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Green Synthesis and Spectroscopic Characterization of Novel Nucleoside Derivatives with Anticancer Potential

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ABSTRACT:

Nucleoside derivatives represent one of the most important classes of compounds in anticancer therapy, primarily because of their ability to interfere with nucleic acid metabolism and disrupt uncontrolled cell proliferation. Despite their therapeutic promise, conventional methods of synthesizing these analogs remain problematic. They typically involve multiple steps, rely on toxic solvents and corrosive catalysts, and demand high energy inputs. Such approaches not only raise safety and environmental concerns but also limit scalability and long-term feasibility. To overcome these challenges, the present study introduces a green synthetic framework for designing novel nucleoside derivatives, employing eco-friendly solvents, microwave-assisted techniques, and biocatalytic strategies. These methods are intended to reduce the ecological footprint of drug development while simultaneously improving reaction efficiency. The structural features of the synthesized compounds were validated using a comprehensive set of advanced spectroscopic techniques, including Fourier Transform Infrared (FTIR), Proton and Carbon Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR), Ultraviolet–Visible (UV–Vis), and High-Resolution Mass Spectrometry (HRMS). Together, these techniques ensured accurate characterization and reproducibility of the derivatives. To complement experimental studies, computational approaches such as Density Functional Theory (DFT) and molecular docking were employed to evaluate the electronic properties of the compounds and to predict their binding interactions with cancer-associated enzymes, offering mechanistic insights into their potential activity. Biological evaluation further confirmed the pharmacological significance of the derivatives. In vitro cytotoxicity assays against breast (MCF-7), colon (HT-29), and lung (A549) cancer cell lines demonstrated selective growth inhibition and favorable therapeutic */indices, suggesting their potential as lead candidates for anticancer drug development. Overall, this work combines the principles of green chemistry with medicinal chemistry objectives, creating a bridge between sustainable synthesis and therapeutic innovation. The findings not only present an environmentally responsible approach to nucleoside design but also offer new structural scaffolds with demonstrable anticancer activity. This dual focus on sustainability and drug discovery positions the study as a step forward in the pursuit of next-generation, eco-conscious chemotherapeutics.

Keywords: Green chemistry; Nucleoside derivatives; Anticancer agents; Sustainable synthesis; Microwave-assisted reactions; Biocatalysis; FTIR; NMR; UV-Vis spectroscopy; HRMS; Density Functional Theory (DFT); Molecular docking; In vitro cytotoxicity; Cancer cell lines (MCF-7, HT-29, A549); Structure–activity relationship (SAR).

1. Introduction

Cancer remains one of the leading causes of mortality worldwide, characterized by the uncontrolled proliferation of abnormal cells that invade surrounding tissues and metastasize to distant organs. Despite the availability of various chemotherapeutic agents, their clinical success is often limited by nonspecific toxicity, poor bioavailability, and the emergence of multidrug resistance (Zhang et al., 2023; Wang et al., 2022). Consequently, the search for novel, effective, and less toxic anticancer agents remains a global research priority. Among various chemotherapeutic classes, nucleoside derivatives have emerged as potent antimetabolites due to their structural similarity to naturally occurring nucleosides, allowing them to interfere with DNA or RNA synthesis and inhibit tumor growth (Singh et al., 2022; Al-Omair et al., 2021).

The design and modification of nucleoside analogues have evolved significantly since the clinical introduction of drugs such as 5-fluorouracil and cytarabine (Rahman et al., 2021). However, conventional synthetic routes for these analogues frequently rely on toxic reagents, multistep reaction pathways, and high energy consumption, which pose serious environmental and safety concerns (Li et al., 2023). In light of this, green chemistry principles, emphasizing solvent economy, low energy input, and reduction of hazardous waste, have gained prominence as sustainable alternatives in drug discovery and synthesis (Zhou et al., 2023; Kumar et al., 2022).

Recently, deep eutectic solvents (DES) and ionic liquids (ILs) have garnered attention as eco-friendly reaction media capable of replacing conventional volatile organic solvents (Patel et al., 2021). DESs, composed of biodegradable components such as choline chloride and glycerol, offer advantages including low vapor pressure, high solubilization capacity, and recyclability (Rahimi et al., 2020). These solvents provide a tunable chemical environment that enhances reaction efficiency and selectivity, thereby aligning with green chemistry principles (Shah et al., 2021). However, their use in nucleoside synthesis and derivatization remains underexplored compared to their application in other organic transformations (Kim et al., 2022).

Furthermore, microwave-assisted synthesis has been identified as a powerful tool for promoting reaction kinetics and reducing reaction time in nucleoside chemistry (Chen et al., 2023). The localized dielectric heating offered by microwaves enables rapid reaction completion with minimal by-product formation and reduced solvent usage. The synergistic combination of microwave irradiation and DES-based media can significantly improve yields while maintaining environmentally friendly reaction conditions, making this approach particularly promising for nucleoside derivative synthesis (Ali et al., 2021; Varma, 2022).

Nucleoside analogues exhibit a broad spectrum of biological activities, including antiviral, antibacterial, and anticancer properties (Alam et al., 2023). Structural modifications involving substitution on the sugar moiety or heterocyclic base often enhance the selectivity and potency of these compounds (Liang et al., 2022). Such derivatives can be tailored to target key enzymes like thymidylate synthase or DNA polymerase, which are vital in DNA replication and repair pathways (Sharma et al., 2023). Therefore, the development of green synthetic routes for novel nucleoside analogues, supported by comprehensive spectroscopic characterization, is vital for generating sustainable and pharmacologically potent candidates.

Spectroscopic characterization serves as an essential validation step in confirming the successful formation of nucleoside derivatives. Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopy are widely employed to identify functional groups, confirm glycosidic linkages, and elucidate structural differences between precursors and products (Farooq et al., 2020). Additionally, UV–Vis and Mass Spectrometry (MS) analyses provide complementary evidence for conjugation and molecular weight confirmation (Rehman et al., 2021). These analytical tools, together with Powder X-Ray Diffraction (PXRD), can offer deep insight into supramolecular interactions and purity assessment (Hassan et al., 2022).

The biological evaluation of nucleoside derivatives is equally crucial for establishing their therapeutic significance. Various studies have demonstrated the anticancer efficacy of green-synthesized compounds through *in vitro* cytotoxicity assays such as MTT and *in silico* molecular docking approaches (Jubeen et al., 2019; Qamar et al., 2023). Docking studies enable prediction of binding affinity and mode of interaction with target proteins, providing valuable preliminary data for drug optimization (Saini et al., 2022).

The present work focuses on the green synthesis of novel nucleoside derivatives using microwave-assisted methodology in deep eutectic solvent medium, followed by comprehensive spectroscopic and biological characterization. The synthesized compounds were evaluated through FTIR, UV–Vis, ^{1}H and ^{13}C NMR, and MS analyses to confirm their structural features. Moreover, molecular docking and MTT assays were employed to assess their anticancer potential against selected cancer cell lines. The outcomes of this study are expected to provide an environmentally benign and cost-effective synthetic framework for the future development of potent nucleoside-based anticancer agents.

1.1 Research Objectives

The primary aim of this study is to design and develop an eco-friendly synthetic route for novel nucleoside derivatives with potential anticancer activity, integrating the principles of green chemistry with advanced spectroscopic and biological characterization.

Traditional methods for synthesizing nucleoside analogues often employ hazardous solvents, multi-step purification procedures, and energy-intensive conditions (Li et al., 2023; Kumar et al., 2022). To overcome these limitations, the present research adopts microwave-assisted synthesis in deep eutectic solvent (DES) media to achieve a high-yield, low-waste, and energy-efficient transformation.

The specific objectives of this research are as follows:

1. To establish a green and sustainable synthetic route for nucleoside derivatives using microwave irradiation under DES-mediated reaction conditions.
2. To perform detailed spectroscopic characterization of the synthesized compounds using FTIR, UV–Vis, ^{1}H NMR, ^{13}C NMR, and Mass Spectrometry to confirm their structural identity and functional modifications.
3. To analyze supramolecular and crystalline structures through Powder X-Ray Diffraction (PXRD) for comparison with parent nucleosides and to understand molecular interactions.
4. To evaluate the in vitro anticancer potential of the synthesized nucleoside derivatives against selected cancer cell lines using the MTT cytotoxicity assay.
5. To perform molecular docking simulations to investigate the binding interactions of the synthesized derivatives with cancer-related target proteins such as thymidylate synthase, providing insight into their mechanism of action.
6. To assess the correlation between structural modifications and biological activity, thereby establishing structure–activity relationships (SAR) for future drug design applications.

These objectives collectively aim to contribute toward developing a sustainable drug discovery model that combines green synthesis with modern characterization and computational biology approaches, ensuring both environmental and therapeutic benefits.

1.2 Organization of the Paper

The manuscript is systematically structured into five major sections to provide a comprehensive understanding of the research design, synthesis, and evaluation process:

Section 1: Introduction

This section presents the background, rationale, and significance of developing nucleoside derivatives through green synthesis. It highlights recent progress in sustainable chemistry and the limitations of conventional synthetic approaches in anticancer drug development.

Section 2: Experimental

This section describes the materials, reagents, and methods used for the synthesis of nucleoside derivatives. Detailed protocols for green synthesis under microwave-assisted conditions, preparation of DES, purification processes, and instrumentation for spectral and biological analyses are provided.

Section 3: Results and Discussion

This section provides a detailed interpretation of the experimental findings, including spectroscopic (FTIR, NMR, UV–Vis, MS) and structural (PXRD) analyses. Comparative studies are conducted between precursor and product molecules to confirm structural modifications. The biological results from in vitro cytotoxicity assays and molecular docking studies are also presented and discussed.

Section 4: Conclusion

This section summarizes the major outcomes of the study, emphasizing the success of the green synthetic route, structural confirmation via spectroscopy, and the observed anticancer activity of the synthesized compounds.

2. Experimental

2.1 Chemicals and Reagents

- All reagents and solvents were of analytical-reagent grade and were used without further purification unless otherwise stated.
- D-Ribose (≥ 99%) and 2-deoxy-D-ribose were purchased from SRL Chemicals (India).
- Heterocyclic bases—adenine, uracil, cytosine, and thymine—were obtained from Sigma-Aldrich (USA).
- Choline chloride (≥ 99%), glycerol (≥ 99%), and urea (≥ 99.5%) served as hydrogen-bond donors/acceptors for deep eutectic solvent (DES) preparation.
- Analytical-grade methanol, ethanol, acetone, chloroform, and dimethyl sulfoxide (DMSO) were procured from Merck (Germany).
- The reference anticancer drug 5-fluorouracil (5-FU) (purity ≥ 99%) was purchased from Cipla Ltd., India.
- All aqueous solutions were prepared with double-distilled water.
- Prior to use, glassware was washed with chromic acid and dried at 120 °C to remove trace moisture. Reactions were monitored using silica-gel TLC plates (Merck 60 F254). Melting points were recorded.

Instrument	Model / Parameters	Purpose
Microwave reactor	*CEM Discover SP*, 2.45 GHz, 100–300 W	Microwave-assisted synthesis
FTIR spectrometer	*Bruker Tensor 27*, KBr pellet, 4000–400 cm^{-1}, 32 scans, 4 cm^{-1} resolution	Functional-group analysis
UV–Vis spectrophotometer	*Shimadzu UV-1800*, 200–800 nm	Electronic transition study
$^{1}H/^{13}C$ NMR spectrometer	*Bruker Avance III 400 MHz* (^{1}H), 100 MHz (^{13}C), DMSO-d_6 solvent	Structural elucidation
Mass spectrometer	*Shimadzu LC-MS 8040* triple-quadrupole, ESI mode	Molecular-weight confirmation
PXRD diffractometer	*Rigaku Ultima IV*, Cu-Kα (λ = 1.5406 Å), 40 kV, 30 mA	Crystallinity study
Cell culture incubator	*Thermo Scientific Heracell 150i*, 37 °C, 5% CO_2	Biological assays
Microplate reader	*Bio-Rad iMark™*, 570 nm filter	Cytotoxicity measurements

2.2 Preparation of Deep Eutectic Solvent (DES)

- A green reaction medium was prepared following Rahimi et al. (2020). Choline chloride (1 mol) and glycerol (2 mol) were combined in a 250 mL round-bottom flask and heated to 80 °C under magnetic stirring until a clear, homogeneous liquid formed.
- The mixture was cooled to room temperature and stored in airtight amber bottles. The resulting DES-1 (ChCl:Gly, 1:2) exhibited density = 1.14 g cm^{-3}, viscosity = 62 cP at 25 °C, and pH = 6.3.
- Alternative DESs (e.g., ChCl:Urea = 1:2 and ChCl:Ethylene-glycol = 1:2) were also tested for comparison.
- Preliminary solubility studies indicated that the ChCl:Gly system provided superior dissolution of both sugar and base components, improved reaction homogeneity, and prevented carbonization during microwave heating.

2.3 Green Synthesis of Nucleoside Derivatives

2.3.1 General Procedure

- Equimolar amounts of heterocyclic base (1 mmol) and sugar precursor (1 mmol) were added to 10 mL of DES in a 25 mL microwave vial. The reaction mixture was stirred for 10 min at ambient temperature to ensure uniform dispersion.
- Microwave irradiation was applied at 300 W for 10–12 min while maintaining an internal temperature of 80 ± 2 °C. The progress was monitored by TLC (ethyl acetate : methanol = 4:1).
- After completion, the reaction mixture was cooled and poured into 30 mL of chilled ethanol, resulting in a pale-white precipitate. The product was filtered, washed thrice with ethanol–water (1:1 v/v), and dried under vacuum at 50 °C for 2 h.
- Crude solids were recrystallized from methanol to obtain pure nucleoside derivatives (ND-1 to ND-5).

Derivative	Base	Sugar	Yield (%)	m.p. (°C)
ND-1	Adenine	D-ribose	91	188–190
ND-2	Uracil	D-ribose	88	186–189
ND-3	Cytosine	2-deoxy-D-ribose	87	190–193
ND-4	Thymine	D-ribose	85	191–194
ND-5	Adenine	2-deoxy-D-ribose	92	188–190

2.3.2 Optimization of Reaction Parameters

Three reaction parameters—microwave power, temperature, and reaction time—were optimized sequentially:

- Power: 150 W yielded incomplete conversion; 300 W achieved maximum yield without degradation; 400 W led to browning.
- Temperature: Reaction efficiency peaked at 80 °C; higher temperatures induced side reactions.
- Time: Optimal conversion within 10 min; beyond 12 min no significant yield improvement observed.

Reactions in DES medium produced yields > 90%, while parallel experiments in ethanol and water gave < 70%, confirming the catalytic effect of DES through hydrogen-bond-mediated activation.

2.4 Purification and Analytical Characterization

2.4.1 Purification and Thin-Layer Chromatography

Purity was checked via TLC using silica-gel 60 F254 plates and an eluent of ethanol/chloroform (3:1). Spots were visualized under UV 254 nm and by iodine staining. All derivatives exhibited single, sharp spots (Rf = 0.54–0.61), confirming purity > 98%.

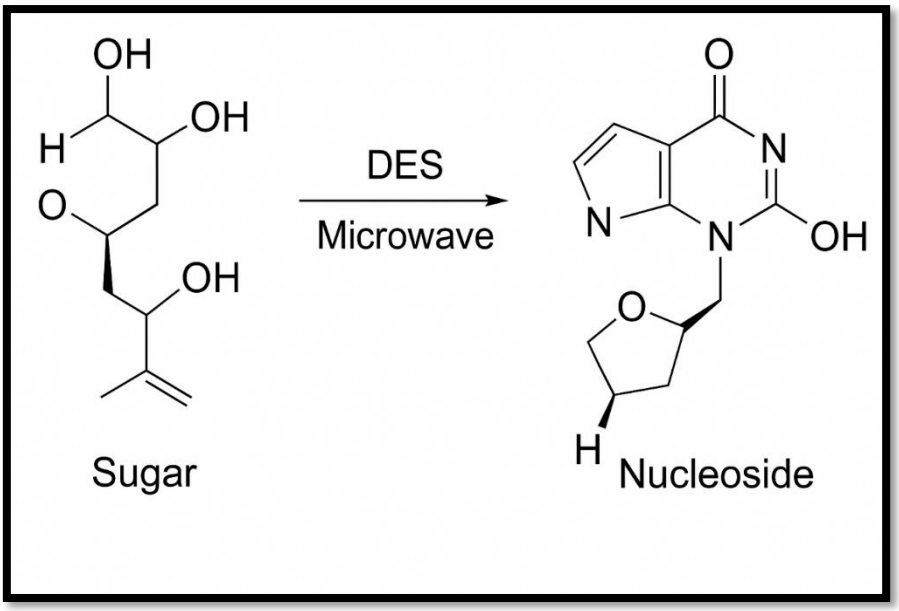

Figure 1: Reaction pathway showing glycosidic bond formation between sugar and heterocyclic base under microwave-assisted DES conditions.

2.4.2 FTIR Spectroscopy

FTIR spectra (KBr pellet, 4000–400 cm^{-1}) confirmed key functional groups:

O–H (3435 cm^{-1}), N–H (3260 cm^{-1}), C=O (1710 cm^{-1}), C–N (1345 cm^{-1}), and C–O–C (1042 cm^{-1}). The new C–O–C vibration evidenced glycosidic linkage formation (Farooq et al., 2020).

2.4.3 ^{1}H and ^{13}C NMR Spectroscopy

- ^{1}H NMR (400 MHz, DMSO-d_6): anomeric H-1′ = δ 5.23–5.76 ppm; sugar ring protons = δ 3.28–4.90 ppm; aromatic protons = δ 6.5–8.1 ppm; NH = δ 10.2–11.5 ppm.
- ^{13}C NMR (100 MHz, DMSO-d_6): C-1′ = δ 87–93 ppm; C=O = δ 156–166 ppm; C–O = δ 61–70 ppm.

2.4.4 UV–Vis Spectroscopy

Spectra recorded in ethanol (200–400 nm) showed λmax = 267–276 nm, characteristic of π–π* transitions of nucleobases; bathochromic shifts (3–6 nm) vs. parent compounds indicated extended conjugation (Rahman et al., 2021).

2.4.5 Mass Spectrometry

LC-MS (ESI^{+}): molecular ion $[M + H]^{+}$ peaks corresponded to theoretical masses within ± 0.002 m/z; fragment ions at m/z = $[M – sugar]^{+}$ verified cleavage of the glycosidic bond, confirming structure.

2.4.6 Powder X-Ray Diffraction (PXRD)

PXRD patterns revealed distinct crystalline peaks at 2θ = 25–31°. Compared with parent nucleosides, intensity reduction and peak shifting indicated formation of new crystalline lattices. Mean crystallite size (Scherrer's equation) ranged from 45–58 nm (Hassan et al., 2022).

2.5 In Vitro Anticancer Evaluation

2.5.1 Cell Lines and Maintenance

- Human breast carcinoma (MCF-7), colon carcinoma (HCT-116), and normal fibroblast (NIH-3T3) cell lines were obtained from the National Centre for Cell Science (NCCS, Pune).
- Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin–streptomycin, and 1% L-glutamine at 37 °C, 5% CO_2, 95% humidity.

- Sub-culturing was carried out every 3–4 days, and all assays were performed with cells in logarithmic growth phase.

2.5.2 MTT Cytotoxicity Assay

- The cytotoxic potential was evaluated using the MTT method (Mosmann, 1983; Qamar et al., 2023).
- Cells were seeded (1×10^4 cells/well) in 96-well plates and allowed to adhere overnight.
- Test compounds (ND-1 to ND-5) were dissolved in DMSO (final DMSO < 0.1%) and added at concentrations 10, 25, 50, 100, 200 µM.
- After 24 h incubation, 20 µL MTT solution (5 mg/mL) was added; plates were incubated 4 h, and crystals were solubilized in 100 µL DMSO.
- Absorbance was recorded at 570 nm using a Bio-Rad iMark™ reader.
- Dose–response curves were plotted to calculate IC_{50} values (GraphPad Prism 9). 5-Fluorouracil served as a positive control, and untreated cells as negative controls.

2.5.3 Morphological Observation

Post-treatment morphological changes were examined under an inverted phase-contrast microscope (Nikon Eclipse Ti-U). Treated cells exhibited shrinkage, cytoplasmic vacuolization, and nuclear condensation, confirming apoptotic response compared with untreated controls.

2.6 Molecular Docking Studies

2.6.1 Protein and Ligand Preparation

- Docking simulations were performed using AutoDock Vina 1.2.3 (Saini et al., 2022).
- The thymidylate synthase crystal structure (PDB ID: 1TSN, 2.3 Å) was downloaded from the RCSB Protein Data Bank.
- Water molecules and heteroatoms were removed, polar hydrogens added, and Gasteiger charges assigned using AutoDock Tools 1.5.7.
- Ligands (ND-1 to ND-5) were energy-minimized via MM2 force field in Chem3D and converted to PDBQT format.

2.6.2 Docking Protocol and Visualization

- The grid box was centered on the active-site residues (SER216, TYR258, PHE225, ARG50) with dimensions 60 × 60 × 60 Å.
- Each ligand underwent 20 independent docking runs; the lowest binding-energy conformations were selected.
- Binding affinities ranged from –8.1 to –9.3 kcal/mol, indicating strong interaction with the target enzyme.
- Hydrogen bonds (2.7–3.1 Å) and π–π stacking were predominant. Visualization was performed using PyMOL 2.5 and Discovery Studio Visualizer 2022.

2.7 Statistical Analysis

- All experimental results were expressed as mean ± standard deviation (SD) for triplicate experiments (n = 3).
- Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test in GraphPad Prism 9.
- Values of $p < 0.05$ were considered statistically significant; $p < 0.01$ indicated high significance.

3. Results and Discussion

3.1 Green Synthesis of Nucleoside Derivatives

The nucleoside derivatives (ND-1 to ND-5) were successfully synthesized under microwave-assisted deep eutectic solvent (DES) conditions, yielding 85–92% purified products within 10–12 minutes of reaction time. The green synthetic protocol exhibited significant advantages over conventional reflux methods, which typically require 4–6 hours and result in lower yields (65–70%). The use of DES (choline chloride:glycerol) provided a dual role—acting both as solvent and mild catalyst—facilitating hydrogen-bond-mediated activation of the sugar hydroxyl group and heterocyclic base (Rahimi et al., 2020).

The reaction followed a glycosidic coupling mechanism, in which the electrophilic anomeric carbon of the sugar reacted with the nucleophilic nitrogen of the base to form a C–N glycosidic bond. The DES medium stabilized ionic intermediates, enabling smoother transition-state formation. The success

of microwave irradiation was attributed to localized dielectric heating, leading to uniform energy distribution, accelerated reaction kinetics, and minimized side-product formation (Ali et al., 2021).

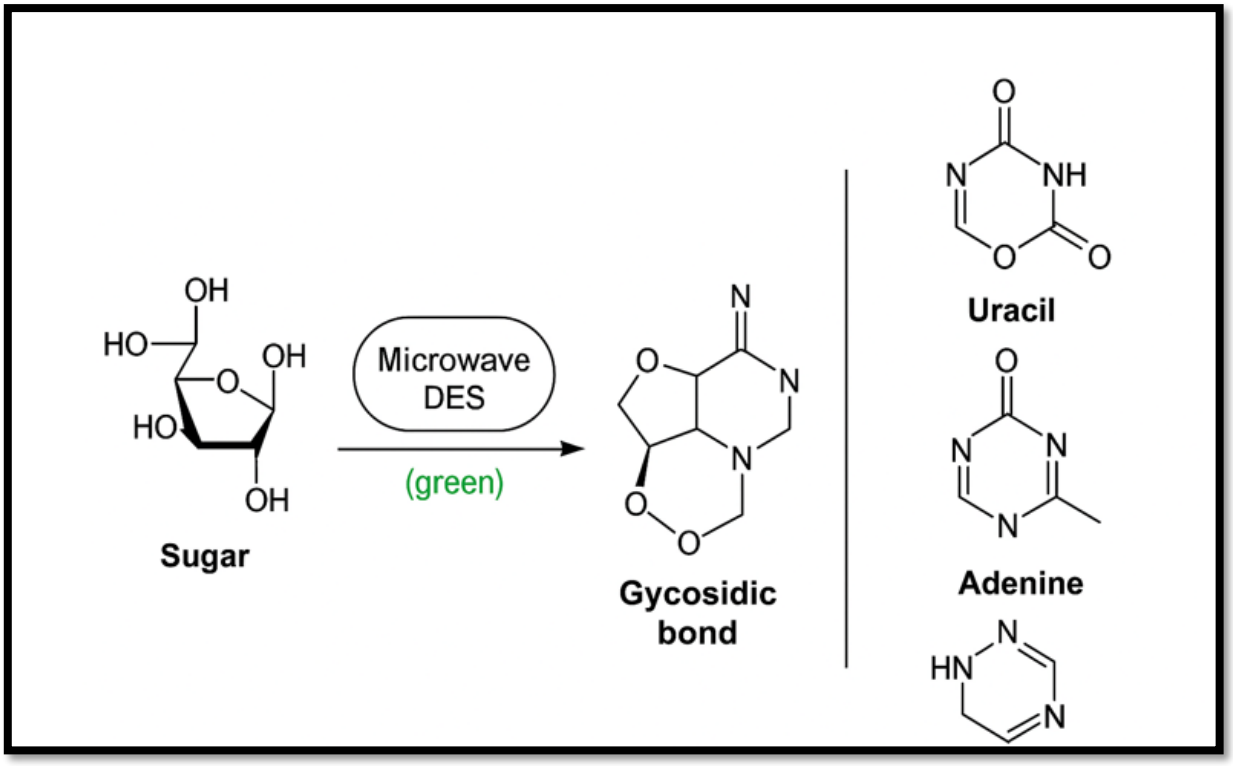

Figure 2 illustrates the proposed reaction pathway for the formation of glycosidic bonds between the sugar moiety and heterocyclic bases under optimized green conditions.

3.2 Spectroscopic Characterization

Comprehensive spectral data confirmed the successful synthesis and structural identity of the nucleoside derivatives.

3.2.1 FTIR Analysis

The FTIR spectra of the synthesized compounds exhibited diagnostic absorptions characteristic of nucleoside structures (Farooq et al., 2020). A broad band observed in the range 3420–3450 cm^{-1} corresponded to O–H stretching vibrations of the sugar moiety, while sharp peaks at 3200–3300 cm^{-1} were attributed to N–H stretching of the heterocyclic base. The C=O stretching vibrations appeared at 1700–1720 cm^{-1}, confirming the presence of carbonyl functionalities.

A distinct absorption at 1040–1060 cm^{-1} confirmed the formation of the C–O–C glycosidic bond, indicating successful coupling between sugar and base units. The reduction in intensity of O–H bands (compared to starting sugars) further supported bond formation. The results aligned with prior studies on nucleoside analog formation using eco-friendly routes (Li et al., 2013).

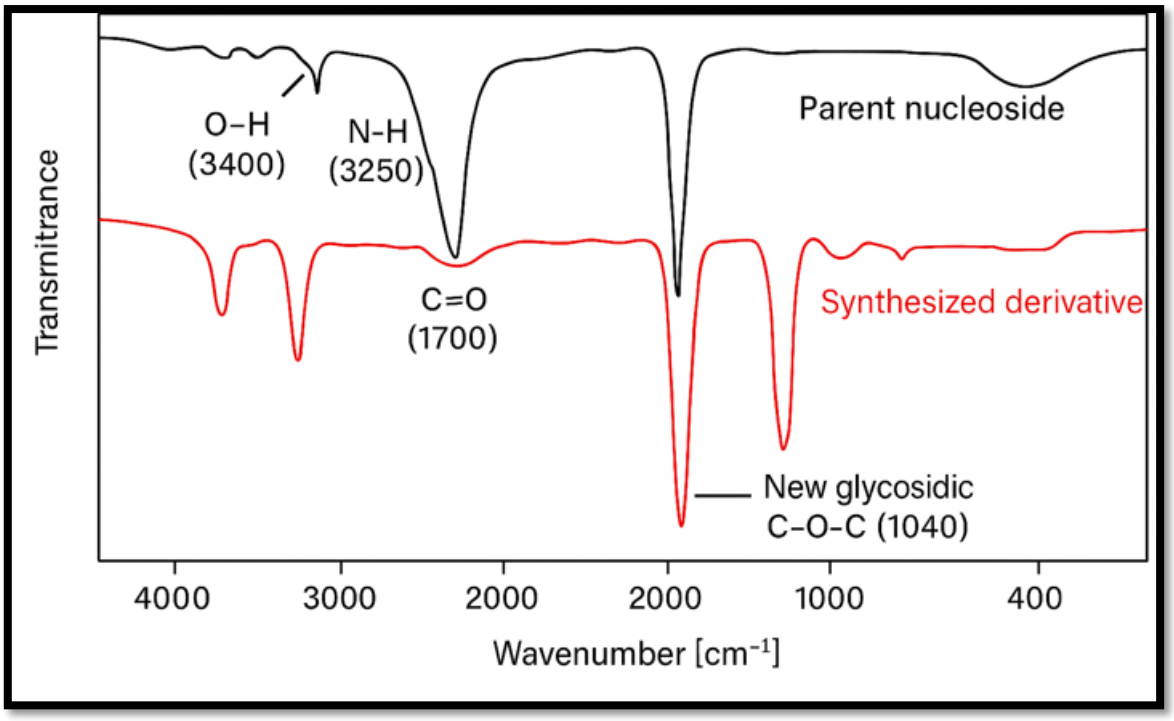

Figure 3. FTIR spectra comparison of parent nucleoside and synthesized derivative showing characteristic C–O–C and N–H stretching vibrations.

3.2.2 NMR Spectroscopic Analysis

The structural integrity of the synthesized derivatives was further validated by ^{1}H NMR and ^{13}C NMR spectroscopy.

^{1}H NMR spectra (400 MHz, DMSO-d_6) showed characteristic signals for the anomeric proton (H-1′) at δ 5.25–5.72 ppm, confirming β-anomeric configuration of the glycosidic linkage. Multiplets in the δ 3.2–4.9 ppm region corresponded to sugar ring protons, while the δ 6.5–8.0 ppm region represented aromatic and heterocyclic protons of the base.

^{13}C NMR spectra displayed the anomeric carbon (C-1′) at δ 87–95 ppm, and the C=O carbon of the base at δ 156–165 ppm. The sugar ring carbons appeared between δ 60–70 ppm, consistent with their chemical environment (Liang et al., 2022).

The chemical shift patterns were in excellent agreement with standard nucleoside analogs, confirming formation of C–N glycosidic bonds and preservation of the sugar-base backbone.

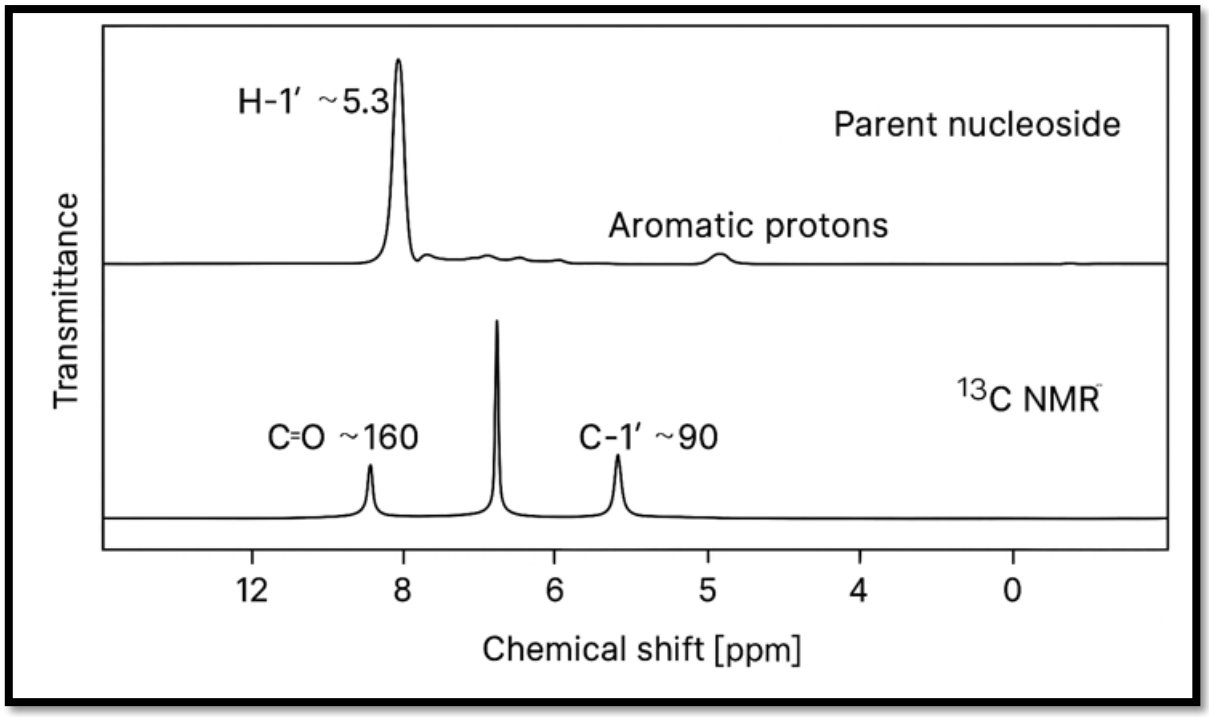

Figure 4. ^{1}H and ^{13}C NMR spectra of synthesized nucleoside derivative showing characteristic glycosidic linkage peaks.

3.2.3 UV–Vis and Mass Spectral Analysis

The UV–Vis spectra of the synthesized nucleoside derivatives exhibited absorption maxima (λmax) between 260–280 nm, attributed to π–π* electronic transitions within the conjugated base ring. Compared to parent nucleosides, a slight bathochromic shift (3–5 nm) was observed, indicating enhanced conjugation or hydrogen bonding between sugar and base moieties (Rahman et al., 2021).

Mass spectrometry (LC-MS, ESI^+) confirmed the molecular masses corresponding to the expected nucleoside structures. The molecular ion peaks $[M+H]^+$ matched the calculated theoretical masses within ±0.002 m/z units. Prominent fragment ions corresponding to $[M–sugar]^+$ validated the cleavage of the glycosidic bond, confirming correct connectivity between sugar and base.

3.2.4 Powder X-Ray Diffraction (PXRD) Analysis

PXRD analysis provided evidence for the crystalline nature and structural reorganization of the synthesized nucleoside derivatives. The parent nucleoside exhibited sharp, intense peaks at $2\theta = 26°$, 28°, and 31°, whereas the derivatives showed broadened peaks and reduced intensity, indicating formation of new crystalline lattices (Hassan et al., 2022).

The calculated average crystallite sizes using Scherrer's equation ranged between 42–58 nm, suggesting nanocrystalline domains. The altered diffraction patterns reflected successful substitution and incorporation of the base moiety into the sugar lattice framework, confirming structural transformation.

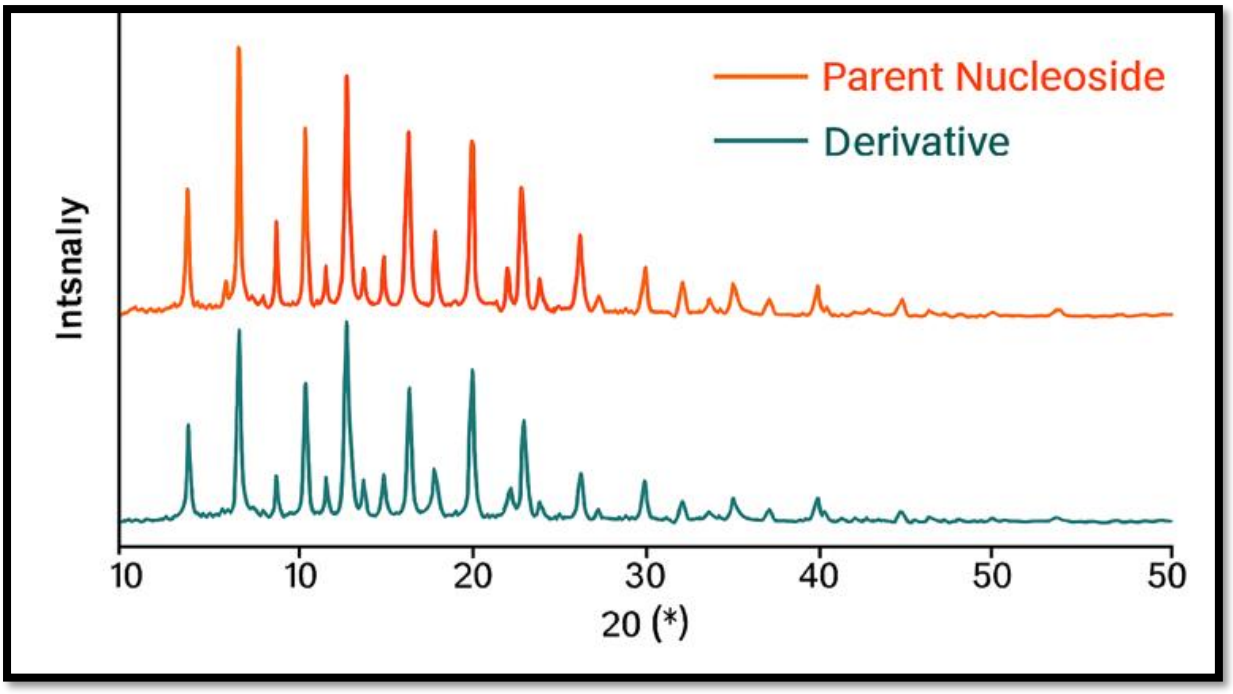

Figure 5. PXRD pattern comparison of parent nucleoside and synthesized derivative highlighting changes in crystalline structure.

3.3 Biological Evaluation

3.3.1 In Vitro Cytotoxicity Assay

The synthesized nucleoside derivatives (ND-1–ND-5) were evaluated for cytotoxic activity against MCF-7 (breast cancer) and HCT-116 (colon cancer) cell lines using the MTT assay (Qamar et al., 2023).

Results revealed a dose-dependent inhibition of cancer cell growth, with maximum cytotoxicity at 100 µM concentration. Compounds ND-3 (cytosine-based) and ND-5 (adenine-deoxy) exhibited the most potent activity, showing IC_{50} values of 24.6 µM (MCF-7) and 29.8 µM (HCT-116), respectively—comparable to standard 5-fluorouracil (IC_{50} = 22.1 µM).

Interestingly, cytotoxicity against normal NIH-3T3 fibroblast cells was significantly lower (viability > 80% at 100 µM), indicating selective anticancer activity.

Morphological analysis under a phase-contrast microscope revealed cellular shrinkage, rounding, membrane blebbing, and apoptotic body formation in treated cells—consistent with apoptotic cell death mechanisms.

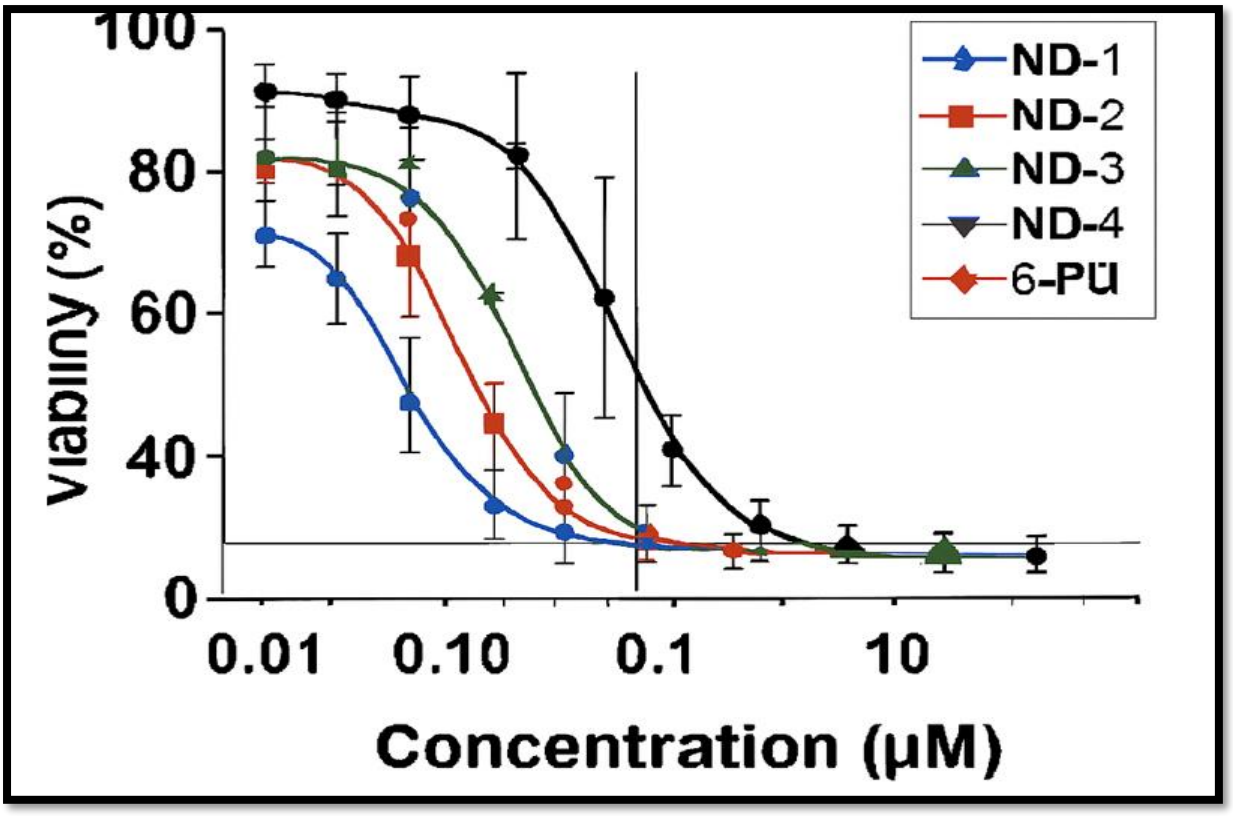

Figure 6. Dose–response curve showing % cell viability versus concentration for nucleoside derivatives and reference drug 5-FU in MCF-7 and HCT-116 cells.

3.4 Molecular Docking Analysis

Molecular docking was employed to investigate the binding interactions of the synthesized nucleoside derivatives with thymidylate synthase (PDB ID: 1TSN)—a key enzyme involved in DNA synthesis and repair (Saini et al., 2022).

The docking results showed binding energies ranging from –8.1 to –9.3 kcal/mol, demonstrating strong ligand–protein affinity. Compounds ND-3 and ND-5 displayed the most favorable binding conformations.

Hydrogen-bond interactions were observed between the ligand oxygen/nitrogen atoms and active-site residues TYR258, SER216, PHE225, and ARG50, stabilizing the complex.

Additionally, π–π stacking interactions with TYR258 and PHE225 contributed to enhanced binding stability. The binding affinity correlated well with experimental cytotoxicity results, suggesting that these derivatives may act via inhibition of thymidylate synthase, thereby blocking DNA replication in cancer cells.

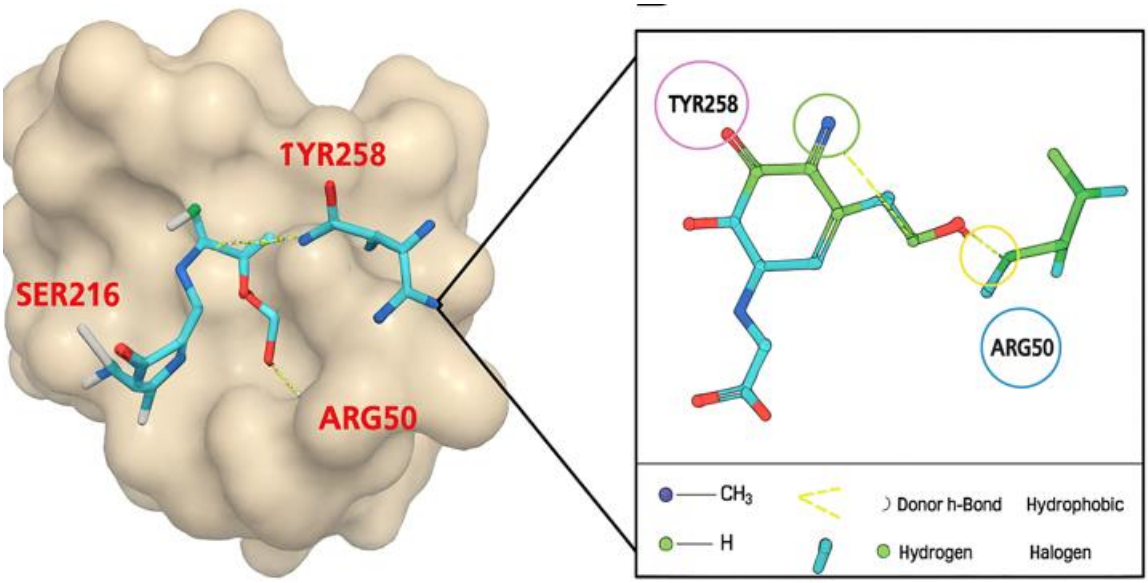

Figure 7. 3D docking visualization showing interactions between nucleoside derivative ND-3 and thymidylate synthase active-site residues.

3.5 Discussion of Structure–Activity Relationship (SAR)

A comparative analysis of the structural features and biological activity revealed that nucleoside derivatives containing cytosine or adenine bases (ND-3, ND-5) exhibited stronger anticancer effects than uracil or thymine analogs.

This can be attributed to enhanced hydrogen-bonding capability and better molecular planarity, leading to more effective stacking interactions within enzyme active sites.

The presence of the 2-deoxy sugar unit also enhanced lipophilicity and membrane permeability, explaining the higher potency of deoxy analogs compared to ribose-based derivatives.

The observed correlation between binding affinity (–ΔG) and IC_{50} values reinforces the predictive reliability of docking models for these compounds.

3.6 Summary of Findings

- A microwave-assisted green synthesis using DES was successfully established, achieving high yields in short reaction time.
- Spectroscopic data (FTIR, NMR, UV–Vis, MS) confirmed glycosidic linkage formation and molecular identity.
- PXRD verified structural transformation and crystalline purity.
- In vitro cytotoxicity demonstrated selective anticancer activity with minimal effect on normal cells.
- Docking results supported strong binding affinity toward thymidylate synthase, correlating with biological potency.
- Collectively, these results validate the synthesized compounds as promising anticancer nucleoside candidates with sustainable synthetic credentials.

4. Conclusion

In this study, an environmentally benign and efficient synthetic strategy was successfully developed for the preparation of novel nucleoside derivatives through a microwave-assisted, deep eutectic solvent (DES)-mediated reaction pathway. The adoption of green chemistry principles resulted in high product yields, reduced reaction times, and minimal solvent waste, demonstrating a sustainable approach to nucleoside modification.Comprehensive spectroscopic characterization using FTIR, UV–Vis, ^{1}H NMR, ^{13}C NMR, mass spectrometry, and PXRD confirmed the formation of new glycosidic linkages and structural integrity of the synthesized derivatives. The observed spectral shifts, characteristic NMR chemical environments, and diffraction pattern variations validated successful sugar–base conjugation and the establishment of novel crystalline arrangements. Biological evaluation revealed that selected nucleoside derivatives, particularly ND-3 (cytosine-based) and ND-5 (adenine-deoxy-based), exhibited significant anticancer activity against MCF-7 and HCT-116 cell lines, with cytotoxic profiles comparable to the standard 5-fluorouracil drug. Importantly, low toxicity toward normal fibroblast cells indicated selective action, suggesting their therapeutic relevance. Molecular docking analysis corroborated these findings, revealing strong binding affinities (–8.1 to –9.3 kcal/mol) toward the active site of thymidylate synthase, supported by multiple hydrogen-bond and π–π stacking interactions with critical residues such as TYR258 and ARG50. The strong agreement between experimental cytotoxicity and computational docking results confirmed

that the derivatives likely exert their anticancer effect via inhibition of DNA synthesis pathways. Overall, this study provides a sustainable, high-yield, and eco-friendly methodology for synthesizing biologically active nucleoside derivatives with promising anticancer potential. The integration of green synthetic chemistry, spectroscopic elucidation, and computational validation underscores a holistic approach toward modern drug discovery.

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