DOI: 10.5961/highereducsci.1697221

Computational Investigation of 5-Ethynyl-2'-deoxyuridine (EdU) as a Biologically Active Nucleoside Analogue: Insights from Molecular Docking, ADMET Profiling, and DFT Analyses

Biyolojik Olarak Aktif Bir Nükleozit Analoğu Olarak 5-Etinil-2'-deoksiüridin'in (EdU) Hesaplamalı İncelenmesi: Moleküler Yerleştirme, ADMETox Değerlendirmesi ve DFT Analizlerinden Elde Edilen Bilgiler

Meryem ALP

ABSTRACT

This study presents a comprehensive computational investigation of the molecule 5-ethynyl-2'-deoxyuridine (EdU), a thymidine analogue of considerable biological importance. The investigation includes quantum chemical analyses, molecular docking, and pharmacokinetic evaluation to assess the structural, electronic, and biological properties of EdU. Initially, the molecular geometry of EdU was optimized using Density Functional Theory (DFT), providing a basis for deeper electronic structure evaluations. Topological analyses, including Electron Localization Function (ELF) and Localized Orbital Locator (LOL), were performed to explore the distribution of electron density and bonding properties within the molecule. These visual and quantitative descriptors contributed to a clearer understanding of the reactivity and stability of the molecule. The pharmacokinetic behavior of EdU and its similarity to the drug was evaluated through in silico ADME (absorption, distribution, metabolism, and excretion) modeling. Using internet-based platforms such as SwissADME and admetSAR, various parameters were evaluated to determine the potential of the molecule as an orally active compound and its compliance with established drug similarity rules. Toxicological properties were further investigated using predictive tools to estimate acute and environmental toxicity risks.

Molecular docking simulations were performed to investigate the interaction between EdU and the selected proteins alpha-amylase and alpha-glucosidase, selected for their endocrinological importance, providing insights into possible binding mechanisms and structural compatibility. Overall, this study uses a multidisciplinary computational approach to provide a detailed theoretical profile of EdU, contributing to the understanding of its chemical behavior and potential applications in biomedical research.

Keywords: 5-Ethynyl-2'-deoxyuridine, Density functional theory, Quantum chemical calculation, Topological analyses, Molecular docking

ÖZ

Bu çalışma, kayda değer biyolojik öneme sahip bir timidin analoğu olan 5-etinil-2'-deoksiüridin (EdU) molekülünün kapsamlı bir hesaplamalı incelemesini sunmaktadır. Araştırma, EdU'nun yapısal, elektronik ve biyolojik özelliklerini değerlendirmek için kuantum

Alp M., (2025). Computational investigation of 5-Ethynyl-2'-deoxyuridine (EdU) as a biologically active nucleoside analogue: Insights from molecular docking, ADMET profiling, and DFT analyses. *Journal of Higher Education and Science/Yükseköğretim ve Bilim Dergisi, 15*(2), 310-321. https://doi.org/10.5961/highereducsci.1697221

Meryem ALP (✉)
ORCID ID: 0000-0001-6821-6252

Gazi University, Faculty of Sciences, Department of Physics, Ankara, Türkiye
Gazi Üniversitesi, Fen Fakültesi, Fizik Bölümü, Ankara, Türkiye
meryem.alp@gazi.edu.tr

Received/*Geliş Tarihi* : 11.05.2025
Accepted/*Kabul Tarihi* : 02.08.2025

kimyasal analizleri, moleküler yerleştirme ve farmakokinetik değerlendirme içermektedir. Başlangıçta, EdU'nun moleküer geometrisi Yoğunluk Fonksiyonel Teorisi (DFT) kullanılarak optimize edilmiş ve daha derin elektronik yapı değerlendirmeleri için bir temel sağlanmıştır. Elektron Lokalizasyon Fonksiyonu (ELF) ve Lokalize Orbital Konumlandırıcı (LOL) dahil olmak üzere topolojik analizler, molekül içindeki elektron yoğunluğu ve bağlanma özelliklerinin dağılımını keşfetmek için yapılmıştır. Bu görsel ve nicel tanımlayıcılar, molekülün reaktivitesinin ve stabilitesinin daha net anlaşılmasına katkıda bulunmuştur. EdU'nun farmakokinetik davranışı ve ilaca benzerliği, in silico ADME (emilim, dağılım, metabolizma ve atılım) modellemesi yoluyla değerlendirilmiştir. SwissADME ve admetSAR gibi internet tabanlı platformlar kullanılarak, molekülün oral olarak aktif bir bileşik olarak potansiyelini ve yerleşik ilaç benzerliği kurallarına uygunluğunu belirlemek için çeşitli parametreler değerlendirilmiştir. Toksikolojik özellikler, akut ve çevresel toksisite risklerini tahmin etmek için öngörücü araçlar kullanılarak daha fazla araştırılmıştır.

EdU ile endokrinolojik öneme sahip olduğu için seçilen alfa-amilaz ve alfa-glukosidaz seçil proteinleri arasındaki etkileşimi araştırmak için moleküler yerleştirme simülasyonları gerçekleştirilerek olası bağlanma mekanizmaları ve yapısal uyumluluk hakkında içgörüler sağlanmıştır. Genel olarak, bu çalışma EdU'nun ayrıntılı bir teorik profilini sunmak için multidisipliner bir hesaplama yaklaşımı kullanmakta, kimyasal davranışının ve biyomedikal araştırmalardaki potansiyel uygulamalarının anlaşılmasına katkıda bulunmaktadır.

Anahtar Sözcükler: 5-Etinil-2, DFT, Kuantum kimyasal inceleme, Topolojik analiz, Moleküler yerleştirme çalışması

INTRODUCTION

Nucleoside derivatives, such as 5-Ethynyl-2'-deoxyuridine (EdU), have been used in DNA biotechnology as potential therapeutics and for modifying DNA structure and function in studies. EdU has been employed in "Click Chemistry," a popular cell tagging technique to track DNA synthesis and look into cell proliferation (Bradford & Clarke, 2011). EdU has been used as an alternative to bromodeoxyuridine (BrdU) to monitor DNA synthesis (Buck et al., 2008; Cieślar-Pobuda & Łos, 2013; Kohlmeier et al., 2013). EdU has been shown to induce DNA damage response and cell death in mESC in culture (Kohlmeier et al., 2013). The dual-pulse method has been done by combining BrdU with iododeoxyuridine or chlorodeoxyuridine, with detection using multiple cross-reacting BrdU antibodies (Bradford & Clarke, 2011). EdU has been used to detect early stages of S phase, or DNA synthesis associated with DNA repair and recombination (Hua & Kearsey, 2011). EdU has been used to label nucleolar RNA transcripts and effectively highlight the nucleolus (Dvořáčková & Fajkus, 2018). The data shown in the Figure reveals that Chk2 and p53 become activated in the cells with the EdU-tagged DNA, particularly 47 h after the pulse of EdU (Zhao et al., 2013). Detection of DNA synthesis in growing organisms is important for biological science including cell proliferation and differentiation, cell cycle dynamics, and carcinogenesis (Seo et al., 2015) 5-Vinyl-2'-deoxyuridine (VdU) is the first reported metabolic probe for cellular DNA synthesis that can be visualized by using an inverse electron demand Diels-Alder reaction with a fluorescent tetrazine(Rieder & Luedtke, 2014). Analogues of the pyrimidine deoxynucleoside thymidine may be inserted into replicating DNA, effectively tagging dividing cells, allowing their characterization (Cavanagh et al., 2011) EdU is a 2'-deoxyribonucleoside derivative of uracil, a pyrimidine base, with an ethynyl group added at position 5. Its molar mass is 244.22 g/mol, and its chemical formula is C11H12N2O5. EdU is a crystalline powder that looks yellowish-white and dissolves in water. It is a helpful tool for tracking DNA synthesis and figuring out cell proliferation due to its capacity to interact with nucleic acids and enzymes inside the cell.

The study of cell proliferation, cell cycle analysis, timing of DNA replication, cell proliferation, and stem cell research are just a few of the many uses for EdU. When DNA is produced in cells, EdU labels it and creates a signal that can be seen in the cells. This characteristic makes it a crucial research tool for tracking cell growth and researching procedures like cell cycle analysis (Sakaue-Sawano et al., 2008). The study of cell proliferation and DNA biotechnology at EdU is significant, and its potential medicinal uses are still being investigated. To contribute to these investigations, the EdU molecule is analysed purely theoretically in this paper. In this study, Computational analysis results are used as an effective tool to predict the structural and electronic properties of the molecule. The article's main body is divided into sections to evaluate the data obtained. The optimized molecular structures of the EdU molecule will be presented in the section on computational methods.

COMPUTATIONAL MODELS and METHODS

All computational calculations were performed using the Gaussian 09 software (Frisch, M.J., 2009). The well-known B3LYP approach was used, which combines Lee et al.'s gradient correlation functional with Becke's three-parameter hybrid density functional. Using the 6-311++G(d,p) basis set(Becke, 1993) in the gas phase, fully optimized geometry and electrical properties were determined. The molecular electrostatic potential (MEP) surface and the frontier molecular orbitals were visualized by GaussView (Dennington et al., 2009). Electronic structure parameters, charges, and quantum chemical properties of the title compounds were calculated by the same method and basis sets to support the hydrogen bonding studies. Multiwfn also performed the electron localization function (ELF) and localized orbital locator (LOL) to analyze the non-bonded interactions (Lu & Chen, 2012).

Molecular docking simulations were employed to investigate the possible binding modes of 5-Ethynyl-2'-deoxyuridine (Edu) with selected protein targets. The three-dimensional structures of the target proteins (PDB IDs: 1B2Y, and 5ZCC) (NAHOUM et al., 2000; Roig-Zamboni et al., 2017; Sendovski

et al., 2011) were retrieved from the RCSB Protein Data Bank (Berman, 2000). Docking calculations were carried out using AutoDock Vina (Trott & Olson, 2010), efficiently predicting the most favorable binding conformations based on binding affinity scores. The resulting docking poses were visualized and analyzed using PyMOL (DeLano, n.d.) to assess the orientation of EdU within the active sites. Furthermore, molecular interactions, such as hydrogen bonding and hydrophobic contacts, were examined in detail using BIOVIA Discovery Studio Visualizer (*BIOVIA, Discovery Studio Visualizer Software*, 2021) to gain insight into the nature and strength of Edu–protein interactions. 5-Ethynyl-2'-deoxyuridine (Edu) was evaluated for its drug-likeness and pharmacokinetic properties using in silico tools. ADME, toxicity, and LD_{50} predictions were carried out via SwissADME (Daina et al., 2017; SwissADME, n.d.) and admetSAR (admetSAR, 2024) to assess its potential as a therapeutic agent.

RESULTS and DISCUSSION

Geometry Optimization

In this study, the input files of the EdU molecule were prepared with the help of GaussView. All calculations were performed in the Gaussian09 package program (Frisch, M.J., 2009). In the first step, systematic calculations of the torsion angles of the molecules were performed to determine the lowest energy state of the EdU structure as initial values. Because there is a C-N bond in the center of the molecule that can rotate. The torsional potential energy was calculated as a function of the rotation angle at 10º intervals between 0º and 360º around the C-N intermediate bond. To find the lowest energy level (-912.1777 Hatree = -24820.99eV), three different potential torsional energy calculations of the molecule were performed. Since the most suitable structure was chosen as the initial structure and the calculations were continued with it. For the optimization of the molecule, calculations were started by considering the geometrical structures at these values. These calculations were performed using the B3LYP/6-311G (d,p) basis set. In this study, the bond length and bond angle values for the most stable structure was calculated in the gas phase. These values were given in Table 1 and the optimized structure of EdU was presented in Figure 1.

Table 1. Optimized Geometrical Parameters of EdU Molecule

Atoms	Bond lenghts	Atoms	Bond angles	Atoms	Bond angles
O_1- C_9	1.42	C_9-O_1- C_{11}	111.04	C_8-C_{11}-H_{23}	108.53
O_1- C_{11}	1.44	C_8-O_2- H_{26}	108.98	C_{12}-C_{11}-H_{23}	108.42
O_2- C_{12}	1.43	C_{12}-O_3- H_{29}	109.28	O_3-C_{12}-C_{11}	109.13
O_2- H_{26}	0.96	C_9-N_6–C_{13}	119.61	O_3-C_{12}-H_{24}	110.80
O_3- C_{12}	1.43	C_9-N_6–C_{14}	118.48	O_3-C_{12}-H_{25}	110.78
O_3- H_{29}	0.96	C_{13}-N_6–C_{14}	121.88	C_{11}-C_{12}-H_{24}	108.79
O_4- C_{14}	1.21	C_{14}-N_7–C_{16}	129.18	C_{11}-C_{12}-H_{25}	108.56
O_5- C_{16}	1.21	C_{14}-N_7–H_{28}	115.04	H_{24}-C_{12}-H_{25}	108.73
N_6-C_9	1.47	C_{16}-N_7–H_{28}	115.77	N_6–C_{13}-C_{15}	123.69
N_6–C_{13}	1.37	O_2-C_8-C_{10}	112.80	N_6–C_{13}- H_{27}	114.77
N_6–C_{14}	1.40	O_2-C_8-C_{11}	106.54	C_{15}–C_{13}-H_{27}	121.54
N_7–C_{14}	1.38	O_2-C_8-C_{19}	110.49	O_4- C_{14}–C_{16}	123.58
N_7–C_{16}	1.41	C_{10}-C_8-C_{11}	103.01	O_4- C_{14}- N_7	122.95
N_7–H_{28}	1.01	C_{10}-C_8-C_{19}	112.09	N_6- C_{14}- N_7	113.47
C_8-C_{10}	1.53	C_{11}-C_8-C_{19}	111.57	C_{13}-C_{15}–C_{16}	118.83
C_8-C_{11}	1.54	O_1- C_9- N_6	108.27	C_{13}-C_{15}–C_{17}	121.66
C_8-C_{19}	1.09	O_1- C_9- C_{10}	105.75	C_{16}-C_{15}–C_{17}	119.51
C_9-C_{10}	1.53	O_1- C_9- H_{20}	110.66	O_5- C_{16}- N_7	120.39
C_9-H_{20}	1.09	N_6–C_9-C_{10}	114.46	O_5- C_{16}-C_{15}	126.67
C_{10}-H_{21}	1.09	N_6–C_9-H_{20}	105.70	N_7- C_{16}-C_{15}	112.95
C_{10}-H_{21}	1.09	C_{10}-C_9-H_{20}	111.99		
C_{11}-C_{12}	1.52	C_8-C_{10}- C_9	102.78		
C_{11}-H_{23}	1.09	C_8-C_{10}-H_{21}	109.50		
C_{12}-H_{24}	1.10	C_8-C_{10}-H_{22}	113.06		
C_{12}-H_{25}	1.10	C_9-C_{10}-H_{21}	110.34		
C_{13}-C_{15}	1.36	C_9-C_{10}-H_{22}	112.14		
C_{13}- H_{27}	1.08	H_{21}-C_{10}-H_{22}	108.90		
C_{15}-C_{16}	1.47	O_1-C_{11}-C_8	106.55		
C_{15}-C_{17}	1.42	O_1-C_{11}-C_{12}	109.64		
C_{17}-C_{18}	1.20	O_1-C_{11}-H_{23}	108.44		
C_{18}-H_{30}	1.06	C_8-C_{11}-C_{12}	115.08		

Frontier Molecular Orbitals

The concepts of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) are fundamental in understanding the electronic properties and reactivity of molecules. The energy difference between these two orbitals, known as the HOMO-LUMO gap (E_{gap} = E_{LUMO} - E_{HOMO}), is a critical parameter that influences various chemical and physical properties of materials, particularly in organic electronics and photochemistry. The electronic characteristics of a compound can be quantitatively gauged by the energies associated with its HOMO and LUMO. An increase in the HOMO energy correlates with enhanced electron-donating capabilities of the molecule, making it more reactive in donating electrons (Honório & Da Silva, 2003). Conversely, a lower LUMO energy indicates a greater ability to accept electrons, which is essential in understanding the redox behavior of the compound (Ahmad, 2020; Honório & Da Silva, 2003). The interplay between the HOMO and LUMO energies can also affect the molecule's response to external stimuli, such as light, influencing properties like absorption spectra and photoconductivity (Liu et al., 2020; Welch & Bazan, 2011).

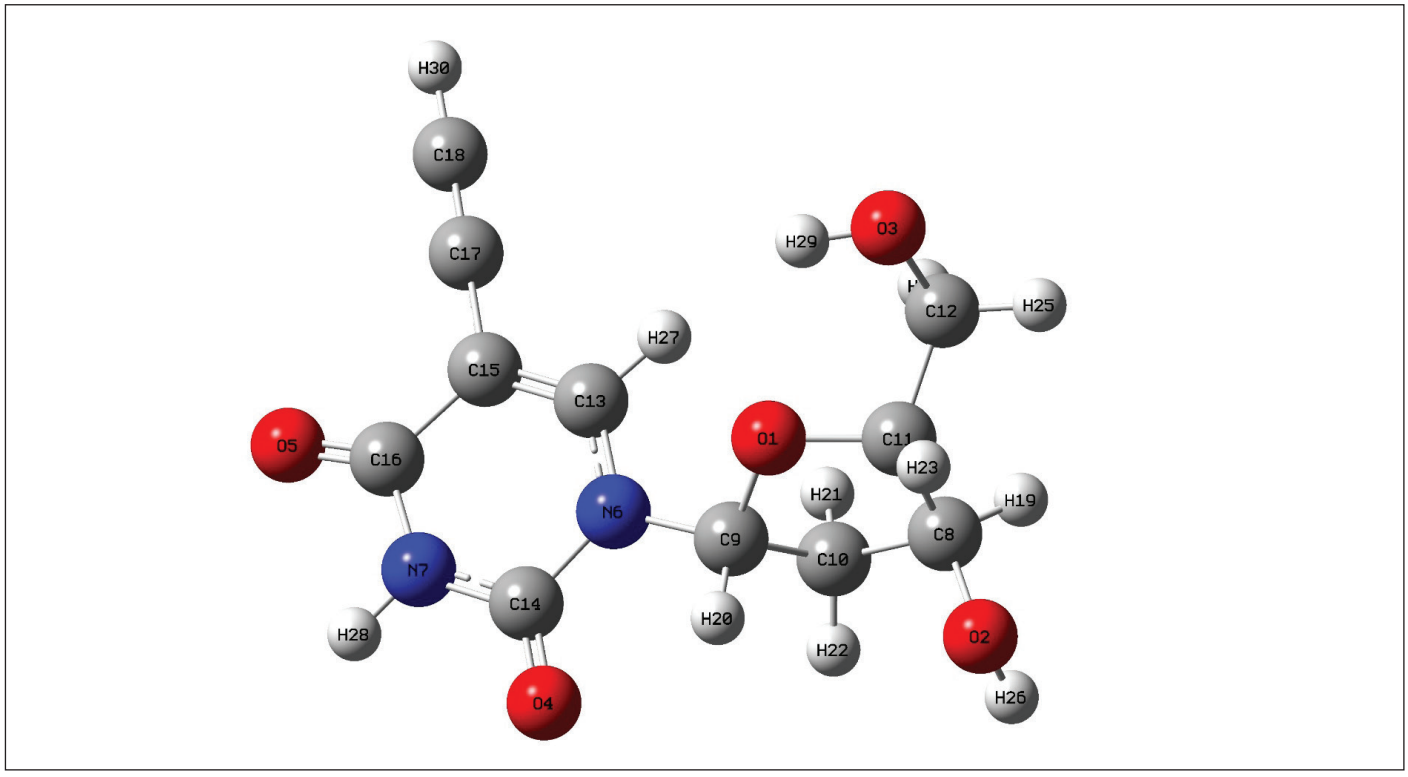

Figure 1. Optimized structure of EdU molecule.

Table 2. The Energy Band Gaps and Quantum Chemical Properties of the EDU Molecule

		(eV)		Eg (eV)	(I)	(A)	(η)	(χ)	(μ_chem)	(σ)	(ω)
1	**H**	-6.59	ΔE_{H-L}	4.84	6.59	1.75	2.42	4.17	-4.17	0.21	3.60
2	**L**	-1.75									
3	$\mathbf{H_{-1}}$	-7.54	$\Delta E_{H\_1-L\_1}$	7.12	7.54	0.42	3.56	3.98	-3.98	0.14	2.23
4	$\mathbf{L_{+1}}$	-0.42									
5	$\mathbf{H_{-2}}$	-7.71	$\Delta E_{H\_2-L\_2}$	7.97	7.71	-0.26	3.99	3.72	-3.72	0.13	1.74
6	$\mathbf{L_{+2}}$	0.26									

The HOMO–LUMO energy gap is an important indicator of molecular chemical reactivity. A small energy gap implies higher chemical reactivity of the molecule (Mary et al., 2019). For the EdU molecule, the calculated band gap energy is approximately 4.84 eV. The HOMO and LUMO molecular orbitals of the structure are presented in Figure 2. In the orbital representations, green regions indicate areas with a higher potential for electron donation, while red regions represent areas with a greater tendency to accept electrons. This energy gap suggests that EdU possesses moderate chemical stability and may exhibit reactive behavior under certain conditions. Furthermore, since this value is lower than that of the urea molecule (ΔE_urea = 6.71 eV), EdU can be considered more chemically reactive in comparison (Soliman et al., 2015).

The calculated orbital energies and selected quantum chemical descriptors of the EdU molecule are summarized in Table 2. Key parameters such as ionization potential (I), electron affinity (A), global hardness (η), chemical potential (μ), softness (σ), and global electrophilicity index (ω) were derived from the HOMO and LUMO energy values using the equations presented in Equation 1 (Parr et al., 1999):

$$I=-E_{HOMO},\quad A=-E_{LUMO},\quad \eta=(-E_{HOMO}+E_{LUMO})/2,\quad \mu=(E_{HOMO}+E_{LUMO})/2,\quad \omega=\mu^2/2\eta \qquad (1)$$

These descriptors are considered valuable for predicting pharmacological characteristics, including drug design efficiency and potential toxicity profiles (Tan et al., 2008).

Molecular Electrostatic Potential and Charge Analysis

Molecular electrostatic potential (MEP) maps serve as an effective tool for evaluating the reactivity profile of a molecule by visualizing the spatial distribution of electrostatic charges. In the case of the EdU molecule, the computed MEP map is

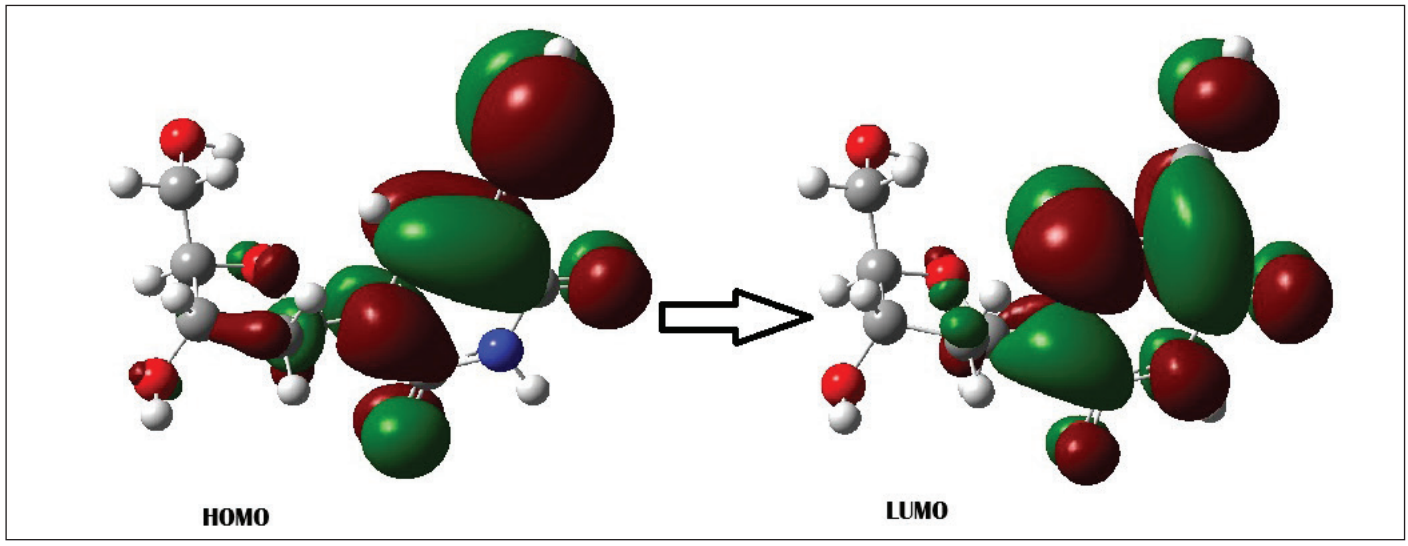

Figure 2. The distribution of HOMO and LUMO pattern of the EdU molecule.

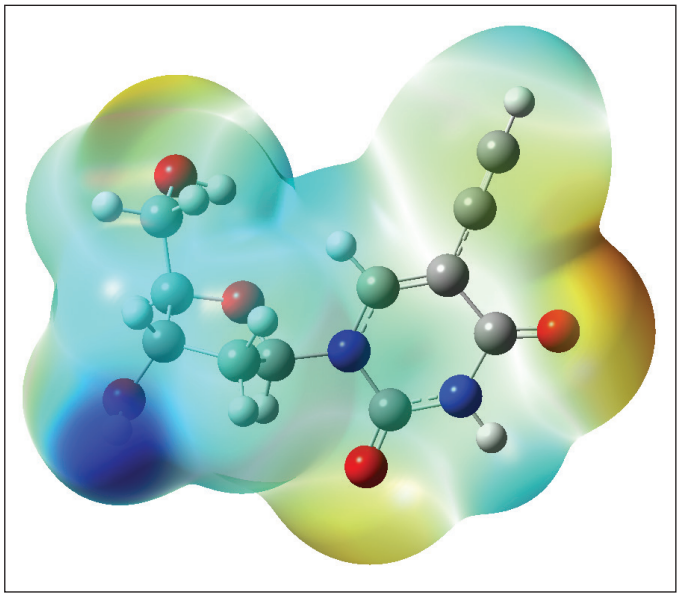

Figure 3. Molecular electrostatic potential (MEP) map of the EdU molecule. The map illustrates electron-rich regions (red to yellow), electron-deficient regions (blue), and neutral zones (green), providing visual insight into potential sites for electrophilic and nucleophilic interactions as well as hydrogen bonding.

presented in Figure 3. These maps provide insight into regions susceptible to electrophilic and nucleophilic interactions, as potential sites for hydrogen bonding. Areas of high electron density are typically represented in red, orange, or yellow, indicating regions prone to nucleophilic attack. Conversely, blue-shaded areas denote electron-deficient regions that are more favorable for electrophilic attack. Zones exhibiting near-neutral charge distributions appear green, reflecting relatively non-reactive sites within the molecular framework (Nakajima et al., 1996; Tasi & Pálinkó, 1995).

The MEP map of the EdU molecule reveals a distinct distribution of electrostatic potential across its structure. The blue region, observed primarily near the terminal acetylene group (close to the oxygen atom), indicates areas of low electron density, suggesting favorable sites for electrophilic attack**.** In contrast, red and yellow regions are located around electronegative atoms such as oxygen**,** particularly in the sugar and uracil moieties, which represent electron-rich sites prone to nucleophilic attack or hydrogen bonding interactions. The green zones illustrate relatively neutral electrostatic regions, reflecting lower reactivity. This electrostatic potential distribution supports the identification of chemically active sites in the EdU molecule, which is essential for understanding its interaction profile in biological systems.

To better understand the interaction profile of the EdU molecule, analyzing its electronic charge distribution is a crucial step. This analysis complements the molecular electrostatic potential (MEP) map by identifying how the distribution of atomic charges influences EdU's reactivity and binding behavior (Sridevi & Velraj, 2012). In the present study, Natural Bond Orbital (NBO) analysis, along with Hirshfeld and Mulliken charge calculations, was employed to examine the electronic structure of EdU in detail (Fedorov, 2019). These approaches provide different yet complementary views of how electrons are distributed throughout the molecule, helping to identify potential reactive or binding sites (Valle et al., 2007). Such charge-based evaluations are particularly important for molecular docking studies, as they directly impact the estimation of binding affinities and the identification of noncovalent interactions critical to drug design (Torres et al., 2019). In this context, scoring functions used in docking rely on accurate electronic characterization to predict the likelihood and strength of EdU's interactions with biological targets (Kumar & Kumar, 2019).

The calculated charge values are given in Figure 4. The graph illustrates variations in charge distribution across individual atoms, highlighting the consistency and differences between

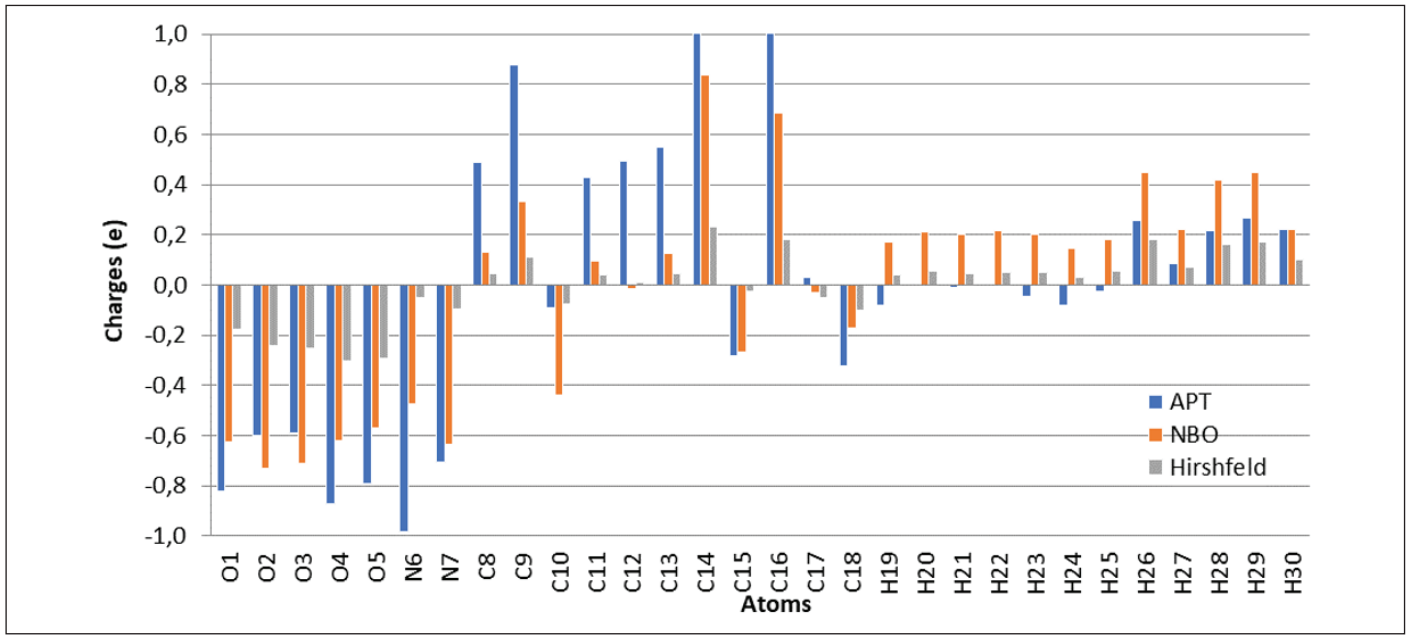

Figure 4. Charge distributions of EdU molecule.

methods. Oxygen and nitrogen atoms generally exhibit more negative charges, indicating regions of potential nucleophilic interaction.

Fukui Functions

Fukui function analysis is a powerful theoretical tool used to investigate the local reactivity of molecular structures. According to the Frontier Molecular Orbital Theory proposed by Fukui, the chemical reactivity of a molecule can be interpreted based on the electron density distributions of its frontier orbitals, namely the HOMO and LUMO. To quantitatively evaluate these reactive sites, Fukui functions were calculated using the finite difference (FD) approach, which involves computing the atomic charges for the neutral, anionic (N+1), and cationic (N–1) species of the EdU molecule at the same optimized geometry (Parr & Yang, 1984; Yang & Mortier, 1986).

The Fukui indices are defined as follows:

$f_k^+ = q_k(N+1) - q_k(N)$ (for nucleophilic attack),

$f_k^- = q_k(N) - q_k(N-1)$ (for electrophilic attack),

$f_k^0 = (1/2)[q_k(N+1) - q_k(N-1)]$ (for neutral attack)

Here, q_k represents the atomic charges on atom k in the specified charge state. The resulting values allow identification of atoms most susceptible to nucleophilic or electrophilic interactions. Additionally, the reactivity descriptors f^+/f^- and f^-/f^+ provide insight into whether a given site is more likely to participate in nucleophilic or electrophilic interactions, respectively (Bultinck et al., 2003; Roy et al., 1998).

In the case of the EdU molecule, the calculated Fukui indices and their ratios are summarized in Table 3. Atom C13 exhibited the highest electrophilic reactivity (f^-=0.15, f^-/f^+=2.44), suggesting a strong tendency to attract nucleophiles. Conversely, C17 was identified as the most nucleophilic site (f^+=0.07, f^+/f^-=2.52). Other atoms, such as O4, N6, and C18, also demonstrated notable dual-reactivity, indicating their importance in potential binding interactions or reaction mechanisms.

Some hydrogen atoms, such as H24 and H29, showed anomalously low or negative Fukui values, which is a common observation due to their limited role in frontier orbital contributions and often negligible involvement in electronic redistribution.

These results support the findings of molecular orbital and charge distribution analyses, confirming EdU's diverse reactive centers. The Fukui function analysis enhances our understanding of its interaction profile, particularly in the context of chemical and biological reactivity.

ELF and LOL Analysis

Electron Localization Function (ELF) and Localized Orbital Locator (LOL) analyses are valuable tools in exploring the electronic structure of molecules. These descriptors help visualize and quantify the distribution of electrons within a molecule, shedding light on the nature of chemical bonding. ELF highlights regions of localized electron pairs, often associated with covalent bonds or lone pairs, while LOL offers a complementary view by mapping areas influenced by localized orbitals (Fuster et al., 2000; Savin et al., 1996). Both analyses are grounded in quantum mechanical principles and typically integrated with density functional theory (DFT) calculations, allowing for detailed interpretation of electron pairing, bond character, and delocalization. Their combined use provides a deeper understanding of molecular stability and reactivity from an electronic perspective (Zhitenev et al., 2000).

The ELF map (Figure 5) indicates well-defined regions of high electron localization, particularly around the heteroatoms and within bonding regions, highlighting strong covalent interac-

Table 3. The Values of Fukui Function Ratios of the Edu Molecule

Label	f+	f-	f0	f+/f-	f-/f+
O_1	0.01	0.00	0.00	-6.80	-0.15
O_2	0.03	0.01	0.02	2.04	0.49
O_3	0.03	0.01	0.02	2.56	0.39
O_4	0.09	0.07	0.08	1.25	0.80
O_5	0.08	0.09	0.09	0.82	1.22
N_6	0.07	0.03	0.05	2.20	0.45
N_7	0.02	0.03	0.02	0.49	2.03
C_8	0.01	0.01	0.01	1.53	0.65
C_9	0.01	0.01	0.01	1.07	0.94
C_{10}	0.01	0.01	0.01	1.72	0.58
C_{11}	0.01	0.01	0.01	1.30	0.77
C_{12}	0.01	0.00	0.01	2.03	0.49
C_{13}	0.06	0.15	0.11	0.41	2.44
C_{14}	0.03	0.03	0.03	0.84	1.19
C_{15}	0.08	0.08	0.08	0.98	1.02
C_{16}	0.02	0.06	0.04	0.39	2.59
C_{17}	0.07	0.03	0.05	2.52	0.40
C_{18}	0.16	0.13	0.15	1.27	0.79
H_{19}	0.02	0.01	0.02	1.23	0.82
H_{20}	0.02	0.02	0.02	0.97	1.03
H_{21}	0.01	0.01	0.01	0.73	1.36
H_{22}	0.01	0.01	0.01	1.08	0.92
H_{23}	0.01	0.01	0.01	1.02	0.98
H_{24}	0.01	0.00	0.00	-9.03	-0.11
H_{25}	0.02	0.01	0.01	1.04	0.96
H_{26}	0.02	0.01	0.01	1.13	0.88
H_{27}	0.03	0.06	0.04	0.46	2.18
H_{28}	0.02	0.03	0.03	0.74	1.36
H_{29}	0.00	0.00	0.00	-2.84	-0.35
H_{30}	0.05	0.05	0.05	1.03	0.97

tions and electron pairing. The three-dimensional ELF plot further supports this, with pronounced peaks corresponding to localized electron density near atoms participating in bonding. Meanwhile, the LOL distribution (Figure 6) confirms these observations by illustrating a consistent pattern of localized orbitals along the molecular framework, particularly in π-bonding regions and lone pair domains. The color gradients in both maps reflect the gradation of electron localization, with red regions signifying high localization (indicative of bonding or lone

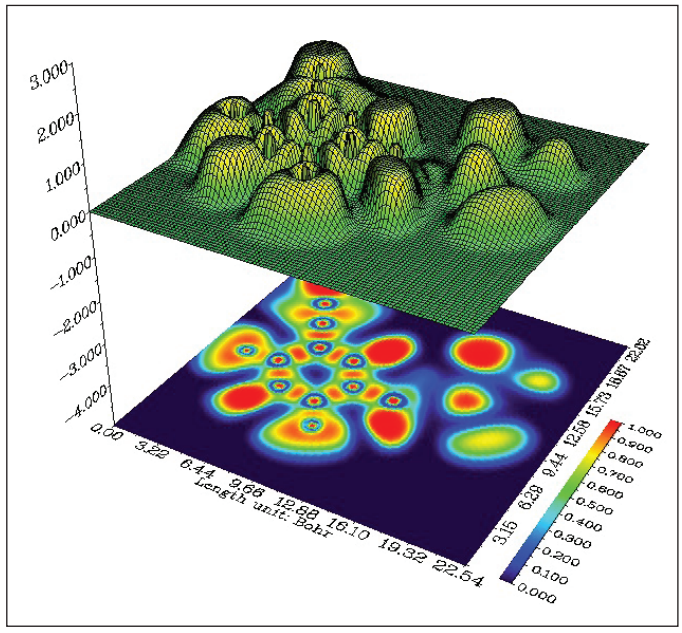

Figure 5. EdU molecule's relief color filled maps of the ELF analysis.

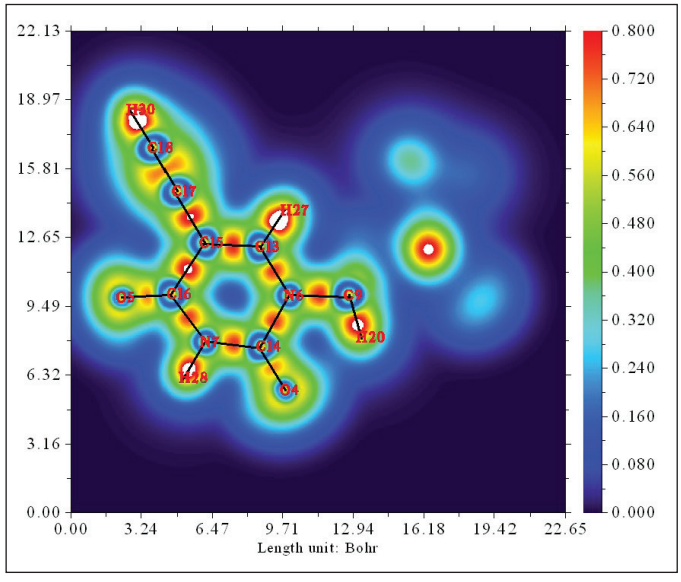

Figure 6. EdU molecule's relief color filled maps of the LOL analysis.

pairs), and blue to green regions marking delocalized electron density. Collectively, these analyses underscore the presence of stable bonding interactions and provide a quantitative and visual understanding of EdU's electronic environment.

Molecular Docking Studies

Molecular docking is one of the most widely applied structure-based computational techniques used to predict the preferred orientation and interaction of small molecules within the binding site of a target protein (Kitchen et al., 2004). Benefiting from advancements in structural biology and the increasing availability of crystallographic data, docking has become a fundamental tool for understanding molecular recognition

and guiding drug discovery processes (Sliwoski et al., 2014). In this study, molecular docking simulations were conducted to investigate the binding behavior of the EdU molecule with three biologically relevant protein targets whose crystal structures are available in the Protein Data Bank (PDB) human pancreatic α-amylase (PDB ID: 1B2Y), and α-glucosidase (PDB ID: 5ZCC). These targets were selected based on their roles in DNA replication and repair mechanisms, which are directly related to the pharmacological activity of nucleoside analogs like EdU. The docking studies aimed to predict the binding affinities and interaction profiles of EdU with each protein, providing insight into its potential inhibitory or modulatory effects at the molecular level. The resulting protein-ligand complexes were further analyzed in terms of binding conformation and key interacting residues, helping to elucidate the structural basis of EdU's biological activity. These findings serve as a valuable foundation for future experimental validations and structure-based drug design efforts targeting nucleotide-processing enzymes (Kitchen et al. 2004; Meng et al. 2011; Ring et al. 1993) we have investigated the use of computer model-built structures for the identification of previously unknown inhibitors of enzymes from two major protease families, serine and cysteine proteases. We have successfully used our model-built structures to identify computationally and to confirm experimentally the activity of nonpeptidic inhibitors directed against important enzymes in the schistosome [2-(4-methoxybenzoyl).

Molecular Docking Analysis of EdU with α-Amylase (PDB ID: 1B2Y)

To explore the potential binding interactions of Edu with the human pancreatic α-amylase enzyme, a molecular docking study was conducted using the crystal structure of α-amylase (PDB ID: 1B2Y). The docking results revealed a stable binding conformation of Edu within the enzyme's active site pocket, where the ligand formed several key interactions critical for molecular recognition and stability.

Three-dimensional visualization of the docking pose showed that Edu is embedded in a well-defined binding cavity surrounded by several amino acid residues capable of forming hydrogen bonds and hydrophobic interactions. As illustrated in the interaction diagram, Edu established conventional hydrogen bonds primarily with GLN A:63 and ASP A:300, which are known to contribute significantly to ligand anchoring. Additional interactions, including π-alkyl contacts with residues such as TRP A:59 and TYR A:62, suggest a complementary hydrophobic environment that enhances the ligand's binding affinity.

Surface representation analysis further demonstrated the distribution of hydrogen bond donor and acceptor sites (indicated in magenta and green, respectively) around the ligand-binding interface, supporting the specificity and directionality of the interaction. Notably, the docking pose exhibited a high degree of geometric and electronic complementarity, indicating a potentially favorable binding free energy.

These preliminary findings support the hypothesis that Edu may serve as a plausible inhibitor or modulator of α-amylase activity. Future studies including binding energy calculations and molecular dynamics simulations are warranted to validate the stability and efficacy of this interaction.

Molecular Docking Analysis of EdU with α-Glucosidase (PDB ID: 5ZCC)

In this targeted molecular docking study, the interaction between EdU and α-glucosidase (PDB ID: 5ZCC) was evaluated. The docking procedure was performed specifically at the enzyme's active site, yielding a binding affinity of –6.8 kcal/mol, which indicates a moderate binding strength between EdU and the target protein.

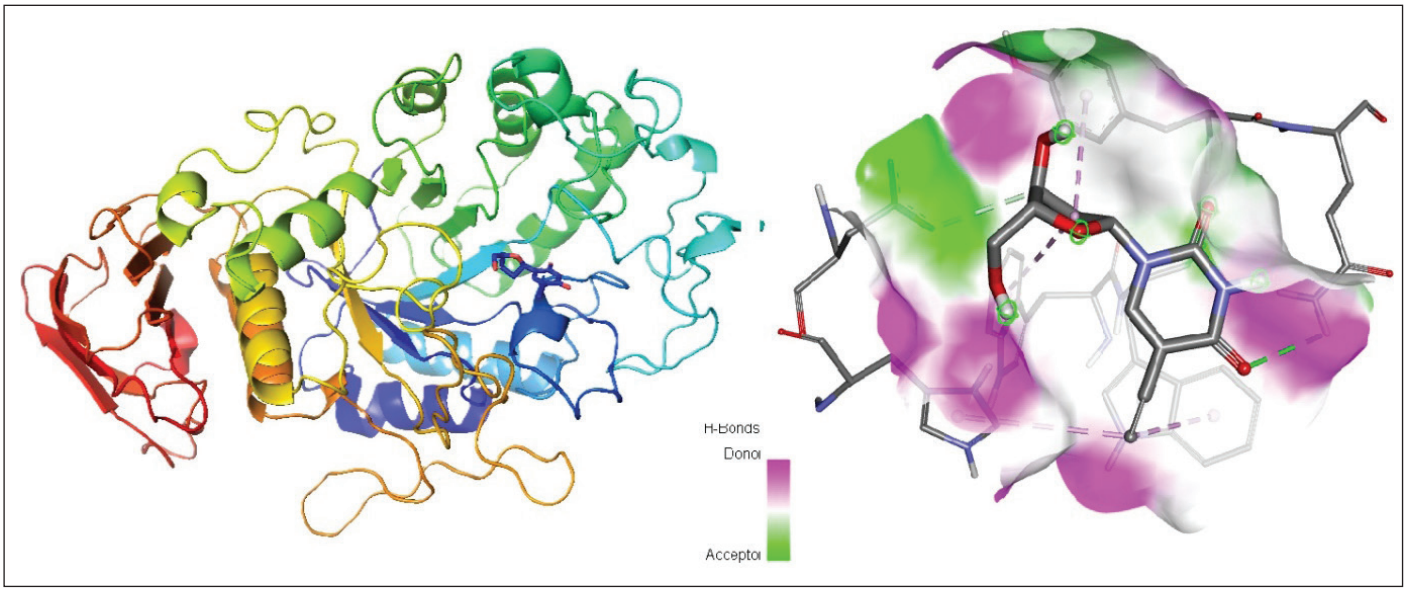

Figure 7. Three-dimensional representation of the binding pose of EdU within the active site of α-amylase (1B2Y) (left), and the hydrogen bond donor/acceptor distribution at the binding interface (right).

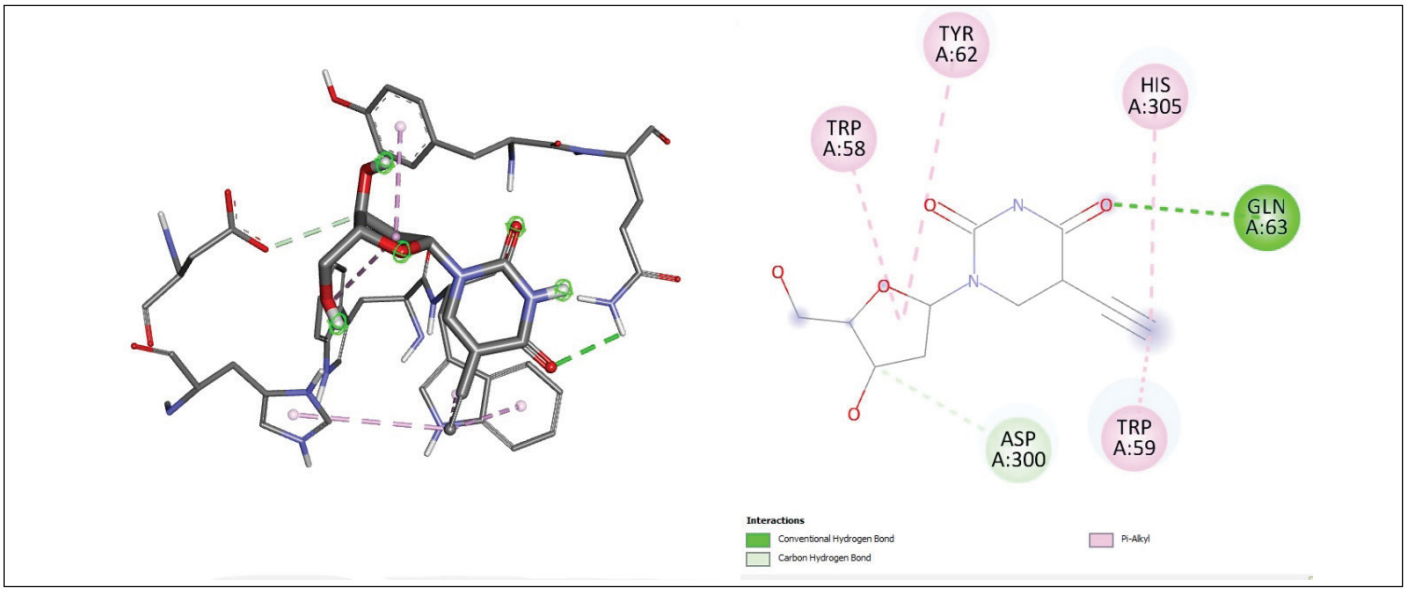

Figure 8. 2D interaction diagram showing the detailed interactions between EdU and surrounding residues of α-amylase' active site. Hydrogen bonds, π-alkyl, and hydrophobic contacts are labeled for clarity.

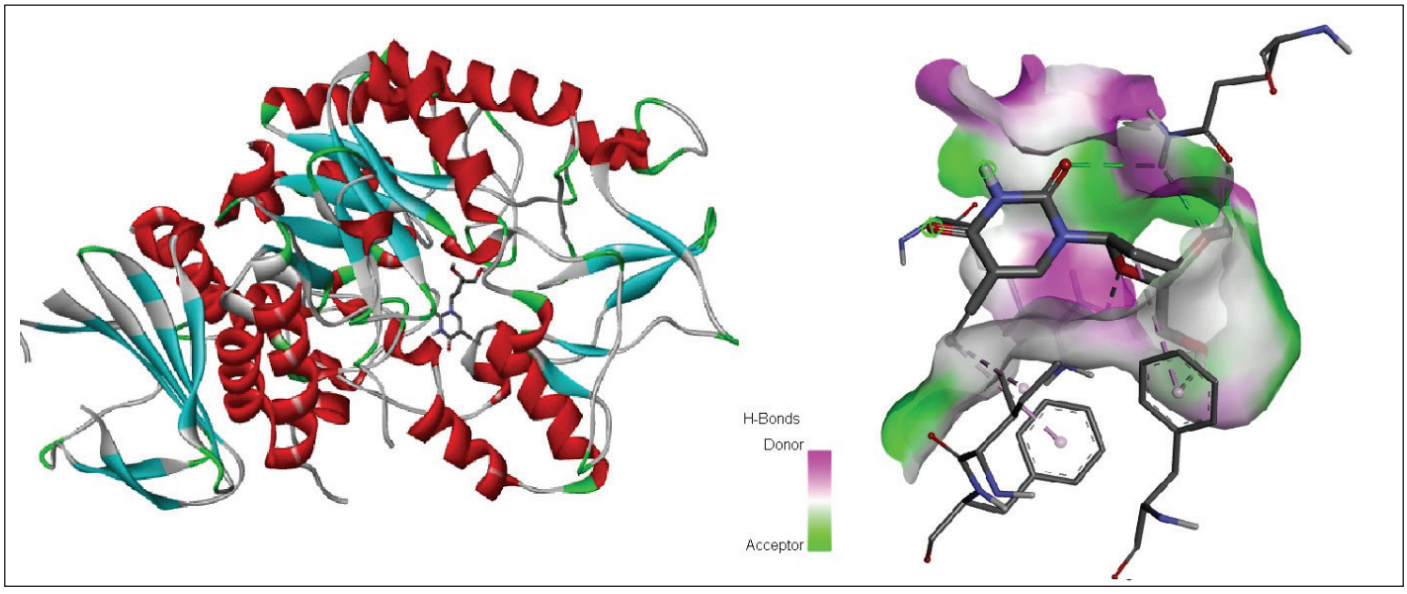

Figure 9. Three-dimensional representation of the binding pose of EdU within the active site of α-glucosidase (5ZCC) (left), and the hydrogen bond donor/acceptor distribution at the binding interface (right).

The molecular interaction analysis revealed several key non-covalent interactions contributing to ligand binding and stabilization:

- Conventional hydrogen bonds were observed with ARG A:411 and GLN A:256, which are likely essential for anchoring the ligand in the active site.
- A combination of **π-donor hydrogen bonding** and **π-alkyl interactions** was detected with PHE A:163, highlighting the involvement of aromatic residues in π-system stabilization.
- Additional alkyl interactions with ILE A:143 and PHE A:144 support hydrophobic stabilization within the binding cleft.

These interactions suggest that EdU has a favorable binding mode within the active site of alpha-glucosidase and may have the potential to affect the enzymatic activity or serve as a structural scaffold for further drug design efforts targeting glycosidase pathways.

ADMETox Study

In early-stage drug discovery, computational approaches are frequently employed to assess the pharmacological poten-

tial of candidate molecules before experimental validation. In this context, 5-Ethynyl-2'-deoxyuridine (Edu) was subjected to in silico evaluations to predict its pharmacokinetic behavior and safety profile. Key parameters were analyzed, including absorption, distribution, metabolism, excretion (ADME), drug-likeness, organ-specific toxicity, and the estimated median lethal dose (LD_{50}). The assessments were performed using SwissADME (Daina et al., 2017; SwissADME, n.d.) and admetSAR (admetSAR, 2024), which are widely accepted platforms for evaluating the suitability of small molecules as potential therapeutic agents. These analyses provided preliminary insight into Edu's viability as a lead compound in pharmaceutical applications targeting the selected protein structures.

The pharmacokinetic properties of the EdU molecule were assessed using key ADME parameters and are presented in Table 4. Evaluation based on Lipinski's rule of five indicates favorable drug-likeness: the molecular weight is 252.22 g/mol, well below the 500 g/mol threshold (Manallack, 2007) the overall proportion of non-ionizable and ionizable compounds for drug-like substances is not well known. Even less well known is the overall distribution of acid and base pK (a; the Log P value is 1.37, indicating balanced hydrophilicity and lipophilicity; and the molecule features three hydrogen bond donors and five acceptors, complying comfortably with the respective upper limits of five and ten (Kujawski et al., 2012). Moreover, the total polar surface area (TPSA) is calculated to be 104.55 Å², remaining within the recommended range for efficient oral bioavailability.

The predicted gastrointestinal absorption is high, which supports the potential for effective oral delivery. The Caco-2 cell permeability was calculated at 0.7707. This value still suggests moderate membrane permeability and does not raise significant concerns regarding intestinal uptake (Marziano et al., 2019). Skin permeability, given by a log Kp value of −8.75 cm/h, indicates a low propensity for dermal absorption, aligning with safety expectations in cases of incidental skin contact (El-Kattan et al., 2000).

Table 4. Predicted ADMETox Properties of EdU Based on in Silico Analyses

ADME*	Parameter
MW (≤ 500 g/mol)	252.22
HBD (≤ 5)	3
HBA (≤ 10)	5
TPSA (≤ 140 Å²)	104.55
Log P (≤ 5)	1.37
GI absorption	High
Skin Permeability (log Kp, cm/hour)	-8.75
Toxicity class	4
Absorption**	**Probability**
BBB (C.brain/C.blood)	0.7408
Caco-2 permeability	0.7707
HIA (%)	0.7330
Toxicity**	**Parameter**
Rat Acute Toxicity (LD_{50}) (mol/kg)	2.0694
Fish Toxicity (pLC_{50}) (mg/L)	2.0276
T. pyriformis Toxicity ($pIGC_{50}$) (mg/L)	0.1085

*: SwissADME; **: admetSAR

The toxicity profile of EdU indicates a moderate level of acute oral toxicity, with a predicted LD_{50} value of 2.0694 mol/kg in rats. This suggests that while the compound is not highly toxic, cautious dose optimization would be required for therapeutic applications. The predicted fish toxicity (pLC_{50}: 2.0276 mg/L) highlights a potential risk to aquatic organisms, suggesting that environmental exposure should be carefully managed. In contrast, EdU exhibits relatively low toxicity against microbial eukaryotes, as indicated by the Tetrahymena pyriformis $pIGC_{50}$ value of 0.1085 µg/L (admetSAR, 2024). Overall, these results underscore the importance of evaluating EdU's safety profile across multiple biological systems to ensure its responsible application in biomedical and environmental contexts.

The molecule's classification under toxicity class 4 (SwissADME, n.d.) suggests it has a relatively low acute toxicity profile. Taken together, these data indicate that EdU exhibits physicochemical and pharmacokinetic properties consistent with orally bioavailable, drug-like molecules.

CONCLUSION and SUGGESTIONS

This study presents a multidisciplinary computational evaluation of 5-Ethynyl-2'-deoxyuridine (EdU), integrating molecular docking, ADMET profiling, electron localization analysis, and drug-likeness assessments to investigate its pharmacological potential and structural properties. The molecular docking results indicated that EdU exhibits stable and specific interactions within the binding pocket of the target protein, primarily through hydrogen bonds and hydrophobic interactions, suggesting potential biological activity.

Pharmacokinetic analyses revealed that EdU complies with Lipinski's rule of five, with a molecular weight of 252.22 g/mol, LogP value of 1.37, and favorable hydrogen bond donor and acceptor counts. The compound showed high gastrointestinal absorption, acceptable Caco-2 permeability, and moderate skin permeability (log Kp = -8.75 cm/h), indicating promising oral bioavailability and low risk of dermal exposure.

Toxicity predictions placed EdU in class IV (according to the OECD classification), with a rat acute toxicity LD50 of 2.0694 mol/kg, and moderate aquatic toxicity as reflected by fish (pLC50 = 2.0276) and Tetrahymena pyriformis (pIGC50 = 0.1085) models. These values suggest a manageable toxicity profile under controlled dosages.

Electron localization function (ELF) and localized orbital locator (LOL) analyses provided a deeper understanding of EdU's bonding nature, highlighting localized electron density regions associated with strong covalent bonding, especially around heteroatoms and aromatic systems. These findings support the compound's electronic stability and contribute to the interpretation of its reactivity patterns.

Altogether, this computational approach emphasizes EdU's potential as a bioactive molecule, demonstrating both pharmacokinetic compatibility and electronic stability. The integrative strategy applied here not only validates EdU's theoretical drug-likeness but also sets a foundation for future experimental investigations and rational drug design efforts involving nucleoside analogues.

Acknowledgements: I would like to express my sincere gratitude to my Ph.D. advisor, Prof. Dr. Şenay Yurdakul, for her valuable scientific guidance and support throughout this study. I also thank Dr. Ceyhun Küçük for his collaboration and assistance during the work.

The Authors Contributions: All stages of this work belong to the author.

Conflict of Interest: There is no conflict of interest.

Refereeing Process: Double-blind refereeing.

REFERENCES

admetSAR. (2024). *A free tool for evaluating ADME and toxicity properties of chemicals*. http://lmmd.ecust.edu.cn/admetsar2/

Ahmad, H. O. (2020). Computational study of optical properties, and enantioselective synthesis of di-substituted esters of hydantoic and thiohydantoic acids. *Zanco Journal Of Pure And Applied Sciences*, *32*(1). https://doi.org/10.21271/ZJPAS.32.1.9

Becke, A. D. (1993). Density-functional thermochemistry. III. The role of exact exchange. *The Journal of Chemical Physics*, *98*(7), 5648–5652. https://doi.org/10.1063/1.464913

Berman, H. M. (2000). The Protein Data Bank. *Nucleic Acids Research*, *28*(1), 235–242. https://doi.org/10.1093/nar/28.1.235

BIOVIA, Discovery Studio Visualizer Software (Version 4.0.). (2021). Dassault Systèmes.

Bradford, J. A., & Clarke, S. T. (2011). Dual-Pulse Labeling Using 5-Ethynyl-2′-Deoxyuridine (EdU) and 5-Bromo-2′-Deoxyuridine (BrdU) in Flow Cytometry. *Current Protocols in Cytometry*, *55*(1). https://doi.org/10.1002/0471142956.cy0738s55

Buck, S. B., Bradford, J., Gee, K. R., Agnew, B. J., Clarke, S. T., & Salic, A. (2008). Detection of S-phase cell cycle progression using 5-ethynyl-2′-deoxyuridine incorporation with click chemistry, an alternative to using 5-bromo-2′-deoxyuridine antibodies. *BioTechniques*, *44*(7), 927–929. https://doi.org/10.2144/000112812

Bultinck, P., Carbó-Dorca, R., & Langenaeker, W. (2003). Negative Fukui functions: New insights based on electronegativity equalization. *Journal of Chemical Physics*, *118*(10), 4349–4356. https://doi.org/10.1063/1.1542875

Cavanagh, B. L., Walker, T., Norazit, A., & Meedeniya, A. C. B. (2011). Thymidine Analogues for Tracking DNA Synthesis. *Molecules*, *16*(9), 7980–7993. https://doi.org/10.3390/molecules16097980

Cieślar-Pobuda, A., & Łos, M. J. (2013). Prospects and limitations of "Click-Chemistry"-based DNA labeling technique employing 5-ethynyl-2′deoxyuridine (EdU). *Cytometry Part A*, *83*(11), 977–978. https://doi.org/10.1002/cyto.a.22394

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, *7*(1), 42717. https://doi.org/10.1038/srep42717

DeLano, W. L. (n.d.). *The PyMOL Molecular Graphics System* (Version 3.0). Schrödinger, LLC.

Dennington, R., Keith, T., & Millam, J. (2009). *GaussView Version 5* (Version 4.1.2.). Semichem Inc.

Dvořáčková, M., & Fajkus, J. (2018). Visualization of the Nucleolus Using Ethynyl Uridine. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.00177

El-Kattan, A., Asbill, C. S., & Haidar, S. (2000). Transdermal testing: practical aspects and methods. *Pharmaceutical Science & Technology Today*, *3*(12), 426–430. https://doi.org/10.1016/S1461-5347(00)00316-3

Fedorov, D. G. (2019). Solvent Screening in Zwitterions Analyzed with the Fragment Molecular Orbital Method. *Journal of Chemical Theory and Computation*, *15*(10), 5404–5416. https://doi.org/10.1021/acs.jctc.9b00715

Frisch, M.J., et. al. (2009). *Gaussian 09*. Gaussian Inc.

Fuster, F., Sevin, A., & Silvi, B. (2000). Topological Analysis of the Electron Localization Function (ELF) Applied to the Electrophilic Aromatic Substitution. *The Journal of Physical Chemistry A*, *104*(4), 852–858. https://doi.org/10.1021/jp992783k

Honório, K. M., & Da Silva, A. B. F. (2003). An AM1 study on the electron-donating and electron-accepting character of biomolecules. *International Journal of Quantum Chemistry*, *95*(2), 126–132. https://doi.org/10.1002/qua.10661

Hua, H., & Kearsey, S. E. (2011). Monitoring DNA replication in fission yeast by incorporation of 5-ethynyl-2′-deoxyuridine. *Nucleic Acids Research*, *39*(9), e60–e60. https://doi.org/10.1093/nar/gkr063

Kitchen, D. B., Decornez, H., Furr, J. R., & Bajorath, J. (2004). Docking and scoring in virtual screening for drug discovery: methods and applications. *Nature Reviews Drug Discovery*, *3*(11), 935–949. https://doi.org/10.1038/nrd1549

Kohlmeier, F., Maya-Mendoza, A., & Jackson, D. A. (2013). EdU induces DNA damage response and cell death in mESC in culture. *Chromosome Research*, *21*(1), 87–100. https://doi.org/10.1007/s10577-013-9340-5

Kujawski, J., Popielarska, H., Myka, A., Drabińska, B., & Bernard, M. (2012). The log P Parameter as a Molecular Descriptor in the Computer-aided Drug Design – an Overview. *Computational Methods in Science and Technology*, *18*(2), 81–88. https://doi.org/10.12921/cmst.2012.18.02.81-88

Kumar, S., & Kumar, S. (2019). Molecular Docking: A Structure-Based Approach for Drug Repurposing. In *In Silico Drug Design* (pp. 161–189). Elsevier. https://doi.org/10.1016/B978-0-12-816125-8.00006-7

Liu, F., Liu, J., & Wang, L. (2020). Panchromatic Organoboron Molecules with Tunable Absorption Spectra. *Chemistry – An Asian Journal*, *15*(20), 3314–3320. https://doi.org/10.1002/asia.202000958

Lu, T., & Chen, F. (2012). Multiwfn: A multifunctional wavefunction analyzer. *Journal of Computational Chemistry*, *33*(5), 580–592. https://doi.org/10.1002/jcc.22885

Manallack, D. T. (2007). The pK(a) Distribution of Drugs: Application to Drug Discovery. *Perspectives in Medicinal Chemistry*, *1*, 25–38. http://www.ncbi.nlm.nih.gov/pubmed/19812734

Mary, Y. S., Mary, Y. S., Resmi, K. S., & Thomas, R. (2019). DFT and molecular docking investigations of oxicam derivatives. *Heliyon*, *5*(7), e02175. https://doi.org/10.1016/j.heliyon.2019.e02175

Marziano, M., Tonello, S., Cantù, E., Abate, G., Vezzoli, M., Rungratanawanich, W., Serpelloni, M., Lopomo, N. F., Memo, M., Sardini, E., & Uberti, D. (2019). Monitoring Caco-2 to enterocyte-like cells differentiation by means of electric impedance analysis on printed sensors. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1863*(5), 893–902. https://doi.org/10.1016/j.bbagen.2019.02.008

Meng, X.-Y., Zhang, H.-X., Mezei, M., & Cui, M. (2011). Molecular Docking: A Powerful Approach for Structure-Based Drug Discovery. *Current Computer Aided-Drug Design*, *7*(2), 146–157. https://doi.org/10.2174/157340911795677602

Nahoum, V., Roux, G., Anton, V., Rougé, P., Puigserver, A., Bischoff, H., Henrissat, B., & Payan, F. (2000). Crystal structures of human pancreatic α-amylase in complex with carbohydrate and proteinaceous inhibitors. *Biochemical Journal*, *346*(1), 201–208. https://doi.org/10.1042/bj3460201

Nakajima, H., Takahashi, O., & Kikuchi, O. (1996). Rapid evaluation of molecular electrostatic potential maps for amino acids, peptides, and proteins by empirical functions. *Journal of Computational Chemistry*, *17*(7), 790–805. https://doi.org/10.1002/(SICI)1096-987X(199605)17:7<790::AID-JCC4>3.0.CO;2-N

Parr, R. G., & Yang, W. (1984). Density functional approach to the frontier-electron theory of chemical reactivity. *Journal of the American Chemical Society*, *106*(14), 4049–4050. https://doi.org/10.1021/ja00326a036

Rieder, U., & Luedtke, N. W. (2014). Alkene-Tetrazine Ligation for Imaging Cellular DNA. *Angewandte Chemie International Edition*, *53*(35), 9168–9172. https://doi.org/10.1002/anie.201403580

Ring, C. S., Sun, E., McKerrow, J. H., Lee, G. K., Rosenthal, P. J., Kuntz, I. D., & Cohen, F. E. (1993). Structure-based inhibitor design by using protein models for the development of antiparasitic agents. *Proceedings of the National Academy of Sciences*, *90*(8), 3583–3587. https://doi.org/10.1073/pnas.90.8.3583

Roig-Zamboni, V., Cobucci-Ponzano, B., Iacono, R., Ferrara, M. C., Germany, S., Bourne, Y., Parenti, G., Moracci, M., & Sulzenbacher, G. (2017). Structure of human lysosomal acid α-glucosidase–a guide for the treatment of Pompe disease. *Nature Communications*, *8*(1), 1111. https://doi.org/10.1038/s41467-017-01263-3

Roy, R. K., Krishnamurti, S., Geerlings, P., & Pal, S. (1998). Local Softness and Hardness Based Reactivity Descriptors for Predicting Intra- and Intermolecular Reactivity Sequences: Carbonyl Compounds. *The Journal of Physical Chemistry A*, *102*(21), 3746–3755. https://doi.org/10.1021/jp973450v

Sakaue-Sawano, A., Kurokawa, H., Morimura, T., Hanyu, A., Hama, H., Osawa, H., Kashiwagi, S., Fukami, K., Miyata, T., Miyoshi, H., Imamura, T., Ogawa, M., Masai, H., & Miyawaki, A. (2008). Visualizing Spatiotemporal Dynamics of Multicellular Cell-Cycle Progression. *Cell*, *132*(3), 487–498. https://doi.org/10.1016/j.cell.2007.12.033

Savin, A., Silvi, B., & Colonna, F. (1996). Topological analysis of the electron localization function applied to delocalized bonds. *Canadian Journal of Chemistry*, *74*(6), 1088–1096. https://doi.org/10.1139/v96-122

Sendovski, M., Kanteev, M., Ben-Yosef, V. S., Adir, N., & Fishman, A. (2011). First Structures of an Active Bacterial Tyrosinase Reveal Copper Plasticity. *Journal of Molecular Biology*, *405*(1), 227–237. https://doi.org/10.1016/j.jmb.2010.10.048

Seo, S., Onizuka, K., Nishioka, C., Takahashi, E., Tsuneda, S., Abe, H., & Ito, Y. (2015). Phosphorylated 5-ethynyl-2′-deoxyuridine for advanced DNA labeling. *Organic & Biomolecular Chemistry*, *13*(15), 4589–4595. https://doi.org/10.1039/C5OB00199D

Sliwoski, G., Kothiwale, S., Meiler, J., & Lowe, E. W. (2014). Computational Methods in Drug Discovery. *Pharmacological Reviews*, *66*(1), 334–395. https://doi.org/10.1124/pr.112.007336

Soliman, S. M., Abu-Youssef, M. A. M., Kassem, T. S., & Assem, R. (2015). Synthesis of two new silver(I) complexes with 3-bromoquinoline: Molecular structure, spectroscopic characterizations and DFT studies. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, *149*, 352–362. https://doi.org/10.1016/j.saa.2015.04.078

Sridevi, C., & Velraj, G. (2012). Investigation of molecular structure, vibrational, electronic, NMR and NBO analysis of 5-chloro-1-methyl-4-nitro-1H-imidazole (CMNI) using ab initio HF and DFT calculations. *Journal of Molecular Structure*, *1019*, 50–60. https://doi.org/10.1016/j.molstruc.2012.03.040

SwissADME. (n.d.). *A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness*. Retrieved May 7, 2025, from http://www.swissadme.ch

Tasi, G., & Pálinkó, I. (1995). *Using molecular electrostatic potential maps for similarity studies* (pp. 45–71). https://doi.org/10.1007/3-540-58672-5_23

Torres, P. H. M., Sodero, A. C. R., Jofily, P., & Silva-Jr, F. P. (2019). Key Topics in Molecular Docking for Drug Design. *International Journal of Molecular Sciences*, *20*(18), 4574. https://doi.org/10.3390/ijms20184574

Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, *31*(2), 455–461. https://doi.org/10.1002/jcc.21334

Valle, F., Sandal, M., & Samorì, B. (2007). The interplay between chemistry and mechanics in the transduction of a mechanical signal into a biochemical function. *Physics of Life Reviews*, *4*(3), 157–188. https://doi.org/10.1016/j.plrev.2007.06.001

Welch, G. C., & Bazan, G. C. (2011). Lewis Acid Adducts of Narrow Band Gap Conjugated Polymers. *Journal of the American Chemical Society*, *133*(12), 4632–4644. https://doi.org/10.1021/ja110968m

Yang, W., & Mortier, W. J. (1986). The use of global and local molecular parameters for the analysis of the gas-phase basicity of amines. *Journal of the American Chemical Society*, *108*(19), 5708–5711. https://doi.org/10.1021/ja00279a008

Zhao, H., Halicka, H. D., Li, J., Biela, E., Berniak, K., Dobrucki, J., & Darzynkiewicz, Z. (2013). DNA damage signaling, impairment of cell cycle progression, and apoptosis triggered by 5-ethynyl-2′-deoxyuridine incorporated into DNA. *Cytometry Part A*, *83*(11), 979–988. https://doi.org/10.1002/cyto.a.22396

Zhitenev, N. B., Fulton, T. A., Yacoby, A., Hess, H. F., Pfeiffer, L. N., & West, K. W. (2000). Imaging of localized electronic states in the quantum Hall regime. *Nature*, *404*(6777), 473–476. https://doi.org/10.1038/35006591