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Novel Alkylaminopyridazine Derivatives: Synthesis and Their Anti-proliferative Effects against MCF-7 Cells

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A series of new 3-alkylamino-6-allylthio-pyridazine derivatives was synthesized through allylthiolation and amino-de-halogenation and were expected to have anti-proliferative activity. 6-Allylthio-3-chloropyridazine was prepared from the reaction of 3,6-dichloropyridazine with allylmercaptan and sodium hydroxide. The alkylamines such as methylamine and the dialkylamines such as dimethylamine were introduced into the 3-position of the pyridazine ring. These new compounds showed anti-proliferative activities against MCF-7 human breast cancer cells in CCK-8 assays. These compounds are thus promising candidates for chemotherapy of breast cancer. Two compounds, **14** and **15**, showed higher potencies for inhibiting growth of breast cancer cells than did 5FU. This suggests the potential anti-proliferative activity of these compounds.

Key Words : Alkylaminopyridazines, Dialkylaminopyridazines, Allylthiopyridazines, Human breast cancer, MCF-7

Introduction

Aminopyridazines are an important pharmacophoric moiety in many drugs that act on various pharmacological targets.[1-5] In particular, the aminopyridazine nucleus is found in dopaminergic, serotoninergic, cholinergic, and GABAergic ligands, as well as in monoamine oxidase and acetylcholinesterase inhibitors.

The allylthio group of allicin and other organosulfur compounds that are isolated from garlic is considered an important pharmacophore, a key structural component for biological activity. Other pyridazine derivatives in which the chlorine atom at the 3-position of the 6-allylthiopyridazinyl chloride is replaced by alkoxy or alkylthio moiety have been synthesized. 3-Alkoxy-6-allylthiopyridazines (K-compounds) and 3-alkylthio-6-allylthiopyridazines (thio-K-compounds) showed especially good hepatoprotective and antitumor activities (Figure 1).[6] The isosteric replacement of the oxygen (or sulfur) by a nitrogen atom yielded the amino-K-compounds (Figure 1). In previous studies, we synthesized *N*-acylated 3-amino-6-chloropyridazine derivatives through amination and acylation.[7] Kwon *et al.* reported the synthesis of 3-allylthio-6-heterocyclylalkylaminopyridazines and their antitumor activities.[8] We have recently reported on allylthioaralkylaminopyridazines and their anticancer activity.[9-11]

We became interested in synthesizing 3-aminopyridazines through coupling of pyridazinyl chloride with known alkyl amines to give new alkylaminopyridazines. Activated aryl halides react well with ammonia and with primary and secondary amines to give the corresponding alkylamine derivatives. The reaction of aryl halide with a secondary amine is not only important for the synthesis of tertiary amines, but is also essential for the preparation of a number of pharmaceuticals. Many reports have been published on the nucleophilic amination of aryl halides. [12]

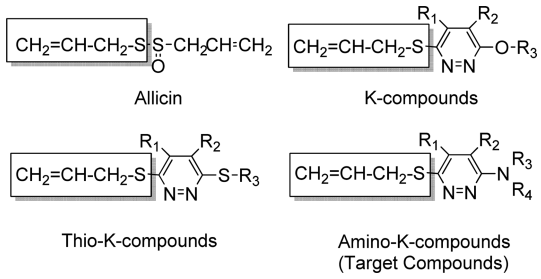


Figure 1. Allicin and target compounds.

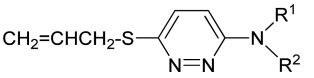
Even though synthetic pathways for 3-aminopyridazines were developed by Wermuth *et al.*,[4] Contreras *et al.*,[2,5b] and Parrot *et al.*,[13] the synthesis of 3-alkylamino-6-allylthiopyridazines has not been reported until now. We applied a general method of preparing aminopyridazines from pyridazinyl halides and alkylamines. The key intermediates in these preparations are pyridazinyl chlorides **2**, which can be readily obtained from the corresponding 3,6-dichloropyridazine **1** by reaction with allylmercaptan. Condensation of the pyridazinyl chlorides **2** with various alkylamines gave the final target products **3-16** (Table 1).

3-Alkylamino-6-allylthiopyridazine derivatives in which the oxygen atom at 6-position of 3-allylthio-6-alkoxypyridazine is replaced by nitrogen (N) were synthesized for development as new anticancer agents. In order to investigate the potential anti-proliferative activity of the synthetic compounds they were examined on MCF-7 breast cancer cells.[14]

Results and Discussion

A series of 3-alkylamino-6-allylthiopyridazines **3-16** were prepared by allylthiolation and nucleophilic substitution. Alkylamines with a nitrogen nucleophile such as methylamines and dimethylamines were introduced into the 3-

Table 1. The reaction times for target 3-amino-6-allylthiopyridazines **3-16** and their anti-proliferative activity in cell lines (MCF-7)



Comp	Time/h	R^1	R^2	IC_{50}/μg/mL[a]
3	24	methyl	H	-
4	48	ethyl	H	219.45
5	48	propyl	H	247.10
6	48	butyl	H	80.39
7	48	pentyl	H	50.05
8	48	hexyl	H	70.73
9	48	heptyl	H	19.61
10	48	octyl	H	19.62
11	24	methyl	methyl	-
12	78	ethyl	ethyl	-
13	48	propyl	propyl	-
14	48	butyl	butyl	17.20
15	51	pentyl	pentyl	17.16
16	48	hexyl	hexyl	-

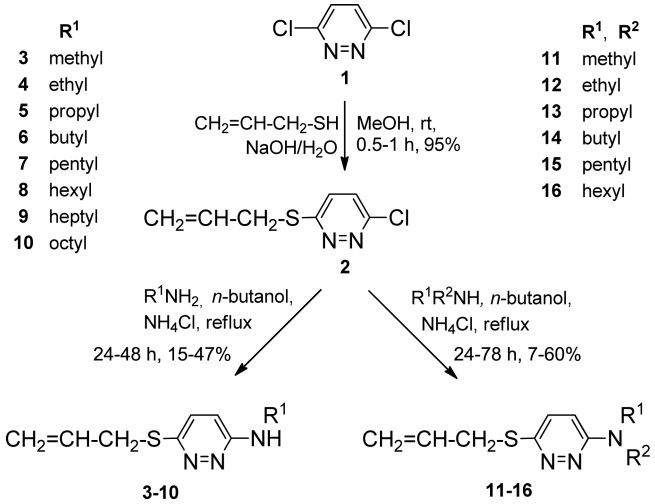
[a]IC_{50} of 5FU as a positive control was 477.47 μg/mL.

position of the pyridazine ring (Scheme 1). Here, we present the amino-de-halogenation of 3-chloro-6-allylthiopyridazine by a nitrogen nucleophile to produce 3-alkylamino-6-allylthiopyridazines. For the synthesis of pyridazine **3-16**, chloropyridazine **2** was converted by nucleophilic aromatic substitution with a nitrogen nucleophile in the presence of ammonium chloride. The ammonium chloride-assisted coupling of various nitrogen nucleophiles with chloropyridazine **2** resulted in nucleophilic amino-de-halogenation. The amination reactions of chloropyridazine **2** with a range of amines are found in Table 1. The alkyl chain on the nitrogen was increased in carbon length to eight: methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl and octyl.

The nucleophilic displacement of chlorine in chloropyridazine **2** required prolonged reaction time at the reflux temperature of *n*-butanol. In a typical reaction, a mixture of alkylamine (12 mmol), chloropyridazine **2** (4 mmol), and ammonium chloride (4 mmol) in *n*-butanol was stirred under reflux for 24-78 h. The reaction was carried out using 1: 3 equivalents of 6-allylthio-3-chloropyridazine: alkylamine.

In the proposed mechanism of the substitution reaction of the amine nucleophile, the addition of alkylamine to the pyridazine nucleus to form a tertiary ammonium intermediate and the proton transfer from nitrogen to chloride produced a hydrochloride. A molecule of hydrochloride was eliminated due to nucleophilic addition at the carbon of the pyridazine nucleus and a new C-N bond formed. For additional amination, halides **2** were converted to compounds **3-16** by eliminating hydrochloride.

Pyridazine halide and dimethylamine were reacted in the presence of ammonium chloride in *n*-butanol to form the corresponding amine products in 60% yield (Compound **11**). Similarly, dibutylamine and dihexylamine were converted into the corresponding aminopyridazine derivatives



Scheme 1. Synthesis of 3-allylthio-6-(mono or dialkylated)aminopyridazines **3-16**.

in somewhat lower yields (Compound **14** and trace amount of **16**) due to steric hindrance between the long carbon chain and the amino moiety.

The formation of a C-N bond in aminopyridazines was accomplished by refluxing with NH_4Cl for 24-78 h in *n*-butanol. Final pyridazines were identified by NMR, IR, and GC-MS. The pyridazine NMR peak of **3-16** appeared at 6.52-6.71 and 7.03-7.08 ppm, and the allyl peak appeared at 5.08-5.09, 5.24-5.26, and 5.94-6.09 ppm. The NH peak appeared at 4.57-4.87 ppm. The pyridazine ^{13}C NMR peak appeared at 118, 129, 151, and 158 ppm. The allylthio ^{13}C NMR peak appeared at 33, 114, and 133 ppm.

In order to investigate the potential anti-proliferative activity of the nine synthetic compounds, the growth-inhibitory effect of these was examined against MCF-7 breast cancer cells. CCK-8 assays were conducted on the cells treated with various concentrations of the compounds. 5-Fluorouracil (5FU), which has previously been shown to have anti-proliferative activity against MCF-7 cells was used as a positive control. We expect that synthesized compound and 5FU have similar mechanism of action. Of nine compounds tested, four (**4**, **6**, **14** and **15**) showed dose-dependent inhibitory effects against the growth of MCF-7 cells (Figure 2). The highest inhibition was observed by **14** and the lowest inhibition by **4**.

We further investigated the anti-proliferative activity of two compounds (**14** and **15**) that caused a higher inhibition of cell growth than the other compounds. As shown in Table 1, these compounds caused a marked inhibition of MCF-7 cell growth in a dose-dependent manner. The IC_{50} values for **14** and **15** for inhibiting MCF-7 cell growth were approximately 17.20 and 17.16 μg/mL, respectively.

In conclusion, we synthesized fourteen new 3-alkylamino-6-allylthiopyridazine derivatives **3-16** in order to discover a potential antitumor candidate. Refluxing 6-allylthio-3-chloropyridazines and the corresponding nucleophilic amines such as alkylamines and dialkylamines for about 24-78 h produced the target amino-k-compounds. Two compounds, **14**

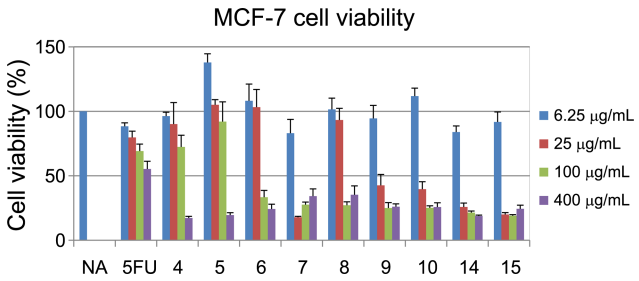


Figure 2. Anti-proliferative activity of synthesized compounds (**4-15**) in MCF-7 breast cancer cells.

and **15**, showed higher potencies (IC_{50} = 17.20 and 17.16 μg/mL) in inhibiting the growth breast cancer cells than did 5FU (IC_{50} = 477.47 μg/mL), suggesting the potential anti-cancer activity of these two compounds.

Experimental

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 on 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 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. Mass fragmentations were recorded using an Agilent 6890 GC and 5973 MS.

General Procedure for the Synthesis of 3-Alkylamino-6-allylthiopyridazine 3-10. A solution of 3-allylthio-6-chloropyridazine (4 mmol) and the appropriate alkylamine (12 mmol) and ammonium chloride (4 mmol) in *n*-butanol (10 mL) were refluxed for 24-48 h. The solvent was evaporated under reduced pressure. The residue was dissolved in 10% K_2CO_3 (50 mL), extracted with ethyl acetate (40 mL × 2), washed with water and brine, and dried over anhydrous Na_2SO_4. After solvent evaporation, the residue was purified by column chromatography on silica gel.

3-Methylamino-6-allylthiopyridazine (3): Yield: 15%, mp 34-36 °C, ^{1}H NMR ($CDCl_3$) δ 7.05 (d, *J* = 9.2 Hz, 1H, pyridazine), 6.56 (d, *J* = 9.2 Hz, 1H, pyridazine), 6.06-5.93 (m, 1H, =CH), 5.26 (d, *J* = 16.9 Hz, 1H, CH_2=), 5.09 (d, *J* = 9.9 Hz, 1H, CH_2=), 4.73 (s, 1H, NH), 3.88 (d, *J* = 6.9 Hz, 2H, SCH_2), 3.02 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 157.83, 150.54, 128.30, 117.69 (pyridazine), 133.74, 114.41, 33.76 (allyl), 28.91 (CH_3). FT-IR (NaCl) cm^{-1}: 3450, 3053, 1602, 1421, 1265. GC-MS *m/z* (%) 181.2 (M+) 166.1 (100.0), 148.1 (17.0), 71.0 (10.6), 167.1 (9.8), 181.1 (8.4).

3-Ethylamino-6-allylthiopyridazine (4): Yield: 19%, mp 39-41 °C, ^{1}H NMR ($CDCl_3$) δ 7.04 (d, *J* = 9.2 Hz, 1H, pyridazine), 6.54 (d, *J* = 9.2 Hz, 1H, pyridazine), 6.04-5.95 (m, 1H, CH=), 5.25 (d, *J* = 17.6 Hz, 1H, CH_2=), 5.08 (d, *J* = 10.0 Hz, 1H, CH_2=), 4.63 (s, 1H, NH), 3.88 (d, *J* = 6.9 Hz, 2H, SCH_2), 3.48-3.39 (m, 2H, CH_2), 1.27 (t, *J* = 7.2 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 157.61, 150.81, 128.69, 118.04 (pyridazine), 134.13, 114.76, 34.13 (allyl), 37.15, 15.13 (ethyl). FT-IR (NaCl) cm^{-1}: 3292, 3050, 1601, 1451, 1265. GC-MS *m/z* (%) 195.2 (M+) 180.1 (100.0), 162.1 (15.0), 181.1 (11.0), 195.1 (9.9), 97.0 (9.2).

3-Propylamino-6-allylthiopyridazine (5): Yield: 36%, mp 52-53 °C, ^{1}H NMR ($CDCl_3$) δ 7.03 (d, *J* = 9.3 Hz, 1H, pyridazine), 6.57 (d, *J* = 9.3 Hz, 1H, pyridazine), 6.04-5.94 (m, 1H, CH=), 5.24 (d, *J* = 16.2 Hz, 1H, CH_2=), 5.08 (d, *J* = 10.2 Hz, 1H, CH_2=), 4.87 (s, 1H, NH), 3.87 (d, *J* = 6.0 Hz, 2H, SCH_2), 3.36 (q, *J* = 6.9 Hz, 2H, -CH_2-), 1.73-1.60 (m, 2H, -CH_2-), 0.98 (t, *J* = 7.2 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 157.81, 150.60, 128.65, 118.02 (pyridazine), 134.13, 114.85, 34.15 (allyl), 44.16, 22.98, 11.86 (propyl). FT-IR (NaCl) cm^{-1}: 3433, 3053, 1602, 1444, 1265. GC-MS *m/z* (%) 209.3 (M+) 194.1 (100.0), 176.2 (15.1), 195.1 (14.5), 209.1 (11.9), 71.1 (10.0).

3-Butylamino-6-allylthiopyridazine (6): Yield: 39%, mp 47-49 °C, ^{1}H NMR ($CDCl_3$) δ 7.04 (d, *J* = 9.2 Hz, 1H, pyridazine), 6.53 (d, *J* = 9.2 Hz, 1H, pyridazine), 6.06-5.93 (m, 1H, CH=), 5.25 (d, *J* = 16.8 Hz, 1H, CH_2=), 5.09 (d, *J* = 9.9 Hz, 1H, CH_2=), 4.59 (s, 1H, NH), 3.88 (d, *J* = 6.9 Hz, 2H, SCH_2), 3.38 (q, *J* = 5.7 Hz, 2H, -CH_2-), 1.67-1.58 (m, 2H, -CH_2-), 1.49-1.36 (m, 2H, -CH_2-), 0.95 (t, *J* = 7.2 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 157.37, 150.39, 128.30, 117.67 (pyridazine), 138.78, 114.25, 33.75 (allyl), 41.82, 31.52, 20.16, 13.83 (butyl). FT-IR (NaCl) cm^{-1}: 3435, 3053, 1598, 1423, 1235. GC-MS *m/z* (%) 223.3 (M+) 208.1 (100.0), 209.1 (13.44), 190.2 (11.8), 223.1 (11.07), 152.1 (9.0).

3-Pentylamino-6-allylthiopyridazine (7): Yield: 37%, mp 39-41 °C, ^{1}H NMR ($CDCl_3$) δ 7.03 (d, *J* = 9.2 Hz, 1H, pyridazine), 6.53 (d, *J* = 9.2 Hz, 1H, pyridazine), 6.06-5.92 (m, 1H, CH=), 5.25 (d, *J* = 16.8 Hz, 1H, CH_2=), 5.08 (d, *J* = 9.3 Hz, 1H, CH_2=), 4.64 (s, 1H, NH), 3.88 (d, *J* = 6.9 Hz, 2H, SCH_2), 3.37 (q, *J* = 6.9 Hz, 2H, -CH_2-), 1.69-1.59 (m, 2H, -CH_2-), 1.47-1.25 (m, 2H×2, -$(CH_2)_2$-), 0.90 (t, *J* = 6.9 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 157.39, 150.36, 128.31, 117.66 (pyridazine), 133.78, 114.28, 33.78 (allyl), 42.09, 29.16, 29.13, 22.43, 14.01 (pentyl). FT-IR (NaCl) cm^{-1}: 3338, 3051, 1601, 1454, 1265. GC-MS *m/z* (%) 237.3 (M+) 222.2 (100.0), 223.2 (14.4), 237.2 (11.2), 204.2 (10.9), 152.0 (8.7).

3-Hexylamino-6-allylthiopyridazine (8): Yield: 20%, mp 48-49 °C, ^{1}H NMR ($CDCl_3$) δ 7.04 (d, *J* = 9.2 Hz, 1H, pyridazine), 6.52 (d, *J* = 9.2 Hz, 1H, pyridazine), 6.06-5.93 (m, 1H, CH=), 5.25 (d, *J* = 16.9 Hz, 1H, CH_2=), 5.08 (d, *J* = 9.9 Hz, 1H, CH_2=), 4.59 (s, 1H, NH), 3.88 (d, *J* = 6.9 Hz, 2H, SCH_2), 3.37 (q, *J* = 5.8 Hz, 2H, -CH_2-), 1.68-1.58 (m, 2H, -CH_2-), 1.43-1.25 (m, 2H×3, -$(CH_2)_3$-), 0.88 (t, *J* = 6.6 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 157.38, 150.39, 128.31, 117.66 (pyridazine), 133.78, 114.22, 33.77 (allyl), 42.13, 31.55, 29.42, 26.68, 22.59, 14.03 (hexyl). FT-IR (NaCl) cm^{-1}: 3338, 3051, 1601, 1449, 1265. GC-MS *m/z* (%) 251.4 (M+) 236.1 (100.0), 237.1 (15.2), 251.1 (15.2), 152.0 (7.1), 238.1 (5.6).

3-Heptylamino-6-allylthiopyridazine (9): Yield: 34%, mp 56-59 °C, ^{1}H NMR ($CDCl_3$) δ 7.03 (d, J = 9.2 Hz, 1H, pyridazine), 6.53 (d, J = 9.3 Hz, 1H, pyridazine), 6.06-5.92 (m, 1H, CH=), 5.25 (d, J = 14.7 Hz, 1H, CH_2=), 5.08 (d, J = 10.3 Hz, 1H, CH_2=), 4.61 (s, 1H, NH), 3.88 (d, J = 7.3 Hz, 2H, SCH_2), 3.37 (q, J = 6.9 Hz, 2H, -CH_2-), 1.68-1.58 (m, 2H, -CH_2-), 1.44-1.23 (m, 2H×4, -$(CH_2)_4$-), 0.88 (t, J = 6.6 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 157.38, 150.37, 128.30, 117.66 (pyridazine), 133.78, 114.25, 33.77 (allyl), 42.13, 31.78, 29.46, 29.03, 26.98, 22.59, 14.07 (heptyl). FT-IR (NaCl) cm^{-1}: 3305, 3050, 1601, 1453, 1235. GC-MS *m/z* (%) 265.4 (M+) 250.1 (100.0), 251.1 (16.6), 265.1 (12.8), 152.0 (7.0), 252.1 (5.8).

3-Octylamino-6-allylthiopyridazine (10): Yield: 47%, mp 40-41 °C, ^{1}H NMR ($CDCl_3$) δ 7.04 (d, J = 9.2 Hz, 1H, pyridazine), 6.52 (d, J = 9.2 Hz, 1H, pyridazine), 6.06-5.93 (m, 1H, CH=), 5.25 (d, J = 16.2 Hz, 1H, CH_2=), 5.09 (d, J = 9.9 Hz, 1H, CH_2=), 4.57 (s, 1H, NH), 3.88 (d, J = 6.9 Hz, 2H, SCH_2), 3.37 (q, J = 6.9 Hz, 2H, -CH_2-), 1.68-1.58 (m, 2H, -CH_2-), 1.40-1.25 (m, 2H×5, -$(CH_2)_5$-), 0.88 (t, J = 6.9 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 157.37, 150.39, 128.31, 117.66 (pyridazine), 133.78, 114.20, 33.76 (allyl), 42.14, 31.80, 29.46, 29.32, 29.23, 27.01, 22.64, 14.09 (octyl). FT-IR (NaCl) cm^{-1}: 3433, 3052, 1601, 1448, 1264. GC-MS *m/z* (%) 279.4 (M+) 264.2 (100.0), 265.2 (17.9), 279.2 (11.5), 266.2 (6.1), 152.0 (5.6).

General Procedure for the Synthesis of 3-Alkylamino-6-allylthiopyridazine 11-16. A solution of 3-allylthio-6-chloropyridazine (4 mmol) and the appropriate dialkylamine (12 mmol) and ammonium chloride (4 mmol) in *n*-butanol (10 mL) were refluxed for 24-48 h. The solvent was evaporated under reduced pressure. The residue was dissolved in 10% K_2CO_3 (50 mL), extracted with ethyl acetate (40 mL × 2), washed with water and brine, and dried over anhydrous Na_2SO_4. After solvent evaporation, the residue was purified by column chromatography on silica gel.

3-Dimethylamino-6-allylthiopyridazine (11): Yield: 60%, Oil, ^{1}H NMR ($CDCl_3$) δ 7.08 (d, J = 9.5 Hz, 1H, pyridazine), 6.71 (d, J = 9.5 Hz, 1H, pyridazine), 6.08-5.94 (m, 1H, =CH), 5.26 (d, J = 17.6 Hz, 1H, CH_2=), 5.09 (d, J = 9.9 Hz, 1H, CH_2=), 3.90 (d, J = 6.9 Hz, 2H, SCH_2), 3.14 (s, 3H×2, CH_3×2) ^{13}C NMR ($CDCl_3$) δ 158.08, 149.20, 127.99, 117.57 (pyridazine), 133.88, 112.26, 33.68 (allyl), 38.18 (dimethyl). FT-IR (NaCl) cm^{-1}: 3450, 3053, 1602, 1421, 1265. GC-MS *m/z* (%) 181.2 (M+) 166.1 (100.0), 148.1 (17.0), 71.0 (10.6), 167.1 (9.8), 181.1 (8.4).

3-Diethylamino-6-allylthiopyridazine (12): Yield: 27%, Oil, ^{1}H NMR ($CDCl_3$) δ 7.05 (d, J = 9.5 Hz, 1H, pyridazine), 6.65 (d, J = 9.5 Hz, 1H, pyridazine), 6.08-5.94 (m, 1H, CH=), 5.26 (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), 3.63 (q, J = 7.0 Hz, 2H×2, CH_2×2), 1.20 (t, J = 7.0 Hz, 3H×2, CH_3×2). ^{13}C NMR ($CDCl_3$) δ 156.28, 148.44, 128.03, 117.50 (pyridazine), 133.93, 111.99, 33.68 (allyl), 42.75, 12.85 (diethyl). FT-IR (NaCl) cm^{-1} 3292, 3050, 1601, 1451, 1265. GC-MS *m/z* (%) 195.2 (M+) 180.1 (100.0), 162.1 (15.0), 181.1 (11.0), 195.1 (9.9), 97.0 (9.2).

3-Dipropylamino-6-allylthiopyridazine (13): Yield: 26%, Oil, ^{1}H NMR ($CDCl_3$) δ 7.05(d, J = 7.5 Hz, 1H, pyridazine), 6.62 (d, J = 6.9 Hz, 1H, pyridazine), 6.07-5.94 (m, 1H, CH=), 5.25 (d, J = 16.2 Hz, 1H, CH_2=), 5.08 (d, J = 9.9 Hz, 1H, CH_2=), 3.91 (d, J = 6.9 Hz, 2H, SCH_2), 3.44 (t, J = 7.5 Hz, 2H×2, -CH_2-×2), 1.69-1.57 (m, 2H×2, -CH_2-×2), 0.93 (t, J = 7.2 Hz, 3H×2, CH_3×2). ^{13}C NMR ($CDCl_3$) δ 156.78, 148.39, 127.94, 117.53 (pyridazine), 133.89, 112.06, 33.75 (allyl), 50.67, 20.67, 11.37 (dipropyl). FT-IR (NaCl) cm^{-1} 3433, 3053, 1602, 1444, 1265. GC-MS *m/z* (%) 209.3 (M+) 194.1 (100.0), 176.2 (15.1), 195.1 (14.5), 209.1 (11.9), 71.1 (10.0).

3-Dibutylamino-6-allylthiopyridazine (14): Yield: 10%, Oil, ^{1}H NMR ($CDCl_3$) δ 7.03 (d, J = 9.5 Hz, 1H, pyridazine), 6.61 (d, J = 9.5 Hz, 1H, pyridazine), 6.07-5.93 (m, 1H, CH=), 5.25 (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), 3.46 (t, J = 7.5 Hz, 2H×2, -CH_2-×2), 1.63-1.53 (m, 2H×2, -CH_2-×2), 1.41-1.31 (m, 2H×2, -CH_2-×2), 0.94 (t, J = 7.2 Hz, 3H×2, CH_3×2). ^{13}C NMR ($CDCl_3$) δ 156.75, 148.35, 127.96, 117.52 (pyridazine), 133.89, 112.03, 33.80 (allyl), 48.70, 29.66, 20.25, 13.99 (dibutyl). FT-IR (NaCl) cm^{-1}: 3435, 3053, 1598, 1423, 1235 GC-MS *m/z* (%) 223.3 (M+) 208.1 (100.0), 209.1 (13.44), 190.2 (11.8), 223.1 (11.07), 152.1 (9.0).

3-Dipentylamino-6-allylthiopyridazine (15): Yield: 28%, Oil, ^{1}H NMR ($CDCl_3$) δ 7.03 (d, J = 9.5 Hz, 1H, pyridazine), 6.61 (d, J = 9.5 Hz, 1H, pyridazine), 6.07-5.93 (m, 1H, CH=), 5.25 (d, J = 14.7 Hz, 1H, CH_2=), 5.08 (d, J = 9.9 Hz, 1H, CH_2=), 3.90 (d, J = 6.9 Hz, 2H, SCH_2), 3.46 (t, J = 7.5 Hz, 2H×2, -CH_2-×2), 1.62-1.55 (m, 2H×2, -CH_2-×2), 1.38-1.25 (m, 2H×4, -CH_2-×4), 0.90 (t, J = 6.8 Hz, 3H×2, CH_3×2). ^{13}C NMR ($CDCl_3$) δ 156.75, 148.35, 127.98, 117.52 (pyridazine), 133.90, 112.03, 33.82 (allyl), 48.91, 29.20, 27.23, 22.61, 14.08 (dipentyl). FT-IR (NaCl) cm^{-1}: 3338, 3051, 1601, 1454, 1265. GC-MS *m/z* (%) 237.3 (M+) 222.2 (100.0), 223.2 (14.4), 237.2 (11.2), 204.2 (10.9), 152.0 (8.7).

3-Dihexylamino-6-allylthiopyridazine (16): Yield: 7%, Oil, ^{1}H NMR ($CDCl_3$) δ 7.03 (d, J = 9.5 Hz, 1H, pyridazine), 6.60 (d, J = 6.6 Hz, 1H, pyridazine), 6.07-5.93 (m, 1H, CH=), 5.25 (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), 3.45 (t, J = 7.7 Hz, 2H×2, -CH_2-×2), 1.61-1.54 (m, 2H×2, -CH_2-×2), 1.34-1.25 (m, 2H×6, -CH_2-×6), 0.88 (t, J = 6.5 Hz, 3H×2, CH_3×2). ^{13}C NMR ($CDCl_3$) δ 156.73, 148.34, 127.98, 117.52 (pyridazine), 133.90, 112.04, 33.82 (allyl), 48.96, 31.73, 27.52, 26.73, 22.67, 14.04 (dihexyl). FT-IR (NaCl) cm^{-1}: 3338, 3051, 1601, 1449, 1265. GC-MS *m/z* (%) 251.4 (M+) 236.1 (100.0), 237.1 (15.2), 251.1 (15.2), 152.0 (7.1), 238.1 (5.6).

Materials and Methods for Bioassays.

Cell Lines Culture Conditions: MCF-7 breast cancer cells were purchased from the ATCC (Manassas, USA), and were maintained at 37 ºC in a humidified atmosphere, with 5% CO_2, in MEM (Gibco-BRL Inc.) medium supplemented with 10% fetal bovin serum (Gibco-BRL Inc., Korea).

Antiproliferative CCK-8 (Cell counting kit-8) Assays.[15] The cytotoxic activity of 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 5 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 cells were treated with various concentrations of synthetic compounds (the final concentrations of **4-15** were 6.25, 25, 100, and 400 μg/mL with MCF-7 cells) 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 3 h. The absorbance at 450 nm was measured by a micro ELISA reader (ASYS Biotech, Cambridge, BK). The cell viability ratio was worked out as follows: (test group A_{450}/control group A_{450}) × 100%. IC_{50} values were determined from three independent experiments.

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