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Synthesis and Anticancer Activities of New 3-Allylthio-6-(mono or disubstituted)aminopyridazines

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A new series of 3-allylthio-6-(mono or disubstituted) aminopyridazines was synthesized by reacting 3-allylthio-6-chloropyridazine with several amines to develop new anticancer agents. These new compounds showed antiproliferative activities against lung cancer (A549), hepatoblastoma (Hep3b), prostate cancer (PC3), colon cancer (SW480) and cervical cancer (HeLa) cells in MTT assays, and could be promising candidates for chemotherapy of carcinomas. Compound **5** (3-allylthio-6-homopiperidinylaminopyridazine) showed higher potencies than 5-FU for inhibiting the growth of these cell lines. This suggests the potential anticancer activity of compound **5**.

Key words: Allylthiopyridazines, Substituted aminopyridazines, Anticancer activities, MTT assay

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. In previous studies, various 3-allylthio-6-alkoxypyridazine derivatives (K-compounds) and 3-allylthio-6-alkylthiopyridazine derivatives (thio-K-compounds) were synthesized (Lee et al., 2001; Kwon, 2002a, 2002b) and their biological activities were tested (Jung et al., 2001). K-Compounds and thio-K-compounds showed especially good hepatoprotective and antitumor activities (Kwon and Moon, 2005) (Fig. 1).

The pyridazine group is an important moiety present in many drugs acting at various pharmacological targets (Tisler and Stanovnik, 1984; Kleeman and Engel, 2001; Song et al., 2008). This moiety has been combined with the allylthio group (Lee et al., 2003; Shin and Kwon, 2003).

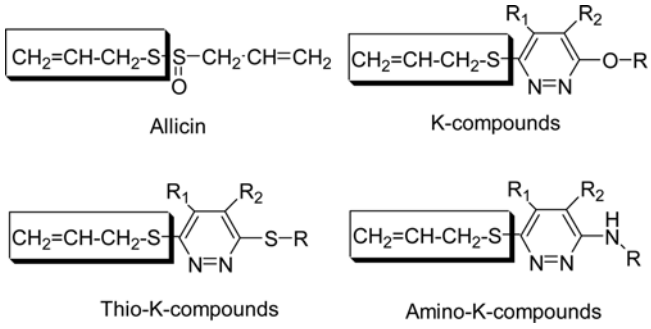

Fig. 1. Allicin and reported allylthiopyridazines

The isosteric replacement of the oxygen (or sulfur) of K-compounds by a nitrogen atom yields the aminopyridazines (Fig. 1). We recently reported the synthesis of heterocyclo(or aryl)alkylaminopyridazines through amination (Lee et al., 2009), and of allylthioheterocyclopyridazines (Park and Park, 2005, 2007). The synthesis of allylthio heterocyclo(or aryl)alkylaminopyridazines and their antitumor activities against SK-Hep-1 human liver cancer cells were also reported (Kwon and Lee, 2005; Lee et al., 2009). We were then interested in synthesizing aminopyridazines through coupling of pyridazinyl chloride with

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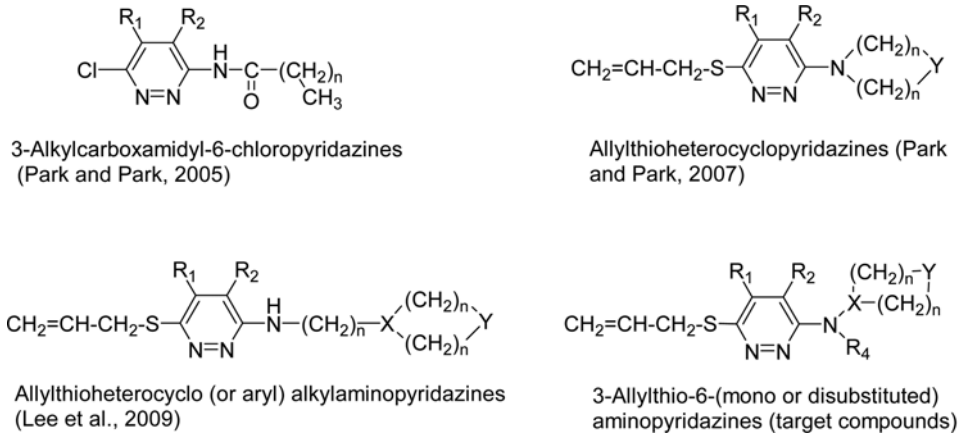


Fig. 2. Derivatives of amino-K-compound

amines known to give new amino-K-compounds (Fig. 2). We also designed new 3-allylthio-6-aminopyridazine derivatives (mono or disubstituted aminopyridazines) **3**-**12** using a modification of this method in order to discover potential anti-tumor candidates. We tested the ability of these synthetic compounds to inhibit the growth of lung cancer (A549), hepatoblastoma (Hep3b), prostate cancer (PC3), colon cancer (SW480) and cervical cancer (HeLa) cell lines.

MATERIALS AND METHODS

Chemicals

Chemicals were supplied by Aldrich, Sigma, Merck, and Tokyo Kasei. Melting points were determined in open 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 3-allylthio-6-heterocyclicaminopyridazines 3-7

A solution of 3-allylthio-6-chloropyridazine **2** (5 mmol), the appropriate amine (10 mmol) and ammonium chloride (5 mmol) in *n*-butanol (16 mL) was refluxed for 4-50 h. The solvent was evaporated under reduced pressure. The residue was extracted with ethyl acetate and dried over Na_2SO_4. After solvent evaporation, the residue was purified by column chromatography on silica gel.

3-Allylthio-6-morpholinylaminopyridazine (3)
Yield: 8%, mp 116-117°C. ^{1}H NMR ($CDCl_3$) δ 7.18 (s, 2H, pyridazine), 5.97-5.92 (m, 1H, =CH), 5.28 (d, J = 15.7 Hz, 1H, CH_2=), 5.10 (d, J = 9.9 Hz, 1H, CH_2=), 3.88 (d, J = 6.9 Hz, 2H, SCH_2), 3.79 (t, J = 4.5 Hz, 4H, CH_2×2), 2.81 (t, J = 4.5 Hz, 4H, CH_2×2), 1.79 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 159.09, 153.03, 133.57, 128.95, 117.86, 114.22, 66.86, 56.59 (morpholine), 33.71. FT-IR (KBr cell) cm^{-1} 3434, 3053, 2986, 1602, 1421, 1265.

3-Allylthio-6-piperidinylaminopyridazine (4)
Yield 13%, ^{1}H NMR ($CDCl_3$) δ 7.06 (d, J = 9.5 Hz, 1H, aromatic), 6.82 (d, J = 9.5 Hz, 1H, aromatic), 6.03 (m, 1H, =CH), 5.30 (d, J = 16.9 Hz, 1H, CH_2=), 5.08 (d, J = 9.9 Hz, 1H, CH_2=), 3.91 (d, J = 6.9 Hz, 2H, SCH_2), 3.58 (t, J = 5.4 Hz, 4H, CH_2×2), 1.66 (m, 6H, piperidine), 1.66 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 158.38, 150.03, 133.81, 127.82, 117.62, 113.75, 46.42, 33.51, 25.32, 24.54. FT-IR (KBr cell) cm^{-1} 3400, 3051, 2981, 2939, 1589, 1433, 1265.

3-Allylthio-6-homopiperidinylaminopyridazine (5)
Yield 11%, ^{1}H NMR ($CDCl_3$) δ 7.06 (d, J = 9.6 Hz, 1H, aromatic), 6.68 (d, J = 9.5 Hz, 1H, aromatic), 6.01 (m, 1H, =CH), 5.26 (d, J = 16.8 Hz, 1H, CH_2=), 5.09 (d, J = 9.9 Hz, 1H, CH_2=), 3.91 (d, J = 7.0 Hz, 2H, SCH_2), 3.67 (t, J = 5.9 Hz, 4H, CH_2×2), 1.77 (m, 4H, CH_2×2), 1.70 (s, 1H, NH), 1.56 (m, 4H, CH_2×2). ^{13}C NMR ($CDCl_3$) δ 157.06, 148.53, 133.95, 128.08, 117.53, 111.89, 47.75, 33.72, 27.57, 27.02. FT-IR (KBr cell) cm^{-1} 3434, 3053, 2985, 2932, 1592, 1429, 1265.

3-Allylthio-6-(4-methylpiperazinyl)aminopyridazine (6)
Yield 28%, ^{1}H NMR ($CDCl_3$) δ 7.11 (d, J = 9.6 Hz, 1H, aromatic), 6.83 (d, J = 9.6 Hz, 1H, aromatic), 6.01 (m, 1H, =CH), 5.28 (d, J = 17.1 Hz, 1H, CH_2=), 5.11 (d, J

= 10.2 Hz, 1H, CH_2=), 3.92 (d, J = 6.9 Hz, 2H, SCH_2), 3.64 (t, J = 5.1 Hz, 4H, CH_2×2), 2.55 (t, J = 5.4 Hz, 4H, CH_2×2), 2.36 (s, 3H, CH_3), 2.17 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 158.21, 151.07, 133.64, 127.96, 117.69, 113.75, 54.51, 46.09, 45.18, 33.41, 30.90. FT-IR (KBr cell) cm^{-1} 3400, 3053, 2985, 2944, 1592, 1431, 1265.

3-Allylthio-6-(4-thiomorpholinyl)aminopyridazine (7)
Yield 12%, mp 116-117°C, ^{1}H NMR ($CDCl_3$) δ 7.22 (d, J = 9.5 Hz, 1H, aromatic), 6.94 (d, J = 9.5 Hz, 1H, aromatic), 6.00 (m, 1H, =CH), 5.32 (d, J = 15.5 Hz, 1H, CH_2=), 5.15 (d, J = 10.1 Hz, 1H, CH_2=), 4.20 (t, J = 5.3 Hz, 4H, CH_2×2), 3.94 (d, J = 6.8 Hz, 2H, SCH_2), 3.11 (t, J = 5.4 Hz, 4H, CH_2×2), 1.60 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 155.84, 153.47, 133.08, 128.63, 118.22, 114.06, 50.78, 44.46, 33.22. FT-IR (KBr cell) cm^{-1} 3421, 3082, 2980, 2922, 1586, 1447, 1279.

General synthetic procedure for the 3-allylthio-6-*N*-alkylanilinopyridazines 8-12

A solution of 3-allylthio-6-chloropyridazine **2** (5 mmol), the *N*-alkyl aniline (10 mmol) and ammonium chloride (5 mmol) in *n*-butanol (16 mL) was refluxed for 6-25 h. The solvent was evaporated under reduced pressure. The residue was extracted with ethyl acetate and dried over Na_2SO_4. After solvent evaporation, the residue was purified by column chromatography on silica gel.

3-Allylthio-6-*N*-methylanilinopyridazine (8)
Yield 64%. ^{1}H NMR ($CDCl_3$) δ 7.42 (t, J = 7.9 Hz, 2H, aromatic), 7.23 (m, 3H, aromatic), 6.92 (d, J = 9.6 Hz, 1H, aromatic), 6.65 (d, J = 9.3 Hz, 1H, aromatic), 6.04 (m, 1H, =CH), 5.31 (d, J = 17.1 Hz, 1H, CH_2=), 5.12 (d, J = 10.7 Hz, 1H, CH_2=), 3.95 (d, J = 6.9 Hz, 2H, SCH_2), 3.57 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 157.61, 151.12, 145.61, 133.72, 130.05, 127.08, 126.35, 117.71, 115.39, 38.96, 33.29. FT-IR (KBr cell) cm^{-1} 3434, 3052, 2984, 2924, 1587, 1444, 1265.

3-Allylthio-6-*N*-ethylanilinopyridazine (9)
Yield 67%. ^{1}H NMR ($CDCl_3$) δ 7.42 (t, J = 7.6 Hz, 2H, aromatic), 7.28 (d, J = 5.9 Hz, 1H, aromatic), 7.20 (d, J = 7.1 Hz, 2H, aromatic), 6.89 (d, J = 9.5 Hz, 1H, aromatic), 6.50 (d, J = 9.5 Hz, 1H, aromatic), 6.02 (m, 1H, =CH), 5.30 (d, J = 15.5 Hz, 1H, CH_2=), 5.12 (d, J = 10.0 Hz, 1H, CH_2=), 4.12 (m, 2H, CH_2), 3.95 (d, J = 6.9 Hz, 2H, SCH_2), 1.26 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 157.15, 150.71, 144.03, 133.75, 130.13, 127.57, 127.22, 126.63, 117.69, 115.54, 45.48, 33.38, 30.95, 12.65. FT-IR (KBr cell) cm^{-1} 3410, 3051, 2981, 1586, 1425, 1265.

3-Allylthio-6-*N*-propylanilinopyridazine (10)
Yield 29%. ^{1}H NMR ($CDCl_3$) δ 7.40 (t, J = 7.9 Hz, 2H, aromatic), 7.27 (d, J = 4.9 Hz, 1H, aromatic), 7.20 (d, J = 7.1 Hz, 2H, aromatic), 6.89 (d, J = 9.5 Hz, 1H, aromatic), 6.49 (d, J = 9.5 Hz, 1H, aromatic), 6.02 (m, 1H, =CH), 5.30 (d, J = 15.6 Hz, 1H, CH_2=), 5.12 (d, J = 10.0 Hz, 1H, CH_2=), 4.02 (t, J = 7.7 Hz, 2H, CH_2), 3.95 (d, J = 6.9 Hz, 2H, SCH_2), 1.72 (m, 2H, CH_2), 0.92 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 157.49, 150.67, 144.33, 133.69, 130.12, 127.51, 126.56, 117.71, 115.42, 52.36, 33.40, 20.53, 11.33. FT-IR (KBr cell) cm^{-1} 3434, 3053, 2985, 2933, 1587, 1421, 1265.

3-Allylthio-6-*N*-butylanilinopyridazine (11)
Yield 45%. ^{1}H NMR ($CDCl_3$) δ 7.40 (t, J = 7.9 Hz, 2H, aromatic), 7.27 (d, J = 5.1 Hz, 1H, aromatic), 7.20 (d, J = 7.1 Hz, 2H, aromatic), 6.89 (d, J = 9.5 Hz, 1H, aromatic), 6.49 (d, J = 9.5 Hz, 1H, aromatic), 6.02 (m, 1H, =CH), 5.29 (d, J = 16.9 Hz, 1H, CH_2=), 5.12 (d, J = 10.6 Hz, 1H, CH_2=), 4.04 (t, J = 7.8 Hz, 2H, CH_2), 3.95 (d, J = 6.9 Hz, 2H, SCH_2), 1.66 (m, 2H, CH_2), 1.36 (m, 2H, CH_2), 0.89 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 157.51, 150.69, 144.35, 133.69, 130.12, 127.52, 126.56, 117.72, 115.42, 50.61, 33.45, 29.56, 20.22, 13.97. FT-IR (KBr cell) cm^{-1} 3412, 3053, 2984, 2932, 1587, 1421, 1265.

3-Allylthio-6-*N*-pentylanilinopyridazine (12)
Yield 40%. ^{1}H NMR ($CDCl_3$) δ 7.42 (t, J = 7.8 Hz, 2H, aromatic), 7.27 (d, J = 4.8 Hz, 1H, aromatic), 7.20 (d, J = 7.2 Hz, 2H, aromatic), 6.89 (d, J = 9.6 Hz, 1H, aromatic), 6.49 (d, J = 9.3 Hz, 1H, aromatic), 6.02 (m, 1H, =CH), 5.29 (d, J = 16.8 Hz, 1H, CH_2=), 5.12 (d, J = 9.9 Hz, 1H, CH_2=), 4.03 (t, J = 7.8 Hz, 2H, CH_2), 3.95 (d, J = 6.9 Hz, 2H, SCH_2), 1.67 (m, 2H, CH_2), 1.31 (m, 4H, CH_2×2), 0.86 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 157.50, 150.67, 144.34, 133.70, 130.12, 127.51, 126.55, 117.71, 115.41, 50.77, 33.46, 29.14, 27.14, 22.59, 14.09. FT-IR (KBr cell) cm^{-1} 3429, 3053, 2985, 2931, 1587, 1421, 1265.

Materials and methods for bioassay

Cell lines and culture conditions. Lung cancer (A549), hepatoblastoma (Hep3b), prostate cancer (PC3), colon cancer (SW480) and cervical cancer (HeLa) cells were purchased from the Korean Cell Line Bank, and were maintained at 37°C in a humidified atmosphere, with 5% CO_2, in Dulbecco's modified Eagle medium (DMEM) (Gibco-BRL Inc.) supplemented with 5% fetal bovine serum (FBS) and 1% penicillin-streptomycin agent (Gibco-BRL Inc.).

MTT assay. Lung cancer (A549), hepatoblastoma (Hep3b), prostate cancer (PC3), colon cancer (SW480)

and cervical cancer (HeLa) cells (5×10^3 cells/well) cultured in 96-well-plates were treated with various concentrations of the synthetic compounds (**3-12**) and allowed to attach for 24 h. Control cells were treated with dimethyl sulphoxide (DMSO) equal to the highest percentage of solvent used in the experimental conditions. 5-FU was used as a positive control. Briefly, after 24 h treatment with the compound (62.5, 125, 250 and 500 μg/mL), 2 mg/mL of 0.5% MTT (3-(4,5-dimethylthiaxol-2-yl)-2,5-diphenyl tetrazolium bromide) was added to the media and the cells were further incubated for 2 h. The supernatant (100 μl) was replaced with the same volume of DMSO, and the absorbance was measured at 540 nm with a micro-ELISA reader (Molecular Devices). The percent of cells surviving was defined as the relative absorbance of treated versus untreated cells.

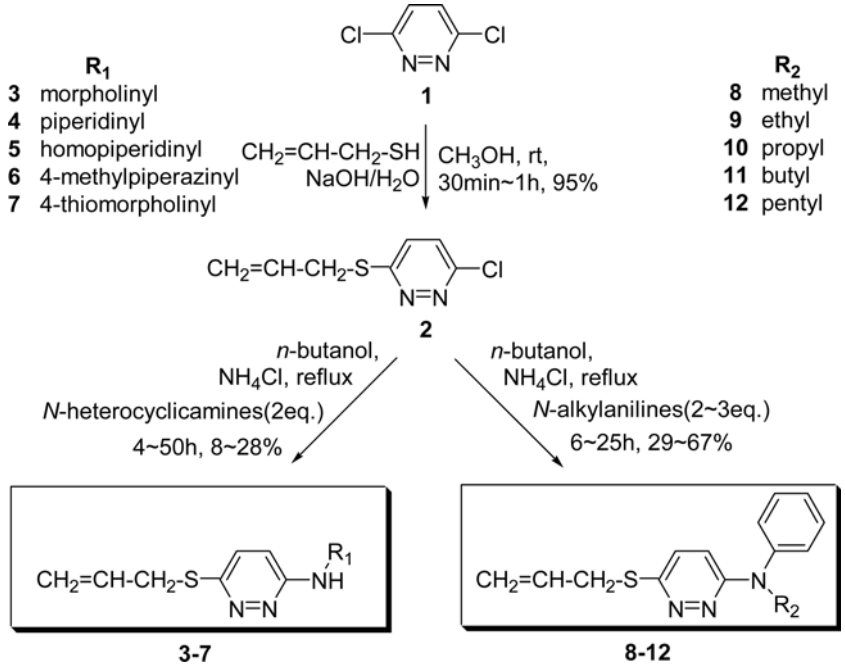
RESULTS AND DISCUSSION

Activated aryl halides react well with amines to give the corresponding arylamines. The reaction of an aryl halide with an amine is not only important for the synthesis of amines, but is also essential for the preparation of pharmaceuticals. Many reports have been published on the nucleophilic amination of aryl halides. Even though a synthetic pathway for 3-aminopyridazines has been developed (Wermuth et al., 1987; Parrot et al., 1999b; Contreras et al., 2001), the synthesis of 3-allylthio-6-(mono or disubstituted) aminopyridazines has not been reported until now. We applied a general method of preparing aminopyridazines from pyridazinyl halides and amines (Wermuth et al., 1989; Contreras et al., 1999; Parrot et al., 1999a).

A series of 3-allylthio-6-(mono or disubstituted) aminopyridazines **3-12** was prepared by allylthiolation and nucleophilic substitution. The allylthio group, a pharmacologically active group, was introduced on one side of the pyridazine ring. The amines with heterocycle such as morpholine, piperidine, homopiperidine, 4-methylpiperazine and 4-thiomorpholine and the *N*-alkylanilines were introduced into the *para*-position of 3-allylthio-6-chloropyridazine **2** (Scheme 1). The key intermediate in these preparations was 3-allylthio-6-chloropyridazine **2**, which could be readily obtained from the corresponding 3,6-dichloropyridazine **1** by reaction with allylmercaptan. Condensation of the 3-allylthio-6-chloropyridazine **2** with various amines gave the final target products **3-12** (Table I).

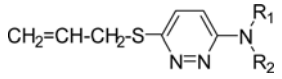
Here, we present the substitution reaction of 3-allylthio-6-chloropyridazine **2** by amines, which produced 3-allylthio-6-(mono or disubstituted) aminopyridazines **3-12**. In Table I, we summarize the physical properties and the reaction conditions for synthesizing compounds **3-12**.

3-Allylthio-6-chloropyridazine **2** was converted to final aminopyridazines **3-12** by nucleophilic aromatic substitution with amines in the presence of ammonium chloride (Scheme 1) as an acid catalyst. The



Scheme 1. Synthesis of 3-allylthio-6-(mono or disubstituted) aminopyridazines **3-12**

Table I. Reaction conditions for the synthesis of target compounds **3**-**12** and antiproliferative activity in each cell lines (A549, Hep3b, PC3, SW480 and HeLa)



Comp	R_1	R_2	Time /h[a]	mp /°C	Yield [b]/%	Molar Ratio[c] Reag./Subs.	Antiproliferative Activity (cells)
3	morpholinyl	H	50	116-117	8	2	-
4	piperidinyl	H	4	oil	13	2	Hep3b, PC3, HeLa
5	homopiperidinyl	H	4	oil	11	2	A549, Hep3b, PC3, SW480, HeLa
6	4-methylpiperazinyl	H	50	oil	28	2	SW480
7	4-thiomorpholinyl	H	16	116-117	12	2	-
8	phenyl	methyl	6	oil	64	2	Hep3b, HeLa
9	phenyl	ethyl	25	57-59	67	3	A549, Hep3b, HeLa
10	phenyl	propyl	25	49-51	29	3	-
11	phenyl	butyl	25	74-75	45	3	-
12	phenyl	pentyl	25	oil	40	2	-

[a] All reactions were performed in *n*-butanol under reflux. [b] Yield referred to isolated product.
[c] Reag./Subs. is the ratio of reagent (alkylamine) to substrate (chloropyridazine **2**).

amination reactions of 3-allylthio-6-chloropyridazine **2** with a range of amines are shown in Table I. The alkyl group at R_2 position was increased in carbon length up to five: methyl, ethyl, propyl, butyl and pentyl.

The nucleophilic displacement of chlorine in 3-allylthio-6-chloropyridazine **2** requires prolonged reaction time at the reflux temperature of *n*-butanol. A typical reaction consisted of a mixture of amine (10 mmol), 6-allylthio-3-chloropyridazine (5 mmol), and ammonium chloride (5 mmol) in *n*-butanol stirred under reflux for 4~50 h. The reaction was usually carried out using 1:2 (or 1:3) equivalents of 3-allylthio-6-chloropyridazine: amine.

The mono-allylthiolation from 3,6-dichloropyridazines **1** to 3-allylthio-6-chloropyridazine **2** produced high yields. Reactions of dichloropyridazines with allylmercaptan occurred in yields of more than 95%. We previously reported the synthesis of 3-allylthio-6-chloropyridazine **2** through allylthiolation (Park and Park, 2007). 3-Allylthio-6-chloropyridazine **2** and *N*-ethylaniline were reacted in the presence of ammonium chloride in *n*-butanol to form the corresponding products in 67% yield (Table I, entry **9**). 4-Aminomorpholine, 1-aminopiperidine, 1-aminohomopiperidine, 1-amino-4-methylpiperidine and 4-aminothiomorpholine were converted into the corresponding aminopyridazine derivatives in lower yields (Table I, entries **3-7**).

The pyridazine NMR peak of **3**-**12** appeared at 6.49-7.42 and 6.94-7.04 ppm, and the allyl peak appeared at 3.88-4.12, 5.08-5.15, 5.26-5.32, and 5.92-6.04 ppm. The NH NMR peak of **3**-**7** appeared at 1.66-2.17 ppm as a broad singlet signal. The alkyl (methyl, ethyl, propyl, butyl and pentyl) NMR peak of **8**-**12** appeared at 1.56-2.36 ppm as splitting signal. The pyridazine ^{13}C NMR peak appeared at 128, 133, 153, and 159 ppm, and the allyl peak appeared at 33, 111-115, and 117-118 ppm. In the FT-IR spectra, the NH absorption band appeared at 3400-3434 cm^{-1}.

Finally, we synthesized ten new 3-allylthio-6-(mono or disubstituted) aminopyridazine derivatives **3**-**12** in order to discover potential anti-tumor candidates. Refluxing of 3-allylthio-6-chloropyridazines **2** and the corresponding amines, such as heterocycloamines and *N*-alkylanilines, for 4~50 h produced the target amino-K-compounds.

In order to investigate the potential anti-cancer activity of the ten synthetic compounds, the growth-inhibitory effect of the synthetic compounds was examined against lung cancer (A549), hepatoblastoma (Hep3b), prostate cancer (PC3), colon cancer (SW480) and cervical cancer (HeLa) cells. MTT assays were conducted on cells treated with various concentrations of the compounds. 5-Fluorouracil (5FU), which produces partial responses in 10% to 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 not determined by the concentration range used in this study.

Of the ten compounds tested, five compounds (**4**, **5**, **6**, **8**, and **9**) inhibited the growth of lung cancer (A549) cells at a high concentration (250 μg/mL) (Fig. 3). We

further investigated the antiproliferative activity of compound **4**, which caused a greater inhibition of cell growth than the other compounds. As shown in Fig. 3, this compound markedly inhibited A549 cell growth in a dose-dependent manner. The greatest inhibition was observed with **5**. Two compounds, **5** and **9**, showed higher potencies than 5FU in inhibiting the growth of A549 cells, suggesting the potential anticancer activity of compounds **5** and **9**.

Of the ten compounds tested, five compounds (**4**, **5**, **8**, **9** and **11**) inhibited the growth of hepatoblastoma (Hep3b) cells at a low concentration (62.5 μg/mL) (Fig. 4). We further investigated the antiproliferative activity of compounds **4**, **5**, **8**, **9** and **11**, which caused greater inhibition of cell growth than the other compounds. As shown in Fig. 4, compounds **4**, **8**, **9** and **11** markedly inhibited Hep3b cell growth in a dose-dependent manner. Four compounds (**4**, **5**, **8** and **9**) showed higher potencies than 5FU in inhibiting the growth of Hep3b cells, suggesting the potential anticancer activity of these compounds (**4**, **5**, **8** and **9**).

Of the ten compounds tested, five compounds (**4**, **5**, **8**, **9** and **10**) inhibited the growth of prostate cancer (PC3) cells at a low concentration (62.5 μg/mL) (Fig. 5). 5FU, a positive control, showed low inhibitory effects on the growth of PC3 cells at standard concentrations (62.5, 125, 250, 500 μg/mL). 5FU is not indicated against prostate cancer. We further investigated the antiproliferative activity of compounds **4**, **5**, **8**, **9** and **10**, which caused greater inhibition of cell growth than the other compounds. As shown in Fig. 5, these compounds markedly inhibited PC3 cell growth in a dose-dependent manner. Two compounds (**4** and **5**) showed higher potencies than 5FU in inhibiting the growth of PC3 cells, suggesting the potential anticancer activity of these compounds (**4** and **5**).

Of the ten compounds tested, five compounds (**4**, **5**, **6**, **8** and **9**) inhibited the growth of colon cancer (SW480) cells at a standard concentration (Fig. 6). We further investigated the antiproliferative activity of compounds **4** and **5**, which caused a greater inhibition of cell growth than the other compounds. As shown in

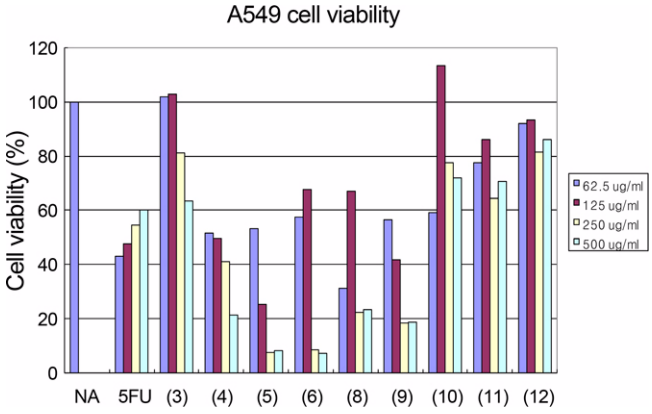


Fig. 3. Anticancer activity of synthesized compounds (**3**-**12**) in A549 lung cancer cells

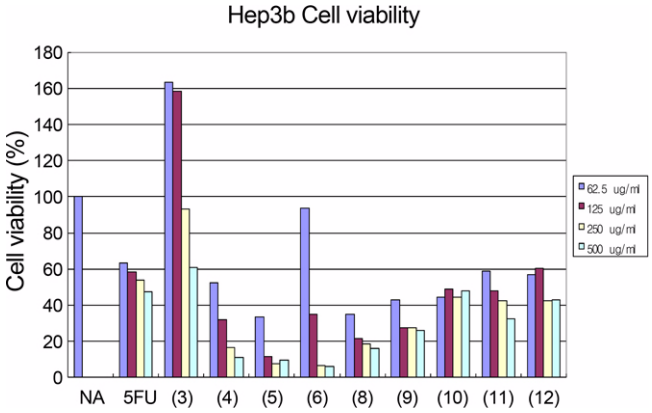


Fig. 4. Anticancer activity of synthesized compounds (**3**-**12**) in Hep3b hepatoblastoma cells

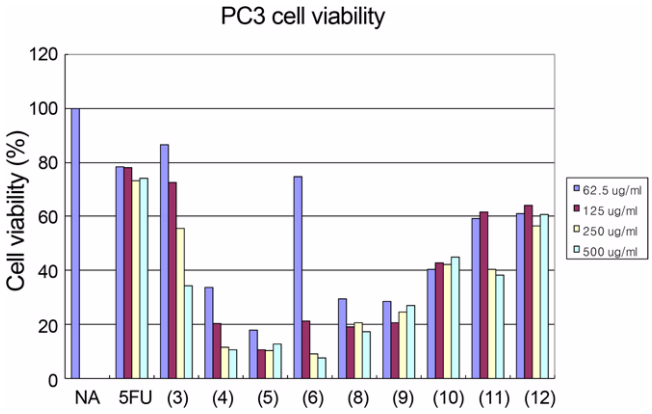


Fig. 5. Anticancer activity of synthesized compounds (**3**-**12**) in PC3 prostate cancer cells

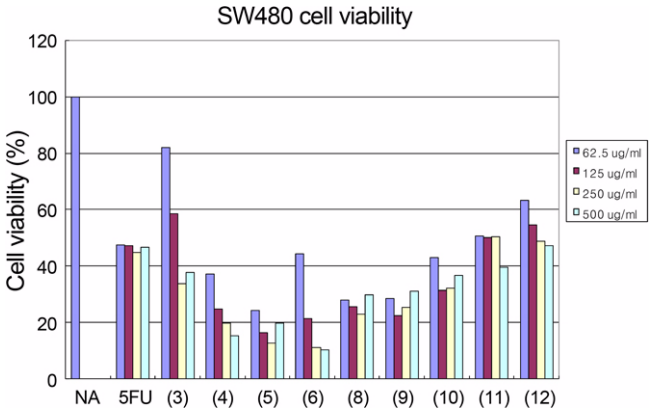


Fig. 6. Anticancer activity of synthesized compounds (**3**-**12**) in SW480 colon cancer cells

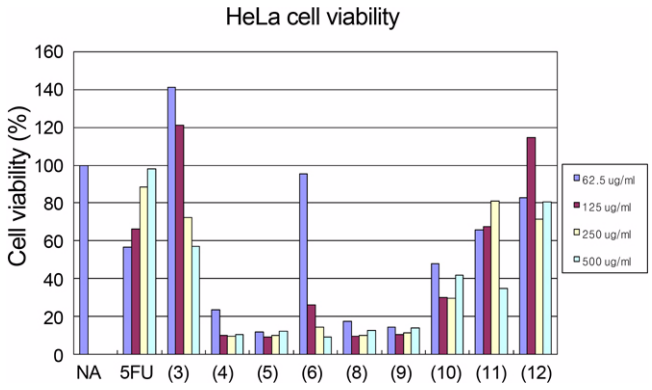


Fig. 7. Anticancer activity of synthesized compounds (**3**-**12**) in HeLa cervical cancer cells

Fig. 6, compounds **4**, **5** and **6** markedly inhibited SW480 cell growth in a dose-dependent manner. Three compounds (**4**, **5** and **6**) showed higher potencies than 5FU in inhibiting the growth of SW480 cells, suggesting the potential anticancer activity of these compounds (**4**, **5** and **6**).

Of the ten compounds tested, four compounds (**4**, **5**, **8** and **9**) inhibited the growth of cervical cancer (HeLa) cells at a low concentration (Fig. 7). We further investigated the antiproliferative activity of compounds **4**, **5**, **8** and **9**, which caused a greater inhibition of cell growth than the other compounds. As shown in Fig. 7, compound **5** markedly inhibited HeLa cell growth. Four compounds (**4**, **5**, **8** and **9**) showed higher potencies than 5FU in inhibiting the growth of HeLa cells, suggesting the potential anticancer activity of these compounds (**4**, **5**, **8** and **9**).

In order to investigate the potential anticancer activity of the ten synthetic compounds we created, the growth-inhibitory effect of these synthetic compounds was examined in lung cancer (A549), hepatoblastoma (Hep3b), prostate cancer (PC3), colon cancer (SW480) and cervical cancer (HeLa) cells. The results revealed that compound **5** had high activity towards A549, Hep3b, PC3, SW480 and HeLa cells. Compounds **4**, **6**, **8**, and **9** had high activity towards A549 cells at a high concentration (250 μg/mL). Compound **3** did not have antiproliferative activity towards any cells. Compounds **10**, **11**, and **12** had low or no antiproliferative activity towards all cells compared to 5FU.

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