

## The assessment of the antioxidant capacity of the selected vanillin-based pyrido-dipyrimidines using DPPH assay: *in silico* approach

Svetlana Jeremić<sup>1\*</sup>, Nenad Janković<sup>2</sup>, Jelena Đorović Jovanović<sup>2</sup>, Zoran Marković<sup>1,2</sup>

<sup>1</sup> The State University of Novi Pazar, Department of Natural Sciences and Mathematics, Vuka Karadžića 9, 36300 Novi Pazar, Serbia; e-mail: [sjeremic@np.ac.rs](mailto:sjeremic@np.ac.rs)

<sup>2</sup> University of Kragujevac, Institute for Information Technologies, Jovana Cvijica bb, 34000 Kragujevac, Serbia; e-mail: [nenad.jankovic@uni.kg.ac.rs](mailto:nenad.jankovic@uni.kg.ac.rs), [jelena.djorovic@uni.kg.ac.rs](mailto:jelena.djorovic@uni.kg.ac.rs), [zmarkovic@uni.kg.ac.rs](mailto:zmarkovic@uni.kg.ac.rs)

\* Corresponding author

DOI: 10.46793/ICCB23.613J

**Abstract:** It is known that pyridine derivatives possess significant biological activity, and that vanillin is a natural aromatic compound with pharmacoeological properties. Earlier research indicated that among the series of synthesized vanillin-based pyrido-dipyrimidine compounds, two of them show significant antioxidative activity. Based on the thermodynamic parameters obtained using the DFT method, the operative mechanism of the reaction of the investigated antioxidants with the DPPH radical was considered. Calculations are done in water, methanol, and benzene, to imitate real experimental conditions, and to examine the reaction mechanisms under the environment of different polarity. HAT and SPLET mechanisms were identified as operative reaction mechanisms. Which reaction pathway is dominant was found to be independent of the nature of the solvent. A molecule with two O-H groups can react through both O-H groups simultaneously, so it shows a higher antioxidant capacity than a molecule with one O-H group, which is in accordance with the experimentally obtained results.

**Keywords:** vanillin-based pyrido-dipyrimidine, DPPH, antioxidant mechanisms, thermodynamic parameters, DFT

### 1. Introduction

Pyrimidine derivatives possess significant biological activity, which is additionally enhanced by the binding of some other heterocyclic molecules to pyrimidine. Thus, it has been proven that molecules with a pyridopyrimidal structure show antimicrobial, anti-inflammatory, antioxidant and anti-carcinogenic activity [1]. Therefore, some of them are officially accepted as drugs in the treatment of some types of cancer [2]. On the other hand, vanillin is a natural compound, which possesses numerous biological and pharmacoeological properties [3]. Due to its specific pleasant aroma, its use in the food and cosmetic industry is large, so today the greatest quantities of vanillin are produced synthetically or biotechnologically. Previously published results indicated remarkably antioxidant activity of vanillin-based pyrido-dipyrimidine [4]. Therefore, the capacity of

selected vanillin-based pyrido-dipyrimidines to neutralize DPPH radical species was investigated, and the obtained results were compared with experimental indicators of antioxidant activity of these compounds determined using the DPPH assay [4].

## 2. Methodology section

Geometry optimization of investigated pyrido-dipyrimidines, their ionic and radical species, as well as neutral and charged species of DPPH is performed using M06-2X/6-311++G(d,p) method implemented in Gaussian09 program package [5,6]. CPCM solvation model was applied to imitate the solvent effect [7]. To examine the reactivity of the considered molecules towards DPPH in polar and non-polar environments, the calculations were performed in water, methanol, and benzene.

The DPPH radical can be neutralized by a transfer of a hydrogen atom from the antioxidant molecule to the DPPH. This reaction can follow several mechanistic pathways, three of which are discussed here: HAT (Hydrogen Atom Transfer), SPLET (Sequential Proton Loss Electron Transfer), and SET-PT (Single-Electron Transfer followed by Proton Transfer) [4,8]. The probability that the reaction follows the HAT mechanism can be estimated based on the  $\Delta G_{BDE}$  value (Bond Dissociation free Energy, Eq. 1) as the corresponding thermodynamic parameter. Whether a SPLET mechanism is the most likely reaction pathway can be discussed based on  $\Delta G_{PA}$  (Proton Affinity, Eq. 2) and  $\Delta G_{ETE}$  (Electron Transfer free Energy, Eq. 3). The possibility that the reaction follows the SET-PT mechanism can be estimated based on  $\Delta G_{IP}$  (Ionization Potential, Eq. 4) and  $\Delta G_{PDE}$  (Proton Dissociation free Energy, Eq. 5).

$$\Delta G_{BDE} = [G(M-O\cdot) + G(DPPH_n)] - [G(M-OH) + G(DPPH_r)] \quad (1)$$

$$\Delta G_{PA} = [G(M-O^-) + G(DPPH_n)] - [G(M-OH) + G(DPPH_a)] \quad (2)$$

$$\Delta G_{ETE} = [G(M-O\cdot) + G(DPPH_a)] - [G(M-O^-) + G(DPPH_r)] \quad (3)$$

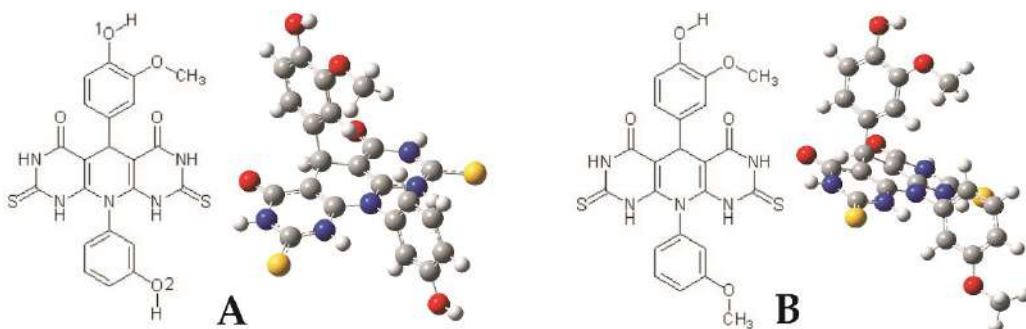
$$\Delta G_{IP} = [G(M-O^{\bullet+}) + G(DPPH_a)] - [G(M-OH) + G(DPPH_r)] \quad (4)$$

$$\Delta G_{PDE} = [G(M-O\cdot) + G(DPPH_n)] - [G(M-O^{\bullet+}) + G(DPPH_a)] \quad (5)$$

In the previous equations  $G(M-OH)$ ,  $G(M-O\cdot)$ ,  $G(M-O^-)$  and  $G(M-O^{\bullet+})$  denote Gibbs free energies of the neutral molecule of antioxidant, its radical, anionic and radical cation moieties respectively, while  $G(DPPH_n)$ ,  $G(DPPH_r)$  and  $G(DPPH_a)$  presents Gibbs free energies of protonated, radical and anionic moieties of DPPH respectively.

## 3. Results and discussions

Geometry optimization of the estimated vanillin-based pyrido-dipyrimidine antioxidants indicated the absence of planarity observing the whole molecule [4]. Earlier investigations indicate a better antioxidant capacity of molecule A than of molecule B, which is explained by the presence of the two O-H groups in molecule A, and only one O-H group in molecule B (Fig. 1), [4].



**Figure 1.** 2D and optimized 3D structures of investigated vanillin-based pyrido-dipyrimidine antioxidants A and B.

Comparing  $\Delta G_{BDE}$  with  $\Delta G_{PA}$  and  $\Delta G_{IP}$  values, as thermodynamic parameters that determine the first step of the reaction, one can conclude that HAT is the most plausible reaction mechanism for O1 position of A and for molecule B. A molecule in position 2 in all three estimated solvents reacts following SPLET as the most plausible mechanistic pathway. The reactivity of B is almost equal to the reactivity of A-O1, and the reactivity of A-O2 is somewhat lower and predominantly follows the other mechanism compared with A-O1 and B. Considering that the reaction is favorable if its Gibbs free energy is  $\leq 40$  kJ/mol [8], SET-PT is not an operative reaction mechanism in any of the observed environments.

**Table 1.** Thermodynamic parameters of investigated mechanisms of antioxidant activity of molecules A and B (see Fig. 1).

Solvent	Molecule	HAT	SPLET		SET-PT	
		$\Delta G_{BDE}$	$\Delta G_{PA}$	$\Delta G_{ETE}$	$\Delta G_{IP}$	$\Delta G_{PDE}$
water	A-O1	19	64	-45	110	-91
	A-O2	139	27	111	110	28
	B	12	55	-43	103	-91
methanol	A-O1	20	66	-47	116	-96
	A-O2	138	27	112	116	22
	B	12	57	-45	109	-97
benzene	A-O1	23	107	-84	221	-198
	A-O2	131	22	110	221	-89
	B	24	99	-74	222	-198

The experimental results obtained by DPPH assay indicate a higher activity of molecule A compared to molecule B. This can be explained by the fact that molecule A

exerts its antioxidant activity via two O-H groups simultaneously. Based on the thermodynamic parameters, it can be concluded that the reactivity of the investigated mechanisms toward the DPPH radical does not depend on the nature of the solvent.

### 3. Conclusions

Examining the reaction mechanism of selected vanillin-based pyrido-dipyrimidine antioxidants A and B in the presence of DPPH radical, we concluded that the HAT is the operative mechanism for A in position 1 and for molecule B, in all estimated solvents. The predominant mechanism for the A molecule at position 2 is SPLET. The experimentally determined high antioxidant activity of molecule A compared to molecule B is probably due to two possible reaction positions in molecule A.

### Acknowledgment

This research is funded by the Ministry of Education and Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grants: No. 451-03-47/2023-01/200252 and 451-03-47/2023-01/200378.

### References

- [1] F. Buron, J.Y. Mérour, M. Akssira, G. Guillaumet, S. Routier, *Recent advances in the chemistry and biology of pyridopyrimidines*, *European Journal of Medicinal Chemistry*, 95 (2015) 76–95.
- [2] S. Wang, X.-H. Yuan, S.-Q. Wang, W. Zhao, X.-B. Chen, B. Yu, *FDA-approved pyrimidine-fused bicyclic heterocycles for cancer therapy: Synthesis and clinical application*, *European Journal of Medicinal Chemistry*, 214 (2021) 113218.
- [3] L. Blaikie, G. Kay, P.K. Thoo Lin, *Synthesis and in vitro evaluation of vanillin derivatives as multi-target therapeutics for the treatment of Alzheimer's disease*, *Bioorganic and Medicinal Chemistry Letters*, 30 (2020) 127505.
- [4] N. Janković, J. Tadić, E. Milović, Z. Marković, S. Jeremić, J. Petronijević, N. Joksimović, T.T. Borović, S.N.A. Bukhari, *Investigation of the radical scavenging potential of vanillin-based pyrido-dipyrimidines: experimental and in silico approach*, *RSC Advances*, 13 (2023) 15236-15242.
- [5] Y. Zhao, D.G. Truhlar, *The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals*, *Theoretical Chemistry Accounts*, 120 (2008) 215-241.
- [6] M.J. Frisch, G.W. Trucks, H.B. Schlegel, et al., *Gaussian 09, Revision A.02*, Gaussian Inc, Wallingford CT, 2009.
- [7] Y. Takano, K.N. Houk, *Benchmarking the Conductor-like Polarizable Continuum Model (CPCM) for Aqueous Solvation Free Energies of Neutral and Ionic Organic Molecules*, *Journal of Chemical Theory and Computation*, 1 (2005) 70–77.
- [8] A. Amić, Z. Marković, J.M. Dimitrić Marković, S. Jeremić, B. Lucić, D. Amić, *Free radical scavenging and COX-2 inhibition by simple colon metabolites of polyphenols: A theoretical approach*, *Computational Biology and Chemistry* 65 (2016) 45-53.