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Synthesis of New Diselenide Compounds as Anti-Inflammatory Agents

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Many diselenide compounds are used as antioxidants, enzyme inhibitors and cytokine inducers. Three new diselenide compounds, bis-(2-hydroxyphenyl) diselenide, bis-(3-hydroxyphenyl) diselenide and bis-(4-hydroxyphenyl) diselenide were designed and synthesized as anti-inflammatory agent. All of them were found to have strong *in vitro* activity in anti-inflammatory assays.

Key words: Organoselenium compound, Bis-(hydroxyphenyl) diselenide, Diselenide, Anti-inflammatory activity

INTRODUCTION

The interest in organoselenium compound chemistry and medicinal biology has increased in the last two decades. Since 1984, when Ebselen (2-phenylbenzisoselenazol-3(2*H*)-one), a nontoxic glutathione peroxidase mimic, was found (Muller *et al.*, 1984; Wendel *et al.*, 1984), a lot of effort has been directed toward the development of stable organoselenium compounds that could be used as antioxidants, enzyme inhibitors, cytokine inducers, immumonodulators and antiradiation agents(Sie and Masumoto, 1997). Other organoselenium compounds like diphenyl diselenide in common with Ebselen, share both the thiolperoxidase like activity and other antioxidant properties (Palmer *et al.*, 1995). Based on the fact that the pharmacological properties of Ebselen are related to its thiolperoxidase-like activity, the pharmacological properties of diphenyl diselenide were investigated. Nogueira *et al.* observed that diselenide caused minimal toxicity when administrated to mice and rats in doses that had anti-inflammatory and antinociception activity (Wilson *et al.*, 1989). Three new diselenide compounds, which demonstrated anti-inflammatory activity, bis-(2-hydroxyphenyl) diselenide, bis-(3-hydroxyphenyl) diselenide and bis-(4-hydroxyphenyl) diselenide, were recently designed and synthesized. Nitric Oxide (NO) is a multifunctional mediator produced by Inducible nitric oxide synthase (iNOS) in inflammatory processes acteing on various cells. Prostaglandings (PGs) are also an inflammatory mediator, produced by cyclo-oxygenase-w (COX-w) and cycloxygenase-2 (COX-2) expression. The novel diselenide compounds represented a new treatment of inflammatory diseases and autoimmune diseases. Here, the activity of the new diselenides compounds on iNOS and COX-2 expression, induced by LPS in Raw 264.7 murine macrophage cells are examined.

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MATERIALS AND METHODS

Synthesis

^{1}H-NMR and ^{13}C-NMR were recorded in the solvent indicated at 200 and 50 MHz, respectively. Chemical shifts are expressed in parts per million downfield from TMS. Mass spectra were recorded by using EI or ES mode. Flash chromatography was performed with 40-63 μm silica gel. TLC was performed on silica gel 60 plates with an F_{254} indicator and visualized under UV light or developed by immersion in a solution of 20% phosphomolybdic acid in ethanol or in a solution of 1.0% $KMnO_4$.

Bromophenoxy-*tert*-butyldiphenylsilane (2a-2c)

2-Bromophenol (**1a**) (1,000 mg, 5.78 mmol) was dissolved in 40 mL of dimethylformamide (DMF). Chloro-*tert*-buthyldiphenylsilane (TBDPSCl) (1.907 mg, 6.94 mmol) and imidazole (787 mg, 11.56 mmol) was added to the solution.

The solution was stirred at room temperature overnight, then diluted with 200mL of CH_2Cl_2, extract six times. The organic layers were combined, dried over $MgSO_4$, then concentrated *in vacuo*. Purification on silica gel (hexane : EtOAc = 40 : 1) provided 2,355mg(99%) of product **2a** as a colorless oil. ^{1}H-NMR(200MHz, $CDCl_3$) : (**2a**) δ1.084(s, 9H), 6.603(J_{ab}=9.2Hz, 2H), 7.144(J_{ab}=9.2Hz, 2H), 7.360 (m, 2H), 7.400(m, 4H), 7.706(m, 4H), (**2b**) δ1.143(s, 9H), 6.484(J_{ab}=8Hz, 1H), 6.671-6.880(m, 3H), 7.329-7.554(m, 6H), 7.719-7.765(m. 4H) and (**2c**) δ1.090(s, 9H), 6.600 (J_{ac}=6Hz, 1H), 6.882(J_{ac}=6Hz, 1H), 6.964-7.025(m, 2H), 7.235-7.477(m, 4H), 7.671-7.728(m, 2H)

Bis-(*tert*-butyldiphenylsilanyloxy)-phenyl diselenide (3a-3c)

To a THF(10mL) solution of **2a** (205.7 mg, 0.5 mmol) at -78°C, a 1.7M pentane solution of *tert*-BuLi (688 μL, 1.17 mmol) was added. The reaction mixture was then stirred at 0°C for 30 min and selenium powder (heated and dried *in vacuo*, 41.8 mg, 0.53 mmol) was added in single portion. The reaction mixture was kept at 0°C for 15min and then at rt for 30 min. Saturated NH_4Cl solution was added slowly, the organic material was extracted with ether three times and the combined organic layers were dried over $MgSO_4$ and concentrated. The residue was dissolved in absolute EtOH(10 mL) and stirred vigorously in the presence of 3 mg of NaOH, for 10 min. The orange solution was concentrated and the resulting material was purified by silica gel (haxane : EtOAc = 15 : 1) to give a yellow oil, contarining the product **3a** (245 mg, 59.7%) ^{1}H-NMR(200MHz, $CDCl_3$) : (**3a**) δ1.079(s, 18H), 6.598 (J_{ab}= 8.8Hz, 4H), 7.147(J_{ab}=8.8Hz, 4H), 7.297-7.437(m, 12H), 7.651-7.724(m, 8H) (**3b**) δ1.077(s, 18H), 6.578 (J_{ac}=7.8 Hz, 2H), 6.834-6.929(m, 4H), 7.039(m, 2H), 7.248-7.433 (m, 12H), 7.644-7.724(m, 8H) and (**3c**) δ1.092(s, 18H), 6.612(J_{ab}=8Hz, 2H), 6.847-6.924(m, 4H), 6.927-7.109(m, 2H), 7.349-7.484(m, 12H), 7.673-7.730(m, 8H)

Bis-(hydroxyphenyl) diselenide (4a-4c)

Compound **3a** (245 mg, 0.30 mmol)was dissolved in 5 mL of distilled THF. The solution was cooled to 0°C and 1.54 mL of 1M solution in THF was added dropwise. The reaction mixture was stirred at 0°C for 30min, diluted with CH_2Cl_2 and washed with water five times. The organic layers were combined, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification on silica gel (hexane : EtOAc = 4 : 1, 2 : 1) provided a yellow solid. The solid was recrystallized from petrolium ether to give a yellow solid **4a** (65 mg, 65%). (**4a**) ^{1}H-NMR(200MHz, $CDCl_3$) : δ6.671-6.790(m, 4H), 7.332-7.492(m, 4H) EI/MS : 57(77), 94(50), 105(60), 145(18), 171(48), 173(80), 342(25), 344(44), 346(46) m/z, (**4b**) EI/MS : 63(50), 65(60), 92(27), 117(17), 143(30), 145(58), 171(50), 173(100), 342(40), 344 (68), 346(78) m/z and (**4c**) ^{1}H-NMR(200MHz, $CDCl_3$) δ6.540 -6.602(m, 2H), δ6.758-7.008(m, 2H), δ7.281-7.590 (m, 2H), δ7.610-7.702(m, 2H).

Bioassay

DMEM medium, fetal bovine serum (FBS), penicillin and streptomycin were obtained from Life Technologies Inc. (Grand Island, NY, USA).

The RAW 264.7 murine macrophage cell line was obtained from the Korean Cell Line Bank (Seoul, Korea). These cells were grown at 37°C in DMEM medium containing 10% heat-inactivated FBS, penicillin (100 units/mL) and streptomycin sulfate (100 μg/mL) in a humidified atmosphere of 5% CO_2. Cells were incubated with diargl diselenide compounds at various concentrations and stimulated with LPS 1 μg/mL.

MTT assay for cell viability

Cytotoxicity studies were performed in a 96-well plate. RAW 264.7 cells were mechanically scraped and plated at 1×10^5/well in 96-well plates containing 100 μL of DMEM medium with 10% heat-inactivated FBS and incubated overnight. Diargl diselenide compounds was dissolved in DMSO, the concentrations of which in all assay did not exceed 0.1%. After overnight incubation, the test material was added and the plates were incubated for 24 h. Cells were washed once before adding 50 μL of FBS-free medium containing MTT 5 mg/mL. After 4 h of incubation at 37°C, the medium was discarded and the formazan blue that formed in the cells was dissolved in DMSO 100 μL. The optical density was measured at 540 nm.

Nitrite determination

The nitrite accumulated in the culture medium was measured as an indicator of NO production according to the Griess reaction. Briefly, 100 μL of cell culture medium was mixed with 100 μL of Griess reagent [equal volumes of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 0.1% (w/v) napthylethylenediamine-HCl], incubated at room temperature for 10 min, and the absorbance at 550 nm was measured in a microplate reader. Fresh culture medium was used as a control in all experiments. The amount of nitrite in the samples was measured with a sodium nitrite serial dilution standard curve and nitrite production was measured.

CONCLUSION

Diselenide compounds have attracted much attention as intermediates in organic synthesis. (Mugesh *et al.*, 2001; Nishibayashi *et al.*, 1995; Pietras *et al.*, 1995; Ethier, 1995) Various methods are available for the synthesis of these

compounds(Hollis Showalter, 1997). Among them, the most important routes involve the reaction of metal diselenides (Wirth and Fragale, 1997) with alkyl halides, dimerisation of selenocyanates(Crich, 1999) and preparation of selenols which can be subjected to further oxidation. Methods involving the use of metal diselenides have generally been carried out in the presence of strong reducing agents and in basic conditions. The first attempt, in this study, to produce diselenide compounds directly by using three bromophenols (*ortho*-, *meta*-, and *para*-), failed to produce the desired compounds. So common reagents for protection of the three bromophenol compounds such as TBDMSCl (Chloro-*tert*-buthyldimethylsilane), BnCl (Benzyl-chloride), PMBCl(*p*-Methoxybenzylchloride) and TBDPSCl (Chloro-*tert*-buthyldimethylsilane), were used. *m*-Bromo-phenol compounds, protected by four kinds of reagents, underwent reactions with selenium powder and a strong reducing agent, which was provided *in situ* using lithium metal and $NaBH_4$ to furnish diaryl diselenides. Results with a low yield were similar to those cases that involved various kinds of side products. A new method of production (Syper and Mlochowski, 1988) of diaryl diselenides, using a catalytic amount of diphenylacetylene, lithium metal and selenium powder, was attempted, to provide the aryl selenol intermediate and obtain diaryl diselenides as further oxidation products. In this study, the new method could not be adopted due to the resulting low yield. Finally, protected bromophenol, treated with *tert*-buthyl lithium and selenium powder, provided dilithium diselenide (Déziel 1996) and subsequently various protected diaryl diselenide compounds were prepared. Among the different protected diselenide compounds, the yield of bis-(*m*-*tert*-buthyldimethylsilyloxiphenyl) diselenide (**3b**) was superior to the others. An appropriative synthesis strategy for the new diselenide compounds was derived from different protected bromophenols using *tert*-buthyldimethylsilane and prepared diaryl diselenide compounds under *tert*-buthyllithium and selenium powder conditions, which when treated with TBAF gave the desired bis-(hydroxy-phenyl) diselenide (Scheme 1).

The cytotoxic effect of bis-(hydroxyphenyl) diselenide was evaluated in the presence or absence of LPS using the MTT assay. To assess the effects of these compounds on NO production in RAW 264.7 cells, the cell culture medium was harvested and the production of nitrite was measured by using the method of Griess. All extracts used in this study showed an inhibitory effect on LPS-induced NO production (Fig. 1). The most active was **4b**, which reduced NO production in a dose-dependent manner with an IC_{50} of 3.4 μM, 8.7 μM and 7.9 μM (Fig. 1).

In the nitric oxide production inhibition experiment (Table I), Bis-(3-hydroxyphenyl) diselenide (**4b**) exhibited the highest NO inhibitory activity (IC_{50} 3.4 mM) against

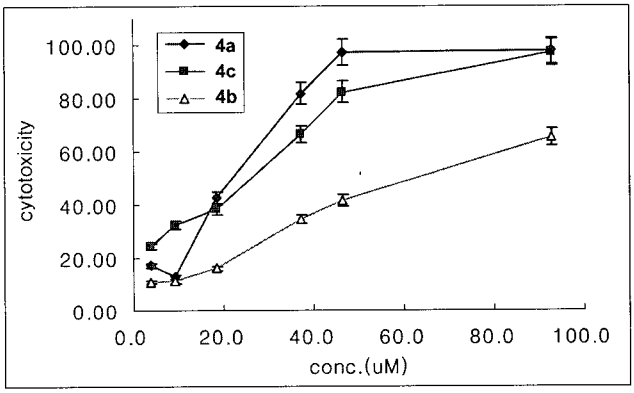
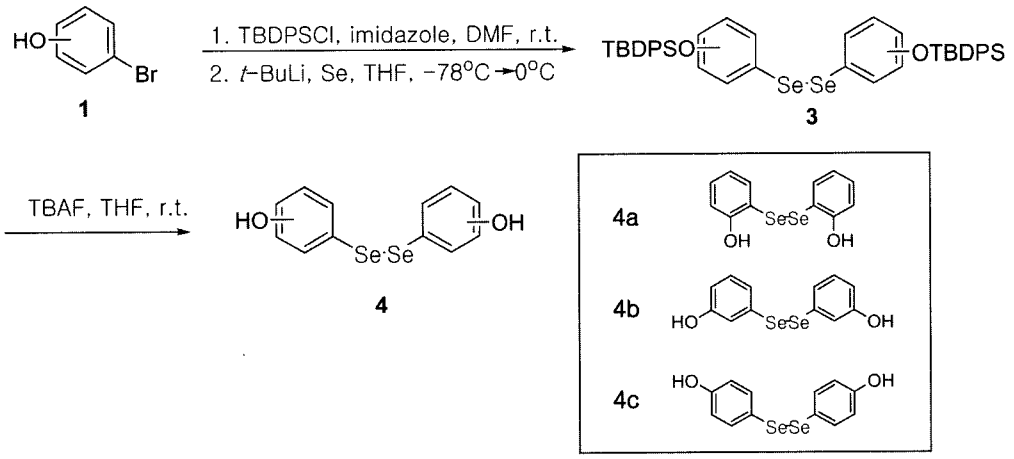


Fig. 1. Effect of various extracts on nitrite production by LPS-induced RAW 264.7 cells. The cells were treated with LPS 1 μg/mL alone or plus different concentrations of various extracts for 24 h. Control (Con) values were obtained in the absence of LPS. The values are the means ± S.D. of three independent experiments.



Scheme 1. Synthesis of bis-(hydroxyphenyl) diselenides

Table I. Effect of diaryl diselenide on NO Production by LPS-induced Raw 264.7 Macrophages.

	$IC_{50}(\mu M)$	
	NO inhibition	Cytotoxicity
4b	3.4	22.1
4c	8.7	26.3
4a	7.9	33.7
L-NIL[a]	19.4	>200

The culture media were collected for nitrite determination. Results are mean from four independant experiments. IC_{50} is defined as the concentration which resulted in 50% decrease in NO production.
[a]L-NIL (L-N^6-1-Iminoethyl)Lysine was used as standard compound.

LPS-induced macrophage cells. It was more potent than the standard compound (L-NIL, IC_{50} 19.4 μM). Bis-(4-hydroxyphenyl) diselenide (**4c**) and bis-(2-hydroxyphenyl) diselenide (**4a**) also showed activity, although less potent than bis-(3-hydroxyphenyl) diselenide (**4b**). In the study of the *in vitro* structure-activity relationship (SAR) of these diaryl diselenide compounds, it can be deduced that the Se-Se sigma bond in bis-(2-hydroxyphenyl) diselenide (**4a**) and bis-(4-hydroxyphenyl) diselenide (**4b**) was resonated by ortho- and parahydroxyl group and produce a more stabilized selenol radical to capture NO· in the cells.

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