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Synthesis and Antioxidant Activity of 1,2-*Bis*(1-ethyl-2-substitutedphenylindolizin-3-yl)diselane

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A series of 1,2-*bis*(1-ethyl-2-substituted phenylindolizin-3-yl)diselane derivatives (**6a-h**) with various acetopheneone substitute were synthesized and evaluated for antioxidant activity by using free radicals (DPPH) method. The structures of compounds were confirmed by elemental analysis, IR and NMR spectral data.

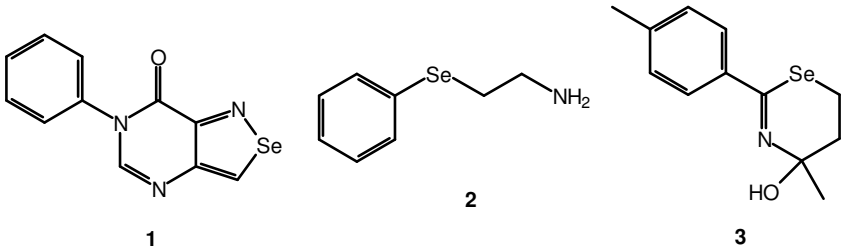
Key Words: Antioxidant, Diselenides, Indolizine, DPPH.

INTRODUCTION

Interest in selenium containing therapeutics has grown over the last thirty years[1]. Simple organoselenium compounds have been prepared (Fig. 1), such as selenazolopyrimidone (**1**) that shows antitumor activity against mouse leukemia[2]. (Aminoethyl)phenylselenide (**2**) shows excellent antihypertensive activity and selenazine derivatives (**3**) show both antibacterial and antitumour activity[3]. Despite this, the major therapeutic benefit that selenium currently offers appears to be in the form of dietary supplements[4]. Selenium is now known to be intimately involved in the activity of enzymes such as glutathione peroxidase and thioredoxin reductase, that catalyze chemistry essential to the protection of biomolecules against oxidative stress and free radical damage[5]. Glutathione peroxidase (GSH Px), a selenoenzyme, is one of the enzymes in the mammalian antioxidant systems, which perform the reduction of H_2O_2 and other hydroperoxides.

It is well accepted that selenium (as selenocysteine), an essential component of the active sites of glutathione peroxidase, is responsible for the scavenging of reactive oxygen species and protecting biomembrane from oxidative stress. Although possessing potent antioxidative activity, like other protein drugs GSH Px is limited for the clinical use because of its instability, poor availability and easy metabolism. Therefore, many efforts have been directed to design and synthesize small selenium-containing organic

molecules with GSH Px activity[6]. Ebselen (2-phenyl-1,2-benzisoselenazole-3(2*H*)-one), a non-toxic low molecular weight selenium-containing heterocyclic compound, has been reported to mediate the reduction of hydroperoxides by glutathione, thereby mimicking the enzymatic activity of GSH Px[7]. In present study, synthesis and antioxidant activity of 1,2-*bis*(1-ethyl-2-substituted phenylindolizin-3-yl)diselane derivatives have been reported.



1 **2** **3**

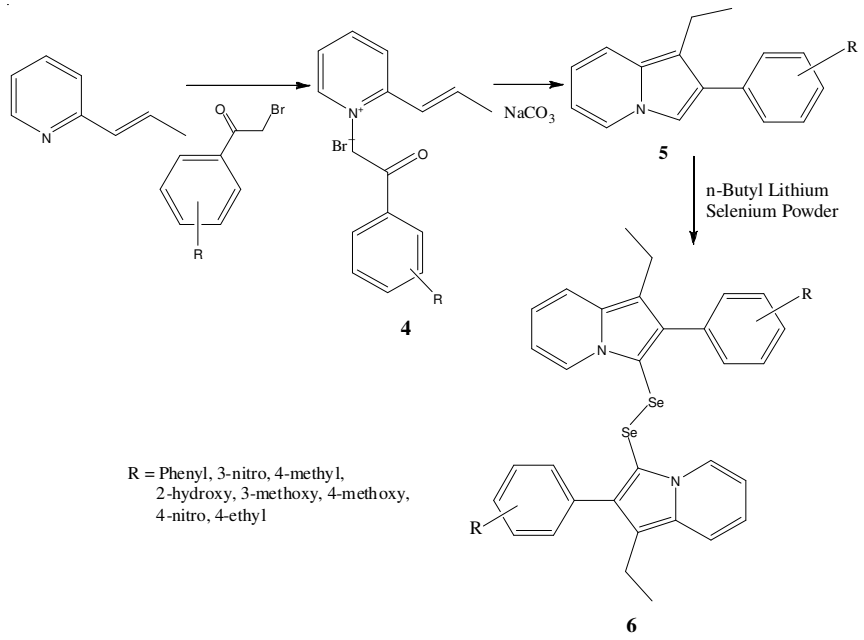
Fig. 1. Structures of simple organoselenium compounds

EXPERIMENTAL

Melting points were determined by open capillary method and are uncorrected. All the reactions were performed under nitrogen atmosphere to prevent oxidation and oxygen-sensitive selenide ions and selenium containing products. IR spectra in KBr (cm^{-1}) were recorded on Perkin Elmer FTIR and ^1H NMR spectra ($CDCl_3$) (in δ ppm) were recorded on Bruker 200 spectrospin spectrometer.

The fusion of 1-ethyl-2-substituted phenylindolizine with selenium results in the formation of 1,2-*bis*(1-ethyl-2-phenylindolizin-3-yl)diselane with the respective substituents (**Scheme-I**). The literature, however, offers no report on the reaction of indolizines with elemental selenium. The formation of diselenide was confirmed by spectral data. The location of the selenium at C(3) was established on the basis of PMR and ^{13}C NMR data (250 and 360 MHz for protons) in a comparison with the original indolizine. The PMR spectrum of diselenide does not show a signal for the 3-H proton, while the remaining indolizine protons are displayed and have chemical shifts differing only slightly from those for the original indolizine. Further the formation of diselenide is conformed by atomic absorption.

Synthesis of phenyl acyl α-propenyl pyridinium bromide (4a-h)[8]: A mixture of 2-propenyl pyridine (10 mmol, 0.93 g) and 6 g (30 mmol) of substituted bromoacetophenone was boiled in 30 mL of ethyl methyl ketone for 10 h. The light yellow crystals of phenyl acyl propenyl pyridinium bromide were separated on cooling. It was filtered and washed with ethyl methyl ketone and ether to yield 60-80 % of product.



Scheme-I: Synthesis of diselenide derivatives

Substituted phenylindolizine (5a-h): A 2.2 g (6 mmol) of phenyl acyl α-propenyl pyridinium bromide (**4a-h**) in 100 mL of a 40 % sodium carbonate solution is boiled for 3 h. The precipitate is separated, washed with 100 mL of water, dried and crystallized from a hexane-ether mixture (1:1). This gives 1.3 g (81 %) of indolizine crystals with ^{1}H NMR: δ 1.25 (3H, s, CH_3), 2.48 (2H, d, CH_2) 6.41 (1H, t, CH), 6.68 (1H, t, CH), 7.11 (1H, d, CH) and 7.44 (6H, m, CH); ^{13}C NMR (DMSO-d_6) 129.5, 127.5, 125.6, 118.8, 117.2, 112.1, 19.3, 15.9; (Found (%): C, 86.84; H, 6.83; N, 6.33; Calcd. (%) for $C_{16}H_{15}N$ (221.00): C, 85.03; H, 6.25; N, 6.98).

Synthesis of 1,2-*bis*(2-phenylindolizin-3-yl)diselane derivatives (6a-h): To a solution of substituted 1-ethyl-2-phenylindolizine (1.75 mL, 1.79 g, 10 mmol) (**5a-h**) in dry hexane (50 mL) was added a 1.6 M solution of *n*-BuLi in hexane (6.8 mL, 11 mmol). After 1 h of stirring at room temper-ature, a white precipitate of the lithiated compound was obtained. The supernatant solvent was removed with a syringe. The white precipitate was dissolved in dry ether, the solution was cooled to 0 ºC and selenium powder (0.8 g, 10 mmol) was added. After 2 h of stirring at this temperature, the resulting solution was filtered and the solvent was evaporated to give a diselenide **6a** (1.29 g, 45 %). The compound was recrystallized from a chloroform-hexane (1:1). The presence of selenium metal was determined from atomic absorption and Se NMR.

1,2-*Bis*(1-ethyl-2-phenylindolizin-3-yl)diselane (6a): m.p. 221 ºC; ^{1}H NMR: ($CDCl_3$) δ 1.20 (3H, s, CH_3), 2.52 (2H, d, CH_2), 6.68 (1H, t, CH), 6.41 (1H, t, CH), 7.11 (1H, d, CH) and 7.42 (6H, m, CH); ^{13}C NMR (DMSO-d_6) 128.5, 127.5, 124.5, 118.1, 116.8, 111.8, 19.1, 15.5; (Found (%): C, 64.22; H, 4.72; N, 4.68; Calcd. (%) for $C_{32}H_{28}N_2Se_2$ (600.00): C, 62.90; H, 4.98; N, 6.32; Se, 24.39.

1,2-*Bis*(1-ethyl-2-(3-nitrophenyl)indolizin-3-yl)diselane (6b): m.p. 196 ºC; ^{1}H NMR: ($CDCl_3$) δ 1.0 (3H, s, CH_3), 2.42 (2H, d, CH_2), 6.61 (1H, t, CH), 6.20 (1H, t, CH), 7.00 (1H, d, CH) and 8.22 (6H, m, CH); ^{13}C NMR (DMSO-d_6) 133.6, 130.5, 123.9 117.0, 116.0, 112.1, 19.0, 16.0; (Found (%): C, 55.82; H, 3.81; N, 8.14; O, 9.30; Calcd. (%) for $C_{32}H_{26}N_4O_4Se_2$ (688.00): C, 58.90; H, 4.80; N, 8.32, O, 9.00); Se, 21.94.

1,2-*Bis*(1-ethyl-2-*p*-tolylindolizin-3-yl)diselane (6c): m.p. 230 ºC; ^{1}H NMR: ($CDCl_3$) δ 3.20 (6H, s, CH_3), 2.00 (2H, d, CH_2), 6.00 (1H, t, CH), 5.85 (1H, t, CH), 7.45 (1H, d, CH) and 8.22 (6H, m, CH); ^{13}C NMR (DMSO-d_6) 131.6, 129.5, 127.4 116.5, 116.0, 110.4, 21.3, 19.5, 17.2; (Found (%): C, 64.71; H, 4.94; N, 4.57; Calcd. (%) for $C_{33}H_{30}N_2Se_2$ (612.00): C, 58.90; H, 4.80; N, 8.32); Se, 24.00.

2,2'-(3,3'-Diselanediylbis(1-ethylindolizin-3,2-diyl))diphenol (6d): mp; 190 ºC; ^{1}H NMR: ($CDCl_3$) δ 1.25 (3H, s, CH_3), 2.0 (2H, d, CH_2), 6.00 (1H, t, CH), 6.41 (1H, t, CH), 6.50 (1H, d, CH), 7.00 (6H, m, CH) and 8.60 (1H, s, OH); ^{13}C NMR (DMSO-d_6) 155.2, 130.0, 124.5, 117.8, 115.3, 112.2, 20.5, 16.0; (Found (%): C, 60.96; H, 4.48; N, 4.44; O, 5.08; Calcd. (%) for $C_{32}H_{28}N_2O_2Se_2$ (630.00): C, 59.55; H, 4.98; N, 5.32; O, 4.00); Se, 23.05.

1,2-*Bis*(1-ethyl-2-(3-methoxyphenyl)indolizin-3-yl)diselane (6e): m.p. 156 ºC; ^{1}H NMR: ($CDCl_3$) δ 1.00 (3H, s, CH_3), 2.45 (2H, d, CH_2), 4.00 (3H, s, CH_3), 7.00 (1H, t, CH), 6.45 (1H, t, CH), 8.00 (1H, d, CH) and 7.55 (6H, m, CH); ^{13}C NMR (DMSO-d_6) 161.1, 129.0, 119.8, 113.3, 118.8, 117.2, 112.5, 55.8, 19.3, 15.0; (Found (%): C, 62.01; H, 4.90; N, 4.25; O, 4.86; Calcd. (%) for $C_{34}H_{32}N_2O_2Se_2$ (658.00): C, 62.90; H, 5.55; N, 8.00; O, 4.50); Se, 24.00.

1,2-*Bis*(1-ethyl-2-(4-methoxyphenyl)indolizin-3-yl)diselane (6f): m.p. 206 ºC; ^{1}H NMR: ($CDCl_3$) δ 1.00 (3H, s, CH_3), 2.45 (2H, d, CH_2), 4.00 (3H, s, CH_3), 7.00 (1H, t, CH), 6.45 (1H, t, CH), 8.00 (1H, d, CH) and 7.55 (6H, m, CH); ^{13}C NMR (DMSO-d_6) 161.1, 130.0, 120.8, 115.3, 112.8, 116.3, 55.8, 19.3, 15.0; (Found (%): C, 62.01; H, 4.90; N, 4.25; O, 4.86; Calcd. for $C_{34}H_{32}N_2O_2Se_2$ (658.00): C, 61.55; H, 4.33; N, 6.00; O, 5.25); Se, 22.00.

1,2-*Bis*(1-ethyl-2-(4-nitrophenyl)indolizin-3-yl)diselane (6g): m.p. 182 ºC; ^{1}H NMR: ($CDCl_3$) δ 1.35 (3H, s, CH_3), 2.95 (2H, d, CH_2), 6.50 (1H, t, CH), 6.80 (1H, t, CH), 7.30 (1H, d, CH) and 8.55 (6H, m, CH); ^{13}C

NMR (DMSO-d_6) 147.9, 128.4, 124.4, 116.55, 118.8, 112.1, 19.3, 15.25; (Found (%): C, 55.82; H, 3.81; N, 8.14; O, 9.30; Calcd. (%) for $C_{32}H_{26}N_4O_4Se_2$ (688.00): C, 55.00; H, 4.10; N, 8.00, O, 8.50); Se, 20.85.

1,2-*Bis*(1-ethyl-2-(4-ethylphenyl)indolizin-3-yl)diselane (6h): m.p. 210 ºC; ^{1}H NMR: ($CDCl_3$) δ 1.25 (6H, s, CH_3), 2.60 (2H, d, CH_2), 6.40 (1H, t, CH), 6.88 (1H, t, CH), 7.15 (1H, d, CH) and 7.32 (6H, m, CH); ^{13}C NMR (DMSO-d_6) 144.3, 129.7, 127.4, 124.5, 118.2, 116.0, 110.5, 28.5, 21.2, 16.5, 14.5; (Found (%): C, 66.05; H, 5.54; N, 4.28; Calcd. (%) for $C_{36}H_{36}N_2Se_2$ (654.00): C, 62.30; H, 5.80; N, 4.22); Se, 22.42.

Determination of radical scavenging activity using DPPH radical method: DPPH assay is the simplest method to measure the ability of antioxidants to intercept free radicals. Antioxidants react with DPPH, which is a stable free radical, then scavenge this radical by converting it in to 1,1-diphenyl-2-picryl hydrazine due to their H-donating ability. The degree of discolouration indicates the scavenging potential of the antioxidant compounds. Experimental procedure was adapted from Kumazawa *et al.*[9]. To 1.0 mL of DPPH radical (0.25 mM) in methanol was added 2.0 mL of the varying concentrations of the test samples (250, 125, 50, 25, 10 and 5 µg/ mL). The reaction mixture was then allowed to stand at room temperature in a dark chamber for 0.5 h. The changes in colour from deep violet to light yellow was measured at 514 nm on a spectrophotometer methanol was used as a blank solution and DPPH$^\bullet$ solution in methanol served as the control. The percentage of remaining DPPH$^\bullet$ was calculated (Table-1) and the radical-scavenging effects of the tested compounds were compared in terms of EC_{50} (the concentration sufficient to elicit 50 % of the initial amount of DPPH$^\bullet$ and expressed as the molar ratio of each compound to the radical). The decrease in absorbance was then converted to percentage antioxidant activity (AA %) using the formula:

TABLE-2
ANTIOXIDANT ACTIVITIES FOR DPPH RADICAL

S. No.	Different derivatives	EC_{50} of different compounds
1	Gallic acid	3.45 (µg/mL)
2	**6a**	9.30 (µg/mL)
3	**6b**	6.70 (µg/mL)
4	**6c**	5.20 (µg/mL)
5	**6d**	15.00 (µg/mL)
6	**6e**	12.80 (µg/mL)
7	**6f**	14.20 (µg/mL)
8	**6g**	6.10 (µg/mL)
9	**6h**	4.90 (µg/mL)

$$AA\ \% = 100 - \frac{Abs_{sample} - Abs_{blank}}{Abs_{control}} \times 100$$

blank = methanol (1.0 mL) plus sample solution (2.0 mL), negative control = DPPH solution (1.0 mL, 0.25 mM) plus methanol (2.0 mL), gallic acid was used as positive control. The EC_{50} is defined as the concentration sufficient to elicit 50 % of the maximum effect estimate in 100 %.

RESULTS AND DISCUSSION

Compounds, **6a-h** were synthesized from 1-ethyl-2-substituted phenylindolizine by treating with *n*-butyl lithium and selenium powder and characterized by physical and spectral analysis and they were screened for their scavenging activity against 1,1-diphenyl-2-pycryl-hydrazyl ($DPPH^{\bullet}$). Compounds **6c** and **6h**, have shown maximum antioxidant activity and compounds, **6a**, **6b** and **6g** have shown moderate antioxidant activity and compound **6d**, **6e** and **6f** have shown poor antioxidant compared to standard drug.

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