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Fungicidal and Bactericidal Activity of 1-[(α-alkoxy-γ-dialkyl- amino) and (α-amido-γ-dialkylamino)propyl)-1*H*-1,2,4-triazole

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Abstract: Some compounds of 1-[(α-alkoxy-γ-dialkylamino) and (α-amido-γ-dialkylamino)propyl)-1H-1,2,4-triazoles were prepared and evaluated against certain phytopathogenic fungi and bacteria. The most promising compounds either as funigicide or bactericide were 3d and 5c. These compounds are easily prepared in one step reaction.

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

INTRODUCTION

During the latest few decades, much attention has been paid to the synthesis of 1*H*-1,2,4-triazole derivatives which possess, antibacterial, antifungal, insecticidal, herbicidal, plant growth regulatory activities and defoliants[7,2].Specifically, 3-amino-1,2,4-triazole is a widely used neutral herbicide and defoliant of cotton, although in low concentrations it may promote growth[7].1-(γ-alkoxy-β-hydroxyalkyl), 1-(γ-alkythio-β-hydroxyalkyl) and 1-(γ-dialkylamino-β-hydroxyalkyl) substituted 1,2,4-triazoles have shown good fungicidal properties[9,3].

Also, the 1*H*,1,2,4-triazole derivatives which comprise three-carbon unit linking an amino group and an ether oxygen atom also leads to high pharmacological activity for many compounds[6,5]. For this reason, we prepared 1-[(α-alkoxy-γ-dialkyl-amino) and (α-amido-γ-dialkylamino)propyl)-1*H*-1,2,4-triazole according to our previously reported procedure[4]. These compounds could have an interesting fungicidal and bactericidal properties against *Helminthosporium sp, Macrofomina phaseolina*, *Diplodia sp*, *Alternaria alternate, Fusarium oxysporum*, *Rhizoctonia solani* and *Erwinia amylovora*, *E. carotovora* sub sp. *carotovora* and *E. carotovora* sub sp. *atroseptica*. under laboratory conditions, discussing aspects of the relationship between chemical structure and antimicrobial activity.

MATERIALS AND METHODS

Synthesis: The sequence of the reactions leading to the synthesis of 1-[(α-alkoxy-γ-dialkylamino) and (α-amido-γ-dialkylamino)propyl)-1*H*-1,2,4-triazole in this study is outlined in Fig. 1. Addition of 1-(N,N-dialkylamino)methyl-1,2-4-triazoles to ethyl vinyl ether or N-vinyl-2-pyrrolidine in presence of catalytic amount of *p*-toluensulfonic acid to give the desired compounds **3** and 5 was carried out according to our previously reported procedure[4].

General experimental procedures: ^{1}H NMR were recorded on a Varian spectrometer at 200 MHz using teteramethylsilane as an internal reference in hexadeuterodimethyl sulfoxide; Melting points were measured with a kofler hot-stage apparatus and were uncorrected. High resolution mass measurements were performed on an AEL MS-30 mass spectrometer.

Preparation of 1-(N,N-dialkylamino)methyl-1,2-4-triazoles (1): Equimolar amounts of 1,2,4-triazole, formaldehyde and a secondary amine were stirred in ethanol at 20 ºC for 24 hrs. The solvent was removed under reduced pressure to afford the crude products, which were purified by recrystalization or distillation to give the desired products.

Addition of 1-(N,N-dialkylamino)methyl-1,2-4-triazoles to ethyl vinyl ether and N-vinyl-2-pyrrolidine. Preparation of compounds (3 and 5)**:** A mixture of 1-(N-pyrrolidinomethyl)-1,2,4-triazole (25 mmol), ethyl vinyl ether (2.52 g, 35 mmoles) or N-vinyl-2-pyrrolidone (3.9 g, 35 mmoles) and a catalytic amount of p-toluenesulfonic acid (10 mg) was heated in a sealed tube at 100-110 ºC for the appropriate time. The mixture was dissolved in 100 ml diethyl ether and washed with saturated sodium carbonate solution. Evaporation of the solvent gave the pure products. The yield and melting points of the compounds 3a-d and 5a-c are given in Table 1.

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Table 1: Characteristics of 1-[(α-alkoxy-γ-dialkylamino) and (α-amido-γ-dialkylamino) propyl)-1*H*-1,2,4-triazole

Compd	R_1	R_2	X	Z	Yield (%)	m.p. (ºC)
3a	H	H	C	0	95	Oil
3b	H	H	C	1	94	Oil
3c	H	H	O	1	95	Oil
3d	$^mCH_3$	$^mCH_3$	O	1	98	Oil
5a	H	H	C	0	95	Oil
5b	H	H	C	1	96	Oil
5c	H	H	O	1	98	Oil

X = Type of atom
Z = Number of carbon atoms

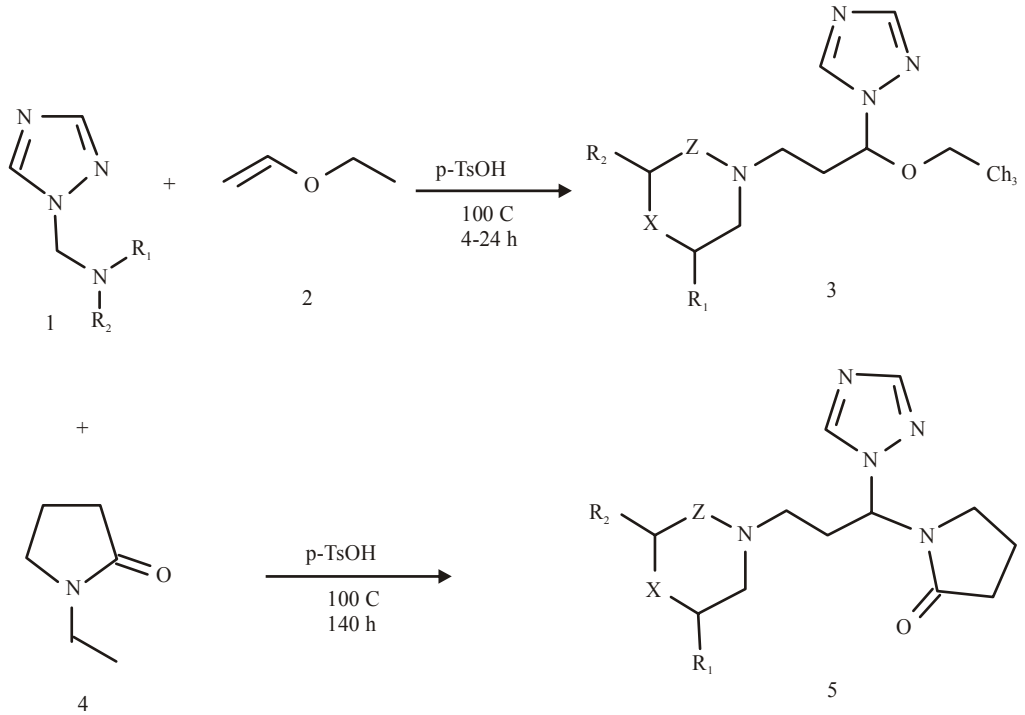

Fig 1: Overall synthesis of 1-[(α-alkoxy-γ-dialkylamino) and (α -amido- γ -dialkylamino)propyl)-1H-1,2,4-triazole

Bioassay:

Test fungi: The five plant pathogenic fungi which are chosen for the study were *Botrydiplodia spp Alternaria tennis*, *Heleminthosporium turicum*, *Fusarium oxysporium* and *F. moniliform*. The cultures of these fungi were supplied by Department of Plant Pathology, Faculty of Agriculture, Alexandria University and maintained during the course of experiments on Czapek-Dox Agar (CDA) medium at 28 ± 1 ºC.

Preparation of Stock Solutions: Stock solutions of each compound were dissolved in a measured amount of dimethyl sulfoxide (DMSO) and incorporated into the molten Czapeck-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*[10] was used for the evaluation. An appropriate volume from the stock solutions of the synthesized compounds was dissolved in DMSO and added to the molten medium (CDA: 15 ml) to obtain the desired concentrations, Five different concentrations, ranged from 100 to 800 µg/ml for each compound were prepared. 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 ± 1 ºC. The antifungal activity was determined by measured the radial growth in terms of diameter (mm) in all treatments at differed intervals till the end of experiment (for the control reach to full growth). Fungitoxicity was expressed as ED_{50} values (mg/ml) and was determined by the probit analysis method of Finney[1]. Five replicates for each treatment were maintained and the entire exercise was repeated three times.

Bacterial strains and media: Three phytopathogenic bacteria: *Erwinia amylovora*, *Erwinia carotovora* sub sp. *carotovora*, *Erwinia 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 ± 1 ºC. 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[8] . One ml bacterial suspension (48 hrs 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 holes 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

Results of compounds of 1-[(α-alkoxy-γ-dialkylamino) and (α-amido-γ-dialkylamino) propyl)-1*H*-1,2,4-triazoles against the tested fungi are shown in Table 2. Compound 3c (R_1 = R_2 = H and X = O) has shown respectful toxicity to *F. moniliform* (ED_{50} = 400 µg/ml) and little toxicity against *F. oxysporium* (ED_{50} = 720 µg/ml) but was inactive against *Botryodiplodia sp.,*

Table 2: Fungicidal activity of 1-[(α-alkoxy-γ-dialkylamino) and (α-amido-γ-dialkylamino) propyl)-1*H*-1,2,4-triazole

					ED_{50} (µg/ml)				
Compd	R_1	R_2	X	Z	BSP	AT	HT	FO	FM
3a	H	H	C	0	> 800	> 800	> 800	800	> 800
3b	H	H	C	1	> 800	> 800	> 800	> 800	760
3c	H	H	O	1	> 800	> 800	> 800	720	400
3d	$^mCH_3$	$^mCH_3$	O	1	560	510	450	560	560
5a	H	H	C	0	500	790	490	600	500
5b	H	H	C	1	> 800	> 800	> 800	760	> 800
5c	H	H	O	1	480	420	450	700	600

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

A. tennis, and *H. tursicum* (ED_{50} >800 µg/ml). Replacement of the hydrogen atom by methyl group as in compound 3d (R_1 = R_2 = CH_3 and X = O), lead to notable improve in the fungicidal activity against all tested fungi and the ED_{50} values were 560, 510, 540, 560 and 560 µg/ml on *Bntryodiplodia sp, A. tennis, H. tursicum. F. oxysporium.and* F. *moniliform* respectively.

Compounds of type 5 showed better fungicidal activity rather than of type 3. The most active compound in this series was 5c (R_1 = R_2 = H, X = O and Z =1 exhibited good fungicidal activity compared to compound 3c that has the same structure except the morpholine moiety. This effect could be attributed to the difference in the ethoxy and 2-pyrolidinone moiety.

Table 3: Bactericidal activity of 1-[(α-alkoxy-γ-dialkylamino) and (α-amido-γ-dialkylamino) propyl)-1*H*-1,2,4-triazole

					ED_{50} (µg/ml)		
Compd	R_1	R_2	X	Z	EA	ECA	ECC
3a	H	H	C	0	>800	>800	>800
3b	H	H	C	1	560	620	600
3c	H	H	O	1	520	720	690
3d	CH_3	CH_3	O	1	300	350	350
5a	H	H	C	0	510	800	710
5b	H	H	C	1	>800	>800	>800
5c	H	H	O	1	530	450	560

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

Generally, the compounds which have morpholine moiety showed good fungicidal activity to all tested fungi more than the other derivatives except compound 5a showed considerably activity.

The results of bactericidal activities of 1-[(α-alkoxy-γ-dialkylamino) and (α-amido-γ-dialkylamino)propyl)-1*H*-1,2,4-triazole on the tested bacteria; *E. amylovora, E. carotovera sub. atroseptica* and *E.caratovera sub caratovera* are recorded in Table 3. Compound 3d where R_1 and R_2 were methyl group, showed the most promising activity against all the tested bacteria with ED_{50} values of 300, 350 and 350 µg/ml against *E. amylovora, E. carotovera sub. atroseptica* and *E. caratovera sub caratovera,* respectively. However, compounds 3c ($R_1 = R_2 = H$, X = O and Z = 1), 3b ($R_1 = R_2 = H$, X = C, Z = 1) and 5c ($R_1 = R_2 = H$, X = O, Z = 1) have exhibited moderate bactericidal activity. Compounds 3a ($R_1 = R_2 = H$, X = C and Z = 0) was non toxic to the tested bacteria.

Finally, the most promising compounds against the tested fungi were compounds 3d and 5c. Importantly, both compounds (3d and 5c) found to be the most active against the tested bacteria. However, more studies are needed to improve the activity of these group of compounds through either structure modification (particularly because that these compounds are easily to prepare just in one step) or adding some synergistic agents.

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