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Synthesis, Characterization and Molecular Docking Studies Of New Erlenmeyer Azlactones

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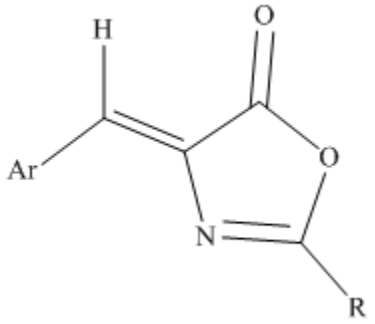
Abstract: *A series of Oxazol-5(4H)-ones are prepared by cyclization, condensation of O-HydroxyHippuric acid with various aromatic aldehydes in presence of acetic anhydride and sodium acetate in catalytic amounts. All the synthesized compounds were confirmed and characterized by using various spectral technique like IR,[1]HNMR, Mass spectral studies and C,H,N Analysis. Molecular Docking studies were carried out on these synthesized compounds and they showed better docking score. Docking studies of (4Z)-4-(arylidene)-2-(2-hydroxyphenyl)oxazol-5(4H)-ones with DNA (PDB ID:1N37)using patch dock server were performed. The aim of molecular docking is to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand such that the free energy of the overall system is minimised. The present focus of molecular docking is to computationally simulate the molecular recognition process. The Azlactone derivatives bind to DNA by intercalating in between the DNA base pairs. These molecules can be considered as good DNA intercalators based on docking score(binding affinity)and Emodel values(Complexation energy).*

Key words: *Azlactones, Docking , DNA intercalation, Hydrogen bonding &Oxazolone.*

I. Introduction

Erlenmeyer azlactones are five membered heterocyclic compounds containing nitrogen and oxygen as hetero atoms.

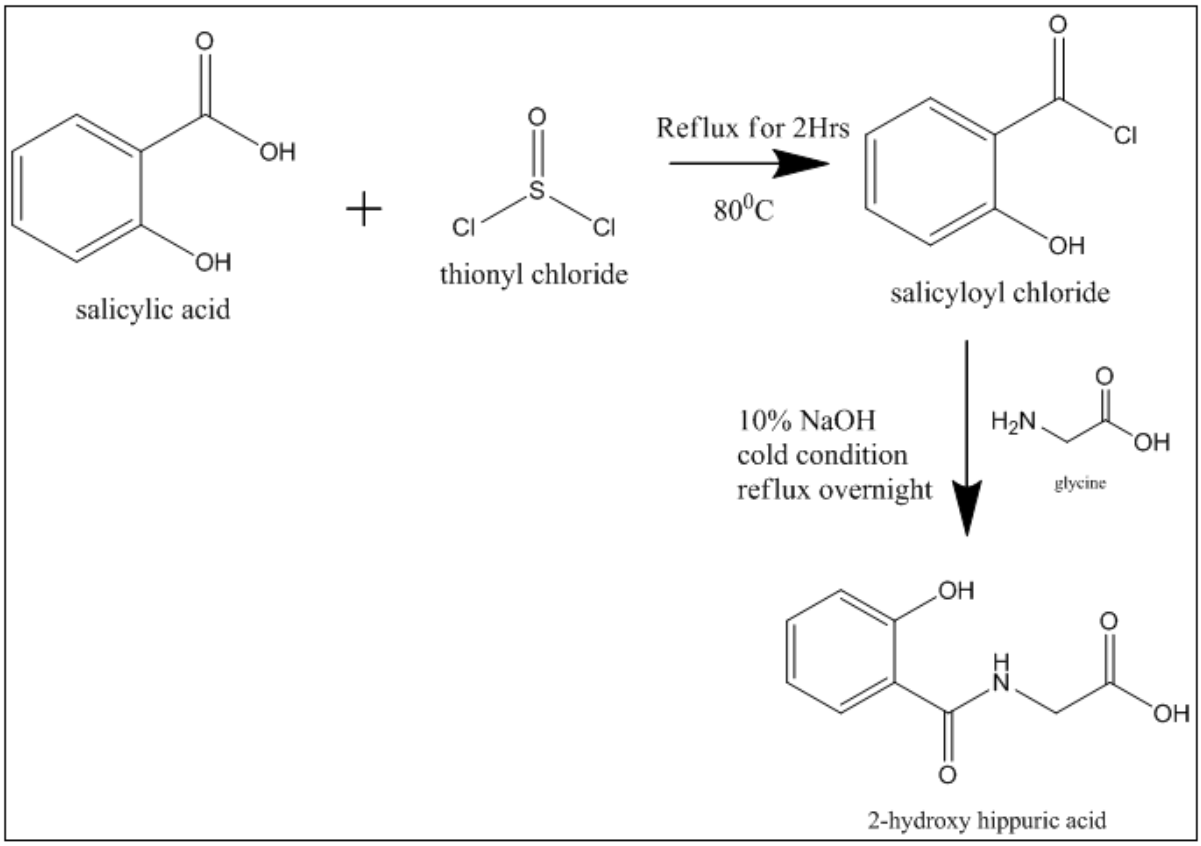


First Plochl [1] reported its formation by the acetic anhydride mediated condensation of hippuric acid with benzaldehyde. Erlenmeyer established the structure and named it as 'azlactone'.

These compounds exhibit important biological activities such as antimicrobial[2], antibacterial[3], analgesic[4], antifungal[5],anticancer[6],[7], anti-inflammatory[8],neuroleptic[9], sedative[10], antidiabetic[11] and antiobesity[12]. Azlactones are important intermediates in the preparation of several chemicals including Aminoacids[13],peptides[14], some heterocyclic precursors[15]as well as coupling and photosensitive devices for proteins[16].They exhibit promising photophysical and photochemical activities [17],[18],[19] and as PH sensors[20]. During the past few decades,Many research papers have been published in the area of Erlenmeyer synthesis by using different methods such as usage of catalysts like Al_2O_3,organic bases, supported heteropolyacids,$Yb(oTf)_3Ca(OAc)_2$,$Bi(OAc)_3$,$H_3PW_{12}O_{40}$[21],[22],[23],[24],[25],[26]. The Erlenmeyer azlactones are 5 membered heterocyclic compounds containing N and O as heteroatoms.The C-2 and C-4 positions of azlactones are crucial for their various biological activities[27].

II. Preparation Of O-HydroxyHippuric Acid (A)

Salicyloyl chloride is prepared according to literature[28].5grams of glycine was dissolved in 50ml of 10%NaOH solution containing in a conical flask and salicyloyl chloride is added in two portions to this solution and shaken vigorously. The solution is poured into crushed ice and refluxed overnight. Conc.HCl is added slowly with stirring until the mixture is red to litmus paper. The resulting 2-hydroxy Hippuric acid is boiled with 10ml CCl_4 and filtered off washed with cold water.[29].



(a)

2.1Characterization Of 2-(2-Hydroxy Benzamido) Acetic Acid(a)

It was a pinkish white solid ;Yield:90%,Solubility:Soluble in Ethyl acetate;M.P 162-165^{0}C;IR ν max^{KBr}Cm^{-1}:1656(C=O),2860(O-H),3233(N-H);^{1}HNMR(400MHz,$CDCl_3$,δ,ppm):3.8(S,2H),6.8(2H,m),7.3(1H,t),7.53 (1H,t), 7.86(1H,d), 8.3(1H,S); Mass(ESI)m/z:195.

2.2 Experimental

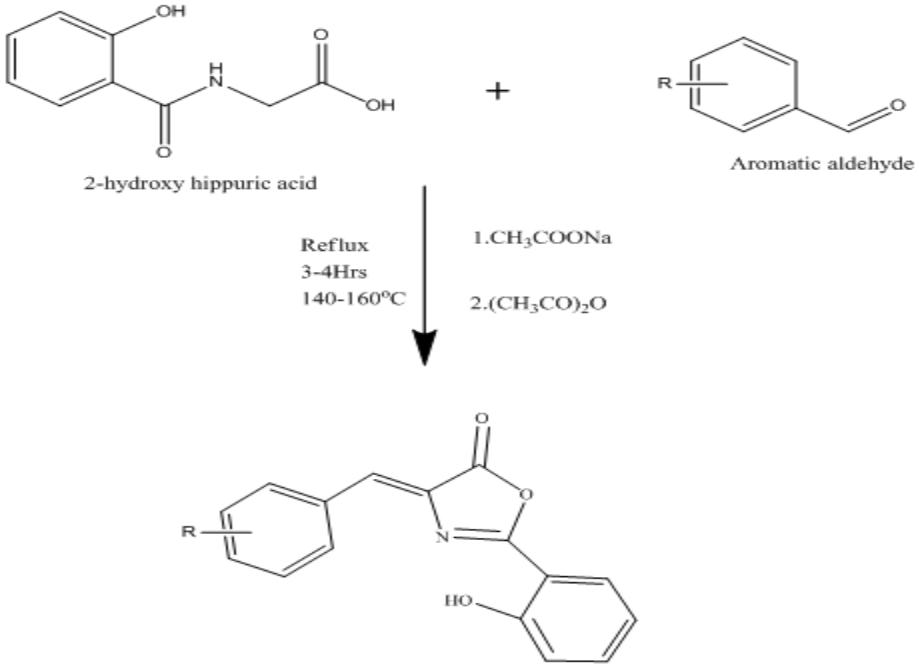
2.2.1 Physical Measurements

All the solvents and chemicals purchased from sigma-Aldrich.Melting points were determined on aElico apparatus and uncorrected. Elemental analysis(C,H,N)were conducted using FLASH EA 1112 Series.The IR spectra were recorded on KBr pellets with IR Prestige 21 shimadzu make and its values are given in Cm^{-1}. ^{1}H NMR spectra were run in $CDCl_3$ on a Bruker Avance-II 400MHz instrument.TMS was used as an internal standard; Mass spectra were recorded on a LCMS 2010 Shimadzu mass spectrometer. Thin layer chromatography sheets coated with silica gel are used to check the progress of the reaction in a U.V Cabinet.

2.2.2General Method For The Preparation Of (4Z)-4-(Arylidene)-2-(2-Hydroxyphenyl)Oxazol-5(4H)-Ones(b-h)

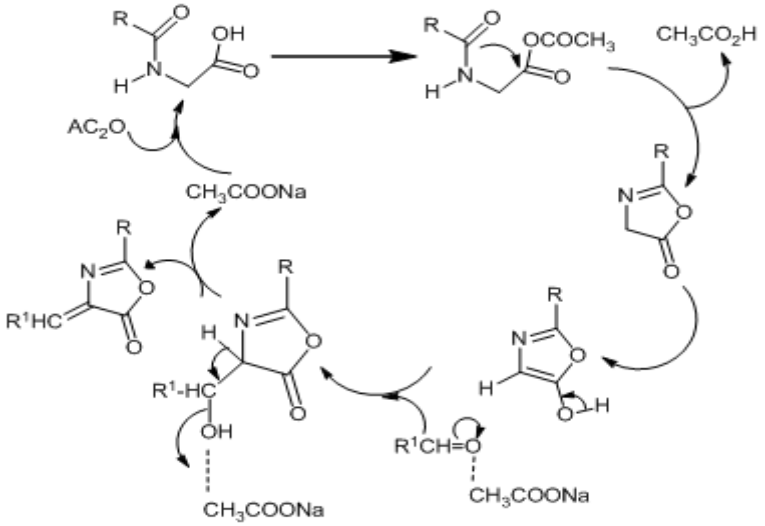
A mixture of 1mmol (0.195 gms)of 2-hydroxy hippuric acid,1mmol(0.185gms)of suitable aldehyde, 3mmol of acetic anhydride and 1 mmol (0.082gms)of fused anhydrous sodium acetate was heated on an oil bath at 140-150^{0}C for 3-4 Hrs and then cooled. Then 5ml of ethanol is added slowly to the contents of the flask and the mixture is allowed to stand overnight. The compound is filtered under suction, washed with 10ml of ice cold ethanol and then with 10ml of boiling water and air dried and recrystallised from Hexane.

Characterization of Molecules in **table1** entry (b-h) are described out of which Entry (b-f) are unreported and entry g, h are reported in the literature[30].



R=O-OH
=P-OCH_3
=P-$N(CH3)_2$
=4-Hydroxy,3,5-dimethoxy
=P-Cl
=p-Br
=m-NO_2

2.2.3 Proposed Mechanism for synthesis of Azlactones:



R=O-hydroxy phenyl
R^1=O-OH phenyl
=P-OCH_3phenyl
=P-$N(CH3)_2$phenyl
=4-Hydroxy,3,5-dimethoxy phenyl
=P-Cl phenyl
=p-Br phenyl
=m-NO_2 phenyl

2.2.4 (Z)-4-(4-chlorobenzylidene)-2-(2-hydroxyphenyl)oxazol-5(4H)-one (b)
Yield:80%;M.P:195-197^{0}C;Anal.calc.for$C_{16}H_{10}ClNO_3$:C,64.12;H,3.36N,4.67;Found: C,64.18;H,3.31;N,4.59;.IR ν max^{KBr} Cm^{-1}:1786(C=O),1653(C=N),1589(C=C),1224(C-O Lactone);^{1}HNMR(400MHz,$CDCl_3$,δ,ppm): 7.28(2H,m),7.35(1H,S),7.48-7.52(3H,m),8.12-8.14(2H,m),8.2(1H,d);Mass(ESI)m/z:300(M+H)$^+$.

2.2.5 (Z)-4-(4-bromobenzylidene)-2-(2-hydroxyphenyl)oxazol-5(4H)-one (c)
Yield:82%;M.P:190-192^{0}C;Anal.calc.for$C_{16}H_{10}BrNO_3$:C,55.84;H,2.93;N,4.07;.Found: C,55.72;H,2.86;N,4.15;.IRνmax^{KBr}Cm^{-1}:1786(C=O),1747(C=N),1653(C=C),1222(C-O Lactone);^{1}HNMR(400MHz,$CDCl_3$,δ,ppm)7.2(2H,m),7.4(1H,S),7.5-7.6(3H,m),7.9-8.1(2H,m),8.3(1H,d);MS(ESI)m/z:345(M+H)$^+$.

2.2.6 (Z)-2-(2-hydroxyphenyl) -4-(3-nitrobenzylidene)oxazol-5(4H)-one (d)
Yield:81%;M.P:185-187^{0}C;Anal.calc.for$C_{16}H_{10}N_2O_5$:C,61.94;H,3.25;N,9.03;.Found: C,61.85;H,3.21;N,9.15;.IR ν max^{KBr} Cm^{-1}:1793(C=O),1654(C=N),1607(C=C),1273(C-O Lactone);^{1}HNMR(400MHz,$CDCl_3$, δ,ppm):7.32(2H,m),7.4(1H,m),7.6(2H,m),7.8(1H,m),8.2(1H,s);MS(ESI)m/z:311(M+H)$^+$.

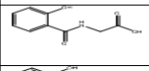
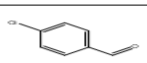
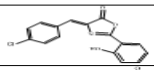
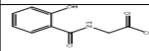
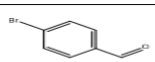
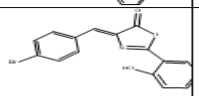
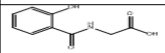
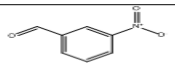
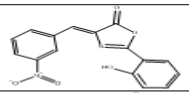
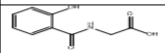
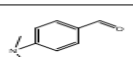
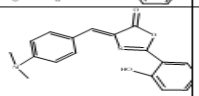
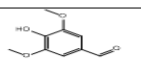
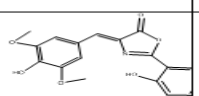
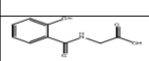
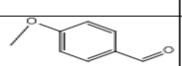
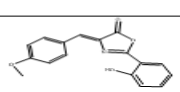
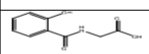
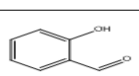
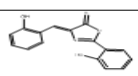
2.2.7 (Z)-4-(4-(dimethylamino)benzylidene)-2-(2-hydroxyphenyl)oxazol-5(4H)-one (e)
Yield:80%;M.P:178-180^{0}C;Anal.calc.for$C_{18}H_{16}N_2O_3$:C,70.12;H,5.23;N,9.09;.Found: C,70.23;H,5.14;N,9.15;.IR νmax^{KBr}Cm^{-1}:1767(C=O),1640(C=N),1597(C=C),1261(C-OLactone);^{1}HNMR(400MHz,$CDCl_3$, δ,ppm):3.3(6H,s),6.7(2H,m),7.3(2H,m),7.5(1H,s),7.7(2H,m),8.2(1H,s);Mass(ESI)m/z:309(M+H)$^+$.

2.2.8(Z)-4-(4-hydroxy-3,5-dimethoxybenzylidene)-2-(2-hydroxyphenyl)oxazol-5(4H)-one (f)
Yield:85%;M.P:180-182^{0}C;Anal.calc.for $C_{18}H_{15}NO_6$:C,63.34;H,4.43;N,4.10;.Found: C,63.21;H,4.49;N,4.18;.IR νmax^{KBr}Cm^{-1}:1672(C=O),1608(C=N),1585(C=C),1251(C-OLactone);^{1}HNMR(400MHz,$CDCl_3$, δ,ppm):3.4(6H,s),6.72(2H,s),7.3(2H,m),7.8(2H,m),8.2(1H,s);Mass(ESI)m/z:342.6(M+H)$^+$.

2.2.9(Z)-2-(2-hydroxyphenyl)-4-(4-methoxybenzylidene)oxazol-5(4H)-one (g)
Yield:82%;M.P:185-187^{0}C;Anal.calc.for $C_{17}H_{13}NO_4$:C,69.15;H,4.44;N,4.74;.Found: C,69.04;H,4.51;N,4.65;.IR νmax^{KBr}Cm^{-1}:1788(C=O),1712(C=N),1651(C=C),1265(C-OLactone);^{1}HNMR(400MHz,$CDCl_3$, δ,ppm):3.7(3H,s),7.0(2H,m),7.3(1H,s),7.8(3H,m),8.3(3H,m);MS(ES+)m/z:296(M+H)$^+$.

2.2.10 (Z)-4-(2-hydroxybenzylidene)-2-(2-hydroxyphenyl)oxazol-5(4H)-one (h)
Yield:85%;M.P:182-184^{0}C;Anal.calc.for $C_{16}H_{11}NO_4$:C,68.32;H,3.94;N,4.98;.Found: C,68.45;H,3.98;N,4.91;.IR νmax^{KBr}Cm^{-1}:1792(C=O),1708(C=N),1600(C=C),1158(C-OLactone);^{1}HNMR(400MHz,$CDCl_3$, δ,ppm):7.2(4H,m),7.5(3H,m),7.9(1H,s),9.8(1H,s),10.5(1H,s);Mass(ESI)m/z:281.9(M+H)$^+$.

Table 1

Entry	Reactant	Aldehyde	Product	Molecular Formula	Reaction time	Temp
b				$C_{16}H_{10}ClNO_3$	4Hrs	145-150°C
c				$C_{16}H_{10}BrNO_3$	4Hrs	150-155°C
d				$C_{16}H_{10}N_2O_5$	3Hrs	140-150°C
e				$C_{18}H_{16}N_2O_3$	3Hrs	140-150°C
f				$C_{18}H_{15}NO_6$	3Hrs	140-145°C
g				$C_{17}H_{13}NO_4$	3Hrs	130-140°C
h				$C_{16}H_{11}NO_4$	3Hrs	140-150°C

III. Molecular Docking Studies

To elucidate the DNA binding ability of synthesized azlactone derivatives, these molecules were subjected to DNA docking using Glide 5.6 module of Schrodinger Suite.

The Present study focuses on MD simulation and docking of ligands with DNA.MD simulation were performed to search for the potential energy surface for energy minima based on the contribution of stretch dihedrals,vanderwaals and electrostatic interactions to molecular energy.

Molecular docking can be thought of as a problem of "lock-and-key", where one is interested in finding the correct relative orientation of the "key" which will open up the "lock".Here, the protein can be thought of as the "lock" and the ligand can be thought of as a "key". Molecular docking may be defined as an optimization problem, which would describe the "best-fit" orientation of a ligand that binds to a particular protein of interest. However, since both the ligand and the protein are flexible, a "hand-in-glove" analogy is more appropriate than "lock-and-key". During the course of the process, the ligand and the protein adjust their conformation to achieve an overall "best-fit" and this kind of conformational adjustments resulting in the overall binding is referred to as "induced-fit".

The focus of molecular docking is to computationally simulate the molecular recognition process. The aim of molecular docking is to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand such that the free energy of the overall system is minimized.

IV. Methodology

Crystal structure of DNA was downloaded from protein data bank (www.rcsb.org) pdb id: 1N37, it was prepared by protein preparation wizard applying OPLS 2005 force field, and a grid was prepared around the intercalation site by selecting the co-crystallized ligand. Azlactone derivatives were constructed in maestro build panel and prepared by Ligprep module. These were docked into DNA intercalation site using Glide 5.6.[31]**.**

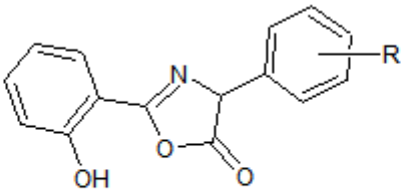
V. Result and Discussion

Docking is a computational technique that places a small molecule (ligand) in the binding site of its macromolecular target (receptor/DNA) and estimates its binding affinity. DNA intercalators usually contain planar polyaromatic systems which form π- π interactions with two flanking bases. These interactions are main driving force of intercalation binding mode, along with hydrogen bond interaction.

Docking of azlactone derivatives into DNA showed hydrogen bond interaction with G5 nucleotide. These molecules were placed in between the base pairs resulting in π- π interaction. The dock score provided in table represents the binding affinity of these derivatives, molecule Aza7 is having highest binding affinity followed by Aza1 and Aza 6. These molecules showed hydrogen bond interaction with G5 and T14 apart from π- π interaction.

3.3 Docking results of azlactones into DNA intercalation site:

Table 2



Compound	R	Dock score (kcal/mol)	Emodel (kcal/mol)
Aza1	2-OH	-6.184	-49.958
Aza2	3-NO_2	-5.149	-52.968
Aza3	4-OCH_3	-3.910	-49.901
Aza4	4-Cl	-5.627	-51.628
Aza5	4-Br	-5.827	-51.329
Aza6	4-OH, 3,5 – OCH_3	-6.326	-50.108
Aza7	4-$N(CH_3)_2$	-8.356	-64.325

Dock pose of Aza 1,Aza 2&Aza 3 in the intercalation site of DNA showing hydrogen bond interaction are shown below as Fig1,Fig 2&Fig 3:

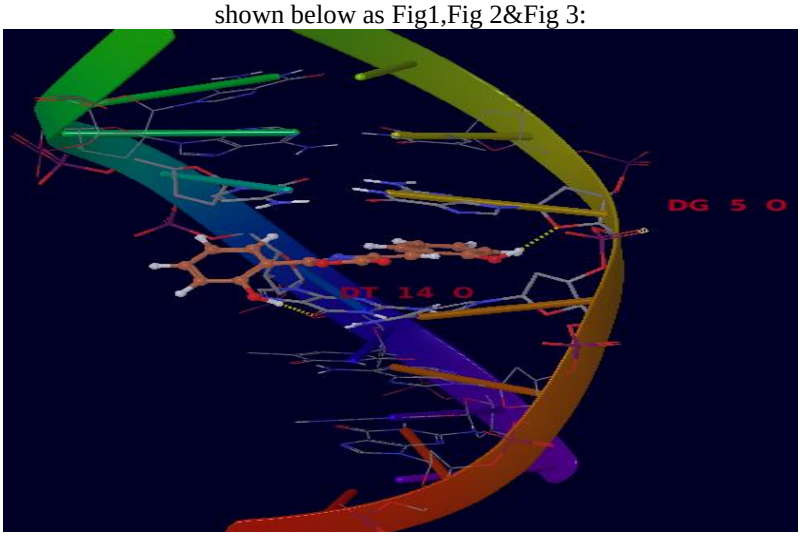


Fig:1

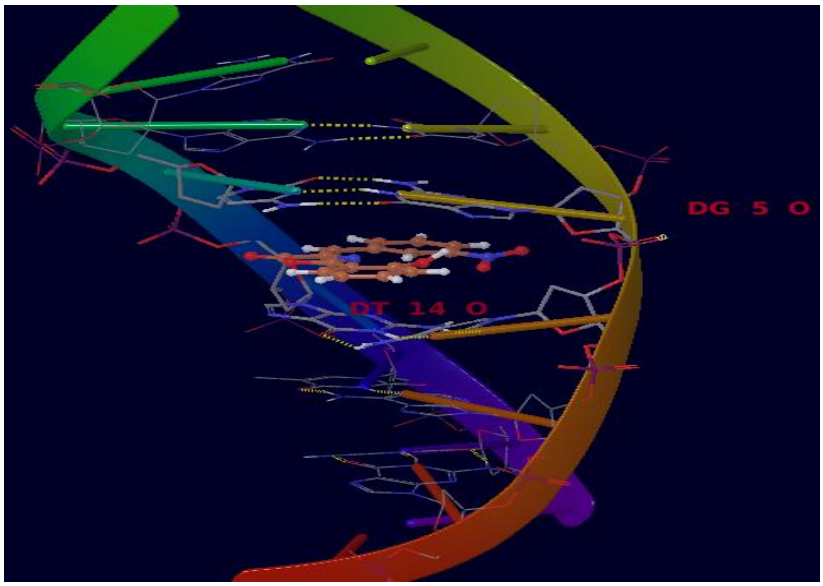


Fig2:

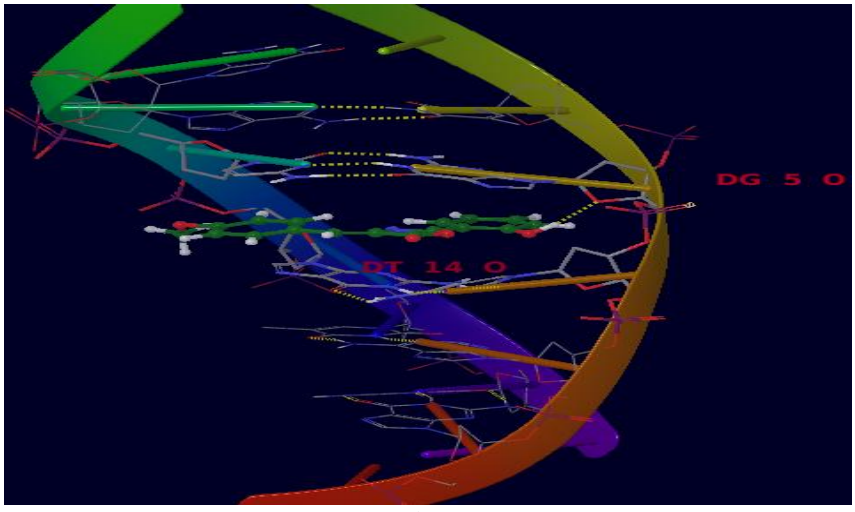


Fig3:

VI. Conclusion

The present work reports synthesis, spectral characterization, molecular docking studies of synthesized azlactones obtained from condensation of aldehydes and 2-hydroxy hippuric acid. The molecular docking studies on these azlactone derivatives show binding to DNA by intercalating in between the DNA base pairs. These molecules can be considered as good DNA intercalators based on docking score (binding affinity) and Emodel values (Complexation energy). The dock score in **table2** represents the binding affinity of molecule Aza7 is having highest binding affinity followed by Aza 1 and Aza 6 which indicate electron releasing groups at para position show greater docking score compared to electron withdrawing groups. The anti microbial activity and anti cancer activity are under study. Docking is frequently used to predict the binding orientation of small drug molecules to their protein targets in order to predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs.

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