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Research Article

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Anti-fungal activity of novel synthesized chiral Schiff bases and their reduction products

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ABSTRACT

*Fungicidal chemicals in crude form have been in use for several decades worldwide. This study aims at evaluating the anti-fungal activity of several novel chiral Schiff bases **3** and their reduction products **4**, which were derived from various salicylaldehydes **1** and naturally occurring amino acid L-valine **2**, against plant and food pathogens. The anti-fungal activity was assessed in vitro using the Poisoned Food Technique. The studied Schiff base derivatives exerted potent anti-fungal activity even at 25 ppm concentration. Some Schiff base derivatives caused 100% inhibition of Rhizoctonia solani and Colletotrichum capsici, two plant fungal pathogens at 1000 ppm concentration. Even at 100 ppm concentration, many Schiff base derivatives caused potent % inhibition of four common plant fungal pathogens (Rhizoctonia solani, Colletotrichum capsici, Phyllosticta sp., and Curvularia lunata). Indeed, Schiff bases and their reduction products displayed differential anti-fungal activities. The minimum inhibitory concentration (MIC) values of the examined Schiff bases and their reduction products against the respective fungal pathogens were also determined. Our study also reveals that the studied Schiff bases and their reduction products display fungicidal efficacy against common food fungal pathogens including Rhizopus sp., Fusarium sp., Penicillium sp., and Botrytis cinerea at 50-100 ppm concentrations. Collectively, our study demonstrates that the studied Schiff bases and their reduction products possess fungicidal properties against several plant and food fungal pathogens at concentrations as low as 25-50 ppm, suggesting that such compounds can serve as broad-spectrum fungicidal agents for the control of common phytopathogenic fungi.*

Keywords: Anti-fungal activity; phytopathogenic fungi; reduction product; Schiff base; fungicides.

INTRODUCTION

Agriculture is the largest and most important sector of the Indian economy, 70% of the population are dependent on it. Production of various agricultural products, contribute to nearly 40% of the national income in India. Plant protection through the usage of fungicides, has reduced losses caused by fungal diseases, and as a result, increased the agricultural production and profit. Fungicidal materials in crude form have been in use for several decades. Fungal diseases encountered by plants are effectively eradicated by synthetic chemicals than by botanical pesticides. The limitations in the use of fungicides are minor when compared to those of pesticides. The environmental hazards of fungicides are comparably less than those caused by pesticides and herbicides[1].The development of tolerance by target fungal pathogens has become a major limitation for the effectiveness of commercially available fungicides.

Therefore, current research endeavors are focused on producing potent, ecologically-friendly and effective synthetic chemicals, asan alternative anti-fungal agents, to protect plants against fungal pathogens.

Generally, biological systems recognize the members of a pair of enantiomers as different substances, and the two enantiomersmay elicit different biochemical and cellular responses[2].One enantiomer may act as an effective therapeutic drug, whereas the other may be toxic or inactivity[3].As a result, the synthesis of chiral compounds is important to allow the identification of the most effective agents. Schiff bases, especially those derived fromvarious salicylaldehydes, have been shown to possess anti-viral and anti-tumor properties [4].Therefore, if chiral amines are used as precursors to synthesize Schiff bases with salicylaldehydes, both goals are attained in a single compound that is active, effective and physiologically significant. As a result, chiral Schiff bases (imine esters) **3** are synthesized from the naturally occurring amino acid (L-valine)**2** and various salicylaldehyde**1**(Scheme 1).Our study also aims at investigating the potential anti-fungal properties of the synthesized novel chiral Schiff bases**3,** and their reduction products (amine esters) **4**against agriculturally important disease-causing fungi.

EXPERIMENTAL SECTION

Materials

Chemicals and solvents

n-Hexane and ethyl acetate for column chromatography were purified by distillation. Silica gel (Fisher Scientific), 70-325 mesh, for column chromatography was used. Benzene and methanol were purchased from Merck.L-valine was purchased from Spectrochem. Salicylaldehyde, thionyl chloride, and triethylamine were purified according to previously developed literature methods[5], and distilled before use. The substituted salicylaldehydes and L-valine alkyl esterswere prepared and purified before use, as previously reported[6-9].

Apparatus

^{1}H-NMR and ^{13}C-NMR spectra were recorded at 200MHz on a Bruker 200MHz spectrometer. Chemical shifts are in parts per million (ppm)and relative to tetramethylsilane (TMS) as an internal reference. $CDCl_3$ was purchased from Aldrich Chemical Co., and was used as a solvent for NMR analyses. The purity of the compounds was checked by HPLC(Shimadzu). IR was recorded on Spectrospin Ag.

Pathogenic fungi

The following fungi were available in the Culture Collection of Mycology Laboratory in T.R. Govindachari Centre for Natural Products (SPIC Science Foundation, Chennai, India): *Rhizoctonia solani*, *Colletotrichum capsici*, *Curvularia lunata*, *Phyllosticta sp.*, *Rhizopus sp.*, *Fusarium sp.*, *Penicillium sp.* and *Botrytis cinerea*. The identification of fungi was confirmed by Dr. J. Muthumary (CAS Botany, University of Madras, Chennai, India), and the specimens were deposited in the culture collection library as voucher specimens. All fungi were stored at 4°C. All fungi were refreshed on Czapek-Dox Agar (CDA) plates and were cultured at room temperature.

Methods

Synthesis of Schiff bases and their reduction products

Schiff bases (imine esters)**3** such as (S)-methyl-2-N(2-hydroxyphenyl methylidene)imino-3-methylbutanoate (**3a)** and their corresponding reduction products (amine esters) **4** such as (S)-methyl-2-N(2-hydroxyphenyl methyl)amino-3-methylbutanoate (**4a**) were prepared, as previously described[10], starting from the various salicylaldehydes and L-valine alkyl esteras shown in Figure 1 and Table 1.

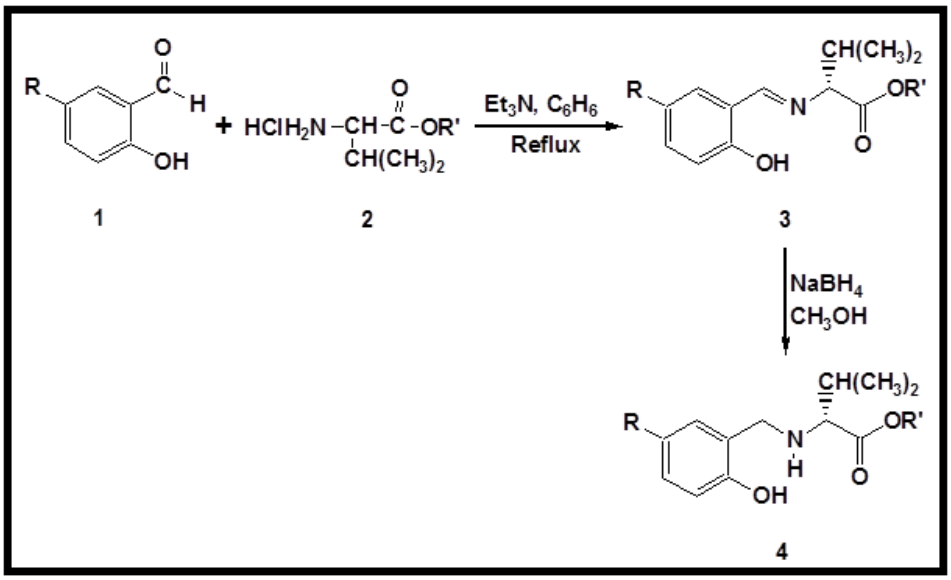

Table 1. Synthesis of Schiff bases3and reduction products 4

Schiff Bases 3	Reduction Products 4	Aldehydes (R =) 1	Amino Acids (R' =) 2
3a	**4a**	H	CH_3
3b	**4b**	Cl	CH_3
3c	**4c**	Br	CH_3
3d	**4d**	*t*-Bu	CH_3
3e	**4e**	OCH_3	CH_3
3f	**4f**	NO_2	CH_3
3g	**4g**	H	CH_2CH_3
3h	**4h**	H	$C(C_6H_5)_2OH$
3i	-	$-(CH)_4-$	CH_3

Preparation of amino acid methyl ester hydrochloride 2

To 50mmol of L-valine in 50mL of methanol (at 0ºC), 50mmol of thionyl chloride was added at room temperature and stirred for 6h. At the end of the reaction, excess amount of methanol and thionyl chloride were removed under reduced pressure. The white solid product was thoroughly dried and stored under nitrogen.

General procedure for the preparation of Schiff bases (imine esters) 3

To 50 mmol of salicylaldehyde**1**in 50mL of benzene, 50mmol of alkyl ester hydrochloride of L-valine **2**and 50 mmol of triethylamine were added and refluxed using Dean-Stark apparatus, until 0.9mL of water separated out. The reaction mixture was then passed through a short silica gel column to remove the triethylamine hydrochloride. Benzene was removed under reduced pressure to furnish imine esters **3**in good isolated yield.

General procedure for the preparation of the reduction products (amine esters) 4

To 25mmol of imine ester**3** in 25mL of methanol, 12.5mmolof sodium borohydride was added and stirred for 15 min. At the end of the reaction, methanol was removed under reduced pressure, and the pH was adjusted to 7-8, by adding 1:1 HCl solution. The amine ester**4** was formed and subsequently was extracted in chloroform and dried over anhydrous sodium sulfate. After removal of the solvent under reduced pressure, the reduction product**4** was purified by flash column chromatography, using hexane: ethyl acetate (98:2) as an eluent.

Poisoned food technique

Poisoned Food Technique was used for assessing the anti-fungal activity of the synthesized compounds as previously described [11]. Briefly, a weighed quantity of each compound was incorporated into the molten Czapek-Dox Agar medium (cooled to approximately45°C) to achieve the desired concentrations of the compounds. Molten medium (15 mL) was poured into each sterile petri dish under aseptic conditions and left to solidify. For initial

screening, 100 mg of selected compounds incorporated in 100 mL of Czapek-Dox Agar medium at the concentration of 1000ppm was used for screening the anti-fungal activity of the compounds. The media supplemented with the compounds served as the treatment samples, while media without the compounds served as a negative control. Discs of fungal pathogens (5 mm) were prepared by punching in the fungal mat grown on Czapek-Dox Agar medium in sterile petri dishes. The fungal pathogen discs were placed in the center of the medium and incubated at room temperature. Radial growth, in terms of diameter (mm), was obtained at 72 h after inoculation and subsequently every 24 h, for a total period of 240 h (10 d). In all cases, five replicates were maintained and the entire experiment was repeated three times.

RESULTS AND DISCUSSION

Initially, the potential anti-fungal activity of some synthesized Schiff base compounds (**3b**, **3c**, **3e**, **3f**, and **3i**) was tested against two common plant fungal pathogens *Colletotrichum capsici* and *Rhizoctonia solani* at 1000ppm concentration. Interestingly, all tested compounds displayed potent anti-fungal activity, with 100% inhibition, against both fungal pathogens(Table 2).

Table 2.Percentage inhibition of *Rhizoctonia solani* and *Colletotrichum capsici* by selected Schiff bases(imine esters) 3 at 1000 ppm concentration

Schiff Bases 3	*Rhizoctonia solani*	*Colletotrichum capsici*
3b	100	100
3c	100	100
3e	100	100
3f	100	100
3i	100	100

Given the potent anti-fungal activity of the selected compounds against *Rhizoctonia solani* and *Colletotrichum capsici*at 1000ppm concentration, we assessed the anti-fungal activity of all synthesized compounds against four common plant fungal pathogens *Rhizoctonia solani*, *Colletotrichum capsici*, *Phyllosticta sp.*, and *Curvularia lunata* at 100ppm concentration. The results are shown in Table 3.

Table 3.Percentage inhibition of *Rhizoctonia solani, Colletotrichum capsici*, *Phyllosticta sp.*, and *Curvularia lunata* by Schiff bases(imine esters) 3 and reduction products(amine esters) 4 at 100 ppm concentration

Schiff Bases 3	Reduction Products 4	Percentage Inhibition*			
		Rhizoctonia solani	*Colletotrichum capsici*	*Curvularia lunata*	*Phyllosticta sp.*
3a		100	22	100	38
	4a	64	24	65	45
3b		100	27	100	76
	4b	55	34	88	66
3c		100	5	100	91
	4c	60	54	91	78
3d		100	51	100	100
	4d	75	50	86	80
3e		65	8	78	57
	4e	79	17	76	18
3f		100	28	100	83
	4f	81	36	86	57
3i		100	100	100	87
3g		97	49	100	62
	4g	72	53	80	51
3h		93	45	100	58
	4h	89	41	85	57

**Mean of three replicates*

Among the synthesized imine esters (3a – 3h), three imine esters 3i, 3d, and 3f showed excellent inhibition against *Rhizoctonia solani*, *Colletotrichum capsici*, and *Curvularia lunata*. The Schiff base imine ester (3d) was the only compound to show 100% inhibition against *Phyllosticta sp.* Among the eight amine esters (4a – 4h), only two (4d and 4c) have a fairly good inhibition quality, against the indicated fungal pathogens. The bromo derivative of imine ester (3c) show a poor inhibition, whereas its reduced form (4c) the amine ester displayed an impressive 54%

inhibition. The t-butyl substituted imine ester (3d) and its amine ester (4d) showed a better inhibition pair of compounds for anti-fungal activity. The inhibition against fungal pathogen *Colletotrichum capsici* was comparatively best exhibited by the reduced form of the imine ester, whereas for other fungal pathogens, the imine esters acted as better inhibitors.

As the synthesized compounds with imine functionality (**3a-i**) showed potent anti-fungal activity (Table 3), the minimum inhibitory concentration (MIC) values of these compounds against the respective fungal pathogens were determined (Table 4).

At concentration as low as 25 ppm, the Schiff base imine esters 3c, 3d, 3f and 3i showed 100% inhibition against *Rhizoctonia solani*. The imine ester (3i) displayed 59% inhibition against *Colletotrichum capsici*, whereas the other compounds show no activity against the pathogen. Similar behavior was observed with imine ester (3d) at 66% inhibition against *Phyllosticta sp.,* at a low concentration of 25 ppm.

Since compounds **3d**, **3f**, and **3i** display potent anti-fungal activity even at lower concentrations (25 ppm), the fungicidal efficacy of these compounds was tested against important common food fungal pathogens (*Rhizopus sp., Fusarium sp., Penicillium sp.,* and *Botrytis cinerea*) (Table 5).

The Schiff base imine ester (3i) showed a better inhibition against the common food pathogens *Rhizopus sp., Fusarium sp., Penicillium sp.,* and *Botrytis cinerea*. Observing the trend in inhibition behavior of the synthesized compounds, the imine esters with β-naph, t-butyl and nitro group as substitution, show an increased percentage in anti-fungal activity. The reduced forms of these imine esters (amine esters) show a fairly good percentage of inhibition.

Table 4.Assessment of the Minimum Inhibitory Concentration (MIC)of Schiff bases (imine esters) 3 against*Rhizoctonia solani, Colletotrichum capsici, Curvularia lunata,* and *Phyllosticta sp*

Schiff Base 3	Percentage Inhibition*							
	Rhizoctonia solani		*Colletotrichum capsici*		*Curvularia lunata*		*Phyllosticta sp.*	
	50 ppm	25 ppm	50 ppm	25 ppm	50 ppm	25 ppm	50 ppm	25 ppm
3a	67	49	-	-	47	36	-	-
3b	90	81	-	-	100	51	-	-
3c	100	91	-	-	65	58	-	-
3d	100	100	-	-	100	56	82	66
3f	100	100	-	-	62	44	-	-
3i	100	100	88	59	89	81	-	-
3g	-	-	-	-	87	57	-	-
3h	-	-	-	-	100	53	-	-

Table 5.Percentage inhibition of common food pathogens (*Rhizopus sp., Fusarium sp.,Penicillium sp.,* and *Botrytis cinerea*) by selectedSchiff bases (Imine Esters) 3 at 100ppm and 50ppm concentrations.

Schiff Base 3	Percentage Inhibition*							
	Rhizopus sp.		*Fusarium sp.*		*Penicillium sp.*		*Botrytis cinerea*	
	100 ppm	50 ppm	100 ppm	50 ppm	100 ppm	50 ppm	100 ppm	50 ppm
3d	68	53	100	58	82	67	90	61
3f	66	55	82	47	81	71	62	34
3i	100	81	100	100	90	75	100	100

***Mean of three replicates*

CONCLUSION

In conclusion, our findings demonstrate that the synthesized novel Schiff base**3** compounds with imine functionality display moderate to high potent anti-fungal activity towards plant and food fungal pathogens, even at lower concentrations of 25-50ppm.This suggests that these novel Schiff base compounds can serve as broad-spectrum fungicidal agents for the control of common phytopathogenic fungi. We anticipate that the studied Schiff base compounds will be employed in the future as alternative ecologically-friendly synthetic agents with potent anti-

fungal activity against plant and food fungal pathogens including *Rhizoctonia solani*, *Colletotrichum capsici*, *Phyllosticta sp.*, *Curvularia lunata*, *Rhizopus sp.*, *Fusarium sp.*, *Penicillium sp.*, and *Botrytis cinerea*.

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