1. Introduction

It has been known for a number of years\(^1\) that bacteria are able to communicate with one another through the use of small molecule signals, and that they employ this communication system to coordinate behavior. Since the initial discovery, it has been established that bacterial communication is a complex and multi-faceted phenomenon, and that bacteria possess a number of mechanisms, by which they communicate. The ability of bacteria to communicate allows the coordination of behavior within a community, resembling complex multicellular organisms. This is advantageous to the bacterial community as a whole, increasing survival by allowing the bacteria to adopt different phenotypes in response to various environmental stressors, and is underpinned by the ability of the bacteria to sense local environmental conditions and alter gene expression accordingly. Intercepting these communication...
pathways with small molecules provides an opportunity to control unwanted bacterial behaviors, such as virulence, biofilm formation, antibiotic resistance, and persister formation as an alternative therapeutic strategy to simply killing bacteria, and may result in a reduced evolutionary pressure for resistance development.12

The most well-studied and arguably best-understood form of bacterial communication is quorum sensing (QS) (reviewed recently by Waters and Bassler),2 in which bacteria produce diffusible small molecules that allow them to sense population density and coordinate gene expression once a critical population is reached. It is generally recognized that two basic forms of QS are employed: distinct chemical signals to transmit messages to other bacteria of the same species (intraspecies communication), or alternate chemical signals to communicate with bacteria of other species (interspecies communication). Intraspecies signals include acyl homoserine lactones (AHLs),3 employed by Gram-negative bacteria and autoinducer peptides (AIPs),4 employed by Gram positive bacteria. Autoinducer-2 (AI-2) molecules derived from 4,5-dihydroxy-2,3-pentanedione (DPD),5–7 are believed to be interspecies signals employed by both Gram-negative and Gram-positive bacteria (Fig. 1).

Recently, it has been posited that the bacterial metabolite indole can also act as both an intra- and interspecies bacterial chemical signal. It has even been demonstrated that indole produced by commensal bacteria can act as an interkingdom signal.10 Indole is produced by upwards of 85 species of bacteria from the action of the enzyme tryptophanase (TnaA)upon tryptophan.11 However, even many bacterial species that lack tryptophanase, and therefore do not produce indole, will exhibit changes in behavior in response to the presence of extracellular indole. Behaviors affected by indole include: biofilm formation,12 motility,12 virulence,13 antibiotic resistance,14 acid tolerance,15 and persister formation.16

In addition to indole itself, many indole derived small molecules (both naturally occurring and synthetic) have been shown to affect bacterial behavior, and the indole scaffold has been exploited for the design of small molecules that have the potential to control bacterial behavior and ultimately lead to the potential discovery of new therapeutics. In this review we aim to give an overview of the phenomenon of indole signaling, including the various behaviors affected by indole and the genes and proteins known to be involved in the process. We will then discuss the control of bacterial behavior with indole-containing small molecules and describe some synthetic approaches taken to access indole-containing molecules that modulate bacterial behavior.

1.1. Indole production

As mentioned above, indole production is widely employed in the bacterial kingdom, with the direct observation of indole production or the presence of tryptophanase in over 85 species of bacteria. Both Gram-negative and Gram-positive bacteria fall within this group, which includes many medically relevant pathogens, such as: Escherichia coli, Klebsiella oxytoca, Shigella dysenteriae, Vibrio cholerae, and Enterococcus faecalis.11 In E. coli, indole is produced when tryptophan is taken up from the environment and utilized as a carbon and nitrogen source following degradation with tryptophanase to indole, pyruvate, and ammonia (Fig. 2), with the yield of indole being dependent on the levels of exogenous tryptophan present in the environment.12 Conversely, tryptophanase can also catalyze the formation of tryptophan from indole and either pyruvate and ammonia, serine or cysteine. The degradation pathway, however, is the favored direction of the enzyme.6,19

Tryptophanase expression is controlled by catabolite repression, and is therefore only transcribed when the bacterium is under carbon limitation, leading to an increase in indole production during the stationary phase of growth.

Fig. 2. Conversion of tryptophan to indole, pyruvate, and ammonia by tryptophanase (TnaA).

1.2. Processes controlled by indole signaling

The role of indole as a chemorepellent in E. coli was initially established in 1974;13 however in the last decade the expanded role of indole in a variety of cellular processes has become apparent (Fig. 3). Interestingly, the effect of indole on these processes is often dependent upon the experimental conditions used, such as temperature and media composition, in addition to the bacterial strain, thus underlying the complexity of the phenomenon of indole signaling.

1.2.1. Biofilm formation. A biofilm is a surface-associated community of bacteria encased within an extracellular matrix, which exhibits distinct phenotypes from free-floating (planktonic) cells, particularly with respect to growth rates, gene expression, and antibiotic tolerance.20 Fig. 4 depicts the five stages of the biofilm life cycle. Di Martino and co-workers showed in 2002 that disruption of tnaA (which encodes tryptophanase) by transposon insertion in the E. coli strain S17–1 generates a bacterial mutant that, in comparison to the wildtype (wt) E. coli strain, shows a significant decrease in biofilm formation in Luria-Bertani (LB) medium at 26 °C, and also a decrease in epithelial cell adherence. Tryptophanase activity, cell adherence, and biofilm formation are restored upon complementation with plasmid encoded wild-type tnaA.21 It was subsequently demonstrated that inactivation of the enzyme with the
tryptophanase inhibitor oxindolyl-1-alanine similarly affects biofilm formation without affecting bacterial growth, and that supplementation with exogenous indole restored biofilm formation.\(^{\text{22}}\)

Conversely, the addition of exogenous indole (500 \(\mu\)M) has been shown to decrease biofilm formation by \(E.\ coli\ K-12\) grown in LB medium supplemented with 0.2\% glucose (LB-Glu) at 30 \(^\circ\)C. In the presence of glucose, indole levels are reduced as a result of catabolite repression of \(tnaA\) and accordingly, deletion of \(tnaA\) did not affect biofilm formation in this medium. Examination of the biofilm architecture of the wild-type \(E.\ coli\) strain revealed that indole effected a change from a typical scattered tower architecture to flatter colonies, while the overall biomass of the biofilm was reduced by 40\% and substratum coverage increased 2.8-fold.\(^{\text{12}}\) In the same report, indole (1 mM) was also shown to decrease biofilm formation in LB at 30 \(^\circ\)C by four additional \(E.\ coli\) strains: ATCC 25404, JM109, TG1, and XL1-Blue.\(^{\text{12}}\)

Further studies with the \(E.\ coli\) K-12 BW25113 strain in LB medium established that the effect of indole on biofilm formation was temperature dependent, such that biofilm formation was reduced by 16-fold at 25 \(^\circ\)C and seven-fold at 30 \(^\circ\)C upon exposure to 1 mM indole. Indole also reduced biofilm formation by a 3(\(\mu\)M) mutant of this strain, though the dependence on temperature was absent, effecting a 10-fold reduction in biofilm formation at both 25 \(^\circ\)C and 30 \(^\circ\)C. Indole exhibited a reduced effect on biofilm formation by either strain in this medium at 37 \(^\circ\)C.\(^{\text{13}}\) Indole has also been shown to decrease biofilm formation by pathogenic enterohemorrhagic \(E.\ coli\) (EHEC) O157:H7.\(^{\text{24,25}}\)

Additional studies in two environmental, nontoxicigenic strains of \(V.\ cholerae\), SIO, and TP have shown that the addition of exogenous indole restored the biofilm forming ability of \(tnaA\) transposon mutants of both strains, thus suggesting that indole signaling also plays a role in biofilm formation by this indole-producing bacterium.\(^{\text{26}}\) As mentioned above, indole can also affect the behavior of non-indole producing bacterial species, for example, indole promotes biofilm formation in \(P.\ aeruginosa\) \(aeR\), increasing the amount of biofilm 1.4-fold at 500 \(\mu\)M and 2.2-fold at 1 mM.\(^{\text{15}}\)

Due to the ubiquity of indole across multiple bacterial species, it has been posited that indole acts not only as an intraspecies signal, but also an interspecies signal. Supporting this hypothesis, it has recently been shown that an \(E.\ coli\) \(tnaA\) mutant was considerably less competitive in a mixed species biofilm with \(P.\ aeruginosa\) than was the wild type strain, and that this lack of competitiveness could be restored by exogenous addition of indole.\(^{\text{27}}\)

1.2.2. Motility. The Wood group have shown that \(E.\ coli\) mutants lacking the genes \(t\), \(t\), or \(t\), which play a role in indole synthesis, have a 10-fold reduction in intracellular indole concentration and exhibit increased motility (3.2-fold and 4.7-fold, respectively). They confirmed that the observed increase in motility in these strains was a result of the reduced indole levels by supplementing with exogenous indole and noting a reduction in motility.\(^{\text{12}}\) It was also shown that indole affects the motility of a strain of the more medically relevant EHEC, decreasing it by 2.8-fold.\(^{\text{28}}\)

Nikaido et al. showed that indole represses the expression of genes involved in bacterial flagella biosynthesis, flagella motor activity, and chemotaxis, including \(fnhC\), a master regulator protein involved in flagellar biogenesis, in \(Salmonella\ enterica\) serovar Typhimurium, a bacterium that does not produce indole.\(^{\text{29}}\) They also showed that this indole-dependent effect was accompanied by a reduction in flagella number as observed by transmission electron microscopy, and a reduction in motility on semi-solid agar.\(^{\text{30}}\) Indole has also been shown to decrease swimming motility and swarming motility, but not twitching motility, in the PAO1 strain of the non-indole producing \(P.\ aeruginosa\).\(^{\text{29}}\)

1.2.3. Virulence. Indole has been shown to restore and enhance secretion of EspA and EspB, type III secretion proteins, which play central roles in EHEC pathogenicity, and similarly restore and enhance the subsequent formation of attaching and effacing (A/E) lesions by an EHEC of the mutant in HeLa cells. Type III secretion genes are located in the locus of enterocyte effacement (LEE), and indole was shown to increase the promoter activity of the LEE operon that encodes EspB and EspA (LEE4).\(^{\text{30}}\) Similarly, indole was shown to complement the downregulation of LEE1 expression in a \(tnaA\) mutant of an enteropathogenic \(E.\ coli\) (EPEC) strain (though indole did not restore the ability of the mutant to paralyze or kill \(Caenorhabditis\ elegans\)).\(^{\text{31}}\)

In contrast, \(S.\ enterica\) serovar Typhimurium cells that had been treated with indole exhibited a reduced rate of invasion of Caco-2 cells in vitro compared to untreated bacterial cells.\(^{\text{28}}\) Indole has also been documented to down-regulate genes involved in the synthesis of several virulence factors in \(P.\ aeruginosa\) PAO1, and to decrease production of the virulence factors pyocyanin, rhamnolipid, PQS, and pyoverdine in this bacterium.\(^{\text{29}}\)

In the Gram-positive \(Staphylococcus\ aureus\), indole decreased production of the virulence factor staphyloxanthin,\(^{\text{22}}\) which confers enhanced oxidant and neutrophil resistance to the bacterium, and has been shown to contribute to virulence in vivo.\(^{\text{32}}\) Indole also downregulated transcription of the virulence genes \(n\)-hemolysin \(hla\) and enterotoxin \(seb\), and decreased the hemolytic activity of \(S.\ aureus\) against human red blood cells.\(^{\text{33}}\)

1.2.4. Antibiotic resistance. Hirakawa and co-workers have shown that indole increases drug resistance in \(E.\ coli\) by inducing a number of xenobiotic exporter genes including: \(acrD\), \(acrE\), \(cub\), \(emrK\), \(mdtA\), \(mdtE\), and \(yceL\), and confers rhodamine 6G and SDS resistance.\(^{\text{34}}\) The Wood group later showed that survival of an \(E.\ coli\) BW25113 \(tnaA\) mutant upon exposure to kanamycin, which was reduced compared to the wild-type, was increased 30-fold in the presence of indole.\(^{\text{35}}\)

Indole has been shown to play a role in population wide antibiotic tolerance in a continuous culture of \(E.\ coli\) exposed to increasing levels of norfloxacin. It was demonstrated that the vast majority of isolates were less resistant than the population as a whole; however a small number of highly resistant mutants produced indole, which induced drug efflux pumps and oxidative stress protective mechanisms in the population as a whole, leading to increased survival of the less resistant cells.\(^{\text{36}}\) Type III secretion genes related to multi-drug efflux in \(S.\ enterica\) serovar Typhimurium including \(acrD\) and \(mdtA\),\(^{\text{14}}\) and \(ramA\) and \(acrB\).\(^{\text{28}}\) It was later shown that indole causes in an increase in tolerance of \(S.\ typhimurium\) to carbenicillin and ciprofloxacin, both when the indole source was exogenously
added indole, and when indole was present from co-culture with *E. coli*. This in vitro result was then recapitulated in vivo in a *C. elegans* model of *S. typhimurium* infection, in which ciprofloxacin was less effective at killing *S. typhimurium* in cultures of *C. elegans* that were fed on a *tauA* *E. coli* mutant in the presence of indole, compared to those in which indole was absent.22

Indole also represses (6–13-fold) the *mexGHI-opmD* multidrug efflux genes in *P. aeruginosa* PA01, and increases resistance to different classes of antibiotics including: tetracycline, gentamicin, kanamycin, and ampicillin.20

1.2.5. Acid tolerance. Indole has been shown to have disparate effects on the ability of *E. coli* to tolerate acidic environments. The Wood group found that indole decreases the acid tolerance of *E. coli*, with 2 mM indole lowering survival at pH 2.5 by 350 to 650-fold in the *E. coli* K12 strain BW25113, and repressing the acid resistance genes gadABCX and *hdeABD*.12 Studies in our laboratory similarly found that the presence of indole at 2 mM decreased survival of this same *E. coli* strain upon exposure to pH 2.5 by 319-fold.13 In contrast, Hirakawa and co-workers found that the same concentration of indole enhanced the acid tolerance of the *E. coli* K12 strain MC4100 at pH 3.5, increasing survival by 6.3-fold, and also found that indole upregulated the acid resistance genes *hdeA*, *hdeB*, *hdeD*, *slp*, and *yihE* (gadE).18

1.2.6. Persister formation. Like much of the biofilm, the phenomenon of bacterial persister formation is a phenotypic type.30 Bacterial persister cells are a sub-population of dormant, non-dividing cells that display a high tolerance to antibiotics, but do not harbor traditional antibiotic resistance genes. Persister cells are thought to be important in chronic and recurrent infections, and also to play a role in the tolerance of biofilm cells to antibiotic treatment.40,41 Recently, indole was reported to play a role in persister cell formation.42 Incubation of *E. coli* with indole increased persistence by at least an order of magnitude upon subsequent exposure to high concentrations of antibiotics from three different classes: ofloxacin, kanamycin, and ampicillin, suggesting that the protective effects of indole are a general phenomenon. Similarly, it was shown that when grown in rich medium where indole signaling is expected to be occurring in the wild-type strain, a *tauA* knockout mutant exhibited decreased persister formation by almost an order of magnitude, and that supplementation with indole restored this deficit. The authors proposed that the mechanism of indole mediated persister formation involves activation of both the OxyR and phage-shock pathways. This is an additional example of bacterial communication via indole-signaling allowing the bacterial population to protect a subpopulation.30

1.2.7. Role as an interkingdom signal. Recently, it was documented that indole can also play a role as an interkingdom messenger between commensal bacteria and the intestinal epithelial cells. It was demonstrated that indole causes increased expression of genes involved in the formation of epithelial cell tight-junctions and actin cytoskeleton, leading to an increase in transepithelial resistance, and also played a role in controlling inflammation by repressing a number of inflammatory cytokines.10

1.3. Proteins/genes involved in indole signaling

The complex pathways, by which indole exerts its myriad of effects, are far from understood, even in the most well studied bacterium, *E. coli*. In this section, we aim to give a brief overview of the current understanding of the proteins and genes involved in the recognition of, and response to, the indole signal.

The majority of the current understanding with regards to indole mediated bacterial communication is derived from studies on the model bacterium *E. coli*. Unlike other Gram-negative bacteria, *E. coli* does not produce AHL quorum sensing signals, leading to the hypothesis that this bacterium might utilize other chemical signals as a means of communication. As mentioned earlier, indole was first identified as a self-produced extracellular signal in *E. coli*, by Wang et al. in 2001,42 and it was reported that both conditioned medium from *E. coli*, and synthetic indole, dose-dependently activated the *astD*, *tauA*, and *gabT* genes, all of which function in the uptake, synthesis, or degradation of amino acids that yield pyruvate and succinate,43 while medium from a mutant lacking *tauA* effected considerably lower activation of these genes.52

While it has since been shown that indole either upregulates or downregulates numerous genes in *E. coli* under a variety of environmental conditions, the direct biological target of indole in *E. coli* (or any other bacterium) has not yet been definitively identified. It is thought that indole may interact with a variety of global regulators.51 A number of genetic studies have given some insight into indole signaling pathways in *E. coli*. For example, the regulation of biofilm formation by indole has been shown to be temperature dependant, with a greater effect observed at lower temperatures (25 °C or 30 °C compared to 37 °C),41 and DNA microarray analysis has been employed to identify the genes affected by the addition of exogenous indole to an *E. coli* taxon mutant at 30 °C.51 Indole (at a concentration of 1 mM) significantly (defined by the authors as more than two-fold) affected the expression of 186 genes in biofilm cells at 30 °C, including upregulation of the biofilm stress regulator *bhsA*, and down-regulation of *hbsR* (*yihH*), which encodes a regulator of biofilm formation.52 Indole has also been shown to induce the expression of the acid resistance operon *yamABC* and repress expression of *ompT* and *ompF*, which encode outer membrane porins. It is known that decreased expression of *ompF* leads to an increase in resistance to a number of antibiotics.44

Studies from the Wood group have established that the transcriptional regulator SdiA is central to the indole signaling cascade in *E. coli*; however indole has never been shown to directly bind SdiA,11 and how indole recognition is integrated into SdiA signaling, is not yet known. SdiA is a homologue of LuxR, the transcription factor, through which other Gram-negative bacteria respond to AHL QS signals, and has been shown to mediate the *E. coli* response to AHLs,12 in addition to repressing pyrimidine biosynthesis genes and inducing genes for purine biosynthesis and curli formation. In response to the addition of exogenous indole (1 mM), the number of genes that were significantly differentially expressed was five-fold lower in an sdiA mutant in comparison to the isogenic *tauA* mutant. In contrast to findings of the Wood group, the Ahmer group reported that the effects of indole on gene expression and biofilm formation in *E. coli* were not sdiA dependent. They did however note that indole did inhibit the response of SdiA to AHLs. Additionally, Lee et al. identified *YmbG* (AprI), which is thought to function as a regulatory protein and bind DNA promoter sequences, as controlling acid resistance in *E. coli* via indole. This protein was also shown to confer hydrogen peroxide resistance and play a role in biofilm formation and motility.45

The stationary phase σ-factor RpoS, GadX—an AraC type transcription factor, and Hfq—a global regulator of sRNA function are required for indole-mediated induction of expression of *mdtEF*, which confers drug tolerance in the stationary phase. This indole-mediated induction of *mdtEF* is brought about by upregulation of *rpoS*, *gadY*, and *gadX*.45 The *mdtEF* operon on the chromosome of *E. coli* via indole. This protein was also shown to confer hydrogen peroxide resistance and play a role in biofilm formation and motility.45

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this gene by indole has been similarly shown occur predominantly via BaeSR by Raffa and Raivio.47

1.4. Modulation of indole controlled phenotypes by native indole derivatives

In addition to indole itself, numerous indole derivatives have been shown to modulate diverse behaviors in several bacterial species. Many bacterial species readily metabolize indole into various derivatives through the action of monoxygenases and dioxygenases,11 and the resulting oxidized indole derivatives have been found to control the behavior of several species of bacteria. For example, *Burkholderia cepacia* G4 readily converts indole into 2-hydroxyindole 1, 3-hydroxyindole 2, 4-hydroxyindole 3, isatin 4, indigo 5, isoindigo 6, and indirubin 7 (Fig. 5), via the action of tol- uene ortho-monoxygenase (TOM).48 Native indole derivatives have been found to modulate a variety of indole-controlled phenotypes, both mimicking and oppose the effects of indole itself without affecting bacterial growth. For example, 5-hydroxyindole 8 and 7-hydroxyindole (7HI) 9 (Fig. 5) decrease biofilm formation by *E. coli* O157:H7 by 11-fold and 27-fold, respectively at 1 mM, compared to an 18-fold reduction brought about by indole at the same concentration. Activity is dependent upon the position and degree of oxidation of the indole derivative, with neither 2-hydroxyindole 1 nor isoindigo 6 displaying any effect on biofilm formation at concentrations of 1 mM and 250 µM, respectively, while isatin 4 increases biofilm formation by EHEC in a dose-dependent manner with a four-fold increase effected at a concentration of 250 µM.25

Oxidized indole derivatives have also been shown to modulate indole-regulated behaviors in non-indole producing bacteria, such as *P. aeruginosa*. For example, both 5-hydroxyindole 8 and isatin 4 increase biofilm formation by *P. aeruginosa* by 20% at 500 µM.25 7HI 9 also increases biofilm formation (by two-fold), and has additionally been shown to increase antibiotic resistance and abolish swarming motility of *P. aeruginosa* PA01. In the presence of 7HI 9, the minimum inhibitory concentration (MIC) of ampicillin against PA01 is increased from 300 µg/mL to 600 µg/mL (compared to 500 µg/mL in the presence of indole). Similar to indole, 7HI 9 decreases production of several *P. aeruginosa* virulence factors, including pyocyanin, rhamnolipid, PQS, and pyoverdine.25

Several tryptophan metabolites also affect indole-dependent bacterial behaviors. Indole-3-acetic acid (IAA) 10 (Fig. 6), which serves as a signaling molecule necessary for the growth and development of plants, has been shown to stimulate production of the extracellular polymeric substance (EPS), which comprises much of the biofilm matrix, and to increase biofilm formation in *E. coli*.11 IAA 10 also suppresses genes encoding the Type III Secretion System (T3SS) in *P. aeruginosa*, an important virulence factor in acute human infections, as does the tryptophan metabolite 3-hydroxykynurenine 11.20

Indole-3-carboxaldehyde (ICA) 12 has been found to decrease biofilm formation in enteraggregative *E. coli* (EAEC) and *P. aeruginosa* by 11-fold and 2.3-fold, respectively. ICA also reduces the production of virulence factors, such as EspA filaments and shiga toxins, inhibits biofilm formation, and decreases motility in the pathogenic *E. coli* strains EPEC and EHEC in a non-toxic manner, exhibiting a greater effect than indole at the same concentration.51

Indole-3-acetaldheyde 13, prevalent in the plant pathogen *Rhodococcus sp* BFI 332, has been shown to decrease biofilm formation by several bacteria including the Gram-positive bacteria *S. aureus* and *S. epidermidis*.52 Indole-3-acetaldheyde was shown to suppress expression of two curli operons, csgBAC and csgDEFG and induce the expression of tryptophanase.52 Another indole derivative, 3-indolylacetoniitrile (IAN) 14, inhibits biofilm formation by *E. coli* by 24-fold at 100 µg/mL, and also exhibits anti-biofilm activity against fungi, inhibiting biofilm formation by *Candida albicans* by five-fold at 1 mM.53,54 IAN has also been shown to decrease the production of the virulence factors pyocyanin and pyoverdine by *P. aeruginosa*.55

Skatole (3-methylindole) 15 is one of the predominant metabolites produced during tryptophan catabolism in the large intestine of humans and animals. Skatole has been shown to inhibit the formation of *E. coli* EHEC biofilm by 52% at 100 µg/mL, via the decrease of catalse activity and the weakening of bacterial surface morphology.52 Finally, indole-3-propionic acid (13PA) 16 and indole-3-carbinol (13C) 17 are naturally occurring indole derivatives that moderately inhibit biofilm formation in *E. coli*.74

1.5. Modulation of indole controlled phenotypes by synthetic indole derivatives and methods to access indole derivatives

Inspired by the finding that many native indole derivatives reduce bacterial virulence, the indole framework has been exploited as a scaffold for the construction of novel compounds designed to interfere with indole signaling and modulate bacterial behaviors. Chemical modification of the indole core at carbon-7 has given compounds that significantly affect phenotypes of pathogenic bacteria. For example, 7-fluoroindole 18 and 7-formylindole 19 (Fig. 7) at 1 mM inhibit biofilm formation by *P. aeruginosa* by four-fold and five-fold, respectively, without inhibiting planktonic cell growth.57 In the same manner as indole, 7-fluoroindole abolishes swarming motility in this bacterium, diminishes protease activity, and inhibits blood hemolytic activity. 7-Fluroindole also reduced the production of virulence factors including 2-heptyl-3-hydroxy-4(1H)-quinolone, pyocyanin, rhamnolipid, and the siderophores pyoverdine and pyochelin.57

7-Benzoylindole (7BOI) 20 has been shown to attenuate the virulence of *S. aureus*.33 Growth of *S. aureus* in the presence of 7BOI revealed a dose-dependent reduction in staphyloxanthin production, hemolytic activity, and H2O2 resistance, with a greater effect at lower concentrations than for indole being noted. Transcriptional analyses have shown that both indole and 7BOI repress the expression of the α-hemolysin gene *hla*.33
The recent high-throughput screening of a 20,014 member small-molecule library for inhibitors of biofilm formation by *S. enterica* serovar Typhimurium led to the discovery of 7-methoxy-4-[4-(3-phenyl-2-propen-1-yl)-1-piperazinyl]-5H-pyrimido[5,4-b] indole 21 (Fig. 7). A structure–activity relationship study conducted upon commercially available analogues revealed that the indole moiety was essential for activity, and the most active compound identified, 8-fluoro-4-[4-(3-phenyl-2-propen-1-yl)-1-piperazinyl]-5H-pyrimido[5,4-b] indole 22, exhibited IC50 values (defined as the concentration needed to inhibit biofilm formation by 50% relative to the untreated control) for inhibition of biofilm formation of 13.69 μM and 26.55 μM at 16 °C and 37 °C, respectively, and also displayed inhibitory effects on preformed biofilms at 37 °C. Compound 22 did not exhibit significant toxic effects on planktonic bacteria, but did promote planktonic growth at 37 °C when present at concentrations above 40 μM.68

Other, more complex compounds with an indole core embedded in their structure have been synthesized and serve as potential candidates to modulate pathogenic behaviors of both Gram-negative and Gram-positive bacteria by interfering with indole signaling at lower concentrations than the relatively high concentrations required by native analogues. The indole-derived flustramine family of natural products, which was isolated from the North Sea bryozoan *Flustra foliacea*,59 consists of six pyrroloindoline- and five indolic alkaloids. Members of this class of small molecules inhibit the colonization and establishment of biofilm-forming bacteria native to the environment the sponge inhabits,60 while it has also been shown that the pyrroloindoline flustramine C (Scheme 1) inhibits biofilm formation by the terrestrial bacterium *Acinetobacter baumannii* by 30% at 100 μM.61

**Scheme 1.** Synthesis of flustramine C 23 developed by Lindel and co-workers. Reagents and conditions: a) GIC2Et, NaOH (aq) CH2Cl2, rt (80%); b) LIAH(H2), 70 °C refux, 1 h (85%); c) Ac2O/HCO2H (3:1) (82%); d) i) BuLi, THF ii) NsCl, THF, −78 °C to rt; (55%) e) HCO2H:H2O (1:3), 150 °C, 5 min; e) CuSO4:5H2O, sodium ascorbate, azide, CH2Cl2, EtOH, H2O, rt. Reagents and conditions for the synthesis of ITA analogues: a) i) BuLi, THF ii) NsCl, THF, −78 °C to rt; (55%) e) HCO2H:H2O (1:3), 150 °C, 5 min; e) CuSO4:5H2O, sodium ascorbate, azide, CH2Cl2, EtOH, H2O, rt; f) PhSH, K2CO3, CH3CN, rt. Reagents and conditions for the synthesis of ITA analogues: a) i) Zn dust, THF, 0 °C to rt; b) CuSO4:5H2O, sodium ascorbate, azide, CH2Cl2, BuOH, H2O, rt.

Synthetic access to flustramine C (Scheme 1) was initiated from tryptamine, which was converted to N-methyltryptamine 25 in two steps via a procedure developed by Sutcliffe and co-workers.62,63 N-Methyltryptamine 25 was then formylated, giving compound 26, and subsequently subjected to Danishefsky’s nucleophilic *tert*-prenylation reaction to yield the reverse prenylated indole 27.64 This indole derivative was then selectively brominated with N-bromosuccinimide (NBS) in the presence of acid to afford the natural product flustrabromine 28.65 The formyl group of flustrabromine was then removed under basic conditions, to deliver a second natural product, desformylflustrabromine (dFBr) 29. Finally, treatment of 29 with NBS in THF induced a facile oxidation, cyclization, [1,5] rearrangement at room temperature to provide flustramine C 23.

Using this natural product scaffold as an inspiration, a library of pyrroloindoline-triazole-amide (PTA) analogues was constructed from the flustramine C derivative 34, in which a propargyl group replaced the reverse prenyl group (Scheme 2).61 The alkyne moiety allowed for the introduction of structural diversity through a Huisgen Cu(I)-catalyzed alkyne/azide cycloaddition (click chemistry) with a series of azide amide moieties. Compound 36 exhibited considerably higher anti-biofilm activity than flustramine C, inhibiting biofilm formation by *A. baumannii* with an IC50 of 193 μM, while compound 37 inhibited biofilm formation by *E. coli* with an IC50 of 36 μM. Compound 38 inhibited biofilm formation by a methicillin resistant *S. aureus* (MRSA) strain with an IC50 of 3.4 μM, while all three lead compounds of this PTA library did not affect planktonic growth at active concentrations.

In another study inspired by flustramine C, the manipulation of a simplified flustramine-based scaffold led to the development of a second family of indole-triazole-amide conjugates.66 These analogues were accessed rapidly in two steps, involving the installation of a propargyl tail at the C3 position of indole via a zinc-mediated Barbier reaction67 to afford intermediate 39, followed by formation of a triazole again using click chemistry (Scheme 2). Interestingly, these analogues either selectively promoted or inhibited biofilm formation by medically relevant Gram-positive and Gram-negative bacteria; for example, compound 40 was found to be a potent inducer of biofilm formation by *E. coli* (50% increase at 82.6 μM), while inhibiting biofilm formation by the pathogens *A. baumannii* and *S. aureus* via a non-microbial mechanism.

As mentioned, the above route to the synthesis of flustramine C passes through the natural product desformylflustrabromine 29. Analogous to the effect of indole itself, desformylflustrabromine has been shown to inhibit biofilm formation by *E. coli* and *S. aureus*, though at considerably lower concentrations, exhibiting IC50 values of 174 μM and 70 μM, respectively.68 This compound exhibited a microbial effect on planktonic bacteria; however, fine tuning of five regions of the dFBr scaffold led to the construction of several libraries of compounds, allowing for the investigation of structural features crucial for biological activity.
Structural modifications to the desformylfluorostabromine (dFBr) scaffold are depicted in Fig. 8. The lead compound of each generated library exhibited an inhibitory effect on biofilm formation by E. coli and S. aureus without affecting planktonic growth. The presence of the bromide at the C6 position in region A was demonstrated to be important for biofilm inhibitory activity against E. coli and S. aureus when the des-bromo dFBr analogue 41 was synthesized and showed only 35% inhibition of S. aureus biofilms at 150 μM and no inhibition of biofilm formation by E. coli.90 Region B was modified by introducing a phenyl group in the 2-position (42) in one step, using a synthetic route developed by Slade and coworkers.90,91 This compound showed moderate activity, inhibiting E. coli and S. aureus biofilm formation by only 20% and 15%, respectively at 200 μM. Region D was modified by simple alkylation of the indolic nitrogen using various alkyl bromides in the presence of sodium hydride in anhydrous DMF. Of these derivatives, analogue 43 was the most active, exhibiting IC50 values of 80 μM and 65 μM for inhibition of biofilm formation by E. coli and S. aureus, respectively.

Fig. 8. Five regions of the dFBr scaffold have been tuned to generate compounds that control bacterial behavior via indole signaling pathways. IC50 values or percentage inhibition for biofilm formation are shown.

Introduction of various alternative secondary amines in region C was employed to probe the importance of the N-methyl secondary amine of dFBr in the context of biofilm inhibition. The anti-biofilm activity of these analogues was dramatically increased by the presence of an aromatic substituent on the aliphatic nitrogen.93 Diversity in this case was installed via alkylation of the nosyl-protected indole derivative 46 in the presence of cesium carbonate in DMF at room temperature (Scheme 3).72,73 Installation of the reverse prenyl group, bromination at the C-6 position and final deprotection using Fukuyama’s conditions delivered each region C analogue for further evaluation. In a manner that parallels indole itself, the lead compound 45 also promotes biofilm formation by P. aeruginosa, represses E. coli and P. aeruginosa motility, decreases acid resistance, and increases antibiotic resistance of E. coli at lower concentrations than indole. Mechanistic studies using knockout E. coli strains demonstrated that identical to the activity of compound 45 is dependent on temperature, mnaA and sdiA, suggesting that this compound acts upon the same pathways as indole.

Finally, modification of region E, allowed for investigation of the impact that the alkyl chain length between the indole core and secondary aliphatic amine had on anti-biofilm properties. The three methylene carbon linker was accessed by treating commercially available 3-indolepropionic acid with ethyl chloroformate and triethylamine in dry THF to yield the corresponding amide 47 (Scheme 3).77 This intermediate was then nosyl-protected, alkylated, prenylated, and brominated by using the same conditions as previously described to generate analogue 44. Compound 44 inhibited biofilm formation by E. coli and S. aureus with IC50 values of 16.3 μM and 13.4 μM, respectively, which compares well to the most potent dFBr derivative 45.37

As mentioned earlier, indole also affects the ability of several bacteria to resist the effects of antibiotics. Several different classes of synthetic indole derivatives have also been investigated for the ability to suppress antibiotic resistance. In this vein, several indole analogues affecting drug resistance in Gram-positive and Gram-negative bacteria have been developed in the past decade. The synthetic 2-aryl-1H-indole class of small molecules (Fig. 9) suppress drug resistance in S. aureus and have been shown to act upon the NorA efflux pump. 5-Nitro-phenyl-1H-indole (INF55) 52 decreased the MIC of the antibacterial alkaloid salt berberine against S. aureus from 125 μg/mL to 0.2 μg/mL.74

Scheme 3. Synthesis of desformylfluorostabromine derivatives. Reagents and conditions for modification of region C. a) NsCl, Et3N, DMAP, CH2Cl2; b) alkyl bromide, Cs2CO3, DMF; c) i) t-BuOOCl, Et3N, THF, 78 °C ii) prenyl 9-BBN −78 °C to rt iii) 3 M aq NaOH, 30% H2O2 0 °C to rt; d) HCO2H:HOAc (1:3), NBS (1 equiv), rt, 35 min; e) PhSH, K2CO3, CH3CN; f) i) CICO2Et, Et3N, THF ii) NH3, MeOH; g)LiAlH4, THF; h) NsCl, Et3N, DMAP, CH2Cl2; i) alkyl bromide, Cs2CO3, DMF; j) i) t-BuOOCl, Et3N, THF, 78 °C ii) prenyl 9-BBN −78 °C to rt iii) 3 M aq NaOH, 30% H2O2 0 °C to rt; k) HCO2H:HOAc (1:3), NBS (1 equiv), rt, 35 min; l) PhSH, K2CO3, CH3CN.

Fig. 9. Promising synthetic efflux pump inhibitors derived from the 2-aryl-1H-indole scaffold. MIC values are indicated with berberine (30 μg/mL) against the wild-type S. aureus strain 8325-4 and the strain overexpressing the NorA pump NorAK2361.
A structure–activity relationship study afforded structural diversity via the introduction of various substituents around the 2-aryl ring and/or at the indole-C5 position (summarized in Fig. 9). Synthetic approaches to introduce structural diversity in the 2-aryl-1H-indole scaffold are depicted in Scheme 4. Replacing the nitro substituent at the indole-C5 position was achieved in two steps from commercially available 2-phenylindole via electrophilic aromatic 5-chlorosulfonation using chlorosulfonic acid at 0 °C to obtain the 5-chlorosulfonyl-2-phenylindole intermediate 53. This was further reacted with diverse nucleophiles to yield 5-sulfonyl-2-phenylindole derivatives. Substitution or removal of the indole 5-nitro group led to loss of activity.72 Functionalization of the 2-aryl substituent was achieved by N-acylation of a preformed indole nucleus 54, followed by a palladium-induced oxidative cyclization and ring opening by amide hydrolysis of the 2-nitro-6H-isoiindole [2,1-a]indol-6-one intermediate 57.73 Reduction of the resulting carboxylic acid 58 accessed the lead compound 59, which increased S. aureus susceptibility to berberine, potentiating the antibacterial agent more than 15-fold against a strain overexpressing the NorA pump at a concentration of 0.8 μg/mL, and also suppressed resistance to fluoroquinolones.74 Introduction of a functionalized 2-aryl group was achieved by direct C2 arylation of the commercially available indole 60 with the aryl iodide 61 via a Rh-catalyzed coupling.76 Further transformations allowed for the introduction of structural diversity leading to the discovery of the most active analogue 62, which was almost equipotent with INF55 against both wild type and NorA overexpressing S. aureus strains.75

Recently, a highly diverse and functionalized polycyclic indoline alkaloid library has been developed, generating molecules that suppress resistance of MRSA to β-lactam antibiotics. Synthesis of these analogues involves a one-pot three-component reaction, in which an alkyl indole species is assembled using an alkyl imine 65, an aryl hydrazine 66, and an activating agent 67.76 (Scheme 5). The indole regioisomers 68 and 69 underwent a gold(I)-catalyzed tandem cyclization developed by Wang and co-workers,77 and the resulting fused 70 or spiro-tetracyclic indoline 71 was modified via alkylation or tandem ring-opening reductive amination to generate the pairs of compounds 72, 74 and 73, 75, respectively. The most active compound of this library, 76, potentiated the activity of methicillin against MRSA (ATCC BAA-44), reducing the MIC from 128 to 8 μg/mL at 20 μM.77 Compound 76 was shown to selectively reseedtize this MRSA strain to all β-lactams tested, including oxacillin, imipenem, and cefazolin, with high fold of potentiation (128-fold reduction in MIC for oxacillin and 32-fold for cefazolin). It was posited that 76 targets genes encoding penicillin binding protein 2a (PBP2a) and β-lactamase enzymes, but the exact mechanism of action is as yet unknown.

2. Conclusions

In conclusion, bacterial communication via indole signaling is a complex and broad-ranging phenomenon that we are only just beginning to understand. It plays a role in the regulation of many important bacterial processes, many of which are important in driving host colonization and pathogenesis. The design of small molecules with the ability to control these processes provides an opportunity to develop new anti-virulence based therapeutics as an alternative approach to controlling bacterial infections in comparison to conventional bactericidal antibiotics, to which resistance typically rapidly arises. In this review, we have described some of the current efforts toward designing such small molecules, and given an overview of the synthetic methods utilized to access a variety of indole-based bioactive compounds.

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References and notes

Biographical sketch

Roberta Melander studied undergraduate chemistry at UMIST (MChem medicinal chemistry, 2004), before obtaining her PhD from the University of Manchester in 2008, studying modified nucleic acids under the supervision of Jason Micklefield. She then carried out post-doctoral studies in medicinal chemistry with David Selwood at the Wolfson Institute for Biomedical Research, University College London. She is currently a Research Assistant Professor at North Carolina State University where her research focuses on small molecule control of bacterial communication and antibiotic resistance.

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