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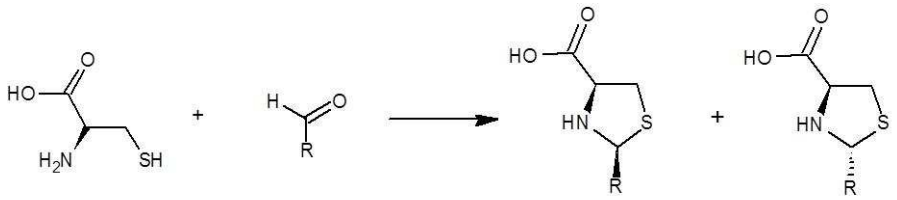

2R, 4R 2S, 4R

R			
Ph-	1		
o-CH_3O-Ph	2	*p*-CH_3O-Ph-	8
o-CH_3-Ph-	3	*p*-CH_3-Ph-	9
o-CN-Ph-	4	*p*-NO_2-Ph-	10
o-Br-Ph-	5	2,6-difluoro-Ph-	11
m-Cl-Ph-	6	2,3-difluoro-Ph	12
p-Cl-Ph-	7	2,3-dichloro-Ph-	13

Antioxidant activity

Cysteine release or NOT ?

The Cysteine Releasing Pattern of Some Antioxidant Thiazolidine-4-carboxylic acids.

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ABSTRACT.

Oxidative stress that corresponds to a significant increase in free radical concentration in cells can cause considerable damage to crucial biological macromolecules if not prevented by cellular defense mechanisms. The low-molecular-weight thiol glutathione (GSH) constitutes one of the main intracellular antioxidants. It is synthesized *via* cysteine, an amino acid found only in limited amounts in cells because of its neurotoxicity. Thus, to ensure an efficient GSH synthesis in case of an oxidative stress, cysteine should be provided extracellularly. Yet, given its nucleophilic properties and its rapid conversion into cystine, its corresponding disulfide, cysteine presents some toxicity and therefore is usually supplemented in a prodrug approach. Here, some thiazolidine-4-carboxylic acids were synthesized and evaluated for their antioxidant properties via the DDPH and CUPRAC assays. Then, the cysteine releasing capacity of the obtained compounds was investigated in aqueous and organic medium in order to correlate the relevant antioxidant properties of the molecules with their cysteine releasing pattern. As a result, the structures' antioxidative properties were not only attributed to cysteine release but also to the thiazolidine cycle itself.

KEYWORDS: oxidative stress, thiazolidine, cysteine, prodrug, heterocycle cleavage.

1. Introduction

Continuous exposure of a cell to high levels of reactive oxygen or nitrogen species causes an imbalance in redox homeostasis [1, 2]. Such a state is better known as an oxidative stress, that can cause various diseases such as atherosclerosis [3-5], cancer [6, 7] or neurodegenerative disorders [8-10].

Thiols are compounds that present a sulfhydryl group on their structures and are largely described for their antioxidant properties. Conversion into disulfides and scavenging radicals constitute the main mechanisms through which thiols act as reducing agents [11-16]. Glutathione, the most abundant thiol-containing small molecule of the cell helps to fight against oxidative stress when converted into its corresponding disulfide in the presence of reactive species [17-19]. Glutathione biosynthesis requires cysteine [20]. Thus, this precursor's intracellular levels should be increased in case of an oxidative stress [21].

As free *L*-cysteine supplementation has been related to toxicity, [22-24] pro-drugs capable of releasing the sulfur-containing amino acid directly or after enzymatic cleavage once administrated have been developed [25-35].

Thiazolidines, five membered heterocycles resulting from the condensation of carbonyl compounds with cysteine are already described for their cysteine releasing capacity and some 2-alkyl and aryl substituted thiazolidine carboxylic acids were evaluated for their antioxidant properties [29-32, 34, 36]. In this study we aimed to generate a series of thiazoldine-4-carboxylic acids as cysteine prodrugs. A series of substituted benzaldehydes comprising diverse electron-withdrawing and electron attracting groups were chosen, so that the impact of substitution on the cysteine releasing pattern and the antioxidant activity could be further discussed. After being synthesized and characterized, the molecules' antioxidative properties were evaluated using radical scavenging methods.

2. Results

2.1 Chemistry

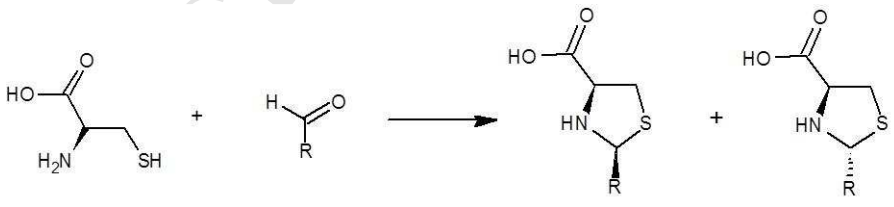
Thiazolidine carboxylic acid derivatives were synthesized with good yields by condensing *L*-cysteine with a series of substituted benzaldehyde derivatives (Table 1). The typical cyclization reaction was carried out in basic conditions in a water/ethanol mixture (50:50, v:v) as suggested in the literature for the preparation of these compounds [5, 31, 37, 38].

	R	Yield (%)	2R,4R/2S,4R ratio
1	Ph-	89	70/30
2	*o*-CH_3O-Ph	65	40/60
3	*o*-CH_3-Ph-	75	60/40
4	*o*-CN-Ph-	62	95/5
5	*o*-Br-Ph-	86	70/30
6	*m*-Cl-Ph-	74	90/10
7	*p*-Cl-Ph-	75	95/5
8	*p*-CH_3O-Ph-	72	50/50
9	*p*-CH_3-Ph-	85	60/40
10	*p*-NO_2-Ph-	80	5/95
11	2,6-difluoro-Ph-	75	40/60
12	2,3-difluoro-Ph	80	40/60
13	2,3-dichloro-Ph-	84	40/60

Table 1. Yields and diastereomeric ratios of compounds **1**-**13**

The structures were analyzed by FT-IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. The formation of the thiazolidine heterocycle was confirmed through the typical signals corresponding to the second position of the ring. While the hydrogen on C-2 gave a distinctive singlet around 5.5 ppm, the signal of the carbon atom appeared as a peak at around 70 ppm.

The ring closure reaction results from two successive nucleophilic attacks of the aldehyde and leads to the generation of a new chiral center in an uncontrolled manner [39, 40]. Thus, thiazolidine derivatives are obtained as diastereomeric mixtures (Figure 1).



2R, 4R 2S, 4R

R			
Ph-	**1**		
o -CH_3O-Ph	**2**	*p* -CH_3O-Ph-	**8**
o -CH_3-Ph-	**3**	*p* -CH_3-Ph-	**9**
o -CN-Ph-	**4**	*p* -NO_2-Ph-	**10**
o -Br-Ph-	**5**	2,6-difluoro-Ph-	**11**
m -Cl-Ph-	**6**	2,3-difluoro-Ph	**12**
p -Cl-Ph-	**7**	2,3-dichloro-Ph-	**13**

Figure 1. Synthesis of substituted 2-phenyl-4-carboxylic acid thiazolidine derivatives Reagents: benzaldehyde derivatives in C_2H_5OH/H_2O (1/1, v/v)

Although clearly distinguishable on ^{1}H NMR *via* the proton on the C-2 carbon of the heterocycle (Figure 2), the isolation of the 2R, 4R and 2S, 4R isomers was not achieved since in fact, there is a rapid interconversion of one diastereomer into the other through the corresponding Schiff base in physiological or basic pH values [41].

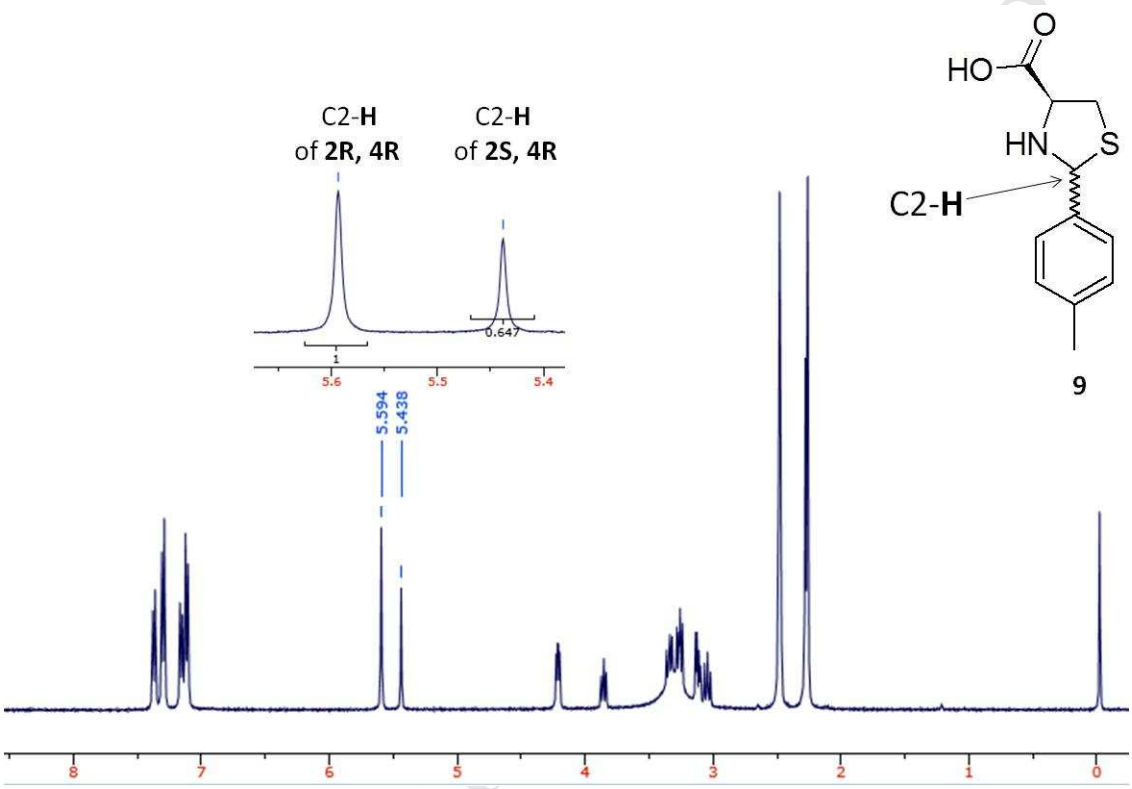


Figure. 2 ^{1}H NMR of compound **9** diastereomeric ratios are determined *via* the C2 protons. The assignment to 2R,4R and 2S,4R diastereomers are given on the basis of NOE experiments detailed in the literature.

Interestingly, nevertheless, the diastereomeric mixture ratios varied according to the substituents of the aromatic cycle (Table 1), the equilibrium being clearly in favor of one of the isomers in the case of **4**, **6**, **7** and **10**. While the *ortho*-cyano, *meta*- and *para*-chloro benzaldehydes definitely orient the reaction to the formation of the 2R, 4R isomer (distinction made through NOE data given in the literature [42]), the *p*-nitrobenzaldehyde led preferably to the 2S, 4R thiazolidine (**7**) molecule. The exact reason for such displacement of the equilibrium remains unknown since we could not precisely correlate the diastereomeric mixture ratios neither with the electron-attracting or withdrawing properties of the substituent nor with its size or position.

2.2 Biology

The synthesized thiazolidine compounds (**1-13**) were evaluated for their antioxidant properties using the classical 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and by determining the cupric reducing antioxidant capacity (CUPRAC) of the compounds.

2.2.1 The DPPH assay

The DPPH assay, a very commonly used methodology for analyzing the antioxidant activity of molecules, consists of the determination of the compound concentration that is capable of scavenging 50% of the DPPH radicals in solution by monitoring the decrease of absorbance at 517 nm that corresponds to the maximum of absorbance wavelength of the stable free DPPH radicals. To ensure a rapid and simultaneous screening of the scavenging capacity of the synthesized thiazolidines, the existing procedure[43, 44] was modified and carried out with 96-well plates.

Aqueous DMSO was chosen as a solvent as samples are not soluble in water at physiological pH. The DMSO concentration was increased up to 5% (v/v) to dissolve a maximum number of thiazolidine carboxylic acids. Yet, even at this elevated DMSO content, six samples (compound **4** and from **6** to **10**) did not dissolve and could not be evaluated. The DPPH radical scavenging ability of the analyzed structures was determined as indicated in the experimental part and results are summarized in Table 2. The concentrations required for scavenging 50% of the DPPH radicals were calculated and indicated as IC_{50} values expressed in micromolars as it is commonly done for DPPH scavenging assays in the literature. The obtained values were compared to the antioxidant capacity of butylated hydroxytoluene (BHT), the classical reference used for DPPH assays and cysteine since the synthesized structures are meant to be cysteine prodrugs.

	BHT	cysteine	1	2	3	4	5	11	12	13
IC_{50} in DMSO (µM)	not soluble	18.4 ±0.1	45.2 ±0.3	29.5 ±0.5	39.0 ±0.3	>1mM	37.7 ±0.1	38.3 ±0.4	46.8 ±0.8	61.7 ±2.3
IC_{50} in methanol (µM)	517 ±25	19.2±0.5	844.2±2.2	342.7±8.4	46.1±0.9	>1mM	39.1±1.3	343.4±12	>1mM	46.2±1.4

Table 2. DPPH scavenging capacity of compounds 1-13: IC_{50} values determined with samples dissolved either in aqueous DMSO (%5, v/v) or in methanol

Cysteine, with an IC_{50} value of 18.4±0.1 µM gave the highest antioxidant capacity, an expected behavior that can be attributed to the thiol function. The thiazolidine compounds were also found to have a greater antioxidant

capacity when compared to BHT. The promising antioxidant property that thiazolidine molecules exhibited strongly suggested a ring opening reaction in aqueous medium that leads to the antioxidant cysteine molecule.

To check that the antioxidant capacity is due to the ring opening reaction that easily occurs in aqueous medium, the DPPH assay was also carried out by dissolving the samples in methanol. The significant increase of the IC_{50} values observed for compounds **1**, **2**, **11** and **12** while cysteines's activity remained constant supported the hypothesis that the high antioxidant activity observed in aqueous DMSO can be attributed to the release of cysteine.

To confirm this hypothesis an HPLC analysis was also carried out with the tested compounds. The analysis was performed either with samples being dissolved in aqueous DMSO or methanol before injection. Given that the DPPH assay requires an incubation time of 50 minutes, HPLC analyses of the samples were also repeated 50 minutes after dissolution. Since the ring opening reaction corresponds to the reverse of the cyclization reaction, cysteine release was controlled by monitoring the peak corresponding to the aldehyde obtained after ring cleavage. Results are summarized in Table 3.

		1			**2**			**3**			**5**			**11**			**12**			**13**		
		0'	**20'**	**50'**	**0'**	**20'**	**50'**	**0'**	**20'**	**50'**	**0'**	**20'**	**50'**	**0'**	**20'**	**50'**	**0'**	**20'**	**50'**	**0'**	**20'**	**50'**
5% aq DMSO	% Thiazolidine	76.3	27.8	13.8	82.8	43.9	26.8	6.8	5.9	5.6	19.0	16.2	14.3	53.8	39.2	38.2	83.7	50.0	28.7	17.8	16.6	15.7
	% Aldehyde	16.5	69.3	84.6	17.2	56.1	73.2	92.3	93.2	93.5	81.0	83.8	85.6	46.1	59.8	60.7	10.6	46.1	67.4	75.3	.76.5	77.4
PBS	% Thiazolidine	78.4	27.8	13,8	82.5	34.0	21.0	15.2	11.3	8.9	11.6	16.3	18.5	79.4	61.1	46.9	92.7	69.3	50.1	15.3	17.4	17.9
	% Aldehyde	21.4	71.9	84.3	17.3	66.0	78.9	84.8	88.6	90.9	88.2	83.5	81.0	6.4	28.4	45.7	7.1	30.7	48.9	80.6	81.7	82.1
pH=2.0	% Thiazolidine	85.0	62.4	49.9	92.9	78.9	67.6	12.9	11.3	10.1	14.1	11.5	9.5	96.2	72.3	54.8	87.3	64.6	43.5	21.7	17.5	14.2
	% Aldehyde	14.7	37.2	49.2	6.5	20.7	30.2	86.4	87.4	88.8	84.3	86.2	88.1	3.1	27.0	44.4	9.0	33.1	54.1	76.7	80.4	83.5
MeOH	% Thiazolidine	96.1	95.7	95.4	100	98.6	97.3	4.0	4.1	4.2	70.5	74	75.6	100	99.4	98.2	100	100	98.8	15.5	25.2	38.2
	% Aldehyde	3.8	4.3	4.6	0	1.4	2.2	95.9	95.8	95.7	22.2	18.2	15.9	0	0.5	1.3	0	0	0.5	83.8	74.1	59.9

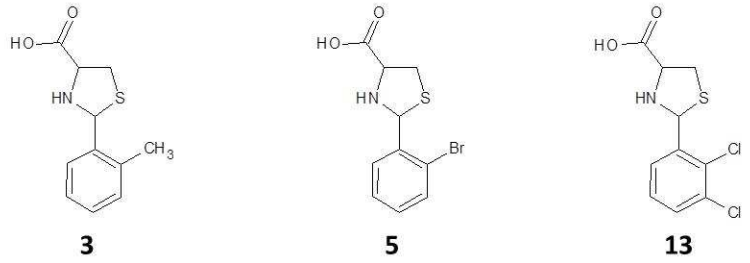
Table 3. HPLC analyses of thiazolidine compounds after dissolution in 5% aqueous DMSO, PBS, phosphoric acid buffer or methanol at time zero, 20 minutes after dissolution and 50 minutes after dissolution

Chromatograms obtained for the samples were consistent with the IC_{50} values obtained with the DPPH assay. First of all, in aqueous DMSO, all of the tested thiazolidines were shown to be progressively converted into cysteine and benzaldehyde since HPLC analyses carried out 50 minutes after dissolution demonstrated a remarkable increase in the benzaldehyde ratio when compared to the one observed at time zero. These results strongly support that the significant antioxidant activity observed for all compounds with the DPPH assay in this solvent is related to the release of cysteine in the medium.

Concerning the results obtained when samples were dissolved in methanol, they indicate a ring opening for compounds **3**, **5** and **13**. For the other tested compounds (**1**, **2**, **11** and **12**) benzaldehyde ratios remained insignificant even for injections realized 50 minutes after compound dissolution, indicating the absence of cleavage for the heterocycles in these conditions. The noticeable increase in the IC_{50} values obtained for compounds **1**, **2**, **11** and **12** can thus be explained by the lack of immediate cysteine release in these conditions.

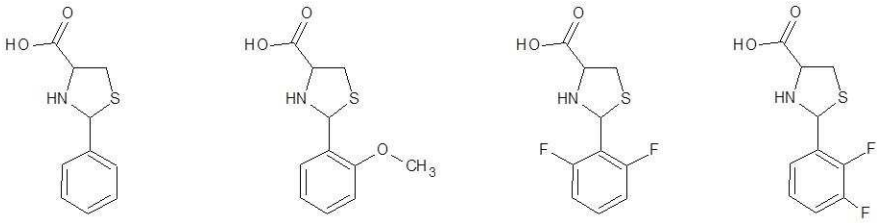
The reason of the differences in cysteine releasing ability of the synthesized thiazolidines observed in methanol remain unexplained, since no obvious relationship between the structures and the ring cleavage phenomenon could be found (Figure 3).

a) Cysteine releasing structures in MeOH



3 **5** **13**

b) Stable thiazolidines in MeOH



1 **2** **11** **12**

Figure. 3 (a) Structures capable of releasing cysteine in methanol (MeOH) (**b**) Structures that are stable and do not open to release cysteine in MeOH

For instance, while the *o,m*-difluoro substituted derivative **12** was shown to be stable in methanol even 50 minutes after dissolution, its close analogue **13** (*o,m*-dichloro substituted thiazolidine) exhibited aldehyde peaks very quickly.

As a result, it is possible to assume that the antioxidant activity determined *via* the DPPH assay is closely related to the cysteine releasing capacity of the thiazolidine compounds.

We also evaluated the cysteine releasing pattern of these compounds in physiological pH (using the PBS phosphate buffer) and in acidic medium (0.1M phosphoric acid, pH=2.0) in order to analyze the stability of the structures in conditions mimicking the stomach environment. For both media, the cysteine releasing pattern was shown to be similar to the one obtained for the studies carried out in water even if a slower ring opening was observed in acidic medium (Table 3).

These results suggested thus, that cysteine should be released when the thiazolidine molecule would be injected in the body.

2.2.2The CUPRAC assay

The antioxidant capacities of the synthesized thiazolidines were also evaluated using the CUPRAC assay. This method developed by Apak *et al.* consists of monitoring the Cu(I)-neocurpine complex formation at 450 nm, a complex that results from the reduction of the Cu(II)-neocuprine complex in an ammonium acetate buffer in the presence of a reducing agent. The antioxidant capacity is determined using the Trolox Equivalent Antioxidant Capacity (TEAC) coefficient, a coefficient that corresponds to the ratio of the molar extinction coefficients of the sample and trolox ($TEAC=\varepsilon_{sample}/\varepsilon_{Trolox}$) [45].

Samples were prepared in aqueous 5% DMSO and methanol and TEAC coefficient were determined for both conditions (Table 4).

	cysteine	**1**	**2**	**3**	**5**	**6**	**7**	**9**	**10**	**11**	**12**	**13**
$TEAC_{MeOH}$	0.63±0.09	1.54 ±0.12	0.96 ±0.06	0.57±0.05	0.80±0.07	1.50 ±0.07	1.52 ±0.01	1.22 ±0.06	0.74 ±0.04	0.83 ±0.09	0.83 ±0.06	0.93 ±0.05
$TEAC_{DMSO}$	0.49±0.03	0.99±0.05	0.79±0.04	0.75±0.04	1.00±0.05	ND	ND	ND	ND	1.05±0.1	0.84±0.05	0.99±0.05

Table 4. TEAC coefficients of samples. Samples were dissolved either in aqueous DMSO (%5, v/v) or in methanol

The $TEAC_{MeOH}$ of compound **4** and **8** and the $TEAC_{DMSO}$ of compounds **4**, **6**-**11** could not be determined due to solubility issues. In both aqueous phase and methanol, the antioxidant capacities of all samples were found to be greater than cysteine, a finding that supports that the antioxidant capacity of thiazolidines should not be related only to cysteine release and that the thiazolidine moiety itself could reduce Cu (II). Moreover in methanol four samples (**1**, **6**, **7**, **9**) were found to have $TEAC_{MeOH}$ coefficient values greater than one, indicating an antioxidant capacity even better than trolox, a property that can again be attributed to the presence of the thiazolidine ring.

3. Discussion

Thiazolidine-4-carboxylic acids have already been described in the literature for their antioxidant activity[29-32, 34, 36] but published studies did not present any data concerning the cysteine releasing capacity of these molecules. In this work we aimed to develop some thiazolidine-4-carboxylic acids from a series of substituted benzaldehydes and analyzed their antioxidant capacities via two methods, the DPPH and CUPRAC assays. These assays were performed in both aqueous and organic media to analyze possible differences in cysteine releasing pattern. The results indicated that in aqueous medium the thiazolidine heterocycle was converted into its corresponding benzaldehyde and cysteine for at least 61% within 50 minutes, the required incubation time for the DPPH assay suggesting strongly that the observed antioxidant activity is attributable to cysteine. This hypothesis can be confirmed with the results obtained when derivatives are dissolved in methanol since then the cysteine releasing capacities of the compounds are shown to be modified along with their antioxidant activity.

Our results demonstrated that cysteine releasing capacity depends on the substitution of the phenyl ring of 2-phenyl-thiazolidine-4-carboxylic acid derivatives but that this ability is not a feature of the position of the substitution since both the stable and unstable structures present substituents at their *ortho*- position. Also, the stability of the heterocycle cannot be related to the electron-withdrawing or electron-donating properties of the substituents since for instance **3** and **5** that are *o*-methyl (an electron-donating group) and *o*-bromo (an electron-withdrawing group) derivatives respectively, are both shown to be unstable. Similarly, the *o*-methoxy **2**(an electron-donating group) and *o,m*-difluoro **12** (electron-withdrawing groups) tend to be stable and not release cysteine when dissolved in methanol. Since the cysteine releasing capacity of thiazolidine rings is a key feature for the development of cysteine prodrugs, we believe, given our results, that the nature of the aldehyde from which the cycle derives should be considered when the prodrugs are designed since its structure can affect the cysteine release pattern of the resulting thiazolidine.

Another finding of our antioxidant analyses results indicate that the antioxidant activity of thiazolidine rings cannot be only attributed to their cysteine releasing property since our CUPRAC assay results demonstrated that antioxidant activity can be observed with stable thiazolidine structures (compound **1**, **6**, **7** and **9**). These data suggest that the thiazolidine ring can be used in antioxidant drug design without being a cysteine prodrug.

4. Conclusion

To conclude, in this work, 2-phenyl thiazolidine carboxylic acid derivatives were synthesized by condensing *L*-cysteine with a series of mono or disubstituted benzaldehyde derivatives. DPPH scavenging and CUPRAC assays were performed and the compounds were found to be capable of reducing the DPPH radical and Cu (II). Also, the antioxidant capacity of the samples was shown to be related not only to the *L*-cysteine release but also to the reducing capacity of the thiazolidine ring itself.

5. Experimental

General procedure for the synthesis of compounds 1 to 13:

L-cysteine hydrochloride monohydrate (1.0 eq) and sodium hydroxide (1.0 eq) were dissolved in water. Then aldehyde (1.0 eq) in ethanol was added and the mixture was stirred at room temperature (RT) for three hours. Solid crystals of the expected product were filtered and washed with dichloromethane. The crystals were dried under *vacuo*.

(*2RS, 4R*)-2-phenyl-1,3-thiazolidine-4-carboxylic acid **(1)**

Yield: %89. m.p.: 163 °C. FT-IR (KBr), cm^{-1}: 3436, 1575, 1435 ^{1}H-NMR (DMSO-d_6) δ: 3.08 (t, *J*=8.8 Hz, 0.3H, NCHC$\underline{H}_2$S); 3.14 (dd, J_1=4.8 Hz, J_2=10.4 Hz, 0.7H, NCHC$\underline{H}_2$S); 3.30 (dd, J_1=7.6 Hz, J_2=10.8 Hz, 0.7H, NCHC$\underline{H}_2$S); 3.38 (dd, J_1=7.6 Hz, J_2=10.4 Hz, 0.3H, NCHC$\underline{H}_2$S); 3.90 (dd, J_1=7.6 Hz, J_2=8.8 Hz, 0.3H, NC$\underline{H}$CH$_2$S), 4.23 (dd, J_1=4.8 Hz, J_2=7.2 Hz, 0.7H, NC$\underline{H}$CH$_2$S); 5.50 (s, 0.3H, NC$\underline{H}$S); 5.67 (s, 0.7H, NC$\underline{H}$S); 7.24 – 7.54 (m, 5H, Harom) ^{13}C NMR (DMSO-d_6) δ: 37.9, 38.3; 64.8, 65.3; 71.0, 71.7; 126.8, 127.2; 127.5, 128.1; 128.2, 128.4; 115.5, 155.7; 172.1, 172.9. Elemental analysis calcd for $C_{10}H_{11}NO_2S$: C, 57.39; H, 6.300; N, 6.690; S, 15.32. Found: C, 57.01; H, 6.164; N, 6.776; S, 14.89.

(*2RS,4R*)-2-(2-methoxyphenyl)-1,3-thiazolidine-4-carboxylic acid **(2)**

Yield: %65. m.p.: 144.1 °C. FT-IR (KBr), cm^{-1}: 3147, 1639, 1490. ^{1}H-NMR (DMSO-d_6) δ: 2.94- 3.00 (m, 1H, NCHC$\underline{H}_2$S); 3.18 (dd, J_1=6.8 Hz, J_2=10.4 Hz, 0.4H, NCHC$\underline{H}_2$S); 3.34 (dd, J_1=6.8 Hz, J_2=10.0 Hz, 0.6H, NCHC$\underline{H}_2$S); 3.80 (s, 1.2H, OC$\underline{H}_3$); 3.81 (s, 1.8H, OC$\underline{H}_3$); 3.85 (dd, *J*=7.2 Hz, 9.2 Hz, 0.4H, NC$\underline{H}$CH$_2$S); 4.17 (t, *J*=12.4 Hz 0.6H, NC$\underline{H}$CH$_2$S); 5.69 (s, 0.6H, NC$\underline{H}$S); 5.86 (s, 0.4H, NC$\underline{H}$S); 6.88–7.06 (m, 2H, Harom); 7.2–7.34(m, 1H, Harom); 7.39 (dd, J_1=1.2 Hz, J_2=7.6 Hz, 0.4H, Harom); 7.51 (dd, J_1=1.6 Hz, J_2=8 Hz, 0.6H, Harom). Elemental analysis calcd for $C_{11}H_{13}NO_3S$: C, 55.21; H, 5.48; N, 5.85; S, 13.40. Found: C, 54.93; H, 5.434; N, 5.943; S, 13.06.

(*2RS, 4R*)-2-(2-methylphenyl)-1,3-thiazolidine-4-carboxylic acid **(3)**

Yield: %75. m.p.: 154.6 °C. FT-IR (KBr), cm^{-1}: 3427, 1666, 1590. ^{1}H-NMR (DMSO-d_6) δ: 2.31 (s, 1.8H, C$\underline{H}_3$); 2.35 (s, 1.2H, C$\underline{H}_3$); 3.05 (dd, J_1=6.8 Hz, J_2=10.2 Hz, 0.4H, NCHC$\underline{H}_2$S); 3.09 (dd, J_1=5.2 Hz, J_2=10.4 Hz, 0.6H, NCHC$\underline{H}_2$S); 3.29 (dd, J_1=6.8 Hz, J_2=10.0 Hz, 0.4H, NC$\underline{H}$CH$_2$S); 3.38 (dd, J_1=7.6 Hz, J_2=10.4 Hz, 0.6H, NC$\underline{H}$CH$_2$S); 3.90 (dd, J_1=6.8 Hz, J_2=7.2 Hz, 0.4H, NC$\underline{H}$CH$_2$S); 4.23 (dd, J_1=5.6 Hz, J_2=7.2 Hz, 0.6H, NC$\underline{H}$CH$_2$S); 5.67 (s, 0.4H, NC$\underline{H}$S); 5.85 (s, 0.6H, NC$\underline{H}$S); 7.12 – 7.84 (m, 4H).

(*2RS-4R*)-2-(2-cyanophenyl)-1,3-thiazolidine-4-carboxylic acid **(4)**

Yield: %62 . FT-IR (KBr), cm^{-1}: 3400, 1613, 1487. ^{1}H-NMR (DMSO-d_6) δ: 3.74 (dd, J_1=7.6 Hz, J_2=12.4 Hz 0.95H, NCHC$\underline{H_2}$S); 3.80 (dd, J_1=8.2 Hz, J_2=12.0 Hz, 0.95H, NCHC$\underline{H_2}$S); 4.47 (t, *J*=7.6 Hz, 0.95H, NC$\underline{H}$CH_2S); 6.39 (s, 0.05H, NC$\underline{H}$S) 6.42 (s, 0.95H, NC$\underline{H}$S); 7.68–7.84 (m, 3H, Harom); 8.20 (d, *J*=8.0 Hz, 0.95H, Harom). Elemental analysis calcd for $C_{11}H_{10}N_2O_2S$: C, 49.24; H, 4.51; N,10.44; S, 11.95. Found: C, 48.9; H, 4.61; N, 10.54; S, 12.17.

(2RS, 4R)-2-(2-bromophenyl)-1,3-thiazolidine-4-carboxylic acid **(5)**

Yield: %86. m.p.: 182.2 °C FT-IR (KBr), cm^{-1}: 3436, 1664 ,1515. ^{1}H-NMR (DMSO-d_6) δ: 2.97 (dd, J_1=6.4 Hz, J_2=10.0 Hz, 0.7H, NCHC$\underline{H_2}$S); 3.06 (t, *J*=9.2 Hz, 0.3H, NCHC$\underline{H_2}$S); 3.25 (dd, J_1=6.4 Hz, J_2=10.0 Hz, 0.7H, NCHC$\underline{H_2}$S); 3.35 (dd, J_1=6.4 Hz, J_2=9.6 Hz, 0.3H, NCHC$\underline{H_2}$S); 3.97 (dd, J_1=6.8 Hz, J_2=9.2 Hz, 0.4H, NC$\underline{H}$CH_2S); 4.19 (t, *J*=6.8Hz, Hz 0.3H, NC$\underline{H}$CH_2S); 5.73 (s, 0.3H, NC$\underline{H}$S); 5.91 (s, 0.7H, NC$\underline{H}$S); 7.24-7.86 (m, 4H). ^{13}C NMR (DMSO-d_6) δ: 37.4, 37.5; 65.3, 65.5; 69.6, 69.8; 122.3, 122.5; 122.3, 127.7, 129.8, 132.4, 138,7, 141.8; 172.1, 172.5. Elemental analysis calcd for $C_{10}H_{10}BrNO_2S$: C, 41.68; H, 3.50; N,4.680; S, 11.10. Found: C, 36.38; H, 4.19; N, 4.938; S, 10.83.

(*2RS, 4R*)- 2-(3-chlorophenyl)-1,3-thiazolidine-4-carboxylic acid **(6)**

Yield: %74. m.p.: 160.4 °C FT-IR (KBr), cm^{-1}: 3438, 1574, 1477. ^{1}H-NMR (DMSO-d_6) δ: 3.09 (dd, J_1=5.2 Hz, J_2=10.8 Hz, 0.9H, NCHC$\underline{H_2}$S); 3.28 (dd, J_1=6.8 Hz, J_2=10.4 Hz, 0.9H, NCHC$\underline{H_2}$S); 4.15 (dd, J_1=4.8 Hz, J_2=6.4Hz, 0.9H, NC$\underline{H}$CH_2S); 5.55 (s, 0.1H, NC$\underline{H}$S); 5.67 (s, 0.9H, NC$\underline{H}$S); 7.28-7.37 (m, 3H, Harom), 7.48 (s, 1H, Harom). Elemental analysis calcd for $C_{10}H_{10}ClNO_2S$: C, 49.28; H, 4.14; N,5.75; S, 13.01. Found: C, 48.98; H, 4.002; N, 5.853; S, 12.90.

(*2RS-4R*)-2-(4-chlorophenyl)-1,3-thiazolidine-4-carboxylic acid (**7**)

Yield: %75. m.p.: 158.8 °C. FT-IR (KBr), cm^{-1}: 3435, 1579, 1491. ^{1}H-NMR (DMSO-d_6) δ: 3.09 (dd, *J*=5.2 Hz, J_2=10.8 Hz 0.95H, NCHC$\underline{H_2}$S); 3.27 (dd, J_1=6.8 Hz, J_2=10.0 Hz, 0.95H, NCHC$\underline{H_2}$S); 4.16 (dd, J_1=4.8 Hz, J_2=6.4 Hz, 0.95H, NC$\underline{H}$CH_2S); 5.49 (s, 0.05H, NC$\underline{H}$S); 5.66 (s, 0.95H, NC$\underline{H}$S); 7.37 (d, *J*=8.8 Hz, 1.9H); 7.44 (d, *J*=8.4 Hz, 1.9H). LC-MS: ELS, rt = 3.80 min., *m*/*z* 244 $[M + H]^+$, 285 $[M + CH_3CN]^+$. Elemental analysis calcd for $C_{10}H_{10}ClNO_2S$: C, 49.28; H, 4.14; N,5.750; S, 13.100. Found: C, 49.20; H, 4.046; N, 5.952; S, 12.91.

(*2RS-4R*)- 2-(4-methoxyphenyl)-1,3-thiazolidine-4-carboxylic acid **(8)**

Yield: %72. m.p.: 159.0 °C. FT-IR (KBr), cm^{-1}: 3435,1586, 1463. ^{1}H-NMR (DMSO-d_6) δ: 3.07 (t, *J*=9.6 Hz, 0.5H, NCHC$\underline{H}_2$S); 3.15 (dd, J_1=4.0 Hz, J_2=10.0 Hz, 0.5H, NCHC$\underline{H}_2$S); 3.29 (dd, J_1=7.2 Hz, J_2=10.4 Hz, 0.5H, NCHC$\underline{H}_2$S); 3.36 (dd, J_1=7.2 Hz, J_2=10.0 Hz, 0.5H, NCHC$\underline{H}_2$S); 3,74 (s, 1.5H, OC$\underline{H}_3$); 3.76 (s, 1.5H, OC$\underline{H}_3$); 3.87 (dd, J_1=7.6 Hz, J_2=9.2 Hz, 0.5H, NC$\underline{H}$CH$_2$S), 4.25 (dd, J_1=4.0 Hz, J_2=6.8 Hz, 0.5H, NC$\underline{H}$CH$_2$S); 5.46 (s, 0.5H, NC$\underline{H}$S); 5.60 (s, 0.5H, NC$\underline{H}$S); 6.89 (d, *J*=8.4 Hz, 1H, Harom); 6.93 (d, *J*=8.8 Hz, 1H, Harom); 7.37 (d, *J*=8.4 Hz, 1H, Harom); 7.44 (d, *J*=8.4 Hz, 1H, Harom). LC-MS: ELSD, rt = 3.47 min., *m*/z 240 $[M + H]^+$, 281 $[M + CH_3CN]^+$. Elemental analysis calcd for $C_{11}H_{13}NO_3S$: C, 55.210; H, 5.480; N,5.850; S, 13.40. Found: C, 55.077; H, 5.213; N, 6.011; S, 13.20.

(*2RS, 4R*)- 2-(4-methylphenyl)-1,3-thiazolidine-4-carboxylic acid **(9)**

Yield: %85. m.p.: 161.8 °C. FT-IR (KBr), cm^{-1}: 3435, 1578, 1473. ^{1}H-NMR (DMSO-d_6) δ: 2.28 (s, 1.8H, C$\underline{H}_3$); 2.50 (s, 1.2H, C$\underline{H}_3$); 3.07 (t, *J*=9.2 Hz, 0.4H, NCHC$\underline{H}_2$S); 3.14 (dd, J_1=4.4 Hz, J_2=10.4 Hz, 0.6H, NCHC$\underline{H}_2$S); 3.28 (dd, J_1=6.8 Hz, J_2=10.0 Hz, 0.6H, NCHC$\underline{H}_2$S); 3.36 (dd, J_1=6.8 Hz, J_2=9.4 Hz, 0.4H, NCHC$\underline{H}_2$S); 3.88 (t, *J*=8.4 Hz, 0.4H, NC$\underline{H}$CH$_2$S); 4.24 (dd, J_1=4.4 Hz, J_2=6.8 Hz, 0.6H, NC$\underline{H}$CH$_2$S); 5.46 (s, 0.4H, NC$\underline{H}$S); 5.62 (s, 0.6H, NC$\underline{H}$S); 7.14 (d, *J*=8.4 Hz, 0.4H, Harom); 7.18 (d, *J*=8.0 Hz, 0.4H, Harom); 7.32 (d, *J*=8.4 Hz, 0.6H, Harom); 7.39 (d, *J*=8.0 Hz, 0.6H, Harom). ^{13}C NMR (DMSO-d_6) δ: 20.5, 20.6; 37.8, 38.6; 64.7, 65.3; 70.9, 71.6 ; 126.8, 127.1; 124.8, 128.7, 128.9; 135.8, 136.8; 137.5, 137.9; 172.1, 172.9. Elemental analysis calcd for $C_{11}H_{13}NO_2S$: C, 59.17; H, 5.870; N,6.27; S, 14.36. Found: C, 59.40; H, 5.889; N, 6.405; S, 14.06.

(*2RS, 4R*)- 2-(4-nitrophenyl)-1,3-thiazolidine-4-carboxylic acid **(10)**

Yield: %80. m.p.: 110 °C. FT-IR (KBr), cm^{-1}: 3407, 1629, 1438. ^{1}H-NMR (DMSO-d_6) δ: 3.12 (dd, J_1=8.8 Hz, J_2=9.6 Hz, 0.95H, NCHC$\underline{H}_2$S); 3.76 (dd, J_1=6.8 Hz, J_2=10.0 Hz, 0.95H, NCHC$\underline{H}_2$S); 3.98 (dd, J_1=7.2 Hz, J_2=8.8 Hz, 0.95, NC$\underline{H}$CH$_2$S); 5.67 (s, 0.95H, NC$\underline{H}$S); 5.87 (s, 0.05H, NC$\underline{H}$S); 7.8 (d, *J*=8.4 Hz, 1.9H, Harom); 8.2 (d, *J*=8.4 Hz, 1.9H, Harom). LC-MS: ELSD, rt = 3.59 min., *m*/*z* 255 $[M + H]^+$, 296 $[M + CH_3CN]^+$. Elemental analysis calcd for $C_{10}H_{10}N_2O_4S$: C, 44.28; H, 4.090; N,10.33; S, 11.80. Found: C, 43.98; H, 4.315; N, 10.33; S, 11.26.

(*2RS, 4R*)-2-(2,6-difluorophenyl)-1,3-thiazolidine-4-carboxylic acid **(11)**

Yield: %75. m.p.: 140.6 °C. FT-IR (KBr), cm^{-1}: 3459, 1656, 1469. ^{1}H-NMR (DMSO-d_6) δ: 3.01 (t, *J*=9.2 Hz, 0.6H, NCHC$\underline{H}_2$S); 3.19 (dd, J_1=3.6 Hz, J_2=10.0 Hz, 0.4H, NCHC$\underline{H}_2$S); 3.33 (dd, J_1=6.8 Hz, J_2=10.4 Hz, 0.4H, NCHC$\underline{H}_2$S); 3.46 (dd, J_1=7.2 Hz, J_2=10.4 Hz, 0.6H, NCHC$\underline{H}_2$S); 3,89 (t, *J*=8.8 Hz, 0.6H, NC$\underline{H}$CH$_2$S); 4.45 (dd,

J_1=3.6 Hz, J_2=6.4 Hz, 0.4H, NC<u>H</u>CH_2S), 5.78 (s, 0.6H, NC<u>H</u>S); 5.98 (s, 0.4H, NC<u>H</u>S); 7.11 (t, *J*=8.8 Hz, 0.8H, Harom); 7.18 (t, *J*=9.0Hz, 1.2.H, Harom); 7.36-7.56 (m, 1H, Harom). Elemental analysis calcd for $C_{11}H_{10}N_2O_2S$: C, 49.24; H, 4.51; N,10.44; S, 11.95. Found: C, 48.59; H, 4.641; N, 10.54; S, 12.57.

(*2RS, 4R*)-2-(2,3-difluorophenyl)-1,3-thiazolidine-4-carboxylic acid **(12)**

Yield: %80. m.p.: 133.5 °C. FT-IR (KBr), cm^{-1}: 3433, 1610, 1426. ^{1}H-NMR (DMSO-d_6) δ: 3.00 (dd, J_1=6.0 Hz, J_2=10.4 Hz, 0.4H, NCHC<u>H</u>$_2$S); 3.05 (t, *J*=9.2 Hz, 0.4H, NCHC<u>H</u>$_2$S); 3.29 (dd, J_1=6.4 Hz, J_2=10.0 Hz, 0.6H, NCHC<u>H</u>$_2$S); 3.35 (dd, J_1=6.4 Hz, J_2=9.6 Hz, 0.6H, NCHC<u>H</u>$_2$S); 3.94 (dd, J_1=6.8 Hz, J_2=8.8 Hz, 0.4H NC<u>H</u>CH_2S); 4.11 (t, *J*=6.0Hz, 0.6H NC<u>H</u>CH_2S); 5.70 (s, 0.6H, NC<u>H</u>S); 5.93 (s, 0.4H, NC<u>H</u>S); 7.12-7.58 (m, 3H, Harom). LC-MS: ELSD, rt = 3.60 min., *m/z* 246 $[M + H]^+$, 287 $[M + CH_3CN]^+$. Elemental analysis calcd for $C_{11}H_{10}N_2O_2S$: C, 48.89; H, 3.70; N,5.710; S, 13.010. Found: C, 48.77; H, 3.684; N, 5.822; S, 12.740.

(*2RS, 4R*)- 2-(2,3-dichlorophenyl)-1,3-thiazolidine-4-carboxylic acid **(13)**

Yield: %84. m.p.: 129.7 °C. FT-IR (KBr), cm^{-1} 3431, 1666, 1418. ^{1}H-NMR (DMSO-d_6) δ: 2.94 (dd, J_1=7.6 Hz, J_2=10.8 Hz, 0.4H, NCHC<u>H</u>$_2$S); 3.05 (t, *J*=9.2 Hz, 0.4H, NCHC<u>H</u>$_2$S); 3.25 (dd, J_1=6.4 Hz, J_2=10.0 Hz, 0.6H, NCHC<u>H</u>$_2$S); 3.34 (dd, J_1=6.8 Hz, J_2=10.4 Hz, 0.6H, NCHC<u>H</u>$_2$S); 4.02 (dd, J_1=7.2 Hz, J_2=9.2 Hz, 0.4H, NC<u>H</u>CH_2S), 4.13 (t, *J*=6.4 Hz, 0.6H, NC<u>H</u>CH_2S); 5.80 (s, 0.4H, NC<u>H</u>S); 5.99 (s, 0.6H, NC<u>H</u>S); 7.36 (t, *J*=8.0 Hz, 0.6H, Harom); 7.41 (t, *J*=8.0 Hz, 0.4H, Harom); 7.53-7.55 (m, 1H, Harom); 7.61 (dd, J_1=1.6 Hz, J_2=7.6 Hz, 0.6H); 7.84 (dd, J_1=1.6 Hz, J_2=7.6 Hz, 0.4H). Elemental analysis calcd for $C_{11}H_{10}N_2O_2S$: C, 38.47; H, 3.55; N,4.49; S, 10.20. Found: C, 38.04; H, 3.559; N, 4.770; S, 10.350.

HPLC assays

The Agilent 1260 series HPLC with a diode array detector and a 5 µm Zorbax Eclipse XDB-C18 column (4.6 mm × 250 mm, 3.5µm) was used to record spectra. Water: acetonitrile (30:70) solvent system was used as the mobile phase with a flow rate of 0.5 $ml.min^{-1}$ at 25°C.Spectra were recorded at 254nm. The injection volume was of 5 µL.

The DPPH assay

The DPPH radical scavenging abilities of the molecules 1-13 were evaluated with respect to the method of Akter *et al.*[46] with few alterations [43]. The assay was performed in 96-well plates. The DPPH solution was prepared (0.1 mM) and sample solutions were prepared by appropriate serial dilution within a range from 6.75 µM to 1000 µM. As a reference, a BHT solution was prepared and used at a concentration range varying between 125

and 2000 µM. In each well 250 µL of DPPH solution and 50 µL of sample or reference solutions were added and incubated in dark at RT for 50 minutes. Each sample was evaluated in triplicate. Absorbance was measured at 517 nm and radical scavenging ability of the molecules was calculated as follows:

DPPH radical - scavenging activity % = $[1-(A_{average} - A_{blank}) / (A_{control}-A_{blank})] \times 100$

The CUPRAC assay

The cupric ion reducing abilities of the molecules **1-13** were evaluated with respect to the method of Apak *et al*.[44] with few alterations [43]. The assay was performed in 96-well plates. Reagent solutions were prepared separately as 10mM $CuSO_4$, 7.5 mM neocuproine and 1M ammonium acetate buffer (pH 7.0). Sample solutions were prepared by appropriate serial dilution within a range from 6.75 µM to 1000 µM. Trolox, a water soluble analogue of vitamin E, was used as reference in the concentration range of 10 µM-70µM. Into each well, equal volumes of each reagent solution 85 µL, 51 µL of H_2O and 43 µL of sample solution were added. Each sample concentration was evaluated triplicate. The plate was incubated in 50°C for 20 minutes and absorbance was measured at 450 nm. Trolox coefficient values (TEAC) were calculated for each sample as TEAC = $\varepsilon_{sample} / \varepsilon_{trolox}$.

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REFERENCES

[1] S.M. Deneke, Thiol-based antioxidants, Curr. Top. Cell. Regul., Vol 36, 36 (2000) 151-180.

[2] M. Valko, D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur, J. Telser, Free radicals and antioxidants in normal physiological functions and human disease, Int. J. Biochem. Cell Biol., 39 (2007) 44-84.

[3] N.S. Dhalla, R.M. Temsah, T. Netticadan, Role of oxidative stress in cardiovascular diseases, J. Hypertens., 18 (2000) 655-673.

[4] C. Napoli, L.J. Ignarro, Nitric oxide and atherosclerosis, Nitric Oxide-Biol. Chem., 5 (2001) 88-97.

[5] S.A. Khan, K. Lee, K.M. Minhas, D.R. Gonzalez, S.V.Y. Raju, A.D. Tejani, D.C. Li, D.E. Berkowitz, J.M. Hare, Neuronal nitric oxide synthase negatively regulates xanthine oxidoreductase inhibition of cardiac excitation-contraction coupling, Proc. Natl. Acad. Sci. U.S.A. , 101 (2004) 15944-15948.

[6] G. Poli, G. Leonarduzzi, F. Biasi, E. Chiarpotto, Oxidative stress and cell signalling, Curr. Med. Chem., 11 (2004) 1163-1182.

[7] M. Valko, C.J. Rhodes, J. Moncol, M. Izakovic, M. Mazur, Free radicals, metals and antioxidants in oxidative stress-induced cancer, Chem. Biol. Interact., 160 (2006) 1-40.

[8] D.A. Butterfield, A. Castegna, C.B. Pocernich, J. Drake, G. Scapagnini, V. Calabrese, Nutritional approaches to combat oxidative stress in Alzheimer's disease, J. Nutr. Biochem, 13 (2002) 444-461.

[9] L. Tretter, V. Adam-Vizi, Generation of reactive oxygen species in the reaction catalyzed by alpha-ketoglutarate dehydrogenase, J. Neurosci., 24 (2004) 7771-7778.

[10] L. Tretter, I. Sipos, V. Adam-Vizi, Initiation of neuronal damage by complex I deficiency and oxidative stress in Parkinson's disease, Neurochem. Res., 29 (2004) 569-577.

[11] N.J. Pace, E. Weerapana, Diverse Functional Roles of Reactive Cysteines, ACS Chem. Biol., 8 (2013) 283-296.

[12] L.A. Komarnisky, R.J. Christopherson, T.K. Basu, Sulfur: Its clinical and toxicologic aspect's, Nutrition, 19 (2003) 54-61.

[13] A. Hollaender, in: D. Doherty (Ed.), Vienna, Austria: International Atomic Energy Agency, 1969.

[14] C.C. Winterbourn, D. Metodiewa, Reactivity of biologically important thiol compounds with superoxide and hydrogen peroxide, Free Radic. Biol. Med., 27 (1999) 322-328.

[15] C.C. Winterbourn, M.B. Hampton, Thiol chemistry and specificity in redox signaling, Free Radic. Biol. Med., 45 (2008) 549-561.

[16] J.P. C., The biochemistry of the SH group, in: Radioprotection by thiols and disulfides, New York: Academic Press, 1972, pp. 94-115.

[17] D.A. Dickinson, H.J. Forman, Cellular glutathione and thiols metabolism, Biochem. Pharmacol., 64 (2002) 1019-1026.

[18] F.Q. Schafer, G.R. Buettner, Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple, Free Radic. Biol. Med., 30 (2001) 1191-1212.

[19] M.P. Murphy, Mitochondrial Thiols in Antioxidant Protection and Redox Signaling: Distinct Roles for Glutathionylation and Other Thiol Modifications, Antioxid. Redox Signal., 16 (2012) 476-495.

[20] G. OW, M. A, Glutathione: interorgan translocation,turnover, and metabolism in, Proc. Natl. Acad. Sci. U.S.A., 1979, pp. 5606-5610.

[21] O.W. Griffith, Biologic and pharmacologic regulation of mammalian glutathione synthesis, Free Radic. Biol. Med., 27 (1999) 922-935.

[22] M. PukaSundvall, P. Eriksson, M. Nilsson, M. Sandberg, A. Lehmann, Neurotoxicity of cysteine: Interaction with glutamate, Brain Res., 705 (1995) 65-70.

[23] R. Janaky, V. Varga, A. Hermann, P. Saransaari, S.S. Oja, Mechanisms of L-cysteine neurotoxicity, Neurochem. Res., 25 (2000) 1397-1405.

[24] S. Park, J.A. Imlay, High levels of intracellular cysteine promote oxidative DNA damage by driving the Fenton reaction, J. Bacteriol., 185 (2003) 1942-1950.

[25] L. Prescott, Oral or intravenous N-acetylcysteine for acetaminophen poisoning?, Ann. Emerg. Med., 2005, pp. 409-413.

[26] S.C. De Rosa, M.D. Zaretsky, J.G. Dubs, M. Roederer, M. Anderson, A. Green, D. Mitra, N. Watanabe, H. Nakamura, I. Tjioe, S.C. Deresinski, W.A. Moore, S.W. Ela, D. Parks, L.A. Herzenberg, N-acetylcysteine replenishes glutathione in HIV infection, Eur. J. Clin. Invest., 30 (2000) 915-929.

[27] M. Kasielski, D. Nowak, Long-term administration of N-acetylcysteine decreases hydrogen peroxide exhalation in subjects with chronic obstructive pulmonary disease, Respir. Med., 95 (2001) 448-456.

[28] R. Tirouvanziam, C.K. Conrad, T. Bottiglieri, L.A. Herzenberg, R.B. Moss, High-dose oral N-acetylcysteine, a glutathione prodrug, modulates inflammation in cystic fibrosis, Proc. Natl. Acad. Sci. U.S.A., 103 (2006) 4628-4633.

[29] J.C. Roberts, H.T. Nagasawa, R.T. Zera, R.F. Fricke, D.J.W. Goon, prodrugs of l-cysteine as protective agents against acetaminophen-induced hepatotoxicity - 2-(polyhydroxyalkyl)thiazolidine-4(R)-carboxylic and 2-(polyacetoxyalkyl)thiazolidine-4(R)-carboxylic acids, J. Med. Chem., 30 (1987) 1891-1896.

[30] H.T. Nagasawa, D.J.W. Goon, R.T. Zera, D.L. Yuzon, Prodrugs of L-cysteine as liver-protective agents - 2(RS)-methylthiazolidine-4(R)-carboxylic acid, a latent cysteine, J. Med. Chem., 25 (1982) 489-491.

[31] H.T. Nagasawa, D.J.W. Goon, W.P. Muldoon, R.T. Zera, 2-substituted thiazolidine-4(r)-carboxylic acids as prodrugs of L-cysteine - protection of mice against acetaminophen hepatotoxicity, J. Med. Chem., 27 (1984) 591-596.

[32] C. Srinivasan, W.M. Williams, H.T. Nagasawa, T.S. Chen, Effects of 2(RS)-n-propylthiazolidine-4(R)-carboxylic acid on extrahepatic sulfhydryl levels in mice treated with acetaminophena, Biochem. Pharmacol., 61 (2001) 925-931.

[33] L.I. Berkeley, J.F. Cohen, D.L. Crankshaw, F.N. Shirota, H.T. Nagasawa, Hepatoprotection by L-cysteine-glutathione mixed disulfide, a sulfhydryl-modified prodrug of glutathione, J. Biochem. Mol. Toxicol., 17 (2003) 95-97.

[34] Y. Yang, W.-S. Liu, B.-Q. Han, H.-Z. Sun, Antioxidative properties of a newly synthesized 2-glucosaminethiazolidine-4(R)-carboxylic acid (GlcNH(2)CYS) in mice, Nutr. Res., 26 (2006) 369-377.

[35] K.R. Atkuri, J.J. Mantovani, L.A. Herzenberg, N-Acetylcysteine - a safe antidote for cysteine/glutathione deficiency, Curr. Opin. Pharmacol., 7 (2007) 355-359.

[36] C. Srinivasan, W.M. Williams, M.B. Ray, T.S. Chen, Prevention of acetaminophen-induced liver toxicity by 2(R,S)-n-propylthiazolidine-4(R)-carboxylic acid in mice, Biochem. Pharmacol., 61 (2001) 245-252.

[37] Y. Liu, F. Jing, Y. Xu, Y. Xie, F. Shi, H. Fang, M. Li, W. Xu, Design, synthesis and biological activity of thiazolidine-4-carboxylic acid derivatives as novel influenza neuraminidase inhibitors, Bioorg. Med. Chem., 19 (2011) 2342-2348.

[38] Y.M. Ha, Y.J. Park, J.Y. Lee, D. Park, Y.J. Choi, E.K. Lee, J.M. Kim, J.-A. Kim, J.Y. Park, H.J. Lee, H.R. Moon, H.Y. Chung, Design, synthesis and biological evaluation of 2-(substituted phenyl)thiazolidine-4-carboxylic acid derivatives as novel tyrosinase inhibitors, Biochimie, 94 (2012) 533-540.

[39] K.R. G., Mechanism of reactions involving Schiff base intermediates. Thiazolidine formation from L-cysteine and formaldehyde, in, J. Am. Chem. Soc., 1971, pp. 6236-6248.

[40] R. Kallen, The mechanism of reactions involving Schiff base intermediates. Thiazolidine formation from L-cysteine and formaldehyde, J. Am. Chem. Soc., 93 (1971) 6236-6248.

[41] L. Terzuoli, R. Leoncini, R. Pagani, R. Guerranti, D. Vannoni, F. Ponticelli, E. Marinello, Some chemical properties and biological role of thiazolidine compounds, Life Sci., 63 (1998) 1251-1267.

[42] Y. Lu, Z. Wang, C.-M. Li, J. Chen, J.T. Dalton, W. Li, D.D. Miller, Synthesis, in vitro structure-activity relationship, and in vivo studies of 2-arylthiazolidine-4-carboxylic acid amides as anticancer agents, Bioorg. Med. Chem., 18 (2010) 477-495.

[43] E. Celep, A. Aydin, E. Yesilada, A comparative study on the in vitro antioxidant potentials of three edible fruits: Cornelian cherry, Japanese persimmon and cherry laurel, Food Chem. Toxicol., 50 (2012) 3329-3335.

[44] N. Gungor, M. Ozyurek, K. Guclu, S.D. Cekic, R. Apak, Comparative evaluation of antioxidant capacities of thiol-based antioxidants measured by different in vitro methods, Talanta, 83 (2011) 1650-1658.

[45] R. Apak, K. Guclu, M. Ozyurek, S.E. Karademir, Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method, J. Agric. Food Chem., 52 (2004) 7970-7981.

[46] M.S. Akter, M. Ahmed, J.-B. Eun, Solvent effects on antioxidant properties of persimmon (Diospyros kaki L. cv. Daebong) seeds, Int.l J. Food Sci. Tech., 45 (2010) 2258-2264.

Highlights

- Thiazolidine carboxylic acids were synthesized with a series of benzaldehydes.
- The antioxidant activity was evaluated *via* the DPPH and CUPRAC assays.
- The cysteine releasing pattern of each thiazolidine compound was analyzed.
- The antioxidant capacity was shown to be related not only to L-cysteine release.
- Thiazolidine heterocycle itself can also be considered as antioxidant.