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Antifungal Activity of Thiophenes from *Echinops ritro*

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Extracts from 30 plants of the Greek flora were evaluated for their antifungal activity using direct bioautography assays with three *Colletotrichum* species. Among the bioactive extracts, the dichloromethane extract of the radix of *Echinops ritro* (Asteraceae) was the most potent. Bioassay-guided fractionation of this extract led to the isolation of eight thiophenes. Antifungal activities of isolated compounds together with a previously isolated thiophene from *Echinops transiliensis* were first evaluated by bioautography and subsequently evaluated in greater detail using a broth microdilution assay against plant pathogens *Colletotrichum acutatum*, *Colletotrichum fragariae*, *Colletotrichum gloeosporioides*, *Botrytis cinerea*, *Fusarium oxysporum*, *Phomopsis viticola*, and *Phomopsis obscurans*. 5′-(3-Buten-1-ynyl)-2,2′-bithiophen (**1**), α-terthienyl (**2**), and 2-[pent-1,3-diynyl]-5-[4-hydroxybut-1-ynyl]thiophene (**5**) at 3 and 30 μM were active against all three *Colletotrichum* species, *F. oxysporum*, *P. viticola*, and *P. obscurans*.

KEYWORDS: ***Echinops ritro*; thiophenes; antifungal activity; *Colletotrichum acutatum*; *Colletotrichum fragariae*; *Colletotrichum gloeosporioides*; *Botrytis cinerea*; *Fusarium oxysporum*; *Phomopsis viticola*; *Phomopsis obscurans***

INTRODUCTION

Increased resistance to commercially available agrochemicals has led to a need for new fungicides. Furthermore, the desire for safer and more effective agrochemicals with reduced environmental and/or mammalian toxicity remains important. Essential to these efforts is the identification of new lead candidates possessing high levels of desirable biological activities, reduced unwanted toxicities, new structural types, and perhaps different modes of action, thereby providing protection from cross-resistance to currently used agrochemicals (*1*). Natural product-based fungicides offer advantages in that they can sometimes be specific to a microbial target species and often have unique modes of action with little mammalian toxicity. An additional benefit is their ability to decompose rapidly, thereby reducing their risk to the environment.

In a program aimed at identifying natural fungicides as alternatives to conventional synthetic agrochemicals, plants from Greece were screened for biological activity in preliminary screens. Growth inhibition of *Colletotrichum acutatum*, *Colletotrichum fragariae*, and *Colletotrichum gloeosporioides* for all extracts was determined using direct bioautography and led to the selection of extracts from the radix of *Echinops ritro* L. (Asteraceae) for further studies.

The genus *Echinops* is represented by 82 species distributed in eastern and southern Europe, tropical and North Africa, and Asia (*2*). Previous phytochemical investigations of *E. ritro* reported on the isolation of quinoline alkaloids (*3*), flavanoids (*4*, *5*), and sesquiterpenes (*6*), as well as fatty acids and alkanes (*7*, *8*). In addition, numerous investigations of the genus *Echinops* have resulted in the isolation of thiophenes (*9*). Thiophenes from *Echinops* have been reported to possess many biological activities including insecticidal (*10*) and fungicidal (*11*). Described below is the bioassay-guided fractionation and isolation of thiophenes from *E. ritro* and antifungal activity measured using a microbioassay method.

MATERIALS AND METHODS

General Experimental Procedures. ^{1}H and ^{13}C NMR (including DEPT 135° and 90°) spectra were recorded in $CDCl_3$ on a Bruker Avance 400 MHz spectrometer (Billerica, MA). Two-dimensional NMR techniques (COSY, NOESY, HMQC, and HMBC) were performed using standard Bruker microprograms. High-resolution mass spectra were obtained on a JEOL AccuTOF (JMS-T100LC) (Peabody, MA). Column chromatography was performed using a Biotage, Inc., Horizon pump (Charlottesville, VA) equipped with a Horizon flash collector and a fixed-wavelength (254 nm) detector. Column size was 25 mm × 15 cm filled with 40 g of silica 60 Å (40−63 μm), and the pressure was 12 mL/min. Vacuum liquid chromatography (VLC) was conducted using silica gel 60H from Merck (20−40 μm) (Whitehouse Station, NJ).

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Table 1. Plant Species of Investigated Greek Flora

name	family	plant part	voucher	origin	collection date
Anchusa cespitosa Lam.[a]	Boraginaceae	whole	KL064	western Crete	May 1999
Aristolochia cretica Lam.[a]	Aristolochiaceae	rhizome	KL001R	eastern Crete	April 2000
		flowers	KL001		
		aerial	KL001Y		
Arum idaeum Coustr. & Gand.[a]	Araceae	aerial	KL003Y	western Crete	May 1999
		bulbs	KL003R		
Asphodeline lutea (L.) Rchb.	Liliaceae	aerial	KL065Y	central Crete	April 1999
		rhizome	KL065R		
Astragalus creticus Lam. subs. *creticus*[a]	Leguminosae	aerial	KL004Y	central Crete	May 1999
		rhizome	KL004R		
Bellis longifolia Boiss. & Heldr. in Boiss.[a]	Compositae	whole	KL068	western Crete	May 1999
Berberis cretica L.	Berberidaceae	radix	KL006R	central Crete	August 1999
		fruits	KL006F		
		aerial	KL006Y		April 1998
Campanula tubulosa Lam.[a]	Campanulaceae	whole	KL008	central Crete	June 1998
Centaurea raphanina Sm. subsp. *raphanina*[a]	Compositae	whole	KL010	central Crete	April 1998
Cistus salvifolius L.	Cistaceae	aerial	KL059	central Crete	June 1997
Cistus creticus L. subsp. *creticus*	Cistaceae	aerial	KL057	central Crete	June 1998
		resin	KL057R		
Cistus monspeliensis L.	Cistaceae	aerial	KL060	eastern Crete	June 1999
Cynoglossum columnae Ten.	Boraginaceae	aerial	KL013b	central Crete	May 1999
Cytinus hypocistis (L.) L. subsp. *hypocystis*	Rafflesiaceae	whole	KL014	central Crete	April 1998
Daphne sericea Vahl	Thymelaeacea	aerial	KL070	western Crete	April 1999
Echinops ritro L.	Compositae	aerial	KL017Y	central Greece	July 2000
		radix	KL017R		
Erodium moschatum (L.) L'Hér.	Geraniaceae	aerial	KL019	central Crete	March 1998
Eryngium amorginum Rech. fil.[a]	Apiaceae	aerial	KL100	eastern Crete	June 2000
Eryngium creticum Lam.	Apiaceae	aerial	KL020	western Crete	May 2000
Inula pseudolimonella Rech.f[a]	Compositae	aerial	KL071	eastern Crete	July 1999
Iris unguicularis Poir. subsp. *cretensis* (Janka) A.P. Davis & Jury[a]	Iridaceae	rhizome	KL024	central Crete	April 1997
Lavandula stoechas L.	Labiatae	aerial	KL099B	western Crete	May 2001
Nepeta melissifolia Lam.[a]	Labiatae	aerial	KL103	eastern Crete	May 2000
Onosma erecta Sm. subsp.*erecta*[a]	Boraginaceae	aerial	KL025	western Crete	May 1999
Petromarula pinnata (L) A.DC.[a]	Campanulaceae	aerial	KL028	central Crete	April 1998
Phlomis cretica C. Presl in J. & C. Presl[a]	Labiatae	aerial	KL029	central Crete	April 1998
Rhamnus lycioides L.subsp. *oleoides* (L.) Jahand & Maire	Rhamnaceae	aerial	KL032	central Crete	June 1998
Sarcopoterium spinosum (L.) Spach	Rosaceae	aerial	KL033	central Crete	April 1998
Staehelina petiolata (L.) Hilliard & B.L. Burtt[a]	Compositae	aerial	KL073	central Crete	May 1999
Styrax officinalis L.	Styracaceae	stems	KL037K	central Crete	April 1998
		flowers	KL037F		

[a] Endemic plants of Greece.

Plant Material and Preparation of Extracts. All plant material was collected in Crete (except *E. ritro*, which was collected in Attica in central Greece) between 1997 and 2001 and was identified by Dr. E. Kalpoutzakis. Specimens are kept in the herbarium of the Laboratory of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, University of Athens, Greece. Botanical name, taxonomic authority, plant part, voucher specimen number, and place and date of collection of each plant are listed in **Table 1**.

Different parts (as specified) of the plant material were reduced to small pieces, dried at room temperature, and powdered in a grinder. The powdered material was then extracted sequentially using solvents of increasing polarity: dichloromethane (CH_2Cl_2, DCM), methanol, and finally water. The extraction procedure was repeated three times for each solvent. The organic solvent was removed by vacuum distillation, whereas aqueous extracts were lyophilized. All residues were then stored in a dry place protected from light.

Extraction and Isolation. The air-dried radix of *E. ritro* L. (350 g) was pulverized using a laboratory mill and extracted with CH_2Cl_2, MeOH, and H_2O (3 × 1.5 L for each solvent). The CH_2Cl_2 extract was evaporated under reduced pressure, and the residue (40 g) was submitted to VLC using CH_2Cl_2/cyclohexane (10:90 to 100:0) and CH_2Cl_2/MeOH (100:0 to 98:2) gradient solutions, which finally gave fractions A1−A10. To determine the antifungal activity of each fraction, direct bioautography was made to a silica gel TLC plate (Merck). Fraction A2 was submitted to column chromatography using a CH_2Cl_2/cyclohexane gradient (0:100 to 50:50) and gave fractions B1−B16. The combined fraction B2−3 afforded compound **1** (53 mg) and combined fraction B5−6, compound **2** (60 mg). Fractions B8−B12 were further fractionated by column chromatography using a CH_2Cl_2/cyclohexane gradient (from 20:80 to 80:20) to yield compounds **3** (15 mg) and **4** (18 mg). Fraction A4 was submitted to column chromatography using CH_2Cl_2/cyclohexane gradient (from 50:50 to 100:0) and gave compounds **5** (28 mg), **6** (12 mg), and **7** (23 mg). Finally, from fraction A7 compound **8** (31 mg) was isolated by column chromatography using first a CH_2Cl_2/cyclohexane gradient (from 60:40 to 100:0) then CH_2Cl_2/MeOH (100:0 to 98:2).

Pathogen Production. Isolates of *C. acutatum* Simmonds, *C. fragariae* Brooks, and *C. gloeosporioides* (Penz.) Penz. & Sacc. in Penz. were obtained from B. J. Smith, Small Fruit Research Station, ARS-USDA, Poplarville, MS. *C. fragariae* (isolate CF63), *C. acutatum* (isolate CAGoff), and *C. gloeosporioides* (isolate CG162) were used for all pathogen and bioautography studies. Isolate CF63 is one of the most virulent isolates at infecting strawberry plants and inducing both crown and fruit rot (*12*). CF63, CAGoff, and CG162 were used as standard test isolates because of our extensive knowledge of these isolates and their known fungicide sensitivity profiles in both bioautography and microtiter formats (*13*).

The three *Colletotrichum* species were isolated from strawberry (*Fragaria* × *ananassa* Duchesne). *Botrytis cinerea* Pers.:Fr was isolated from commercial grape (*Vitis vinifera* L.) and *Fusarium oxysporum* Schlechtend:Fr from orchid (*Cynoches* sp.). *Phomopsis viticola* (Sacc.) Sacc. and *P. obscurans* (Ellis & Everh.) Sutton were obtained from Mike A. Ellis, Ohio State University, Wooster, OH. Fungi were grown on potato dextrose agar (PDA, Difco, Detroit, MI) in 9-cm Petri dishes and incubated in a growth chamber at 24 ± 2 °C and under cool-white fluorescent lights (55 ± 5 μmol·m^{-2}·s^{-1} light) with a 12-h photoperiod.

Inoculum Preparation. Conidia were harvested from 7−10-day-old cultures by flooding plates with 5 mL of sterile distilled water and

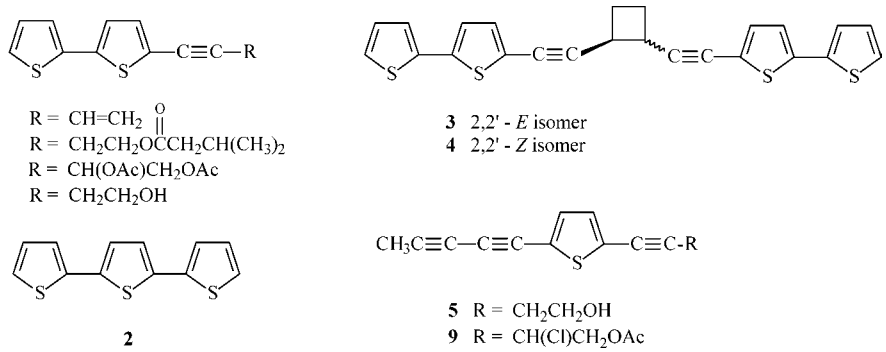

Figure 1. Structures of thiophenes **1**–**8** from *E. ritro* and compound **9** from *E. transiliensis*.

dislodging conidia by softly brushing the colonies with an L-shaped glass rod. Conidial suspensions were filtered through sterile miracloth (Calbiochem-Novabiochem Corp., La Jolla, CA) to remove mycelia. Conidia concentrations were determined photometrically (*14*) from a standard curve based on the percent of transmittance (%T) at 625 nm, and suspensions were then adjusted with sterile distilled water to a concentration of 1.0×10^6 conidia/mL.

Bioautography. Extracts and fractions containing antifungal compounds were indicated by clear zones of fungal growth inhibition directly on silica gel chromatography plates using modifications of thin-layer chromatography (TLC) bioautographic assays (*15*, *16*). TLC plates for bioautography assays were spotted with 80 μg samples of crude plant extracts or fractions and chromatographed in one dimension. Fungicide technical grade standards benomyl, cyprodinil, captan (Chem Service, Inc., West Chester, PA), and azoxystrobin (Syngentia, Greensboro, NC) were used as controls at 2 μg in 4 μL of acetone.

To detect biological activity directly on the TLC plate, silica gel plates were sprayed with one of the three spore suspensions adjusted to a final concentration of 3.0×10^5 conidia/mL with liquid potato dextrose broth (PDB, Difco) and 0.1% Tween-80. Using a 50 mL chromatographic sprayer, each TLC plate with a fluorescent indicator (250 μm, silica gel GF Uniplate, Analtech, Inc., Newark DE) was sprayed lightly (to a damp appearance) three times with the conidial suspension. Inoculated plates were then placed in a $30 \times 13 \times 7.5$ cm moisture chamber (398-C, 100% relative humidity, Pioneer Plastics, Inc., Dixon, KY) and incubated in a growth chamber at 24 ± 1 °C and a 12-h photoperiod under 60 ± 5 μmol·m^{-2}·s^{-1} light. Inhibition of fungal growth was measured 4 days after treatment. The sensitivity of each fungal species to each test compound was determined by comparing size of inhibitory zones.

Microtiter Assay. A standardized 96-well microdilution assay developed for the discovery of natural product fungicidal agents (*17*) was used to evaluate naturally occurring antifungal agents from *Echinops* sp. A microdilution assay was used to determine sensitivity of *B. cinerea*, *C. acutatum*, *C. fragariae*, *C. gloeosporioides*, *F. oxysporum*, *P. viticola*, and *P. obscurans* to the various antifungal agents in comparison with known fungicidal standards (*13*). Technical grade thiophenes (National Center for Natural Products Research, University, Mississippi) and commercial fungicides [captan, benomyl, vinclozolin (Chem Service, Inc.), and azoxystrobin (Syngentia)] were dissolved in 95% ethanol at 12 mM, sonicated for 10 min, and then diluted 1:4 with RPMI buffered with 3-[*N*-morpholino]propanesulfonic acid (MOPS) (34.5 g/L; Sigma, St. Louis, MO). The commercial fungicides represent different modes of action and were used as standards in these experiments. Each fungus was challenged in a dose−response format using test compounds at final treatment concentrations of 0.3, 3.0, and 30.0 μM. Microtiter plates (Nunc MicroWell, untreated; Roskilde, Denmark) were covered with a plastic lid and incubated in a growth chamber as described previously for fungal growth. Growth was then evaluated by measuring the absorbance of each well at 620 nm using a microplate photometer (Packard Spectra Count, Packard Instrument Co., Downers Grove, IL). The inhibitory concentration required to produce 50% growth inhibition (IC_{50}) of each fungal isolate was subsequently established for the three most active compounds using a dose−response format in which the final treatment concentrations of test compounds were 1.55, 3.1, 6.25, 12.5, 25.0, and 50.0 μM.

Microdilution Assay Experimental Design. Sixteen wells containing broth and inoculum served as positive controls; eight wells containing solvent at the appropriate concentration and broth without inoculum were used as negative controls. 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 and 144 h.

RESULTS AND DISCUSSION

Screening of Plant Extracts. With the aim of identifying bioactive extracts against plant pathogenic fungi, 30 plant species of the Greek flora—among them 14 endemic to Greece—were investigated (**Table 1**). Different plants parts were extracted to produce 123 extracts. Stock solutions of dried extracts were prepared in DCM or MeOH at a concentration of 20 mg/mL.

All extracts were tested against *C. acutatum*, *C. fragariae*, and *C. gloeosporioides*, and fungal growth inhibition was determined using direct bioautography assays. Eighteen extracts were active against only one or two fungal species, 12 extracts were active in all three species, and 92 extracts were weakly suppressive (data not shown). The most active plant extracts were from the aerial parts of *Eryngium creticum* Lam., *Eryngium amorginum* Rech. fil., and *Arum idaeum* Coustr. & Gand. and the radix of *Aristolochia cretica* Lam. and *E. ritro* L. Among the aforementioned extracts, the extract from the radix of *E. ritro* was the most potent antifungal and thus was chosen for further studies.

Isolation and Identification of Active Compounds. The dichloromethane, methanol, and water extracts of the radix of *E. ritro* were chromatographed on precoated silica gel plates with 80 μg of crude extract for bioautography assays against *C. acutatum*, *C. fragariae*, and *C. gloeosporioides*. Antifungal activity was observed in each extract, but the largest and most well-defined inhibitory zones were associated with the dichloromethane radix extract (data not shown). Hence, this extract was fractionated by VLC, and isolation of active compounds was guided by bioautography, yielding eight compounds (**Figure 1**). The following compounds were identified according to identity in NMR data: **1**, 5′-(3-buten-1-ynyl)-2,2′-bithiophene (*18*); **2**, α-terthienyl (*19*); **3** and **4**, cardopatine and isocardopatine, respectively (*20*); **5**, 2-[pent-1,3-diynyl]-5-[4-hydroxybut-1-ynyl]thiophene (*21*); **6**, 5′-(4-isovaleroyloxybut-1-ynyl)-2,2′-bithiophene; **7**, 5′-(3,4-diacetoxybut-1-ynyl)-2,2′-bithiophene; and finally **8**, 5′-(4-hydroxybut-1-ynyl)-2,2′-bithiophene (*22*).

Antifungal Activity of Compounds. The antifungal activity of compounds **1**−**8** and **9** was examined in detail using the microtiter assay in a three-point dose−response format. Compound **9**, 2-[pent-1,3-diynyl]-5[4-acetoxy-3-chlorobut-1-ynyl]-thiophene, was included in the antifungal evaluation study due to its similarity with compound **5**. Compound **9** had been

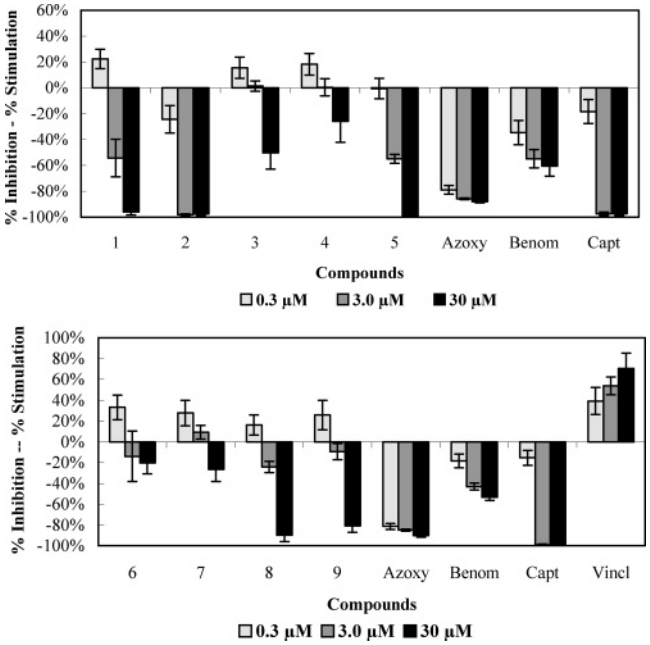

Figure 2. Growth inhibition/stimulation of *C. gloeosporioides* after 48 h using a 96-well mictotiter dose–response format to compounds **1**–**9** and commercial fungicide standards azoxystrobin, benomyl, and captan. Means from percent growth inhibition were pooled from two experiments replicated in time. Error bars represent standard error of the mean percent inhibition/stimulation. Commercial fungicide standards used for comparion were azoxystrobin (Azoxy), benomyl (Benom), captan (Capt), and vinclozolin (Vincl).

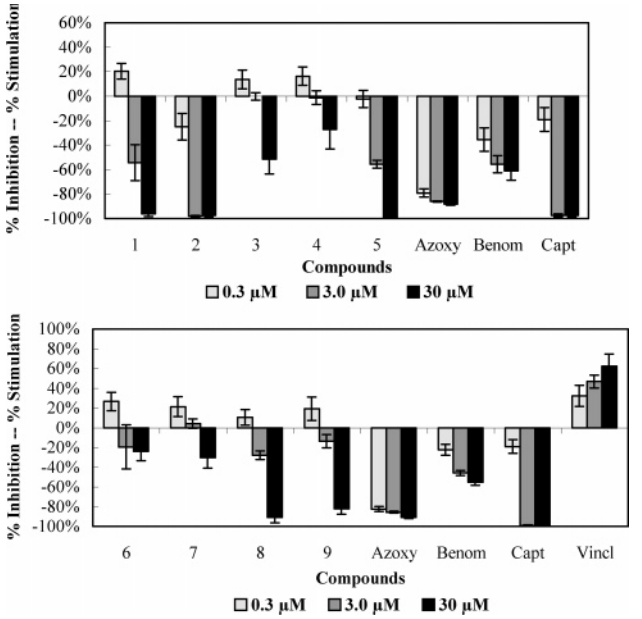

Figure 3. Growth inhibition/stimulation of *F. oxysporum* after 48 h using a 96-well mictotiter dose–response format to compounds **1**–**9** and commercial fungicide standards azoxystrobin, benomyl, and captan. Means from percent growth inhibition were pooled from two experiments replicated in time. Error bars represent standard error of the mean percent inhibition/stimulation. Commercial fungicide standards used for comparion were azoxystrobin (Azoxy), benomyl (Benom), captan (Capt), and vinclozolin (Vincl).

isolated from *Echinops transiliensis* Golosh in a manner similar to that which has been described previously (*23*). Three of the most active compounds (**1**, **2**, and **5**) were further evaluated in a six-point dose−response format to generate IC_{50} values.

Compounds **1**, **2**, **5**, **8**, and **9** possessed the highest levels of antifungal activity (**Figures 2** and **3**), whereas compounds **3**, **4**, **6**, and **7** possessed insignificant antifungal activity. Compound **2** showed potent activity with 98% growth inhibition of *C. gloeosporioides* (**Figure 2**) at 3 μM with similar levels of activity againt *C. fragariae* and *C. acutatum* (data not shown). *F. oxysporum* is one of the most chemically insensitive species in our repository. Compound **2** also inhibited the growth of *F. oxysporium* 98% at 3 μM and 25% at 0.3 μM (**Figure 3**). Thiophenes **1** and **5** had comparable activity at 30 μM; **1** produced 96% and **5** produced 100% growth inhibition against *C. gloeosporioides* and *F. oxysporum*. Thiophenes were overall less active against *B. cinerea*, *P. obscurans*, and *P. viticola* (data not shown). The chlorinated thiophene, compound **9**, possessed its greatest activity at 30 μM and inhibited the growth of *C. gloeosporioides* and *F. oxysporium* 81 and 82%, respectively.

The three most active compounds identified were subsequently evaluated in a six-point dose−response format at 48 and 72 h. The concentrations required to induce 50% fungal growth (IC_{50}) are listed in **Table 2**. Compound **2** appears to be relatively selective to *Colletotrichum* species, exhibiting a high level of activity against *C. gloeosporioides* ($IC_{50} < 1.6$ μM) and moderate activity against *C. acutatum* ($IC_{50} = 3.0$) and *C. fragariae* ($IC_{50} = 4.9$). The activity of compound **2** at 1.6 μM against *C. gloeosporioides* at 48 and 72 h is comparable to that of the positive control, captan. IC_{50} results confirm the relative chemical insensitivities of *B. cinerea*, *P. obscurans*, *P. viticola*, and *F. oxysporum* that were demonstrated in the three-point assay. Compound **5** appeared to demonstrate selective antifungal activity toward both *Phomopsis* species with moderate activity

Table 2. IC_{50} Values for Compounds Tested against *C. gloeosporioides*, *C. acutatum*, *C. fragariae*, *P. viticola*, *P. obscurans*, *F. oxysporum*, *and B. cinerea* at 48 and 72 h

	compound (μM)							
	1		**2**		**5**		captan	
fungal isolate	48 h	72 h	48 h	72 h	48 h	72 h	48 h	72 h
C. gloeosporioides	*4.2*	*10.2*	*<1.6*	*<1.6*	2.6	6.0	<1.6	<1.6
C. acutatum	*7.6*	*28.5*	*3.0*	*5.3*	3.5	19.2	<1.6	2.1
C. fragariae	28.0	46.0	4.9	9.0	4.4	12.5	<1.6	2.1
P. viticola	19.5	22.0	<6.3	<6.3	<1.6	4.0	18.0	20.0
P. obscurans	>50.0	>50.0	7.2	9.6	2.9	13.5	2.4	3.9
F. oxysporum	>50.0	>50.0	5.0	>50.0	9.5	20.0	2.2	5.8
B. cinerea	>50.0	>50.0	>50.0	>50.0	29.4	41.0	<1.6	<1.6

against *P. obscurans* ($IC_{50} = 2.9$ μM) and *P. viticola* ($IC_{50} < 1.6$ μM). The activity of compound **5** toward *F. oxysporum* is of interest because *Fusarium* species are seldom sensitive to chemicals below 30 μM in our assays and the level of activity ($IC_{50} = 9.5$ μM) observed is unusual. Compound **1** was the least active antifungal compound, with its greatest inhibition against *C. gloeosporioides* ($IC_{50} = 4.2$ μM) and *C. acutatum* ($IC_{50} = 7.6$ μM). The selective activities of compound **2** toward *Colletotrichum* species and compound **5** toward *Phomopsis* species are worthy of more study as potential plant protectants.

Our discussion of structure−activity relationships will be focused primarily on differences among closely related compounds. Beginning with the bithiophene derivatives (**1**, **6**, **7**, and **8**), compound **1** at a concentration of 3.0 μM is 2.2 times more active than compound **8** in *C. gloeosporioides* and compound **6** is 1.5 times more active than compound **7** in the same strain. The same trend is observed in *F. oxysporum*. It is also clear from **Figures 2** and **3** that compounds **1** and **8** are much more active than their ester derivatives, compounds **6** and **7**. It appears that the terminal olefin in compound **1** is necessary

to maintain the highest level of activity, because compounds **6**−**8** are all further substituted. In addition, the higher level of activity observed for compounds **1** and **8** as compared to compounds **6** and **7**, as well as that observed between compounds **6** and **7**, suggests that esterification results in less activity. This same phenomenon is observed between compounds **5** and **9**. Compound **5** is 5.7 and 4.1 times more active than compound **9** on *C. gloeosporioides* and in *F. oxysporum*, respectively. Indeed, the esterification of the OH group and the addition of chlorine seems to be responsible for reducing the activity. Finally, between the two isomers **3** and **4**, the *E* isomer is apparently more active.

Compounds **1**, **2**, and **5** had significant activity against *Colletotrichum* species and *Fusarium*; however, the potential drawback of these compounds may be the fact that their activity appears to peak between 60 and 80% growth inhibition, and none of the three compounds in the IC_{50} assay caused 100% growth inhibition at 50 μM. In particular, α-terthienyl (**2**) can be used as a lead compound for the future development of semisynthetic derivatives with improved activity against commercial pathogenic fungi.

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