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Synthesis and evaluation of novel ferrocene-substituted triadimenol analogues

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In search of potent 1*H*-1,2,4-triazole derivatives with improving antifungal activity, a class of novel ferrocene–triadimenol analogues was synthesized and their biological potential evaluated. Screening data revealed that these new derivatives did not have the antifungal activities of parent compounds, but showed unexpectedly promising plant growth regulatory activity. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: ferrocene; triadimenol; antifungal activity; plant growth regulatory activity

INTRODUCTION

Recently, increasing interest has been focused on developing metallocenic organometallic compounds as alternatives to chemotherapy due to drug resistance in cancer and tropical diseases such as malaria.[1–6] Ferrocene, because of its stablity, non-toxicity, membrane permeation, the accessibility of a large variety of derivatives, as well as its favorable electrochemical properties, has been introduced into several biological molecules for more potent activity than the relative parent compounds.[7,8] Several structural modifications of established drugs with ferrocenyl moiety have been reported, such as ferrocene fluconazole, ferrocene aspirin, the anti-malarial drugs chloroquine (ferroquine), quinine, mefloquine and artemisinin, and the anti-cancer drug tamixofen (ferrocifen).[1]

1*H*,2,4-Triazole derivatives constitute a class of biologically significant substances, such as agrochemicals triadimefon **1**, triademenol **2**, flusilazole, cyproconazole and clinical drugs fluconazole **3** and itraconazole **4** (Fig. 1), which are effective antifungal agents widely used clinically and in agriculture.[9–12] Their antifungal activity arises from inhibiting cytochrome P-450-dependent 14α-sterol demethylase (P-450$_{DM}$), an important enzyme in ergosterol biosynthesis in fungi and cholesterol synthesis in mammalian cells.[13,14] In addition, some of them also show predominant plant growth regulatory activity on a variety of agricultural and horticultural species.[9,10,15,16]

The commercial 1*H*-1,2,4-triazole fungicides triadimefon and triadimenol[17] are important two systemic agents with a broad spectrum of fungicidal activity against plant pathogens, especially powdery mildew, loose smut and rust of cereals and other crops. In particular, triadimenol, major metabolite of triadimfon in mammals, plants and soil,[18] is also an effective plant growth regulator at low concentrations and has no distinct species specificity.

With extensive application in agriculture and horticulture, however, these triazole compounds are suspected of having teratogenic potential such as craniofacial and axial skeletal defects on the basis of developmental toxicity studies on rodent mammals.[19–21] It has been ascertained that triadimefon and triadimenol can also produce a neurotoxic syndrome in rats characterized by increased motor activity, stereotyped behavior and altered monoamine metabolism due to inhibition of dopamine uptake.[22] In addition, triadimefon had a significant stimulating side effect on respiration at a concentration of 1–100 mg/kg and on inhibition of nitrification in soil at a concentration of 100 mg/kg.[23]

As part of our ongoing project devoted to synthesis of novel biological molecules containing 1*H*-1,2,4-triazole moiety possessing antifungal and plant growth regulatory activity, a class of triadimenol analogues structurally modified by ferrocenyl moiety was designed and synthesized for improving biological behavior (Fig. 2). We herein reported their chemistry and biological screening results involving antifungal and plant growth regulatory activity.

EXPERIMENTAL

Material and methods

All reactions were carried out under nitrogen atmosphere and monitored by conventional TLC. THF was distilled on CaH_2 and acetonitrile was distilled on anhydrous $CaCl_2$ prior to

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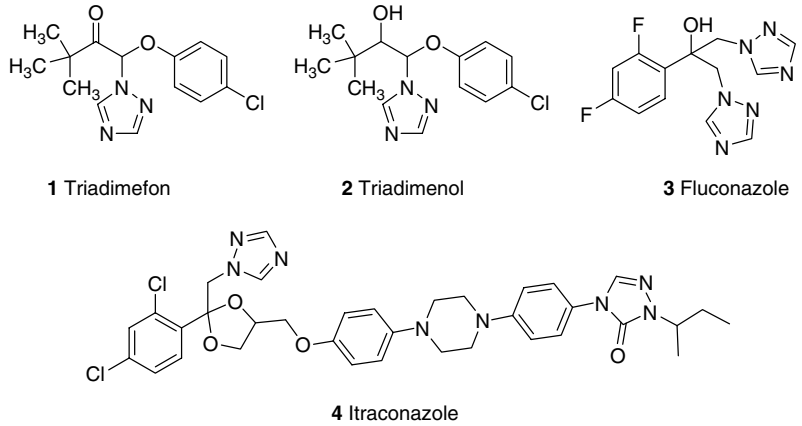


Figure 1. Some commercial 1*H*-1,2,4-triazole fungicides.

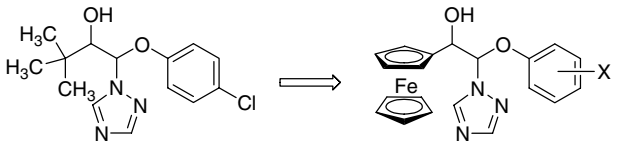


Figure 2. Designed target molecules.

use. All melting points were determined on a Taike hotplate melting apparatus and thermometer was uncorrected. The ^{1}H NMR spectra were measured on a Bruker Ultra-300 spectrometer in d_6-DMSO solution with TMS as internal standard. Elemental analyses were determined on an MT-3 elemental analyser within ±5‰ of the theoretical values. Mass spectra were recorded on a HP-5988A GC-MS instrument at 70 eV, and the temperature of ionization was 200 °C.

Preparation of the intermediate ferrocene-triadimefon analogues (3a–j)

Syntheses and characterization of the intermediates ferrocene–triadimefon analogues **3a–j** referred to our ongoing work as outlined in the Scheme 1.[24]

Preparation of the ferrocene-triadimenol analogues (4a–j)

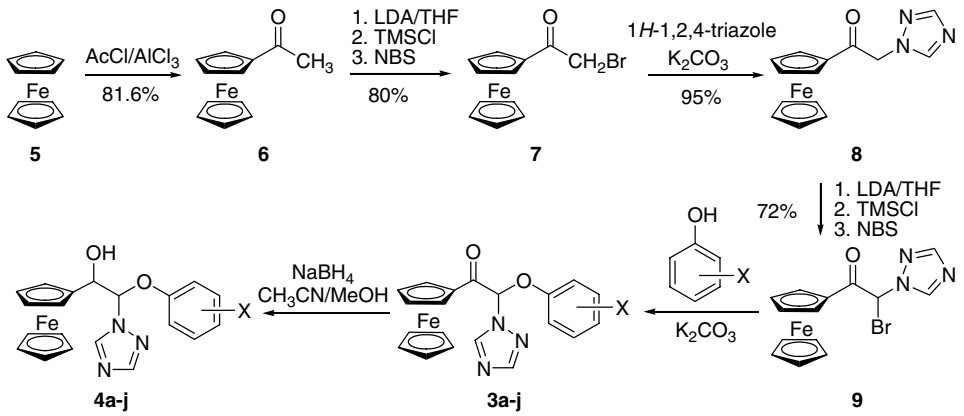
*General procedure for syntheses of 2-aryloxy-1-ferrocenyl-2-(1H-1,2,4-triazol-1-yl)ethanols (**4a–j**)*

To a stirred solution of 2-aryloxy-1-ferrocenyl-2-(1*H*-1,2,4-triazol-1-yl)ethanone **3** (3 mmol) in acetonitrile (5 ml) and methanol (5 ml) below 0 °C was added sodium boronhydride (6 mmol) in small portions. After stirring at room temperature for 30 minutes, the reaction mixture was poured into cooled water (50 ml) and neutralized to pH 7 with 5% diluted hydrochloric acid. The product was precipitated and collected by filtration. Recrystallization from ethyl acetate gave the title compounds **4a–j** as an analytically pure sample. All compounds were thus obtained as a yellow solid in moderate yield. Physical and analytical data of these derivatives are outlined in Table 1.

Biological evaluation

Fungicidal activity

The title compounds **4a–j** were assayed for antifungal activities against powdery mildew and brown rust on intravital wheat plants at the Biological Assay Centre, Nankai



Scheme 1. Synthesis of the target compounds.

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Table 1. Physical and elemental analysis data of the title compounds **4**

Compound	X (Aryl)	Yield (%)	M.p. (°C)	Elemental analysis: found/calcd (%)		
				C	H	N
4a	4-Cl	64	149–151	56.70/56.70	4.19/4.28	10.10/9.92
4b	2,4-Cl_2	63	152–155	52.40/52.43	3.86/3.74	9.22/9.17
4c	2,5-Cl_2	71	166–169	52.43/52.43	3.75/3.74	9.06/9.17
4d	2,4,5-Cl_3	60	163–165	48.76/48.77	3.33/3.27	8.56/8.53
4e	2,4,6-Cl_3	59	173–175	48.70/48.77	3.27/3.27	8.68/8.53
4f	4-NO_2	66	168–173	55.16/55.32	4.08/4.18	13.04/12.90
4g	1-Naphthyl	51	177–180	65.74/65.62	4.72/4.82	9.58/9.57
4h	2-Naphthyl	61	178–180	65.62/65.62	4.88/4.82	9.59/9.57
4i	3-Me	58	165–168	62.43/62.55	5.43/5.25	10.68/10.42
4j	H	65	170–172	61.59/61.72	5.01/4.92	10.96/10.80

University according to procedures described previously.[25] The five selected fungi included *Isariopsis clavispora*, *Bremia lactucae*, *Cladosporium fulvum*, *Erysiphe graminis* and *Alternaria mali*.

Plant growth regulatory activity

The plant growth regulatory activity of compounds **4a–j** was screened using cucumber cotyledon rhizogenesis at the same department as above. All compounds were tested at a same concentration of 10 mg/l. The detailed procedure was described as follows. After dipping in distilled water for 1 h at 23 °C, the cucumber seeds (Jinke, no. 4, commercial availably) were then sown into soil with 0.7% agar on a covered porcelain enamel plate and incubated at 26 °C in a darkroom for 3 days. The same size cotyledons were carefully selected to subsequent biological assay. The tested compound (3 mg) was resolved in *N*,*N*-dimethyl formamide (3 ml) and this solution was then diluted to 10% concentration with distilled water. A sample solution (0.3 ml) was sprayed over a 6 cm diameter filter paper and solvent was volatilized to dryness on air. The filter paper thus prepared was placed into a 6 cm diameter incubation vessel and soaked with 10 cm distilled water. Finally, 10 pieces of cotyledon of the same size were added into incubation vessel. These cotyledons were incubated at 26 °C in a darkroom for 5 days. Then the rhizogenesis numbers of every 10 pieces of hypocotyls were measured. Each sample was repeated twice. In contrast, the distilled water was used as a control experiment. The relative ratios of cucumber cotyledon rhizogenesis were calculated according to the following formula:

$$\text{Relative ratio } \% = (N_S - N_C)/N_C \times 100\%$$

where N_S and N_C are the numbers of cucumber cotyledon rhizogenesis of tested compound and control experiment, respectively.

RESULTS AND DISCUSSION

Chemistry

Starting from commercial ferrocene **5**, the target compounds **4a–j** were prepared as illustrated in the Scheme 1. The intermediates acetylferrocene **6**, α-bromoacetylferrocene **7** and α-(1*H*-1,2,4-triazol-1-yl)acetylferrocene **8** were synthesized according to established methods in yields of 81.6, 80 and 95%, respectively.[26–28]

α-(1*H*-1,2,4-triazol-1-yl)acetylferrocene **8** was brominated to deliver key intermediate bromide **9** in satisfactory yield under analogous conditions described by Tarraga and co-workers.[27] Originally, substitution reactions of bromide **9** by various phenols were conducted utilizing sodium hydride as base in anhydrous tetrahydrofuran (THF), and low yields were reached because of insolubility of sodium phenoxyl in THF. Instead of sodium hydride, anhydrous potassium carbonate was thus used as base and reactions were carried out in anhydrous acetonitrile at elevated temperature. Ferrocene–triadimefon analogues **3a–j** were readily obtained in good to excellent yields after purification by flash chromatography. Triadimefon analogues **3a–j** were ultimately reduced to target ferrocene–triadimenol analogues **4a–j** by sodium boronhydride in acetonitrile–methanol (V : V 1 : 1) system.

^{1}H NMR spectra

The structures of target compounds **4a–j** were well supported by ^{1}H NMR and EIMS spectra, which are shown in the Table 2. The ^{1}H NMR spectra of all compounds showed a set of signals compatible with an 1*H*-1,2,4-triazole. The chemical shifts of the triazole group appear between 7.90 and 8.90 ppm as two single peaks, while signals of single-substituted ferrocene were found in the range 4.00–5.00 ppm in good agreement with those results in previous work.[28] Two conjoint methine protons (—CH) appeared between 4.97–5.36 and 6.07–6.36 ppm, respectively, with the same coupling constant (7 Hz).

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Table 2. ^{1}H NMR and EIMS spectra of compounds **4**

Compound	X	^{1}H NMR δ (PPM, 300 MHz, d_6-DMSO)	EIMS (m/z, M^+)
4a	4-Cl	8.810 (1H, s, H3′, triazole), 8.014 (1H, s, H5′, triazole), 7.304–6.963 (4H, m, H2″, H3″, H5″ and H6″, aryl), 6.100 (1H, d, J = 7 Hz, C*H*-Tr), 4.988 (1H, d, J = 7 Hz, C*H*-OH), 4.324 (2H, s, metallocene), 4.207 (5H, s, metallocene), 4.177 (1H, s, metallocene), 4.118 (1H, s, metallocene)	423
4b	2,4-Cl_2	8.445 (1H, s, H3′, triazole), 8.002 (1H, s, H5′, triazole), 7.585–7.223 (3H, m, H3″, H5″ and H6″, aryl), 6.210 (1H, d, J = 7 Hz, C*H*-Tr), 5.051 (1H, d, J = 7 Hz, C*H*-OH), 4.194 (5H, s, metallocene), 4.237 (2H, d, J = 45 Hz, metallocene), 3.870 (2H, d, J = 69 Hz, metallocene)	458
4c	2,5-Cl_2	8.469 (1H, s, H3′, triazole), 8.016 (1H, s, H5′, triazole), 7.466–7.084 (3H, m, H3″, H4″ and H6″, aryl), 6.315 (1H, d, J = 7 Hz, C*H*-Tr), 5.049 (1H, d, J = 7 Hz, C*H*-OH), 4.197 (5H, s, metallocene), 4.150 (2H, d, J = 35 Hz, metallocene), 3.850 (2H, d, J = 75 Hz, metallocene)	458
4d	2,4,5-Cl_3	8.862 (1H, s, H3′, triazole), 8.029 (1H, s, H5′, triazole), 7.792 (1H, s, H3″, aryl), 7.497 (1H, s, H6″, aryl), 6.292 (1H, d, J = 7 Hz, C*H*-Tr), 5.058 (1H, d, J = 7 Hz, C*H*-OH), 4.199 (5H, s, metallocene), 4.358–4.093 (4H, m, metallocene)	493
4e	2,4,6-Cl_3	8.596 (1H, s, H3′, triazole), 7.947 (1H, s, H5′, triazole), 7.632 (2H, s, H3″ and H5″, aryl), 6.063 (1H, d, J = 7 Hz, C*H*-Tr), 5.326 (1H, d, J = 7 Hz, C*H*-OH), 4.215 (5H, s, metallocene), 4.086–4.014 (4H, m, metallocene)	493
4f	4-NO_2	8.892 (1H, s, H3′, triazole), 8.048 (1H, s, H5′, triazole), 8.149 (2H, d, J = 9 Hz, H3″, H5″, aryl), 7.220 (2H, d, J = 9 Hz, H2″, H6″, aryl), 6.358 (1H, d, J = 7 Hz, C*H*-Tr), 5.045 (1H, d, J = 7 Hz, C*H*-OH), 4.346 (2H, s, metallocene), 4.211 (5H, s, metallocene), 4.207 (1H, s, metallocene), 4.108 (1H, s, metallocene)	434
4g	1-Naphthyl	8.840 (1H, s, H3′, triazole), 8.002 (1H, s, H5′, triazole), 8.161–6.983 (7H, m, H2″, H3″, H4″, H5″, H6″, H7″, H8″, aryl), 6.286 (1H, d, J = 7 Hz, C*H*-Tr), 5.172 (1H, d, J = 7 Hz, C*H*-OH), 4.428 (2H, s, metallocene), 4.280 (1H, s, metallocene), 4.237 (5H, s, metallocene), 4.120 (1H, s, metallocene)	439
4h	2-Naphthyl	8.908 (1H, s, H3′, triazole), 8.015 (1H, s, H5′, triazole), 7.866–7.158 (7H, m, H1″, H3″, H4″, H5″, H6″, H7″, H8″, aryl), 6.278 (1H, d, J = 7 Hz, C*H*-Tr), 5.060 (1H, d, J = 7 Hz, C*H*-OH), 4.383 (2H, s, metallocene), 4.261 (1H, s, metallocene), 4.221 (5H, s, metallocene), 4.111 (1H, s, metallocene)	439
4i	3-Me	8.822 (1H, s, H3′, triazole), 8.009 (1H, s, H5′, triazole), 7.131–6.733 (4H, m, H2″, H4″, H5″ and H6″, aryl), 6.074 (1H, d, J = 7 Hz, C*H*-Tr), 4.969 (1H, d, J = 7 Hz, C*H*-OH), 4.324 (2H, s, metallocene), 4.206 (5H, s, metallocene), 4.175 (1H, s, metallocene), 4.113 (1H, s, metallocene), 2.207 (3H, s, methyl)	403
4j	H	8.831 (1H, s, H3′, triazole), 8.013 (1H, s, H5′, triazole), 7.269–6.938 (4H, m, H2″, H4″, H5″ and H6″, aryl), 6.098 (1H, d, J = 7 Hz, C*H*-Tr), 4.987 (1H, d, J = 7 Hz, C*H*-OH), 4.335 (2H, s, metallocene), 4.206 (5H, s, metallocene), 4.176 (1H, s, metallocene), 4.114 (1H, s, metallocene)	389

Biology

Fungicidal activity

The antifungal activity and plant growth regulatory activity of compounds **3** have been reported in our prior research paper.[24] Biological evaluation results of compounds **4** were listed in the Table 3.

In our previous studies, we have confirmed that a linear linkage between the triazole ring and substituted benzene ring via no more than two single or double bond(s) is essential for their fungicidal activity.[28,29] The replace of *tert*-butyl moiety by the ferrocenyl group should not influence their biological activity because of the stability of the above-mentioned functional group after structure modification. To our disappointment, however, all the tested compounds were inactive against all selected mycelium as the same as their ketone precursors.[24] It is well known that the antifungal activities of the triazole derivatives are related to their interference with steroid biosynthesis and fungal cell-wall formation mediated by ferrous cytochrome-P450 enzymes.[14] It was hypothesized that binding of the triazole to ferrous of cytochrome-P450 enzymes was replaced by binding of the triazole to the ferrous atom of the ferrocene group; as a result, the antifungal activities of the title compounds **4a–j** was retarded.

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Table 3. Biological evaluation data of compounds **4**

Compound	Fungicidal activities (relative inhibitory ratio, %)[a]					Plant growth regulatory activity (%)[b,c]
	I. clavispora	*B. lactucae*	*C. fulvum*	*E. graminis*	*A. mali*	
4a	33.3	0	20.0	16.7	0	+118.9
4b	30.0	0	0	0	0	+43.7
4c	20.0	0	16.7	0	0	+135.2
4d	33.3	16.7	33.3	0	10.0	+200.6
4e	16.7	0	0	0	0	+79.7
4f	25.0	0	0	0	0	+266.3
4g	16.7	0	0	0	0	+93.9
4h	30.0	16.7	0	16.7	33.3	+119.8
4i	10.0	0	0	0	0	+279.3
4j	0	0	0	0	0	+309.4
Triadimefon	65.0	45.9	89.9	65.0	90.0	+106.8
Triadimenol	75.0	65.6	85.0	88.0	95.0	+205.2

[a] Fungicidal activities of all compounds were tested at a concentration of 500 mg/ml.
[b] Plant growth regulatory activity of all compounds was tested at a concentration of 10 mg/l and showed various promoting activity.
[c] Relative ratio of promoting cucumber cotyledon rhizogenesis.

Plant growth regulatory activity

Plant growth regulatory activities of new ferrocene-triadimenol derivatives were tested using cucumber cotyledon rhizogenesis method (see Experimental section). The plant growth regulatory activity data for compounds **4a–j** are also shown in Table 3.

Surprisingly, although these novel triadimenol analogues did not exhibit any antifungal activities against various fungi, the screening data showed that all of them had excellent plant growth regulatory activity. Compared with their ketone precursors **3**, these triadimenol analogues **4** showed higher plant growth regulatory activity.[24] The target compounds **4a–j** obvious promoted cotyledon rhizogenesis of cucumber seed at a concentration of 10 mg/l. Except for compounds **4b, 4e** and **4g**, the rest ferrocene analogues had a higher plant growth regulatory activity than Triadimefon. In comparison with parent triadimenol, the ferrocene analogue **4d** had a comparable activity while compounds **4f, 4i** and **4j** displayed higher activity. To verify the plant growth regulatory activity of these ferrocene-modified analogues, further biological evaluations such as wheat gemmale elongation experiments were carried and the results will be reported in the future.

With respect to ineffective antifungal activity and promising plant growth regulatory activity of these ferrocene-triadimenol derivatives, it could be inferred that these triazole derivatives might share a different action mechanism between fungicidal activity and plant growth regulator activity. However, to prove this possibility, further chemical and biological investigation is required to clarify the challenging plant growth regulatory activity of these novel ferrocene-triadimenol analogues.

CONCLUSION

In summary, we synthesized a series of ferrocene analogues of a commercial antifungal agent, triadimenol. Biological screening data revealed that these novel organometallic derivatives lost the antifungal activities of the parent compound, while exhibiting unexpectedly promising plant growth regulatory activity. Further structural modification to restore their fungicidal activity will be the next challenge.

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