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Design and synthesis of 1,2-dithiolane derivatives and evaluation of their neuroprotective activity

Maria Koufaki,[a,*] Christina Kiziridi,[a] Faidra Nikoloudaki[b] and Michael N. Alexis[b]

[a]*National Hellenic Research Foundation, Institute of Organic and Pharmaceutical Chemistry, 48 Vas. Constantinou Avenue, 116 35 Athens, Greece*

[b]*Institute of Biological Research and Biotechnology, 48 Vas. Constantinou Avenue, 116 35 Athens, Greece*

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Abstract—We designed and synthesized new analogues containing 1,2-dithiolane-3-alkyl and protected or free catechol moieties connected through heteroaromatic rings such as triazole, 1,2,4-oxadiazole, 1,3,4-oxadiazole, tetrazole or thiazole in order to explore the influence of the bioisosteric replacement of the amide group on the neuroprotective activity of the lipoic acid/dopamine conjugate. Evaluation of the activity of the new compounds, using glutamate-challenged hippocampal HT22 cells, showed that incorporation of heteroaromatic rings in the alkyl-1,2-dithiolane moieties in conjunction with another antioxidant, in this case catechol, may result in strong neuroprotective activity.

Oxidative stress is a ubiquitously observed hallmark of neurodegenerative disorders.[1] Neuronal cell dysfunction and cell death due to oxidative stress may causally contribute to the pathogenesis of progressive neurodegenerative disorders, as well as of acute syndromes of neurodegeneration.[2–5] The pathways to nerve cell death induced by a diverse array of neurotoxins, including peptides, excitatory amino acids, and cytokines, commonly share oxidative downstream processes which can cause oxidative destruction of key regulators of cell survival and/or activation of secondary events leading to apoptosis.[6–8]

Elevated levels of the excitatory amino acid glutamate are thought to be responsible for CNS disorders through various mechanisms causing oxidative stress *via* depletion of intracellular glutathione (GSH). Maintenance of normal GSH levels within the brain may hold an important key to the prevention and therapy for neurodegenerative diseases. In this respect, neuroprotective antioxidants are considered a promising approach to slowing the progression and limiting the extent of neuronal cell loss in these disorders. However, clinical evidence demonstrating that antioxidant compounds can act as a means to prevent and/or treat neurodegenerative disease is still relatively scarce.[9]

α-Lipoic acid (LA)[10–12] was first isolated in 1951 and is known by a diversity of names including 1,2-dithiolane-3 pentanoic acid, 1,2-dithiolane-3 valeric acid, and thioctic acid. The naturally occurring *R*-enantiomer of LA is an essential cofactor in α-ketoacid dehydrogenase complexes and is known to be covalently attached to the amino group of a lysine residue. Exogenously supplied LA is transported to tissues and reduced to dihydrolipoic acid (DHLA). LA and DHLA were found to be highly reactive against a variety of ROS in vitro. The antioxidant activity of LA is also attributed to its capacity to regenerate intracellular GSH, vitamin C, and vitamin E.[13–15] Numerous studies have reported that LA is beneficial in a number of oxidative stress models of cell death pertinent to ischemia–reperfusion injury, neurodegeneration, diabetes, inflammation, and radiation injury. Thus, LA possesses the potential to intervene in various therapeutically interesting pathways.[16–20] However, it should be noted that LA reportedly exerts most of its effects at high micromolar concentrations; that amides of LA exhibit higher biological activity than the parent compound;[21,22] and that molecular combinations obtained by coupling LA with an amino-substituted bioactive moiety possess multifunctional activity.[23–31]

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* Corresponding author. Tel.: +30 210 7273818; fax: +30 210 7273831; e-mail: mkoufa@eie.gr

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The present work involves replacement of the amide functionality by heterocyclic five-membered rings. Organic compounds containing five-membered aromatic heterocyclic rings are widely distributed in nature and often play an important role in various biochemical processes. Moreover, heteroaromatic rings serve as bioisosteres of several substituents such as amides, esters, phenyl rings and can contribute substantially higher pharmacological activity to the resulting compounds.[32–34] In the light of this, they are often incorporated into new chemical entities by medicinal chemists.

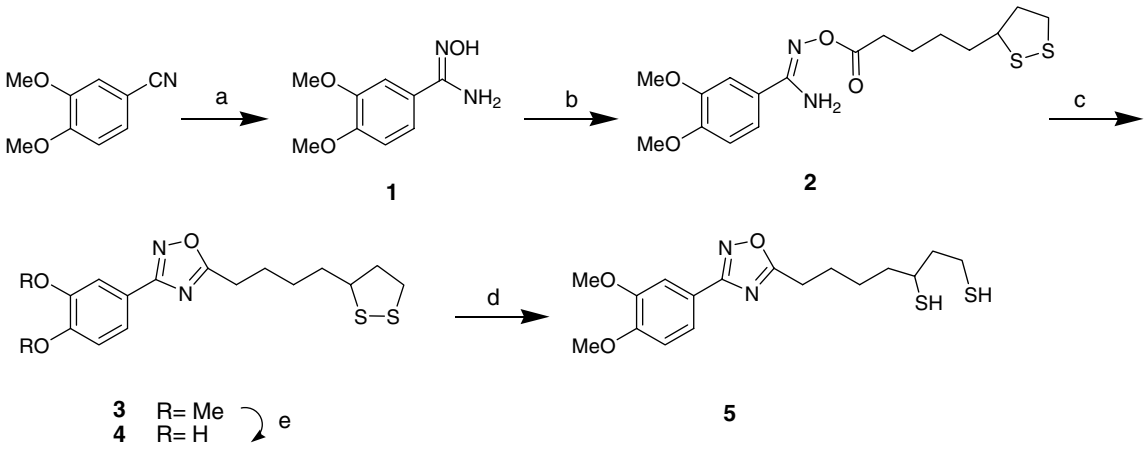
We designed and synthesized new LA analogues containing 1,2-dithiolane-3-alkyl and protected or free catechol moieties connected through heteroaromatic rings such as triazole, 1,2,4-oxadiazole, 1,3,4-oxadiazole, tetrazole or thiazole. The aim of this work is to compare the neuroprotective activity of the new compounds with that of LA as well as its amide with dopamine. The neuroprotective activity of the new derivatives was evaluated using glutamate-challenged HT22 mouse hippocampal cells.[35,36]

The synthesis of 1,2,4-oxadiazole containing derivatives is illustrated in Scheme 1. 3,4-Dimethoxybenzonitrile was converted to *N*-hydroxy-amidine **1** by treatment with hydroxylamine hydrochloride. Reaction of **1** with LA in the presence of DCC afforded the acyl amidoxime **2.** Intramolecular cyclization in the presence of tetrabutylammonium fluoride[37] gave the oxadiazole analogue **3** which was deprotected using $BF_3(SMe)_2$. Reduction of the 1,2-dithiolane ring of **3** using $NaBH_4$ afforded dithiol **5**.

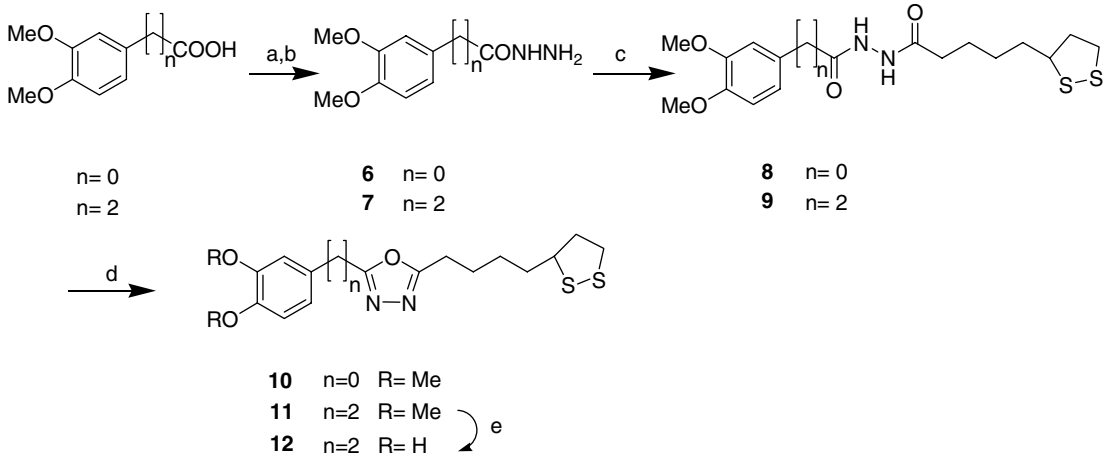
1,3,4-Oxadiazole containing analogues were synthesized as shown in Scheme 2. Hydrazides **6** and **7** were prepared from the corresponding esters and then reacted with *N*-hydroxysuccinimide-activated LA to give **8** and **9**. Cyclodehydration in boiling $POCl_3$[38] afforded **10** and **11**.

Triazoles (Scheme 3) were synthesized by cycloaddition[39] of lipoylazide and 3,4-dimethoxyphenylacetylene. Azide **14** was prepared by reduction of LA with catechol borane, conversion of the resulting alcohol to mesylester **13** and then to azide **14** by treatment with sodium azide.

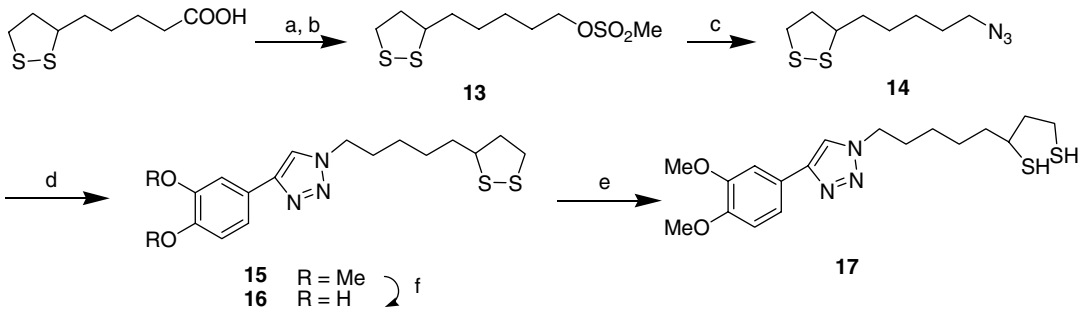
The synthesis of tetrazole derivatives is depicted in Scheme 4. *N*-Hydroxysuccinimide-activated LA reacted



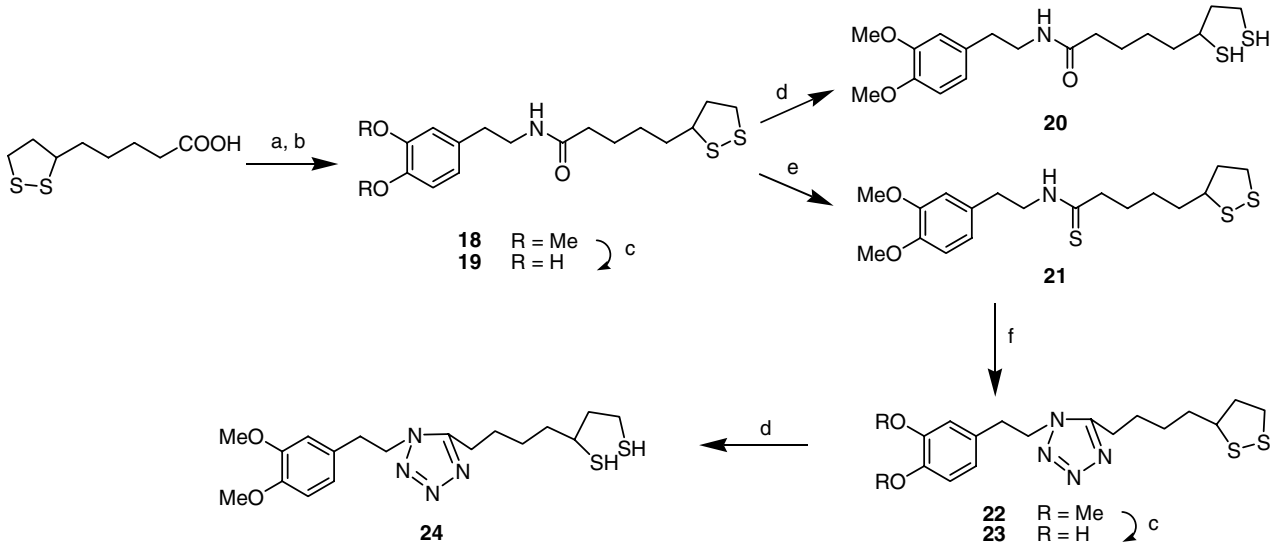
Scheme 1. Reagents: (a) $NH_2OH{\cdot}HCl$, Et_3N; (b) lipoic acid, DCC, THF; (c) TBAF, THF; (d) $NaBH_4$, EtOH; (e) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2.



Scheme 2. Reagents and condition: (a) dimethyl sulfate, K_2CO_3, TBAI, acetone; (b) $NH_2NH_2{\cdot}H_2O$, MeOH; (c) *N*-hydroxysuccinimide-activated LA, THF; (d) $POCl_3$, reflux; (e) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2.



Scheme 3. Reagents: (a) catechol borane, CH_2Cl_2; (b) CH_3SO_2Cl, CH_2Cl_2; (c) NaN_3, DMF; (d) 3,4-dimethoxyphenylacetylene, $CuSO_4{\cdot}5H_2O$, sodium ascorbate; (e) $NaBH_4$, EtOH; (f) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2.



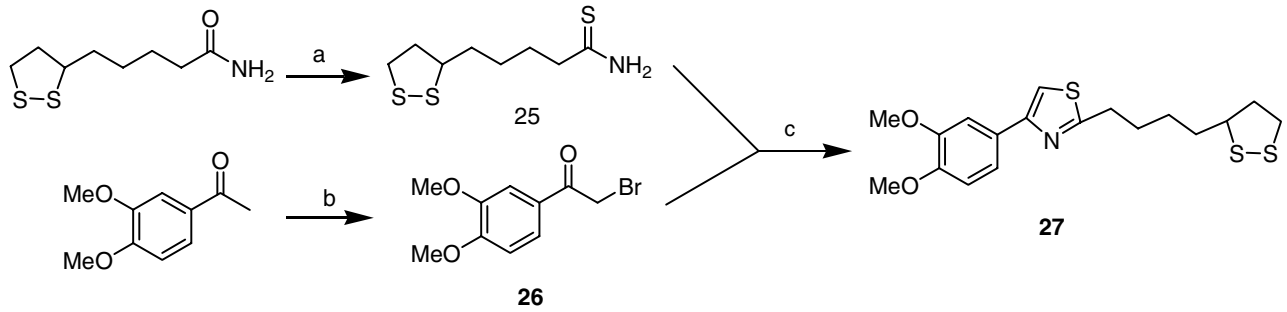
Scheme 4. Reagents and condition: (a) NHS, DCC, dioxane; (b) 3,4-dimethoxyphenethylamine, CH_2Cl_2; (c) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2; (d) $NaBH_4$, EtOH; (e) Lawesson's reagent, THF, reflux; (f) DIAD, TMSA, Ph_3P, THF.

with 3,4-dimethoxyphenethylamine to afford amide **18** which in turn converted to thioamide **21** by treatment with Lawesson's reagent. Tetrazole **22** was obtained by reaction of **21** with trimethylsilyl azide (TMSA), in the presence of triphenylphosphine and diisopropylazodicarboxylate (DIAD).[40] Deprotection of the catechol moiety, by treatment with $BF_3{\cdot}S(Me)_2$, gave analogue **23**.

Thiazole **27** was prepared from lipoamide and 3,4-dimethoxyacetophenone as depicted in Scheme 5.

HT22 cells suffer cell death within 24 h following exposure to 1–5 mM glutamate. The cells lack ionotropic glutamate receptors (that could mediate excitotoxicity) and are known to succumb to acute oxidative stress due to glutamate inhibition of cystine uptake and the ensuing depletion of intracellular GSH.[35,41,42]

The new compounds were tested at concentrations 0.01–10 μM. As expected, LA was inactive at these low μM concentrations. The presence of catechol group (**4**, **12**,



Scheme 5. Reagents and condition : (a) Lawesson's reagent, THF; (b) pyridinium tribromide, $CHCl_3$, EtOH; (c) EtOH, reflux.

Table 1. Neuroprotective activity of the new compounds, using glutamate-challenged hippocampal HT22 cells[43]

Compound	EC_{50} (μM) (means ± SEM)	Compound	EC_{50}[a] (μM) (means ± SEM)
3	>10	**17**	>10
4	3.63 ± 0.33	**18**	>10
5	>10	**19**	>10
10	>10	**20**	>10
11	>10	**22**	>10
12	4.21 ± 0.40	**23**	2.99 ± 0.14
15	>10	**24**	>10
16	6.23 ± 0.97	**27**	>10

[a] EC_{50} values are compound concentrations required to secure a viability in the glutamate-exposed cells equal to 50% of that of the non-exposed cells calculated as described in the Experimental section. Values are means ± SEM of at least three independent experiments.

16, **23**) was requisite for neuroprotective activity in all the LA derivatives we tested, except in the case of the dopamine conjugate **19**, which did not show any activity at the concentrations tested (Table 1). Interestingly, however, its analogue **23**, in which the amide functionality was replaced by the tetrazole ring, ranked as the strongest neuroprotectant, while the 1,3,4-oxadiazole derivative **12** was somewhat less potent. Thus, it appears that the replacement of the amide functionality by the aromatic heterocycles conveys greater neuroprotective activity to the resulting compounds. As regards compounds in which the heterocyclic ring is directly attached to the catechol moiety, 1,2,4-oxadiazole analogue **4** is almost two times more potent than the triazole derivative **16**. From the present data one can assume that the presence of an alkyl chain between catechol and the heteroaromatic ring has little influence on the activity, since oxadiazoles **4** and **12** have very similar activity. By comparison, the nature of heterocycle seems to have a somewhat higher effect on the activity (tetrazole derivative **23** is more active than the oxadiazole derivative **12** and the oxadiazole derivative **4** is more potent than the triazole analogue **16**).

Although the presence of a free catechol moiety was found to be requisite for the activity of the new molecules, reduction of dithiolane ring did not improve its neuroprotective effect. A possible explanation is that the dithiol group of the analogues **5**, **17**, **20**, and **24** is either readily reoxidized to dithiolane in the culture medium and/or incompatible with compound entry into the cell.

These present data show that LA and its conjugates with protected or free dopamine (**18**,**19**) are inactive at low μM concentrations against glutamate-induced damage in HT22 cells. Comparison of the EC50 values of compounds **19** and **23** suggests that the presence of the heteroaromatic ring is associated with threefold increase in antioxidant activity at the least. Similarly, comparison of compounds **22** and **23** suggests that the presence of the catechol moiety is also associated with threefold increase in antioxidant activity at the least. Thus, it appears that in the case of **23** the effect of housing a heteroaromatic ring and a catechol moiety in one molecule is synergistic rather than additive.

In conclusion, analogues possessing strong neuroprotective activity can be obtained by inserting heteroaromatics in the alkyl-1,2-dithiolane moieties in conjunction with the incorporation of another antioxidant entity, in this case the catechol moiety.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.05.036.

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