Asian Journal of Chemistry Vol. 19, No. 1, (2007), 550–554

Antimicrobial Effect on Bacterial and Yeast Cells of Phenylselenocyanates and Comparison with Some Antibiotics

SERVET ÇETE*, HAMDI ÖZKAN, FATMA ARSLAN, YILMAZ YILDIRIR and AHMET YAŞAR

Department of Chemistry, Faculty of Arts and Sciences, Gazi University
06500 Teknikokullar, Beşevler/Ankara, Turkey
Fax: (90)(312)2122279; Tel: (90)(312)2126030/2713; E-mail: scete@gazi.edu.tr

In this study, antimicrobial effect of *p*-bromophenylselenocyanate and *p*-nitrophenylselenocyanate were investigated against three strains of bacteria and one yeast. Antimicrobial effects were examined in 1 mg/mL concentrations of these compounds. Antimicrobial effects of *p*-bromophenylselenocyanate and *p*-nitrophenylselenocyanate were observed on the bacterial and yeast strain in the studied concentration. When the *p*-bromophenylselenocyanate and *p*-nitrophenylselenocyanate are compared with some antibiotics, it is seen that these compounds have the same antimicrobial effect as some other antibiotics.

Key Words: Antimicrobial effect, Bacterial and yeast cells, Phenylselenocyanates.

INTRODUCTION

Recently, various chemicals have been used for treatment and protection against some diseases. The use of many chemicals is common in food industries to enhance the safety of many foods. Chemical preservatives can be described according to their ability of killing microorganisms.

There are some studies concerning antimicrobial and biological activity of various organic and inorganic substances in literature[1]. Various experimental models show that selenium inhibits tumorigenesis[2]. Low serum selenium levels are associated with an increased risk of developing cancer at several sites, especially cancers of the stomach and lungs in human beings[3].

Selenium, an essential trace element involved in many physiological functions, is known to have an antioxidant effect as well as being an immune system modulator. Selenium has an important role in intestinal mucosal morphology, lipid peroxidation and bacterial translocation[4].

The essential trace mineral, selenium, is of fundamental importance for human health. As a constituent of selenoproteins, selenium has structural and enzymic roles (*e.g.* GSH-Px), in the latter context being best known as an antioxidant and catalyst for the production of active thyroid hormone. Selenium is needed for the proper functioning of the immune system and appears to be a key nutrient in counteracting the development of virulence and inhibiting HIV progression to AIDS. Selenium bioeffects are mainly involved in immune function, reproduction, mood, thyroid function, cardiovascular diseases, cancer, viral infection, metal toxicity and other functions. Recent evidence has reinforced the importance to

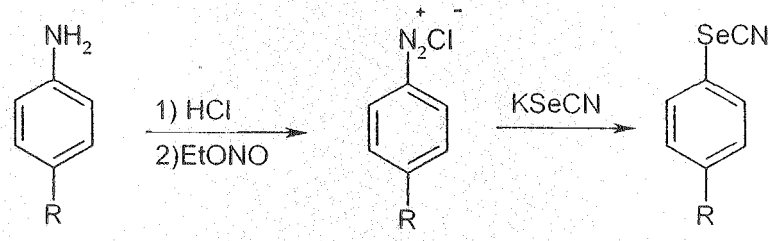
health of adequate selenium states[5]. Selenium species have occasionally been determined by making use of a few of its biological properties[6]. Seleno-azoles have antifungal, antibacterial, antiviral and cytotoxic activity[7].

More recently extensive studies on the chemistry and biology of organoselenium and its analogues demonstrated their antiinflammatory, antisclerotic and cytoprotective properties. Other benziselenoazolones and diaryl diselenides exhibited inhibitory activity against strains of bacteria and fungi[8].

Organoselenium compounds have a potential peroxidase like activity[9]. Thus, many organoselenium compounds have been synthesized[10, 11]. Some substituted phenylselenoglycollic acids have been synthesized by using Grignard reagent **(Scheme-1)** (yields 20–25%) and from diazonium salts[12, 13]. In studies on diazonium salts, substituted anilines were diazotized in aqueous medium, followed by addition. In this study, *p*-bromophenylselenocyanate and *p*-nitrophenylselenocyanate were synthesized and antimicrobial effects of these compounds were determined against clinically important pathogens.

EXPERIMENTAL

Preparation of 4-Bromophenylselenocyanate (3a): 4-Bromoaniline (1.72 g, 0.01 mol) and conc. HCl (2.7 mL, 0.04 mol) were mixed slowly. The anilinium bromide was collected, air-dried and dissolved in 30 mL of absolute alcohol. The stirred solution was cooled (–5 to 0°C) and diazotized by careful dropwise addition (over a period of 30 min) of ethyl nitrite (1 mL, 0.006 mol). Both the solution and ethyl nitrite (b.p. 17°C) must be cold (–5 to 0°C) during the addition. Then a solution of KSeCN (1.44 g, 0.01 mol) in 30 mL of absolute alcohol was added into the solution of the diazonium salt. The precipitate which formed was collected, air dried and recrystallized from absolute alcohol[14].



1 2 3

a) R = Br b) R = NO_2

Scheme-1

TABLE-1
ANALYTICAL AND SPECTROSCOPIC DATA FOR COMPOUNDS **3a–b**

Compound	m.p. (°C)	lit. m.p. (°C)	Yield (%)	ν_{max} (KBr, cm^{-1})
3a	48	–	75	C≡C—H: 3040, CN: 2138
3b	135	138	80	C≡C—H: 3125, CN: 2143

Bacterial and yeast strains

Bacterial cells: *Bacillus subtilis* ATCC 6633; *Bacillus cereus* RSKK 863; *Micrococcus luteus* NRLL B-4375.

Yeast cell: *Candida albicans* (ATCC 10239).

Preparation of microbial culture media

Bacterial strains were inoculated into nutrient broth (Difco) and incubated at 30 ± 0.1°C for 24 h. Yeasts cells were inoculated into YPD broth (Difco) and incubated at 30 ± 0.1°C for 48 h. In order to test the antimicrobial effects, *p*-bromophenylselenocyanate and *p*-nitrophenylselenocyanate and 15 mL of Mueller Hinton agar (Merck) were placed in petri dishes, which were then inoculated with strains of bacteria by taking 100 µL from cell culture media. In order to test the antimicrobial effects, *p*-bromophenylselenocyanate and *p*-nitrophenylselenocyanate and 15 mL of YPD agar (Merck) were placed in petri dishes, which were then inoculated with strains of yeast by taking 100 µL from cell culture media. It was left to solidify at room temperature for a while and then holes were made on top with a sterile stick. Solutions of quantities stated above were then added to these holes. Petri dishes were left at 4°C for 2 h. The bacterial cultures were incubated at 34 ± 0.1°C for 24 h and yeast cultures were incubated at 30 ± 0.1°C for 72 h. At the end of incubation time, the inhibition zones on the bacterial and yeast nutrient media were measured.

RESULTS AND DISCUSSION

p-Bromophenylselenocyanate and *p*-nitrophenylselenocyanate have antimicrobial effect on all the studied microorganisms in 1 mg/mL concentration. When their antimicrobial effects were compared, it was observed that *p*-nitrophenylselenocyanate has more effect than *p*-bromophenylselenocyanate in these conditions. This state may occur because the nitro group has more effect than bromo group on the studied microorganisms (Table-2). But, *p*-bromophenylselenocyanate has more effect than *p*-nitrophenylselenocyanate on yeast cell. The result can explain the difference of bacteria and yeast (Table-2).

TABLE-2
ANTIMICROBIAL EFFECT *p*-BROMOPHENYLSELENOCYANATE AND *p*-NITROPHENYLSELENOCYANATE COMPOUND ON THE BACTERIAL AND YEAST CELLS (INHIBITION ZONE = mm)

	p-Bromophenyl selenocyanate	*p*-Nitrophenyl selenocyanate
Bacterial strains		
Bacillus subtilis (ATCC 6633)	5.0	22.0
Bacillus cereus (RSKK 863)	10.0	17.0
Micrococcus luteus (NRLL, B-4375)	8.0	72.0
Yeast strain		
Candida albicans (ATCC 10239)	14.0	10.0

Besides antimicrobial effects of some antibiotics were investigated on the studied microorganisms in this study. The present findings clearly demonstrate that the compounds investigated have same antimicrobial effect as some other antibiotics (Table-3).

TABLE-3
ANTIMICROBIAL EFFECT OF SOME ANTIBIOTICS ON THE BACTERIAL AND YEAST CELLS (Inhibition Zone = mm)[15]

Antibiotics	*Bacillus subtilis* (ATCC 6633)	*Bacillus cereus* (RSKK 863)	*Micrococcus luteus* (NRLL, B-4375)
Cefadroxil (30 μg)	27.10	23.00	40.00
Ampicillin (10 μg)	8.70	19.70	33.60
Tetracycline (30 μg)	30.00	22.00	30.40
Linkomisin (2 μg)	0.00	8.70	27.60
Erythromycin (15 μg)	24.50	19.00	32.00
Vankomycin (30 μg)	22.00	17.00	28.30
Azithromycin (15 μg)	33.40	19.20	38.00
Amoxycillin/Clavulinic acid (20 μg)	19.40	0.00	48.00
Penicillin-G (10 units)	0.00	0.00	5.00
Chloramphenicol (30 μg)	22.70	22.70	38.50
Cephalothin (30 μg)	18.20	19.20	23.40
Polymyxcine-B (300 units)	0.00	1.60	0.00
Cefoxitin (30 μg)	16.00	7.40	24.20
Antifungal	Yeast strain Candida albicans (ATCC 10239)		
Oxiconazole			
0.20 μg/mL	17 mm		
0.10 μg/mL	17 mm		
0.05 μg/mL	14 mm		
İsoconazole			
0.20 μg/mL	17 mm		
0.10 μg/mL	14 mm		
0.05 μg/mL	14 mm		

Selenium in low concentrations may have anticarcinogenic effect whereas in high concentrations it can be genotoxic and carcinogenic. When the structure of these compounds was compared with each other, it was observed that these compounds have different functional groups in their structure skeleton (halogen and nitro group). The antimicrobial effect of these compounds may be due to nitro group and bromide atom in their structure skeletons because these groups have different antimicrobial effects on the microbial growth media[16]. The present compounds can inhibit the development of microorganisms in different ways. These compounds can destroy the structure of cell and inhibit some enzymes and can destroy DNA or stop the development of microorganisms by inhibition of protein synthesis. The compounds studied may follow different mechanisms that were mentioned above.

From these studies, it is suggested that selenocyanate may be useful bactericidal agents.These compounds may also be used in medical processes because they have antimicrobial effects in low concentration. The concept that selenium-containing molecules may be better nucleophiles than classical antioxidants has led to the design of synthetic organoselenium drugs.

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(*Received*: 15 December 2005; *Accepted*: 24 June 2006) AJC-4976