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Indole-Based Novel Small Molecules for the Modulation of Bacterial Signalling Pathways

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Abstract

Gram-negative bacteria such as *Pseudomonas aeruginosa* use *N*-acylated L-homoserine lactones (AHLs) as autoinducers (AIs) for quorum sensing (QS), a major regulatory and cell-to-cell communication system for social adaptation, virulence factor production, biofilm formation and antibiotic resistance. Some bacteria use indole moieties for intercellular signaling and as regulators of various bacterial phenotypes important for evading the innate host immune response and antimicrobial resistance. A range of natural and synthetic indole derivatives have been found to act as inhibitors of QS-dependent bacterial phenotypes, complementing the bactericidal ability of traditional antibiotics. In this work, various indole-based AHL mimics were designed and synthesized *via* the 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl) and *N,N*'-dicyclohexylcarbodiimide (DCC) mediated coupling reactions of a variety of substituted or unsubstituted aminoindoles with different alkanoic acids. All synthesized compounds were tested for QS inhibition using a *P. aeruginosa* QS reporter strain by measuring the amount of green fluorescent protein (GFP) production. Docking studies were performed to examine their potential to bind and therefore inhibit the target QS receptor protein. The most potent compounds **11a**, **11d** and **16a** showed 44 to 65% inhibition of QS activity at 250 μM concentration, and represent promising drug leads for the further development of anti-QS antimicrobial compounds.

Introduction

Bacterial infection is a leading cause of hospitalization and death throughout the world, and has primarily been treated with antibiotics over the past half-century.[1] However, history has shown that the introduction of any novel antibiotic is quickly followed by the evolution of clinically-significant resistant bacterial strains within a few years of introduction.[2,3] The rapid increase of multidrug resistance strains worldwide coupled with the slowing pace of discovery of new antibiotics represent an urgent need to develop new and effective antibacterial therapies.[4] One approach has involved the structural modification of existing drugs such as antifungal azoles, antibacterial β-lactams and quinolones.[5] However, this strategy merely delays the development of bacterial resistance, without total eradication of the infectious species. Basically, traditional antibiotics ultimately kill or inhibit the growth of the bacteria. They may exert their antimicrobial action by the disruption of cell walls, inhibition of DNA or RNA synthesis, inhibition of protein synthesis, inhibition of folate synthesis, or depolarization of membrane potential.[6] Since traditional antibiotics cause death of bacterial cells, this exerts selective pressure on bacteria and encourages the development and spread of antibiotic drug resistance *via* different defensive phenotypes such as biofilm formation.[7] In this context, it is increasingly important to understand bacterial virulence and survival mechanisms in order to identify new therapeutic approaches to combat bacterial infection.

Bacteria use various autoinducers (AIs) chemical signals such as *N*-acylated L-homoserine lactones (AHLs) **1, 2, 3** (Figure 1) to monitor and coordinate their genome expression in a cell density-dependent manner, which is a mechanism known as quorum sensing (QS).[8] This population-dependent regulatory system plays an important role in the expression of genes and phenotypes responsible for overcoming the host immune response and regulating pathogenicity. Some typical

QS controlled behaviors include the control of biofilm formation, production of virulence and defense factors, conjugation, sporulation and swarming motility.[8,9] The QS system of *Pseudomonas aeruginosa*, a causative agent of nosocomial infections and infections of cystic fibrosis patients, has been widely studied and it is clear that QS controls the expression of a broad range of virulence determinants such as exotoxin, pyocyanin, pyoverdin etc.[10] Importantly, QS and the phenotypes controlled by this pathway are not essential genes and thus the inhibition of QS is not lethal. Hence, blocking or interfering with the communication between bacteria to prevent the production of virulence factors or biofilm formation represents an attractive alternative strategy to combat bacterial infections while reducing the likelihood of engendering bacterial resistance.[11,12]

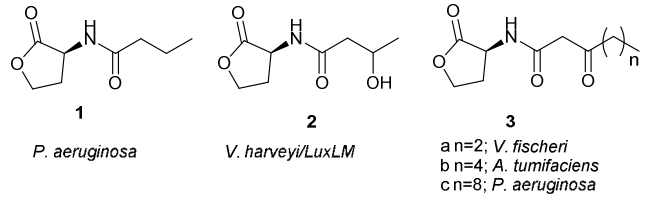


Figure 1. Different types of natural *N*-acylated L-homoserine lactones (AHLs).

Numerous attempts have been directed towards producing synthetic analogues of QS signaling molecules as antagonists of bacterial communication systems. In *P. aeruginosa*, synthetic AHLs, which mimic the structures of natural AHL molecules can compete with their binding to the LasR receptor of the LAS QS system, thereby inhibiting signal transduction and gene transcription processes required for biofilm formation and virulence expression.[13] Research groups have previously studied the effect of various structural modifications of natural AHLs on receptor binding and AHL inhibitory activity, including variations in the length and substitution pattern of the acyl chain, as well as in the nature of the substituents on the lactone ring.[14-21] However, homoserine lactones are prone to non-enzymatic hydrolysis at physiological pH and are also readily degraded by mammalian lactonases, producing ring-opened products that are inactive against QS.[22] While various small heterocycles and natural products have also been investigated for QS inhibitory activity over the past few decades, the application of indole heterocycles for this purpose has been comparatively less explored.

Indole is an intercellular signaling molecule employed by diverse bacteria that regulates various bacterial behaviors in a QS-dependent fashion, including antibiotic resistance, virulence and biofilm formation.[23-27] Further, bacterial oxygenases can readily oxidize indole into various metabolites, such as isatin and isoindigo, that have also been shown to play a role in the control of biofilm formation *via* QS mechanisms.[28] Consequently, small molecules based on the indole scaffold could potentially be developed as inhibitors of bacterial QS systems. Indole-derived plant secondary metabolites such as indol-3-yl-acetonitrile (IAN) **4** and indole-3-carboxaldehyde (I3CA) **5** were found to be active against biofilm formation in enterohemorrhagic *Escherichia coli* (EHEC) (Figure 2).[29] The synthetic indole derivative 7-fluoroindole **6** has also been reported to reduce biofilm formation by four-fold and hemolytic activity by 14-fold at a concentration of 1 mM in *P. aeruginosa*, and also suppressed swarming motility, protease activity, extracellular polymeric matrix production and QS-regulated virulence factors in this species.[30] Recently, it has been reported that the Gram-positive bacteria *Staphylococcus intermedius* and *Staphylococcus delphini* excreted an indole-based molecule *N*-[2-(1*H*-indol-3-yl)ethyl]-urea **7**, termed yayurea A, that interfered with the QS system of a range of Gram-negative bacteria.[31]

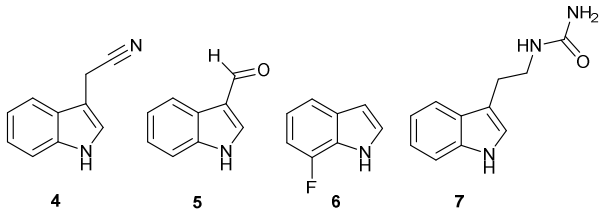


Figure 2. Indole derivatives that interfere with QS.

Inspired by these recent studies, we sought to examine the LAS-dependent QS modulatory activity of novel indole-based small molecules. We describe herein the design and synthesis of AHL mimics containing indole-based, non-lactone head groups and substituted or unsubstituted aliphatic tail groups. The synthesized compounds were tested for QS inhibitory activity in the *P. aeruginosa* MH602 *lasB* reporter strain. Additionally, docking studies were performed to examine their binding potential with the target receptor.

Results

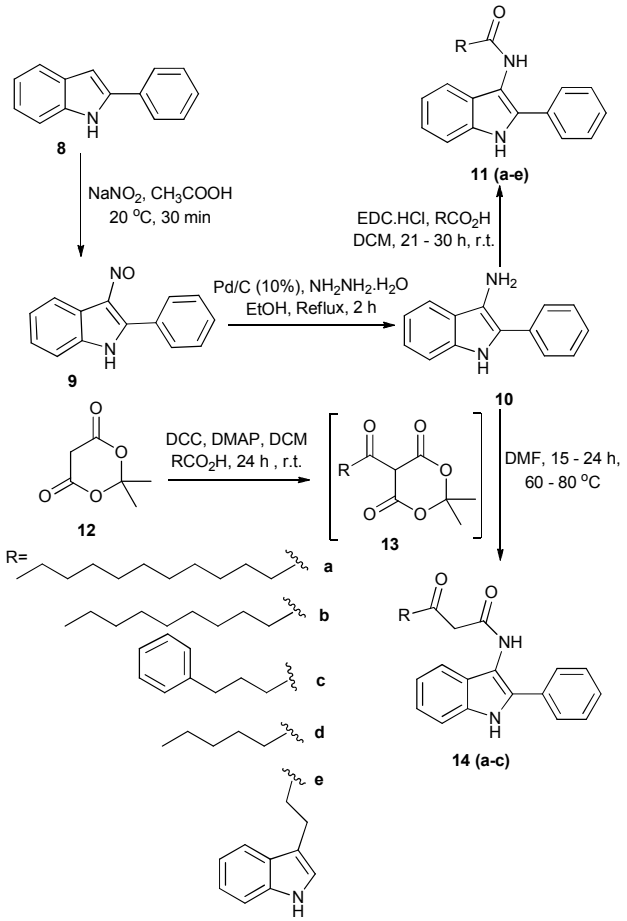
Synthetic Schemes

The synthesis of the indole-based AHL mimics involved the coupling of a variety of substituted or unsubstituted aminoindoles with different alkanoic acids. The indole amines chosen in the study were 3-amino, 5-amino and 7-amino indole derivatives. Additionally, various alkyl chains ranging from 6 to 12 carbon atoms in length and mimicking natural autoinducer chains, such as decyl, hexyl or phenylbutyl were explored.

To synthesize AHL mimics based on 3-amino-2-phenylindole, the commercially available 2-phenylindole **8** was first converted to 3-nitroso-2-phenylindole **9** by nitrosation, followed by reduction to 3-amino-2-phenylindole **10** using hydrazine with a palladium catalyst.[32,33] EDC.HCl mediated coupling of indole amine **10** with carboxylic acids at room temperature yielded the targeted amides **11a-e** in 25–82% yield.[34] Meanwhile, for the synthesis of the 3-oxo-substituted amides **14a-c**, the desired 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) conjugate **13** was obtained by the coupling reaction between Meldrum's acid **12** and alkanoic acids using DCC. Compound **13** was directly used for the next step and was

reacted with 3-amino-2-phenylindole **10** in DMF to give the 3-oxo-substituted amides **14a-c** in 33–39% (Scheme 1).[34]

For the synthesis of AHL mimics based on 5-amino-2-methylindole, the commercially available 2-methyl-5-aminoindole **15** was used as the starting material. Using the conditions described above, the targeted amides **16a-e** and 3-oxo-substituted amides **17a-c** were synthesized in 46–63% and 20–30% yields, respectively (Scheme 2).



Scheme 1. Synthesis of AHL mimics based on 3-amino-2-phenylindole.

For the synthesis of AHL mimics bearing the 7-aminoindole scaffold, the commercially available 7-nitroindole **18** was first reduced to 7-aminoindole **19** using hydrazine with a palladium catalyst.[33] Using similar amide coupling reactions, the targeted amide compounds **20a-c** and 3-oxo substituted amides **21a-c** were synthesized in 39–92% and 19–22% yields, respectively (Scheme 3).

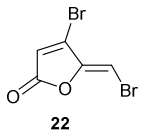
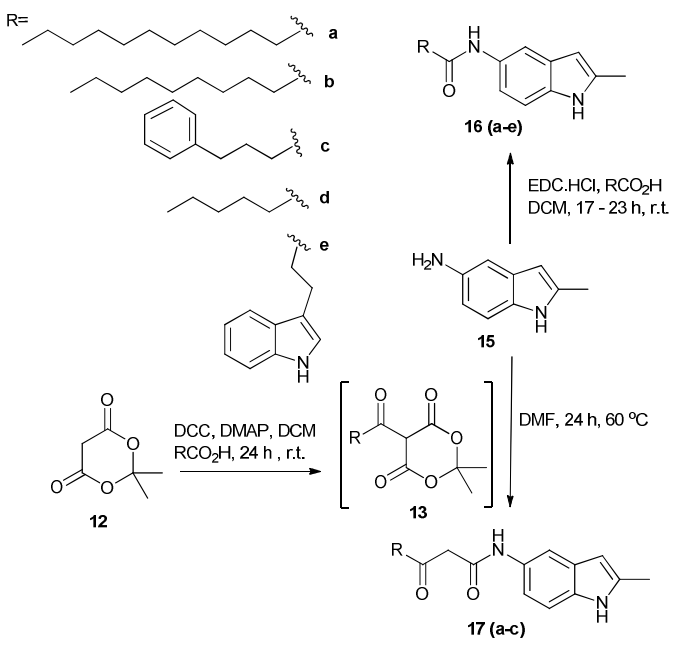
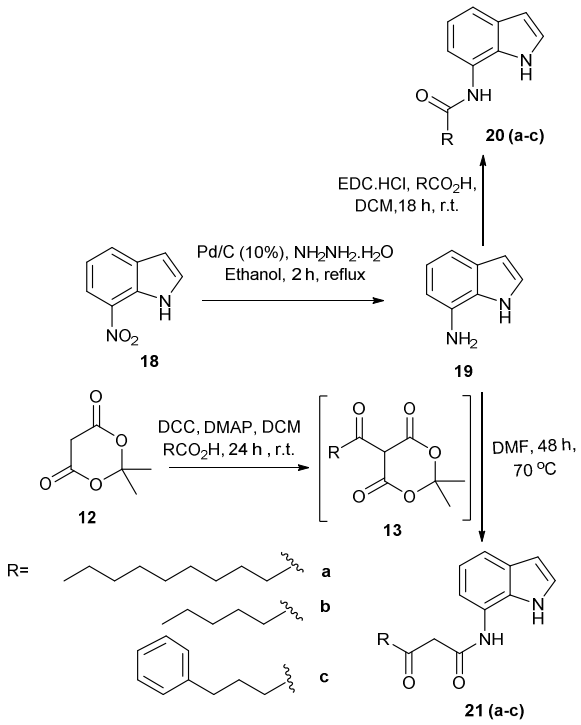


Figure 3. Furanone 30 (control).



Scheme 2. Synthesis of AHL mimics based on 2-methyl-5-aminoindole.



Scheme 3. Synthesis of AHL mimics based on 7-aminoindole.

Quorum sensing inhibition assay

The assay was performed using the *P. aeruginosa* MH602 *lasB* reporter strain (P_{lasB}::*gfp*(ASV)) following the protocol developed by Hentzer *et al*.[35] The production of AHL signals by this reporter strain leads to an increase of unstable green fluorescent protein (GFP-ASV) production as a function of an active QS system. Compounds that inhibit bacterial QS systems

would be expected to reduce the expression of GFP in the reporter strain.

In this assay, *P. aeruginosa* MH602 cultures were incubated with various concentrations of the synthesized compounds at 37 ºC. The fluorescence of GFP at λ = 535 nm and the optical density at 600 nm (OD) of the cultures were recorded every 30 min. Data for the most potent compound **11d** are presented in Figure 4. The percentage QS inhibition of the compounds was calculated as the percentage difference of GFP intensity between the sample and the control at the time point when the fluorescence reached its maximum value in the control. The optical density (OD) of the cultures was utilized as a measure of bacterial cell growth. A well-known QS inhibitor, furanone 30, **22** (Figure 3) was used as a positive control to validate the assay protocol.

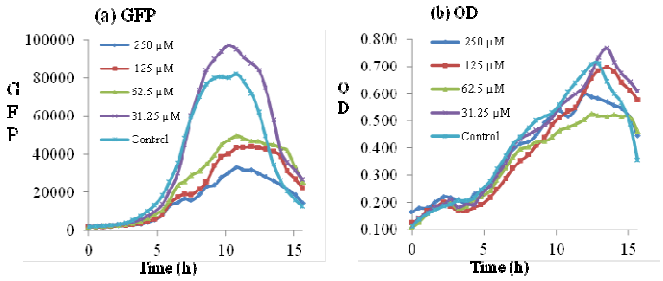


Figure 4. QS inhibition assay for compound **11d** in the P. aeruginosa MH602 lasB reporter strain (PlasB::gfp(ASV)). (a) GFP fluorescence as a function of time. (b) OD as a function of time.

The results showed that the synthesized compounds exhibited promising QS inhibitory activity as measured by the reduction in GFP fluorescence, with the most potent compound **11d** inhibiting QS activity by 65% at 250 µM (Table 1). The GFP inhibitory activities of the tested compounds were found to be concentration-dependent. Additionally, the positive control compound furanone 30 reduced bacterial cell growth by 32% at 250 µM concentration, whereas the synthesized compounds showed less than 15% inhibition of bacterial cell growth at the same concentration (supplementary information).

Docking studies

In order to understand the nature of the interaction between the novel indole-based AHL mimics and the QS receptor, docking studies were performed. The crystal structure of the QS signal receptor protein LasR with the agonist *N*-(3-oxododecanoyl) L-homoserine lactone (OdDHL, **3c**) was used for this study (PDB code, 2UV0, resolution 1.8 Å).[36] The Gold algorithm was used *via* the Accelrys Discovery Studio interface to examine the ability of the compounds to bind to the receptor protein LasR. The receptor agonist OdDHL was docked back into the protein as a control in order to determine the reliability of the docking method. The docking runs were analyzed for the predicted binding interactions, including hydrogen bonding, electrostatic and hydrophobic interactions, between the compounds and the LasR receptor in the best scoring pose and are presented in Table 2. The docking results suggested that hydrogen bonding and hydrophobic interactions to amino acid residues such as Thr75, Tyr56, Ser129, Asp 73 and Val76,

Table 1. Percentage inhibition of QS activity by the indole-based AHL mimics as determined by the reduction of GFP fluorescence at λ = 535 nm in the *P. aeruginosa* MH602 *lasB* reporter strain (P_{lasB}::*gfp*(ASV)).

Entry	Concentrations (µM)			
	250	125	62.5	31.3
11a	44.1 ± 3.9[a]	31.8 ± 4.7	18.4 ± 4.4	5.5 ± 7.3
11b	37.9 ± 4.3[a]	25.7 ± 2.5	18.5 ± 3.4	4.3 ± 1.9
11c	31.2 ± 3.7[a]	22.2 ± 2.2	14.2 ± 4.9	NA
11d	65.0 ± 3.9[a]	54.2 ± 2.9	39.3 ± 6.5	NA
11e	40.5 ± 0.5[a]	16.6 ± 5.1	14.3 ± 3.5	2.6 ± 0.6
14a	7.5 ± 0.8[a]	NA	NA	NA
14b	22.2 ± 1.7[a]	4.1 ± 1.8	NA	NA
14c	9.9 ± 3.5[a]	NA	NA	NA
16a	45.0 ± 2.5[a]	28.9 ± 4.4	19.8 ± 4.0	7.6 ± 1.3
16b	31.8 ± 2.7[a]	17.3 ± 1.9	NA	NA
16c	20.5 ± 5.1[a]	10.0 ± 2.5	NA	NA
16d	20.9 ± 3.2[a]	9.2 ± 5.1	NA	NA
16e	12.8 ± 0.5[a]	1.4 ± 0.2	NA	NA
17a	14.2 ± 2.1[a]	NA	NA	NA
17b	9.1 ± 3.5[a]	NA	NA	NA
17c	30.0 ± 3.5[a]	14.0 ± 6.4	9.0 ± 7.6	5.6 ± 2.5
20a	NA	NA	NA	NA
20b	NA	NA	NA	NA
20c	NA	NA	NA	NA
21a	NA	NA	NA	NA
21b	31.3 ± 4.3[a]	NA	NA	NA
21c	12.5 ± 3.6[a]	9.0 ± 1.6	NA	NA
22	89.7 ± 4.6[b]	64.2 ± 3.5	41.4 ± 2.7	26.0 ± 5.2

[a]Growth inhibition less than 15%; [b]Growth inhibition greater than 30%; ± standard deviation of the mean from at least two independent experiments. In each independent experiment, compounds were tested in duplicate. NA = no activity.

Gly126, Ala50, Leu40, Tyr47, Leu36, Ala127, Cys79 respectively played an important role in the binding of the compounds to the LasR receptor. The indole rings and phenyl-substituted tails of the tested compounds were able to form multiple hydrophobic interactions that led to high docking scores, which did not necessarily relate to activity. Therefore, prudent analysis of docking scores and interactions was required to understand the docking results with respect to activity. It was observed that the orientation of the docked ligand is crucial for inhibitory activity and an orientation similar to the natural ligand OdDHL in the receptor pocket was observed for synthesized compounds with high activity. The amino acid sequence of LasR ligand binding region (36-129), the docking poses of OdDHL and of the most potent compound **11d** are shown in Figure 5(a-c) respectively.

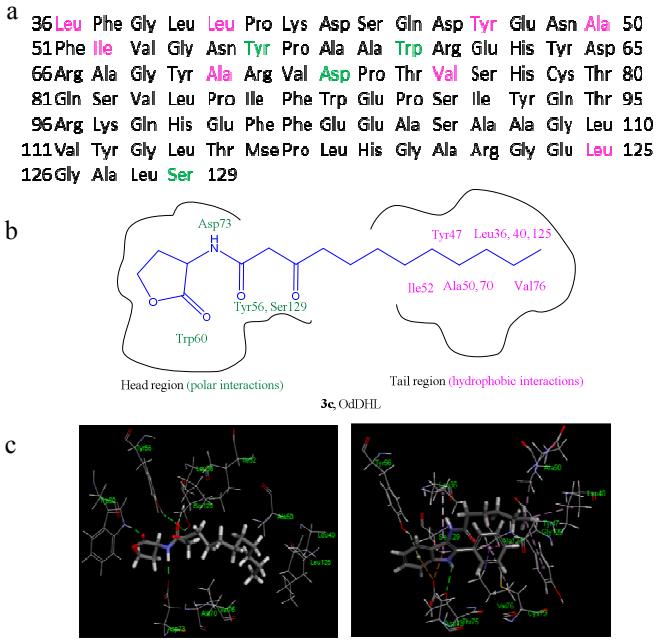


Figure 5. (a) Amino acid sequence for the LasR ligand binding region (amino acids color coded according to the nature of interaction). **(b)** Schematic representation of the natural ligand OdDHL (**3c**). **(c)** Docking interaction of natural ligand OdDHL (left) and Compound **11d** (right) with LasR.

Discussion

Bacteria communicate with each other using different autoinducer molecules, such as AHLs, to regulate virulence factor production, biofilm formation and antibiotic resistance in a population-dependent manner. Many bacteria such as *E. coli* use indole as an intercellular signaling molecule for QS-dependent phenotypes. Thus, AHL mimics bearing an indole moiety as the head group and an acyl moiety as a tail have the potential to inhibit QS. These synthetic AHL compounds with similar structures to natural AHL molecules can compete with their binding to the LasR protein. Binding of these artificial signaling molecules to the LuxR receptor prevents signal transduction from leading to gene transcription, thereby inhibiting virulence expression and biofilm formation. In this work, 22 indole-based AHL mimics were synthesized using facile coupling reactions as shown in Schemes 1-3. The amide series compounds generally resulted in good yields and the 3-oxo analogues generally gave moderate yields. The synthesized compounds were analyzed by docking studies for their interaction with the LasR protein and further tested for QS inhibitory activity against the *P. aeruginosa* MH602 *lasB* reporter strain.

Table 2. Docking of the indole-based AHL mimics to the LasR receptor protein

Entry	Docking score	H Bonds	Π and hydrophobic interactions	Unfavorable interactions
11a	52.20	Arg61, Thr75, Ser129	Tyr64, Tyr56, Val76, Cys79, Leu125, Ala127	Thr75, Tyr56
11b	50.85	Tyr64	Tyr47, Ala105, Trp88, Tyr93, Phe102, Val76, Cys79, Leu125, Ala127, Ala50, Ile52	-
11c	55.43	-	Ile52, Ala70, Ala50, Val76, Cys79, Ala127	-
11d	61.58	Thr75, Tyr56, Ser129	Val76, Gly126, Ala50, Leu40, Tyr47, Leu36, Ala127, Cys79	-
11e	60.26	-	Phe101, Tyr56, Val76, Cys79, Leu125, Ala127, Ala50, Ile52	-
14a	38.55	Tyr56, Ser129,	Try47, Trp88, Tyr93, Phe102, Val76, Ala127, Ala50, Ile52, Ala70	-
14b	60.25	Cys79, Ser129, Asp73	Tyr64, Trp88, Leu40, Leu125, Leu110, Leu36, Ala70	-
14c	58.93	Tyr64	Trp88, Tyr47, Phe101, Ile52, Ala70, Ala50, Val76, Cys79, Leu125, Ala127, Ala105, Leu110	Asp73, Thr75
16a	60.97	Tyr56, Ser129, Leu110, Asp73	Trp88, Phe101, Ala127, Cys79, Leu125, Ala105, Leu110	Tyr56
16b	68.13	Arg61, Tyr56,	Trp88, Phe101, Ala127, Val76,	-

		Ser129, Trp88	Cys79, Leu125, Leu36, Leu110	
16c	71.02	Tyr56, Trp88	Tyr56, Ala105, Leu110, Leu40, Ala50, Ala127	-
16d	60.87	Tyr56, Trp88	Tyr56, Ala50, Leu40, Leu36, Leu110	-
16e	73.26	Tyr56	Tyr56, Phe101, Leu36, Ala50, Ala70, Val76, Leu40, Leu125	Arg 61
17a	70.03	Arg61, Tyr56, Trp88	Tyr56, Val76, Cys79, Leu125, Ala105, Leu110	-
17b	67.86	-	Tyr56, Ala127, Leu125, Leu36	Arg61
17c	74.63	Arg61, Tyr56, Trp88	Phe101, Ala105, Leu110, Val76, Cys79, Leu125, Ala127	-
20a	58.48	Tyr56, Ser129, Asp73	Trp88, Phe101, Leu40, Leu125, Ala105, Leu110	Tyr64
20b	52.59	-	Leu110, Tyr56, Trp88, Ala50, Val76, Cys79, Ala127	Thr75
20c	62.48	Tyr56	Tyr56, Ala105, Leu110, Leu40, Ala50, Ala127	-
21a	68.34	Tyr56, Asp73	Trp88, Phe101, Val76, Cys79, Leu125, Ala105, Leu110	Asp73
21b	62.25	Thr115, Ser129	Ala105, Leu110, Trp88, Leu40, Ala50, Val76, Cys79, Ala127	-
21c	71.40	Tyr56, Ser129, Asp73	Tyr47, Trp88, Phe101, Ala105, Leu110, Leu40, Ala50, Val76	Tyr64
OdDHL (**3c**)	61.70	Asp73, Tyr56, Trp60, Arg61, Ser129, Tyr93	Trp88	Tyr64

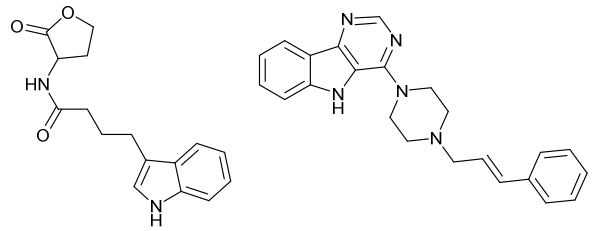
The results from the QS inhibition assay (Table 1) indicated that for the 3-amino-2-phenylindole-based compounds, the amide derivatives **11a-e** were more active compared to the 3-oxo-amide compounds **14a-c**. Furthermore, compound **11d** bearing a six-carbon acyl chain was the most active compound in the series. In contrast, AHL mimics (**11a-b** and **14a-c**) containing longer hydrophobic chains showed lower activity. This is consistent with the results of the molecular docking study, which showed compound **11d** having the highest docking score of 61.58 out of all the 3-amino-2-phenyl-based compounds (Table 2). Moreover, compound **11d** was predicted to form both hydrogen bonding and hydrophobic interactions with LasR at the binding site. Additionally, the orientation of the docked molecule appears to be crucial for the activity of compounds **11a-e**, as only **11a** and **11d** docked in a similar head to tail orientation compared to the natural agonist OdDHL, and also showed better QS inhibitory activity in the QS assay. Even though the compounds with opposite orientation may show higher docking scores, this orientation generally does not appear to facilitate QS inhibitory activity against *P. aeuroginosa* in the series.

For the 5-amino-2-methylindole-based compounds, the amide analogues **16a-e** again showed better activity compared to their 3-oxo amide **17a-c** counterparts. However, in contrast to the previous results, 5-amino-2-methylindole-based compounds **16a** and **16b** containing longer acyl chains were the most active, suggesting that more hydrophobic acyl chains could be beneficial for indole mimics lacking a bulky substituent at the 2-position. In the docked poses, the orientation of the head and tail of **16** and **17** in a similar fashion to OdDHL, as well as the orientation of the amide bond between indole and the linker and its H-bonding propensity increases the docking score and supports the observed activity. In the case of 7-aminoindole, a drastic loss in activity was observed for the amide analogues **20a-c**, while only minimal activity was seen for the 3-oxo amide analogues **21a-c**. Although the docking scores were higher for these series of compounds, it was observed that the proximity of the two '*NH*' groups the indole '*NH*' and the 7-substituted '*NH*', led to unfavorable clashes with the receptor protein. These indicate that 7-amino substitution is not ideal for QS inhibitory activity.

Comparing across the three series of compounds, AHL amide mimics based on the 3-amino-2-phenylindole scaffold were more active than those based on 5-amino-2-methyl or 7-amino indoles. A trend between activity and docking score could be observed for the 3-amino-2-phenyl compounds, but not for the other two series. For the 3-oxo mimics, the 5-amino-2-methyl compounds **17a-c** and the 7-aminoindole compound **21b** were more active than the 3-amino-2-phenyl- compounds **14a-c**. Thus, in the case of 3-oxo compounds, less bulky or no substitution was preferable for activity. Overall, AHL mimics based on 3-amino-2-phenylindole and 5-amino-2-methylindole bearing different acyl or aryl tail groups were found to be moderate QS inhibitors.

The Blackwell group synthesized the AHL mimic **23** (Fig 6) with an indole tail and in qualitative experiments on engineered *P. aeruginosa* at 50 μM concentration found significant GFP inhibition followed by biofilm reduction.[14,37] A recently isolated indole based natural compound *N*-[2-(1*H*-indol-3-yl)ethyl]-urea **7**, or yayurea A from the Gram-positive bacteria *Staphylococcus intermedius* and *Staphylococcus delphini* also

interferes with the QS mediated pyocyanin production in *P. aeruginosa,* but this effect was found to be associated with about 50% bacterial cell killing compared to the QS inhibitory effect.[31] Some attempts to modify indoles have been made by substitution at different positions of the indole ring and reported as QS inhibitors[38] (one representative and the most potent QS inhibitory compound is **24** (Fig 6). However, to date, this study is the first attempt to make AHL mimics bearing indole as a head group as QS inhibitors. The current work may provide an opportunity to develop new QS based antimicrobial therapeutics without affecting normal bacterial growth and thus avoiding resistance to antibiotics.



23 24

Figure 6. Indole-based representative QS inhibitors.

Conclusion

In this work, twenty two different AHL mimics based on indoles have been designed, synthesized, biologically evaluated, and docked into the LasR receptor to study the possible interactions such as hydrogen bonding, electrostatic and hydrophobic interactions. Biological assays (QS inhibition) were performed using a *P. aeruginosa* MH602 reporter strain. *N*-(2-Phenyl-1*H*-indol-3-yl)hexanamide **11d** showed 65% QS inhibitory activity at 250 μM concentration, which makes it the most potent compound studied. This is also supported by the results of the molecular docking study, where compound **11d** showed the highest docking score of the 3-amino-2-phenyl-based compounds (Table 3), and was predicted to form both hydrogen bonding and hydrophobic interactions with LasR at the binding site. An optimized chain length for AHLs bearing indole as a head group was found to be 6 carbons. All synthesized compounds were found to be non-toxic to bacterial cells (≤15% cell death) which may overcome the main drawbacks of traditional antibiotics (antibiotic resistance). This study could further assist in the development of novel indole-based antibacterials (QS inhibitors) without killing bacteria and thereby not enhancing resistance.

Materials and methods

General chemistry details

All chemical reagents were purchased from commercial sources (Alfa-Aesar and Sigma Aldrich) and used without further purification. Solvents were used from commercial sources and used as obtained. Reactions were performed using oven-dried glassware under an atmosphere of nitrogen and in anhydrous conditions (as required). Room temperature refers to the ambient temperature (22-24 °C). Yields refer to chromatographically and spectroscopically pure compounds unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) precoated with Merck silica gel 60 F254. Visualization was performed by the quenching of short or long wavelength UV fluorescence or by staining with potassium permanganate or ninhydrin solution. Flash chromatography was carried out using Grace Davison LC60A 6-35 μm silica gel. Preparative thin layer chromatography was carried out on 3 x 200 x 200 mm glass plates coated with Merck $60GF_{254}$ silica gel. Infrared spectra were recorded using a Cary 630 FTIR spectrophotometer. Ultraviolet spectra were measured using a Cary 100 Bio UV-visible spectrophotometer in the designated solvents and data reported as wavelength (λ) in nm and absorption coefficient (ε) in $M^{-1}cm^{-1}$. High-resolution mass spectrometry was performed by the Bioanalytical Mass Spectrometry facility, UNSW. Melting points were obtained using Mel-Temp melting point apparatus and are uncorrected. Proton and Carbon NMR was recorded in designated solvents using Bruker DPX 300 or a Bruker Avance 400 spectrometer as designated. Chemical shifts (δ) are quoted in parts per million (ppm), to the nearest 0.01 ppm and internally referenced relative to the solvent nuclei. ^{1}H NMR spectral data are reported as follows: [chemical shift in ppm; multiplicity in br, broad; s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; sext, sextet; sept, septet; m, multiplet; or as a combination of these (*e.g*. dd, dt *etc*.)]; coupling constant (*J*) in hertz, integration, proton count and assignment.

3-Nitroso-2-phenyl-1*H*-indole (9)

A solution of 2-phenyl-1-*H*-indole (5.00 g, 25.87 mmol) in acetic acid (50 ml) was cooled to 18 °C and a solution of sodium nitrite (1.60 g, 23.19 mmol) in water (3 ml) was added drop-wise, while keeping the temperature of the reaction mixture at 20 °C. The resulting reaction mixture was stirred at room temperature for 30 min and then diluted with ice water (250 ml). The precipitate was filtered and the resulting yellow solid was washed with water and then with methanol to yield the desired product as yellow solid (4.50g, 78%); mp 263-265 °C; UV (MeOH): λ_{max} 254 nm (ε 25231 M^{-1} cm^{-1}), 210 (29910); IR (neat): ν_{max} 3400, 2845, 1610, 1548, 1435 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.33 (t, *J* = 7.4 Hz, 1H, ArH), 7.45-7.57 (m, 5H, ArH), 8.12 (d, *J* = 7.4, 1H, ArH), 8.25 (dd, *J* = 7.4, 1.7 Hz, 2H, ArH), 13.79 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 127.7 (3 x ArCH), 129.0 (3 x ArC), 129.9 (3 x ArCH), 131.4 (3 x ArCH); HRMS (+ ESI): Found *m/z* 223.0862, $[M + H]^+$, $C_{14}H_{11}N_2O$ requires 223.0863.

2-Phenyl-1*H*-indol-3-amine (10)

To a boiling solution of 3-nitroso-2-phenyl-1*H*-indole (1.00 g, 4.50 mmol) in absolute ethanol (50 ml) was added 10% Pd/C (0.10 g), followed by the addition of hydrazine hydrate (2.25 g, 45.00 mmol) drop-wise over a period of 15 min. The mixture was heated under reflux for further 2 h, followed by filtration through a filter column of silica. The ethanol was removed by

rotary evaporation and the residue was dissolved in dichloromethane (50 ml). The organic layer was then washed with water (50 ml x 3) and then with brine, dried over sodium sulfate and evaporated in vacuo to yield the title compound an white solid (0.65g, 69%); mp 119 °C; UV (MeOH): λ_{max} 254 nm (ε 25231 M^{-1} cm^{-1}), 205 (27310); IR (neat): ν_{max} 3400, 2850, 1610 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.46 (s, NH_2), 6.90 (t, J = 7.5 Hz, 2H, ArH), 7.04 (t, J = 7.5 Hz, 2H, ArH), 7.16-7.25 (m, 2H, ArH), 7.43 (t, J = 7.4 Hz, 2H, ArH), 7.65 (d, J = 7.6 Hz, 2H, ArH), 7.79 (d, J = 7.4 Hz, 2H, ArH), 10.47 (s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 111.2 (ArCH), 117.8 (ArCH), 118.7 (ArC), 119.3 (ArCH), 122.4 (ArCH), 123.4 (ArCH), 123.4 (ArCH), 125.4 (ArCH), 125.5 (2 x ArC), 125.5, 129.1 (2 x ArCH), 134.0 (ArC), 135.4 (ArC); HRMS (+ESI): Found *m/z* 209.1069 $[M + H]^+$, $C_{14}H_{13}N_2$ requires 209.1070.

_N_-(2-Phenyl-1_H_-indol-3-yl)dodecanamide (11a)

To a solution of 2-phenyl-1*H*-indol-3-amine (0.17 g, 0.83 mmol) in dry dichloromethane (15 ml) was added dodecanoic acid (0.15 g, 0.75 mmol) and EDC.HCl (0.23 g, 1.20 mmol). The reaction mixture was stirred at room temperature for 30 h and then further diluted with dichloromethane (20 ml). The mixture was washed with water (40 ml x 3), saturated potassium bisulfate solution, sodium bicarbonate solution and then brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as green solid (0.10g, 37%); mp 92-94 °C; UV (MeOH): λ_{max} 305 nm (ε 20231 M^{-1} cm^{-1}), 236 (21910), 205 (29213); IR (neat): ν_{max} 3315, 3215, 3054, 2917, 2847, 2338, 2106, 1881, 1729, 1641, 1456, 1258, 1070, 1020, 798 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.84-0.88 (m, 3H, CH_3), 1.27-1.33 (m, 16H, CH_2), 1.65 (t, J = 6.9 Hz, 2H, CH_2), 2.37 (t, J = 7.1 Hz, 2H, CH_2), 6.97-7.79 (m, 9H, ArH), 9.36 (s, 1H, NH), 11.36 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH_3), 22.6 (CH_2), 26.0 (CH_2), 29.2 (2 x CH_2), 29.3 (2 x CH_2), 29.5 (2 x CH_2), 31.8 (CH_2), 36.0 (CH_2), 111.3 (ArC), 111.8 (2 x ArCH), 119.5 (ArCH), 122.4 (ArCH), 126.7 (ArC), 127.2 (ArCH), 127.2 (ArCH), 127.8 (ArCH), 129.0 (ArCH), 129.0 (ArCH), 131.8 (ArC), 132.1 (ArC), 135.0 (ArC), 173.1 (CO); HRMS (+ESI): Found *m/z* 413.2563 $[M + Na]^+$, $C_{26}H_{34}N_2ONa$ requires 413.2569.

_N_-(2-Phenyl-1_H_-indol-3-yl)decanamide (11b)

To a solution of 2-phenyl-1*H*-indol-3-amine (0.22 g, 1.04 mmol) in dry dichloromethane (15 ml) was added decanoic acid (0.15 g, 0.80 mmol) and EDC.HCl (0.21 g, 1.12 mmol). The reaction mixture was stirred at room temperature for 23 h and then further diluted with dichloromethane (20 ml). The mixture was washed with water (40 ml x 3), saturated potassium bisulfate solution, sodium bicarbonate solution and then brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as green solid (0.11g, 29%); mp 64 °C; UV (MeOH): λ_{max} 305 nm (ε 24179 M^{-1} cm^{-1}), 236 (35707); IR (neat): ν_{max} 3244, 3054, 2918, 2849,2339, 2100, 1874, 1636, 1487, 1453, 1337, 1238, 1182, 1106, 918, 737, 690 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.85-0.89 (m, 3H, CH_3), 1.27-1.33 (m, 12H, CH_2), 1.65 (t, J = 7.2 Hz, 2H, CH_2), 2.36 (t, J = 7.3 Hz, 2H, CH_2), 6.97-7.78 (m, 9H, ArH), 9.35 (s, 1H, NH), 11.35 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH_3), 22.6 (CH_2), 26.0 (CH_2), 29.2 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 31.8 (CH_2), 36.0 (CH_2), 111.3 (ArC), 111.8 (ArCH), 118.8 (ArCH), 119.5 (ArCH), 122.4 (ArCH), 126.7 (ArC), 127.2 (ArCH), 127.2 (ArCH), 127.8 (ArCH), 129.0 (ArCH), 129.0 (ArCH), 131.8 (ArC), 132.1 (ArC), 135.0 (ArC), 173.1 (CO); HRMS (+ESI): Found *m/z* 385.2249 $[M + Na]^+$, $C_{24}H_{30}N_2ONa$ requires 385.2250.

4-Phenyl-_N_-(2-phenyl-1_H_-indol-3-yl)butanamide (11c)

To a solution of 2-phenyl-1*H*-indol-3-amine (0.23 g, 1.10 mmol) in dry dichloromethane (15 ml) was added 4-phenyl butyric acid (0.20 g, 1.20 mmol) and EDC.HCl (0.34 g, 1.76 mmol). The reaction mixture was stirred at room temperature for 24 h and then further diluted with dichloromethane (20 ml). The mixture was washed with water (40 ml x 3), saturated potassium bisulfate solution, sodium bicarbonate solution and then brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as a greenish solid (0.10g, 25%); mp 139 °C; UV (MeOH): λ_{max} 305 nm (ε 16014 M^{-1} cm^{-1}), 236 (17334), 205 (28717); IR (neat): ν_{max} 3251, 3023, 2921, 2340, 2110, 1880, 1637, 1490, 1451, 1336, 1239, 1143, 1027, 910, 824, 737, 692 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.90-2.00 (m, 2H, CH_2), 2.40 (t, J = 7.9 Hz, 2H, CH_2), 2.68 (t, J = 7.8 Hz, 2H, CH_2), 6.97-7.79 (m, 14H, ArH), 9.40 (s, 1H, NH), 11.36 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 27.8 (CH_2), 35.2 (CH_2), 35.4 (CH_2), 111.2 (ArC), 111.8 (ArCH), 118.8 (ArCH), 119.5 (ArCH), 122.4 (ArCH), 126.3 (ArCH), 126.7 (ArC), 127.2 (ArCH), 127.2 (ArCH) 127.9 (ArCH), 128.5 (ArCH), 128.8 (ArCH), 128.8 (ArCH), 128.8 (ArCH), 129.1 (ArCH), 129.1 (ArCH), 131.9 (ArC), 132.2 (ArC), 135.0 (ArC), 142.3 (ArC), 172.8 (CO); HRMS (+ESI): Found *m/z* 377.1625 $[M + Na]^+$, $C_{24}H_{22}N_2ONa$ requires 377.1629.

_N_-(2-Phenyl-1_H_-indol-3-yl)hexanamide (11d)

To a solution of 2-phenyl-1*H*-indol-3-amine (0.16 g, 0.77 mmol) in dry dichloromethane (15 ml) was added hexanoic acid (0.099 g, 0.85 mmol) and EDC.HCl (0.24 g, 1.23 mmol). The reaction mixture was stirred at room temperature for 21 h and then further diluted with dichloromethane (20 ml). The mixture was washed with water (40 ml x 3), saturated potassium bisulfate solution, sodium bicarbonate solution and then brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as an off-white solid (0.19g, 82%); mp. 120–121 °C; UV (MeOH): λ_{max} 305 nm (ε 25186 M^{-1} cm^{-1}), 236 (27269), 205 (37319); IR (neat): ν_{max} 3235, 3055, 2925, 2855, 2340, 2108, 1883, 1638, 1506, 1453, 1341, 1240, 1189, 969, 916, 842, 738, 690 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.88-0.93 (m, 3H, CH_3), 1.32-1.38 (m, 2H,

CH_2), 1.65 (t, J = 7.1 Hz, 2H, CH_2), 2.36 (t, J = 7.2 Hz, 2H, CH_2), 6.97-7.78 (m, 9H, ArH), 9.35 (s, 1H, NH), 11.35 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH_3), 22.4 (CH_2), 25.6 (CH_2), 31.5 (CH_2), 36.0 (CH_2), 111.3 (ArC), 111.8 (ArCH), 118.8 (ArCH), 119.5 (ArCH), 122.4 (ArCH), 126.7 (ArC), 127.2 (ArCH), 127.2 (ArCH), 127.9 (ArCH), 129.1 (ArCH), 129.1 (ArCH), 131.8 (ArC), 132.1 (ArC), 135.0 (ArC), 173.1 (CO); HRMS (+ESI): Found *m/z* 329.1625 $[M + Na]^+$, $C_{20}H_{22}N_2ONa$ requires 329.1629.

3-(1*H*-Indol-3-yl)-*N*-(2-phenyl-1*H*-indol-3-yl)propanamide (11e)

To a solution of 2-phenyl-1*H*-indol-3-amine (0.20 g, 0.96 mmol) in dry dichloromethane (15 ml) was added 3-(1*H*-indol-3-yl)propanoic acid (0.20 g, 1.06 mmol) and EDC.HCl (0.29 g, 1.54 mmol). The reaction mixture was stirred at room temperature for 24 h and then further diluted with dichloromethane (20 ml). The mixture was washed with water (40 ml x 3), saturated potassium bisulfate solution, sodium bicarbonate solution and then brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as an off-white solid (0.14g, 37%); mp 187-188 °C; UV (MeOH): λ_{max} 304 nm (ε 28686 M^{-1} cm^{-1}), 222 (77598), 204 (66214); IR (neat): ν_{max} 3331, 3042, 2921, 2776, 2340, 2113, 1859, 1736, 1648, 1482, 1453, 1331, 1248, 1157, 1096, 1004, 912, 828, 732, 685 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.78 (t, J = 7.4 Hz, 2H, CH_2), 3.09 (t, J = 7.4 Hz, 2H, CH_2), 6.96-7.71 (m, 14H, ArH), 9.41 (s, 1H, NH), 10.84 (s, 1H, indole NH), 11.33 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.6 (CH_2), 36.7 (CH_2), 111.3 (ArC), 111.8 (2 x ArCH), 114.3 (ArC), 118.7 (ArCH), 118.9 (ArCH), 119.0 (ArCH), 119.4 (ArCH), 121.4 (ArCH), 122.3 (ArCH), 122.8 (ArCH), 126.6 (ArC), 127.1 (2 x ArCH), 127.6 (ArC), 127.8 (ArCH), 129.1 (2 x ArCH), 131.6 (ArC), 132.1 (ArC), 135.0 (ArC), 136.8 (2 x ArC), 172.8 (CO); HRMS (+ESI): Found *m/z* 402.1576 $[M + Na]^+$, $C_{25}H_{21}N_3ONa$ requires 402.1579.

3-Oxo-*N*-(2-phenyl-1*H*-indol-3-yl)dodecanamide (14a)

To a solution of decanoic acid (0.25 g, 1.44 mmol) in dry dichloromethane (20 ml) was added DCC (0.34g, 1.63 mmol), 4-dimethylaminopyridine (DMAP) (0.19g, 1.54 mmol) and Meldrum's acid (0.21 g, 1.44 mmol) under a N_2 atmosphere. The reaction mixture was stirred at room temperature for 24 h and then filtered through filter paper. The filtrate was evaporated by rotary evaporation and the residue was dissolved in dimethylformamide (DMF) (7 ml). 2-Phenyl-1*H*-indol-3-amine (0.20 g, 0.96 mmol) was added and the mixture was heated at 80 °C for 15 h. After cooling to room temperature, water (20 ml) was added and the mixture was extracted with ethyl acetate (30 ml x 3), washed with brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as a green solid (0.15g, 39%); mp 79-81 °C; UV (MeOH): λmax 279 nm (ε 38836 M^{-1} cm^{-1}); IR (neat): ν_{max} 3250, 3054, 2920, 2848, 2318, 2106, 1882, 1713, 1625, 1568, 1522, 1452, 1310, 1239, 1084, 918, 840, 738, 690 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.83-0.88 (m, 3H, CH_3), 1.25-1.73 (m, 16H, CH_2), 2.59 (t, J = 7.4 Hz, 2H, CH_2), 3.59 (s, 2H, CH_2), 7.01-7.82 (m, 9H, ArH), 9.61 (s, 1H, NH), 11.40 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH_3), 23.5 (CH_2), 24.9 (CH_2), 25.8 (CH_2), 29.0 (CH_2), 29.2 (CH_2), 29.4 (CH_2), 33.8 (CH_2), 51.2 (CH_2), 110.8 (ArC), 111.9 (ArCH), 119.0 (ArCH), 119.5 (ArCH), 122.5 (ArCH), 126.4 (ArC), 127.2 (2 x ArCH), 128.0 (ArCH), 129.11 (2 x ArCH), 131.7 (ArC), 132.0 (ArC), 134.5 (ArC), 166.9 (CO), 205.5 (CO); HRMS (+ESI): Found *m/z* 427.2358 $[M + Na]^+$, $C_{26}H_{32}N_2O_2Na$ requires 427.2356.

3-Oxo-*N*-(2-phenyl-1*H*-indol-3-yl)octanamide (14b)

To a solution of hexanoic acid (0.17 g, 1.44 mmol) in dry dichloromethane (20 ml) was added DCC (0.34 g, 1.63 mmol), DMAP (0.19 g, 1.54 mmol) and Meldrum's acid (0.21 g, 1.44 mmol) under a N_2 atmosphere. The reaction mixture was stirred at room temperature for 24 h and then filtered through filter paper. The filtrate was evaporated by rotary evaporation and the residue was dissolved in DMF (7 ml). 2-Phenyl-1*H*-indol-3-amine (0.20 g, 0.96 mmol) was added and the mixture was heated at 60 °C for 24 h. After cooling to room temperature, water (20 ml) was added and the mixture was extracted with ethyl acetate (30 ml x 3), washed with brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as an off-white powder (0.12g, 36%); mp 145-147 °C; UV (MeOH): λ_{max} 279 nm (ε 21603 M^{-1} cm^{-1}); IR (neat): ν_{max} 3337, 3188, 3048, 2928, 2850, 2112, 1718, 1646, 1541, 1448, 1325, 1196, 1085, 963, 891, 827, 737, 682 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.84-0.89 (m, 3H, CH_3), 1.24-1.59 (m, 6H, CH_2), 2.58 (t, J = 7.3, 2H, CH_2), 3.60 (s, 2H, CH_2), 7.01-7.82 (m, 9H, ArH), 9.60 (s, 1H, NH), 11.39 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.3 (CH_3), 23.1 (CH_2), 24.9 (CH_2), 33.8 (CH_2), 42.7 (CH_2), 51.2 (CH_2), 110.8 (ArC), 111.8 (ArCH), 119.0 (ArCH), 119.5 (ArCH), 122.5 (ArCH), 126.4 (ArC), 127.2 (2 x ArCH), 128.0 (ArCH), 129.1 (2 x ArCH), 131.7 (ArC), 132.0 (ArC), 134.5 (ArC), 166.9 (CO), 205.5 (CO); HRMS (+ESI): Found *m/z* 371.1731 $[M + Na]^+$, $C_{22}H_{24}N_2O_2Na$ requires 371.1730.

3-Oxo-6-phenyl-*N*-(2-phenyl-1*H*-indol-3-yl)hexanamide (14c)

To a solution of 4-phenyl butyric acid (0.24 g, 1.44 mmol) in dry dichloromethane (20 ml) was added DCC (0.34 g, 1.63 mmol), DMAP (0.19 g, 1.54 mmol) and Meldrum's acid (0.21 g, 1.44 mmol) under a N_2 atmosphere. The reaction mixture was stirred at room temperature for 24 h and then filtered through filter paper. The filtrate was evaporated by rotary evaporation and the residue was dissolved in DMF (7 ml). 2-Phenyl-1*H*-indol-3-amine (0.20 g, 0.96 mmol) was added and the mixture was heated at 60 °C for 24 h. After cooling to room temperature, water (20 ml) was added and the mixture was extracted with ethyl acetate (30 ml x 3), washed with brine, dried over sodium sulfate and the solvent was evaporated in

vacuo. Purification of the residue by silica gel chromatography afforded the title compound as an off-white powder (0.127g, 33%); mp 118-120 °C; UV (MeOH): λ_{max} 279 nm (ε 24581 M^{-1} cm^{-1}); IR (neat): ν_{max} 3386, 3222, 3052, 2915, 2328, 117, 1874, 1713, 1636, 1452, 1307, 1258, 1100, 1006, 919, 843, 737, 687 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.83-1.89 (m, 2H, CH_2), 2.56-2.64 (m, 4H, CH_2), 3.61 (s, 2H, CH_2), 6.99-7.82 (m, 14H, ArH), 9.61 (s, 1H, NH), 11.40 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 25.4 (CH_2), 34.8 (CH_2), 42.2 (CH_2), 51.2 (CH_2), 110.7 (ArC), 111.8 (ArCH), 119.1 (ArCH), 119.6 (ArCH), 122.5 (ArCH), 123.5 (ArCH), 126.3 (ArCH), 126.4 (ArC), 127.2 (2 x ArCH), 128.0 (ArCH), 128.8 (3 x ArCH), 129.1 (2 x ArCH), 131.7 (ArC), 132.0 (ArC), 135.0 (ArC), 142.2 (ArC), 166.9 (CO), 205.3 (CO); HRMS (+ESI): Found *m/z* 419.1736 $[M + Na]^+$, $C_{26}H_{24}N_2O_2Na$ requires 419.1730.

_N_-(2-Methyl-1_H_-indol-5-yl)dodecanamide (16a)

To a solution of 2-methyl-1*H*-indol-5-amine (0.20 g, 1.38 mmol) in dry dichloromethane (15 ml) was added dodecanoic acid (0.30 g, 1.52 mmol) and EDC.HCl (0.42 g, 2.21 mmol). The reaction mixture was stirred at room temperature for 21 h and then further diluted with dichloromethane (20 ml). The mixture was washed with water (40 ml x 3), saturated potassium bisulfate solution, sodium bicarbonate solution and then brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as a brown solid (0.26g, 57%); mp 108 °C; UV (MeOH): λ_{max} 238 nm (ε 36462 M^{-1} cm^{-1}); IR (neat): ν_{max} 3404, 3223, 3044, 2912, 2846, 2340, 1638, 1539, 1468, 1303, 1226, 1094, 1028, 971, 882, 775, 698 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.86 (t, *J* = 7.0 Hz, 3H, CH_3), 1.24-1.29 (m, 16H, CH_2), 1.59 (t, *J* = 7.1 Hz, 2H, CH_2), 2.27 (t, *J* = 7.2 Hz, 2H, CH_2), 2.35 (s, 3H, indole ring CH_3), 6.03 (s, 1H, ArH), 7.07-7.16 (m, 2H, ArH), 7.70 (s,1H, ArH), 9.55 (s, 1H, NH), 10.75 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 14.4 (CH_3), 22.6 (CH_2), 25.8 (CH_2), 29.2 (2 x CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (2 x CH_2), 31.8 (CH_2), 36.9 (CH_2), 99.6 (ArCH), 110.3 (ArCH), 110.5 (ArCH), 114.1 (ArCH), 128.9 (ArC), 131.6 (ArC), 133.2 (ArC), 136.5 (ArC), 170.9 (CO); HRMS (+ESI): Found *m/z* 351.2403 $[M + Na]^+$, $C_{21}H_{32}N_2ONa$ requires 351.2407.

_N_-(2-Methyl-1_H_-indol-5-yl)decanamide (16b)

To a solution of 2-methyl-1*H*-indol-5-amine (0.18 g, 1.24 mmol) in dry dichloromethane (15 ml) was added decanoic acid (0.23 g, 1.36 mmol) and EDC.HCl (0.38 g, 1.98 mmol). The reaction mixture was stirred at room temperature for 23 h and then further diluted with dichloromethane (20 ml). The mixture was washed with water (40 ml x 3), saturated potassium bisulfate solution, sodium bicarbonate solution and then brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as an off-white solid (0.17g, 46%); mp 98 °C; UV (MeOH): λ_{max} 238 nm (ε 60207 M^{-1} cm^{-1}); IR (neat): ν_{max} 3405, 3223, 3044, 2912, 2846, 2340, 2108, 1638, 1538, 1470, 1303, 1258, 1028, 971, 882, 775, 698 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.85 (t, *J* = 6.9 Hz, 3H, CH_3), 1.25-1.29 (m, 12H, CH_2), 1.59 (t, *J* = 7.0 Hz, 2H, CH_2), 2.26 (t, *J* = 7.2 Hz, 2H, CH_2), 2.35 (s, 3H, indole ring CH_3), 6.03 (s, 1H, ArCH), 7.07-7.16 (m, 2H, ArCH), 7.71 (s, 1H, ArCH), 9.55 (s, 1H, NH), 10.75 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 14.4 (CH_3), 22.6 (CH_2), 25.8 (CH_2), 29.2 (2 x CH_2), 29.3 (CH_2), 29.4 (CH_2), 31.8 (CH_2), 36.9 (CH_2), 99.6 (ArCH), 110.3 (ArCH), 110.5 (ArCH), 114.1 (ArCH), 128.9 (ArC), 131.6 (ArC), 133.2 (ArC), 136.5 (ArC), 170.9 (CO); HRMS (+ESI): Found *m/z* 323.2093 $[M + Na]^+$, $C_{19}H_{28}N_2ONa$ requires 323.2094.

_N_-(2-Methyl-1_H_-indol-5-yl)-4-phenylbutanamide (16c)

To a solution of 2-methyl-1*H*-indol-5-amine (0.20 g, 1.38 mmol) in dry dichloromethane (15 ml) was added 4-phenyl butyric acid (0.25 g, 1.52 mmol) and EDC.HCl (0.42 g, 2.21 mmol). The reaction mixture was stirred at room temperature for 17 h and then further diluted with dichloromethane (20 ml). The mixture was washed with water (40 ml x 3), saturated potassium bisulfate solution, sodium bicarbonate solution and then brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as a white solid (0.25g, 63%); mp 118-120 °C; UV (MeOH): λ_{max} 239 nm (ε 59156 M^{-1} cm^{-1}); IR (neat): ν_{max} 3402, 3225, 3021, 2918, 2857, 2341, 2109, 1857, 1632, 1534, 1477, 1372, 1273, 1247, 1205, 1132, 1030, 968, 883, 779, 739, 697 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): 1.86-1.91 (m, 2H, CH_2), 2.30 (t, *J* = 7.5 Hz, 2H, CH_2), 2.34 (s, 3H, indole ring CH_3), 2.62 (t, *J* = 7.6 Hz, 2H, CH_2), 6.03 (s, 1H, ArH), 7.07-7.32 (m, 7H, ArH), 7.70 (s, 1H, ArH), 9.58 (s, 1H, NH), 10.75 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 27.5 (CH_2), 35.2 (CH_2), 36.3 (CH_2), 99.6 (ArCH), 110.3 (ArCH), 110.5 (ArCH), 114.1 (ArCH), 126.2 (ArCH), 128.8 (4 x ArCH), 128.9 (ArCH), 128.9 (ArC), 131.6 (ArC), 133.2 (ArC), 136.5 (ArC), 170.6 (CO); HRMS (+ESI): Found *m/z* 315.1468 $[M + Na]^+$, $C_{19}H_{20}N_2ONa$ requires 315.1469.

_N_-(2-Methyl-1_H_-indol-5-yl)hexanamide (16d)

To a solution of 2-methyl-1*H*-indol-5-amine (0.18 g, 1.24 mmol) in dry dichloromethane (15 ml) was added hexanoic acid (0.16 g, 1.36 mmol) and EDC.HCl (0.38 g, 1.98 mmol). The reaction mixture was stirred at room temperature for 21 h and then further diluted with dichloromethane (20 ml). The mixture was washed with water (40 ml x 3), saturated potassium bisulfate solution, sodium bicarbonate solution and then brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as an off-white solid (0.16g, 53%); mp 144 °C; UV(MeOH): λ_{max} 238 nm (ε 48486 M^{-1} cm^{-1}); IR (neat): ν_{max} 3302, 3079, 2951, 2852, 2340, 2099, 1838, 1627, 1540, 1481, 1217, 957, 868, 773, 678 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.88 (t, *J* = 7.1 Hz, 3H, CH_3), 1.28-1.32 (m, 4H, CH_2), 1.57-1.61 (m, 2H, CH_2), 2.26 (t, *J* = 7.2 Hz, 2H, CH_2), 2.34 (s, 3H, indole ring CH_3), 6.04 (t, *J*

= 0.93 Hz, 1H, ArH), 7.06-7.16 (m, 2H, ArH), 7.70 (s, 1H, ArH), 9.55 (s, 1H, NH), 10.75 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 14.4 (CH_3), 22.4 (CH_2), 25.5 (CH_2), 31.5 (CH_2), 36.8 (CH_2), 99.6 (ArCH), 110.3 (ArCH), 110.5 (ArCH), 114.1 (ArCH), 128.9 (ArC), 131.6 (ArC), 133.2 (ArC), 136.5 (ArC), 171 (CO); HRMS (+ESI): Found *m/z* 267.1467 $[M + Na]^+$, $C_{15}H_{20}N_2ONa$ requires 267.1468.

3-(1*H*-Indol-3-yl)-*N*-(2-methyl-1*H*-indol-5-yl)propanamide (16e)

To a solution of 2-methyl-1*H*-indol-5-amine (0.20 g, 1.38 mmol) in dry dichloromethane (15 ml) was added 3-(1*H*-indol-3-yl)propanoic acid (0.29 g, 1.52 mmol) and EDC.HCl (0.42 g, 2.21 mmol). The reaction mixture was stirred at room temperature for 22 h and then further diluted with dichloromethane (20 ml). The mixture was washed with water (40 ml x 3), saturated potassium bisulfate solution, sodium bicarbonate solution and then brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as light purple solid (0.24g, 54%); mp 179 °C; UV (MeOH): λ_{max} 226 nm (ε 11496 M^{-1} cm^{-1}); IR (neat): ν_{max} 3387, 3183, 3034, 2913, 2343, 2118, 1877, 1635, 1543, 1452, 1337, 1218, 1094, 1007, 879, 799, 740 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.34 (s, 3H, indole ring CH_3), 2.63 (t, *J* = 7.2 Hz, 2H, CH_2), 3.02 (t, *J* = 7.3 Hz, 2H, CH_2), 6.04 (s, 1H, ArH), 6.97-7.16 (m, 5H, ArH), 7.32 (d, *J* = 7.4 Hz, 1H, ArH), 7.58 (d, *J* = 7.5 Hz, 1H, ArH), 7.71 (s, 1H, ArH), 9.62 (s, 1H, NH), 10.76 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 21.5 (CH_2), 37.7 (CH_2), 99.6 (ArCH), 110.3 (ArCH), 110.5 (ArCH), 111.8 (ArC), 114.1 (ArC), 114.4 (ArC), 118.6 (ArC), 118.9 (ArC), 121.4 (ArC), 122.6 (ArC), 127.6 (ArC), 128.9 (ArC), 131.6 (ArC), 133.2 (ArC), 136.6 (ArC), 136.7 (ArC), 170.7 (CO); HRMS (+ESI): Found *m/z* 340.1433 $[M + Na]^+$, $C_{20}H_{19}N_3ONa$ requires 340.1430;

***N*-(2-Methyl-1*H*-indol-5-yl)-3-oxododecanamide (17a)**

To a solution of decanoic acid (0.33 g, 1.93 mmol) in dry dichloromethane (20 ml) was added DCC (0.46 g, 2.21 mmol), DMAP (0.25 g, 2.07 mmol) and Meldrum's acid (0.28 g, 1.93 mmol) under a N_2 atmosphere. The reaction mixture was stirred at room temperature for 24 h and then filtered through filter paper. The filtrate was evaporated by rotary evaporation and the residue was dissolved in DMF (7.0 ml). 2-Methyl-1*H*-indol-5-amine (0.20 g, 1.38 mmol) was added and the mixture was heated at 60 °C for 24 h. After cooling to room temperature, water (20 ml) was added and the mixture was extracted with ethyl acetate (30 ml x 3), washed with brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound an off-white powder (0.09g, 20%); mp 88 °C; UV (MeOH): λ_{max} 213 nm (ε 33768 M^{-1} cm^{-1}); IR (neat): ν_{max} 3381, 3276, 2915, 2847, 2341, 2114, 1867, 1719, 1626, 1544, 1449, 1408, 1306, 1241, 1183, 1044, 874, 782 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.85 (t, *J* = 7.1 Hz, 3H, CH_3), 1.21-1.33 (m, 12H, CH_2), 1.48 (t, *J* = 7.1 Hz, 2H, CH_2), 2.34 (s, 3H, CH_3), 2.56 (t, *J* = 7.2, 2H, CH_2), 3.48 (s, 2H, CH_2), 6.05 (s, 1H, ArH), 7.04-7.08 (m, 1H, ArH), 7.16 (d, *J* = 8.5 Hz, 1H, ArH), 7.69 (s, 1H, ArH), 9.81 (s, 1H, NH), 10.80 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 14.4 (CH_3), 22.6 (CH_2), 23.4 (CH_2), 29.0 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 31.8 (CH_2), 42.6 (CH_2), 52.1 (CH_2), 99.7 (ArCH), 110.4 (ArCH), 110.6 (ArCH), 114.0 (ArCH), 128.9 (ArC), 131.2 (ArC), 133.4 (ArC), 136.7 (ArC), 164.7(CO), 205.6 (CO); HRMS (+ESI): Found *m/z* 365.2206 $[M + Na]^+$, $C_{21}H_{30}N_2O_2Na$ requires 365.2200.

***N*-(2-Methyl-1*H*-indol-5-yl)-3-oxooctanamide (17b)**

To a solution of hexanoic acid (0.24 g, 2.07 mmol) in dry dichloromethane (20 ml) was added DCC (0.49 g, 2.35 mmol), DMAP (0.27 g, 2.21 mmol) and Meldrum's acid (0.30 g, 2.07 mmol) under a N_2 atmosphere. The reaction mixture was stirred at room temperature for 24 h and then filtered through filter paper. The filtrate was evaporated by rotary evaporation and the residue was dissolved in DMF (7 ml). 2-Methyl-1*H*-indol-5-amine (0.20 g, 1.38 mmol) was added and the mixture was heated at 60 °C for 24 h. After cooling to room temperature, water (20 ml) was added and the mixture was extracted with ethyl acetate (30 ml x 3), washed with brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as an off-white powder (0.11g, 30%); mp 92-94 °C; UV (MeOH): λ_{max} 216 nm (ε 50066 M^{-1} cm^{-1}); IR (neat): ν_{max} 3380, 3269, 3085, 2919, 2341, 2115, 1906, 1634, 1542, 1442, 1345, 1246, 1182, 1112, 1033, 965, 880, 777 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.11-1.14 (m, 3H, CH_3), 1.16-1.29 (m, 4H, CH_2), 1.49-1.74 (m, 2H, CH_2), 2.36 (s, 3H, CH_3), 3.43 (s, 2H, CH_2), 6.07 (s, 1H, ArH), 7.14-7.20 (m, 2H, ArH), 7.74 (s, 1H, ArH), 9.90 (s, 1H, NH), 10.82 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (2 x CH_3), 24.9 (CH_2), 25.8 (CH_2), 33.8 (2 x CH_2), 48.0 (CH_2), 99.7 (ArCH), 110.2 (ArCH), 110.7 (ArCH), 113.9 (ArCH), 128.9 (ArC), 131.3 (ArC), 133.4 (ArC), 157.1 (CO), 165.5 (CO); HRMS: (+ESI): Found *m/z* 309.3598 $[M + Na]^+$, $C_{17}H_{22}N_2O_2Na$ requires 309.3596.

***N*-(2-Methyl-1*H*-indol-5-yl)-3-oxo-6-phenylhexanamide (17c)**

To a solution of 4-phenyl butyric acid (0.34 g, 2.07 mmol) in dry dichloromethane (20 ml) was added DCC (0.49 g, 2.35 mmol), DMAP (0.27 g, 2.21 mmol) and Meldrum's acid (0.30 g, 2.07 mmol) under a N_2 atmosphere. The reaction mixture was stirred at room temperature for 24 h and then filtered through filter paper. The filtrate was evaporated by rotary evaporation and the residue was dissolved in DMF (7 ml). 2-Methyl-1*H*-indol-5-amine (0.20 g, 1.38 mmol) was added and the mixture was heated at 60 °C for 24 h. After cooling to room temperature, water (20 ml) was added and the mixture was extracted with ethyl acetate (30 ml x 3), washed with brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as a brown oil (0.08g, 20%); UV

(MeOH): λ_{max} 262 nm (ε 35299 M^{-1} cm^{-1}); IR (neat): ν_{max} 3247, 2923, 2848, 2328, 2116, 1644, 1569, 1425, 1363, 1272, 1200, 1084, 1018, 928, 826, 777, 699 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.74-1.84 (m, 2H, CH_2), 2.35 (s, 3H, CH_3) 2.54-2.61 (m, 4H, CH_2), 3.49 (s, 2H, CH_2), 6.06 (s, 1H, ArCH), 7.04-7.29 (m, 9H, ArH), 9.81 (s, 1H, NH), 10.80 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 25.3 (CH_2), 34.8 (CH_2), 42.1 (CH_2), 52.1 (CH_2), 99.7 (ArCH), 110.4 (ArCH), 110.7 (ArCH), 114.0 (ArCH), 126.3 (ArCH), 128.8 (4 x ArCH), 128.9 (ArC), 131.2 (ArC), 133.4 (ArC), 136.8 (ArC), 142.2 (ArC) 164.7 (CO), 205.4 (CO); HRMS (+ESI): Found *m/z* 357.1569 $[M + Na]^+$, $C_{21}H_{22}N_2O_2Na$ requires 357.1573.

1*H*-Indol-7-amine (19)

To a boiling solution of 7-nitroindole (1.00 g, 6.17 mmol) in ethanol (40 ml) was added Pd/C (10%) (0.18 g) and hydrazine hydrate (3.09 g, 61.73 mmol) under inert atmosphere. The reaction mixture was heated under reflux for 2 h, cooled, and filtered through a small amount of silica in a filter column. The ethanol was evaporated, dichloromethane (20 ml) was added and the solution was washed with water (40 ml x 3). The organic layer was then washed with brine and dried over sodium sulfate. Evaporation of the solvent afforded the title compound as a red-brown solid (0.57g, 70%); mp 95-96 °C; UV (MeOH): λ_{max} 278 nm (ε 24231 M^{-1} cm^{-1}), 286 (30120); IR(neat): ν_{max} 3300, 3000, 1850, 1610 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.99 (s, 2H, NH_2), 6.29-6.33 (m, 2H, ArH), 6.73 (t, *J* = 7.6 Hz, 1H, ArH), 6.82-6.99 (m, 1H, ArH), 7.23 (t, *J* = 2.8 Hz, 1H, ArH), 10.61 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 101.9 (ArCH), 105.1 (ArCH), 109.1 (ArCH), 120.4 (ArCH), 124.2 (ArCH), 126.1 (ArC), 128.7 (ArC), 134.2 (ArC); HRMS (+ESI): Found *m/z* 165.1419[M + Na]$^+$, $C_8H_8N_2Na$ requires 165.1418.

***N*-(1*H*-Indol-7-yl)decanamide (20a)**

To a solution of 1*H*-indol-7-amine (0.18 g, 1.36 mmol) in dry dichloromethane (15 ml) was added decanoic acid (0.26 g, 1.50 mmol) and EDC.HCl (0.42 g, 2.18 mmol). The reaction mixture was stirred at room temperature for 18 h and then further diluted with dichloromethane (20 ml). The mixture was washed with water (40 ml x 3), saturated potassium bisulfate solution, sodium bicarbonate solution and then brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as a light pink solid (0.36g, 92%); mp 80 °C; UV (MeOH): λ_{max} 262 nm (ε 13174 M^{-1} cm^{-1}); IR (neat): ν_{max} 3389, 3251, 3025, 2916, 2847, 2723, 2340, 2111, 1895, 1655, 1581, 1533, 1488, 1430, 1337, 1184, 1105, 960, 887, 795 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.86 (t, *J* = 6.9 Hz, 3H, CH_3), 1.21-1.31 (m, 12H, CH_2), 1.65 (t, *J* = 7.1 Hz, 2H, CH_2), 2.41 (t, *J* = 7.2 Hz, 2H, CH_2), 6.42 (dd, *J* = 3.0, 2.1, 1H, ArH), 6.92 (t, *J* = 7.8 Hz, 1H, ArH), 7.29-7.35 (m, 2H, ArH), 7.41 (d, *J* = 7.6 Hz, 1H, ArH), 9.66 (s, 1H, NH), 10.73 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH_3), 22.6 (CH_2), 25.7 (CH_2), 29.2 (2 x CH_2), 29.4 (2 x CH_2), 31.8 (CH_2), 36.7 (CH_2), 102.0 (ArCH), 113.7 (ArCH), 116.7 (ArCH), 119.3 (ArCH), 124.1 (ArC), 125.4 (ArCH), 128.5 (ArC), 129.5 (ArC), 171.8 (CO); HRMS (+ESI): Found *m/z* 309.1941 $[M + Na]^+$, $C_{18}H_{26}N_2ONa$ requires 309.1939.

***N*-(1*H*-Indol-7-yl)hexanamide (20b)**

To a solution of 1*H*-indol-7-amine (0.16 g, 1.21 mmol) in dry dichloromethane (15 ml) was added hexanoic acid (0.16 g, 1.33 mmol) and EDC.HCl (0.37 g, 1.94 mmol). The reaction mixture was stirred at room temperature for 18 h and then further diluted with dichloromethane (20 ml). The mixture was washed with water (40 ml x 3), saturated potassium bisulfate solution, sodium bicarbonate solution and then brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as a light brown solid (0.11g, 61%); mp 108-110 °C; UV (MeOH): λ_{max} 256 nm (ε 11791 M^{-1} cm^{-1}); IR (neat): ν_{max} 3363, 3275, 3068, 2921, 2856, 2339, 2108, 1891, 1647, 1541, 1432, 1341, 1256, 1198, 1052, 949, 844, 769, 723 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.89 (t, *J* = 7.3 Hz, 3H, CH_3), 1.32-1.35 (m, 4H, CH_2), 1.64-1.68 (m, 2H, CH_2), 2.40 (t, *J* = 7.5 Hz, 2H, CH_2), 6.43 (dd, *J* = 3.1, 2.1 Hz, 1H, ArH), 6.93 (t, *J* = 7.6 Hz, 1H, ArH), 7.30-7.40 (m, 3H, ArH), 9.62 (s, 1H, NH), 10.66 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH_3), 22.4 (CH_2), 25.4 (CH_2), 31.4 (CH_2), 36.6 (CH_2), 102.0 (ArCH), 113.8 (ArCH), 116.7 (ArCH), 119.3 (ArCH), 124.1 (ArC), 125.4 (ArCH), 128.6 (ArC), 129.6 (ArC), 171.7 (CO); HRMS (+ESI): Found *m/z* 253.1315 $[M + Na]^+$, $C_{18}H_{26}N_2ONa$ requires 253.1311.

***N*-(1*H*-Indol-7-yl)-4-phenylbutanamide (20c)**

To a solution of 1*H*-indol-7-amine (0.16 g, 1.21 mmol) in dry dichloromethane (15 ml) was added 4-phenyl butyric acid (0.22 g, 1.33 mmol) and EDC.HCl (0.37 g, 1.94 mmol). The reaction mixture was stirred at room temperature for 18 h and then further diluted with dichloromethane (20 ml). The mixture was extracted with water (40 ml x 3) and washed with saturated potassium bisulfate solution, sodium bicarbonate solution and then brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as a light brown solid (0.21g, 39%); mp 130-132 °C; UV (MeOH): λ_{max} 262 nm (ε 15030 M^{-1} cm^{-1}); IR (neat): ν_{max} 3291, 3022, 2918, 2685, 2339, 2112, 1874, 1685, 1623, 1540, 1431, 1339, 1252, 1213, 1116, 1022, 947, 841, 770 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.92-2.02 (m, 2H, CH_2), 2.43 (t, *J* = 7.6 Hz, 2H, CH_2), 2.68 (t, *J* = 7.7 Hz, 2H, CH_2), 6.43 (dd, *J* = 3.1, 2.1 Hz, 1H, ArH), 6.94 (t, *J* = 7.8 Hz, 1H, ArH), 7.17-7.40 (m, 8H, ArH), 9.66 (s, 1H, NH), 10.67 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 27.4 (CH_2), 35.2 (CH_2), 36.0 (CH_2), 102.0 (ArCH), 114.0 (ArCH), 116.8 (ArCH), 119.4 (ArCH), 124.0 (ArC), 125.5 (ArCH), 126.3 (ArCH), 128.7 (ArCH), 128.8 (2 x ArCH), 128.9 (ArCH), 129.6 (ArC), 142 (ArC), 142.2 (ArC), 171.4 (CO); HRMS (+ESI): Found *m/z* 301.1312 $[M + Na]^+$, $C_{18}H_{18}N_2ONa$ requires 301.1311.

***N*-(1*H*-Indol-7-yl)-3-oxododecanamide (21a)**

To a solution of decanoic acid (0.35 g, 2.04 mmol) in dry dichloromethane (20 ml) was added DCC (0.48 g, 2.31 mmol), DMAP (0.27 g, 2.18 mmol) and Meldrum's acid (0.29 g, 2.04 mmol) under a N_2 atmosphere. The reaction mixture was stirred at room temperature for 24 h and then filtered through filter paper. The filtrate was evaporated by rotary evaporation and the residue was dissolved in DMF (7.0 ml). 1*H*-Indol-7-amine (0.18 g, 1.36 mmol) was added and the mixture was heated at 70 °C for 48 h. After cooling to room temperature, water (20 ml) was added and the mixture was extracted with ethyl acetate (30 ml x 3), washed with brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as an off-white powder (0.10g, 22%); mp 108-110 °C; UV (MeOH): λ_{max} 263 nm (ε 10805 M^{-1} cm^{-1}); IR (neat): ν_{max} 3330, 3082, 2915, 2848, 2338, 2106, 1713, 1646, 1543, 1434, 1329, 1259, 1116, 1044, 941, 890, 782, 721 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.86 (t, J = 6.8 Hz, 3H, CH_3), 1.21-1.31 (m, 12H, CH_2), 1.51 (t, J = 6.9 Hz, 2H, CH_2), 2.63 (t, J = 7.0 Hz, 2H, CH_2), 3.61 (s, 2H, CH_2), 6.45 (dd, J = 3.0, 2.1 Hz, 1H, ArH), 6.95 (t, J = 7.8 Hz, 1H, ArH), 7.34-7.38 (m, 3H, ArH), 9.87 (s, 1H, NH), 10.59 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.4 (CH_3), 22.6 (CH_2), 23.4 (CH_2), 26.6 (CH_2), 29.0 (CH_2), 29.2 (CH_2), 29.4 (CH_2), 31.8 (CH_2), 42.7 (CH_2), 51.7 (CH_2), 102.1 (ArCH), 114.2 (ArCH), 117.3 (ArCH), 119.4 (ArCH), 123.5 (ArC), 125.6 (ArCH), 128.8 (ArC), 129.6 (ArC), 165.6 (CO), 205.3 (CO); HRMS (+ESI): Found m/z 351.2051 $[M + Na]^+$, $C_{20}H_{28}N_2O_2Na$ requires 351.2049.

***N*-(1*H*-Indol-7-yl)-3-oxooctanamide (21b)**

To a solution of hexanoic acid (0.24 g, 2.04 mmol) in dry dichloromethane (20 ml) was added DCC (0.48 g, 2.31 mmol), DMAP (0.27 g, 2.18 mmol) and Meldrum's acid (0.29 g, 2.04 mmol) under a N_2 atmosphere. The reaction mixture was stirred at room temperature for 24 h and then filtered through filter paper. The filtrate was evaporated by rotary evaporation and the residue was dissolved in DMF (7 ml). 1*H*-Indol-7-amine (0.18 g, 1.36 mmol) was added and the mixture was heated at 70 °C for 48 h. After cooling to room temperature, water (20 ml) was added and the mixture was extracted with ethyl acetate (30 ml x 3), washed with brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as a brown powder (0.08g, 20%); mp 38-40 °C; UV (MeOH): λ_{max} 262 nm (ε 12028 M^{-1} cm^{-1}); IR (neat): ν_{max} 3321, 2923, 2254, 2126, 1649, 1554, 1430, 1341, 1293, 994, 822, 760, 723, 648 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.86 (t, J = 7.0 Hz, 3H, CH_3), 1.24-1.28 (m, 4H, CH_2), 1.47-1.57 (m, 2H, CH_2), 2.62 (t, J = 7.1 Hz, 2H, CH_2), 3.62 (s, 2H, CH_2), 6.45 (dd, J = 3.0, 2.1 Hz, 1H, ArH), 6.95 (t, J = 7.7 Hz, 1H, ArH), 7.31-7.38 (m, 3H, ArH), 9.86 (s, 1H, NH), 10.59 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.3 (CH_3), 22.4 (CH_2), 23.1 (CH_2), 31.2 (CH_2), 42.7 (CH_2), 51.7 (CH_2), 102.1 (ArCH), 114.2 (ArCH), 117.3 (ArCH), 119.4 (ArCH), 123.5 (ArC), 125.6 (ArCH), 128.8 (ArC), 129.6 (ArC), 165.6 (CO), 205.3 (CO); HRMS (+ESI): Found m/z 295.1422 $[M + Na]^+$, $C_{16}H_{20}N_2O_2Na$ requires 295.1420.

***N*-(1*H*-Indol-7-yl)-3-oxo-6-phenylhexanamide (21c)**

To a solution of 4-phenyl butyric acid (0.34 g, 2.04 mmol) in dry dichloromethane (20 ml) was added DCC (0.48 g, 2.31 mmol), DMAP (0.27 g, 2.18 mmol) and Meldrum's acid (0.29 g, 2.04 mmol) under a N_2 atmosphere. The reaction mixture was stirred at room temperature for 24 h and then filtered through filter paper. The filtrate was evaporated by rotary evaporation and the residue was dissolved in DMF (7 ml). 1*H*-Indol-7-amine (0.18 g, 1.36 mmol) was added and the mixture was heated at 70 °C for 48 h. After cooling to room temperature, water (20 ml) was added and mixture was extracted with ethyl acetate (30 ml x 3), washed with brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Purification of the residue by silica gel chromatography afforded the title compound as an off-white solid (0.08g, 19%); mp 130-132 °C; UV (MeOH): λ_{max} 262 nm (ε 12601 M^{-1} cm^{-1}); IR (neat): ν_{max} 3325, 3025, 2923, 2847, 2329, 2112, 1716, 1627, 1543, 1434, 1327, 1245, 1117, 953, 889, 781, 723, 676 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.77-1.87 (m, 2H, CH_2), 2.55-2.67 (m, 4H, CH_2), 3.62 (s, 2H, CH_2), 6.45 (dd, J = 3.0, 2.1 Hz, 1H, ArH), 6.95 (t, J = 7.7 Hz, 1H, ArH), 7.10-7.37 (m, 8H, ArH), 9.87 (s, 1H, NH), 10.59 (s, 1H, indole NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.8 (CH_2), 34.8 (CH_2), 42.2 (CH_2), 51.7 (CH_2), 102.1 (ArCH), 114.3 (ArCH), 117.3 (ArCH), 119.4 (ArCH), 123.5 (ArC), 125.7 (ArCH), 126.3 (ArC), 128.7 (2 x ArCH), 128.8 (3 x ArCH), 129.6 (ArC), 142.2 (ArC), 165.5 (CO), 205.2 (CO); HRMS (+ESI): Found m/z 343.1425 $[M + Na]^+$, $C_{20}H_{20}N_2O_2Na$ requires 343.1421.

Quorum sensing inhibition assay

To evaluate the effect of the synthesized compounds on QS signaling, the *P. aeruginosa* MH602 P_{lasB}::*gfp*(ASV) reporter strain, which harbors a chromosomal fusion of the *lasB* promoter to an unstable *gfp* gene and responds to the AHL 3-oxo-dodecanoyl homoserine lactone (3oxo-C12-HSL), was used.[35] To each well of the top row in a 96-well plate, 160 µL of Luria-Bertani (LB_{10}) broth media and 40 µL of 5 mM test compound's solution in DMSO were added. This was followed by 2 times dilution each time in LB_{10} broth media in all subsequent wells. Then 100 µL of a diluted (100 times diluted in LB_{10} broth) overnight culture of *P. aeruginosa* MH602 were added to all wells and the final volume in each well was 200 µL. The plates were incubated for 15 h in a microplate reader (Wallac Victor, Perkin-Elmer) heated at 37 ºC and every 30 min were briefly shaken and measured for GFP expression (fluorescence: excitation 485 nm, emission 535 nm) and cell growth (OD 600). The inhibitory effect of a DMSO control (1% of total volume) was examined in similar fashion but no inhibitory effect either for GFP or OD was observed.

Docking

Possible binding sites and poses of the compounds within the quorum sensing receptor LasR were predicted by docking these compounds into the LasR receptor (PDB code, 2UV0, resolution 1.8 Å) using Ligand Docking (GOLD) (Cambridge Crystallography Data Centre, UK) in its implementation through the Discovery Studio (Accelrys) interface. Hydrogens were added to all ligands and the receptor prior to performing the docking runs. All ligands were also minimised under the CHARMm forcefield. The binding pocket was defined from the binding site of agonist OdDHL in the crystal structure. The number of docking runs was set to 10, the "Detect Cavity" and "Early Termination" options were set to be "False". All other parameters were left at their default values. Gold scores, hydrogen bonds, and π-interactions of the ligands were analysed for the first pose with the highest Gold score.

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Graphical abstract: