A Synthetic Entry to Isoxazolo[5,4-*d*]pyrimidine-4(5*H*)thione and Isothiazolo[4,3-*d*]isoxazole

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4-Thiocarbamoyl-5-aminoisoxazole **4** was used as a convenient starting material for preparing both isoxazolo[5,4-*d*]pyrimidine-4(5*H*)thiones **5** and 4-aminoisothiazolo[4,3-*d*]isoxazole **6**. The structure of the latter compound was definitively assigned by X-ray analysis.

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Within the framework of investigations on biologically active heterocycles, we found that pyrazolo[3,4-*d*]pyrimidine-4(5*H*)thiones **1** and 4-aminopyrazolo[3,4-*c*]isothiazoles **2** are potent fungicides [1-4] (Figure 1). After biological tests proved that the activity of the above compounds was comparable or superior to that of certain reference fungicides, the synthesis of a series of analogues was planned so that further S.A.R. information could be generated. The purpose of the present work was to synthesize analogs of **1** and **2** whereby the pyrazole moiety, would be replaced with the isosteric isoxazole ring.

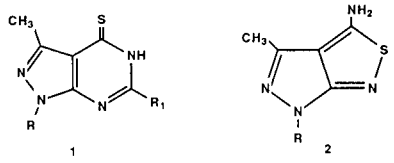
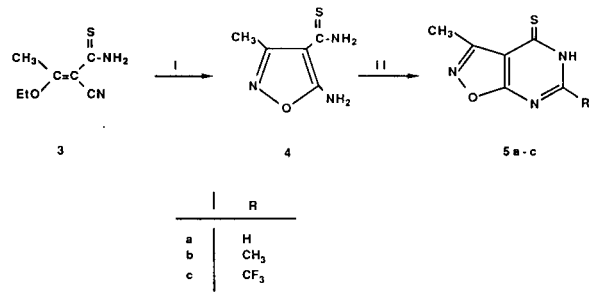


Figure 1

The preparation of bicyclic ring systems, such as the desired isoxazolo[5,4-*d*]pyrimidine-4(5*H*)thione **5** (Scheme 1) and isothiazolo[4,3-*d*]isoxazole **6** (Scheme 2) was achievable from either of two approaches. The pyrimidine or the isothiazole ring could be prepared first and the isoxazole ring closed in the final step of the synthetic pathway; alternatively, the isoxazole ring could be prepared initially and the second heterocyclic ring attached in the final stage. We chose the latter approach because of our previous experience in the cyclization of 4-thiocarbamoyl-5-aminopyrazole to **1** and **2** [4,5]. The key intermediate in this synthetic procedure was 3-methyl-4-thiocarbamoyl-5-aminoisoxazole (**4**).

Scheme 1

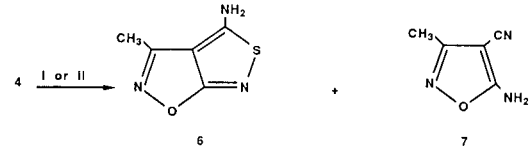


I - hydroxylamine. II - triethyl orthoformate, triethyl orthoacetate, trifluoroacetic acid/trifluoroacetic anhydride.

The intermediate **4** was obtained in good yield by reacting 2-cyano-3-ethoxythiocrotonamide (**3**) [6] with the free base form of hydroxylamine. Treatment of **4** in acidic medium with orthoesters, *i.e.* triethyl orthoformate and triethyl orthoacetate, gave the corresponding isoxazolo[5,4-*d*]pyrimidine-4(5*H*)thiones **5a-b**. The homologue **5c** was obtained by reacting **4** with a mixture of trifluoroacetic acid and trifluoroacetic anhydride. All these reactions proceeded smoothly at room temperature.

The synthesis of 3-methyl-4-aminoisothiazolo[4,3-*d*]-isoxazole **6** was accomplished according to the procedure for the preparation of 3-amino-2,1-benzisothiazoles [7]. This procedure involves the concomitant oxidation of both the amino and thiocarbamoyl functions of the aromatic ring, and leads to ring closure with formation of the fused isothiazole. A possible drawback of this method is the competitive dehydrosulfurization effected by the oxidant on the thiocarbamoyl function, which is thereby converted into a cyano group [8].

Scheme 2



I - hydrogen peroxide. II - iodine.

We found that the oxidation of **4** afforded a mixture of 3-methyl-4-aminoisothiazolo[4,3-*d*]isoxazole (**6**) and 3-methyl-4-cyano-5-aminoisoxazole (**7**) under all the experimented conditions which we employed. Obtaining either **6**

or **7** as the main product depended on the choice of the oxidizing agent. The best yields of **6** were obtained by treating **4** with hydrogen peroxide in pyridine at 0° whereas the oxidation of **4** with iodine gave the cyano derivative **7** as the major reaction product.

Analytical and spectral data of compounds **4-6** agree with the proposed structures (see Experimental). However, as compound **6** represents the first example of isothiazole-isoxazole fusion, the definitive assignment of this structure was made by a single crystal X-ray analysis on a sample of **6** grown from ethanol.

Crystal Structure Determination.

The compound **6** crystallizes in the orthorombic space group $Pca2_1$ with unit cell dimensions a = 12.867(2), b = 6.366(1), c = 7.696(2) Å; V = 630.4(2) Å^3, and d calc = 1.65 g cm^{-3} for Z = 4. A colorless crystal of 0.12 x 0.24 x 0.33 mm was used for X-ray analysis. Intensity data were collected at room temperature on an Enraf-Nonius CAD4 diffractometer with graphite monochromated Mo-Kα radiation and $\omega/2\theta$ scan technique. Cell parameters were obtained from least-squares refinement of the setting angles of 25 centered reflections in the range $8<\theta<12°$. 736 independent reflections were measured in the range $2\leq\theta\leq27°$. Intensities were corrected for Lorentz and polarization. Scattering factors were taken from reference [9]. The intensities of three standard reflections measured after every 2 hours showed no significant variation during data collection. The structure was solved by direct methods (MULTAN 81) [10] and refined by full-matrix least-squares analysis using 605 reflections having $I\geq3\sigma(I)$ with anisotropic temperature factors for all non H atoms and isotropic ones for hydrogens. Weights were applied according to the scheme: $w = 4Fo^2/[\sigma^2(Fo^2) + (0.05\ Fo^2)^2]$ and final statistical parameters: $R = \Sigma|\Delta Fo|/\Sigma|Fo|$ and $R_w = (\Sigma w|\Delta Fo|^2/\Sigma w|Fo|^2)^{1/2}$ were 0.030 and 0.038, respectively. The error of an observation of unit weight was 1.19, and the largest peak in the final Difference Map was of 0.17 e Å^{-3}. Final positional and equivalent isotropic vibrational parameters are reported in Table 1. All calculations were done using the CAD4-SDP system of programs [11] and PARST [12]. Bond distances and angles are reported in Tables 2 and 3, respectively. An ORTEP view [13] of the molecule with the atom-labelling scheme is shown in Figure 2. The two rings constituting the molecule are essentially planar; for C2-C3-C4-N2-O [$\Sigma(\Delta/\sigma)^2$ = 8.2] maximum displacement from the least-squares plane is of 0.007 Å and for C1-C2-C3-N1-S [$\Sigma(\Delta/\sigma)^2$ = 5.8] is of 0.006 Å, the dihedral angle between the two planes being 1.7 (1)°. The packing is mainly controlled by Van der Waals interactions and two weak hydrogen bonds, N3-H1--N1 ($x-\frac{1}{2}, -y, z$) and N3-H2---N2 ($-x+\frac{1}{2}, y+1, z-\frac{1}{2}$) with the following parameters: N3-H1 = 0.90(4), N3----N1 = 3.037(4); H1--N1 = 2.16(4)Å, N3-HI--NI = 163(3)° and N3-H2 = 0.78(3), N3---N2 = 3.127(4), H2---N2 = 2.34(3)Å N3-H2---N2 = 176(3)°.

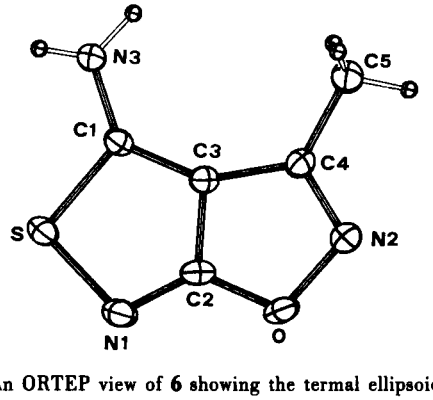


Figure 2. An ORTEP view of **6** showing the termal ellipsoid at 30% probability.

Table 1

Positional Parameters and Their Estimated Standard Deviations

Atom	x	y	z	B (Å^2)
S	0.37993 (5)	0.1390 (1)	0.000	3.22 (1)
O	0.3894 (2)	-0.3641 (3)	0.2435 (4)	3.31 (5)
N1	0.4464 (2)	-0.0560 (4)	0.0964 (4)	3.22 (5)
N2	0.2866 (2)	-0.4490 (4)	0.2758 (4)	3.06 (5)
N3	0.1701 (2)	0.1470 (3)	-0.0047 (5)	2.96 (4)
C1	0.2557 (2)	0.0443 (4)	0.0431 (4)	2.23 (5)
C2	0.3750 (2)	-0.1813 (5)	0.1564 (5)	2.69 (6)
C3	0.2689 (2)	-0.1412 (4)	0.1325 (4)	2.17 (5)
C4	0.2188 (2)	-0.3201 (4)	0.2109 (4)	2.45 (5)
C5	0.1069 (3)	-0.3673 (4)	0.2181 (5)	3.20 (6)
H1	0.107 (3)	0.092 (5)	0.020 (6)	3.8 (7)*
H2	0.178 (2)	0.247 (5)	-0.063 (4)	2.7 (6)*
H51	0.072 (3)	-0.277 (6)	0.257 (5)	4.3 (8)*
H52	0.085 (3)	-0.407 (6)	0.100 (6)	6 (1)*
H53	0.100 (3)	-0.465 (5)	0.316 (5)	3.7 (8)*

Starred atoms were refined isoptropically. Anisotropically refined atoms are given in the form of isotropic equivalent thermal parameters defined as: $B_{eq} = 4/3\Sigma_i\Sigma_j\beta_{ij}\hat{a}_i\hat{a}_j$.

Table 2

Bond distances (Å) with e.s.d.'s in parentheses

Atom 1	Atom 2	Distance
S	N1	1.680 (3)
S	C1	1.740 (3)
O	N2	1.451 (3)
O	C2	1.356 (4)
N1	C2	1.302 (4)
N2	C4	1.297 (4)
N3	C1	1.333 (4)
N3	H1	0.90 (3)
N3	H2	0.78 (3)
C1	C3	1.378 (4)
C2	C3	1.401 (4)
C3	C4	1.441 (4)
C4	C5	1.472 (4)
C5	H51	0.79 (4)
C5	H52	0.98 (5)
C5	H53	0.98 (4)

Table 3

Bond angles (°) with e.s.d.'s in parentheses

Atom 1	Atom 2	Atom 3	Angle
N1	S	C1	97.3 (1)
N2	O	C2	106.2 (2)
S	N1	C2	104.5 (2)
O	N2	C4	108.1 (2)
C1	N3	H1	120. (2)
C1	N3	H2	117. (2)
H1	N3	H2	123. (3)
S	C1	N3	122.5 (2)
S	C1	C3	106.2 (2)
N3	C1	C3	131.3 (3)
O	C2	N1	127.2 (3)
O	C2	C3	110.8 (2)
N1	C2	C3	122.0 (3)
C1	C3	C2	110.0 (3)
C1	C3	C4	146.3 (3)
C2	C3	C4	103.7 (2)
N2	C4	C3	111.1 (3)
N2	C4	C5	120.9 (3)
C3	C4	C5	128.0 (3)
C4	C5	H51	115. (3)
C4	C5	H52	107. (3)
C4	C5	H53	105. (2)
H51	C5	H52	112. (4)
H51	C5	H53	97. (3)
H52	C5	H53	121. (3)

EXPERIMENTAL

Melting points were determined using a Büchi capillary apparatus and are uncorrected. The ir spectra were recorded from potassium bromide discs on a Hitachi-Perkin 157G spectrometer. The uv spectra (ethanol) were determined with a Jasco UVIDEC 510 spectrophotometer. The ^{1}H-nmr and ^{13}C-nmr spectra were recorded on a Bruker AC 200 spectrometer. Chemical shifts (δ) are given in parts per million (ppm) relative to tetramethylsilane as internal standard and coupling constants in Hz. For column chromatography, silica gel (Kieselgel 60 Merck, 70-230 mesh ASTM) was used.

3-Methyl-4-thiocarbamoyl-5-aminoisoxazole (**4**).

Tetramethylguanidine (3.45 g, 30 mmoles) was added to a suspension of hydroxylamine hydrochloride (2.08 g, 30 mmoles) in chloroform (60 ml). After 15 minutes stirring, 2-cyano-3-ethoxythiocrotonamide (**3**, 5.10 g, 30 mmoles) was added portionwise to the solution and stirring was continued for 2 hours. The resulting precipitate was collected and washed with cold ethanol to yield 3.4 g, 72% of **4**, mp 173-175° (ethanol); ir (potassium bromide): cm^{-1} 3420, 3340, 3295, 3200, 1640, 1610, 1550, 840; ^{1}H-nmr (hexadeuteriodimethyl sulfoxide): δ 2.33 (s, 3H, CH_3), 7.8 (br, 1H, CSNH), 8.5 (br, 2H, NH_2), 9.0 (br, 1H, CSNH) (the absorptions at 7.8, 8.5 and 9.0 are deuterium oxide exchangeable); ^{13}C-nmr (hexadeuteriodimethyl sulfoxide): δ 11.98 (q, J = 128 Hz, CH_3), 92.7 (s, C-4), 154.85 (s, C-3), 171.83 (s, C-5), 188.50 (s, C=S).

Anal. Calcd. for $C_5H_7N_3OS$: C, 38.20; H, 4.49; N, 26.73; S, 20.40. Found: C, 38.58; H, 4.46; N, 26.81; S, 20.50.

3-Methylisoxazolo[5,4-*d*]pyrimidine-4(5*H*)thione (**5a**).

A few drops of 37% hydrogen chloride and triethyl orthoformate (4.44 g, 30 mmoles) were added to a solution of 3-methyl-4-thiocarbamoyl-5-aminoisoxazole (**4**, 0.94, 6 mmoles) in tetrahydrofuran (47 ml). The mixture was stirred at room temperature for 20 hours, the solvent was removed and the residue was taken up with ethyl ether. The precipitate was crystallized from toluene to yield 0.81 g, 80% of **5a**, mp 195-197°; ir (potassium bromide): cm^{-1} 3160, 3010, 2940, 1610, 1565, 1530, 1510, 1190; ^{1}H-nmr (hexadeuteriodimethyl sulfoxide): δ 2.61 (s, 3H, CH_3), 8.44 (s, 1H, CH), 14.3 (br, 1H, NH, deuterium oxide exchangeable); ^{13}C-nmr (hexadeuteriodimethyl sulfoxide): δ 11.95 (q, J = 130 Hz, CH_3), 112.66 (s, CH_3-C-*C*), 151.43 (d, J = 210 Hz, CH), 158.84 (s, CH_3-*C*), 170.03 (s, C-O), 179.95 (s, C=S).

Anal. Calcd. for $C_6H_5N_3OS$: C, 43.10; H, 3.01; N, 25.13; S, 19.17. Found: C, 43.23; H, 2.98; N, 25.10; S, 18.92.

3,6-Dimethylisoxazolo[5,4-*d*]pyrimidine-4(5*H*)thione (**5b**).

A solution of **4** (0.94 g, 6 mmoles) in a 1:1 mixture (45 ml) of triethyl orthoacetate and acetic acid was stirred at room temperature for 2 hours. The suspension was cooled and the precipitate was collected and washed with ethyl ether to yield 0.85 g, 78% of **5b** mp 255-256° (ethyl acetate/light petroleum ether); ir (potassium bromide): cm^{-1} 3200, 3080, 1600, 1570, 1535, 1180, 960, 775; ^{1}H-nmr (hexadeuteriodimethyl sulfoxide): δ 2.51 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 14.0 (s, 1H, NH, deuterium oxide exchangeable); ^{13}C-nmr (hexadeuteriodimethyl sulfoxide): δ 12.10 (q, J = 130 Hz, CH_3), 21.39 (q, J = 130 Hz, CH_3), 110.65 (s, CH_3-C-*C*), 158.75 (s, CH_3-*C*-C), 162.32 (s, CH_3-*C*-N), 170.51 (s, O-C-N), 180.62 (s, C=S).

Anal. Calcd. for $C_7H_7N_3OS$: C, 46.39; H, 3.89; N, 23.19; S, 17.69. Found: C, 46.50; H, 3.78; N, 23.39; S, 17.46.

3-Methyl-6-trifluoromethylisoxazolo[5,4-*d*]pyrimidine-4(5*H*)thione (**5c**).

Trifluoroacetic anhydride (4.2 g, 20 mmoles) was added to a solution of **4** (1.57 g, 10 mmoles) in trifluoroacetic acid (10 ml). The mixture was stirred at room temperature for 15 hours, made neutral with ammonium hydroxide and extracted with ethyl acetate (3 x 50 ml). The combined extracts were dried over magnesium sulfate, evaporated to a solid residue which was purified by column chromatography (eluent 8:2 ethyl acetate/light petroleum ether) to give 1.16 g, 40% of **5c**, mp 107-108° (ethyl acetate); ir potassium bromide): cm^{-1} 3440 (br), 1640, 1600, 1535, 1430, 1320, 1210, 1150, 1020; ^{1}H-nmr (hexadeuteriodimethyl sulfoxide): δ 2.63 (s, 3H, CH_3), 3.43 (s, 6H, $3H_2O$), 6.00 (br, 1H, NH, deuterium oxide exchangeable); ^{13}C-nmr (hexadeuteriodimethyl sulfoxide): δ 12.81 (q, J = 129.5 Hz, CH_3), 113.30 (s, CH_3-C-*C*), 120.25 (q, J_{CF} = 275 Hz, CF_3), 152.68 (q, J_{CF} = 33.9 Hz, *C*-CF_3), 158.65 (s, CH_3-*C*), 172.03 (s, C-O), 191.04 (s, C=S).

Anal. Calcd. for $C_7H_4F_3N_3OS \cdot 3H_2O$: C, 29.07; H, 3.49; F, 19.71; N, 14.53; S, 11.08. Found: C, 29.02; H, 3.28; F, 19.51; N, 14.83; S, 10.96.

Oxidation of **4** with Hydrogen Peroxide.

Hydrogen peroxide (40%) (0.06 ml, 7 mmoles) was added to an ice-cooled solution of 3-methyl-4-thiocarbamoyl-5-aminoisoxazole (**4**, 0.79 g, 5 mmoles) in pyridine (5 ml). The mixture was stirred at 0° for 24 hours and the precipitate (0.25 g of **6**) was collected. The filtrate was evaporated to a solid residue which, on separation by column chromatography (eluent 1:1 ethyl acetate/light petroleum ether) afforded a further crop (0.18 g) of **6** and 3-methyl-4-cyano-5-aminoisoxazole **7** (yield 0.093 g, 15%, mp 222-224°, identical with a sample prepared by the reported method [14]).

Compound **6** was obtained in 55% yield, mp 201-202° (ethyl acetate); ir (potassium bromide): cm^{-1} 3340 (br), 3150 (br), 1650, 1600, 1530, 1520, 1110 (br); uv (ethanol): nm λ max 302 (ε = 8966), λ max 257 (ε = 9878); ^{1}H-nmr (hexadeuteriodimethyl sulfoxide): δ 2.32 (s, 3H, CH_3), 8.03 (s, 2H, NH_2, deuterium oxide exchangeable); ^{13}C-nmr (hexadeuteriodimethyl sulfoxide): δ 10.94 (q, J = 129 Hz, CH_3), 103.90 (s, CH_3-C-*C*), 152.26 (s, CH_3-*C*), 167.52 (s, C-NH_2), 175.87 (s, O-C=N).

Anal. Calcd. for $C_5H_5N_3OS$: C, 38.70; H, 3.25; N, 27.08; S, 20.66. Found: C, 38.51; H, 3.26; N, 27.03; S, 20.69.

Oxidation of **4** with Iodine.

Iodine (1*N*) (8 ml, 4 mmoles) was added dropwise to a solution of **4** (0.63 g, 4 mmoles) and sodium hydrogen carbonate (0.68 g, 8 mmoles) in a 5:1 mixture (48 ml) of tetrahydrofuran and water. After the addition was completed (about 30 minutes) the solvent was removed and the residue was washed with water. After being dried *in vacuo* over phosphorus pentoxide the crude solid was purified by column chromatography (eluent 1:1 ethyl acetate/light petroleum ether) affording **7** (yield 0.33 g, 65%) and **6** (yield 0.026 g, 4%).

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