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Biological Evaluation of Chiral Amides Synthesized from Diacetyl-L-tartaric Acid and Aromatic Amines

MAHRUKH MALIK[1], SHER WALI KHAN[1], MUHAMMAD ARFAN[2], JAVID HUSSAIN ZAIDI[1], ASGHARI BANO[3,*] and FAIZAN ULLAH[3]

[1]Department of Chemistry, Quaid-i-Azam University, Islamabad, Pakistan
[2]Department of Chemistry, COMSATS Institute of Information Technology, Abbottabad, Pakistan
[3]Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan

*Corresponding author: E-mail: banoasghari@gmail.com

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L-Tartaric acid is chiral compound and commercially available was conveniently converted into diacetyl-L-tartaric acid anhydride. Diacetyl-L-tartaric acid anhydride was then made to form half ester of diacetyl-L-tartaric acid anhydride which was then reacted with substituted anilines to yield respected chiral amides. These chiral amides were further purified and were characterized by using ^{1}H NMR. The compound E11 [methyl-2,3-diacetoxy-4-oxo-4-(2'-methoxyphenylamino)butanoate] showed greater antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*. However, maximum antifungal activity was recorded for **E3** [methyl-2, 3-diacetoxy-4-oxo-4-(4'-bromophenyl amino)butanoate]. The compounds **E2** [methyl-2,3-diacetoxy-4-oxo-4-(2'-bromophenyl amino)butanoate] and **E3** completely inhibited the germination of canola seeds, whereas, the compounds **E1** [methyl-2,3-diacetoxy-4-oxo-4-(4'-methyl phenyl amino)butanoate] and **E11** [methyl-2,3-diacetoxy-4-oxo-4-(2'-methoxyphenylamino)butanoate] caused 40 % inhibition of seed germination.

Key Words: Chiral amides, Synthesis, Antimicrobial, Phytotoxicity.

INTRODUCTION

The amide functionality is a common feature in small or complex synthetic or natural molecules. For example, it is ubiquitous in life, as transport/storage (haemoglobin), immune protection (antibodies) and mechanical support (collagen), proteins play a crucial role in virtually all biological processes such as enzymatic catalysis (nearly all known enzymes are proteins). This can be expected, since carboxamides are neutral, are stable and have both hydrogen-bond accepting and donating properties. Medicinal Chemistry database revealed that the carboxamide group appears in more than 25 % of known drugs[1].

Amides bear a variety of biological activities such as antibacterial[2,3], antifungal[4,5], antidiabetic, cytotoxic[6,7] and phytotoxic, antioxidant *etc*. Bacterial resistance to antibiotics is an increasing problem that concern clinicians, the pharmaceutical industry and chemists. The multi drug resistance bacteria are the major cause of failure in the treatment of infectious diseases. Therefore, the current investigation was to synthesize and biologically evaluate some chiral amides and study their potential as antimicrobial and phytotoxic agents.

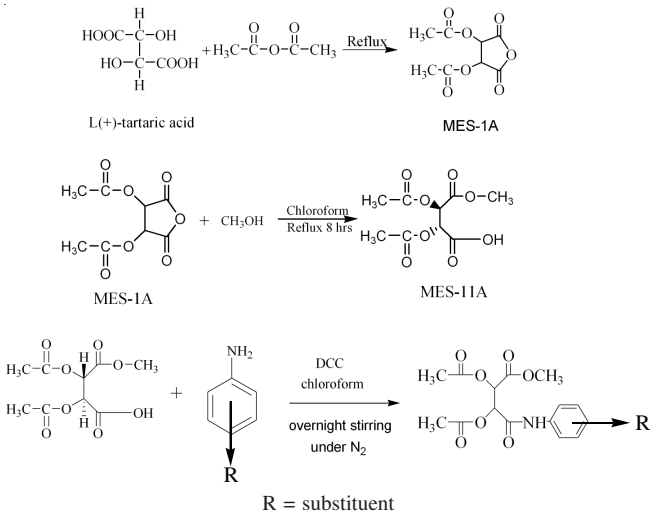
EXPERIMENTAL

Synthesis: In view of importance of chiral compounds in organic synthesis in recent years some new biologically active chiral compounds were synthesized. L-Tartaric acid is chiral compound and commercially available was conveniently converted into diacetyl-L-tartaric acid anhydride (MES-1A). Diacetyl-L-(+)-tartaric acid anhydride was synthesized by refluxing L-(+)-tartaric acid with acetic anhydride. Diacetyl-L-tartaric acid anhydride was then made to form half ester (MES-11A) by refluxing diacetyl-L-tartaric acid anhydride with methanol and then subsequently reacted with substituted anilines to yield respected chiral amides.

General procedure: In a (100 mL) round bottom flask, (0.02 mol, 4.32 g) of 2,3-diacetoxy-4-methoxy-4-oxo-butanoic acid (MES-11A) and DCC (1.2 eq, 4.29 g) were placed under nitrogen. 40 mL of chloroform was added as a solvent. After 0.5 h substituted anilines (0.02 mol) were added. The reaction mixture was stirred under nitrogen atmosphere for 8 h, so that amide was synthesized. Dicyclohexyl urea was removed by extraction the reaction mixture with ethyl acetate or chloroform and water. Product was purified by column chromatography. ^{1}H NMR spectra were recorded on NMR Bruker apparatus at 300 MHz in $CDCl_3$.

Antibacterial assay: Agar diffusion method (well diffusion method) or (cylinder plate method) was used for antibacterial activity. Wells were made in seeded agar and the test sample (3 mg/mL of each compound) was then introduced directly into these wells. After incubation, the diameter of the

clear zones around each well was measured and compared against zone of inhibition of the known concentrations of the standard antibiotic chloramphenicol.



Synthesis of substituted amides

Four strains of bacteria were used; two were gram positive, which were *Staphylococcus aureus* (ATCC 6538) and *Bacillus subtilis* (ATCC 6633) and two were gram negative, which were *Pseudomonas aeruginosa* (ATTC 9721) and *Klebsiella pneumoniae*. The organisms were maintained on nutrient agar medium at 4 ºC. Twenty-four hours old culture in nutrient broth (MERCK) of selected bacterial strain was mixed with physiological saline (0.9 % NaCl w/v) and turbidity was corrected by adding sterile physiological saline until a McFarland 0.5 $BaSO_4$ turbidity standard [10^6 colony forming unit (CFU) per mL density was obtained. Then this inoculum was used for seeding the nutrient agar.

Antifungal assay: The agar tube dilution method was used for determination of the antifungal activity. The fungal strains were used *i.e.*, (1) *Aspergillus niger* (0198), (2) *Aspergillus flavus* (0067), (3) *Aspergillus fumigates* (0066).

Each fungal strain was maintained on sabouraud dextrose agar medium at 4 ºC. 12 mg of the each compound was dissolved in 1 mL of DMSO to prepare the initial stock solution which was further diluted to get final concentration of 200 µg/mL. Solution of antibiotic terbinafine 12 mg/mL in DMSO was prepared for positive control. Pure DMSO was used as negative control. The test tubes were incubated at 28 ºC for 7 days and the inhibition of growth was calculated with reference to negative control. Percentage inhibition of fungal growth was determined by the following formula:

Inhibition of fungal growth =

$$100 - \frac{\text{Linear grwoth in test (mm)}}{\text{Linear growth in control (mm)}} \times 100$$

Phytotoxicity assay: Seeds of canola (*Brassica napus* L.) cv. Pakola were obtained from National Agriculture Research Centre, Islamabad, surface sterilized with 10 % chlorox solution and washed three times with autoclaved distilled water and finally dried with sterilized blotting paper. 10 mg/mL solution of each compound was prepared separately in methanol. Autoclaved distilled water and pure methanol were used as positive and negative control, respectively.

0.5 mL of each compound solution was put separately in a sterilized (autoclaved) 10 cm petriplate containing a sterilized filter paper (Whatman No. 1). Methanol was vacuum evaporated and then 5 mL autoclaved distilled water was added to each petriplate. Three replicates were prepared for each concentration. For negative control, 5 mL methanol was added to the plate, it was vacuum evaporated and then 5 mL autoclaved distilled water was added to it. For positive control only 5 mL autoclaved distilled water was added to each plate. Three replicates were prepared for each control. Sterilized 10 canola seeds were placed at sufficient distances with sterilized forecep in each plate. Petri plates were incubated in dim light at 25 ºC.

$$\text{Percentage germination } \frac{\text{Germinated seeds}}{\text{Total seeds}} \times 100$$

RESULTS AND DISCUSSION

Table-1 shows the physicochemical properties of the amides synthesized like physical appearance, melting point, yield and α-value. The details of NMR spectra data is given in Table-2.

The results presented in Table-3 revealed that maximum bactericidal activity (12 mm zone of inhibition) against *Staphylococcus aureus* was exhibited by **E11** followed by **E4**, **E3** and **E8**, respectively. Higher inhibitory activity against *Bacillus subtilis* was shown by **E4** showing 14 mm zone of inhibition. The compounds **E8** and **E11** showed 11 and 9 mm zones of inhibition, respectively. *Pseudomonas aeruginosa* was the sole bacterial strain that showed complete resistance to all tested compounds. *Klebsiella pneumoniae* showed susceptibility only to compounds **E11** and **E4**; maximum susceptibility being observed to **E11** showing 12 mm zone of

TABLE-1
PHYSICO-CHEMICAL CHARACTERIZATION OF THE SUBSTITUTED AMIDES

Compounds	Physical appearance	m.p. (ºC)	Yield (%)	α-Value (15 mg/ 20 mL)
E1	White solid	89-90	78	+1.08
E2	Pink solid	112-113	76	+1.24
E3	Light green solid	98-99	78	+0.95
E4	Light yellow solid	87-88	77	+1.14
E8	White solid	112-113	79	+1.02
E11	Light Yellow solid	92-94	78	+0.11

Compounds: Methyl-2,3-diacetoxy-4-oxo-4-(4'-bromophenyl amino)butanoate (MES-**E3**), methyl-2,3-diacetoxy-4-oxo-4-(4'-trifluoromethyl phenyl amino)butanoate (MES-**E8**), methyl-2,3-diacetoxy-4-oxo-4-(2'-trifluoromethyl phenyl amino) butanoate (MES-**E4**), methyl-2,3-diacetoxy-4-oxo-4-(2'-bromophenyl amino)butanoate (MES-**E2**), methyl-2,3-diacetoxy-4-oxo-4-(4'-methyl phenyl amino)butanoate (MES-**E1**), methyl-2,3-diacetoxy-4-oxo-4-(2'-methoxyphenylamino)butanoate (MES-**E11**).

TABLE-2
^{1}H-NMR SPECTRAL DATA AT 300 MHz IN $CDCl_3$; δ; ppm)

Compound	δ (ppm)
E1	7.49(dd, *J* = 7.2, 2.8 Hz, 2H, Ar), 7.06 (dd, *J* = 7.1, 2.7 Hz, 2H, Ar,), 4.72 (d, *J* = 7.1 Hz, 1H, -CH-CON), 4.86(d, *J* = 7.1 Hz, 1H,-CH-$COOCH_3$), 1.44 (s, 3H, -CH_3), 1.42 (s, 3H, -CH_3), 3.65 (s, 3H, OCH_3), 8.07, (s, 1H, -NH-), 2.34 (s, 3H, *p*-CH_3),
E2	7.6-7.01(m, 4H, Ar), 4.86 (d, *J* = 7.1 Hz, 1H, -CH-CON), 4.96 (d, *J* = 7.0 Hz, 1H, -CH-$COOCH_3$), 1.6 (s, 3H, -CH_3), 1.58 (s, 3H, -CH_3), 3.69 (s, 3H, -OCH_3), 9.7 (s, 1H, -NH-)
E3	7.7 (d, *J* = 7.2 Hz, 2H, Ar), 7.41 (d, *J* = 7.0 Hz, 2H, Ar,) 4.87 (d, *J* = 7.1 Hz, 1H, -CH-CON), 4.95 (d, *J* = 7.1 Hz, 1H, -CH-$COOCH_3$), 1.58 (s, 3H, -CH_3), 1.56 (s, 3H, -CH_3), 3.72 (s, 3H, -OCH_3), 9.9 (s,1H, -NH-)
E4	7.69-7.45 (m, 4H, Ar), 5.87 (d, *J* = 7.1 Hz, 1H, -CH-CON), 5.70 (d, *J* = 7.0 Hz, 1H, -CH-$COOCH_3$), 2.07 (s, 3H, -CH_3), 2.33 (s, 3H, -CH_3), 3.68 (s, 3H, -OCH_3), 9.8 (s, 1H, -NH-)
E8	7.69 (d, 2H, Ar, 7.2),7.98 (d, 2H, Ar,7.1), 5.88 (d, *J* = 7.2 Hz, 1H,-CH-CON), 5.71(d, *J* = 7.0 Hz, 1H, -CH-$COOCH_3$), 2.06 (s, 3H, -CH_3), 2.34 (s, 3H, -CH_3), 3.67 (s, 3H, -OCH_3), 9.98 (s, 1H, NH-)
E11	7.53-6.65 (m, 4H, Ar), 4.77 (d, *J* = 7.2 Hz, 1H, -CH-CON), 4.87 (d, *J* = 7.1 Hz, 1H,-CH-$COOCH_3$), 1.45 (s, 3H, -CH_3), 1.44 (s, 3H, -CH_3), 3.65 (s, 3H, -OCH_3), 8.1 (s, 1H, -NH-), 3.67 (s, 3H, *o*-OCH_3)

Compounds: Methyl-2,3-diacetoxy-4-oxo-4-(4'-bromophenyl amino)butanoate (MES-**E3**), methyl-2,3-diacetoxy-4-oxo-4-(4'-trifluoromethyl phenyl amino)butanoate (MES-**E8**), methyl-2,3-diacetoxy-4-oxo-4-(2'-trifluoromethyl phenyl amino) butanoate (MES-**E4**), methyl-2,3-diacetoxy-4-oxo-4-(2'-bromophenyl amino)butanoate (MES-**E2**), methyl-2,3-diacetoxy-4-oxo-4-(4'-methyl phenyl amino)butanoate (MES-**E1**), methyl-2,3-diacetoxy-4-oxo-4-(2'-methoxyphenylamino)butanoate (MES-**E11**).

TABLE-3
ANTIBACTERIAL ACTIVITY OF SYNTHETIC COMPOUNDS. THE CRYSTALS WERE APPLIED AT 3 mg/mL CONCENTRATION. ANTIBIOTIC CHLORAMPHENICOL WAS USED AS POSITIVE CONTROL WHILE PURE DMSO WAS USED AS NEGATIVE CONTROL. THE ZONE OF INHIBITION IS MEAN OF THREE REPLICATES

Compound	*Staphylococcus aureus* (ATTC 6538)	*Bacillus subtilis* (ATCC 6633)	*Pseudomonas aeruginosa* (ATTC 9721)	*Klebsiella pneumoniae*
	Mean zone of inhibition (mm)	Mean zone of inhibition (mm)	Mean zone of inhibition (mm)	Mean zone of inhibition (mm)
E1	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
E2	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
E3	6 ± 0.122	7 ± 0.159	0 ± 0.00	0 ± 0.00
E4	8 ± 0.154	14 ± 0.129	0 ± 0.00	6 ± 0.164
E8	4 ± 0.210	11 ± 0.128	0 ± 0.00	0 ± 0.00
E11	12 ± 0.173	9 ± 0.121	0 ± 0.00	12 ± 0.210
Chloramphenicol	25 ± 0.0100	28 ± 0.126	21 ± 0.171	23 ± 0.169

Compounds: Methyl-2,3-diacetoxy-4-oxo-4-(4'-bromophenyl amino)butanoate (MES-**E3**), methyl-2,3-diacetoxy-4-oxo-4-(4'-trifluoromethyl phenyl amino)butanoate (MES-**E8**), methyl-2,3-diacetoxy-4-oxo-4-(2'-trifluoromethyl phenyl amino) butanoate (MES-**E4**), methyl-2,3-diacetoxy-4-oxo-4-(2'-bromophenyl amino)butanoate (MES-**E2**), methyl-2,3-diacetoxy-4-oxo-4-(4'-methyl phenyl amino)butanoate (MES-**E1**), methyl-2,3-diacetoxy-4-oxo-4-(2'-methoxyphenylamino)butanoate (MES-**E11**).

inhibition. The compounds coded as **E1** and **E2** did not show any bactericidal activity against all the tested bacterial strains. The antifungal activity of the compounds was checked against three fungal strains *viz*., *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus* by using agar tube dilution method. All the compounds showed fungicidal activity against the tested fungal strains. However, **E3** was observed as the most effective compound showing 95, 99 and 90 % inhibition of growth against *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus*, respectively. Similarly, the compound **E8** inhibited the growth of *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus* by 23, 20 and 22 %, respectively (Table-4). This indicated that compound (**E3**) has greater fungicidal properties that are comparable to the standard terbenafine (fungicide). It will be obvious from current findings that such compound will be target for clinical trials because *Aspergillus fumigatus* is a common mould pathogen of human causing persistent disease in immuno compromised patients. The 4 % of patients dying in modern European teaching hospitals have persistent aspergillosis and it is the leading transferable cause of death in leukaemia patients[8]. *A. niger* causes black rot of onion which causes major losses of onion bulbs in the field and storage. These results are also in confirmation with previous findings of Narasimhan *et al*.[9] and Priya *et al*.[10] that amides bear antimicrobial properties. Bacterial resistance to antibiotics is an increasing problem that concern clinicians, the pharmaceutical industry and chemists. The multidrug resistance bacteria are the major cause of failure in the treatment of infectious diseases. Thus the need for novel antibiotics is more and more important.

Phytotoxicity is defined as a delay of seed germination, inhibition of plant growth or any adverse effect on plants caused by specific substances. The results of the current investigation showed that compounds **E4** and **E8** did not show any phytotoxic effects on canola seeds germination. However, the compounds **E2** and **E3** completely inhibited the germination. The compounds **E1** and **E11** caused 40 % inhibition of seed germination in canola (Fig. 1).

The results obtained during present investigation could help to understand phytotoxicity of various chiral amides and could be helpful in terms of use and disposal of these chemical compounds. Further investigation should be carried out to determine the effect of these chemical compounds on rhizosphere nutrient content, on root surfaces and their effects on beneficial microbes.

TABLE-4
ANTIFUNGAL ACTIVITY (% INHIBITION OF LINEAR GROWTH) OF SYNTHETIC COMPOUNDS. THE COMPOUNDS WERE APPLIED AT 12 mg/mL WITH FINAL CONCENTRATION OF 200 µg/mL. PURE DMSO WAS USED AS NEGATIVE CONTROL WHILE FUNGICIDE TERBENAFINE WAS UTILIZED AS POSITIVE CONTROL. THE DATA REPRESENTED IS MEAN OF THREE REPLICATES

Crystal	*Aspergillus niger* (0198)	*Aspergillus fumigatus* (0066)	*Aspergillus flavus* (0067)
E1	10 ± 0.210	20 ± 0.194	10 ± 0.178
E2	10 ± 0.223	20 ± 0.210	20 ± 0.183
E3	95 ± 0.321	99 ± 0.267	90 ± 0.237
E4	10 ± 0.197	20 ± 0.271	10 ± 0.213
E8	23 ± 0.169	20 ± 0.189	22 ± 0.184
E11	13 ± 0.234	14 ± 0.192	18 ± 0.321
Terbenafine (Fungicide)	100 ± 0.00	100 ± 0.009	100 ± 0.00

Compounds: Methyl-2,3-diacetoxy-4-oxo-4-(4'-bromophenyl amino)butanoate (MES-**E3**), methyl-2,3-diacetoxy-4-oxo-4-(4'-trifluoromethyl phenyl amino)butanoate (MES-**E8**), methyl-2,3-diacetoxy-4-oxo-4-(2'-trifluoromethyl phenyl amino) butanoate (MES-**E4**), methyl-2,3-diacetoxy-4-oxo-4-(2'-bromophenyl amino)butanoate (MES-**E2**), methyl-2,3-diacetoxy-4-oxo-4-(4'-methyl phenyl amino)butanoate (MES-**E1**), methyl-2,3-diacetoxy-4-oxo-4-(2'-methoxyphenylamino)butanoate (MES-**E11**).

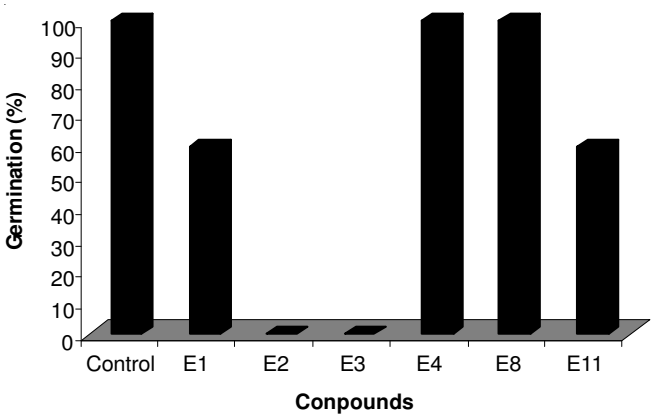


Fig. 1. Phytotoxicity of synthetic compounds on canola (*Brassica napus* L.) seed germination. Compounds: Methyl-2,3-diacetoxy-4-oxo-4-(4'-bromophenyl amino)butanoate (MES-**E3**), methyl-2,3-diacetoxy-4-oxo-4-(4'-trifluoromethyl phenyl amino) butanoate (MES-**E8**), methyl-2,3-diacetoxy-4-oxo-4-(2'-trifluoromethyl phenyl amino)butanoate (MES-**E4**), methyl-2,3-diacetoxy-4-oxo-4-(2'-bromophenyl amino)butanoate (MES-**E2**), methyl-2,3-diacetoxy-4-oxo-4-(4'-methyl phenyl amino)butanoate (MES-**E1**), methyl-2,3-diacetoxy-4-oxo-4-(2'-methoxyphenylamino)butanoate (MES-**E11**)

Conclusion

It is inferred that chiral amides particularly **E11**, **E3** and **E2** could be helpful in formulation of some novel antimicrobials, pesticides and herbicides. However, future researches in these directions are needed.

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