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IDENTIFICATION OF SUBSTITUTED 4, 7-DIHYDROY-8-(4-METHYL-1H-BENZO [B] [1, 4] DIAZEPIN-2-YL)-3-PHENYL-CHROMEN-2-ONE ANALOGUES AS ANTIMICROBIAL AGENTS, MOLECULAR DOCKING AND PHARMACOKINETIC EVALUATION

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ABSTRACT:
Antibacterial activity of a set of eleven (11) substituted benzodiazapine- chromene-2-ones on gram-positive bacteria (*Bacillus licheniformis, Bacillus subtilis, and Staphylococcus aureus)* and gram-negative bacteria *(Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa)* were performed. The compounds were also tested on fungal species *(Aspergilusniger, Candida albicans, Fusariumoxysporum, Fusariumsolani).* Docking analysis was accomplished to expertise the formational features and binding mechanism of synthesized different 4, 7-Dihydroy-8-(4-Methyl-1h-Benzo [B] [1, 4] Diazepin-2-yl)-3-phenyl-chromen-2-one analogues and were docked against the structures of *Staphylococcus aureus* DNA gyrase (PDB ID: 3G7B) and *Candidapespsin-5* through *Candida albicans* (PDB ID: 2QZX). The structures of derivatives were sketched using Chemdraw Ultra 12.0.2 and they are converted to pdb format using babel conversion tool. Autodock Vina of PyRx was used for molecular docking studies. All synthesized derivatives had shown interactions with binding energies. Pharmacokinetic evaluation results indicate the compounds possess drug-likeness properties.

KEY WORDS: PyRx, Benzodiazepine-chromene-2-ones, Antibacterial, Molecular docking studies

INTRODUCTION:

Due to their potent pharmacological properties, 1, 5-Benzodiazepines and their analogues have been studied widely by chemists [i]. They are mostly employed as antianxiety, antidepressive, anticonvulsant, hypnotic, analgesic, anti-inflammatory and sedative agents [ii]. On the other hand, 1, 5-benzodiazepine analogues are vital building blocks which can be employed for the formation of various fused cyclic ring compounds like pyrolo-, oxadiazolo-, triazolo-, oxazino- or furanobenzodiazepines [iii]. Hence, the research studies in this field are still ongoing actively aiming for the preparation of novel molecules with increased therapeutical properties [iv]. The

benzodiazepine analogue Lofendazam [v] in which nitrogen atoms are located at 1 and 5 positions in ring. The lofendazam exhibits close similarity with the different 1, 5-benzodiazepine like clobazam [vi, vii]. The drug Lofendazam exhibits sedative and anziolytic properties like other benzodiazepine analogues. It functions as an active metabolite compound for arfendazam, another benzodiazepine [viii]. The benzodiazepines are a set of molecules which act upon CNS. They specifically act on the gamma-amino butyric acid-A receptors present in the brain. This increases response to neurotransmitter GABA, making nerve cell to acquire negative charge and tolerant to excitation [ix]. The coumarine molecules are the main class of natural molecules and set up a group of medicinally active agents.

Most coumarin analogs have pharmacological properties in a broad range like anti-helminthic, hypnotic, anti-insecticidal, anti-coagulant & anticoronary vasodilator. The substituted coumarin molecules exhibits antibacterial [x-xii], antioxidant [xiii-xiv], antinflammatory [xv-xvi] and antitumor properties [xvii-xviii]. The derivatives of coumarins have also exhibited CNS depressant and anti-HIV [xix]. Thus, the focus of the current research was to examine the bio reactivity of an eleven (11) novel benzidiazapin-chromene-2-ones on gram-positive bacteria (GPB) and gram-negative bacteria (GNB).

The compounds were also tested on fungal species. The aimed and newer molecules (11a-k) were also docked against the structure of Staphylococcus aureus DNA gyrase crystal (PDB ID: 3G7B) and candidapespsin-5 from Candida albicans (PDB ID: 2QZX), thus, affording information for a better optimization of these classes of compounds as potential antimicrobial agents.

In addition to our research on the discovery of bioactive heterocyclic molecules and their biological evaluation of coumarin-based derivatives [xx, xxi] and their activity against antimicrobial activity, we are making efforts on research and studies of many new molecules providing evidence that the introduction of moieties like substituted aryl groups increases the antimicrobial activity by increasing the lipophilicity of the molecule, resulting in greater penetration into cells.

EXPERIMENTAL:

In vitro Anti-microbial assay.

A solution (1mg/mL) of test compound in DMSO was infused on sterilized standard discs of filter paper. The size of each filter paper disc was 5mm. The discs of filter paper were immersed with the aimed compound and were added to an agar plate inserted onto the aimed organism. Triplicate was maintained for each test. *Ciprofloxacin* was used as a standard anti-bacterial medicine & *Nystatin* as a standard anti-fungal medicine. Only DMSO was employed as control at the same concentrations. All the petri plates were allowed to incubate at 37^0C for a period of one to five days. The microbial culture sensitive to a compound will not show growth around the disc impregnated with that compound. This is observed as a zone of inhibition around the disc. The antimicrobial property was evaluated by determining the zone diameter listed in Table 1 & 3 respectively. The aimed molecules (discs) which exhibited significant zones of inhibitions around them (300) were further analyzed to determine their MIC measurement.

MIC measurement

To determine the microbial MIC vulnerability, tests on nutrients and dextrose broths were employed. The solvent DMSO was used to prepare the stock solutions (0.001g/ml) of *Ciprofloxacin* (standard antibacterial agent), *Nystatin* (standard antifungal agent) & aimed compounds. The dilute solutions were prepared at concentrations ranging from 25 to 250µg per ml. The microbial suspension cultures were immunized on to the plate of agar and then the disc of the aimed compound & control was placed on the surface of agar listed in Table 2 & 4 respectively for selective compounds.

Molecular Docking Studies

Molecular docking is a computer-aided tool for recognizing the binding correlations between a ligand and receptor. In the current research, the aimed molecules were evaluated for molecular docking studies and PyRx [xxii] virtual screening tool was used for docking studies. The PyRx wizard uses Autodock 4.2 and Autodock Vina docking tools which are embedded in it. It contributes to higher docking efficiency and accuracy. Autodock Vina uses a scoring function with efficient optimization and multithreading [xxiii].

Hardware and Software: PyRx virtual screening tool is software installed in a computer system configured with Intel(R) Core(TM) i5-8250U CPU @ 1.60GHz 1.80 GHz processor and RAM capacity of 8.00GB.The aimed molecules were drawn using software ChemSketch in .mol format, and converted to PDB format using a tool Open Babel GUI.

To recognize the extent of binding between aimed molecules and target proteins, the structure of Staphylococcus aureus DNA gyrase (PDB ID: 3G7B) and candidapespsin-5 from Candida albicans (PDB ID: 2QZX) crystals were taken from the protein database. At first, protein molecules were prepared with the Biovia discovery studio; water molecules and existing ligands in the active pocket were removed and saved to the PDB file. Then the target proteins are loaded into the PyRx tool and saved as a PBDQT file using the Autodock command. The ligands were loaded into PyRx using Open Babel GUI input wizard. The energies of ligands were minimized and converted to PDBQT file format. The AutodockVina wizard was used to perform docking simulations after setting up a grid box onto the active site of the target molecule. The active site pocket of target molecules was determined from the inhibitors demonstrated in the crystal structures. From the docking procedure, conformations were ranked according to their binding energy and the confirmation with the lowest binding energy was considered as the best docking score. The docking results were visualized using Pymol and Biovia Discovery Studio Visualizer.

Pharmacokinetics

SwissADME web server protocol [xxiv] was used for pharmacokinetics evaluation of compounds **11a-k**.

RESULTS AND DISCUSSION:

Antibacterial activity of compounds 11a-k

Antibacterial properties of the newly prepared derivatives **(11a-k)** were tested on Gram-Positive bacteria (GPB) (*Bacillus licheniformis, Bacillus subtilis, and Staphylococcus aureus)* and Gram-Negative bacteria (GNB) *(Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa)*. Ciprofloxacin is used as standard medicine.

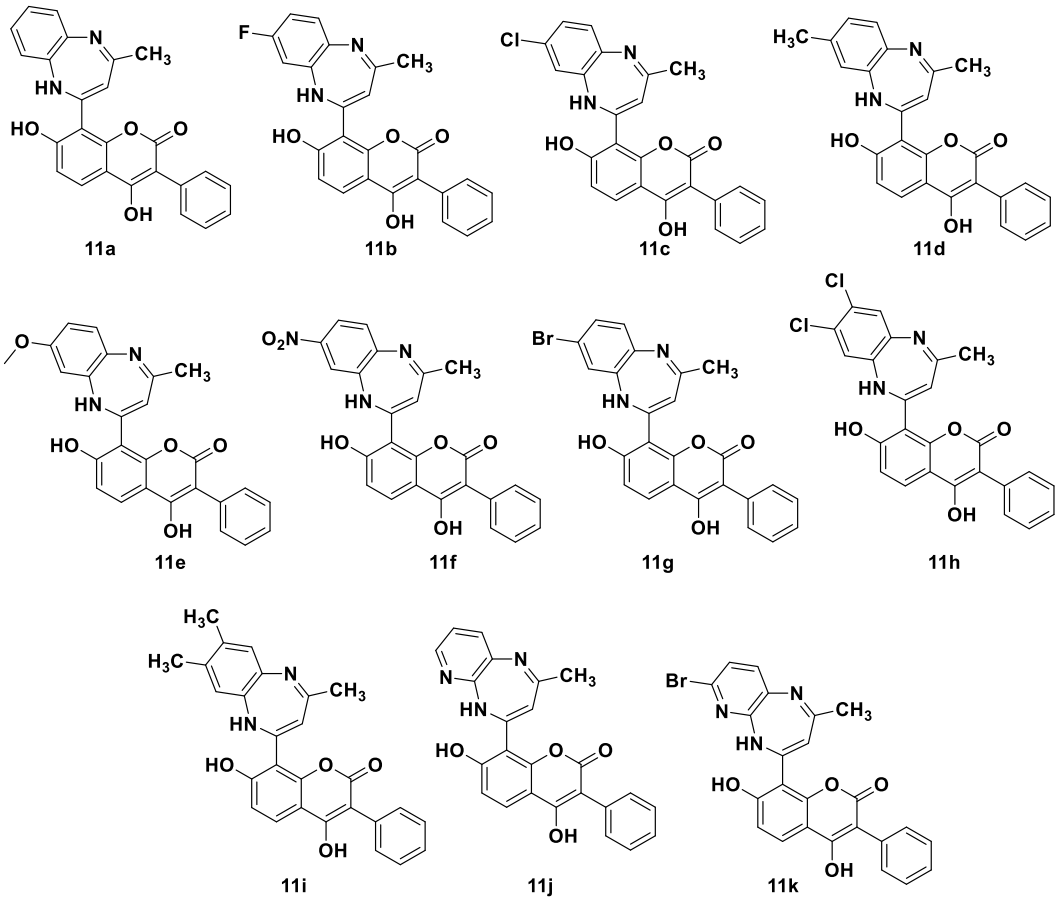

Fig 1. Structures of compounds evaluated.

TABLE 1*: Inhibition Zone (in 10^{-3}m) (antibacterial activity) values for compounds 11a- k*

	Inhibition Zone (in 10^{-3}m)					
	Gram -Negative bacteria			Gram-Positive bacteria		
Test compound Code	*Escherichia Coli*	*Klebsiella pneumonia*	*Pseudomonasaeruginosa*	*Bacilluslichenifo rms*	*Bacillus subtilis*	*Staphylococcus aureus*
11a	14	23	12	23	27	23
11b	22	14	20	22	20	53
11c	10	13	00	04	21	06
11d	11	11	16	15	12	12
11e	12	11	05	14	12	16
11f	00	00	00	05	13	00
11g	22	22	22	23	25	20
11h	09	13	14	05	12	15
11i	22	22	20	23	21	26
11j	21	22	12	13	23	20
11k	20	09	00	00	00	12

Ciprofloxacin (std)	26	24	25	24	23	25
Contro (1%DMSO)	00	00	00	00	00	00

TABLE 2: *Minimal inhibitory concentrations (MICs) (in 10^{-6}g/mL) values for compounds 11-a/b/g/i/j/k.*

	Minimal inhibitory concentrations (MICs) (in 10^{-6}g/mL)					
	Gram-negative bacteria			**Gram-positive bacteria**		
Test Compound Code	***Escherichia Coli***	***Klebsiella pneumonia***	***Pseudomonas aeruginosa***	***Bacillus licheniforms***	***Bacillus subtilis***	***Staphylococcus aureus***
11a	43	76	152	104	62	73
11b	22	52	23	23	23	75
11g	23	23	53	23	22	53
11i	152	103	53	146	132	83
11j	24	43	30	24	26	73
11j	22	09	00	00	00	12
***Ciprofloxacin* (std)**	25	25	25	25	25	25
Control (1%DMSO)	00	00	00	00	00	00

Compounds **11a**, **11b** and **11g** exhibited potent anti-bacterial activity (>20mm) on all the six bacteria (Table 1). From the screening results, it is revealed that the presence of chloro and fluoro groups of the phenyl ring enhances the antibacterial activity. However, compound 11a also showed good activity. The compounds **11a** and **11i** showed six times more activity against the *Pseudomonasaeruginosa* and *Escherichia Coli* respectively. The compound **11g** showed better activity against *Bacillus licheniforms, Bacillus subtilis*, and *Pseudomonas aeruginosa* may be attributed to the liphophlic group. The compound **11c** is shown good to moderate antibacterial activity (>20mm) against all six bacterial strains. The compounds **11c** and **11f** were not shown any activity against *Pseudomonas aeruginosa, Escherichia Coli, Klebsiella pneumonia, Staphylococcus aureus, Bacillus licheniforms & Bacillus subtilis*. The minimal inhibitory concentrations of molecules (11a) and (11g) are good potent (200-25 μg/ml) showed in Table 2.*3.2.*

Anti-fungal activity of compounds (11a-k)

TABLE-3: *Inhibition zone (in 10^{-3}m) (anti-fungal activity) values for compounds (11a-k)*

Inhibition zone (in 10^{-3}m) (anti-fungal activity)				
Test compound Code	***Aspergillus niger***	***Candida albicans***	***Fusarium oxysporum***	***Fusarium Solani***
11a	05	15	17	04
11b	23	15	22	15
11c	04	12	06	15
11d	12	03	11	00
11e	00	04	00	06
11f	07	13	04	03
11g	22	21	24	23
11h	18	08	10	09
11i	08	04	14	06
11j	08	06	12	08
11k	05	09	03	06
Nystatin	22	22	23	20
Control (1%DMSO)	00	00	00	00

TABLE 4: *Minimal inhibitory concentrations (MICs) (in 10^{-6}g/mL) values for compounds 11 (a, b, g, i, j)*

Minimal inhibitory concentrations (MIC) values (in 10^{-6}g/mL)				
Compound Code	***Aspergillus niger***	***Candida albicans***	***Fusarium oxysporum***	***Fusarium Solani***
11a	122	1325	153	153
11b	21	22	24	53
11g	133	173	172	102
Nystatin	25	25	25	25
Control (1%DMSO)	00	00	00	00

The aimed molecules (**11a-k**) were examined on fungal species (*Aspergilus Niger, Candida albicans, Fusarium oxysporum, Fusarium solani*) using Nystatin as a reference drug and the inhibition values of the zone are presented in Table 3. Out of eleven products tested for antifungal activity, the compounds with more electronegative substituents were (11g) shown higher activity against *Fusarium oxysporum* and *Candida albicance* than the standard of Nystatin. The compounds with fluoro (11b) also exhibited maximum activity against *Fusarium Solani* and *Candida albicance* respectively. Nearly all the tested compounds from 11a-k displayed potent activity. It may be attributed that the whole molecular system is responsible for the activity. The compounds (11e) have not shown any activity against *Aspergilusniger, Fusarium Oxysporum* and the activity of all target test compounds are revealed in Table 3. The

Minimam inhibitory concentration values of products (11a), (11g) (200-25 μg/ml) were shown in Table 4.

Antibacterial Docking Study

Staphylococcus aureus DNA gyrase (PDB ID: 3G7B) is chosen as an important target for drug discovery as it plays an important role in the replication of bacterial DNA [xxv]. The 3G7B active site pocket is occupied by amino acid residues, namely Asn54, Asp81, Arg84, Gly85, Ile86, Pro87, Ile102, Arg144 and Thr173[xxvi]. The grid box was configured with dimensions of 17.204 x 23.169 x 19.309 A^0 (X, Y, Z) and grid coordinates 48.135, -5.647 and 19.453 A^0 were assigned to cover all the active sites on DNA gyrase. To understand ligand – receptor binding interactions, all newly synthesized molecules and the standard molecule ciprofloxacin were docked into the binding pocket of a DNA gyrase. Each newly synthesized molecule and the standard compound ciprofloxacin were dumped into the binding pocket of the DNA gyrase to understand the ligand - receptor binding interactions. All the compounds have exemplified better docking results and binding interactions than reference drug ciprofloxacin. The binding affinity scores of aimed molecules **11a-k** were ranging from -7.5 to -8.6 Kcal /mol whereas ciprofloxacin scored about -7.7 Kcal /mol (table 5).

TABLE 5**:** *Binding affinities of compounds **(11a-k)** and interacting amino acids of staphylococcus aureus DNA gyrase (PDB ID: 3G7B)*

Compound	Binding Affinity (Kcal/mol)	Interacting amino acids	
		H-bond	Hydrophobic
11a	-8.0	Glu58, Ile86, Thr173	Ile51, Asp57, Ile86, Leu103, Ile175
11b	-8.1	Asn54, Ser55	Ile51, Asp81, Ile86, Pro87, Ile102, Leu103
11c	-8.3	Asn54	Asn54, Glu58, Arg84, Ile86, Pro87, Ile102, Leu103, Ser129
11d	-8.6	--	Asn54, Glu58, Arg84, Ile86, Pro87, Ile102, Leu103
11e	-7.8	Asn54, Ser55	Ile51, Asp57, Glu58, Asp81, Ile86, Pro87, Ile175
11f	-8.4	Asn54, Val130, Val131	Asp57, Ile86
11g	-7.7	Asn54	Asp57, Ile86, Val130, Val131
11h	-8.5	Asp57	Ile51, Glu58, Arg84, Ile86, Pro87, Ile102, Leu103, Ser129, Ile175
11i	-7.5	Ser128	Gly85, Ile86, Pro87, Ile102, Leu103
11j	-7.8	Asn54	Glu50, Ile86, Ile102, Val130
11k	-7.8	Asn54	Asn54, Asp57, Ile86, Val131
Ciprofloxacin	-7.7	--	Glu58, Asp81, Ile86, Pro87

Molecule **11d** scored the highest binding affinity, about -8.6 Kcal/mol and it, by only hydrophobic interactions with DNA gyrase (PDB ID: 3G7B). The amino acids Asn54, Glu58, Arg84, Ile86, Pro87, Ile102 and Leu103 of DNA gyrase were involved in the hydrophobic interactions (fig 2, 3).

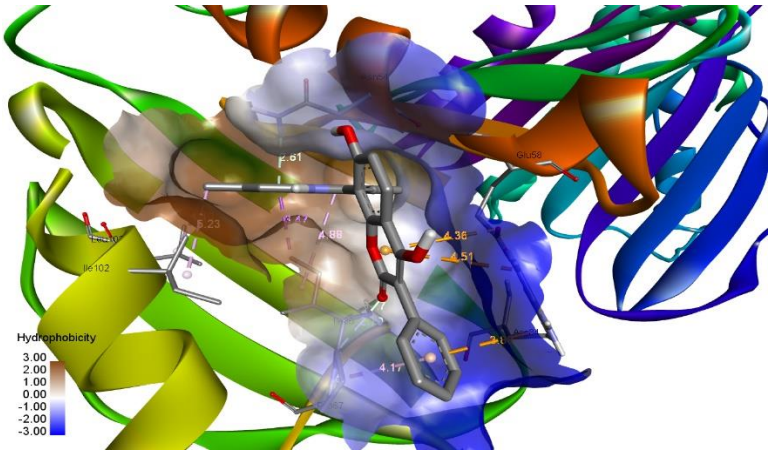

FIGURE 2: Hydrophobic surface and docking pose of hydrophobic surface of **molecule 11d** with DNA gyrase (PDB ID: 3G7B)

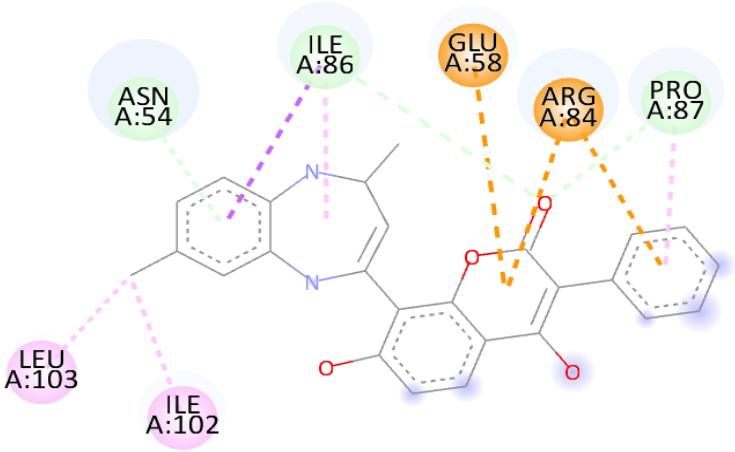

FIGURE 3: 2D reciprocity of **molecule 11d** with DNA gyrase (PDB ID: 3G7B)

Molecule **11h** scored the second highest binding affinity at about -8.5 Kcal/mol and the H-bond interaction was demonstrated with amino acid Asp57 and hydrophobic interactions with Ile51, Glu58, Arg84, Ile86, Pro87, Ile102, Leu103, Ser129, Ile175 of DNA gyrase (PDB ID: 3G7B)(fig4, 5).

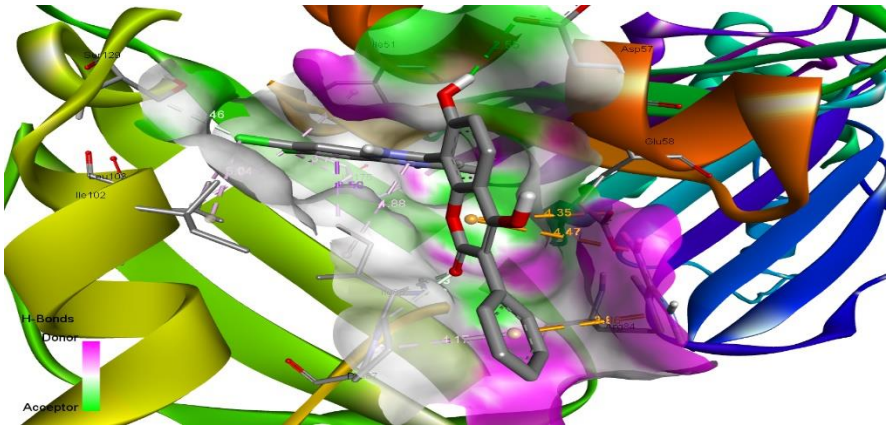

FIGURE 4: H-bond surface and docking pose **molecule 11h** with DNA gyrase (PDB ID: 3G7B)

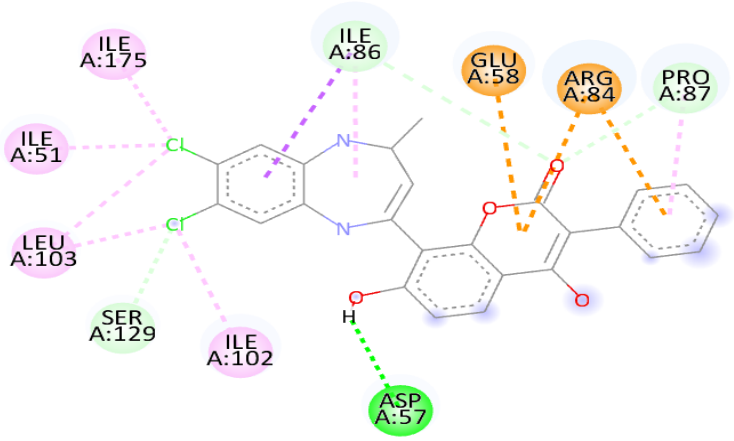

FIGURE 5: 2D reciprocity of **molecule 11h** with DNA gyrase (PDB ID: 3G7B).

The standard reference *Ciprofloxacin* scored a binding affinity value of about -7.7 Kcal/mol and demonstrated only hydrophobic interactions with the amino acids Glu58, Asp81, Ile86 and Pro87 of DNA gyrase (fig6, 7).

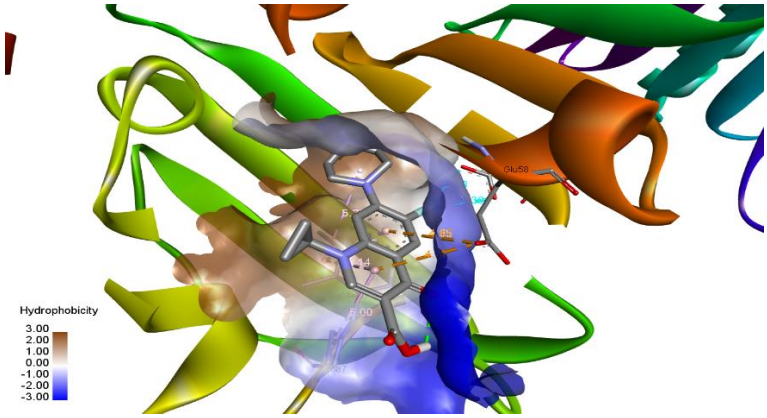

FIGURE 6: Hydrophobic surface and docking pose of ***Ciprofloxacin*** with DNA gyrase (PDB ID: 3G7B).

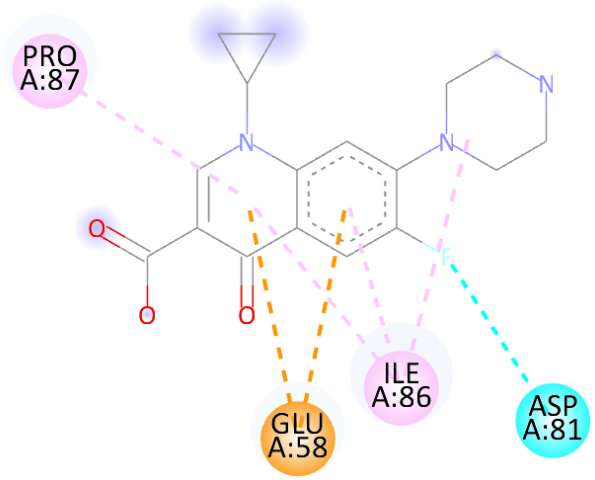

FIGURE 7: 2D reciprocity of ***Ciprofloxacin*** with DNA gyrase (PDB ID: 3G7B)

Antifungal Docking Study

Candida pepsins play an important role in proteolytic activity of C. albicans and hence it was chosen as target [xxvii]. The active site pocket of candida pespsin-5 was well defined by the inhibitor pepstatin (PRD-000557) present in the crystal structure. It demonstrated H-bond

interactions with amino acids Asp32, Gly34, Lys83, Gly85, Asp86, Asp218, Gly220, Thr222 and hydrophobic interactions with Ile82, Tyr84, Ile123, Lys192, Lys193, Tyr225 of candidapespin-5 (fig8).

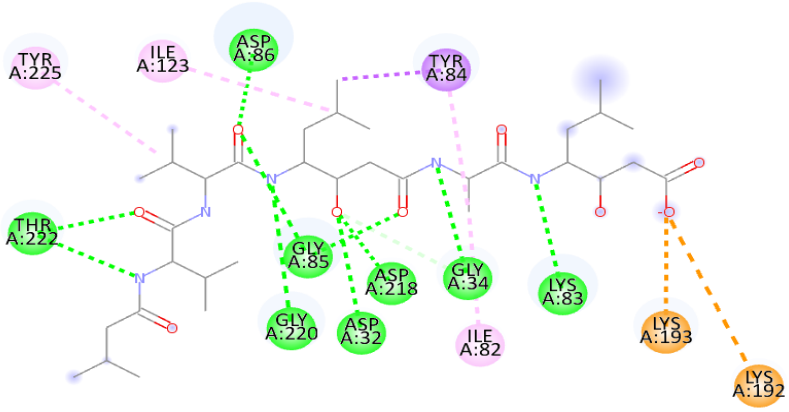

FIGURE 8: 2D reciprocity of pepstatin in the crystal structure of candidapespsin-5 (PDB ID: 2QZX)

The grid box was configured with dimensions of 25.968 x 23.434 x 26.359 A^0 (X, Y, Z) and grid coordinates 5.337, 33.300 and 26.396 A^0 were assigned to cover all the active sites on candidapepsin-5. All the newly synthesized molecules and reference drug nystatin were docked into the active site pocket of 2QZX. Except **molecule 11k**, all the molecules have exhibited better docking scores than reference compound nystatin. The docking scores of **molecules 11a – k** were ranging from -9.7 to -10.8 Kcal /mol whereas the nystatin scored about -9.8 Kcal /mol (table-6).

TABLE 6: *Binding affinities of compounds (11a-k) and interacting amino acids of candidapespin-5 (PDB ID: 2QZX)*

Compound	Binding Affinity (Kcal/mol)	Interacting amino acids	
		H-bond	Hydrophobic
11a	-10.4	Asp32, Gly34, Tyr225	Gly85, Asp86, Asp218, Tyr225
11b	-10.6	Asp32, Asp86, Tyr225	Gly85, Asp86, Asp218, Tyr225
11c	-10.7	Asp32, Asp86, Tyr225	Ile12, Trp51, Gly85, Asp86, Lys193, Asp218, Tyr225
11d	-10.7	Gly34, Tyr225	Ile12, Trp51, Gly85, Asp86, Lys193, Asp218, Tyr225
11e	-10.3	Asp32, Asp86, Tyr225	Ile12, Trp51, Gly85, Asp86, Lys193, Asp218, Tyr225
11f	-10.5	Asp32, Trp51, Asp86, Tyr225	Gly85, Asp86, Asp218, Tyr225
11g	-10.6	Asp86, Tyr225	Ile12, Trp51, Gly85, Asp86, Lys193, Asp218, Tyr225
11h	-10.8	Asp32, Asp86, Tyr225	Ile12, Trp51, Gly85, Asp86, Lys193, Asp218, Tyr225
11i	-10.8	Asp32, Asp86, Tyr225	Ile12, Trp51, Gly85, Asp86, Lys193, Asp218, Tyr225
11j	-10.2	Gly34, Tyr225	Gly85, Asp86, Lys193, Asp218, Tyr225
11k	-9.7	Gly220, Thr222	Ile30, Gly85, Asp86, Ala119, Arg120, Arg123, Thr221, Tyr225

Nystatin	-9.8	Thr13, Thr222, Tyr225	Trp51, Arg120, Ile223

Molecules **11h and 11i** scored the highest binding affinity, about -10.8 Kcal/mol towards candidapepsin-5. They demonstrated H-bond interactions with the amino acids Asp32, Asp86, Tyr225 and hydrophobic interactions with Ile12, Trp51, Gly85, Asp86, Lys193, Asp218, Tyr225 of 2QZW (fig9,10,11,12).

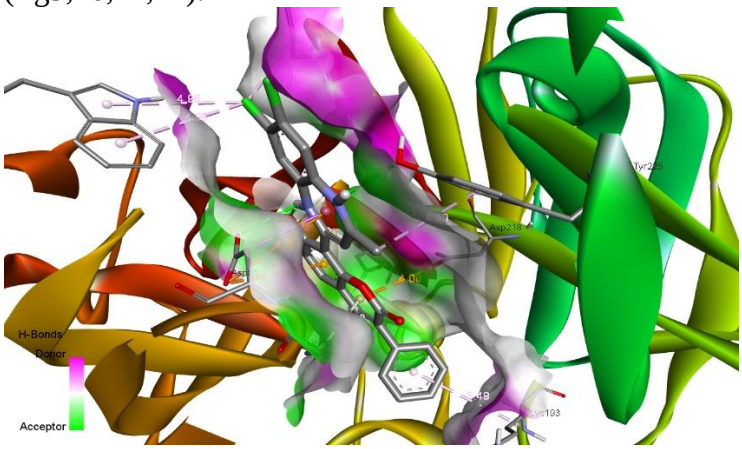

FIGURE 9: H-bond surface and Docking pose of **molecule 11h** with candidapespsin-5 (PDB ID: 2QZX)

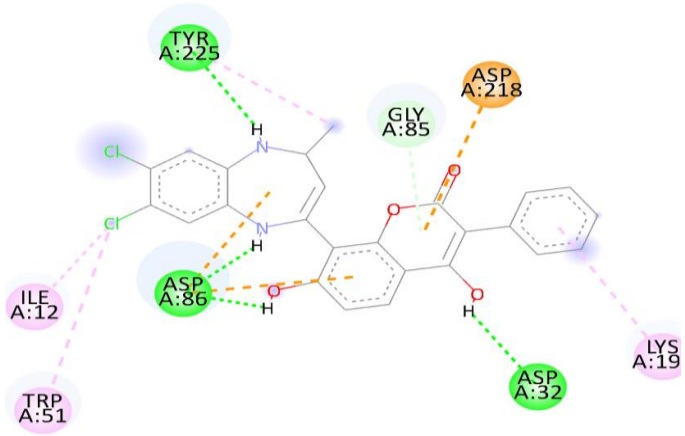

FIGURE 10: 2D reciprocity of **molecule 11h** with candidapespsin-5 (PDB ID: 2QZX)

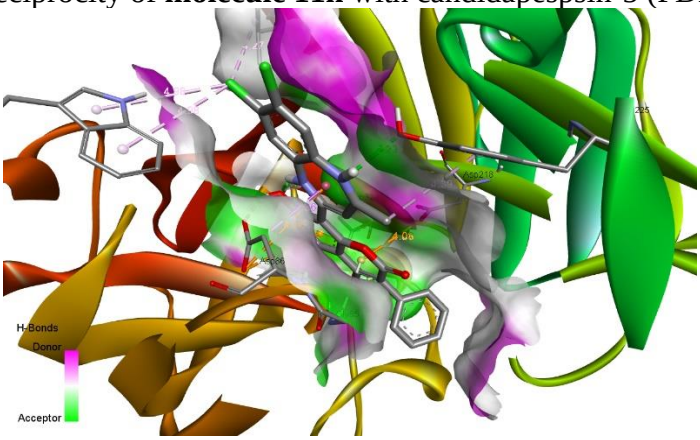

FIGURE 11: H-bond surface and Docking pose of **molecule 11i** with candidapepsin-5 (PDB ID: 2QZX)

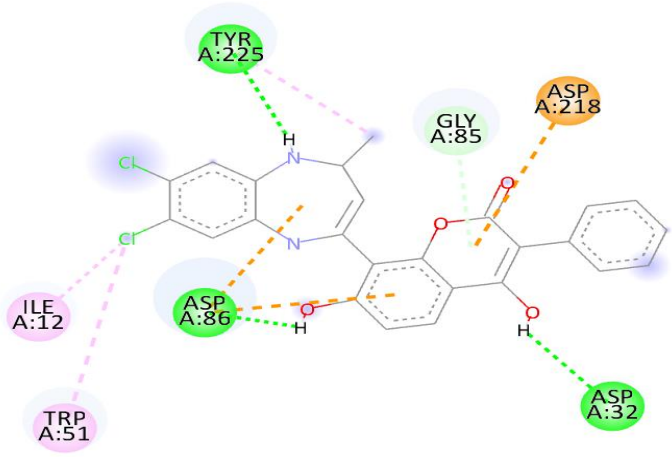

FIGURE 12: 2D reciprocity of **molecule 11i** with candidapepsin-5 (PDB ID: 2QZX)

The reference drug nystatin was scored binding affinity value about -9.8 Kcal/mol and it was demonstrated H-bond interactions with amino acids Thr13, Thr222, Tyr225 and hydrophobic interactions with Trp51, Arg120, Ile223 of candidapespin-5 (fig13,14).

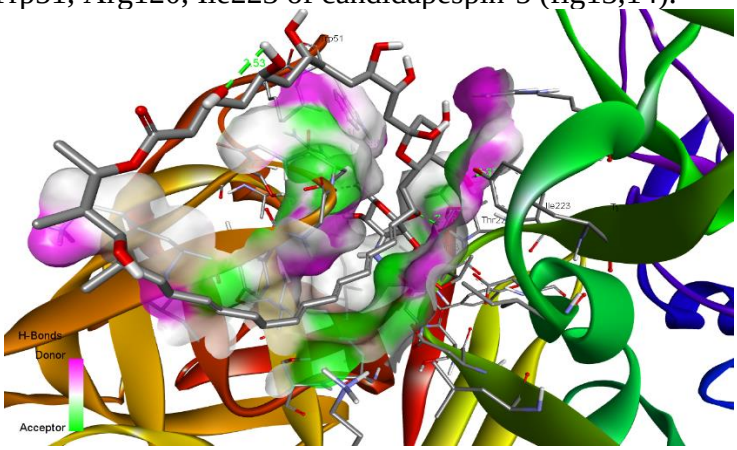

FIGURE 13**:** H-bond surface and Docking pose of ***Nystatin*** with candidapepsin-5 (PDB ID: 2QZX)

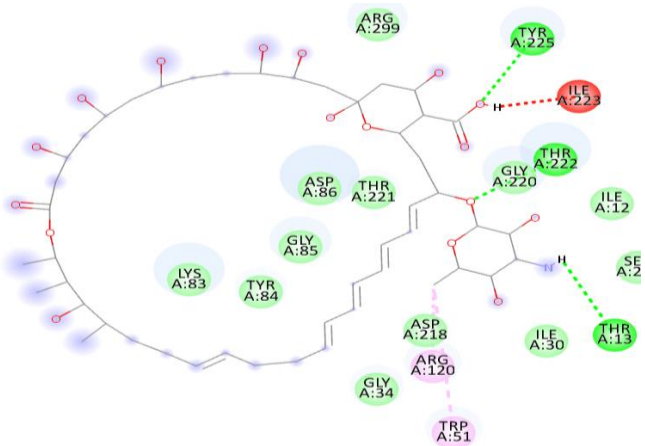

FIGURE 14: 2D reciprocity of ***Nystatin*** with candidapepsin-5 (PDB ID: 2QZX)

Pharmacokinetics

Oral bioactivity such as Absorption, Distribution, Metabolism and Excretion properties study is important for development of new drug candidates. The calculated drug-likeness properties of the studied compounds **11a-k** shown in **Table-7.** All the tested compounds have molecular

weight below 410.42 – 490.31 g/mol. The molecular weight characteristics of these molecules suggested that they can easily be transported, diffused, and absorbed in the body in a significant manner [xxviii]. The Log P value of the compounds were found to be in the range of 2.7 – 3.36, which meet the essential conditions of the Lipinski's rule of five [xxix]. The calculated number of H-bond acceptors of all the molecules were less than ten which is in accordance with ADME as the number of hydrogen bond acceptors must be <10. Bioavailability score of 0.55 suggested that these molecules can be absorbed and used by body [xxx]. Synthetic accessibility scores recommended the ease of synthesis of these molecules [xxxi].

TABLE-7**:** Drug-likeness properties of compounds **11a-k**

Compound	Molecular Weight	Rotatable bonds	H-bond acceptors	H-bond donors	Molar Refractivity	TPSA	iLOGP	ESOL Log S	GI absorption	Lipinski violations	Bioavailability Score	Synthetic Accessibility
11a	410.42	2	5	3	129.05	95.06	2.95	-5.17	High	0	0.55	4.2
11b	428.41	2	6	3	129.00	95.06	3.01	-5.33	High	0	0.55	4.15
11c	444.87	2	5	3	134.06	95.06	3.2	-5.77	High	0	0.55	4.16
11d	424.45	2	5	3	134.01	95.06	3.52	-5.47	High	0	0.55	4.3
11e	440.45	3	6	3	135.54	104.29	3.66	-5.24	High	0	0.55	4.32
11f	455.42	3	7	3	137.87	140.88	2.7	-5.23	High	0	0.55	4.31
11g	489.32	2	5	3	136.75	95.06	3.34	-6.08	High	0	0.55	4.24
11h	479.31	2	5	3	139.07	95.06	3.56	-6.36	High	0	0.55	4.16
11i	438.47	2	5	3	138.98	95.06	3.43	-5.77	High	0	0.55	4.41
11j	411.41	2	6	3	126.84	107.95	2.9	-5.09	High	0	0.55	4.18
11k	490.31	2	6	3	134.54	107.95	3.55	-6.21	High	0	0.55	4.21

CONCLUSION:

Compounds **11a**, **11b** and **11g** exhibited potent antimicrobial activity against both bacteria and fungi comparable to standard drugs ciprofloxacin and nystatin. Docking scores of all synthesized compounds are higher than the reference compounds ciprofloxacin and nystatin and well in agreement with cell line studies. The best confirmers of **11d, 11h, 11f** and **11c** have exhibited best docking scores and binding interactions with *Staphylococcus aureus* DNA gyrase. The best confirmers of **11h, 11i, 11c, 11d, 11b** and **11f** were demonstrated H-bond and hydrophobic interactions with candidapepsin-5 of *Candida Albicans.* The pharmacokinetic evaluation revealed that these compounds have favorable drug-likeness properties to be considered for investigation.

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