



## CHAPTER III

### EXPERIMENTS

#### Instruments

1. Elemental Analyser : Perkin Elmer 240 C (The Scientific and Technological Research Equipment Center, Chulalongkorn University)
2. Infared Spectrophotometer : Shimaszu IR - 440 (The Scientific and Technological Research Equipment Center, Chulalongkorn University)
3. Nuclear Magnetic Resonance Spectrophotometer : Jeol FX 90 Q (90 MHz) (The Scientific and Technological Research Equipment Center, Chulalongkorn University)
4. Mass Spectrometer : Jeol FX 3000 double focusing (The Scientific and Technological Research Equipment Center, Chulalongkorn University)
5. Melting Point : Buchi capillary melting point apparatus

#### Chemicals

1. Ethyl cyanoacetate (Wako Pure Chemical industries Ltd.)
2. Phenyl isothiocyanate (Sigma Chemical Company)
3. Methyl isothiocyanate (Fluka Chemie Co.)
4. Dimethyl sulphate (Farmitalia Carlo Erba)

The solvents used were either BP. or Laboratory grade.

3-Amino-4-carbethoxy-5-phenylaminoisothiazole

2.83 g (0.0250 mole) of ethyl cyanoacetate was added to a solution of sodium ethoxide, which was prepared by dissolving 0.60 g (0.0250 mole) of sodium metal in 25 ml. absolute ethanol. Then, the mixture was stirred until the cloudiness was noted. Then, 3.38 g (0.0250 mole) of phenyl isothiocyanate was followed. The mixture was refluxed for 10 minutes, and allowed to stir at room temperature for 12 hours. The solution was added to cold aqueous chloramine solution, which was prepared from 50 ml of 5% sodium hypochlorite solution in 100 g of crushed ice and 10 ml of 25% ammonium hydroxide. The mixture was stirred overnight and off white precipitate formed. The product was collected by filtration, then washed and dried. Recrystallisation from ethanol yield 5.89 g (85% yield) crystals, mp. 159-160 °C (23,36).

The product obtained was confirmed by comparing with authentic sample using thin-layer chromatography and mix melting point method.

3-Amino-4-carbethoxy-5-methylaminoisothiazole

2.83 g (0.0250 mole) of ethyl cyanoacetate was added to a solution of sodium ethoxide, which was prepared

by dissolving 0.60 g (0.0250 mole) sodium metal in 25 ml of absolute ethanol. Then, the mixture was stirred until the cloudiness was noted. Then, 1.85 g (0.0250 mole) of methyl isothiocyanate was followed. The mixture was refluxed for 10 minutes, and allowed to stir at room temperature for 12 hours. The solution was added to cold aqueous chloramine solution, which prepared from 50 ml of 5% sodium hypochlorite solution in 100 g of crushed ice and 10 ml of 25% ammonium hydroxide. The reaction mixture was stirred at room temperature for 12 hours and the solid obtained was filtered, washed and dried. Recrystallisation from ethanol yield 2.44 g (48% yield) crystals, mp. 151-152 °C (23,36).

The product obtained was confirmed by comparing with authentic sample using thin-layer chromatography and mix melting point method.

5-Methyl-3-phenylaminoisothiazolo [3,4-d] pyrimidine-4-one-6(7H)-thione

To a solution of 0.53 g (0.0020 mole) of 3-amino-4-carbethoxy-5-phenylaminoisothiazole in 10 ml pyridine, 0.12 g (0.0020 mole) of methyl isothiocyanate was added. The solution was refluxed for 6 hours. After cooling the reaction, the mixture was poured into crushed ice. Separated the solid, washed with water, dried, then added ethanol 20 ml and separated the solid

obtained from boiling ethanol. The product were recrystallised from diluted sodium hydroxide solution and then were dried in a desicator over anhydrous calcium chloride. The total yield was 0.23 g (40% yield), mp. over 260 °C (23).

5-Phenyl-3-phenylaminoisothiazolo [3,4-d] pyrimidine-4-one-6(7H)-thione

Phenylisothiocyanate 0.23 g (0.0020 mole) was added to a solution of 0.53 g (0.0020 mole) of 3-amino-4-carbethoxy-5-phenylaminoisothiazole in 10 ml pyridine. The solution was refluxed for 6 hours. After cooling the reaction, the mixture was poured into the crushed ice. Separated the solid, collected, washed with water, dried, then added ethanol 20 ml and separated the solid obtained from boiling ethanol. The product were recrystallised from dilute sodium hydroxide solution and then were dried in a desicator over anhydrous calcium chloride. The total yield was 0.23 g (32% yield), mp. over 260 °C (23).

5-Methy-3-methylaminoisothiazolo [3,4-d] pyrimidine-4-one-6(7H)-thione

To a solution of 0.40 g (0.0020 mole) of 3-amino-4-carbethoxy-5-methylaminoisothiazole in 10 ml pyridine, 0.12 g (0.0020 mole) of methyl isothiocyanate was added. The solution was refluxed for 6 hours. After

cooling the reaction, the mixture was poured into the crushed ice. Separated the solid, collected, washed with water, dried, then added ethanol 20 ml and separated the solid obtained from boiling ethanol. The product were recrystallised from dilute sodium hydroxide solution and then were dried in a desicator over anhydrous calcium chloride. The total yield was 0.23 g (50% yield), mp. over 260 °C (23).

5-Phenyl-3-methylaminoisothiazolo [3,4-d] pyrimidine-4-one-6(7H)-thione

To a solution of 0.53 g (0.0020 mole) of 3-amino-4-carbethoxy-5-methylaminoisothiazole in 10 ml pyridine, 0.23 g (0.0020 mole) of phenyl isothiocyanate was added. The solution was refluxed for 6 hours. After cooling the reaction, the mixture was poured into crushed ice. Separated the solid, collected, washed with water, dried, then added ethanol 20 ml and separated the solid obtained from boiling ethanol. The product were recrystallised from dilute sodium hydroxide solution and then were dried in a desicator over anhydrous calcium chloride. The total yield was 0.20 g (35% yield), mp. over 260 °C (23).

5-Methyl-6-methylthio-3-phenylaminoisothiazolo [3,4-d] pyrimidine-4-one and 5,7-Dimethyl-3-phenylaminoisothiazolo [3,4-d] pyrimidine-4-one-6-thione

To a solution of 1.00 g (0.0035 mole) of 5-methyl-3-phenylaminoisothiazolo [3,4-d] pyrimidine-4-one-6(7H)-thione in 150 ml of 20% sodium hydroxide solution, 1.32 g (0.0105 mole) of dimethyl sulphate was added dropwise with stirring at 50-60°C for one hour. The separated product was filtered, washed with water, dried, and recrystallised in ethanol. Two forms of crystal appeared and were separated by heating in ethanol until the shot light needle crystals were dissolved. Filtered the remaining bigger needle crystals. Recrystallisation from ethanol to yield 0.66 g (62% yield) of 5-methyl-6-methylthio-3-phenylaminoisothiazolo [3,4-d] pyrimidine-4-one. mp. 182-183 °C.

IR (KBr)  $\checkmark$  : 3210 (NH) ; 1660 (C=O) ;  
 1600-1500 (aromatic) ;  
 1460 (SCH<sub>3</sub>) ;  
 750-690 (CH in aromatic)  
 (Figure 23)

<sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  : 2.66 (s, 3H, -SCH<sub>3</sub>) ;  
 3.52 (s, 3H, -NCH<sub>3</sub>) ;  
 7.22 (m, 5H, -NH-C<sub>6</sub>H<sub>5</sub>) ;  
 9.84 (Br, 1H, -NH-C<sub>6</sub>H<sub>5</sub>)  
 (Figure 5)



Analysis for  $C_{13}H_{12}ON_4S_2$  :

Calcd.	C = 51.32	H = 3.95	N = 18.42
Found	C = 51.49	H = 3.92	N = 18.34

The filtrate was allowed to stand until crystals of 5,7-dimethyl-3-phenylaminoisothiazolo [3,4-d] pyrimidine-4-one-6-thione was obtained. Recrystallisation from ethanol yields 0.23 g (22% yield), mp. 196-197 °C.

IR (KBr)  $\nu$  : 3250 (NH) ; 1660 (C=O) ;  
1350 (C=S) ; 1600-1500 (aromatic) ;  
745, 680 (CH in aromatic)  
(Figure 22)

$^1H$ -NMR ( $CDCl_3$ ) $\delta$  : 3.78 (s, 3H, >N-CH<sub>3</sub>) ;  
3.97 (s, 3H, >N-CH<sub>3</sub>) ;  
7.20 (m, 5H, -NH-C<sub>6</sub>H<sub>5</sub>) ;  
9.95 (Br, 1H, -NH-C<sub>6</sub>H<sub>5</sub>)  
(Figure 4)

Analysis for  $C_{13}H_{12}ON_4S_2$  :

Calcd.	C = 51.32	H = 3.95	N = 18.42
Found	C = 51.10	H = 4.07	N = 18.49

5-Phenyl-6-methylthio-3-phenylaminoisothiazolo [3,4-d]  
pyrimidine-4-one

To a solution of 1.23 g (0.0035 mole) of 5-phenyl-3-phenylaminoisothiazolo [3,4-d] pyrimidine-4-one

-6(7H)-thione in 150 ml of 20% sodium hydroxide solution, 1.32 g (0.0105 mole) of dimethyl sulphate was added dropwise with stirring at 50-60°C for one hour. The separate product was filtered, washed with water, dried, and recrystallised by ethanol to yield a mixture of 5-phenyl-6-methylthio-3-phenylaminoisothiazolo [3,4-d] pyrimidine-4-one and 7-methyl-5-phenyl-3-phenylamino isothiazolo [3,4-d] pyrimidine-4-one-6-thione in the total yield of 0.94 g (73% yield). Recrystallisation with ethanol for several times yields 5-phenyl-6-methylthio-3-phenylaminoisothiazolo [3,4-d] pyrimidine-4-one, mp. 248-249 °C.

IR (KBr)  $\nu$  : 3250 (NH) ; 1680 (C=O) ;  
 1600-1500 (aromatic) ;  
 1460, 1340 (SCH<sub>3</sub>) ;  
 730, 720, 700, 690 (CH in aromatic)  
 (Figure 24)

<sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  : 2.53 (s, 3H, -SCH<sub>3</sub>) ;  
 7.20 (m, 10H, -NH-C<sub>6</sub>H<sub>5</sub>, >N-C<sub>6</sub>H<sub>5</sub>) ;  
 9.81 (Br, 1H, -NH-C<sub>6</sub>H<sub>5</sub>) (Figure 6)

Analysis for C<sub>18</sub>H<sub>14</sub>ON<sub>4</sub>S<sub>2</sub>

Calcd.	C = 59.02	H = 3.82	N = 15.30
Found	C = 58.99	H = 3.81	N = 15.26



5-Methyl-6-methylthio-3-methylaminoisothiazolo [3,4-d]  
pyrimidine-4-one and 5,7-Dimethyl-3-methylaminoisothiazolo  
[3,4-d] pyrimidine-4-one-6-thione

To a solution of 0.80 g (0.0035 mole) of 5-methyl-3-methylaminoisothiazolo [3,4-d] pyrimidine-4-one-6(7H)-thione in 150 ml of 20% sodium hydroxide solution, 1.32 g (0.0105 mole) of dimethyl sulphate was added dropwise with stirring at 50-60 °C for one hour. The separated product was filtered, washed with water, dried, and recrystallised in ethanol. Two forms of crystals appeared and were separated by heating in ethanol until the lighter needle crystals was dissolved. Filtered the remaining bigger needle crystals. Recrystallisation from ethanol yields 5-methyl-6-methylthio-3-methylaminoisothiazolo [3,4-d] pyrimidine 0.54 g (64% yield), mp. 235-236 °C.

IR (KBr)  $\checkmark$  : 3200 (NH) ; 1660 (C=O) ;  
 1600-1500 (aromatic) ;  
 1415, 1330 (SCH<sub>3</sub>)  
 (Figure 26)

<sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  : 2.63 (s, 3H, -SCH<sub>3</sub>) ;  
 3.07 (d, 3H, -NH-CH<sub>3</sub>) ;  
 3.47 (s, 3H, >N-CH<sub>3</sub>) ;  
 7.30 (Br, 1H, -NH-CH<sub>3</sub>)  
 (Figure 8)

(CDCl<sub>3</sub>+D<sub>2</sub>O) : (Figure 9)

Analysis for  $C_8H_{10}ON_4S_2$

Calcd. C = 39.67    H = 4.13    N = 23.14

Found    C = 40.35    H = 4.07    N = 23.33

The filtrate was allowed to stand until the crystal of 5,7-dimethyl-3-methylaminoisothiazolo [3,4-d] pyrimidine-4-one-6-thione was obtained. Recrystallisation from ethanol yield 0.19 g (23% yield), mp.248-249 °C.

IR (KBr)  $\checkmark$  : 3320 (NH) ; 1680 (C=O) ;  
1600-1450 (aromatic) ; 1350 (C=S)  
(Figure 25)

$^1H$ -NMR ( $CDCl_3$ )  $\delta$  : 3.08 (d, 3H, -NH-CH<sub>3</sub>) ;  
3.74 (s, 3H, >N-CH<sub>3</sub>) ;  
3.94 (s, 3H, >NCH<sub>3</sub>) ;  
7.52 (Br, 1H, -NH-CH<sub>3</sub>)  
(Figure 7)

Analysis for  $C_8H_{10}ON_4S_2$

Calc. C = 39.67    H = 4.13    N = 23.14

Found C = 40.02    H = 4.18    N = 23.39

5-Phenyl-6-methylthio-3-methylaminoisothiazolo [3,4-d] pyrimidine-4-one and 7-Methyl-5-phenyl-3-methylaminoisothiazolo [3,4-d] pyrimidine-4-one-6-thione

To a solution of 1.01 g (0.0035 mole) of 5-phenyl-3-methylaminoisothiazolo [3,4-d] pyrimidine-4-one

-6(7H)-thione in 150 ml of 20% sodium hydroxide solution, 1.32 g (0.0105 mole) of dimethyl sulphate was added dropwise with stirring at 50-60 °C for one hour. The separated product was filtered, washed with water, dried, and recrystallised in ethanol. Two products were obtained in the total yield of 0.81 g (76% yield). The two products were separated using preparative thin layer chromatography using silica gel 60 GF254 as stationary phase and the system of chloroform:acetone (50:1) as the mobile phase. Recrystallised each product with ethanol.

5-Phenyl-6-methylaminoisothiazolo [3,4-d] pyrimidine  
-4-one melts at 249-250 °C.

IR (KBr)  $\nu$  : 3340 (NH) ; 1670 (C=O) ;  
1415, 1340 (SCH<sub>3</sub>) ;  
720, 699 (CH in aromatic)  
(Figure 28)

<sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  : 2.50 (s, 3H, -SCH<sub>3</sub>) ;  
3.01 (d, 3H, -NH-CH<sub>3</sub>) ;  
7.30 (m, 6H, >NH-C<sub>6</sub>H<sub>6</sub>, -NH-CH<sub>3</sub>)  
(Figure 11)

Analysis for C<sub>13</sub>H<sub>12</sub>ON<sub>4</sub>S<sub>2</sub>

Calcd. C = 51.32 H = 3.95 N = 18.42

Found C = 51.17 H = 3.83 N = 18.30

7-Methyl-5-phenyl-3-methylaminoisothiazolo [3,4-d]  
pyrimidine-4-one-6-thione melts at 258-259 °C.

IR (KBr)  $\checkmark$  : 3350 (NH) ;  
1680 (C=O) ;  
1350 (C=S) ;  
730, 690 (CH in aromatic)  
(Figure 27)

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 3.04 (d, 3H, -NH-CH<sub>3</sub>) ;  
3.97 (s, 3H, >N-CH<sub>3</sub>) ;  
7.40 (m, 6H, >N-C<sub>6</sub>H<sub>6</sub>, -NH-CH<sub>3</sub>)  
(Figure 10)

Analysis for C<sub>13</sub>H<sub>12</sub>ON<sub>4</sub>S<sub>2</sub>

Calcd.	C = 51.32	H = 3.95	N = 18.42
Found	C = 51.43	H = 4.01	N = 18.17

5-Methyl-3-phenylamino-6-hydroxyisothiazolo [3,4-d]  
pyrimidine-4-one

A mixture of 25 ml of 6N hydrochloric acid and 1.06 g (0.0035 mole) of 5-methyl-6-methylthio-3-phenyl aminoisothiazolo [3,4-d] pyrimidine-4-one was heated under refluxed condition for 16 hours. The precipitated product was filtered, washed with water and dried. The precipitated were recrystallised from chloroform to afford 0.60 g (63% yield) of 5-methyl-3-phenylamino-6-hydroxyisothiazolo [3,4-d] pyrimidine-4-one as white needle crystals, mp. over 300 °C.

IR (KBr)  $\checkmark$  : 3200-3400 (OH) ; 1720 (C=O) ;  
 1600-1500 (aromatic) ; 1190 (C-O) ;  
 750, 690 (CH in aromatic)  
 (Figure 29)

$^1\text{H-NMR}$  ( $\text{C}_5\text{D}_5\text{N}$ ) : (Figure 12)

Analysis for  $\text{C}_{12}\text{H}_{10}\text{O}_2\text{N}_4\text{S}$

Calcd.	C = 52.55	H = 3.65	N = 20.40
Found	C = 52.35	H = 3.53	N = 20.37

5-Phenyl-3-phenylamino-6-hydroxyisothiazolo [3,4-d]  
 pyrimidine-4-one

A mixture of 25 ml of 6N hydrochloric acid and 1.28 g (0.0035 mole) of 5-phenyl-6-methylthio-3-phenylaminoisothiazolo [3,4-d] pyrimidine-4-one was heated under refluxed condition for 16 hours. The precipitated product was filtered, washed with water and dried. The precipitated were recrystallised from chloroform to afford 0.72 g (61% yield) of 5-phenyl-3-phenylamino-6-hydroxyisothiazolo [3,4-d] pyrimidine-4-one as white needle crystals, mp. over  $300^\circ\text{C}$ .

IR (KBr)  $\checkmark$  : 3400-3500 (OH) ; 1710 (C=O) ;  
 1600-1500 (aromatic) ;  
 1190 (C-O) ;  
 725, 695 (CH in aromatic)  
 (Figure 30)

$^1\text{H-NMR}$  ( $\text{C}_5\text{D}_5\text{N} + \text{CDCl}_3$ ) : (Figure 13)

Analysis for  $\text{C}_{17}\text{H}_{12}\text{O}_2\text{N}_4\text{S}$

Calcd.	C = 60.71	H = 3.57	N = 16.66
Found	C = 60.72	H = 3.52	N = 16.63

5-Methyl-3-methylamino-6-hydroxyisothiazolo [3,4-d]  
pyrimidine-4-one

A mixture of 25 ml of 6N hydrochloric acid and 0.85 g (0.0035 mole) of 5-methyl-6-methylthio-3-methyl aminoisothiazolo [3,4-d] pyrimidine-4-one was heated under refluxed condition for 16 hours. The precipitated product was filtered, washed with water and dried. The precipitated were recrystallised from chloroform to afford 0.48 g (65% yield) of 5-methyl-3-methylamino-6-hydroxyisothiazolo [3,4-d] pyrimidine-4-one as white needle crystals, mp. over 300 °C.

IR (KBr)  $\checkmark$  : 3200-3400 (OH) ; 1705 (C=O) ;  
1200 (C-O)  
(Figure 31)

$^1\text{H-NMR}$  ( $\text{C}_5\text{D}_5\text{N}$ ) : (Figure 14)

$^1\text{H-NMR}$  ( $\text{C}_5\text{D}_5\text{N} + \text{CDCl}_3$ ) : (Figure 15)

Analysis for  $\text{C}_7\text{H}_8\text{O}_2\text{N}_4\text{S}$

Calcd.	C = 39.62	H = 3.77	N = 26.42
Found	C = 39.54	H = 3.76	N = 26.32

5-Methyl-3-methylamino-6-hydroxyisothiazolo [3,4-d]  
pyrimidine-4-one

A mixture of 25 ml of 6N hydrochloric acid and 1.06 g (0.0035 mole) of 5-methyl-6-methylthio-3-methylamino isothiazolo [3,4-d] pyrimidine-4-one was heated under refluxed condition for 16 hours. The precipitated product was filtered, washed with water and dried. The precipitated were recrystallised from chloroform to afford 0.56 g (59% yield) of 5-phenyl-3-methylamino-6-hydroxyisothiazolo [3,4-d] pyrimidine-4-one as white needle crystals, mp. over 300 °C.

IR (KBr)  $\nu$  : 3400-3500 (OH) ; 3350 (NH) ;  
1710 (C=O) ; 1600-1500 (aromatic) ;  
1200 (C-O) ; 770, 690 (CH in aromatic)  
(Figure 32)

$^1\text{H-NMR}$  ( $\text{C}_5\text{D}_5\text{N}$ ) : (Figure 16)

$^1\text{H-NMR}$  ( $\text{C}_5\text{D}_5\text{N} + \text{CDCl}_3$ ) : (Figure 17)

Analysis for  $\text{C}_{12}\text{H}_{10}\text{O}_2\text{N}_4\text{S}$

Calcd. C = 52.55 H = 3.65 N = 20.44

Found C = 52.63 H = 3.51 N = 20.72

5-Methyl-3-methylthio-6-(1'-piperidino)isothiazolo [3,4-d]  
pyrimidine-4-one

A mixture of 3 ml of piperidine and 0.20 g

(0.0080 mole) of 5-methyl-6-methylthio-3-methylamino isothiazolo [3,4-d] pyrimidine-4-one was heated at 140 °C for 24 hours in a sealed tube. Then, evaporated the unreacted piperidine and recrystallised the precipitated with hexane to afford 0.17 g (75% yield) of 5-methyl-3-methylamino-6-(1'-piperidino)isothiazolo [3,4-d] pyrimidine-4-one, mp. 189-190 °C.

IR (KBr)  $\nu$  : (Figure 33) 3400

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) 8 : (Figure 18)

3.35 (s, 3H,  $>\text{NCH}_3$ )

3.23 (s, 3H,  $-\text{NHCH}_3$ )

3.18 (s, 4H,  $2'\text{H}$ ,  $6'\text{H}$ )

1.66 (br, 6H,  $3'\text{H}$ ,  $4'\text{H}$ ,  $5'\text{H}$ )

$^1\text{H-NMR}$  ( $\text{CDCl}_3 + \text{D}_2\text{O}$ ) : (Figure 19)

$^{13}\text{C}$  NMR : (Figure 20, 21)

190.37 (s, C4)

165.39 (s, C6)

163.23 (s, C7a)

157.19 (s, C3)

92.75 (s, C3a)

49.90 (t,  $\text{C}2'$  and  $\text{C}6'$ )

33.86 (q,  $\text{CH}_3$  at C5)

31.75 (q,  $\text{NHCH}_3$ )

25.41 ( $\text{C}3'$  and  $\text{C}5'$ )

24.16 ( $\text{C}4'$ )