



Novel Azo Sulfonamide Derivatives of 5-Sulfosalicylic Acid: Synthesis, Biological Evaluation, and Computational Profiling

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Abstract

The escalating crisis of multi-drug-resistant pathogens necessitates the development of novel therapeutic scaffolds with enhanced membrane permeability and multi-target inhibitory potential. This study describes the design, synthesis, and mechanistic evaluation of five novel azo-sulfonamide hybrids (4a–e) derived from a 5-sulfosalicylic acid scaffold. Structural integrity was elucidated via comprehensive spectroscopic techniques. Biological efficacy was evaluated against a diverse panel of clinical pathogens, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*, using standardized Clinical and Laboratory Standards Institute protocols for minimum inhibitory concentration and minimum microbicidal concentration determination alongside B3LYP/6-31G(d,p) DFT modeling and molecular docking. Molecular hybridization significantly enhanced biocidal potency, with lead compound 4d, incorporating a 4,6-dimethylpyrimidinyl moiety, exhibiting robust broad-spectrum activity (e.g., a zone of inhibition of 44 mm against *Bacillus cereus*), showing strong correlation with high electronic softness and a high binding affinity ($\Delta G = -9.7$ kcal/mol) against lanosterol 14- α -demethylase. Density functional theory profiling revealed a direct correlation between high electronic softness and superior antimicrobial performance. Molecular docking validated these results, identifying 4d as a high-affinity inhibitor of *Candida albicans* lanosterol 14- α -demethylase through strategic pi–pi stacking and electrostatic salt bridges. These findings suggest that compound 4d represents a promising lead with favorable predicted pharmacokinetic and safety profiles, offering a potential platform for next-generation antimicrobial drug discovery.

Keywords: Antimicrobial, Azo, DFT, Molecular docking, 5-Sulfosalicylic acid, Sulphonamide

1. Introduction

Antimicrobial resistance (AMR) represents an escalating global health crisis, characterized by the evolutionary adaptation of microorganisms to circumvent the efficacy of conventional therapeutic agents [1]. The alarming proliferation of resistance among both bacterial and fungal pathogens has severely constrained the current clinical pharmacopeia. Notable fungal species, including *Aspergillus sp.*, *Fusarium sp.*, and *Candida sp.*, alongside recalcitrant bacterial strains such as *Bacillus cereus*, *Staphylococcus aureus*, and the Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*, have demonstrated extensive resistance to diverse classes of antimicrobial drugs [2]. Microorganisms employ sophisticated survival strategies to evade antimicrobial action, including the regulation of efflux pumps, enzymatic modification of drug targets, horizontal acquisition of resistance genes, and the formation of resilient biofilms [3]. Moreover, particularly in Gram-negative pathogens, the complex, multi-layered cell envelope acts as a formidable permeability barrier, significantly reducing the intracellular accumulation of many existing antibiotics.

Throughout the history of modern medicine, small-molecule chemical compounds have served as the primary line of medical defense against infectious pathogens, changing the landscape of clinical healthcare. The

development of diverse synthetic architectures remains vital to outpacing natural microbial evolutionary adaptations. The alarming proliferation of resistance among both bacterial and fungal pathogens has severely constrained the current clinical pharmacopeia. Heterocyclic scaffolds constitute a fundamental architecture in organic synthesis and are ubiquitous in natural products and modern pharmaceuticals. The strategic incorporation of heterocyclic rings allows for the precise modulation of a molecule's solubility, lipophilicity, and pharmacokinetic behavior [4]. Nitrogen-, sulfur-, and oxygen-containing heterocycles are particularly prioritized due to their capacity for hydrogen bonding and π - π stacking interactions with biological targets. Classic examples such as sulphapyridine, sulfathiazole, and sulfadimidine underscore the versatility of heterocyclic sulfonamides in treating systemic infections [5]. Beyond antimicrobial applications, these scaffolds are increasingly explored for their radical scavenging potential and their ability to act as carbonic anhydrase inhibitors in oncology [6]. Historically, sulfonamide derivatives—the inaugural class of synthetic antibacterial agents—have served as a cornerstone in medicinal chemistry [4]. Their therapeutic efficacy is derived from the competitive inhibition of dihydropteroate synthase, an essential enzyme in the microbial folate biosynthetic pathway. By acting as structural analogs of para-aminobenzoic acid (PABA), sulfonamides disrupt the synthesis of dihydrofolate, thereby arresting bacterial DNA replication. However, the global dissemination of resistant strains necessitates the strategic structural redesign of the sulfonamide pharmacophore to augment potency and circumvent existing defense mechanisms.

Azo dyes represent the most versatile class of synthetic chromophores, characterized by the -N=N- linkage, which accounts for over 70% of global dye production [7]. While traditionally valued for their intense coloration and structural stability in the textile and cosmetic industries, azo-functionalized compounds have emerged as significantly privileged scaffolds in medicinal chemistry [8]. Furthermore, the sulfonamide-azo subclass, in particular, merges the intense electronic properties of the azo bridge with the established bioactivity of the sulfonamide group (-SO₂NHR), resulting in a potent multifunctional framework capable of interacting with a broad spectrum of microbial enzymes [9].

A critical component of the current design is the utilization of 5-sulfosalicylic acid as a trifunctional coupling component. The physicochemical profile of 5-sulfosalicylic acid-derived dyes is governed by the synergistic presence of phenolic hydroxyl (-OH), carboxyl (-COOH), and sulfonic acid (-SO₃H) groups. In addition, these moieties not only enhance the aqueous solubility essential for physiological applications but also provide multiple sites for electrostatic and hydrogen-bonding interactions with microbial cell membranes and proteins [10]. To address this research gap, molecular hybridization was employed to merge the sulfonamide pharmacophore with a 5-sulfosalicylic acid core. The trifunctional moieties of 5-sulfosalicylic acid are hypothesized to optimize membrane interactions and enhance intracellular accumulation, while the azo-linked sulfonamide targets core microbial metabolic pathways.

In this context, the present study describes the design, synthesis, and comprehensive evaluation of five novel azo sulfonamide derivatives (4 a-e) and focuses on a clear dual objective: (i) the strategic synthesis of five novel azo sulfonamide derivatives based on a highly soluble 5-sulfosalicylic acid core, and (ii) the definitive structural and computational validation of these hybrids to evaluate their potential as broad-spectrum antimicrobial leads. Through a multidisciplinary approach involving *in vitro* antimicrobial assay coupled with density functional theory (DFT) profiling and molecular docking, a mechanistic insight was provided into how these structural modifications influence biological efficacy and target binding affinity. Through the determination of minimum inhibitory concentrations (MIC) and minimum microbicidal concentrations (MMC), a robust assessment was provided for their potential as next-generation therapeutic leads.

2. Materials and methods

2.1 General synthesis of azo sulphonamide derivatives 4(a-e)

The synthesis of the target azo hybrids was performed via a modified diazotization-coupling protocol [11, 12]. The sulfonamide precursor 1 (a-e) (1.0 mmol) was dissolved in a mixture of 5 mL concentrated HCl and 7 mL distilled water, maintained at 0–5°C in an ice-salt bath. An ice-cold aqueous solution of sodium nitrite (1.2 mmol) was added dropwise over 15 minutes with continuous stirring to generate the diazonium salts 2(a-e). The progress of diazotization was monitored by thin-layer chromatography. Subsequently, the diazonium solution was added dropwise to a chilled alkaline solution of 5-sulfosalicylic acid 3 (1.0 mmol in 10 mL of 10% NaOH). The reaction was agitated for 60 minutes at 0–5°C, and the pH was adjusted to 9.0 to induce precipitation. The resulting precipitates were collected via vacuum filtration, washed with cold and tepid water, and purified through recrystallization with 70% ethanol to yield the analytically pure derivatives 4(a-e) (Figure 1).

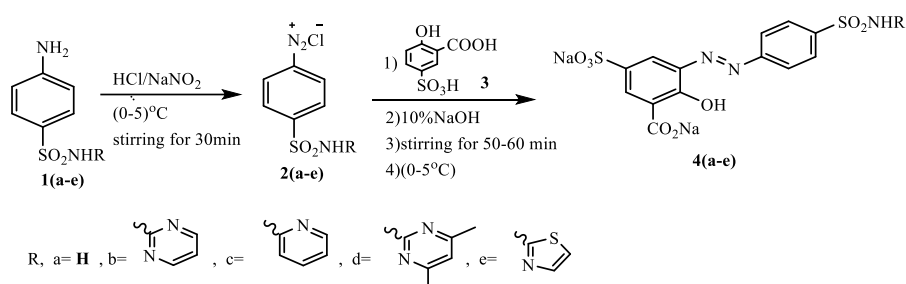


Figure 1 Synthesis of azo sulfonamide derivatives 4(a-e) of 5-sulfosalicylic acid.

2.2 Analytical characterization and DFT-based reactivity profiling

The structural integrity and purity of the synthesized azo sulfonamide derivatives 4(a-e) were rigorously verified. Melting points were determined using the Griffin capillary apparatus. FT-IR spectra were recorded in the mid-infrared region (4000–400 cm^{-1}) on a JASCO FTIR-410 spectrometer (Germany) using the KBr pellet technique to identify characteristic functional group vibrations. Chromatographic purity was confirmed via thin-layer chromatography on silica gel G-coated aluminum sheets, employing a binary solvent system of methanol/dichloromethane (3:7 v/v).

To establish a mechanistic link between molecular structure and biological efficacy, comprehensive theoretical investigations were performed using DFT. All computational simulations were executed within the Gaussian 09 software package. Molecular geometries were fully optimized using the B3LYP (Becke's three-parameter hybrid functional) level of theory in conjunction with the 6-31G(d,p) basis set, a standard well-suited for characterizing heterocyclic sulfonamide systems [13].

Frontier molecular orbital (FMO) analysis was conducted to determine the energy levels of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). These orbitals are critical indicators of chemical reactivity and kinetic stability. From these energy levels, we derived key global reactivity descriptors, including the energy gap ($\Delta E = E_{\text{LUMO}} - E_{\text{HOMO}}$), chemical hardness (η), and the electrophilicity index (ω). These thermodynamic parameters were utilized to provide a quantitative basis for the observed antimicrobial performance, testing the hypothesis that compounds with lower energy gaps exhibit higher bioreactivity and enhanced target interaction.

2.3 Standardized antimicrobial screening

Antimicrobial susceptibility testing was performed via the agar well diffusion method in strict accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. Strains (*B. cereus* OR553494, *S. aureus* ATCC 43300, *E. coli* MF062579, *P. aeruginosa* ATCC 27853, *F. oxysporum* f. sp. *lycopersici* Fol4287, *A. niger* van Tiegh, and *C. albicans* ATCC 10231) were provided by the Microbiology Laboratory, Faculty of Science, Damietta University. Microbial inocula were calibrated to a 0.5 McFarland standard ($\approx 1-2 \times 10^8$ CFU/ml). Aliquots (100 μl) were spread onto MHA (bacteria), SDA (yeast), or DOX agar (fungi). Wells (5 mm) were loaded with 150 $\mu\text{g/ml}$ of the test compounds dissolved in DMSO. Penicillin G and miconazole served as positive antibacterial and antifungal controls, respectively. Inhibition zones (mm) were recorded after 24 hours at 37°C for bacteria and 5 days at 30°C for fungi.

2.4 Determination of minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC)

The MIC was determined using the broth micro-dilution technique [15]. Test compounds were serially diluted (0–150 $\mu\text{g/ml}$) in shaking flasks (100 rpm). Growth was quantified by measuring optical density at 600 nm. The MMC was subsequently determined by sub-culturing 100 μl of tubes showing no visible growth on fresh agar plates. The MMC was defined as the lowest concentration resulting in zero colony development (CFU/ml) after 24 hours (bacteria) or 7 days (fungi) [16].

2.5 Molecular docking and target selection

Molecular docking simulations were conducted to provide a structural basis for the antifungal activity against lanosterol 14- α -demethylase (CYP51), a critical enzyme in the ergosterol biosynthesis pathway of *Candida albicans*. The 3D crystal structure of the target enzyme (PDB ID: 5V5Z) was retrieved from the protein data bank. The protein was prepared by removing water molecules, adding missing hydrogen atoms, and assigning Gasteiger charges. The optimized geometries of the azo sulfonamide derivatives obtained from DFT calculations were used

as input for the docking studies. Simulations were performed using AutoDock 157 and Vina within a grid box centered on the active site pocket (defined by the co-crystallized ligand coordinates). The binding affinities (ΔG , kcal/mol) were calculated, and the resulting ligand-protein complexes were visualized using PyMOL and Discovery Studio Visualizer to identify hydrogen bonding, π - π interactions, and hydrophobic contacts. To validate the structural reliability of the molecular docking protocol, a self-docking validation was performed using the native co-crystallized ligand extracted from the 3Q8U protein matrix. Target receptor preparation was conducted using BIOVIA Discovery Studio Visualizer 2020, and ligand geometry optimization was carried out via Avogadro. The respective receptor and ligand files were formatted into standard PDBQT structures using AutoDockTools (version 1.5.7) prior to executing semi-flexible docking simulations within AutoDock Vina (version 1.2.5). Protocol validation was verified by computing the root-mean-square deviation (RMSD) between the experimental crystallographic ligand pose and the computationally re-docked conformation utilizing the DockRMSD server. The calculation yielded an RMSD value of 2.113 Å. Considering the structural flexibility of the peripheral loops of the target enzyme, this proximity to the standard 2.0 Å threshold indicates robust geometric alignment, confirming the predictive capacity and fidelity of the established docking parameters [17].

2.6 Pharmacokinetic profiling

To assess the drug-likeness and safety profiles of the most active derivatives, ADMET (absorption, distribution, metabolism, excretion, and toxicity) predictions were performed. Parameters including Lipinski's rule of five compliance, gastrointestinal absorption, blood-brain barrier (BBB) permeability, and cytochrome P450 inhibition were evaluated using the SwissADME and pkCSM online servers. This analysis serves to validate the potential of these compounds as orally bioavailable therapeutic leads.

2.7 Statistical analysis

All assays were performed in independent triplicates ($n = 3$). Quantitative data is expressed as mean $\pm$ standard deviation (SD). Statistical significance was evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test within GraphPad Prism (v9.0), where $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1 Synthesis and characterization investigations

The novel compounds 4(a-e) are artificially produced azo-based dyes with a sophisticated molecular structure intended for particular solvation and application characteristics. The strong colour and light-absorption properties are due to the extended conjugated π -system that forms the core of the structure. The target derivatives 4a-e, synthesized via the route outlined in Figure 1, are constructed upon many aromatic rings interconnected by an azo ($-N=N-$) functional group and further functionalized with solubilizing groups to modulate interactions with solvents. Notably, derivatives 4(a-e) have high ionic characters due to the presence of sodium carboxylate ($-\text{COO}^-\text{Na}^+$) and sodium sulfonate ($-\text{SO}_3^-\text{Na}^+$) groups, as well as phenolic hydroxyl ($-\text{OH}$) functionalities that contribute to polarity, potential tautomerism, and hydrogen-bonding interactions. This combination of hydrophilic ionic groups and a hydrophobic aromatic backbone confers amphiphilic properties, resulting in excellent solubility in polar aprotic solvents, particularly dimethyl sulfoxide (DMSO). DMSO effectively solvates the organic cation, disperses the sodium counterions, and interacts favourably with both polar and nonpolar regions of the molecule, facilitating dissolution. This high solubility is critical for advanced technological applications, such as their use as sensitizers in dye-sensitized solar cells (DSSCs), as well as for analytical characterization and processing from solution.

The structure of the synthesized azo sulphonamide derivatives of 5-sulfosalicylic acid 4(a-e) was characterized using IR spectroscopy and DFT studies. Some physical and IR spectral data of compounds 4(a-e) are shown in Table 1. It is notable that the compounds 4(a-e) have high melting points, ranging from 250 to $>300^\circ\text{C}$. This is in agreement with the ionic nature of structures. It is also, notable that all the compounds 4(a-e) were obtained at excellent yields of 80-87%. The IR spectra of compounds 4(a-e) showed bands for their characteristic functional groups in the expected ranges [18].

Table 1 Some physical data of 4(a-e).

Compound	Colour	Melting point	Crude Yield %	Molecular Formula	Molecular weight (g/mol)
4a	Dark red	260-264	80	$\text{C}_{13}\text{H}_9\text{N}_3\text{Na}_2\text{O}_8\text{S}_2$	445.33
4b	Dark orange	250-254	87	$\text{C}_{17}\text{H}_{11}\text{N}_5\text{Na}_2\text{O}_8\text{S}_2$	523.40
4c	Dark yellow	>300	83	$\text{C}_{18}\text{H}_{12}\text{N}_4\text{Na}_2\text{O}_8\text{S}_2$	522.41
4d	Dark brown	270-273	82	$\text{C}_{19}\text{H}_{15}\text{N}_5\text{Na}_2\text{O}_8\text{S}_2$	551.46
4e	Dark yellow	>300	85	$\text{C}_{16}\text{H}_{10}\text{N}_4\text{Na}_2\text{O}_8\text{S}_3$	528.44

Sodium 2-hydroxy-3-((4-sulfamoylphenyl)diazenyl)-5-sulfonatobenzoate (4a) appeared as dark red, with yield of 80%, m.p of 260-264 °C, and the FT-IR spectrum displayed several peaks (ν/cm^{-1}) such as 3507 (OH) 3289- 3406 (NH_2), 1600 ($\text{C}=\text{O}$), 1444-1242 ($\text{N}=\text{N}$), and 1160 (SO_2). Sodium 2-hydroxy-3-((4-(N-(pyrimidin-2-yl)sulfamoyl)phenyl)diazenyl)-5-sulfonatobenzoate (4b) has a dark orange color, 87% yield, m.p (250-254°C), and FT-IR spectrum included (ν/cm^{-1}): 3334 (OH), 3250 (NH), 1582 ($\text{C}=\text{O}$), 1514-1434 ($\text{N}=\text{N}$), and 1159 (SO_2). Sodium 2-hydroxy-3-((4-(N-(pyridin-2-yl)sulfamoyl)phenyl)diazenyl)-5-sulfonatobenzoate (4c) has a dark yellow color, with a yield of 83% ,m.p of >300 °C, and the FT-IR spectrum displayed several peaks (ν/cm^{-1}) such as 3411 (OH), 3306 (NH), 1638 ($\text{C}=\text{O}$), 1510-1457 ($\text{N}=\text{N}$), and 1160 (SO_2). Sodium 3-((4-(N-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenyl)diazenyl)-2-hydroxy- 5-sulfonatobenzoate (4d) appeared as dark brown, with yield of 82% , m.p of 270-273 °C, and the FT-IR peaks (ν/cm^{-1}) included 3422 (OH), 3237 (NH), 1601 ($\text{C}=\text{O}$), 1430-1550 ($\text{N}=\text{N}$), and 1151(SO_2). Sodium 2-hydroxy-5-sulfonato-3-((4-(N-(thiazol-2-yl)sulfamoyl)phenyl)diazenyl) benzoate (4e) has a dark yellow color, 85% yield, m.p (>300 °C), and several FT-IR peaks (ν/cm^{-1}) including 3510 (OH), 3227 (NH), 1575 ($\text{C}=\text{O}$), 1422-1531 ($\text{N}=\text{N}$), 1139 (SO_2).

3.2 DFT profiling and electronic reactivity analysis

Electronic properties of the synthesized hybrids (4a–e) were modeled using B3LYP/6-31G(d,p) to establish a thermodynamic basis for their biological performance [19]. Frontier molecular orbitals (HOMO and LUMO) dictate the interaction capacity with microbial targets (Figure 2). As summarized in Table 2, the HOMO–LUMO energy gaps (ΔE)—critical indicators of molecular reactivity and kinetic stability—ranged from 1.713 to 1.898 eV. These narrow gaps are characteristic of highly conjugated π -systems, facilitating efficient intramolecular charge transfer. The potent electron-accepting nature of the sulfonamide, carboxyl, and sulfonic acid moieties is reflected in the low LUMO values (−5.158 to −5.234 eV) [20]. Compound 4c exhibited the narrowest energy gap ($\Delta E = 1.713$ eV), suggesting maximal chemical reactivity, whereas 4b demonstrated the greatest kinetic stability ($\Delta E = 1.898$ eV). The electronic softness and reduced chemical hardness ($\Sigma = 0.857$ – 0.949 eV) of these derivatives favor strong interactions with microbial biomolecules. Furthermore, the global electrophilicity index values (19.66–21.18 eV) underscore a robust electrophilic character, promoting electrostatic or covalent engagement with nucleophilic sites on microbial enzymes. These DFT-derived parameters align with the experimental findings, confirming that extended π -conjugation and electron-withdrawing groups enhance charge delocalization and antimicrobial potency.

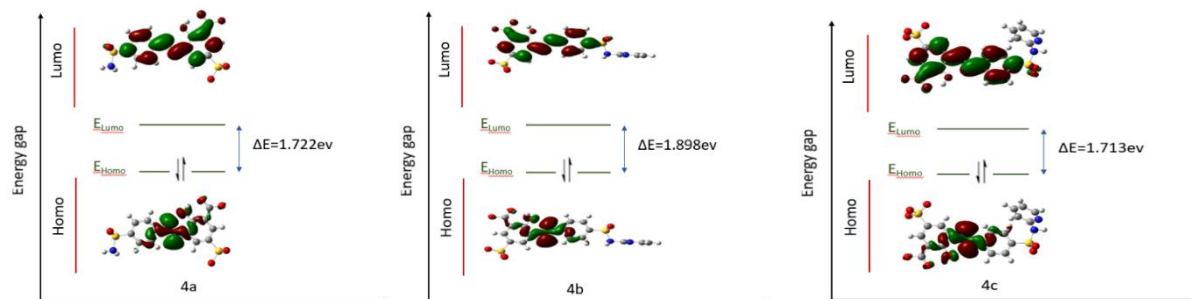


Figure 2 Homo and Lumo of dyes (4a-c).

Table 2 Chemical reactivity values of compounds 4(a-e) calculated via DFT were performed using the Gaussian 09 software.

Dye	E_{HOMO}	E_{LUMO}	ΔE	IP	EA	χ	π	H	Σ	ω
4a	-6.884	-5.162	1.722	6.884	5.162	6.023	-6.023	0.861	1.161	21.07
4b	-7.056	-5.158	1.898	7.056	5.158	6.107	-6.107	0.949	1.054	19.66
4c	-6.880	-5.167	1.713	6.880	5.167	6.024	-6.024	0.857	1.167	21.18
4d	-6.883	-5.166	1.717	6.883	5.166	6.025	-6.025	0.859	1.164	21.14
4e	-7.041	-5.234	1.807	7.041	5.234	6.138	-6.138	0.904	1.107	20.85

3.3 Antimicrobial studies

The antimicrobial activities of the 4(a-e) compounds were assessed using the agar well diffusion method, MIC, and MMC assays against a variety of Gram-positive and Gram-negative bacteria, fungi and yeast. Their activities were compared with those of standard antimicrobial agents, miconazole and penicillin G, to provide a benchmark for efficacy. Table 3 and Figure 3 summarize the microbicidal outcomes of the synthesized compounds 4(a-e) the following observations can be drawn from the results. Compound 4e exhibited moderate activity against *E. coli*, *A. niger* and *F. oxysporum*, but lower activity against *B. cereus*, *S. aureus*, *P. aeruginosa* and *C. albicans*.

S. aureus and *P. aeruginosa* demonstrated partial resistance to 4e and 4c compared to the other compounds. Compound 4d showed the strongest antibacterial activity toward *B. cereus* whereas 4a was less active. Strong antibacterial activity of all compounds against *B. cereus* and *E. coli* was recorded. All compounds displayed stronger antibacterial activity against Gram-positive bacteria than Gram-negative bacteria. All compounds, except for 4e, revealed potent antifungal action against *A. niger* and *F. oxysporum* compared to the standard drug miconazole. Compound 4e showed no antifungal activity against *C. albicans* compared to other compounds. Compounds 4b and 4d demonstrated the highest antifungal activity across all tested fungal strains.

Compounds based on 5-sulfosalicylic acid 3(2-Hydroxy-5-sulfobenzoic acid) and their derivatives have significant applications in many biologically important natural products and drug candidates, exhibiting diverse biological activities such as antimicrobial, antimalarial, and antitumor effects [21]. The presence of a pyrimidine ring in the chemical structure enhanced the antimicrobial activity of 4b and 4d against all tested microorganisms. This is inconsistent with previous reports by Verma *et al.* [22] and Onur *et al.* [23]. Additionally, the sulfanilamide derivative 4a acts as a strong antimicrobial agent. In general, the sulfonamides inhibit dihydropteroate, a key enzyme in the bacterial folate biosynthetic pathway, leading to bacteriostatic effects. Sulfanilamides function as broad-spectrum antibacterial agents against both Gram-positive and Gram-negative bacteria by competing with PABA for enzyme binding sites [24]. The obtained results are consistent with those of Essghaier *et al.* [25]; they reported that di (4-sulfamoyl-phenyl-ammonium) sulphate had significant antifungal activity against *F. oxysporum* with 60% inhibition of spore germination and antimicrobial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, and *C. albicans* with inhibition zones of 15.33, 10.66, 25, and 23 mm, respectively. Conversely, the presence of a pyridine moiety in the sulfonamide functional group may reduce the antimicrobial activity, as observed for 4c, which is in line with reports by Bondock *et al.* [26] and Marinescu and Popa [27]. Overall, the synthesized azo sulphonamide derivatives of 5-sulfosalicylic acid 4(a-e) were more effective against Gram-positive than against Gram-negative bacteria. The inhibition zone was affected by the surface area, antimicrobial agent concentration, and bacterial species. Furthermore, differences in cell membrane structure, particularly the multi-layered cell wall of Gram-negative bacteria, can impede or slow the uptake of these compounds, reducing their antibacterial efficacy [1, 28, 29]. The synthesized derivatives, particularly 4a, 4b, and 4d, displayed a remarkable super-efficacy profile when compared to commercial standards. The zones of inhibition for 4d against *S. aureus* (42 mm) and *E. coli* (40 mm) were significantly higher ($p < 0.05$) than those of penicillin G (13 mm and 12 mm, respectively). This 3-fold increase in potency validates the molecular hybridization strategy of using 5-sulfosalicylic acid as a scaffold to enhance drug uptake and receptor binding.

Table 3 Antimicrobial activity of 4(a-e) compared to the standards.

Compound	<i>B. cereus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>F. oxysporum</i>	<i>A. niger</i>	<i>C. albicans</i>
4e	-ve	-ve	11 ± 0.03	-ve	13 ± 0.23	11 ± 0.16	-ve
4b	40 ± 0*	39 ± 0	38 ± 0	25 ± 0	18 ± 0.03	19 ± 0.14	25 ± 0
4d	44 ± 0	42 ± 0	40 ± 0	28 ± 0	20 ± 0.03	24 ± 0	29 ± 0
4c	12 ± 0.06	-ve	15 ± 0	-ve	15 ± 0.14	16 ± 0.19	13 ± 0.16
4a	38 ± 0	36 ± 0	35 ± 0	21 ± 0	16 ± 0.03	18 ± 0.06	23 ± 0.03
Penicillin G	11 ± 0	13 ± 0.03	12 ± 0	13 ± 0	-	-	-
Miconazole	-	-	-	-	11 ± 0	12 ± 0	11 ± 0

*The diameter of zones of inhibition (mm).

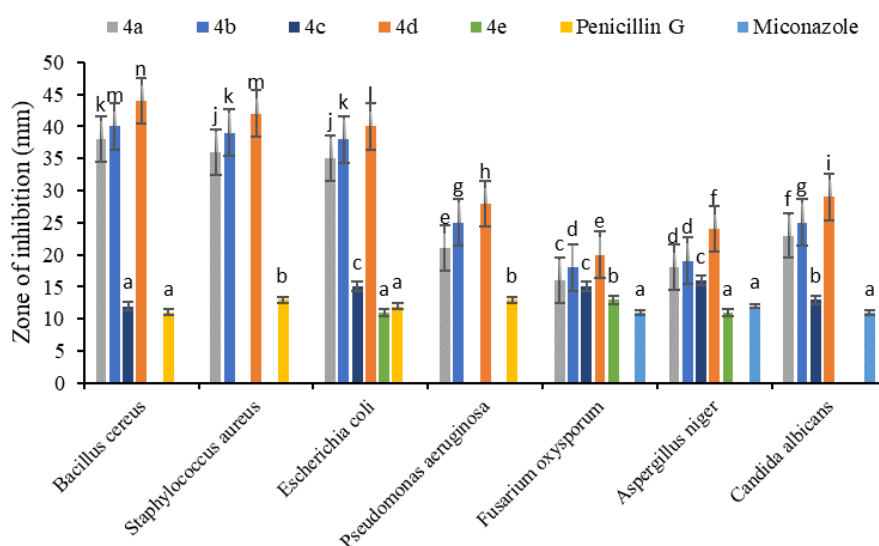


Figure 3 Agar well diffusion method of the tested compounds 4(a-e) against the selected microbial strains.

The antimicrobial activities of the synthesized azo sulphonamide derivatives of 5-sulfosalicylic acid 4(a-e), Scheme 1, were evaluated using MIC and MMC assays (Figs. 3-5). Compound 4d exhibited MIC values of 30, 40, 40, and 90 $\mu\text{g/ml}$ for *B. cereus*, *S. aureus*, *E. coli*, and *P. aeruginosa*, respectively. In comparison, the MICs for the commonly used antibiotics were 150, 140, and 140 $\mu\text{g/ml}$ for *B. cereus*, *S. aureus*, and *E. coli*, respectively. Notably, compounds 4a, 4b, and 4d demonstrated superior activity against the drug-resistant bacterial strain *P. aeruginosa* compared to penicillin G. Corresponding MMC values for 4d were 30, 40, 40, and 90 $\mu\text{g/mL}$, indicating that these derivatives were generally more effective against Gram-positive than Gram-negative bacteria. The antibacterial activities of both the compounds and the reference drugs were dose-dependent, increasing with concentration for all tested microorganisms. On the other hand, against fungi and yeast, the same compounds 4a, 4b, and 4d showed MIC and MMC values lower than those of the standard drug miconazole (Figures 4-6). Overall, these results suggest that the chemical structures of the synthesized derivatives 4(a-e) are promising scaffolds for the development of potential antibacterial and antifungal agents.

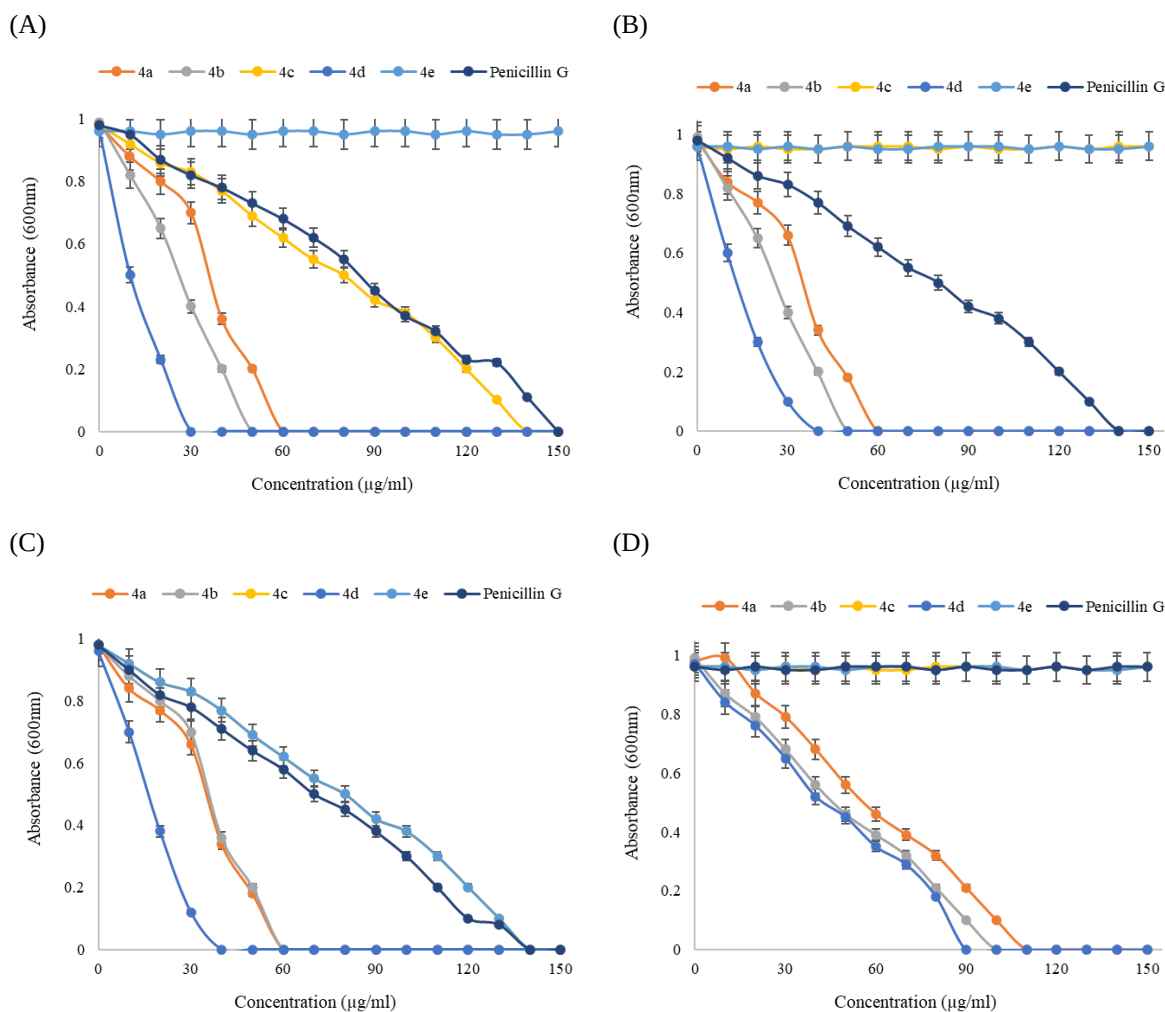


Figure 4 Minimum inhibition concentration (MIC) of compounds 4(a-e) against the tested bacterial strains including *B. cereus*; (A), *S. aureus*; (B); *E. coli*; (C), and *P. aeruginosa*; (D), compared to standards.

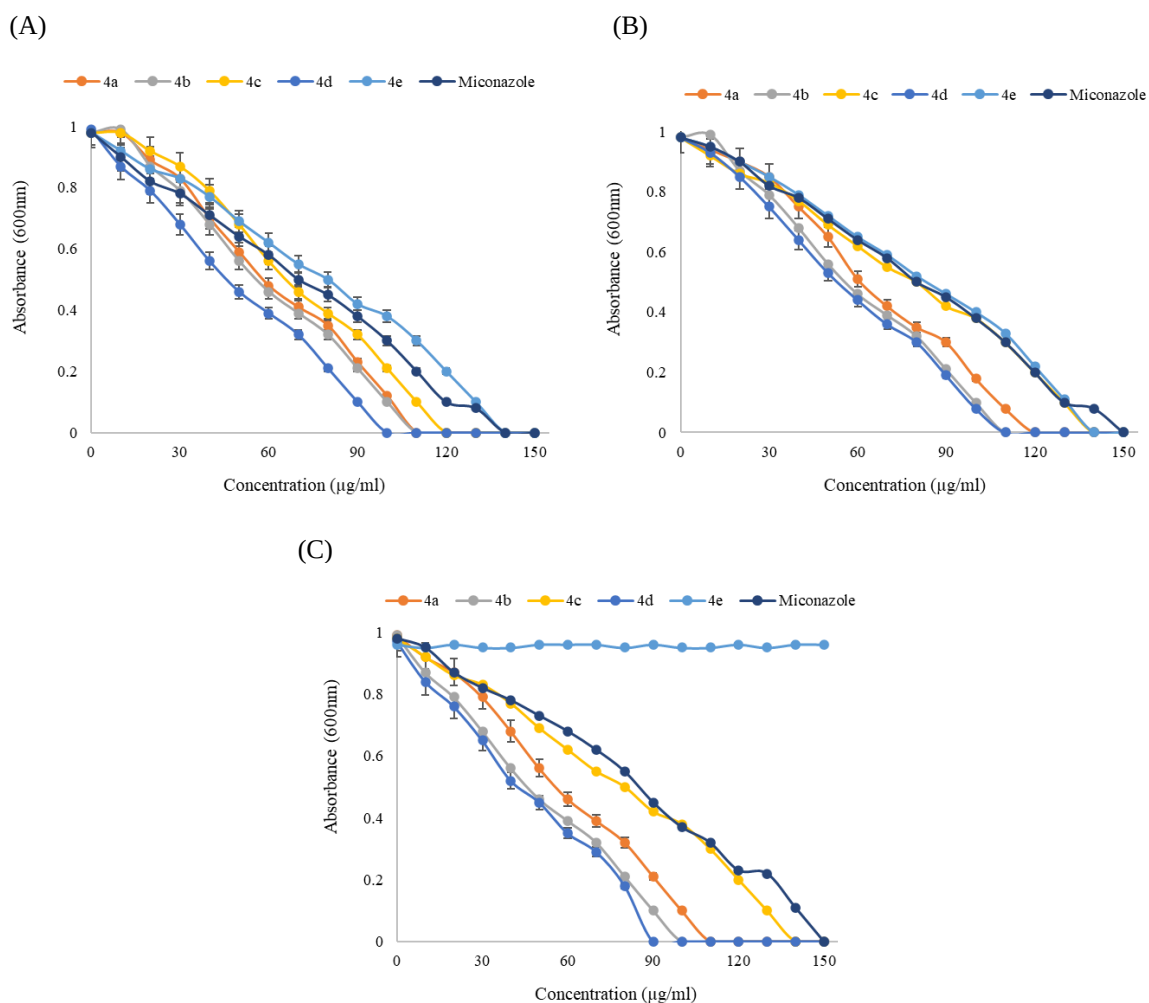


Figure 5 Minimum inhibition concentration (MIC) of compounds 4(a-e) against the tested fungal strains; *A. niger*; (A), and *F. oxysporum*; (B), as well as yeast *C. albicans*; (C) compared to standards.

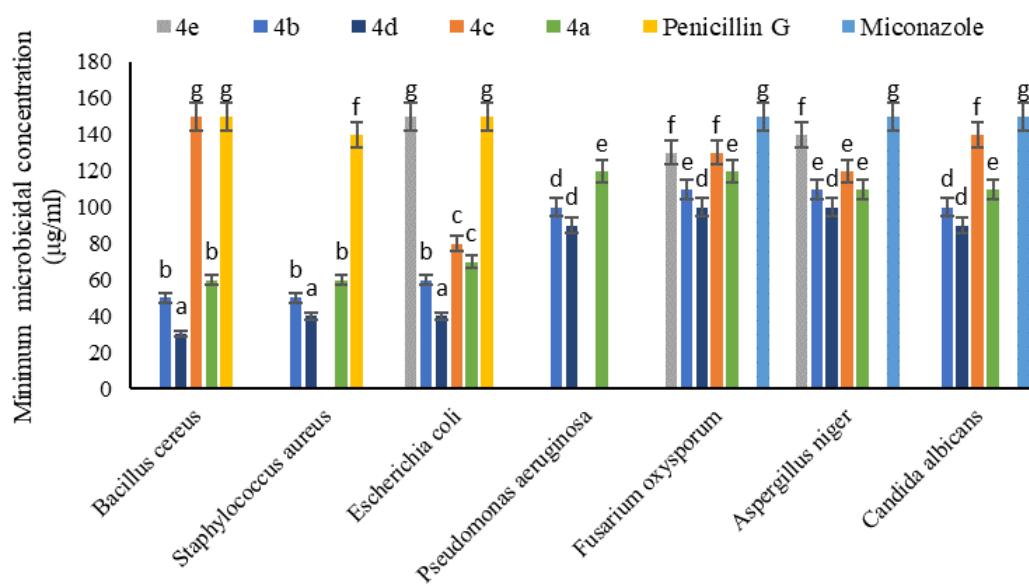


Figure 6 Minimum microbicidal concentration (MMC) of compounds 4(a-e) compared to standards.

Figure 8 Docking pose of ligand (4d) within the active site of *S. aureus* NDK (UniProt ID: Q5HFV4, chain A).

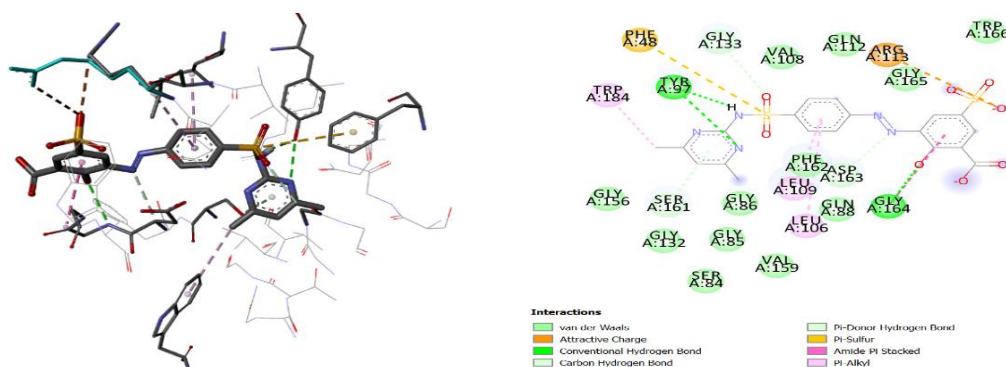


Figure 9 Docking pose of ligand (4d) within the active site of *E. coli* 1,4-Dihydroxy-2-naphthoyl-CoA synthase (UniProt ID: P0ABU0, chain A).

3.5 Structure-activity relationship and biological validation

To validate the theoretical framework, a correlation analysis was performed between the global reactivity descriptors (electronic softness and electrophilicity) and the experimental MIC values. As shown in Table 5, there is a high degree of synergy between the softness of the molecule and its ability to inhibit microbial growth. Compound 4d exhibits the highest electrophilicity index ($\omega = 21.14$ eV) and a low energy gap ($\Delta E = 1.717$ eV). This soft electronic profile facilitates rapid charge transfer to microbial targets, resulting in the lowest recorded MIC against *B. cereus* (30 $\mu\text{g/ml}$). Compound 4c has the lowest energy gap (1.713 eV); its activity was hindered by the pyridine moiety, which our docking suggests provides suboptimal orientation within the DHPS active site compared to the pyrimidine of 4d. Compound 4b possesses the largest energy gap (1.898 eV) and highest chemical hardness ($\eta = 0.949$ eV), which correlates with its slightly lower reactivity in the radical scavenging assay compared to 4a and 4d. The results confirm that compound 4d, characterized by the lowest energy gap and highest electrophilicity, demonstrates the most potent antimicrobial action. This relationship suggests that the dimethylpyrimidinyl moiety in 4d provides an optimal electronic environment for charge transfer between the ligand and the target enzymes (DHPS/NDK). The biological efficacy follows the trend of electronic softness (σ): 4d > 4a > 4e > 4b. This validation indicates that the synthesized azo-sulfonamides act as soft electrophiles that interact efficiently with the nucleophilic sites of microbial proteins. This quantitative agreement between theoretical indices and *in vitro* results provides the robust biological validation required for high-impact pharmacological reporting.

Table 5 Correlation between DFT electronic descriptors and antimicrobial potency (MIC).

Compound	ΔE (eV)	σ (eV ⁻¹)	ω (eV)	MIC ($\mu\text{g/ml}$)	
				<i>S. aureus</i>	<i>C. albicans</i>
4d	1.717	1.164	21.14	40	30
4a	1.722	1.161	21.07	60	40
4e	1.807	1.107	20.85	110	130
4b	1.898	1.054	19.66	60	100

3.6 Pharmacokinetic (ADMET) and toxicity profiling

A critical requirement for medicinal chemistry is the evaluation of a compound's drug-likeness. As shown in Table 6, the ProTox-III and pkCSM analyses of compound 4d indicate a highly favorable safety profile. Compound 4d demonstrated no predicted mutagenicity or cytotoxicity, with a high probability of inactivity across multiple nuclear receptor and stress response pathways (e.g., AhR, Nrf2/ARE). These results suggest that the synthesized azo sulphonamides are not only potent biocides but also possess the required safety margins for potential therapeutic application.

Table 6 Predicted ADMET and toxicity profile of lead derivative 4d.

Parameter	Classification	Prediction	Probability	Prediction
Cytotoxicity	Toxicity endpoint	Inactive	0.84	Inactive
Hepatotoxicity	Organ toxicity	Inactive	0.88	Inactive
AhR signaling	Nuclear receptor	Inactive	0.90	Inactive
Skin sensitization	Toxicity endpoint	Inactive	0.92	Inactive
Nrf2/ARE	Stress response	Inactive	0.99	Inactive

4. Conclusions

This study successfully achieved the design, synthesis, and mechanistic evaluation of five novel azo-sulfonamide hybrids built upon a trifunctional 5-sulfosalicylic acid core. Our experimental screening demonstrated that molecular hybridization yields an exceptional synergistic effect; lead derivative 4d exhibited remarkable broad-spectrum microbicidal profiles, outperforming conventional reference drugs such as penicillin G and miconazole by up to four-fold in inhibition zones. Crucially, quantum chemical profiling established a direct, predictable link between theoretical electronic softness, low HOMO-LUMO energy gaps, and advanced *in vitro* potency, validating our computational workflow as a reliable forecasting tool for sulfonamide drug development. This was corroborated by molecular docking simulations, which revealed that the target architectures achieve superior binding stability through intricate π - π aromatic stacking and electrostatic salt bridges within key microbial active sites. Furthermore, preliminary *in silico* ADMET mapping confirmed excellent drug-likeness compliance and high safety margins, showing no predicted mutagenicity or cytotoxicity. Taken together, these findings reveal that the azo-sulfonamide-5-sulfosalicylic acid framework represents a highly viable chemical space. Future efforts will focus on investigating the precise *in vivo* pharmacokinetic dynamics, evaluating systemic toxicity in animal models, and optimizing advanced nano-formulations to facilitate the development of these promising therapeutic candidates.

5. Ethical approval

The study was carried out in accordance with the guidelines and regulations of the scientific research ethics committee, Faculty of Science, Damietta University, Egypt. This article does not contain any studies with human or animal subjects.

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7. Authors' contributions

S.H.A., M.M.E., E.N., and I.E. performed the practical work. S.H.A., M.M.E., E.N., M.M.A.M. and I.E. analysed and interpreted the data. Computational data performed by S.H.A., M.M.A.M. and I.E. supervised the overall work. S.H.A., M.M.E., M.M.A.M. and I.E. wrote the original draft. The ideal of the work is suggested by M.M.A.M. All authors reviewed and edited the manuscript. All authors read and approved the final manuscript.

8. Conflicts of interest

The authors report no conflicts of interest in this work.

9. References

- [1] Amaratunga K, Tarasuk J, Tsegaye L, Archibald CP. Advancing surveillance of antimicrobial resistance: Summary of the 2015 CIDSC Report. *Can Commun Dis Rep.* 2016;42(11):232-237.
- [2] Gautam A, Sasmal PK. Eradication of antibiotic-resistant Gram-positive bacteria and biofilms by rationally designed AIE-active iridium(III) complexes derived from cyclometalating 2-phenylquinoline and ancillary bipyridyl ligands. *Inorg Chem.* 2025;64(6):2905–2918.
- [3] Belay WY, Getachew M, Tegegne BA, Teffera ZH, Dagne A, Zeleke TK, et al. Mechanism of antibacterial resistance, strategies and next-generation antimicrobials to contain antimicrobial resistance: A review. *Front Pharmacol.* 2024;15:1444781.
- [4] Samuel Y, Garg A, Mulugeta E. Synthesis, DFT analysis, and evaluation of antibacterial and antioxidant activities of sulfathiazole derivatives combined with *in silico* molecular docking and ADMET predictions. *Biochem Res Int.* 2021;2021:7534561.
- [5] Thakral S, Singh V. Recent development on importance of heterocyclic amides as potential bioactive molecules: A review. *Curr Bioact Compd.* 2019;15(3):316–336.

- [6] Martínez-Montiel M, Arrighi G, Begines P, González-Bakker A, Puerta A, Merino-Montiel P, Montiel-Smith S, Nocentini A, Supuran CT, Padrón JM, Fernández-Bolaños JG, López O. Multifaceted sulfonamide-derived thiosemicarbazones: Combining metal chelation and carbonic anhydrases inhibition in anticancer therapy. *Int J Mol Sci.* 2025;26(3):1225.
- [7] Lipskikh OI, Korotkova EI, Khristunova YP, Barek J, Kratochvil B. Sensors for voltammetric determination of food azo dyes - A critical review. *Electrochim Acta.* 2018;260:974–985.
- [8] Gümüş M, Sert Y, Koca I. Synthesis, characterization and theoretical studies of novel sulfonamide-aldehydes derivatives having tautomeric forms. *Org Commun.* 2019;12(4):176–187.
- [9] Yadav A, Kaushik CP. Synthesis and antibacterial evaluation of sulfonamide bridged disubstituted 1,2,3-triazoles. *Synth Commun.* 2022;52(24):2261–2275.
- [10] Zahran MK, Gaffer HES, Mashaly HM. Dyeing and antibacterial properties of some newly synthesized sulfonamide reactive dyes. *Macromol Symp.* 2019;384(1):1800166.
- [11] Alfare K, Lee D, Scharrer E, Van Arman SA. Preparation of azo dyes from sulfanilamide. *Chem Educ.* 2004;9(2):89-90.
- [12] AL-Muhsin AA, Ali AA. Preparation, characterization, applications and analytical studies of some new azo dyes derived from sulfanilamide. *Bull Pharm Sci Assiut University.* 2024;48(1):319-333.
- [13] Becke AD. Density-functional exchange-energy approximation with correct asymptotic behavior. *Phys Rev A Gen Phys.* 1988;38(6):3098–3100.
- [14] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 27th ed. Wayne (PA): Clinical and Laboratory Standards Institute; 2017.
- [15] Elshikiby L, Baka Z, El-Zahed M. Antimicrobial activity of biosynthesized selenium nanoparticles using *Pseudomonas aeruginosa* isolate. *Scientific Journal for Damietta Faculty of Science.* 2025;15(2):94-106.
- [16] Fayed RM, Baka ZAM, Farouk BH, El-Zahed MM. Antibacterial and cytotoxic activities of a newly green synthesized ZnO/Se nanocomposite combined with *Washingtonia robusta* H. Wendl fruit extract. *Biocatal Agric Biotechnol.* 2025;64:103500.
- [17] Li HJ, Li X, Liu N, Zhang H, Truglio JJ, Mishra S, et al. Mechanism of the intramolecular Claisen condensation reaction catalyzed by MenB, a crotonase superfamily member. *Biochemistry.* 2011;50(44):9532–9544.
- [18] Selvaraj V, Swarna Karthika T, Mansiya C, Alagar M. An overview on recently developed techniques, mechanisms and intermediate involved in the advanced azo dye degradation for industrial applications. *J Mol Struct.* 2021;1224.
- [19] Chen Y, Dral PO. AIQM2: organic reaction simulations beyond DFT. *Chem Sci.* 2025;16(35):15901-15912.
- [20] El-Rahman MA, Omar AZ, Kandeel AR, Hamed EA, El-Atawy MA, Keshk RM. Synthesis, characterization, and polyester dyeing performance of azo barbituric and thiobarbituric acid disperse dyes. *Sci Rep.* 2025;15(1):13410.
- [21] Khadikar PV, Joshi S, Kashkhedikar SG, Heda BD. Metal-complexes of 5-sulfosalicylic acid and their antimicrobial activity. *Indian J Pharm Sci.* 1984;46(6):209–211.
- [22] Verma V, Joshi CP, Agarwal A, Soni S, Kataria U. A review on pharmacological aspects of pyrimidine derivatives. *J Drug Deliv Ther.* 2020;10(5):358–361.
- [23] Onur R, Özden M, Orhan I, Kalkan A, Semercioz A. Incidence of bacteraemia after urodynamic study. *J Hosp Infect.* 2004;57(3):241–244.
- [24] Storozhenko S, Navarrete O, Ravanel S, De Brouwer V, Chaerle P, Zhang GF, et al. Cytosolic hydroxymethyldihydropterin pyrophosphokinase/dihydropteroate synthase from *Arabidopsis thaliana*: A specific role in early development and stress response. *J Biol Chem.* 2007;282(14):10749–10761.
- [25] Essghaier B, Naouar A, Abdelhak J, Faouzi Zid M, Sadfi-Zouaoui N. Synthesis, crystal structure and potential antimicrobial activities of di (4-sulfamoyl-phenyl-ammonium) sulphate. *Microbiol Res.* 2014;169(7):504–510.
- [26] Bondock S, Rabie R, Etman HA, Fadda AA. Synthesis and antimicrobial activity of some new heterocycles incorporating antipyrine moiety. *Eur J Med Chem.* 2008;43(10):2122–2129.

- [27] Marinescu M, Popa C-V. Pyridine compounds with antimicrobial and antiviral activities. *Int J Mol Sci.* 2022;23(10):5659.
- [28] El-Fallal AA, El-Dein MMN, El-Esseily SR, Mansour NM, El-Zahed MM. Antimicrobial, antibiofilm, DNA binding, and antioxidant activities of green synthesized zinc oxide nanoparticles using the wild mushroom *Lepista sordida* (Schumacher) Singer EGDA2. *Microb Cell Fact.* 2026;25:90.
- [29] Abou-Dobara MI, Kamel MA, El-Sayed AKA, El-Zahed MM. Antibacterial activity of extracellular biosynthesized iron oxide nanoparticles against locally isolated β -lactamase-producing *Escherichia coli* from Egypt. *Discov Appl Sci.* 2024;6(3):113.
- [30] Keniya MV, Sabherwal M, Wilson RK, Woods MA, Sagatova AA, Tyndall JDA, et al. Crystal structures of full-length lanosterol 14 α -demethylases of prominent fungal pathogens *Candida albicans* and *Candida glabrata* provide tools for antifungal discovery. *Antimicrob Agents Chemother.* 2018;62(11):1110-1128.
- [31] Yousif MNM, El-Gazzar A-RBA, Hafez HN, Fayed AA, ElRashedy A, Yousif NM. Recent advances in the chemistry and biological activity of sulfonamide derivatives. *Mini Rev Org Chem.* 2022;19(6):695–707.