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Synthesis, anti-bacterial evaluation and in silico ADMET study of novel sydnones

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Abstract

In the present study, a series of novel sydnone-based derivatives (AS_{a-e}) incorporating chalcone and substituted arylamide/morpholine moieties were synthesized and evaluated for their antibacterial activity and pharmacokinetic characteristics. The target compounds were synthesized through a multi-step reaction sequence and characterized by physicochemical parameters, thin-layer chromatography (TLC), Fourier-transform infrared (FTIR) spectroscopy, and ^{1}H NMR spectroscopy. Antibacterial activity was assessed against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method at concentrations ranging from 25 to 100 µg/mL. All synthesized compounds exhibited concentration-dependent antibacterial activity, with the halogen-substituted derivatives showing superior efficacy compared to non-halogenated analogues. Among the tested compounds, AS_d, containing a bromo-substituted phenyl group, demonstrated the highest antibacterial activity, producing inhibition zones of up to 19 mm against *E. coli* and 16 mm against *S. aureus*. In silico ADMET and physicochemical assessments revealed that all compounds complied with Lipinski's rule of five, possessed acceptable bioavailability profiles, and exhibited favorable pharmacokinetic characteristics. The findings suggest that sydnone-chalcone hybrids, particularly halogenated derivatives, represent promising lead molecules for the development of new antibacterial agents and warrant further biological investigation.

Keywords: Sydnone, chalcone, anti-bacterial, mesoionic, *in-silico*

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Introduction

The rising occurrence of infectious illnesses and fast advancement of antibiotic resistance have become significant concerns to the world's health care systems. The overuse of antibiotics has resulted in the creation of resistant bacterial strains, limiting the efficacy of conventional antimicrobial treatments. Therefore, there is an urgent need for discovery and development of new chemotherapeutic drugs with increased antibacterial activity, good pharmacokinetic qualities and low toxicity. Heterocyclic compounds remain a major focus of medicinal chemistry due to their wide range of biological activity and potential as adaptable scaffolding for drug discovery [1].

Sydnones are an important class of mesoionic chemicals with a distinctive 1,2,3-oxadiazolium-5-olate ring structure among the diverse heterocyclic systems. Sydnone derivatives have a wide variety of pharmacological actions such as antibacterial, anti-inflammatory, analgesic, anticancer, antiviral and antioxidant effects due to their unique electronic structure. The sydnone nucleus has been extensively studied by structural alteration to develop novel derivatives with increased biological activity and drug-like properties. The potential of sydnones to interact with many biological targets makes them intriguing candidates for the creation of novel therapeutic treatments [2,5].

Chalcones are natural and synthetic compounds possessing an α,β-unsaturated ketone moiety, which is an essential pharmacophoric unit in medicinal chemistry. Several chalcone compounds have been reported to exhibit promising antibacterial, antifungal, anti-inflammatory, antioxidant and anticancer effects. The conjugated structure of chalcones allows it to interact with different microbial enzymes and cellular components, causing disruption of vital biological processes. The pharmacological properties of chalcone-containing compounds have attracted substantial attention in the hunt for novel antibacterial medicines.

Molecular hybridization technique, whereby two or more bioactive pharmacophores are incorporated into a single molecular framework has emerged as a viable approach for the production of potent medicinal medicines. The sydnone scaffold combined with a chalcone moiety is intended to generate synergistic effects by combining the favourable biological properties of both pharmacophores. In addition, the presence of various amine and halogen-substituted aromatic groups can strongly influence the lipophilicity, membrane permeability, target affinity and hence the total biological activity. Halogen substitution has been shown to increase antibacterial potency via increasing molecule stability and interactions with microbial targets.

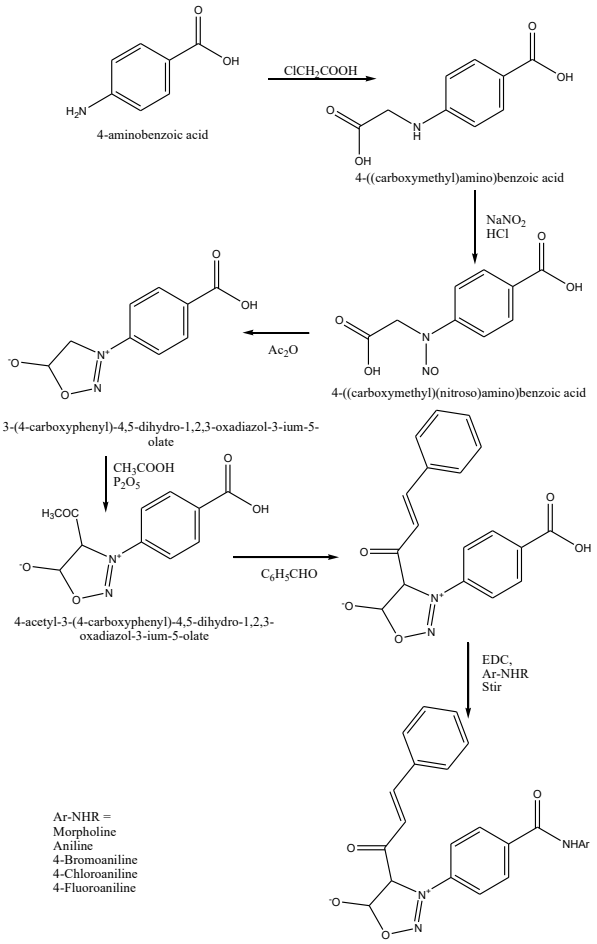
In recent years, computational methods have become a crucial tool in early drug development. In silico ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) prediction may

give a good understanding of the pharmacokinetic behaviour, drug-likeness and safety profile of freshly synthesized compounds before thorough biological examination. Such prediction investigations are useful in identifying prospective lead molecules and in rational design of drugs with better therapeutic potential.

Thus, the present study was aimed to synthesis a variety of new sydnone derivatives bearing chalcone and modified arylamide/morpholine functionalities. The compounds were synthesized and characterized by physicochemical and spectroscopic techniques and screened for their antibacterial activity against selected Gram-positive and Gram-negative bacterial strains, besides being subjected to *in silico* ADMET studies for their drug-likeness and pharmacokinetic properties. The results of this study may provide a basis for the creation of novel antimicrobial drugs derived from sydnone against the increasing problem of bacterial resistance.

Material and Methods

The experimental scheme was adapted from the previous reports by modification in conditions and optimized for obtaining highest yield (Scheme 1) [6-8].



Scheme 1

The synthesis has been achieved into 6 steps involving synthesis of glycine derivative in the first step, followed by its nitroso salt formation in the second step; the third step involved the cyclization to sydnone nucleus followed by acylation of the sydnone ring in the fourth step, preparation of imine derivative in the fifth step and finally reaction of the carboxyl group with various anilines in the sixth step leading to the formation of target compounds **(AS_{a-e})**.

Synthesis of 4-((caboxymethyl)amino)benzoic acid

Chloroacetic acid (0.6 mol), and 4-aminobenzoic acid (0.5 mol) in 60 ml of ethanol were refluxed for 6 h. The reaction mixture was left overnight at room temperature and poured into ice-cold water; a precipitate of 4-((carboxymethyl)amino)benzoic acid. To the precipitate was added sodium hydroxide (0.5 mol) in 150 ml of water and the mixture was refluxed for 0.5 h. After cooling, the reaction mixture was acidified to pH 2 using hydrochloric acid in an ice bath. The precipitate of 4-((carboxymethyl)amino)benzoic acid was obtained, filtered and dried [9].

Synthesis of 4-((carboxymethyl)(nitroso)amino)benzoic acid

A suspension of 4-((carboxymethyl)amino)benzoic acid (0.05 mol) in water (20 ml) was cooled in an ice bath to 0°C. To the suspension was added dropwise concentrated hydrochloric acid (0.05 mol) over a duration of 0.5 h under stirring. The stirring was continued for 2.5 h and then a solution of sodium nitrite (0.05 mol) in water (20 ml) was added dropwise to it, maintaining the temperature to 0°C. The mixture was stirred for 1h and dichloromethane (20 mL) was added to it and stirred for 15 min. The organic layer was dried over sodium sulphate and evaporated to obtain 4-((carboxymethyl) (nitroso)amino)benzoic acid [10].

Synthesis of Sydnone

The 4-((carboxymethyl)(nitroso)amino)benzoic acid was dissolved in acetic anhydride (25 mL) and the reaction mixture was left in a dark overnight at room temperature. Then the reaction mixture was poured onto cold water (50 mL) with cooling and forceful stirring and was neutralized with sodium bicarbonate. The product was extracted thrice with dichloromethane (20 mL) and the organic layer was dried over sodium sulphate, filtered and evaporated to obtain the sydnone afford the crude sydnone [11].

Synthesis of 4-acetyl-3-(4-carboxyphenyl)-4,5-dihydro-1,2,3-oxadiazol-3-ium-5-olate

To a suspension of (0.015 mol) of phosphorus pentoxide in 125 mL of benzene in a three-necked round bottom flask fitted with a reflux condenser equipped with a calcium chloride drying tube was added (0.005 mol) of sydnone. The magnetically stirred mixture was heated to reflux and

glacial acetic acid (0.005 mol) was added dropwise, through a dropping funnel, over a 10 minute period. The stirred reaction mixture was refluxed for 5 hours during which the resultant clear solution turned brown-black. After cooling to room temperature, the benzene was decanted and the remaining black residue washed twice with 20 mL of benzene. The combined washings and decants were evaporated to dryness to yield a solid which was recrystallized from ethanol to obtain 4-acetyl-3-(4-carboxyphenyl)-4,5-dihydro-1,2,3-oxadiazol-3-ium-5-olate [12].

Synthesis of 3-(4-carboxyphenyl)-4-cinnamoyl-4,5-dihydro-1,2,3-oxadiazol-3-ium-5-olate

To a suspension of 4-acetyl-3-(4-(methoxycarbonyl)phenyl)-4,5-dihydro-1,2,3-oxadiazol-3-ium-5-olate (0.005 mol) in ethanol (20 mL) was added sodium hydroxide (10% w/v, 10 mL). The solution was cooled in an ice bath to 5-10°C and benzaldehyde (0.005 mol) was added to it with stirring. The stirring was continued for up to 4 h and the precipitate obtained was filtered off and recrystallized from ethanol-ethylacetate (1:1) to obtain 3-(4-carboxyphenyl)-4-cinnamoyl-4,5-dihydro-1,2,3-oxadiazol-3-ium-5-olate [13].

General method for synthesis of target compounds, AS_{a-e}

The measured quantity of 3-(4-carboxyphenyl)-4-cinnamoyl-4,5-dihydro-1,2,3-oxadiazol-3-ium-5-olate (0.001 mol) and appropriate amine (0.001 mol) were dissolved in dichloromethane. To the reaction mixture was added EDC (0.0012 mol) and the mixture was stirred vigorously at 40°C under reflux. The precipitate was filtered and the solvent was evaporated under reduced pressure to obtain the target compopunds [14].

Characterization of compounds

The synthesized compounds were subjected to characterization for solubility, melting point, yield, ^{1}H NMR study, and IR spectral study [15,16].

Thin Layer Chromatograph (TLC)

A diluted sample of the compounds was spotted on a precoated TLC plate at distance one cm above the bottom of the plate. The plate was dipped in a selected solvent system and the solvent was allowed to climb up the plate till one cm from the top of the plate. The plate was removed from the solvent systems and allowed to air dry for few seconds. The plate was visualized in a UV cabinet.

Antimicrobial Study

The microorganisms used for the antimicrobial study were procured from Institute of Microbial Technology, Chandigarh (MTCC). *Escherichia coli* (MTCC 40), and *Staphylococcus aureus* (MTCC 3160) were used for the present investigation. The lyophilized cultures obtained from IMT, Chandigarh were revived by adding 0.3 mL of nutrient broth to the culture ampoules to obtain a suspension of the bacteria.

Agar plates were prepared by pouring the sterilized medium into sterilized petridishes suitably marked and labelled. The plates were allowed to solidify in the laminar flow bench and stored packed for culturing with microbes and antimicrobial screening.

Preparation of test compounds

The synthesized compounds **As**$_{a\text{-}e}$ were dissolved in DMSO to obtain the solutions of 25, 50, 75 & 100 μg/mL. These solutions were used as the test samples.

Determination of zone of inhibition

About 3 mm thick pre-poured nutrient agar plates were inoculated with a few drops of the bacterial suspension by swabbing on the surface of agar. The antimicrobial action was screened using disc diffusion method [17]. Wells were bored into the agar plate at equal distances using cork borer (10mm) and 200μL of the sydnone derivative (25, 50, 75 & 100 μg/mL) were placed in each hole. The plates were incubated for 24h at 37 ± 0.1°C to allow for microbial growth. The zone of inhibition in each plate was measured in millimeters.

***In silico* ADMET study**

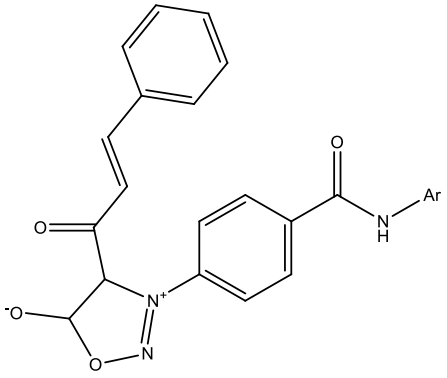
The 2D structures of the ligands were sketched on ChemDraw Ultra 12.0 and then transferred on Chem 3D to create the three-dimensional structures. The canonical SMILES (simplified molecular-input line-entry system) were generated using ChemDraw and then submitted to SwissADME online tools for the pharmcokinetic analysis, the prediction of physicochemical parameters and the drug-likeness using the Lipinski rule of five. The so-called Rule-of-five of Lipinski delineated the relationship between pharmacokinetic and physicochemical parameters.

Results and Discussion

The sydnone-morpholine derivatives (**AS**$_{a\text{-}e}$) were synthesized in 6 steps involving synthesis of glycine derivative in the first step, followed by its nitroso salt formation in the second step; the third step involved the cyclization to sydnone nucleus followed by acylation of the sydnone ring

in the fourth step, preparation of imine derivative in the fifth step and finally reaction of the carboxyl group with amine in the sixth step leading to the formation of target compounds **(AS_{a-e})** (Table 1).

The synthesized conjugates were characterized by determining the practical yield, melting point, solubility (Table 2) and spectral studies.



General structure of the AS_{a-e}

Table 1. Yield and color of As_{a-e}

Compound code	Ar	Molecular formula	Yield (%)	Color
AS_a	Morpholine	$C_{22}H_{21}N_3O_5$	69	Yellow
AS_b	Aniline	$C_{24}H_{19}N_3O_4$	68	Off-white
AS_c	4-chloroaniline	$C_{24}H_{18}ClN_3O_4$	71	Dark Yellow
AS_d	4-bromoaniline	$C_{24}H_{18}BrN_3O_4$	65	Pale Yellow
AS_e	4-fluoroaniline	$C_{24}H_{18}FN_3O_4$	63	Dark Yellow

Table 2. Physical Properties of the synthesized compounds As_{a-e}

Compound code	Molecular Weight	R_f Value	Melting point (°C)
AS_a	407.15	0.67	198-200
AS_b	413.43	0.58	214-216
AS_c	447.87	0.71	200-202
AS_d	492.35	0.69	232-234
AS_e	431.42	0.59	209-211

All the compounds were soluble in methanol, chloroform and dimethylsulfoxide.

The confirmation of the structures of the compounds was done by ^{1}H-NMR and FT-IR spectral study, which validated the presence of key functional groups such as sydnone protons (δ 5.48–6.02 ppm), aromatic and chalcone protons (δ 6.8–7.9 ppm), and characteristic IR stretches for carbonyl (1653–1718 cm^{-1}), imine (1436–1461 cm^{-1}), and carbamoyl linkages. The halogenated derivatives (**AS_c–AS_e**) additionally exhibited diagnostic absorptions for C–Cl, C–Br, and C–F bonds, confirming successful substitution. These structural features are directly relevant to antimicrobial potency, as halogen substitution often enhances lipophilicity, membrane penetration, and target affinity.

4-cinnamoyl-3-(4-(morpholine-4-carbonyl)phenyl)-4,5-dihydro-1,2,3-oxadiazol-3-ium-5-olate, AS_a

^{1}HNMR ($CDCl_3$, δ ppm) – 5.48, 6.02 (C-H, sydnone), 7.38 to 7.97 (C-H, aromatic), 6.89, 7.59 (C-H, chalcone), 3.64, 3.52 (C-H, morpholine); FT-IR (cm^{-1}) – 1718.3 cm^{-1} (C=O), 1448.1 cm^{-1} (C=N), 1170.4 cm^{-1} (C-N) and bending vibrations at around 743.6 cm^{-1} (C-N)

4-cinnamoyl-3-(4-(phenylcarbamoyl)phenyl)-4,5-dihydro-1,2,3-oxadiazol-3-ium-5-olate, AS_b

^{1}HNMR ($CDCl_3$, δ ppm) – 9.33 (NH), 8.06 (CH, ring attached to sydnone), 5.48 (C-H, sydnone), 6.02 (CH, chalcone), 6.89 to 7.68 (C-H, aromatic); FT-IR (cm^{-1}) – 3298 cm^{-1} (N-H), 1461 cm^{-1} (C=N), 1177 cm^{-1} (C-N) and bending vibrations at around 751 cm^{-1} (C-N)

3-(4-((4-chlorophenyl)carbamoyl)phenyl)-4-cinnamoyl-4,5-dihydro-1,2,3-oxadiazol-3-ium-5-olate, AS_c

^{1}HNMR ($CDCl_3$, δ ppm) – 8.91 (NH), 8.06 (CH, ring attached to sydnone), 5.48 (C-H, sydnone), 6.02 (CH, chalcone), 6.89 to 7.71 (C-H, aromatic); FT-IR (cm^{-1}) – 3337 cm^{-1} (N-H), 1653 (C=O), 1459 cm^{-1} (C=N), 1127 cm^{-1} (C-N), 812 (C-Cl), and bending vibrations at 749 cm^{-1} (C-N)

3-(4-((4-bromophenyl)carbamoyl)phenyl)-4-cinnamoyl-4,5-dihydro-1,2,3-oxadiazol-3-ium-5-olate, AS_d

^{1}HNMR ($CDCl_3$, δ ppm) – 8.91 (NH), 8.06 (CH, ring attached to sydnone), 5.48 (C-H, sydnone), 6.02 (CH, chalcone), 6.89 to 7.64 (C-H, aromatic); FT-IR (cm^{-1}) – 3371 cm^{-1} (N-H), 1718 cm^{-1} (C=O), 1436 cm^{-1} (C=N), 1170 cm^{-1} (C-N), C-Br stretch below 700 and bending vibrations at 775 cm^{-1} (C-N)

4-cinnamoyl-3-(4-((4-fluorophenyl)carbamoyl)phenyl)-4,5-dihydro-1,2,3-oxadiazol-3-ium-5-olate, AS_d

^{1}HNMR ($CDCl_3$, δ ppm) – 9.01 (NH), 8.06 (CH, ring attached to sydnone), 5.48 (C-H, sydnone), 6.02 (CH, chalcone), 6.89 to 7.59 (C-H, aromatic); FT-IR (cm^{-1}) – 3363 cm^{-1} (N-H), 1653 cm^{-1} (C=O), 1459 cm^{-1} (C=N), 1170 cm^{-1} (C-N), 1172 (C-F) and bending vibrations at 812 cm^{-1} (C-N)

Antimicrobial activity

The zone of inhibition was measured to assess the preliminary antibacterial activity of the sydnone-morpholine derivatives. Four concentrations of the sydnone derivatives were tested for antibacterial action (Table 3).

Table 3. Zone of inhibition exhibited by compounds

Compound Code	Zone of Inhibition (mm)*							
	E. coli				*S. aureus*			
	25µg	50µg	75µg	100µg	25µg	50µg	750µg	100µg
AS_a	10	10	14	16	10	10	11	12
AS_b	10	10	12	13	10	10	11	12
AS_c	10	10	15	17	10	10	12	13

AS_d	10	12	**17**	**19**	10	10	**15**	**16**
AS_e	10	10	15	16	10	10	13	14

The antibacterial screening against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) revealed concentration-dependent activity across all compounds (Table 3.5). At lower concentrations (25–50 μg), the inhibition zones were modest (10–12 mm), indicating baseline activity. However, at higher concentrations (75–100 μg), significant enhancement was observed, particularly for halogenated derivatives. **AS_a and AS_b** showed moderate activity, with inhibition zones reaching 14–16 mm (*E. coli*) and 12–13 mm (*S. aureus*). Their morpholine and phenylcarbamoyl substitutions provided solubility advantages but comparatively weaker penetration. **AS_c** (chloro derivative) exhibited stronger activity (15–17 mm against *E. coli*, 12–13 mm against *S. aureus*), consistent with the electron-withdrawing effect of chlorine enhancing target binding. **AS_d** (bromo derivative) demonstrated the highest potency, with inhibition zones up to 19 mm (*E. coli*) and 16 mm (*S. aureus*). The larger, more polarizable bromine atom likely facilitated stronger hydrophobic interactions with bacterial membranes. **AS_e** (fluoro derivative) showed balanced activity (15–16 mm against *E. coli*, 13–14 mm against *S. aureus*), reflecting fluorine's ability to modulate lipophilicity and electronic distribution without severely compromising solubility.

In silico physicochemical properties

The SMILES of the synthesized derivatives **AS_{a-e}** was generated using chemdraw ultra 12.0 software. The physicochemical properties of the compounds predicted *in silico* by SwissADME are presented in table 4.

Table 4. Physicochemical properties of AS_{a-e}

Compound Code	HBD	HBA	Log P	NRB	PSA (A)	MR	Log S
AS_a	0	6	2.02	6	94.27	117.76	-4.29
AS_b	1	5	3.48	7	93.83	121.58	-5.50
AS_c	1	5	4.08	7	93.83	126.59	-6.10
AS_d	1	5	4.16	7	93.83	129.28	-6.41
AS_e	1	6	3.84	7	93.83	121.54	-5.66

All the compounds exhibited drug likeliness and obeyed the Lipinski's rule of five. The compounds had bioavailability score of 0.55 and possessed high gastric absorption and ability to permeate the blood brain barrier.

The physicochemical profiling of compounds **AS$_{a-e}$** provides valuable insights into their potential antimicrobial efficacy. All derivatives obeyed Lipinski's rule of five, confirming drug-likeness and supporting oral bioavailability. The hydrogen bond donor (HBD) and acceptor (HBA) counts (0–1 and 5–6, respectively) are consistent with favorable interactions at microbial enzyme active sites, particularly those involved in folate biosynthesis and cell wall synthesis. Such balanced hydrogen bonding capacity facilitates both target binding and membrane permeability.

The log P values (2.02–4.16) indicate moderate lipophilicity, which is crucial for antimicrobial activity. Compounds with higher log P (**AS$_c$, AS$_d$**) are more likely to penetrate bacterial membranes, enhancing intracellular accumulation and target inhibition. However, excessive lipophilicity correlates with reduced aqueous solubility (Log S −6.10 to −6.41), which may limit bioavailability. Conversely, **AS$_a$**, with lower log P (2.02) and better solubility (−4.29), may exhibit improved systemic distribution but comparatively weaker membrane penetration.

The polar surface area (PSA) values (~94 Å^2) fall within the optimal range (<140 Å^2) for passive diffusion, supporting both gastrointestinal absorption and microbial cell wall penetration. The number of rotatable bonds (NRB) (6–7) suggests moderate conformational flexibility, which can enhance adaptability to diverse microbial targets without compromising binding affinity. The molar refractivity (MR) values (117–129) reflect suitable molecular size and polarizability, favoring interactions with bacterial enzymes and receptors.

Conclusion

The present investigation successfully demonstrated the synthesis, characterization, and preliminary antimicrobial evaluation of novel sydnone derivatives. Spectral analyses (FT-IR, ^{1}H-NMR) confirmed the structural integrity of the synthesized compounds, while physicochemical profiling established their compliance with Lipinski's rule of five, supporting drug-likeness and oral bioavailability.

In vitro antibacterial screening revealed that all compounds exhibited measurable activity against *E. coli* and *S. aureus*, with inhibition zones increasing in a concentration-dependent manner. Notably, halogen-substituted derivatives (**AS$_c$–AS$_e$**) showed enhanced potency compared to non-halogenated analogues, highlighting the importance of electronic and lipophilic modulation in antimicrobial efficacy. Among them, **AS$_d$** (bromo derivative) emerged as the most promising

candidate, displaying the largest inhibition zones and strong bacterial membrane penetration potential.

Overall, the study establishes sydnone–chalcone scaffolds as viable leads for antimicrobial drug development. The balance between solubility, lipophilicity, and bioavailability underscores the need for rational optimization, while the observed structure–activity relationship provides a foundation for further SAR-guided design, advanced biological assays, and formulation strategies to enhance therapeutic potential.

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