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GALLIC ACID HYDRAZONES: *IN SILICO* INHIBITION OF THIOREDOXIN REDUCTASE

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Abstract:

Gallic hydrazones, as gallic acid derivatives, are known as pharmacophores of numerous multipotent agents. Among them, antiproliferative activity is one of the most important. On the other hand, thioredoxin reductase (TrxR1) is a part of the thioredoxin system, one of the most important systems responsible for maintaining the redox equilibrium inside the cell. It is overexpressed in different forms of tumors. Bearing this in mind, TrxR1 is a valid target for the development of compounds with potential antiproliferative activity. For this purpose, eight gallic acid-based hydrazones are selected and examined *in silico* for their potential inhibitory activity towards TrxR1.

Keywords: Gallic acid, hydrazones, molecular docking, TrxR1

1. Introduction

Gallic acid (GA) also known as 3,4,5-trihydroxybenzoic acid is well known polyphenolic compound widely presented in different plants. It can be found in foods such as berries, pomegranates, grapes, nuts, honey, vegetables, as well as in beverages such as wine, tea, etc. [1]. GA can be obtained by alkaline/acid hydrolysis of hydrolysable tannins, naturally occurring polymers of galloyl moieties and a glucose molecule, which are presented in plants in large amounts [1,2]. GA as a naturally existing antioxidant [3], exerts various biological activities such as anti-inflammatory [4], anticancer [5,6], antimutagenic [7], etc.

This prominent polyphenolic compound can protect biological cells, tissues, and organs from disorders caused by oxidative stress due to strong antioxidative and free radical scavenging properties [8]. Additionally, GA exerts pro-oxidant property in presence of metal ions which has been recognized as the apoptosis inducer in cancer cell lines [9]. GA has been reported as selectively cytotoxic agent on various cancer cell lines and less toxic for normal cells [10]. In addition, gallic acid exhibits neuroprotective effects in treating nervous system diseases such as Alzheimer's disease, Parkinson's disease, ischemia, depression, and anxiety [11].

Bearing all these in mind, the availability of gallic acid in nature and its bioactivity present a significant basis in designing new potent pharmacophores [12,13]. Recently, the development of synthetic gallic acid derivatives has led to the synthesis of many biologically and pharmacologically active compounds [2]. Gallic hydrazones (**GAH**), as gallic acid derivatives, have been reported as pharmacophores of numerous multipotent agents [14]. **GAH** were examined for their antiproliferative activity on nine human tumor cell lines. In particular, the derivatives that possess phenyl, *p*-hydroxyphenyl, and dimethylaminophenyl group attached to the galloyl hydrazide moiety, displayed cytotoxic efficacy against the ovarian cell line [15].

Thioredoxin reductase (TrxR) presents a selenocysteine-containing enzyme responsible for maintaining redox homeostasis in cells. Also, TrxR is a part of the thioredoxin system, one of the most important systems responsible for maintaining the redox equilibrium inside the cell [16]. Three mammalian TrxR isoforms have been identified: a cytosolic (TrxR1), a mitochondrial (TrxR2), and

thioredoxin-glutathione reductase (TGR/TrxR3) [17,18]. Physiologically, TrxR protects normal cells from carcinogenesis, but if carcinogenesis still occurs, it can also promote cancer progression [19]. TrxR is overexpressed in different forms of tumors, and this is of pathological significance in the maintenance of tumor phenotypes, such as a high level of intracellular reactive oxygen species (ROS), resistance to apoptosis, and uncontrolled proliferation. According to this, interest in the development of cancer therapeutic agents that target TrxR is increased [20].

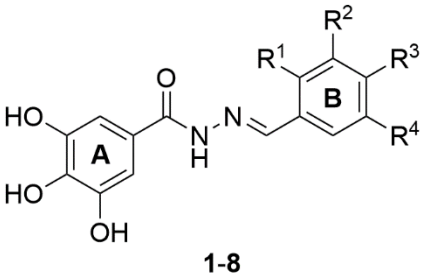
Here, gallic acid hydrazones are examined as TrxR1 inhibitors since they have been described as promising agents which exhibit cytotoxic efficacy against the various cancer cell lines.

2. Computational details

The equilibrium geometries of compounds **1-8** were calculated using density functional theory (B3LYP functional in conjunction with the 6-311+G(d, p) basis set). Molecular docking was performed using Autodock Vina software. The cuboid grid box was set to embrace all the minimized inhibitors spanning 10 Å in all three dimensions around the active site.

3. Results and discussion

Selected gallic hydrazones (**GAH**s **1**-**8**) for evaluation of their potential inhibitory activity against the TrxR1 enzyme (PDB ID: 2j3n) are presented in Figure 1. It is important to emphasize that selected **GAH**s possess two aromatic rings **A** and **B**. Ring **A** is originating from GA, (acyl moiety), while ring **B** presents substituted benzylidene pharmacophore.



1-8

1: R^1 = H, R^2 = H, R^3 = H, R^4 = H
2: R^1 = OH, R^2 = H, R^3 = H, R^4 = H
3: R^1 = H, R^2 = H, R^3 = OH, R^4 = H
4: R^1 = OH, R^2 = OH, R^3 = H, R^4 = H
5: R^1 = H, R^2 = OCH_3, R^3 = OH, R^4 = H
6: R^1 = H, R^2 = OCH_3, R^3 = OH, R^4 = OCH_3
7: R^1 = OH, R^2 = H, R^3 = OH, R^4 = H
8: R^1 = H, R^2 = OH, R^3 = OH, R^4 = H

Fig. 1. The structures of selected **GAH**s for evaluation of their inhibitory activity against TrxR1.

The screening of **GAH**s potential inhibitory activity was done using Autodock Vina software. The docking box was picked to take the active site of the enzyme. Based on the values of the obtained binding affinities (Table 1), compounds **2**, **4,** and **6**, stand out as the most potent *in silico* inhibitors of TrxR1. It is important to emphasize that obtained values of the binding affinities are comparable to those obtained for standard inhibitors [21]. Potential bioactive conformations of compounds **1**-**8** are depicted in Figure 2 (left), as well as the potential bioactive conformation of the highest binding affinity compound **4** (Figure 2, right). All compounds are positioned within the TrxR1 so that both aromatic rings are oriented towards side chains of amino acids which form hydrophobic chambers. In all cases, ring **A** is positioned in the chamber formed by PHE406 and VAL478, both interacting via π-alkyl interaction. In addition, one of the *m*-OH groups of the ring **A** forms hydrogen bond with the peptide NH of TRP407, while the second one with peptide carbonyl groups of VAL474, and CYS475. Moreover, the carbonyl group of all compounds is hydrogen-bonded to SER404. The ring **B** is stacked within the hydrophobic chamber of ILE347 (π-alkyl interaction) and HIS472 (π-π stacked interaction). Unlike others, compounds **2** and **4** possess *o*-OH group on the ring **B**, which enables the formation of hydrogen bonds with the peptide carbonyls of GLU477, THR480, and THR481. Based on this, one can say that this feature is responsible for higher binding affinities of these compounds. Moreover, compound **4** bears an additional *m*-OH group on the ring **B**, which establishes an additional hydrogen bond with the THR480, obviously responsible for the highest binding affinity of compound **4**.

Table 1. Binding affinities of **GAH**s **1**-**8** towards TrxR1 enzyme (kcal/mol).

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Binding affinity	-6.8	-7.4	-6.9	-7.7	-6.8	-7.5	-6.8	-6.7

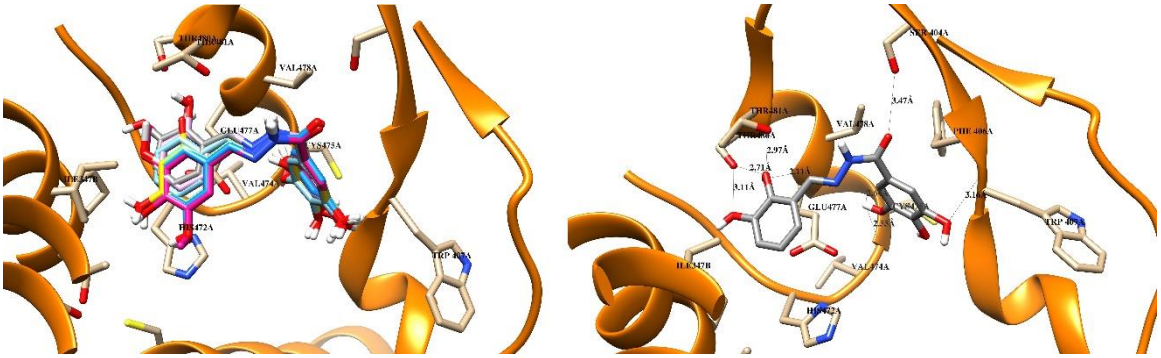


Fig. 2. Left: potential bioactive conformations of compounds **1**-**8**. Right: bioactive conformation of the highest binding affinity compound **4**.

4. Conclusions

Eight gallic acid-based hydrazones (**GAH**s **1**-**8**) are selected for the examination of their potential inhibitory activity towards the TrxR1 enzyme. *In silico* obtained binding affinities pointed compounds **2**, **4**, and **6** as the most potent inhibitors for further evaluations. Particularly, compound **4** exerted the highest binding affinity, owing to the favorable positioning of the -OH groups on the ring **B**.

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