Supporting Information

Synthesis and evaluation of indole-based autoinducers on quorum sensing in *Vibrio cholerae*

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General information

All reactions were performed under open air unless mentioned otherwise. All reagents and solvents were purchased from commercial sources and used without further purification.

Flash column chromatography was performed using silica gel, 60-Å pore size, 40-63 μm, standard grade. All of the compounds were identified by ^{1}H-NMR and ^{13}C-NMR and showed purity of >95%.

All of the biological experiment were performed in triplicates at least (biofilm formation in quintuplicates at least). T-Tests were performed for cholera toxin production and biofilm formation.

V. cholerae strains that were used in the experiments were generously provided by Bonnie L. Bassler.

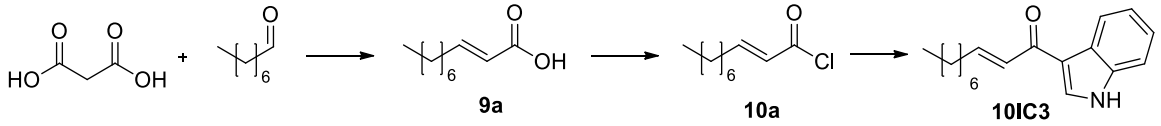
MM920 - *V. cholerae* double mutant reporter strain *ΔcqsAΔluxPQ* - unable to produce CAI-1 and does not have LuxPQ receptor for CAI-1 detection, coupled with *luxCDABE* operon, resistant to tetracycline.

MM825 - *V. cholerae* double mutant reporter strain *ΔcqsSΔluxPQ* -does not have the CqsS receptor for detection of CAI-1 and LuxPQ receptor for detection of AI-2, and is thus used as a control strain to examine interaction with the CqsS receptor. The strain contains the *luxCDABE* operon and is resistant to tetracycline.

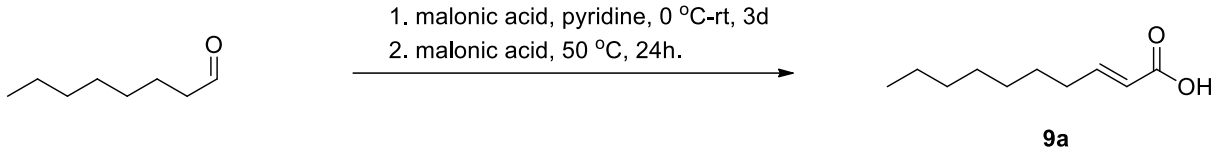
VC1 – *V. cholerae* wild type

BB120 – *V. harveyi* wild type

Synthesis of 10IC3:



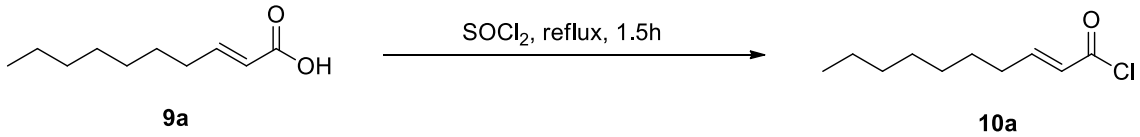
Procedure for synthesis of (E)-dec-2-enoic acid (**9a**):



Pyridine (19.5 mmol, 2.5 eq) was slowly added to malonic acid (8.6 mmol, 1.1 eq) and the solution was cooled to 0 ºC. Right afterwards octanal (7.8 mmol, 1 eq) was added and the solution was stirred at room temperature for 3 days. More of the malonic acid (2.3 mmol, 0.3 eq) was added and the reaction was heated to 50 ºC for 24 h. The reaction was quenched with HCl 2M, extracted with DCM, washed with brine, dried with $MgSO_4$ and concentrated under reduced pressure. The product was purified by silica gel chromatography (20% ethyl acetate in hexane) to give a yellow oil.

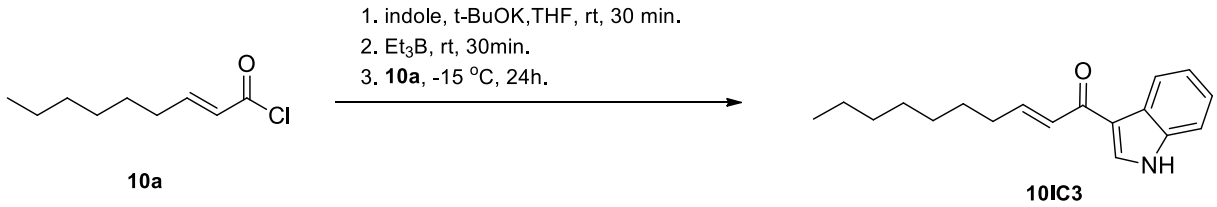
9a: Yellow oil, 85% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.09 (dt, *J* = 15.4, 7.0 Hz, 1H), 5.82 (dt, *J* = 15.6, 1.3 Hz, 1H), 2.30 – 2.15 (m, 2H), 1.46 (m, 2H), 1.29 (m, 8H), 0.88 (t, J = 6.8 Hz, 3H). **^{13}C NMR** (101 MHz, CDCl3) δ 172.39, 152.21, 120.77, 32.25, 31.74, 29.13, 29.06, 27.87, 22.60, 13.94.

Procedure for synthesis of (E)-dec-2-enoyl chloride (**10a**):



(E)-dec-2-enoic acid (1.53 mmol, 1 eq) and thionyl chloride (31 mmol, 20 eq) were refluxed for 1.5h. The product was concentrated under reduced pressure and was used without further purification.

Procedure for synthesis of (E)-1-(1H-indol-3-yl) dec-2-en-1-one (**10IC3**):

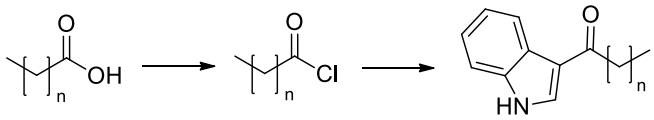


To a solution of indole (1.1 mmol, 1 eq) in THF under N_2 atmosphere was added t-BuOK (1.2 mmol, 1.1 eq) and the mixture was stirred for 30 min at room temperature. Et_3B (1.2 mmol, 1.1 eq) was added and the reaction mixture was stirred for an additional 30 min. The solution was cooled to at -15 ºC and

freshly prepared (E)-dec-2-enoyl chloride (**10a**) (1.2 mmol, 1.1 eq) was added dropwise and the mixture was stirred at -15 ºC for 24h, quenched with NH_4Cl and diluted with water; the organic phase was extracted with EtOAc, dried with Na_2SO_4, concentrated and purified by HPLC 20%-90% gradient ACN in water to give brown solid.

10IC3a: Brown solid, 56% yield: **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.47 (d, J = 5.1 Hz, 1H), 7.90 (s, 1H), 7.47 – 7.37 (m, 1H), 7.33 – 7.27 (m, 2H), 7.12 – 6.97 (dd, J = 15.4 Hz, J = 8 Hz, 1H), 6.77 (d, J = 15.2 Hz, 1H), 2.30 (d, J = 7.2 Hz, 2H), 1.54 – 1.51 (m, 2H), 1.31 – 1.29 (m, 8H), 0.89 (t, J = 5.9 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 186.17, 146.32, 136.69, 132.03, 127.18, 126.04, 123.80, 122.64, 122.55, 118.33, 111.72, 32.65, 31.80, 29.73, 29.28, 29.15, 28.45, 22.68, 14.13.

Synthesis of 6-10IC3a

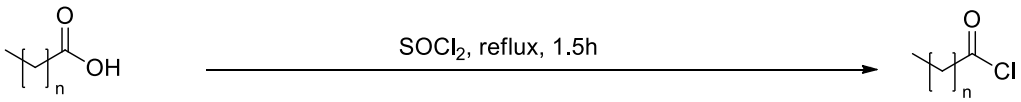


4a-e

4a: n=4
4b: n=5
4c: n=6
4d: n=7
4e: n=8

6-10IC3

6IC3a: n=4
7IC3a: n=5
8IC3a: n=6
9IC3a: n=7
10IC3a: n=8



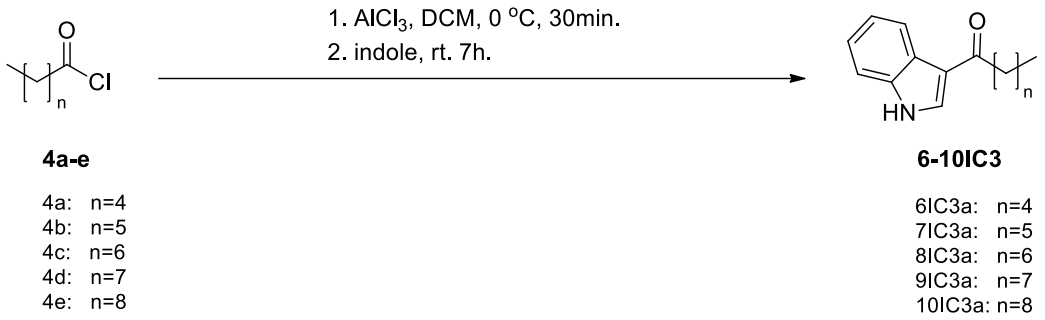
4a-e

4a: n=4
4b: n=5
4c: n=6
4d: n=7
4e: n=8

<u>General procedure for synthesis of acyl chlorides (**4a-e**):</u>

The corresponding carboxylic acid (5.8 mmol, 1 eq) and thionyl chloride (120 mmol, 20 eq) were refluxed for 1.5h. The product was concentrated under reduced pressure and was used without further purification.

General procedure for synthesis of 1-(1H-indol-3-yl) alka-1-one (**6-10IC3a**):



$AlCl_3$ (5.13 mmol, 0.66 eq) and freshly prepared corresponding acyl chloride (7.7 mmol, 1 eq) were suspended in precooled to 0ºC DCM and the mixture was stirred at 0 ºC for 30 min. Indole (2.6 mmol, 0.33 eq) was added to the mixture and stirred for 7h at room temperature. The reaction was diluted with DCM and quenched dropwise with water, the organic phase washed with brine, dried over Na_2SO_4 and concentrated. The product purified by silica gel chromatography (30% ethyl acetate in hexane) to give white solid.

6IC3a: White solid, 88% yield. **^{1}H NMR** (400 MHz, Acetone) δ 8.37 – 8.33 (m, 1H), 8.22 (d, *J* = 2.8 Hz, 1H), 7.49 (d, *J* = 7.0 Hz, 1H), 7.23 – 7.18 (m, 2H), 2.87 (t, *J* = 7.4 Hz, 3H), 2.06 – 2.04 (m, 2H), 1.76 – 1.70 (m, 2H), 1.38 – 1.34 (m, 5H), 0.89 (t, *J* = 6.7 Hz, 4H). **^{13}C NMR** (101 MHz, Acetone) δ 195.25, 137.00, 132.48, 125.98, 122.85, 121.63, 117.36, 111.74, 39.05, 31.56, 24.80, 22.39, 13.42.

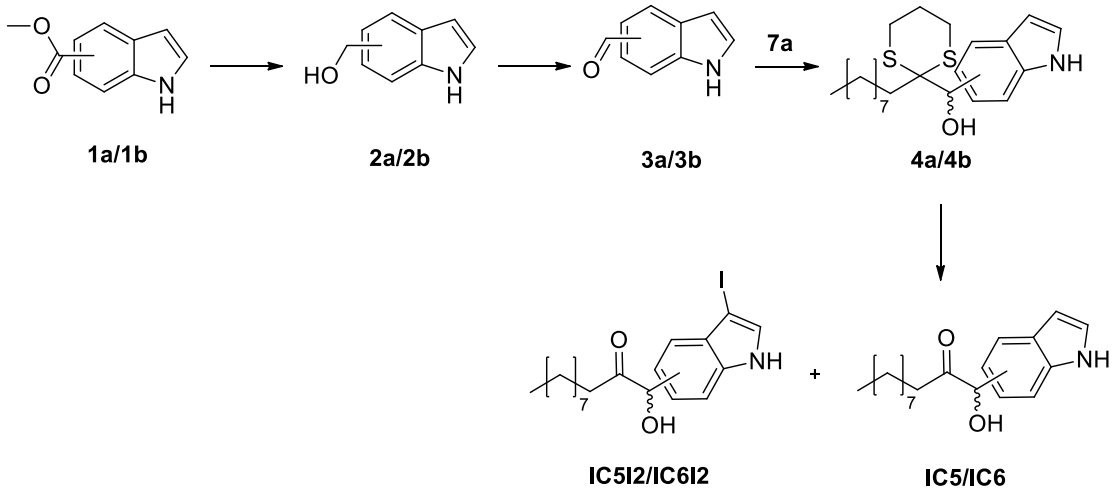
7IC3a: White solid, 76% yield. **^{1}H NMR** (400 MHz, Acetone) δ 8.35 (d, J = 6.4 Hz, 1H), 8.23 (d, J = 2.3 Hz, 1H), 7.50 – 7.48 (m, 1H), 7.21 – 7.19 (m, 2H), 2.87 (t, J = 7.4 Hz, 2H), 1.78 – 1.67 (m, 2H), 1.46 – 1.25 (m, 7H), 0.89 (d, J = 6.2 Hz, 3H). **^{13}C NMR** (101 MHz, Acetone) δ 195.22, 137.00, 132.47, 125.99, 122.84, 122.00, 121.63, 117.36, 111.74, 39.11, 31.66, 25.07, 22.38, 13.47.

8IC3a: White solid, 70% yield. **^{1}H NMR** (400 MHz, Acetone) δ 8.35 (dd, J = 7.6, 1.6 Hz, 1H), 8.23 (d, J = 3.1 Hz, 1H), 7.51 – 7.48 (m, 1H), 7.22 – 7.18 (m, 2H), 2.90 – 2.83 (t, J = 7.4, 2H), 1.73 – 1.71 (m, 2H),1.39 - 1.36 (m, 3H), 1.31 – 1.28 (m, 3H), 0.88 (t, J =6.7 Hz, 3H). **^{13}C NMR** (101 MHz, Acetone) δ 195.72, 137.51, 132.96, 126.50, 123.37, 122.53, 122.15, 117.90, 112.25, 39.62, 32.19, 25.63, 22.94, 13.99.

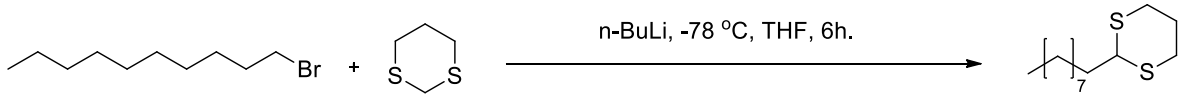
9IC3a: White solid, 91% yield. **^{1}H NMR** (400 MHz, Acetone) δ 8.38 – 8.30 (m, 1H), 8.22 (d, *J* = 2.8 Hz, 1H), 7.48 (d, *J* = 7.0 Hz, 1H), 7.23 – 7.15 (m, 2H), 2.86 (t, *J* = 7.4 Hz, 2H), 1.72 (d, *J* = 7.2 Hz, 2H), 1.36 (m, 9H), 0.88 (d, *J* = 6.6 Hz, 3H). **^{13}C NMR** (101 MHz, Acetone) δ 196.12, 137.87, 133.35, 133.18, 126.85, 123.72, 122.87, 122.51, 118.23, 112.62, 39.93, 32.43, 25.68, 23.26, 14.29.

10IC3a: White solid, 81% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.44 – 8.37 (m, 1H), 7.88 (d, J = 2.9 Hz, 1H), 7.48 – 7.37 (m, 1H), 7.29 (m, 2H), 2.92 – 2.81 (m, 2H), 1.77 (dd, J = 14.9, 7.4 Hz, 2H), 1.27 (m, 12H), 0.87 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 196.61, 136.27, 130.75, 125.52, 123.65, 122.60, 122.55, 118.32, 111.22, 40.04, 31.89, 29.58, 29.31, 25.19, 22.67, 14.12.

Synthesis of IC5, IC6, IC5I2, IC6I2:



Synthesis of 2-nonyl-1,3-dithiane (**7a**):

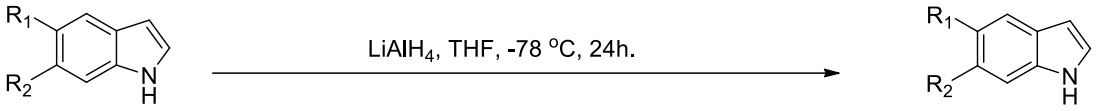


7a

1,3-dithiane (1.25 mmol, 1.3 eq) was suspended in freshly dried THF (0.5M) and cooled to -78 ºC. n-BuLi (1.25 mmol, 1.3 eq) was added dropwise and was stirred at -5 ºC for 1.5h, then cooled back to -78 ºC and bromononane (0.9 mmol, 1 eq) in THF (2M) was added dropwise. The mixture was stirred for 6h, quenched with NH_4Cl and extracted with EtOAc, dried over $MgSO_4$ and concentrated under reduced pressure. The product was purified by silica gel chromatography (20% toluene in hexane) to give colorless oil.

7a: Colorless oil, 82% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.04 (t, *J* = 6.9 Hz, 1H), 2.92 – 2.77 (m, 4H), 2.11 (d, *J* = 14.0 Hz, 1H), 1.85 (d, *J* = 14.0 Hz, 1H), 1.77 – 1.70 (m, 2H), 1.53 – 1.45 (m, 4H), 1.35 – 1.20 (m, 10H), 0.87 (t, *J* = 6.9 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 47.69, 35.47, 31.88, 30.52, 29.50, 29.39, 29.30, 29.24, 26.62, 26.08, 22.68, 14.13.

General procedure for synthesis of (1H-indole-5/6-yl) methanol (**2a-b**):



1a: R_1=$COOCH_3$, R_2=H
1b: R_1=H, R_2=$COOCH_3$

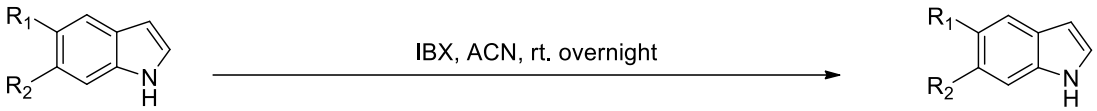
2a: R_1=CH_2OH, R_2=H
2b: R_1=H, R_2=CH_2OH

$LiAlH_4$ (3.4 mmol, 3 eq) was suspended in precooled to -78 ºC THF (2M) and methyl 1H-indole-5-carboxylate (1.14 mmol, 1 eq) in THF was added dropwise. The mixture was stirred for 24h allowed to rise to room temperature and was quenched with ice cold water. The product was extracted with EtOAc (x3), the combined organic layers dried over Na_2SO_4, concentrated and purified by silica gel chromatography (30% EtOAc in hexane) to give white solid.

2a: White solid, 64% yield. **^{1}H NMR** (400 MHz, MeOD) δ 7.54 (d, *J* = 0.7 Hz, 1H), 7.36 (d, *J* = 8.3 Hz, 1H), 7.20 (d, *J* = 3.1 Hz, 1H), 7.13 (d, *J* = 8.3 Hz, 1H), 6.42 (dd, *J* = 3.1, 0.7 Hz, 1H), 4.66 (s, 2H). **^{13}C NMR** (101 MHz, MeOD) δ 137.13, 132.90, 129.35, 125.92, 122.44, 120.31, 112.08, 102.39, 66.34.

2b: White solid, 64% yield. **^{1}H NMR** (400 MHz, MeOD) δ 7.51 (d, *J* = 8.1 Hz, 1H), 7.38 (s, 1H), 7.20 (d, *J* = 3.1 Hz, 1H), 7.02 (dd, *J* = 8.1, 1.4 Hz, 1H), 6.41 (dd, *J* = 3.1, 1.2 Hz, 1H), 4.68 (s, 2H). **^{13}C NMR** (101 MHz, MeOD) δ 137.61, 135.47, 128.81, 125.80, 121.09, 119.99, 111.04, 102.18, 66.17.

Synthesis of 1H-indole-5/6-carbaldehyde (**3a-b**):



2a: R_1=CH_2OH, R_2=H
2b: R_1=H, R_2=CH_2OH

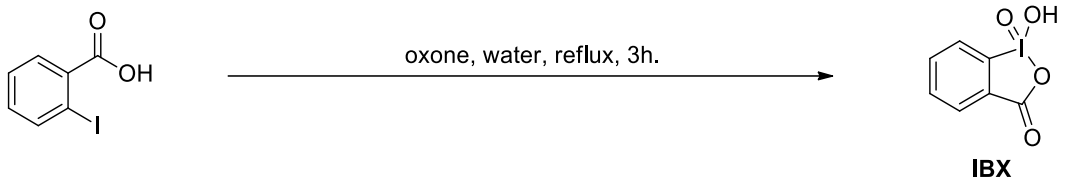
3a: R_1=CHO, R_2=H
3b: R_1=H, R_2=CHO

IBX (2 eq) was added to mixture of corresponding alcohol (1 eq) in ACN and was stirred under reflux overnight. The reaction was quenched with water and extracted with EtOAc. The combined organic phases were dried over Na_2SO_4, concentrated under reduced pressure and purified by silica gel chromatography (20% EtOAc in hexane) to red solid.

3a: Red solid, 49% yield. **^{1}H NMR** (400 MHz, MeOD) δ 9.92 (s, 1H), 8.16 (d, J = 0.9 Hz, 1H), 7.70 – 7.66 (d, J = 8.5 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H), 7.37 (d, J = 3.2 Hz, 1H), 6.65 (dd, J = 3.2, 0.7 Hz, 1H). **^{13}C NMR** (101 MHz, MeOD) δ 195.61, 142.14, 131.18, 130.06, 128.73, 128.35, 123.16, 113.73, 105.33.

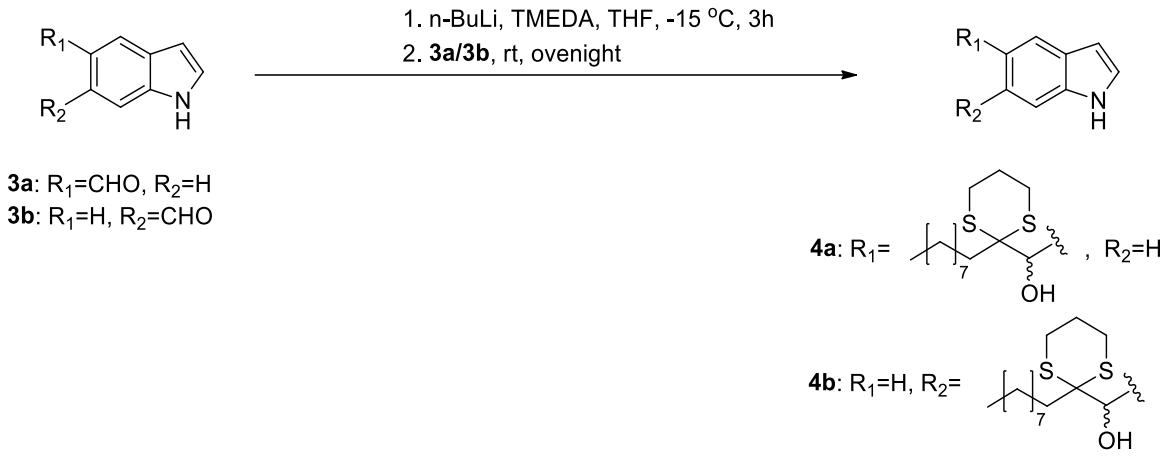
3b: Red solid, 45% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.04 (s, 1H), 8.52 (s, 1H), 8.19 (s, 1H), 7.88 – 7.72 (m, 1H), 7.49 (d, *J* = 8.5 Hz, 1H), 7.39 – 7.29 (m, 1H), 6.72 (s, 1H). **^{13}C NMR** (101 MHz, MeOD) δ 194.74, 137.05, 134.84, 131.89, 130.88, 121.63, 120.55, 116.38, 103.29.

Synthesis of **IBX**:



2-iodobenzoic acid (4 mmol, 1 eq) and oxone (12 mmol, 3 eq) was suspended in water (40 mL). The mixture was refluxed at 70 ºC for 2-3h and then stirred for 30 min at 0 ºC. The created white crystals were filtrated and washed with water and acetone. The product was ready to use without further purification.

IBX: White powder 60% yield. **^{1}H NMR** (400 MHz, DMSO) δ 8.11 (t, *J* = 9.4 Hz, 1H), 8.05 – 7.95 (m, 2H), 7.83 (t, *J* = 7.3 Hz, 1H). **^{13}C NMR** (101 MHz, DMSO) δ 167.53, 146.55, 133.43, 132.98, 131.44, 130.12, 125.02.



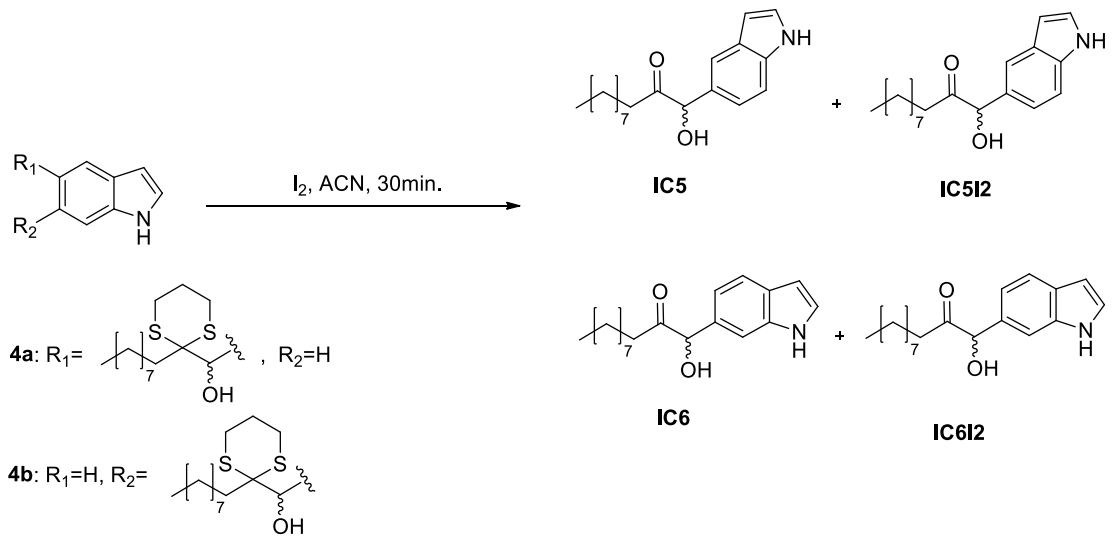
General procedure for synthesis of (1H-indol-5/6-yl) (2-nonyl-1,3-dithian-2-yl) methanol (**4a-b**):

n-BuLi (2.1 mmol, 1.5 eq) was added dropwise to a precooled to -78 ºC mixture of TMEDA (1.38 mmol, 1 eq) and 2-nonyl-1,3-dithiane (3.5 mmol, 2.5 eq) in THF (0.2M). The mixture was stirred at -15 ºC for 3h and after addition of 1H-indole-5-carbaldehyde (1.38 mmol, 1 eq) in THF (0.2M) the mixture was stirred overnight allowing it to rise to room temperature. The reaction was quenched with NH_4Cl, extracted with diethyl ether, dried over $MgSO_4$ and concentrated under reduced pressure. The product was purified by silica gel chromatography (20% EtOAc in hexane) to give white solid.

4a: White solid, 81% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.75 (s, 1H), 7.33 (s, 2H), 7.19 (s, 1H), 6.53 (s, 1H), 5.27 (s, 1H), 3.21 (t, J = 12.4 Hz, 1H), 3.02 (t, J = 12.7 Hz, 1H), 2.77 – 2.59 (m, 2H), 2.09 (s, 1H), 1.88 (s, 2H), 1.43 (m Hz, 1H), 1.21 (m, 14H), 0.85 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ138.11, 135.53, 129.02, 127.10, 124.30, 122.90, 109.67, 102.88, 74.66, 59.74, 34.81, 31.59, 30.06, 29.50, 29.25, 26.65, 25.50, 22.66, 14.12.

4b: White solid, 81% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.64 (d, J = 8.1 Hz, 1H), 7.33 (s, 1H), 7.25 (d, J = 8.1 Hz, 1H), 7.03 (d, J = 8.1 Hz, 1H), 6.55 (s, 1H), 5.17 (s, 1H), 4.42 (s, 1H), 2.34 (d, J = 11.6 Hz, 2H), 1.61 (m, 4H), 1.25 (m, 4H), 1.15 (m, 12H), 0.86 (t, J = 7.0 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 135.24, 131.55, 127.52, 124.58, 120.88, 119.06, 111.18, 102.14, 74.58, 59.61, 34.88, 31.78, 30.54, 29.43, 29.18, 26.53, 25.41, 22.59, 14.04.

General procedure for synthesis of 1-hydroxy-1-(1H-indol-5/6-yl) undecan-2-one and 1-hydroxy-1-(3-iodo-1H-indol-5/6-yl) undecan-2-one:



To a solution at 0 ºC of (1H-indol-5-yl) (2-nonyl-1,3-dithian-2-yl) methanol (0.3 mmol, 1 eq) in ACN (0.4M) iodine crystals (1.3 mmol, 4 eq) were added and the mixture was stirred for 30 min at room temperature. The reaction was diluted with diethyl ether and quenched with aqueous solution of $Na_2S_2O_3$ and saturated $NaHCO_3$. The aqueous layer was extracted with diethyl ether, dried over $MgSO_4$ and concentrated under reduced pressure. The products were separated by HPLC 30%-90% ACN in water to give brown solids.

IC5: Brown solid, 33% yield. **MS** (HRMS) m/z = 324.1932 $[M+Na]^+$ (calculated = 324.1934). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.60 (s, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.25 (m, 1H), 7.09 – 7.04 (m, 1H), 6.56 (s, 1H), 5.18 (s, 1H), 2.37 – 2.30 (m, 2H), 1.51 – 1.48 (m, 2H), 1.25 – 1.14 (m, 12H), 0.85 (t, J = 7.0 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 210.60, 159.92, 156.49, 135.87, 125.03, 121.17, 120.45, 111.69, 102.87, 80.13, 37.92, 31.83, 29.72, 29.33, 29.22, 28.99, 23.77, 22.66, 14.12.

IC5I2: Brown solid, 29% yield. **MS** (HRMS) m/z = 450.0899 $[M+Na]^+$ (calculated = 450.0906). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.45 (s, 1H), 7.37 (s, 1H), 7.33 – 7.24 (m, 1H), 7.22 (s, 1H), 7.06 (d, J = 8.4 Hz, 1H), 5.18 (s, 1H), 2.49 – 2.09 (m, 3H), 1.50 – 1.43 (m, 2H), 1.21 (m, 2H), 1.13– 1.10 (m, 9H), 0.81 (t, J = 7.0 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 210.26 (s), 135.75 (s), 130.80 (s), 130.13 (s), 129.30 (s), 122.24 (s), 120.73 (s), 112.04 (s), 79.93 (s), 57.64 (s), 37.90 (s), 31.80 (s), 29.29 (s), 29.17 (s), 28.94 (s), 23.73 (s), 22.62 (s), 14.08 (s).

IC6: Brown solid, 30% yield. **MS** (HRMS) m/z = 324.1937 $[M+Na]^+$ (calculated = 324.1934). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.60 (s, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.24 (t, J = 2.7 Hz, 1H), 7.08 – 7.02 (m, 1H), 6.56 (s, 1H), 5.18 (s, 1H), 2.45 – 2.20 (m, 4H), 1.59 – 1.40 (m, 2H), 1.25 – 1.09

(m, 10H), 0.85 (t, J = 7.0 Hz, 3H). **^{13}C NMR** (101 MHz, CDCl3) δ 210.28, 135.95, 132.01, 125.13, 121.31, 119.41, 111.83, 110.20, 102.69, 80.04, 37.93, 31.83, 23.73, 22.65, 14.10.

IC6I2: Brown solid, 35% yield. **MS** (HRMS) m/z = 450.0897 $[M+Na]^+$ (calculated = 450.0906). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.46 (d, J = 8.2 Hz, 1H), 7.34 – 7.27 (m, 2H), 7.13 (d, J = 8.2 Hz, 1H), 5.17 (d, J = 3.6 Hz, 1H), 2.45 – 2.19 (m, 2H), 1.52 – 1.44 (m, 2H), 1.25 (t, J = 9.5 Hz, 2H), 1.22 – 1.08 (m, 10H), 0.86 (t, J = 6.9 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 209.98, 135.77, 129.40, 127.93, 127.27, 121.61, 120.32, 110.34, 79.78, 57.38, 37.87, 31.80, 29.30, 29.16, 28.92, 23.66, 22.63, 14.09.

Bioluminescence assays:

Both of *V. cholerae* bacteria reporter strains (MM920 *ΔcqsaΔluxp*, MM825 *ΔcqssΔluxp*) coupled with the luxCDABE luminescence system were cultured separately overnight in 5 mL Difco™ LB Broth, Lennox with tetracycline 10 mg/mL at 30 ºC.
The bacteria were diluted with fresh medium to OD_{600}≈0.05 and the analysed molecules dissolved in DMSO to 10mM concentration and were added to the bacterial cultures in corresponding concentrations (DMSO<1%) into white 96-well clear bottom plates. The final sample volume was 200 µL. The plates were incubated at 30 ºC, and every 20 minutes OD_{600} and luminescence measurements were taken for 8 hours. Data were analysed for several different time points approximately around the point that measurements yield 75% of the maximum bacterial response.

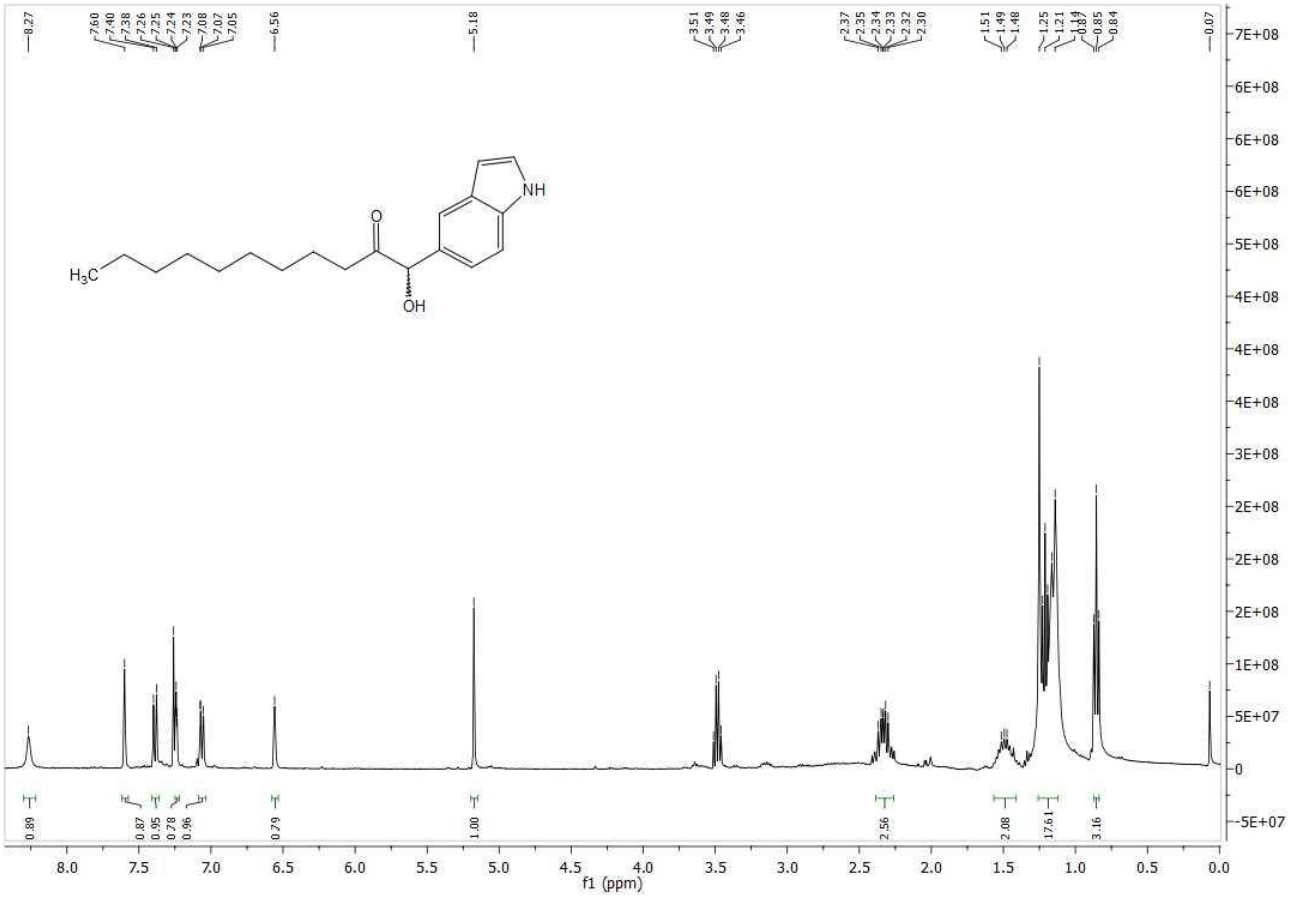
Biofilm assay:

Wild type *V. cholerae* (VC1) was cultured overnight in in 10 mL Difco™ LB Broth, Lennox at 30 ºC. The next day the culture was diluted to OD_{600}≈0.05 with fresh medium and the tested molecules dissolved in DMSO were added to the cultures (DMSO<1%) in corresponding concentrations into 96 well MBEC™ Biofilm Inoculator plates with final volumes of 150 µL per well. The plates were incubated for an additional 12 hours at the same temperature and at the end the pins lid was transferred and shaken for few minutes in a new 96 well plate with 200 µL PBS. The pins lid was transferred to a new 96 well plate with 200 µL of crystal violet solution 1 mg/mL in 10% ethanol in water and was left shaking for an additional 10 minutes. After this, the pins lid was transferred to a new 96 well plate with 200 µL PBS and left shaken for 10 minutes. The last transfer of the pins lid was to a clear 96-well plate with 200 µL acetic acid solution of 6.3 gr/mL in water. The plate was left shaking for 20 minutes (or until no crystal violet was left on the pins) and OD_{570} measurements were taken. At the last step of the experiment, OD_{570} of the original acetic acid solution was taken and was subtracted from the final OD results during data analysis.

Cholera toxin assay:

The assay was performed according to an established cholera toxin ELISA protocol.

Wild type *V. cholerae* (VC1) was cultured overnight in 10 mL DifcoTM LB Broth, Lennox at 30 ºC. The next day, the culture was diluted to OD600≈0.05 with fresh medium and the tested molecules dissolved in DMSO were added to 1 mL of the bacteria (DMSO<1%) in corresponding concentrations. The bacteria were incubated in open air for an additional night at 30 ºC. The next day the cultures were spun down and 50 µL of the supernatant wad diluted in PBS-BSA (4mg/mL) solution in ratio of 1:2. Next, 100 µL of the diluted supernatant moved to a GM_1 ganglioside coated clear bottom white 96 well plate and instantly shaken under 37 ºC for at least 30 min. The plate was washed 3 times with PBS and 200 µL of 1:1000 dilution of Anti-Cholera Toxin antibody produced in rabbit in PBS-BSA added to the wells of the same plate. The plate was incubated in the same condition described previously and washed three times with PBS. Next, 200 µL of 1:5000 diluted anti-rabbit IgG – alkaline phosphatase in PBS-BSA added to each well and incubated and washed afterwards as described previously. At last, 80 µL of chemoluminescence ELISA substrate were added to each well of the plate and luminescence emission measurement took place in a plate-reader.
Wild type *V. harveyi* underwent the exact same assay and was used as a control strain as it is a *Vibrio* species that does not produce the cholera toxin.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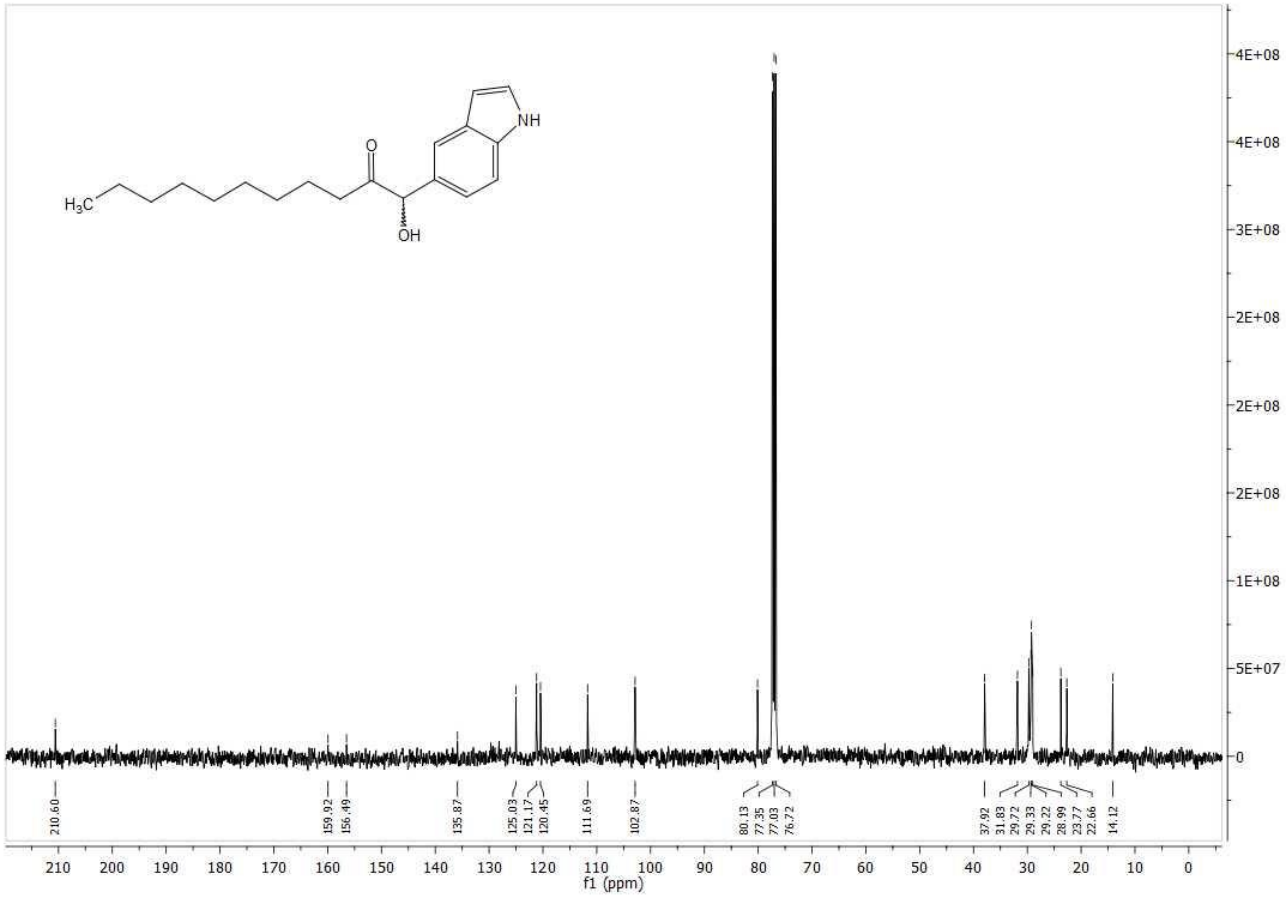
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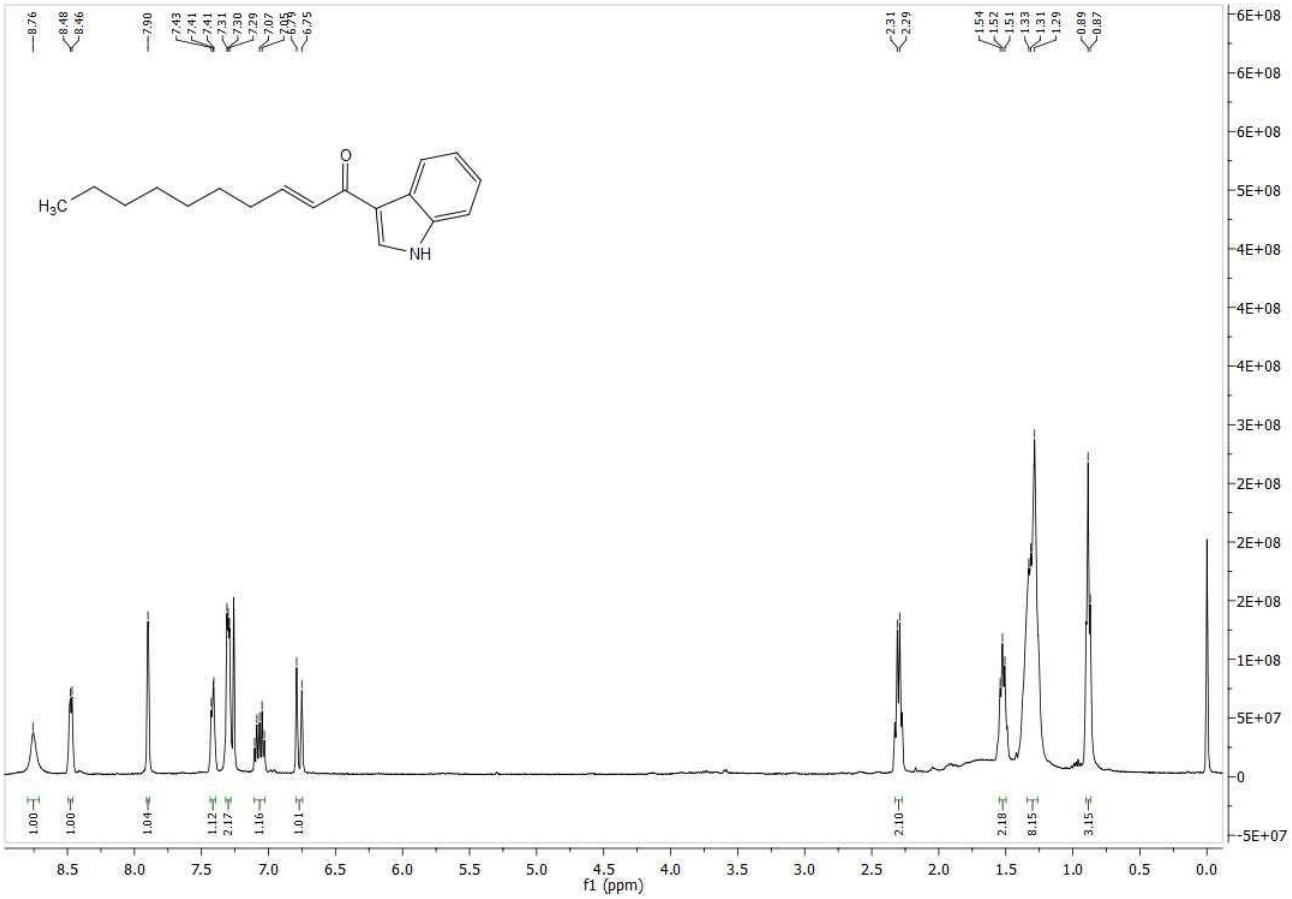
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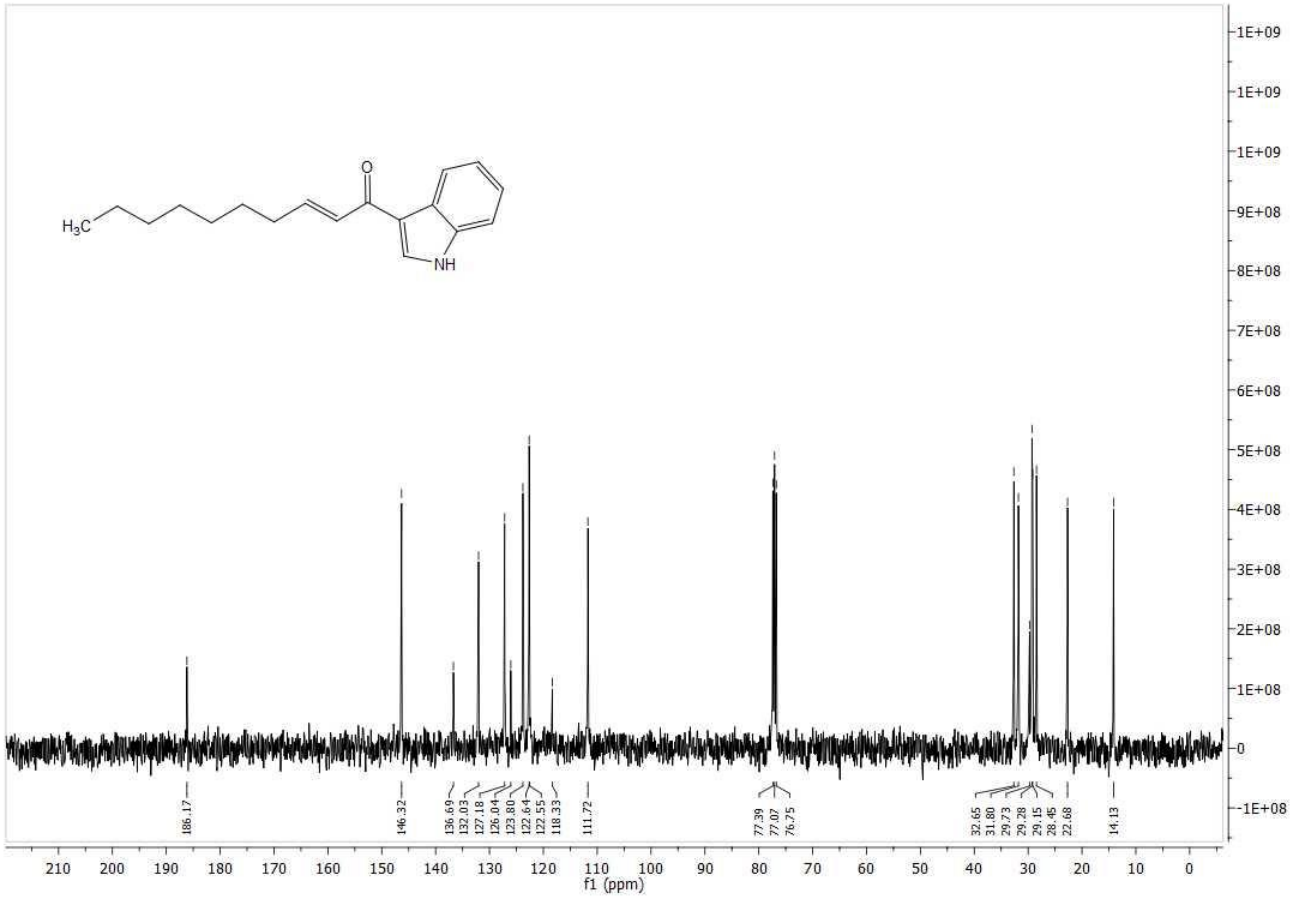
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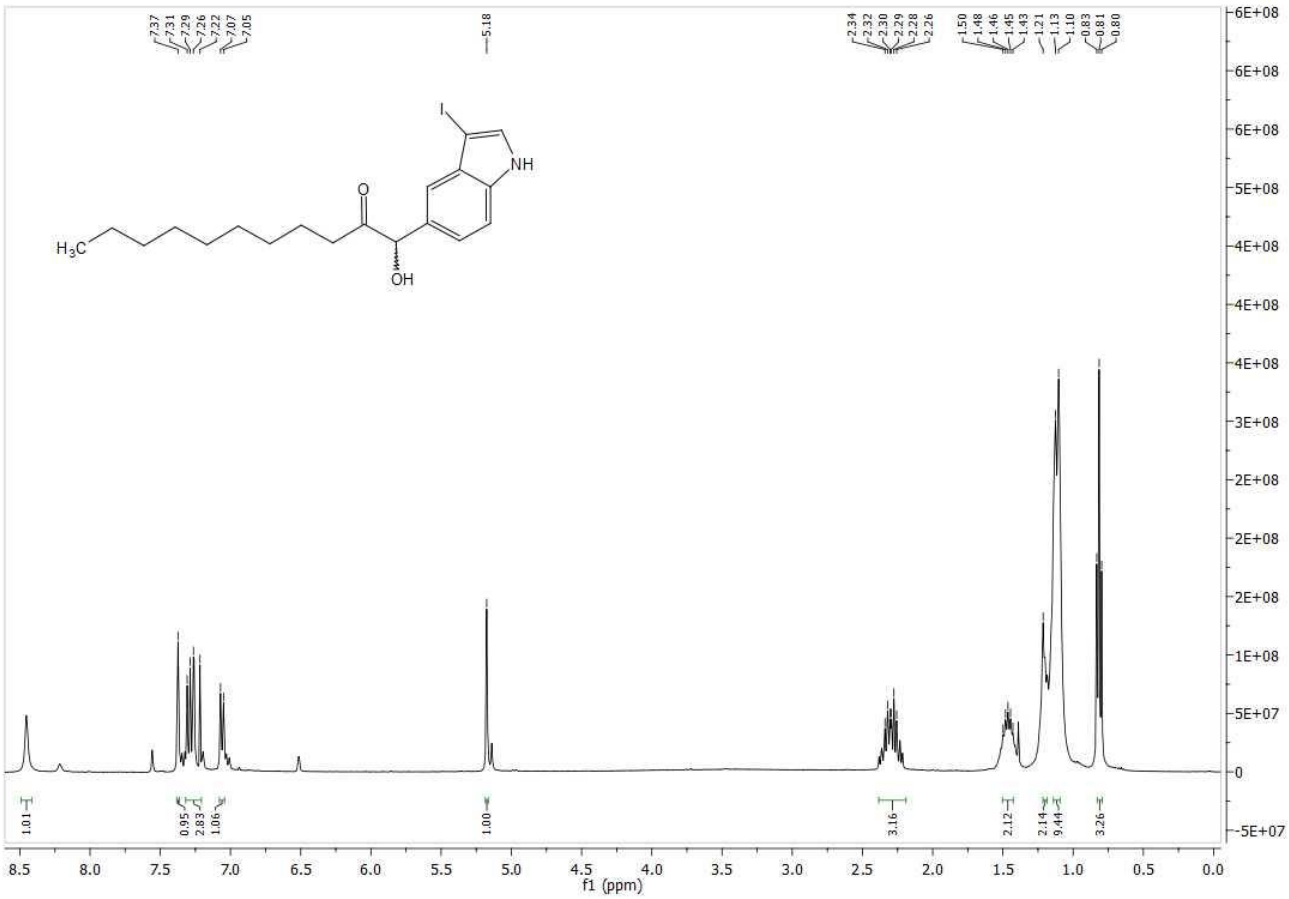
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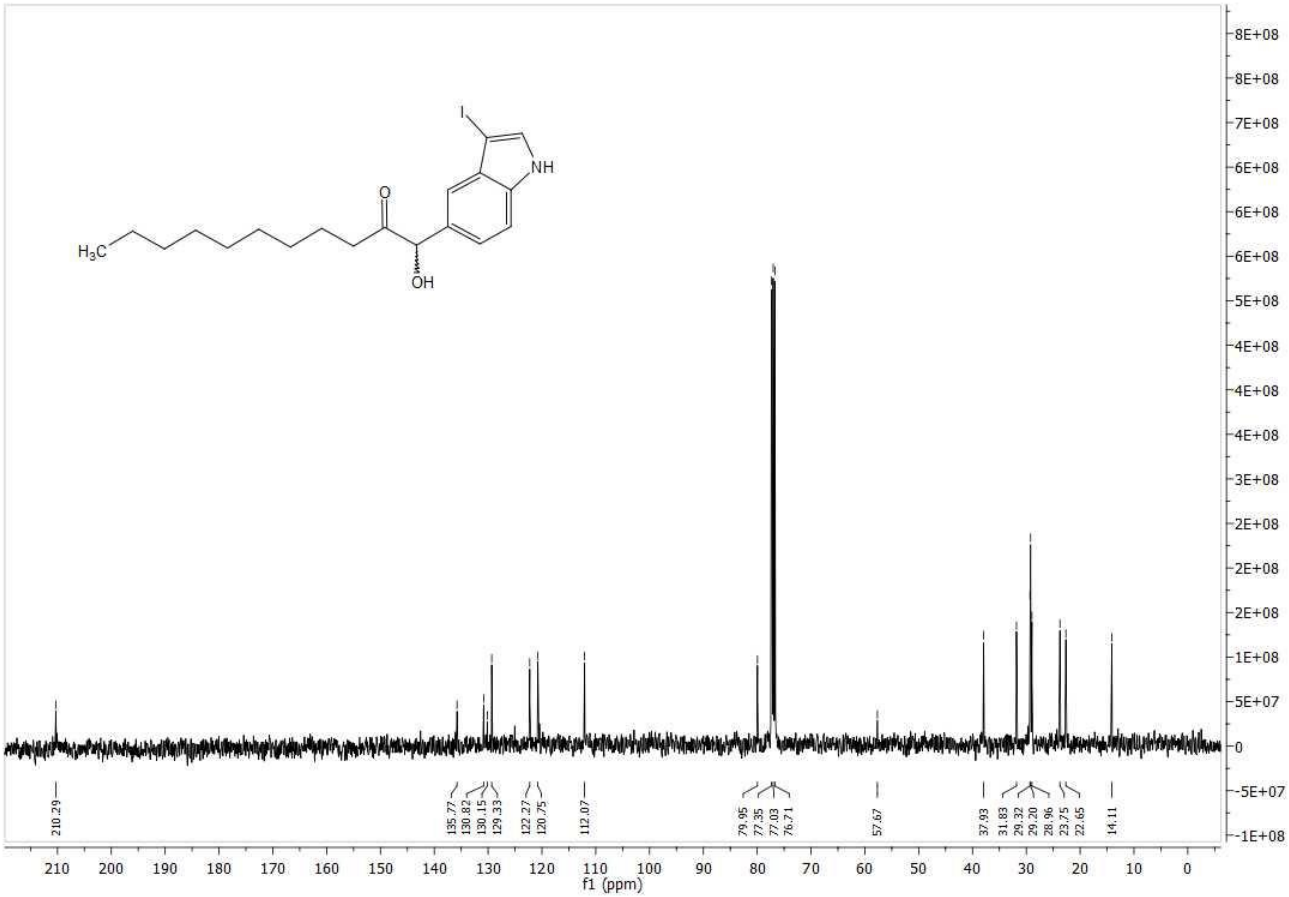
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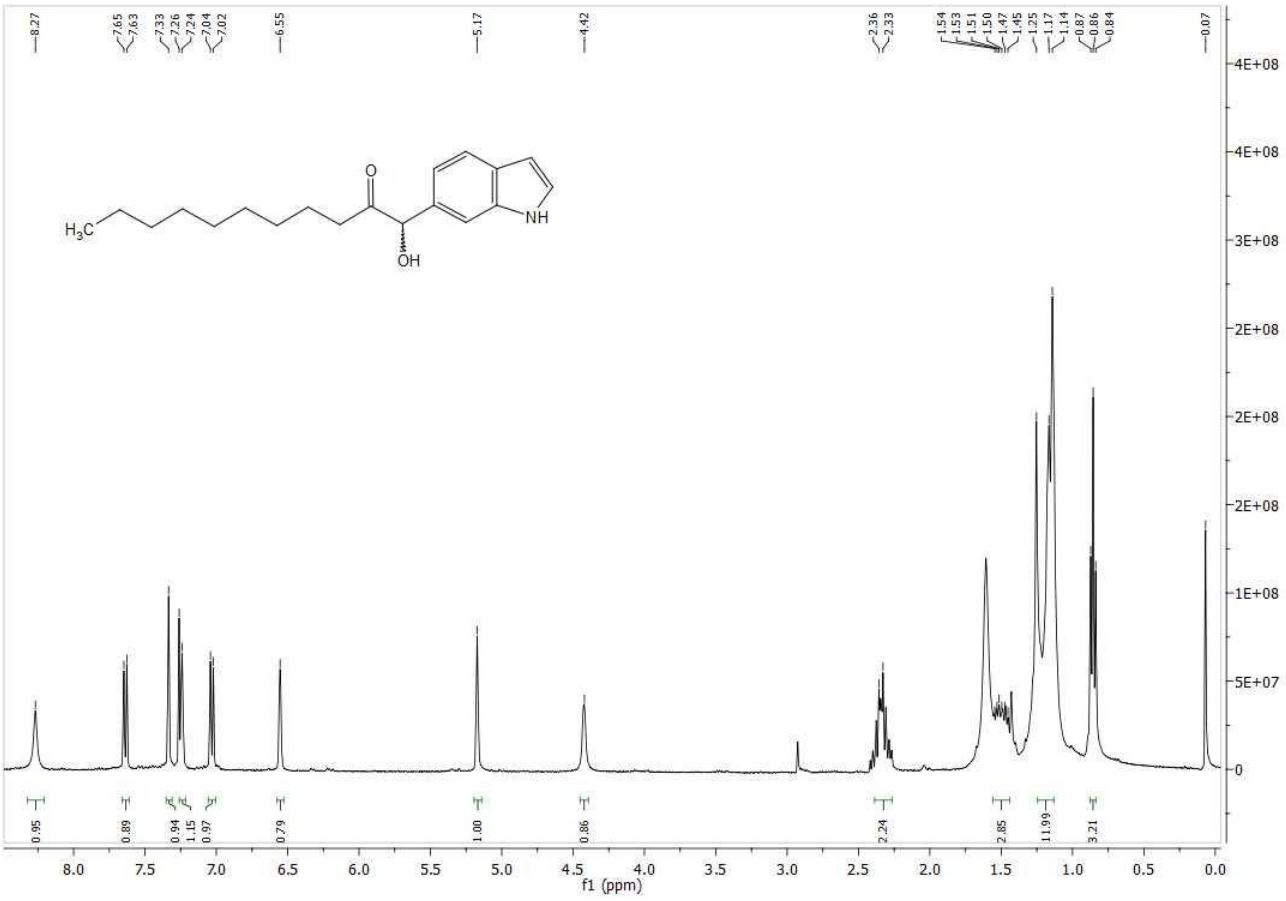
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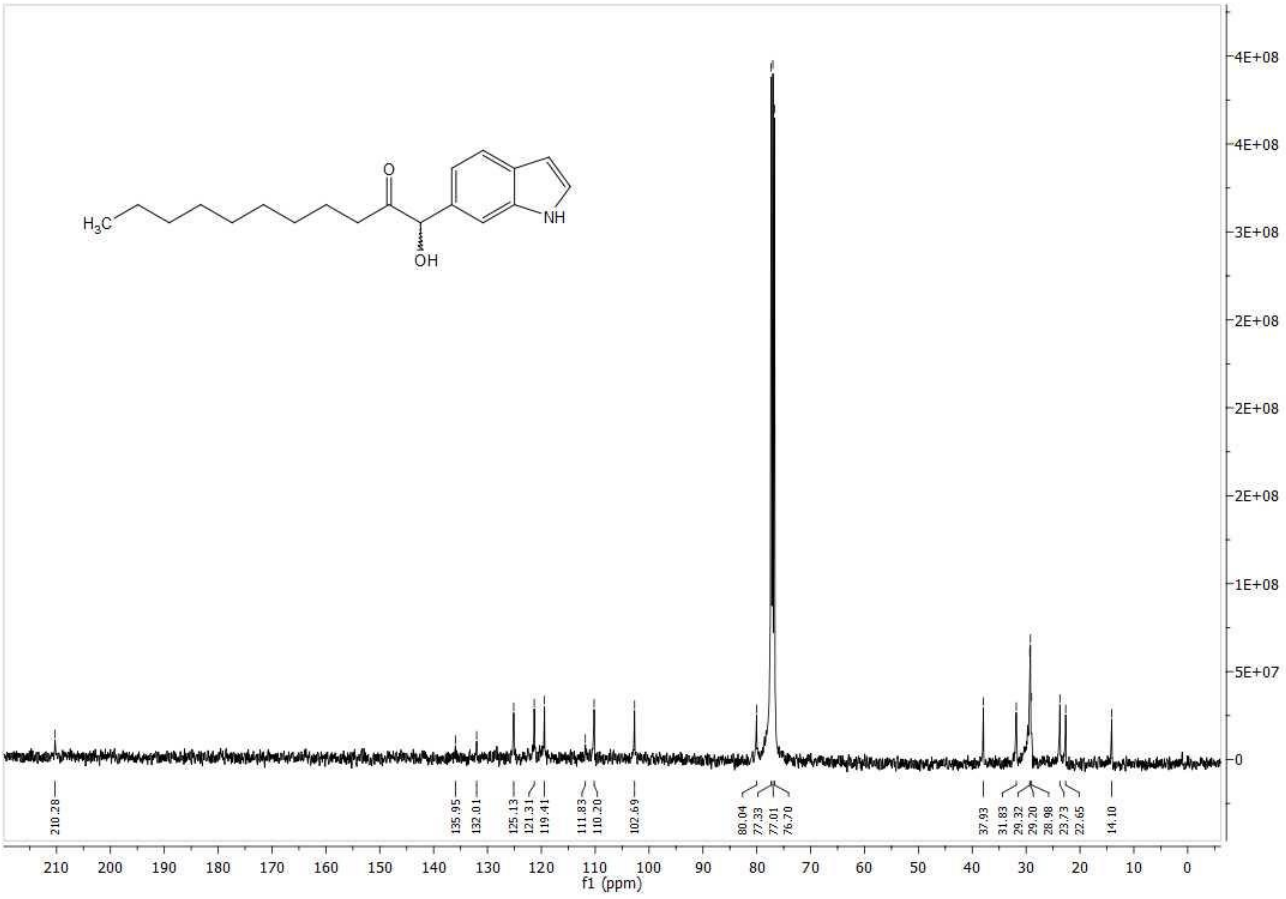
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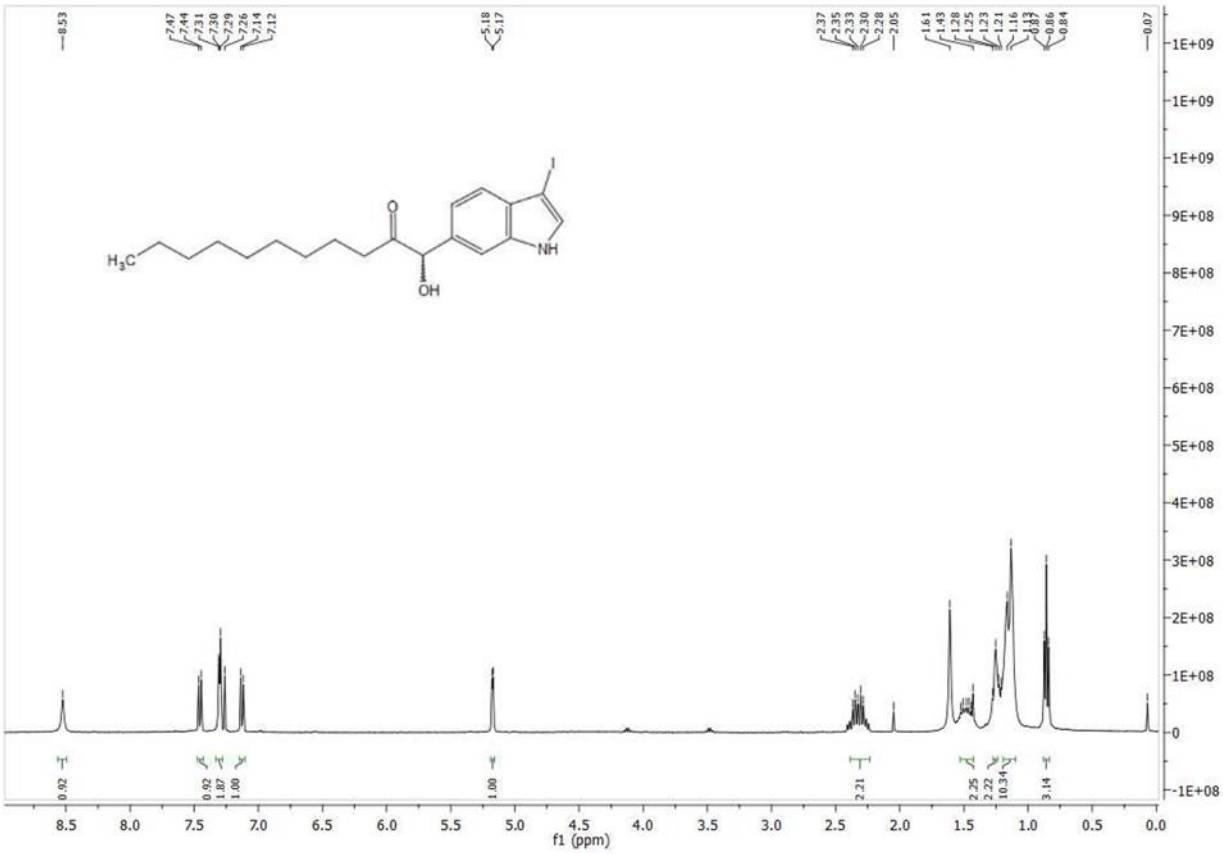
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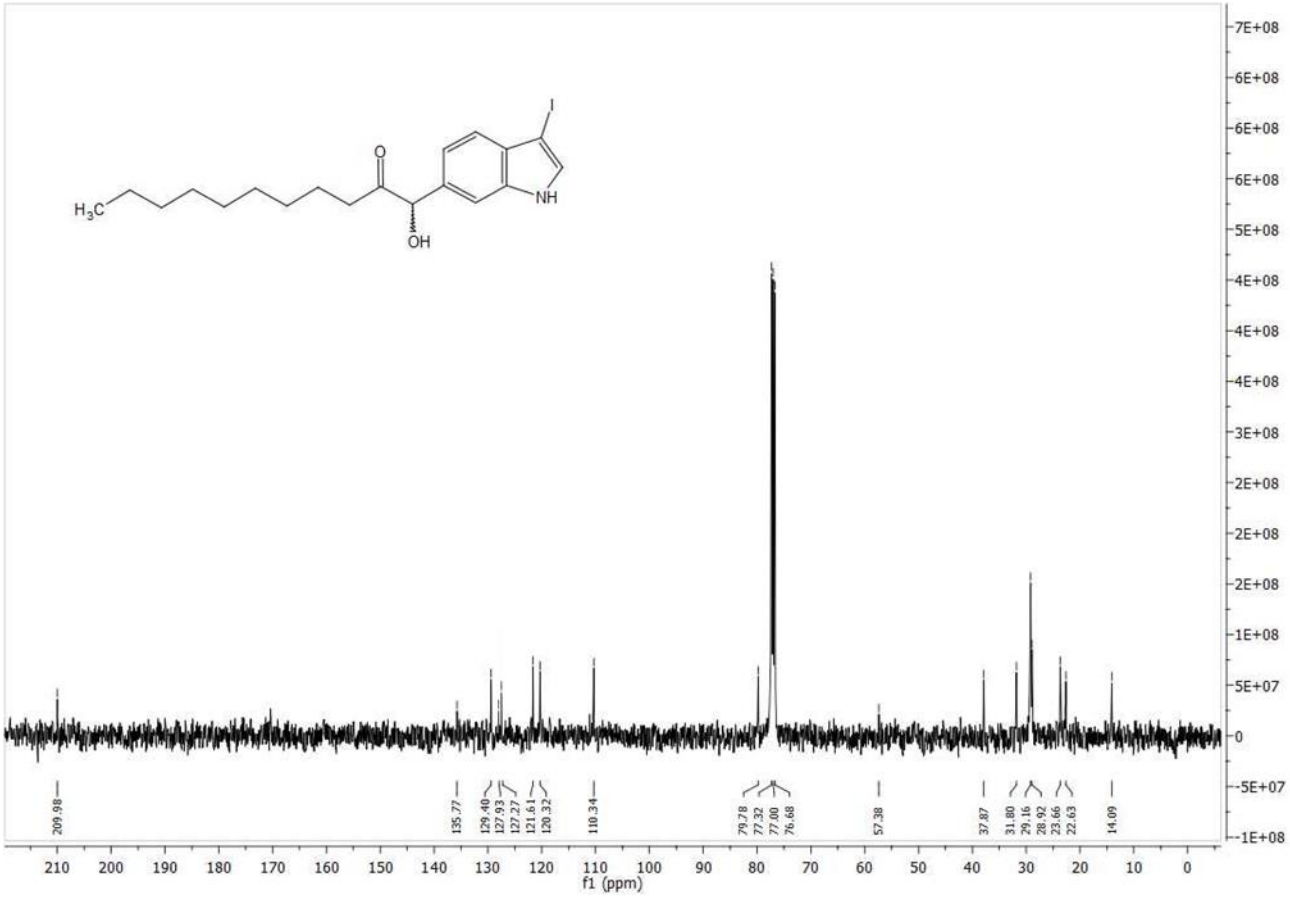
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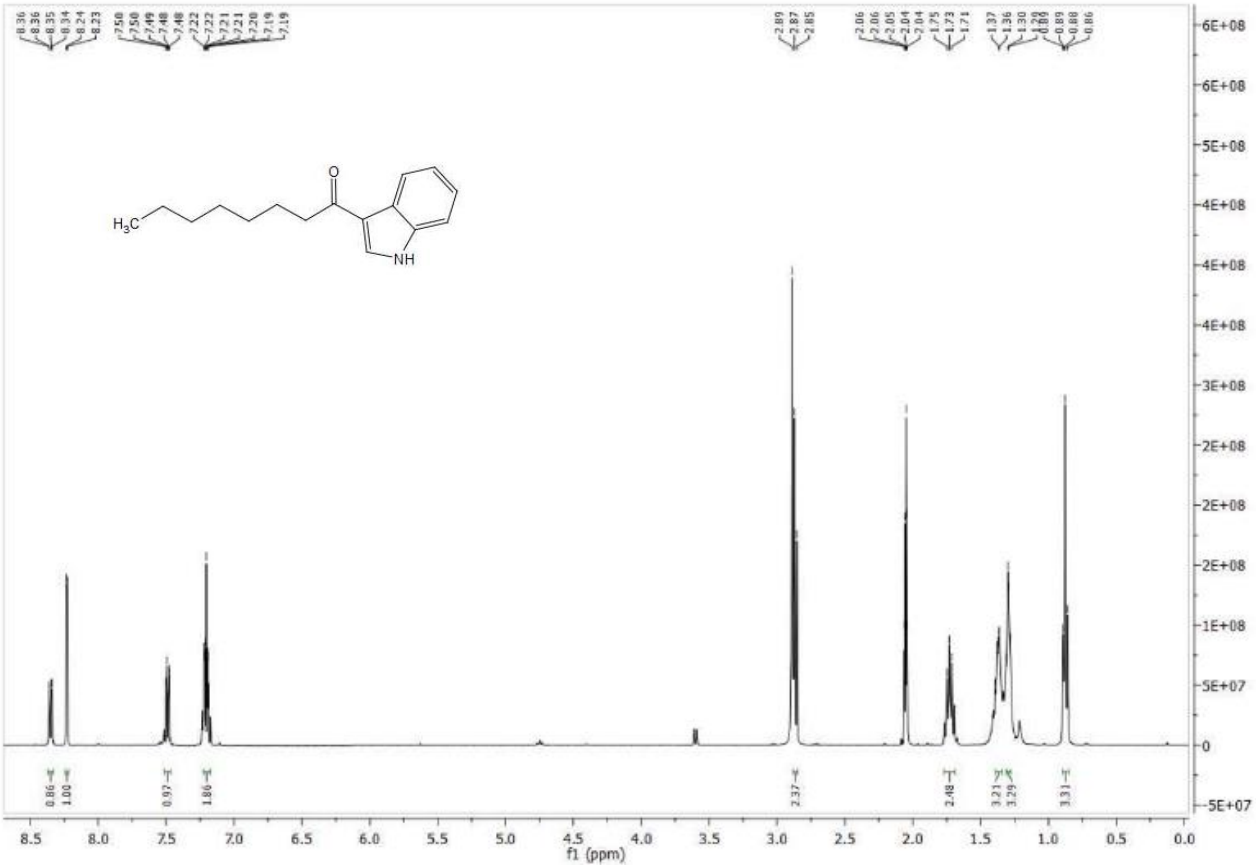
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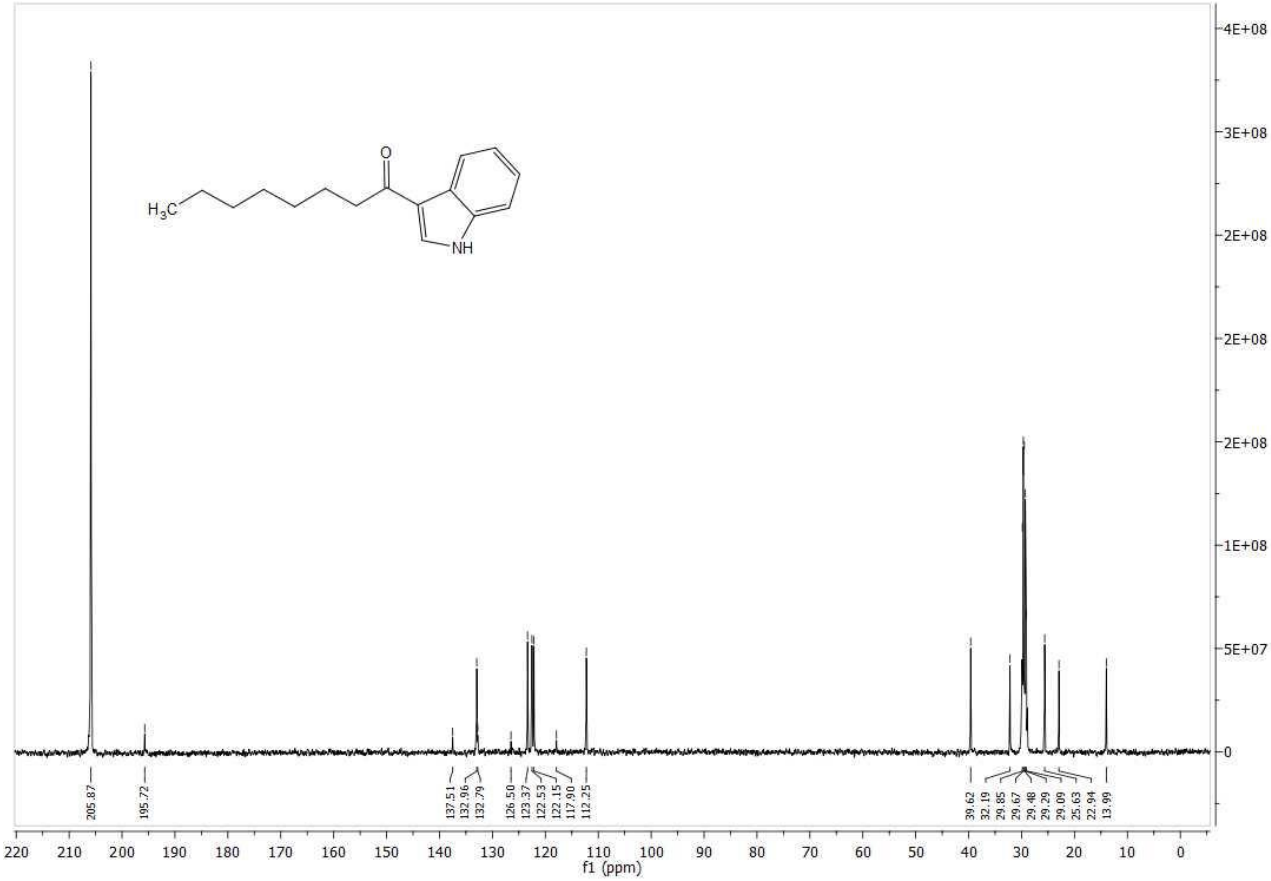
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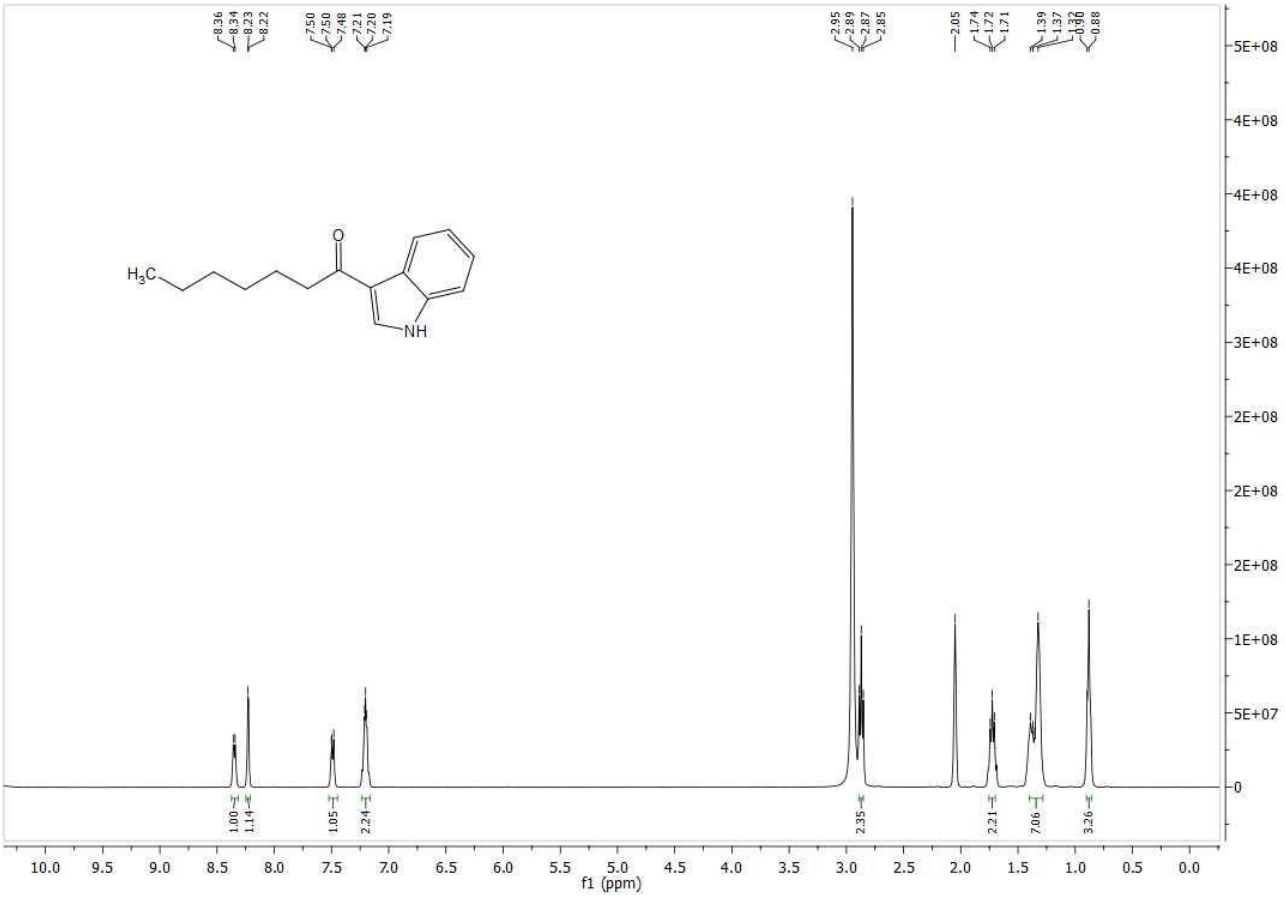
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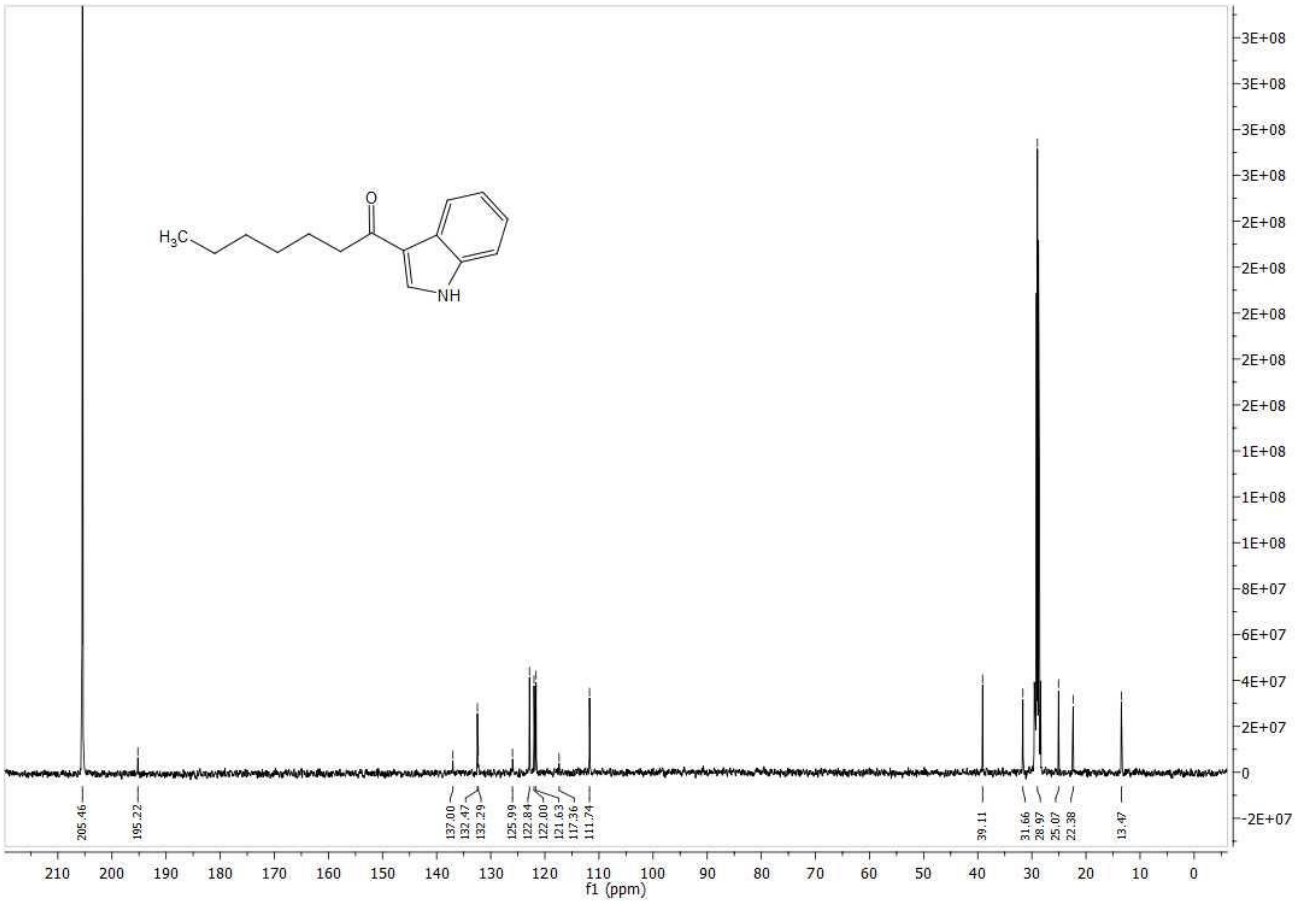
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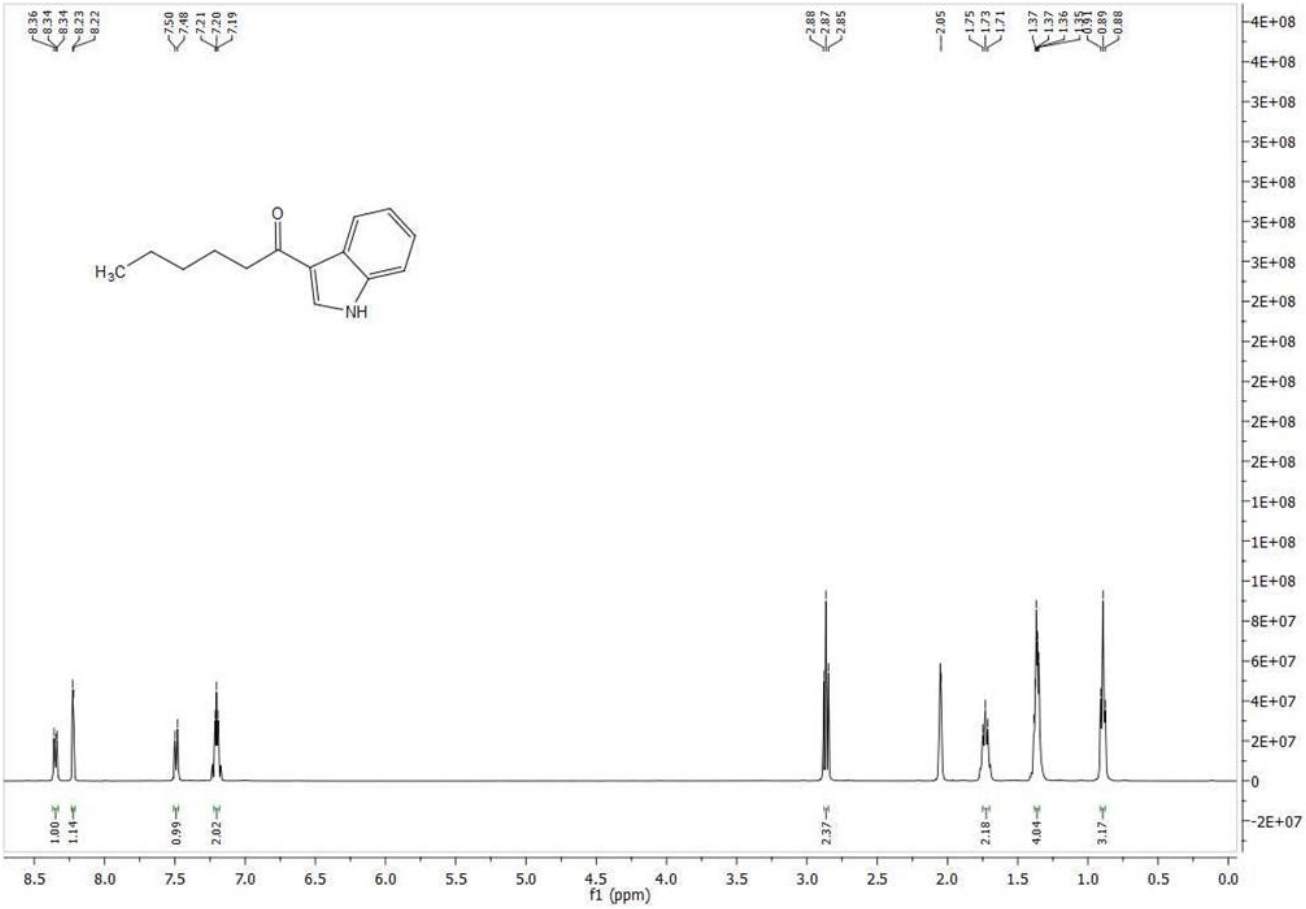
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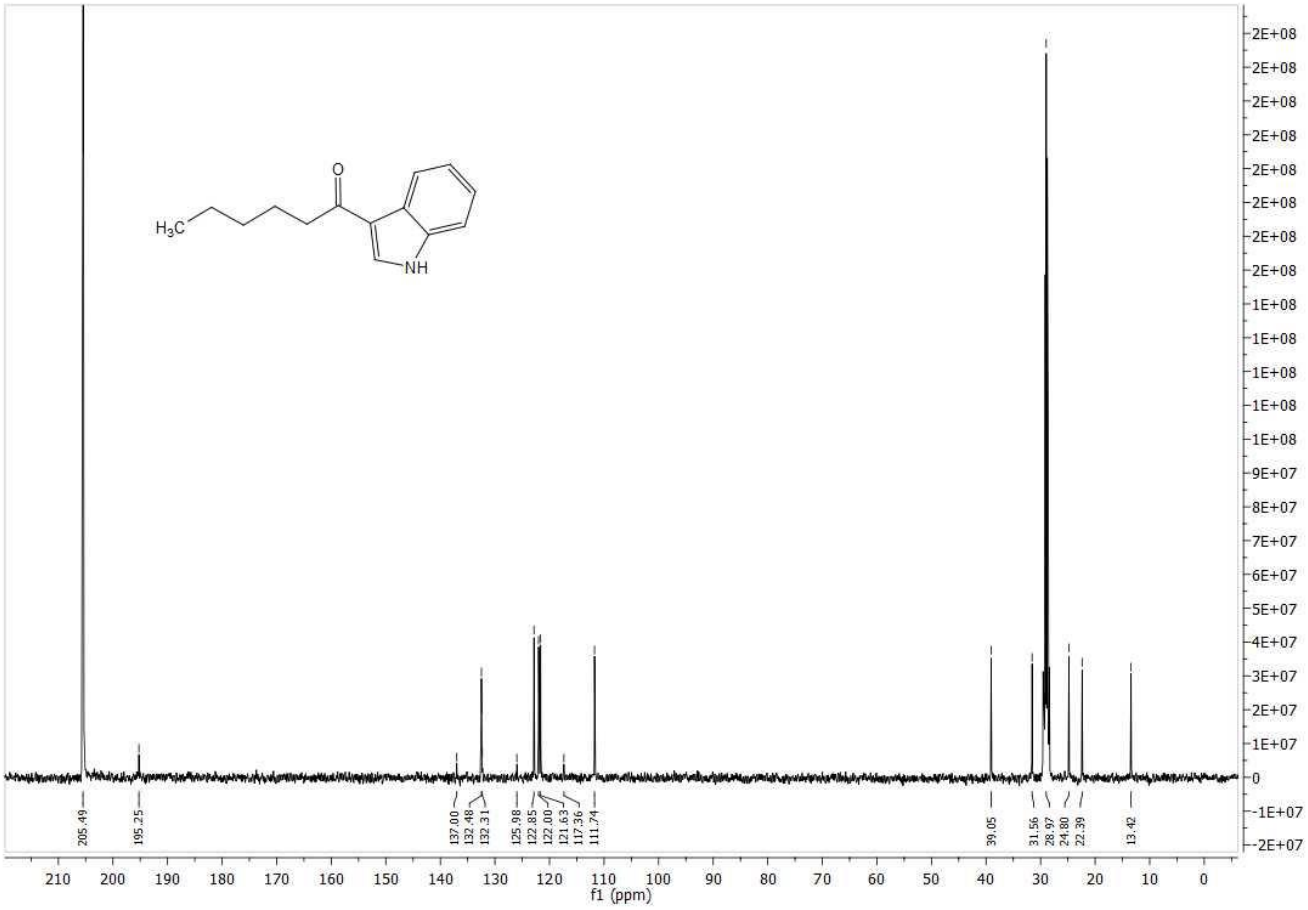
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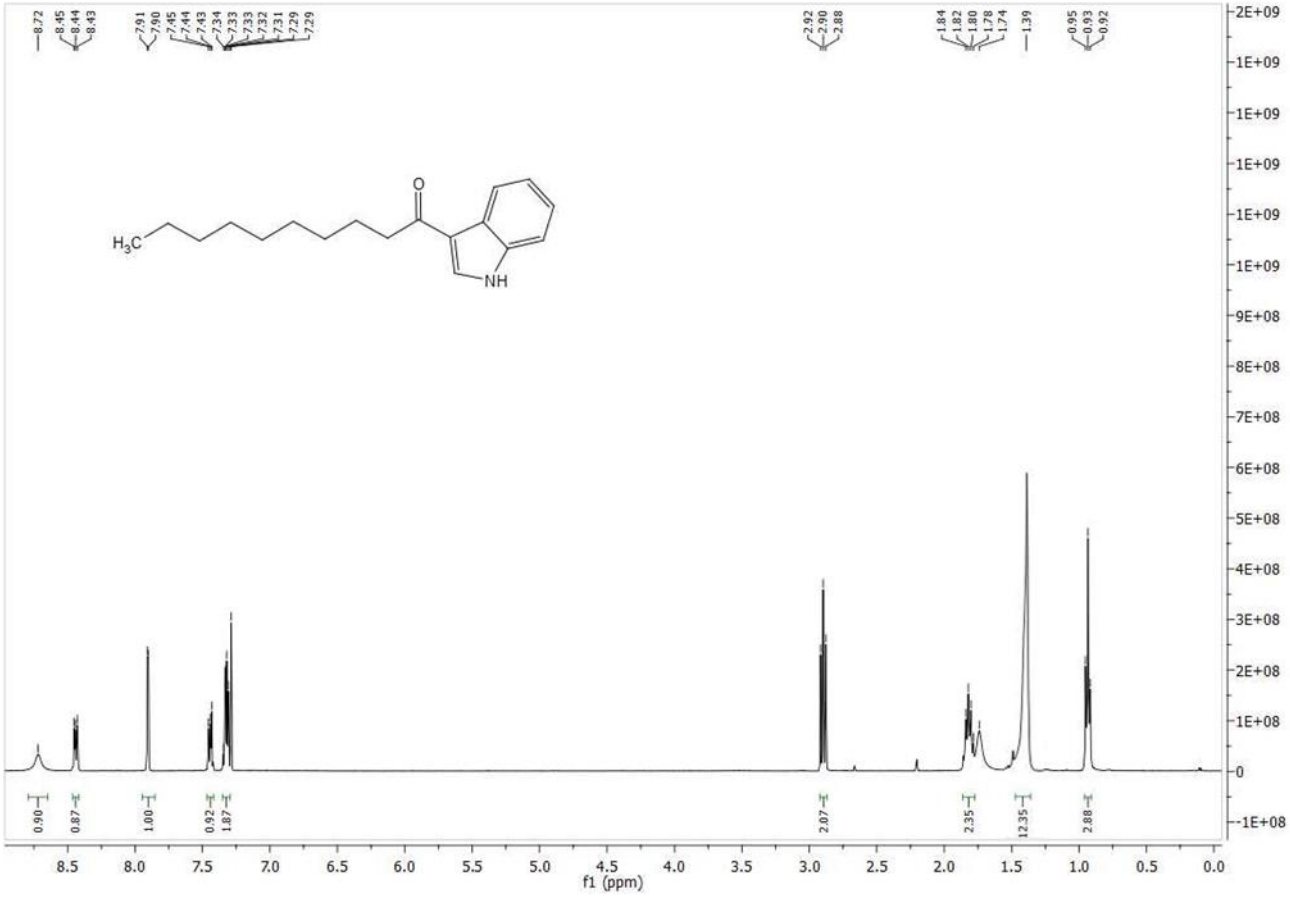
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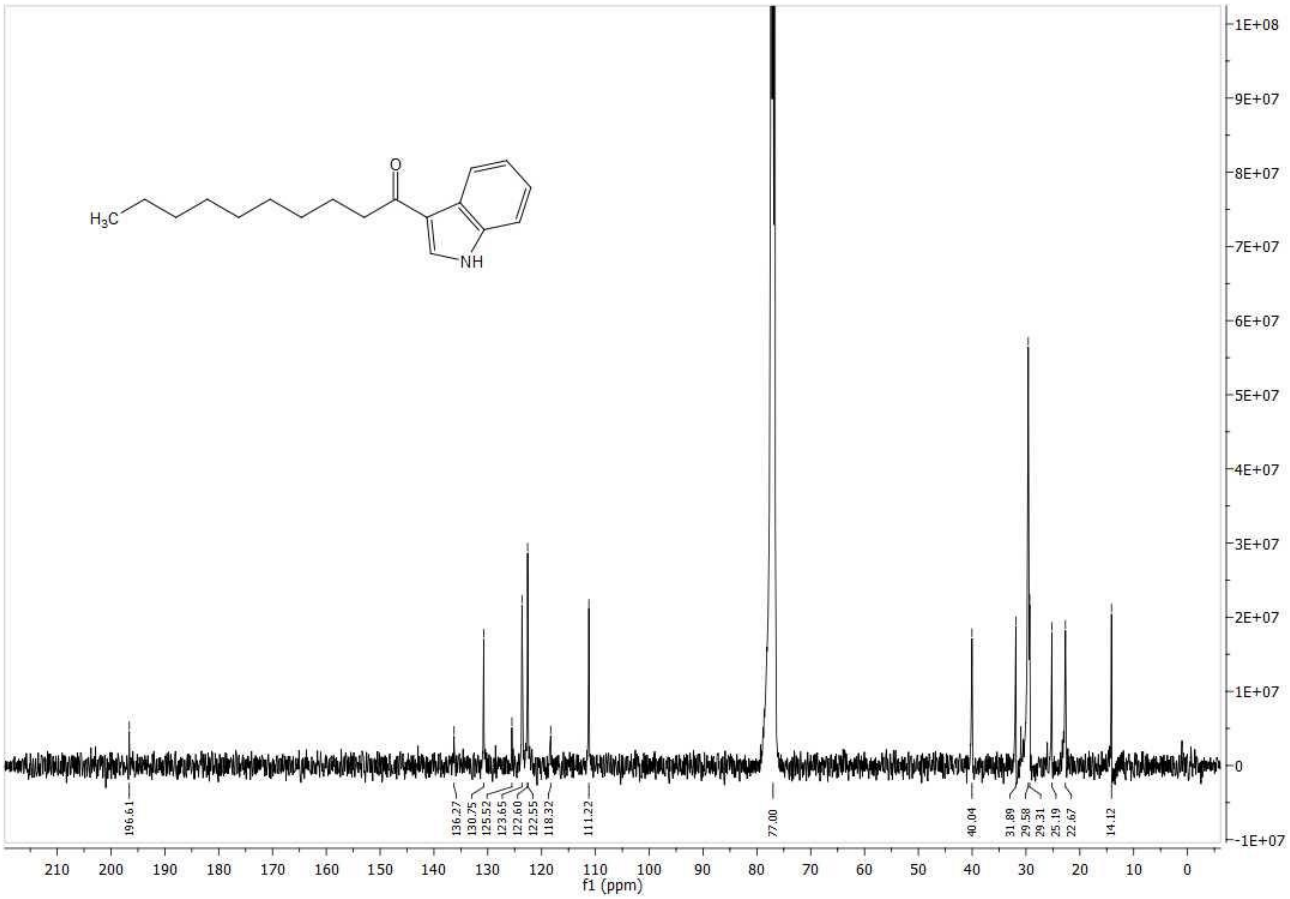
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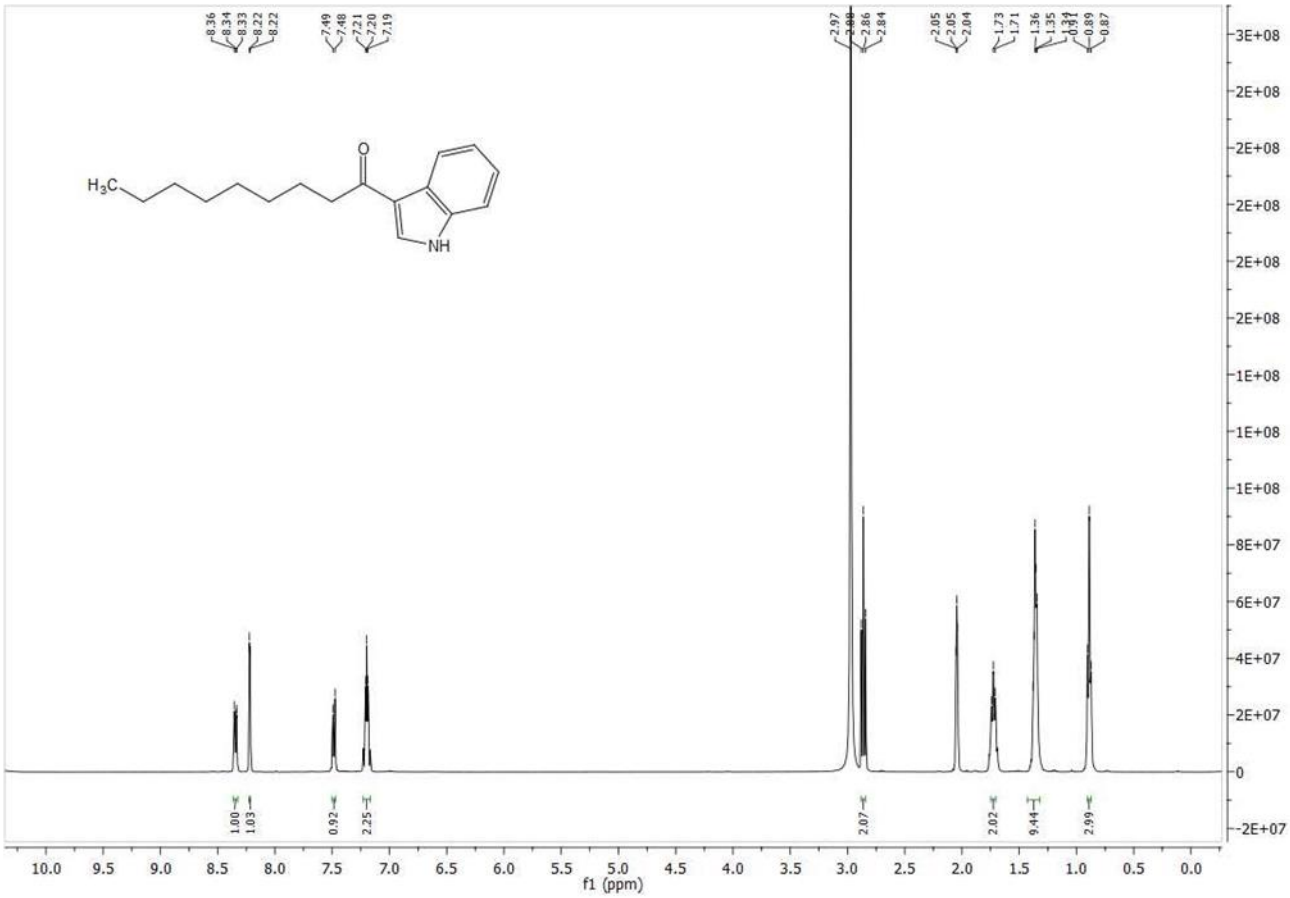
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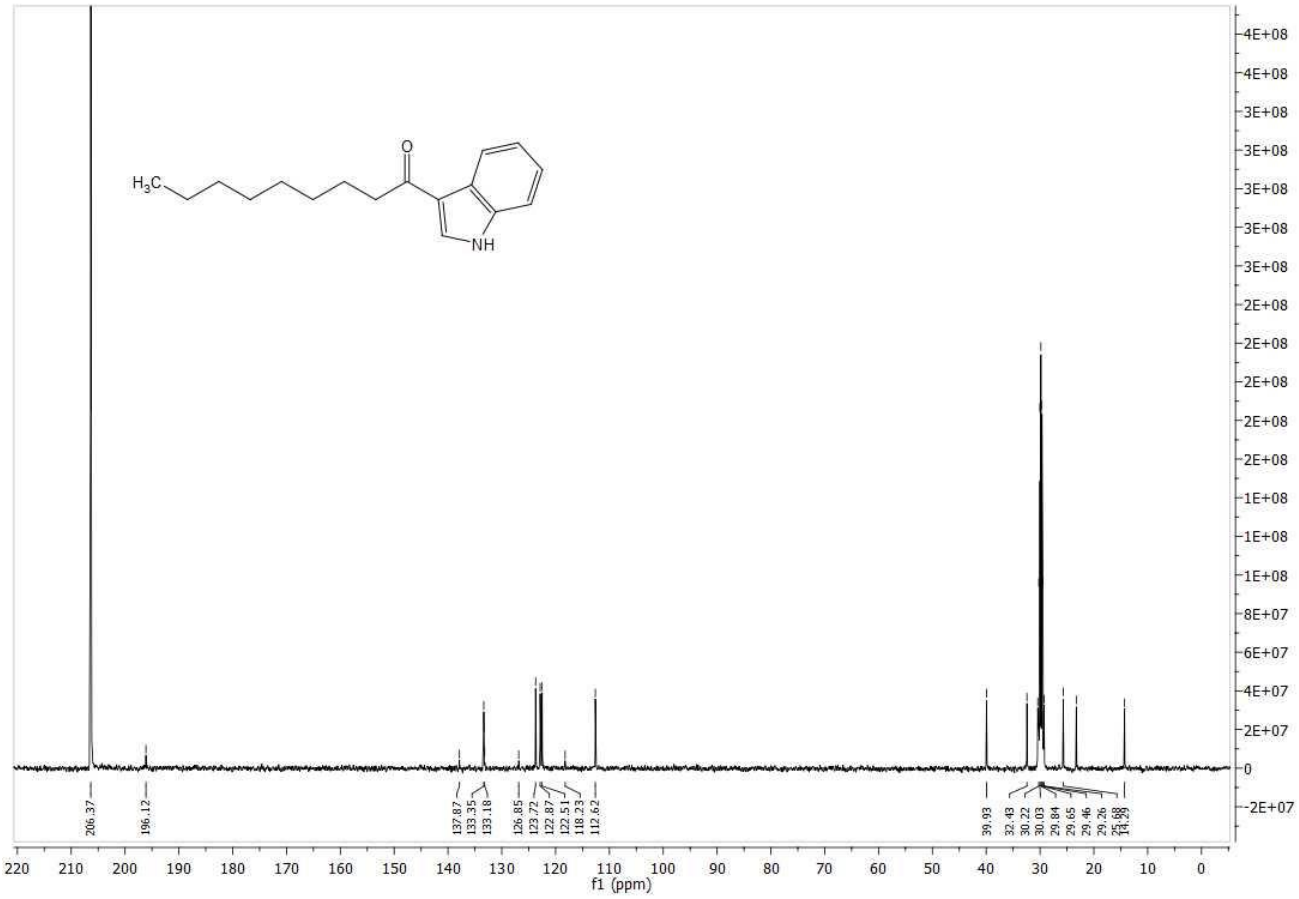
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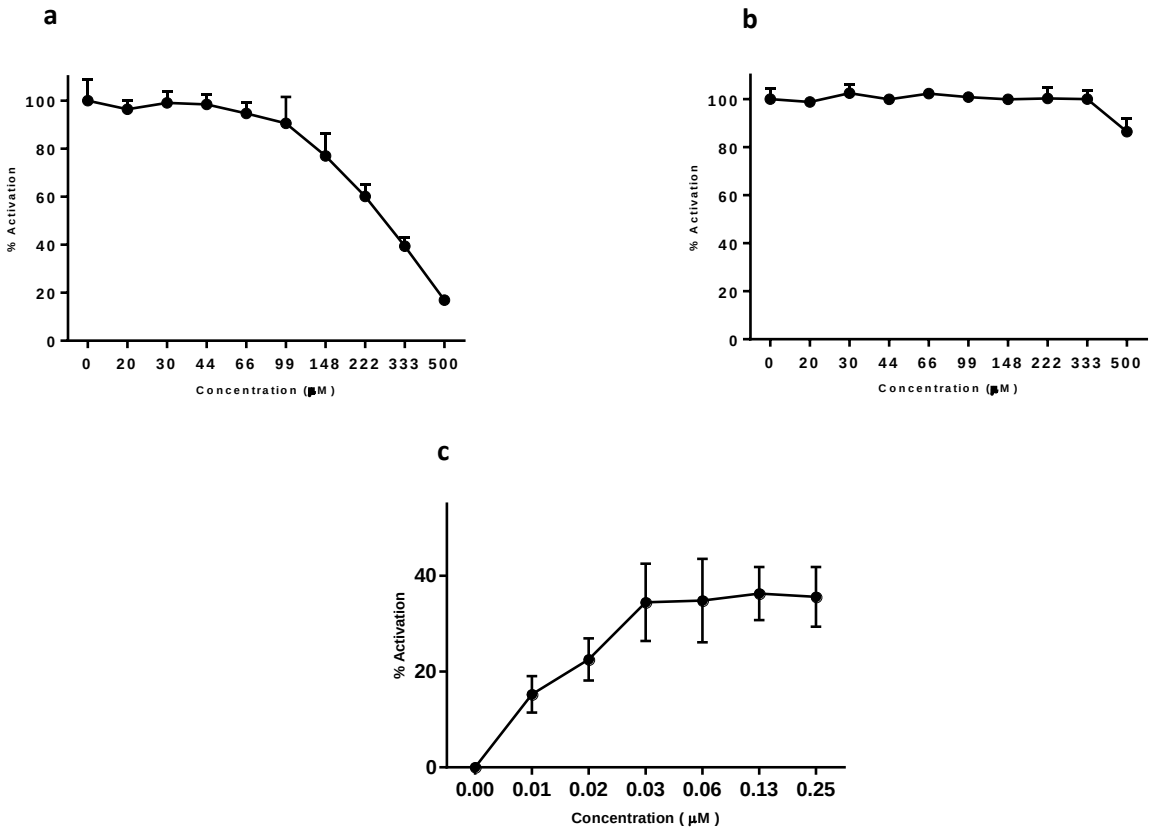

Figure S1: Antagonist competitive assay with indole on (a) MM920 and (b) MM825 *Vibrio cholerae* strain. (c) agonist assay on MM920 *V. cholerae* strain with indole.

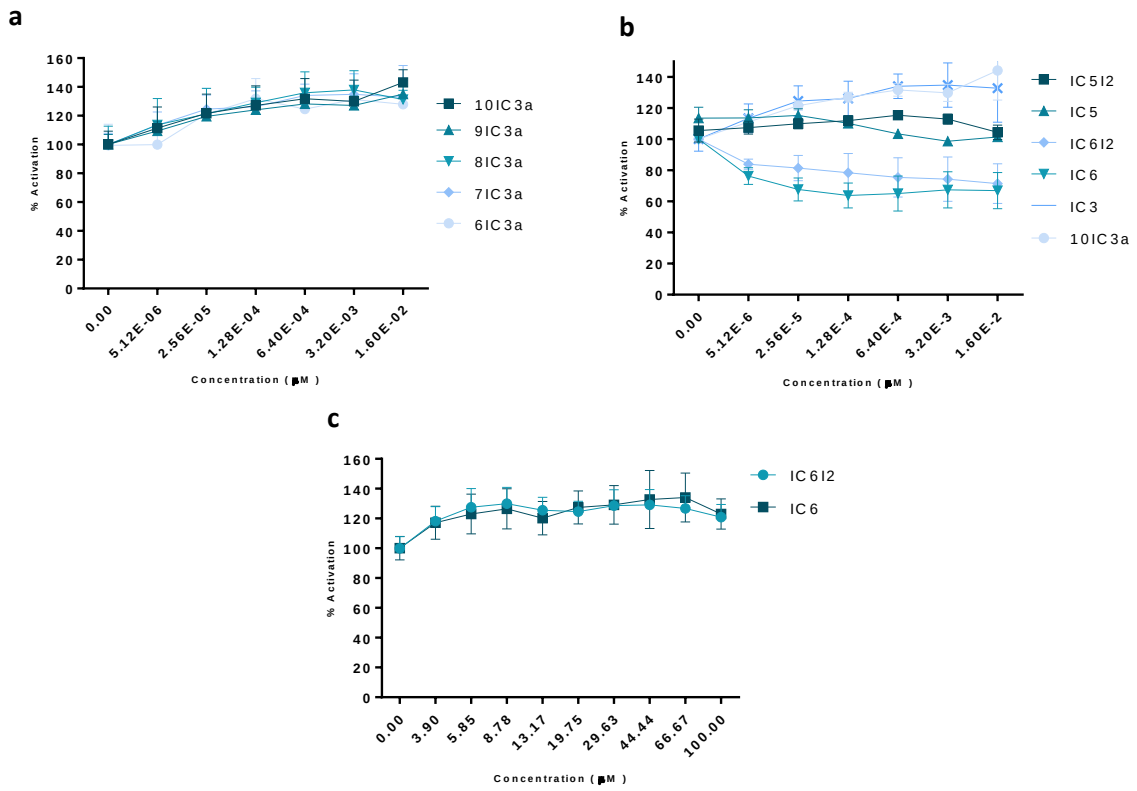

Figure S2: Agonist assay on MM920 reporter strain with (a) 6-10IC3a analogs (b) IC5, IC5I2, IC6, IC6I2, IC3 and IC3a. (c) Effect of IC6 and IC6I2 on MM825 *Vibrio cholerae* strain.

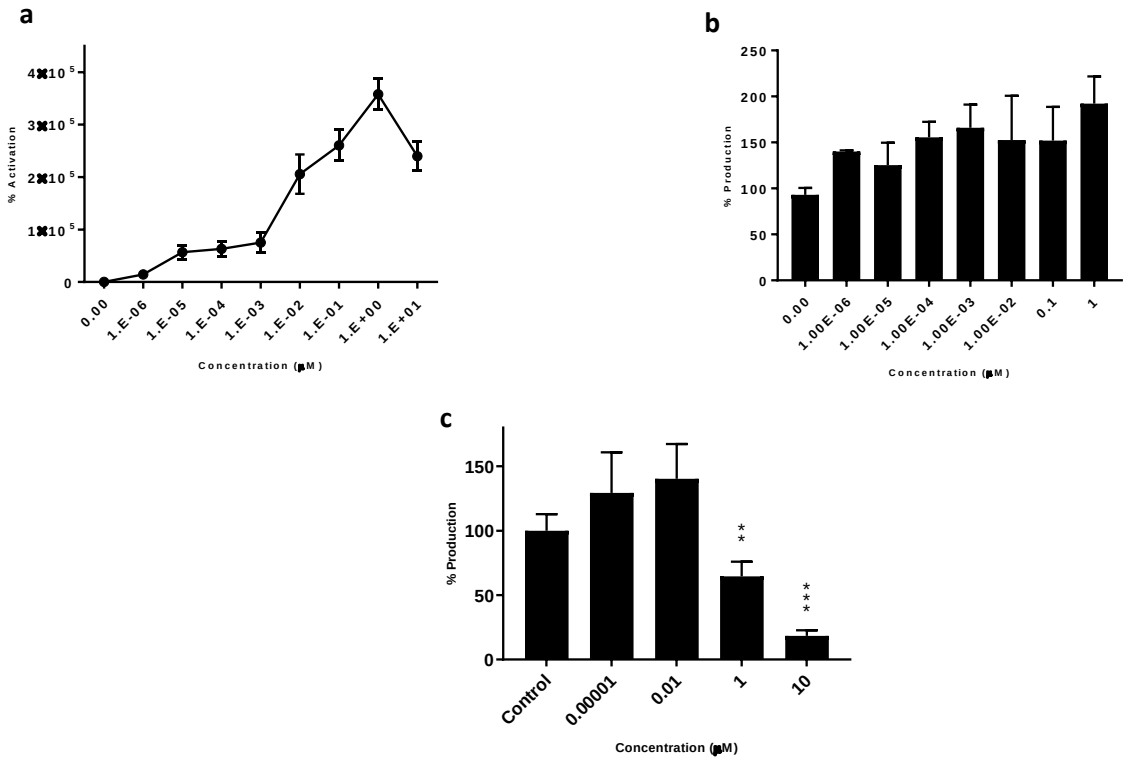

Figure S3: (a) Agonist assay on MM920 reporter strain with 3-acylpyrrole. Effect of 3-acylpyrrole on (b) biofilm production (c) cholera toxin production in WT bacteria.
* P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

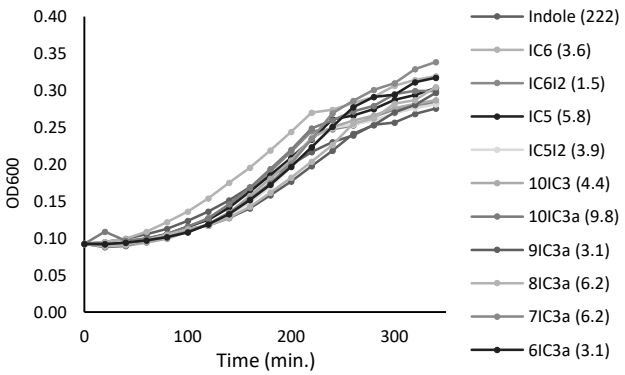

Figure S4: Growth curves for all of the tested molecules (tested molecules' concentrations are shown in parentheses [µM]). The IC_{50} values were measured relying on the values obtained after 280-320 minutes.