International Journal of Education and Science Research Review
Volume-11, Issue-2 March-April-2024 E-ISSN 2348-6457
( Peer-Reviewed, Open Access, Fully Refereed International Journal) P-ISSN 2349-1817
www.ijesrr.org Impact Factor - 7.02 Email- editor@ijesrr.org

# Antimicrobial Evaluation and Structure-Activity Relationship of Novel Isatin Derivatives

- Neelima Tripathi, Research Scholar, Glocal School of Pharmacy, The Glocal University, Mirzapur Pole, Saharanpur (Uttar Pradesh) India
- Dr. Yash Pratap Singh Rana, Professor & Research Supervisor, Glocal School of Pharmacy, The Glocal University, Mirzapur Pole, Saharanpur (Uttar Pradesh) India

**Abstract**

The search for new antimicrobial agents is critical in the face of growing antimicrobial resistance (AMR). Isatin derivatives have emerged as potential candidates due to their diverse biological activities. In this study, we evaluated the antimicrobial activity of a series of novel isatin derivatives synthesized through structural modifications of the isatin core. The antimicrobial efficacy was assessed against both bacterial and fungal strains using the Minimum Inhibitory Concentration (MIC) method, and the results were compared with standard drugs such as ciprofloxacin and fluconazole. A structure-activity relationship (SAR) analysis was conducted to investigate the impact of various functional groups on the antimicrobial properties of the compounds. The results demonstrated that the synthesized derivatives possess broad-spectrum antimicrobial activity, with several compounds showing MIC values comparable to standard antimicrobial agents. This study highlights the potential of isatin derivatives as promising candidates for the development of new antimicrobial therapies.

## Introduction

The increasing incidence of antimicrobial resistance (AMR) poses a significant threat to public health worldwide. The discovery of new antimicrobial agents with novel mechanisms of action is essential to combat this issue. Isatin derivatives, known for their broad range of biological activities, have attracted attention in drug discovery efforts. The aim of this study is to investigate the antimicrobial activity of newly synthesized isatin derivatives and analyze the structure-activity relationship (SAR) to determine how various structural modifications influence antimicrobial potency.

Isatin's discovery was a major turning point in organic chemistry history. In addition, did the process of oxidising indigo yield it, but it also revealed new synthetic routes and shed light on its chemical structure. The oxidation process of indigo, a naturally existing dye that was utilised extensively in ancient civilisations, allowed scientists to understand the intricate nature of the compounds that might be made from it. The chemical and historical significance of indigo is carried on by its derivative, isatin. Its processes for synthesis have improved throughout time, increasing more streamlined and effective, enabling deeper investigation of its distinctive features and applicability.

With its dual carbonyl groups and indole backbone, isatin's molecular architecture enables it to take part in a variety of chemical processes. It is a flexible building block that is often employed in the manufacturing of organic compounds to produce more complex ones. Isatin's capacity to participate in both nucleophilic and electrophilic reactions makes it a useful precursor in chemical manufacturing. Utilising its reactivity, several medications, agrochemicals, and colours have been created. In addition, because of the compound's structural flexibility, scientists have been able to alter it in ways that enhanced its biological action, which has led to in the creation of a large number of derivatives that are biologically active as well.

Isatin and its derivatives have attracted a lot of interest in medicinal chemistry because of their possible therapeutic applications. Their biological actions encompass a wide range, encompassing antibacterial, antiviral, anticancer, anti-inflammatory in nature and anticonvulsant effects. Isatin interacts with an assortment of biological targets, which explains its broad spectrum of actions. The compound's medicinal potential is highlighted by its interactions with digestive enzymes, receptors, and other biomolecules; more study is being done to fully understand its pharmaceutical profile. To further progress drug discovery, a significant amount of research has been done on creating isatin derivatives with increased activity and selectivity targeting certain biological molecules.

Beyond its synthetic and pharmacological applications, isatin has a function in biological systems. It is an endogenous molecule that is involved in many different species' typical metabolic activities. Microorganisms, plants, and human tissues have all been shown that they contain it, demonstrating its broad biological relevance. The substance's capacity to modify biological activities has been related to a number of physiological processes, and it is believed to be involved in cellular signalling networks. Isatin's endogenous roles remain under investigation, with the goal of clarifying how it operates in both health and sickness. The biological activity of isatin has been investigated recently, and this has revealed the substance's potential as a medicinal agent. For instance, studies on the anticancer potential of isatin derivatives have shown encouraging findings. The compound's interaction with important enzymes involved in cell survival and proliferation has been connected to its capacity to limit the development of cancer cells. Furthermore, molecules based on isatin have demonstrated promise as antiviral medicines, especially in the management of viral diseases like hepatitis and HIV. These results highlight the compound's therapeutic adaptability and potential to treat a variety of illnesses.

Since its first discovery, there has been a substantial evolution in the synthesis of isatin and its derivatives. With the use of contemporary synthetic methods, a wide variety of isatin-based molecules with various structures and purposes may be produced. Not only have these synthetic developments made it easier to research isatin's chemical characteristics, but they have also increased the number of sectors in which it may be used.

Isatin is a useful precursor for the synthesis of heterocyclic compounds in the context of organic synthesis. It is an essential component in the synthesis of several significant compounds due to its capacity to produce stable intermediates during chemical processes. Research has been focused on creating effective synthetic pathways to isatin and its

derivatives, with the goal of increasing yields, decreasing reaction times, and using fewer dangerous chemicals. These developments have made it possible to use isatin in more industrial operations and scholarly study. Isatin has a wide range of uses, although its chemistry and biology are still not completely understood in certain cases. Research is still being done to find out more about how it works, especially in relation to different research.

With the aim of expanding treatment options for a range of disorders, the creation of novel isatin-based molecules with enhanced effectiveness and selectivity remains a prominent topic of focus. Isatin is a chemical that has significant applications in both biology and chemistry, to sum up. There is constant interest in this topic in scientific research because of its distinct chemical structure, lengthy history, and wide variety of uses. Isatin is a useful and versatile molecule that was first discovered as a derivative of indigo. It is now used in medical chemistry. Its significance in the scientific community is further cemented by the prospect of fresh discoveries and applications resulting from the ongoing investigation of its characteristics and functions.

Isatin's discovery's historical background is strongly related to the research of indigo, a naturally occurring dye that has been utilised for ages. An important discovery made in the 19th century gave early scientists important knowledge about the chemical makeup of natural dyes: the oxidation of indigo to form isatin. This finding opened up new avenues for investigating the characteristics and possible uses of isatin.

Isatin has been synthesised in the contemporary era using a range of techniques, each with a unique set of benefits for yield and efficiency. Isatin can now be produced on both small and big scales thanks to these synthetic processes, opening up a wide range of possibilities for it. Because of its adaptability, the compound is a preferred option for different things.

Isatin's reactivity is one of the main reasons it is used so widely. Isatin may take part in a wide range of chemical reactions because it has carbonyl groups at the second and third positions of the indole scaffold. Numerous compounds with increased biological activity have been created by using this reactivity. Isatin's structure may be changed to accomplish particular objectives, which makes it a useful tool in drug development and other fields of study.

**Review of Literature**

**"Bioactive Heterocycles: Synthesis and Antimicrobial Studies"**

*Author:* Mohammed K. Azhar

Bioactive Heterocycles: Synthesis and Antimicrobial Studies" by Mohammed K. Azhar offers a deep dive into the synthesis of bioactive heterocyclic compounds, emphasizing the significant role of isatin derivatives in antimicrobial research. The exploration of heterocyclic chemistry, which is fundamental in pharmaceutical and medicinal chemistry, has opened new avenues for drug development, especially in the fight against infectious diseases. Azhar's book is a valuable resource for anyone interested in understanding the relationship between chemical structure and biological activity, specifically regarding isatin derivatives and their effectiveness as antimicrobial agents.

Heterocyclic compounds are molecules that contain a ring structure composed of at least one atom other than carbon, such as nitrogen, oxygen, or sulfur. These compounds are prevalent in many biologically active molecules, including antibiotics, antivirals, and anticancer agents. The importance of heterocyclic chemistry lies in the ability of these ring structures to engage in a variety of chemical reactions and interactions with biological targets, making them versatile scaffolds in drug design. Azhar's work underscores the diverse applications of heterocycles, particularly isatin-based structures, in the development of antimicrobial agents.

Isatin, or 1H-indole-2,3-dione, is a well-known heterocyclic compound that has been widely studied due to its broad range of biological activities. Isatin derivatives, with their unique indole-based core, have shown potential as antimicrobial agents, making them a focal point in the synthesis of new therapeutic compounds. The chemical structure of isatin is highly amenable to modifications, allowing researchers to create a variety of derivatives with enhanced biological properties. Azhar's book delves into the methods used to synthesize these derivatives, offering a detailed analysis of the synthetic routes and chemical reactions involved.

One of the key features of isatin derivatives is their ability to inhibit the growth of bacteria and fungi. Bacterial resistance to existing antibiotics has become a global health crisis, and the need for new antimicrobial agents is more urgent than ever. Azhar discusses the mechanisms by which isatin derivatives exert their antimicrobial effects, including the disruption of bacterial cell walls, inhibition of enzyme activity, and interference with DNA replication. By targeting multiple pathways within the microbial cell, isatin derivatives offer a promising solution to the problem of antibiotic resistance.

In addition to their antibacterial properties, isatin derivatives have also shown activity against fungi, which are responsible for a range of serious infections, particularly in immunocompromised individuals. Fungal infections, such as those caused by Candida and Aspergillus species, are becoming increasingly difficult to treat due to the emergence of resistance to antifungal drugs. Azhar's book highlights the potential of isatin derivatives as antifungal agents, with specific derivatives demonstrating the ability to inhibit the growth of pathogenic fungi by interfering with cell membrane integrity and enzyme activity.

The synthesis of bioactive isatin derivatives involves various chemical transformations and reaction mechanisms. Azhar provides a detailed description of the methods used to modify the isatin core, such as nucleophilic substitution, condensation reactions, and cyclization processes. These reactions allow for the introduction of different functional groups into the isatin molecule, resulting in a wide range of derivatives with diverse biological activities. For example, the introduction of halogens or alkyl groups into the isatin structure can enhance the compound's lipophilicity, improving its ability to penetrate microbial cell membranes and increasing its antimicrobial potency.

Azhar also explores the concept of structure-activity relationships (SAR) in the design of antimicrobial agents. SAR studies are crucial for understanding how the chemical structure of a compound influences its biological activity. By analyzing the effects of different substituents on the isatin core, researchers can identify which modifications lead to enhanced antimicrobial activity. Azhar's book includes numerous examples of isatin derivatives with varying degrees of antimicrobial activity, providing insights into the structural features that contribute to their effectiveness.

One of the challenges in developing new antimicrobial agents is ensuring that they are selective for microbial cells while minimizing toxicity to human cells. Azhar addresses this issue by discussing the pharmacokinetics and pharmacodynamics of isatin derivatives. He explains that while many isatin derivatives show potent activity against bacteria and fungi in vitro, their effectiveness in vivo depends on factors such as solubility, stability, and metabolic clearance. By optimizing the pharmacokinetic properties of isatin derivatives, researchers can improve their efficacy and safety as therapeutic agents.

In recent years, there has been a growing interest in the use of green chemistry principles in drug synthesis. Green chemistry aims to reduce the environmental impact of chemical processes by minimizing the use of hazardous reagents and reducing waste. Azhar's book highlights the application of green chemistry techniques in the synthesis of bioactive isatin derivatives. For example, the use of microwave-assisted synthesis has been shown to reduce reaction times and improve yields, making the synthesis of isatin derivatives more efficient and environmentally friendly. By incorporating green chemistry principles into the synthesis of antimicrobial agents, researchers can develop more sustainable methods for drug production.

Azhar also emphasizes the importance of drug repurposing in the development of new antimicrobial agents. Drug repurposing involves identifying new therapeutic uses for existing drugs, which can significantly reduce the time and cost associated with drug development. Isatin derivatives, originally studied for their anticancer and antiviral properties, have been repurposed as antimicrobial agents due to their ability to inhibit the growth of bacteria and fungi. By repurposing isatin derivatives, researchers can leverage existing safety and pharmacokinetic data, accelerating the process of bringing new antimicrobial therapies to market.

The book also discusses the challenges associated with antimicrobial resistance and the strategies used to overcome this issue. Antimicrobial resistance occurs when microorganisms develop mechanisms to evade the effects of drugs, rendering them ineffective. Azhar explains that one of the key strategies to combat resistance is the development of antimicrobial agents that target multiple pathways within the microbial cell. Isatin derivatives, with their ability to inhibit various enzymes and disrupt cell membranes, are particularly well-suited to this approach. By targeting multiple pathways, these compounds reduce the likelihood of resistance development, making them effective against resistant strains of bacteria and fungi.

Azhar's book also covers the role of computational chemistry in the design of antimicrobial agents. Computational tools, such as molecular docking and virtual screening, allow researchers to predict how a compound will interact with its target before it is synthesized. This in silico approach can significantly reduce the time and cost of drug development, as it allows researchers to prioritize the most promising compounds for further testing. Azhar provides examples of how these techniques have been applied to the design of isatin derivatives, helping to identify key structural features that contribute to their antimicrobial activity.

Another important aspect covered in the book is the role of molecular hybridization in the development of new antimicrobial agents. Molecular hybridization involves the combination of two or more pharmacophores into a single molecule to create hybrid compounds with enhanced biological activity. In the case of isatin derivatives, researchers have successfully combined the isatin nucleus with other bioactive moieties, such as quinolones or thiazoles, to create hybrid molecules with potent antimicrobial properties. These hybrid compounds often exhibit a synergistic effect, where the combination of two pharmacophores results in greater antimicrobial activity than either compound would achieve alone.

The book also explores the use of isatin derivatives in combination therapy, where multiple antimicrobial agents are used together to enhance their effectiveness. Combination therapy is particularly useful in the treatment of infections caused by multidrug-resistant bacteria, as it allows for the simultaneous targeting of different microbial pathways. Azhar discusses how isatin derivatives can be used in combination with other antimicrobial agents, such as β-lactams or aminoglycosides, to achieve synergistic effects and overcome resistance.

In conclusion, "Bioactive Heterocycles: Synthesis and Antimicrobial Studies" by Mohammed K. Azhar provides a comprehensive and insightful overview of the role of isatin derivatives in antimicrobial drug development. Through a detailed examination of their synthesis, structure-activity relationships, and mechanisms of action, Azhar highlights the potential of these compounds as valuable tools in the fight against resistant infections. With a focus on green chemistry, computational methods, and molecular hybridization, the book offers a forward-looking perspective on the future of antimicrobial drug discovery, emphasizing the importance of innovation and collaboration in addressing the global challenge of antimicrobial resistance.

International Journal of Education and Science Research Review
Volume-11, Issue-2 March-April-2024 E-ISSN 2348-6457 P-ISSN 2349-1817
www.ijesrr.org Email- editor@ijesrr.org

**"Isatin: A Versatile Molecule in Drug Design"**

*Author:* Ayesha Salim

Isatin, also known as 1H-indole-2,3-dione, has garnered significant attention in drug discovery and design due to its unique chemical structure and broad spectrum of biological activities. It serves as a versatile building block in medicinal chemistry, and its ability to interact with various biological targets makes it a key component in the design of numerous therapeutic agents. Isatin's diverse bioactivity has been explored extensively, particularly for its antimicrobial, antiviral, and anticancer properties, making it a molecule of interest in the pharmaceutical industry.

The structural framework of isatin is characterized by an indole ring fused with a keto group at the second and third positions, which contributes to its high reactivity and ability to form derivatives with potent biological activities. Researchers have synthesized a vast array of isatin derivatives by modifying its core structure, yielding molecules with enhanced or novel pharmacological profiles. These derivatives have shown efficacy in treating infections, cancers, and viral diseases, among other conditions.

Isatin's antimicrobial activity has been well-documented. Bacterial infections pose a serious threat to public health, particularly with the rise of multidrug-resistant strains. The search for new antibiotics has led scientists to explore isatin derivatives as potential antibacterial agents. Isatin's mechanism of action against bacteria typically involves the inhibition of key enzymes essential for bacterial survival and replication. Various studies have reported the successful synthesis of isatin derivatives that exhibit strong antibacterial activity against both Gram-positive and Gram-negative bacteria. These compounds often target bacterial enzymes such as DNA gyrase and topoisomerase IV, which are crucial for bacterial DNA replication and cell division.

In addition to its antibacterial properties, isatin has also demonstrated significant antifungal activity. Fungal infections, especially in immunocompromised individuals, can lead to severe and sometimes life-threatening conditions. Isatin derivatives have shown potential in inhibiting the growth of various pathogenic fungi. The antifungal activity of isatin is often attributed to its ability to interfere with the synthesis of fungal cell walls or membranes, thereby disrupting their structural integrity. Researchers continue to explore new isatin-based antifungal agents to combat resistant fungal strains and improve treatment outcomes for patients with fungal infections.

Another area where isatin has shown promise is in antiviral drug development. Viral infections, ranging from the common cold to more severe diseases like HIV and hepatitis, have long been a challenge for the medical community. The ability of viruses to rapidly mutate and develop resistance to existing treatments necessitates the development of novel antiviral compounds. Isatin and its derivatives have been investigated for their antiviral properties, and many have demonstrated potent activity against a variety of viruses. For example, isatin derivatives have shown inhibitory effects against the replication of human immunodeficiency virus (HIV), hepatitis C virus (HCV), and influenza viruses. These

compounds often work by targeting viral enzymes or proteins essential for viral replication, such as reverse transcriptase in the case of HIV or the NS5B polymerase in HCV.

The anticancer potential of isatin has also been a focal point of research. Cancer remains one of the leading causes of death worldwide, and the search for effective, targeted treatments continues to be a top priority. Isatin derivatives have shown remarkable activity against various cancer cell lines, including breast, lung, colon, and prostate cancers. The anticancer activity of isatin is primarily due to its ability to induce apoptosis (programmed cell death) in cancer cells, inhibit angiogenesis (the formation of new blood vessels that feed tumors), and interfere with key signaling pathways involved in cancer cell proliferation. Several isatin-based compounds have been identified as potent inhibitors of kinases, proteases, and other enzymes that play critical roles in tumor growth and survival.

One notable isatin derivative, sunitinib, is an FDA-approved anticancer drug that has been successfully used in the treatment of renal cell carcinoma and gastrointestinal stromal tumors. Sunitinib works by inhibiting multiple receptor tyrosine kinases (RTKs), which are involved in tumor growth and angiogenesis. This highlights the therapeutic potential of isatin-based molecules in cancer treatment, especially in cases where conventional therapies may fail or become ineffective due to resistance.

Beyond its antimicrobial, antiviral, and anticancer activities, isatin and its derivatives have been explored for other pharmacological properties as well. For instance, isatin has shown promise in neuroprotection, where it may help protect neurons from damage caused by oxidative stress or neurodegenerative diseases such as Alzheimer's and Parkinson's. The antioxidant activity of isatin derivatives has been attributed to their ability to scavenge free radicals and reduce oxidative damage in neuronal cells. Furthermore, isatin-based compounds have been investigated for their anti-inflammatory and analgesic properties, making them potential candidates for treating inflammatory diseases and pain management.

The versatility of isatin as a scaffold in drug design can be attributed to its ability to undergo various chemical modifications, allowing for the synthesis of derivatives with different biological activities. The keto group at position 2 and 3 of the indole ring is highly reactive and can participate in numerous reactions, including condensation, alkylation, and acylation. This enables medicinal chemists to introduce a wide range of functional groups into the isatin structure, which can enhance its bioactivity, improve its pharmacokinetic properties, or reduce toxicity. As a result, isatin derivatives have become an essential part of drug development programs targeting a variety of diseases.

The success of isatin-based drugs in preclinical and clinical studies has further spurred interest in this molecule. Researchers are continuously working to identify new isatin derivatives with improved efficacy and safety profiles. The design of multi-targeted isatin derivatives is an emerging trend, where a single compound is engineered to interact with multiple biological targets, thereby enhancing its therapeutic potential and minimizing the risk of resistance. For instance,

some isatin derivatives have been designed to exhibit both antibacterial and anticancer activities, offering a dual therapeutic benefit in cases where infections and cancer coexist.

In drug design, the ability to optimize the pharmacokinetic and pharmacodynamic properties of a compound is crucial for its success as a therapeutic agent. Isatin derivatives have shown favorable pharmacokinetic profiles, with good absorption, distribution, metabolism, and excretion (ADME) properties. Researchers are also exploring the use of prodrug strategies to enhance the bioavailability of isatin derivatives. A prodrug is an inactive form of a drug that is metabolized in the body to release the active compound. This approach can improve the solubility, stability, and targeted delivery of isatin-based drugs, thereby increasing their therapeutic efficacy.

Despite the promising pharmacological activities of isatin and its derivatives, there are still challenges that need to be addressed in drug design and development. One of the key challenges is the potential for off-target effects, where isatin-based compounds may interact with unintended biological targets, leading to adverse side effects. Toxicity is another concern, as some isatin derivatives have been found to exhibit cytotoxicity in healthy cells at higher concentrations. To overcome these challenges, researchers are employing structure-activity relationship (SAR) studies to optimize the chemical structure of isatin derivatives, aiming to maximize their therapeutic activity while minimizing toxicity.

In conclusion, isatin is a versatile molecule with immense potential in drug design. Its unique chemical structure and ability to form derivatives with diverse biological activities make it a valuable scaffold in the development of new therapeutic agents. The antimicrobial, antiviral, and anticancer properties of isatin derivatives have been extensively studied, and several compounds have shown promising results in preclinical and clinical trials. The ongoing research into isatin-based drug design holds great promise for the development of novel treatments for a wide range of diseases, including infectious diseases, cancer, and neurodegenerative disorders. As medicinal chemistry continues to evolve, isatin and its derivatives will likely play a pivotal role in shaping the future of pharmaceutical innovation.

## Materials and Methods

### Synthesis of Novel Isatin Derivatives

Novel isatin derivatives were synthesized by introducing different substituents at various positions on the isatin core. The reactions were carried out using standard organic synthesis techniques, and the products were purified and characterized using Nuclear Magnetic Resonance (NMR) and Infrared Spectroscopy (IR) to confirm their structure and purity.

### Antimicrobial Activity Assay

The antimicrobial activity of the synthesized compounds was tested using the Minimum Inhibitory Concentration (MIC) method. The bacterial strains used included *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and

*Bacillus subtilis*. Fungal strains such as *Candida albicans* and *Aspergillus niger* were also tested. The standard antimicrobial agents ciprofloxacin (for antibacterial activity) and fluconazole (for antifungal activity) were used as controls for comparison.

**Results and Discussion**

The synthesized isatin derivatives exhibited a broad spectrum of antimicrobial activity. Several compounds showed potent activity against both Gram-positive and Gram-negative bacteria, as well as fungal strains. Derivatives with halogen substituents, particularly chlorine and fluorine, displayed enhanced antibacterial activity, especially against Gram-positive bacteria like *S. aureus*. In contrast, derivatives with hydroxyl and methoxy groups demonstrated higher efficacy against fungal strains such as *C. albicans*.

The structure-activity relationship (SAR) analysis revealed that the nature and position of the functional groups significantly influenced the antimicrobial properties of the compounds. Electron-withdrawing groups, such as halogens, increased antibacterial potency, while electron-donating groups enhanced antifungal activity.

**Conclusion**

This study demonstrates the successful synthesis of novel isatin derivatives with potent antimicrobial activity. The structure-activity relationship (SAR) analysis provided valuable insights into how specific functional groups influence antimicrobial efficacy. These findings suggest that isatin derivatives have great potential as antimicrobial agents, particularly in addressing the growing issue of antimicrobial resistance. Future work should focus on optimizing the structure of these compounds and exploring their potential for use in combination therapies.

The docking results also highlighted the importance of water molecules in mediating some of the interactions between carbazide derivatives and DNA gyrase. Water molecules are often found within protein binding sites, where they can bridge interactions between the ligand and the protein. In the case of the carbazide derivatives, some of the docking poses revealed that water molecules acted as mediators of hydrogen bonds between the ligand and the DNA gyrase binding site. These water-mediated hydrogen bonds contributed to the stability of the ligand-protein complex, further enhancing the binding affinity of the carbazide derivatives. The involvement of water molecules in the binding process underscores the complexity of the interactions between the carbazide derivatives and DNA gyrase, as the binding affinity is not solely determined by direct interactions with the protein but also by the surrounding environment, including solvent molecules.

Another key aspect of the docking analysis was the clustering and ranking of docking poses based on their FullFitness scores. FullFitness scores provide a measure of the overall binding energy of a ligand within the protein binding site, with lower scores indicating more favorable interactions and higher binding affinities. The clustering of docking results allowed for the identification of groups of similar binding poses, each of which represents a potential binding mode of

the carbazide derivatives to DNA gyrase. These clusters provide valuable information about the diversity of possible binding modes and help identify the most energetically favorable poses.

Clusters with lower average FullFitness scores were considered more promising, as they indicated more stable and energetically favorable binding poses. By analyzing the clustering results, it was possible to identify the carbazide derivatives that exhibited the most stable binding to DNA gyrase, as well as the specific binding modes that contributed to this stability. The ranking of these clusters based on their FullFitness scores provided a clear hierarchy of the most promising carbazide derivatives for further experimental validation. The derivatives that were ranked highest in this analysis are likely to have the greatest potential as inhibitors of DNA gyrase, making them strong candidates for further development as antibacterial agents.

The clustering and ranking of docking results also provided insight into the structural features of the carbazide derivatives that contributed to their binding affinity. By comparing the binding poses within different clusters, it was possible to identify common structural motifs or functional groups that were associated with high binding affinity. For example, carbazide derivatives that formed strong hydrogen bonds with key residues in the DNA gyrase binding site were often found in the top-ranked clusters. Similarly, derivatives with bulky hydrophobic groups that interacted with non-polar regions of the binding site were also frequently found in clusters with low FullFitness scores.

This analysis of clustering and ranking also highlighted the diversity of potential binding modes for the carbazide derivatives. While some derivatives bound to the DNA gyrase binding site in a single, well-defined orientation, others exhibited multiple binding modes, each with similar binding affinities. This diversity of binding modes is advantageous in drug development, as it provides flexibility in optimizing the structure of the carbazide derivatives to improve their binding affinity and specificity for DNA gyrase. By identifying the most favorable binding modes, it is possible to design new derivatives that enhance these interactions and improve the overall efficacy of the compounds as inhibitors of DNA gyrase.

**References**

- Ahmed, M., & Patel, J. A. (2018). Design and synthesis of isatin derivatives with antimicrobial activity. *Journal of Medicinal Chemistry, 61*(2), 891-903. doi:10.1021/acs.jmedchem.7b00843
- Ali, M. S., Khedher, N. B., & Ghorbel, A. (2019). Novel isatin derivatives as potential antimicrobial agents: Synthesis, characterization, and biological evaluation. *European Journal of Medicinal Chemistry, 175*, 1-15. doi:10.1016/j.ejmech.2019.04.037

- Anwar, J., Zafar, A., & Hussain, H. (2017). Synthesis and biological evaluation of isatin derivatives as antimicrobial agents. *Bioorganic & Medicinal Chemistry Letters, 27*(18), 4110-4115. doi:10.1016/j.bmcl.2017.08.030
- Barik, K., Ghosh, N., & Ghosh, P. (2020). Antimicrobial activity of isatin and its derivatives: A review. *Antibiotics, 9*(3), 112. doi:10.3390/antibiotics9030112
- Bhat, S. R., & Khan, M. N. (2021). Synthesis, characterization, and antimicrobial activity of novel isatin derivatives. *Journal of Enzyme Inhibition and Medicinal Chemistry, 36*(1), 123-135. doi:10.1080/14756366.2021.1860819
- Choudhury, M., & Singh, M. (2018). Isatin derivatives: Synthesis, antimicrobial activity, and structure-activity relationship studies. *Pharmaceutical Research, 35*(9), 165-178. doi:10.1007/s11095-018-2440-5
- Das, S., Sharma, S., & Prakash, M. (2019). Design, synthesis, and biological evaluation of new isatin derivatives. *Journal of Chemical Research, 43*(5), 346-354. doi:10.1177/1747519818823232
- Das, S. K., & Choudhury, A. (2017). Synthesis and antimicrobial activity of isatin-based compounds. *Molecules, 22*(11), 1993. doi:10.3390/molecules22111993
- Faria, D. A., & Alvarenga, L. S. (2020). Novel isatin derivatives: Synthesis, characterization, and biological evaluation. *Journal of Pharmaceutical and Biomedical Analysis, 186*, 113301. doi: 10.1016/j.jpba.2020.113301
- Gupta, R., & Kumar, S. (2019). Isatin derivatives as potential antimicrobial agents: Design, synthesis, and evaluation. *ChemBioChem, 20*(2), 231-245. doi:10.1002/cbic.201800496
- Hameed, M. S., & Ahmed, A. (2018). Synthesis and antimicrobial evaluation of isatin derivatives. *Journal of Chemical Research, 42*(4), 235-248. doi:10.1177/1747519818778525
- Hasan, M., & Ali, N. (2019). Synthesis and antimicrobial activity of novel isatin derivatives. *RSC Advances, 9*(28), 15941-15949. doi:10.1039/C9RA01321E
- Jaiswal, A., & Kumar, P. (2017). Synthesis and antimicrobial evaluation of isatin derivatives. *European Journal of Medicinal Chemistry, 130*, 170-182. doi: 10.1016/j.ejmech.2017.01.009
- Jang, M. K., & Lee, K. H. (2020). Design and synthesis of isatin derivatives with potent antimicrobial activity. *Bioorganic & Medicinal Chemistry, 28*(22), 115214. doi: 10.1016/j.bmc.2020.115214