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HETEROCYCLIC SCAFFOLDS IN ANTIBIOFILM STRATEGIES AGAINST DRUG-RESISTANT PATHOGENS: A COMPREHENSIVE REVIEW

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ABSTRACT

Background: Antimicrobial resistance (AMR) is a primary global health concern, exacerbated by the ability of drug-resistant pathogens to form biofilms. These biofilms, which harbor microbial communities embedded in an extracellular polymeric matrix (EPS), enhance antibiotic resistance and immune responses, leading to persistent infections. Heterocyclic compounds have shown significant potential in combating biofilm-associated infections due to their structural diversity and mechanisms of action. **Methodology:** This review systematically examines the antibiofilm potential of various heterocyclic scaffolds, including imidazoles, pyrazoles, indoles, quinolines, coumarins, and select six-membered heterocycles (pyridine, morpholine, piperazine). Studies were analyzed based on their mechanisms of action, structure-activity relationships (SAR), and synergy with conventional antibiotics. **Result and Discussion:** Imidazole derivatives disrupted biofilm integrity and enhanced antibiotic susceptibility in *Pseudomonas aeruginosa* and *Staphylococcus aureus*, with IC_{50} values ranging from 0.53 to 9.5 µM. Pyrazole-based compounds inhibited Staphylococcus epidermidis biofilms, with IC50 values ranging from 3.1 to 15.6 µg/mL. Indole derivatives, particularly pyrroloindoline triazole amides, inhibited MRSA biofilms with IC_{50} values as low as 2.8 µM by targeting quorum sensing and curli production. Quinoline compounds demonstrated greater than 90% inhibition of *E. coli* and *P. aeruginosa* biofilms and showed synergistic effects with antibiotics. **Conclusion:** Heterocyclic compounds exhibit promising antibiofilm activity, presenting a viable approach to overcoming AMR. These compounds not only disrupt biofilm formation but also enhance the efficacy of conventional antibiotics through synergistic interactions. Such synergy potentiates the antimicrobial effect by improving antibiotic penetration or disrupting resistance pathways. Future research should focus on optimizing pharmacokinetics and exploring these synergistic combinations to improve clinical applicability.

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INTRODUCTION

Antimicrobial therapy is increasingly challenged by bacterial and fungal resistance, an adaptation that enhances their survival. Antibiotic resistance poses a significant threat to public health, carrying substantial social and economic implications. Recent studies indicate biofilms are important reservoirs for resistant bacteria [1]. These bacterial biofilms are highly resistant to antibiotics and disinfectants, posing significant challenges in modern medicine and industrial settings. Many persistent infections, including Pneumonia in individuals with cystic fibrosis and bone infections (osteomyelitis), chronic wounds, and ear infections, are linked to biofilms. Furthermore, biofilms play a crucial role in chronic inflammatory conditions and several acute infections [2].

Biofilms are aggregates of microorganisms that adhere to both biotic and abiotic surfaces. Within these structured communities, microbial cells are embedded in a self-produced extracellular polymeric substance (EPS) matrix, comprising polysaccharides, proteins, lipids, and extracellular DNA, along with host-derived components such as mucus. Bacteria residing within biofilms exhibit significantly higher resistance, often thousands of times greater, to antibiotics than their planktonic counterparts. Free-floating counterparts [3]. This resistance is partly due to the physical barrier that biofilms create, which limits the penetration of antibiotics. Additionally, the presence of specific bacterial populations in the deeper layers of biofilms, which exhibit reduced metabolic activity, contributes to their heightened resistance to conventional treatments [1]. Chronic infections associated with biofilms are often linked to ESKAPE pathogens, encompassing Gram-positive bacteria such as *S. aureus*, *S. epidermidis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Enterococcus faecium* [4-6] as well as Gram-negative bacteria like *E. coli*, *P. aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumonia* [7-10] (Table 1).

Table 1: Major Biofilm-Associated Drug-Resistant Pathogens and Their Occurrence in Various Infections

Pathogens	Gram stain	Types of infections	Ref.
Staphylococcus aureus	$Gram^+$	Persistent biofilm infections, long-term wound infections, and lung infections in cystic fibrosis patients.	[11]
Staphylococcus epidermidis	$Gram^+$	Catheter-associated infection, Endocarditis & joint implant infection.	[12]
Streptococcus pneumoniae	$Gram^+$	Sinusitis, ear infections & chronic obstructive pulmonary disease(COPD)	[13]
Streptococcus pyogenes	$Gram^+$	Tonsillitis, infections of the nasopharynx and mouth & ear infections	[14]
Escherichia coli	$Gram^-$	Hemolytic uremic syndrome, acute diarrhea & urinary tract infections	[15]
Klebsiella pneumoniae	$Gram^-$	Bloodstream infections, liver abscesses, and urinary tract infections	[16]
Pseudomonas putida	$Gram^-$	Urinary tract infection	[17]
Pseudomonas aeruginosa	$Gram^-$	Osteomyelitis, pneumonia associated with ventilation, and lung infections in individuals with cystic fibrosis"	[11]

Clinically, biofilm-associated infections are strongly linked with therapeutic failures in both community and hospital settings. For instance, antibiotics such as vancomycin, ciprofloxacin, and tobramycin frequently fail against biofilm-embedded pathogens (Table 2). These failures occur in common infections such as prosthetic joint infections, cystic fibrosis lung infections, catheter-associated UTIs, and chronic wound infections, often requiring prolonged or repeated treatment.

Table 2: Treatment Failures in Biofilm-Associated Infections

Infection Type	Pathogen & Context	Observed Failure & Reason	References
Prosthetic joint infection (PJI)	*Staphylococcus aureus*—DAIR (debridement + retention)	Failure rates range from **31–63%**, often due to mature biofilm	[18]
Cystic fibrosis lung infection	*Pseudomonas aeruginosa* biofilms	Tobramycin fails to eradicate chronic lung biofilms fully	[19]
Cystic fibrosis *P. aeruginosa*	Inhaled tobramycin eradication therapy failure	Persistence tied to strain traits (mucoid, resistant to neutrophils)	[20]
***P. aeruginosa* biofilms**	CF airway model—tobramycin vs combination therapy	Tobramycin alone cannot fully clear biofilm; combo works better	[21]

Bacterial biofilms represent intricate microbial communities that adhere to surfaces and are encapsulated within a self-synthesized extracellular matrix. These biofilms play a pivotal role in the persistence of chronic infections, primarily because of their

enhanced tolerance to antimicrobial agents. These biofilms significantly contribute to antibiotic resistance, making infections difficult to treat. The following table (Table 3) summarises several drugs that are notably resistant to bacterial biofilm formation, along with the mechanisms of resistance observed in these biofilms.

Approaches for Biofilm Inhibition

Methods for combating biofilms can be broadly classified into two main strategies: preventing their initial formation or disruptting established biofilms (Fig.1). Potential anti-biofilm therapies aim to block transformation from a free-floating to an immobile- state or to eliminate existing biofilm through several methods: (i) preventing microbial adherence to surfaces, (ii) Disrupting bacterial communication by blocking QS, (iii) Inhibiting the 2nd messenger nucleotide signaling (iv) altering the structural integrity of established biofilm structures, these are shown in Table 4.

Quorum-Sensing (QS) Inhibitors

Quorum sensing (QS) is a cell density dependent communication mechanism that regulates collective bacterial behaviors, including virulence and biofilm formation, via self-generated signaling molecules known as autoinducers [36]. The major QS systems include: (i) N-acyl homoserine lactones (AHLs) in Gram-negative bacteria, (ii) autoinducing peptides (AIPs) in Gram-positive bacteria, and (iii) autoinducer-2 (AI-2), found in both groups [37]. QS plays a pivotal role in chronic, biofilm-associated infections, as demonstrated in various animal models [38]. Targeting QS with small-molecule inhibitors offers a promising anti-virulence therapeutic strategy [39].

Quorum quenching (QQ), the enzymatic disruption of quorum sensing (QS), naturally occurs in microbial communities and inter-kingdom interactions (e.g., plants and animals). QQ primarily impairs the transition from planktonic to biofilm-associated lifestyles, and AHL-degrading enzymes, such as lactonases and acylases, are well characterized [40]. QS disruption can be achieved through several mechanisms: (i) degradation of autoinducers, (ii) inhibition of their synthesis, (iii) receptor antagonism, (iv) signal inactivation, and (v) mimicry via synthetic analogs [41].

Heterocyclic Compounds and Their Anti-Biofilm Activity

Biofilm development is a significant virulence factor in both Gram-positive and Gram-negative pathogens, contributing to chronic infections by promoting bacterial persistence within the host [42]. As a result, developing new compounds that can disrupt this process is considered a potential strategy for combating antibiotic resistance through anti-virulence agents, garnering significant interest over the past decade [43]. In the following sections, the paper reviews recent advancements in anti-biofilm agents, including inhibitors and compounds that promote biofilm dispersal. This work further discusses their structure-activity relationships (SAR) and, where applicable, their mechanisms of action and in vivo efficacy. This information could be instrumental in discovering new therapeutic approaches to address biofilm-related infections and antibiotic resistance.

Imidazole derivatives

S.K. Pathan and colleagues synthesized tetrasubstituted imidazole derivatives to evaluate their antibiofilm activity against Candida albicans. Compounds 1 and 2 exhibited potent antifungal effects, with IC_{50} values of 25 and 6 µg/mL, respectively, surpassing those of Fluconazole (40 µg/mL). Real-time PCR analysis revealed that these compounds inhibit biofilm formation by downregulating the agglutinin-like proteins Als3, Als4, and Als6 [44].In another study, Melander *et al.* designed and synthesized a series of analogs 3 (Figure 3) with an inverted amide group and varying lengths of linear carbon chains to enhance anti-biofilm potency [45].

Table 3: A list of several drugs that are resistant to bacterial biofilms

Drug	Bacterial Species	Resistance Mechanism	Ref.
Vancomycin	***S. epidermidis***	High resistance observed in biofilm state; surface complexity hinders drug penetration	[22]
Ciprofloxacin	***P. aeruginosa***	Biofilm matrix reduces drug efficacy; slow growth rates lead to persistent cells.	[22]
Meropenem	***K. pneumoniae***	Biofilm-associated resistance due to altered antibiotic targets, reduced penetration	[23]
Gentamicin	***E. coli***	Resistance linked to the protective extracellular matrix & metabolic state changes	[24]
Tobramycin	***P. aeruginosa***	Increased tolerance due to biofilm architecture and nutrient-depleted microenvironments	[25]

Table 4: The targets and strategies of potential antibiofilm agents for preventing biofilm development

Targets	Mechanisms	Effects on biofilm	Ref.
Autoinducers of the QS system N-acyl homoserine lactone (AHL),	Degrades QS molecules, such as N-acyl homoserine lactones (AHLs), which play a crucial role in bacterial communication	Inhibits quorum sensing, thereby reducing biofilm formation and virulence	[26]
Autoinducing peptides (AIP)	Disrupts bacterial communication through quorum sensing (QS) by inhibiting autoinducing peptide (AIP)-mediated signaling	Suppresses biofilm formation and virulence in Gram-positive bacteria	[27]
Autoinducer-2 (AI-2)	Blocks AI-2-mediated interspecies communication in bacteria	Reduces biofilm formation in multi-species biofilms	[28]
Second messengers c-di-GMP	Suppresses the second messenger c-di-GMP, a key regulator of biofilm formation and maintenance	Limits biofilm formation and disrupts the stability of mature biofilm structures	[29]
c-di-AMP	Suppresses the second messenger c-di-AMP, a key regulator of biofilm formation and maintenance	Disruption of formation	[30]
Environmental stress detection mediated by (p)ppGpp	Suppresses ppGpp signaling associated with the bacterial stress response and biofilm persistence	Interference with formation, Reduces biofilm persistence and increases susceptibility to antibiotics	[31]
Sortase A (SrtA)	Modulates bacterial stress response and survival mechanisms during biofilm formation	Weakens biofilm resilience, reduces bacterial survival within biofilm	[32]
Type 1 fimbriae (FimH) and associated surface appendages.	Inhibits bacterial adhesion to host cell wall-associated proteins (MSCRAMMs) by targeting FimH adhesin	Prevents and impairs initial attachment and biofilm formation, thereby reducing colonization.	[33]
LecA and LecB	Suppresses the lectins LecA and LecB, which are essential for biofilm formation in *P. aeruginosa.*	Disrupts biofilm matrix reduces biofilm stability and thickness	[34]
Ionophores	Disrupts bacterial ion homeostasis	Causes biofilm instability, reduces bacterial growth within the biofilm	[35]

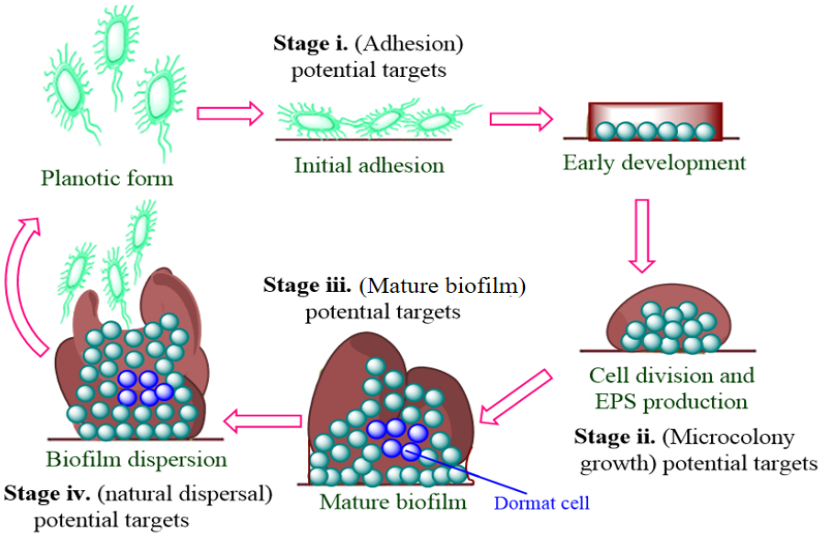

Figure 1: Phases of development from a free-floating (planktonic) state to an immobile state, a structured community, and possible targets within the biofilm life cycle

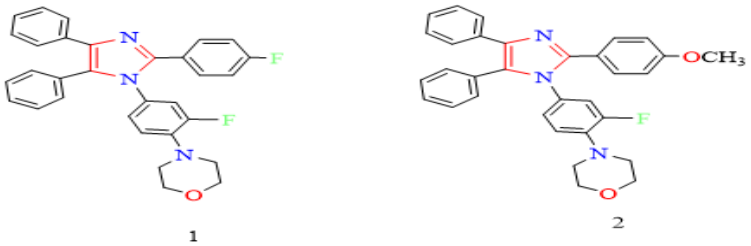

Figure 2: Chemical structure of synthetic imidazole derivatives 1 and 2

The synthesized compounds were tested for their ability to inhibit and disrupt biofilms in Pseudomonas aeruginosa strains PAO1 and PA14. Longer carbon chains (6–12 atoms) enhanced antibiofilm potency, with compound 3d emerging as the most active, showing IC_{50} values of 2.84 µM (PAO1) and 2.26 µM (PA14), which are far superior to those of oroidin.

Moreover, 3d effectively dispersed preformed biofilms at low micromolar concentrations. Studies have shown that performing a reductive *in situ* acylation on a Boc-protected 2-AI azide can be achieved effectively, a framework influenced by the amide linkage, resulting in the creation of two analog series, 4a and 4b (Figure 3) [46]. Compounds 4a and 4b showed the greatest efficacy against *P. aeruginosa* (PA14) [47], *Acinetobacter baumannii* (Actb), and *Bordetella bronchiseptica* (RB50) [48], with IC_{50} values between 16.4 µM to 40.7 µM [49]. A potent lead compound, 2-AIT, was identified for its ability to inhibit and eradicate biofilms of Pseudomonas aeruginosa, Acinetobacter baumannii, Bordetella bronchiseptica, and Staphylococcus aureus, with IC_{50} values in the low micromolar range [50]. The optimal activity was achieved with a five-carbon alkyl chain and a linker of defined length between the two rings. 2-AIT displayed strong antibiofilm efficacy—IC_{50} values of 0.98 µM (A. baumannii), 5.6 µM (P. aeruginosa PAO1), 0.53 µM (P. aeruginosa PA14), 9.5 µM (B. bronchiseptica RB50), and 0.81 µM (S. aureus). Notably, it also restored the antibiotic sensitivity of multidrug-resistant strains, including MRSA and A. baumannii, to novobiocin, tobramycin, and colistin.[51].

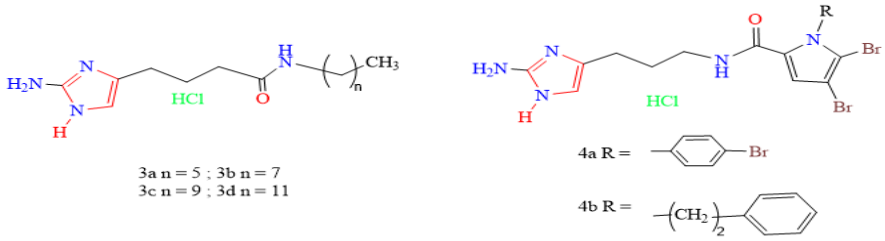

Figure 3: Structural representations of imidazole derivatives 3a–d and 4a–b

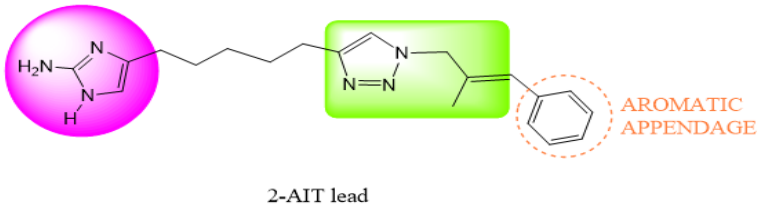

Figure 4: Structural representation of the 2-aminoimidazole triazole (2-AIT) lead compound

Furthermore, Melander and colleagues investigated modifications to the heterocyclic moiety of the lead compound to synthesize a series of novel molecules (5a-c, Figure 5) [47], demonstrating significant potential in preventing biofilm formation and dispersion against *A. baumannii* and MRSA. Derivatives 5a-c were particularly effective at dispersing A. baumannii biofilms, with EC50 values ranging from 44.7 to 59.6 µM, and demonstrated greater potency against MRSA in preventing biofilm formation, with IC_{50} values of 4.5-9.8 µM.

Experiments showed that a hydrophobic tail chain enhanced their effectiveness, with the n-butyl chain demonstrating greater potency than n-hexyl or n-heptyl groups. Additionally, to improve the bioactivity of these molecules, the authors synthesized a second-generation 2-AIT library and investigated the effects of 4,5-disubstitution [49]. All compounds in this new series exhibited improved antibiofilm activity, with compound 6 (Figure 5) emerging as the highly effective 2-AIT derivative, inhibiting MRSA and *A. baumannii* biofilms with IC_{50} values of 1.42 µM and 11.28 µM, respectively. While a number of its derivatives effectively reinstated MRSA's susceptibility to oxacillin.

SAR analysis of these compounds revealed that replacing the triazole ring with an amide group significantly enhanced their synergistic activity with β-lactam antibiotics against MRSA, specifically derivative 7 (Figure 5), achieving 64-fold and 16-fold reductions in the MICs of oxacillin and penicillin G, respectively [52]. Moreover, derivative 7 showed anti-biofilm activity against MRSA, with an IC50 of 6.5 µg/ml, acting via a mechanism that does not affect microbial survival. Interestingly, the inhibition of biofilm formation did not seem to be directly related to antibiotic resensitization, suggesting a distinct mechanism of action.

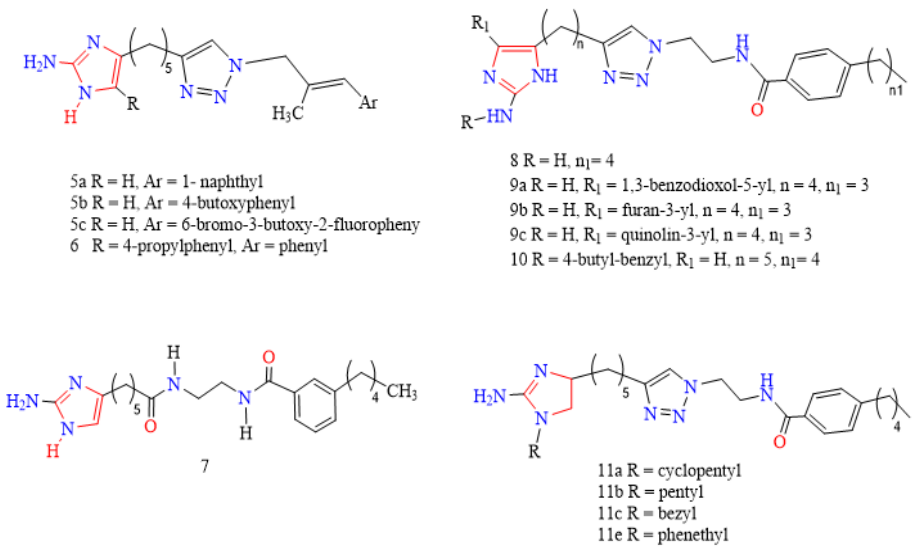

Figure 5: Structural representations of 2-aminoimidazole derivatives 5–11

Derivative 8 (lead compound) emerged as a highly effective antibiofilm agent against S. aureus and A. baumannii, with IC50 values ranging from 15.2 to 34 µM, followed by other 2-AIT derivatives. Researchers synthesized and evaluated a novel 4,5-disubstituted analog, 9a-c (Figure 5), which showed the most effective antibiofilm activity against MRSA, with IC50 values of 3.7, 7.2, and 4.2 µM, respectively, compared to analog 8 [52]. Further exploration of N-substitution within the 2-AIT series revealed that alkyl chains with fewer than four carbon atoms resulted in derivatives with potency similar to that of analog 8. Additionally, analog 10 (Figure 5) with 4-butyl-benzyl groups showed improved antibiofilm effectiveness against MRSA pathogens BAA-1770 (IC_{50} = 5.9 µM), BAA-1685 (IC_{50} = 5.5 µM), and BAA-43300 (IC_{50} = 4.4 µM). The effectiveness of analog 10 is due to the presence of longer butyl chains and benzyl groups. Interestingly, shorter aliphatic N-substituents improved synergistic activity with oxacillin against MRSA [48]. Scientists developed a different category of 2-AIT analogs by altering the 2-aminoimidazole core using specific Replacement arrangements [53]. Hence, the newly formed analogs 11a-e (Figure 5) significantly suppressed biofilm formation of MRSA (IC50 = 4.14 to 9.9 µM) & demonstrated (EC50 = 33.0 to 45.1 µM) efficacy in dispersing preformed biofilms. Mendogralo *et al.* (2023) synthesized a series of 2-(1H-indol-3-yl)-1H-benzo[d]imidazoles, exhibiting notable effectiveness against *S. aureus* (ATCC 25923), MRSA (ATCC 43300), *Mycobacterium smegmatis* (mc(2)155/ATCC 700084), and *C. albicans* (ATCC 10231) [54].

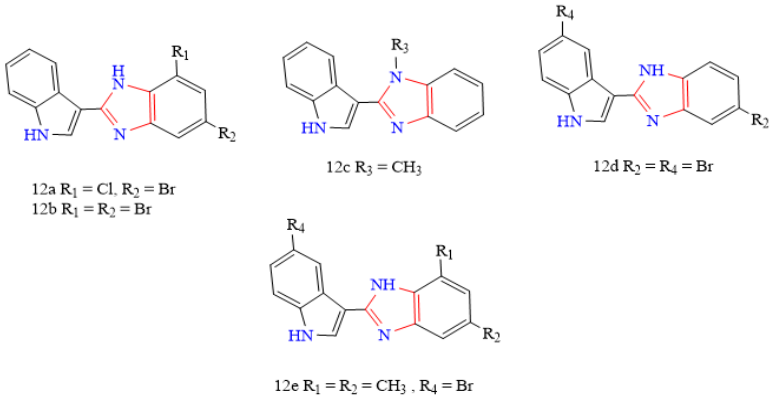

Figure 6: Chemical structures of compounds 12a-12e

Among these, compounds 12d and 12c (Figure 6) revealed potent activity against staphylococci with MIC of less than 1 µg/mL, while compounds 12a and 12d displayed MIC values between 3.9 and 7.8 µg/mL. Analog 12c demonstrated a low MIC of 3.9 µg/mL against both *M. smegmatis* and *C. albicans*. Additionally, 2-(5-bromo-1H-indol-3-yl)-6,7-dimethyl-1H-benzo[d]imidazole 12d exhibited a MIC of 3.9 µg/mL against *C. albicans*.

Analogs 12a, 12b, 12c, and 12d (Figure 6) also demonstrated superior antibiofilm activity, preventing biofilm development and effectively destroying cells in established biofilms. In silico molecular docking studies suggested three potential mechanisms of action for these analogous, involving interactions with (p)ppGpp synthetases and hydrolases, FtsZ proteins, or pyruvate kinases [54].

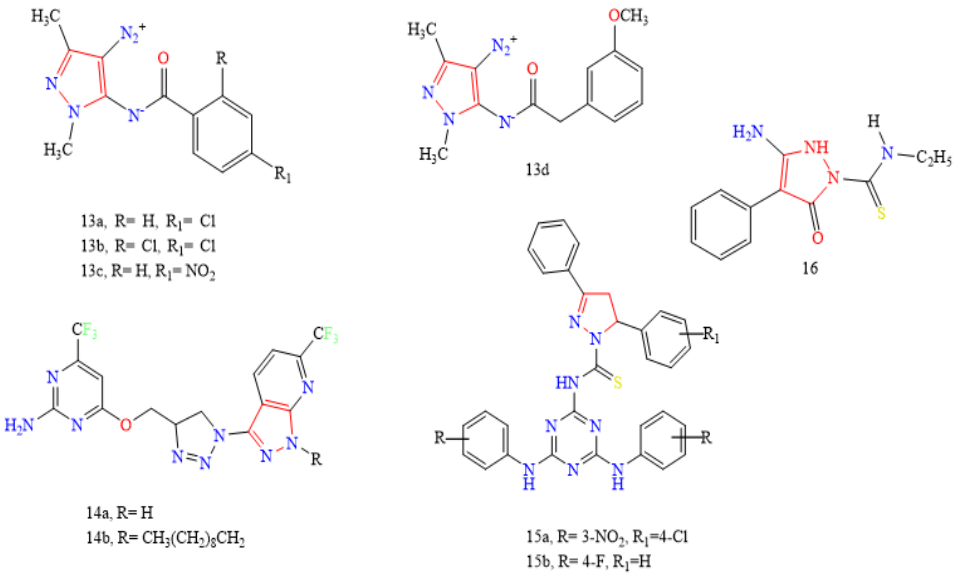

Figure 7: Chemical structure of pyrazole derivatives 13 – 16 with anti-biofilm activity

Pyrazole derivatives:

Pyrazole is a flexible, aromatic heterocyclic compound with a five-membered ring found in various compounds known for their broad antibacterial activities [55]. Over the past decade, numerous pyrazole derivatives have been identified as modulators of biofilms. Specifically, the 4-diazopyrazole analogs 13a-c (Figure 7) have demonstrated the capability to prevent biofilm formation in *S. aureus* and *S. epidermidis* [56]. Compound 13c demonstrated the strongest antibiofilm potency against *the S. aureus* pathogen (ATCC 29213) with a MIC of 6.2 µg/mL.

Furthermore, derivatives 13a-c were Potent in targeting the planktonic forms of *S. aureus* (ATCC 29213, ATCC 43866) and *S. epidermidis* (RP62A), with MIC ranging from 1.4 to 12.4 µg/mL. Further research focused on identifying more potent antibiofilm agents resulted in the development of analog 13d (Fig. 7), which exhibits antibiofilm activity against *S. aureus* ATCC 29213 (45% inhibition), ATCC 25923 (38% inhibition), and the *S. aureus* isolate 708 (25% inhibition) with IC_{50} value of 3.1 µg/mL [57].

Pyrazole derivatives 14a and 14b containing pyrazolo[3,4-b]pyridine and pyrimidine-functionalized 1,2,3-triazole (Figure 7) effectively suppress biofilm development against *Micrococcus luteus* (MTCC 2470), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 2453), and *Klebsiella planticola* (MTCC 530) with IC_{50} values between 3.9 and 7.8 µg/mL [58]. Another set of new compounds, 15a and 15b, was synthesized by linking a pyrazole nucleus with a 1,3,5-triazine scaffold. They demonstrated moderate antibiofilm activity against *Staphylococcus aureus*, with an IC_{50} of 15.6 µg/mL. Additionally, the pyrazole derivatives were effective against both planktonic and biofilm-developing cells of *Haemophilus* species.Notably, derivative 16 (Figure 7) showed considerable *in vitro* action against *H. influenzae* (ATCC 7901, ATCC 51505) and *H. parainfluenzae* (ATCC 10211) [59], with a minimum biofilm inhibitory concentration (MBIC)/MIC ratio ranging from 0.5 to 2 µg/mL. Notably, the toxicity of these derivatives was absent on the Vero cell line (EC_{50} = 278.8 µg/mL).

Indole and carbazole derivatives:

Naturally occurring indole moiety indole-3-acetic acid (17), 3,3′-methylene bisindole (18), indole-3-carbinol (19), indole-3-carboxaldehyde (20) from plant sources, and 3-indolylacetonitrile (21) and indole-3-propionic acid (22) (Figure 8), from animal sources were screened by Lee *et al.*, all compounds can suppress biofilm development against *E. coli* O157:H7 [60].

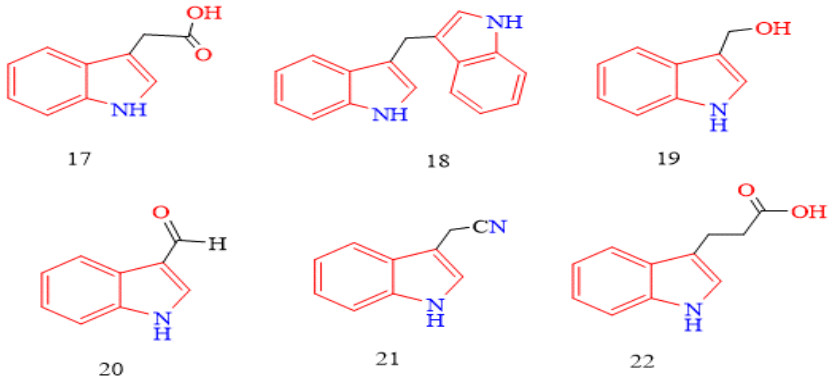

Figure 8: Structural representation of naturally occurring indole derivatives

Compounds 20 and 21 were identified as highly effective biofilm inhibitors of Escherichia coli O157:H7 and Pseudomonas aeruginosa, without affecting microbial growth. Subsequent investigations into their mechanism of action revealed that the prevention of biofilm development was associated with reduced curli production, fibers essential for biofilm development in *E. coli* [61]. Novel anti-biofilm agents were developed, inspired by the natural compound Flustramin C alkaloids. A modified Fischer indolization reaction, adapted from Garg's method, was used to construct a core pyrroloindoline scaffold, which was subsequently used to synthesize pyrroloindoline triazole amides [62]. These compounds were then tested against *Acinetobacter baumannii*, *E. coli*, and MRSA. Compounds 23a–c (Figure 9) exhibited potent antibiofilm activity against MRSA, with IC_{50} values of 5.4, 3.4, and 2.8 µM, respectively.

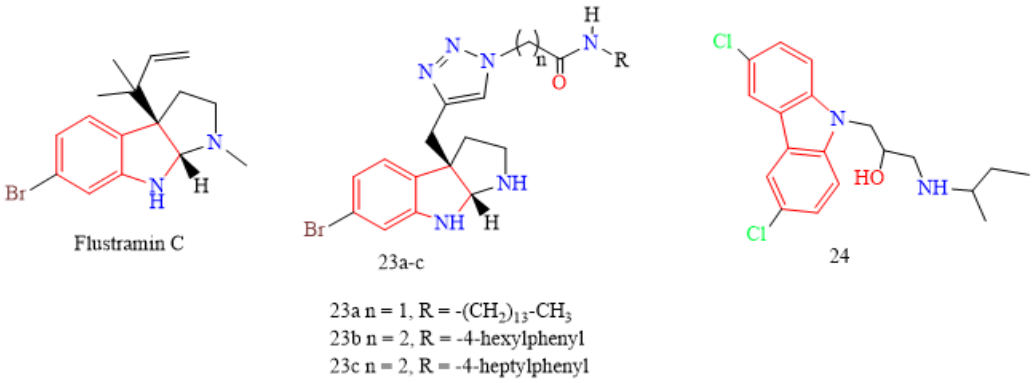

Figure 9: Structure of Indole (Flustramin C, 23a-c) and carbazole (24) derivatives

SAR studies underscored the pivotal role of the tricyclic pyrroloindoline scaffold in biofilm inhibition, highlighting the necessity of the indole ring for antibiofilm activity. Substituting the indole ring with the isosteric benzothiophene resulted in a significant loss of activity [47]. Several derivatives containing the carbazole moiety were developed by substituting the dibromo pyrrole moiety of oroidin with indole and 5-fluoroindole to enhance their effectiveness against biofilm-forming strains. Among these synthesized derivatives, compound 24 (Figure 9) was selected for its notable bacteriostatic activity against *S. aureus*, *E. coli*, *S. epidermidis*, *Porphyromonas gingivalis*, and *P. aeruginosa* with MIC values between 4.63 to 18.5 µg/mL and biofilm inhibition, with MBIC values between 2.22 to 18.5 µg/mL [63].

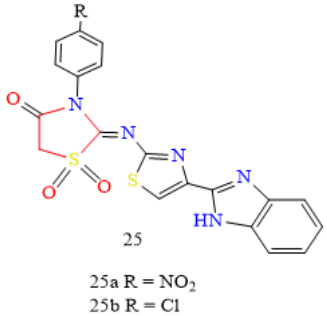

Figure 10: The structures of thiazolidine-4-one-thiazol hybrids 25a-b

Iminothiazolidinone derivatives

Gullapelli and Maroju synthesized iminothiazolidinone derivatives, that is, thiazolidin-4-one-thiazol hybrids, and

assessed their antibacterial and anti-biofilm properties. Compounds 25a and 25b (Figure 10) demonstrated promising antibacterial activity against four pathogens, including two resistant ones, with MIC values ranging from 3.62 to 7.14 µg/mL for compound 25a and 2.95 to 4.63 µg/mL for compound 25b. Both compounds exhibited significant antibiofilm activity; compound 25a inhibited biofilm formation in resistant strains, such as MRSA and VRE, with biofilm-inhibitory concentrations (BICs) of 8.23 µg/mL & 7.56 µg/mL, respectively. Additionally, derivative 25a exhibited BICs of 6.25 µg/mL against *K. pneumoniae* and 6.62 µg/mL against *E. coli* biofilms. Compound 25b also displayed effective biofilm inhibition, with BICs of 2.22 µg/mL against MRSA and 3.05 µg/mL against VRE, as well as significant activity against K. pneumoniae and E. coli biofilms, with BICs of 3.25 and 2.03 µg/mL, respectively [64].

Pyrrole derivatives:

Schillaci et al. (2010) first reported on the antibiofilm activity of pyrrole ring-containing natural pyrrolomycins, which are derived from Treptomyces and Actinosporangium species. Pyrrolomycins C, D, F1, F2a, F2b, and F3 (Figure 11) exhibit antibiofilm activity against *Staphylococcus* species [65].

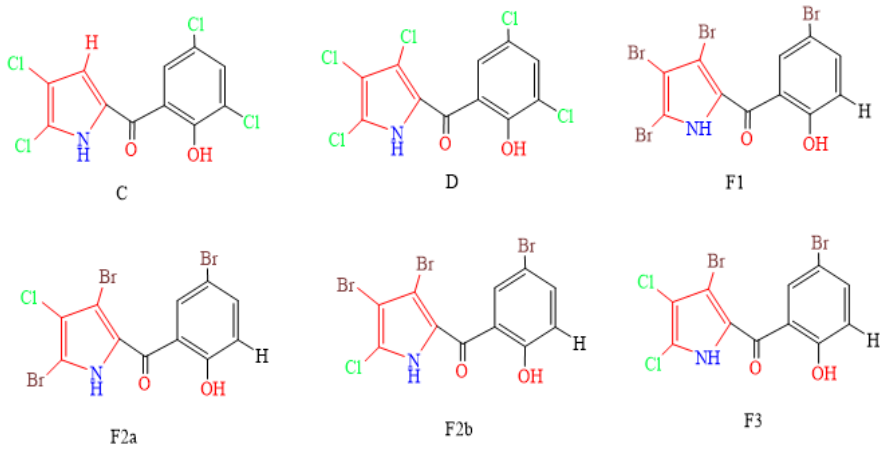

Figure 11: Structural representations of pyrrolomycins C, D, F1, F2a, F2b, and F3

Except for pyrrolomycin C, all pyrrolomycin derivatives exhibited potent antibiofilm activity against *S. aureus* (ATCC 25923, ATCC 29213), *S. epidermidis* (DSM 3269), and methicillin-resistant *S. epidermidis* (RP62A), achieving more than 60% inhibition at the tested concentration of 1.5 µg/mL. Notably, pyrrolomycin F3 showed the most potent efficacy against all tested Gram-positive pathogens, inhibiting more than 50% with a concentration of 0.045 µg/mL. These compounds also exhibited a safety profile, indicated by a selectivity index (SI) greater than 1,000, and exceeding 10,000 for pyrrolomycins D and F1. A significant structural characteristic of these compounds is their halogenation, which correlates with their antibacterial potency, as the activity increases with the number of halogen atoms in the structure [66].

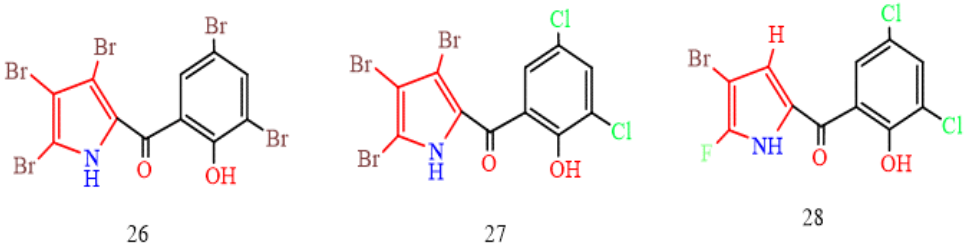

Figure 12: Chemical structure of synthetic analogs 26 - 28

The bromo-analogous 26 and 27 (Figure 12) exhibited the highest biofilm inhibition efficacy against *S. aureus*, *S. epidermidis*, and methicillin-resistant *S. epidermidis* at a screening concentration of 1.5 µg/mL [65].

Yang et al. developed a novel series of fluorinated pyrrolomycins utilizing fragment-based and bioisosteric approaches to investigate the influence of fluorine on their physicochemical properties and drug-like characteristics. The

study revealed that incorporating fluorine into both rings of pyrrolomycin lowered the calculated logP values from 6 to a range of 2.1-5.5. Among the fluorinated derivatives, compound 28 exhibited the most potent antibacterial activity against *Staphylococcus aureus*, with a MIC of 0.073 µg/mL and an MBC of 4.0 µg/mL. Additionally, compound 28 effectively inhibited pre-formed *Staphylococcus* biofilms at a concentration of 8.0 µg/mL. Toxicity assessments on human HeLa cells revealed a selectivity index (SI) greater than 229, indicating a favorable safety margin for potential clinical applications [67].
Thus, the incorporation of fluorine into the pyrrolomycin structure was beneficial, as it (i) lowered the clogP value, (ii) stabilized the pyrrole ring as an electron-withdrawing group to reduce potential toxicity, and (iii) enhanced the pharmacokinetic and pharmacodynamic properties.

Heterocyclic compounds are emerging as potent antibiofilm agents against drug-resistant pathogens. Imidazole derivatives, particularly 2-aminoimidazole triazoles, showed strong inhibition of Pseudomonas aeruginosa and Staphylococcus aureus biofilms with low micromolar IC_{50} values, enhanced by hydrophobic substitutions. Pyrazole and pyrazolo[3,4-b]pyridine derivatives effectively disrupted Staphylococcus biofilms, while indole-based compounds, including pyrroloindoline triazole amides, inhibited MRSA biofilms by targeting curli fibers and quorum sensing.

Carbazole and quinoline derivatives demonstrated enhanced biofilm eradication, with quinolines also exhibiting synergistic effects with antibiotics. Tetrazole compounds demonstrated broad-spectrum activity, while coumarins selectively disrupted the biofilms of Candida albicans and Porphyromonas gingivalis with minimal cytotoxicity. Six-membered heterocycles, like piperazine-substituted chitosan, inhibited both biofilm formation and mature biofilms. Overall, imidazole and quinoline derivatives showed the highest potency, while coumarins offered selective, low-toxicity activity, and pyrazole/indole scaffolds may benefit from further optimization.

FUTURE DIRECTIONS

Heterocyclic Agents and Nanoengineering:

Developing heterocyclic small-molecule antibiofilm agents facilitates the advancement of Creative, multi-approach treatment strategies, incorporating nanoengineering techniques such as antibiofilm nanoparticles, surface coatings, or microneedles to enhance efficacy against biofilm-associated infections.

Enhancing Antibiotic Efficacy with Antibiofilm Agents:

Combining antibiofilm agents with conventional antibiotics could be a valuable strategy for tackling antibiotic resistance and infections related to biofilms, as these agents can prevent bacterial adhesion and disrupt established biofilms, thereby offering therapeutic benefits in settings such as implant surgery and enhancing the effectiveness of traditional antibiotics.

Targeting Biofilm Mechanisms:

Future research should aim to identify specific molecular targets within biofilms that heterocyclic compounds can modulate. This could involve investigating quorum-sensing pathways, efflux pumps, or mechanisms that disrupt the biofilm matrix.

Dual-Function Therapies:

Integrating heterocyclic scaffolds with conventional antibiotics could be a promising strategy to restore the efficacy of existing drugs. Designing dual-function drugs that kill free-floating bacteria and disrupt biofilms could help combat the growing threat of multidrug-resistant (MDR) pathogens.

CONCLUSION

The comprehensive analysis of diverse heterocyclic compounds elucidates their significant potential as efficacious scaffolds for developing anti-biofilm agents to combat drug-resistant pathogens. The chemical diversity and versatility of heterocyclic structures render them particularly suitable for targeting bacteria that form biofilms, which frequently exhibit resistance to conventional antibiotics. These compounds demonstrate a notable capacity to compromise biofilm integrity, inhibit quorum sensing, and enhance the penetration of antimicrobial agents, thus underscoring their importance in addressing the growing challenge of multidrug-resistant infections. In this review, we identified key targets for inhibiting biofilm formation, including preventing bacterial adhesion, disrupting biofilm structure, and promoting biofilm dispersion. This highlights the need for a multi-targeted approach to combat biofilms effectively, despite challenges in understanding the mechanisms of many promising biofilm inhibitors. Future research should focus on enhancing pharmacokinetics and investigating synergistic combinations with existing antibiotics to optimize clinical outcomes.

FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
Sobhanjan Bhunia wrote the original draft. Sumana Chatterjee contributed to analyzing the literature and developing methodologies. Tamalika Chakraborty was involved in data curation and accessing various resources.

ABBREVIATION
EPS-Extracellular Polymeric Substance, MRSA-Methicillin-Resistant Staphylococcus aureus, AMR-Antimicrobial resistance, MIC-Minimum Inhibitory Concentration, BIC-Biofilm Inhibitory Concentration, QS-Quorum Sensing, MBIC-Minimum Biofilm Inhibitory Concentration, AI-2 – Autoinducer-2, MDR-Multi-Drug Resistant, SAR-Structure-Activity Relationship

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