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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

# Synthesis and structure–activity relationships of aliphatic isothiocyanate analogs as antibiotic agents

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**Abstract** Isothiocyanates (ITCs) are one of the many classes of breakdown products of glucosinolates found in plants and exhibit biologic activity against various pathogens. In this work, aliphatic isothiocyanates were prepared and the antimicrobial activities against plant pathogenic fungi and bacteria were tested to understand the structure–activity relationships. The results indicated that longer-chain derivatives exert a steric inhibition on toxicity of ITCs against *Rhizoctonia solani* because of steric hindrance and the order of the eight aliphatic ITCs was ethyl > *n*-propyl > methyl > *n*-hexyl > *n*-octyl > *n*-butyl > *n*-heptyl > *n*-pentyl. Because the hydrophobicity of ITCs was enhanced by increasing alkyl chain length, the antibacterial activity of ITCs against *Erwinia carotovora* was moderately intense with an increase in hydrophobicity and the order was *n*-octyl > *n*-pentyl > *n*-heptyl > *n*-hexyl > *n*-propyl > *n*-butyl > methyl > ethyl. The present study revealed that some of the compounds exhibited promising antimicrobial activity and could be used as an acceptable alternative to the traditional synthetic fungicides for controlling *R. solani* and *E. carotovora*.

**Keywords** Aliphatic isothiocyanate · Synthesis · Biologic activity · In vitro studies · Structure–activity relationships

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## Introduction

In recent years, as a result of the usage of antimicrobial agents at high concentrations, the risk of developing pathogenic microorganism resistance to antimicrobial agents has increased in many countries (Mueller *et al.*, 2002; Ma *et al.*, 2009). Studies have focused on the development of new antimicrobial agents from various natural sources including medicinal plant extracts, herb oils, and spices (Mohn *et al.*, 2007; Wu *et al.*, 2009). Isothiocyanates (ITCs) are one of the many classes of breakdown products of glucosinolates found in plants such as broccoli, brussels sprouts, cabbage, canola, cauliflower, kale, radish, turnip, watercress, and various mustards (Cinciripini *et al.*, 1997; Drewnowski and Gomez-Carneros, 2000; Fahey *et al.*, 2001; Chuanphongpanich *et al.*, 2006). ITCs exhibit biologic activity against various pathogens including bacteria (Jang *et al.*, 2010), viruses (Mochida and Ogawa, 2008), fungi (Smolinska and Horbowicz, 1999), insects, and other pests (Bennett and Wallgrove, 1994; Gamage *et al.*, 2009). Kurt *et al.* (2011) determined the toxicity of individual pure aliphatic and aromatic ITCs to different fungal growth parameters, such as mycelial growth, sclerotial viability, and carpogenic germination of *Sclerotinia sclerotiorum*. The result showed that methyl, allyl, and benzyl ITC were the most fungitoxic of the tested compounds in bioassays (Kurt *et al.*, 2011). Troncoso-Rojas *et al.* (2005) tested the efficacy of benzyl isothiocyanate (BITC) against *Alternaria alternata* growth. The results showed that the minimal inhibitory concentration of BITC in vitro was 0.1 mg/mL and suggested that BITC can be used as a post-harvest treatment to control *Alternaria* rot in tomato fruit without detrimental effects on the tomato post-harvest quality (Troncoso-Rojas *et al.*, 2005). Kim and Lee (2009) tested the growth-inhibiting activities of phenethyl isothiocyanate

(PITC) and its derivatives against intestinal bacteria. The study displayed that PITC strongly inhibited the growth of *Clostridium difficile* and *Clostridium perfringens* at 1 mg/disk and moderately inhibited the growth of *Escherichia coli* at a dose of 2 mg/disk (Kim and Lee, 2009). Tajima *et al.* (2007) examined the efficacy of hydroxyl isothiocyanates against a bacterial virus. The test demonstrated that ITCs with a hydroxyl-substituted phenyl structure exhibited remarkable antiviral activities (Tajima *et al.*, 2007). Liblikas *et al.* (2003) compared the activity of nine ITC compounds to the beetle genera *Phyllotreta* species. The results illustrated that butenyl isothiocyanate and butenyl thiocyanate were the most effective at controlling *Phyllotreta* spp (Liblikas *et al.*, 2003).

Studies have shown that the structural identities of ITCs appear to play an important role in the degree of eliciting antimicrobial properties. Minor changes in ITC structure have been shown to impact in vitro bioactivities significantly (Saksena, 1985; Schultz *et al.*, 2007). However, limited study regarding to the growth-inhibiting activities of aliphatic ITCs analogous against plant pathogenic microorganisms has been reported. In this study, to understand the structure–activity relationships and determine the optimal chain length for maximal antimicrobial activity of aliphatic ITCs, we prepared eight aliphatic ITCs and evaluated the antimicrobial activity against *Rhizoctonia solani* and *Erwinia carotovora*.

## Results and discussion

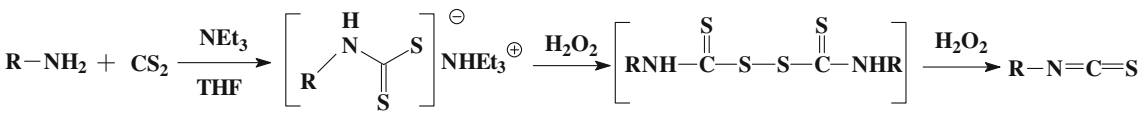
### Synthesis and characterization of ITCs

A series of ITCs were synthesized as per the route outlined in Fig. 1. The method for the preparation of ITCs involved reaction of amines with carbon disulfide in the presence of triethylamine and then treated the salts with hydrogen peroxide. Excess carbon disulfide was generally required. The factors of reaction (temperature, time, and solvent) were investigated and optimized to establish the optimum reaction conditions. The parameters were evaluated by computing the yields of the compounds. The values ranging from 20 to 80 °C were performed to find the best reaction temperature during the dropwise addition of hydrogen peroxide. The result showed that the yields of the target compounds were lower when the reaction temperatures exceeded 50 °C. In order to eliminate the possibility of ITCs directly reacting with amines, the reaction temperature should not exceed 50 °C. In this work, the effect of mixing time followed by dropwise addition of hydrogen peroxide on the yields of the compounds was also studied over a period of 10–60 min. It was found that the yields of the compounds increased quickly as time went on and reached a plateau at 30 min. Therefore, a reaction time of 30 min was chosen. The various solvents (MeOH, EtOH, DMF, and THF) were tested. The best results were obtained when the reaction took place in THF. The study found that the yields of the compounds were falling with the increase in chain length under the same conditions. It may be an increase in chain length led to the increase in steric hindrance and to reduction of the compounds' reactivity. In the present study, the yields of the compounds were the mean values of at least three experiments. The compounds were characterized by elemental analyses, mass spectra, IR, and NMR (Table 1). Mass spectra of all of the compounds showed the molecular ion peaks. Elemental analyses were found within ±0.4 % of the theoretic values.

### ITCs efficacy against mycelial growth of *R. solani*

The efficacies of five concentrations of each ITC against mycelial growth of *R. solani* are shown in Table 2. The results showed that ethyl ITC was the most active against *R. solani*. The percentage inhibitions of mycelial growth were 58.82 and 91.83 % at a concentration of 5.0 and 100.0 μg/mL, respectively. At the highest concentrations, all ITCs exhibited significant fungistatic effects and the percentage inhibition of mycelial growth ranged from 84.25 to 97.90 %.

In the present study, an examination of toxic potency in relationship to the number of carbon atoms for the straight-chain derivatives was done to compare analog $EC_{50}$ values. $EC_{50}$ values determined for the reduction in mycelial growth of *R. solani* are given in Table 3. For the mycelial growth, ethyl ITC illustrated the lowest $EC_{50}$ value (3.52 μg/mL), which means the most activity to the *R. solani*. The $EC_{50}$ values of other ITCs against *R. solani* were higher than 10.0 μg/mL. Compared with the toxic potency of the longer-chain derivatives (C4, C5, C6, C7, C8) against *R. solani*, the shorter-chain derivatives (C1, C2, C3) exhibited potent fungistatic effects. The order of eight aliphatic ITCs




**R: $CH_3$, $CH_3CH_2$, $CH_3(CH_2)_2$, $CH_3(CH_2)_3$, $CH_3(CH_2)_4$, $CH_3(CH_2)_5$, $CH_3(CH_2)_6$, $CH_3(CH_2)_7$**

**Fig. 1** Synthetic route of the compounds

**Table 1** Characterization data of the synthesized compounds

| Comp. | R | Molecular formula | Molecular weight | Yield (%) | Elemental analysis (calcd./found) | | | EI-MS $[M]^+$ | $^1$H NMR (300 MHz, $CDCl_3$) | IR (KBr) ν/cm$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | C | H | N | | | |
| 1 | Methyl | $C_2H_3NS$ | 73.12 | 92 | 32.85/32.81 | 4.14/4.13 | 19.16/19.18 | 72.0 | δ: 3.32 (s, 3 H) | 2,983, 2,932 ($CH_2$) 2,108 (NCS) 1,407 (CS) |
| 2 | Ethyl | $C_3H_5NS$ | 87.15 | 91 | 41.35/41.29 | 5.78/5.76 | 16.07/16.09 | 87.0 | δ: 3.58 (q, 2 H), 1.42 (t, 3 H) | 2,983, 2,936 ($CH_2$) 2,103 (NCS) 1,388 (CS) |
| 3 | *n*-Propyl | $C_4H_7NS$ | 101.75 | 89 | 47.49/47.31 | 6.97/6.94 | 13.84/13.80 | 101.0 | δ: 3.45 (t, 2 H), 1.71 (sext, 2 H), 1.01 (t, 3 H,) | 2,981, 2,887 ($CH_2$) 2,096 (NCS) 1,448 (CS) |
| 4 | *n*-Butyl | $C_5H_9NS$ | 115.20 | 85 | 52.13/52.25 | 7.87/7.83 | 12.16/12.12 | 115.0 | δ: 3.54 (t, 2H), 1.64–1.74 (m, 2 H), 1.39–1.52 (m, 2 H), 0.96 (t, 3 H) | 2,972, 2,867 ($CH_2$) 2,105 (NCS) 1,456 (CS) |
| 5 | *n*-Pentyl | $C_6H_{11}NS$ | 129.22 | 82 | 55.77/55.87 | 8.58/8.54 | 10.84/10.81 | 129.0 | δ: 3.56 (d, 2 H), 1.51 (q, 2 H),1.35 (m, 4H), 0.91 (t, 3 H) | 2,945, 2,874 ($CH_2$) 2,092 (NCS) 1,465 (CS) |
| 6 | *n*-Hexyl | $C_7H_{13}NS$ | 143.25 | 78 | 58.69/58.53 | 9.15/9.16 | 9.78/9.74 | 142.0 | δ: 3.45 (t, 2 H), 1.46 (m, 2 H), 1.39 (m, 4 H), 1.31 (q, 2 H), 0.88 (t, 3 H) | 2,947, 2,864 ($CH_2$) 2,107 (NCS) 1,461 (CS) |
| 7 | *n*-Heptyl | $C_8H_{15}NS$ | 157.28 | 81 | 61.09/61.08 | 9.61/9.59 | 8.91/8.92 | 157.0 | δ: 3.67 (t, 2 H), 1.31 (m, 4 H), 1.29 (m, 6H), 0.87 (t, 3 H) | 2,963, 2,887 ($CH_2$) 2,087 (NCS) 1,445 (CS) |
| 8 | *n*-Octyl | $C_9H_{17}NS$ | 171.30 | 73 | 63.10/63.04 | 10.00/10.03 | 8.18/8.22 | 170.0 | δ: 3.52 (t, 2 H), 1.48 (q, 4 H), 1.31 (m, 8 H), 0.85 (t, 3 H) | 2,933, 2,867 ($CH_2$) 2,105 (NCS) 1,458 (CS) |

**Table 2** Percentage inhibition of mycelial growth of *Rhizoctonia solani* by ITCs

| Methyl | | Ethyl | | *n*-Propyl | | *n*-Butyl | | *n*-Pentyl | | *n*-Hexyl | | *n*-Heptyl | | *n*-Octyl | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc. (μg/mL) | Inhibition (%) | Conc. (μg/mL) | Inhibition (%) | Conc. (μg/mL) | Inhibition (%) | Conc. (μg/mL) | Inhibition (%) | Conc. (μg/mL) | Inhibition (%) | Conc. (μg/mL) | Inhibition (%) | Conc. (μg/mL) | Inhibition (%) | Conc. (μg/mL) | Inhibition (%) |
| 10.0 | 41.50a[a] | 5.0 | 58.82a | 5.0 | 21.36a | 5.0 | 3.72a | 10.0 | 16.01a | 5.0 | 11.73a | 10.0 | 21.56a | 10.0 | 21.79a |
| 15.0 | 49.67a | 10.0 | 61.11ab | 10.0 | 50.13b | 10.0 | 15.49a | 50.0 | 34.91b | 10.0 | 29.66b | 50.0 | 46.61b | 50.0 | 66.14b |
| 25.0 | 66.34b | 50.0 | 67.32b | 50.0 | 67.98c | 50.0 | 57.74b | 100.0 | 44.62b | 50.0 | 70.61c | 100.0 | 61.99c | 100.0 | 74.80bc |
| 50.0 | 77.45c | 70.0 | 73.86b | 100.0 | 84.25d | 100.0 | 68.24c | 300.0 | 69.03c | 100.0 | 82.42c | 300.0 | 75.11d | 300.0 | 82.15c |
| 100.0 | 84.64c | 100.0 | 91.83c | 300.0 | 93.71d | 300.0 | 91.60d | 500.0 | 87.40d | 300.0 | 97.90d | 500.0 | 89.14e | 500.0 | 84.25c |

[a] Mean values followed by *same letters* within the same column were not significantly different according to the LSD test ($P < 0.05$)

**Table 3** The $EC_{50}$ values of ITCs against *Rhizoctonia solani* and *Erwinia carotovora* and hydrophobicity of ITCs

| ITCs | log P[a] | $EC_{50}$ (μg/mL) | |
|---|---|---|---|
| | | *Rhizoctonia solani* | *Erwinia carotovora* |
| Methyl | 1.13 | 12.33b[b] | 38.24d |
| Ethyl | 1.64 | 3.52a | 41.65d |
| *n*-Propyl | 2.15 | 11.97b | 20.65c |
| *n*-Butyl | 2.66 | 42.83e | 26.15c |
| *n*-Pentyl | 3.17 | 89.95g | 2.03a |
| *n*-Hexyl | 3.68 | 22.76c | 8.73b |
| *n*-Heptyl | 4.19 | 54.47f | 7.51b |
| *n*-Octyl | 4.70 | 34.70d | 1.17a |

$EC_{50}$ (μg/mL) for ITCs causing 50 % inhibition of mycelial growth or the number of bacteria

[a] Hydrophobicity

[b] Mean values followed by *different letters* within the same column were significantly different according to the LSD test ($P < 0.05$)

for fungicidal activity was ethyl > *n*-propyl > methyl > *n*-hexyl > *n*-octyl > *n*-butyl > *n*-heptyl > *n*-pentyl.

In general, toxicity for a group of chemicals is related to a steric term, a hydrophobicity term and an electronic term (Etzenhouser *et al.*, 2001). We hypothesize that all the ITCs are Michael-type acceptors, which can set off Michael addition reaction with the cellular thiols as the molecular mechanism of action. For that matter, the longer-chain derivatives should be less reactive and toxic than shorter-chain derivatives because of steric hindrance. By comparing molecular structure, it is obvious that a longer-chain exerts a steric inhibition on thiol reactivity with aliphatic ITCs. This suggests that the differences of toxicity of the candidate aliphatic ITCs against *R. solani* are mainly due to the differences of steric hindrance at the reaction center, possibly leading to alteration in entropy of activation. The interpretation of steric hindrance focuses on the ability of the alkyl groups to adopt conformations, which may be adverse to the attacking thiol nucleophile. To a great extent, those obstructive conformations are likely. The alkyl can obstruct the electrophile from reaching the reactive centers with the alkyl group increasing and the reduction of chain length will be beneficial to nucleophilic attack. In the case of shorter-chain ITCs, there is less steric hindrance to nucleophilic attack. The results suggest that longer-chain derivatives exert a steric inhibition on toxicity of ITCs against *R. solani* because of steric hindrance.

## Antibacterial activity of ITCs against *Envinia carotovora*

The efficacies of five concentrations of each ITC on the growth–inhibition activity of *E. carotovora* are shown in Table 4. Among eight screened compounds, *n*-octyl ITC

**Table 4** Antibacterial activity of ITCs against *Erwinia carotovora* by the turbidimetric method

| Methyl | | Ethyl | | *n*-Propyl | | *n*-Butyl | | *n*-Pentyl | | *n*-Hexyl | | *n*-Heptyl | | *n*-Octyl | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc. (μg/mL) | Inhibition (%) | Conc. (μg/mL) | Inhibition (%) | Conc. (μg/mL) | Inhibition (%) | Conc. (μg/mL) | Inhibition (%) | Conc. (μg/mL) | Inhibition (%) | Conc. (μg/mL) | Inhibition (%) | Conc. (μg/mL) | Inhibition (%) | Conc. (μg/mL) | Inhibition (%) |
| 10.0 | 10.88a[a] | 10.0 | 6.74a | 10.0 | 29.04a | 0.05 | 6.38a | 0.02 | 2.04a | 1.0 | 1.52a | 5.0 | 28.79a | 0.01 | 6.40a |
| 20.0 | 29.93b | 20.0 | 11.65a | 20.0 | 47.04b | 0.2 | 10.74a | 0.2 | 22.30b | 2.0 | 3.70a | 10.0 | 68.05b | 0.5 | 24.68b |
| 50.0 | 61.11c | 30.0 | 48.51b | 50.0 | 76.26c | 5.0 | 31.66b | 0.5 | 26.23b | 5.0 | 13.93b | 20.0 | 84.12c | 1.0 | 47.84c |
| 80.0 | 71.50d | 50.0 | 64.63c | 80.0 | 85.97d | 10.0 | 43.87b | 2.0 | 36.05b | 10.0 | 39.75c | 30.0 | 94.37d | 5.0 | 70.58d |
| 100.0 | 81.33e | 70.0 | 94.76d | 100.0 | 90.65d | 20.0 | 84.36c | 5.0 | 72.34c | 20.0 | 94.64d | 50.0 | 97.04d | 10.0 | 83.06e |

[a] Mean values followed by *same letters* within the same column were not significantly different according to the LSD test ($P < 0.05$)

showed the highest activity, and the percentage inhibitions against *E. carotovora* were 83.06 % at a concentration of 10.0 μg/mL. At their highest concentration, all the tested compounds exhibited significant antibacterial activity and the percentage inhibition ranged from 72.34 to 97.04 %.

$EC_{50}$ values determined for the reduction in the concentration of *E. carotovora* are given in Table 3. For the *E. carotovora*, *n*-octyl ITC had the lowest $EC_{50}$ value with a concentration of 1.17 μg/mL. However, the $EC_{50}$ values of *n*-pentyl, *n*-hexyl, *n*-heptyl, and *n*-octyl ITC against *E. carotovora* were less than 10.0 μg/mL. The order of eight aliphatic ITCs for antibacterial activity was *n*-octyl > *n*-pentyl > *n*-heptyl > *n*-hexyl > *n*-propyl > *n*-butyl > methyl > ethyl. Compared with the toxic potency of the different length of the straight-chain derivatives against *E. carotovora*, the longer-chain derivatives (C5, C6, C7, C8) exhibited a significant antibacterial effect. The antimicrobial activity of the compounds depended critically on their hydrophobic content, which is typically quantified by the 1-octanol/water partition coefficient (Kuroda *et al.*, 2009). The hydrophobicity (log P) of ITCs was measured by an octanol-aqueous shake-flask method (Screnci *et al.*, 2000). Log P values of eight screened ITCs ranged from 1.13 to 4.70, and are shown in Table 3. The results showed that the hydrophobicity of ITCs was enhanced by increasing alkyl chain length. In the present study, the activity of ITCs against *E. carotovora* was moderately intense with an increase in hydrophobicity. These findings indicated that hydrophobicity favored the antibacterium activity of ITCs against *E. carotovora*, and suggested that the transport of ITCs to the target sites of *E. carotovora* might involve hydrophobicity transport mechanisms.

The present study indicated that the activity of ITCs against *R. solani* in general reduced with increasing chain length. However, the activity of ITCs against *E. carotovora* was general intensive when the chain length was increased. These differences were not only related to steric hindrance and hydrophobicity of ITCs but also related to the differences in the biochemistry and physiology of the fungi and bacteria. For this reason, the results suggested that more studies should be taken to investigate the possible roles of differences in the biochemistry and physiology of fungi and bacteria in their differential sensitivity to ITCs.

## Experimental methods

### Chemicals and microorganisms

Methylamine, ethylamine, *n*-propylamine, *n*-butylamine, *n*-pentylamine, *n*-hexylamine, *n*-heptylamine, *n*-octylamine, carbon disulfide, hydrogen peroxide, triethylamine, agar powder, proteose peptone, and yeast extract were purchased from Sigma. HPLC grade methanol was supplied by J.T. Baker. Ultrapure water from a Milli-Q system (Millipore, Billerica, MA, USA). All other chemicals used in this study were of reagent grade. The fungus *R. solani* and bacteria *E. carotovora* were supplied by the Laboratory of Seed Pathology and Fungicide Pharmacology at China Agricultural University.

### Instruments

All reactions were monitored using a HPLC system, which comprised two LC-10ATvp pumps, a SPD-10Avp ultraviolet detector (Shimadzu, Japan) and a reversed-phase kromasil ODS $C_{18}$ column (AkzoNobel, Amsterdam, ZZ, Netherlands, 250 × 4.6 mm, 5 μm, 100 Å). The mass spectra were performed on a JEOL JMS-AX500 mass spectrometer and elemental analyses were conducted using a LECO-183 CHNS analyzer. Infrared (IR) spectra were recorded on a Jasco FT-IR 5300 spectrophotometer. NMR spectra were acquired on Varian Unity Inova 300 MHz spectrometers with TMS as an internal standard. The numbers of the bacteria *E. carotovora* were determined using an Agilent 8453 UV–Visible spectrophotometer.

### Synthesis of ITCs

General procedure for the preparation of ITCs was followed the literature method (Li *et al.*, 1997). Carbon disulfide (0.02 mol) was added dropwise to a stirred mixture of aliphatic amine (0.02 mol), triethylamine (0.02 mol), and THF solution (100 mL) at 2–5 °C over a period of 30 min. Agitation was continued for 30 min, followed by the dropwise addition of hydrogen peroxide (30 %, 0.03 mol) with a controlled reaction temperature of 0–40 °C. Then, hydrochloric acid was added to the mixture to neutralize the reaction solution. The reaction mixture was evaporated under reduced pressure and extracted with ethyl acetate. Then, the ester was removed by evaporation under reduced pressure. The remaining residue was a yellowish oily liquid. Compounds were purified by silica gel column chromatography.

### Characterization of ITCs

For HPLC characterization, methanol solutions of the compounds were injected into a HPLC using a SPD-10Avp ultraviolet detector at 225 nm. Compounds were further characterized by mass spectra, elemental analyses, NMR, and IR spectra. NMR spectra were acquired with TMS as internal reference; the chemical shifts were reported in ppm. IR spectra were obtained as KBr pellets. Mass spectra (70 eV electron impact [EI]) were obtained using mass spectrometer.

Evaluation of ITC analogs against *R. solani*

In this experiment, the efficacy of ITCs against *R. solani* was determined by growth rate method. On the basis of the preparation experiment, appropriate concentrations (μg/mL) of ITCs were determined, and the blank flat and the flat with the only solvent were used as the control. After rejuvenation re-identification, fungus cake of *R. solani* (diameter 5 mm) was vaccinated in the toxic flat, and cultured in a constant temperature incubator at 24–28 °C. The percentage of relative inhibition of ITCs against fungus was determined by the comparing colonial diameter after the culture.

Evaluation of ITC analogs against *Envinia carotovora*

The synthesized compounds were tested for antimicrobial activity against *E. carotovora* by the turbidimetric method. An aliquot (0.1 mL) of the bacterial suspension ($10^9$ CFU/mL) was incorporated into a conical flask (35 mL) containing 10 mL of lysogeny broth (LB), which contained with ITCs at different concentrations (μg/mL) using DMSO as solvent. DMSO concentrations in both control and assay were below 0.1 % (v/v). The mixtures were inoculated at 30 °C for 12 h. The turbidity of the bacteria solution was examined with the turbidimetric method using an Agilent 8453 UV–Visible spectrophotometer. A sample (1 mL) was taken from each conical flask, and the optical density (OD) value of the growth of the bacteria was measured through spectrophotometric colorimetry (the wave length was 600 nm). The relation of the OD value to number of viable bacteria was predetermined, and the actual concentration of the microorganism with the different concentrations of ITCs was determined. All growth inhibition assays were repeated three times. Finally, the percent inhibition of bacterial growth was calculated in comparison with controls that did not have ITCs.

Data analysis

The half maximal effective concentration ($EC_{50}$) values were calculated by the linear regression of the probit % of inhibition of mycelial growth or the number of bacteria as a function of the log of inhibitor concentrations. All data were subjected to one-way ANOVA by SPSS 17.0 statistical analysis software (SPSS, Chicago, IL, USA). The confidence limits used in this study were based on 95 % ($P < 0.05$).

## Conclusions

A series of aliphatic ITCs were prepared by reacting aliphatic amines with carbon disulfide resulting in precipitation of dithiocarbamate derivatives, which were then treated with hydrogen peroxide to yield corresponding ITC derivatives. Structure–activity relationships for the newly synthesized compounds against plant pathogenic fungi and bacteria were evaluated and discussed. The results indicated that the activity of ITCs against *R. solani*, in general, reduced when the chain length was increased, while the activity of ITCs against *E. carotovora* was generally intensive with increasing chain length. The present study revealed that some of the compounds exhibited promising antimicrobial activity and could be used as an acceptable alternative to the traditional synthetic fungicides for controlling *R. solani* and *E. carotovora*.

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## References

Bennett RN, Wallgrove RM (1994) Secondary metabolites in plant defence mechanisms. New Phytol 127:617–633

Chuanphongpanich S, Phanichphant S, Bhuddasukh D, Suttajit M, Sirithunyalug B (2006) Bioactive glucosinolates and antioxidant properties of broccoli seeds cultivated in Thailand. Songklanakarin J Sci Technol 28:55–61

Cinciripini PM, Hecht SS, Henningfield JE, Manley MW, Kramer BS (1997) Tobacco addiction: implications for treatment and cancer prevention. J Natl Cancer Inst 89:1852–1867

Drewnowski A, Gomez-Carneros C (2000) Bitter taste, phytonutrients, and the consumer: a review. Am J Clin Nutr 72:1424–1435

Etzenhouser B, Hansch C, Kapur S, Selassie CD (2001) Mechanism of toxicity of esters of caffeic and dihydrocaffeic acids. Bioorg Med Chem 9:199–209

Fahey JW, Zalcmann AT, Talalay P (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56:5–51

Gamage GR, Park HJ, Kim KM (2009) Effectiveness of antimicrobial coated oriented polypropylene/polyethylene films in sprout packaging. Food Res Int 42:832–839

Jang M, Hong E, Kim GH (2010) Evaluation of antibacterial activity of 3-butenyl, 4-pentenyl, 2-phenylethyl, and benzyl isothiocyanate in *brassica* vegetables. J Food Sci 75:M412–M416

Kim MG, Lee HS (2009) Growth-inhibiting activities of phenethyl isothiocyanate and its derivatives against intestinal bacteria. J Food Sci 74:M467–M471

Kuroda K, Caputo GA, DeGrado WF (2009) The role of hydrophobicity in the antimicrobial and hemolytic activities of polymethacrylate derivatives. Chem Eur J 15:1123–1133

Kurt Ş, GÜneŞ U, Soylu EM (2011) In vitro and in vivo antifungal activity of synthetic pure isothiocyanates against *Sclerotinia sclerotiorum*. Pest Manage Sci 67:869–875

Li G, Tajima H, Ohtani T (1997) An improved procedure for the preparation of isothiocyanates from primary amines by using hydrogen peroxide as the dehydrosulfurization reagent. J Org Chem 62:4539–4540

Liblikas I, MÖttus E, Borg-Karlson AK, Kuusik S, Ojarand A, Kännaste A, Tanilsoo J (2003) Flea beetle (Coleoptera: Chrysomelidae) response to alkyl thiocyanates and alkyl isothiocyanates. Agron Res 1:175–184

Ma HX, Chen Y, Wang JX, Yu WY, Tang ZH, Chen CJ, Zhou MG (2009) Activity of carbendazim, dimethachlon, iprodione, procymidone and boscalid against Sclerotinia stem rot in Jiangsu Province of China. Phytoparasitica 37:421–429

Mochida K, Ogawa T (2008) Anti-influenza virus activity of extract of Japanese wasabi leaves discarded in summer. J Sci Food Agric 88:1704–1708

Mohn T, Cutting B, Ernst B, Hamburger M (2007) Extraction and analysis of intact glucosinolates-A validated pressurized liquid extraction/liquid chromatography-mass spectrometry protocol for *Isatis tinctoria*, and qualitative analysis of other cruciferous plants. J Chromatogr A 1166:142–151

Mueller DS, Dorrance AE, Derksen RC, Ozkan E, Kurle JE, Grau CR, Gaska JM, Hartman GL, Bradley CA, Pedersen WL (2002) Efficacy of fungicides on *Sclerotinia sclerotiorum* and their potential for control of sclerotinia stem rot on soybean. Plant Dis 86:26–31

Saksena NK (1985) Fungicidal action and structure correlation of monosubstituted phenyl isothiocyanates. Folia Microbiol 30: 359–362

Schultz TW, Yarbrough JW, Pilkington TB (2007) Aquatic toxicity and abiotic thiol reactivity of aliphatic isothiocyanates: effects of alkyl-size and -shape. Environ Toxicol Pharmacol 23:10–17

Screnci D, McKeage MJ, Galettis P, Hambley TW, Palmer BD, Baguley BC (2000) Relationships between hydrophobicity, reactivity, accumulation and peripheral nerve toxicity of a series of platinum drugs. Br J Cancer 82:966–972

Smolinska U, Horbowicz M (1999) Fungicidal activity of volatiles from selected cruciferous plants against resting propagules of soil-borne fungal pathogens. J Phytopathology 147:119–124

Tajima H, Nakamoto Y, Taketo A (2007) Effect of synthetic hydroxy isothiocyanates on a bacterial virus and DNA. Biosci Biotechnol Biochem 71:1094–1097

Troncoso-Rojas R, Sánchez-Estrada A, Ruelas C, García HS, Tiznado-Hernández ME (2005) Effect of benzyl isothiocyanate on tomato fruit infection development by *Alternaria alternata*. J Sci Food Agric 85:1427–1434

Wu H, Zhang GA, Zeng SY, Lin KC (2009) Extraction of allyl isothiocyanate from horseradish (*Armoracia rusticana*) and its fumigant insecticidal activity on four stored-product pests of paddy. Pest Manage Sci 65:1003–1008