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New α, β and γ semicarbazone and thiosemicarbazone 1,3-dithiolanes as radioprotectors. Anticonvulsant activity

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Summary — Ten semicarbazone and nine thiosemicarbazone 1,3-dithiolanes (α, β and γ) were prepared and tested as radioprotector agents. Dithiolane is well known for its radioprotective properties; this compound protects 78% of mice if 500 mg/kg is administered 15 min before a 850 cGy irradiation dose [1]. For the first time, the combination of a semicarbazone or thiosemicarbazone group with a dithiolane moiety has been achieved within a single molecule. We also studied the potential anticonvulsant activities of these compounds, since benzodiazepines have been observed to greatly decrease radioinduced convulsions. Among the tested compounds, the correlation between the anticonvulsant activity and the radioprotective effect was not systematic.

dithiolane / semicarbazone / thiosemicarbazone / radioprotector /anticonvulsant activity

Introduction

Some compounds with a linear SCCS or SCCN link have shown significant radioprotector activity. The radioprotector effect has also been observed when the former moiety is included in a ring such as 1,3-dithiolane [1–3]. We have recently reported that mixed compounds with a dithiolane ring, like γ-thiazolidine-1,3-dithiolanes and oximinoether-1,3-dithiolanes, are indeed radioprotectors [4, 5]. In the present work we have achieved the first combination of a semicarbazone or thiosemicarbazone group and a dithiolane moiety within a single molecule. We decided to include a semicarbazone or thiosemicarbazone group, because irradiation with a lethal dose is followed by an increase in cerebral excitability manifested by cerebral electrical activity alterations, such as spikes and spikes–waves [6], and some compounds that bear these carbazone groups are anticonvulsants [7–9].

Since anticonvulsant benzodiazepines greatly decrease radioinduced convulsions [10], we thought it was of interest to study the potential anticonvulsant activities of these compounds. We also tested compound **6**, which has been reported previously [5].

Chemistry

Our strategy for the synthesis of the target compounds was the construction of α-, β- and γ-keto-1,3-dithiolane skeletons followed by attachment of a semicarbazone or thiosemicarbazone moiety in the last stage. The syntheses are summarized in scheme 1. α- and β-diketones are commercially available, and γ-diketones were obtained from commercially-available 1-buten-3-one **1** by condensation with the corresponding aldehyde and 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride as catalyst. Keto-1,3-dithiolanes **3** were obtained by reacting one equivalent of corresponding α-, β- or γ-diketone **2** with one equivalent of 1,2-ethanedithiol with paratoluenesulfonic acid. The compounds **3'** and **3''** were separated by chromatography on a silica column and their structures have been determined by ^{1}H-NMR studies [5, 11].

α-, β- and γ-semicarbazone-1,3-dithiolanes **4** were prepared by reacting **3** with semicarbazide chloride in aqueous sodium acetate at room temperature for 3 h, and α-, β- or γ-thiosemicarbazone-1,3-dithiolanes **5**

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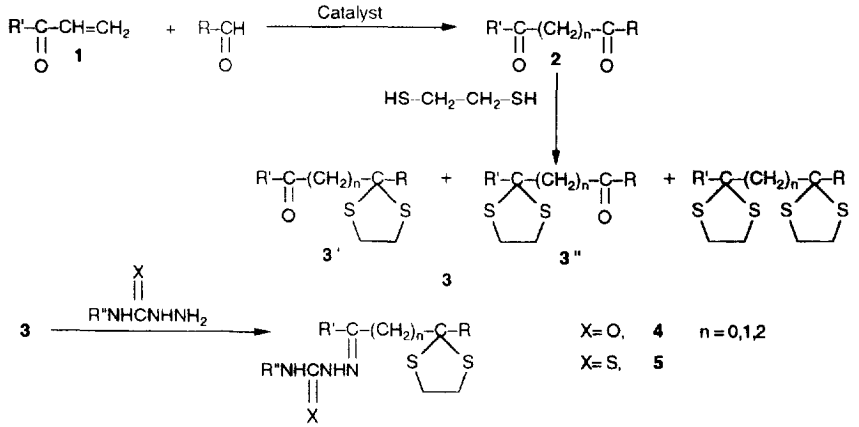


Scheme 1.

was prepared by reacting **3** with thiosemicarbazide chloride in ethanol followed by heating to reflux for 12 h.

Pharmacological results and discussion

The results are shown in table I.

Radioprotective effect

It is important to note that there were difficulties dissolving several of the compounds. Some could not be dissolved even at low concentrations (**4b** and **5f**). Others were only soluble up to a certain concentration so that the toxicity could not be exactly determined (**4f** and **5a**,**c**,**d**,**e**,**i**). On the whole, the compounds were not very toxic and there were no significant differences between semicarbazones and thiosemicarbazones.

Six compounds, **4e**,**f**,**g**, **5a**,**b** and **5c**, have some radioprotective activity. The three semicarbazones have an *n* value equal to 2 and short substituents R and R'. The three thiosemicarbazones have an *n* value equal to zero and short substituents R and R'. These observations confirm the hypothesis that the radioprotective activity disappears as the length of the substituent increases. The decrease in radioprotective effectiveness with an increase in chain length is well known; in particular, it has been described for aminothiols [12].

The radioprotective activity was never very significant and disappeared quickly with an increase in the irradiation dose (**4e**,**f** and **5a**). The greater effectiveness of compound **4e** at 100 rather than 400 mg/kg is at first sight surprising. However, there are several examples of this kind in pharmacology. It may be due to a different mechanism. As a matter of fact, the most interesting result is the prolonged activity, over 90 min. After this delay, the radioprotective effect was often higher than at 15 min (**4f**,**g**, **5a** and **5b**). This observation confirms the notion that the opening of the dithiolane ring, as well as the thiazolidine ring, plays an important role in the radioprotective effect.

We can remark that a difference of 10% is not significant. The variation of LD_{100} at 30 days (LD_{100}/30d) had no adverse result on the estimation of the radioprotective effect. A control group of untreated mice was irradiated under the same conditions as the treated ones and their survival curve regularly determined with the evaluation of LD_{50}/30d and LD_{100}/30d.

The radioprotective effect was never important, so it was not necessary to have a precise LD_{50}/30d determination for treated mice. For instance, if 8 Gy gave a survival rate of 80% and 10 Gy a survival rate of 10%, it could be concluded that the LD_{50}/30d was roughly 9 Gy.

Anticonvulsant activity

Oxime derivatives **4**, **5** and **6**, which showed radioprotective activities or not, were screened for their anticonvulsant activities in order to detect a possible correlation between these two activities. The experimental values are given in table II.

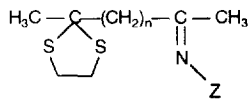
The capacity of a compound to prevent or delay convulsions induced by sc administration of pentetrazole (PTZ) was correlated with an increased threshold of excitability of the central nervous tissue. It is agreed that a positive response corresponds to a potential effect in the 'absence state'. Benzodiazepines (and particularly Diazepam) are considered references for this test. However the problem resides in the major differences between the central nervous

Table I. Data for compounds **4** and **5**.

Compound	*n*	*R*	*R'*	*R''*	*Mp (°C)*	*Yield (%)*	*LD_{50} (mg/kg)*	*Irrad dose (cGy)*	*Delay (min)*	*Admin dose (mg/kg)*	*Survival (%)*	*MST 30d*
4a	0	CH_3	CH_3	H	210	89	>200	775	15	200	0	10.8
								775	90	200	0	8.6
4b	0	CH_3	CH_3	C_6H_5	191	84	[a]					
4c	1	CH_3	CH_3	H	178	94	>800	850	15	200	10	13.5
4d	1	CH_3	CH_3	C_6H_5	173	85	800	850	15	400	0	11
								850	15	100	10	14.2
								1050	15	400	0	9.8
4e	2	CH_3	CH_3	H	164	96	800	850	15	400	20	15.6
								850	15	100	70	25.3
								850	90	400	30	18.3
								1050	15	400	0	9.6
4f	2	CH_3	CH_3	C_6H_5	147	83	>800	850	15	800	60	23.5
								850	15	200	30	19.2
								850	90	800	70	25.3
								1050	15	800	0	9.9
4g	2	C_2H_5	CH_3	H	161	90	800	850	15	400	10	12.7
								850	15	100	0	12.5
								850	90	400	90	27.1
								1050	15	400	0	11.3
4h	2	CH_3	C_2H_5	C_6H_5	132	92	800	900	15	400	0	9.3
4i	2	CH_3	C_2H_5	H	159	94	NT					
4j	2	CH_3	*i*-Pr	H	167	89	NT					
5a	0	CH_3	CH_3	H	110	41	>400	775	15	400	60	24.3
								775	90	400	90	28.4
								775	60	400	30	17.6
								975	60	400	0	8.1
								775	90	400	70	24.3
								975	90	400	0	9.4
								775	120	400	0	10.0
5b	0	CH_3	CH_3	CH_3	136	47	400	775	15	200	10	12.2
								775	90	200	70	24.9
5c	0	CH_3	CH_3	C_6H_5	151	52	>400	775	15	400	50	21.0
								775	90	400	0	7.0
5d	1	CH_3	CH_3	H	131	53	>800	800	15	800	30	10.1
								800	15	200	20	16.4
5e	1	CH_3	CH_3	CH_3	115	78	>400	775	15	400	10	13.4
								775	90	400	40	19.5
5f	1	CH_3	CH_3	C_6H_5	149	85	[a]					
5g	2	CH_3	CH_3	H	163	63	200	900	15	100	0	11.9
								900	15	25	0	9.9
								900	90	100	0	11.5
								1100	15	100	0	10.4
5h	2	CH_3	CH_3	CH_3	74	56	350	775	15	175	30	18.1
								775	15	43.7	0	10.2
								775	90	175	10	13.9
								950	15	175	0	8.6
5i	2	CH_3	CH_3	C_6H_5	124	69	>400	750	15	400	40	19.2
								750	90	400	20	17.5

[a]Insoluble in miglyol; NT: not tested.

Table II. Anticonvulsants tests (realized to 250 mg/kg).



	n	*Z*	*Clonic seizures*		*Tonic seizures*		*Death*	
			Delay (s)	*Incidence (%)*	*Delay (s)*	*Incidence (%)*	*Delay (s)*	*Incidence (%)*
PTZ			763.5 ± 151.3	100	1997.0 ± 727.0	20	2938.7 ± 621.9	30
PTZ/DMSO			860.6 ± 185.2	90	1670.0 ± 0.0	10	2814.2 ± 246.6	60
Diazepam			0.0*	0*	0.0	0	0.0	0*
6a	1	OH	1313.3 ± 352.0	90	3340.0 ± 442.5	50	3676.3 ± 563.0	40
6b	1	OCH_3	927.5 ± 166.0	80	0.0	0	4615.0 ± 750.0	20*
6c	1	OCH_2-Ph	631.1 ± 90.9	100	0.0	0	3327.5 ± 797.5	20
6d	2	OH	760.0 ± 135.3	100	0.0	0	4945.0 ± 0.0	10*
6e	2	OCH_3	1165.8 ± 196.0	60	0.0	0	0.0	0*
6f	2	OCH_2-Ph	641.0 ± 68.3	100	5835.0 ± 0.0	10	5556.7 ± 1351.2	30
4c	1	$NHCONH_2$	661.3 ± 133.6	80	1846.7 ± 125.6	60	2658.0 ± 178.5	100
4d	1	NHCONHPh	762.8 ± 135.8	90	1776.7 ± 672.5	30	2447.5 ± 586.8	40
4e	2	$NHCONH_2$	475.0 ± 78.2	90	1102.5 ± 2.5	20	2293.0 ± 467.9	50
4f	2	NHCONHPh	456.4 ± 57.0	90	1603.8 ± 370.6	40	2496.0 ± 497.9	50
5a	0	$NHCSNH_2$	313 ± 44	100	0.0	0	1567 ± 266	90
5d	1	$NHCSNH_2$	734.5 ± 96.5	100	1832.5 ± 14.8	40	3112.9 ± 381.4	70
5g	2	$NHCSNH_2$	823.0 ± 120.5	100	960.0 ± 141.7	100	1720.5 ± 131.8	100

*$p < 0.05$.

systems of rodents and humans, rendering experimental models far from the clinical situation [13]. Compounds **6b,d** and **6e** displayed significant anticonvulsant activity. The most interesting compound was **6e**, which gave complete protection against tonic seizures and lethality as compared with control animals. Clonic seizures were notably reduced to 60% at 250 mg/kg. Although compounds **6b** and **6d** notably reduced tonic seizures and lethality, they were poorly effective against clonic seizures. Indeed **6b** became active at doses higher than 1000 mg/kg. It is worth noting that the semicarbazone **4c** and the thiosemicarbazone **5g** exhibit agonist activity with regard to PTZ. These results show that only compounds which possess an oxime function or derivative are anticonvulsant. Among these compounds the correlation with the radioprotective activity is not systematic.

Conclusion

New α-, β- or γ-semicarbazone and thiosemicarbazone 1,3-dithiolanes appear to be interesting series with a prolonged radioprotective effect over 90 min for some molecules. The correlation between anticonvulsant activity and the radioprotective effect was not systematic.

Experimental protocols

Chemistry

Melting points were determined on a Kofler bench and are uncorrected. Analyses (C, H, N) were within ±0.4% of the theoretical values for all the compounds. Thin-layer chromatography was performed with Merck silica-gel plates and neutral aluminium oxide. Compounds were detected under UV light or by exposure to iodine vapour. Aluminium oxide 90 (Merck) was used for column chromatography.

IR spectra were recorded in KBr discs or films on a Philips SP3-100 spectrometer. NMR spectra were obtained on a Bruker AC200 machine. Chemical shifts are reported relative to tetramethylsilane. Splitting patterns are designated as follows: s = singlet; d = doublet; dd = doublet of doublets; t = triplet; q = quartet; and m = multiplet.

We describe, as examples, synthesis of the semicarbazone **4e** and the thiosemicarbazone **5g**.

2-Methyl-2-(3-semicarbazonyl butyl)-1,3-dithiolane ***4e***
γ-Keto-1,3-dithiolane (0.016 mol) was reacted with 0.022 mol semicarbazide hydrochloride, 4.5 g sodium acetate and 25 mL H_2O. The mixture was emulsified by stirring vigorously for 3 h at room temperature. The precipitated semicarbazone was filtered, then recrystallized in a mixture of H_2O/ethanol, 3:7. Yield: 96%; mp: 147 °C.

2-Methyl-2-(3-thiosemicarbazonyl butyl)-1,3-dithiolane ***5g***
γ-Keto-1,3-dithiolane (0.018 mol), 0.018 mol thiosemicarbazide, 1.5 mL concentrated HCl and 15 mL ethanol were stirred and refluxed for 12 h. The solvent was evaporated and the residue dissolved in dichloromethane. The organic layer was washed with $NaHCO_3$ solution then with water, and dried with $MgSO_4$. The compound was recrystallized in ethylacetate. Yield: 63%; mp: 163 °C.

2-Methyl-2-acetylsemicarbazonyl-1,3-dithiolane ***4a***. IR (ν cm^{-1}, KBr 2%): NH 3475; NH_2 3200; C=O 1720; C=N 1700. ^{1}H-NMR (δ (ppm), DMSO, 200 MHz): 1.90 (3H, s, CH_3 α position of the ring); 1.97 (3H, s, CH_3CN); 3.34 (4H, m, $2CH_2$ dithiolane ring); 6.22 (2H, s, NH_2); 9.09 (H, s, N*H*). ^{13}C-NMR (δ (ppm), DMSO, 50 MHz): 13.9; 31.3; 40.9; 69.5; 148.2; 157.0.

2-Methyl-2-(4N-phenylacetylsemicarbazonyl)-1,3-dithiolane ***4b***. IR (ν cm^{-1}, KBr 2%): NH 3400; NH-Ph 3200; C=O 1710; C=N 1690. ^{1}H-NMR (δ (ppm), $CDCl_3$, 200 MHz): 2.05 (3H, s, CH_3 α position of the ring); 2.16 (3H, s, CH_3CN); 3.45 (4H, m, $2CH_2$ dithiolane ring); 7.07 (1H, m, *H* in para); 7.32 (2H, m, 2*H* in meta); 7.52 (2H, m, 2*H* in ortho); 8.20 (1H, s, N*H*Ph); 8.78 (1H, s, NN*H*). ^{13}C-NMR (δ (ppm), $CDCl_3$, 50 MHz): 13.9; 30.9; 41.2; 69.6; 119.2; 123.2; 128.9; 148.2; 157.0.

2-Methyl-2-(2-semicarbazonyl propyl)-1,3-dithiolane ***4c***. IR (ν cm^{-1}, KBr 2%): NH 3350; NH_2 3200; C=O 1690; C=N 1590. ^{1}H-NMR (δ (ppm), $CDCl_3$, 200 MHz): 1.81 (3H, s, CH_3 α position of the ring); 1.91 (3H, s, CH_3CN); 2.96 (2H, s, CH_2); 3.30 (4H, m, $2CH_2$ dithiolane ring); 8.48 (H, s, N*H*). ^{13}C-NMR (δ (ppm), $CDCl_3$, 50 MHz): 16.8; 32.5; 39.3; 53.1; 63.9; 146.3; 157.9.

2-Methyl-2-[2-(4N-phenylsemicarbazonyl)propyl]-1,3-dithiolane ***4d***. IR (ν cm^{-1}, KBr 2%): NH 3350; NH-Ph 3200; C=O 1690; C=N 1600. ^{1}H-NMR (δ (ppm), $CDCl_3$, 200 MHz): 1.69 (3H, s, CH_3 α position of the ring); 1.99 (3H, s, CH_3CN); 3.04 (2H, s, CH_2); 3.36 (4H, m, $2CH_2$ dithiolane ring); 7.04 (1H, m, *H* in para); 7.31 (2H, m, 2*H* in meta); 7.57 (2H, m, 2*H* in ortho); 8.55 (1H, s, N*H*Ph); 8.80 (1H, s, N*H*). ^{13}C-NMR: (δ (ppm), $CDCl_3$, 50 MHz): 16.9; 32.7; 39.8; 53.0; 63.8; 119.0; 122.9; 138.5; 146.3; 153.9.

2-Methyl-2-(3-semicarbazonyl butyl)-1,3-dithiolane ***4e***. IR (ν cm^{-1}, KBr 2%): NH 3450; NH_2 3200; C=O 1700; C=N 1590. ^{1}H-NMR (δ (ppm), $CDCl_3$, 200 MHz): 1.78 (3H, s, CH_3 α position of the ring); 1.84 (3H, s, CH_3CN); 2.13 (2H, m, CH_2 α position of the ring); 2.49 (2H, m, CH_2 β position of the ring); 3.31 (4H, m, $2CH_2$ dithiolane ring); 8.18 (H, s, N*H*). ^{13}C-NMR (δ (ppm), $CDCl_3$, 50 MHz): 15.7; 32.6; 36.3; 40.0; 41.3; 66.1; 149.6; 157.8.

2-Methyl-2-[3-(4N-phenylsemicarbazonyl)butyl]-1,3-dithiolane ***4f***. IR (ν cm^{-1}, KBr 2%): NH 3350; NH_2 3200; C=O 1690; C=N 1600. ^{1}H-NMR (δ (ppm), $CDCl_3$, 200 MHz): 1.82 (3H, s, CH_3 α position of the ring); 1.92 (3H, s, CH_3CN); 2.19 (2H, m, CH_2 α position of the ring); 2.57 (2H, m, CH_2 β position of the ring); 3.35 (4H, m, $2CH_2$ dithiolane ring); 7.05 (1H, m, *H* para); 7.31 (2H, m, 2*H* meta); 7.50 (2H, m, 2*H* ortho); 8.19 (H, s, N*H*); 8.25 (H, s, N*H*Ph). ^{13}C-NMR (δ (ppm), $CDCl_3$, 50 MHz): 15.8; 32.8; 36.4; 40.1; 41.4; 66.1; 119.4; 123.1; 128.8; 149.8; 153.6.

2-Ethyl-2-(3-semicarbazonyl butyl)-1,3-dithiolane ***4g***. IR (ν cm^{-1}, KBr 2%): NH 3450; NH_2 3200; C=O 1700; C=N 1600. ^{1}H-NMR (δ (ppm), $CDCl_3$, *J* (Hz), 200 MHz): 1.06 (3H, t, CH_2CH_3, *J* = 7); 1.84 (3H, s, CH_3CN); 1.97 (2H, q, CH_2CH_3, *J* = 7); 2.11 (2H, m, CH_2 α position of the ring); 2.46 (2H, m, CH_2 β position of the ring); 3.26 (4H, m, $2CH_2$ dithiolane ring); 8.12 (H, s, N*H*). ^{13}C-NMR (δ (ppm), $CDCl_3$, 50 MHz): 11.0; 15.7; 35.9; 36.7; 38.6; 39.7; 71.6; 149.7; 157.7.

2-Methyl-2-[3-(4N-phenylsemicarbazonyl)pentyl]-1,3-dithiolane ***4h***. IR (ν cm^{-1}, KBr 2%): NH 3350; NH-Ph 3200; C=O 1690; C=N 1600. ^{1}H-NMR (δ (ppm), $CDCl_3$, 200 MHz): 1.11 (3H, t, CH_2CH_3, *J* = 7); 1.92 (3H, s, CH_3CN); 2.01 (2H, q, CH_2CH_3, *J* = 7); 2.16 (2H, m, CH_2 α position of the ring); 2.54 (2H, m, CH_2 β position of the ring); 3.29 (4H, m, $2CH_2$ dithiolane ring); 7.05 (1H, m, *H* para); 7.31 (2H, m, 2*H* meta); 7.50 (2H, m, 2*H* ortho); 8.21 (H, s, N*H*). ^{13}C-NMR (δ (ppm), $CDCl_3$, 50 MHz): 11.1; 15.8; 35.9; 36.9; 38.7; 39.8; 71.6; 119.3; 123.1; 128.8; 150.0; 153.6.

2-Methyl-2-(3-semicarbazonyl pentyl)-1,3-dithiolane ***4i***. IR (ν cm^{-1}, KBr 2%): NH 3450; NH_2 3200; C=O 1700; C=N 1600. ^{1}H-NMR (δ (ppm), $CDCl_3$, 200 MHz): 1.08 (3H, t, CH_2CH_3, *J* = 4); 1.78 (3H, s, CH_3CN); 2.02 (2H, m, CH_2 α position of the ring); 2.24 (2H, q, CH_2CH_3, *J* = 4); 2.42 (2H, m, CH_2 β position of the ring); 3.35 (4H, m, $2CH_2$ dithiolane ring); 8.05 (2H, s, NH_2); 8.12 (H, s, N*H*). ^{13}C-NMR (δ (ppm), $CDCl_3$, 50 MHz): 10.5; 15.7; 22.6; 32.7; 33.7; 40.3; 41.2; 66.7; 153.3; 157.5.

2-Methyl-2-(4-methyl-3-semicarbazonyl pentyl)-1,3-dithiolane ***4j***. IR (ν cm^{-1}, KBr 2%): NH 3390; NH_2 3200; C=O 1690; C=N 1630. ^{1}H-NMR (δ (ppm), $CDCl_3$, 200 MHz): 1.11 (6H, d, $CH(CH_3)_2$, *J* = 7); 1.79 (3H, s, CH_3CN); 2.00 (2H, m, CH_2 α position of the ring); 2.41 (2H, m, CH_2 β position of the ring); 2.48 (1H, q, $CH(CH_3)_2$, *J* = 7); 3.37 (4H, m, $2CH_2$ dithiolane ring); 8.10 (H, s, N*H*). ^{13}C-NMR (δ (ppm), $CDCl_3$, 50 MHz): 20.0; 25.9; 31.5; 35.9; 40.3; 40.3; 66.9; 156.1; 157.3.

2-Methyl-2-acetylthiosemicarbazonyl-1,3-dithiolane ***5a***. IR (ν cm^{-1}, KBr 2%): NH 3460; NH_2 3200; C=N 1600; C=S 1310. ^{1}H-NMR (δ (ppm), DMSO, 200 MHz): 1.92 (3H, s, CH_3 α position of the ring); 2.10 (3H, s, CH_3CN); 3.45 (4H, m, $2CH_2$ dithiolane ring); 7.42 (1H, s, NH_2); 8.28 (1H, s, NH_2); 10.01 (H, s, N*H*). ^{13}C-NMR (δ (ppm), DMSO, 50 MHz): 14.6; 31.1; 41.2; 69.5; 152.7; 179.1.

2-Methyl-2-(4N-methylacetylthiosemicarbazonyl)-1,3-dithiolane ***5b***. IR (ν cm^{-1}, KBr 2%): NH 3350; NH-CH_3 3250; C=N 1560; C=S 1410. ^{1}H-NMR (δ (ppm), $CDCl_3$, 200 MHz): 1.97 (3H, s, CH_3 α position of the ring); 2.02 (3H, s, CH_3CN); 3.22 (3H, d, $HNCH_3$, J = 5); 3.43 (4H, m, $2CH_2$ dithiolane ring); 7.40 (1H, s, $NHCH_3$); 8.45 (1H, s, N*H*). ^{13}C-NMR (δ (ppm), $CDCl_3$, 50 MHz): 13.7; 30.8; 31.2; 41.4; 69.4; 150.8; 179.2.

2-Methyl-2-(4N-phenylacetylthiosemicarbazonyl)-1,3-dithiolane ***5c***. IR (ν cm^{-1}, KBr 2%): NH 3325; C=N 1605; C=S 1350. ^{1}H-NMR (δ (ppm), $CDCl_3$, 200 MHz): 2.03 (3H, s, CH_3 α position of the ring); 2.17 (3H, s, CH_3CN); 3.46 (4H, m, $2CH_2$ dithiolane ring); 7.32 (1H, m, *H* in para); 7.40 (2H, m, 2*H* in meta); 7.66 (2H, m, 2*H* in ortho); 8.55 (1H, s, N*H*); 9.25 (1H, s, N*H*Ph). ^{13}C-NMR (δ (ppm), $CDCl_3$, 50 MHz): 14.0; 30.7; 41.4; 69.4; 123.8; 126.0; 128.7; 151.2; 176.5.

2-Methyl-2-(2-thiosemicarbazonyl propyl)-1,3-dithiolane ***5d***. IR (ν cm^{-1}, KBr 2%): NH 3350; NH_2 3250–3200; C=N 1610; C=S 1310. ^{1}H-NMR (δ (ppm), DMSO, 200 MHz): 1.71 (3H, s, CH_3 α position of the ring); 1.96 (3H, s, CH_3CN); 2.93 (2H, s, CH_2); 3.33 (4H, m, $2CH_2$ dithiolane ring); 7.42 (H, s, N*H*H); 8.23 (H, s, NH*H*); 10.16 (H, s, N*H*). ^{13}C-NMR (δ (ppm), DMSO, 50 MHz): 17.9; 31.9; 38.7; 52.3; 64.0; 150; 178.8.

2-Methyl-2-(2-(4N-methylthiosemicarbazonyl)propyl)-1,3-dithiolane ***5e***. IR (ν cm^{-1}, KBr 2%): NH 3350; C=N 1550; C=S 1350. ^{1}H-NMR (δ (ppm), $CDCl_3$, 200 MHz): 1.78 (3H, s, CH_3 α position of the ring); 1.95 (3H, s, CH_3CN); 2.96 (2H, s, CH_2); 3.21 (3H, d, $NHCH_3$, J = 5); 3.30 (4H, m, $2CH_2$ dithiolane ring); 7.26 (H, s, $NHCH_3$); 8.45 (H, s, N*H*). ^{13}C-NMR (δ (ppm), $CDCl_3$, 50 MHz): 16.9; 31.2; 32.6; 39.4; 53.0; 63.8; 147.4; 178.9.

2-Methyl-2-[2-(4N-phenylthiosemicarbazonyl)propyl]-1,3-dithiolane ***5f***. IR (ν cm^{-1}, KBr 2%) : NH 3300; NH-Ph 3250; C=N 1600; C=S 1370. ^{1}H-NMR (δ (ppm), $CDCl_3$, 200 MHz): 1.86 (3H, s, CH_3 α position of the ring); 2.01 (3H, s, CH_3CN); 3.06 (2H, s, CH_2); 3.33 (4H, m, $2CH_2$ dithiolane ring); 7.21 (1H, m, *H* in para); 7.39 (2H, m, 2*H* in meta); 7.75 (2H, m, 2*H* in ortho); 8.60 (H, s, N*H*); 9.56 (H, s, N*H*Ph). ^{13}C-NMR (δ (ppm), $CDCl_3$, 50 MHz): 17.1; 32.7; 39.4; 52.9; 63.6; 123.4; 125.8; 128.6; 147.8; 176.0.

2-Methyl-2-(3-thiosemicarbazonylbutyl)-1,3-dithiolane ***5g***. IR (ν cm^{-1}, KBr 2%): NH 3400; NH_2 3200–3150; C=N 1600; C=S 1310. ^{1}H-NMR (δ (ppm), DMSO, 200 MHz): 1.70 (3H, s, CH_3 α position of the ring); 1.89 (3H, s, CH_3CN); 2.12 (2H, m, CH_2 α position of the ring); 2.42 (2H, m, CH_2 β position of the ring); 3.36 (4H, m, $2CH_2$ dithiolane ring); 8.03 (2H, s, NH_2); 9.90 (H, s, N*H*). ^{13}C-NMR (δ (ppm), DMSO, 50 MHz): 16.8; 32.1; 36.0; 39.4; 40.5; 66.3; 153.7; 178.4.

2-Methyl-2-[3-(4N-methylthiosemicarbazonyl)butyl]-1,3-dithiolane ***5h***. IR (ν cm^{-1}, KBr 2%): NH 3350; NH_2 3225; C=N 1640; C=S 1360. ^{1}H-NMR (δ (ppm), $CDCl_3$, 200 MHz): 1.78 (3H, s, CH_3 α position of the ring); 1.97 (3H, s, CH_3CN); 2.12 (2H, m, CH_2 α position of the ring); 2.51 (2H, m, CH_2 β position of the ring); 3.19 (3H, d, $NHCH_3$, J = 5); 3.32 (4H, m, $2CH_2$ dithiolane ring); 7.26 (1H, s, $NHCH_3$); 8.37 (1H, s, N*H*). ^{13}C-NMR (δ (ppm), $CDCl_3$, 50 MHz): 15.8; 31.0; 32.7; 36.3; 40.3; 41.2; 65.9; 150.9; 178.8.

2-Methyl-2-[3-(4N-phenylthiosemicarbazonyl)butyl]-1,3-dithiolane ***5i***. IR (ν cm^{-1}, KBr 2%): NH 3325; NH_2 3225; C=N 1660; C=S 1370. ^{1}H-NMR (δ (ppm), $CDCl_3$, 200 MHz): 1.79 (3H, s, CH_3 α position of the ring); 1.95 (3H, s, CH_3CN); 2.17 (2H, m, CH_2 α position of the ring); 2.60 (2H, m, CH_2 β position of the ring); 3.33 (4H, m, $2CH_2$ dithiolane ring); 7.21 (1H, m, *H* para); 7.37 (2H, m, 2*H* meta); 7.67 (2H, m, 2*H* ortho); 8.56 (1H, s, N*H*); 9.23 (1H, s, N*H*Ph). ^{13}C-NMR (δ (ppm), $CDCl_3$, 50 MHz): 16.0; 32.8; 36.3; 40.3; 41.0; 65.9; 123.9; 125.6; 128.5; 151.7; 178.

Pharmacological protocols

Radiobiological protocol

Six-week-old male CD1 mice (Charles River, France) were used. Their weight was 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 made initially, followed by a more precise determination.

The radioprotective effect was evaluated by determining the survival rate observed 30 days 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}/30d of control mice (between 7.75 and 9 Gy according to the date of the test) or 10.5 Gy. When necessary, other irradiation doses (between 9.75 and 11 Gy) were tested in order to evaluate the irradiation LD_{50}/30d of protected mice. The LD_{50}/30d value was evaluated from the survival rates obtained with the different irradiation doses, using the Karber method (calculated or graphical) [14, 15]. The LD_{50}/30d irradiation of the untreated control mice was monitored every 90 days via the survival rate of different groups of mice and that of control mice was hence calculated. The mean survival time after 30 days (MST 30) was determined for each test.

Whole-body irradiation was performed with a ^{60}Co γ-ray source (610^{13} Bq). The dose rate was 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 28.5 x 28.5 cm field. Dosimetry was carried out by means of ionization-chamber and lithium fluoride thermoluminescent dosimeters. Each irradiation session involved a group of 20 mice irradiated at the LD_{100}/30d 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 days. Furthermore, a group of unirradiated 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, these animals were alive 30 days after injection.

Anticonvulsant protocol

Although PTZ produces reversible convulsivant effects, this drug was used in a first screening approach. The PTZ test, as described initially by Krall [16], was used on groups of ten female mice OF1 (IFFA CREDO) weighing 22.9 ± 0.1 g. Compounds under study were administered po in DMSO/H_2O (1:1) solutions at a dose of 250 mg/kg. A positive test group was given 2.5 mg/kg of diazepam po. After 15 min, an alcoholic solution (10%) of PTZ (100 mg/kg) was injected sc in the

scruff of the neck of the animal. The following parameters were monitored: latent period before clonic seizures, tonic seizures and death, as well as the incidence of these events.

Statistical analysis was based on the comparison of results to those of control animals, treated by the Mann–Whitney U test [17]; $p < 0.05$ was considered significant.

References

1 Fatome M, Poutrain P, Granger R et al (1970) *Chim Ther* 5, 327–331
2 Grassy G, Terol A, Belly A et al (1975) *Eur J Med Chem* 10, 14–18
3 Robbe Y, Fernandez JP, Dubief R et al (1982) *Eur J Med Chem* 17, 235–243
4 Ho-Law, Péra MH, Taillandier G, Fatome M, Laval JD, Leclerc G, (1994) *Eur J Med Chem* 29, 121–125
5 Taroua M, , Péra MH, Taillandier G, Fatome M, Laval JD, Leclerc G, (1994) *Eur J Med Chem* 29, 621–625
6 Court I, Bassant MH, Dufour R et al (1966) *Br J Cancer* 53, 236–231
7 Dimmock JR, Doreen CS, Brenner JM et al (1986)) *Eur J Med Chem* 21, 187–192
8 Dimmock JR, McColl JM, Wonko SL et al (1991) *Eur J Med Chem* 26, 529–534
9 Singh SP, Misra RS, Parmar SS et al (1975) *J Pharm* 64, 1245–1247
10 Fatome M, Gaudefroy A, Laval JD, Andrieu L, Sentenac-Roumanou H, (1977) *Eur J Med Chem* 12, 93–95
11 Ho-Law, Péra MH, Taillandier G, (1993) *Sulfur Lett* 16, 4, 157–163
12 Bacq ZM (1965) CC Thomas, Springfield (IL)
13 Meldrum BS, Horton RW, Taseland PA, (1975) *Arch Neurol* 32, 489–494
14 Kimbal AW, Burnett WT, Doherty DG (1957) *Radiat Res* 7, 1–11
15 Oiry J, Pue JY, Imbach JL et al (1988) *J Med Chem* 29, 2217–2225
16 Krall RL (1978) *Epilepsia* 19, 409–428