Synthesis of Novel Allylthio Heterocyclo(or aryl)alkylaminopyridazines and Their Anticancer Activity against SK-Hep-1 Cells

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To develop new anticancer agents, 3-allylthio-6-aminopyridazine derivatives were synthesized from maleic anhydrides or phthalic anhydrides by formation of a pyridazine nucleus, dichlorination, allylthiolation and amination. The pyridazine nuclei were obtained by condensing a hydrazine monohydrate with maleic anhydride. An allylthio group as a pharmacologically active group was introduced into one side of a pyridazine ring. Arylalkylamines with benzene or pyridine moieties or heterocycloalkylamines with heterocycle moieties such as morpholine, piperidine, or pyrrolidine were also introduced into the *para*-position of allylthio pyridazine. These new compounds showed antiproliferative activities against SK-Hep-1 human liver cancer cells in MTT assays. These compounds are thus promising candidates for chemotherapy of hepatocellular carcinomas. Two compounds, **20c** and **22a**, showed higher potencies for inhibiting growth of hepatocellular carcinoma cells than did K6 (ID_{50}=1.08 mM). This suggests the potential anticancer activity of these two compounds.

Key Words: Allylthiopyridazines, Aminopyridazines, Amination, Anticancer activity, SK-Hep-1

Introduction

The allylthio group of allicin and other organosulfur compounds that are isolated from garlic is considered to be an important pharmacophore, a key structural component of the molecules that are 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 and their biological activities tested.[1a-1d] K-Compounds and thio-K-compounds showed especially good hepatoprotective and antitumor activities (Fig. 1).[1d]

The pyridazine group is an important moiety present in many drugs acting at various pharmacological targets.[2-6] This moiety was combined with an allylthio group. We also developed 3- allylthio-6-aminopyridazine derivatives (amino-K-compounds) **19~23** using a modification of this method. We tested the ability of our synthetic compounds to inhibit the growth of a SK-Hep-1 cell line.

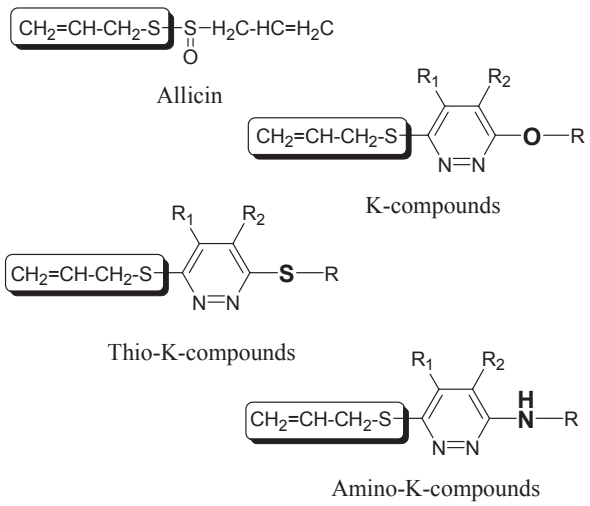


Figure 1. Reported alkoxy(or alkylthio)allylthiopyridazines

The isosteric replacement of the exo oxygen (or sulfur) of K-compounds by a nitrogen atom yields the aminopyridazines (Fig. 1). We have recently reported the synthesis of 3-alkylcarboxamidyl-6-chloropyridazines through amination and acylation,[7a] and allylthioheterocyclopyridazines (as tertiary amines).[7b]

Kwon *et al.* reported the synthesis of 3-allylthio-6-heterocyclylaminopyridazines and their antitumor activities against SK-Hep-1 human liver cancer cells.[8] We then became interested in synthesis of aminopyridazines through coupling of pyridazinyl chloride with amines known to give new amino-K-compounds (Fig. 2).

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

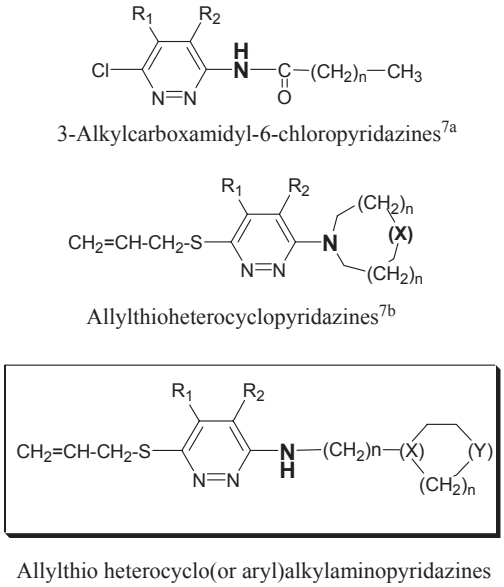


Figure 2. Amino-K-compounds and Target Compounds

essential for the preparation of pharmaceuticals. Many reports have been published on the nucleophilic amination of aryl halides.[10]

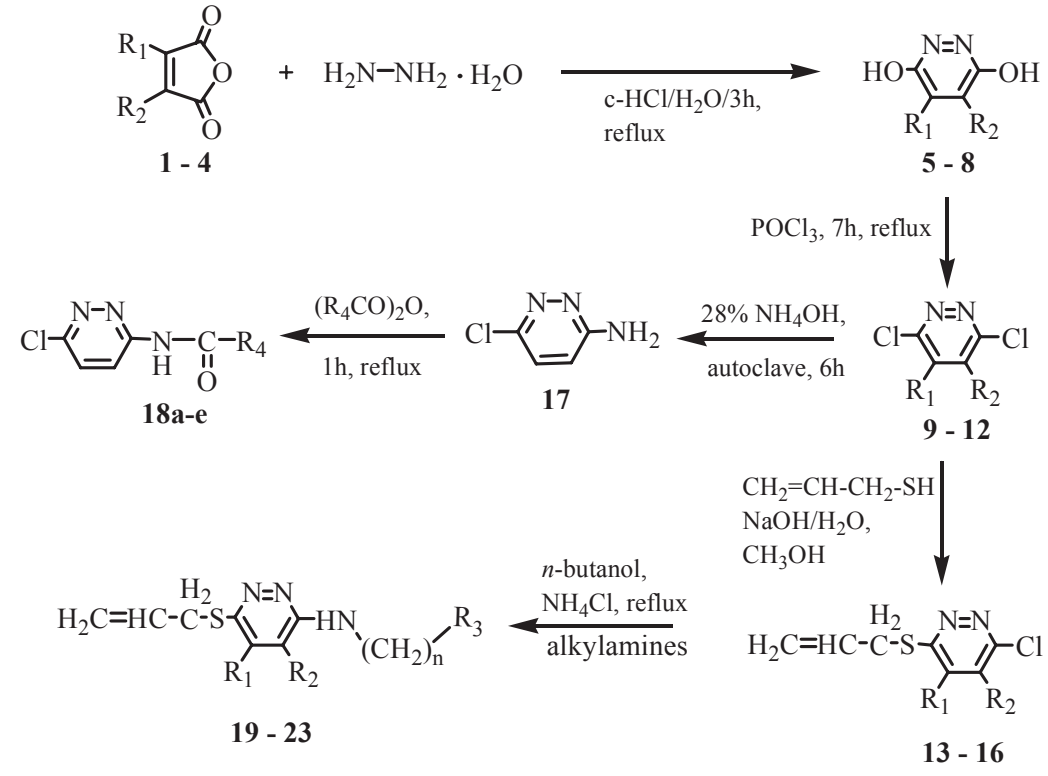
Even though the synthetic pathway for 3-aminopyridazines was developed by Wermuth *et al*.,[5] Contreras *et al*.,[3,6b] and Parrot *et al*.,[9] the synthesis of heterocyclo(or aryl)alkylaminopyridazines has not been reported until now. We applied a general method of preparing aminopyridazines from pyridazinyl halides and amines.[5] The key intermediates in these preparation are pyridazinyl chlorides **13~16**, which can be readily obtained from the corresponding 3,6-dichloropyridazine **9~12** by reaction with allylmercaptan. Condensation of the pyridazinyl chlorides **13~16** with various amines gave the final products **19~23** (Table 1). In order to investigate the potential anticancer activity of the twentytwo synthetic compounds we created, the growth-inhibitory effect of these synthetic compounds was examined on SK-Hep-1 hepatocarcinoma cells. *N*-Substituted-3-amino-6-chloropyridazines **18a~e** were prepared through amination and acylation from 3, 6-dichloropyridazine.[7a]

Results and Discussion

A series of 3-allylthio-6-heterocycloalkylaminopyridazines **19~23** was prepared by formation of a pyridazine nucleus, dichlorination, allylthiolation and nucleophilic substitution. The alkylamines with benzene, pyridine and heterocycle -such as pyridine, morphorine, piperidine and pyrrolidine- were introduced into the 6-position of the pyridazine ring (Scheme 1). Here, we present our results concerning the substitution reaction of 3-allylthiopyridazine by alkylamines, which produced 3-allylthio-6-heterocycloalkylaminopyridazines **19~23**. In Table 1, we summarize the physical properties and the optimal condition for synthesizing compounds **19~23.**

3-Allylthio-6-chloropyridazines **13~16** were converted to final alkylaminopyridazines **19~23** by nucleophilic aromatic substitution with alkylamines in the presence of ammonium chloride (Scheme 2). The ammonium chloride assisted coupling of various alkylamines with 3-allylthio-6-chloropyridazines **13~16** resulted in nucleophilic substitution. The amination reactions of chloropyridazine **13~16** with a range of amines are shown in Table 1. The alkyl ligand between the nitrogen and the ring cycle was increased in carbon length to four; methyl, ethyl, propyl and butyl.

The nucleophilic displacement of chlorine in 3-allylthio-6-chloropyridazine **13~16** requires a prolonged reaction time at the reflux temperature of *n*-butanol. A typical reaction was that a mixture of amines (12mmol), 6-allylthio-3-chloro-



Comp.	R_1	R_2
19a-b, 20a-b, 21a-d, 22a, 23a	H	H
19c-d, 20c-d, 21e-h, 22b, 23b	CH_3-	CH_3-
19e-f, 20e-f, 21i-l, 22c	-$(CH_2)_4$-	
21m-n	-$(CH)_4$-	

R_3 = pyridine, morpholine, benzene, piperidine, pyrrolidine

Comp.	R_1	R_2
1, 5, 9, 13	H	H
2, 6, 10, 14	CH_3-	CH_3-
3, 7, 11, 15	-$(CH_2)_4$-	
4, 8, 12, 16	-$(CH)_4$-	

R_4 = methyl, ethyl, propyl, butyl, pentyl

Scheme 1. Synthesis of 3-allylthio-6-aminopyridazine derivatives **19-23**

Table 1. Target Compounds **19a-f～23a-b** and Optimal Reaction Conditions.

Comp.	R_1	R_2	R_3	n	Time / h[a]	mp / ℃	Yield / %[b]	Molar Ratio[c] Reag./Subs.
19a	H	H	pyridine	1	48	94-98	20	2
19b	H	H	pyridine	2	48	oil	8	2
19c	CH_3	CH_3	pyridine	1	24	97-98	60	3
19d	CH_3	CH_3	pyridine	2	65	oil	9	3
19e	-$(CH_2)_4$-		pyridine	1	48	60-64	74	3
19f	-$(CH_2)_4$-		pyridine	2	48	oil	15	2.8
20a	H	H	morpholine	2	48	57-59	16	3
20b	H	H	morpholine	3	30	49-51	14	3
20c	CH_3	CH_3	morpholine	2	72	74-75	12	2
20d	CH_3	CH_3	morpholine	3	72	oil	33	2
20e	-$(CH_2)_4$-		morpholine	2	48	67-69	47	3
20f	-$(CH_2)_4$-		morpholine	3	48	oil	56	3
21a	H	H	benzene	1	48	66-67	29	3
21b	H	H	benzene	2	30	48-50	39	3
21c	H	H	benzene	3	48	39	36	2.1
21d	H	H	benzene	4	48	56-57	42	3
21e	CH_3	CH_3	benzene	1	72	oil	15	3
21f	CH_3	CH_3	benzene	2	72	oil	37	3
21g	CH_3	CH_3	benzene	3	96	oil	6	2
21h	CH_3	CH_3	benzene	4	96	30-31	46	3
21i	-$(CH_2)_4$-		benzene	1	48	92-93	67	3
21j	-$(CH_2)_4$-		benzene	2	48	57-59	60	3
21k	-$(CH_2)_4$-		benzene	3	48	oil	51	3
21l	-$(CH_2)_4$-		benzene	4	48	oil	50	3
21m	-$(CH)_4$-		benzene	1	48	oil	13	3
21n	-$(CH)_4$-		benzene	2	48	oil	11	3
22a	H	H	piperidine	2	48	34-36	27	3
22b	CH_3	CH_3	piperidine	2	48	oil	23	3
22c	-$(CH_2)_4$-		piperidine	2	48	58-59	25	3.4
23a	H	H	pyrrolidine	2	48	70-75	18	3
23b	CH_3	CH_3	pyrrolidine	2	72	51-54	62	3

[a]All reactions were performed in *n*-butanol under reflux. [b]Yields referred to isolated product. [c]Reag./Subs. is the ratio of reagent (alkylamine) to substrate (chloropyridazines **13-16**).

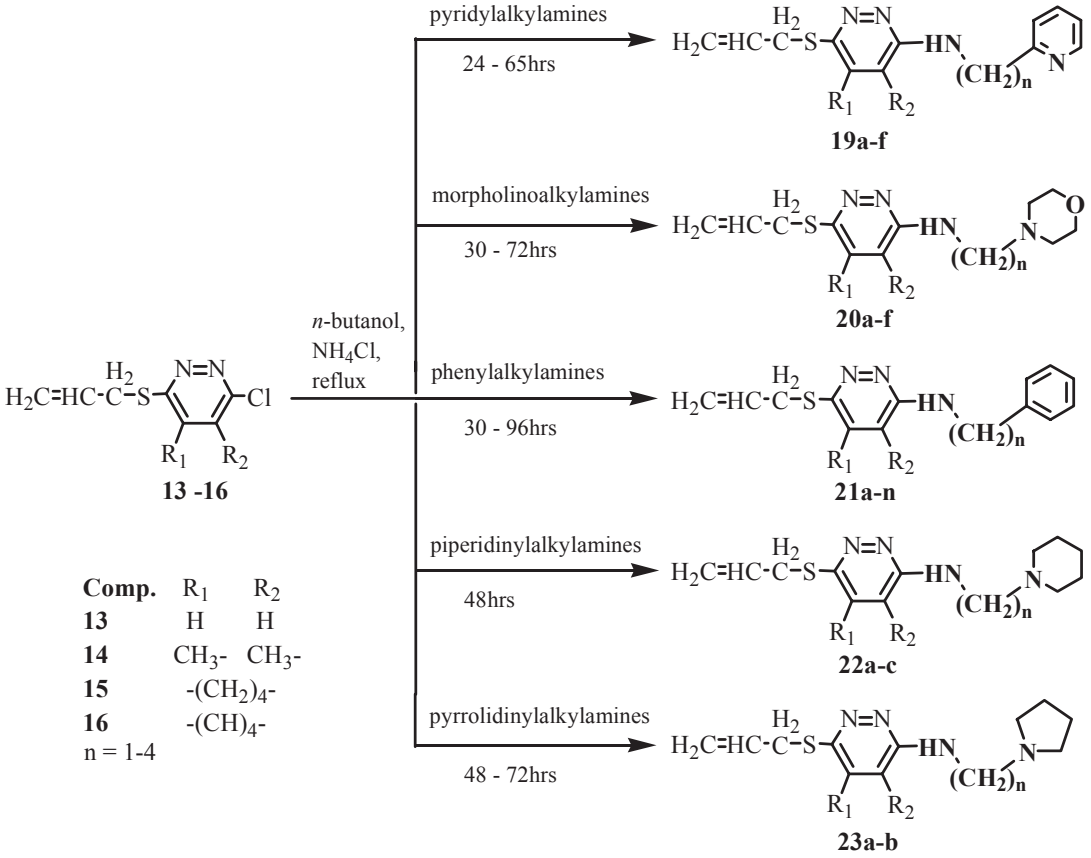
pyridazine (4mmol), and ammonium chloride (4mmol) in *n*-butanol were stirred under reflux for 24-96h. The reaction was usually carried out using 1: 3 equivalents of 3-allylthio- 6-chloropyridazine: alkylamines.

In the proposed mechanism-a substitution reaction of an amine nucleophile-the amine is added to the pyridazine nucleus to form a tertiary ammonium intermediate and proton transfer from nitrogen to chloride produces 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 **13~16** were converted to compounds **19~23** by eliminating the hydrochloride.

The mono-allylthiolation from 3,6-dichloropyridazines **9~12** to 3-allylthio-6-chloropyridazine **13~16** gave high yields. Reactions of dichloropyridazines with allylmercaptan occurred in yields of more than 91%. Pyridazine halides and pyridylalkylamines were reacted in the presence of ammonium chloride in *n*-butanol to form the corresponding products in yields of 74% (Table 1, entry **19e)**. Similarly, morpholinoalkylamines, phenylalkylamines, piperidinylalkylamines and pyrrolidinylamines were converted into the corresponding aminopyridazine derivatives in somewhat lower yields (Table 1, entries **20~23**).

The pyridazine NMR peak of **19~23** appeared at 6.45-6.66 and 6.94-7.04 ppm, and the allyl peak appeared at 3.87-3.96, 5.08-5.10, 5.25-5.31, and 5.92-6.15 ppm. The NH NMR peak appeared at 5.29-5.88 ppm (**19a~19f),** 4.48-5.80 ppm (**20a~20f**), 4.04-4.99 ppm (**21a~21n**), 5.08-5.40 ppm (**22a~22c**) and 5.08-5.20 ppm (**23a~23b**) as a broad singlet signal. The pyridazine ^{13}C NMR peak appeared at 128, 136, 151, and 156 ppm, and the allyl peak appeared at 46, 115, and 133 ppm. In the FT-IR spectrum, an NH absorption band appeared at 3367~3400 cm^{-1}.

Finally, we synthesized new 3-allylthio-6-alkylaminopyridazine derivatives in order to discover a potential antitumor candidate. The refluxing of 3-allylthio-6-chloropyridazines and the corresponding amines such as pyridylalkylamines, morpholinoalkylamines, phenylalkylamines, piperidinylalkylamines and pyrrolidinylalkylamines for about 24-96h produced the



Scheme 2. Synthesis of allylthioaminopyridazines **19-23** from allylthiochloropyridazines **13-16.**

target amino-K-compounds (Scheme 2).

In order to investigate the potential anti-cancer activity of the twenty-two synthetic compounds, the growth-inhibitory effect of the synthetic compounds was examined against SK-Hep-1 hepatocarcinoma cells. MTT assays were conducted on the cells treated with various concentrations of the compounds. K6, which has previously been shown to have anti-proliferative activity against SK-Hep-1 cells[1d,1e] was used as a positive control. Of twenty-two compounds tested, seven (**18b, 19c, 20a, 20b, 20c, 22a** and **23a**) showed dose-dependent inhibitory effects against the growth of SK-Hep-1 cells (Fig. 3).

We further investigated the anti-proliferative activity of four compounds (**20a, 20c, 22a** and **23a**) that caused a higher inhibition of cell growth than the other compounds. As shown in Fig. 4, these compounds caused a marked inhibition of SK-Hep-1 cell growth in a dose-dependent manner. The highest inhibition was observed by **20c** and the lowest inhibition by **20a**. The IC_{50} values for **20c, 22a**, and **23a** for inhibiting

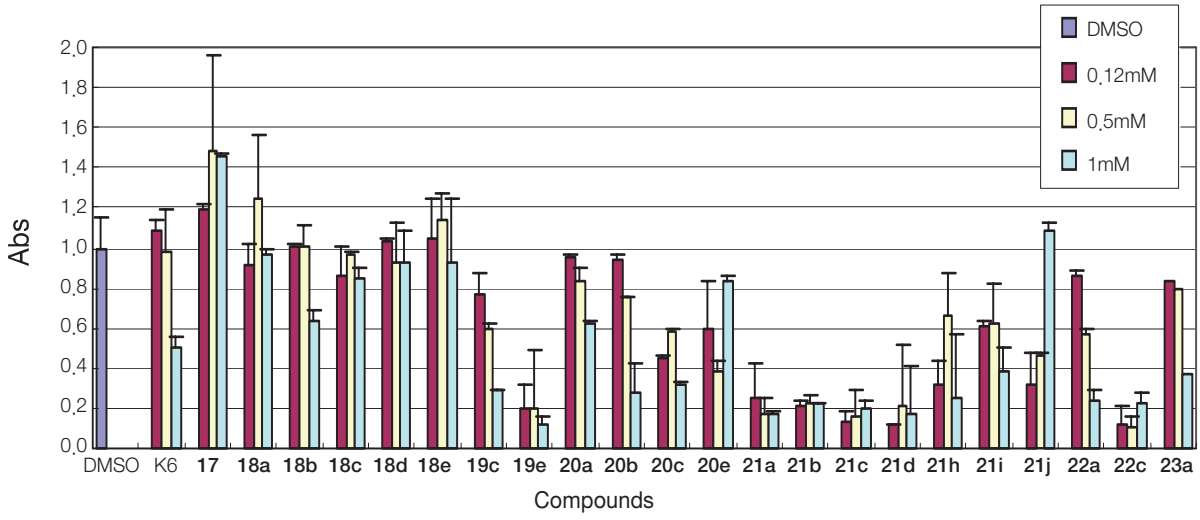


Figure 3. Anticancer activity of synthesized compounds

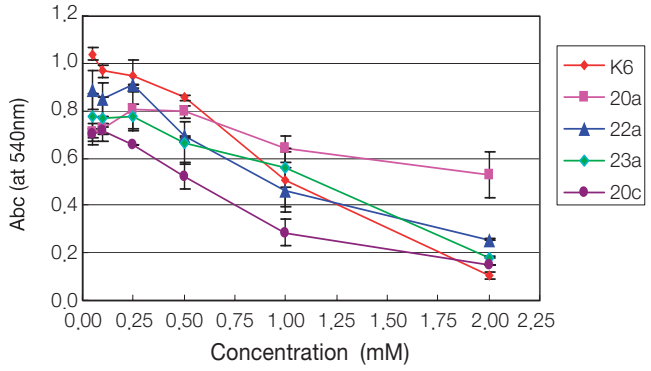


Figure 4. Comparison of K6 and allylthioaminoalkylpyridazine compounds

SK-Hep-1 cell growth were approximately 0.56, 0.92 and 1.11 mM, respectively. The IC_{50} value of **20a** could not be determined by the concentration range used in this study. Two compounds, **20c** and **22a**, showed higher potencies in inhibiting the growth hepatocellular carcinoma cells than did K6 (1.08 mM), suggesting the potential anticancer activity of these two compounds.

Experimental Section

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

General Synthetic Procedure for Compounds 19a-f ~ 23a-b. A solution of 3-allylthio-6-chloropyridazine (1.12g, 6mmol) and the appropriate amine (12mmol) and ammonium chloride (0.32g, 6mmol) in *n*-butanol (30mL) was refluxed for 24-96h. The solvent was evaporated under reduced pressure. The residue was resolved with 10% K_2CO_3 solution and extracted with ethyl acetate. The organic layer was extracted with 10% citric acid solution. After the water layer was neutralized by pH 8-9 with K_2CO_3, 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-[(2-pyridyl)methylamino]pyridazine (19a): Yield: 20%, mp 94-98℃. ^{1}H NMR ($CDCl_3$) δ 8.55 (d, *J*=4.5 Hz, 1H, CH, pyridine), 7.66 (t, *J*=7.5Hz, 1H, CH, pyridine), 7.29 (d, *J*=7.8Hz, 1H, CH, pyridine), 7.20 (t, *J*=6.0Hz, 1H, CH, pyridine), 7.04 (d, *J*=9.0Hz, 1H, CH, pyridazine), 6.66 (d, *J*=9.3Hz, 1H, CH, pyridazine), 5.93-6.08 (m, 1H, =CH), 5.88 (s, 1H, NH), 5.27 (d, *J*=16.9Hz, 1H, CH_2=), 5.10 (d, *J*=10.2Hz, 1H, CH_2=), 4.78 (d, *J*=5.1Hz, 2H, CH_2), 3.90 (d, *J*=6.9Hz, 2H, SCH_2). ^{13}C NMR ($CDCl_3$) δ 156.93, 148.92, 122.33, 122.25, 117.70 (pyridine), 156.62, 151.07, 136.73, 128.11 (pyridazine), 133.76 (=CH), 115.62 (CH_2=), 46.54 (SCH_2), 33.58 ($NHCH_2$). FT-IR (NaCl) cm^{-1} 3399 (N-H), 3053 (aromatic), 1594 (N=N), 1421 (C=N), 1265 (C-N). GC-MS *m/z*(%) 93.10 (100.00), 243.00 (54.92), 39.10 (49.07), 92.10 (37.73), 107.10 (27.91).

3-Allylthio-6-[(2-pyridyl)ethyl)amino]pyridazine (19b): Yield: 8%. ^{1}H NMR ($CDCl_3$) δ 8.51 (d, *J*=4.5Hz, 1H, CH, pyridine), 7.59 (d, *J*=7.8Hz, 1H, CH, pyridine), 7.01-7.19 (m, 1H×2, CH×2, pyridine), 6.94 (d, *J*=9.1Hz, 1H, CH, pyridazine), 6.56 (d, *J*=9.3Hz, 1H, CH, pyridazine), 5.92-6.04 (m, 1H, =CH), 5.59 (s, 1H, NH), 5.25 (d, *J*=17.4Hz, 1H, CH_2=), 5.08 (d, *J*=10.2Hz, 1H, CH_2=), 3.84-3.89 (m, 2H×2, CH_2×2), 3.12 (t, *J*=6.6Hz, 2H, SCH_2). ^{13}C NMR ($CDCl_3$) δ 159.699, 149.11, 123.56, 121.52, 117.63 (pyridine), 156.99, 149.43, 136.61, 128.06 (pyridazine), 1343.73 (=CH), 115.27 (CH_2=), 41.24 (SCH_2), 36.55, 33.60 (CH_2×2).

3-Allylthio-4,5-dimethyl-6-[(2-pyridyl)methylamino]pyridazine (19c): Yield: 60%, mp 97-98℃. ^{1}H NMR ($CDCl_3$) δ 8.57 (d, *J*=4.7Hz, 1H, CH, pyridine), 7.63-7.68 (m, 1H, CH, pyridine), 7.33 (d, *J*=7.6Hz, 1H, CH, pyridine), 7.20 (t, *J*=2.7Hz, 1H, CH, pyridine), 6.05-6.07 (m, 1H, =CH), 5.79 (s, 1H, NH), 5.31 (d, *J*=16.9Hz, 1H, CH_2=), 5.10 (d, *J*=9.9Hz, 1H, CH_2=), 4.84 (d, *J*=4.4Hz, 2H, CH_2), 3.96 (d, *J*=6.9Hz, 2H, SCH_2), 2.18 (s, 3H, CH_3), 2.06 (s, 3H, CH_3) . ^{13}C NMR ($CDCl_3$) δ 157.52, 152.12, 122.76, 122.58, 122.52 (pyridine), 156.11, 152.12, 136.92, 133.95 (pyridazine), 134.48 (=CH), 117.83 (CH_2=), 46.98 ($NHCH_2$), 33.58 (SCH_2), 14.86 (CH_3), 12.26 (CH_3). FT-IR (NaCl) cm^{-1} 3400 (N-H), 3053 (aromatic), 1543 (N=N), 1265 (C-N). GC-MS *m/z*(%) 271.10 (100.00), 93.10 (64.93), 107.10 (62.22), 272.10 (45.86), 92.10 (45.43).

3-Allylthio-4,5-dimethyl-6-[(2-pyridyl)ethylaminopyridazine (19d): Yield: 9%. ^{1}H NMR ($CDCl_3$) δ 8.53(d, *J*=4.1Hz, 1H, CH, pyridine), 7.60(m, 1H, CH, pyridine), 7.12-7.20(m, 1H×2, CH×2, pyridine), 6.00-6.10(m, 1H, =CH), 5.44(s, 1H, NH), 5.29(d, *J*=16.9Hz, 1H, CH_2=), 5.09(d, *J*=9.5Hz, 1H, CH_2=), 3.91-3.95(m, 2H×2, CH_2×2), 3.15(t, *J*=6.1Hz, 2H, CH_2), 2.18 (s, 3H, CH_3), 2.05 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 160.88, 149.31, 123.90, 122.43, 121.84(pyridine), 156.42, 151.61, 137.05, 133.72(pyridazine), 134.50(=CH), 117.73(CH_2=), 41.91 ($NHCH_2$), 36.88(CH_2), 33.56(SCH_2), 14.81(CH_3), 12.11(CH_3). FT-IR (NaCl) cm^{-1} 3367(N-H), 3052(aromatic), 1550(N=N), 1265(C-N). GC-MS *m/z*(%) 192.10(100.00), 285.20(100.00), 106.10(87.90), 93.10(52.25), 286.10(40.66).

1-Allylthio-4-[(2-pyridyl)methylamino]-5,6,7,8-tetrahydrophthalazine (19e): Yield: 74%, mp 60-62℃. ^{1}H NMR ($CDCl_3$) δ 8.54(s, 1H, CH, pyridine), 7.63(t, *J*=2.4Hz, 1H, CH, pyridine), 7.31(t, *J*=5.4Hz, 1H, CH, pyridine), 7.17(t, *J*=3.3Hz, 1H, CH, pyridine), 6.00-6.10(m, 1H, =CH), 5.73(s, 1H, NH), 5.29(d, *J*=17.1Hz, 1H, CH_2=), 5.09(d, *J*=9.3Hz, 1H, CH_2=), 4.84(d, *J*=3.9Hz, 2H, $NHCH_2$), 3.95(d, *J*=5.7Hz, 2H, SCH_2), 2.46(s, 2H×2, CH_2×2), 1.80(s, 2H×2, CH_2×2). ^{13}C NMR ($CDCl_3$) δ 157.55, 155.63, 136.86, 135.11(pyridazine), 151.72, 149.13, 123.69, 122.67, 122.46(pyridine), 134.54(=CH), 117.72(CH_2=), 46.72($NHCH_2$), 33.00(SCH_2), 22.02, 22.81, 21.66, 21.57(CH_2×4). FT-IR (NaCl) cm^{-1} 3395(N-H), 3051(aromatic), 1265(C-N)). GC-MS *m/z*(%) 297.10(100.00), 272.10(96.43), 107.10(62.28), 93.10(60.71), 92.10(41.43).

1-Allylthio-4-[(2-pyridyl)ethylamino]-5,6,7,8-tetrahydrophthalazine (19f): Yield: 15%. ^{1}H NMR ($CDCl_3$) δ 8.53 (d,

J=3.9Hz, 1H, CH, pyridine), 7.59(t, *J*=3.9Hz, 1H, CH, pyridine), 7.13-7.20(m, 1H×2, CH×2, pyridine), 6.04-6.15 (m, 1H, =CH), 5.29(d, *J*=16.8Hz, 1H×2, CH+NH), 5.09(d, *J*=10.2Hz, 1H, CH), 3.93(t, *J*=6.9Hz, 4H, NH-CH_2×2), 3.15(t, *J*=6.0Hz, 2H, CH_2), 1.75(s, 2H×2, CH_2×2). ^{13}C NMR ($CDCl_3$) δ 160.84, 155.95, 137.03, 134.98(pyridazine), 151.30, 149.35, 123.91, 123.53, 121.83(pyridine), 134.59(=CH), 117.68 (CH_2=), 41.68($NHCH_2$), 36.99(CH_2), 33.02(SCH_2), 25.01, 22.71, 21.72, 21.55(CH_2×4). FT-IR (NaCl) cm^{-1} 3368(N-H), 3053(aromatic), 1551(N=N), 1265(C-N). GC-MS *m/z*(%) 106.10(100.00), 311.10(82.28), 93.10(51.67), 286.10(49.46), 218.10(44.78).

3-Allylthio-6-morpholinoethylaminopyridazine (20a): Yield: 16%, mp 57-59℃. ^{1}H NMR ($CDCl_3$) δ 7.03(d, *J*=9.3Hz, 1H, CH, pyridazine), 6.60(d, *J*=6.6Hz, 1H, CH, pyridazine), 5.94-6.00(m, 1H, =CH), 5.22-5.29(m, 2H, NH+CH_2=), 5.08 (d, *J*=9.9Hz, 1H, CH_2=), 3.87(d, *J*=6.9Hz, 2H, SCH_2), 3.70 (m, 2H×2, CH_2×2, morpholine), 3.51(q, *J*=11.7Hz, 2H, CH_2), 2.64(t, *J*=6.0Hz, 2H, CH_2), 2.48(m, 2H×2, CH_2×2, morpholine). ^{13}C NMR ($CDCl_3$) δ 156.38, 133.69, 117.65 (pyridazine), 128.10(=CH), 115.25(CH_2=), 66.85, 53.30 (morpholine), 56.92 (CH_2), 37.80(CH_2), 33.63(SCH_2), 14.84(CH_3), 12.13(CH_3). FT-IR (NaCl) cm^{-1} 3434(N-H), 3054(aromatic), 1550(N=N), 1421(C=N), 1265(C-N). GC-MS *m/z*(%) 100.10 (100.00), 56.10(27.64), 42.10(22.97), 70.10(22.62), 113.10 (16.27).

3-Allylthio-6-morpholinopropylaminopyridazine(20b): Yield: 14 %, mp 49-51℃. ^{1}H NMR ($CDCl_3$) δ 7.02(d, *J*=9.3 Hz, 1H, CH, pyridazine), 6.52(d, *J*=9.0Hz, 1H, CH, pyridazine), 5.93-6.06(m, 1H, =CH), 5.78(s, 1H, NH), 5.25(d, *J*=16.2Hz, 1H, CH_2=), 5.08(d, *J*=9.9Hz, 1H, CH_2=), 3.88(d, *J*=7.2Hz, 2H, SCH_2), 3.72(m, 2H×2, CH_2×2, morpholine), 3.46-3.53 (m, 2H, CH_2), 2.45-2.52(m, 2H×2, CH_2×2, morpholine), 1.78-1.86(m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ 157.23, 150.27, 133.77, 117.64(pyridazine), 128.17(=CH), 114.75(CH_2=), 67.07, 53.73(morpholine), 57.54(CH_2), 41.50(CH_2), 25.03CH_2), 33.69(SCH_2). FT-IR (NaCl) cm^{-1} 3435(N-H), 3053(aromatic), 1552(N=N), 1421(C=N), 1265(C-N). GC-MS *m/z*(%) 100.10 (100.00), 166.00(67.51), 42.10(63.16), 56.10(56.40), 41.20 (49.08).

3-Allylthio-4,5-dimethyl-6-morpholinoethylaminopyridazine (20c): Yield: 12%, mp 74-75℃. ^{1}H NMR ($CDCl_3$) δ 6.00-6.09(m, 1H, =CH), 5.30(d, *J*=16.8Hz, 1H, CH_2=), 5.10 (d, *J*=10.8Hz, 1H, CH_2=), 4.93(s, 1H, NH), 3.94(d, *J*=6.9Hz, 2H, SCH_2), 3.71(m, 2H×2, CH_2×2, morpholine), 3.59(q, *J*=11.1Hz, 2H, CH_2), 2.68(t, *J*=5.8Hz, 2H, CH_2), 2.50(m, 2H×2, CH_2×2, morpholine), 2.19(s, 3H, CH_3), 2.05(s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 156.46, 151.96, 133.83, 122.23 (pyridazine), 134.46(=CH), 117.80(CH_2=), 67.52, 53.59 (morpholine), 57.11(CH_2), 38.33(CH_2), 33.54 (SCH_2), 14.84(CH_3), 12.13(CH_3). FT-IR(NaCl) cm^{-1} 3400 (N-H), 3053(aromatic), 1265(C-N). GC-MS *m/z*(%) 100.20 (100.00), 196.10(68.35), 180.10(53.25), 113.10(40.03), 293.10 (35.98).

3-Allylthio-4,5-dimethyl-6-morpholinopropylaminopyridazine (20d): Yield: 33%. ^{1}H NMR ($CDCl_3$) δ 5.98-6.12 (m, 1H, =CH), 5.69(s, 1H, NH), 5.29(d, *J*=16.8Hz, 1H, CH_2=), 5.09(d, *J*=9.9Hz, 1H, CH_2=), 3.94(d, *J*=6.9Hz, 2H, SCH_2), 3.73(m, 2H×2, CH_2×2, morpholine), 3.62(s, 2H, $NHCH_2$), 2.51-2.63(m, 2H×2, CH_2×2, morpholine), 2.18(s, 3H, CH_3), 2.06(s, 3H, CH_3), 1.83-1.91(m, 2H, -CH_2-). ^{13}C NMR ($CDCl_3$) δ 156.62, 151.49, 133.62, 122.05(pyridazine), 134.53(=CH), 117.73(CH_2=), 67.28, 54.37(morpholine), 59.22(CH_2), 43.17(CH_2), 24.72(CH_2), 33.55(SCH_2), 14.83(CH_3), 12.88(CH_3). FT-IR (NaCl) cm^{-1} 3053(aromatic), 1550(N=N), 1265(C-N). GC-MS *m/z*(%) 196.10(100.00), 307.10(72.27), 100.10(49.39), 222.10 (48.80), 196.10(23.90).

1-Allylthio-4-morpholinoethylamino-5,6,7,8-tetrahydrophthalazine (20e): Yield: 47%, mp 67-69℃. ^{1}H NMR ($CDCl_3$) δ 6.00-6.06(m, 1H, =CH), 5.29(d, *J*=16.5Hz, 1H, CH_2=), 5.09 (d, *J*=9.9Hz, 1H, CH_2=), 4.88(s, 1H, NH), 3.94(d, *J*=7.2Hz, 2H, SCH_2), 3.71(m, 2H×2, CH_2×2, morpholine), 3.59(q, *J*=5.7Hz, 2H, $NHCH_2$), 2.67(t, *J*=6.0Hz, 2H, CH_2-N), 2.49 (m, 2H×2, CH_2×2, morpholine), 2.31(t, *J*=5.7Hz, 2H, CH_2), 1.78-1.86(m, 2H×2, CH_2×2). ^{13}C NMR ($CDCl_3$) δ 155.97, 151.56, 135.04, 123.38(pyridazine), 134.53(=CH), 117.73 (CH_2=), 67.47, 53.59(morpholine), 57.16(CH_2), 38.06 (CH_2), 32.97(SCH_2), 25.00, 22.73, 21.70, 21.55(CH_2×4). FT-IR (NaCl) cm^{-1} 3400(N-H), 3052(aromatic), 1543(N=N), 1265 (C-N). GC-MS *m/z*(%) 100.20(100.00), 222.10(62.73), 206.10 (56.53), 319.20(34.36), 234.10(25.25).

1-Allylthio-4-morpholinopropylamino-5,6,7,8-tetrahydrophthalazine (20f): Yield: 56%. ^{1}H NMR ($CDCl_3$) δ 6.00- 6.06 (m, 1H, =CH), 5.60(s, 1H, NH), 5.29(d, *J*=16.2Hz, 1H, CH_2=), 5.08(d, *J*=9.9Hz, 1H, CH_2=), 3.94(d, *J*=7.2Hz, 2H, SCH_2), 3.73(m, 2H×2, CH_2×2, morpholine), 3.61(q, *J*=11.1Hz, 2H, $NHCH_2$), 2.46-2.55(m, 2H×2, CH_2×2, morpholine), 2.31(t, *J*=5.7Hz, 2H, CH_2), 1.77-1.90(m, 2H×3, CH_2×3). ^{13}C NMR ($CDCl_3$) δ 156.14, 151.13, 134.82, 123.16(pyridazine), 134.60 (=CH), 117.67(CH_2=), 67.33, 54.34(morpholine), 59.10 (CH_2), 42.82(CH_2), 33.00(SCH_2), 24.97(CH_2), 24.76, 23.65, 21.67, 21.58(CH_2×4). FT-IR (NaCl) cm^{-1} 3292(N-H), 3051 (aromatic), 1551(N=N), 1265 (C-N). GC-MS *m/z*(%) 333.20 (100.00), 222.10(69.58), 100.10(57.65), 248.10(43.65), 222.10 (30.99).

3-Allylthio-6-phenylmethylaminopyridazine (21a): Yield: 29%, mp 66-67℃. ^{1}H NMR ($CDCl_3$) δ 7.26-7.35(m, 5H, -C_6H_5), 7.03(d, *J*=9.3Hz, 1H, CH, pyridazine), 6.53(d, *J*=9.3Hz, 1H, CH, pyridazine), 5.92-6.06(m, 1H, =CH), 5.26(d, *J*=17.1Hz, 1H, CH_2=), 5.09(d, *J*=10.5Hz, 1H, CH_2=), 4.95(s, 1H, NH), 4.60(d, *J*=5.7Hz, 2H, SCH_2). ^{13}C NMR ($CDCl_3$) δ 157.06, 151.12, 138.51, 133.66(pyridazine), 128.73, 128.29, 127.70, 117.75(C_6H_5), 127.52(=CH), 114.54(CH_2=), 46.13(CH_2), 33.66 (SCH_2). FT-IR (NaCl) cm^{-1} 3435(N-H), 3054(aromatic), 1421(C=N), 1265(C-N)). GC-MS *m/z*(%) 93.10(100.00), 243.00 (66.74), 39.10(48.63), 92.10(39.72), 41.20(30.36).

3-Allylthio-6-phenylethylaminopyridazine (21b): Yield: 39%, mp 48-50℃. ^{1}H NMR ($CDCl_3$) δ 7.20-7.31(m, 5H, -C_6H_5), 7.02(d, *J*=9.3Hz, 1H, CH, pyridazine), 6.46(d, *J*=9.3Hz, 1H, CH, pyridazine), 5.96-6.05(m, 1H, =CH), 5.27(d, *J*=17.1Hz, 1H, CH_2=), 5.10(d, *J*=9.9Hz, 1H, CH_2=), 4.48(s, 1H, NH), 3.90(d, *J*=7.2Hz, 2H, SCH_2), 3.71(q, *J*=12.6Hz, 2H, CH_2), 2.96(t, *J*=6.9Hz, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ 156.95, 150.84, 139.04, 133.72(pyridazine), 128.86, 128.65, 128.22, 117.72 (C_6H_5), 126.51(=CH), 114.75 (CH_2=), 42.95(CH_2), 35.36(CH_2), 33.65(SCH_2). FT-IR (NaCl) cm^{-1} 3434(N-H), 3053(aromatic), 1554(N=N), 1423(C=N), 1265(C-N). GC-MS *m/z*(%) 256.00 (100.00), 91.10(27.48), 105.10(20.34), 79.10(19.86), 152.00 (18.86).

3-Allylthio-6-phenylpropylaminopyridazine (21c): Yield: 36%, mp 39℃. ^{1}H NMR ($CDCl_3$) δ 7.17-7.28(m, 5H, -C_6H_5), 7.02(d, *J*=9.0Hz, 1H, CH, pyridazine), 6.45(d, *J*=9.3Hz, 1H, CH, pyridazine), 5.95-6.01(m, 1H, =CH), 5.26(d, *J*=16.8Hz, 1H, CH_2=), 5.09(d, *J*=9.9Hz, 1H, CH_2=), 4.52(s, 1H, NH), 3.89(d, *J*=6.9Hz, 2H, SCH_2), 3.43(q, *J*=12.9Hz, 2H, CH_2), 2.73(t, *J*=7.8Hz, 2H, CH_2), 1.93-2.03(m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ 157.19, 150.60, 141.47, 133.74(pyridazine), 128.47, 128.40, 128.27, 117.69(C_6H_5), 126.02(=CH), 114.37(CH_2=), 41.58(CH_2), 33.69(CH_2), 33.29(SCH_2), 30.93(CH_2). FT-IR (NaCl) cm^{-1} 3272(N-H), 3052(aromatic), 1601(C=C), 1453 (C=N), 1265(C-N). GC-MS *m/z*(%) 270.10(100.00), 91.10 (49.90), 271.10(19.06), 166.00(15.89), 41.20(10.57).

3-Allylthio-6-phenylbutylaminopyridazine (21d): Yield: 42%, mp 56-57℃. ^{1}H NMR ($CDCl_3$) δ 7.15-7.27(m, 5H, -C_6H_5), 7.02(d, *J*=9.3Hz, 1H, CH, pyridazine), 6.48(d, *J*=9.0Hz, 1H, CH, pyridazine), 5.92-6.06(m, 1H, =CH), 5.26(d, *J*=16.8Hz, 1H, CH_2=), 5.08(d, *J*=9.9Hz, 1H, CH_2=), 4.55(s, 1H, NH), 3.88(d, *J*=6.9Hz, 2H, SCH_2), 3.41(q, *J*=12.3Hz, 2H, CH_2), 2.65(t, *J*=6.9Hz, 2H, CH_2), 1.65-1.73 (m, 2H×2, CH_2×2). ^{13}C NMR ($CDCl_3$) δ 157.21, 150.50, 142.10, 133.75(pyridazine), 128.40, 128.35, 128.26, 117.68 (C_6H_5), 125.83(=CH), 114.41 (CH_2=), 41.91(CH_2), 35.56 (CH_2), 33.70(SCH_2), 29.00(CH_2), 28.77(CH_2). FT-IR (NaCl) cm^{-1} 3291(N-H), 3051(aromatic), 1601(C=C), 1452(C=N), 1265(C-N). GC-MS *m/z*(%) 284.10 (100.00), 91.10(41.47), 285.10(19.51), 152.00(10.69), 299.10 (7.31).

3-Allylthio-4,5-dimethyl-6-phenylmethylaminopyridazine (21e): Yield: 15%. ^{1}H NMR ($CDCl_3$) δ 7.29-7.39(m, 5H, -C_6H_5), 6.01-6.10(m, 1H, =CH), 5.30(d, *J*=16.9Hz, 1H, CH_2=), 5.11(d, *J*=9.7Hz, 1H, CH_2=), 4.73(d, *J*=5.1Hz, 2H, CH_2), 4.23(s, 1H, NH), 3.96(d, *J*=6.9Hz, 2H, SCH_2), 2.19(s, 3H, CH_3), 2.03(s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 155.71, 152.10, 139.33, 134.00(pyridazine), 133.69, 128.66, 128.18, 121.69 (C_6H_5), 127.42(=CH), 117.52(CH_2=), 46.47(CH_2), 33.21(SCH_2), 14.52(CH_3), 11.84(CH_3). FT-IR(NaCl) cm^{-1} 3467(N-H), 3053 (aromatic), 1265(C-N). GC-MS *m/z*(%) 91.10(100.00), 270.20 (100.00), 271.10(57.36), 285.10(35.60), 106.10(31.23).

3-Allylthio-4,5-dimethyl-6-phenylethylaminopyridazine (21f): Yield: 37%. ^{1}H NMR ($CDCl_3$) δ 7.19-7.30(m, 5H, -C_6H_5), 6.01-6.06(m, 1H, =CH), 5.30(d, *J*=17.1Hz, 1H, CH_2=), 5.10 (d, *J*=10.2Hz, 1H, CH_2=), 4.04(s, 1H, NH), 3.95(d, *J*=7.2Hz, 2H, SCH_2), 3.83(s, 2H, $NHCH_2$), 2.98(t, *J*=6.9Hz, 2H, CH_2), 2.16(s, 3H, CH_3), 1.89(s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 155.73, 151.67, 139.61, 134.02(pyridazine), 133.55, 128.86, 128.54, 121.74(C_6H_5), 126.33(=CH), 117.48(CH_2=), 42.92 (CH_2), 35.24(CH_2), 33.23(SCH_2), 14.48(CH_3), 11.60(CH_3). FT-IR (NaCl) cm^{-1} 3462(N-H), 3053(aromatic), 1265(C-N). GC-MS *m/z*(%) 284.20(100.00), 285.10(84.26), 180.10(56.97), 299.10(43.41), 91.10(37.45).

3-Allylthio-4,5-dimethyl-6-phenylpropylaminopyridazine (21g): Yield: 6%. ^{1}H NMR ($CDCl_3$) δ 7.17-7.30(m, 5H, -C_6H_5), 6.00-6.09(m, 1H, =CH), 5.29(d, *J*=16.9Hz, 1H, CH_2=), 5.10(d, *J*=9.9Hz, 1H, CH_2=), 3.94(d, *J*=6.9Hz, 2H, SCH_2), 3.61(q, *J*=11.8Hz, 2H, $NHCH_2$), 2.74(t, *J*=7.5Hz, 2H, CH_2), 2.16(s, 3H, CH_3), 1.94-2.08(m, 2H, $CH_2C_6H_5$), 1.90(s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 156.18, 151.87, 142.30, 134.46(pyridazine), 133.82, 128.84, 128.82, 121.91 (C_6H_5), 126.31(=CH), 117.83(CH_2=), 42.36(CH_2), 34.12(CH_2), 33.59(SCH_2), 31.35 (CH_2), 14.87(CH_3), 12.00(CH_3). FT-IR (NaCl) cm^{-1} 3053 (aromatic), 1552(N=N), 1265(C-N). GC- MS *m/z*(%) 298.10 (100.00), 91.10(23.19), 299.10(20.14), 194.10(10.98), 313.10 (7.52).

3-Allylthio-4,5-dimethyl-6-phenylbutylaminopyridazine (21h): Yield: 46%, mp 30-31℃. ^{1}H NMR ($CDCl_3$) δ 7.15-7.28(m, 5H, -C_6H_5), 5.99-6.08(m, 1H, =CH), 5.28(d, *J*=16.2Hz, 1H, CH_2=), 5.08(d, *J*=9.9Hz, 1H, CH_2=), 3.94(d, *J*=6.9Hz, 2H, SCH_2), 3.56(s, 2H, CH_2), 2.64(s, 2H, CH_2), 2.13(s, 3H, CH_3), 1.97(s, 3H, CH_3), 1.71(t, *J*=3.6Hz, 2H×2, CH_2×2). ^{13}C NMR ($CDCl_3$) δ 155.88, 151.32, 142.28, 134.08(pyridazine), 133.38, 128.38, 128.27, 121.56(C_6H_5), 125.70(=CH), 117.36 (CH_2=), 42.02(CH_2), 35.61(CH_2), 33.17(SCH_2), 29.14 (CH_2), 28.94(CH_2), 14.45(CH_3), 11.72(CH_3). FT-IR(NaCl) cm^{-1} 3459 (N-H), 3052(aromatic), 1265(C-N). GC-MS *m/z*(%) 312.20 (100.00), 313.10(64.45), 91.10(53.48), 180.10(21.76), 327.20 (21.43).

1-Allylthio-4-phenylmethylamino-5,6,7,8-tetrahydrophthalazine (21i): Yield: 67%, mp 92-93℃. ^{1}H NMR ($CDCl_3$) δ 7.30-7.38(m, 5H, -C_6H_5), 5.99-6.10(m, 1H, =CH), 5.30(d, *J*=16.8Hz, 1H, CH_2=), 5.10(d, *J*=9.9Hz, 1H, CH_2=), 4.73(d, *J*=5.1Hz, 2H, CH_2), 4.20(s, 1H, NH), 3.96(d, *J*=7.2Hz, 2H, SCH_2), 2.50(s, 2H, CH_2), 2.28(s, 2H, CH_2), 1.78-1.85(m, 2H×2, CH_2×2). ^{13}C NMR ($CDCl_3$) δ 155.67, 152.13, 139.75, 135.30(pyridazine), 134.50(=CH), 129.27, 129.15, 129.05, 128.56, 127.80, 123.22(C_6H_5), 117.85(CH_2=), 46.60 ($NHCH_2$), 33.06(SCH_2), 25.07, 22.86, 21.67, 21.53(CH_2×4). FT-IR (NaCl) cm^{-1} 3459(N-H), 3052(aromatic), 1550(N=N), 1265 (C-N). GC-MS *m/z*(%) 296.10(100.00), 91.10(37.75), 297.10 (24.59), 106.10(17.99), 271.10(13.52).

1-Allylthio-4-phenylethylamino-5,6,7,8-tetrahydrophthalazine (21j): Yield: 60%, mp 57-59℃. ^{1}H NMR ($CDCl_3$) δ 7.16-7.30(m, 5H, -C_6H_5), 6.03-6.06(m, 1H, =CH), 5.28(d, *J*=16.8Hz, 1H, CH_2=), 5.08(d, *J*=10.2Hz, 1H, CH_2=), 4.04(s, 1H, NH), 3.95(d, *J*=6.9Hz, 2H, SCH_2), 3.80(q, *J*=12.3Hz, 2H, $NHCH_2$), 2.96(t, *J*=6.6Hz, 2H, CH_2), 2.44(s, 2H, CH_2), 2.08(s, 2H, CH_2), 1.72(s, 2H×2, CH_2×2). ^{13}C NMR ($CDCl_3$) δ 155.69, 151.63, 139.98, 135.19(pyridazine), 134.51(=CH), 129.56, 129.21, 129.01, 128.90, 126.98, 123.29(C_6H_5), 117.78 (CH_2=), 43.11($NHCH_2$), 35.67 (CH_2), 33.06(SCH_2), 25.05, 22.58, 21.63, 21.47(CH_2×4). FT-IR (NaCl) cm^{-1} 3442(N-H), 3048(aromatic), 1551(N=N), 1265(C-N). GC-MS *m/z*(%) 181.10(100.00), 194.10(100.00), 310.10(100.00), 285.10(72.24), 91.10(41.69).

1-Allylthio-4-phenylpropylamino-5,6,7,8-tetrahydrophthalazine (21k): Yield: 51%. ^{1}H NMR ($CDCl_3$) δ 7.15-7.28(m, 5H, -C_6H_5), 6.00-6.05(m, 1H, =CH), 5.28(d, *J*=17.1Hz, 1H, CH_2=), 5.08(d, *J*=10.2Hz, 1H, CH_2=), 3.94(d, *J*=6.9Hz, 3H, NH+SCH_2), 3.59(q, *J*=12.9Hz, 2H, $NHCH_2$), 2.72(t, *J*=7.5Hz, 2H, CH_2), 2.44(t, *J*=5.1Hz, 2H, CH_2), 2.01- 2.12(m, 2H×2, CH_2×2), 1.69-1.98(m, 2H×2, CH_2×2). ^{13}C NMR ($CDCl_3$) δ 155.76, 151.42, 142.29, 135.00(pyridazine), 134.75(=CH), 128.80, 126.24, 123.12(C_6H_5), 117.75 (CH_2=), 42.08(CH_2), 34.07(CH_2), 33.04(SCH_2), 31.32(CH_2), 25.02, 22.62, 21.50, 21.45(CH_2×4). FT-IR(NaCl) cm^{-1} 3444(N-H), 3049(aromatic), 1551(N=N), 1265(C-N). GC-MS *m/z*(%) 195.10(100.00), 324.10(96.71), 299.10(56.58), 91.10(55.13), 180.10(37.75).

1-Allylthio-4-phenylbutylamino-5,6,7,8-tetrahydrophthalazine (21l): Yield: 52%. ^{1}H NMR ($CDCl_3$) δ 7.12-7.28(m, 5H, -C_6H_5), 5.98-6.08(m, 1H, =CH), 5.26(d, *J*=16.8Hz, 1H, CH_2=), 5.06(d, *J*=9.9Hz, 1H, CH_2=), 4.08(s, 1H, NH), 4.04(d, *J*=5.7Hz, 2H, SCH_2), 3.53(s, 2H, CH_2), 2.61(s, 2H, CH_2), 2.56(s, 2H, CH_2), 2.18(s, 2H, CH_2), 1.69(s, 2H×4, CH_2×4). ^{13}C NMR ($CDCl_3$) δ 155.85, 151.29, 142.67, 134.95 (pyridazine), 134.59(=CH), 128.77, 128.66, 126.09, 123.13 (C_6H_5), 117.69(CH_2=), 42.18($NHCH_2$), 36.03(CH_2), 33.04 (SCH_2), 29.56(CH_2), 29.37(CH_2), 25.02, 22.74, 21.67, 21.49 (CH_2×4). FT-IR(NaCl) cm^{-1} 3447(N-H), 3049(aromatic), 1550 (N=N), 1265(C-N). GC-MS *m/z*(%) 338.20(100.00), 91.10 (51.77), 313.10(46.17), 181.10(39.34), 222.10(32.55).

1-Allylthio-4-phenypropylaminophthalazine (21m): Yield: 13 %. ^{1}H NMR ($CDCl_3$) δ 8.00-8.03(m, 1H, CH, phthalazine), 7.67-7.76(m, 1H×2, CH×2, phthalazine), 7.50-7.53(m, 1H, CH, phthalazine), 7.18-7.29(m, 5H, C_6H_5), 6.04-6.13(m, 1H, =CH), 5.33(d, *J*=16.8Hz, 1H, CH_2=), 5.12(d, *J*=9.9Hz, 1H, CH_2=), 4.99(s, 1H, NH), 4.06(d, *J*=6.6Hz, 2H, SCH_2), 3.70-3.76(m, 2H, CH_2), 2.78(t, *J*=7.5Hz, 2H, CH_2), 2.06-2.16(m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ 152.70, 150.08, 142.25, 131.75, 121.21, 118.76(phthalazine), 134.24(=CH), 131.68, 128.92, 128.86, 126.89, 126.89, 126.34, 124.84(C_6H_5), 118.13(CH_2=), 33.18(SCH_2), 42.31, 34.19, 31.10(CH_2×3). FT-IR (NaCl) cm^{-1} 3448(N-H), 3053(aromatic), 1547(N=N), 1265(C-N).

1-Allylthio-4-phenylbutylaminophthalazine (21n): Yield: 11%. ^{1}H NMR ($CDCl_3$) δ 7.99-8.03(m, 1H, CH, phthalazine), 7.70-7.78(m, 1H×3, CH×3, phthalazine), 7.10-7.28 (m, 5H, C_6H_5), 6.07-6.09(m, 1H, =CH), 5.33(d, *J*=17.1Hz, 1H, CH_2=), 5.12(d, *J*=9.9Hz, 1H, CH_2=), 4.05(d, *J*=6.9Hz, 2H, SCH_2), 3.70(s, 2H, CH_2), 2.66(t, *J*=6.9Hz, 2H, CH_2), 1.71-1.81(m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ 152.28, 149.65, 142.25, 131.48, 121.05, 118.43(phthalazine), 133.80(=CH), 131.40, 128.42, 128.31, 126.49, 125.75, 124.45(C_6H_5), 117.77(CH_2=), 32.77 (SCH_2), 41.99, 35.60, 28.96, 28.90 (CH_2×4). FT-IR(NaCl) cm^{-1} 3391(N-H), 3051(aromatic), 1265(C-N)).

3-Allylthio-6-piperidinylethylaminopyridazine (22a): Yield: 27%, mp 34-36℃. ^{1}H NMR ($CDCl_3$) δ 7.01(d, *J*=9.3Hz, 1H, CH, pyridazine), 6.58(d, *J*=9.3Hz, 1H, CH, pyridazine), 5.93-6.06(m, 1H, =CH), 5.39(s, 1H, NH), 5.26(d, *J*=17.7Hz, 1H, CH_2=), 5.08(d, *J*=10.2Hz, 1H, CH_2=), 3.88(d, *J*=6.9Hz, 2H, SCH_2), 3.49(q, *J*=11.1Hz, 2H, CH_2), 2.59(t, *J*=6.0Hz, 2H, CH_2), 2.43(s, 2H×2, CH_2×2, piperidine), 1.54-1.60(m, 2H×2, CH_2×2, piperidine), 1.44-1.46(m, 2H, CH_2, piperidine). ^{13}C NMR ($CDCl_3$) δ 157.08, 150.29, 128.00, 117.59 (pyridazine), 133.18(=CH), 115.29(CH_2=), 57.13(CH_2), 54.27, 24.32, 21.04 (piperidine), 38.16(CH_2), 33.61(SCH_2). FT-IR (NaCl) cm^{-1} 3391(N-H), 3053(aromatic), 1544(N=N), 1265(C-N). GC-MS *m/z*(%) 98.20(100.00), 111.10(23.94), 41.20(11.57), 99.10 (9.69), 55.20(9.66).

3-Allylthio-4,5-dimethyl-6-piperidinylethylaminopyridazine (22b): Yield: 23%. ^{1}H NMR ($CDCl_3$) δ 6.00-6.09(m, 1H, =CH), 5.29(d, *J*=16.8Hz, 1H, CH_2=), 5.09(d, *J*=10.5Hz, 1H, CH_2=), 5.19(s, 1H, NH), 3.94(d, *J*=6.9Hz, 2H, SCH_2), 3.56(q, *J*=11.1Hz, 2H, CH_2), 2.64(t, *J*=6.0Hz, 2H, CH_2), 2.44(s, 2H×2, CH_2×2, piperidine), 2.18(s, 3H, CH_3), 2.05(s, 3H, CH_3), 1.55-1.60(m, 2H×2, CH_2×2, piperidine), 1.25(s, 2H, CH_2, piperidine). ^{13}C NMR ($CDCl_3$) δ 156.25, 151.26, 133.30, 122.04(pyridazine), 134.18(=CH), 117.33(CH_2=), 56.82(CH_2), 38.30(CH_2), 54.09, 26.14, 24.37(piperidine), 33.17(SCH_2), 14.44(CH_3), 11.74(CH_3). FT-IR (NaCl) cm^{-1} 3390(N-H), 3053(aromatic), 1539(N=N), 1265(C-N)). GC-MS *m/z*(%) 98.30(100.00), 111.20(100.00), 112.20(33.54), 99.20(32.38), 96.20(27.73).

1-Allylthio-4-piperidinylethylamino-5,6,7,8-tetrahydrophthalazine (22c): Yield: 25%, mp 58-59℃. ^{1}H NMR ($CDCl_3$) δ 6.03-6.06(m, 1H, =CH), 5.29(d, *J*=18.6Hz, 1H, CH_2=), 5.08(d, *J*=9.3Hz, 2H, NH+CH_2=), 3.94(d, *J*=7.2Hz, 2H, SCH_2), 3.55(q, *J*=10.5Hz, 2H, $NHCH_2$), 2.41-2.47(m, 6H, N-CH_2×2+CH_2, piperidine), 2.31(m, 2H, -CH_2-), 1.79-1.84 (m, 4H, CH_2×2, piperidine), 1.44-1.58(m, 6H, CH_2×3). ^{13}C NMR ($CDCl_3$) δ 156.11, 151.18, 134.92, 134.70 (pyridazine), 123.46(=CH), 117.59(CH_2=), 57.23($NHCH_2$), 54.43, 26.54, 24.97(morpholine), 38.44(CH_2), 32.95(SCH_2), 24.77, 22.67, 21.72, 21.56(CH_2×4). FT-IR (NaCl) cm^{-1} 3379(N-H), 3052 (aromatic), 1541(N=N), 1265(C-N). GC-MS *m/z*(%) 98.20 (100.00), 111.20(79.12), 222.10(35.98), 112.20(25.04), 317.20 (23.26).

3-Allylthio-6-pyrrolidinylethylaminopyridazine (23a): Yield: 18%, mp 70-75℃. ^{1}H NMR ($CDCl_3$) δ 7.01(d, *J*=9.3Hz, 1H, CH, pyridazine), 6.55(d, *J*=9.3Hz, 1H, CH, pyridazine), 5.94-6.07(m, 1H, =CH), 5.26(d, *J*=17.1Hz, 1H, CH_2=), 5.20(s, 1H, NH), 5.09(d, *J*=10.2Hz, 1H, CH_2=), 3.89(d, *J*=6.9Hz, 2H, SCH_2), 3.52(q, *J*=11.7Hz, 2H, CH_2), 2.74(t, *J*=6.0Hz, 2H, CH_2), 2.52-2.57(m, 2H×2, CH_2×2, pyrrolidine), 1.76-1.81(m, 2H×2, CH_2×2, pyrrolidine). ^{13}C NMR ($CDCl_3$) δ 157.05, 150.41, 128.00, 117.63(pyridazine), 133.80(=CH), 115.32 (CH_2=), 54.58(CH_2), 53.86, 23.46(pyrrolidine), 40.25(CH_2), 33.59(SCH_2). FT-IR (NaCl) cm^{-1} 3070(aromatic), 1500(N=N). GC-MS *m/z*(%) 84.10(100.00), 97.10(32.89), 42.10(22.48), 96.10(10.60), 41.20(9.23).

3-Allylthio-4,5-dimethyl-6-pyrrolidinylethylaminopyridazine (23b): Yield: 62%, mp 51-54 ℃. ^{1}H NMR ($CDCl_3$) δ 6.00-6.09(m, 1H, =CH), 5.29(d, *J*=17.1Hz, 1H, CH_2=), 5.08-5.11(m, 2H, NH+CH_2=), 3.94(d, *J*=6.9Hz, 2H, SCH_2), 3.16-3.65(m, 2H, $NHCH_2$), 2.81(d, *J*=5.7Hz, 2H, CH_2-N), 2.60(s, 2H×2, CH_2×2, pyrrolidine), 2.17(s, 3H, CH_3), 2.05 (s, 3H, CH_3), 1.80(s, 2H×2, CH_2×2, pyrrolidine). ^{13}C NMR ($CDCl_3$) δ 156.58, 151.73, 133.73, 122.51(pyridazine), 134.49(=CH), 117.74(CH_2=), 55.06($NHCH_2$), 40.79(CH_2-N), 54.24, 23.89 (pyrrolidine), 14.82(CH_3), 12.24(CH_3). GC-MS *m/z*(%) 84.10 (100.00), 97.10(21.84), 42.20(18.99), 55.10 (8.16), 196.10(7.18).

Materials and Methods for Bioassays

Cell lines and Culture Conditions. SK-Hep-1 hepatocarcinoma cells were purchased from the Korean Cell Line Bank (Seoul, Korea), and were maintained at 37°C in a humidified atmosphere, with 5% CO_2, in DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin.

MTT Assay. SK-Hep-1 cells (1×10^5 cells/well) cultured in a 96 well-plate were treated with various concentrations of the synthetic compounds (**17, 18a-e, 19c, 19e, 20a-c, 20e, 21a-d, 20h-j, 22a, 22c, 23a**) for 48 hr. Control cells were treated with dimethyl sulphoxide (DMSO) equal to the highest percentage of solvent used in the experimental conditions. K6 (3-methoxy-

6-allylthio-pyridazine) was used as a positive control. Briefly, 25 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 4 hr. After the supernatant (100 μl) was replaced with the same volume of DMSO, absorbance was measured at 540 nm with a micro-ELISA reader (Molecular Devices, Sunnyvale, CA). The percent of cells surviving was defined as the relative absorbance of treated versus untreated cells.

References

1. (a) Lee, J. I.; Park, H.; Yun, Y. S.; Kwon, S. K. *J. Kor. Chem. Soc.* **2001**, *45*, 386. (b) Kwon, S. K.; Kim, M. K. *Yakhak Hoeji* **2002**, *46*, 89. (c) Kwon, S. K. *Yakhak Hoeji* **2002**, *46*, 155. (d) Kwon, S. K.; Moon, A. R. *Arch. Pharm. Res.* **2005**, *4*, 391. (e) Jung, M. Y.; Kwon, S. K.; Moon, A. *Eur. J. Cancer* **2001**, *37*, 2104.
2. (a) Tisler, M.; Stanovnik, B. *Advances in Heterocyclic Chemistry*; Katritzky & Boulton, Eds.; 1984; Vol. 3, pp 1-56. (b) Song, J. H.; Kim, S. G.; No, Z. S.; Hyun, Y. L.; Jeon, D. J.; Kim, I. *Bull. Korean Chem. Soc.* **2008**, *29*, 1467
3. (a) Contreras, J. M.; Parrot, I.; Sippl, W.; Rival, Y. M.; Wermuth, C. G. *J. Med. Chem.* **2001**, *44*, 2707. (b) Parrot, I.; Wermuth, C. G.; Hibert, M. *Tetrahedron Lett.* **1999**, *40*, 7975.
4. (a) Wermuth, C. G.; Bourguignon, J.; Schlewer, G.; Gies, J.; Schoenfelder, A.; Melikian, A.; Bouchet, M. *J. Med. Chem.* **1987**, *30*, 239. (b) Shin, H. S.; Kwon, S. K. *Arch. Pharm. Res.* **2003**, *5*, 351.
5. Wermuth, C. G.; Schlewer, G.; Bourguignon, J. J.; Maghiros, G.; Bouchet, M. J.; Moire, C.; Kan, J. P.; Worms, P.; Biziere, K. *J. Med. Chem.* **1989**, *32*, 528.
6. (a) Kleemann, A.; Engel, J. *Pharmaceutical Substances,* 4th ed.; 2001; pp 1340-1342. (b) Contreras, J. M.; Rival, Y. M.; Chayer, S.; Bourguignon, J. J.; Wermuth, C. G. *J. Med. Chem.* **1999**, *42*, 730.
7. (a) Park, E. H.; Park, M. S. *Yakhak Hoeji* **2005**, *49*(1), 56. (b) Park, E. H.; Park, M. S. *J. Kor. Chem. Soc.* **2007**, *51*(3), 244.
8. Kwon, S. K.; Lee, M. S. *Yakhak Hoeji* **2005**, *49*(6), 505.
9. Parrot, I.; Rival, Y.; Wermuth, C. G. *Synthesis* **1999**, *7*, 1163.
10. (a) Lu, Z.; Twieg, R. J. *Tetrahedron* **2005**, *61*, 903. (b) Eldrup, A. B.; Dahl, O.; Nielsen, P. *J. Am. Chem. Soc.* **1997**, *119*, 1116. (c) Beletskaya, I.; Bessmertnykh, G.; Guilard, R. *Tetrahedron Lett.* **1999**, *40*, 6393. (d) Tewari, A.; Hein, M.; Zapt, A.; Beller, M. *Tetrahedron* **2005**, *61*, 9705. (e) Yokoyama, R.; Ito, S.; Okujima, T.; Kubo, T.; Yasunami, M.; Tajiri, A.; Morita, N. *Tetrahedron* **2003**, *59*, 8191.