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Synthesis of New Organoselenium Compounds Containing Nucleosides as Antioxidant

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ABSTRACT

Selenium containing nucleosides derived from some heterocyclic moieties such as Pyridineselenol, and pyridazineselenol is described herein. Ribosylation of selenol compounds were prepared in good yield by silyation of selenol derivatives with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose followed by debenzoylation to afford the corresponding free N-nucleosides β and α-1-(2,3,5-trihydroxy-β-D-ribofuranosyl)-2-seleno-4,6-dimethylpyridine-3-carbonitrile (6a,7a); *b* and *a*-1-(2,3,5-trihydroxy-β-D-ribofuranosyl)-3-seleno-5,6-diphenylpyridazine-4-carbonitrile (6b,7b). Newly synthesized compounds were characterized using the well known spectroscopic tools (IR, ^{1}HNMR, ^{13}CNMR and mass spectroscopy). Antioxidant activity of six selenonucleoside compounds (1a; 6a; 7a; 1b; 6b and 7b) was evaluated by animal assay model using experimental mice. The resulted data revealed that compounds 6a and 7b showed to be more active as antioxidant with a better performance of scavenging ability than the other compounds.

Key words: 1-*O*-Acetyl-2,3,5-trihydroxy-β-D-ribofuranose; Nucleosides; Selenium ; Pyridineselenol; pyridazineselenol; antioxidants

INTRODUCTION

From the literature survey indicates that few publications have mentioned the incorporation of selenium atom into nucleosides[1,2]. In this paper[1] describe the synthesis of selenium- and tellurium-containing nucleosides, derived from uridine which was prepared in a concise and short synthetic route in good yields, by nucleophilic substitution of a tosylate group by organoselenium nucleophiles. On the other hand in previous work in our laboratory[2] describes the synthesis of selenium-containing nucleoside analogues, derived from some heterocyclic moieties such as pyridineselenol, pyridazineselenol and quinolineselenol derivatives which indicated that one of pyridine-moiety 2-(1,3-dihydroxypropylselenyl)-4,6-dimethylpyridine-3-carbonitrile which bearing two hydroxyl groups, and

three of pyridazine-moieties 3-(3-hydroxypropyl selenyl)-5,6-diphenylpyridazine-4-carbonitrile, 3-(1,3-dihydroxy propyl selenyl)-5,6-diphenylpyridazine-4-carbonitrile and 3-(oxiran-2-ylmethyl selenyl)-5,6-diphenyl- pyridazine-4-carbonitrile respectively have definite antioxidant effect.

Stimulated by our recent work on the synthesis of selenium containing nucleoside analogues[2], sulfa drugs[3], and the synthesis of selenium containing amino acid analogues[4], we decided to expand our interest to the introduction of an organoselenium compounds in the nucleoside framework and screened their biological activity as antioxidants.

RESULTS AND DISCUSSION

Chemistry

Ribosylation of 1a, b were achieved by refluxing in hexamethyldisilazane (HMDS) to give the silylated derivatives 2a, b. The latter was stirred with 1-*O*-acetyl-2,3,5-*O*-benzoyl-β-D-ribofuranose (3) in the presence of dry 1,2-dichloroethane as a solvent using trimethylsilyl trifluoromethane sulfonate (TMS Triflate) ($CF_3SO_2OSiMe_3$) as a catalyst as according to the method of Vorbruggen *et al.,*[5] to give the corresponding mixture of β-anomeric protected *N*-nucleoside derivatives and *a*-anomeric protected *N*-nucleoside. Separation of the mixture of β and *a* anomers (4a, 5a) and (4b, 5b) was carried out by column chromatography in yields ranging from 25-75 % of the corresponding benzoylated nucleosides (4a, 5a, 4b, and 5b) respectively (Scheme 1).

Debenzoylation of compounds (4a, 5a, 4b and 5b) were performed by using methanolic sodium methoxide solution following Zemplen *et al*. method[6] to afford the free nucleosides (6 a, 7a, 6b and 7b) respectively.

The chemical structures of the nucleoside derivatives 4a, 4b, 5a, 5b, 6a, 6b, 7a and 7b were established and confirmed on the basis of their elemental analyses and spectral data (IR, ^{1}H and ^{13}C NMR) (see the Experimental section).

The IR spectra of compounds (4a, 5a, 4b and 5b) were observed at ν 2210 cm^{-1} due to CN group and stretching vibration frequencies of the benzoyl carbonyl groups C=O appeared at ν 1740, 1730, 1727 and 1724 cm^{-1} of compounds 4a, 5a,

Table 1: Effects of synthesized compounds (1a; 6a; 7a; 1b; 6b and 7b) on SOD; GST activities and GSH-Rd levels

Design of treatment	SOD (Units/mg protein)	GST (µmol /mgprotein)	GSH-Rd (mg/g protein)
Untreated group,Group 1	35.13 ± 1.25^{a,b}	5.13 ± 0.11^{a,b}	4.21 ± 0.21^{a,b}
Compound 1a (100 mg/kg) (Group 2)	35.87 ± 0.51	5.84 ± 0.11	4.10 ± 0.10
Compound 1a (200 mg/kg) (Group 3)	32.21 ± 1.93	6.13 ± 0.25	5.01 ± 0.32
Compound 1b (100 mg/kg) (Group 4)	34.39 ± 1.13	5.10 ± 0.61	4.91 ± 0.27
Compound1b (200 mg/kg) (Group 5)	33.90 ± 1.21	5.82 ± 0.18	5.11 ± 0.22
Compound 6a (100 mg/kg) (Group 6)	39.01 ± 1.15^{a,b,}	3.91 ± 0.26^{a,b}	5.85 ± 0.13^{a,b}
Compound 6a (200 mg/kg) (Group 7)	38.90 ± 1. 01^{a,b,}	6.19 ± 0.10^{a,b}	5.39 ± 0.11^{a,b}
Compound 6b (100 mg/kg) (Group 8)	32.49 ± 2.10	5.01 ± 0.72^{b}	4.01 ± 0.12
Compound 6b (200 mg/kg) (Group 9)	33.54 ± 1.42	4.45 ± 0.11	3.92 ± 0.15
Compound 7a (100 mg/kg) (Group 10)	34.54 ± 2.10	5.12 ± 0.32	4.78 ± 0.17
Compound 7a (200 mg/kg) (Group 11)	31.97 ± 1.79	5.16 ± 0.40	4.98 ± 0.13
Compound 7b (100 mg/kg) (Group 12)	41.54 ± 1.15^{a,b}	7.12 ± 0.81^{a,b}	6.78 ± 0.14^{a,b}
Compound 7b (200 mg/kg) (Group1 3)	39.17 ± 1.02^{a,b}	6.82 ± 0.42^{a,b}	6.18 ± 0.08^{a,b}
Vitamin E (100 mg/ kg) (Group 14)	45.17 ± 1.13	8.12 ± 0.11	7.37 ± 0.31

Values are mean ± SD, n = 6, $^{a\,b}$ the difference is significant ($p>0.05$) in a column between treated and untreated control group1.

4b and 5b respectively. In addition signals at ν1625, 1620 cm^{-1} for the C=N group of compounds 4a, 5a, and at ν 1630 cm^{-1} of compounds 4b and 5b.

The IR spectra and the most important peaks for compounds (6a, 7a, 6b and 7b) were observed at ν 3400-3450 cm^{-1} due to (OH group) for compounds 6a and 6b respectively and signals at ν 3380 cm^{-1} due to (OH group) for compounds 7a and 7b.

The 1H NMR spectra of compounds (4a, 5a, 4b and 5b) showed a doublet ranging from ν 5.55 - 6.39 ppm , with spin–spin coupling constant ($\nu_{1',2'}$) equal to 7.5 Hz for proton (H-1') which confirms the β-anomeric configuration of compound 4a and 4b respectively, while the data showed that a doublet signals at δ ranging from 5.80 - 6.39 ppm , with spin–spin coupling constant (J1',2) ranging from 4.5-5 Hz for proton (H-1') assigned to the *a* - anomeric configuration of compound 5a and 5b respectively.

The 1H NMR spectra of compounds (**6a, 7a, 6b** and 7b) showed a doublet ranging

From δ 6.20-6.08 ppm with ($J_{1',2'}$) equal to 7-7.5 Hz for for proton (H-1') which confirms the ²-anomeric configuration of compound 6a and 6b. While signals at 5ØÿÞ ranging from 6.08 - 6.22 ppm, with spin–spin coupling constant ($J_{1',2'}$) ranging from 5.5-5 Hz for proton (H-1') assigned to the *a* - anomeric configuration of compound 7a and 7b respectively.

The 13C NMR spectrum of compounds (4a, b, 5a, b, 6a, b and 7a, b) showed the most signals at δ C 13.4 and 18.6 for two groups of CH_3 for compounds 4a and 5a. The five signals at δ C 93.2, 84.2, 78.1, 71.4 and 59.2 were assigned to C-1', C-2' , C-3' , C-4', and C-5' of the sugar moiety, respectively. Data showed that at δ C 118.2 due to (CN), 120.0–135.0 (Ar), 151.2, 160.2, 168.0 (C=O); 175.0 (C=Se) of compound 4a and 175.9 (C=Se) of compound 4b. The 13C NMR spectrum of compounds 7a and 7b showed signals: 22.63, 29.70, 52.15, 63.42, 76.12, 79.11, 84.71, 117.786, 128.39, 129.74, 132.09, 133.26, 169.79 for 7a and signals at δ 52.12, 64.50, 76.12, 79.11, 89.51, 117.53, 127.34, 128.28, 128.36, 128.65, 128.88, 128.99, 129.57, 132.06, 132.92, 133.23, 134.13, 167.72, 169.27 for 7b

Finally the Mass spectra and Elemental analysis are in agreement and confirmed of all new compounds.

Biological activity

Toxicity studies

Toxicity parameters including LD_{50}; GPT

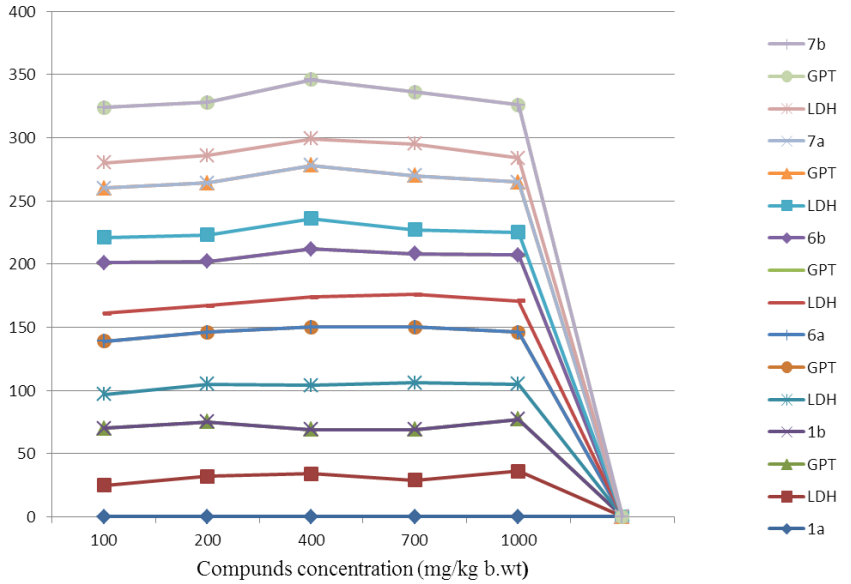

Fig. 1: Effects of synthesized compounds on activities of GPT and LDH enzymes

and LDH activities were determined and ranged in normal limits compared to infected untreated group with concentrations range up to 1000 mg kg- b.wt. The GPT, an enzyme which allows determining the liver function as indicator on liver cells damage and LDH enzyme is often used as a marker of tissue breakdown (Butt *et al*., 2002) [6].

Antioxidant activity evaluation

Hepatic GSH-Rd and serum activities of SOD, GSH-S-transferase levels were measured as an indicator of antioxidant activity and result are present in Table 1. SOD and GSH-S-transferase are antioxidant enzymes that protect cells from oxidative stress of highly reactive free radicals and induces on the generation of free radicals in living cells. Result indicated that significant increasing ($p < 0.05$) in SOD, and GST activities in the treated groups at doses of 100 and 200 mg/kg compared to un-treated control group. No significant deference found between used doses (100 and 200 mg/kg) with all tested compounds. The highest SOD and GST, activities rather than GSH-Rd levels was monitored in animals treated with compounds 6a and 7b. (See Table 1, Fig. 1).

EXPERIMENTAL

General

Melting points were determined by using the Kofler melting point apparatus, and were uncorrected. IR (KBr, cm^{-1}) spectra were recorded on a Pye-Unicam SP3–100 instrument at Taif University. 1H NMR spectra were obtained on a Varian (400 MHz) EM 390 USA instrument at King Abdel-Aziz University by using TMS as internal reference.13 C NMR spectra were recorded on a JNM-LA spectrometer (100 MHz) at King Abdel-Aziz University, Saudi Arabia. Elemental analyses were obtained on an Elementar Vario EL 1150C analyzer. Mass spectra were recorded on a JEOL-JMS-AX 500 at Cairo National Research Center, Cairo, Egypt. Purity of the compounds was checked by thin layer chromatography (TLC) using silica gel plates.

Synthesis of a and b-1-(-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)seleno derivatives (4a,b , 5a,b)

Ribosylation of 2-seleno-4,6-dimethylpyridine-3-carbonitrile and 3-seleno-5,6-diphenylpyridazine-4-carbonitrile (1a,b). Synthesis of *b* and *a*-1-(2,3,5-tri-*O*-benzoyl-δ-D-ribofuranosyl) -2-seleno-4,6-dimethylpyridine-3-carbonitrile (4a, 5a); *b* and *a*-δ1-(2,3,5-tri-*O*-benzoyl-5ØýÞ-D-ribofuranosyl)-3-seleno-5,6-diphenylpyridazine-4-carbonitrile (4b, 5b).

General Procedure. A mixture of 2-seleno-4,6- dimethylpyridine-3-carbonitrile or 3-seleno-5,6-diphenylpyridazine-4-carbonitrile (1a,b) (0.02 mol) and hexamethyl di- silazane (20 ml) was heated under reflux for 24h with a catalytic amount of ammonium sulfate (0.01g). After that, the clear solution was cooled and evaporated till dryness to give the silyated derivative (2a,b), which directly was dissolved in 20 ml of dry 1,2-dichloroethane and then 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose (3) (5.05 g, 0.01 mol) was added. The mixture was added dropwise onto a mixture of (10 ml trimethylsilyl trifluoromethanesulfonate (Triflate) in dry 1,2-dichloroethane (50 ml)). All mixture was stirred at room temperature for 24 h, and then washed with a saturated solution of aqueous sodium bicarbonate (3 × 50 ml), washed with water (3 × 50 ml), and dried over anhydrous sodium sulfate. The solvent was removed in vacuum and the residue was chromatographic on silica gel with chloroform: ethyl acetate (9: 1) as eluent to afford a white crystal from pure anomeric *b* and colorless crystals from *a* anomeric (4a,b and 5a,b) respectively.

Compound (pydBzb) (4a)

Yield (42%), m.p. 160-161°C; IR (KBr) β cm^{-1}: 2217 (CN), 1730 (CO). ^{1}H NMR ($CDCl_3$): β 3.19 (s, 3H, CH_3); 2.20 (s, 3H, CH_3); 3.98-4.10 (m, 2H, 2H-5'); 4.90 (m, 1H, 1H-4'), 5.25–5.30 (m, 1H, H-3'), 5.38–5.40 (m, 1H, H-2'), 5.55 (d, 1H, H-1', $\beta=J_{1',2'}$ = 7.5Hz), 6.32 (s, 1H, CH-pyridine), 7.33–8.05 (m, 15H, aromatic protons); ^{13}CNMR ($CDCl_3$): 16.2, 20.3, 59.2, 71.4, 78.1, 84.2, 93.2, 118.2, 120.0–135.0, 151.2,160.2, 168.0; 175.0 (C=Se). Anal. Calcd. for $C_{34}H_{28}N_2O_7Se$ (655.56): C, 62.29; H, 4.31; N, 4.27 (%); Found: C, 62.00; H, 4.11; N, 4.14 (%).

Compound (pydBza) (**5a**)

Yield (28%), m.p. 203-204°C; IR (KBr) *ν* cm^{-1}: 2210 (CN), 1730 (CO). ^{1}H NMR ($CDCl_3$): 3.12 (s, 3H, CH_3); 2.23 (s, 3H, CH_3); 3.46-3.48 (m, 2H, 2H-5'), 4.60–4.95 (m, 1H, H-4'), 5.15-5.30 (m, 1H,

H-3'), 5.40–5.60 (m, 1H, H-2'), 5.80-6.94 (d, 1H, H-1', $J_{1',2'}$ = 4.5Hz), 6.30 (s, 1H, CH-pyridine), 8.00–7.45 (m, 15H, Ar-H); ^{13}C NMR ($CDCl_3$): 13.4, 18.6, 59.5, 71.2, 78.1, 84.0, 93.2, 121.4, 125.0–140.2, 151.2, 159.2, 168.0, 175.2 (C=Se); Anal. Calcd. for $C_{34}H_{28}N_2O_7Se$ (655.56): C, 62.29; H, 4.31; N, 4.27 (%); Found: C, 62.15; H, 4.22; N, 4.10 (%).

Compound (PydzBzβ) (4b)

Yield (47%), m.p. 185–187°C; IR (KBr) ν cm^{-1}: 2210 (CN), 1730(CO), 1630 (C=N); ^{1}H NMR ($CDCl_3$): δ 4.23 (d, 1H, H-4' β=$J_{1',2'}$ = 7.5Hz); 4.58-4.57 (m, 2H,H-5'), 4.64 (d, 1H,H-3', 5Ø=Ü1',2'= 7.5Hz); 5.65 (d, 1H, H-2', $J_{1',2'}$= 7.5Hz), 6.39 (d, 1H, H-1', $J_{1',2'}$= 7.5Hz), 7.53-7.00-7.45 (d, 10H,aromatic protons, J= 8.0Hz), 8.05–7.81 (m, 15H, Ar-H); ^{13}CNMR ($CDCl_3$): 59.1, 71.5, 78.3, 84.2, 93.0, 121.2, 125.0–140.0,151.2, 162.1, 168.4, 175.9 (C=Se); Anal. Calcd. for $C_{43}H_{31}N_3O_7Se$ (780.68): C, 66.15; H, 4.00; N, 5.38 (%); Found: C, 66.08; H, 3.99; N, 5.10 (%).

Compound (PydzBza) (5b)

Yield (30%), m.p. 168-169°C; IR (KBr) νcm^{-1}: 2210(CN), 1724 (CO), 1630 (C=N); ^{1}H NMR ($CDCl_3$): δ 4.23 (d, 1H, H-4' 5Ø=$Ü_{1',2'}$ = 7.5Hz); 4.58-4.57 (m, 2H,H-5'), 4.64 (d, 1H,H-3', 5Ø=Ü1',2'= 7.5Hz); 5.65 (d, 1H, H-2', $J_{1',2'}$= 7.5Hz), 6.39 (d, 1H, H-1', $J_{1',2'}$= 5.0 Hz), 7.53-7.00-7.45 (d, 10H,aromatic protons, J = 8.0Hz), 8.05–7.81 (m, 15H, Ar-H).^{13}C NMR ($CDCl_3$): 35.2, 59.1, 71.0, 78.3, 84.4, 93.2, 121.0, 125.1–140.0, 151.3, 160.4, 168.1, 175.5 (C=Se); Anal. Calcd. for $C_{43}H_{31}N_3O_7Se$(780.68): C, 66.15; H, 4.00; N, 5.38 (%); Found: C, 66.00; H, 3.98; N, 5.30 (%).

Deprotection of 4 a,b and 5a, b. Synthesis of nucleosides 6a,b and 7a,b respectively.

General Procedure

A mixture of each protected nucleoside 4a,b and 5a, b (0.001 mol for each), absolute methanol (20 ml) and sodium methoxide (0.055 g, 0.001mol) was stirred at room temperature for 48 h. The solvent was evaporated under vacuum to give a colorless solid, which was dissolved in hot water and neutralized with acetic acid. The precipitate compound was chromatographic on silica gel with chloroform: ethyl acetate (9: 1) as eluent to afford colorless and white crystals of the corresponding nucleosides *6 a,b* and *7a,b* respectively.

Compound (PydOHb)(6a)

Yield (35%), m.p. 210-212°C; IR (KBr) νcm^{-1}: 3400 (OH), 2215 (CN); ^{1}H NMR (DMSO-5ØQÜ6): 5ØÿÞ 3.27 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 3.55-3.65 (m, 2H, 5'-H), 3.92-3.90 (m, 1H, 4'-H), 4.15-4.25 (m, 1H, 3'-H), 4.53-4.54 (m, 1H, 2'-H), 4.75-5.85 (s, 3H, 3-OH), 6.20 (d, 1H, $J_{1',2'}$= 7.5 Hz, 1'-H), 6.30 (s, 1H, CH-pyridine). ^{13}C NMR ($CDCl_3$): 20.26, 24.63, 61.33, 71.2, , 78.1, 84.0, 109.63, 115.786, 122.79, 127.34, 132.04, 151.95, 162.48; Anal.Calcd. for $C_{13}H_{16}N_2O_4Se$ (343.24): C, 45.49; H, 4.70; N, 8.16 (%); Found: C, 45.15; H, 4.50; N, 8.07 (%).

Compound (PydOHa)(7a)

Yield (27%), m.p. 215-217°C; IR (KBr) ν^{-1}: 3380 (OH), 2217 (CN); ^{1}H NMR (DMSO-d6): δ3.25 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 3.47-3.48 (m, 2H, 5'-H), 3.9-3.92 (m, 1H, 4'-H), 4.05–4.12 (m, 1H, 3'-H), 4.35-4.45 (m, 1H, 2'-H), 4.75-5.85 (s, 3H, 3-OH, 6.22 (d, 1H, $J_{,2'}$= 5.5 Hz, 1'-H), 6.35 (s, 1H, CH-pyridine). ^{13}C NMR ($CDCl_3$): 22.63, 29.70, 52.15, 63.42, 76.12, 79.11, 84.71, 117.786, 128.39, 129.74, 132.09, 133.26, 169.79; Anal. Calcd. for $C_{13}H_{16}N_2O_4Se$ (343.24): C, 45.49; H, 4.70; N, 8.16; Found: C, 45.20; H, 4.29; N, 8.10(%).

Compound (PydzOHb)(6b)

Yield (64%), m.p.230-232°C; IR(KBr) ν cm^{-1}: 3450(OH), 2210 (CN), 1630 (C=N); ^{1}H NMR (DMSO-d6): δ1.25 (s, 1H,5'-OH), 1.66 (s, 1H, 3'-OH), 3.92 (s, 1H, 2'-OH), 4.28 (m, 2H, 5'-H); 4.38 (m, 1H, 4'-H); 4.96 (t, 1H, 3'-H), 5.65 (d, 1H, 2'-H),6.08 (d, 1H, $J_{1',2'}$ = 7.0 Hz, 1'-H), 7.26-8.05 (m, 10H, aromatic protons). ^{13}C NMR ($CDCl_3$): 66.81, 72.74, 82.90, 88.52, 91.42, 117.78, 128.54, 128.59, 129.40, 129.45,129.55,130.11,130.38, 131.14, 132.83, 133.19, 134.75,138.90, 167.77 ,168.52; Anal. Calcd. for $C_{22}H_{19}N_3O_4Se$(468.36): C, 56.42; H, 4.09; N,8.97 (%); Found: C, 55.95; H, 3.97; N, 8.72(%).

Compound (PydzOHα)(7b)

Yield (75%), m.p. 235-237°C; IR (KBr) νcm^{-1}: 3380 (OH),2210 (CN), 1630 (C=N). ^{1}H NMR (DMSO-δ): δ1.25 (s, 1H,5'-OH), 1.66 (s, 1H, 3'-OH), 3.92 (s, 1H, 2'-OH), 4.28 (s, 2H, 5'-H); 4.38 (m, 2H, 4'-H); 4.96 (t, 1H, 3'-H), 5.65 (d, 1H, 2'-H), 6.08 (d, 1H, $J_{1',2'}$ = 5.0 Hz, 1'-H), 7.26-8.05 (m, 10H, aromatic protons). ^{13}C NMR ($CDCl_3$): 52.12, 64.50, 76.12, 79.11, 89.51, 117.53, 127.34, 128.28, 128.36,

128.65, 128.88, 128.99, 129.57, 132.06, 132.92, 133.23, 134.13, 167.72, 169.27; Anal. Calcd. $C_{22}H_{19}N_3O_4Se$ (468.36): C, 56.42; H, 4.09; N, 8.97 (%); Found: C, 56.10; H, 4.00; N, 8.50 (%).

Biological experiments.

Chemicals

Dimethyl sulfoxide (DMSO) and vitamin E were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All enzymatic kits were purchased from Bioassays system, USA.

Experimental animals

Male Albino mice (20 ± 2 g) were obtained from department of animal science, cairo university and animals were handled under standard laboratory conditions with a 12-h light/dark cycle in a temperature of 25 ± C and a relative humidity of 55 ± 5 % controlled room. The basal diet used in these studies was certified feed to research laboratories animals. Food and water were available adlibitum. Cairo university animal care and use committee approved all protocols for the animal studies research.

Toxicity experiment

Male Albino mice of 6 animals per group and weighing between 25± 5 g were administered after overnight fasting with graded doses of (100-1000) mg kg^{-1}b.wt. intra peritoneal of each individual synthesized compounds suspended in DMSO. The toxicological effects were observed after 72 h of treatment in terms of mortality and expressed as LD_{50} (Ghosh, 1984) [8]. Others biochemical parameters determined after 14 days of administration according to methods of Reitman and Frankel (1957) [9] for GPT activity and Bergmeyer (1974) [10]for LDH activity

Bioassays model design

The animals were randomly divided into fourteen groups of 6 mice each. The first group served as untreated normal control. Group 2 to Group 13 on 7th day, animals were pre-treated with individual synthesized compounds at 100 and 200 mg/ kg b, wt, per day p.o., respectively for 7 days. Group 14, animals were pre-treated with standard drug Vitamin E (100 mg/ kg b, wt, per day p.o) for 7 days. (Rai *et al*., 2006)[11].

Twenty-four hours after the last administration, mice were sacrificed. Blood samples were collected and centrifuged at 4000×g at 4æ%C for 10 min for serums preparation. The liver was removed rapidly, washed and homogenized in ice-cold physiological saline to prepare 10% (w/v) homogenate. Then, the homogenate was centrifuged at 4000×g at 4æ%C for 10 min to remove cellular debris, and the supernatant was collected for biochemical analysis.

The biochemical assays.

Measurement of Glutathione-S-Transferase activity (GST)

GST activity was determined as described by Habig *et al*., (1974)[12]. Reaction mixture containing 50 mM phosphate buffer, pH 7.5, 1 mM of 1-chloro-2, 4 dinitrobenzene (CDNB) and a appropriate volume of compound solution. The reaction was initiated by the addition of reduced glutathione GSH) and formation of S-(2, 4-dinitro phenyl) glutathione (DNP-GS) was monitored as an increase in absorbance at 334 nm. The result was expressed as µmol of CDNB conjugation formed /mg protein /min.

Measurement of Super Oxide Dismutase (SOD) activity

SOD activity was measured through the inhibition of hydroxylamine oxidation by the superoxide radicals generated in the xanthine–xanthine oxidase system. (*Kakkar et al*. 1972) [13]. The results were expressed in units/mg protein.

Measurement of Glutathione Reduced (GSH-Rd) levels

GSH in liver and kidney tissues was determined according to the Ellman method (Ellaman, 1959)[14], which measures the reduction of 5,50-dithio-bis (2-nitrobenzoic acid) (DTNB) (Ellman's reagent) by sulfhydryl groups to 2-nitro-5-mercaptobenzoic acid, which has an intense yellow color. The results were expressed in mg per g protein (mg/g protein).

Measurement of Protein content

Protein levels were determined spectrophotometrically at 595 nm, using comassie blue G 250 as a protein binding dye (Bradford, 1976)[15]. Bovine serum albumin (BSA) was used as a protein standard.

CONCLUSION

Silyation of selenol derivatives with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose followed by debenzoylation affording the corresponding free N-nucleosides compounds β and α-1-(2,3,5-trihydroxy-*b*-D-ribofuranosyl)-2-seleno-4,6-dimethylpyridine-3-carbonitrile (6a,7a); β and α-1-(2,3,5-trihydroxy-*b* -D-ribofura- nosyl)-3-seleno-5,6-diphenylpyridazine-4-carbonitrile (6b,7b) respectively. Compounds 6a and 7b showed to be more active as antioxidant with a better performance of scavenging ability than other compounds. The SOD activity of these molecules was compared with standard antioxidant (vitamin E). Selenonucleoside compounds are active sites of a large number of selenium dependent enzymes, such as antioxitant enzymes [Spallholz J.E 1994] [7]. The configuration structure of compounds 6a and 7b may more suitable for SOD and GST enzymes active center, so these compounds induce the antioxidants enzymes activity.

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