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Insecticidal, repellent and fungicidal properties of novel trifluoromethylphenyl amides ☆

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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_{50} 1940 nM), while against adults *N*-(2,6-dichloro-4-(trifluoromethyl)phenyl)-2,2,2-trifluoroacetamide (**1c**) was most active (24 h LD_{50} 19.182 nM, 0.5 μL/insect). However, the 24 h LC_{50} and LD_{50} 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 LC_{50} values of 5.6 and 4.9 μg/cm^2 for the Oregon-R and 1675 strains, respectively. Fipronil had LC_{50} values of 0.004 and 0.017 μg/cm^2 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^2 compared to DEET (MED of 0.091 μmol/cm^2). Compound *N*-(2-(trifluoromethyl)phenyl)hexanamide (**4a**) had an MED of 0.091 μmol/cm^2 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.

1. Introduction

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

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

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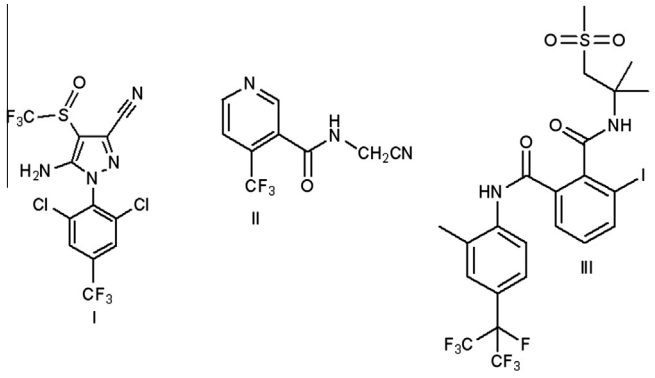

Fig. 1. Structures of (I) fipronil, (II) flonicamid, and (III) flubendiamide.

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 (Et_3N) 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 $CDCl_3$ or DMSO-d_6 with TMS (tetramethylsilane) as the internal standard for ^1H (500 MHz) and $CDCl_3$ or DMSO-d_6 as the internal standard for ^{13}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 Et_3N (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. $NaHCO_3$ (3 × 60 mL) and the organic layer dried over anhydrous Na_2SO_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. $NaHCO_3$ (3 × 60 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent and recrystallization from ethanol resulted in compounds **1b**, **1d** and **1e** with yields of 72–80%.

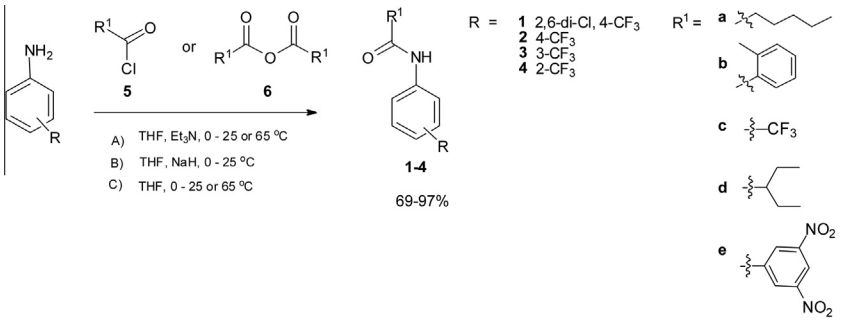

Fig. 2. Synthesis of trifluoromethylphenyl amides 1–4.