4-Amino triazole 5a-b was obtained by heating the oxadiazole 4a-b with hydrazine hydrate under reflux conditions for 10 h according to reaction condition. The product formed crystalline fibres and had a slightly higher melting point 122°C. The ^1H-NMR spectrum showed signals at 13.79 ppm (NH_2), 8.76 ppm (SH) and 3.65 ppm for OH. The IR, MS spectrum and elemental analysis were in accordance with the structure for 5.

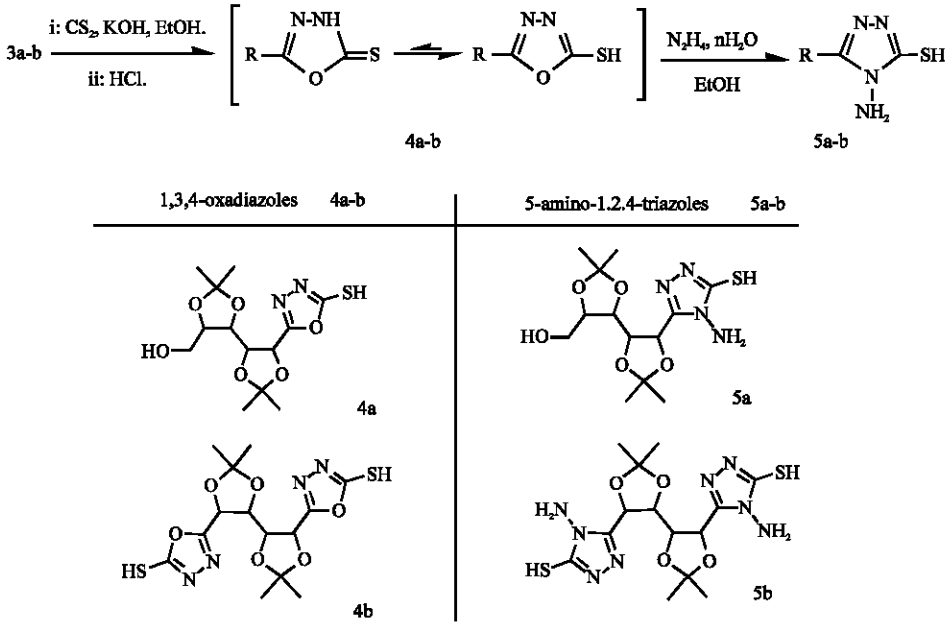
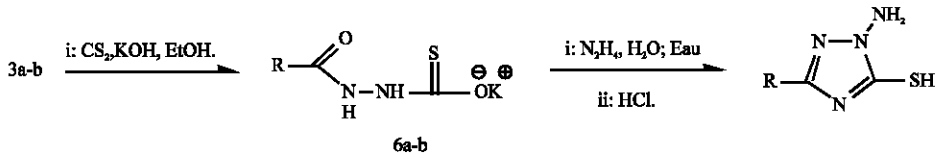


Fig. 3: Synthesis of 1,3,4-oxadiazoles 4a-b and 5-amino-1.2.4-triazoles 5a-b derivative from hydrazides 3a-b



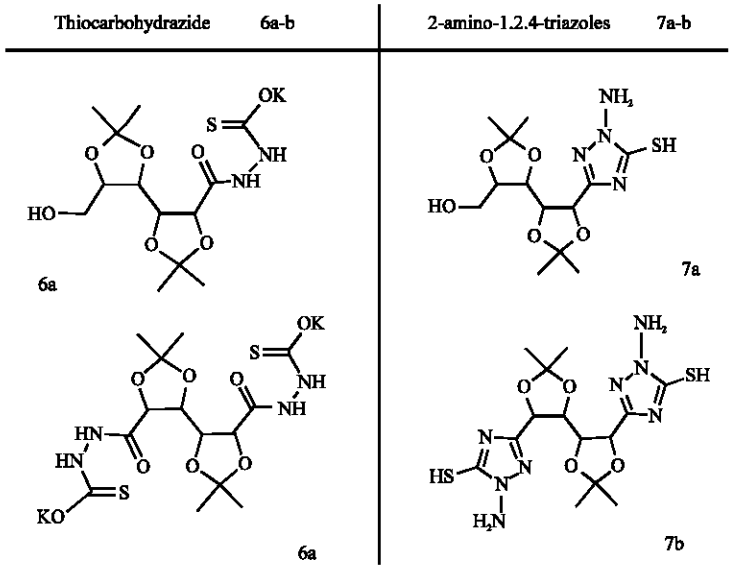


Fig. 4: Synthesis of 2-amino-1.2.4-triazoles 7a-b derivative from hydrazides 3a-b

The compound 7a-b was also prepared by second way (Fig. 4) according to the method by Clanceska-Ragenovie *et al.* (2001) in two steps.

This involved the treatment of the hydrazide 3a-b with KOH/CS_2 in ethanol (first step) and they reported the formation of the thiocarbazinic sel-acid 6a-b.However, in our hands we isolated the novel potassium thiocarbazinic sel-acid 6 as a yellowish crystalline with melting point 196-197°C in a yield of 68%. The IR spectrum of the product 6a-b showed characteristic absorption bands at 3120 cm^{-1} (broad) for free and bonded N-H, at 1667 cm^{-1} for CO-N and at 1225 cm^{-1} for C = S.

The mass spectrum showed a molecular ion (M+) at m/z 388.07 and a fragmentation ion at m/z 172 which relates to R-CONHNHCSOK$^+$.

Treatment of compound (6a-b) with hydrazine hydrate (second step) is expected to give 7a-b; the product was a crystalline solid which showed a lower melting point (119-122°C). The IR for the product 7 was similar to that of 5, but some differences were observed in the ^{1}HNMR spectrum. The NH_2 signal showed at 13.15 ppm, a signal at 10.05 ppm for SH.

On the basis of the mass spectrum which showed a molecular ion (M+) at m/z 346.13 and other spectral and elemental analysis data of 1-aminotriazole 7 was proposed. Formation of compound 5 might have resulted from the thermal rearrangement of the NH_2 group in position N-4 of 7 to position N-1 of 5, as a similar phenomenon was reported before in the literature (Jorgensen *et al.*, 2001). Molecular models of 7 suggest that the molecule prefers to exist in a coplanar form, whereas 5 prefer the non-coplanar form. Finally, the crystal structures of 7 and 5 corresponded to the thione form, but they showed thiol-thione tautomerism in solution.

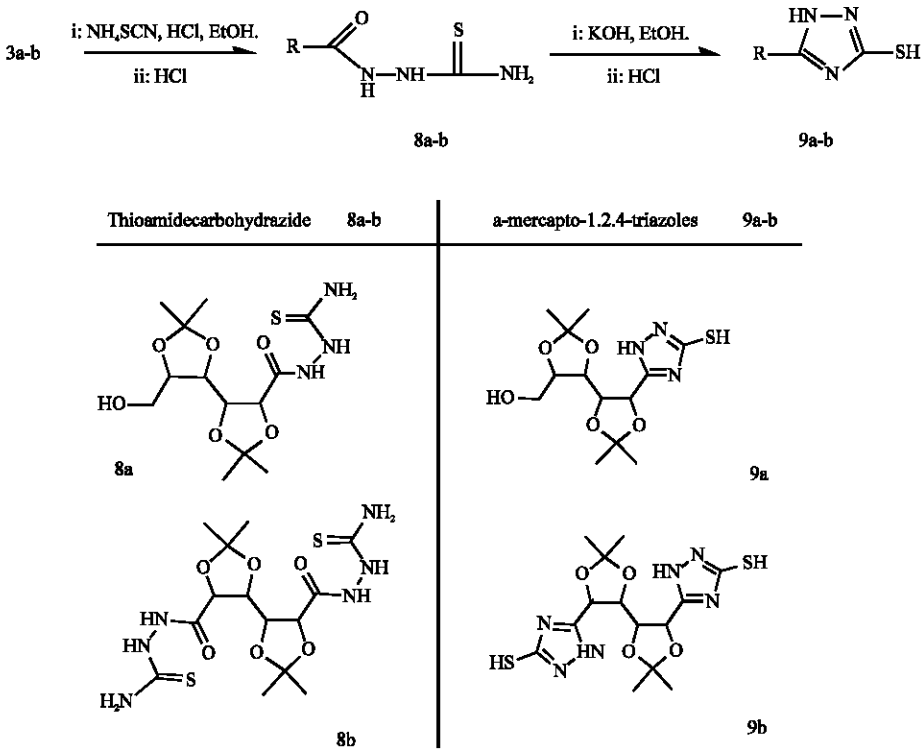


Fig. 5: Synthesis of 4-mercapto-1.2.4-triazoles 9a-b derivative from hydrazides 3a-b

The synthesis of the triazole 9a-b (third way) was accomplished by two steps from the hydrazide 3 (Fig. 5).

The treatment of 3 (first step) with ammonium thiocyanate and HCl for 18 h in max under reflux conditions to give the thiosemicarbazide derivative 8a-b as a yellow crystalline product in good yield (94%). The IR spectrum in CCl_4 solution showed a broad absorption in the region 3300-3100 cm^{-1} due to free and bonded OH and NH. The peak at 1660 cm^{-1} was assigned to CO-N and the peak at 1241 cm^{-1} was assigned to C = S (Shawali *et al*., 2002). The mass spectrum showed the molecular ion fragments at m/z, 231.12 for R-dioxolane, 118 for amide and 90.13 for N-aminothiourea.

The second step was achieved by heating 8a-b in ethanolic KOH under reflux conditions followed by removal of the ethanol by vacuum distillation. A solid product was extracted with ethyl acetate from the excess aqueous KOH layer. The extract give the triazole 9a-b in 80% after evaporation of solvent. The IR spectra in THF solution showed characteristic absorptions at 3330-3200, 2820 cm^{-1} for SH and 1671 cm^{-1} for C = N although some tautomerism to thione might have taken place (Zhang *et al*., 2006). The ^{1}H-NMR spectrum showed signals at 13.29 ppm and 10.25 ppm for NH and SH, respectively.

Biological screening: Almost all the major classes of antibiotics have encountered resistance in clinical application (Hamilton-Miller, 2004). The emergence of bacterial resistance to β-lactam antibiotics, macrolides, quinolones and vancomycin is becoming a major worldwide health problem (Hanaki *et al*., 2004). In particular, antibiotic resistance among Gram-positive bacteria is becoming increasingly serious (Nunez *et al*., 2003).

In order to overcome these emerging resistance problems, there is an urgent need to discover novel antibacterial agents in structural classes distinct from existing antibiotics. These finding have inspired us to synthesize new class of potential such as 1,3,4-oxadiazole 5 and 1,2,4-triazole 9. Compounds 3, 4, 5, 7 and 9 were tested for antibacterial using various strains with the microdilution method (Fadda *et al*., 2004).

For the determination of antibacterial activity, *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, *P. aeruginosa* ATCC 27833 and *E. coli* ATCC 25882 strains were utilized. All the compounds were also tested for in vitro antifungal activity against *C. albiccans* ATCC 64550 and *C. krusei* ATCC 14243 strains. Ampicillin, ketoconazole and fluconazole were used as reference compounds.

The MIC values of gluconic acid 1a and glucaric acid 1b derivatives (3a-b, 5a-b, 7a-b and 9a-b) are given as μg mL^{-1} in Table 1 and 2.

The results of this study showed that 2,3:4,5-di-O-isopropylidene-D-gluconic acid hydrazide 3a had significant antibacterial activity. The activity of compounds 4a and 7a is equal to that of ampicillin in terms of antibacterial activity against *S. aureus*.

Entire derivatives of Gluconic acid 1a had unpronounced antifungal activity and indifferent that of ketoconazole, which was used as the reference compound. It is well known that the antifungal drug ketoconazole is used clinically to treat or suppress various fungal infections (Abraham *et al*., 2003).

These results suggest that derivative of compound 3a may be worth studying further in terms of their antifungal activity.

As seen in Table 2, the oxadiazole thione 4b, the 1-aminotriazole 7b and triazolo glucaric acid 9b exhibited an important antibacterial activity against *P. aeruginosa* and *E. coli* (Bercin *et al*., 1995).

It appears a methyl substitutent on the side chain of 1b derived compound reduced antibacterial activity among Gram-positive bacteria. The oxadiazole derivatives of glucaric acid 4b were assayed for antifungal activity against different fungi, using fluconazole as the reference compound. All the derivatives of glucaric acid are more active than fluconazole.

As there has been no detailed study regarding the antibacterial properties of carboxylic acids 1a-b, in this study we investigated the antibacterial and antifungal activities of these compounds and their derivatives 3, 4, 5, 7 and 9 (Valdez *et al*., 2002).

Table 1: The MIC (μg mL^{-1}) values of Gluconic acid derived compounds

	Antibacterial activities				Antifungal activities	
	Gram positive		Gram negative		Yeast	Fungi
Comp.	*S. aureus*	*E. faecalis*	*P. aerugino*	*E. coli*	*Cand. albicans*	*Cand. krusei*
3a	15.6	15.6	15.6	15.6	3.9	3.9
4a	3.9	15.6	15.6	15.6	3.9	3.9
5a	15.6	7.8	15.6	15.6	3.9	3.9
7a	3.9	15.6	15.6	15.6	3.9	3.9
9a	7.8	7.8	15.6	15.6	3.9	3.9
AMP	3.9	3.9	3.9	3.9	-	-
FLUC	-	-	-	-	1.9	1.9
KET	-	-	-	-	7.8	7.8

Key to the inhibition zones activities: Highly active = Inhibition zone >12 mm; Moderately active = Inhibition zone 9-12 mm. Slightly active = Inhibition zone 6-9 mm; Inactive = inhibition zone <6 mm

Table 2: The MIC (μg mL^{-1}) values of Glucaric acid derived compounds

	Antibacterial activities				Antifungal activities	
	Gram positive		Gram negative		Yeast	Fungi
Comp.	*S. aureus*	*E. faecalis*	*P. aerugino*	*E. coli*	*Cand. albicans*	*Cand. krusei*
3b	15.6	15.6	31.2	31.2	15.6	15.6
4b	15.6	15.6	62.5	62.5	31.2	31.2
5b	15.6	15.6	31.2	31.2	15.6	15.6
7b	7.8	7.8	62.5	62.5	15.6	15.6
9b	7.8	7.8	62.5	62.5	15.6	15.6
AMP	3.9	3.9	3.9	3.9	-	-
FLUC	-	-	-	-	1.9	1.9
KET	-	-	-	-	7.8	7.8

Key to the inhibition zones activities: Highly active = Inhibition zone >12 mm; Moderately active = Inhibition zone 9-12 mm. Slightly active = Inhibition zone 6-9 mm; Inactive = inhibition zone <6 mm

The screening results in Table 1 and 2 indicates that the hydrazides 3a-b showed weak effects against Gram-positive bacteria (*S. aureus* and *E. faecalis),* but showed a moderate inhibition effect against the Gram-negative bacteria (*E. coli* and *P. aeruginosa*) (Andrzejewska *et al.*, 2004).

The oxadiazoles have shown generally higher activities against both Gram-positive and Gram-negative bacteria. Our synthesized oxadiazoles 4a-b exhibited higher inhibition effects against Gram-negative bacteria (Climesova *et al.*, 2002).

Compounds 5a-b and 7a-b have a moderately active effect on the Gram-negative bacteria (*E. coli*), while they exhibited slight activity against the Gram-positive bacteria.

The triazole 9a-b has a weak inhibition effect than the Ampicillin next to Gram-positive and negative.

CONCLUSION

Development of bacterial resistance has led to the synthesis of newer and more potent nucleosides. As detailed above the carboxylic acid derivatives have been designed, synthesized, characterized and evaluated for their biological activities in order to discover potent agent against Gram-positive and Gram negative bacteria. It was observed that when an aromatic amino group was introduced to the carboxylic side a significant enhancement of potency against organisms appears as potential anticancer agent.

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