Design, synthesis, and biological evaluation of abiotic, non-lactone modulators of LuxR-type quorum sensing

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Supplementary Material.

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General instrumentation and analytical methods.

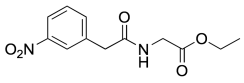
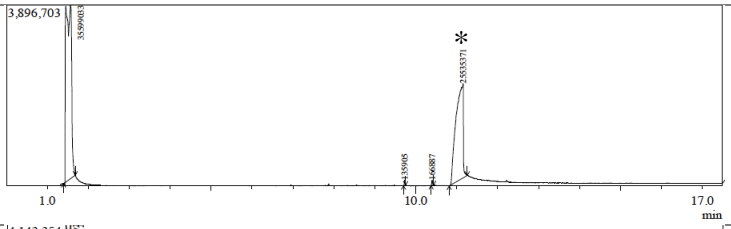
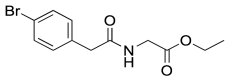
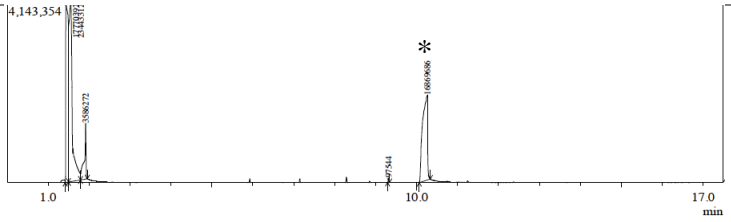
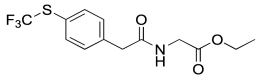
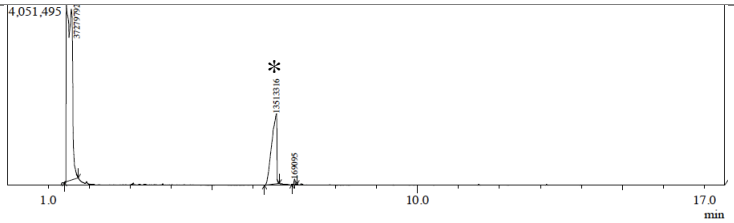
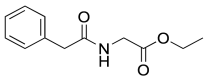
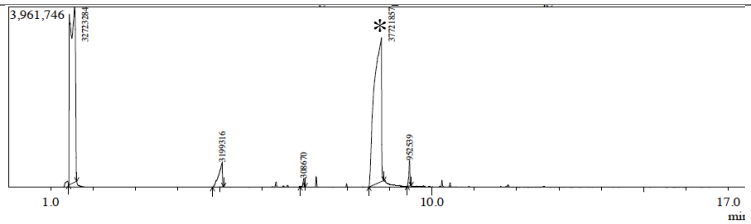
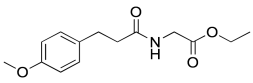
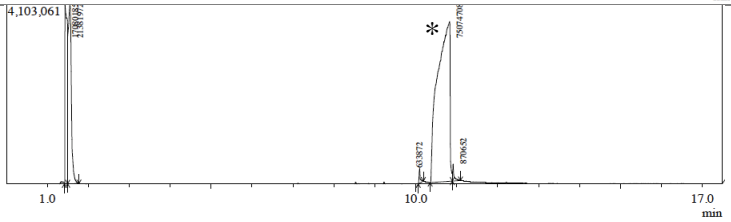
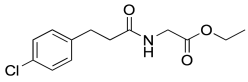
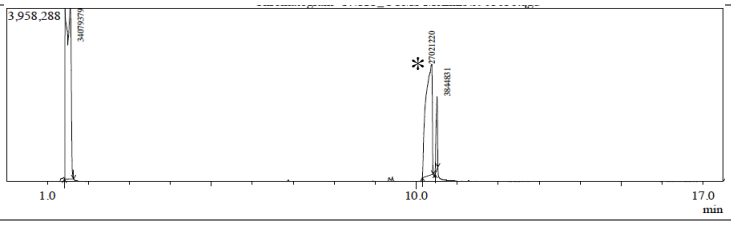
^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-300 spectrometer in $CDCl_3$ at 300 MHz at room temperature. Chemical shifts are reported in parts per million (ppm). Tetramethyl silane (TMS) was used as an internal reference (0.0 ppm) for ^{1}H NMR spectra. The chloroform carbons were used as an internal reference for ^{13}C NMR spectra. Couplings are reported in hertz. Electrospray ionization (ESI) MS data were obtained using a Shimadzu LCMS-2010 system (Columbia, MD) equipped with two pumps (LC-10ADvp), controller (SCL-10Avp), autoinjector (SIL-10Avp), UV diode array detector (SPD-M10Avp), and a single quadrupole analyzer. Electron impact (EI) MS data were obtained on the Shimadzu single quadrupole GCMS-QP2010S. Infrared (IR) measurements were recorded using a Bruker Equinox 55 ATR-FT-IR using a germanium crystal.

Gas chromatography (GC) data were obtained using a Shimadzu GC-2010 system. A Shimadzu SHRXI-5MS capillary column (dimensions: 30 m x 0.25 mm x 0.25 μm) was used for all GC work. The standard GC method was as follows: injection temperature 275 °C; initial oven temperature 100 °C; hold 5 min; ramp at 10 °C/min to 300 °C; hold 5 min for a total run time of 30 min.

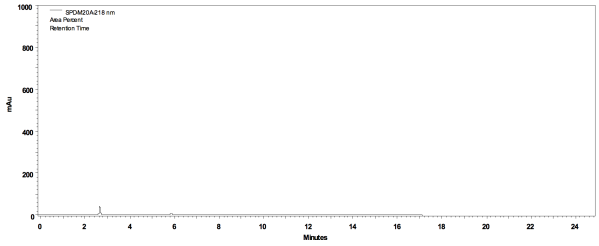
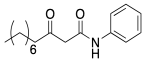
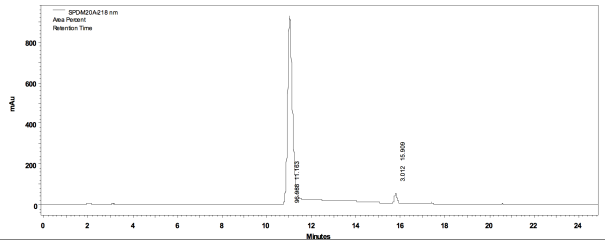
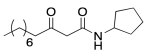
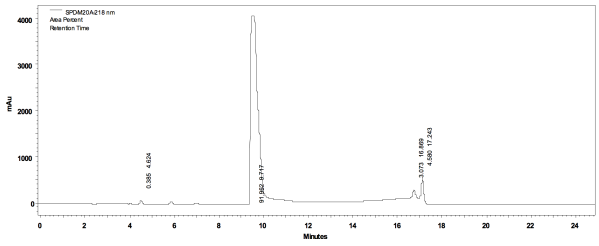
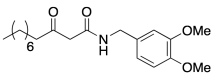
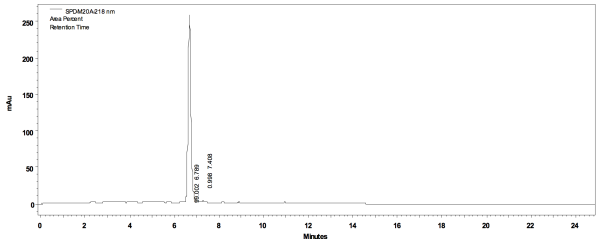
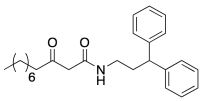
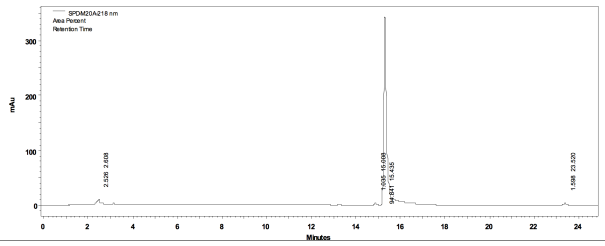
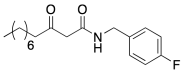
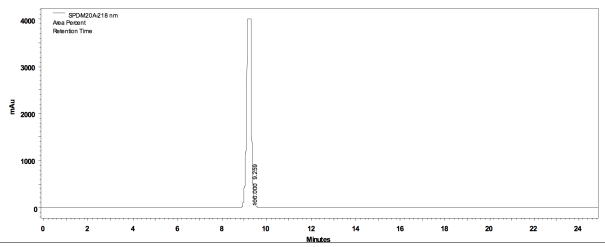
Certain compounds containing the 3-oxo group decomposed when subjected to the high temperatures required for GC. We therefore used reversed-phase high performance liquid chromatography (RP-HPLC) to analyze these compounds. All RP-HPLC data was obtained on a Shimadzu HPLC equipped with a single pump (LC-10Atvp), solvent mixer (FCV-10Alvp), controller (SCL-10Avp), autoinjector (SIL-10AF), and UV diode array detector (SPD-M10Avp). A Shimadzu Premier 25 cm x 4.6 mm C-18 reverse-phase column was used for all analytical HPLC work. A ramp from 70–95% acetonitrile in water over 20 min was used to obtain optimal separation.

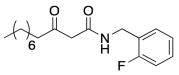
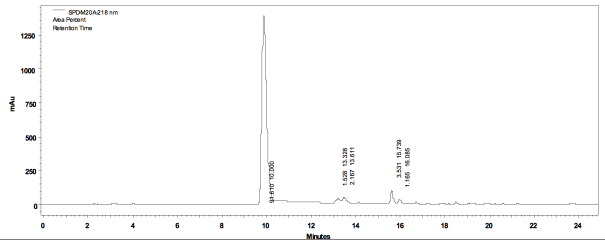
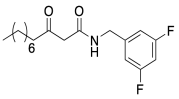
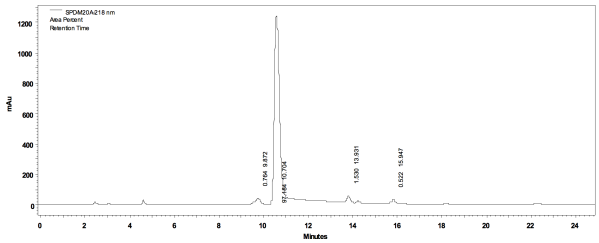
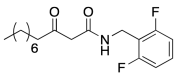
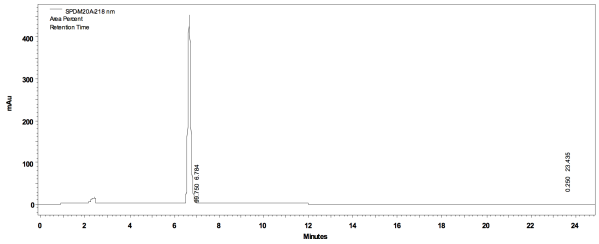
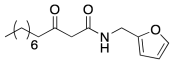
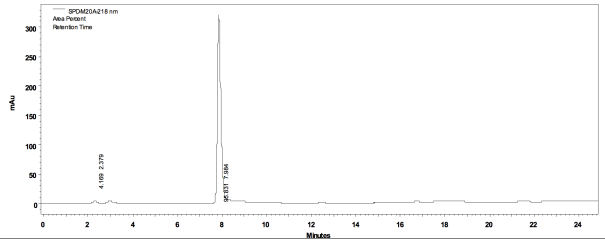
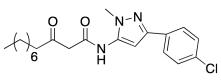
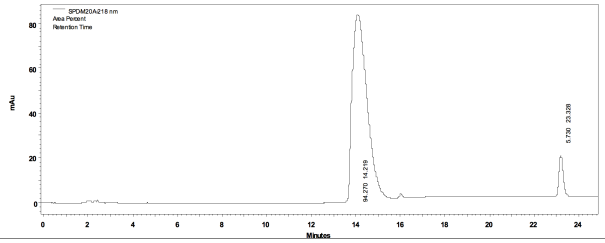
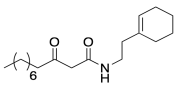
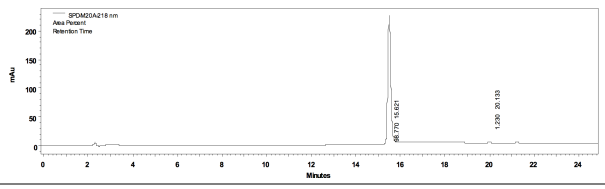
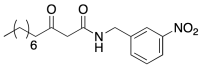
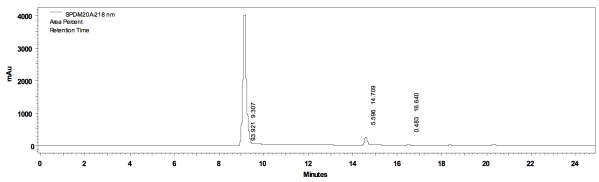
GC compound purity data.

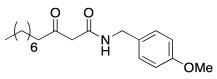
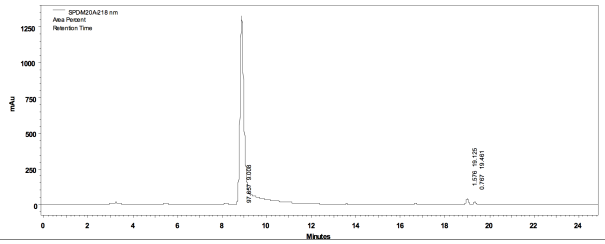
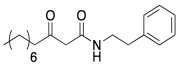
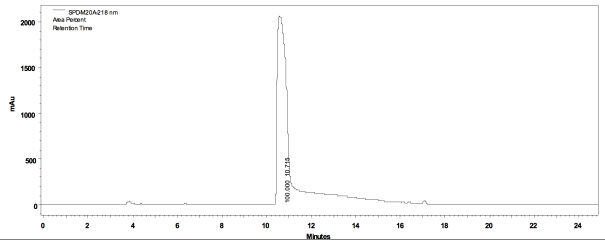
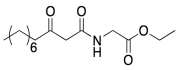
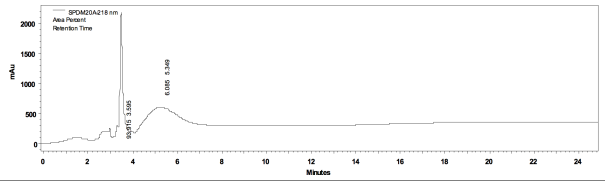
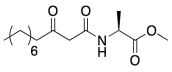
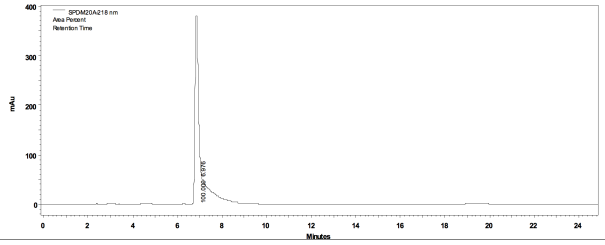
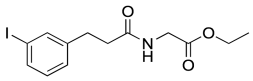
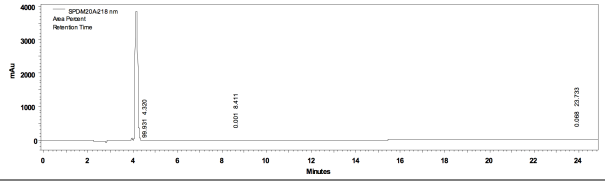
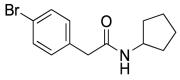
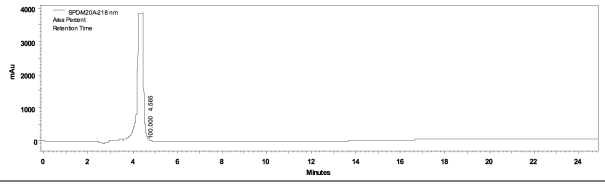
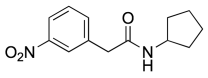
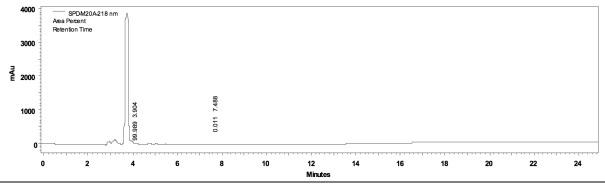
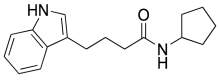
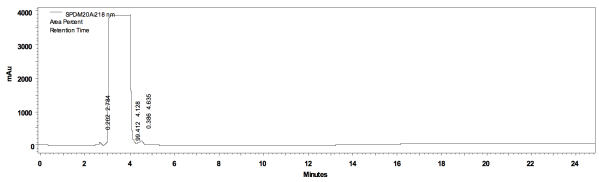
The compound peak is marked with an asterisk.

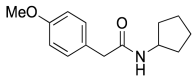
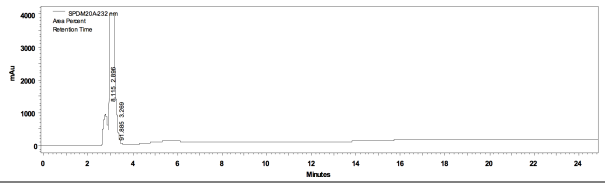
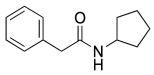
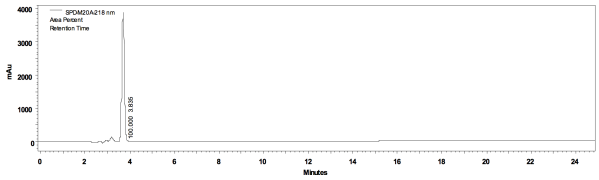
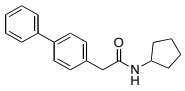
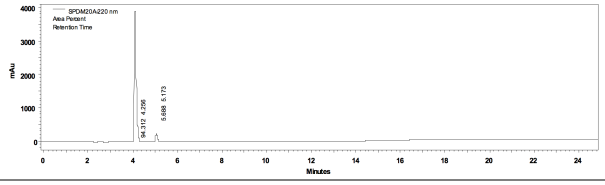
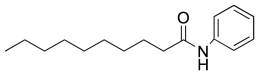
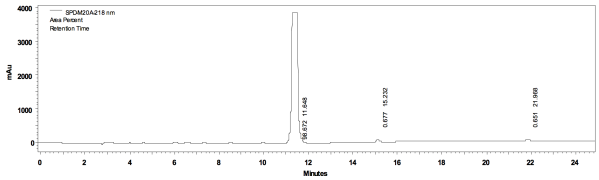
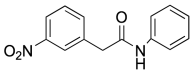
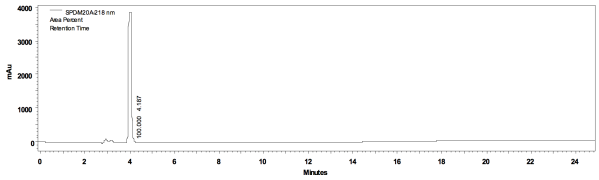
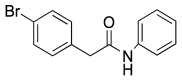
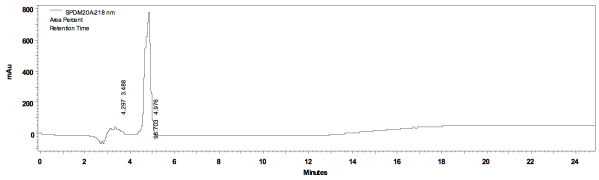
Compound Purity	Structure	GC chromatogram
28 99%		
29 99%		
30 99%		
32 90%		
33 99%		
34 89%		

HPLC compound purity data.

Compound Purity	Structure	HPLC chromatogram
Methanol Blank for HPLC	--	
12 97%		
13 92%		
14 99%		
15 94%		
16 99%		

17 92%		
18 97%		
19 99%		
20 96%		
21 94%		
22 99%		
23 94%		

24 98%		
25 99%		
26 94%		
27 99%		
31 99%		
35 99%		
36 99%		
37 99%		

38 92%		
39 99%		
40 94%		
41 99%		
42 99%		
43 96%		

Characterization data for Libraries A and B.

^{1}H NMR and MS (ESI or EI) data were obtained for all compounds. IR and ^{13}C NMR data were obtained for the most active compounds.

12: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 9.15 (NH), 7.46 (Ar-H, d, J = 7.8 Hz, 2H), 7.22 (Ar-H, t, J = 8.7 Hz, 2H), 7.01 (Ar-H, t, J = 6.8 Hz, 1H), 3.46 (CH_2, s, 2H), 2.47 (CH_2, t, J = 7.8 Hz, 2H), 1.51 (CH_2, m, 2H), 1.19 (CH_2, m, 12H), 0.80 (CH_3, t, J = 6.8 Hz, 3H); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 208.2, 163.7, 137.8, 129.2, 124.7, 120.3, 49.1, 44.4, 32.0, 29.6, 29.5, 29.4, 29.2, 23.6, 22.9, 14.3; ESI: expected m/z = 289, observed [M+Na^+] = 312.3; IR (cm^{-1}): 3304, 2921, 2852, 1710, 1652, 1604, 1555, 1442, 750.

13: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 6.99 (NH), 4.21 (NC_2H, sex, J = 7.0 Hz, 1H), 3.37 (CH_2, s, 2H), 2.53 (CH_2, t, J = 8.2 Hz, 2H), 1.97 (CH_2, m, 2H), 1.66 (CH_2, broad m, 6H), 1.42 (CH_2, m, 2H), 1.27 (CH_2, m, 13H), 0.89 (CH_3, t, J = 7.0 Hz, 3H); ^{13}C NMR: (300 MHz, $CDCl_3$) ∂ 207.8, 165.2, 51.4, 49.1, 44.2, 33.2, 32.0, 29.6, 29.5, 29.4, 29.2, 23.9, 23.6, 22.9, 14.3; ESI: expected m/z = 282.24, observed [M+H^+] = 283.3; IR (cm^{-1}): 3265, 2925, 2855, 1715, 1639, 1557, 1338.

14: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 6.82 (Ar-H, m, 3H), 5.64 (NH), 4.38 (CH_2, d, J = 5.7 Hz, 2H), 3.87 (CH_2, s, 2H), 2.70 (CH_2, t, J = 9.1 Hz, 2H), 1.63 (CH_2, m, 2H), 1.25 (CH_2, m, 12H), 0.87 (CH_3, t, J = 7.9 Hz, 3H); ESI: expected m/z = 364.2, observed [M+Na^+] = 387.4

15: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.24 (Ar-H, m, 10H), 3.97 (CH, t, J = 8.0 Hz, 1H), 3.34 (CH_2, s, 2H), 3.25 (CH_2, dt, J = 6.3, 7.7 Hz, 2H), 2.51 (CH_2, t, J = 7.4 Hz, 2H), 2.29 (CH_2, t, J = 7.3 Hz, 2H), 1.57 (CH_2, m, 2H), 1.27 (CH_2, m, 12H), 0.89 (CH_3, t, J = 3.1 Hz, 3H); ESI: expected m/z = 407.3, observed [M+H^+] = 408.4

16: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.45 (NH), 7.26 (Ar-H, m, 2H), 7.02 (Ar-H, m, 2H), 4.43 (CH_2, d, J = 5.8 Hz, 2H), 3.44 (CH_2, s, 2H), 2.52 (CH_2, t, J = 7.3 Hz, 2H), 1.56 (CH_2, m, 2H), 1.25 (CH_2, m, 12H), 0.87 (CH_3, t, J = 7.1 Hz, 3H); ESI: expected m/z = 321.4, observed [M+Na^+] = 344.4

17: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.46 (NH), 7.28 (Ar-H, m, 2H), 7.07 (Ar-H, m, 2H), 4.51 (CH_2, d, J = 4.5 Hz, 2H), 3.43 (CH_2, s, 2H), 2.51 (CH_2, t, J = 6.3 Hz, 2H), 1.57 (CH_2, m, 2H), 1.26 (CH_2, m, 12H), 0.88 (CH_3, t, J = 7.2 Hz, 3H); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 207.5, 129.6, 129.5, 124.5, 124.5, 115.8, 115.5, 58.3, 48.8, 44.2, 37.7, 32.0, 29.6, 29.5, 29.4, 29.2, 23.6, 22.9, 14.3; ESI: expected m/z = 321.4, observed [M+Na^+] = 344.3; IR (cm^{-1}): 3261, 2919, 2851,1716, 1632, 755.

18: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.62 (NH), 6.81 (Ar-H, m, 2H), 6.71 (Ar-H, 1H), 4.46 (CH_2, d, J = 5.7 Hz, 2H), 3.49 (CH_2, s, 2H), 2.54 (CH_2, t, J = 7.2 Hz, 2H), 1.59 (CH_2, m, 2H), 1.26 (CH_2, m, 12H), 0.88 (CH_3, t, J = 6.4 Hz, 3H); ESI: expected m/z = 340.4, observed [M+Na^+] = 363.4

19: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.42 (NH), 7.29 (Ar-H, m, 1H), 6.96 (Ar-H, m, 2H), 4.64 (CH_2, d, J = 5.3 Hz, 2H), 3.47 (CH_2, s, 2H), 2.57 (CH_2, t, J = 7.4 Hz, 2H), 1.62 (CH_2, m, 2H), 1.32 (CH_2, m, 12H), 0.95 (CH_3, m, J = 7.1 Hz, 3H); ESI: expected m/z = 340.2, observed [M+Na^+] = 363.4

20: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.48 (NH), 7.38 (CH-vinyl, dd, J = 1.9, 0.9 Hz, 1H), 6.33 (CH-vinyl, dd, J = 3.2, 1.9 Hz, 1H), 6.25 (CH-vinyl, dd, J = 3.2, 0.7 Hz, 1H), 4.48 (CH_2, d, J = 5.7 Hz, 2H), 3.46 (CH_2, s, H), 2.531 (CH_2, t, J = 7.2 Hz, 2H), 1.58 (CH_2, m, 2H), 1.27 (CH_2, m (appears broad s), 12H), 0.89 (CH_3, t, J = 6.2 Hz, 3H); ESI: expected m/z = 294.3, observed [M+Na^+] = 317.3

21: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 9.77 (NH), 7.71 (Ar-H, d, J = 8.4 Hz, 2H), 7.35 (Ar-H, d, J = 8.4 Hz, 2H), 6.70 (CH-vinyl, s, 1H), 3.86 (CH_3, s, 3H), 3.65 (CH_2, s, 2H), 2.61 (CH_2, t, J = 6.7 Hz, 2H), 1.65 (CH_2, m, 2H), 1.28 (CH_2, m, 12H), 0.89 (CH_3, m, 3H); ESI: expected m/z = 404.2, observed [M+H^+] = 405.4

22: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 6.87 (NH), 5.45 (CH, m, 1H), 3.36 (CH_2, s, 2H), 3.34 (CH_2, ddd, J = 6.8, 5.7, 5.6 Hz, 2H), 2.51 (CH_2, t, J = 7.4 Hz, 2H), 2.14 (CH_2, t, J = 7.0 Hz, 2H), 2.01 (CH_2, m, 2H), 1.92 (CH_2, m, 2H), 1.61 (CH_2, m, 8H), 1.26 (CH_2, m, 12H), 0.88 (CH_3, t, J = 6.4 Hz, 3H); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 207.4, 165.5, 134.6, 124.0, 49.1, 44.2, 37.7, 37.6, 32.1, 29.6, 29.5, 29.4, 29.2, 28.1, 25.5, 23.6, 23.0, 22.9, 22.6, 14.3; ESI: expected m/z = 321, observed [M+H^+] = 322.4; IR (cm^{-1}):3292, 2925, 2854, 1708, 1643, 1555.

23: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 8.13 (Ar-H, m, 2H), 7.68 (NH), 7.63 (Ar-H, m, 1H), 7.51 (Ar-H, m, 1H), 4.57 (CH_2, d, J = 5.8 Hz, 2H), 3.50 (CH_2, s, 2H), 2.54 (CH_2, t, J = 7.3 Hz, 2H), 1.59 (CH_2, m, 2H), 1.26 (CH_2, m, 12H), 0.88 (CH_3, t, J = 6.4 Hz, 3H); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 207.6, 166.2, 148.7, 140.6, 133.9, 129.8, 122.5, 48.4, 44.3, 42.8, 32.0, 29.6, 29.5, 29.4, 29.2, 23.6, 22.9, 14.3; ESI: expected m/z = 348.2, observed [M+Na^+] = 371.3; IR (cm^{-1}): 3271, 2920, 2852, 1716, 1642, 1528, 1350.

24: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.21 (Ar-H, d, J = 7.5 Hz, 2H), 6.86 (Ar-H, d, J = 8.7 Hz, 2H), 4.39 (CH_2, d, J = 6.2 Hz, 2H), 3.80 (CH_3, s, 3H), 3.42 (CH_2, s, 2H), 2.52 (CH_2, t, J = 7.3 Hz, 2H), 1.25 (CH_2, m, 12H), 0.88 (CH_3, t, J = 7.3 Hz, 3H); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 207.4, 165.6, 159.2, 130.2, 129.3, 114.3, 55.5, 48.9, 44.2, 43.3, 32.0, 29.6, 29.5, 29.4, 29.2, 23.6, 22.9, 14.3; ESI: expected m/z= 334.47, observed [M+H^+]= 335.3; IR (cm^{-1}): 3277, 2922, 2851, 1712, 1645, 1515, 1251.

25: ^{1}H NMR: (300.1 MHz, $CDCl_3$) δ 7.25 (Ar-H, m, 5H), 6.98 (NH), 3.53 (CH_2, dd, J = 7.2, 5.9 Hz, 2H), 3.36 (CH_2, s, 2H), 2.83 (CH_2, t, J = 7.2 Hz, 2H), 2.49 (CH_2, t, J = 7.5 Hz, 2H), 1.56 (CH_2, m, 2H), 1.26 (CH_2, m, 12H), 0.88 (CH_2, t, J = 7.0 Hz, 3H); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 206.1, 164.5, 137.7, 127.7, 127.6, 125.5, 47.8, 43.0, 39.7, 34.6, 30.8, 28.4, 28.3, 28.2, 28.0, 22.4, 21.6, 13.1; ESI: expected m/z=318.2, observed [M+H^+]= 319.4

26: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 4.22 (CH_2, q, J = 7.5 Hz, 2H), 4.05 (CH_2, d, J = 5.8 Hz, 2H), 3.45 (CH_2, s, 2H), 2.53 (CH_2, t, J = 7.7 Hz, 2H), 1.59 (CH_2, t, J = 7.2 Hz, 2H), 1.26 (CH_2, m, 13H), 0.88 (CH_3, t, J = 7.0 Hz, 3H); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 206.8, 169.7, 166.2, 111.3,

61.7, 48.7, 44.1, 41.6, 32.0, 29.6, 29.5, 29.4, 29.2, 23.6, 22.8, 14.3; ESI: expected m/z = 299.4, observed [M+Na^+] = 322.3; IR (cm^{-1}): 3293, 2926, 2853, 1711, 1644, 1217.

27: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.46 (NH, d, J = 6.0 Hz, 1H), 4.59 (CH, dt, J = 7.4, 7.2 Hz, 1H), 3.75 (CH_3, s, 3H), 3.42 (CH_2, s, 2H), 2.53 (CH_2, t, J = 7.2 Hz, 2H), 1.58 (CH_2, t, J = 7.4 Hz, 2H), 1.43 (CH_3, d, J = 7.2 Hz, 3H), 1.26 (CH_2, m, 10H), 0.88 (CH_3, t, J = 6.9 Hz, 3H); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 206.8, 173.3, 165.5, 52.6, 48.9, 48.3, 44.0, 32.0, 29.6, 29.5, 29.4, 29.2, 23.6, 22.8, 18.3, 14.3; ESI: expected m/z = 299.4, observed [M+H^+] = 300.4; IR (cm^{-1}): 3307, 2926, 2854, 1747, 1649, 1212, 1164; $[\alpha]_D$ ($CHCl_3$, c=0.104g/ml) = -12.6

28: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 8.16 (Aryl, d, J = 8.0 Hz, 2H), 7.65 (Aryl, t, J = 9.0 Hz, 1H), 7.53 (Aryl, t, J = 8.5 Hz, 1H), 6.13 (NH), 4.22 (CH_2, q, J = 7.4 Hz, 1H), 4.04 (CH_2, d, J = 5.8 Hz, 1H), 3.75 (CH_2, d, J = 19.6 Hz, 2H), 3.49 (CH_2, s, 2H), 1.28 (CH_3, t, J = 6.9 Hz, 3H); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 169.9, 148.6, 136.9, 135.9, 129.9, 124.5, 122.5, 61.9, 42.6, 41.8, 29.9, 14.3; EI: expected m/z = 266.1, observed [M] = 266.1

29: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.48 (Aryl, d, J = 8.1 Hz, 2H), 7.17 (Aryl, d, J = 8.1 Hz, 2H), 5.94 (NH), 4.187 (CH_2, q, J = 7.6 Hz, 2H), 4.00 (CH_2, d, J = 5.4 Hz, 2H), 3.57 (CH_2, s, 2H), 1.27 (CH_3, t, J = 6.8 Hz, 3H); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 170.7, 170.0, 168.7, 133.6, 132.3, 132.0, 131.3, 121.7, 61.8, 43.0, 41.7, 14.3; EI: expected m/z = 299.0, observed [M] = 298.9

30: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.63 (Aryl, d, J = 8.4 Hz, 2H), 7.36 (Aryl, d, J = 8.0 Hz, 2H), 6.12 (NH), 4.12 (CH_2, q, J = 7.3 Hz, 2H), 4.02 (CH_2, d, J = 5.5 Hz, 2H), 3.64 (CH_2, s, 2H), 1.26 (CH_3, t, J = 7.1 Hz, 3H); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 176.6, 171.5, 136.7, 136.6, 130.8, 123.7, 60.7, 40.8, 21.2, 14.4; EI: expected m/z = 321.3, observed [M] = 320.9

31: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.54 (Aryl, m, 2H), 7.17 (Aryl, d, J = 8.0 Hz, 1H), 7.01 (Aryl, t, J = 8.0 Hz, 1H), 6.12 (NH), 4.20 (CH_2, q, J = 7.1 Hz, 2H), 4.01 (CH_2, d, J = 5.3 Hz, 2H), 2.91 (CH_2, t, J = 8.3 Hz, 2H), 2.52 (CH_3, t, J = 8.3 Hz, 2H), 1.27 (CH_3, t, J = 7.1 Hz, 3H); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 172.0, 170.2, 143.4, 137.5, 135.6, 130.5, 127.9, 94.7, 61.8, 41.6, 37.8, 31.1, 14.4; EI: expected m/z = 361.2, observed [M] = 361.0

32: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.32 (Aryl, m, 5H), 5.89 (NH), 4.18 (CH_2, q, J = 7.2 Hz, 2H), 4.00 (CH_2, d, J = 5.9 Hz, 2H), 3.63 (CH_2, s, 2H), 1.26 (CH_3, t, J = 7.1 Hz, 3H); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 171.4, 170.0, 134.7, 129.7, 129.2, 127.7, 61.7, 43.7, 41.7, 14.3; EI: expected m/z = 221.2, observed [M] = 221.0

33: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.10 (Aryl, d, J = 8.5 Hz, 2H), 6.80 (Aryl, d, J = 8.5 Hz, 2H), 6.08 (NH), 4.18 (CH_2, q, J = 7.2 Hz, 2H), 3.98 (CH_2, d, J = 5.2 Hz, 2H), 3.76 (OMe, s, 2H), 2.90 (CH_2, t, J = 7.9 Hz, 2H), 2.50 (CH_2, t, J = 7.9 Hz, 2H), 1.26 (CH_3, t, J = 7.2 Hz, 3H); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 172.6, 170.3, 158.3, 133.0, 129.5, 114.2, 61.7, 55.4, 41.6, 38.5, 30.8, 14.3; EI: expected m/z = 265.1, observed [M^+] = 265.0

34: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.24 (Aryl, d, J = 8.4 Hz, 2H), 7.13 (Aryl, d, J = 8.4 Hz, 2H), 6.08 (NH), 4.20 (CH_2, q, J = 7.0 Hz, 2H), 4.00 (CH_2, d, J = 5.6 Hz, 2H), 2.94 (CH_2, t, J = 7.7 Hz,

2H), 2.52 (CH_2, t, J = 7.7 Hz, 2H), 1.27 (CH_3, t, J = 7.0 Hz, 3H); EI: expected m/z = 269.7, observed [M] = 268.9 and 271.0 due to Cl isotopes.

35: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.47 (Aryl, d, J = 8.4 Hz, 2H), 7.13 (Aryl, d, J = 8.4 Hz, 2H), 5.23 (NH), 3.47 (CH_2, s, 2H), 1.95 (CH_2, m, 2H), 1.56 (CH_2, m, 5H), 1.27 (CH_2, m, 2H); ESI: expected m/z = 281.0, observed [M+Na^+] = 304.2

36: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 8.15 (Aryl, m, 2H), 7.66 (Aryl, d, J = 7.3 Hz, 1H), 7.52 (Aryl, t, J = 8.4 Hz, 1H), 5.38 (NH), 3.60 (CH_2, s, 2H), 1.99 (CH_2, m, 2H), 1.62 (CH_2, m, 5H), 1.33 (CH_2, m, 2H); ESI: expected m/z = 248.1, observed [M+Na^+] = 271.2

37: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.99 (NH), 7.60 (Aryl, d, J = 7.5 Hz, 1H), 7.35 (Aryl, d, J = 8.5 Hz, 1H), 7.19 (Aryl, t, J = 8.0 Hz, 1H), 7.10 (Aryl, t, J = 7.5 Hz, 1H), 6.97 (Aryl, d, J = 7.0 Hz, 1H), 5.29 (NH), 4.18 (CH, q, J = 7.4 Hz, 1H), 2.82 (CH_2, dd, J = 8.0, 7.5 Hz, 2H), 2.44 (CH_2, q, J = 8.0 Hz, 1H), 2.20 (CH_2, t, J = 7.5 Hz, 1H), 2.01 (CH_2, m, 2H), 1.60 (CH_2, m, 4H), 1.31 (CH_2, m, 2H); ESI: expected m/z = 270.2, observed [M+H^+] = 271.3

38: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.15 (Aryl, d, J = 8.9 Hz, 2H), 6.88 (Aryl, d, J = 8.9 Hz, 2H), 5.23 (NH), 3.81 (OMe, s, 3H), 4.18 (CH, q, J = 7.4 Hz, 1H) 3.48 (CH_2, s, 2H), 1.92 (CH_2, m, 2H), 1.55 (CH_2, m, 4H), 1.23 (CH_2, m, 2H); ESI: expected m/z = 233.1, observed [M+Na^+] = 256.3

39: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.32 (Aryl, m, 5H), 5.23 (NH), 4.18 (CH, q, J = 6.8 Hz, 1H), 3.55 (CH_2, s, 2H), 1.92 (CH_2, m, 2H), 1.55 (CH_2, m, 4H), 1.23 (CH_2, m, 2H); ESI: expected m/z = 203.2, observed [M+H^+] = 204.2

40: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.58 (Aryl, m, 5H), 7.43 (Aryl, d, J = 7.9 Hz, 2H), 7.37 (Aryl, d, J = 7.7 Hz, 2H), 5.32 (NH), 4.21 (CH, q, J = 6.8 Hz, 1H), 3.58 (CH_2, s, 2H), 1.95 (CH_2, m, 2H), 1.56 (CH_2, m, 4H), 1.26 (CH_2, m, 2H); ESI: expected m/z = 279.2, observed [M+Na^+] = 302.3

41: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.51 (Aryl, d, J = 7.4 Hz, 2H), 7.31 (Aryl, t, J = 8.5 Hz, 2H), 7.09 (Aryl, t, J = 7.4 Hz, 1H), 2.35 (CH_2, t, J = 8.0 Hz, 2H), 1.72 (CH_2, m, 2H), 1.27 (CH_2, m, 12H), 0.88 (CH_3, t, J = 6.9 Hz, 3H); ESI: expected m/z = 247.2, observed [M+Na^+] = 270.3

42: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 8.20 (Aryl, t, J = 8.4 Hz, 2H), 7.73 (Aryl, d, J = 7.9 Hz, 1H), 7.56 (Aryl, t, J = 8.4 Hz, 1H), 7.47 (Aryl, d, J = 7.4 Hz, 2H), 7.32 (Aryl, t, J = 8.4 Hz, 2H), 7.19 (NH), 7.12 (Aryl, t, J = 6.8 Hz, 1H), 3.82 (CH_2, s, 2H); ESI: expected m/z = 256.1, observed [M+ H^+] = 257.1

43: ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.53 (Aryl, d, J = 8.0 Hz, 2H), 7.43 (Aryl, d, J = 7.6 Hz, 2H), 7.31 (Aryl, d, J = 7.1 Hz, 1H), 7.23 (Aryl, d, J = 8.0 Hz, 2H), 7.10 (Aryl, t, J = 7.6 Hz, 2H), 4.83 (NH), 3.69 (CH_2, 2H); ESI: expected m/z = 289.0, observed [M+Na^+] = 312.2

Biological screening protocols and supplementary assay data.

Compound handling and reagents. Stock solutions of synthetic compounds (10 mM) were prepared in DMSO and stored at room temperature (rt) in sealed vials. Solvent resistant polypropylene (Corning Costar cat. no. 3790), clear polystyrene (Corning Costar cat. no. 3997), black polystyrene clear bottom (Corning Costar cat. no. 3603), or white polystyrene clear bottom (Corning Costar cat. no. 3610) 96-well microtiter plates were used as appropriate. All biological reagents were purchased from Fisher and used according to enclosed instructions. Buffers, media, and solutions were prepared as previously described[1] for *E. coli* DH5α (pJN105L + pSC11), *V. fischeri* ESI 114 (Δ-LuxI), and *A. tumefaciens* WCF (pCF372). Medium for *P. aeruginosa* PA01 MW1 assays was prepared as follows: 20 g/L Luria-Bertani broth (Sigma) and 10.4 g/L 3-(*N*-morpholino)propanesulfonic acid (MOPS, Acros) were dissolved in distilled water, and the pH was adjusted to 7 prior to autoclaving. Immediately before using the medium, carbenicillin (300 μg/mL, Sigma) was added.

Instrumentation. Absorbance and fluorescence measurements were obtained using a PerkinElmer Wallac 2100 EnVision multilabel plate reader running Wallac Manager v1.03 software. A 600 nm filter was used for reading bacterial cell density. Filters of 420 nm and 550 nm were used for Miller-type absorbance assays. Filters of 485 nm (excitation) and 535 nm (emission) were used for evaluating the production of yellow fluorescent protein (YFP) in fluorescence assays.

Reporter gene assays. Reporter gene assays for *E. coli* DH5α (pJN105L + pSC11),[2] *V. fischeri* ESI 114 (Δ-LuxI),[3] and *A. tumefaciens* WCF (pCF372)[4] were conducted as previously described.[1] The assay using *P. aeruginosa* PA01 MW1 was modified from a literature procedure.[5] In brief, cultures were prepared by adding one PA01 MW1 colony to 5 mL of prepared medium and growing the culture overnight at 30 °C. A subculture was prepared the following morning (~16 h later) by making a 1:10 dilution of the overnight culture. This subculture was incubated with shaking at 37 °C until it reached an optical density of 0.25–0.30. The subculture was then plated into microtiter plate wells. OdDHL was added directly to the subculture for antagonist assays for a final concentration of 1 μM; agonists assays contained only the compound of interest. The outer wells of the compound plate were filled with subculture minus compound to circumvent problems due to evaporation. After plating was complete, plates were incubated with shaking at 37 °C for 8 h. The optical density and fluorescence of the wells were read thereafter.

Table S-1. Antagonism and agonism assay data for Library A in LuxR and TraR.[a]

	V. fischeri LuxR		*A. tumefaciens* TraR	
Compound	Antagonism [%] [b]	Agonism [%] [c]	Antagonism [%] [b]	Agonism [%] [c]
12	12	2	-33	1
13	89	4	-3	-1
14	-14	1	-10	0
15	-39	6	-6	-1
16	-35	1	-11	0
17	-27	2	-11	-1
18	-33	3	-10	-1
19	-24	5	-15	-1
20	-4	4	1	-1
21	2	3	-25	-2
22	35	3	-22	-1
23	44	2	-6	0
24	15	2	-8	0
25	7	1	-4	0
26	-22	8	-3	2
27	24	4	-4	0

[a] All synthetic compounds were screened at 10 μM. All assays were preformed in triplicate; error did not exceed ± 10%. Negative controls contained neither synthetic nor native ligand, and were subtracted from each sample to account for background. Negative inhibition values indicate that the compound activates at the tested concentration. [b] Antagonism assays were preformed against the native ligand at its EC_{50} value for each strain: *V. fischeri* ESI 114 (Δ-LuxI) = 2 μM OHHL; *A. tumefaciens* WCF (pCF372) = 200 nM OOHL. [c] Agonism assays were normalized to that for the native ligand at 100 μM in each strain.

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