Med Chem Res
DOI 10.1007/s00044-015-1415-8

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Discovery and structure activity relationships of 2-pyrazolines derived from chalcones from a pest management perspective

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Received: 2 April 2015 / Accepted: 11 July 2015
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Abstract Synthesis of chalcones and 2-pyrazoline derivatives has been an active field of research due to the established pharmacological effects of these compounds. In this study, a series of chalcone (**1a–i**), 2-pyrazoline-1-carbothioamides (**2a–i**), and 2-pyrazoline-1-carboxamide derivatives (**3a–g**) were synthesized and screened for their potential pesticide activities. The proposed structures of all the synthesized compounds were confirmed using the elemental analysis, UV, IR, ^{1}H-NMR, and mass spectroscopy. Among the total of 25 tested compounds, compounds **1g** and **2a** and **2e** with Biting Deterrence Index (BDI) values of 0.85, 0.83, and 0.8, respectively, at 25 nmol/cm^2 showed the highest biting deterrent activity against *Aedes aegypti*, which was comparable to *N*,*N*-diethyl-3-methylbenzamide (DEET). Compounds **1g**, **2a** and **2e** were subsequently tested in human-based repellent bioassays, and they showed MED (minimum effective dose) values of 0.375, 0.094, and 0.375 mg/cm^2, respectively. Compound **1e** was the most toxic compound (LC_{50} = 2.58 ppm), followed by **1f** (LC_{50} = 5.69 ppm) and **2g** (LC_{50} = 15.14 ppm), against 1-day-old *Ae. aegypti* larvae. Compounds **1f** and **2h** **1f** and **2h** showed the greatest growth inhibition against *Colletotrichum gloeosporioides* (97.6 and 98.5 %, respectively) at the lowest dose (0.3 μM), which was greater antifungal activity than with standard commercial fungicides captan and azoxystrobin. Compounds **2d**, **2g** and **2h** produced 79.5, 98.3, and 82.3 % growth inhibition, respectively, at 30.0 μM against *Botrytis cinerea*, which was similar to captan in the antifungal activity. The active fungicidal compounds (**2d**, **2g**, and **2h**) were weaky phytotoxic, with little or no phytotoxicity at concentrations that were fungitoxic. Compound **2h** stimulated the growth of *Lemma paucicostata* at concentrations that are fungitoxic to several plant pathogens.

Keywords Chalcones · 2-Pyrazolines · Carbothioamide · Carboxamide · *Aedes aegypti* · *Colletotrichum gloeosporioides* · *Botrytis cinerea* · *Lactuca sativa* · *Agrostis stolonifera* · *Lemna paucicostata*

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Introduction

The pyrazole moiety is present in several pharmaceuticals and agrochemicals. Changes in pyrazole structure offer a high degree of diversity which is useful for the development of new therapeutic agents (Meegalla *et al.*, 2004; Rueger *et al.*, 2012; Desai *et al.*, 2013). Many pyrazole derivatives are known to possess a wide range of bioactivities and are employed as anti-inflammatory (Keche *et al.*, 2012; Bansal *et al.*, 2011), antidepressant (Kaplancıkli *et al.*, 2010; Karuppasamy *et al.*, 2010), antimicrobial (Pathak *et al.*, 2012; Mamolo *et al.*, 2001; Bondock *et al.*, 2011), antiviral (El-Sabbagh *et al.*, 2009), and anticancer (Peng-Cheng *et al.*, 2010; Banday *et al.*, 2010) agents. Furthermore,

Published online: 18 July 2015

1-phenylpyrazoles with alkyl, acyl, thioalkyl, or cyano substituents at the position 4 exhibit potent insecticidal activity (Zhong-Zheng and Guang-Fu, 2006; Meegalla *et al*., 2004; Jiang *et al*., 2009). Moreover, 5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoro-methanesulfinyl-1*H*-pyrazole-3-carbonitrile (fipronil) is one of the most commercially successful insecticides (Ozoe *et al*., 2000; Davari *et al*., 2007). Pyrazoles block the γ-aminobutyric acid (GABA)-regulated chloride channel in insect neurons (Singh and Bhati, 2011). Fipronil shows excellent insecticidal activity against *Plutella xylostella* and has low toxicity to mammals (Nash and Hoffmann, 2012). In recent years, a large number of active compounds have been obtained by modifying fipronil at its 1-(3-chloropyridyl)pyrazole moiety, substituted phenyl moiety, aliphatic amide moiety, and amide bridge (Hainzl and Casida, 1996). Furthermore, amide bonds are a prevalent motif in natural products as well as other biologically active compounds. Sedaxane is the mixture of two *cis*-isomers 2′-[(1RS, 2RS)-1,1′-bicycloprop-2-yl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxanilide and two trans-isomers 2′-[(1RS,2SR)-1,1′-bicycloprop-2-yl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxanilide which has insecticidal activity (Kang *et al*., 2013), but sedaxane is sold as a fungicide on the market (Lamberth *et al*., 2013). Chalcones are the versatile intermediates for the synthesis of various heterocyclic systems such as oxazoline, thiazine, oxazine as well as pyrazoline. The formation of these nucleuses is mainly constituted with the reaction of unsaturated carbonyl unit of chalcone (Singh and Bhati, 2011). Chalcone is a unique template that is associated with several promising biological activities such as antimicrobial (Ahmed *et al*., 2015), anti-inflammatory (Sashidhara *et al*., 2011), antimalarial (Pingaew *et al*., 2014), anticancer (Görgülü *et al*., 2015; Shankaraiah *et al*., 2015), antileishmanial (Boeck *et al*., 2006), and antimalarial (Tadigoppula *et al*., 2013). Moreover, chalcones find several applications in agriculture; for example, insect antifeedant (Thirunarayanan and Vanangamudi, 2014, Kumar *et al*., 2012) and larvicidal (Pasquale *et al*., 2012; Satyavani *et al*., 2015) activities have also been shown by chalcone derivatives.

On the basis of historical backgrounds of the pesticide activity of chalcone and pyrazole derivatives, we synthesized different substituted pyrazoles derived from chalcones and evaluated their activity as insect repellents, insecticides, fungicides, and herbicides.

Results and discussion

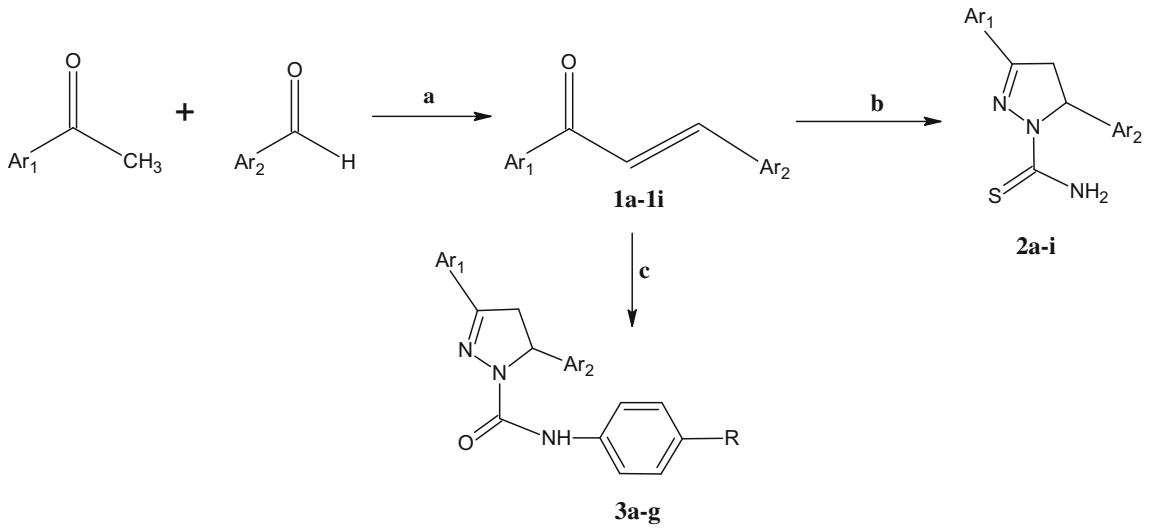
Chemistry

The synthetic route to the target compounds is outlined in Scheme 1. The intermediate chalcones (**1a–i**) were prepared by reacting equimolar aldehyde and ketone in the presence of a base by conventional Claisen–Schmidt condensation. 3,5-Disubstituted-4,5-dihydro-1*H*-pyrazole-1-carbothioamides (**2a–i)** were synthesized by refluxing compounds **1a–e** and thiosemicarbazide in the presence of alkaline medium. Physicochemical and spectroscopic characterization of the 2-pyrazoline derivatives **1a–i** and **2a–i** has been previously described (Beyhan *et al*., 2015). The purities of the synthesized compounds were checked by reversed-phase HPLC (Chromasil C_{18} 3.6 × 150 mm column using acetonitrile and water (50:50 v/v) as the eluent) and elemental analysis. All compounds showed a single and sharp peak with a retention time of 4.129–5.690 min, and also, elemental analysis results were calculated within 0.3 %. *N*,3,5-trisubstituted-4,5-dihydro-1*H*-pyrazole-1-carboxamides (**3a–g)** were synthesized by refluxing selected chalcones with substituted semicarbazides in alkaline medium. All the compounds were isolated in satisfactory yields (30–65 %) and purified by recrystallization from ethanol. Structures of the synthesized compounds were confirmed by elemental analysis and spectral (UV, IR, ^{1}H-NMR, ^{13}C-NMR, and mass) data, which were in line with the proposed structures. The IR spectra of the compounds **3a–g** afforded pyrazoline C=N stretching (1589–1573 cm^{-1}), carboxamide group N–H stretching (3462–3232 cm^{-1}), and C=O stretching (1373–1348 cm^{-1}) bands. The CH_2 protons of the pyrazoline ring resonated as a pair of doublets of doublets at 3.10–3.22 ppm (H_A) and 3.90–4.00 ppm (H_B). The CH (H_X) proton appeared as a doublet of doublets at 5.50–6.32 ppm due to vicinal coupling with the two magnetically non-equivalent protons of the methylene group at position 4 of the pyrazoline ring (J_{AB}: 17.72–18.40, J_{AX}: 5.10–5.50 Hz, J_{BX}: 12.01–12.95 Hz). N–H protons of the carboxamide group for compounds **3a–g** were seen at 9.10–9.37 ppm as singlet. The protons belonging to the aromatic ring and the other aliphatic groups are observed with the expected chemical shift and integral values. Mass spectra (ESI–MS) of compounds showed a $[MH]^+$ peak, in line with their molecular formula.

Biological activities

Mosquito activity

The biting deterrent activity of 25 tested compounds against *Ae. aegypti* is given in Table 1. The chalcone derivative **1g** carrying 4-methylsulfonylphenyl and 4-metoxyphenyl ring and the 2-pyrazoline-1-carbothioamide derivatives **2a** having 4-trifluoromethylphenyl with 4-methoxyphenyl and **2e** having thiophen-2-yl with 2,6-dichlorophenyl rings in 2-pyrazoline moiety showed the highest deterrent activity



1a, 2a: Ar_1=4-Trifluoromethylphenyl, Ar_2=4-Methoxyphenyl

1b, 2b: Ar_1=5-Chlorothiophen-2-yl, Ar_2=2,6-Dichlorophenyl

1c, 2c: Ar_1 =5-Bromothiophen-2-yl, Ar_2=2,6-Dichlorophenyl

1d, 2d: Ar_1=4-Methylsulfonylphenyl, Ar_2=2,6-Dichlorophenyl

1e, 2e: Ar_1=Thiophen-2-yl, Ar_2=2,6-Dichlorophenyl

1f, 2f : Ar_1=Phenyl, Ar_2=2,6-Dichlorophenyl

1g, 2g: Ar_1=4-Methylsulfonylphenyl, Ar_2=4-Metoxyphenyl

1h, 2h: Ar_1=Thiophen-2-yl, Ar_2=3,4-Dimetoxyphenyl

1i, 2i: Ar_1= Thiophen-2-yl, Ar_2=4-Dimethylaminophenyl

3a: Ar_1=4-Trifluoromethylphenyl, Ar_2=4-Methoxyphenyl (R:4-Cl)

3b: Ar_1=4-Trifluoromethylphenyl, Ar_2=4-Methoxyphenyl (R:4-CH_3S)

3c: Ar_1=4-Trifluoromethylphenyl, Ar_2=4-Methoxyphenyl (R:4-CF_3)

3d: Ar_1=4-Trifluoromethylphenyl, Ar_2=4-Methoxyphenyl (R:3-Cl)

3e: Ar_1=4-Methylsulfonylphenyl, Ar_2=4-Methoxyphenyl (R:4-Cl)

3f: Ar_1=4-Methylsulfonylphenyl, Ar_2=4-Methoxyphenyl (4-CH_3S)

3g: Ar_1=4-Methylsulfonylphenyl, Ar_2=4-Methoxyphenyl (R:4-CF_3)

Scheme 1 Synthetic pathway for compounds **1a**–**i**, **2a**–**i**, and **3a**–**g**. Reagents and conditions: *a* NaOH, MeOH; *b* $NH_2CSNHNH_2$, C_2H_5OH, NaOH *c* 4-substituted phenyl semicarbazide (**S1**–**S4**), NaOH

with BDI (Biting Deterrent Index) values of 0.85, 0.83, and 0.80 nmol/cm^2 at 25 nmol/cm^2 which was closed to positive control, *N*,*N*-diethyl-3-methylbenzamide (DEET) (BDI = 1 nmol/cm^2). Compounds **1g**, **2a** and **2e** were evaluated using human-based cloth-based repellent assay to determine minimum effective dosage (MED) for repellency.

However, these compounds did not repel *Ae. aegypti* at 0.375, 0.094, and 0.375 mg/cm^2, respectively, compared with the reference standard, DEET (0.004 ± 0.002 mg/cm^2). In larvicidal bioassays, only chalcone derivatives **1e**, **1f** and 2-pyrazoline-1-carbothioamide derivative **2g** showed larvicidal activity, whereas all the other compounds did not show any larvicidal activity at the highest screening dose of 100 ppm. Based on the calculated LC_{50} values (Table 2), chalcone derivative **1e** carrying thiophene and 2,6-dichlorophenyl was the most toxic compound (LC_{50} = 2.58 ppm) against 1-day-old *Ae. aegypti* larvae, followed by **1f** carrying phenyl and 2,6-dichlorophenyl (LC_{50} = 5.69 ppm) and **2g** having 4-methylsulfonylphenyl and 4-methoxyphenyl rings on the 2-pyrazoline moiety (LC_{50} = 15.1 ppm).

Table 1 Mosquito biting deterrent effects of synthesized compounds against seven- to 13-day-old mated females of *Ae. aegypti*

Compound	Concentration (nmol/cm^2)	BDI value (±SE) (A)
1a	25	0.57 ± 0.093
1b	25	0.48 ± 0.114
1c	25	0.53 ± 0.058
1d	25	0.64 ± 0.094
1e	25	0.64 ± 0.048
1f	25	0.64 ± 0.048
1g	25	0.85 ± 0.145
1h	25	0.7 ± 0.099
1i	25	0.75 ± 0.097
2a	25	0.83 ± 0.044
2b	25	0.49 ± 0.071
2c	25	0.63 ± 0.070
2d	25	0.79 ± 0.064
2e	25	0.8 ± 0.089
2f	25	0.73 ± 0.086
2g	25	0.71 ± 0.078
2h	25	0.57 ± 0.086
2i	25	0.62 ± 0.101
3a	25	0.61 ± 0.070
3b	25	0.58 ± 0.107
3c	25	0.61 ± 0.070
3d	25	0.56 ± 0.063
3e	25	0.64 ± 0.048
3f	25	0.58 ± 0.071
3g	25	0.69 ± 0.087

SE Standard error of the mean

Values within a column not followed by the same letter are significantly different ($P < 0.05$, DMRT). A Biting Deterrence Index (BDI) value of 1 is similar to DEET, whereas a value of 0 is similar to solvent control at 25 nmol/cm^2

Antifungal activity

A total of 25 compounds were evaluated against seven important plant pathogens, and the active compounds are given in Fig. 1. At the lowest dose, 0.3 μM, chalcone derivative **1f** and 2-pyrazoline-1-carbothioamide derivative **2h** having thiophen and 3,4-dimethoxyphenyl rings showed 97.6 and 98.5 % inhibition, respectively, against *C. gloeosporioides* after 48 h. Compound **2e** possessed good antifungal activity with 64.6 % growth inhibition of *C. gloeosporioides* at 30 μM, although it showed weak antifungal activity against *C. acutatum* and *C. fragariae* at the highest concentration. Compound **2h** showed a decrease in activity against *C. gloeosporioides* at 30 μM; this lower antifungal activity at higher concentration was due to precipitation in the aqueous growth medium. Out of the 25 compounds, only **1f**, **2e,** and **2h** demonstrated weak antifungal activity at 30 μM with 59.0, 41.9, and 73.3 % inhibition, respectively, against *F. oxysporum*. Compounds **2e** and **2h** exhibited the most antifungal activity against *Phomopsis* species with 83.5 and 72.3 %, respectively, growth inhibition of *P. obscurans* at 30 μM, respectively.

At 30 μM, compounds **1f** and **2a** and **2g** showed greater fungal growth inhibition than positive control captan against *B. cinerea.* The other compounds, **1c**, **1g**, **1i**, **2d**, **2e**, and **2 h** exhibited fungal growth inhibition that was similar or slightly higher than positive control azoxystrobin (64.1 %) at the 30 μM (Fig. 1). 2-Pyrazoline-1-carboxamide derivatives (**3a**–**g**) were found to be less active than chalcones (no data were shown).

Phytotoxicity activity

The most active antifungal compounds (**2d**, **2g**, **2h**) were evaluated for their phytotoxicity because, to be used as fungicides for plant protection, they should have little or no phytotoxicity. A dose–response experiment was performed using 33, 100, 333, and 1000 μM of each compound against lettuce and agrostis (Fig. 2). None of the compounds were significantly phytotoxic to lettuce at the highest concentration. Compounds **2g** and **2h** inhibited the growth of bentgrass at concentrations of 333 μM and higher. Compounds **2g** and **2h** were phytotoxic to *L. paucicostata* (Fig. 3). **2g** completely inhibited growth at 100 μM and higher, whereas **2h** was only effective at 333 μM and higher. Both compounds stimulated growth at subtoxic concentrations (hormesis), with remarkable hormesis using **2h** at 33 μM. Hormesis caused by natural phytotoxins is a common phenomenon (Belz *et al.*, 2007). At concentrations needed for fungicidal activity, these compounds were not phytotoxic and may have growth stimulatory effects.

Table 2 Toxicity of the compounds **1e**, **1f,** and **2g** against 1-day-old *Ae. aegypti* 24 h after treatment

Compound	LC_{50}	(95 % CI)[a]	LC_{90}	(95 % CI)[a]	χ^2	DF[b]
1e	2.58	(2.27–2.90)	4.63	(3.97–5.81)	59.9	48
1f	5.69	(5.0–6.46)	11.14	(9.44–14.04)	81.0	48
2g	15.14	(10.93–19.78)	51.12	(36.43–90.64)	37.7	48

[a] LC_{50} and LC_{90} values are given in ppm (95 % confidence interval)

[b] DF refers to degree of freedom

Conclusion

A series of chalcones (**1a–i**), 2-pyrazoline-1-carbothioamides (**2a–i**), and 2-pyrazoline-1-carboxamide derivatives (**3a–g**) have been synthesized and screened for the first time for their pesticidal activities. 2-Pyrazoline-1-carboxamide derivatives (**3a–g**) are new compounds and synthesized for the first time. The chalcone derivative **1g** carrying 4-methylsulfonylphenyl and 4-metoxyphenyl ring and the 2-pyrazoline-1-carbothioamide derivatives **2a** having 4-trifluoromethylphenyl with 4-methoxyphenyl and **2e** having thiophen-2-yl with 2,6-dichlorophenyl rings in 2-pyrazoline moiety showed the highest deterrent activity with Biting Deterrent Index (BDI) values of 0.85, 0.83, and 0.80 nmol/cm^2 at 25 nmol/cm^2 which was closed to positive control, *N*,*N*-diethyl-3-methylbenzamide (DEET, BDI = 1 nmol/cm^2). Based on the calculated LC_{50} values, chalcone derivative **1e** carrying thiophene and 2,6-dichlorophenyl was the most toxic compound against 1-day-old *Ae. aegypti* larvae. The chalcone derivative **1f** and 2-pyrazoline-1-carbothioamide derivative **2h** showed the highest antifungal activity, each with similar or greater activity than commercial fungicides. The most active fungicidal compounds (**2d**, **2g**, and **2h**) were weakly phytotoxic, with little or no phytotoxicity at concentrations that were highly fungitoxic. Compound **2h** stimulated the growth of *L. paucicostata* at concentrations that are fungitoxic to several plant pathogens.

The results of bioassays indicated that chalcones (**1a–i**) and 2-pyrazoline-1-carbothioamides (**2a–i**) derivatives possessed weak-to-excellent activity against mosquito and plant pathogens. However, 2-pyrazoline-1-carboxamide derivatives (**3a–g**) showed weak biting deterrent activity at 25 nmol/cm^2 against adult female *Ae. aegypti* and showed no mortality at 100 pm against 1-day-old *Ae. aegypti* larvae and **3a–g** compounds also demonstrated insignificant antifungal activity at a highest concentration of 30 mM against *Colletotrichum acutatum*, *C. fragariae, C. gloeosporioides*, *Botrytis cinerea, Phomopsis viticola, P. obscurans,* and *Fusarium oxysporum.* Based on these results, we conclude that the position of the substituent present on the ring affects the activity, and active molecules can be utilized for the design of new molecules to find new derivatives for controlling pest and pathogenic fungi.

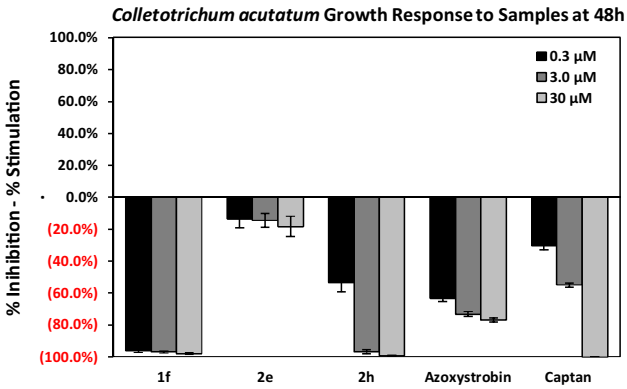
Experimental

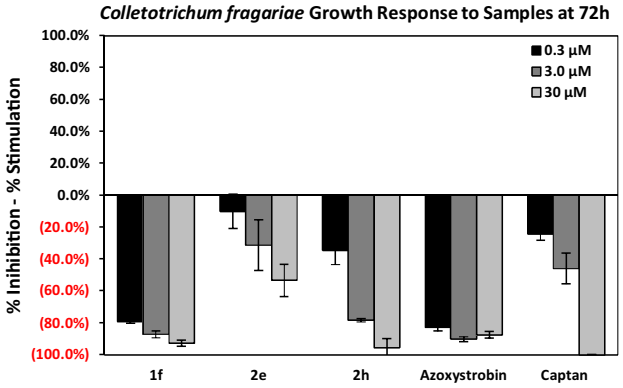
All the reagents were obtained commercially and used by further purification using standard procedures. Melting points were determined by an open capillary method and are uncorrected. The IR spectra were recorded on a PerkinElmer (Spectrum ONE) FT-IR Spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded (in DMSO-d_6) with a Bruker NMR 400 and 100-MHz spectrometer, respectively. The chemical shift values are expressed in ppm (δ scale) using tetramethylsilane as an internal standard. The mass spectral measurements were taken by electrospray method on LC–MS-Agilent 1100. Elemental analysis was performed on Leco 215 CHNS-932 analyzer. All the compounds gave C, H, and N analyses within ±0.4 %.

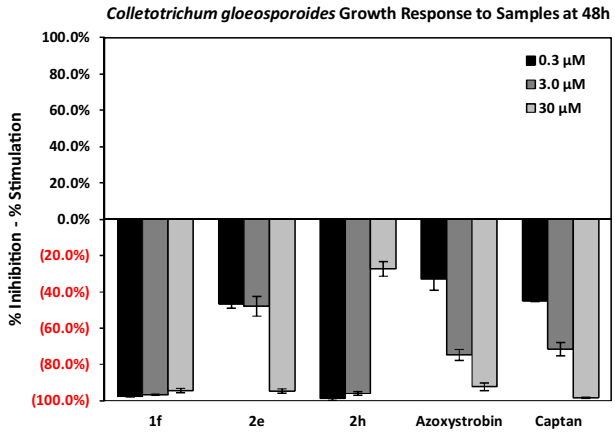
The intermediated chalcones (**1a–i**) were prepared by reacting equimolar aldehyde and ketone in the presence of a sodium hydroxide and methanol through Claisen–Schmidt condensation. 3,5-disubstituted-4,5-dihydro-1*H*-pyrazole-1-carbothioamides (**2a–i)** were synthesized by refluxing compounds **1a–i** and thiosemicarbazide in the presence of alkaline medium. Physicochemical and spectroscopic characterization of the 2-pyrazoline derivatives **1a–i** and **2a–i** has been previously described (Beyhan *et al*., 2015).

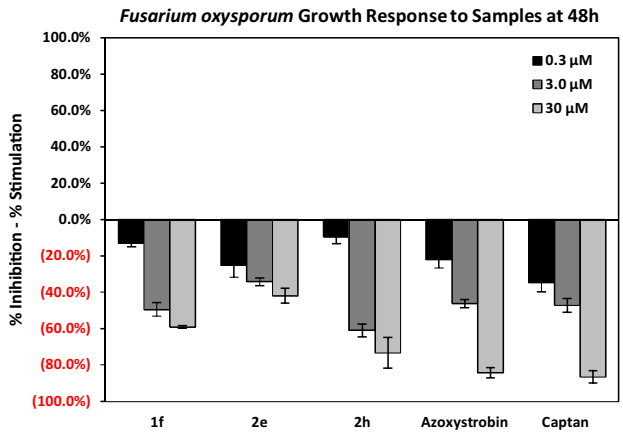
General synthesis methods of *N*,3,5-trisubstituted-4,5-dihydro-1*H*-pyrazole-1-carboxamides (3a–g)

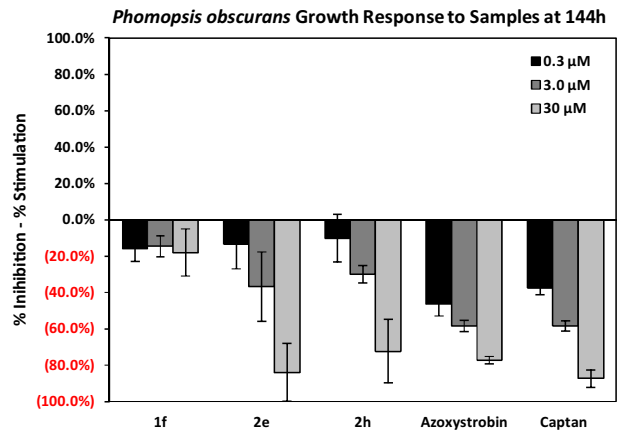
The 5 mmol 4-substituted phenylisothiocyanates were dissolved in diethyl ether. In order to stir the mixture, 5 mmol hydrazine hydrate was added dropwise and stirred for 15 min. The obtained 4-substituted phenyl semicarbazides (**S1–S4**) were filtered, dried, and washed with petrol ether. 2 mmol chalcone (**1a–1i)** was dissolved in ethanol (10 ml), and then, semicarbazides (**S1–S4**) were added. Finally, solution of 5 mmol NaOH in 1 ml water

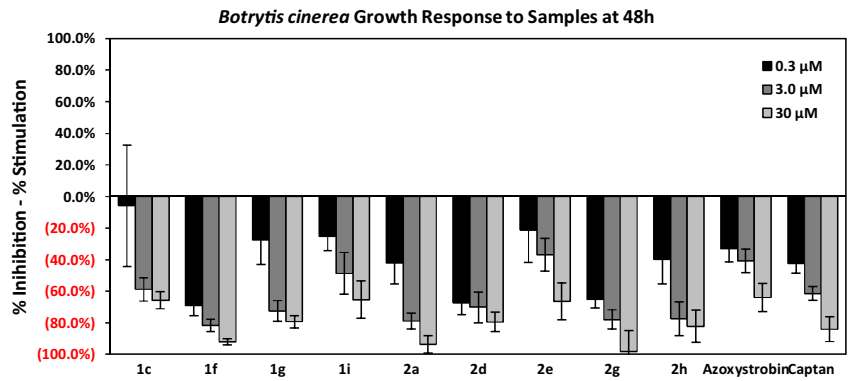

Colletotrichum acutatum Growth Response to Samples at 48h
% Inihibition - % Stimulation
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
(20.0%)
(40.0%)
(60.0%)
(80.0%)
(100.0%)
0.3 µM
3.0 µM
30 µM
1f
2e
2h
Azoxystrobin
Captan


Colletotrichum fragariae Growth Response to Samples at 72h
% Inihibition - % Stimulation
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
(20.0%)
(40.0%)
(60.0%)
(80.0%)
(100.0%)
0.3 µM
3.0 µM
30 µM
1f
2e
2h
Azoxystrobin
Captan


Colletotrichum gloeosporoides Growth Response to Samples at 48h
% Inihibition - % Stimulation
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
(20.0%)
(40.0%)
(60.0%)
(80.0%)
(100.0%)
0.3 µM
3.0 µM
30 µM
1f
2e
2h
Azoxystrobin
Captan


Fusarium oxysporum Growth Response to Samples at 48h
% Inihibition - % Stimulation
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
(20.0%)
(40.0%)
(60.0%)
(80.0%)
(100.0%)
0.3 µM
3.0 µM
30 µM
1f
2e
2h
Azoxystrobin
Captan


Phomopsis obscurans Growth Response to Samples at 144h
% Inihibition - % Stimulation
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
(20.0%)
(40.0%)
(60.0%)
(80.0%)
(100.0%)
0.3 µM
3.0 µM
30 µM
1f
2e
2h
Azoxystrobin
Captan


Botrytis cinerea Growth Response to Samples at 48h
% Inihibition - % Stimulation
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
(20.0%)
(40.0%)
(60.0%)
(80.0%)
(100.0%)
0.3 µM
3.0 µM
30 µM
1c
1f
1g
1i
2a
2d
2e
2g
2h
Azoxystrobin
Captan

◀**Fig. 1** Growth inhibition of *Colletotrichum*, *Fusarium*, *Phomopsis*, and *Botrytis* species using 96-well microdilution broth assays in a dose–response experiment to tested compounds with the commercial fungicide standard azoxystrobin and captan. Only active compounds are presented here

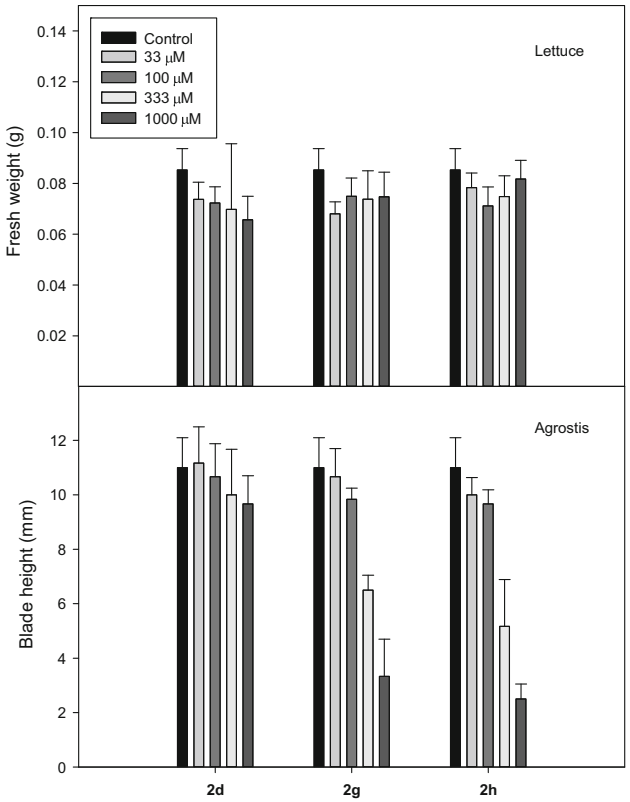


Fig. 2 Growth effects of **2d**, **2g**, **2h** on *Lactuca sativa* and *Agrostis stolonifera*

was added. After the completion of the reaction by TLC, the reaction mixture was poured into ice, and the precipitate was filtered, dried, and crystallized from ethanol.

*N-(4-Chlorophenyl)semicarbazide (**S1**)*

Yield 98 %, mp 282–284 °C (EtOH), UV (EtOH), λ_{max} (EtOH) (nm): 204, 247. IR ν_{max} (cm^{-1}): 3330, 3292, 3218, 3098, 2980, 2924, 1667, 1592, 1530 1491, 1455, 1405, 1093.

*N-[4-(Methylsulphanyl)phenyl]semicarbazide (**S2**)*

Yield 92 %, mp 251–252 °C (EtOH), λ_{max} (EtOH) (nm): 206, 240, 269. IR ν_{max} (cm^{-1}): 3248, 3209, 3095, 2976, 2914, 1664, 1587, 1525, 1491, 1402.

*N-(4-Trifluoromethlphenyl)semicarbazide (**S3**)*

Yield 90 %, mp 240–242 °C (EtOH), λ_{max} (EtOH) (nm): 204, 245. IR ν_{max} (cm^{-1}): 3335, 3280, 3220, 3100, 2980, 2925, 1665, 1590, 1540 1495, 1450, 1409.

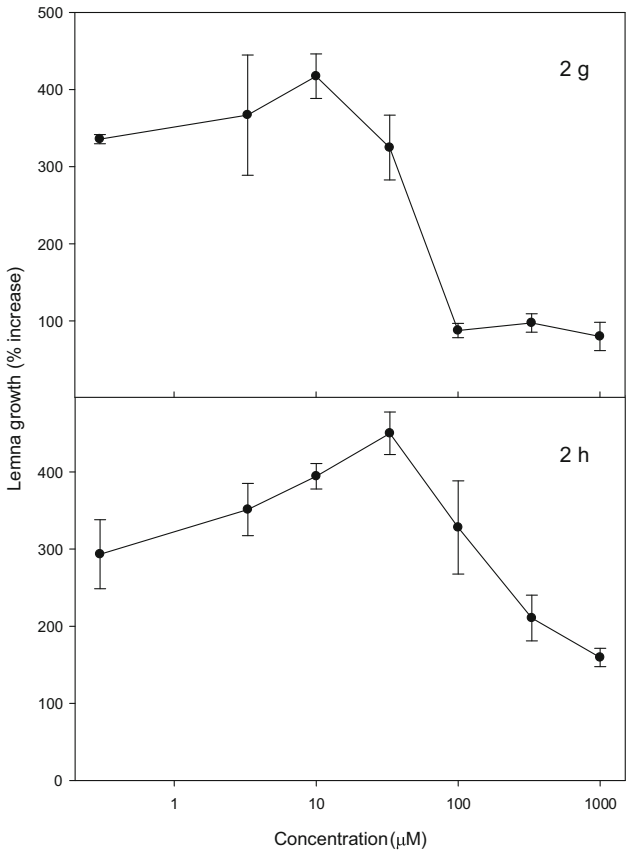


Fig. 3 Dose–growth response curve of the effects of the compounds **2g** and **2h** on *Lemna pauciscostata* growth 7 days after treatment. The lowest concentration is zero

*N-(3-Chlorophenyl)semicarbazide (**S4**)*

Yield 90 %, mp 260–262 °C (EtOH), UV (EtOH), λ_{max} (EtOH) (nm): 204, 247. IR ν_{max} (cm^{-1}): 3330, 3290, 3220, 3100, 2980, 2924, 1665, 1590, 1530 1490, 1455, 1400, 1093.

N-(4-Chlorophenyl)-5-(4-methoxyphenyl)-3-[4-(trifluoromethylphenyl)]-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3a)

Yield 30 %, mp 183–185 °C (EtOH), UV (EtOH), λ_{max} (logε): 204 (4.00), 225 (4.12), 269 (4.22), 323 (4.25). IR ν_{max} (cm^{-1}): 3385(NH), 1676 (C=O), 1585 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6/TMS), δ, ppm (J, Hz): 3.21 (1H, dd, H_A, J_{AB}: 17.72 Hz, J_{AX}: 5.58 Hz); 3.72 (3H, s, OCH_3); 3.90 (1H, dd, H_B, J_{BA}: 17.72 Hz, J_{BX}: 12.01 Hz); 5.53 (1H, dd, H_X, J_{XA}: 5.35 Hz, J_{XB}: 12.01 Hz); 6.90 (2H, d, J: 8.67 Hz, 4-methoxyphenyl H3, H5); 7.18 (2H, d, J: 8.64 Hz, 4-methoxyphenyl H2, H6); 7.31 (2H, d, J: 8.86 Hz, 4-chlorophenyl H3, H5); 7.66 (2H, d, J: 8.91 Hz, 4-chlorophenyl H2, H6); 7.84 (2H, d, J: 8.42 Hz,

4-trifluoromethylphenyl H2, H6); 8.14 (2H, d, *J*: 8.27 Hz, 4-trifluoromethylphenyl H3, H5); 9.29 (1H, s, NH). ^{13}C-NMR (100 MHz), (DMSO-d_6/TMS), δ (ppm): 42.55 (pyrazole C4); 60.49 (pyrazole C5); 116.37, 129.15, 129.91, 130.59, 131.89, 132.16, 133.31, 135.59, 136.06, 137.02 (aromatic C and CF_3); 149.98 (pyrazole C3); 164.18 (C=O). Anal. Calcd. for $C_{24}H_{19}ClF_3N_3O_2$ (%); C, 59.67; H 3.89; N 8.86. Found: C, 59.83; H, 3.90; N, 8.84, ESI–MS: *m/z* 474 (MH^+, 32 %), 347 (15 %), 319 (100 %), 305 (40 %), 212 (10 %).

N-(4-(Methylsulfanyl)phenyl)-5-(4-methoxyphenyl)-3-[4-(trifluoromethylphenyl)]-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3b)

Yield 46 %, mp 166–167 °C (EtOH), UV (EtOH), λ_{max} (logε): 204 (4.70), 225 (4.47), 323 (4.45), IR ν_{max} (cm^{-1}): 3383, 3325 (NH), 1678 (C=O), 1579 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6/TMS), δ, ppm (*J*, Hz): 2.44 (3H, s, SCH_3); 3.22 (1H, dd, H_A, J_{AB}: 18.10 Hz, J_{AX}: 5.50 Hz); 3.72 (3H, s, OCH_3); 3.90 (1H, dd, H_B, J_{BA}: 18.10 Hz, J_{BX}: 12.04 Hz); 5.50 (1H, dd, H_X, J_{XA}: 5.47 Hz, J_{XB}: 12.04 Hz); 6.90 (2H, d, *J*: 8.60 Hz, 4-methoxyphenyl H3, H5); 7.19 (4H, dd, J_1: 6.44 Hz, J_2: 6.37 Hz, 4-methoxyphenyl H2, H6 and 4-methylsulfanylphenyl H2, H6); 7.58 (2H, d, *J*: 8.66 Hz, 4-methylsulfanylphenyl H3, H5); 7.84 (2H, d, *J*: 8.40 Hz, 4-trifluoromethylphenyl H2, H6); 8.15 (2H, d, *J*: 8.27 Hz, 4-trifluoromethylphenyl H3, H5); 9.19 (1H, s, NH). ^{13}C-NMR (100 MHz), (DMSO-d_6/TMS), δ (ppm): 16.41 (SCH_3); 42.55 (pyrazole C4); 55.76 (OCH_3); 60.73 (pyrazole C5); 130.11, 130.43, 131.32, 135.53, 135.97, 137.57, 151.14 (aromatic C and CF_3); 151.83 (pyrazole C3); 159.16 (C=O). Anal. Calcd. for $C_{25}H_{22}F_3N_3O_2S$ (%); C, 61.84; H, 4.57; N, 8.65; S 6.60. Found: C, 61.86; H, 4.53; N, 8.62; S, 6.61. ESI–MS: *m/z* 486 (MH^+, 20 %), 319 (100 %), 305 (40 %), 212 (10 %), 166 (25 %).

N-(4-Trifluoromethylphenyl)-5-(4-methoxyphenyl)-3-[4-(trifluoromethylphenyl)]-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3c)

Yield 55 %, mp 252–254 °C (EtOH), UV (EtOH), λ_{max} (logε): 206 (4.10), 224 (4.12), 338 (4.20), IR ν_{max} (cm^{-1}): 3360, 3248 (NH), 1670 (C=O), 1590 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6/TMS), δ, ppm (*J*, Hz): 3.20 (1H, dd, H_A, J_{AB}: 18.40 Hz, J_{AX}: 5.20 Hz); 4.00 (1H, dd, H_B, J_{BA}: 18.40 Hz, J_{BX}: 12.80 Hz); 6.30 (1H, dd, H_X, J_{XA}: 5.20 Hz, J_{XB}: 12.80 Hz); 6.90 (2H, d, *J*: 8.60 Hz, 4-methoxyphenyl H3, H5); 7.19 (4H, dd, J_1: 6.44 Hz, J_2: 6.37 Hz, 4-methoxyphenyl H2, H6); 7.58–8.18 (8H, m aromatic protons); 9.25 (1H, s, NH), ^{13}C-NMR (100 MHz), (DMSO-d_6/TMS), δ (ppm): 40.93 (pyrazole C4); 60.34 (pyrazole C5); 128.76, 129.14, 129.85, 130.58, 130.75, 131.35, 133.30, 134.26, 135.61, 137.14 (aromatic C and CF_3); 150.92 (pyrazole C3); 166.10 (C=O): Anal. Calcd. for $C_{25}H_{19}F_6N_3O_2$ (%); C, 59.17; H, 3.77; N, 8.28. Found: C, 59.52; H, 3.78; N, 8.30. ESI–MS: *m/z* 508 (MH^+, 30 %), 347 (25 %) 319 (100 %), 305 (32 %), 212 (10 %), 160 (25 %).

N-(3-Chlorophenyl)-5-(4-methoxyphenyl)-3-[4-(trifluoromethylphenyl)]-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3d)

Yield 30 %, mp 183–185 °C (EtOH), UV (EtOH), λ_{max} (logε): 204 (4.00), 225 (4.12), 269 (4.22), 323 (4.25), IR ν_{max} (cm^{-1}): 3385(NH), 1676 (C=O), 1585 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6/TMS), δ, ppm (*J*, Hz): 3.21 (1H, dd, H_A, J_{AB}: 17.76 Hz, J_{AX}: 5.35 Hz); 3.72 (3H, s, OCH_3); 3.90 (1H, dd, H_B, J_{BA}: 17.76 Hz, J_{BX}: 12.01 Hz); 5.53 (1H, dd, H_X, J_{XA}: 5.35 Hz, J_{XB}: 12.01 Hz); 6.90 (2H, d, *J*: 8.67 Hz, 4-methoxyphenyl H3, H5); 7.18 (2H, d, *J*: 8.64 Hz, 4-methoxyphenyl H2, H6); 7.31 (1H, s, 3-chlorophenyl H2); 7.66 (3H, m, 3-chlorophenyl H4, H5, H6); 7.84 (2H, d, *J*: 8.42 Hz, 4-trifluoromethylphenyl H2, H6); 8.14 (2H, d, *J*: 8.27 Hz, 4-trifluoromethylphenyl H3, H5); 9.29 (1H, s, NH). ^{13}C-NMR (100 MHz), (DMSO-d_6/TMS), δ (ppm): 42.55 (pyrazole C4); 60.49 (pyrazole C5); 116.37, 129.15, 129.91, 130.59, 131.89, 132.16, 133.31, 135.59, 136.06, 137.02 (aromatic C and CF_3); 149.98 (pyrazole C3); 164.18 (C=O). Anal. Calcd. for $C_{24}H_{19}ClF_3N_3O_2$ (%); C, 59.67; H, 3.89; N, 8.86, Found: C, 59.65; H, 3.90; N, 8.84. ESI–MS: *m/z* 474 (MH^+, 20 %), 347 (27 %), 305 (100 %), 212 (10 %), 127 (25 %).

N-(4-Chlorophenyl)-5-(4-methoxyphenyl)-3-[4-(methylsulfonylphenyl)]-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3e)

Yield 45 %, mp 256–257 °C (EtOH), UV (EtOH), λ_{max} (logε): 203 (4.05), 248 (4.15), 347 (4.20), IR ν_{max} (cm^{-1}): 3440, 3220 (NH), 1690 (C=O), 1580 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6/TMS), δ, ppm (*J*, Hz): 3.17 (1H, dd, H_A, J_{AB}: 18.07 Hz, J_{AX}: 5.10 Hz); 3.20 (3H, s, $-SO_2CH_3$); 3.96 (1H, dd, H_B, J_{BA}: 18.06 Hz, J_{BX}: 12.95 Hz); 6.31 (1H, dd, H_X, J_{XA}: 5.10 Hz, J_{XB}: 12.95 Hz); 6.90 (2H, d, *J*: 8.67 Hz, 4-methoxyphenyl H3, H5); 7.16 (2H, d, *J*: 8.66 Hz, 4-methoxyphenyl H2, H6); 7.30 (2H, d, *J*: 8.90 Hz, 4-chlorophenyl H3, H5); 7.64 (2H, d, *J*: 8.90 Hz, 4-chlorophenyl H2, H6); 7.97 (2H, d, *J*: 8.60 Hz, 4-methylsulfonylphenyl H2, H6); 8.12 (2H, d, *J*: 8.20 Hz, 4-methylsulfonylphenyl H3, H5); 9.10 (1H, s, NH). ^{13}C-NMR (100 MHz), (DMSO-d_6/TMS), δ (ppm): 40.34 (pyrazole C4); 44.00 (SO_2CH_3); 60.0 (pyrazole C5); 129.28, 129.73, 131.17, 131.68, 133.09, 133.91, 134.01, 136.21, 137.62 (aromatic C); 151.20 (pyrazole C3); 164.20 (C=O). Anal. Calcd. for $C_{24}H_{12}ClN_3O_4S$ (%); C, 60.07; H,

4.53; N, 8.66; S 6.60. Found: C, 60.02; H 4.54; N 8.68; S 6.61. ESI–MS: *m/z* 484 (MH^+, 18 %), 358 (30 %), 329 (100 %), 224 (10 %), 127 (20 %).

N-(4-(Methylsulfanyl)phenyl)-5-(4-methoxyphenyl)-3-[4-(methylsulfonylphenyl)]-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3f)

Yield 53 %, mp 158–160 °C (EtOH), UV (EtOH), λ_{max} (logε): 202 (4.14), 221 (4.10), 250 (4.20), 348 (4.10), IR ν_{max} (cm^{-1}): 3400, 3250 (NH), 1686 (C=O), 1580 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6/TMS), δ, ppm (*J*, Hz): 3.22 (1H, dd, H_A, J_{AB}: 17.43 Hz; J_{AX}: 5.50 Hz); 3.25 (3H, s, $-SO_2CH_3$); 3.98 (1H, dd, H_B, J_{BA}: 17.43 Hz, J_{BX}: 12.90 Hz); 6.32 (1H, dd, H_X, J_{XA}: 5.50 Hz, J_{XB}: 12.90 Hz); 6.91 (2H, d, *J*: 8.67 Hz, 4-methoxyphenyl H3, H5); 7.18 (2H, d, *J*: 8.64 Hz, 4-methoxyphenyl H2, H6); 7.19 (2H, d, *J*: 8.64 Hz, 4-methylsulfanylphenyl H2, H6); 7.58 (2H, d, *J*: 8.66 Hz, 4-methylsulfanylphenyl H3, H5); 7.97 (2H, d, *J*: 8.60 Hz, 4-methylsulfonylphenyl H2, H6); 8.12 (2H, d, *J:* 8.20 Hz, 4-methylsulfonylphenyl H3, H5); 9.28 (1H, s, NH). ^{13}C-NMR (100 MHz), (DMSO-d_6/TMS), δ (ppm): 16.40 (SCH_3); 40.34 (pyrazole C4); 44.02 (SO_2CH_3); 60.49 (pyrazole C5); 116.37, 129.15, 129.91, 130.59, 131.89, 132.16, 133.31, 135.59, 136.06, 137.02 (aromatic C); 149.98 (pyrazole C3); 164.18 (C=O). Anal. Calcd. for $C_{25}H_{25}N_3O_4S_2$ (%); C, 61.14; H, 5.10; N, 8.91; S, 12.36. Found: C, 61.12; H, 5.08; N, 8.88; S, 12.34. ESI–MS: *m/z* 496 (MH^+, 18 %), 358 (25 %), 329 (100 %), 166 (25 %).

N-(4-Trifluoromethylphenyl)-5-(4-methoxyphenyl)-3-[4-(methylsulfonylphenyl)]-4,5-dihydro-1*H*-pyrazole-1-carboxamide (3g)

Yield 65 %, mp 281–283 °C (EtOH), UV (EtOH), λ_{max} (logε): 204 (4.04), 250 (4.17), 342 (4.22), IR ν_{max} (cm^{-1}): 3430, 3225 (NH), 1695 (C=O), 1580 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6/TMS), δ, ppm (*J*, Hz): 3.12 (1H, dd, H_A, J_{AB}: 18.07 Hz, J_{AX}: 5.50 Hz); 3.24 (3H, s, $-SO_2CH_3$); 3.94 (1H, dd, H_B, J_{BA}: 18.06 Hz, J_{BX}: 12.90 Hz); 6.30 (1H, dd, H_X, J_{XA}: 5.50 Hz, J_{XB}: 12.90 Hz); 6.92 (2H, d, *J*: 8.66 Hz, 4-methoxyphenyl H3, H5); 7.18 (2H, d, *J*: 8.66 Hz, 4-methoxyphenyl H2, H6); 7.82 (2H, d, *J*: 8.42 Hz, 4-trifluoromethylphenyl H2, H6); 8.10 (2H, d, *J*: 8.27 Hz, 4-trifluoromethylphenyl H3, H5); 7.97 (2H, d, *J*: 8.60 Hz, 4-methylsulfonylphenyl H2, H6); 8.12 (2H, d, *J*: 8.20 Hz, 4-methylsulfonylphenyl H3, H5); 9.12 (1H, s, NH). ^{13}C-NMR (100 MHz), (DMSO-d_6/TMS), δ (ppm): 40.30 (pyrazole C4); 44.01 (SO_2CH_3); 60.05 (pyrazole C5); 129.30, 129.74, 131.15, 131.56, 133.02, 133.84, 134.00, 136.19, 137.02 (aromatic C); 151.10 (pyrazole C3); 164.40 (C=O). Anal. Calcd. for $C_{25}H_{22}F_3N_3O_4S$ (%); C, 58.42; H, 4.27; N, 8.22; S 6.22. Found: C 58.46; H 4.28; N 8.20; S 6.21. ESI–MS: *m/z* 518 (MH^+, 22 %), 358 (25 %), 329 (60 %), 305 (100 %), 160 (21 %).

Biological activities

Insects

The *Ae. aegypti* (L.) mosquitoes used in these studies were from a laboratory colony maintained since 1952, originally from Orlando, FL, and now at the Mosquito and Fly Research Unit at the Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, in Gainesville, Florida, USA. For biting deterrence bioassays, eggs were hatched, and the insects were reared to the adult stage in a laboratory maintained at 27 ± 2 °C and 60 ± 10 % RH with a photoperiod regimen of 12:12 h (L–D), and eight- to 18-day-old adult females were used. For larval bioassays, the eggs were hatched, and the larvae were maintained at the above temperature.

Mosquito biting bioassay

Experiments were conducted by using a six-celled in vitro Klun & Debboun (K&D) module bioassay system developed by Klun *et al*. (2005) for quantitative evaluation of biting deterrent properties of candidate compounds. Briefly, the assay system briefly consists of a six-well reservoir with each of the 3 × 4 cm wells containing 6 ml of blood. As described by Ali *et al*. (2012), a feeding solution consisting of CPDA-1 and ATP was used instead of blood. Green fluorescent tracer dye (www.blacklightword.com) was used to determine feeding by the females. Chalcone and pyrazole derivatives were tested in this study. Samples were applied at concentrations of 25 nmol/cm^2, and DEET (97 %, *N*,*N*-diethyl-3-methylbenzamide) (Sigma-Aldrich, St. Louis, MO) at 25 nmol/cm^2 was used as positive control. All treatments were freshly prepared in molecular biology grade 100 % ethanol (Fisher Scientific Chemical Co. Fair Lawn, NJ) at the time of bioassay. Temperature of the solution in the reservoirs was maintained at 37 °C by continuously passing warm water through the reservoir using a circulatory bath. Reservoirs were covered with a layer of collagen membrane (Devro, Sandy Run, SC). Test compounds were randomly applied to six 4 × 5 cm areas of organdy cloth and positioned over the membrane-covered CPDA-1 + ATP solution with a Teflon separator placed between the treated cloth and the six-celled module to prevent the contamination of the module. A six-celled K&D module containing five female mosquitoes per cell was positioned over the cloth treatments covering the six CPDA-1 + ATP solution membrane wells, and trap doors were opened to

expose the treatments to these females. The number of mosquitoes biting through cloth treatments in each cell was recorded after a 3-min exposure, and mosquitoes were prodded back into the cells to check the actual feeding. Mosquitoes were squashed, and the presence or absence of green fluorescent tracer dye in the gut was used as an indicator of feeding. A replicate consisted of six treatments: four test materials, DEET (commercial biting deterrent standard), and ethanol-treated organdy as solvent control applied randomly. One set of five replications each with five females per treatment was conducted using a newly treated organdy and a new batch of females in each replication. Treatments were replicated five times.

Mosquito repellent bioassay

Repellency was determined as the minimum effective dosage (MED), which is the minimum threshold surface concentration necessary to prevent mosquitoes from biting through the treated surface (US Department of Agriculture, 1977). Approximately 500 (±10 %) mosquitoes were collected and loaded into a test cage (size of 45 cm × 37.5 cm × 5 cm) and held in the cage for 25 (± 2.5) min before initiating repellency assays. Serial dilutions were then made such that the concentrations on the cloth for the remaining 1 mL solution were 0.375 and 0.094 mg/cm^2. Each concentration was tested to determine the point where the repellent failed for each of the volunteers in the study; this concentration was averaged and reported. Each test was conducted by having a volunteer affix the treated cloth onto a plastic sleeve to cover a 32-cm^2 window previously cut into the sleeve. Each of the volunteers wore this sleeve/cloth assembly above a nylon stocking covering their arm, with their hands protected by a glove (Katritzky *et al*., 2010). The arm with the sleeve/-cloth assembly was inserted into a cage in which approximately 500 female *Ae. aegypti* mosquitoes (aged 6–10 days) had been preselected as host-seeking using a draw box (Posey and Schreck 1981). Failure of the repellent treatment was 1 % bite through, i.e., the volunteer received five bites through the cloth over the sleeve window during the 1-min assay. There were three human volunteers in this study, and all three provided written informed consent to participate in this study as part of a protocol (636-2005) approved by the University of Florida Human Use Institutional Review Board (IRB-01).

Larvicidal bioassay

Bioassays were conducted to test chalcone and pyrazole compounds for their larvicidal activity against *Ae. aegypti* by using the bioassay system described earlier (Pridgeon *et al*., 2009). Five *Ae. aegypti* larvae were added in a droplet of water to each well of 24-well plates (BD Labware, Franklin Lakes, NJ) by use of a disposable 22.5-cm Pasteur pipette. Fifty microliters of larval diet (2 % slurry of 3:2 beef liver powder (Now Foods, Bloomingdale, Illinois) and Brewer's yeast (Lewis Laboratories Ltd., Westport, CT) was added to each well by using a Finnpipette stepper (Thermo Fisher, Vantaa, Finland). All chemicals tested were diluted in ethanol. Eleven microliters of the test chemical was added to the labeled wells, and 11 µL of ethanol was added to control treatments. After the treatment application, the plates were swirled in clockwise and counterclockwise motions and front to back and side to side five times to ensure even mixing of the chemicals. Larval mortality was recorded 24 h post-treatment. Larvae that showed no movement in the well after manual disturbance of the water were recorded as dead. Compounds that showed larvicidal activity in screening bioassays were followed by dose–response bioassays. A series of five concentrations ranging between 100 and 6.25 ppm were used in each treatment to obtain a range of mortality. Treatments were replicated 10 times for each compound. Permethrin (46.1 % *cis*–53.2 % *trans*, Chemical Service, West Chester, PA) at 0.025 ppm was used as positive control. Larval mortality was recorded 24 h post-treatment.

Statistical analyses for mosquito biting and larvicidal bioassays

As the K&D module bioassay system can handle only four treatments along with negative and positive controls, in order to make direct comparisons among more than four test compounds and to compensate for variation in overall response among replicates, biting deterrent activity was quantified as Biting Deterrence Index (BDI) (Ali *et al*., 2012). The BDIs were calculated using the following formula:

$$\left[\mathrm{BDI}_{i,j,k}\right] = \left[\frac{\mathrm{PNB}_{i,j,k} - \mathrm{PNB}_{c,j,k}}{\mathrm{PNB}_{d,j,k} - \mathrm{PNB}_{c,j,k}}\right]$$

where $\mathrm{PNB}_{i,j,k}$ denotes the proportion of females not biting when exposed to test compound i for replication j and day k ($i = 1$–4, $j = 1$–5, $k = 1$–1), $\mathrm{PNB}_{c,j,k}$ denotes the proportion of females not biting the solvent control "c" for replication j and day k ($j = 1$–5, $k = 1$–1), and $\mathrm{PNB}_{d,j,k}$ denotes the proportion of females not biting in response to DEET "d"(positive control) for replication j and day k ($j = 1$–5, $k = 1$–1). This formula makes an adjustment for inter-day variation in response and incorporates information from the solvent control as well as the positive control.

A BDI value of 0 indicates an effect similar to ethanol, whereas a value significantly greater than 0 indicates biting deterrent effect relative to ethanol. BDI values not

significantly different from 1 are statistically similar to DEET. BDI values were analyzed using the SAS Proc ANOVA procedure of SAS [single factor: test compound (fixed)] (SAS Institute, 2007), and means were separated using the Ryan–Einot–Gabriel–Welsch multiple range test. To determine whether confidence intervals included the values of 0 or 1 for treatments, Scheffe's multiple comparison procedure with the option of CLM was used. LC_{50} values for larvicidal data were calculated by using the PROBIT procedure of SAS. Control mortality was corrected by using Abbott's formula (Abbott 1925). Toxicity was compared among treatments based on non-overlapping 95 % CIs (Savin *et al*., 1977).

Antifungal activity against plant pathogens

A standardized 96-well microdilution broth assay was used to evaluate antifungal activity of compounds toward *B. cinerea*, *C. acutatum, C. fragariae, C. gloeosporioides, P. viticola, P. obscurans,* and *F. oxysporum* (Wang *et al*., 2011; Sobolev *et al*., 2011). The fungicides azoxystrobin (>98 %, Syngenta, Greensboro, NC) and captan (> 98 %, Chem Service, Inc., West Chester, PA) were used as internal fungicide standards in all assays. Each fungus was grown in 0.3, 3.0, and 30.0 μM solutions of each test compound. Microtiter plates (Nunc MicroWell, untreated; Roskilde, Denmark) were covered with a plastic lid and incubated in a growth chamber as described previously (Wang *et al*., 2013; Radwan *et al*., 2014). Fungal growth was then evaluated by measuring the absorbance of each well at 620 nm using a microplate photometer (BioTek Instruments, Winooski, VT, USA). Each chemical was evaluated in duplicate at doses 0.3, 3.0, and 30.0 μM. Sixteen wells containing broth and inoculum served as positive growth controls. Eight wells containing solvent at the appropriate concentration and broth without inoculum were used as negative growth controls. The experiments were repeated three times over a period of time. Mean absorbance values and standard errors were used to evaluate fungal growth at 48 and 72 h except for *P. obscurans* and *P. viticola* where the data were recorded at 144 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. The SAS, Proc ANOVA procedure of SAS, was used to identify significant factors, and Fisher's protected LSD was used to separate means (Steel and Torrie, 1980).

Phytotoxicity activity in *Lactuca sativa* (lettuce) and *Agrostis stolonifera* (bentgrass) bioassays

Bioassays were performed in a similar manner to Dayan *et al*. (2000). A filter paper (Whatman grade 1, 1.5 cm) was placed in each well of the 24-well plates (Costar 3524, Corning Incorporated). Test fractions were dissolved in MeOH with the final concentration being 1 mg/ml. To each test well was added 180 μL distilled deionized (DDI) H_2O and 20 μL appropriate dilution of the sample. Only 20 μL MeOH and 180 μL DDI H_2O were added to each control well as control 1, while 200 μL DDI H_2O was added to each control well as control 2. The solvent was evaporated in a fume hood. Five lettuce or 10 mg of bentgrass seeds was placed into each well of the 24-well plates. The plates were covered, sealed with Parafilm, and incubated in a Percival Scientific CV-36L5 incubator under light conditions at 26 °C and 120.1 μmol/s/m^2 average light intensity for at least 7 days. Phytotoxicity data were quantitatively evaluated on lettuce by blotting and weighing the plants after 7 days and on bentgrass by measuring average shoot height after 7 days. Treatments were replicated so that standard errors of the means could be calculated.

Bioassay against *Lemna paucicostata*

The bioassay used was almost identical to that of Michel *et al*. (2004). This analysis determines the growth of *L. paucicostata* by total frond area. *L. paucicostata* stocks were grown from a single colony consisting of a mother and two daughter fronds in a beaker in Hoagland's No. 2 Basal Salt Mixture (Sigma H2395) (1.6 g/L) with added iron (1 mL of 1000X FeEDTA solution to 1 L of Hoagland media). The 1000X iron solution contained 18.36 g/L of Fe-EDTA. The pH of the media was adjusted to 5.5 with 1 N NaOH. The media was filter-sterilized using a 0.2-μm filter and stored in sterile 1-L bottles. The *L. paucicostata* stocks were grown in approximately 100 mL of media in sterile jars with vented lids in a Percival Scientific CU-36L5 incubator under continuous light conditions at 26 °C and 120 μmol/s/m^{-2} average PAR. Media were changed every 2–3 days, or new stocks were prepared in fresh media. Plant doubling time was approximately 24–36 h. Both screening and replicate series tests were conducted using non-pyrogenic polystyrene sterile six-well plates (CoStar 3506, Corning Incorporated). Each well contained 4950 μL of the Hoagland medium plus 50 μL of water, or the solvent, or the compound dissolved in the appropriate solvent (at a concentration of 100×). Final concentration of the solvent was therefore approximately 1 % by volume. A graphic template of the six-well plates was used for LemnaTec image analysis software (LemnaTec, Würselen, Germany). Each well was inoculated with two 3-frond plants of the same age (four- to five-day olds) and approximate size. All six-well plates were incubated in the Percival incubator as described above at 26 °C and 120 μmol/s/m^2 average PAR.

Acknowledgments This study was supported by Marmara University Scientific Research Projects Commission (BAPKO, Grant No. SAG-A-040609-0140) for the financial support and in part by USDA/ARS Grant No. 56-6402-1-612, Deployed War-Fighter Protection Research Program Grant funded by the US Department of Defense through the Armed Forces Pest Management Board. We thank Jessie Linda Robertson, Ramona Pace, Robert Johnson, Dr. Maia Tsikolia, Natasha M. Agramonte, Nathan Newlon, and Greg Allen for performing bioassays for this study and Dr. James J. Becnel, USDA-ARS, Gainesville, FL, for supplying mosquito eggs.

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