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RESEARCH ARTICLE

Synthesis of novel 3-allylseleno-6-alkylthiopyridazines: their anticancer activity against MCF-7 cells

Chaewon Kim · Saet-Byeul Kim · Myung-Sook Park

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Abstract A new series of 3-allylseleno-6-alkylthiopyridazines **6a–6g** was synthesized by two synthetic routes from 3,6-dichloropyridazine to develop new anticancer agents. These new compounds showed antiproliferative activities against breast cancer (MCF-7) cells in CCK-8 assays, and could be promising candidates for chemotherapy of carcinomas. Compound **6e** (3-allylseleno-6-pentylthiopyridazine) showed higher potency than 5FU for inhibiting the growth of these cell lines. This suggests the potential anticancer activity of compound **6e**.

Keywords Allylselenopyridazines · Pyridazines · Organoseleniums · Anticancer activities · MCF-7 cells

Introduction

The allylthio group of allicin and other organosulfur compounds that are isolated from garlic is considered an important pharmacopore (Cavallito et al. 1944; Block et al. 1986; Yamasaki et al. 1991; Agarwal 1996), i.e., a key structural component of the molecules that is responsible for their antitumor activities. Garlic is said to contain selenoproteins (Wang et al. 1989) and a selenopolysaccharide (Yang et al. 1992). Selenium plays an important role in biology. It is required for the correct functioning of the immune system and for cellular defense against oxidative damage, and may play a role in the prevention of cancer and premature aging (Block 2010).

In previous studies, various allylthioalkoxypyridazines and allylthioalkylthiopyridazines were synthesized (Lee et al. 2001; Kwon 2002a, b) and their biological activities were tested (Jung et al. 2001; Kwon and Moon 2005) (Fig. 1). Allylthioheterocyclo(or aryl)alkylaminopyridazines and allylthioaminopyridazines showed especially good anticancer activities (Lee et al. 2009; Won and Park 2010).

The pyridazine group is an important moiety present in many drugs acting at various pharmacological targets (Kleemann and Engel 2001; Song et al. 2008; Cha et al. 2012). The pyridazine moiety has been combined with the allylthio group (Lee et al. 2003; Shin and Kwon 2003).

We recently reported the synthesis of allylthioheterocyclopyridazines (Park and Park 2005, 2007). The synthesis of allylthioheterocyclo(or aryl)alkylaminopyridazines and their antitumor activities against SK-Hep-1 human liver cancer cells were also reported (Kwon and Lee 2005). More recently, the synthesis of 3-(6-arylamino)pyridazinylamino benzoic acids and their antitumor activities against HT-29 colon cancer cells were investigated (Abouzid et al. 2013). The synthesis of isoxazolo (or thiazolo) [4,5-*d*]pyridazines and their antimicrobial activities were also reported (Faidallah et al. 2013).

The isosteric replacement of the sulfur of allylthiopyridazines by a selenium atom yields the allylselenopyridazines (Fig. 1). We designed new 3-allylseleno-6-alkylthiopyridazine derivatives **6a–6g** in order to discover potential anti-tumor candidates. We tested the ability of these synthetic compounds to inhibit the growth of breast cancer MCF-7 cell lines.

Materials and methods

Chemicals

Chemicals were supplied by Aldrich, Sigma, Merck, and Tokyo Kasei. Melting points were determined in open

C. Kim · S.-B. Kim · M.-S. Park (✉)
College of Pharmacy, Duksung Women's University, Seoul 132-714, Korea
e-mail: mspark@duksung.ac.kr

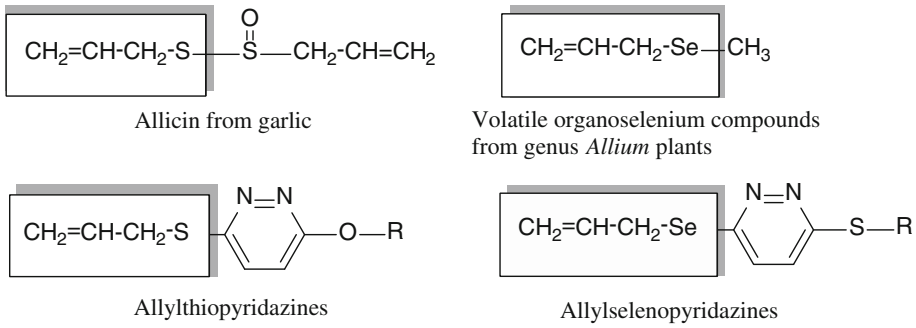


Fig. 1 Allicin, organoseleniums, reported allylthiopyridazines and allylselenopyridazines as target compounds

capillary tubes on a Büchi 535 melting point apparatus and were uncorrected. NMR spectra were recorded using a Bruker 300 MHz NMR spectrometer. Chemical shifts are reported in parts per million and were recorded in chloroform-d or dimethyl-d_6 sulfoxide with tetramethylsilane as the internal standard. NMR spin multiplicities are indicated by the symbols: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). IR spectra were recorded on a Perkin-Elmer 16F PC FT-IR spectrometer using NaCl discs and pellets.

General synthetic procedure
for the dialkylthiopyridazinyl diselenides **5a**–**5e**

To a vigorously stirred mixture of powdered sodium hydroxide (0.3 g, 7.4 mmol), elemental selenium (0.39 g, 4.93 mmol) and dimethylformamide (DMF) (20 mL), 100 % hydrazine hydrate (0.16 mL, 4.93 mmol) was added slowly. After stirring for nearly 6 h at rt, 6-alkylthiopyridazinyl chloride **4a**–**4e** (4.93 mmol) was added slowly. The mixture was refluxed for 1 h. When the solution turned to red black from green black, it was cooled to rt. After the excess selenium was filtered, the solution was diluted with cold water (40 mL). The mixture was left for 48 h at 4 °C. The yellow solid was formed in aqueous solution. The crude product was filtered to afford **5a**–**5e**.

*Dimethylthiopyridazinyl diselenide (**5a**)*

Yield: 42 %; mp 181 °C; ^{1}H NMR ($CDCl_3$) δ 7.76 (d, $J = 9.0$ Hz, 2H, CH, pyridazine), 7.23 (d, $J = 9.0$ Hz, 2H, CH, pyridazine), 2.70 (s, 6H, SCH_3, methyl); ^{13}C NMR ($CDCl_3$) δ 161.89, 154.46, 127.23, 127.07 (pyridazine), 13.35 (methyl).

*Diethylthiopyridazinyl diselenide (**5b**)*

Yield: 55 %; mp 159–162 °C; ^{1}H NMR ($CDCl_3$) δ 7.74 (d, $J = 9.0$ Hz, 2H, CH, pyridazine), 7.18 (d, $J = 9.0$ Hz, 2H, CH, pyridazine), 3.31 (q, $J = 7.2$ Hz, 4H, SCH_2, ethyl), 1.41 (t, $J = 7.2$ Hz, 6H, CH_3, ethyl); ^{13}C NMR ($CDCl_3$) δ 161.70, 154.39, 127.33, 127.32 (pyridazine), 24.72, 14.22 (ethyl).

*Dipropylthiopyridazinyl diselenide (**5c**)*

Yield: 51 %; mp 137–140 °C; ^{1}H NMR ($CDCl_3$) δ 7.73 (d, $J = 9.0$ Hz, 2H, CH, pyridazine), 7.18 (d, $J = 9.0$ Hz, 2H, CH, pyridazine), 3.28 (t, $J = 7.2$ Hz, 4H, SCH_2, propyl), 1.84–1.72 (m, 4H, CH_2, propyl), 1.04 (t, $J = 7.5$ Hz, 6H, CH_3, propyl); ^{13}C NMR ($CDCl_3$) δ 161.86, 154.36, 127.38, 127.27 (pyridazine), 32.23, 22.40, 13.35 (propyl).

*Dibutylthiopyridazinyl diselenide (**5d**)*

Yield: 38 %; mp 128–133 °C; ^{1}H NMR ($CDCl_3$) δ 7.74 (d, $J = 9.0$ Hz, 2H, CH, pyridazine), 7.19 (d, $J = 9.0$ Hz, 2H, CH, pyridazine), 3.32 (t, $J = 7.5$ Hz, 4H, SCH_2, butyl), 1.80–1.73 (m, 4H, CH_2, butyl), 1.55–1.42 (m, 4H, CH_2, butyl), 0.95 (t, $J = 7.2$ Hz, 6H, CH_3, butyl); ^{13}C NMR ($CDCl_3$) δ 161.91, 154.36, 127.36, 127.26 (pyridazine), 31.02, 30.04, 21.99, 13.53 (butyl).

*Dipentylthiopyridazinyl diselenide (**5e**)*

Yield: 60 %; mp 148–152 °C; ^{1}H NMR ($CDCl_3$) δ 7.72 (d, $J = 9.0$ Hz, 2H, CH, pyridazine), 7.17 (d, $J = 9.0$ Hz, 2H, CH, pyridazine), 3.29 (t, $J = 7.5$ Hz, 4H, SCH_2, pentyl), 1.80–1.70 (m, 4H, CH_2, pentyl), 1.47–1.28 (m, 8H, CH_2, pentyl), 0.89 (t, $J = 7.2$ Hz, 6H, CH_3, pentyl); ^{13}C NMR ($CDCl_3$) δ 161.91, 154.35, 127.35, 127.26 (pyridazine), 31.02, 30.32, 28.64, 22.18, 13.84 (pentyl).

General synthetic procedure for the 3-allylseleno-6-alkylthiopyridazines **6a**–**6g**

Method A

To a stirred mixture of powdered sodium hydroxide (89 mg, 2.23 mmol), water and absolute methanol (30 mL), 3-allylseleno-6-chloropyridazine 3 (0.5 g, 2.23 mmol) was added at rt. Sodium hydroxide and related thiols were useful reagents for generating the alkylthioxide in the case of long chain thiols such as ethanethiol, propanethiol, butanethiol, pentanethiol, hexanethiol and heptanethiol as shown in

Table 1. Related thiol (2.23 mmol) was dropwised to reaction mixture at 0 °C. The mixture was stirred for 1 ~ 17 h at 0 °C. Upon completion, the reaction was stopped and the excess thiol was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (50 mL). The organic layer was washed with water (50 mL × 2) and then dried over anhydrous Na_2SO_4. After solvent evaporation, the residue was purified by column chromatography (hexanes: EtOAc) on silica gel to afford **6a**–**6g**.

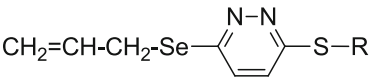
Method B

To a stirred mixture of dipyridazyinyl diselenide **5a**–**5e** (5.2 mmol), powdered sodium hydroxide (1.03 g, 26 mmol) and tetrabutylammonium bromide (0.34 g, 1.04 mmol) in absolute THF (30 mL), was added a solution of hydrazine monohydrate (0.054 mL, 1.73 mmol) at rt. After 1 h of stirring, the color of the reaction mixture turned to orange red from pale yellow. The reaction mixture was then cooled to 0 °C, allyl bromide (0.86 mL, 10.4 mmol) was added slowly dropwise and stirring continued for 1 h at 0 °C until the color of the reaction mixture turned to yellow from orange red. After the reaction was complete, the solvent THF was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (50 mL). The organic layer was washed with water (50 mL × 4) and then dried over anhydrous Na_2SO_4. After solvent evaporation, the residue was purified by column chromatography (hexanes: EtOAc) on silica gel to afford **6a**–**6e**.

*3-Allylseleno-6-methylthiopyridazine (**6a**)*

Yield: 19 % (method B); mp 31–32 °C; ^{1}H NMR ($CDCl_3$) δ 7.23 (d, J = 9.1 Hz, 1H, CH, pyridazine), 7.12 (d, J = 9.1 Hz, 1H, CH, pyridazine), 6.18–6.02 (m, 1H, CH, allyl), 5.31 (d, J = 16.3 Hz, 1H, =CH_2, allyl), 5.11 (d, J = 10.0 Hz, 1H, =CH_2, allyl), 4.02 (d, J = 7.5 Hz, 2H, $SeCH_2$), 2.72 (s, 3H, SCH_3, methyl); ^{13}C NMR ($CDCl_3$) δ 160.21, 154.60, 127.90, 125.24 (pyridazine), 134.00, 117.79, 28.70 (allyl), 13.24 (SCH_3).

Table 1 Totals yields of two synthetic routes for target compounds **6a**–**6e** and their antiproliferative activity against breast cancer cell lines (MCF-7) in CCK-8 assays



Compound	R	Total yields[a] (%)	Total yields[b] (%)	Mean IC_{50} (μM)
6a	Methyl	1.19	5.77	842
6b	Ethyl	9.13	7.11	766
6c	Propyl	3.26	7.85	1,512
6d	Butyl	7.83	6.38	1,122
6e	Pentyl	1.30	5.98	207
5FU				6,112

[a] Total yields referred to isolated product (method A)

[b] Total yields referred to isolated product (method B)

*3-Allylseleno-6-ethylthiopyridazine (**6b**)*

Yield: 28 % (method A), 19 % (method B); oil; ^{1}H NMR ($CDCl_3$) δ 7.19 (d, J = 9.0 Hz, 1H, CH, pyridazine), 7.04 (d, J = 9.0 Hz, 1H, CH, pyridazine), 6.12–6.03 (m, 1H, CH, allyl), 5.48 (d, J = 16.9 Hz, 1H, =CH_2, allyl), 5.06 (d, J = 10.0 Hz, 1H, =CH_2, allyl), 3.99 (d, J = 7.5 Hz, 2H, $SeCH_2$), 3.33 (q, J = 7.4 Hz, 2H, SCH_2, ethyl), 1.44 (t, J = 7.4 Hz, 3H, CH_3, ethyl); ^{13}C NMR ($CDCl_3$) δ 159.98, 154.57, 128.07, 125.65 (pyridazine), 134.02, 117.81, 28.37 (allyl), 24.51 (SCH_3), 14.36 (ethyl); FT-IR (NaCl) cm^{-1} 3081 (aromatic), 2926 (aromatic), 1562 (N=N), 1396 (CH_2), 1129 (CH_3), 988, 916 (allyl double band), 821 (CSe); GC–MS *m/z* (%) 260 (M+), 245.0 (100.0), 260.0 (76.0), 243.0 (49.7), 258.0 (37.6), 118.1 (36.7).

*3-Allylseleno-6-propylthiopyridazine (**6c**)*

Yield: 10 % (method A), 22 % (method B); mp 58 °C; ^{1}H NMR ($CDCl_3$) δ 7.26 (d, J = 9.0 Hz, 1H, CH, pyridazine), 7.05 (d, J = 9.0 Hz, 1H, CH, pyridazine), 6.11–6.02 (m, 1H, CH, allyl), 5.25 (d, J = 17.1 Hz, 1H, =CH_2, allyl), 5.05 (d, J = 10.1 Hz, 1H, =CH_2, allyl), 3.99 (d, J = 7.5 Hz, 2H, $SeCH_2$), 3.30 (t, J = 7.2 Hz, 2H, SCH_2, propyl), 1.86–1.73 (m, 2H, CH_2, propyl), 1.05 (t, J = 7.3 Hz, 3H, CH_3, propyl); ^{13}C NMR δ 160.56, 154.92, 128.41, 126.07 (pyridazine), 134.40, 118.20, 28.76 (allyl), 32.40, 22.78, 13.89 (propyl); FT-IR (NaCl) cm^{-1} 3034 (aromatic), 2925 (aromatic), 1566 (N=N), 1393 (CH_2), 1150 (CH_3), 1014, 896 (allyl double band), 849 (CSe); GC–MS *m/z* (%) 274 (M+), 259.0 (100.0), 274.1 (78.1), 257.0 (50.5), 272.1 (38.7), 118.1 (34.3).

*3-Allylseleno-6-butylthiopyridazine (**6d**)*

Yield: 24 % (method A), 24 % (method A); mp 34–36 °C; ^{1}H NMR ($CDCl_3$) δ 7.19 (d, J = 9 Hz, 1H, pyridazine), 7.05 (d, J = 9 Hz, 1H, pyridazine), 6.14–6.00 (m, 1H, CH, allyl), 5.25 (d, J = 16.4 Hz, 1H, =CH_2, allyl), 5.05 (d, J = 9.9 Hz, 1H, =CH_2, allyl), 3.98 (d, J = 7.4 Hz, 2H, $SeCH_2$), 3.32 (t, J = 7.2 Hz, 2H, SCH_2), 1.79–1.69 (m, 2H, CH_2, butyl), 1.55–1.45 (m, 2H, CH_2, butyl), 0.95 (t, J = 7.5 Hz, 3H, CH_3, butyl); ^{13}C NMR ($CDCl_3$) δ 160.64, 154.91, 128.39, 126.03 (pyridazine), 134.39, 118.18, 28.76 (allyl), 31.35, 30.25, 22.45, 14.06 (butyl); FT-IR (NaCl) cm^{-1} 3080 (aromatic), 2957 (aromatic), 1561 (N=N), 1384 (CH_2), 1130 (CH_3), 987, 916 (allyl double band), 823

(CSe); GC–MS *m/z* (%) 288 (M+), 272.9 (100.0), 288.0 (68.7), 271.0 (48.7), 118.0 (46.0), 119.0 (43.1).

*3-Allylseleno-6-pentylthiopyridazine (**6e**)*

Yield: 4 % (method A), 16 % (method B); mp 52–54 °C; ^{1}H NMR ($CDCl_3$) 7.18 (d, $J = 8.9$ Hz, 1H, pyridazine), 7.04 (d, $J = 8.9$ Hz, 1H, pyridazine), 6.11–6.02 (m, 1H, CH, allyl), 5.25 (d, $J = 17.5$ Hz, 1H, $=CH_2$, allyl), 5.05 (d, $J = 9.9$ Hz, 1H,$=CH_2$, allyl), 3.98 (d, $J = 7.0$ Hz, 2H, $SeCH_2$), 3.31 (t, $J = 7.3$ Hz, 2H, SCH_2), 1.79–1.74 (m, 2H, CH_2, pentyl), 1.46–1.31 (m, 4H, CH_2, pentyl), 0.91 (t, $J = 7.1$ Hz, 3H, CH_3, pentyl); ^{13}C NMR ($CDCl_3$) δ 160.28, 154.53, 128.03, 125.66 (pyridazine), 134.02, 117.81, 28.64 (allyl), 31.08, 30.16, 28.40, 22.28, 13.97 (pentyl); FT-IR (NaCl) cm^{-1} 3036 (aromatic), 2925 (aromatic), 1565 (N=N), 1034, 1018 (allyl double band), 845 (CSe). GC–MS *m/z* (%) 302 (M+), 146.0 (100.0), 148.0 (59.7), 147.0.0 (50.4), 160.0 (41.0), 173.0 (31.3).

*3-Allylseleno-6-hexylthiopyridazine (**6f**)*

Yield: 4 % (method A); mp 42–44 °C; ^{1}H NMR ($CDCl_3$) δ 7.18 (d, $J = 9.0$ Hz, 1H, pyridazine), 7.04 (d, $J = 9.0$ Hz, 1H, pyridazine), 6.14–6.00 (m, 1H, CH, allyl), 5.25 (d, $J = 17.1$ Hz, 1H, $=CH_2$, allyl), 5.05 (d, $J = 9.9$ Hz, 1H, $=CH_2$, allyl), 3.98 (d, $J = 7.2$ Hz, 2H, $SeCH_2$), 3.31 (t, $J = 7.3$ Hz, 2H, OCH_2), 1.80–1.70 (m, 2H, CH_2, hexyl), 1.50–1.40 (m, 2H, CH_2, hexyl), 1.35–1.25 (m, 4H, CH_2, hexyl), 0.89 (t, $J = 7.0$ Hz, 3H, CH_3, hexyl); ^{13}C NMR ($CDCl_3$) δ 160.27, 154.51, 128.01, 125.64 (pyridazine), 134.03, 117.81, 28.87 (allyl), 31.38, 30.18, 28.61, 28.39, 22.55, 14.03 (hexyl); FT-IR (NaCl) cm^{-1} 3046 (aromatic), 2920 (aromatic), 1549 (N=N), 988, 915 (allyl double band), 830 (CSe); GC–MS *m/z* (%) 316 (M+), 234.0 (100.0), 150.1 (66.4), 85.2 (54.7), 117.1 (40.0), 55.1 (23.0).

*3-Allylseleno-6-heptylthiopyridazine (**6g**)*

Yield: 3 % (method A); mp 37–38 °C; ^{1}H NMR ($CDCl_3$) δ 7.18 (d, $J = 9.0$ Hz, 1H, pyridazine), 7.04 (d, $J = 9.0$ Hz, 1H, pyridazine), 6.11–6.01 (m, 1H, CH, allyl), 5.25 (d, $J = 17.0$ Hz, 1H, $=CH_2$, allyl), 5.05 (d, $J = 9.9$ Hz, 1H, $=CH_2$, allyl), 3.98 (d, $J = 7.2$ Hz, 2H, $SeCH_2$), 3.31 (t, $J = 7.2$ Hz, 2H, SCH_2), 1.80–1.70 (m, 4H, CH_2, heptyl), 1.48–1.40 (m,2H, CH_2, heptyl), 1.37–1.27 (m, 4H, CH_2, heptyl), 0.90 (t, $J = 4.7$ Hz, 3H, CH_3, heptyl); ^{13}C NMR ($CDCl_3$) δ 160.69, 154.92, 128.41, 126.04 (pyridazine), 134.41, 118.19, 28.79 (allyl), 32.11, 30.56, 29.31, 29.29, 29.25, 22.99, 14.46 (heptyl); FT-IR (NaCl) cm^{-1} 3100 (aromatic), 2920 (aromatic), 1556 (N=N), 987, 915 (allyl double band), 830 (CSe); GC–MS *m/z* (%) 330 (M+), 315.0 (100.0), 313.0 (50.8), 330.0 (44.4), 119.0 (26.4), 118.0 (24.8).

Materials and methods for bioassays: cell line culture conditions

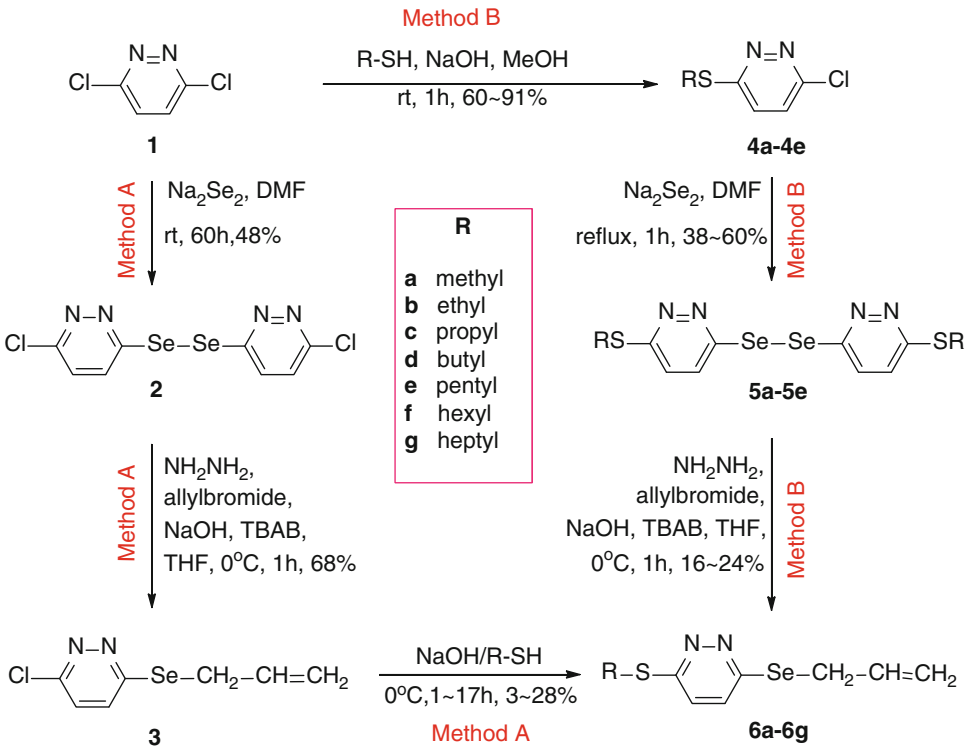
MCF-7 breast cancer cells were purchased from the ATCC (Manassas, USA) and maintained at 37 °C in a humidified atmosphere, with 5 % CO_2, in MEM (Gibco-BRL Inc.) medium supplemented with 10 % fetal bovine serum (Gibco-BRL Inc., Korea).

Antiproliferative CCK-8 (cell counting kit-8) assays (Jung et al. 2012)

The cytotoxic activity of the compounds was determined in vitro using the CCK-8 assay kit (Dojindo, Korea). The human breast cancer cells were seeded in 96-well plates at densities of 5000 cells/well with five replicates for each drug concentration and maintained at 37 °C in a 5 % CO_2 humidified incubator for 24 h. Control cells were treated with dimethyl sulfoxide (DMSO) equal to the highest percentage of solvent used in the experimental conditions. 5FU was used as a positive control. Then, the MCF-7 cells were treated with various concentrations of synthetic compounds (the final concentrations of **6a**–**6e** were 6.25, 25, 100, and 400 μg/mL) for 24 h. 10 μL of Cell Counting Kit-8 solution were added into each well (containing 100 μL), and the plates were further incubated for 2.5 h. The absorbance at 450 nm was measured by a micro ELISA reader (ASYS Biotech, Cambridge, BK). The cell viability ratio was calculated as follows: (test group A_{450}/control group A_{450}) × 100 %. IC_{50} values were determined from three independent experiments.

Results and discussion

Activated aryl halides react well with thiols to give the corresponding thioethers. The SN reaction of an aryl halide with a thiol is not only important for the synthesis of thioethers, but is also essential for the preparation of pharmaceuticals. Many reports have been published on the nucleophilic thiolation of aryl halides. Even though a synthetic pathway for 3-allylthiopyridazines has been developed (Kwon 2002a; Kwon and Lee 2005; Kwon and Moon 2005), the synthesis of 3-allylselenopyridazines has not been reported until now. We applied a general method of preparing allylthiopyridazines from pyridazinyl halides and thiols (Kwon 2002b; Lee et al. 2003) such as synthetic method A. Also, a method of splitting the selenium–selenium bond, allylation of dipyridazinyl diselenide and allyl bromide such as synthetic method B was applied (Bhasin



Scheme 1 Synthetic routes for target 3-allylseleno-6-alkylthiopyridazines **6a–6g** through method A and method B

and Singh 2002; Bhasin et al. 2004; Scianowski et al., Scianowski 2006; Lee and Park 2011; Lee et al. 2011).

A series of 3-allylseleno-6-alkylthiopyridazines **6a–6g** was prepared by diselenylation, allylation and alkylthiolation as shown in method A (Scheme 1). The allylseleno group, a pharmacologically active group, was introduced on one side of the pyridazine ring. Also, an alkylthio moiety such as methylthio, ethylthio, propylthio, butylthio, pentylthio, hexylthio and heptylthio was introduced into the *para*-position of pyridazines **3**. The key intermediates in these preparations were dichloropyridazinyl diselenide **2**, which could be obtained from the corresponding 3,6-dichloropyridazine **1** by reaction with sodium selenide. Dichloropyridazinyl diselenide **2** was converted to allylselenopyridazinyl chloride **3** by allylation using hydrazine hydrate and allyl bromide. Condensation of the allylselenopyridazinyl chloride **3** with various thiols gave the final target products **6a–6g** (Table 1). On the other hand, **6a–6e** were also prepared by alkylthiolation, diselenylation and allylation as shown in method B. The allylthio group was introduced on one side of the 3,6-dichloropyridazine ring. An alkylthio moiety such as methylthio, ethylthio, propylthio, butylthio and pentylthio was introduced into the C6-position of pyridazines for alkylthiopyridazinyl chloride **4a–4e**. Condensation of the 3,6-dichloropyridazine **1** with various thiols gave **4a–4e**. The key intermediates in these synthetic routes were dipyridazinyl diselenides **5a–5e**, which could be readily obtained from the corresponding **4a–4e** by diselenylation with sodium selenide. Diselenides **5a–5e** was converted to final allylselenopyridazines **6a–6e** by allylation using hydrazine hydrate and allyl bromide.

Here, we present the synthesis of new 3-allylseleno-6-alkylthiopyridazine **6a–6g** by two synthetic routes. In Table 1, we summarize the total yield of two method and the antiproliferative activity for compounds **6a–6e**. 3-Allylseleno-6-chloropyridazine **3** was converted to final **6a–6g** by nucleophilic aromatic substitution with thiols in the presence of sodium hydroxide (Scheme 1). The nucleophilic displacement of chlorine in 3-allylseleno-6-chloropyridazine **3** requires prolonged the reaction time at 0 °C. A typical reaction consisted of a mixture of thiols (2.23 mmol), 6-allylthio-3-chloropyridazine (2.23 mmol), and sodium hydroxide (2.23 mmol) in absolute methanol stirred at 0 °C for 1–17 h. The reaction was usually carried out using 1: 1 equivalents of 3-allylseleno-6-chloropyridazine **3**: thiols. **3** were converted into the corresponding pyridazines **6a–6g** in low yields (3–28 %).

Thus we designed synthetic method B to improve the low yield. Method B differed from method A in the order of reactions. The mono-alkylthiolation from 3,6-dichloropyridazines **1** to **4a–4e** produced high yields (60–91 %). We previously reported the synthesis of 3-allylthio-6-chloropyridazine in 95 % yield through allylthiolation (Park and Park 2007). 3-Alkylthio-6-chloropyridazine **4a–4e** and sodium selenide were reacted in DMF to form the corresponding diselenides **5a–5e** in 38–60 % yield (Scheme 1). Diselenides **5a–5e** were converted into the

corresponding compounds **6a–6e** in lower yields (16–24 %). But, the total average yield of method B was better than that of method A.

The pyridazine NMR peak of **5a–5e** appeared at 7.17–7.23 and 7.72–7.76 ppm. The alkyl (methyl, ethyl, propyl, butyl and pentyl) NMR peak of **5a–5e** appeared at 0.89–3.33 ppm as splitting signal. The pyridazine ^{13}C NMR peak appeared at 127.07–127.32, 127.23–127.38, 154.35–154.46 and 161.70–161.91 ppm, and the alkyl peak appeared at 13.75–32.23 ppm.

The pyridazine NMR peak of **6a–6g** appeared at 7.04–7.12 and 7.18–7.23 ppm, and the allyl peak appeared at 3.98–4.02, 5.05–5.11, 5.25–5.48, and 6.00–6.18 ppm. The alkyl (methyl, ethyl, propyl, butyl, pentyl, hexyl and heptyl) NMR peak of **6a–6g** appeared at 0.89–3.33 ppm as splitting signal. The pyridazine ^{13}C NMR peak appeared at 125.24–126.07, 127.9–128.41, 154.51–154.92 and 159.98–160.69 ppm, and the allyl peak appeared at 28.37–28.87, 117.79–118.20, and 134.00–134.41 ppm.

In order to investigate the potential anti-cancer activity of the seven synthetic compounds, the growth-inhibitory effect of the synthetic compounds was examined against breast cancer (MCF-7) cells. CCK-8 assays were conducted on cells treated with various concentrations of the compounds. 5-Fluorouracil (5FU), which produces partial responses in 10–20 % of patients with metastatic colon carcinomas, upper gastrointestinal tract carcinomas, and breast carcinomas (Brunton et al. 2006), was used as a positive control. The IC_{50} values for these compounds were determined from the concentration range used in this study.

Of the seven target compounds, five compounds (**6a–6e**) inhibited the growth of breast (MCF-7) cells at standard concentrations (6.25, 25, 100, 400 μg/mL) (Fig. 2). 5FU, a positive control, showed inhibitory effects on the growth of MCF-7 cells at standard concentrations (6.25, 25, 100, 400 μg/mL). We further investigated the antiproliferative activity of compound **6e**, which caused greater inhibition of cell growth than the other compounds. As shown in Fig. 2,

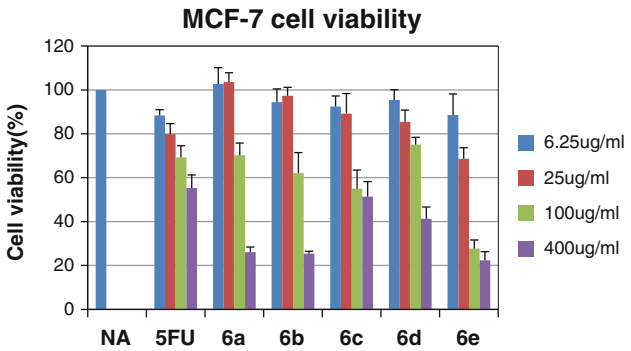


Fig. 2 Antiproliferative activity of synthesized compounds (**6a–6e**) in MCF-7 breast cancer cells

compound **6e** markedly inhibited MCF-7 cell growth at IC_{50} (207 μM) in a dose-dependent manner at a low concentration (6.25 μg/mL). Five compounds (**6a–6e**) showed higher potencies than 5FU at IC_{50} (6,112 μM) in inhibiting the growth of MCF-7 cells, suggesting the potential anticancer activity of these compounds (Table 1.). The results revealed that compound **6e** had high activity towards MCF-7 cells.

Finally, we synthesized new dipyridazinyl diselenides **5a–5e** and 3-allylseleno-6-alkylthiopyridazines **6a–6g** in order to discover potential anticancer candidates. Compound **6e** (3-allylseleno-6-pentylthiopyridazine) showed higher potency than 5-FU for inhibiting the growth of these MCF-7 cell lines.

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