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Quantum-chemical Study on the Thermodynamical Aspect of Competitive Inhibition of Ribonucleotide Reductase by *trans*-Resveratrol, *trans*-Piceatannol and Hydroxyurea

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Abstract: Ribonucleotide reductase is an enzyme that catalyzes the formation of deoxyribonucleotides from ribonucleotides. We present for the first time a quantum-chemical study of the thermodynamics of reactions of naturally occurring potent inhibitors (*trans*-resveratrol, *trans*-piceatannol and hydroxyurea) of ribonucleotide reductase with tyrosine radical and cysteine radical, scavenging of which is crucial for the inhibition of the enzyme. Density functional theory has been applied to compute the Gibbs free enthalpy changes for these reactions in the gas phase and in the presence of water medium. Various reaction pathways have been analyzed. The results obtained prove that *trans*-resveratrol 4'-OH group is mainly responsible for effective reaction with these enzymatic free radicals. In water medium, the reactions studied are characterized by more negative values of the Gibbs free enthalpy changes than in vacuum. It was found that *trans*-resveratrol and *trans*-piceatannol may be efficient inhibitors of the enzyme (*trans*-piceatannol is more efficient than *trans*-resveratrol). Because inhibition of ribonucleotide reductase is essential for blockage of the cancer development pathways, the polyphenols studied may block diverse processes (including cancerogenesis) by inhibition of free radical reaction steps that occur during the catalytic action of this enzyme.

Keywords: Enzyme inhibition, ribonucleotide reductase, *trans*-resveratrol, *trans*-piceatannol.

1. INTRODUCTION

Ribonucleoside reductase (RR) (Fig. **2**) is an enzymatic complex that is responsible for the transformation of ribonucleosides into deoxyribonucleosides and plays a crucial role, especially in the process of DNA synthesis [1]. In view of this, the RR has become a target for many anticancer agents researches and its selective inhibition in cancer tissues is one of the main fields the investigation of this enzyme is focused on. Although in many living organisms this enzyme differs slightly in construction, numerous studies have shown that all of its forms execute similar enzymatic paths and their tertiary structures prove that they are homologous. Structural investigation has revealed that the enzyme always consists of two dimeric subsystems: R1 and R2 [1].

On the basis of a detailed analysis of the mechanism by which the RR works, we can claim that the catalytic cycle starts from an electron transfer from a cysteine residue to the tyrosine radical (Fig. **1**) which results in the creation of a very reactive cysteine thiol radical within the subsystem R1. The radical is characterized by the presence of an unpaired electron on a sulfur atom [1] - its very high reactivity is caused by the lack of possibility of resonance stabilization. The radical then detaches a hydrogen atom from the C3' carbon atom of ribose – carbon radical is created and it further induces removal of a hydroxyl anion from the C2' carbon atom of ribose. The mechanism of the RR action shows that the tyrosine radical plays a pivotal role in the whole reaction as it works as a catalytic cycle initiator. Therefore, its scavenging by antioxidant compounds may lead to an effective inhibition of the enzyme [2, 3]. Recent studies have found that *trans*-resveratrol and *trans*-piceatannol, hydroxyurea (amide form) (Fig. **1**) are very active inhibitors of this enzyme [4, 5] and that their activity is caused by the ability to scavenge tyrosine radical.

The main aim of this research is to assess the thermodynamical preference of the first step of reaction of competitive inhibition of the ribonucleotide reductase by *trans*-resveratrol, *trans*-piceatannol, and hydroxyurea (amide form), providing additional knowledge about the enzyme inhibition ability of these compounds.

2. QUANTUM-CHEMICAL COMPUTATIONS

All quantum calculations were performed within the GAUSSIAN 09 computational package [6]. The total molecular energy calculations and structure optimizations have been carried out for the chemical systems considered in vacuum and in the presence of water medium without symmetry constraints in the ground state. In computations, the amide form of hydroxyurea was used since in the media studied it is its most energetically stable tautomeric form (each tautomeric form of the hydroxyurea had been fully optimized at the same level as other compounds and the most energeti-

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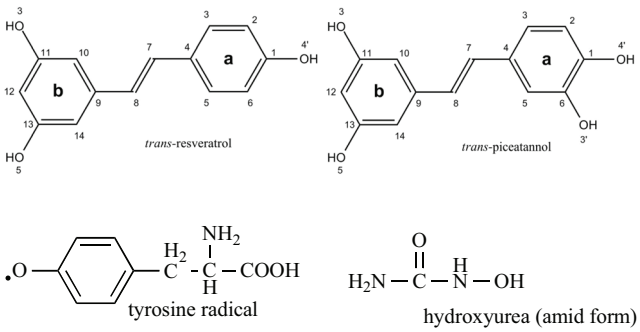


Fig. (1). Molecular structures of the *trans*-resveratrol, *trans*-piceatannol, tyrosine radical and hydroxyurea (amide form).

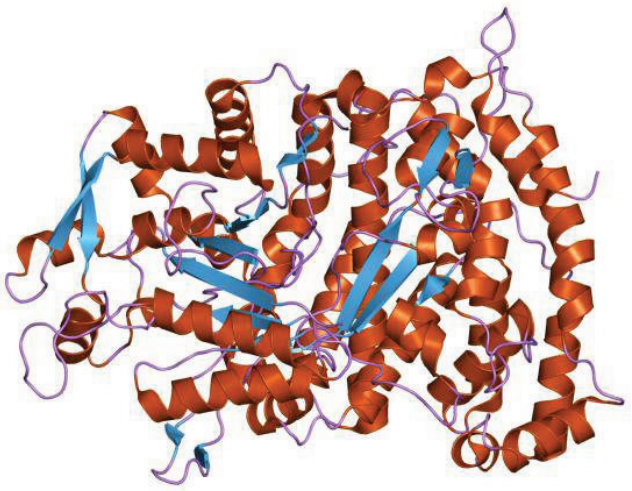


Fig. (2). The molecular structure of the ribonucleotide reductase enzyme.

cally stable form had been chosen). For the optimization of geometries and energies of *t*-RES (*trans*-resveratrol), *t*-RES phenoxyradical, *t*-PIC (*trans*-piceatannol), hydroxyurea, hydroxyurea oxyradical, tyrosine, tyrosine radical, tyrosine anion, tyrosine anion radical, cysteine and cysteine thiol radical in their ground states, the restricted Becke's three-parameter hybrid functional B3LYP with the gradient-corrected correlation functional by Lee *et al.* [7] and 6-311++G(3df,2p) basis-set were employed. To confirm the positions of the minima, geometry optimization was performed, starting from the most stable conformations obtained from the potential energy surface (PES). Potential energy surfaces were built by changing the dihedral angles α (C_5-C_4-C_7-C_8) and θ (C_7-C_8-C_9-C_{10}) in *trans*-resveratrol and *trans*-piceatannol. The conformational energy maps were obtained through discrete rotation of these selected dihedral angles, in 10° increments from 0° to 180°. At each point, the total energy of *t*-RES and *t*-PIC was computed at the B3LYP/6-311++G(3df,2p) level of theory. Afterwards, the most stable structures obtained from the energy profiles were fully optimized without any geometrical and symmetry constraints around each potential minimum. This computational procedure has been employed in order to obtain the equilibrium structures of the chemical systems studied in their energy minima. For each fully optimized structure, the frequency analysis at the same level of theory was used to verify if the structure corresponds to a stationary point on the potential energy surface. Thermodynamical preference of the reactions of electron transfer and hydrogen transfer from *t*-RES to tyrosine radical was determined by means of the Gibbs free enthalpy changes of the reactions. The same parameters were computed for the reaction of hydroxyurea with tyrosine radical in order to compare the inhibition potential of *t*-RES and hydroxyurea and for the reactions of *t*-RES and hydroxyurea with cysteine thiol radical with the latter being created during the catalytic cycle. The Gibbs free enthalpy change of the reaction of *t*-RES with tyrosine anion radical was computed as the tyrosine (with $pI = 5.64$) occurs mainly in the anion form (deprotonated carboxyl group) in neutral pH. Gibbs free enthalpy changes of the reactions were computed as the Gibbs free enthalpy differences of products and reactants under standard conditions $\left(T = 298{,}15K; p = 1{,}00 \cdot 10^5 Pa\right)$. The solvation effects were considered by means of the *Conductor-like Polarizable Continuum Model* (CPCM) that is based on the COSMO model [8] implemented in GAUSSIAN 09 package. The CPCM computations were performed with tesserae of 0.2 Å average size. The polarizable dielectric medium is described by a dielectric constant of the solvent (78.39 for water). The solvation effects on the values of the Gibbs free enthalpy changes of the reactions were estimated for the vacuum equilibrium geometries.

3. RESULTS AND DISCUSSION

All of the chemical reactions investigated are presented in Figs. (**3, 4** and **5**). The corresponding Gibbs free enthalpy changes of the reactions for vacuum and water medium are shown consecutively in Tables **1**, **2** and **3**.

All of the first-step reactions of ribonucleotide reductase competitive inhibition were found to be more preferred thermodynamically (to have lower Gibbs free enthalpy change magnitude) in water medium than in vacuum. Each chemical reaction of the potential inhibitor with tyrosine or cysteine radical, the products of which are inhibitor radical and neutral molecule of tyrosine or cysteine correspondingly (**Reactions R1, R2, H1, P1, P2, P3**; Figs. (**3, 4** and **5**)), is fully allowed in the thermodynamical sense. It is important, that the reaction of hydroxyurea has the lowest and the reactions of *trans*-resveratrol have the highest Gibbs free enthalpy change from among the reactions proceeding by this pathway. We claim that the relatively small size of the hydroxyurea molecule and the presence of the hydroxyamide group are the two main factors that are responsible for the lower free enthalpy change of the hydroxyurea reaction with tyrosine radical. On the basis of this we can claim that hydroxyurea is a stronger inhibitor of the ribonucleotide reductase than *trans*-resveratrol and *trans*-piceatannol, whilst *trans*-piceatannol has higher inhibiting potential than *trans*-resveratrol.

On the other hand, most of the reactions in which the tyrosine anion radical takes place are not preferred (they are preferable when hydroxyurea takes part in the reaction (**Reaction H2**) and not preferable when *trans*-resveratrol (**Reactions R3** and **R4**) or *trans*-picetannol (**Reactions R3, R4** and **R5**) takes part). This difference in the Gibbs free enthalpy

Table 1. The respective Gibbs free enthalpy changes [kcal*mol^{-1}] of the possible first-step competitive inhibition reactions of the RR by *trans*-resveratrol and the reaction of deprotonation of the *t*-RES (Reaction R6) presented in the Fig. (3) obtained at the B3LYP/6-311++G(3df,2p) level of theory in vacuum and water medium.

Reaction	Vacuum Gibbs Free Enthalpy Change	Water Medium Gibbs Free Enthalpy Change
R1	-3.175	-5.764
R2	-0.432	-4.321
R3	5.609	2.321
R4	6.145	3.208
R5	3.372	1.012
R6	-3.432	-5.897
R7	-4.301	-6.008
R8	-0.260	-6.768

Table 2. The respective Gibbs free enthalpy changes [kcal*mol^{-1}] of the possible first-step competitive inhibition reactions of the RR by the amide form of the hydroxyurea and the reaction of deprotonation of the hydroxyurea (Reaction H4) presented in the Fig. (4) obtained at the B3LYP/6-311++G(3df,2p) level of theory in vacuum and water medium.

Reaction	Vacuum Gibbs Free Enthalpy Change	Water Medium Gibbs Free Enthalpy Change
H1	-12.248	-15.679
H2	-10.235	-13.453
H3	4.723	1.479
H4	-5.231	-8.210
H5	-12.834	-15.987

change can be caused by the distinction between stabilities of tyrosine radical and tyrosine anion radical. In the first case the species are only stabilized by delocalization of the unpaired electron over the phenyl ring (resonance stabilization). In the second case the species are additionally stabilized by delocalization of the negative charge over the carboxyl group. Therefore, the free energy of the reactants is lower in the reactions of tyrosine anion radical and the Gibbs free enthalpy change is higher for those reactions. That is the reason why they are not preferred thermodynamically.

Additionally, we can notice that all of the reactions of tyrosine radical in which cation radicals of potential inhibitors are formed are not thermodynamically preferred in the media studied (apart from the **Reaction P7** of *trans*-piceatannol with tyrosine radical in the water medium). We claim that this can be caused by a higher acidity of the inhibitors studied than tyrosine radical – that is the reason why transfer of

Table 3. The respective Gibbs free enthalpy changes [kcal*mol^{-1}] of the possible first-step competitive inhibition reactions of the RR by the *trans*-piceatannol and the reaction of deprotonation of the *t*-PIC (Reaction P8) presented in the Fig. (5) obtained at the B3LYP/6-311++G(3df,2p) level of theory in vacuum and water medium.

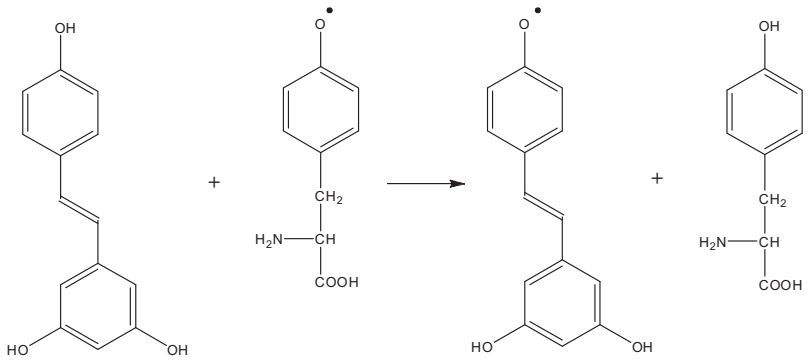
Reaction	Vacuum Gibbs Free Enthalpy Change	Water Medium Gibbs Free Enthalpy Change
P1	-4.445	-6.830
P2	-3.913	-5.841
P3	-1.115	-5.450
P4	3.263	1.403
P5	4.720	2.625
P6	3.924	1.823
P7	1.266	-0.842
P8	-4.524	-6.112
P9	-6.112	-8.941
P10	-5.844	-7.890
P11	-1.093	-7.552

the proton from the tyrosine to the inhibitor is not preferred. All reactions of the tyrosine and cysteine thiol radicals that lead to creation of inhibitor radical (not inhibitor cation radical) are fully preferred thermodynamically.

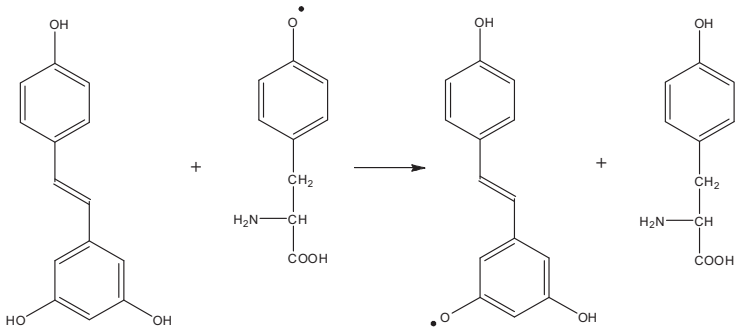
Reactions R6, H4 and **P8** of deprotonation of the inhibitors studied are fully allowed thermodynamically which means that they proceed spontaneously.

The reactions of the 4'-OH group of *t*-RES and *t*-PIC with the tyrosine radical are preferred thermodynamically rather than the reactions in which the 3-OH group takes part. Moreover, also the reactions of these inhibitors with tyrosine anion radical are more preferred when in their proceeding the unpaired electron is localized on the 4'-O atom than when it is created on the 3-O atom (even though these reactions are not thermodynamically allowed) – (compare the Gibbs free enthalpy changes of the **Reactions R3** and **R4** and those of the **Reactions P4** and **P6)**. This is caused by a higher resonance stabilization of the 4'-O-radical than 3-O-radical. For this type of reactions in which *t*-PIC takes part, the most stable radical is the one with the unpaired electron localized on the 4'-O atom and the least stable – the one with the unpaired electron localized on the 3'-O atom (**Reactions P4-P6**). Also this result is provided by diverse extent of resonance stabilization in different unpaired electron locations in radicals. *t*-PIC reactions result in the creation of inhibitor cation radicals because Gibbs free enthalpy change magnitudes are in opposition to those obtained from the analysis of the reactions that result in the creation of inhibitor radicals. It was found that in the latter case the compound in which the unpaired electron is localized on the 3'-O atom is more stable than the one in which it is localized on the 3-O atom.

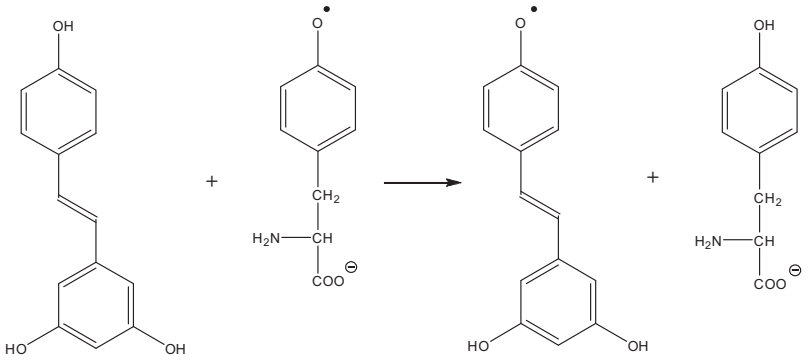
R1



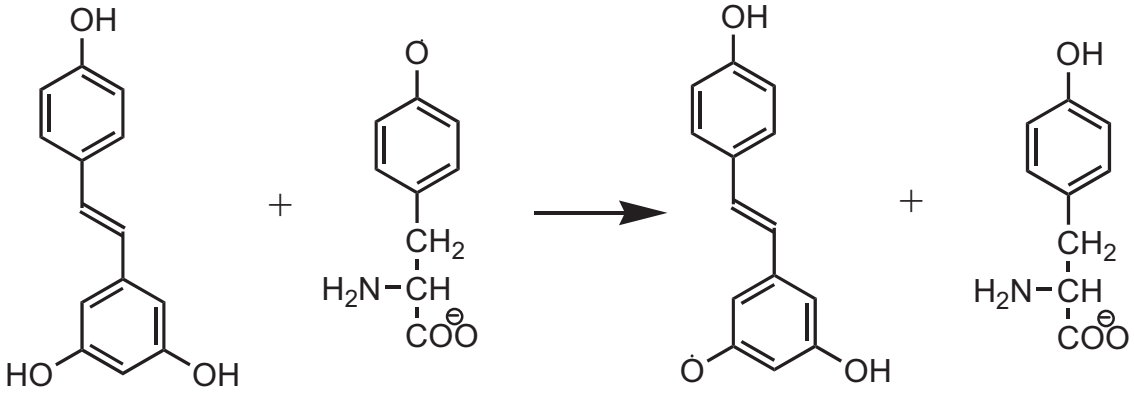
R2



R3



R4



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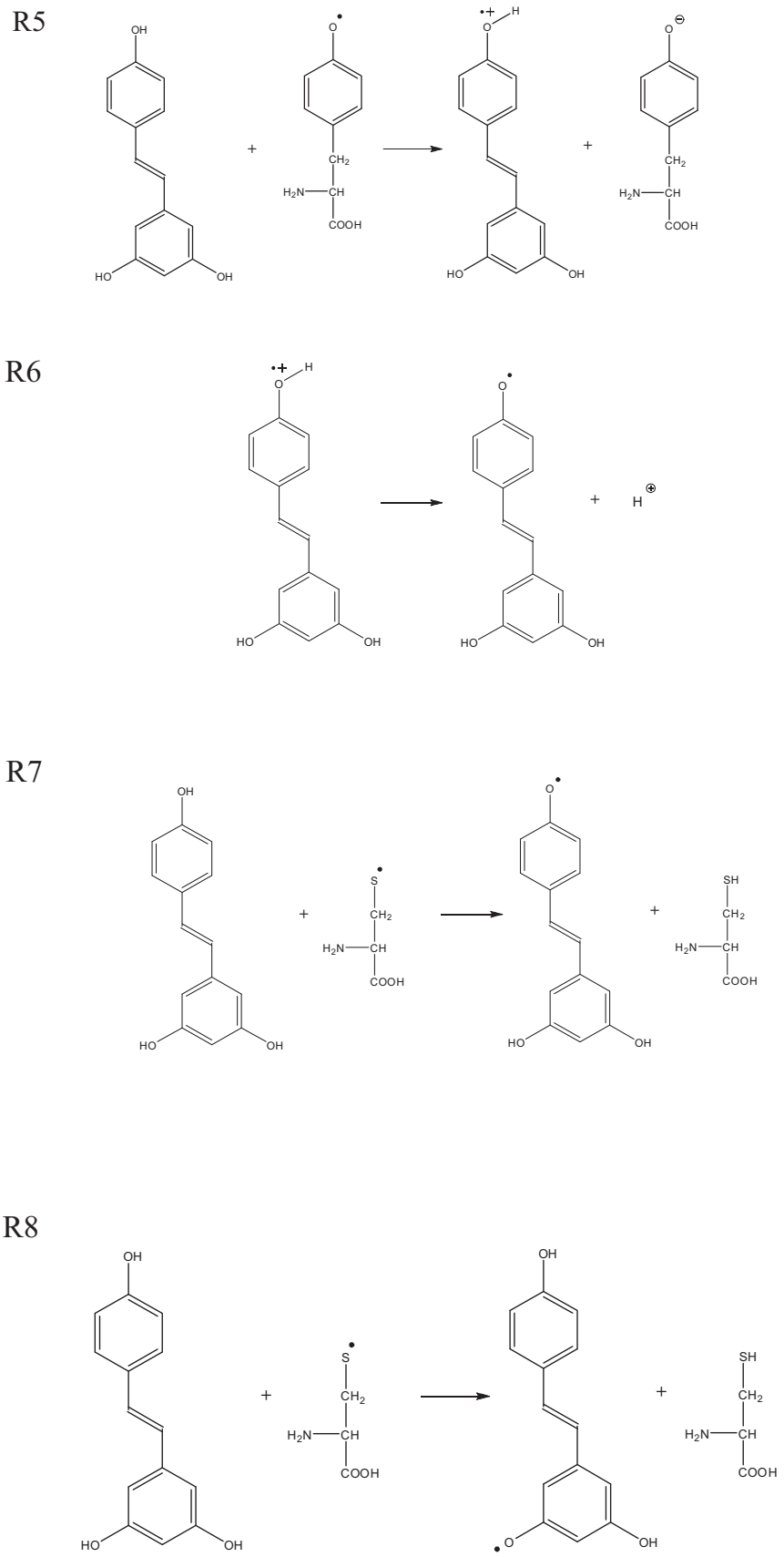
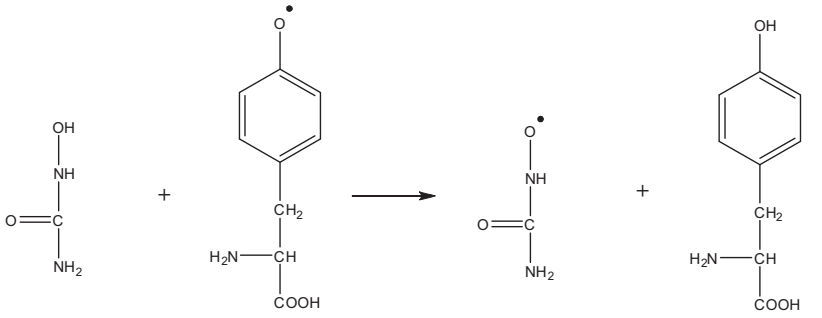
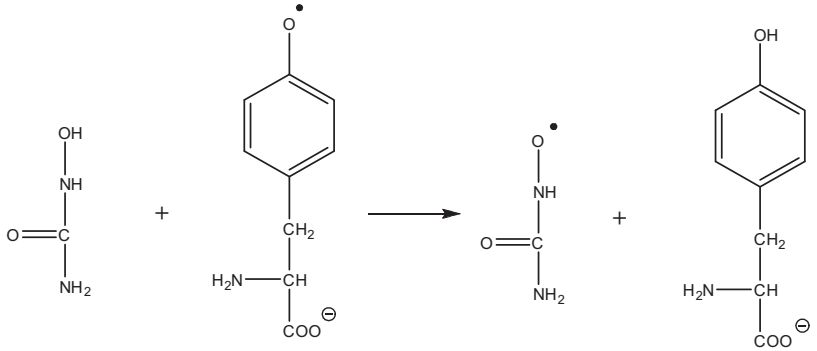


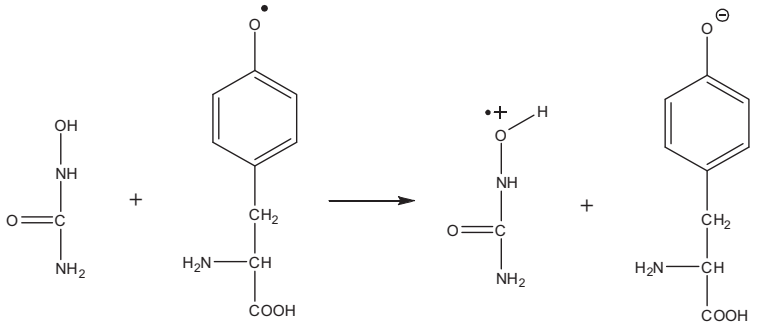
Fig. (3). The schemes of the possible first-step competitive inhibition reactions of the RR by *trans*-resveratrol and the reaction of deprotonation of the *t*-RES (reaction **R7**).



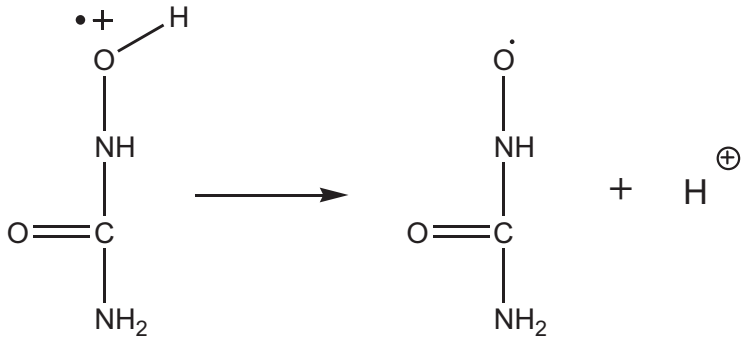
H1



H2



H3



H4

Fig. (**4**). Contd….

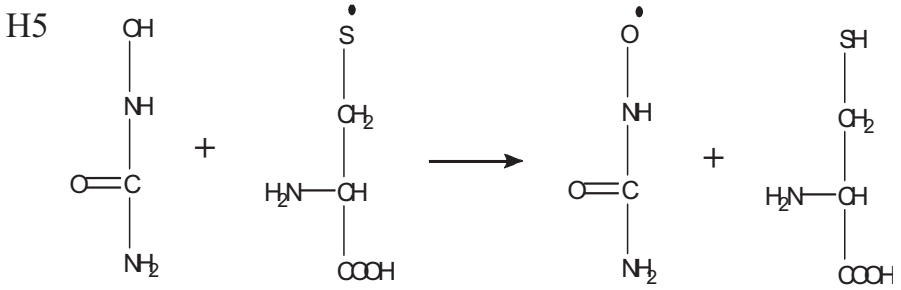
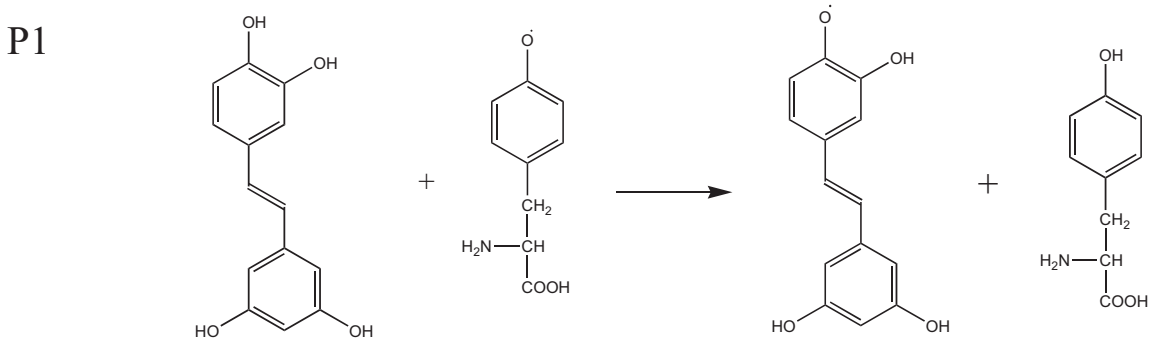
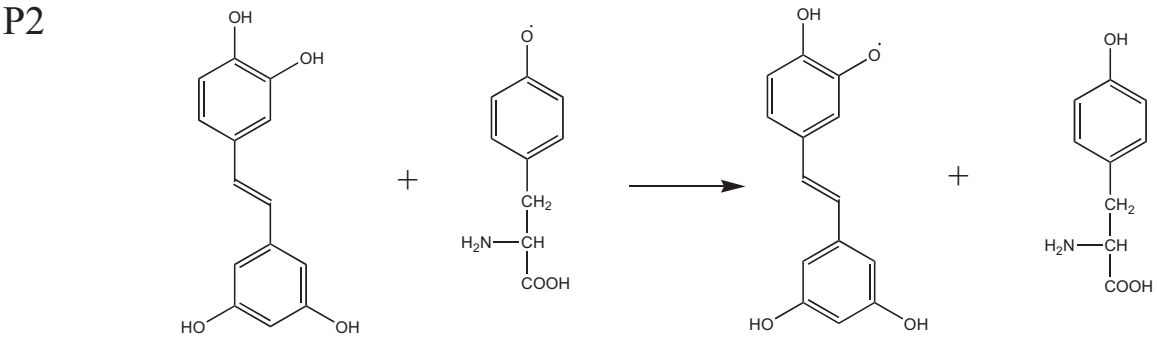
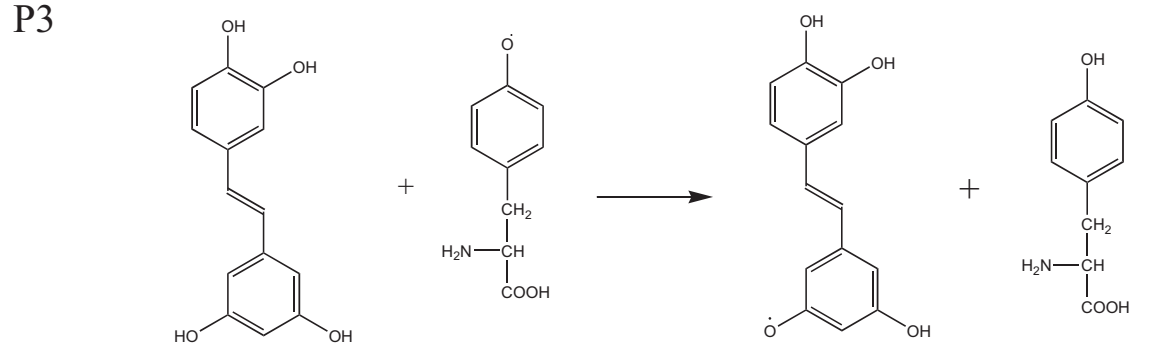


Fig. (4). The schemes of the possible first-step competitive inhibition reactions of the RR by the amid form of the hydroxyurea and the reaction of deprotonation of the hydroxyurea (reaction **H5**).







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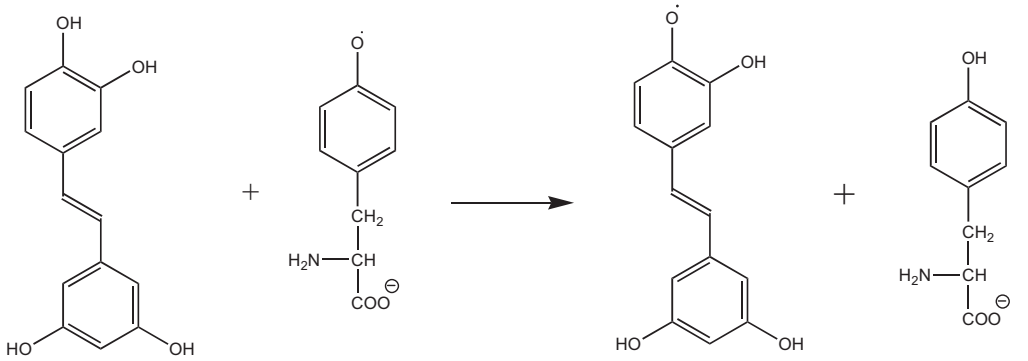
P4



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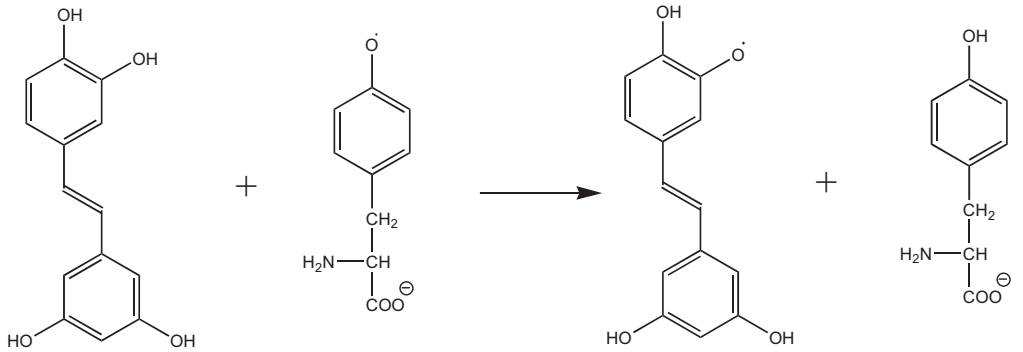

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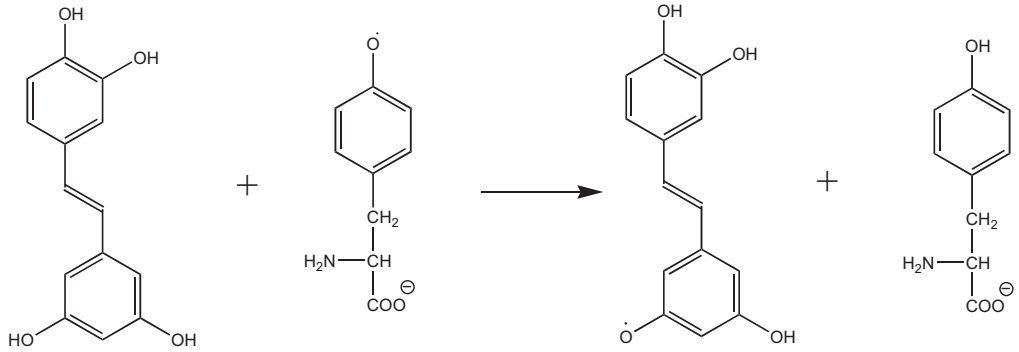
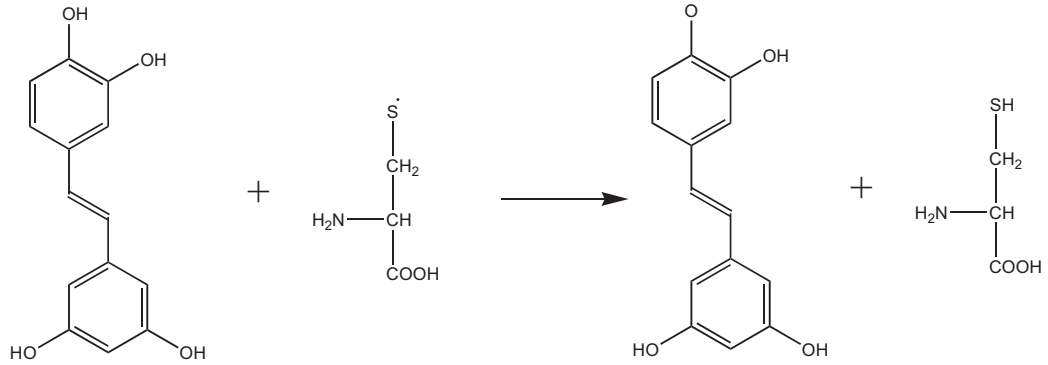


P7



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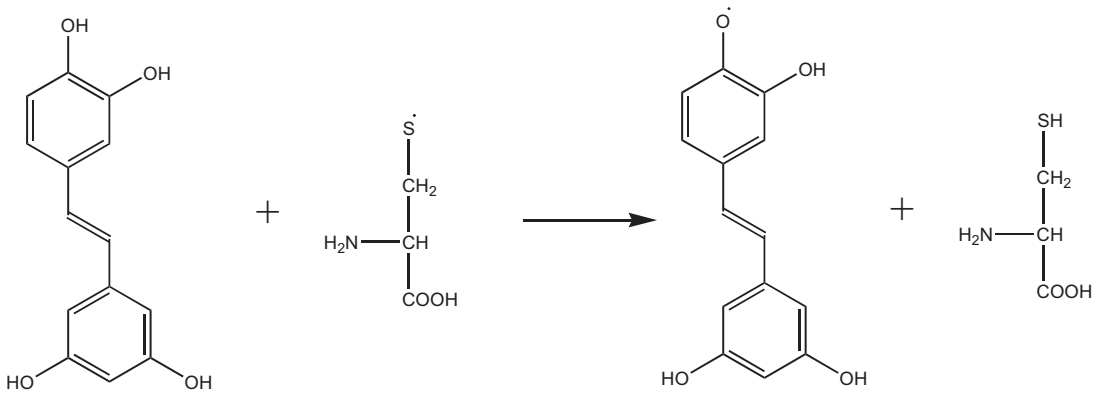
P8



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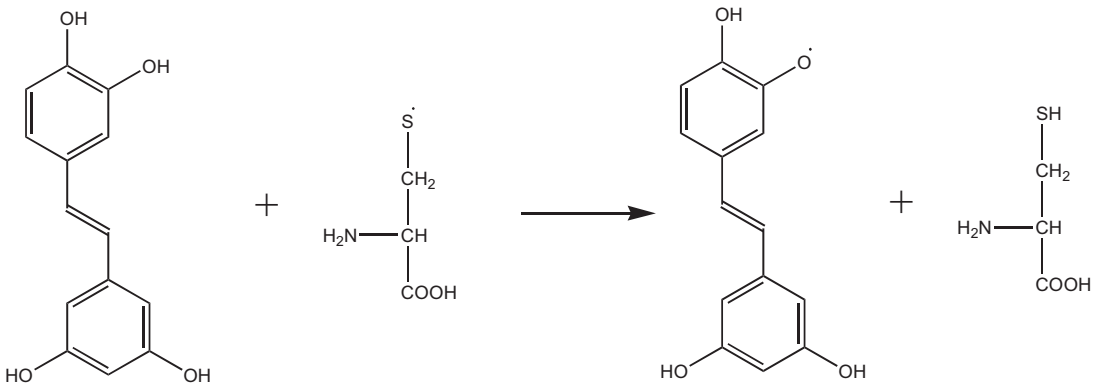

P10

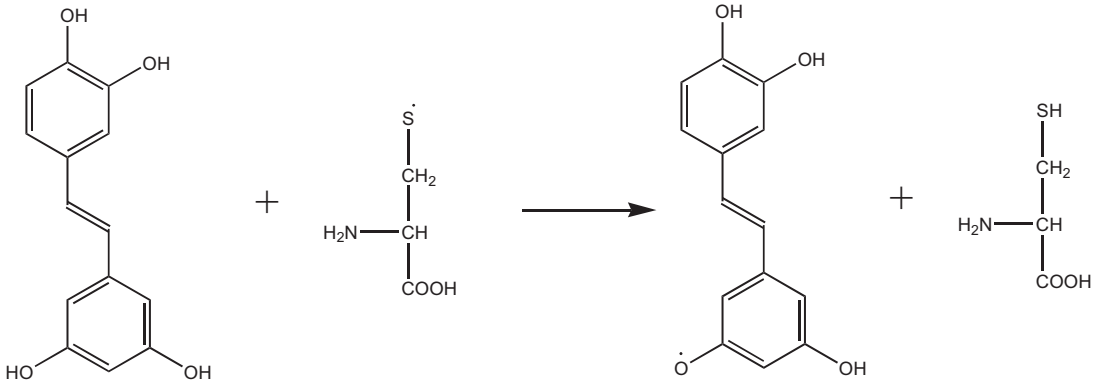

Fig. (5). The schemes of the possible first-step competitive inhibition reactions of the RR by the *trans*-piceatannol and the reaction of deprotonation of the *t*-PIC (reaction **P7**).

As it is not associated with any chemical justification we claim that this small difference is caused by the specifics of the quantum method applied.

The reactions of *t*-RES and *t*-PIC with tyrosine radical and cysteine thiol radical are characterized by similar magnitudes of the free enthalpy change which are negative. These results suggest that *t*-RES and *t*-PIC can effectively scavenge both tyrosine radical and cysteine thiol radical, and therefore are strong chemotherapeutic compounds, which effectively inhibit the process of deoxyribonucleosides creation and can be used in antitumor therapies.

CONCLUSION

- All of the reactions proceeding in water medium were found to have lower Gibbs free enthalpy change than the ones proceeding in vacuum.
- All of the reactions during which inhibitor radicals are created were found to be allowed thermodynamically both in vacuum and in water medium**.**
- Hydroxyurea (amide form) was found to be a stronger inhibitor of the ribonucleotide reductase than the *t*-RES and *t*-PIC (the first-step inhibition reactions in which hydroxyurea takes part have lower Gibbs free enthalpy changes); *t*-PIC was found to be a stronger inhibitor than *t*-RES.
- It was found that *t*-RES and *t*-PIC can effectively scavenge tyrosine radical and cysteine thiol radical and therefore inhibit the process of catalytic production of deoxyribonucleosides by the RR enzymatic complex.
- The reactions in the proceeding of which inhibitor cation radicals are created are not preferred and the reactions of cation radical deprotonation are preferred both in vacuum and water medium, which means that most probably the first step of the inhibition process of the ribonucleotide reductase by the compound studied proceeds with the creation of inhibitor radical.
- It was shown that the most stable structures of *t*-RES and *t*-PIC radicals obtained in those reaction are the ones in which the unpaired electron is localized on the 4'-O atom.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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