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Research Article

Zinc Acetate as a Catalyst: Improved Method of Protection of Amino Group in Synthesis of Nucleobase Derivatives and its Biological Applications

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ABSTRACT

2-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-1H-benzoisoquinoline-1,3(2H)-dione was synthesized by reacting 5-amino uracil with 1,8-naphthalic anhydride in the presence of zinc acetate as a catalyst and screened for the *in-silico/in-vitro* antimicrobial activities. All the analyzed physicochemical descriptors (*viz.* drug-likeness, bioactivity score, and absorption, distribution, metabolism, excretion, and toxicity (ADMET)) of the compound provide an impetus to the docking results obtained by simulating against the catalytic site of *Escherichia coli* peptide deformylase enzyme (PDF, PDB ID: 1G2A) and allosteric site of human immunodeficiency viruses (HIV) reverse transcriptase enzyme (PDB ID: 3MEC). Docking results revealed that the compound has interacted well with the amino acid residues Lys103, Lys101, and Pro 236 of non-invasive blood pressure (NIBP) of human immunodeficiency viruses (HIV) reverse transcriptase (RT) and Ile44, Gly89, Leu91, and Glu133 of peptide deformylase (PDF). The predicted free binding energy ΔG (-6.83 kcal/mol) showed that the compound formed a stable complex with PDF protein receptor, thus, can behave as a probable drug regimen in antibiotics. Whereas, the calculated total binding energy (-38.87 kcal/mol) alluded to the anti-HIV property of the compound as probable nucleoside reverse transcriptase inhibitor (NNRTI). *In-vitro* antibacterial screening, results exhibited promising inhibitory activity against *Pseudomonas aeruginosa and E. coli* (MIC value, 12.5 and 25 µg/mL, respectively).

INTRODUCTION

An infectious disease that occurs due to viruses and bacteria is a serious matter of concern to human health, despite a great development in medicinal chemistry. The easy outspread of the microorganism and its high-intensity effects are the major cause of infectious diseases affecting millions of people with fluctuation in the death rate. Nowadays, one of the fundamental tasks of medicinal chemistry research is the discovery and development of small and novel antimicrobial agents along with new structure designs and varied molecular targets. The most serious challenge in novel drug development is the emergence of multi-drug resistance pathogenic viruses/bacteria, as the microorganism is getting resistant by a mutation which changed the membrane permeability and daughter particles forming tendency. The microorganism can withstand the potency by adapting to the already present drug. Therefore, due to the limited treatment options of multi-resistant isolates, the discovery and development of new treatment options and the synthesis of novel, more potent compounds with high efficacy are still in demand to overcome these problems.[1] In this series, the pharmaceutical application of compounds formed as intermediates in several organic synthesis can prove to be a highly cheap, cost-effective, easy, and less time-consuming step in novel treatment options.

Nucleobases are of great importance to biologists and chemists as it is a chemically useful moiety with various

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biological activities such as antibacterial, anti-fungal, and anti-HIV (human immunodeficiency viruses), antimalarial, anti-inflammatory, anticancer, antihypertensive.[2-6] Some prominent antibacterial agents having nucleobases are 8-hydroxyquinoline (8HQ), ciprofloxacin, primaquine, tafenoquin (8AQ), sitamaquine and clotrimazole.

Similarly, naphthalic anhydride derivatives have been the focus of attention for a long time due to their wide array of biological activities.[7-12] These derivatives are known to subdue bacterial growth by targeting several pathways, leading to the inhibition of the biosynthesis of essential amino acids and ultimately preventing the growth and reproduction of microorganisms.

Owing to the such significance and keeping in view the wide range of pharmaceutical activities of nucleobases.[13-16] and anhydride scaffolds, in this report, we expect that the incorporation of these two in the same scaffold structure may lead to better activity and elevate the antiviral/antibacterial potential. Focusing our research on the search for new antibacterial and antiviral agents, we have designed and developed anhydride-protected uracil derivatives and subjected them to *in silico* studies against HIV-1 (human immunodeficiency virus-1) reverse transcriptase and bacterial *E. coli* - PDF (*Escherichia coli* - peptide deformylase). *In-vitro* screening was also performed on the synthesized compound, and other *in-silico* pharmacokinetic assessments were also performed on this molecule.

The present study owns a significant pharmaceutical advantage as it provides insight into the development and utilization of stable reaction intermediates of great biological importance, formed during multistep organic oligonucleotide synthesis as probable drug regimen.

MATERIALS AND METHODS

Design, Physicochemical Descriptors, Bioactivity Score, and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) Properties of the Compound

ChemDraw and molinspiration software were used to predict the drugs like characteristics such as molecular weight, hydrogen bond donors, hydrogen bond acceptors, log Portable bonds and TPSA (total polar surface area), and bioactivity scores of the compound.[17] The molecule was assessed in the eyes of Lipinski's rule of five.[18]ADMET and other pharmaceutically relevant properties like aqueous solubility (Log S), skin permeability (Log Kp), and synthetic accessibility score of the designed compound were evaluated with the help of SwissADME and pkCSM online available software.[19-20]

Molecular Docking Studies

The designed compound was optimized and prepared for molecular simulations using BIOVIA/Discovery Studio 2016 Client (DS 16.1.0.15350 version) protocol default parameters.[21] The 3D X-ray crystal structure of docking receptors, viz. catalytic core of peptide deformylase enzyme (PDF, PDB ID: 1G2A) and allosteric site of human immunodeficiency viruses reverse transcriptase enzyme (HIV-1 RT, PDB ID: 3MEC), were retrieved as an adduct from research laboratory for structural bioinformatics (RCSB, www.rcsb.org) protein data bank.[20, 22] All missing hydrogens were updated and the excess water molecules were removed.[19] Further, proteins were optimized by applying CHARMmforcefield [17, 23] and the adopted basis set newton raphson (ABNR) method, available in the DS protocol. Moreover, the ligand was prepared accordingly and docked by using the top-ranked key interacted ligand docking pose. Overall, the standard measures were employed for preparing, docking, and scoring proteins with ligands.[24-29]

Chemistry

All the chemicals and reagents of the analytical grade were purchased from a commercial supplier (Sigma Aldrich chemical company, USA, and E. Merck India Ltd, India). Dried-distilled solvents and oven-dried apparatus were used for all the chemical reactions. The course of the reaction till completion was routinely monitored by thin-layer chromatography (TLC) on Merck pre-coated silica gel 60F254 aluminium plates and the separated compound was visualized by illumination under UV (ultraviolet-visible) lamp at 254 nm.[19] Evaporations were carried out under reduced pressure (Rotary evaporator) with bath temperature below 40°C unless specified otherwise. The melting point was determined in an open capillary with the help of an electrochemical apparatus. Electrospray ionization (ESI) mass spectra was obtained by Micromass Q-Tof (ESI-HRMS), the positive ion is reported as *m/z*, and nuclear magnetic resonance (NMR) spectra was recorded in DMSO-d_6 (^{1}H at 400 MHz and^{13} C at 100 MHz) on a BRUKER-AV400 spectrometer (Bruker Co., Faellanden, Switzerland) with solvent signals allotted as internal standard. Chemical shifts (δ) are expressed in parts per million (ppm) relative to the standard chemical shift of Tetramethylsilane (TMS) and coupling constants (J) values in Hz. Multiplicities are indicated as singlet (s), doublet (d), double doublet (dd), triplet (t), quartet (q), and multiplet (m). Elemental analysis for the compound was performed by Perkin-Elmer 240-C analysis equipment and agreed within 0.4% of the theoretical values.[20, 30]

Experimental Procedure for Synthesis of 2-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-1H-benzoisoquinoline-1,3(2H)-dione

In a 100 mL evacuated round-bottom flask equipped with a Claisen adapter and stirrer, 1,8 – naphthalic anhydride (0.504 mmol) was suspended in dry pyridine. Then 5 amino-uracil (0.252 mmol) was added to it and allowed to stir for 10 minutes. After that, zinc acetate was added as a catalyst. The reaction mixture was allowed to be stirred for 5 hourse at 120°C.[31] Progress of the reaction was monitored at an interval of 1 hours through thin-layer chromatography (TLC) with ethyl acetate/hexane as the developing solvent, and the newly formed product appeared as a bright pink spot on TLC when exposed to UV light at 365 nm. On completion of the reaction, the reaction mixture was cooled to room temperature and evaporated under reduced pressure. The reaction mixture was then partitioned into ethyl acetate and water. Organic extracts were then dried over anhydrous sodium sulphate, filtered, and the filtrate was evaporated under reduced pressure. The final product was purified through column chromatography using ethyl acetate and hexane as an eluting solvent and recrystallized using ethanol to yield 87%.

2-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-1*H*-benzoisoquinoline-1,3(2*H*)-dione

Yield 87%, greyish solid; m.p. 236-238°C;R_f -0.58 (Ethyl acetate:Hexane::3:7); ^{1}H-NMR (400 MHz, DMSO-d_6) δppm:10.52 (s, 1H, -N-H), 11.23 (s, 1H,-N-H), 8.47-8.44 (m, 4H, Ar-H), 7.84 (dd, 2H, J = 7.98 Hz, Ar-H), 7.80 (s, 1H, Ar-H);^{13}C NMR (100 MHz, DMSO-d6) δppm:164.2, 161.3, 150.7, 137.7, 137.5, 129.4, 128.6, 128.1, 125.6, 123.6, 108.3; HRMS m/z $(M+H)^+$: 308, Calcd. For $C_{16}H_9N_3O_4$: 307.05, Anal. Calcd. for $C_{16}H_9N_3O_4$: C, 62.57; H, 2.97; N, 13.69, O, 20.86. Found: C, 62.54; H, 2.94; N, 13.68; O, 20.83.

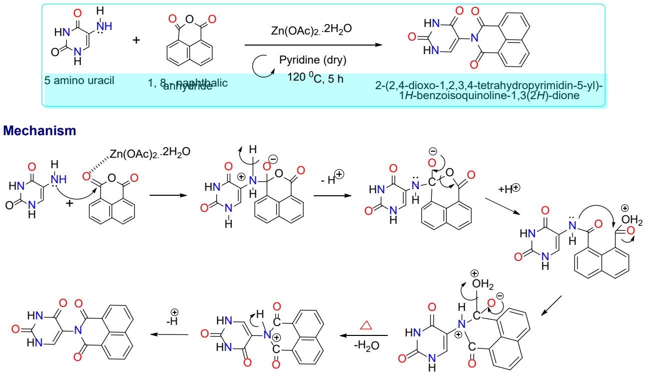
Antibacterial Assay

Based on the National Committee for Clinical Laboratory Standards recommendation, the broth microdilution susceptibility test with minor modifications was employed to screen the synthesized compound for its antibacterial activity.[19,26,32] For this, the nutrient broth was taken as media. Synthesized compound was screened against most pathogenic, two gram-positive (*viz. Bacillus cerus* NCIN-2156 and *Staphylococcus Aureus* NCIM-2079) and two gram-negative (*viz. E. coli* : NCIM-2065 and *Pseudomonas aeruginosa*: NCIM-2036) bacterial strains, to evaluate their antibacterial activity in the form of minimum inhibitory concentration (MIC, µg/mL). In this study, chloramphenicol was employed as the standard reference. A stock solution of the test compound and reference drug were prepared in dimethyl sulfoxide (DMSO) at 100 µg/mL concentration. Ten test tubes in triplicate, two sets for the test compound and one set for reference control, were prepared to screen the compound at a given concentration. Test tubes 2–9 contained 0.8 mL of nutrient broth. Tube 1 (negative control- without bacteria and antibiotic) contained 1-mL of nutrient broth, and tube 10 (positive control- with bacteria) contained 0.9 mL of nutrient broth and 0.1 mL of tested bacteria. Previously prepared stock solution of test compound (100 µg/mL) was serially diluted in tubes 2–9 and the obtained concentrations of compound were 100 µg/mL (tube 2), 50 µg/mL (tube 3), 25 µg/mL (tube 4), 12.5 µg/mL (tube 5), 6.25 µg/mL (tube 6), 3.12 µg/mL (tube 7), 1.56 µg/mL (tube 8) and 0.78 µg/mL (tube 9). Test bacteria (0.1 mL) with a concentration of 0.5 McFarland scale (9×108 cells/mL) was added to test tubes 2–9. Test tubes were covered with cotton plugs in sterilized condition and incubated at 37°C for 24 hours in a humid chamber to observe the macroscopic growth or inhibitory potency. After 24 hours, the lowest concentration of the test compound was noted as the MIC (minimum inhibitory concentration) value on which no visible turbidity, i.e., no visible macroscopic growth of bacteria, was found.[20,27]

Table 1: Physicochemical Analysis

S. No.	*Descriptor*	*Value*
1	Log P	1.07
2	TPSA	104.80
3	MW	304.26
4	H-A	7
5	H-D	2
6	No. of violations	0
7	N rot b	1
8	Molar Refractivity	85.75
9	N–atoms	23
10	Volume	247.29

Log P: Predicted octanol-water partition coefficient; **TPSA**: Total polar surface area; **N- atom** = Total number of atoms; **MW**: Molecular weight; **H-A**: Number of H bond acceptors; **H-D**: Number of H bond donors; **N rot b** = Number of rotatable bonds; **volume** = Molecular volume of distribution (Å^3).



Scheme 1: Synthesis of compound 2-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-1H benzoisoquinoline-1,3(2H)-dione

RESULT AND DISCUSSION

Analysis of Physicochemical Properties of the Compound

The compound has been designed and analyzed for its pharmaceutical properties in the eyes of Lipinski's rule of five using the molinspiration, Swiss ADME, and ChemDraw online software. According to Lipinski's rule of five, the

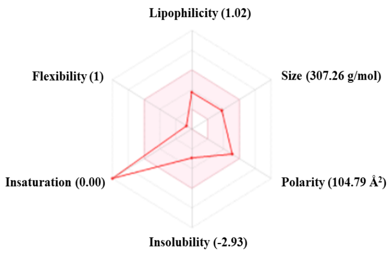
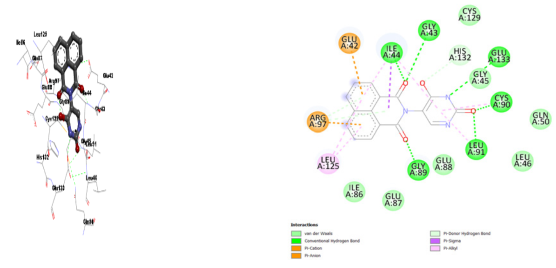


Fig. 1: Radar graph of compound showing drug-likeness characteristics (pink area focuses the permissible values of drug-likeness characteristics of the molecule), (LIPO - Lipophilicity, SIZE - Molecular weight, FLEX - Rotatable bonds, INSOLU - Insolubility, INSATU – Insaturation, fraction Csp3)

Table 2: Bioactivity Score

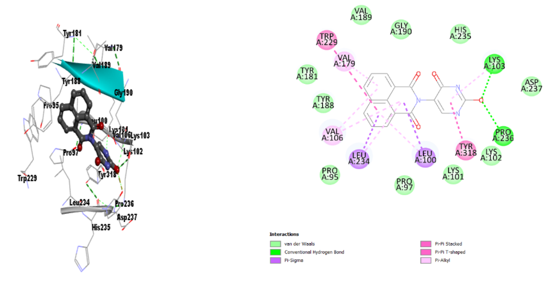
S. No.	*Physicochemical Descriptor*	*Value*
1.	GPCR	-0.16
2.	Ion channel modulator	-0.45
3.	Kinase inhibitor	-0.14
4.	Nuclear receptor ligand	-0.27
6.	Protease inhibitor	-0.39
7.	Enzyme inhibitor	-0.07

GPCR (G-protein coupled receptor-ligand): (-0.62 to -0.39, moderate activity), **Ion channel modulator:** (0.27 to 0.05, significant activity), **Protein kinase inhibitors:** (0.40 to 0.18, significant activity), **Nuclear receptor:** (-0.63 to -0.31, moderately active), **Protease-activated receptors:** (-0.15 to 0.00, moderate activity) and **Enzyme inhibitor:** (0.61 to 0.48, significant activity)



Compound with PDF receptor (3D)

Compound with PDF receptor (2D)



Compound with HIV RT receptor (3D)

Compound with HIV RT receptor (2D)

Fig. 2: Docking interactions of the designed compound with *E. coli* PDF and HIV-RT in ligand-receptor docked complex (2D and 3D diagram)

Table 3: ADMET Analysis

ADME Result			
S. No.	*Descriptor*	*Value*	*Category*
1.	Pure_water_solubility_mg_L	1.55809	Absorption
2.	HIA	95.20991	
3.	Skin_Permeability	-4.51749	
4.	Plasma Protein_Binding	63.231231	Distribution
5.	BBB	0.116633	
6.	Caco2	18.3175	
7.	MDCK	1.125	
8.	Pgp_inhibition	No	Metabolism
9.	CYP_2C19_inhibition	No	
10.	CYP_2C9_inhibition	Inhibitor	
11.	CYP_2D6_inhibition	No	
12.	CYP_2D6_substrate	No	
13.	CYP_3A4_inhibition	No	
14.	CYP_3A4_substrate	No	
15.	Total clearance (logCLtot)	0.286	Excretion
Toxicity Result			
16.	Ames_test	Mutagen	Toxic
17.	Carcino_Mouse	Positive	
18.	Carcino_Rat	Positive	
19.	Daphnia_at	0.378367	Non-Toxic
20.	hERG_inhibition	medium_risk	
21.	Minnow_at	0.571115	

Water solubility = < 4 soluble; **HIA (Human Intestinal Absorption)** = below 30 indicates poor absorbance; **Skin permeability** = < -2.5 considered to be permeable; **Blood brain barrier permeability** = < -1 considered poorly distributed to the brain; **Total clearance (logCLtot)** = Lower value indicates high drug half lifetime.

hydrogen bond acceptor should be ≤ 10; the hydrogen bond donor should be ≤ 5, the molecular weight of the compound should be less than ≤ 500, lipophilicity indicated by Log P should be less than ≤ 5, and total polar surface area (TPSA) should be ≤ 140. Here, the designed compound follows this rule very strictly. The lower value of lipophilicity (Log P, 1.07) displayed better cell internalization than the standard drugs.[17-20, 30-34] The number of rotatable bonds < 5, considered that compound must have drug like characteristics. The physicochemical data of compound and reference drugs are shown in Table 1 and as a radar graph in Fig. 1.

Similarly, the bioactivity scores viz. ion channel modulator, nuclear receptor ligand, a kinase inhibitor, and G-protein coupled receptor (GPCR) have also been calculated to predict more drug-like properties. It was considered that the compound with a bioactivity score less than -0.50 might be found pharmaceutically inactive, and those

Table 4: Docking scores of ligand-receptor docked complexes

Complex	*PLP1*	*PLP2*	*PMF*	*Lib-DS*	*EE*	*Ludi2*	*Ludi3*	*ΔG*	*EC_{50} (Predicted)*
Comp. + PDF	92.89	88.00	71.33	127.76	-31.08	482	557	-6.83	2.69×10^{-6}
Comp. + HIV RT	88.85	87.16	103.83	111.60	-31.08	474	541	-6.72	3.89×10^{-6}
Chloramphenicol	64.14	63.73	-15.49	97.57	477	477	505	-6.76	8.90×10^{-5}
Nevirapine	66.92	69.08	85.81	86.57	-4.32	425	728	-6.02	5.24×10^{-8}

PLP = Piecewise Linear Potential, **PMF** = Potential of Mean Force, **Lib-DS** = Dock Score, **EE** = Electrostatic energy, **Ludi2** and **Ludi3** = Empirical scoring functions derived from the Ludi algorithm, **ΔG** = Predicted binding free energy (kcal/mol), **Predicted EC_{50}** = Predicted 50% effective concentration of given compound essential to reduce bacterial growth (μM).

Table 5: Docking scores of ligand-receptor docked complexes

Complex	*Vander Waals Energy*	*Bond Energy*	*Binding Energy*	*Complex Energy*	*Total Binding Energy*	*Complex Entropy*	*Ligand Conf. Entropy*	*Ligand RMSD Ref.*
Comp. + PDF	-591.93	3.11	-26.55	-2481.87	-26.14	-29.499	0.4106	0.3916
Comp. + HIV RT	7.464	1.329	-38.87	-8358.02	-38.87	-32.91	0	0.2464
Chloramphenicol	-2.98	0.75	-33.72	-2680.85	-31.39	-29.50	1.15	0.52
Nevirapine	-0.618	0.53	--	--	--	--	--	--

Conf. = Conformational, **RMSD =** Root mean square deviation

with a bioactivity score greater than -0.50 should be pharmaceutically active. Here, all the scoring parameters of the compound were found within an acceptable range of standard drugs[20,34] and the results are summarized in Table **2**.

The ADMET properties of the compound have been presented in Table **3**.

Molecular Docking Studies of Designed Compound

Molecular docking is a computational approach to analyzing designed compounds' binding ability and interaction with the target protein.[17, 22] The compound was docked into the active site of the *E. coli* - PDF enzyme to analyze the antibacterial activity and at the allosteric site of the HIV-RT enzyme to analyze its antiviral activity. Several scoring functions (Dock score, piecewise linear potentials, binding energy, electrostatic energy, predicted $EC_{50,}$ etc.) were generated from the molecular docking to predict the stability of complexes formed between compound and protein.[23-29]

A higher piecewise linear potential (PLP) value displayed the potent binding affinity of the ligand with the target protein. Here, the compound showed a strong binding affinity with receptor protein, as their PLP1 and PLP2 scores were 92.89 and 88.00 against the PDF receptor and 88.85 and 87.16 against the HIV-RT receptor, subsequently. Ligand internal energy (LIE) is the addition of electrostatic and van der waals (vdW) energy, which is used to know ligand binding efficiency with the target protein.[23] The lower value of the ligand's internal energy indicates the higher stability of the protein-ligand complex. A deep analysis of the result displayed that compound had a lower value of internal ligand energy (LIE) than the reference drug against both targeted receptors (PDF and HIV RT).

Ludi2 and Ludi3 values are very helpful in predicting the exact conformation of the complex formed between the ligand and protein.[28,29] Binding energy indicated by ΔG is calculated by the Ludi2 and predicted EC_{50} values of the compound are calculated by the Ludi3.[32-34] It is worth mentioning that a higher affinity for forming stable protein-ligand complexes is associated with higher -ΔG values, which may lower EC_{50} values. Results presented in Table 4 revealed that the compound›s binding energy and predicted EC_{50} value were found to be better than the reference drugs Chloramphenicol-antibacterial and nevirapine-anti-HIV.

Dock Score (DS) predicts the strength of the binding affinity of the ligand with the target protein. The compound displayed a value of dock score comparable to the reference drug.[20]

From the result of docking simulations, it was confirmed that the compound had a virtuous binding affinity and exhibited a better mode of interactions with amino acid residues of PDF[32-34] and HIV-RT receptor proteins[18,30] as compared to the reference drugs (chloramphenicol–antibacterial and nevirapine–anti-HIV) used for analysis results are presented in Table 5.

The compound's interactions were explained as hydrogen bonds/electrostatic interactions and hydrophobic interactions. A close analysis displayed that compound formed 4 H-bonds with Ile 44 (2.90 Å), gly 89 (2.94 Å), leu 91 (2.97 Å), and glu 133 (3.12 Å) amino acid residue at the catalytic site of PDF protein receptor,[27-29]and 4 H-bonds with lys 103 (2.82 and 2.65 Å), lys 101 (2.73 Å) and pro 236 (2.59 Å) amino acid residue at the allosteric site of HIV-RT protein receptor.[18,30] The distinctive alignment of the compound implied that it got accommodated well within the binding pockets and thus provided the basis for the inhibitory action of the compound against targeted proteins.

Table 6: Docking interactions of the compound with amino acid residues of targeted receptors (bacterial Peptide deformylase enzyme/ HIV reverse transcriptase enzyme)

Complex	Hydrogen bonds							Electrostatic/Hydrophobic interaction						
	No. of H-B	*Types of bonds*	*A-A in H-B*	*H-B Type*	*D(▯)*	*D-A*	*A-A*	*No. of Bonds*	*Type of Bonds*	*A-A in Bond*	*H-B Type*	*D(▯)*	*D-A*	*A-A*
Comp. + PDF	10	CHB	Ile 44	Ile 44:HN - **M:1**:O15	2.90	Ile44:HN(N)	**M:1**:O15	9	E(Pi-Cation)	Arg97	Arg97:NE – **M:1**	4.60	Arg97:NE (+ve)	**M:1**(Pi-O)
		CHB	Gly89	Gly89:N - **M:1**:O14	2.94	Gly89:N(N)	**M:1**:O14		E(Pi-Anion)	Glu42	Glu42:OE2 – **M:1**	4.06	Glu42:OE2 (-ve)	**M:1**(Pi-O)
		CHB	Leu91	Leu91:N - **M:1**:O23	2.97	Leu91:N	**M:1**:O23		(Pi-Sigma)	Ile44	Ile44:CD1 – **M:1**	3.80	Ile44:CD1	**M:1** (Pi-O)
		CHB	Glu133	**M:1**:N18–Glu133:OE2	3.12	**M:1**:N18	Glu133:OE2		(Pi-Alkyl)	Leu125	**M:1 –** Leu125	5.18	**M:1** (Pi-O)	Leu125 (Alkyl)
		CHB	Gly43	Gly43:N - **M:1**:O15	3.39	Gly89:N	**M:1**:O15		(Pi-Alkyl)	Ile44	**M:1-** Ile44	5.02	**M:1** (Pi-O)	Ile44 (Alkyl)
		CHB	Cys90	Cys90:SG – **M:1**:O23	3.67	Cys90:SG	**M:1**:O23		(Pi-Alkyl)	Leu125	**M:1 –** Leu125	5.39	**M:1** (Pi-O)	Leu125 (Alkyl)
		(Pi-Do)HB	Arg97	Arg97:NH2-**M:1**	4.16	Arg97:NH2	**M:1**		(Pi-Alkyl)	Ile44	**M:1-** Ile44	4.90	**M:1** (Pi-O)	Ile44 (Alkyl)
		(Pi-Do)HB	Arg97	Arg97:NH2-**M:1**	3.99	Arg97:NH2	**M:1**		(Pi-Alkyl)	Cys90	**M:1-** Cys90	5.12	**M:1** (Pi-O)	Cys90 (Alkyl)
		(Pi-Do)HB	Arg97	Arg97:NH2-**M:1**	3.88	Arg97:NH2	**M:1**		(Pi-Alkyl)	Leu91	**M**:1 – Leu91	5.14	**M:1** (Pi-O)	Leu91 (Alkyl)
		(Pi-Do)HB	His132	**M:1:O22-** His132	3.52	**M:1:**O22	His132							
Comp. + HIV-RT	4	CHB	Lys103	Lys103:N - **M:1**:O23	2.82	Lys103:N	**M:1**:O23	10	(Pi-Sigma)	Leu100	Leu100:CD1 **- M:1**	3.94	Leu100:CD1	**M:1**(Pi-O)
		CHB	Lys101	**M:1:N20** - Lys101:O	2.73	**M:1:N20**	Lys101:O		(Pi-Sigma)	Leu234	Leu234:CD2 – **M:1**	3.68	Leu234:CD2	**M:1**(Pi-O)
		CHB	Lys103	**M:1:O23** - Lys103:O	2.65	M:1:O23	Lys103:O		(Pi-Pi Stack)	Tyr318	Tyr318 – **M:1**	3.58	Tyr318 (Pi-O)	**M:1** (Pi-O)
		CHB	Pro236	**M:1:O23** – Pro236:O	2.59	M:1:O23	Pro236:O		(Pi-Pi T-Shaped)	Trp229	Trp229 – **M:1**	5.29	Trp229 (Pi-O)	**M:1 (**Pi-O)
									(Pi-Alkyl)	Leu100	**M:1-** Leu100	4.99	**M:1 (**Pi-O)	Leu100 (Alkyl)
									(Pi-Alkyl)	Val106	**M:1** – Val106	5.29	**M:1** (Pi-O)	Val106 (Alkyl)
									(Pi-Alkyl)	Leu234	**M:1 –** Leu234	5.08	**M:1** (Pi-O)	Leu234 (Alkyl)
									(Pi-Alkyl)	Val106	**M:1** – Val106	5.06	**M:1** (Pi-O)	Val106 (Alkyl)
									(Pi-Alkyl)	Val179	**M:1 –** Val179	4.53	**M:1** (Pi-O)	Val179 (Alkyl)
									(Pi-Alkyl)	Lys103	**M:1** – Lys103	4.60	**M:1** (Pi-O)	Lys103 (Alkyl)
Ref. 1 + PDF	9	CHB	Gln50	Gln50:H21 - **Ref.1**:O19	2.32	Gln50:H21	**Ref.1**:O19(H)	--	--	--	--	--	--	--
		CHB	Gly89	Gly89:HN - **Ref.1**:O15	1.88	Gly89:HN(H)	**Ref.1**:O15(H)							
		CHB	Cys90	Cys90:H -**Ref.1**:O20	2.20	Cys90:H	**Ref.1**:O20(H)							
		CHB	Leu91	Leu91:HN - **Ref.1**:O20	2.00	Leu91:HN(H)	**Ref.1**:O20(H)							
		CHB	Glu133	Glu133:H2 - **Ref.1**:O19	2.65	Glu133:H2	**Ref.1**:O19(H)							
		CHB	Gly89	**Ref.1**:H31 - Gly89:O	1.90	**Ref.1**:H31	Gly89:O(H)							
		CHB	Glu87	**Ref.1**:H37 - Glu87:O	1.81	**Ref.1**:H37	Glu87:O(H)							
		CHB	Leu46	Leu46:H - **Ref.1**:O19	2.45	Leu46:H	**Ref.1**:O19(H)							
		CHB	Cys90	Cys90:H - **Ref.1**:O20	2.73	Cys90:H	**Ref.1**:O2(H)							

To be continued...

Ref. 2 + HIV-RT	2	CHB	Tyr188	**M:1**:O20 – Tyr188:O	2.80	**M:1**:O20	Tyr188:O	13	(Pi-Sigma)	Leu100	Leu100:CD1-**M:1**	3.96	Leu100:CD1	**M:1**(Pi-O)
		Carbon HB	Tyr318	**M:1**:C17-Tyr318:OH	3.52	**M:1**:C17(H)	Tyr318:OH		(Pi-Sigma)	Leu234	Leu234:CD2- **M:1**	3.87	Leu234:CD2	**M:1**(Pi-O)
									Pi-Pi T-shaped	Trp229	Trp229- **M:1**	5.18	Trp229(Pi-O)	**M:1**(Pi-O)
									Pi-Pi T-shaped	Trp229	Trp229- **M:1**	5.25	Trp229(Pi-O)	**M:1**(Pi-O)
									Pi-Pi T-shaped	Tyr318	Tyr318-**M:1**	5.04	Tyr318(Pi-O)	**M:1**(Pi-O)
									(Alkyl)	Val106	Val106-**M:1**	4.14	Val106(Alkyl)	**M:1**(Alkyl)
									(Alkyl)	Lys103	**M:1**:C16-Lys103	4.13	**M:1**:C16(Alkyl)	Lys103(Alkyl)
									(Alkyl)	Val179	**M:1**:C16 – Val179	3.76	**M:1**:C16(Alkyl)	Val179(Alkyl)
									(Alkyl)	Leu234	**M**:1 – Leu234	5.49	**M:1**(Alkyl)	Leu234(Alkyl)
									(Pi-Alkyl)	Phe227	Phe227 – **M:1**	4.33	Phe227(Pi-O)	**M:1**(Alkyl)
									(Pi-Alkyl)	Tyr318	Tyr318- **M:1**	5.34	Tyr318(Pi-O)	**M:1**(Alkyl)
									(Pi-Alkyl)	Val106	**M:1 –** Val106	5.15	**M:1**(Pi-O)	Val106(Alkyl)
									(Pi-Alkyl)	Val106	**M:1 –** Val106	5.45	**M:1**(Pi-O)	Val106(Alkyl)

H-B= Hydrogen bonds, **A-A in H-B**= Amino acid involved in Hydrogen bond, **D (Å)**= Distance in **Å**, **D-A**= Donor atom, **A-A**= Acceptor atom, **Ref. 1**= Chloramphenicol (antibacterial), **Ref. 2**= Nevirapine (anti-HIV)

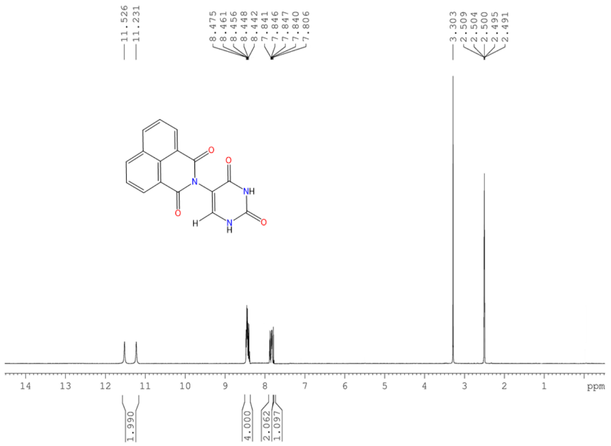


Fig. 3 : ^{1}H NMR of compound

Docking interactions of the compound and reference drugs with PDF and HIV-RT enzymes are summarized in Table 6, and resultant images are presented in Fig. 2.

Chemistry

The synthetic route for synthesizing 2-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-1H-benzoisoquinoline-1,3(2H)-dione is shown in Scheme 1. The compound has been synthesized by the reaction of 5-amino uracil with naphthalic anhydride in the presence of zinc acetate as a catalyst.

In previously published work the naphthaloyl group was found to be a selective amino protecting group for deoxycytidine, deoxyadenosine, and deoxyguanosine in oligodeoxyribonucleotide synthesis. Where triethylamine was used as a catalyst, but the yield was not satisfactory.[31]

Therefore, zinc acetate has been used as a catalyst in the present study to get an improved % yield.

Characterization data of the compound have been provided in Fig. 3.

Reagents and condition: Zinc acetate as a catalyst, pyridine as a solvent, temp 120°C, stirred for 5 hours

Antibacterial Studies

In-vitro antibacterial activity of the compound was performed by broth micro-dilution susceptibility test in Müller Hinton Broth against different strains of gram +ve bacteria, *viz. B. cerus* and *S. aureus,* and gram -ve bacteria, *viz. E. coli* and *P. aeruginosa*. Chloramphenicol was used as a standard control drug for antibacterial activity assessment. The MIC values of the compound are presented in Table 7.

The antibacterial screening studies revealed that the synthesized compound showed good to poor antimicrobial activity against the tested microorganisms. It is noticeable that the molecule exhibited good inhibitory activity against *P. aeruginosa*, moderate activity against *E. coli,* and poor inhibitory activity against *B. cerus* and *S. aureus* bacterial strains.[19,20,27,32-34]

Table 7: Antibacterial screening result of the compound against tested microorganisms.

S. No.	*Type of strain*	*Name of bacteria*	*MIC value in µg/mL*	*Inhibitory activity criteria*
1.	Gram + ve	*B. cerus*	50	Poor
2.		*S. aureus*	100	Poor
3.	Gram - ve	*E. coli*	25	Moderate
4.		*P. aeruginosa*	12.5	Good

MIC = Minimum Inhibitory Concentration in µg/mL

CONCLUSION

The compound 2-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-1H-benzoisoquinoline-1,3(2H)-dione has been designed and synthesized in a single step as one-pot synthesis. By analyzing several physicochemical/pharmaceutical/pharmacokinetics properties, it was observed that the compound is biologically active and can act as a drug-like agent. Moreover, to analyze the compound's antibacterial and antiviral activity, it was subsequently docked against *E. coli* peptide deformylase and HIV-RT enzyme receptors. The obtained result of calculated total binding energy clearly shows that the compound formed a comparatively robust complex with targeted HIV-RT receptor than the peptide deformylase enzyme receptor and also shows several hydrophilic/electrostatic and hydrophobic interactions with the amino acid residues present at the active site of the receptor. The *in-vitro* antibacterial screening result revealed that the compound has appreciable inhibitory potential against tested microorganisms and showed more potency against gram -ve bacterial strains. The reason behind the biological activity of the compound might be the presence of several heteroatoms (N, O). So, in conclusion, we can say that the designed compound, apart from being a protected nucleoside derivative that can be further employed for oligonucleotide synthesis, also has some drug-like characteristics. It was thus proved by docking simulation results against *E. coli* peptide deformylase enzyme and HIV-1 RT enzyme receptors. Thus, the study established a strong correlation between *in-silico* and *in-vitro* screening results. Hence, the compound can behave as a good antimicrobial agent. The present study also gave an insight into the improved method of protection of nucleoside and its derivatives with naphthalic anhydride.

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