Synthesis of 2,2'-Dithiobis- and 2,2'-Trithiobis-3-pyridinecarboxylic Acid Derivatives as New Potential Radiosensitizers/Radioprotectors

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This paper reports the synthesis of dithio and trithio derivatives of 1,2-dihydro-2-thioxopyridine, starting with 1,2-dihydro-2-thioxo-3-pyridine carboxylic acid **1**.

This compound reacted with thionyl chloride to give the respective dithiobis (acyl chloride) **2**, which hydrolyzed to the corresponding dithio acid **3**. On the other hand, **1** reacted with sulfur dichloride to give trithio acid **4**, which on treatment with thionyl chloride gave the trithiobis (acyl chloride) **5**. Treatment of **2** and **5** with ethanol/pyridine, gave **8** and **12** respectively. Compounds **2-5**, **8** and **12** were unstable in alkaline medium and they were degraded to **1**.

The bis(acyl chloride) **2** and **5** reacted with ammonia and primary and secondary amines to give the respective bis(amide) **9** and **13**. Most of these amides (R = H, alkyl, aryl, R' = H) were found to be unstable in the presence of bases such as triethylamines, pyridine or excess of the starting amine, which promotes disproportion to give (see Scheme 3) the respective *N*-substituted (R) 1,2-dihydro-2-thioxo-3-pyridinecarboxamide **10** and the respective 2-substituted (R)-3-oxo-isothiazolo[5,4-*b*]pyridine **11**. On the other hand, compounds **11** were unstable to strong bases and they were transformed into the respective compounds **10** by an unknown mechanism. Boiling **11f** with sodium hydroxide in ethanol gave **10f** (50%). According to these last results, boiling **9h** or **13d** with sodium hydroxide in ethanol **10h** (68%) and **10d** (70%) were obtained.

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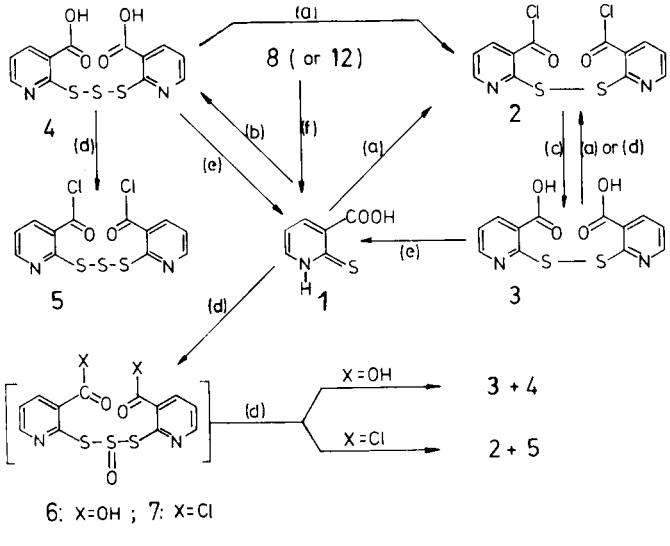
In recent years, a lot of work has been carried out on the synthesis and biological activity of organic compounds as radiosensitizers or radioprotectors in connection with the cancer therapy [1,2]. The effectiveness of sulfur derivatives of basic organic systems as radioprotectors is well established [2]. This activity is generally attributed [3,4] to the ability of such compounds to trap free radicals generated by irradiation. Organic disulfides [5,6] and polysulfides [7] are particularly effective radioprotector agents. However, to our knowledge, pyridine polysulfide derivatives have not yet been studied as radioprotectors.

In this paper we report the synthesis of a series of 2,2'-dithiobis and 2,2'-trithiobis(3-pyridinecarboxylic acid) derivatives for their further study as radioprotector agents.

Schemes **1**, **2** and **3** summarize our results and illustrate the structures of the new products obtained from 1,2-dihydro-2-thioxo-3-pyridinecarboxylic acid **1**.

Scheme 1

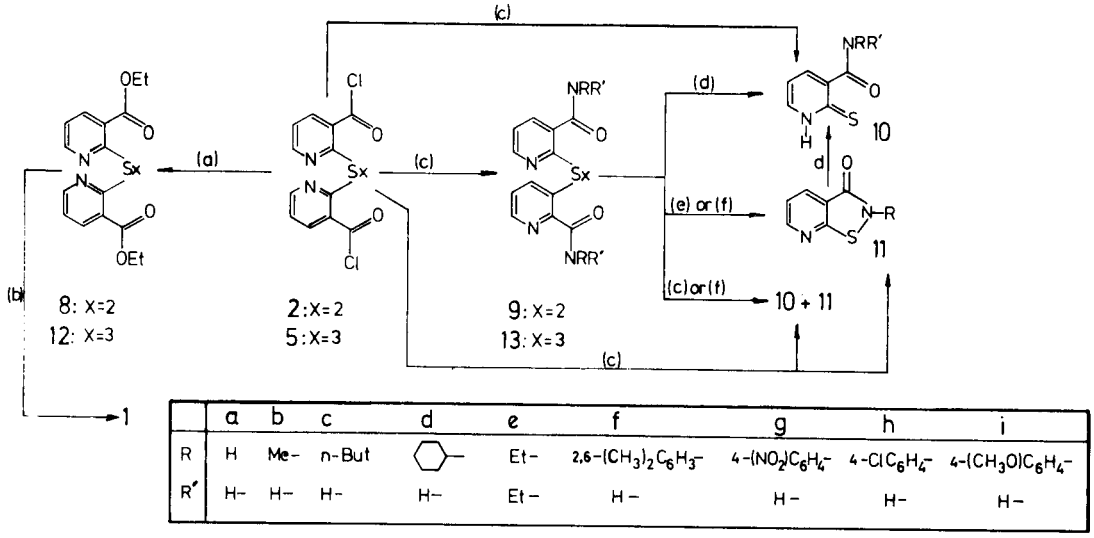


(a) $SOCl_2$ (toluene)/reflux; (b) S Cl_2/pyridine ($CHCl_3$); (c) H_2O/pyridine ($CHCl_3$); (d) $SOCl_2$/pyridine (CH_2Cl_2); (e) 1, NaOH; 2, H^+; (f) 1, NaOH/methanol; 2, H^+.

Organic disulfides are generally obtained by oxidation of the respective thiol derivatives; most frequently used oxidants are iodine [8,9], hydrogen peroxide [10], dimethylsulfoxide [11], or thionyl chloride [12-14]. This last reagent was particularly interesting for our work due to the possibility to obtain 2,2'-dithiobis(3-pyridinecarbonyl chloride) **2** directly from **1**, as previously reported by us [15]. However, in that paper [15], **2** was prepared by boiling a solution of **1**, thionyl chloride and pyridine in chlorofrom and the compound was not characterized, but used as a crude product for the preparation of some amides of **2** and some 2-substituted 3-oxoisothiazolo[5,4-*b*]pyridines.

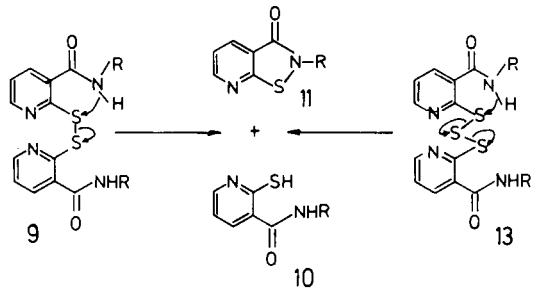
Compound **2** has now been obtained in good yield

Scheme 2



(a) EtOH/pyridine; (b) NaOH/heat; (c) HNRR'; (d) NaOH/EtOH/reflux; (e) $SOCl_2$/pyridine; (f) NEt_3 (CH_2Cl_2 or $CHCl_3$).

Scheme 3



(~75%) by boiling a solution of **1**, **3**, or **4** and thionyl chloride in toluene. The product, which crystallizes on cooling the reaction mixture, was characterized by elemental analysis and ir spectra.

The reaction of thiols with thionyl chloride seems to be complex and the products obtained depend mainly on the reaction conditions. Field and Lacefield [14] have obtained organic dithiosulfites by the reaction of thiols with thionyl chloride in the presence of pyridine. Dithiosulfites are unstable compounds, thermally discomposing to a mixture of respective dithio and trithio derivatives in proportions dependent on reaction conditions and sulfur dioxide, which catalyzes the reaction.

We have also studied the reaction of **1** with thionyl chloride and pyridine in methylene dichloride at 0°, with reagents in different proportion. Employing a molar ratio of **1**/thionyl chloride/pyridine of 2:1:2, we obtained a mixture of about 50% of the dithio and trithio acids **3** and **4**, respectively. By recrystallization from acetone/*N*,*N*-dimethylformamide, a sample of pure **4** was obtained.

On the other hand, with a molar ratio of **1**/thionyl chloride/pyridine of 2:3:4, we obtained a mixture of about 50% of the acyl chlorides **2** and **5** of the dithio and trithioacids **3** and **4**, respectively. By extraction of a sample of this mixture with pyridine/methylene dichloride, a pure sample of the most insoluble compound **5** was obtained. We could'nt obtain evidence for the formation of the respective dithiosulfites **6** and **7** as hypothetical intermediates in any case, as determined by ir spectroscopy of the reaction mixtures. Dithiosulfites show a strong and characteristic band for the group S=O at about 1110-1145 cm^{-1} [14,16,17]. However, it is interesting to indicate that a synthesis of bis(2-pyridyl)dithiosulfite [1,3-bis(2-pyridyl)trisulfan 2-oxide] has been reported [18].

Hydrolysis of **2** with aqueous chloroform/pyridine gave **3** (85%). Treatment of **3** with boiling thionyl chloride in toluene or thionyl chloride/pyridine in methylene dichloride at about 0° gave **2** in 70% and 85% yields respectively.

On the other hand, the reaction of **1** with sulfur dichloride/pyridine [19] at room temperature gave the acid **4** (70%), as expected. Treatment of **4** with thionyl chloride/pyridine at room temperature gave the acyl chloride **5**.

The acyl chlorides **2** and **5** react easily with ethanol in the presence of pyridine at room temperature to give in good yields (90%, 92%) the esters **8** and **12**, respectively.

All the above reported new compounds were characterized by ir spectra and elemental analysis. Compounds **3**, **4** and **8** were also characterized by ^1H nmr spectroscopy. However, the ^1H nmr spectra of these compounds gave little information, because with the exception of compounds **8** and **12**, only three protons of the pyridine ring can be observed. Consequently all the registered ^1H nmr spectra

are very similar.

Compounds **2-5**, **8** and **12** were very unstable in alkaline medium, and degraded to give **1**. Treating **3** or **4** with 1*M* sodium hydroxide at room temperature we obained **1** (70%). Similarly treatment of **8** or **12** with 1*M* sodium hydroxide in methanol at room temperature gave **1** (50%).

We have not studied the mechanism of these degradations. However, it is well knwon [20], that organic disulfides, particularly aromatic disulfides, are degraded by nucleophilic attack by hydroxide ion to give the thiolate and the corresponding sulphenate, which disproportionates to the respective thiolate and sulphinate.

The acyl chloride **2** reacts with amines to give the respective amides **9**. Compounds **9h** and **9i** and some other amides **9** obtained from aromatic amines were previously reported by us [15] by the reaction of **2** with the respective aromatic amine in boiling toluene. Compound **9f** is obtained by the reaction of **2** with 2,6-dimethylaniline in the presence of pyridine and chloroform as the solvent, at room temperature. Under similar conditions **9h** and **9i** were also obtained in yields of 10-20% higher than previously reported [15].

Nevertheless, by the addition of **2** to a stirred chloroform, chloroform/pyridine or chloroform/triethylamine solution of 4-nitroaniline at room temperature we obtained **10g** (30%) and **11g** (41%), but no **9g**. In a similar manner, we could not obtain in pure form the respective amides **9** with ammonia, methylamine or cyclohexylamine. These compounds were unstable under the reaction or recrystallization conditions, and they disproportionated to the respective 1,2-dihydro-2-thioxo-3-pyridinecarboxamide **10** and the 3-oxoisothiazolo[5,4-*b*]pyridine **11**, possibly by the path illustrated in Scheme 3.

This disproportionation of the amides **9** is favored by the presence of bases. Thus compounds **10i** (35%) and **11i** (30%), reported previously [15], were obtained by disproportionation of **9i** when treated with triethylamine in methylene dichloride solution at room temperature.

The acyl chloride **5** reacts with amines to give the respective amides **13**. However, these compounds seem to be at least as unstable as the amides **9**, and they also disproportionate, possibly as illustrated in Scheme 3, to give the respective compounds **10** and **11**. However, we can obtain in pure form (45-47%) the compounds **13c**, **13d** and **13e**, but attempts to obtain **13a**, **13b**, **13f-13i** and the respective amides with aniline and *p*-toluidine were fruitless. In some of these cases, in which we carried out the reactions in the presence of pyridine and chloroform as solvent at low temperature (−20°), we could observe by ir spectroscopy the presence of the respective amide, but the compound disproportionated during isolation.

The reaction of **5** with ammonium hydroxide gave **10a** (25%) and **11a** (27%). Similarly, the reaction of **5** with methylamine in chloroform at room temperature gave **10b** (35%) and **11b** (30%), and the reaction of **5** with 2,6-dimethylaniline in the presence of pyridine gave **10f** (35%) and **11f** (40%). Also the reaction of **5** with *p*-nitroaniline gave **10g** (30%) and **11g** (43%). All these compounds were characterized by elemental analyses and ir spectra and, in some cases (**10f**, **11f**, **11g**), by ^{1}H nmr, which are described in the experimental. In our previous paper [15], we reported that some carboxamides **10** were obtained by the reaction of the acyl chloride from **1** with aromatic amines, by reduction of the carboxamides **10** with sodium bisulphite and by other procedures. We have also reported [15] the syntheses of some 2-aryl-3-oxoisothiazolo[5,4-*b*]pyridines **11** by oxidation of **9** (R = H, R′ = aryl) or **10** (R = H, R = aryl) with thionyl chloride/pyridine, and the reduction of **11** to **10** with sodium bisulphite.

Compound **11d** (85%) was obtained by treating **13d** with thionyl chloride/pyridine, in a similar reaction to that reported [15] for the oxidation of **9** to **11**.

When **11f** was boiled with sodium hydroxide in 96% ethanol **10f** (50%) was obtained. The new compound was characterized by elemental analysis, ir, and ^{1}H nmr spectroscopy.

When **9h** or **13d** were boiled with sodium hydroxide in 96% ethanol, **10h** (68%) and **10d** (70%) were obtained respectively. Compound **10d** was characterized by elemental analysis and ir spectra and compound **10h** had been previously reported by us [15].

Work is in progress to evaluate the radioprotective properties of some of the compounds reported in this and in our previous paper [15].

EXPERIMENTAL

Melting points were determined in a Kofler apparatus and are uncorrected. Elemental analyses were obtained from vacuum-dried samples (over phosphorus pentoxide at 3-4 mm Hg, 2-3 hours, at about 60-70°). Infrared spectra were recorded on a Perkin-Elmer 681 apparatus, using potassium bromide tablets for solid products and sodium chloride plates for liquid products; the frequencies are expressed in cm^{-1}. The ^{1}H nmr spectra were obtained on a Perkin-Elmer R-32(90 MHz) instrument, with tetramethylsilane as the internal reference, at a concentration of about 0.1 g/ml and dimethylsulfoxide-d_6 as solvent; the chemical shifts are reported in ppm from tetramethylsilane and are given in δ units, and the abbreviations are the usual. The ms spectra were recorded in a VG 12-250 instrument.

Thin-layer chromatography (tlc) was carried out in silica gel (DSF-5, Cammaga 0.3 mm. thickness) with benzene:dioxane:acetic acid (90:25:4) as solvent and the plates were scanned under ultraviolet light, λ = 254 and 366 nm.

Solvents were usually removed under vacuum, when stated, in a rotatory evaporator.

Materials.

1,2-Dihydro-2-thioxo-3-pyridinecarboxylic Acid (1).

Compound **1** (mp 244-246°) was prepared from 2-chloronicotinic acid as previously reported [15]. In addition, **1** was obtained as a degradation product of compounds **3**, **4**, **8** and **12**, according to the following methods.

From **4**.

Compound **4** (0.68 g, 2 mmoles), was added to sodium hydroxide (10 ml, 1*M*) and the solution stirred at room temperature for one hour. The solution was acidified to *p*H 2 by slow addition of hydrochloric acid (1*M*), and the suspension left for 3-4 hours. The yellow solid material was collected by filtration, washed with water, dried and recrystallized, mp 244-246° (methanol/*N,N*-dimethylformamide), yield 0.43 g (70%).

From **3**.

In a similar way to that described above for **4**, but with a reaction time of 24 hours, yield 0.40 g (65%).

From **8** (or **12**).

Compound **8** (or **12**) (1 mmole), was added to a solution of sodium hydroxide (5 ml, 1*M*) and methanol, and the mixture stirred at room temperature for 24 hours. Methanol was removed under vacuum, the residual solution diluted with water (5 ml) and acidified with hydrochloric acid (1*M*) to *p*H 2. The yellow solid material was collected by filtration, washed with water, dried and recrystallized as indicated above, yield 0.15 g (50%).

2,2'-Dithiobis(3-pyridinecarbonyl Chloride) (**2**).

From **1**.

A mixture of **1** (1.0 g, 6.45 mmoles), toluene (10 ml) and thionyl chloride (3 ml) was boiled for one hour. The yellow product which crystallized on cooling the solution was collected and washed with carbon disulfide and then with chloroform, mp 212-214° dec, yield 0.83 g (75%); ir: 1725 (s, CO).

Anal. Calcd. for $C_{12}H_6Cl_2N_2O_2S_2$: C, 41.74; H, 1.74; Cl, 20.58; N, 8.11; S, 18.55. Found: C, 41.84; H, 1.77; Cl, 20.60; N, 8.18; S, 18.52.

For most of the reactions carried out in our laboratory, crude **2** was used. To obtain crude **2**, the solvent was removed under vacuum from the above reaction mixture, and the residual material treated with toluene and the solvent removed under vacuum. This treatment was repeated two or three more times to obtain a yellow solid with an ir spectra identical to that of the crystalline product, mp 211-213° dec, yield 1.04 g (100%).

From **3**. Method a.

A mixture of **3** (1.0 g, 3.25 mmoles), toluene (10 ml) and thionyl chloride (3 ml) was treated in a similar manner to that described above from **1**, yield 0.78 g (70%).

Method b.

Compound **3** (1.0 g, 3.25 mmoles) was added to an ice-cold solution of methylene chloride (15 ml), pyridine (3 ml) and thionyl chloride (3 ml) was added. The mixture was stirred at about 0° for one hour. Solvents were removed under vacuum to yield the yellow solid residue as described above from **1**, yield 0.95 g (85%).

From **4**.

A mixture of **4** (1.0 g, 2.94 mmoles), toluene (10 ml) and thionyl chloride (3 ml) was treated in a similar manner as described for **1**, yield 0.71 g (70%).

2,2'-Dithiobis(3-pyridinecarboxylic Acid) (**3**).

Crude **2** was obtained as described above for **1** (1.0 g, 6.45 mmoles) and dissolved in chloroform (10 ml) and pyridine (1 ml). After the addition of water (5 ml), the mixture was stirred at room temperature for 5 hours. Water (10 ml) was added and then hydrochloric acid (1*M*) to bring the *p*H of the aqueous portion to **2**. The solid material was collected by filtration, washed with water and recrystallized, mp 218-220° dec (ethanol/*N,N*-dimethylformamide), yield 0.85 g (85%); ir: 3400-2400 (broad, NH^+), 1690 (CO); ^1H nmr: 5.50 (bs, 2H, NH^+), 7.28 (c, 2H, H-5), 8.25 (dd, 2H, H-4), 8.50 (dd, 2H, H-6), $J_{4,5} = 7.5$, $J_{5,6} = 5$; ms: 155 (M^+-153, 86%), 153 (M^+-155, 100%), 111 (M^+-197, 63%), 109 (M^+-199, 40%).

Anal. Calcd. for $C_{12}H_8N_2O_4S_2$: C, 46.75; H, 2.60; N, 9.09; S, 20.78. Found: C, 46.77; H, 2.65; N, 9.05; S, 20.77.

2,2'-Trithiobis(3-pyridinecarboxylic Acid) (**4**).

To an ice-cold and stirred mixture of **1** (8.0 g, 51.6 mmoles) and chloroform (30 ml), a solution of sulfur dichloride (2.66 g, 25.8 mmoles) in chloroform (10 ml) was added. The mixture was stirred for 0.5 hours and then a solution of pyridine (4.08 g, 51.6 mmoles) in chloroform (20 ml) was added to the reaction mixture. The ice bath was removed and the mixture stirred for 9 hours at room temperature. The solid material was collected by filtration, successively washed with several portions of chloroform and carbon disulfide and recrystallized, mp 221-223° dec (acetone/*N,N*-dimethylformamide), yield 6.2 g (70%) as white crystals; ir: 3300-2400 (broad, COOH), 1690 (CO); ^1H nmr: 5 (bs, 2H, NH^+), 7.50 (c, 2H, H-5), 8.40 (dd, 2H, H-4), 8.90 (dd, 2H, H-6).

Anal. Calcd. for $C_{12}H_8N_2O_4S_3$: C, 42.35; H, 2.35; N, 8.23; S, 28.23. Found: C, 42.37; H, 2.38; N, 8.27; S, 28.20.

2,2'-Trithiobis(3-pyridinecarbonyl Chloride) (**5**).

To an ice-cold and stirred solution of thionyl chloride (3 ml), pyridine (3 ml) and methylene dichloride (13 ml), **4** (1.0 g, 2.94 mmoles) was added in small portions. The solution was stirred for 0.5 hours, the solid material collected by filtration and successively washed with several portions of chloroform and carbon disulfide, mp 212-214° dec, as a white powder, yield 0.8 g (72%); ir: 1725 (CO).

Anal. Calcd. for $C_{12}H_6Cl_2N_2O_2S_3$: C, 38.19; H, 1.59; Cl, 18.83; N, 7.42; S, 25.46. Found: C, 38.02; H, 1.65; Cl, 18.95; N, 7.40; S, 25.22.

The Reactions of **1** with Thionyl Chloride and Pyridine.

Method A.

To an ice-cold and stirred solution of thionyl chloride (0.38 g, 3.22 mmoles), pyridine (0.51 g, 6.45 mmoles) and methylene dichloride (15 ml), **1** (1.0 g, 6.45 mmoles) was added in small portions and the mixture stirred for one hour at about 0°. The solid material was collected by filtration and successively washed with several portions of chloroform and carbon disulfide. Elemental analysis (C, 44.05; H, 2.50; N, 8.52; S, 24.12) and ir spectra of this product showed it was a mixture at about 50% of **3** and **4**. By recrystallization from acetone/*N,N*-dimethylformamide) a sample of pure **4** was obtained.

Method B.

To an ice-cold and stirred solution of thionyl chloride (1.16 g, 9.68 mmoles), pyridine (1.04 g, 12.9 mmoles) and methylene dichloride (15 ml), **1** (1.0 g, 6.45 mmoles) was added in small portions and the mixture treated as in method 1. Elemental analysis (C, 39.18; H, 1.70; N, 7.62; S, 21.51) and the ir spectra of the product showed it was a mixture at about 50% of **2** and **5**. A sample of this product (0.1 g) was stirred with methylene dichloride (10 ml) and pyridine (1 ml) at room temperature for 2 days. The insoluble material was collected and identified as pure **5**.

2,2'-Dithiobis(ethyl 3-Pyridinecarboxylate) (**8**).

To crude **2**, prepared from **1** (1.0 g, 6.45 mmoles) as above described, ethanol (10 ml) and pyridine (2 ml) were added and the mixture was stirred for one hour at room temperature. Solvents were removed in vacuum and the solid residue washed with water and recrystallized, mp 163-165° dec (ethanol), yield 1.18 g (93%); ir: 1690 (CO); ^1H nmr: 1.35 (t, 6H, CH_3), 4.40 (dd, 4H, CH_2), 7.32 (c, 2H, H-5), 8.27 (dd, 2H, H-4), 8.52 (dd, 2H, H-6), $J_{4,5} = 7.5$, $J_{5,6} = 5$.

Anal. Calcd. for $C_{16}H_{16}N_2O_4S_2$: C, 52.74; H, 4.39; N, 7.69. Found: C, 52.70; H, 4.35; N, 7.68.

2,2'-Dithiobis[*N*-(2,6-dimethylphenyl)-3-pyridinecarboxamide] (**9f**).

Crude **2** was prepared from **1** (1.0 g, 6.45 mmoles) as described above and suspended in chloroform (10 ml) and pyridine (3 ml); to the stirred mixture a solution of 2,6-dimethylaniline (0.78 g, 6.45 mmoles) in chloroform (5 ml) was added slowly. The reaction mixture was stirred for 4 hours at room temperature, and then the solvents were removed under vacuum. The residual material was dispersed in dilute ammonium hydroxide (30 ml, 2 *M*) and the solid material collected, washed with water,

dried and recrystallized, mp 177-179° dec (methanol), yield 0.66 g (40%); ir: 3300 (NH), 1645 (CO); ^{1}H nmr: 2.22 (s, 12H, CH_3), 7.00-7.40 (m, 8H, H-5, H-3', H-4', H-5'), 8.15 (dd, 2H, H-4), 8.48 (dd, 2H, H-6), $J_{4,5}$ = 7.5, $J_{5,6}$ = 5, 10.10 (s, 2H, NH).

Anal. Calcd. for $C_{28}H_{26}N_4O_2S_2$: C, 65.37; H, 5.06; N, 10.89. Found: C, 65.35; H, 5.12; N, 10.83.

2,2'-Trithiobis(ethyl 3-Pyridinecarboxylate) (**12**).

To a stirred solution of pyridine (0.21 g, 2.65 mmoles) and ethanol (3 ml) in methylene dichloride (10 ml), **5** (0.5 g, 1.32 mmoles) was added in small portions. The mixture was stirred for one hour at room temperature. Solvents were removed in vacuum and the solid residue collected, washed with water, dried and recrystallized as white crystals, mp 134-136° dec (ethanol), yield 0.47 g (90%); ir: 1695 (CO).

Anal. Calcd. for $C_{16}H_{16}N_2O_4S_3$: C, 48.48; H, 4.04; N, 7.07. Found: C, 48.42; H, 4.05; N, 6.99.

2,2'-Trithiobis(3-pyridinecarboxamides) (**13**).

To a stirred and ice-cold solution of the respective amine (5.3 mmoles), triethylamine (or pyridine) (5.3 mmoles) in chloroform (10 ml), **5** (1.0 g, 2.65 mmoles) were added in small portions, and the mixture was stirred for 4 hours. A new portion of chloroform (50 ml) was added and the organic solution washed successively with several portions of sodium hydroxide (1*M*), hydrochloric acid (1*M*) and water, and dried over anhydrous sodium sulfate. Solvent was removed in vacuum and the solid residue recrystallized. In this way the following compounds were obtained.

2,2'-Trithiobis[*N*-(butyl)-3-pyridinecarboxamide] (**13c**).

From butylamine and triethylamine this compound had mp 124-126° dec (methanol/ethyl ether), yield 0.71 g (60%); ir: 3300 (NH), 1635 (CO).

Anal. Calcd. for $C_{20}H_{26}N_4O_2S_3$: C, 53.33; H, 5.78; N, 12.44. Found: C, 53.20; H, 5.80; N, 12.41.

2,2'-Trithiobis[*N*-(cyclohexyl)-3-pyridinecarboxamide] (**13d**).

From cyclohexylamine and triethylamine this compound had mp 206-208° dec (methanol), yield 1.0 g (75%); ir 3300 (NH), 1630 (CO).

Anal. Calcd. for $C_{24}H_{30}N_4O_2S_3$: C, 57.37; H, 5.97; N, 11.15. Found: C, 57.40; H, 6.01; N, 11.17.

2,2'-Trithiobis[*N,N*-(diethyl)-3-pyridinecarboxamide] (**13e**).

From diethylamine and triethylamine this compound had mp 142-144° dec (methanol/ethyl ether), yield 0.5 g (45%); ir: 1625 (CO).

Anal. Calcd. for $C_{20}H_{26}N_2O_2S_3$: C, 53.33; H, 5.78; N, 12.44. Found: C, 53.28; H, 5.80; N, 12.42.

1,2-Dihydro-2-thioxo-3-pyridinecarboxamides **10**.

1,2-Dihydro-2-thioxo-3-pyridinecarboxamide (**10a**).

To a stirred solution of ammonium chloride (1.0 g) in 20% ammonium hydroxide (10 ml), **2** (or **5**) (2.65 mmoles) were added in small portions, and the mixture stirred at room temperature for 2 hours. To the solution 1*N* hydrochloric acid was added to *p*H 8 and the mixture stirred for one hour. The solid material was collected, washed with water and recrystallized, mp 228-230° dec (acetone/methanol), yield 0.2 g (24%) of **10a**; ir: 3300-2700 (several bands, NH, SH), 1670 (CO); ^{1}H nmr: 7.02 (c, 1H, H-5), 7.90 (dd, 1H, H-6), 8.48 (dd, 1H, H-4), $J_{4,5}$ = 8, $J_{5,6}$ = 5, 7.65-8.00 (bs, 1H), 10.2 (bs, 1H), 14.1 (bs, 1H) for NH_2 and SH.

Anal. Calcd. for $C_6H_6N_2OS$: C, 46.75; H, 3.89; N, 18.18. Found: C, 46.71; H, 3.95; N, 18.21.

Other compounds **10** were obtained according to the following methods.

Method 1.

A solution of **13** (or **9**, or **11**) (1.0 mmole), and sodium hydroxide (0.4 g, 10 mmoles) in 96% ethanol (10 ml) was stirred for one hour at room temperature and then boiled for 15 minutes. Solvent was removed in vacuum and the residue diluted with water (15 ml) and the solution filtered. To the filtrate, hydrochloric acid (1*M*) was added to *p*H 7 and the precipitate collected. The filtrate was extracted with chloroform and the solid recovered from the extract, after removing the solvent in vacuum, mixed with the precipitate and the mixture recrystallized.

In this manner the following compounds **10** were obtained.

N-(Cyclohexyl)-1,2-dihydro-2-thioxo-3-pyridinecarboxamide (**10d**).

From **13d** this compound had mp 184-186° dec (ethyl ether), yield 0.16 g (70%); ir: 2700-3200 (several bands, NH, SH), 1645 (CO).

Anal. Calcd. for $C_{12}H_{16}N_2OS$: C, 61.02; H, 6.77; N, 11.86. Found: C, 61.08; H, 6.80; N, 11.88.

N-(2,6-Dimethylphenyl)-1,2-dihydro-2-thioxo-3-pyridinecarboxamide (**10f**).

From **11f** this compound had mp 228-230° (d) (methanol), yield 0.18 g (70%); ir: 2700-3200 (several bands, NH, SH), 1645 (CO); ^{1}H nmr: 2.22 (s, 6H, CH_3), 7.00-7.25 (m, 4H, H-5, H-3', H-4', H-5'), 8.00 (bs, 1H, H-6), 8.54 (dd, 1H, H-4), $J_{4,5}$ = 7.5, 12.2 (s, 1H), 14.1 (bs, 1H) for NH, SH.

Anal. Calcd. for $C_{14}H_{14}N_2OS$: C, 65.12; H, 5.43; N, 10.85. Found: C, 65.08; H, 5.47; N, 10.84.

N-(4-Chlorophenyl)-1,2-dihydro-2-thioxo-3-pyridinecarboxamide (**10h**).

From **9h** [15] this compound had mp 226-228° (acetone/methanol), yield 0.18 g (68%), reported [15], mp 226-228°.

Method 2.

The reaction mixture was similar to that described above for the preparation of **13**, but the isolation procedure was different. The method is illustrated for the preparation of **10b**.

N-(Methyl)-1,2-dihydro-2-thioxo-3-pyridinecarboxamide (**10b**).

To a stirred and an ice-cold mixture of 40% aqueous methylamine (10.6 mmoles), and chloroform (10 ml), **5** (1.0 g, 2.65 mmoles) was added in small portions. The mixture was stirred for 4 hours at room temperature. Solvent was removed under vacuum and the residue extracted with sodium hydroxide (10 ml, 5*M*). The insoluble material was collected by filtration, washed with water and used to obtain **11b** (see below). To the filtrate, hydrochloric acid (1*M*) was added to bring the *p*H 7 and the precipitate collected, washed with water and recrystallized, mp 208-210° dec (ethanol/acetone), yield 0.31 g (35%); ir: 2600-3200 (several bands, SH, NH), 1645 (CO).

Anal. Calcd. for $C_7H_8N_2OS$: C, 49.99; H, 4.76; N, 16.66. Found: C, 50.00; H, 4.80; N, 16.65.

N-(4-Nitrophenyl)-1,2-dihydro-2-thioxo-3-pyridinecarboxamide (**10g**).

Compound **10g** was obtained from *p*-nitroaniline (1.29 g, 10 mmoles) and **2** (1.11 g, 3.22 mmoles) in chloroform (10 ml), mp >250° (*N,N*-dimethylformamide), yield 0.53 g (30%); ir: 2750-3200 (several bands, SH, NH), 1680 (CO).

Anal. Calcd. for $C_{12}H_9N_3O_3S$: C, 52.36; H, 3.27; N, 15.27. Found: C, 52.43; H, 3.40; N, 15.25.

Method 3.

N-(4-Methoxyphenyl)-1,2-dihydro-2-thioxo-3-pyridinecarboxamide (**10i**).

A solution of **9i** (0.52 g, 1.0 mmoles) [15] and triethylamine (1.0 ml) in methylene dichloride (10 ml) was stirred at room temperature for 2 hours. The solvents were removed under vacuum and the product isolated as described in Method 2, mp 204-206° (acetone/methanol), yield 0.18 g (35%), reported mp 204-206° [15]. The insoluble material in sodium hydroxide (5*M*), was collected, washed with water and used to recover **11i** (see below).

2,3-Dihydro-3-oxoisothiazolo[5,4-*b*]pyridines **11**.

2,3-Dihydro-3-oxoisothiazolo[5,4-*b*]pyridine (**11a**).

To an ice-cold and stirred solution of ammonium chloride (1.0 g) in ammonium hydroxide (10 ml, 20%), **2** (or **5**) (2.65 mmoles) was added in small portions and the mixture stirred at room temperature for 2 hours. Hydrochloric acid (1*M*) was added to *p*H 8 and the mixture stirred for

one hour and filtered. To the filtrate more hydrochloric acid (1*M*) was added to bring the *p*H at about 6. The precipitate was collected and the solution extracted several times with chloroform. The residual solid obtained after removing the solvent under vacuum was combined with the collected precipitate and the mixture was recrystallized, mp 222-224° (d) (acetone/methanol), yield 0.22 g (27%); ir: 2700-3150 (several bands, NH, SH), 1650-1690 (several bands, C=O); ^{1}H nmr: 7.52 (d, 1H, H-5), 8.34 (dd, 1H, H-4), 8.82 (dd, 1H, H-6), $J_{4,5} = 8$, $J_{5,6} = 5$, 12.0 (bs, 1H, NH); ms: 152 (M^+, 100%); 97 ($M^+ - 55$, 15%), 77 ($M^+ - 75$, 15%).

Anal. Calcd. for $C_6H_4N_2OS$: C, 47.37; H, 2.63; N, 18.42. Found: C, 47.35; H, 2.67; N, 18.40.

Other compounds **11** were obtained according to the following methods:

Method 1.

2-(4-Methoxyphenyl)-2,3-dihydro-3-oxoisothiazolo[5,4-*b*]pyridine (**11i**).

A solution of **9i** (0.52 g, 1.0 mmoles) [15], triethylamine (1 ml) in methylene dichloride (10 ml) was stirred for 2 hours at room temperature. Solvent was removed in vacuum and the solid residue (a mixture of **10i** and **11i**) was extracted with sodium hydroxide (10 ml, 5*M*) and the insoluble material collected, washed with water and recrystallized, mp 164-166° (methanol/acetone), yield 0.15 g (30%), reported [15]. From the alkaline filtrate **10i** was recovered as described above.

Method 2.

The reaction mixture was similar to that reported above to obtain compounds **13**, starting with **5** or **2**, triethylamine (or pyridine) and the respective amine in chloroform, except in the case of **11b** in which the reaction mixture was the same as described for **10b**. In each case, the reaction mixture was stirred at room temperature for 4 hours. Solvent was removed under vacuum and the solid residue extracted with sodium hydroxide (10 ml, 5*M*), and the insoluble material collected by filtration, washed with water and recrystallized. From the alkaline filtrate the respective compound **10** was generally recovered.

In this way the following compounds **11** were obtained.

2-(Methyl)-2,3-dihydro-3-oxoisothiazolo[5,4-*b*]pyridine (**11b**).

This compound was obtained from 40% aqueous methylamine (10.6 mmoles), **5**, (1.0 g, 2.65 mmoles) in chloroform (10 ml), mp 128-130° (isooctane/ethanol), yield 0.26 g (30%); ir: 1640 (CO).

Anal. Calcd. for $C_7H_6N_2OS$: C, 50.60; H, 3.61; N, 16.86. Found: C, 50.59; H, 3.65; N, 16.87.

From the alkaline filtrate **10b** was obtained as described above.

2-(2,6-Dimethylphenyl)-2,3-dihydro-3-oxothiazolo[5,4-*b*]pyridine (**11f**).

From **5** (1.0 g, 2.65 mmoles), 2,6-dimethylaniline (0.64 g, 5.3 mmoles) and pyridine (1 ml), mp 110-112° (isooctane/ethyl ether), yield 0.54 g (40%); ir: 1680 (CO); ^{1}H nmr (deuteriochloroform): 2.32 (s, 6H, CH_3), 7.10-7.40 (m, 3H, H-3', H-4', H-5'), 7.52 (dd, 1H, H-5), 8.45 (dd, 1H, H-4), 8.87 (dd, 1H, H-6).

Anal. Calcd. for $C_{14}H_{12}N_2OS$: C, 65.62; H, 4.69; N, 10.93. Found: C, 65.65; H, 4.71; N, 10.90.

From the alkaline filtrate, **10f** was obtained as described above.

2-(4-Nitrophenyl)-3-oxoisothiazolo[5,4-*b*]pyridine (**11g**).

From **2** (0.91 g, 2.65 mmoles), *p*-nitroaniline (10.6 mmoles) in chloroform (10 ml) **11g** was obtained mp >250° (*N,N*-dimethylformamide), yield 0.62 g (43%); ir: 1690 (CO); ^{1}H nmr (trifluoroacetic acid-5%): 8.07 (d, 2H, H-2', H-6'), 8.27 (dd, H-5), 8.62 (d, 2H, H-3', H-5'), 8.20-8.50 (m, 2H, H-4, H-6).

Anal. Calcd. for $C_{12}H_7N_3O_3S$: C, 52.74; H, 2.56; N, 15.38. Found: C, 52.63; H, 2.59; N, 15.35.

Method 3.

2-(Cyclohexyl)-2,3-dihydro-3-oxoisothiazolo[5,4-*b*]pyridine (**11d**).

A mixture of **13d** (0.5 g, 1.0 mmoles), chloroform (10 ml), thionyl chloride (1 ml) and pyridine (2 ml) was stirred for 10 minutes at room temperature. Solvents were removed under vacuum and the residue treated with sodium hydroxide (10 ml, 5*M*) and the solid material collected, washed with water and recrystallized, mp 135-137° (methanol), yield 0.38 g (82%); ir: 1675, 1645 (CO).

Anal. Calcd. for $C_{12}H_{14}N_2OS$: C, 61.53; H, 5.98; N, 11.96. Found: C, 61.50; H, 6.01; N, 11.95.

Compound **11d** was also obtained as reported above, method 2, from **2** (2.22 g, 6.45 mmoles), cyclohexylamine (1.28 g, 12.9 mmoles) and triethylamine (1.30 g, 12.9 mmoles) on chloroform (10 ml), yield 0.7 g (46%).

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