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Synthesis, antioxidant properties and radioprotective effects of new benzothiazoles and thiadiazoles

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ABSTRACT

In this work, we report the synthesis and characterization of new compounds derived from benzothiazoles and thiadiazoles. We observed that structural modifications on these skeletons affected the antioxidant activity. Thiol and aminothiol compounds derived from thiadiazoles and benzothiazoles showed an interesting antioxidant property. The radioprotective activity has also been evaluated in mice. Some of these compounds could be good radioprotectors.

1. Introduction

The potential application of radioprotective chemicals in the event of various human radiation exposures has been investigated since nuclear exposure from accidents or warfare became possible.[1] However, in recent years, another application of such radioprotective compounds has been in the treatment of cancer patients during radiotherapy in order to protect normal tissues adjacent to the treated tumor.

In 1949, the ability of a chemical compound to protect from the toxic effects of radiation was reported for the first time by Patt et al.[2] It was demonstrated that the administration of the sulfydryl amino acid cysteine to rats before an 8 Gy whole-body X-irradiation, significantly increased the animal's resistance to cytotoxic effects of the exposure. Since this initial finding, new agents have been developed like mercaptans, di- and trisulfides, phosphorothioates, alcohols, acid hydrazides, imidazoles, benzofurans, amine oxides and thiazolidines.[3] However, most of these compounds are toxic and so there is still a need to develop new radioprotective compounds that are more effective and less toxic.

It is also well known that the biological effects of ionizing radiation are linked to the production of reactive oxygen species in organisms which induce cellular DNA damage which leads to mutations and chromosomal aberrations.[4] Moreover, it is generally accepted that endogenous antioxidants such as cellular non-protein thiols (glutathion...) and antioxidant enzymes provide some degree of protection. Even if a variety of other radioprotective mechanisms have been proposed to explain prophylactic and therapeutic effects of a large number of agents, antioxidants are still considered a main class of radioprotectors.[3,5,6] We supposed that a direct link between a thiol function that catches radicals and an aromatic group that traps them by potential degradation of the aromatic structure, and that this is the way the antioxidant properties perform. Furthermore, the synthesis of N-alkylated aromatic heterocycles by well known antioxidant or radioprotective functions can have an interesting effect on such properties. Since the radioprotective activity of thiosulfonic acids and phosphorothioates is well-known, we speculated that the introduction of these important functional groups into new heterocycles like benzothiazole and thiadiazole might provide potent activity.[7–12] More specifically, heterocycles such as benzimidazole, imidazole and thiazolidines were studied in this area.[13–15]

So, in this work, we report the synthesis of *N*-alkylbenzothiazole and thiadiazole substituted by amide, thiosulfonic acid,

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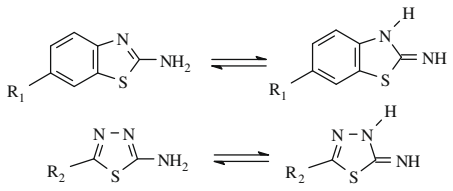


1a: R_1= H, **1b**: R_1 = CH_3, **1c**: R_1 = CH_3CH_2O
1d: R_2 = CH_3CH_2S, **1e**: R_2 = CH_3CH_2

Scheme 1. Structures of benzothiazole and thiadiazole derivatives **1**(**a**–**e**).

thiobenzothiazole, thiothiadiazole and phosphorothioate groups. We then present their free radical scavenging properties measured in vitro and their radioprotective activity studied in mice. Finally, we discuss about these in vitro and in vivo results.

2. Results and discussion

2.1. Synthesis

We have focused our work on the development of two new classes of radioprotector agents that are benzothiazole and thiadiazole derivatives. Both types of molecules are characterized by their tautomeric forms (Scheme 1).

Therefore the synthesis of compounds which differ in the position of the alkyl chain should be interesting. Indeed, by different methods, the alkylation could occur either on the endocyclic (**6–9**) or on the exocyclic nitrogen atom (**2** and **5**).[16]

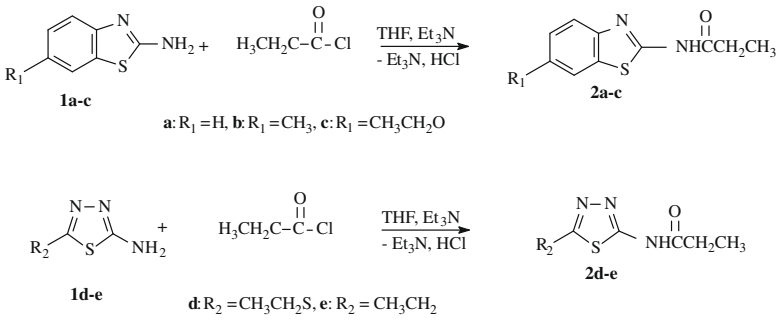
Amides derivatives **2(a–e)**—The reaction of propionyl chloride with amines **1(a–e)** in tetrahydrofurane (THF) in the presence of triethylamine leads to amide derivatives (Scheme 2).

Thiols **4(a–e)** and aminothiols derivatives **5(a–e)**—They were synthesized in two steps. Firstly, the amine function of aminobenzothiazole and aminothiadiazole derivatives **1(a–e)** was substituted by chloride. The treatment of **1(a–e)** with 37% hydrogen chloride solution in presence of copper powder and sodium nitrite leads to halogenated derivatives **3(a–e)** (Scheme 3).

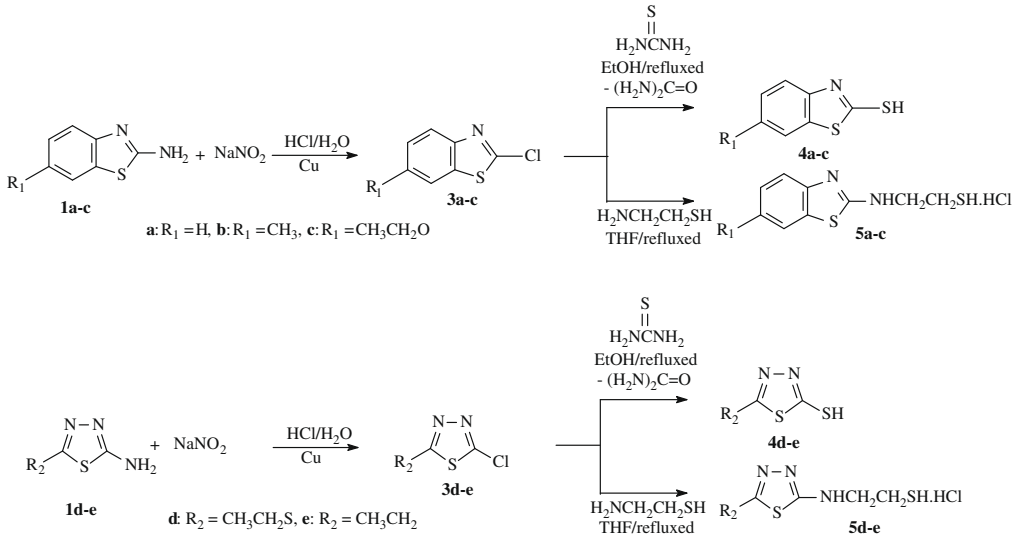
Thiol derivatives **4(a–e)** were obtained in a second step by reaction of thiourea in ethanol under reflux. This method has been previously reported in the literature.[17] Compounds **3(a–b), 3d** and **4a** have been already obtained by other methods described previously.[18–20]

Finally, hydrochlorides of the aminothiols **5(a–e)** were synthesized under reflux in THF, by reaction of halogenated derivatives **3(a–e)** with cysteamine (Scheme 3). Compound **5a** has been already synthesized by another method.[21]

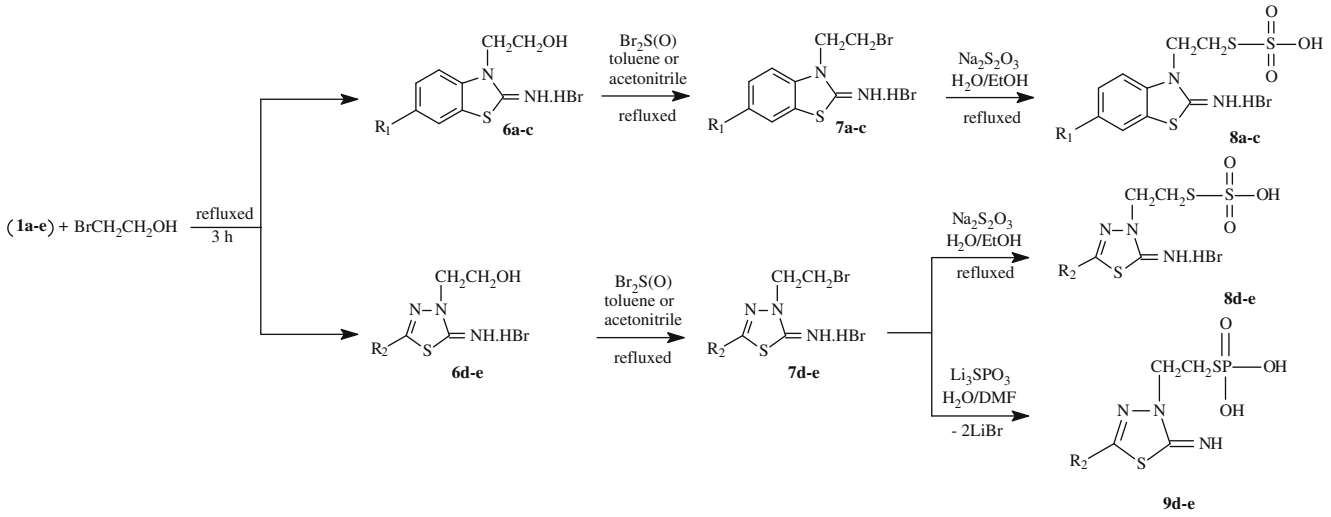
Thiosulfonic acids **8(a–e)** and phosphorothioates derivatives **9(a–e)** were obtained by three steps. First, we synthesized alcohol derivatives **6(a–e)** by treatment of bromoethanol solution with aminobenzothiazole and aminothiadiazole. In a second step, the



Scheme 2. Synthesis of amide derivatives **2**(**a**–**e**).



Scheme 3. Synthesis of thiol **4**(**a**–**e**) and aminothiol **5**(**a**–**e**) derivatives.



Scheme 4. Synthesis of thiosulfonic acids **8**(**a**–**e**) and phosphorothioates derivatives **9**(**a–e**).

alcohol group was substituted by bromine by reaction with thionyl bromide to give compounds **7(a–e**).

In the last step, thiosulfate derivatives **8(a–e**) were obtained by treatment of an aqueous solution of sodium thiosulfate with corresponding hydrobromides **7(a–e**) in a mixture of water/ethanol, at reflux (Scheme 4).

Finally, lithium hydrogen phosphorothioates **9(d–e**) were prepared from lithium thiophosphate and the appropriate hydrobromide **7(d–e**) by a standard reaction procedure (Scheme 4).[22,23] Indeed, treatment of an aqueous solution of lithium thiophosphate with hydrobromides followed by dilution of the aqueous reaction mixture with dimethylformamide and through washing of the resulting precipitate obtained after concentration with acetone and methanol yielded a solid which gave the expected ^{31}P NMR resonance chemical shift.

The use of trilithium rather than trisodium phosphorothioate makes the isolation of phosphorothioates from thiadiazole easier **9(d–e**).

The preparation of the corresponding phosphorothioate from benzothiazole by a similar procedure leads to three compounds (Scheme 5). One of these three compounds is an annulated derivative which was characterized by ^{31}P NMR and mass spectrum. The two other compounds correspond to *O*-alkylphosphorothioate with a chemical shift at 37.23 ppm and *S*-alkylphosphorothioate with a chemical shift at 15.85 ppm in ^{31}P NMR.[24] Only the annulated (benzothiazol-2-yliden-amino) phosphorothioate was isolated. *O*-alkylphosphorothioate and *S*-alkylphosphorothioate seem to undergo a rearrangement to the annulated form which is more stable in solution (Scheme 5).

3. Biological studies

3.1. Antioxidant properties

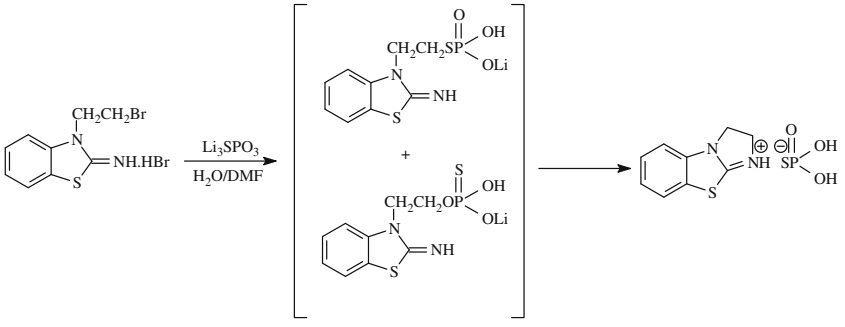
The majority of these compounds were subjected to antioxidant activity screening by determining the DPPH or ABTS free radical scavenging using simple UV spectroscopic methods.[25,26] Precursor compounds **1(a–e**) have been also tested to demonstrate the effect of the structural modifications on antioxidant activity in comparison to amifostine (WR-2721) or ascorbic acid.

Indeed, for many of them it was not possible to calculate an IC_{50} which was superior to 4 mM. As shown in Table 1, amines and amides derivatives did not display free radical scavenging activity towards DPPH. This low activity is most probably due to the disability of amine and amide to catch radicals.

The aminoalcohols derivatives **6c**, **6d** and **6e,** exhibit an IC_{50} lower than 4 mM but these values are still very high compared to WR-2721 (0.1 mM) or thiols and aminothiols. This bears out the higher efficiency of thiol and phosphorothioates over alcohols.

This in vitro evaluation has been previously reported for thiol and aminothiol derivatives.[27] It shows that thiol derivatives of benzothiazole and thiadiazole are the best antioxidant compounds toward the DPPH radical. Their radical scavenging is nearly the same as WR-2721's for compounds **4a**, **4b**, **4e**. Compounds **4c** (IC_{50} = 0.046 mM) and **4d** (IC_{50} = 0.053 mM) show a strong antioxidant property which is around half more than WR-2721. But, ascorbic acid remains the best antioxidant compound.

This good activity of thiol derivatives of benzothiazole and thiadiazole shows that the hypothesis of a direct link between thiol



Scheme 5. Rearrangement of *O*- and *S*-alkylphosphorothioates.

Table 1
Free radical scavenging activity of compounds for DPPH radical

Chemical family		Compound	IC_{50}[a] (mM)	Inhibition[b] (%)
		WR-2721	0.100 ± 0.005	—
		Ascorbic acid	0.020 ± 0.004	—
		Trolox	0.034 ± 0.006	—
		Glutathion	0.096 ± 0.008	—
Amine derivatives of	Benzothiazole	**1a**	>4	6.03 ± 0.57
		1b	>4	5.57 ± 0.98
		1c	>4	9.03 ± 1.01
	Thiadiazole	**1d**	>4	9.57 ± 0.68
		1e	>4	4.8 ± 0.78
Amide derivatives of	Benzothiazole	**2a**	>4	19.1 ± 0.11
		2b	>4	2.99 ± 0.15
	Thiadiazole	**2c**	>4	5.49 ± 0.89
		2d	>4	7.21 ± 0.56
		2e	>4	2.62 ± 0.76
Thiols derivatives of	Benzothiazole	**4a**	0.092 ± 0.006	—
		4b	0.091 ± 0.004	—
		4c	0.046 ± 0.005	—
	Thiadiazole	**4d**	0.053 ± 0.006	—
		4e	0.084 ± 0.005	—
Aminothiols derivatives of	Benzothiazole	**5a**	1.39 ± 0.49	—
		5b	3.17 ± 0.85	—
	Thiadiazole	**5d**	0.11 ± 0.20	—
Aminoalcool derivatives of	Benzothiazole	**6a**	>4	—
		6b	>4	—
		6c	1.4 ± 0.5	—
	Thiadiazole	**6d**	3.5 ± 0.8	—
		6e	2.4 ± 0.6	—

[a] IC_{50}: Concentration of test compounds needed to reduce DPPH absorption by 50% at 516 nm. Values are means of three independent determinations.
[b] Inhibition (%) indicates the percent inhibition at 4 mM of test compounds.

function and an aromatic ring was a good one. The thiol catches the radical and after, the aromatic ring permits the trapping of this radical. Moreover, aminothiol derivatives of benzothiazole **5a** and **5b** (IC_{50} = 1.39 mM and IC_{50} = 3.17 mM respectively) do not seem to be strong radical scavengers but the aminothiol derivative of thiadiazole **5d** (IC_{50} = 0.11 mM) shows a better activity than WR-2721 (0.1 mM). So, the aromatic ring interferes with the radical scavenging: that thiadiazoles are usually better scavengers than benzothiazoles may be due to the efficiency of the trapping.

Table 2
Free radical scavenging activity of compounds for ABTS radical

Chemical family		Compound	IC_{50}[a] (μM)
		Ascorbic acid	46.46 ± 0.33
Thiols derivatives of	Benzothiazole	**4a**	79.84 ± 3.55
		4b	76.66 ± 5.97
		4c	88.35 ± 3.28
	Thiadiazole	**4d**	23.08 ± 2.17
		4e	21.26 ± 1.41
Aminothiols derivatives of	Benzothiazole	**5a**	1520 ± 90
		5b	4040 ± 62
	Thiadiazole	**5d**	390 ± 30
Thiosulfonic acids derivatives of	Thiadiazole	**8d**	8.32 ± 0.12
		8e	51.85 ± 3.06
Phosphorothioates derivatives of	Thiadiazole	**9d**	76.87 ± 0.99
		9e	90.33 ± 2.56

[a] IC_{50}: Concentration of test compounds needed to reduce ABTS absorption by 50% at 732 nm. Values are means of three independent determinations.

These observations on thiols and aminothiols are corroborated by the ABTS test. Toward the $[ABTS]^{+\cdot}$ radicals, thiols **4(a–e)** are always good antioxidants with the same tendency for thiadiazole ring which is more effective than benzothiazole ring. Futhermore, aminothiols stay less efficient than thiols.

Under our experimental conditions, it was not possible to evaluate free radical scavenging activity towards the DPPH radical of thiosulfonic acids and phosphorothioates because of the lack of solubility of these compounds in ethanol. As a result, we used the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation discoloration assay (ABTS test).

As shown in Table 2, thiosulfonic acids and phosphorothioates compounds displayed various degrees of free radical scavenging activity towards the ABTS radical, with decreasing activity in the following order: **8d** > **8e** > **9d** > **9e**. The most potent compound is the thiosulfonic acid **8d** with an antiradical effect (IC_{50} = 8.32 μM) higher than ascorbic acid (IC_{50} = 46.46 μM). Other compounds demonstrated potent antioxidant activity comparable to that of ascorbic acid for **8e** (IC_{50} = 51.85 μM), or lower for compounds **9d** (IC_{50} = 76.87 μM) and **9e** (IC_{50} = 90.33 μM). These results demonstrate that thiosulfonic acids are more effective antioxidants than the corresponding phosphorothioates. This could be explained by the lack of alkaline phosphatase in the medium that is required to convert phosphorothioate to the corresponding thiol active form

Table 3
Radioprotective effect of some thiol and aminothiol derivatives

Compound	$LD_{50tox/48H}$ (mg kg^{-1})	Posology (mg kg^{-1})	Animal model	Irr. dose (Gy)	Survival at day 30 (%) [*t*, min][a]
4a	>960	480	B6D2F1 mice	8.8	100 [15]
			CD1 mice	8.2	60 [90]
				10.2	0 [90]
		240	B6D2F1 mice	8.8	90 [15]
4b	>520	260	B6D2F1 mice	8.8	100 [15]
				10.8	0 [15]
			CD1 mice	8.2	80 [90]
				10.2	0 [90]
4d	698	349	CD1 mice	8.2	0 [90]
				10.2	0 [90]
4e	>680	340	CD1 mice	8.2	30 [90]
				10.2	0 [90]
5a	>616	308	CD1 mice	8.2	60 [90]
				10.2	0 [90]
5d	>736	368	CD1 mice	8.2	0 [90]
				10.2	0 [90]
WR-2721	800	400	B6D2F1 mice	8.8	100 [15]
				10.8	60 [15]
			CD1 mice	8.2	50 [90]
				10.2	20 [90]

[a] Time elapsed between injection of the drug and the beginning of the irradiation.

as is well known for WR-2721. IC_{50} of compounds **8(a–c)** were not determined because of lack of solubility.

3.2. In vivo evaluation

In vitro studies show that compounds **4(a–e), 5(a,b,d)**, 6(**c,d,e**) **and 8d** exhibit an antioxidant effect. Compounds **6(c,d,e)** are low radical quenchers and this function usually not give good radioprotection compared to thiols or phosphorothioates. Another test on irradiated plasmids[27] seems to show compounds **4(a–e)** and **5d** (total DNA protection for concentration lower than 200 μM) and, in a merest way **5a** (more than 50% of DNA protection for concentration lower than 200 μM), as very good candidates for in vivo studies. So, in order to complete the study of these compounds, we tested the toxicity and the radioprotective effectiveness of these compounds in vivo on two strains of mice.

In a preliminary study, we determined lethal doses for gamma irradiations (^{60}Co, 300 mGy min^{-1}) on 6 weeks old male mice: CD1 mice and B6D2F1 mice (Janvier, France). $LD_{50/30days\text{-}IRR}$ and $LD_{99.9/30days\text{-}IRR}$ defined as irradiation doses which kill, respectively 50% and 99.9% of the animals 30 days after exposure, were determined by probit analysis. For these determinations, 20 mice of each strain and for each irradiation dose were placed in a Plexiglas box divided into 30 individual cells in an homogeneous field 28.5 cm × 28.5 cm in size, and exposed at different doses from 6.5 to 10 Gy by 0.5 Gy step. Dosimetry was checked with an ionization chamber dosimeter (Dosimentor PTW, USA). In these conditions, $LD_{50/30days\text{-}IRR}$ and $LD_{99.9/30days\text{-}IRR}$ on CD1 mice were estimated at 6.8 Gy and 8.2 Gy, respectively. The irradiation dose on B6D2F1 mice was 7.4 and 8.8 Gy, respectively.

In a second experiment, for each of the tested molecules, the acute (48 h) toxicity was evaluated in male CD1 mice. The molecules were injected intraperitoneally at different concentrations to determine the $LD_{50tox/48H}$, defined as the concentration which kills 50% of the animals at 48 h.

Then, compounds were injected intra peritoneally to 10 CD1 mice at the maximum tolerated dose (defined as $1/2LD_{50tox/48H}$) 90 min before radiation exposure at doses $LD_{99.9/30days\text{-}IRR}$ (8.2 Gy) and $LD_{99.9/30days\text{-}IRR}$ + 2 Gy (10.2 Gy). Survival rate was determined 30 days after exposure. WR 2721 (at 1/2 $LD_{50tox/48H}$) was used as a reference. To confirm results obtained in CD1 mice, compounds **4(a–b)**, and WR-2721 were tested in another experimental model: they were administered 15 min before irradiation (instead of 90 min) in B6D2F1 mice exposed at doses of 8.8 Gy or 10.8 Gy.

The results are reported in Table 3.

The first comment is that our tested compounds are non toxic (in most case, LD_{50} cannot be determined because of lack of solubility) which indicates the possibility of development of radioprotective drugs to prevent accidental irradiation.

Considering thiol compounds **4(a,b,d,e)**, this study reveals that benzothiazole derivatives are better radioprotectors than thiadiazole derivatives even if thiadiazole **4d** presents the best antioxidant activity measured in vitro. What's more, the tendency observed during the antioxidant screening in which thiadiazoles are better than benzothiazoles is not observed in vivo. In fact, here, that benzothiazoles are in a global way better than thiadiazoles may be due to better vectorisation of the drug.

Comparing thiol and aminothiol derivatives of benzothiazole, in vitro tests show that thiol derivative **4a** ($IC_{50\ DPPH}$ = 92 μM) is a better antioxidant compound than aminothiol derivative **5a** ($IC_{50\ DPPH}$ = 1390 μM) but these two compounds present a similar in vivo radioprotective effect in CD1 mice. Note that compound **4a** is effective even at $1/4LD_{50tox/48H}$.

Moreover, the thiol derivative **4b** presents a very interesting radioprotective effect in mice exposed to $LD_{99.9/30days\text{-}IRR}$ with a survival rate of 80% in CD1 mice treated 90 min before irradiation and 100% in B6D2F1 treated 15 min before exposure. This good radioprotective effect has been suggested by results of the in vitro DPPH and ABTS studies (IC_{50} = 92 μM and 77 μM, respectively) or plasmide test.[27] However no compound has a radioprotective efficacy at $LD_{99.9/30days\text{-}IRR}$ + 2 Gy whatever the mice strain and the delay of administration.

4. Conclusion

In conclusion, we have synthesized new derivatives from aminobenzothiazole and thiadiazole. Results of the antioxidant activity evaluation have demonstrated that only thiols, thiosulfonic acids and phosphorothioates exhibit evident antioxidant activity. The antioxidant properties have led us to expect that these compounds could be potent radioprotective agents in comparison with WR-2721.

In vivo tests showed an efficient radioprotective effect at $LD_{99.9/30days\text{-}IRR}$ for the compounds **4a** and **5a** and particularly for 2-mercapto-6-methylbenzothiazole **4b** compared to WR-2721.

So, the new hypothesis of a direct link between the thiol function for catching the radical and the aromatic ring for the trapping of this radical, gives very encouraging results for radioprotection.

Modifications of these structures are in progress in order to increase the radioprotective effect of benzothiazole derivatives.

5. Experimental

Most reagents were purchased and all solvents were freshly distilled from sodium benzophenone or P_2O_5 before use. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AC 200 and 300 NMR spectrometers operating at 200.1, 300.1 MHz and 75.4 MHz (chemical shifts in ppm relative to TMS as internal standard), ^{31}P NMR spectra were recorded on an Bruker AC 300 spectrometer operating at 121.5 MHz (chemical shifts in ppm relative to phosphorothioic acid as internal standard). Mass spectra under electron impact (EI) conditions were obtained on a Nermag R10-10H spectrometer. Mass spectra under electrospray (ESI) and fast atom bombardment (FAB) conditions were obtained on Qtrap and API365 (Applied Biosystems). IR spectra were recorded on Perkin–Elmer 1760 FT-IR. Melting points were taken uncorrected on a Leitz Biomed hot-plate microscope apparatus or, in capillary tubes, on a digital electrothermal apparatus. Elemental analyses (C, H and N) were performed at the « Ecole Nationale Supérieure de Chimie », Toulouse, France.

5.1. Syntheses of the amides derivatives 2(a–e)

5.1.1. *N*-Benzothiazol-2-yl-propylamide (2a)

To a solution of 2-aminobenzothiazole (1.54 g, 10.26 mmol) and freshly distilled triethylamine (2.02 g, 20 mmol) in tetrahydrofurane (50 ml) was added a solution of 1.85 g of propionyl chloride (20 mmol) in THF (20 ml). The reaction mixture was stirred at room temperature for 3 h. The solvent was evaporated under reduced pressure, afforded a solid residue which was washed with water (30 ml) and hexane (30 ml). The solid obtained was crystallized in methanol to give compound **2a** (0.74, 36%). Mp: 198–190 °C. ^1H NMR (DMSO-d_6, 300.1 MHz): 1.12 (3H, t, $^3J_{H-H}$ = 9.0 Hz, CH_2CH_3), 2.50 (2H, q, $^3J_{H-H}$ = 9.0 Hz, CH_2CH_3), 7.27–7.98 (4H, m, C_6H_4). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 9.42 (CH_2CH_3), 28.93 (CH_2CH_3), 120.90 (C-aryl), 122.13 (C-aryl), 123.87 (C-aryl), 126.51 (C-aryl), 131.90 (C-aryl), 149.04 (C-aryl), 158.44 (C–NH), 173.47 (C=O). Mass spectrum: m/z = 206 $[M-56]^+$. Anal. Calcd for $C_{10}H_{10}N_2OS$: C, 58.23; H, 4.89; N, 13.58. Found: C, 58.02; H, 4.64; N, 14.04%.

Compounds **2**(**b**–**e**) was also prepared using the general procedure described for **2a**.

5.1.2. *N*-(6-Methylbenzothiazol-2-yl)-propylamide (2b)

Yield 52%. Mp: 221–222 °C. ^{1}H NMR (DMSO-d_6, 300.1 MHz): 1.11 (3H, t, $^3J_{H-H}$ = 9.0 Hz, CH_2CH_3), 2.40 (3H, q, $^3J_{H-H}$ = 9.0 Hz, CH_2CH_3 and CH_3), 7.22–7.74 (3H, m, C_6H_3), 12.21 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 9.46 (CH_2CH_3), 21.45 (CH_3), 28.90 (CH_2CH_3), 120.55 (C-aryl), 121.74 (C-aryl), 127.82 (C-aryl), 132.05 (C-aryl), 133.30 (C-aryl), 147.01 (C-aryl), 157.57 (C–NH), 173.32 (C=O) ppm. Mass spectrum: m/z = 220 $[M-56]^+$. Anal. Calcd for $C_{11}H_{12}N_2OS$: C, 59.97; H, 5.49; N, 12.72. Found: C, 59.59; H, 5.15; N, 12.56.

5.1.3. *N*-(6-Ethoxybenzothiazol-2-yl)-propylamide (2c)

Yield 53%. Mp: 206–208 °C. ^{1}H NMR (DMSO-d_6, 300.1 MHz): 1.11 (3H, t, $^3J_{H-H}$ = 9.0 Hz, –(CO)CH_2CH_3), 1.34 (3H, t, J = 9.0 Hz, –O–CH_2CH_3), 2.45 (2H, q, $^3J_{H-H}$ = 9.0 Hz, –(CO)CH_2CH_3), 4.06 (2H, q, $^3J_{H-H}$ = 9.0 Hz, –O–CH_2CH_3), 6.98–7.61 (3H, m, C_6H_3), 12.14 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 9.47 [(C=O)CH_2CH_3], 15.18 (OCH_2CH_3), 28.85 [(C=O)CH_2CH_3], 64.05 (OCH_2CH_3), 105.82 (C-aryl), 115.62 (C-aryl), 121.47 (C-aryl), 133.18 (C-aryl), 143.05 (C-aryl), 155.74 (C-aryl), 156.40 (C–NH), 173.15 (C=O). Mass spectrum: m/z = 250 $[M-56]^+$. Anal. Calcd for $C_{12}H_{14}N_2O_2S$: C, 57.58; H, 5.64; N, 11.19. Found: C, 57.31; H, 5.16; N, 11.07.

5.1.4. *N*-[5-(Ethylthio)-1,3,4-thiadiazol-2-yl]-propylamide (2d)

Yield 96%. Mp: 163–165 °C. ^{1}H NMR (DMSO-d_6, 300.1 MHz): 1.09 (3H, t, $^3J_{H-H}$ = 9.0 Hz, –(O=C)CH_2CH_3), 1.33 (3H, t, $^3J_{H-H}$ = 9.0 Hz, –SCH_2CH_3), 2.47 (2H, q, $^3J_{H-H}$ = 9.0 Hz, –SCH_2CH_3), 3.21 (2H, q, $^3J_{H-H}$ = 9.0 Hz, –(O=C)CH_2CH_3), 12.53 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 9.42 [(C=O)CH_2CH_3], 15.22 (SCH_2CH_3), 28.52 [(C=O)CH_2CH_3], 28.57 (SCH_2CH_3), 158.76 (C_5), 159.08 (C_2), 172.79 (C=O). Mass spectrum: m/z = 217 $[M-56]^+$. Anal. Calcd for $C_7H_{11}N_3OS_2$: C, 38.69; H, 5.10; N, 19.34. Found: C, 38.70; H, 4.91; N, 19.16.

5.1.5. *N*-(5-Ethyl-1,3,4-thiadiazol-2-yl)propylamide (2e)

Yield 96%. Mp: 224–226 °C. ^{1}H NMR (DMSO-d_6, 300.1 MHz): 1.09 (3H, t, $^3J_{H-H}$ = 9.0 Hz, (–(C=O)CH_2CH_3), 1.29 (3H, t, $^3J_{H-H}$ = 9.0 Hz, CH_3CH_2), 2.47 (2H, q, $^3J_{H-H}$ = 9.0 Hz, CH_3CH_2), 2.97 (2H, q, $^3J_{H-H}$ = 9.0 Hz, –(C=O)CH_2CH_3), 12.31 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 9.53 [(C=O)CH_2CH_3], 14.29 (CH_2CH_3), 23.10 [(C=O)CH_2CH_3], 28.66 (CH_2CH_3), 158.59 (C_5), 165.64 (C_2), 172.54 (C=O). Mass spectrum: m/z = 185 $[M-56]^+$. Anal. Calcd for $C_7H_{11}N_3OS$: C, 45.39; H, 5.99; N, 22.68. Found: C, 45.10; H, 5.48; N, 22.32.

5.2. Syntheses of the thiols and aminothiols derivatives 4(a–e) and 5(a–e)

5.2.1. 2-Chlorobenzothiazole (3a)

To a stirred solution of hydrogen chloride (105 ml, 1.06 mol) with copper powder (1.70 g, 26.75 mmol) in 45 ml of water, a mixture of 2-aminobenzothiazole (5 g, 33.3 mmol) and an excess of $NaNO_2$ (6.83 g, 100 mmol), was added slowly at −5 °C. The reaction mixture was stirred for 2 h at room temperature and heated at 55 °C until the evolution of gas ceased. The reaction mixture was extracted by $CHCl_3$ (3 × 200 ml), the combined organic extracts were washed with sulfuric acid (10%, 100 ml), dried over Na_2SO_4, filtered and concentrated in vacuo. Compound **3a** is a red oil (5.15 g, 94%). ^{1}H NMR ($CDCl_3$, 300.1 MHz): 7.34–7.95 (4H, m, C_6H_4). ^{13}C NMR ($CDCl_3$, 75.4 MHz): 121.84 (C-aryl), 123.55 (C-aryl), 126.50 (C-aryl), 127.42 (C-aryl), 150.71 (C-aryl), 153.56 (C-aryl), 172.75 (C–Cl). Mass spectrum: m/z = 169 $[M]^{+\cdot}$. Anal. Calcd for C_7H_4ClNS: C, 49.56; H, 2.38; N, 8.26. Found: C, 49.08; H, 2.11; N, 9.01.

General procedure described for **3a** was used for the synthesis of compounds **3(b–e)**.

5.2.2. 2-Chloro-6-methylbenzothiazole (3b)

Yield 53%. Mp: 50–52 °C. ^{1}H NMR ($CDCl_3$, 300.1 MHz): 2.32 (3H, s, CH_3), 7.01–7.81 (4H, m, C_6H_3). ^{13}C NMR ($CDCl_3$, 75.4 MHz): 21.53 (CH_3), 120.82 (C-aryl), 122.34 (C-aryl), 128.19 (C-aryl), 136.02 (C-aryl), 149.03 (C-aryl), 152.04 (C-aryl), 172.04 (C–Cl). Mass spectrum: m/z = 184 $[M+1]^+$. Anal. Calcd for C_8H_6ClNS: C, 52.32; H, 3.29; N, 7.63. Found: C, 52.04; H, 3.34; N, 7.58.

5.2.3. 2-Chloro-6-ethoxybenzothiazole (3c)

Yield 64%. Mp: 54–56 °C. ^{1}H NMR ($CDCl_3$, 300.1 MHz): 1.35 (3H, t, $^3J_{H-H}$ = 6.0 Hz, CH_3), 4.07 (2H, q, $^3J_{H-H}$ = 6.0 Hz, OCH_2), 7.09–7.85 (3H, m, C_6H_3). ^{13}C NMR ($CDCl_3$, 75.4 MHz): 15.00 (CH_3), 64.23 (CH_2), 106.05 (C-aryl), 116.78 (C-aryl), 123.43 (C-aryl), 137.57 (C-aryl), 145.04 (C-aryl), 149.73 (C-aryl), 157.37 (C–Cl). Mass spectrum: m/z = 214 $[M+1]^+$. Anal. Calcd for C_9H_8ClNOS: C, 50.59; H, 3.77; N, 6.55. Found: C, 49.17; H, 3.76; N, 6.80.

5.2.4. 2-Chloro-5-(ethylthio)-1,3,4-thiadiazole (3d)

Yield 56%. ^{1}H NMR ($CDCl_3$, 300.1 MHz): 1.37 (3H, t, $^3J_{H-H}$ = 6.0 Hz, CH_3), 3.24 (2H, q, $^3J_{H-H}$ = 6.0 Hz, OCH_2). ^{13}C NMR ($CDCl_3$, 75.4 MHz): 14.44 (CH_3), 28.38 (CH_2CH_3), 152.55 (C_5), 168.15 (C_2). Mass spectrum: 181 $[M+1]^+$. Anal. Calcd for $C_4H_5ClN_2S_2$: C, 26.59; H, 2.79; N, 15.50. Found: C, 26.44; H, 2.57; N, 16.61.

5.2.5. 2-Chloro-5-ethyl-1,3,4-thiadiazole (3e)

Yield 79%. ^{1}H NMR ($CDCl_3$, 300.1 MHz): 1.31 (3H, t, $^3J_{H-H}$ = 6.0 Hz, CH_3), 3.00 (2H, q, $^3J_{H-H}$ = 6.0 Hz, OCH_2). ^{13}C NMR ($CDCl_3$, 75.4 MHz): 14.32 (CH_3), 24.35 (CH_2CH_3), 153.85 (C_5), 175.05 (C_2). Mass spectrum: m/z = 149 $[M+1]^+$. Anal. Calcd for $C_4H_5ClN_2S$: C, 32.33; H, 3.39; N, 18.85. Found: C, 32.56; H, 3.45; N, 18.56.

5.2.6. 2-Mercaptobenzothiazole (4a)

A mixture of **3a** (2.50 g, 14.79 mmol) and excess thiourea (3.03 g, 47.33 mmol) in 36 ml of ethanol was refluxed for 3 h. The mixture was cooled down to room temperature and a solution of HCl 37% (5 ml) in 50 ml of water was added dropwise under stirring, the solid formed was isolated by filtration and the aqueous layer was extracted by $CHCl_3$ (2 × 100 ml). The combined organic layers were dried on Na_2SO_4. The solvent was removed in vacuo. Recrystallization from ethanol gave **4a** as a white solid (0.70 g, 28%). Mp 170–172 °C. IR (KBr): ν_{SH} = 2664 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300.1 MHz): 3.49 (1H, s, SH), 7.15–7.48 (4H, m, C_6H_4). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 112.35 (C-aryl), 121.37 (C-aryl), 124.70 (C-aryl), 127.23 (C-aryl), 130.02 (C-aryl), 140.33 (C-aryl), 190.92 (C-SH). Mass spectrum: m/z = 167 $[M]^{+\cdot}$. Anal. Calcd for $C_7H_5NS_2$: C, 50.27; H, 3.01; N, 8.37. Found: C, 50.55; H, 2.82; N, 8.86.

Compound **4(b-e**) was obtained by using the same procedure described for **4a.**

5.2.7. 2-Mercapto-6-methylbenzothiazole (4b)

Yield 87%. Mp: 150–152 °C. IR (KBr): ν_{SH} = 2509 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300.1 MHz): 2.33 (3H, s, CH_3), 3.59 (1H, s, SH), 7.16–8.05 (4H, m, C_6H_3). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 21.19 (CH_3), 112.68 (C-aryl), 123.74 (C-aryl), 128.55 (C-aryl), 130.56 (C-aryl), 134.46 (C-aryl), 151.73 (C-aryl), 184.33 (C-SH). Mass spectrum: m/z = 182 $[M+1]^+$. Anal. Calcd for $C_8H_7NS_2$: C, 53.00; H, 3.89; N, 7.73. Found: C, 52.60; H, 4.03; N, 7.99.

5.2.8. 2-Mercapto-6-ethoxybenzothiazole (4c)

Yield 63%. Mp: 180–182 °C. IR (KBr): ν_{SH} = 2509 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300.1 MHz): 1.32 (3H, t, $^3J_{H-H}$ = 6.0 Hz, CH_3), 3.56 (1H, s, SH), 4.0 (2H, q, $^3J_{H-H}$ = 6.0 Hz, CH_2), 6.94–7.31 (4H, m, C_6H_4). ^{13}C NMR (DMSO-d_6, 75.4 MHz): δ = 15.05 (CH_3), 64.19 (CH_2), 106.93 (C-aryl), 113.55 (C-aryl), 115.63 (C-aryl), 131.14 (C-aryl),

135.58 (C-aryl), 156.31 (C-aryl), 188.75 (C-SH). Mass spectrum: *m/z* = 212 $[M+1]^+$. Anal. Calcd for $C_9H_9NOS_2$: C, 51.16; H, 4.29; N, 6.63. Found: C, 51.03; H, 3.85; N, 7.20.

5.2.9. 2-Mercapto-5-(ethylthio)-1,3,4-thiadiazole (4d)

Yield 73%. Mp: 102–104 °C. IR (KBr): ν_{SH} = 2528 cm^{-1}. ^1H NMR (DMSO-d_6, 300.1 MHz): 1.31 (3H, t, $^3J_{H-H}$ = 9.0 Hz, CH_3), 3.16 (2H, q, $^3J_{H-H}$ = 9.0 Hz, CH_2), 7.03 (1H, s, SH). ^{13}C NMR (DMSO-d_6, 75.4 MHz,): 14.94 (CH_3), 28.17 (CH_2), 162.13 (C_5), 188.32 (C_2). Mass spectrum: *m/z* = 179 $[M+1]^+$. Anal. Calcd for $C_4H_6N_2S_3$: C, 26.95; H, 3.39; N, 15.71. Found: C, 25.73; H, 3.56; N, 16.77.

5.2.10. 2-Mercapto-5-ethyl-1,3,4-thiadiazole (4e)

Yield 34%. Mp: 68–70 °C. IR (KBr): ν_{SH} = 2523 cm^{-1}. ^1H NMR (DMSO-d_6, 300.1 MHz): 1.19 (3H, t, $^3J_{H-H}$ = 9.0 Hz, CH_3), 2.00 (2H, q, $^3J_{H-H}$ = 9.0 Hz, CH_2), 7.03 (1H, s, SH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 12.69 (CH_3), 24.40 (CH_2), 165.98 (C_5), 189.35 (C_2). Mass spectrum: *m/z* = 146 $[M+1]^+$. Anal. Calcd for $C_4H_6N_2S_2$: C, 32.85; H, 4.14; N, 19.16. Found: C, 32.59; H, 4.04; N, 19.52.

5.2.11. 2-(Benzothiazol-2-ylamino)ethanethiol hydrochloride (5a)

Solution of cysteamine (0.7 g, 9.00 mmol) in THF (15 ml) was added to a stirred suspension of **3a** (1.5 g, 8.87 mmol) in THF (30 ml). After heating under argon for 3 h, the solvent was evaporated in vacuo and the yellow solid residue was washed with THF/pentane (1/3; 30 ml). Drying in vacuo afforded pure **5a** (0.65 g, 35%). Mp: 174–176 °C. IR (KBr): ν_{SH} = 2446 cm^{-1}, ν_{NH} = 3444 cm^{-1}. ^1H NMR (DMSO-d_6, 300.1 MHz): 3.18 (2H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2SH), 3.36 (1H, s, SH) 3.58 (2H, t, $^3J_{H-H}$ = 6.0 Hz, $NHCH_2$), 7.29–7.98 (4H, m, C_6H_4), 8.49 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 29.46 ($NHCH_2$), 38.84 (CH_2SH), 120.9 (C-aryl), 125.09 (C-aryl), 125.75 (C-aryl), 134.58 (C-aryl), 152.22 (C-aryl), 164.54 (C–NH). Mass spectrum: *m/z* = 211 $[M+1]^+$. Anal. Calcd for $C_9H_{11}ClN_2S_2$: C, 43.80; H, 4.49; N, 11.35. Found: C, 43.67; H, 4.75; N, 11.22.

Using the same procedure described for **5a**, compounds **5(b–e)** were obtained.

5.2.12. 2-(6-Methylbenzothiazol-2-ylamino)ethanethiol hydrochloride (5b)

Yield 16%. mp 170–172 °C. IR (KBr): ν_{SH} = 2580 cm^{-1}, ν_{NH} = 3434 cm^{-1}. ^1H NMR (DMSO-d_6, 300.1 MHz): 2.43 (3H, s, CH_3), 3.26 (2H, t, $^3J_{H-H}$ = 6.0 Hz, $NHCH_2$), 3.36 (1H, s, SH), 3.64 (2H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2SH), 7.30–7.83 (4H, m, C_6H_3), 8.47 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 21.40 (CH_3), 30.36 ($NHCH_2$), 38.64 (CH_2SH), 121.25 (C-aryl), 121.98 (C-aryl), 128.24 (C-aryl), 134.88 (C-aryl), 135.32 (C-aryl), 151.12 (C-aryl), 164.35 (C–NH). Mass spectrum: *m/z* = 225 $[M+1]^+$. Anal. Calcd for $C_{10}H^{13}ClN_2S_2$: C, 46.05; H, 5.02; N, 10.74. Found: C, 45.90; H, 4.95; N, 10.96.

5.2.13. 2-(6-Ethoxybenzothiazol-2-ylamino)ethanethiol hydrochloride (5c)

Yield 33%. ^1H NMR (DMSO-d_6, 300.1 MHz): 1.34 (3H, t, CH_3), 3.19 (2H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2SH), 3.34 (1H, s, SH), 3.56 (2H, t, $^3J_{H-H}$ = 6.0 Hz, $NHCH_2$), 4.05 (2H, q, CH_3CH_2), 7.02–7.84 (4H, m, C_6H_3), 8.42 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 15.07 (CH_3), 31.24 ($NHCH_2$), 38.44 (CH_2SH), 64.18 (CH_3CH_2), 106.01 (C-aryl), 115.98 (C-aryl), 122.17 (C-aryl), 136.66 (C-aryl), 147.38 (C-aryl), 156.52 (C-aryl), 162.36 (C_2–NH). Mass spectrum: *m/z* = 225 $[M+1]^+$. Anal. Calcd for $C_{11}H_{15}ClN_2OS_2$: C, 45.43; H, 5.20; N, 9.63. Found: C, 45.29; H, 5.30; N, 9.67.

5.2.14. 2-[5-(Ethylthio)-1,3,4-thiadiazol-2-ylamino]ethanethiol hydrochloride (5d)

Yield 16%. IR. (KBr): ν_{SH} = 2539 cm^{-1}, ν_{NH} = 3375 cm^{-1}. ^1H NMR (DMSO-d_6, 300.1 MHz): 1.38 (3H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2SH), 3.19 (2H, t, $^3J_{H-H}$ = 6.0 Hz, $NHCH_2$), 3.29 (2H, q, $^3J_{H-H}$ = 6.0 Hz, CH_2CH_3), 3.36 (1H, s, SH), 3.55 (2H, t, $^3J_{H-H}$ = 6.0 Hz, $NHCH_2$), 8.32 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 14.97 (CH_3), 31.21 (CH_2S), 38.55 ($NHCH_2$), 39.07 (CH_2SH), 164.10 (C_5), 166.23 (C_2). Mass spectrum: *m/z* = 222 $[M+1]^+$. Anal. Calcd for $C_6H_{12}ClN_3S_3$: C, 27.95; H, 4.69; N, 16.30. Found: C, 27.78; H, 4.75; N, 16.41.

5.2.15. 2-[5-(Ethyl)-1,3,4-thiadiazol-2-ylamino]ethanethiol hydrochloride (5e)

Yield 44%. IR. (KBr): ν_{SH} = 2036 cm^{-1}. ν_{NH} = 3372 cm^{-1}. ^1H NMR (DMSO-d_6, 300.1 MHz): 1.37 (3H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2SH), 3.06 (2H, t, $^3J_{H-H}$ = 6.0 Hz, $NHCH_2$), 3.43 (2H, q, $^3J_{H-H}$ = 6.0 Hz, CH_2CH_3), 3.61 (1H, s, SH), 3.80 (2H, t, $^3J_{H-H}$ = 6.0 Hz, $NHCH_2$), 8.62 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 13.99 (CH_3), 23.82 (CH_2S), 32.92 ($NHCH_2$), 39.78 ($NHCH_2$), 164.29 (C_5), 172.78 (C_2). Mass spectrum: *m/z* = 188 $[M+1]^+$. Anal. Calcd for $C_6H_{12}ClN_3S_2$: C, 31.92; H, 5.36; N, 18.61. Found: C, 31.68; H, 5.55; N, 18.66.

5.3. Syntheses of the thiosulfonic acids 8(a–e) and phosphorothioates derivatives 9(d–e)

5.3.1. 2-Iminobenzothiazol-3-yl-ethanol hydrobromide (6a)

A mixture of 2-aminobenzothiazole (10.19 g, 65.89 mmol) and 2-bromoethanol (45.76 g, 366.16 mmol) was heated under reflux for 3 h. After one more hour stirring at room temperature 200 ml of diethyl ether were added. The solid formed was isolated by filtration, washed with 50 ml of acetone and 100 ml of diethyl ether. Recrystallization in ethanol gave compound **6a** as a white solid (11.47 g, 90%). Mp: 200–202 °C (dec). ^1H NMR (DMSO-d_6, 300.1 MHz): 3.79 (2H, t, $^3J_{H-H}$ = 6.0 Hz, NCH_2), 4.41 (2H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2OH), 4.71 (1H, s, OH), 7.23–8.03 (4H, m, C_4H_6), 10.13 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 48.13 ($NHCH_2$), 58.41 (CH_2-OH), 114.18 (C-aryl), 122.74 (C-aryl), 123.54 (C-aryl), 125.40 (C-aryl), 127.86 (C-aryl), 139.12 (C-aryl), 168.86 (C=NH). Mass spectrum: *m/z* = 195 $[M+1]^+$. Anal. Calcd for $C_9H_{11}BrN_2OS$: C, 39.28; H, 4.03; N, 10.18. Found: C, 39.43; H, 4.10; N, 9.96.

Using the same operating conditions described for **6a** compounds **6(b–e)** were obtained.

5.3.2. (2-Imino-6-methylbenzothiazol-3-yl)ethanol hydrobromide (6b)

Yield 76%. Mp: 232–234 °C (dec). ^1H NMR (DMSO-d_6, 300.1 MHz,): 2.39 (3H, s, CH_3), 3.77 (2H, t, $^3J_{H-H}$ = 6.0 Hz, $NHCH_2$), 4.39 (2H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2OH), 4.69 (1H, s, OH), 7.24–7.81 (3H, m, C_6H_3), 10.07 (1H, s, NH). ^{13}C NMR (75.4 MHz, DMSO-d_6): 21.16 (CH_3), 48.14 (NCH_2), 58.42 (CH_2OH), 113.89 (C-aryl), 122.67 (C-aryl), 123.33 (C-aryl), 128.74 (C-aryl), 135.22(C-aryl), 137.00 (C-aryl), 168.18 (C=NH). Mass spectrum: *m/z* = 209 $[M+1]^+$. Anal. Calcd for $C_{10}H_{13}BrN_2OS$: C, 41.53; H, 4.53; N, 9.69. Found: C, 41.22; H, 4.74; N, 9.79.

5.3.3. (2-Imino-6-ethoxybenzothiazol-3-yl)ethanol hydrobromide (6c)

Yield 86%. Mp: 225–227 °C (dec). ^1H NMR (DMSO-d_6, 300.1 MHz): 1.35 (3H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2CH_3), 3.76 (2H, t, $^3J_{H-H}$ = 6.00 Hz, $NHCH_2$), 4.06 (2H, q, $^3J_{H-H}$ = 6.0 Hz, CH_2CH_3), 4.37 (2H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2OH), 4.91 (1H, s, OH), 7.07–7.67 (3H, m, C_6H_3), 9.98 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 15.01 (CH_3), 48.15 ($NHCH_2$), 62.01 (CH_2OH), 64.40 (CH_2–O), 108.89 (C-aryl), 115.14 (C-aryl), 115.47 (C-aryl), 123.98 (C-aryl), 132.99 (C-aryl), 156.52 (C-aryl), 167.79 (C=NH). Mass spectrum: *m/z* = 239 $[M+1]^+$. Anal. Calcd for $C_{11}H_{15}BrN_2O_2S$: C, 41.39; H, 4.74; N, 8.78. Found: C, 41.19; H, 4.80; N, 8.92.

5.3.4. (2-Imino-5-ethylthio-1,3,4-thiadiazol-3-yl)ethanol hydrobromide (6d)

Yield 59%. Mp: 136–138 °C (dec). ^{1}H NMR (DMSO-d_6, 300.1 MHz,): 1.36 (3H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2CH_3), 3.22 (2H, q, $^3J_{H-H}$ = 6.0 Hz, CH_2CH_3), 3.77 (2H, t, $^3J_{H-H}$ = 6.0 Hz, $NHCH_2$), 4.25 (2H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2OH), 4.90 (1H, s, OH), 9.96 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 14.78 (CH_3), 28.39 (CH_2CH_3), 53.44 ($NHCH_2$), 58.47 (CH_2OH), 154.11 (C_5), 167.97 (C_2). Mass spectrum: m/z = 206 $[M+1]^+$. Anal. Calcd for $C_6H_{12}BrN_3OS_2$: C, 25.18; H, 4.23; N, 14.68. Found: C, 25.25; H, 4.12; N, 14.72.

5.3.5. (2-Imino-5-ethyl-1,3,4-thiadiazol-2-yl)ethanol hydrobromide (6e)

Yield 56%. Mp: 150–152 °C (dec). ^{1}H NMR (DMSO-d_6, 300.1 MHz): 1.22 (3H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2CH_3), 2.90 (2H, q, $^3J_{H-H}$ = 6.0 Hz, CH_2CH_3), 3.74 (2H, t, $^3J_{H-H}$ = 6.0 Hz, $NHCH_2$), 4.23 (2H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2OH), 5.45 (1H, s, OH), 9.81 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 12.80 (CH_2CH_3), 23.86 (CH_2CH_3), 53.00 ($NHCH_2$), 58.47 (CH_2OH), 122.67 (C_5), 159.58 (C_2). Mass spectrum: m/z = 174 $[M+1]^+$. Anal. Calcd for $C_6H_{12}BrN_3OS$: C, 28.36; H, 4.76; N, 16.53. Found: C, 28.42; H, 4.55; N, 16.68.

5.3.6. 3-Bromoethylbenzothiazole-2-imine hydrobromide (7a)

Thionyl bromide (1.2 ml, 15.45 mmol) was added to a stirred suspension of **6a** (1 g, 5.15 mmol) in 80 ml of anhydrous toluene. The reaction mixture was heated under reflux until the evolution of gas ceased. After cooling down to room temperature, a solid was isolated by filtration and washed with 50 ml of acetone. The product was recrystallized from methanol and provided (0.96 g, 72%) of **7a**. Mp: 205–207 °C (dec). ^{1}H NMR (DMSO-d_6, 300.1 MHz): 3.87 (2H, t, $^3J_{H-H}$ = 6.0 Hz, $NHCH_2$), 4.78 (2H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2Br), 7.11–8.05 (4H, m, C_6H_4), 10.41 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 28.63 (CH_2Br), 46.00 ($NHCH_2$), 114.20 (C-aryl), 122.60 (C-aryl), 124.13 (C-aryl), 125.80 (C-aryl), 128.22 (C-aryl), 138.42 (C-aryl), 168.91 (C=NH). Mass spectrum: m/z = 259 $[M+1]^+$. Anal. Calcd for $C_9H_{10}Br_2N_2S$: C, 31.98; H, 2.98; N, 8.29. Found: C, 31.79; H, 3.04; N, 8.42.

5.3.7. 3-Bromoethyl-6-methylbenzothiazole-2-imine hydrobromide (7b)

Thionyl bromide (1.6 ml, 20.61 mmol) was added to a stirred suspension of **6a** (1.5 g, 7.21 mmol) in 80 ml of anhydrous acetonitrile. The solution was heated until the evolution of gas ceased. The reaction mixture was cooled down to room temperature and the solid formed was filtered and washed with 50 ml of diethyl ether. Recrystallization from methanol gave **7b** as a white solid (1.5 g, 80%). Mp: 219–221 °C (dec). ^{1}H NMR (DMSO-d_6, 300.1 MHz): 2.38 (3H, s, CH_3), 3.86 (2H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2Br), 4.77 (2H, t, $^3J_{H-H}$ = 6.0 Hz, $NHCH_2$), 7.34–7.82 (4H, m, C_6H_3), 10.37 (1H, s, NH). ^{13}C NMR (75.4 MHz, DMSO-d_6): 21.27 (CH_3), 28.71 (CH_2Br), 46.02 ($NHCH_2$), 113.91 (C-aryl), 123.88 (C-aryl), 125.09 (C-aryl), 129.00 (C-aryl), 135.52 (C-aryl), 136.31 (C-aryl), 168.49 (C=NH). Mass spectrum: m/z = 273 $[M]^{+\cdot}$. Anal. Calcd for $C_{10}H_{12}Br_2N_2S$: C, 34.11; H, 3.44; N, 7.96. Found: C, 34.46; H, 3.36; N, 7.69.

Using the same operating conditions described for **7b** compounds **7c–e** were obtained.

5.3.8. 3-Bromoethyl-6-ethoxybenzothiazole-2-imine hydrobromide (7c)

Yield 73%. Mp: 225–227 °C (dec). ^{1}H NMR (DMSO-d_6, 300.1 MHz): 1.34 (3H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2CH_3), 3.86 (2H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2Br), 4.05 (2H, q, $^3J_{H-H}$ = 6.0 Hz, CH_2CH_3), 4.74 (2H, t, $^3J_{H-H}$ = 6.0 Hz, $NHCH_2$), 7.67–7.09 (4H, m, C_6H_3), 10.22 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 15.01 (CH_3), 28.86 (CH_2Br), 46.06 ($NHCH_2$), 64.42 (CH_2CH_3), 109.11 (C-aryl), 114.99 (C-aryl), 115.61 (C-aryl), 123.79 (C-aryl), 132.06 (C-aryl), 156.70 (C-aryl), 168.10 (C=NH). Mass spectrum: m/z = 303 $[M+1]^+$. Anal. Calcd for $C_{11}H_{14}Br_2N_2OS$: C, 34.58; H, 3.69; N, 7.33. Found: C, 34.62; H, 3.87; N, 7.11.

5.3.9. 3-Bromoethyl-5-ethylthio-1,3,4-thiadiazole-2-imine hydrobromide (7d)

Yield 63%. Mp: 140–142 °C (dec). ^{1}H NMR (DMSO-d_6, 300.1 MHz): 1.35 (3H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2CH_3), 3.22 (2H, q, $^3J_{H-H}$ = 6.0 Hz, CH_2CH_3), 3.88 (2H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2Br), 4.61 (2H, t, $^3J_{H-H}$ = 6.0 Hz, $NHCH_2$), 10.18 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 14.72 (CH_3), 28.34 (CH_2S), 29.70 (CH_2Br), 51.63 (CH_2NH), 154.77 (C_5), 168.01 (C_2). Mass spectrum: m/z = 269 $[M]^{+\cdot}$. Anal. Calcd for $C_6H_{11}Br_2N_3S_2$: C, 20.64; H, 3.18; N, 12.04. Found: C, 20.52; H, 3.23; N, 12.11.

5.3.10. 3-Bromoethyl-5-ethyl-1,3,4-thiadiazole-2-imine hydrobromide (7e)

Yield 30%. Mp: 206–208 °C (dec). ^{1}H NMR (DMSO-d_6, 300.1 MHz): 1.23 (3H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2CH_3), 2.92 (2H, q, $^3J_{H-H}$ = 6.0 Hz, CH_2CH_3), 3.86 (2H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2Br), 4.60 (2H, t, $^3J_{H-H}$ = 6.0 Hz, $NHCH_2$), 10.11 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 12.76 (CH_3), 23.91 (CH_2CH_3), 29.74 (CH_2Br), 51.27 (CH_2NH), 160.14 (C_5), 168.01 (C_2). Mass spectrum: m/z = 237 $[M+1]^+$. Anal. Calcd for $C_6H_{11}Br_2N_3S$: C, 22.73; H, 3.50; N, 13.25. Found: C, 22.46; H, 3.71; N, 13.31.

5.3.11. S-2-(2-iminobenzothiazol-3-yl-ethyl)thiosulfonic acid hydrobromide (8a)

A solution of 3-bromoethyl- benzothiazole-2-imine (1.00 g, 3.89 mmol) in ethanol/water (20/10) was added dropwise to a stirred solution of sodium thiosulfate (1.02 g, 4.13 mmol) in water (42 ml). The mixture was heated under reflux for 1 h 30 min and than cooled down to 4 °C. The solid residue formed gave after filtration and recrystallization from ethanol/water **8a** (0.21 g, 19%). Mp: 230–232 °C (dec). ^{1}H NMR (DMSO-d_6, 300.1 MHz): 3.24 (2H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2S), 4.56 (2H, t, $^3J_{H-H}$ = 6.0 Hz, NCH_2), 7.34–7.94 (4H, m, C_6H_4), 10.01 (1H, s, NH). ^{13}C NMR (DMSO-d_6: 75.4 MHz): 31.39 (CH_2–S), 45.13 (CH_2–N), 113.99 (C-aryl), 122.74 (C-aryl), 123.96 (C-aryl), 125.74 (C-aryl), 128.36 (C-aryl), 138.87 (C-aryl), 168.97 (C=NH). Mass spectrum: m/z = 291 $[M+1]^+$. Anal. Calcd for $C_9H_{11}BrN_2O_3S_3$: C, 29.11; H, 2.99; N, 7.54. Found: C, 29.29; H, 3.05; N, 7.30.

Following the same procedure as the preceding preparation **8(b–e**) were obtained.

5.3.12. S-2-(2-Imino-6-methylbenzothiazol-3-yl)-ethyl thiosulfonic acid hydrobromide (8b)

Yield 27%. Mp: 233–235 °C (dec). ^{1}H NMR (DMSO-d_6, 300.1 MHz): 2.49 (3H, s, CH_3), 3.27 (2H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2S), 4.57 (2H, t, $^3J_{H-H}$ = 6.0 Hz, N–CH_2), 7.37–7.77 (3H, m, C_6H_3), 9.99 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 20.68 (CH_3), 30.98 (CH_2-S), 44.63 (CH_2-N), 113.24 (C_8-aryl), 123.25 (C_5-aryl), 124.51 (C-aryl), 128.72 (C-aryl), 135.04 (C-aryl), 136.29 (C-aryl), 168.10 (C=NH). Mass spectrum: m/z = 305 $[M+1]^+$. Anal. Calcd for $C_{10}H_{13}BrN_2O_3S_3$: C, 31.17; H, 3.40; N, 7.27. Found: C, 30.99; H, 3.59; N, 7.26.

5.3.13. S-2-(2-Imino-6-ethoxybenzothiazol-3-yl)-ethyl thiosulfonic acid hydrobromide (8c)

Yield 34%. Mp: 256–258 °C (dec). ^{1}H NMR (DMSO-d_6, 300.1 MHz): 1.36 (3H, t, $^3J_{H-H}$ = 6.0 Hz, CH_3), 3.27 (2H, t, $^3J_{H-H}$ = 6.0 Hz, CH_2S), 4.08 (2H, q, $^3J_{H-H}$ = 6.0 Hz, CH_2CH_3), 4.56 (2H, t, $^3J_{H-H}$ = 6.0 Hz, N–CH_2), 7.12–7.76 (3H, m, C_6H_3), 9.91 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 14.98 (CH_3), 31.46 (CH_2-S), 45.12 (CH_2-N), 64.43 (CH_2CH_3), 109.01 (C-aryl), 114.70 (C-aryl), 115.71 (C-aryl), 123.89 (C-aryl), 132.50 (C-aryl), 156.70 (C-aryl),

167.99 (C=NH). Mass spectrum: $m/z = 334$ $[M+1]^+$. Anal. Calcd for $C_{11}H_{17}BrN_2O_4S_3$: C, 31.81; H, 3.64; N, 6.74. Found: C, 31.70; H, 3.84; N, 6.65.

5.3.14. *S*-2-(2-Imino-5-ethylthio)-1,3,4-thiadiazol-ethyl thiosulfonic acid hydrobromide (8d)

Yield 11%. Mp: 180–182 °C (dec). ^{1}H NMR (DMSO-d_6, 300.1 MHz): 1.33 (3H, t, $^3J_{H-H} = 6.0$ Hz, CH_2CH_3), 3.17 (2H, q, $^3J_{H-H} = 6.0$ Hz, CH_2CH_3), 3.32 (2H, t, $^3J_{H-H} = 6.0$ Hz, CH_2-S), 4.44 (2H, t, $^3J_{H-H} = 6.0$ Hz, N–CH_2), 9.90 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 13.75 (CH_3), 28.59 (CH_2CH_3), 32.54 (CH_2-S), 49.81 (CH_2-N), 132.60 (C_5), 173.68 (C_2). Mass spectrum: $m/z = 300$ $[M-1]^+$. Anal. Calcd for $C_6H_{12}BrN_3O_3S_4$: C, 18.85; H, 3.16; N, 10.99. Found: C, 18.63; H, 3.32; N, 11.05.

5.3.15. *S*-2-(2-Imino-5-ethyl)-1,3,4-thiadiazol-3-yl-ethyl thiosulfonic acid hydrobromide (8e)

Yield 14%. Mp: 212–214 °C (dec). ^{1}H NMR (DMSO-d_6, 300.1 MHz): 1.23 (3H, t, $^3J_{H-H} = 6.0$ Hz, CH_2CH_3), 2.95 (2H, q, $^3J_{H-H} = 6.0$ Hz, CH_2CH_3), 3.07 (2H, t, $^3J_{H-H} = 6.0$ Hz, CH_2-S), 4.0 2 (2H, t, $^3J_{H-H} = 6.0$ Hz, N-CH_2), 9.90 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): 12.79 (CH_3), 24.10 (CH_2CH_3), 35.39 (CH_2-S), 49.29 (CH_2-N), 159.72 (C_5), 168.00 (C_2). Mass spectrum: $m/z = 174$ $[M+1]^+$. Anal. Calcd for $C_6H_{12}BrN_3O_3S_3$: C, 20.57; H, 3.45; N, 12.00. Found: C, 20.34; H, 3.65; N, 12.03.

5.3.16. *S*-2-(2-Imino-5-ethylthio)-1,3,4-thiadiazol-ethyl lithium hydrogen phosphorothioate (9d)

3-Bromoethyl-5-ethylthio-1,3,4-thiadiazol-2-imine **7d** (1.92 g, 5.50 mmol) was added in portions to a stirred solution of Li_3S-$PO_3{\cdot}5H_2O$ (0.99 g, 5.32 mmol) in water (24 ml). 12 ml of DMF were added and the solution was stirred at room temperature for 3 h. The solvent was removed in vacuo. The residual was washed in methanol and dried under reduced pressure to give **9d** (0.32 g, 20%). Mp: 250–252 °C (dec). ^{1}H NMR (D_2O, 300.1 MHz): 1.31 (3H, t, $^3J_{H-H} = 9.0$ Hz, CH_2CH_3), 3.00 (4H, m, CH_3CH_2–S and CH_2SP), 4.28 (2H, t, $^3J_{H-H} = 9.0$ Hz, N-CH_2), 7.80 (1H, s, NH). ^{13}C NMR (D_2O, 75.4 MHz): 11.78 (CH_3), 27.76 (CH_2CH_3), 28.54 (CH_2–S), 50.68 (CH_2–N), 158.73 (C_5), 168.45 (C_2). ^{31}P NMR (D_2O, 121.5 MHz): 15.80. Mass spectrum: $m/z = 308$ $[M+1]^+$. Anal. Calcd for $C_6H_{11}LiN_3O_3PS_3$: C, 23.45; H, 3.61. N, 13.67. Found: C, 23.06; H, 3.89; N, 13.78.

5.3.17. *S*-2-(2-Imino-5-ethyl)-1,3,4-thiadiazol-ethyl lithium hydrogen phosphorothioate (9e)

N-3-Bromoethyl-5-ethylthio-1,3,4-thiadiazol-2-imine **7e** (2.13 g, 6.72 mmol) was added in portions to a stirred solution of Li_3S-$PO_3{\cdot}5H_2O$ (1.20 g, 6.45 mmol) in water (30 ml). 15 ml of DMF were added and the solution was stirred at room temperature for 3 h. The solvents were removed in vacuo. The residual was washed in methanol (20 ml) and dried under reduced pressure to give **9e** (0.6 g, 32%). Mp: 250–252 °C (dec) ^{1}H NMR (D_2O, 300.1 MHz): 1.25 (3H, t, $^3J_{H-H} = 9.0$ Hz, CH_2CH_3), 2.81 (2H, q, $^3J_{H-H} = 9.0$ Hz, CH_2CH_3), 3.06 (2H, m, $^3J_{H-H} = 9.0$ Hz, CH_2-S), 4,32 (2H, t, $^3J_{H-H} = 9.0$ Hz, N-CH_2), 7.80 (1H, s, NH). ^{13}C NMR (D_2O, 75.4 MHz): 11.83 (CH_3), 23.74 (CH_2CH_3), 27.90 (CH_2–S), 49.59 (CH_2–N), 158.73 (C), 167.82 (C) ppm. ^{31}P NMR (D_2O, 121.5 MHz): 15.80. Mass spectrum: $m/z = 275$ $[M+1]^+$. Anal. Calcd for $C_6H_{11}LiN_3O_3PS_2$: C, 26.18; H, 4.03; N, 15.27. Found: C, 25.99; H, 4.43; N, 15.06.

5.4. In vitro antioxidant activity

5.4.1. DPPH test

First, the antiradical activity was measured as the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH·) free radical following the method described by Molineux (2003).[17] In its radical form, DPPH· has an absorption band at 516 nm which disappears upon reduction by an antiradical compound. The reaction mixture contained in 3 ml of ethanol, 80 μM DPPH· and test compounds at different concentrations. After 30 min at room temperature, the absorbance was recorded at 516 nm on an Agilent 8453. All experiments were carried out in triplicate. The percentage of remaining DPPH· (DPPH· $_{REM}$) was calculated as follows:

$$\%\mathrm{DPPH}^{\cdot}_{\mathrm{REM}} = [\mathrm{DPPH}^{\cdot}]_t/[\mathrm{DPPH}^{\cdot}]_0 \times 100$$

For all derivatives, IC_{50} that is, the concentration of test compound needed to reduce DPPH· absorption by 50% at 516 nm was determined.

5.4.2. ABTS test

Secondly, 2,2′-azinobis(3-ethylbenz-thiazoline-6-sulfonic acid) radical cation discoloration assay (ABTS test) was used. The $ABTS^{+\cdot}$ radical cation scavenging activity of thiols (**4–5**), thiosulfonic acids and phosphorothioates (**8–9**), and ascorbic acid was determined according to Re et al. (1999).[18] Briefly, to an aqueous solution of ABTS 7.0 mM, potassium persulfate 140 mM was added to obtain a final solution of 3.5 mM potassium persulfate. The mixture was kept overnight in the dark to yield the $ABTS^{+\cdot}$ radical cation. Prior to use in the assay, the $ABTS^{+\cdot}$ radical cation was diluted with ethanol to give an initial absorbance of 0.70 at 734 nm, at a controlled temperature of 30 °C. Free radical scavenging activity was assessed by mixing 1.5 ml diluted $ABTS^{+\cdot}$ radical cation with 10 μL of test compound at different concentrations and monitoring the change in absorbance at 0, 0.5, 1 min after the addition and again at 5 min intervals until a steady state was achieved. The antioxidant capacity of test compounds was expressed as IC_{50}, the concentration necessary to obtain 50% reduction of $ABTS^{+\cdot}$ radical cation.

5.5. In vivo radioprotective effectiveness

It is essential to establish certain criteria in order to evaluate radioprotective effectiveness and toxicity of compounds. For each of the molecules we tested in mice, first we evaluated its acute (48 h) toxicity in male six weeks old Swiss CD1 mouse (Janvier, France). The molecule was injected intraperitonally at different concentrations to determine the $LD_{50tox/48H}$, defined as the concentration which kills 50% of the animals at 48 h.

Then radioprotective effectiveness was determined in the same animal model, then in male six week old B6D2F1 mice (Janvier, France). Compounds were dissolved in Mygliol 812 and administered (ip, 20 ml/kg) at the maximum tolerated dose (defined as $1/2\ LD_{50tox/48H}$). In Swiss CD1 mice, compounds were injected 90 min before radiation; in B6D2F1 mice they were injected 15 min before exposure. Gamma irradiation (^{60}Co) was delivered at doses $LD_{100irr/30days}$ and $LD_{100irr/30days} + 2$ Gy. $LD_{100irr/30days}$ is defined as the irradiation dose which kills 100% of the animals 30 days after exposure.

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