Original Article

Design, Synthesis and Antibacterial Activity of Certain Novel Indole-glyoxylamide Conjugates

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ABSTRACT

Objectives: To synthesize 2-Aryl 3-substituted indole-glyoxylamides to evaluate their antibacterial activity on target protein (4DH6). **Materials and Methods:** Computational techniques: The fused heterocyclic compound indole linked with glyoxylamide was designed by using computational techniques and also energy minimized. The molecular property and biological activities is determined by mol inspiration tool. The antibacterial target receptor/protein (PDB ID: 4DH6) have been selected for the docking studies. Experimental work: Synthesis of 2-aryl indol-3-yl glyoxylamide derivatives by reaction of 2-phenylindole condensed with oxalyl chloride and different amines in one pot reaction. Spectral characterization and antibacterial activity: The synthesized lead molecules were characterized by means of FTIR, ESI-Mass spectral analysis and tested for their antibacterial activity by using agar well plate diffusion method. **Results:** The lead molecules were selected based on high binding affinity on target receptor 4DH6. The selected lead molecule was synthesized and the functional group, molecular weight, number of proton is identified by FTIR, ESI-Mass and 1HNMR respectively. The purity of the compound was determined by melting point and TLC. Antibacterial activities of synthesized lead compounds were active against gram positive bacteria *Bacillus subtilis* and gram-negative bacteria *Escherichia coli* at a concentration of 100μg/ml. **Conclusion:** In the present work indicates that nitro (NO_2) and chloro (Cl) substituted 2 –Aryl indole 3-Glyoxylamide derivatives showed potent antibacterial activity against both gram positive *Bacillus subtilis and* gram negative *E.coli.* Hence this study will used to design of more potent antibacterial agents for therapeutic use.

Keywords: Auto dock, Molinspiration FTIR, ESI-MASS, Antimicrobial activity.

INTRODUCTION

Bacteria capable of causing disease usually enter into the body through the eyes, mouth, nose, or urogenital openings, or through wounds or bites that breach the skin barrier. Symptoms of a bacterial infection are fever chills and sweats, swollen lymph nodes, headache, skin flushing, swelling, or soreness and gastrointestinal symptoms, such as nausea. vomiting, diarrhea, abdominal or rectal pain. The inflammatory response (inflammation) occurs when tissues are injured by bacteria, trauma, toxins, heat, or any other cause. The damaged cells release chemicals including histamine, bradykinin, and prostaglandins. Also bacterial infection reduces the immune system for individuals having AIDS, cancer or that undergoing organ transplantation. (Figure 1)

Due to this design and development of new lead molecules against bacterial infection and with different modes of action rather than existing drugs is necessary for clinical needs.[1-4]

MECHANISM AGAINST BACTERIAL INFECTION (FIGURE 2)

- Inhibition of Cell Wall Synthesis.
- Inhibition of Protein Synthesis (Translation)
- Alteration of Cell Membranes.
- Inhibition of Nucleic Acid Synthesis.
- Antimetabolite Activity.

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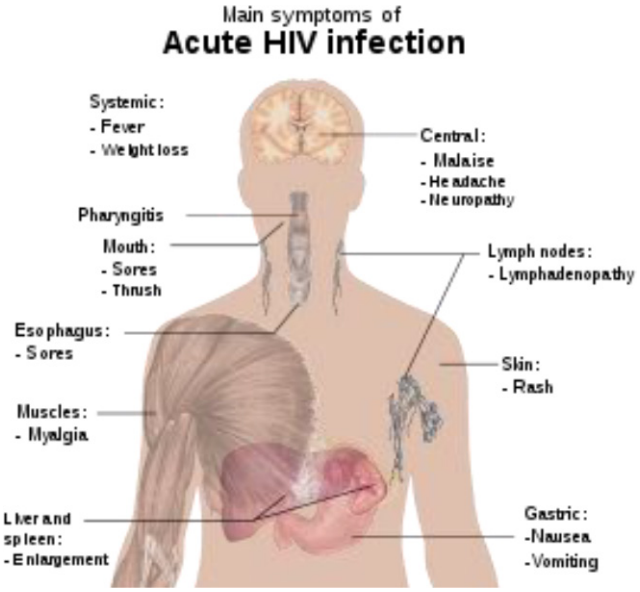


Figure 1: Bacterial infection.

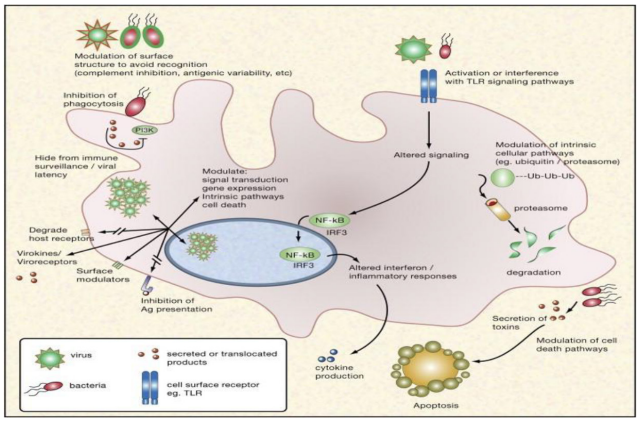


Figure 2: Mechanism.

Infection process	Host defense	Bacterial evasion mechanisms
Attachment to host cells	Blockage of attachment by secretory IgA antibodies	Secretion of proteases that cleave secretory IgA dimers (*Neisseria meningitidis, N. gonorrhoeae, Haemophilus influenzae*) Antigenic variation in attachment structures (pili of *N. gonorrhoeae*)
Proliferation	Phagocytosis (Ab- and C3b-mediated opsonization)	Production of surface structures (polysaccharide capsule, M protein, fibrin coat) that inhibit phagocytic cells Mechanisms for surviving within phagocytic cells Induction of apoptosis in macrophages (*Shigella flexneri*)
	Complement-mediated lysis and localized inflammatory response	Generalized resistance of gram-positive bacteria to complement-mediated lysis Insertion of membrane-attack complex prevented by long side chain in cell-wall LPS (some gram-negative bacteria)
Invasion of host tissues	Ab-mediated agglutination	Secretion of elastase that inactivates C3a and C5a (*Pseudomonas*)
Toxin-induced damage to host cells	Neutralization of toxin by antibody	Secretion of hyaluronidase, which enhances bacterial invasiveness

INDOLE

The word indole is a trivial name for benzo[b]pyrrole obtained from the combination of indigo and oleum. In 1866, Baeyer and Knop obtained two products, dihydroxyindole and oxindole from the reduction of indigo which they considered as derivative of C8H7N, later they proposed name-Indole.

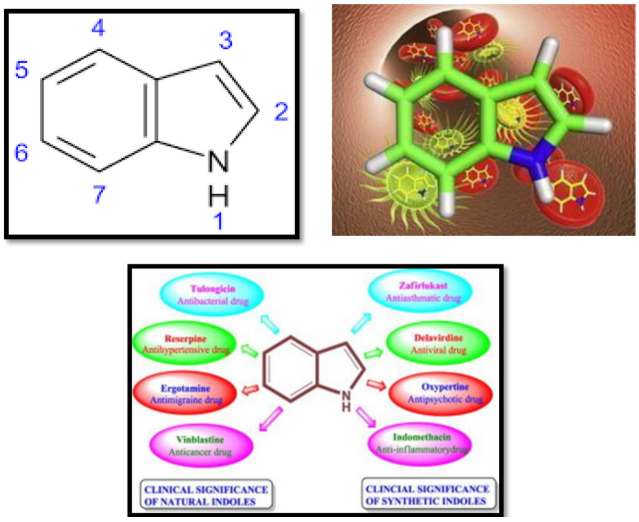


Figure 3: Activity of Indole.

A heterocyclic compound contains different pharmacological activities. Nitrogen, sulphur or oxygen atom substituted five or six membered and fused heterocyclic compounds are important in the field of medicinal chemistry and drug discovery.[5-7] Indole nucleus is a fused heterocyclic compound contains six membered benzene ring with five membered pyrole ring. Most of the marketed available drugs are nitrogen containing heterocyclic compounds. Indole nucleus present in the derivatives constitute an important in the field of medicinal chemistry including pharmacological action such as antitumor, antiviral, antihypertensive, anti-inflammatory, analgesic, antimicrobial, antifungal activities and anti-depressant. (Figure 3)

Indole is a important nirogen containing heterocyclic nucleus and also back bone of the neutrotransmitter serotonin, natural hormone melatoin and essential amino acid tryptophan.The indole derivatives have been synthesized or isolated from nature sources. Vincristine vinblastine and mitomycin is an indole alkaloids and its analogues are used in cancer chemotherapy and antibacterial activity respectively.[8] An indole-containing derivatives makes them suitable lead for the development of new antibacterial and antifungal agents.

2-phenyl 3- substituted indole derivatives shown to exhibit pharmacological activity. Indole fused with glyoxylamide compounds are designed by computational technique's and calculated the drug likeness property.

The glyoxyamides side chain contains two carbonyl groups with different spatial arrangement and potentially

enhances their hydrogen bonding interaction with target receptor and also enhancing their biological activities.

Also glyoxylamide derivatives are important in organic chemistry and they are fused in bioactive molecules which produce number of pharmacological activity.[9-12] Based on this concept to planned to design indole fused glyxylamide derivatives and synthesized by the reaction of 2-phenyl indole derivatives with oxalyl chloride and different amine in one pot reaction. The purity of synthesized lead molecules was identified by thin layer chromatography, melting point and characterized with help of FTIR, NMR and Mass spectral data.

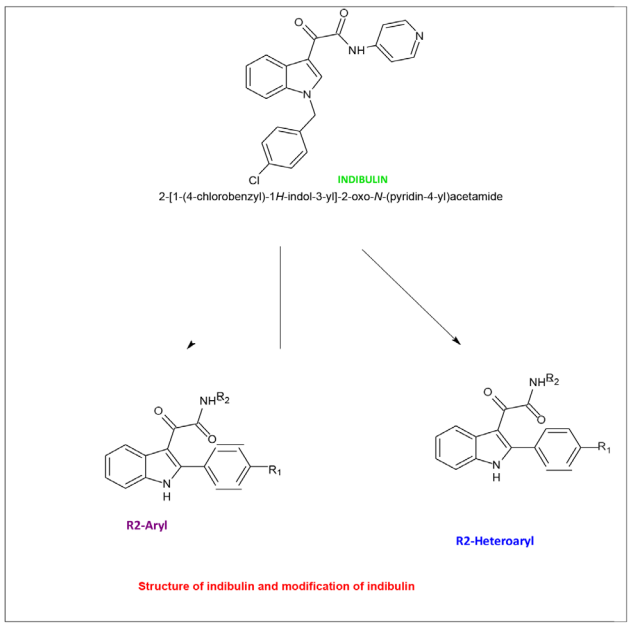
In this present study the antimicrobial activity has been studied in detail. The antimicrobial activity was determined using cup-plate agar well diffusion method against *Bacillus subtilis* and *Escherichia coli.*

EXPERIMENTAL WORK

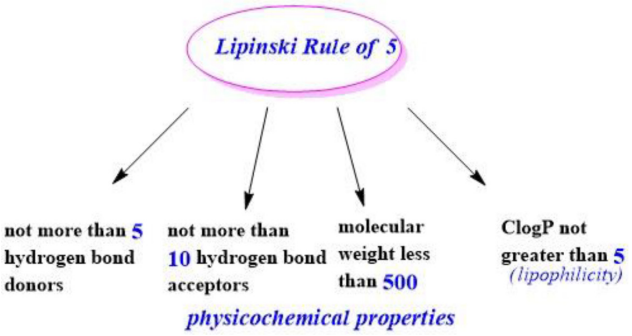
In silico Molecular Docking Studies

Pharmacophore Analysis

The computationally designed 2-phenyl indol-3-yl glyoxyamide (IG-1 to IG-70) derivatives were used for physical and biochemical characterization. The pharmacophore analysis of these derivatives was studied with aromatic and heterocyclic amine substituents at 2nd and 3rd position of indole respectively. The designed derivatives were drawn using chemdraw ultra 8.0. All the above designed derivatives are obeying the Lipinski rule of five. The above selected lead molecules such as aryl and heteroaryl substituted indol-3-yl glyoxylamide have been shown potent antibacterial activity against target protein.[13-19]



Structure of indibulin and modification of indibulin



Molecular Descriptors Analysis

Molecular descriptor or chemical descriptor are readily calculated a chemical and physical information of lead molecules. It is plays important role in the field of quantitative structure activity relationship and quantitative structure property relationship.

Lipinski's rule of five analysis

Lipinski's rule stated that an orally active drug molecule has no violation of the following criteria.[20,21]

Chem Draw Ultra

The computationally designed lead molecules were drawn by using Chem Draw Ultra 8.0. developed by Cambridge Pvt.Ltd.[22]

Protein selection

The selected protein/receptor target which has the specific biological activity was downloaded in the RCSB PDB format using respective PDB ID 4DH6 from Protein Data Bank (www.rcsb.org)

Preparation of protein

By the protein crystal structures are prepared prior to docking to add hydrogen atoms, optimize hydrogen bonding and water molecules and ligands are removed from protein, saved in PDB format.

Ligand preparation

The lead molecules that are docked must have been good representation of the actual ligand are docked in a protein-ligand complex in order to give the better binding energy. For this the lead molecules must show following condition.

- Must be prepared in PDB format and must have all hydrogens.
- It must contain a single molecule that has no covalent bonds to the receptor, with no fused fragments such as counter ions and solvents.
- Must contain realistic bond lengths and bond angles.

Docking

After making a protein and ligand to pdbqt format, the grid was made to maximum. Then docking was done to obtain the binding energy.

Visualizing docking results

PyMol was used for visualizing ligand-protein interaction.[23]

MATERIALS AND METHODS

Materials

To synthesize structurally distinct 2-phenyl indol-3yl-glyoxylamides.

2-phenyl indol-3yl- glyoxylamide derivatives prepared from substituted acetophenone and phenyl hydrazine was condensed with ethanol for 2-4 hr.

Chemicals Used	Apparatus used
Phenyl hydrazine substituted acetophenone	Beaker, thermometer,
Ethanol and sulphuric acid	Reflux condenser
Oxalyl chloride, diethyl ether	Round bottom flask
Toluene magnesium sulphate	Glassrod, Funnel and magnetic stirrer
Aniline, P-Cl aniline P- OH aniline	Beaker, thermometer,
Sulphanilic acid, Sulphanilamide	
P-amino benzoic acid	
2-aminopyridine, 4-amino pyridine	
2-amino, piperazinamine	
Phenyl hydrazine substituted acetophenone	

The experiment was carried out in the Department of Pharmaceutical Chemistry, Grace College of Pharmacy, Palakkad.

Methods

Melting points was determined by using melting point apparatus MP-DS TID 2000V.scientific and were uncorrected. Reactions were monitored by TLC on pre coated silica G plates using iodine vapors as visualizing agents by using dichloromethane/methanol = 9:1. IR Spectra were recorded on JASCO FT/IR-140 Spectrophotometer in the Department of Pharmaceutical Analysis, Grace College of Pharmacy, Palakkad.

^{1}H NMR was recorded with DMSO as internal standard on a Bruker spectrometer at 400MHz and 100 MHz respectively. Antimicrobial activity was evaluated using cup plate method.

Standard Drug used: Gentamycin were taken as reference standards and the concentration of standard drugs were prepared in DMSO to give 100µg/ml.

Chemical Synthesis[24-26]

STEP-I Substituted 2-Phenyl-1H-indoles

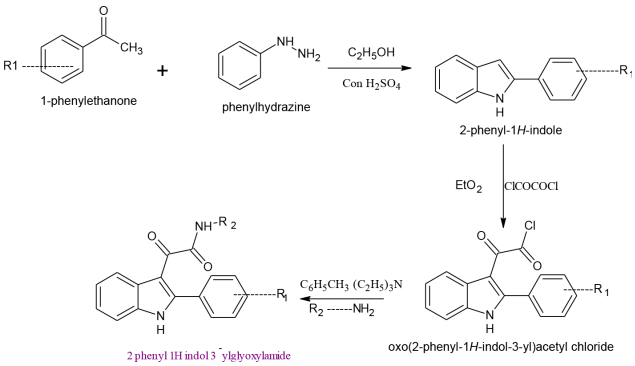
A mixture of acetophenone substituted derivatives (1 mmol) and phenyl hydrazine (1 mmol) was condensed in 10ml ethanol for 2 to 4 hr. The hydrazones formed were poured into 5ml sulfuric acid. The reaction mixture was stirred and heated for additional 25-30 min. After completion of reaction (monitored by TLC), the reaction mixture was added to ice cold water. The solid product obtained was filtered, dried and recrystallized from ethanol to get the product with high purity. M.P-212°C – 214°C

STEP-II Oxo (2-phenyl-1*H*-indol-3-yl)acetyl chloride

An oxalyl chloride (0.18 ml, 2.0 mmol) was added drop wise at 0°C to a solution of compound 1 in anhydrous diethyl ether (10 ml). The mixture was maintained at room temperature for 2 hr. The solid precipitate obtained, was collected by vacuum filtration, and immediately used in the subsequent reaction.

STEP-III 2-phenyl 1-H indole 3yl glyoxylamide

To a solution of compound 2 (0.205 g, 0.60 mmol) in anhydrous toluene (5 ml), was added to the appropriate amine (0.70 mmol) at 0°C in 1ml of the same solvent. Triethylamine (0.80 mmol) was added to the mixture and the reaction was stirring at room temperature for 4 hr (TLC analysis: dichloromethane/methanol = 9:1). The toluene solution was removed under reduced pressure, and the residue was extracted with dichloromethane and evaporated to dryness.



R1	P-OH, P-Cl, P-NO2, P-NH2, P-OCH3, 2,6 Dihydroxy Acetophenone
R2	**Aryl** -Aniline,4-chloro,p-OHaniline suphanilic acid, sulphanilamide,p-Amino phenol, p-amino benzoic acid. **Heteroaryl**- 2-Amino pyridine, 4-Amino pyridine & 2- Amino thiazole

BIOLOGICAL SCREENING

Antimicrobial Activities[27-30]

Antibacterial activities of synthesized lead compounds were active against gram positive bacteria *Bacillus subtilis* and gram-negative bacteria *Escherichia coli* at a concentration of 100μg/ml. DMSO act as a control and similar conditions using Gentamycin as standard for comparison. A nutrient agar was prepared by dissolving a weighed ingredient in water and incubated at 35-37°C for 24 hr. Each sterile nutrient agar plate was then flooded with the corresponding peptone culture of the test organism, dried for 30 min. and after drying of the flooded plates, wells were made using a cork borer (size3) on the solidified medium. Prepared wells were filled with dilution of 100μg/ml of respective test and standard compounds. All the flooded plates were incubated at 35-37°C for 24 hr for bacterial strains. The zones of inhibition were measured in mm and their means were compared accordingly.

RESULTS AND DISCUSSION

In the current study, a new lead 2-aryl indol-3- yl glyoxylamide derivatives are design and minimize the energy by computational techniques. Furthermore, the synthesized lead were docked to the active site of antibacterial (PDB ID: 4DH6) target protein using the docking program Auto dock. The least binding energy was found to be more potent anti-bacterial agents than standard. These lead molecules were investigated for drug like properties by calculating Lipinski's rule of five using molinspiration. Lipinski's rule stated that an orally active drug molecule has no violation which indicates good bioavailability. Selection of lead molecules was synthesized characterized and evaluated antimicrobial activity.

In-silico Drug Design

In-silico drug design was successfully carried out with commercially available freesoftware as well as online tools. A series of derivatives were designed using thesesoftware's.

Molecular Descriptors Analysis

Molecular descriptor or chemical descriptor are readily calculated a chemical and physical information of lead molecules.

Molecular descriptors of selected lead molecules generated by using ACD Chem Sketch and the results are shown in Table 1.

Analysis of Lipinski's rule of five

The selected lead molecules were obeying the Lipinski rule of five which indicates that the lead molecule have good pharmacological and biological activity that would make it a likely orally active drugs.

The analysis was performed by using molinspiration software and the results are shown in the Table 2.

Prediction of Drug Likeness

Prediction of drug likeness property used in drug design for how-drug like a substance and determined

Table 1: Molecular descriptors of selected lead molecules.

Compound code	Molar Refractivity (cm^3)	Molar Volume (cm^3)	Refractive Index	Parachor (cm^3)	Polarizability 10-24 (cm^3)	Surface Tension (dyne/cm)
IG-21	108.90 ± 0.3	271.4 ± 3.0	1.713 ± 0.02	784.0 ± 4.0	43.17 ± 0.5 10^{-24}	69.5 ± 3.0
IG-22	113.79 ± 0.3	283.4 ± 3.	1.735 ± 0.02	19.8 ± 4.0	45.11 ± 0.5 10^{-24}	70.0 ± 3.0
IG-25	119.26 ± 0.4	300.4 ± 3.0	1.724 ± 0.02	899.1 ± 6.0	47.28 ± 0.5 10^{-24}	80.2 ± 3.0
IG-27	106.99 ± 0.3	264.7 ± 3.0	1.742 ± 0.02	778.1 ± 4.0	42.41 ± 0.5 10^{-24}	74.6 ± 3.0
IG-30	106.13 ± 0.4	268.9 ± 5.0	1.719 ± 0.03	803.1 ± 6.0	42.07 ± 0.5 10^{-24}	79.5 ± 5.0
IG-31	109.03 ± 0.3	283.6 ± 3.0	1.695 ± 0.02	785.2 ± 4.0	43.22 ± 0.5 10^{-24}	58.7 ± 3.0
IG-37	107.12 ± 0.3	276.8 ± 3.0	1.700 ± 0.02	779.3 ± 4.0	42.46 ± 0.5 10^{-24}	62.7 ± 3.0
IG-40	106.46 ± 0.4	279.5 ± 5.0	1.686 ± 0.03	804.6 ± 6.0	42.20 ± 0.5 10^{-24}	68.6 ± 5.0
IG-55	118.05 ± 0.4	300.5 ± 3.0	1.714 ± 0.02	879.2 ± 6.0	46.80 ± 0.5 10^{-24}	73.2 ± 3.0
IG-56	114.18 ± 0.3	284.1 ± 3.0	1.736 ± 0.02	826.5 ± 4.0	45.26 ± 0.5 10^{-24}	71.6 ± 3.0
IG-58	105.34 ± 0.3	264.8 ± 3.0	1.726 ± 0.02	758.5 ± 4.0	41.76 ± 0.5 10^{-24}	67.3 ± 3.0
IG-59	103.72 ± 0.3	254.3 ± 3.0	1.751 ± 0.02	741.7 ± 4.0	41.12 ± 0.5 10^{-24}	72.3 ± 3.0
IG-64	114.76 ± 0.4	284.7 ± 3.0	1.739 ± 0.02	857.3 ± 6.0	45.49 ± 0.5 10^{-24}	82.2 ± 3.0
IG-65	116.84 ± 0.4	290.8 ± 3.	1.736 ± 0.02	870.0 ± 6.0	46.32 ± 0.5 10^{-24}	80.0 ± 3.0
IG-68	104.68 ± 0.3	255.1 ± 3.0	1.757 ± 0.02	748.5 ± 4.0	41.50 ± 0.5 10^{-2}	74.0 ± 3.0

Table 2: Physico chemical properties of lead molecules.

COM P	Log P	TPSA	MW	No of Hydrogenbond acceptor	No of Hydrogen bond donor	Violation	No of Rotatable bond	Molar volume
IG-21	4.05	107.78	385.38	7	2	0	5	329.59
IG-22	4.72	107.78	419.82	7	2	0	5	343.08
IG-25	2.74	167.45	464.60	10	4	0	6	372.01
IG-27	3.15	120.68	386.37	8	2	0	5	325.38
IG-30	1.65	123.05	393.40	9	3	0	5	339.69
IG-31	4.14	71.19	370.41	5	2	0	5	331.75
IG-37	3.24	84.09	371.40	6	2	0	5	327.60
IG-40	1.75	86.46	378.43	7	3	0	5	341.90
IG-55	3.46	122.12	453.91	7	4	0	5	362.46
IG-56	4.68	99.26	418.89	6	3	0	5	346.75
IG-58	3.48	74.85	375.81	5	1	0	4	315.59
IG-59	3.82	74.85	381.84	5	2	0	4	306.30
IG-64	0.15	142.35	435.48	8	5	0	5	356.94
IG-65	1.86	148.15	434.48	8	6	0	5	360.22
IG-68	1.87	100.88	356.38	6	4	0	4	313.34

Table 3: Biological activities of selected lead molecules.

Comp	GPCR	Ion channel	Kinase Inhibitor	Nuclear receptor	Protease inhibitor	Enzyme Inhibitor
IG-21	-0.07	-0.14	0.03	-0.28	-0.29	-0.08
IG-22	-0.07	-0.40	0.01	-0.29	-0.32	-0.11
IG-25	-0.13	-0.20	0.00	-0.43	-0.13	0.02
IG-27	0.04	-0.09	0.22	-0.34	-.21	0.00
IG-30	0.08	-0.13	0.83	-0.44	-0.14	-0.05
IG-31	0.02	-0.17	0.13	-0.22	-0.23	-0.04
IG-37	0.14	-0.11	0.33	-0.26	-0.13	0.06
IG-40	0.19	-0.15	0.13	-0.38	-0.66	0.01
IG-55	-0.03	-0.18	0.11	-0.39	-0.07	0.08
IG-56	0.07	-0.11	0.11	-0.09	-0.18	0.03
IG-58	0.14	-0.62	0.30	-0.26	-0.16	0.06
IG-59	-0.03	-0.20	0.23	-0.41	-0.29	-0.01
IG-64	0.18	0.62	0.13	-0.43	-0.07	0.20
IG-65	0.01	-0.13	0.19	-0.40	0.62	0.17
IG-68	0.18	0.64	0.41	-0.29	-0.04	0.10

from the chemical structure before the molecules is even synthesized and tested. Table 3 shows the analysis of drug likeness of the designed lead molecules.

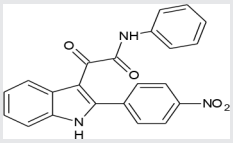
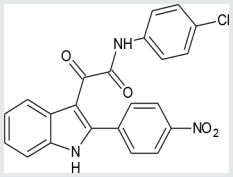
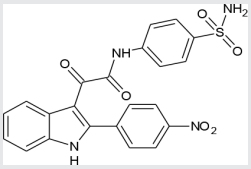
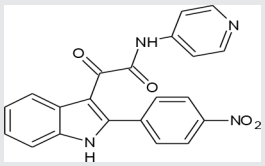
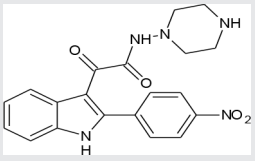
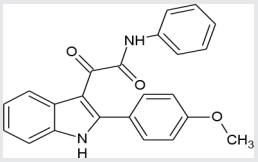
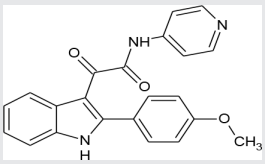
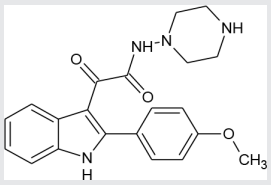
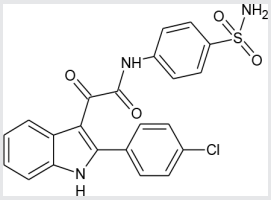
Docking Studies

Molecular docking was carried out to calculate the binding affinities and interaction modes between lead molecule and the target protein using the auto dock. The selected lead molecules were interacted in the active site of the antibacterial agents (PDB ID: 4DH6). The negative energy of the least binding energy is a better binding affinity. Thus, the computationally designed lead molecules showed least binding energy ranged from-9.56 to – 5.88 kcal/mol Table 4.

Experimental Work

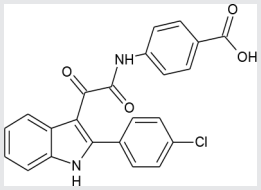
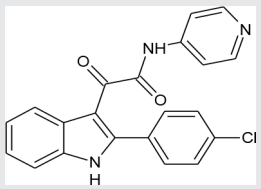
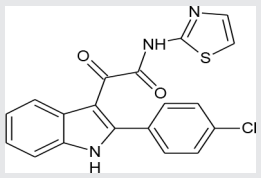
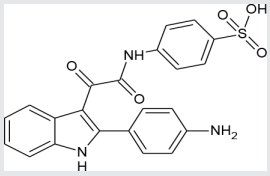
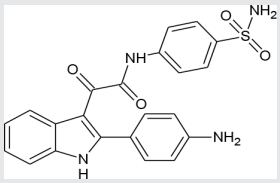
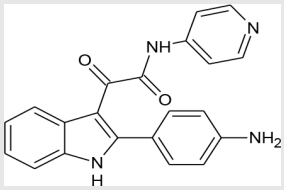
Selected leads were synthesized

In the current work, sixteen new lead molecules were synthesized based on least binding energy and availability of reagents. Conventional method was used for the synthesis of selected lead molecules.

Table 4: *In silico* Docking Results against 4DH6 Affinity (Kcal/mol).			
Compound code	**Lead compounds**	**Binding Affinity (Kcal/mol)**	**Aminoacid interaction**
IG-21		-9.56	ARG95 GLN72 HIS392 MET79 PHE255 CYS394
IG-22		-7.87	PHE265 GLY257 ALA256 LEU321 PRO320
IG-25		-6.96	ARG96 HIS392 TYR76 LEU324 VAL396
IG-28		-7.06	LEU815 PHE387 ALA400 PRO385 GLY388 CYS394
IG-30		-7.50	ALA124 GLU125 TYR122 GLU196 ASP138 ASN148
IG-31		-7.04	THR 76 TYR 78 SER 37 LYS 832 GLY36
IG-37		-6.10	THR72 TYR71 SER35 LYS822 GLY34 ASP228
IG-40		-6.52	VAL291 THR292 ASN293 MET379 GLU380
IG-55		-6.23	LYS142 VAL141 THR144 ASN148

continued...

Table 4: Cont'd.

Compound code	Lead compounds	Binding Affinity (Kcal/mol)	Aminoacid interaction
IG-56		-6.89	LYS107 GLN73 ILE110 PHE108 LYS832
IG-58		-7.63	ARG 144 PRO192 MET288 GLU293
IG-59		-5.98	LEU84 THR94 GLN143
IG-64		-6.14	ALA168 ARG30 SER10 LYS32 GLY11 ASN 112
IG-65		5.88	TRP147 ASP223 LYS824 TYR198 PRO70 THR329
IG-68		6.62	ARG54 GLN55 TYR60 ASP62 SER57
Standard	Gentamycin	-8.26	ARG 54 TRP 146 ASP 224 LYS 824 TYR 198

Characterization of synthesized compounds

Physical characterization

The purity of the synthesized lead molecule was determined by melting point and thin layer chromatography.

The physical characterizations of synthesized compounds are shown in the Table 5.

Spectral characterization of synthesized compounds[31,32]

The functional group, molecular weight and number of proton of synthesized compound is identified by FTIR, ESI-Mass and 1HNMR respectively. Spectral Studies of the synthesized lead molecules are shown in the Table 6.

Antimicrobial Activity

Table 7 shows Antibacterial activity of synthesised lead molecules against G (-ve) and G (+ve).

CONCLUSION

- The lead molecules 2-aryl indol-3- yl glyoxylamide derivatives were designed and minimize the energy by computational techniques. Drug likeness

Table 5: Physical characterization of synthesized compounds.

Sl. No	Comp code	Colour and appearance	Molecular formula	Molecular weight	% yield	Melting Point	Rf Value	Solubility
1	**IG-21**	Red Powder	$C_{22}H_{15}N_3O_4$	385.3722	78%	164°C	0.74	DMSO/Ethanol
2	**IG-22**	Red Powder	$C_{22}H_{14}ClN_3O_4$	419.81	80%	160°C	0.56	DMSO/Ethanol
3	**IG-25**	Red Powder	$C_{22}H_{16}N_4O_6S$	464.45	76%	162°C	0.64	DMSO/Ethanol
4	**IG-28**	Red Powder	$C_{21}H_{14}N_4O_4$	386.36	84%	158°C	0.58	DMSO/Ethanol
5	**IG-30**	Red Powder	$C_{20}H_{19}N_5O_4$	393.39	82%	160°C	0.52	DMSO/Ethanol
6	**IG-31**	Yellow powder	$C_{23}H_{18}N_2O_3$	370.40	80%	152°C	0.62	DMSO/Ethanol
7	**IG-38**	Yellow powder	$C_{22}H_{17}N_3O_3$	371.38	84%	168°C	0.58	DMSO/Ethanol
8	**IG-40**	Yellow powder	$C_{21}H_{22}N_4O_3$	378.42	80%	152°C	0.62	DMSO/Ethanol
9	**IG-55**	Yellowpowder	$C_{22}H_{16}Cl\ N_3O_4$	453.89	82%	166°C	0.56	Ethanol DMSO/
10	**IG-56**	Yellowpowder	$C_{23}H_{15}Cl\ N_2O_4$	418.82	80%	158°C	0.62	DMSO/Ethanol
11	**IG-58**	Yellowpowder	$C_{21}H_{14}ClN_3O_2$	375.80	84%	162°C	0.56	DMSO/Ethanol
12	**IG-59**	Yellowpowder	$C_{19}H_{12}ClN_3O_2S$	381.83	82%	156°C	0.66	DMSO/Ethanol
13	**IG-64**	Redpowder	$C_{22}H_{17}N_3O_5S$	435.45	86%	154°C	0.52	DMSO/Ethanol
14	**IG-65**	Redpowder	$C_{22}H_{18}N_4O_4S$	434.46	80%	150°C	0.72	DMSO/Ethanol
15	**IG-68**	Redpowder	$C_{21}H_{16}N_4O_2$	356.37	84%	152°C	0.62	DMSO/Ethanol

Table 6: Spectral characterizations of synthesized compounds.

Lead Compound	FT-IR	H^1 NMR	MASS
IG-21	NH(Indolering)3420.80 CH(str) Aromatic3224.72 C-N(str) 1128.54 C=O(stramide) 1621.52 C=O(str) 1655.39 N-O(str) 1528.32	1H NMR DMSO δ11.58 (s 1H) 9.14 (s 1H) 8.16- 8.28 (d 3H) 7.99 (s 1H) 7.43-7.62 (m 3H) 7.20-7.49 (m 5H) 7.08(s 1H)	386
IG-22	NH(Indolering)3446.79 CH(str) Aromatic3230.77 C-N(str) 1118.71 C=O(stramide) 1616.35 C=O(str) 1675.69 C-Cl 605.05 N-O(str) 1548.84	^{1}H NMR DMSO δ 11.82 (s 1H) 9.20(s 1H) 8.10-.35 (d 3H) 8.00(s 1H) 7.70-7.84 (s 2H) 7.29- 7.60 (m 6H)	418
IG-25	NH(Indolering)3415.93 CH(str)Aromatic3375.43 C-N(str) 1134.14 C=O(stramide) 1616.35 C=O(str) 1637.69 N-O(str) 1577.77 S=O - (str) -1370.26	^{1}H NMR DMSO δ12.50 (s 1H) 9.15 (s 1H) 8.16-8.28 (d 6H) 7.98 (s 1H) 7.79-7.90 (d 2H) 7.49-7.55 (d 3H) 7.29 (s 1H)	465
IG-28	NH(Indolering)3367.71 CH(str)Aromatic3176.76 C-N(str) 1120.64 C=O(stramide) 1629.04 C=O(str) 1624.06 N-O(str) 1577.77 C=N (str)-1250.24	^{1}H NMR DMSO δ12.46 (s 1H) 9.25 (s 1H) 8.16-8.40 (d 4H) 8.00 (s 1H) 7.69-7.75 (d 2H) 7.49-7.55 (d 3H) 7.29(s 1H)	388
IG-30	NH(Indolering)3265.52 CH(str)Aromatic3136.26 C-N(str) 1140.64 C=O(stramide) 1615.04 C=O(str) 1634.10 N-O(str) 1556.77 C-NH(str)-1482.24	^{1}H NMR DMSO δ11.62 (s 1H)9.05(s 1H) 8.24 (d 2H) 8.14(s 1H) 7.75 (s 2H) 7.51(s 1H) 7.39 (s 1H) 7.33 (s 1H) 2.77 (d 4 H) 2.72 (d 4H) 2.18 (s 1H)	393.39
IG-31	NH(Indolering)3217 CH(str)Aromatic 2931.80 C-N(str)1126.43 C=O(stramide)1627.92 C=O(str)1685.69 C-O(str)1149.57 Ar-O-CH3-2836.50	^{1}H NMR DMSO δ12.31 (s 1H) 9.14 (s1H) 7.89 (s 1H) 7.00- 7.14 (d 3H) 7.16-7.40 (m 6H) 7.58-7.60 (d 3H) 3.86 (s 3H)	371

continued...

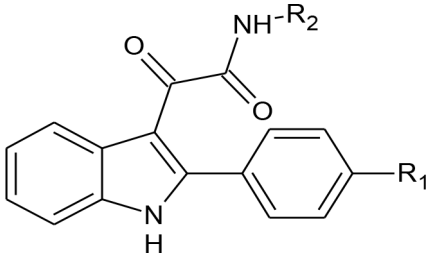
Table 6: Cont'd.			
Lead Compound	**FT-IR**	**H[1] NMR**	**MASS**
IG-38	NH(Indolering)3320.80 CH(str) Aromatic3124.62 C-N(str) 1126.44 C=O(stramide) 1612.34 C=O(str) 1645.52 N-O(str) 1542.12 C-O-CH3-2835.24	^{1}H NMR DMSO δ12.62 (s 1H) 9.56 (s 1H) 8.54 (s 2H) 7.99 (s 1H) 7.88 (s 1H) 7.58 (m 3H) 7.23 (s 2H) 7.05 (s 2H) 3.78 (s 3H)	372
IG-40	NH(Indolering)3430.52 CH(str)Aromatic3224.42 C-N(str) 1118.42 C=O(stramide) 1616.52 C=O(str) 1665.34 C-O-CH3-2826.84	^{1}H NMR DMSO δ11.56 (s 1H) 9.24 (s 1H) 7.89(s 1H) 7.60 (s 2H) 7.58 (s 1H) 7.15-7.24 (m 4H) 3.90 (s 3H) 2.74(s 4H) 2.76 (s 4H) 2.24 (s 1H)	380
IG-55	NH(Indolering)3320.56 CH(str)Aromatic3214.54 C-N(str) 1112.52 C=O(stramide) 1618.28 C=O(str) 1662.46 (str) S=O -1372.366 C-Cl- 796.26	^{1}H NMR DMSO δ11.36(s 1H) 9.12(s 1H) 8.19 (d 3H) 7.99 (s 1H) 7.85 (d 2H)7.62 (s 1H) 7.22-7.38 (m 4H) 7.08 (d 2H)	454
IG-56	NH(Indolering) 3326. 64 CH(str)Aromatic3210.42 C-N(str) 1114.24 C=O(stramide) 1624.26 C=O(str) 1668.5 C-Cl-780.24	^{1}H NMR DMSO δ12.46 (s 1H) 10.26 (s 1H) 9.24 (s 1H) 7.99- 8.05(d 3H) 7.63(s 1H) 7.24-7.37(m 6 H) 7.09 (d 2H)	420
IG-58	NH(Indolering) 3356.62 CH(str)Aromatic3226.18 C-N(str) 1118.56 C=O(stramide) 1634.18 C=O(str) 1672.50 C-Cl-786.28 C-N- 1324.36	^{1}H NMRDMSO δ12.24 (s 1H) 9.18 (s 1H) 8.54 (s 2H) 7.99 (d 3H) 7.63 (s 1H) 7.20 – 7.38 (m 4H) 7.07 (d 2H)	376
IG-59	NH(Indolering) 3298.24 CH(str)Aromatic3210.42 C-N(str) 1124.36 C=O(stramide) 1628.58 C=O(str) 1668.34 C-Cl- (str)- 769.28 C-N-(str- 1380.2 C-S -(str)-720.36	^{1}H NMR DMSO δ11.86 (s 1H) 9.12 (s 1H)7.99 (s 1H) 7.63 (s 1H) 7.20- 7.40 (m 5H) 7.08 (d 2H) 6.88 (s 1H)	380
IG-64	NH(Indolering)3315.56 CH(str)Aromatic3256.45 C-N(str) 1127.64 C=O(stramide) 1632.28 C=O(str) 1684.68 (str) S=O(str)- 1352.64 C-NH2(str)- 134.63	^{1}H NMR DMSO δ11.92 (s 1H)9.26 (s1H) 8.20 (d 2H) 7.98 (d 2H) 7.84 (s 1H) 7.50- 7.60(m 3H) 7.10- 7.20(d 2H) 7.32 (s 1H) 6.93 (d 2H) 5.20 (s 1H)	436
IG-65	NH(Indolering)3360.32 CH(str)Aromatic3190.54 C-N(str) 1182.52 C=O(stramide) 1636.82 C=O(str) 1672.68 (str) S=O (str)1372.34 C-NH2-1338.24	^{1}H NMR DMSO δ12.20 (s 1H) 9.26 (s 1H) 8.19 (d 2H) 7.84 (s 1H) 7.50- 7.60 (d 3H) 7.10- 7.25 (d 2H) 6.86 (s 1H) 6.82 (d 2H) 5.18 (s 1H)	433
IG-68	NH(Indolering)3296.16 CH(str)Aromatic3172..54 C-N(str) 1146.36 C=O(stramide) 1627.48 C=O(str) 1668.54 (str) C-N (str)-1336.42 C-NH2-1256.46	^{1}H NMR DMSO δ12.28 (s 1H) 9.20 (s 1H) 8.52 (d 2H) 7.98 (s 2H) 7.85 (d 1H) 7.50- 7.60 (m 3H) 7.10- 7.30 (d 2H) 6.93 (d 2H) 5.22 (s 1H)	358

Table 7: Antibacterial activity of synthesised lead molecules against G (-ve) and G (+ve).

Compound	Antibacterial activity	
	Gram positive *Bacillussubtilis (NCIM2063)*	Gram negative *E. coli (NCIM2064)*
	Zone of inhibition (mm)	
IG-21	20	12
IG-22	18	09
IG-25	17	10
IG-28	18	09
IG-30	19	11
IG-31	18	09
IG-38	15	06
IG-40	14	07
IG-55	15	06
IG-56	17	09
IG-58	18	10
IG-59	17	08
IG-64	16	07
IG-65	12	07
IG-68	14	09
Gentamycin	22	16
Control	00	00

property of lead molecules was calculated by online software tool molinspiration. There is the designed lead molecules does not containing any violation of rule five which indicates that the lead molecule contains good bioavailability.

- Molecular docking studies indicated that the selected lead molecules showed least energy towards the target protein / receptor ranging from-5.88 to-9.56 kcal/mol.



- The substitution in aromatic ring at C-2 position of indol-3-yl-glyoxylamide derivatives may increase their binding energy. Aromatic/ heterocyclic 5-membered and 6-membered amine derivatives substituted at C-3 position of indol-3yl-glyoxylamide.
- The *in silico* docking study of-2-aryl indol-3-yl glyoxylamides were revealed that the compound IG-21, IG-22, IG-25, IG-28, IG-30, IG-31, IG-37, IG-40, IG-55, IG-56, IG-58, IG-59IG-64, IG-66 and IG-68 found to bind efficiently with 4DH6 protein.
- Antibacterial assay performed in this study showed that compound IG-21, IG-22, IG-25, IG-28, IG-30 with nitro group displayed good inhibition of bacterial growth. Whereas the compounds without a EWG (Cl or NO_2) Showed weak antibacterial activity. This may be due to the fact that EWG substituents increase the liphophilicity of the compound which leads to higher partitioning of such compounds into the lipophilic phase of a microbial membrane.2-Aryl 4-methoxy indole 3yl –glyoxylamide IG-31 IG-38 and IG-40 showed significant antibacterial activity.
- Five membered heterocyclic amine substituted in 3rd position of indole 3- glyoxylamide IG-59 showed potent antibacterial activity.
- In the present work reveals that NO_2 and Cl substituted 2 –Aryl indole 3- Glyoxylamide derivatives showed potent antibacterial activity against both gram positive *Bacillus subtilis and* gram-negative *E. coli*. Hence this study will used to design of more potent antibacterial agents for therapeutic use.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publications of this paper.

ABBREVIATIONS

4DH6: Beta Secretase; **PDB:** Protein Data Bank; **FTIR:** Fourier Transform Infrared; **ESI MASS:** Electro Spray Ionization Mass Spectrometry; **1HNMR:** Proton Nuclear Magnetic Resonance; **TLC:** Thin Layer Chromatography; ***E.coli*:** *Escherichia coli*; **AIDS:** Acquired Immune Deficiency Syndrome; **RCSB:** Research Collaboratory for Structural Bioinformatics; **DMSO:** Dimethy Sulphoxide; **MHz:** Mega hertz; **MP:** Melting Point; **ACD:** Advanced Chemistry Development; **G (+ve):** Gram Positive; **G (-ve):** Gram Negative.

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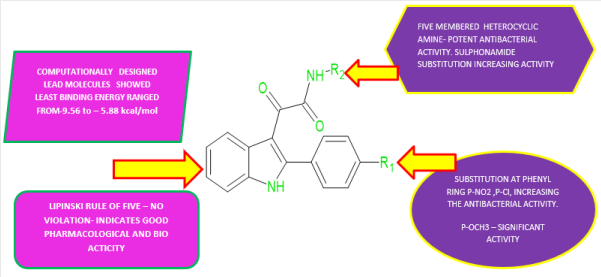
PICTORIAL ABSTRACT



SUMMARY

- Drug design methodology is used to a screening lead has resulted in the identification of clinical candidate. The main goal of this study was to investigate the effects of novel 2-aryl indole 3-glyoxylamide compounds against gram +ve and gram –ve bacteria. Among these most of the compounds have shown a broad spectrum of antibacterial activity.
- Hence this study will further widen the scope for the development of similar new 2-aryl indole3-glyoxylamide as possible potential antimicrobial agents.

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