CHEMISTRY & BIODIVERSITY

Accepted Article

Title: Fungicidal properties of some novel trifluoromethylphenyl amides

Authors: Maia Tsikolia, Ulrich R. Bernier, David E. Wedge, Nurhayat Tabanca, Khalil A. Abboud, and Kenneth J. Linthicum

To be cited as: *Chem. Biodiversity* 10.1002/cbdv.201800618

Link to VoR: http://dx.doi.org/10.1002/cbdv.201800618

10.1002/cbdv.201800618

Fungicidal properties of some novel trifluoromethylphenyl amides

Maia Tsikolia[a]*, Ulrich R. Bernier[b], David E. Wedge[c], Nurhayat Tabanca[d], Khalil A. Abboud[e], and Kenneth J. Linthicum[b]

[a] Emerging Pathogens Institute, Department of Entomology and Nematology, University of Florida, Gainesville, FL 32610-0009 USA

[b] U.S. Department of Agriculture-Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL, 32608 USA

[c] United States Department of Agriculture, Agricultural Research Service, Natural Products Utilization Research Unit, The University of Mississippi, University, MS 38677 USA

[d] U.S. Department of Agriculture-Agricultural Research Service, Subtropical Horticulture Research Station, 13601 Old Cutler Rd., Miami, FL 33158 USA

[e] Center for X-ray Crystallography, Department of Chemistry, University of Florida, Gainesville, FL 32611 USA

Abbreviations: TFMPA, trifluoromethylphenyl amides; THF, tetrahydrofuran; CH_2Cl_2, dichloromethane; Et_3N, triethylamine; TLC, thin layer chromatography; SI, Supporting Information.

* Correspondence to: Maia Tsikolia, Email: m.tsikolia@gmail.com; Address: Emerging Pathogens Institute, 2055 Mowry Road, Department of Entomology and Nematology, University of Florida, Gainesville, FL 32610-0009.

Abstract

Trifluoromethylphenyl amides (TFMPAs) were designed and synthesized as potential pesticides. Thirty three structures were evaluated for fungicidal activity against three *Colletotrichum* species using direct bioautography assays. Active compounds were subsequently tested against *C. fragariae, C. gloeosporioides, C. acutatum, Phomopsis obscurans, P. viticola, Botrytis cinerea* and *Fusarium oxysporium*. The study identified 2-chloro-*N*-(2,6-dichloro-4-(trifluoromethyl)phenyl)acetamide (**7a)** as showing the strongest antifungal activity, and the broadest activity spectrum in this set against *Colletotrichum acutatum* (at 48 and 72 h) and *Phomopsis viticola* (at 144 h). The presence of triethylamine in its complex with *N*-(2,6-dichloro-4-(trifluoromethyl)phenyl)-2,2,3,3,3-pentafluoropropanamide (**7b′**) played an important role in the bioactivity, and depending on the concentration or fungal species it showed higher or lower activity than the parent amide. X-Ray crystallography have shown that the complex (**7b′**) is an ion pair, $(C_{10}H_2Cl_2F_8NO)^-$ $(C_6H_{16}N)^+$, where a proton is transferred from the amide nitrogen to the triethylamine nitrogen and then connected by hydrogen bonding to the acyl oxygen [N-H 0.893 Å; H…..O 1.850 Å; N…..O 2.711 Å; N-H…..O 161.2(13)°]. Although none of these compounds were better than standards, this work revealed some potential lead structures for further development of active novel compounds.

Keywords: *Colletotrichum acutatum, C. fragariae, C. gloeosporioides, Botrytis cinerea, Phomopsis viticola, P. obscurans, micro*-dilution broth assay, bioautography assay, X-ray crystallography.

Introduction

Fungi are the main cause of plant diseases and result in significant loss of agricultural production worldwide.[1,2] Fungicides thus are an important component of the agrochemical armamentarium.[3,4] The resistance to fungicides remains one of the major problems in crop protection programs,[4] and development of new fungicides is an important research objective.[5] Amide derivatives, such as mandipropamid, dimethomorph, flumorph, mepronil, tiadinil etc. have been developed over the last several decades as chemicals that kill or inhibit the growth of fungi.[6,7] The presence of fluorine can increase the bioactivity of potential insecticides and fungicides[8-10] and can make them highly effective. Flutolanil, fluopyram, fluoxastrobin and fluxapyroxad are examples of fluorine-containing amide fungicides mainly used in plant protection[11-13] with a medium to high risk of resistance, except fluopicolide, for which resistance formation has not yet been observed.

In our previous work[14] we studied the fungicidal activity of twenty trifluoromethylphenyl amides (TFMPAs 1-4, Figure 1) that were designed following an extensive literature search for compounds with insect repellent or pesticidal activity using the SciFinder database.[15] These twenty TFMPAs were evaluated against three *Colletotrichum* species using direct bioautography assays and the active compounds were subsequently tested against *C. fragariae, C. gloeosporioides, C. acutatum, Phomopsis obscurans, P. viticola, Botrytis cinerea* and *Fusarium oxysporium* using a 96-well micro-dilution broth assay. 2,2,2-Trifluoro-*N*-(2-(trifluoromethyl)phenyl)acetamide (**4c**), was the most potent fungicide against *P. obscurans* within this original set[14] (Figure 1). The initial twenty TFMPAs[14] were subsequently used as the basis for designing additional fluorine-containing amides (**5-12**) as potential leads for broad-spectrum pesticides and repellents[16] (Figure 2, Table 1).

All 33 structures were evaluated for pesticidal properties, which included evaluation of insecticidal activity against mosquito species, as well as fungicidal effects against plant pathogens. The present work describes the fungicidal activity studies for these 33 TFMPAs. An X-ray crystallography study was performed for complex *N*-(2,6-dichloro-4-(trifluoromethyl)phenyl)-2,2,3,3,3-pentafluoropropanamide with triethylamine (**7b′,** Figure 3), which was formed in an effort to synthesize amide **7b**.

Results and Discussion

Design and Synthesis

Fluorine-containing *N*-(substituted)phenyl amide derivatives were designed with the aim to develop novel compounds as the broad-spectrum activity pesticides. Substituents[14-23] were selected by structural similarity to the active compounds from our previous work[14] (Figure 2, Table 1), and also using a literature search of the SciFinder database.[15] Previous reports on the biological activity gave important information on the impact of the substituted groups on the properties of the tested compounds. There are many reports on the pesticidal activity of compounds with trifluoromethyl groups attached to the phenyl ring or fluoroalkyl substituents attached to the amide carbonyl carbon. The amide groups can improve stability and provide intermolecular hydrogen bonds with biological targets.[14]

Out of 33 TFMPAs sixteen compounds were synthesized (**7b′**, **8-12**, Figure 2, Table 1) twelve of which were novel structures. Fourteen compounds (**5-7)** were prepared previously[16] and three compounds (**8a, 8f** and **11a)** were purchased from commercial sources. Acid chlorides were prepared *in situ* by overnight reaction of the corresponding carboxylic acids with a 20–25% excess of thionyl chloride at 25 °C.[14,16] Anhydrides were purchased from commercial sources. Reaction of 1.05 equivalents of acid chloride or acid anhydride with one equivalent of

corresponding trifluoromethylphenyl amines in tetrahydrofuran (THF) (10 mL) at 0-25 °C (**8b-e**, **8g-h, 9e-f, 10a, 11b**), or at 66 °C (**9a-d**), or in toluene at 110 °C (**12a**) led to the production of TFMPAs **8-12** in yields of 68-99% (Figure 2). Triethylamine (Et_3N) was used as a base for the preparation of amide **12a** (see Supporting Information (SI) for compounds yields, melting points, NMR, mass spectral data).

X-Ray crystallography

Complex **7b′** was formed in an attempt to synthesize **7b** using triethylamine (Et_3N) as the base. We found it interesting to study the crystal structure of **7b′** along with the pesticidal activity of this complex in comparison with the activity of free **7b** and other compounds. A complex ratio of 1:1 was confirmed by X-ray crystallography and NMR analysis (see SI text and Figures S1 and S2 for ^{1}H and ^{13}C NMR of **7b′** in comparison with **7b**). According to the X-ray crystallography results, **7b′** is an ion pair, $(C_{10}H_2Cl_2F_8NO)^-(C_6H_{16}N)^+$, where proton (H2) is transferred from the amide nitrogen (N1) to the nitrogen (N2) of triethylamine and then connected by hydrogen bonding to the acyl oxygen (O1); H2 is involved in strong H-bonding with O1 of the molecule [N2-H2 0.893(15) Å; H2…..O1 1.850(15) Å; N2…..O1 2.711(1) Å; N2-H2…..O1 161.2(13)°] (Figures 3 and S3, Tables S1-S6).

Direct bioautography assay for activity against plant pathogenic fungi

All 33 TFMPAs were pre-screened against three *Colletotrichum* species using a matrix bioautography technique. Bioautography of compounds **5-7** indicated that **6a**, **7a**, **7b** and **7b′** had antifungal activity against *Colletotrichum* test species, while compounds **5a-f** and **6b-f** were inactive against these species. Compounds possessing antifungal activity produced a clear zone of inhibition and different compounds showed different sizes of the inhibitory zone (Table 2). Compound **6a** exhibited good, clear antifungal zones against *C. acutatum* and *C. fragariae*, while it had diffuse zones against *C. gloeosporioides*, which indicated potentially selective

activity. Bioautography of compounds **8a-12a** revealed four compounds **8f**, **8h**, **10a** and **11a** with only mild antifungal activity (Table 2).

Microdilution broth assay

Active antifungal compounds **6a, 7a, 7b, 7b′**, **8f**, **8h**, **10a** and **11a** were subjected to a secondary screening using a liquid broth culture bioassay (micro-dilution broth assay) in a concentration-response format (Figures 4 and 5). Against *C. acutatum* at 48 h, compounds **7a** and 7**b** were more active at all concentrations than the azoxystrobin standard, but less active than the captan standard (Figure 4A). At 0.3 μM concentration, compound **7a** showed higher activity than both azoxystrobin and captan standards. Against *B. cinerea* at 72 h, at all concentrations, compounds **6a**, **7a, 7b** and **7b′** had little or no activity (Figure 4B). Compounds **7a** and **7b′** produced 95% and 89% fungal growth inhibition of *P. viticola* at 72 h, respectively, at 30 μM and were comparable to the captan and azoxystrobin standards (88% and 100% inhibition, respectively, Figure 4C, Table 3). It should be noted that in our previous work[14] the most potent fungicide 2,2,2-trifluoro-*N*-(2-(trifluoromethyl)phenyl)acetamide (**4c,** Figure 1), with ~44% growth inhibition was less active than both standards at 120 h, at 30.0 μM concentration against *P. viticola*. Compounds **8f**, **8h, 10a** and **11a** were less active than the standards, against *P. obscurans* at 144 h (Figure 5), and poorly active against the other test fungi (data not shown). In our previous research[14] compound **4c (**Figure 1) was comparable to captan standard against *P. obscurans,* producing ~72% inhibition at 3.0 μM, at 120 h exposure. Against *C. fragariae* (Table 3) at 48 h, at 30.0 μM, **7a, 7b** and **7b′** showed higher activity (~35-55% inhibition) than the azoxystrobin standard (~29 % inhibition), but they were much less active than the captan standard (~98% inhibition). Against *C. gloeosporioides* and *F. oxysporum* (data not shown), tested compounds (**6a, 7a, 7b, 7b′**, **8f**, **8h**, **10a** and **11a)** were less active than both standards at 30 μM or showed no activity (Table 3).

It appears from Figure 4A that as concentration of **6a** increases from 3.0 to 30.0 μM, growth inhibition decreases (fungal growth increases). Such a response is typical for compounds from the solution as the concentration increases. This is a misinterpretation of spectrophotometer measured turbidity caused by the molecule as it precipitates out of solution. The growth of the organism may or may not be increased due to loss of solubility from the aqueous broth and, accordingly, only data at concentrations below 30.0 μM is valid. Also, there was at least one observation when **7b** demonstrated the solubility problems associated with testing lipophilic compounds.

Fungal growth inhibition against three *Colletotrichum* species and *F. oxysporium* (data not shown) generally decreased with time for **6a, 7a, 7b** and **7b′**. The fungi may metabolize these compounds, or these compounds are not stable in the bioassay system, or there could be other reasons (an effect of adaption, or fungi can induce defense mechanisms such as increased efflux etc.). Further study is needed to identify the inhibition mechanism for these species. *B. Cinerea* and the two *Phomopsis* species do not demonstrate this effect.

Structure activity relationship

Active compounds were not only those with the halogenated acyl (**6a**, **7a**, **7b, 7b′, 8f** and **8h),** but also those with aliphatic acyl substituents (**10a** and **11a**). On the other hand, 2,6-dichloro-4-(trifluoromethyl)phenyl) amide moiety produced most potent compounds (**7a**, **7b**, **7b′**); Acording to our previous work,[14] 2,6-dichloro-4-(trifluoromethyl)phenyl) amide **1c** (figure 1), which is structurally similar to **7b**, but with a shorter trifluoroacetyl substituent, was not active against *Colletotrichum* species. *N-ortho*-(Trifluoromethyl)phenyl amide **4c** with the same trifluoroacetyl group (figure 1) was the best fungicide according to our previous work;[14] while, in this study, *N-ortho*-(trifluoromethyl)phenyl amide **5b**, with a longer fluorinated chain, pentafluoropropionyl, attached to the nitrogen atom, was not active in bioautography assay

against *Colletotrichum* species. Also, none of the *N-ortho*-(trifluoromethyl)phenyl amides **5a-f** and **9a-f** were active, as well.

The presence of triethylamine in **7b′** resulted in significant alteration of the biological properties of the active ingredient **7b.** As shown on Figure 4A and B, at 0.3 μM **7b′** promoted fungal growth in *C. acutatum* and *B. cinerea* while **7b** inhibited growth. Against *C. acutatum* at 30 μM both **7b** and **7b′** showed almost the same activity; at 3 μM **7b** remained active while activity of **7b′** dramatically decreased; at 3 and 30 μM concentrations against *P. viticola* **7b′** was more active than **7b** (Figure 4C); at 0.3 μM **7b′** showed less activity than **7b** although **7b′** was more potent than captan standard at all concentrations. Thus, the presence of triethylamine in the **7b′** complex significantly influenced the bioactivity and depending on the concentration or fungal species it performed better or was less active than the original amide (**7b**).

Conclusions

2,6-Dichloro-4-trifluoromethyl phenyl amides **7a**, **7b** and **7b′** with the pentafluoropropionyl or chloroacetyl substituents showed the best activity out of 33 compounds, and against *Colletotrichum* and *Phomopsis* they were comparable to standards. For amides: **7b** and **1c**,[14] **5b** and **4c**,[14] this study, once more, showed that a small change in the structure can produce a significant change in the biological (in this case fungicidal) properties of the molecule. The fungicidal activity of the complex **7b′** differ from the parent molecule **7b**. Overall, this study revealed several 2,6-dichloro-4-trifluoromethyl phenyl amides with the antifungal activity and broad antifungal spectrum with potential for further study and development of the lead compounds for the control of *Phomopsis* infections. The synthesis of a larger number of structural analogues is needed to establish proper structure-activity relationships for these leads.

Supporting Information

Supporting Information is available for compounds yields, melting points, NMR, mass spectral and X-ray crystallography data.

Experimental Section

Synthesis

Sixteen compounds (**7b′**, **8-12**, twelve novel) were synthesized. Three Compounds were purchased from commercial sources: **8a, 8f** (SynQuest Labs, Inc. Alachua, FL, USA) and **11a** (Matrix Scientific, Columbia, SC, USA). Fourteen compounds (groups **5-7,** Table 1) we synthesized previously.[16]

General methods and materials

Melting points were determined on a hot-stage apparatus and are uncorrected. Nuclear Magnetic Resonance (NMR) analyses were performed at NMR Facility of the University of Florida in Gainesville, FL, USA. NMR spectra were recorded in $CDCl_3$ or acetone-d_6 with TMS (tetramethylsilane) as the internal standard for ^1H (500 MHz) and $CDCl_3$ or acetone-d_6 as the internal standard for ^{13}C (125 MHz). Accurate masses were measured at the Mass Spectrometry Facility of the Department of Chemistry, University of Florida, using a 6220 TOF-MS (Agilent Technologies) equipped with an electrospray and atmospheric pressure chemical ionization source. Direct analysis in real time (DART) was performed with the aid of an Ionsense DART source (Ionsense, inc). Samples were dissolved in dichloromethane and solutions introduced via direct injection. All reactions were carried out under argon atmosphere in anhydrous THF, CH_2Cl_2 or toluene, from Acros Organics, NJ, USA. The progress of a reaction was monitored by thin layer chromatography (TLC).

*Preparation of **7b′***

Route 1: To a solution of 2,6-dichloro-4-(trifluoromethyl)phenyl amine (10 mmol) in THF (12 mL), acid anhydride (10.5 mmol) was added at 0 °C in the presence of Et_3N (10.1 mmol) and the mixture was stirred continuously for 2 h at 0-25 °C. The reaction mixture was diluted and extracted with ethyl acetate (40 mL), washed with sat. aq. $NaHCO_3$ (3 x 60 mL) and the organic layer dried over anhydrous Na_2SO_4. Evaporation of the solvent and recrystallization from hexane/ethyl acetate gave the product *N*-(2,6-dichloro-4-(trifluoromethyl)phenyl)-2,2,3,3,3-pentafluoropropanamide with Et_3N (**7b′)** in 96% yield. **Route 2:** To a solution of N-(2,6-dichloro-4-(trifluoromethyl)phenyl)-2,2,3,3,3-pentafluoropropanamide (0.5 mmol) in THF or CH_2Cl_2 (1 mL) was added Et_3N (0.55 mmol) and stirred continuously at 25 °C. The mixture was concentrated and recrystallized from hexane/ethyl acetate to give desired product *N*-(2,6-dichloro-4-(trifluoromethyl)phenyl)-2,2,3,3,3-pentafluoropropanamide with triethylamine (**7b′**). According to the NMR analysis and X-ray crystallography data, ratio for this **7b′** complex [**7b**:Et_3N] is 1:1 (Figures 3, S1-S3, SI text).

*Preparation **of 8b-e**, **8g-h**, **9e-f**, **10a**, **11b***

To a solution of trifluoromethylphenyl amine (10 mmol) in THF (12 mL), acid chloride (10.5 mmol, for **8c**-**e**, **9e**, **9f**, **10a** ,**11b**) or acid anhydride (10.5 mmol, for **8b**, **8g**-**h**) was added at 0 °C and the mixture was stirred continuously for 0.2-4 h at 25 °C (Figure 2, route A). The reaction mixture was diluted and extracted with ethyl acetate (40 mL), washed with sat. aq. $NaHCO_3$ (3 x 60 mL) and the organic layer was dried over anhydrous Na_2SO_4. Evaporation of the solvent and recrystallization from hexane (for **9f**, **11b**) or hexane/ethyl acetate (for **8b**, **8d-e**, **8g-h**), or purification by silica gel column chromatography using hexane/ethyl acetate as a eluent (for **8c**, **10a**) gave pure compounds **8b-e**, **8g-h**, **9e-f**, **10a**, **11b** in yields of 70-86 %.

*Preparation of **9a-d***

To a solution of 2-trifluoromethylphenyl amine (10 mmol) in THF (12 mL), acid anhydride (10.5 mmol) was added at 0°C, the mixture was stirred continuously and then refluxed (66 °C) for 16 h (Figure 2, route B). The reaction mixture was diluted and extracted with ethyl acetate (40 mL), washed with sat. aq. $NaHCO_3$ (3 x 60 mL) and the organic layer dried over anhydrous Na_2SO_4. Evaporation of the solvent and recrystallization from hexane/ethyl acetate (**9a**, **9c**) hexane (**9b**), or purification by silica gel column chromatography using hexane/ethyl acetate as a eluent (**9d**) gave the pure compounds **9a-d** in yields of 73-97 %.

*Preparation of **12a***

To a solution of 2,6-Dichloro-4-(trifluoromethyl)aniline (10 mmol) in toluene (12 mL), 3-cyclohexylpropanoyl chloride (10.5 mmol) was added in the presence of Et3N (10.5 mmol) and stirring continued for 6 h under reflux (110°C) (Figure 2, route C). The reaction mixture was diluted and extracted with ethyl acetate (40 mL), washed with sat. aq. $NaHCO_3$ (3 x 60 mL) and the organic layer dried over anhydrous Na_2SO_4. Evaporation of the solvent and recrystallization from hexane resulted in compound **12a** in 76% yield. See the Supporting Information for yields, melting points, NMR and mass spectral data.

X-ray crystallography

Crystals were grown using the following procedure: complex **7b′** (20 mg) was dissolved in hot hexane (0.5 mL) in a 3 mL vial and left at 23°C with a loose lid for slow evaporation of solvent from the solution of the compound until saturation was reached and crystals formed.

The structure (**7b′**) was solved and refined in SHELXTL2014,[24] using full-matrix least-squares refinement (Bruker-AXS, Madison, Wisconsin, USA). The non-H atoms were refined with anisotropic thermal parameters and all of the H atoms were calculated in idealized positions and refined riding on their parent atoms. The amine proton H2 was obtained from a Difference

Fourier map and refined freely. In the final cycle of refinement, 4598 reflections (of which 4144 are observed with $I > 2\sigma(I)$) were used to refine 269 parameters and the resulting R_1, wR_2 and S (goodness of fit) were 2.45%, 6.33% and 1.051, respectively.

Fungicidal bioassay

Isolates of *Colletotrichum acutatum* Simmonds, *C. fragariae* Brooks, and *C. gloeosporioides* (Penz.) Penz. and Sacc. were obtained from B. J. Smith, (USDA, ARS, Poplarville, MS). Cultures of *Phomopsis viticola* (Sacc.) Sacc. and *P. obscurans* (Ellis & Everh.) were obtained from Mike Ellis (The Ohio State University, Columbus, OH), and *B. cinerea* Pers. and *F. oxysporum* Schlechtend were isolated at USDA-ARS, NPURU (Oxford, MS). The three *Colletotrichum* species and *P. obscurans* were isolated from strawberry (*Fragaria x ananassa* Duchesne), while *P. viticola* and *B. cinerea* were isolated from commercial grape (*Vitis vinifera* L.) and *F. oxysporum* from orchid (*Cynoches* sp.).

Direct bioautography assay for activity against plant pathogenic fungi

Matrix bioautography was used to screen of TFMPAs onto a silica plate. *Colletotrichum* species were used as test organisms to identify the antifungal activity. Conidia of *C. fragariae, C. acutatum and C. gloeosporioides* suspensions were adjusted to $3.0x10^5$ conidia/mL with liquid potato-dextrose broth (PDB, Difco, Detroit, MI) and 0.1% Tween-80. Using a 50 mL chromatographic sprayer, each glass silica gel thin layer chromatography (TLC) plate with fluorescent indicator (250 mm, Silica Gel GF Uniplate, Analtech, Inc., Newark, DE) was sprayed lightly (until damp) three times with the conidial suspension. Inoculated plates were placed in a 30 x 13 x 7.5 cm moisture chamber (398-C, Pioneer Plastics, Inc. Dixon, KY) and incubated in a growth chamber at 24 ± 1^oC with 12 h photoperiod under 60 ± 5 $\mu mols \bullet m^{-2} \bullet sec^{-1}$ light with a Li-Cor Quantum/Radiometer/Photometer (Model LI-250 Light Meter, Lincoln, NE USA).

279 The diameter of the clear zones of fungal growth inhibition were measured 4 d after
280 treatment.[14] Clear zones appearing against a dark background on the TLC plate represent
281 regions where fungal mycelia or reproductive stroma are not present. Direct bioautography is an
282 effective technique in evaluating extracts or pure compounds that are lipophilic.[25] Sensitivity of
283 each fungal species in response to each test compound was determined by comparing the mean
284 diameter of the inhibitory zones.

285 *Microdilution broth assay*

286 A standardized 96-well micro-dilution broth assay developed by Wedge and Kuhajek
287 (1998)[26] and validated by Abril et al.[27] was used to evaluate antifungal activity towards *B.*
288 *cinerea, C. acutatum, C. fragariae, C. gloeosporioides, P. viticola, P. obscurans* and *F.*
289 *oxysporum*. Azoxystrobin and captan are registered for control of anthracnose (caused by
290 *Colletotrichum* spp.) and Botrytis fruit rot (caused by *B. cinerea*).[28] For this reason, captan
291 (Phthalimide, multi-site inhibitor) and azoxystrobin (strobilurin class, Quinone outside inhibitor
292 (QoI) with different modes of action were used as internal fungicide standards in all assays.

293 Each test fungus was challenged in a dose-response format using test compounds where the
294 final treatment concentrations were 0.3, 3.0 and 30.0 µM. Sixteen wells containing broth and
295 inoculum served as positive controls. Eight wells containing solvent at the appropriate
296 concentration and broth without inoculum were used as negative controls. The experiments were
297 repeated three times. Fungal growth was then evaluated by measuring absorbance of each well
298 at 620 nm using a microplate photometer (Packard Spectra Count, Packard Instrument Co.,
299 Downers Grove, IL). Mean absorbance values and standard errors were used to evaluate fungal
300 growth at 48 h and 72 h except for *P. obscurans* and *P. viticola* the data were recorded at 144 h.
301 Means for percent inhibition of each fungus at each dose of test compound relative to untreated
302 positive growth controls were used to evaluate fungal growth. The SAS system analysis of

variance procedure (Statistical Analysis System, Cary, North Carolina) was used to identify significant factors, and Fisher's protected LSD was used to separate means.[29]

Acknowledgements

We thank J. Linda Robertson and Ramona Pace for assistance with the bioautography and microtiter assays as well as the Nucleic Magnetic Resonance, Mass Spectrometry facilities and the Center for X-Ray Crystallography of the University of Florida. K. A. A. wishes to acknowledge the National Science Foundation and the University of Florida for funding of the purchase of the X-ray equipment.

This work was supported by the Deployed War-Fighter Protection Research Program and funded by the United States Department of Defense through the Armed Forces Pest Management Board, Agreement 60-0208-4-001 and under USDA Specific Cooperative Agreements 58-0208-0-068 and 58-0208-5-001.

Author Contribution Statement

M. T., N. T., K. A. A. and D. E. W. performed the experiments, analyzed the data and wrote the paper. U. R. B and K. J. L. contributed samples/reagents/materials/analysis tools and analyzed the data. M. T., N. T. and U. R. B conceived and designed the experiments.

References

[1] D. Ivic, in 'Fungicides', Ed. O. Carisse, IntechOpen, 2010, p.3. https://doi.org/10.5772/13766

[2] L. P. Gianessi, N. Reigner, 'The Value of Fungicides in U.S. Crop Production', Croplife foundation, Crop Protection Research Institute (CPRI), 2005.

Available at: https://croplifefoundation.org/wp-content/uploads/2016/11/fungicide-full-report.pdf; Accessed March 19, 2019.

[3] M. T. McGrath, 'What are Fungicides?', *The Plant Health Instructor* **2004**. Posted in Topics in Plant Pathology, Introductory Plant Pathology, APSnet Education Center. https://doi:10.1094/phi-i-2004-0825-01;

[4] J. P. Damicone, 'Fungicide Resistance Management', Division of Agricultural Sciences and Natural Resources, Oklahoma State University, USA, **2009**, *EPP-7663*, 1 - 8. Available at: http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Version-6953/EPP-7663web.pdf; Accessed March 19, 2019.

[5] D. E. Wedge, J. C. G. Galindo, F. A. Macias, 'Fungicidal Activity of Natural and Synthetic Sesquiterpene Lactone Analogs', *Phytochemistry* **2000**, *53*, 747 - 757. http://dx.doi.org/10.1016/s0031-9422(00)00008-x

[6] U. Gisi, M. Waldner, N. Kraus, P. H. Dubuis, H. Sierotzki, 'Inheritance of Resistance to Carboxylic Acid Amide (CAA) Fungicides in *Plasmopara viticola*', *Plant Pathol.* **2007**, *56*, 199 - 208. https://doi.org/10.1111/j.1365-3059.2006.01512.x

[7] R. Tang, L. Jin, C. Mou, J. Yin, S. Bai, D. Hu, J. Wu, S. Yang, B. Song, 'Synthesis, Antifungal and Antibacterial Activity for Novel Amide Derivatives Containing a Triazole Moiety', *Chem. Cent. J.* **2013** *7*, p. 30. https://doi.org/10.1186/1752-153X-7-30

[8] P. Maienfisch, R. G. Hall, 'The Importance of Fluorine in the Life Science Industry', *Chimia* **2004**, *58*, 93 – 99. http://dx.doi.org/10.2533/000942904777678091

[9] W. K. Hagmann, 'The Many Roles for Fluorine in Medicinal Chemistry', *J. Med. Chem.* **2008**, *51*, 4359 - 4369. https://pubs.acs.org/doi/10.1021/jm800219f

[10] G. Theodoridis, in 'Advances in Fluorine Science (Fluorine and the Environment: Agrochemicals, Archaeology, Green Chemistry and Water)', ed. A. Tressaud, University

Bordeaux, Bordeaux, France, 2006, p. 121 - 175. http://dx.doi.org/10.1016/s1872-0358(06)02004-5

[11] F. Araki, K. Yabutani, 'Development of a Systemic Fungicide, Flutolanil', *J. Pestic. Sci.* **1993**, *18*, 2, S69 - S77. https://doi.org/10.1584/jpestics.18.2_S69

[12] L. D. Thiessen, J. E. Woodward, 'Diseases of Peanut Caused by Soilborne Pathogens in the Southwestern United States', *ISRN Agron.* **2012**, ID 517905. http://dx.doi.org/10.5402/2012/517905

[13] http://www.phi-base.org/images/fracCodeList.pdf; Accessed March 19, 2019.

[14] M. Tsikolia, U. R. Bernier, M. R. Coy, K. C. Chalaire, J. J. Becnel, N. M. Agramonte, N. Tabanca, D. E.Wedge, G. G. Clark, K. J. Linthicum, D. R. Swale, J. R. Bloomquist, 'Insecticidal, Repellent and Fungicidal Properties of Novel Trifluoromethylphenyl Amides', *Pestic. Biochem. Physiol.* **2013**, *107*, 138 - 147. https://doi.org/10.1016/j.pestbp.2013.06.006

[15] http://www.cas.org/products/scifinder; Accessed April 11, 2016.

[16] M. Tsikolia, U. R. Bernier, N. M. Agramonte, A. S. Estep, J. J. Becnel, N. Tabanca, K. J. Linthicum, A. D. Gross, P. M. Guerin, T. Kröber, J. R. Bloomquist, 'Insecticidal and Repellent Properties of Novel Trifluoromethylphenyl Amides II', *Pestic. Biochem. Physiol.* **2018**, *151*, 40 - 46. https://doi.org/10.1016/j.pestbp.2018.08.006

[17] H. C. Stecker, 'Bistrifluoromethyl Anilides', U.S. Pat. *3331874A*, 1967.

[18] S. Hwang, S. Y. Choi, J. H. Lee, S. Kim, J. In, S. K. Ha, E. Lee, T-Y. Kim, S. Y. Kim, S. Choi, S. Kim, 'Identification of a Potent and Noncytotoxic Inhibitor of Melanin Production', *Bioorg. Med. Chem.* **2010**, *18*, 5602 - 5609. https://doi.org/10.1016/j.bmc.2010.06.034

[19] H. C. Stecker, 'Germicidal Poly(trifluoromethyl)anilides', Fr. Pat. 1372475, 1964.

[20] T. Kuragano, S. Nakamura, K. Minami, I. Minamida, T. Okauchi, 'Preparation of β-(Phenylamino)styrene and Analogs as Insecticides and Fungicides', *Jpn. Kokai Tokkyo Koho*. Jpn. Pat. 08295663 A, 1996.

[21] B. D. Andrews, I. D. Rae, B. E. Reichert, 'Intramolecular Hydrogen Bonding in 2'-Substituted Anilides', *Tetrahedron Lett.* **1969**, *23*, 1859 - 1862. https://doi.org/10.1016/S0040-4039(01)88032-1

[22] K. Fukui, H. Kitano, R. Ijiri, Y. Inamoto, T. Matsufuji, 'Studies on Aromatic Fluorine Compounds. II. Preparations of Fluorin-Containing Anilines and their Derivatives', *Nippon. Kagaku. Zasshi*. **1958**, *79*, 889 - 894.

[23] R. G. Jones, '*Ortho* and *Para* Substituted Derivatives of Benzotrifluoride', *J. Am. Chem. Soc.* **1947**, *69*, 2346 - 2350. https://pubs.acs.org/doi/abs/10.1021/ja01202a028

[24] G. M. Sheldrick, 'A short history of SHELX', *Acta Crystallogr., Sect. A: Found. Crystallogr*. **2008**, *64*, 112 - 122. https://doi.org/10.1107/S0108767307043930

[25] N. Tabanca, D. E. Wedge, X. Wang, B. Demirci, K. H. Baser, L. Zhou, S. J. Cutler, 'Chemical Composition and Antifungal Activity of *Angelica sinensis* Essential Oil Against Three Colletotrichum species', *Nat. Prod. Commun.* **2008**, *3*, 1073 - 1078. https://pubag.nal.usda.gov/download/55176/PDF

[26] D. E. Wedge, J. M. Kuhajek, 'A Microbioassay for Fungicide Discovery', *SAAS Bull. Biochem. Biotechnol.* **1998**, *11*, 1 - 7. https://www.ars.usda.gov/research/publications/publication/?seqNo115=91373

[27] M. Abril, K. J. Curry, B. J. Smith, D. E. Wedge, 'Improved Microassays Used to Test Natural Product-Based and Conventional Fungicides on Plant Pathogenic Fungi', *Plant Dis.* **2008**, *92*, 106 - 112. http://dx.doi.org/10.1094/PDIS-92-1-0106

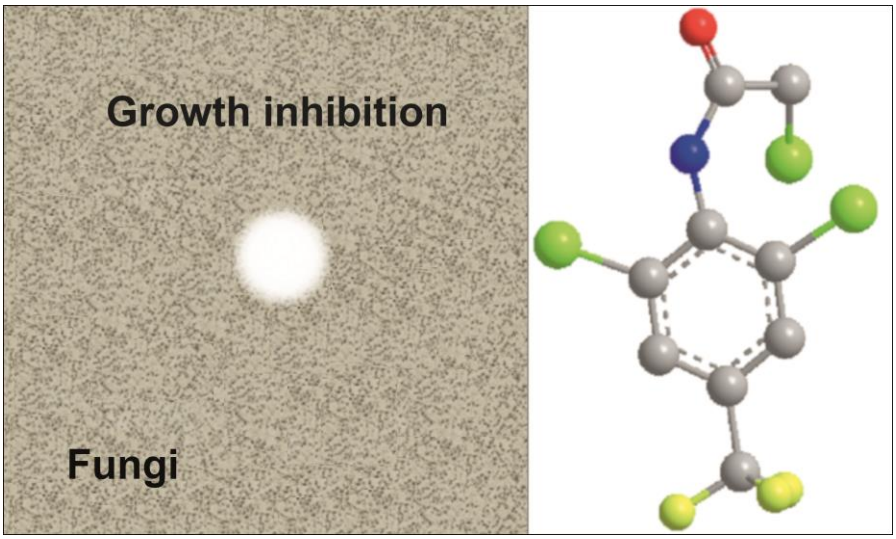
[28] D. E. Wedge, B. J. Smith, J. P. Quebedeaux, R. J. Constantin, 'Fungicide Management Strategies for Control of Strawberry Fruit Rot Diseases in Louisiana and Mississippi', *Crop Prot.*, **2007**, *26*, 1449 - 1458. https://doi.org/10.1016/j.cropro.2006.12.007

[29] R. G. D. Steel, J. H. Torrie, in 'Principles and Procedures of Statistics: A Biometrical Approach', ed. C. Napier, J. W. Maisel, 2nd ed.; McGraw-Hill: New York, NY, USA, 1980, p. 172. https://trove.nla.gov.au/work/9171434?q&sort=holdings+desc&_=1552932007108&versionId=49088515

10.1002/cbdv.201800618

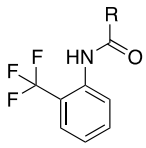
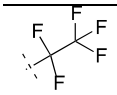
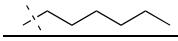
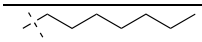
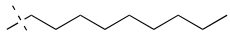
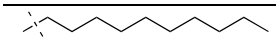
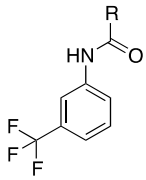
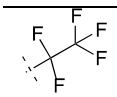
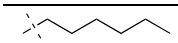
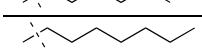
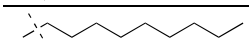
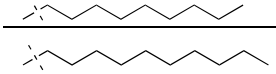
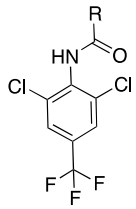
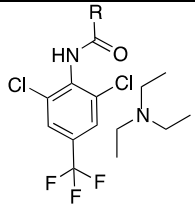
Graphical abstract:

Development of new fungicides is an important research objective. This work revealed some lead structures for further development of active novel compounds.



10.1002/cbdv.201800618

436 **Table 1.** The 33 trifluoromethylphenyl amides used in this study.
437

Entry	ID	Compound name	Structure	R
1.	**5a**	2-chloro-*N*-(2-(trifluoromethyl)phenyl)acetamide		
2.	**5b**[a]	2,2,3,3,3-pentafluoro-N-(2-(trifluoromethyl)phenyl)propanamide		
3.	**5c**	*N*-(2-(trifluoromethyl)phenyl)heptanamide		
4.	**5d**	*N*-(2-(trifluoromethyl)phenyl)octanamide		
5.	**5e**	*N*-(2-(trifluoromethyl)phenyl)decanamide		
6.	**5f**[a]	*N*-(2-(trifluoromethyl)phenyl)undecanamide		
7.	**6a**	2-chloro-*N*-(3-(trifluoromethyl)phenyl)acetamide		
8.	**6b**[a]	2,2,3,3,3-pentafluoro-*N*-(3-(trifluoromethyl)phenyl)propanamide		
9.	**6c**	*N*-(3-(trifluoromethyl)phenyl)heptanamide		
10.	**6d**[a]	*N*-(3-(trifluoromethyl)phenyl)octanamide		
11.	**6e**	*N*-(3-(trifluoromethyl)phenyl)decanamide		
12.	**6f**[a]	*N*-(3-(rifluoromethyl)phenyl)undecanamide		
13.	**7a**	2-chloro-*N*-(2,6-dichloro-4-(trifluoromethyl)phenyl)acetamide		
14.	**7b**	*N*-(2,6-dichloro-4-(trifluoromethyl)phenyl)-2,2,3,3,3-pentafluoropropanamide		
15.	**7b′**[a]	*N*-(2,6-dichloro-4-(trifluoromethyl)phenyl)-2,2,3,3,3-pentafluoropropanamide with triethylamine		

438
439
440
441
442
443
444
445
446
447
448
449
450

10.1002/cbdv.201800618

Table 1. The 33 trifluoromethylphenyl amides used in this study (contd).

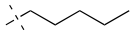
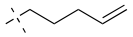
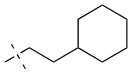
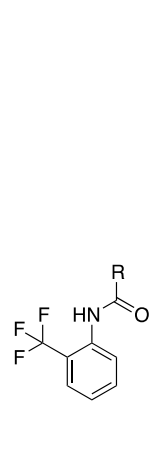
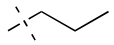
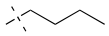
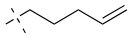
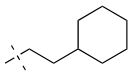
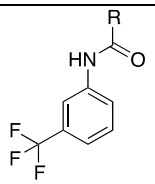
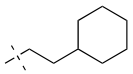
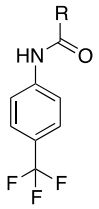
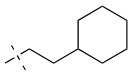
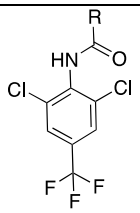
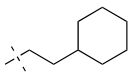
Entry	ID	Compound name	Structure	R
16.	**8a**	*N*-(3,5-bis(trifluoromethyl)phenyl)acetamide		
17.	**8b**[a]	*N*-(3,5-bis(trifluoromethyl)phenyl)hexanamide		
18.	**8c**[a]	*N*-(3,5-bis(trifluoromethyl)phenyl)hex-5-enamide		
19.	**8d**[a]	*N*-(3,5-bis(trifluoromethyl)phenyl)-3-cyclohexylpropanamide		
20.	**8e**	*N*-(3,5-bis(trifluoromethyl)phenyl)-2-methylbenzamide		
21.	**8f**	*N*-(3,5-bis(trifluoromethyl)phenyl)-2-chloroacetamide		
22.	**8g**	*N*-(3,5-bis(trifluoromethyl)phenyl)-2,2,2-trifluoroacetamide		
23.	**8h**	*N*-(3,5-bis(trifluoromethyl)phenyl)-2,2,3,3,3-pentafluoropropanamide		

Table 1. The 33 trifluoromethylphenyl amides used in this study (contd).

Entry	ID	Compound name	Structure	R
24.	**9a**	*N*-(2-(trifluoromethyl)phenyl)acetamide		
25.	**9b**	*N*-(2-(trifluoromethyl)phenyl)propionamide		
26.	**9c**	*N*-(2-(trifluoromethyl)phenyl)butyramide		
27.	**9d**	*N*-(2-(trifluoromethyl)phenyl)pentanamide		
28.	**9e**[a]	*N*-(2-(trifluoromethyl)phenyl)hex-5-enamide		
29.	**9f**[a]	3-cyclohexyl-*N*-(2-(trifluoromethyl)phenyl)propanamide		
30.	**10a**[a]	3-cyclohexyl-*N*-(3-(trifluoromethyl)phenyl)propanamide		
31.	**11a**	*N*-[4-(trifluoromethyl)phenyl]acetamide		
32.	**11b**[a]	3-cyclohexyl-*N*-(4-(trifluoromethyl)phenyl)propanamide		
33.	**12a**[a]	3-cyclohexyl-*N*-(2,6-dichloro-4-(trifluoromethyl)phenyl)propanamide		

[a]Novel compounds. See references for other compounds: **5-7**;[14] **8a**;[17] **8e**;[18] **8f,g**;[19] **8h**;[20] **9a**;[21] **9b,c,d**;[22] **11a**.[23]

10.1002/cbdv.201800618

Table 2. Antifungal activity results of amides **6a, 7a, 7b, 8f, 8h, 10a** and **11a** using direct bioautography against three *Colletotrichum* Species.

Compounds	**Mean zone diameter of fungal growth inhibition in mm (SD)** [a]					
	C. acutatum		***C. fragariae***		***C. gloeosporioides***	
Concentration	**12 mM**		**12 mM**		**12 mM**	
Applied	**4 µL**	**8 µL**	**4 µL**	**8 µL**	**4 µL**	**8 µL**
6a	5.5 (0.7)	13.0 (0.7)	6.0 (0)	8.0 (1.4)	Diffuse	Diffuse
7a	9.5 (0.7)	11.0 (1.4)	9.5 (2.1)	12.5 (0.7)	11.5 (0.7)	Diffuse
7b	9.0 (0)	10.5 (0.7)	10.0 (1.4)	12.5 (0.7)	9.00 (0)	12.5 (0.7)
7b′	10.5 (0.7)	12 (0)	8.5 (0.7)	11.5 (0.7)	8.5 (0.7)	8.5 (0.7)
8f	2.0 (0)	3.0 (0)	2.7 (0)	4.3 (0.6)	2.0 (0)	3.0 (0)
8h	2.0 (0)	2.3 (0.5)	3.0 (0)	3.0 (0)	2.0 (0)	3.0 (0)
10a	2.0 (0)	3.0 (0)	2.0 (0)	3.3 (0.5)	2.0 (0)	3.7 (1.1)
11a	2.0 (0)	3.0 (0)	2.0 (0)	4.3 (1.5)	Diffuse	Diffuse
Agrochemical standards						
	C. acutatum		***C. fragariae***		***C. gloeosporioides***	
Concentration	2 mM		2 mM		2 mM	
Applied	**2 µL**		**2 µL**		**2 µL**	
Benomyl	Diffuse		21.5 (2.1)		Diffuse	
Captan	12.5 (0.7)[b]		13.5 (0.7)		15.0 (4.2)	
Azoxystrobin	Diffuse		28.5 (0.7)		Diffuse	

[a] Mean inhibitory zone diameter and standard deviation (SD) were used to determine the level of antifungal activity against each fungal species,

Diffuse: Diffuse zone is indicated by the growth inhibitory zone that appears thinly populated with mycelia and reproductive hyphae and the zone margin is not sharply contrasted.

10.1002/cbdv.201800618

Table 3. Mean fungal growth inhibition (%) of compounds **6a, 7a**, **7b**, **7b′ 8f**, **8h**, **10a** and **11a** against fungal plant pathogens at 30 µM.

Compound	Mean fungal growth inhibition, % (SEM)[a]					
	C. acutatum **at 48 h**	***B. cinerea*** **at 72 h**	***P. viticola*** **at 144 h**	***P. obscurans*** **at 144 h**	***C. fragariae*** **at 48 h**	***C. gloeosporioides*** **at 48 h**
6a	29.2 (8.2)	40.0 (13.1)	40.4 (11.0)	45.5 (34.2)	18.4 (5.1)	0.0 (3.9)
7a	66.8 (5.4)	49.0 (9.0)	95.3 (1.9)	59.7 (22.6)	35.4 (4.8)	4.2 (2.2)
7b	44.5 (5.9)	23.2 (33.7)	51.4 (4.1)	58.6 (25.7)	42.1 (2.1)	7.5 (2.8)
7b′	47.3(4.1)	12.8 (16.2)	89.5 (2.6)	57.6 (20.5)	54.7 (1.2)	17.3 (11.3)
8f	[b]NA	NA	20.9 (2.3)	42.9 (16.9)	NA	NA
8h	NA	NA	18.0 (2.9)	59.4 (3.4)	NA	NA
10a	NA	NA	17.4 (0.9)	63.6 (4.0)	NA	NA
11a	NA	NA	17.4 (0.9)	62.4 (3.9)	NA	NA
Azoxystrobin	41.8 (10.6)	96.2 (0.8)	99.9 (0.5)	98.0 (9.4)	29.0 (3.1)	31.3 (6.3)
Captan	86.9 (12.6)	98.4 (1.2)	88.4 (8.8)	95.9 (1.7)	97.8 (6.4)	81.5 (8.9)

[a]SEM, Standard error of the mean.

[b]NA, not applicable

10.1002/cbdv.201800618

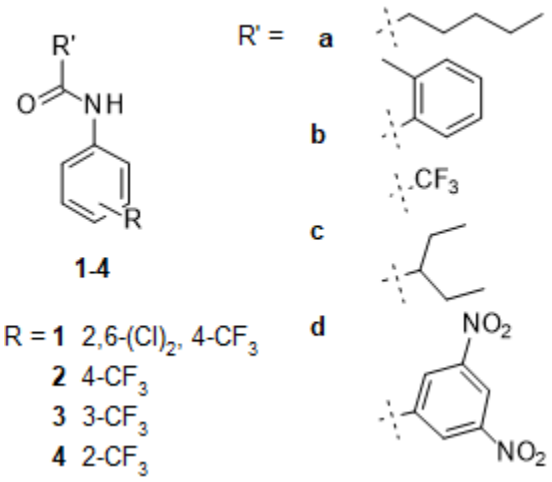


Figure 1. TFMPAs **1-4** we synthesized and tested in our previous work.[14]

10.1002/cbdv.201800618

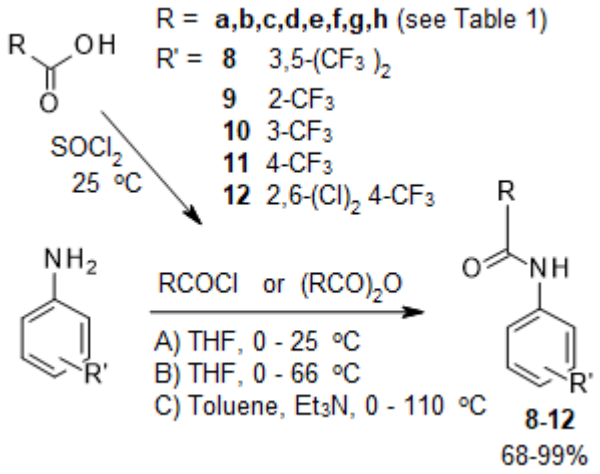


Figure 2. Synthesis of TFMPAs **8-12**.

10.1002/cbdv.201800618

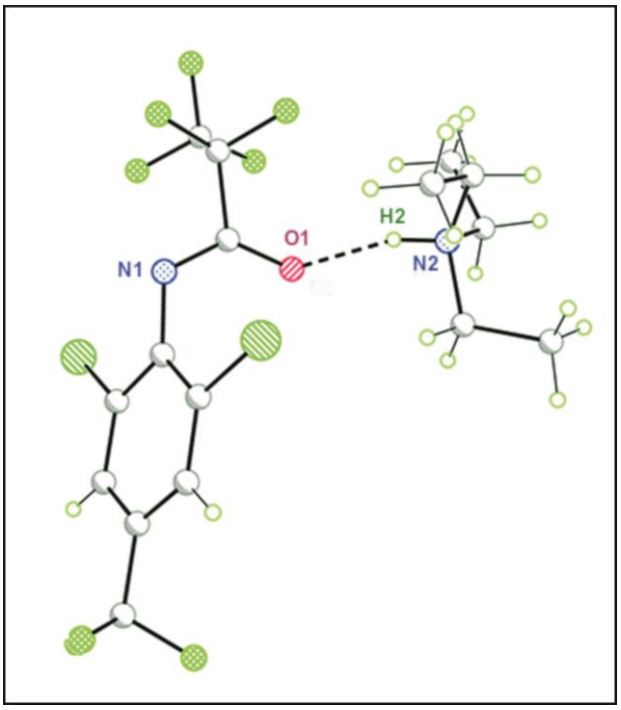


Figure 3. X-ray crystal structure for the complex **7b′**.

10.1002/cbdv.201800618

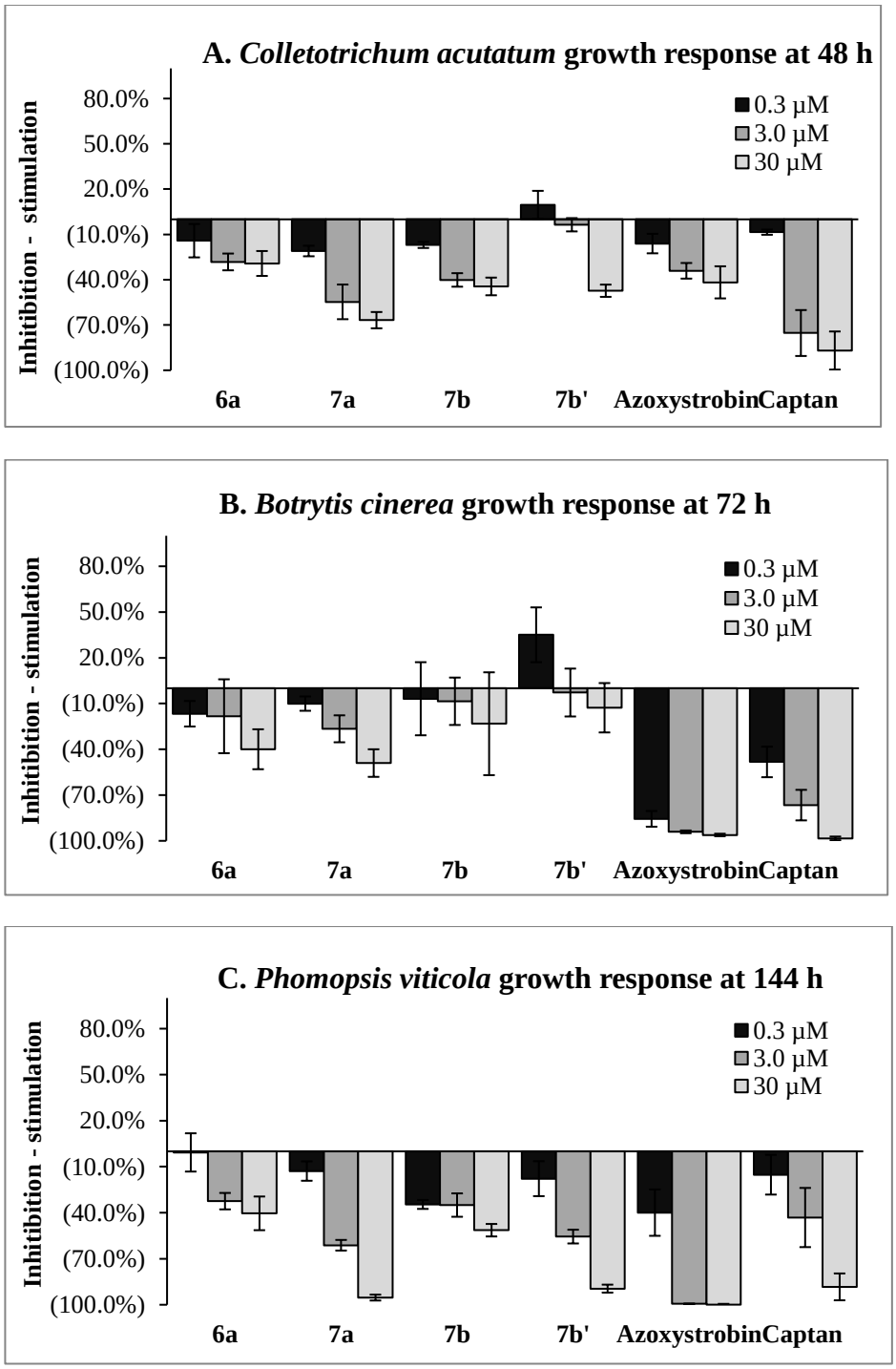


Figure 4. Growth inhibition of various fungi using a 96 well micro-dilution broth assay in a 3-point dose response to **6a**, **7a**, **7b** and **7b′** compared to the commercial fungicide standards Azoxystrobin and captan. *Colletotrichum acutatum* (A), *Botrytis cinerea* (B), and *Phomopsis viticola* (C) exposures.

10.1002/cbdv.201800618

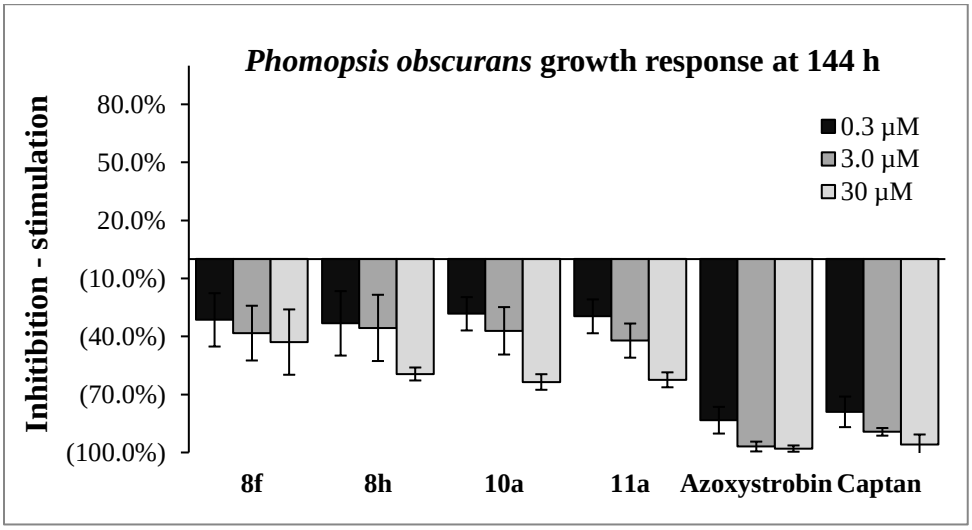


Figure 5. Growth inhibition of *Phomopsis obscurans* at 144 h using a 96 well micro-dilution broth assay in a 3-point dose response to **8f, 8h, 10a** and **11a** compared to the commercial fungicide standards azoxystrobin and captan.