Supporting Information

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

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Chemistry

General. Tetrahydrofuran was distilled from sodium-benzophenone immediately prior to use. The ^{77}Se chemical shifts were expressed in ppm deshielded with respect to neat Me_2Se in $CDCl_3$. $^1J(^{77}Se\text{-}^1H)$ values are observed as ^{77}Se satellites of the ^{1}H NMR spectra.

Preparation of lithium phenylselenorate (**2).** To THF solution (25 mL) of phenylacetylene **1** (10 mmol), *n*-BuLi (12 mmol) was added at 0°C, and the mixture was stirred for 15 min before elemental selenium powder (10 mmol) was added. The reaction mixture was warmed to room temperature and stirred for 30 min under argon atmosphere. The obtained **2**, formed *in situ*, was then ready for further reaction.

Synthesis of 1,5-diphenyl-3-selena-4-pentyn-1-one (4a). 2-Penetynyl bromide **3a**

(10 mmol) was added to the lithium phenylselenorate **2** (10 mmol) in THF solution (25 mL). The reaction mixture was stirred at -78°C for 1 h under argon atmosphere. The mixture was extracted with diethyl ether and washed with saturated NaCl solution, and the organic layer was separated, dried over Na_2SO_4, and evaporated. The residue was subjected to flash column chromatography on silica gel using *n*-hexane : Et_2O (10:1) as the eluent, giving 1,5-diphenyl-3-selena-4-pentyn-1-one **4a** (2.65 g, 89%) as yellow crystals; Mp: 48.7-50.3°C; IR (KBr): 1664 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.31 (2H, s), 7.22-7.95 (10H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 34.5, 68.9, 101.3, 123.1-135.1, 194.5; ^{77}Se NMR (76 MHz, $CDCl_3$): δ 175.1; MS (CI): *m/z* = 301 [M^++1]; HRMS: Calcd. for $C_{16}H_{12}NOSe$: 299.2307. Found: 299.2322

1-(4-Methoxyphenyl)-5-phenyl-3-selena-4-pentyn-1-one (4b). Yield: 82%, yellow crystals; Mp: 48.5-50.0°C; IR (KBr): 1654 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.86 (3H, s), 4.31 (2H, s), 6.92-7.99 (9H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 34.5, 55.5, 69.7, 101.3, 113.9-164.0, 193.0; ^{77}Se NMR (76 MHz, $CDCl_3$): δ 172.8; MS (CI): *m/z* = 330 [M^++1]; HRMS: Calcd. for $C_{17}H_{14}O_2Se$: 329.2570. Found: 329.2584.

1-(4-Chlorophenyl)-5-phenyl-3-selena-4-pentyn-1-one (4c). Yield: 97%, yellow crystals; Mp: 61.2-62.0°C; IR (KBr): 1663 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.30 (2H, s), 7.30-7.95 (9H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 33.8, 69.0, 101.7, 122.9-143.0, 193.2; ^{77}Se NMR (76 MHz, $CDCl_3$): δ 184.0; MS (CI): *m/z* = 335 [M^++1]; HRMS: Calcd. for $C_{16}H_{11}OSeCl$: 333.6754. Found: 333.6747.

Synthesis of 2,6-diphenyl-1,4-oxaselenin (5a). $AgNO_3$ (1.1 mmol) was added to **4a** (1.0 mmol) in THF (20 mL). The reaction mixture was stirred at 0°C for 1 h under

argon atmosphere. After cooling to -78°C, 1.2 mmol of LDA was added to the mixture, and it was stirred at the same temperature for 1 h, then at -20°C for 3 h. The reaction mixture was extracted with diethyl ether and washed with saturated NaCl solution, and the organic layer was separated, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel using *n*-hexane : Et_2O (10:1) as the eluent, giving 2,6-diphenyl-1,4-oxaselenin **5a** (0.15 g, 52%) as yellow crystals; Mp: 81.5-83.0°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.10 (2H, s), 7.25-7.39 (6H, m), 7.62-7.65 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 89.8, 124.8-134.1, 151.4; ^{77}Se NMR (76 MHz, $CDCl_3$): δ 192.3; MS (CI): *m/z* = 301 [M^++1]; Anal. Calcd for $C_{16}H_{12}OSe$: C, 64.22; H, 4.04. Found: C, 64.25; H, 4.02.

2-(4-Methoxyphenyl)-6-phenyl-1,4-oxaselenin (5b) Yield: 35%, yellow crystals; Mp: 94.5-95.2°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.82 (3H, s), 5.94 (1H, d, *J* = 1.2 Hz), 6.11 (1H, d, *J* = 1.6 Hz), 6.88-7.63 (9H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 55.3, 87.3, 90.0, 113.8-134.2, 151.3, 159.9; ^{77}Se NMR (76 MHz, $CDCl_3$): δ 188.9; MS (CI): *m/z* = 331 [M^++1]; Anal. Calcd for $C_{17}H_{14}O_2Se$: C, 62.01; H, 4.29. Found: C, 62.25; H, 4.23.

2-(4-Chlorophenyl)-6-phenyl-1,4-oxaselenin (5c). Yield: 53%, yellow crystals; Mp: 102.8-103.5°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.09 (2H, dd, J = 1.2, 5.2 Hz), 7.26-7.40 (5H, m), 7.55-7.61 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 89.8, 90.5, 124.7-134.3, 150.3, 151.3; ^{77}Se NMR (76 MHz, $CDCl_3$): δ 196.0; MS (CI): *m/z* = 335 [M^++1]; Anal. Calcd for $C_{16}H_{11}OSeCl$: C, 57.59; H, 3.32. Found: C, 57.66; H, 3.28.

Biology

Cell culture and treatment

Two human uterine cervical cancer cell lines including SiHa and HeLa and one human ovarian cancer cell line, SK-OV-3, were obtained from the Cancer Research Institute, College of Medicine, Seoul National University, Korea. SiHa was cultured in minimum essential medium (Life Technologies, Inc), HeLa was cultured in Dulbecco's modified Eagle's medium (Life Technologies, Inc), and SK-OV-3 was cultured in RPMI 1640 (Life Technologies, Inc). The cultured cells were supplemented with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT, USA), and 1% penicillin/streptomycin (Life Technologies, Inc). The cells were grown to approximately 85 to 90% of confluence in a humidified atmosphere of 5% CO_2 at 37°C. SiHa, HeLa and SK-OV-3 were incubated with various concentrations (10 - 60 μM) of **5a-c** in culture medium for 24 h. Compounds, **5a-c**, were dissolved in dimethyl sulfoxide (DMSO) and diluted with culture media (final concentration of DMSO, 0.2%).

Inhibitory effect of 1,4-oxaselenines on cancer cell proliferation

The inhibition effects of **5a-c** on the cell growth were assessed with cytotox-96 assay (Promega, Medison, MI, USA). In brief, 24 h after the cell seeding at 5 x 10^4 cells/ml in 96-well plates in complete growth medium, the medium were removed, replaced with serum free media, and cultured cells were treated with **5a-c** for 24 h. Then, aliquots of the cultured medium were transferred new plates and estimated lactate dihydrogenase (LDH)[1] on cytotox-96 equipped with a 490nm ELISA reader.

Inducing effects on early stages of apoptosis

Apoptosis effects were evaluated by combining annexin V-FITC[2] and Propidium Iodide (PI) labeling with flow cytometric analysis according to the instruction of the manufacturer (Becton Dickinson, USA). Annexin-V (Pharmigen, Becton Dickinson, USA) is an endogenous human lipocortin that has a high affinity for phosphatidyl serine (PS) exposed on the cell surface during apoptotic cell death. All assays were carried out by plating cells in 24-well plates at a cell density of 1×10^6 cells/ml in complete medium. After 24 h, medium was replaced with fresh complete medium and treated with **5** for 24h. And then each medium was discarded, cells were collected by trypsinization, and washed thoroughly with ice-cold PBS and annexin binding buffer. After washing, the cells were stained and with annexin V-FITC and PI, and analyzed on FACScan flow cytometry (FACScalibur, Becton Dickinson, USA).

References

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