Bioassay-Guided Investigation Of Inhibitors Of Pierce's Disease And Citrus Greening Disease

Connor A. Brandenburg[1], Jonathan W. Lockner[1], Katherine N. Maloney[1], Lane W. Votapka[1], Marc C. Perry[1], Claudia A. Castro[2], Alex A. Blacutt[2], M. Caroline Roper[2], Philippe E. Rolshausen[3], Elizabeth A. Costa[4]

[1]*Department of Chemistry, Point Loma Nazarene University, San Diego, CA, United States*

[2]*Department of Plant Pathology and Microbiology, University of California, Riverside, CA, United States*

[3]*Department of Botany and Plant Sciences, University of California, Riverside, CA, United States*

[4]*Pfizer Inc., La Jolla, CA, United States*

ABSTRACT: Pierce's Disease, threatening grapevines in California, and Citrus Greening Disease, plaguing commercial citrus trees in Florida, are caused by bacterial plant pathogens. *Xylella fastidiosa* and *Candidatus* liberibacter asiaticus (*C*Las) are the respective agents of these destructive diseases. Both reside in the plant vascular system, leading to dwarfed plants with dehydrated fruits that are ultimately worthless for the production of wine, juice, and oil, among other goods. If left untreated, these pathogens ultimately kill their host plants. Potent antibacterial agents, then, are desirable to prevent both the loss of these plants, and their products at market. Here, we explored the effectiveness of different compounds against *X. fastidiosa* and *Liberibacter crescens*, a culturable surrogate for *C*Las. Using an agar diffusion bioassay, we quantified the antibacterial activity of a variety of compounds, identified common structural motifs of these inhibitors, and presented possible modes of antibacterial action. Of the compounds tested, it was determined that Michael acceptors possess the greatest capacity for bacterial inhibition.

Bacterial plant pathogens of economically important crops pose a serious risk to the availability of such crops and their products, as well as to the income of their farmers. While the specifics of these diseases can often be very different, and range in discrepancies such as the mode of transmission to the mode of bacterial growth in planta, two bacteria in the modern-day United States share a remarkably similar story.

Pierce's Disease (PD), caused by the gram-negative bacterium *Xylella fastidiosa*, resides in the xylem of grapevines.[1] Citrus Greening Disease (also referred to as Huanglongbing; HLB) is caused by the gram-negative bacterium *Candidatus* liberibacter asiaticus (*C*Las), and resides in the phloem of citrus trees.[2] Both diseases restrict the flow of nutrients and water throughout infected plants, leading to slowed plant growth, browned and dead leaves, and dehydrated fruits. These important agricultural crops, then, become unusable and unprofitable for their cultivators. Further, when left untreated, PD and HLB ultimately kill their host plants.

Both infections are transmitted swiftly by invasive insect vectors; the glassy-winged sharpshooter is responsible for transmitting PD, and the Asian citrus psyllid disseminates HLB (Fig. 1). While PD has been observed in the southwestern United States, HLB is found in the southeastern United States.

Figure 1. The glassy-winged sharpshooter (at left) is responsible for spreading *X. fastidiosa*, while the Asian citrus psyllid (at right) transmits *C*Las.

Notably, in both diseases, it has been observed that disease-symptomatic plants live in close proximity to plants that are non-symptomatic (Fig. 2). All plants within a single orchard or vineyard are clonally propagated; thus, it can be inferred that a non-genetic factor is at work in minimizing symptoms of the diseases in plants that appear to be healthy. Previous work has showed that a natural product from fungi found in disease-escaped vines inhibits the causative bacterium.[1] This work suggested that the natural products produced by endophytic microorganisms might play a role in the health of the disease-escaped plants. Radicinin (**1**), a fungal metabolite originally isolated from *Alternaria radicina* in 1953 and reisolated from disease-escaped vines[3], has shown antibacterial activity against *X. fastidiosa*. **1** has also been isolated from a variety of other fungi, including *Cochliobolus* sp.[1], *Alternaria* sp.[4], and *Curvularia* sp.[5]

Figure 2. Symptomatic plants (at left in each picture) are observed living in close proximity to genetically identical disease-escaped plants (at right in each picture).

A total organic chemical synthesis of **1** was first reported in 1969, detailing an eleven step route to the racemic natural product from 3-oxoglutaric acid.[6] Recent attempts at improving similar syntheses include a four step chemical synthesis of **1**'s biogenetic precursor deoxyradicinin (**2**), published in 2012.[7] One of the caveats, however, of this 2012 approach is the relatively high cost of the starting material. Despite this contemporary work, published syntheses suffer poor yields while employing expensive reagents, and a more environmentally benign route is desired. A focus of our work remains improving on these reactions by utilizing a more direct synthesis and more recently published chemistry (Scheme 1).

The development of potent antibacterial agents toward both *X. fastidiosa* and *C*Las, respectively, is needed. An ideal inhibitor of each bacterium would be cost-effective to produce, soluble in water, non-toxic to both flora and fauna, and easy to administer to ailing plants. Here, the antibacterial activity of various synthetic, commercially available, and naturally occurring compounds (Fig. 3) was quantified by an inhibitory in

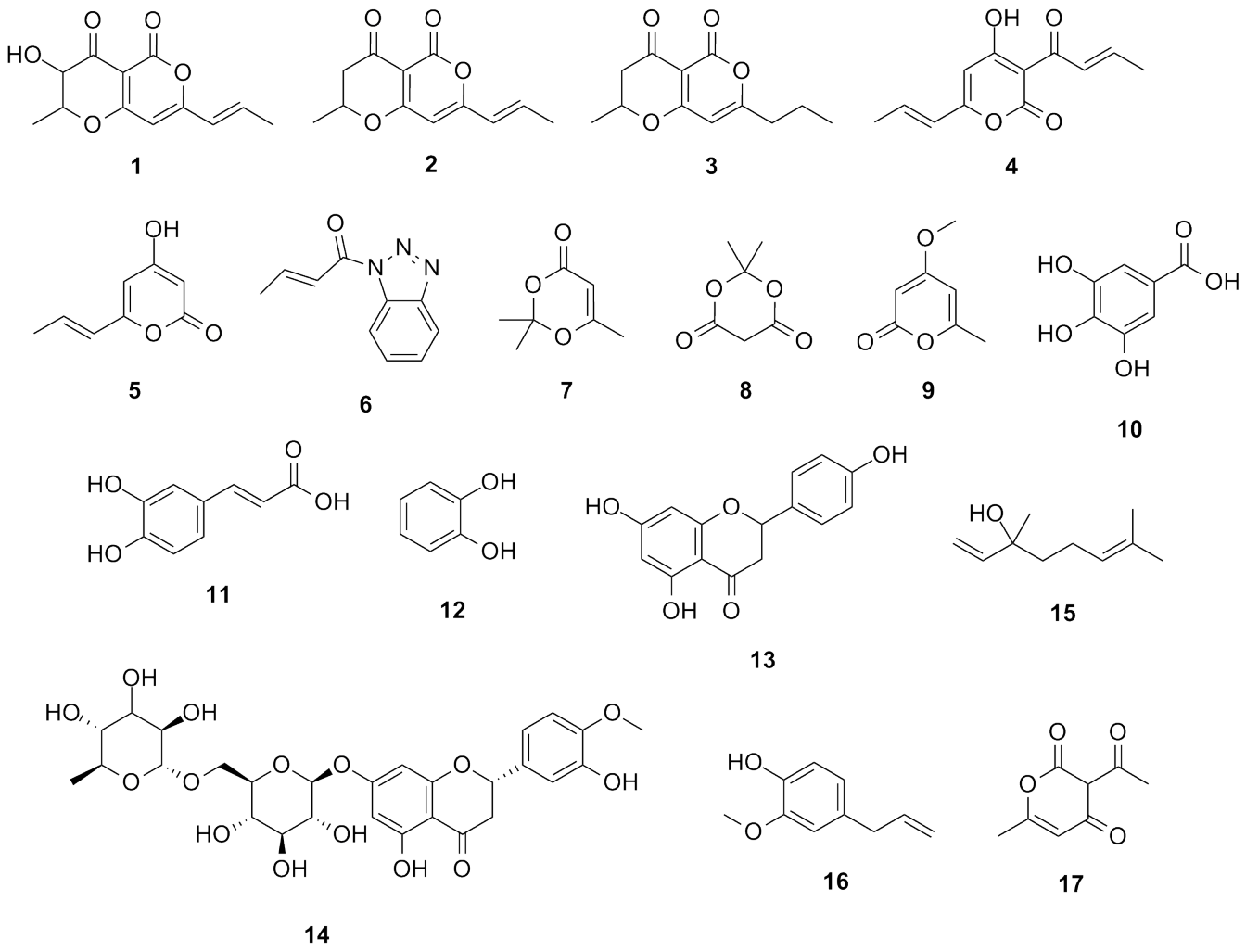
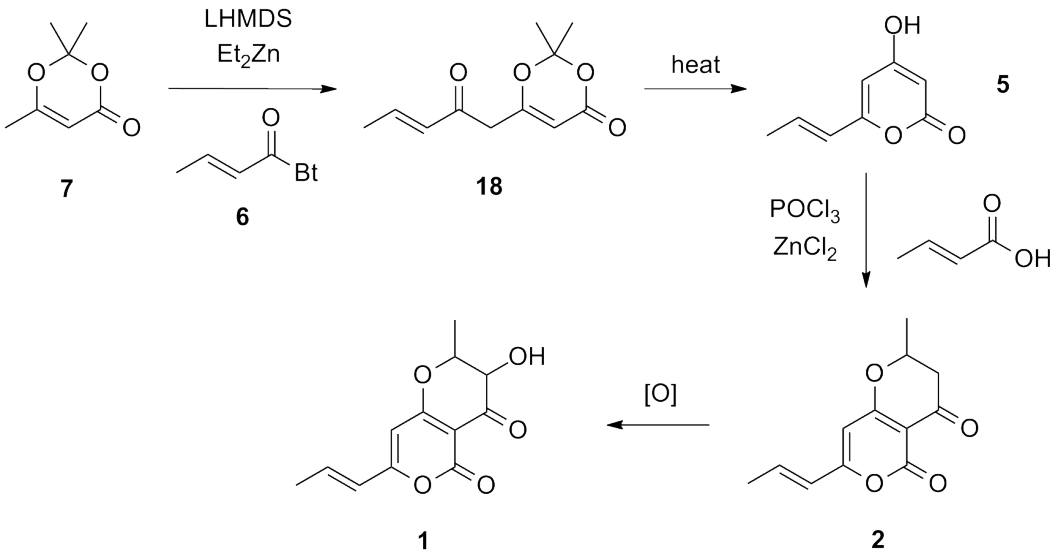


Figure 3. Compounds selected for bioassay against *X. fastidiosa* and *L. crescens*.



Scheme 1. A succinct total organic synthesis of radicinin (**1**) and deoxyradicinin (**2**), inhibitors of *X. fastidiosa* and *L. crescens*.

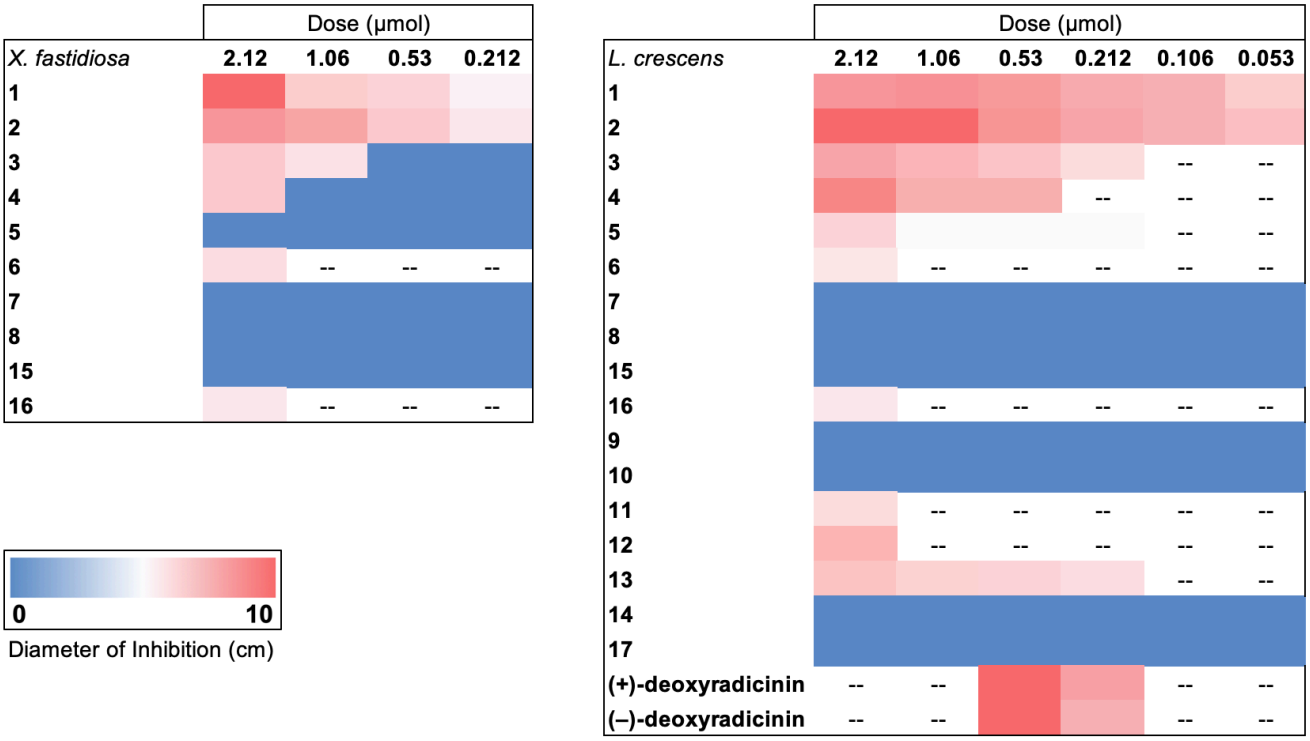


Table 1. Heat map showing inhibition of *X. fastidiosa* and *L. crescens* by various compounds. Boxes marked with a dashed line notation ("--") indicate that data was not obtained.

vitro agar diffusion bioassay technique (Fig. 4) against *X. fastidiosa* and *L. crescens*, a culturable surrogate for *C*Las. We seek to identify common structural motifs of inhibitors, and explore possible antibacterial mechanisms of action.

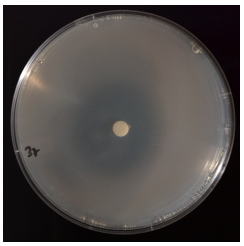


Figure 4. The agar diffusion bioassay technique shows a clear zone of inhibition of *L. crescens* by a given compound on the filter disc placed at the center of the agar plate. A similar method was followed to evaluate the inhibition of *X. fastidiosa*.

RESULTS AND DISCUSSION

Rationale for Compound Selection. Compounds selected for bioassay (Fig. 3) were chosen based on a variety of factors, including literature precedents, availability, and prior work completed in our lab. Compound **1** was known to inhibit *X. fastidiosa* in a dose-dependent fashion[1], and we decided to test several of its synthetic analogs: deoxyradicnin (**2**), dihydrodeoxyradicinin (**3**), a ring-opened derivative of deoxyradicinin (**4**), and a monocyclic synthetic intermediate (**5**). Crotonyl benzotriazole (**6**) was selected due to its propenyl functional group, its facile preparation, and availability as a precursor for the synthesis of **1** and **2**. A dioxinone (**7**) and Meldrum's acid (**8**) were selected to examine if the conjugation of a ring system contributed to antibacterial activity. **9** was selected in the interest of its single-ring, conjugated system. Gallic acid (**10**), caffeic acid (**11**), catechol (**12**), and naringenin (**13**) were selected for their known potent activity against *X. fastidiosa*.[8] Hesperidin (**14**) was selected due to its function as a phytoanticipin in *X. fastidiosa*, present in plants before infection by microorganisms.[9] The monoterpene linalool (**15**) was selected for its presence in certain species of citrus, and its known antibacterial activity.[10] Eugenol (**16**) was selected for its propenyl and phenolic moieties.[10] For our purposes, some compounds that were known to be biologically active against *X. fastidiosa* were tested against *L. crescens*, and vice versa. Owing to the high potency of our racemic compound **2** against *L. crescens* (vide infra), we performed chiral supercritical fluid chromatography (SFC) to separate the enantiomers of (–)-deoxyradicinin and (+)-deoxyradicinin.

Dose Response Curves. For all compounds tested, a higher antibacterial response is indicated at a higher dose. As expected, as the dose decreases, the antibacterial effect decreases as well, as seen in a smaller diameter of inhibition (Table 1).

For *X. fastidiosa*, **1** and **2** evidence a consistent, somewhat linear relationship between compound dose and bacterial response (Fig. 5). While **2** possesses greater activity at lower doses as compared to **1**, and more of a linear dose response overall, **1** shows complete inhibition (diameter = 10 cm) at the highest dose tested (2.12 μmol). Notably, **3** evidences activity only at doses greater than 0.53 μmol, and at higher doses, its potency is trivial with 3.4 cm of inhibition at 2.12 μmol.

For *L. crescens*, **1**, **3**, and **4** display notable linear relationships between compound dose and bacterial response (Fig. 3). Compound **13** possesses a somewhat inconsequential dose response, with inhibition increasing only from 2 cm to 3.92 cm over a tenfold increase in compound dose from 0.212 μmol to 2.12 μmol. Compound **2** is the most potent inhibitor of *L. crescens* tested in our lab, with notable inhibition (diameter = 4.0 cm) at the lowest tested concentration of 0.053 μmol. As the dose of **2** increased to 1.06 μmol and higher,

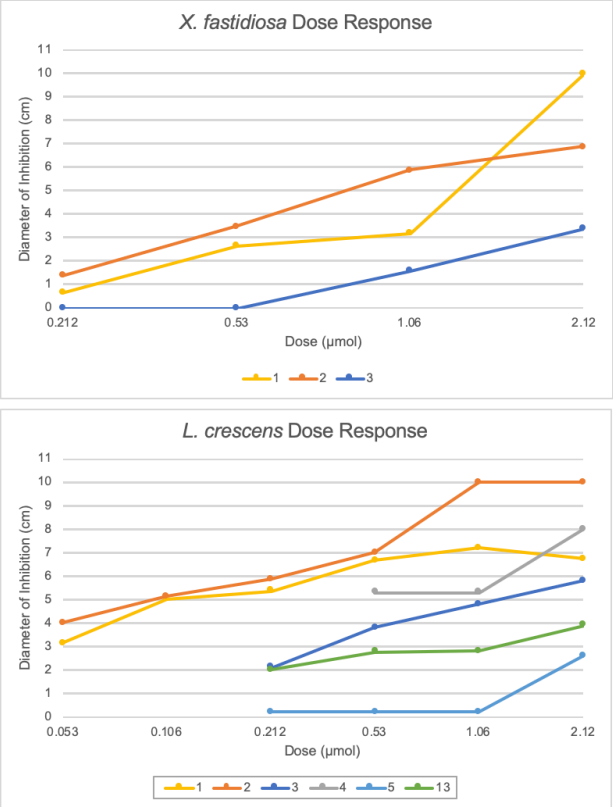


Figure 5. Dose response curves for *L. crescens* and *X. fastidiosa* show a dose-dependent antibacterial effect for various compounds.

total inhibition of the bacterium was observed. Compound **5** showed a non-linear, negligible antibacterial activity at lower concentrations (diameter = 0.2 cm), with higher concentrations showing slightly more activity (diameter = 2.6 cm).

New Inhibitors of *L. crescens*. While **1** and its analogs have previously been reported to possess antibacterial activity against *X. fastidiosa*[1], their capacity to inhibit *L. crescens* has not been detailed. Here, we report inhibitors of *L.crescens* that have not been previously reported: **1**, **2**, **3**, **4**, **5**, as well as **13**. As a note, the sample of **1** tested is mostly, if not exclusively, (–)-radicinin, whereas compounds **2** and **3** were synthesized as racemic mixtures. The literature reports the ability of specific, structure-based polycyclic compounds to inhibit *L. crescens*' protein translocase ATPase subunit SecA[11], as well as the keto-enol insecticide spirotetramat to inhibit the bacterium's acetyl CoA carboxylase[12], and tolfenamic acid to inhibit the a transcriptional accessory protein in the bacterium.[13] Further, several tetracycline antibiotics and penicillin derivatives have shown nearly complete inhibition of *L. crescens*.[14] Additional efforts towards developing targeted antibacterial agents for HLB is desired.

Discussion. Compounds **1**, **2**, **4**, **5**, and **16** each possess a sterically unhindered electrophilic site for Michael addition. Previous research has ascribed this functional group's ability to inactivate *X. fastidiosa* proteases by the enzyme's nucleophilic attack at the compound's alkene.[1] Thus, other Michael acceptors were expected to show inhibitory effects on *X. fastidiosa*; the bioassay data support this claim that Michael acceptors are important motifs in antibacterials for *X. fastidiosa* and *L. crescens* (Table 1). Interestingly, the high degree of similarity in the inhibition of *L. crescens* by enantiopure samples of (+)- and (–)-deoxyradicinin supports a non-enantioselective mode of action.[15]

Molecules **1**, **2**, **3**, **4**, **7**, **11**, **13**, **14**, and **17** all possess an enone moiety, though most antibacterially active compounds possess this motif inside of greater conjugated systems. Compounds with an enone group in smaller or non-conjugated systems show little activity, seen in **7**, **14**, and **17** (Table 1).

Previous literature details the ability of phenolic compounds to inhibit *X. fastidiosa* in vitro.[8] While it was determined that the number of hydroxy substitutions on a ring structure does not correlate with antibacterial activity, it was found that the presence of hydroxy groups at the meta-position of a benzene ring appear to contribute to activity. Here, we report similar findings with **4**, **5**, **11**, and **16**, all possessing propenyl moieties meta to hydroxy groups. We do note, however, that their antibacterial effects on both *X. fastidiosa* and *L. crescens* are minor when compared to polycyclic, Michael acceptor-possessing, highly conjugated systems. While the literature precedents detail conflicting activity of the ability of **10** to inhibit *X. fastidiosa*[8,16], our work shows no activity against *L. crescens*. **11** has shown activity against *X. fastidiosa*[8,16], and our work corroborates its antibacterial effects in activity against *L. crescens*, although they are minimal (diameter = 2.2 cm).

Compound **14**, a citrus bioflavonoid with known antibacterial and antioxidant properties[17], is known to combat *X. fastidiosa* when in complex with a polypyridine ligand.[18] Further, increased concentrations of **14** in the leaves and stems of citrus have been indicated in *X. fastidiosa* infections, with the compound possibly acting as a phytoanticipin.[9] Based on our data, it was determined that **14** fails to inhibit *L. crescens*.

Compound **13**, another citrus bioflavonoid with known antibacterial and antifungal properties[19], is also known to inhibit *X. fastidiosa* both in a synthetic complex[18] and by itself.[8] Our work presents **13** as a new inhibitor of *L. crescens* in a dose-dependent fashion (Table 1, Fig. 5). As **13** is difficult to quantify due to its limited aqueous solubility, it has not been determined if an increased amount of **13** is present in symptomatic citrus trees[20], which would indicate the plant's use of the compound as a bacterial defense mechanism. Several proposed mechanisms of action for flavonoids as antibacterial agents have been presented[21]: damage to the cytoplasmic membrane[22], inhibition of DNA synthesis[23], and inhibition of electron transport in the bacterial respiratory chain.[24] Further research in this area could determine the specific mode of action by which **13** acts on bacteria.

CONCLUSION

Plant pathogenic bacteria pose a great threat to the availability of economically important crops like grapes and oranges. Among a variety of commercially available, synthesized, and natural product antibacterial molecules, it was determined that Michael acceptors possess the greatest capacity for antibacterial inhibition of PD and HLB.

In looking toward a succinct total organic synthesis of radicinin, we began with a commercially available starting material that is less than 1% of the cost per gram when compared to the route published in 2012, and aimed to avoid the use of toxic metals like selenium dioxide and lead tetraacetate, used in the 2012 and 1969 syntheses, respectively. While we explored modern oxidation tactics for the final conversion of deoxyradicinin to radicinin, our efforts thus far have been unsuccessful for this last step, and future work on its development remains.

EXPERIMENTAL SECTION

Compound Collection. A pure sample of radicinin (**1**) (Maloney Lab, Point Loma Nazarene University, San Diego, CA, USA) was obtained from the fermentation of *Alternaria radicina* (ATCC strain AR96831) shaken at 190 rpm in potato dextrose broth for 24 days. **12** (Sigma-Aldrich, St. Louis, MO,

USA) was recrystallized from toluene. **8** was obtained from Oakwood Chemical (Estill, SC, USA). Pure samples of **10**, **11**, **13**, and **14** were obtained from Tokyo Chemical Industry Co., Ltd. (Portland, OR, USA). **16** and 2,2,6-Trimethyl-4H-1,3-dioxin-4-one were obtained from ACROS Organics (Morris Plains, NJ, USA). **15** and **17** were obtained from Sigma-Aldrich (St. Louis, MO, USA). MeOH, DMSO, and EtOAc (Sigma-Aldrich, St. Louis, MO, USA) were used as solvents for filter disc preparation.

Preparation of 6.[25] To a solution of benzotriazole (28.6 g, 240.4 mmol) in CH_2Cl_2 (300 mL) was added $SOCl_2$ (7.1 g, 60.1 mmol) at 25 °C with stirring. After 0.5 h, crotonic acid (5.2 g, 60.1 mmol) was added in one portion and stirring was continued for 2 h. The white precipitate was filtered off and washed with CH_2Cl_2 (2 × 50 mL). The combined organic solution was washed with 2 M NaOH (3 × 360 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (silica gel; CH_2Cl_2) to yield (*E*)-1-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)but-2-en-1-one. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, *J* = 8.2 Hz, 1H), 8.12 (d, *J* = 8.5 Hz, 1H), 7.73-7.59 (m, 1H), 7.57-7.43 (m, 3H), 2.12 (d, *J* = 5.5 Hz, 3H).

Preparation of 2,2-dimethyl-6-[(3*E*)-2-oxopent-3-en-1-yl]-2,4-dihydro-1,3-dioxin-4-one.[26,29] To a solution of anhydrous THF (84 mL) was added hexamethyldisilazane (8.8 mL, 42 mmol) at –78 °C with stirring. *n*-Butyllithium (16.8 mL, 42 mmol, 2.5 M in hexanes) was added dropwise over 10 min. After 20 min, **7** (4 mL, 30 mmol) in THF (12 mL) was added dropwise over 10 min. After 1 h, diethyl zinc (42 mL, 42 mmol, 1.0 M in hexanes) was slowly added. After 20 min, the mixture was allowed to warm to –20 °C. A solution of **6** (6.75 g) in THF (18 mL) was added and stirring was continued for 2 h. The reaction was quenched with 1 M HCl (240 mL), and the aqueous layer was acidified to pH 1-2 using 1 M HCl. The product was extracted with EtOAc (2 x 300 mL), the organic layers were combined and dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (silica gel; 3:1 hexanes:EtOAc) to yield 2,2-dimethyl-6-[(3*E*)-2-oxopent-3-en-1-yl]-2,4-dihydro-1,3-dioxin-4-one. ^{1}H NMR (400 MHz, $CDCl_3$) 6.92 (dq, *J* = 15.6, 6.9 Hz, 1H), 6.16 (m, 1H), 5.36 (s, 1H), 3.44 (s, 2H), 1.94 (m, 3H), 1.69 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.46, 165.19, 160.97, 145.63, 130.90, 107.39, 96.82, 44.72, 25.09, 18.50.

Preparation of 5.[6] To a solution of anhydrous toluene was added 0.1 M 2,2-dimethyl-6-[(3*E*)-2-oxopent-3-en-1-yl]-2,4-dihydro-1,3-dioxin-4-one at 130 °C with stirring under reflux for 10 min. The white precipitate was filtered off and washed with toluene (2 × 10 mL), yielding (*E*)-4-hydroxy-6-(prop-1-en-1-yl)-2*H*-pyran-2-one. ^{1}H NMR (400 MHz, DMSO-D_6) δ 11.63 (s, 1H), 6.46 (dq, *J* = 15.6, 6.9 Hz, 1H), 6.15 (dd, *J* = 15.6, 1.4 Hz, 1H), 5.98 (d, *J* = 1.8 Hz, 1H), 5.22 (d, *J* = 1.8 Hz, 1H), 1.82 (dd, *J* = 6.9, 1.4 Hz, 3H).

Preparation of 2.[27] To a solution of $POCl_3$ (0.7 g, 4.4 mmol) and $ZnCl_2$ (0.3 g, 2.5 mmol) was added crotonic acid (0.1 g, 0.6 mmol) and **5** (0.1 g, 0.6 mmol) at 85 °C with stirring for 4 h. The reaction was quenched with a small handful of ice and Na_2CO_3 (10% aq w/v%, 15 mL). The product was extracted with CH_2Cl_2 (2 x 40 mL), the organic layers were combined and dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (silica gel; CH_2Cl_2 followed by 10:1 CH_2Cl_2:MeOH) to afford a dark brown solid, (*E*)-2-methyl-7-(prop-1-en-1-yl)-2,3-dihydropyrano[4,3-*b*]pyran-4,5-dione. ^{1}H NMR data is consistent with published results.

Preparation of butyryl benzotriazole.[25] To a solution of benzotriazole (28.6 g, 240.4 mmol) in CH_2Cl_2 (300 mL) was added $SOCl_2$ (7.1 g, 60.1 mmol) at 25 °C with stirring. After 0.5 h, butyric acid (5.3 g, 60.1 mmol) was added in one portion and stirring was continued for 2 h. The white precipitate was filtered off and washed with CH_2Cl_2 (2 × 50 mL). The combined organic solution was washed with 2 M NaOH (3 × 360 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (silica gel; CH_2Cl_2) to yield 1-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)butan-1-one. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (d, *J* = 8.2 Hz, 1H), 8.11 (d, *J* = 8.2 Hz, 1H), 7.65 (t, *J* = 7.8 Hz, 1H), 7.50 (t, *J* = 7.8 Hz, 1H), 3.40 (t, *J* = 7.3 Hz, 2H), 1.94 (m, 2H), 1.10 (t, *J* = 7.5 Hz, 3H).

Preparation of LDA.[26] To a solution of diisopropylamine (3.2 mL, 22.5 mmol) in anhydrous THF (60 mL) was added *n*-butyllithium (2.5 M in hexanes, 9.9 mL, 24.8 mmol) dropwise over 20 min at −78 °C with stirring.

Preparation of 2,2-dimethyl-6-(2-oxopentyl)-4*H*-1,3-dioxin-4-one.[26,29] To the prepared LDA solution was added **7** (2.4 mL, 17 mmol) in THF (60 mL) dropwise over 12.5 min at −78 °C with stirring. After 1.5 h, a solution of butyryl benzotriazole (2.9 g, 15 mmol) in THF (60 mL) was added and stirring was continued overnight as the reaction warmed to room temperature. The reaction was quenched with a saturated NH_4Cl solution (6 mL), and was condensed under reduced pressure to a golden brown syrup. H_2O (300 mL) was added to the reaction mixture and it was transferred to a separatory funnel for extraction with EtOAc (3 x 150 mL). The organic layers were combined, then washed with a saturated Na_2CO_3 solution (300 mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure, and residue was purified by flash chromatography (silica gel; 1:1 hexanes:EtOAc; R_f = 0.5) to yield 2,2-dimethyl-6-(2-oxopentyl)-4*H*-1,3-dioxin-4-one. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34 (s, 1H), 3.31 (s, 2H), 2.48 (t, *J* = 7.2 Hz, 2H), 1.71 (s, 6H), 1.67 – 1.61 (m, 2H), 0.93 (t, *J* = 7.4 Hz, 3H).

Preparation of 4-hydroxy-6-propyl-2*H*-pyran-2-one.[6] To a solution of anhydrous toluene was added 0.1 M 2,2-dimethyl-6-(2-oxopentyl)-4*H*-1,3-dioxin-4-one at 120 °C with stirring under reflux for 1 h. The white precipitate was filtered off and washed with toluene (2 × 10 mL), yielding 4-hydroxy-6-propyl-2*H*-pyran-2-one. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.97 (s, 1H), 5.64 (d, *J* = 1.4 Hz, 1H), 2.46 (t, *J* = 7.5 Hz, 2H), 1.68 (m, 2H), 0.92-1.00 (m, 4H).

Preparation of 3.[27] To a solution of $POCl_3$ (2.4 g, 15.3 mmol) and $ZnCl_2$ (1.2 g, 8.8 mmol) was added crotonic acid (0.2 g, 2.19 mmol) and 4-hydroxy-6-propyl-2*H*-pyran-2-one (0.3 g, 2.2 mmol) at 85 °C with stirring for 4 h. The reaction was quenched with a small handful of ice and Na_2CO_3 (10% aq w/v%, 50 mL). The product was extracted with CH_2Cl_2 (2 x 100 mL), the organic layers were combined and dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (silica gel; 10:1 CH_2Cl_2:MeOH; R_f = 0.4) to afford a dark brown solid, 2-methyl-7-propyl-2,3-dihydropyrano[4,3-*b*]pyran-4,5-dione. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.88 (s, 1H), 4.79 – 4.72 (m, 1H), 2.71 – 2.58 (m, 2H), 2.47 (t, *J* = 7.5 Hz, 2H), 1.71 (sextet, *J* = 7.2 Hz, 2H), 1.54 (d, *J* = 6.3 Hz, 3H), 0.98 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.64, 176.00, 172.33, 158.05, 99.96, 99.11, 43.81, 36.36, 20.42, 19.94, 13.53.

Preparation of 4.[28] To a solution of KOH (0.0056 g, 0.10 mmol) in MeOH (2 mL) at 0 °C with stirring was added **2** (0.022 g, 0.01 mmol) over 10 minutes. (Diacetoxyiodo)benzene (0.0064 g, 0.02 mmol) was added over 10 minutes, and the reaction mixture was stirred overnight and allowed to warm to room temperature. The reaction was quenched with 0.1 M HCl (0.2 mL), and was extracted with CH_2Cl_2 (2 x 10 mL). The organic layers were combined and dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (1:1 hexanes:acetone) to afford 3-((*E*)-but-2-enoyl)-4-hydroxy-6-((*E*)-prop-1-en-1-yl)-2*H*-pyran-2-one. ^{1}H NMR (400 MHz, $CDCl_3$)

δ 7.64 (d, *J* = 15.3 Hz, 1H), 7.34 – 7.23 (m, 1H), 6.92 (dq, *J* = 15.2, 7.0 Hz, 1H), 6.03 (d, *J* = 15.5 Hz, 1H), 5.88 (s, 1H), 2.02 (d, *J* = 6.9 Hz, 3H), 1.96 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.0, 183.2, 163.4, 160.8, 147.4, 140.2, 128.3, 122.9, 100.8, 99.6, 19.1, 18.9.

NMR Analysis. All compounds were analyzed using a Jeol 400 MHz nuclear magnetic resonance spectrometer (Jeol, Ltd., Akishima, Tokyo, Japan). ^{1}H nuclei were observed at 400 MHz. $CDCl_3$ (Cambridge Isotope Laboratories, Inc., Tewksbury, MA, USA) and DMSO-D_6 (Sigma-Aldrich, St. Louis, MO, USA) were used as solvents.

Preparation of Inhibition Assays. Compounds were evaluated using an in vitro assay for their availability to inhibit *X. fastidiosa* and *L. crescens* growth. Compounds to be tested were dissolved in MeOH, DMSO, or EtOAc and applied to sterile filter discs (Difco) to achieve desired doses. Refer to the specific assay techniques for each bacterium below. After incubation of the bacterium with the compound-loaded filter disc at 28 °C for 7 days, the zone diameters of clear zones of inhibition were recorded. Each assay was performed in triplicate, and the average diameter of inhibition is reported in this paper. Pure compounds that showed activity at higher concentrations were tested at lower doses to observe the dose response.

***X. fastidiosa* Inhibition Assay.** *X. fastidiosa* was harvested from 6 day old cultures on PD3 plates using liquid PD3 and the OD_{600} was adjusted to 0.1. To 3 mL of PD3 top agar (0.8% agar) was added 300 µL of this culture, and the resulting solution was poured on top of a plate containing 20 mL of solidified PD3 agar. After two days of incubation at 28 °C, compound-loaded filter discs were placed in the center of the plate.

***L. crescens* Inhibition Assay.** mBM7 top agar (0.8% agar) was prepared and cooled to 60 °C, and amended 10% v/v with a four-day *L. crescens* liquid culture (mBM7, 28 °C, 180 rpm shaking). This amended top agar was then dispensed to evenly coat previously poured mBM7 agar plates, and compound-loaded filter discs were placed in the center of freshly prepared *L. crescens* top-agar plates.

ASSOCIATED CONTENT

Supporting Information

Additional material as described in the text: NMR spectra for all compounds and details of chiral SFC separation of **2**. This material in print is available free of charge by contacting Connor Brandenburg.*

AUTHOR INFORMATION

Corresponding Author

*E-mail: cbrandenburg4710@pointloma.edu

Notes

The authors declare no competing financial interest.

ACKNOWLEDGEMENTS

We extend our thanks to the Point Loma Nazarene University Department of Chemistry, the Point Loma Nazarene University Research Associates, the California Department of Food and Agriculture, the Pierce's Disease/Glassy-Winged Sharpshooter Board, the United States Department of Agriculture, Lia Lozano and Jennifer Cordoza of Point Loma Nazarene University for providing a quantity of radicinin (**1**) for bioassay, Elizabeth Costa of Pfizer Inc. for performing chiral SFC, and Dr. Chambers Hughes and Trevor Purdy of the Scripps Institute of Oceanography for aid in acquiring mass spectrometric data.

REFERENCES

(1) Aldrich, T. J.; Rolshausen, P. E.; Roper, M. C.; Reader, J. M.; Steinhaus, M. J.; Rapicavoli, J.; Vosburg, D. A.; Maloney, K. N. *Phytochemistry* **2015**, *116*, 130–137.

(2) Grafton-Cardwell, E. Center for Invasive Species Research, University of California Riverside. https://cisr.ucr.edu/citrus_greening.html (accessed Jan 29, 2019).

(3) Clarke, D.; Nord, F. *Arch. Biochem. Biophys.* **1953**, *45* (2), 469–470.

(4) Nukina, M.; Marumo, S. *Tetrahedron Lett.* **1977**, *18* (37), 3271–3272.

(5) Robeson, D. J.; Strobel, G. A. *Phytochemistry* **1982**, *21* (7), 1821–1823.

(6) Kato, K.; Hirata, Y.; Yamamura, S. *J. Chem. Soc. C* **1969**, No. 15, 1997–2002.

(7) Suzuki, M.; Sakuno, E.; Ishihara, A.; Tamura, J.-I.; Nakajima, H. *Phytochemistry* **2012**, *75*, 14–20.

(8) Maddox, C. E.; Laur, L. M.; Tian, L. *Curr. Microbiol.* **2009**, *60* (1), 53–58.

(9) Soares, M. S.; Silva, D. F. D.; Forim, M. R.; Maria Fátima Das Graças Fernandes Da Silva; Fernandes, J. B.; Vieira, P. C.; Silva, D. B.; Lopes, N. P.; Carvalho, S. A. D.; Souza, A. A. D.; Machado, M. A. *Phytochemistry* **2015**, *115*, 161–170.

(10) Killiny, N.; Valim, M. F.; Jones, S. E.; Omar, A. A.; Hijaz, F.; Gmitter, F. G.; Grosser, J. W. *Plant Physiol. Biochem.* **2017**, *116*, 36–47.

(11) Akula, N.; Trivedi, P.; Han, F. Q.; Wang, N. *Eur. J. Med. Chem.* **2012**, *54*, 919–924.

(12) Lümmen, P.; Khajehali, J.; Luther, K.; Leeuwen, T. V. *Insect Biochem. Mol. Biol.* **2014**, *55*, 1–8.

(13) Gardner, C. L.; Pagliai, F. A.; Pan, L.; Bojilova, L.; Torino, M. I.; Lorca, G. L.; Gonzalez, C. F. *Front. Microbiol.* **2016**, *7*.

(14) Turpen, T. Discussion of Penicillin, Antimicrobial R&D and Bactericide Now Initiative. (accessed Mar 18, 2019).

(15) Chiral SFC separation of synthetic racemic deoxyradicinin (**2**) was performed by Pfizer Inc. See Supporting Information for details.

(16) Bleve, G.; Gallo, A.; Altomare, C.; Vurro, M.; Maiorano, G.; Cardinali, A.; D'Antuono, I.; Marchi, G.; Mita, G. *FEMS Microbiol. Lett.* **2017**, *365* (5).

(17) Corciova, A.; Ciobanu, C.; Poiata, A.; Mircea, C.; Nicolescu, A.; Drobota, M.; Varganici, C.-D.; Pinteala, T.; Marangoci, N. *J. Inclusion Phenom. Macrocyclic Chem.* **2014**, *81* (1-2), 71–84.

(18) Alves de Souza, A. et al. Bactericides for control of Xylella fastidiosa and method for in-vitro and in-vivo bacterial inhibition, February 21, 2017.

(19) Rauha, J.-P.; Remes, S.; Heinonen, M.; Hopia, A.; Kähkönen, M.; Kujala, T.; Pihlaja, K.; Vuorela, H.; Vuorela, P. *Int. J. Food Microbiol.* **2000**, *56* (1), 3–12.

(20) Dagulo, L.; Danyluk, M. D.; Spann, T. M.; Valim, M. F.; Goodrich-Schneider, R.; Sims, C.; Rouseff, R. *J. Food Sci.* **2010**, *75* (2).

(21) Babii, C.; Mihalache, G.; Bahrin, L. G.; Neagu, A.-N.; Gostin, I.; Mihai, C. T.; Sârbu, L.-G.; Birsa, L. M.; Stefan, M. *PLOS ONE* **2018**, *13* (4).

(22) Ikigai, H.; Nakae, T.; Hara, Y.; Shimamura, T. *Biochim. Biophys. Acta, Biomembr.* **1993**, *1147* (1), 132–136.

(23) Mori, A.; Nishino, C.; Enoki, N.; Tawata, S. *Phytochemistry* **1987**, *26* (8), 2231–2234.

(24)Haraguchi, H.; Tanimoto, K.; Tamura, Y.; Mizutani, K.; Kinoshita, T. *Phytochemistry* **1998**, *48* (1), 125–129.

(25)Katritzky, A. R.; Zhang, Y.; Singh, S. K. *Synthesis* **2003**, No. 18, 2795–2798.

(26)Katritzky, A. R.; Wang, Z.; Wang, M.; Hall, C. D.; Suzuki, K. *J. Org. Chem.* **2005**, *70* (12), 4854–4856.

(27)Zehnder, L. R.; Dahl, J. W.; Hsung, R. P. *Tetrahedron Lett.* **2000**, *41* (12), 1901–1905.

(28)Moriarty, R. M.; Hu, H.; Gupta, S. C. *Tetrahedron Lett.* **1981**, *22* (14), 1283–1286.

(29)Patel, B. H.; Mason, A. M.; Barrett, A. G. M. *Org. Lett.* **2011**, *13* (19), 5156–5159.