

Dereplicating and Spatial Mapping of Secondary Metabolites from Fungal Cultures *in Situ*

Vincent P. Sica, Huzefa A. Raja, Tamam El-Elimat, Vilmos Kertesz, Gary J. Van Berkel, Cedric J. Pearce, and Nicholas H. Oberlies

Figure S1. (A) The guttate (red circle) and mycelium (blue circle) of fungal code G100, *Clohesyomyces aquaticus*, were sampled using the droplet-LMJ-SSP. A clearer picture of the guttates from a different culture of G100 is shown on the right. (B) The base peak chromatogram displayed phomopsinone A in the guttate (red). (C) The same peak was not apparent in the mycelium (blue). The XIC for phomopsinone A (m/z 225.11 at 4.43 min) was over two orders of magnitude higher in counts per second (cps) for (D) the guttate (4.8×10^5 cps) over (E) the mycelium (1.4×10^3 cps).

Figure S2. Comparison of the MS/MS fragmentation for (A) compound **5** with (B) its standard and (C) compound **6** with its (D) standard.

Figure S3. The extracted ion chromatogram (XIC) of m/z (A) 381.1099 ± 5 ppm and (B) 399.1204 ± 5 ppm. (C) At 3.47 min, the accuracy for $[M + H]^+$ between the observed and calculated was 0.3 ppm (399.1204 observed vs. 399.1205 calculated for $[C_{19}H_{24}O_7Cl + H]^+$ with multiple indicators suggesting for the parent ion. The HCD fragmentation (NCE =23) of molecular ions with m/z (D) 381.11 and (E) 399.12 detected at 3.47 min. (F) Proposed structure based off of assumed biosynthetic route.

Figure S4. (A) Contaminated fungal culture coded MSX19583 with the “green” fungus indicated by the green circles and the “purple” fungus indicated by the purple circles. (B) Isolated fungal cultures of MSX19583 (green), *Aspergillus sydowii*, (C) and the contaminant (purple), *Chaetomium* sp.

Figure S5. (A) Structure of a diketopiperazine dimer (**9**) detected on the green fungal culture coded MSX19583 (Figure S4A). (B) A comparison of the extracted ion chromatograms (XIC) from the fungal culture (top) and the standard (bottom) for the accurate mass of compound **9**. (C) A comparison of the HRMS and the MS/MS for the fungal culture and the standard for compound **9**.

Figure S6. (A) Structure of cyclo-(L-phenylalaninyl-L-tryptophanyl) (**10**) detected on the green fungal culture coded MSX19583 (Figure S4A). (B) A comparison of the extracted ion chromatograms (XIC) from the fungal culture (top) and the standard (bottom) for the accurate mass of compound **10**. (C) A comparison of the HRMS and the MS/MS for the fungal culture and the standard for compound **10**.

Figure S7. (A) Image of pure culture MSX19583 with yellow crosshairs indicating sampled areas. (B) Heat map of compound **9** as sampling from the contaminant to the culture. (C) Heat map of compound **8** as sampling from the contaminant to the culture. (D) The color scale and diameter of spot indicate the relative amount of signal detected for the given analytes.

Figure S8. 3D model created using SketchUp Make of the custom tray designed to hold a Petri dish and a solvent vial (A). Photograph of the printed tray using the F306 3D printer (B).

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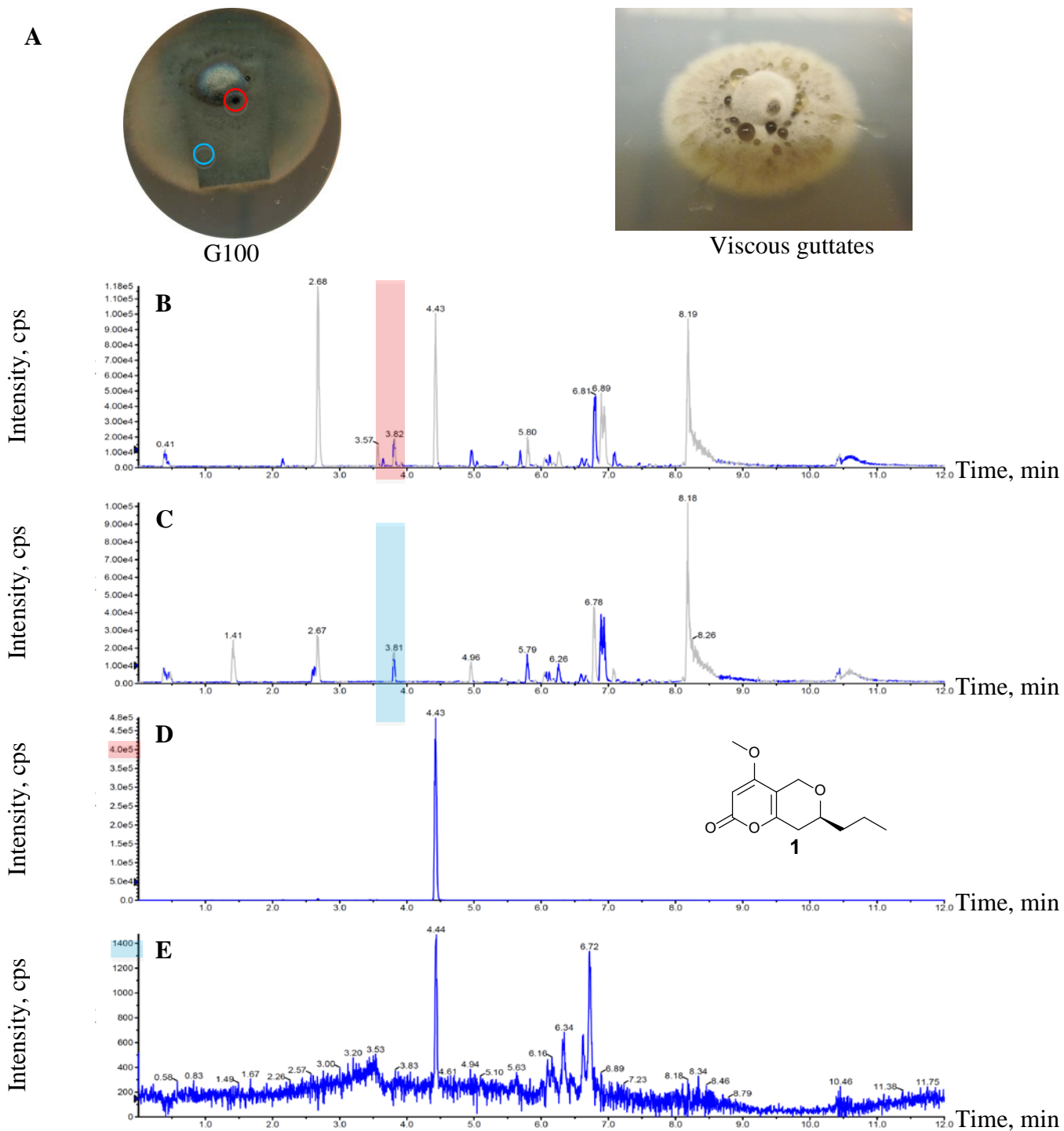


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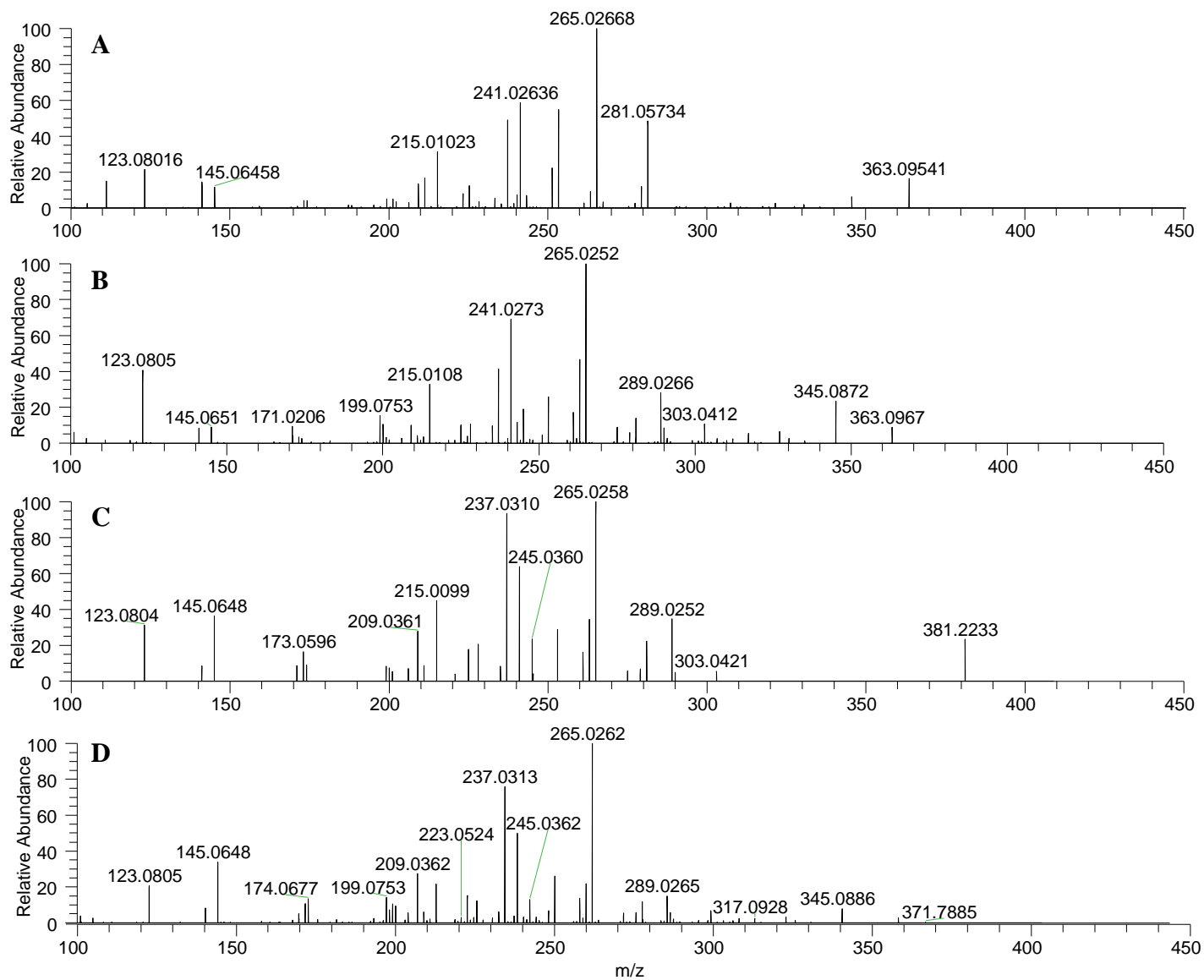


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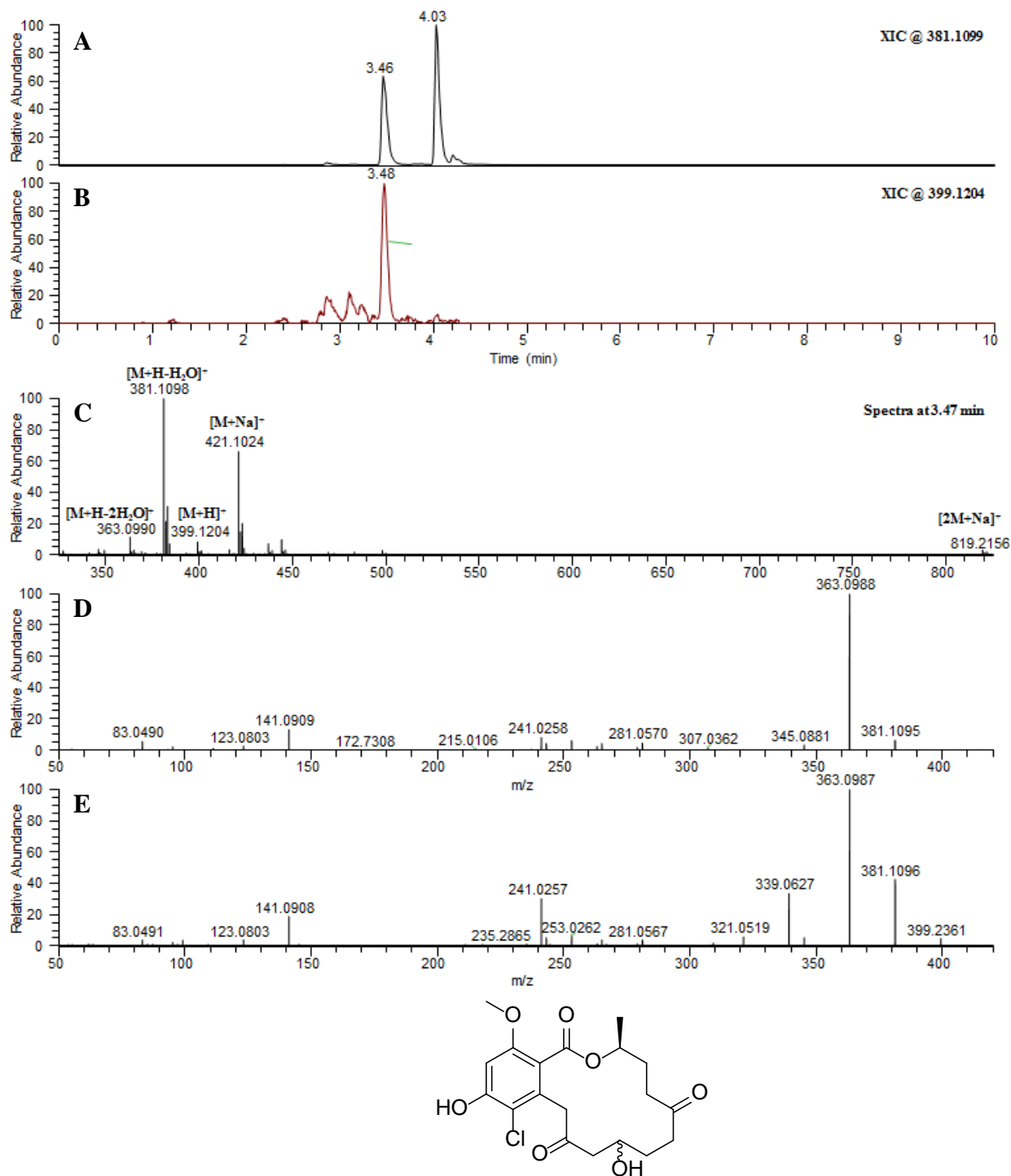


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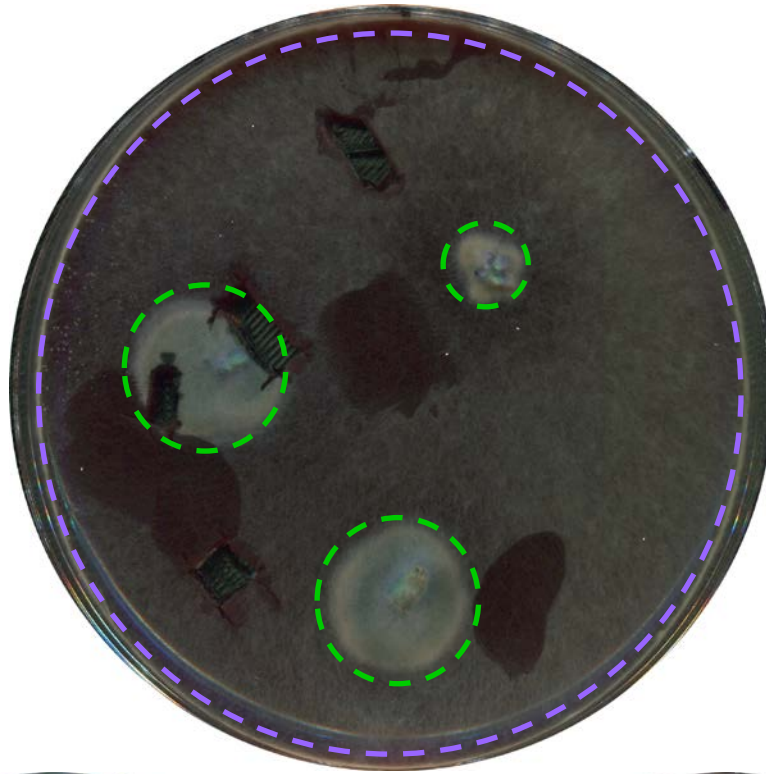
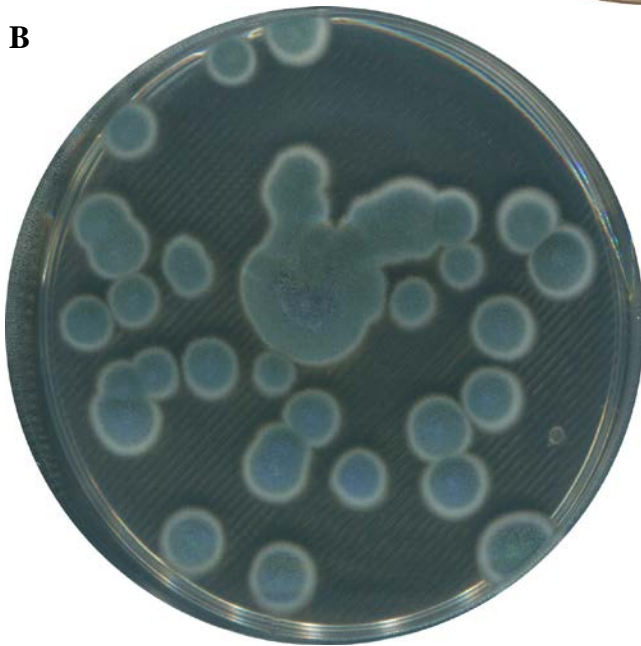
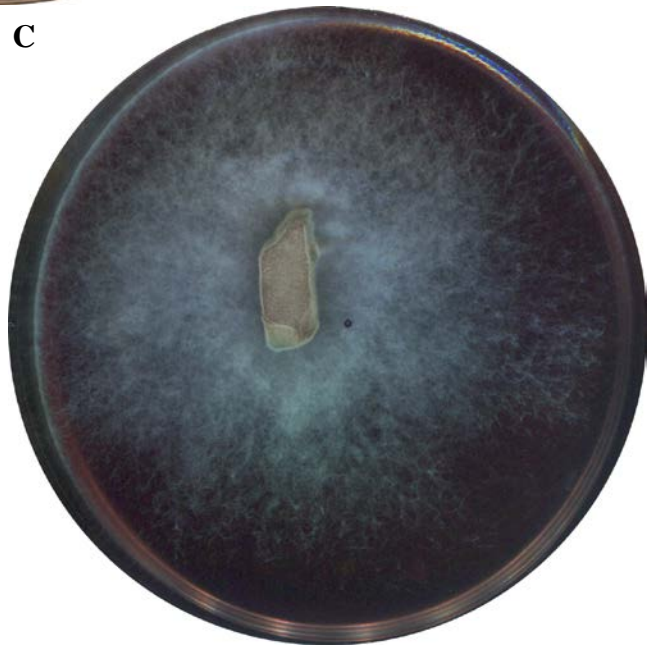
A**B****C**

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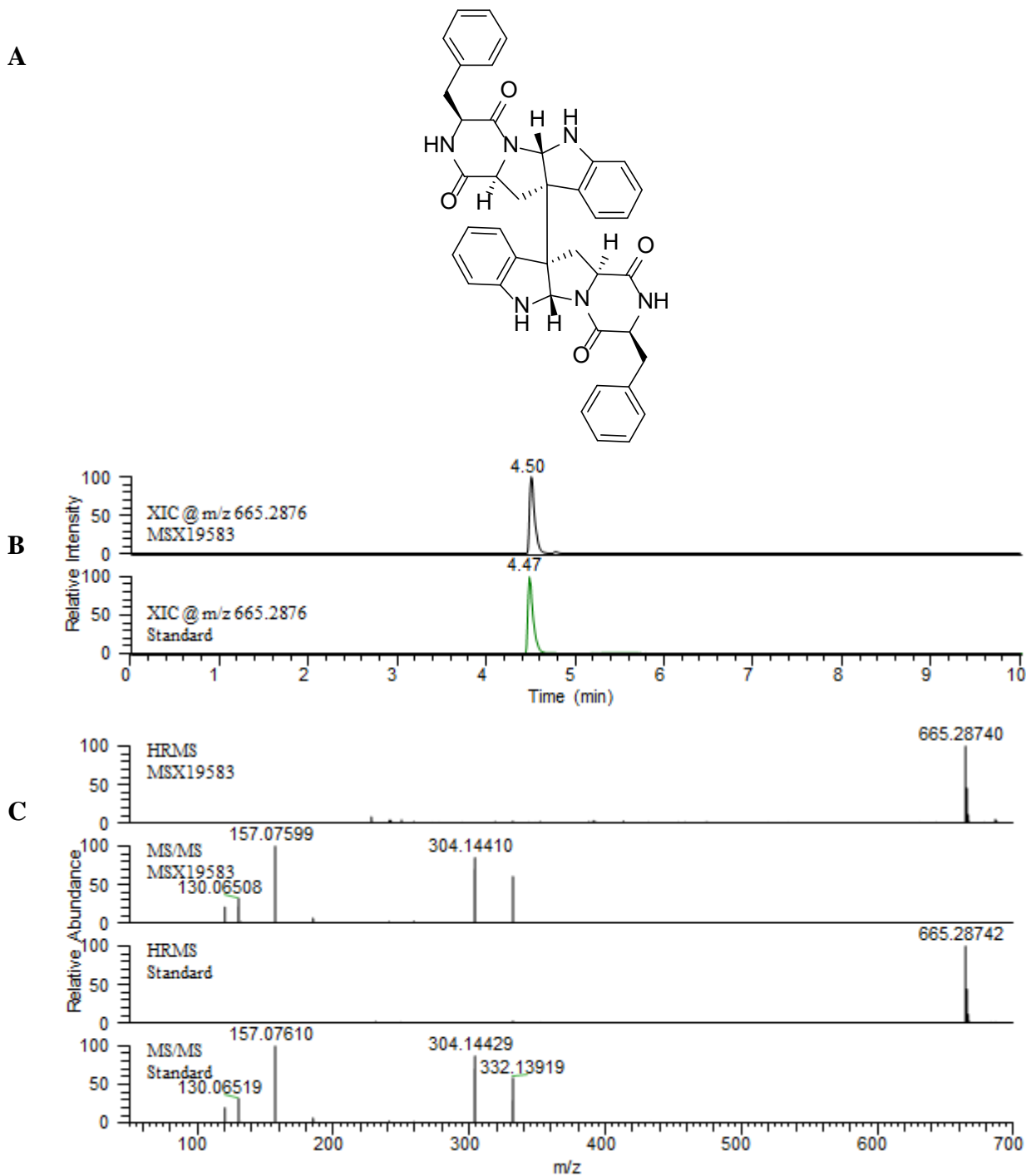


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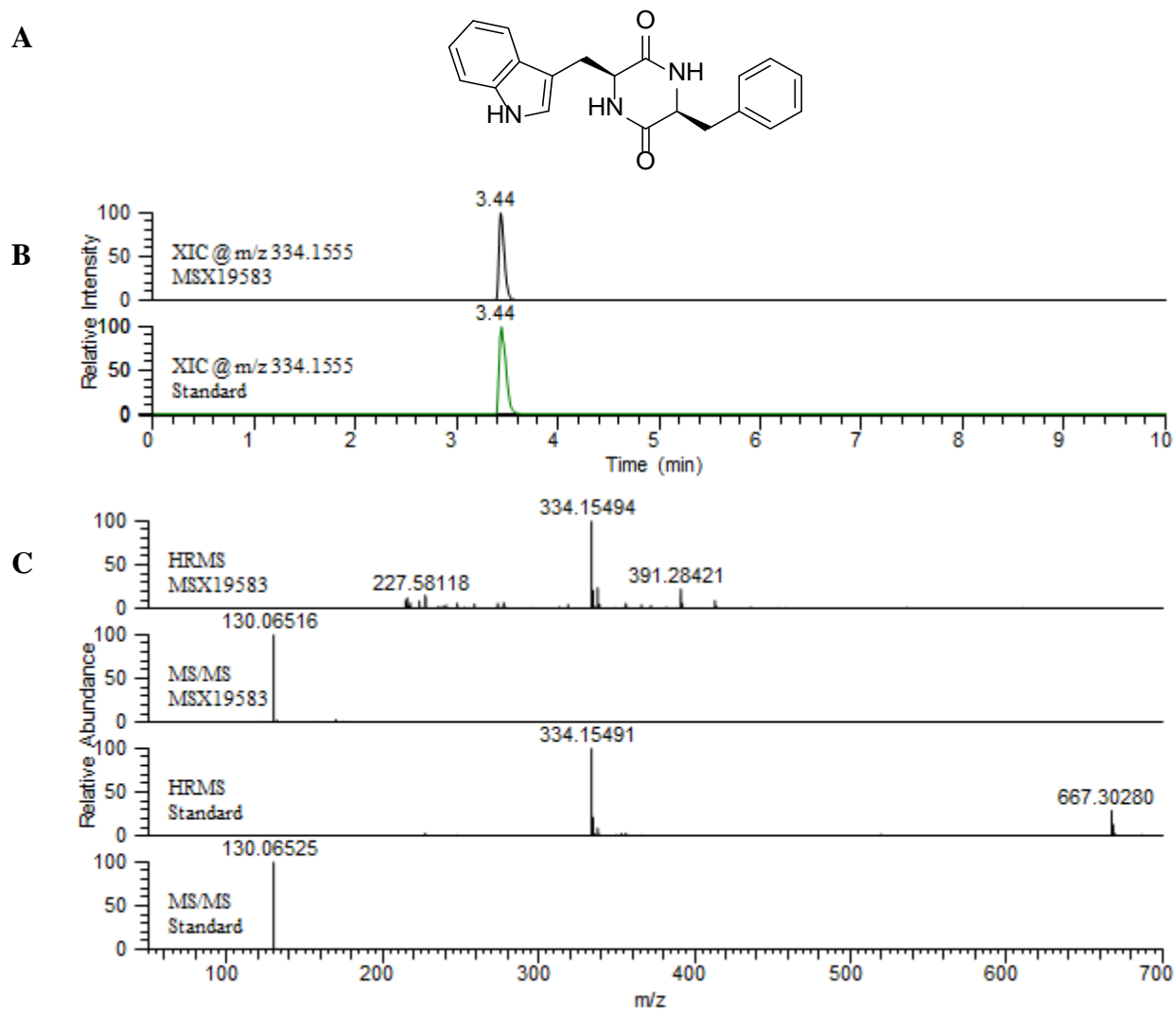


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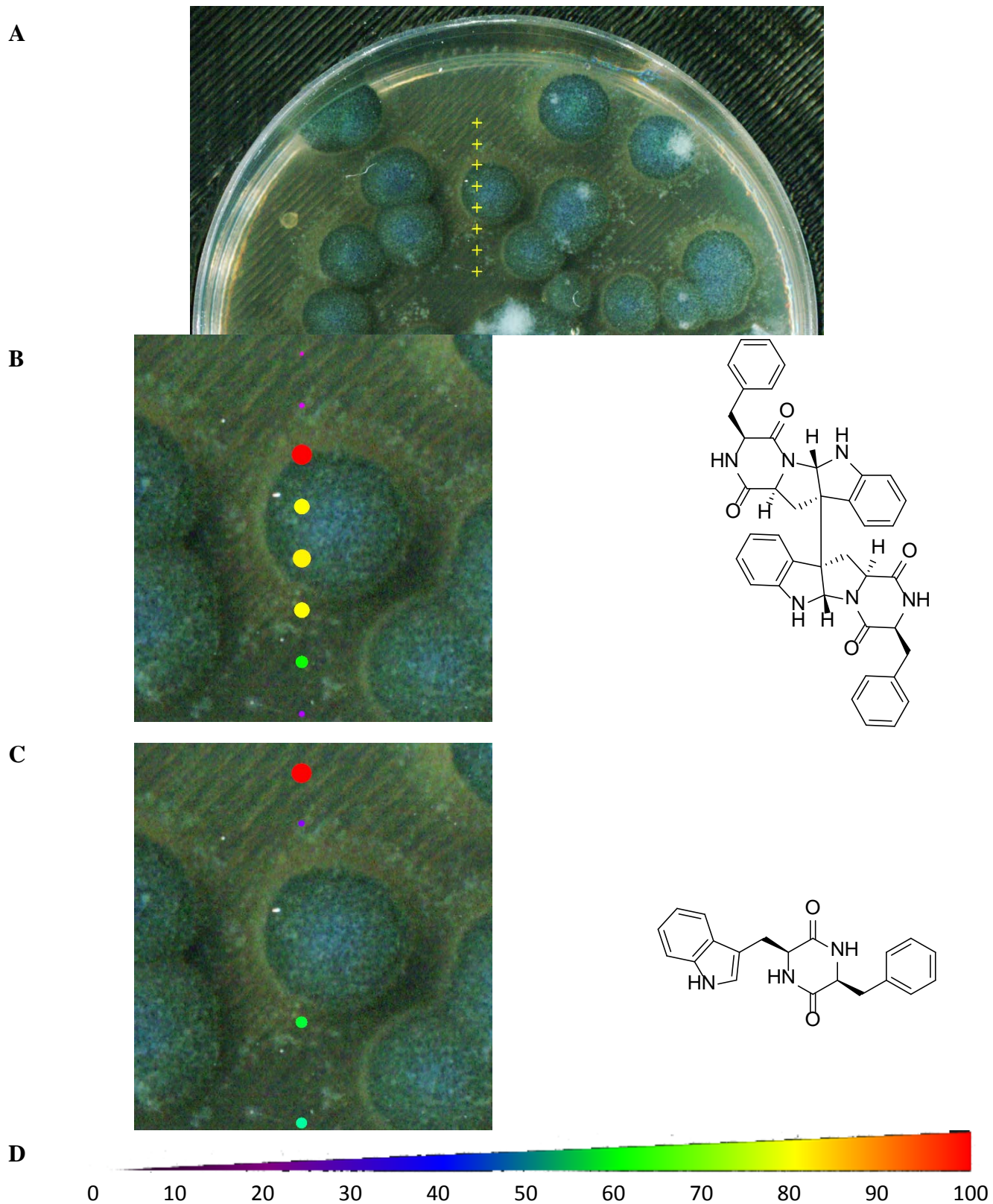


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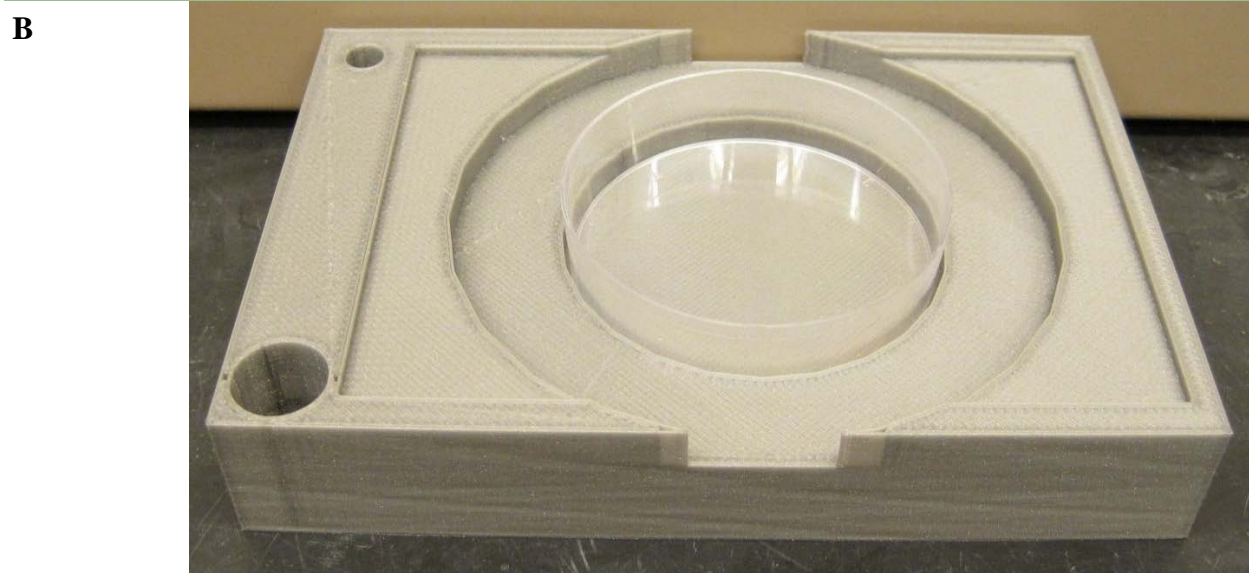
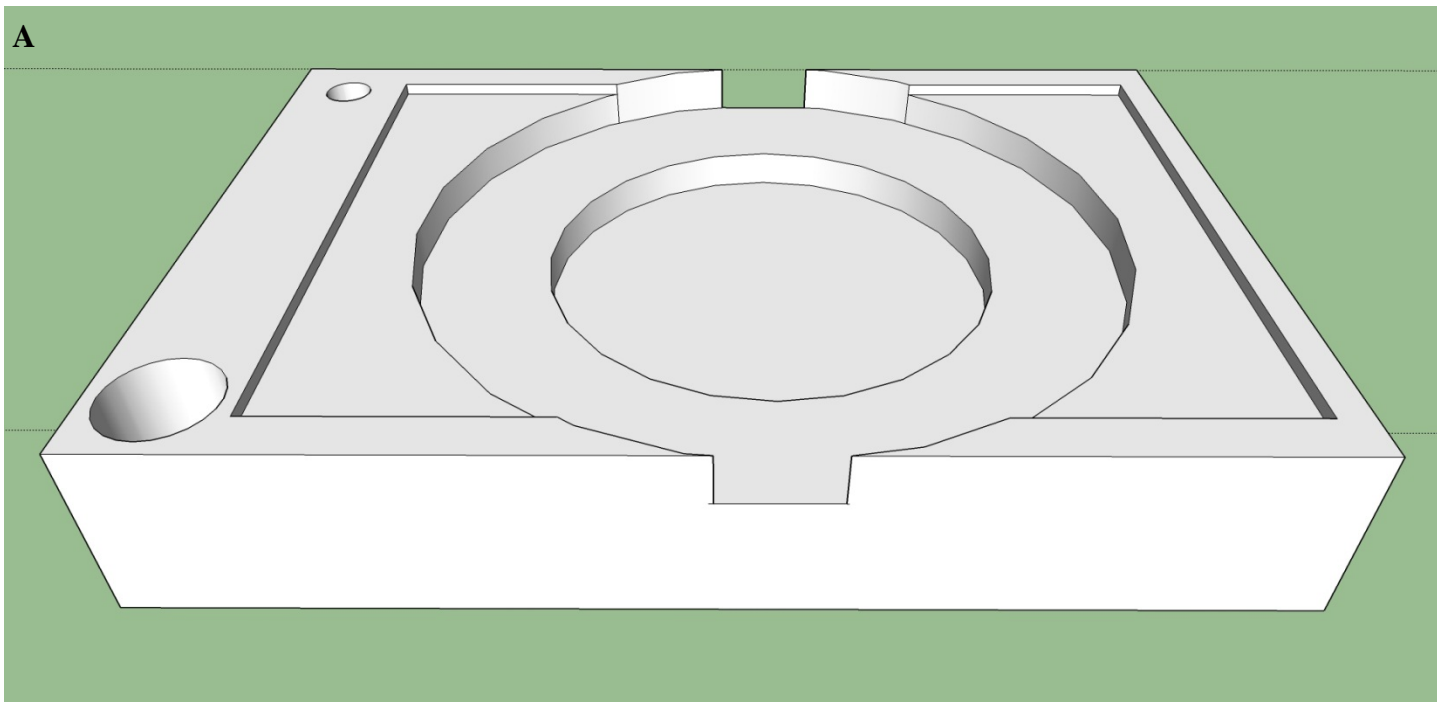

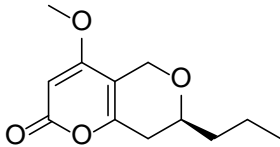
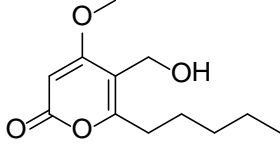
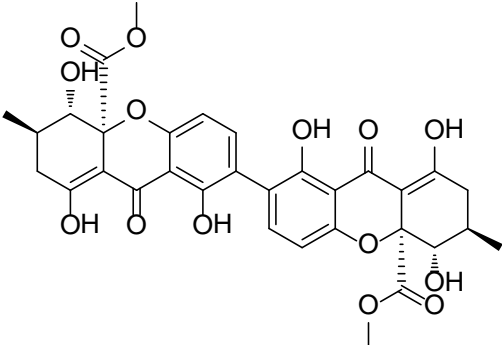
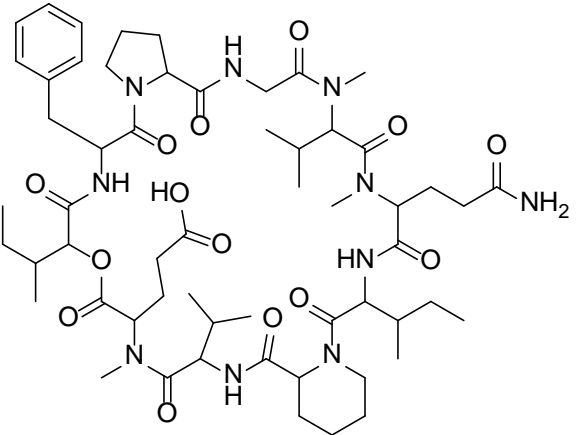

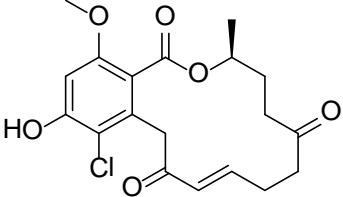
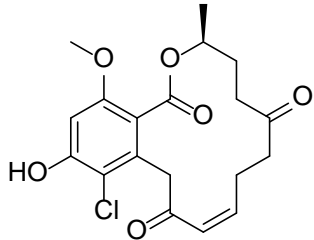
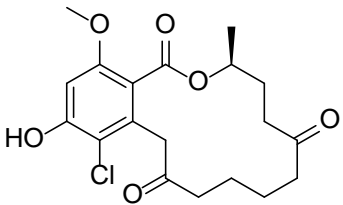
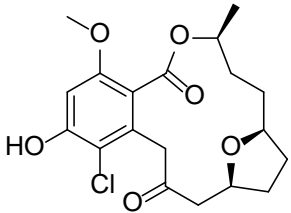



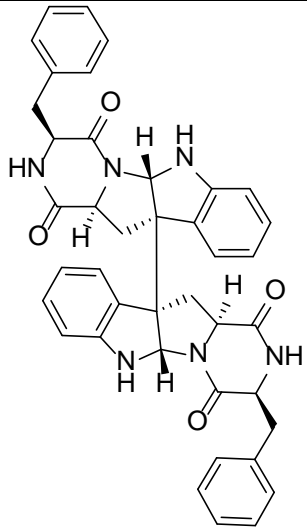
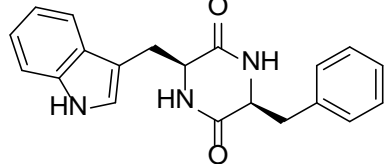
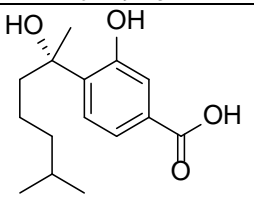
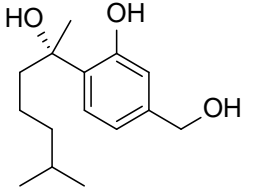
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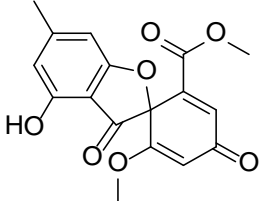
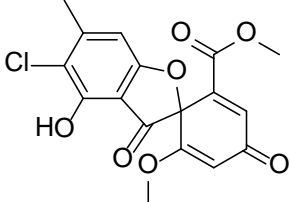
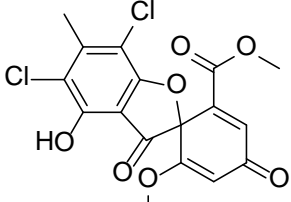
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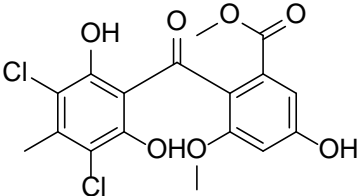

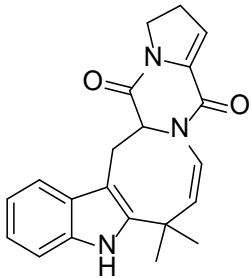
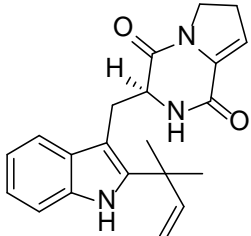
#	Chemical Structure and Chemical formula	Rt (min)	UV (nm)	Positive Ionization Mode	
				[M+H] ⁺	MS/MS
		G100¹³ <i>Clohesyomyces aquaticus</i> GenBank # KM589854 Freshwater fungus isolated from submerged wood			
1	 Phomopsinone A <chem>C12H16O4</chem>	4.36	205 221 284	225.1120 (-0.6)	153.0546 111.0440, 135.0440 125.0597, 97.0646 79.0178, 121.0283
2	 Pyrenocine M <chem>C12H18O4</chem>	3.62	208 221 283	227.1278 (+0.1)	209.1171 167.0703, 131.0854 93.0699, 79.0541 197.1172, 139.0390
3	 Secalonic acid A <chem>C32H30O14</chem>	5.50	193 222 264 337	639.1713 (+0.7)	183.0651, 561.1390 193.0495, 501.1177 579.1493, 483.1071 589.1340, 377.0652 169.0495, 455.0979 543.1285, 151.0390


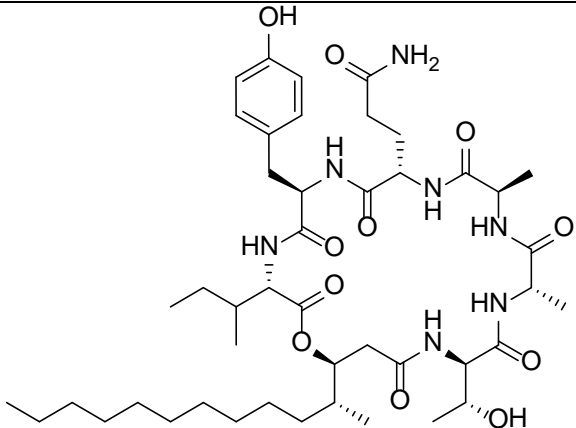

4	 <p style="text-align: center;">Sch 217048 C₅₇H₈₈N₁₀O₁₄</p>	4.98	192 216	1137.6570 (+1.4)	86.0963, 268.1651 256.1653, 228.1704 350.2066, 39.1958 377.2065, 405.2017 211.1438, 286.1754 484.2432, 155.0813 654.3506, 559.2763
		<p>G87³¹ <i>Halenospora aff. varia</i> GenBank # KJ803850 Fungus isolated from submerged wood in fresh water</p>			
#	Chemical Structure and Chemical formula	Rt (min)	UV (nm)	Positive Ionization Mode	
5	 <p style="text-align: center;">Greensporone A C₁₉H₂₁O₆Cl</p>	4.03	222 294	381.1099 (0.0)	265.0259, 237.0310 241.0261, 123.0804 145.0648, 215.0102 253.0260, 263.0466 209.0364, 345.0883 363.0987, 281.0574 303.0415, 289.0260

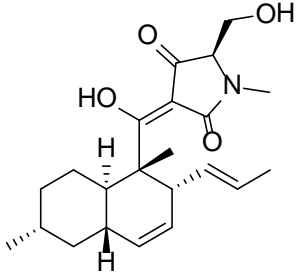

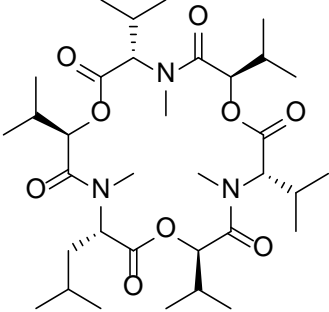
6	 <p>Greensporone B C₁₉H₂₁O₆Cl</p>	4.20	238 291	381.1099 (0.0)	265.0262, 237.0312 241.0261, 253.0261 145.0648, 209.0362 123.0805, 215.0104 289.0265, 345.0886 363.0995, 303.0793
7	 <p>8,9-Dihydrogreensporone A C₁₉H₂₃O₆Cl</p>	4.45	218 291	383.1256 (0.0)	265.0619, 263.0472 215.0104, 125.0961 83.0491, 171.0209 224.9948, 243.0054 253.0261, 237.0673 227.0624, 293.0577 305.0581, 329.0937 347.1045, 365.1153
8	 <p>Greensporone F C₁₉H₂₃O₆Cl</p>	4.70	217 290	383.1255 (-0.2)	241.0252, 107.0857 79.0543, 97.1014 125.0958, 211.0159 224.9953, 253.0249 265.0253, 293.0578 309.0531, 347.1031 365.1136, 329.0913
		<p>MSX19583³² <i>Aspergillus sydowii</i> GenBank # ITS: KP702233; RPB1: KP702234; RPB2: KP702231, KP702232 Fungus from MSX library</p>			
#	Chemical Structure and Chemical formula	Rt (min)	UV (nm)	Positive Ionization Mode	
				[M+H] ⁺	MS/MS

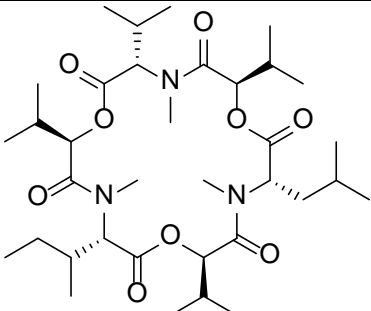
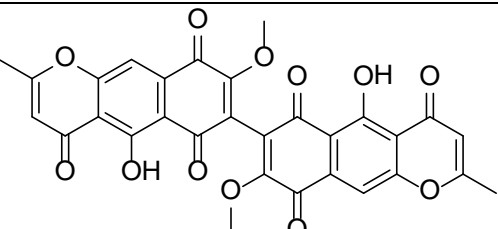
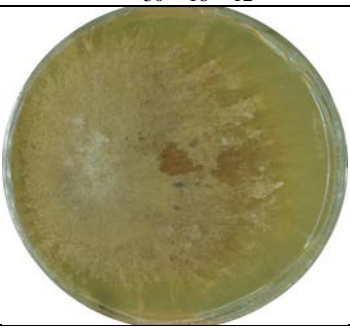
9	 <p>Diketopiperazine dimer C₄₀H₃₆N₆O₄</p>	4.48	192 208 300	665.2877 (+0.9)	157.0763, 304.1448 332.1401, 130.0652 120.0811, 185.0715 241.0845, 433.1996 259.1226, 286.1351
10	 <p>Cyclo-(L-phenylalaninyl-L-tryptophanyl) C₂₀H₁₉N₃O₂</p>	3.46	191 218 279 289 364	334.1549 (-0.3)	130.0652, 170.0596 120.0810, 159.0914 175.0863, 234.1275 205.0968, 289.1338 261.1387, 91.0543
11	 <p>S-sydonic acid C₁₅H₂₂O₄</p>	5.07	212 240 300	[M+H-H ₂ O] ⁺ 249.1486 (+0.3)	165.0549, 151.0386 69.0699, 107.0490 121.0646, 179.0704 193.0865, 231.1387 85.1010, 213.1267
12		4.96	198 221 278	[M+H-H ₂ O] ⁺ 235.1694 (+0.6)	151.0755, 137.0601 121.0647, 161.0956 217.1589, 107.0490 175.1121, 147.0808 205.1593, 69.0700

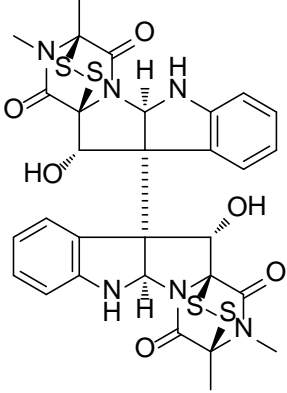
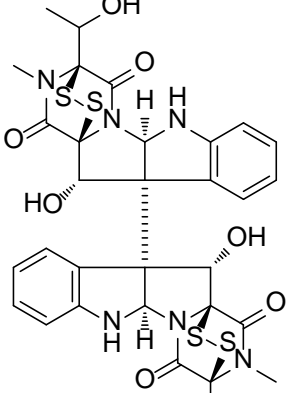

		S-sydonol $C_{15}H_{24}O_3$							
				<p style="text-align: center;">G77³³ <i>Aspergillus izukae</i> GenBank # AB859956 Endophytic fungus isolated from surface sterilized leaves of <i>Silybum marianum</i></p>					
#	Chemical Structure and Chemical formula	Rt (min)	UV (nm)	Positive Ionization Mode					
				[M+H] ⁺	MS/MS				
13	 Bisdechlorogedin $C_{17}H_{14}O_7$	4.06	218 284	331.0810 (-0.7)	272.0681, 299.0535 275.0446, 284.0308 287.0917, 255.0649 244.0726, 228.0408				
14	 Antibiotic SS 19508D; SS 19508D $C_{17}H_{13}O_7Cl$	4.52	214 284	365.0423 (+0.1)	306.0286, 333.0149 291.0060, 317.9919 321.0529, 286.0828 263.0098, 277.0258 286.0844, 271.0587				
15	 Geodin $C_{17}H_{12}O_7Cl_2$	5.15	220 284 352	399.0034 (+0.3)	339.9913, 324.9667 366.9775, 351.9529 296.9696, 310.9878 268.9758, 295.9627 355.0136, 320.0448				

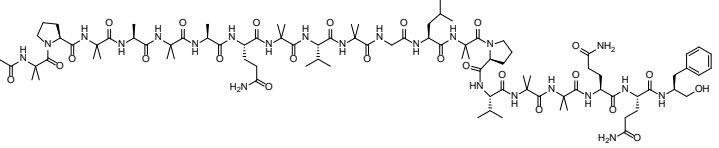

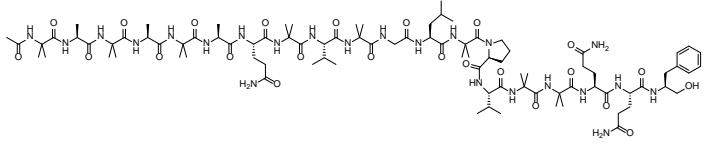

16	 <p>Dihydrogeodin C₁₇H₁₄O₇Cl₂</p>	5.26	220 281 347	401.0190 (+0.2)	209.0444, 237.0391 277.0262, 341.9870 219.0286, 151.0389 326.9632, 368.9863 179.0337, 86.9995 305.0213, 297.9612
		<p>G324 <i>Penicillium</i> sp. GenBank # KM215636 Endophytic fungus isolated from surface sterilized leaves of <i>Silybum marianum</i></p>			
#	Chemical Structure and Chemical formula	Rt (min)	UV (nm)	Positive Ionization Mode	
				[M+H] ⁺	MS/MS
17	 <p>10, 20-dehydro[12,13-dehydropropyl-2-(1,1-dimethylallyl) tryptophyl] diketopiperazine C₂₁H₂₁N₃O₂</p>	3.56	222 270	348.1708 (+0.4)	196.1120, 292.1077 182.0964, 264.1128 237.1020, 306.1232 331.1448, 280.1080 169.0759, 130.0652
18		4.09	217 288 437	350.1864 (+0.3)	130.0651, 198.1276 153.0657, 69.0700 238.1338, 294.1234 282.1232, 144.0807 221.1075, 183.1042 170.0598, 151.0500

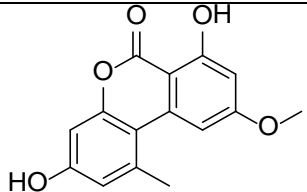
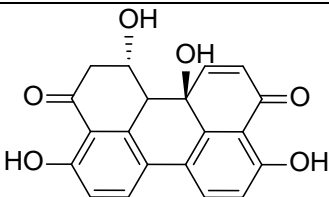
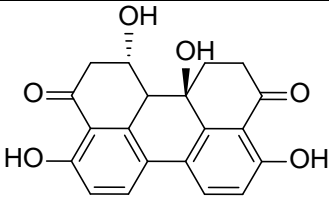
	<p>12,13-Dehydroprolyl-2-(1,1-dimethylallyltryptophyl)diketopiperazine $C_{21}H_{23}N_3O_2$</p>				
		<p>G142³⁴ <i>Fusicolla</i> sp. GenBank # AB858346 Fungus isolated from submerged wood in fresh water</p>			
#	Chemical Structure and Chemical formula	Rt (min)	UV (nm)	Positive Ionization Mode	
				[M+H] ⁺	MS/MS
19	 <p>Acuminatum B $C_{45}H_{73}N_7O_{11}$</p>	6.57	193 224 277	888.5443 (+0.2)	306.2430, 324.2522 377.2790, 101.0708 136.0760, 86.0964 395.2893, 349.2832 143.0818, 296.2573 448.3170, 466.3271 576.3774, 778.0210
		<p>G121 <i>Cylindrocarpon</i> sp. GenBank # KM816764 Endophytic fungus isolated from surface sterilized roots of <i>Yerba mansa</i></p>			

#	Chemical Structure and Chemical formula	Rt (min)	UV (nm)	Positive Ionization Mode	
				[M+H] ⁺	MS/MS
20	 <p>Equisetin C₂₂H₃₁NO₄</p>	7.08	224 295	374.2328 (+0.6)	175.1475, 71.0700 119.0853, 188.0562 105.0701, 83.0855 133.1012, 147.1166 170.0806, 200.0913 302.1758, 356.2210
		<p>G168 <i>Fusarium</i> sp. GenBank # KP897159 Endophytic fungus isolated from surface sterilized stems of <i>Hedera helix</i></p>			
#	Chemical Structure and Chemical formula	Rt (min)	UV (nm)	Positive Ionization Mode	
				[M+H] ⁺	MS/MS
21	 <p>Enniatin D (Analogue) C₃₄H₅₉N₃O₉</p>	7.87	210	654.4301 (-3.5)	196.1325, 214.1431 86.0962, 100.1117 186.1486, 314.1964 210.1487, 228.1586 328.2092, 414.2442

22	 <p>Enniatin E (Analogue) C₃₅H₆₁N₃O₉</p>	8.24	210	668.4458 (-3.4)	210.1489, 100.1117 228.1586, 196.1325 86.0962, 328.2093 200.1639, 217.1433 186.1487, 314.1956 441.2947, 598.7202
23	 <p>Aurofusarin C₃₀H₁₈O₁₂</p>	5.05	223	571.0860 (-2.6)	485.0493, 556.0576 541.0351, 528.0696 496.0432, 456.0803 441.0589, 571.0832 513.0442, 511.0640 231.0291, 297.0352
		<p>MSX59553³⁵ Hypocreales sp. GenBank # JQ749725 Fungus from MSX library</p>			
#	Chemical Structure and Chemical formula	Rt (min)	UV (nm)	Positive Ionization Mode	
				[M+H] ⁺	MS/MS

24	 <p>Verticillin A C₃₀H₂₈N₆O₆S₄</p>	5.63	212 303	697.1026 (0.0)	169.0603, 383.1494 232.0990, 615.1462 284.1017, 551.2020 401.1592, 266.0911 465.1022, 348.0461
25	 <p>Sch 52900 C₃₁H₃₀N₆O₇S₄</p>	5.44	210 300	727.11334 (0.3)	169.0671, 199.0712 232.0991, 284.1025 268.1075, 296.1021 314.1130, 383.1498 413.1608, 266.0918
		<p>MSX70741³⁶ Hypocreales sp. GenBank # JN377382 Fungus from MSX library</p>			
#	Chemical Structure and Chemical formula	Rt	UV (nm)	Positive Ionization Mode	

		(min)		[M+H] ⁺	MS/MS
26	 <p>Alamethicin F50 C₉₂H₁₅₁N₂₃O₂₄</p>	6.38	217	1963.13428 (-1.6)	931.5340, 750.4118 849.4814, 466.2643 537.3010, 1189.6916 991.5539, 623.3485 381.2119, 744.4483
		<p>MSX57715³⁶ Hypocreales sp. GenBank # JN377381 Fungus from MSX library</p>			
#	Chemical Structure and Chemical formula	Rt (min)	UV (nm)	Positive Ionization Mode	
				[M+H] ⁺	MS/MS
27	 <p>Trichokonin VI C₉₀H₁₄₉N₂₃O₂₄</p>	6.09	222	1937.12146 (-0.2)	908.5174, 724.3960 823.4647, 440.2488 511.2867, 774.4476 1163.6768, 335.1964 639.3439, 965.5388 1078.6225, 284.1588
		<p>G169 Alternaria sp. GenBank # KP897160 Endophytic fungus isolated from surface sterilized stems of <i>Hedera helix</i></p>			
#	Chemical Structure and Chemical formula	Rt (min)	UV (nm)	Negative Ionization Mode	
				[M-H] ⁻	MS/MS

28	 <p>Alternariol monomethyl ether C₁₅H₁₂O₅</p>	5.03	222 255 287 298 332 341	271.06070	256.0372, 250.9916 210.9987, 190.9926 231.0053, 204.9888 59.0135, 154.9923
29	 <p>Alterperyleneol C₂₀H₁₄O₆</p>	3.89	216 257 285 356	349.0708 (+0.4)	303.0653, 261.0557 331.0616, 285.0551 313.0494, 275.0694 287.0371, 317.0466 301.0532, 338.3737
30	 <p>Dihydroalterperyleneol C₂₀H₁₆O₆</p>	3.81	218 258 283 356	351.0863 (0.0)	315.0647, 333.0765 305.0819, 263.0714 301.0494, 289.0500 285.0555, 298.0639 297.0555, 287.0700