



## Insecticidal, repellent and fungicidal properties of novel trifluoromethylphenyl amides <sup>☆</sup>

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### ABSTRACT

Twenty trifluoromethylphenyl amides were synthesized and evaluated as fungicides and as mosquito toxicants and repellents. Against *Aedes aegypti* larvae, *N*-(2,6-dichloro-4-(trifluoromethyl)phenyl)-3,5-dinitrobenzamide (**1e**) was the most toxic compound (24 h LC<sub>50</sub> 1940 nM), while against adults *N*-(2,6-dichloro-4-(trifluoromethyl)phenyl)-2,2,2-trifluoroacetamide (**1c**) was most active (24 h LD<sub>50</sub> 19.182 nM, 0.5 µL/insect). However, the 24 h LC<sub>50</sub> and LD<sub>50</sub> values of fipronil against *Ae. aegypti* larvae and adults were significantly lower: 13.55 nM and  $0.787 \times 10^{-4}$  nM, respectively. Compound **1c** was also active against *Drosophila melanogaster* adults with 24 h LC<sub>50</sub> values of 5.6 and 4.9 µg/cm<sup>2</sup> for the Oregon-R and 1675 strains, respectively. Fipronil had LC<sub>50</sub> values of 0.004 and 0.017 µg/cm<sup>2</sup> against the two strains of *D. melanogaster*, respectively. In repellency bioassays against female *Ae. aegypti*, 2,2,2-trifluoro-*N*-(2-(trifluoromethyl)phenyl)acetamide (**4c**) had the highest repellent potency with a minimum effective dosage (MED) of 0.039 µmol/cm<sup>2</sup> compared to DEET (MED of 0.091 µmol/cm<sup>2</sup>). Compound *N*-(2-(trifluoromethyl)phenyl)hexanamide (**4a**) had an MED of 0.091 µmol/cm<sup>2</sup> which was comparable to DEET. Compound **4c** was the most potent fungicide against *Phomopsis obscurans*. Several trends were discerned between the structural configuration of these molecules and the effect of structural changes on toxicity and repellency. *Para*- or *meta*- trifluoromethylphenyl amides with an aromatic ring attached to the carbonyl carbon showed higher toxicity against *Ae. aegypti* larvae, than *ortho*- trifluoromethylphenyl amides. *Ortho*- trifluoromethylphenyl amides with trifluoromethyl or alkyl group attached to the carbonyl carbon produced higher repellent activity against female *Ae. aegypti* and *Anopheles albimanus* than *meta*- or *para*- trifluoromethylphenyl amides. The presence of 2,6-dichloro- substitution on the phenyl ring of the amide had an influence on larvicidal and repellent activity of *para*- trifluoromethylphenyl amides.

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### 1. Introduction

The goal of this research is to discover new mosquito insecticides, repellents, and fungicides by synthesizing inexpensive novel

Abbreviations: DEET, *N,N*-diethyl-*m*-toluamide; MED, minimum effective dosage; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide; TLC, thin layer chromatography.

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compounds which may be active or lead to the discovery of additional active compounds based on structure–activity analysis. Compounds with a broad spectrum of activities would be ideal and could result in new products for eventual commercial use. Our approach is to evaluate a set of compounds with similar chemical base structures and varied substitutions. In this study, fluorine-containing chemicals were the focus because over the past decade they have become increasingly important in controlling agricultural pests. Compounds within this class are effective insecticides and fungicides [1]. Examples of pesticides that contain fluorine as a trifluoromethyl group include fipronil, flonicamid, and flubendiamide (Fig. 1). The inclusion of fluorine atoms or a trifluoromethyl group into small molecules can significantly increase their biological activity by promoting electrostatic interactions with biological

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| 14. ABSTRACT<br><p><b>Twenty trifluoromethylphenyl amides were synthesized and evaluated as fungicides and as mosquito toxicants and repellents. Against Aedes aegypti larvae, N-(2,6-dichloro-4-(trifluoromethyl)phenyl)-3,5-dinitrobenzamide (1e) was the most toxic compound (24 h LC50 1940 nM), while against adults N-(2,6-dichloro-4-(trifluoromethyl)phenyl)-2,2,2-trifluoroacetamide (1c) was most active (24 h LD50 19.182 nM, 0.5 IL/insect). However, the 24 h LC50 and LD50 values of fipronil against Ae. aegypti larvae and adults were significantly lower: 13.55 nM and 0.787 10 4 nM, respectively. Compound 1c was also active against Drosophila melanogaster adults with 24 h LC50 values of 5.6 and 4.9 lg/cm2 for the Oregon- R and 1675 strains, respectively. Fipronil had LC50 values of 0.004 and 0.017 lg/cm2 against the two strains of D. melanogaster, respectively. In repellency bioassays against female Ae. aegypti, 2,2,2-trifluoro-N-(2-(trifluoromethyl)phenyl)acetamide (4c) had the highest repellent potency with a minimum effective dosage (MED) of 0.039 lmol/cm2 compared to DEET (MED of 0.091 lmol/cm2). Compound N-(2-(trifluoromethyl)phenyl)hexanamide (4a) had an MED of 0.091 lmol/cm2 which was comparable to DEET. Compound 4c was the most potent fungicide against Phomopsis obscurans. Several trends were discerned between the structural configuration of these molecules and the effect of structural changes on toxicity and repellency. Para- or meta- trifluoromethylphenyl amides with an aromatic ring attached to the carbonyl carbon showed higher toxicity against Ae. aegypti larvae, than ortho- trifluoromethylphenyl amides. Ortho- trifluoromethylphenyl amides with trifluoromethyl or alkyl group attached to the carbonyl carbon produced higher repellent activity against female Ae. aegypti and Anopheles albimanus than meta- or para- trifluoromethylphenyl amides. The presence of 2,6-dichloro- substitution on the phenyl ring of the amide had an influence on larvicidal and repellent activity of para- trifluoromethylphenyl amides.</b></p> |                            |                                                     |
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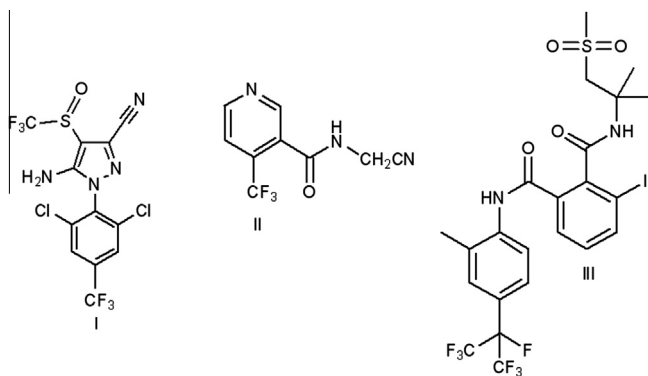


Fig. 1. Structures of (I) fipronil, (II) flonicamid, and (III) flubendiamide.

targets, increasing metabolic stability, and improving cellular membrane permeability and bioavailability [2–8].

The design of target molecules was based on an extensive literature search [9]. Previous reports of compounds with insecticidal, mosquito repellent or fungicidal activity provided valuable information on potential base structures. We then synthesized compounds comprised of trifluoromethylphenyl moieties attached to the amide nitrogen of the base structures. The trifluoromethyl groups were located in the *ortho*-, *meta*-, or *para*- positions on the *N*-phenyl ring, since there are reports describing promising insecticidal and repellent properties in all three different ring substitution positions. The amide groups within the molecule were retained since they are known to improve stability and provide the ability to establish intermolecular hydrogen bonds with biological targets. The addition of a fluorine or trifluoromethyl on the aryl ring increases lipophilicity and can strongly polarize the parent structure [5,10], and thus should significantly influence the biological activity of the molecule. A total of 20 trifluoromethylphenyl amides (14 of which were novel) were designed and synthesized based on the aforementioned criteria.

All compounds were evaluated for toxicity against *Aedes aegypti* larvae and adults, for repellency against adult female *Ae. aegypti* and *Anopheles albimanus*, and for fungicidal activity against *Colletotrichum fragariae*, *C. gloeosporioides*, *C. acutatum*, *Phomopsis obscurans*, *P. viticola*, *Botrytis cinerea* and *Fusarium oxysporum*. Selected compounds were evaluated for toxicity against *Drosophila melanogaster*.

## 2. Materials and methods

### 2.1. Synthesis of trifluoromethylphenyl amides 1–4

Twenty compounds were synthesized (Fig. 2, Table 1). Acid chlorides **5** were either commercially available or prepared *in situ* by overnight reaction of the corresponding carboxylic acid

with 20–25% excess of thionyl chloride at 20 °C. Acid anhydrides **6** were purchased from commercial sources. Reaction of 1.05 equivalent acyl chloride **5** or acid anhydride **6** with one equivalent of corresponding trifluoromethylphenyl amines in tetrahydrofuran (THF) (12 mL) at 0–25 °C led to the production of trifluoromethylphenyl amides **1–4** in yields of 69–97% (Fig. 2). Triethylamine ( $\text{Et}_3\text{N}$ ) for **1a** and **1c** (Fig. 2, route A) and sodium hydride (NaH), 60% for **1b**, **1d**, and **1e** (Fig. 2, route B) were used as the bases.

#### 2.1.1. General methods and materials

Melting points were determined on a hot-stage apparatus and are uncorrected. Nuclear Magnetic Resonance (NMR) analyses were performed at the NMR Facility of the University of Florida in Gainesville, FL, USA. NMR spectra were recorded in  $\text{CDCl}_3$  or  $\text{DMSO-d}_6$  with TMS (tetramethylsilane) as the internal standard for  $^1\text{H}$  (500 MHz) and  $\text{CDCl}_3$  or  $\text{DMSO-d}_6$  as the internal standard for  $^{13}\text{C}$  (125 MHz). Accurate masses were measured at the Mass Spectrometry Facility of the University of Florida, using a 6220 TOF-MS (Agilent Technologies) equipped with an electrospray and atmospheric pressure chemical ionization source. Samples were dissolved in dichloromethane and solutions introduced via direct injection. All reactions were carried out under argon atmosphere in anhydrous THF obtained from Acros Organics, NJ, USA. The progress of a reaction was monitored by thin layer chromatography (TLC).

#### 2.1.2. Procedures for the preparation of trifluoromethylphenyl amides 1–4

**2.1.2.1. Preparation of 1a and 1c.** To a solution of 2,6-dichloro-4-(trifluoromethyl)phenyl amine (10 mmol) in THF (12 mL), acid anhydride **6** (10.5 mmol) was added at 0 °C in the presence of  $\text{Et}_3\text{N}$  (10.1 mmol) and stirred continuously for 32 h at 65 °C (**1a**) and 24 h at 25 °C (**1c**) (Fig. 2, route A). The reaction mixture was diluted and extracted with ethyl acetate (40 mL), washed with sat. aq.  $\text{NaHCO}_3$  ( $3 \times 60$  mL) and the organic layer dried over anhydrous  $\text{Na}_2\text{SO}_4$ . Evaporation of the solvent and recrystallization from hexane/ethyl acetate (**1a**) or ethanol (**1c**) resulted in compound yields of 85% and 69%, respectively.

**2.1.2.2. Preparation of 1b, 1d, and 1e.** To a solution of 2,6-dichloro-4-(trifluoromethyl)phenyl amine (10 mmol) in THF (12 mL), NaH (10.4 mmol) was added and stirred continuously for 40 min at 0 °C (Fig. 2, route B). Acid chloride **5** (10.5 mmol) was then added and stirred continuously for 48–72 h at 25 °C. The reaction was quenched with water (10 mL), extracted with ethyl acetate (40 mL), washed with sat. aq.  $\text{NaHCO}_3$  ( $3 \times 60$  mL) and dried over anhydrous  $\text{Na}_2\text{SO}_4$ . Evaporation of the solvent and recrystallization from ethanol resulted in compounds **1b**, **1d** and **1e** with yields of 72–80%.

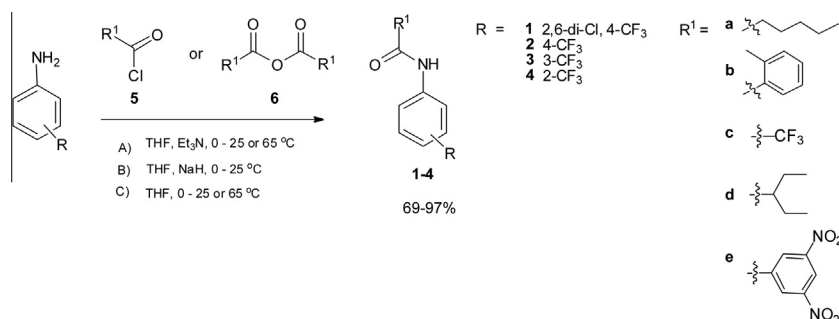


Fig. 2. Synthesis of trifluoromethylphenyl amides 1–4.

**Table 1**Average mortality of first instar *Ae. aegypti* larvae at 24, 48, and 72 h post-exposure to 100  $\mu$ M test compounds **1–4**.

| Compound ID           | Structure | R <sup>1</sup> | Mortality (SEM) <sup>b</sup> (%) |             |            |
|-----------------------|-----------|----------------|----------------------------------|-------------|------------|
|                       |           |                | 24 h                             | 48 h        | 72 h       |
| <b>1a<sup>a</sup></b> |           |                | 3.3 (3.3)                        | 3.3 (3.3)   | 3.3 (3.3)  |
| <b>1b<sup>a</sup></b> |           |                | 33.3 (6.7)                       | 100 (0)     | 100 (0)    |
| <b>1c</b>             |           |                | 0 (0)                            | 0 (0)       | 3.3 (3.3)  |
| <b>1d<sup>a</sup></b> |           |                | 0 (0)                            | 0 (0)       | 13.3 (8.8) |
| <b>1e<sup>a</sup></b> |           |                | 100 (0)                          | 100 (0)     | 100 (0)    |
| <b>2a<sup>a</sup></b> |           |                | 0 (0)                            | 40.0 (5.8)  | 80.0 (5.8) |
| <b>2b<sup>a</sup></b> |           |                | 6.7 (3.3)                        | 60.0 (11.5) | 100 (0)    |
| <b>2c</b>             |           |                | 3.3 (3.3)                        | 3.3 (3.3)   | 6.7 (3.3)  |
| <b>2d<sup>a</sup></b> |           |                | 40.0 (10)                        | 56.7 (14.5) | 73.3 (3.3) |
| <b>2e<sup>a</sup></b> |           |                | 83.3 (12)                        | 96.7 (3.3)  | 100 (0)    |
| <b>3a<sup>a</sup></b> |           |                | 36.7 (12)                        | 100.0 (0)   | 100.0 (0)  |
| <b>3b</b>             |           |                | 93.3 (6.7)                       | 100 (0)     | 100 (0)    |
| <b>3c</b>             |           |                | 3.3 (3.3)                        | 6.7 (3.3)   | 6.7 (3.3)  |
| <b>3d</b>             |           |                | 10.0 (5.8)                       | 36.7 (8.8)  | 53.3 (3.3) |
| <b>3e<sup>a</sup></b> |           |                | 86.7 (3.3)                       | 100 (0)     | 100 (0)    |
| <b>4a<sup>a</sup></b> |           |                | 0 (0)                            | 0 (0)       | 3.3 (3.3)  |
| <b>4b<sup>a</sup></b> |           |                | 6.7 (3.3)                        | 16.7 (3.3)  | 23.3 (3.3) |
| <b>4c</b>             |           |                | 3.3 (3.3)                        | 3.3 (3.3)   | 3.3 (3.3)  |
| <b>4d<sup>a</sup></b> |           |                | 3.3 (3.3)                        | 3.3 (3.3)   | 3.3 (3.3)  |
| <b>4e<sup>a</sup></b> |           |                | 26.7 (12)                        | 36.7 (14.5) | 50.0 (5.8) |
| DMSO                  |           |                | 3.3 (3.3)                        | 3.3 (3.3)   | 3.3 (3.3)  |
| Fipronil              |           |                | 100 (0)                          | 100 (0)     | 100 (0)    |
| Untreated             |           |                | 0 (0)                            | 0 (0)       | 0 (0)      |

For known compounds, see references: **1c** and **2c**: [11], **3b**: [12], **3c** and **4c**: [13], **3d**: [14].<sup>a</sup> Novel compounds.<sup>b</sup> Standard error of the mean.

**2.1.2.3. Preparation of 2a–2e, 3a–3e and 4a–4e.** To a solution of trifluoromethylphenyl amine (10 mmol) in THF (12 mL), acid chloride **5** or acid anhydride **6** (for **2a,c**, **3a,c** and **4a,c**) (10.5 mmol) was added at 0 °C and stirred continuously for 1–2 h at 25 °C to produce compounds **2b–2e**, **3b–3e** and **4b–4e**, and for 16–24 h at 65 °C to produce compounds **2a**, **3a** and **4a** (Fig. 2, route C). The reaction mixture was diluted and extracted with ethyl acetate (40 mL), washed with sat. aq. NaHCO<sub>3</sub> (3 × 60 mL) and the organic layer dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent and recrystallization from ethanol or ethanol/water gave pure compounds **2a–2e**, **3b–3e** and **4a–4e** in 84–97% yields and chromatography on silica gel using hexanes/ethyl acetate as eluent gave pure compound **3a** in 89% yield.

**2.1.2.4. N-(2,6-Dichloro-4-(trifluoromethyl)phenyl)hexanamide (1a).** Colorless crystals after recrystallization from hexane/ethyl acetate; mp 63–64 °C; yield 85%; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.57 (s, 2H), 7.51 (br s, 1H), 2.40 (t, *J* = 7.6 Hz, 2H), 1.72 (quintet, *J* = 7.4 Hz, 2H), 1.42–1.24 (m, 4H), 0.90 (t, *J* = 7.0 Hz, 3H). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  171.7, 135.7, 134.3, 130.7, 125.4 (q, *J* = 3.6 Hz), 122.5 (q, *J* = 272.8 Hz), 36.3, 31.3, 25.1, 22.3, 13.9. ESI-TOF-MS Calcd for C<sub>13</sub>H<sub>14</sub>Cl<sub>2</sub>F<sub>3</sub>NO [M + H]<sup>+</sup>: *m/z* 328.0477. Found: 328.0472.

**2.1.2.5. N-(2,6-Dichloro-4-(trifluoromethyl)phenyl)-2-methylbenzamide (1b).** Colorless crystals after recrystallization from ethanol; mp 125–127 °C; yield 77%; mixture of rotamers: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.74–7.65 (m, 2H), 7.62 (s, 2H), 7.62–7.56 (m, 1H), 7.50 (br s,

1H), 7.40–7.32 (m, 2H), 7.24–7.21 (m, 3H), 7.17–6.94 (m, 3H), 2.50 (s, 3H), 2.36 (s, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  167.6, 138.5, 137.4, 135.9, 135.5–135.4 (m), 134.4, 134.3, 131.6–131.3 (m), 131.3, 131.0–130.9 (m), 130.9, 126.2–126.0 (m), 127.4, 127.1, 126.2–125.9 (m), 125.6 (q,  $J$  = 3.7 Hz), 124.9, 124.7 (q,  $J$  = 279.1 Hz), 19.9, 19.8. ESI-TOF-MS Calcd for  $\text{C}_{15}\text{H}_{10}\text{Cl}_2\text{F}_3\text{NO}$  [ $\text{M} + \text{H}$ ] $^+$ :  $m/z$  348.0164. Found: 348.0156.

**2.1.2.6. *N*-(2,6-Dichloro-4-(trifluoromethyl)phenyl)-2,2,2-trifluoroacetamide (1c).** Colorless crystals [11] after recrystallization from ethanol; mp 61–62 °C; yield 69%; mixture of rotamers:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  11.37 (br s, 1H), 7.90–7.73 (m, 2H), 7.68–7.50 (m, 2H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  155.6 (q,  $J$  = 37.6 Hz), 133.4, 132.4, 129.2, 127.0, 126.7 (q,  $J$  = 5.1 Hz), 125.2, 123.8 (q,  $J$  = 272.2 Hz), 122.2 (q,  $J$  = 30.1 Hz), 122.0, 121.9, 115.8 (d,  $J$  = 288.2 Hz).

**2.1.2.7. *N*-(2,6-Dichloro-4-(trifluoromethyl)phenyl)-2-ethylbutanamide (1d).** Colorless needles after recrystallization from ethanol; mp 147–150 °C; yield 72%;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.62 (s, 2H), 7.12 (br s, 1H), 2.28–2.15 (m, 1H), 1.85–1.68 (m, 2H), 1.67–1.54 (m, 2H), 1.04 (t,  $J$  = 7.3 Hz, 6H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  173.7, 135.6, 134.0, 130.5 (q,  $J$  = 34.2 Hz), 129.3, 120.6, 125.5 (q,  $J$  = 3.7 Hz), 51.6, 25.8, 12.1. ESI-TOF-MS Calcd for  $\text{C}_{13}\text{H}_{14}\text{Cl}_2\text{F}_3\text{NO}$  [ $\text{M} + \text{H}$ ] $^+$ :  $m/z$  328.0477. Found: 328.0463.

**2.1.2.8. *N*-(2,6-Dichloro-4-(trifluoromethyl)phenyl)-3,5-dinitrobenzamide (1e).** Colorless needles after recrystallization from ethanol; mp 235–237 °C; yield 80%;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  11.39 (br s, 1H), 9.24–9.18 (m, 2H), 9.09–9.05 (m, 1H), 8.14 (s, 2H).  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  162.1, 149.2, 137.1, 135.7, 135.4, 131.0, 128.7, 126.6 (q,  $J$  = 3.8 Hz), 122.6. ESI-TOF-MS Calcd for  $\text{C}_{14}\text{H}_6\text{Cl}_2\text{F}_3\text{N}_3\text{O}_5$  [ $\text{M} + \text{Na}$ ] $^+$ :  $m/z$  445.9529. Found: 445.9540.

**2.1.2.9. *N*-(4-(Trifluoromethyl)phenyl)hexanamide (2a).** Colorless needles after recrystallization from ethanol; mp 105–107 °C; yield 96%;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.69–7.51 (m, 4H), 7.49 (br s, 1H), 2.38 (t,  $J$  = 7.6 Hz, 2H), 1.73 (quintet,  $J$  = 7.1 Hz, 2H), 1.41–1.22 (m, 4H), 0.90 (t,  $J$  = 6.1 Hz, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  171.8, 141.0, 126.2 (d,  $J$  = 3.7 Hz), 124.1 (q,  $J$  = 271.4 Hz), 119.3, 37.8, 31.4, 25.1, 22.4, 13.9. ESI-TOF-MS Calcd for  $\text{C}_{13}\text{H}_{16}\text{F}_3\text{NO}$  [ $\text{M} + \text{Na}$ ] $^+$ :  $m/z$  282.1076. Found: 282.1078.

**2.1.2.10. 2-Methyl-*N*-(4-(trifluoromethyl)phenyl)benzamide (2b).** Colorless needles after recrystallization from ethanol; mp 127–129 °C; yield 95%;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.88 (br s, 1H), 7.79–7.68 (m, 2H), 7.67–7.55 (m, 2H), 7.44 (d,  $J$  = 7.7 Hz, 1H), 7.41–7.32 (m, 1H), 7.31–7.17 (m, 2H), 2.46 (s, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  168.5, 141.0, 136.5, 135.7, 131.3, 130.6, 128.1, 126.6, 126.2 (q,  $J$  = 3.7 Hz), 125.9, 124.1 (q,  $J$  = 272.3 Hz), 119.5, 19.7. ESI-TOF-MS Calcd for  $\text{C}_{15}\text{H}_{12}\text{F}_3\text{NO}$  [ $\text{M} + \text{H}$ ] $^+$ :  $m/z$  280.0944. Found: 280.0936.

**2.1.2.11. 2,2,2-trifluoro-*N*-(4-(trifluoromethyl)phenyl)acetamide (2c).** Colorless crystals [11] after recrystallization from ethanol; mp 126–128 °C; yield 84%;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  10.24 (br s, 1H), 7.79 (d,  $J$  = 8.3 Hz, 2H), 7.55 (d,  $J$  = 8.3 Hz, 2H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  155.7 (q,  $J$  = 38.2 Hz), 139.5, 127.6 (q,  $J$  = 32.8 Hz), 126.3 (q,  $J$  = 3.8 Hz), 124.1 (q,  $J$  = 272.8 Hz), 120.9, 115.9 (q,  $J$  = 285.8).

**2.1.2.12. 2-Ethyl-*N*-(4-(trifluoromethyl)phenyl)butanamide (2d).** Colorless needles after recrystallization from ethanol; mp 123–124 °C; yield 92%;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.68 (d,  $J$  = 8.5 Hz, 2H), 7.55 (d,  $J$  = 8.5 Hz, 2H), 2.12–2.05 (m, 1H), 1.80–1.64 (m, 1H), 1.63–1.52 (m, 4H), 0.94 (t,  $J$  = 7.1 Hz, 6H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  174.9, 141.0, 126.1 (q,  $J$  = 3.7 Hz), 124.1 (q,  $J$  = 270.2 Hz), 119.5, 52.2, 25.7, 12.0. ESI-TOF-MS Calcd for  $\text{C}_{13}\text{H}_{16}\text{F}_3\text{NO}$  [ $\text{M} + \text{Na}$ ] $^+$ :  $m/z$  282.1076. Found: 282.1081.

**2.1.2.13. 3,5-Dinitro-*N*-(4-(trifluoromethyl)phenyl)benzamide (2e).** Colorless crystals after recrystallization from ethanol/water; mp 195–196 °C; yield 95%;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  11.11 (br s, 1H), 9.22–9.13 (m, 2H), 9.06–8.96 (m, 1H), 8.05–7.95 (m, 2H), 7.81–7.71 (m, 2H).  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  161.8, 148.1, 141.9, 137.0, 128.2, 126.0 (q,  $J$  = 3.7 Hz), 124.9–124.4 (m), 124.2 (q,  $J$  = 272.3 Hz), 121.3, 120.6. ESI-TOF-MS Calcd for  $\text{C}_{14}\text{H}_8\text{F}_3\text{N}_3\text{O}_5$  [ $\text{M} + \text{H}$ ] $^+$ :  $m/z$  354.0354. Found: 354.0343.

**2.1.2.14. *N*-(3-(Trifluoromethyl)phenyl)hexanamide (3a).** Colorless oil after chromatography on silica gel using hexanes/ethyl acetate as eluent (100/1–10/1, v/v); yield 89%;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.97 (br s, 1H), 7.83 (s, 1H), 7.72 (br d,  $J$  = 6.6 Hz, 1H), 7.46–7.28 (m, 2H), 2.37 (t,  $J$  = 7.4 Hz, 2H), 1.79–1.69 (m, 2H), 1.40–1.21 (m, 4H), 0.95–0.80 (m, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  172.4, 138.5, 131.2 (q,  $J$  = 32.6 Hz), 129.4, 123.8 (q,  $J$  = 272.4 Hz), 123.0, 120.6 (q,  $J$  = 3.5 Hz), 116.6 (q,  $J$  = 3.6 Hz), 37.5, 31.3, 25.2, 22.3, 13.9. ESI-TOF-MS Calcd for  $\text{C}_{13}\text{H}_{16}\text{F}_3\text{NO}$  [ $\text{M} + \text{Na}$ ] $^+$ :  $m/z$  282.1086. Found: 282.1076.

**2.1.2.15. 2-Methyl-*N*-(3-(trifluoromethyl)phenyl)benzamide (3b).** Colorless crystals [12] after recrystallization from ethanol; mp 105–106 °C; yield 90%;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.91 (br s, 1H), 7.83–7.64 (m, 2H), 7.52–7.32 (m, 4H), 7.30–7.20 (m, 2H), 2.48 (s, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  168.2, 138.5, 136.6, 135.7, 131.4, 129.6, 126.6, 126.0, 124.9 (q,  $J$  = 272.2 Hz), 122.9, 121.0 (q,  $J$  = 3.7 Hz), 116.6 (q,  $J$  = 3.5 Hz), 19.8.

**2.1.2.16. 2,2,2-Trifluoro-*N*-(3-(trifluoromethyl)phenyl)acetamide (3c).** White crystals [13] after recrystallization from ethanol; mp 61–62 °C; yield 88%;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.27 (br s, 1H), 7.86 (s, 1H), 7.83–7.73 (m, 1H), 7.53–7.45 (m, 2H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  155.2 (q,  $J$  = 38.1 Hz), 135.6, 131.9 (q,  $J$  = 33.0 Hz), 130.0, 123.8–123.6 (m), 123.4 (q,  $J$  = 272.8 Hz), 123.1 (q,  $J$  = 3.7 Hz), 117.5 (q,  $J$  = 3.9 Hz), 115.7 (q,  $J$  = 288.6 Hz).

**2.1.2.17. 2-Ethyl-*N*-(3-(trifluoromethyl)phenyl)butanamide (3d).** Colorless crystals [14] after recrystallization from ethanol; mp 75–76 °C; yield 94%;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.86 (s, 1H), 7.81–7.60 (m, 2H), 7.49–7.30 (m, 2H), 2.18–2.00 (m, 1H), 1.81–1.63 (m, 2H), 1.62–1.45 (m, 2H), 0.94 (t,  $J$  = 6.6 Hz, 6H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  177.8, 138.4, 131.8–130.7 (m), 129.4, 125.6 (q,  $J$  = 3.3 Hz), 123.8 (q,  $J$  = 272.3 Hz), 123.0, 120.7, 116.7, 52.2, 25.8, 12.0.

**2.1.2.18. 3,5-Dinitro-*N*-(3-(trifluoromethyl)phenyl)benzamide (3e).** Colorless crystals after recrystallization from ethanol; mp 204–206 °C; yield 97%;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  9.19–9.16 (m, 2H), 9.02–8.98 (m, 1H), 8.19 (br s, 1H), 8.07 (d,  $J$  = 8.21 Hz, 1H), 7.64 (t,  $J$  = 8.0 Hz, 1H), 7.52 (d,  $J$  = 7.8 Hz, 1H).  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  161.6, 148.1, 139.1, 136.9, 130.1, 129.4 (q,  $J$  = 31.6 Hz), 128.0, 124.2, 124.1 (q,  $J$  = 271.8 Hz), 121.3, 120.8 (q,  $J$  = 3.9 Hz), 116.8 (q,  $J$  = 3.9 Hz). ESI-TOF-MS Calcd for  $\text{C}_{14}\text{H}_8\text{F}_3\text{N}_3\text{O}_5$  [ $\text{M}-\text{H}$ ] $^-$ :  $m/z$  354.0343. Found: 354.0349.

**2.1.2.19. *N*-(2-(Trifluoromethyl)phenyl)hexanamide (4a).** Colorless crystals after recrystallization from ethanol; mp 43–45 °C; yield 89%; mixture of rotamers:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.16 (br s, 1H), 7.68–7.37 (m, 3H), 7.33–7.11 (m, 1H), 2.52–2.22 (m, 2H), 1.82–1.49 (m, 2H), 1.46–1.14 (m, 4H), 1.02–0.72 (m, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  171.8–171.4 (m), 135.4–135.2 (m), 132.8, 126.2–125.8 (m), 125.1–124.2 (m), 122.3–122.2 (m), 37.7, 35.2, 33.9, 31.0, 25.0, 24.4, 23.8, 22.3, 22.2, 13.8. ESI-TOF-MS Calcd for  $\text{C}_{13}\text{H}_{16}\text{F}_3\text{NO}$  [ $\text{M} + \text{Na}$ ] $^+$ :  $m/z$  282.1076. Found: 282.1086.

**2.1.2.20. 2-Methyl-*N*-(2-(trifluoromethyl)phenyl)-benzamide (4b).** Colorless crystals after recrystallization from ethanol; mp 123–124 °C; yield 95%;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.39 (m, 1H), 7.80 (br s, 1H), 7.65 (d,

$J = 7.6$  Hz, 1H), 7.61 (t,  $J = 7.9$  Hz, 1H), 7.51 (d,  $J = 7.3$  Hz, 1H), 7.38 (t,  $J = 7.3$  Hz, 1H), 7.32–7.24 (m, 3H), 2.53 (s, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  168.1–167.9 (m), 136.8, 135.6, 135.3, 132.9, 131.4, 130.8, 126.6–125.9 (m), 124.8–124.3 (m), 124.1 (q,  $J = 273.2$  Hz), 120.4 (q,  $J = 30.1$  Hz), 19.8. ESI-TOF-MS Calcd for  $\text{C}_{15}\text{H}_{12}\text{F}_3\text{NO}$   $[\text{M}]^+$ :  $m/z$  279.0871. Found: 279.0868.

**2.1.2.21. 2,2,2-Trifluoro-N-(2-(trifluoromethyl)phenyl)acetamide (4c).** Colorless crystals [13]; mp 56–57 °C; yield 98%;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.19 (br s, 1H), 8.13 (d,  $J = 8.4$  Hz, 1H), 7.72–7.58 (m, 2H), 7.39 (t,  $J = 7.7$ , 0.9 Hz, 1H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  155.4 (q,  $J = 37.5$  Hz), 133.3, 132.3 (q,  $J = 1.7$  Hz), 126.7, 126.6 (q,  $J = 5.2$  Hz), 124.7, 123.7 (q,  $J = 273.6$  Hz), 121.6 (q,  $J = 30.3$  Hz), 115.6 (q,  $J = 287.2$  Hz).

**2.1.2.22. 2-Ethyl-N-(2-(trifluoromethyl)phenyl)butanamide (4d).** Colorless crystals after recrystallization from ethanol; mp 97–98 °C; yield 94%;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.24 (d,  $J = 8.8$  Hz, 1H), 7.61–7.50 (m, 2H), 7.46 (br s, 1H), 7.25–7.18 (m, 1H), 2.17–2.02 (m, 1H), 1.80–1.66 (m, 2H), 1.65–1.51 (m, 2H), 0.98 (t,  $J = 7.4$  Hz, 6H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  174.3, 135.6–135.0 (m), 132.8, 125.9 (q,  $J = 5.2$  Hz), 124.4 (q,  $J = 21.7$  Hz), 124.0 (q,  $J = 273.2$ ), 52.7, 25.8, 11.9. ESI-TOF-MS Calcd for  $\text{C}_{13}\text{H}_{16}\text{F}_3\text{NO}$   $[\text{M} + \text{Na}]^+$ :  $m/z$  282.1076. Found: 282.1085.

**2.1.2.23. 3,5-Dinitro-N-(2-(trifluoromethyl)phenyl)benzamide (4e).** Colorless crystals after recrystallization from ethanol/water; mp 203–205 °C; yield 92%;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  10.91 (br s, 1H), 9.25–9.12 (m, 2H), 9.08–9.00 (m, 1H), 7.91–7.75 (m, 2H), 7.68–7.55 (m, 2H).  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  162.7 (m), 148.3, 136.4, 134.7, 133.4, 131.2, 128.2, 127.9, 126.7 (q,  $J = 4.7$  Hz), 123.5 (q,  $J = 272.4$  Hz), 121.4. ESI-TOF-MS Calcd for  $\text{C}_{14}\text{H}_8\text{F}_3\text{N}_3\text{O}_5$   $[\text{M}-\text{H}]^-$ :  $m/z$  354.0343. Found: 354.0341.

## 2.2. Biological testing

### 2.2.1. Larval and adult bioassays with *Ae. aegypti* mosquitoes

The larvae and mosquitoes used were from the *Ae. aegypti* strain originally established in Orlando, FL, USA (1952), and maintained at the Mosquito and Fly Research Unit at the United States Department of Agriculture-Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology (USDA-ARS CMAVE) in Gainesville, FL, USA. Preliminary screening bioassays of compounds against first instar *Ae. aegypti* larvae were performed as described by Pridgeon et al. [15]. Five larvae were placed into individual wells of a 24-well plate containing 950  $\mu\text{L}$  of deionized, sterile water and 40  $\mu\text{L}$  of a 2:1 aqueous suspension of alfalfa:pot belly pig chow. Ten  $\mu\text{L}$  of 10 mM of each test chemical was solubilized in dimethyl sulfoxide (DMSO) (Sigma, St. Louis, MO, USA), and added to the wells for a final concentration of 100  $\mu\text{M}$ . Mortality was recorded at 24, 48, and 72 h post-exposure. Controls included 10  $\mu\text{L}$  of the following: water (untreated), DMSO (carrier) and fipronil (100  $\mu\text{M}$  concentration, purity 97.5%) (Sigma, St. Louis, MO, USA). For  $\text{LC}_{50}$  estimates, assays were set up as described above with serial dilutions of test chemical.

Adult topical assays were performed against female *Ae. aegypti*. The mosquito rearing and application methods were conducted as previously described by Pridgeon et al. [16]. Prior to the application of the test compounds, 5- to 8-day-old adult mosquitoes were collected from a screened cage using a vacuum aspirator (BioQuip Products, Rancho Dominguez, CA, USA) and cold anesthetized at 4 °C for an hour. Ten female mosquitoes were sorted into 3.5 oz clear plastic cups (Solo Cup Company, Lake Forest, IL, USA) using pointed featherweight forceps (BioQuip Products, Rancho Dominguez, CA, USA). The opening to each cup was sealed with a double layer of mesh tulle fabric and secured with a rubber elastic band.

Mosquitoes were provided with 10% sucrose solution and held overnight at  $26 \pm 1$  °C and  $80 \pm 1\%$  RH.

The majority of the chemicals were solubilized in DMSO to a 4 M stock solution, except for **1a**, **1e**, **2e**, and **4e**, which were solubilized at 2 M, and **1d** and **3e** which were solubilized at 1 M. The stock solutions were diluted with acetone to produce a 5% DMSO/acetone treatment solution and 0.5  $\mu\text{L}$  was applied to the thorax of each mosquito using a 700 series syringe and a PB 600 repeating dispenser (Hamilton, Reno, NV, USA). Ten mosquitoes each were treated with one of the four concentrations that were tested for each chemical. After the topical application, mosquitoes were transferred back into a plastic assay cup, held as described above, and provided with a 10% sucrose solution daily. Mortality data was recorded at 24, 48 and 72 h post topical application for determination of  $\text{LD}_{50}$ . Fipronil (50 nmol) was used as a positive control, while acetone and untreated mosquitoes were used as negative controls. Three replicates were completed and the data were analyzed using PoloPlus probit analysis software v2.0 (LeOra Software, Petaluma, CA, USA) to calculate the  $\text{LC}_{50}$  and  $\text{LD}_{50}$ .

### 2.2.2. Contact toxicity bioassays with *D. melanogaster*

Susceptible (Oregon-R) and cyclodiene-resistant (*rdl*; 1675) strains of *D. melanogaster* were used to determine the contact toxicity of the trifluoromethylphenyl amides. The Oregon-R strain was originally donated by Doug Knipple, Cornell University, Ithaca NY, USA, and has been maintained in culture at the University of Florida since 2009. The *rdl* strain, 1675, was purchased from the Bloomington Drosophila Stock Center at Indiana University, Bloomington, IN. Both strains were reared in plastic vials on artificial media purchased from Carolina Biological Supply, Burlington, NC. Toxicity bioassays used a surface-contact method in which compounds were dissolved in acetone and a 100  $\mu\text{L}$  aliquot was applied to glass vials (40  $\text{cm}^2$ ) that were evenly coated by manual rotation of the vial for a duration of 2 min. Twenty female flies were then added to the vials, which were stoppered with cotton balls containing 10% sucrose solution. Ethanol was used as the negative control and fipronil was used as the positive control. Fipronil produced 100% mortality at concentrations equal or less than that of the experimental inhibitors. Mortality was determined at 24 h post treatment and analyzed by PoloPlus. Six compound concentrations were used in triplicate to construct dose-response curves to determine one  $\text{LC}_{50}$  value.  $\text{LC}_{50}$  values were averaged ( $n = 3$ ) using GraphPad InStat™ (GraphPad Software, San Diego, CA, USA) to determine mean  $\text{LC}_{50}$  values for each compound to *D. melanogaster*. The mean  $\text{LC}_{50}$  values were statistically analyzed using an unpaired t-test (two tail) with significance being represented by  $P < 0.05$ . Statistical analyses were performed using GraphPad InStat™.

### 2.2.3. Repellency bioassays with *Ae. aegypti* mosquitoes

The mosquito species used for testing were *Ae. aegypti* (Orlando strain, 1952) and *An. albimanus* (El Salvador, 1974) from colonies maintained at USDA-ARS CMAVE in Gainesville, FL. Newly emerged mosquitoes were maintained on 10% sugar water and kept in laboratory cages at an ambient temperature of  $28 \pm 1$  °C and RH of 35–60%. Nulliparous 6- to 8-day-old female mosquitoes were pre-selected from stock cages using a hand-draw box and trapped in a collection trap [17]. After 500 ( $\pm 10\%$ ) females were collected in the trap, they were transferred to a test cage (approximately 59,000  $\text{cm}^3$  with dimensions 45  $\times$  37.5  $\times$  35 cm) and allowed to acclimate for 17.5 ( $\pm 2.5$ ) min before initiating testing [18].

To evaluate the minimum effective dosage (MED) [19], a 1 mL solution of an appropriate concentration of each amide was transferred into a 2-dram vial containing a muslin cloth patch (5  $\times$  10 cm). The MED is a measurement used to estimate the concentration level of repellent, which fails to prevent mosquito bites,

equivalent to an ED<sub>99</sub>. There were two series of dosages used. The series of high dosage was 25,000, 12,500, 6,250, and 3,125  $\mu\text{mol}/\text{cm}^2$ . The lower dosage series consisted of cloths treated with 2,500, 1,250, 0.625, 0.313, 0.156, 0.078, 0.039, and 0.020  $\mu\text{mol}/\text{cm}^2$ . Prior to the start of testing, the cloth was removed from the vial and affixed with staples onto two sections of card stock (5 × 2.5 cm). Approximately 5 cm of masking tape was affixed to the edges of the card stock. After the cloth and card stock were treated, they were placed on a drying rack and allowed to dry for at least 3 min prior to testing. The MED calculation was initiated using the middle range (0.313  $\mu\text{mol}/\text{cm}^2$ ) treated cloth and followed by use of higher or lower dosage treatments as necessary until all subjects had evaluated the cloths and pinpointed the dosage at the 1% (5 bites) failure point. If the 2,500  $\mu\text{mol}/\text{cm}^2$  cloth was not efficacious (>5 bites per min), then a higher dosage series was used to determine the MED. There were 3 volunteers (all male) that tested each cloth. During each test, all volunteers wore a patch treated with a specific compound and tested it for a 1 min interval. Patches were then rotated among the volunteers. DEET was the positive control for these tests and cloth treated with acetone, which was the solvent used in this bioassay, served as the negative control. No patch was evaluated more than 10 min after the 3 min drying period in order to avoid any bias that may result from evaporative loss of treatment from the cloth. All procedures were approved by the University of Florida Human Use Institutional Review Board and informed consent was provided by all participants (Project #636-2005).

Each volunteer participating in the bioassay test wore a specially designed sleeve that exposed only a small area of the forearm to the mosquitoes. The hand of each human volunteer was protected by a powder-free latex glove (Diamond Grip, Microflex Corporation, Reno, NV). The gloved hand and arm were then placed inside a knee-high stocking (Leggs Everyday Knee Highs, Winston-Salem, NC). A plastic sleeve constructed of polyvinyl was then placed over the arm and stocking. The sleeve had a lengthwise Velcro seam to allow sealing over the arm. There was a window cut into the sleeve (4 × 8 cm opening) approximately half way between the wrist and elbow. This window allowed odors from the volunteer's skin surface to escape from the sleeve through the opening, over which the treated cloth was placed.

The arm, sleeve and cloth were inserted into the mosquito cage for 1 min to determine if the compound and dosage on cloth were repellent to the mosquitoes. The number of fed mosquitoes was determined by shaking the arm briskly after 1 min and counting the number of mosquitoes that remained biting through the cloth. During the testing process, no more than 10 compounds were assayed in succession with a caged population of test mosquitoes before allowing a 15 min recovery period. This was necessary because following prolonged and repeated repellent exposure, mosquitoes fatigue and exhibit decreased response to attractant (skin) odors.

## 2.2.4. Fungicidal bioassay

### 2.2.4.1. Direct bioautography assay for activity against plant pathogenic fungi.

Pure compounds were initially evaluated for their antifungal activity against three important plant pathogenic fungi (*Colletotrichum* species) using direct-bioautography. Pathogen production and bioautography procedures described by Wedge et al. [20], were used to evaluate antifungal activity against fungal plant pathogens. Technical grade commercial fungicides azoxystrobin, and captan (Chem Service, Inc., West Chester, PA, USA) were used as fungicide standards at 2 mM in 2  $\mu\text{L}$  of 95% ethanol. The test compounds and commercial fungicides were applied onto TLC plates at 12 mM in 4  $\mu\text{L}$  of 95% ethanol and tested against all three *Colletotrichum* species. Conidia of *C. fragariae*, *C. acutatum* and *C. gloeosporioides* suspensions were adjusted to  $3.0 \times 10^5$  conidia/

mL with liquid potato-dextrose broth (PDB, Difco, Detroit, MI, USA) and 0.1% Tween-80. Using a 50 mL chromatographic sprayer, each glass silica gel TLC plate treated with fluorescent indicator (250 mm, Silica Gel GF Uniplate) (Analtech, Inc., Newark, DE, USA) 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, USA) and incubated in a growth chamber at  $24 \pm 1$  °C with 12 h photoperiod under  $60 \pm 5$   $\mu\text{mol}/\text{m}^2 \text{ sec}^{-1}$  light. Inhibition of fungal growth was measured 4 d after treatment. Sensitivity of each fungal species to each test compound was determined by comparing the size of inhibitory zones. Fungal growth inhibition means for extracts and pure compounds were analyzed separately by ANOVA using SAS software, Ver. 8 (Statistical Analysis System, Cary, NC, USA). Mean separations were performed based on Fisher's Protected Least Significant Difference (LSD) ( $P = 0.05$ ). Statistical comparisons were made for fungal growth across compounds, and for each compound across fungal growth. Means and standard deviations of inhibitory zone size were used to evaluate antifungal activity of pure compounds.

### 2.2.4.2. Microdilution broth assay.

A standardized 96-well microdilution broth assay developed by Wedge and Kuhajek [20] was used to evaluate antifungal activity towards *B. cinerea*, *C. acutatum*, *C. fragariae*, *C. gloeosporioides*, *P. viticola*, *P. obscurans* and *F. oxysporum*. The commercial fungicides captan and azoxystrobin were used as the positive controls and 95% ethanol as the negative control in all assays. Solutions of tested compounds and positive controls were prepared in 95% ethanol. Each fungus was challenged in a dose–response format using test compounds where the final treatment concentrations were 0.3, 3.0 and 30.0  $\mu\text{M}$  in microtiter plates (Nunc MicroWell, untreated; Fisher Scientific, Roskilde, Denmark) covered with a plastic lid and incubated in a growth chamber, as described previously [21]. Sixteen wells containing broth and inoculum served as growth controls; eight wells containing solvent at the appropriate concentration and broth without inoculum were used as negative controls. Experiments were conducted in triplicate. Mean absorbance values and standard errors were used to evaluate fungal growth at 48 and 72 h except for *P. obscurans* and *P. viticola*, for which the data were recorded at 120 h. Means for percent inhibition of each fungus at each dose of test compound relative to the untreated positive growth controls were used to evaluate fungal growth inhibition. The SAS system analysis of variance procedure was used to identify significant factors, and Fisher's protected LSD was used to separate means [22]. Fungal growth was then evaluated by measuring absorbance of each well at 620 nm using a microplate photometer (Packard Spectra Count; Packard Instrument Co., Downers Grove, IL, USA).

## 3. Results and discussion

Trifluoromethylphenyl amides were synthesized by treatment of trifluoromethylphenyl amines in THF with acid chlorides **5** or acid anhydrides **6** (**1a,c**, **2a,c**, **3a,c**, **4a,c**), in the presence of Et<sub>3</sub>N (**1a**, **1c**) or NaH, 60% (**1b**, **1d**, **1e**) in 69–97% yields (Fig. 2, Table 1).

Compounds **1e** and **3b** produced 93–100% mortality in first instar *Ae. aegypti* larvae after a 24 h exposure at 100  $\mu\text{M}$  concentration. Another four compounds, **1b**, **2e**, **3a** and **3e** produced 100% mortality after 48 h and compound **2b** exhibited 100% mortality after 72 h at the same concentration (Table 1). The LC<sub>50</sub> was evaluated for **1a–1e** at 24 h post-exposure against first instar *Ae. aegypti* larvae (Table 2). Compound **1e** was most active with a LC<sub>50</sub> of 1940 nM, compared to the LC<sub>50</sub> for fipronil of 13.55 nM. Some generalized trends were apparent. *Meta*- or *para*- trifluoromethylphenyl amides, with an aromatic substituent at the carbonyl

**Table 2**

Determination of LC<sub>50</sub> (24 h) for compounds **1e–4e** against first instar *Ae. aegypti* larvae (time point).

| Compound ID | LC <sub>50</sub> <sup>a</sup> (nM; 95% CI) | n <sup>b</sup> | Slope (SEM) <sup>c</sup> | χ <sup>2</sup> | df <sup>d</sup> |
|-------------|--------------------------------------------|----------------|--------------------------|----------------|-----------------|
| <b>1e</b>   | 1940 (1820–2080)                           | 360            | 5.25 (0.53)              | 6.34           | 10              |
| <b>2e</b>   | 7630 (6750–8560)                           | 360            | 4.99 (0.61)              | 9.83           | 7               |
| <b>3e</b>   | 190160 (15,912–23,202)                     | 360            | 2.22 (0.26)              | 2.63           | 7               |
| <b>4e</b>   | >1500000 (nd)                              | 360            | nd                       | nd             | nd              |
| Fipronil    | 13.55 (12.75–14.26)                        | 360            | 7.69 (1.16)              | 4.75           | 6               |

nd, not determined.

<sup>a</sup> LC<sub>50</sub> is an estimate, and therefore a range is given.

<sup>b</sup> Number of insects tested in total.

<sup>c</sup> Slope standard error of the mean.

<sup>d</sup> Degrees of freedom.

carbon produced higher larvicidal activity (**1b**, **1e**, **2b**, **2e**, **3b**, **3e**) (60.0–100.0% mortality at 48–72 h) than the *ortho*-trifluoromethyl amides (**4b**, **4e**) (0.0–50.0% mortality at 48–72 h). The presence of 2,6-dichloro- substitution on the phenyl ring of *para*-trifluoromethylphenyl amides with alkyl group attached to the carbonyl carbon produced lower mortality (**1a** and **1d**) (0.0–13.3%, at 48–72 h) than *para*-trifluoromethylphenyl amides without a 2,6-dichloro- substitution (**2a** and **2d**) (40.0–80.0%, at 48–72 h). The presence of 2,6-dichloro- substitution on the phenyl ring of *para*-trifluoromethylphenyl amides with an aromatic group attached to the carbonyl carbon generated higher mortality (**1b** and **1e**) (100.0%, at 48–72 h) than compounds without the 2,6-dichloro-substitution (**2b** and **2e**) (60.0–100.0%, at 48–72 h). LC<sub>50</sub> values presented in Table 2 also show the higher larvicidal activity of **1e** compared to **2e**.

In toxicity bioassays against *Ae. aegypti* adults, compounds **2a–2e** and **4a–e**, demonstrated ≤10% mortality at any time point and in series **1a–e** and **3a–e** only **1c** and **3b** showed some activity. The controls: water and DMSO were zero and fipronil was always 100% at any time point. Compound **1c** at 100 and 50 nmol doses resulted

in approximately 80%, 90%, and 100% mortality after 24, 48, and 72 h, respectively (Table 3), and the 100 nmol dose of compound **3b** produced 30%, 70%, and 80% mortality after 24, 48, and 72 h post-application, respectively. The LD<sub>50</sub> values for **1c** were 19.182 nM for 24 h, 13.389 nM for 48 h, and 12.077 nM for 72 h, compared to fipronil which was determined to have a 24 h LD<sub>50</sub> of 0.787 10<sup>−4</sup> nM (Table 4). Because only compound **1c** produced sufficient mortality in screening assays to merit evaluation of the LD<sub>50</sub>, additional adult topical testing was not conducted on the remaining compounds.

The experimental trifluoromethylphenyl amides were found to be minimally toxic to *D. melanogaster* through contact exposure. Compounds **1c** and **4c** were the only compounds found to be toxic to either strain, Oregon-R or the GABA receptor mutant, 1675 (Table 5). Compound **1c** was found to have near identical LC<sub>50</sub> values for the Oregon-R and 1675 strains of 5.6 and 4.9 μg/cm<sup>2</sup>, respectively. Compound **4c** was found to have LC<sub>50</sub> values of 15.3 μg/cm<sup>2</sup> and 20.6 μg/cm<sup>2</sup> for the Oregon-R and 1675 strains, respectively. The mean LC<sub>50</sub> values for compounds **1c** and **4c** were not significantly different (*P* > 0.05) between the two fly strains. LC<sub>50</sub> values of other experimental compounds were found to be greater than 25 μg/cm<sup>2</sup> and were deemed to be non-toxic. Fipronil was highly toxic with LC<sub>50</sub> values of 0.004 and 0.017 μg/cm<sup>2</sup> for the Oregon-R and 1675 strain, respectively, a statistically significant 4-fold difference between fly strains. These data demonstrate that the toxicity of experimental compounds **1c** and **4c** are able to circumvent resistance from the GABA receptor mutation present in 1675 (*rdl*).

The toxicity bioassays with *Ae. aegypti* adults (Table 3) and *D. melanogaster* (Table 5) resulted in poor to no activity for most of the compounds. In both sets of assays, the most active compound was **1c**. This compound has a 2,6-dichloro- substitution, a trifluoromethyl group located in the *para*- position relative to the amide

**Table 3**

Average mortality of female *Ae. aegypti* at 24, 48, and 72 h post-exposure to a range of test compounds **1** and **3**, with concentrations starting at 100 nmol.

| Compound ID | Time Post Exposure (h) | Mortality (SEM) <sup>a</sup> (%) |            |            |             |             |
|-------------|------------------------|----------------------------------|------------|------------|-------------|-------------|
|             |                        | 100 (nmol)                       | 50 (nmol)  | 25 (nmol)  | 12.5 (nmol) | 6.25 (nmol) |
| <b>1a</b>   | 24                     |                                  | 0 (0)      | 0 (0)      | 0 (0)       | 0 (0)       |
|             | 48                     |                                  | 0 (0)      | 0 (0)      | 3.3 (0.1)   | 0 (0)       |
|             | 72                     |                                  | 0 (0)      | 0 (0)      | 3.3 (0.1)   | 3.3 (0.1)   |
| <b>1b</b>   | 24                     | 3.3 (0.1)                        | 3.3 (0.1)  | 6.7 (0.1)  | 0 (0)       |             |
|             | 48                     | 10.0 (0.2)                       | 3.3 (0.1)  | 6.7 (0.1)  | 0 (0)       |             |
|             | 72                     | 10.0 (0.2)                       | 3.3 (0.1)  | 6.7 (0.1)  | 0 (0)       |             |
| <b>1c</b>   | 24                     | 80.0 (0.2)                       | 77.0 (0.4) | 33.3 (0.1) | 30.0 (0.4)  |             |
|             | 48                     | 90.0 (0.2)                       | 90.0 (0.2) | 43.3 (0.1) | 23.3 (0.4)  |             |
|             | 72                     | 100 (0)                          | 100 (0)    | 73.3 (0.1) | 30.0 (0.5)  |             |
| <b>1d</b>   | 24                     |                                  |            | 6.7 (0.2)  | 0 (0)       | 3.3 (0.1)   |
|             | 48                     |                                  |            | 6.7 (0.2)  | 0 (0)       | 6.7 (0.1)   |
|             | 72                     |                                  |            | 10.0 (0.2) | 0 (0)       | 6.7 (0.1)   |
| <b>1e</b>   | 24                     |                                  | 3.3 (0.1)  | 3.3 (0.1)  | 0 (0)       | 0 (0)       |
|             | 48                     |                                  | 3.3 (0.1)  | 3.3 (0.1)  | 3.3 (0.1)   | 0 (0)       |
|             | 72                     |                                  | 10.0 (0.2) | 3.3 (0.1)  | 3.3 (0.1)   | 0 (0)       |
| <b>3a</b>   | 24                     | 13.3 (0.1)                       | 0 (0)      | 0 (0)      | 0 (0)       |             |
|             | 48                     | 13.3 (0.1)                       | 6.7 (0.2)  | 6.7 (0.1)  | 0 (0)       |             |
|             | 72                     | 13.3 (0.1)                       | 10.0 (0.3) | 6.7 (0.1)  | 0 (0)       |             |
| <b>3b</b>   | 24                     | 30.0 (0.3)                       | 23.3 (0.1) | 3 (0.1)    | 3.3 (0.1)   |             |
|             | 48                     | 70.0 (0.2)                       | 43.3 (0.6) | 6.7 (0.1)  | 3.3 (0.1)   |             |
|             | 72                     | 80.0 (0.2)                       | 50.0 (0.5) | 6.7 (0.1)  | 3.3 (0.1)   |             |
| <b>3c</b>   | 24                     | 16.7 (0.1)                       | 3.3 (0.1)  | 0 (0)      | 10.0 (0.2)  |             |
|             | 48                     | 16.7 (0.1)                       | 3.3 (0.1)  | 3.3 (0.1)  | 16.7 (0.1)  |             |
|             | 72                     | 16.7 (0.1)                       | 3.3 (0.1)  | 3.3 (0.1)  | 16.7 (0.1)  |             |
| <b>3d</b>   | 24                     | 10.0 (0)                         | 0 (0)      | 3.3 (0.1)  | 3.3 (0.1)   |             |
|             | 48                     | 16.7 (0.1)                       | 3.3 (0.1)  | 3.3 (0.1)  | 6.7 (0.2)   |             |
|             | 72                     | 16.7 (0.1)                       | 3.3 (0.1)  | 3.3 (0.1)  | 3.7 (0.2)   |             |
| <b>3e</b>   | 24                     |                                  | 0 (0)      | 0 (0)      | 0 (0)       | 0 (0)       |
|             | 48                     |                                  | 0 (0)      | 0 (0)      | 0 (0)       | 0 (0)       |
|             | 72                     |                                  | 0 (0)      | 0 (0)      | 0 (0)       | 0 (0)       |

<sup>a</sup> Standard error of the mean.

**Table 4**Estimates of LD<sub>50</sub> for compound 1c against female *Ae. aegypti* 24, 48, and 72 h post topical application.

| Compound and exposure Time | LD <sub>50</sub> <sup>a</sup> (nM; 95% CI)               | n <sup>b</sup> | Slope (SEM) <sup>c</sup> | χ <sup>2</sup> | Df <sup>d</sup> |
|----------------------------|----------------------------------------------------------|----------------|--------------------------|----------------|-----------------|
| 1c (24 h)                  | 19.182 (14.402–25.145)                                   | 450            | 2.56 (0.20)              | 4.89           | 3               |
| 1c (48 h)                  | 13.389 (10.179–16.882)                                   | 450            | 2.67 (0.23)              | 3.80           | 3               |
| 1c (72 h)                  | 12.077 (10.388–13.809)                                   | 450            | 2.56 (0.23)              | 1.63           | 3               |
| Fipronil (24 h)            | 0.787 10 <sup>−4</sup> (0.685–0.903 × 10 <sup>−4</sup> ) | 450            | 4.89 (0.38)              | 37.21          | 3               |

Mosquitoes were treated with a 0.5 μL solution of each concentration.

<sup>a</sup> LD<sub>50</sub> is an estimate, and therefore a range is given.<sup>b</sup> Number of insects tested in total.<sup>c</sup> Slope standard error of the mean.<sup>d</sup> Degrees of freedom.**Table 5**Contact toxicity (24 h) of selected trifluoromethylphenyl amides against *D. melanogaster*.

| Compound | OR strain                                                   | 1675 strain                               |                                                             |                                           |
|----------|-------------------------------------------------------------|-------------------------------------------|-------------------------------------------------------------|-------------------------------------------|
|          | LC <sub>50</sub> <sup>a</sup> (μg/cm <sup>2</sup> ; 95% CI) | Slope (SEM) <sup>b</sup> , χ <sup>2</sup> | LC <sub>50</sub> <sup>a</sup> (μg/cm <sup>2</sup> ; 95% CI) | Slope (SEM) <sup>b</sup> , χ <sup>2</sup> |
| 1c       | 5.6 (2–9) <sup>A</sup>                                      | 5.9 (0.8), 1.1                            | 4.9 (1.0–9.0) <sup>A</sup>                                  | 2.9 (0.3), 12.0                           |
| 1e       | >25                                                         | –                                         | >25                                                         | –                                         |
| 2b       | >25                                                         | –                                         | >25                                                         | –                                         |
| 2e       | >25                                                         | –                                         | >25                                                         | –                                         |
| 3b       | >25                                                         | –                                         | >25                                                         | –                                         |
| 3e       | >25                                                         | –                                         | >25                                                         | –                                         |
| 4a       | >25                                                         | –                                         | >25                                                         | –                                         |
| 4c       | 15.3 (7–24) <sup>A</sup>                                    | 4.7 (0.9), 14.7                           | 20.6 (13–27) <sup>A</sup>                                   | 8.1 (1.5), 0.8                            |
| Fipronil | 0.004 (0.002–0.006) <sup>A</sup>                            | 4.1 (0.6), 9.2                            | 0.017 (0.008–0.025) <sup>B</sup>                            | 5.2 (0.8), 7.2                            |

Upper case letters after 95% CI values represent statistical significance for LC<sub>50</sub> values between the two strains of fly. Values for each compound not labeled by the same letter represent statistical significance at *P* < 0.05.<sup>a</sup> LC<sub>50</sub> values are represented as means (*n* = 3).<sup>b</sup> Slope standard error of the mean.

group and a trifluoromethyl group attached to the carbonyl carbon of the amide group.

Compound **4c** showed the greatest repellency against female *Ae. aegypti* with an MED of 0.039 μmol/cm<sup>2</sup> which in this study was lower than for DEET (0.091 μmol/cm<sup>2</sup>). Compound **4a** had an MED comparable to that of DEET (Table 6). None of these compounds were more repellent than DEET (0.401 μmol/cm<sup>2</sup>) in assays with *An. albimanus*. Against this anopheline, compounds **1c**, **3c**, **4a**, **4c** were the most potent (1.407–3.335 μmol/cm<sup>2</sup>) with compound **4c** having the lowest MED. The structural arrangement that produced the most potent repellency against *Ae. aegypti* (MED 0.39–0.469 μmol/cm<sup>2</sup>) and *An. albimanus* (MED 1.407–3.125 μmol/cm<sup>2</sup>) was the location of a trifluoromethylphenyl group in *ortho*- position to the amide with either a trifluoromethyl- or an alkyl- group attached to the carbonyl carbon (**4a**, **4c**, **4d**). None of the compounds with aromatic substituents at the carbonyl carbon, except **1e** (MED 1.875 μmol/cm<sup>2</sup> against *Ae. aegypti*), were repellent, even when tested at the highest concentration in this study (25.000 μmol/cm<sup>2</sup>) against female *Ae. aegypti* and *An. albimanus*. The presence of a 2,6-dichloro- substitution on the phenyl ring increased the repellent activity of *para*- trifluoromethylphenyl amide with the trifluoromethyl group attached to the carbonyl carbon (**1c**) compared to **2c**, and, in contrast, decreased the repellent activity of the amide with alkyl group attached to the carbonyl carbon (**1a**) compared to **2a** (Table 6).

Bioautography indicated that six trifluoromethylphenyl amides had antifungal activity against *C. acutatum*, *C. fragariae* and *C. gloeosporioides* (Table 7). Antifungal activity was evident by the presence of clear zones with a dark background where fungal mycelia or reproductive stroma were not present on the TLC plate. Compounds **1e**, **3a** and **4c** appeared to be the most effective against all three *Colletotrichum* species and generated clear zones of fungal growth inhibition. Some of the compounds showed diffuse zones in those regions on the bioautography plate where the fungal growth is visually interspersed with few mycelia. Compounds **2a**, **2b** and

**3b** showed selective activity against *C. gloeosporioides*. The six most active antifungal compounds identified by bioautography were subsequently evaluated using a 96-well microbioassay for activity against *C. acutatum*, *C. fragariae*, *C. gloeosporioides*, *B. cinerea*, *P. obscurans*, *P. viticola* and *F. oxysporum*. Compound **4c** had the most fungicidal activity with 70% growth inhibition at 3.0 μM against *P. obscurans* (Fig. 3). Compounds **1e**, **3b** and **4c** at 30 μM reduced *P. obscurans* growth by more than 60%. Secondary screening of active compounds using this microbioassay system showed

**Table 6**MED for repellency values for amides **1–4** against *Ae. aegypti* and *An. albimanus*.

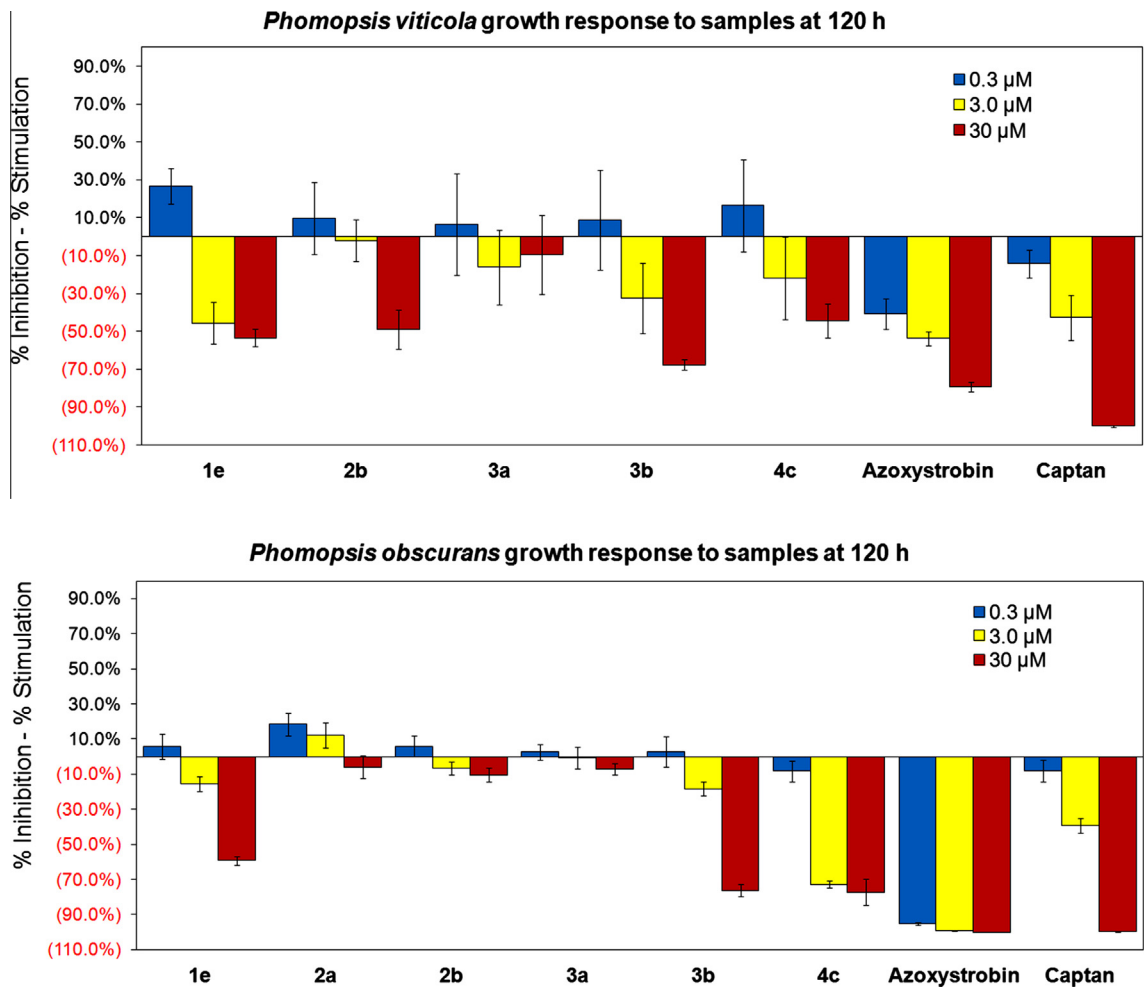
| Compound ID | Average MED (SD) <sup>a</sup> (μmol/cm <sup>2</sup> ) |                      |
|-------------|-------------------------------------------------------|----------------------|
|             | <i>Ae. aegypti</i>                                    | <i>An. albimanus</i> |
| DEET        | 0.091 (0.060)                                         | 0.417 (0.180)        |
| 1a          | nr                                                    | nr                   |
| 1b          | nr                                                    | nr                   |
| 1c          | 2.083 (0.722)                                         | 3.130 (0.000)        |
| 1d          | nr                                                    | nr                   |
| 1e          | 1.875 (0.884)                                         | nr                   |
| 2a          | 16.667 (7.217)                                        | 12.500 (0.000)       |
| 2b          | nr                                                    | nr                   |
| 2c          | 9.167 (13.712)                                        | nr                   |
| 2d          | nr                                                    | nr                   |
| 2e          | nr                                                    | nr                   |
| 3a          | 18.750 (10.825)                                       | 25.000 (0.000)       |
| 3b          | nr                                                    | nr                   |
| 3c          | 0.417 (0.180)                                         | 3.335 (2.818)        |
| 3d          | nr                                                    | nr                   |
| 3e          | nr                                                    | nr                   |
| 4a          | 0.091 (0.060)                                         | 3.125 (2.864)        |
| 4b          | nr                                                    | nr                   |
| 4c          | 0.039 (0.098)                                         | 1.407 (1.546)        |
| 4d          | 0.469 (0.221)                                         | nr                   |
| 4e          | nr                                                    | nr                   |

nr, not repellent up to 25.000 μmol/cm<sup>2</sup>.<sup>a</sup> Standard deviation.

**Table 7**  
Antifungal activity results of 20 trifluoromethylphenyl amides using direct bioautography with three *Colletotrichum* test species.

| Sample       | Test concentration (µg) | Mean Fungal Growth Inhibition (SEM) <sup>a</sup> (mm) |                     |                          |
|--------------|-------------------------|-------------------------------------------------------|---------------------|--------------------------|
|              |                         | <i>C. acutatum</i>                                    | <i>C. fragariae</i> | <i>C. gloeosporoides</i> |
| 1a           | 15.8                    | n/a                                                   | dz                  | dz                       |
| 1b           | 16.7                    | n/a                                                   | dz                  | dz                       |
| 1c           | 15.6                    | n/a                                                   | n/a                 | n/a                      |
| 1d           | 15.8                    | dz                                                    | dz                  | n/a                      |
| 1e           | 20.4                    | 5.00 (0.57)                                           | 5.33 (0.57)         | 5.00 (0.57)              |
| 2a           | 12.4                    | n/a                                                   | dz                  | 6.60 (1.15)              |
| 2b           | 13.4                    | dz                                                    | dz                  | 7.00 (1.00)              |
| 2c           | 12.3                    | dz                                                    | dz                  | dz                       |
| 2d           | 12.4                    | dz                                                    | dz                  | dz                       |
| 2e           | 17.1                    | dz                                                    | dz                  | dz                       |
| 3a           | 12.4                    | 11.00 (1.00)                                          | 11.66 (0.57)        | 11.66 (0.57)             |
| 3b           | 13.4                    | dz                                                    | dz                  | 10.33 (0.57)             |
| 3c           | 12.3                    | n/a                                                   | n/a                 | n/a                      |
| 3d           | 12.4                    | dz                                                    | dz                  | dz                       |
| 3e           | 17.1                    | dz                                                    | dz                  | dz                       |
| 4a           | 12.4                    | n/a                                                   | n/a                 | n/a                      |
| 4b           | 13.4                    | dz                                                    | dz                  | dz                       |
| 4c           | 12.3                    | 11.33 (0.57)                                          | 17.66 (0.57)        | 6.66 (0.57)              |
| 4d           | 12.4                    | n/a                                                   | n/a                 | n/a                      |
| 4e           | 17.1                    | n/a                                                   | dz                  | dz                       |
| Captan       | 1.2                     | 14.67 (0.33)                                          | 15.00 (0)           | 10.33 (0.33)             |
| Azoxystrobin | 1.61                    | 24.33 (0.33)                                          | 19.33 (0.33)        | 28.33 (0.33)             |

dz, diffuse zone.  
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.  
n/a, not applicable.  
<sup>a</sup> Mean inhibitory zones and standard error of the mean (SEM) were used to determine the level of antifungal activity against each fungal species.



**Fig. 3.** Growth inhibition of *P. obscurans* and *P. viticola* after 120 h using 96 well microdilution broth assay in a dose–response format using the commercial fungicide azoxystrobin and captan as standards.

that five out of six amides, except **3a**, inhibited *P. viticola* growth by 50% at 30  $\mu$ M, after 120 h exposure (Fig. 3). *P. obscurans* appeared to be approximately 10 times more sensitive to **4c**, because **4c** produced 72.8% inhibition at 3.0  $\mu$ M in *P. obscurans* and in *P. viticola* **4c** at 30.0  $\mu$ M produced only 44.3% growth inhibition. While **4c** was more active than captan at 3.0  $\mu$ M, it did not produce 100% growth inhibition even at the higher concentration of 30.0  $\mu$ M. Captan is an excellent fungicide with a multisite mode of action that is applied to crops such as strawberry at relative high rates, in the range of 1.64 kg (ai/ha), and azoxystrobin, a QoI (quinone outside inhibitor) is applied at 0.131 oz (ai/ha) [23]. Therefore, the most active compound in this series, **4c**, is currently not suitable for commercial application with its therapeutic threshold of 3.0  $\mu$ M; however, it is the analog of choice for further structural activity studies against *P. obscurans*. Compound **4c** contains two trifluoromethyl groups: one in *ortho*-position of the phenyl ring and another attached to the carbonyl carbon. The same compound was the most active repellent against female *Ae. aegypti* and *An. albimanus*.

#### 4. Conclusions

Twenty trifluoromethylphenyl amides (14 of which were novel) were designed, synthesized and evaluated for insecticidal, repellent and fungicidal activity.

Seven compounds **1b**, **1e**, **2b**, **2e**, **3a**, **3b**, and **3e** produced 100% mortality in first instar *Ae. aegypti* larvae at a concentration of 100  $\mu$ M after 24–72 h, although the  $LC_{50}$  for the most active **1e** was ~143 times higher than for fipronil. Compound **1c** was the most active compound in this series against female *Ae. aegypti*, but the  $LD_{50}$  for this compound at 24 h was ~23,055 times higher than that for fipronil. The same compound, **1c**, had highest activity against *D. melanogaster*; the  $LC_{50}$  for the 1675 strain was ~288 times and the  $LC_{50}$  for the OR strain ~1,400 times higher than that for fipronil. However, unlike fipronil, there was no cross resistance to **1c** and **4c** in the *rdl* strain of *D. melanogaster*.

Compound **4c** was the most potent repellent against *Ae. aegypti* with an MED 2.3 times lower than that of DEET; although 3.5 times higher than DEET for *An. albimanus*. Compound **4a** had an MED comparable to DEET against *Ae. aegypti*.

None of the active trifluoromethylphenyl amides produced inhibition in the range of azoxystrobin, and none of them showed significant inhibition against *C. acutatum*, *C. fragariae*, *C. gloeosporioides*, *B. cinerea* and *F. oxysporum*. We found, that compounds **3b** and **4c** have the potential to control *Phomopsis* species.

The structure–activity relationships based on the bioassay results against *Ae. aegypti* larvae showed that the presence of a trifluoromethyl group in the *para*- or *meta*- positions of the phenyl ring of amides increased their larvicidal activity, compared to *ortho*- trifluoromethylphenyl amides. The presence of a 2,6-dichloro- substitution in *para*- trifluoromethylphenyl amides increased the larvicidal activity of amides with an aromatic group attached to the carbonyl carbon, and decreased the larvicidal activity of compounds with an alkyl group attached to the carbonyl carbon.

According to repellency bioassay results against female *Ae. aegypti* and *An. albimanus*, *ortho*- trifluoromethylphenyl amides with a trifluoromethyl or an alkyl group attached to the carbonyl carbon produced higher repellent activity than *meta*- or *para*- trifluoromethylphenyl amides. Addition of a 2,6-dichloro- substitution decreased the repellent activity of *para*- trifluoromethylphenyl amide with alkyl group attached to the carbonyl carbon and increased the repellent activity of *para*- trifluoromethylphenyl amide with trifluoromethyl group attached to the carbonyl carbon.

Although none of the novel compounds synthesized and evaluated were potent insecticides, this study revealed the potential structures that could serve as the basis for further design to find

new derivatives with a broad spectrum of activity for controlling pest insects and pathogenic fungi.

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