Supplemental information

Evidence that Indo-Pacific bottlenose dolphins self-medicate with invertebrates in coral reefs

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Supplementary Information

Table S1 Compilation of the organisms explicitly accessed by the dolphins for repeated rubbing behaviour. Sampling data on the three different organisms collected from 30\textsuperscript{th} July 2019 to 1\textsuperscript{st} August 2019 according to the standards of recreational and scientific diving (VDST, CMAS) and according to Egyptian regulations, Related to Figure 1.

| Marine organism | ID  | Sample place       | Depth [m] |
|-----------------|-----|--------------------|-----------|
|                 | G1  | Shaab El Fanous West | 9.5       |
|                 | G2  | Shaab El Fanous East | 12.0      |
|                 | G3  | Shaab El Erg        | 10.5      |
|                 | G4  | Shaab El Erg        | 10.6      |
|                 | G5  | Shaab El Erg        | 10.8      |
|                 | G6  | Shaab El Erg        | 10.0      |
|                 | G7  | Shaab El Erg        | 10.5      |
|                 | G8  | Shaab El Erg        | 10.6      |
|                 | G9  | Shaab El Erg        | 10.8      |
|                 | G10 | Shaab El Erg        | 10.0      |
| *Rumphella aggregata* | | | |
|                 | S1  | Shaab El Fanous East | 12.7      |
|                 | S2  | Shaab El Fanous East | 12.8      |
|                 | S3  | Shaab El Erg        | 8.3       |
|                 | S4  | Shaab El Erg        | 9.7       |
|                 | S5  | Shaab El Erg        | 7.8       |
|                 | S6  | Shaab El Erg        | 7.1       |
|                 | S7  | Shaab El Erg        | 8.3       |
|                 | S8  | Shaab El Erg        | 9.7       |
|                 | S9  | Shaab El Erg        | 7.8       |
|                 | S10 | Shaab El Erg        | 7.1       |
| *Ircinia sp.*    | | | |
|                 | L1  | Shaab El Erg        | 10.5      |
|                 | L2  | Shaab El Erg        | 12.0      |
|                 | L3  | Shaab El Erg        | 9.2       |
|                 | L4  | Shaab El Erg        | 9.4       |
|                 | L5  | Shaab El Erg        | 9.8       |
|                 | L6  | Shaab El Erg        | 9.2       |
|                 | L7  | Shaab El Erg        | 9.2       |
|                 | L8  | Shaab El Erg        | 9.4       |
|                 | L9  | Shaab El Erg        | 9.8       |
|                 | L10 | Shaab El Erg        | 9.2       |

*Sarcophyton sp.*
Table S2 Sample preparation of the three different organisms collected. Related to Figure 1.

| Sponge ID | Treated in | Same as | Tube no. | Extraction solvent | Solvent volume [µL] | ad volume [µL] | Sample weight [mg]² |
|-----------|------------|---------|----------|---------------------|---------------------|-----------------|---------------------|
| S1        | Lab        | 1       | Methanol | 400                 | -                   | 1352            |
|           |            | 2       | Methanol | 400                 | -                   | 1242            |
| S11       |            | 3       | n-Hexane | 400                 | 1350                | 1465            |
| S2        | Lab        | 1       | Methanol | 400                 | -                   | 1738            |
|           |            | 2       | Methanol | 400                 | -                   | 1389            |
| S12       |            | 3       | n-Hexane | 400                 | 1350                | 1122            |
| S3        | Lab        | 1       | Methanol | 400                 | -                   | 1216            |
|           |            | 2       | Methanol | 400                 | -                   | 1463            |
| S13       |            | 3       | n-Hexane | 400                 | 1350                | 1240            |
| S4        | Lab        | 1       | Methanol | 400                 | -                   | 875             |
|           |            | 2       | Methanol | 400                 | -                   | 1088            |
| S14       |            | 3       | n-Hexane | 400                 | 1350                | 949             |
| S5        | Lab        | 1       | Methanol | 400                 | -                   | 1139            |
|           |            | 2       | Methanol | 400                 | -                   | 1314            |
| S15       |            | 3       | n-Hexane | 400                 | 1350                | 1131            |
| S6        | Lab        | 1       | Methanol | 400                 | -                   | 1369            |
|           |            | 2       | Methanol | 400                 | -                   | 1452            |
| S16       |            | 3       | n-Hexane | 400                 | 1350                | 1463            |
| S7        | Boat       |         | Methanol | 1000                | -                   | 600             |
| S8        | Boat       |         | Methanol | 1000                | -                   | 897             |
| S9        | Boat       |         | Methanol | 1000                | -                   | 970             |
| S10       | Boat       |         | Methanol | 1000                | -                   | 897             |

| Gorgonian coral ID | Treated in | Same as | Tube no. | Extraction solvent | Solvent volume [µL] | After addition³ | Sample weight [mg]² |
|--------------------|------------|---------|----------|---------------------|---------------------|-----------------|---------------------|
| G1                 | Lab        | 1       | Methanol | 800                 | -                   | 1224            |
|                    |            | 2       | Methanol | 800                 | -                   | 1258            |
| G11                |            | 3       | n-Hexane | 800                 | 1500                | 892             |
| G2                 | Lab        | 1       | Methanol | 800                 | -                   | 1285            |
|                    |            | 2       | Methanol | 800                 | -                   | 1090            |
| G12                |            | 3       | n-Hexane | 800                 | 1500                | 671             |
| G3                 | Lab        | 1       | Methanol | 800                 | -                   | 1088            |
|                    |            | 2       | Methanol | 800                 | -                   | 1138            |
| G13                |            | 3       | n-Hexane | 800                 | 1500                | 730             |
| G4                 | Lab        | 1       | Methanol | 800                 | -                   | 1231            |
|                    |            | 2       | Methanol | 800                 | -                   | 1379            |
| G14                |            | 3       | n-Hexane | 800                 | 1500                | 685             |
| G5                 | Lab        | 1       | Methanol | 800                 | -                   | 1146            |
|                    |            | 2       | Methanol | 800                 | -                   | 1016            |
| G15                |            | 3       | n-Hexane | 800                 | 1500                | 605             |
| G6                 | Lab        | 1       | Methanol | 800                 | -                   | 1043            |
|                    |            | 2       | Methanol | 800                 | -                   | 1049            |
| G16                |            | 3       | n-Hexane | 800                 | 1500                | 458             |
| G7                 | Boat       |         | Methanol | 1000                | -                   | 813             |
| G8                 | Boat       |         | Methanol | 1000                | -                   | 967             |
| G9                 | Boat       |         | Methanol | 1000                | -                   | 735             |
| G10                | Boat       |         | Methanol | 1000                | -                   | 1046            |
| Leather coral ID | Treated in | Same as | Tube no. | Extraction solvent | Solvent volume [µL] | After addition [µL] | Sample weight [mg] |
|-----------------|------------|---------|---------|-------------------|---------------------|-------------------|------------------|
| L1              | Lab        |         | 1       | Methanol          | 200                 | -                 | 853              |
| L11             | Lab        |         | 2       | Methanol          | 200                 | -                 | 936              |
|                 |            |         | 3       | n-Hexane          | 200                 | 1100              | 915              |
| L2              | Lab        |         | 1       | Methanol          | 200                 | -                 | 899              |
| L12             | Lab        | L7      | 2       | Methanol          | 200                 | -                 | 952              |
|                 |            |         | 3       | n-Hexane          | 200                 | 1100              | 915              |
| L3              | Lab        | L7      | 1       | Methanol          | 200                 | -                 | 925              |
|                 |            |         | 2       | Methanol          | 200                 | -                 | 887              |
|                 |            |         | 3       | n-Hexane          | 200                 | 1100              | 823              |
| L4              | Lab        | L8      | 1       | Methanol          | 200                 | -                 | 786              |
|                 |            |         | 2       | Methanol          | 200                 | -                 | 984              |
|                 |            |         | 3       | n-Hexane          | 200                 | 1100              | 915              |
| L5              | Lab        | L9      | 1       | Methanol          | 200                 | -                 | 1002             |
|                 |            |         | 2       | Methanol          | 200                 | -                 | 933              |
|                 |            |         | 3       | n-Hexane          | 200                 | 1100              | 1180             |
| L6              | Lab        | L10     | 1       | Methanol          | 200                 | -                 | 770              |
|                 |            |         | 2       | Methanol          | 200                 | -                 | 933              |
|                 |            |         | 3       | n-Hexane          | 200                 | 1100              | 722              |
| L7              | Boat       |         |         | Methanol          | 1000                | -                 | 972              |
| L8              | Boat       |         |         | Methanol          | 1000                | -                 | 994              |
| L9              | Boat       |         |         | Methanol          | 1000                | -                 | 687              |
| L10             | Boat       |         |         | Methanol          | 1000                | -                 | 843              |

1. 200 µL n-Hexane and 750 µL acetone were added.

2. The extractant volumes in the Eppendorf tubes were 200, 400, 800 and 1000 µL of n-hexane or methanol as specified. In Germany, the weight of the samples was determined approximately: three empty Eppendorf tubes with the respective extractant were weighed and averaged. This mean value was subtracted from the respective sample tube weight.

3. 200 µL n-Hexane and 500 µL acetone were added.

4. 200 µL n-Hexane and 700 µL acetone were added.
Table S3 Ten different assays applied for effect-directed profiling. Organisms used in the effect-directed assays and respectively generated responses for detection of bioactive metabolites in the three selected substrates. Related to Figure 2.

| Biological assay | Final response of the band | Interpretation |
|------------------|---------------------------|----------------|
| **Gram-negative bacteria** | | |
| *Aliivibrio fischeri* | Dark, Bright | Anthimicrobials and bioactive compounds - Reduction of bioluminescence related to decrease of energetic cell metabolism - Increase of bioluminescence related to improved energetic cell metabolism |
| *Salmonella typhimurium TA1535/pSK1002* | Blue fluorescent | Genotoxic effect |
| **Gram-positive bacteria** | | |
| *Bacillus subtilis* | Colourless | Anthimicrobial |
| **Yeast cells** | | |
| *Saccharomyces cerevisiae BJ3505* | Blue fluorescent | Estrogen-like effect |
| | Reduction of blue fluorescence | Antiestrogen-like effect |
| *Saccharomyces cerevisiae BJ1991* | Blue fluorescent | Androgen-like effect |
| | Reduction of blue fluorescence | Antiandrogen-like effect |
| **Biochemical assay** | | |
| **Enzymes** | | |
| Acetylcholinesterase inhibition assay | Colourless | Neurotoxins or inhibitors of the degrading of the neurotransmitter acetylcholine in the nerve synapses, thus improving cholinergic neurotransmission |
| Butyrylcholinesterase inhibition assay | Colourless | Neurotoxins or inhibitors decreasing the increased number of neuritic plaques in demented brains, acting non-specific in plasma and tissue |
| **Chemical assay** | | |
| Chemical | | |
| 2,2-Diphenyl-1-picrylhydrazyl (DPPH•) | Yellow | Radical scavengers |
Table S4 Compilation of activities described in literature. Bioactive metabolites already isolated and described for the three different marine organisms. Related to Figures 3–5.

| Species          | Substance                                | Molecular formula | Weight (Da) | Effect                                                                 | Literature |
|------------------|------------------------------------------|-------------------|-------------|------------------------------------------------------------------------|------------|
| *Ircinia* sp.    | 7-Methyl-9-oxo-dec-7-eneoic acid         | C_{15}H_{29}O_3   | 198.26      | Active in Alzheimer’s diseases                                         | (Tatli et al. 2008) |
|                  | 2-Hexaprenylhydroquinone                 | C_{36}H_{54}O_2   | 518.81      | Reverse transcriptase and HIV-integrase inhibitor, bacteriostatic       | (Bifulco et al. 1995; Loya et al. 1997) |
|                  | Pentaprenylhydroquinone 4-sulfate         | C_{33}H_{44}O_5S  | 530.77      | Neuropeptide Y receptor, tyrosine protein kinase and HIV-integrase inhibitor | (Bifulco et al. 1995) |
|                  | Hexaprenylhydroquinone 4-sulfate          | C_{36}H_{54}O_2S  | 598.89      | Neuropeptide Y receptor, tyrosine protein kinase and HIV-integrase inhibitor | (Bifulco et al. 1995) |
|                  | Heptaprenylhydroquinone 4-sulfate         | C_{41}H_{62}O_5S  | 667.01      | Neuropeptide Y receptor, tyrosine protein kinase and HIV-integrase inhibitor | (Bifulco et al. 1995) |
|                  | Variabilin                               | C_{25}H_{34}O_4   | 398.54      | Antibacterial                                                          | (Faulkner 1973) |
|                  | Fasciculatin                             | C_{25}H_{34}O_4   | 398.54      | Inosine monophosphate dehydrogenase inhibitor                          | (Cafieri et al. 1972; Rifai et al. 2005) |
|                  | Palinurin                                | C_{25}H_{34}O_4   | 398.54      | Cytotoxic                                                              | (Martí et al. 2003) |
|                  | Tedanolide C                             | C_{32}H_{56}O_{11} | 610.73     | Cytotoxic                                                              | (Blunt et al. 2008) |
|                  | Irciniasulfonic acid B1                  | C_{36}H_{60}NO_6S | 639.97      | Reversing multi-drug resistance in cancer cells                        | (Chung et al. 2008b) |
|                  | Irciniasulfonic acid B2                  | C_{38}H_{62}NO_6S | 613.93      | Reversing multi-drug resistance in cancer cells                        | (Blunt et al. 2008) |
|                  | Irciformonin C                          | C_{23}H_{34}O_7   | 422.51      | Moderately cytotoxic                                                   | (Chung et al. 2008c) |
|                  | Irciformonin D                          | C_{23}H_{34}O_7   | 422.51      | Moderately cytotoxic                                                   | (Chung et al. 2008d) |
|                  | Ircinolin A                              | C_{21}H_{32}O_6   | 382.49      | Cytotoxic                                                              | (Mioso et al. 2017) |
|                  | 15-Acetylicriciformonin B                | C_{34}H_{44}O_6   | 418.52      | Cytotoxic                                                              | (Chung et al. 2014a) |
|                  | 10-Acetylicriciformonin B                | C_{34}H_{44}O_6   | 418.52      | Cytotoxic                                                              | (Chung et al. 2014b) |
|                  | Irciformonin B                          | C_{23}H_{34}O_{5} | 376.49      | Cytotoxic                                                              | (Chung et al. 2014a) |
|                  | Irciformonin F                          | C_{23}H_{34}O_{5} | 376.49      | Cytotoxic                                                              | (Chung et al. 2014b) |
| *Rumphella* sp.  | Fucoxanthin                              | C_{42}H_{68}S_6  | 659.91      | Cytotoxic                                                              | (Chung et al. 2014b) |
|                  | 5α,8α-Epidoxystergosta-6,9(11)-dieno-3β-ol | C_{28}H_{42}O_3  | 428.33      | Moderately cytotoxic                                                   | (Yin et al. 2020) |
|                  | Antipacid A                              | C_{15}H_{22}O_3   | 252.17      | Anti-inflammatory                                                      | (Chung et al. 2014a) |
|                  | Rumphellolide L                         | C_{30}H_{48}O_4   | 472.36      | Anti-inflammatory                                                      | (Chung et al. 2014c) |
|                  | Rumphellaic acid A                       | C_{32}H_{44}O_2   | 236.35      | Anti-inflammatory                                                      | (Chung et al. 2014a) |
|                  | Rumphellol A                             | C_{32}H_{44}O_2   | 236.35      | Anti-inflammatory                                                      | (Chung et al. 2014c) |
|                  | Rumphellol B                             | C_{31}H_{40}O_2   | 266.22      | Anti-inflammatory                                                      | (Chung et al. 2014c) |
|                  | Rumphellaic acid C                       | C_{32}H_{44}O_2   | 268.17      | Anti-inflammatory                                                      | (Chung et al. 2014b) |
|                  | 2β-Acetoxyvovann-9α-ol                    | C_{37}H_{52}O_3   | 280.20      | Anti-inflammatory                                                      | (Chung et al. 2013) |
|                  | 9α-Acetoxyvovann-2β-ol                    | C_{32}H_{28}O_3   | 280.20      | Anti-inflammatory                                                      | (Chung et al. 2013) |
|                  | Rumphellolovane B                        | C_{33}H_{32}O_3   | 252.17      | Anti-inflammatory                                                      | (Chung et al. 2011) |
|                  | Rumphellaic acid A                       | C_{32}H_{32}O_3   | 252.17      | Moderately cytotoxic                                                   | (Chung et al. 2010) |
| **Sarcophyton sp.** | **Chemical** | **Formula** | **Molecular Weight** | **Activity** |
|---------------------|--------------|-------------|----------------------|-------------|
| Rumphellolide A     | C_{24}H_{22}O_3 | 238.32      | Anti-inflammatory     | (Sung et al. 2009) |
| Rumphellolide B     | C_{24}H_{22}O_3 | 274.78      | Antibacterial         | (Sung et al. 2007a) |
| Rumphellolide C     | C_{25}H_{24}O_3 | 252.17      | Antibacterial         | (Sung et al. 2007b) |
| Rumphellolide D     | C_{24}H_{22}O_3 | 238.32      | Antibacterial         |                |
| Rumphellolide E     | C_{24}H_{22}O_3 | 238.32      | Antibacterial         |                |
| Rumphellolide F     | C_{24}H_{22}O_2 | 222.23      | Antibacterial         |                |
| Sarcophyne          | C_{25}H_{22}O_3 | 376.49      | Cytotoxic             | (Blunt et al. 2008) |
| Sarcophytolide      | C_{24}H_{28}O_3 | 316.44      | Cytotoxic, antimicrobial properties | |
| Sarcophytolide B    | C_{24}H_{28}O_3 | 316.44      | Cytotoxic             | (Bowden et al. 1979) |
| Sarcophytolide C    | C_{24}H_{28}O_3 | 316.44      | Cytotoxic             |             |
| Sarcophytolol       | C_{24}H_{24}O_2 | 306.48      | Cytotoxic             |             |
| Isosarcophytolide   | C_{24}H_{20}O_2 | 302.45      | Antimicrobial         |             |
| Sarcophytol B       | C_{24}H_{20}O_2 | 304.47      | Cytotoxic             | (Kobayashi et al. 1979) |
| 24¢-Methylcholestone-3β,5α,6β,25-tetraol 25-monoacetate | C_{30}H_{52}O_5 | 492.38 | Cytotoxic | (Zubair et al. 2016; Ahmed, M.M.A., Albadry, M.A., Ragab, E.A., El-Ghaly, E.M., Kotb, S.E., Khan, S.I., Chittiboyina, A.G. & Khan, I.A. 2019) |
| (24S)-Methylcholestone-3β,5α,6β,12β,25-pentol 25-monoacetate | C_{30}H_{52}O_6 | 508.38 | Cytotoxic | |
| (24S)-Methylcholestone-3β,5α,6β,25-tetraol 25-monoacetate | C_{30}H_{52}O_5 | 492.38 | Cytotoxic | |
| (24S)-24-Methylcholestone-3β,5α,6β-triol | C_{28}H_{56}O_3 | 434.38 | Cytotoxic | |
| (24S)-Ergostan-3β,5α,6β,18,25-pentaoiland,18,25-diacetate | C_{31}H_{52}O_7 | 536.37 | Cytotoxic | |
| Sarcoaldesterol A   | C_{30}H_{52}O_4 | 476.39      | Antibacterial         | |

*Note: The activities include antibacterial, antifungal, and antimicrobial properties.*
| **Chemical** | **Formula** | **M.Wt** | **Activity** |
|-------------|-------------|----------|--------------|
| Sarcoaldesterol B | C_{28}H_{50}O_{4}  | 450.37 | Antibacterial  |
|  |  |  | - *Escherichia coli*  |
|  |  |  | - *Bacillus megaterium*,  |
|  |  |  | and antifungal  |
|  |  |  | - *Microbotryum violaceum*  |
|  |  |  | - *Septoria tritici*  |
| 11α-Acetoxy-16β-methoxy-23,24-dimethylcholesterol-17(20)-en-3β,5α,6β-triol | C_{32}H_{64}O_{6}  | 534.39 | Cytotoxic  |
| (24R)-Gorgost-25-en-3β,5α,6β,11α-tetraol | C_{36}H_{66}O_{4}  | 474.37 | Cytotoxic  |
| (24S)-11α-Acetoxy-ergost-3β,5α,6β-triol | C_{30}H_{56}O_{5}  | 490.37 | Cytotoxic  |
| (24R)-Methylcholesterol-7-en-3β,5α,6β-triol | C_{28}H_{44}O_{3}  | 432.36 | Moderately cytotoxic,  |
|  |  |  | antiviral  |
|  |  |  | - H1N1 IAV  |
| (24S)-24-Methylcholen-1b,3b,5a,6b,25-pentaol-25-monoacetate | C_{30}H_{52}O_{6}  | 508.38 | Cytotoxic, anti-inflammatory,  |
|  |  |  | antibacterial  |
|  |  |  | - *Staphylococcus aureus*  |
| Gorgostane-1α,3β,5α,6β,11α-pentaol | C_{30}H_{52}O_{5}  | 492.38 | Moderately antibacterial,  |
|  |  |  | antifungal  |
| 11α-Acetoxycholesterol-24-en-1α,3β,5α,6β-tetraol | C_{28}H_{44}O_{6}  | 492.35 | Cytotoxic  |
| Sarcopanol A | C_{30}H_{56}O_{6}  | 506.36 | Anti-inflammatory  |
| Sarcomilasterol | C_{28}H_{48}O_{4}  | 448.68 | Anti-osteoporotic  |
| (24S)-24-Methylcholestan-3β,6β,25-triol-25-O-acetate | C_{30}H_{52}O_{4}  | 476.39 | Antibacterial  |
|  |  |  | - *Staphylococcus aureus*  |
Table S5 Assignment of the NP-HPTLC–HESI–HRMS signals of bioactive zones 1–10. Main signals in bold, Related to Figures 4 and S12.

| Zone | Sample | Bioactivity | Fig. | Mass signal m/z | Assignment | Molecular formula | Δ ppm | Tentative molecule |
|------|--------|-------------|------|-----------------|------------|-------------------|-------|-------------------|
| 1    | G9     | Metabolism enhancing | 5D   | 138.0551 160.0370 | [M1+H]^+  [M1+Na]^+ | C7H7NO2  C7H7NO3 | -0.36 -0.44 |  |
|      |        |             |      | 182.0789     | [M2+Na]^+  | C7H13NO3 | -0.44 |  |
|      |        |             |      | 220.0614     | [M3+Na]^+  | C6H15NO4S | 0.14  | Cyclohexylamine sulfate |
|      |        |             |      | 124.0073     | [M4−H]^-  | Unknown | - |  |
|      |        |             |      | 181.0718 217.0483 | [M5−H]^-  [M5+Cl]^− | C6H14O6  C6H14O6 | 0.00 0.51 | Hexane-hexol (e.g., sorbitol) |
| 2    | S10    | Metabolism enhancing | 5D   | 203.0526 215.0327 | [M6+Na]^+  [M6+Cl]^− | C6H12O6  C6H12O5 | -0.25 -0.29 | Glucose |
|      |        |             |      | 124.9913     | C2H5O4S | 0.56  | Ethyl sulfate |
| 4    | S12    | Antibacterial | 5D   | 105.0702     | [M8+H]^+  | C8H8 | -3.52 |  |
|      |        |             |      | 122.0966     | C8H11N | -1.64 |  |
|      |        |             |      | 173.0421     | C6H10O5 | -0.52 |  |
| 6    | G16    | Metabolism enhancing | 5D   | 243.0621     | [M11−H]^- | C9H12N2O6 | 0.53  |  |
| 8    | S12    | Antibacterial | 5D   | 265.0795 241.0830 | [M12+Na]^+  [M12−H]^- | C10H14N2O5  C8H6O2 | 0.08 -0.12 |  |
|      |        |             |      | 111.0199     | C8H6N2O2 | 0.54  |  |
|      |        |             |      | 287.0885     | C11H16N2O7 | -0.14 |  |
| 9    | S10    | Antibacterial, weakly AChE/BChE inhibiting | 5D   | 399.2531 421.2349 397.2383 795.4845 | [M15+H]^+  [M15+Na]^+  [M15−H]^-  [2M15−H]^- | C25H32O4  C25H32O4  C25H32O4  C25H32O4 | -0.35 -0.35 -0.35 -0.35 | Fasciculatin, variabilin, palinurin |
| 10   | L8     | Antibacterial, AChE/BChE inhibiting | S12  | 339.1931 655.3972 315.1966 | [M16+Na]^+  [2M16+Na]^+  [M16−H]^-  | C20H28O3  C20H28O3  C20H28O3 | 0.24 0.24 0.24 | Sarcophine, sarcophytolide, sarcophytolide B, sarcophytolide C |
|      |        |             |      | 331.1915     | C20H28O4 | 0.03  |  |
|      |        |             |      | 333.2072     | C20H30O4 | -0.11 |  |
|      |        |             |      | 347.1863     | C20H28O5 | 0.26  |  |
|      |        |             |      | 325.2138 641.4181 | [M17+Na]^+  [2M17+Na]^+ | C20H30O2  C20H30O2 | 0.28 -0.41 | Isosarcophytolide |
|      |        |             |      | 303.2329     | C20H32O2 | 0.03  | Sarcophytol B |
|      |        |             |      | 118.9419     | C20H32O2 | - |  |
Table S6 Assignment of the NP-HPTLC– pYES/pYAAS–RP-HPLC–PDA–HESI-MS signals of bioactive zones 11–15. Main signals in bold, Same signals same colour, Related to Figures 5 and S13.

| Zone | Sample | Bioactivity | Fig. | Mass signal \(m/z\) | Assignment | RT [min] | \(\lambda_{\text{max}}\) [nm] |
|------|--------|-------------|------|----------------------|------------|----------|------------------|
| 11   | L8     | Estrogen-like | 6E   | 315.18               | \([M20−H_2O+H]^+\) | 8.13/8.24 | 276              |
|      |        |             |      | 333.29               | \([M20+H]^+\)    |          |                  |
|      |        |             |      | 350.27               | \([M20+NH_4]^+\)  |          |                  |
|      |        |             |      | 355.21               | \([M20+Na]^+\)    |          |                  |
|      |        |             |      | 371.05               | \([M20+K]^+\)     |          |                  |
|      |        |             |      | 331.38               | \([M20−H]^−\)     |          |                  |
|      |        |             |      | 377.32               | \([M20+HCOO]^−\)  |          |                  |
|      |        |             |      | 391.32               | \([M20+H_3C-COO]^−\)|          |                  |
| 12   | L8/L13 | Estrogen-like | 6F   | 229.35               | \([M21+H]^+\)    | 7.07     |                  |
|      |        |             |      | 246.07               | \([M21+NH_4]^+\)  |          |                  |
|      |        |             |      | 267.10               | \([M21+K]^+\)     |          |                  |
|      |        |             |      | 375.29               | \([M22+H]^+\)    | 8.24     | 226              |
|      |        |             |      | 392.46               | \([M22+NH_4]^+\)  |          |                  |
|      |        |             |      | 397.08               | \([M22+Na]^+\)    |          |                  |
|      |        |             |      | 413.42               | \([M22+K]^+\)     |          |                  |
|      |        |             |      | 299.28               | \([M23−H_2O+H]^+\) |          |                  |
|      |        |             |      | 317.20               | \([M23+H]^+\)    | 8.54     | 231              |
|      |        |             |      | 334.43               | \([M23+NH_4]^+\)  |          |                  |
|      |        |             |      | 339.12               | \([M23+Na]^+\)    |          |                  |
|      |        |             |      | 355.15               | \([M23+K]^+\)     |          |                  |
|      |        |             |      | 315.24               | \([M23−H]^−\)     |          |                  |
|      |        |             |      | 333.29               | \([M24−H_2O+H]^+\) |          |                  |
|      |        |             |      | 373.07               | \([M24+Na]^+\)    | 8.75     |                  |
|      |        |             |      | 389.23               | \([M24+K]^+\)     |          |                  |
| 13   | L8/G11 | Estrogen-like | 6G   | 331.27               | \([M25−2H_2O+H]^+\)|          |                  |
|      |        |             |      | 349.32               | \([M25−H_2O+H]^+\) |          |                  |
|      |        |             |      | 366.36               | \([M25]^+\)      | 6.72/7.07 |                  |
|      |        |             |      | 384.29               | \([M25+NH_4]^+\)  |          |                  |
|      |        |             |      | 389.16               | \([M25+Na]^+\)    |          |                  |
|      |        |             |      | 405.19               | \([M25+K]^+\)     |          |                  |
|      |        |             |      | 365.35               | \([M25−H]^−\)     |          |                  |
|      |        |             |      | 401.20               | \([M25+Cl]^−\)    |          |                  |
|      |        |             |      | 411.27               | \([M25+HCOO]^−\)  |          |                  |
|      |        |             |      | 425.27               | \([M25+H_3C-COO]^−\)|          |                  |
|      |        |             |      | 313.15               | \([M26−2H_2O+H]^+\)|          |                  |
|      |        |             |      | 331.27               | \([M26−H_2O+H]^+\) |          |                  |
|      |        |             |      | 349.32               | \([M26+H]^+\)    | 7.44     |                  |
|      |        |             |      | 366.36               | \([M26+NH_4]^+\)  |          |                  |
|      |        |             |      | 387.20               | \([M26+K]^+\)     |          |                  |
|      |        |             |      | 347.23               | \([M26−H]^−\)     |          |                  |
|      |        |             |      | 393.28               | \([M26+HCOO]^−\)  |          |                  |
|      |        |             |      | 380.61               | \([M27+H]^+\)    |          |                  |
|      |        |             |      | 403.42               | \([M27+Na]^+\)    |          |                  |
|      |        |             |      | 419.12               | \([M27+K]^+\)     |          |                  |
|      |        |             |      | 415.20               | \([M27+Cl]^−\)    |          |                  |
|      |        |             |      | 425.08               | \([M27+HCOO]^−\)  |          |                  |
|      |        |             |      | 439.01               | \([M27+H_3C-COO]^−\)|          |                  |
| 14 | L8 | Antiandrogenic | 7E |
|----|----|----------------|----|
| 297.00 | [M20–2H₂O+H]^+ | 8.12/8.23 | 275 |
| 315.05 | [M20–2H₂O+H]^+ | | |
| 332.15 | [M20]^+ | | |
| 350.27 | [M20+NH₄]^+ | | |
| 355.27 | [M20+Na]^+ | | |
| 370.98 | [M20+K]^+ | | |
| 299.28 | [M23–H₂O+H]^+ | 8.54 | 225 |
| 317.20 | [M23+H]^+ | | |
| 334.24 | [M23+NH₄]^+ | | |
| 338.99 | [M23+Na]^+ | | |
| 355.15 | [M23+K]^+ | | |
| 655.45 | [2M23+Na]^+ | | |
| 14 | L8 | Antiandrogenic | 7F |
| 331.33 | [M27–H₂O+H]^+ | 7.44 | - |
| 349.38 | [M27+H]^+ | | |
| 366.36 | [M27+NH₄]^+ | | |
| 387.26 | [M27+K]^+ | | |
| 347.36 | [M27–H]^− | | |
| 403.16 | [M28+Na]^+ | | |
| 419.44 | [M28+K]^+ | | |
| 415.32 | [M28+Cl]^− | | |
| 425.02 | [M28+HCOO]^− | | |
| 439.33 | [M28+H₂C-COO]^− | | |
| 315.18 | [M21–2H₂O+H]^+ | 8.12/8.23 | 275 |
| 332.34 | [M21]^+ | | |
| 350.08 | [M21+NH₄]^+ | | |
| 355.08 | [M21+Na]^+ | | |
| 371.11 | [M21+K]^+ | | |
| 15 | L13 | Antiandrogenic | 7F |
| 331.20 | [M25–2H₂O+H]^+ | 6.73/7.09 | - |
| 349.26 | [M25–H₂O+H]^+ | | |
| 366.23 | [M25]^+ | | |
| 384.29 | [M25+NH₄]^+ | | |
| 389.35 | [M25+Na]^+ | | |
| 405.32 | [M25+K]^+ | | |
| 365.22 | [M25–H]^− | | |
| 401.33 | [M25+Cl]^− | | |
| 411.27 | [M25+HCOO]^− | | |
| 425.14 | [M25+H₂C-COO]^− | | |
| 299.28 | [M23–H₂O+H]^+ | 8.51 | 208 |
| 317.14 | [M23+H]^+ | | |
| 334.31 | [M23+NH₄]^+ | | |
| 339.25 | [M23+Na]^+ | | |
| 355.34 | [M23+K]^+ | | |
| 655.51 | [2M23+Na]^+ | | |
Figure S1. Study site of sampling

Study map of the Indo-Pacific bottlenose dolphins around Hurghada in the Northern Red Sea, Egypt ranges from the reefs of Shaab Umm Usk in the North to the Abu Hashish reefs in the south (yellow boxes). Organism samples were taken at the two reef sites Shaab El Erg and Shaab El Fanous (red circles), which are regularly visited by the dolphins for resting, socializing and rubbing, Related to Figure 1.
Figure S2. Rubbing behaviour of a group of Indo-Pacific bottlenose dolphins (I–IV), queuing up to rub against the gorgonian coral (gorgoning)

Dolphins queue up behind each other to wait their turn: (A) An Indo-Pacific bottlenose dolphin glides towards and (B) rubs its skin on the gorgonian coral *Rumphella aggregata* as documented in Shaab El Erg and Shaab El Fanous, Red Sea, Egypt (details in Videos S1 and S2). The individual either rubs its ventral, lateral or dorsal body part on the gorgonian coral; its head, pectoral fins and fluke often touch the gorgonian too. This is often repeated so that all body areas are rubbed. When in groups, dolphins queue up behind each other to wait their turn for their next approach. In general, the soft gorgonian coral polyps start to close and retract when dolphins rub on them. This inherent coral protection mechanism can support abrasion, as it allows the dolphins’ skin to come into contact with secondary metabolites. A larger mucus secretion from the branches of the coral during the rubbing behaviour has been observed, Related to Figure 1.
Figure S3. Underwater photo documentation of the gorgonian coral sampling

(A) Individual gorgonian coral *Rumphella aggregata*, (B) overview image, (C) detail image with scale bar and (D) sampling process, Related to Figure 1. Scientific diver Jennifer Tersteegen consents to the use of the image.
Figure S4. Underwater photo documentation of the leather coral sampling

(A) Individual leather coral *Sarcophyton* sp. with scale bar, (B) overview image, (C) detail image and (D) sampling process, Related to Figure 1. Scientific diver Jennifer Tersteegen consents to the use of the image.
Figure S5. Underwater photo documentation of the sponge sampling

(A) Individual sponge *Ircinia* sp. with scale bar, (B) overview image, (C) detail image and (D) sampling process, Related to Figure 1. Scientific diver Jennifer Tersteegen consents to the use of the image.
Figure S6. HPTLC instrumentation used for the effect-directed profiling

(A) Application, (B) development, (C) piezoelectric spraying of assay solutions or suspensions, (D) UV/Vis/FLD detection and (E) bioluminescence detection; all in operation in Video S6, Related to Figure 2.
Figure S7. Chromatograms at FLD 366 nm before the *A. fischeri* (A) and *B. subtilis* bioassays (B)

Chromatograms of the three different substrate extracts developed on HPTLC plates silica gel 60 with ethyl acetate – methanol – water 15:3:1 (V/V/V) and detected at FLD 366 nm before the bioassay performance, Related to Figures 3 and 4.
Figure S8. Chromatograms at FLD 366 nm before application of the respective assays

Separation of the \textit{n}-hexane and methanol boat extracts of the three different substrates; respective UV 254 nm chromatograms did not show additional compounds and Vis chromatograms showed orange chlorophylls near the front only (both not depicted), Related to Figures 5 and S9.
Figure S9. AChE/BChE inhibiting, hormonal and genotoxic compounds

(A) AChE and (B) BChE inhibiting compound zones 9 and 10 evident as colourless/white bands (L7/15 and L4/7, respectively; zone 9 with halo-effect), (C/D) estrogenic (11–13), (D) antiestrogenic (14/15), (E/F) androgenic (none), (F) antiandrogenic (14/15), and (G/H) genotoxic (16/17) compound zones, evident as (C–H) blue fluorescence or (D/F) fluorescence reduction in the n-hexane and methanol boat extracts of the three different substrates (C–H 10 µL/band each as listed at bottom; up to 10 µg/band) developed on HPTLC plates silica gel 60 (except for H on RP-18 W plates) with A/B/H ethyl acetate – methanol – water 15:3:1 (V/V/V), C–F n-hexane – ethyl acetate 3:4 (V/V) and G 1:4 (V/V), detected at A/B white light illumination and C–H FLD 366 nm (respective pre-assay FLD 366 nm chromatograms), Related to Figures 5 and S8.
Figure S10. Radical scavenging assay and physico-chemical detection of the chromatograms including derivatization

Chromatograms of the methanol and n-hexane extracts of the three different substrates (10 µL each) developed on HPTLC plates silica gel 60 with ethyl acetate – methanol – water 15:3:1 (V/V/V) and detected mainly the more apolar bioactive zones 9 and 10 at Vis and FLD 366 nm after the (A) 2,2-diphenyl-1-picrylhydrazyl (DPPH•) assay for detection of radical scavengers (antioxidants), as well as after derivatization with (B) 2% diethylamine aniline sulphuric acid reagent for detection of glycosides or lactones; (saccharides are supposed to be in the start region for the given mobile phase); (C) 1% vanillin sulfuric acid reagent for more universal detection of organic compounds like sequiterpene derivatives. Related to Figure 2.
**Figure S11 Physico-chemical detection of the chromatograms after primuline reagent**

Chromatograms of the methanol and *n*-hexane extracts of the three different substrates (10 µL each) developed on HPTLC plates silica gel 60 with ethyl acetate – methanol – water 15:3:1 (V/V/V) and detected at UV 254 nm (native UV-absorbance of the bioactive zones 9 and 10) and at FLD 366 nm before and after physisorption of the primuline reagent for detection of lipophilic compounds as blue fluorescent bands. Related to Figure 2.
Figure S12. Proof of proper positioning and mass spectra recorded for zone 10

(A) FLD 366 nm chromatogram developed on HPTLC plates silica gel 60 with ethyl acetate – methanol – water 15:3:1 (V/V/V) each of a methanol boat (L15) and n-hexane extract (L7) of the leather coral, (B) respective acetyl- and butyrylcholinesterase (AChE/BChE) inhibition autogram, and (C) plate duplicate used for the recording of mass spectra followed by post-MS BChE assay application to prove the proper positioning of the elution head on the zones (fully automated operation in Video S7). (D) Mass signal assignment of zone 10. Related to Figure 3.
Figure S13. Characterization of the antiandrogenic zones 14 and 15 directly from the pYAAS bioautogram

(A) Chromatogram at FLD 366 nm on HPTLC plate silica gel 60 with ethyl acetate – methanol – water 15:3:1 (V/V/V) of the methanol boat and n-hexane extracts of the three distinct substrates, (B) respective pYAAS bioautogram and (C) elution head imprint, verifying proper positioning of the elution head on the zones. (D) Schematic overview of the zone characterization by NP-HPTLC–pYAAS bioassay–RP-HPLC–DAD–HESI–MS and recorded PDA or MS–TIC chromatograms as well as extracted mass spectra (same colour) with assigned mass signals. Related to Figures 5 and S9.