Synthesis of 2-(Allylthio)pyrazines As a Novel Cancer Chemopreventive Agent

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2-(Allylthio)pyrazine derivatives were designed as a novel cancer chemopreventive agent that functions through selective inhibtion of cytochrome P-450 and induction of phase II enzymes involved in the detoxification of carcinogens. A practical preparation method of 2-(allylthio) pyrazine derivatives was established by the reaction of 2-mercaptopyrazine and allylbromides in the presence of a catalytic antioxidant, DABCO (1,4-diazabicyclo[2,2,2] octane), in dimethylformamide at below 50°C.

Key words: 2-Mercaptopyrazine, 2-(Allylthio)pyrazine, Cancer chemopreventive agents, CYP2E1

INTRODUCTION

Chemoprevention is the process of inhibiting, delaying, or reversing the process of carcinogenesis (Morse and Stoner, 1993; Kelloff *et al.*, 1994). Organosulfur compounds have been found to possess protective effects against experimental carcinogenesis and mutagenesis. Examples include diallylsulfide present in garlic and onion (Sparmins *et al.*, 1988, Hong *et al.*, 1992; Wargowich *et al.*, 1992). Diallylsulfide inhibits expression of cytochrome P450 2E1 (CYP2E1) (Brady *et al.*, 1991; Haber *et al.*, 1995), and also induces Phase II detoxification enzymes such as glutathione S-transferases and NAD(P)H:quinone oxidoreductase (Sparmin *et al.*, 1988; Reddy *et al.*, 1993; Eaton and Gallagher, 1994). Besides naturally-occurring organosulfur compounds, several synthetic sulfur-containing substances including oltipraz have been shown to exert chemopreventive properties against chemical-induced carcinogenesis in many animal models (Sparmins *et al.*, 1988; Haber *et al.*, 1995; Helzlsouer and Kensler. 1933; Maxuitenko *et al.*, 1993). Studies in this laboratory have shown that organosulfur compounds allylsulfide, allylmercaptan and allymethylsulfide are effective in suppressing both constitutive and chemical-inducible CYP 2E1 expression (Kwak *et al.*, 1994), and inducing glutathione S-transferases (GSTs) and microsomal epoxide hydrolase(mEH) and their mRNA levels in rats (Kim *et al.*, 1994; Kim *et al.*, 1995). Certain nitrogen-containing heterocycles such as pyridine, thiazole and pyrazine inhibit CYP2E1-catalyzed metabolic activities in vitro and induce CYP2E1 in vivo after multiple treatment (Kim and Novak, 1993). Interestingly, certain CYP2E1 inducers and inhibitors of CYP2E1 expression may serve as inhibitors of CYP 2E1 metabolic activities in vitro, as observed with nitrogen heterocycles of certain organosulfur compounds. Based on these previous observations, a series of pyrazine derivatives covalently linked with allymercaptan or structurally-related organosulfur compounds was synthesized in this study.

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MATERIALS AND METHODS

General experimental

Melting points were uncorrected. The infrared spectra of solids (potassium bromide) and liquid (paraffin oil) were recorded on a Shimadzu IR-435 spectrometer (Shimazu Corporation, Japan). The ^{1}H-NMR spectra were recorded on a Varian VXR 5200S 200MHz instrument (Varian Associates Inc., CA, USA) in deuteriochloroform or deuteriodimethylsulfoxide solution and chemical shifts (δ) are expressed in ppm relative to tetramethylsilane as an internal standard. Thin layer chromatography was performed on precoated plates of silica gel 60 F 254 (Merck KGaA, Germany) and the spots visualized using an ultraviolet lamp or iodine vapor. E. Merck silica gel 60 F (70-230 mesh) was used for silica-gel column chromatography. All chemicals and solvents were purchased from Aldrich Co. (Aldrich Chemical Company Inc., Wisconsin, USA and used without further purification.

Preparation of 2-mercaptopyrazine (2a)

To a solution of 2-chloropyrazine (5.0 g) in 40 ml DMF was added $NaSH.xH_2O$ (5.0 g). The mixture was stirred for 2 h at 60°C, and then cooled to 5°C. The resulting precipitates were collected and washed with 30 ml of ethyl ether. The resulting solid was dried in vacuo to give the title compound (3.0 g). mp: 165°C (dec.) IR(KBr): 1650, 1570, 1562, 1425 cm^{-1}. NMR(DMSO-d_6, δ): 7.60(d, 1H), 7.80(d, 1H), 8.55(s, 1H), 14.35(bs, 1H).

Preparation of 2-(allylthio)pyrazine (3a)

To a solution of 2-mercaptopyrazine (6.57 g) in 80 ml DMF and 8.6 ml of triethylamine was added allyl bromide (6.84 ml). After 2 h stirring at 50°C, 500 ml of ice water were added to the reaction mixture. The organic phase was extracted with ethyl ether and concentrated in vacuo. The residue was distilled under 0.5 torr to give the title compound (7.85 g, 85%) as a pale yellow oil. bp: 0.5 torr/68-69°C. TLC: Rf = 0.5 (EtOAc:n-Hx=1:4). IR(paraffin): 1139, 1376, 1456, 1506 cm^{-1}. NMR(DMSO-d_6, δ): 3.77(d, 2H), 5.10(d, 1H), 5.30(d, 1H), 5.81-6.02(m, 1H), 8.33(s, 1H), 8.48(s, 1H), 8.60(s, 1H).

Preparation of 2-benzylthiopyrazine (3b)

To a solution of 2-mercaptopyrazine (2.5 g) and 1,4-diazabicyclo[2,2,2]octane (1.46 g) in 20 ml DMF was added benzyl bromide (1.13 ml). The mixture was stirred for 2 hr at room temperature, and then was poured into 50 ml of ice water. The organic phase was extracted with ethyl ether and concentrated in vacuo. The residue was purified with silica-gel column chromatography (EtOAc:n-Hx=1:5) to provide a white solid of the title compound (1.8 g). mp: 66-67°C. TLC: Rf = 0.4 (EtOAc:n-Hx=1:4).IR(KBr): 1500, 1455, 1380 cm^{-1}. NMR($CDCl_3$, δ): 4.45(s, 2H), 7.2-7.35(m, 5H), 8.2-8.35 (m, 3H).

***In situ* preparation of alkyl bromide and preparation of 2-(alkylthio)pyrazine**

2-(3-Methyl-2-butenylthio)pyrazine (3c)

To a solution of 0.58 g of 3-methyl-2-butenol (0.58 g) and triphenylphosphine (2.89 g) in 50 ml of dichloromethane was added *N*-bromosuccinimide (1.96 g). After stirring at room temperature for 30 min, a solution of 2-mercaptopyrazine (1.0 g) and triethylamine(1.0 ml) in DMF (5.0 ml) was added to the above solution. The resulting mixture was stirred for 1 h at room temperature, and then poured into 100 ml of water. The organic layer was separated and concentrated to dryness. The residue was purified by column chromatography on silica gel using ethyl acetate : *n*-hexane=1:1 (v/v) as an eluent to give 0.6 g of the title compound as an oil.
TLC: Rf = 0.55 (EtOAc:n-Hx=1:4). IR(paraffin): 1501, 1375 cm^{-1}. NMR($CDCl_3$, δ): 1.74(s, 6H), 3.81(d, 2H), 5.35(m, 1 H), 8.21-8.41(m, 3H).

2-(Cinnamylthio)pyrazine (3d)

mp: 65-68°C. TLC: Rf = 0.40 (EtOAc:n-Hx=1:4). IR(KBr): 1490, 1440, 1380 cm^{-1}. NMR($CDCl_3$, δ): 4.04(d, 2H), 6.35 (t, 1H), 6.6(d, 1H), 7.2-7.4(m, 5H), 8.21-8.47(m, 3H).

2-(Furfurylthio)pyrazine (3e)
TLC: Rf = 0.30 (EtOAc:n-Hx=1:4). IR(paraffin): 1501, 1456, 1382 cm^{-1}. NMR($CDCl_3$, δ): 4.46(s, 2H), 6.26(m, 2H), 7.34 (d, 1H), 8.21-8.45(m, 3H).

2-(3-Methyl-3-butenylthio)pyrazine (3f)

TLC: Rf = 0.35 (EtOAc:n-Hx=1:4). IR(paraffin): 1500, 1450, 1370 cm^{-1}. NMR($CDCl_3$, δ): 1.80(s, 3H), 2.42(t, 2H), 3.30(t, 2H), 4.8 (d, 2H), 8.20-8.45(m, 3H).

2-(2-Butenyl-1-thio)pyrazine (3g)

TLC: Rf = 0.45 (EtOAc:n-Hx=1:4).IR(paraffin): 1502, 1455, 1381 cm^{-1}. NMR($CDCl_3$, δ): 1.70(d, 3H), 3.8(d, 2H), 5.6(m, 2H), 8.20-8.45(m, 3H).

2-(3-Butenyl-2-thio)pyrazine (3h)

TLC: Rf = 0.50 (EtOAc:n-Hx=1:4).IR(paraffin): 1502, 1475, 1380 cm^{-1}. NMR($CDCl_3$, δ): 1.40(d, 3H), 4.4(m, 1H), 5.0(d, 1H), 5.2 (d, 1H), 5.9(m, 1H), 8.14-8.35(m, 3H).

Preparation of 2,3-di-(allylthio)pyrazine (3i)

2,3-Pyrazinedithiol (2b)

To a solution of 2,3-pyrazinediol (4 g) in toluene (20 ml) was added Lawessons ragent (1.1 eq) at room temperature. The reaction mixture was refluxed for 24 h. After cooling to room temperature, 2N-HCl (10 ml) was added and extracted with ethyl acetate. The organic phase was concentrated in vacuo. The residue was triturated several times with acetone. The resulting solid was collected by filtration, and dried in vacuo to give 2,3-pyrazinedithiol (1.0 g, 20%) as a brownish solid.

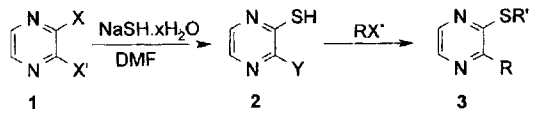
2,3-Di-(allylthio)pyrazine (3i)

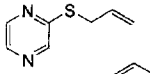
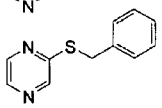
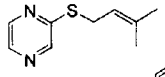
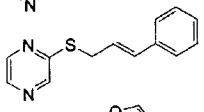
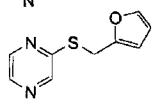
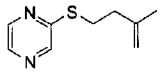
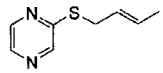
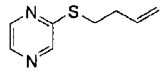
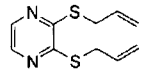
To a solution of 2,3-pyrazinedithiol (0.2 g) in DMF (10 ml) and triethylamine (2.1 eq) was added allyl bromide (2.2 eq). After stirring for 1 h at room temperature, 20 ml of ice water was added to the reaction mixture. The organic phase was extracted with ethyl ether and concentrated in vacuo. The residue was purified by silica-gel column chromatography to afford 2,3-di-(allylthio)pyrazine as an oil.
TLC: Rf = 0.55 (EtOAc:n-Hx=1:9).IR(paraffin): 1630, 1488, 1425 cm^{-1}.NMR(DMSO-d6, δ): 3.85(d, 4H), 5.10(d, 2H), 5.30(d, 2H), 5.81-6.02(m, 2H), 8.0 (s, 2H).

RESULTS AND DISCUSSION

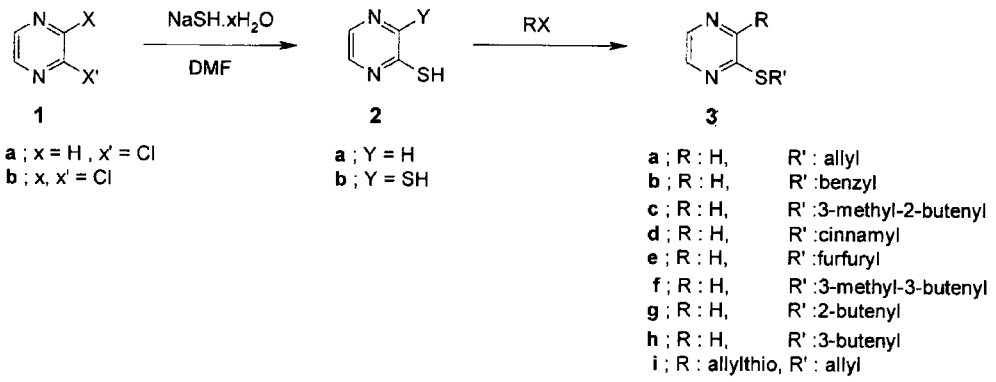
It is known that 2-mercaptopyrazine or 2,3-dimercaptopyrazine were prepared by the reaction of the corresponding chlorides with hydrogen sulfide or sodium thiosulfate in *N,N*-dimethylformamide in moderate yields (Barlin *et al.*, 1986; Foye *et al.*, 1980). However, these known methods have some disadvantages. The former method has to use highly toxic hydrogen sulfide gas and give low yield. Even the latter (use of sodium thiosulfate) was more useful than hydrogen sulfide method but work-up procedure was so complicate. Therefore, we optimized the reaction by taking the advantage of each methods. As shown Scheme 1, sodium hydrosulfide was used instead of hydrogen sulfide gas or sodium thiosulfate. 2-Mercaptopyrazine and 2,3-dimercaptopyrazine (**2**) was readily prepared from 2-chloropyrazine and 2,3-dichloropyrazine (**1**), respectively, with sodium hydrosulfide. Allylthiopyrazine derivatives (**3**) were also prepared from mercaptopyrazine (**2**) and allyl halides. Treatment of chloropyrazines (**2a**) with excess of sodium hydrosulfide hydrate in DMF gave mercaptopyrazines in high yield by routine operations. Mercaptopyrazines were easily isolated by the precipitation of reaction mixture into water followed by filtration. Since mercaptopyrazines is very poor soluble in protic solvents such as ethyl acetate, acetone, dichloromethane, acetonitrile and ethanol, precipitation in water and washing with organic solvent provided pure enough product being capable of using in the next alkylation step without further purification. It was found that there was a great difference in the yields of alkylation step depending on reaction solvents. For instance, when the reaction was carried out in methanol, the yield was very low and some unknown side products were appeared. However, the yield was excellent in DMF without other side products. It was assumed that sodium hydrosulfide could be decomposed in polar protic solvent such as methanol. It was also recognized that polarity of DMF was a major contribution factor in S_N2 type alkylation. In the presence of triethylamine as a base, alkylations were carried out with mercaptopyrazine and alkyl or allyhalides in various solvents and a range of temperatures from room temperature to 60°C. Alkyl halide or allylhalide were prepared by *in situ* bromination of the correspond-

Table I. The yields of alkylthiopyrazines from mercaptopyrazines and alkylhalides prepared *in situ*

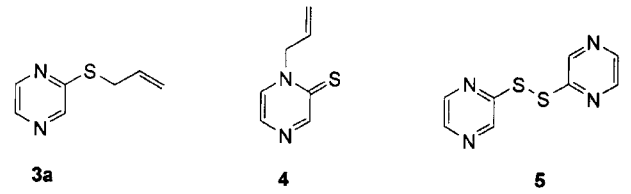


	Product,3	Yield(%)**
a		65
b		85
c		80
d		77
e		86
f		92
g		90
h		89
i		83

*Alkyl bromides were prepared from the bromination of the corresponding alcohol using NBS/TPP and were used *in situ*
**Isolated yields



Scheme 1. Synthesis of allylthiopyrazine derivatives



3a 4 5

Fig. 1. Isolated products from the reaction of thiopyrazine and allylbromide.

ing alcohol using *N*-bromosuccinimide and triphenylphosphine. As shown in Table I, S-substituted thiopyrazines from mercaptopyrazine and alkyl or allyhalides were prepared in moderate yields. 2-Benzylthiopyrazine and 2-(cinnamylthio)pyrazine obtained as solid state were purified by recrystallization from a cosolvent of *n*-hexane/ethyl acetate (3/1, v/v). All of the other compounds were isolated as oily products, which were purified by fractional distillation under reduced pressure. All the products had some typical characteristics like sulfur including odor and yellow color. Interestingly, the reaction of allylbromide and 2-mercaptopyrazine in DMF at a temperature range from 38°C to 50°C provided 2-(allylthio)pyrazine (**3**) as a major product. However, as shown in Fig. 1, the reaction at above 60°C provided the product as a mixture of 2-(allylthio)pyrazine (**3a**), 1-allylpyrazine-2-thione (**4**) and dipyrazinedisulfide (**5**) as a ratio of 55:15:30, respectively (Dlabal *et al.*, 1990). This ratio was largely affected from the kind of solvents and temperature. Moreover, 1-allylpyrazine-2-thione (**4**) was obtained from a condition of more polar solvent and higher temperature. From the above results, we presumed that 2-(allylthio)pyrazine formation was kinetically controlled and 1-allylpyrazin-2-thione formation was thermodynamically controlled. Although, formation mechanism of 1-allylpyrazin-2-thione was not clear yet, the rearrangement of 2-(allylthio)pyrazines had to be considered. The other product, dipyrazindisulfide (**5**) was also considered to be produced by the oxidation of 2-mercaptopyrazine. The characteristic R_f values of 2-(allylthio)pyrazine (**3a**), 1-allylpyrazin-2-thione (**4**) and dipyrazinedisulfide (**5**) in a thin layer silica-gel chromatography (*n*-hexane:ethyl acetate = 4:1, v/v) were 0.1, 0.45, and 0.5, respectively. However, the formation of the dipyrazinedisulfide and 1-allylpyrazin-2-thione were able to be controlled by using an antioxidant or performing the reaction at low temperature. The range of 40-50°C was favorable temperature to avoid the formation of the side products. Moreover, 2-(allylthio)pyrazine was generated in high yield by the addition of DABCO(1,4-Diazabicyclo[2,2,2]octane) as an antioxidant. In conclusion, we established a practical preparation method of 2-(allylthio)pyrazine derivatives by the reaction of 2-mercaptopyrazine and allyl bromides in the presence of a catalytic antioxidant, DABCO(1,4-Diazabicyclo[2,2,2] octane), in DMF at below 50°C.

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