ICSSR Sponsored
ISSN: 2319-9997

Journal of Nehru Gram Bharati University, 2025; Vol. 14 (I):39-50

Synthesis, Molecular Docking and DFT of 6-Amino-5-(2-Bromobenzyl)-1,3-Dimethylpyrimidine-2,4(1H,3H)-dione: A Study of its Binding Affinity and Structure-Activity Relationship

Radha Singh[1], Shainda Laeeq[2], Anita Singh[1] and Anoop Kumar Pandey[3]*

[1]Department of Chemistry, Nehru Gram Bharati (Deemed to be University), Jamunipur, Kotwa Dubawal, Prayagraj, Uttar Pradesh, India, 221505

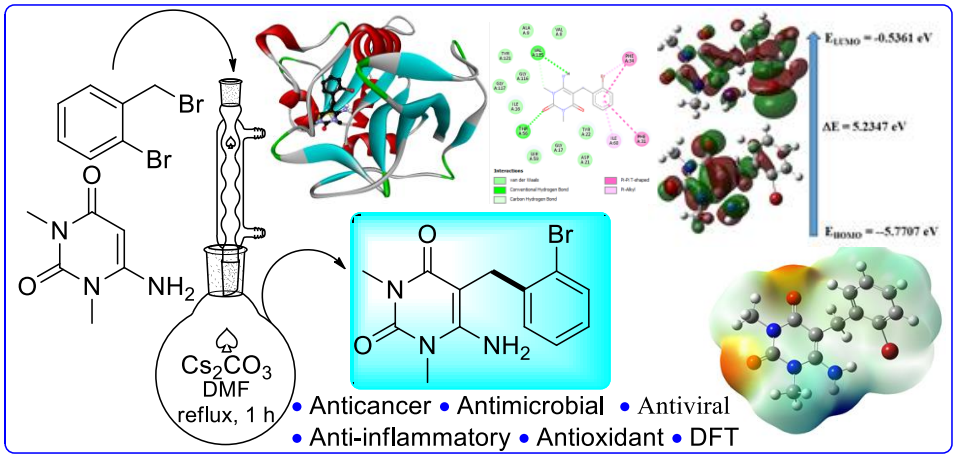
[2]Department of Pharmaceutical Chemistry, Maharana Pratap College of Pharmaceutical Sciences Kanpur, Uttar Pradesh, India, 209217

[3]Department of Pharmaceutical Chemistry, Maharana Pratap College of Pharmacy Kanpur, Uttar Pradesh, India, 209217

***Corresponding author:** pandeyanoop2014@gmail.com,
ORCID: https://orcid.org/0000-0003-4581-1002

Received: 04.12.2024 Revised:31.05.2025 Accepted: 05.06.2025

Graphical Abstract



Abstract

*In present study, base-mediated 6-amino-5-(2-bromobenzyl)-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (**ABDD**) was synthesized from 6-amino-1,3-dimethyl uracil and 2-bromobenzyl bromide. NMR, FT-IR, and HRMS characterized **ABDD**. DFT has performed calculations for HOMO, LUMO, MESP, and other factors. Pyrimidine derivatives have*

potential applications in drug design and medicinal chemistry. In addition, molecular docking shows the theoretical possibility of binding ability with various proteins. The results suggested that this compound holds promise as a therapeutic scaffold for developing novel pharmacological agents focusing on its antimicrobial, anticancer, and antioxidant properties.

Keywords: *Pyrimidine, 2-bromobenzyl bromides, 6-amino-uracil, molecular docking, DFT, HOMO-LUMO, MESP, bioactive molecule.*

Introduction

Pyrimidine derivatives are heterocyclic compounds that are widely studied and versatile in medicinal chemistry. This is largely due to their structural role in nucleic acids, DNA, and RNA, as well as their broad range of biological activities (Nerkar 2021, Patil 2023, Islam *et al.* 2024, N & Goudgaon 2021). With their unique electronic structure and ease of modification, pyrimidine derivatives provide an ideal framework for synthesizing novel pharmacologically active compounds (Nadar & Khan, 2021; Chiacchio *et al.*, 2018; Bhatnagar & Pemawat, 2024). Researchers have recognized 6-Aminouracil as a particularly advantageous scaffold among pyrimidine derivatives. 6-Aminouracil is structurally characterized by an amino group at the C-6 location and a ketone group at the C-2 and C-4 locations, which facilitates further functionalization and derivatization (Alhilal *et al.*, 2021; Pałasz & Cież, 2014; El-Kalyoubi *et al.*, 2021).

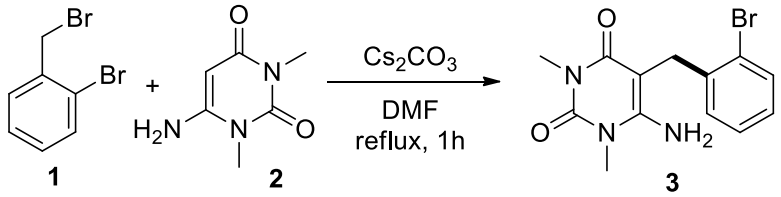
This chemical allows for a range of substitutions and modifications, enhancing the compound's bioavailability, stability, and binding affinity toward biological targets. For instance, studies have demonstrated that 6-aminouracil derivatives exhibit antimicrobial properties, frequently by inhibiting enzymes or disrupting cell membrane integrity in pathogens (Hussain *et al.,* 2022). Adding a halogenated, alkyl, or aryl group to the structure of 6-aminouracil has also been shown to improve its ability to bind, which often leads to better pharmacokinetic and pharmacodynamic properties of the compounds that are made (Cawrse *et al.*, 2017; Tylińska *et al.*, 2021).

In line with our ongoing work on pyrimidine derivative synthesis (Ali *et al.*, 2023; Panday *et al.*, 2023; Ali *et al.*, 2020; Panday *et al.*, 2020; Mahata *et al.*, 2020; Jana *et al.,* 2019; Panday *et al.*, 2018), In this investigation, present the preparation, analysis, DFT calculation and molecular docking of a pyrimidine derivative that originates from 6-aminouracil. **ABDD 3** was synthesized and structurally characterized to explore its potential pharmacological properties.

Results and Discussion

Materials: The solvents and reagents were purchased from commercial sources and used without further purification. Thin-layer chromatography (TLC) was used to monitor all of the reactions, and afterward, the reactions were studied under ultraviolet (UV) light and/or in an iodine chamber in order to observe reaction spots. Column chromatography was carried out using silica gel and a solvent combination of ethyl acetate and hexane in different ratios.

Procedure: A combination of 2-bromobenzyl bromide **1** (0.5 mmol) and 6-amino-1,3-dimethyluracil **2** (0.5 mmol) in 2 ml of DMF, with the addition of Cs_2CO_3 (1.0 mmol), was stirred under reflux for a time frame of 1 hours. Upon the completion of the reaction, the mixture was placed in icy water, and the crude product was subsequently isolated through the process of simple filtration. After this, **ABDD 3** underwent purification through column chromatography employing a hexane/ethyl acetate solvent system (Scheme 1).



Scheme 1: Preparation of **ABDD 3**.

Characterization:

Panday et al., (2018): White solid; M.P. 255-256 °C. Yield 85%; ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6) δ 7.47 (d, J = 8.0 Hz, 1H), 7.14 (t, J = 8.0 Hz, 1H), 7.02-6.95 (m, 2H), 6.21 (s, 2H), 3.66 (s, 2H), 3.36 (s, 3H), 3.17 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ 161.9, 152.4, 151.0, 138.7, 131.8, 128.0, 127.2, 127.1, 124.5, 82.8, 29.8, 29.6, 27.5 ppm. IR (ATR) 3374, 3219, 2925, 2856, 1668, 1594, 1502, 1458, 1372, 1212, 1026, 968, 827 cm^{-1}. HRMS (ESI-TOF): m/z $[M+H]^+$ calculated for $C_{13}H_{14}BrN_3O_2Na$ $[M + Na]^+$ 346.0162; observed 346.0159.

Molecular Docking Studies:

Evaluations are predicated on the target-specific ligand, which serves as a condensed representation of the salient characteristics of the target system or ligand. These evaluations rely on two-dimensional property profiles, detailed three-dimensional structural modeling of receptor-ligand interactions, and assessments based on the target-specific ligand. A target-based docking technique was performed for the lead

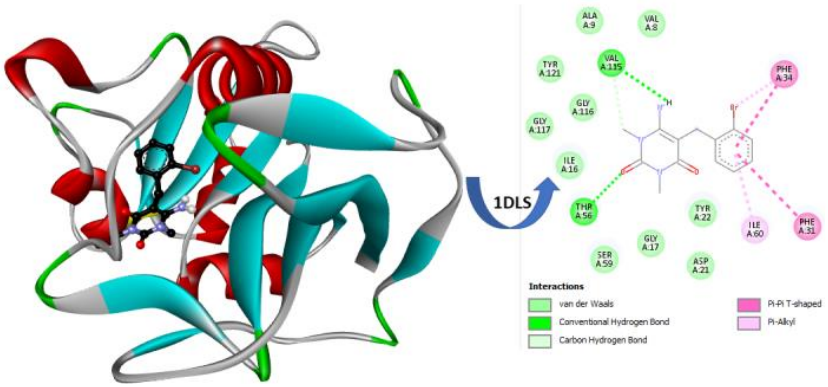
compound **ABDD 3**, which interacts with the 1DLS, 1J31, 5IKR, 5MTR, 1M17, 3K5A, 1DNU, 1HVR, 6LU7, 3EML, 1VJF, 4ZK2, and 1CA2 receptors.

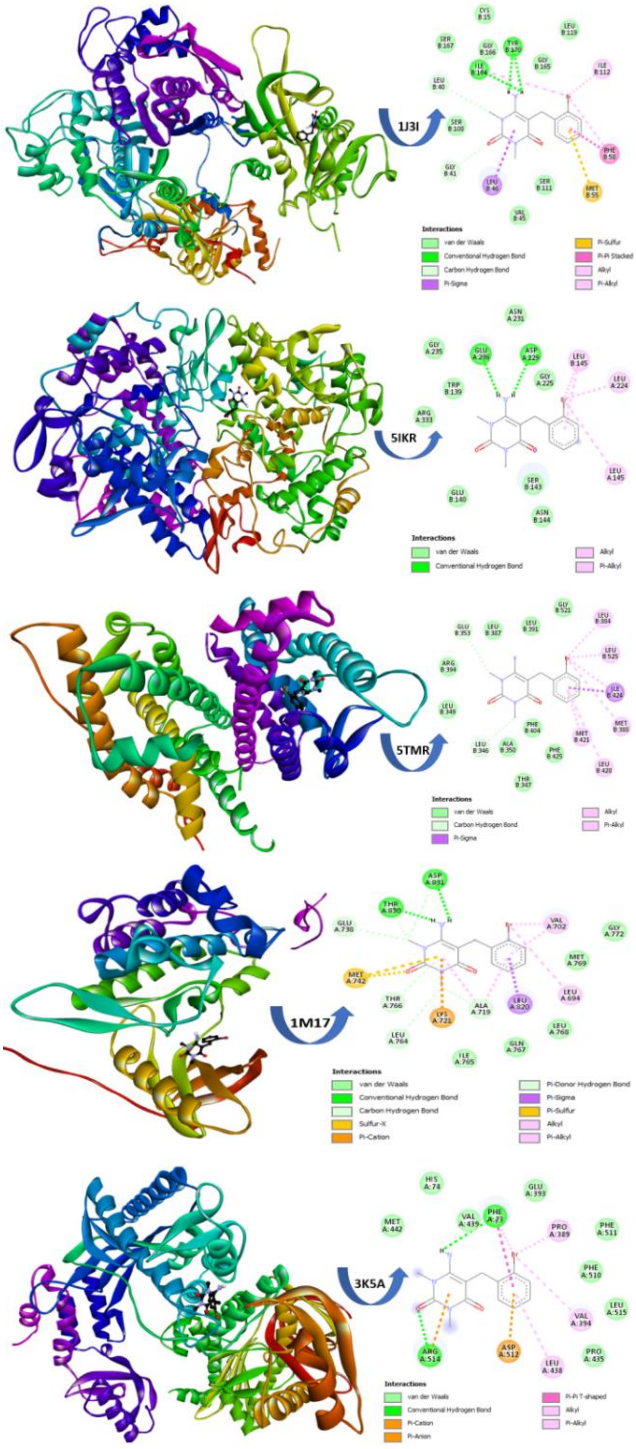
Procedure for Molecular Docking:

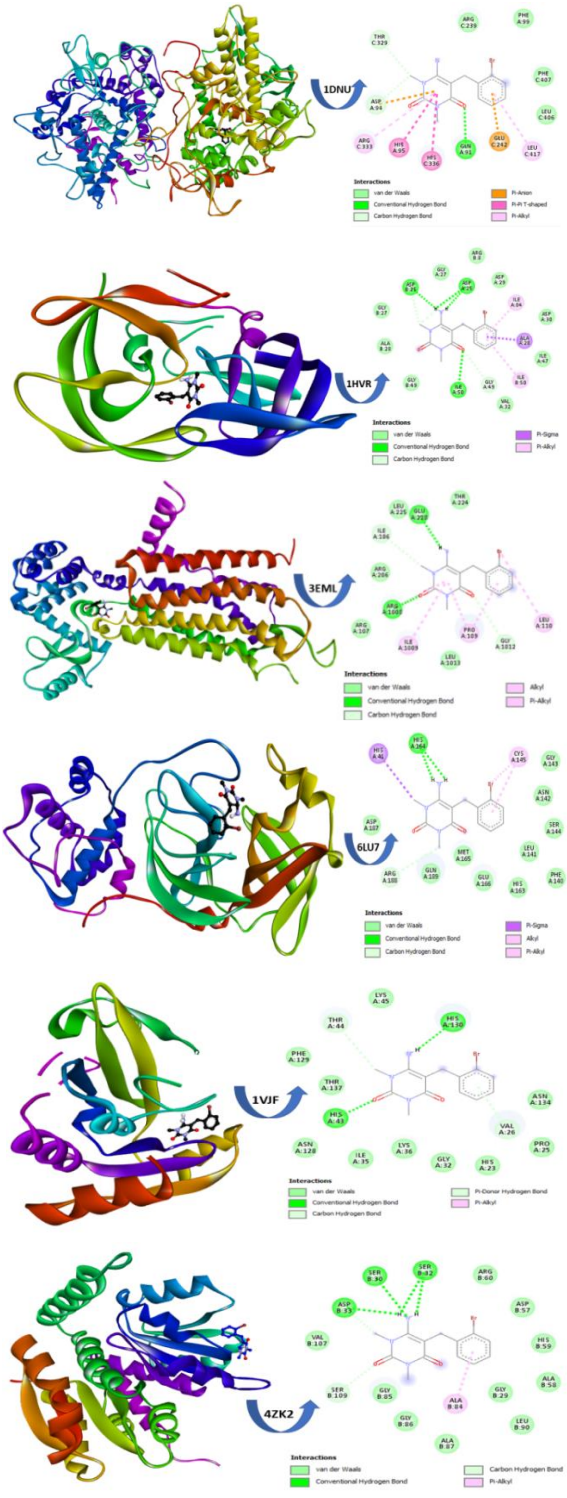
A ligand termed **ABDD** was developed using Chem Draw Ultra 12.0, adhering to Lipinski's rule of five. Precautions were taken to exclude heavy or carcinogenic elements from the molecule. The ligand was synthesized using the Open Babel GUI software. The ligand structure was saved in .mol format and converted to .pdb format. A selection of protein PDB IDs: 1DLS, 1J31, 5IKR, 5MTR, 1M17, 3K5A, 1DNU, 1HVR, 6LU7, 3EML, 1VJF, 4ZK2, and 1CA2 were obtained from the Protein Data Bank website (http://www.rcsb.org).

The retrieved proteins of interest were initially prepared by eliminating the ligand and water, followed by the addition of polar hydrogen to the protein structure. Only the protein devoid of bound ligand and water was preserved as a PDB file (protein). Similarly, all the proteins were preserved in .pdb format. In AutoDock Vina program, each protein was selected as .pdb file and subsequently input the ligand. AutoDock Vina was used for the ligand and minimized receptors.

The receptor-ligand complex was examined to evaluate the potential of the docked molecules. The conformation of the protein was evaluated and displayed with the ligand using Biovia Discovery Studio Visualizer, presented in 2D and 3D diagrams as depicted in Figure 1. **ABDD** demonstrates a range of actions, including anticancer, antimalarial, antibacterial, anti-inflammatory, antioxidant, and antiviral properties, with favorable binding scores presented in Table 1, against diverse proteins.




1J3I
5IKR
5TMR
1M17
3K5A


1DNU
1HVR
3EML
6LU7
1VJF
4ZK2

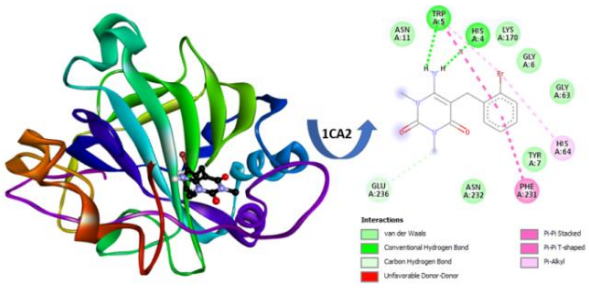


Figure 1. 2D and 3D Protein – Ligand interaction of proteins with PDB id :1DLS, 1J31, 5IKR, 5MTR, 1M17, 3K5A, 1DNU, 1HVR, 6LU7, 3EML, 1VJF, 4ZK2, 1CA2 (top to bottom, left to right).

Table 1. Binding Scores of **ABDD** with Different Proteins Showing Their Activity.

Protein ID	Binding Affinity	Activity	Organism
1DLS	-8.1	Anticancer and antimicrobial DHFR inhibitor	Homo sapiens
1J3I	-7.7	Antimalarial DHFR inhibitor	Plasmodium falciparum
5IKR	-7.7	Anti-Inflammatory	Homo sapiens
1M17	-7.4	Anticancer (lung and breast cancer) transferase Inhibitor	Homo sapiens
1DNU	-7.3	Antioxidant activity	Homo sapiens
3K5A	-7.3	Anticancer (Protein /kinase Inhibitor)	Escherichia coli K-12
5TMR	-7.3	Anticancer	Homo sapiens
1HVR	-6.6	Antiviral (Hydrolase Inhibitor)	Human immunodeficiency virus 1
6LU7	-6.6	Antiviral	Severe acute respiratory syndrome coronavirus 2, synthetic construct
3EML	-6.5	Anti-inflammatory and neuroprotective	Homo sapiens, Tequatrovirus T4
4ZK2	-6.3	Isomerase Inhibitor	Acetobacter aceti 1023
1CA2	-6	Antiglaucoma, antiepileptic anticancer (pH regulation.	Homo sapiens
1VJF	-6	Anticancer (Protein /kinase Inhibitor)	Caulobacter vibrioides CB15

Adme Analysis: ADME analysis was conducted an in-silico evaluation of pharmacokinetics using pkCSM analysis

(**https://biosig.lab.uq.edu.au/pkcsm/ prediction_single/ adme_ 1730955537.45**). The compound **ABDD** shows good gastrointestinal

absorption and does not cross BBB as shown in the BOILED-Egg Model Figure 2. As it is inhibiting CYP1A2 and CYP3A4 enzymes it can lead to good cancer chemopreventive agents.

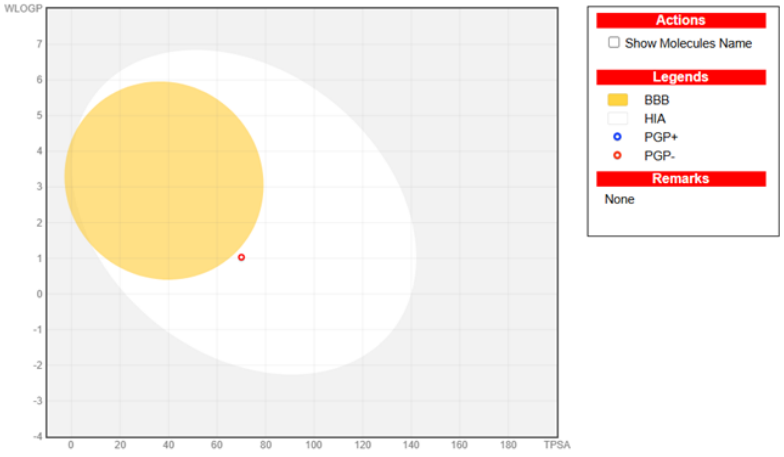


Figure 2. BOILED-Egg Model of **ABDD**.

Structure-Activity Relationship (SAR): Ligand receptor interaction shows that –NH group shows conventional bonding with THR A:44, THR A:56, THR C:329, THR A:830, GLU A:228, ASP B:33, ASP A:94, ASP B:25, ASP A:25, ASP A:831, GLU A:738, ILE A:50, ILE B:164, TYR B:130, TYR B:170, HIS A:164, HIS A:4, VAL A:115, TRP A: 5, VAL A:439, ARG A:514, -CH group shows covalent bond with LEU B:40, LEU B:152, LEU A:764, GLY B:41, GLY A:1012, ARG B:44, ARG C:333, CYS B:41, CYS A:145, GLU A:738, GLU A:236, GLU B:353, LEU B:346, THR A:830, THR A:766, THR A:44 , ALA A:719, ASP A:831, ASP A:94, ASP B:33, HIS A:95, HIS C:336, ARG A:188, ILE A:106, VAL A:26, SER B:109, pi-pi alkyl bond with PHE A:231, PHE A:73, PHE A:34, PHE A:31, PHE B:58, ILE A:60, ILE A:84, ILE A:1009, ILE B:50, ILE B:112, MET B:55, MET B:421, MET B:388, VAL A:394, VAL A:702, VAL B:46, CYS B:47, PRO B:153, LEU A:110, LEU A:694, LEU A:438, LEU B:384, LEU B:525, LEU B:428, LEUC:417, ALA A:719, ALA B:84, LYS A:721, ARG A:514, PRO A:389, PRO A:109, GLU C:242, ASP B:57, HIS B:59, HIS A:64 and Sigma Bond with LEU A:820, LEU B:46, ILE B:424, ASPA:512, ALA A: 28, HIS A:41 amino acids of the protein ids docked. This infers that **ABDD** structure has a good binding activity with amino acids in cancer chemoprevention, anti-inflammatory, antimicrobial, and antiviral activity.

DFT Analysis

The structure of the **ABDD** was optimized by DFT calculations using the

B3LYP hybrid functional with 6-31g basis set (Frisch et al, 2016). The value of HOMO and LUMO, energy difference, chemical hardness, electronegativity, chemical potential, chemical softness, and global electrophilicity index are summarized in Table 2.

The Mulliken charge distributions of the molecule have been calculated using 6-31g level shown in Table 3.

Table 2. The Energy Values of Global Reactivity Descriptors.

Parameter	**Value (eV)**
E_{LUMO}	-0.5361
E_{HOMO}	-5.7707
ΔE	5.2347
Chemical Hardness (η)	2.3493
Electronegativity (χ)	3.1534
Chemical Potential (μ)	-3.1534
Chemical Softness (s)	1.1746
Global electrophilicity index (ω)	11.6806

Table 3: Atomic Charge Analysis for **ABDD**.

Atom	**Mulliken Charge**	**Atom**	**Mulliken Charge**	**Atom**	**Mulliken Charge**
C1	-0.119	C12	0.733	H23	0.181
C2	-0.123	N13	-0.65	H24	148
C3	-0.082	C14	0.49	H25	0.198
C4	-0.342	O15	-0.496	H26	0.34
C5	0.167	N16	-0.795	H27	0.329
C6	-0.155	C17	-0.28	H28	0.166
C7	-0.429	O18	-0.46	H29	0.288
Br9	0.094	C19	-0.235	H30	0.165
C9	0.063	H20	0.137	H31	0.197
C10	0.603	H21	0.136	H32	0.171
N11	-0.699	H22	0.149	H33	0.169

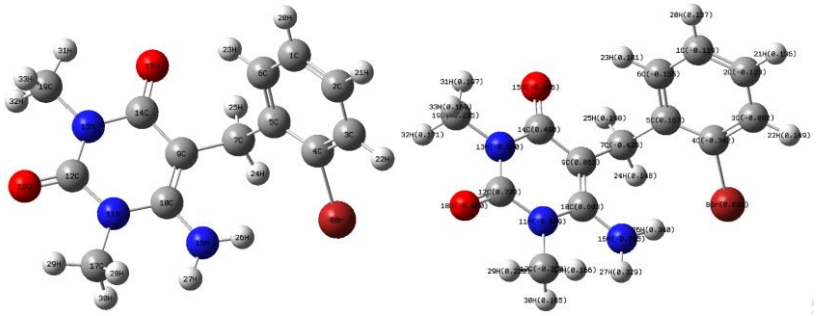


Figure 3. DFT optimized molecular structure and Mulliken charge distributions of **ABDD**.

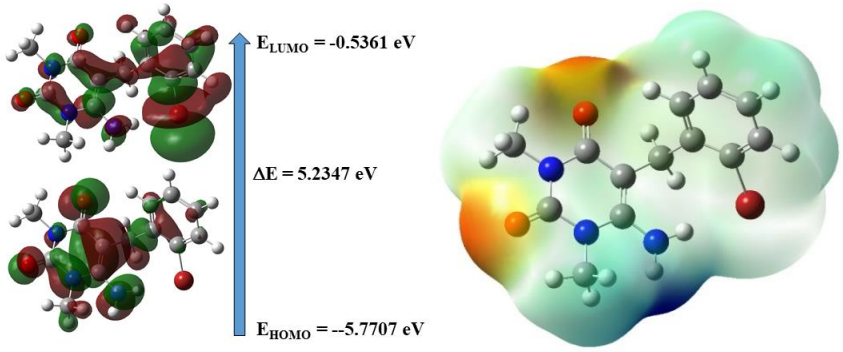


Figure 4. HOMO-LUMO orbitals and MEP surface of the of **ABDD**.

Conclusion

Synthesized molecule exhibits promising anticancer activity and antimicrobial, suggesting it as a valuable scaffold for further drug development. The combination of experimental data and computational insights indicates potential pharmacological benefits and supports further exploration of pyrimidine-based compounds in medicinal chemistry.

Acknowledgment

We are thankful to N.G.B. University for providing basic facilities to Radha Singh. The authors are also grateful to Maharana Pratap Group of Institutions Kanpur, Uttar Pradesh, and SAIF-IIT Patna, for providing NMR, and HRMS data.

Notes: The authors declare no competing financial interest.

References

Nerkar, A. U. (2021). Use of Pyrimidine and Its Derivative in Pharmaceuticals: A Review. *Journal of Advanced Chemical Sciences*, *7*(2), 729–732.

Patil, S. B. (2023). Recent medicinal approaches of novel pyrimidine analogs: A review. *Heliyon*, *9*(6), e16773.

Islam, M. W., Islam, M. M., Akter, R., Limon, T. R., Vasquez, E. S., Shaikh, M. a. A., & Habib, A. (2024). A review on pyrimidine-based derivatives: Synthesis and their biological application. *Journal of Heterocyclic Chemistry*, *61*(7), 1159–1179.

N, J. B., &Goudgaon, N. M. (2021). A comprehensive review on pyrimidine analogs-versatile scaffold with medicinal and biological potential. *Journal of Molecular Structure*, *1246*, 131168.

Islam, M. W., Islam, M. M., Akter, R., Limon, T. R., Vasquez, E. S., Shaikh, M. a. A., & Habib, A. (2024). A review on pyrimidine-based derivatives: Synthesis and their biological application. *Journal of Heterocyclic Chemistry*, *61*(7), 1159–1179.

Manna, T., Maji, S., Maity, M., Debnath, B., Panda, S., Khan, S. A., Nath, R., & Akhtar, M. J. (2024). Anticancer potential and structure activity studies of purine and pyrimidine derivatives: an updated review. *Molecular Diversity*. https://doi.org/10.1007/s11030-024-10870-4

Mahapatra, A., Prasad, T., & Sharma, T. (2021). Pyrimidine: a review on anticancer activity with key emphasis on SAR. *Future Journal of Pharmaceutical Sciences*, *7*(1). https://doi.org/10.1186/s43094-021-00274-8

Nadar, S., & Khan, T. (2021). Pyrimidine: An elite heterocyclic leitmotif in drug discovery-synthesis and biological activity. *Chemical Biology & Drug Design*, *100*(6), 818–842.

Chiacchio, M. A., Iannazzo, D., Romeo, R., Giofrè, S. V., &Legnani, L. (2018). Pyridine and Pyrimidine Derivatives as Privileged Scaffolds in Biologically Active Agents. *Current Medicinal Chemistry*, *26*(40), 7166–7195.

Bhatnagar, A., &Pemawat, G. (2024). Anticancer and antibacterial Activeness of fused Pyrimidines: Newfangled Updates. *Bioorganic Chemistry*, *153*, 107780.

Alhilal, M., Sulaiman, Y. a. M., Alhilal, S., Gomha, S. M., &Ouf, S. A. (2021). Synthesis of Novel Acyclic Nucleoside Analogue Starting From 6-Aminouracil as Potent Antimicrobial Agent. *Polycyclic Aromatic Compounds*, *42*(9), 6463–6474.

Pałasz, A., &Cież, D. (2014). In search of uracil derivatives as bioactive agents. Uracils and fused uracils: Synthesis, biological activity and applications. *European Journal of Medicinal Chemistry*, *97*, 582–611.

El-Kalyoubi, S., Agili, F., Adel, I., &Tantawy, M. A. (2021). Novel uracil derivatives depicted potential anticancer agents: In Vitro, molecular docking, and ADME study. *Arabian Journal of Chemistry*, *15*(4), 103669.

Hussain, Z., Ibrahim, M. A., El-Gohary, N. M., &Badran, A. (2022). Synthesis, characterization, DFT, QSAR, antimicrobial, and antitumor studies of some novel pyridopyrimidines. *Journal of Molecular Structure*, *1269*, 133870.

Cawrse, B. M., Lapidus, R. S., Cooper, B., Choi, E. Y., &Seley-Radtke, K. L. (2017). Anticancer Properties of Halogenated Pyrrolo[3,2-d]pyrimidines with Decreased Toxicity via N5 Substitution. *ChemMedChem*, *13*(2), 178–185.

Tylińska, B., Wiatrak, B., Czyżnikowska, Ż., Cieśla-Niechwiadowicz, A., Gębarowska, E., &Janicka-Kłos, A. (2021). Novel Pyrimidine Derivatives as Potential Anticancer Agents: Synthesis, Biological Evaluation and Molecular Docking Study. *International Journal of Molecular Sciences*, *22*(8), 3825.

Ali, D., Mondal, N., Panday, A. K., & Choudhury, L. H. (2023). Synthesis of Selenocyanates and Selenoethers of Amino Pyrazoles and Amino Uracils by In Situ Triselenium Dicyanide from Malononitrile and Selenium Dioxide. *ACS*

Omega, *8*(28), 25349–25357.

Panday, A. K., Ali, D., Parvin, T., & Choudhury, L. H. (2023). Metal-Free Synthesis of Pyrimidine and Naphthoquinone-Fused Pyrroles from Arylglyoxal-Based Domino Reactions. *ChemistrySelect*, *8*(14). https://doi.org/10.1002/slct.202300158

Ali, D., Panday, A. K., & Choudhury, L. H. (2020). Hydrogen Peroxide-Mediated Rapid Room Temperature Metal-Free C(sp2)-H Thiocyanation of Amino Pyrazoles, Amino Uracils, and Enamines. *The Journal of Organic Chemistry*, *85*(21), 13610–13620.

Panday, A. K., Ali, D., & Choudhury, L. H. (2020). One-pot synthesis of pyrimidine linked naphthoquinone-fused pyrroles by iodine-mediated multicomponent reactions. *Organic & Biomolecular Chemistry*, *18*(26), 4997–5007.

Mahata, A., Bhaumick, P., Panday, A. K., Yadav, R., Parvin, T., & Choudhury, L. H. (2020). Multicomponent synthesis of diphenyl-1,3-thiazole-barbituric acid hybrids and their fluorescence property studies. *New Journal of Chemistry*, *44*(12), 4798–4811.

Jana, A., Bhaumick, P., Panday, A. K., Mishra, R., & Choudhury, L. H. (2019). I2/DMSO mediated multicomponent reaction for the synthesis of 2-arylbenzo[d]imidazo[2,1-b] thiazole derivatives. *Organic & Biomolecular Chemistry*, *17*(21), 5316–5330.

Panday, A. K., Mishra, R., Jana, A., Parvin, T., & Choudhury, L. H. (2018). Synthesis of Pyrimidine Fused Quinolines by Ligand-Free Copper-Catalyzed Domino Reactions. *The Journal of Organic Chemistry*, *83*(7), 3624–3632.

M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, Williams, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman and D. J. Fox, Gaussian 16, Revision B.01, Gaussian, Inc., Wallingford CT, 2016. R.