Synthesis and Evaluation of Novel 3-Allylseleno-6-Alkylsulfonylpyridazine Derivatives with Potential Anticancer Properties

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A new series of 3-allylseleno-6-alkylsulfonylpyridazines and 3-allylseleno-6-alkylsulfinylpyridazines were synthesized from 3,6-dichloropyridazine for anticancer agent development. The inhibitory effects of 3-allylseleno-6-alkylsulfonylpyridazines on human cancer cell lines were investigated. The synthesis involved thiolation, oxidation, selenylation, and Se-allylation of alkylthiols. That is, sodium methanethiolate, ethanethiol, propanethiol, butanethiol, pentanethiol, and hexanethiol were inserted into the 6-position of the pyridazine nucleus. These new synthetic compounds exhibited antiproliferative activity against human breast cancer (MCF-7), hepatocarcinoma (Hep3B), and human colon carcinoma (RKO) cells in CCK-8 assays, and are potential candidates for cancer chemotherapy.

Keywords: Organoselenium Compounds, 3-Allylseleno-6-alkylsulfonylpyridazines, Antiproliferative, Apoptosis

Introduction

The pyridazine nucleus is a feature of many drugs that act on a variety of biological targets.[1] Several modifications of the alkylselenopyridazine pharmacophore have been reported. Allylselenoalkoxypyridazines,[2] allylselenoalkylthiopyridazines,[3] alkylselenoalkylamino-pyridazines,[4] and bis(alkylselanyl)pyridazines[5] have been shown to have good antiproliferative effects on breast cancer MCF-7 cells.

Lee *et al.*[6] developed synthetic routes for alkylselenopyridazine derivatives but the synthesis of allylselenoalkylsulfonylpyridazines has not been reported. The aim of the present study was to develop new 3-allylseleno-6-alkylsulfonylpyridazine derivatives with stronger anticancer effects.

The pyridazine nucleus was combined with a seleno group. The replacement of the oxygen (or sulfur or nitrogen or selenium) of 6-substituted 3-allyl (or alkyl)selenopyridazine derivatives by a sulfonyl (or sulfinyl) moiety yielded 3-allylseleno-6-alkylsulfonyl (or sulfinyl) pyridazines (Figure 1). The allylseleno group, a pharmacologically active group, was substituted on the 3-position of the pyridazine ring. An alkylsulfonyl (or sulfinyl) moiety, such as methylsulfonyl, ethylsulfonyl, *n*-propylsulfonyl (or sulfinyl), *n*-butylsulfonyl, *n*-pentylsulfonyl, and *n*-hexylsulfonyl, was inserted into the 6-position of the pyridazine ring.

Through previous studies, the allylthiopyridazines were converted to allylselenopyridazines. As a continuation of earlier studies, 3-allylselenopyridazine derivatives with electron donating groups were transformed into 3-allylselenopyridazine derivatives with electron withdrawing groups. This paper describes the design and synthesis of target 3-allylseleno-6-alkylsulfonyl (or alkylsulfinyl) pyridazines and their anticancer activities.

Experimental

Chemicals. The melting points (uncorrected) were determined on a Büchi 545 melting point apparatus in open capillary tubes. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) data were analyzed using a Bruker 300 MHz NMR spectrometer, which were recorded in $CDCl_3$ or DMSO-d_6 using tetramethylsilane (TMS) as the reference; the values are reported as chemical shifts in parts per million (ppm). The FT-IR spectra were recorded on a Perkin-Elmer 16 F PC FT-IR spectrometer from KBr discs. High and low resolution mass spectroscopy (HRMS and LRMS) was performed on a JMS-700 Mass spectrometer.

Materials and Methods for Cell Culture. MCF-7, Hep3B, and RKO cells from the ATCC (Manassas, VA, USA) were used and retained at 37°C in a humidified atmosphere containing 5% CO_2 in Minimum Essential Medium (MEM; Gibco-BRL Inc., Grand Island, USA), Dulbecco's Modified Eagle's Medium (DMEM; Gene depot, USA) medium containing 10% fetal bovine serum (FBS; Gibco-BRL Inc., Grand Island, USA), and 0.01% penicillin–streptomycin.

Antiproliferative CCK-8 Assay[7]. The antiproliferative activities were evaluated using a CCK-8 assay kit (Dojindo, Kumamoto, Japan). MCF-7, Hep3B, and RKO cells were seeded in 96-well plates at 5×10^3 (3×10^3 in the case of RKO) cells/well. The assay was conducted in triplicate for

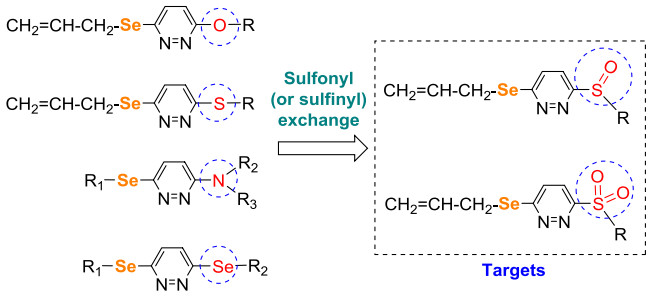


Figure 1. Reported allyselenoalkoxy (or alkylthio, alkylamino, alkylseleno)pyridazines and structural modification for target 3-allylseleno-6-alkylsulfonyl(or sulfinyl)pyridazines.

each compound/concentration combination. The plates were cultured in a humidified 5% CO_2 incubator for 24 h at 37°C. The control cells were added to dimethyl sulfoxide (DMSO) at the highest concentration administered to the experimental cells. The cells were treated with different concentrations (0.1, 1, 10, 50, or 100 μM, respectively) of **8a–8f** for 72 h. The CCK-8 reagent solution was added to each well in 10 μL portions, and the plates were incubated for 3 h.

The absorbance at 450 nm was measured using a micro ELISA reader (ASYS Biotech, Cambridge, UK). The cell viabilities were calculated using the following equation: viability = test group A_{450}/ control group $A_{450} \times 100\%$. The half-maximal inhibitory concentrations (IC_{50}) of the six synthesized compounds were determined by averaging the values of three independent experiments. Table 1 lists the results.

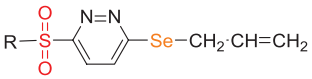
Cell Apoptosis Assay[8]. Apoptosis was observed using the FITC Annexin-V Apoptosis Detection Kit (BD Pharmingen, San Diego, CA, USA), as described previously. Briefly, MCF-7 or Hep3B cells were placed in 6-well plates at a density of 1×10^6 cells/mL and cultivated for 24 h. The cells were treated with **8c** (0, 50 μM) for 24 h, cleaned with PBS (phosphate buffered saline) (at 0°C), re-suspended in 500 μL of Annexin-V-FLUOS buffer, and treated with an Annexin V and PI solution. They were then left to stand for 20 min in the dark at room temperature, and measured by flow cytometry (Novocyte Flow Cytometer, ACEA Biosciences, Inc., San Diego, CA, USA).

Nuclear-DAPI Staining[9]. The MCF-7, Hep3B, and RKO cells were covered at a density of 5×10^4 cells/well per 24-well plate and placed on an incubator at 37°C for proliferation. The cells were cleaned with PBS after 24 h of incubation and treated with different concentrations (0, 50, and 100 μM, respectively) of **8c**, **8d** for 24 h. Subsequently, it was washed with PBS and fixed with a 2% p-formaldehyde solution for 20 min and exposed to DAPI (4′,6-diamidino-2-phenylindole dichloride) for 10 min at a final concentration of 4 μg/mL. The cells were then rinsed with PBS after 10 min, and examined by fluorescence microscopy (confocal microscope, Zeiss LSM700, Carl Zeiss, Germany) to observe the apoptosis behavior.

General Synthetic Procedure for 3-Allylseleno-6-Alkylsulfinylpyridazine 7c, and 3-Allylseleno-6-Alkylsulfonylpyridazines 8a–8f. A mixture of dialkylsulfonylpyridazyinyl diselenide **6a** (1.4 mmol),[10] powdered sodium hydroxide (7 mmol), tetrabutylammonium bromide (TBAB) (0.3 mmol), and hydrazine hydrate (0.3 mmol) in distilled Tetrahydrofuran (THF) (15 mL) was stirred at room temperature (RT). After cooling to 0°C, allyl bromide (2.8 mmol) with the same equivalent THF was added dropwise to the solution under reaction. The final mixture was stirred for at 0°C for 2 h. The resulting mixture was concentrated after the reaction. The residue was dissolved in ethyl acetate (50 mL) and quenched with water (15 mL × 3). The organic ethyl acetate layer was collected and the trace water was removed by drying with sodium sulfate (Na_2SO_4) and filtered. The product was refined by column chromatography (hexanes/EtOAc 3:1, 2:1) on silica gel to obtain **8a**.

3-Allylseleno-6-*n*-propylsulfinylpyridazine (7c): Yield: 11%, Oil. ^{1}H NMR ($CDCl_3$) δ 7.85 (d, J = 8.8 Hz, 1H, pyridazine), 7.67 (d, J = 8.8 Hz, 1H, pyridazine), 6.13–5.99 (m, 1H, CH, allyl), 5.33 (d, J = 16.8 Hz, 1H, =CH_2, allyl), 5.12 (d, J = 9.9 Hz, 1H, =CH_2, allyl), 4.08 (d, J = 7.3 Hz, 2H, $SeCH_2$), 3.54 (t, J = 7.9 Hz, 2H, $SOCH_2$, propyl), 1.90–1.78 (m, 2H, CH_2, propyl), 1.06 (t,

Table 1. The inhibitory results of **8a–8f** on the cancer cell lines for 72 h.



Comp. No.	R	IC_{50} μM/MCF-7	IC_{50} μM/Hep3B	IC_{50} μM/RKO
8a	Methyl	44.5	34.1	108.5
8b	Ethyl	44.0	32.9	80.3
8c	*n*-Propyl	33.8	30.7	55.6
8d	*n*-Butyl	35.4	30.9	62.9
8e	*n*-Pentyl	37.8	36.0	58.3
8f	*n*-Hexyl	39.9	45.3	>200
5-FU	—	56.9	44.9	36.6

J = 7.4 Hz, 3H, CH_3, propyl). ^{13}C NMR ($CDCl_3$) δ 164.34, 159.48, 132.93, 118.87 (pyridazine), 129.80, 123.05, 28.87 (allyl), 54.06, 16.00, 12.95 (propyl).

3-Allylseleno-6-methylsulfonylpyridazine (8a): Yield: 32%, m.p.: 86–88°C. ^1H NMR ($CDCl_3$) δ 7.86 (d, J = 8.8 Hz, 1H, pyridazine), 7.70 (d, J = 8.8 Hz, 1H, pyridazine), 6.16-6.02 (m, 1H, CH, allyl), 5.36 (d, J = 16.9 Hz, 1H, $=CH_2$, allyl), 5.15 (d, J = 9.9 Hz, 1H, $=CH_2$, allyl), 4.10 (d, J = 7.3 Hz, 2H, $SeCH_2$), 3.42 (s, 3H, SO_2CH_3, methyl). ^{13}C NMR ($CDCl_3$) δ 164.60, 160.16, 132.93, 118.97 (pyridazine), 129.99, 122.23, 28.92 (allyl), 40.38 (methyl). FT-IR (NaCl) cm^{-1} 3060, 3026 (aromatic), 2924, 2849 (CH_2) 1601, 1372 (C–N), 1154 (SO_2), 1028 (SO), 700 (C–Se). HRMS (FAB, M + H) Calcd for 278.9706, found 278.9704.

3-Allylseleno-6-ethylsulfonylpyridazine (8b): Yield: 16%, m.p.: 90–94°C. ^1H NMR ($CDCl_3$) δ 7.85 (d, J = 8.8 Hz, 1H, pyridazine), 7.67 (d, J = 8.8 Hz, 1H, pyridazine), 6.13–5.99 (m, 1H, CH, allyl), 5.33 (d, J = 16.9 Hz, 1H, $=CH_2$, allyl), 5.12 (d, J = 9.9 Hz, 1H, $=CH_2$, allyl), 4.08 (d, J = 7.3 Hz, 2H, $SeCH_2$), 3.59 (q, J = 7.4 Hz, 2H, SO_2CH_2, ethyl), 1.37 (t, J = 7.4 Hz, 3H, CH_3, ethyl). ^{13}C NMR ($CDCl_3$) δ 164.43, 159.04, 132.95, 118.91 (pyridazine), 129.83, 123.24, 28.90 (allyl), 47.00, 6.89 (ethyl). HRMS (FAB, M + H) Calcd for 292.9863, found 292.9850.

3-Allylseleno-6-*n*-propylsulfonylpyridazine (8c): Yield: 12%, Oil. ^1H NMR ($CDCl_3$) δ 7.86 (d, J = 8.8 Hz, 1H, pyridazine), 7.69 (d, J = 8.8 Hz, 1H, pyridazine), 6.14–6.00 (m, 1H, CH, allyl), 5.34 (d, J = 16.8 Hz, 1H, $=CH_2$, allyl), 5.12 (d, J = 9.9 Hz, 1H, $=CH_2$, allyl), 4.08 (d, J = 7.2 Hz, 2H, $SeCH_2$), 3.54 (t, J = 7.9 Hz, 2H, SO_2CH_2, propyl), 1.88–1.76 (m, 2H, CH_2, propyl), 1.07 (t, J = 7.4 Hz, 3H, CH_3, propyl). ^{13}C NMR ($CDCl_3$) δ 164.34, 159.47, 132.94, 118.91 (pyridazine), 129.83, 123.24, 28.83 (allyl), 54.10, 15.98, 12.92 (propyl). FT-IR (NaCl) cm^{-1} 3060, 3026 (aromatic), 2923, 2849 (CH_2) 1601, 1373 (C–N), 1154 (SO_2), 1028 (SO), 697 (C–Se). HRMS (FAB, M + H) Calcd for 307.0019, found 307.0023.

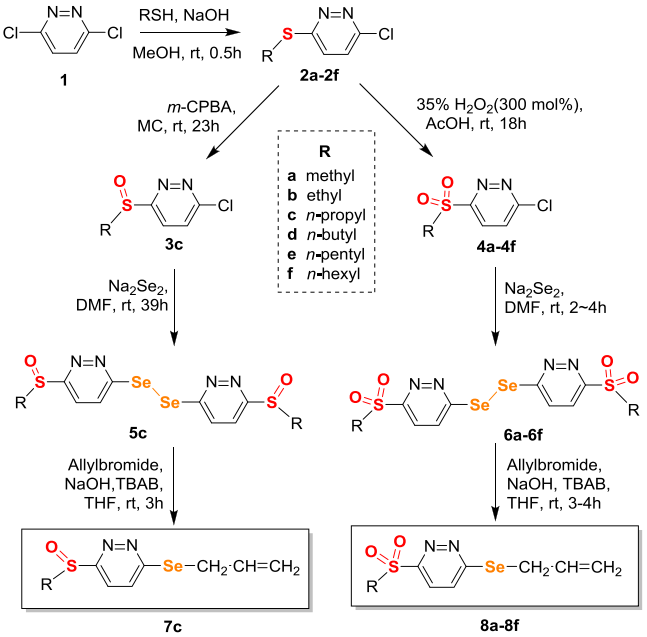
3-Allylseleno-6-*n*-butylsulfonylpyridazine (8d): Yield: 18%, Oil. ^1H NMR ($CDCl_3$) δ 7.86 (d, J = 8.8 Hz, 1H, pyridazine), 7.67 (d, J = 8.8 Hz, 1H, pyridazine), 6.13–5.99 (m, 1H, CH, allyl), 5.33 (d, J = 16.8 Hz, 1H, $=CH_2$, allyl), 5.12 (d, J = 9.9 Hz, 1H, $=CH_2$, allyl), 4.08 (d, J = 7.3 Hz, 2H, $SeCH_2$), 3.55 (t, J = 8.0 Hz, 2H, SO_2CH_2, butyl), 1.83–1.73 (m, 2H, CH_2, butyl), 1.52–1.40 (m, 2H, CH_2, butyl), 0.93 (t, J = 7.4 Hz, 3H, CH_3, butyl). ^{13}C NMR ($CDCl_3$) δ 164.36, 159.43, 132.94, 118.85 (pyridazine), 129.84, 123.12, 28.85 (allyl), 52.22, 23.99, 21.56, 13.43 (butyl). HRMS (FAB, M + H) Calcd for 321.0176, found 321.0178.

3-Allylseleno-6-*n*-pentylsulfonylpyridazine (8e): Yield: 36%, m.p.: 52–55°C. ^1H NMR ($CDCl_3$) δ 7.86 (d, J = 8.8 Hz, 1H, pyridazine), 7.69 (d, J = 8.8 Hz, 1H, pyridazine), 6.16–5.99 (m, 1H, CH, allyl), 5.36 (d, J = 16.8 Hz, 1H, $=CH_2$, allyl), 5.13 (d, J = 9.9 Hz, 1H, $=CH_2$, allyl), 4.10 (d, J = 7.2 Hz, 2H, $SeCH_2$), 3.57 (t, J = 7.9 Hz, 2H, SO_2CH_2, pentyl), 1.85–1.77 (m, 2H, CH_2, pentyl), 1.46–1.26 (m, 4H, CH_2x2, pentyl), 0.90 (t, J = 7.1 Hz, 3H, CH_3, pentyl). ^{13}C NMR ($CDCl_3$) δ 164.29, 159.45, 132.96, 118.94 (pyridazine), 129.87, 123.12, 28.92 (allyl), 52.41, 30.41, 22.08, 21.75, 13.69 (pentyl). FT-IR (NaCl) cm^{-1} 3060, 3026 (aromatic), 2924, 2850 (CH_2) 1601, 1372 (C–N), 1154 (SO_2), 1028 (SO), 699 (C–Se). HRMS (FAB, M + H) Calcd for 335.0332, found 335.0323.

3-Allylseleno-6-*n*-hexylsulfonylpyridazine (8f): Yield: 38%, m.p.: 83–85°C. ^1H NMR ($CDCl_3$) δ 7.84 (d, J = 8.8 Hz, 1H, pyridazine), 7.66 (d, J = 8.8 Hz, 1H, pyridazine), 6.13–6.00 (m, 1H, CH, allyl), 5.34 (d, J = 16.9 Hz, 1H, $=CH_2$, allyl), 5.12 (d, J = 9.9 Hz, 1H, $=CH_2$, allyl), 4.08 (d, J = 6.0 Hz, 2H, $SeCH_2$), 3.55 (t, J = 7.9 Hz, 2H, SO_2CH_2, hexyl), 1.74–1.85 (m, 2H, CH_2, hexyl), 1.38–1.48 (m, 2H, CH_2, hexyl), 1.25–1.30 (m, 4H, CH_2x2, hexyl), 0.87 (t, J = 7.0 Hz, 3H, CH_3, hexyl). ^{13}C NMR ($CDCl_3$) δ 164.29, 159.55, 132.94, 118.83 (pyridazine), 129.77, 123.03, 28.86 (allyl), 52.43, 31.04, 27.93, 22.19, 21.97, 13.78 (hexyl). FT-IR (NaCl) cm^{-1} 3060, 3026 (aromatic), 2924, 2850 (CH_2) 1601, 1372 (C–N), 1154 (SO_2), 1028 (SO), 701 (C–Se). HRMS (FAB, M + H) Calcd for 349.0489, found 349.0485.

Results and Discussion

As shown in Scheme 1, condensation of dichloropyridazine **1** with various thioles, such as ethanethiol, propanethiol, butanethiol, pentanethiol, and hexanethiol, at room



Scheme 1. Synthesis of target 3-allylselenyl-6-alkylsulfonyl (or sulfinyl) pyridazine derivatives (**7c**, and **8a–8f**)

temperature in methanol gave alkylthiopyridazines **2a–2f**.[11,12] **3c** and **4a–4f** were also prepared by oxidation with 35% hydrogen peroxide or *m*-CPBA.[13] The intermediates in these synthetic routes were diselenide **5c** and **6a–6f**, which could be obtained from the corresponding alkylsulfonylchloropyridazines **3c** and **4a–4f** via a diselenylation reaction with disodium diselenide.[14,15] The characterization data of intermediates **5c**, and **6a–6f** are reported elsewhere.[16] Diselenides **5c** and **6a–6f** were converted to allylselenopyridazines **7c** and **8a–8f** by Se-allylation using allyl bromide.

The target compounds were synthesized by alkylthiolation, oxidation, diselenylation, and Se-allylation of 3,6-dichloropyridazine, and the inhibition of cellular proliferation of synthetic compounds against three human cancer cell lines, *i.e.*, MCF-7 (breast cancer), Hep3B (hepatocarcinoma), and RKO (colon carcinoma) cells, was examined using a CCK-8 assay. DAPI nuclear staining revealed a morphological change in the compounds, and a flow cytometry assay confirmed the induced apoptosis.

CCK-8 assays were performed with various concentrations of synthetic compounds. 5-Fluorouracil (5-FU), which is used as a therapeutic agent of metastatic colorectal cancer, gastrointestinal tract cancer, and breast cancer, was applied as a positive control.[17] The IC_{50} values were determined within the concentration range tested in the paper.

Among the target compounds, three compounds (**8c**, **8d**, and **8e**) with IC_{50}s below 40 μM prevented the development of MCF-7 cells at the concentrations tested (0, 0.1, 1, 10, 50, and 100 μM) (Figure 2).

The half-maximal inhibitory concentration (IC_{50}) of all the synthesized compounds **8a–8f** was determined; 5-FU was used as the standard anticancer agent. The antiproliferative activities of the allylselenopyridazine derivatives, which were measured relative to that of 5-FU, were 33.8 ~ 44.5 μM. Furthermore, the synthetic compounds displayed more potent activities than 5-FU. The *n*-propylsulfonyl substituted pyridazine derivative **8c** (IC_{50} = 33.8 μM) was more potent than the methyl, ethyl, *n*-butyl, *n*-pentyl, and *n*-hexylsulfonyl substituted pyridazine compounds (**8a**, **8b**, **8d**, **8e**, and **8f**, respectively) (Figure 2). Compound **8c** showed the highest relative inhibitory activity against MCF-7 cells.

Among the six compounds assessed, five compounds (**8a–8e**) showed better potency than 5-FU (IC_{50} = 44.9 μM) against Hep3B cells. The ethyl, *n*-propyl, and *n*-butylsulfonyl substituted pyridazine derivatives (IC_{50} of **8b** = 32.9 μM, IC_{50} of **8c** = 30.7 μM, IC_{50} of **8d** = 30.9 μM) were more potent than the methyl, *n*-pentyl, and *n*-hexylsulfonyl substituted pyridazine compounds (**8a**, **8e**, and **8f**, respectively) (Figure 3). Therefore, these compounds are potential anticancer drugs (Table 1).

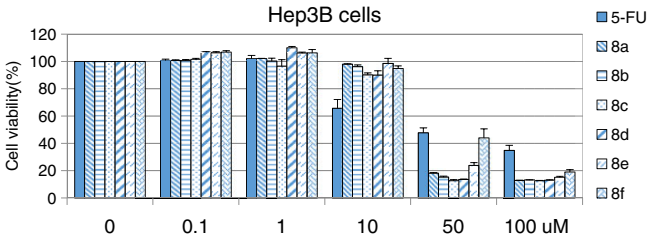


Figure 3. The antiproliferative activity of **8a–8f** on Hep3B.

In general, the inhibitory activities of all the compounds were less than that of 5-FU (IC_{50} = 36.6 μM) against colon cancer (RKO) cells. On the other hand, there was decrease in antiproliferative activity of five compounds (**8a–8e**) (IC_{50} = 58.3 ~ 108.5 μM) (Figure 4). Moreover, the antiproliferative activity of the *n*-hexylsulfonyl substituted pyridazine derivatives **8f** was very low.

As shown in Table 1, most compounds had moderate antiproliferative effects; they displayed IC_{50} values in the micromolar concentration range (IC_{50} < 45 μM) against both MCF-7 and Hep3B cell lines. Generally, most *n*-propyl and *n*-butylsulfonyl substituted pyridazine derivatives (**8c** and **8d**) possess better anti-breast cancer and anti-hepatocarcinoma activities than the corresponding methyl, ethyl, *n*-pentyl, and *n*-hexyl substituted ones. As a result, the length of the alkyl group affects the activity.

Table 2 lists the IC_{50} values of four synthetic allylselenopyridazine derivatives of **8e**, **A**,[2] **B**,[3] and **C**.[4] The synthetic compounds of 3-allylseleno-6-pentyloxypyridazine (**A**), 3-allylseleno-6-pentylthiopyridazine (**B**), and 3-allylseleno-6-pentylaminopyridazine (**C**) were recently reported.[2–4] *In vitro* assays revealed the IC_{50} of these compounds (**8e** = 292.61 μM, **A** = 218.55 μM, **B** = 207.00 μM, and **C** = 861.01 μM). The CCK-8 assays showed that among the four compound types (*n*-pentyloxy, *n*-pentylthio, *n*-pentylamino, and *n*-pentylsulfonyl moieties), the *n*-pentylthio compound (3-allylseleno-6-*n*-pentylthiopyridazine **B**) had potent antiproliferative activity against MCF-7 cells. The IC_{50} of compound **8c** against MCF-7 cells was 292.61 μM

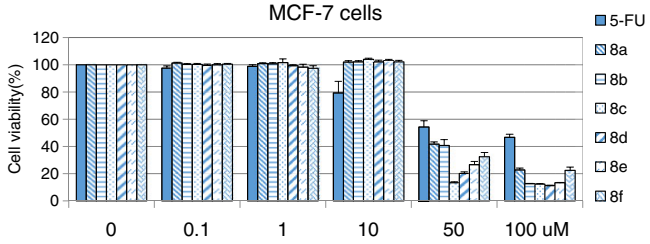


Figure 2. The antiproliferative activity of **8a–8f** on MCF-7.

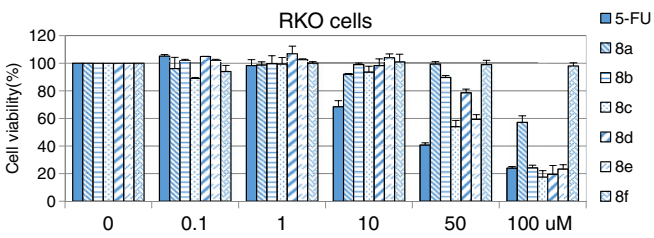
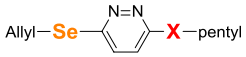


Figure 4. The antiproliferative activity of **8a–8f** on RKO.

Table 2. Comparison of the IC_{50} values of four 3-allylselenopyridazines (replacement, O, S, N, SO_2) with MCF-7 for 24 h.



Comp. No.	X	IC_{50} μM/MCF-7
A[2]	O	218.55
B[3]	S	207.00
C[4]	N	861.01
8e	SO_2	292.61

for 24 h, whereas it was 37.8 μM for 72 h (Table 1), despite the same compound being used against the same cell line. The difference in IC_{50} values in both experiments indicates that the effect of the drug is time-dependent. A comparison of the antiproliferative activity of four compounds with different substituents on MCF-7 cells showed that the electronic effect of the substituents on the ring was not proportional to the inhibition of cell proliferation.

DAPI staining is a method of nuclear staining, which can verify apoptosis by combining DAPI with DNA. The apoptosis signal increases the cell membrane permeability, DAPI binding ability, and the intensity of light, and alters the cell shape. When MCF-7, Hep3B, and RKO cells were treated with **8c** or **8d** (0, 50, 100 μM) for 24 h, the decrease in the number of cells was concentration-dependent, which was in contrast to the control, and morphological changes were observed (Figure 5). The fluorescence of the cells treated with synthetic compound **8c** (or **8d**) was stronger than that of the normal cells. The cytoplasm of the cells treated with the synthetic compound **8c** (or **8d**) was destroyed, and the round nucleus collapsed. The light intensity increased after exposure to compound **8c** (or **8d**), highlighting its effects on the apoptosis of cancer cells.

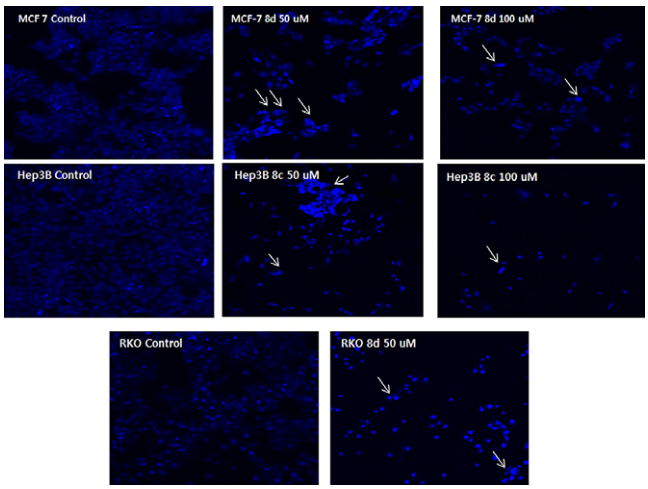


Figure 5. Morphological changes after exposing the Hep3B cells to **8c** and MCF-7, RKO cells to **8d**.

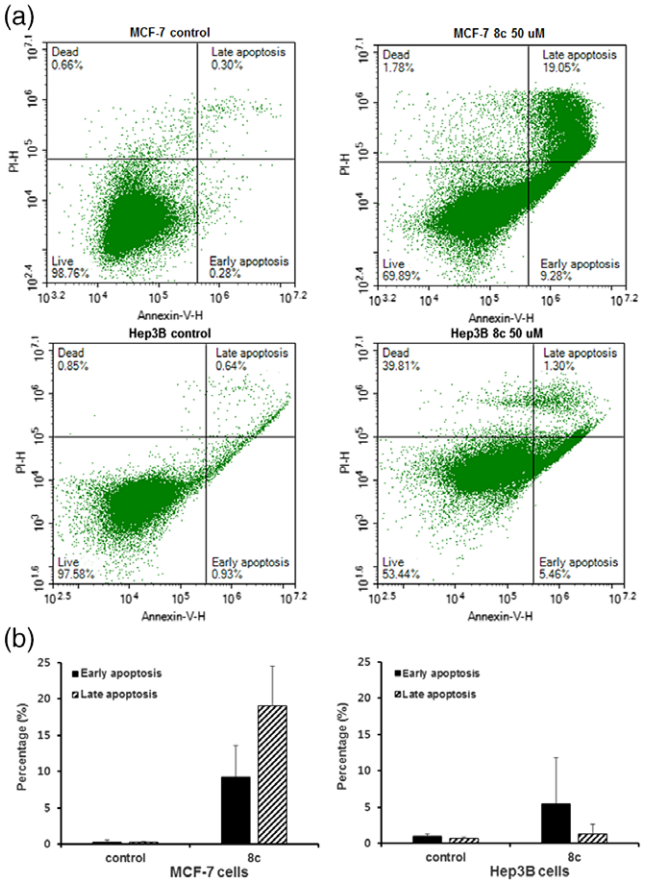


Figure 6. Effects of **8c** on MCF-7 and Hep3B cell apoptosis. The cells were treated with **8c** (0, 50 μM) for 24 h. Apoptosis was verified by Annexin V-FITC/PI double staining assay. The results are presented in (a). In (b), the percentage of apoptosis is indicated as a bar graph. The data is expressed as the mean ± SD (n = 3).

The mechanism of **8c**, the compound with the best antiproliferative activity among the synthetic compounds, was investigated. The aim was to determine if the cell proliferation inhibitory power was derived from apoptosis or necrosis. Figure 6 shows that compound **8c** induced apoptosis in MCF-7 and Hep3B cells. The effects of compound **8c** on cell apoptosis were examined by Annexin V-FITC and propidium iodide (PI) staining. Annexin V staining was positive for both apoptosis and necrosis, and PI staining was used to detect necrotic cells. The number of Annexin V^+ PI^- cells, which are only apoptotic cells, not necrotic cells, were counted. As shown in Figure 6(a) and (b), 50 μM of **8c** induced early apoptosis in 9.28 and 5.46% of MCF-7 and Hep3B cells, respectively, which suggests that **8c** has higher potency in the induction of apoptosis in MCF-7 than Hep3B.

Conclusion

A series of new allylselenopyridazines were synthesized and assessed for their ability to inhibit cell proliferation against human MCF-7 breast cancer, Hep3B hepatocarcinoma, and

RKO colon cancer cell lines. Two compounds (**8c**, **8d**) had a better effect on the developmental inhibition of tumor cell lines than 5-FU. This shows that 3-allylseleno-6-*n*-propylsulfonylpyridazine **8c** has good results towards MCF-7 and Hep3B. This study proposes the potential anticancer effect of compounds **8c** and **8d**. The potent biological activities highlight 3-allylseleno-6-*n*-alkylsulfonylpyridazines as a new anticancer candidate.

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