Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 38 (2003) 1–11
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Original article

Synthesis and pharmacological evaluation of new 1,2-dithiolane based antioxidants

Claude Guillonneau [a], Yves Charton [a], Yves-Michel Ginot [b], Marie-Victoire Fouquier-d'Hérouël [b], Marc Bertrand [b], Brian Lockhart [c], Pierre Lestage [c], Solo Goldstein [a,*]

[a] *Chemistry Research Division A, institut de recherches Servier, 11, rue des Moulineaux, 92150 Suresnes, France*
[b] *Technologie Servier, 25-27, rue Eugène Vignat, 45007 Orléans, France*
[c] *Cerebral Pathology Division, institut de recherches Servier, 125, chemin de Ronde, 78290 Croissy-Sur-Seine, France*

Received 9 April 2002; received in revised form 3 October 2002; accepted 3 October 2002

Abstract

Molecules containing a dithiolane moiety are widely investigated due to their antioxidant properties. The archetypal representative of this class of compounds is lipoic acid and indeed the lipoic acid–dihydrolipoic acid couple is part of the antioxidant defence system of the cell. In the course of a program aiming to find improved antioxidants effective in vivo, we designed, synthesised and pharmacologically investigated new lipoic acid analogs. The salient feature of these structures is the connection, via a thioamide or a thiocarbamate, of a 1,2-dithiolane moiety bearing a carbon chain and a *N*-alkyl-substituted morpholine ring. It was expected that the antioxidant and chelating properties of these functional groups combined with the basicity of the morpholine ring will impact on the antioxidant as well as on the partition and solubility characteristics of the compounds. Indeed in vitro and in vivo pharmacological investigation showed that these new molecules and especially those containing a thiocarbamate linker possess superior antioxidant properties compared with α-lipoic acid and to the amide or carbamate linker analogs. In particular, some of these compounds efficiently cross the blood brain barrier (BBB) thus providing efficient protection from lethality in a situation of induced oxidative stress. Moreover the absence of the 1,2-dithiolane moiety does not completely abolish antioxidant effects thus demonstrating that these compounds are distinct new chemical entities and not merely lipoic acid prodrugs. The chemical and pharmacological features of these new antioxidants are presented and discussed in the following paper.

Keywords: Antioxidant; Dithiolanes; Oxidative stress

1. Introduction

The deleterious effects of an imbalance between reactive oxygen species (ROS) production and the available antioxidant defence capacity, termed oxidative stress, as well as its role in the aggravation of a plethora of pathological conditions, are widely documented in the literature [1].

A variable sensitivity to this excessive oxidative state is observed between tissues and organs, the brain being the most vulnerable due to its high oxygen consumption, high content of unsaturated membrane fatty acids and low level of antioxidant enzymes or vitamins [2].

In the last decade much effort has been directed towards finding molecules capable of opposing the oxidative stress challenge [3]. Thus one of the natural molecules known to prevent or retard oxidation is α-lipoic acid and, therefore, the α-lipoic acid (LA)–dihydrolipoic acid (DHLA) redox couple (Fig. 1) has received considerable attention. Indeed, beneficial ef-

Abbreviations: BBB, blood brain barrier; DHLA, dihydrolipoic acid; DCC, dicyclohexylcarbodiimide; GSH, reduced glutathione; HOBT, 1-hydroxybenzotriazole; i.p., intraperitoneal; p.o., per os; i.c.v., intracerebroventricular; i.v., intravenous; LA, α-lipoic acid; NADH, reduced nicotinamide adenine dinucleotide; ROS, reactive oxygen species; TBTU, *O*-(1H-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyl uronium tetrafluoroborate; *t*-BuOOH, *tert*-butylhydroperoxide.

* Correspondence and reprints.
E-mail address: solo.goldstein@fr.netgrs.com (S. Goldstein).

0223-5234/02/$ - see front matter
PII: S0223-5234(02)01424-1

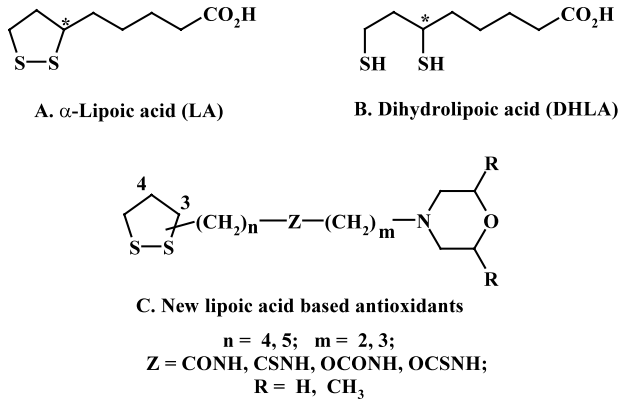


Fig. 1. Structures of dithiolane based antioxidants.

fects were demonstrated in a number of paradigms implicating oxidative stress such as ischemia-reperfusion, diabetes, cataract-formation, HIV activation, and neurodegeneration [4].

Growing evidence suggests that these compounds, initially known as cofactors of α-keto-acid dehydrogenase complexes, are part of the antioxidant defence system of the cell [5]. It is well-known that LA is readily absorbed from diet and also crosses the blood brain barrier (BBB) [6,7]; the intracellular reduction by lipoamide dehydrogenase (which is NADH-dependent) affords DHLA and since mammalian cells are capable of releasing it, the effects of LA and DHLA might be present both intra and extracellularly even if LA alone is administered extracellularly [8].

LA and DHLA are capable of quenching a number of radicals both in a lipid and in an aqueous environment [9–11]. However, it also appears that LA and DHLA act as antioxidants not only by direct radical trapping and/or metal chelation but also by recycling other antioxidants (ascorbate, vitamin E) and also by inducing increased cellular levels of reduced glutathione (GSH). Indeed DHLA prevents lipid peroxidation by reducing glutathione, which in turn recycles vitamin E [12,13]. The pro-oxidant effects of DHLA obtained under certain circumstances raised some concerns but the results obtained are controversial [13,14].

There is strong evidence that the neuroprotective effects of LA and DHLA are mediated by antioxidant and free radical scavenging mechanisms. However, LA exerts its neuroprotective activity only after a long period of treatment suggesting that endogenous reduction to DHLA has to occur in order to diminish neuronal damage [15]. LA contains an asymmetric centre but no clear difference between (*R*) and (*S*) enantiomers in terms of neuroprotective activity could be observed. However, it is known that (*R*)-LA is transformed by lipoamide dehydrogenase to DHLA with a higher rate than (*S*)-LA. Also (*R*)-LA is more effectively incorporated into mitochondrial enzyme complexes. Therefore, (*S*)-LA which is converted into DHLA at a slower rate is also less efficiently incorporated into the enzyme complexes and might be fully available for radical scavenging or other actions which would explain the observed pharmacological results (i.e. no difference between the enantiomers) [16–19].

Our research on novel antioxidants focused on structures which, besides the 1,2-dithiolane moiety, also contained a thioamide or a thiocarbamate functional groups as well as a morpholine ring (Fig. 1) [20].

It was expected that the metal chelating and antioxidant properties of these two functional groups combined with the basicity of the morpholine ring will positively modulate the antioxidant as well as the partition and solubility characteristics of the compounds. Moreover based on calculated partition coefficients (log $P \sim 2$–4) it was also expected that these compounds will exhibit good BBB crossing capabilities. The pharmacological profiles have been compared with those obtained for lipoic acid, for amide and carbamate linker analogs as well as for an analog lacking the 1,2-dithiolane moiety (vide infra).

2. Chemistry

The synthetic methodologies employed for the preparation of amide and thioamide containing structures are summarised in Fig. 2. Commercially available α-lipoic acid (racemic mixture) served as a convenient starting material. The presence of the morpholino group in all end-products allowed easy transformation into the corresponding hydrochlorides, which could be crystallised (see Section 6).

Thus HOBT–TBTU mediated coupling [21] of the above acid with the commercially available 2-morpholin-4-yl-ethylamine proceeds smoothly in tetrahydrofuran to afford the desired amide containing structure while the corresponding hydrochloride **1** is obtained by treatment with 2 N HCl.

The thioamide analog **2** was obtained by reaction of **1a** (free base) with Lawesson's reagent in toluene at 80 °C. (*R*,*S*) Lipolol, the alcohol obtained by catecholborane reduction of (*R*,*S*) LA [22], was employed as starting material for the obtention of carbamate and thiocarbamate containing members of the series (Fig. 3). Thus triethylamine promoted reaction with *N*,*N*′-disuccinimidyl carbonate in an acetonitrile–toluene mixture afforded derivative **3a** that was used as such for the next step. Carbamate **3** was obtained by reacting **3a** with 2-morpholin-4-yl-ethylamine [23] in the presence of triethylamine in dichloromethane followed by acid treatment to yield the corresponding hydrochloride (Fig. 3A).

The thiocarbamate analog **4** was obtained by reacting racemic lipolol with a tin-oxide reagent followed by further reaction of the crude **4a** with an isothiocyanate

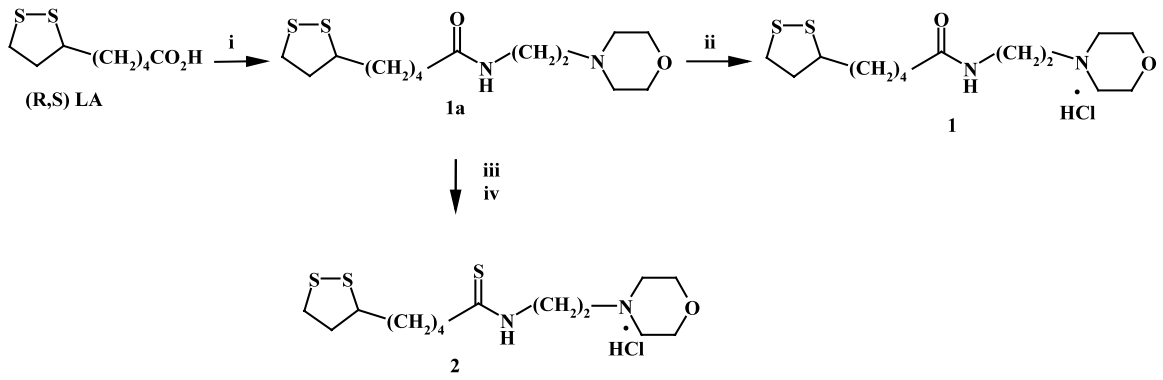


Fig. 2. Conditions: (i) HOBT–TBTU–ethyl diisopropylamine–H_2N-$(CH_2)_2$–N⟨O⟩, THF, 20 °C, 88%; (ii) 2 N HCl–ethanol; (iii) Lawesson's reagent, toluene, 80 °C, 31%; (iv) 4 N HCl–dioxane, ethanol.

derivative [24] and then hydrochloride formation as usual (Fig. 3B).

Enantiomers **5** and **6** (Table 1) of the racemic thiocarbamate **4** were obtained by the same reaction sequence but using optically pure (*R*) and (*S*) α-lipoic acid that could be reduced to the corresponding optically pure lipolol (see Section 6).

Furthermore, compounds **7**–**10** (Table 1) were also prepared following the same procedure as employed for compound **4**.

4-(2-Isothiocyanato-ethyl)-2,6-dimethyl-morpholine **11** and 5-[1,2] dithiolan-4-yl-pentan-1-ol (**12**), used for preparation of compounds **9** and **8**, respectively, were prepared as indicated in Figs. 4 and 5, respectively. For compound **11**, the procedure given in the Section 6 is the same as a typical procedure previously described [32]. For compound **12**, the procedure is given in the Section 6.

3. Pharmacology

The compounds described above were examined in a series of pharmacological models in order to evaluate their antioxidant protective effects both in vitro and in vivo.

Thus the capacity to inhibit Fe^{2+}–H_2O_2 induced lipid peroxidation was studied in fresh cortical membrane preparations, by comparing percentage inhibition in the presence or absence of antioxidant (see Section 6). Only the global effect was considered and no attempt was made to analyse the result in terms of metal chelating, radical scavenging and/or other effects. However, we also included in our study, compound **10** which does not contain the dithiolane moiety.

Experimentation with antioxidants has shown that in vitro effects as observed in lipid peroxidation or other tests, do not necessarily reflect the in vivo profile [25]. Therefore, in order to pharmacologically characterise our compounds in vivo, several pharmacological models were employed. Thus intracerebroventricular (i.c.v.) administration of *tert*-butylhydroperoxide to mice induces a direct oxidative stress leading to an apoptotic type neurodegeneresence and ultimately to animal death. The protective effect of our compounds was evaluated by the lethality observed in animals who received the antioxidant agent comparative to those receiving vehicle alone (see Section 6). It was expected that the protective potency observed in this model will

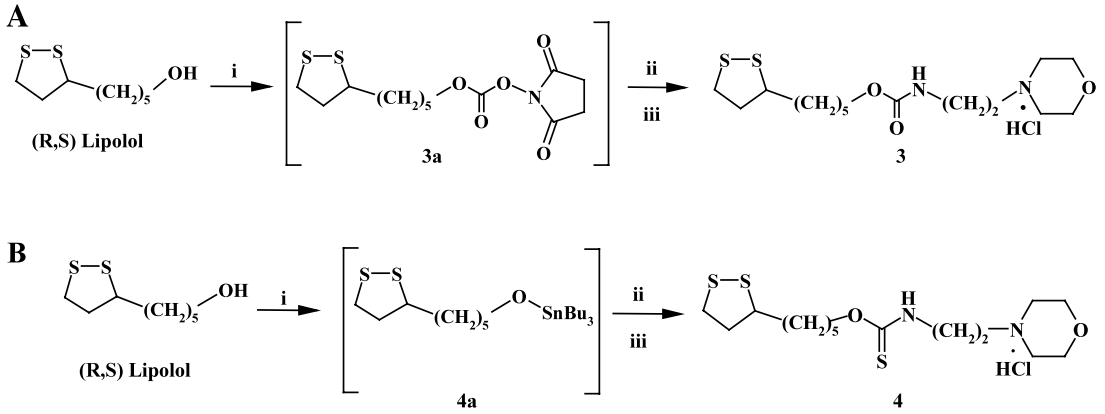
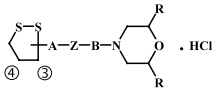
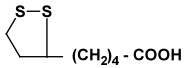
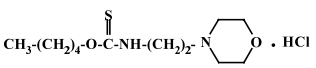


Fig. 3. (A) Conditions, i: *N*,*N*′ disuccinimidyl carbonate, Et_3N, CH_3CN, toluene, 20 °C; ii: $H_2N(CH_2)_2$–N⟨O⟩, Et_3N, CH_2Cl_2, 20 °C, 78%; iii: 4 N HCl–dioxane. (B) Conditions: i: $(Bu_3Sn)_2O$, toluene; ii: O⟨N⟩–$(CH_2)_2$–N=C=S, toluene 60 °C; iii: 4 N HCl–dioxane, ethanol, 41%.

Table 1
Pharmacological profile of dithiolane-based antioxidants



Compounds	A	Z	B	R	Enantiomer	Log D pH 7.4	Lipid Peroxydation % Inhibition (5 mM) vs control *P<0.05; **P<0.01; (Student's t-test)	t-BuOOH 150 mg/kg i.p. % survival at 2 h $^{+}$50 mg/kg i.p.	Alloxan 1 h 400 mg/kg p.o. Hyperglycemia Inhibition % $^{+}$300 mg/kg p.o. $^{++}$100 mg/kg p.o. vs control *P<0.05; **P<0.01; P<0.001 (Student's t-test)	Δ-T°C 150 mg/kg i.p. $^{+}$50 mg/kg i.p.
					R, S	- 0.4	82**	toxic $^{+}$0	57***	2.0
1	$(CH_2)_4$-③		$(CH_2)_2$	H	R, S	1.8	31*	toxic $^{+}$40	48**	toxic $^{+}$0.4
2	$(CH_2)_4$-③		$(CH_2)_2$	H	R, S	2.9	84**	20 $^{+}$0	$^{+}$96***	6.15
3	$(CH_2)_5$-③		$(CH_2)_2$	H	R, S	2.9	45*	toxic	$^{++}$25*	toxic
4	$(CH_2)_5$-③		$(CH_2)_2$	H	R, S	3.8	90**	100	100***	4.6
5	$(CH_2)_5$-③		$(CH_2)_2$	H	R	3.3	91**	80	85***	4.2
6	$(CH_2)_5$-③		$(CH_2)_2$	H	S	3.3	91**	100	93***	4.36
7	$(CH_2)_5$-③		$(CH_2)_3$	H	R, S	3.8	83**	20	100***	ND
8	$(CH_2)_5$-④		$(CH_2)_2$	H	R, S	3.8	98**	100	100***	3.96
9	$(CH_2)_5$-③		$(CH_2)_2$	CH_3	R, S	4.2	96**	80	87***	4.05
10						2.8	70**	50	33*	2.7

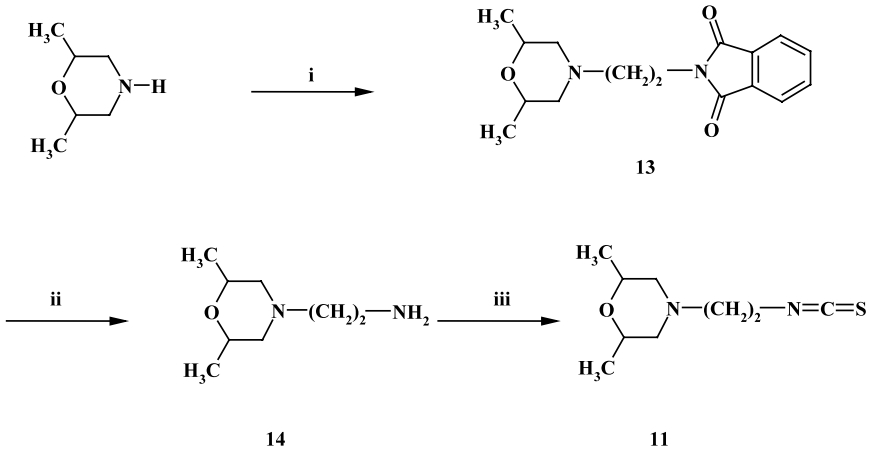


Fig. 4. Conditions: (i) *N*-(2-Bromoethyl) phtalimide, acetonitrile, 49%; (ii) Hydrazine hydrate, methanol, 79 °C; (iii) DCC, carbondisulphide, diethylether, 45%.

be strongly influenced by the BBB permeability of the investigated compound.

Compounds were also evaluated in an additional model whereby a peripheral oxidative stress created by alloxan selectively damages β cells of the pancreas via a redox cycling mechanism, thus inducing a type I diabetes in mice. Indeed evidence of oxidative stress in diabetic rat tissues has been reported in the literature [26]. Moreover it is known that free radicals are increasingly formed in diabetes mellitus by the autooxidation of glucose and glycosilated proteins [27]. The potency of our compounds to inhibit alloxan-induced hyperglycemia in mice was evaluated, in comparison with lipoic acid.

The in vivo antioxidant pattern as observed in the above tests could be compared with the in vitro results, concerning lipid peroxidation (vide infra). Moreover in order to further characterise the in vivo behaviour of our compounds, the whole body temperature of animals was also measured.

4. Results and discussion

Investigation of our compounds, in the above mentioned models, resulted in the pharmacological profiles summarised in Table 1. Also indicated are octanol–

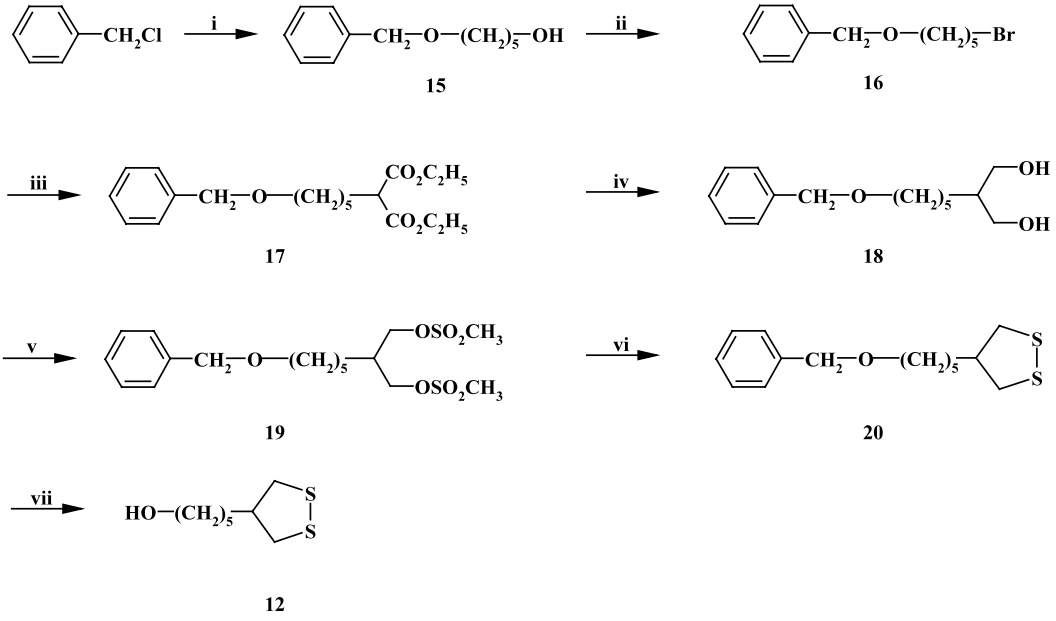


Fig. 5. Conditions: (i) 1,5-pentanediol, NaOH, H_2O, 31%; (ii) $P(C_6H_5)_3$, Br_2, CH_3CN, 71%; (iii) sodium ethoxyde, diethylmalonate, 72%; (iv) $LiA1H_4$, diethylether, 67%; (v) $N(C_2H_5)_3$, CH_2Cl_2, methanesulfonylchloride, 91%; (vi) sulphur, Na_2S, DMF, 73%; (vii) boron tribromide–methyl sulphide complex, 1,2-dichloethane, 73%.

water partition coefficients measured at pH 7.4 (log D), using a Sirius apparatus (see Section 6).

For comparative purposes, commercially available lipoic acid was also studied in the same models. Thus, in our hands, this compound provides at a 5 mM concentration, a high percentage of lipid peroxidation inhibition (no inhibition was observed at 2 mM).

However, at a dose of 150 mg kg^{-1} intraperitoneally (i.p.), no evidence of protection against *t*-BuOOH mediated lethality was observed, only toxic effects (convulsion, morbidity) being apparent in this case. No toxic effects but also no reduction in *t*-BuOOH mediated lethality were observed at a lower dose (50 mg kg^{-1} i.p.). On the other hand, a moderate effect in reducing alloxan induced hyperglycemia was evidenced after oral administration (400 mg kg^{-1}).

As mentioned above, the chemical structure of the new antioxidants is characterised by the presence of a 1,2-dithiolane moiety bearing a carbon chain and a *N*-alkyl-substituted morpholine ring, both these units being connected via one of amide, thioamide, carbamate or thiocarbamate linker (Z). It should be pointed out that even so compounds including similar connectivities have been claimed in the recent patent literature, no integrated in vivo tests have been employed in their characterisation [28,29].

In our case it was found that the nature of the linker (Z) as well as the length of the alkyl chains (A and B), have an impact on the pharmacological results. Thus, the mere change of the linker from amide to thioamide increases the lipid peroxidation inhibition (compare **1** and **2**) to a level comparative to the one observed for lipoic acid. However, no real reduction in lethality is observed for both **1** and **2** in the *t*-BuOOH model at 50 mg or 150 mg kg^{-1} i.p. (toxic effects are observed for **1** at the higher dose). Interestingly, however, compound **2** totally inhibits alloxan induced hyperglycemia when administered orally at a dose of 300 mg kg^{-1} while lipoic acid shows a lower effect at a higher dose. Unfortunately **2** also displays at 150 mg kg^{-1} i.p. a more marked hypothermic effect higher than the one observed for lipoic acid.

The change of the linker from carbamate to thiocarbamate also leads to an improvement in the pharmacological profile (compare **3** and **4**). Indeed, while **3** shows very weak activities or even toxic effects in the investigated models, **4** shows excellent levels of activity both in the in vitro (lipid peroxidation inhibition) and in vivo tests. These results indicate an impact of the thiocarbamate function on the biological behaviour of our compounds and are also in line with known literature data indicating that thiocarbamates alone exhibit antioxidant properties [30].

In order to further substantiate these facts, compound **10** lacking the 1,2-dithiolane ring was synthesised and tested. As can be seen from Table 1, this compound shows a very weak level of activity in the alloxan model but moderate to good activities in the *t*-BuOOH and lipid peroxidation tests, respectively. Also, the hypothermia effect induced by this compound is lower than the one usually observed in this series and is similar to the one observed for lipoic acid. These results tend to demonstrate, at least partly, that the investigated compounds are not merely lipoic acid prodrugs but new chemical entities per se. Nevertheless and despite the fact that specific enzymes for carbamate and thiocarbamates are not known, this does not imply complete resistance of these functions to esterases.

As already documented in the literature for the *R* and *S* enantiomers of lipoic acid, in our case too, no difference has been observed between racemic compound **4** and its enantiomers **5** and **6**. However, lengthening the chain B by one carbon atom has a negative impact on the protective effect (compare **4** and **7** in the *t*-BuOOH model).

It is worthwhile to stress again that not all compounds that are effective in the lipid peroxidation model (in vitro test) display activities in the in vivo tests, especially in what the protection against *t*-BuOOH induced lethality model is concerned. Indeed while compound **4** and lipoic acid behave similarly in the lipid peroxidation test, only the former provides an excellent protective action against *t*-BuOOH induced lethality. Also compound **4** is more efficient than lipoic acid in what hyperglycemia inhibition is concerned. Another striking example is provided by compounds **2** and **7** both inhibiting the lipid peroxidation but being completely inactive in the *t*-BuOOH test. However, both these compounds are active and better than lipoic acid in the alloxan test.

Albeit a genuine correlation between measured partition data (log *D*) and protective effect in the *t*-BuOOH test cannot be established for this series, it might be observed that a good BBB crossing is probably linked to a distribution coefficient higher than 3 as can be judged from the protection against *t*-BuOOH induced lethality. Compound **7** is an outlayer in this respect at least if a passive mechanism for the BBB crossing is assumed. This observation tends to indicate that transport mechanisms and/or partial metabolisation cannot be completely ruled out as factors influencing the pharmacological results in this model.

The obtained results seem to indicate a tendency of higher hypothermia with increasing log *D*.

An excellent level of activity in all examined models is obtained for the 1,2-dithiolane regioisomer **8** and the morpholine substituted analog **9**.

In summary, we presented in this study new 1,2-dithiolane derivatives, which demonstrate in vivo pharmacological profiles superior to those of lipoic acid. Moreover some of our data indicate the possibility of modulating peripheric (alloxan test) vs. central (*t*-

BuOOH test) effects. These compounds might be valuable starting points for drugs acting in situations of oxidative stress.

5. Conclusion

New antioxidants containing an alkylated 1,2-dithiolane unit connected via an amide, thioamide, carbamate or thiocarbamate linker to a *N*-alkylated morpholine unit, have been synthesised and tested. The thioamide and thiocarbamate linker analogs demonstrate superior in vivo pharmacological profiles in comparison to their amide and carbamate counterparts and to the archetypal lipoic acid. The stereochemistry of the 1,2-dithiolane unit does not influence the obtained pharmacological profile, both possible enantiomers being equally active.

Ongoing studies aiming to further characterise this class of compounds are in course.

6. Experimental

6.1. Chemistry

Compounds prepared in this publication were named using AUTONOM 2 Software.

All reagents were fresh, commercial grade chemicals.

Melting points (m.p.) were determined on a Meltemps capillary apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker AC 200 or 300 spectrometer in DMSO-d_6 solutions.

Chemical shifts are expressed in ppm downfield from a tetramethylsilane internal standard. Significant ^{1}H-NMR data are reported in the following order: multiplicity (bs, broad signal; d, doublet; t, triplet; m, multiplet; bd, broad triplet; dd, double doublet), number of protons. Elemental analyses were performed on a Carlo Erba analyser 1108. Column chromatography was performed using Merck Silica gel (0.040–0.063 mm) under a 1 bar nitrogen pressure (flash chromatography). All reactions were carried out under a nitrogen atmosphere.

Separation of α-lipoic acid enantiomers was achieved by salt formation with α-methylbenzylamine followed by crystallisation from toluene [31].

Elemental analyses indicated by the symbols of the elements were within $\pm$0.4% of the theoretical values.

Dicyclohexylcarbodiimide (DCC), *O*-(1H-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′ tetramethyl uronium tetrafluoroborate (TBTU), 1-hydroxybenzotriazole (HOBT), Lawesson's reagent, bis (tributyltin) oxide, *N*,*N*′-disuccinimidyl carbonate, malonic acid diethyl ester, triphenylphosphine, *n*-pentanol and *N*-(2-bromoethyl) phtalimide were obtained from Aldrich.

2-(4-Morpholino) ethyl isothiocyanate, 3-(4-morpholino) propyl isothiocyanate and 2,6-dimethylmorpholin were obtained from Lancaster.

Racemic α-lipoic acid (LA) was obtained from Sigma. All compounds, prepared in this publication, containing the [1,2-dithiolan-3-yl] or [1,2-dithiolan-4-yl] moiety were dried at 30 °C maximal temperature due to their sensitivity to heat induced polymerisation.

6.1.1. (*R,S*)-5-[1,2] dithiolan-3-yl-pentanoic acid (2-morpholin-4-yl-ethyl)-amide hydrochloride (**1**)

To a stirred solution of racemic α-lipoic acid (6.2 g, 30 mmol) in THF (180 mL) were added HOBT (4.5 g, 33.3 mmol), TBTU (10.5 g, 32.7 mmol), ethyl-diisopropylamine (7.8 g, 60 mmol) and 2-morpholin-4-yl-ethylamine (3.9 g, 30 mmol). The mixture was stirred at 20 °C for 20 h and concentrated under vacuum at a temperature below 30 °C. The residue was taken up in dichloromethane followed by washing with a solution of sodium hydrogencarbonate in water. The organic layer was dried (Na_2SO_4) and concentrated to dryness. Column chromatography of the residue (5% methanol in dichloromethane) afforded 5-[1,2] dithiolan-3-yl pentanoic acid (2-morpholin-4-yl-ethyl)-amide **1a** which was dissolved in ethanol. A small excess of a 2 N solution of HCl in ethanol was then added and the mixture was cooled to 5 °C. The precipitated solid was collected by filtration, washed with ethanol and dried at 30 °C under vacuum to afford compound **1** (8.4 g). Yield (88%); m.p. 80–82 °C. ^{1}H-NMR (200 MHz): $\delta =$ 11.1 (bs, 1H, exchanged by D_2O); 8.2 (t, 1H, exchanged by D_2O); 3.9 (m, 4H); 3.6 (m, 1H); 3.45 (m, 4H); 3.15 (m, 6H); 2.4 (m, 1H); 2.1 (m, 2H); 1.9 (m, 1H); 1.5 (m, 4H); 1.35 (m, 2H). Anal. $C_{14}H_{26}N_2O_2S_2$, HCl (C, H, N, S, Cl).

6.1.2. (*R,S*)-5-[1,2] Dithiolan-3-yl pentanethioic acid (2-morpholin-4-yl-ethyl)-amide hydrochloride (**2**)

A mixture of **1a** (8.3 g, 26 mmol), Lawesson's reagent (5.3 g, 13 mmol) and toluene (250 mL) was stirred at 80 °C during 4 h. After cooling, the mixture was extracted with an aqueous solution of 1 N HCl. The aqueous layer was brought to a basic pH with sodium carbonate and then extracted with ethyl acetate. The organic layer was dried (Na_2SO_4) and concentrated under vacuum. The residue was taken up in ethanol, a small excess of a 2 N HCl solution in ethanol was added and the mixture was concentrated under vacuum. The residue was stirred in ether and the solid collected by filtration was washed with ether and dried at 30 °C under vacuum to afford compound **2** (3.0 g). Yield (31%); m.p. 98–100 °C. ^{1}H-NMR (200 MHz): $\delta = 11.4$ (bs, 1H, exchanged by D_2O); 10.4 (t, 1H, exchanged by D_2O); 3.95 (m, 6H); 3.6 (m, 1H); 3.45 (m, 4H); 3.15 (m, 4H); 2.6 (m, 2H); 2.4 (m, 1H); 1.9 (m, 1H); 1.65 (m, 4H);

1.35 (m, 4H). Anal. $C_{14}H_{26}N_2O\,S_3$, HCl (C, H, N, S, Cl).

*6.1.3. (R,S)-(2-Morpholin-4-yl-ethyl)-carbamic acid 5-[1,2] dithiolan-3-yl pentyl ester hydrochloride (**3**)*

N,*N*′-disuccinimidyl carbonate (11.5 g, 0.045 mol) and triethylamine (9 g) were added to a stirred suspension of (*R*,*S*) lipolol (5.77 g, 0.03 mol) in toluene (50 mL) and acetonitrile (150 mL). After 3 h of stirring, a further amount of *N*,*N*′-disuccinimidyl carbonate (2.5 g) and triethylamine (2 g) was added. The mixture was stirred for 16 h at 20 °C, concentrated under vacuum at a temperature below 30 °C, the residue was taken up in an aqueous solution of sodium chloride, dried (Na_2SO_4) and concentrated under vacuum at 30 °C. Column chromatography of the residue using dichloromethane as eluent afforded carbonic acid 2,5-dioxo-pyrrolidin-1-yl ester 5-[1,2] dithiolan-3-yl pentyl ester **3a**. The crude residue was dissolved in dichloromethane (60 mL) and added over 30 min to a stirred solution of 2-morpholin-4-yl-ethylamine (4.68 g, 0.036 mol) and triethylamine (6 g) in dichloromethane (100 mL). The mixture was stirred for 18 h at 20 °C, washed with an aqueous solution of sodium chloride, dried (Na_2SO_4) and concentrated under vacuum at a temperature below 30 °C. Chromatography of the residue using a mixture of 4% methanol in dichloromethane as eluent gave (*R*,*S*)-(2-morpholinyl-4-yl-ethyl)-carbamic acid 5-[1,2] dithiolan-3-yl pentyl ester which was dissolved in ethanol; the solution was treated with a small excess of 4 N HCl in dioxane and concentrated under vacuum at a temperature below 30 °C. The residue was stirred in ether, the crystals were collected by filtration and dried under vacuum at 30 °C to afford compound **3** (8.2 g). Yield (71%); m.p. 113–115 °C. ^{1}H-NMR (300 MHz); $\delta = 10.5$ (bs, 1H, exchanged by D_2O); 7.35 (t, 1H, exchanged by D_2O); 3.75 (m, 2H); 3.6 (m, 1H); 3.4 (m, 4H); 3.15 (m, 6H); 2.4 (m, 1H); 1.9 (m, 1H); 1.65 (m, 1H); 1.55 (m, 3H); 1.35 (m, 4H). Anal. $C_{15}H_{28}N_2O_3S_2$, HCl (C, H, N, S, Cl).

*6.1.4. (R,S)-(2-Morpholin-4-yl-ethyl)-thiocarbamic acid O-(5-[1,2] dithiolan-3-yl pentyl) ester hydrochloride (**4**)*

Racemic lipolol (9.6 g, 0.05 mol) and bis-(tributyltin)-oxide (30 g, 0.05 mol) in toluene (400 mL) were stirred at a reflux temperature for 20 h. The reaction mixture was cooled to 60 °C and 2-(4-morpholinyl) ethyl isothiocyanate (17.2 g, 0.1 mol) were added dropwise; the mixture was maintained at 40 °C overnight. After cooling, the mixture was washed with an aqueous solution of sodium carbonate and concentrated to 100 mL under vacuum. Column chromatography of the solution eluting with a mixture 4% THF in dichloromethane afforded (*R*,*S*)-(2-morpholin-4-yl-ethyl)-thiocarbamic acid *O*-(5-[1,2] dithiolan-3-yl pentyl) ester as the free base. The above product was dissolved in ethanol and treated with a small excess of 4 N HCl in dioxane followed by concentration of the solution under vacuum. The residue was stirred in ether, the crystals were collected by filtration, washed with ether and hexane and then dried under vacuum at 30 °C, affording compound **4** (8.2 g). Yield (41%); m.p. 105–110 °C. ^{1}H-NMR (300 MHz): $\delta = 10.75$ (bs, 1H, exchanged by D_2O); 9.25 (t, 1H, exchanged by D_2O); 4.3 (t, 2H); 3.95 (m, 2H); 3.75 (m, 4H); 3.6 (m, 1H); 3.45 (m, 2H); 3.2 (m, 2H); 3.15 (m, 4H); 2.4 (m, 1H); 1.9 (m, 1H); 1.65 (m, 4H); 1.4 (m, 4H). Anal. $C_{15}H_{28}N_2O_2S_3$, HCl (C, H, N, S, Cl).

*6.1.5. (R)-(2-Morpholin-4-yl-ethyl)-thiocarbamic acid 0-(5-[1,2] dithiolan-3-yl-pentyl) ester hydrochloride (**5**)*

(*R*)-Lipolol (5.8 g, 0.03 mol) used as starting material afforded compound **5** (4.3 g) by the same method as described for **4**. Yield (36%); m.p. 108–110 °C. ^{1}H-NMR (300 MHz): $\delta = 10.75$ (bs, 1H, exchanged by D_2O); 9.25 (t, 1H, exchanged by D_2O); 4.3 (t, 2H); 3.95 (m, 2H); 3.75 (m, 4H); 3.6 (m, 1H); 3.45 (m, 2H); 3.2 (m, 2H); 3.15 (m, 4H); 2.4 (m, 1H); 1.9 (m, 1H); 1.65 (m, 4H); 1.4 (m, 4H). Anal. $C_{14}H_{26}N_2O_2S_2$, HCl (C, H, N, S, Cl).

*6.1.6. (S)-(2-Morpholin-4-yl-ethyl)-thiocarbamic acid 0-(5-[1,2] dithiolan-3-yl-pentyl) ester hydrochloride (**6**)*

(*S*)-Lipolol (5.8 g, 0.03 mol) used as starting material afforded compound **6** (4.7 g) by the same method as described for **4**. Yield (39%); m.p. 118–120 °C.^{1}H-NMR (300 MHz): $\delta = 10.75$ (bs, 1H, exchanged by D_2O); 9.25 (t, 1H, exchanged by D_2O); 4.3 (t, 2H); 3.95 (m, 2H); 3.75 (m, 4H); 3.6 (m, 1H); 3.45 (m, 2H); 3.2 (m, 2H); 3.15 (m, 4H); 2.4 (m, 1H); 1.9 (m, 1H); 1.65 (m, 4H); 1.4 (m, 4H). Anal. $C_{15}H_{28}N_2O_2S_3$, HCl (C, H, N, S, Cl).

*6.1.7. (R,S)-(3-Morpholin-4-yl-propyl)-thiocarbamic acid 0-(5-[1,2] dithiolan-3-yl-pentyl) ester hydrochloride (**7**)*

3-(4-Morpholinyl)propylisothiocyanate (8.4 g, 0.045 mol) and racemic lipolol (5.8 g, 0.03 mol) used as starting materials afforded compound **7** (1.55 g) by the same method as described for **4**. Yield (11%); m.p. 86–88 °C. ^{1}H-NMR (300 MHz): $\delta = 10.65$ (bs, 1H, exchanged by D_2O); 9.15 (t, 1H, exchanged by D_2O); 4.3 (t, 2H); 3.9 (m, 2H); 3.75 (m, 2H); 3.6 (m, 1H); 3.4 (m, 2H); 3.3 (m, 2H); 3.15 (m, 6H); 2.4 (m, 1H); 1.9 (m, 3H); 1.65 (m, 4H); 1.4 (m, 4H). Anal. $C_{15}H_{28}N_2O_2S_3$, HCl (C, H, N, S, Cl).

*6.1.8. (2-Morpholin-4-yl-ethyl)-thiocarbamic acid 0-5-[1,2] dithiolan-4-yl-pentyl) ester hydrochloride (**8**)*

5-[1,2] dithiolan-4-yl-pentan-1-ol (**12**) (5.8 g, 0.03 mol) used as starting material afforded compound **8** (3.0 g) by the same method as described for **4**. Yield (25%); m.p. 80–85 °C. ^{1}H-NMR (300 MHz): $\delta = 11.05$ (bs, 1H,

exchanged by D_2O); 9.25 (t, 1H, exchanged by D_2O); 4.3 (t, 2H); 3.95 (m, 2H); 3.8 (m, 4H); 3.4 (m, 2H); 3.25 (m, 3H); 3.05 (m, 2H); 2.8 (m, 2H); 2.5 (m, 2H); 1.65 (m, 2H); 1.4 (m, 6H). Anal. $C_{15}H_{28}N_2O_2S_3$, HCl (C, H, N, S, Cl).

*6.1.9. (R,S)-[2-(2,6-Dimethyl-morpholin-4-y)l-ethyl]-thiocarbamic acid O-(5-[1,2] dithiolan-3-yl-pentyl) ester hydro-chloride (**9**)*

4-(2-Isothiocyanato-ethyl)-2,6-dimethyl-morpholine (**11**) (11.2 g, 0.06 mol) and racemic lipolol (5.8 g, 0.03 mol) used as starting materials afforded compound **9** (2.9 g) by the same method as described for **4**. Yield (23%); m.p. 85–88 °C. ^{1}H-NMR (300 MHz): $\delta = 11.3$ (bs, 1H, exchanged by D_2O); 9.25 (t, 1H, exchanged by D_2O); 4.3 (t, 2H); 3.95 (m, 2H); 3.8 (m, 2H); 3.6 (m, 1H); 3.45 (m, 2H); 3.15 (m, 4H); 2.65 (m, 2H); 2.4 (m, 1H); 1.9 (m, 1H); 1.65 (m, 4H); 1.4 (m, 4H); 1.15 (d, 6H). Anal. $C_{17}H_{32}N_2O_2S_3$, HCl (C, H, N, S, Cl).

*6.1.10. (2-Morpholin-4-yl-ethyl)-thiocarbamic acid O-pentyl ester (**10**)*

n-Pentanol (4.3 g, 0.042 mol) used as starting material afforded compound **10** (4.5 g) by the same method as described for **4**. Yield (31%); m.p. 108–110 °C. ^{1}H-NMR (300 MHz): $\delta = 11.0$ (bs, 1H, exchanged by D_2O); 9.25 (t, 1H, exchanged by D_2O); 4.3 (t, 2H); 3.95 (m, 2H); 3.8 (m, 4H); 3.45 (m, 2H); 3.25 (m, 2H); 3.1 (m, 2H); 1.65 (m, 2H); 1.3 (m, 4H); 0.9 (m, 3H). Anal. $C_{12}H_{24}N_2O_2S$, HCl (C, H, N, S, Cl).

*6.1.11. 2-[2-(2,6-Dimethyl-morpholin-4-yl)-ethyl]-isoindole-1,3-dione (**13**)*

N-(2-Bromoethyl)-phtalimide (25.4 g, 0.1 mol) and 2,6-dimethylmorpholine (23 g, 0.2 mol) in acetonitrile (250 mL) are stirred at reflux temperature for 16 h. The mixture was concentrated under vacuum to dryness. The residue was dissolved in diethylether, washed successively with aqueous solutions of sodium carbonate and sodium chloride, dried (Na_2SO_4) and concentrated to dryness. Chromatography of the residue on silicagel eluting with 4% tetrahydrofuran in dichloromethane gave compound **13** (14 g, 49%).

*6.1.12. 2-(2,6-Dimethyl-morpholin-4-yl)-ethylamine (**14**)*

2-[2-(2,6-Dimethyl-morpholin-4-yl)-ethyl]-isoindole-1,3-dione (**13**) (69 g, 0.239 mol) was dissolved in methanol (1 L). Hydrazine hydrate (13 mL) was added and the mixture was stirred under reflux for 6 h and at 20 °C for 16 h. The mixture was cooled at 5 °C and an aqueous solution of 4 N hydrochloric acid (200 mL) was added. The solid was collected by filtration, washed with water and the combined filtrates concentrated to dryness. The residue was taken up with an aqueous solution of 4 N sodium hydroxyde, extracted with dichloromethane, the organic layer dried (Na_2SO_4) and concentrated to dryness. Distillation of the residue gave compound **14** (29.8 g, 79%): b.p. 50 °C/0.1 mm.

*6.1.13. 4-(2-Isothiocyanato-ethyl)-2,6-dimethyl-morpholine (**11**)*

2-(2,6-Dimethyl-morpholin-4-yl)-ethylamine (**14**) (20 g, 0.126 mol) and DCC (26 g, 0.126 mol) were dissolved in diethyl ether (200 mL). Carbon disulphide (9.6 g, 0.126 mol) in diethyl ether (7.6 mL) was added over a 30 min period. The mixture was stirred for 16 h at 20 °C. The precipitate was filtered and the filtrate concentrated to dryness. Chromatography of the residue on silicagel eluting with dichloromethane gave compound **11** (11.4 g, 45%). ^{1}H-NMR (300 MHz): $\delta = 3.72$ (m, 2H); 3.61 (t, 2H); 2.74 (m, 2H); 2.68 (t, 2H); 1.91 (t, 2H); 1.22 (d, 6H).

*6.1.14. 5-Benzyloxy-pentan-1-ol (**15**)*

Benzyl chloride (506 g, 4 mol) was added to a stirred solution of 1,5-pentanediol (416 g, 4 mol) and a 12 N sodium hydroxide aqueous solution (510 mL). The mixture was stirred at 90 °C for 16 h. After cooling, it was filtered, the organic layer obtained from the filtrate, dried (Na_2SO_4) and concentrated to dryness. The residue was taken up with toluene, concentrated and distilled under vacuum to give compound **15** as an oil (240 g, 31%): b.p. 124–130/0.1 mm.

*6.1.15. (5-Bromo-pentyloxymethyl)-benzene (**16**)*

Bromine (4.9 mL) and 4-benzyloxy-pentan-1-ol (**15**) (19.4 g, 0.1 mol) in acetonitrile (150 mL) was added successively to a stirred solution of triphenylphosphine (25 g) in acetonitrile (200 mL) at a temperature below 10 °C. The mixture was stirred 2 h at 10 °C and 1 h at 20 °C. Triphenylphosphine oxide was filtered and the filtrate concentrated under vacuum. Column chromatography of the residue eluting with a mixture of 15% dichloromethane in cyclohexane gave compound **16** (18.3 g, 71%).

*6.1.16. 3-(5-Benzyloxy-pentyl)-pentanedioic acid diethyl ester (**17**)*

Sodium (11 g, 0.48 mol) was added over 20 min to ethanol (400 mL) to give sodium ethoxide. Malonic acid diethyl ester (80 g, 0.5 mol) was added and the mixture stirred for 10 min and concentrated under vacuum to dryness. The residue was taken up with toluene and the suspension concentrated under vacuum to dryness. The residue was dissolved in dimethylformamide (1 L), (5-bromo-pentyloxymethyl)-benzene **16** (123 g, 0.48 mol) was added, the mixture stirred for 4 days at 20 °C and concentrated to dryness under vacuum. The residue was dissolved in ether, washed successively with aqueous solutions of sodium hydrogencarbonate and lithium chloride, dried (Na_2SO_4), concentrated and distilled

under reduced pressure to give compound **17** as an oil (116 g, 72%): b.p. 175 °C/0.1 mm.

*6.1.17. 2-(5-Benzyloxy-pentyl)-propane-1,3-diol (**18**)*

3-(5-Benzyloxy-pentyl)-pentanedioic acid diethyl ester (**17**) (14.5 g, 0.043 mol) dissolved in diethyl ether (50 mL) was added dropwise to a lithium aluminium hydride in diethyl ether (50 mL) suspension. The mixture was stirred for 2 h at reflux temperature and 20 h at 20 °C. After cooling at 5 °C, water (2.2 mL), 2 N aqueous solution of sodium hydroxyde (4.4 mL) and water (2.2 mL) was successively added. The suspension was filtered, the solid washed with diethylether and the combined filtrates dried (Na_2SO_4) and concentrated under vacuum. Chromatography of the residue on silicagel (1 L) eluting with a mixture 20% tetrahydrofuran in dichloromethane gave compound **18** (7.3 g, 67%).

*6.1.18. Methanesulfonic acid 7-benzyloxy-2-methanesulfonyloxymethyl-heptyl-ester (**19**)*

Methanesulfonyl chloride (20.7 g, 0.18 mol) was added at 5 °C to a stirred solution of 2-(5-benzyloxy-pentyl)-propane-1,3-diol (**18**) (7 g, 0.028 mol) and triethylamine (56 mL) in dichloromethane (115 mL). The mixture was stirred at 5 °C for 5 h and at 20 °C for 20 h. Dichloromethane (200 mL) was added and the mixture washed with an aqueous solution of sodium hydrogencarbonate, dried (Na_2SO_4) and concentrated to dryness under vacuum. Chromatography of the residue on silicagel (1.2 L) eluting with 5% ethyl acetate in dichloromethane gave compound **19** (10 g, 91%).

*6.1.19. 4-(5-Benzyloxy-pentyl)-[1,2] dithiolane (**20**)*

Methane sulfonic acid 7-benzyloxy-2-methanesulfonyloxymethyl-heptyl ester (**19**) (36.5 g, 0.092 mol) was dissolved in dimethylformamide (360 mL). Sulphur (2.95 g) and sodium sulphide monohydrate (22 g) was added and the mixture was stirred at 85 °C for 4 h. Diethylether (1 L) was added and the mixture was washed with an aqueous solution of sodium chloride, dried (Na_2SO_4) and concentrated under vacuum to dryness. Chromatography of the residue on silicagel eluting with a mixture of 50% cyclohexane in dichloromethane gave compound **20** (19 g, 73%).

*6.1.20. 5-[1,2] Dithiolan-4-yl-pentan-1-ol (**12**)*

4-(5-Benzyloxy-pentyl)-[1,2] dithiolane (**20**) (0.3 g, 0.00106 mol) was dissolved in 1,2-dichloroethane (3 mL). A 1 M solution of boron tribromide-methylsulphide complex in dichloromethane (3 mL) was added and the mixture stirred at 20 °C for 2 h, poured in an aqueous solution of sodium hydrogencarbonate and extracted with diethyl ether. The combined organic layers was dried (Na_2SO_4) and concentrated to dryness at a temperature below 30 °C. Chromatography of the residue on silicagel eluting with dichloromethane gave compound **12** (0.15 g, 73%). ^{1}H-NMR (300 MHz): $\delta =$ 4.29 (bt, 1H, exchangeable by D_2O); 3.33 (d, 2H); 3.23 (dd, 2H); 2.78 (dd, 2H); 2.45 (m, 1H); 1.48–1.2 (m, 8H).

6.2. Lipophilicity

Octanol–water (0.15 M KCl) distribution coefficients (log D) at pH 7.4 were measured by a potentiometric method using a Sirius GlpKa apparatus [33,34]. The experiments were carried out under strictly controlled conditions of temperature (25 °C) and ionic strength (0.15 M KCl). At least two different $V_{octanol}/V_{aqueous}$ phase ratios were examined. Log D values for investigated compounds are indicated in Table 1.

6.3. Pharmacology

6.3.1. Peroxidation assay [35]

Fresh mice cortical membrane preparations (1 g per 20 mL, 20 mM) Tris–HCl, pH 7.4) were preincubated for 15 min at 27 °C with the tested antioxidant agent (5 mM). Membranes were then treated for 15 min with $FeSO_4$ (100 μM)–ascorbic acid (1 mM)–H_2O_2 (1 mM).

The reaction was stopped by addition of trichloroacetic acid (20% v/v) at +4 °C, followed by centrifugation for 5 min at +4 °C (1500 g). After addition of 1 mL thiobarbituric acid (0.67%), the supernatants were incubated at 100 °C for 20 min. The reaction was stopped in ice and tissue concentration of malonodialdehyde (expressed as TBARS formation) were measured at 532 nm (Beckman DU 640 spectrophotometer; a standard curve of malonodialdehyde, 0–40 μM, was established for each experiment). Percentage inhibition of lipid peroxidation was expressed in drug treated animals relative to controls ($FeSO_4$–ascorbic acid–H_2O_2 treated animals).

6.3.2. t-Butylhydroperoxide mediated lethality [36]

Based on the method previously described by Adams et al., male NMRI (28–35 g) mice ($n = 10$ per group) were injected i.p. with the compound under study (150 mg kg^{-1} in 0.2 mL Tween-saline solution, gauge needle 4.48 mm) 30 min before an i.c.v. injection of a 70% solution of t-BuOOH (22 mg kg^{-1} per 1 μL saline). Lethality was assessed 2 h after administration of t-BuOOH and was expressed as the percent survival relative to the lethality observed in t-BuOOH plus Tween-saline vehicle-treated animals.

6.3.3. Alloxan-induced hyperglycemia [37]

Fasted (> 18 h) male NMRI (28–35 g) mice ($n = 10$ per group) were administered by oral route with the compound under study (400 mg kg^{-1} in Tween-water; 20 mL kg^{-1}), 60 min before an intravenous (i.v.) injection of alloxan monohydrate (40 mg kg^{-1}). Ani-

mals were sacrificed at 24 h, the plasma recovered and D-glucose levels were evaluated by assay in a Roche Cabas–Fara Analyser. The protection against hyperglycemia was estimated as the difference between alloxan drug-treated animals relative to alloxan-treated animals.

6.3.4. Effect on whole body temperature

The whole body temperature of male NMRI mice ($n = 10$ per group) was measured with a rectal probe (Physitemp, Bat-12) at $t = 0$ h.

Animals (NMRI) were then treated with either vehicle (20 mL kg^{-1}) or the compound under study, by intraperitoneal administration. Rectal temperature was assessed every 30 min for up to 2 h post-injection. Results are expressed as the mean maximal temperature difference, at a given time, between control saline-treated animals and drug-treated mice.

Acknowledgements

We thank Dr. J.P. Volland and his staff for analytical and NMR data, the staff of Cerebral Pathology Division for the pharmacological testing and Mrs. F. Chevereau for the typing of the manuscript.

References

[1] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, 3rd ed, Oxford, London, 1999, pp. 246–350.
[2] K. Hensley, Q.N. Pye, T. Tabatabaie, C.A. Stewart, R.A. Floyd, in: P.L. woods (Ed.), Neuroinflammation: Mechanism and Management, Humana Press, Totowa, NJ, 1998, pp. 265–276.
[3] H. Sies (Ed.), Antioxidants in Disease, Mechanisms and Therapy, Academic Press, London, 1997.
[4] J. Fuchs, L. Packer, G. Zimmer (Eds.), Lipoic Acid in Health and Disease, Marcel Dekker, New York, 1997.
[5] S. Roy, C.K. Sen, H.J. Tritschler, L. Packer, Biochem. Pharmacol. 53 (1997) 393–399.
[6] M. Podda, H.J. Tritschler, H. Ulrich, L. Packer, Biochem. Biophys. Res. Commun. 204 (1994) 98–104.
[7] M. Panigrahi, Y. Sadguna, B.R. Shivakumar, S.V. Kolluri, S. Roy, L. Packer, V. Ravindranath, Brain Res. 717 (1996) 184–188.
[8] G.J. Handelman, D. Han, H.J. Tritschler, L. Packer, Biochem. Pharmacol. 47 (1994) 1725–1730.
[9] Y.J. Suzuki, M. Tsuchiya, L. Packer, Free Radic. Res. Commun. 15 (1991) 255–263.
[10] L.J. Yan, M.G. Traber, H. Kobuchi, S. Matsugo, H.J. Tritschler, L. Packer, Arch. Biochem. Biophys. 327 (1996) 330–334.
[11] V.E. Kagan, A. Shvedova, E. Serbinova, S. Khan, C. Swanson, R. Powell, L. Packer, Biochem. Pharmacol. 44 (1992) 1637–1649.
[12] D.P. Xu, W.W. Wells, J. Bioenerg. Biomembr. 28 (1996) 78–85.
[13] A. Bast, G.R.M.M. Haenen, Biochim. Biophys. Acta 963 (1988) 558–562.
[14] B.C. Scott, O.I. Aruoma, P.J. Evans, C. O'Neill, A. van der Vliet, C.E. Cross, H. Tritschler, B. Halliwell, Free Radic. Res. 20 (1994) 119–133.
[15] L. Packer, H.J. Tritschler, K. Wessel, Free Radic. Biol. Med. 22 (1997) 359–378.
[16] U. Müller, J. Krieglstein, Brain Res. 678 (1995) 265–268.
[17] P. Wolz, J. Krieglstein, Neuropharmacology 35 (1996) 369–375.
[18] S. Löffelhardt, C. Bonaventura, M. Locher, H.O. Borbe, H. Bisswanger, Biochem. Pharmacol. 50 (1995) 637–646.
[19] H. Schempp, H. Ulrich, E.F. Elstner, Z. Naturforsh 49 (1994) 691–692.
[20] S. Goldstein, C. Guillonneau, Y. Charton, P. Lestage, B. Lockhart, 1,2 Dithiolane derivatives, their process of preparation and pharmaceutical compositions containing them EP 1010698 ADIR & Cie, 92415 Courbevoie, France, 2001.
[21] O. Frey, M. Hoffmann, H. Kessler, Angew. Chem. Int. Ed. Engl. 34 (1995) 2026–2028.
[22] M. Saah, W.M. Wu, K. Eberst, E. Marvanyos, N. Bodor, J. Pharm. Sci. 85 (1996) 496–504.
[23] A.K. Ghosh, T.T. Duong, S.P. McKee, W.J. Thompson, Tetrahedron Lett. 33 (1992) 2781–2784.
[24] K. Nishiyama, M. Oba, Tetrahedron Lett. 34 (1993) 3745–3748.
[25] T.L. Yue, J.L. Gu, P.G. Lysko, H.Y. Cheng, F.C. Barone, G. Feuerstein, Brain Res. 574 (1992) 193–197.
[26] B. Matkovics, M. Sasvari, M. Kotorman, I.S. Varga, D.Q. Hai, C. Varga, Acta Physiol. Hung. 85 (1998) 183–192.
[27] R.V. Aruna, B. Ramesh, V.N.R. Kartha, Indian J. Exp. Biol. 37 (1999) 399–401.
[28] T. Fujita, T. Yokoyama, Dithiolan derivatives, their preparation and their therapeutic effect EP0869126 Sankyo Company Ltd., Tokyo 103-8426, Japan, 1998.
[29] L. Packer, O. Tirosh, C.K. Sen, S. Roy, Lipoic acid analogs WO 99/45922 The Regents of the University of California, Oakland, CA 94612-3550, USA, 2000.
[30] R. Schreck, R. Grassmann, B. Fleckenstein, P.A. Baeuerle, J. Virol. 66 (1992) 6288–6293.
[31] H. Bethge, R. Möller, T. Deibvvenger, K. Huthmacker, G. Blaschke, Preparation and use of enantiomerically pure α-Lipoic acid salts Deutsches Petentamt DE 4137773A1, 1994.
[32] J.P. Hénichart, J.L. Bernier, R. Houssin, Synthesis (1980), 311–312.
[33] F.H. Clarke, N.M. Cahoon, J. Pharm. Sci. 76 (1987) 611–619.
[34] B. Slater, A. McCormack, A. Avdeef, J.E.A. Comer, J. Pharm. Sci. 83 (1994) 1280–1283.
[35] M. Mihara, M. Uchiyama, Anal. Biochem. 86 (1978) 271–278.
[36] J.D. Adams, Jr., L.K. Klaidman, Y.M. Huang, J.J. Cheng, Z.J. Wang, M. Nguyen, B. Knusel, A. Kuda, Mol. Chem. Neuropathol. 22 (1994) 123–142.
[37] L.W. Oberley, Free Radic. Biol. Med. 5 (1988) 113–124.