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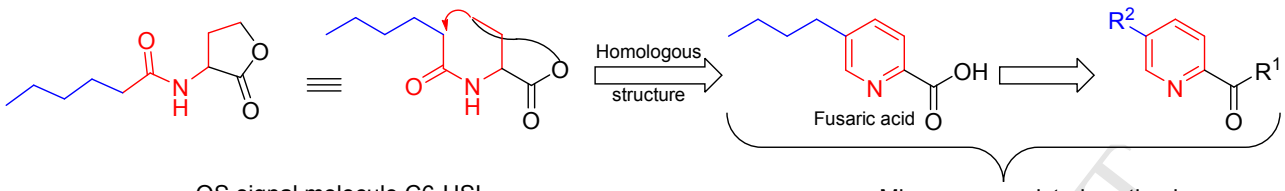

QS signal molecule C6-HSL

- Microwave-assisted synthesis
- QS inhibitors, SAR

Fusaric acid and analogues as gram-negative bacterial quorum sensing inhibitors

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Abstract: Taking advantage of microwave-assisted synthesis, efficient and expedite procedures for preparation of a library of fusaric acid and 39 analogues are reported. The fusaric acid analogues were tested in cell-based screening assays for inhibition of the *las* and *rhl* quorum sensing system in *Pseudomonas aeruginosa* and the *lux* quorum sensing system in *Vibrio fischeri*. Eight of the 40 compounds in the library including fusaric acid inhibited *lux* quorum sensing and one compound inhibited activity of the *las* quorum sensing system. To our delight, none of the compounds showed growth inhibitory effects in the tested concentration ranges.

Keywords: fusaric acid, quorum sensing inhibitors, microwave-assisted synthesis.

1. Introduction

The mycotoxin fusaric acid (**FA**, **Fig. 1**) was originally isolated by Yabuta in 1934 from the fungus *Fusarium heterosporum* [1]. Later studies have revealed that the compound is produced by a large number of species belonging to the *Fusarium* genus and maybe even by all *Fusarium* species. In some cultures, more than 1 mg of **FA** per gram medium is produced. The agent was the first compound shown to have a pivotal role in plant diseases such as tomato, cucumber and banana wilt [2, 3]. In addition to the involvement in plant diseases [2-4], **FA** has a number of pharmacological properties even though only in moderate to high doses [5, 6] and possesses the ability to augment the effects of other mycotoxins. Despite a number of effects in ranging from alteration of membrane activity, decreased mitochondrial activity, inhibition of ATP synthesis and reduced production of polyphenol oxidase and peroxidases, the mechanism of **FA** is still not fully delineated [2]. **FA** is also reported to chelate divalent cations and inhibit of dopamine β-hydroxylase [7a-b]. Over the year, **FA** has undergone several preclinical and clinical trials for treatment of diseases such as mania, cancer and hypertension [7].

We hypothesized that **FA** can be considered a bioequivalent to *N*-hexanoyl-L-homoserine lactone, a signal molecule involved in qorum sensing (QS) in bacteria. In addition, **FA** like some QS inhibitors (QSIs) possesses a pyridine ring (**Fig. 1c**). QSIs are very encouraging for design of new antimicrobial drugs acting through a hitherto untapped bacterial pathway. No new molecular entities are currently in clinical trials for interfering with QS in patients. Notably however, azithromycin, one of the best-selling antibiotics, has been demonstrated to inhibit virulence and cooperation of *Pseudomonas aeruginosa* through QS mechanism [8a]. Recently, hamamelitannin analogues were suggested to increase the susceptibility of a methicillin-resistant *Streptococcus aureus* strain through QS inhibition [8b].

Fig. 1

During QS bacteria use small signal molecules or autoinducers for cell-cell communication. QS enables bacteria to coordinate a collective response such as biofilm formation as a protection towards external factors. Therefore, compounds possessing QS inhibitory ability but void of bactericidal or bacteriostatic activity inactivate bacteria defense systems, reduce the production of toxins and most importantly are less prone to selection for resistance than traditional cytotoxic remedies [9, 10]. In Gram-negative bacteria, the QS signal compounds are *N*-Acyl-L-homoserine lactones (AHLs) [9-11]. Many QSIs based on structure of AHLs have been designed and tested [12]. As shown in **Fig. 2 FA** might be considered bioequivalent to the AHL.

Fig. 2

FA comprises of a pyridine nucleus decorated with a carboxylic group at C-2 and an *n*-butyl moiety protruding from C-5. Overlay of the ester carbonyl of the two molecules, C-3 of homoserine lactone with C-2 of **FA**, and the methylene groups in the side chains of the two

molecules gives a good match indicating that these groups might be bioequivalent **(Fig. 2**). In addition, molecules containing a pyridine ring (4-NPO, IC_{50} of 24 µg/ml or 171 µM [12c, 13]) and these structural features are found to be QSIs **(Fig. 1b, c**). Bloemberg et al. [14] have also reported that **FA** prevents the production of the signaling molecule *N*-hexanoyl-L-homoserine lactone (C6-HSL) but no **FA** analogues were synthesized and evaluated to get structure-activity relationship and thereby maybe hint to understand the mechanism of action. Based on these arguments, **FA** was chosen as a suitable template for construction of a library of potential QSIs [15].

2. Results and discussion

2.1. Strategy for design of library

Fig. 3

The strategy behind the design of the library to get structure-activity relationship and thereby to identify pharmacophore descriptors is illustrated in **Fig. 3**. To further elaborate on these structural features the compounds shown in **Table 1** were designed, prepared and screened as QSI. The importance of the substituent at C-5 was investigated by decorating with a broad plethora of substituents with different lengths, aromatic rings and functional groups.

2.2. Microwave assisted synthesis of fusaric acid and analogues

Only a few analogues of **FA** have been reported [16-19]. Common for all reported protocols, however, are that they only afford a limited spectrum of analogues. No biological studies have been performed on these compounds and none of the reported methods describe microwave-

assisted synthesis. Taking advantage of microwave methodology, we have developed a protocol for fast preparation of **FA** and a diverse library of analogues (**Scheme 1**).

Scheme 1

Treatment of 2,5-dibromopyridine (**1**) with $TMSCH_2Li$-LiDMAE in toluene at 0 ^oC for 30 min afforded the 2-lithiated intermediate selectively [20]. Addition of methyl or ethyl formate at -78 ^oC and stirring for 3h followed by oxidation with iodine in methanol or ethanol introduced an ester group at C-2 in medium yield to give methyl 5-bromopicolinate (**2a**) or ethyl 5-bromopicolinate (**2b**), respectively.

FA and analogues can be prepared from key intermediate **2a** or **2b**. In pathway 1, compound **2a** or **2b** is converted into methyl or ethyl 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)picolinate (**3a** or **3b**, respectively) by treatment with bis(pinacolato)diboron. The reaction was accelerated by irradiation with microwave and catalyzed with 10 mol % of $Pd(dppf)Cl_2$. After just 15 min, all starting material was converted into the target compound **3** (in situ) as observed by TLC and UPLC (compared to 12-36 h in traditional heating). Crude compound **3a** or **3b** (without purification) was subsequently treated with different bromo- or chloroalkanes or with halogenated heterocycles to afford esters **4** in excellent yield. An alternative successful pathway (Pathway 2) consisted in treatment of key intermediate **2** with various boronate esters using the same condition as stated above for Pathway 1, under which conditions esters **4** were formed in good yield. All esters **4** were converted to corresponding acids **5** by saponification (**Scheme 1**).

The alkoxy groups at C-5 (**7**) were installed via a Williamson ether synthesis from the reaction of methyl 5-hydroxypicolinate (**6**) with an alkyl halide under basic condition at room temperature for 18 - 48 h. The use of microwave-assisted synthesis reduced the reaction time to

only 30 to 180 min. Saponification **7** afforded the carboxylic acids **8**. The ester methyl 5-(2-oxo-2-(phenylamino)ethoxy)picolinate (**7c**) was completely hydrolyzed in 4 h to give 5-(2-oxo-2-(phenylamino)ethoxy)picolinic acid (**8c**). Remarkably, the instability of **8c** was illustrated by complete conversion into 5-(carboxymethoxy)picolinic acid (**8c'**) if work up of the reaction was postponed until the next day. Compound *N*-propyl-5-(propylamino)picolinamide (**10a**) was obtained directly from **2a** by reaction with propylamine in the presence of 6 mol % Pd(dppf)Cl_2 under microwave irradiation. Under these conditions substitution of the ester as well as the 5-bromo group occurred to give **10a** in good yield. Ethyl 5-(chloromethyl)picolinate (**9**) was successfully converted into ethyl 5-((ethylthio)methyl)picolinate (**10b**) by treatment with ethanethiol at room temperature (**Scheme 2**).

Scheme 2

Overall, the broad spectrum of **FA** and analogues can be prepared from the key intermediate **2a**/**2b** using our microwave-assisted synthesis method. This protocol can be used for fast expansion of the library compounds for biological studies in the future.

2.3. Synthesis of fusaric acid

The microwave irradiation condition enabled synthesis of ester of **FA** in 90 % yield via Pathway 2 using *n*-butylboronic acid pinacol ester and **2b** as starting materials to give ethyl 5-butylpicolinate (**FAE**). Saponification of **FAE** afforded 75 % of **FA** (**Scheme 3**).

Scheme 3

Pathway 1 using **2b** converted into ethyl 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)picolinate and n-butyl bromide as starting materials for preparation of **FAE** succeeded in 70 % yield from **2b** over 2 steps (**Scheme 3**).

In summary, **FA** and a library of 39 analogues was prepared by an efficient microwave-assisted synthesis. The members of the library are depicted in **Table 1**.

Table 1

2.4. Biological assays

Fig. 4

Table 2

Three cell-based biological screens were used to test for QSI activity. The first two reporter systems, *lasB-gfp* [21] and *rhlA-gfp* [22] in *P. aeruginosa* background contain fusions of the QS-controlled *lasB* promoter or *rhlA* promoter to Green Fluorescence Protein ((*gfp*) (*ASV*)) encoding an unstable GFP variant. The third QS reporter system, *luxI-gfp* [23] in an *Escherichia coli* background contains the promoter region of the *luxI* fused to *gfp* (*ASV*) and a *luxR* gene. With these reporter systems activation of QS can be measured by an increase in fluorescence. Presence of a QSI will therefore results in a decrease in GFP expression. Bacterial growth was measured simultaneously to be certain the test compounds do not affect the growth rate.

Activities as inhibitors of the *Lux* QS system were found for compound **4b**, **4g**, **7a**, **7b**, **7c**, **FAE** and **FA** (**Fig. 4, Table 2**). To our delight, none of the compounds affected the bacterial growth in the tested concentration ranges except for **FA**. No QSI activity was found against two QS reporter strains *lasB-gfp* and *rhlA-gfp* in concentrations up to 100 µg/ml. Three compounds

5c, **8a**, **8b** reduced the GFP expression, however that reduction was potentially generated by a simultaneous growth inhibition meaning that it cannot be regarded as a QSI (**Fig. 5**). **FA** seem to delay the bacterial growth in concentrations ranging from 6.2 to 100 µg/ml, whereas at a concentration of 3.1 µg/ml a small reduction in GFP expression was seen with no simultaneous inhibition of growth. The ester **FAE** showed clearly QSI activity in concentrations from 6.2 to 50 µg/ml. All compounds with QSI activity are shown in **Fig. 4**. (See supporting information for screening results of other compounds). The larger number of compounds targeting the *lux* QS system compared to the *las* and *rhl* systems are potentially because of a higher binding affinity for the *Lux* receptor protein and the use of different background strains in the monitor systems.

Fig. 5

2.5. Structure-activity relationship

Except for our lead compound **FA**, all the QSIs **4b**, **4g**, **7a**, **7b**, **7c**, and **FAE** are esters. The ester group might simulate the intermolecular interaction solicited by the lactone moiety in structures of current QSIs (**Fig. 1b**). Another possibility is that the zwitterionic character of the free acids prevents penetration over the cell wall of plasma membrane. This latter hypothesis, however, is unsubstantiated by the observation that all the growth inhibitors **5c**, **8a**, **8b** are acids (**Fig. 5**).

The QS inhibitory activities of **4b**, **4g** and **7c** possessing 5-methoxy-2-pyridyl-, phenyl- and 2-oxo-2-(phenylamino)- ethoxy-, respectively, reveals that a lipophilic alkyl group in the 5 position as in the case for **FA** as well as AHL is not mandatory (**Fig. 4**). In contrast the ester moiety at C-2 position seems to be favorable for QS inhibition. In addition, **FA** is delaying the growth of bacterial as did some of the other carboxylic acids.

Compounds **2a**, **4j**, **5e**, **5k**, **5l**, **8c**, **8c´** did not show QS inhibitory activity or growth inhibitory effect in concentrations up to 100 µg/ml. At concentrations above 125 µg/ml compound **2a** inhibited *lasB* expression in the *lasB-gfp* reporter without affecting growth (**Fig. 6**).

Fig. 6

In summary, the observed structure-activity relationship for inhibition of QS suggested by this study is depicted in **Fig. 7**.

Fig. 7

2.6. Screening for the inhibition of plasma membrane H^+-ATPase (Pma1)

Reduced stomatal [24] conductance has been observed for banana plants infected by *Fursarium spp.* [4, 25] suggesting that the agent might stimulate the H^+-ATPase. The possibility of inhibition of H^+-ATPase [26, 27] inspired us to test our library towards the pump, but no compounds showed any significant activity (data not shown).

3. Conclusions

An efficient, facile and expedite microwave-assisted synthesis of **FA** and analogues have been developed and used to prepare a library of 40 analogues. The compounds were tested in three QS screening systems, *lasB-gfp* (*P. aeruginosa*), *rhlA-gfp* (*P. aeruginosa*) and *luxI-gfp* (*E. coli*). In the *luxI-gfp* system compounds **4b**, **4g**, **7a**, **7b**, **7c** and **FAE** revealed QS inhibitory activity in the concentration range 6.25 to 100 µg/ml. Compound **2a** showed good inhibitory activity in concentration from 125 µg/ml toward *lasB-gfp*. In the *luxI-gfp* system, **FA** showed a little QS inhibition activity at 3.13 µg/ml while at higher concentrations from 6.25 to 100 µg/ml

the delaying of cell growth were observed along with QS inhibitory activity. Structure-activity relationship studies suggest that the ester group at C-2 is essential for QS inhibition. In contrast, the alkyl substituent at the 5-position can be exchanged with an alkoxy or aromatic/heterocyclic aromatic ring. Even though the compounds reveal a modest QS inhibitory activity, **FA** analogues have not previously been found to possess this system making it a new scaffold for developing QSIs. Moreover, we have demonstrated that a natural product scaffold with homologous structure toward the QS signals could serve successfully as a starting point for design of a focused library of new compounds with QS activities.

4. Experimental section

4.1. Biology

4.1.1. QSI screening Assay

The QS reporter strains *lasB-gfp* (*P. aeruginosa*) [21], *rhlA-gfp* (*P. aeruginosa*) [22] and *luxI-gfp* (*E. coli*) [23] were used for measuring inhibition of QS. The following growth medium was used for bacterial growth; BT minimal medium (B medium plus 2.5 mg thiamine L^{-1}) supplemented with 10% A10, 0.5% (wt/vol) glucose, 0.5% (wt/vol) Casamino Acids. An overnight culture of each reporter strain was grown for 16 h at 37 °C with shaking at 180 rpm. The overnight cultures were diluted to a final optical density at 450 nm (OD450) of 0.1. The biological assays were conducted in 96-well microtiter dishes (Black Isoplate®, Perkin Elmer®, Waltham Massachusetts, USA). Test compounds, growth media and reporter strains were added to the microtiter dishes. The corresponding signal molecule 3-oxo-C6-HSL was added in a final concentration of 10 nM to the screen with the reporter strain, *luxI-gfp*. Growth and green fluorescent protein (GFP) expression

was monitored using Victor™ X4 multilabel plate reader (Perkin Elmer®, Waltham Massachusetts, USA) set at a constant temperature of 34 °C measuring every 15 min over a time course of 14 h. GFP expression was measured as fluorescence at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. Please see [28] for full protocol.

4.1.2. Pma1 ATPase Screening Assay

Yeast plasma membrane protein Pma1 was prepared from *Saccharomyces cerevisiae* as previously described [29a]. The protein activity was measured based on Baginski method [29b], by using 0.5 µg of purified Pma1 protein in 300 µL of reaction mixture containing 5 mM NaN_3, 3.5 mM sodium molybdate, 50 mM KNO_3, 10 mM $MgSO_4$, 5mM ATP, 20 mM MES-KOH adjusted to pH 5.9. Pma1 was first pre-incubated with 100 µM of compounds for 30 min at room temperature. Reactions were stopped after 30 min by adding 300 µL of an ice-cold stop solution (containing 93 mM ascobic acid, 0.273 N HCl, 0.059 % SDS, 5 mM ammonium heptamolybdate). The tubes were continuously incubated on ice for 10 min to allow formation of the phosphate-molybdate complex. Excess molybdate was complexed by the addition of 450 µL of an arsenite-solution (containing 154 mM $NaAsO_2$, 68 mM trisodium citrate, 350 mM acetic acid). After 60 min of incubation at room temperature, the absorbance was determined spectrophotometrically at 860 nm.

4.2. **Chemistry**

4.2.1. General information

Commercially available reagents (Aldrich) were used without further purification unless otherwise noted. Microwave reactions were carried out in an automatic Biotage® Initiator equipped with robot sixty (an automated 60-position system) and using high precision microwave vials and aluminum seals. NMR-spectra were acquired using a 400 or 600 MHz Bruker Avance III HD equipped with a cryogenically cooled 5 mm dual probe optimized for ^{13}C and ^{1}H. Samples were dissolved in one of following solvent: DMSO-d_6, $CDCl_3$, MeOH-d_4 and analyzed at 300K. ^{1}H, COSY, HMBC, and HSQC spectra were acquired at 400 or 600 MHz. ^{13}C spectra were recorded at 151 MHz or 101 MHz. Chemical shifts (δ) are reported in ppm relative to the residual solvent peak (^{1}H NMR) or the solvent peak (^{13}C NMR) as the internal standard. The coupling constant (J) are reported in Hertz. Solvents used for the synthesis were of analytical grade, dried over activated 4 Å molecular sieves when necessary (all solvents used under dry conditions had a water content <25 ppm). Analytical TLC was performed using pre-coated silica gel 60 F_{254} plates and visualized using UV light. Flash column chromatography was performed using Merk silica 60. Melting points were determined on a Mettler Toledo MP70 Melting Point system. All tested compounds possess ≥95% purity (purified by preparative RP-HPLC for compounds less than 90% purity). Purity determinations were performed on a Waters 2795 system equipped with a Waters 996 PDA detector and a Waters Symmetry C18 Column (2.1 × 50 mm, 3.5 lm) with a flow of 0.2 ml min^{-1}. 100% → 0% A 0–10 min (A: 0.1% aq formic acid, B: 95% CH_3CN in 0.05% aq formic acid). High-resolution mass spectral (HRMS) data were obtained on an electrospray (ESI) or a MALDI-TOF mass spectrometer.

*4.2.2. General synthetic procedure for compounds **2a–b***

N,*N*-Dimethyl aminoethanol (0.85 ml, 8.5 mmol, 1 equiv.) and (trimethylsilyl)methyllithium 1M (25.3 ml, 25.3 mmol, 3 equiv.) was added dropwise to 25 ml of dry toluene at 0 °C and the solution was stirred for 30 min to form $TMSCH_2Li$-LiDMAE (in situ). Then, A solution of 2,5-dibromopyridine **1** (2 g, 8.5 mmol, 1 equiv.) in 9 ml of dry toluene was added dropwise. The reaction mixture was stirred for 30 min, cooled to -78 °C and added methyl formate (5.2 ml, 85 mmol, 10 equiv.) or ethyl formate (6.9 ml, 85 mmol, 10 equiv.) in 10 ml of toluene. The reaction mixture stirred for 3 hours, and I_2 (6.4 g, 25.5 mmol, 3 equiv.), K_2CO_3 (5.8 g, 42.5 mmol, 5 equiv.) and 20 ml methanol or ethanol were added. The mixture was stirred at room temperature for 18 hours and added 10 ml of a sat. aqueous Na_2SO_3 solution. Water (10 ml) was added and the mixture extracted with dichloromethane (3 x 40 ml), washed with water (1 x 25 ml) and brine (1 x 25 ml). The residue was purified by silica-gel flash column chromatography (eluent: heptane/diethyl ether = 2 : 1) to obtain **2a–b**.

4.2.2.1. *Methyl 5-bromopicolinate (**2a**)*.

From **1** (2 g) gave **2a** (997 mg, 55%); white solid; >99% purity; mp 103 – 104 °C (reported 98.0-102.0 °C); ^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.77 (dd, J = 0.74, 0.71 Hz, 1H, H2), 8.01 (dd, J = 0.84, 0.79 Hz, 1H, H5), 7.98 (dd, J = 2.3, 2.2 Hz, 1H, H6);^{13}C NMR (100 MHz, $CDCl_3$): δ 165.0(C=O, C-7), 151.0(C-2), 146.3(C-4), 139.7(C-6), 126.3(C-5), 125.1(C-1); HRMS (ESI) m/z $[M+H]^+$ calcd for $C_7H_7BrNO_2^+$ 215.9660, found 215.9659. Data was in accordance with those reported [30a].

4.2.2.2. *Ethyl 5-bromopicolinate (**2b**)*

From **1** (2 g) gave **2b** (889 mg, 46%); yellow solid; >99% purity; mp 59.3–60.4 ˚C (reported 63-64 °C [102]); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.77 (dd, J = 2.2 Hz, J = 0.79, 0.75 Hz, 1H, H2), 7.99 (dd, J = 0.73, 0.74 Hz, 1H, H5), 7.95 (dd, J = 2.2, 2.2 Hz, 1H, H6), 4.45, (q, J = 7.1 Hz, 2H, H11), 1.40 (t, J = 7.1 Hz, 3H; H12);^{13}C NMR (100 MHz, $CDCl_3$): δ 164.6(C=O, C-8), 151.1(C-2), 146.7(C-4), 139.7(C-6), 126.3(C-5), 124.9(C-1), 62.2(C-11), 14.3(C-12); HRMS (ESI) m/z $[M+H]^+$ calcd for $C_8H_9BrNO_2^+$ 229.9817, found 229.9807. Data was in accordance with those reported [30a-b].

*4.2.3. General synthetic procedure for compounds **4a**–**m**.*

4.2.3.1. Method

4.2.3.1.1. Method 1.

A mixture of **2a** or **2b** (1 g, 1 equiv.), bis(pinacolato)diboron (1.2 equiv.), [1,1′-bis(diphenylphosphino)ferrocene] dichloropalladium(II) complex with dichloromethane (10 mol %), potassium acetate (2 equiv.), 1,4-dioxane (15 ml) and water (7.5 ml) was sealed in a 20 ml microwave reaction vial (Biotage). The vial was irradiated in a microwave apparatus at 110 °C, high absorption for 15 min. The reaction mixture was cooled to room temperature (afforded crude **3a** or **3b**) and used to the next step without purification. An appropriate alkyl halide (0.95 equiv.), cesium carbonate (2.0 equiv) and water (4 ml) were added directly to the reaction mixture. The vial was sealed and irradiated in a microwave apparatus at 110 °C, normal absorption for 30-90 min. After cooling to room temperature, water (100 ml) was added and the mixture was extracted with ethyl acetate (4 x 100 ml). The organic layer was washed with brine (200 ml), dried with anhydrous Na_2SO_4(s) and concentrated. The residue was purified by silica-gel flash

column chromatography (eluent: ethyl acetate/heptane = 1:1) to obtain the esters **4a** and **4j-m**.

4.2.3.1.2. Method 2.

A mixture **2a** or **2b** (1 g, 1 equiv.), an appropriate pinacol boronate ester (1.2 equiv.), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (10 mol %), cesium carbonate (2.0 equiv.), 1,4-dioxane (8 ml) and water (4 ml) was sealed in a 20 ml microwave reaction vial (Biotage). The vial was irradiated in a microwave apparatus at 110 °C, normal absorption for 30-90 min. The reaction mixture was cooled to room temperature and work up was performed as described in *method 1* to obtain the esters **4b-i**.

*4.2.3.2. Ethyl 5-butylpicolinate (**FAE**).*

Method 2; **2b** (1 g) gave **FAE** (810 mg, 90%). If method 1 was used the yield was 70% over 2 steps; yellow oil; >98% purity; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.57 (d, J = 2.2 Hz, 1H, H2), 8.05 (d, J = 7.8 Hz, 1H, H5), 7.63 (dd, J = 2.2, 7.8 Hz, 1H, H6), 4.47 (q, J =7.1 Hz, 2H, H14), 2.69 (t, J = 7.6 Hz, 2H, H10), 1.62 (m, 2H, H11), 1.44 (t, J = 7.1, 3H, H15), 1.37 (qt, J =7.3, 7.6 Hz, 2H, H12), 0.94 (t, J = 7.3 Hz, 3H, H13);^{13}C NMR (151 MHz, $CDCl_3$) δ 165.4(C=O, C-7), 150.1(C-2), 145.9(C-4), 141.9(C-1), 136.5(C-6), 124.8(C-5), 61.7(C-14), 32.9(C-10), 32.7(C-11), 22.2(C-12), 14.4(C-15), 13.8(C-13); HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{12}H_{18}NO_2^+$ 208.1338, found 208.1340. Data was in accordance with those reported [30c].

4.2.3.3. *Methyl 5-benzylpicolinate (**4a**).*

Method 1; **2a** (1 g) gave **4a** (925 mg, 88%); white solid; >98% purity; mp 66–67°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.63 (dd, *J* = 1.6, 1.6 Hz, 1H, H9), 8.05 (d, *J* = 7.3 Hz, 1H, H12), 7.59 (dd, *J* = 2.4, 2.4 Hz, 2H, H13), 7.30-7.32 (m, 2H, H4, H6), 7.23-7.27 (m, 1H, H2), 7.15-7.17 (d, *J* = 7.3 Hz, 2H, H1, H3), 4.05 (s, 2H, H7), 3.99 (s, 3H, H17); ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.7(C=O, C-14), 150.2(C-9), 146.0(C-8), 140.6(C-11), 138.8(C-5), 137.2(C-13), 128.8(C-4, C-6), 128.8(C-1, C-3), 126.8(C-2), 125.1(C-12), 52.8(C-7), 39.0(C-17); HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{14}H_{14}NO_2^+$ 228.1025, found 228.1025.

4.2.3.4. *Characterization data of compounds **4b-4m**:* see supporting information.

4.2.4. *General synthetic procedure for compounds **FA**, **5a–m** and **2´**.*

Ester (**FAE**, **4a-m, 2a or 2b**) (500 mg scale, 1 equiv.) was dissolved in 6 ml THF at 0°C in a 25 ml round-bottom flask. Then NaOH(aq) (5 equiv.) was added dropwise and stirred for 15h at room temperature. After starting materials were consumed (by TLC), water (20 ml) was added. The reaction mixture was washed with ethyl acetate (2 x 20 ml). The aqueous solution was acidified (pH 2-3) with 1 M HCl(aq) causing precipitation of a solid, which was filtered and dried under vacuum. Recrystallization in ethanol afford clean compound.

*4.2.4.1. Fusaric acid (**FA**).*

FAE (500 mg) gave **FA** (324 mg, 75%); slightly yellow solid; >99% purity after recrystallization from ethanol; mp 96.9-98.0°C (reported 96-99°C[172]); ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.46 (d, *J* = 2.2 Hz, 1H, H2), 8.16 (d, *J* = 8.1 Hz, 1H, H5), 7.76 (dd, *J* = 8.1, 2.2 Hz, 1H, H6), 2.74 (t, *J* = 7.7x(2) Hz, 2H, H10), 1.66 (m, 2H, H11), 1.4 (tq, *J* = 7.3x(3), 7.7x(2) Hz, 2H, H12), 0.96 (t, *J* = 7.3, 7.3 Hz, 3H, H13); ^{13}C NMR (151 MHz,

DMSO-d_6) δ 164.2(C=O, C-7), 148.2(C-2), 143.8(C-4), 143.5(C-1), 138.1(C-6), 123.4(C-5), 32.9(C-11), 32.8(C-10), 22.2(C-12), 13.8(C-13); HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{10}H_{14}NO_2^+$ 180.1025, found 180.1025. Data was in accordance with those reported [19].

4.2.4.2. *5-Benzylpicolinic acid (**5a**).*

4a (500 mg) gave **5a** (432 mg, 92%); white solid; >98% purity; mp 147–148.9 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.09 (s, 1H, OH), 8.64 (d, *J* = 2.2 Hz, 1H, H9), 7.98 (d, *J* = 7.9 Hz, 1H, H12), 7.79 (dd, *J* = 2.3, 2.2 Hz, 2H, H13), 7.31-7.34 (m, 2H, H4, H6), 7.27-7.28 (br, 2H, H2, H3), 7.21 (br, 1H, H2), 4.07 (s, 2H, H7); ^{13}C NMR (151 MHz, DMSO-d6) δ 166.5(C=O, C-14), 150.1(C-9), 146.8(C-8), 141.0(C-11), 140.3(C-5), 137.7(C-13), 129.2(C-4, C-6), 129.1(C-1, C-3), 126.9(C-2), 125.0(C-12), 38.3(C-7); HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{13}H_{12}NO_2^+$ 214.0868, found 214.0868.

4.2.4.3. *Characterization data of compounds **5b-m** and **2´**:* see supporting information.

*4.2.5. General synthetic procedure for compounds **7a–c**.*

A mixture of methyl 5-hydroxypicolinate **6** (1.0 g, 4.6 mmol, 1 equiv.), the appropriate alkyl halide (5.06 mmol, 1.1 equiv.) potassium carbonate (1.3 g, 9.2 mmol, 2 equiv.) and DMF (5 ml) was stirred at room temperature for 18 - 48 hours. Water (100 ml) was added and the mixture was extracted with EtOAc (4 x 100 ml). The organic layer was washed with brine (200 ml) dried with anhydrous Na_2SO_4(s) and concentrated. The residue was purified by silica-gel flash column chromatography (eluent: ethyl acetate/heptane = 1:1) to give **7a–c**.

4.2.5.1. *Methyl 5-propoxypicolinate (**7a**).*

From **6** (1 g) gave **7a** (1.13 g, 89%); yellow semi-solid; >98% purity; mp 38–40 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.39 (d, *J* = 2.8 Hz, 1H; H2), 8.10 (d , *J* = 8.7 Hz, 1H, H5), 7.25 (dd, *J* = 2.9, 2.9 Hz, 1H, H6), 4.03 (t, *J* = 6.5 Hz, 2H, H12), 3.98 (s, 3H, H10), 1.83-1.88 (m, 2H, H13), 1.07 (t, *J* = 7.4, 3H, H14); ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.5(C=O, C-7), 157.8(C-4), 139.9(C-1), 138.6(C-2), 126.5(C-6), 120.2(C-5), 70.2(C-12), 52.6(C-10), 22.4(C-13), 10.4(C14); HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{10}H_{14}NO_3^+$ 196.0974, found 196.0974.

4.2.5.2. *Methyl 5-(cyclopropylmethoxy)picolinate (**7b**).*

From **6** (1 g) gave **7b** (1.10 g, 79%); white solid; >98% purity; mp 53.9–54.8 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.39 (d, *J* = 6 Hz, 1H, H2), 8.08 (dd, *J* = 0.6, 0.6 Hz, 1H, H5), 7.25 (dd, *J* = 2.9, 2.9 Hz, 1H, H6), 3.98 (s, 3H, H10), 3.93 (d, J = 6.7 Hz, 2H, H12), 1.27-1.33 (m, 1H, H13), 0.67-0.70 (m, 2H, H14, H16), 0.39-0.41 (m, 2H, H14, H16); ^{13}C NMR (151 MHz, $CDCl_3$) 165.4(C=O, C-7), 157.7(C-4), 139.9(C-1), 138.6(C-2), 126.5(C-6), 120.2(C-5), 73.4(C-12), 52.5(C-10), 9.99(C-13), 3.3(C-14, C-16); HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{11}H_{14}NO_3^+$ 208.0974, found 208.0974.

4.2.5.3. *Methyl 5-(2-oxo-2-(phenylamino)ethoxy)picolinate (**7c**).*

From **6** (1 g) gave **7c** (1.5 g, 83%); yellow solid; >98% purity; mp 185–186 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.54 (d, *J* = 2.9 Hz, 1H, H13), 8.18 (d, *J* = 8.8 Hz, 2H, H16, H17), 7.59 (d, *J* = 7.9 Hz, 2H, H4, H6), 7.37 (dd, *J* = 2.6, 6.5 Hz, 3H, H1, H2, H3), 7.18 (t, *J* = 7.4 Hz, 1H, NH), 4.72 (s, 2H, H9), 4.00 (s, 3H, H21); ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.0(C=O, C-8), 164.5(C=O, C-18), 155.6(C-12), 141.9(C-15), 138.7(C-13), 136.4,

129.2 (C-4, C-6), 126.7(C-5), 125.3, 120.9(C-2), 120.3(C-4, C-6), 67.6(C-9), 52.9(C-21); HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{15}H_{15}N_2O_4^+$ 287.1032, found 287.1030.

4.2.6. *Synthetic procedure for compounds **8a-c***.

Compounds **8a-c** were synthesized from **7a-c** (200 mg scale) using the same procedure as described for **5a-m**.

4.2.6.1. *5-Propoxypicolinic acid (**8a**).*

From **7a** (200 mg) gave **8a** (149 mg, 80%); white solid; >98% purity; mp 128.9–129.2 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.82 (s, 1H, OH), 8.35 (t, J = 2.4 Hz, 1H, H2), 8.01 (dd , J = 1.7, 1.7 Hz, 1H, H5), 7.48 (dt, J = 2.5, 2.6 Hz, 1H, H6), 4.03 (td, J = 1.7, 1.7, 1.7 Hz, 2H, H12), 1.73-1.79 (m, 2H, H13), 0.98 (td, J =1.7, 1.7, 1.7, 3H, H14); ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.2(C=O, C-11), 157.7(C-4), 140.7(C-1), 138.3(C-2), 126.7(C-6), 121.1(C-5), 70.2(C-12), 22.3(C-13), 10.7(C-14); HRMS (ESI) m/z $[M+H]^+$ calcd for $C_9H_{12}NO_3^+$ 182.0817, found 182.0815.

4.2.6.2. *5-(Cyclopropylmethoxy)picolinic acid (**8b**).*

From **7b** (200 mg) gave **8b** (131 mg, 70%); white solid; >98% purity; mp 138–139.5 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.81 (s, 1H, OH), 8.36 (d, J = 2.7 Hz, 1H, H2), 8.01 (dd, J = 0.5, 0.5 Hz, 1H, H5), 7.48 (dd, J = 2.9, 2.9 Hz, 1H, H6), 3.99 (d, J = 7.1 Hz, 2H, H11), 1.23-1.29 (m, 1H, H12), 0.59-0.62 (m, 2H, H13, H14), 0.35-0.38 (m, 2H, H13, H14); ^{13}C NMR δ (151 MHz, DMSO-d_6) 166.2(C=O, C-7), 157.7(C-4), 140.7(C-1), 138.4(C-2), 126.6(C-6), 121.2(C-5), 73.4(C-11), 10.3(C-12), 3.6 (C-13, C-14); HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{10}H_{12}NO_3^+$ 194.0817, found 194.0816.

4.2.6.3. *5-(2-Oxo-2-(phenylamino)ethoxy)picolinic acid (**8c**).*

For 4 h of reaction time of hydrolysis **7c** (200 mg) gave **8c** (139 mg, 73%); yellow solid; >98% purity; mp 225–226 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.9 (s, 1H, OH), 10.18 (s, 1H, NH), 8.45 (d, *J* = 2.9 Hz, 1H, H13), 8.04 (d, J = 8.7 Hz, 1H, H16), 7.62 (dbr, 2H, H4, H6), 7.53 (dd, J = 2.9, 2.9 Hz, 1H, H17), 7.31-7.35 (m, 2H, H1, H3), 7.09 (m, 1H, H2), 4.91 (s, 2H, H9); ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.1(C=O, C-12), 166.1(C=O, C-7), 157.0(C-4), 141.5(C-1), 138.7(C-6), 138.6(C-13), 129.2(C-1, C-3), 126.6(C-16), 124.3(C-2), 121.6(C-17), 120.2(C-4, C-6), 67.6(C-9); HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{14}H_{13}N_2O_4^+$ 273.0875, found 273.0873.

4.2.6.4. *5-(Carboxymethoxy)picolinic acid (**8c´**).*

Compound **8c´** was obtained from **7c** after 24h of hydrolysis. **7c** (200 mg) gave **8c´** (127 mg, 92%); >98% purity; mp 263.9–264.5 °C; yellow solid; ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.04 (br, 2H, OH), 8.38 (d, *J* = 2.9 Hz, 1H, H2), 8.02 (d, *J* = 8.7 Hz, 1H, H5), 7.48 (dd, *J* = 2.9, 3.0 Hz, 3H, H6), 4.90 (s, 2H, H11); ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.9(C=O, C-12), 166.1(C=O, C-7), 156.9(C-1), 141.3(C-4), 138.4(C-2), 126.5(C-5), 121.5(C-6), 65.2(C-11); HRMS (ESI) m/z $[M+H]^+$ calcd for $C_8H_8NO_5^+$ 198.0402, found 198.0401.

4.2.7. *N-Propyl-5-(propylamino)picolinamide (**10a**).*

A mixture of **2a** (500 mg, 2.3 mmol), propylamine (0.3 ml, 3.45 mmol, 1.5 equiv.), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (168 mg, 6 mol %), cesium carbonate (2.2 g, 6.9 mmol, 2.0 equiv.) and toluene (10 ml) were added to a 20 ml microwave reaction vial (Biotage). The vial was

sealed and irradiated in a microwave apparatus at 110 °C, high absorption for 45 min. The reaction mixture was cooled to room temperature; added 100 ml of water and the resultant mixture was extracted with ethyl acetate (4 x 100 ml). The organic layer was washed with brine (2 x 200 ml), dried with anhydrous Na_2SO_4(s). The residue after concentration was purified by silica-gel flash column chromatography (eluent: ethyl acetate/heptane = 1:1) to give 372 mg of **10a**. Yield 73 %; colorless oil; >98% purity; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.99 (d, J = 8.6 Hz, 1H, H2), 7.87 (d , J = 2.7 Hz, 1H, H5), 7.78 (br, 1H, NH), 6.90 (dd, J = 2.8, 2.8 Hz, 2H, H6), 4.09 (br, 1H), 3.41 (dd, J = 6.8, 6.4 Hz, 2H, H14), 3.14 (t, J = 7.1, 2H, H10) 1.60-1.71 (m, 4H, H11, H15), 0.96-1.03 (m, 6H; H12, H16); ^{13}C NMR (151 MHz, $CDCl_3$) δ 164.9(C=O, C-7), 146.3(C-1), 139.1(C-4), 133.6(C-5), 123.3(C-2), 117.7(C-6), 45.1(C-10), 41.0(C-14), 23.1(C-15), 22.5(C-11), 11.5(C-12), 11.5(C-16); HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{12}H_{20}N_3O^+$ 222.1606, found 222.1606.

4.2.8. *Ethyl 5-((ethylthio)methyl)picolinate (**10b**).*

A solution of ethyl 5-(chloromethyl)picolinate **9** (500 mg, 2.5 mmol, 1 equiv.), ethanethiol (233 mg, 3.75 mmol, 1.5 equiv.) and potassium carbonate (690 mg, 5 mmol, 2 equiv.) in 5 ml DMF was transferred to a 20 ml round-bottom flask. The mixture was stirred overnight at room temperature. When starting materials were consumed 100 ml of water was added and the mixture was extracted with ethyl acetate (4 x 100 ml). The organic layer was washed with brine (2 x 200 ml) and dried with anhydrous Na_2SO_4(s). The residue after concentration was purified by silica-gel flash column chromatography (eluent: ethyl acetate/heptane = 1:1) to obtain **10b**. Alternatively a microwave reactor was used. By transferring, then sealed and irradiated in a microwave apparatus at 60 °C, high

absorption for 30 min. Workup procedure is the same as solution phase stated above of method 1 to obtain 366 mg of **10b**. Yield 65%; yellow oil; >98% purity; ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.66 (d, J = 2.2 Hz, 1H, H2), 8.10 (d , J = 7.2 Hz, 1H, H5), 7.83 (dd, J = 2.3, 2.2 Hz, 1H, H6), 4.48 (dd, J = 7.1, 7.1 Hz, 2H, H10), 3.76 (s, 2H, H11), 2.43 (dd, J = 7.4, 7.4 Hz, 2H, H13), 1.48 (t, J = 7.2, 3H, H15), 1.23 (t, J = 7.4 Hz, 3H, H14); ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.1(C=O, C-7), 150.0(C-2), 147.0(C-4), 138.3(C-1), 137.2(C-6), 125.0(C-5), 61.9(C-10), 32.8(C-11), 25.4(C-13), 14.4(C-15), 14.3(C-14); HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{11}H_{16}NO_2S^+$ 226.0902, found 226.0903.

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ABBREVIATIONS

AHLs, *N*-Acyl-L-homoserine lactones; MW, Microwave; 4-NPO, 4-nitropyridine-*N*-oxide; Pma1, plasma membrane H^+-ATPase; QS, quorum sensing; QSI, quorum sensing inhibitor; TLC, thin layer chromatography; UPLC, ultra performance liquid chromatography.

Figure, scheme and table captions

Fig. 1: Homologous structure of **FA** and analogues toward the QS signal molecule *N*-hexanoyl-L-homoserine lactone (**a**); QSI containing structural features of QS signal molecule 4-bromo-5-[1-bromo-meth-(*Z*)-ylidene]-3-butyl-5*H*-furan-2-one (**b**) and reported QSI containing pyridine 4-NPO (4-nitropyridine-*N*-oxide) (**c**)

Fig. 2: Overlap between *N*-hexanoyl-L-homoserine lactone and **FA**

Fig. 3: **FA** and strategy for design of library

Fig. 4: QSI activity of compounds **FAE**, **FA**, **4b**, **4g**, **7a**, **7b** and **7c** tested with the QS monitor *luxI-gfp*. GFP fluorescence (RFU), GFP expression is controlled by the QS controlled *luxI* promoter. None of the compounds showed growth inhibitory effects in the tested concentration ranges, except for **FA**. All Compounds were tested in triplicate in 2-fold dilutions from 100 μg/ml.

Fig. 5: QSI activity of compounds **5c**, **8a** and **8b** tested in the QS monitor *luxI-gfp*. a) Bacterial growth (OD450). b) GFP fluorescence (RFU), GFP expression is controlled by the QS controlled *luxI* promoter. All Compounds were tested in triplicate in 2-fold dilutions from 100 μg/ml.

Fig. 6: QSI activity of compound **2a** tested with the *lasB-gfp* monitor. GFP fluorescence (RFU), GFP expression is controlled by the QS controlled *lasB* promoter. No growth inhibitory effects in the tested concentration ranges. Compound was tested in triplicate and in 2-fold dilutions from 1000 μg/ml.

Fig. 7: Simplified structure-activity relationship based on our study

Scheme 1: Synthetic pathway for preparation of a library containing 5-substituted picolinic acids and esters thereof. a) i) $TMSCH_2Li$-LiDMAE, toluene, 0 ^oC, 30min .ii) $HCOOCH_3$ or $HCOOC_2H_5$, -78 ^oC, 3h. iii) I_2, K_2CO_3, methanol or ethanol; b) bis(pinacolato)diboron, postassium acetate, 10 mol % Pd(dppf)Cl_2 complex with chloroform, MW, 30 min; c) R^2-Cl or R^2-Br for Pathway 1 and substituted boronate ester for Pathway 2, Pd(dppf)Cl_2 complex with chloroform 10 %, Cs_2CO_3, 1,4-Dioxane/water (1/0.5), MW, 90 min; d) NaOH(aq) (5 equiv.), rt, 12h.

Scheme 2: Synthesis of the different alkyls side chain. a) R^3-Cl or R^3-Br, DMF, rt, 18 - 48 h or MW, 180 min; b) NaOH(aq) (5 equiv.), rt, 12h; c) propyl amine, 6 mol % Pd(dppf)Cl_2 complex with dichloromethane, Cs_2CO_3 (2 equiv.), toluene, MW, 90min; d) ethanethiol, K_2CO_3, DMF, rt, 5h.

Scheme 3: Fast synthesis of **FA** using microwave-assisted methodogy. a) n-butylboronic acid pinacol ester, 10 mol % Pd(dppf)Cl_2 complex with chloroform, Cs_2CO_3 (2 equiv.), 1,4-Dioxane/water (1/0.5), MW, 90 min; b) NaOH(aq) (5 equiv.), rt, 12h; c) bis(pinacolato)diboron, postassium acetate, 10 mol % Pd(dppf)Cl_2 complex with chloroform, MW, 30 min; d) n-butyl bromide, 10 mol %,Pd(dppf)Cl_2 complex with chloroform, Cs_2CO_3, 1,4-dioxane/water (1/0.5), MW, 90 min.

Table 1: Structures of tested analogues of **FA** and yield$^+$

Table 2: IC_{50} values for QSI activity of compounds **FAE**, **FA**, **4b**, **4g**, **7a**, **7b** and **7c** tested with the *luxI-gfp* monitor screens.

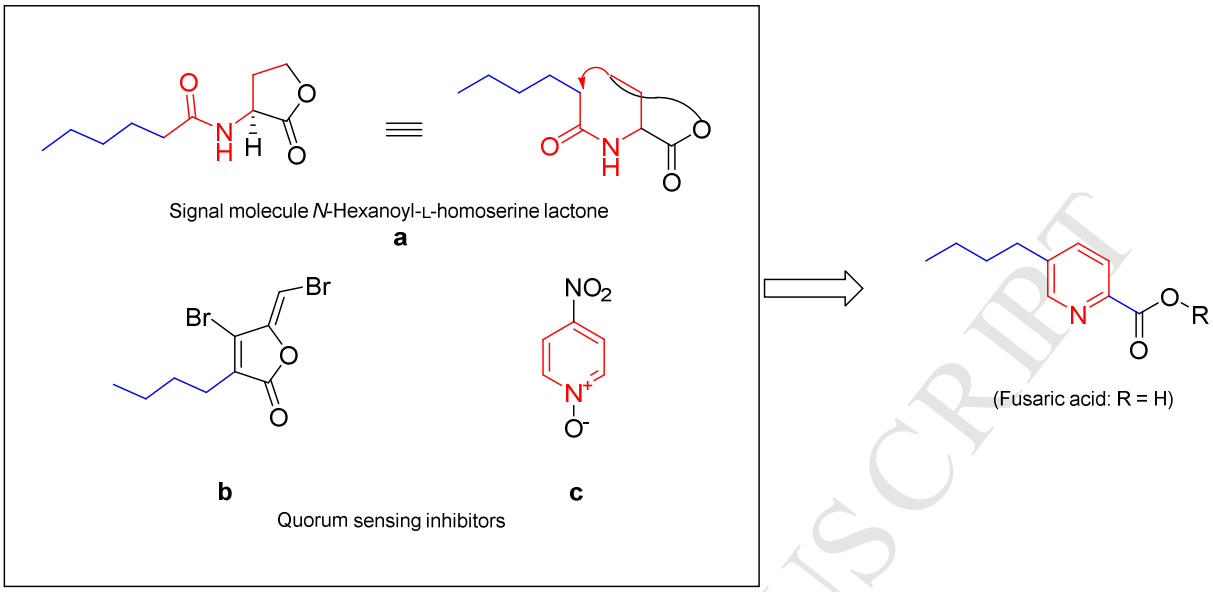


Fig. 1

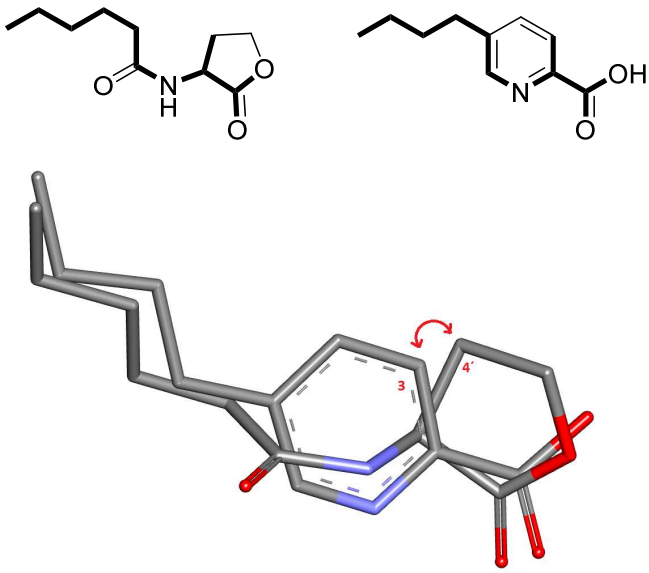


Fig. 2

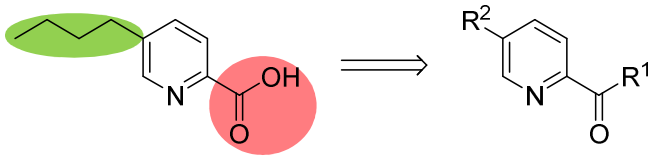


Fig. 3

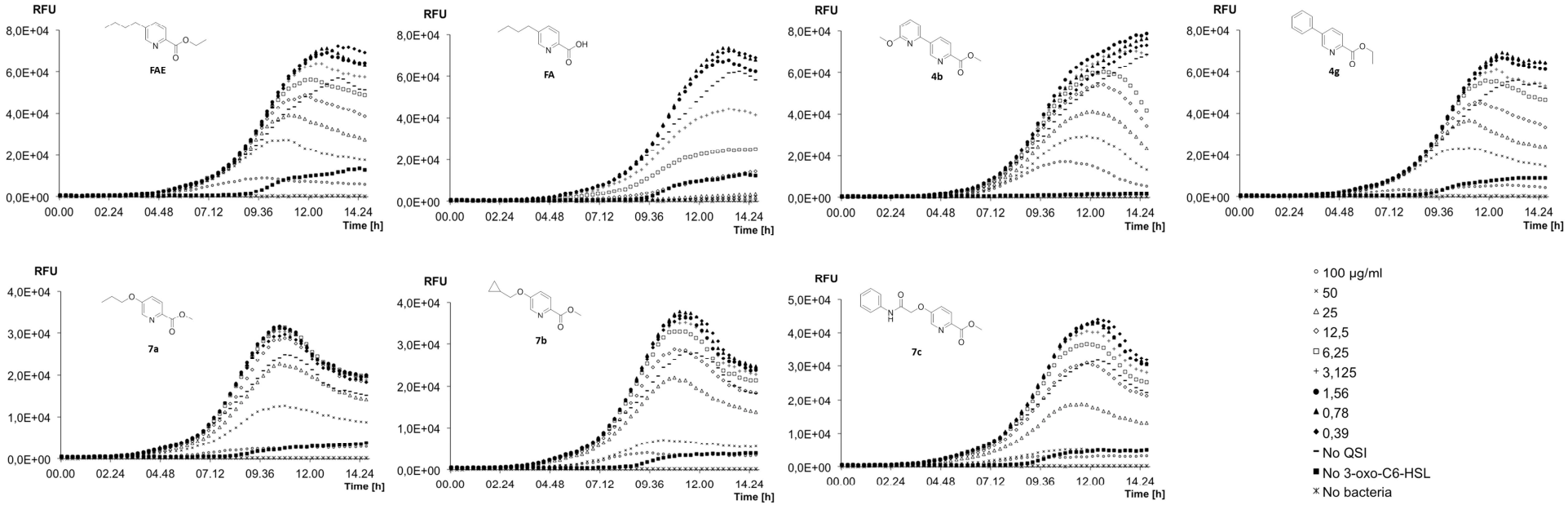


Fig. 4

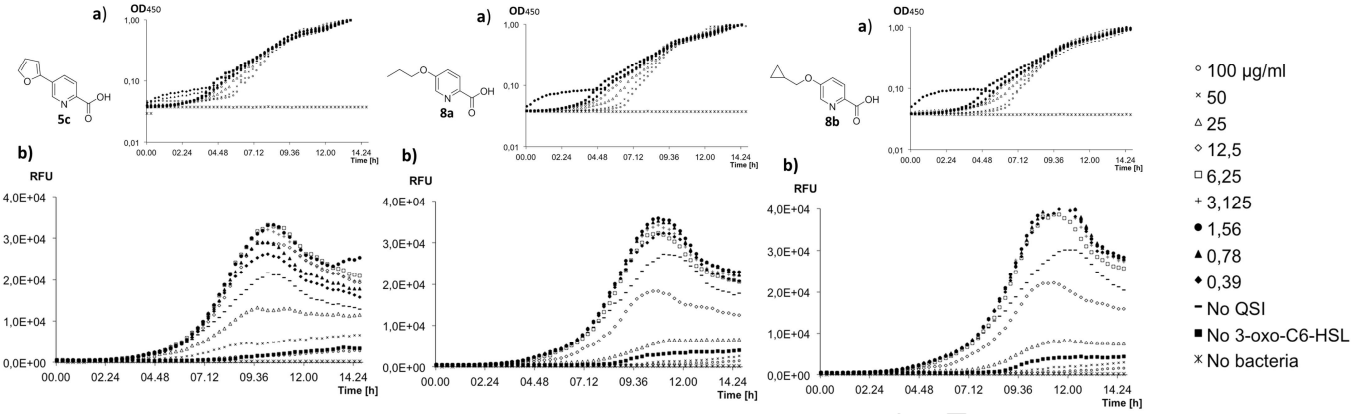


Fig. 5

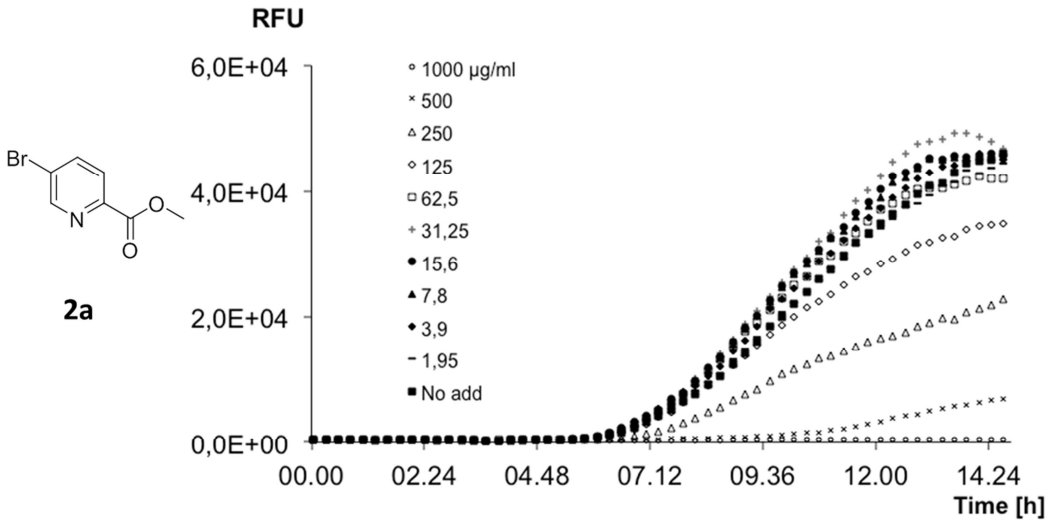


Fig. 6

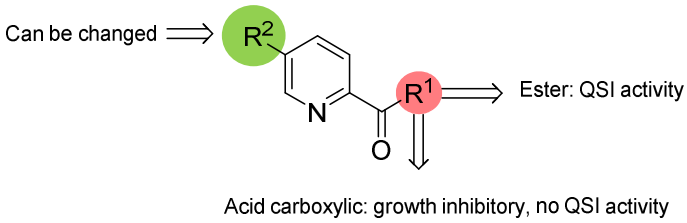
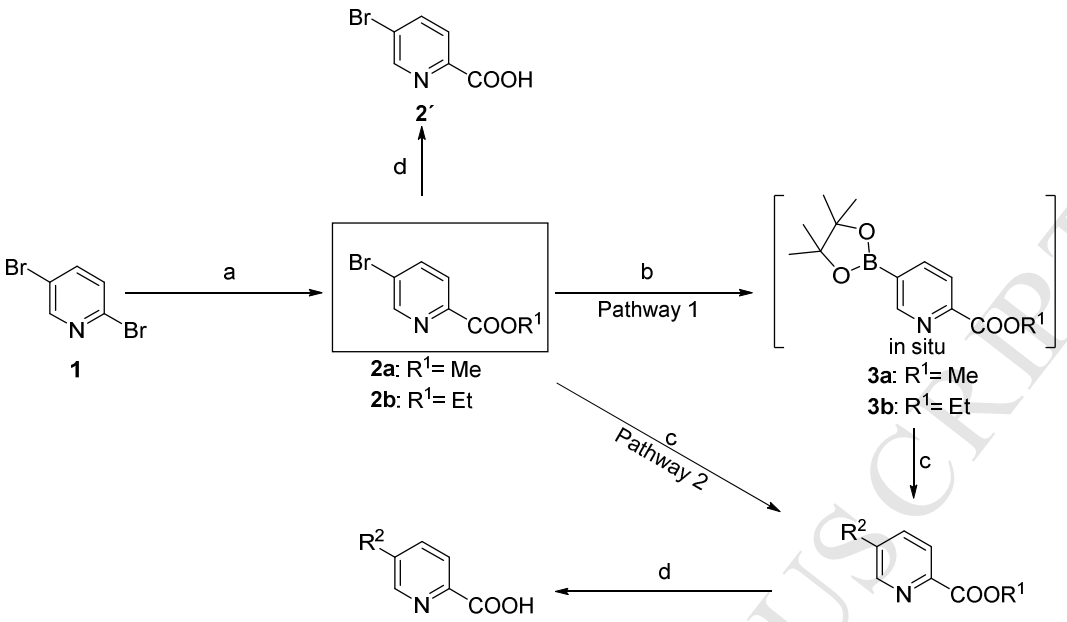


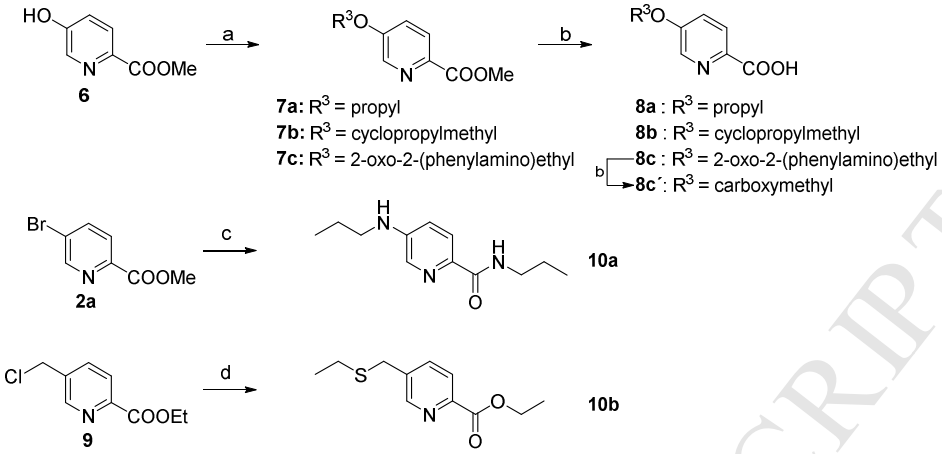
Fig. 7



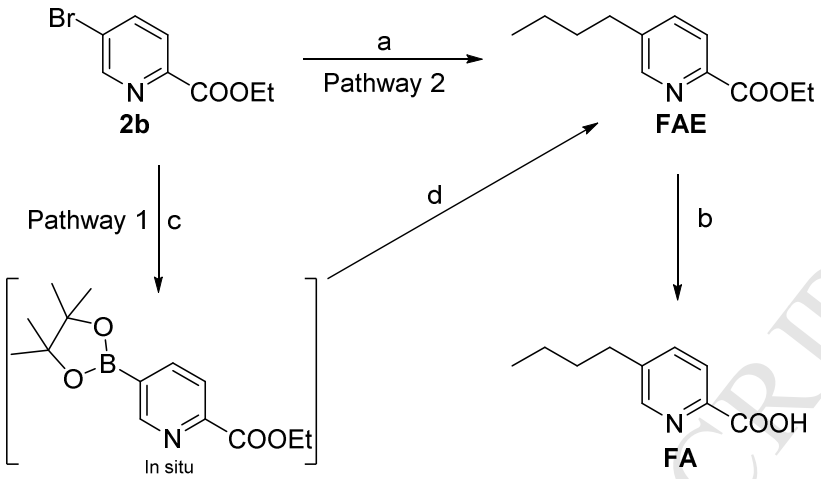
5a: R^2 = benzyl
5b: R^2 = 6-methoxypyridine-2-yl
5c: R^2 = furan-2-yl
5d: R^2 = 5-methylthiophen-2-yl
5e: R^2 = thiazol-2-yl
5f: R^2 = 2-methyl-2*H*-indazol-6-yl
5g: R^2 = phenyl
5h: R^2 = 4-methylphenyl
5i: R^2 = naphthalen-1-yl
5j: R^2 = quinolin-4-yl
5k: R^2 = 2-methylquinolin-4-yl
5l: R^2 = 6-methoxyquinolin-4-yl
5m: R^2 = 7-methoxyquinolin-4-yl

4a: R^1= Me, R^2 = benzyl
4b: R^1= Me, R^2 = 6-methoxypyridine-2-yl
4c: R^1= Me, R^2 = furan-2-yl
4d: R^1= Me, R^2 = 5-methylthiophen-2-yl
4e: R^1= Me, R^2 = thiazol-2-yl
4f: R^1= Me, R^2 = 2-methyl-2*H*-indazol-6-yl
4g: R^1= Et, R^2 = phenyl
4h: R^1= Et, R^2 = 4-methylphenyl
4i: R^1= Et, R^2 = naphthalen-1-yl
4j: R^1= Me, R^2 = quinolin-4-yl
4k: R^1= Me, R^2 = 2-methylquinolin-4-yl
4l: R^1= Me, R^2 = 6-methoxyquinolin-4-yl
4m: R^1= Me, R^2 = 7-methoxyquinolin-4-yl

Scheme 1

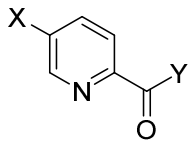


Scheme 2



Scheme 3

Table 1: Structures of tested analogues of **FA** and yield[+]



Cpds	X	Y	Method	Yield[+]
2a	Br	OMe	-	55
2b	Br	OEt	-	46
2´	Br	OH	saponification	90
4a	benzyl	OMe	1	88
4b	6-methoxypyridine-2-yl	OMe	2	92
4c	furan-2-yl	OMe	2	77
4d	5-methylthiophen-2-yl	OMe	2	82
4e	thiazol-2-yl	OMe	2	78
4f	2-methyl-2H-indazol-6-yl	OMe	2	80
4g	phenyl	OEt	2	80
4h	4-methylphenyl	OEt	2	70
4i	naphthalen-1-yl	OEt	2	82
4j	quinolin-4-yl	OMe	1	78
4k	2-methylquinolin-4-yl	OMe	1	88
4l	6-methoxyquinolin-4-yl	OMe	1	81
4m	7-methoxyquinolin-4-yl	OMe	1	77
5a	benzyl	OH	saponification	92
5b	6-methoxypyridine-2-yl	OH	saponification	92
5c	furan-2-yl	OH	saponification	80
5d	5-methylthiophen-2-yl	OH	saponification	62
5e	thiazol-2-yl	OH	saponification	69
5f	2-methyl-2H-indazol-6-yl	OH	saponification	90
5g	phenyl	OH	saponification	81

5h	4-methylphenyl	OH	saponification	91
5i	naphthalen-1-yl	OH	saponification	89
5j	quinolin-4-yl	OH	saponification	80
5k	2-methylquinolin-4-yl	OH	saponification	83
5l	6-methoxyquinolin-4-yl	OH	saponification	78
5m	7-methoxyquinolin-4-yl	OH	saponification	90
7a	propyl	OMe	Williamson	89
7b	cyclopropylmethyl	OMe	Williamson	79
7c	2-oxo-2-(phenylamino)ethyl	OMe	Williamson	83
8a	propyl	OH	saponification	80
8b	cyclopropylmethyl	OH	saponification	70
8c	2-oxo-2-(phenylamino)ethyl	OH	saponification	73[#]
8c´	carboxymethyl	OH	saponification	92[##]
10a	propylamino	propylamino	-	73
10b	(ethylthio)methyl	OEt	-	65
FAE	butyl	OEt	2	90
FA	butyl	OH	saponification	75

[+]Isolated yield (%) after Suzuki or Williamson or Saponification reaction as described in method. [#]**8c** was synthesized from **7c** in 4 hours. [##]**8c´** was obtained by let the hydrolysis of **7c** overnight. – see experimental section for method.

Table 2: IC_{50} values for QSI activity of compounds **FAE**, **FA**, **4b**, **4g**, **7a**, **7b** and **7c** tested with the *luxI-gfp* monitor screens.

No.	Compounds	IC_{50} (µg/ml)
1	**FAE**	55
2	**FA**	*ND*[a]
3	**4b**	95
4	**4g**	12
5	**7a**	*ND*[a]
6	**7b**	14
7	**7c**	64
8	**Ajoene***	4

*Ajoene [15g] was used as positive control.
[a]Not determined.
Half-maximal inhibitory concentration (IC_{50}) values were calculated from curves showing the GFP expression

Highlights

- 40 novel FA analogues were prepared efficiently by microwave-assisted synthesis
- Fusaric acid is bioequivalent with the endogenous QS signal molecules
- Natural product scaffolds as a starting point for developing QSIs
- Eight compounds inhibit *lux* QS system and one compound inhibit *las* QS system
- Structure-activity relationships have been established