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Synthesis of zinc-naphthol azo dye complex

Dhanashree Desale[1], Dr. Rahul Lokhande[2], Dr. Vijaykumar Wakale[3], Dr. Kuldeep Ramteke[4]
[1,2,3]Samarth institute of pharmacy Belhe, Tal -Junnar, Dist- Pune

Abstract - Antimicrobial resistance (AMR) poses a growing threat to global health, necessitating the exploration of novel therapeutic agents with enhanced efficacy and reduced toxicity. This study investigates the synthesis, characterization, and antimicrobial evaluation of an azo dye and its corresponding zinc metal complex. Azo dyes, recognized for their structural versatility and vivid coloration, possess promising biological properties owing to the presence of the azo (- N=N-) chromophore. In this work, the azo dye was synthesized via diazotization of sulphanilic acid followed by coupling with β-naphthol, yielding a reddish-brown crystalline compound. The resulting ligand was then complexed with Zn (II) ions under alkaline conditions to obtain a stable azo-metal complex. Comprehensive physicochemical and spectroscopic analyses, including melting point determination, TLC, UV-Visible, FTIR, and NMR spectroscopy, confirmed the successful synthesis and structural attributes of the dye and its metal complex. Notably, UV-Vis spectra revealed a bathochromic shift upon metal coordination, indicative of altered electronic transitions. FTIR analysis showed characteristic bands for the azo, hydroxyl, and metal-ligand interactions, affirming complex formation. The synthesized compound displayed desirable solubility, moderate stability, and a pH around 8.4, aligning with conditions suitable for biological applications. Preliminary antimicrobial testing demonstrated that the azo-metal complex exhibited superior antibacterial and antifungal activities compared to the free ligand, highlighting the enhanced bioactivity imparted by metal coordination. This can be attributed to improved cellular uptake, metal-induced oxidative stress, and interactions with microbial enzymes and DNA. These findings align with literature reports supporting the potential of metal complexes in overcoming microbial resistance. This research underscores the significance of structure–activity relationships in the development of effective antimicrobial agents and positions azo-metal complexes as promising candidates for further pharmacological exploration. Future work may involve evaluating cytotoxicity, mechanism of action studies, and incorporation into nanocarrier systems for targeted delivery. The study contributes to the ongoing efforts in designing eco-friendly and potent antimicrobial compounds to combat resistant pathogens.

Keywords: Azo Dye, Azo-Zinc Complex, Coupling, Complexation, anti-microbial, UV-vis spectroscopy, TLC, FT-IR, 1H NMR, A. Niger, S. aureus, E. coli, reddish brown dye.

I.INTRODUCTION

Antimicrobial resistance is a global public health threat, exacerbated by irrational use and inadequate prevention and control. Pathogens evolve through genetic changes, acquiring new resistance mechanisms that render existing antimicrobials ineffective. This vulnerability is heightened by novel resistance mechanisms, multi-drug resistance, and horizontal transfer of resistance genes between diverse bacterial species. Effective infection management relies on both host immunity and antimicrobial treatment, but pathogens have developed strategies to evade immune responses. The efficacy of antimicrobial agents can vary depending on the infection site, concurrent infections, and interactions with the micro biome. Antimicrobial resistance is a significant challenge in medicine, and azo dyes and their metal complexes have emerged as promising candidates due to their antimicrobial properties. This research explores the synthesis and application of these compounds for antimicrobial therapies. The chemical structure of azo derivatives allows for modifications that enhance their antimicrobial efficacy, making them promising candidates for pharmaceutical development against microbial infections. The study provides a comprehensive understanding of antimicrobial action mechanisms, enzyme inhibition, membrane disruption, and reactive oxygen species generation. Metal ions play a significant role in enhancing structural stability, reactivity, and targeting microbial enzymes effectively. Novel azo-metal complexes show significant antifungal and antibacterial activities. The research emphasizes the importance of structure-activity relationships and their potential sensing ability to detect and identify microorganisms in infection management. Azo dyes have antimicrobial properties through their effectiveness depends on factors like ligand type, metal ion geometry, lipophilicity, and pharmacokinetics.

1) Azo dye : The history of dyeing can be divided into two great periods, the "pre-aniline," extending to 1856and the "post-aniline" period. First, azo dyes were first introduced by Griess in 1865. In the beginning, the dye industry obtained its supply of dyes by taking advantage of natural resources such as plants and insects. The term "Azo" finds its etymological roots in the French word "azote," historically associated with nitrogen. Azo compounds are the most extensively used and structurally diverse category of synthetic dyes, finding applications across textiles, pharmaceuticals, and food industries. In 1991, the U.S. Food and Drug Administration (FDA) approved using over 3,000 tonnes of azo dyes in food, pharmaceuticals, and cosmetics. However, due to the presence of cytotoxic aromatic amines in their synthesis process, the European Union and the state of California have enacted regulations restricting the use of azo dyes.

2) Chemistry of Azo dye : Azo compounds represent a significant class of organic compounds. Azo compounds are chemical substances that contain a special group (-N=N-) called an azo group, which has shown promising industrial and biological properties and good results in killing bacteria and other microbes. The azo compounds exhibit distinct colour because they have chromophore group (- N=N-) attach to aromatic or heterocycle systems. They have lot of brightly coloured, particularly the oranges, yellows, and reds. Azo dyes are the most significant class of synthetic colorants and the largest class of dye in use. The main viable techniques for synthesizing azo compounds -are Diazotization and azo coupling reaction. Most aromatic azo compounds are prepared by the reaction of a diazonium salt with an organic substance that contains easily replaced hydrogen atoms. They are significant and frequently used as colouring agents in the textile and leather industries. Also, Azo dyes have been extensively studied in various applications, including biological studies, coordination polymers, textile industry, solvent extraction, and spectrophotometric determination. It has several additional uses such as colouring fibre, printing systems, photo electronics, Polymer additives, and storage, and providing resistance to solvents, water, light, and weather.

3) Azo-Metal complex : Azo-metal complexes have garnered increasing attention in recent years due to their remarkable antimicrobial potential and structural versatility. These compounds, formed through the coordination of azo dyes with transition metals such as copper (Cu), cobalt (Co), zinc (Zn), iron (Fe), silver (Ag), and nickel (Ni), exhibit significantly improved biological activity compared to their non-metalized counterparts. The azo group (–N=N–), which is a defining feature of these compounds, provides a site for metal coordination, resulting in stable complexes that often display planar, tetrahedral, or octahedral geometries. The ability of the azo group to conjugate with various aromatic systems allows for structural modification, enabling finetuning of both chemical properties and biological efficacy. Upon complexation, the metal ion contributes to the biological activity by enhancing the reactivity of the ligand, facilitating better cellular uptake, and possibly targeting essential microbial enzymes and nucleic acids. In parallel, the ligand stabilizes the metal ion, improving its bioavailability and modulating its release in a biological environment. Several studies have highlighted the superior antimicrobial activity of these complexes. For instance, Khanum et al. (2023) reviewed synthetic methods for azo dyes and their metal complexes, emphasizing their growing application in biomedical sciences. The studies shows that metalation significantly amplifies antimicrobial potential by introducing new pathways for microbial inhibition, such as oxidative stress induction and membrane disruption. Pervaiz et al. (2021) further demonstrated that Schiff base ligands containing azo and azomethine moieties exhibited enhanced antibacterial and antifungal activity upon metal coordination. Their subsequent study in 2022 emphasized the importance of structural geometry, illustrating how spatial configuration directly impacts microbial target binding and overall bioactivity. Complexes exhibiting octahedral or square planar geometry, in particular, were found to be more effective due to their optimal spatial arrangement for interacting with biological molecules. In another

notable example, Aravindhan et al. (2014) explored the antimicrobial effects of embelin-azo metal complexes and found that these compounds exhibited a marked increase in efficacy compared to the free azo ligands. Embelin, a naturally bioactive compound, served as a powerful scaffold for azo conjugation, and its subsequent metalation yielded derivatives with potent antimicrobial properties. These findings underscore the synergistic effects achieved through the integration of bioactive ligands and metal ions, which collectively enhance stability, reactivity, and target specificity.

4) Chemistry of Azo-Metal complex: The coordination chemistry of azo dyes with metals typically involves stoichiometric ratios of 1:1 or 1:2 between the metal ion and the ligand, with resulting complexes adopting tetrahedral or octahedral configurations depending on the metal and ligand structure. These geometries play a crucial role in determining solubility, bioavailability, and microbial interaction. The mordant process, well-known in textile dyeing, serves as a valuable model for forming such complexes. In biological systems, this coordination enhances not only the physicochemical properties of the compound but also its pharmacodynamic behaviour. The redox properties of the metal centre, for example, can generate reactive oxygen species that contribute to microbial cell death, while the azo ligand ensures targeted delivery and controlled release. Despite these advantages, some limitations persist. Azo-metal complexes can exhibit poor light fastness, meaning they degrade or lose colour upon exposure to light, and their appearance is often less vibrant than the uncoordinated dyes. This could restrict certain industrial applications, particularly in textiles or cosmetics. Nevertheless, ongoing research is actively addressing these issues, exploring safer metals and developing green synthesis techniques that minimize environmental and health risks. Recent advancements in spectroscopic techniques, including UV-Vis, FTIR, NMR, and X-ray diffraction, have greatly facilitated the characterization and understanding of these complexes. Additionally, computational chemistry and molecular docking studies are being increasingly employed to elucidate the structure–activity relationships (SAR) that underpin their biological functions. Such insights are crucial for designing targeted antimicrobial agents with enhanced efficacy and reduced resistance development. Furthermore, there is a growing interest in incorporating these complexes into nanocarrier systems such as liposomes or polymeric nanoparticles to improve their solubility, stability, and site-specific delivery. Overall, the continued exploration of azo-metal complexes represents a promising direction in antimicrobial research. By optimizing synthetic strategies, manipulating structural features, and understanding the underlying mechanisms of action, these compounds have the potential to be developed into effective antimicrobial agents capable of addressing the growing threat of resistant infections.

5) Anti-microbial Activity: Azo dyes exhibit antimicrobial activity through various mechanisms, including DNA intercalation, membrane disruption, enzyme inhibition, and reactive oxygen species (ROS) generation. However, the effective- ness of these compounds can vary based on factors such as the type of ligand, metal ion and the geometry of metal complexes, the lipophilicity and pharmacokinetic factors. a) DNA intercalation: The planar structures enable them to intercalate into microbial DNA, causing structural distortions that hinder replication and transcription. This intercalation leads to partial unwinding of the double helix and local distortions, affecting hydrogen bonding and base pair stacking interactions. b) Membrane disruption: The amphipathic nature of azo dyes allows them to interact with the lipid bilayer, causing physical disruption and increased permeability dyes can also bind to the active sites of microbial enzymes, inhibiting their catalytic activity and disrupting critical metabolic pathways. c) Oxidative stress: Azo dyes can undergo redox cycling, generating ROS like superoxide anions, hydrogen peroxide, and hydroxyl radicals, which damage cellular components such as lipids, proteins, and DNA. The oxidative stress caused by these ROS results in membrane damage, enzyme inactivation, and DNA strand breaks, ultimately leading to cell death.

6) Antifungal mechanism of Azo compounds : From the literature review it has been evidenced that novel azo dyes have been effectively exhibited inhibitory potential against most of the fungal infection in human. The antifungal activity of azo com- pounds is primarily attributed to their ability to interfere with critical biological processes to impair the growth and

reproduction of the fungus through induction of oxidative stress, disruption cell membrane and damaging cellular components such as DNA, proteins, and lipids. Additionally, azo compounds can penetrate fungal cells and bind to intracellular targets, such enzymes and proteins, which are vital for the survival and proliferation of the fungus. By inhibiting these enzymes, azo compounds can obstruct essential metabolic pathways, including those involved in the synthesis of ergosterol, a crucial component of the fungal cell membrane. The disruption of ergosterol synthesis compromises the cell membrane's structure and function, making the fungal cells more susceptible to environmental stress and osmotic imbalance. Additionally, azo compounds may interfere with the fungal cell cycle, leading to cell cycle arrest and apoptosis (programmed cell death). The ability of azo compounds to act on multiple targets and disrupt various cellular processes makes them potent antifungal agents with a broad spectrum of activity. The synthetic procedure of azo dyes significantly influences their antibacterial activity due to variations in structural attributes and the presence of specific functional groups. The diazotization-coupling pro- cess used in the synthesis can be tailored to introduce different substituents on the aromatic rings, which can enhance or diminish the dye's ability to interact with microbial cells. Additionally, the choice of intermediates and reaction conditions, such as pH, temperature, and catalysts, can influence the dye's final structure, including its charge distribution, hydrophobicity, and potential to form stable complexes with microbial enzymes or DNA. Thus, precise control over the synthetic procedure allows for the optimization of azo dyes' antibacterial properties, tailoring them for specific applications and microbial targets.

The unusual chemical characteristics of azo dye have demonstrated significant promise as a possible candidate for antifungal action. The capacity to impede the growth of fungi and effectively address diverse fungal infections renders it a prospective choice for the advancement of novel antifungal medications. Furthermore, empirical research has provided evidence indicating that azo dye displays minimal toxicity toward human cells, hence emphasizing its promise as a secure and efficacious antifungal drug. The evaluation of antifungal activity will involve the utilization of established drugs such as Fluconazole, Amphotericin-B, and Neomycin against different fungal strains. The fungus will be cultivated in an appropriate growth medium and there- after subjected to varying quantities of the established pharmaceutical compounds. The assessment of antifungal activity will involve the measurement of the inhibition zone or the determination of the mini- mum inhibitory concentration, of each medicine against the fungus. Fungal infections have the potential to afflict individuals indiscriminately, manifesting on various anatomical regions.

In this study, an azo dye was synthesized through the coupling of sulphanilic acid and βnaphthol. Subsequently, azo-metal complexes were prepared by coordinating the synthesized dye with selected metal ions using simple ligand-metal interactions. The chemical structures of both the azo dye and its corresponding metal complexes were investigated to understand their coordination behaviour and electronic properties. Particular attention was given to the shift in absorption maxima observed from the free ligands to their metal complexes, highlighting the influence of metal coordination on the electronic transitions within the dye structure.

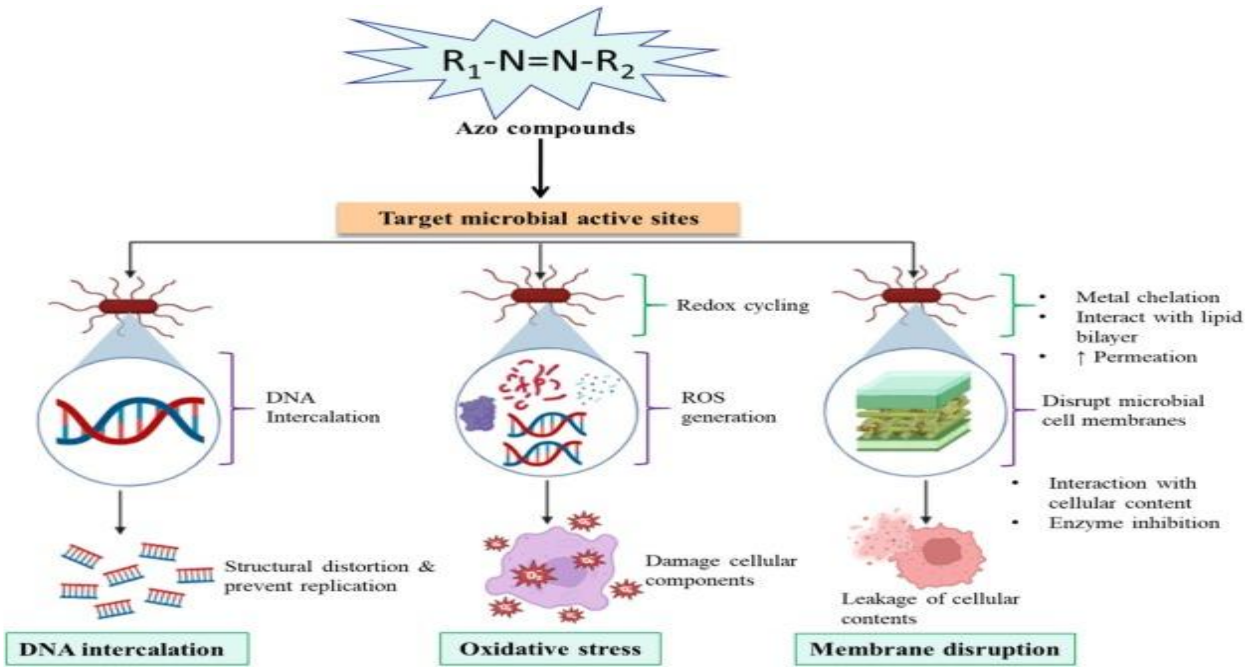

Figure: Mechanism of antimicrobial activity of azo compound

METHODOLOGY

1. Synthesis of Azo ligand:

a. Diazotisation reaction: Take 5.2g sulphanilic acid and 1.35g of anhydrous sodium carbonate in conical flask and add 50 ml distilled water and warm the solution until the solution becomes clear. Measure 1.8g of sodium nitrite and dissolve in 5ml of distilled water. Add sodium nitrite solution in above conical flask. Take 5.25ml of conc. HCL and add to 30g of crushed ice. Pour this solution in conical flask and complete the diazotization reaction to form diazonium salt.

b. Coupling reaction: Dissolve 3.6g of 2-napthol in 20 ml of cold 10% NaOH. Cool the solution up to 5°C.Transfer the diazotised sulphanilic salt into this solution slowly while stirring. Maintain the temperature at 5°C. Keep it on room temperature for 30 minutes to complete the reaction. Filter to get the crude product wash with ethanol and dry the product.

General reaction:

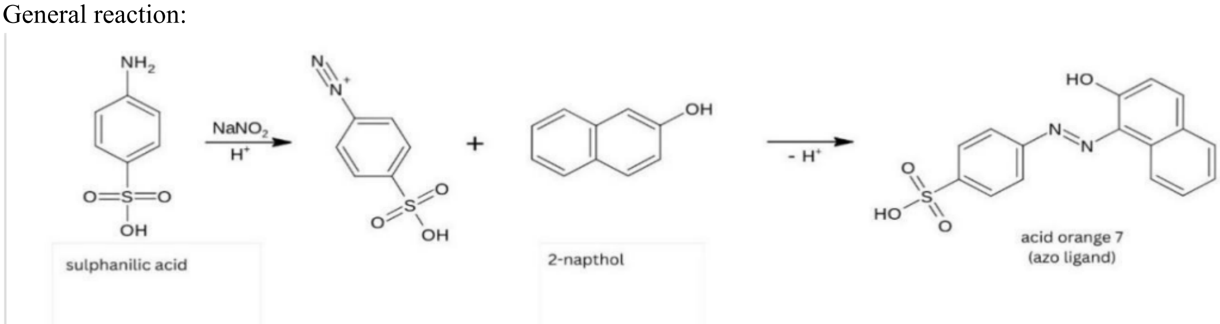

Figure: Synthesis of azo ligand

2. Synthesis of Azo-Metal Complex:

The Ethanolic solution of Azo ligand containing 0.280g azo ligand in 10 ml of pure ethanol, added to the metalsalt solution containing 0.140g Zn (II) dissolved in pure ethanol at 2:1 molar ratio. The pH of the mixture was maintained at 8.0-9.0 using 10% NaOH solution. The mixture was heated for 2 hrs at 65°C, then cooled in an ice bath till precipitation occurred, before being left overnight. The solid complexes were separated and rinsed with distilled water and a small amount of heated ethanol to eliminate any unreacted components. Finally, vacuum desiccators were used to dry the complexes.

General reaction:

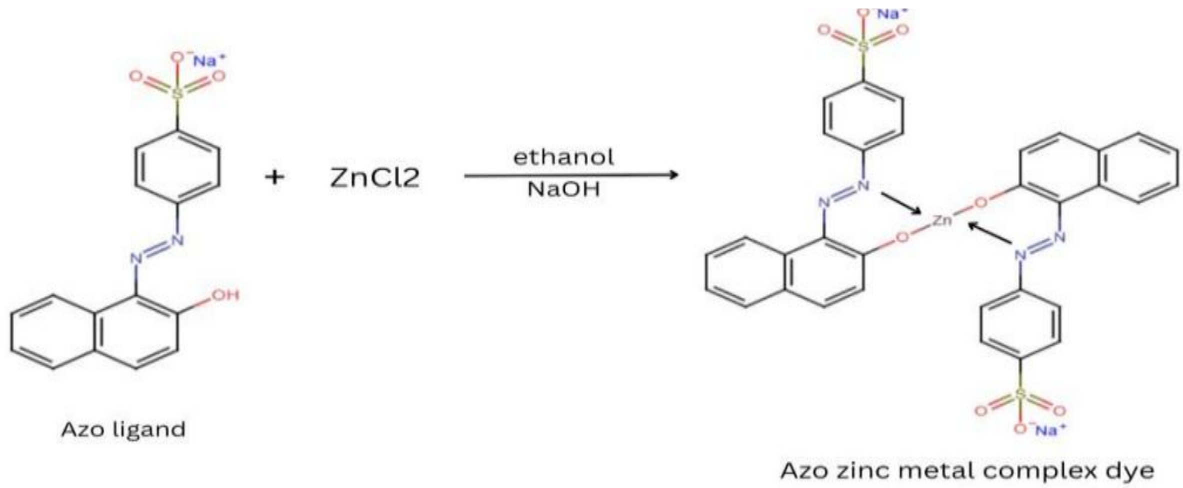

Figure : Synthesis of Azo Zinc Metal Complex

3. Sar of Azo-Zinc Metal Complex:

The antimicrobial efficacy and mechanisms of action of azo com-pounds depend on their structure and functional groups. This section aims to provide a perspective on SAR from the reviewed literature, offering a better understanding of the structural effects on the biological activity potential of the compounds.

a. Azo Group (-N=N-): Key chromophore responsible for coloration. It is an Electron-donating groups (e.g., -OH, -NH_2) on the aromatic rings. It Improves dyeing strength and can affect antimicrobial activity due to electron flow.

b. Aromatic Rings: It is a Larger or more electron-rich aromatic systems increase resonance, stabilizing the molecule and enhancing both colour and binding to substrates (like fabrics or microbial membranes). It Boosts affinity for microbial surfaces or Fiber matrices.

c. Phenolic -OH Groups (coordinated with Zn): It Serve as coordination sites for zinc. It is Essential for complex formation; substitution affects complex stability and biological activity. Stronger ligands improve complexation, thus enhancing antimicrobial activity and dye stability.

d. Zinc Metal Ion (Zn^{2+}): It is a Central coordination metal, bridges two ligand molecules. The Zinc coordination increases molecular rigidity and electron delocalization. It Enhances antimicrobial properties (Zn^{2+} known for antimicrobial action), improves thermal and photostability of the dye.

e. Sulfonate Groups (-SO_3Na): It Increase water solubility and ionic character. It also Facilitates dye diffusion and binding in aqueous environments; affects cell membrane interaction.

4) Advantages of Azo Dyes:

a. Textile materials can be dyed in a variety of physical shapes using a variety machinery, or even in small quantities with household items in the absence machinery

b. It is easy for synthesis processes, high solubility, and high substrate absorption.

c. It is feasible to get dark green, orange, and red hues economically

d. The reproducibility of dyes is quite good.

e. Commercial economic sustainability and low cost.
f. Dischargeable combinations are the majority.

g. Overall, the fastness characteristics are good.

5) Disadvantages of Azo Dyes:

a. The Application process is difficult and drawn out.

b. Chemicals must be used and preserved. Difficulties handling the constituent compounds under common chemical processing applications particularly the diazotization of aromatic amines' rapid bases.

c. Available hues are a few.

d. It has always been difficult to make azoic- colored material fast to rubbing. To produce good rubbing fastness, it is crucial to effectively remove surplus naphthol from the surface of the treated material, have a high coupling component substantivity, and thoroughly soap the material after development.

e. Since there would be cross-coupling between different naphthols and diazonium salt, compound hues as generated with other dye classes by mixing three or four dyes are difficult to make with azoic dyes and the combined shades are unpredictable and nonreproducible.

f. Only after the coupling stage is the final shade apparent. As a result, any unevenness that results from naphtholation may be unnoticed and only become apparent after coupling, when shade correction is hard.

PHARMACOLOGICAL ACTIVITY :

1. Anti bacterial activity: Azo dye compounds are synthetic organic molecules containing an azo linkage (–N=N–) between aromatic rings, and many of these compounds exhibit significant antibacterial activity against both Gram-positive and Gram-negative bacteria. Their antibacterial effect is mainly due to their ability to disrupt bacterial cell membranes, inhibit essential enzymes, interfere with DNA replication, and block protein synthesis. The activity of azo dyes depends on their chemical structure, especially the presence of substituent groups such as –Cl, $–NO_2$, –OH, and heterocyclic rings, which can enhance their biological effectiveness. Some azo dye derivatives also form metal complexes that increase antibacterial potency and stability. These compounds have shown activity against bacteria such as Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. Due to their broad-spectrum antibacterial properties, azo dye compounds are being studied for applications in pharmaceuticals, antimicrobial textiles, medical coatings, and disinfectant formulations.

2. Anti microbial activity: many of these compounds possess important antimicrobial activity against bacteria and fungi. Their antimicrobial effect is mainly due to their ability to disrupt microbial cell membranes, inhibit essential enzymes, interfere with DNA replication, and prevent protein synthesis in microorganisms. The activity of azo dyes depends on their chemical structure, nature of substituent groups, and presence of heterocyclic rings or metal complexes, which often enhance their biological effectiveness. Electron-withdrawing groups such as –Cl and $–NO_2$ generally increase antimicrobial potency. Azo dye derivatives have shown activity against microorganisms such as Escherichia coli, Staphylococcus aureus, and Candida albicans. Because of their broad-spectrum antimicrobial properties, azo dye compounds are widely studied in medicinal chemistry for the development of new antimicrobial drugs, antimicrobial textiles, medical coatings, and disinfectant agents.

3. Anti fungal Activity: Azo dye compounds are synthetic organic compounds containing an azo linkage (–N=N–) between aromatic rings, and many of these compounds exhibit significant antifungal activity. Their antifungal action occurs through disruption of fungal cell membranes, inhibition of essential fungal enzymes, interference with nucleic acid synthesis, and blockage of metabolic pathways necessary for fungal growth. The antifungal effectiveness of azo dyes is influenced by the presence of electron-withdrawing groups, hydroxyl groups, heterocyclic rings, and metal complexes, which can enhance their biological activity. Several azo dye derivatives have shown strong activity against fungi such as Candida albicans, Aspergillus niger, and Trichophyton rubrum. Due to their broad antifungal properties and easy structural modification, azo dye compounds are widely investigated in pharmaceutical and medicinal chemistry for the development of new antifungal

agents, antimicrobial coatings, and protective textile materials.

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