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**(2R)- and (2S)- 2-hydroxy Hexanoyl and Octanoyl-L-Homoserine Lactones: new highly
potent Quorum Sensing modulators with opposite activities**

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Abstract – The synthesis and the QS modulation activity of diastereoisomerically pure 2-hydroxy acyl-L-homoserine lactones (2-OH-AHLs) are unveiled. (2*R*)- and (2*S*)- 2-Hydroxy hexanoyl-L-homoserine lactone and 2-hydroxy octanoyl-L-homoserine lactone have been identified as very potent QS agonists and antagonists on the *Vibrio fischeri*-quorum sensing system with opposite activities depending on the configuration of the carbon atom with the hydroxyl group. Flexible molecular docking showed that the (2*R*)-OH configuration in the antagonist isomer induces new hydrogen bonds with Tyr70 and Asp79, two importantly conserved residues in the LuxR protein family, while the (2*S*)-OH agonist configuration exhibits a binding mode comparable to the natural ligand 3-oxo-hexanoyl-L-homoserine lactone (OHHL). For the analogs with long alkyl chain **3a** and **3b** and aromatic analogs, all are antagonists with no effect of the configuration at C-2.

Keywords – Quorum sensing, 2-Hydroxy AHL, Flexible docking

Introduction

Quorum sensing (QS) is a bacterial system of communication [1] allowing bacteria to communicate with each other and synchronize their behavior according to their population density, notably by regulating the expression of genes including those responsible for the virulence. The modulation of QS opens therefore new perspectives to decrease bacterial virulence in biotechnologies as well as in medicine [2-6].

The QS process is based on small diffusible molecules called autoinducers capable of binding to specific protein receptors, which are transcription factors allowing the regulation of target genes encoding for particular phenotypes. An important approach to interfere with QS is the development of molecules able to bind the same receptors [7-9]. In terms of chemical structure, several types of autoinducers are found, such as acyl homoserine lactones (AHLs) (AI-1) in Gram negative bacteria [10], such as for example the pathogenic *Pseudomonas aeruginosa*, cyclic peptides in Gram positive bacteria such as *Staphylococcus aureus* [11], or dihydroxypentanedione (DPD, AI-2) in both Gram positive and Gram negative bacteria [12]. With respect to AHL, their structure differs according to bacterial strains and their general structure is depicted in Figure 1. Structural variations such as the modifications of the amide functional group with bioisosteres, or modification of the lactone part or of the side-chain have been shown to lead to different biological responses [7-9]. A structural feature of AHLs is the possible presence of a 3-oxo functional group (for *Vibrio fischeri* or *Pseudomonas aeruginosa*) or of a (3*R*)-hydroxyl functional group (for *Vibrio harveyi*) [13] on the acyl chain. It has been shown that the oxo group interfere within the binding site of the some receptors in the LuxR protein family by bridging one water molecule [14]. 2-Oxo analogs have been reported twice and found inactive [15, 16].

In the pathogen *Acinetobacter baumannii*, among (3*R*) and (3*S*) hydroxyl C12 homoserine-L-lactone diastereoisomers, both have been found to exert agonistic activities, the (3*R*) isomer being the most active and probably the natural autoinducer [17]. If a few studies discuss the biological properties of analogs of 3-oxo and 3-hydroxy derivatives [15, 17], no systematic study of enantiomerically pure 2-hydroxy AHL analogs has ever been reported. Only a mixture of four possible isomers of 2-hydroxybutanoyl homoserine lactone was tested and found inactive on *Vibrio harveyi* QS [18]. In the context of our work on QS chemical modulation [19-24], we present herein the first synthesis, biological evaluation and docking studies of enantiomerically pure AHL analogs with a hydroxyl group in position 2 of the acyl chain.

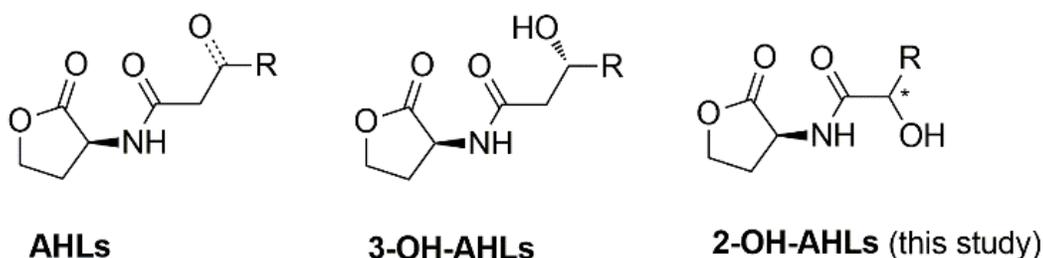


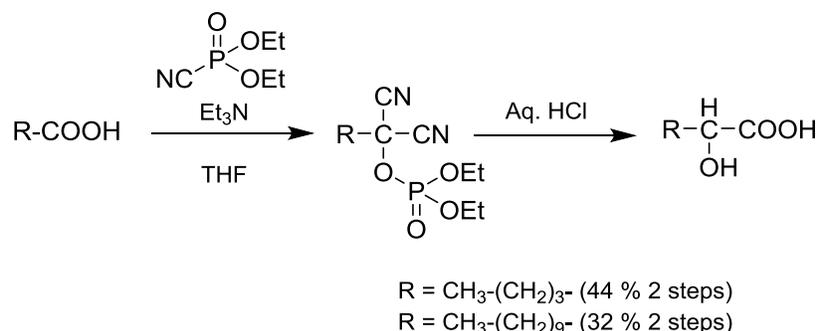
Fig. 1 Structure of AHLs as autoinducers in Gram negative bacteria.

Results and discussion

Synthesis

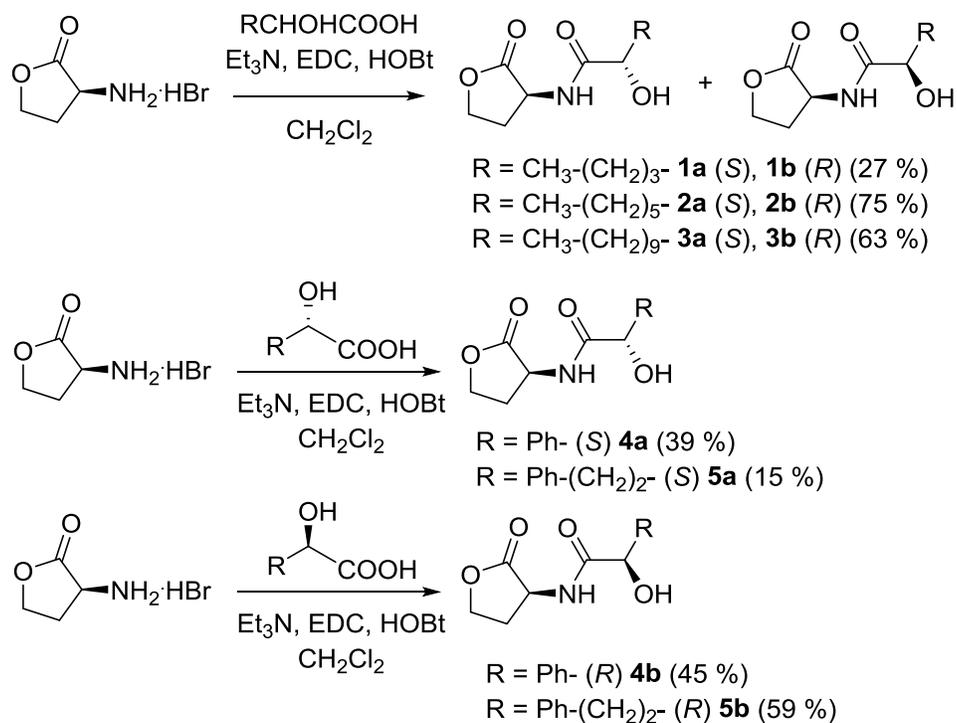
The synthesis of 2-hydroxy acylhomoserine lactones was achieved by coupling L-homoserine lactone and 2-hydroxy carboxylic acids using 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (EDC) as a coupling agent in the presence of 1-hydroxybenzotriazole (HOBt) [25]. Some 2-hydroxy carboxylic acids were commercially available, such as (*R* or *S*)-mandelic acid, (*R* or *S*)-2-hydroxy-4-phenylbutyric acid and (\pm)-2-hydroxy-octanoic acid. The (\pm)-2-hydroxy hexanoic or

dodecanoic acids, were prepared from pentanoic and undecanoic acids by reaction with 2 equivalents of diethyl phosphorocyanidate (scheme 1) [26]. In this reaction, the intermediate dicyanophosphate is isolated then submitted to acidic hydrolysis under reflux which provokes subsequent decarboxylation providing the corresponding racemic α -hydroxy carboxylic acid.



Scheme 1. Synthesis of (\pm)-2-hydroxy hexanoic or dodecanoic acids.

The coupling reaction to obtain 2-hydroxy acylhomoserine lactones (scheme 2) uses the classical HOBt and EDC reagents starting from pure L-homoserine lactone. For 2-hydroxyacids available under their pure enantiomeric form, such as (*R* or *S*)-mandelic acid and (*R* or *S*)-2-hydroxy-4-phenylbutyric acid, the reaction provides directly each single 2-OH epimer as a single isomer. For 2-hydroxyacids available under their racemic mixture, such as (\pm)-2-hydroxy-hexanoic, -octanoic and -dodecanoic acids, the reaction with L-homoserine lactone gives two diastereoisomers. Pure 2(*R*) and 2(*S*)-hydroxy-L-homoserine lactones diastereoisomers were finally obtained after silica gel chromatography purification.



Scheme 2 Synthesis of 2-hydroxy acylhomoserine-L-lactones.

The absolute configuration at C-2 was assigned first for the 2-hydroxyoctanoyl AHL **2a** as *S* by X-ray diffraction (XRD) analysis (Fig. 2). The structure indeed shows clearly the α -hydroxy amide functional group with the *S* configuration at C-2.

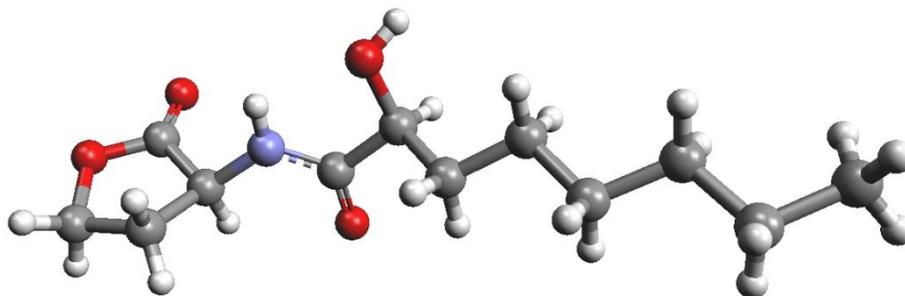


Fig. 2 Tridimensional structure of diastereoisomer **2a** obtained from XRD.

Then, we could assign the *R/S* configuration at C-2 in the series **1-3** by unambiguous comparison of ¹H NMR data (see ESI). Having in hands pure **2a** and **2b**, distinctive chemical shifts for each diastereoisomer were found, notably CHNH at δ 4.74 ppm for the *S* isomer **2a** and δ 4.55 ppm for the *R* isomer **2b**. The same patterns were clearly observed also in both isomers of **1ab** and **3ab**, respectively, allowing to assign the *S* configuration to **1a** and **3a**, and the *R* one to **1b** and **3b**. Fully consistent differences for the CHOH of signals of both diastereoisomers were also found in the series **1-3**.

Biological evaluation

All compounds were evaluated for their agonistic and antagonistic activity. For these experiments, the *Escherichia coli* strain NM522 equipped with the sensor plasmid pSB401 was used to monitor the bioluminescence induced by increasing concentrations of an AHL analog (agonistic activity). For the antagonistic activity, the bioluminescence was induced by 200 nM of 3-oxo-hexanoyl-L-homoserine lactone (OHHL) and the residual bioluminescence was measured for increasing concentrations of AHL analogs [27]. The results are given in Table 1.

Compounds	IC ₅₀ (μM)	EC ₅₀ (μM)
2 <i>S</i> -OH-HHL (1a)	> 100	15 ± 6
2 <i>R</i> -OH-HHL (1b)	11 ± 3	^a
2 <i>S</i> -OH-OHL (2a)	> 100	16 ± 3

2 <i>R</i> -OH-OHL (2b)	4 ± 3	^a
2 <i>S</i> -OH-dDHL (3a)	72 ± 9	^a
2 <i>R</i> -OH-dDHL (3b)	64 ± 3	^a
2 <i>S</i> -OH-PAHL (4a)	> 100	^a
2 <i>R</i> -OH-PAHL (4b)	84 ± 4	^a
2 <i>S</i> -OH-PBHL (5a)	17 ± 2	^a
2 <i>R</i> -OH-PBHL (5b)	15 ± 6	^a

Table 1 IC₅₀ and EC₅₀ values for compounds **3-5**. ^a Too weak activity for accurate and meaningful EC₅₀ measurement

An obvious difference in the properties between short chain compounds (**1** and **2**), and longer chain ones (compounds **3-5**) is observed. For the short hexanoyl and octanoyl hydroxyl-AHLs **1a**, **1b** and **2a**, **2b**, the two isomers in each pair exert an opposite biological activity according to the configuration of the carbon atom C-2 bearing the hydroxyl functional group. 2-Hydroxy hexanoyl-*l*-homoserine **1a** (2-OH-HHL) and 2-hydroxy octanoyl-*l*-homoserine **2a** (2-OH-OHL), both with an *S* configuration at C-2, were found to be strong agonists with an EC₅₀ value of 15 ± 6 and 16 ± 3 μM, respectively. In contrast, the other diastereoisomers **1b** and **2b** (with an *R* configuration at C-2) were found to be extremely weaker agonist as illustrated in fig. 3. However, these latter were found to be highly potent antagonist with IC₅₀ value of 11 ± 3 and 4 ± 3 μM respectively.

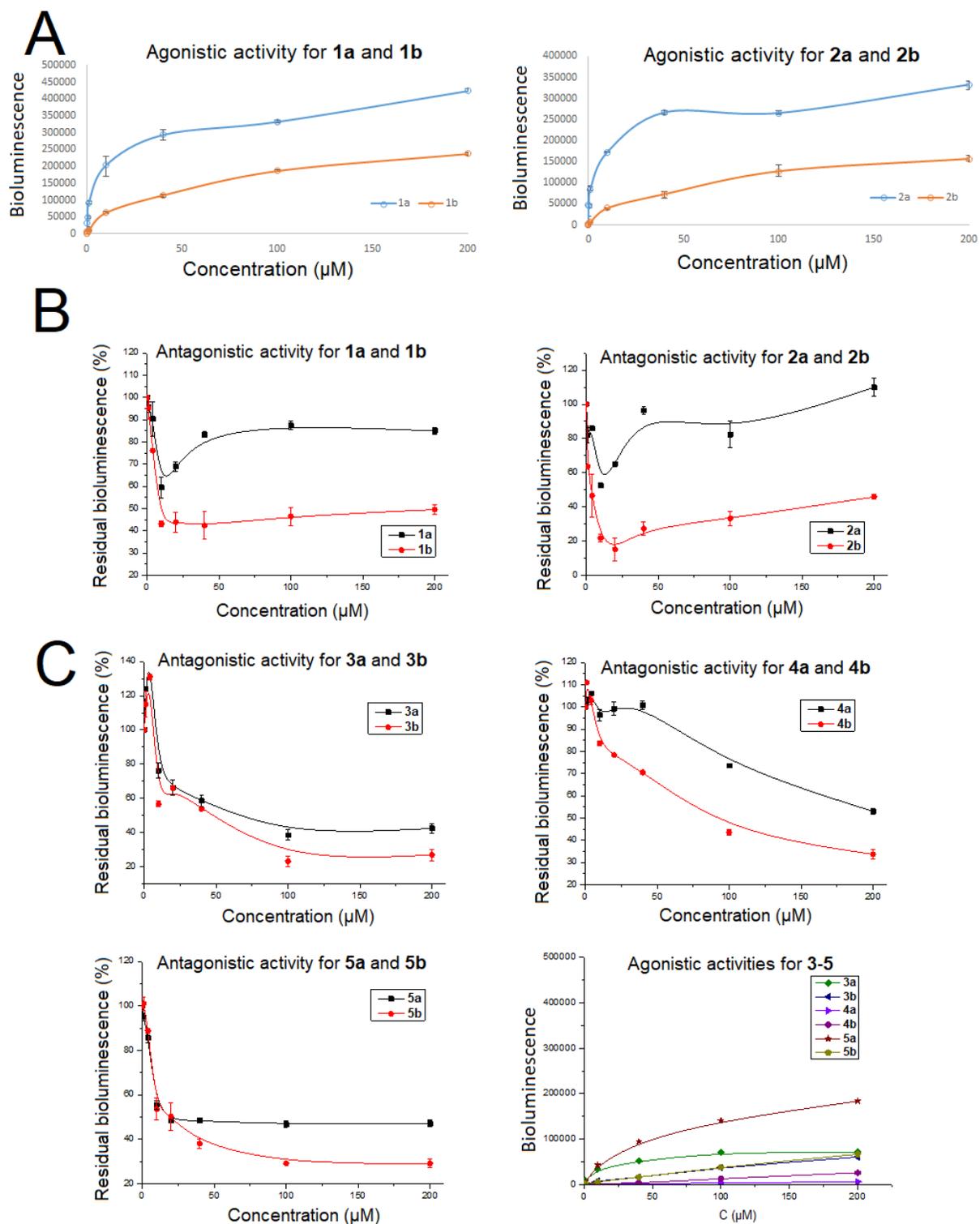


Fig. 3 Agonistic (A) and antagonistic (B) activities for compounds **1a**, **1b** and **2a**, **2b**. (C) Agonistic and antagonistic activities for compounds **3-5**.

For analogs with longer chains, both diastereoisomers of compounds **3-5** were found to exert antagonistic activity with IC₅₀ values ranging from 15 μM to 84 μM whatever their configuration at C-2 (Table 1). Actually, only compounds **5ab** exert significant activity, with antagonistic activity close to that of the hexanoyl analog **1b**, but not as potently as octanoyl compounds **2b**. Very weak agonistic activity was also observed however with levels preventing to get meaningful and accurate EC₅₀ values (Fig. 3).

No effect of the configuration at C-2 was observed for these analogs which are less structurally close to the natural ligand of LuxR OHHL. Their effect on QS is therefore governed by the presence of hydrophobic side chains, with or without aromatic moieties, consistently with the reported effect of chain length for 3-oxo AHL and non-oxo analogs with long alkyl chain displaying antagonist activity in contrast with shorter chain [15].

Docking studies

The structural difference in the series of 2-hydroxy-AHL analogs was then investigated through docking studies using the LuxR model [21, 28] by comparing their binding mode to that of the natural ligand OHHL. This latter, depicted in fig. 4 [21], shows several hydrogen bonds (Table 2) between the C=O of the lactone and Trp66, the NH of the amide functional group and Asp79, between the C=O of the amide functional group and Tyr62 and also between the 3-oxo and a water molecule. The amide functional group is located in a polar area between Asp79 and Tyr62 whereas the alkyl chain is located in the hydrophobic pocket.

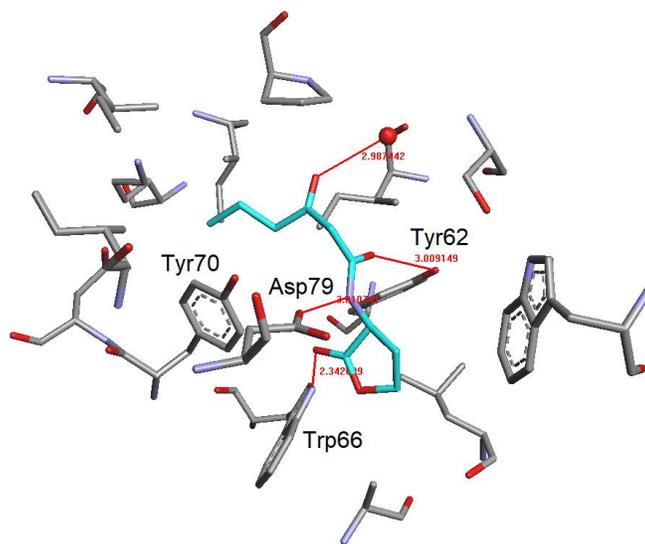


Fig. 4 Binding mode of the natural ligand within the binding site of the LuxR model (hydrogen bonds are represented with red lines).

All 2-OH AHL analogs of this study were docked within the binding site of LuxR and the docking results show a consistent hydrogen-bond networks in the series **1a-5a** (configuration *S* at C-2) and in the series **1b-5b** (configuration *R* at C-2) (Table 2 and ESI). For the *S* series **1a-5a**, the hydrogen bond network is comparable to that of OHHL, notably exhibiting a hydrogen bond between the 2-OH and the water molecule imprisoned within the binding site. In the *R* series **1b-5b**, two new hydrogen bonds involve the 2-OH group and Tyr70 and Asp79 respectively.

Compounds	Hydrogen bonds network				Interactions in the hydrophobic pocket
	C=O lactone	NH amide	C=O amide	2-OH	Side chain
1a (strong agonist)	Trp66	Asp79	Tyr62	H ₂ O	Weak
1b (strong antagonist)	Trp66	Asp79	Tyr62	Asp79 Trp70	Weak
2a (strong agonist)	Trp66	Asp79	Tyr62	H ₂ O	Weak
2b (strong antagonist)	Trp66	Asp79	Tyr62	Asp79 Trp70	Weak
3a (antagonist)	Trp66	Asp79	Tyr62	H ₂ O	Strong
3b (antagonist)	Trp66	Asp79	Tyr62	Asp79 Trp70	Strong
4a (antagonist)	Trp66	Asp79	Tyr62	H ₂ O	Strong + aromatic
4b (antagonist)	Trp66	Asp79	Tyr62	Asp79 Trp70	Strong + aromatic
5a (antagonist)	Trp66	Asp79	Tyr62	H ₂ O	Strong + aromatic
5b (antagonist)	Trp66	Asp79	Tyr62	Asp79 Trp70	Strong + aromatic

Table 2 Hydrogen bonds network in the series **1-5** and interactions in the hydrophobic pocket.

The binding mode of both isomers (*S*)-2-OH-HHL (**1a**) and (*R*)-2-OH-HHL (**1b**) are depicted in Fig 5A and 5B respectively, together with a superimposition with the natural ligand OHHL for easy comparison. It is seen that the (*S*)-isomer binds the key residues of the binding site in a very close way as compared to the natural ligand, with the same hydrogen bonds network, and, importantly showing the hydroxyl group being involved in a hydrogen bond with the water molecule like the 3-oxo function does in OHHL. Binding exactly like the natural ligand, it is therefore anticipated to act as an agonist, consistently with the observed biological activity. In contrast, the (*R*)-isomer gave a completely different result, with the hydroxyl group being

involved in new hydrogen bonds with Tyr70 and Asp79, two important residues strictly conserved in the LuxR family [28]. These new interactions can be therefore suggested to be responsible for the antagonistic activity of the *R* isomer in this series of short chain analogs. Identical observations were made in the case of the octanoyl compounds **2a** and **2b**.

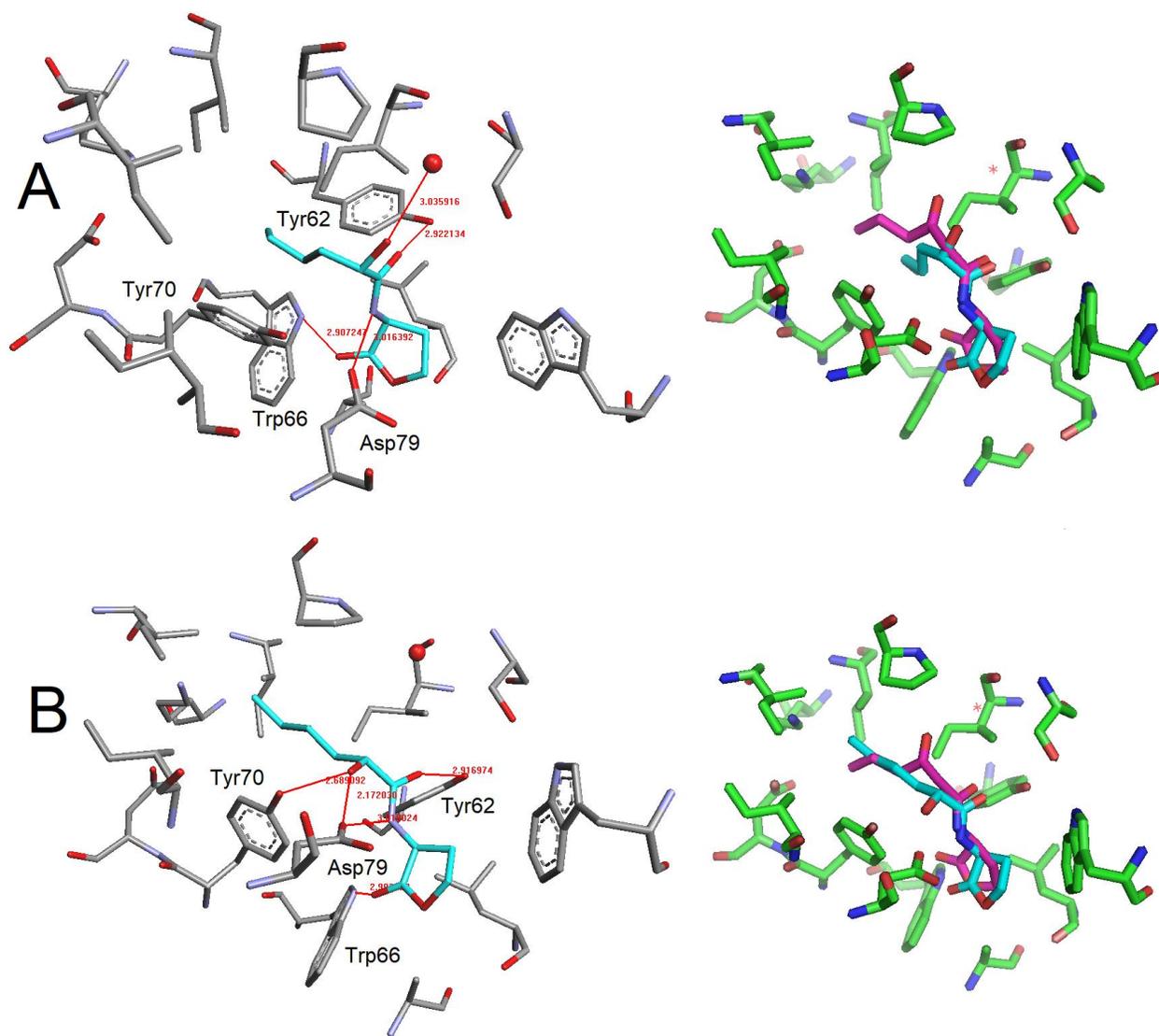


Fig. 5 Docking results of the diastereoisomers (*S*)-2-OH-HHL (A), (*R*)-2-OH-HHL (B) within the binding site of the LuxR model (left). Superimposition (right) of (*S*)-2-OH-HHL, (*R*)-2-OH-HHL (in cyan) with the natural ligand OHHL (in magenta) within the binding site of the LuxR model.

For the longer chain compounds **3a** and **3b**, the long alkyl chain interacts deeply in the hydrophobic pocket for both isomers (Table 2). For aromatic compounds **4-5**, attractive interactions are observed resulting from their aromaticity and the presence of aromatic residues in the binding site, namely Tyr62 and Tyr70 (Table 2). These interactions are observed whatever the configuration at C-2. It is therefore observed that, as soon as hydrophobic interactions exist, these interactions become pre-eminent in orienting the activity towards an antagonistic activity whatever the configuration at C-2, whereas when weaker, the effect of the configuration becomes critical in orienting towards either agonistic or antagonistic activity.

Conclusions

In summary, we have unveiled that 2-hydroxy AHL analogs are *Vibrio fischeri*-quorum sensing modulators. This systematic study includes enantiomerically and diastereoisomerically pure analogs with different alkyl chains as well as aromatic compounds. Several clear trends can be established in this series, with respect to the influence of the configuration at C-2, and to the structure of the side chains.

The very potent 2-OH-HHL and 2-OH-OHL with short alkyl chain display either agonistic or antagonistic activity according to the configuration of the carbon atom with the hydroxyl functional group. For analogs with long alkyl chain or aromatic analogs, they exert antagonistic activity whatever the configuration at C-2. Flexible docking experiments performed with all compounds showed that the (2*R*)-OH configuration induces new hydrogen bonds with Tyr70 and Asp79, two importantly conserved residues in the LuxR protein family, while the (2*S*)-OH configuration

exhibits a binding mode comparable to the natural ligand 3-oxo-hexanoyl-L-homoserine lactone (OHHL). For the 2-OH-HHL and 2-OH-OHL (short alkyl chain), these key interactions are proposed to govern the agonistic (*S* configuration) or antagonistic (*R* configuration). For the analogs with long alkyl chain **3a** and **3b** and aromatic analogs, strong hydrophobic interactions or attractive interactions are responsible of the antagonistic activity whatever the configuration at C-2.

Experimental section

Synthesis

L-homoserine lactone hydrobromide was synthesized as previously reported [29].

General procedure for the synthesis of 2-hydroxy carboxylic acid (route A):

To a solution of undecanoic acid or pentanoic acid (2.0 mmol) and diethyl phosphorocyanidate (DEPC) (90 %, 4.2 mmol) in THF (4 mL) was added triethylamine (2.1 mmol) at -20°C. The reaction mixture was stirred for 3 h at -20°C. The mixture was diluted with ether or EtOAc (30 mL) and washed with water (2 x 20 mL), the organic phase was collected, dried over anhydrous Na₂SO₄, concentrated and purified by flash chromatography to get the intermediate product dicyanophosphate. A mixture of dicyanophosphate (0.7 mmol) and HCl (37 %, 2.9 mL) was heated at reflux for 12 h. The reaction mixture was cooled, then extracted with ether (3 x 20 mL), and the organic phase was dried over anhydrous Na₂SO₄, concentrated and purified by flash chromatography to get the desired product 2-hydroxy acid.

General procedure for the synthesis of 2-hydroxy acylhomoserine lactones (route B):

To a solution of 2-hydroxy acid (0.5 mmol) and L- α -amino- γ -butyrolactone hydrobromide (0.6 mmol) in CH₂Cl₂ (4 mL) were added triethylamine (1.1 mmol) and 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDC) (0.55 mmol) and 1-hydroxybenzotriazole (HOBt) (0.5 mmol) at 0°C. The reaction mixture was stirred for 1 h at 0°C and overnight at room temperature. The solvent was evaporated and EtOAc (20 mL) was added. The mixture was washed respectively with brine, 1 % HCl, 5 % NaHCO₃, and brine, dried over anhydrous Na₂SO₄, concentrated and purified by flash chromatography to get the desired product.

(S)-2-hydroxy-N-((S)-2-oxotetrahydrofuran-3-yl)hexanamide (1a) (2-OH-HHL). From pentanoic acid following the general procedure for route A. Flash chromatography of the crude product (2:1 EtOAc-pentane) afforded 2-hydroxyhexanoic acid (44 % for two steps) as a white solid. ¹H NMR data were consistent with the literature [30]. According to the general procedure for route B, flash chromatography of the crude product (3:1 EtOAc-pentane) afforded **1a** and **1b** (27 %) as a white solid. ¹H NMR (300 MHz, Chloroform-*d*) δ 7.38 (d, *J* = 7.9 Hz, 1H, NH), 4.77 (dt, *J* = 11.7, 8.4 Hz, 1H, CH-NH), 4.46 (td, *J* = 9.1, 1.3 Hz, 1H, OCHH-lactone), 4.29 (ddd, *J* = 11.2, 9.2, 6.0 Hz, 1H, OCHH-lactone), 4.17 (dt, *J* = 8.3, 4.1 Hz, 1H, CH-OH), 3.75 (d, *J* = 4.8 Hz, 1H, OH), 2.69 (m, 1H, CHH-lactone), 2.34 – 2.14 (m, 1H, CHH-lactone), 1.81 (m, 1H, CH-CHH), 1.60 (m, 1H, CH-CHH), 1.45 – 1.26 (m, 4H, 2 \times CH₂), 0.84 (t, *J* = 7.1 Hz, 3H, CH₃). ¹³C NMR (75 MHz, Chloroform-*d*) δ 175.9 (CO), 175.5 (CO), 72.2 (CHOH), 66.0 (OCH₂-lactone), 48.2 (CH-NH), 34.1 (CHCH₂), 29.7 (CH₂-lactone), 27.2 (CH₂), 22.4 (CH₂), 14.0 (CH₃). ESI-HRMS(M+Na)⁺: 238.1050; found: 238.1052. [α]_{24 D} = -60 (c=0.5, acetone).

(R)-2-hydroxy-N-((S)-2-oxotetrahydrofuran-3-yl)hexanamide (1b) (2-OH-HHL). ¹H NMR (300 MHz, Chloroform-*d*) δ 7.24 (d, *J* = 7.5 Hz, 1H, NH), 4.55 (ddd, *J* = 11.1, 8.7, 6.9 Hz, 1H, CH-NH), 4.49 (td, *J* = 9.0, 1.5 Hz, 1H, OCHH-lactone), 4.29 (ddd, *J* = 10.9, 9.2, 6.2 Hz, 1H,

O*CHH*-lactone), 4.14 (dt, $J = 8.1, 4.1$ Hz, 1H, **CH**-OH), 3.12 (s, 1H, OH), 2.84 – 2.63 (m, 1H, *CHH*-lactone), 2.27 (m, 1H, *CHH*-lactone), 1.84 (m, 1H, CH-*CHH*), 1.65 (m, 1H, CH-*CHH*), 1.48 – 1.23 (m, 4H, 2×CH₂), 0.91 (t, $J = 7.1$ Hz, 3H, CH₃). ¹³C NMR (75 MHz, Chloroform-*d*) δ 175.4 (CO), 174.9 (CO), 72.2 (CHOH), 66.1 (OCH₂-lactone), 48.8 (CH-NH), 34.2 (CHCH₂), 29.6 (CH₂-lactone), 27.1 (CH₂), 22.4 (CH₂), 13.9 (CH₃). HRMS(M+Na)⁺: 238.1050; found: 238.1047 [α]₂₄ D = +9.6 (c=0.415, acetone).

(S)-2-hydroxy-N-((S)-2-oxotetrahydrofuran-3-yl)octanamide (2a) (2-OH-OHL). From commercial available 2-hydroxyoctanoic acid following the general procedure for route B, flash chromatography of the crude product (3:1 EtOAc-pentane) afforded **2a** and **2b** (75 %) as a white solid. ¹H NMR (500 MHz, Chloroform-*d*) δ 7.25 (d, $J = 6.0$ Hz, 1H, NH), 4.74 (ddd, $J = 11.8, 8.7, 7.5$ Hz, 1H, **CH**-NH), 4.47 (td, $J = 9.0, 1.2$ Hz, 1H, O*CHH*-lactone), 4.30 (ddd, $J = 11.2, 9.3, 6.0$ Hz, 1H, O*CHH*-lactone), 4.18 (ddd, $J = 8.5, 5.0, 3.7$ Hz, 1H, **CH**-OH), 3.32 (d, $J = 5.0$ Hz, 1H, OH), 2.74 (m, 1H, *CHH*-lactone), 2.22 (m, 1H, *CHH*-lactone), 1.83 (m, 1H, CH-*CHH*), 1.62 (m, 1H, CH-*CHH*), 1.41 (p, $J = 7.1, 6.6$ Hz, 2H, CH₂), 1.36 – 1.20 (m, 6H, 3×CH₂), 0.87 (t, $J = 7.0$ Hz, 3H, CH₃). ¹³C NMR (126 MHz, Chloroform-*d*) δ 175.6 (CO), 175.2 (CO), 72.3 (CHOH), 66.0 (OCH₂-lactone), 48.3 (CH-NH), 34.5 (CHCH₂), 31.7 (CH₂), 29.9 (CH₂-lactone), 29.0 (CH₂), 25.0 (CH₂), 22.9 (CH₂), 14.1 (CH₃). ESI-HRMS(M+Na)⁺: 266.1363; found: 266.1364. [α]₂₄ D = -47.3 (c=0.55, acetone).

(R)-2-hydroxy-N-((S)-2-oxotetrahydrofuran-3-yl)octanamide (2b) (2-OH-OHL). ¹H NMR (500 MHz, Chloroform-*d*) δ 7.24 (d, $J = 6.4$ Hz, 1H, NH), 4.55 (ddd, $J = 11.3, 8.8, 6.9$ Hz, 1H, **CH**-NH), 4.49 (td, $J = 9.1, 1.4$ Hz, 1H, O*CHH*-lactone), 4.29 (ddd, $J = 10.6, 9.2, 6.1$ Hz, 1H, O*CHH*-lactone), 4.14 (ddd, $J = 8.7, 5.3, 3.8$ Hz, 1H, **CH**-OH), 3.11 (d, $J = 5.0$ Hz, 1H, OH), 2.73 (m, 1H, *CHH*-lactone), 2.27 (m, 1H, *CHH*-lactone), 1.85 (m, 1H, CH-*CHH*), 1.65 (m, 1H, CH-

CHH), 1.42 (m, 2H, CH₂), 1.36 – 1.22 (m, 6H, 3×CH₂), 0.87 (t, *J* = 6.7 Hz, 3H, CH₃). ¹³C NMR (126 MHz, Chloroform-*d*) δ 175.4 (CO), 174.9 (CO), 72.2 (CHOH), 66.1 (OCH₂-lactone), 48.8 (CH-NH), 34.5 (CHCH₂), 31.7 (CH₂), 29.6 (CH₂-lactone), 29.0 (CH₂), 24.9 (CH₂), 22.6 (CH₂), 14.1 (CH₃). ESI-HRMS(M+Na)⁺: 266.1363; found: 266.1361. [α]₂₄ D = +6.2 (c=0.325, acetone).

(*S*)-2-hydroxy-*N*-((*S*)-2-oxotetrahydrofuran-3-yl)dodecanamide (3a) (2*S*-OH-dDHL) : From undecanoic acid following the general procedure for route A. Flash chromatography of the crude product (1:1 EtOAc-pentane) afforded 2-hydroxydodecanoic acid (32 % for two steps) as a white solid. ¹H NMR data were consistent with the literature [30]. According to the general procedure for route B, flash chromatography of the crude product (1:2 EtOAc-pentane) afforded **3a** and **3b** (63 %) as a white solid. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.30 (d, *J* = 7.6 Hz, 1H, NH), 4.75 (dt, *J* = 11.7, 8.2 Hz, 1H, *CH*-NH), 4.47 (td, *J* = 9.0, 1.2 Hz, 1H, *OCHH*-lactone), 4.29 (ddd, *J* = 11.2, 9.2, 5.9 Hz, 1H, *OCHH*-lactone), 4.17 (dd, *J* = 8.2, 3.7 Hz, 1H, *CH*-OH), 3.24 (br, 1H, OH), 2.72 (m, 1H, *CHH*-lactone), 2.22 (m, 1H, *CHH*-lactone), 1.81 (m, 1H, CH-*CHH*), 1.61 (m, 1H, CH-*CHH*), 1.46 – 1.20 (m, 16H, 8×CH₂), 0.87 (t, *J* = 8.0 Hz, 3H, CH₃). ¹³C NMR (101 MHz, Chloroform-*d*) δ 175.7 (CO), 175.3 (CO), 72.3 (CHOH), 66.0 (OCH₂-lactone), 48.3 (CH-NH), 34.5 (CHCH₂), 31.9 (CH₂), 29.8 (CH₂), 29.6 (CH₂), 29.5 (2×CH₂), 29.4 (CH₂), 29.3 (CH₂), 25.1 (CH₂), 22.7 (CH₂), 14.1 (CH₃). ESI-HRMS(M+Na)⁺: 322.1989; found: 322.1976. [α]₂₄ D = -36 (c=0.39, acetone).

(*R*)-2-hydroxy-*N*-((*S*)-2-oxotetrahydrofuran-3-yl)dodecanamide (3b) (2*R*-OH-dDHL): ¹H NMR (400 MHz, Chloroform-*d*) δ 7.21 (d, *J* = 6.9 Hz, 1H, NH), 4.55 (ddd, *J* = 11.4, 8.8, 6.9 Hz, 1H, *CH*-NH), 4.49 (td, *J* = 9.1, 1.5 Hz, 1H, *OCHH*-lactone), 4.29 (ddd, *J* = 11.0, 9.3, 6.2 Hz, 1H, *OCHH*-lactone), 4.14 (dd, *J* = 8.2, 3.8 Hz, 1H, *CH*-OH), 3.04 (br, 1H, OH), 2.74 (m, 1H, *CHH*-

lactone), 2.26 (m, 1H, *CHH*-lactone), 1.85 (m, 1H, CH-*CHH*), 1.64 (m, 1H, CH-*CHH*), 1.47 – 1.20 (m, 16H, 8×CH₂), 0.87 (t, *J* = 8.0 Hz, 3H, CH₃). ¹³C NMR (101 MHz, Chloroform-*d*) δ 175.4 (CO), 174.9 (CO), 72.3 (CHOH), 66.1 (OCH₂-lactone), 48.8 (CH-NH), 34.5 (CHCH₂), 31.9 (CH₂), 29.6 (CH₂), 29.6 (2×CH₂), 29.5 (CH₂), 29.3 (2×CH₂), 25.0 (CH₂), 22.7 (CH₂), 14.1 (CH₃). ESI-HRMS(M+Na)⁺: 322.1989; found: 322.1985. [α]₂₄ D = 0 (c=0.62, acetone).

(*S*)-2-hydroxy-*N*-((*S*)-2-oxotetrahydrofuran-3-yl)-2-phenylacetamide (4a) (2*S*-OH-PAHL).

From commercial available (*S*)-2-hydroxy-2-phenylacetic acid following the general procedure for route B, flash chromatography of the crude product (4:1 EtOAc-pentane) afforded **4a** (39 %) as a white solid. ¹H NMR (300 MHz, Acetone-*d*₆) δ 7.97 (d, *J* = 8.0 Hz, 1H, NH), 7.59 – 7.44 (m, 2H, Ph), 7.42 – 7.19 (m, 3H, Ph), 5.40 (d, *J* = 4.6 Hz, 1H, OH), 5.12 (d, *J* = 4.4 Hz, 1H, CH-OH), 4.72 (ddd, *J* = 11.2, 9.0, 8.1 Hz, 1H, CH-NH), 4.40 (td, *J* = 8.9, 1.8 Hz, 1H, O*CHH*-lactone), 4.29 (ddd, *J* = 10.7, 8.9, 6.3 Hz, 1H, O*CHH*-lactone), 2.53 (m, 1H, *CHH*-lactone), 2.36 (m, 1H, *CHH*-lactone). ¹³C NMR (101 MHz, Acetone-*d*₆) δ 174.7 (CO), 172.3 (CO), 140.8 (Ph), 128.0 (2C, Ph), 127.6 (Ph), 126.8 (2C, Ph), 73.9 (CHOH), 65.2 (OCH₂-lactone), 48.0 (CH-NH), 28.7 (CH₂-lactone). ESI-HRMS(M+Na)⁺: 258.0737; found: 258.0735. [α]₂₄ D = +14 (c=0.425, acetone).

(*R*)-2-hydroxy-*N*-((*S*)-2-oxotetrahydrofuran-3-yl)-2-phenylacetamide (4b) (2*R*-OH-PAHL).

From commercial available (*R*)-2-hydroxy-2-phenylacetic acid following the general procedure for route B, flash chromatography of the crude product (4:1 EtOAc-pentane) afforded **4b** (45 %) as a white solid. ¹H NMR (300 MHz, Acetone-*d*₆) δ 8.06 (d, *J* = 8.2 Hz, 1H, NH), 7.57 – 7.46 (m, 2H, Ph), 7.43 – 7.29 (m, 3H, Ph), 5.43 (d, *J* = 4.5 Hz, 1H, OH), 5.11 (d, *J* = 4.4 Hz, 1H, CH-OH), 4.71 (ddd, *J* = 11.1, 9.1, 8.2 Hz, 1H, CH-NH), 4.39 (td, *J* = 8.9, 1.9 Hz, 1H, O*CHH*-lactone), 4.28 (ddd, *J* = 10.6, 8.9, 6.4 Hz, 1H, O*CHH*-lactone), 2.54 (m, 1H, *CHH*-lactone), 2.35

(m, 1H, **CHH**-lactone). ^{13}C NMR (75 MHz, Acetone- d_6) δ 174.7 (CO), 172.4 (CO), 140.8 (Ph), 128.1 (2C, Ph), 127.7 (Ph), 127.0 (2C, Ph), 74.0 (CHOH), 65.2 (OCH₂-lactone), 48.1 (CH-NH), 28.5 (CH₂-lactone). ESI-HRMS(M+Na)⁺: 258.0737; found: 258.0740. $[\alpha]_{24}^D = -59$ (c=0.61, acetone).

(S)-2-hydroxy-N-((S)-2-oxotetrahydrofuran-3-yl)-4-phenylbutanamide (5a) (2S-OH-PBHL).

From commercial available (S)-2-hydroxy-4-phenylbutanoic acid following the general procedure for route B, flash chromatography of the crude product (5:1 EtOAc-pentane) afforded **5a** (15 %) as a white solid. ^1H NMR (300 MHz, Chloroform- d) δ 7.34 (d, $J = 7.9$ Hz, 1H, NH), 7.31 – 7.27 (m, 1H, Ph), 7.26 – 7.14 (m, 4H, Ph), 4.75 (ddd, $J = 11.8, 8.8, 7.9$ Hz, 1H, **CH**-NH), 4.46 (td, $J = 9.1, 1.3$ Hz, 1H, **OCHH**-lactone), 4.28 (ddd, $J = 11.2, 9.3, 6.0$ Hz, 1H, **OCHH**-lactone), 4.18 (dt, $J = 8.0, 3.8$ Hz, 1H, **CH**-OH), 3.72 (d, $J = 4.3$ Hz, 1H, OH), 2.85 – 2.73 (m, 2H, **CH**₂), 2.73 – 2.60 (m, 1H, **CHH**-lactone), 2.31 – 2.08 (m, 2H, **CHH**-lactone, **CHCHH**), 2.02 – 1.86 (m, 1H, **CHCHH**). ^{13}C NMR (75 MHz, Chloroform- d) δ 175.8 (CO), 175.1 (CO), 141.2 (Ph), 128.5 (2C, Ph), 128.5 (2C, Ph), 126.1 (Ph), 71.5 (CHOH), 66.0 (OCH₂-lactone), 48.3 (CH-NH), 36.0 (CH₂), 31.3 (CH₂), 29.7 (CH₂-lactone). ESI-HRMS(M+Na)⁺: 286.1050; found: 286.1050. $[\alpha]_{24}^D = -35$ (c=0.46, acetone).

(R)-2-hydroxy-N-((S)-2-oxotetrahydrofuran-3-yl)-4-phenylbutanamide (5b) (2R-OH-PBHL).

From commercial available (R)-2-hydroxy-4-phenylbutanoic acid following the general procedure for route B, flash chromatography of the crude product (5:1 EtOAc-pentane) afforded **5a** (59 %) as a white solid. ^1H NMR (300 MHz, Acetone- d_6) δ 7.85 (d, $J = 8.1$ Hz, 1H, NH), 7.35 – 7.08 (m, 5H, Ph), 4.88 (s, 1H, OH), 4.71 (ddd, $J = 11.2, 9.0, 8.2$ Hz, 1H, **CH**-NH), 4.41 (td, $J = 8.9, 1.8$ Hz, 1H, **OCHH**-lactone), 4.30 (ddd, $J = 10.6, 8.9, 6.3$ Hz, 1H, **OCHH**-lactone), 4.10 (dd, $J = 7.7, 4.0$ Hz, 1H, **CH**-OH), 2.83 – 2.70 (m, 2H, **CH**₂), 2.56 (m, 1H, **CHH**-lactone), 2.48 – 2.32

(m, 1H, **CHH**-lactone), 2.17 – 2.07 (m, 1H, **CHCHH**), 1.98 – 1.79 (m, 1H, **CHCHH**). ¹³C NMR (75 MHz, Acetone-d₆) δ 174.7 (CO), 174.0 (CO), 142.1 (Ph), 128.4 (2C, Ph), 128.3 (2C, Ph), 125.7 (Ph), 70.8 (CHOH), 65.2 (OCH₂-lactone), 47.9 (CH-NH), 36.7 (CH₂), 30.9 (CH₂), 28.6 (CH₂-lactone). ESI-HRMS(M+Na)⁺: 286.1050; found: 286.1052. [α]_D²⁴ = -8.3 (c=0.48, acetone).

Biological evaluation

Escherichia coli strain NM522 equipped with the sensor plasmid pSB401 was grown in Luria broth (50 mL) at 30°C. After 18 h, this bacterial culture was diluted 10 times and 100 μL per well (96 wells plates) were added. For agonistic activity, compounds were tested at increasing concentrations in DMSO (max 2 % in a total volume of 200 μL of LB). For antagonistic activity, compounds were tested at increasing concentrations in DMSO (max 2 % in a total volume of 200 μL) in competition with OHHL (200 nM). Bioluminescence was measured with a Tecan spark luminometer.

Molecular modeling

Compounds were drawn with Vega ZZ and 3D structures were generated and minimized using the SP4 force field (Gasteiger charges assignment) [31-33]. The LuxR model reported in previous study was employed for docking experiments. The docking box centered on the natural ligand (15×15×15 Å) was created by selecting residues of the binding site. Docking experiments were achieved using Arguslab [34] with the genetic algorithm engine [35] with default parameters.

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Conflicts of interest

There are no conflict to declare.

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