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# Combination of pseudo-LC-NMR and HRMS/MS-based molecular networking for the rapid identification of antimicrobial metabolites from *Fusarium petroliphilum*

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16 **Keywords:** *Posidonia oceanica*, fungal endophytes, *Fusarium petroliphilum*, UHPLC-  
17 **HRMS/MS molecular networking**, high resolution semi-preparative liquid chromatography, LC-  
18 **NMR coupling, antimicrobial natural products, anti-quorum sensing assays**

## Abstract

19 An endophytic fungal strain isolated from a seagrass endemic to the Mediterranean Sea (*Posidonia*  
20 *oceanica*) was studied in order to identify its antimicrobial constituents and further characterize the  
21 composition of its metabolome. It was identified as *Fusarium petroliphilum* by in depth  
22 phylogenetic analyses. The ethyl acetate extract of that strain exhibited antimicrobial activities as  
23 well as an ability to inhibit quorum sensing of *Staphylococcus aureus*. To perform this study with a  
24 few tens of mg of extract, an innovative one step generic strategy was devised. On one side, the  
25 extract was analyzed by UHPLC-HRMS/MS molecular networking for dereplication. On the other  
26 side semi-preparative HPLC using a similar gradient profile was used for a single step high  
27 resolution fractionation. All fractions were systematically profiled by <sup>1</sup>H-NMR. The data were  
28 assembled into a 2D contour map, that we call "*pseudo*-LC-NMR", and combined with those of  
29 UHPLC-HRMS/MS. This further highlight connection within structurally related compounds,  
30 facilitates data interpretation and provides an unbiased quantitative profiling of the main extract  
31 constituents. This innovative strategy led to an unambiguous characterization of all major  
32 specialized metabolites of that extract and to the localization of its bioactive compounds. Altogether  
33 this approach identified 22 compounds, 13 of which being new natural products and 6 being  
34 inhibitors of the quorum sensing mechanism of *S. aureus* and *Pseudomonas aeruginosa*. Minor  
35 analogues were also identified by annotation propagation through the corresponding HRMS/MS  
36 molecular network which enable a constituent annotation of 27 additional metabolites. This  
37 approach was designed to be generic and applicable to natural extracts of the same polarity range.

38

## 39 1 Introduction

40 The rapid and efficient identification of novel natural products (NPs) in complex biological systems  
41 is a priority for the search for new lead compounds. In this context, the development of approaches  
42 that allow both rapid and unambiguous identification of natural extracts constituents as well as  
43 estimation of their biological activity are key to NP research. This is particularly true in the context  
44 of the search for antibacterial/anti-infective compounds from microorganism cultures that are often  
45 obtained on a small scale in screening programs dedicated to finding new molecules to combat the  
46 challenging resistance problems in the field (Paytubi et al., 2017).

47 Following a physical isolation, a classical approach for the structural determination of a metabolite  
48 naturally consists of combining high-resolution mass spectrometry (HRMS) and nuclear magnetic  
49 resonance (NMR) data to obtain a definitive identification (Yu et al., 2019). To speed up this  
50 process, HPLC coupling with NMR has proven to be an interesting alternative to work directly in  
51 mixtures, but also has some limitations. On-line and at-line LC-<sup>1</sup>H-NMR hyphenations have  
52 permitted to partly characterize natural extracts metabolome (Dias and Urban, 2009; Vásquez-  
53 Ocmín et al., 2016; Hammerl et al., 2019). However, they are limited by their low sensitivity and  
54 resolution, in addition to practical issues such as solvent compatibility and solvent suppression. One  
55 way to solve these problems and to improve both spectral quality and sensitivity has been the  
56 development of LC-SPE-NMR approaches (Exarchou et al., 2005; Richard et al., 2013). Several  
57 recent works demonstrate the interest of this approach to work on natural extracts (Cardoso et al.,  
58 2016; Zebiri et al., 2016; Gad et al., 2018). A limitation, however, may be that this approach often  
59 requires repeated collection of chromatographic peaks in order to yield sufficient amounts of  
60 metabolites from given LC peaks of interest.

61 In this study, we developed an alternative approach to obtain consecutive NMR spectra of all  
62 fractions from a single high-resolution semi-preparative HPLC injection which is hereafter defined  
63 as *pseudo*-LC-NMR. This approach enables NMR analyses obtained at the semi-preparative level to  
64 be directly linked to UHPLC-HRMS metabolite profiling at the analytical scale with high spectral  
65 quality data on both NMR and MS dimensions. The combination of these MS, HRMS and NMR  
66 data, fraction by fraction, often allows an unambiguous identification of the metabolites present and  
67 an estimation of their amounts in parallel to biological activity tests. On the other hand, working on  
68 a semi-preparative scale enables a collection of numerous metabolites in the low mg range, a scale  
69 compatible with different types of biological tests. In this study, such an approach was applied to a  
70 fungus of marine origin (*Fusarium petroliphilum*) isolated from *Posidonia oceanica*. It allowed the  
71 identification of the metabolites responsible for antibacterial activity and obtaining a good overview  
72 of the metabolome composition of this endophytic strain.

73 The proposed method in this work was applied as part of a research effort to encounter new  
74 antibiotics. The emergence of resistant bacterial strains against the classical antibiotics represents  
75 indeed a major health issue (Hernando-Amado et al., 2019). New chemical entities with original  
76 activity profiles are particularly needed in drug discovery to fight such multi-resistant pathogenic  
77 bacterial strains (Brown and Wright, 2016). Among these multi-resistant bacteria, special attention  
78 must be paid to methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*  
79 (PA). The gram-positive bacterium *S. aureus* causes superficial and potentially fatal infections,  
80 such as sepsis and pneumonia (Holden et al., 2013; Foster et al., 2014). The gram-negative  
81 bacterium *P. aeruginosa* is an opportunistic pathogen considered to be life-threatening to  
82 immunocompromised patients and to cystic fibrosis patients, in addition, it is a major cause of  
83 sepsis upon burn injuries (Church et al., 2006).<sup>4</sup> Unfortunately, currently available antibiotics are  
84 often ineffective against multi-resistant bacterial strains due to the loss of their efficacy against  
85 what are now called "superbugs" (Cordell, 2000; Foster et al., 2014). In this respect, the discovery  
86 of molecules which are capable of blocking quorum sensing (QS) could offer promising alternative  
87 to current antibiotics. Indeed, QS or cell-to-cell communication in bacteria is a regulatory process,  
88 governed by chemical signaling, that ensures sufficient cell density before inducing the expression  
89 of certain genes, at the same time throughout the bacterial population. In the case of pathogens,  
90 these genes often code for virulence factors. The disruption of this system can therefore limit the  
91 virulence of pathogenic bacteria (Bassler and Losick, 2006; Ng and Bassler, 2009; Saeki et al.,  
92 2020).

93 In this context, NPs and their derivatives represent a historical source of unique chemical scaffolds  
94 with potential anti-infective properties. They represent 55% of FDA-approved antibiotics  
95 introduced in the period 1981-2019 (Newman and Cragg, 2020). This underlines the importance of  
96 NPs, always valuable as a source of chemical entities for new treatments of bacterial diseases. At  
97 present, the majority of naturally originated antibiotics have been isolated from soil  
98 microorganisms. Thus, investigation of specialized metabolites from marine microbial strains in  
99 this regard is an expanding field (Blunt et al., 2018; Sun et al., 2019).

100 Microbial communities which often exist in competitive environments with other strains are  
101 evolving specialized metabolites pathways to produce a wide range of chemical entities which  
102 could be an interesting source of novel NPs with antibiotic activity (Peric-Concha and Long, 2003).  
103 In this relation, metabolomics study of endophytes communities is a first step to orient further drug  
104 discovery approaches on such sources. Endophytes are organisms, often fungi and bacteria that live  
105 inside plant tissues (Nisa et al., 2015). They establish different relationships with plants that vary

106 from symbiotic to border on pathogenic. Endophytes have shown promising potential as source of  
107 new and bioactive NPs by having evolved a diversity of specialized metabolites (Singh et al., 2017).  
108 Among the possible sources of NPs from endophytes, the marine-derived fungus *F. petroliphilum*  
109 isolated from *Posidonia oceanica* (Posidoniaceae) was chosen in this work. *P. oceanica* is an  
110 underwater sea grass endemic to the Mediterranean Sea, it forms dense meadows from the surface  
111 down to 40 m depth. *P. oceanica* is known for its longevity and being potentially the host for a  
112 diverse endophyte community. The species is endangered due to intense human activities. *P.*  
113 *oceanica* plays an important role at the ecological and sedimentary level (Vassallo et al., 2013).

## 114 2 Results

115 In order to select a bioactive fungal strain and to rapidly identify the metabolites responsible from  
116 this activity, the following procedure was applied in this study. 1) A bioactivity screen was  
117 performed on a set of *Posidonia* fungal endophytes. 2) The most active strain was prioritized for the  
118 study. 3) An aliquot from the extract was subjected to UHPLC-HRMS and automated data-  
119 dependent acquisition MS/MS for metabolite profiling followed by dereplication of known  
120 compounds through molecular network analysis. 4) Since the annotated compounds were not  
121 reported to act as QS inhibitors, their unambiguous characterization had to be performed. 5) In  
122 order to localize and identify the bioactive compounds, the fungal extract was thoroughly analyzed  
123 by *pseudo*-LC-NMR (Figure 1F). The *pseudo*-LC-NMR process was developed for the purpose of  
124 this study but was intended to be generic in order to rapidly provide complementary NMR  
125 information to the LC-MS metabolite profiling of crude extracts available in limited amounts.

126 The key steps for this approach were as follow: 1) An optimized geometrical transfer was applied  
127 from the scale of UHPLC to analytical HPLC then to semi-preparative HPLC (Figure 1A, D and E).  
128 2) The crude extract was injected by LC at the semi-preparative scale with automated fraction  
129 collection each 30 seconds. 3) All fractions were submitted to <sup>1</sup>H-NMR analyses followed by LC-  
130 HRMS/MS. 4) <sup>1</sup>H-NMR spectra were stacked and plotted in 2D map sequenced according to  
131 retention time generating the *pseudo*-LC-NMR plot. 5) The concomitant processing of the *pseudo*-  
132 LC-NMR with the LC-MS profile allowed to increase the level of annotation and to conduct  
133 specific 2D-NMR experiments when needed.

134 In order to localize the bioactivity, the fractionation strategy had to be adapted to the sensitivity of  
135 the assays. In this specific study, the measurement of antibacterial and anti-QS activity required  
136 larger quantities than those obtained from the 30 seconds/fraction at the semi-preparative scale  
137 described above. Thus, a pooling of fractions was designed to highlight the area of chromatographic  
138 activity (Figure 1D,E). This finally permitted conducting a specific targeted purification of  
139 bioactive compounds on enriched extract with the same experimental setting.

### 140 2.1 Identification of the fungal strains

141 The identification of the marine strain *F. petroliphilum* that is the subject of this study proved to be  
142 challenging as this species is part of a species complex of *Fusarium solani*. Its identification  
143 required its placement within this complex in which terrestrial species are also found.

144 Although ITS is presently the best barcode sequence for fungi (Schoch et al., 2012), it did not allow  
145 the assignment of the selected fungal strain, FEP 16, at species rank. BLAST top score results in  
146 GenBank (GB) indicated that the FEP 16 ITS sequence was 100% similar with a 100% coverage, to  
147 the sequences of several species: *F. petroliphilum* (Q.T. Chen & X.H. Fu) D. Geiser, O'Donnell,

148 Short & Zhang 2013 (GB accession number: LC512834), *F. macroceras* Wollenweber & Reinking  
149 1925 (MH854821) and *F. solani* Mart. 1842 (MH855493). These species, based on ITS BLAST  
150 results, belong to the *F. solani* species complex in which many species are still not formally  
151 described (Short et al., 2013; Chehri et al., 2015; O'Donnell, 2019). Trying to determine further to  
152 which species FEP 16 belongs, we sequenced four more loci for that strain (see experimental  
153 section) and combined these data with data sampled in Bohni et al. (2016). Unfortunately for *F.*  
154 *macroceras*, only ITS and part of the nuclear large subunit (28S) were available in GB. After  
155 similarity search of the 28S (MH866321) of *F. macroceras* in GB, that sequence appeared 100%  
156 similar with several sequences for *F. solani* (i.e. AY097317) with 100% of sequence coverage but  
157 also with four sequences for *F. petrophilum* (i.e. MH874378) but with 90% of sequence coverage.  
158 Consequently, these two taxa are likely to be identical but without sequences for other more  
159 variable loci (*RPB2*, beta-tubulin and calmodulin) for *F. macroceras* but it is not possible to clarify  
160 the situation without type sequences for these species. Combined analyses for the *Fusarium* 5  
161 locus-81 taxa (Figure 2) (Supplementary Table S1) allowed to identify FEP 16 as *F. petrophilum*.

## 162 2.2 Evaluation of the antibacterial activity of the crude extracts

163 The minimum inhibitory concentration (MIC) of the extracts of all strains were tested against a  
164 methicillin-resistant strain of *Staphylococcus aureus* (ATCC 33591-MRSA) and a strain of  
165 *Pseudomonas aeruginosa* (ATCC 27853) (Supplementary Table S2). The crude extract of the  
166 fungus *F. petrophilum* (FEP 16) presented an antibacterial activity against MRSA with a MIC at  
167 32 µg/mL but no activity against Gram-negative *P. aeruginosa* strain.

168 In order to further verify if activity could be revealed at the level of quorum sensing (QS) inhibition  
169 for *P. aeruginosa*, we performed a reporter assay based on percentage of fluorescence to evaluate  
170 the potential of the crude extract on QS (Hentzer et al., 2002). Interestingly, the crude extract  
171 displayed about 80% reduction of the fluorescence level of 2 reporter genes (*pqsA* and *lasB*)  
172 normally induced by QS in *P. aeruginosa* (Table 1). The *pqsA* gene encodes an enzyme involved in  
173 the synthesis of PQS which is a signaling molecule of QS, while the *lasB* gene encodes an  
174 important elastase enzyme (Jimenez et al., 2012). These primary bioassay results encouraged us to  
175 go for further investigations.

## 176 2.3 Metabolites dereplication by UHPLC-HRMS/MS

177 In order to have a primary overview of the crude extract chemical content, it was analyzed by  
178 UHPLC-HRMS/MS in positive (PI) and negative (NI) ionization modes, MS/MS spectra of all  
179 detected features were recorded by data dependent analysis (DDA). Feature-based molecular  
180 networks (Nothias et al., 2020) for both modes were built to arrange the extracted ions (precursor  
181 ions) into clusters based on MS/MS similarity. This process was done by filtering ions below an  
182 intensity threshold at 1E6 and yielded 1900 features in PI mode, and 2270 in NI mode for building  
183 the corresponding MNs (Supplementary Figures S1,S2). The precursor masses and their associated  
184 MS/MS spectra were matched against experimental data from GNPS as well as predicted spectra  
185 obtained by *in silico* MS/MS fragmentation database (ISDB-DNP) (Allard et al., 2016).

186 For each node of the MN, possible structure candidates were listed according to MS/MS similarity  
187 (initial rank) up to a maximum of top 50 compounds reported to occur in fungi. To increase the  
188 level of confidence in annotation, a reweighting step based on taxonomy was performed. This step  
189 takes into consideration the matching between the biological source reported in the DNP at level of  
190 species > genus > family, resulting in a maximum of top 5 candidate compounds (final rank) for

191 each annotated node (Rutz et al., 2019). As a result, in PI mode, 823 of the 1900 detected features  
192 were annotated, 214 of them were found in family Nectriaceae, 148 of them present in genus  
193 *Fusarium* and 28 features reported in closely related species *F. solani*. In NI mode, 705 features  
194 were annotated out of 2720, 200 of them belonging to Nectriaceae, 135 of them were reported in the  
195 same genus and 19 of them have been found in *F. solani*. The annotated metabolites corresponding  
196 to the most intense MS peaks detected in both PI (threshold > 5E7) and NI (threshold > 1E7) are  
197 presented in Table 2. Their annotation was further refined by taxonomical ranking and by structural  
198 consistency of corresponding clusters. Compounds not previously found at least in the Nectriaceae  
199 family were not considered in this LC-MS dereplication process. The full MNs with the complete  
200 annotation is deposited in Figshare (<https://doi.org/10.6084/m9.figshare.14706198>). The same MN will  
201 be used later on this study to apply annotation propagation through location of isolated molecules in  
202 the MN.

203 Most of the annotated metabolites belong to pyran and pyrone derivatives, furan lactones,  
204 naphthoquinones, isocoumarins, terpenes and sterols. Such data are consistent with previously  
205 reported studies on the chemical content of *Fusarium* species (Wei and Wu, 2020). In particular, the  
206 presence of some frequently reported compounds in *Fusarium*, such as, gibepyrone D (Wang et al.,  
207 2011), aloesol (Kashiwada et al., 1984), fusarubin (Tatum and Baker, 1983), anhydrofusarubin  
208 (Shao et al., 2010) and bostrycoidin (Arsenault, 1965) which were highlighted in Table 2. As shown  
209 in Table 2, more than 30% of the most intense MS peaks could not be annotated through this  
210 process which could be either unknown compounds or compounds never reported in the  
211 Nectriaceae family.

212 Based on these dereplication results and the bioactivity data measured on the extract, this prompted  
213 us to establish an efficient approach that should provide complementary NMR data in line with  
214 metabolite profiling. This workflow is designed to run with few tens of milligrams of active extract,  
215 which corresponds to the usual amount obtained at laboratory scale using conventional Petri dishes  
216 (about 100 plates).

#### 217 **2.4 Culture scale-up and semi-preparative HPLC fractionation**

218 In order to obtain enough material to continue our proposed workflow, the culture of *F.*  
219 *petroliphilum* was scaled up to 100 petri dishes under the conditions described in materials and  
220 methods. This yielded 300 mg of ethyl acetate crude extract which exhibited bioactivity results  
221 comparable to those obtained during screening (Table 1).

222 To effectively link the expected fractionation of this extract with the metabolite profiling data (see  
223 above), a chromatographic gradient transfer method was used to find the correct separation  
224 parameters, ideally for a single separation at the semi-preparative level. In practice, an intermediate  
225 step at the analytical HPLC level (Figure 1D) was necessary on a column having the exact same  
226 phase chemistry as the one used in the semi-preparative level (see experimental section). The  
227 optimum HPLC conditions were determined with UV monitoring at 254 nm. This latter linear  
228 gradient method was then geometrically transferred to the semi-preparative scale. (Guillarme et al.,  
229 2008)

230 In order to obtain an efficient high-resolution separation of this complex mixture and to avoid any  
231 loss of chromatographic resolution, the crude extract (40 mg) was introduced into a dry loading cell  
232 according to our previously published protocol (Queiroz et al., 2019). Using this approach, it was  
233 possible to obtain equivalent separations at analytical and semi-preparative scales (Figure 1A, D

234 and E). To match the high chromatographic resolution that was obtained, 135 fractions were  
235 automatically collected on the basis of one fraction per 30 seconds. All fractions were immediately  
236 dried under vacuum and weighted.

## 237 **2.5 Combination of NMR spectra for an overview as a *pseudo*-LC-NMR plot**

238 With the idea to obtain a comprehensive NMR profile of all fractions and be able to align all spectra  
239 over the extended polarity range of the various metabolites separated, each fraction was diluted in  
240 600  $\mu\text{L}$  of  $\text{DMSO-}d_6$  and submitted to  $^1\text{H-NMR}$  analysis.  $\text{DMSO-}d_6$  was selected since it is known  
241 to have good solubility properties and for its compatibility with bioassays. The  $^1\text{H-NMR}$  spectra of  
242 all fractions were obtained by automated acquisition (29 hours of total acquisition). In order to  
243 visualize all  $^1\text{H-NMR}$  signals from the 135 fractions (F1-F135), a 2D plot was generated. For this,  
244 all individual spectra were binned and combined into a single matrix (ppm vs. RT or fraction  $N^\circ$ ).  
245 This plot simulates the actual output of a classic on-flow LC-NMR analysis (Queiroz et al., 2002).  
246 The chromatographic dimension of the plot was expressed either as fraction number or retention  
247 time since all spectra were stacked according to their elution order, which allows a straightforward  
248 correlation with the corresponding analytical HPLC-PDA trace (Figure 1F). An interactive version  
249 of the plot can be explored here: ([https://oolonek.github.io/pseudo\\_lcnmr\\_plotter/2dNMR.html](https://oolonek.github.io/pseudo_lcnmr_plotter/2dNMR.html)).

250 The generated 2D plot from collected and dried micro-fractions has several advantages over  
251 classical LC-NMR. In this case, all  $^1\text{H-NMR}$  signals are perfectly aligned, since all spectra are  
252 recorded with the same solvent across the whole separation ( $\text{DMSO-}d_6$ ). By comparing our  
253 approach to on-flow LC-NMR, no solvent suppression is necessary. In addition, compared to at-line  
254 LC-NMR detection methods, such as LC-SPE-NMR (Chang et al., 2020), the spectra obtained are  
255 recorded from a single LC separation and multiple injections for sample enrichment are not  
256 necessary. We define this workflow as a *pseudo*-LC-NMR analysis which can be viewed either as a  
257 2D plot (Figure 1F) or as a stacked view (Figure 3A) for a comprehensive analysis of the evolution  
258 of the  $^1\text{H}$  signals across the chromatographic dimension. Since the NMR response is directly  
259 proportional to the amounts of compounds, the overall observation of the 2D plot provides in a first  
260 instance an unbiased view of the molar ratios between constituents. However, this of course also  
261 related to the number of magnetically diverse protons for each constituent.

262 In order to determine molar ratios between fractions, a histogram was created based on  $^1\text{H-NMR}$   
263 peak integration which represents total proton intensities of each recorded spectrum  
264 (Supplementary Figure S3A). This histogram was rather similar to the one obtained by weighed dry  
265 fractions (Supplementary Figure S3B) which shows mainly major apolar constituents eluting at the  
266 end of the chromatogram between fractions F110 and F132. Analysis of the related  $^1\text{H-NMR}$   
267 spectra revealed signal patterns which mainly correspond to fatty acids, i.e., a methyl group at  $\delta_{\text{H}}$   
268 0.5 and an intense signal from methylene protons around  $\delta_{\text{H}}$  1.25. A specific histogram based on the  
269 methylene chain signal found typically in fatty acids was built across all fractions, it allows to  
270 highlight their presence mainly in fractions F110, 111, 114, 115, 117, 118, 119, 122 and 123  
271 (Supplementary Figure S3C). This trace matches well with the ELSD detection as shown in  
272 Supplementary Figure S3D and demonstrates that the crude extract was dominated by apolar  
273 constituents. Most of these fatty acids were found to be unsaturated fatty acids and exhibited a  
274 characteristic signal of their ethylenic protons around  $\delta_{\text{H}}$  5.3 whereas fractions F117, 122 and 123  
275 contain saturated fatty acids.



276 In addition to fatty acids, steroids and triterpenes were also identified in this chromatographic  
277 region. For example, ergosterol (**22**) a main constituent of fungal membrane, could be identified in  
278 F132 yielding an intense signal in the histogram (Supplementary Figure S3A). Its <sup>1</sup>H-NMR  
279 spectrum was in good agreement with reported data (Kwon et al., 2002; Yang et al., 2003) and  
280 confirming the dereplication results (Table 2). Moreover cerevisterol (**20**), could be confirmed in  
281 F116 by comparing its <sup>1</sup>H-NMR spectrum with literature (Kawagishi et al., 1988). Based on the  
282 extraction procedure, the presence of these main common apolar fungal constituents is not  
283 surprising. The analysis of the *pseudo*-LC-NMR 2D plot facilitated their identification and  
284 estimations of the ratio in an unbiased manner.

285 Further inspection of this *pseudo*-LC-NMR plot revealed the presence of specialized metabolites  
286 with aromatic signals (between  $\delta_H$  6.5 and 8.0) which were easily identified at the beginning of the  
287 chromatogram (F1-27). In this part of the semi-preparative HPLC chromatogram, the LC-UV  
288 profile exhibited main peaks that were mostly not fully separated (Figure 1E). The inspection of the  
289 2D plot as well as the related <sup>1</sup>H-NMR profile of each fraction was in line with this observation and  
290 exhibited spectra with overlapping signals. In this polar region, several compounds dereplicated by  
291 HRMS/MS (Table 2) could be confirmed by NMR upon comparison with reported data of the  
292 literature: adenosine (**1**) (Ciuffreda et al., 2007) was identified in fraction F4, gibepyrone D (**4**)  
293 (Wang et al., 2011) in fraction F20, aloesol (**5**) (Kashiwada et al., 1984) in fraction F26. In this  
294 region of the chromatogram arthrospolide A (Ayer et al., 1992) (Table 2) was dereplicated by  
295 HRMS/MS but in this case it could not be confirmed by <sup>1</sup>H-NMR, this could indicate that this  
296 common *Fusarium* metabolite is most likely present but in quantities below the NMR detection  
297 limit.

298 In the intermediate region of the chromatogram (fractions F28-F109), three known compounds  
299 which are common to *Fusarium* were dereplicated by HRMS/MS, bostrycoidin (**11**) (Arsenault,  
300 1965), fusarubin (**7**) and 3-*O*-methylfusarubin (**8**) (Tatum and Baker, 1983) which were confirmed  
301 by <sup>1</sup>H-NMR in F60 for **11** and in mixture in F43 and F44 for **7** and **8** respectively. Gibepyrone A  
302 (Westphal 2018) was dereplicated by HRMS/MS and confirmed by some of its characteristic <sup>1</sup>H-  
303 NMR signals in fraction F42, this compound was however present in very low concentration. In  
304 addition, minor metabolites dihydroanhydrojavanicin (Tatum et al., 1989) and solaniol (Niu et al.,  
305 2019) were dereplicated by HRMS/MS only but could not be found in NMR (Table 2). In this  
306 medium polarity region, several fractions (F51-56 and F91-93) seem to have <sup>1</sup>H signals in common  
307 particularly in the regions between  $\delta_H$  5.5 and 5.7, and between  $\delta_H$  6.0 and 6.3 (Figure 3A,  
308 highlighted in green), indicating that these fractions may contain structures with close skeletons (**9**,  
309 **10**, **16**, and **17**). Another class of molecules (cyclohexanones) could be highlighted in fractions F64-  
310 65 and F103-104 which were characterized by their signals between  $\delta_H$  5.2 and 5.3 (orange). The  
311 analysis of NMR spectra of these fractions, for which no confident HR-MS/MS annotation were  
312 obtained, revealed 3 new compounds (**13**, **14**, and **18**), Figure 4.

313 Since all NMR spectra were recorded in DMSO-*d*<sub>6</sub> the low field region ( $\delta_H$  11.0 -14.0) of the  
314 *pseudo*-LCNMR 2D plot highlighted characteristic deshielded mobile protons. Among them the  
315 acidic protons of fatty acids were clearly seen ( $\delta_H$  12.0) in F113-119 and quinonic phenolic groups  
316 of fusarubin (**7**) and 3-*O*-methylfusarubin (**8**) were observed (Figure 1F). Several similar types of  
317 signals were linked to the unknown metabolites in F35 and F69.

318 These selected examples show a good complementarity of NMR and HRMS/MS data for the  
319 dereplication of main constituents and help highlighting new *Fusarium* metabolites. For full *de*

320 *novo* structure identification 2D-NMR spectra were recorded for the compounds of interest and  
321 those exhibiting sufficient S/N ratio in the <sup>1</sup>H-NMR spectra.

## 322 2.6 Structure elucidation of new compounds

323 Careful interpretation of the HRMS/MS and <sup>1</sup>H-NMR data resulted in the identification of twenty-  
324 two compounds in one-step (**1-22**), among them, 13 are original NPs described here for the first  
325 time (**2a/2b, 3, 6, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19**) and presented in Figure 5. The known  
326 dereplicated compounds described above were also confirmed, when necessary, by additional 2D-  
327 NMR data and were all previously reported in *Fusarium* species: adenosine (**1**) (Hou et al., 2015),  
328 gibepyrone D (**4**) (Wang et al., 2011), aloesol (**5**) (Kashiwada et al., 1984), fusarubin (**7**) and 3-*O*-  
329 Methylfusarubin (**8**) (Tatum and Baker, 1983), bostrycoidin (**11**) (Yamamoto et al., 2002),  
330 cerevisterol (**20**) (Wang et al., 2011), 6-Dehydrocerevisterol (**21**) (Qiao et al., 2017) and ergosterol  
331 (**22**) (Thammawong et al., 2011).

332 Compound **6** (F35) was isolated as a pale rose amorphous solid. The HRMS/MS spectrum showed a  
333 molecular ion at *m/z* 288.0846 [M + H]<sup>+</sup> (calculated for C<sub>15</sub>H<sub>14</sub>NO<sub>5</sub>, 288.0866). No valid  
334 annotations based on MS could be obtained for this compound among all Nectriaceae described  
335 metabolites. The <sup>1</sup>H and edited-HSQC NMR spectra showed 3 exchangeable protons at δ<sub>H</sub> 5.06  
336 (1H, t, *J*=5.6 Hz, OH-15), 11.39 (1H, s, NH-13) and 12.88 (1H, s, OH-5), two aromatic protons at  
337 δ<sub>H</sub> 6.23 (1H, s, H-3) and 6.44 (1H, dd, *J*=2.1, 1.1 Hz, H-11), an oxymethylene at δ<sub>H</sub> 4.75 (2H, d,  
338 *J*=5.5 Hz, H<sub>2</sub>-15) and a methoxy group at δ<sub>H</sub> 3.89 (3H, s, H<sub>3</sub>-16) and a methyl at δ<sub>H</sub> 2.44 (3H, d,  
339 *J*=1.0 Hz, H<sub>3</sub>-14). A methoxy naphthoquinone moiety such as that found in fusarubin (**7**) was  
340 identified, thanks to the HMBC correlations from H-3 to the carbonyl C-4 (δ<sub>C</sub> 191.0) and C-1 (δ<sub>C</sub>  
341 179.5) to the quaternary carbons C-2 (δ<sub>C</sub> 160.5) and C-10 (δ<sub>C</sub> 107.4), and from the methoxy group  
342 to C-2. The methoxy signals were clearly shared in the *pseudo*-LC-NMR plot between compound **6**  
343 (F35) and the two furarubins **7** and **8** (F43-F44) this was also clearly visible for the common H-3  
344 aromatic signal of these molecules (Figure 4). The HMBC correlations from the deshielded  
345 hydroxyl OH-5 to C-10, C-5 (δ<sub>C</sub> 152.1) and C-6 (δ<sub>C</sub> 126.8) and from the oxymethylene H<sub>2</sub>-15 to C-  
346 5, C-6 and C-7 allowed to position these two groups. Then, a 2-methyl-pyrrole group was placed in  
347 C7-C8 according to the HMBC correlations from the aromatic proton H-11 to C-6, C-7 and C-8 (δ<sub>C</sub>  
348 130.5), from the methyl protons H<sub>3</sub>-14 to C-11 (δ<sub>C</sub> 99.8) and C-12 (δ<sub>C</sub> 156.7) and from NH-13 to C-  
349 7, C-8, C-11 and C-12. The ROESY correlations from H-3 to the methoxy H<sub>3</sub>-16 and from H-11 to  
350 H<sub>2</sub>-15 and H<sub>3</sub>-14 confirmed the structure. Compound **6** was thus identified as 5-hydroxy-4-  
351 (hydroxymethyl)-8-methoxy-2-methyl-1H-benzo[*g*]indole-6,9-dione.

352 The HRMS/MS spectrum of **9** (F51) displayed a molecular ion at *m/z* 295.1542 [M + H]<sup>+</sup>  
353 corresponding to the molecular formula C<sub>16</sub>H<sub>22</sub>O<sub>5</sub>. The NMR spectra showed 3 aromatic protons at  
354 δ<sub>H</sub> 5.52 (1H, d, *J*=1.8 Hz, H-3), 6.11 (1H, d, *J*=1.8 Hz, H-5) and 6.15 (1H, dd, *J*=9.8, 1.4 Hz, H-8),  
355 2 methylenes at δ<sub>H</sub> 1.21 (1H, dt, *J*=13.4, 7.4 Hz, H-10''), 1.34 (1H, dt, *J*=13.4, 6.8 Hz, H-10'), 1.96  
356 (1H, dd, *J*=15.0, 8.5 Hz, H-12'') and 2.25 (1H, m, H-12'), a methine at δ<sub>H</sub> 1.79 (1H, dqd, *J*=8.5, 6.8,  
357 5.1 Hz, H-11), 3 methyl groups at δ<sub>H</sub> 0.89 (3H, d, *J*=6.6 Hz, H<sub>3</sub>-14), 0.96 (3H, d, *J*=6.6 Hz, H<sub>3</sub>-15)  
358 and 1.86 (3H, d, *J*=1.4 Hz, H<sub>3</sub>-16) and a methoxy signal at δ<sub>H</sub> 3.88 (3H, s, H<sub>3</sub>-17). A 4-methoxy-6-  
359 substituted- $\alpha$ -pyrone was identified from the HMBC correlations between H-5 and C-3 (δ<sub>C</sub> 88.7),  
360 C-6 (δ<sub>C</sub> 159.6) and C-7 (δ<sub>C</sub> 127.3), between H-3 and C-5 (δ<sub>C</sub> 108.7), C-4 (δ<sub>C</sub> 167.2) and C-2 (δ<sub>C</sub>  
361 180.2), and between H<sub>3</sub>-17 and C-4. An alkyl side chain was attached in C-7, the HMBC  
362 correlations from H<sub>3</sub>-16 to C-6, C-7 and C-8 (δ<sub>C</sub> 140.0), from H<sub>3</sub>-15 to C-8, C-9 (δ<sub>C</sub> 29.9) and C-10  
363 (δ<sub>C</sub> 43.3), from H<sub>3</sub>-14 to C-10, C-11 (δ<sub>C</sub> 27.6) and C-12 (δ<sub>C</sub> 40.9), and from H<sub>2</sub>-12 to C-13 (δ<sub>C</sub> 173.7)

364 allowed to unambiguously assigned this 3,5,7-trimethylhept-6-enoic acid side chain. The *E*  
365 configuration of the double bond of the side chain was deduced from the ROE correlation between  
366 H<sub>3</sub>-16 and H-9. As discussed previously on the *pseudo*-LC-NMR plot some of the <sup>1</sup>H-NMR signals  
367 of **9** were common to other metabolites (**10**, **16** and **17**) found in F55, F91 and F93 suggesting a  
368 common pyrone moiety (Figure 4). Compound **9** was identified as (6*E*)-7-(4-methoxy-6-oxo-6H-  
369 pyran-2-yl)-3,5-dimethyloct-6-enoic acid.

370 The NMR data of **10** (F55) exhibited indeed close similarities to those of **9**, they share the same 4-  
371 methoxy- $\alpha$ -pyrone and the difference lies in the side chain. One of the methyl doublet signals  
372 present in **9** was replaced in **10** by a methyl triplet at  $\delta_{\text{H}}$  0.81 (3H, t, *J*=7.3 Hz, H<sub>3</sub>-13). An additional  
373 oxymethylene was observed at  $\delta_{\text{H}}$  3.25 (1H, dt, *J*=10.2, 5.0 Hz, H-14") and 3.30 (1H, dt, *J*=10.2, 5.0  
374 Hz, H-14') in addition to a hydroxyl at  $\delta_{\text{H}}$  4.34 (1H, t, *J*=5.2 Hz, OH-14) whereas the carbonyl of  
375 the acid group was no longer observed. The 2D-NMR experiments were in good agreement with the  
376 structure presented in Figure 4 and the ion at *m/z* 281.1744 [*M* + H]<sup>+</sup> confirmed the structure as 6-  
377 ((*E*)-6-ethyl-7-hydroxy-4-methylhept-2-en-2-yl)-4-methoxy-2H-pyran-2-one.

378 Compound **16** which has MF of C<sub>16</sub>H<sub>24</sub>O<sub>3</sub> and ionized at *m/z* 265.1816 [*M* + H]<sup>+</sup> in F91 shares the  
379 same skeleton as **9** and **10**. The carboxyl group presented at the end of the side chain of **9** was  
380 replaced in this case by a methyl group at  $\delta_{\text{H}}$  0.82 (3H, t, *J*=7.4 Hz, H<sub>3</sub>-13). Compound **16** was thus  
381 identified as 4-methoxy-6-((*E*)-4,6-dimethyloct-2-en-2-yl)-2H-pyran-2-one.

382 Compound **17** which has MF of C<sub>17</sub>H<sub>26</sub>O<sub>3</sub> and ionized at *m/z* 279.1956 [*M* + H]<sup>+</sup> in F93 belongs  
383 also to the same family. The side chain was the same as that of **16** but an additional methyl group  
384 was observed in C-3 at  $\delta_{\text{H}}$  1.69 (3H, s, H<sub>3</sub>-18). The HMBC correlations from the methyl H<sub>3</sub>-18 to  
385 the ester carbon C-2 ( $\delta_{\text{C}}$  179.7), the olefinic carbon C-3 ( $\delta_{\text{C}}$  98.9) and the oxygenated olefinic  
386 carbon C-4 ( $\delta_{\text{C}}$  162.0) confirmed this structure as 4-methoxy-3-methyl-6-((*E*)-4,6-dimethyloct-2-  
387 en-2-yl)-2H-pyran-2-one. A zoom in the stacked plot also clearly highlights this similarity on the  
388 side chain (Figure 3D).

389 Compound **12** in F62 was found to possess a molecular formula of C<sub>28</sub>H<sub>40</sub>O<sub>6</sub> with 8 degrees of  
390 unsaturation, as evidenced by PI HRMS/MS at *m/z* 473.2877 (calcd for C<sub>28</sub>H<sub>41</sub>O<sub>6</sub>). The <sup>1</sup>H-NMR  
391 data of **12** showed typical signals of an oxygenated steroid: 2 methyl singlets at  $\delta_{\text{H}}$  0.77 (3H, s, H<sub>3</sub>-  
392 18) and 0.89 (3H, s, H<sub>3</sub>-19), 3 methyl doublets at  $\delta_{\text{H}}$  0.85 (3H, d, *J*=7.0 Hz, H<sub>3</sub>-28), 0.90 (3H, d,  
393 *J*=6.8 Hz, H<sub>3</sub>-26) and 1.73 (3H, d, *J*=1.2 Hz, H<sub>3</sub>-21), 4 oxygenated methines at  $\delta_{\text{H}}$  3.27 (1H, d,  
394 *J*=3.7 Hz, H-6), 3.59 (1H, tq, *J*=10.6, 5.0 Hz, H-3), 4.18 (1H, t, *J*=8.5 Hz, H-23) and 5.53 (1H, dd,  
395 *J*=5.2, 3.7 Hz, H-7), an oxygenated methylene at  $\delta_{\text{H}}$  3.31 (1H, overlapped, H-27") and 3.93 (1H, dd,  
396 *J*=8.2, 6.4 Hz, H-27'), an ethylenic proton at  $\delta_{\text{H}}$  5.22 (1H, d, *J*=8.4 Hz, H-22) and a series of  
397 methines and methylenes between  $\delta_{\text{H}}$  1.20 and 2.63. The HMBC also indicated the presence of two  
398 oxygenated quaternary carbon at  $\delta_{\text{C}}$  68.1 (C-5) and 74.8 (C-9), two quaternary *sp*<sup>2</sup> carbons at  $\delta_{\text{C}}$   
399 134.1 (C-20) and 142.8 (C-14), and a carbonyl at  $\delta_{\text{C}}$  206.7 (C-15). HMBC correlations from H<sub>3</sub>-19  
400 to C-1 ( $\delta_{\text{C}}$  25.6), C-5, C-9 and C-10 ( $\delta_{\text{C}}$  37.0), from H<sub>3</sub>-18 to C-12 ( $\delta_{\text{C}}$  32.1), C-13 ( $\delta_{\text{C}}$  43.1), C-14  
401 and C-17 ( $\delta_{\text{C}}$  51.5), from the methylene H<sub>2</sub>-16 at  $\delta_{\text{H}}$  2.16 (1H, dd, *J*=18.3, 7.3 Hz, H-16 $\alpha$ ) and 2.63  
402 (1H, m, H-16 $\beta$ ) to C-15 allowed to identify the four member rings of sterol in compound **12** which  
403 was identical to 5 $\beta$ ,6 $\beta$ -epoxy-3 $\beta$ ,7 $\alpha$ ,9 $\alpha$ -trihydroxy-(22*E*,24*R*)ergosta-8(14),22-dien-15-one  
404 previously isolated by Wang et al. (2012) from the culture of the Basidiomycete *Polyporus ellisii*.  
405 The chain attached to C-17 was found to be new, its linkage was established by the HMBC  
406 correlations from the methyl H<sub>3</sub>-21 to C-17 ( $\delta_{\text{C}}$  51.5), C-20 and C-22 ( $\delta_{\text{C}}$  128.1) and thus the  
407 presence of a double bond between C-20 and C-22. The HMBC correlations from H<sub>3</sub>-28 to C-23 ( $\delta_{\text{C}}$

408 79.7), C-24 ( $\delta_C$  42.9) and C-25 ( $\delta_C$  35.9), from H<sub>3</sub>-26 to C-24, C-25 and C-27 ( $\delta_C$  73.7) and from  
409 H<sub>2</sub>-27 to C-23 indicated the formation of a furan ring. The ROESY correlations from H<sub>3</sub>-18 to H<sub>3</sub>-  
410 21 and H-11 $\beta$ , from H<sub>3</sub>-19 to H-11 $\beta$  and H-2 $\beta$ , from H-4 $\alpha$  to H-3 and H-6, from OH-7 to H-6, from  
411 H-1 $\alpha$  to OH-9, from H-23 to H<sub>3</sub>-21 and H<sub>3</sub>-28, and from H-22 to H-17 and H-24 allowed to  
412 determine the relative configuration of this new sterol (Figure 5). Compound (**12**) was characterized  
413 as 5 $\beta$ ,6 $\beta$ -23,26-diepoxy-3 $\beta$ ,7 $\alpha$ ,9 $\alpha$ -trihydroxy-(20Z,23S,24S,25R) ergosta-8(14),20-dien-15-one.

414 As discussed above, compounds **13**, **14**, and **18** sharing many common <sup>1</sup>H-NMR signals in the  
415 *pseudo*-LC-NMR plot were assigned to the same structural type. Among them, **18** exhibited the  
416 most intense signals and were analyzed in depth first.

417 Compound **18** in F103 was isolated as a pale yellow amorphous solid. The HRMS/MS spectrum  
418 showed a protonated ion at *m/z* 333.2441 corresponding to a molecular formula of C<sub>21</sub>H<sub>32</sub>O<sub>3</sub>. The  
419 <sup>1</sup>H and HSQC NMR spectra displayed two ethylenic protons at  $\delta_H$  5.25 (1H, dd, *J*=15.2, 7.9 Hz, H-  
420 15) and 5.29 (1H, dd, *J*=15.2, 7.0 Hz, H-16), five methylenes at  $\delta_C$  27.2 (C-16), 27.3 (C-15), 30.9  
421 (C-7), 32.7 (C-11), 35.8 (C-12), four methine at  $\delta_C$  32.4 (C-25), 38.1 (C-20), 42.0 (C-24), 55.4 (C-  
422 17), four methyl doublets at  $\delta_H$  0.81 (3H, d, *J*=6.9 Hz, H<sub>3</sub>-27), 0.83 (3H, d, *J*=6.9 Hz, H<sub>3</sub>-26), 0.91  
423 (3H, d, *J*=6.7 Hz, H<sub>3</sub>-28), 1.03 (3H, d, *J*=6.6 Hz, H<sub>3</sub>-21) and one methyl singlet at  $\delta_H$  1.06 (3H, s,  
424 H<sub>3</sub>-18). The HMBC correlations from methyl H<sub>3</sub>-18 to the methylene C-12, the quaternary carbon  
425 C13 ( $\delta_C$  44.6), the *sp*<sup>2</sup> carbon C-14 (175.2) and the methine C-17, from the methylene H<sub>2</sub>-7 to C-14,  
426 the *sp*<sup>2</sup> carbon C-8 ( $\delta_C$  125.0), the carbonyl C-9 ( $\delta_C$  196.3) and to the carboxyl C-6 ( $\delta_C$  171.7), from  
427 the methylene H<sub>2</sub>-11 ( $\delta_H$  2.22 and 2.56) to C-6 and H<sub>2</sub>-15 ( $\delta_H$  2.34 and 2.46) to C-14 and C-17 in  
428 combination with COSY correlation from H<sub>2</sub>-11 to H<sub>2</sub>-12 ( $\delta_H$  1.79 and 2.15) and H-17 ( $\delta_H$  1.42) to  
429 H<sub>2</sub>-16 ( $\delta_H$  1.51 and 1.78) allowed to identify a fused six- and five-membered rings. The side chain  
430 in C-17 was identified and positioned thanks to the HMBC correlation from the methyl H<sub>3</sub>-21 to the  
431 methines C-17 and C-20 and the olefinic carbon C-22 ( $\delta_C$  134.7), from the methyl H<sub>3</sub>-28 to the  
432 olefinic carbon C-23 ( $\delta_C$  132.0), the methines C-24 and C-25 and from the methyls H<sub>3</sub>-26 and H<sub>3</sub>-  
433 27 to the methines C-24 and C-25. This compound corresponds to a highly degraded ergostane-type  
434 steroid identified as 2-(2,3,5,6,7,7a-hexahydro-7a-methyl-1-((*E*)-5,6-dimethylhept-3-en-2-yl)-5-  
435 oxo-1H-inden-4-yl)acetic acid.

436 The NMR signals of the isomeric **13** and **14**, both possess MF of C<sub>21</sub>H<sub>32</sub>O<sub>4</sub> and protonated ions at  
437 *m/z* 349.2349, showed great similarities with those of **18** in F103 (C<sub>21</sub>H<sub>32</sub>O<sub>3</sub>), except for the  
438 terminal part of the side chain. Compound **13** contains an additional hydroxy group in C-25  
439 evidenced by the HMBC correlations from the methyls H<sub>3</sub>-26 and H<sub>3</sub>-27 ( $\delta_H$  1.03 and 0.99,  
440 respectively) to the oxygenated quaternary carbon C-25 ( $\delta_C$  70.5) and the methine C-24 ( $\delta_C$  47.1).  
441 In compound **14**, the terminal methyl H<sub>3</sub>-27 was hydroxylated and replaced by an oxygenated  
442 methylene at  $\delta_H/\delta_C$  3.17/64.4.

443 Compound **15** (F69) was found to possess a molecular formula of C<sub>14</sub>H<sub>13</sub>NO<sub>4</sub> and showed a  
444 deprotonated ion at *m/z* at 258.0771 [M - H]<sup>-</sup>. It was isolated as a pale yellow amorphous solid, its  
445 <sup>1</sup>H and HSQC NMR spectra indicated the presence of two mobile protons at  $\delta_H$  11.39 (1H, s, NH-  
446 11) and 12.84 (1H, s, OH-6), two aromatic protons at  $\delta_H$  6.21 (1H, s, H-3) and 6.28 (1H, s, H-9),  
447 one methoxy group at  $\delta_H$  3.89 (3H, s, H<sub>3</sub>-14) and two methyl groups at  $\delta_H$  2.36 (3H, s, H<sub>3</sub>-15), 2.44  
448 (3H, s, H<sub>3</sub>-16). The HMBC correlation from the methyl H<sub>3</sub>-16 to only two olefinic carbons C-9 and  
449 C-10 at  $\delta_C$  98.9 and 146.2, respectively indicated that it was adjacent to a nitrogen whose presence  
450 was demonstrated by HRMS/MS. The correlations from the mobile proton NH-11 to the olefinic  
451 carbons C-9, C-8 ( $\delta_C$  137.6) and C-12 ( $\delta_C$  129.8) and from the aromatic proton H-9 to C-8, C-10

452 and C-12 allowed to identify a pyrrole group. The HMBC correlation from methyl H<sub>3</sub>-15 to the  
453 olefinic carbons C-8, C-7 ( $\delta_C$  123.9) and C-6 ( $\delta_C$  152.2) and from the hydroxy group OH-6 to C-7,  
454 C-6 and C-5 ( $\delta_C$  107.3) linked the pyrrole cycle to a phenol moiety. The HMBC correlations from  
455 the aromatic proton H-3 to C-5, C-4 ( $\delta_C$  160.5) and the ester carbon C-2 ( $\delta_C$  179.1) and from the  
456 methoxy to C-4 allowed to position a 2-pyrone cycle. The ROESY correlations from H-9 to the  
457 methyls H<sub>3</sub>-16 and H<sub>3</sub>-15 and from H-3 to the methoxy H<sub>3</sub>-14 were in good agreement with 5-  
458 hydroxy-4-methoxy-6,8-dimethylpyrano[3,2-*g*]indol-2(9H)-one (Figure 4).

459 Compound **19** in F107, showed a Na<sup>+</sup> adduct ion at *m/z* 599.4380 which correlated to C<sub>35</sub>H<sub>60</sub>O<sub>6</sub>.  
460 The NMR spectra of **19** indicated the presence of stigmast-8-en-3-ol with typical signals like two  
461 methyl singlets at  $\delta_H$  0.65 (3H, s, H<sub>3</sub>-18) and 0.96 (3H, s, H<sub>3</sub>-19), three methyl doublets at  $\delta_H$  0.79  
462 (3H, d, *J*=6.9 Hz, H<sub>3</sub>-27), 0.82 (3H, d, *J*=6.9 Hz, H<sub>3</sub>-26), 0.90 (3H, d, *J*=6.5 Hz, H<sub>3</sub>-21), one methyl  
463 triplet at  $\delta_H$  0.82 (3H, t, *J*=7.3 Hz, H<sub>3</sub>-29), one oximethine at  $\delta_H$  3.46 (1H, tt, *J*=11.2, 4.3 Hz, H-3)  
464 and one olefinic carbons detected on the HMBC spectrum from the correlations of methyl H<sub>3</sub>-18 to  
465 C-9 ( $\delta_C$  140.4). Additional signals corresponding to a glucose unit were detected at  $\delta_H$  2.89 (1H, td,  
466 *J*=8.4, 4.8 Hz, H-2'), 3.01 (1H, td, *J*=9.2, 5.0 Hz, H-4'), 3.06 (1H, m, H-5'), 3.12 (1H, td, *J*=8.9, 4.8  
467 Hz, H-3'), 3.40 (1H, m, H-6'b), 3.64 (1H, dd, *J*=11.1, 6.2 Hz, H-6'a), 4.22 (1H, d, *J*=7.8 Hz, H-1').  
468 Due to the very small amount of isolated compound and the presence of an overlapping fatty acid in  
469 the fraction, it was not possible to obtain a complete assignment of the molecule. However, the  
470 ROESY correlation between the H-1' proton of glucose and the H-3 proton of the stigmasteryl  
471 skeleton allowed the positioning of glucose in C-3 and to identify **19** as 3-*O*- $\beta$ -D-glucopyranoside-  
472 stigmast-8-en-3-ol.

473 In addition to the compounds described in the polar part of the chromatogram (F1-27), F10  
474 exhibited a <sup>1</sup>H-NMR spectrum of possibly 2 to 3 metabolites. The HRMS/MS data confirmed the  
475 presence of two ions at *m/z* 199.0965 and 155.0703 [M + H]<sup>+</sup> which are typical for C<sub>10</sub>H<sub>15</sub>O<sub>4</sub> and  
476 C<sub>8</sub>H<sub>11</sub>O<sub>3</sub> respectively.

477 A detailed 2D-NMR analysis of the fraction revealed the presence of 3 compounds which share a 3-  
478 methyl-pyran-2-one moiety similar to the one of **4** as indicated by the aromatic H-4 and H-5 at  
479  $\delta_H/\delta_C$  7.35-7.36/140.4-140.5 and 6.26-6.33/100.4-101.4, respectively and the methyl at  $\delta_H/\delta_C$  1.95-  
480 1.96/16.0-16.2. Compounds **2a** and **2b** (C<sub>10</sub>H<sub>15</sub>O<sub>4</sub>) were diastereoisomers with a 2,3-  
481 dihydroxybutan-2-yl side chain characterized by a methyl doublet (*J*=6.3 Hz, H<sub>3</sub>-9) at  $\delta_H$  0.97 and  
482 1.03, an oxygenated methine (m, H-8) at  $\delta_H$  3.73 and 3.74, and a methyl singlet (H<sub>3</sub>-10) at  $\delta_H$  1.37  
483 and 1.24 for **2a** and **2b**, respectively. The hydroxylation in C-7 and the linkage of the 2,3-  
484 dihydroxybutyl chain in C-6 was confirmed by the HMBC correlations from the methyl H<sub>3</sub>-10 to C-  
485 6 ( $\delta_C$  167.0 and 167.2 for **2a** and **2b**, respectively), C-7 ( $\delta_C$  74.5 and 75.0 for **2a** and **2b**,  
486 respectively) and C-8 ( $\delta_C$  70.2 and 70.4 for **2a** and **2b**, respectively) and from the aromatic protons  
487 H-4 and H-5 to C-6. On the other hand, the 3-methyl-pyran-2-one of **3** (C<sub>8</sub>H<sub>11</sub>O<sub>3</sub>) was substituted  
488 by a hydroxyethyl group in C-6 as indicated by the methyl doublet at  $\delta_H$  1.30 (3H, d, *J*=6.6 Hz, H<sub>3</sub>-  
489 8), the methine at  $\delta_H$  4.41 (1H, q, *J*=6.6 Hz, H-7), and the HMBC correlation from the methyl to C-  
490 6 ( $\delta_C$  165.6) and C-7 ( $\delta_C$  64.9).

491 Overall, the combination of the LC-HRMS/MS data and *pseudo*-LC-NMR plot together with in  
492 depth 2D-NMR analysis of selected peaks provided a good overview of all main constituents of  
493 fractions F1-135 in a single semi-preparative HPLC separation. As many of the fractions were in  
494 very small quantities (in the sub-mg range), we had to find a strategy to collect enough compounds

495 for the bioassays which could only be conducted, after optimization, with at least 500 µg of pure  
496 compound.

## 497 **2.7 Determination of bioactive zones**

498 Following our workflow, to ensure accumulating enough material required for bioassays, the 135  
499 collected fractions were pooled into 14 chromatographic zones (Z), where each zone represents 5  
500 minutes window of elution time (Figure 6A). Submitting of the pooled fractions to bioactivity tests  
501 gives an approximate location of the active compounds and facilitates targeting them in an  
502 additional chromatographic separation. Since the extract was active against a methicillin-resistant  
503 *Staphylococcus aureus* (MRSA), the 14 zones were subjected firstly to MIC tests against this strain.  
504 As a result, only zone 9 (Z9) showed significant inhibitory effect at 32 µg/ml which suggested the  
505 presence of an antibacterial compound in fractions F81-91. In parallel, the 14 zones were evaluated  
506 for anti-QS of PA in the same way as for the crude extract and on the same reporter genes (*pqsA*  
507 and *lasB*).

508 For this purpose, we consider zones with bioactive candidates, those that have shown values of 70%  
509 or less in fluorescence level for at least one of the reporters. The value of 70% was chosen since  
510 each zone is still a mixture of several constituents and to avoid missing pure bioactive candidates.  
511 As a result, 7 zones from Z3-Z9, which represent the mid-polarity region, presented activity as QS  
512 inhibitors. Z5 was the most active one and significantly reduced the fluorescence level of gene *pqsA*  
513 to 31% and *lasB* to 43% (Table 3). In order to determine the specific molecules responsible for  
514 these activities, an additional LC-peak targeted chromatographic separation at semi-preparative  
515 level was performed, and an enrichment step was designed to remove the very lipophilic zones  
516 which clearly were not responsible for bioactivity.

## 517 **2.8 Enrichment of the crude extract for targeted purification of bioactive compounds**

518 In order to increase the concentration of the bioactive compounds in Z3-Z9, the ethyl acetate crude  
519 extract was submitted to liquid-liquid separation using water/methanol in ratio (7:3) and hexane.  
520 This yielded approximately 70 mg of hydroalcoholic fraction and 90 mg of hexane fraction from  
521 160 mg of crude extract. As seen in Figure 6B-C, all zones of interest (Z3-9) are retained in the  
522 hydroalcoholic part except Z9 and the ELSD traces highlight well the efficiency of the enrichment  
523 procedure.

524 Taking into account the enrichment factor (2.3 folds), a single semi-preparative HPLC fractionation  
525 was carried on 30 mg of hydroalcoholic fraction in the same conditions as for the crude extract  
526 (Supplementary Figure S4). This yielded a good baseline separation in most compounds that ease  
527 the peak targeted collection. Purity of collected fractions were checked by <sup>1</sup>H-NMR and LC-ELSD-  
528 MS (data not shown) and enabled the bioactivity assessment of compounds **1-14**. Since Z9 was also  
529 a bioactive target, the apolar hexane fraction was purified similarly. Interestingly, by comparing the  
530 dry weights of collected fractions from enriched extract with the equivalent ones from crude extract  
531 all compounds were collected in amounts higher than 500 µg which was the threshold for the  
532 bioassay we selected. This grants supplementary amounts to perform further bioassay experiments  
533 such as quantitative PCR.

## 534 **2.9 Biological assay of pure compounds**

535 In order to assign compounds which are responsible for the antibacterial and anti-QS activity,  
536 purified compounds (**4-16**) that belong to Z3-Z9 were submitted to the same biological tests as  
537 described before.

538 For the QS test on *P. aeruginosa*, compounds **5**, **10**, **13** and **16**, presented moderate to weak activity  
539 profiles as they did not reach values under 30% in fluorescence reduction (Table 3) at 128 µg/ml.  
540 However, a mixture of the known *Fusarium* quinones fusarubin (**7**) and 3-*O*-methylfusarubin (**8**)  
541 which previously located in the active Z5, presented an enhanced and significant QS inhibition  
542 (12% and 15%) in pqs and las systems respectively at 128 µg/ml. To further confirm these results,  
543 we performed quantitative RT-PCR analyses on QS regulated genes *pqsA*, *lasB*, and *rhIA* involved  
544 in rhamnolipids production (Van Gennip et al., 2009). The relative expression of QS regulated gene  
545 *pqsA* in the presence of **7** and **8** was two times less expressed than the reference (Figure 7B).  
546 However, relative expression of *lasB* and *rhIA* did not show significant effects (data not shown)  
547 unlike results obtained by *gfp* transcriptional fusions (Table 3). In addition, since the zone Z9 was  
548 the only one showing MRSA inhibition activity, its main compound is one of the new pyrones **16**  
549 was evaluated against MRSA following the protocol of Wiegand et al. (2008). Compound **16**  
550 presented MIC at 32 µg/mL (Table 3).

551 On the whole series of the isolated compounds anti-QS assay for *S. aureus* on the reporter strain  
552 *rnaIII-lacZ* targeting the reporter gene *agr* were also performed following the method of (Nielsen et  
553 al., 2010). Compounds **5**, **10**, **13** and **16** presented a QS *rnaIII-lacZ* inhibition at 32 µg/ml showing  
554 fluorescence values less than 20% (Table 3). These results were confirmed by real time quantitative  
555 reverse transcription (RT-qPCR) in gene expression of *hla* and *hld*, both coding for QS regulated  
556 exotoxins (Figure 7A). Compared to the positive control, the antagonist auto-inducing peptide  
557 (AIP) from *Staphylococcus caprae*, all of these compounds show a better inhibition on *hla* gene  
558 expression which codes for the alpha-hemolysin. The tendency is the same for *hld* gene expression  
559 which codes for the delta-hemolysin, except for **16** that shows a less efficient effect with an  
560 inhibition of only 2-folds (Figure 7A).

### 561 **3 Discussion**

562 This comprehensive study of *F. petroliphilum* enables an unambiguous characterization of 22  
563 compounds based on one single high resolution semi-preparative HPLC separation of the crude  
564 extract.

565 On the chemical profiling aspect, this typical fungal extract (ethyl acetate extract from solid culture)  
566 allows us to devise an integrated workflow that bridges in depth LC-HRMS/MS molecular  
567 networking with subsequent NMR analysis. This was achieved by systematic collection of NMR  
568 data from fractions of the semi-preparative HPLC separation. We define this process as “pseudo-  
569 LC-NMR” since it enables combining proton NMR spectra recorded in a common solvent in a  
570 single 2D plot. This way of processing highlights relationships between metabolites through  
571 common chemical shifts and generates plots that also provide semi-quantitative estimation of their  
572 amount in the extract. In a first instance, The <sup>1</sup>H-NMR data acquired for all fractions correlated well  
573 with the dereplication results obtained from the molecular networks. For most of the unannotated  
574 metabolites, full *de novo* structure assignment based on 2D-NMR and MS data obtained from the  
575 profiling efficiently enables the identification of new fungal NPs. The unambiguous structural  
576 assignment of most of the main metabolites also provides a valid set of standards which allows  
577 more extended annotations to be performed through molecular networking mainly based on

578 relationships that could be established between analogues (MN annotation propagation). However,  
579 in its current state, the proposed workflow still requires partial manual inspection/processing of  
580 both NMR and MS data. Future development of algorithms for connecting NMR information into  
581 molecular network would facilitate the efficiency of the full metabolome composition assessment  
582 process. Our study mainly highlights the potential of such data integration and demonstrates that  
583 with well optimized chromatographic conditions at the semi-preparative HPLC level, high quality  
584 spectral data can be efficiently generated in a restricted laboratory time frame.

585 On the bioactivity aspects, we could demonstrate that it is possible to take advantage of the same  
586 fractionation to evaluate bioactivity on several complementary assays for assessing anti-bacterial  
587 and anti-QS activity. In our case, one of the limiting factors being the sensitivity of the bioassay, an  
588 enrichment of the extract had to be performed to meet the requirements of the tests. The method  
589 proposed is however generic and could be applied to various natural extracts of medium polarity  
590 and to diverse types of bioassays. It enables the characterization of specific bioactive compounds  
591 and provides at the same time valuable data for in depth metabolome characterization of a given  
592 organism.

593 In the case of this specific study, among the 22 compounds unambiguously identified, 13 were  
594 never reported to our knowledge. This allowed a better characterization of composition of *F.*  
595 *petroliphilum* which is a rarely chemically studied member of the *F. solani* species complex. Our  
596 genetic investigation of this endophyte also enables its unambiguous identification and position this  
597 strain as a member of *F. solani* species complex. Several of the identified metabolites exhibited  
598 weak to moderate MIC value on a gram-positive MRSA and no growth inhibition on the gram-  
599 negative *P. aeruginosa*. However, an in-depth study of the QS activity of both strains through our  
600 selected assays revealed significant QS inhibition for some of the metabolites especially for the  
601 known fusarubins for which anti-QS activities were never reported. Using our workflow, we were  
602 also able to show that the generic fractionation obtained by semi-preparative HPLC allowed a  
603 consistent concentration of activity from the broad chromatographic zones to the active ingredients.  
604 This further demonstrates the advantage of such high-resolution fractionation profiling for tracking  
605 bioactivity. Based on this approach, we plan to further study the different endophytes found in  
606 *Posidonia*, which we identify as an interesting model for the study of the endophytic community.

## 607 **4 Materials and Methods**

### 608 **4.1 Plant Material, fungal endophytes isolation and identification**

609 *Posidonia oceanica* shoots were collected from the shores of Banyuls-sur-Mer in France at five to  
610 ten meters depth. Fresh plant parts (leaves, roots and rhizomes) were cleaned under stream water  
611 and then dipped into 70% ethanol for 3 minutes. Samples of all plant parts were cut into 1 cm<sup>2</sup>  
612 pieces and placed in a culture plate containing potato dextrose agar (PDA). Fungal tips were  
613 transferred to a new PDA culture plate as soon as they appeared and left to grow for 30 days.

614 Samples of the fungal cultures were sent to Bio2Mar, France (<http://bio2mar.obs-banyuls.fr>) who  
615 performed the amplification and sequencing of the internal transcribed spacers plus the 5.8S (ITS).  
616 After the removal of small and large subunit ITS flanking regions, a first identification of this  
617 fungal isolate was performed searching for similarity of that ITS sequences with those deposited in  
618 GenBank (National Center for Biotechnology Information;  
619 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5753231/>). Sequence similarity search (BLAST;



620 [https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE\\_TYPE=BlastSearch#](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch#)) in GenBank used the ‘blastn’  
621 (Megablast) option excluding “uncultured/environmental sample sequences”.

622 To identify more precisely the selected *Fusarium* strain FEP 16, DNA was extracted from a sample  
623 of the fungal culture placed in 500 µL of cetyl-trimethyl-ammonium bromide buffer (CTAB 1x).  
624 DNA extraction was performed according to Hofstetter et al. (2002). Four more loci were amplified  
625 and sequenced: part of the transcription elongation factor 1-alpha (*TEF1-α*) using primers EF1-1F  
626 and EF1-1R (Morehouse et al., 2003), part of the RNA polymerase II second largest subunit [*RPB2*]  
627 using primers fRPB2-5F and fRPB2-7cR for region 5-7 and fRPB2-7cF and fRPB2-11aR for region  
628 7-11 (Liu et al., 1999), part of β-tubulin using primers Bt2a and Bt2b (Glass and Donaldson, 1995),  
629 and part of calmodulin with primers CAL-228F and CAL-737R (Carbone and Kohn, 2019).  
630 Amplification of these loci used reagents and conditions of the Taq PCR core kit (Qiagen Inc.,  
631 Valencia, CA, USA). Sanger sequencing was performed with the amplification primers by Fasteris  
632 SA (Life Science Genesupport, Geneva, Switzerland). The obtained sequences were assembled in  
633 Sequencher v. 4.9 (Gene Codes Corp., USA). These sequences were combined with sequences  
634 sampled from Bohni et al. (2016). The alignments of sequences were done in MacClade v. 4.08a  
635 (Maddison and Maddison, 1989). Ambiguously aligned regions (mostly spliceosomal introns in  
636 protein-coding genes and gap regions in ribosomal loci) were excluded from phylogenetic analyses.

637 Searches for the most likely tree included three independent runs conducted in PhyML v. 3.0  
638 (Guindon and Gascuel, 2003), with evolutionary model = GTR and other parameters estimated  
639 during the search. Bootstrap values (BS) were inferred based on 500 replicates using the same  
640 settings as for the search of the most likely tree. Branch support was considered significant when  
641 BS values were  $\geq 70\%$  (Alfaro et al., 2003).

#### 642 **4.2 Cultivation and extraction of *Fusarium petroliphilum***

643 Small scale (10 plates) and large scale (100 plates) cultivations were done in identical conditions.  
644 The fungi *F. petroliphilum* strain was cultivated in the laboratory using Sabouraud dextrose agar  
645 (CM0041, Oxoid), suspended in artificial sea water, then laid down on 8.5 cm Petri dishes at room  
646 temperature for 18 days (Ingredients in Supplementary Table S3) (Kour et al., 2007). After 18 days,  
647 the fresh agar was cut into 1 cm squares and directly agitated with ethyl acetate overnight. Agitation  
648 was repeated with fresh solvent three times followed by 20 minutes ultrasound sonication. The  
649 solvent was filtered then evaporated to dryness under vacuum to yield 300 mg of extract.

#### 650 **4.3 General Experimental Procedures**

651 The NMR spectroscopic data were recorded on a Bruker Avance III HD 600 MHz. NMR  
652 spectrometer equipped with a QCI 5mm Cryoprobe and a SampleJet automated sample changer  
653 (Bruker BioSpin, Rheinstetten, Germany). Chemical shifts are measured in parts per million ( $\delta$ )  
654 using the residual DMSO-*d*<sub>6</sub> ( $\delta_{\text{H}}$  2.50;  $\delta_{\text{C}}$  39.5) as internal standard for <sup>1</sup>H and <sup>13</sup>C, respectively, and  
655 coupling constants (*J*) are reported in Hz. Complete assignments were performed based on 2D-  
656 NMR experiments (COSY, ROESY, HSQC, and HMBC). Optical rotations were measured with a  
657 Jasco P-2000 polarimeter (JASCO Corporation, Tokyo, Japan). UV absorbance was measured with  
658 a JASCO FT/IR-4100 spectrometer (JASCO Corporation) equipped with a PIKE MIRacle™  
659 (JASCO Corporation).

#### 660 **4.4 UHPLC-PDA-MS and UHPLC-HRMS/MS Analyses**

661 UHPLC-PDA-MS analyses were performed on a Waters Acquity UHPLC, equipped with PDA and  
662 QDA detectors supplemented with electrospray ionization source (ESI). For the profile of the ethyl  
663 acetate extract, 3  $\mu$ l was injected through a Waters® column BEH C18 (100 x 2.1 mm i.d., 1.7  $\mu$ m,  
664 Waters, Milford, MA, USA). Solvent system was MeOH (B) and H<sub>2</sub>O (A). The optimized  
665 conditions were as follows: 34% to 100% of B in 16.57 min., followed by 3 minutes to wash at  
666 100% of B. Flow rate was 0.3 mL/min.

667 UHPLC-HRMS/MS analyses was performed on Thermo Dionex Ultimate 3000 UHPLC system  
668 interfaced with a Q Exactive Plus MS (Thermo Scientific, Bremen, Germany) supplemented with  
669 heated electrospray Ionization source (HESI-II). For the profile of the ethyl acetate extract, 2  $\mu$ l of  
670 the extract was injected through a Waters® column BEH C18 100 x 2.1 mm, 1.7  $\mu$ m i.d. Solvent  
671 system was MeCN (B) and H<sub>2</sub>O (A), both containing 0.1% FA. Gradient mode was as follows: 5%  
672 to 100% of B in 18 min, at flow rate of 0.6 mL/min. followed by 4 min. for washing and  
673 reconditioning. For the short run analyses of all fractions, 2  $\mu$ l was injected through a Waters®  
674 column BEH C18 (50 x 2.1 mm i.d., 1.7  $\mu$ m, Waters, Milford, MA, USA). Solvent system was  
675 MeCN (B) and H<sub>2</sub>O (A), both containing 0.1% FA. Gradient mode was 5% to 100% of B in 4 min.,  
676 followed by 2 min. for washing and reconditioning.

#### 677 4.5 Generation of molecular networks

678 Raw spectral data of the extract were analyzed by MZmine 2.53 (Pluskal et al., 2010). The  
679 parameters were adjusted as follows: Mass detection performed as MS level 1 (noise level at  $1e^6$ )  
680 and MS level 2 (noise level at 0). ADAP chromatogram builder was used with threshold set to  $4e^5$ .  
681 Chromatogram deconvolution (algorithm ADAP) set within its default parameters except for RT  
682 wavelet range set as 0 to 0.1. The most intense isotopes were kept through isotope peak grouper.  
683 Adduct search was performed for (Na<sup>+</sup>, K<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, ACN<sup>+</sup>) in PI and (Na<sup>-</sup>, K<sup>-</sup>) in NI. Custom  
684 database search restricted to *Fusarium* was performed using the Dictionary of Natural Products.  
685 Peak alignment was performed using join aligner method where absolute RT tolerance at 0.03  
686 minutes. Molecular networks built through GNPS (<https://gnps.ucsd.edu/>) and visualized using  
687 Cytoscape version 3.8.

#### 688 4.6 HPLC-PDA analysis

689 HPLC-PDA analyses were conducted on a HP 1260 system equipped with a photodiode array  
690 detector (Agilent Technologies, Santa Clara, CA, USA). The HPLC conditions were as follows: X-  
691 Bridge C18 column (250 x 4.6 mm i.d., 5  $\mu$ m, Waters, Milford, MA, USA); solvent system MeOH  
692 (B) and H<sub>2</sub>O (A), both containing 0.1% FA. The optimized separation was performed in gradient  
693 mode as follows: 34% to 100% of B in 60 min. Flow rate 1 mL/min; injection volume 10  $\mu$ L,  
694 sample concentration 10 mg/mL in the mobile phase. The ELSD detection parameters, pressure 3.4  
695 bar, 45 °C, split to provide a 500  $\mu$ L/min flow rate, gain 8.

#### 696 4.7 Generation of 2D plot

697 The aligned <sup>1</sup>H-NMR spectra of all 135 fractions were divided into equally sized bins (0.01 ppm) in  
698 a range from -1 to 15 ppm then exported as excel file (.csv). The file was loaded into an R script  
699 which was written to create an interactive 2D plot that combines all binned spectra in a matrix  
700 (sample Vs. ppm). The retention time was added manually to the plot. The following R packages  
701 were used: “plotly”, “stringr”, “reshape2”, “dplyr” and “readr”. The R script is freely available here  
702 ([https://github.com/oolonek/pseudo\\_lcnmr\\_plotter/blob/main/src/plotter/NMR\\_data\\_plotter.Rmd](https://github.com/oolonek/pseudo_lcnmr_plotter/blob/main/src/plotter/NMR_data_plotter.Rmd)).

#### 703 4.8 Liquid/liquid extraction and extract purification at semi-preparative scale

704 The crude was subjected to liquid/liquid extraction, 160 mg was suspended in 50 ml 7:3  
705 water/methanol, the same volume of hexane was added to the suspension and gently shaken, the  
706 apolar phase was transferred to new flask, the later step was repeated 4 times. Both fractions were  
707 dried under vacuum, the hydroalcoholic fraction yield was 70 mg and 90 mg for hexane fraction.  
708 Both fractions plus the original crude extract were purified using a semi-preparative HPLC-UV  
709 equipment (Shimadzu® SPD-20A, Kyoto, Japan) through an X-Bridge RP C18 column (250 x 19  
710 mm i.d., 5 µm. To transfer from analytical to semi-preparative HPLC, the conditions were  
711 geometrically transferred by selecting a column with a similar stationary phase and gradient system  
712 (Guillarme et al., 2008). The flow rate was set at 17 mL/min. In order to avoid loss of resolution,  
713 the sample was introduced into the column through a homemade dry load cell (Queiroz et al.,  
714 2019). All peaks have been collected automatically by UV threshold at 254 nm or ELSD trace.  
715 After collection, each fraction was evaporated to dryness using a high-performance evaporation  
716 system (HT-4X Genevac®, Stone Ridge, NY, USA).

#### 717 4.9 Description of the isolated compounds

718 **Adenosine (1)**: white amorphous solid (0.3 mg); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ 3.55 (1H, dd,  
719 *J*=12.1, 3.5 Hz, H-5'b), 3.67 (2H, dd, *J*=12.1, 3.5 Hz, H-5'a), 3.96 (2H, q, *J*=3.4 Hz, H-4'), 4.14 (1H,  
720 dd, *J*=4.8, 3.1 Hz, H-3'), 4.60 (1H, t, *J*=5.6 Hz, H-2'), 5.87 (1H, d, *J*=6.3 Hz, H-1'), 7.33 (1H, s, OH-  
721 5), 8.13 (1H, s, H-7), 8.34 (1H, s, H-2); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 151 MHz) δ 61.6 (C-5'), 70.6 (C-3'),  
722 73.4 (C-2'), 85.8 (C-4'), 87.8 (C-1'), 119.3 (C-4), 139.8 (C-2), 156.1 (C-5). HRMS/MS *m/z*  
723 268.1033 [M + H]<sup>+</sup> (calcd for C<sub>10</sub>H<sub>14</sub>N<sub>5</sub>O<sub>4</sub>, 268.1045).

724 **6-(2,3-dihydroxybutan-2-yl)-3-methyl-2H-pyran-2-one (2)**: white amorphous solid; **2a**: <sup>1</sup>H-NMR  
725 (DMSO-*d*<sub>6</sub>, 600 MHz) δ 0.97 (3H, d, *J*=6.3 Hz, H<sub>3</sub>-9), 1.31 (3H, s, H<sub>3</sub>-10), 1.95 (3H, d, *J*=1.2 Hz,  
726 H<sub>3</sub>-11), 3.73 (1H, m, H-8), 4.67 (1H, d, *J*=6.1 Hz, OH-8), 5.19 (1H, s, OH-7), 6.32 (1H, d, *J*=5.7  
727 Hz, H-5), 7.35 (1H, m, H-4); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 151 MHz) δ 16.0 (C-11), 17.4 (C-9), 21.8 (C-  
728 10), 70.2 (C-8), 74.5 (C-7), 101.4 (C-5), 121.3 (C-3), 140.4 (C-4), 162.7 (C-2), 167.0 (C-6); **2b**: <sup>1</sup>H-  
729 NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ 1.03 (2H, d, *J*=6.4 Hz, H<sub>3</sub>-9), 1.24 (3H, s, H<sub>3</sub>-10), 1.95 (3H, d, *J*=1.2  
730 Hz, H<sub>3</sub>-11), 3.74 (1H, m, H-8), 4.59 (1H, d, *J*=5.8 Hz, OH-8), 5.09 (1H, s, OH-7), 6.33 (1H, d,  
731 *J*=5.8 Hz, H-5), 7.35 (1H, m, H-4); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 151 MHz) δ 16.0 (C-11), 17.1 (C-9), 22.6  
732 (C-10), 70.4 (C-8), 75.0 (C-7), 101.4 (C-5), 120.9 (C-3), 140.4 (C-4), 162.7 (C-2), 167.2 (C-6).  
733 HRMS/MS *m/z* 199.0962 [M + H]<sup>+</sup> (calcd for C<sub>10</sub>H<sub>15</sub>O<sub>4</sub>, 199.0966).

734 **6-(1-hydroxyethyl)-3-methyl-2H-pyran-2-one (3)**: white amorphous solid; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>,  
735 600 MHz) δ 1.30 (3H, d, *J*=6.6 Hz, H<sub>3</sub>-8), 1.96 (3H, d, *J*=1.2 Hz, H<sub>3</sub>-9), 4.41 (1H, q, *J*=6.6 Hz, H-  
736 7), 5.56 (1H, d, *J*=4.4 Hz, OH-7), 6.26 (1H, dd, *J*=6.7, 0.8 Hz, H-5), 7.36 (1H, m, H-4); <sup>13</sup>C-NMR  
737 (DMSO-*d*<sub>6</sub>, 151 MHz) δ 16.2 (C-9), 21.5 (C-8), 64.9 (C-7), 100.4 (C-5), 122.0 (C-3), 140.5 (C-4),  
738 162.7 (C-2), 165.6 (C-6). HRMS/MS *m/z* 155.0703 [M + H]<sup>+</sup> (calcd for C<sub>8</sub>H<sub>11</sub>O<sub>3</sub>, 155.0708).

739 **Gibepyrone D (4)**: pale yellow amorphous solid (0.5 mg); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ 2.03  
740 (3H, d, *J*=1.3 Hz, H<sub>3</sub>-10), 2.27 (3H, s, H<sub>3</sub>-11), 6.43 (1H, s, H-8), 6.72 (1H, d, *J*=6.9 Hz, H-5), 7.44  
741 (1H, dd, *J*=6.9, 1.3 Hz, H-4); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 151 MHz) δ 12.9 (C-11), 16.2 (C-10), 105.6  
742 (C-5), 125.8 (C-3), 139.8 (C-4), 156.6 (C-6), 161.4 (C-2). HRMS/MS *m/z* 195.1016 [M + H]<sup>+</sup>  
743 (calcd for C<sub>10</sub>H<sub>11</sub>O<sub>4</sub>, 195.0657).

744 **Aloesol (5)**: milk white crystals (0.3 mg); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ 1.14 (3H, d, *J*=6.2 Hz, H<sub>3</sub>-14), 2.54 (1H, dd, *J*=14.3, 7.7 Hz, H-12b), 2.60 (1H, dd, *J*=14.3, 5.2 Hz, H-12a), 2.65 (3H, s, H<sub>3</sub>-11), 4.02 (1H, dq, *J*=7.6, 5.5 Hz, H-13), 4.83 (1H, d, *J*=5.1 Hz, OH-13), 5.96 (1H, s, H-3), 6.60 (1H, d, *J*=2.4 Hz, H-6), 6.62 (1H, d, *J*=2.4 Hz, H-8); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 151 MHz) δ 22.3 (C-11), 23.3 (C-14), 42.7 (C-12), 63.9 (C-13), 100.4 (C-8), 111.4 (C-3), 114.3 (C-10), 116.3 (C-6), 141.3 (C-5), 159.1 (C-7), 160.8 (C-9), 164.6 (C-2). HRMS/MS *m/z* 235.0963 [M + H]<sup>+</sup> (calcd for C<sub>13</sub>H<sub>15</sub>O<sub>4</sub>, 235.0965).

751 **5-hydroxy-4-(hydroxymethyl)-8-methoxy-2-methyl-1H-benzo[*g*]indole-6,9-dione (6)**: pale rose amorphous solid (0.2 mg); UV λ<sub>max</sub> 213, 280 nm; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ 2.44 (3H, d, *J*=1.0 Hz, H<sub>3</sub>-14), 3.89 (3H, s, H<sub>3</sub>-16), 4.75 (2H, d, *J*=5.5 Hz, H<sub>2</sub>-15), 5.06 (1H, t, *J*=5.6 Hz, OH-15), 6.23 (1H, s, H-3), 6.44 (1H, dd, *J*=2.1, 1.1 Hz, H-11), 11.39 (1H, s, H-13), 12.88 (1H, s, OH-5); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 151 MHz) δ 13.8 (C-14), 55.1 (C-15), 56.6 (C-16), 99.8 (C-11), 107.4 (C-10), 109.0 (C-3), 126.8 (C-6), 130.5 (C-8), 137.5 (C-7), 146.7 (C-12), 152.1 (C-5), 160.5 (C-2), 179.5 (C-1), 191.0 (C-4); HRMS/MS *m/z* 288.0846 [M + H]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>14</sub>NO<sub>5</sub>, 288.0866).

758 **Fusarubin (7)**: Reddish rose crystals; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ 1.48 (3H, s, H<sub>3</sub>-11), 2.59 (1H, m, H-4''), 2.78 (1H, dd, *J*=18.0, 1.9 Hz, H-4'), 3.91 (3H, s, H<sub>3</sub>-12), 4.67 (1H, dt, *J*=17.5, 2.4 Hz, H-1''), 4.72 (1H, d, *J*=17.5 Hz, H-1'), 6.13 (1H, d, *J*=1.6 Hz, OH-3), 6.46 (1H, s, H-8), 12.49 (1H, s, OH-5), 13.00 (1H, s, OH-10); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 151 MHz) δ 28.4 (C-11), 32.8 (C-4), 57.0 (C-12), 57.3 (C-1), 92.9 (C-3), 107.1 (C-9a), 109.7 (C-8), 133.3 (C-4a), 136.7 (C-10a), 156.3 (C-10), 159.6 (C-5), 160.7 (C-7), 177.8 (C-6), 184.7 (C-9). HRMS/MS *m/z* 307.0823 [M + H]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>15</sub>O<sub>7</sub>, 307.0812).

765 **3-O-methylfusarubin (8)**: Reddish rose crystals; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ 1.47 (3H, s, H<sub>3</sub>-11), 2.67 (1H, dt, *J*=18.0, 1.9 Hz, H-4''), 2.85 (1H, dd, *J*=18.0, 2.0 Hz, H-4'), 3.20 (3H, s, H<sub>3</sub>-13), 3.91 (3H, s, H<sub>3</sub>-12), 4.44 (1H, dt, *J*=17.7, 2.5 Hz, H-1''), 4.78 (1H, d, *J*=17.7 Hz, H-1'), 6.45 (1H, s, H-8), 12.49 (1H, s, OH-5), 12.99 (1H, s, OH-10); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 151 MHz) δ 22.4 (C-11), 32.5 (C-4), 48.3 (C-13), 57.0 (C-12), 57.9 (C-1), 96.3 (C-3), 109.7 (C-8), 132.1 (C-4a), 135.7 (C-10a), 155.4 (C-10), 158.7 (C-5), 160.7 (C-7), 178.4 (C-6), 185.1 (C-9). HRMS/MS *m/z* 319.0976 [M - H]<sup>-</sup> (calcd for C<sub>16</sub>H<sub>15</sub>O<sub>7</sub>, 319.0817).

772 **(6E)-7-(4-methoxy-6-oxo-6H-pyran-2-yl)-3,5-dimethyloct-6-enoic acid (9)**: Red amorphous solid (0.4 mg); [α]<sup>20</sup><sub>D</sub> +22.3 (c 0.04, MeOH); UV (DAD) λ<sub>max</sub> 218, 269; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ 0.89 (3H, d, *J*=6.6 Hz, H<sub>3</sub>-14), 0.96 (3H, d, *J*=6.6 Hz, H<sub>3</sub>-15), 1.21 (1H, dt, *J*=13.4, 7.4 Hz, H-10''), 1.34 (1H, dt, *J*=13.4, 6.8 Hz, H-10'), 1.79 (1H, dqd, *J*=8.5, 6.8, 5.1 Hz, H-11), 1.86 (3H, d, *J*=1.4 Hz, H<sub>3</sub>-16), 1.96 (1H, dd, *J*=15.0, 8.5 Hz, H-12''), 2.25 (1H, m, H-12'), 2.67 (1H, m, H-9), 3.88 (3H, s, H<sub>3</sub>-17), 5.52 (1H, d, *J*=1.8 Hz, H-3), 6.11 (1H, d, *J*=1.8 Hz, H-5), 6.15 (1H, dd, *J*=9.8, 1.4 Hz, H-8), 11.99 (1H, s, COOH); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 151 MHz) δ 12.3 (C-16), 19.8 (C-14), 19.9 (C-15), 27.6 (C-11), 29.9 (C-9), 40.9 (C-12), 43.3 (C-10), 56.4 (C-17), 88.7 (C-3), 108.7 (C-5), 124.3 (C-7), 140.0 (C-8), 159.6 (C-6), 167.2 (C-4), 173.7 (C-13), 180.2 (C-2). HRMS/MS *m/z* 295.1542 [M + H]<sup>+</sup> (calcd for C<sub>16</sub>H<sub>23</sub>O<sub>5</sub>, 295.1545).

782 **6-((E)-6-ethyl-7-hydroxy-4-methylhept-2-en-2-yl)-4-methoxy-2H-pyran-2-one (10)**: Red amorphous solid (0.7 mg); [α]<sup>20</sup><sub>D</sub> +82.7 (c 0.03, MeOH); UV (DAD) λ<sub>max</sub> 286, 422; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ 0.81 (3H, t, *J*=7.3 Hz, H<sub>3</sub>-13), 0.98 (3H, d, *J*=6.6 Hz, H<sub>3</sub>-15), 1.22 (1H, m, H-10''), 1.23 (1H, m, H-11), 1.27 (2H, m, H<sub>2</sub>-12), 1.35 (1H, m, H-10'), 1.86 (3H, d, *J*=1.3 Hz, H<sub>3</sub>-16), 2.69 (1H, m, H-9), 3.25 (1H, dt, *J*=10.2, 5.0 Hz, H-14''), 3.30 (1H, dt, *J*=10.2, 5.0 Hz, H-14'),

787 3.88 (3H, s, H<sub>3</sub>-17), 4.34 (1H, t, *J*=5.2 Hz, OH-14), 5.52 (1H, d, *J*=1.8 Hz, H-3), 6.10 (1H, d, *J*=1.8  
788 Hz, H-5), 6.14 (1H, dd, *J*=9.9, 1.5 Hz, H-8); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 151 MHz) δ 10.5 (C-13), 12.1  
789 (C-16), 20.4 (C-15), 22.6 (C-12), 29.9 (C-9), 37.8 (C-10), 39.1 (C-11), 56.3 (C-17), 62.9 (C-14),  
790 88.6 (C-3), 108.6 (C-5), 124.1 (C-7), 140.4 (C-8), 159.6 (C-6), 167.2 (C-4), 180.2 (C-2).  
791 HRMS/MS *m/z* 281.1745 [M + H]<sup>+</sup>, calcd for C<sub>16</sub>H<sub>25</sub>O<sub>4</sub>, 281.1747.

792 **Bostrycoidin (11)**: Dark red amorphous solid (0.5 mg); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ 2.73  
793 (3H, s, H<sub>3</sub>-15), 3.98 (3H, s, H<sub>3</sub>-16), 7.04 (1H, s, H-7), 7.98 (1H, s, H-4), 9.33 (1H, s, H-1), 12.87  
794 (1H, s, OH-5/8), 13.54 (1H, s, OH-5/8); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 151 MHz) δ 24.5 (C-15), 56.7 (C-  
795 16), 107.8 (C-7), 117.5 (C-4), 124.0 (C-13), 138.4 (C-14), 147.9 (C-1), 157.6 (C-6), 164.8 (C-3).  
796 HRMS/MS *m/z* 286.0715 [M + H]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>12</sub>NO<sub>5</sub>, 286.0716).

797 **5β,6β-23,26-diepoxy-3β,7α,9α-trihydroxy-(20Z,23S,24S,25R)ergosta-8(14),20-dien-15-one (12)**:  
798 pale yellow amorphous solid (0.4 mg); [α]<sup>20</sup><sub>D</sub> +18.9 (c 0.04, MeOH); UV λ<sub>max</sub> 248, 229, 229; <sup>1</sup>H-  
799 NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ 0.77 (3H, s, H<sub>3</sub>-18), 0.85 (3H, d, *J*=7.0 Hz, H<sub>3</sub>-28), 0.89 (3H, s, H<sub>3</sub>-  
800 19), 0.90 (3H, d, *J*=6.8 Hz, H<sub>3</sub>-26), 1.20 (1H, dt, *J*=13.6, 3.3 Hz, H-4α), 1.45 (3H, m, H-1β, H-2β,  
801 H-11α), 1.66 (2H, m, H<sub>2</sub>-12), 1.69 (1H, m, H-11β), 1.73 (3H, d, *J*=1.2 Hz, H<sub>3</sub>-21), 1.85 (2H, m, H-  
802 2α, H-24), 2.00 (1H, td, *J*=14.4, 4.0 Hz, H-1α), 2.06 (1H, dd, *J*=13.6, 11.5 Hz, H-4β), 2.16 (1H, dd,  
803 *J*=18.3, 7.3 Hz, H-16α), 2.26 (1H, m, H-25), 2.35 (1H, m, H-17), 2.63 (1H, m, H-16β), 3.27 (1H, d,  
804 *J*=3.7 Hz, H-6), 3.59 (1H, tq, *J*=10.6, 5.0 Hz, H-3), 3.93 (1H, dd, *J*=8.2, 6.4 Hz, H-27'), 4.18 (1H, t,  
805 *J*=8.5 Hz, H-23), 4.77 (1H, d, *J*=5.0 Hz, OH-3), 4.77 (1H, d, *J*=2.3 Hz, OH-9), 4.91 (1H, d, *J*=5.2  
806 Hz, OH-7), 5.22 (1H, d, *J*=8.4 Hz, H-22), 5.53 (1H, dd, *J*=5.2, 3.7 Hz, H-7); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>,  
807 151 MHz) δ 11.2 (C-28), 13.5 (C-26), 17.6 (C-18), 18.8 (C-21), 19.8 (C-19), 25.6 (C-1), 26.7 (C-  
808 11), 30.2 (C-2), 32.1 (C-12), 35.9 (C-25), 37.0 (C-10), 39.5 (C-16), 39.9 (C-4), 42.9 (C-24), 43.1  
809 (C-13), 51.5 (C-17), 59.7 (C-7), 60.4 (C-6), 66.5 (C-3), 68.1 (C-5), 73.7 (C-27), 74.8 (C-9), 79.7  
810 (C-23), 128.1 (C-22), 134.1 (C-20), 142.8 (C-14), 206.7 (C-15). HRMS/MS *m/z* 473.2877 [M + H]<sup>+</sup>  
811 (calcd for C<sub>28</sub>H<sub>41</sub>O<sub>6</sub>, 473.2903).

812 **2-(2,3,5,6,7,7a-hexahydro-1-((E)-6-hydroxy-5,6-dimethylhept-3-en-2-yl)-7a-methyl-5-oxo-1H-**  
813 **indén-4-yl)acetic acid (13)**: pale yellow amorphous solid (0.4 mg); [α]<sup>20</sup><sub>D</sub> +21.5 (c 0.04, MeOH);  
814 UV λ<sub>max</sub> 247, 207; <sup>1</sup>H-NMR (DMSO, 600 MHz) δ 0.92 (3H, d, *J*=7.0 Hz, H<sub>3</sub>-21), 0.99 (3H, s, H<sub>3</sub>-  
815 20), 1.03 (3H, d, *J*=7.1 Hz, H<sub>3</sub>-14), 1.03 (3H, s, H<sub>3</sub>-19), 1.06 (3H, s, H<sub>3</sub>-12), 1.42 (1H, m, H-11),  
816 1.50 (1H, m, H-10''), 1.78 (1H, m, H-10'), 1.79 (1H, m, H-6''), 2.01 (1H, p, *J*=7.0 Hz, H-17), 2.16  
817 (1H, m, H-6'), 2.17 (1H, m, H-13), 2.19 (1H, m, H-5''), 2.22 (1H, m, H-5'), 2.99 (1H, d, *J*=16.5 Hz,  
818 H-2''), 3.02 (1H, d, *J*=16.5 Hz, H-2'), 5.26 (1H, dd, *J*=15.3, 8.7 Hz, H-15), 5.41 (1H, dd, *J*=15.3, 7.0  
819 Hz, H-16); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 151 MHz) δ 14.9 (C-21), 16.2 (C-12), 20.8 (C-14), 26.0 (C-19),  
820 27.0 (C-10), 28.2 (C-20), 30.8 (C-2), 32.7 (C-5), 35.8 (C-6), 38.1 (C-13), 44.7 (C-7), 47.1 (C-17),  
821 55.5 (C-11), 70.5 (C-18), 131.1 (C-16), 135.0 (C-15), 171.7 (C-1), 175.2 (C-8). HRMS/MS *m/z*  
822 349.2383 [M + H]<sup>+</sup> (calcd for C<sub>21</sub>H<sub>33</sub>O<sub>4</sub>, 349.2378).

823 **2-(2,3,5,6,7,7a-hexahydro-1-((E)-7-hydroxy-5,6-dimethylhept-3-en-2-yl)-7a-methyl-5-oxo-1H-**  
824 **indén-4-yl)acetic acid (14)**: pale yellow amorphous solid (0.3 mg); [α]<sup>20</sup><sub>D</sub> +19.8 (c 0.07, MeOH);  
825 UV λ<sub>max</sub> 245, 212; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ 0.75 (3H, d, *J*=6.9 Hz, H<sub>3</sub>-19), 0.94 (3H, d,  
826 *J*=7.0 Hz, H<sub>3</sub>-21), 1.03 (3H, d, *J*=6.7 Hz, H<sub>3</sub>-14), 1.06 (3H, s, H<sub>3</sub>-12), 1.41 (1H, m, H-11), 1.43 (1H,  
827 m, H-18), 1.51 (1H, m, H-10''), 1.78 (2H, m, H-6'', H-10'), 2.17 (3H, m, H-6', H-13, H-17), 2.22  
828 (1H, m, H-5''), 2.98 (1H, d, *J*=16.4 Hz, H-2''), 3.02 (1H, d, *J*=16.4 Hz, H-2'), 3.17 (1H, m, H-20),  
829 5.27 (2H, m, H-15, H-16); <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 151 MHz) δ 13.1 (C-19), 16.3 (C-12), 18.3 (C-  
830 21), 21.0 (C-14), 27.3 (C-10), 30.9 (C-2), 32.7 (C-5), 35.9 (C-6), 37.0 (C-17), 38.2 (C-13), 40.6 (C-

831 18), 44.6 (C-7), 55.5 (C-11), 64.4 (C-20), 130.9 (C-16), 135.0 (C-15), 171.8 (C-1), 175.2 (C-8).  
832 HRMS/MS  $m/z$  349.2349  $[M + H]^+$  (calcd for  $C_{21}H_{33}O_4$ , 349.2378).

833 **5-hydroxy-4-methoxy-6,8-dimethylpyrano[3,2-g]indol-2(9H)-one (15)**: pale yellow amorphous  
834 (0.1 mg) solid; UV  $\lambda_{max}$  280, 239;  $^1H$ -NMR (DMSO- $d_6$ , 600 MHz)  $\delta$  2.36 (3H, s, H<sub>3</sub>-15), 2.44 (3H,  
835 s, H<sub>3</sub>-16), 3.89 (3H, s, H<sub>3</sub>-14), 6.21 (1H, s, H-3), 6.28 (1H, s, H-9), 11.39 (1H, s, NH-11), 12.84  
836 (1H, s, OH-6);  $^{13}C$ -NMR (DMSO- $d_6$ , 151 MHz)  $\delta$  12.0 (C-15), 13.8 (C-16), 56.6 (C-14), 98.9 (C-  
837 9), 107.3 (C-5), 108.8 (C-3), 123.9 (C-7), 129.8 (C-12), 137.6 (C-8), 146.2 (C-10), 152.2 (C-6),  
838 160.5 (C-4), 179.1 (C-2). HRMS/MS  $m/z$  258.0771  $[M + H]^+$ , calcd for  $C_{14}H_{12}NO_4$ , 258.0766.

839 **4-methoxy-6-((E)-4,6-dimethyloct-2-en-2-yl)-2H-pyran-2-one (16)**: light brown amorphous solid  
840 (0.1 mg);  $[\alpha]^{20}_D +17.5$  (c 0.04, MeOH); UV  $\lambda_{max}$  220,274;  $^1H$ -NMR (DMSO- $d_6$ , 600 MHz)  $\delta$  0.82  
841 (3H, t,  $J=7.4$  Hz, H<sub>3</sub>-13), 0.84 (3H, d,  $J=6.3$  Hz, H<sub>3</sub>-14), 0.96 (3H, d,  $J=6.5$  Hz, H<sub>3</sub>-15), 1.08 (1H,  
842 m, H-12"), 1.14 (1H, m, H-10"), 1.32 (3H, m, H-10', H-11, H-12'), 1.86 (3H, d,  $J=1.2$  Hz, H<sub>3</sub>-16),  
843 2.66 (1H, m, H-9), 3.88 (3H, s, H<sub>3</sub>-17), 5.52 (1H, d,  $J=1.8$  Hz, H-3), 6.10 (1H, d,  $J=1.8$  Hz, H-5),  
844 6.16 (1H, dq,  $J=9.8, 1.2$  Hz, H-8);  $^{13}C$ -NMR (DMSO- $d_6$ , 151 MHz)  $\delta$  11.0 (C-13), 12.3 (C-16),  
845 19.3 (C-14), 20.1 (C-15), 28.6 (C-12), 30.1 (C-9), 31.5 (C-11), 43.5 (C-10), 56.3 (C-17), 88.8 (C-3),  
846 108.9 (C-5), 124.2 (C-7), 140.5 (C-8), 159.7 (C-6), 167.4 (C-4). HRMS/MS  $m/z$  265.1816  $[M +$   
847  $H]^+$ , calcd for  $C_{16}H_{25}O_3$ , 265.1803.

848 **4-methoxy-3-methyl-6-((E)-4,6-dimethyloct-2-en-2-yl)-2H-pyran-2-one (17)**: light brown  
849 amorphous solid (0.1 mg);  $[\alpha]^{20}_D +19.7$  (c 0.04, MeOH); UV  $\lambda_{max}$  218, 252;  $^1H$ -NMR (DMSO- $d_6$ ,  
850 600 MHz)  $\delta$  0.83 (3H, t,  $J=7.4$  Hz, H<sub>3</sub>-13), 0.85 (3H, d,  $J=6.1$  Hz, H<sub>3</sub>-14), 0.98 (3H, d,  $J=6.7$  Hz,  
851 H<sub>3</sub>-15), 1.24 (1H, m, H-12"), 1.34 (2H, m, H-11, H-12'), 1.69 (3H, s, H<sub>3</sub>-18), 1.88 (3H, d,  $J=1.3$  Hz,  
852 H<sub>3</sub>-16), 2.66 (1H, m, H-9), 4.02 (3H, s, H<sub>3</sub>-17), 6.17 (1H, s, H-5), 6.19 (1H, d,  $J=9.4$  Hz, H-8);  $^{13}C$ -  
853 NMR (DMSO- $d_6$ , 151 MHz)  $\delta$  6.5 (C-18), 11.1 (C-13), 12.3 (C-16), 19.3 (C-14), 20.1 (C-15), 28.7  
854 (C-12), 30.1 (C-9), 31.4 (C-11), 43.5 (C-10), 56.0 (C-17), 98.9 (C-3), 108.3 (C-5), 124.4 (C-7),  
855 140.1 (C-8), 158.3 (C-6), 162.0 (C-4), 179.7 (C-2). HRMS/MS  $m/z$  279.1967  $[M + H]^+$  (calcd for  
856  $C_{17}H_{27}O_3$ , 279.1955).

857 **2-(2,3,5,6,7,7a-hexahydro-7a-methyl-1-((E)-5,6-dimethylhept-3-en-2-yl)-5-oxo-1H-inden-4-**  
858 **yl)acetic acid (18)**: pale yellow amorphous solid (0.6 mg);  $[\alpha]^{20}_D +14.9$  (c 0.05, MeOH); UV  
859  $\lambda_{max}$  246, 239;  $^1H$ -NMR (DMSO- $d_6$ , 600 MHz)  $\delta$  0.81 (3H, d,  $J=6.9$  Hz, H<sub>3</sub>-20), 0.83 (3H, d,  $J=6.9$   
860 Hz, H<sub>3</sub>-19), 0.91 (3H, d,  $J=6.7$  Hz, H<sub>3</sub>-21), 1.03 (3H, d,  $J=6.6$  Hz, H<sub>3</sub>-14), 1.06 (3H, s, H<sub>3</sub>-12), 1.42  
861 (1H, m, H-11), 1.48 (1H, m, H-18), 1.51 (1H, m, H-10"), 1.78 (1H, m, H-10'), 1.79 (1H, m, H-6"),  
862 1.88 (1H, m, H-17), 2.15 (1H, m, H-6'), 2.18 (1H, m, H-13), 2.22 (1H, m, H-5"), 2.34 (1H, m, H-  
863 9"), 2.46 (1H, m, H-9'), 2.56 (1H, m, H-5'), 2.98 (1H, d,  $J=16.5$  Hz, H-2"), 3.02 (1H, d,  $J=16.5$  Hz,  
864 H-2'), 5.25 (1H, dd,  $J=15.2, 7.9$  Hz, H-15), 5.29 (1H, dd,  $J=15.2, 7.0$  Hz, H-16);  $^{13}C$ -NMR (DMSO-  
865  $d_6$ , 151 MHz)  $\delta$  16.3 (C-12), 17.3 (C-21), 19.4 (C-20), 19.7 (C-19), 20.9 (C-14), 27.2 (C-10), 27.3  
866 (C-9), 32.4 (C-18), 32.7 (C-5), 35.8 (C-6), 38.1 (C-13), 42.0 (C-17), 44.6 (C-7), 55.4 (C-11), 125.0  
867 (C-3), 132.0 (C-16), 134.7 (C-15), 171.7 (C-1), 175.2 (C-8), 196.3 (C-4). HRMS/MS  $m/z$  333.2426  
868  $[M + H]^+$  (calcd for  $C_{21}H_{33}O_3$ , 333.2429).

869 **3-O- $\beta$ -D-glucopyranoside-Stigmast-8-en-3-ol (19)**: pale yellow amorphous solid (0.1 mg);  
870  $[\alpha]^{20}_D +6.2$  (c 0.08, MeOH); UV  $\lambda_{max}$  234, 207;  $^1H$ -NMR (DMSO- $d_6$ , 600 MHz)  $\delta$  0.65 (3H, s, H<sub>3</sub>-  
871 18), 0.79 (3H, d,  $J=6.9$  Hz, H<sub>3</sub>-27), 0.82 (3H, d,  $J=6.9$  Hz, H<sub>3</sub>-26), 0.82 (3H, t,  $J=7.3$  Hz, H<sub>3</sub>-29),  
872 0.88 (1H, m, H-5), 0.90 (3H, d,  $J=6.5$  Hz, H<sub>3</sub>-21), 0.91 (1H, m, H-24), 0.96 (3H, s, H<sub>3</sub>-19), 0.97  
873 (2H, m, H-1b, H-14), 1.01 (1H, m, H-22b), 1.09 (1H, m, H-17), 1.20 (1H, m, H-28b), 1.25 (1H, m,

874 H-28a), 1.30 (1H, m, H-22a), 1.33 (1H, m, H-20), 1.47 (1H, m, H-2b), 1.63 (1H, m, H-25), 1.79  
875 (1H, m, H-1a), 1.80 (3H, s), 2.12 (1H, t,  $J=12.5$  Hz, 4"), 1.81 (1H, m, H-2a), 2.12 (1H, m, H-4b),  
876 2.37 (1H, m, H-4a), 2.89 (1H, td,  $J=8.4, 4.8$  Hz, H-2'), 3.01 (1H, td,  $J=9.2, 5.0$  Hz, H-4'), 3.06 (1H,  
877 m, H-5'), 3.12 (1H, td,  $J=8.9, 4.8$  Hz, H-3'), 3.40 (1H, m, H-6'b), 3.46 (1H, tt,  $J=11.2, 4.3$  Hz, H-3),  
878 3.64 (1H, dd,  $J=11.1, 6.2$  Hz, H-6'a), 4.22 (1H, d,  $J=7.8$  Hz, H-1'), 4.42 (1H, t,  $J=5.8$  Hz, OH-6'),  
879 4.85 (1H, d,  $J=5.0$  Hz, OH-4'), 4.86 (1H, d,  $J=4.8$  Hz, OH-2'), 4.88 (1H, d,  $J=4.8$  Hz, OH-3');  $^{13}\text{C}$ -  
880 NMR (DMSO- $d_6$ , 151 MHz)  $\delta$  11.6 (C-18), 11.8 (C-29), 18.6 (C-21), 19.0 (C-27), 19.1 (C-19), 19.6  
881 (C-26), 22.5 (C-28), 28.7 (C-25), 29.3 (C-2), 33.3 (C-22), 35.4 (C-20), 36.3 (C-10), 36.7 (C-1), 38.3  
882 (C-4), 39.1 (C-12), 41.8 (C-13), 45.1 (C-24), 49.6 (C-5), 55.4 (C-17), 56.1 (C-14), 61.0 (C-6'), 70.1  
883 (C-4'), 73.4 (C-2'), 76.7 (C-5'), 76.8 (C-3, C-3'), 100.7 (C-1'), 140.4 (C-9). HRMS/MS  $m/z$  599.4280  
884  $[\text{M} + \text{Na}]^+$ , calcd for  $\text{C}_{35}\text{H}_{59}\text{O}_6\text{Na}$ , 599.4287.

885 **Cerevisterol (20)**: White amorphous solid (0.9 mg)  $^1\text{H}$ -NMR (DMSO- $d_6$ , 600 MHz)  $\delta$  0.55 (3H, s,  
886  $\text{H}_3$ -18), 0.80 (3H, d,  $J=7.3$  Hz,  $\text{H}_3$ -27), 0.81 (3H, d,  $J=7.3$  Hz,  $\text{H}_3$ -26), 0.88 (3H, d,  $J=6.8$  Hz,  $\text{H}_3$ -  
887 28), 0.90 (3H, s,  $\text{H}_3$ -19), 0.99 (3H, d,  $J=6.5$  Hz,  $\text{H}_3$ -21), 3.37 (1H, t,  $J=5.5, 4.8$  Hz, H-6), 3.59 (1H,  
888 s, OH-5), 3.76 (1H, tq,  $J=10.4, 5.0$  Hz, H-3), 4.22 (1H, d,  $J=5.6$  Hz, OH-3), 4.49 (1H, d,  $J=5.5$  Hz,  
889 OH-6), 5.08 (1H, dt,  $J=4.8, 2.2$  Hz, H-7), 5.17 (1H, dd,  $J=15.3, 8.3$  Hz, H-22), 5.25 (1H, dd,  
890  $J=15.3, 7.4$  Hz, H-23);  $^{13}\text{C}$ -NMR (DMSO- $d_6$ , 151 MHz)  $\delta$  12.1 (C-18), 17.2 (C-28), 17.7 (C-19),  
891 19.5 (C-26), 19.8 (C-27), 20.9 (C-21), 21.3 (C-16), 22.5 (C-15), 27.6 (C-11), 31.1 (C-2), 32.4 (C-1,  
892 C-25), 36.6 (C-10), 38.9 (C-12), 39.9 (C-20), 40.1 (C-4), 42.0 (C-24), 42.2 (C-9), 42.9 (C-13), 54.1  
893 (C-14), 55.3 (C-17), 65.9 (C-3), 72.1 (C-6), 74.4 (C-5), 119.5 (C-7), 131.4 (C-23), 138.4 (C-22),  
894 139.6 (C-8). HRMS/MS  $m/z$  453.3397  $[\text{M} + \text{Na}]^+$ , calcd for  $\text{C}_{28}\text{H}_{46}\text{O}_3\text{Na}$  453.3345.

895 **6-Dehydrocerevisterol (21)**: White amorphous solid (0.6 mg);  $^1\text{H}$ -NMR (DMSO- $d_6$ , 600 MHz)  $\delta$   
896 0.55 (3H, s,  $\text{H}_3$ -18), 0.78 (3H, s,  $\text{H}_3$ -19), 0.81 (3H, d,  $J=7.0$  Hz,  $\text{H}_3$ -27), 0.80 (3H, d,  $J=7.0$  Hz,  $\text{H}_3$ -  
897 26), 0.89 (3H, d,  $J=6.8$  Hz,  $\text{H}_3$ -28), 1.00 (3H, d,  $J=6.6$  Hz,  $\text{H}_3$ -21), 3.71 (1H, tq,  $J=7.0, 5.5$  Hz, H-  
898 3), 4.40 (1H, d,  $J=5.5$  Hz, OH-3), 5.12 (1H, s, OH-5), 5.18 (1H, dd,  $J=15.3, 8.4$  Hz, H-22), 5.25  
899 (1H, dd,  $J=15.3, 7.4$  Hz, H-23), 5.39 (1H, t,  $J=2.4$  Hz, H-7);  $^{13}\text{C}$ -NMR (DMSO- $d_6$ , 151 MHz)  $\delta$   
900 12.4 (C-18), 15.8 (C-19), 17.3 (C-28), 19.5 (C-27), 19.7 (C-26), 20.9 (C-21), 21.2 (C-11), 22.0 (C-  
901 15), 27.6 (C-16), 30.0 (C-1), 30.4 (C-2), 32.5 (C-25), 35.9 (C-4), 38.1 (C-12), 39.8 (C-20), 40.1 (C-  
902 10), 42.0 (C-24), 43.2 (C-9), 44.0 (C-13), 54.8 (C-14), 55.2 (C-17), 65.5 (C-3), 76.0 (C-5), 119.3  
903 (C-7), 131.6 (C-23), 135.1 (C-22), 163.3 (C-8), 198.6 (C-6). HRMS/MS  $m/z$  429.3387  $[\text{M} + \text{H}]^+$ ,  
904 calcd for  $\text{C}_{28}\text{H}_{45}\text{O}_3$ , 429.3368.

905 **Ergosterol (22)**: White amorphous solid (0.4 mg);  $^1\text{H}$ -NMR (DMSO- $d_6$ , 600 MHz)  $\delta$  0.59 (3H, s,  
906  $\text{H}_3$ -18), 0.80 (3H, d,  $J=6.9$  Hz,  $\text{H}_3$ -27), 0.81 (3H, d,  $J=7.0$  Hz,  $\text{H}_3$ -26), 0.86 (3H, s,  $\text{H}_3$ -19), 0.89 (3H,  
907 d,  $J=6.8$  Hz,  $\text{H}_3$ -28), 1.01 (3H, d,  $J=6.6$  Hz,  $\text{H}_3$ -21), 3.36 (1H, overlapped, H-3), 4.40 (1H, d,  $J=4.4$   
908 Hz, OH-3), 5.18 (1H, dd,  $J=15.3, 8.1$  Hz, H-22), 5.24 (1H, dd,  $J=15.3, 7.2$  Hz, H-23), 5.32 (1H, dt,  
909  $J=5.0, 2.1$  Hz, H-7), 5.47 (1H, dd  $J=5.5, 2.1$  Hz, H-7);  $^{13}\text{C}$ -NMR (DMSO- $d_6$ , 151 MHz)  $\delta$  11.8 (C-  
910 18), 16.0 (C-19), 17.3 (C-28), 19.5 (C-26), 19.8 (C-27), 20.6 (C-11), 21.0 (C-21), 22.6 (C-15), 27.9  
911 (C-16), 31.8 (C-2), 32.5 (C-25), 36.6 (C-10), 37.9 (C-1), 38.4 (C-12), 39.9 (C-20), 40.7 (C-4), 42.0  
912 (C-24), 42.3 (C-13), 45.7 (C-9), 53.9 (C-14), 55.0 (C-17), 68.6 (C-3), 116.2 (C-7), 118.7 (C-6),  
913 131.4 (C-23), 135.4 (C-22), 140.2 (C-8), 140.6 (C-5). HRMS/MS  $m/z$  395.3325  $[\text{M} - \text{H}]^-$ , calcd for  
914  $\text{C}_{28}\text{H}_{45}\text{O}$ , 395.3313.

#### 915 4.10 Minimum inhibitory concentration test

916 Methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 33591) and *Pseudomonas aeruginosa*  
917 (ATCC 27853) strains were used for the antibacterial assay. The minimum inhibitory concentration  
918 (MIC) of the extract and the isolated compounds were determined in triplicates according to  
919 Wiegand et al. (2008) in Mueller-Hinton medium (MH). After the incubation of the inoculated 96-  
920 well plates at 37°C for 24h, iodinitrotetrazolium chloride (INT, Sigma-Aldrich) was added to each  
921 well at a final concentration of 0.2 mg/mL and incubated for 20 minutes (Eloff, 1998). The highest  
922 dilution of a compound in which no growth appears corresponds to its MIC. Gentamicin for *P.*  
923 *aeruginosa* and chloramphenicol for *S. aureus* were used as controls.

#### 924 **4.11 Anti-quorum sensing assay for *Pseudomonas aeruginosa***

925 The assay was performed according to the protocol proposed by Hentzer et al. (2002) on a black 96  
926 well plate with clear bottom. Reporter strain PPAO1 *pqsA::gfp*, was constructed using the  
927 following primers GCTCTAGATCGAGCAAGGGTTGTAACGGTTTTTG and  
928 GCTGCTGCATGCGACAGAACGTTCCCTCTTCAGCGA to amplify *pqsA* gene promoter and  
929 cloned by usual molecular methods into XbaI-SphI sites of *lasB::gfp* plasmid (Hentzer et al.,  
930 2002), in place of the *lasB* promoter. Reporter strains containing the *lasB::gfp* or the *pqsA::gfp*  
931 plasmid were grown at starting OD600 of 0.05 in PTSB (5% peptone, 0.25% trypticase soy broth)  
932 supplied with gentamicin 50 µg/ml and each sample at 128 µg/ml. Azithromycin 2 µg/ml was used  
933 as positive control. Plates were incubated at 37°C, 160 rpm. After 15 hours, OD600 and  
934 fluorescence at 480/520 nm were measured using a microplate reader (SynergyHT BioTek). Results  
935 are represented in percentage of fluorescence compared to the solvent control (DMSO) fixed at  
936 100%.

#### 937 **4.12 Anti-quorum sensing assay for *Staphylococcus aureus***

938 The assay was performed according to the protocol proposed by Nielsen et al. (2010). In a 96 well  
939 plate, the reporter strain *rnaIII::lacZ* (Nielsen et al., 2010) was grown at a starting OD600 of 0.05  
940 in MH, supplied with erythromycin 5 µg/ml and each sample at 128 µg/ml. *Staphylococcus caprae*  
941 autoinducing peptide (AIP) (Paharik et al., 2017) at concentration of 1 µM was used as positive  
942 control. After 6 hours at 37°C, 160 rpm, incubation was stopped and OD600 value was read. Then,  
943 10 µl of freshly made 4-methylumbelliferyl-β-D-galactopyranoside (MUG) 10 mg/ml was added to  
944 each well and left to incubate for 1 hour at room temperature. Reaction was stopped by addition of  
945 100 µl of Na<sub>2</sub>CO<sub>3</sub> 0.4M and fluorescence was read using a microplate reader (SynergyHT BioTek)  
946 at 360/460 nm. Results are represented in percentage of fluorescence compared to the solvent  
947 control (DMSO) fixed at 100%.

#### 948 **4.13 Quantitative qRT-PCR**

949 Cultures were grown in triplicates during 4 hours in presence of the compound of interest at 128  
950 µg/ml. Then, 1 ml of bacteria was treated with RNA protect Bacteria Reagent (Qiagen) before  
951 centrifugation and storage at -20°C. Pellets were resuspended in 100 µl TE pH8, 2.5 µl of  
952 lysostaphin 10 mg/ml for *S. aureus* PR01 and incubated 10 min. at 37°C, or 100 µl TE pH8,  
953 lysozyme 1 mg/ml and incubated 5 min. at RT for *P. aeruginosa* PAO1. RNA was extracted by  
954 RNeasy kit (Qiagen) according to the manufacturer's protocol. RNA was eluted in 40 µl of RNase-  
955 free water, quantified using Qubit 2.0 fluorometer (Invitrogen) and DNase treated with RQ1 RNase-  
956 free DNase (Promega) according to manufacturer's instructions. Then, 500 ng of RNA were reverse  
957 transcribed into cDNA using random primers (Promega) and Improm-II reverse transcriptase  
958 (Promega) according to the manufacturer. qPCR was performed using SYBR select master mix



959 (Thermo Fisher). Primers for the amplification of target genes are listed in Supplementary Table S4.  
960 *HU* and *oprF* genes were used for normalization for *S. aureus* and *P. aeruginosa*, respectively.

## 961 **5 Abbreviations**

962 <sup>1</sup>H-NMR, Proton nuclear magnetic resonance; DMSO, dimethyl sulfoxide; F, fraction; FA, formic  
963 acid; GNPS, global natural products social molecular networking; LC-NMR, liquid  
964 Chromatography with Nuclear Magnetic Resonance Spectroscopy; *m/z*, mass to charge ratio; PQS,  
965 *Pseudomonas* quinolone signal; SPE, solid phase extraction; UHPLC-MS/MS, ultra-high  
966 performance liquid chromatography tandem mass spectrometry; UV, Ultra-violet; Z,  
967 chromatographic zones.

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972 collection and maintaining the *Fusarium* strains.

## 973 **7 Data Availability Statement**

974 The raw data of this work can be requested from the authors.

## 975 **8 Author Contributions**

976 JLW and EFQ supervised and designed the research. AA, LM, VH, KG, SL, and PMA conducted  
977 the experiments. AA, LM and VH analyzed the data and wrote the manuscript. EFQ, KP, DS, and  
978 JLW assisted the editing of the manuscript. All authors read and approved the manuscript.

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## 986 **10 Conflict of Interest**

987 The authors declare that the research was conducted in the absence of any commercial or financial  
988 relationships that could be construed as a potential conflict of interest.

## 989 **11 Supplementary Materials**

990 The Supplementary Materials for this article can be found online at:

991 **Figure Captions**

992 **Figure 1.** Schematic overview of the workflow of this study. **(A)** Optimized UHPLC-PDA-MS  
993 profile of the ethyl acetate crude extract (base peak intensity (BPI), PI mode) and UV trace (254  
994 nm). **(B)** HRMS/MS spectra of the LC peaks from the UHPLC-HRMS/MS extract profile  
995 corresponding to the isolated compounds **9** and **10**. **(C)** Selected part of a molecular network cluster  
996 highlighting the features of **9**, **10**, **16**, and **17** (series of structurally related pyrone derivatives).  
997 Some of the other features were dereplicated based on MN annotation propagation see Table 2. **(D)**  
998 HPLC-PDA chromatogram after gradient transfer from (A). Z1 to Z14 indicate chromatographic  
999 zones combined in semi-preparative HPLC for the bioassay monitoring, shown on (D) for a clearer  
1000 display. **(E)** semi-preparative HPLC-UV chromatogram after gradient transfer from (D) and dryload  
1001 sample introduction; vertical lines represent collected fractions. **(F)** Pseudo-LC-NMR obtained by a  
1002 combination of the <sup>1</sup>H-NMR spectra of all fractions from (E) into a single matrix (ppm vs. RT or  
1003 fraction N°). **(G)** <sup>1</sup>H-NMR spectra of fractions F51 and F55 highlighted in the 2d plot (F).

1004 **Figure 2.** Most likely tree (-ln = 20922.71682) inferred from the *Fusarium* 5 locus-64 taxa dataset  
1005 (alignment length after exclusion of ambiguously aligned regions = 2442 characters (char);  
1006 calmodulin = 142 char, beta-tubulin = 262 char, ITS = 364 char, *RPB2* = 1500 char, TEF-1 = 174  
1007 char). The fungal collection newly sequenced for that study is in blue. Branches that received  
1008 significant bootstrap (BS) values ( $\geq 70\%$ ) are in bold with BS values reported along the branches.

1009 **Figure 3.** **(A)** Stacked view of the <sup>1</sup>H-NMR spectra of all fractions ordered by retention time from  
1010 top to bottom, all recorded in DMSO-*d*<sub>6</sub> as bridging solvent. Green shadow highlights common  
1011 signals in fractions F51-56 in the regions of  $\delta_H$  5.5 to 5.7, and  $\delta_H$  6.0 to 6.3. Orange shadow  
1012 highlights common signals in fractions F64-65 and F103-104 in the region of  $\delta_H$  5.2 to 5.3. Yellow  
1013 shadow highlights the presence of fatty acids. **(B)** UV trace of the semi-preparative HPLC  
1014 chromatogram for LC peaks localization (Figure 1E). **(C)** Inset showing characteristic signals of the  
1015 series across a set of fractions F51-56 and 90-93. **(D)** Inset showing characteristic signals of the  
1016 series across a set of fractions F64-65 and 103-104.

1017 **Figure 4.** Structures of isolated compounds **1-22**.

1018 **Figure 5.** **(A)** Structure of **12**. **(B)** ROESY NMR spectrum of **12** in DMSO-*d*<sub>6</sub> **(C)** Observed  
1019 ROESY correlations for compound **12**.

1020 **Figure 6.** HPLC-PDA-ELSD analysis highlighting the effect of the extract enrichment and location  
1021 of various bioactivities monitoring. **(A)** Chromatogram of the crude extract, dashed lines represent  
1022 the 14 chromatographic zones. Highlighted zones represent the part of the chromatogram for  
1023 possible bioactivity. The square in the ELSD highlights the presence of fatty acids. **(B)**  
1024 chromatogram of the hydroalcoholic part. **(C)** chromatogram of the hexane. Compounds that  
1025 possess bioactivity are annotated by colored circles and the process demonstrates that they can be  
1026 precisely localized after enrichment.

1027 **Figure 7.** **(A)** Quantitative PCR test of *S. aureus* QS regulated genes *hla* and *hld*. AIP (autoinducer  
1028 from *S. caprae*) is used as positive inhibitor control **(B)** Quantitative PCR test of *P. aeruginosa* for  
1029 QS regulated gene *pqsA*. Fold induction is represented relative to DMSO alone (fixed at 1).

1030

1031 **Table 1.** Minimum Inhibitory Concentration (MIC) and quorum sensing inhibition (QS) assays of  
 1032 crude extract compared to several antibiotics.

1033

	MIC ( $\mu\text{g/ml}$ )		QS (% of fluorescence)	
	MRSA	PA	PA ( <i>pqsA</i> )	PA ( <i>lasB</i> )
Crude extract (small scale)	32	> 128	21%	19%
Crude extract (large scale)	32	> 128	20%	25%
Azithromycin	NA	10	32%	39%
Gentamicin	1	NA		
Tetracycline	16			
Erythromycin	16			
Chloramphenicol	32			
<p><i>Values show the mean of triplicates. Test results were compared to DMSO fixed at 100%. MRSA, methicillin resistant Staphylococcus aureus; PA, Pseudomonas aeruginosa; NA, not applicable.</i></p>				

1034

**Table 2.** Annotated compounds by UHPLC-HRMS/MS in both PI and NI modes in the crude extract of *F. petroliphilum*.

N° F.	N° C.	RT	C. Index	Isolated as	Annotated as	T. score	Chemical class	MF	I. mode	<i>m/z</i>	Error (ppm)
F4	1	1.03	Sgl	Adenosine	Adenosine	3	Purine Nucleoside	C <sub>10</sub> H <sub>13</sub> N <sub>5</sub> O <sub>4</sub>	[M+H] <sup>+</sup>	268.1039	-1.49
F10	3	2.31	19	6-(1-hydroxyethyl)-3-methyl-2H-pyran-2-one	-	3	Pyrone	C <sub>8</sub> H <sub>10</sub> O <sub>3</sub>	[M+H] <sup>+</sup>	155.0703	-3.22
F10	2a	2.21	155	6-(2,3-dihydroxybutan-2-yl)-3-methyl-2H-pyran-2-one	Fusanolide B	3	Pyrone	C <sub>10</sub> H <sub>14</sub> O <sub>4</sub>	[M+H] <sup>+</sup>	199.0967	-2.51
F10	2b	2.27	155	Isomer of 2a	Fusanolide B	3	Pyrone	C <sub>10</sub> H <sub>14</sub> O <sub>4</sub>	[M+H] <sup>+</sup>	199.0872	-1.51
-	-	2.45	3	-	Arthrospolide A	2	Polyketide	C <sub>13</sub> H <sub>14</sub> O <sub>5</sub>	[M-H] <sup>-</sup>	249.0766	-1.2
-	-	2.47	145	-	Fusaquinone A	2	Naphthofuran	C <sub>16</sub> H <sub>18</sub> O <sub>6</sub>	[M+H] <sup>+</sup>	307.1178	-1.17

-	-	2.56	145	-	Fusarnaphthoquinone B	2	Naphthofuran	C <sub>15</sub> H <sub>16</sub> O <sub>5</sub>	[M+H] <sup>+</sup>	277.1066	-3.61
-	-	2.59	9	-	Diaporthin	1	Benzopyone	C <sub>13</sub> H <sub>14</sub> O <sub>5</sub>	[M+H] <sup>+</sup>	251.0911	-3.65
F20	4	2.65	329	Gibepyrone D	Gibepyrone D	2	Pyrone	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	[M-H] <sup>-</sup>	193.0498	-1.55
F26	5	2.71	37	Aloesol	Aloesol	2	Benzopyran	C <sub>13</sub> H <sub>14</sub> O <sub>4</sub>	[M+H] <sup>+</sup>	235.0963	-2.97
			Sgl						[M-H] <sup>-</sup>	233.0821	3.42
-	-	2.74	Sgl	-	Cladobotrin V	2	Pyranone	C <sub>10</sub> H <sub>12</sub> O <sub>4</sub>	[M-H] <sup>-</sup>	195.0655	-0.85
-	-	2.95	222	-	Javanicin O-De-Me	2	Naphthoquinone	C <sub>14</sub> H <sub>12</sub> O <sub>6</sub>	[M-H] <sup>-</sup>	275.0564	3.08
-	-	3.01	Sgl	-	Gibepyrone A	3	Pyranone	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	[M-H] <sup>-</sup>	163.0753	-0.22
-	-	3.2	19	-	Fusarpyrone A	3	Pyran	C <sub>10</sub> H <sub>12</sub> O <sub>3</sub>	[M+H] <sup>+</sup>	181.0858	0.8

-	-	3.23	222	-	5,8-Dihydroxy-6-hydroxymethyl-7-(2-hydroxypropyl)-2-methoxy-1,4-naphthoquinone	3	Naphthoquinone	C <sub>15</sub> H <sub>15</sub> O <sub>7</sub>	[M-H] <sup>-</sup>	307.0825	2.44
-	-	3.24	9	-	Fusarubin 5-deoxy	3	Benzoquinone	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	[M+H] <sup>+</sup>	291.086	-2.8
-	-	3.37	207	-	Bostrycoidin 6-Deoxy	3	Quinone	C <sub>15</sub> H <sub>11</sub> NO <sub>4</sub>	[M+H] <sup>+</sup>	270.0759	-2.76
F35	6	6.63	207	5-hydroxy-4-(hydroxymethyl)-8-methoxy-2-methyl-1H-benzo[g]indole-6,9-dione	-	3	Quinone	C <sub>15</sub> H <sub>13</sub> NO <sub>5</sub>	[M+H] <sup>+</sup>	288.0868	-1.38
-	-	3.37	5	-	Sescandelin 1'-Ketone	1	Isocoumarin	C <sub>11</sub> H <sub>8</sub> O <sub>5</sub>	[M-H] <sup>-</sup>	219.0295	1.6
-	-	3.85	306	-	5-Acetyl-1,2,4,6-tetrahydroxyanthraquinone 2-Me ether	2	Quinone	C <sub>17</sub> H <sub>12</sub> O <sub>7</sub>	[M+H] <sup>+</sup>	329.0636	2.6
F43	7	3.86	9	Fusarubin	Fusarubin	3	Benzoquinone	C <sub>15</sub> H <sub>14</sub> O <sub>7</sub>	[M+H] <sup>+</sup>	307.0823	1.62

F44	8	3.86	-	3-O-Methylfusarubin	3-O-Methylfusarubin	3	Quinone	C <sub>16</sub> H <sub>16</sub> O <sub>7</sub>	[M-H] <sup>-</sup>	319.0825	2.19
-	-	3.86	45	-	Anhydrofusarubin	3	Quinone	C <sub>15</sub> H <sub>12</sub> O <sub>6</sub>	[M+H] <sup>+</sup>	289.0715	1.03
-	-	3.87	54	-	3-Acetyl-2,5,8-trihydroxy-6-methoxy naphthoquinone	3	Naphthoquinone	C <sub>14</sub> H <sub>12</sub> O <sub>7</sub>	[M-H] <sup>-</sup>	291.0511	1.04
F51	9	3.96	25	(6E)-7-(4-methoxy-6-oxo-6H-pyran-2-yl)-3,5-dimethyloct-6-enoic acid	7S-Hydroxy-O-demethylasiodiplodin	3	Pyran	C <sub>16</sub> H <sub>22</sub> O <sub>5</sub>	[M+H] <sup>+</sup>	295.1544	-3.72
-	x	3.97	54	-	Rhodolamprometrin	2	Anthracene	C <sub>16</sub> H <sub>10</sub> O <sub>7</sub>	[M-H] <sup>-</sup>	313.0352	1.13
F55	10	4.4	29	6-((E)-6-ethyl-7-hydroxy-4-methylhept-2-en-2-yl)-4-methoxy-2H-pyran-2-one	-	3	Pyrone	C <sub>16</sub> H <sub>24</sub> O <sub>4</sub>	[M+H] <sup>+</sup>	281.1745	-2.13
-	-	4.54	45	-	Solaniol or Karuquinone C	3	Naphthalene	C <sub>15</sub> H <sub>16</sub> O <sub>6</sub>	[M+H] <sup>+</sup>	293.1017	-2.73
		4.55	54						[M-H] <sup>-</sup>	291.0885	4.8

-	-	4.61	45	-	Dihydroanhydrojavanicin	3	Naphthofuran	C <sub>15</sub> H <sub>14</sub> O <sub>5</sub>	[M+H] <sup>+</sup>	275.0912	-1.89
F60	11	5.63	19	Bostrycoidin	Bostrycoidin	3	Quinone	C <sub>15</sub> H <sub>11</sub> NO <sub>5</sub>	[M+H] <sup>+</sup>	286.0695	-0.34
F62	12	5.34	Sgl	5β,6β-23,26-diepoxy-3β,7α,9α-trihydroxy-(20Z,23S,24S,25R)ergosta-8(14),20-dien-15-one	-	3	Steroid	C <sub>28</sub> H <sub>40</sub> O <sub>6</sub>	[M+Na] <sup>+</sup>	495.2706	-3.23
F64	13	5.44	86	2-(2,3,5,6,7,7a-hexahydro-1-((E)-6-hydroxy-5,6-dimethylhept-3-en-2-yl)-7a-methyl-5-oxo-1H-inden-4-yl)acetic acid	-	3	Cyclohexenone	C <sub>21</sub> H <sub>32</sub> O <sub>4</sub>	[M+H] <sup>+</sup>	349.2378	-0.28
		5.44	Sgl						[M-H] <sup>-</sup>	347.2228	1.72
F66	14	5.68	86	2-(2,3,5,6,7,7a-hexahydro-1-((E)-7-hydroxy-5,6-dimethylhept-3-en-2-yl)-7a-methyl-5-oxo-1H-inden-4-yl)acetic acid	-	3	Cyclohexenone	C <sub>21</sub> H <sub>32</sub> O <sub>4</sub>	[M+H] <sup>+</sup>	349.2386	2
			Sgl						[M-H] <sup>-</sup>	347.2232	2.87



F69	15	3.38	Sgl	5-hydroxy-4-methoxy-6,8-dimethylpyrano[3,2-g]indol-2(9H)-one	-	3	Pyrone	C <sub>14</sub> H <sub>13</sub> NO <sub>4</sub>	[M-H] <sup>-</sup>	258.0771	1.82
-	-	7.05	173	-	kauranoic acid ent-16β-Hydroxy-19	2	Fatty acid	C <sub>20</sub> H <sub>32</sub> O <sub>3</sub>	[M+H] <sup>+</sup>	321.2442	2.1
-	-	7.81	19	-	Fusarone	2	Cyclopentanone	C <sub>14</sub> H <sub>22</sub> O <sub>3</sub>	[M+H] <sup>+</sup>	239.1661	0.5
F91	16	8.89	29	4-methoxy-6-((E)-4,6-dimethyloct-2-en-2-yl)-2H-pyran-2-one	-	3	Pyrone	C <sub>16</sub> H <sub>24</sub> O <sub>3</sub>	[M+H] <sup>+</sup>	265.1805	0.37
F93	17	9.6	Sgl	4-methoxy-3-methyl-6-((E)-4,6-dimethyloct-2-en-2-yl)-2H-pyran-2-one	-	3	Pyrone	C <sub>17</sub> H <sub>26</sub> O <sub>3</sub>	[M+H] <sup>+</sup>	279.1956	-1.43
F103	18	11.34	Sgl	2-(2,3,5,6,7,7a-hexahydro-7a-methyl-1-((E)-5,6-dimethylhept-3-en-2-yl)-5-oxo-1H-inden-4-yl)acetic acid	-	3	Cyclohexenone	C <sub>21</sub> H <sub>32</sub> O <sub>3</sub>	[M+H] <sup>+</sup>	333.2426	0.3
									[M-H] <sup>-</sup>	331.228	2.11

107	19	14.5	-	3-O-β-D-glucopyranoside-Stigmast-8-en-3-ol	-	3	Steroid	C <sub>35</sub> H <sub>60</sub> O <sub>6</sub>	[M+Na] <sup>+</sup>	599.428	-1.16
F116	20	14.6	-	24R/24S Cerevisterol	-	3	Steroid	C <sub>28</sub> H <sub>46</sub> O <sub>3</sub>	[M+Na] <sup>+</sup>	453.3397	11.6
F117	21	14.27	-	6-Dehydrocerevisterol	-	3	Steroid	C <sub>28</sub> H <sub>44</sub> O <sub>3</sub>	[M+H] <sup>+</sup>	429.3387	4.42
F122	x	16.48	-	-	3-Acetoxy-2,3-dihydropiptoporic acid	1	Fatty acid	C <sub>23</sub> H <sub>28</sub> O <sub>5</sub>	[M-H] <sup>-</sup>	383.1898	1.8
F132	22	13.62	-	Ergosterol	Ergosterol	3	Steroid	C <sub>28</sub> H <sub>44</sub> O	[M-H] <sup>-</sup>	395.3325	2.78

*N° F.*, = Number of semi-preparative fraction; *N° C.* = Isolated compounds **1–22**; *RT* = retention time in UHPLC-HRMS/MS analysis of the extract; *C. index* = component index (cluster number in MN); *Sgl* = singleton node; *T. score* = taxonomically informed score; *I.mode* = ionization mode; 1 = family (Nectriaceae); 2 = genus (*Fusarium*); 3 = species (*F. solani*); *MF* = molecular formula.

**Table 3.** Minimum Inhibitory Concentration (MIC) and quorum sensing inhibition (QS) assays of zones of fractions and their corresponding compounds compared to several positive controls.

Zone	% PA ( <i>pqsA</i> )	% PA ( <i>lasB</i> )	Compound	% PA ( <i>pqsA</i> )	% PA ( <i>lasB</i> )	% SA ( <i>lacZ</i> )
Control	32 ± 0.2	39 ± 2.9	NA	32 ± 0.2	39 ± 2.9	27 ± 1.6
Z_3	68 ± 0.0	85 ± 0.2	(4)	60 ± 4.5	76 ± 8.0	79 ± 0.7
			(5)	40 ± 8.5	51 ± 7.7	<b>11 ± 5.0</b>
Z_4	48 ± 2.6	65 ± 1.2	(6)	59 ± 2.7	69 ± 0.9	37 ± 0.0
Z_5	31 ± 1.6	43 ± 0.4	(7/8)	<b>12 ± 0.6</b>	<b>15 ± 1.0</b>	<b>3 ± 0.5*</b>
Z_6	45 ± 0.7	60 ± 0.9	(9)	58 ± 3.6	68 ± 2.7	90 ± 0.0
			(10)	36 ± 2.2	41 ± 0.4	<b>17 ± 4.2</b>
Z_7	60 ± 4.3	75 ± 0.4	(11)	66 ± 10.1	71 ± 0.5	121 ± 22.0
			(12)	58 ± 3.6	68 ± 2.7	79 ± 0.7
			(13)	34 ± 2.6	<b>39 ± 0.4</b>	<b>13 ± 0.2</b>
Z_8	64 ± 0.3	82 ± 1.8	(14)	72 ± 6.5	73 ± 0.2	31 ± 1.2

			<b>(15)</b>	57 ± 2.9	63 ± 0.1	36 ± 3.3
Z_9 (MIC MRSA at 32 µg/ml)	63 ± 0.6	82 ± 0.0	<b>(16)</b> (MIC MRSA at 32 µg/ml)	37 ± 2.1	39 ± 6.9	<b>15 ± 11.0</b>

*Values show the mean of triplicates ± SD. Values in **bold** are lower than the corresponding control. Positive controls of QS assays are, azithromycin at 2 µg/ml for PA, and S. caprae AIP 1 µM for SA. Results were compared to DMSO fixed at 100%. MRSA, methicillin resistant Staphylococcus aureus; PA, Pseudomonas aeruginosa; NA, not applicable. \*Fluorescence was biased due to the natural coloration of the compound.*

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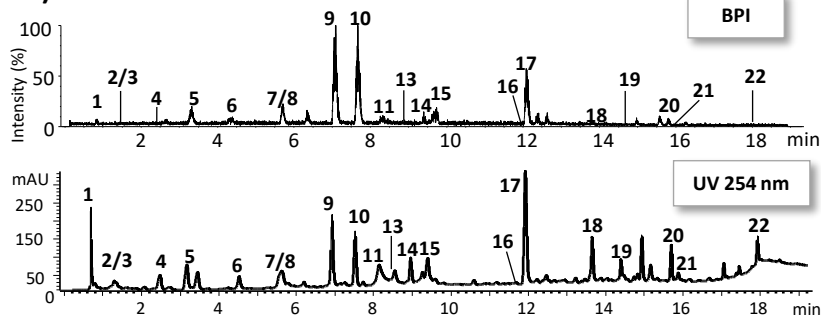
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Figure 1

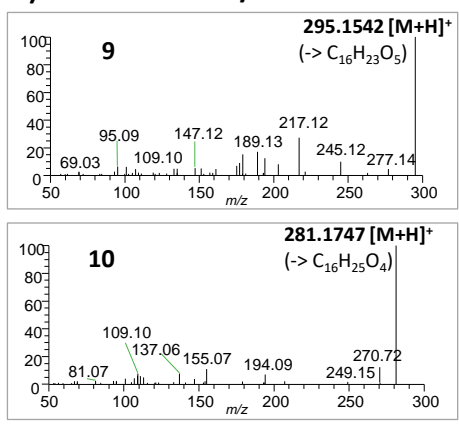
Aligned chromatograms through gradient transfer

UHPLC-HRMS/MS

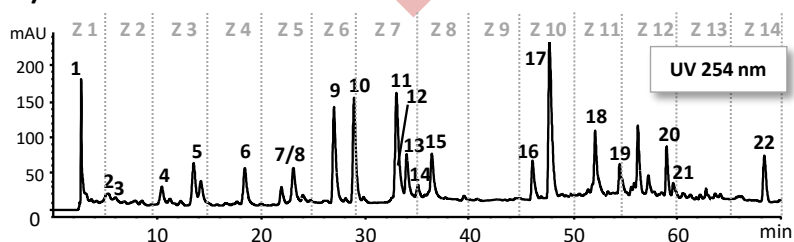
A) UHPLC-PDA-MS



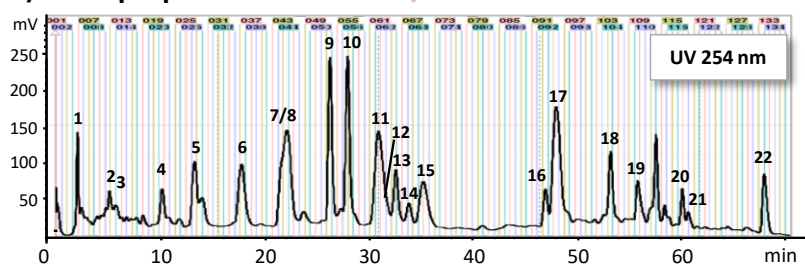
B) Tandem HRMS/MS



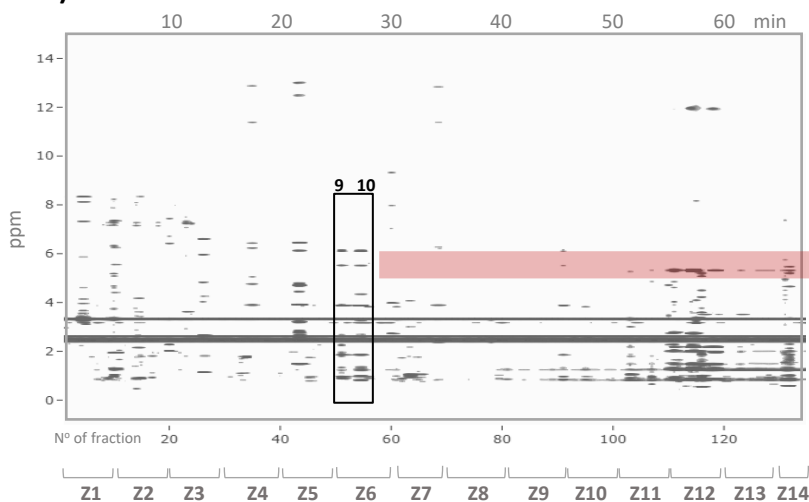
D) HPLC-PDA



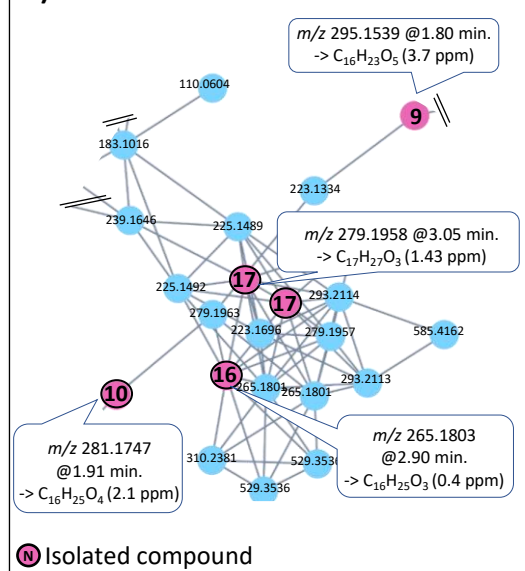
E) Semi-prep HPLC-UV



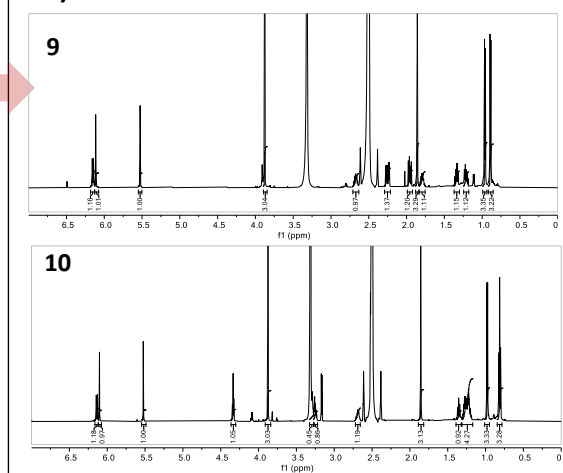
F) Pseudo 2D LC-<sup>1</sup>H NMR



C) Molecular Network



G) <sup>1</sup>H NMR and 2D NMR



14 Zones of fractionation to biological assay evaluation

Figure 2

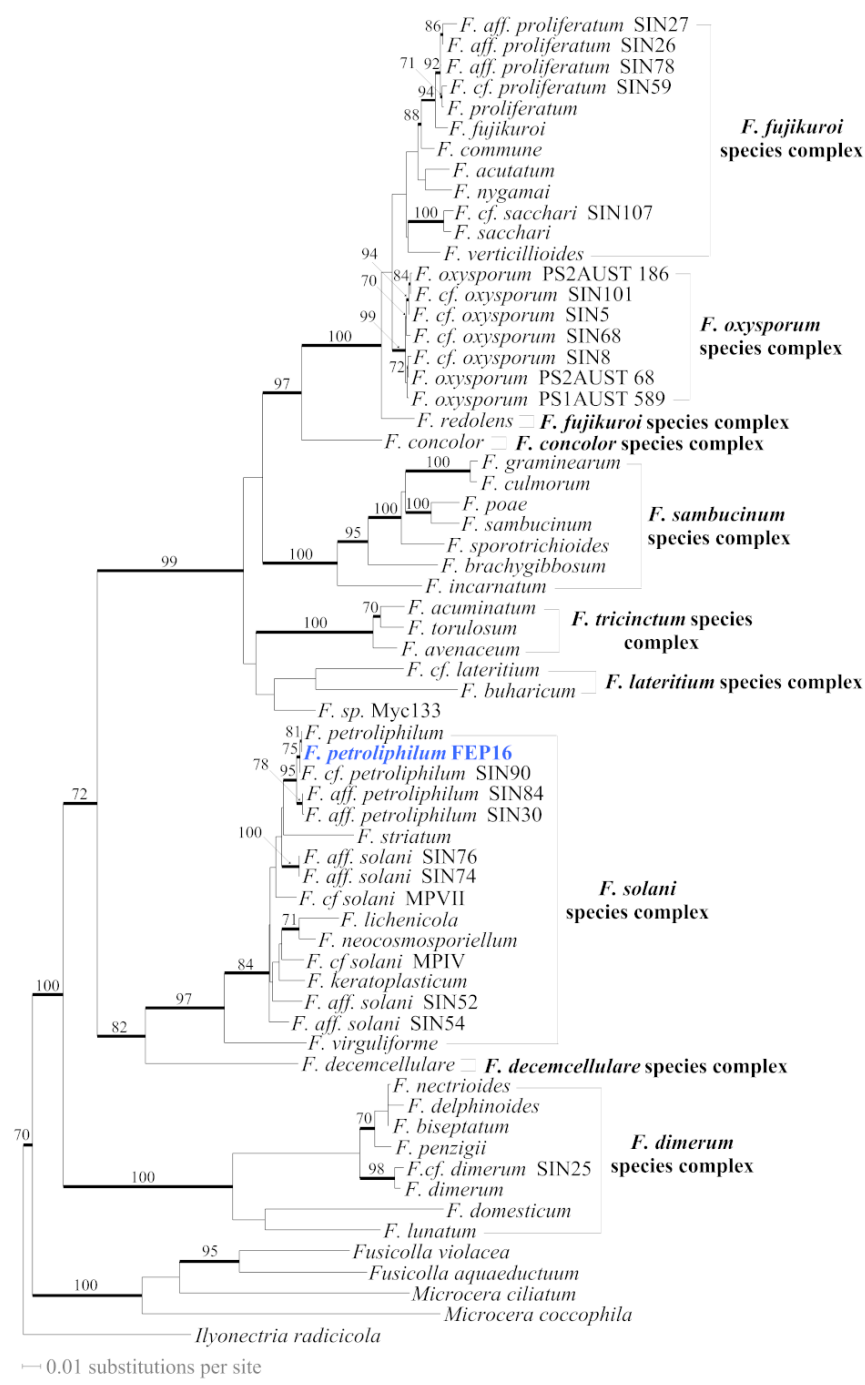


Figure 3

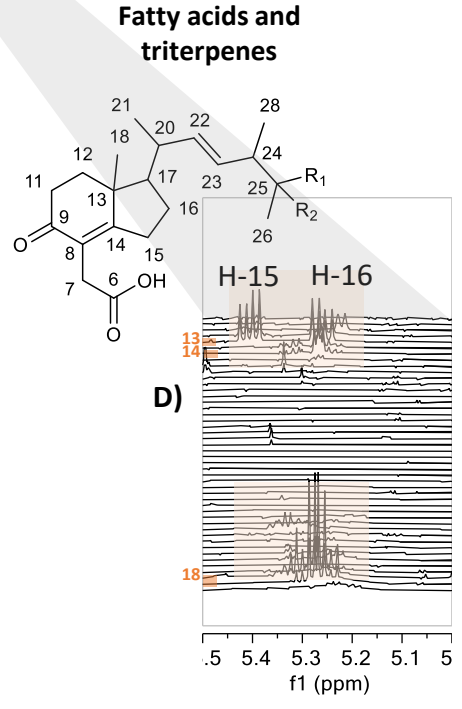
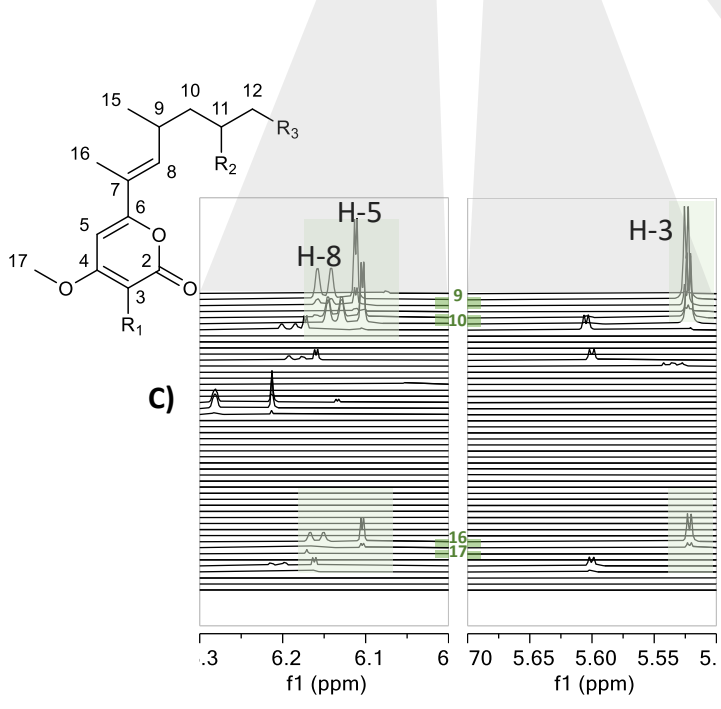
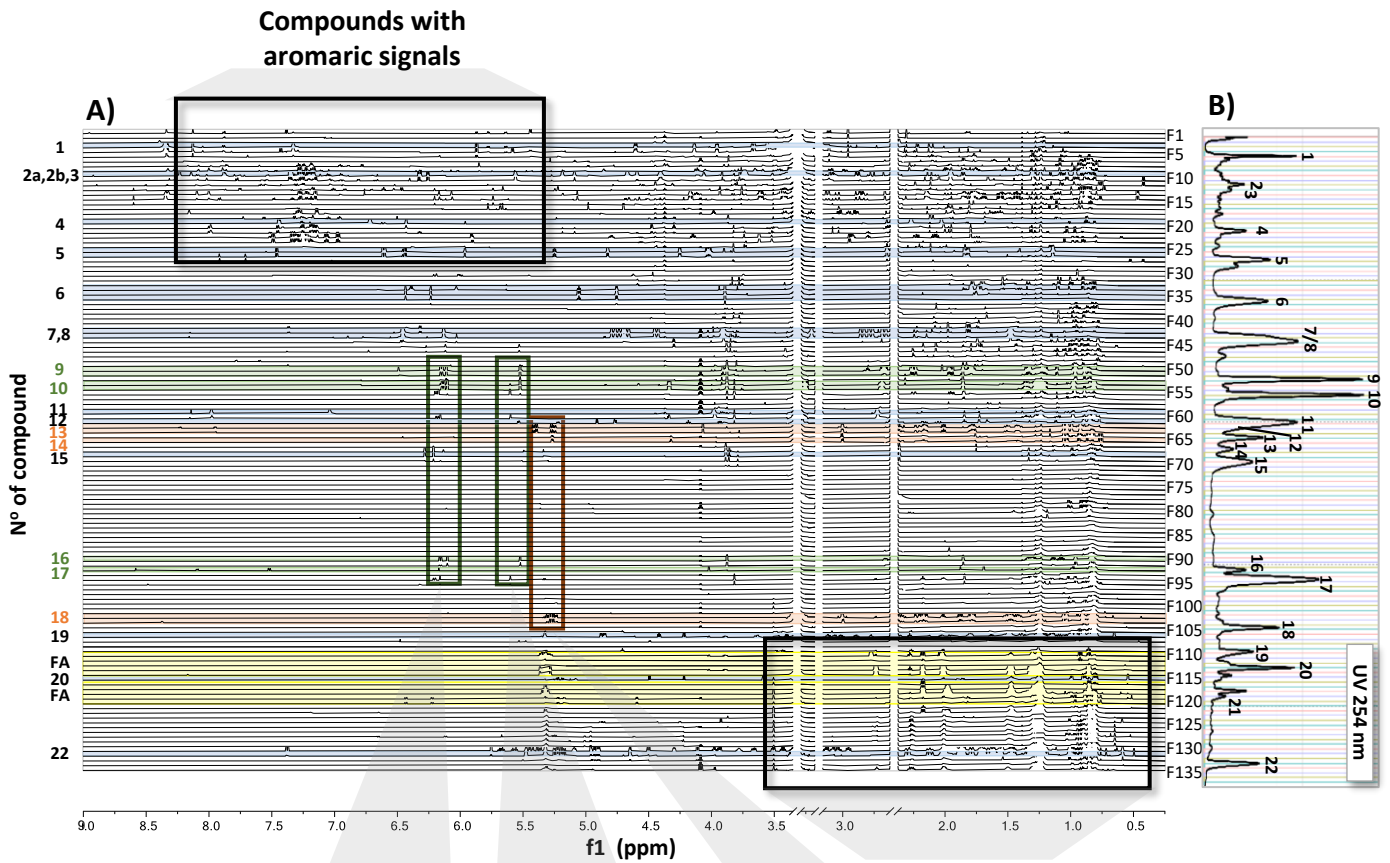
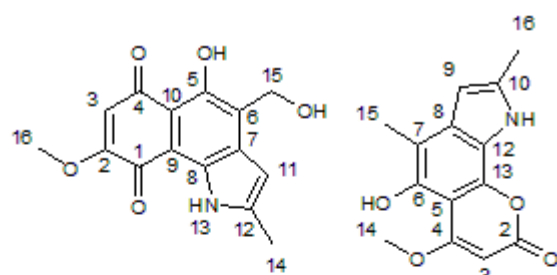
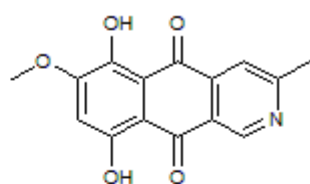
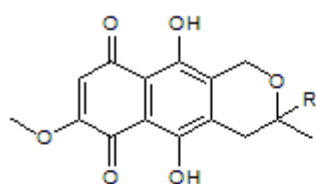


Figure 4

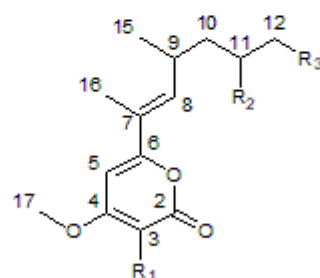
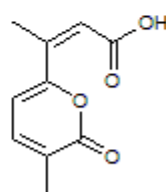
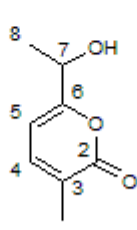
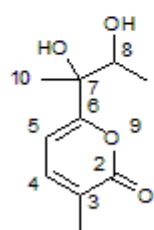


7. R=OH Fusarubin (F43)  
8. R=OCH<sub>3</sub> 3-O-methylfusarubin (F43)

11. Bostrycoidin (F60)

6. (F35)

15. (F69)



2a/2b. (F10)

3. (F10)

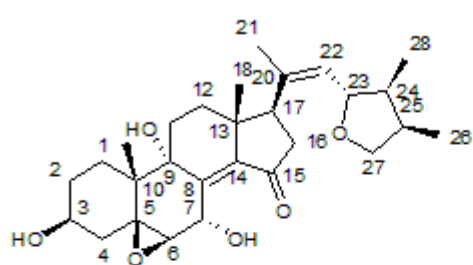
4. Gibepyrone D (F20)

9. R<sub>1</sub> = H R<sub>2</sub> = CH<sub>3</sub> R<sub>3</sub> = COOH (F51)

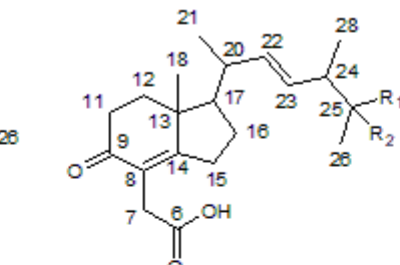
10. R<sub>1</sub> = H R<sub>2</sub> = CH<sub>2</sub>CH<sub>3</sub> R<sub>3</sub> = OH (F55)

16. R<sub>1</sub> = H R<sub>2</sub> = CH<sub>3</sub> R<sub>3</sub> = CH<sub>3</sub> (F91)

17. R<sub>1</sub> = CH<sub>3</sub> R<sub>2</sub> = CH<sub>3</sub> R<sub>3</sub> = CH<sub>3</sub> (F93)



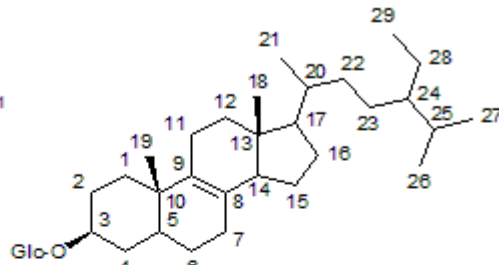
12. (F62)



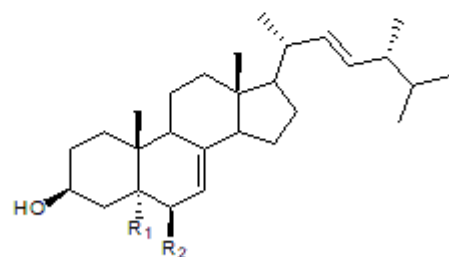
13. R<sub>1</sub> = CH<sub>3</sub> R<sub>2</sub> = OH (F64)

14. R<sub>1</sub> = CH<sub>2</sub>OH R<sub>2</sub> = H (F66)

18. R<sub>1</sub> = CH<sub>3</sub> R<sub>2</sub> = H (F103)



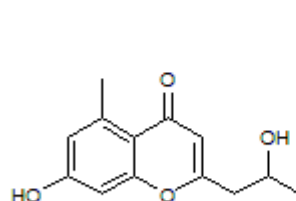
19. (F107)



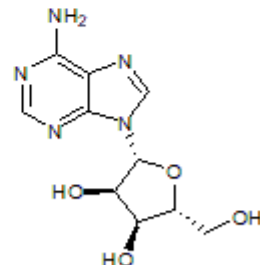
20. R<sub>1</sub> = OH R<sub>2</sub> = OH Cerevisterol (F116)

21. R<sub>1</sub> = OH R<sub>2</sub> = H 6-Dehydrocerevisterol (F36 HX)

22. R<sub>1</sub>, R<sub>2</sub> C=C Ergosterol (F132)



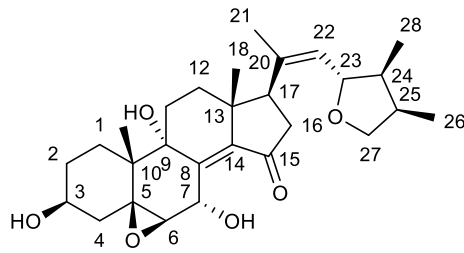
5. Aloesol (F26)



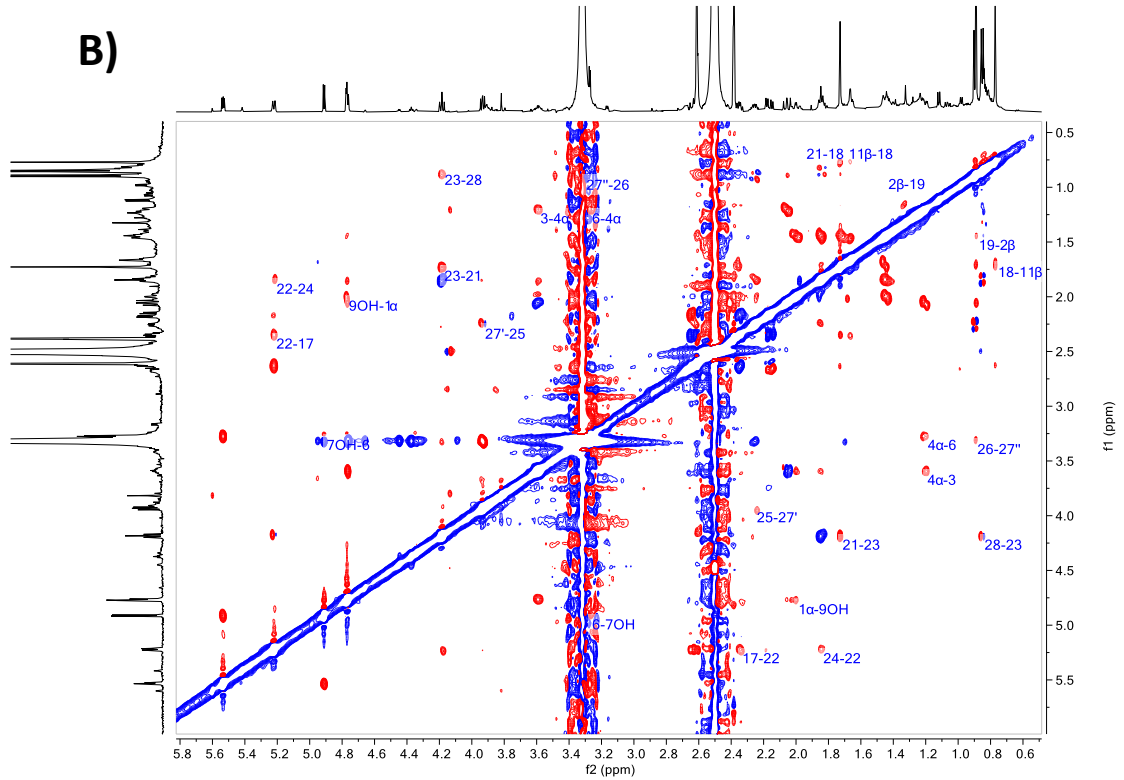
1. Adenosine (F4)

Figure 5

A)



B)



C)

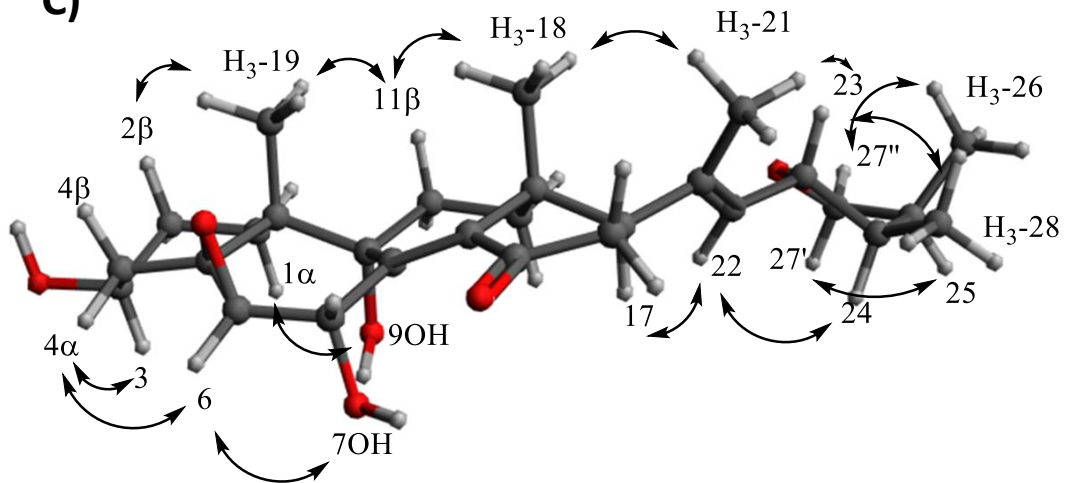
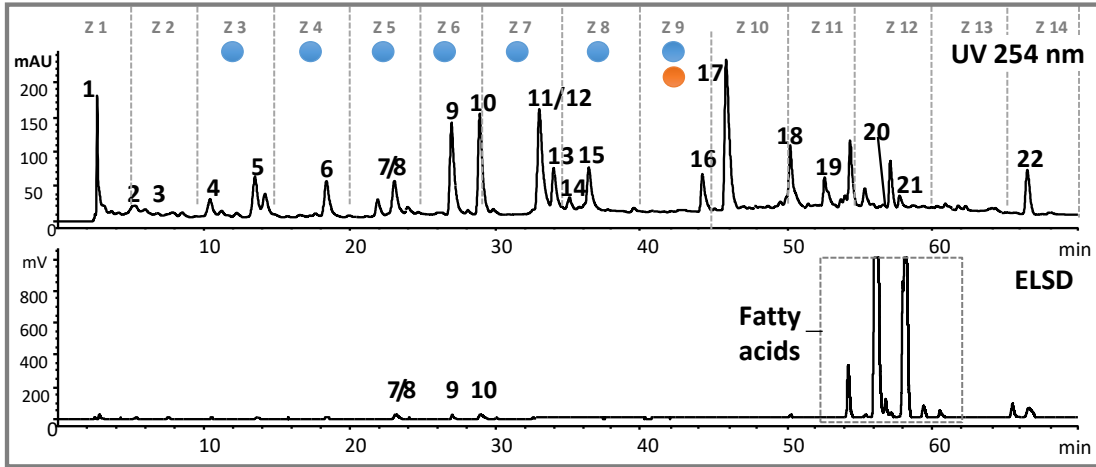
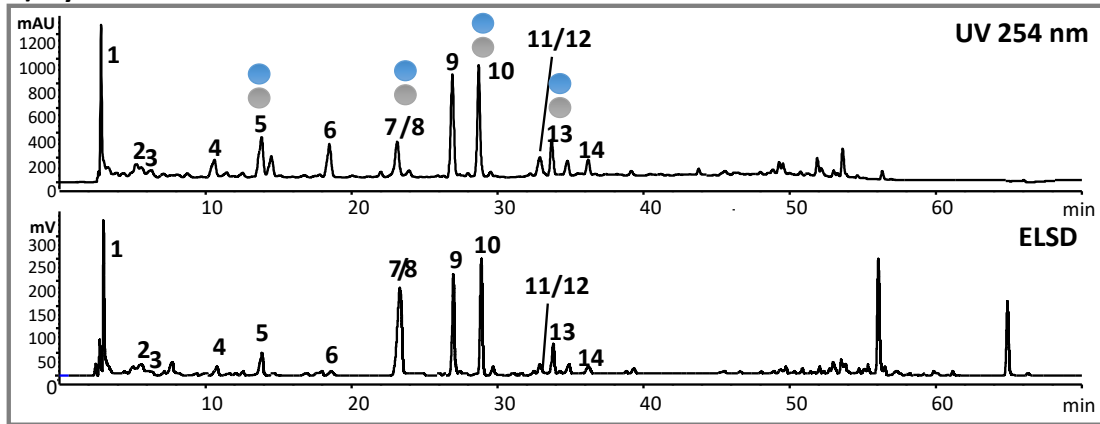


Figure 6

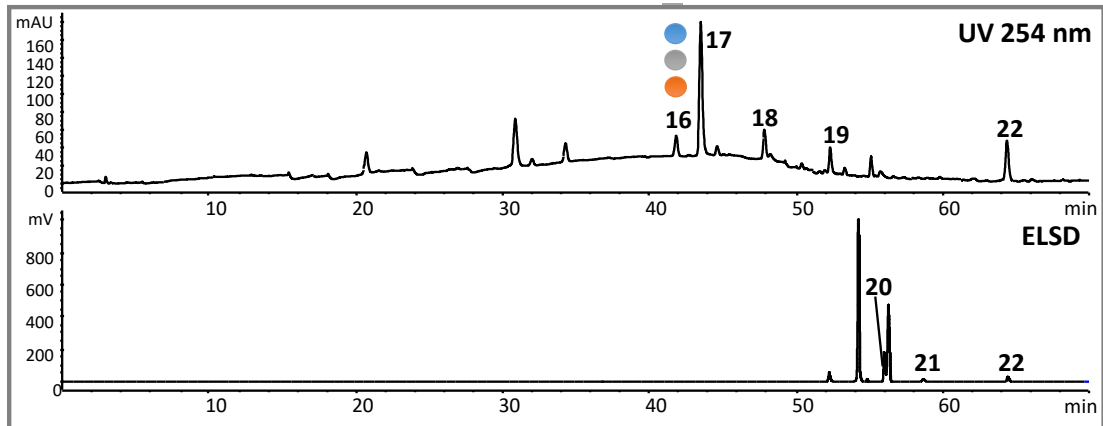
A) AcOEt crude extract



B) Hydroalcoholic fraction



C) Hexane fraction



Liquid/Liquid partition

● QSI of *P. aeruginosa*      ● QSI of *S. aureus*      ● MRSA inhibition



Figure 7

