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Synthesis and antimicrobial activity of novel mancozeb derivatives containing 1,3,4-thiadiazole

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Abstract Four mancozeb derivatives containing 1,3,4-thiadiazole, **5a**–**5d**, were successfully synthesized, then characterized by IR, ^{1}H NMR, and ^{13}C NMR spectroscopy and elemental analysis. Their antimicrobial activity against paddy fusarium, *Botrytis cinerea*, cucumber fusarium, tomato gibberella, and grape white rot was tested in vitro by use of the filter paper disk-diffusion technique. Compounds **5a**–**5d** had moderate to excellent antimicrobial activity in comparison with mancozeb, **6**. Among these compounds, **5a** had the best inhibitory activity.

Keywords Mancozeb · 1,3,4-Thiadiazole · Synthesis · Antimicrobial activity

Introduction

1,3,4-Thiadiazole compounds have been of much interest in synthetic chemistry and as pesticides because of their unique biological properties. Recently, 1,3,4-thiadiazole derivatives have been shown to have a variety of biological activity, for example as fungicides [1], plant growth regulators [2, 3], insecticides [4], herbicides [5], and anticholinergic [6] and anticonvulsant [7] compounds. Therefore, these compounds attract much attention as novel pesticides. Dithiocarbamate salts are widely used in the petroleum [8], agriculture [9], pharmaceutical [10], environmental protection [11], and rubber [12] industries. However, because resistance to Zineb has been observed, development of novel potent antimicrobial agents with superior bioavailability is

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highly desirable. Herein, we describe the synthesis and antimicrobial activity of four novel mancozeb derivatives containing 1,3,4-thiadiazole (Scheme 1).

Results and discussion

All the reagents described in this paper were commercially available, and all solvents were freshly distilled before use. Melting points were recorded on an X-4 microscopic melting point apparatus, and are uncorrected. Elemental analysis (C, H, and N) was performed with a VarioEL III. IR spectra, as KBr pellets, were recorded on a Vector-22 instrument. ^{1}H NMR and ^{13}C NMR spectra were recorded on an Avance III 400 MHz instrument, with $CDCl_3$ as solvent and $SiMe_4$ as internal reference.

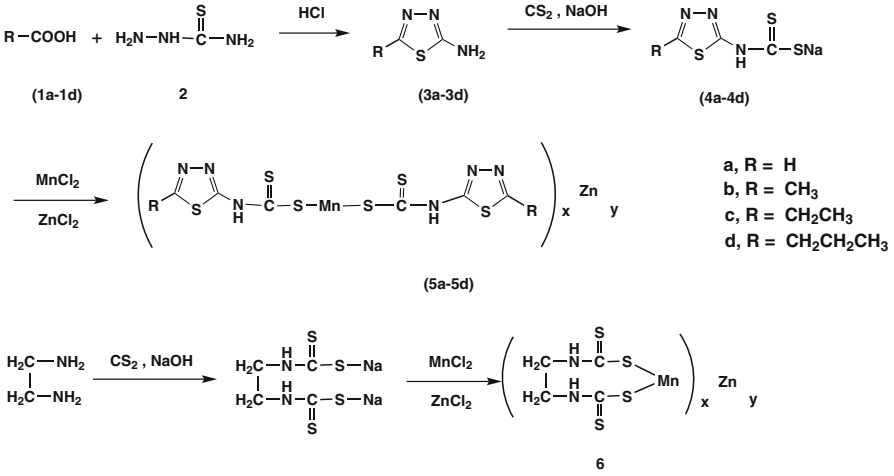
Synthesis of 2-amino-1,3,4-thiadiazole derivatives

A mixture of 20 mL concentrated hydrochloric acid, 0.026 mol carboxylic acid, and 0.022 mol thiosemicarbazide was heated under reflux for 5 h. After cooling to room temperature, the mixture was adjusted to pH 8 with 40 % sodium hydroxide solution. The white solid powder obtained was isolated by filtration, washed with water, and finally recrystallized from 30 % ethanol.

Results from characterization of 2-amino-1,3,4-thiadiazole derivatives **3a**–**3d** are listed in Tables 1, 2, and 3.

Synthesis of mancozeb and 1,3,4-thiadiazole derivatives of mancozeb

A mixture of 0.010 mol 1,2-ethanediamine or 2-amino-1,3,4-thiadiazole derivative and 0.010 mol sodium hydroxide aqueous solution was stirred, heated to 30 °C, then 0.020 mol carbon disulfide was slowly added dropwise to the mixture. The



Scheme 1 Synthetic routes to **5a**–**5d** and **6**

Table 1 Yield, melting point, and results from elemental analysis of compounds **3a–3d**

Compound	Molecular formula	Yield (%)	Melting point (°C)	Elemental analysis found (calculated) (%)
3a	$C_2H_3N_3S$	91.6	192–194	C: 23.68 (23.75), H: 3.05 (3.00), N: 41.43 (41.56)
3b	$C_3H_5N_3S$	89.5	236–239	C: 31.27 (31.28), H: 4.43 (4.38), N: 36.28 (36.49)
3c	$C_4H_7N_3S$	90.3	205–212	C: 37.42 (37.18), H: 5.13 (5.27), N: 32.48 (32.53)
3d	$C_5H_9N_3S$	84.1	212–218	C: 41.81 (41.83), H: 6.30 (6.35), N: 29.31 (29.34)

Table 2 Characteristic IR spectral data for compounds **3a–3d**

Compound	IR (ν_{max}, KBr, cm^{-1})
3a	3,288, 3,082 ($\nu_{N–H}$, s); 1,618, 1,611 ($\nu_{C=N}$, s); 1,021 ($\nu_{=C–S–C=}$, s); 680 ($\nu_{=C–S–C=}$, m)
3b	3,324, 3,100 ($\nu_{N–H}$, s); 2,939 ($\nu_{C–H}$, s); 1,618, 1,520 ($\nu_{C=N}$, s); 1,445, 1,376 ($\nu_{C–H}$, w); 1,022 ($\nu_{=C–S–C=}$, s); 685 ($\nu_{=C–S–C=}$, m)
3c	3,287, 3,104 ($\nu_{N–H}$, s); 2,974, 2,929, 2,870 ($\nu_{C–H}$, s); 1,678, 1,637 ($\nu_{C=N}$, s); 1,489, 1,453, 1,368 ($\nu_{C–H}$, w); 1,027, 823, 742, 691, 482 ($\nu_{N–C–S}$, w)
3d	3,433 ($\nu_{N–H}$, s); 2,987, 2,927, 2,760 ($\nu_{C–H}$, s); 1,639, 1,527 ($\nu_{C=N}$, s); 1,498, 1,451, 1,407($\nu_{C–H}$, w); 1,027, 820, 740, 687, 587($\nu_{N–C–S}$, w)

Table 3 Characteristic ^{1}H NMR and ^{13}C NMR data for compounds **3a–3d**

Compound	^{1}H NMR ($CDCl_3$, TMS, ppm)	^{13}C NMR ($CDCl_3$, TMS, ppm)
3a	6.96 (s, 2H, $–NH_2$); 9.16 (s, 1H, N=C–H)	152.0; 161.7
3b	2.64 (s, 3H, $–CH_3$); 6.96 (s, 2H, $–NH_2$)	19.5; 142.7; 161.5
3c	1.25 (t, 3H, $–CH_3$); 3.07 (q, 2H, $–CH_2–$); 6.96 (s, 2H, $–NH_2$)	13.4; 24.3; 161.6; 168.5
3d	0.91 (t, 3H, $–CH_3$); 1.67 (m, 2H, $–CH_2–$); 2.86 (t, 2H, $–CH_2–$); 6.97 (s, 2H, $–NH_2$)	13.7; 23.8; 30.7; 161.6; 168.9

reaction was monitored until completion then cooled to room temperature. Manganous chloride (0.01 mol) aqueous solution was slowly added dropwise, the mixture was stirred for 0.5 h, then 0.01 mol zinc chloride aqueous solution was slowly added dropwise, followed by stirring for 1.5 h. Mancozeb was obtained as a gray solid powder after filtration and drying.

Results from characterization of mancozeb, **6**, and 1,3,4-thiadiazole derivatives of mancozeb, **5a–5d**, are listed in Tables 4, 5, and 6.

Antimicrobial activity of 1,3,4-thiadiazole derivatives of mancozeb

Antimicrobial activity was determined by use of the filter paper disk method [13]. Paddy fusarium, *Botrytis cinerea*, cucumber fusarium, tomato gibberella, and grape white rot were selected for the test.

Table 4 Yield and results from elemental analysis of compounds **5a–5d** and **6**

Compound	Molecular formula	Yield (%)	Elemental analysis found (calculated) (%)
6	$C_4H_6MnN_2S_2Zn$	96.5	C: 18.79(18.02), H: 2.19(2.27), N: 10.56(10.51)
5a	$C_6H_4MnN_6S_6Zn$	84.9	C: 15.29(15.24), H: 0.78(0.85), N: 17.72(17.77)
5b	$C_8H_8MnN_6S_6Zn$	85.2	C: 19.09(19.18), H: 1.55(1.61), N: 16.83(16.78)
5c	$C_{10}H_{12}MnN_6S_6Zn$	82.1	C: 22.74(22.71), H: 2.31(2.29), N: 15.80(15.89)
5d	$C_{12}H_{16}MnN_6S_6Zn$	79.4	C: 25.81(25.88), H: 2.88(2.90), N: 15.12(15.09)

Table 5 Characteristic IR spectral data for compounds **5a–5d** and **6**

Compound	IR (ν_{max}, KBr, cm^{-1})
6	3,425 (ν_{N-H}); 1,609 ($\nu_{C=N}$); 1,442 (ν_{N-C}); 1,125 ($\nu_{C=S}$); 1,075 (ν_{C-S-C}); 956 (ν_{C-S}); 550 (ν_{S-X})
5a	3,410 (ν_{N-H}); 1,615 ($\nu_{C=N}$); 1,442 (ν_{N-C}); 1,128 ($\nu_{C=S}$); 1,007 (ν_{C-S-C}); 920 (ν_{C-S}); 529 (ν_{S-X})
5b	3,411 (ν_{N-H}); 1,618 ($\nu_{C=N}$); 1,431 (ν_{N-C}); 1,233 ($\nu_{C=S}$); 1,075 (ν_{C-S-C}); 865 (ν_{C-S}); 658 (ν_{S-X})
5c	3,443 (ν_{N-H}); 1,622 ($\nu_{C=N}$); 1,429 (ν_{N-C}); 1,230 ($\nu_{C=S}$); 1,013 (ν_{C-S-C}); 930 (ν_{C-S}); 576 (ν_{S-X})
5d	3,421 (ν_{N-H}); 1,625 ($\nu_{C=N}$); 1,433 (ν_{N-C}); 1,119 ($\nu_{C=S}$); 1,078 (ν_{C-S-C}); 944 (ν_{C-S}); 530 (ν_{S-X})

Table 6 Characteristic ^1H NMR and ^{13}C NMR data for compounds **5a–5d** and **6**

Compound	^1H NMR ($CDCl_3$, TMS, ppm)	^{13}C NMR ($CDCl_3$, TMS, ppm)
6	2.00(t, 1H, –NH–), 2.93(d, 2H, $–CH_2–$)	47.6; 200.5
5a	4.00 (s, 1H, –NH–); 9.16 (s, 1H, N=C–H)	152.0; 152.9; 196.1
5b	2.65 (s, 3H, $–CH_3$); 4.05 (s, 1H, –NH–)	19.5; 142.9; 152.5;198.1
5c	1.25 (t, 3H, $–CH_3$); 3.07 (q, 2H, $–CH_2–$); 3.98(s, 1H, –NH–)	13.2; 24.4; 152.4; 168.5; 196.0
5d	0.91 (t, 3H, $–CH_3$); 1.68 (m, 2H, $–CH_2–$); 2.86 (t, 2H, $–CH_2–$); 4.01 (s, 1H, –NH–)	13.9; 23.9; 30.7; 152.5; 167.3; 196.5

The samples (1,3,4-thiadiazole derivatives of mancozeb **5a–5d**) were separately dissolved in an appropriate solvent at a concentration of 10 mg/mL. Compound **6** was used as the standard. Cultures of known inoculum size (10^6 cells/mL) of the tested strains were spread on nutrient agar plates. Sterile filter paper disks 6 mm in diameter was placed on the plates and the 0.5 ml of 10 mg/mL solutions of the mancozeb derivatives were applied to the filter disks. The plates were incubated for three days at 28 °C. Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition; the results are summarized in Table 7.

Investigation of the antimicrobial screening data revealed that all the novel mancozeb derivatives **5a–5d** had moderate to excellent antimicrobial activity

Table 7 Antimicrobial activity of mancozeb derivatives **5a–5d** and **6**

Compound (10 mg/mL)	Zone of Inhibition (mm)				
	Paddy fusarium	*Botrytis cinerea*	Cucumber fusarium	Tomato gibberella	Grape white rot
5a	19.6	14.5	8.1	20.2	14.3
5b	13.8	23.6	7.8	12.7	15.4
5c	14.3	14.3	15.1	12.4	13.5
5d	12.5	7.8	13.6	8.9	11.4
6	14.2	25.6	7.6	8.1	8.0

The results in the table are average values from three experiments; the diameter of the filter paper was 6 mm

against the five strains tested. Compound **5b** had the best inhibitory activity against *Botrytis cinerea*, besides the standard **6**; its zone of inhibition reached 23.6 mm. Compounds **5c** and **5d** had better inhibitory activity than standard **6** against cucumber fusarium; their zones of inhibition were 15.1 mm and 13.6 mm, respectively. All these novel mancozeb derivatives had stronger inhibitory activity than the standard **6** against grape white rot. Among these mancozeb derivatives, compound **5a** had the most broad spectrum and strongest inhibitory activity.

From the discussion above we can propose a speculative mechanism of the antimicrobial activity of these novel mancozeb derivatives. Mancozeb had a relatively strong inhibitory antimicrobial effect and mancozeb containing the 1,3,4-thiadiazole group had stronger antimicrobial activity than mancozeb because the 1,3,4-thiadiazole group is a fat-soluble functional group. Different 1,3,4-thiadiazole-containing derivatives of mancozeb had different antimicrobial activity against the five strains tested. Because of less steric hindrance, compound **5a** had high activity against paddy fusarium, tomato gibberella, and grape white rot.

Conclusion

Novel mancozeb derivatives containing the 1,3,4-thiadiazole group were synthesized in good yield. Their antimicrobial activity was tested. Compound **5a** had the best inhibitory activity. This work is an excellent basis for discovery of potent antimicrobial agents.

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