

# Synthesis and Antibacterial Activity of Novel Organoselenium Compounds

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## Abstract

Organoselenium compounds are known to possess antioxidant, anticancer and antibacterial activities. In the present study, 3-[(phenylcarbonyl)selenyl]propanoic acid was synthesized and different ester derivatives of 3-[(phenylcarbonyl)selenyl]propanoic acid and 2-[(phenylcarbonyl)selenyl]acetic acid (benzoylselenoglycolic acid) were synthesized and characterized using IR, <sup>1</sup>HNMR and Mass spectral methods. Minimum Inhibitory Concentration (MIC) of the newly synthesized compounds was determined against gram positive and gram negative bacteria. Some of the compounds showed moderate to significant activity against *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli* and *Bacillus subtilis*.

**Keywords:** Organoselenium compounds, Antibacterial activity, Esters

## 1. Introduction

Selenium, an essential trace mineral (Rayman, 2002) is a vital component of the selenoproteins specifically glutathione peroxidase mainly required for normal health and reproduction (Rayman, 2000). Selenium was discovered in 1818 by Swedish chemist Berzelius. It was considered as a poison until it was identified as a micronutrient for bacteria, mammals and birds (Schwarz & Foltz, 1957). Selenium is essential for the efficient and effective operation of the immune system in both animals and humans (Arthur *et al.*, 2003). Organoselenium compounds have substantially greater bioavailability than that of inorganic selenium (Cantor *et al.*, 1975). More importantly, organic selenium is usually found to be less toxic than inorganic forms (Bendsleve *et al.*, 1988; Khalil, 1989; Khalil, 1994).

Organoselenium compounds have been tested as antibacterial, antiviral, antifungal, antiparasitic, antihistamine and anticancer agents (Athayde-Filho *et al.* 2004). During the last few years, tremendous effort has been directed towards the synthesis of stable organoselenium compounds that could be used as antioxidants, enzyme modulators, antitumors, antivirals, antimicrobials, antihypertensive agents and cytokine inducers (Mugesh *et al.*, 2001; Garcia, 2004; Carland & Fenner, 2005). Though several organoselenium compounds are known, they are less explored for their antibacterial properties. Some novel organoselenium compounds were synthesized and their antibacterial activity was tested.

One of the reagents adopted to synthesize organoselenium compounds is sodium hydrogen selenide, which can be prepared by the action of sodium borohydride on metallic selenium in protic solvents (Klayman & Griffin, 1973). Aqueous solution of sodium hydrogen selenide has been effectively used to synthesize benzoylselenoglycolic acid (**1**) (Athayde-Filho *et al.*, 2004). In the present work benzoylselenoglycolic acid was synthesized as per the reported method and 3-[(phenylcarbonyl) selenyl]propanoic acid (**2**) was synthesized with slight modification (Scheme 1). Several esters of compound **1** and **2** were also synthesized (Scheme 2).

## 2. Experimental Section

### 2.1 Chemistry material

Sodium borohydride, hexane, ethyl acetate were procured from RFCL India, selenium metal from Sigma Aldrich, benzoyl chloride and 3-chloropropanoic acid from Merck. Reaction was monitored using silica gel 60 F<sub>254</sub> TLC plates from Merck. Melting points were taken in open capillary tubes and are uncorrected. All the compounds were characterized by <sup>1</sup>HNMR, IR, and Mass spectral methods. The IR spectra were recorded on Bruker IR spectrophotometer. <sup>1</sup>HNMR in CDCl<sub>3</sub> was recorded on Bruker 200MHz instrument using TMS as internal standard and mass spectra was recorded using Shimadzu LCMS 2010A system.

### 2.2 Synthesis of 3-[(Phenylcarbonyl)selenyl] propanoic acid (2)

Selenium metal (1g, 12.66 mmol) was suspended in 12 mL of water. Sodium borohydride (1g, 26.5 mmoles) was dissolved in 12 mL of water and added slowly to the selenium suspension under nitrogen atmosphere. The reaction was exothermic with vigorous evolution of hydrogen gas. Benzoyl chloride (1.8g, 12.8 mmol) was then added slowly at 30°C. A yellow colored solution was obtained. 3-Chloropropanoic acid (1.4g, 13 mmol) was added slowly to the above reaction mass at 65-70°C and solid precipitated out towards the end of the addition. The solid was filtered and dissolved in 20 mL of dichloromethane and extracted into 5% sodium bicarbonate solution. The pH of the collected sodium bicarbonate layer was adjusted to 4-5 using 5% hydrochloric acid. The product was extracted back into dichloromethane and concentrated under vacuum to obtain white solid. Yield: 80%.

Melting point: 84-88°C. IR ( $\gamma_{\max}$  cm<sup>-1</sup>): 1697(-COOH) 1665(-Se-CO-).

MS: m/z: 257 (base peak) (calculated for C<sub>10</sub>H<sub>10</sub>O<sub>3</sub>Se : 257.14).

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  2.92(t, 2H, -CH<sub>2</sub>-CO-), 3.28(t, 2H, -Se-CH<sub>2</sub>), 7.42–7.91(5H, Ar-H).

### 2.3 General procedure for the preparation of esters (3a-3d)

Benzoylselenoglycolic acid (**1**) (2.43g, 10 mmol) was dissolved in corresponding alcoholic solvent. Thionyl chloride (1.78g, 15 mmol) was added slowly at 55- 60°C. Reaction mass was stirred for 30 minutes at 50-55°C. The solvent was distilled out completely and the residue was dissolved in 15 mL of dichloromethane. Dichloromethane layer was washed with 10 mL of 5% sodium bicarbonate solution and then with water. The organic layer was collected and dried over anhydrous sodium sulfate. Solvent was distilled under vacuum and the product was obtained in the form of oil which was further purified using silica gel column. Combination of hexane and ethyl acetate was used as eluant by progressively increasing the polarity. Finally the desired compounds were eluted by hexane: ethyl acetate in the ratio of 85:15.

Similarly ester derivatives of Compound **2** (**3e-3h**) were also prepared using the same method.

### 2.3.1 Yield and Characterization Data

#### **3a**

Yield: 60%

IR ( $\gamma_{\max}$   $\text{cm}^{-1}$ ): 1720(-COO-), 1678(-Se-CO-).

MS: m/z: 259(base peak,  $[\text{M}+\text{H}]^+$ ) (calculated for  $\text{C}_{10}\text{H}_{10}\text{O}_3\text{Se}$  : 257.14).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  3.74(s, 3H, -OCH<sub>3</sub>), 3.85(s, 2H, -Se-CH<sub>2</sub>), 7.43–7.91(5H, Ar-H).

#### **3b**

Yield: 70%

(IR ( $\gamma_{\max}$   $\text{cm}^{-1}$ ): 1731(-COO-), 1678(-Se-CO-).

MS: m/z: 273(base peak,  $[\text{M}+\text{H}]^+$ ) (calculated for  $\text{C}_{11}\text{H}_{12}\text{O}_3\text{Se}$  : 271.17).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.28(t, 3H, -CH<sub>3</sub>), 3.84(s, 2H, -Se-CH<sub>2</sub>), 4.19(quartet, 2H, -OCH<sub>2</sub>), 7.26–7.92(5H, Ar-H).

#### **3c**

Yield: 70%

IR ( $\gamma_{\max}$   $\text{cm}^{-1}$ ): 1725(-COO-), 1677(-Se-CO-).

MS: m/z: 287(base peak,  $[\text{M}+\text{H}]^+$ ) (calculated for  $\text{C}_{12}\text{H}_{14}\text{O}_3\text{Se}$  : 285.19).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.24(d, 6H, -C(CH<sub>3</sub>)<sub>2</sub>), 3.81(s, 2H, -Se-CH<sub>2</sub>), 5.05 (m, 1H, -OCH), 7.39–8.06( 5H, Ar-H).

#### **3d**

Yield: 75%

IR ( $\gamma_{\max}$   $\text{cm}^{-1}$ ): 1730(-COO-), 1677(-Se-CO-).

MS: m/z: 301 (base peak,  $[\text{M}+\text{H}]^+$ ) (calculated for  $\text{C}_{13}\text{H}_{16}\text{O}_3\text{Se}$  : 299.22).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  0.92(t, 3H, -CH<sub>3</sub>), 1.44(m, 2H, -CH<sub>2</sub>), 1.56(quintet, 2H, -CH<sub>2</sub>), 3.84(s, 2H, -Se-CH<sub>2</sub>), 4.14(t, 2H, -OCH<sub>2</sub>), 7.43-7.91(5H, Ar-H).

#### **3e**

Yield: 75%

IR ( $\gamma_{\max}$   $\text{cm}^{-1}$ ): 1735(-COO-), 1668(-Se-CO-).

MS: m/z: 273 (base peak,  $[\text{M}+\text{H}]^+$ ) (calculated for  $\text{C}_{11}\text{H}_{12}\text{O}_3\text{Se}$  : 271.17).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  2.86(t, 2H, -CH<sub>2</sub>-CO), 3.29(t, 2H, -Se-CH<sub>2</sub>), 3.71(s, 3H, -OCH<sub>3</sub>), 7.41–7.90 (5H, Ar-H).

#### **3f**

Yield: 75%

IR ( $\gamma_{\max}$   $\text{cm}^{-1}$ ): 1731(-COO-), 1669(-Se-CO-).

MS: m/z: 287(base peak,  $[\text{M}+\text{H}]^+$ ) (calculated for  $\text{C}_{12}\text{H}_{14}\text{O}_3\text{Se}$  : 285.19).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.27(t, 3H, -CH<sub>3</sub>), 2.84(t, 2H, -CH<sub>2</sub>-CO), 3.29(t, 2H, -Se-CH<sub>2</sub>), 4.15(quartet, 2H, -OCH<sub>2</sub>), 7.41 –7.91(5H, Ar-H).

#### **3g**

Yield: 85%

IR  $\gamma_{\max}$   $\text{cm}^{-1}$ : 1727(-COO-), 1670(-Se-CO-).

MS: m/z: 301 (base peak,  $[\text{M}+\text{H}]^+$ ) (calculated for  $\text{C}_{13}\text{H}_{16}\text{O}_3\text{Se}$  : 299.22).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.26(d, 6H, -C(CH<sub>3</sub>)<sub>2</sub>), 2.85(t, 2H, -CH<sub>2</sub>-CO-), 3.29(t, 2H, -Se-CH<sub>2</sub>), 5.04(m, 1H, -OCH), 7.40–7.90 (5H, Ar-H).

#### **3h**

Yield: 87%

IR ( $\gamma_{\max}$   $\text{cm}^{-1}$ ): 1735(-COO-), 1673(-Se-CO-).

MS: m/z: 315 (base peak,  $[M+H]^+$ ) (calculated for  $C_{14}H_{18}O_3Se$  : 313.25).

$^1H$  NMR ( $CDCl_3$ ):  $\delta$  0.94(t, 3H,  $-CH_3$ ), 1.39(m, 2H,  $-CH_2$ ), 1.62(quintet, 2H,  $-CH_2$ ), 2.84(t, 2H,  $-CH_2-CO-$ ), 3.29(t, 2H,  $-Se-CH_2$ ), 4.03(t, 2H,  $-OCH_2$ ), 7.41–7.90(5H, Ar-H).

#### 2.4 Antibacterial activity

Benzoylselenoglycolic acid, 3-[(phenylcarbonyl) selenyl] propanoic acid and their esters were assayed *in vitro* for their antibacterial activity against a panel of selected gram positive and gram negative bacteria. They were screened for their antibacterial activity against *Escherichia coli* (ATCC 9637), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29737) and *Salmonella typhimurium* (ATCC 23564) (recultured) bacterial strains which were obtained from National Chemical Laboratory, Pune, India.

The testing was done according to the reported method (Rose & Miller, 1939). Samples were prepared in methanol and serially diluted to determine the minimum inhibitory concentration that inhibited the growth of the tested microorganisms (MIC). After 24 hours of incubation at  $37^\circ C$ , the zone of inhibition was measured in mm. A control was also prepared using only methanol. Doxycycline hydrochloride which is commonly used as an antibiotic in animal health industry was used as positive control. The results obtained are tabulated in Table 1.

### 3. Results and Discussion

One pot synthesis of organoselenium compounds reported in the literature for synthesizing benzoylselenoglycolic acid was used effectively to synthesize 3-[(phenylcarbonyl) selenyl] propanoic acid with slight modification in the reaction temperature. The structure of newly synthesized compound was established on the basis of spectral (IR,  $^1H$  NMR, and mass) data. The IR spectrum of compound **2** showed a band in the region of  $1697\text{cm}^{-1}$  which is characteristic of carbonyl stretching of  $-COOH$  group and  $1665\text{cm}^{-1}$  corresponding to  $-Se-CO$  stretching. In the  $^1H$  NMR spectrum the  $-Se-CH_2-$  proton came into resonance at  $\delta$  3.28 as triplet,  $-CH_2-CO$  protons appeared at  $\delta$  2.92 as triplet. Aromatic protons resonated between  $\delta$  7.42–7.91. The signal corresponding to  $-COOH$  proton was not visible. However presence of  $-COOH$  group was confirmed through esterification reactions. The mass spectrum of this compound showed molecular ion peak at m/z 257 which is also the base peak.

Esters of compounds **1** and **2** were synthesized by the method described above and the confirmation of the structure was obtained by spectral (IR,  $^1H$  NMR, and mass) methods. IR spectrum of compound **3b** showed a band in the region of  $1731\text{cm}^{-1}$  which is characteristic of carbonyl stretching of  $-COO$  group. In the  $^1H$  NMR spectrum the  $-Se-CH_2-$  protons came into resonance at  $\delta$  3.84 as singlet and  $-OCH_2$  protons resonated at  $\delta$  4.19 as quartet. The  $-CH_3$  protons appeared as triplet at  $\delta$  1.28. Aromatic protons resonated between  $\delta$  7.26–7.92. The mass spectrum of this compound showed  $[M+H]^+$  peak at m/z 273 which is also the base peak. The mass spectra of the compounds (**3b-3h**) showed a common fragment peak at m/z 169. A typical pattern which is characteristic of selenium isotopic abundance (Block *et al*, 1996; Ip *et al*, 2000) was observed in the mass spectra of the compounds.

All the newly synthesized compounds were screened for their antibacterial activity. The investigation of antibacterial screening data revealed that majority of the tested compounds (**1, 2 and 3a-3h**) showed moderate to good inhibition against the tested bacterial species. Among all these compounds **3b** and **3d** showed significant inhibitory activity at lower concentrations (less than 0.1 mg/mL) against both gram positive and gram negative bacteria except *S. typhimurium*. Compounds **3a, 3e and 3g** were effective in the concentration range of 0.1-1 mg/mL against all the bacterial species tested. Compounds **2** and **3h** were effective against *S. typhimurium* at slightly higher concentration of 1-10mg/mL. However compounds **3c** and **3d** were less active against *S. typhimurium*.

### 4. Conclusion

This research study reports the successful synthesis and testing for antimicrobial activity of novel organoselenium compounds (**2** and **3a-3h**). These compounds showed moderate to significant antibacterial activity against selected pathogenic gram positive and gram negative bacteria and these results indicate the probable role of organoselenium compounds (**1-3h**) against the tested pathogenic bacterial species. In conclusion, most of the target compounds exhibited *in vitro* antibacterial activity. Further biological evaluation of these compounds is in progress.

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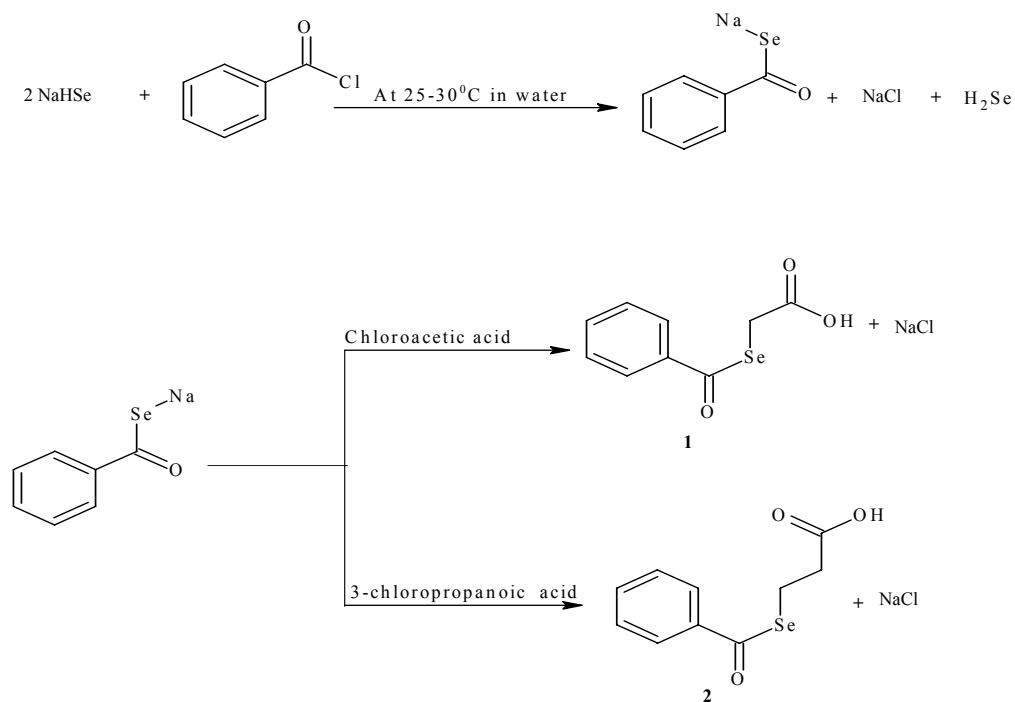
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Table 1. Antibacterial activities of compounds **1**, **2**, **3a** – **3h**

Compound	<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhimurium</i>	<i>B. subtilis</i>
1	+++++	++++	+++	+++++
2	+++++	+++++	++++	+++++
3a	+++++	+++++	+++++	+++++
3b	+++++	+++++	+++++	+++++
3c	+++++	+++++	++	+++++
3d	+++++	+++++	+	+++++
3e	+++++	+++++	+++++	+++++
3f	++++	++++	+++	++++
3g	+++++	+++++	+++++	+++++
3h	+++++	+++++	++++	+++++
Doxycyline Hydrochloride	+++++	+++++	+++++	+++++

Less than 0.1 mg/mL = ++++++, 0.11- 1 mg/mL = +++++, 1-10mg/mL = +++++, 10.1-50mg/mL = +++, 51-100 mg/mL = ++, above 100mg/mL = +.



Scheme 1. Synthesis of compounds 1 and 2



3a R=CH<sub>3</sub> (n=1), 3b R=C<sub>2</sub>H<sub>5</sub> (n=1), 3c R=CH (CH<sub>3</sub>)<sub>2</sub> (n=1) and 3d R=CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub> (n=1)  
 3e R=CH<sub>3</sub> (n=2), 3f R=C<sub>2</sub>H<sub>5</sub> (n=2), 3g R=CH (CH<sub>3</sub>)<sub>2</sub> (n=2) and 3h R=CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub> (n=2)

Scheme 2. Synthesis of esters 3a-3h