STM JOURNALS

Research & Reviews: A Journal of
Pharmaceutical Science

ISSN: 2229-7006
Volume 16, Issue 2, 2025
May–August
DOI (Journal): 10.37591/RRJoPS

https://journals.stmjournals.com/rrjops

Research RRJoPS

Synthesis, Characterization, Biological Evaluation, and in Silico Study of Aniline Derivatives

Alka Tyagi*

Abstract

In many hospitals worldwide, bacterial infections brought on by resistant strains are wreaking havoc, particularly in patients who are already impaired by age, disease, and other conditions. Due to the bacterial resistance, it is required to search for newer antibacterial drugs or molecules. In the current study, we aimed to develop aniline derivatives, followed by their anti-bacterial and in silico studies. Out of all the synthesized derivatives, compound 4d was found to be most active, which is confirmed by both in silico as well as anti-bacterial studies of the synthesized compounds. The PDB ID of the protein is 2Y2T. The phenyl ring of alanine interacts with Leu 214A and Arg 211A in the binding pocket. The isatin ring interacts with Ala 3A of the protein. Both in vitro and in silico tests revealed that all the compounds had good to moderate antibacterial activity and resembled lead. As a result, there is a correlation between in-silico and in vivo research.

Keywords: Alanine derivatives, anti-bacterial activity, in silico study, docking, antibiotic resistance

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 knowledge of resistance mechanisms [1, 2]. As a result, it is necessary to synthesize fresh types of antibiotics with unique mechanisms of action. Therefore, it may be possible to use the inhibition of microbial growth under normal circumstances to illustrate the therapeutic effectiveness of antibiotics. Therefore, it may be possible to use the inhibition of microbial growth under normal circumstances to illustrate the therapeutic effectiveness of antibiotics. any alteration to the antibiotic molecule that affects the antimicrobial activity, but chemical approaches may not be able to detect it. Therefore, the microbiological assays are very useful for resolving doubts regarding the possible loss of potency of antibiotics [3–10]. Aromatic amine derivatives are used for antimicrobial activities. In the case of aromatic_amines (for example, aniline derivatives), the basicity is increased by an electron-repelling group, such as − OCH_3, for example (increasing the electron density in the nitrogen atom), and is decreased by an electron-withdrawing group (such as a nitrogenous group) [11]. Understanding molecular characteristics and the unique behavior or nature of drug-receptor interactions at the molecular level has improved thanks to in-silico approaches. As a crucial tool in the drug development toolbox, molecular docking has grown in appeal among researchers and the industry due to its ease of use and relative affordability [12]. 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 antibacterial activity.

***Author for Correspondence**
Alka Tyagi
E-mail: alka.tyagi35@gmail.com

Associate Professor, Shri Pandit Baburao Chaughule College of Pharmacy, Thane, Maharashtra, India

Received Date: March 25, 2025
Accepted Date: April 12, 2025
Published Date: April 26, 2025

Citation: Alka Tyagi. Synthesis, Characterization, Biological Evaluation, and in Silico Study of Aniline Derivatives. Research & Reviews: A Journal of Pharmaceutical Science. 2025; 16(2): 25–32p.

MATERIALS AND METHODS

Materials

Chemicals, like aniline, isatin, benzoquinone, acetone, acetophenone, benzophenone, and *E. coli* strains, were purchased from research lab.

Method

- *Procedure:* Aniline (1) was subjected to reaction with sodium cyanate and ethanol to give derivative (2), which was further allowed to react with hydrazine hydrate to yield derivative (3). The compound (D) was allowed to react with different ketones to give final products 4(a–d) (Figure 1).

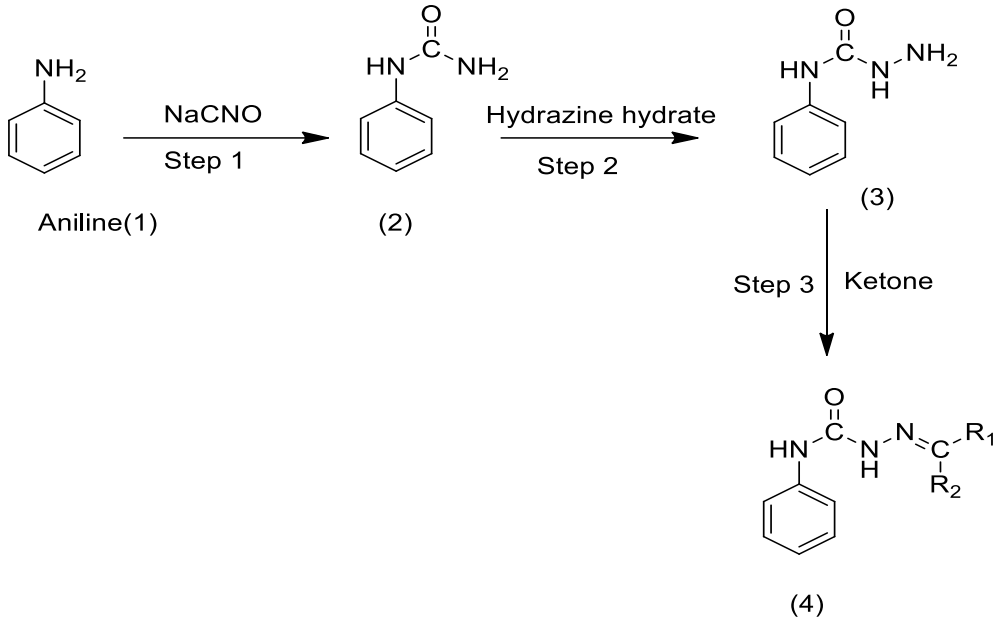


Figure 1. Scheme: reagents-sodium cyanate, ethanol, hydrazine hydrate, and different suitable ketones.

Biological Evaluation

Antibacterial Activity

Utilizing the Kirby-Bauer method, also known as the zone inhibition method, the antibacterial activity was evaluated. Before inserting the discs with 10 µl of varying concentrations (0 to 100 mg/ml), the MHA plates were inoculated with 100 µl of bacterial culture, *E. coli* (adjusted to 0.5 McFarland unit-approx. densities of cells (1.5 x 108 CFU/mL). The positive control was a ciprofloxacin disc (10 µg), while the vehicle control was a single disk filled with solvent alone in each plate. The *S. aureus* plates (Basil Scientific Corp., India) were incubated at 37°C for a whole day [13–16]. Measurements were taken.

In silico-Research

Swiss ADME

- *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.
- *Lipophilicity:* The M logP value is taken into consideration from the different Log P values.
- *Pharmacokinetic Parameters:* These include P-glycoprotein substrate or inhibitor, blood-brain barrier penetration, and gastrointestinal absorption.
- *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 is not ideal and needs to be changed to better fit the target.

- To demonstrate the compound's absorption in the GIT or its capacity to cross the BBB, create a model of a boiled egg.

Molecular Docking

ChemDraw version 12.0 was used to draw the structures of the ligand molecules, and ChemDraw Biochem 3D was used to convert them to a 3D conformation. The PDB database was used to download the standard (ciprofloxacin) structure. AutoDock v.4.0, protein plus pose view software, and one-click docking were used for molecular docking experiments. RCBS: PDB was used to import the 3D X-ray crystal structures of the enzyme *E. coli* (PDB ID: 2y2t Additionally, the Protein Data Bank (PDB) provided the receptor grid along with its ligand information. The enzyme was ready for research on docking.

RESULT AND DISCUSSION

The compounds procured from the lab were tested using in silico and antibacterial methods. The synthesized compounds were characterized using physical characterization (Table 1) and spectroscopic methods (Table 2).

Table 1. Physical characteristics data of compounds 4(a–e).

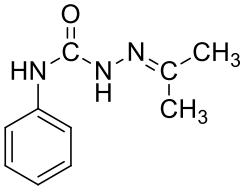
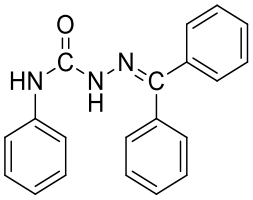
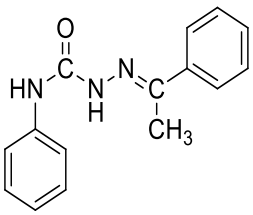
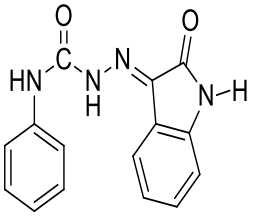
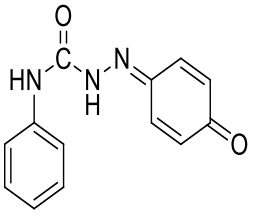
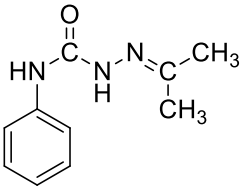
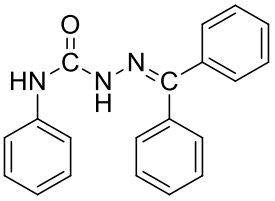
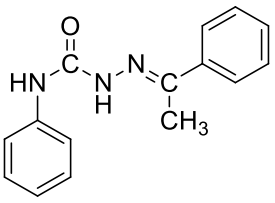
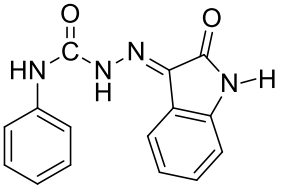
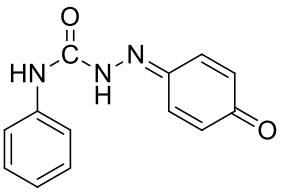
S.N.	Compound Code	Compound Structure	IUPAC Name	Chemical Formula	Mol wt	Solubility
1.	4a		N-phenyl-2-(propan-2-ylidene) hydrazinecarboxamide	$C_{10}H_{13}N_3O$	191.23	ethanol
2.	4b		2-(diphenylmethylene)-N-phenylhydrazinecarboxamide	$C_{20}H_{17}N_3O$	315.37	ethanol
3.	4c		(E)-N-phenyl-2-(1-phenylethylidene) hydrazinecarboxamide	$C_{15}H_{15}N_3O$	253.30	ethanol
4.	4d		E)-2-(2-oxoindolin-3-ylidene)-N-phenylhydrazinecarboxamide	$C_{15}H_{12}N_4O_2$	280.28	ethanol
5.	4e		2-(4-oxocyclohexa-2,5-dien-1-ylidene)-N-phenylhydrazinecarboxamide	$C_{13}H_{11}N_3O_2$	241.25	ethanol

Table 2. Characterization data of compounds 4(a–e).

S.N.	Compound Code	Structure	IR	NMR	Mass	Elemental Analysis
1.	4a		(C=O) =16 48–1720; (N=NH) =3100–3450	1H NMR (500MHZ, CDCI3-d) δ (7.19–7.61 4H Aniline), (6.00 NH-aniline), (7.00 NH-N), (1.94, 6 H, methyl)	191.11 (100.0%), 192.11 (11.0%), 192.10 (1.1%)	C, 62.81; H, 6.85; N, 21.97; O, 8.37
2.	4b		(C=O) = 16 15–1700; (N=NH) =3260–3500	1H NMR (500MHZ, CDCI3-d) δ (7.19–7.61 4H Aniline), (6.00 NH-aniline), (7.00 NH-N), (7.58–7.97, 10 H, phenyl)	315.14 (100.0%), 316.14 (21.9%), 317.14 (2.7%), 316.13 (1.1%)	C, 76.17; H, 5.43; N, 13.32; O, 5.07
3.	4c		(C=O) =1590–1784; (N=NH) = 3220–3400	1H NMR (500MHZ, CDCI3-d) δ (7.19–7.61 4H Aniline), (6.00 NH-CO), (7.00 NH-N) (7.52–7.94, 5 H, phenyl, (2.32,3H methyl)	253.12 (100.0%), 254.12 (17.3%), 255.13 (1.5%)	C, 71.13; H, 5.97; N, 16.59; O, 6.32
4.	4d		(C=O) =1600–1700; (N=NH) =3130–3630	1H NMR (500MHZ, CDCI3-d) δ (7.19–7.61 4H Aniline), (6.00 NH-CO), (7.00 NH-N)), (7.26–7.86, 3H, isatin), (8.00,1H, isatin	280.10 (100.0%), 281.10 (16.4%), 282.10 (1.9%), 281.09 (1.5%)	C, 64.28; H, 4.32; N, 19.99; O, 11.42
5.	4e		(C=O) =16 48–1720; (N=NH) =3100–3450	1H NMR (500MHZ, CDCI3-d) δ (7.19–7.61 4H Aniline), (6.00 NH-CO), (7.00 NH-N), (6.30–8.61, 3 H, hydroquinone)	241.09 (100.0%), 242.09 (14.3%), 243.09 (1.5%), 242.08 (1.1%)	C, 64.72; H, 4.60; N, 17.42; O, 13.26

Anti-Bacterial Activity

Based on the study's findings, the greatest zone of inhibition (ZI) was calculated as shown in Table 3 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 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 a specific test organism is vulnerable to the effects of a given antimicrobial drug.

Table 3. Anti-bacterial activity of tested compounds 4a–e.

S.N.	Sample Code	Max. ZI (mm) Avg	Conc. (μg)
1	Ciprofloxacin (pc)	26.66	10
2	4a	22.70	30
3	4b	22.88	30
4	4c	17.40	30
5	4d	22.20	30
6	4d	19.24	30

In-silico Studies

Swiss ADME was used for the compounds' in-silico examination (Tables 4 & 5) and boiled egg representation data (Figure 2). Swiss ADME: Studies conducted by Swiss ADME indicated that the substances had good pharmacokinetic characteristics.

Table 4. Swiss ADME study data of aniline derivatives.

Molecule Code	Heavy Atoms	Aromatic Heavy Atoms	Rotatable Bonds	H-bond acceptors	H-bond donors	Ali Class	GI absorption	BBB permeant	Bioavailability Score	Leadlikeness violations
4a	14	6	4	2	2	Very soluble	High	Yes	0.55	1
4b	24	18	6	2	2	Moderately soluble	High	Yes	0.55	1
4c	19	12	5	2	2	Soluble	High	Yes	0.55	0
4d	21	12	4	3	3	Soluble	High	No	0.55	0
4e	18	6	4	3	2	Soluble	High	Yes	0.55	1

Boiled Egg Representation

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 –7.05 to –5.22, respectively. Most of the compounds' dock scores were comparable to those of the common ciprofloxacin. The best docking score and binding interactions were shown by compound 4d. The 3D interaction of compound 4d is shown in Figure 3**,** and the 2D interaction is shown in Figure 4. The PDB ID of the protein is 2Y2T. The phenyl ring of alanine interacts with Leu 214A and Arg 211A in the binding pocket. The isatin ring interacts with Ala 3A of the protein.

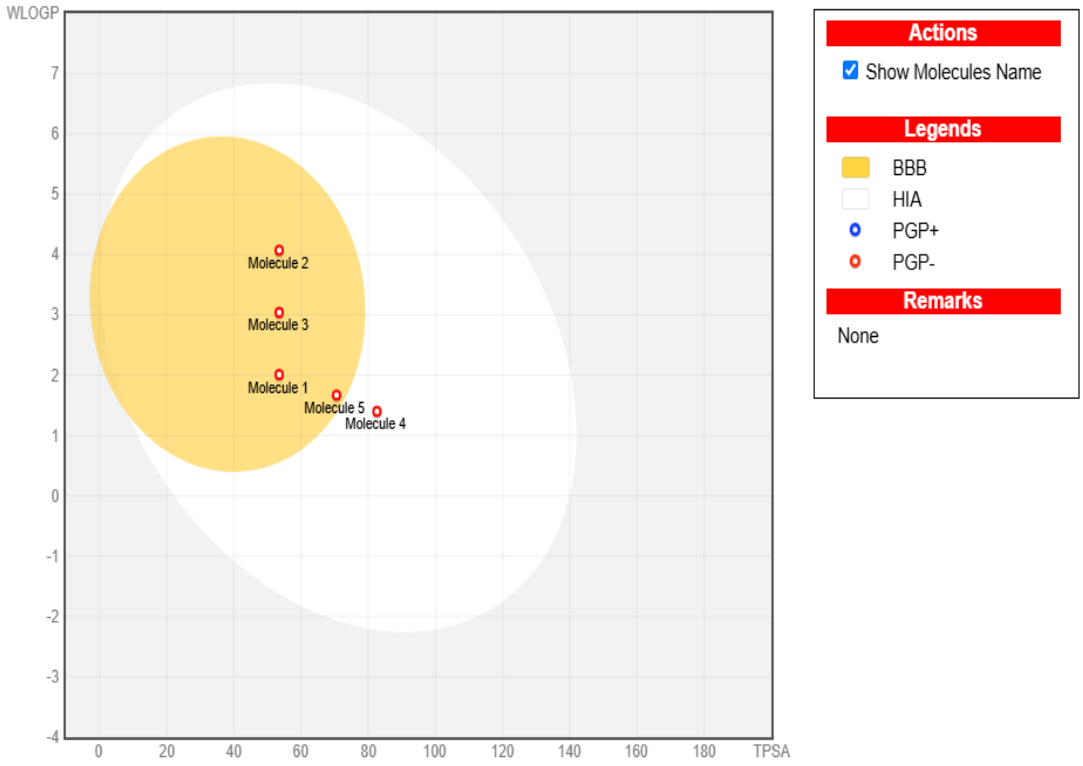
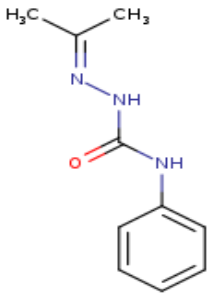
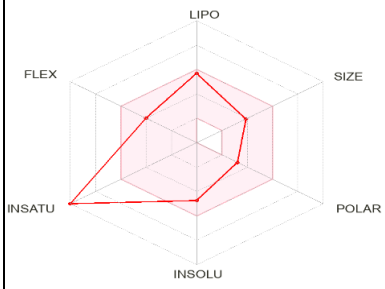
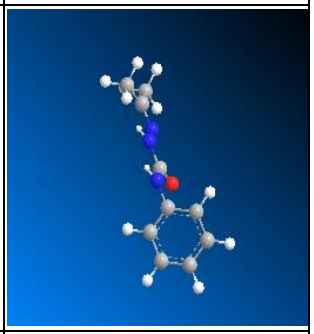
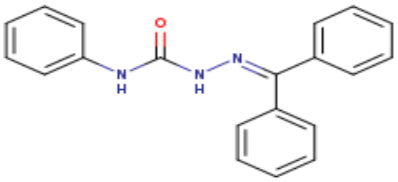
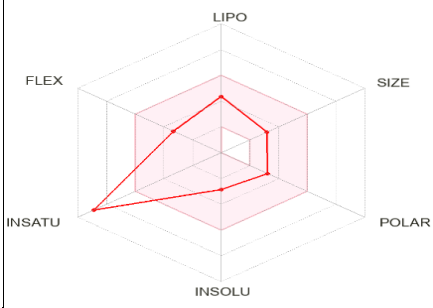
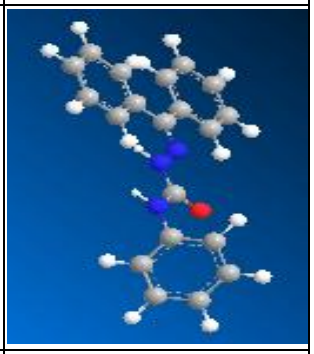
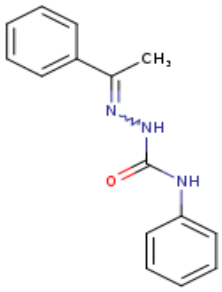
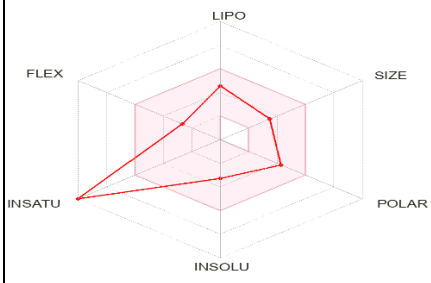
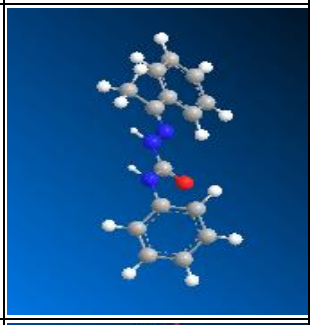
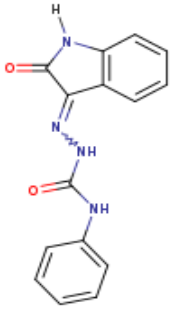
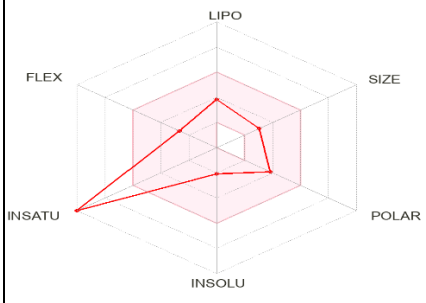
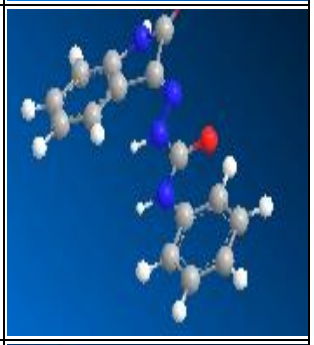
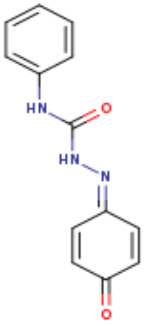
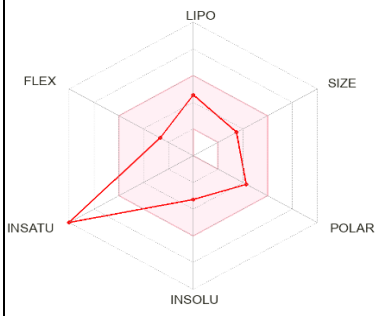
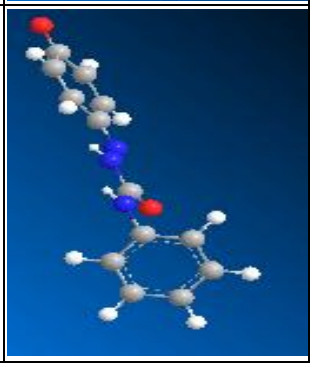


Figure 2. Boiled egg representation of compounds 4(a–e).

Table 5. ADME properties and 3D structures of synthesized compounds.

S. N.	Compound Structure	Lead Likeness Properties	3D Structure
1.			
2.			
3.			
4.			
5.			

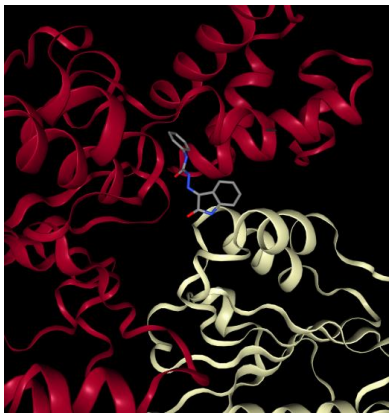


Figure 3. 3D interaction of the compound 4din binding pocket of protein.

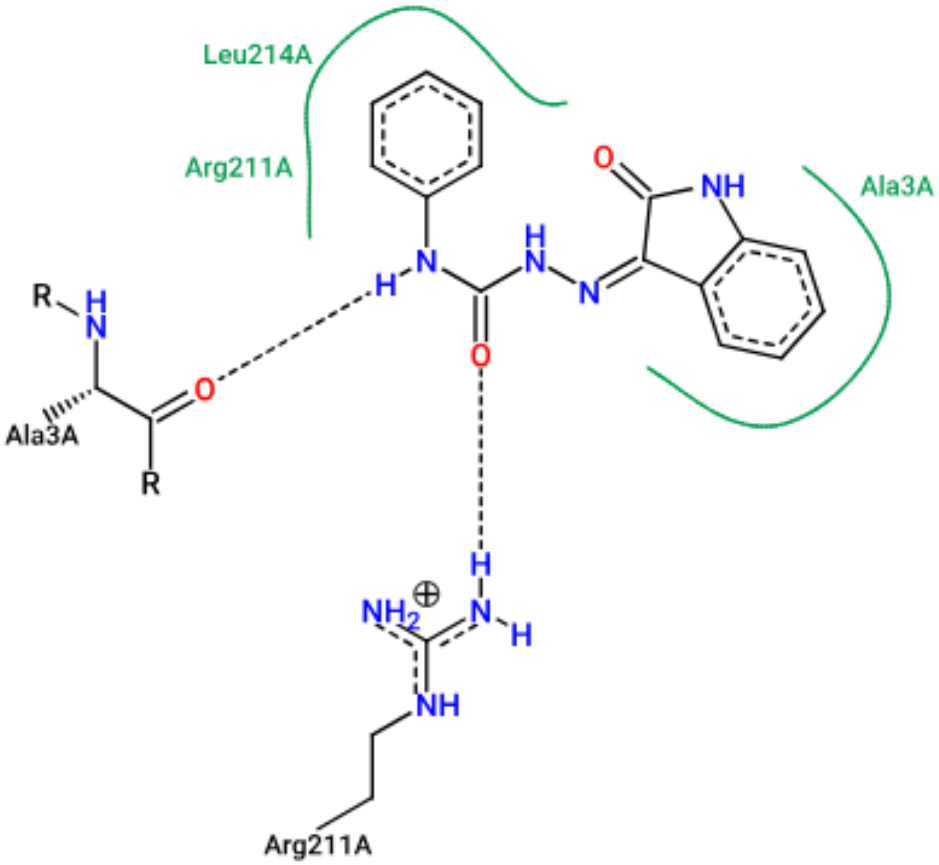


Figure 4. 2D interaction of the compound 4din binding pocket of protein.

CONCLUSIONS

The Swiss ADME study states that the chemicals in series (4a–e), aniline derivatives had favorable pharmacokinetic characteristics. They are BBB permeant, as demonstrated by the boiled-egg characteristic. The compounds displayed lead resemblance and complied with Lipinski's guideline without any infractions. On Auto Dock v.4.0, the molecular docking analysis of two targets revealed

good to moderate binding scores. When these compounds were tested against *E. coli* in vitro, they were similarly shown to have good to moderate activity. We can draw the conclusion from the aforementioned data that these compounds exhibited lead to similarity and strong antibacterial activity in both vitro and in silico investigations. As a result, there is a correlation between in-silico and in vivo research. In the search for novel antibiotics, these moieties can be investigated further.

Statements and Declaration

The author's relevant financial and non-financial interests do not have to be disclosed.

Funding

None.

Acknowledgments

The authors would like to thank the management of Shri. Pandit Baburao Chaughule College of Pharmacy, Bhiwandi, Rahanal, India, for providing research facilities.

REFERENCES

1. Bhamidipati M, Swathi K. Screening of 4-([1,3,4]oxadiazinoa6,5-b]indole-3-yl)aniline derivatives for anti-bacterial activity by in-silico and in-vitro method. Int J Pharm Sci Res. 2019;10(10):4799–4805.
2. Sahoo S, Mahendrakumar CB, Setty C. Synthesis, anti-inflammatory and antibacterial activities of some substituted isatin and isatin fused with 3 substituted 4-amino-5-mercapto-1,2,4-triazoles. Int J Chem Sci. 2015;13:613–624.
3. Swathi K, Manasa C, Sarangapani M. Development of novel indole molecules for the screening of anti-inflammatory activity. In: Vlamos P, editor. GeNeDis 2016. Adv Exp Med Biol. 2016;988.
4. Blessi P, Pogula M, Suresh S, Thirupathi K, Sammaiah G. Synthesis and characterization of new indole derivatives for analgesic activity. Int J Pharm Pharm Sci. 2012;4:231–233.
5. Sriram D, Yogeeswari P, Meena K. Synthesis, anti-HIV and antitubercular activities of isatin derivatives. Pharmazie. 2006;61:274–277.
6. Deweshri K, Menghani S, Khedekar P. Synthesis, characterization, antidepressant activity and docking studies of some novel indole bearing azetidinone derivatives. Indian J Pharm Educ Res. 2018;52:110–121.
7. Prakash CR, Raja S, Saravanan G. Synthesis, characterization and anticonvulsant activity of novel Schiff base of isatin derivatives. Int J Pharm Pharm Sci. 2010;2:177–181.
8. Patel AA. Antimicrobial Activity. In: Chapter IV. 2(1):69–71.
9. Erdmann OL. Untersuchungen über den Indigo. J Prakt Chem. 1841;24:1–18.
10. Ali KA, et al. Insight into the mechanism of steroidal and non-steroidal anti-inflammatory drugs. Sci Direct. 2023;61–94.
11. Ibrahim MM, Elsaman T, Al-Nour MY. Synthesis, anti-inflammatory activity, and in-silico study of novel diclofenac and isatin conjugates. Int J Med Chem. 2018;2018:1–11.
12. Alkhaldi AAM, Abdelgawad MA, Youssif BGM. Synthesis, antimicrobial evaluation and docking studies of new pyrazolone derivatives. Trop J Pharm Res. 2018;17(11):2235–2241.
13. Ezeokonkwo MA, Ogbonna ON, Okafor SN, Nwakwasi EG, Ibeanu FN, Okoro UC. Angular phenoxazine ethers as potent multi-microbial targets inhibitors: Design, synthesis, and molecular docking studies. Front Chem. 2017;5:1–15.
14. Shrivastava S, Ahuja D. Antimicrobial activity of Mannich bases of 1H-indole-2,3-dione: A review. World J Pharm Res. 2021;10(11):734–755.
15. Pandeya SN, Yadav V, Tyagi A, Nath G. Molecular modification of quinoline derivatives and their antibacterial activity. J Sci Res Pharm. 2012;1(3):16–18.
16. Saravana KN, Pradeep T, Jani G, Divya S, Vijaya KB. Design, synthesis, and antimicrobial screening of novel pyridyl-2-amidrazone incorporated isatin Mannich bases. J Adv Pharm Technol Res. 2012;3:57–61.

