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p-Aromatic Isothiocyanates: Synthesis and Anti Plant Pathogen Activity[1]

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Abstract—In this study, a series of *p*-aromatic isothiocyanates are prepared by reacting *p*-aromatic amines with carbon disulphide and further treating with molecular iodine to yield corresponding isothiocyanate derivatives. The structures of newly synthesized compounds are confirmed by IR, NMR, and MS data. Activity of the products against plant pathogenic fungi and bacteria is tested and the structure-activity relationship is approached. *p*-Nitrophenyl isothiocyanate most efficiently inhibits *Rhizoctonia solani* and *Erwinia carotovora*. The order of seven aromatic isothiocyanates antifungicidal activity is following: *p*-nitrophenyl > *p*-methoxyphenyl > *p*-chlorophenyl > *p*-methylphenyl > *p*-ethylphenyl > phenyl > *p*-fluorophenyl. For antibacterial activity, the order was *p*-nitrophenyl > *p*-chlorophenyl > *p*-methylphenyl > *p*-ethylphenyl > *p*-fluorophenyl > phenyl > *p*-methoxyphenyl. The present study indicates that some of the compounds exhibit promising antimicrobial activity and can be used as an alternative to the traditional synthetic fungicides for controlling *R. solani* and *E. carotovora*.

Keywords: aromatic isothiocyanates, antimicrobial activity, structure-activity relationship

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INTRODUCTION

Over the recent years, the new strategies for plant disease management have led to development of botanical fungicides [1–4]. Glucosinolates (GLs), the secondary metabolites of Brassicales plants, and their enzymatic hydrolysis attracted close attention [5, 6]. Isothiocyanates (ITCs) exhibit biological activity against various bacteria [7], fungi [8], nematodes [9], viruses [10], insects, and weeds [11, 12]. Minor changes in ITCs structures can result in significant impact on their bioactivity [13–16]. Hansch and co-authors [17] studied structure-activity relationships for ITC and indicated that substituents could influence isothiocyante "function" [17]. It was determined that *n*-pentyl-ITC was 22-fold more toxic than its highly branched isomer [18]. Toxicity of *p*-butylphenyl and *p*-bromophenyl ITCs was more pronounced than that of phenyl ITC [19]. *p*-Hydroxyphenyl ITC demonstrated higher antiviral activity than phenyl ITC [20].

Rhizoctonia solani is a plant pathogenic fungus with a wide host range all over the world. It causes serious plant loss by attacking primarily roots and lower stems of plants [21, 22]. *Erwinia carotovora* is a Gram-negative rod-shaped bacterium that thrives alone or aggregates into pairs and chains [23]. Li and co-workers [16] determined that longer-chain ITC derivatives toxicity was influenced by steric hindrance. Antibacterial activity of ITCs was moderately influenced by increased hydrophobicity [16].

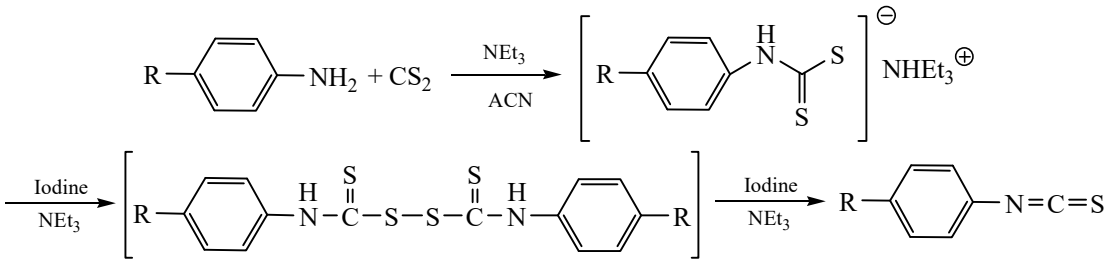
Up to date, little research is performed on activity of different *para* substituted aromatic ITCs against plant pathogenic bacteria and fungi. The current study targeted creating a highly active antimicrobial compound and an efficient approach to structure-activity relationship. For this purpose seven new aromatic ITCs were synthesized and tested for antifungal and antibacterial activities against *R. solani* and *E. carotovora*.

RESULTS AND DISCUSSION

Synthesis of ITCs involved reaction of *p*-aromatic amines with carbon disulfide in the presence of TEA and treatment of the salts thus obtained by iodine (Scheme 1). The process conditions (temperature, time of iodine addition and solvents) were optimized. It was determined that the optimal reaction temperature was

[1] The text was submitted by the authors in English.

Scheme 1. Synthetic route to isothiocyanates.



R = H, CH_3, CH_3CH_2, OCH_3, F, Cl, NO_2.

below 5°C. In order to avoid ITCs direct reacting with amines, the process temperature should not exceed 50°C. The effect of iodine addition time on the yield of the products was studied over a period of 5 to 60 min. Iodine addition to the suspension of dithiocarbamate salt must be carried out over a period of 20 min. Among the solvents, ACN, DMF, MeOH, and EtOH, the most efficient one was determined to be ACN. Aromatic substrates containing various substituents in para position gave ITCs in high yields (89–98%), even with electron-withdrawing substituents (NO_2, F, Cl) attached to the aromatic ring. Structures of the synthesized compounds were characterized by IR, NMR and mass spectra (Table 1).

Efficacy of ITCs against mycelial growth of *R. solani*. According to the present study *p*-nitrophenyl ITC displayed the highest activity against *R. solani* (Table 2). The percent inhibition of mycelial growth was 37.92 and 94.17% at concentrations of 5.0 and 25.0 μg/mL, respectively. *p*-Fluorophenyl ITC demonstrated the lowest activity, 50.3% at 100.0 μg/mL. At

Table 1. Characteristic data for the synthesized compounds

R	Molecular formula	Molecular weight	Yield, %	Found, %			Calculated, %			EI-MS $[M]^+$	^{1}H NMR, δ, ppm	IR, ν, cm^{-1}
				C	H	N	C	H	N			
Phenyl	C_7H_5NS	135.19	98	61.95	3.72	10.31	62.19	3.73	10.36	135.0	7.13–7.37 m (5H)	3062, 2936 (CH), 2098(NCS), 1615 (CS)
p-Methylphenyl	C_8H_7NS	149.21	95	64.20	4.72	9.37	64.39	4.73	9.39	149.0	2.32 d (3H), 7.13 m (5H)	3061, 2930 (CH), 2175 (NCS), 1493 (CS)
p-Methoxyphenyl	C_8H_7NOS	165.21	92	57.93	4.26	8.45	58.16	4.27	8.48	165.0	3.78 s (3H), 6.83 m (2H), 7.13 m (2H)	3066, 2905 (CH), 2115 (NCS), 1503 (CS)
p-Ethylphenyl	C_9H_9NS	163.24	94	66.24	5.54	8.61	66.22	5.56	8.58	163.0	1.37 t (3H), 2.62 d (2H), 7.14 t (2H), 7.42 t (2H)	3012, 2957 (CH), 2098(NCS), 1501 (CS)
p-Fluorophenyl	C_7H_4FNS	153.18	93	54.79	2.62	9.11	54.89	2.63	9.14	153.0	7.09–7.37 m (4H)	2125 (NCS), 1487 (CS)
p-Chlorophenyl	C_7H_4ClNS	169.63	91	49.38	2.37	8.23	49.56	2.38	8.26	169.0	7.09–7.37 m (4H)	3021 (CH_2), 2125 (NCS), 1501 (CS), 726 (Ph)
p-Nitrophenyl	$C_7H_4N_2O_2S$	180.18	89	46.54	2.23	15.51	46.66	2.24	15.55	180.0	7.37 d (2H), 8.25 d (2H)	2930 (Ar-H), 2135 (NCS), 1530 (CS), 1145 (NO)

Table 2. Percentage inhibition of mycelial growth of *R. solani* by ITCs

Phenyl		*p*-Methylphenyl		*p*- Methoxyphenyl		*p*-Ethylphenyl		*p*-Fluorophenyl		*p*-Chlorophenyl		*p*-Nitrophenyl	
concentration, μg/mL	inhibition, %	concentration, μg/mL	inhibition, %	concentration, μg/mL	inhibition, %	concentration, μg/mL	inhibition, %	concentration, μg/mL	inhibition, %	concentration, μg/mL	inhibition, %	concentration, μg/mL	inhibition, %
20.0	12.03a[a]	5.0	12.29a	5.0	17.92a	10.0	12.65a	15.0	12.92a	10.0	10.56a	5.0	37.92a
50.0	34.91b	10.0	13.56a	10.0	38.33b	20.0	37.16b	25.0	27.08b	20.0	19.10b	10.0	75.83b,c
80.0	54.81c	20.0	28.07b	15.0	52.92c	50.0	67.06c	50.0	32.92b	50.0	52.24c	15.0	82.83c
100.0	68.31d	50.0	68.63c	25.0	69.17d	100.0	78.32d	100.0	50.30c	80.0	93.62d	20.0	91.40d
120.0	84.01e	100.0	79.25c	50.0	86.67e	150.0	84.29d	150.0	70.70d	100.0	98.59d	25.0	94.17d

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

the highest concentrations, all screened compounds exhibited significant fungistatic efficiency ranged from 70.70 to 98.59%.

The toxic potency related to various aromatic substituent attached to the aromatic ring was determined to have the following order: *p*-nitrophenyl > *p*-methoxyphenyl > *p*-chlorophenyl > *p*-methylphenyl > *p*-ethylphenyl > phenyl > *p*-fluorophenyl (Table 3).

In general, toxicity of chemicals is related to a hydrophobicity term, an electronic term and a steric term [24]. We hypothesize that all the ITCs are Michael-type acceptors, that can set off the Michael addition reaction with the cellular thiols, and this can be considered as the molecular mechanism of action. Antimicrobial activity of the compounds depends critically on their hydrophobic content, which is typically quantified by the *n*-octanol/water partition coefficient [25]. Hydrophobicity (log *P*) of ITCs was measured using an octanol-aqueous shake-flask method [26]. log *P* Values of seven screened ITCs ranged from 3.20 to 4.43 (Table 3). In the present study, activity of ITCs against *R. solani* became moderately intensive with the hydrophobicity increasing. This indicated that hydrophobicity favored antifungal activity of ITCs against *R. solani*, and suggested that the transport of ITCs to the target sites of *R. solani* could involve hydrophobicity transport mechanisms.

Table 3. EC_{50}[a] values of ITCs against *R. solani* and *E. Carotovora*

ITCs	log P[b]	EC_{50}, μg/mL	
		R. solani	*E. carotovora*
Phenyl	3.20	62.49d[c]	24.05a,b
p-Fluorophenyl	3.28	80.79e	20.01a,b
p-Methoxyphenyl	3.58	13.55b	32.36c
p-Nitrophenyl	3.62	5.63a	13.18a
p-Chlorophenyl	3.91	30.39c	18.25a
p-Methylphenyl	3.92	33.23c	18.32a
p-Ethylphenyl	4.43	35.51d	19.98a

[a] EC_{50} for ITCs causing 50% inhibition of mycelial growth or the number of bacteria. [b] Hydrophobicity. [c] Mean values followed by same letters within the same column were not significantly different according to the LSD test ($P < 0.05$).

The derivatives with an electron-withdrawing substituent should be more activity against *R. solani* than those with electron-donating substituent, because of electronic effect. Comparison of the molecular structures indicated that the nitro substituent exerted a more powerful electronic effect on thiol reactivity with aromatic ITCs than other compounds. It can be concluded that inhibition of mycelial growth of *R. solani* depended mainly on the hydrophobicity of the ITCs and electronic nature of the substituents.

Antibacterial activity of ITCs against *E. carotovora.* The effect of five concentrations of each ITC on growth-inhibition activity of *E. carotovora* are presented in Table 4. *p*-Methoxyphenyl ITC displayed the lowest activity against *E. carotovora*, percentage inhibition was only 68.08% at 50.0 μg/mL. *p*-Nitrophenyl ITC demonstrated the highest activity against *E. carotovora* (20.23 and 89.91% at the concentration of 5.0 and 50.0 μg/mL, respectively). At the highest concentration, all tested compounds exhibited significant antibacterial activity and their percentage inhibition ranged from 68.08 to 98.81%.

Table 4. Antibacterial activity of ITCs against *E. carotovora* by ITCs

Phenyl		*p*-Methylphenyl		*p*- Methoxyphenyl		*p*-Ethylphenyl		*p*-Fluorophenyl		*p*-Chlorophenyl		*p*-Nitrophenyl	
concentration, μg/mL	inhibition, %	concentration, μg/mL	inhibition, %	concentration, μg/mL	inhibition, %	concentration, μg/mL	inhibition, %	concentration, μg/mL	inhibition, %	concentration, μg/mL	inhibition, %	concentration, μg/mL	inhibition, %
15.0	17.41a[a]	10.0	24.97a	10.0	14.37a	5.0	13.25a	1.0	10.12a	5.0	12.32a	5.0	20.23a
20.0	26.39b	20.0	67.07b	15.0	23.67b	10.0	36.13b	5.0	14.27a	10.0	14.97a	10.0	22.93a
25.0	53.31c	50.0	73.53b	25.0	36.94c	20.0	48.07c	10.0	28.30b	15.0	52.21b	15.0	40.38b
30.0	54.77c	80.0	84.68c	30.0	46.50d	25.0	59.31d	25.0	50.83c	20.0	67.20c	25.0	73.68c
50.0	98.81d	100.0	92.83c	50.0	68.08e	50.0	72.61e	50.0	79.49d	50.0	81.04d	50.0	89.91d

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

EC_{50} values determined for reduction of *E. carotovora* growth are presented in Table 3. The order of ITCs antibacterial activity was *p*-nitrophenyl > *p*-chlorophenyl > *p*-methylphenyl > *p*-ethylphenyl > *p*-fluorophenyl > phenyl > *p*-methoxyphenyl. Due to electron-donating nature of the methoxy group reaction activity of ITC with the cellular thiols was the lowest.

Hydrophobicity of the substituents favored the antibacteria activity of ITCs against *E. carotovora*, and suggested that it might be more beneficial for the transport of ITCs to the target sites. Activity of ITCs containing the nitro group against *E. carotovora* was more pronounced than that of other compounds, which was most likely due to its electron-withdrawing nature. It was also beneficial for setting off the Michael addition reaction with the cellular thiols than the electron-donating substituent. Thus, the electronic effects influenced upon the antibacteria activity of ITCs against *E. carotovora*.

Possibly the sulfur containing fungicides act as hydrogen acceptors in metabolic systems and disturbed the normal hydrogenation and dehydrogenation reactions in cells [27]. Activity of ITCs against *R. solani* and *E. carotovora* was not exactly in accordance with the change of electronic effects and hydrophobicity of ITCs. Probably the activity could be entirely related to the electronic effects and hydrophobicity of ITCs, but also be related to the differences in the steric factors and differences in biochemistry and physiology of the fungi and bacteria. For this reason, more studies should be carried out on the possible influence of these factors on the antimicrobial activity of ITCs.

EXPERIMENTAL

The reagent grade chemicals were purchased from Sigma. HPLC grade methanol was supplied from J.T. Baker and ultrapure water from a Milli-Q system (Millipore, Billerica, MA, USA). The fungus *R. solani* and bacteria *E. carotovora* were supplied from the Laboratory of Seed Pathology and Fungicide Pharmacology at China Agricultural University.

Synthesis of isothiocyanates. Synthetic approach to ITCs followed the developed earlier method [28].

Characterization of isothiocyanates. HPLC was carried out for methanol solutions of the compounds using a SPD-20Avp UV detector at 225 nm. NMR spectra were measured on a Varian Unity Inova 300 MHz spectrometers using TMS or the solvent ($CHCl_3$) as an internal reference. IR spectra were recorded for KBr pellets on a Jasco FT-IR 5300 spectrophotometer. Mass spectra (70 eV electron impact [EI]) were measured on a JEOL JMS-AX500 mass spectrometer. Elemental analyses was carried out with a LECO-183 CHNS analyzer.

Evaluation of isothiocyanate derivatives against *R. solani*. Antifungal activity of ITCs against *R. solani* was tested by the growth rate method. ITCs were dissolved in DMSO. Appropriate concentrations (μg/mL) of ITCs were determined by preliminary tests. The blank flat and the flat with solvent were used as the controls. After rejuvenation, fungus cake of *R. solani* (diameter 5 mm) was inoculated in the toxic flat and cultured at 24–28°C for 2–3 days. The percentage of relative inhibition of ITCs against fungus was calculated by comparing the colony diameter with the control.

Evaluation of isothiocyanate derivatives against *E. carotovora*. The synthesized compounds were tested for their antibacterial activity against *E. carotovora* using the turbidimetric method. An aliquot (0.1 mL) of the bacterial suspension (10^9 CFU/mL) was added into a conical flask (35 mL) containing 10 mL of lysogeny broth (LB), which contained ITCs at different concentrations (μg/mL) dissolved in DMSO. The amount of DMSO in both control and assay was below 0.1% (v/v). The mixtures were cultivated in a shaker at 30°C for 12 h. After cultivation, the turbidity of the bacteria solution was determined by using an Agilent 8453 UV-Vis spectrophotometer. A sample (1 mL) was taken from each conical flask, and optical density (OD) value was measured at the wave length of 600 nm. The relation of OD value with the predetermined concentration of bacteria was used for determining the actual concentrations of bacteria. Inhibition rates of ITCs against bacteria were calculated by comparing OD values with controls.

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 using 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 aromatic ITCs were prepared by reacting *p*-aromatic amines with carbon disulphide and further treating with molecular iodine to yield corresponding isothiocyanate derivatives. Structure-activity relationships for the newly synthesized compounds against plant pathogenic fungi and bacteria were evaluated. The results indicated that *p*-nitrophenyl ITC was the most effective in inhibiting *R. solani* and *E. carotovora*. The present study revealed that some of the compounds exhibited promising antimicrobial activities and could be used as an acceptable alternative to the traditional synthetic fungicides in controlling *R. solani* and *E. carotovora*.

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