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Preparation of 1,4-Oxaselenin from $AgNO_3$/LDA-Assisted Reaction of 3-Selena-4-pentyn-1-one as Potential Antitumor Agents

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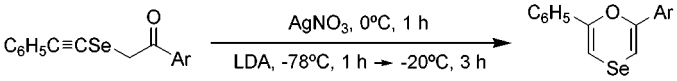
ABSTRACT



1,4-Oxaselenins were synthesized from 3-selena-4-pentyn-1-ones by the use of $AgNO_3$ and LDA. One of the obtained oxaselenins, 2-(4-chlorophenyl)-6-phenyl-1,4-oxaselenin 5c, showed an inhibitory effect against the proliferation of human cancer cells and inducing effects on the early stage of apoptosis.

The literature shows many selenium-containing heterocyclic compounds,[1] and of these, many are potential pharmaceutical agents.[2] Among the oxaselenins, the syntheses of 1,3-oxaselenin-2-one,[3] 1,2-oxaselenin,[4] and 3,1-benzooxaselenin-4-one[5] have been extensively reported, while preparations of 1,4-oxaselenins are much rarer.[6] We have recently reported that the reaction of alkynyl propargyl selenide with primary amines afforded 2-imino-2*H*-5,6-dihydroselenines.[7] The use of alkynyl selenide is one of the most efficient methods for the synthesis of heterocyclic compounds containing selenium.[8] Herein, we describe the preparation of three 1,4-oxaselenins from 3-selena-4-pentyn-1-one by treatment of 2-bromoacetophenones with $AgNO_3$ and LDA. We also present their evaluation as potential antitumor agents.

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The synthesis of 5-phenyl-3-selena-4-pentyn-1-ones **4a**−**c** using lithium phenylselenorate **2** was achieved by the following one-pot procedure (Scheme 1). Bromoacetophenones **3a**−**c** were added to the lithium phenylethyneselenolate **2** in THF, generated in situ from phenylacetylene **1** and elementary selenium, and stirred at −78 °C for 1 h. Three 5-phenyl-3-selena-4-pentyn-1-ones, **4a**−**c**, were obtained in high yield. Several attempts to determine the optimal activator for the preparation of 2,6-diphenyl-1,4-oxaselenin **5a** from 1,5-diphenyl-3-selena-4-pentyn-1-one **4a** showed that the best result was obtained from the combination of $AgNO_3$[9] and LDA (Table 1). In this procedure, $AgNO_3$ was added to a THF solution of 1,5-diphenyl-3-selena-4-pentyn-1-one **4a** at 0 °C, and the mixture was stirred for 1 h. Subsequently, LDA was added at −78 °C, and the mixture was stirred for 1 h, warmed to 0 °C, and further stirred for 3 h. This condition afforded 2,6-diphenyl-1,4-oxaselenin **5a** in a 37% yield (run 1). The structure of **5a** was determined from its ^{1}H NMR spectra, which showed a $^1J(^{77}Se-^1H)$ value of J = 21.6 Hz at the H3 and H5 protons of product **5a**. Other combinations of $AgNO_3$, Cu(I)I, LDA, and *n*-BuLi gave no identifiable products. Only the corresponding starting material could be isolated (runs 2−4). Though copper(I) has been commonly used to catalyze cyclizations[10] and the substitutions of nonactivated aryl bromides or iodides,[11] it did not provide the desired product in the present reaction. It was anticipated that activation of alkyne by silver-induced nucleophilic attack of the oxygen in **4a** was followed by dehydrogenated by LDA to afford **5a**. Similarly, the use of one base only (e.g., LDA, *n*-BuLi, NaH, or EtONa) also only gave mixtures instead of **5a** (runs 5, 7, and 8) or provided no products other than recovered starting material (runs 6 and 9). Next, we investigated various reaction conditions to improve the yield of **5a** from the treatment with $AgNO_3$ and LDA (Table 2). The addition of $AgNO_3$ at 0 °C followed by LDA at −78 °C, stirring for 1 h, and furtherstirring at −20 °C for 3 h gave **5a** in the highest yield (run 1). For the addition of $AgNO_3$, 0 °C provided better yields than room temperature (runs 1 and 5 or 2 and 6). For LDA addition, both higher and lower temperature failed to improve the yields (runs 1−4). Three 1,4-oxaselenins, **5a**−**c**, were obtained from the corresponding 5-phenyl-3-selena-4-pentyn-1-ones **4a**−**c** in 35−53% yield (Scheme 2), respectively. According to Baldwin's rules for ring closure,[12] the present reaction is a *favored* 6-*End-Digonal system*, which affords a six-membered ring heterocyclic product. For comparison,

Scheme 1

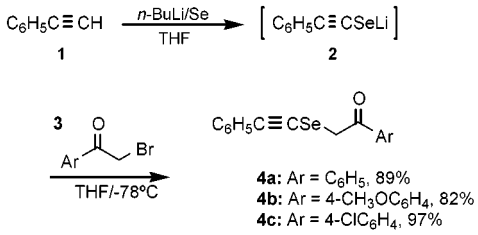
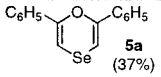
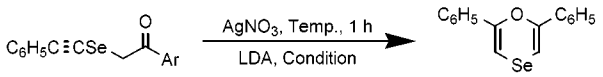


Table 1. Evaluation of Activators for Optimizing the Preparation of 2,6-Diphenyl-1,4-oxaselenin **5a** from 1,5-Diphenyl-3-selena-4-pentyn-1-one **4a**

run	activators	result
1	$AgNO_3$/LDA[a]	 **5a** (37%)
2	Cu(I)I/LDA[a]	mixture
3	$AgNO_3$/*n*-BuLi[a]	mixture
4	Cu(I)I/*n*-BuLi[a]	mixture
5	LDA[b]	mixture
6	LDA[c]	no reaction
7	*n*-BuLi[d]	mixture
8	NaH[e]	mixture
9	EtONa[e]	no reaction

[a] $AgNO_3$ or Cu(I)I (0 °C/1 h)/ LDA or *n*-BuLi (−78 °C/1 h → 0 °C/3 h). [b] -78 °C/1 h → rt/20 h. [c] -78 °C/1 h → −0 °C/5 h. [d] -78 °C/6 h. [e] 0 °C/3 h.

Table 2. Investigations of Optimal Condition for the Preparation of 1,4-Oxaselenin



4a **5a**

run	temp (°C)	conditions	yield (%)[a]
1	0	−78 °C/1 h → −20 °C/3 h	52
2	0	−78 °C/1 h → 0 °C/3 h	37
3	0	−78 °C/6 h	7
4	0	0 °C/2 h	13
5	rt	−78 °C/1 h → −20 °C/3 h	35
6	rt	−78 °C/1 h → 0 °C/3 h	21

[a] Isolated yield.

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(9) The use of other Ag(I) reagents could not improve the yield of **5a**.

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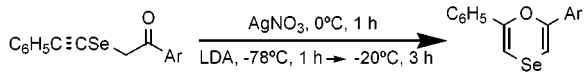
Scheme 2



4a: Ar = C_6H_5
4b: Ar = 4-$CH_3OC_6H_4$
4c: Ar = 4-ClC_6H_4

5a: 52%
5b: 35%
5c: 53%

a ring closure of propargyloxime would yield isoxazole,[13] a five-membered ring compound, by a 5-*Exo-Digonal system*. The reason was thought to be the different character of the linkers (e.g., trajectories, bond angles, and length of the linking chain) between selenium and nitrogen atom.

The antitumor effects of the three 1,4-oxaselenins **5a**−**c** were also investigated.[14] The proliferative inhibitory effects of **5a**−**c** against human uterine cervical cancer cells, such as SiHa and HeLa cells, and human ovarian cancer cell, SK-OV-3, are shown in Table 3. All three selenium compounds exhibited growth inhibition against SiHa and SK-OV-3 cells.

Table 3. 1,4-Oxaselenin **5** Induced Cytotoxicity against Human Tumor Cell Lines in Vitro[a]

	IC_{50} (μM)		
human tumor cells	**5a**	**5b**	**5c**
SK-OV-3 (ovarian cancer cell)	>100	>100	37.5 ± 6.8
SiHa (uterine cervical cancer cell)	>100	>100	45.1 ± 9.1
HeLa (uterine cervical cancer cell)	*b*	*b*	*b*

[a] Dose-dependent inhibitory effect of **5** on the growth of SK-OV-3, SiHa, and HeLa cells. The cancer cells (1×10^4 cells per well) were seeded into 96-well plates and preincubated for 24 h. The complete medium was removed and replaced with a serum-free media, and the cultured cells were treated with various concentrations (10−60 μM) of **5** in the serum-free medium for 24 h. Then, aliquots of the cultured medium were transferred to new plates and the amount of lactate dehydrogenase (LDH) estimated on a cytotox-96 equipped with a 490 nm ELISA reader. Data from three independent cultures (triplicate wells for each condition) are expressed at the mean ±SD. [b] No inhibition.

Compound **5c** showed the greatest growth inhibition with IC_{50}s of 45.1 and 37.5 μM, respectively. Compounds **5a** and **5b** exhibited only weak inhibitory activities, with IC_{50}s of >100 μM. All three compounds showed no inhibitory effects on proliferation of HeLa cells (Table 3). To determine the mechanism of cytotoxicity for **5**, the inducing effect of the early stage of apoptosis was studied by the method of combined annexin V-FITC and PI labeling with flow cytometric analysis on SK-OV-3 cells. As we predicted, **5a** and **5b** did not show any inducing effect of apoptosis on cancer cells at doses of 15−60 μM. Compound **5c** showed inducing effects on the early stage of apoptosis of 11%, 15%, and 19% at doses of 15, 30, and 60 μM, respectively. These results showed that a 4-chlorophenyl group at the C_2 position of **5** was more effective than a 4-methoxyphenyl group or an unsubstituted 4-phenyl group at showing cytotoxicity and apoptosis induction against the cancer cells. These facts also suggest that chlorination on the phenyl group at the C_2 position is important for the cytotoxic activity. On the basis of these data, further structure−activity relationship studies are needed.

In conclusion, we have demonstrated a novel method of preparing 1,4-oxaselenins from 5-phenyl-3-selena-4-pentyn-1-ones by treatment with $AgNO_3$ and LDA and a useful basis for the development of potential antitumor agents based on 1,4-oxaselenin.

Supporting Information Available: Synthetic procedures for the compounds prepared in this Letter, including spectral characterization. Condition of cancer cell culture and assay systems for the measurement of antitumor effects of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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