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YEDITEPE UNIVERSITY

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DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

THIAZOLIDINE CARBOXYLIC ACID DERIVATIVES AS ANTIOXIDANT MOLECULES; SYNTHESIS AND EVALUATION

MASTER of CHEMISTRY THESIS

REYHAN KAHVECİ ULUGÖL

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

Date :

Signature :

Name Surname :

dedicated to my son, Kaya…

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LIST OF SYMBOLS AND ABBREVIATIONS

A	Absorbace
AIDS	Acquired immune deficiency syndrome
APAP	N-acetyl-p-aminophenol
Aq	Aqueous
ATP	Adenosine triphosphate
BBB	Blood brain barrier
BHT	Buthylated hydroxytoluene
CUPRAC	Cupric reducing antioxidant capacity
Cys	Cysteine
d	Doublet
dd	Doublet of doublet
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EtOH	Ethanol
FT-IR	Fourier transform infrared
GCS	γ-*L*-glutamyl-*L*-cysteine
GS	Glutathione synthetase
GSH	Glutathione
GSH-Px	Glutathione peroxidase
GSSG	Glutathione disulfidie
HPLC	High performance liquid chromatography
IR	Infra red
LC-MS	Liquid chromatography-mass spectrometery
m	Multiplet
MeOH	Methanol
NAC	N-acetylcysteine
Nc	Neocuproine
NMR	Nuclear magnetic resonance
NOE	Nuclear overhauser effect
PBS	Phosphate buffered saline
Ppm	Parts-per-million

PTCA	Propyl-thiazolidine carboxylic acid
ROS	Reactive oxygen species
RT	Room temperature
Rt	Retention time
s	Singlet
S	Sulfur
SAM	S-adenosylmethionine
t	Triplet
TCA	Thiazolidine carboxylic acid
TEAC	Trolox equivalent antioxidant coefficient
TLC	Thin layer chromatography
Tlx	Trolox
Trx	Thioredoxin
UV	Ultra violet
v/v	Volume/volume
w/v	Weight/volume
ε	Molar extinction coefficient

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ABSTRACT

ULUGOL, R. K. Thiazolidine carboxylic acid derivatives as antioxidant molecules; synthesis and evaluation. Yeditepe University Institute of Health Science, Thesis on Pharmaceutical Chemistry Master of Science Degree Programme, Istanbul, 2015.

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 is usually supplemented in a prodrug approach. In this study, the antioxidant properties of *o, m* or *p* substituted (2*RS*,4*R*)-2-phenyl-thiazolidine-4-carboxylic acid derivatives (**1-12**), heterocycles that potentially convert into cysteine in physiological conditions, were investigated. (2*RS*,4*R*)-2-phenyl-thiazolidine-4-carboxylic acid derivatives were synthesized by condensing *L*-cysteine with a series of mono or di substituted benzaldehydes. Compounds were characterized by LC-MS, FT-IR , ^{1}H NMR, ^{13}C NMR and elemental analisis. *In vitro* antioxidant properties were evaluated using the classical 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and by determining the cupric reducing antioxidant capacity (CUPRAC).

Compounds are shown to exhibit relevant antioxidative properties that are not only attributed to cysteine release but also to the direct antioxidant capacity of the thiazolidine cycle itself.

Keywords: Thiazolidine carboxylic acid derivatives, antioxidant, *L*-cysteine, free radical, DPPH, CUPRAC.

ÖZET

ULUGÖL, R. K. Antioksidan etki gösteren bazı tiyazolidin karboksilik asit türevlerinin sentez ve değerlendirilmesi. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü Farmasötik Kimya Programı Bilim Uzmanlığı Tezi, İstanbul 2015.

Oksidatif stres, hücre içerisindeki serbest radikal konsantrasyonunda önemli bir artışa sebep olur. Bu artış hücresel savunma mekanizmaları tarafından önlenemediği taktirde hayati önem taşıyan biyolojik makromoleküllerin zarar görmesi söz konusu olur. Düşük moleküler ağırlıklı tiyoller grubunda bulunan glutatyon (GSH) molekülü, hücre içerisindeki başlıca antioksidan yapılardan birisidir. GSH molekülü, nörotoksik olduğu için hücre içerisinde sınırlı miktarda bulunan sistein aminoasidi kullanılarak sentezlenir. Oksidatif stres durumunda yeterli GSH sentezinin sağlanması için sistein hücre dışından temin edilmelidir. Ancak sistein aminoasidinin nükleofilik yapısı ve disülfit formu olan sistin molekülüne hızlıca dönüşebiliyor olması glutatyon sentezi için sisteinin organizmaya önilaç formunda verilmesini gerektirmektedir. Bu çalışmada, fizyolojik ortamda potansiyel olarak sisteine dönüşen *o, m* veya *p* sübstitüe (2*RS*,4*R*)-2-fenil-tiyazolidin-4-karboksilik asit türevlerinin **(1-12)** antioksidan özellikleri incelenmiştir. (2*RS*,4*R*)-2-fenil-tiyazolidin-4-karboksilik asit türevleri, bir dizi mono veya disübstitüe benzaldehit molekülünün *L*-sistein ile kondenzasyonu sonucunda sentezlenmiştir. Elde edilen bileşikler LC-MS, FT-IR , ^{1}H NMR, ^{13}C NMR ve elemental analiz yöntemleri kullanılarak karakterize edilmiştir. *In vitro* antioksidan aktiviteler 1,1-difenil-2-pikrilhidrazil (DPPH) radikalini süpürme kapasitesinin belirlenmesi ve indirgenmiş bakırda antioksidan kapasitenin saptanması (CUPRAC) yöntemleri kullanılarak tayin edilmiştir.

Yapılan bu çalışma ile bileşiklerin gösterdiği kayda değer miktardaki antioksidatif özelliğin, sadece sistein salımı ile değil tiyazolidin halkasının direkt olarak antioksidan özellik göstermesi ile de sağlandığı gösterilmiştir.

Anahtar kelimeler: tiyazolidin karboksilik asit türevleri, antioksidan, *L*-sistein, serbest radikal, DPPH, CUPRAC

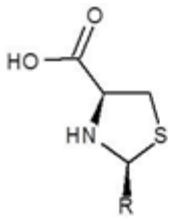
1. INRODUCTION AND AIM

Production of free radicals is a consequence of the continuous oxidative reactions that take place in aerobic organisms. Biological systems have strong enzymatic and nonenzymatic defense mechanisms in order to cope with free radicals and there is a balance between radicals and the defense mechanisms. However these defense mechanisms remain unsufficient when a significant increase in the free radical concentration occur which then cause considerable damage to crucial biological macromolecules such as DNA, proteins, carbohydrates and lipids. Such a situation is called oxidative stress (1).

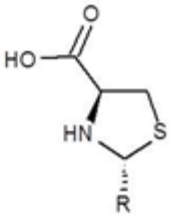
GSH is one of the most important low molecular weight thiol which acts directly or indirectly as an antioxidant when the concentration of free radicals rises in the cells and in the extracellular environment. GSH is composed of cysteine, glutamate and glycine moiety and its synthesis is limited by intracellular cysteine concentration. Since it is toxic, cysteine is found only in very small amounts in cells. Therefore, increasing the intracellular cysteine concentration boosts GSH levels and protects cells against oxidative damage (2).

Besides boosting GSH levels in the cells, cysteine can also act as a direct radical scavenger due to its thiol function (3-6). Yet, because of its toxicity, cysteine supplementation should be administrated as a prodrug (7). Thiazolidine-4-carboxylic acid (TCA) derivatives are reported to release cysteine by both enzymatic and spontaneous mechanisms (8-14). In this study, (2RS,4R)-2-phenyl-thiazolidine-4-carboxylic acid derivatives (**1-12**) were synthesized with a series of aromatic aldehydes in order to evaluate their *in vitro* antioxidant properties.

The compounds **1-12** were synthesized using cysteine and different aromatic aldehydes as shown in Scheme 1. Types of the aromatic aldehydes were chosen in order to discuss the effect of the substituents on the phenyl ring. Position, electron withdrawing or donating properties of the substituents on the phenyl ring of the aldehydes were considered.



2R, 4R



2S, 4R

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

Scheme 1. (2*RS*,4*R*)-2-pheny-thiazolidine-4-carboxylic acid derivatives **1-12**

It is reported in the literature that the compounds **1-12** were previously synthesized with similar synthetic pathway as given in the material method and some of them were used as important intermediates (15-32). Compounds **1, 4, 5, 6, 7, 8** and **9** were previously evaluated for several biologic activities including antivirutic (21), antifungal (33), antibacterial (34), urease inhibition (35), tyrosinase inhibition (36), anticancer activity (37), hepatoprotectivity (38), and prevention of the β-amyloid peptide aggregation activitiy (39). In this study, the *in vitro* antioxidant properties of the compounds **1-12** were evaluated for the first time.

2. GENERAL INFORMATION

2.1. Sulfur Element

Sulfur is found in 6A group of the periodic table and have both oxidizing and reducing ability (40). It can oxidize carbon and some metals while it can reduce oxygen. As it is compared to oxygen, sulfur have less electronegativity according to its location in the periodic table. Depending on the compound; sulfur can take oxidation numbers between 2- and 6+ as shown in Tables 1, 3 and 4. Oxidation of carbon by sulfur ends up with a negatively charged sulfur residue, as a result of that, sulfur compounds in living cells are found in the oxidation number 2- (R-S-R' , R-S-S-R' or R–SH) (41). Electronegativity difference between oxygen and sulfur leads thiols to have weaker hydrogen bonds than alcohols, the result is thiols have lower pKa values in comparison with alcohols (42). Sulfur is a vital non-metal element for all living organisms regarding the very important properties it owns. It participates to the structure of many biological components like proteins or vitamins (43). Totally, in human body, we have sulfur as the third most often encountered element and in breastmilk sulfur is the sixth most abundant element (44). Plants are one of the most substantial sulfur reserve for animals (45). Sulfur cycle in biological organisms is shown in Figure 1.

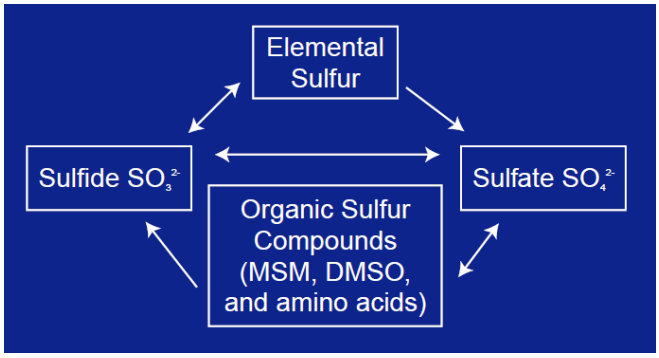


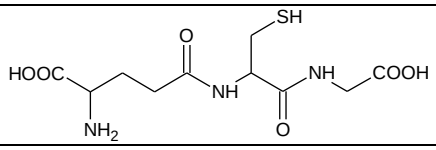
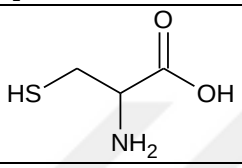
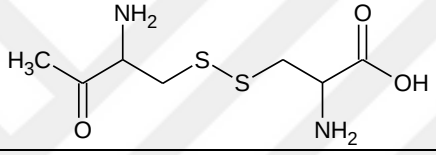
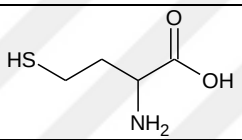
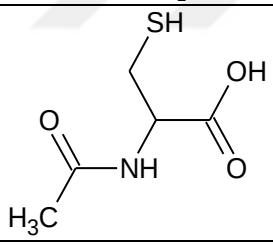
Figure 1. Sulfur cycle in biological organisms.

The side chain of almost every protein structure contains sulfur in form of methionine, cysteine and cystine. Generally, organosulfur compounds in metabolism can be classified in three groups; thioether molecules, thiol/disulfide molecules and other types of sulfur containing molecules like xenobiotics, glycosaminocans and vitamins (46).

2.2. Organosulfur Compounds In Human Metabolism

2.2.1. Thiols (Sulfhydryls) / Disulfides

Table 1. Oxidation numbers and pKa values of some thiols and disulfides.

Name	Structure	pKa	Oxidation # of S
GSH		8.6	2-
Cysteine		8.3	2-
Cystine		7.75	1-
Homocysteine		8.8	2-
N-Acetylcysteine		9.5	2-

Thiols, that have several important functions like protein folding, redox signaling and supporting the defense mechanism of cells against oxidative stress, can be found both in cells and plasma (4, 47-54). Cysteine, cysteinylglycin, homocysteine, glutathione, γ-glutamiylcysteine and hydrogen sulfide are simple thiol compounds and thiol derivatives which can either exist in free reduced forms, or be integrated in low molecular weight disulfides or protein mixed disulfides as shown in Table 2 (49).

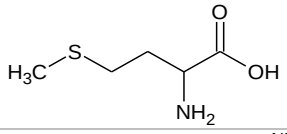
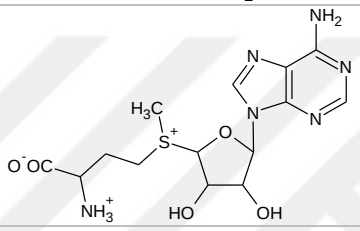
Table 2. Plasma concentrations of some low molecular weight thiols and disulfides.

Species	Plasma Concentration (μM)
Total albumin	527-783
Protein thiols	400-600
Albumin thiol	422± 52
Cysteine	
Total	202-281
Reduced	8.3-10.7
LMW disülfite	41-63
Protein mixed disulfite	145-176
Homocysteine	
Total	6.5-11.9
Reduced	0.17-0.32
LMW disülfite	1.0-1.2
Protein mixed disulfite	7.3-10.4
Thiolactone	0-34.8
Glutathione	
Total	4.9-7.3
Reduced	2.0-5.1
LMW disülfite	0.7-1.6
Protein mixed disulfite	0.7-1.9
γ-Glutamiylcysteine	
Total	3.1-5.4
Reduced	0.06
Hydrogen sulfide	
Protein mixed disulfite	1×10^{-4}

2.3. Thiomethyl compounds

The most important thiomethyl compounds are methionine and S-adenosylmethione, both are crucial for thiol homeostasis.

Table 3. Oxidation number of some thiomethyl compounds.

Name	Structure	Oxidation # of S
Methionine		2-
SAM S-Adenosylmethionine		2-

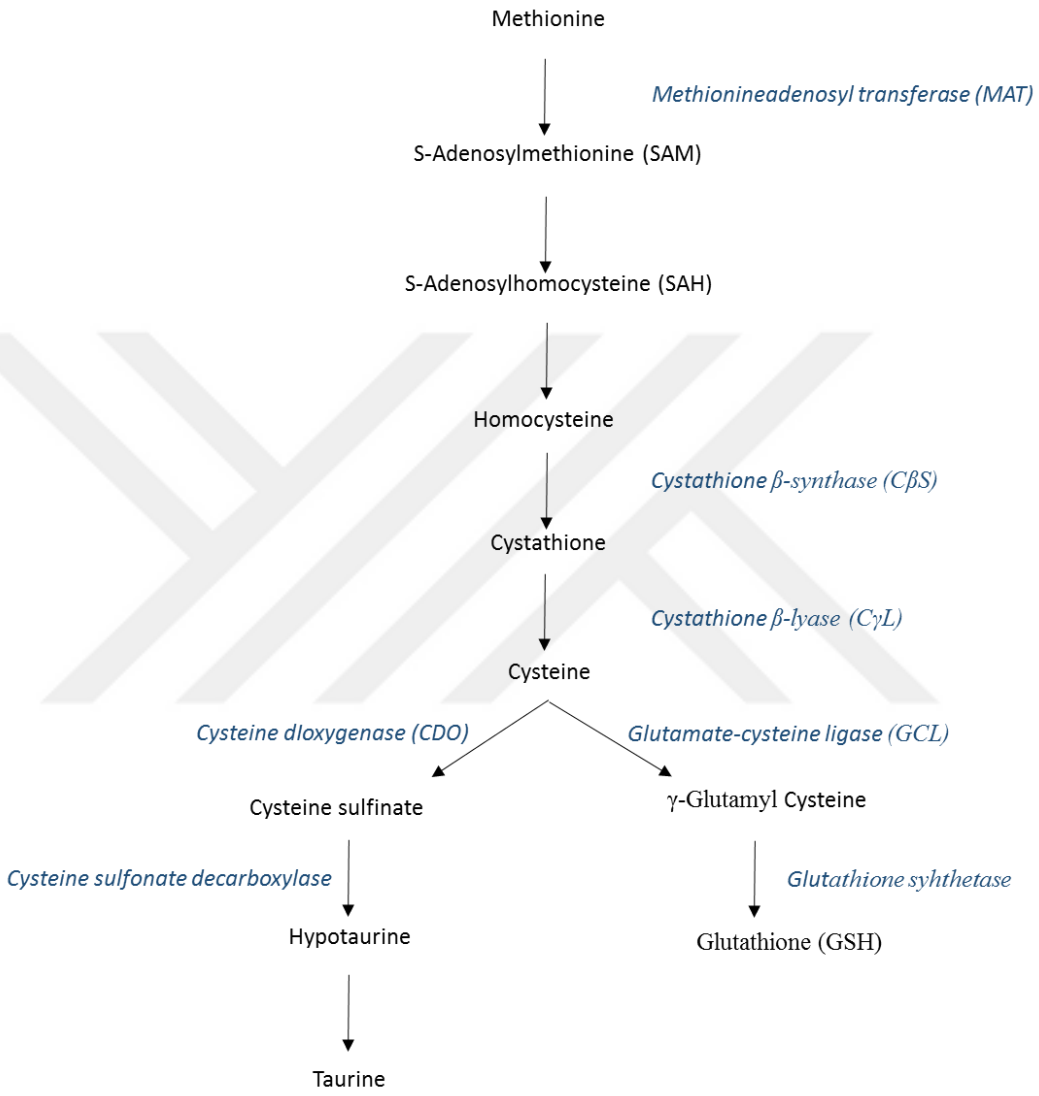
2.3.1.1. Methionine

Hydrophobicity of methionine comes from the sulfur moiety $–SCH_3$ (42). As being a very hydrophobic aminoacid; methionine is usually present in the lipid part of the cell membrane. As a consequence of its hydrophobicity, methionine starts protein synthesis; binding met-$tRNA^{met}$ to ribosomal subunit (56). In some proteins, zones with methionine can be prone to interact with cell surface, as a result of that, methionine can be oxidized to methionine sulfoxide. This kind of methionine parts are called endogenous antioxidants (57). A very important semi-essential aminoacid cysteine can be syntheised from methionine in human metabolism as it is illustrated in Scheme 2 (57).

2.3.1.2. S-adenosylmethionine (SAM)

Enzymatic reaction of *L*-methionine and adenosinetriphosphate (ATP) ends up with SAM production.

SAM is a very important co-substrate which has various functions in transmethylation, transsulfuration and aminopropylation processes as shown in Scheme 2 (58).



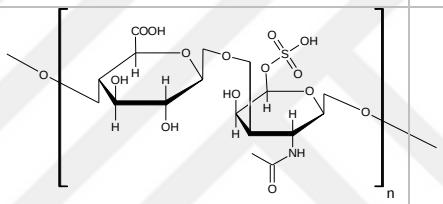
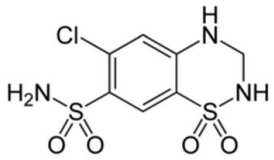
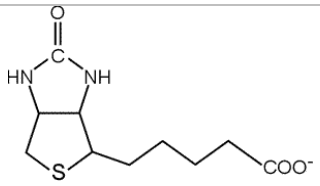
Scheme 2. Transsulfuration pathway.

Transsulfuration process ends up with the synthesis of aminoacid cysteine which is an absolute must in order to synthesize glutathione (2, 57).

2.3.2. Sulfur containing other important molecules

Drugs, glycosaminoglycans and vitamins are important sulfur containing compound groups, these groups are exemplified in Table 4. Hydrochlorothiazide is a sulfur containing drug molecule used for the treatment of hypertension (59). As an example of glycosaminoglycans; chondroitin-6-sulfate is an important component of joints which provides strength to cartilages (60). Biotin is an important sulfur containing vitamin which takes place in the synthesis of fatty acids, isoleucine, and valine.

Table 4. Examples of some sulfur compounds in metabolism.

Name	Structure	Oxidation # of S
Chondroitin-6-sulfate		6+
Hydrochlorothiazide		4+
Biotin		2-

2.4. Reactive and Unreactive Thiols

Thiols can be divided into two groups according to their reactivity as reactive and unreactive. Two important factors determine the reactivity of a cysteine residue. First factor is accesibility. If the cysteine is embedded inside the protein structure, where it is highly hydrophobic, it is usually accepted as unreactive. Its role is limited to conformational regulations via disulfide bonds (Figure 2) (61). While peptide bonds bind aminoacids together and form protein backbone; disulfide bonds occur between the thiol groups of cysteines.

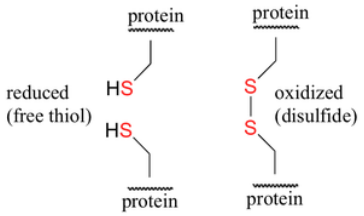


Figure 2. Reduced thiol and oxidized disulfide forms in proteins.

Interchain and intrachain disulfide bonds provide conformation and functionalize the protein skeletal. As the proteins become folded and acquire their three dimensional conformation, they are ready to accomplish their biological roles in the metabolism (62). For instance, the insulin protein has four chains (the A, B, C and D chains). Figure 3 illustrates A and B chain that are bound to each other through three disulfide bonds with two interchain and one intrachain disulfides. These chemical modifications provide the protein's quaternary structure.

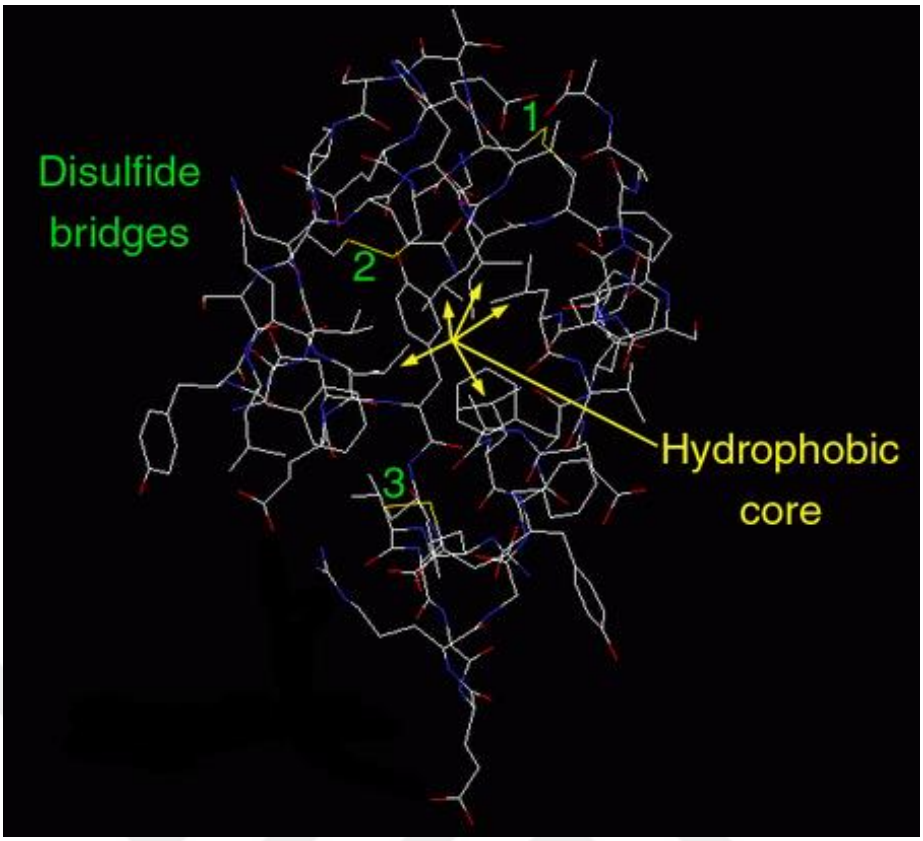


Figure 3. Intramolecular and intermolecular disulfide bond illustration of one monomer of insulin molecule.

Unreactive thiols are reported to be acting very slowly against thiol binding reagents in comparison with reactive thiols or simple thiols. Hemoglobin has totally 6 cysteine residues, 4 of them are unreactive and form 2 disulfide bonds. The remaining 2 other are reactive and are prone to be oxidized by any thiol binding reagent as shown in Figure 5. This proves the participation and stability of unreactive thiols in disulfide bonds (63).

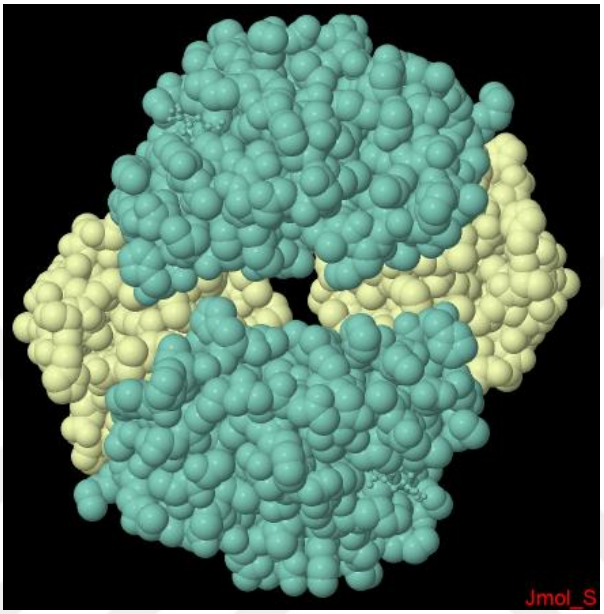


Figure 4. Deoxyhemoglobin PDBID: 2HBS.

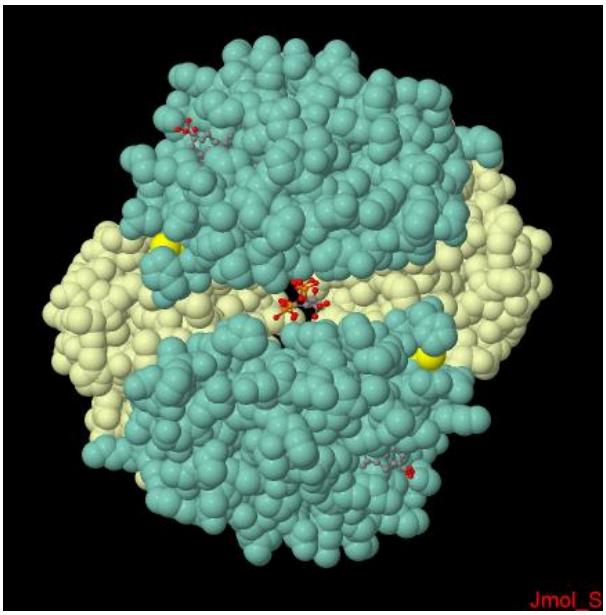


Figure 5. Deoxyhemoglobin-SH drug complex PDBID: 3WCP. Two reactive cysteine residues of hemoglobin are illustrated in yellow.

Second factor is pKa. Thiols with low pKa values and that can easily lose their proton in physiological pH values are identified as reactive thiols (61). The cysteine aminoacid has a thiol function which has pKa 8.3, it is relatively closer to biological pH value comparing with other aminoacids. Even a slight increase in the environmental pH may lead cysteine residues to behave as nucleophiles or sudden charge interchanges may cause regional molecular surface alterations that can effect the interactions of a protein with the environment.

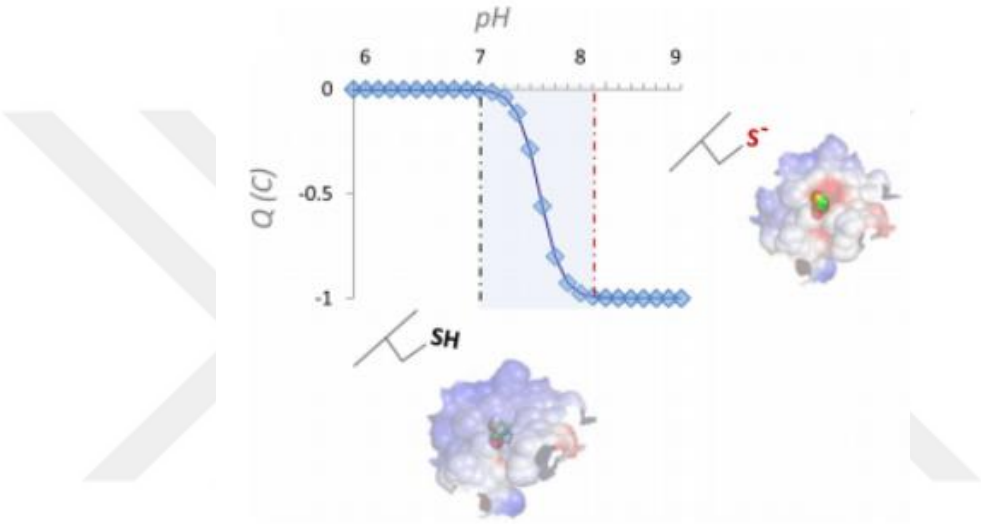


Figure 6. Relationship between pH and thiolate occurance.

In slightly alkaline medium thiols tend to loose their proton and thiolate form occurs as shown in Figure 6.

According to the pH values of the cell compartments that are shown in Figure 7A, the pH in the cells have a reducing effect on thiols, while in plasma oxidized forms are more common as it is showed in Table 2 (64, 65).

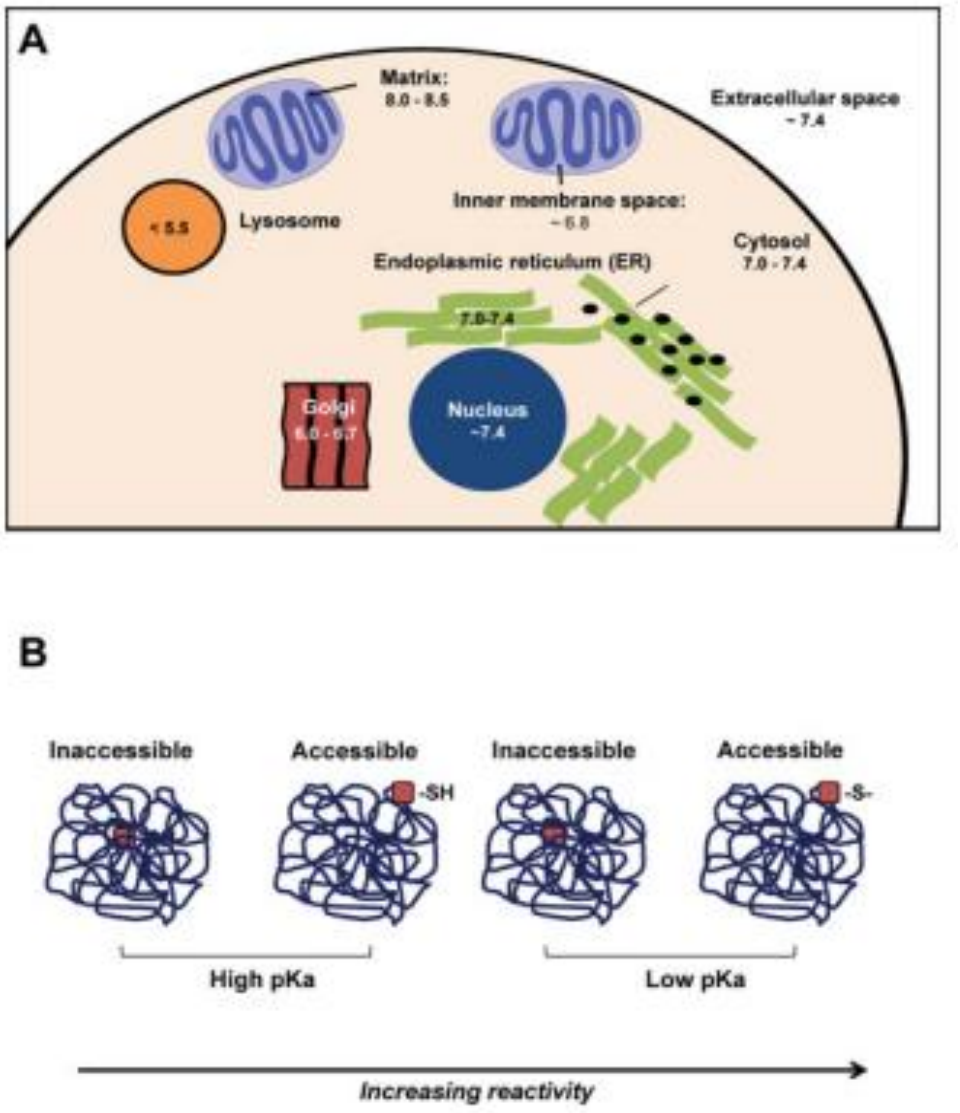
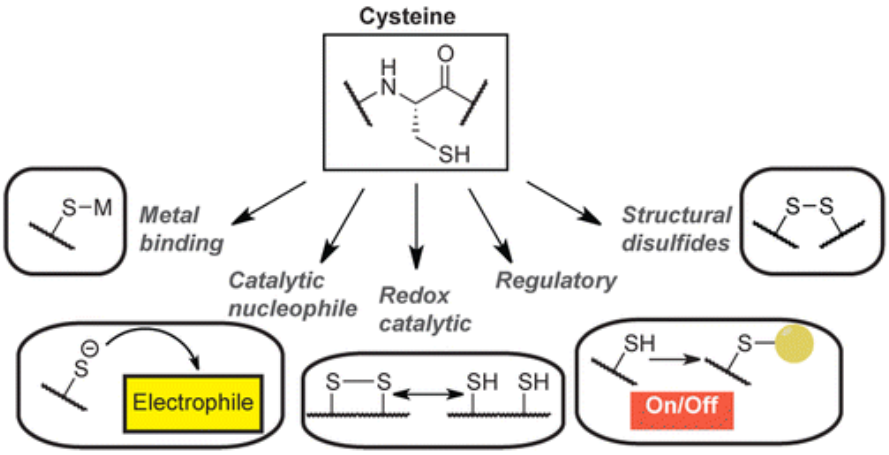


Figure 7. A. pH values of cell compartments and extracellular space. B. Effect of steric hindrence and pKa on reactivity.

2.5. Biological Functions of Cysteine Thiols



Scheme 3. Various functions of cysteine thiols.

The thiol function of cysteine has diverse functional roles as shown in Scheme 3. While structural and metal binding cysteines bring structural persistence, or conformational diversity to proteins, redox catalytic and regulatory cysteines act in order to decrease the damaging effects of oxidative stress (54).

2.5.1. Metal binding cysteine residues

Metal-cysteine complexes, for instance zinc finger motifs (Figure 8), can work in order to stabilize protein structure or act as redox switches (66-69).

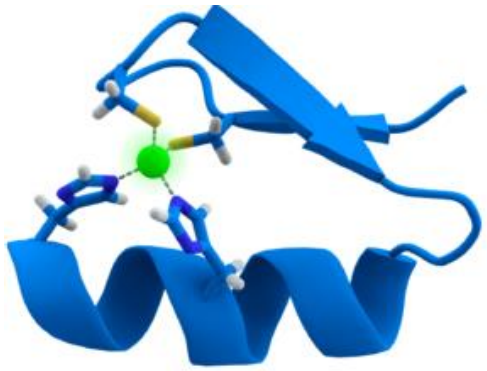


Figure 8. Zinc finger motif with 2 cysteines and 2 histidines.

2.5.2. Catalytic nucleophilicity of cysteine thiols

Cysteine is found as the central aminoacid in the active sites of some enzymes. For instance in thiol protease enzyme class, cysteine behaves as the catalytic nucleophile and forms enzyme-substrate acyl intermediate (70).

2.5.3. Regulatory functions of cysteine thiols

2.4.3.1 Oxidative stress

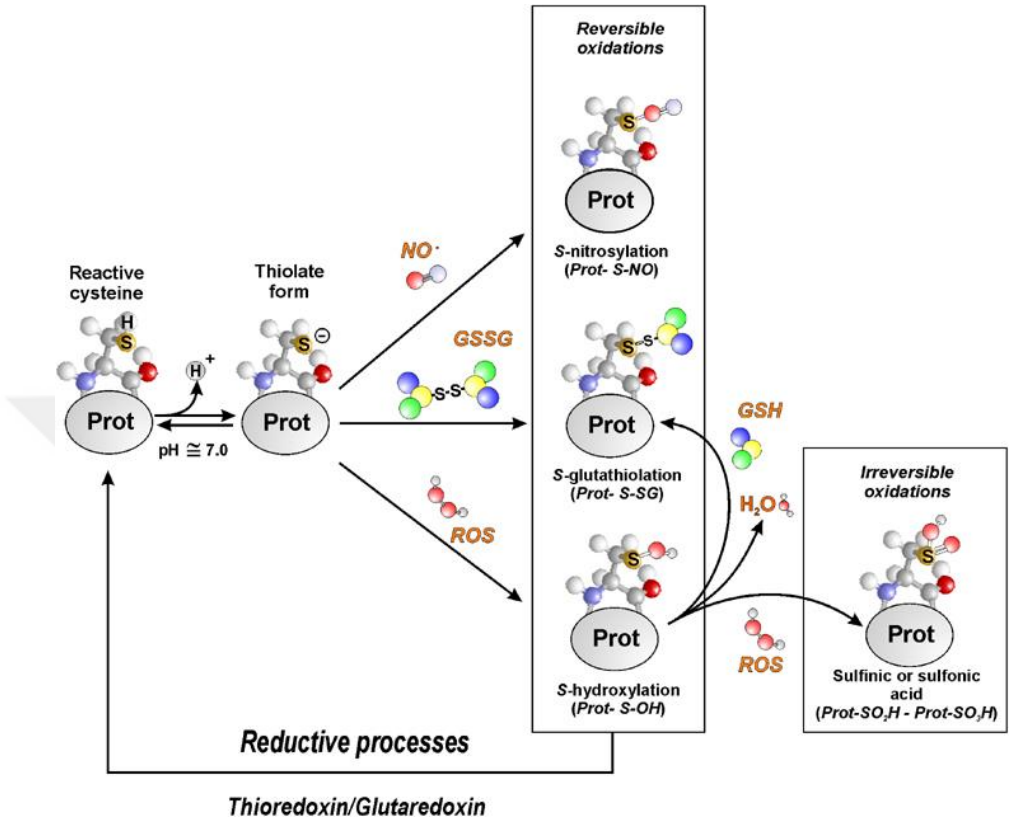
Oxidative stress can be defined as the situation where free radicals defeat antioxidant mechanism of the cells and damage several important parts like DNA, lipids and proteins. The consequences of oxidative damage are proved to cause many diseases as illustrated in Scheme 4 (71).

Scheme 4. Diseases caused by oxidative stress.

2.4.3.2 S-nitrosylation, S-hydroxylation and S-glutathiolation

Cell signaling process can be activated as a consequence of reactions of protein binded thiolate anion with reactive oxygen species (ROS), nitric oxide (NO) radical or glutathione disulfide (GSSG) molecule in order to cope with oxidative stress as shown in Scheme 5. Engagement of thiolate with NO and GSSG is revocable while binding of ROS can be irrevocable. Engagement of GSH with a S-OH is a kind of protection

mechanism which prevents enzyme active sites from possible permanent damage of ROS (53).



Scheme 5. S-nitrosylation, S-hydroxylation and S-glutathiolation processes.

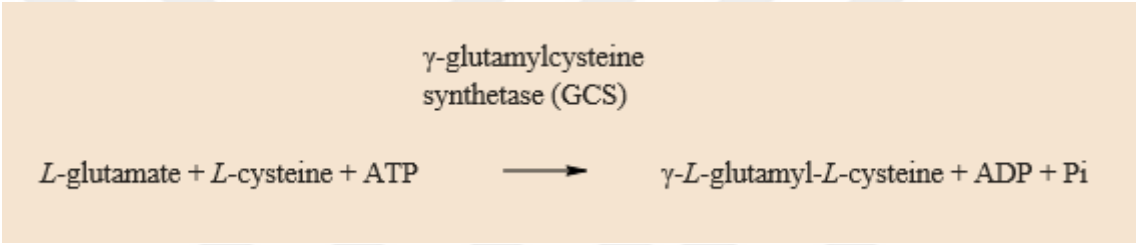
2.5.4. Redox catalytic functions of cysteine thiols

The equilibrium between GSH and GSSG represents the redox state of cells. Protein binded reactive thiols are more common than GSH molecule in the cells. In case of oxidative stress these reactive thiols are easily oxidized, GSH can recover these protein thiols and prevents irreversible oxidative damage by S-glutathiolation, which is a regulatory mechanism as mentioned in Scheme 5. Normally GSSG is found in cells but in small amounts like %1 of GSH concentration. The ascent in the concentration of GSSG indicates the exposure to oxidative stress since it binds with protein thiols and forms more stable molecules than GSSG itself, these molecules contribute cellular signaling. GSSG can be reduced back to GSH by the activity of glutathione reductase enzyme (48).

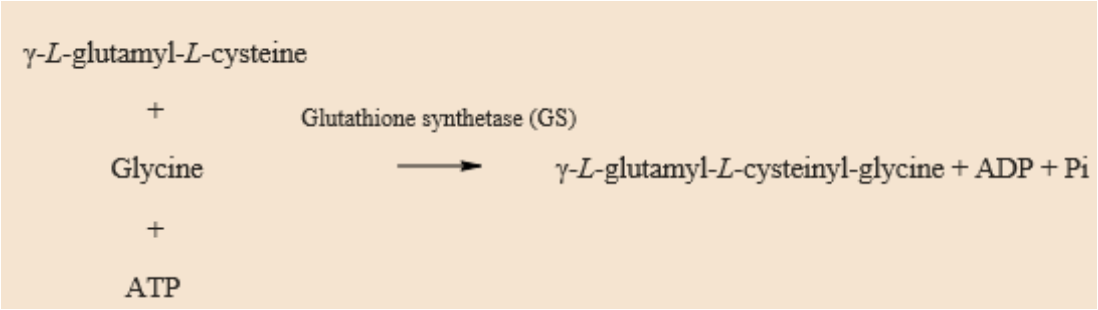
2.6. Glutathione (GSH)

2.6.1. Structure of GSH

GSH is one of the most important low molecular weight thiol which can work directly or indirectly when the concentration of free radicals rise in the cells and extracellular environment. Its constituents are cysteine, glutamate and glycine. The two enzymes which takes part in the synthesis process depend on ATP in order to work, γ-*L*-glutamyl-*L*-cysteine synthesis is the rate limiting step as shown in Scheme 6, so the concentration of *L*-cysteine can limit or boost GSH synthesis according to the circumstances (48).



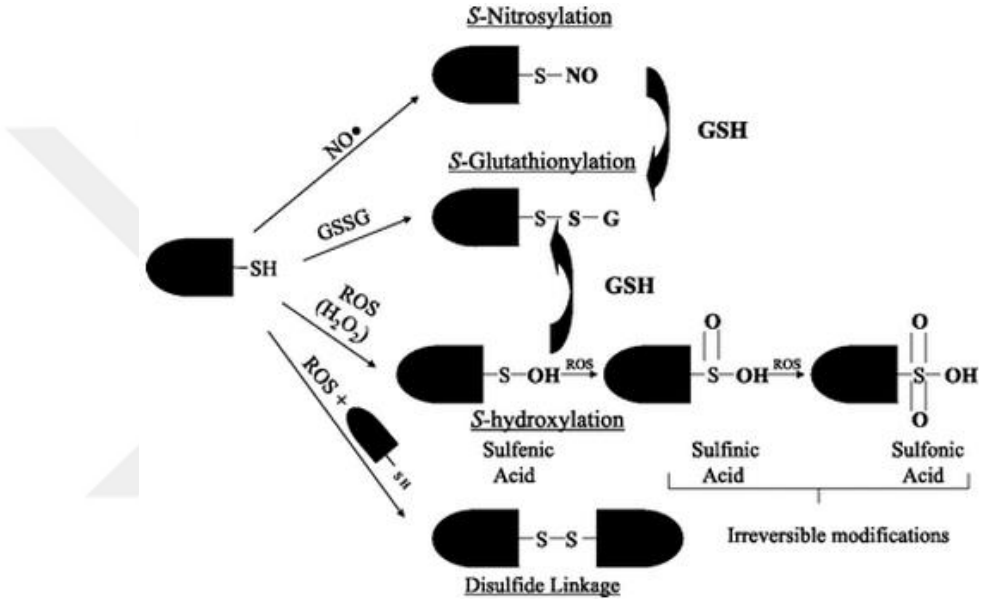
Scheme 6. Rate limiting step of GSH synthesis.



Scheme 7. Second step of glutathione synthesis.

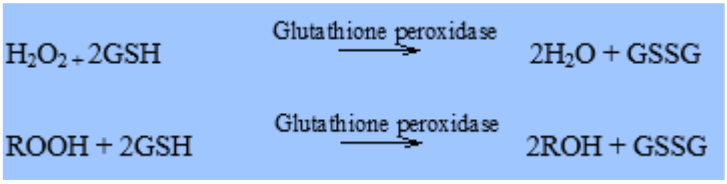
2.6.2. Functions of GSH

In case of a stress caused by ROS and RNS, S-nitrosylation, S-glutathiolation and S-hydroxylation processes begin as shown in Scheme 8, and results with regulatory signals in order to control free radical attack as it is mentioned before. GSH attempts to recover S-nitrosylated or S-hydroxylated thiols by S-glutathionation and this gives rise to GSH levels to decrease. GSH can be re-synthesized by recovery of its constituents but the main path is syhthesing GSH initially (72).



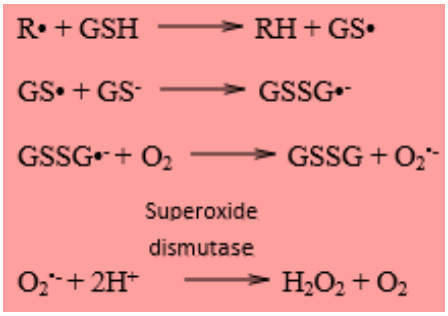
Scheme 8. Redox catalytic functions of protein thiols and GSH.

During the inactivation process of hydroperoxides, glutathione peroxidase converts two GSH molecules to a GSSG using hydroperoxides as its substrate. This is an indirect mechanism of GSH to cope with hydroperoxides as shown in Scheme 9.



Scheme 9. Inactivation of hydroperoxides by glutathione peroxidase.

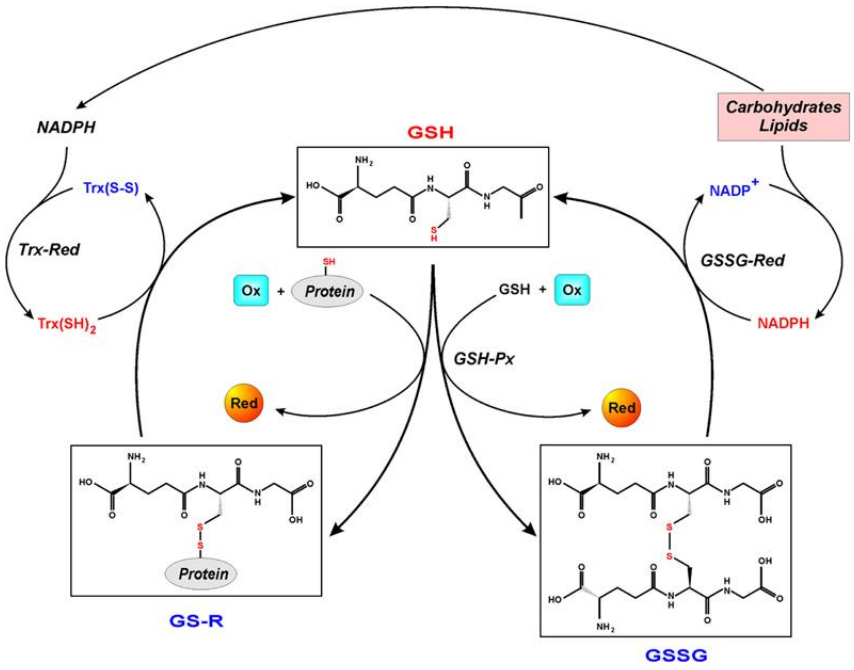
GSH can also directly inactivate carbon based radicals and helps cells to cope with oxidative stress in a direct way as shown in Scheme 10 (73).



Scheme 10. Direct inactivation of carbon based radicals by glutathione.

2.6.3. Consumption and recovery of GSH

Consumption and regeneration of GSH is depicted in Scheme 11. Although it is possible for the metabolism to recover GSH, some of the conjugations can be irreversible and GSH amount can be decreased. GSH synthesis is regulated with the reactions which are shown in Scheme 6 and 7. In Scheme 6, GSH amount is the factor which controls GCS enzyme. As the GSH amount decreases reaction rate of the synthesize will increase but this will be a temporary effect (74).



Scheme 11. Glutatione (GSH) oxidation and reduction cycle.

2.6.4. The importance of cysteine abundancy in GSH recovery

The abundancy glutamate, glycine, cysteine and glutamate in the cells are shown in Figure 9. Both glycine and glutamate are above their Michalis Menten constant (*Km)* in sometic cells while free cysteine can be found approximately around its *Km.* Amounts of free cysteine in neurons is limited because of its neurotoxic behavior (75-77).

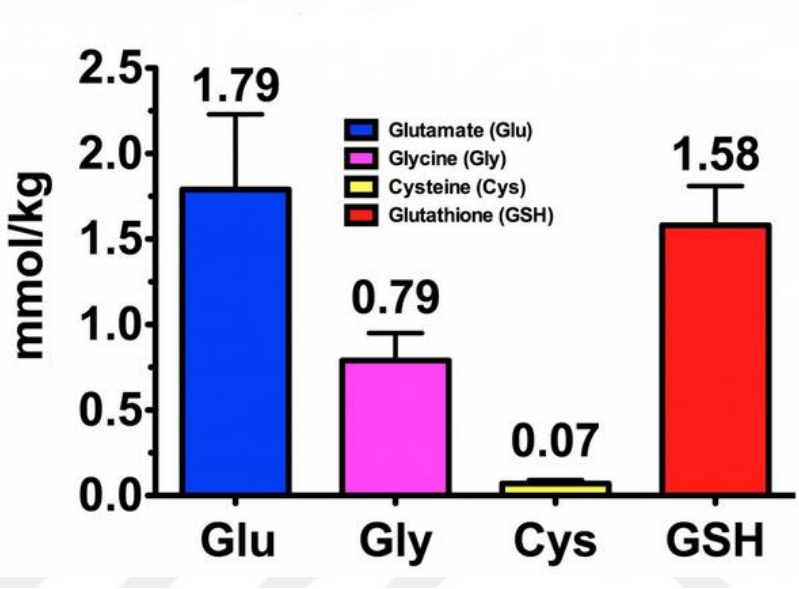


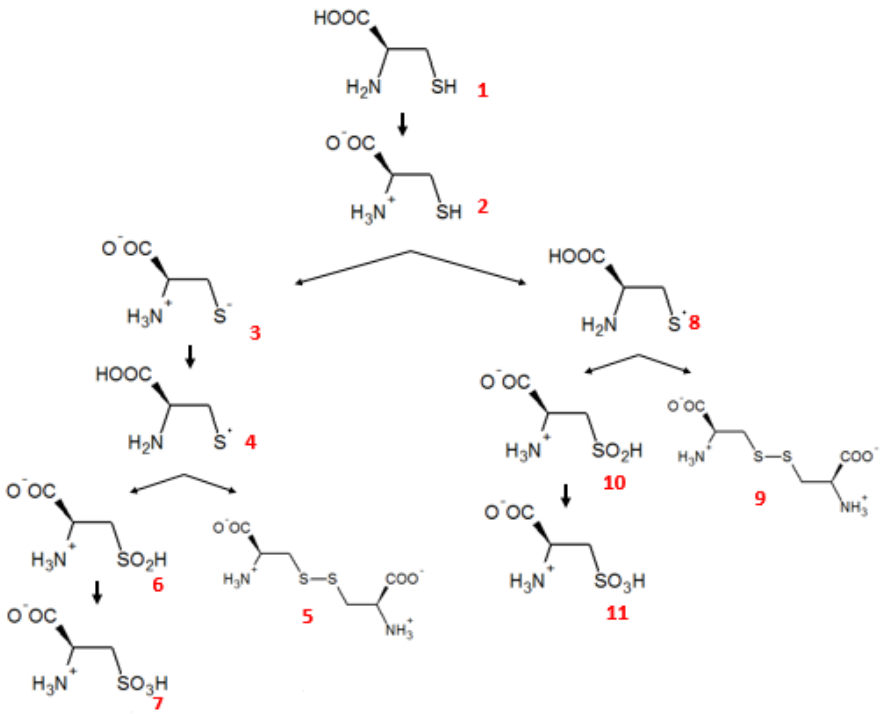
Figure 9. Glutathione, glycine, cysteine and glutathione concentrations in the cells.

This makes cysteine aminoacid the limiting compound for glutathione synthesis. Although sulfhydryl groups usually accepted to be acting like antioxidants, high amounts of cysteine can be cytotoxic for the cells because of its nucleophilic behavior, its interaction with trace metals and its rapid transformation into its less soluble disulfide form cystine so it can not be used as a drug in order to support GSH synthesis (7).

2.7. Some Mechanisms Regarding the Cysteine Reactivity

Possible forms and reactions of free cysteine is depicted in Scheme 12. When cysteine is dissolved in water, its carboxyl and amino groups are ionised (2). Small portions of cysteines also lose their hydrogen from their thiol group and highly reactive thiolate occurs through dissolvation. In a slightyl basic medium of the cells, thiolate occurence will increase to six percent (%6) (3). Nucleophilic behaviour (3) or one electron transfer to metals (4) are possible paths. Cysteine thiolate gives one of its electrons to Cu^{2+} or Fe^{3+} or Fe^{2+}, two thiyl radicals combine and forms less soluble cystine (5). Under heavy oxidative stress consecutively *L*-cysteine sulfinic acid and sulfonic acid occurs (6,7) (77-79).

Free radicals can break of the hydrogen bond of thiol leaving one electron to the sulfur (8). Under mild conditions cystine occurs (9) while heavy oxidative stress results with consequently *L*-cysteine sulfinic and sulfonic acids (10,11) (80).



Scheme 12. Fate of *L*-cysteine in biological environment.

2.8. Neurotoxicity of Cysteine

In neurons, interactive relation of thiolate and metals results with neurotoxicity. There are some hypothesis for the toxicity mechanisms. First one is that copper helps sulfur transferring one of its electron to oxygen, subsequently superoxide, hydrogenperoxide and hydroxyl radical occurs (78, 81). Secondly sulfur transfers one of its electron to cell-free Fe^{3+}, but not physiologic Fe^{3+}, and cause Fe^{2+} formation which can oxidize hydrogen peroxide to hydroxyl radical that can cause brain damage (82).

2.9. Antioxidant Capacity of Cysteine

Cysteine can exhibit antioxidant properties by two different mechanisms. First mechanism is to support GSH synthesis and second one is to act like radical scavenger itself. When the cysteine concentration is low; first mechanism will be dominant. As the cysteine concentration rises, second mechanism can be active too (2, 3).

Direct oxidation of *L*-cysteine by free (Cu^{2+}, Fe^{2+} or Fe^{3+}) or biological sourced (hemin) metals is possible. Being target molucule for some metals protects important components of the cells from oxidation. As *L*-cysteine thiolate gives one of its electrons to Cu^{2+} or Fe^{3+} (hemin) and Fe^{2+}, low density lipoproteins (LDL) are protected as the Cu^{2+} or Fe^{2+} concentration are decreased by cysteine (4-6).

Irradiation also results with oxidation of *L*-cysteine to its sulfonic acid form. *L*-Cysteine also reacts with molecular oxygen(O_2) and Hydrogen peroxide(H_2O_2) in order to protect cells from oxidation (80).

2.10. Some Important Cysteine Prodrugs

GSH transmission through blood brain barrier (BBB) is poor due to its hydrophobicity. Reduced/total cysteine concentrations in Table 2 demonstrates that plasma have oxidizing effect on thiols (49). Cystine, oxidized form of cysteine (Table 1), will be reduced to cysteine in less alkaline cytosol medium. Since cystine can pass BBB, it is the cysteine source for the biosynthesis of GSH in neurons. In order to support GSH synthesis cystine is not convenient as a prodrug since it is hydrophobic (7).

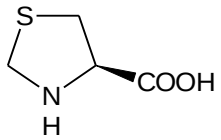
2.10.1. N-acetylcylsteine (NAC)

Cysteine is the most reactive thiol comparing to structurally similar low molecular weight thiols. As it is seen in Table 1, the difference between NAC and cysteine is that NAC have an acetyl binded to its amino group. This structural difference leads to a less reactive thiol. This makes NAC a more stable and safe resource of cysteine (83).

When NAC is taken orally, nearly all of it will be transformed subsequently into cysteine and GSH within the first pass effect in kidneys and liver (84, 85). The primary antioxidant effect is increasing GSH levels in order to support metabolism against several diseases such as acetaminophen toxicity, AIDS, cystic fibrosis, chronic obstructive pulmonary disease and diabetes (86-92).

NAC also has the ability to inactivate free radicals directly but with a low reaction rate (93, 94).

2.10.2. Thiazolidine derivatives (TCA)



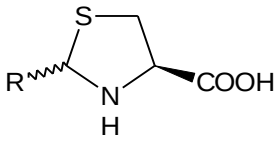
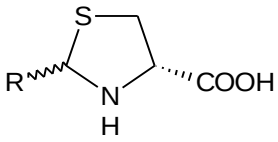
Thiazolidine-4(R)-carboxylic acid (TCA)

Thiazolidine-4(R)-carboxylic acid (TCA) is a five membered heterocyclic compound which is proved to be hepatoprotective. It supports GSH synthesis in liver by releasing *L*-cysteine. A mitochondrial enzyme, proline oxidase metabolises most of the TCA (8, 95).

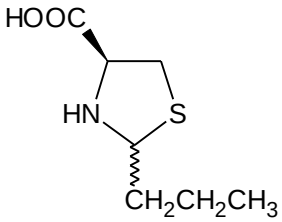
It is reported that 2-substituted alkyl dervatives of TCA reveals *L*-cysteine without a need of enzymatic reactions (9-11, 13, 14, 96).

Nagasawa *et al*. synthesized a series of 2-alkyl and aryl substituted TCA molecules (Table 5). Molecules 1b, 1d and 1e protect hepatic cells against acetaminophen better than molecules 1c, 1g, 1f, molecule 2b, *S* conformation of molecule 1b, did not show any protection effect (38).

Table 5. 2-substituted-alkyl and aryl derivatives of TCA as prodrugs of cysteine

COMPOUND		R groups
1 *R* conformation 	2 *S* conformation 	
1a		H
1b	2b	CH_3
1c		C_2H_5
1d		n-C_3H_7
1e		n-C_5H_{11}
1f		C_6H_5
1g		4.C_5H_4N

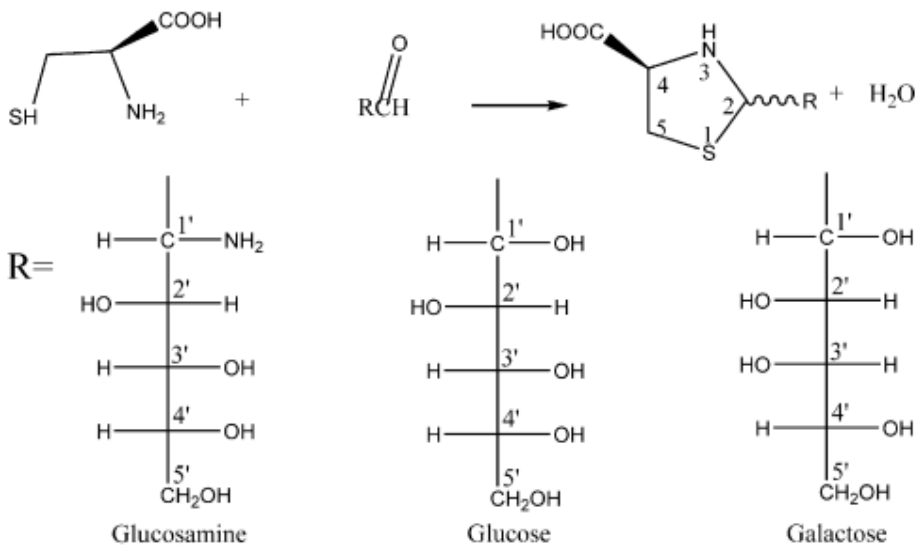
Srinivasan *et al.* synthesized and evaluated hepatoprotective actions of 2(*RS*)-n-propylthiazolidine-4*R*-carboxylic acid (PTCA) against analgesic and antipyretic drug N-acetyl-p-aminophenol (APAP). APAP is toxic in high doses.



2(*RS*)-n-propylthiazolidine-4R-carboxylic acid (PTCA)

It is stated that PTCA exhibits its protection mechanism by revealing *L*-cysteine through solvation which increase GSH synthesis (97, 98). In order to exhibit its activity must be in *L* form (98).

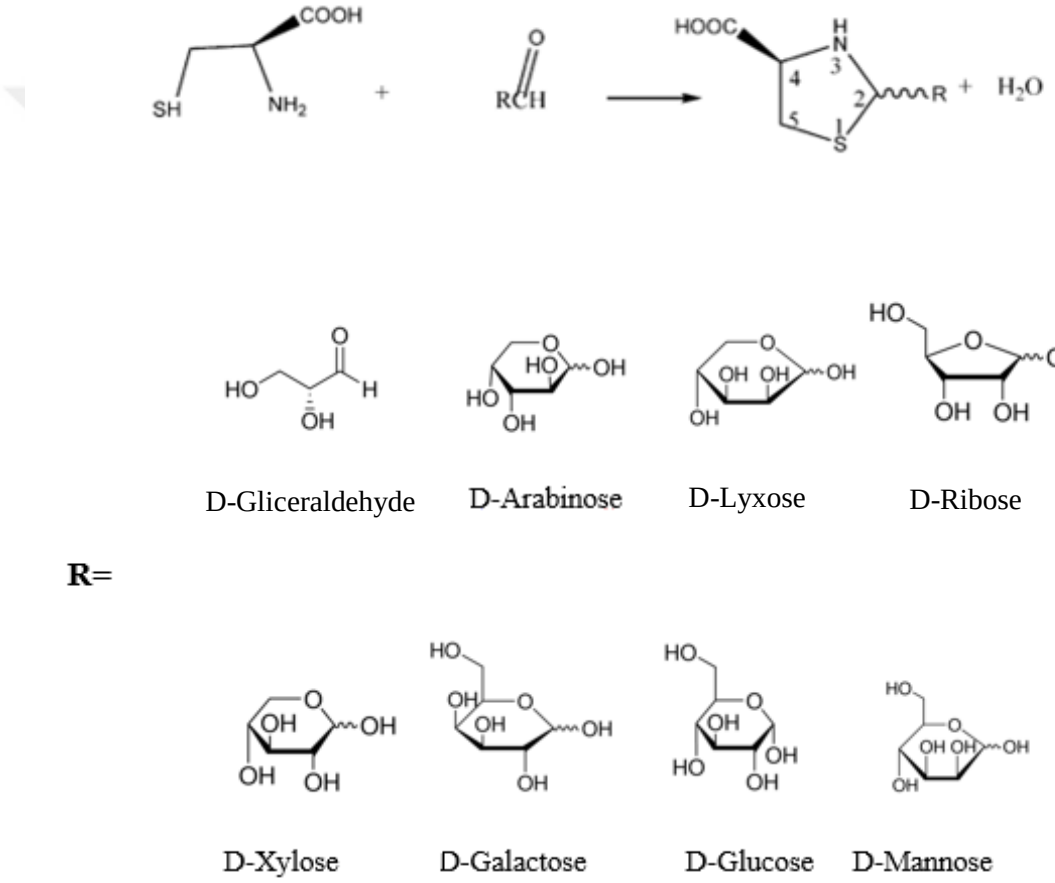
Yan *et al.* obtained the thiazolidine by condensation reaction of *L*-cysteine with glucosamine glucose and galactose sugar molecules (Scheme 13).



Scheme 13. 2-Substituted TCA derivatives with the corresspponding sugars.

2-Glucosaminethiazolidine-4(*R*)-carboxylic acid ($GlcNH_2Cys$), 2-galactoseaminethiazolidine-4(*R*)-carboxylic acid (GalCys) and 2-glucosethiazolidine-4(*R*)-carboxylic acid are obtained. These TCA molecules which are derivatized with sugar give *L*-cysteine and the relevant sugar when they are hydrolysed. $GlcNH_2Cys$ GalCys, GluCys and $GlcNH_2Cys$ molecules evaluated *in vivo* and *in vitro*. *In vitro* studies shows that reducing power of $GlcNH_2Cys$ is as strong as ascorbic acid at the same concentration and the reducing ability is directly proportional with the concentration. Strong chelating activity is explained with the glucosamine binding to ferrous. In comparison with mannitol, •OH scavenging activity is found significantly better. GluCys, glucosamine, $GlcNH_2Cys$, and GalCys protected deoxyribose 79%, 35%, 60%, and 74.5% when the molecules are in a concentration of 2 mg/mL. When the TCA molecules and glucosamine are in a concentration 2.8 mg/ mL, glucosamine, GalCys, GluCys, and $GlcNH_2Cys$ show 38%, 60%, 70%, and 80% comparing to control so it is suggested that they might protect cell membrane against oxidative damage. It is also showed that $GlcNH_2Cys$ molecule decreased the oxidative damage of proteins which is caused by •OH %49. *In vivo* activity of $GlcNH_2Cys$ increased –SH levels of the cells against APAP induced acetaminophen toxicity (99).

Roberts *et al.* obtained 2-(polyhydroxyalkyl)thiazolidine-4(*R*)-carboxylic acids with naturally occurring aldose monosaccharides containing three, five, and six carbon atoms that are capable of releasing *L*-cysteine and the sugars by nonenzymatic ring opening and hydrolysis (Scheme 14). By this way toxic effect of the released aldehydes were prevented. When added to rat hepatocyte preparations *in vitro*, these TCAs (1.0 mM) raised cellular glutathione (GSH) levels 1.2-2.1-fold relative to controls. TCA molecules were also tested as protective agents against acetaminophen-induced hepatotoxicity in mouse model. The TCA derived from *D*-ribose and *L*-cysteine (RibCys) showed the greatest therapeutic effect of the series (95).

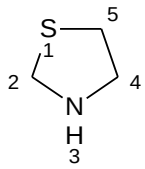


Scheme 14. 2-Substituted TCA derivatives with the correspponding aldose monosaccarides.

2.11. Thiazolidines: structure, synthesis, NMR characterization and biological activity

2.11.1. Structure

Thiazolidine is a heterocycle with a sulfur and a nitrogen at its 1st and 3rd positions. Thiazolidines are known compounds and very useful synthetic intermediates that are widely used.



Thiazolidine ring

2.11.2. Synthesis of Thiazolidine-4-carboxylic acid

It can be synthesized through condensation of an aldehyde or a ketone with cysteine. It is a reversible reaction, both aldehyde or ketone and cysteine can be obtained back. As the condensation reaction is done with optically pure cysteine, the resulting thiazolidine will be a diasteroemeric mixture: using *L*-cysteine gives the 2R, 4R and 2S, 4R molecules however *D*-cysteine leads to the 2R, 4S and 2S, 4S compounds (Figure 10).

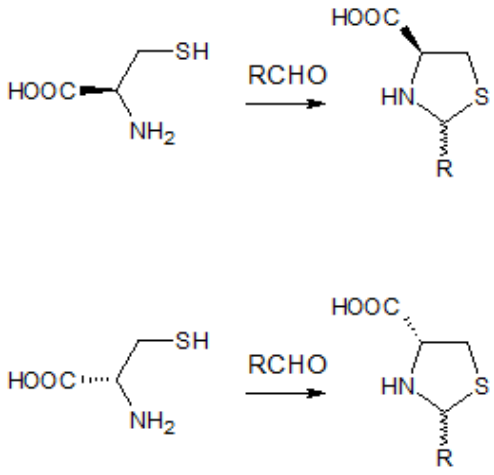
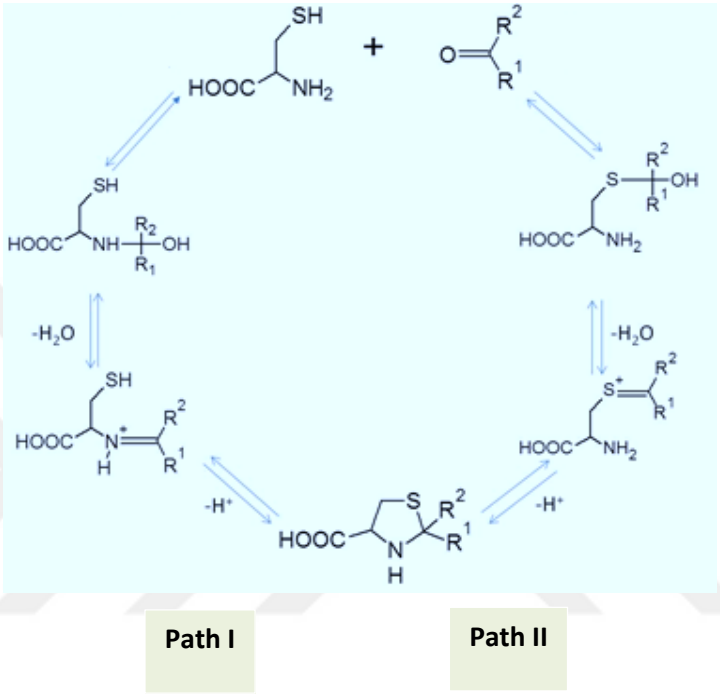


Figure 10. 2-Substituted TCA molecule as a diastereomeric mixture.

Thiazolidine ring can occur by two different pathways according to environmental pH (Scheme 15). In the first path; a cationic schiff base forms with an addition-elimination sequence and subsequently thiazolidine cycle occurs. In alkali circumstances first pathway will take place. In the second path sulfonium ion intermediate occurs firstly as the sulfur atom attacks to the carbonyl (100).



Scheme 15. TCA synthesis pathways in acidic (path II) and basic (path I) environment.

In the literature following methods were given for the synthesis of 2-substituted TCAs.

Table 6. Synthethic pathways for the synthesis of 2-substituted TCA derivatives.

Cysteine	Aldehyde	Base	Solvent	Conditions	Yield %	Ref.
L-Cysteine hydrochloride monohydrate	Benzaldehyde *o*-Hydroxy, *o*-nitro, *p*-Cyano, *2*-Hydroxy-4-methoxy, benzaldehydes.	$NaHCO_3$	EtOH:H_2O 50:50	RT	80-95	(21)
L-Cysteine	2-pyridine carboxaldehyde, 3-pyridine carboxaldehyde, 4-pyridine carboxaldehyde, Benzaldehyde, *p*-nitro, *p*-chloro, *p*-methyl, 4-(dimethylamino), benzaldehydes	$NaHCO_3$	EtOH:H_2O 50:50	RT	80-90	(39)

Table 7. Synthethic pathways for the synthesis of 2-substituted TCA derivatives

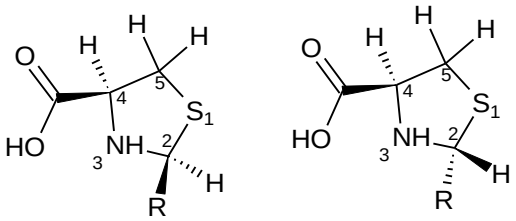
Cysteine	Aldehyde	Base	Solvent	Conditions	Yield %	Ref.
L-Cysteine hydrochloride monohydrate	*p*-methyl benzaldehyde	KOAc	EtOH:H_2O 60:40	RT	89	(37)
L-Cysteine	*p*-Hydroxy *p*-methoxy, *o*-Hydroxy, 3-Hydroxy-*4*-methoxy, 3-methoxy-*4*-Hydroxy, 3-ethoxy-*4*-Hydroxy, 3,4,5-trimethoxy, 2,4-dimethoxy, 3,4-dimethoxy, 3,5-dimethoxy-4-hydroxy, benzaldehydes.	-	EtOH	Under reflux condenser	40-77	(36)

Table 8. Synthethic pathways for the synthesis of 2-substituted TCA derivatives.

Cysteine	Aldehyde	Base	Solvent	Conditi-ons	Yield %	Ref
L-Cysteine hydrochloride	Benzaldehyde *m*-chloro, *m*-Hydroxy, *p*-Hydroxy *p*-Cyano, *p*-methoxy, *p*-nitro, 3-methoxy-*4*-Hydroxy, 3,4,5-trimehoxy,	NaOAc	EtOH:H_2O 60:40	RT, In ice water	62-98	(35)
L-Cysteine	Benzaldehyde *p*-chloro, *p*-floro, *p*-hydroxy, *p*-metoxy, 2,5-dimethoxy, 2,4,5-trimethoxy, benzaldehydes.	-	EtOH	RT	85-94	(33)
L-Cysteine hydrochloride monohydrate	Benzaldehyde	NaOH	EtOH:H_2O 50 : 50	RT	98	(19)

2.11.3. ^{1}H NMR Characterization

For the identification of 2-substituted TCA ring C2-H, C4-H and C5-H peaks must be examined. It is important to consider that the two hydrogens of the 5th carbon are not equivalent.



2-Substituted TCA ring in 2R, 4R and 2S, 4R configurations

The signals of both diastereomers (2S, 4R and 2R, 4R) can be clearly observed with the ratio of occurance. C2-H signals are observed as two separate singlets at around 5.58 and 5.32 ppm. The C4 protons are observed as doublet of doublets around 4.33 and 3.90 ppm which couple with both of the unequivalent C5. Four groups of doublet of doublets represent C5 protons. Peaks for the major isomer are located around 3.41 whereas peak at 3.90 ppm indicated for the minor isomer, C5-H dd signals are located around 3.30 and 3.12 ppm.

The 2S, 4R diastereomer is expected to be the major one since in this configuration the two massive groups, R group and the carboxylic acid function, locates in the opposite sides of the thiazolidine ring. Yu *et al.* proved this distribution by 1D nuclear Overhauser effect (NOE) experiments as illustrated in Figure 11; while the irradiation of the signal of the C4-H of the major isomer (peak at around 4.33 ppm) did not generated any NOE signal with the corresponding C2-H peak (around 5.32 ppm), the C2-H (around 5.58 ppm) of the minor isomer gave a strong NOE signal when the corresponding C4-H (around 3.90 ppm) was irradiated (24).

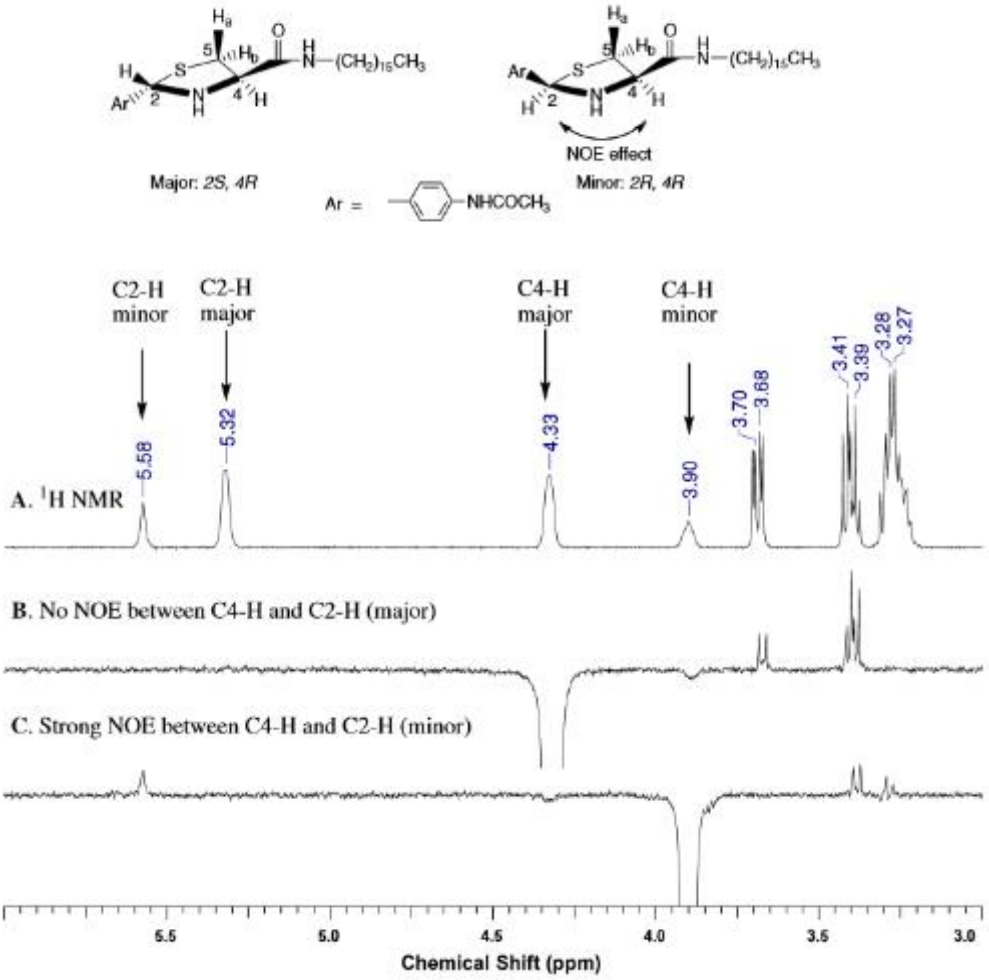


Figure 11. A. ^{1}H NMR of 2-Aromatic thiazolidine-4-carboxyamide ring B. and C. NOE between C4-H and C2-H major and minor peaks

2.11.4. Biological activities of 2-phenyl thiazolidine carboxylic acid derivatives

Some of the compounds (**1, 4, 5, 6, 7, 8** and **9)** were previously evaluated for several biologic activities including antivirutic, antifungal, antibacterial, antioxidant, urease inhibition, tyrosinase inhibition, anticancer and prevention of the β-amyloid peptide aggregation activities, the results are shown in Tables 9 and 10.

Table 9. Biological activities of the compounds 1, 4, 5 and 6 reported in the literature.

Compound	Activity	Potency				Refence
1	Antivirutic activity	IC_{50} = 21,3l μM				(21)
	Prevention of the β-amyloid peptide aggregation	Signicificant activity				(39)
	Urease inhibition	IC_{50} = 58,32 μM				(35)
	Antifungal activity	*CA*	*CN*	*TM*	*AF*	(33)
		I	0,62 μM	0,62 μM	I	
	Antibacterial activity	*BS*	*SA*	*PA*	*EC*	(34)
		3,125 μM	12,5 μM	>50 μM	25 μM	
	Antioxidant activity (in vivo)	Active				(38)
4	Urease inhibition	IC_{50} = 48,91 μM				(35)
5	Urease inhibition	IC_{50} = 10,62 μM				(35)
6	Prevention of the β-amyloid peptide aggregation	Signicificant activity				(39)

Table 10. Biological activities of the compounds 7, 8 and 9 reported in the literature.

Compound	Activity	Potency				Ref.
7	Tyrosinase inhibition	%21 inhibition				(36)
	Urease inhibition	IC_{50} = 12,08 μM				(35)
	Antifungal activity	*CA*	*CN*	*TM*	*AF*	(33)
		I	0,62 μM	0,62 μM	I	
	Antibacterial activity	*BS*	*SA*	*PA*	*EC*	(34)
		12,5 μM	25 μM	>50 μM	>50 μM	
8	Prevention of the β-amyloid peptide aggregation	Signicificant activity				(39)
	Antitumor activity	Inactive				(37)
9	Prevention of the β-amyloid peptide aggregation	Signicificant activity				(39)
	Urease inhibition	IC_{50} = 39,28 μM				(35)

AF : Aspergillus fumigatus, BS : Bacillus subtilis, CA : Candida albicans, CN : Cryptococcus neoformans, EC : Escherichia coli, PA : Pseudomonas aeruginosa, SA : Pseudomonas aeruginosa, TM : Tricophyton mentagrophytes.

3. MATERIALS AND METHODS

3.1. Chemicals

All starting materials were commercially available used without further purification. *L*-cysteine hydrochloride monohydrate is purchased from Merck KGaA and the aldehydes; 4-chlorobenzaldehyde, 2,3-dichlorobenzaldehyde, 2,3-difluorobenzaldehyde, 3-chlorobenzaldeyde, 2,6-difluorobenzaldehyde, 4-methoxybenzaldehyde, 2-bromobenzaldehyde, 4-methylbenzaldehyde, 4-nitrobenzaldehyde, 2-methylbenzaldehyde, 2-meyhoxybenzaldehyd were purchased from Sigma–Aldrich.

3.2. Analytical Methods

3.2.1. General Procedure : Preparation of 2(*RS*)-(phenyl) thiazolidine-4(*R*)-carboxylic acid derivatives (1-12)

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 temparature (RT) for three hours. EtOH : H_2O ratio is arranged as 1:1. Solid cyristals of the expected compounds were filtered and washed with water. The crystals were dried under *vacuo* (19).

3.2.2. Melting point determination

Melting points of the compounds were determined by an electrothermal melting point apparatus (Mettler Toledo FP62) in open capillary tubes.

3.2.3. Controls with thin layer chromatography (TLC) system

Plates: During this work, kieselgel 60 F_{254} (Merck) silica gel plaques were used for thin layer chromatography. Completion of the reactions were observed by the chromatographic monitoring of aldehydes.

Solvent systems: Ethyl acetate: *n*-hexane (30 : 70) solvent system was used for chromatographic controls in this work.

Elution conditions: Solvent system was poured to chambers ans waited for saturation. Synthesized compounds and their starting materials were dissolved in

appropriate solvents, applications were made on thin layer chromatography (TLC) plaques and dragged along at room temparature.

Identification of TLC spots: UV light (254 nm) was used for the detection of the spots

3.2.4. Spectrometric analysis

3.2.4.1. Infrared (IR) spectra

Infrared (IR) spectra (10T/cm^3 pressure applied potassium bromide discs) were recorded on a Perkin Elmer FT-IR 1720X spectrometer and the frequencies were expressed in cm^{-1}.

3.2.4.2. HPLC Spectra

Agilent 1260 series HPLC with diode array dedector was used to record spectras. The chromatography column used was 5 μm Zorbax Extend RP-18 column (4.6 mm × 250 mm), as the mobile phase; water : acetonitrile (50 : 50) solvent system was used with the flow rate of 0.5 ml/min.

3.2.4.3. UV spectrophotometric determination

The UV spectrophotometric determinations were done with a Bandelin Sonorex Microplate Photometer during the CUPRAC assay and a Synergy Htx Multiplate Reader during DPPH assay.

3.2.4.4. ^{1}H-NMR Spectra

The NMR spectra were recorded with a Bruker AC 400 Hz spectrometer using tetramethylsilane as the internal reference, dimethylsulfoxide-d_6 (DMSO) as solvent and chemical shifts were reported in parts per million (ppm).

3.2.4.5. ^{13}C-NMR Spectra

^{13}C-NMR Spectra of some compounds were recorded with a Bruker AC 400 Hz spectrometer.

3.2.4.6. LC-MS spectra

Spectra were recorded with a Waters 2695 Alliance Micromass ZQ LC-MS.

3.3. *In vitro* Activity Test Procedures

3.3.1. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical scavenging abilities of the compounds **1-12** were evaluated with respect to the method of Akter *et al*. (100) with few alterations (101). The DPPH assay is a very commonly used methodology for analyzing the antioxidant activity of molecules, it 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 520 nm that belongs to the stable free radicals.

The assay was performed in 96-well plates. The DPPH solution was prepared (0.1mM or 0.004 g / 0.1L) and sample solutions were prepared by appropiate serial dilution within a range from 1000µM to 6.75 µM. As a reference solution, butyl hydroxytoluene (BHT) was prepared and used at a concentration range varying between 2000 and 125 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 were calculated as follows;

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

$A_{control}$ is the absorbance value of the BHT reference solutions and A_{sample} is the absorbance of the samples.

3.3.2. Cupric reducing antioxidant capacity (CUPRAC)

The cupric ion reducing abilities of the compounds **1-12** were evaluated with respect to the method of Apak *et al*. (103) with few alterations (102). Reacting with trace metals and protecting lipids and some parts of the cells from oxidative damage is known as an antioxidant mechanism. As the antioxidant molecule reacts with Cu(II)-neocuproine complex, the complex will be transformed into Cu(I)-neocuproine.

This method is based on the spectrophotometric determination of the yellow Cu(I)-neocuproine complex at 450 nm.

The assay was performed in 96-well plates. Reagent solutions were prepared separately as 10mM $CuSO_4$, 7.5mM neocuproine and 1M ammonium acetate buffer (pH 7.0). Sample solutions were prepared by appropiate serial dilution within a range from 1000µM to 6.75 µM. Trolox, a water soluble analogue of vitamin E, was used as reference in the concentration range of 10µM-70µM.

Into each well, equal volumes of each reagent solution 85µL, 51µL of H_20 and 43µL of sample solution were added. Each sample concentration was evaluated triplicate. The plate was incubated in 50^oC for 20 minutes and absorbance values were measured at 450 nm.

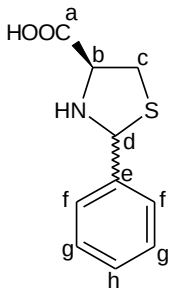
Trolox coefficient values (TEAC) were calculated for each sample as follows;

$$TEAC = \varepsilon_{sample} / \varepsilon_{trolox}$$

4. EXPERIMENTAL

Spectral data is given for the molecules 1-12.

4.1. (2*RS*-4*R*)-2-phenyl-1,3-thiazolidine-4-carboxylic acid (Compound 1) (21, 33-35, 38, 39)



0.028 mol *L*-Cysteine hydrochloride monohydrate and 0. 028 mol benzaldehyde were reacted as described in the general procedure and precipitates were washed with water and dried under *vacuo* to yield 89%. The compound is in white solid form and soluble in pure DMSO.

m.p. : 163ºC , m.p. ^{literature} (21) : 159ºC

FT-IR (KBr), cm^{-1}: 3200-3450 (O-H carboxylic acid), 1575 (C=O), 1435 (C=C, aromatic)

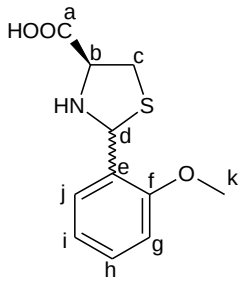
^{1}H-NMR (DMSO-d_6) δ: 3.08 (t, *J*=19.2 Hz, 0.3H, H_c); 3.14 (dd, J_1=4.8 Hz, J_2=10.4 Hz, 0.7H, H_c); 3.30 (dd, J_1=7.6 Hz, J_2=10.8 Hz, 0.7H, H_c); 3.38 (dd, J_1=7.6 Hz, J_2=10.4 Hz, 0.3H, H_c); 3.90 (dd, J_1=7.6 Hz, J_2=8.8 Hz, 0.3H, H_b), 4.23 (dd, J_1=4.8 Hz, J_2=7.2 Hz, 0.7H, H_b); 5.50 (s, 0.3H, H_d); 5.67 (s, 0.7H, H_d); 7.24 – 7.54 (m, 5H, C_{arm})

^{13}C NMR (DMSO-d_6) δ: 37.9, 38.3 (C_c); 64.8, 65.3 (C_b); 71.0, 71.7 (C_d); 126.8, 127.2 (C_h); 127.5, 128.1 (C_g); 128.2, 128.4 (C_f); 115.5, 155.7 (C_f); 172.1, 172.9 (C_a).

Elemental analysis for $C_{10}H_{11}NO_2S$ (M_W = 209.26 g.mol^{-1})

	C %	H %	N %	S %
Calculated	57.39	6.30	6.69	15.32
Found	57.01	5.16	6.78	14.89

4.2. (2*RS*-4*R*)-2-(2-methoxyphenyl)-1,3-thiazolidine-4-carboxylic acid (Compound 2) Cas no : 1029800-35-8



0.028 mol *L*-Cysteine hydrochloride monohydrate and 0.028 mol *o*-methoxy benzaldehyde were reacted as described in the general procedure and precipitates were washed with water and dried under *vacuo* to yield 65%. The compound is in white solid form and soluble in pure DMSO.

m.p. : 144.1ºC

FT-IR (KBr), cm^{-1}: 3100-3300 (O-H carboxylic acid), 3021 (C-H sp^2), 1639 (C=O), 1490 (C=C, aromatic)

^{1}H-NMR (DMSO-d_6) δ: 2.94 - 3 (m, 1H, H_c); 3.18 (dd, J_1=6.8 Hz, J_2=10.4 Hz, 0.4H, H_c); 3.34 (dd, J_1=6.8 Hz, J_2=10 Hz, 0.6H, H_c); 3.80 (s, 1,2H H_k); 3.81 (s, 1.8H, H_k); 3.85 (dd, J=7.2 Hz, 9.2 Hz, 0.4H, H_b); 4.17 (t, J=12.4 Hz 0.6H, H_b); 5.69 (s, 0.6H, H_d); 5.86 (s, 0.4H, H_d); 6.88 – 7.06 (m, 2H, H_{arm}); 7.2 – 7.34 (m, 1H, H_{arm}); 7.39 (dd, J_1=1.2 Hz, J_2=7.6 Hz, 0.4H, H_g); 7.51 (dd, J_1=1.6 Hz, J_2=8 Hz, 0.6H, H_g)

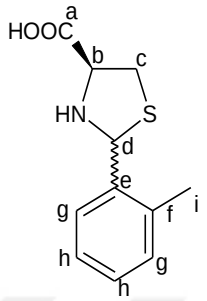
Elemental analysis for $C_{11}H_{13}NO_3S$ (M_W = 239.29 $g.mol^{-1}$)

	C %	H %	N %	S %
Calculated	55.21	5.48	5.85	13.40
Found	54.93	5.43	5.94	13.06

4.3. (2*RS*-4*R*)-2-(2-methylphenyl)-1,3-thiazolidine-4-carboxylic acid (Compound 3) Cas no: 1290211-93-6

$C_{11}H_{13}NO_2S$

M_W = 223.29 g.mol^{-1}



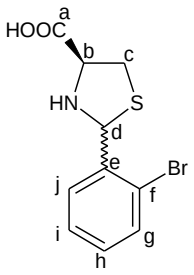
0.028 mol *L*-Cysteine hydrochloride monohydrate and 0.028 mol *o*-tolyl benzaldehyde were reacted as described in the general procedure and precipitates were washed with water and dried under *vacuo* to yield 75%. The compound is in white solid form and soluble in pure DMSO.

m.p. : 154.6ºC

FT-IR (KBr), cm^{-1}: 3210-3430 (O-H carboxylic acid), 3021 (C-H sp^2), 1666 (C=O), 1590 (C=C, aromatic)

^{1}H-NMR (DMSO-d$_6$) δ: 3.05 (dd, J_1=9.2 Hz, J_2=10.2 Hz, 0.4H, H$_c$); 3.09 (dd, J_1=5.2 Hz, J_2=10.4 Hz, 0.6H, H$_c$); 3.29 (dd, J_1=6.8 Hz, J_2=10 Hz, 0.4H, H$_c$); 3.38 (dd, J_1=7.6 Hz, J_2=10.4 Hz, 0.6H, H$_c$); 3.90 (dd, J_1=7.6 Hz, J_2=*8.8* Hz, 0.4H, H$_b$); 4.23 (dd, J_1=5.6 Hz, J_2=7.2 Hz, 0.6H, H$_b$); 5.67 (s, 0.4H, H$_d$); 5.85 (s, 0.6H, H$_d$); 7.12 – 7.84 (m, 4H, H$_{arm}$)

4.4. (2*RS*-4*R*)-2-(2-bromophenyl)-1,3-thiazolidine-4-carboxylic acid (Compound 4) (35)



0.028 mol *L*-Cysteine hydrochloride monohydrate and 0.028 mol *o*-bromo benzaldehyde were reacted as described in the general procedure and precipitates were washed with water and dried under *vacuo* to yield %86. The compound is in white solid form and soluble in pure DMSO.

m.p. : 182.2ºC

FT-IR (KBr), cm^{-1}: 3260-3450 (O-H carboxylic acid), 3060 (C-H sp^2), 1664 (C=O), 1515 (C=C, aromatic)

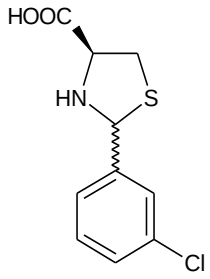
^{1}H-NMR (DMSO-d_6) δ: 2.97 (dd, J_1=6.4 Hz, J_2=10 Hz, 0.7H, H_c); 3.06 (t, J=19.2 Hz, 0.3H, H_c); 3.25 (dd, J_1=6.4 Hz, J_2=10 Hz, 0.7H, H_c); 3.35 (dd, J_1=6.4 Hz, J_2=9.6 Hz, 0.3H, H_c); 3.97 (dd, J_1=6.8 Hz, J_2=9.2 Hz, 0.4H, H_b); 4.19 (t, J=13.2 Hz 0.3H, H_b); 5.73 (s, 0.3H, H_d); 5.91 (s, 0.7H, H_d); 7.24-7.86 (m, 4H, H_{arm})

^{13}C NMR (DMSO-d_6) δ: 37.4, 37.5 (C_c); 65.3, 65.5 (C_b); 69.6, 69.8 (C_d); 122.3, 122.5 (C_h); 122.3, 127.7, 129.8, 132.4, 138,7, 141.8 (C_{arom}); 172.1, 172.5 (C_a).

Elemental analysis for $C_{10}H_{10}BrNO_2S$ (M_W = 288.16 g.mol^{-1})

	C %	H %	N %	S %
Calculated	41.68	3.50	4.86	11.10
Found	36.38	4.20	4.94	10.83

4.5. (2*RS*-4*R*)- 2-(3-chlorophenyl)-1,3-thiazolidine-4-carboxylic acid (Compound 5) (35)



0.028 mol *L*-Cysteine hydrochloride monohydrate and 0.028 mol *m*-chloro benzaldehyde were reacted as described in the general procedure and precipitates were washed with water and dried under *vacuo* to yield 74%. The compound is in white solid form and soluble in pure DMSO.

m.p. : 160.4ºC

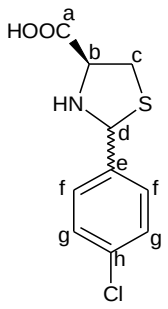
FT-IR (KBr), cm^{-1}: 3220-3450 (O-H carboxylic acid), 3044 (C-H sp^2), 1574 (C=O), 1477 (C=C, aromatic)

^{1}H-NMR (DMSO-d_6) δ: 3.09 (dd, J_1=5.2 Hz, J_2=10.8 Hz, 0.9H, H_c); 3.28 (dd, J_1=6.8 Hz, J_2=10.4 Hz, 0.9H, H_c); 4.15 (dd, J_1=4.8 Hz, J_2=6.4 Hz, 0.9H H_b,); 5.67 (s, 0.9H, H_d); 7.28-7.51 (m, 4H, H_{arm})

Elemental analysis for $C_{10}H_{10}ClNO_2S$ (M_W = 243.71 g.mol^{-1})

	C %	H %	N %	S %
Calculated	49.28	4.14	5.75	13.01
Found	48.98	4.00	5.85	12.90

4.6. (2*RS*-4*R*)-2-(4-chlorophenyl)-1,3-thiazolidine-4-carboxylic acid (Compound 6) (39)



0.028 mol *L*-Cysteine hydrochloride monohydrate and 0.028 mol *p*-chloro benzaldehyde were reacted as described in the general procedure and precipitates were washed with water and dried under *vacuo* to yield 75%. The compound is in white solid form and soluble in pure DMSO.

m.p. : 158.8 ºC

FT-IR (KBr), cm^{-1}: 3280-3450 (O-H carboxylic acid), 1579 (C=O), 1491 (C=C, aromatic)

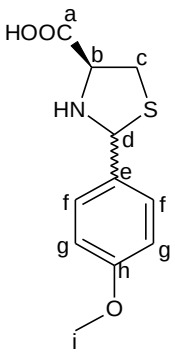
^{1}H-NMR (DMSO-d_6) δ: 3.09 (dd, *J*=5.2 Hz, J_2=10.8 Hz 0.95H, H_c); 3.27 (dd, J_1=6.8 Hz, J_2=10 Hz, 0.95H, H_c); 4.16 (dd, J_1=4.8 Hz, J_2=6.4 Hz, 0.95H, H_b); 5.66 (s, 0.95H, H_d); 7.37 (d, *J*=8.8 Hz, 1.9H, H_g); 7.44 (d, *J*=8.4 Hz, 1.9H, H_f).

LC-MS: ELS, rt = 3.80 min., *m*/*z* 244 $[M + H]^+$, 285 $[M + CH_3CN]^+$.

Elemental analysis for $C_{10}H_{10}ClNO_2S$ (M_W = 243.71 $g.mol^{-1}$)

	C %	H %	N %	S %
Calculated	49.28	4.14	5.75	13.10
Found	49.20	4.05	5.95	12.91

4.7. (2*RS*-4*R*)- 2-(4-methoxyphenyl)-1,3-thiazolidine-4-carboxylic acid (Compound 7) (33-36)



0.028 mol *L*-Cysteine hydrochloride monohydrate and 0.028 mol *p*-methoxy benzaldehyde were reacted as described in the general procedure and precipitates were washed with water and dried under *vacuo* to yield 72%. The compound is in white solid form and soluble in pure DMSO.

m.p. : 159ºC , m.p. ^{literature} (36) : 157.6 - 158.2 ºC

FT-IR (KBr), cm^{-1}: 3270-3450 (O-H carboxylic acid), 3061 (C-H sp^2), 1586 (C=O), 1463 (C=C, aromatic)

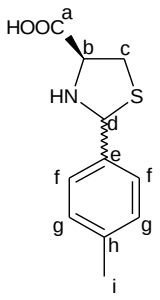
^{1}H-NMR (DMSO-d_6) δ: 3.07 (6t, *J*=9.6 Hz, 0.5H, H_c); 3.15 (dd, J_1=4.0 Hz, J_2=10.0 Hz, 0.5H, H_c); 3.29 (dd, J_1=7.2 Hz, J_2=10.4 Hz, 0.5H, H_c); 3.36 (dd, J_1=7.2 Hz, J_2=10.0 Hz, 0.5H, H_c); 3,74 (s, 1.5H, H_i); 3.76 (s, 1.5H, H_i); 3.87 (dd, J_1=7.6 Hz, J_2=9.2 Hz, 0.5H, H_b), 4.25 (dd, J_1=4.0 Hz, J_2=6.8 Hz, 0.5H, H_b); 5.46 (s, 0.5H, H_d); 5.60 (s, 0.5H, H_d); 6.89 (d, *J*=8.4 Hz, 1H, H_g); 6.93 (d, *J*=8.8 Hz, 1H, H_g); 7.37 (d, *J*=8.4 Hz, 1H, H_f); 7.44 (d, *J*=8.4 Hz, 1H, H_f).

LC-MS: ELSD, rt = 3.47 min., *m*/*z* 240 $[M + H]^+$, 281 $[M + CH_3CN]^+$.

Elemental analysis for ($C_{11}H_{13}NO_3S$ M_W = 239.29 $g.mol^{-1}$)

	C %	H %	N %	S %
Calculated	55.21	5.48	5.85	13.40
Found	55.08	5.21	6.01	13.20

4.8. (2*RS*-4*R*)- 2-(4-methylphenyl)-1,3-thiazolidine-4-carboxylic acid (Compound 8) (37, 38)



0.028 mol *L*-Cysteine hydrochloride monohydrate and 0.028 mol *p*-tolyl benzaldehyde were reacted as described in the general procedure and precipitates were washed with water and dried under *vacuo* to yield 85%. The compound is in white solid form and soluble in pure DMSO.

m.p. : 161.8ºC

FT-IR (KBr), cm^{-1}: 3250-3470 (O-H carboxylic acid), 1578 (C=O), 1473 (C=C, aromatic)

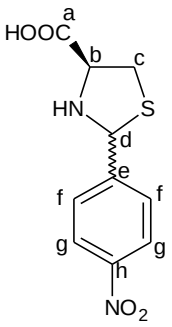
^{1}H-NMR (DMSO-d_6) δ: 2.28 (s, 1.8H, H_i); : 2.50 (s, 1.2H, H_i); 3.07 (t, *J*=18.4 Hz, 0.4H, H_c); 3.14 (dd, J_1=4.4 Hz, J_2=10.4 Hz, 0.6H, H_c); 3.28 (dd, J_1=6.8 Hz, J_2=10 Hz, 0.6H, H_c); 3.36 (dd, J_1=6.8 Hz, J_2=9.6 Hz, 0.4H, H_c); 3.88 (t, *J*=16 Hz, 0.4H, H_b); 4.24 (dd, J_1=4.4 Hz, J_2=6.8 Hz, 0.6H, H_b); 5.46 (s, 0.4H, H_d); 5.62 (s, 0.6H, H_d); 7.14 (d, *J*=8.4 Hz, 0.4H, H_g); 7.18 (d, *J*=8 Hz, 0.4H, H_i); 7.32 (d, *J*=8.4 Hz, 0.6H, H_g); 7.39 (d, *J*=8 Hz, 0.6H, H_i).

^{13}C NMR (DMSO-d_6) δ: 20.5, 20.6 (Ci); 37.8, 38.6 (C_c); 64.7, 65.3 (C_b); 70.9, 71.6 (C_d); 126.8, 127.1 (C_f); 124.8, 128.7, 128.9 (C_g); 135.8, 136.8 (Ch); 137.5, 137.9 (Ce) 172.1, 172.9 (C_a).

Elemental analysis for $C_{11}H_{13}NO_2S$ (M_W = 223.29 $g.mol^{-1}$)

	C %	H %	N %	S %
Calculated	59.17	5.87	6.27	14.36
Found	59.40	5.89	6.41	14.06

4.9. (2*RS*-4*R*)-2-(4-nitrophenyl)-1,3-thiazolidine-4-carboxylic acid (Compound 9) (35, 39)



0.028 mol *L*-Cysteine hydrochloride monohydrate and 0.028 mol *p*-nitro benzaldehyde were reacted as described in the general procedure and precipitates were washed with water and dried under *vacuo* to yield 80%. The compound is in white solid form and soluble in pure DMSO.

m.p. : 110ºC

FT-IR (KBr), cm^{-1}: 3200-3400 (O-H carboxylic acid), 3017 (C-H sp^2), 1629 (C=O), 1526 (NO_2), 1438 (C=C, aromatic)

^{1}H-NMR (DMSO-d_6) δ: 3.12 (dd, J_1=8.8 Hz, J_2=9.6 Hz, 0.95H, H_c); 3.76 (dd, J_1=6.8 Hz, J_2=10 Hz, 0.95H, H_c); 3.98 (dd, J_1=8.8 Hz, J_2=9.6 Hz, 0.95H H_b,); 5.67 (s, 0.95H, H_d); 7.8 (d, J=8.4 Hz, 1.9H, H_g); 8.2 (d, J=8.4 Hz, 1.9H, H_f).

LC-MS: ELSD, rt = 3.59 min., *m*/*z* 255 $[M + H]^+$, 296 $[M + CH_3CN]^+$.

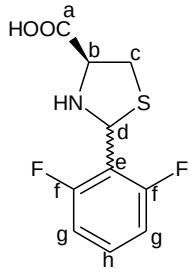
Elemental analysis for $C_{10}H_{10}N_2O_4S.H_20$ (M_W = 254.26 $g.mol^{-1}$)

	C %	H %	N %	S %
Calculated	44.28	4.09	10.33	11.80
Found	43.98	4.32	10.33	11.26

4.10. (2*RS*-4*R*)-2-(2,6-difluorophenyl)-1,3-thiazolidine-4-carboxylic acid (Compound 10) Cas no : 1290229-85-4

$C_{10}H_9F_2NO_2S$

M_W = 245.25 g.mol^{-1}



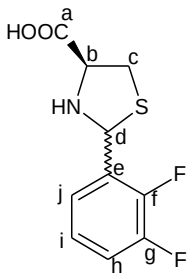
0.028 mol *L*-Cysteine hydrochloride monohydrate and 0.028 mol 2,6-difloro benzaldehyde were reacted as described in the general procedure and precipitates were washed with water and dried under *vacuo* to yield 75%. The compound is in white solid form and soluble in pure DMSO.

m.p. : 140.6ºC

FT-IR (KBr), cm^{-1}: 3200-3460 (O-H carboxylic acid), 1656 (C=O), 3081 (C-H sp^2) 1469 (C=C, aromatic)

^{1}H-NMR (DMSO-d$_6$) δ: 3.01 (t, *J*=18.8 Hz, 0.6H, H$_c$); 3.19 (dd, J_1=3.6 Hz, J_2=10.0 Hz, 0.4H, H$_c$); 3.33 (dd, J_1=6.8 Hz, J_2=10.4 Hz, 0.4H, H$_c$); 3.46 (dd, J_1=7.2 Hz, J_2=10.4 Hz, 0.6H, H$_c$); 3,89 (t, *J*=16.4 0.6H, H$_b$); 4.45 (dd, J_1=3.6 Hz, J_2=6.4 Hz, 0.4H, H$_b$), 5.78 (s, 0.6H, H$_d$); 5.98 (s, 0.4H, H$_d$); 7.11 (t, *J*=17.2, 0.4H, H$_b$); 7.18 (t, *J*=18, 0.6H, H$_b$); 7.36-7.56 (m, 2H, H$_g$).

4.11. (2*RS*-4*R*)-2-(2,3-difluorophenyl)-1,3-thiazolidine-4-carboxylic acid (Compound 11) Cas no : 1344968-75-7



0.028 mol *L*-Cysteine hydrochloride monohydrate and 0.028 mol 2,3-difloro benzaldehyde were reacted as described in the general procedure and precipitates were obtained as washed with water and dried under *vacuo* to yield 80%. The compound is in white solid form and soluble in pure DMSO.

m.p. : 133.5ºC

FT-IR (KBr), cm^{-1}: 3200-3450 (O-H carboxylic acid), 1610 (C=O), 1426 (C=C, aromatic)

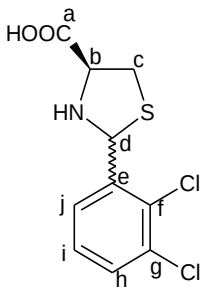
^{1}H-NMR (DMSO-d_6) δ: 3.00 (t, *J*=10 Hz, 0.4H, H_c); 3.05 (t, *J*=18.8 Hz, 0.6H, H_c); 3.29 (dd, J_1=3.6 Hz, J_2=10 Hz, 0.6H, H_c); 3.35 (dd, J_1=3.2 Hz, J_2=9.6 Hz, 0.4H, H_c); 3.94 (dd, J_1=6.8 Hz, J_2=8.8 Hz, 0.4H, H_b); 4.11 (t, *J*=12.8 Hz, 0.6H, H_b); 5.70 (s, 0.6H, H_d); 5.93 (s, 0.4H, H_d); 7.12-7.58 (m, 3H, H_{arm}).

LC-MS: ELSD, rt = 3.60 min., *m*/*z* 246 $[M + H]^+$, 287 $[M + CH_3CN]^+$.

Elemental analysis for $C_{10}H_9F_2NO_2S$ (M_W = 245.25 $g.mol^{-1}$)

	C %	H %	N %	S %
Calculated	48.89	3.70	5.71	13.01
Found	48.77	3.69	5.82	12.74

4.12. (2*RS*-4*R*)- 2-(2,3-dichlorophenyl)-1,3-thiazolidine-4-carboxylic acid (Compound 12) Cas no : 1212481-14-5



0.0028 mol *L*-Cysteine hydrochloride monohydrate and 0.028 mol 2,3-diclorobenzaldehyde were reacted as described in the general procedure and precipitates were washed with water and dried under *vacuo* to yield 80%. The compound is in white solid form and soluble in pure DMSO.

m.p. : 129.7ºC

FT-IR (KBr), cm^{-1} 3200-3450 (O-H carboxylic acid), 3163 (C-H sp^2), 1666 (C=O), 1418 (C=C, aromatic)

^{1}H-NMR (DMSO-d_6) δ: 2.94 (dd, J_1=7.6 Hz, J_2=10.8 Hz, 0.6H, H_c); 3.05 (t, J=19.2 Hz, 0.4H, H_c); 3.25 (dd, J_1=6.4 Hz, J_2=10 Hz, 0.4H, H_c); 3.34 (dd, J_1=6.8 Hz, J_2=10.4 Hz, 0.6H, H_c); 4.02 (dd, J_1=7.2 Hz, J_2=9.2 Hz, 0.4H, H_b), 4.13 (t, J=13.2 Hz, 0.6H H_b); 5.80 (s, 0.4H, H_d); 5.99 (s, 0.6H, H_d); 7.36 (t, J=16 Hz, 0.6H, H_i); 7.41 (t, J=15.6 Hz, 0.4H, H_i); 7.53 (d, J=2.8 Hz, 0.6H H_h); 7.55 (d, J=2.4 Hz, 04H, H_h); 7.61 (dd, J_1=1.2 Hz, J_2=7.6 Hz, 0.6H, H_j); 7.84 (t, J=7.6 Hz, 0.4H, H_j).

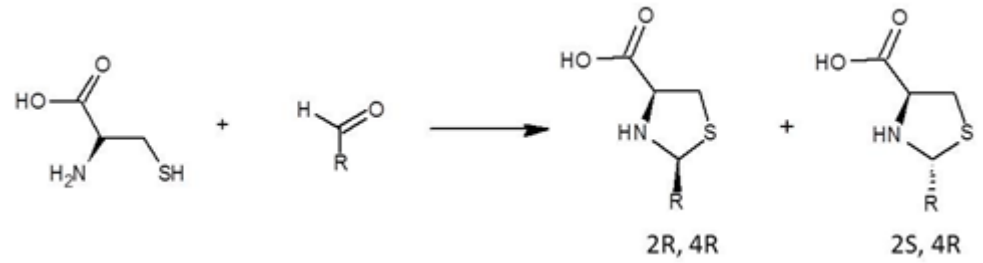
Elemental analysis for $C_{10}H_9Cl_2NO_2S.2H_20$ (M_W = 278.16 $g.mol^{-1}$

	C %	H %	N %	S %
Calculated	38.47	3.550	4.490	10.200
Found	38.04	3.559	4.770	10.350

5. DISCUSSION AND CONCLUSION

5.1. Chemistry

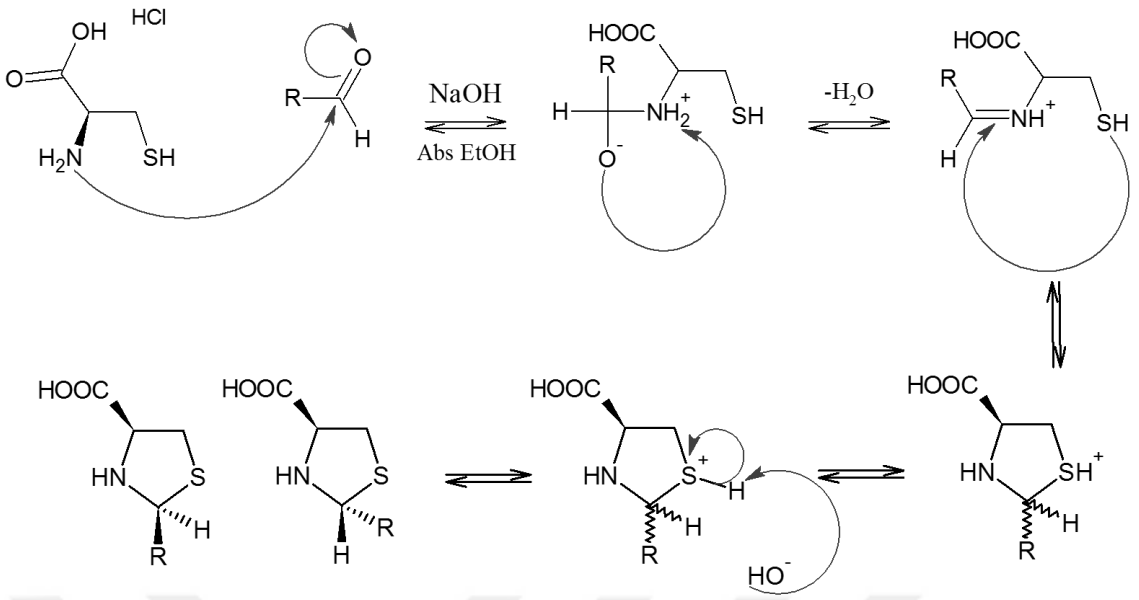
In this study; 2-substituted TCA derivatives (**1-12**) were synthesized as shown in Scheme 16 in order to evaluate their *in vitro* antioxidant capacities which rises from their spontaneous *L*-cysteine release or thiazolidine ring. The final products present two main parts: thiazolidine ring, and mono or di substituted phenyl group at the second position of the thiazolidine ring.



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

Scheme 16. Synthesis of substituted 2-phenyl-4-carboxylic acid thiazolidine derivatives **1-12**.

TCA derivatives were synthesized with good to excellent yields by condensing *L*-cysteine with a series of substituted benzaldehyde derivatives as shown in Scheme 16. The typical cyclization reaction was carried out in basic conditions in a water/ethanol mixture (50:50, v:v) as shown in Scheme 17 (100).



Scheme 17. Reaction mechanism of the compounds **1-12** as diastereomeric mixture.

The synthesis of thiazolidine carboxylic acids were achieved using conditions optimized by Onen Bayram et al. (19). Indeed, syntheses carried out with cysteine hydrichlorate salt require the use of a base fot the neutralization of the thiol compound before its cyclization and the hydroxide ion was found to be suitable for such a purpose. Also, the reaction solvent was chosen to be a mixture of ethanol and water to ensure the proper soubilization of both cysteine hydrochlorate (water soluble) and the benzaldehyde derivatives (soluble in polar protic solvents). Finally temperature was maintained around 20°C since room temperature was found to be enough for obtaining thiazolidine compounds with satisfactory yields.

The structures of the synthesized compounds were elucidated by FT-IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. The formation of the thiazolidine ring was confirmed through the typical signals corresponding to the second position of the cycle. As it is illustrated in Figure 13 the hydrogen on C-2 gave a typical singlet around 5.5 ppm, and according to Figure 14 the signal of the C- 2 carbon atom appeared as a peak at around 71 ppm.

The ring closure reaction results from two successive nucleophilic attacks to the aldehyde and leads to the generation of a new chiral center in an uncontrolled manner. Thus, thiazolidine derivatives are obtained as diastereomeric structures (Scheme 17). Although clearly distinguishable on ^{1}H NMR *via* the proton on the C-2 carbon of the heterocycle [24], the isolation of the 2*R*, 4*R* and 2*S*, 4*R* isomers were 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 (100).

The *RS/RR* ratios were determined by integrating the C-2 protons of the each diastereomer. According to Figure 13, the peak at 5.6 ppm belongs to *RR* diastereomer and the peak at 5.4 ppm belongs to *RS* diastereomer.

Interestingly, nevertheless, the diastereomeric mixture ratios varied according to the substituents of the aromatic cycle (Table 11), the equilibrium being clearly in favor of one of the isomers in the compounds **5**, **6** and **9**. While the *ortho*-cyano, *meta*- and *para*-chloro benzaldehydes definitely orient the reaction to the formation of the 2*S*, 4*R* isomer (distinction made through NOE data given in the literature) (24), the *p*-nitrobenzaldehyde led preferably to the 2*R*, 4*R* thiazolidine derivative (**6**). 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.

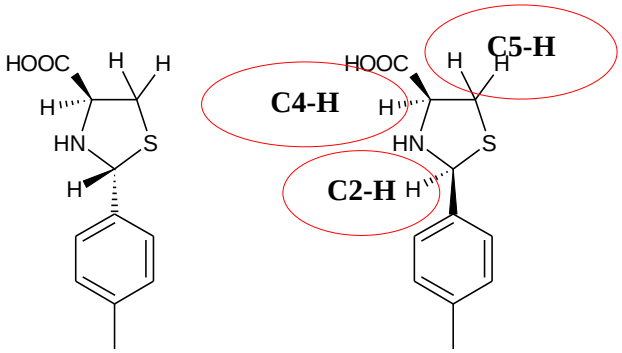


Figure 12. *2S,4R* (major) and *2R,4R* (minor) diastereoisomers of compound **8.**

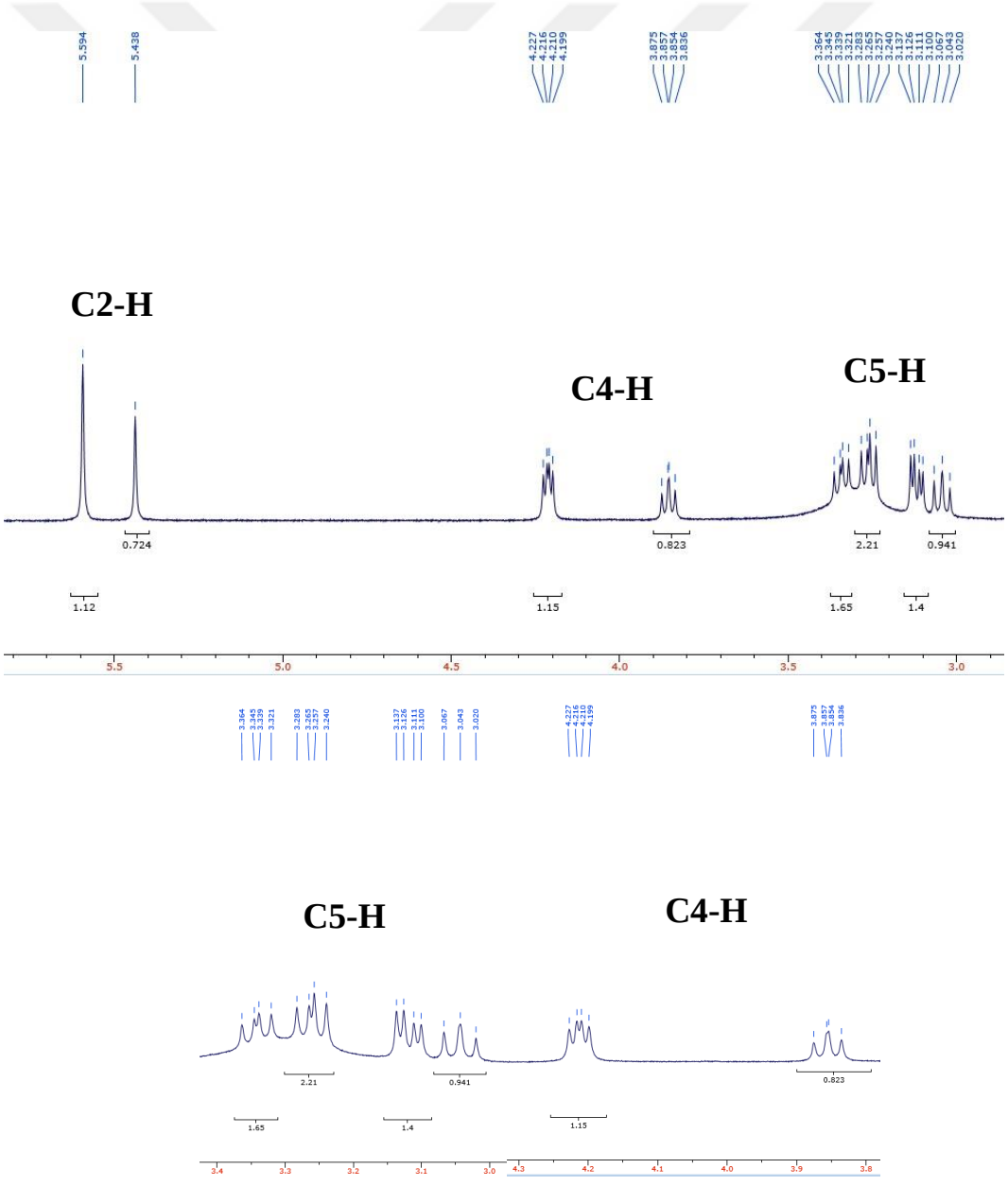


Figure 13. NMR spectrum of the compound **8.**

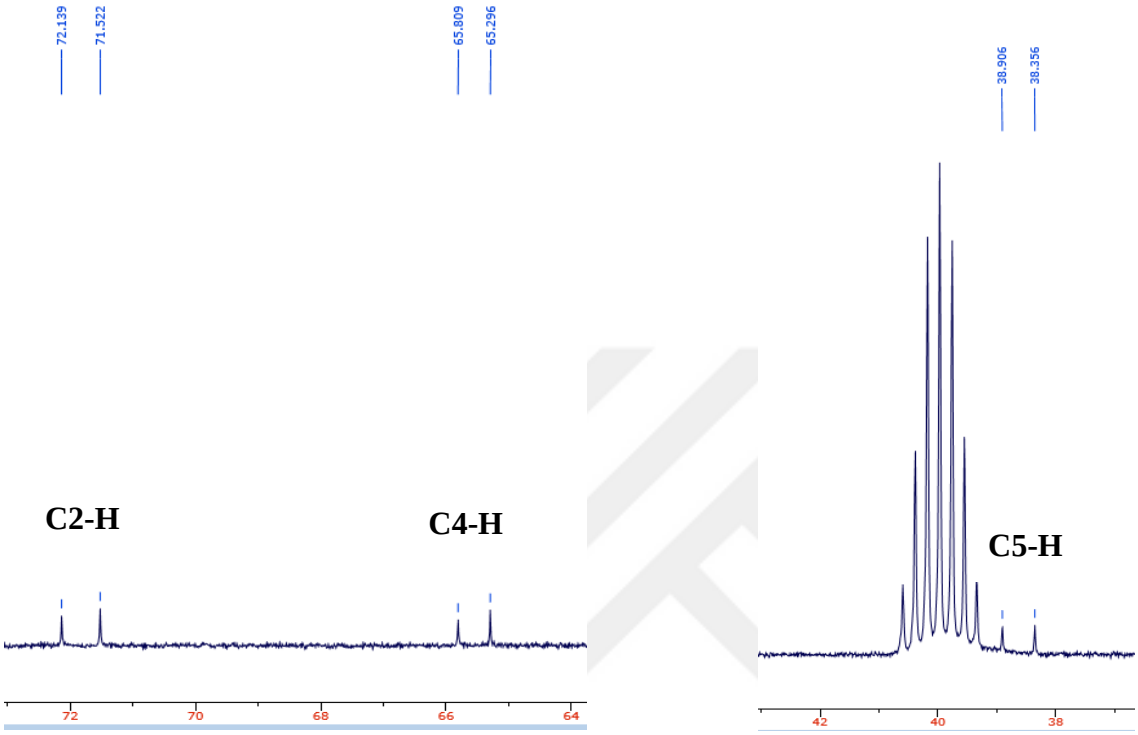


Figure 14. ^{13}C spectrum of compound **8.**

Table 11. *RS/RR* Ratios of compounds 1-12.

Compound	2S,4R / 2R,4R
1	70 : 30
2	40 : 60
3	60 : 40
4	70 : 30
5	90 : 10
6	95 :5
7	50 : 50
8	60 : 40
9	5 : 95
10	40 : 60
11	40 :60
12	40 : 60

5.2. Biology

The synthesized thiazolidine compounds 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.

5.2.1. The DPPH assay

The DPPH assay, a very commonly used methodology for analyzing the antioxidant activity of molecules, consists on 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 belongs to the stable free radicals.

Aqueous DMSO was chosen as a solvent, as the compounds 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, five compounds (from **5** to **9**) did not dissolve and could not be evaluated. IC_{50} values were determined (Table 12) and compared to the antioxidant capacity of butylated hydroxytoluene (BHT) which is classical reference used for DPPH assays and cysteine since the synthesized structures are meant to be cysteine prodrugs.

IC_{50} values were calculated by plotting the DPPH scavenging capacity versus compound concentration using a logarithmic scale. The characteristic graphic is a sigmoid curve with two plateaus: the first one corresponding to low and the second to high compound concentrations. The half inhibition concentration (IC_{50}) of a compound is the concentration obtained for 50% of scavenging. This value can be easily determined graphically using the sigmoidal curve as shown in Figure 15.

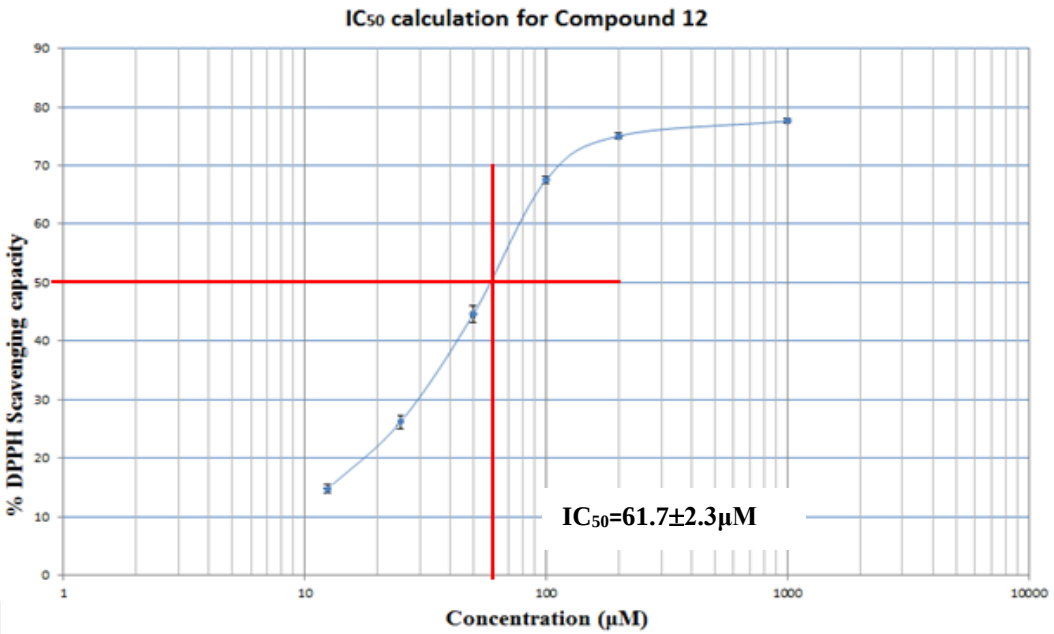


Figure 15. IC_{50} determination of compound **12**.

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 its 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**, **10** and **11** 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 (Table 12).

Table 12. IC_{50} values of the compounds 1-4 and 10-12 against BHT molecule in DPPH assay.

	BHT	cysteine	1	2	3	4	10	11	12
IC_{50} in DMSO (mM)	not soluble	18.4 ±0.1	45.2 ±0.3	29.5 ±0.5	39.0 ±0.3	37.7 ±0.1	38.3 ±0.4	46.8 ±0.8	61.7 ±2.3
IC_{50} in methanol (mM)	517 ±25	19.2±0.5	844.2±2.2	342.7±8.4	46.1±0.9	39.1±1.3	343.4±12	>1mM	46.2±1.4

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 13.

Table 13. HPLC analysis of the compounds 1-4 and 10-12 after dissolution in 5% aqueous DMSO or methanol at time zero, 20 minutes after dissolution and 50 minutes after dissolution.

Compound		**1**			**2**			**3**			**4**		
Time (min.)		**0 '**	**20 '**	**50 '**	**0 '**	**20 '**	**50 '**	**0 '**	**20 '**	**50 '**	**0 '**	**20 '**	**50 '**
5% aq DMSO	% Thiazolidine	76.3	27.8	14	83	44	27	6,8	5,9	5,6	19.0	16	14
	% Aldehyde	17	69	85	17	56	73	92	93	94	81.0	84	86
MeOH	% Thiazolidine	96,1	95,7	95,4	100	99	97	4.0	4,1	4,2	71	74	76
	% Aldehyde	3,8	4,3	4,6	0	1,4	2,2	96	96	96	22	18	16
Compound		**10**			**11**			**12**					
Time (min.)		**0 '**	**20 '**	**50 '**	**0 '**	**20 '**	**50 '**	**0 '**	**20 '**	**50 '**			
5% aq DMSO	% Thiazolidine	54	39	38	84	50	29	84	57	12			
	% Aldehyde	46	60	61	11	46	67	8,8	39	86			
MeOH	% Thiazolidine	100	99	98	100	100	99	16	25	38			
	% Aldehyde	0	0,5	1,3	0	0	0,5	84	74	60			

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**, **4** and **12**. For the other tested compounds (**1**, **2**, **10** and **11**) 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**, **10** and **11** 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 16).

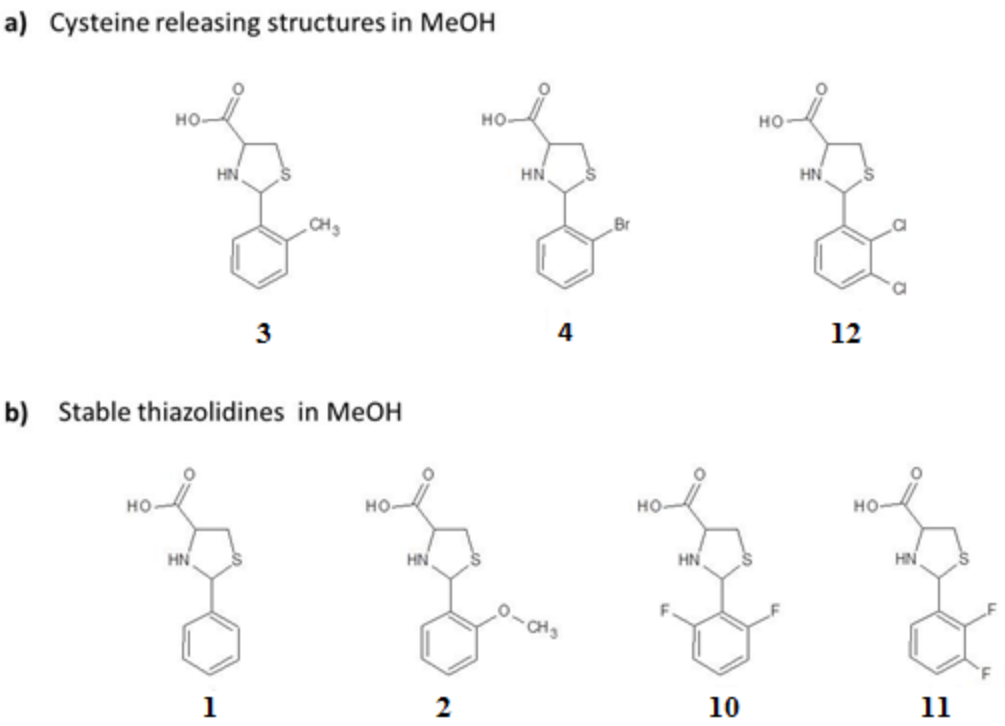


Figure 16. (a) Structures capable of releasing cysteine in methanol (MeOH). (b) Structures that are stable and do not open to release cysteine in MeOH.

For instance, while the *o,m*-difluoro substituted derivative **11** was shown to be stable in methanol even 50 minutes after dissolution, its close analogue **12** (*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.

5.2.2. The Cupric ion reducing antioxidant capacity (CUPRAC) assay

The antioxidant capacities of the synthesized thiazolidines were also evaluated using the CUPRAC assay. This method developed by Apak *et al.* (103) consists of monitoring the Cu(I)-neocuproine complex formation at 450 nm, a complex that results from the reduction of the Cu(II)-neocuproine complex in an ammonium acetate buffer in the presence of a reducing agent. To determine the antioxidant capacity of the authors suggest to calculate the trolox equivalent antioxidant capacity, namely the TEAC coefficient, a coefficient that corresponds to the ratio of the molar extinction coefficients of the sample and trolox ($TEAC=\varepsilon_{sample}/\varepsilon_{Trolox}$).

Samples were prepared in aqueous 5% DMSO and methanol and TEAC coefficient were determined for both conditions (Table 14).

Table 14. TEAC coefficients of the compounds 1-6, 8-12 and cysteine which were dissolved either in aqueous DMSO (%5, v/v) or in MeOH.

Compound	**cysteine**	**1**	**2**	**3**	**4**	**5**	**6**
$TEAC_{MeOH}$	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
$TEAC_{DMSO}$	0.49±0.03	0.99±0.05	0.79±0.04	0.75±0.04	1.00±0.05	ND	ND
Compound	**cysteine**	**8**	**9**	**10**	**11**	**12**	
$TEAC_{MeOH}$	0.63±0.09	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	ND	ND	1.05±0.1	0.84±0.05	0.99±0.05	

The $TEAC_{MeOH}$ of compound **7** and the $TEAC_{DMSO}$ of compounds **5-10** 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 compounds (**1**, **5, 6, 8**) 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.

5.3. Conclusion

In this work, a series of 2-phenyl thiazolidine carboxylic acid derivatives (**1-12**) were synthesized by condensing *L*-cysteine with a series of mono or disubstituted benzaldehyde derivatives by known methods in the literature [19] . The compounds **1-12** were known compounds on the other hand they were tested for the *in vitro* antioxidant activity for the first time. The antioxidative properties of these compounds were investigated using the DPPH scavenging and CUPRAC assays.

DPPH scavenging assays were performed by dissolving the molecules in two different solvents: 5% DMSO and methanol. Samples dissolved in aqueous medium demonstrated a high antioxidant capacity whereas only compound **3, 4** and **12** exhibited similar results when dissolved in MeOH. The differences in the biological activities were attributed to the capacity of cysteine release of the molecules. Indeed, an HPLC analysis carried out by dissolving the compounds in 5% DMSO and MeOH revealed that the cysteine releasing is solvent dependent, a property that has been closely related to the drop of activity observed for compounds **1, 2, 10** and **11** when they were dissolved in MeOH. Thus, the significant antioxidant activity with the DPPH assay can be related to the release of cysteine in the medium.

CUPRAC assay were also carried out in both solvents. Results indicated that antioxidant activities better than that of Trolox control compound, an antioxidant analog of vitamin E, can give are obtained in conditions for which thiazolidines were shown to

remain cyclic (**1** and **2**). These findings suggested that the thiazolidine ring itself can also have antioxidant properties *via* pathways that might be different from cysteine's.

Thus, this study shows that the antioxidant capacity of thiazolidine derivatives is related not only to the *L*-cysteine release but also to the reducing capacity of the thiazolidine ring itself.

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Bildiği Yabancı Diller	**Yabancı Dil Sınav Notu**
İnglizce	KPDS : 85/100
Fransızca	B+

İş Deneyimi

Görev	**Kurum**	**Süre**
Araştırma Görevlisi	Yeditepe Üniversitesi	3 yıl
Kimyasal Analiz Sorumlusu	Yeditepe Üniversitesi	3 yıl 6 ay

Bilgisayar Bilgisi

Program	**Kullanma Becerisi**
Microsoft Office Programları	Çok iyi
Molekül Çizim Programları	Çok iyi