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# STRUCTURE - ACTIVITY RELATIONSHIP (SAR) TRENDS OF AZO BASED MEDICINES: MEDICINAL CHEMISTRY PERSPECTIVES AND EMERGING APPLICATIONS

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## ABSTRACT

Azo based compound, characterized by the presence of the -N=N- azo linkage, represent a unique and versatile class of molecules in medicinal chemistry. Although historically associated with dyes and pigments, azo derivatives have demonstrated significant pharmacological potential, including antimicrobial, anti inflammatory, antioxidant, anticancer, and targeted prodrug applications. The biological performance of azo compound is strongly governed by their structural features, including electronic substituent effects, aromaticity, heterocyclic integration, steric modulation, and metabolic reducibility. This review comprehensively examines structure-activity relationship (SAR) trends governing azo based medicinal agents. Emphasis is based on electronic and steric influences, heteroaromatic scaffolds, redox behavior, enzyme-mediated bioreduction, metal coordination, and emerging photopharmacological strategies. Toxicological considerations and computational SAR correlations are also discussed. BY consolidating recent advances, this review provides rational design principles for developing safer and more effective azo-based therapeutics.

**KEYWORDS:** Azo compounds, Structure-activity relationship, Medicinal chemistry, prodrugs, azoreductase, antimicrobial agents, photopharmacology.

## INTRODUCTION

The azo functional group occupies a distinctive position in organic and medicinal chemistry due to its conjugated electronic structure, reversible redox behaviour, and capacity for metabolic transformation.[1] Azo compounds were initially developed for industrial dye applications because of their intense coloration arising from extended π conjugation.[2] However, early discoveries such as Prontosil, the first commercially successful antibacterial drug, revealed the therapeutic relevance of azo linkages. Prontosil itself is pharmacologically inactive but undergoes in vivo reductive cleavage to yield sulfanilamide, establishing the concept of azo compounds as prodrugs.[3]

Over the past two decades, renewed interest in azo-based medicines has emerged due to advances in medicinal chemistry, enzymology, and computational drug design. The ability of azo compounds to undergo selective enzymatic reduction, particularly by bacterial azoreductase in anaerobic environments such as the colon, has been exploited for targeted drug delivery.[4] Additionally, azo derivatives have shown intrinsic biological activities independent of cleavage, including antimicrobial, antioxidant, anticancer, and eznyme - inhibitory effects.[5] Structure-Activity relationship studies are central to understanding how molecular modifications influence biological activity, selectivity, metabolism, and toxicity.[6] Given the dual nature of azo compounds- acting both as intact pharmacophores and as metabolically activated prodrugs - SAR analysis must integrate chemical structure, biological environment, and metabolic fate.[7] This review systematically review systematically examines SAR trends of azo based medicines, highlighting molecular features responsible for pharmacological performance.

### Chemical and structural Characteristics of Azo compounds

#### Fundamental azo scaffold

The azo linkage is formed by two $sp^2$-hybridized nitrogen atoms joined by a double bond (-N=N-), usually bridging two aromatic or heteroaromatic rings. This arrangement gives the azo group a planar or nearly planar geometry, which promotes strong orbital overlap and efficient π electron delocalization. The extended conjugation across the azo bridge and the attached rings leads to distinctive electronic properties, such as a reduced HOMO - LUMO gap and absorption in the visible region. These electronic features are responsible for the intense colors observed in many azo dyes and pigments. The planar, conjugated structure also contributes to moderate chemical stability under neutral conditions, although the -N=N- bond can undergo reduction or photoisomerization under specific stimuli. In biological systems, the planarity and aromatic character of azo compounds enable interactions with biomolecules such as DNA or proteins, influencing binding and activity. Overall, the azo linkage's geometry and conjugation underlie both its functional versatility in materials and its role in certain drug and dye applications.[8]

#### Electronic distribution and resonance

The azo bond engages in extensive resonance with adjacent aromatic systems, allowing π electron density to delocalize across the entire conjugated framework. Electron-donating substituents increase electron density along the azo linkage, enhancing its nucleophilic character and influencing its spectral properties. In contrast, electron-withdrawing substituents stabilize the azo bond through both inductive and mesomeric effects, often making it less prone to reduction. These electronic effects directly modulate the redox potential and the ease with which enzymes can reduce the azo bond in biological environments. They also affect the molecule's hydrogen bonding capability by altering the polarity and charge istribution around the azo group. Furthermore, changes in electron density and planarity influence

π-π stacking interactions with biomolecules such as DNA and proteins, thereby impacting binding and biological activity.[9,10]

**Geometric isomerism**

Azo compounds are typically found in the E-configuration, which is the thermodynamically favored form due to lower steric strain and better orbital overlap. In this configuration, the two aromatic rings lie on opposite sides of the azo bond, resulting in a relatively linear and planar molecular geometry. Upon exposure to light, the azo bond can undergo reversible E to Z isomerization, which changes the spatial arrangement of the substituents. This photoisomerization significantly alters the overall molecular shape, dipole moment, and conformational flexibility. As a result, the biological activity of the compound can be switched on or off depending on the isomeric stste. Because of this light-controlled behavior, azo compounds have become important tools in photopharmacology for designing stimuli-responsive drugs.[11]

**Biological fate of azo compounds**

**Enzymatic reduction**

Azo compounds undergo enzymatic reduction catalyzed by azoreductase enzymes, which are predominantly present in the intestinal microbiota and some mammalian tissues.These enzymes transfer electrons to the azo bond, leading to its cleavage. The reduction process typically generates two aromatic amine metabolites from a single azo compound. Depending on their chemical structure, these aromatic amines can exhibit pharmacological activity and contribute to the therapeutic effect of certain prodrugs. In other cases, the amines may be toxic or carcinogenic, raising safety concerns for some azo dyes and drugs. Therefore, the enzymatic reduction of azo compounds plays a key role in both drug activation and potential toxicity.[12]

**Key SAR implication**

Substituents that increase steric hindrance around the azo bond can shield it from the active site of azoreductase enzymes. This steric shielding reduces the accessibility of the azo linkage to the enzyme, making it more difficult for reduction to occur. As a result, the rate of enzymatic cleavage is slowed, which can prolong the lifetime of the intact azo compound in biological systems. Slower cleavage may delay the release of aromatic amine metabolites, thereby affecting the onset and duration of pharmacological or toxic effects. Bulky groups near the azo bond can also influence the overall molecular conformation and planarity, further modulating enzyme recognition. In structure-activity relationship (SAR) studies, such steric effects are often exploited to tune the metabolic stability of azo based prodrugs. Therefore, controlling steric hindrance around the azo bond is a key strategy for optimizing both efficacy and safety in azo compound design.

**Redox behaviour and ROS modulation**

Some azo derivatives are capable of participating in redox cycling, in which they undergo repeated oxidation and reduction reactions within biological systems. This redox cycling can influence cellular oxidative stress pathways by altering the balance of reactive oxygen species (ROS). Depending on their structure, certain azo compounds may either promote or suppress ROS levels, thereby modulating oxidative damage. Phenolic azo compounds, in particular, often act as radical scavengers because the phenolic hydroxyl group can donate hydrogen atoms to reactive radicals. The resulting phenoxyl radicals are stabilized by resonance delocalization across the azo linkage and the aromatic rings. This stabilization enhances the ability of phenolic azo derivatives to terminate radical chain reactions and protect

biomolecules from oxidation Overall, the redox behaviour of azo compounds makes them important players in ROS modulation and potential candidates for antioxidant therapeutic strategies.[13]

**SAR Trends in Antimicrobial azo compounds**

**Aromatic Substitution Effects**

The antibacterial effect of azo compound is often enhanced when one aromatic ring bears a heterocyclic such as thiazole, pyrimidine, or quinoloine. These heterocyclic systems can improve target binding and influence electronic properties around the azo linkage. Para substitution on the aromatic rings tends to improve molecular planarity, which can enhance stacking interactions and membrane permeability. Moderate lipophilicity is crucial, as it facilitates penetration through the bacterial cell wall and access to intracellular targets. Together, these aromatic substitution effects allow fine tuning of potency, selectivity, and pharmacokinetic behaviour in antibacterial azo derivatives.[14]

**Table 1: SAR Trends in Antimicrobial azo derivatives.**

| Structural Feature | Effect on Activity |
|---|---|
| Para halogen substitution | Increased MIC potency and broader spectrum |
| Ortho halogen substitution | Reduce potency due to steric hindrance |
| Nitro group on aromatic ring | Increased antibacterial activity, especially against Gram-positive strains |
| Hydroxyl group | Enhanced radical scavenging and modertae activity modulation |
| Azo-metal complexes | Boosted antimicrobial potency |
| Heteroaromatic Ring | Improved enzyme binding |
| Bulky ortho groups | Reduced enzymatic reducibility and slower cleavage |

**Mechanistic Insights**

Docking studies indicate that azo compounds can bind to bacterial enzymes by engaging aromatic residues in the active site. The planar, conjugated structure of the azo linkage facilitates π-π stacking interactions with these aromatic amino acid side chains. These stacking interactions help anchor the molecule in a favorable orientation within the binding pocket. In addition, the lone pairs on the azo nitrogen atoms can participate in hydrogen bonding with polar residues or backbone amides.Hydrogen bonding further stabilizes the enzyme-ligand complex and may influence catalytic activity. The combination of π-π stacking and hydrogen bonding pften enhances binding activity and selectivity for specific bacterial targets. Together, these interactions provide a plausible mechanistic basis for the antimicrobial activity observed with many azo derivatives.[15]

**Azo Compunds as Prodrugs**

**Colon-Targeted Drug Delivery**

Sulfasalazine is widely regarded as the prototypical colon-targeted azo prodrug, where the azo bond links a pharmacophore (5-aminosalicylic acid) to a carrier moiety (sulfapyridine). SAR studies indictae that the distance or spacer length between the pharmacophore and the azo linkage is critical for optimal enzymatic reduction and site-specific drug release. Proper spacing ensures that the azo bond is accessible to colonic bacterial azoreductases while maintaining sufficient stability in the upper gastrointestinal tract. Electron-withdrawing substituents on the aromatic rings tend to increase the reduction rate of the azo bond, accelerating the liberation of the active drug in the colon.In contrast, excessive steric bulk near the azo linkage can shield the bond from enzymatic attack, thereby delaying or impairing drug release, These electronic and steric effects together determine the balance between systematic stability and localized activation in the colon. As a result, fine-tuning the substituents and linker design around the azo bond is a key strategy in developing effective colon-targeted azo prodrugs.[16]

**Design principles for Prodrug Optimization**

Design principles for azo prodrug optimization emphasize a careful balance between systematic stability and efficient microbial activation in the target site. Research shows that the prodrug should remain largely intact during transit through the upper gastrointestinal tract but be readily cleaved by bacterial azoreductases inn the colon or other hypoxic niches. A key concern is avoiding the formation of carcinogenic aromatic amines upon reduction, which has driven the selection of safer amine metabolites in modern prodrug design. Incorporating polar or ionizable substituents can limit systematic absorption and promote local retention, thereby improving site-specific delivery. Overall, successful prodrug optimization integrates electronic, steric, and solubility-modifying features to achieve controlled activation while minimizing toxicity and off target exposure.[17]

**SAR in Antioxidant and Anti-Inflammatory Azo Agents**

Phenolic azo compounds display strong antioxidant and anti-inflammatory activity, largely because extended conjugation stabilizes the phenoxy radical formed after hydrogen atom donation. Research shows that the synergy between the phenolic OH group and the azo linkage enhances radical scavenging capacity by lowering the O-H bond dissociation energy and improving electron delocalization. Para nitro and para fluoro substitutions have been reported to increase radical scavenging potency, likely by tuning the electron deficient character of the aromatic ring and facilitating electron transfer pathways. Ortho hydroxyl groups promote intramolecular hydrogen bonding with the azo nitrogen or adjacent acceptors, which stabilizes the radical intermediate and can improve both antioxidant efficacy and metabolic stability. Taken together, SAR studies on antioxidant and anti inflammatory azo agents highlight that careful placemnet of electron withdrawing halogens, nitro groups, and ortho hydroxyl substituents optimizes radical scavenging behavior while maintaining favorable physicochemical and biological profiles.[18]

**Anticancer activity and SAR trends**

**Metal-Azo complexes**

Complexation of azo ligands with transition metal ions such as Zn2+,Cu2+, and Ni2+ significantly alters their biological activity compared with the free ligand. Research shows that metal coordination often increases molecular planarity, which enhances the ability of the complex to intercalate into DNA. The more planar, rigid structure facilitates stronger stacking intercalations with DNA base pairs, leading to higher binding constants and improved DNA targeting potential. In addition, the redox active nature of metals like Cu2+ and Ni2+ introduces enhanced redox properties that can promote the generation of reactive oxygen species (ROS). Elevated ROS levels can trigger oxidative stress in cancer or microbial cells, thereby inducing apoptosis or cell cycle arrest. Studies on azo Schiff base and azo thiosemicarbazone metal complexes report that Cu(II) and Ni(II) derivatives frequently exhibit superior cytotoxic or antimicrobial effects relative to their Zn(II) analogues. The combination of improved DNA binding, altered electronic structure, and metal mediated redox cycling makes metal azo complexes a promising candidates for anticancer and antimicrobial agents. Overall, SAR work on these systems highlights that careful choice of metal ion and ligand geometry is critical for optimizing DNA interaction, redox behaviour, and apoptopic potency.[19, 20]

**Table 2: SAR effects of metal coordination.**

| Metal Ion | Observed Effect |
|---|---|
| Zn(II) | Improved cytotoxicity |
| Cu(II) | ROS-mediated apoptosis |
| Ni(II) | Moderate DNA binding |

**Selectivity Considerations**

Selective cytotoxicity in azo based or metal azo systems can be achieved by introducing bulky substituents that hinder uptake into normal cells while still allowing entry into target cancer or microbial cells. As highlighted in the review by Sudhindra pete et, al on ruthenium metalloid drugs, controlling lipophilicity is a key strategy to bias cellular accumulation toward tumor cells and away from healthy tissues. Bulky groups can reduce passive diffusion through membranes of normal cells, thereby lowering off target toxicity and improving the therapeutic index. At the same time, moderate lipophilicity is maintained to ensure sufficient uptake into diseased cells, where the compound can exert its cytotoxic effect. The balance between steric bulk and lipophilicity thus directly influences both cellular selectivity and overall cytotoxic potency. By applying these principles, azo or metal azo agents can be tuned to display preferential activity against pathological cells while sparing normal ones.[21]

**Photopharmacology and Azo-Based Drugs**

Azo compounds are uniquely suited for light controlled therapy because their reversible E-Z isomerization allows precise spatiotemporal modulation of biological activity. As discussed by Flavia et al, the photoisomerization efficiency and spectral properties of azobenzenes can be rationally tuned by structural design. Substituents near the azo bond strongly influence isomerization kinetics and quantum yield, with steric and electronic effects dictating how readily the molecule switches between isomers. Push-pull electronic systems, in which one ring bears an electron donating group and the other an electron withdrawing group, enhance intramolecular charge transfer and increase photo switching speed. These design principles enable red shifted absorption and improved photoconversion, which are critical for practical photopharmacology in biological settings. Overall, SAR guided optimization of substituents and electronic asymmetry allows azo based drugs to achieve fast, efficient and wavelength seective photoswitching for targeted therapeutic action.[22]

**Computational binding and QSAR models**

**Docking based SAR**

Docking studies reveal a clear correlation between calculated binding energy and the electronic nature of substituents on the ligand scaffold. As shown by Michali et al, more electron donating or electron withdrawing groups can either stabilize or destabilize the ligand protein complex, thereby modulating affinity. The aromatic surface area of the ligand also strongly influences binding, with larger conjugated systems generally yielding more favorable interaction energies. Increased aromatic surface area enhances π stacking and π-alkyl contacts with aromatic residues in the binding pocket. These non covalent π interactions contribute significantly to the overall binding profile and can be tuned by modifying ring size, substituent position, and planarity. The authors further demonstrate that combining favorable electronics with extended aromatic surfaces improves target-specific binding and selectivity for the intended protein. Taken together, their docking analyses highlight that both substituent electronics and aromatic surface area are key SAR parameters for optimizing ligand affinity. This systematic approach provides a rational framework for designing benzosuberane based compounds with enhanced binding and biological activity.[23]

**Table 3: QSAR Descriptors Relevant to Azo Drugs.**

| Descriptor | SAR Coorelation |
|---|---|
| HOMO-LUMO gap | Redox activity |
| Molecular volume | Enzyme accessibility |
| TPSA | Bioavailability |

**Toxicologiocal SAR considerations**

While medicinal azo compounds offer valuable therapeutic benefits, their potential toxicity remains a significant concern in drug design. Benzidine like aromatic amines and unsubstituted biphenyl systems are particularly problematic because they can be metabolized to genotoxic and carcinogenic intermediates. These high risk features underscore the need for careful SAR driven structural optimization to minimize adverse effects. One effective risk reduction strategy is to introduce steric shields around the azo bond and its cleavahe products, which can hinder metabolic activation and reduce interaction with DNA. Replacing benzene rings with heteroaromatic systems can also lower the propensity to form toxic aromatic amines while maintaining desired pharmacological activity. Additionally, designing compounds that yield polar, readily excreted metabolites helps limit systematic accumulation and long term toxicity. Hence, integrating these toxicological SAR considerations enables the development of safer azo based agents that balance efficacy with improved safety profiles.[24]

**Future Perspectives**

Integration of AI driven SAR prediction to accelerate the identification of potent and selective azo based drug candidates, Development of safer, non carcinogenic azo prodrugs by avoiding genotoxic aromatic amine metabolites and favoring benign cleavage products, Expansion of photoresponsive therapeutics using azo photo switches for spatiotemporaly controlled drug activation in oncology, neurology, and opthalmology, Green synthesis of azo compounds through atom economical, metal free, and catalytic methods that minimize hazardous reagents and waste, adoption of sustainable medicinal azo chemistry, including recyclable catalysts, aqueous or biobased solvents, and energy efficient reaction conditions, exploration of hybrid azo systems that combine metal coordination, heterocyclic scaffolds, or peptide motifs to enhance target specificity and reduce off target toxicity, application of multi omics and systems toxicology approaches to map the metabolic fate and long term safety of azo drugs in vivo, design of stimuli responsive azo formulations for targeted delivery to the colon, tumors, or microbial biofilms.

## CONCLUSION

Structure - Activity relationship analysis reveals that azo-based medicines are highly tunable systems whose biological properties depends on subtle structural modifications.Electronic effects, steric control, heterocyclic incorporation, and metabolic behavior collectively define activity and safety. Advances in computational chemistry and photopharmacology continue to expand the therapeutic scope of azo compounds, reinforcing their relevance in modern medicinal chemistry.

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