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Synthesis and Antioxidant Activity of 3,3′-Diselanediylbis (N,N-Disubstituted Indolizine-1-carboxamide) and Derivatives

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ABSTRACT

A series of novel diselenides 5a–e **were synthesized from α-haloketones, α-picoline alkenes and various secondary amines under a dry nitrogen atmosphere. The structures of these compounds were established by means of their spectral data and they were screened for scavenging activity against 1,1-diphenyl-2-picryl-hydrazyl (DPPH•). The results of this research showed that two of these compounds (**5e **and** 5d**) exhibited reasonable antioxidant activity. The intermediate, methyl 5-methylindolizine-1-carboxylate was prepared by condensation of methyl acrylate with 1-(carboxymethyl)-2-methylpyridinium halide, which was prepared from α-picoline and chloroacetic acid by using the Tschischibabin reaction.**

KEYWORDS

Antioxidants, diselenides, indolizine, free radical scavenger, 1,1-DPPH•.

1. Introduction

Aerobic cells are inevitably exposed to reactive oxygen species (ROS) formed as oxygen metabolites.[1] ROS such as hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^•$) and superoxide anion ($O_2^{-•}$) may degenerate various biomacromolecules (DNA and proteins), resulting in oxidative and genotoxic stress.[2–4] Biological systems use enzymatic (glutathione peroxidase, thioredoxin reductase superoxide dismutase, etc.) and nonenzymatic (uric acid, creatinine, polyamine, retinal, etc.) antioxidant systems to prevent oxidative stress. However, once the systems are disturbed the uncontrolled oxidative stresses initiate a series of harmful biochemical events, or generate them as a consequence of earlier tissue injury, thus aggravating the final damages. Such damages include brain dysfunction, cancer, cardiovascular disease and inflammation.[5,6]

Glutathione peroxidases (GPx) are antioxidant selenoenzymes protecting various organisms from oxidative stresses by catalysing the reduction of hydroperoxides at the expense of glutathione.[7,8] After the discovery of selenium as selenocysteine (SeCys) in the active site of GPx,[9,10] there has been a growing interest in the biochemistry of selenium. Other selenium-containing enzymes have been identified recently.[11,12] X-ray diffraction studies on GPx have shown that the two nitrogen atoms of Gln-70 and Trp-148 are located very near the selenium atom of SeCys-35 in the active site.[13,14] Based on this observation, various organoselenium compounds containing other hetero-atoms have been synthesized to mimic the active site of GPx.[15–21] In the present study, we have synthesized 3,3′-diselenobis(N,N-disubstituted-indolizine-1-carboxamide) derivatives and have investigated their antioxidant activity.

2. Experimental

Melting points were determined by using the open capillary method and are uncorrected. All the reactions were run under nitrogen atmosphere to prevent oxidation and the formation of oxygen-sensitive selenide ions and selenium-containing products. The purity of the compounds was routinely checked by TLC on silica gel plates using 20 % EtOAc in petroleum ether (60–90 °C) 1:4 solvent systems. IR spectra (in KBr) were recorded on a Perkin Elmer FTIR spectrometer and ^{1}H NMR spectra (in $CDCl_3$) were recorded on a Bruker 200 Spectrospin spectrometer.

2.1. Synthesis of Methyl 5-Methylindolizine-1-carboxylate[22] (3)

A suspension of 1-(carboxymethyl)-2-methylpyridinium halide (**2**, 10 mmol), methyl acrylate (50 mmol), triethyl amine (1.5 mL) and MnO_2 (80 mmol) in toluene (80 mL) was refluxed and stirred for 5 h (monitored by TLC). The reaction mixture was then cooled to room temperature, filtered and washed with acetone. The combined filtrates were evaporated to yield a residue **3**, which was purified by column chromatography (silica gel, 20 % EtOAc in petroleum ether (60–90 °C), yield 60 %, δ_H (200 MHz, DMSO-d_6) 2.3 (3H, s, CH_3), 3.8 (3H, s, OCH_3), 6.5 (1H, t, CH), 6.7 (1H, t, CH), 7.5 (1H, d, CH), 7.9 (1H, d, CH) and 8.1 ppm (1H, d, CH); δ_C (75 MHz, DMSO-d_6) 162.8, 132.1, 130.2, 119.0, 117.9, 110.8, 64.4 and 19.3 ppm; (Found: C, 69.83; H, 5.86; N, 7.40; O, 16.91 %; Calc. for $C_{11}H_{11}NO_2$ (189.00): C, 70.03; H, 5.25; N, 6.98; O, 17.15 %).

2.2. General Procedure: Synthesis of 3,3′-Diselanediylbis (5-methyl-N,N-substituted indolizine-1-carboxamide) (5a–e)

To a solution of indolizine derivative (1.75 mL, 1.79 g, 10 mmol) in dry hexane (50 mL) was added a 1.6 mol L^{-1} solution of n-BuLi in hexane (6.8 mL, 11 mmol). After 1 h of stirring at room temperature, a white precipitate of the lithiated compound was obtained. The supernatant solvent was removed with a syringe. The white precipitate was dissolved in dry THF, the solution was cooled to 0 °C and selenium powder (0.8 g, 10 mmol) was added. Stirring was continued for an additional 1 h at 0 °C and 4 h at room temperature. The resulting solution was filtered through celite, and the solvent was evaporated to give a diselenide **5a** (1.29 g, 45 %). The presence of selenium metal was determined from atomic absorption and ^{77}Se NMR.

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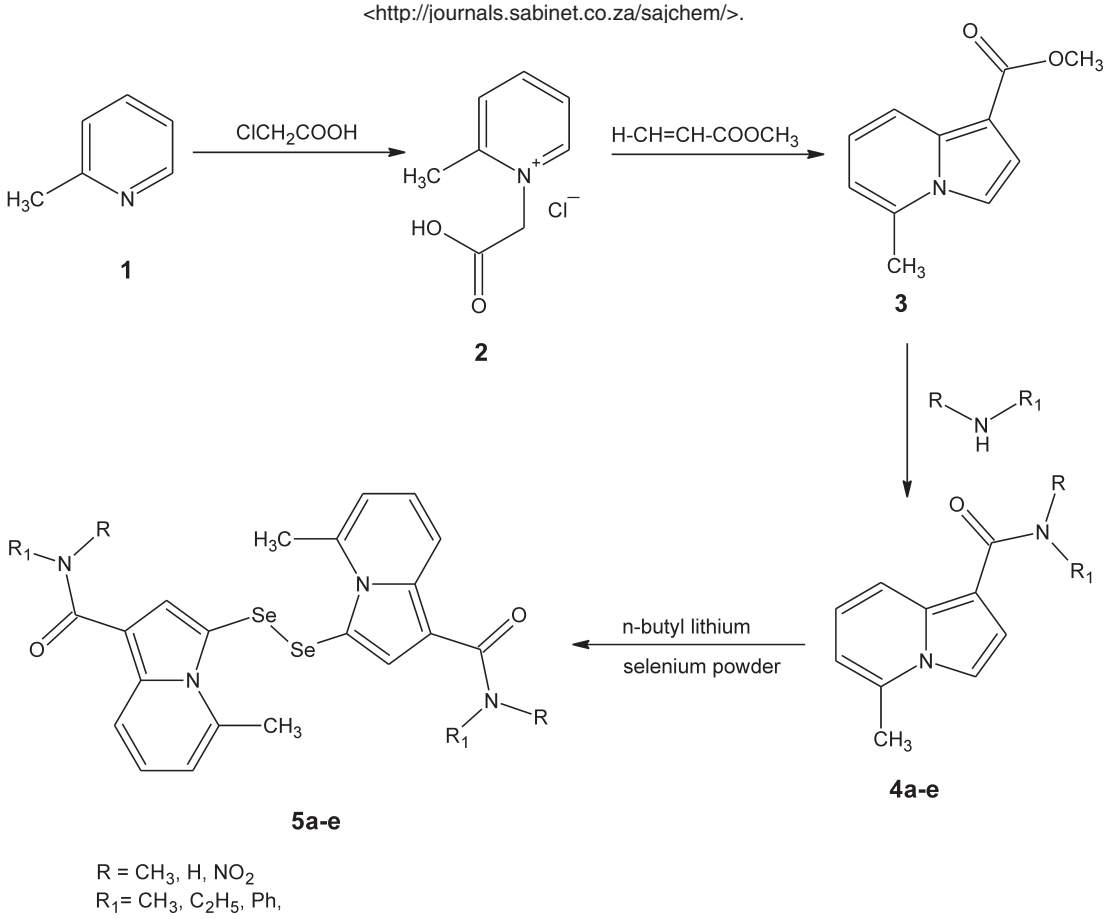

Scheme 1
Synthesis of diselenide derivatives.

2.2.1. *Synthesis of 3,3′-Diselanediylbis(N,N,5-trimethylindolizine-1-carboxamide)* (**5a**)

M. p.: 167–168 °C; IR (KBr): 1210 (C-N amide), 1552 (C=C), 1635 cm^{-1} (C=O). δ_H (200 MHz, $CDCl_3$): 2.3 (3H, s, CH_3), 2.9 (6H, s, CH_3), 6.2 (1H, d, CH), 6.4 (1H, d, CH) and 8.35 ppm (1H, t, CH); δ_C (75 MHz, $CDCl_3$): 168.8, 132.3, 117.4, 116.2, 112.2, 37.5 and 20.2 ppm; (Found: C, 50.90; H, 4.98; N, 9.32; O, 5.00 %; Calc. for $C_{24}H_{26}N_4O_2Se_2$ (560.00); C, 51.44; H, 4.68; N, 10.00; O, 5.71 %). Atomic absorption Se-27.25 %. ^{77}Se NMR ($CDCl_3$) δ 897 ppm. MS (*m*/*z*): 560 (M^+).

2.2.2. *Synthesis of 3,3′-Diselanediylbis(N-ethyl-5-methylindolizine-1- carboxamide)* (**5b**)

M. p.: 154–155 °C; IR (KBr): 1210 (C-N amide), 1546 (C=C), 1627 (C=O), 3366 cm^{-1} (N-H amide). δ_H (200 MHz, $CDCl_3$): 1.0 (3H, t, CH_3), 2.35 (3H, s, CH_3), 3.0 (3H, s, CH_3), 6.0 (1H, d, CH), 6.3 (1H, d, CH), 8.3 (1H, t, CH) and 8.5 ppm (1H, d, H); δ_C (75 MHz, $CDCl_3$): 163.9, 133.3, 118.4, 116.9, 111.0, 34.5, 19.2 and 15.0 ppm; (Found: C, 50.00; H, 4.25; N, 9.02; O, 4.85 %; Calc. for $C_{24}H_{26}N_4O_2Se_2$ (559.75.): C, 51.44; H, 4.68; N, 10.00; O, 5.71 %). Atomic absorption Se-26.85 %. ^{77}Se NMR ($CDCl_3$) δ 796 ppm. MS (*m*/*z*): 558 (M^+).

2.2.3. *Synthesis of 3,3′-Diselanediylbis(N,5-dimethylindolizine-1-carboxamide)* (**5c**)

M. p.: 205–208 °C; IR (KBr): 1221 (C-N amide), 1580 (C=C), 1666 (C=O), 3359 cm^{-1} (N-H amide). δ_H (200 MHz, $CDCl_3$): 2.4 (3H, s, CH_3), 2.85 (3H, s, CH_3), 6.3 (1H, d, CH), 6.5 (1H, d, CH), 8.4 (1H, s, H) and 8.5 ppm (1H, t, CH) ; δ_C (75 MHz, $CDCl_3$): 167.8, 132.1, 117.1, 116.8, 111.2, 26.5 and 19.6 ppm; (Found: C, 48.54; H, 4.00; N, 9.91; O, 6.52 %; Calc. for $C_{22}H_{22}N_4O_2Se_2$ (532.36): C, 49.64; H, 4.17; N, 10.52; O, 6.01 %). Atomic absorption Se-28.21 %. ^{77}Se NMR (95.3 MHz) δ 960.3 ppm. MS (*m*/*z*): 532 (M^+).

2.2.4. *Synthesis of 3,3′-Diselanediylbis(N,5-dimethyl-N-phenyl-indolizine-1-carboxamide)* (**5d**)

M. p.: 192–193 °C; IR (KBr): 1226 (C-N amide), 1556 (C=C), 1671 cm^{-1} (C=O). δ_H (200 MHz, $CDCl_3$): 2.35 (3H, s, CH_3), 6.1 (1H, d, CH), 6.3 (1H, d , CH), 7.4 (5H, m, C_6H_5), 8.0 (1H, t, CH) and 10.0 ppm (1H, s, H); δ_C (75 MHz, $CDCl_3$): 164.5, 137.9, 131.3, 128.9, 128.0, 121.6, 118.8, 117.6, 110.3 and 20.6 ppm; (Found: C, 57.94; H, 4.99; N, 7.23; O, 4.17 %; Calc. for $C_{32}H_{26}N_4O_2Se_2$ (656.49): C, 58.54; H, 3.99; N, 8.53; O, 4.87 %). ^{77}Se NMR (95.3 MHz) δ 796 ppm. Atomic absorption Se-24.66 %. MS (*m*/*z*): 654 (M^+).

2.2.5. *Synthesis of 3,3′-Diselanediylbis(5-methyl-N-nitro-N-phenyl-indolizine-1-carboxamide)* (**5e**)

M. p.: 223–224 °C; IR (KBr): 1226 (C-N amide), 1588 (C=C), 1671 cm^{-1} (C=O). δ_H (200 MHz, $CDCl_3$): 2.1 (3H, s, CH_3), 6.4 (1H, d, CH), 6.6 (1H, d, CH), 7.6 (5H, m, C_6H_5) and 7.8 ppm (1H, t, CH); δ_C (75 MHz, $CDCl_3$): 137.3, 133.3, 131.4, 128.9, 128.0, 124.6, 117.1, 117.4, 111.5 and 19.5 ppm; (Found: C, 50.21; H, 4.11; N, 10.63; O, 10.17 %; Calc. for $C_{32}H_{24}N_6O_6Se_2$ (746.49): C, 51.49; H, 3.24; N, 11.26; O, 12.86 %). Atomic absorption Se-22.03 %. ^{77}Se NMR (95.3 MHz) δ 742 ppm. MS (*m*/*z*): 746 (M^+).

3. Pharmacological Activity

3.1. Determination of Radical Scavenging Activity using the DPPH$^\bullet$ Method

DPPH$^\bullet$ assay is the simplest method to measure the ability of antioxidants to intercept free radicals. Antioxidants react with DPPH$^\bullet$, which is a stable free radical, then scavenge this radical by converting it to 1,1-diphenyl-2-picryl hydrazine due to their H-donating ability. The degree of discoloration indicates the scavenging potential of the antioxidant compounds. The experi-

Table 1 Antioxidant activities for DPPH• radical.

Compound	DPPH• model IC_{50}/mmol L^{-1}
5a	10.69 ± 0.50
5b	6.09 ± 0.24
5c	7.059 ± 0.30
5d	5.69 ± 0.55
5e	4.11 ± 0.05
Ascorbic acid	12.56 ± 0.50

mental procedure was adapted from Kumazawa *et al.*[23] To 1.0 mL of DPPH• (0.25 mmol L^{-1}) in methanol was added 2.0 mL of the varying concentrations of the test samples (250, 125, 50, 25, 10, 5, 2 and 1 μg mL^{-1}). The reaction mixture was then allowed to stand at room temperature in a dark chamber for 30 min. The change in colour from deep violet to light yellow was measured at 514 nm using a spectrophotometer. Methanol was used as a blank solvent and a fresh DPPH• solution in methanol served as the control. The percentage of remaining DPPH• was calculated (Table 1), and the radical-scavenging effects of the tested compounds were compared in terms of IC_{50} (the concentration needed to reduce 50 % of the initial amount of DPPH• which was 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 equation:

$$AA\% = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100]/Abs_{control}\}$$

blank = methanol (1.0 mL) plus sample solution (2.0 mL), negative control = DPPH• solution (1.0 mL, 0.25 mmol L^{-1}) plus methanol (2.0 mL). Ascorbic acid was used as positive control. IC_{50} is defined as the concentration sufficient to elicit 50 % of a maximum effect estimate in 100 %.

4. Results and Discussion

In an effort to develop a novel antioxidant, a series of 3,3′-diselanediylbis (N,N-disubstituted indolizine-1-carboxamide) derivatives were synthesized and evaluated for their antioxidant activities. Compounds **5a–e** were synthesized from indolizine-1-carboxamide by treating it with n-butyl lithium and selenium powder and characterized by physical and spectral analysis. These compounds were screened for their scavenging activity against 1,1-diphenyl-2-picryl-hydrazyl (DPPH•). DPPH• tests the ability of a compound to act as donor for hydrogen atoms or electrons, which was measured spectrophotometrically and listed in Table 1. Recently, a number of studies have shown that di-indolylmethane derivatives, indolizine and organoselenium compounds show promising antioxidant activity.[20,24,25] It has been reported that the introduction of coordinating amino or other groups to the thiols would enhance the GPx-like catalytic activity of ebselen and other related organoselenium compounds.[26,27] In this study various substitutions were made to the nitrogen of the above-mentioned carboxamide group. Among the diselenide derivatives (**5a–e**), the N-aryl substituent exhibited higher antioxidant activity in comparison with compounds bearing N-alkyl and N,N-dialkyl substituents. This is true in the case of ebselen, where the experimental and theoretical results suggest that the presence of a phenyl substituent is important for the antioxidant activity. These findings suggest that N-aryl substituents might be more important for antioxidant activity than N-alkyl chains. This clearly revealed that most of the compounds used in the study possess significant DPPH• scavenging activity, in all cases even superior to ascorbic acid. Compound **5e** appears to be the best among those tested having a nitrophenyl group. The presence of the polar group NO_2 led to an interesting modification of the pharmacological properties. This may warrant further in-depth biological evaluations. Work is in progress to design, synthesize and evaluate additional compounds in this and related systems.

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