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ORIGINAL ARTICLE **OPEN ACCESS**

Design, Synthesis, *In Silico* and *In-Vitro* Anti-Bacterial Screening of Some Novice Cinnoline Derivatives

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ABSTRACT

Designing and synthesis of lead molecules are one of the main challenges in drug discovery, so synthesis of 1, 2-benzodiazine derivatives possessing prominent biological activities like antibacterial is important. Owing to their biological importance, synthesis of various 1, 2-benzodiazine derivatives was done and evaluated for antibacterial activity. A novel series of substituted 1, 2-benzodiazene was synthesized as potential antibacterial agents via intra molecular cyclization reaction. In Silico is a term used to mean which is a computer simulation or a method done in the computer. A Method which is used to identify the fit between a receptor and a potential legend is called docking. In this research work in silicon design of substituted 1, 2-Benzodiazine derivatives was carried out Molegro virtual docker software for antimicrobial activity. New compounds are synthesized and antibacterial activity is evaluated. The antibacterial proteins were excluded from Protein Data Bank and docked with eight derivatives of by using glide. Synthesized title molecules are interpreted by 1H-NMR, 13C-NMR, and IR spectroscopy. A new series of compounds was synthesized by selecting an appropriate scheme, submitted for Docking studies of large molecule revealed that compound 1, 2, 4 had shown considerable interaction with the active site of the acyl enzyme of E. coli (Code: 3ITA) and DHFR (2W9S) is complexes with trimethoprim of ligand 4.

Keywords: *1, 2-benzodiazine, anti-bacterial, Cinnoline, trimethoprim, Molegro docking software, docking studies, drug target.*

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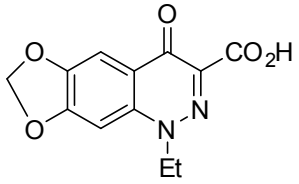
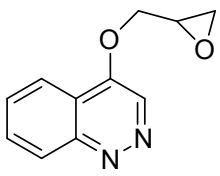
INTRODUCTION

Treatment of disease by newly developing methods is devoted by practice of medicinal chemistry. 1, 2-benzodiazine is a potential lead molecule with commercial interest that has been investigated due to its important pharmacological activities. The structure of 1, 2-benzodiazine resembles the structure of Quinoline is having potent antibacterial activity. The nitrogenous base in the nucleus is responsible for the activity. Heterocyclic Compounds containing 1,2-benzodiazine (cinnoline) as their basic structure is Cinoxacin (1) and Schizocommunin the Benz [c] pyridazine nucleus, is also known as cinnoline, and its derivatives have acquired great interest due to its wide range of pharmacological importance, e.g., antibacterial (1), Antitumor (2) antifungal(3,4)and anti-inflammatory activities(5). This ring is reactive due to attack the electrophilic attack. 1, 2-benzodiazine are six member rings with two nitrogen as hetero atom. It is also called as cinnoline or benzopyridazine or 1,-diazanaphalene or phenodiazine (6). The main intention of organic and medicinal chemistry is to synthesis of compounds by selecting a scheme and characterizing using spectrophotometry and evaluation of pharmacological high therapeutic and efficient molecules. (7-9) Helen Giamarellou (1975) synthesized cinoxacin and evaluated anti bacterial activity which played an important role in chemotherapy (10). Schofield K reported on the synthesis and reactivity of 4-(Oxiran-2-ylmethoxy) cinnoline was prepared by treating 4-chloro cinnoline with glycidol /sodium hydride in the presence of DMF. Derivatives are subject for NMR studies (11).



(Figure-1)



(Figure-2)

There are an infinite number of various types of bacteria in the world around where most of them are not harmful to mankind, some of the bacteria cause dangerous diseases of all kinds, and a few are panic like bacterial pneumonia was mostly the leading chance of death among the elderly so there is need to synthesize potent anti bacterial compounds so our investigation. In addition 1,2-benzothiazines have been seen to show marked activity against *B. subtilis* [17-20]. In view of the interest in activity and profile of 1, 2 benzodiazine new series of compounds are synthesised and characterized, purified using suitable solvent and screened for activity.

MATERIAL AND METHODS

Materials

Phenyl hydrazine (Merck), thenyol chloride (Lobachemie PVT.LTD.107), chloro acetyl chloride (Otto chem.LTD), chloro acetic acid (Lobachemie PVT.LTD.107), guanidine (Lobachemie PVT.LTD.107), amino guanidine (Otto chem.LTD), agar, bees wax, tragacanth gum. Melting points were recorded by open capillaries by WRS-1B (Germany) digital melting point apparatus and were corrected. IR spectra (KBr) were noted on SHIMADZU IR-470 spectrophotometer. ^{1}HNMR spectra were recorded by Bruker 400 ultra shield instrument using deuteron DMSO (d6-DMSO) as (TMS) as international reference standard. Purity of compound is determined by (TLC) chemicals are from sigma-Aldrich Chemical Corporation"

Scheme-1

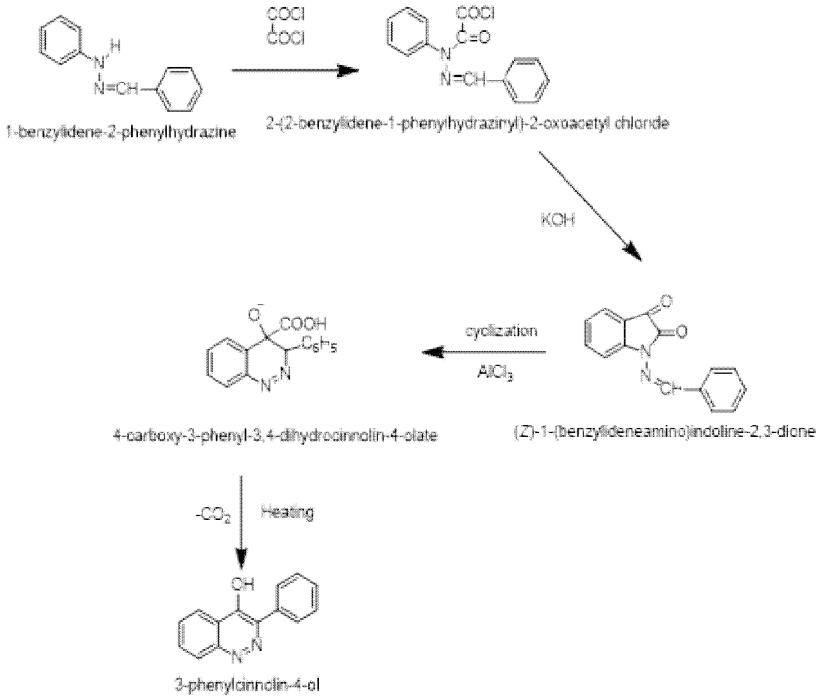


Table-1: list of synthesized compounds

Compounds	R
1	
2	
3	
4	

Scheme-2

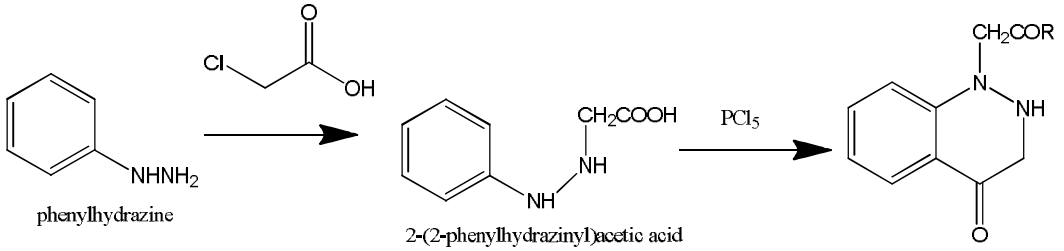
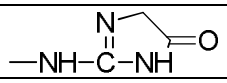
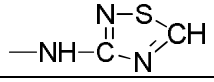
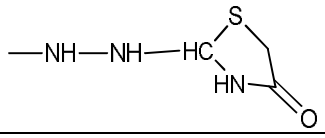


Table: 2 List of compounds

Compound	R
6	
7	
8	

DOCKING STUDY

Preparation of Ligand

To investigate the detailed intermolecular interactions between the analogues it was planned to carry out docking of molecules using Molegro Virtual Docker (MVD), Ligand structures were drawn and optimized using MM2 force field by using Chem3D Ultra 8.0 and saved in mol format. The ligand are imported to the workspace and preparation of them is done. The docking scores of the active constituents are compared against the standard drugs (Acarbose) obtained from the drug bank in .mol format.

Preparation of Protein target

The target for docking studies is selected as α - Amylase. Docking analysis is done by initially selecting the target for the disease and followed by obtaining the 3D structure of α - Amylase (3M07) D from protein data bank in the pdb format [12]. It is well known that PDB files often have poor or missing assignments of explicit hydrogen's, and the PDB file format cannot accommodate bond order information. Therefore, proper bonds, bond orders, hybridization, and charges were assigned using the MVD. The potential binding sites of both the targets were calculated using the built-in cavity detection algorithm implemented in MVD. The search space of the simulation exploited in the docking studies was studied as a subset region of 25.0 Angstroms around the active side cleft. The water molecules are also taken into consideration and the replaceable water molecules were given a score of 0.50.

Molegro Virtual Docker's docking search algorithms and scoring functions

Ligand docking studies were performed by which has recently been introduced and gained attention among medicinal chemists. MVD is a fast and flexible docking program that gives the most likely conformation of ligand binding to a macromolecule. MolDock software is based on a new heuristic search algorithm that combines differential evolution with a cavity prediction algorithm [13]. It has an interactive optimization technique inspired by Darwinian Evolution Theory (Evolutionary Algorithms - EA), in which a population of individuals is exposed to the competitive selection that weeds out poor solutions. Recombination and mutation are used to generate new solutions. The scoring function of Mol-Dock is based on the Piecewise Linear Potential (PLP), which is a simplified potential whose parameters are fit to protein ligand structures and a binding data scoring function [28,29] that is further extended in GEMDOCK (Generic Evolutionary Method for molecular DOCK) with a new hydrogen bonding term and charge schemes.

ANTI BACTERIAL ACTIVITY [16]

Preparation of Test Solutions:

Concentrations of Compounds were prepared to get 250 and 500 µg/ml by dissolved in minimum amount of Dimethyl Sulfoxide (DMSO) and the finally volume is made with DMSO. Standard solution is prepared by taking Ampicillin as the reference for *E.coli* and Trimethoprim for *Staphylococcus aureus*. Petri plates are sterilized and prepared agar medium was transferred to petriplates (thickness of 5-6mm). Test Organisms used is *Escherichia coli. (NCIM 2607) Gram* $^{-ve}$ *Staphylococcus aureus Gram* $^{+ve.}$ The sample

messed in Dimethyl sulphoxide and standard solution for respective references of 10 µg discs is introduced in to agar medium and incubated at 37°C for 24hrs. The sample is measured after incubation and the anti bacterial activity is determined by calculating the zone of inhibition in mm and MIC is determined by serial dilution method. Preparation of standard solutions is done by taking Ampicillin and Trimethoprim as the reference standard and the drug prepared in DMSO to get 250 & 500 µg/ml. Test Organisms was *Escherichia coli. (NCIM 2607)* - *Gram -ve Staphylococcus aureus* - *Gram +ve.*

Preparation of Peptone water media was prepared done by taking following ingredients. Beef extract – 20 g ,Peptone – 20g Sodium chloride – 10 g ,Agar - 40g,Distilled water – Q.S. to 1000 ml. The above shown quantities of different ingredients were accurately weighed and dissolved in 1000ml of distilled water. 250ml of each of the media was distributed in to 4 conical flasks. Media was prepared then sterilized by autoclaving at 15 Lbs/Sq. inch for 15 minutes. Inoculums were prepared by sterilizing the peptone water medium autoclaved at 15 Lbs/Sq.inch for 15 minutes. Loop full organisms were transferred from a laboratory maintained culture in to a conical flask (250 ml) containing sterilized peptone water medium. The flask was incubated for 24 hours at 37°C.

Procedure for Microbial Assay:

Each conical flask with the medium was cooled to 46°C and inoculated with test organism (20 ml of subculture medium per 100ml of the assay medium). To 20 ml each of inoculated media was distributed into petri plates and maintained at room temperature (each reading was taken in triplicate). When media was solidified, four cups (8 mm diameter) were made using sterile cork borer. Into these cups 10µl of test and standard solutions were placed under aseptic conditions. Dimethyl Sulfoxide was under as control. The petri plates were kept in the refrigerator for 2 hrs to allow the uniform diffusion of drug into the agar medium. All the petri plates were then incubated at 37°C for 24 hours and zones of inhibition (in mm) were measured.

Table-3: List of newly synthesized compounds

Compound	IUPAC NAME
1	4-(5-methyl-1H-pyrazol-1-yl)-3-phenylcinnoline
2	2-((3-phenylcinnolin-4-yl)amino)-1H-imidazol-5(4H)-one
3	N-(3-phenylcinnolin-4-yl)-4H-1,2,5-thiadiazin-3-amine
4	2-(2-(3-phenylcinnolin-4-yl)hydrazinyl)thiazolidin-4-one
5	2,3-dihydrocinnolin-4(1H)-one
6	2-(4-oxo-3,4-dihydrocinnolin-1(2H)-yl)-N-(5-oxo-4,5-dihydro-1H-imidazol-2-yl)acetamide
7	2-(4-oxo-3,4-dihydrocinnolin-1(2H)-yl)-N-(1,2,4-thiadiazol-3-yl)acetamide
8	2-(4-oxo-3,4-dihydrocinnolin-1(2H)-yl)-N'-(4-oxothiazolidin-2-yl)acetohydrazide

Chemical synthesis:

3-phenylcinnolin-4-ol

Weigh accurately 0.15 mole of benzaldehyde into 250ml beaker, then add glacial acetic acid to 15ml to the mixture and stir well heat the mixture on the water bath now add 0.1 mole of phenyl hydrazine slowly to the reaction mixture with continuous stirring. Heat the reaction mixture for 1hr on water bath and pour into ice cold water, filter and dry the product name as 1-benzylidene-2-phenylhydrazine then dissolve in 15ml of glacial acetic acid add oxaolyl chloride of 15ml to the reaction mixture and kept for reflection for 3hr then pour the mixture into crushed ice and filter the precipitate and now treat the mixture with 30%KOH heat the mixture on water bath for 1hr and filter the precipitate, dried and examine by TLC, and named as 3-phenylcinnolin-4-ol.

IR (KBr Pellet, υ in cm^{-1}):3389.32 (OH Str), 1543.01(N=N Str of charecterist of cinnoline), 1457.52(C=C Str of aromatic ring), 1392.85(C-N Str), 685.24(Aryl O-di substituted),

^{1}HNMR (DMSO-d6, δinppm):6.034(s,1H,CH),7.152(s,1H,CH),7.272(s,1H,CH),7.41-7.369(d,J=8.2 Hz,2H,CH), 7.516(s,2H,CH), 7.672(s,1H,CH), 7.798(s,2H,CH)

4-(5-methyl-1H-pyrazol-1-yl)-3-phenylcinnoline (2)

3-phenylcinnolin-4-ol of 0.001 moles is taken in clean beaker submitted for chlorination in presence of phosphorous trichloride 15ml and the intermediate formed is treated with hydrazine hydrate 12ml in presence of ethyl acetone acetate 20ml to give 4-(5-methyl-1H-pyrazol-1-yl)-3-phenylcinnoline

IR (KBr Pellet, υ in cm^{-1}):3391.03(N-H, str), 3055.33(Ar-CH, str), 3025.31 (C-H Str), 1597.31 (C=C Str), 1651.91 (C=O Str), 1460.17(C-N str), 1418.41(N=N charecterist of cinnoline), 1107.91(C-S str) 759.00(Aryl O-di substituted

^{1}HNMR (DMSO-d6, δ in ppm): 2.256(s,2H,CH_2), 4.082(s,1H,C-NH), 7.142(s,1H,CH), 7.349-7.412(d,J=8.2Hz,2H,CH),7.515(s,2H,CH),7.668(s,1H,CH).7.519(s,2H,CH),7.792(s,2H,CH), 7.9524(s,1H,CH)

2-((3-phenylcinnolin-4-yl) amino)-1H-imidazol-5(4H)-one (3)

4-chloro-3-phenylcinnoline of 0.005 mole is treated with 2.5gm of guanidine in presence of methanol and refluxed for 2hrs and the intermediate formed is treated with Chloro acetyl chloride to give 2-((3-phenylcinnolin-4-yl) amino)-1H-imidazol-5(4H)-one

IR (KBr Pellet, υ in cm^{-1}): 3316.39(N-HStr), 3204(NHstrCONHNH$_{2)}$, 2925 C-H,str(OCH_2),2855 (CHstr,thidiazole), 1836(C-HstrArylovertone)1545.81(C=C Str), 1686.89(C=O str Amide),1622.58 (C=NStr), 1060.85(C-Ostr,ether), 728.93 (C-S Str), 685.24(Aryl O-di substituted),

^{1}HNMR (DMSO-d6, δ in ppm): 1.406(s,2H,CH_2),4.238(s,1H,NH), 7.349-7.410(d,J=8.2 Hz,s,2H,CH),7.490-7.528(J=7.6 Hz,d,3H,CH), 7.662(s,1H,CH). 7.795(s,2H,CH), 7.952(s,1H,CH).

N-(3-phenylcinnolin-4-yl)-4H-1, 2, 5-thiadiazin-3-amine (4)

4-chloro-3-phenylcinnoline of 0.005 moles is treated with 2.5gm of guanidine in the presence of methanol and reflexed for 2hrs and the intermediate formed is treated potassium hydroxide 3.5 Gms and carbon disulphide 15ml to give N-(3-phenylcinnolin-4-yl) -4H-1, 2, 5-thiadiazin-3-amine

IR (KBr Pellet, υ in cm^{-1}): 3316.39(N-HStr), 3204(NHstrCONHNH$_{2)}$, 2925 C-H,str(OCH_2),2855(CHstr,thidiazole),1836(C-HstrArylovertone)1545.81(C=C Str), 1686.89(C=O str Amide),1622.58(C=NStr), 1060.85(C-Ostr,ether), 728.93 (C-S Str), 685.24(Aryl O-di substituted),

^{1}HNMR (DMSO-d6, δ in ppm): 2.3(s,1H,NH), 3.950-3.939(d,J=2.2,Hz,2H,CH),4.1(s,1H,NH), 5.771(s,1H,CH), 7.141(s,1H,CH), 7.349-7.410(d J=8.2 Hz,2H,CH),7.514(s,2H,CH), 7.795(s,2H,CH), 7.95-8.01(d,2H,CH)

2-(2-(3-phenylcinnolin-4-yl) hydrazinyl) thiazolidin-4-one (5)

2-(3-phenylcinnolin-4-yl) hydrazinecarbothioamide is treated with cloroacetyl chloride 15ml in presence of 25ml methanol refluxed for 3hrs to give 2-(2-(3-phenylcinnolin-4-yl) hydrazinyl) thiazolidin-4-one

IR (KBr Pellet, υ in cm^{-1}):3391.03(N-H str), 3055.33(Ar-CH str),3025.31 (C-H Str),1597.31 (C=C Str), 1683.91 (C=N Str 1460.17(C-N str),1418.41(N=N charecterist of cinnoline), 1107.91(C-S str)759.00(Aryl O-di substituted).

^{1}HNMR (DMSO-d6, δ in ppm) 2.160(s,1H,N-N), 3.914(s,1H,CH_2), 4.108(s,1H,C-NH), 6.958-7.021(d,J=8,2H,CH), 7.568(s,1H,CH), 7.723(s,1H,CH),

Scheme-2

EXPERIMENTAL METHODOLOGY GENERAL PROCEDURE FOR SYNTHESIS

STEP-1 SYNTHESIS OF PHENYL HYDRAZON

Weigh accurately 0.1 mole of phenyl hydrazine, taken into clean beaker add 15ml of glacial acetic acid slowly with continuous stirring then add 0.1 mole of Chloro acetic acid slowly into the reaction mixture, heat the mixture on water bath for 2hr at 70c,finally pour the reaction mixture into the crushed ice and filter the precipitate

STEP-2: SYNTHESIS OF CINNOLIN-4(1H)-ONE

The intermediate compound which was prepared in the first step was dissolved in 20 ml of diethyl ether with stirring and then add phosphorous penta chloride of 3gm stir well kept aside for half an hour then add crushed ice into the reaction mixture then finally filter the precipitate, and kept for air-dry,cinnolin-4(1H)-one

2-(4-oxo-3, 4-dihydrocinnolin-1(2H)-yl)-N-(5-oxo-4, 5-dihydro-1H-imidazol-2-yl) acetamide (6)

2,3-dihydrocinnolin-4(1H)-one of 0.005 mole is treated with 20ml of Chloro acetic acid for acetylating then treated with 2.5gm of guanidine in presence of methanol and refluxed for 2hrs and the intermediate formed is treated with Chloro acetyl chloride to give 2-(4-oxo-3,4-dihydrocinnolin-1(2H)-yl)-N-(5-oxo-4,5-dihydro-1H-imidazol-2-yl)acetamide

IR (KBr Pellet, υ in cm^{-1}): 3316.39(N-HStr), 3204(NHstrCONHNH$_{2)}$, 2925 C-H,str(OCH_2),2855(CHstr,thidiazole),1836(C-HstrArylovertone)1545.81(C=C Str), 1686.89(C=O str Amide),1622.58(C=NStr), 1060.85(C-Ostr,ether), 728.93 (C-S Str), 685.24(Aryl O-di substituted),

^{1}HNMR (DMSO-d6, δ in ppm): 2.202(s,1H,N-H), 3.91-3.93,-3.95(t,6H,CH_2), 7.728(s,1H,CH),6.949-7.010 (d,J=12.2Hz ,2H,CH), 7.562(s,1H,CH), 7.729(s,1H,CH), 8.022(s,2H,NH),

2-(4-oxo-3, 4-dihydrocinnolin-1(2H)-yl)-N-(1, 2, 4-thiadiazol-3-yl) acetamide (7)

2,3-dihydrocinnolin-4(1H)-one of 0.005 moles is treated with Chloro acetic acid and 2.5gm of guanidine in presence of methanol and refluxed for 2hrs and the intermediate formed is treated potassium hydroxide with carbon disulphide 20ml to yield 2-(4-oxo-3, 4-dihydrocinnolin-1(2H)-yl)-N-(1, 2, 4-thiadiazol-3-yl) acetamide

^{1}HNMR (DMSO-d6, δ in ppm): 2.**104(s,1H,N-NH),3.919-3.951(d,J=6.4,4H,CH_2),6.949-7.010(d,2H,CH), 7.57-7.719(s,1H,CH), 8.026(s,1H,NH),9.102(s,1H,CH).**

2-(4-oxo-3, 4-dihydrocinnolin-1(2H)-yl)-N'-(4-oxothiazolidin-2-yl) acetohydrazide (8)

2,3-dihydrocinnolin-4(1H)-one of 0.005 moles is treated with chloroacetic acid and then treated with amino guanidine 2.5gm of guanidine in presence of methanol and refluxed for 2hrs and the intermediate

formed is treated with carbon disulphide 20ml to give 2-(4-oxo-3, 4-dihydrocinnolin-1(2H)-yl)-N'-(4-oxothiazolidin-2-yl) acetohydrazide

^{1}HNMR (DMSO-d6, δ in ppm): 2.022(s,2H,N-NH),3.95-3.85(d,1H,CH_2),3.912-3.950(d,4H,CH_2), 6.942-7.013(d,2H,CH), 7.563-7.713(s,1H,CH),

Table-4: List of compounds with IUPAC names

Compound	IUPAC NAME
1	**4-(5-methyl-1H-pyrazol-1-yl)-3-phenylcinnoline**
2	**2-((3-phenylcinnolin-4-yl)amino)-1H-imidazol-5(4H)-one**
3	**N-(3-phenylcinnolin-4-yl)-4H-1,2,5-thiadiazin-3-amine**
4	**2-(2-(3-phenylcinnolin-4-yl)hydrazinyl)thiazolidin-4-one**
5	**2,3-dihydrocinnolin-4(1H)-one**
6	**2-(4-oxo-3,4-dihydrocinnolin-1(2H)-yl)-N-(5-oxo-4,5-dihydro-1H-imidazol-2-yl) acetamide**
7	**2-(4-oxo-3,4-dihydrocinnolin-1(2H)-yl)-N-(1,2,4-thiadiazol-3-yl)acetamide**
8	**2-(4-oxo-3,4-dihydrocinnolin-1(2H)-yl)-N'-(4-oxothiazolidin-2-yl)acetohydrazide**

Table-5: *In-silico* docking analysis of designed molecules ranking based on Mol Dock Score and H-Bond Interaction

Ligand	Molecular dock score	H-bond interactions
1	**-110.633**	**-1.9186**
2	**-82.4779**	**-82.4779**
3	**-86.5191**	**-0.65447**
4	**-97.2528**	-1.7974
5	**-54.6095**	-2.26009
8`	**-89.128**	0.033998

Table-6: *In-silico* docking analysis of designed molecules ranking based on Mol Dock Score and H-Bond Interaction

Ligand	Molecular dock score	H-bond interactions
1	-124.725	0
2	-91.5215	-0.59635
3	-115.468	-2.74114
4	-139.082	-9.63919
5	-70.2666	-7.50377
8	-122.486	-7.25822

Table-7: Docking score and Hydrogen binding interactions of derivatives with trimethoprim

S.No	compound	Docking score	Binding interactions
1	**1**	**-110.633**	**Tyrosine-21**
2	**2**	**-82.4779**	**Phenyl,tyrosine-98**
3	**4**	**-97.2528**	**Threonine 121** **Arginine 44**

Table-8: Docking score and Hydrogen binding interactions of derivatives with Amphicillin

S.No	compound	Docking score	Binding interactions
1	**1**	-124.725	**Tyrosine-21**
2	**4**	-139.082	**Histidine-170,tyrosine**

Table-9: Docking code of proposed Target for antibacterial activity

Targets & PDB ID
acyl enzyme(*E.coli*) 3ITA **DHFrase A (*S.Aureus*) 2W9S**

Molecular virtual docker was used to perform all docking simulation. A set of new 1,2-benzodiazine derivatives was subjected to dock with acyl enzyme (PDB ID: 3ITA) and DHFR (PDB ID: 2W9S). From the Protein Data Bank (RCSB) (http://www.rcsb.org/pdb), the acyl enzyme and DHFR were retrieved. To carry out *in- silico* studies, the 2D structures of the synthesized ligands CN-1(a-c), CN-2(a–d) were drawn and converted to energy minimized 3D structures in the pdb file format using Marvin Sketch (Chem Axon). By removing the hetero atoms, water molecule and cofactors, the target protein file was prepared by leaving the associated residue with protein by using Molecular virtual docker. Preparation of target

protein file Molecular virtual docker tool has been done, which involves the assignment of Gasteiger charges for all the atoms of molecules converting into AD4 type. Docking simulation for the compounds CN-1(a-c), CN-2(a–d) was performed against the active site of an acyl enzyme and DHFR. Binding energy of compounds is calculated finally. Docking studies of large molecule revealed that compound CN-1, CN-2, CN-4 had shown considerable interaction with Ampicillin in the active site of an acyl-enzyme of E.coli (code: 3ITA) and DHFR (2W9S) is complexes with trimethoprim in *S.aureus.*

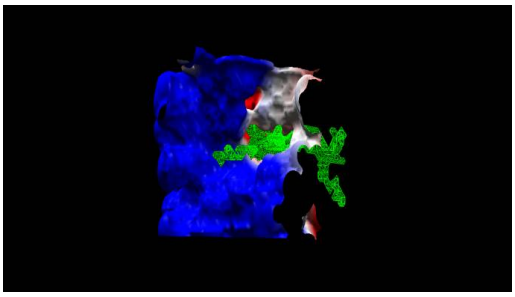


Figure-3.Binding pocket in acyl enzyme

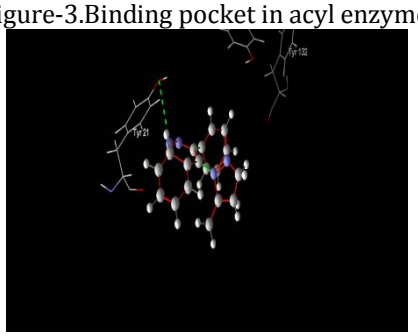


Figure-4.Structure of protein acyl enzyme (3ITA) binding with target of ligand-1

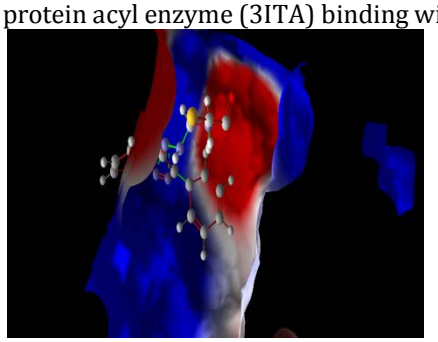


Figure-5.Structure of protein acyl enzyme (3ITA) binding with target of ligand-4

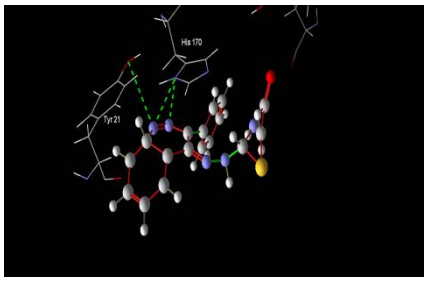


Figure-6.Structure of protein acyl enzyme (3ITA) binding with target of ligand-4

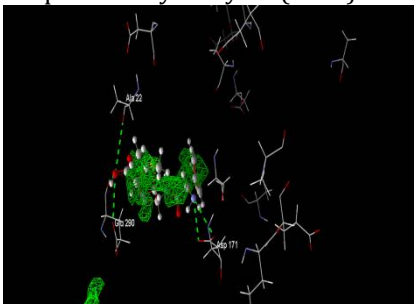


Figure-7 HB interactions of standard drugs with protein

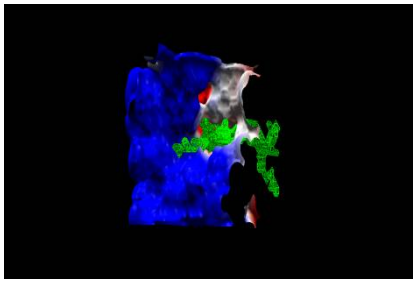


Figure-8.Binding pocket in DHRF

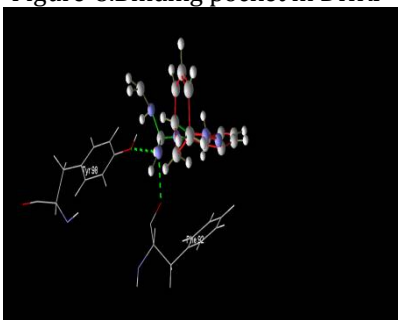


Figure-9. Structure of protein DHFR (**2W9S**) binding with target of ligand-2

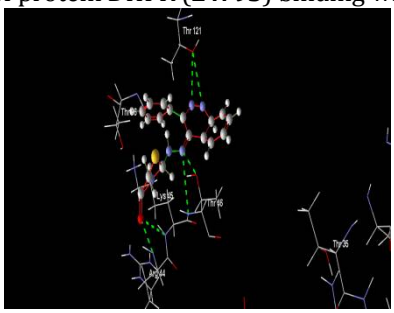


Figure-10. Structure of protein DHFR (**2W9S**) binding with target of ligand-4

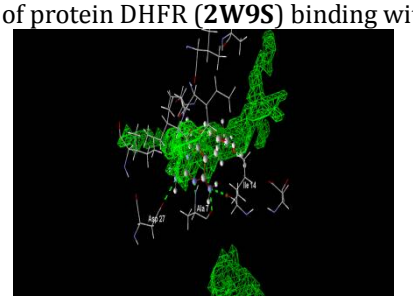


Figure-11.HB interactions of standard drugs with protein

ANTI BACTERIAL ACTIVITY

Preparation of Test Solutions:

Concentrations of Compounds were prepared to get 250 and 500 µg/ml be dissolved in a minimum amount of Dimethyl Sulfoxide (DMSO) and finally volume is made with DMSO. Standard solution is prepared by taking Streptomycin as the reference. Petri plates are sterilized and prepared agar medium was transferred to petriplates (thickness of 5-6mm) .Test Organisms used is *Escherichia coli*. (NCIM 2607) Gram –ve *Staphylococcus aureus* Gram +ve. The sample messed in dimethyl sulphoxide and standard streptomycin 10 µg discs are introduced in to agar medium and incubated at 37oC for 24hrs.the sample is measured after incubation and the anti bacterial activity is determined by calculating the zone of inhibition in mm and MIC is determined by serial dilution method. Preparation of standard solutions is done by taking Streptomycin as the reference standard drug prepared in DMSO to get 250 500 µg/Ml. Test Organisms was *Escherichia coli*. (NCIM 2607) - Gram -ve *Staphylococcus aureus* - Gram +ve.

Preparation of Peptone water media was prepared done by taking following ingredients Beef extract – 20 g ,Peptone – 20g Sodium chloride – 10 g ,Agar - 40g,Distilled water – Q.S. to 1000 ml The above shown quantities of different ingredients were accurately weighed and dissolved in 1000ml of distilled Water. 250ml of each of the media were distributed in about 4 conical Flasks. Media was prepared then sterilized by autoclaving at 15 Lbs/Sq. inch for 15 Minutes. Inoculum was prepared by sterilizing the peptone water

medium autoclaved at 15 Lbs/Sq.inch of 15 MM Minutes. Loop full organisms were transferred from a laboratory maintained culture in to conical flask (250 ml) containing sterilized peptone water Medium. The flask was incubated for 24 hours at 37°C.

Procedure for Microbial Assay:

Each conical flask with the medium was cooled to 46°C and inoculated with the test organism (20 ml of subculture medium per 100ml of the assay Medium). To 20 ml of each of inoculated media was distributed into petri plates and maintained at room temperature (each reading was taken in triplicate). When media were solidified. Four cups (8mm diameter) were made using sterile cork borer. Into these cups 10µl of test and standard solutions were placed under aseptic Conditions. Dimethyl Sulfoxide was under as control. The petri plates were kept in the refrigerator for 2 hrs to allow the uniform diffusion of the drug into the agar medium. All the petri plates were then incubated at 37°C for 24 hours and zones of inhibition (in mm), were measured.

Table-11: Antibacterial Activity of Synthesized Compound

S.NO	Compound Code	Zone of Inhibition (mm)			
		Gram $^{+Ve}$		Gram $^{-Ve}$	
		S.aureus		*E.Coli*	
		250µg/ml	500µg/ml	250µg/ml	500µg/ml
1	CN-1	14	11	17	18
2	CN-2	11	8	16	18
3	CN-3	8	11	9	12
4	CN-4	8	12	13	16
5	CN-5	10	9	14	12
6	CN-6	10	8	9	11
7	CN-7	8	12	8	8
8	CN-8	8	11	9	7
Control	DMSO	8		8	
Standard		20(trimethoprim)		21(Amphicillin)	

The compounds synthesized are screened for anti bacterial activity against Gram negative *(Escherichia coli* ATCC 8739) and Gram positive bacteria (*Staphylococcus aureus* ATCC25923). Compounds **-1** and Compound-2 and Compound-4 showed fairly good anti-bacterial activity when compared to Streptomycin. Among the test derivatives, Compound **-1** had shown significant antibacterial property against gram-positive **(***Staphylococcus aureus***)** compounds 1,2 & 4 is potent against gram-negative (*Escherichia coli)* due to presence of nitrogenous base The compounds activity is assumed to be due to presence of Quinoline moiety which is a potent anti bacterial agent.

CONCLUSION

Synthesized characterization and evaluation of anti-bacterial activity of substituted benzodiazine derivatives were evaluated with physical and biological methods. Docking studies of large molecule revealed that compound CN-1, CN-2, CN-4 had shown considerable interaction with Ampicillin in the active site of an acyl enzyme of *E. coli* (code: 3ITA) and DHFR (2W9S) is complexes with trimethoprim in *S. aureus*. Study on this particular nucleus is planned for further development in the field of research. All the synthesized molecules were achieved in excellent yields by following a simple method. The projected structures of synthesized compounds were well supported by the spectral characterization data by IR, ^{1}H-NMR and EI-MS. Antibacterial potential of the parent compound 3, and its derivatives 5a-e, revealed that none of the compounds were active against *S. aureus* and *E. coli***.** Moreover, 2-(4-oxo-3, 4-dihydrocinnolin-1(2H)-yl)-N'-(4-oxothiazolidin-2-yl) acetohydrazide (8) did not show any inhibitory potential against any bacterial strain. Overall, 3-phenylcinnolin-4-ol (1) was the only compound which showed maximum inhibition against *S. aureus and E***.** coli**.** However, all compounds showed potent inhibitory action except 2-(4-oxo-3, 4-dihydrocinnolin-1(2H)-yl)-N-(1, 2, 4-thiadiazol-3-yl) acetamide (7), 2-(4-oxo-3, 4-dihydrocinnolin-1(2H)-yl)-N'-(4-oxothiazolidin-2-yl) acetohydrazide (8) which displayed weak inhibition against gram –ve bacteria .On the basis of aforesaid results, the synthesized cinnolines may provide an overall indispensable basis to introduce new drug candidates for the cure of inflammatory and other associated diseases.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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