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Synthesis and Docking Studies of Cinnoline Derivatives for Enhanced Anti-Bacterial Activity

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ABSTRACT: This study involves the synthesis and evaluation of a series of cinnoline derivatives for their biological activities. The compounds were synthesized through a multi-step process, including the preparation of benzene diazonium chloride, formation of phenyl hydrazoneacetylacetone, synthesis of 4-methyl-3-acetyl cinnoline, and subsequent reactions with various amines. These compounds were characterized using Thin Layer Chromatography (TLC), melting point determination, solubility tests, and advanced spectroscopic techniques, including Infrared (IR) and Proton Nuclear Magnetic Resonance (1H-NMR) spectroscopy, to confirm their structures.The compounds demonstrated good solubility in common solvents, and their yields varied depending on the reaction steps. Antibacterial testing was conducted using the cup plate method against Staphylococcus aureus and Escherichia coli, revealing moderate to promising antibacterial activity, with Compound G showing superior activity compared to others, particularly Compound A.In addition, docking studies indicated that some of these cinnoline derivatives exhibited favorable binding energies, especially in comparison to the standard antibiotic Ciprofloxacin, against targets such as dihydrofolatereductase and DNA gyrase. These findings suggest that cinnoline derivatives hold potential as antibacterial agents. Further optimization and pharmacological evaluation are recommended for these compounds.

Keywords: Cinnoline, Anti-Bacterial Agent

I. INTRODUCTION:

Drug discovery is an iterative process which uses a variety of strategies but essentially comprises a few discrete stages: target identification and validation; pharmacological screening; hits and lead identification; lead optimization; preclinical and clinical evaluation; industrial development. Medicinal chemistry plays its role essentially in the phases of hits and lead discovery and lead optimization, building structure-activity relationships (SAR) and identifying drug candidates. Toward this end, understanding the nature and structure of drug targets as well as knowledge of the mechanism of action and of the pharmacokinetic and toxicological behavior of putative dugs is of utmost importance.

1.1 Cinnoline:

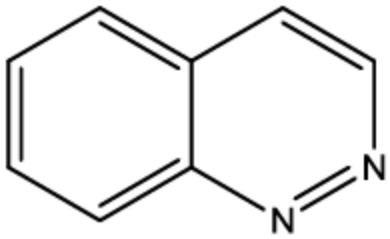

Figure 1: Structure OfCinnoline

- **Molecular Weight:** 134.15 g/mol.
- **Chemical Formula:** $C_8H_6N_2$
- **Appearance**: Cinnoline is typically found as a yellow to brown solid.
- **Melting Point**: The melting point of cinnoline is around 59-61 °C.
- **Boiling Point**: Its boiling point is approximately 220 °C.
- **Density**: The density of cinnoline is about 1.25 g/cm³.
- **Structure**: Cinnoline consists of a fused ring system, featuring two nitrogen atoms in a five-membered ring structure.
- **Solubility**: Cinnoline is generally soluble in organic solvents such as ethanol, ether, and chloroform, but has low solubility in water due to its hydrophobic nature.

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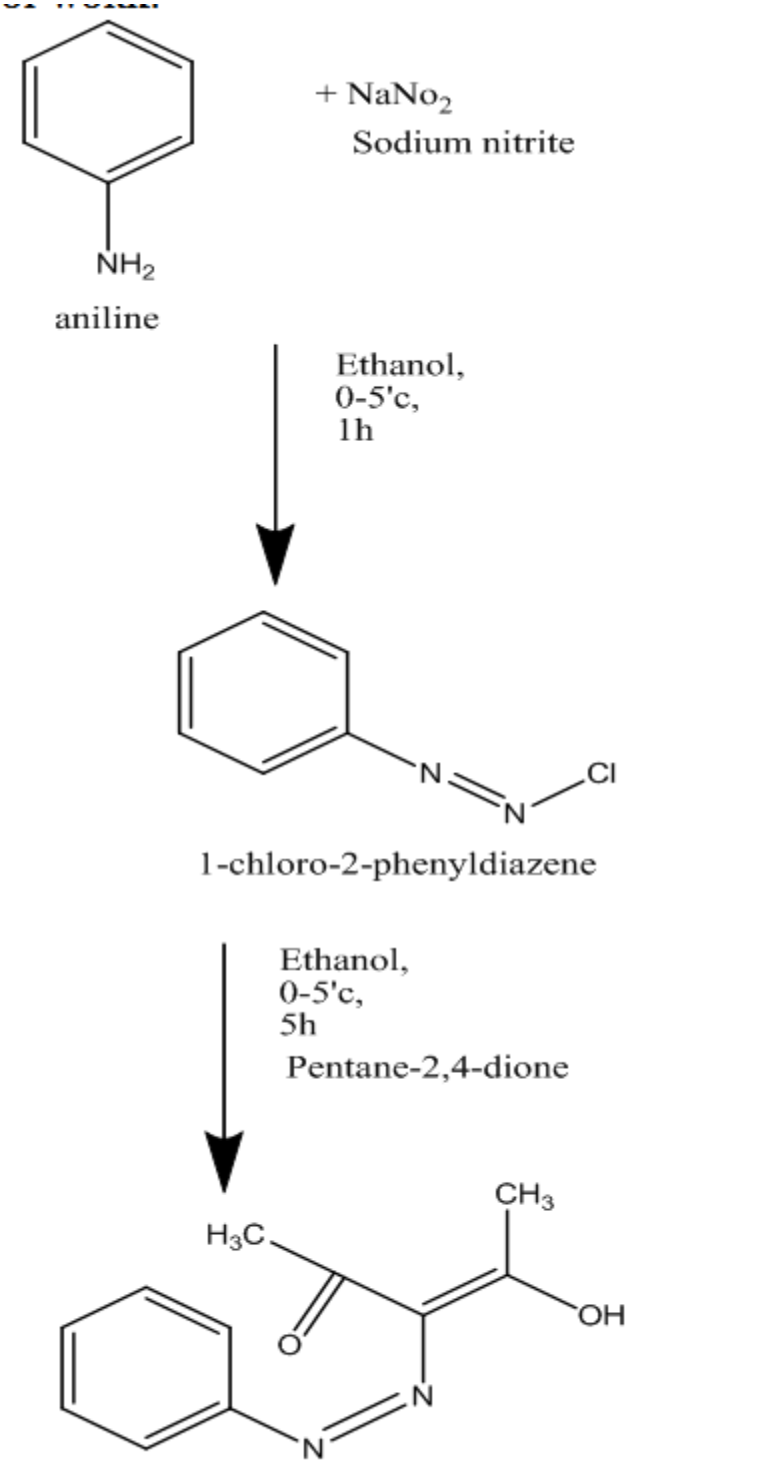
II. MATERIALS AND METHODS:

2.1 SCHEME OF WORK:



(*Z*)-4-hydroxy-3-((*Z*)-phenyldiazenyl)pent-3-en-2-one

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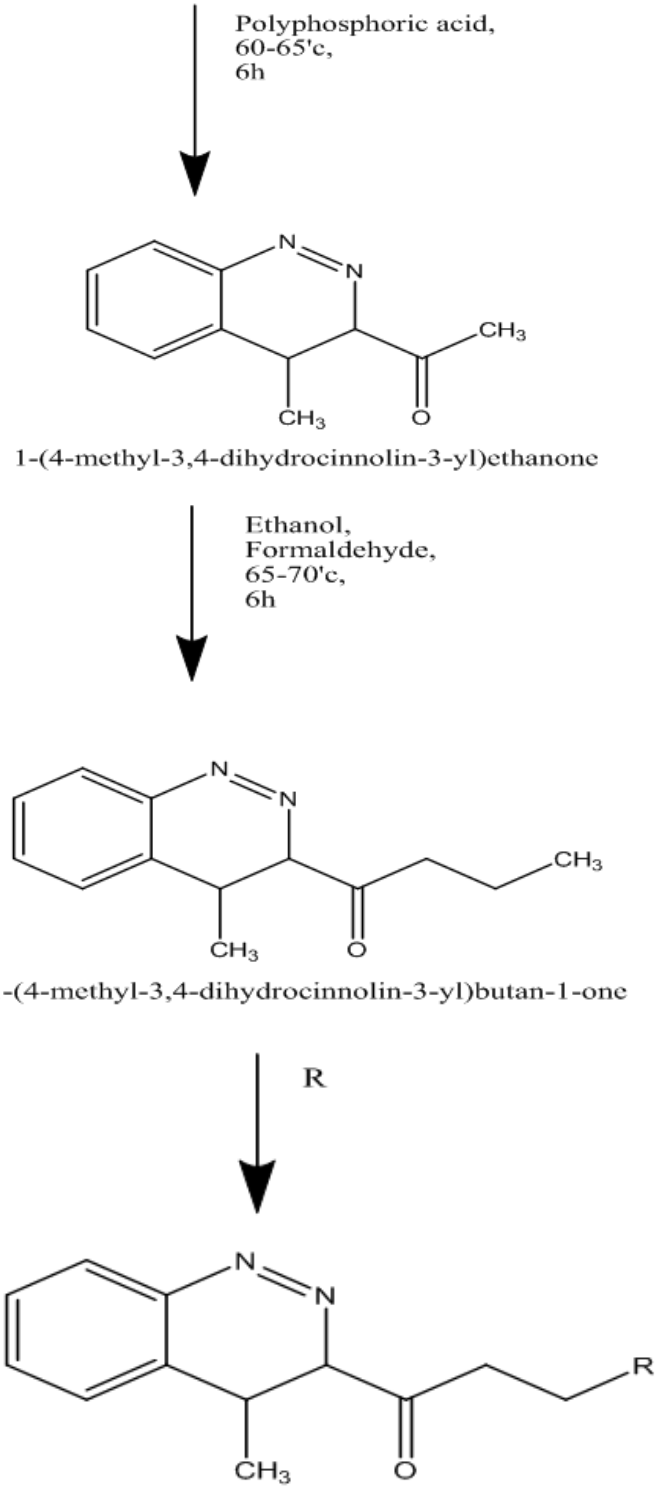

Figure 2: Scheme Of Work

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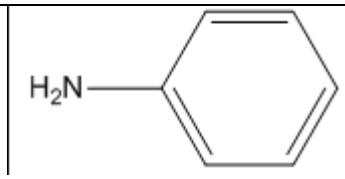
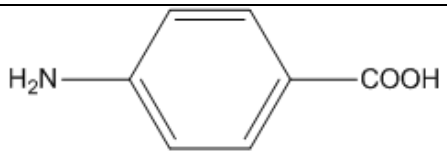
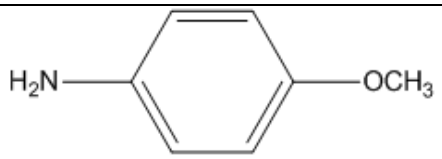
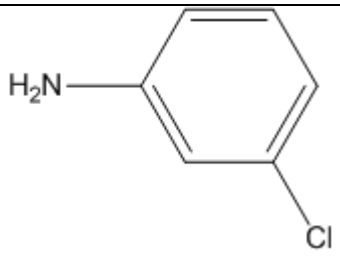
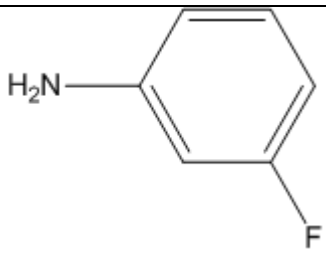
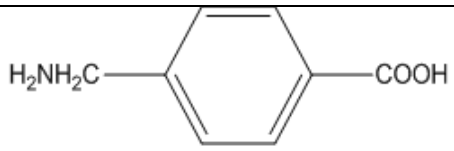
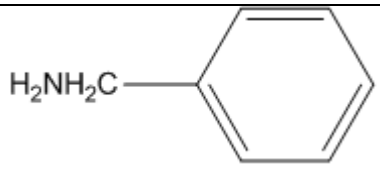
S.No	Compound Code	R1	IUPAC Name	Mol. Formula (Mol. Wt.)
1	A		Aniline	C_6H_7N 93.13
2	B		4-Aminobenzoic Acid	$C_7H_7NO_2$ 137.14
3	C		4-Methoxyaniline	C_7H_9NO 123.15
4	D		3-Chloroaniline	C_6H_6ClN 127.57
5	E		3-Fluoroaniline	C_6H_6FN 111.12
6	F		4- Amino Methyl Benzoic Acid	$C_8H_9NO_2$ 151.16
7	G		Phenylmethanamine	C_7H_9N 107.15

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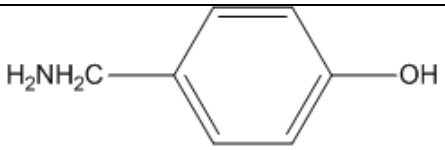
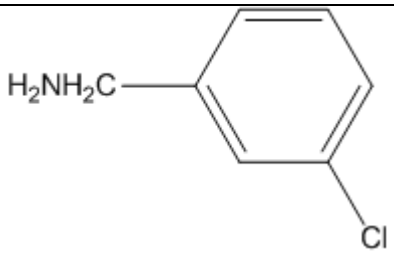
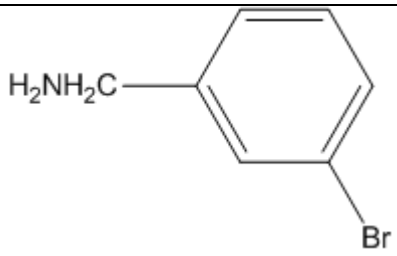
8	**H**		**4 Aminomethyl Phenol**	**C_7H_9NO 123.15**
9	**I**		**3-Chlorophenyl Methanamine**	**C_7H_8ClN 141.60**
10	**J**		**3-Bromophenyl Methanamine**	**C_7H_8BrN 186.05**

Table 1: Synthesis Used For Compounds

2.2 MOLECULAR DOCKING STUDY:

The ligands were drawn in Marvin sketch ,Chem draw 2014 assigned with proper 3D orientation and the structure of each compound was analyzed for connection error in bond order. OSIRIS, an ADMET based Java library layer that provides reusable chem informatics functionality which is an entirely in-house developed drug discovery informatics system was used to predict the total drug score via in silico.The energy minimized compounds were then read as input for Auto Dock 4.2, in order to carry out the docking simulation .All the heteroatoms were removed from the1DLS and 2XCO .pdb, to make complex receptor free of any ligand before docking.

The Graphical User Interface program ‘‘Auto-Dock Tools’’ was used to prepare, run, and analyze the docking simulations. Kollman united atom charges, salvation parameters and polar hydrogen’s were added to the receptor for the preparation of protein in docking simulation. Since ligands are not peptides, Gasteiger charge was assigned and then non-polar hydrogens were merged. AutoDock requires pre-calculated grid maps, one for each atom type; present in the ligand being docked as it stores the potential energy arising from the interaction with macromolecule. This grid must surround the region of interest (active site) in the macromolecule.

In the present study, the binding site was selected based on the amino acid residues, which are involved in binding with the staphylococcus aureus DNA gyrase (pdb cord: 2XCO) and di hydrofolatereductase (pdb cord: 1DLS) as obtained from PDB the1DLS and 2XCO respectively which would be considered as the best accurate active region as it is solved by experimental crystallographic data. Grid box for docking simulations were constructed with 60 points in x, y, and z direction to be centered in the active site using Autogrid utility of the Autodockprogramme Docking software AutoDock 4.2 Program supplied with AutoGrid 4.0 and AutoDock 4.0 was used to produce grid maps. The Lamarckian Genetic Algorithm (LGA) was chosen to search for the best conformers. During the docking process, a maximum of 20 conformers was considered for each compound. All the AutoDock docking runs were performed in Intel Centrino Core2Duo CPU @ 2.20 GHz of IBM system origin, with 2 GB DDR2 RAM. AutoDock 4.0 was compiled and run under Microsoft Windows 10 operating system.

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2.2.1 PROTEIN: STAPHYLOCOCCUS AUREUS DNA GYRASE: DNA gyrase and topoisomerase IV are the biological targets of the quinolones in bacterial cells. These enzymes are heterotetrameric (A_2B_2), type II DNA topoisomerases that play essential roles in bacterial DNA replication, chromosome segregation, recombination, repair, and transcription. In addition to inhibiting the catalytic activities of the enzymes, quinolones induce the formation of stable, covalent protein-DNA complexes.

The antibacterial activity of quinolones is thought to be derived, in part, from their ability to induce enzyme-mediated double-strand DNA breaks, which ultimately lead to lethal DNA damage . The 2-pyridones, of which ABT-719 is an example, are a related class of agents that also target DNA gyrase and topoisomerase IV. They are a potent series of compounds which demonstrate a broad spectrum of antibacterial activity. For instance, ABT-719 is highly active against ciprofloxacin-resistant Staphylococcus aureus, including ciprofloxacin-resistant methicillin-resistant S. aureus .

Interesting differences in the mechanisms of quinolone resistance have been observed in gram-positive and gram-negative bacteria. In general, the majority of first-step mutations conferring quinolone resistance in gram-negative organisms arise in the gyrA gene encoding the A subunit of gyrase . First-step mutations in gram-positive species are generally found in the gene encoding the corresponding subunit of topoisomerase IV (e.g., grlA in S. aureus and parC in Streptococcus pneumoniae) . Thus, while gyrase appears to be the primary target of quinolones in gram-negative organisms , topoisomerase IV is the primary target in gram-positive organisms . Biochemical analyses also suggest that the molecular basis of these differences in resistance mechanisms likely lies in the relative sensitivities of the enzymes from various species to any given quinolone.

In Escherichia coli, gyrase is more sensitive than topoisomerase IV to inhibition of catalytic activity by quinolones as well as to induction of cleavage complex formation . Conversely, in S. aureus, topoisomerase IV is more sensitive than gyrase to both inhibition of catalytic activity and induction of cleavage complex formation .In an effort to further characterize the effect of selected quinolones and 2-pyridones, we have cloned, expressed, and purified individual subunits of S. aureus gyrase and topoisomerase IV. During characterization of the enzyme preparations, we discovered conditions whereby cleavage complex formation with S. aureus gyrase could be readily detected by using plasmid DNA as the substrate. We also examined the cleavage complex-stimulating activities of selected quinolones and the 2-pyridone ABT-719 against both S. aureus DNA gyrase and topoisomerase IV.

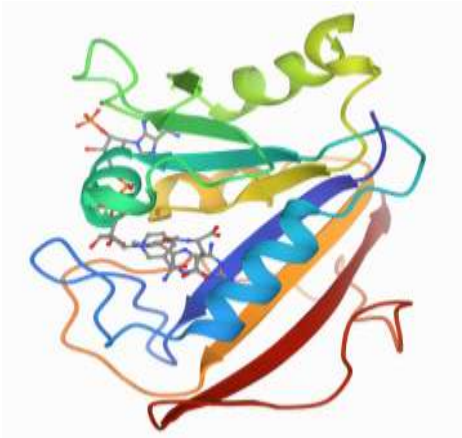

Figure 3: 1DLS = Di HydrofolateReductase

DI HYDROFOLATE REDUCTASE: Dihydrofolatereductase (DHFR) is an enzyme which uses the co-factor NADPH as electron donor which converts it to NADP. It catalyzes the reduction of dihydrofolic acid to tetrahydrofolic acid (folate). The folate is a form of the essential vitamin B9. Trimethoprim (TMP) is a known inhibitor of DHFR. DHFR forms a complex with thymidylate synthase (TS). Both enzymes participate in the biosynthesis of pyrimidine.DHFR is a ubiquitous enzyme found in all organisms. The primary physiological role of DHFR is maintenance of the intracellular levels of tetrahydrofolate, a precursor of cofactors required for the biosynthesis of purines, pyrimidines, and several amino acids.

The enzyme, which is the sole source of tetrahydrofolate catalyzes the reduction of 7,8-dihydrofolate (DHF) (magenta) to 5,6,7,8-tetrahydrofolate (THF) by stereospecific hydride transfer from the NADPH (blue) cofactor to the C6 atom of the pterin ring with concomitant protonation at N5. Being the sole source of THF it is an Achilles' heel of rapidly proliferating cells, making it an attractive target of several important anticancer and antimicrobial drugs such as methotrexate, trimethoprim, and pyrimethamine. E. coli DHFR is a small (18 kD) protein with an α/β

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structure consisting of a central eight-stranded β-sheet and four flanking α-helices. The active site cleft divides the protein into two structural subdomains: the Adenosine binding subdomain (red) and the major subdomain (green).

The adenosine binding subdomain is the smaller of the two subdomains and provides the binding site for the adenosine moiety of the cofactor. The major subdomain consists of ~100 residues from the N and C termini and is dominated by a set of three loops on the ligand binding face that surround the active site. In terms of sequence length, these loops make up approximately 40% - 50% of the major subdomain; hence it is sometimes called the "loop" subdomain. The loops are termed Met20, F-G, and G-H loops. Hinge bending motions about Lys38 and Val88 allow the adenosine binding domain to move relative to the major domain upon binding of various ligands, resulting in closure of the active site cleft.

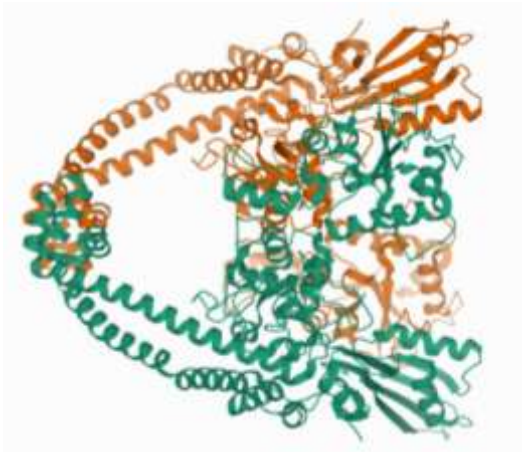

Figure 4: 2XCO = Staphylococcus Aureus DNA Gyrase

2.3 SYNTHESIS:

General Procedure for Synthesis:

STEP : 1 Synthesis of Benzene Diazonium Chloride:

Equimolar mixture of Benzene diazoniumchloride was prepared by dissolving Sodium nitrite (7.4 g, 0.1 mole) in 26ml of water and added drop wise to a solution of aniline(10 g in 1N HCl) at 0 0C under Stirring for about 10mins.Then orange coloured precipitate was collected by filtration and dried.

STEP : 2 Synthesis of Phenyl Hydrazone Acetyl Acetone:

The synthesized benzene diazonium was added to stirred solution of ethanol 30ml, water 500ml and acetyl acetone at 0 0C under continuous stirring. Then sodium acetate was added to keep the reaction alkaline to litmus after 3hrs under stirring at 0-5 0C A crude product was obtain that is filtered and washed with water and air dried. Later it is recrystallised from ethanol in order to get the pure form of yellow needles i.e., Phenyl hydrazoneacetylcaetone crystals

STEP : 3 Synthesis of 4-Methyl-3-Acetyl Cinnoline:

Phenyl hydrazoneacetylacetone (10g, 0.05 mole) and polyphosphoric acid (16 g 7.216 ml) in small lots over 30 mins and the temperature was maintained at 60-65 0C ,the reaction was kept for additional 2 hrs. Reaction was monitored by TLC. After completion of reaction chilled water (200 ml) was added carefully to decompose the black residue at 0-5 0c. Later the product was then extracted with ethyl acetate. Ethyl acetate layer was treated with charcoal and concentrated to get the crude product as brownish black residue .Then recrystallize from methanol will give light yellow crystals.

STEP : 4 4-Methyl-3-Acetylcinnoline Reacts with Amine - General Procedure:

4-methyl-3-acetyl cinnoline was dissolved in a suitable solvent such as methanol and taken in a RBF along with a secondary amine such as aniline ,phenylmethanamineindividually and formaldehyde solution in the same proportion and then reflux for 2hrs by maintaining 60-65 0C.After refluxing the contents are taken in china dish and dried there a brownish yellow colour crystal.

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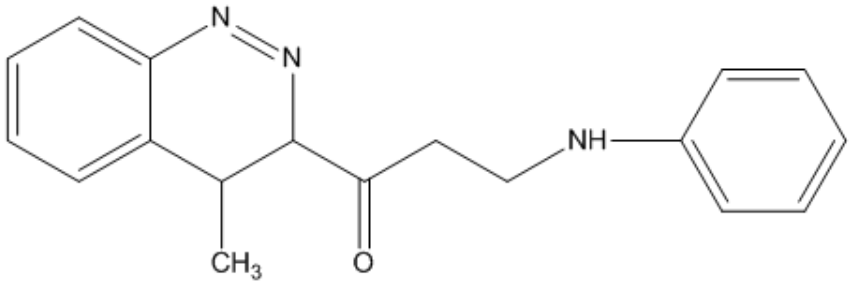
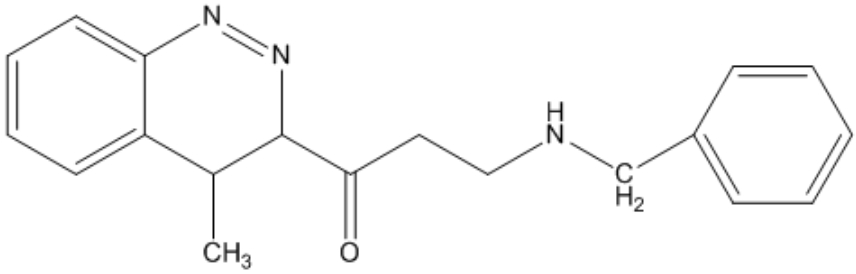
S.NO	VARIOUS COMPOUNDS	STRUCTURE OF THE COMPOUNDS
1.	**Aniline (A)**	 **1-(4-methyl-3,4-dihydrocinnolin-3-yl)-3-(phenylamino)propan-1-one**
2.	**Phenylmethanamine (G)**	 **3-(benzylamino)-1-(4-methyl-3,4-dihydrocinnolin-3-yl)propan-1-one**

Table 2: List Of Synthesized Compounds

2.4 PHYSICAL CHARACTERISTICS:

2.4.1 Thin Layer Chromatography:

The purity of the compound was ascertained by TLC.

Absorbent used: Silica gel G

Developing solvent: Benzene : Phenol (4:6)

Detecting agent: Iodine vapour

Color of the spot : Brown (iodine vapour)

2.4.2 Melting Point:

Melting point of the synthesized compounds was determined by open capillary tube method. The melting points of the synthesized compounds were shown in the Table No. 6

2.4.3 Solubility:

At room temperature solubility of the synthesized compounds were determined. The solubility of the synthesized compounds was shown in the Table No. 6

2.5 SPECTRAL ANALYSIS:

The objectives of the spectral analysis is to confirm the chemical structures of the synthesized compounds and the various functional groups in final compounds. The IR spectra of the starting compounds and intermediates where taken to confirm the changes at the reactive functional groups and then final compounds were confirmed by UV, IR, NMR analysis.

2.6 EVALUATION OF ANTI BACTERIAL ACTIVITY:

Biological evaluation: In view of varied biological importance of difference series of Cinnoline derivatives, it is felt worthwhile in evaluate them for possible activities. The newly formed synthesized compounds were screened for Anti Bacterial activity.

Antibacterial activity: The antibacterial activity of newly synthesized phenothiazine was conducted against Gram Positive bacteria i.e Staphylococcus aureus and Gram Negative bacteria i.e E. coli using cup plate method. Amoxicillin was employed as

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reference standard to compare the results. Nutrient broth was used for the preparation of inoculation of the bacteria and nutrient agar was used for the screening method. Each test compounds (5 mg) was dissolved in DMSO (5ml) at a concentration of 1000µg/ml. Amoxicillin solution were also prepared at a concentration of 1000µg/ml in sterilized distilled water. All the compounds were tested at a concentration of 0.05ml (50µg) and 0.1ml (100µg) level and DMSO used as a control. The solutions of each test compound, control and reference standards (0.05 and 0.1 ml) were added separately in the cups and plate were kept undistributed for at least 2 hours in refrigerator to allow diffusion of the solution properly into nutrient agar medium. Petri dish were subsequently incubated at 37±1°C for 24 hours. After incubation, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an anti-biotic zone reader. All the experiments were carried out in triplicates and compared to control.

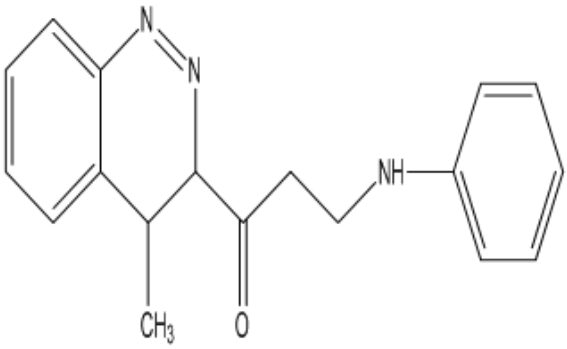
III. RESULTS AND DISCUSSION:

3.1 DOCKING RESULTS:

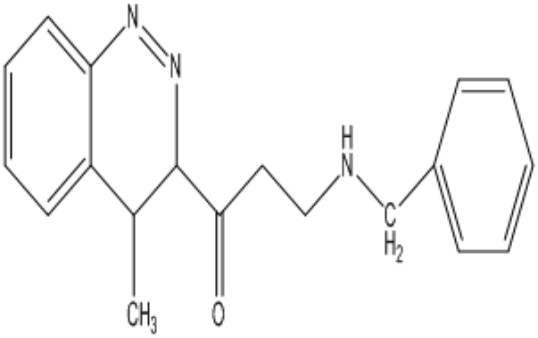
S.No	Compound Code	1DLS		2XCO	
		Binding Energy (kJ mol^{-1})	Inhibition Constant (µM)	Binding Energy (kJ mol^{-1})	Inhibition Constant (µM)
1.	**A**	-12.71	11.25	-8.68	5.95
2.	**B**	-10.45	4.04	-6.31	4.35
3.	**C**	-10.66	15.99	-6.58	2.8
4.	**D**	-8.10	6.99	-6.83	9.8
5.	**E**	-10.57	17.95	-6.54	16.02
6.	**F**	-8.30	819.47	-5.91	46.81
7.	**G**	-11.45	9.04	-8.32	41.62
8.	**H**	-6.08	8.98	-5.79	1.94
9.	**I**	-7.17	2.46	-5.14	31.63
10.	**J**	-7.83	4.24	-6.1	33.87
11.	**Gefitinib**	-5.14	169.43	----------	----------
12.	**Rosmarinic acid**	----------	----------	-5.08	188.13

Table 3: Binding Energy & Inhibition Constant Of TheCompounds & The Standard Drug

SELECTED COMPOUNDS



COMPOUND A



COMPOUND G

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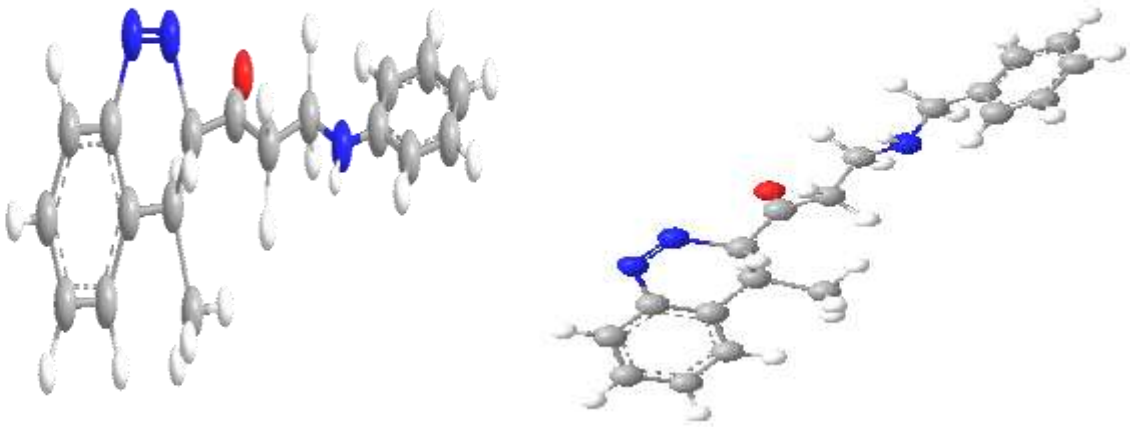
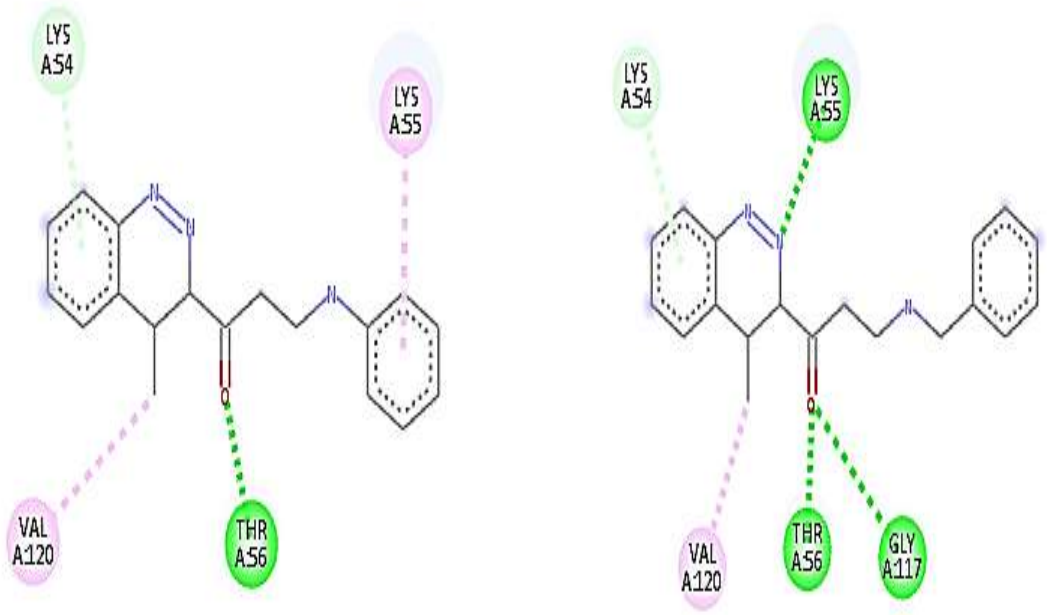
3D MODELS OF SELECTED COMPOUNDS



COMPOUND A **COMPOUND G**

Figure 5: Structure & 3D Models



COMPOUND A **COMPOUND G**

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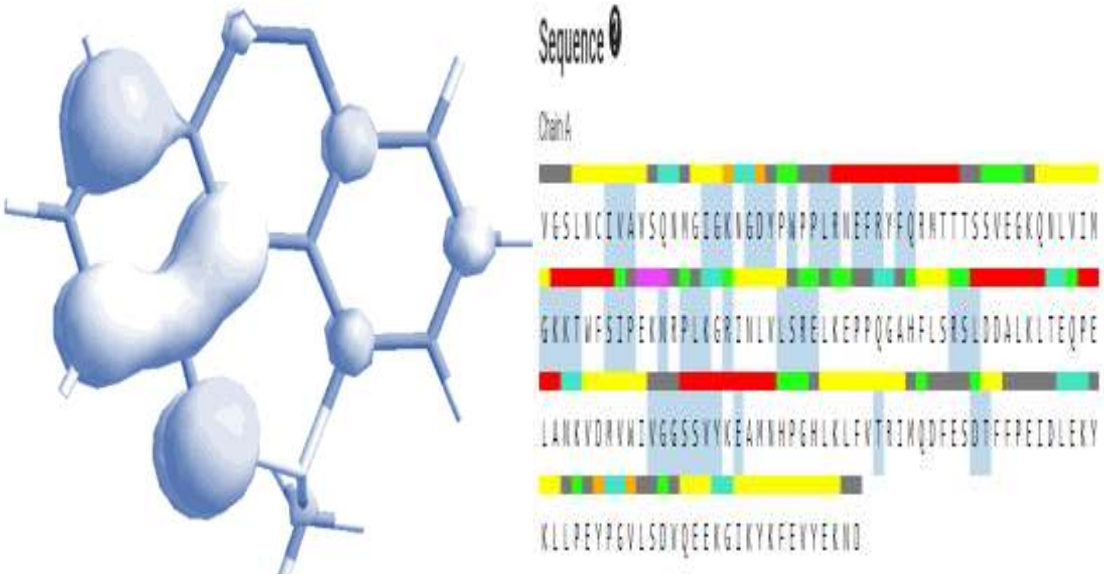
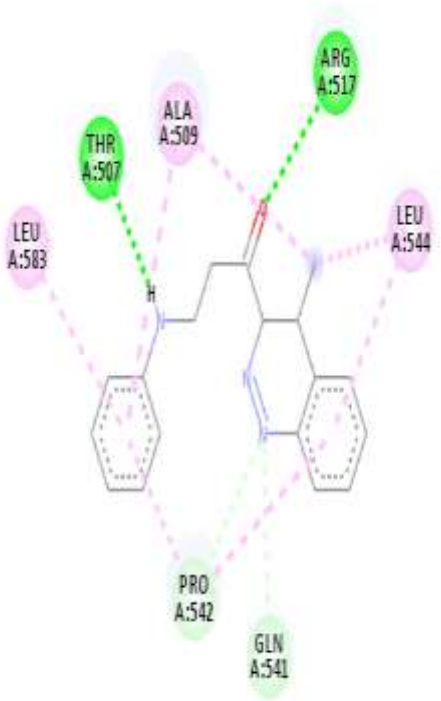
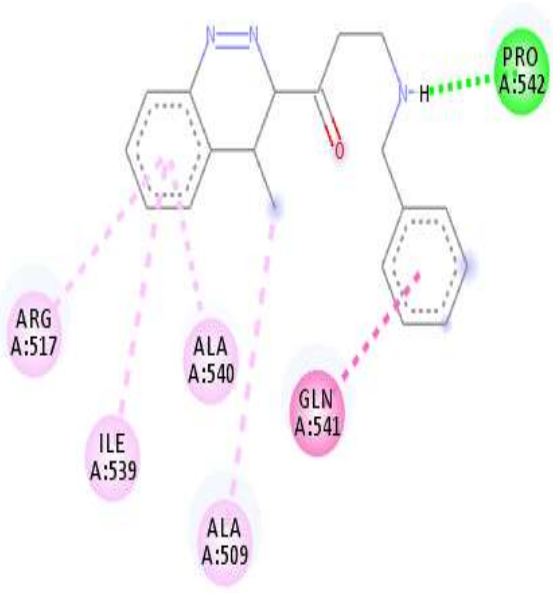


Figure 6: Compound A & G Docking (2XCO = Staphylococcus AureusDnaGyrase)



COMPOUND A



COMPOUND G

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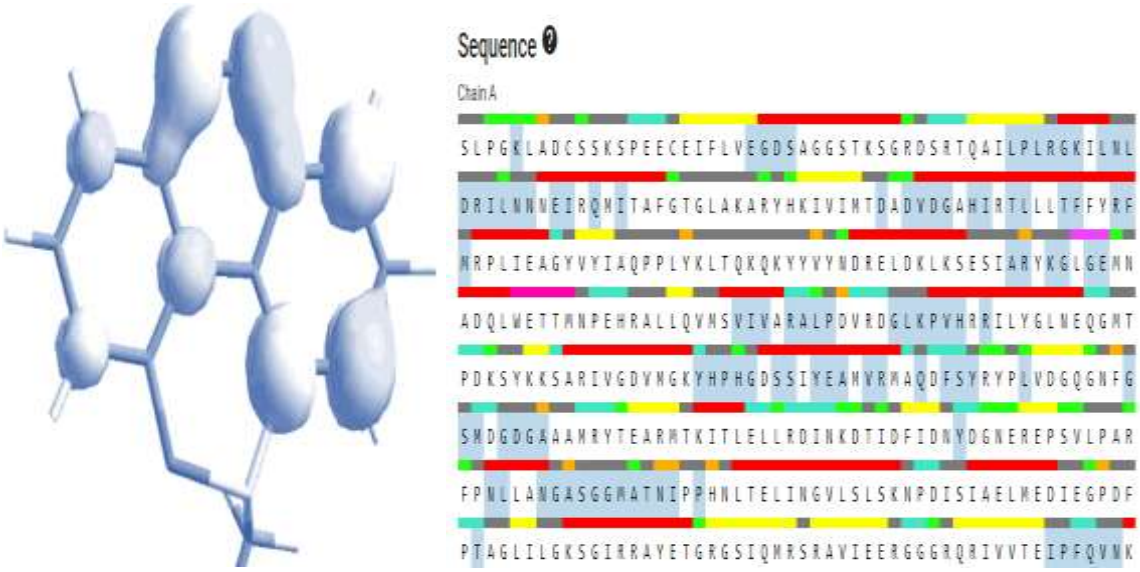

Figure 7: Compound A & G Docking (1DLS = Di HydrofolateReductase)

S.No	Compound code	Molecular weight	No. Of Hba	No. Of Hbd	ClogP	No. Of Rot.b	n violation
1	**A**	473.32	2	2	3.604	1	0
2	**B**	398.41	0	2	2.647	1	0
3	**C**	394.41	2	2	2.647	1	0
4	**D**	394.41	2	3	2.647	1	0
5	**E**	397.45	1	2	3.777	2	0
6	**F**	413.86	3	4	4.467	2	0
7	**G**	459.86	4	4	4.467	4	0
8	**H**	413.86	0	5	4.967	3	0
9	**I**	458.31	2	5	4.467	4	0
10	**J**	458.31	1	5	4.497	3	0

Table 4: Analysis Of Lipinski Rule Of 5 For The Novel Proposed Analogues

3.2 PHYSICAL CHARACTERISTCS:

S.No	Code	Molecular Weight	Color	Nature	% Yield
1.	**1**	140.57	whitish Yellow	Powder	-
2.	**2**	106.13	light Yellow	Crystal	81.05%
3.	**3**	188.23	Orange	Crystal	95.12%
4.	**4**	216.28	Yellow	Crystal	96.33%
5.	**A**	293.36	Yellow	Crystal	89.23%
6.	**G**	307.39	Yellow	Crystal	85.78%

Table 5: Physical Data Of Synthetic Compounds

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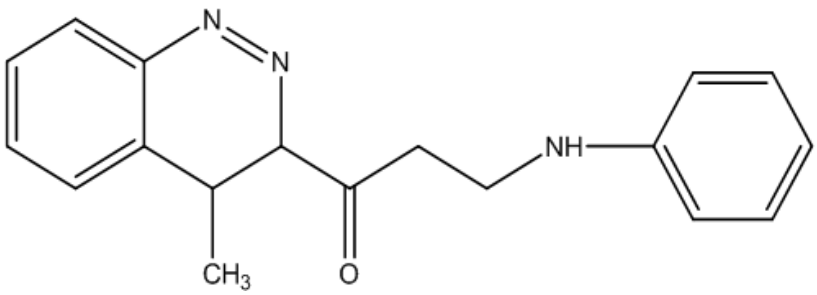
3.3 PHYSICAL DATA OF SYNTHETIC COMPOUNDS:

S.No	Code	Solubility					Melting Point(°c)	Rf value
		CHCl3	DMF	DMSO	Me-OH	Et-OH		
1.	**1**	+	+	+	+	+	120-125	0.76
2.	**2**	+	+	+	+	+	127-130	0.54
3.	**3**	+	~ +	~ +	~ +	~ +	145-148	0.83
4.	**4**	+	~ +	~ +	~ +	~ +	136-140	0.65
5.	**A**	+	+	~ +	+	+	135-140	0.71
6.	**G**	+	+	~ +	+	+	145-150	0.77

Where + → soluble,
-→ insoluble,
~ +→ slightly soluble

Table 6: Solubility Profile

3.4 SPECTRUM ANALYSIS OF COMPOUND A:



1-(4-methyl-3,4-dihydrocinnolin-3-yl)-3-(phenylamino)propan-1-one
Chemical Formula: C18H19N3O
Exact Mass: 293.15
Molecular Weight: 293.36

m/z: 293.15 (100.0%), 294.16 (19.7%), 295.16 (2.0%), 294.15 (1.1%)
Elemental Analysis: C, 73.69; H, 6.53; N, 14.32; O, 5.45

3.4.1 UV Analysis:

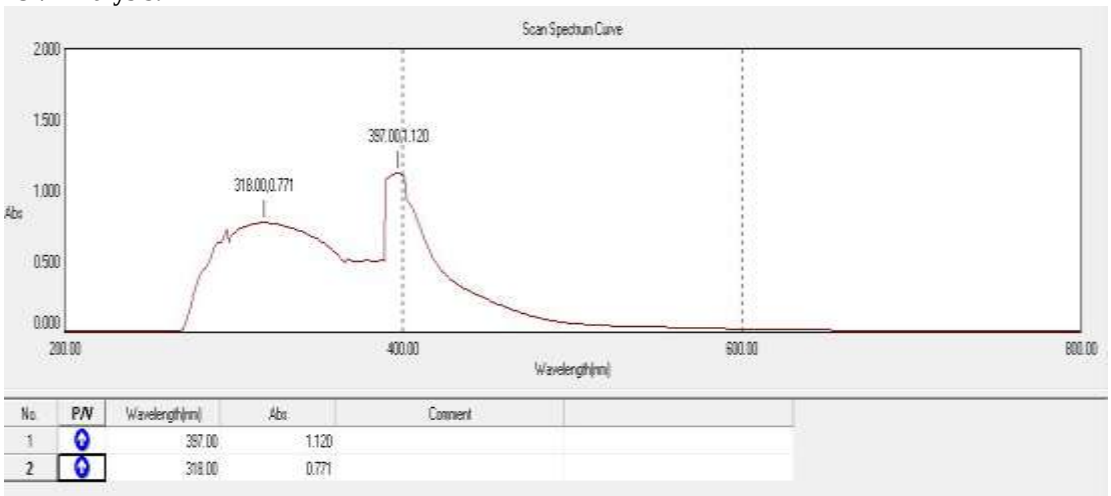


Figure 8: UV Spectrum Of Sample A

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3.4.2 IR Analysis:

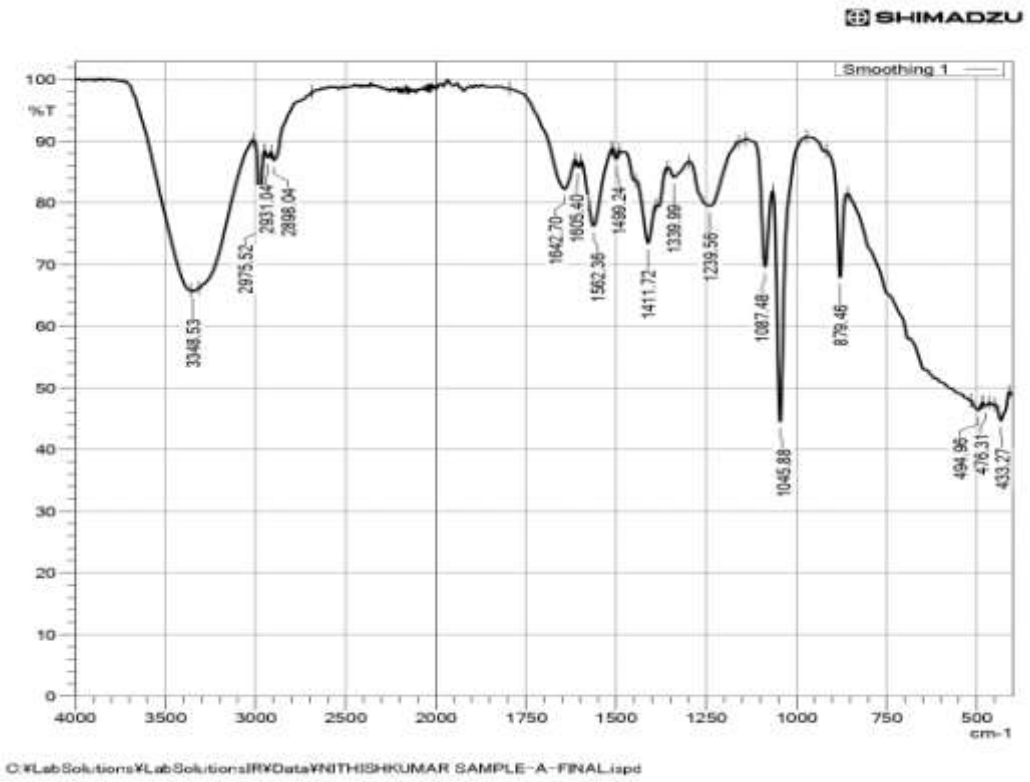

Figure 9: IR Spectrum Of Sample A

3.4.3 H1 NMR Analysis:

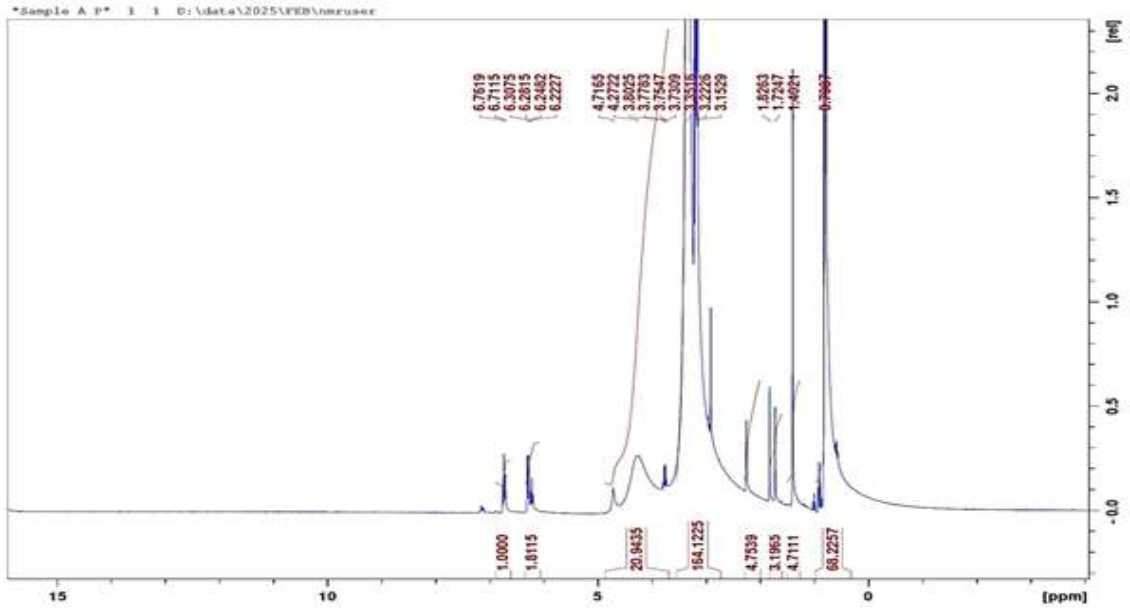

Figure 10: NMR Spectrum Of Sample A

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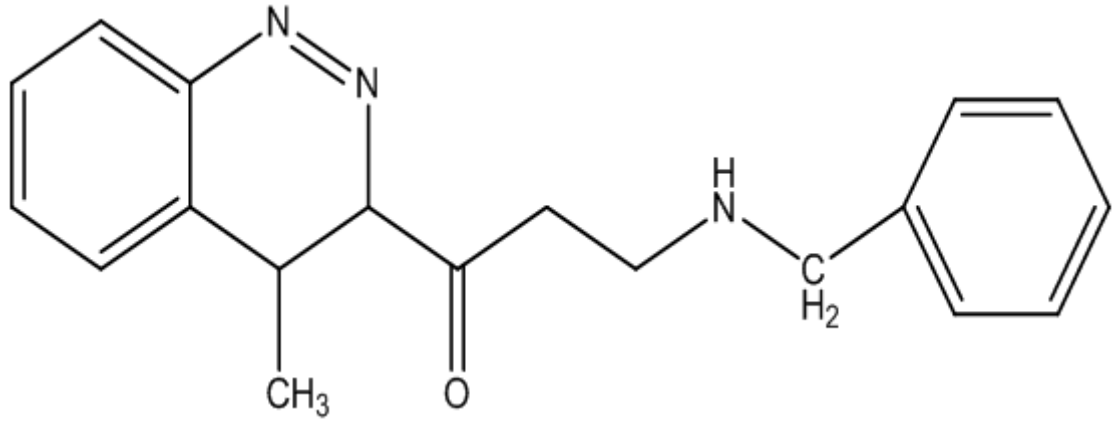
3.5 SPECTRUM ANALYSIS OF COMPOUND G:



3-(benzylamino)-1-(4-methyl-3,4-dihydrocinnolin-3-yl)propan-1-one
Chemical Formula: C19H21N3O
Exact Mass: 307.17
Molecular Weight: 307.39

m/z: 307.17 (100.0%), 308.17 (21.9%), 309.18 (2.1%)
Elemental Analysis: C, 74.24; H, 6.89; N, 13.67; O, 5.20

3.5.1 UV Analysis:

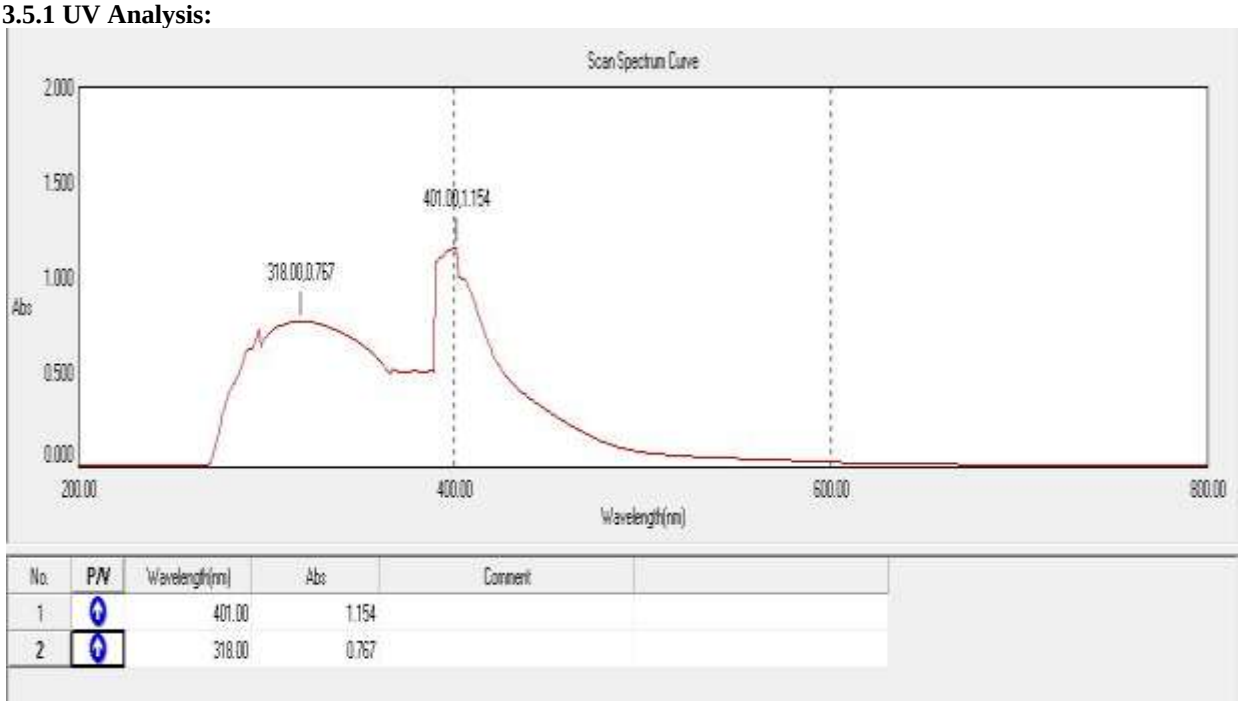


Figure 11: UV Spectrum Of Sample G

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3.5.2 IR Spectrum:

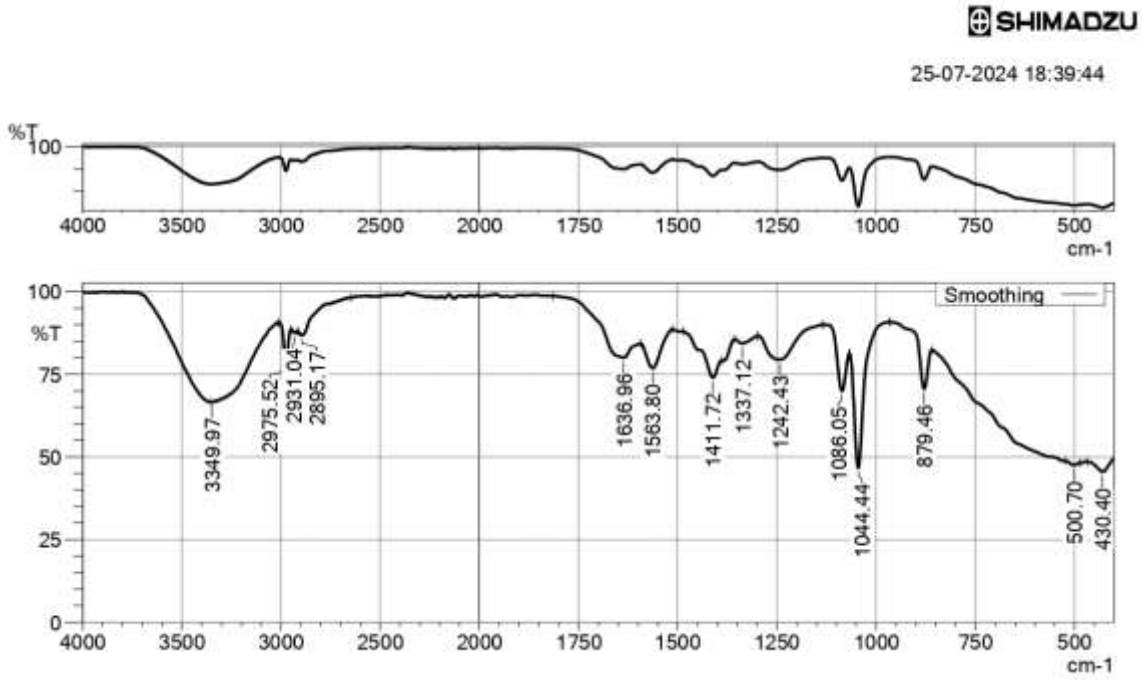

Figure 12: IR Spectrum Of Sample G

3.5.3H1 NMR Analysis:

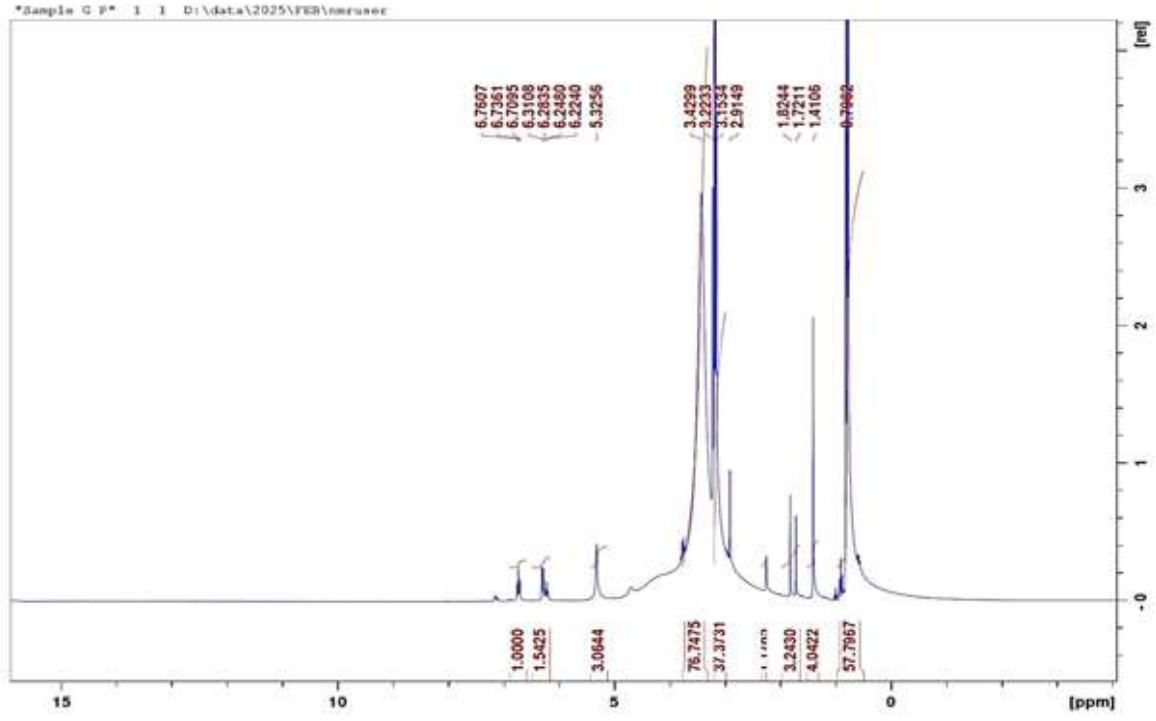

Figure 13:H1 NMR Spectrum Of Sample G

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3.6 ANTIBACTERIAL ACTIVITY:

S.NO.	Microorganisms	CONTROL	A	G	Ciprofloxacin
		Zone of inhibition in mm			
1.	Staphylococcus aureus	-	-	-	45
2.	Escherichia coli	-	20	17	35

Table 7: Antibacterial Activity

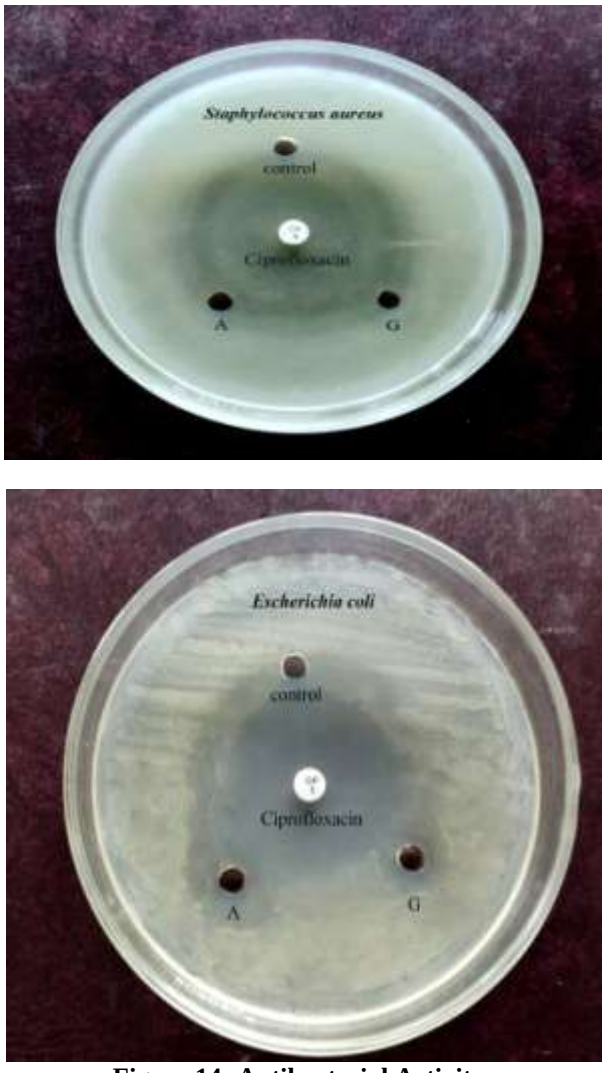

Figure 14: Antibacterial Activity

IV. DISCUSSION:

DOCKING ANALYSIS: The docking scores were obtained from the analogues against di hydrofolatereductase (pdb cord: 1DLS) and staphylococcus aureus DNA gyrase (pdb cord: 2XCO). The output of all ligands was given by energy values in kcal/mol. All the compounds show good docking scores when compared to standard drugs. Docking score of the compounds di hydrofolatereductase (pdb cord: 1DLS) was compared with the score of the drug Ciprofloxacin which is used as a potent drug for the bacterial toleranceand docking score of the compounds targeted staphylococcus aureus DNA gyrase (pdb cord: 2XCO)was compared with the score of the drug Ciprofloxacin which is used as drugs to

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treatment of bacterial infection . In Auto dock, compound A shows the highest docking score than the standard drugs against the receptor di hydrofolatereductase (pdb cord: 1DLS) and staphylococcus aureus DNA gyrase (pdb cord: 2XCO) than the standard drugs, Ciprofloxacin Next comes, all the 9 analogues with high docking score against the receptor di hydrofolatereductase (pdb cord: 1DLS) cinnoline derivatives were docked with the crystallographic structures of the targets by Autodock version 4.2 screening programme .The analogues were examined for their binding energies and hydrogen bonding.

The conformations with highest binding energies and greater number of hydrogen bonds of all the ligands were taken in consideration for ranking the analogues. All the analogues show higher docking scores when compared to standard drugs. compound A and G and A shows higher docking scores with both di hydrofolatereductase (pdb cord: 1DLS) and staphylococcus aureus DNA gyrase (pdb cord: 2XCO). Studies have proved that compounds showing good bacterial activitycan also be considered as good agents for anti bacterial therapy. The interactions were stronger (energetically lesser) for all the ligands which are used for docking simulation.

SYNTHESIS: In the present work two difference Cinnoline derivatives were synthesized.

PHYSICAL CHARACTERISATION: Melting point of the synthesized compound was taken in open capillary tubes and was uncorrected and were found to be in the range 130-150°C**.** TLC was performed using pre coated silica gel plates of 0.25 mm thickness eluted used were , benzene ; phenol (1:4) spots were visualized in UV light.At room temperature solubility of newly synthesized compounds were determined by various organic solvents and it was found that all compounds were freely soluble in methanol, ethanol, DMSO and DMF

STRUCTURAL CONFIRMATION:

The IR spectroscopy was performed with KBr on SHIMADZU spectrum instrument. ^{1}HNMR spectroscopy was recorded on Burker 400 MZs Avance .

ANTI-BACTERIAL ACTIVITY:Cup Plate Method: The Anti-bacterial activity of newly synthesized phenothiazine was conducted against gram positive bacteria i.e Staphylococcus aureus and gram negative bactreriai.eE.coli by using cup plate method .**Discussion:** The Anti-bacterial activity results were presented in. In particular, compound A possessed maximum activity which may due to presence aromatic ring of cinnoline. Other compounds also showed mild to moderate activity at 0.1ml concentration level on all organisms.**Conclusion:**The result of presence study indicates that compound Compound A possess maximum Anti-bacterial activity against Negative bacteria (E.Coli), or new synthesized products of Cinnoline .

V. CONCLUSION:

In this study, a series of cinnoline derivatives were successfully synthesized through a multi-step process involving the preparation of benzene diazonium chloride, the formation of phenyl hydrazoneacetylacetone, and the synthesis of 4-methyl-3-acetyl cinnoline, followed by reaction with amines. The synthesized compounds were characterized by a variety of techniques including Thin Layer Chromatography (TLC), melting point determination, solubility testing, and advanced spectroscopic methods (IR and NMR) to confirm their structure.

The synthesized compounds displayed good solubility in common solvents and exhibited varied yields depending on the chemical reaction steps. The antibacterial activity of these compounds was evaluated against Staphylococcus aureus and Escherichia coli, revealing moderate to promising activity, particularly for compound G. Furthermore, docking studies demonstrated that some compounds exhibited better binding energies compared to the standard antibiotic, Ciprofloxacin, against targets like dihydrofolatereductase and DNA gyrase. This suggests that these cinnoline derivatives may be promising candidates for further research as potential antibacterial agents.

Future studies could focus on optimizing these compounds for improved activity and exploring their broader pharmacological potential. The result of presence study indicates that compound Compound A possess maximum Anti-bacterial activity against Negative bacteria (E.Coli), or new synthesized products of Cinnoline .

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