Eur. J. Med. Chem. **23** (1988) 211—216

Original paper

N-Heterocyclic compounds as radioprotectors II. Derivatives of pyridine and pyrimidine containing thiol precursors

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(Received March 10, 1986, accepted November 2, 1987)

Summary — Pyridine and 4-substituted pyrimidine derivatives incorporating a sulphur functional side-group were evaluated as potential radioprotective agents by screening tests in mice. 2-(2-Pyridyl)ethanethiol and its derivatives showed some radioprotective action when administered intraperitoneally, but all were inferior to 2-pyridinemethanethiol. They showed no protection when administered orally. Substitution of the pyridine ring by chlorine gave compounds which also showed inferior radioprotective properties to those demonstrated by 2-pyridinemethanethiol. Corresponding phosphorothioates also exhibited little activity. The pyrimidine derivatives showed protection of a low order.

Résumé — **Composés *N*-hétérocycliques radioprotecteurs II. Dérivés pyridiniques et pyrimidiniques contenant des précurseurs soufrés.** *Des dérivés pyridiniques et pyrimidiniques substitués en 4 avec un groupement fonctionnel soufré ont été testés comme agents radioprotecteurs potentiels chez la Souris. Le pyridyl-2-éthane-2-thiol et ses dérivés ont présenté une action radioprotectrice après leur administration par voie intra-péritonéale mais elle a toujours été inférieure à celle du pyridine-2-méthanethiol. Ils n'ont présenté aucune protection quand ils ont été administrés par voie orale. La substitution du noyau pyridine par le chlore a fourni des composés qui ont également présenté des propriétés radioprotectrices inférieures à celles du pyridine-2-méthanethiol. Les phosphorothioates correspondants ont été également peu actifs. Les dérivés de pyrimidine ont présenté une protection négligeable.*

pyridine derivatives / pyrimidine derivatives / thiol / thiol precursor / radioprotectors

Introduction

In a previous paper describing the synthesis and testing of 2-pyridinemethanethiol, 2-pyrazinemethanethiol and related compounds [1], these were evaluated as potential radioprotective agents related to the classical radioprotective compound 2-aminoethanethiol. The novel structural feature of these compounds, having the nitrogen atom and the adjacent carbon atom of the 2-aminoethanethiol moiety incorporated into the pyridine ring, was introduced with the objective of obtaining a new class of compounds having improved radioprotective properties, and in order to examine the relationship between structure and biological properties amongst compounds within this group. Those modifications of 2-aminoethanethiol previously described [2—4] have been directed primarily at either the thiol or amino group, but the design of thiol-covering functions, *i.e.*, phosphorothioates, which may be cleaved enzymatically in the tissues to release the free thiol were found to offer marked advantages [5]. Consideration of the results published in the initial paper [1] indicated that further study of structurally related compounds would be beneficial. So, 6-chloro-2-pyridinemethanethiol, 2-(2-pyridyl)ethanethiol, the corresponding disulphides and phosphorothioates, together with 4-chloro-2-pyridine phosphorothioate were prepared, and their radioprotective activities compared with those of the corresponding pyridine methanethiols and derivatives.

Moreover, a few derivatives of 4-thiomethylpyrimidine were prepared and tested for comparison. A study of pyrimidine analogues would be of interest, particularly since the pyrimidine ring is found in several biologically important molecules. We could not prepare 2-chloromethylpyrimidine by analogous reactions used in the preparation of the 4-isomer [7].

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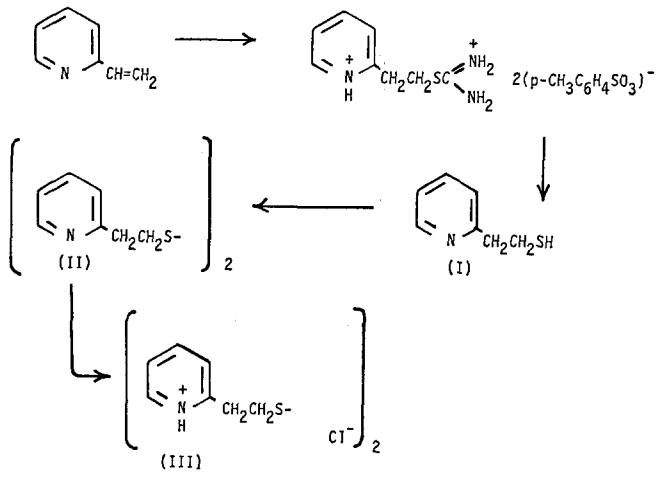
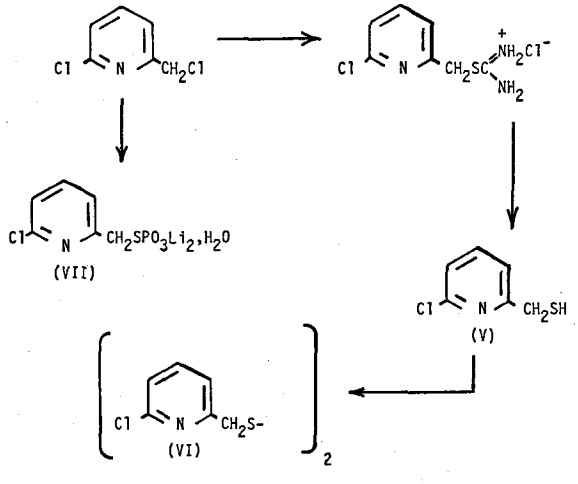
Chemistry



The general synthetic route to the preparation of 2-(2-pyridyl)ethanethiol **I** and related compounds **II** and **III** is outlined above, full synthetic details being given under Experimental protocols. 2-Vinylpyridine reacted with thiourea to give an isothiouronium salt, which was then cleaved, under conditions of mild alkaline hydrolysis, to give the thiol **I**. The latter was oxidised with iodine in chloroform to the disulphide **II**, which, because of its insolubility in water, was converted into the corresponding hydrochloride salt **III**, using ethereal hydrogen chloride. 4-Chloro-2-chloromethylpyridine was prepared as described previously [6] and converted into the corresponding hydrochloride salt using the same reagent. This hydrochloride salt reacted in aqueous solution with trilithium phosphorothioate to give dilithium *S*-(4-chloro-2-pyridylmethyl)-phosphorothioate **IV**.



The general synthetic route to the preparation of such compounds as 6-chloro-2-pyridinemethanethiol **V**, and related derivatives **VI** and **VII** is as shown. 6-Chloro-2-chloromethylpyridine was prepared as described previously [6] and reacted with thiourea in a well-documented preparation to give an isothiouronium salt which was then hydrolysed under strongly basic conditions to give the thiol **V**. The corresponding disulphide **VI** was obtained by oxidation with iodine in chloroform, and the phosphorothioate **VII** was prepared from 6-chloro-2-chloromethylpyridine by reaction with trilithium phosphorothioate in a dimethylformamide/water solution.

4-Pyridiniummethylisothiouronium chloride hydrochloride **VIII** was prepared by the reaction of 4-chloromethylpyridine hydrochloride with thiourea. The isothiouronium salt gave very little of the corresponding thiol under conditions of both mild and strong alkaline hydrolysis. However, hydrolysis of the isothiouronium salt with ammonium hydroxide followed by immediate oxidation of the isolated oil with iodine in chloroform gave the disulphide **IX**. Due to the latter's insolubility in all solvents used for biological screening, the disulphide was converted into the corresponding hydrochloride salt **X**. Reaction of 4-chloromethylpyridine hydrochloride in aqueous solution with trilithium phosphorothioate gave the phosphorothioate salt **XI**.

4-Methylpyrimidine underwent chlorination of the alkyl side-group by a free-radical mechanism following treatment with *N*-chlorosuccinimide in dry carbon tetrachloride. Benzoyl peroxide was used to initiate the free-radical reaction [8]. The product, 4-chloromethylpyrimidine **XII**, was treated immediately upon isolation with trilithium phosphorothioate [9] to give dilithium *S*-(4-methylpyrimidine)phosphorothioate **XIII**. Reaction of 4-chloromethylpyrimidine **XII** with thiourea in methanol gave the corresponding isothiouronium salt **XIV** [10] which was hydrolysed by weak alkali to give 4-thiomethylpyrimidine **XV** in low yield. Because of the difficulties encountered in the vacuum distillation of the thiol **XV** the corresponding hydrochloride salt **XVI** was prepared. Bis(4-methylpyrimidine) disulphide **XVIII** was prepared by oxidation of 4-thiomethylpyrimidine **XV** with iodine in chloroform [10] and the disulphide then converted into the corresponding hydrochloride salt **XVIII**, as previously described.

Results of radioprotection studies and Discussion

The LD_{50} values of 2-(2-pyridyl)ethanethiol and 2-pyridylmethanethiol are similar (400 mg/kg and 500 mg/kg, respectively), yet 2-(2-pyridyl)ethanethiol is far less radioprotective (*cf.* 2-pyridylmethanethiol has a dose reduction factor (*DRF*) of approximately 1.45). The same observation is noted for corresponding disulphides. These results confirm the fact that the increase (from two to three) in the number of $-CH_2-$groups between the amine and the thiol function of aminothiols and derivatives lowers the radioprotective activity [11].

Of interest is the fact that the LD_{50} values of 2-(2-pyridyl)-ethanethiol (**I**) and the corresponding disulphide (**II**) are the same, and the protective effects in equal dosages are of approximately the same order. This would tend to suggest that the disulphide (**II**) is enzymatically cleaved to the thiol (**I**) *in vivo*. (It is known that cystamine is reduced in this way.)

The fact that 2-(2-pyridylethyl)disulphide dihydrochloride **III** was found to be less radioprotective than the corresponding disulphide **II** may be due to the solvent used (water and miglyol, respectively).

Introduction of a chlorine atom into the 6-position of the pyridine ring in 6-chloro-2-pyridinemethanethiol **V**, and bis(6-chloro-2-pyridylmethyl) disulphide **VI** demonstrates that the chlorine substituent markedly reduces the radioprotective action.

The properties of the two isomeric chlorine-substituted phosphorothioates dilithium *S*-(4-chloro-2-pyridylmethyl)-phosphorothioate and dilithium *S*-(6-chloro-2-pyridylmethyl)-phosphorothioate **VII** may be compared. Whilst the 4-chloro compound **IV** shows no protection whatsoever, the 6-chloro compound **VII**, which is considerably more toxic (*viz*, LD_{50} 200 mg/kg and 500 mg/kg), shows significant protection when the interval between injection and radiation exposure is 2 h. As the only structural difference between the two compounds is the position of the chlorine substituent on the pyridine ring, the effect of this is to render the nitrogen of the 6-chloro compound less basic than that of the corresponding 4-chloro derivative, and so the differences in the biological properties of the two compounds *may* be attributable to this difference in basicity. Since the 6-chloro compound shows significant protection after a 2 h interval (but not after 15 min), it may be that the phosphorothioate group is only very slowly hydrolysed to the corresponding thiol in the tissues. Moreover, *S*-(2-pyridylmethyl)phosphorothioate and even *S*-(4-pyridylmethyl)phosphorothioate showed a slight activity, compared to that of linear phosphorothioates, such as **WR 2721** ($DRF = 2.5$). So, it can be argued that the passage of these four pyridine phosphorothioates through the cell membrane and their hydrolysis to the corresponding thiol are too low to obtain an important protection.

Although, 4-pyridylmethanethiol could not be isolated for biological screening, bis(4-pyridylmethyl)disulphide dihydrochloride **X** was prepared. Moderate protective action was shown by i.p. administration when the dosage was 1/2 or 1/8 the LD_{50}. A low radioprotective action was also observed when the compound was given 90 min before irradiation.

The disulphides **II**, **III** and **X** were tested orally and showed a lack of radioprotective activity. Oral administration of radioprotective compounds, such as cysteamine or phosphorothioate **WR 2721** leads to a too low drug level in plasma to obtain the high radioprotective activity observed after intraperitoneal injection [3—15].

Bis(4-methylpyrimidine)disulphide and 4-methylpyrimidine phosphorothioate are more toxic and less active than the pyridine analogues. The corresponding thiol also shows a lack of activity.

In conclusion, the different new compounds cannot be considered as promising radioprotectors. They have a lower activity than either 2-pyridinemethanethiol or cysteamine ($DRF = 1.5$). During the pharmacokinetic stage, metabolic alteration may occur and indeed may be of paramount importance for the disulphides and phosphorothioate salts so that the intracellular thiol concentration is certainly too low to obtain an important radioprotective activity.

Experimental protocols

Chemistry

Melting points were recorded using a Gallenkamp apparatus, and are uncorrected. Infrared spectra were recorded as thin liquid films using potassium bromide plates in a Perkin—Elmer 577 Grating Infrared Spectrophotometer. Microanalytical data was supplied by Dr. F. Strauss (Oxford) and the Butterworth Microanalytical Laboratory (Teddington).

2-(2-Pyridyl)ethanethiol I
This compound was prepared from 2-vinylpyridine as described earlier [12]. bp: 58—59°C, 0.2 mm Hg (lit. [12] 57—58°C, 0.15 mm Hg). IR (liquid film): 2540 cm^{-1} (weak, broad, —SH). Found: C: 60.2; H: 6.5; N: 10.0%. Calc. for C_7H_9NS: C: 60.4; H: 6.5; N: 10.1%.

2-(2-Pyridylethyl)disulphide II
2-(2-Pyridyl)ethanethiol (18.94 g, 0.14 mol) was dissolved in $CHCl_3$ (30 ml) and the solution warmed. Iodine (20.0 g, 0.16 g atom) in chloroform (400 ml) was added dropwise to the thiol chloroform solution until oxidation was complete as indicated by a permanent brown colouration [10]. The white solid which formed during the course of the oxidation dissolved on shaking in 10% sodium hydroxide solution (50 ml). The chloroform layer was washed with water (2 × 50 ml) and dried (Na_2SO_4). The crude disulphide was vacuum distilled to give a yellow oil. bp: 177°C, 0.4 mm Hg. Found: C: 60.8; H: 6.0; N: 10.3%. Calc. for $C_{14}H_{16}N_2S_2$: C: 60.8; H: 5.8; N: 10.1%.

2-(2-Pyridylethyl)disulphide dihydrochloride III
To 2-(2-pyridyl)ethyldisulphide (10.2 g, 0.04 mol) dissolved in dry ether (25 ml), was added an excess of ethereal hydrogen chloride forming a pale yellow gelatinous precipitate. Excess ethereal hydrogen chloride was decanted off, a little dry ether was added and the gelatinous solid agitated, to give a free-flowing white solid. This was collected and dried (P_2O_5). The crude hydrochloride salt was recrystallised from absolute ethanol/ether. mp: 140°C. Found: C: 48.0; H: 5.2; N: 8.1%. Calc. for $C_{14}H_{18}N_2S_2Cl_2$: C: 48.1; H: 5.2; N: 8.0%.

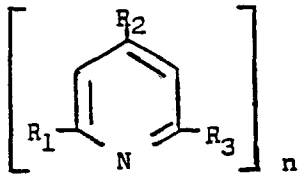
Dilithium S-(4-chloro-2-pyridylmethyl)phosphorothioate IV
4-Chloromethylpyridine was prepared as described previously [6] and converted into the corresponding hydrochloride salt with ethereal hydrogen chloride. The crude hydrochloride salt was recrystallised from absolute ethanol/diethyl ether. mp: 145°C (dec.).

4-Chloro-2-chloromethylpyridine hydrochloride (20.0 g, 0.1 mol) was added to a solution of trilithium phosphorothioate (16.8 g, 0.1 mol) in water (210 ml)/dimethylformamide (105 ml) [9]. The pH of the solution was adjusted to 9.5 by the addition of lithium hydroxide solution and stirred for 2 h by which time the silver ion test for unreacted phosphorothioate ion was negative. The solution was concentrated to a small volume and added to rapidly-stirred acetone (500 ml) to form a white solid, which was collected, and dried (silica gel). The compound was purified by reprecipitation from acetone [13]. IR (nujol mull): 1118 cm^{-1} (P=O); 768 cm^{-1} (P—S); 720 cm^{-1} (P—S); 648 cm^{-1} (C—S). Found: C: 26.7; H: 2.1; N: 5.2; S: 11.4; Cl: 12.9%. Calc. for $C_6H_7NClSPO_4Li_2$: C: 26.7; H: 2.6; N: 5.2; S: 11.9; Cl: 13.1%.

6-Chloro-2-pyridinemethanethiol V
6-Chloro-2-chloromethylpyridine (19.50 g, 0.12 mol), prepared as described previously [6] was dissolved in ethanol (65 ml). Thiourea (9.52 g, 0.12 mol) in ethanol (125 ml) was added and the solution refluxed for 2 h. On cooling, the solution was concentrated to give the isothiouronium salt as yellow platelets. mp: 186—188°C. Found: C: 35.5; H: 3.8; N: 17.4%. Calc. for $C_7H_9N_3Cl_2S$: C: 35.3; H: 3.8; N: 17.6%.

6-Chloro-2-pyridiniummethylisothiouronium chloride (23.8 g, 0.1 mol) was dissolved in water (150 ml) and a solution of sodium hydroxide (12.20 g dissolved in 90 ml water) was added. The solution was refluxed for 1 h under an N_2 atmosphere, cooled, and the pH adjusted with hydrochloric acid to 6. The solution was extracted

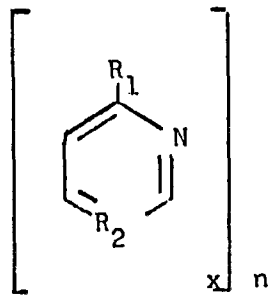
Table I. Toxicity and radioprotective activity of pyridine derivatives in mice.



Compd.	R_1	R_2	R_3	n	Solvent[a]	LD_{50} (mg/kg)	Dose (mg/kg)	Time interval (min)	Dose of γ-radiation (Gy)	Survival on day 30
I	H	H	CH_2—CH_2—SH	1	M	400	50	15	8.5	3/10
							200	15	8.5	5/15
							200	15	10.5	0/10
							200	120	8.5	1/10
V	Cl	H	CH_2—SH	1	M	400	50	15	9.5	5/20
							200	15	9.5	6/20
							200	15	11.5	0/10
*	H	H	CH_2—SH	1	M	500	250	15	8.5	15/20
							250	15	10.5	14/20
							250	15	11.5	0/10
II	H	H	CH_2—CH_2—S—	2	M	400	50	15	9.5	7/20
							200	15	9.5	10/20
							200	15	11.5	0/20
							200	120	9.5	0/20
III	H	H	CH_2—CH_2—S—, 2 HCl	2	W	500	62.5	15	9.5	1/20
							250	15	9.5	5/20
							250	15	11.5	0/20
							250	120	9.5	0/10
VI	Cl	H	CH_2—S—	2	M	1 200	150	15	8.5	0/10
							600	15	8.5	4/10
*	H	H	CH_2—S—	2	M	350	175	15	9.5	20/20
							175	15	9.5	0/20
VII	Cl	H	$CH_2SPO_3Li_2$, H_2O	1	W	200	25	15	9.5	0/10
							100	15	9.5	0/10
							100	15	11.5	0/10
							100	120	9.5	7/10
IV	H	Cl	$CH_2SPO_3Li_2$, H_2O	1	W	500	62.5	15	9.5	0/15
							250	15	9.5	0/20
							250	15	11.5	0/10
							250	120	9.5	0/10
*	H	H	$CH_2SPO_3Li_2$, H_2O	1	W	700	350	15	9.5	6/20
X	H	CH_2—S—	H	2	W	200	25	15	9.5	12/20
							100	15	9.5	8/20
							100	15	11.5	0/10
							100	120	9.5	6/10
XI	H	CH_2—S—PO_3Li_2, 3 H_2O	H	1	W	800	100	15	9.5	0/20
							400	15	9.5	17/20
							400	15	11.5	0/10
							400	120	9.5	0/10

[a]M: miglyol 812; W: water.
*Results given in [1] and reported here to facilitate comparison.

Table II. Toxicity and radioprotective activity of pyrimidine derivatives in mice.



Compd.	R_1	R_2	x	*n*	Solvent	LD_{50} (mg/kg)	Dose (mg/kg)	Time internal (min)	Dose of γ-radiation (Gy)	Survival on day 30
V	CH_2SH	N^+ H	Cl^-	1	W	150	18.7	15	8.5	0/10
							75	15	8.5	1/15
							75	15	10.5	0/10
							75	120	8.5	4/10
VII	CH_2—S—	N^+ H	Cl^-	2	W	100	12.5	15	9.0	0/10
							50	15	9.0	0/15
							50	15	11.0	0/10
							50	120	9.0	2/10
II	CH_2—S—PO_3Li_2, H_2O	N	—	1	W	350	43.7	15	9.0	0/10
							175	15	9.0	2/15
							175	15	11.0	0/10
							175	120	9.0	0/10

with chloroform (8×50 ml) and the extracts were dried (Na_2SO_4). After removal of solvent, the crude thiol was purified by vacuum distillation. bp: 90—91°C, 1.2 mm Hg. Found: C: 45.0; H: 3.8; N: 9.0; Cl: 22.6; S: 20.1%. Calc. for C_6H_6NClS: C: 45.1; H: 3.8; N: 8.8; Cl: 22.2; S: 20.1%.

Bis(6-chloro-2-pyridylmethyl)disulphide VI
6-Chloro-2-pyridinemethanethiol (2.5 g, 0.008 mol) dissolved in chloroform (25 ml), was oxidised by the addition of iodine (5.0 g, 0.04 g atom) in the same solvent (80 ml) as described previously [10] to give a pale yellow solid. Found: C: 45.4; H: 3.5; N: 8.9; S: 19.9; Cl: 22.3%. Calc. for $C_{12}H_{10}N_2S_2Cl_2$: C: 45.4; H: 3.2; N: 8.8; S: 20.2; Cl: 22.3%.

Dilithium S-(6-chloro-2-pyridylmethyl)phosphorothioate VII
6-Chloro-2-chloromethylpyridine (4.93 g, 0.03 mol) dissolved in dimethylformamide was added dropwise to a rapidly-stirred solution of trilithium phosphorothioate (6.75 g, 0.03 mol) dissolved in a mixture of water (100 ml) and dimethylformamide (50 ml) [9]. The pH of the solution was adjusted to 10 with a lithium hydroxide solution and the course of the reaction was monitored by the silver ion test for free phosphorothioate. On completion of reaction, the solution was concentrated and added to rapidly-stirred acetone (500 ml) to give a white solid. This was collected and dried (silica gel). The phosphorothioate salt was purified by reprecipitation from acetone [18]. IR (nujol mull): 1125 cm^{-1} (P=O); 748 cm^{-1} (P—S); 720 cm^{-1} (P—S); 635 cm^{-1} (C—S). Found: C: 26.7; H: 2.1; N: 5.2; S: 11.7; P: 11.5; Cl: 12.9%. Calc. for $C_6H_7NClSPO_4Li_2$: C: 26.7; H: 2.6; N: 5.2; S: 11.9; P: 11.5; Cl: 13.1%.

4-Pyridiniummethylisothiouronium chloride hydrochloride VIII
4-Chloro-methylpyridine hydrochloride (32.8 g, 0.2 mol) dissolved in methanol (250 ml) was added to thiourea (16.0 g, 0.21 mol) dissolved in 150 ml of the same solvent, and the resulting solution was refluxed for 1 h. Cooling to 4°C gave the isothiouronium salt as a yellow crystalline solid, which was collected and dried (P_2O_5). The filtrate was concentrated to yield a second crop of crystals. Recrystallisation was from methanol. mp: 214°C. Found: C: 35.1; H: 4.6; N: 17.7; S: 13.2; Cl: 29.3%. Calc. for $C_7H_{11}N_3SCl_2$: C: 35.0; H: 4.6; N: 17.5; S: 13.3; Cl: 29.5%.

Alkaline hydrolysis of 4-pyridiniummethylisothiouronium chloride hydrochloride, in an identical manner to that described for the corresponding 2-isomer, gave the thiol (identified by IR and ^1H NMR) in very low yield (5.4%), together with a solid (identified by ^1H NMR and mp as the disulphide), suggesting that the conditions of hydrolysis are far too aggressive, the thiol being very easily oxidised to the corresponding disulphide.

Bis(4-pyridylmethyl)disulphide IX
A solution of ammonium hydroxide (about 35% NH_3, 295 ml) and 4-pyridiniummethylisothiouronium chloride hydrochloride (70.6 g, 0.3 mol) dissolved in water (88 ml) was heated on a steam bath for 0.5 h. On cooling, the pH was adjusted to 6.5 with hydrochloric acid with deposition of a brown oil. The latter was extracted with chloroform (5×150 ml), the extracts were dried (Na_2SO_4) and most of the solvent was removed to give a solution of the thiol in chloroform (approx. 50 ml). Iodine (36.0 g, 0.29 atom) in chloroform (700 ml) was added to the thiol oxidising it to the corresponding disulphide [10]. The crude disulphide was twice recrystallised from absolute ethanol prior to drying (P_2O_5). mp: 154°C. Found: C: 58.1; H: 4.9; N: 11.2; S: 25.8%. Calc. for $C_{12}H_{12}N_2S_2$: C: 58.0; H: 4.9; N: 11.3; S: 25.8%.

Bis(4-pyridylmethyl)disulphide dihydrochloride X
To bis(4-pyridylmethyl) disulphide (6.0 g, 0.02 mol) dissolved in butan-1-ol (75 ml) was added an excess of ethereal hydrogen chloride. A white solid formed immediately, which was collected, and dried (P_2O_5). Recrystallisation was from methanol. mp: 206°C. Found: C: 44.6; H: 4.3; N: 8.6; S: 19.5; Cl: 21.9%. Calc. for $C_{12}H_{14}N_2S_2Cl_2$: C: 44.9; H: 4.4; N: 8.7; S: 20.0; Cl: 22.1%.

Dilithium S-*(4-pyridylmethyl)phosphorothioate* ***XI***
To trilithium phosphorothioate (16.8 g, 0.1 mol) dissolved in water (150 ml) was added 4-chloromethylpyridine hydrochloride (20.0 g, 0.12 mol) with constant stirring. The pH of the solution was adjusted to 11 with lithium hydroxide solution, with the course of the reaction being monitored by the silver ion test for unreacted phosphorothioate [9]. After 2 h, on completion of reaction, the solution was filtered to remove slight turbidity and the filtrate was added to rapidly-stirred *n*-propanol (500 ml) to give a pink solid. The latter was collected and dried (silica gel). Purification was by reprecipitation from water/acetone (1:5) [13]. IR (nujol mull): 1123 cm^{-1} (P=O); 748 cm^{-1} (P—S); 722 cm^{-1} (P—S); 639 cm^{-1} (C—S). Found: C: 26.6; H: 2.8; N: 5.0; S: 11.8; P: 11.3%. Calc. for $C_6H_{12}NSPO_6Li_2$: C: 26.6; H: 4.5; N: 5.2; S: 11.8; P: 11.4%.

Dilithium S-*(4-methylpyrimidine)phosphorothioate* ***XIII***
4-Methylpyrimidine (37.0 g, 0.39 mol), *N*-chlorosuccinimide (67.0 g, 0.5 mol) and benzoyl peroxide (0.6 g, 0.0025 mol) were dissolved in carbon tetrachloride (1110 ml) and the solution refluxed at 78°C for 20 h [8]. On cooling, the succinimide was filtered off and the filtrate dried (Na_2SO_4). Removal of carbon tetrachloride gave a yellow liquid which was shown to contain three compounds: unreacted 4-methylpyrimidine, the required 4-chloromethylpyrimidine **XII** and 4-dichloromethylpyrimidine. Because of the instability of 4-chloromethylpyrimidine [14], it was treated immediately upon isolation with trilithium phosphorothioate [9], which was precipitated from aqueous solution by pouring into rapidly-stirred acetone. Previously, ethanol had been used; however, this solvent led to gel formation making the isolation of trilithium phosphorothioate extremely difficult. The phosphorothioate was reprecipitated from water:acetone (1:5) [13] and dried (silica gel). Found: S: 13.8; P: 13.4%. Li_3SPO_3, 5.5 H_2O requires: S: 13.9; P: 13.4%.

Trilithium phosphorothioate (5.0 g, 0.02 ml) dissolved in water (25 ml) was added to rapidly-stirred 4-chloromethylpyrimidine **XII**. The course of the reaction was followed by the silver ion test for unreacted phosphorothioate [9], the pH of the solution being maintained at approximately 10 by the addition of lithium hydroxide solution. The slightly turbid solution was filtered and poured into rapidly-stirred acetone (500 ml) to give a fine precipitate, which was collected and dried (silica gel). Purification of the phosphorothioate salt **XIII** was by reprecipitation from water:acetone (1:5) [13]. mp: >270°C (dec.). IR (nujol mull): 1122 cm^{-1} (P=O); 720 cm^{-1} (P—S); 643 cm^{-1} (C—S). Found: C: 25.4; H: 3.0%. $C_5H_5N_2SPO_3Li_2 \cdot H_2O$ requires: C: 25.2; H: 2.8%.

4-Thiomethylpyrimidine hydrochloride ***XVI***
4-Chloromethylpyrimidine **XII** was prepared as described [8] and dissolved in methanol (50 ml). Thiourea (20.0 g, 0.26 mol) in methanol (200 ml) was added dropwise to the gently refluxing solution. After complete addition, the solution was refluxed for 1.5 h before cooling. The volume of solution was reduced to approximately 75 ml and refrigerated. The resultant brown solid was collected and dried (P_2O_5). The hygroscopic 4-pyrimidineisothiouronium salt **XIV** was purified by recrystallisation from absolute ethanol, and dried (P_2O_5).

To 4-methylpyrimidineisothiouronium chloride **XIV** (22 g, 0.1 mol) in water (100 ml), was added an aqueous solution of sodium hydroxide (8.60 g, 0.22 mol) and the solution was refluxed under an N_2 atmosphere for 1.5 h. On cooling, the pH of the solution was adjusted to approximately 6 by the addition of hydrochloric acid prior to extraction with chloroform (5×80 ml). The chloroform extracts were combined and dried (Na_2SO_4). Removal of chloroform gave an oil, in low yield (15%). IR (liquid film): 2535 cm^{-1} (S—H).

To 4-thiomethylpyrimidine **XV** (1.80 g, 0.015 mol) in methanol (20 ml) was added methanolic hydrogen chloride in excess. 4-Thiomethylpyrimidine hydrochloride **XVI** precipitated from solution on the addition of dry diethyl ether. The hygroscopic solid was collected and dried (P_2O_5); purification was by dissolving in absolute ethanol and reprecipitating by the careful addition of dry diethyl ether. Found: C: 37.1; H: 4.2; N: 17.9%. $C_5H_7N_2SCl$ requires: C: 36.9; H: 4.3; N: 17.2%.

Bis(4-methylpyrimidine)disulphide dihydrochloride ***XVIII***
4-Thiomethylpyrimidine **XV** (4.0 g, 0.03 mol) in chloroform (25 ml) was oxidised to the corresponding disulphide by the dropwise addition of iodine (5.0 g, 0.04 g atom) in chloroform (100 ml) as described previously [10] to give bis(4-methylpyrimidine)disulphide **XVII** as a yellow viscous liquid. To bis(4-methylpyrimidine)disulphide **XVII** (4.52 g, 0.018 mol) in absolute ethanol (50 ml) was added ethanolic hydrogen chloride in excess with the immediate formation of a precipitate. This hygroscopic solid was collected and dried (silica gel). The crude hydrochloride salt **XVIII** was purified by recrystallisation from absolute ethanol prior to drying (silica gel). mp: 130°C (dec.). Found: C: 37.3; H: 4.1; N: 17.1; S: 19.0; Cl: 22.3%. $C_{10}H_{12}N_4S_2Cl_2$ requires: C: 37.2; H: 3.7; N: 17.3; S: 19.8; Cl: 21.9%.

Radiobiological protocol

This has been reported in detail in [1]. The radioprotective activity was evaluated by the determination of the survival rate 30 days after whole-body irradiation with a dose equal to the LD_{100} (30 days) of unprotected mice (3 months old, of pure XVII strain). The compounds were injected i.p. at doses equal to 1/2 or 1/8 of their LD_{50} toxicity, 15 min or 2 h before irradiation delivered with a ^{60}Co source with a dose—rate equal to 0.30—0.35 $Gy \cdot min^{-1}$ according to the date of experiment.

At each irradiation session, a group of 10 mice was irradiated at the LD_{100} (30 days) without injection of a compound. A 100% 30-day lethality was always observed, with a mean survival time of 12 ± 1 days. Furthermore, for each compound, a group of 8 unirradiated mice received an i.p. dose of ½ of the LD_{50}; all these animals were alive 30 days after injection. The mean survival time of unprotected mice irradiated at the LD_{100} (30 days) + 2 Gy was equal to 9 ± 1 days. This dose, which was used in the tests, still gives a haemopoietic death, and permits the rapid assessment of compounds of low *DRF*.

Acknowledgments

We thank Professor F. R. Hartley for valuable discussions during the course of this research, and the Ministry of Defence (P.E.) for financial support.

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