Synthesis, antioxidant and anti-microbial properties of two organoselenium compounds

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Abstract: The aim of this study is synthesis of two different series of organoselenium compounds and available *in vitro* antioxidant and antimicrobial properties of these synthetic compounds. The synthetic compounds were identified by ^{1}H-NMR (300 MHz), ^{13}C-NMR (75.5 MHz), FT-IR spectroscopic techniques and micro analysis. Antioxidant properties of two synthetic organoselenium compounds were determined by 1,1- diphenyl-2-picrylhydrazyl (DPPH) radical method, reducing power assay and β-carotene bleaching method as *in vitro*. Antimicrobial effects of samples were assessed by the agar dilution procedure and using gram positive and gram-negative bacteria and yeast strains. Although 1,3-di-*p*-methoxybenzylpyrimidine-2-selenone showed better antiradical activity in DPPH test and higher protective activity on β-carotene, 1-isopropyl-3-methylbenzimidazole-2-selenone was found to be better in reducing power and antimicrobial activity.

Keywords: Synthetic organoselenium compounds, antioxidant activity, antimicrobial activity.

INTRODUCTION

Reactive oxygen species (ROS) are highly reactive O_2 metabolites that include superoxideradical ($O^{\bullet-}_2$), hydrogen peroxide (H_2O_2), andhydroxyl radical ($OH^{\bullet-}$). The ROS causes considerable injury to DNA, protein and lipid and it is claimed that this injury is a main reason to aging and deteriorating disorders of aging such as cancer (Khan *et al.,* 2013).

Selenium is component of the enzyme glutathione peroxidase (GSH-Px). Selenium is known to be closely concerned in the activity of enzymes glutathione peroxidase and thioredoxin reductase, which catalyse chemistry vital to defensing of biomolecules against oxidative stress and free radical destruction (McKenzie *et al.,* 2002). Selenium including molecules, which has brought about synthetic organoselenium compounds might excel in classical antioxidants (Das *et al.,* 2004; Zwolak *et al.,* 2009). Most of studies in recently years have been described deal with activity of organoselenium compounds such as: anticancer, as enzyme inhibitors, enzyme mimetics. Organoselenium compounds have great action against gram-positive and gram-negative bacteria and fungi (Braga *et al.,* 2010; Alberto *et al.,* 2011). Derivatives of benzimidazole and benzylpyrimidine have widely interest in due to their different biological actions and clinical functions (Kaliranjan *et al.,* 2011).

Newly plenty of synthetic organocompounds have been organized in our laboratory for their antimicrobial and anti-oxidant features. In this study, it was planned to modify the position of the methyl and methoxy on structure of the benzimidazole and benzylpyrimidine in order to develop new anti-microbial and antioxidant agents. We showed new data on the anti-microbial and antioxidant activities of synthetic organoselenium compounds.

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MATERIAL AND METHODS

Structure of synthetic organoselenium compounds

The organoselenium compounds (Se I and Se II) were synthesized in our laboratories (fig. 1 (a) and 1 (b). Se I and Se II were prepared according to literatures (Aygun *et al.,* 2003; Gok *et al.,* 2004). The synthetic compounds were identified by ^{1}H-NMR (300 MHz), ^{13}C-NMR (75.5 MHz), FT-IR spectroscopic techniques and microanalysis.

Synthesis of 1-isopropyl-3-methylbenzimidazolium iodide (I)

To asolution of 1-*iso*propylbenzimidazole (5.20g; 32.50 mmol) in toluene (20mL) methyl iodide (3mL; 48.18 mmol) was inserted and the mixture mixed at room tempareture 12 h. Et_2O (10mL) was inserted the reaction mixture. A white solid accelerated in this duration. The mixing was filtered, washed two times with dried Et_2O and dried in vacuo. Yield: 92%, mp. 195-196^{o}C.*Anal.* Found For: C, 43.75, H, 5.02, N, 9.30. Cald: C, 43.70, H, 4.96, N, 9.27. ^{1}H NMR ($CDCl_3$) δ: 1.85 (d, 6H, *J*=6.7 Hz, CH (CH_3)$_2$), 4.25 (s, 3H, CH_3), 5.09 (hep., 1H, *J*=6.7 Hz, CH(CH_3)$_2$), 7.78-7.79 (m, 4H, Ar-*H*), 9.45 (s, 1H, 2-C*H*). ^{13}C NMR ($CDCl_3$) δ: 18.2 (CH(CH_3)$_2$), 30.4 (CH(CH_3)$_2$), 49.1 (CH_3), 110; 110.3; 110.4; 113.1; 116; 125 (Ar-*C*), 135.7 (2-*C*H).

Bis-[(1-isopropyl-3-methyl) benzimidozolidin-2-ylidene] (II)

1-*iso*propyl-3-methylbenzimidazolium iodide (8 g; 26.49 mmol) was added to a suspension of sodium hydride (1.10 g; 45.83 mmol) in THF (30mL). The mixing was mixed at 20°C for 12h after heated at 60°C for 1 h, and next the volaties were removed under reduced pressure. Toluene (20mL) was inserted to the last product oily residue and the suspension was filtered. After removal of the solvent, the oily residue was recrystallized from a mixture of toluene (5mL) and *n*-hexane (10mL) at -20°C. Nevertheless, the bis-[(1-*iso*propyl-3-methyl) benzimidozolidin-2-ylidene]'s obtained in this study could not be characterized by elemental analyses or NMR spectroscopy due to their air sensitivity.

Synthesis of 1-isopropyl-3-methylbenzimidazol-2-selenone (III; Se I)

Bis-[(1-*iso*propyl-3-methyl) benzimidozolidin-2-ylidene] (0.5g; 1.33mmol) was heated with elemental selenium (0.25 g; 3.16 mmol) in refluxing toluene (15mL) for 2h. The last product solution was cooled to room temperature and then filtered for remove the excess selenium. The last volume was reduced to ca. 10mL and *n*-hexane (10mL) was added. Upon cooling the solution to -20°C cream crystals of the title compound were obtained. Yield: 61%, mp. 96-97°C. *Anal.* Found For: C, 51.86, H, 5.44, N, 11.12. Cald: C, 51.96, H, 5.55, N, 11.02. IR, ν: 1408 (C=Se). ^{1}H NMR ($CDCl_3$) δ: 1.61 (d, 6H, *J*=7 Hz, CH(C*H*$_3$)$_2$), 3.91 (s, 3H, C*H*$_3$), 5.70 (hep., 1H, *J*=7 Hz, C*H*(CH$_3$)$_2$), 7.23-7.50 (m, 4H, Ar-*H*). ^{13}C NMR ($CDCl_3$) δ: 20 (CH(*C*H$_3$)$_2$), 33.5 (*C*H(CH$_3$)$_2$), 51.6 (*C*H$_3$), 109.7; 111.2; 122.8; 123; 131.2; 134.2 (Ar-*C*), 167.3 (*C*=Se).

Synthesis of 1,3-bis(p-methoxybenzylideneamino) propane (IV)

1,3-diaminoprophane (1.0mmol) was joined drop wise to *p*-methoxybenzaldehyde (2.0mmol) in 20mL of absolute alcohol, and the mixture was heated under reflux. After 1h, the clear solution was cooled to 25°C. The light yellow crystals obtained were filtered off and washed with Et_2O (3 x 20 mL). Yield: 80%, mp. 76-78°C.^{1}H NMR ($CDCl_3$) δ: 2.18 (quin., 2H, *J*=6 Hz, NCH$_2$C*H*$_2$CH$_2$N), 3.74 (t, 4H, *J*=6 Hz, NC*H*$_2$CH$_2$C*H*$_2$N), 3.90 (s, 6H, OCH$_3$), 7.02 and 7.80 (d, 8H, *J*=8 Hz, Ar-*H*) 8.35 (s, 2H, N=C*H*).

Synthesis of 1,3-bis(p-methoxybenzylamino) propane (V)

1,3-bis (*p*-methoxybenzylideneamino) propane (5.5g), Pd/C (5%) and dry toluene were put in a reactor and H_2 gas was applied at 340psi pressure. Decantation at Pd/C was followed by distillation of the excess toluene. Oily residue was distilled under vacuum or recrystallized from toluene/n-hexane (5/10mL). Yield: 65%, bp. 160-165 (0.3 mmHg). ^{1}H NMR ($CDCl_3$) δ: 1.30 (s, 2H, N*H*), 1.59 (quin., 2H, *J*=6 Hz, NCH$_2$C*H*$_2$CH$_2$N), 2.56 (t, 4H, *J*=6 Hz, NC*H*$_2$CH$_2$C*H*$_2$N), 3.56 (s, 2H, NC*H*$_2$), 3.67 (s, 6H, OCH$_3$), 6.68 and 7.10 (d, 8H, *J*=8 Hz, Ar-*H*).

Synthesis of bis[(1,3-di-p-methoxybenzyl)-1,3-pyrimidin-2-ylidene] (VI)

A mixed solution of *N*,*N*-dimethylformamide dimethyl acetal (1.0mmol) and 1,3-bis(*p*-methoxybenzylamino) propane (1.0mmol) in dry toluene (20mL) was heated for 3 h at 90°C under an argon atmosphere. The reaction mixture was then heated for 1h at 120°C under distillation situations, allowing the produced dimethylamine and methanol to escape. From the resultant product, unreacted starting materials were annihilated in vacuo. The white solid was recrystallized from a mixture of toluene (5mL) and *n*-hexane (10mL) at -20°C. However, the bis[(1,3-di-*p*-methoxybenzyl)-1,3-pyrimidin-2-ylidene]'s acquired in this work could not be characterized by elemental analyses or NMR spectroscopy due to their air susceptibility.

Synthesis of 1,3-di-p-methoxybenzylpyrimidin-2-selenone (VII; Se II)

Bis[(1,3-di-*p*-methoxybenzyl)-1,3-pyrimidin-2-ylidene] (1.0mmol) was heated with elemental selenium (2.0 mmol) in refluxing toluene (20mL) for 2h. The resulting solution was cooled to room temperature and then filtered to allay the extreme selenium. The volume of the filtrate was reduced to ca. 10mL and *n*-hexane (10mL) was added. Upon cooling the solution to -20°C cream crystals of the title compound were recovered.

Yield: 79 %, mp.164-165 °C. *Anal.* Found For: C, 59.39, H, 6.18, N, 6.99. Cald.: C, 59.42, H, 6.37, N, 7.17. IR, ν: 1511 (C=Se). ^{1}H NMR ($CDCl_3$) δ: 1.74 (quin., 2H, *J*=5.9 Hz, NCH$_2$C*H*$_2$CH$_2$N), 3.12 (t, 4H, *J*=5.9 Hz, NC*H*$_2$CH$_2$C*H*$_2$N), 3.70 (s, 6H, OCH$_3$), 5.34 (s, 4H, NC*H*$_2$), 6.79 and 7.28 (d, 8H, *J*=8.8 Hz, Ar-*H*). ^{13}C NMR ($CDCl_3$) δ: 21.2; 46 (NCH$_2$*C*H$_2$*C*H$_2$N), 55.7 (O*C*H$_3$), 61.7 (N-*C*H$_2$), 114.4; 129.4; 129.5; 159.6 (Ar-*C*), 180.8 (*C*=Se).

Radical scavenging power

Radical scavenging power (RSP) of synthetic organoselenium compounds was assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical method (Guo *et al.,* 1999). 3mL reaction mixture, which is containing 2.9mL DPPH ($1x10^{-4}$ M) and 0.1mL test compounds at various concentrations. In control, ethanol was used in as pattern. Cuvettes were left in dark at room temperature for 15min and the resulting color was measured spectrophotometrically at 520 nm against blanks. A decreasing density of violet color was related to higher RSP percentage, which was calculated using the following equation:

$$RSP = [1-(\frac{A_{S:15}}{A_{B:15}})]\times 100$$

where, $A_{S:15}$ is absorbance of pattern and $A_{B:15}$ is absorbance of blank at 15 mi n reaction time.

Reducing power

Reducing power of samples was assigned according to the method of Oyaizu (Oyaizu, 1986). Various amounts of sample contents 0, 0.1, 0.25, 0.50mg organoselenium compounds solutions were put into tubes and volume was arranged to 1mL with ethanol. 2.5mL 0.2M phosphate buffer (pH 6.6) and 2.5mL 1% potassium ferricyanide were added into these tubes and it was mixed gently. The mixtures were incubated at 50°C in a water bath for 20 min. 2.5mL of 10% trichloroacetic acid (TCA) was added to the tubes and the mixtures were centrifuged at 6 000 rpm for 10min. From the top layer of supernatant 2.5mL was transferred into tubes containing 2.5mL distilled water and 0.5mL 0.1% ferric chloride ($FeCl_3.6H_2O$). The color intensity was read at 700nm against blanks after shaking and has it rest for 5 min. The higher absorbance and the better reducing power of the sample is recognized.

β-carotene bleaching test

Anti-lipid peroxidative activities of organoselenium compounds were assigned by using β-carotene bleaching method (Hammerschmidt and Pratt, 1978). 2mg of crystalline β-carotene was dissolved in 10mL chloroform and to 1mL of this solution in round-bottom flasks 20µg of linoleic acid and 200µL of Tween-20 (Merck) were added. Chloroform was eliminated in rotary evaporator under vacuum at 40°C for 5 min and 50mL of distilled water was added with vigorous stirring to form an emulsion. 4,9mL of this emulsion was added into each tube which is containing 0,1mL of sample solution (containing 100µg of compounds). Tubes were placed in a water bath at 50°C and absorbance was at 470 nm recorded in 10 min intervals during 90 min incubation.

Antimicrobial activity tests of synthetic organoselenium compounds

Antimicrobial activities of the synthetic organoselenium compounds (Se I and Se II) were assigned using the agar dilution procedure suggested by the Clinical and Laboratory Standards Institute (Wayne, PA, USA, 2002; Wayne, PA, USA, 2003). Minimal inhibitory concentrations for each compound were studied against standard bacterial strains; *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 were acquired from American Type Culture Collection (Rockville, MD) and the fungal strains of *Candida albicans* and *Candidatropicalis* acquired from the Department of Microbiology, Faculty of Medicine, Ege University (Turkey). Bacterial strains were subcultured on Muller Hinton Broth (HiMedia Laboratories Pvt. Ltd. Mumbai-India) and fungal strains were also on RPMI 1640 Broth (Sigma-Aldrich Chemie GmbH Taufkirchen, Germany). Their turbidites were synchronized with McFarland no. 0.5 turbidity Standard (Hindler and Hochstein, 1992). The stock solution of all compounds was prepared in dimethyl sulfoxide (DMSO). Distilled water was used for all of the dilutions. The compounds of Se I and Se II were prepared 800, 400, 200, 100, 50, 25, 12.5 and 6.25µg/mL concentrations. Ampicillin and ciprofloxacin were used as antibacterial standard drugs, while fluconazole was used as antifungal standard drug, which were provided minimum inhibitory concentration (MIC) values. A loopful (0.01mL) of the standardised inoculums of the bacteria and yeasts (106 CFUs/mL) was disseminated over the surface of agar plates. All the inoculated plates were incubated at 35°C and results were evaluated after 16-20 h of incubation for bacteria and 48h for yeasts. The lowest concentration of the compounds repressed visible growth. As a result of that was evaluated to be the minimal inhibitory concentration (MIC).

STATISTICAL ANALYSIS

Statistical analyses were evaluated using the SPSS 12.0 software. To identify correlations between the data were analyzed by bivariate correlation using the Pearson

Table 1: Minimum inhibitory concentration (µg/mL) of synthetic organoselenium compounds (Se I and Se II) tested against bacterial and fungus

Compounds	*E. coli*	*S. aureus*	*E. faecalis*	*P. aeruginosa*	*C. albicans*	*C. tropicalis*
Se-I	800	800	800	800	25	25
Se-II	800	>800	800	800	400	200

Fig. 1: Structure of synthetic organoselenium compounds
(a) 1-*iso*propyl-3-methylbenzimidazole-2-selenone (Se I), (b) 1, 3-di-*p*-methoxybenzylpyrimidine-2-selenone (SeII)

correlation test. Values of $P < 0.05$ were evaluated to be statistically significant.

RESULTS

Radical scavenging power

DPPH is a synthetic radical, which commonly used in *in vitro* determination of antiradical activity. It was found that antiradical activity of Se II compound higher than Se I at all concentrations (fig. 2).

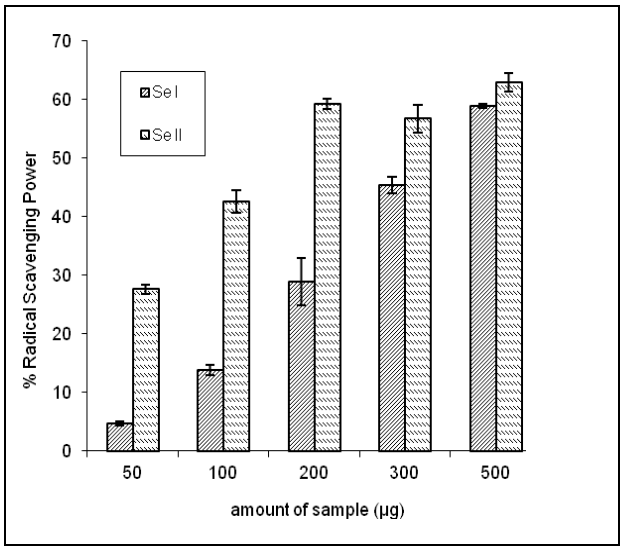


Fig. 2: 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity.

Reducing power

The using concentrations are 100µg, 250µg, and 500µg for reductive power. According to our results, Se I was found to be stronger reductive compound than Se II in 500µg concentration. Unlike antiradical test, differences were observed in reducing power of two organoselenium compounds in higher concentrations (fig. 3).

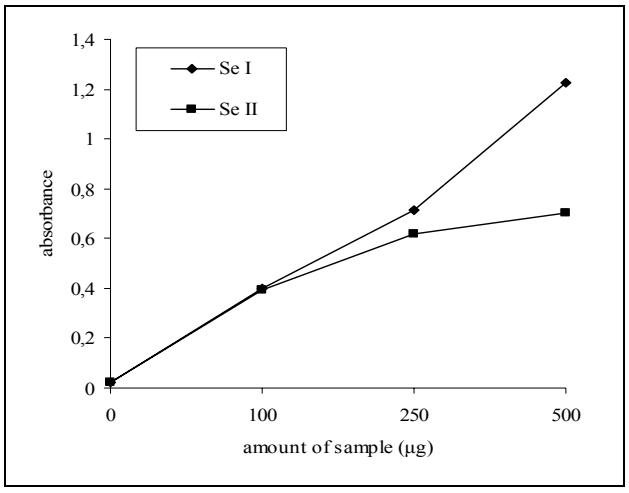


Fig. 3: Reducing power of synthetic organo selenium compounds (Se I and Se II**)** in different concentrations.

β-carotene bleaching test

Anti-per oxidative capacity of test compounds (Se I and Se II) against lipid per oxidation was carried out by "β-carotene Bleaching Method". There is a linear relation between anti-lipid per oxidative effect and preserving the characteristic colour of β-caroten, which was measured at 470 nm. Based on this principal, it is clear that Se II has relatively higher anti-lipid per oxidative effect than Se I (fig. 4).

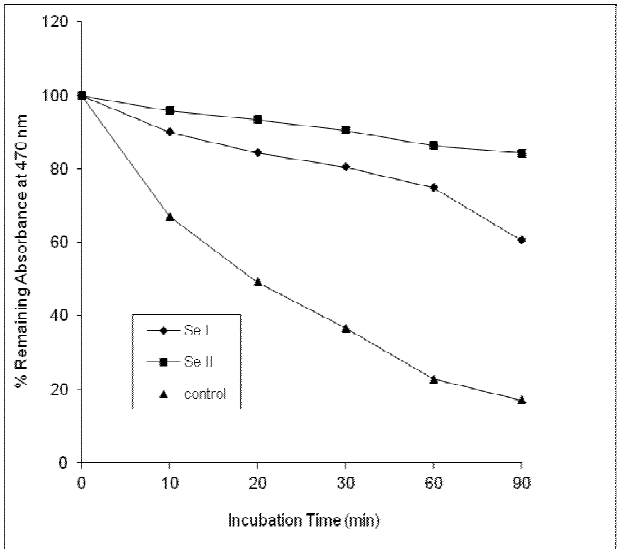


Fig. 4: Anti-peroxidative capacity of synthetic organo selenium compounds (Se I and Se II) against lipid peroxidation was carried out by β-carotene bleaching method.

Anti-microbial activity tests

The anti-microbial activity was showed in terms of the minimum inhibitory concentration (MIC) values, which are described as the lowest concentration of an anti-microbial. Anti-microbial visibly blocks the development of the bacteria after an overnight incubation (Pernak and Skrzypczak, 1996). The usefulness of Se I and Se II compounds as anti-microbial factors was evaluated. The test organism, which are laboratory strains used to test a range of concentration of the organo selenium compounds for minimum inhibitory concentration determination. The antibacterial actions of benzimidazole (Se I) and benzylpyrimidin (Se II) derivatives were first time tested by using agar dilution procedure against Gram-positive and Gram-negative bacteria. The minimum inhibitory concentration (MIC) of synthesized organoselenium compounds against Gram positive, Gram-negative bacteria and fungus are showed in table 1.

DISCUSSION

The differences in antiradical efficiency of the two selenium compounds decreased with gradually increasing concentrations of test compounds. Se II showed ~6 fold

better antiradical activity at the lowest concentration. Between Se I and Se II anti-radical efficiency decreased at highest concentration. The differences between Se I and Se II are significant when they were used at low concentration (50-200µg), but the concentrations of 300µg or 500µg is not significant (fig. 2). Antiradical activity of Se II didn't change significantly in three highest concentrations. This was probably due to the saturation of the reaction mixture to H^+ that was given by Se II. In further concentrations of Se II was not increased the antiradical power. So the lower concentrations may be comparatively more reliable in predicting the antiradical power.

Unlike antiradical test, differences were obtained in reducing power of two organoselenium compounds in higher concentrations (fig. 3).

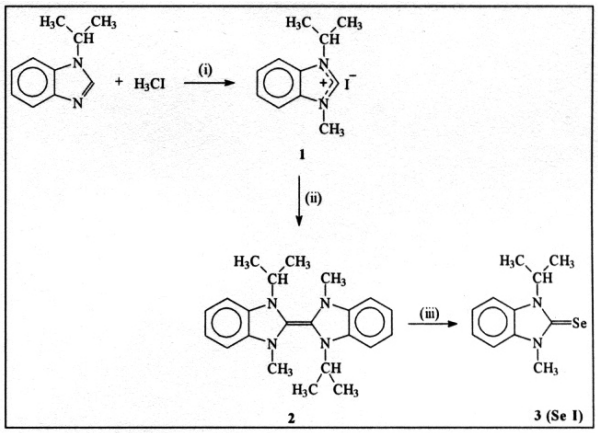
Se II has relatively higher anti-lipid per oxidative effect than Se I (fig. 4). In both mixures in which organo selenium compounds exist, exhibited higher absorbances than control sample at 470 nm through incubation period. These results obtained by *in vitro* studies (radical scavenging power, reducing power, β-carotene bleaching) it was showed that Se I and Se II compounds has chemo preventive potential.

As demonsrated in the table 1, antimicrobial activities against bacteria and fungi were observed in the organoselenium compounds tested at >800-25µg/mL concentrations. The new compounds displayed efficient events against Gram-positive, Gram-negative bacteria and fungi. The complexes were obtained influential for preventing the development of Gram-positive and Gram-negative bacteria with MICs values between 800–>800 µg/mL. The tested compounds showed antifungal activities with a range of the MICs between 25 and 400µg/mL. Se I generally was found to be better inhibitor on these microbial strains than Se II. SeI showed high activities against *C. albians* and *C. tropicalis* with MIC 25µg/mL.

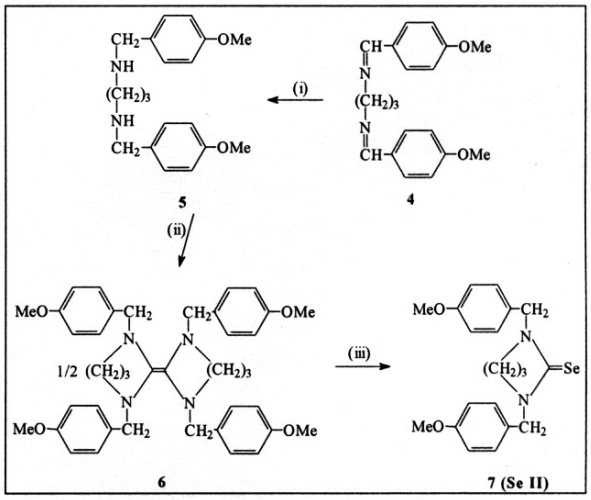
S.aureus was inhibited by Se I more than Se II. Both selenium compounds showed remarkable antimicrobial effects on microbial strains. Se I showed better inhibitory on *C.albicans* than Se II.

The results of these studies are summarized in table 1. This study emphasizes a novel group of vigorous, wide spectrum anti-microbial compounds. These findings are also confirmed by other work (Kazimierczuk *et al.,* 2002; Siddiqui *et al.,* 2013; Khalid *et al.,* 2013).

Selenium compounds have high toxicity. But organic reproduces of selenium have been synthesized as anti-cancer and for other medicinal treatments, which are biologically active agents exhibiting antiviral, antibacterial, anti-hypertensive, and fungicidal features. Organoselenium compounds have great actions against gram-positive and gram-negative bacteria and fungi.



Scheme 1: Synthesis of 1-*iso*propyl-3-methylbenzimidazol-2-selenone (Se I). Reaction conditions: (i) CH_3-I, toluene, 25°C; (ii) NaH, THF, 25°C; (iii) Se, toluene, 110°C.



Scheme 2: Synthesis of 1,3-di-*p*-methoxybenzylpyrimidin-2-selenone (Se II). Reaction conditions: (i) EtOH, 76°C; (ii) Pd/C (5%), H_2; (iii) CH $(OMe)_2NMe_2$, 100-130°C; (iv) Se, toluene, 110°

CONCLUSION

The synthetic compounds (Se I and Se II) were identified by ^{1}H-NMR (300 MHz), ^{13}C-NMR (75.5 MHz), FT-IR spectroscopic techniques and microanalysis. The results show the chemo preventive and antioxidant potency of Se I and Se II compounds. The synthetic organo selenium compounds are useful antioxidant and anti-microbial chemicals. Anti-oxidant activities of these synthetic compounds indicate the certain potential to reduce oxidative stress and consequent health benefits. Also, this study implies a novel class of wide spectrum anti-microbial and potent antioxidant matters.

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REFERENCES

Alberto EE, Rossato LL, Alves SH, Alves D and Braga AL (2011). Imidazolium ionic liquids containing selenium: synthesis and anti-microbial activity. *Biomol. Chem.,* **9**: 1001-1003.

Aygun M, Cetinkaya E, Gok Y, Kendi E and Cetinkaya B (2003). Synthesis and crystal structure of hexahydrobis [(1,3-p-dimethylaminobenzyl)-1,3- diazepine]-2-selenone, $C_{23}H_{32}N_4Se$. *Anal. Sci.,* **19**: 1093-1094.

Braga HC, Stefani HA, Paixao MW, Santos FW and Lüdtke DS (2010). Synthesis of 5-seleno xylofuranosides. *Tetrahedron,* **66**: 3441-3446.

Clinical and Laboratory Standards Institute (2002). Reference Method for Broth Dilution Anti-fungal Susceptibility Testing of Yeasts: Approved Standard-Second Edition: NCCLS document M27-A2 (ISBN 1-56238-469-4). NCCLS, Wayne, PA, 19087-1898 USA.

Clinical and laboratory standards institute (2003). Methods for dilution anti-microbial susceptibility Tests for Bacteria that Grow Aerobically: Approved Standard-Seventh Edition; CLSI Document. M7-A7, Wayne, PA, 19087-1898 USA.

Das RK, Ghosh S, Sengupta A, Das S and Bhattacharya S (2004). Inhibition of DMBA/croton oil-induced two-stage mouse skin carcinogenesis by diphenylmethyl selenocyanate. *Eur. J. Cancer. Prev.,* **13**: 411-417.

Gok Y, Cetinkaya E, Ozdemir I, Cetinkaya B and Lappert MF (2004). Synthesis and characterisation of N-functionalized enetetramines, and their properties. *Acta Chim. Slov.,* **51**: 437-446.

Guo Q, Zhao B, Shen S, Hou J and Xin W (1999). ESR study on the structure-anti-oxidant activity relationship of tea catechins and their epimers. *Biochim. Biophys. Acta.,* **1427**: 13-23.

Hammerschmidt PA and Pratt DE (1978). Phenolic antioxidants of dried soybeans. *J. Food Sci.,* **43(**2): 556-559.

Hindler J, Hochstein L and Howell A (1992). In Clinical Microbiology Procedures Handbook: Isenberg, HD, Ed: American Society for Microbiology (ASM). Washington, DC, USA, **1**:5.19.1–5.19.6.

Kaliranjan R, Leela R, Jubie S, Gowramma B, Gomathy S and Shakar S (2011). Microwave assisted synthesis of pyrazole substituted benzimidazoles and evaluation of their biologicals activities. *Indian J. Chem.,* **50**B: 1794-1799.

Kazimierczuk Z, Jacqueline A, Upcroft P, Górska A, Starooeciak B and Laudy A (2002). Synthesis, anti-protozoal and anti-bacterial activity of nitro- and halogeno-substituted benzimidazole derivatives. *Acta. Biochimica. Polonica.,* **49**(1): 185-195.

Khalid H, Rehman A, Abbasi MA, Khan KM, Ashraf M, Ahmad I and Ejaz SA (2013). Synthesis of biologically active *O*-substituted derivatives of 1-[(3, 5-dichloro-2-hydroxyphenyl) sulfonyl]piperidine. *Pak. J. Pharm. Sci.,* **26**(3): 479-485.

Khan RA, Khan MR and Sahreen S (2013). Protective effects of *Sonchus asper* (L.) against KBrO3-induced oxidative stress in rat testis. *Pak. J. Pharm. Sci.,* **26**(3): 567-570.

McKenzie RC, Arthur JR and Beckett GJ (2002). Selenium and the regulation of cell signaling, growth, and survival: Molecular and mechanistic aspects. *Antiox. Redox. Signal,* **4**: 339-351.

Oyaizu M (1986). Antioxidant activity of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography. *Nippon Shokuhin Kogyo Gakkaishi,* **35**: 771-775.

Pernak J and Skrzypczak A (1996). 3-Alkylthiomethyl-1-ethylimidazolium chlorides. Correlation between critical micelle concentrations and minimum inhibitory concentrations. *Eur. J. Med. Chem.,* **31**: 901-903.

Siddiqui SZ, Rehman A, Abbasi MA, Abbas N, Khan KM, Ashraf M and Ejaz SA (2013). Synthesis, characterization and biological screening of *N*-substituted derivatives of 5-benzyl-1,3,4-oxadiazole-2yl-2″-sulfanyl acetamide. *Pak. J. Pharm. Sci.,* **26**(3): 455-463.

Zwolak I and Zaporowska H (2009). Preliminary studies on the effect of zinc and selenium on vanadium-induced cytotoxicity *in vitro*. *Acta Biol. Hung.,* **60**(1): 55-67.