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New *N*-ethanethiol pyrroles as radioprotectors

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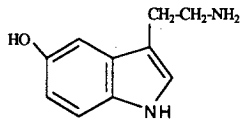
Summary — Seventeen *N*-ethanethiol pyrroles have been prepared and tested as radioprotective agents. This is the first time that pyrrole compounds with radioprotective activity have been reported. The dimethyl pyrrole derivative **3a** and its corresponding disulfur **4a** exhibited strong long-lasting effects.

***N*-ethanethiol / pyrrole / radioprotectors**

Introduction

The development of nuclear techniques for civilian or military purposes renders the research into efficient radioprotective agents highly necessary. The radiation accidents which occurred at Tchernobyl or Goïana (Brazil) have focussed attention on this problem. These compounds could also improve cancer treatment by radiotherapy. Most radioprotective drugs reported in the literature derive from sulfur structures [1]. So far, the most active compounds contain either the S-C-C-S moiety [2] or the S-C-C-N moiety, *eg* cysteamine hydrochloride [3], cysteamine derivatives [4, 5] and phosphorothioates, such as S-2-(3-aminopropyl)aminoethyl phosphorothioate (**WR 2721**) or H_2N-$(CH_2)_3$-NH-CH_2-CH_2-SPO_3H_2, HCl [6,7]. Among the scarce non-sulfurated radioprotective agents, one of the most efficient is serotonin or 5-hydroxytryptamine:

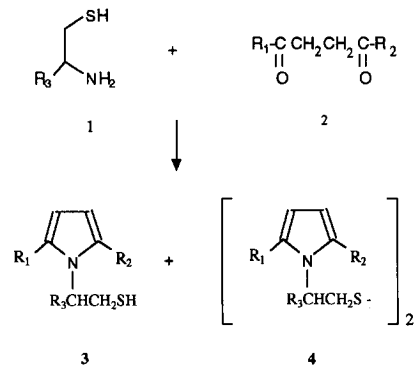


which contains a substituted pyrrole ring [8]. We therefore undertook the preparation of *N*-ethanethiol pyrroles which contain the N-CH_2-CH_2-SH moiety and incorporate a pyrrole ring, the radioprotective potential of which has not yet been reported.

We thus prepared 17 new compounds including 14 *N*-ethanethiol pyrroles and 3 disulfur derivatives and tested them as radioprotectors.

Chemistry

Pyrrole derivatives were obtained following the Paal–Knorr synthesis (scheme 1). One equivalent of a γ-diketone reacted with one equivalent of cysteamine, or cysteine ethylester (base or hydrochloride) in pyridine. The reactions were conducted under N_2 for 10 h



Scheme 1.

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Table I. Structure and radioprotective activity of *N*-ethanethiol pyrroles.

No	R_1	R_2	R_3	*Bp* ($°C_{mmHg}$)	*Mp* (°C)	*Yield* (%)	LD_{50} (mg/kg)	*Irrad. dose (cGy)*	*Delay (min)*	*Admin. dose (mg/kg)*	*Survival (%)*	*MST (30 d)*
3a	CH_3	CH_3	H	$76_{0.2}$		75	1200	900	15	600	90	28.4
								900	15	150	80	26.4
								900	120	600	60	24.5
								1100	15	600	0	10.1
4a	CH_3	CH_3	H		96	63	1500	900	15	1000	70	25.6
								900	15	250	90	28.8
								900	120	1000	90	28.2
								1100	15	1000	10	13
3b	CH_3	C_2H_5	H	$81_{0.2}$		60	1200	950	15	600	0	9.3
								950	90	600	0	9.6
3c	CH_3	C_3H_7	H	$90_{0.2}$		68	400	950	15	200	0	9
								950	90	200	0	8.4
3d	CH_3	*i*–C_3H_7	H	$96_{0.2}$		73	600	900	15	300	30	18
								900	90	300	0	11.7
4d*	CH_3	*i*–C_3H_7	H		171	15						
3e	CH_3	C_4H_9	H	$95_{0.2}$		67	1600	850	15	800	0	13.1
								850	15	200	20	18.9
								850	90	800	20	15.3
3f	CH_3	C_5H_{11}	H	$100_{0.4}$		71	1500	950	15	1000	0	11.3
								950	90	1000	0	9.2
3g	CH_3	$(C_2H_5)_2CH$	H	$79_{0.2}$		71	1000	900	15	500	0	9.9
								900	90	500	10	14
3h	CH_3	*i*–C_4H_9	H	$84_{0.2}$		65	700	900	15	350	0	10.7
								900	90	350	0	11.8
3i	CH_3	$C_4H_9(CH)C_2H_5$	H	$97_{0.2}$		6	800	900	15	400	0	11.6
								900	90	400	0	8.3
3j	CH_3	C_6H_5	H	$119_{0.2}$		78	1000	900	15	500	10	12.6
								900	90	500	0	9.1
3k	CH_3	2'-Cl-C_6H_4	H	$122_{0.2}$		76	1500	900	15	750	0	8.7
								900	90	750	0	9.1
31*	CH_3	4'-OCH_3-C_6H_4	H	$144_{0.3}$		67						
3m	C_6H_5	C_6H_5	H		71	56	1200	950	15	600	0	10.6
								950	15	150	20	14.4
								950	90	600	10	12.9
4m**	C_6H_5	C_6H_5	H		156	40						
3n	CH_3	CH_3	COOEt	$98_{1.5}$		65	1500	900	15	1000	0	12.2

*Not tested. **No solubility in miglyol.

at 60°C, followed by 2 h under reflux. *N*-Ethanethiol pyrroles **3** were obtained with their corresponding disulfurs **4**.

Compounds **3** and **4** were isolated by fractional distillation followed by alumina column chromatography and elution with cyclohexane–ethylacetate (9:1), except for **4a** which was crystallized in CH_3OH, **4d** in cyclohexane, **3m** and **4m** in CH_3OH / cyclohexane.

For compounds **3**, yields attained 70% except for **3i** which contained a bulky substituent.

Pharmacological results and discussion

The aim of this study was to prepare compounds possessing good radioprotective activity and a long duration of action. The toxicity and radioprotective results obtained with the different compounds have been summarized in table I. The radiobiological protocol has been detailed in the *Experimental protocols*.

The LD_{50} values of each compound indicated that they were weakly toxic (400–1600 mg/kg). Under the same conditions, they were less toxic than serotonin (LD_{50} 225 ± 25 mg/kg), and generally less toxic than cysteamine (LD_{50} 450 ± 40 mg/kg) or cystamine (LD_{50} 600 ± 50 mg/kg).

Two molecules appeared worthy of interest: **3a** and the corresponding disulfur **4a**. At low doses **3a** and **4a** exhibited a rapid and long-lasting effect. The latter could be accounted for by the cleavage of the disulfur bond giving rise to thiol derivatives. It should be noted that **4a** still protected mice irradiated at 1100 cGy, corresponding to a dose reduction factor (DRF) of 1.3.

Several compounds in the series exhibited weak radioprotective effects: quick-acting and of short duration with **3d**; or sustained with **3e**, although this effect could only be observed at low doses (**3m**).

The presence of one ester group in R_3 on **3a** (molecule **3n**), resulted in loss of activity. In conclusion, only **3a** and **4a** are worthy of interest as radioprotective agents. When the size of R_1 and R_2 was increased, activity decreased markedly or was abolished.

Thus the pyrrole itself may be a good candidate for radioprotective activity with DRF values, under the same conditions, comparable to those of known references. This was the case of compounds **4a** and cystamine. However, when comparing compound **3a** to cysteamine, which is rapidly degraded in the organism, or compound **3a** to serotonin, the activity of pyrrole does not seem so satisfactory.

	3a	**4a**	Cysteamine	Cystamine	Serotonin
DRF ($p < 0.05$)	1.25 ± 0.1	1.3 ± 0.1	1.6 ± 0.05	1.2 ± 0.05	1.5 ± 0.1

Experimental protocols

Chemistry

Material

Melting points were determined on a Köfler bench and are uncorrected. Analyses (C, H, N) were within ±0.4% of the theoretical values for all compounds. Thin-layer chromatography was performed with silica gel 60f 254 Merck plates and neutral aluminium oxide.The compounds may be detected under UV light or by exposure to iodine vapour. Aluminium oxide 90 (Merck) was used for column chromatography. IR spectra were recorded from KBr discs or film on a Philips SP3-100 spectrometer. NMR spectra were obtained on a Bruker AC200. The chemical shifts have been reported relative to tetramethylsilane. Splitting patterns have been designated as follows: s = singlet; d = doublet; dd = doublet of doublet; t = triplet; q = quartet; m = multiplet.

Synthesis of compounds

The γ-diketones were prepared according to procedures reported in the literature [9–17].

N-ethanethiol pyrrole. For example, the synthesis of 2,5-dimethyl *N*-ethanethiol pyrrole **3a** and that of its disulfur **4a** is illustrative.

To a pyridine solution (90 ml), 0.1 mol 2,5-hexanedione was added to 0.1 mol (11.3 g) cysteamine hydrochloride.The reaction mixture was stirred at rt under an N_2 atmosphere overnight. It was then warmed to 90–100°C for 2 h. The solvent was evaporated *in vacuo*, and the reaction mixture treated with 10% sodium bicarbonate, then extracted with ether. The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was distilled *in vacuo*. Pyrrole **3a** was purified from the disulfur **4a** on an alumina column using cyclohexane/ethylacetate (9:1) as eluent.

*2,5-Dimethyl N-ethanethiol pyrrole **3a***

IR: (ν cm^{-1}, film): HC 3100; SH 2550; C-N 1400; ^{1}H-NMR: δ (ppm), *J* (Hz), $CDCl_3$, 200 MHz: 1.4 (1H, t, SH, *J* = 8.5); 2.24 (6H, s, $2CH_3$); 2.84 (2H, m, CH_2-S, *J* = 6.2 and 8.5); 4.01 (2H, m, CH_2-N, *J* = 6.2); 5.88 (2H, s, H_3 H_4). ^{13}C-NMR: (δ ppm, $CDCl_3$, 200 MHz): 12.40; 24.60; 46.40; 105.5; 127.

*Di-(2,5–dimethyl N-ethanethiol pyrrole) **4a***

IR: (ν cm^{-1}; KBr 2%): HC 3100; no SH free 2250; C-N 1400; ^{1}H-NMR: δ (ppm), *J* (Hz), $CDCl_3$, 200 MHz: 2.28 2(6H, s, $2CH_3$); 2.88 2(2H, m, CH_2-S, *J* = 8); 4.12 2(2H, m, CH_2-N, *J* = 8); 5.82 2(2H, s, H_3H_4).^{13}C-NMR: (δ ppm, $CDCl_3$, 200 MHz): 12.50; 24.55; 42.95; 105.65; 127.20.

*2,5–Ethyl methyl N-ethanethiol pyrrole **3b***

IR: (ν cm^{-1}, film): HC 3100; SH 2550; C-N 1400; ^{1}H-NMR: δ (ppm), *J* (Hz), $CDCl_3$, 200 MHz: 1.28 (3H, t, CH_2-CH_3); 1.38 (1H, t, SH, *J* = 8.5); 2.26 (3H, s, CH_3); 2.58 (2H,q, CH_2-CH_3); 2.72 (2H, m, CH_2-SH, *J* = 7 and 8.5); 3.94 (2H, m, CH_2-N, *J* = 7.2); 5.63 (2H, s, H_3H_4). ^{13}C-NMR: (δ ppm, $CDCl_3$, 200 MHz): 12.26; 12.69; 19.65; 24.58; 46.27; 103.43; 105.54; 127.05; 133.55.

*2-Methyl 5-propyl N-ethanethiol pyrrole **3c***

IR: (ν cm^{-1}, film):HC 3100; SH 2550; C-N 1400; ^{1}H-NMR: δ (ppm), *J* (Hz), $CDCl_3$, 200 MHz: 1.02 (3H, t, CH_3-CH_2); 1.37 (1H, t, SH, *J* = 8.5);1.65 (2H, m, CH_2-CH_2-CH_3); 2.24 (3H, s, CH_3); 2.51 (2H, t, CH_2-CH_2-CH_3); 2.74 (2H, m, CH_2 SH, *J* =

7.2 and 8.5); 3.93 (2H, m, CH_2-N, J = 7.2); 5.81 (2H, s, H_3H_4). ^{13}C-NMR: (δ ppm, $CDCl_3$, 200 MHz): 12.23; 13.90; 21.87; 24.56; 28.62; 46.21; 104.21;105.52;126.77;131.85.

2-Isopropyl 5-methyl N-ethanethiol pyrrole ***3d***
IR: (ν cm^{-1}, film): HC 3100; SH 2550; C-N 1400; ^{1}H-NMR: δ (ppm), J (Hz), $CDCl_3$, 200 MHz: 1.25 (6H, d, $CH(CH_3)_2$, J = 7); 1.37 (1H, t, SH, J = 8.5); 2.25 (3H, s, CH_3); 2.70 (2H, m, CH_2-SH, J = 7.2 and 8.5); 2.88 (1H, q, $CH(CH_3)_2$, J = 7.0); 3.96 (2H, m, CH_2N, J = 7.2); 5.83 (2H, s, H_3H_4). ^{13}C-NMR: (δ ppm, $CDCl_3$, 200 MHz): 12.43; 23.66; 25.03; 25.82; 46.39; 102.04; 105.89; 126.88; 138.95.

Di-(2-isopropyl 5-methyl N-ethanethiol pyrrole) ***4d***
IR: (ν cm^{-1}, KBr 2%): HC 3050; no SH free 2550; C-N 1360; ^{1}H-NMR: δ (ppm), J (Hz), $CDCl_3$, 200 MHz: 1.25 2[6H,d,$CH(CH_3)_2$]; 2.25 2(3H,s, CH_3); 2.82 to 2.98 2[2H and 1H, m, CH_2S(J = 8.0), and $CH(CH_3)_2$]; 4.11 2[2H, m, CH_2-N, J = 8.0]; 5.83 2(2H, s, H_3H_4). ^{13}C-NMR: (δ ppm, $CDCl_3$, 200 MHz): 12.26; 23.57; 25.70; 37.78; 42.72; 102.08; 105.90; 126.69; 138.67.

2-Butyl 5-methyl N-ethanethiol pyrrole ***3e***
IR: (ν cm^{-1}, film): HC 3100; SH 2550; C-N 1400; ^{1}H-NMR: δ (ppm), J (Hz), $CDCl_3$, 200 MHz: 0.95 (3H, t, CH_2-CH_3); 1.37 (1H, t, SH, J = 8.5); 1.44 (2H, m, -CH_2-CH_2-CH_2-CH_3); 1.56 (2H, m, CH_2-CH_2-CH_2-CH_3) 1.63 (2H, m, CH_2-CH_2-CH_2-CH_3); 2.24 (3H, s, CH_3); 2.53 (2H, q, CH_2-CH_3); 2.70 (2H, m, CH_2-SH, J = 7.8 and 8.5); 3.93 (2H, m, CH_2N, J = 7.8); 5.80 (2H, s, H_3H_4). ^{13}C-NMR: (δ ppm, $CDCl_3$, 200 MHz): 12.39; 13.87; 22.53; 24.72; 26.33; 30.93; 46.40; 104.24; 105.63; 127.00; 132.25.

2-Methyl 5-pentyl N-ethanethiol pyrrole ***3f***
IR: (ν cm^{-1}, film): HC 3100; SH 2550; C-N 1400; ^{1}H-NMR: δ (ppm), J (Hz), $CDCl_3$, 200 MHz: 0.96 (3H, t, CH_2-CH_3); 1.37 (1H, t, SH, J = 8.5); 1.43 (4H, m, CH_2-CH_2-CH_2-CH_2-CH_3); 1.6 (2H, m, CH_2-CH_2-CH_2-CH_2-CH_3); 2.28 (3H, s, CH_3); 2.56 (2H, t,CH_2-CH_2-CH_2-CH_2-CH_3); 2.74 (2H, m, CH_2SH, J = 7.2 and 8.5); 3.96 (2H, m, CH_2N, J = 7.2); 5.84 (2H, s, H_3H_4). ^{13}C-NMR: (δ ppm, $CDCl_3$, 200 MHz): 12.40; 13.93; 22.47; 24.75; 26.63; 28.47; 31.68; 46.43; 104.25; 105.66; 127.03; 132.34.

2-(1-Ethylpropyl) 5-methyl N-ethanethiol pyrrole ***3g***
IR: (ν cm^{-1}, film): HC 3100; SH 2550; C-N 1400; ^{1}H-NMR: δ (ppm), J (Hz), $CDCl_3$, 200 MHz: 0.85 (6H, t, CH (CH_2-CH_3)$_2$); 1.39 (1H, t, SH, J = 8.5); 1.59 (4H, m, $CH(CH_2$-$CH_3)_2$); 2.24 (3H, t, CH_3); 2.43 (1H, q, $CH(CH_2$-$CH_3)_2$); 2.68 (2H, m, CH_2-SH, J = 8 and 8.5); 3.94 (2H, m, CH_2N, J = 8.0); 5.81 (2H, dd, H_3H_4, J = 3.1). ^{13}C-NMR: (δ ppm, $CDCl_3$, 200 MHz): 11.63; 12.40; 24.90; 28.27; 39.54; 46.04; 103.08; 105.81; 126.12; 135.62.

2-Isobutyl 5-methyl N-ethanethiol pyrrole ***3h***
IR: (ν cm^{-1}, film): HC 3100; SH 2550; C-N 1400; ^{1}H-NMR: δ (ppm), J (Hz), $CDCl_3$, 200 MHz: 0.96 (6H, d, CH_2-$CH(CH_3)_2$); 1.36 (1H, t, SH, J = 8.5) ; 1.86 (1H, q, CH_2-$CH(CH_3)_2$; 2.24 (3H, s, CH_3) ; 2.40 (2H, d, CH_2-$CH(CH_3)_2$); 2.69 (2H, m, CH_2-SH, J = 7.2 and 8.5); 3.92 (2H, m, CH_2-N, J = 7.2); 5.80 (2H, m, H_3H_4, J = 3.1). ^{13}C-NMR: (δ ppm, $CDCl_3$, 200 MHz): 12.30; 22.48; 24.58; 28.13; 35.84; 46.26; 105.34; 105.55; 126.64; 130.87.

2-(1-Ethylpentyl) 5-methyl N-ethanethiol pyrrole ***3i***
IR: (ν cm^{-1}, film): HC 3100; SH 2550; C-N 1400; ^{1}H-NMR: δ (ppm), J (Hz), $CDCl_3$, 200 MHz: 0.83 (3H, d, CH_2-CH_3); 0.98 (3H, t, CH_2-CH_3) ; 1.25 (2H, m, CH_2-CH_2-CH_2-CH_3) ; 1.36 (1H, t, SH, J = 8.5); 1.58 (4H, m, CH_2-CH_2-CH_2-CH_3); 2.24 (3H, s, CH_3); 2.47 (1H, q, CH-); 2.66 (2H, m, CH_2-SH, J = 8 and 8.5); 3.92 (2H, m, CH_2N, J = 8.0); 5.79 (2H, dd, H_3H_4, J = 3.5). ^{13}C-NMR: (δ ppm, $CDCl_3$, 200 MHz): 11.67; 12.48; 13.88; 22.85; 24.95; 28.66; 29.60; 35.46; 38.15; 46.18; 103.03; 105.83; 126.16; 136.16.

2-Methyl 5-phenyl N-ethanethiol pyrrole ***3j***
IR: (ν cm^{-1}, film): HC 3100; SH 2550, C-N 1400; ^{1}H-NMR: δ (ppm), J (Hz), $CDCl_3$, 200 MHz: 1.23 (lH, t, SH, J = 8.5); 2.42 (3H, s, CH_3); 2.66 (2H, m, CH_2-SH, J = 7.2) and 8.5); 4.15 (2H, m,CH_2N, J = 7.2); 6.07 (1H, d, H_3, J = 3.1); 6.21 (1H, d, H_4, J = 3.1); 7.47 (5H, m, 5 Ar-H). ^{13}C-NMR: (δ ppm, $CDCl_3$, 200 MHz): 12.66; 24.69; 46.85; 107.02; 108.36; 126.72; 128.33; 128.61; 129.69; 133.41; 133.81.

2-(2-Chlorophenyl) 5-methyl N-ethanethiol pyrrole ***3k***
IR: (ν cm^{-1}, film): HC 3100; SH 2550; C-N 1400; ^{1}H-NMR: δ (ppm), J (Hz), $CDCl_3$, 200 MHz: 1.15 (1H, t, SH, J = 8.5); 2.37 (3H, s, CH_3); 2.52 (2H, m, CH_2SH, J = 7.2 and 8.5); 3.93 (2H, m, CH_2N, J = 7.2); 6.03 (1H, d, H_3, J = 3.0); 6.10 (1H, d, H_4, J = 3.0); 7.36 (3H, m, 3 Ar-H); 7.50 (1H, m, 1 Ar-H). ^{13}C-NMR: (δ ppm, $CDCl_3$, 200 MHz): 12.69; 24.87; 47.10; 106.62; 109.05; 110; 126.54; 129.11; 129.17; 129.58; 132.90; 132.97; 134.91,

2-(4 Methoxyphenyl) 5-methyl N-ethanethiol pyrrole ***3l***
IR: (ν cm^{-1}, film): HC 3100 ; SH 2550 ; C-N 1400; ^{1}H-NMR: δ (ppm), J (Hz), $CDCl_3$, 200 MHz: 1.21 (1H, t, SH, J = 8.5); 2.37 (3H, s, CH_3);2.58 (2H, m, CH_2-SH, J = 7.2 and 8.5); 3.88 (3H, s, CH_3O); 4.06 (2H, m, CH_2N, J = 7.2); 5.99 (1H, d, H_3, J = 3.1); 6.10 (1H, d, H_4, J = 3.1); 6.99 (2H, m, 2 Ar-*H*); 7.33 (2H, m, 2 Ar-*H*). ^{13}C-NMR (δ ppm, $CDCl_3$, 200 MHz): 12.69; 24.72; 46.86; 55.13; 106.76; 107.78; 113.82; 126.38; 129.06; 130.12; 133.17; 158.64.

2,5-Diphenyl N-ethanethiol pyrrole ***3m***
IR: (ν cm^{-1}; KBr 1%): HC 3100; SH 2550; C-N 1400; ^{1}H-NMR: δ (ppm), J (Hz), $CDCl_3$, 200 MHz: 0.86 (1H, t, SH, J = 8.5); 2.27 (2H, m, CH_2-SH, J = 7.2 and 8.5); 4.26 (2H, m, CH_2N, J = 7.2); 6.29 (2H, s, H_3H_4); 7.43 (10H, m, 10 Ar-H). ^{13}C-NMR: (δ ppm, $CDCl_3$, 200 MHz): 36.01; 44.27; 109.81; 127.08; 128.47; 128.93; 133.44; 136.28.

Di-(2,5-diphenyl N-ethanethiol pyrrole) ***4m***
IR: (ν cm^{-1}; KBr 2%): HC 3050, no SH free 2550; C-N 1360; ^{1}H-NMR: δ (ppm), J (Hz), $CDCl_3$, 200 MHz: 1.85 2(2H, m, CH_2-S, J = 7.8); 4.09 2(2H, m, CH_2N, J = 7.8); 6.26 2(2H, s, H_3H_4); 7.32 2(10H, m, 10 ArH). ^{13}C-NMR: (δ ppm, $CDCl_3$, 200 MHz) 36.01; 44.27; 109.81; 127.08; 128.47; 128.93; 133.44; 136.28.

2,5-Dimethyl-(3' ethylcarboxylate N-ethanethiol) pyrrole ***3n***
IR: (ν cm^{-1}, film): HC 3100; SH 2550; CN 1400; CO 1740; ^{1}H-NMR: δ (ppm), J (Hz), $CDCl_3$, 200 MHz: 1.22 (3H, t, CH_3CH_2, J = 7.0); 1.26 (lH, t, SH, J = 5.0); 2.21 (6H, s, $2CH_3$); 3.12 (2H, m, CH_2S, J = 5.0 and J = 6.2); 4.24 (2H, q, CH_2CH_3, J = 7.0); 4.81 (1H, m, CH_2N, J = 6.2); 5.81 (2H, s, H_3H_4). ^{13}C-NMR: (δ ppm, $CDCl_3$, 200 MHz): 13.24; 14.05; 26.06; 60.07; 61.83; 106.93; 128.29; 169.50.

Radiobiological protocol

Six-week-old male CD1 mice (Charles–River France) were used weighing 25 ± 1 g. Compounds were injected ip in

Miglyol 812 solution. The toxicity of each compound was expressed as LD_{50}. A rough evaluation was initially made followed by a more precise determination.

The radioprotective effect was evaluated by determining the survival rate observed 30 d after irradiation in different groups of 20 mice receiving an ip injection of the tested compound 15 or 90 min before whole-body irradiation. The irradiation dose was equal to the LD_{100}/30 d of control mice (between 8.5–9.5 Gy according to the date of the test) or to this dose + 2 Gy. When necessary, other irradiation doses (between 9–13 Gy) were tested in order to evaluate the irradiation LD_{50}/30 d of protected mice. This was found to be the case for compounds **3a** and **4a**. The LD_{50}/30 d were evaluated from the survival rates obtained with the different irradiation doses by the Karber method (calculated or graphic) [18, 19]. They were equal to 9.6 ± 0.3 Gy for compound **3a** and 10 ± 0.4 Gy for compound **4a**.

The irradiation LD_{50}/30 d of the untreated control mice was monitored regularly using the survival rate of different groups of mice irradiated between 5–10 Gy. The LD_{50} was generally equal to 7.7 ± 0.2 Gy ($p \leq 0.05$).

The dose reduction factor (DRF), which is defined as the ratio between irradiation LD_{50}/30 d of treated mice and that of control mice, was therefore calculated. The mean survival time after 30 d (MST 30) was determined for each test. Whole-body irradiation was performed with a ^{60}Co γ-ray source (6×10^{13} Bq). The dose rate was between 0.6–0.7 Gy/min according to the date of the test. For exposure, mice were positioned in an Altuglass box divided into 30 cells in a homogeneous field 28.5 cm x 28.5 cm. Dosimetry was carried out by means of ionization chamber dosimeters and lithium fluoride thermoluminescent dosimeters.

Each irradiation session included a group of 20 mice irradiated at the LD_{100}/30 d of controls 15 min after an ip injection of the solvent alone. 100% lethality was always observed with a mean survival time of 11 ± 1 d. Furthermore, a group of non-irradiated mice received the test compound with a dose equal to half of its LD_{50}, in order to check for toxic lethality among the injected and irradiated mice. For all the compounds, the test animals were alive 30 d after injection.

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