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Fungicidal Properties of Some Novel Trifluoromethylphenyl Amides

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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 oxysporum*. 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 has 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*, *Colletotrichum fragariae*, *Colletotrichum gloeosporioides*, *Botrytis cinerea*, *Phomopsis viticola*, *Phomopsis obscurans*, micro-dilution broth assay, bioautography assay, X-ray crystallography, biological activity.

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

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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

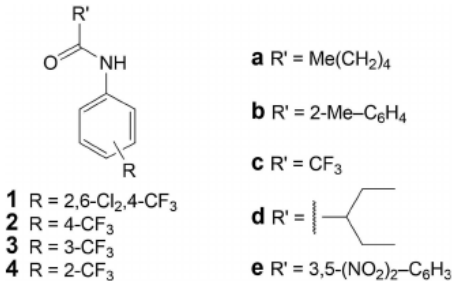
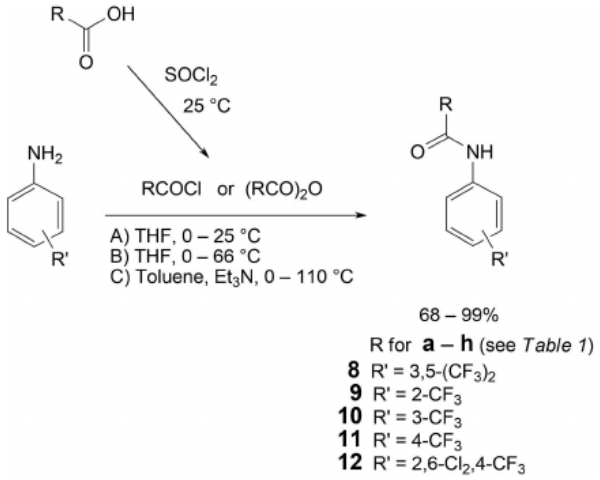


Figure 1. TFMPAs **1–4** synthesized and tested in our previous work.[14]

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 oxysporum* 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] (*Scheme 1*, *Table 1*).



Scheme 1. Synthesis of TFMPAs **8–12**.

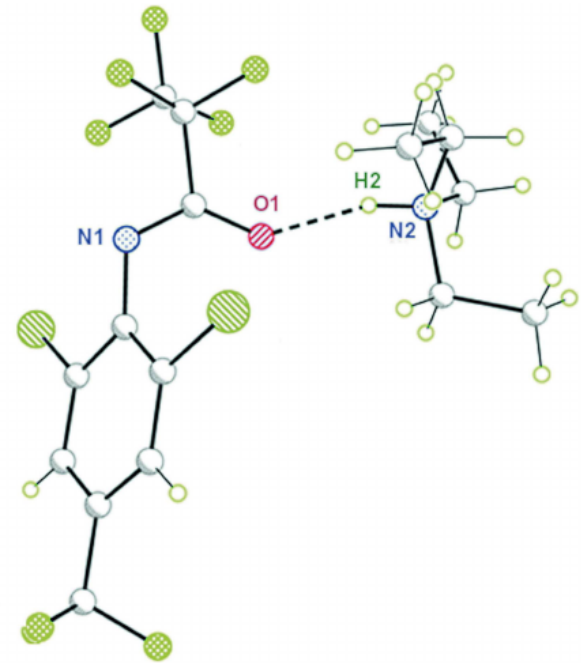


Figure 2. X-ray crystal structure for complex **7b**′.

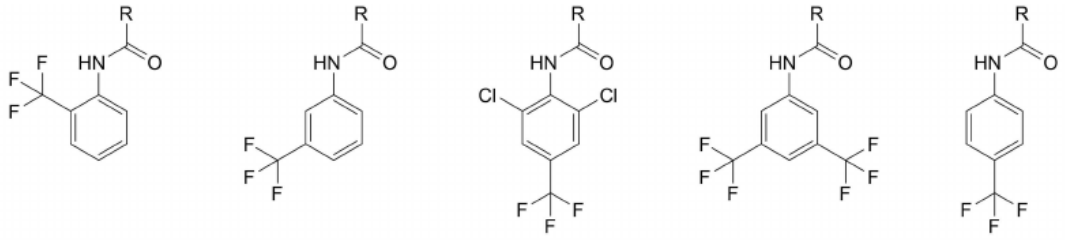
All 33 structures were evaluated for pesticidal properties, which included evaluation of insecticidal activity against mosquito species, as well as fungicidal effects against plant pathogens. The present work describes the fungicidal activity studies for these 33 TFMPAs. An X-ray crystallography study was performed for complex *N*-[2,6-dichloro-4-(trifluoromethyl) phenyl]-2,2,3,3,3-pentafluoropropanamide with triethylamine (**7b**′, *Figure 2*), 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] (*Scheme 1*, *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

Table 1. The 33 (trifluoromethyl)phenyl amides used in this study.



5, 9 6, 10 7, 12 8 11

Entry	ID	Compound name	R
1	**5a**	2-Chloro-*N*-[2-(trifluoromethyl)phenyl]acetamide	$ClCH_2$
2	**5b**[a]	2,2,3,3,3-Pentafluoro-*N*-[2-(trifluoromethyl)phenyl]propanamide	CF_3CF_2
3	**5c**	*N*-[2-(Trifluoromethyl)phenyl]heptanamide	$Me(CH_2)_5$
4	**5d**	*N*-[2-(Trifluoromethyl)phenyl]octanamide	$Me(CH_2)_6$
5	**5e**	*N*-[2-(Trifluoromethyl)phenyl]decanamide	$Me(CH_2)_8$
6	**5f**[a]	*N*-[2-(Trifluoromethyl)phenyl]undecanamide	$Me(CH_2)_9$
7	**6a**	2-Chloro-*N*-[3-(trifluoromethyl)phenyl]acetamide	$ClCH_2$
8	**6b**[a]	2,2,3,3,3-Pentafluoro-*N*-[3-(trifluoromethyl)phenyl]propanamide	CF_3CF_2
9	**6c**	*N*-[3-(Trifluoromethyl)phenyl]heptanamide	$Me(CH_2)_5$
10	**6d**[a]	*N*-[3-(Trifluoromethyl)phenyl]octanamide	$Me(CH_2)_6$
11	**6e**	*N*-[3-(Trifluoromethyl)phenyl]decanamide	$Me(CH_2)_8$
12	**6f**[a]	*N*-[3-(Trifluoromethyl)phenyl]undecanamide	$Me(CH_2)_9$
13	**7a**	2-Chloro-*N*-[2,6-dichloro-4-(trifluoromethyl)phenyl]acetamide	$ClCH_2$
14	**7b**	*N*-[2,6-Dichloro-4-(trifluoromethyl)phenyl]-2,2,3,3,3-pentafluoropropanamide	CF_3CF_2
15	**7b′**[a]	*N*-[2,6-Dichloro-4-(trifluoromethyl)phenyl]-2,2,3,3,3-pentafluoropropanamide with triethylamine	CF_3CF_2
16	**8a**	*N*-[3,5-Bis(trifluoromethyl)phenyl]acetamide	Me
17	**8b**[a]	*N*-[3,5-Bis(trifluoromethyl)phenyl]hexanamide	$Me(CH_2)_4$
18	**8c**[a]	*N*-[3,5-Bis(trifluoromethyl)phenyl]hex-5-enamide	$CH_2=CH(CH_2)_3$
19	**8d**[a]	*N*-[3,5-Bis(trifluoromethyl)phenyl]-3-cyclohexylpropanamide	$cHexCH_2CH_2$
20	**8e**	*N*-[3,5-Bis(trifluoromethyl)phenyl]-2-methylbenzamide	$2\text{-}Me\text{-}C_6H_4$
21	**8f**	*N*-[3,5-Bis(trifluoromethyl)phenyl]-2-chloroacetamide	$ClCH_2$
22	**8g**	*N*-[3,5-Bis(trifluoromethyl)phenyl]-2,2,2-trifluoroacetamide	CF_3
23	**8h**	*N*-[3,5-Bis(trifluoromethyl)phenyl]-2,2,3,3,3-pentafluoropropanamide	CF_3CF_2
24	**9a**	*N*-[2-(Trifluoromethyl)phenyl]acetamide	Me
25	**9b**	*N*-[2-(Trifluoromethyl)phenyl]propanamide	Et
26	**9c**	*N*-[2-(Trifluoromethyl)phenyl]butanamide	Pr
27	**9d**	*N*-[2-(Trifluoromethyl)phenyl]pentanamide	Bu
28	**9e**[a]	*N*-[2-(Trifluoromethyl)phenyl]hex-5-enamide	$CH_2=CH(CH_2)_3$
29	**9f**[a]	3-Cyclohexyl-*N*-[2-(trifluoromethyl)phenyl]propanamide	$cHexCH_2CH_2$
30	**10a**[a]	3-Cyclohexyl-*N*-[3-(trifluoromethyl)phenyl]propanamide	$cHexCH_2CH_2$
31	**11a**	*N*-[4-(Trifluoromethyl)phenyl]acetamide	Me
32	**11b**[a]	3-Cyclohexyl-*N*-[4-(trifluoromethyl)phenyl]propanamide	$cHexCH_2CH_2$
33	**12a**[a]	3-Cyclohexyl-*N*-[2,6-dichloro-4-(trifluoromethyl)phenyl]propanamide	$cHexCH_2CH_2$

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

intermolecular hydrogen bonds with biological targets.[14]

Out of 33 TFMPAs, 16 compounds were synthesized (**7b′**, **8–12**, *Scheme 1*, *Table 1*), 12 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) at 0–25 °C (**8b–8e**, **8g**, **8h**, **9e**, **9f**, **10a**, **11b**), or at 66 °C (**9a–9d**), or in toluene at 110 °C (**12a**) led to the production of

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

Compound	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[b]		21.5 (2.1)		Diffuse	
Captan	12.5 (0.7)		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. [b] 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.

TFMPAs **8–12** in yields of 68–99% (*Scheme 1*). Triethylamine (Et_3N) was used as a base for the preparation of amide **12a** (see the *Supporting Information* for compounds yields, melting points, NMR and mass spectral data).

X-Ray Crystallography

Complex **7b′** was formed in an attempt to synthesize **7b** using 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 the *Supporting Information* and *Figures S1* and *S2* for ^{1}H- and ^{13}C-NMR data 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 2* 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–5f** and **6b–6f** 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

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	NA[b]	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.

(*Figures 3* and *4*). 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 3A*). 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 3B*). 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 3C*, *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 4*), 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 3A* 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. oxysporum* (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′**); according to our previous work,[14] 2,6-dichloro-4-(trifluoromethyl)phenyl amide

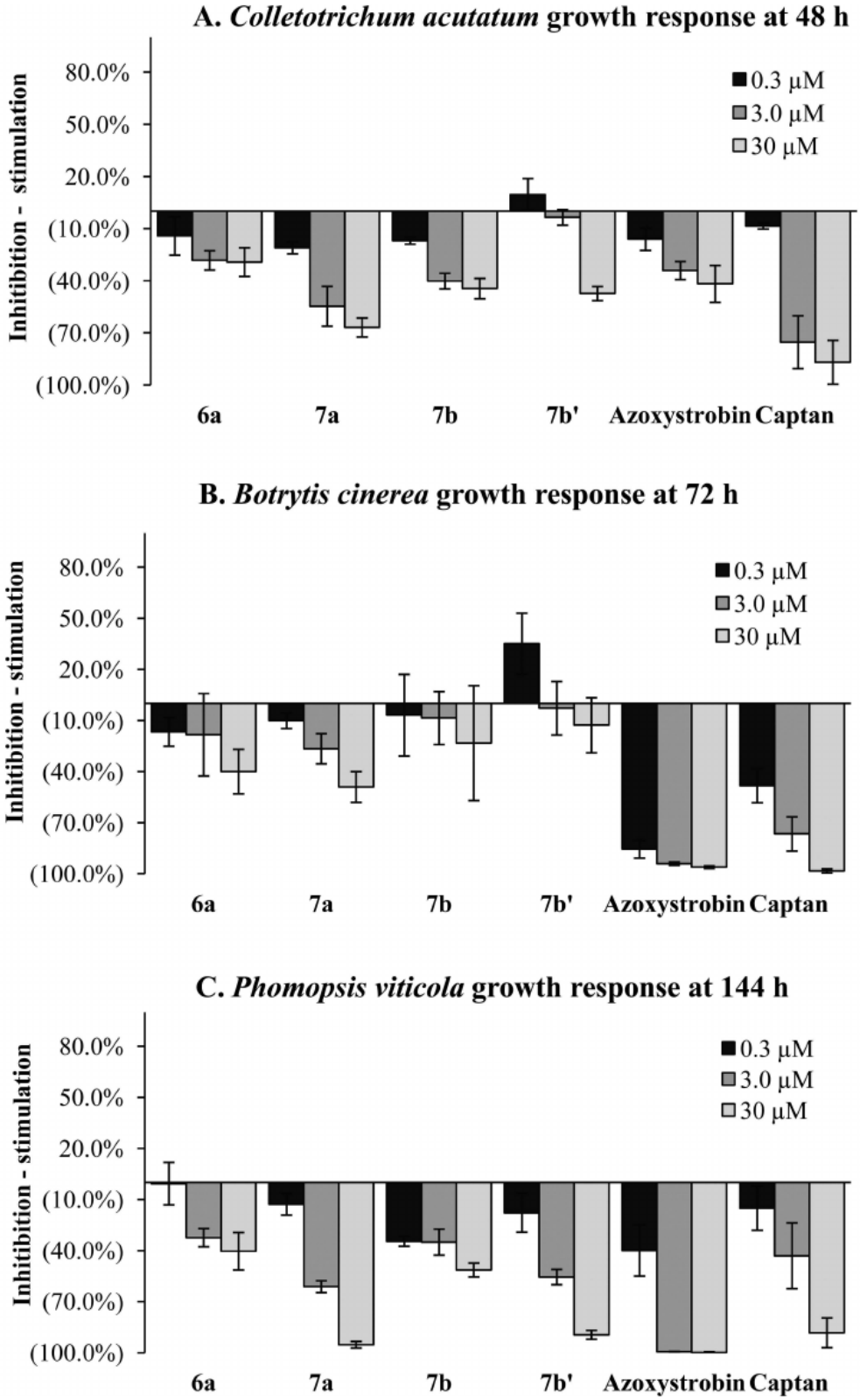


Figure 3. Growth inhibition of various fungi using a 96-well micro-dilution broth assay in a 3-point dose response to **6a**, **7a**, **7b** and **7b**′ compared to the commercial fungicide standards azoxystrobin and captan. A) *Colletotrichum acutatum*, B) *Botrytis cinerea*, and C) *Phomopsis viticola* exposures. Data are presented as means ± SEM, $n = 6$.

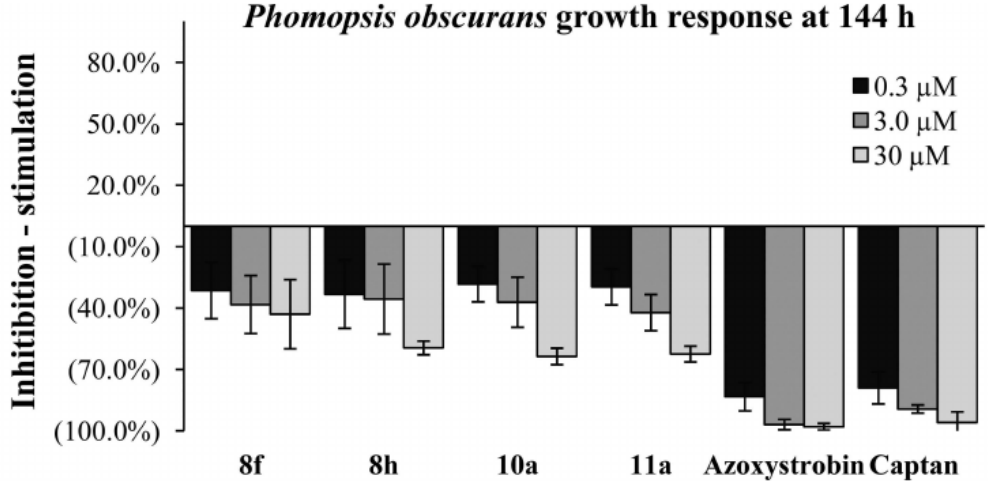


Figure 4. Growth inhibition of *Phomopsis obscurans* at 144 h using a 96-well micro-dilution broth assay in a 3-point dose response to 8f, 8h, 10a and 11a compared to the commercial fungicide standards azoxystrobin and captan. Data are presented as means ± SEM, $n = 6$.

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**–**5f** and **9a**–**9f** 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 in *Figure 3A* 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 3C*); 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-trifluoromethylphenyl 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-trifluoromethylphenyl 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 analogs is needed to establish proper structure–activity relationships for these leads.

Experimental Section

General

Melting points were determined on a hot-stage apparatus and are uncorrected. NMR analyses were performed at NMR Facility of the University of Florida in Gainesville, FL, USA. NMR spectra were recorded in $CDCl_3$ or (D_6)acetone with TMS (tetramethylsilane) as the internal standard for ^1H (500 MHz) and $CDCl_3$ or (D_6)acetone as the internal standard for ^{13}C (125 MHz); δ in ppm relative to Me_4Si as internal standard, J in Hz.

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 (Acros Organics, NJ, USA). The progress of a reaction was monitored by thin layer chromatography (TLC).

Synthesis

Sixteen compounds (**7b′**, **8–12**, 12 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*) were synthesized previously.[16]

Preparation of **7b′**

Route 1: To a solution of 2,6-dichloro-4-(trifluoromethyl)phenylamine (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 mixture was diluted and extracted with ethyl acetate (40 mL), washed with saturated aqueous $NaHCO_3$ (3×60 mL), and the organic layer was 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 2* and *S1–S3*, *Supporting Information*).

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

To a solution of trifluoromethylphenylamine (10 mmol) in THF (12 mL), acid chloride (10.5 mmol, for **8c – 8e**, **9e**, **9f**, **10a**, **11b**) or acid anhydride (10.5 mmol, for **8b**, **8g**, **8h**) was added at 0 °C and the mixture was stirred continuously for 0.2–4 h at 25 °C (*Scheme 1*, route A). The mixture was diluted and extracted with ethyl acetate (40 mL), washed with saturated aqueous $NaHCO_3$ (3×60 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**, **8e**, **8g**, **8h**), or purification by silica gel column chromatography using hexane/ethyl acetate as an eluent (for **8c**, **10a**) gave pure compounds **8b–8e**, **8g**, **8h**, **9e**, **9f**, **10a**, **11b** in yields of 70–86%.

Preparation of **9a–9d**

To a solution of 2-trifluoromethylphenylamine (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 (*Scheme 1*, route B). The mixture was diluted and extracted with ethyl acetate (40 mL), washed with saturated aqueous $NaHCO_3$ (3×60 mL) and the organic layer was 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 an eluent (**9d**) gave the pure compounds **9a–9d** 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 Et_3N (10.5 mmol) and stirring continued for 6 h under reflux (110 °C; *Scheme 1*, route C). The mixture was diluted and extracted with ethyl acetate (40 mL), washed with saturated aqueous $NaHCO_3$ (3×60 mL) and the organic layer was 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 of **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* J.H. Simmonds, *C. fragariae* A.N. Brooks, and *C. gloeosporioides* (Penz.) Penz. & 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* Schlecht. were isolated at USDA-ARS, NPURU (Oxford, MS). The three *Colletotrichum* species and *P. obscurans* were isolated from strawberry (*Fragaria* × *ananassa* Duchesne), while *P. viticola* and *B. cinerea* were isolated from commercial grape (*Vitis vinifera* L.) and *F. oxysporum* from orchid (*Cycnoches* sp.).

Direct Bioautography Assay for Activity against Plant Pathogenic Fungi

Matrix bioautography was used to screen 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 3.0×10^5 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 30×13×7.5 cm moisture chamber (398-C, Pioneer Plastics, Inc., Dixon, KY) and incubated in a growth chamber at 24±1 °C with 12 h photoperiod under 60±5 μmol $m^{-2} sec^{-1}$ light with a Li- Cor Quantum/Radiometer/Photometer (Model LI-250 Light Meter, Lincoln, NE, USA).

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

Microdilution Broth Assay

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

Each test fungus was challenged in a dose-response format using test compounds where the final treatment concentrations were 0.3, 3.0 and 30.0 μM. Sixteen wells containing broth and inoculum served as positive controls. Eight wells containing solvent at the appropriate concentration and broth without inoculum were used as negative controls. Standard errors of the means (SEM) are calculated from a sample size of $n = 6$. Samples were run in duplicate in independent experiments performed three times in time. Error bars in each figure (*Figures 3* and *4*) are graphical representation of the SEM and overlapping error bars for each compound at each concentration indicates means are not significantly different. Fungal growth was then evaluated by recording absorbance of each well at 620 nm using a microplate photometer (Packard Spectra Count, Packard Instrument Co., Downers Grove, IL). Mean absorbance values and standard

errors were used to evaluate fungal growth at 48 h and 72 h except for *P. obscurans* and *P. viticola* the data were recorded at 144 h. Means for percent inhibition of each fungus at each dose of test compound relative to untreated 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]

Supporting Information

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

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Author Contribution Statement

M.T., N.T., K.A.A. and D.E.W. performed the experiments, analyzed the data and wrote the article. 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.

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