

## DFT-based theoretical investigation of hydroxyl radical-induced degradation mechanisms of antineoplastic drugs in aqueous media

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Accepted: August 17, 2025

Within the first 48 hours following chemotherapy, small quantities of cytostatic drugs are excreted *via* urine, vomit, and other bodily secretions. These residues pose potential risks to human and environmental health, highlighting the need for their effective removal from wastewater streams. In this study, the degradation pathways of epirubicin and methotrexate—two commonly used antineoplastic agents—were explored using density functional theory (DFT). Optimized molecular geometries were obtained using the B3LYP functional with the 6-31G(d) basis set *via* Gaussian 09 software. Fragmentation patterns and degradation mechanisms initiated by hydroxyl radical attacks were evaluated in both gas and aqueous phases, incorporating solvation effects using the COSMO model. The findings serve as a theoretical framework to inform and support experimental degradation studies.

**Keywords:** Antineoplastic drugs, hydroxyl radicals, DFT, wastewater treatment, epirubicin, methotrexate

### INTRODUCTION

Pharmaceutical compounds, particularly cytostatic drugs such as epirubicin and methotrexate, are frequently detected in aquatic environments due to their widespread and repeated use in cancer therapy. These compounds are frequently excreted in unmetabolized form, subsequently entering municipal wastewater systems, posing a significant risk to both aquatic life and human health due to their high bioactivity and persistence in the environment [1, 2].

Advanced oxidation processes (AOPs), especially those involving hydroxyl radicals ( $\bullet\text{OH}$ ), have gained attention as effective methods for degrading pharmaceuticals in water. Hydroxyl radicals exhibit strong oxidative potential, reacting rapidly and non-selectively with a broad spectrum of organic molecules, including aromatic rings and aliphatic side chains commonly found in cytostatic drugs [3, 4]. These radicals can initiate degradation pathways *via* hydrogen abstraction, hydroxylation, and electron transfer mechanisms, often resulting in fragmentation into smaller, less harmful species such as  $\text{CO}_2$ ,  $\text{NH}_4^+$ , or  $\text{NO}_3^-$  [5].

In recent years, density functional theory (DFT) has emerged as a powerful computational tool to model and predict molecular-level interactions during oxidation processes. Compared to traditional quantum mechanical methods, DFT offers a balance between computational efficiency and accuracy,

making it particularly suitable for exploring the degradation mechanisms of complex drug molecules in aqueous media [6, 7]. The B3LYP functional combined with the 6-31G(d) basis set has been shown to reliably predict bond dissociation energies, reaction pathways, and transition states in systems involving free radicals [8].

In this study, DFT was used to investigate the structural properties and fragmentation patterns of epirubicin and methotrexate under attack by hydroxyl radicals. The reaction energetics were evaluated in both gas phase and aqueous environments using the COSMO solvation model. This theoretical approach provides valuable insights into the degradation mechanisms of these pharmaceuticals and supports the development of more effective treatment strategies for contaminated wastewater.

Recent studies further support the relevance of computational chemistry in pharmaceutical degradation. Hadidi *et al.* [9] demonstrated the application of DFT in modeling the degradation of anticancer drug carmustine. Zhou *et al.* [10] and Gogate & Pandit [11] provided comprehensive reviews of AOPs' effectiveness in degrading pharmaceuticals, confirming the central role of hydroxyl radicals. Additionally, the works of Basaleh *et al.* [12] and Kumar *et al.* [13] highlight the importance of theoretical predictions in guiding experimental validation and environmental impact assessments.

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## METHODOLOGY

The degradation pathways of epirubicin and methotrexate under the influence of photo-generated hydroxyl radicals ( $\bullet\text{OH}$ ) were modeled using quantum chemical calculations. Initial molecular geometries were constructed using GaussView 5.0 and subsequently optimized using the B3LYP hybrid functional and 6-31G(d) basis set within the Gaussian 09W software suite. Both gas-phase and aqueous-phase environments were considered, with solvation effects modeled using the COSMO continuum solvation approach.

Each molecule's geometry was analyzed with respect to average bond lengths, bond angles in aromatic rings, and hybridization patterns. Particular attention was given to the transition from  $\text{sp}^2$  to  $\text{sp}^3$  hybridization that occurs upon hydroxyl radical attack. The addition of  $\bullet\text{OH}$  to unsaturated carbon centers, abstraction of hydrogen atoms, and electron transfer mechanisms were all considered plausible reaction pathways.

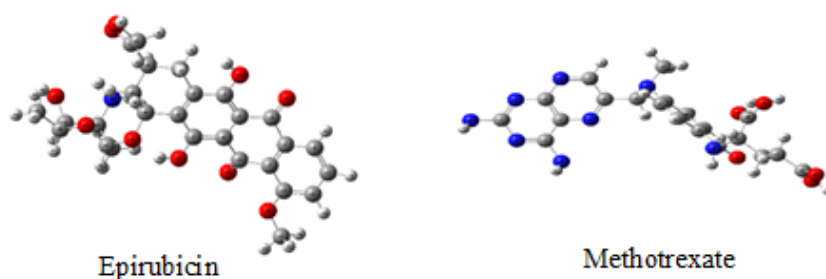
Frequency calculations were performed to confirm that all optimized structures corresponded to true minima on the potential energy surface. Theoretical reaction enthalpies and Gibbs free energies ( $\Delta\text{H}$  and  $\Delta\text{G}$ ) were computed to evaluate the energetic favorability of possible degradation

routes. This computational protocol allowed for the identification of the most reactive molecular sites and fragmentation products resulting from hydroxyl radical interaction [14-17].

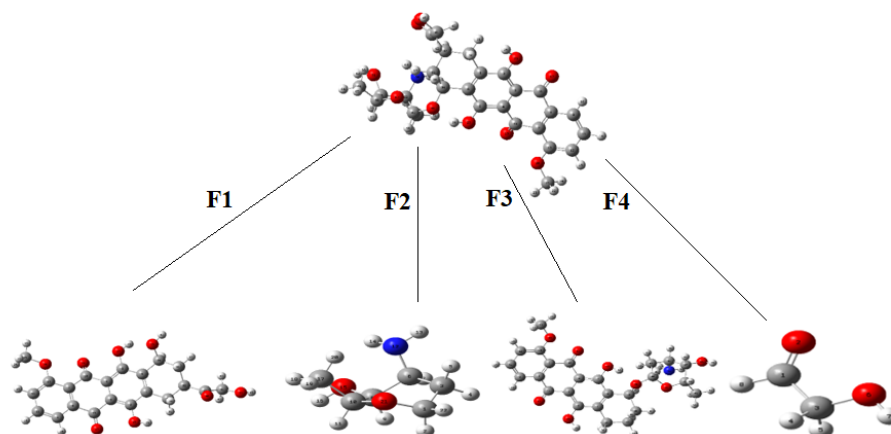
## RESULTS AND DISCUSSION

The optimized geometries of epirubicin and methotrexate were obtained and analyzed to identify the molecular regions most susceptible to  $\bullet\text{OH}$  radical attack. As shown in Figure 1, the electron-rich aromatic systems and side chains were prominent reactive sites. The reaction pathways were simulated to predict the most probable fragmentation patterns initiated by hydroxyl radicals.

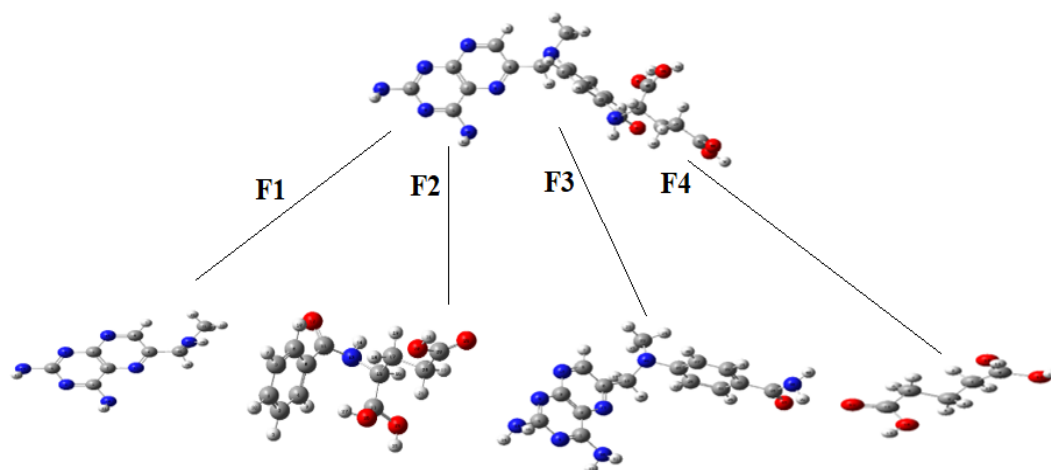
Figs. 2 and 3 illustrate potential degradation pathways for epirubicin and methotrexate, respectively. The degradation was modeled by examining the bond dissociation energies and Gibbs free energy changes ( $\Delta\text{G}$ ) for specific fragmentation reactions. Lower  $\Delta\text{G}$  values indicated energetically favorable reactions. These computational results were in agreement with degradation pathways proposed in literature for structurally similar compounds.



**Fig. 1.** Optimized geometric structure of epirubicin and methotrexate molecules *via* DFT method. (grey, C; white, H; red, O; blue, N)



**Fig. 2.** Possible pathways for the photocatalytic degradation of epirubicin (grey, C; white, H; red, O; blue, N)



**Fig. 3.** Possible pathways for the photocatalytic degradation of methotrexate (grey, C; white, H; red, O; blue, N)

**Table 1.** Constant energy, enthalpy and Gibbs free energy values according to the DFT method.

| Molecules  | Phase        | $\Delta E$<br>Energy<br>(kcal mol <sup>-1</sup> ) | $\Delta H$<br>Enthalpy<br>(kcal mol <sup>-1</sup> ) | $\Delta G$<br>Gibbs free energy<br>(kcal mol <sup>-1</sup> ) |
|------------|--------------|---|---|--|
| Epirubicin | Gas          | -1852.666498                                      | -1852.665554  | -1852.770413   |
|            | <i>COSMO</i> | -1852.706760                                      | -1852.705816  | -1852.809057   |
| F1         | Gas          | -1412.423582                                      | -1412.422638  | -1412.505501   |
|            | <i>COSMO</i> | -1412.458387                                      | -1412.457443  | -1412.539994   |
| F2         | Gas          | -441.420941                                       | -441.419997   | -441.464388  |
|            | <i>COSMO</i> | -441.431062                                       | -441.430118   | -441.474112  |
| F3         | Gas          | -1624.879322                                      | -1624.878378  | -1624.971809   |
|            | <i>COSMO</i> | -1624.911163                                      | -1624.910218  | -1625.002645   |
| F4         | Gas          | -228.961057                                       | -228.960113   | -228.992735  |
|            | <i>COSMO</i> | -228.972708                                       | -228.971764   | -229.004268  |

**Table 2.** Constant energy, enthalpy and Gibbs free energy values according to the DFT method.

| Molecules    | Phase        | $\Delta E$<br>Energy<br>(kcal mol <sup>-1</sup> ) | $\Delta H$<br>Enthalpy<br>(kcal mol <sup>-1</sup> ) | $\Delta G$<br>Gibbs free energy<br>(kcal mol <sup>-1</sup> ) |
|--------------|--------------|---|---|--|
| Methotrexate | Gas          | -1589.617330                                      | -1589.616386  | -1589.718600   |
|              | <i>COSMO</i> | -1589.654479                                      | -1589.653535  | -1589.758458   |
| F1           | Gas          | -694.534243                                       | -694.533299   | -694.588342  |
|              | <i>COSMO</i> | -694.551097                                       | -694.550152   | -694.606003  |
| F2           | Gas          | -896.256056                                       | -896.255111   | -896.322768  |
|              | <i>COSMO</i> | -896.273932                                       | -896.272988   | -896.343253  |
| F3           | Gas          | -1094.172616                                      | -1094.171672  | -1094.246764   |
|              | <i>COSMO</i> | -1094.200041                                      | -1094.199097  | -1094.274502   |
| F4           | Gas          | -496.637390                                       | -496.636445   | -496.683615  |
|              | <i>COSMO</i> | -496.647881                                       | -496.646937   | -496.697138  |

Tables 1 and 2 summarize the total electronic energy, enthalpy, and Gibbs free energy of the parent compounds and their major fragmentation products in both gas and COSMO aqueous phases. The data show that degradation is more thermodynamically favorable in the aqueous phase, supporting the hypothesis that water and its reactive species facilitate drug decomposition.

The modeled degradation products included small molecules such as CO<sub>2</sub>, NO<sub>3</sub><sup>-</sup>, and NH<sub>4</sub><sup>+</sup>, indicating mineralization processes. The fragmentation patterns identified can help inform experimental setups aimed at validating these degradation routes and quantifying end-products in real wastewater samples.

## CONCLUSIONS

This study presents a theoretical investigation into the degradation mechanisms of epirubicin and methotrexate, two widely used antineoplastic agents, in the presence of hydroxyl radicals. Using density functional theory (DFT), the reactivity of different molecular regions was explored, and potential degradation pathways were proposed based on energetic parameters. The results reveal that hydroxyl radical attack initiates fragmentation reactions that are more favorable in aqueous environments. These findings not only contribute to the understanding of drug breakdown in natural and engineered water systems but also offer a valuable foundation for the design of effective advanced oxidation processes (AOPs). Further experimental studies are encouraged to validate these theoretical predictions and assess the formation of degradation by-products under real-world conditions.

**Acknowledgement:** The authors greatly appreciate Tekirdag Namik Kemal University Research Foundation for financial support. Project number: NKUBAP.01.GA.22.437.

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