Research Article

Design, Synthesis, Characterization, Biological Evaluation and Docking Studies of Mono/Dichloro Aniline Derivatives

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ABSTRACT

Purpose: In many hospitals worldwide, bacterial infections brought on by resistant strains are wreaking havoc. Due to the bacterial resistance, it is required to search newer anti-bacterial drugs or molecules.

Methods: In the current study, we aimed to develop aniline derivatives followed by their *in vitro* anti-bacterial and *in silico* studies. The PDB id of E. coli protein used is 2Y2T. The docking was done using Glide and Auto dock tools and comparative analysis was done.

Results: Out of all the 21 synthesized derivatives, compounds 12e were found to be most active, which is confirmed by both in silico as well as anti-bacterial study. Docking studies using Glide show that in most potent compound 2e, the hydroxyl group of coumarin moiety forms H- bond with GLN 94 in binding pocket. The hydrophobic interactions are shown by TRP95, ILE 6, PHE 8 and PRO 97 while Polar interactions are shown by THR10, ASN 9, THR 11, THR 7, GLN 93. Similarly, Auto dock studies show that in compound 2e, chloride of dichloro aniline moiety forms pi-alkyl bond with PRO 97 and pi-pi stacking with PHE. Nitrogen atoms of NH-C=O bridge forms conventional H- bond with ILE 6, GLN 93 and GLN 5 while hydroxy group of coumarin forms conventional H- bond with SER 3.

Conclusion: Both *in vitro* and *in silico* tests revealed that all of the compounds had good to moderate antibacterial activity and resembled lead. As a result, there is a correlation between in-silico and in vitro research.

Keywords: Chloro-aniline, anti-bacterial, *in silico* study, Carbazide.

INTRODUCTION

Antibiotic resistance has grown in importance as a health issue during the last 20 years. In many hospitals worldwide, resistant strains of bacteria are wreaking havoc, particularly in patients who are already impaired by age, disease, or immunosuppressive medication. In this regard, developing medications with potential action against these infections requires a deeper understanding of resistance mechanisms [1,2].

Semi carbazide often undergoes a condensation reaction with aldehydes and ketones to yield semi carbazones. This is an illustration of how a primary amine reacting with a carbonyl group might generate an imine [3]. Semi carbazones have garnered a lot of interest in the drug discovery process to fight microbial infections, and numerous derivatives of this family have been shown to exhibit promising bioactivity. Several studies on the synthesis and biological testing of bioactive heterocyclic compounds in the aryl-containing semi carbazide and thiosemicarbazone derivatives have been published [4].

The use of these compounds in organic synthesis has become a standard method for producing a variety of heterocycles. Their reactions with compounds with C=O and C=N groups are common way to make biologically active compounds [5,6].

Chloroaniline, a compound consisting of an aniline ($C_6H_5NH_2$) structure with one or more chlorine atoms substituting hydrogen atoms on the benzene ring, has been studied for its antibacterial properties. Chloro-aniline derivatives are used as anti-bacterial agents [7-9]. Numerous biological uses, including antibacterial, antifungal, anticancer, analgesic, and anti-inflammatory properties, have been identified for aniline derivatives, which are the condensed products of aromatic amines with suitable ketones [10-11]. Numerous derivatives have been found to have fungicidal, anti-inflammatory, antiviral, antibacterial, antioxidant and anticancer [12-18].

The in-silico techniques have enhanced the understanding of molecular properties and the specific behavior or nature of drug-receptor interaction at molecular level. Molecular docking has become an important component of the drug discovery toolbox, and its relative low-cost implications and ease of use have stimulated ever-increasing popularity in the industry and amongst researchers [19].

This article describes the design, synthesis, and assessment of a range of aniline derivatives with the carbonyl group that have a variety of aliphatic/aromatic substitutions for anti-bacterial activity.

MATERIALS AND METHODS

Materials:

The semi carbazide and carbazide derivatives were prepared using substituted aniline compounds. All the Chemicals were purchased from Research Lab. The chemicals and apparatus used is given in **Table 1**.

Table 1: Chemicals and apparatus used

Chemicals Used	
3-Chloro Aniline	Glacial Acetic Acid
2,3 Dichloro Aniline	Distilled Water
2,5 Dichloro Aniline	Ethanol
Isatin	Sodium Cynate
P- Benzo Quinone	Hydrazine Hydrate
4-Methyl 7- Hydroxyl Coumarin	Sodium Acetate
Acetophenone	Sodium Hydroxide

Method:

Each reaction's progress was monitored by TLC. The melting values were checked. The spectrum of the FT-IR was recorded using an Alpha ECO-ATR Spectrophotometer. Thirteen C NMR (125 MHz) and one H NMR (500 MHz) spectra were checked with a Bruker FT-NMR spectrophotometer. There is indication of the chemical shifts (d, ppm) and coupling constants (J, Hz). Elements were studied with an EXETER CE-440 elemental analyzer. Antimicrobial activity was evaluated using cup plate method. Ciprofloxacin was used as standard drug.

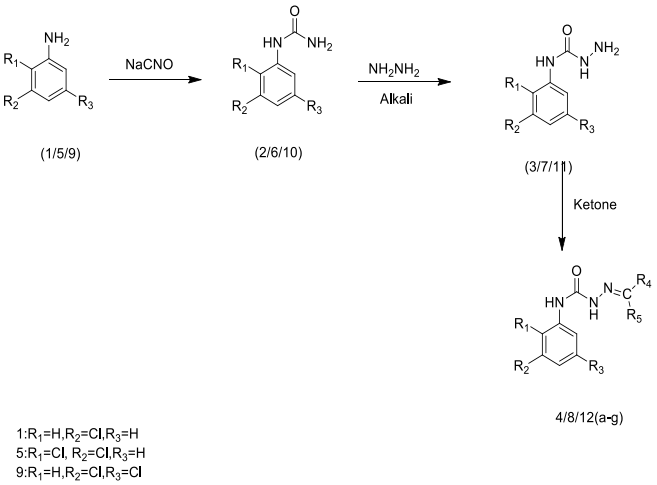
General method for preparation of compounds

Step 1: Synthesis of urea (2/6/10): Glacial acetic acid (10 ml) was used to dissolve chloroaniline (0.1 mol), which was then diluted with 50 ml of water. An equimolar (0.1mol) amount of sodium cyanate in warm water was stirred into this solution. Following the compound's recrystallization from ethanol, the reaction mixture was left to stand for 30 minutes before being filtered, cleaned with water, and dried and collected.

Step2: Synthesis of Semi carbazide (3/7/11): An equimolar amount of hydrazine hydrate was added to an aqueous solution of aryl urease (0.1mol). This solution was mixed with two milliliters of ethanol. After 30 minutes of refluxing, the reaction mixture was chilled in ice. Before refluxing, 4g of NaOH was added to make the mixture alkaline. After being suction-filtered, the product (Semi carbazide) was recrystallized from ethanol.

Step 3: Synthesis of carbazide (4/8/12): An equimolar amount of Semi carbazide derivative was added in ethanol followed by addition of 1-2 ml of glacial acetic acid to maintain pH between 5-6. Equimolar ketone was added in mixture and refluxed for 1 hour. After being suction-filtered, the product (carbazide) was recrystallized from ethanol.

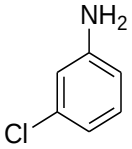
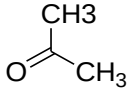
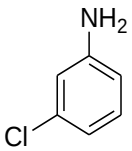
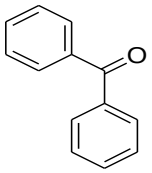
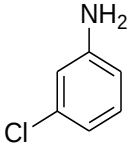
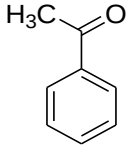
Scheme:

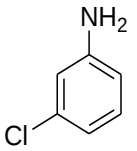
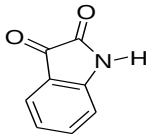
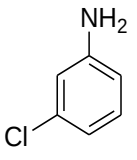
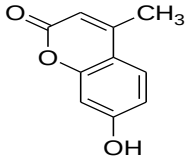
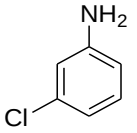
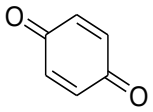
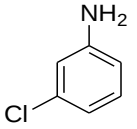
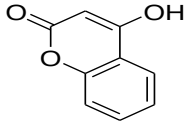
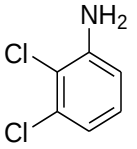
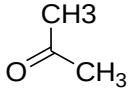
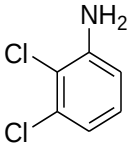
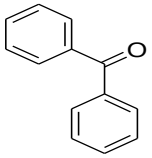
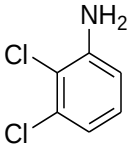
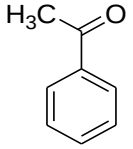
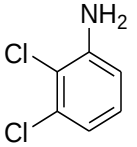
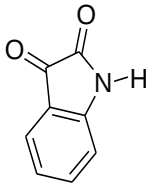
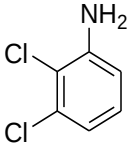
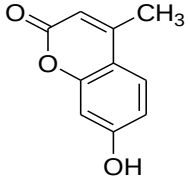
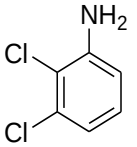
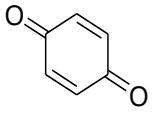
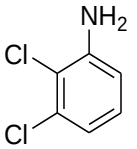
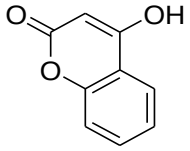


Scheme: Procedure for making the suggested compounds. Reagents and conditions (i) Ethanol, sodium cynate; (ii) aqueous NaOH Hyrazine hydrate; (iii) Appropriate ketone, glacial acetic acid ethanol.

The final synthesized compounds were evaluated for their Physical Characteristics as given in **Table 2** while spectroscopic data of compounds is given in **Table 3.**

Table 2: Physical Characteristics data of synthesized compounds

S.No.	Compound code	Aniline derivative	Ketone	Mol.wt.	Mol. formula	% Yield
1.	4a			225	$C_{10}H_{12}ClN_3O$	87
2.	4b			349.10	$C_{20}H_{16}ClN_3O$	90
3.	4c			287.08	$C_{15}H_{14}ClN_3O$	81

4.	4d			314.06	$C_{15}H_{11}ClN_4O_2$	89
5.	4 e			343.07	$C_{17}H_{14}ClN_3O_3$	92
6.	4 f			275.05	$C_{13}H_{10}ClN_3O_2$	85
7.	4g			329.06	$C_{16}H_{12}ClN_3O_3$	86
8.	8a			260.12	$C_{10}H_{12}Cl_2N_3O$	83
9.	8b			383.06	$C_{20}H_{15}Cl_2N_3O$	88
10.	8c			321.04	$C_{15}H_{13}Cl_2N_3O$	90
11.	8d			348.02	$C_{15}H_{10}Cl_2N_4O_2$	81
12.	8e			377.03	$C_{17}H_{13}Cl_2N_3O_3$	83
13.	8f			309.01	$C_{13}H_9Cl_2N_3O_2$	78
14.	8g			363.02	$C_{16}H_{11}Cl_2N_3O_3$	80

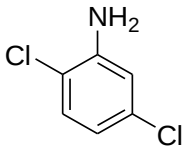
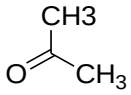
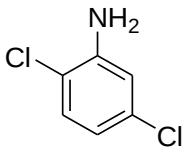
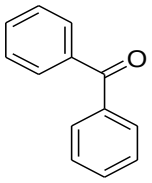
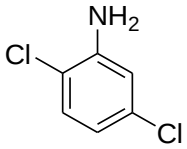
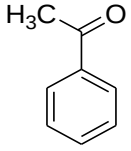
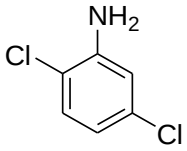
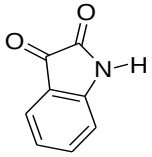
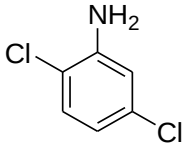
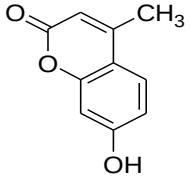
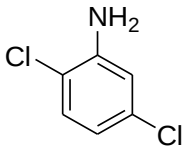
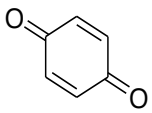
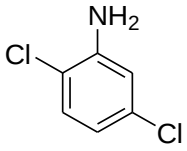
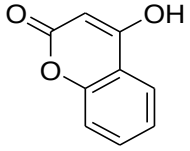
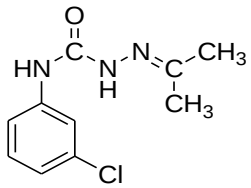
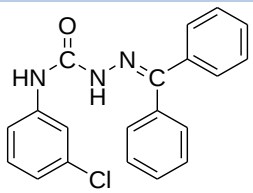
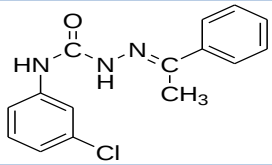
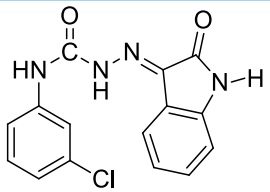
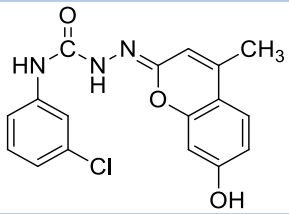
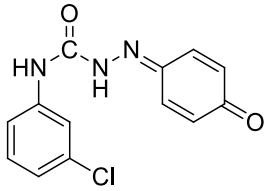
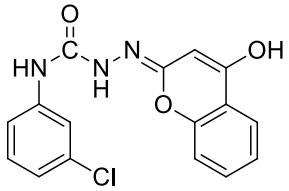
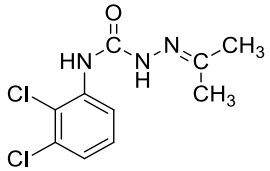
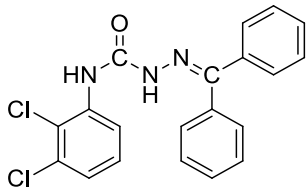
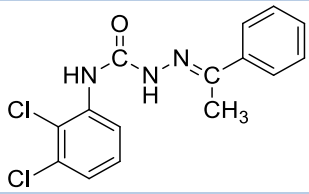
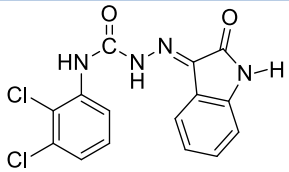
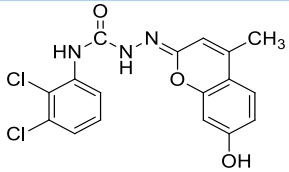
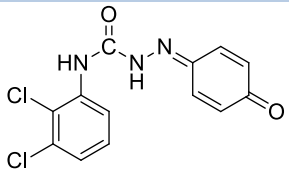
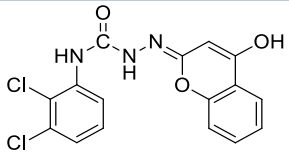
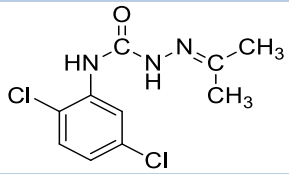
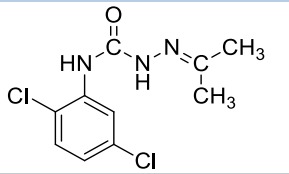
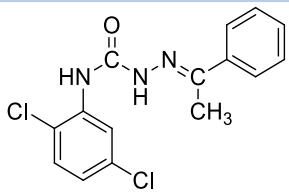
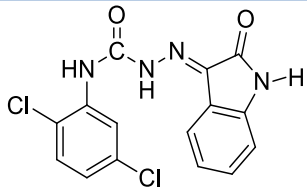
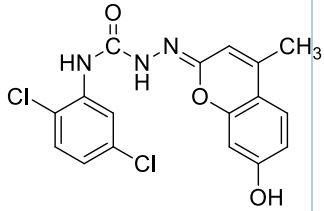
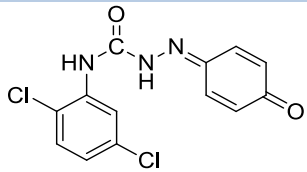
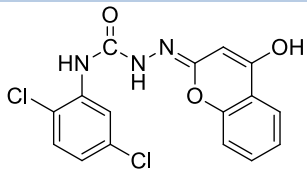
15.	12a			259.03	$C_{10}H_{11}Cl_2N_3O$	90
16.	12b			259.03	$C_{10}H_{11}Cl_2N_3O$	80
17.	12c			321.04	$C_{15}H_{13}Cl_2N3O$	89
18.	12d			348.02	C15H10Cl2N4O2	86
19.	12e			377.03	$C_{17}H_{13}Cl_2N_3O_3$	88
20.	12f			309.01	$C_{13}H_9Cl_2N_3O_2$	92
21.	12g			363.02	$C_{16}H_{11}Cl_2N_3O_3$	95

Table 3: Characterization Data of compounds 4a-12e

S. NO	Compd code	Ligand	IR	NMR[1]H NMR (500MHZ, CDCl3-d)	Elemental Analysis
1.	4a		(C=O) =1648-1720; (N=NH) =3100-3450	δ (7.23-7.99 4H Aniline), (6.00 NH-aniline), (7.00 NH-N), (1.94, 6 H, methyl)	C, 53.22; H, 5.36; Cl, 15.71; N, 18.62; O, 7.09
2.	4b		(C=O) = 1615-1700; (N=NH) =3260-3500	δ (7.23-7.99 4H Aniline), (6.00 NH-aniline), (7.00 NH-N), (7.57-7.58, 5 H, phenyl,7.62-7.97,5H phenyl)	C, 68.67; H, 4.61; Cl, 10.13; N, 12.01; O, 4.57
3.	4c		(C=O) =1590-1784; (N=NH) = 3220-3400	δ (7.23-7.99 4H Aniline), (6.00 NH-CO), (7.00 NH-N) (7.52-7.94, 5 H, phenyl, (2.32,3H methyl)	C, 62.61; H, 4.90; Cl, 12.32; N, 14.60; O, 5.56.

4.	4d		(C=O) =1600-1700; (N=NH) =3130-3630	δ (7.23-7.99 H Aniline), (6.00 NH-CO), (7.00 NH-N)), (7.26-7.86, 3H, isatin), (8.00,1H, isatin	C, 57.24; H, 3.52; Cl, 11.26; N, 17.80; O, 10.17.
5.	4e		(C=O) =1648-1720; (N=NH) =3100-3450	δ (7.23-7.99 4H Aniline), (6.00 NH-CO), (7.00 NH-N), (2.42-7.04, 3 H, hydroquinone)	C, 59.40; H, 4.10; Cl, 10.31; N, 12.22; O, 13.96
6.	4f		(C=O) =1648-1720; (N=NH) =3100-3450	δ (7.23-7.99 4H Aniline), (6.00 NH-CO), (7.00 NH-N,6.30-8.61 ,4-H Quinone),	C, 56.64; H, 3.66; Cl, 12.86; N, 15.24; O, 11.61.
7.	4g		(C=O) =1640-1710; (N=NH) =3200-3350	δ (7.23-7.99 4H Aniline), (6.00 NH-CO), (7.00 NH-N ,6.21-16.77 6H cumarin),	C, 58.28; H, 3.67; Cl, 10.75; N, 12.74; O, 14.56
8.	8a		(C=O) = 1615-1710; (N=NH) =3250-3600	δ (7.25-7.92 3H 2,3 di chloro aniline,) (6.00 NH-CO), (7.00 NH-N, 1.94, 6 H, methyl)	C, 46.17; H, 4.26; Cl, 27.26; N, 16.15; O, 6.15
9.	8b		(C=O) =1580-1780; (N=NH) = 3210-3420	δ (7.25-7.92 3H 2,3 di chloro aniline,) (6.00 NH-CO), (7.00 NH-N, 1.94. 7.57-7.58, 5 H, phenyl,7.62-7.97,5H phenyl)	C, 62.51; H, 3.93; Cl, 18.45; N, 10.94; O, 4.16.
10.	8c		(C=O) =1630-1720; (N=NH) =3140-3640	δ (7.25-7.92 3H 2,3 di chloro aniline,) (6.00 NH-CO), (7.00 NH-N, 1.94, (7.52-7.94, 5 H, phenyl, (2.32,3H methyl)	C, 55.92; H, 4.07; Cl, 22.01; N, 13.04; O, 4.97
11.	8d		(C=O) =1640-1720; (N=NH) =3140-3420	δ (7.25-7.92 3H 2,3 di chloro aniline,) (6.00 NH-CO), (7.00 NH-N, 1.94, (7.26-7.86, 3H, isatin), (8.00,1H, isatin).	C, 51.60; H, 2.89; Cl, 20.31; N, 16.05; O, 9.16.
12.	8e		(C=O) =1648-1730; (N=NH) =3100-3350	δ (7.25-7.92 3H 2,3 di chloro aniline,) (6.00 NH-CO), (7.00 NH-N, 1.94, (.2.42-7.04, 3 H, hydroquinone)	C, 53.99; H, 3.46; Cl, 18.75; N, 11.11; O, 12.69.
13.	8f		(C=O) = 1625-1750; (N=NH) =3260-3500	δ (7.25-7.92 3H 2,3 di chloro aniline,) (6.00 NH-CO), (7.00 NH-N, 1.94, 6.30-8.61 ,4-H Quinone), .	C, 50.35; H, 2.92; Cl, 22.86; N, 13.55; O, 10.32.

14.	8g		(C=O) =1590-1780; (N=NH) = 3210-3430	δ (7.25-7.92 3H 2,3 di chloro aniline,) (6.00 NH-CO), (7.00 NH-N, 1.94, 6.21-16.77 6H cumarin),.	C, 52.77; H, 3.04; Cl, 19.47; N, 11.54; O, 13.18.
15.	12a		(C=O) =1648-1720; (N=NH) =3150-3450	δ (7.47-8.18 3H 2,5 di chloro aniline,) (6.00 NH-CO), (7.00 NH-N, 1.94, 6 H, methyl)	C, 46.17; H, 4.26; Cl, 27.26; N, 16.15; O, 6.15
16.	12b		(C=O) = 1615-1700; (N=NH) =3220-3320	δ (7.47-8.18 3H 2,5 di chloro aniline,) (6.00 NH-CO), (7.00 NH-N, 1.94. 7.57-7.58, 5 H, phenyl,7.62-7.97,5H phenyl)	C, 46.17; H, 4.26; Cl, 27.26; N, 16.15; O, 6.15
17.	12c		(C=O) =1590-1784; (N=NH) = 3220-3440	δ (7.25-7.92 3H 2,5 di chloro aniline,) (6.00 NH-CO), (7.00 NH-N, 1.94, . (7.52-7.94, 5 H, phenyl, (2.32,3H methyl)	C, 55.92; H, 4.07; Cl, 22.01; N, 13.04; O, 4.97
18.	12d		(C=O) =1600-1700; (N=NH) =3130-3620	δ (7.47-8.18 3H 2,5 di chloro aniline,) (6.00 NH-CO), (7.00 NH-N, 1.94, (7.26-7.86, 3H, isatin), (8.00,1H, isatin).	C, 51.60; H, 2.89; Cl, 20.31; N, 16.05; O, 9.16.
19.	12e		(C=O) =1658-1760; (N=NH) =3160-3444	δ(7.47-8.18 3H 2,5 di chloro aniline,) (6.00 NH-CO), (7.00 NH-N, 1.94, (.2.42-7.04, 3 H, hydroquinone)	C, 53.99; H, 3.46; Cl, 18.75; N, 11.11; O, 12.69.
20.	12f		(C=O) =1635-1710; (N=NH) =3105-3405	δ (7.47-8.18 3H 2,5 di chloro aniline,) (6.00 NH-CO), (7.00 NH-N, 1.94, 6.30-8.61 ,4-H Quinone), .	C, 50.35; H, 2.92; Cl, 22.86; N, 13.55; O, 10.32.
21.	12g		(C=O) = 1615-1704; (N=NH) =3260-3540	δ (7.47-8.18 3H 2,5 di chloro aniline,) (6.00 NH-CO), (7.00 NH-N, 1.94, 6.21-16.77 6H cumarin),.	C, 52.77; H, 3.04; Cl, 19.47; N, 11.54; O, 13.18

BIOLOGICAL ACTIVITY

At a dosage of 100µg/ml, the produced lead compounds exhibited antibacterial activity against the gram-negative bacteria Escherichia coli and the gram-positive bacteria Bacillus subtilis. Gentamycin is used as a reference for comparison, and DMSO serves as a control under comparable circumstances. A weighed component was dissolved in water to create a nutrient agar, which was then incubated for 24 hours at 35–37°C. Following a 30-minute drying period, the matching peptone culture of the test organism was added to each sterile nutrient agar plate. A cork borer (size 3) was then used to create wells in the solidified medium. A dilution of 100 µg/ml of the corresponding test and standard chemicals was added to the prepared wells. For 24 hours, all of the flooded plates were cultured for bacterial strains at 35–37°C. Following a 30-minute drying period, the matching peptone culture of the test organism was added to each sterile nutrient agar plate. A cork borer (size 3) was then used to create wells in the solidified medium. A dilution of 100 µg/ml of the corresponding test and standard chemicals was added to the prepared wells. For 24 hours, all of the flooded plates were cultured for bacterial strains at 35–37°C. The zones of

inhibition were measured in millimeters, and the corresponding means were compared [20,21].

***In silico*-Studies**

SwissADME:

i. Physicochemical Parameters: The molecular characteristics that influence the mixture. For example, the number of rotatable bonds (n rot bonds), the molecular weight, H-bond donors (H donor/acceptor) etc. ii. Lipophilicity: The M logP value is taken into consideration from the different Log P values. iii. Pharmacokinetic Parameters: These include P-glycoprotein substrate or inhibitor, blood-brain barrier penetration, and gastrointestinal absorption. iv. Lead likeness: In drug discovery, a lead compound is a chemical compound with biological or pharmacological activity that is probably going to be therapeutically beneficial, but it may also have a structure that isn't ideal and needs to be changed to better fit the target. v. To demonstrate the compound's absorption in the GIT or its capacity to cross the BBB, create a model of a boiled egg.

Computational studies

In silico studies were done using Schrödinger 2024 module and Autodock. Using in silico techniques, the interactions between the most active compounds and the enzyme active site were investigated (PDB Code: 2Y2T). We used the Schrödinger 2024 module to create the protein's crystal structure. Prime was used to add the missing side chains and loops. Protonation states were assigned using Epik after all water molecules were removed at neutral pH. Following that, protein design was optimized using the PROPKA method at neutral, keeping the convergence-heavy atoms' RMSD at 0.30Å during restricted minimization. To locate active areas, a receptor grid was made using the obtained protein structure. The LigPrep module was used to create the stable conformers of *E.coli* with most active compounds which were then docked by the Schrödinger Maestro 2024. The Glide XP Visualizer program and Discover studio were used to analyze the interactions in detail. Molecular docking studies were performed using AutoDock v.4.0. The enzyme was prepared for docking studies. It involves i) Removal of a ligand molecule and other heteroatoms from the enzyme active site. ii) Addition of polar hydrogens and charges to the structure with their standard geometry iii) The obtained model was used in predicting the ligand enzyme interaction at the active site. The prepared ligands and target molecules were docked, and the results are given in the table as best pose binding energy scores

RESULT AND DISCUSSION

Chemistry

Schemes 1/2/3 illustrates how chloroaniline (1/5/9), is converted into the suggested aniline carbazide 4a-g/8a-g/12a-g. By using the recrystallization method, the synthesized compounds were obtained. By performing spectroscopic and elemental investigations, the structures of the compounds were verified. Compound 12e was formed, as indicated by the emergence of a (–NHCONH-) proton peak at 6.00 ppm in the ^{1}H NMR spectra. The assessment peaks of the coumarin's methyl and hydroxyl groups appeared at 2.42 ppm and 5.35ppm respectively. Similarly, the spectral analysis was done for all synthesized compounds.

Anti-bacterial Activity

Acquired Based on the study's findings, the greatest zone of inhibition (ZI) was calculated as shown in **Table 4** for test organisms treated with varying amounts of sample on agar plates in contrast to the positive control, against the test pathogen E. coli. The information is also given in Bar graph form (**Fig.1**) The region surrounding a disc on an agar plate where no bacterial growth is seen because of the presence of an antimicrobial agent is known as the zone of inhibition. It is employed to ascertain whether or not a specific test organism is vulnerable to the effects of a given antimicrobial drug.

Table 4: Antimicrobial evaluation of aniline carbazides against E. coli

S.no	Compound code	Max.ZOI(mm)avg	CONC. (μg)
1.	4a	11.11	30
2.	4b	11.25	30
3.	4c	12.10	30
4.	4d	17.44	30
5.	4e	18.16	30
6.	4f	11.82	30
7.	4g	17.45	30
8.	8a	13.55	30
9.	8b	13.95	30
10.	8c	14.10	30
11.	8d	22.24	30
12.	8e	23.92	30
13.	8f	14.88	30
14.	8g	22.68	30
15.	12a	14.89	30
16.	12b	14.55	30
17.	12c	16.82	30
18.	12d	24.24	30
19.	12e	25.45	30
20.	12f	15.67	30
21.	12g	24.67	30
22.	Ciprofloxacin	26.66	10

In-silico Studies: Swiss ADME tool was used for the compounds' in-silico study (**Table 5**) and boiled Egg representation (**Fig 2)**. Swiss ADME: Studies conducted by Swiss ADME indicated that the substances had good pharmacokinetic characteristics.

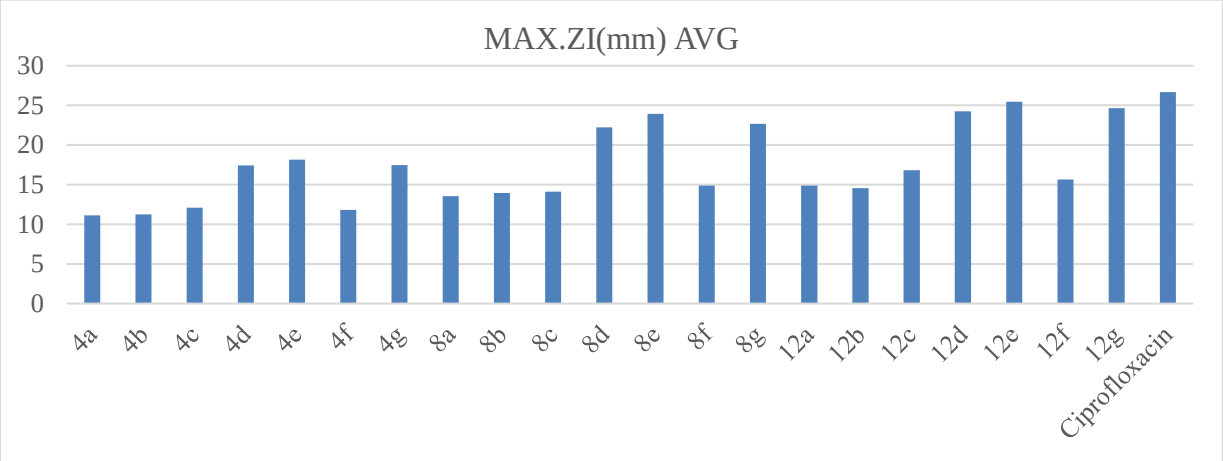


Figure 1: Bar graph showing Antimicrobial evaluation of aniline carbazides

Table 5: Swiss ADME study data of 9 most active Chloro aniline derivatives

Molecule code	Heavy atoms	Rotatable bonds	H-bond acceptors	H-bond donors	GI absorption	BBB permeant	Leadlikeness violation	Bioavailability Score
4d	22	4	3	3	High	No	1	0.55
4e	24	4	4	3	High	No	1	0.55
4g	23	4	4	3	High	No	1	0.55
8d	23	4	3	3	High	No	0	0.55
8e	25	4	4	3	High	No	0	0.55
8g	24	4	4	3	High	No	1	0.55
12d	23	4	3	3	High	No	1	0.55
12e	25	4	4	3	High	No	1	0.55
12g	24	4	4	3	High	No	1	0.55

Boiled Egg Representation:

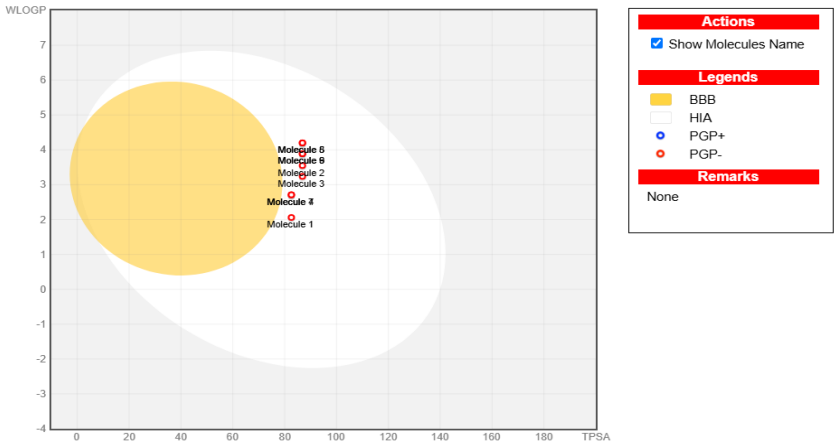
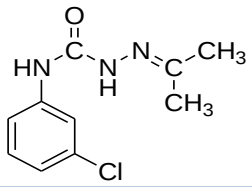
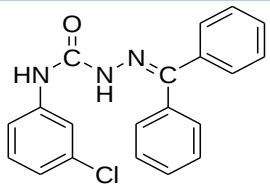
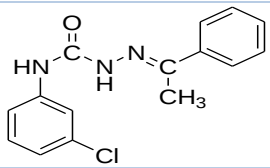
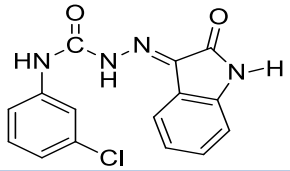
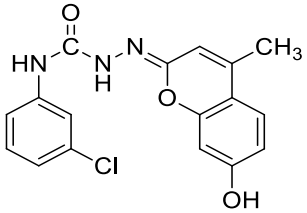
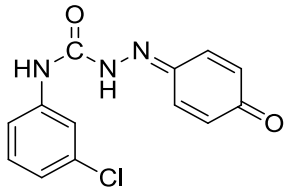
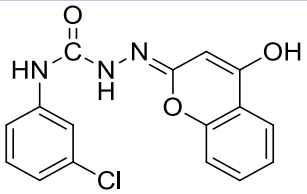
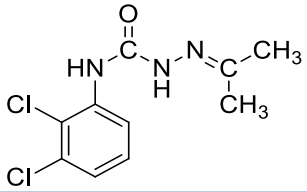
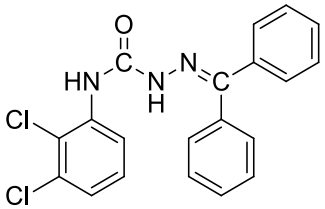
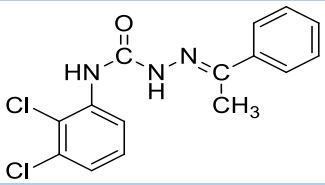
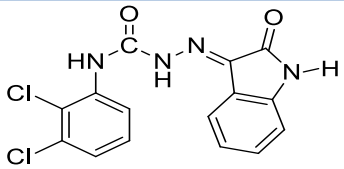
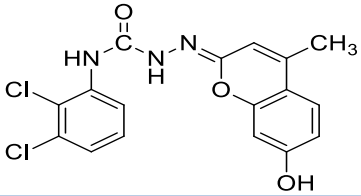
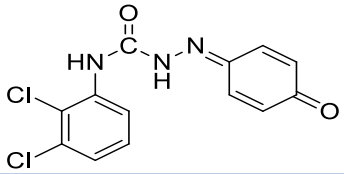
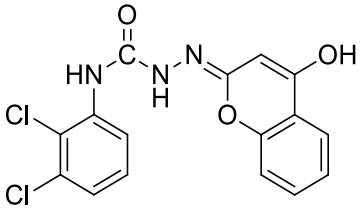
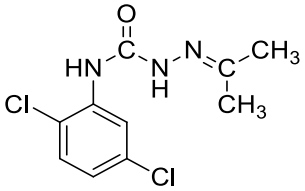
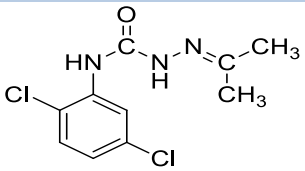
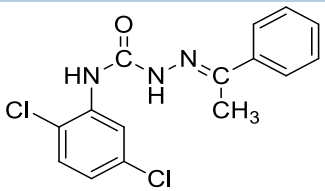
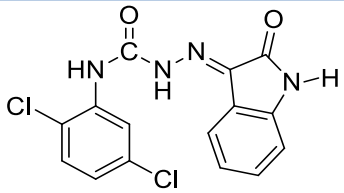
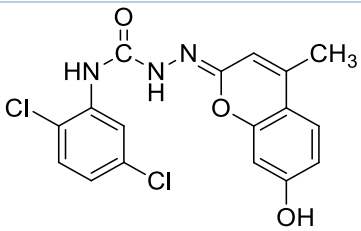


Figure 2: Boiled Egg Representation of most active compounds

Molecular Docking: The compounds' docking outcomes were noted. Every Compound displayed docking scores that ranged from good to moderate. The compounds had the best posture binding energy values, which ranged from -3.11 to -6.95 respectively using both the tools. The binding energies of synthesized compounds is given in **Table 6**. The majority of the compounds' dock scores were comparable to those of the common ciprofloxacin. The best docking score and binding interactions were showed by compound 12e. Further, good scores were shown by 12d/12g/84/8g/8e compounds. The PDB id is 2Y2T. Glide tool was used to study 2 D interactions of ciprofloxacin and other compounds (**Fig.3**). In most active compound 2e, the hydroxyl group of coumarin moiety forms H- bond with GLN 94 in binding pocket. The hydrophobic interactions are shown by TRP95, ILE 6, PHE 8 and PRO 97 while Polar interactions are shown by THR10, ASN 9, THR 11, THR 7, GLN 93. Auto dock tool was used to study 2 D interactions of ciprofloxacin and other compounds. compounds. In compound 2e, chloride of dichloro aniline moiety forms pi-alkyl bond with PRO 97 and pi-pi stacking with PHE. Nitrogen atoms of NH-C=O bridge forms conventional H- bond with ILE 6, GLN 93 and GLN 5 while hydroxy group of coumarin forms conventional H-bond with SER 3. The Study of 2D Interactions by most active compounds(12d/e/g) using both the tools is given in **Table 7.**

Table 6. Binding Energies of most active compounds using both the tools

S.no.	Compound code	Ligand	Binding Energy Glide	Binding Energy Auto dock 4
1.	4a		4.82	4.22
2.	4b		4.21	4.15
3.	4c		4.88	4.29
4.	4d		5.80	5.42
5.	4e		5.95	5.28
6.	4f		3.98	3.44
7.	4g		5.91	5.21
8.	8a		3.42	3.11
9.	8b		4.34	3.98

10.	8c		4.66	4.15
11.	8d		6.60	6.52
12.	8e		6.62	6.56
13.	8f		4.74	4.14
14.	8g		6.61	6.50
15.	12a		3.99	3.12
16.	12b		3.88	3.44
17.	12c		3.55	3.45
18.	12d		6.80	6.70
19.	12e		6.95	6.72

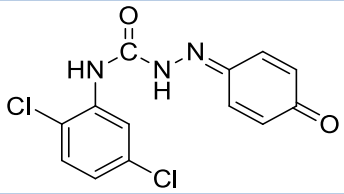
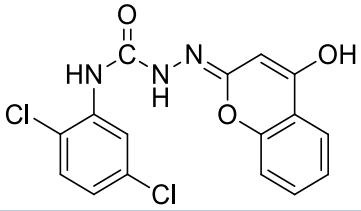
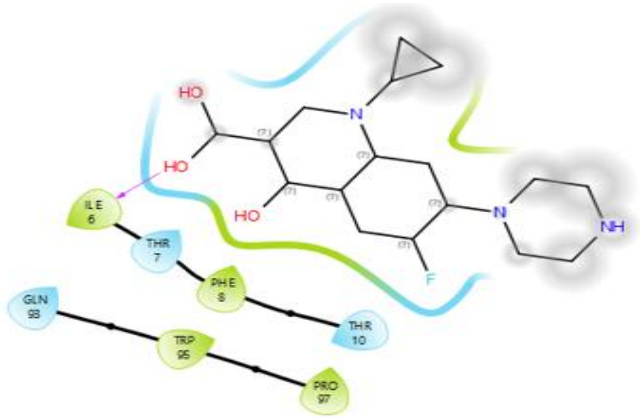
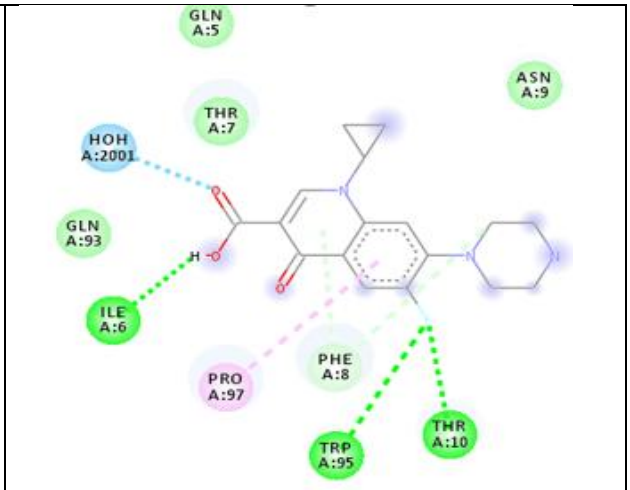
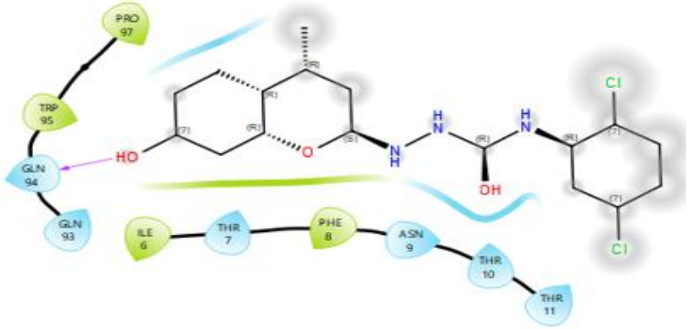
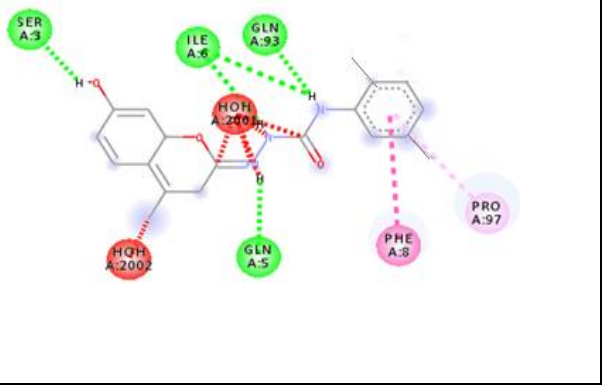
20.	12f		4.38	3.98
21.	12g		6.90	6.80
22.	Ciprofloxacin		7.2	7.2

Table 7: Study of 2D Interactions by most active compounds using both the tool

S.no	Compd code	Glide			Autodock 4	
		Interactions	hydrophobic	Polar	H-bond	Pi-pi/pi-alkyl
1.	12d	GLN 94	ILE6, PHE8, PRO97, LEU 91	GLN 93, GLN 94, THR 7, GLN5, SER 4	ILE 6, GLN 93	ILE 91
2.	12e	GLN 94	TRP 95, ILE 6, PHE 8, PRO 97	THR 10, ASN 9, THR 11, THR7, GLN 93	SER 3, ILE6, GLN 93, GLN 5	PRO 97, PHE 8
3.	12g	GLN 5	LEU 91, TRP 95, PRO 97, PHE 8, ILE6	GLN 93, SER 4, GLN 5, THR 7	GLU 94, ILE6	PRO 97
4.	Cipro floxacin	ILE6	ILE6, PHE 8, TRP 95, PRO 97	THR 10, THR 7, GLN 93	ILE 6, TRP 95, THR 10, GLN 93	PRO 97

2D Interactions of ciprofloxacin and most active synthesized compounds

	Glide	Autodock
Ciprofloxacin		
12e		

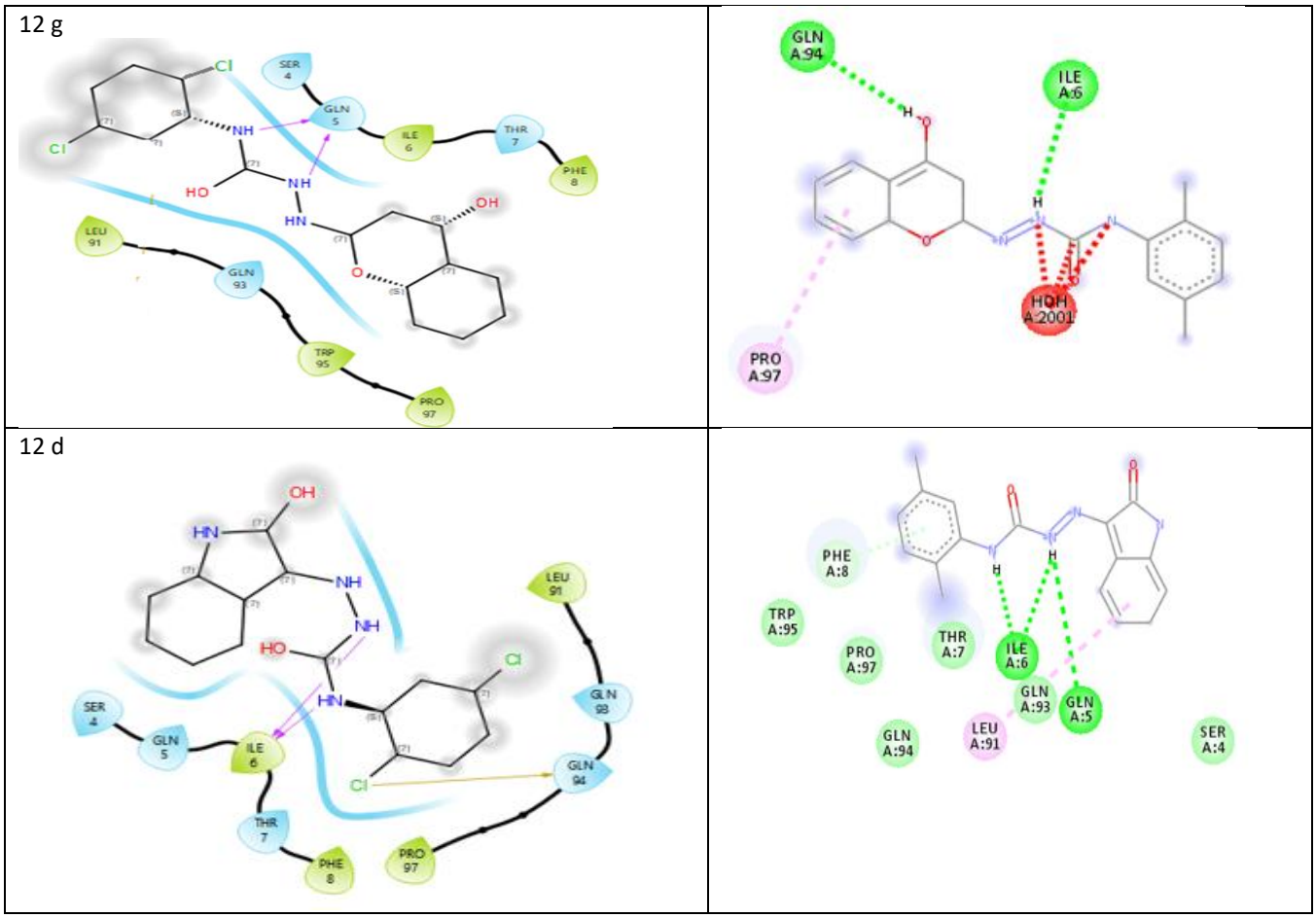


Figure 3: 2D Interactions of ciprofloxacin and most active synthesized compounds

CONCLUSION

The Swiss ADME study states that the chemicals in three series of (4a-g/8a-g/12a-g) aniline derivatives had favorable pharmacokinetic characteristics. They are not BBB permeant, as demonstrated by the boiled-egg characteristic. The compounds displayed lead resemblance. All the compounds showed good to moderate binding affinities using both the docking tools but results of Schrodinger module showed better results (binding affinities and interactions) which were well correlated with actual biological study. On Auto Dock v.4.0, the molecular docking analysis of targets revealed good to moderate binding scores. When these compounds were tested against E. coli in vitro, they similarly showed good to moderate activity. We can draw the conclusion from the aforementioned data that these compounds exhibited lead similarity and moderate to strong antibacterial activity in both in vitro and in silico investigations. As a result, there is a correlation between in-silico and in vitro research. In the search for novel antibiotics, these moieties can be investigated further.

Statements and Declaration

It is not necessary to disclose the author's pertinent financial or non-financial interests.

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REFERENCES

1. Bhamidipati M and Konda S. Screening of 4-([1,3,4]oxadiazino[6,5-b] indole-3-yl)aniline derivatives for anti-bacterial activity by in-silico and in-vitro method. IJPSR. 01 October 2019;10(10):4799-4805.
2. Sahoo S, Mahendrakumar CB and Setty C. Synthesis, antiinflammatory and antibacterial activities of some substituted isatin and isatin fused with 3 substituted 4- amino-5-mercapto 1, 2, 4-triazoles. International Journal of Chemical Sciences, 2015;13:613-24.
3. McMurry J. Organic Chemistry. (1984). Brooks/Cole. p. 676.
4. Mohammad A, Alghamdi S. Chemical and Biological potentials of semicarbazide and thiosemicarbazide derivatives and their metals complexes. Advanced Journal of Chemistry, Section B, 2021;3(3):243-270.

5. Siwek A, Stefanska J, Dzitko K, Ruszezak A. Antifungal effect of 4-arylthiosemicarbazides against Candida species. Search for molecular basis of antifungal activity of thiosemicarbazide derivatives J. Mol. Model, 2012;18:4159-4170.
6. A. Del Corso, U.C. Mura. Thiol dependent oxidation of enzymes: the last chance against oxidative stress. Int. J. Biochem., 1994;26:745-750.
7. Patel, R. P. "Antimicrobial activity of chloroaniline derivatives: Mechanism of action and structure-activity relationships." *International Journal of Antimicrobial Agents*, 2006;28(3): 232-239. [DOI: 10.1016/j.ijantimicag.2006.04.008]
8. Singh, B. R., et al. "Synthesis, antimicrobial activity, and SAR of chloroaniline derivatives." *Medicinal Chemistry Research*, 2011;20(12):1692-1700. [DOI: 10.1007/s00044-011-9489-4]
9. Srinivasan, P., et al. "Antimicrobial activity and the mechanism of action of chloroaniline derivatives." *Pharmaceutical Biology*, 2010;48(10):1130-1136. [DOI: 10.3109/13880201003746201]
10. Kumar S, Niranjan MS, Chaluvaraju KC, Jamakhandi CM, Dayanand K. Synthesis and Antimicrobial Study of Some Schiff Bases of Sulfonamides. J Curr Pharm Res. 2010;01:39-42.
11. Mistry KM, Desai KR. Synthesis of Novel Heterocyclic 4-Thiazolidinone Derivatives and their Antibacterial Activity. E-J Chem. 2004;1(4):189-193.
12. Pandey, V.K., Yadava, S., Chandra, K., Joshi, M.N. and Bajpai, S.K. Antiviral studies of 7-arylamido/imidoalkyl-2,3 dihydro-2, 3- diphenyl-1, 3 benzoxazine-4-ones. Indian Drugs, 1999;36:532-534.
13. Vazzanaa, I. Terranovaa, E. Mattiolib, F. and Sparatorea, F. Aromatic Schiff Bases and 2.3-disubstituted-1.3 thiazolidin-4-one Derivatives as Antiinflammatory Agents, Arkivoc (v): 2004;364-374.
14. Nair, V. R. Soni, M. Baluja, S. Synthesis, Structural Determination and Anti bakterial Activity of compounds derived from vanillin and 4-aminoantipyrine, J. Serb. Chem. Soc. 2004;69:991-998.
15. Garg, H. G. Kaur, M. Synthesis of N`-substituted Arylsulfonylpyrazoles, their anthelmintic Activity, and the Cytotoxicity of some Hydrazides, J. Med. Chem. 1972;15(5):554-555.
16. Ambroziak, K. Rozwadowski, Z Dziembowska, T. and Bieg, B. Synthesis and spectroscopic Study of Schiff bases derived from trans-1,2-diaminocyclohexane. Deuterium isotope shift ob 13C chemical shift, J. Mol. Struct., 2002;615:105-120.
17. Desai, S. B., Desai, P. B. & Desai, K. R. Synthesis of some Schiff Bases,Thiazolidones, and Azetidinones derived from 2.6-diaminobenzo[1.2-d.4,5-d`]bisthiazole and their Anticancer Activities, Heterocycl. Commun, 2001;7(1):83-90.
18. Samadhiya, S., Halve, A. 2001. Synthetic Utility of Schiff Bases as potential herbicidal Agents, Orient. J. Chem. 17:119-122. 12. More, P. G., Bhalvankar, R. B. & Pattar, S. C., Synthesis and biological activity of schiff bases of aminothiazoles, J. Indian Chem. Soc., 2001;78:474-475.
19. Ezeokonkwo MA, Ogbonna ON, Okafor SN, GodwinNwakwasi EU, Ibeanu FN and Okoro UC: Angular phenozaxine ethers as potent multi-microbial targets inhibitors: Design, synthesis, and molecular docking studies. Frontiers in Chemistry 2017;5: 1-15.
20. Aniyery R, Gupta A and Singh P: In-vitro and in silico antimicrobial study of stannane of pyridoxal 5-phosphate. Int J of Pharmacy and Pharm Sci, 2016;9:145-53.
21. Vijesh AM and Isloor AM: Molecular docking studies of some new imidazole derivatives for antimicrobial properties. Arabian J of Chemistry, 2013;6(2): 197-04.

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