Supporting Information

A modular library of small molecule signals regulates social behaviors in
*Caenorhabditis elegans*

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Supporting Methods

1. Calculation of number of molecules of icas#3 in one L4 worm volume at 100 fM.

\[ V_{YA} : \text{volume of } C.\ elegans \text{ young adult } [1]; c:\text{ concentration (100 fM)}; N_A: \text{ Avogadro’s number.} \]

\[ V_{YA} = 3 \times 10^6 \, \mu m^3 = 3 \times 10^{-9} \, L; \, c = 10 \, fM = 10^{-14} \, M \]

\[ n = V_{YA} \times c \times N_A = 3 \times 10^{-9} \, L \times 10^{-14} \, \text{mol*L}^{-1} \times 6.022 \times 10^{23} \, \text{mol}^{-1} = 18 \, \text{molecules.} \]

Result: there are 18 icas#3 molecules contained in one worm volume of agar at an icas#3 concentration of 10 fM (femtomolar).

2. Synthesis of indole ascarosides

2.1 Synthesis of ascr#9.

(R)-hex-5-en-2-ol 2 (32 mg, 0.32 mmol, prepared as described [2]) was coupled to trichloroacetimide 1 (150 mg, 0.3 mmol, [2]) using the conditions described for the synthesis of ascr#6. The resulting glycoside 3 (92 mg, 0.21 mmol) was dissolved in acetone (2 ml) and treated with 2 ml of a 1 M solution of potassium permanganate. After 30 min, the reaction mixture was poured into a mixture of ice-cold saturated aqueous sodium chloride solution (5 ml), acetic acid (0.1 ml), and dichloromethane (5 ml). The organic phase was separated and the aqueous phase extracted with two 5 ml-portions of dichloromethane. The combined organic extracts were dried over sodium sulfate, evaporated to dryness and re-dissolved in a mixture of 0.5 M aqueous lithium hydroxide (2 ml) and dioxane (6 ml). The mixture was stirred for 3 h at 70 ºC, then cooled to 23 ºC and acidified with 0.2 M aqueous hydrochloric acid. The mixture was evaporated to dryness and purified via Combiflash column.
chromatography using a methanol-dichloromethane solvent gradient, yielding 15.6 mg (0.063 mmol) of pure ascr#9 as a viscous oil.

**Spectroscopic data for ascr#9.**

$^1$H (600 MHz) and $^{13}$C (126 MHz) NMR spectroscopic data for ascr#9 were acquired in methanol-$d_4$. Chemical shifts were referenced to (CD$_2$HOD) = 3.31 ppm and (CD$_2$HOD) = 49.05 ppm. Coupling constants are given in Hertz [Hz]. $^1$H NMR (600 MHz, CD$_3$OD): δ 4.65 (s, 1H), 3.84 (m, 1H), 3.72 (m, 1H), 3.61 (dq, 1H, $J = 9.4$ Hz, $J = 6.1$ Hz), 3.51 (ddd, 1H, $J = 11.2$ Hz, $J = 9.5$ Hz, $J = 4.5$ Hz), 2.43 (m, 2H), 1.95 (dt, 1H, $J = 13.1$ Hz, $J = 3.5$ Hz), 1.71-1.87 (m, 3H), 1.22 (d, 3H, $J = 6.1$ Hz), 1.15 (d, 3H, $J = 6.1$ Hz) ppm; $^{13}$C NMR (126 MHz, CD$_3$OD): δ 174.5, 97.3; 71.5, 71.4, 69.9, 68.4, 36.0, 33.5, 31.3, 19.1, 18.1 ppm; ESI-MS ($m/z$): [M–H] 247.2.

**2.2 Synthesis of ascr#10.**

A stirred solution of ascr#3 (3.2 mg, 10.6 µmol) [2] in 10 ml of ethanol was hydrogenated using palladium on activated carbon (10% Pd, 1 atm H$_2$ pressure) at 23 ºC for 18 h. After completion, the reaction was evaporated to dryness, and the residue filtered over a short pad of silica using a 1:8 (v/v) mixture of methanol and dichloromethane yielding 3.0 mg (9.9 µmol) of pure ascr#10.

**Spectroscopic data for ascr#10.**

$^1$H (600 MHz) and $^{13}$C (126 MHz) NMR spectroscopic data for ascr#10 were acquired in methanol-$d_4$. Chemical shifts were referenced to (CD$_2$HOD) = 3.31 ppm and (CD$_2$HOD) = 49.05 ppm. Coupling constants are given in Hertz [Hz]. $^1$H NMR (600 MHz, CD$_3$OD): δ 4.64 (s, 1H), 3.78 (m, 1H), 3.71 (m, 1H), 3.63 (dq, 1H, $J = 9.3$ Hz, $J = 6.2$ Hz), 3.51 (ddd, 1H, $J = 11.2$ Hz, $J = 9.3$ Hz, $J = 4.6$ Hz), 2.27 (t, 2H, $J = 7.4$ Hz), 1.94 (dt, 1H, $J = 13.0$ Hz, $J = 3.7$ Hz), 1.77 (ddd, 1H, $J = 13.3$ Hz, $J = 11.5$ Hz, $J = 3.0$ Hz), 1.61 (m, 2H), 1.56 (m, 1H), 1.46 (m, 1H), 1.32-1.38 (m, 6H), 1.21 (d, 3H, $J = 6.2$ Hz), 1.12 (d, 3H, $J = 6.1$ Hz) ppm; $^{13}$C NMR (126 MHz, CD$_3$OD): δ 177.7, 97.3, 72.3, 71.0, 69.8, 68.1, 38.1, 38.2, 35.7, 34.9, 30.0, 26.5, 26.0, 19.0, 18.0 ppm; ESI-MS ($m/z$): [M–H] 303.2.
2.3 Synthesis of icas\#1.

Conversion of ascr\#1 into the corresponding methyl ester. Ascr\#1 (10 mg, 0.036 mmol), prepared using previously described methods [2], was dissolved in a mixture of toluene (1 mL) and methanol (1 mL), and a solution of trimethylsilyldiazomethane (2 M solution in hexane, 50 µL, 0.1 mmol) was added. After stirring for 20 min at 23 °C, excess trimethylsilyldiazomethane was destroyed by addition of acetic acid (40 µL) and solvents were removed in vacuo, yielding ascr\#1 methyl ester (10.3 mg, 0.035 mmol) as a viscous oil.

Preparation of a solution of indole-3-carbonyl chloride. A well-stirred suspension of indole-3-carboxylic acid (67.7 mg, 0.42 mmol) in dry dichloromethane (2 ml) containing a small amount of dimethylformamide (20 µL) was treated with oxalyl chloride (0.84 mmol, 107 mg, 72 µL) at 0 °C. Following addition to the oxalyl chloride, the mixture was stirred for 20 min at 23 °C, which produced a clear, slightly yellow solution. This solution was evaporated to dryness in vacuo at 0.1 Torr to ensure removal of excess oxalyl chloride and subsequently re-dissolved in 2 ml of dry dichloromethane.

Preparation of icas\#1. The sample of ascr\#1 methyl ester (10.3 mg, 0.035 mmol) was dissolved in 1 ml of dry dichloromethane and diisopropylethylamine was added (129 mg, 1 mmol). The resulting solution was equipped with an effective stir bar and cooled to -20 °C. Subsequently, the above solution of indole-3-carboxylic acid chloride was added drop wise over a period of 10 min with vigorous stirring. The well-stirred reaction was gradually warmed to -7 °C at which temperature ice-cold saturated aqueous sodium bicarbonate solution (2 ml) was added. The biphasic mixture was allowed to warm to 20 °C and extracted three times with ethyl acetate. The combined ethyl acetate extracts were evaporated in vacuo and subjected to column chromatography on silica gel using 0-10% methanol in dichloromethane. Fractions containing the bis-2,4-O-(indole-3-carbonyl)-derivative of the ascr\#1 methyl ester were combined, evaporated to dryness and treated with a mixture of 3 ml aqueous 0.5 M lithium hydroxide solution and 7 ml dioxane at 67 °C for 3 h. Subsequently, the reaction mixture was cooled to 23 °C, neutralized by addition of 0.2 M aqueous hydrochloric acid and evaporated in vacuo. The residue was purified by HPLC, using the Agilent 1100 Series HPLC system equipped with an Agilent Eclipse XDB C-18 column (25 cm x 9.4 mm, 5 µm particle
diameter). Acetonitrile and 0.1% aqueous acetic acid were used as solvents, increasing the percentage of acetonitrile from 15% at 0 min to 85% at 30 min. Icas#1-containing fractions were evaporated yielding 5.8 mg (0.014 mmol) of the target compound as a wax-like white solid.

**Spectroscopic data of icas#1.** $^1$H (600 MHz), $^{13}$C (126 MHz), and HMBC NMR spectroscopic data for icas#1 in methanol-$d_4$. Chemical shifts were referenced to (CD$_3$HOD) = 3.31 ppm and (CD$_2$HOD) = 49.05 ppm. Coupling constants are given in Hertz [Hz]; *: interchangeable.

| Position | $\delta^{13}$C [ppm] | $\delta^1$H [ppm] | $^1$H-$^1$H-coupling constants |
|----------|---------------------|-----------------|-----------------------------|
| 1        | 177.6               | 2.35            | $J_{2,3} = 7.4$              |
| 2        | 35.1                | 2.35            |                             |
| 3        | 26.6*               | 1.45-1.70       |                             |
| 4        | 26.2*               | 1.45-1.70       |                             |
| 5        | 38.1                | 1.45-1.70       |                             |
| 6        | 72.7                | 3.86            |                             |
| 7        | 19.4                | 1.17            | $J_{6,7} = 6.1$              |
| 1*       | 97.7                | 4.75            |                             |
| 2*       | 69.6                | 3.79            |                             |
| 3*       | 33.5                | 2.01 (ax)       | $J_{3',ax,3',eq} = 13.0$, $J_{3',ax,4'} = 11.4$, $J_{2',3',ax} = 2.9$ |
|          |                     | 2.21 (eq)       | $J_{2',3',eq} = 3.2$, $J_{3',eq,4'} = 4.7$ |
| 4*       | 70.6                | 5.12            | $J_{4',5'} = 9.6$            |
| 5*       | 68.8                | 4.05            | $J_{5',6'} = 6.3$            |
| 6*       | 18.3                | 1.24            |                             |
| 2**      | 133.5               | 7.97            |                             |
| 3**      | 108.4               |                 |                             |
| 3''-COO  | 166.5               |                 |                             |
| 3a**     | 127.3               |                 |                             |
| 4**      | 121.9               | 8.02            | $J_{4'',5''} = 7.2$          |
| 5**      | 122.7               | 7.16            |                             |
| 6**      | 123.8               | 7.29            |                             |
| 7**      | 113.1               | 7.44            | $J_{6'',7''} = 7.9$          |
| 7a**     | 138.2               |                 |                             |
2.4 Synthesis of icas\#3.

Conversion of ascr\#3 into the corresponding methyl ester. Ascr\#3 (5.2 mg, 0.017 mmol), prepared as described previously [2], was dissolved in a mixture of toluene (1 mL) and methanol (1 mL). To this mixture, a solution of trimethylsilyldiazomethane (2 M solution in hexane, 25 \( \mu \)L, 0.05 mmol) was added. After stirring for 20 min at 23 °C, excess trimethylsilyldiazomethane was destroyed by addition of acetic acid (30 \( \mu \)L) and solvents were removed in vacuo, yielding ascr\#3 methyl ester (5.3 mg, 0.017 mmol) as a viscous oil.

Preparation of icas\#3. The sample of ascr\#3 methyl ester (10.3 mg, 0.035 mmol) was reacted with indole carbonyl chloride as describes above for the preparation of icas\#1, using proportionally smaller amounts of all reagents. Purification of the crude reaction products via HPLC using the conditions described above yielded icas\#3 (2.3 mg, 5.2 \( \mu \)mol) as a colorless oil. NMR spectroscopic data are listed below. For copies of the \(^1\)H NMR, HSQC, and HMBC spectra, see section 4 below “Supporting NMR spectra”.

**Spectroscopic data of icas\#3.** \(^1\)H (600 MHz), \(^13\)C (126 MHz), and HMBC NMR spectroscopic data for icas\#3 in methanol-\(d_4\). Chemical shifts were referenced to (CD\(_2\)HOD) = 3.31 ppm and (CD\(_2\)HOD) = 49.05 ppm. Coupling constants are given in Hertz [Hz].

| Position | \(\delta\) \(^{13}\)C [ppm] | \(\delta\) \(^1\)H [ppm] | \(^1\)H-\(^1\)H-coupling constants | Relevant HMBC correlations |
|----------|-----------------|-----------------|---------------------------------|--------------------------|
| 1        | 173.7           | 5.82            | \(J_{2,3} = 15.1\)              | C-1, C-4, C-5            |
| 2        | 126.7           | 6.79            | \(J_{3,4} = 6.9\)              | C-2, C-3, C5, C-6        |
| 3        | 147.8           | 2.23            |                                 | C-4, C-6                 |
| 4        | 33.6            | 1.54            |                                 |                          |
| 5        | 29.9            | 1.51            |                                 |                          |
| 6        | 27.2            | 1.51            |                                 |                          |
| 7        | 38.7            | 1.53            | C-5, C-6, C-8                   |                          |
| 8        | 73.1            | 3.83            | C-6, C-7, C-9                   |                          |
| 9        | 19.9            | 1.15            | \(J_{8,9} = 6.1\)              | C-7, C-8                 |
| 1\(^{-}\) | 98.0            | 4.73            | C-3\(^{-}\), C-5\(^{-}\), C-8   |                          |
| 2\(^{-}\) | 70.1            | 3.77            | C-4\(^{-}\)                    |                          |
| 3\(^{-}\) | 33.8            | 1.98 (ax)       | \(J_{3ax,3eq} = 13.0\)         |                          |
### 2.5 Synthesis of icas#7.

A standard sample of icas#7 (120 µg) was obtained from ascr#7 (0.5 mg) [2] as described above for the preparation of icas#1 from ascr#1.

**Spectroscopic data of icas#7.** $^1$H (600 MHz) NMR spectroscopic data for **icas#7** were obtained using methanol-$d_4$. Chemical shifts were referenced to (CD$_3$HOD) = 3.31 ppm. Coupling constants are given in Hertz [Hz].

| Position | $^1$H [ppm] | $^1$H-$^1$H-coupling constants |
|----------|-------------|---------------------------------|
| 2        | 5.84        | $J_{3,3} = 15.3$                |
| 3        | 6.99        | $J_{3,4} = 6.8$                 |
| 4        | 2.40        |                                 |
| 5        | 1.70        |                                 |
| 6        | 1.65        |                                 |
| 7        | 3.91        |                                 |
| 7a       | 1.18        | $J_{6,7} = 6.1$                 |
| 1*  | 4.75 |
|-----|------|
| 2*  | 3.80 |
| 3*  | 2.03 (ax) | $J_{3'ax,3'eq} = 13.0$, $J_{3'ax,4'} = 11.4$, $J_{2',3'ax} = 2.9$ |
|     | 2.21 (eq) | $J_{2',3'eq} = 3.2$, $J_{3'eq,4'} = 4.7$ |
| 4*  | 5.12 |
| 5*  | 4.07 | $J_{4',5'} = 9.6$ |
| 6*  | 1.24 |
| 2** | 7.97 |
| 4** | 8.04 | $J_{4'',5''} = 7.5$ |
| 5** | 7.15 |
| 6** | 7.16 |
| 7** | 7.43 | $J_{6'',7''} = 7.9$ |

2.6 Synthesis of icas#9.

Icas#9 was obtained from ascr#9 as described above for the preparation of icas#1 from ascr#1. NMR-spectroscopic data are in agreement with published data [3].
3. References cited in “Supporting Methods”

1. Knight CG, Patel MN, Azevedo RB, Leroi AM (2002) A novel mode of ecdysozoan growth in *Caenorhabditis elegans*. Evol Dev 4: 16-27.

2. Pungaliya C, Srinivasan J, Fox BW, Malik RU, Ludewig AH, et al. (2009) A shortcut to identifying small molecule signals that regulate behavior and development in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 106: 7708-7713.

3. Butcher RA, Ragains JR, Clardy J (2009) An indole-containing dauer pheromone component with unusual dauer inhibitory activity at higher concentrations. Org Lett 11: 3100-3103.

4. Supporting NMR Spectra

**NMR Spectrum 1.** Wild-type (N2) metabolome fraction containing *daf*-22 dependent indole derivatives (red boxes), central section of dqfCOSY NMR spectrum (CDCl₃, 600 MHz).

**NMR Spectrum 2.** Synthetic icas#3, ¹H NMR spectrum (CD₃OD, 600 MHz).

**NMR Spectrum 3.** Synthetic icas#3, ¹H, ¹³C-HSQC spectrum (CD₃OD, 600 MHz).

**NMR Spectrum 4.** Synthetic icas#3, ¹H, ¹³C-HMBC spectrum (CD₃OD, 600 MHz).
NMR Spectrum 1.
Wild-type (N2) metabolome fraction containing *daf-22* dependent indole derivatives (red boxes), central section of dqfCOSY NMR spectrum (CDCl$_3$, 600 MHz).
NMR Spectrum 2.
Synthetic icas#3, $^1$H NMR spectrum (CD$_3$OD, 600 MHz)
NMR Spectrum 3.
Synthetic icas#3, $^1$H, $^{13}$C-HSQC spectrum (CD$_3$OD, 600 MHz)
NMR Spectrum 4.
Synthetic icas#3, $^1$H, $^{13}$C-HMBC spectrum (CD$_3$OD, 600 MHz)