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Synthesis and Fungicidal Activity of Chiral *N*-(S,S)-3,5-Bis-(1-methoxyethyl)-1,2,4-triazol-4-yl]arylimines and 4-(*S*- or *R*-α-substituted phenyl-p-methylbenzylamino)-(S,S)-3,5-bis(1-meth-oxyethyl)-1,2,4-triazole Derivatives

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ABSTRACT

A series of chiral *N*-[(*S,S*)-3,5- bis (1- methoxyethyl) - 1,2,4 – triazol -4-yl] arylimines and 4-(*S*- or *R*-α-substituted phenyl-p-methylbenzylamino) - (*S,S*) - 3,5 – bis - (1-methoxyethyl) -1,2,4 -triazole derivatives was synthesized and their fungicidal activity against two soil born fungi; *Rhizoctonia solani, Fusarium oxysporium,* and two air born fungi; *Diplodia sp.,* and *Aternaria alternata,* was investigated under laboratory conditions using the poison food technique. Some of these compounds showed very promising fungicidal activity.

Keywords: chiral 1,2,4-triazole, Fungicidal activity, *Rhizoctonia solani, Fusarium oxysporium, Diplodia sp., Alternaria alternata,*

INTRODUCTION

1,2,4-Triazoles have achieved potential importance as substructure of numerous biologically active agents such as fungicides (Popkov *et al,* 1997 and Gotoda *et al.* 1998), insecticides (Bosch *et al,* 1980), herbicides (Polya, 1984), or molluscicides (El-Zemity and Radwan 1999 a; El-

Zemity and Radwan 1999 b) and hundred of patents have revealed the extraordinarily high potency of these compounds. Many of these compounds which are used in agrochemicals contain chiral centers and are produced, and used as racemic mixtures. Consequently, a lot of environmental and economic problems are encountered. For example, half the product may be useless, while costs have been incurred in its production, besides, it may posses a load on the system to which it is applied and may react with a different cellular receptor to cause an unwanted, and potentially damaging, side-effect (Williams, 1996).

Having the above aspects in mind, our attention has been drawn towards using the known *C2*-symmetric chiral (*S,S*)-4-amino-3,5-bis (1-hydroxyethyl) - 1,2,4 - triazole (Martinez - Diaz , 1994) after the successful utilization of some symmetrical 3,5-disubstituted 1,2,4-triazoles in agriculture (Inaba *et al* 1990) has been reported. Thus, we have synthesized a series of Chiral 4-(*S*- or *R*-α-substituted phenyl-p-methylbenzylamino) - (*S,S*) - 3,5 – Bis - (1-methoxyethyl) - 1,2,4 - triazole according to our recently reported and versatile procedure (Katritzy *et al*, 1996) in order to evaluate their possible fungicidal activity under laboratory conditions by poison food technique, discussing aspects of the relationship between the chemical structure and the fungicidal activity.

MATERIALS AND METHODS

1. General experimental procedures :

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR Gemini 200 MHz spectrometer; J values are given in Hz. Toluene was predried and freshly distilled from sodium and benzophenone. DMF was dried over molecular sieves. Column chromatography was carried out on MCB silica gel (120-230 mesh).

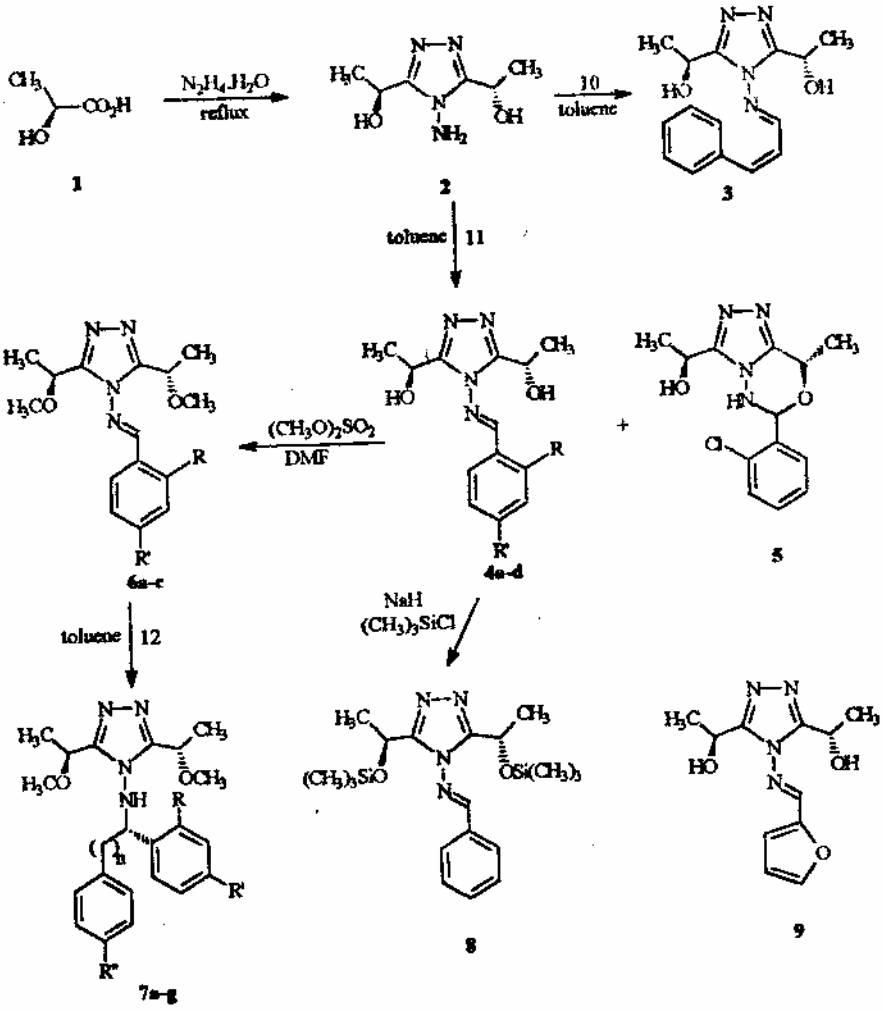
2. SYNTHESIS :

The sequence of the reaction leading to the synthesis of N-[(S,S)-3,5-bis- (1-methoxyethyl)-1,2,4-triazol-4-yl]arylimines followed by the protection of the hydroxyl groups and then, reaction of the protected imine 6 with Grignard reagent is outlined in Fig 1. The entiomerically pure (*S,S*)-4-amino-3,5-bis(1-hydroxyethyl)-1,2,4-triazole was prepared according to the procedure of Martinez-Diaz *et al.* from (*S*)-lactic acid (1) in good yield (75-80%). Chiral 4-amino-1,2,4-triazole **(2)** was then reacted with the corresponding benzaldehyde **10** or **11** in the presence of a catalytic amount of *p*-toluenesulphonic acid in refluxing toluene, to afford the highly crystalline Schiff bases 3, **4a-f** and **9** as single geometrical isomers in excellent yields (90-98%).

Protection of the free hydroxyl groups was successfully accomplished by reacting the hydrazones **4** with sodium hydride and dimethyl sulphate using DMF as a solvent, to generate the corresponding protected chiral hydrazones **6** in excellent yields 88-98% (Fig 1). Treatment of the protected hydrazones **6a-c** with various aryl alkyl Grignard reagents (3-4 equivalents) at –78 °C, using toluene as the solvent, afforded the resulting amines **7a-g** in 60-76% yields with diastereoselectivities ranging from 70% to ≥ 99%.

4-Amino-3,5-bis(1-hydroxyethyl)-1,2,4-triazole 2.,This compound was prepared according to the literature procedure described by Martinez-Diaz *et al.* (m.p. 130-132 C; lit m.p. 130-132 °C).

General procedure for the condensation of aromatic aldehydes with (S,S)-4-amio-3,5-bis(1-hydroxyethyl)-1,2,4-triazole: synthesis of compounds **3**, **4a-f** and 9. (*S,S*)-4-Amino-3,5-bis(1-hydroxyethyl)-1,2,4-triazole 2 (4.3 g, 25 mmol), the corresponding aromatic aldehyde (25 mmol) and a catalytic amount of *p*-toluenesulfonic acid (30 mg) were heated in toluene (25 ml) under reflux for 7 h. The solvent was removed under reduced pressure to give a solid yield which was recrystallized from



Reagents: 10 $C_6H_5(CH)_2CHO$, 11 a) C_6H_5CHO, b) 4-$CH_3C_6H_4CHO$, c) 4-ClC_6H_4CHO,
d) 2,4-$Cl_2C_6H_3CHO$, 12 a) 4-$CH_3C_6H_4MgBr$, b) C_6H_5MgBr, c) $C_6H_5CH_2MgBr$,
d) $C_6H_5(CH_2)_2MgBr$

Figure 1. Overall synthetic routes to chiral 4-(*S*- or *R*-α -substituted phenyl-*p*-meth benzylamino)-(*S*,*S*)-3,5-bis-(1-methoxyethyl)-1,2,4-triazole derivatives.

ethanol.

Synthesis of compound **5**. This compound was obtained by cyclization reaction during the preparation of compound **4b**. This procedure gave compound **4b** in 60% yield and compound **5** in 30% yield. The solid product was separated by column chromatography using hexane + ethyl acetate (2 + 5).

General procedure for the protection of the hydroxyl groups in compounds **4**: synthesis of compounds **6a-c** and **8**. To a well stirred suspension of 95% sodium hydride (1.6 g, 60 ml, 24 equiv.) in dry DMF (50 ml) under argon, hydrazine **4** (25 ml) was added. The reaction mixture was stirred at rt. for 10 min. before adding dimethyl sulfate (chlorotrimethylsilaine in the case of compound **8**, 2.4 equiv.) and stirring the reaction mixture at rt. for 5 h. This was then quenched with water (50 ml) and extracted with ethyl acetate (3 × 30 ml) to give compounds **6** and **8** in (88-98%) yield.

General procedure for the reaction of the protected imines **6** *with Gringard reagents: synthesis of compounds* **7a-g** ,The protected imine **4** (3 ml/mmol) was dissolved in toluene (30-50) ml/mmol) and cooled to -78 °C. At this temperature 3-5 equiv. of Grignard reagent in THF (1 ml/ mmol) were added dropwise to the solution at a rate of 20 ml/h. The reaction mixture was then stirred for 1 h at -78 °C before being allowed to warm to room temperature overnight. This was then quenched with water (50 ml) and resulting emulsion was extracted with ethyl acetate (3 × 30 ml). The organic extracts were then dried over anhydrous $MgSO_4$, filtered and evaporated to dryness to give the pure products.

Fungicidal activity:

<u>Test fungi :</u>

The plant pathogens chosen for the study are two of the soil born fungi; *Rhizoctonia solani* and *Fusarium oxysporium*, (Cause the damping [illegible] in tomato and cotton plants), and two of the air born fungi; *Alternaria alternata* (causes Alternaria spot and brown spot in potato and tomato, leaf spot in squash and apple, Also, it has a role in the postharvest diseases in orange, lemon and potato), *Diplodia sp.* (causes Diplodia spot in sweet potato and banana).

Table 1. Characteristics of Optically active 1,2,4-Triazole Derivatives

Compd	R	R'	R"	n	Yield (%)	mp (°C)
2	-	-	-	-	75-80	130-132 Li
3	-				85	188-189
4a	-	H	-	-	90	203-205
4b	Cl	H	-	-	60	160-161
4c	H	Cl	-	-	95	192-193
4d	Cl	Cl	-	-	83	149-150
4e	H	F	-	-	95	
4f	OH	H	-	-	86	
5	Cl	-	-	-	30	171-172
6a	-	H	-	-	97	57-58
6b	-	CH_3	-	-	88	57-58
6c	-	Cl	-	-	98	73-74
7a	-	H	CH_3	0	67	183-185
7b	-	CH_3	H	0	76	134-135
7c	-	Cl	CH_3	0	70	161-162
7d	-	H	H	1	71	151-153
7e	-	Cl	H	1	69	146-147
7f	-	H	H	2	65	154-155
7g	-	Cl	H	2	60	150-151
8	-	-	-	-	94	Oil
9	-	-	-	-	90	

Test chemicals :

Stock solutions of each compound, including thiophenate-methyl as a reference, were dissolved in a measured amount of dimethyl sulfoxide (DMSO) and incorporated into the molten Czapek-Dox Agar (CDA) medium (ca. 45 °C) to give the desired concentration of the compound.

Bioassay technique :

Molten medium (6 ml) which contains the test compound was poured into each sterile Petri Plate under aseptic conditions and left to settle. Addition of dimethyl sulfoxide alone to the medium without the compound served as control. Circular blocks (5 mm diam.) of the test fungal mats from the growing tips (punched in fungal mat grown on CDA medium in sterile Petri plates) were placed in the centre of the settled medium and left to grow. The entire procedure was done under aseptic conditions. Radial growth in terms of diameter (mm) was noted at different intervals till the complete growth of the control. In all cases, five replicates were maintained and the entire exercise was repeated three times.

RESULTS AND DISCUSSION

The fungicidal activities of the aforementioned synthesized chiral 1,2,4-triazole derivatives were determined against four economic plant pathogens, two air born fungi; *A. alternata* and *Diplodia sp* and two soil born fungi; *R. solani, F. oxysporium* using the radial growth technique and are expressed as ED_{50} (Tables 2 and 3), compared with thiophenate-methyl as a standard.

The fungicidal activity of these compounds against *A. alternata* and *Diplodia sp* are shown in Table 2. The results show that the activity depends on the linked group to chiral 1,2,4-triazole moiety. Therefore, Compound **2** showed low fungicidal activity against the two tested fungi.

Table:2 Fungicidal Activity of N-[(S,S)-3,5-Bis-(1-methoxyethyl)-1,2,4-triazol-4-yl]aryl imines derivatives against two air born fungi

Compd	ED_{50} (μg ml^{-1}) (± *SE*)	
	Diplodia sp.	*Aternaria alternata*
2	> 1000	> 1000
3	504.69 (1.51± 0.031)	> 1000
4a	> 1000	> 1000
4b	172.53 (3.09 ± 0.061)	326.84 (1.91± 0.033)
4c	291.98 (2.34 ± 0.037)	381.18 (2.44 ± 0.041)
4d	069.22 (6.99 ± 1.020)	103.84 (2.20 ± 0.073)
4e	715.39 (3.49 ± 0.120)	> 1000
4f	987.46 (9.62 ± 1.940)	> 1000
5	057.57 (4.03 ± 1.490)	173.18 (1.56 ± 0.030)
6a	182.75 (1.53 ± 0.030)	957.82 (1.60 ± 0.042)
6b	169.67 (2.23 ± 0.039)	551.76 (0.97 ± 0.027)
6c	225.02 (1.77 ± 0.031)	823.16 (1.40 ± 0.035)
7a	> 1000	> 1000
7b	353.58 (2.56 ± 0.042)	368.87 (1.38 ± 0.029)
7c	235.19 (0.82 ± 0.026)	280.06 (0.53 ± 0.025)
7d	> 1000	> 1000
7e	215.34 (0.45 ± 0.025)	374.19 (0.76 ± 0.026)
7f	360.72 (0.90 ± 0.026)	> 1000
7g	463.97 (0.35 ± 0.025)	> 1000
8	> 1000	> 1000
9	> 1000	> 1000
Thiophenate-methyl	267.06 (0.8 ± 0.15)	466.43 (0.51 ± 0.23)

Table:3 Fungicidal Activity of N-[(S,S)-3,5-Bis-(1-methoxyethyl)-1,2,4-triazol-4-yl]aryl imines derivatives against two soil born fungi

Compd	ED_{50} ($\mu g\ ml^{-1}$) ($\pm$ *SE*)	
	Rhizoctonia solani	*Fusarium oxysporium*
2	> 1000	> 1000
3	> 1000	> 1000
4a	> 1000	> 1000
4b	756.29 (2.32 ± 0.059)	970.76 (1.02 ± 0.030)
4c	586.24 (2.05 ± 0.042)	310.57 (7.85 ± 0.59)
4d	103.03 (4.39 ± 0.38)	077.86 (4.33 ± 0.22)
4e	> 1000	> 1000
4f	> 1000	> 1000
5	458.30 (3.12 ± 0.063)	352.43 (0.90 ± 0.026)
6a	> 1000	> 1000
6b	205.68 (1.11 ± 0.027)	812.46 (0.99 ± 0.029)
6c	> 1000	> 1000
7a	062.84 (0.55 ± 0.026)	> 1000
7b	062.38 (0.83 ± 0.028)	343.76 (1.13 ± 0.027)
7c	273.79 (2.48 ± 0.039)	> 1000
7d	556.36 (1.27 ± 0.030)	> 1000
7e	210.89 (0.48 ± 0.025)	914.35 (0.81 ± 0.028)
7f	> 1000	> 1000
7g	> 1000	> 1000
8	136.47 (0.78 ± 0.026)	> 1000
9	> 1000	> 1000
Thiophenate-methyl	3.71 (10.44 ± 0.87)	4.31 (3.29 ± 0.08)

The ED_{50} of cinnamaldehyde against *A. alternata* and *Diplodia sp* was 70 and 18.5 µg ml.$^{-1}$ These activity patterns were encouraging to synthesize the new chiral imine **3** by the condensation of cinnamaldehyde with compound **2** in order to form a potent compound. Unfortunately, compound **3** was as inactive as compound **2** and **4a** compared to cinnamaldehyde. These results indicate that the exerted activity of cinnamaldehyde may be due to the free aldehyde group and therefore any further improvement of the activity has to be on the double bond or the aromatic ring.

Inserting chlorine into the structure as shown in compounds **4b-c** enhanced the fungicidal activity. The substitution of chlorine in *o*-position lead to a better fungicidal activity than *p*-position (**4b** versus **4c**). The results attracted our attention to synthesize the new imine **4d** which is *o,p*-dichloro-substitution. Surprisingly, the fungicidal activity of compound **4d** was very promising and gave 100 % inhibition at 100 ppm. During the reaction leading to compound **4b** formation , cyclization reaction has taken place and compound **5** was formed which showed higher fungicidal activity than **4b**.

Converting the free hydroxyl groups in compound **4a** and **4c** into methoxy groups as in compounds **6a** and **6b** respectively, lead to an increase in the fungicidal activity against the air born fungi. However, converting the hydroxyl groups with trimethyl silane (**4a** versus **8**) did not affect the activity against *Diplodia sp* and *Alternaria alternata*. Compounds of type **7** showed lower fungicidal activity against the two tested fungi

The fungicidal activity of the synthesized compounds against the two soil born fungi, *R. solani* and *F. oxysporium* are shown in Table 3. The activity of the unalkylated 1,2,4-triazole compound **2** as well as the alkylated compounds **3** and **4a** was very low. The activity was slightly improved by adding chlorine as in compounds **4b-d** which was optimum in compound **4d**. Substitution with hydroxyl group or fluorine was inactive against the two tested fungi. The cyclized compound **5** was much active than its mimic **4b** as it was against the air born fungi, *Diplodia sp.* and *A. alternata*. Compounds of type **6** were inactive except in compound **6b**.

Compounds of type **7** were more active than their protected imines **6** and the unprotected compounds **4**. The fungicidal activity of compound **7a** reached the maximum. Interestingly, the structurally analogue **7b** exerted higher fungicidal activity against the two tested fungi. Moreover, adding chlorine into compound **7a** as represented by compound **7c** improved the fungicidal activity. Lengthening the carbon chain by adding a methylene group in compound **7d** (n = 1) did not enhance the fungitoxicity. However, the introduction of chlorine into the structure (**7d** versus **7e**) highly increased the fungicidal activity. Furthermore, adding more carbon into the structure as in compounds 7d versus **7f** and **7e** versus **7g** reduced the fungicidal activity against all the tested fungi.

In conclusion: Introducing chlorine into the chemical structure has lead to a high increase in the fungicidal activity as a toxiphoric moiety. This was observed with the most potent compound in this chiral series **4d** which showed very high fungicidal activity against the four tested fungi. Moreover, compounds **7a** and **7b** exerted promising fungicidal activity against *Rhizoctonia solani*. However, lengthening the hydrocarbon chain with one or two methylene group as in compounds **7d-g** reduced the fungicidal activity. This might suggest that the relatively more polar derivatives with lower molecular weight favour fungitoxicity.

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الملخص العربى

تخليق سلسلة من مشتقات ن-(اس،اس) ثنائى (١-ميثوكسى ايثيل)-١و٢و٤-ثلاثى الازول-٤-يل] اريل امين و ٤-(اس او ار الفا مستبدل فينيل بارا ميثيل بنزيل امينو)-(اس،اس)٣،٥ -ثنائى (١-ميثوكسى)-١و٢و٤-ثلاثى الازول النشطة ضوئيا

سعد رشاد الزميتى، سعاد محمد احمد و نبيلة سعيد بكرى

قسم كيمياءالمبيدات - كلية الزراعة (الشاطبى) - جامعة الاسكندرية

تم تخليق سلسلة من مشتقات ان-(اس،اس) ثنائى (١-ميثوكسى ايثيل)-١و٢و٤-ثلاثى الازول-٤-يل] اريل امين و ٤-(اس او ار الفا مستبدل فينيل بارا ميثيل بنزيل امينو)-(اس،اس)٣ ،٥ -ثنائى (١-ميثوكسى)-١و٢و٤-ثلاثى الازول النشطة ضوئيا واختبار تأثيرها الأبادى الفطرى على اربعة اجناس مختلفة من الفطريات منهما اثنين من فطريات التربة (ريزوكتونيا سولانى، فيوزاريم اكسىسبورم) و اثنين من الفطريات الهوائية (الديبلوديا، ألترناريا الترناتا) وذلك تحت الظروف المعملية باستخدام تجارب الطعام المسمم. وقد اظهرت بعض هذه المركبات نشاطا فطريا جيدا.