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Research paper

The cysteine releasing pattern of some antioxidant thiazolidine-4-carboxylic acids

F. Esra Önen Bayram [a, *], Hande Sipahi [b], Ebru Türköz Acar [c], Reyhan Kahveci Ulugöl [a], Kerem Buran [a], Hülya Akgün [a]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Yeditepe University, Istanbul, Turkey
[b] Department of Toxicology, Faculty of Pharmacy, Yeditepe University, Istanbul, Turkey
[c] Department of Analytical Chemistry, Faculty of Pharmacy, Yeditepe University, Istanbul, Turkey

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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.

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

* Corresponding author.
E-mail address: filizesraonen@gmail.com (F.E. Önen Bayram).

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

	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

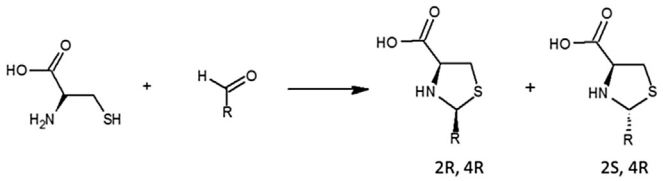
benzaldehyde derivatives (Table 1). The typical cyclization reaction was carried out in basic conditions in a water/ethanol mixture (50:50, v:v) as suggested in the literature for the preparation of these compounds [5,31,37,38].

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 (Fig. 1).

Although clearly distinguishable on ^{1}H NMR *via* the proton on the C-2 carbon of the heterocycle (Fig. 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].

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.



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

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

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.

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 min, HPLC analyses of the samples were also repeated 50 min 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.

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 min 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