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Generation of Stilbene Antimicrobials Against Multi-resistant Strains of *Staphylococcus aureus* Through Biotransformation by the Enzymatic Secretome of *Botrytis cinerea*

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ABSTRACT: The biotransformation of a mixture of resveratrol and pterostilbene was performed by the protein secretome of *Botrytis cinerea.* Several reaction conditions were tested to overcome solubility issues and to improve enzymatic activity. Using MeOH as co-solvent, a series of unusual methoxylated compounds was generated. The reaction was scaled-up and the resulting mixture purified by semi-preparative HPLC-PDA-ELSD-MS. Using this approach, 15 analogues were isolated in one step. Upon full characterization by NMR and HRMS analyses, eight of the compounds were new. The antibacterial activities of the isolated compounds were evaluated in vitro against the opportunistic pathogens, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The selectivity index (SI) was calculated based on cytotoxic assays performed against human liver carcinoma cells (HepG2) and human breast epithelial cell line (MCF10A). Some compounds revealed remarkable antibacterial activity against multidrug-resistant strains of *S. aureus* on the background of the moderate human cell line cytotoxicity.

The overuse and misuse of antibiotics in the past decades resulted in the rapid emergence of resistant bacterial strains worldwide.[1,2] Novel compounds with original scaffolds are particularly needed in drug discovery to fight such multi-resistant pathogenic bacterial strains. Among these multi-resistant bacteria, particular attention should be given to the Gram-positive bacterium *Staphylococcus aureus* which causes superficial and invasive infections, potentially fatal, such as sepsis and pneumonia.[3-5] Current classical antibiotic treatments are ineffective against the methicillin-resistant strains of *S. aureus* (MRSA).[4, 6, 7]

The exploitation of natural products is of great interest in the search for new antimicrobial lead compounds.[8] Plants produce a vast variety of natural products with huge structural diversity, some of which confer selective advantages against microbial attack.[9] The exploitation of natural products of plant origin by alternative approaches is of interest to discover new therapeutic agents since plant extracts are known to contain a wide variety of antimicrobial compounds.[10, 11]

Recently, the application of an enriched fraction of enzymes from fungi ('fungal secretome') for the transformation of conventional natural products was effective to create complex chemically diverse compounds with enhanced biological activity.[12] This approach takes advantage of the catalytic promiscuity of various enzymes constituting the secretome.[13] In comparison to the pure enzyme, the enzymatic diversity of a secretome produced by fungi for the degradation and/or transformation of various substrates, has the potential to catalyze different chemical reactions at the same time.[12] The use of secretomes for biotransformation thus has great potential to transform various types of substrates with less specificity than pure enzymes[14] and is probably well suited to generate high chemodiversity by the transformation of related rather simple initial natural compound substrates.

The majority of studies using enzymes were performed using water as the reaction medium. However, enzymatic reactions can occur in the presence of organic solvents and this could lead

to numerous advantages.[15] For example, organic solvents can improve solubility in aqueous media and also allow a relatively easy product recovery. In addition, in this unnatural milieu, enzymes can acquire unusual properties such as enhanced stability, radically altered substrate and enantiomeric specificities, molecular memory, and the ability to catalyze unusual reactions.[15-18]

As a continuation of our proof of concept study,[12] in the present work the biotransformation of a mixture of resveratrol and pterostilbene was optimized by using different percentages of organic solvent in the reaction mixture in view of improving solubility and increasing the chemodiversity of compounds generated. The choice to work with a mixture of these two stilbenes instead of using only one was motivated by the need to obtain heterodimers in addition to homodimers and thus increase chemical diversity. This yielded 15 structurally complex stilbenes, eight being new. These derivatives together with those obtained in previous studies were evaluated for their antimicrobial properties against Gram-positive and Gram-negative pathogens.

RESULTS AND DISCUSSION

In our previous work, a series of biotransformation reactions were performed on resveratrol, pterostilbene, and a mixture of both by using the secretome of *B. cinerea*.[12] Although a series of new active compounds were obtained, we often observed poor solubility of the stilbenes (substrates) in the aqueous media required for the enzymatic reaction. To overcome this problem and also generate additional chemodiversity, a series of reactions was performed on a mixture of resveratrol and pterostilbene as a substrate in the presence of different amounts of organic solvents used as co-solvent with water.[15-18]

First, the solubility of resveratrol and pterostilbene at the concentration of 0.5 mg/mL (0.25 mg/mL of each compound) was tested in aqueous solutions containing 2, 10, 30, 50, 70, 90, and 98% of an organic solvent (acetone or MeOH). At concentrations of 2 and 10% of organic solvents, the compounds were not completely soluble, resulting in a "bleached" solution, while at concentrations of 30-98%, the compounds were fully soluble (Figure S1, Supporting Information). In a second step, resveratrol and pterostilbene (concentration of 0.5 mg/mL; 0.25 mg/mL of each compound) were diluted according to the ratios of organic solvent (acetone or MeOH) and H_2O, before incubation with the secretome of *B. cinerea* over 24 hours. Each reaction was monitored by UHPLC-PDA-ELSD-MS (Figures S2-S4, Supporting Information). Based on the semi-quantitative ELSD response the increase in the percentage of acetone (2 to 50%) seemed to improve the yield of the reaction. With 70, 90, and 98% of acetone the reactions did not occur suggesting enzyme denaturation (Figure S2, Supporting Information).

The same patterns were observed using increasing amounts of MeOH while at 90 and 98% of MeOH the reactions did not occur (Figure S3, Supporting Information). However, starting from 10% MeOH, peaks that were not observed in the proof of concept study[12] appeared clearly after 24 hours and became intense at 50 and 70% MeOH. In comparison with the previous results[12] MS deprotonated formic acid adduct ions at *m/z* 559 $[M+FA-H]^-$ and *m/z* 587 $[M+FA-H]^-$ were detected, suggesting the presence of new dimeric analogs (Figure S4, Supporting Information).

To isolate these compounds, the biotransformation reaction was scaled up using a solution with 50% of MeOH and 50 mg of each substrate (see Experimental section). After 24 hours, the reaction was monitored by UHPLC-PDA-ELSD-MS. As expected, the resveratrol and pterostilbene substrates were totally consumed demonstrating a good enzymatic activity under such optimized conditions.

For an efficient direct targeted isolation of the biotransformed natural products, the analytical conditions used for monitoring were transposed to a high-resolution semi-preparative HPLC-UV system hyphenated to an evaporative light scattering detector (ELSD) and a single quadrupole MS detector.[19] A geometrical transfer of the analytical HPLC conditions to the semi-preparative HPLC was performed, using the same reversed-phase column chemistry to obtain the same chromatographic selectivity (Figures 1A and 1B). The method takes advantage of HPLC modeling based on generic linear gradients at the analytical level to maximize the separation of the compounds of interest in a given reaction mixture (Figures 1B and 1C).[20, 21] To avoid loss of resolution caused by the organic solvent during the sample injection, a sample dry load was used instead of a conventional loop injection, following a protocol recently developed in our laboratory.[22] Using this approach, the separations obtained at the semi-preparative HPLC scale perfectly matched those obtained at the analytical scale, demonstrating the efficiency of the gradient transfer method. The use of the ELSD detector gave precious information about the relative amount of each compound in the crude reaction mixture (Figure 1C). Finally, MS detection enabled an easy localisation of the minor compounds (e.g. **7** and **13**) within the fractions obtained. This is illustrated by the extracted-ion chromatogram (XIC) obtained at the semi-preparative scale (Figure 1D).

Using this approach, 15 compounds were isolated in one step. Seven compounds were identified by comparison with the literature as pallidol (**1**),[23] parthenostilbenin A/B (**2**)[24] resvepterol A/B (**3**),[12] *trans*-δ-viniferin (**6**), 11',13'-di-*O*-methyl-*trans*-δ-viniferin (**11**),[12] 11,13-di-*O*-methyl-*trans*-δ-viniferin (**14**),[12] and pterostilbene-*trans*-dehydromer (**15**) (Figure 2).[23] Eight other compounds are new biotransformed natural products and their structure identification is described hereafter.

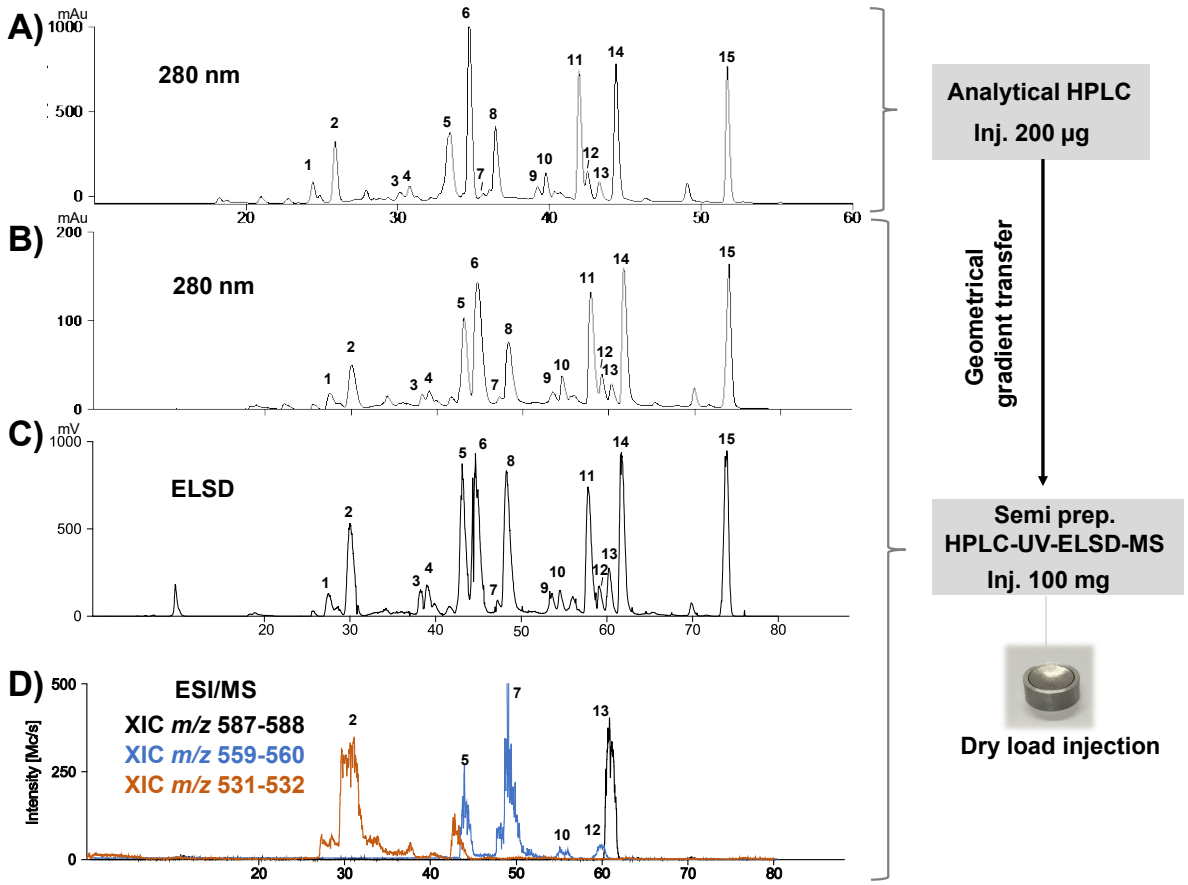

Figure 1. The biotransformation reaction of resveratrol and pterostilbene with the secretome of *B. cinerea* was followed A) by HPLC-UV analysis in analytical scale, B and C) by HPLC-UV-ELSD-MS in semi-preparative scale for isolation of compounds from the scaled-up reaction. D) Extracted-ion chromatogram (XIC-MS) traces recorded by MS during the semi-preparative separation.

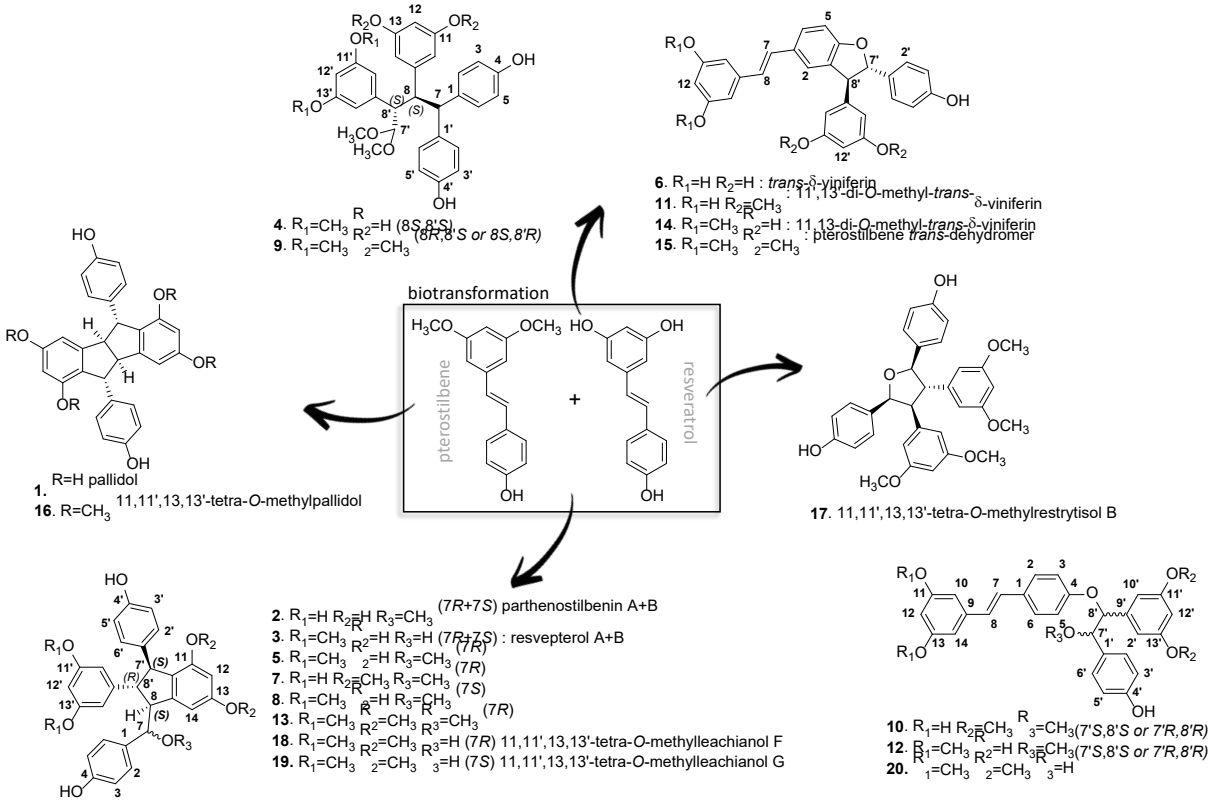

Figure 2. Compounds identified from the biotransformation of resveratrol and pterostilbene with the secretome of *B. cinerea* in 50% MeOH/H_2O and/or from the biotransformation of pterostilbene in 2% of acetone.

Compound **4** was isolated as an amorphous solid. The HRMS spectrum showed a deprotonated formic acid adduct ion at *m/z* 591.2253 $[M+FA-H]^-$, (calcd. for $C_{33}H_{35}O_{10}$, 591.2230, $\Delta = 3.9$ ppm). The ^1H NMR spectrum presented 14 aromatic protons, distributed over four aromatic rings according to the COSY spectrum. This corresponded to the coupling of either two pterostilbene units, two resveratrol units, or a pterostilbene and a resveratrol unit. The ^1H NMR and COSY spectra showed the sequence of four methine protons at δ_H 3.75 (1H, d, J = 12.8 Hz, H-7), 4.06 (1H, dd, J = 12.8, 2.9 Hz, H-8), 3.00 (1H, dd, J = 9.4, 2.9 Hz, H-8') and 4.43 (1H, d, J = 9.4 Hz, H-7'). The HMBC correlations from H-2/H-6 and H-2'/H-6' (δ_H 6.88 and 7.20, respectively) to C-7 (δ_C 51.0), from H-8 to the aromatic carbons at δ_C 109.3 (C-10/C-14) and from H-8' to the aromatic carbons at δ_C 108.1 (C-10'/C-14') allowed the

positioning of all the aromatic rings (Figure 3). The HMBC spectrum also showed correlations between the two methoxy groups at δ_H 3.57 (6H, s, CH_3O-11', CH_3O-13') and the aromatic carbon at δ_C 158.8 (C-11'/C-13') and between the two remaining methoxy groups at δ_H 2.84 and 3.36 and the carbon at δ_C 104.2 suggesting that the first two methoxy group belong to a pterostilbene unit whereas the last two methoxy groups were solvent (MeOH) related and were attached to C-7'. The small coupling constant between H-8 and H-8' ($^3J_{H,H}$ = 2.9 Hz) and the NOESY correlations between H-7 and H-8', H-8 and H-8', H-7' and H-8, and between H-7' and H-10/H-14 allowed the assignment of the relative configuration at C-8, C-8' to be 8*R*,8'*R* or 8*S*,8'*S* (Figure 3). Comparison of the TDDFT calculated ECD spectra of these two possible stereoisomers (8'*S*,8*S* and 8'*R*,8*R*) (Figure 3) with the experimental data of **4**, indicated that the most probable configuration of **4** was (8'*S*,8*S*). Based on the HRMS and NMR data, resvepterodimer A (**4**) was established as a new heterodimer comprised of pterostilbene and resveratrol.

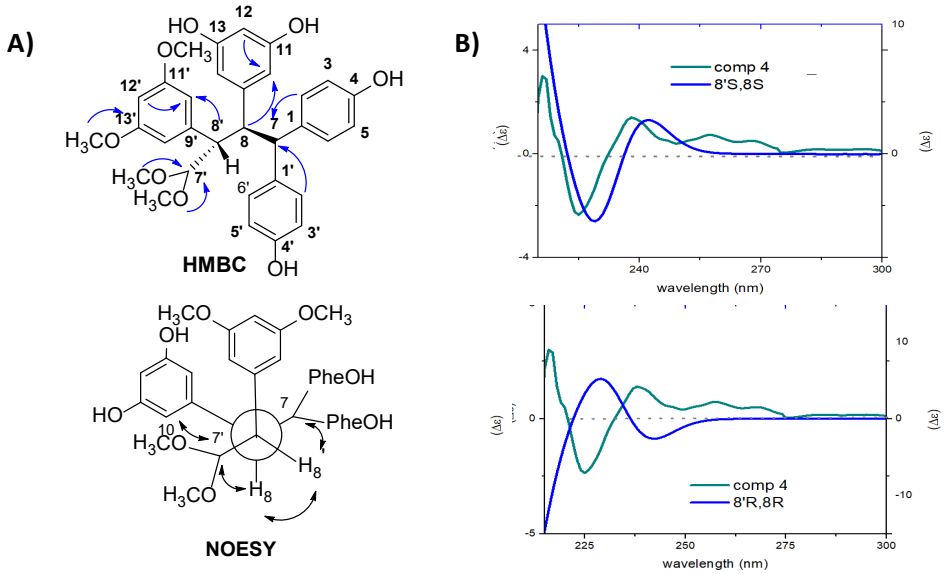

Figure 3. A) HMBC and NOESY correlations observed for compound **4**. B) Experimental (green) and TDDF calculated (blue) ECD spectra of the two enantiomers (8'*S*,8*S* and 8'*R*,8*R*) of compound **4** at the CAM-B3LYP/6-31G** level.

The HRMS data of **5** showed a deprotonated formic acid adduct ion at *m/z* 559.1995 [M+FA-H]$^-$, (calcd. for $C_{32}H_{31}O_9$, 591.1968, Δ = 4.8 ppm). The NMR data were closely related to those of resvepterol A, previously isolated from the biotransformation of pterostilbene,[12] except for the presence of one additional methoxy group at δ_H 2.97 (3H, s, CH_3O-7). This was in good agreement with the molecular formula of **5** which corresponded to the coupling of a resveratrol and a pterostilbene unit with an additional methoxy group. The position of this methoxy at C-7 is shown by its HMBC correlation with C-7 at δ_C 86.6. The NOESY correlations and the chemical shifts of H-14 (deshielded) and H-8', H-10'/H-14' (shielded) being very similar to those of resvepterol A, leachianol F, 11,11',13,13'-tetra-*O*-methylleachianol F and parthenostilbenin B, the configuration of which was deduced as the same as these compounds.[12] Compound 7-*O*-methylresvepterol B (**5**) was identified as a new *O*-methyl derivative of leachianol F.

Compound **7** had the same molecular formula as **5**, suggesting that this compound is also composed by the coupling of resveratrol and pterostilbene moieties with an additional methoxy group. The NMR data of **7** showed high similarity with those of **5**, and confirmed this hypothesis. While in **5** the two methoxy groups positioned at C-11' and C-13" were equivalent (δ_H 3.57), they were not equivalent in **7** (δ_H 3.58 and 3.79) because they were linked to C-11 and C-13. This is confirmed by the dipolar interaction from H-12 to CH_3O-11 and CH_3O-13 and from H-14 to CH_3O-13. The configuration of **7** was determined to be the same as **5** (7'*S*,8'*R*,7*R*,8*S*). The ECD calculations for this configuration were also in good agreement with the experimental ECD spectrum (Figure S2, Supporting Information). 7-*O*-methylisoresvepterol B (**7**) was identified as a new *O*-methyl derivative of leachianol F.[12]

Compound **8** showed the same molecular formula as **5** and **7**. The NMR data of **8** were close to those of **5** with one methoxy signal at δ_H 3.66 for the two methoxy groups at C-11' and C-13'. However, the NOESY correlations and the chemical shifts of H-14 (shielded) and H-8', H-

10'/H-14' (deshielded) were closed to those of resvepterol B, leachianol G, 11,11',13,13'-tetra-*O*-methylleachianol G and parthenostilbenin A, indicating that the C-7 configuration was (7*S*). This was also confirmed by ECD calculations (Figure S2, Supporting Information). 7-*O*-Methylresvepterol A (**8**) was identified as the new *O*-methyl derivative of leachianol G.[12]

The NMR data of compound **9** showed close similarity to those of **4** with an additional singlet at δ_H 3.53 integrating for 6 methoxy group hydrogens. The HRMS data of **9** (*m/z* 619.2560) confirmed the presence of two additional methoxy groups. The HMBC correlation from this singlet and the triplet at δ_H 6.16 (H-12) with the aromatic carbons at δ_C 159.3 and 159.6 (C-11/C-13) confirmed the position of these methoxy groups on the second trisubstituted aromatic unit. The small coupling constant between H-8 and H-8' ($^3J_{8,8'}$ = 2.8 Hz) and the NOESY correlations between H-7 and H-8', H-8 and H-8', and between H-7' and H-7 permitted assignment of the relative configurations of C-8, C-8' as (8*R*,8'*S*) or (8*S*,8'*R*). The other NMR data were identical to those of **4**. Pterodimer C (**9**) was thus identified as a new dimeric form of pterostilbene.

The HRMS data of **10** displayed an $[M+FA-H]^-$ ion at *m/z* 559.1981, which is in agreement with the molecular formula $C_{32}H_{31}O_9$ (calcd for $C_{32}H_{31}O_9$, 559.1968, Δ = 2.3 ppm). Based on this information, **10** is also formed by the coupling of a resveratrol and a pterostilbene moiety with an extra methoxy group. The NMR data were closely related to those of the known pterostilbene acyclic dimer (*E*)-4-{2-(3,5-dimethoxyphenyl)-2-[4-(3,5-dimethoxystyryl)phenoxy]-1-hydroxyethyl}phenol (**20**),[25] except for the presence of a methoxy group at C-7' (δ_H 3.15) and the absence of two methoxy group at C-11 and C-13. The coupling constant between H-7' and H-8' ($^3J_{7',8'}$ = 6.8 Hz) and the NOESY correlations between H-7' and H-8', H7' and H-10'/14', and H-8' and H-2'/6' indicated that the configurations at C-7', C-8' was (7'*S*,8'*S*) or (7'*R*,8'*R*). The experimental ECD spectrum did not allow differentiation of these two enantiomers, probably because they comprised a scalemic mixture as indicated by the near-

zero specific rotation ($[\alpha]^{23}_{D}$ -1.3). Compound **10** was identified as a 7'-*O*-methylresveptero acyclic dimer named (*E*)-5-{4-[1-(3,5-dimethoxyphenyl)-2-(4-hydroxyphenyl)-2-methoxyethoxy]styryl}benzene-1,3-diol.

Compound **12** had the same molecular formula as **10** and close NMR data. The difference between **10** and **12** was due to the position of the methoxy groups at C-11' and C-13' for **10** and at C-11 and C-13 for **12**. The coupling constant between H-7' and H-8' ($^{3}J_{7',8'}$ = 6.6 Hz) and the NOESY correlations being the same as for **10**, the configuration was established as (7'*S*,8'*S*) or (7'*R*,8'*R*). As for **10**, the experimental ECD spectrum did not allow discrimination of these two enantiomers probably because it was a racemic mixture. Compound **12** was identified as (*E*)-5-{1-[4-(3,5-dimethoxystyryl)phenoxy]-2-(4-hydroxyphenyl)-2-methoxyethyl}benzene-1,3-diol (**12**).

The NMR data of **13** were closely related to that of 11,11',13,13'-tetra-*O*-methylleachianol F,[12] except for the presence of an additional methoxy group at δ_H 3.00 (3H, s, CH_3O-7) which was in good agreement with such substitution ($C_{34}H_{35}O_9$ ($[M+FA-H]^-$)). The NOESY correlations, coupling constants, and the chemical shifts of H-14 (deshielded) and H-8', H-10'/H-14' (shielded) were similar to those of resvepterol A, leachianol F, 11,11',13,13'- tetra-*O*-methylleachianol F, and parthenostilbenin B.[12] The experimental and calculated ECD spectra confirmed the (7'*S*,8'*R*,8*R*,7*R*) absolute configuration of **13** (Figure S2, Supporting Information). Based on the HRMS and NMR data, **13** was identified as the new *O*-methyl derivative of leachianol F and named 7,11,11',13,13'-penta-*O*-methylleachianol F (**13**).

The biotransformation of the mixture of resveratrol and pterostilbene using 50% MeOH as solvent afforded a series of dimeric methoxylated compounds. As expected, biotransformations with the secretome of *B. cinerea* generated a wide diversity of stilbene analogues. All compounds seem to be obtained by dimerization of the stilbene units through a coupling of phenoxy radicals probably generated by oxidation via laccases or peroxidases present in the

fungal secretome.[26, 27] The occurrence of the methoxylated derivatives could be explained by the use of MeOH as a solvent. A mechanism for these chemical reactions is proposed in Figures S5 and S6 (Supporting Information). This is the first demonstration that *B. cinerea* enzymes can remain operational even in high concentrations of organic solvents.

The use of MeOH as a co-solvent in biotransformation reactions did not appear to increase the overall reaction efficiency (sum of the mass of all compounds obtained). However, the number of derivatives obtained was slightly higher in the reaction with 50% MeOH (15 compounds, total yield of 63%) compared to the reaction in 2% of acetone (12 compounds, total yield of 58%). The number of compounds obtained for the "leachianol" series (compounds **2**, **3**, **5**, **7**, **8**, and **13**) was higher in the reaction with 50% MeOH while the compound pallidol **1** (1 mg) was obtained in much smaller quantities compared to the reaction in 2% of acetone (17.4 mg). This can be explained by the fact that the methoxylation of leachianol at C-7 blocks the formation of pallidol (Figure S5, Supporting Information).

All derivatives obtained (**1**-**15**) together with the compounds obtained from our previous study (**16**-**20**)[12] were tested against the Gram-negative microorganism *Pseudomonas aeruginosa* (ATCC 27853) and Gram-positive methicillin-resistant of *Staphylococcus aureus* (MRSA, ATCC 33591) strains (Table 1). No activity was observed against *P. aeruginosa* at 256 µg/mL, the maximum concentration tested (data not shown).

The antibacterial activity of the initial compound used for the biotransformation, resveratrol (MIC > 561 µM), was considered as not active while pterostilbene showed a moderate activity against the wild strain of *S. aureus* (MIC of 31 µM). These results confirmed the antibacterial properties already described for pterostilbene.[28]

Table 1. Minimum Inhibitory Concentration (MIC) of the Isolates Against the Methicillin (MRSA 33591) and Vancomycin (VRSA 510) resistant strains of *Staphylococcus aureus*.

Compounds	*S. aureus* MRSA ATCC 33591 (μM)	*S. aureus* VRSA 510 (μM)
Resveratrol	>561	-
Pterostilbene	31	-
1	>282	-
2	>263	-
3	>256	-
4	>234	-
5	>249	-
6	35	-
7	>249	-
8	>249	-
9	14	-
10	62	-
11	4	4
12	8	4
13	15	-
14	2	2
15	>251	-
16	31	-
17	121	-
18	121	-
19	121	-
20	>242	-
Vancomycin[Ref.]	0.69	22

- = Not tested. Ref.= reference compound.

Compounds **11**, **12**, and **14** showed significant antibacterial activities against *S. aureus* MRSA strain with a minimum inhibitory concentration (MIC) of 4, 8 and 2 μM, respectively. It was also interesting to observe that **6**, **9**, **13**, and **16** also exhibited antibacterial activities against the MRSA strain with MIC values of 14 to 35 μM, while compounds **10**, **17**, **18**, and **19** showed moderate activity against both strains with MIC values between 32 to 121 μM.

The three most active compounds (**11**, **12**, and **14**) against MRSA were selected and tested against vancomycin-resistant strains of *S. aureus* (VRSA 510).[29] All compounds showed remarkable activities with MIC values of 4, 4 and 2 μg/mL, respectively.

To check if the active compounds showed a selective antibacterial activity, they were additionally submitted to a general cytotoxicity assay. This was performed against human liver carcinoma cells (HepG2) and a human breast epithelial cell line (MCF10A) (Table 2). These assays revealed that the most active compounds **11**, **12**, and **14** were cytotoxic but in higher

concentration when compared to those used for the antibacterial activity. For the most active compounds **11** and **12** the selectivity index, defined as a ratio of cytotoxic IC_{50} against MCF10A cell line to MIC of MRSA or VRSA strains, was low (SI=7.2 and 3.4, respectively) while it was moderate for **14** (SI=32).

Table 2. Cytotoxic Activity of the Derivatives Against Human Liver Carcinoma Cells (HepG2) and Human Breast Epithelial Cell Line (MCF10A) and the Selectivity Indices.

Compounds	IC_{50} cytotoxicity against HepG2 cells (μM)	IC_{50} cytotoxicity against MCF10A cells (μM)	Selectivity Index-SI (MCF10A vs MRSA activity)	Selectivity Index-SI (MCF10A vs VRSA activity)
6	48±9	48±2	1.4	NA
7	NT	NT	0	NA
8	NT	NT	0	NA
9	19±2	19±2	1.4	NA
10	29±4	23±2	0.4	NA
11	27±2	29±2	7.2	7.2
12	23±2	27±2	3.4	6.9
13	15±1	17±1	1.1	NA
14	27±4	66±8	32.0	32.0
15	29±2	25±2	<0.1	NA
16	22±2	24±6	0.7	NA
17	>95	>95	NA	NA
Doxorubicin*	1.18±0.13	0.31±0.07	NA	NA

*reference compound. NA = Not available.

Previous studies reported antimicrobial effects of ε-viniferins against fungi and bacteria.[30-33] This study is the first one that demonstrates that δ-viniferin derivatives show strong antibacterial activities against vancomycin and methicillin-resistant strains of *S. aureus.*

In the same way, the presence of methoxy groups is crucial for the antibacterial effect. For example, **16** exhibited activity against the methicillin-resistant strain of *S. aureus* (MIC 31 μM), while its analogue **1** (pallidol), was inactive and this is in good agreement with previous results for pallidol.[34]

The linear type dimers **10**, **12**, and **20** exhibit particular structural features that modulate their antibacterial properties. Compound **12** obtained from the dimerization of pterostilbene and

resveratrol, possessing three methoxy and three hydroxy groups was the most active compound with a MIC value of 8 μM. When this dimerization occurs in the opposite way (pterostilbene at C-8'), the activity decreases as observed for **10**, MIC of 62 μM. As already observed for other dimers, **20** obtained from dimerization of pterostilbene was inactive (MIC > 242 μM).

Similarly, the addition of *O*-methyl groups to δ-viniferin, in the case of **11** and **14** greatly increases the antibacterial activity. These dimers of pterostilbene and resveratrol possessed two methoxy and three hydroxy groups and were the most active compounds with MIC values of 4 and 2 μM, respectively while δ-viniferin (**6**) having four free hydroxy groups had a MIC of 35 μM. Recently, it was reported that δ-viniferin displayed antimicrobial activity against Gram-positive bacteria, including *S. aureus* with a MIC value of 62.0 μM.[34] This compound acts as membrane disrupter and in the inhibition of DNA synthesis.[34] On the other hand, the completely methylated derivative of δ-viniferin (**15**) was found to be inactive (MIC >242 μM) suggesting that a too high degree of methoxylation is not favorable for the antibacterial activity. Further investigations are underway to establish the antibacterial mechanism of action of the most active compounds **11**, **12**, and **14**. As shown the approach developed has generated a large and diverse series of analogues and additional chemical modifications on such scaffolds worth to be performed for finding compounds with even better activities and selectivity indices.

EXPERIMENTAL SECTION

General Experimental Procedures. The optical rotations were measured in MeOH on a JASCO P-1030 polarimeter (Loveland, CO, USA) in a 1 cm tube. UV spectra were measured on a HACH UV-vis DR/4000 instrument (Loveland, CO, USA). The ECD spectra were recorded on a JASCO J-815 spectrometer (Loveland, CO, USA) in MeCN. NMR data were recorded on a Bruker Avance III HD 600 MHz NMR spectrometer equipped with a QCI 5mm Cryoprobe and a SampleJet automated sample changer (Bruker BioSpin, Rheinstetten,

Germany). Chemical shifts are reported in parts per million (δ) using the residual DMSO-d_6 signal (δ_H 2.50; δ_C 39.5) as internal standards for ^{1}H and ^{13}C NMR, respectively, and coupling constants (*J*) are reported in Hz. Complete assignments were obtained based on 2D NMR experiments (COSY, NOESY, HSQC, and HMBC). HRMS data were obtained on a Micromass-LCT Premier TOF mass spectrometer with an electrospray (ESI) interface (Waters, Milford, MA, USA). The biotransformation reactions were controlled on a multidetection UHPLC-PDA-ELSD-MS (Waters, Milford, MA, USA) platform fit with on a single quadrupole detector (QDa) using heated electrospray ionization. Analytical HPLC was carried out on an HP 1260 Agilent system equipped with a photodiode array detector (Agilent technologies, Santa Clara, CA, USA). Semi-preparative HPLC was performed on a modular semi-preparative HPLC-ELSD-UV-MS system (Puriflash-MS 4250, Interchim, Montluçon, France) equipped with a quaternary pump, a UV detector module, a fraction collector and an ESI-single-quadrupole mass spectrometer (Advion, Ithaca, NY, USA). The split system consisted of a make-up pump combined to a dilution pump (Dynamic split, Interchim, Montluçon, France). Resveratrol (99% purity) and pterostilbene (99% purity) were purchased from Hangzhou Apichem Technology Co. Ltd. (Hangzhou, Zhejiang, China).

Secretome Isolation from *Botrytis cinerea* Culture. The *Botrytis cinerea* Pers.:Fr., isolate K16 was obtained from naturally sporulation grape berries from the Changins Agroscope experimental vineyards in 2015. The strain was purified, determined phenotypically as well as by molecular tools (sequencing of the ITS regions), and maintained by regular transplanting. The fungus was grown on oatmeal agar medium (OMA, Difco), conidia are sampled by vacuum aspiration and stored dry at -80 °C until use.[35] *B. cinerea* was cultivated in 1.5 L liquid medium (in 5L bottles) at 22°C under alternating light and dark (12h/12h). The medium was filtrated through a double layer of folded filter paper (diameter 500 mm, Prat Dumas, France). The filtrate was brought to 80% saturation [$(NH_4)_2SO_4$, 24 hours at 4 °C] and centrifuged (4200 x

g, 4 °C, 2h). The supernatant was discarded and the resulting pellet was solubilized in nanopure water (Evoqua water technologies, 4.21 µS cm^{-1}) (the protein crude extract) and submitted to a dialysis (Spectra/Por 1 dialysis membrane, 6-8 kD, diameter 14.6 mm) against nanopure water overnight at 4 °C. The resulting protein extract was concentrated against polyethylene glycol beads (PEG 20'000) in the dialyzed tube. The protein content was determined by the method of Bradford, using a Bio-Rad protein assay kit with BSA as a standard.[36] The volume of the extract was adjusted to obtain a final protein concentration of 2 µg/µL. The resulting extract (considered as secretome) was aliquoted to 1 mL and stored at -20 °C until use.

Biotransformation of the Mixture of Resveratrol/Pterostilbene at Analytical Scale. The biotransformations of the mixture of resveratrol and pterostilbene at analytical scale were performed in Eppendorf tubes using a total concentration of 0.5 mg/mL (0.25 mg/mL of each compound). For every condition (2-98% of acetone or MeOH), the total volume was kept constant at 1 mL as well as the *B. cinerea* secretome percentage at 2%. The only variable parameter was the percentage of organic solvent, which was increased from 2 to 98%. First, a stock solution with a concentration of 20 mg/mL (10 mg/mL of each compound) was prepared in acetone. 25 µL of this solution (0.5 mg of total mass) was placed in each Eppendorf tube and the solvent was evaporated to give a dry deposit. The required volume of organic solvent was added (from 20 µL for 2% to 980 µL for 98%) and the volume was adjusted to 980 µL with water (from 960 µL for 2% to 0 µL for 98%). The 20 µL (2%) of secretome was added at the end and are considered for our calculations as a volume of water. The composition of each reaction mixture is described in Table S1, Supporting Information. Once prepared, the mixtures were incubated at 22 °C in the dark under constant agitation. Reactions were stopped after 24 hours by drying under vacuum in a centrifugal evaporator and analysed by UHPLC-PDA-ELSD-MS.

UHPLC-PDA-ELSD-MS Analysis of the Crude Biotransformation Reactions. Aliquots (0.25 mL) of the biotransformed reactions were analyzed by UHPLC-PDA-ELSD-MS. ESI conditions were as follows: capillary voltage 800 V, cone voltage 15 V, source temperature 120 °C, probe temperature 600 °C. Detection was performed in negative ion mode (NI) with *m/z* range of 150-1000 Da. The separation was done on an Acquity UPLC BEH C_{18} column (50 x 2.1 mm i.d., 1.7 µm; Waters, Milford, MA, USA) using a linear gradient (0.6 mL/min, 40°C) with H_2O (A) and MeCN (B) both containing 0.1% formic acid with the following gradient: from 5 to 100% of B from 0 to 7 min, 1 min at 100% B and a re-equilibration step of 2 min. The ELSD was set at 45°C, with a gain of 9. The PDA data were acquired in the range from 190 to 500 nm, with a resolution of 1.2 nm. Sampling rate was set at 20 points/sec.

UHPLC-HRMS Analysis of the Pure Compounds. Aliquots (0.1 mL) of isolated compounds were analyzed by UHPLC-TOF-ESI-HRMS using the followed conditions: ESI conditions were as follows: capillary voltage 2400 V, cone voltage 40 V, MCP detector voltage 2500 V, source temperature 120 °C, desolvation temperature 300 °C, cone gas flow 20 L/h, and desolvation gas flow 700 L/h. Detection was performed in negative ion mode (NI) with *m/z* range of 100–1300 Da and a scan time of 0.5 s in the W-mode. The MS was calibrated using sodium formate, and leucine encephalin (Sigma-Aldrich, Steinheim, Germany) was used as an internal reference at 2 µg/mL and infused through a Lock Spray™ probe at a flow rate of 10 µL/min with the help of a second LC pump. The separation was performed on an Acquity UPLC BEH C_{18} column (150 x 2.1 mm i.d., 1.7 µm; Waters, Milford, MA, USA) using a linear gradient (solvent system: A) 0.1% formic acid-H_2O, B) 0.1% formic acid-MeCN; gradient: 5-95% B in 30 min, then 95% B for 10 min re-equilibration step of 10 min at 5% B; flow rate 0.46 mL/min). The temperature was set to 40 °C. The injected volume was 2 µL.

Large Scale Biotransformation of the Mixture of Pterostilbene/Resveratrol Purification of the Crude Reaction Mixture by Semi-preparative HPLC-UV-ELSD-MS. The

biotransformation of pterostilbene and resveratrol was performed using 50 mg of each compound. First, the two compounds were solubilized in 100 mL of MeOH. This solution was diluted in 90 mL of water and 10 mL of the enzymatic extract was added. This mixture was incubated for 24 hours at 22°C in the dark under constant gentle agitation. After evaporation of the MeOH under vacuum with a rotary evaporator, the solution was lyophilized to give 107.5 mg of extract. This crude mixture was purified by semi-preparative HPLC-UV-MS. Waters X-Bridge C_{18} column (250 x 4.6 mm i.d., 5 µm; Waters, Milford, MA, USA) equipped with a Waters C_{18} pre-column cartridge holder (10 x 2.1 mm i.d.); solvent system MeOH (B) and H_2O (A), both containing 0.1% of formic acid. The column was equilibrated with 20% of B for 15 min. The separation was performed in the gradient mode as follows: 20% of B for 10 min, followed by a gradient of 20 to 100% of B in 70 min. Flow rate 1 mL/min; injection volume 10 µL; sample concentration 10 mg/mL in MeOH. The UV absorbance was measured at 254 nm and the UV-PDA spectra were recorded between 190 and 600 nm (step 2 nm). The optimized HPLC analytical conditions were geometrically transferred by gradient transfer to the semi-preparative HPLC scale.[20] The crude reaction extract (100 mg) was fractionated using reverse-phase semi-preparative HPLC with a Waters X-Bridge C_{18} column (250 x 19 mm i.d., 5 µm) equipped with a Waters C_{18} pre-column cartridge holder (10 x 19 mm i.d.). The separation was performed with a gradient of MeOH (B) and H_2O (A) with 0.1% formic acid. The method started with 20% of B for 10 min, followed by a gradient of 20 to 100% of B in 70 min. The flow rate was set to 17 mL/min, and UV detection 210, 254, and 366 nm. The ESI-MS conditions were as follows: capillary voltage, 180 V; capillary temperature, 250°C; source voltage offset, 30 kV; source voltage span, 30 kV; source gas temperature, 280°C. The isolation was followed in the ESIMS negative ion mode in the 400-600 amu range with an acquisition time of 1 s. The MS-detection of specific extracted ion chromatogram (XIC) was acquired over three *m/z* ranges: 531-532, 559-560, and 587-588. The conditions for the split system were as

follows: the flow rate of the make-up pump was fixed at 0.1 mL/min, the dilution pump at 0.05 mL/min, and the valve rotation period at 1.5 sec. Both pumps delivered MeCN with 0.1% formic acid. The extract (100 mg) was introduced on the semi-preparative HPLC column using a dry load methodology recently developed in our laboratory.[22] The separation yielded 120 fractions (12 mL each). The semi-preparative HPLC fractions F34 and F35 afforded **1** (1 mg), F38-F40 afforded **2** (9.1 mg), F54 **3** (1.9 mg), F55 **4** (1.1 mg), F60 and F61 **5** (8.7 mg), F63 and F64 **6** (10.2 mg), F67 **7** (1.3 mg), F69 and F70 **8** (11.1 mg), F72 **9** (1.7 mg), F79 and F80 **10** (3.1 mg), F85 **11** (1.5 mg), F87 **12** (1.8 mg), F89 **13** (2.5 mg), F91 and F92 **14** (2.9 mg), and F111 and F112 afforded **15** (5.3 mg).

*Resvepterodimer A (**4**).* Amorphous solid; $[\alpha]^{23}_{D}$ +18 (*c* 0.02, MeCN); UV (MeOH) λ_{max} (log ε) 216 (sh) (4.38), 277 (3.27) nm; ECD (c = 9.2 × 10^{-5} M, MeCN) $[\theta]_{210}$ -4.7, $[\theta]_{214}$ +0.8, $[\theta]_{225}$ -0.5, $[\theta]_{239}$ +0.3; ^{1}H NMR (DMSO-*d*$_6$, 600 MHz) δ 7.20 (2H, d, *J*=8.4 Hz, H-2', 6'), 6.88 (2H, d, *J*=8.6 Hz, H-2, 6), 6.74 (2H, d, *J*=8.4 Hz, H-3', 5'), 6.37 (2H, d, *J*=8.6 Hz, H-3, 5), 6.28 (1H, t, *J*=2.3 Hz, H-12'), 5.88 (1H, t, *J*=2.2 Hz, H-12), 5.60 (2H, brs, H-10', 14'), 5.38 (1H, brs, H-10, 14), 4.43 (1H, d, *J*=9.4 Hz, H-7'), 4.06 (1H, dd, J=12.8, 2.9 Hz, H-8), 3.75 (1H, d, J=12.8 Hz, H-7), 3.57 (6H, s, CH_3O-11', CH_3O-13'), 3.36 (3H, s, CH_3O-7'a), 3.00 (1H, dd, J=9.4, 2.9 Hz, H-8'), 2.84 (3H, s, CH_3O-7'b); ^{13}C NMR (DMSO-*d*$_6$, 151 MHz) δ 158.8 (C-11', 13'), 156.6 (C-11, 13), 155.5 (C-4'), 154.5 (C-4), 140.4 (C-9), 139.3 (C-9'), 135.5 (C-1), 135.2 (C-1'), 128.9 (C-2', 6'), 128.3 (C-2, 6), 115.3 (C-3', 5'), 114.7 (C-3, 5), 109.3 (C-10, 14), 108.1 (C-10', 14'), 104.2 (C-7'), 100.4 (C-12), 99.3 (C-12'), 54.6 (CH_3O-11', CH_3O-13'), 52.5 (CH_3O-7'b), 52.3 (CH_3O-7'a), 51.0 (C-7), 48.2 (C-8'), 47.2 (C-8); HRESIMS *m/z* 591.2253 $[M+FA-H]^-$ (calcd for $C_{33}H_{35}O_{10}$, 591.2230, Δ = 3.9 ppm).

*7-O-Methylresvepterol B (**5**).* Amorphous solid; $[\alpha]^{23}_{D}$ -5 (*c* 0.1, MeCN); UV (MeOH) λ_{max} (log ε) 223 (sh) (4.05), 283 (3.41), 306 (3.17) nm; ^{1}H NMR (DMSO-*d*$_6$, 600 MHz) δ 6.67 (2H, d, *J*=8.5 Hz, H-2', 6'), 6.66 (2H, d, *J*=8.5 Hz, H-2, 6), 6.62 (2H, d, *J*=8.5 Hz, H-3', 5'), 6.59 (2H,

d, *J*=8.5 Hz, H-3, 5), 6.45 (1H, d, *J*=2.0 Hz, H-14), 6.20 (1H, t, *J*=2.2 Hz, H-12'), 6.17 (1H, d, *J*=2.0 Hz, H-12), 5.72 (2H, d, *J*=2.2 Hz, H-10', 14'), 4.05 (1H, d, *J*=3.5 Hz, H-7'), 3.82 (1H, d, *J*=9.1 Hz, H-7), 3.57 (6H, s, CH_3O-13', CH_3O-11'), 3.15 (1H, dd, *J*=9.1, 3.5 Hz ,H- 8), 2.97 (3H, s, CH_3O-7), 2.67 (1H, t, *J*=3.5 Hz, H-8'); ^{13}C NMR (DMSO-d_6, 151 MHz) δ 160.1 (C-11', 13'), 157.8 (C-13), 156.8 (C-4), 155.2 (C-4'), 154.0 (C-11), 149.3 (C-9'), 147.1 (C-9), 136.0 (C-1'), 130.3 (C-1), 128.7 (C-2, 6), 128.1 (C-2', 6'), 120.9 (C-10), 114.7 (C-3, 5), 114.7 (C-3', 5'), 104.5 (C-10', 14'), 104.4 (C-14), 101.5 (C-12), 97.2 (C-12'), 86.6 (C-7), 59.6 (C-8), 57.8 (C-8'), 55.9 (CH_3O-7), 54.8 (CH_3O-11', CH_3O-13'), 54.2 (C-7'); HRESIMS *m/z* 559.1995 $[M+FA-H]^-$ (calcd for $C_{32}H_{31}O_9$, 559.1968, Δ = 4.8 ppm).

7-O-Methylisoresvepterol B (7). Amorphous solid; $[\alpha]^{23}_D$ -2 (*c* 0.1, MeCN); UV (MeOH) λ_{max} (log ε) 223 (sh) (4.12), 283 (3.49) nm; ECD (c = 9.7 × 10−5 M, MeCN) $[\theta]_{212}$ -0.4, $[\theta]_{228}$ +0.3, $[\theta]_{262}$ -0.1; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 6.64 (1H, d, *J*=2.0 Hz, H-14), 6.62 (8H, m, H-2, 3, 5, 6, 2', 3', 5', 6'), 6.45 (1H, d, *J*=2.0 Hz, H-12), 5.93 (1H, t, *J*=2.2 Hz, H-12'), 5.59 (2H, d, *J*=2.2 Hz, H-10', 14'), 4.00 (1H, d, *J*=2.8 Hz, H-7'), 3.83 (1H, d, *J*=8.8 Hz, H-7), 3.79 (3H, s, CH_3O-13), 3.58 (3H, s, CH_3O-11), 3.21 (1H, dd, *J*=8.8, 2.8 Hz, H-8), 2.96 (3H, s, CH_3O-7), 2.67 (1H, t, *J*=2.8 Hz, H-8'); ^{13}C NMR (DMSO-d_6, 151 MHz) δ 160.3 (C-13), 158.1 (C-11', 13'), 156.7 (C-4), 156.4 (C-11), 155.2 (C-4'), 149.4 (C-9'), 147.3 (C-9), 135.8 (C-1'), 130.1 (C-1), 128.4 (C-2, 6), 127.8 (C-2', 6'), 123.9 (C-10), 114.8 (C-3, 5, 3', 5'), 104.2 (C-10', 14'), 103.0 (C-14), 100.2 (C-12'), 97.3 (C-12), 86.2 (C-7), 59.5 (C-8), 57.3 (C-8'), 56.1 (CH_3O-7), 55.2 (CH_3O-13), 55.1 (CH_3O-11), 54.8 (7'); HRESIMS *m/z* 559.1981 $[M+FA-H]^-$ (calcd for $C_{32}H_{31}O_9$, 559.1968, Δ = 2.3 ppm).

*7-O-Methylresvepterol A (**8**).* Amorphous solid; $[\alpha]^{23}_D$ +0.5 (*c* 0.6, MeCN); UV (MeOH) λ_{max} (log ε) 223 (sh) (4.06), 283 (3.30) nm; ECD (c = 9.7 × 10−5 M, MeCN) $[\theta]_{212}$ +1.4, $[\theta]_{229}$ -0.2, $[\theta]_{236}$ 0, $[\theta]_{247}$ -0.1; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 6.92 (2H, d, *J*=8.5 Hz, H-2, 6), 6.71 (2H, d, *J*=8.6 Hz, H-2', 6'), 6.67 (2H, d, *J*=8.5 Hz, H-3, 5), 6.60 (2H, d, *J*=8.6 Hz, H-3', 5'), 6.29 (1H,

t, *J*=2.3 Hz, H-12'), 6.12 (2H, d, *J*=2.3 Hz, H-10', 14'), 6.07 (1H, d, *J*=2.1 Hz, H-12), 5.57 (1H, d, *J*=2.1 Hz, H-14), 4.04 (1H, d, *J*=3.2 Hz, H-7'), 3.83 (1H, d, *J*=7.3 Hz, H-7), 3.66 (6H, s, CH_3O-13', CH_3O-11'), 3.30 (2H, m, H-8, 8'), 2.86 (3H, s, CH_3O-7); ^{13}C NMR (DMSO-d_6, 151 MHz) δ 160.3 (C-11', 13'), 157.4 (C-13), 156.8 (C-4), 155.1 (C-4'), 154.1 (C-11), 149.6 (C-9'), 145.3 (C-9), 136.3 (C-1'), 129.8 (C-1), 129.1 (C-2, 6), 128.1 (C-2', 6'), 121.4 (C-10), 114.7 (C-3, 5), 114.6 (C-3', 5'), 105.0 (C-10', 14'), 103.8 (C-14), 101.5 (C-12), 97.2 (C-12'), 85.6 (C-7), 59.4 (C-8), 57.7 (C-8'), 55.8 (CH_3O-7), 55.0 (CH_3O-11', CH_3O-13'), 54.7 (C-7'); HRESIMS *m/z* 559.1979 $[M+FA-H]^-$ (calcd for $C_{32}H_{31}O_9$, 559.1968, Δ = 2.0 ppm).

*Pterodimer C (**9**).* Amorphous solid; UV (MeOH) λ_{max} (log ε) 226 (sh) (4.72), 280 (4.19), 324 (4.00) nm; ^1H NMR (DMSO-d_6, 600 MHz) δ 7.27 (2H, d, *J*=8.3 Hz, H-2', 6'), 6.93 (2H, d, *J*=8.8 Hz, H-2, 6), 6.76 (2H, d, *J*=8.3 Hz, H-3', 5'), 6.35 (2H, d, *J*=8.8 Hz, H-3, 5), 6.32 (1H, t, *J*=2.3 Hz, H-12'), 6.16 (1H, t, *J*=2.3 Hz, H-12), 5.60 (2H, brs, H-10', 14'), 5.53 (2H, brs, H-10, 14), 4.40 (1H, d, *J*=9.4 Hz, H-7'), 4.19 (1H, dd, *J*=12.8, 2.8 Hz, H-8), 3.85 (1H, d, *J*=12.8 Hz, H-7), 3.58 (6H, s, CH_3O-11', CH_3O-13'), 3.53 (6H, s, CH_3O-11, CH_3O-13), 3.39 (3H, s, CH_3O-7'a), 3.07 (1H, dd, *J*=9.4, 2.8 Hz, H-8'), 2.87 (3H, s, CH_3O-7'b); ^{13}C NMR (DMSO-d_6, 151 MHz) δ 159.6 (C-11/13), 159.3 (C-11/13), 158.9 (C-11', 13'), 155.5 (C-4'), 154.5 (C-4), 141.0 (C-9/9'), 139.1 (C-9/9'), 135.4 (C-1), 134.9 (C-1'), 128.9 (C-2', 6'), 128.5 (C-2, 6), 115.4 (C-3', 5'), 114.7 (C-3, 5), 104.1 (C-7'), 99.0 (C-12'), 98.0 (C-12), 54.8 (CH_3O-11, CH_3O-13), 54.7 (CH_3O-11', CH_3O-13'), 52.9 (CH_3O-7'a), 52.5 (CH_3O-7'b), 51.0 (C-7), 48.2 (C-8'), 47.8 (C-8); HRESIMS *m/z* 619.2560 $[M+FA-H]^-$ (calcd for $C_{35}H_{39}O_{10}$, 619.2543, Δ = 2.7 ppm).

*(E)-5-{4-[1-(3,5-Dimethoxyphenyl)-2-(4-hydroxyphenyl)-2-methoxyethoxy]styryl}benzene-1,3-diol (**10**).* Amorphous solid; $[\alpha]^{23}_D$ -1 (*c* 0.1, MeCN); UV (MeOH) λ_{max} (log ε) 223 (sh) (4.24), 305 (3.61), 322 (3.59) , 285 (3.58) nm; ^1H NMR (DMSO-d_6, 600 MHz) δ 7.38 (2H, , d, *J*=8.6 Hz, H-2, 6), 7.00 (2H, d, *J*=8.5 Hz, H-2', 6'), 6.88 (2H, d, *J*=16.5 Hz, H-7), 6.88 (2H, d, *J*=8.6 Hz, H-3, 5), 6.82 (1H, d, *J*=16.5 Hz, H-8), 6.62 (2H, d, *J*=8.5 Hz, H-3', 5'), 6.36 (2H, d,

J=2.2 Hz, H-10, 14), 6.30 (2H, d, *J*=2.3 Hz, H-10', 14'), 6.23 (1H, t, *J*=2.3 Hz, H-12'), 6.10 (1H, t, *J*=2.2 Hz, H-12), 5.33 (1H, d, *J*=6.8 Hz, H-8'), 4.43 (1H, d, *J*=6.8 Hz, H-7'), 3.59 (6H, s, CH_3O-11', CH_3O-13'), 3.15 (3H, s, CH_3O-7'); ^{13}C NMR (DMSO-d_6, 151 MHz) δ 159.8 (C-11', 13'), 158.5 (C-11, 13), 157.4 (C-4), 156.8 (C-4'), 140.4 (C-9'), 139.0 (C-9), 129.7 (C-1), 129.1 (C-2', 6'), 127.9 (C-1'), 127.5 (C-2, 6), 127.4 (C-7), 126.7 (C-8), 116.0 (C-3, 5), 114.6 (C-3', 5'), 105.7 (C-10', 14'), 104.4 (C-10, 14), 101.9 (C-12), 99.0 (C-12'), 85.9 (C-7'), 82.3 (C-8'), 56.3 (CH_3O-7'), 55.0 (CH_3O-11', CH_3O-13'); HRESIMS *m/z* 559.1981 $[M+FA-H]^-$ (calcd for $C_{32}H_{31}O_9$, 559.1968, Δ = 2.3 ppm).

*(E)-5-{1-[4-(3,5-Dimethoxystyryl)phenoxy]-2-(4-hydroxyphenyl)-2-methoxyethyl}benzene-1,3-diol (**12**).* Amorphous solid; $[\alpha]^{23}_D$ -1 (*c* 0.1, MeCN); UV (MeOH) λ_{max} (log ε) 223 (sh) (4.24), 305 (3.80), 322 (3.79) , 285 (3.74) nm; ^1H NMR (DMSO-d_6, 600 MHz) δ 9.31 (1H, s, 4'OH), 9.05 (2H, s, 11'OH, 13'OH), 7.41 (2H, d, *J*=8.9 Hz, H-2, 6), 7.13 (1H, d, *J*=16.4 Hz, H-7), 7.00 (2H, d, *J*=8.5 Hz, H-2', 6'), 6.95 (1H, d, *J*=16.4 Hz, H-8), 6.86 (2H, d, *J*=8.9 Hz, H-3, 5), 6.70 (2H, d, *J*=2.3 Hz, H-10, 14), 6.62 (2H, d, *J*=8.5 Hz, H-3', 5'), 6.36 (1H, t, *J*=2.3 Hz, H-12), 6.05 (2H, d, *J*=2.2 Hz, H-14', 10'), 5.97 (1H, t, *J*=2.2 Hz, H-12'), 5.17 (1H, d, *J*=6.6 Hz, H-8'), 4.35 (1H, d, *J*=6.6 Hz, H-7'), 3.75 (6H, s, CH_3O-11, CH_3O-13), 3.13 (3H, s, CH_3O-7'); ^{13}C NMR (DMSO-d_6, 151 MHz) δ 160.6 (C-11, 13), 157.7 (C-4), 157.7 (C-11', 13'), 156.7 (C-4'), 140.1 (C-9'), 139.4 (C-9), 129.4 (C-1), 129.1 (C-2', 6'), 128.5 (C-7), 128.0 (C-1'), 127.6 (C-2, 6), 126.1 (C-8), 115.9 (C-3, 5), 114.6 (C-3', 5'), 105.7 (C-10', 14'), 104.1 (C-10, 14), 101.8 (C-12'), 99.5 (C-12), 86.0 (C-7'), 82.4 (C-8'), 56.4 (CH_3O-7'), 55.2 (CH_3O-11, CH_3O-13); HRESIMS *m/z* 559.1973 $[M+FA-H]^-$ (calcd for $C_{32}H_{31}O_9$, 559.1968, Δ = 0.9 ppm).

*7,11,11',13,13'-Penta-O-methylleachianol F (**13**).* Amorphous solid; $[\alpha]^{23}_D$ -0.3 (*c* 0.3, MeCN); UV (MeOH) λ_{max} (log ε) 223 (sh) (4.06), 283 (3.32) nm; ECD (c = 9.2 × 10−5 M, MeCN) [θ]214 +0.8, [θ]232 -0.1, [θ]255 +0.1; ^1H NMR (DMSO-d_6, 600 MHz) δ 6.70 (2H, m, H-2, 6), 6.69 (1H, d, *J*=2.2 Hz, H-14), 6.62 (6H, m, H-3, 5, 2', 3', 5', 6'), 6.46 (1H, d, *J*=2.2 Hz,

H-12), 6.21 (1H, t, *J*=2.3 Hz, H-12'), 5.72 (2H, d, *J*=2.3 Hz, H-10', 14'), 4.09 (1H, d, *J*=3.9 Hz, H-7'), 3.90 (1H, d, *J*=8.9 Hz, H-7), 3.78 (3H, s, CH_3O-13), 3.58 (6H, s, CH_3O-13', CH_3O-11'), 3.56 (3H, s, CH_3O-11), 3.26 (1H, dd, *J*=8.9, 3.9 Hz, H-8), 3.00 (3H, s, CH_3O-7), 2.74 (1H, t, *J*=3.9 Hz, 8'); ^{13}C NMR (DMSO-d_6, 151 MHz) δ 160.4 (C-11), 160.1 (C-11', 13'), 156.9 (C-4), 156.3 (C-13), 155.3 (C-4'), 148.9 (C-9'), 146.9 (C-9), 135.7 (C-1'), 130.0 (C-1), 128.7 (C-2, 6), 127.9 (C-2', 6'), 123.9 (C-10), 114.8 (C-3, 5), 114.8 (C-3', 5'), 104.5 (C-10', 14'), 102.9 (C-14), 97.5 (C-12), 97.3 (C-12'), 86.3 (C-7), 59.6 (C-8), 57.8 (C-8'), 56.0 (CH_3O-7), 55.2 (CH_3O-13), 55.1 (CH_3O-11), 54.8 (CH_3O-11', CH_3O-13'), 54.6 (C-7'); HRESIMS *m/z* 587.2258 $[M+FA-H]^-$ (calcd for $C_{34}H_{35}O_9$, 587.2281, Δ = -3.9 ppm).

Computational Details. Conformational analysis of compound **4** was carried out using Macro Model 9.1 software (Schrödinger, LLC, New York, USA) by applying the OPLS-2005 force field in H_2O. The selected conformers were subjected to geometrical optimization using DFT with the CAM-B3LYP functional and the 6-31G**basis-set as implemented with the Gaussian 09 program package.[37] Vibrational analysis was performed at the same level to confirm the stability of the minima. Time-dependent density function theory calculation at the TDDFT/CAM-B3LYP/6-31G** level in MeOH using the "self-consistent reaction field" method with the conductor-like polarizable calculation model was employed to calculate excitation energy (denoted by wavelength in nm) and rotatory strength in dipole velocity (Rvel) and dipole length (Rlen) forms. ECD curves were calculated based on rotatory strengths using a half bandwidth of 0.3 eV with SpecDis version 1.61.[38]

Antibacterial Assay. Methicillin-resistant *S. aureus* (MRSA, ATCC 33591) and vancomycin-resistant *S. aureus* (VRSA 510) were used for the antibacterial assay. The minimum inhibitory concentration (MIC) of the different compounds were determined in triplicate using the broth dilution method in 96-well microtiter plates as previously described (Wiegand et al., 2008). Briefly, compounds were suspended at 10.24 mg/mL in DMSO and

serially diluted in Mueller−Hinton broth (MHB, Oxoid). The maximum initial concentration used for this assay was 256 µg/mL for *P. aeruginosa* and 128 µg/mL for *S. aureus*. After incubation of 24h at 37°C, iodonitrotetrazolium chloride (INT, Sigma-Aldrich) was added to each well, as growth indicator, and incubated for several hours (Eloff, 1998). The highest dilution of a compound in which no growth appears corresponds to its MIC. Gentamicin sulfate (Applichem) and vancomycin hydrochloride (Sigma-Aldrich) were used as control of inhibition for *P. aeruginosa* and *S. aureus*, respectively, and compared to the reference values.[39-41]

Compound Cytotoxicity Test. 50 µL of indicated cell lines re-suspended at 60000 cells/mL were added into each well of a transparent 384-well plate. The cells were maintained in DMEM/F12 (MCF-10A) or low glucose MEM (HepG2) containing 10% FBS and after seeding incubated at 37°C, 5% CO_2 overnight. The next day medium of each well was replaced by 50 µL of fresh medium containing serial dilution of compounds starting from 50 µg/mL. After incubation for 3 days, the medium in each well was replaced by 50 µL of 0.5 mg/mL Thiazolyl blue (Carl Roth) solution in 1 x PBS. The plates were incubated for 3h at 37°C, the solution was removed, and 30 µL DMSO was added into each well. Absorbance at 570 nm was measured in the plate reader (Tecan Infinite MPlex). Selectivity index (SI) of a compound was calculated as a ratio between its IC_{50} for human breast epithelial MCF-10A line and MIC for and MRSA or VRSA.

ASSOCIATED CONTENT

Supporting Information

Figures S1−S54 showing UHPLC-PDA-ELSD-MS analysis of the biotransformation reactions, NMR and ECD spectra for the new compounds, and some reaction mechanism hypothesis. The raw data files for the UHPLC-PDA-ELSD-MS analysis of the biotransformation reactions,

NMR and HRMS data of the isolated compounds are available at the following link: DOI: 10.26037/yareta:ksyiya3d4nfy3n7iutqh6hwdia.

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Notes

The authors declare no competing financial interest.

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