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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis and activity evaluation study of novel substituted *N*-sulfonyl homoserine lactone derivatives as bacterial quorum sensing inhibitors

Qi Sun[1] · Mingming Zhao[2] · Jingwei Liang[1] · Junhai Xiao[3] · Fanhao Meng[1]

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Abstract A novel series of *N*-sulfonyl homoserine lactone derivatives **7a–7m** has been designed, synthesized, and evaluated for quorum sensing inhibitory activities through the violacein inhibition in *Chromobacterium violaceum* CV026. Compound **7e** displayed the high level of inhibitory activity among all the compounds synthesized. Studies of structure-activity relationship indicated that compounds with thiophene group in side chain showed better activity than those substituted by furan, pyrrole, pyridyl, and phenethyl group. Thiophene substituted compounds which connected electron withdrawing group exhibited better inhibitory activity relate to those connected electron donating group. Further analysis indicated that compound bearing an electron withdrawing substituent at the position 2 of their thiophene ring exhibited superior activity against violacein production to those bearing the substituent at the position 3 and 4. Compound **7e** in particular, with IC_{50} value of 6.19 μM, were identified as promising lead compounds for further development.

Keywords Bacterial quorum sensing inhibitor · Acylhomoserine lactone · Design · Synthesis · Activity evaluation

✉ Junhai Xiao
13611090335@139.com

✉ Fanhao Meng
fhmeng@cmu.edu.cn

Qi Sun
qsun@cmu.edu.cn

Mingming Zhao
zhaomingmingbest@163.com

Jingwei Liang
liangjw89@sina.com

[1] School of Pharmacy, China Medical University, 110122 Liaoning, China

[2] School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, 110016 Liaoning, China

[3] Laboratory of Computer-Aided Drug Design and Discovery, Beijing Institute of Pharmacology and Toxicology, 100850 Beijing, China

Introduction

In the growth process of bacteria, they can produce some chemical signal molecules called auto-inducers and release them into the surrounding environment. When the concentration of auto-inducers reaches to a certain threshold, a variety of specific receptors (genes) associated with transcription signals will be regulated to adapt the environmental changes, which is known as the bacterial quorum sensing (Swift et al. 2001; Yang et al. 2010). Several important phenotypes are regulated by quorum sensing, including biofilm formation and bioluminescent (Kim et al. 2009; Steven et al. 2001). Currently, quorum sensing has been observed in more than 50 kinds of bacteria, including *Pseudomonas aeruginosa* and *Escherichia coli* (Bassler 2002; Choudhary and Schmidt–Dannert 2010). Quorum sensing inhibitors make pathogens lose pathogenicity by preventing the expression of harmful genes, which do not interfere with the normal physiological processes of bacteria, allowing for the discovery of new direction for the development of novel antibacterial agents (Stewart and William 2001; Galloway et al. 2011; Ng and Bassler 2009; Reverchon et al. 2002).

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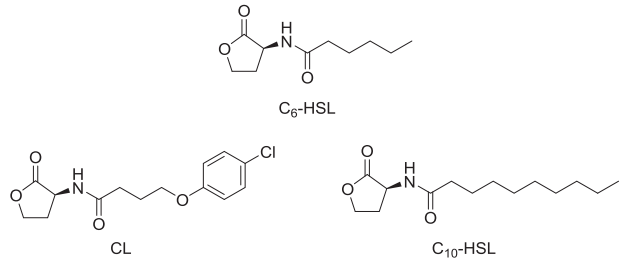


Fig. 1 Structures of C_6-HSL, CL and C_{10}-HSL

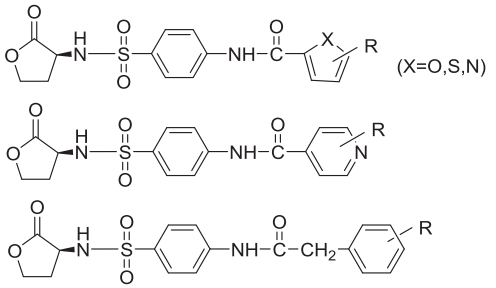


Fig. 2 Design of the target compounds

Chromobacterium violaceum is a gram-negative bacteria that synthesizes the violet pigment violacein as the result of quorum sensing using *N*-acyl homoserine lactone (C_6-HSL, Fig. 1) as its auto-inducer (Choo et al. 2006). *C. violaceum* CV026 (CV026) has been widely used as a bacterial model to screen new quorum sensing inhibitors, since the phenomenon of its quorum sensing system is easily observed and facilitate quantitative analysis (Durán and Menck 2001).

A great number of *N*-acyl homoserine lactone (AHL) derivatives have been developed on the basis of their inhibition of violacein production. Chloro Lactone (CL) and C_{10}-HSL (Fig. 1) are the effective quorum sensing inhibitors reported to date (Reverchon et al. 2002; Chen et al. 2011; Fuqua et al. 2001). AHLs can freely access cell membrane and combine with receptor protein because they possess a hydrophilic homoserine lactone ring together with a hydrophobic aliphatic amide side chain. CviR is a canonical transcription factor protein from *C. violaceum*. The mechanism of inhibiting violacein production was that C_{10}-HSL allowed DNA binding but reduced or eliminated transcriptional activation, suggesting that the CviR-C_{10}-HSL complex could not productively interact with RNA polymerase. But CL prevented CviR from binding DNA (Swem et al. 2009).

Among a number of AHLs quorum sensing inhibitors, relatively few studies have focused on the amide moiety between homoserine lactone nucleus and acyl side chain. To research the function of the amide groups, ureidos, sulfonamides and heterocyclic rings were replaced (Frezza et al. 2006; Sabbah et al. 2012; Castang et al. 2004). Using bioisosterism, the amide group was replaced by ureido group. The resulting compounds exhibited good quorum sensing inhibitory activity in *Vibrio fischeri* (Frezza et al. 2006). Furthermore, heterocyclic rings like triazoles and tetrazoles replaced the amide group because they showed some similarity with amide bonds (Sabbah et al. 2012). Replacing amide group with sulfonamide, Castang et al. synthesized a series of *N*-sulfonyl homoserine lactone analogs which displayed quorum sensing inhibitory activity in *V. fischeri* because of the widespread biological activity of sulfonamides. Furthermore, *N*-sulfonyl homoserine lactone analogs bearing phenyl group in the tail chain possessed superior quorum sensing inhibitory activity than bearing aliphatic group (Castang et al. 2004). Based on above, in order to increase the inhibitory activity, we designed to replace the amide group with 4-aminobenesulfonyl moiety between the nucleus and the acyl side chain, and the compound should have aryl group in tail chain.

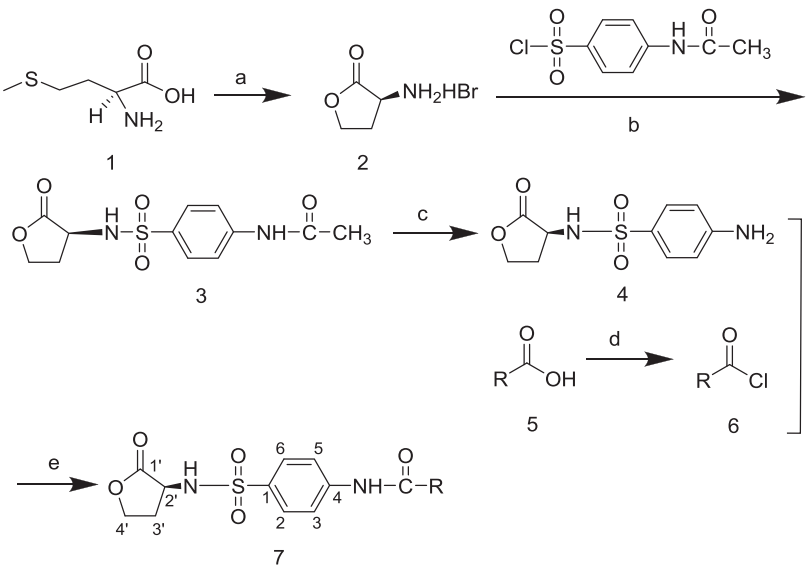
To research the inhibitory activities of novel AHLs quorum sensing inhibitors against violacein production, we have made several changes to the structures of the existing inhibitors CL and C_{10}-HSL, while reserving the key homoserine lactone nucleus which is the active essential group of quorum sensing inhibitory activity (McClean et al. 1997). A 4-aminobenzenesulfonyl moiety was introduced between nucleus and acyl side chain, which could improve hydrophobicity of the compounds and exhibit broad biological activity (Castang et al. 2004). In order to explore the inhibitory activity effects of the five-aromatic, six-aromatic heterocyclic group and phenethyl group in the tail chain following the introduction of the 4-aminobenzenesulfonyl moiety, a series of novel *N*-sulfonyl homoserine lactone derivatives **7a–7m** (Fig. 2) was designed, synthesized and valuated as potential quorum sensing inhibitors on the basis of their ability to inhibit the production of violacein, and a preliminary analysis of the structure-activity relationship (SAR) study of these target compounds was conducted.

Results and discussion

Chemical synthesis

All the agents were commercially available and were directly used without further purification unless mentioned otherwise. The syntheses of the intermediates and target compounds were completed according to the steps demonstrated in Scheme 1. Commercially available *L*-methionine **1** was reacted with bromoacetic acid at reflux to give homoserine lactone hydrobromide **2**, which was subsequently reacted with 4-acetylamino-benzenesulfonyl chloride to give compound **3**. Using 95% ethanol as the

Scheme 1 Synthesis of target compounds **7a–m**. Reaction conditions and reagents: **a** bromoacetic acid, acetic acid, H_2O, 2-propanol, reflux, then concentrated HCl, 55 °C, 30 min; **b** 4-acetylamino-benzenesulfonyl chloride, TEA, EtOH, 0 °C to rt., overnight; **c** 6 mol/L HCl, EtOH, reflux, 4 h; **d** oxalyl chloride, CH_2Cl_2, 0 °C to rt., overnight; **e** CH_2Cl_2, 0 °C to rt., overnight



R=2-thienyl(**7a**); 5-chloro-2-thienyl(**7b**); 4-methyl-2-thienyl(**7c**); 3-methyl-2-thienyl(**7d**); 3-chloro-2-thienyl(**7e**); 3-bromo-2-thienyl(**7f**); 2-furyl(**7g**); 3-furyl(**7h**); 5-bromo-2-furyl(**7i**); *1H*-2-pyrrolyl(**7j**); 4-pyridyl(**7k**); 2-methyl -phenethyl(**7l**); 2-chloro-phenethyl(**7m**)

solvent, compound **3** which treated with refluxing in 6 mol/L HCl for 2 h, produced the hydrolyzate compound **4**. With dichloromethane as the solvent, N,N-Dimethylformamide (DMF) as a catalyst, a series of five or six membered aromatic carboxylic acid **5** produced the corresponding acid chloride intermediate **6** under the action of oxalyl chloride. Ultimately, according to amide Schotten–Baumann reaction, compound **4** was reacted with intermediate **6** to give the desired target compounds **7a–7m**. The structures of all the target compounds were characterized by methods of MS and ^{1}H-NMR.

Evaluation of biological activity figures

The biological activity of target compounds were evaluated through the violacein inhibition in *C. violaceum* CV026. Since quorum sensing inhibition is focused on the inhibition of bacterical quorum sensing signal molecules and not on the interference of the normal physiological activity of bacteria, we designed to exclude the effect of growth inhibition activity on quorum sensing before the evaluation of inhibitory activity. The spot test method was performed to test growth inhibition activity of compound **7a–7m** against *C. violaceum* CV026. The results showed the white colonies in purple background of flatbeds (plates) and no transparent growth inhibition zone even in the highest concentration of compound **7e** (Fig. 3a), and the same phenomenon had been observed on other compound. As compared with them, Fig. 3b showed the transparent growth inhibition zone with a sharp and regular edge. The above results indicated that

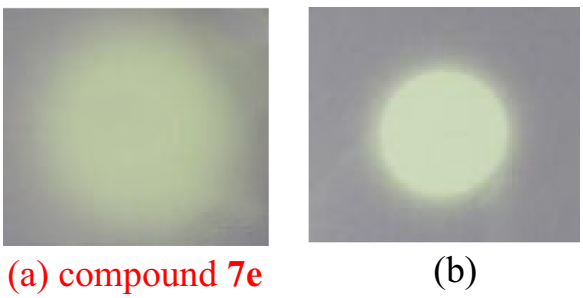


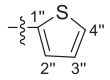
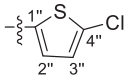
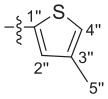
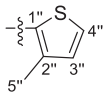
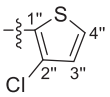
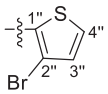
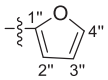
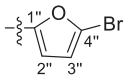
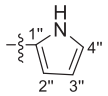
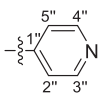
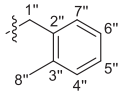
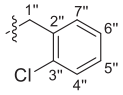
Fig. 3 Growth inhibition spot tests of compound **7e**

target compound **7a–7m** have no effect on the normal growth of *C. violaceum* CV026 but inhibit bacterial QS system. The violacein was produced by inducer C_6-HSL.

The quorum sensing inhibitory activity of target compounds **7a–7m** were evaluated through the inhibition of the violacein production in CV026, with compound C_{10}-HSL being used as a positive control. The experimental results showed the white colonies in purple background of plates were observed in all target compounds, which indicated compounds **7a–7m** have varying range of quorum sensing inhibitory activity. The IC_{50} values for each synthesized compound have been summarized in Table 1.

As shown in Table 1, compounds **7a–7b** and **7d–7f** exhibited considerable levels of quorum sensing inhibitory activity against the production of violacein, with IC_{50} values ranging from 6.19–130.74 μM. Among these newly synthesized compounds **7e** exhibited the best inhibitory activity with IC_{50} value of 6.19 μM, which showed the potential as lead compound for further development of

Table 1 The substituents and IC_{50} values of **7a–7m** for inhibiting violacein production

Compound	R	IC50(μM)
7a		130.74 ± 1.33
7b		102.69 ± 2.76
7c		NA[a]
7d		49.22 ± 1.42
7e		6.19 ± 0.34
7f		13.51 ± 0.54
7g		NA[a]
7h		NA[a]
7i		NA[a]
7j		NA[a]
7k		NA[a]
7l		NA[a]
7m		NA[a]
C_{10}-HSL		0.66 ± 0.07

[a] NA means no significant inhibition

novel quorum sensing inhibitors. Considering the SAR studies derived from these synthesized compounds suggested that the introduction of 4-aminobenzenesulfonyl moiety between the homoserine lactone nucleus and acyl side chain of CL and C_6-HSL contributed to the enhancement of inhibitory activity against violacein production. The introduction of thiophene group at the R position provided better inhibitory activity relate to the furan, pyrrole, pyridyl, and phenethyl group, as exemplified by the comparison of compound **7a** and **7g**, **7j**, **7k**, **7l**. Thiophene substituted compounds which connected electron withdrawing group exhibited better inhibitory activity relate to those connected electron donating group, as the example of the comparison of **7e** and **7d**. Further analysis indicated that compound bearing an electron withdrawing substituent at the position 2 of their thiophene ring exhibited superior activity against violacein production to those bearing the substituent at the position 3 and 4. Compound **7e** containing position 2 substituted thiophene group at the R position exhibited the highest level of inhibitory activity against violacein production of all of the analogs.

Molecular modeling

A molecular docking study was conducted with the CviR structure (PDB entry 3QP1), which C_6-HSL and compound **7e** were docked into the structure using molecular operating environment (MOE) 2014.09. C_6-HSL could bind to the CviR from bacteria *C. violaceum* to regulate the production of violacein as AI. As shown in Fig. 4, compound **7e** occupied the space to that of the natural ligand C_6-HSL. Both of them bound to CviR through hydrogen bonding interactions with the Asp 97 and Trp 84 residues.

C_6-HSL and compound **7e** have been shown as pink and green stick models, respectively. The key amino acid residues which provided interactions with C_6-HSL and compound **7e** have been drawed as their respective chemical

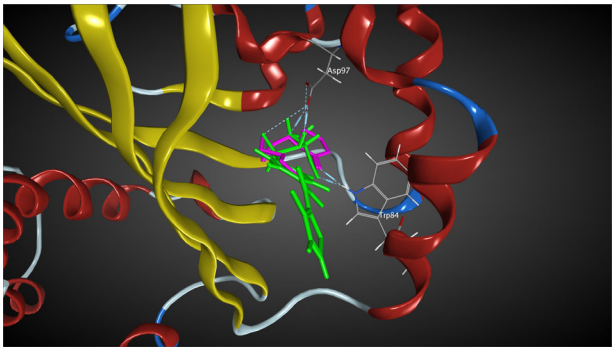


Fig. 4 Molecular model of CviR with C_6-HSL and compound **7e** bound to the active site

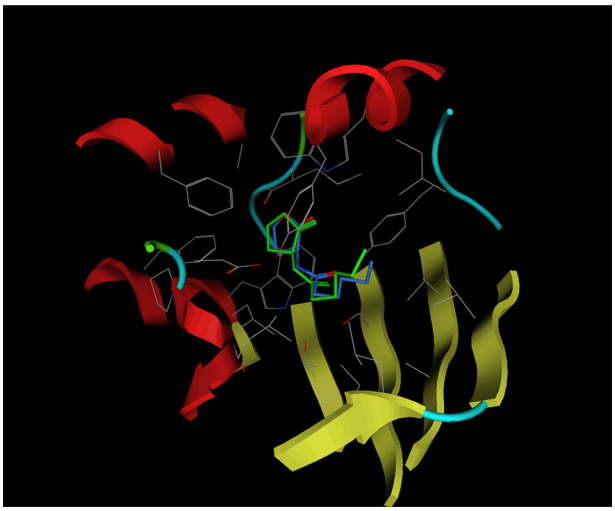


Fig. 5 Comparison of C_6-HSL molecular docking conformation and eutectic conformation

structures. *Cyan dotted lines* represented the hydrogen bonding interactions between the compounds and the amino acid residues (Trp 84 and Asp 97).

In order to validate the docking protocol,a self-docking of C_6-HSL into the binding pocket was firstly performed. As shown in Fig. 5, C_6-HSL docking pose stacked well with the crystallographic ligand (RMSD = 0.9429), and the H-bonds predicted by MOE were similar to those found in the crystal structure.

Materials and methods

General procedures

The melting points of intermediates and target compounds were determined with a YRT-4 melting point detector (P.I. F. Tianjin University,Tianjin, China) and have been reported as uncorrected values. Optical rotations were measured with a Polar 3005 polarimeter (Optical Activity Ltd., Cambridgeshire, UK). The mass spectra were determined using an API-3000 mass spectrometer. The mass spectra were determined using an Agilent 5875 (EI) spectrometer (Palo Alto, CA, USA). The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded with a Bruker ARX 400 spectrometer (Karlsruhe, Germany), using TMS as a reference material and DMSO as solvent. Thin—layer chromatography silica gel GF254 (Qingdao Marine Chemical Products) was used to monitor the extent of reaction. All solvents and reagents were purchased from commercial sources and used without further purification.

Synthesis of compound 2–4

*(S)-3-aminodihydrofuran-2(3H)-one hydrobromide (**2**)*

Compound **2** was synthesized with a well-established literature procedure (Nielsen and Givskov 2003). It was obtained as a white solid;Yield 55%; mp 220–224 °C; $[\alpha]_D^{25} = -24.2°$ ($c = 0.1$, H_2O); ^{1}H-NMR (400 Hz, DMSO-d_6) δ: 8.76 (s, 3H), 4.46 (t, 1H, $J = 8.0$ Hz,$J = 7.6$ Hz), 4.35–4.31 (m,2H), 2.56–2.51 (m,1H), 2.30–2.27 (m,1H); EI-MS *m/z*: 102.1 $[M + H]^+$.

*(S)-N-(4-(N-(2-oxotetrahydrofuran-3-yl)sulfamoyl)phenyl) acetamide (**3**)*

L-homoserine lactone hydrobromide **2** (10.9 g, 60 mmol) and triethylamine (25.5 mL, 120 mmol) were dissolved in ethanol (120 mL). 4-acetylaminobenzenesulfonyl chloride (16.8 g, 72 mmol) was added at 0 °C in a portion-wise manner. The resulting mixture was stirred overnight at ambient temperature. The next day, the reaction solution was concentrated to 1/3 volume and poured into ice-cold water (200 mL) with vigorous stirring for 1 h. Then the white solid precipitate was collected by filtration. The filter cake was washed with ice water and dried under vacuum before being recrystallized from ethaol to give white solid **3**. (12 g, 67%); mp 174–176 °C; ^{1}H-NMR (400 Hz, DMSO-d_6) δ: 10.33 (s, 1H), 8.16 (d, 1H, $J = 8.0$ Hz), 7.75 (s, 4H), 4.34–4.30 (m, 1H), 4.21 (m, 1H), 4.08 (m, 1H), 2.08–2.06 (m, 4H), 1.80(m, 1H); EI-MS *m/z*: 299.34 $[M + H]^+$.

*(S)-4-amino-N-(2-oxotetrahydrofuran-3-yl) benzenesulfonamide (**4**)*

6 mol/L HCl (20 mL) was added with stirring to a solution of intermediate 3(10 g, 33 mmol) in ethanol (40 mL). After heating at relux for 4 h, the reaction mixture was then cooled and the solvent was evaporated to dryness in vacuo to give the crude product, which was dissolved in water (100 mL). The pH of the solution was adjusted to 8–9 with 1 mol/L NH_4OH. After stirring for 1 h, the resulting white

solid was filtrated under vacuum. The white solid was collected by filtration. Then the filter cake was washed with ice-cold water and dried under vacuum before being recrystallied from ethaol to give product **4** (5.2 g, 57%); mp 163–165 °C; ^{1}H-NMR (400 Hz, DMSO-d_6) δ: 7.72 (d, 1H, $J = 9.2$ Hz), 7.45 (d, 2H, $J = 8.4$ Hz), 6.61 (d, 2H, $J = 8.8$ Hz), 5.95 (m, 2H), 4.21 (m, 2H), 4.09 (m, 1H), 2.06(m, 1H), 1.78 (m, 1H); EI-MS *m/z*: 256.4 $[M + H]^+$.

Synthesis of different aromatic chain substituted chloride 6

A five or six membered aromatic carboxylic acid **5** was dissolved in CH_2Cl_2 with dropping three drop DMF. 1.5 times the amount of oxalylchloride was cautiously added into the reaction solution at 0 °C. After stirring at ambient temperature for 3 h, the reaction mixture was concentrated under reduced pressure to give the corresponding acid chloride intermediate **6**. The remaining acid chloride were synthesized according to the method described above with a yield of 83–92%.

General procedure for the preparation of compound 7

*(*S*)-*N*-(4-(*N*-(2-oxotetrahydrofuran-3-yl)sulfamoyl)phenyl) thiophene-2-carboxamide(**7a**)*

Thiophene-2-carbonyl chloride (0.36 g, 2.44 mmol) was cautiously added into a solution of compound **4** (0.52 g, 2.03 mmol) and triethylamine (0.41 g, 4.06 mmol) in dry CH_2Cl_2 (15 mL) at 0 °C in a portion-wise manner, and the reaction system was stirred at ambient temperature overnight. The next day, the reaction mixture was filtrated under reduced pressure before the filtrate was washed with water. Then the yellow solid was precipitated from the organic phase and was filtrated under *vacuum* to provide the desired product **7a** (0.44 g, 60%); mp 114–119 °C; ^{1}H-NMR (400 Hz, DMSO-d_6) δ: 10.57 (s, 1H), 8.25 (1H, d, $J = 8.0$ Hz), 8.22 (d, 1H, $J = 8.0$ Hz), 8.07 (d, 1H, $J = 8.0$ Hz), 7.93 (m, 3H), 7.82 (d, 2H, $J = 8.0$ Hz), 7.26 (d, 1H, $J = 8.0$ Hz), 4.37 (m, 1H), 4.23 (m, 1H), 4.11 (m, 1 H), 2.14 (m, 1H), 1.84 (m, 1H); ^{13}C-NMR (DMSO-d_6) δ: 174.6(C-1′), 160.3 (C = O), 142.5(C-4), 139.4(C-1″), 135.7(C-1), 132.7(C-4″), 129.9(C-2″), 128.3(C-3″), 127.6(C-2,C-6) 119.9(C-3, C-5), 65.2(C-4′), 51.4(C-2′), 29.5(C-3′); EI-MS *m/z*: 366.9 $[M + H]^+$.

*(*S*)-5-chloro-*N*-(4-(*N*-(2-oxotetrahydrofuran-3-yl) sulfamoyl)phenyl)thiophene-2-carboxamide (**7b**)*

Compound **7b** was obtained as a yellow solid (0.45 g, 55% yield) from compound **4** according to the same procedure used for the synthesis of **7a**. mp 243–249 °C; ^{1}H-NMR (400 Hz, DMSO-d_6) δ: 10.64 (s, 1H), 8.21 (d, 1H, $J = 8.0$ Hz), 7.94 (m, 3H), 7.82 (d, 2H, $J = 8.0$ Hz), 7.32 (s, 1H), 4.37 (m, 1H), 4.22 (m, 1H), 4.10 (m, 1H), 2.14 (m, 1H), 1.83 (m, 1H); ^{13}C-NMR (DMSO-d_6) δ: 174.6(C-1′), 159.3(C = O), 142.2(C-4), 138.5(C-4″), 135.9(C-1), 134.7 (C-1″), 130.0(C-2″), 128.5(C-3″), 127.7(C-2,C-6), 119.9 (C-3,C-5), 65.2(C-4′), 51.4(C-2′), 29.5(C-3′); EI-MS *m/z*: 401.1 $[M + H]^+$.

*(*S*)-4-methyl-*N*-(4-(*N*-(2-oxotetrahydrofuran-3-yl) sulfamoyl)phenyl)thiophene-2-carboxamide (**7c**)*

Compound **7c** was obtained as a pale yellow solid (0.38 g, 50% yield) from compound **4** according to the same procedure used for the synthesis of **7a**. mp 187–188 °C; ^{1}H-NMR (400 Hz, DMSO-d_6) δ: 10.50 (s, 1H), 8.23 (d, 1H, $J = 8.0$ Hz), 7.94 (m, 3H), 7.81 (d, 2H, $J = 8.0$ Hz), 7.51 (d, 1H, $J = 8.0$ Hz), 4.37 (m, 1H), 4.23 (m, 1H), 4.11 (m, 1H), 2.14 (m, 1H), 1.83 (m, 1H); ^{13}C-NMR (DMSO-d_6) δ: 174.6 (C-1′), 160.3(C = O), 142.6(C-4), 138.9(C-1″), 138.2(C-4″), 135.6(C-1), 131.7(C-2″), 128.1(C-3″), 127.6(C-2,C-6), 119.7(C-3,C-5), 65.2(C-4′), 51.4(C-2′), 29.5(C-3′), 15.5(C-5″); EI-MS *m/z*: 381.1$[M + H]^+$.

*(*S*)-3-methyl-*N*-(4-(*N*-(2-oxotetrahydrofuran-3-yl) sulfamoyl)phenyl)thiophene-2-carboxamide (**7d**)*

Compound **7d** was obtained as a white solid (0.40 g, 53% yield) from compound **4** according to the same procedure used for the synthesis of **7a**. mp 176–178 °C; ^{1}H-NMR (400 Hz, DMSO-d_6) δ: 10.36 (s, 1H), 8.23 (d, 1H, $J = 8.0$ Hz), 7.88 (d, 2H, $J = 8.0$ Hz), 7.79 (d, 2H, $J = 8.0$ Hz), 7.72 (m, 1H), 7.06 (d, 1H, $J = 8.0$ Hz), 4.36 (m, 1H), 4.23 (m, 1H), 4.11 (m, 1H), 2.14 (m, 1H), 1.83 (m, 1H); ^{13}C-NMR (DMSO-d_6) δ: 174.6(C-1′), 161.8(C = O), 142.7(C-4), 141.6(C-1″), 135.6(C-1), 131.6(C-4″), 130.9(C-2″), 128.5 (C-3″), 127.6(C-2,C-6), 119.9(C-3,C-5), 65.2(C-4′), 51.4(C-2′), 29.5(C-3′), 15.5(C-5″); EI-MS *m/z*: 381.0$[M + H]^+$.

*(*S*)-3-chloro-*N*-(4-(*N*-(2-oxotetrahydrofuran-3-yl) sulfamoyl)phenyl)thiophene-2-carboxamide (**7e**)*

Compound **7e** was obtained as a white solid (0.42 g, 52% yield) from compound **4** according to the same procedure used for the synthesis of **7a**. mp 219–221 °C; ^{1}H-NMR (400 Hz, DMSO-d_6) δ: 10.63 (s, 1H), 8.23 (d, 1H, $J = 8.0$ Hz), 7.96 (m, 1H),7.87 (d, 2H, $J = 8.0$ Hz), 7.82 (d, 2H, $J = 8.0$ Hz), 7.24(d, 1H, $J = 8.0$ Hz), 4.37 (m, 1H), 4.23 (m, 1H), 4.11 (m, 1H), 2.13 (m, 1H), 1.83 (m, 1H); ^{13}C-NMR (DMSO-d_6) δ: 174.6(C-1′), 160.1(C = O), 142.7(C-4), 136.1(C-1), 134.7(C-1″), 132.6(C-2″), 130.5(C-4″), 127.6 (C-2,C-6),119.7(C-3,C-5), 110.7(C-3″), 65.2(C-4′), 51.4(C-2′), 29.5(C-3′); EI-MS *m/z*: 401.3$[M + H]^+$.

*(S)-3-bromo-*N*-(4-(*N*-(2-oxotetrahydrofuran-3-yl) sulfamoyl)phenyl)thiophene-2-carboxamide (**7 f**)*

Compound **7 f** was obtained as a yellow solid (0.52 g, 58% yield) from compound **4** according to the same procedure used for the synthesis of **7a**. mp 151–154 °C; ^{1}H-NMR (400 Hz, DMSO-d_6) δ: 10.71 (s, 1H), 8.24 (d, 1H, $J = 8.0$ Hz), 7.92 (m, 1H),7.87 (d, 2H, $J = 8.0$ Hz), 7.83 (d, 2H, $J = 8.0$ Hz), 7.27 (d, 1H, $J = 8.0$ Hz), 4.37 (m, 1H), 4.23 (m, 1H), 4.11 (m, 1H), 2.13 (m, 1H), 1.83 (m, 1H); ^{13}C-NMR (DMSO-d_6) δ: 174.6(C-1′), 159.8(C = O), 142.1 (C-4), 136.2(C-1), 132.8(C-1″), 131.5(C-2″), 130.5(C-4″), 127.7(C-2,C-6), 119.7(C-3,C-5), 111.7(C-3″), 65.2(C-4′), 51.4(C-2′), 29.5(C-3′); EI-MS *m/z*: 444.9$[M + H]^+$.

*(S)-*N*-(4-(*N*-(2-oxotetrahydrofuran-3-yl)sulfamoyl)phenyl) furan-2-carboxamide (**7 g**)*

Compound **7 g** was obtained as a yellow solid (0.46 g, 65% yield) from compound **4** according to the same procedure used for the synthesis of **7a**. mp 195–196 °C;^{1}H-NMR (400 Hz, DMSO-d_6) δ: 10.49 (s, 1H), 8.18 (d, 1H, $J = 8.0$ Hz), 7.96 (m, 3H),7.82 (d, 2H, $J = 8.0$ Hz), 7.40 (d, 1H, $J = 8.0$ Hz), 6.72(d, 1H, $J = 8.0$ Hz), 4.36 (m, 1H), 4.23 (m, 1H), 4.11 (m, 1H), 2.17 (m, 1H), 1.86 (m, 1H); ^{13}C-NMR (DMSO-d_6) δ: 174.6(C-1′), 156.5(C = O), 147.0(C-1″), 146.3(C-4″), 142.4(C-4), 135.7(C-1), 127.6(C-2,C-6), 119.9 (C-3,C-5), 115.7(C-2″), 112.4(C-3″), 65.2(C-4′), 51.4(C-2′), 29.5(C-3′); EI-MS *m/z*: 351.1$[M + H]^+$.

*(S)-*N*-(4-(*N*-(2-oxotetrahydrofuran-3-yl)sulfamoyl)phenyl) furan-3-carboxamide (**7 h**)*

Compound **7 h** was obtained as a pale yellow solid (0.40 g, 58% yield) from compound **4** according to the same procedure used for the synthesis of **7a**. mp 202–209 °C; ^{1}H-NMR (400 Hz, DMSO-d_6) δ: 10.28 (s, 1H), 8.44 (s, 1H), 8.21 (d, 1H, $J = 8.0$ Hz), 7.92 (m, 5 H), 7.02 (d, 1H, $J = 8.0$ Hz), 4.36 (m, 1H), 4.23 (m, 1H), 4.10 (m, 1H), 2.13 (m, 1H), 1.82 (m, 1H); ^{13}C-NMR (DMSO-d_6) δ: 174.6(C-1′), 160.9(C = O), 146.4(C-4″), 144.5(C-3″), 142.7(C-4), 135.5 (C-1), 127.6(C-2,C-6), 122.7(C-1″), 119.7(C-3,C-5), 109.3 (C-2″), 65.2(C-4′), 51.4(C-2′), 29.5(C-3′); EI-MS *m/z*: 351.1 $[M + H]^+$.

*(S)-5-bromo-*N*-(4-(*N*-(2-oxotetrahydrofuran-3-yl) sulfamoyl)phenyl)furan-2-carboxamide (**7i**)*

Compound **7i** was obtained as a pale yellow solid (0.53 g, 61% yield) from compound **4** according to the same procedure used for the synthesis of **7a**. mp 190–196 °C; ^{1}H-NMR (400 Hz, DMSO-d_6) δ: 10.56 (s, 1H), 8.24 (d, 1H, $J = 8.0$ Hz), 7.94 (d, 2H, $J = 8.0$ Hz), 7.81 (d, 2H, $J = 8.0$ Hz), 7.45 (d, 1H, $J = 8.0$ Hz), 6.87 (d, 1H, $J = 8.0$ Hz), 4.36 (m, 1H), 4.23 (m, 1H), 4.10 (m, 1H), 2.13 (m, 1H), 1.83 (m, 1H); ^{13}C-NMR (DMSO-d_6) δ: 174.6(C-1′), 155.4(C = O), 148.8(C-4″), 142.1(C-4), 135.9(C-1), 127.6(C-2,C-6), 126.1 (C-1″), 119.9(C-3,C-5), 118.1(C-2″), 114.5(C-3″), 65.2(C-4′), 51.4(C-2′), 29.5(C-3′); EI-MS *m/z*: 453.1$[M + Na]^+$.

(S)- N*-(4-(*N*-(2-oxotetrahydrofuran-3-yl)sulfamoyl)phenyl) -*1H*-pyrrole-2-carboxamide (**7j**)*

Compound **7j** was obtained as a pale yellow solid (0.34 g, 50% yield) from compound **4** according to the same procedure used for the synthesis of **7a**. mp 170–174 °C; ^{1}H-NMR (400 Hz, DMSO-d_6) δ: 11.79 (d, 1H, $J = 8.0$ Hz), 10.10 (s, 1H),8.17 (d, 1H, $J = 8.0$ Hz), 7.95 (d, 2H, $J = 8.0$ Hz), 7.80 (d, 2H, $J = 8.0$ Hz), 7.12 (d, 1H, $J = 8.0$ Hz), 7.02 (d, 1H, $J = 8.0$ Hz), 6.20 (d, 1H, $J = 8.0$ Hz), 4.36 (m, 1H), 4.23 (m, 1H), 4.11 (m, 1H), 2.13 (m, 1H), 1.83 (m, 1H); ^{13}C-NMR (DMSO-d_6) δ: 174.6(C-1′), 161.3(C = O), 142.3 (C-4), 135.6(C-1), 127.6(C-2,C-6), 123.7(C-1″), 120.3(C-4″), 119.7(C-3,C-5), 110.5(C-3″), 65.2(C-4′), 51.4(C-2′), 29.5(C-3′); EI-MS *m/z*: 365.0$[M + H]^+$.

*(S)-*N*-(4-(*N*-(2-oxotetrahydrofuran-3-yl)sulfamoyl)phenyl) isonicotinamide (**7k**)*

Compound **7k** was obtained as a white solid (0.32 g, 45% yield) from compound **4** according to the same procedure used for the synthesis of **7a**. mp 174–185 °C; ^{1}H-NMR (400 Hz, DMSO-d_6) δ: 10.86 (s, 1H), 8.81 (m, 2H), 8.23 (d, 1H, $J = 8.0$ Hz), 7.97 (d, 2H, $J = 8.0$ Hz), 7.86 (m, 4H), 4.36 (m, 1H), 4.23 (m, 1H), 4.11 (m, 1H), 2.14 (m, 1H), 1.83 (m, 1H); ^{13}C-NMR (DMSO-d_6) δ: 174.6(C-1′), 164.7 (C = O), 150.4(C-3″,C-4″), 142.3(C-4), 151.6(C-1″), 136.2 (C-1), 127.6(C-2,C-6), 121.7(C-2″,C-5″), 120.12(C-3, C-5), 65.2(C-4′), 51.4(C-2′), 29.5(C-3′); EI-MS *m/z*: 362.1 $[M + H]^+$.

*(S)-*N*-(4-(*N*-(2-oxotetrahydrofuran-3-yl)sulfamoyl)phenyl)-2-(*o*-tolyl)acetamide (**7l**)*

Compound **7 l** was obtained as a white solid (0.36 g, 46% yield) from compound **4** according to the same procedure used for the synthesis of **7a**. mp 191–193 °C; ^{1}H-NMR (400 Hz, DMSO-d_6) δ: 10.56 (s, 1H), 8.17 (d, 1H, $J = 8.0$ Hz), 7.78 (m, 4H), 7.16 (m, 4H), 4.35 (m, 1H), 4.21 (m, 1H), 4.08 (m, 1H), 3.73 (s, 2H), 2.10 (m, 1H), 1.81 (m, 1H); ^{13}C-NMR (DMSO-d_6) δ: 174.6(C-1′), 169.8(C = O), 142.9 (C-4), 136.8(C-1), 135.2(C-2″), 134.3(C-3″), 130.1(C-4″), 130.0(C-7″), 127.8(C-2,C-6), 126.9(C-5″), 125.9(C-6″), 118.8(C-3,C-5), 65.2(C-4′), 51.4(C-2′), 41.0(C-1″), 29.5(C-3′), 19.5(C-8″); EI-MS *m/z*: 389.2$[M + H]^+$.

(S)-2-(2-chlorophenyl)-N-*(4-(*N-*(2-oxotetrahydrofuran-3-yl)sulfamoyl)phenyl)acetamide (**7m**)*

Compound **7 m** was obtained as a white solid (0.43 g, 52% yield) from compound **4** according to the same procedure used for the synthesis of **7a**. mp 223–228 °C; ^{1}H-NMR (400 Hz, DMSO-d_6) δ: 10.64 (s, 1H), 8.18 (d, 1H, $J = 8.0$ Hz), 7.78 (m, 4H), 7.44 (m, 4H), 4.33 (m, 1H), 4.21 (m, 1H), 4.08 (m, 1H), 3.89 (s, 2H), 2.11 (m, 1H), 1.81 (m, 1H); ^{13}C-NMR (DMSO-d_6) δ: 174.6(C-1′), 168.7(C = O), 142.8 (C-4), 135.2(C-1), 133.8(C-3”), 133.6(C-2”), 132.4(C-7”), 129.1(C-4”), 128.8(C-5”), 127.8(C-2,C-6), 127.2(C-6”), 118.7(C-3,C-5), 65.2(C-4′), 51.4(C-2′), 40.9(C-1”), 29.5(C-3′); EI-MS *m/z*: 409.2$[M + H]^+$.

Evaluation of the biological activity

Through the primary screening of the synthesized compounds, the inhibitory activities against violacein production were determined according to a spot test method with compound C_{10}-HSL being used as a positive control. Specifically, CV026 (400 μL) was cultured in molten semi-solid medium. The phosphate buffered solution (PBS) solution of C_6-HSL (15 μL, 0.125 mM) was then added and the mixture was uniformly mixed with the semi-solid LB agar (5 mL). The resulting mixture was subsequently dumped on the surface of the solid LB agar. Upon solidification of the mixture plate, the test compounds were spotted on the plates. The plates were then placed in an oven and being cultured for 16–18 h at 30℃. The inhibitory activities of the compounds towards violacein production were determined by the presence of white colonies in purple background of the plates. If the transparent growth inhibition zone were observed, it would suggest that the compounds had the growth-inhibitory effect.

For the further screening of the compounds which inhibited violacein production, CV026 were cultured to log phase in LB medium and then placed in 12-well plates with the LB medium. The PBS solution of C_6-HSL (15 μL, 0.125 mM) and different concentrations of the compounds which showed inhibitory activities in the primary screening were added to the wells. After an incubation period of 16–18 h, a portion (1 mL) of cultures was removed from each mixture to Eppendorf tube at 12,470 × g for 10 min to precipitate the insoluble violacein and the culture supernatant was discarded. Following DMSO (500 μL) was added to each Eppendorf tube to completely dissolve the violacein, the resulting mixtures were centrifuged at 12,470 × g for 10 min to remove cells. The upper layer of the above violacein solution (200 μL) was then removed and placed into a 96-well microplate, and its absorbance value at 585 nm was measured(Olivero et al. 2011). The final results were expressed as IC_{50} (half maximum inhibitory concentration) and were taken to be average of three determinations. All the IC_{50} values were calculated with the Origin 8.0 software.

Conclusions

A novel series of *N*-sulfonyl homoserine lactone derivatives **7a–7m** has been designed, synthesized and evaluated for quorum sensing inhibitory activities against violacein production. Preliminary SAR studies indicated that compounds with thiophene groups at the R position showed better inhibitory activity than those substituted by furan, pyrrole, pyridyl and phenethyl groups at the same position. Thiophene substituted compounds which connected electron withdrawing substituent displayed better levels of inhibitory activity relate to those connected electron donating substituent. Of the compounds synthesized, compound **7e** exhibited promising levels of inhibitory activity towards violacein production, with IC_{50} values of 6.19 μM and are currently being identified as promising lead compound for further structural transformation studies.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no competing interests.

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