Research Journal of Agriculture and Biological Sciences, 2(6): 380-383, 2006

Fungicidal and Bactericidal Properties of Chiral *N*-[(S,S)-3,5-bis(1-methoxyethyl) -1,2,4-triazol-4-yl]arylimines

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Abstract: Some chiral 1,2,4-triazole derivatives were prepared and evaluated against phytopathogenic fungi and bacteria. (R)-configuration (compound 5b) has shown better fungicidal activity against the tested fungi than (*S*)-configuration (compound 5a). The presence of chlorine atom as in compound 5c and 5e improved their fungicidal activity. Lengthen the carbon chain (n = 1 and/ or 2) reduced the fungicidal activity as in compound 5d and 5f. The bactericidal activity of the new chiral 1,2,4-triazoles was moderate against all the tested bacteria. Compound 5b showed reasonable effect where the ED_{50} values were 250, 370 and 380 against *E. caratovera sub caratovera*, *E. amylovora* and *E. caratovera sub atroseptica*, respectively.

Key words: Chiral 1,2,4-triazole derivatives, fungicidal activity, bactericidal activity, structure-activity relationship, plant pathogenic fungi, plant pathogenic bacteria

INTRODUCTION

Among the large number of heterocyclic compounds that have received the most attention during the last two decades as potential antimicrobial agents, substituted-1,2,4-triazoles. They are associated with diverse biological activities such as, fungicidal, antimicrobial and antiviral activity[13,5,3,9,12]. However, 1,2,4-triazole derivatives are usually used as racemic mixture in pharmaceuticals and / or agrochemicals but only one of the chiral pairs is the bioactive isomer which is selectively engaged in biochemical interactions with receptors, differentiating it from the other isomer. Therefore, several reviews and papers focus on the perspective of the optically active materials in agrochemicals[4,14,7].

The economic manufacture of a single pure isomer depends upon availability of the appropriate building blocks and the reaction consequences. It is certain that the full panoply including the revenue side (expensive intermediates) and the capital side (more complex plant) of these has not yet been defined and / or economically optimized. Still the impact of chirality is less clear on fungicidal activity with 1,2,4-triazole fungicides than with the pyrethroid insecticides or phenoxy herbicide classes. For example, cyproconazole and diniconazole are implied to have differential activity between their isomers by the fact that each of them has CAS registration number.

Having the above aspects in mind, and as a part of our program in pursuit of novel biological molecules containing chiral 1H-1,2,4-triazole moiety, our attention has been drawn toward using (*S,S*)-4-amino-3,5-bis(1-hydroxyethyl)-1,2,4-triazole (2)[1,10] as a building block and chiral auxiliary for improving the antimicrobial potential of 1,2,4-triazole derivatives. We have synthesized some of chiral 1,2,4-triazoles according to our previously published procedure[8] and evaluated against phytopathogenic fungi and bacteria.

MATERIALS AND METHODS

Synthesis: *Preparation of (S,S)-4-amino-3,5-bis(1-hydroxyethyl)-1,2,4-triazole 2*

It was prepared according to the procedure described by Alonso *et al*.[1].

Condensation of Aromatic Aldehydes with (S,S)-4-amino-3-5-bis(1-hydroxyethyl)-1,2,4-triazole (3a-c): (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 hrs. The solvent was removed under reduced pressure to give a solid which was recrystallized from ethanol.

Protection of the Hydroxyl Groups in Compounds 4a-c: To a well stirred suspension of 95% sodium hydride (1.6 g, 60 mmol, 2.4 equiv.) in dry dimethylformamide (50 ml) under argon was added hydrazone 3 (25 mmol). The reaction mixture was stirred at R.T. for 10 min. before adding methyl tosylate (11.16 g, 60 mmol, 2.4 equiv.) and stirring the reaction mixture at R.T. for

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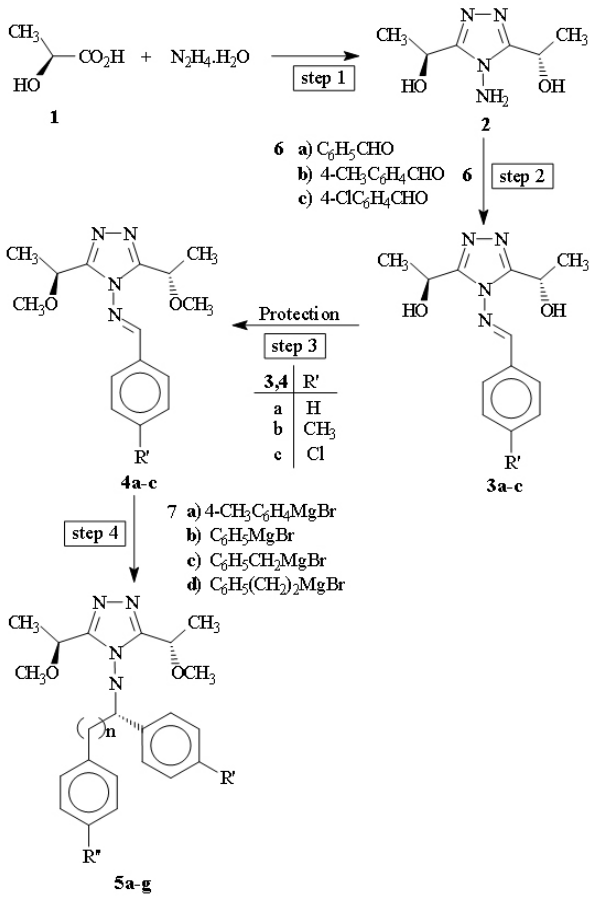

Fig. 1: Overall synthetic routes to Chiral N-[(S,S)-3,5 (1-methoxyethyl)-1,2,4-trizol-4-yl]ary limines

5 hrs. This was then quenched with water (50 ml) and extracted with ethyl acetate (3 x 30 ml). The solvent was removed under reduced pressure to give the desired compounds 4a-c in 88-98 % yield (Fig. 1).

Reaction of the Protected Imines 4 with Grignard Reagents (5a-g): The protected imines 4 (3 ml/mmol) was dissolved in toluene (30-50 ml/mmol) and cooled to -78 ºC. 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/hrs. The reaction mixture was then stirred for 1 hrs at – 78 ºC before being allowed to warm to room temperature overnight. This was then quenched with water (50 ml) and the resulting emulsion was extracted with ethyl acetate (3 x 30 ml). The organic extracts were then dried over anhydrous magnesium sulfate, filtered and evaporated to dryness to give the pure products 5a-g. The sequence of the reactions leading to the target compounds 5a-g in this study is outlined in Fig.1. The yields and melting points of the compounds 5a-g are given in Table 1.

Table 1: Charateristics of chiral N-[(S,S)-3,5-Bis(1-methoxy-ethyl)-1,2,4-triazol-4-yl]arylimines.

Compd	n	R'	R"	Yield (%)	m.p. (ºC)
5a	0	H	CH_3	67	183-185
5b	0	CH_3	H	76	134-135
5c	0	Cl	CH_3	70	161-162
5d	1	H	H	71	151-153
5e	1	Cl	H	69	146-147
5f	2	H	H	65	154-155
5g	2	Cl	H	60	150-151

Bioassay:

Test Fungi: The five plant pathogenic fungi: *Botrydiplodia spp, Alternaria tennis, Heleminthosporium turicum*, *Fusarium oxysporium* and *Fusarium moniliform* were used in this study. Cultures of test fungi were provided by Plant Pathology Department, Faculty of Agriculture, Alexandria University and maintained during the course of experiments on Czapic Dox Agar (CDA) medium at 28±1ºC.

Preparation of Stock Solutions: Stock solutions of each compound were dissolved in a measured amount of dimethyl sulfoxide (DMSO) and incorporated into the molten Czapek-Dox Agar medium (ca. 45 ºC) to give the desired concentrations (i.e. 100, 200, 400, 600 and 800 mg / ml).

Measurement of Antifungal Activity: The radial growth method of Zambonelli *et al*.,[16]. was used for the evaluation. An appropriate volume from the stock solutions of the synthesized compounds was dissolved in dimethyl sulfoxide (DMSO) and added to the molten medium (CDA: 15 ml) to obtain seven different concentrations, ranged from 50 to 1000 ug ml $^{-1}$ for each compound. The fungal media which contains the test compound was poured into each sterile Petri dish (90 mm diameter) at 40-45 ºC under aseptic conditions and left to settle. Addition of DMSO alone to the medium was served as control. Mycelial discs (5 mm diameter) of the plant pathogenic fungi, 8-day-old were transferred aseptically to the center of Petri dishes after solidification of the medium. The treatments were incubated at 28±1C. The antifungal activity was determined by measured the radial growth in terms of diameter (mm) in all treatments at different intervals till the end of experiment (for the control reach to full growth). Fungitoxicity was expressed as ED_{50} values (mg ml $^{-1}$) and was determined by the probit analysis method of Finney[6]. Five replicates for each treatment were maintained and the entire exercise was repeated three times

Bacterial Strains and Media: Three phytopathogenic bacteria: *Erwinia amylovora*, *E. carotovora* sub sp. *carotovora, E. carotovora* sub sp. *atroseptica* were provided by the Department of Plant Pathology, Faculty

of Agriculture, University of Alexandria, Egypt. The bacterial strains were cultured in glycerol agar medium at 28±1ºC. The medium contained peptone (5 g), beef extract (3 g), glycerol (20 ml) and agar (15 g) in distilled water up to 1 liter.

Preparation of Stock Solutions: Stock solutions of each tested compound were initially dissolved in DMSO to enhance the solubility, then serially diluted further with distilled water to achieve the desired concentration (100, 200, 400, 600 and 800 mg/ml). The final DMSO concentration in the experiments never exceeded 0.5 % (v/v), and an equal amount was added to the control.

Measurement of Bacterial Growth Inhibition: The *in vitro* measurement of growth inhibition was carried out according to the method of Staskawicz and Panopoulos[11]. One ml bacterial suspension (48 hours old), yielding an approximate inoculum size of 10^8 colony forming units [CFU]/ml was inoculated into 14 ml of glycerol agar medium in a glass Petri dish (90 mm diameter). After the layer of agar and bacterial suspension has been solidified, three hols of 10 mm diameter for each are punched into each plate by a flamed corkborer. The agar was sucked out of the holes by a glass tube connected to an aspirator. Each hole was filled with 50 µl from each concentration (tested compound). Each treatment was replicated three times and the experiment was repeated twice. The Petri dishes were allowed to pre-diffuse for 5 hrs at 4 ºC followed by incubation for 48 hrs at 28 ±1 ºC. Inhibition zone (mm) were determined by measuring the clear zone of growth inhibition on a surface around the holes. Two negative controls, water and DMSO, were performed and did not display any inhibitory effect on the growth of bacterial cultures.

RESULTS AND DISCUSSIONS

The results of chiral N-(S,S)-3,5-bis (1-methoxyethyl)-1,2,4- triazol-4-ylimines 5a-g against the tested fungi are presented in Table 2. Compound 5a which is supposed to be (S) - configuration (R_1 = H, R_2 = CH_3, and n = 0) has shown moderate fungicidal activity. However, the chiral 1,2,4-triazole derivative 5c showed better fungitoxic action than that of compound 5a. The reasonable activity obtained may be due to the presence of chlorine atom as in compound 5c where R_1 = Cl and R_2 = CH_3 which always shows notable activity as reported in the literature. Changing the alkyl group position as in compound 5b (R1 = CH_3, R_2= H and n = 0) which is presumably (*R*)-configuration affected positively on the fungicidal activity in comparison to (*S*)-configuration (5a).

Table 2: Fungicidal activity of chiral N-[(*S,S*)-3,5-bis(1-methoxyl)]-1,2,4-triazol-4-yl]arylimines.

				ED_{50} (µg ml $^{-1}$)				
Entry	R_1	R_2	N	BSP	AT	HT	FO	FM
5a	H	CH_3	0	360	360	590	520	560
5b	CH_3	H	0	300	245	180	380	260
5c	Cl	CH_3	0	350	200	260	510	260
5d	H	H	1	310	450	800	560	580
5e	Cl	H	1	490	160	640	500	590
5f	H	H	2	>800	>800	>800	>800	>800
5g	Cl	H	2	340	195	710	510	510

BSP=*Botrydiplodia spp*, AT=*Alternaria tennis*, HT=*Heleminthosporium turicum*, FO=*Fusarium oxysporium*, FM=*Fusarium moniliform*.

Table 3: Bactericidal activity of chiral N-[(S,S)-3,5-bis (1-methoxyl)]-1,2,4-triazol-4-yl]arylimines.

				ED_{50} (µg ml $^{-1}$)		
Entry	R_1	R_2	N	EA	ECA	ECC
5a	H	CH_3	0	430	440	500
5b	CH_3	H	0	370	380	250
5c	Cl	CH_3	0	520	480	480
5d	H	H	1	580	680	600
5e	Cl	H	1	470	630	580
5f	H	H	2	660	430	370
5g	Cl	H	2	480	500	450

EA=*Erwinia amylovora*, ECA=*E. caratovera sub atroseptica* ECC=*E. caratovera sub caratovera*,

Increasing the carbon chain by adding a methylene group (n = 1) reduced the fungicidal activity as in compound 5d and 5e. However, the activity turned to be somewhat better by replacing the hydrogen atom with chlorine atom as in compound 5e (R_1 = Cl). Similarly, in compound 5f, the fungitoxicity remarkably reduced by adding one more carbon to the chain (n = 12). The most important observation was the chlorine substitution which has enhanced the fungitoxicity. This could refer to an increase in the lipophilicity of such compounds[15,2]. However compound 5b which is (R)-configuration was the most important one compared to (S)-configuration in these series.

Results of the bactericidal activities of chiral N-[S,S)-3,5-bis(1-methoxyethyl)]-1,2,4-triazol-4-ylimines 5a-g against *E*. amylovora, *E. carotovera sub. atroseptica*, and *E. caratovera* are recorded in Table 3. The results showed that the bactericidal activities was in the same manner exhibiting moderate effect against all of the tested bacteria except compound 5b (R_1= CH_3, R_2= H and n =0), which showed better bactericidal activities with ED_{50} values 250, 370 and 380 on *E. caratovera sub caratovera*, *E. amylovora* and *E. carotovera sub. Atroseptica*, respectively.

It is quite important to observe that the same compound 5b has showed also remarkable fungicidal activities more than any of the rest compounds of the same group. Generally, the bactericidal pattern was

parallel to the fungicidal effect in all members of this group. The structure variety in both the heterocyclic rings and lipophilic substituents may account for their variation of the antifungal spectrum. These results may be attributed to the difference in the sensitivity between the tested fungi and bacteria.

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