Synthesis, Characterization, Antifungal and Antibacterial Activities of Novel Amide Derivatives of 1,3-Dioxolane

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Abstract

L-Tartaric acid which is chiral compound and commercially available, was converted into 1,3-dioxolane. In the synthetic sequence, 1,3-dioxolane was first formed via protection and partial hydrolysis of L-tartaric acid. Treatment with altered substituted aromatic amines, 1,3-dioxolane gave the desired amides i.e. *ethyl 2,2-dimethyl-5-(phenylcarbamoyl)-1,3-dioxolane-4-carboxylate 3a, 2-(4-(ethoxycarbonyl)-2,2-dimethyl-1,3-dioxolane-5-carboxamido)benzoic acid 3b, ethyl 5-(4-iodophenylcarbamoyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylate 3c, ethyl 5-(2-chlorophenylcarbamoyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylate 3d and ethyl 5-(2,4-dichlorophenylcarbamoyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylate 3e*. These chiral derivatives were purified through column chromatographic technique and characterized by different spectroscopic techniques i.e IR, ^{1}H NMR, ^{13}C NMR and EIMS. The antimicrobial activities of these compounds were determined at different concentrations against different strains of bacteria and fungi.

Keywords: L-tartaric acid, protection, partial hydrolysis, amides, antimicrobial activity

1. Introduction

Tartaric acid is an important chiral compound. Many studies for example selective ion complexation (Leapine, 2005), asymmetric oxidation of prochiral sulfides (Schitter, 1998) and as bio-inspired antifreeze additives (Ilmarinen, 2001) have been carried out on derivatives of tartaric acid. The synthetic development of macrocyclic peptide antibiotics such as biphenomycin B (Yavuz, 2006) and vancomycine type glycopeptides antibiotics (Cupido, 2007) have brought some dramatic changes in the last few years. Synthesis of carbazolophane amides along with their antibacterial and antifungal activities has also been reported (Ghose, 1999). Derivatives of tartaric acid with amide and ester bond, possess interesting biological activities such as anti-inflammatory (Dai, 2011) antifungal (Behr, 2003) antimicrobial (Kucuk, 2011), thrombin inhibition (Dahlgren, 2002) inhibitors of chitin synthase (Xiea, 2012) and β-secretase inhibitor (Li, 2012).

In the continuation of our research work on organic compounds (Khalid, 2009, sher, 2013, Khalid, 2010) and due to the amide's importance in organic synthesis, we synthesized some novel amides from 1,3-dioxolane of L-tartaric acid. All the compounds were purified via column chromatography and were characterized with the help of spectro analytical techniques including IR, ^{1}H-NMR, ^{13}C-NMR and EIMS. The amides (**3a-e**) were tested for their antimicrobial activities at different concentrations against different strains of bacteria and fungi. All the compounds showed good to significant activities against all the three fungal as well as bacterial strains.

2. Results and Discussion

Chemistry

Tartaric acid is a very important compound and has been used in stereochemical studies due to its optically active nature. Many tartaric acid derivatives have broad applications in numerous chiral compounds preparation and is too used as a parent compound of chiral auxiliaries, chiral ligands, and in the synthesis of bioactive molecule (Gawronski, 1999). In organic synthesis, owing to the grandness of chiral substrates, we designed the synthesis of some novel chiral derivatives with L-tartaric acid backbone. In this research work, L-tartaric acid was selected which is cheapest and enantiomerically pure available compound. L-tartaric acid is a chiral, polar and poly-functional molecule having two asymmetric atoms of carbon (2R,3R) with absolute configuration possessing $C2$-axis of symmetry (Figure 1).
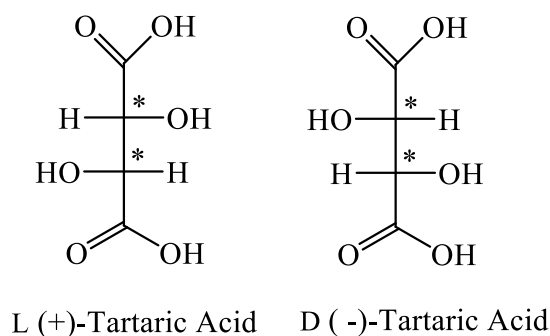

L (+)-Tartaric Acid   D (-)-Tartaric Acid

Figure-1

Protection and partial hydrolysis of bifunctional L-tartaric acid was carried out to get the monoester 3 as shown in Scheme-1.

Synthesis of diethyl 2,2-dimethyl-1,3-dioxolane-4,5-dicarboxylate (2) and monoester (3)

The first step for synthesis of L-tartaric acid derivatives was the protection of the diol as 1,3-dioxolane and diacid as diethyl ester. To avert the abdicable side reactions of the -OH groups various protecting groups can be used. In order to prepare 1,3-dioxolane, 2-methoxypropene was selected as a protecting group for the diol group. 1,3-dioxolanes have wide applications, as protecting groups, for 1,2-diols, aldehydes, and ketones in the synthesis of natural products. In basic medium it is stable, very important intermediate, and the end-product of various fragrance, polymer and pharmaceutical industries (Greene, 1991). By using 2,2-dimethoxy propane, Musich *et al.* reported the 1,3-dioxolane derivatives of 'L and D' tartaric acid (Musich, 1978). Diethyl-L-tartate was first synthesized from L-tartaric acid and ethanol and then 1,3-dioxolane diethyl-L-tartrate (2) synthesis was done (Kozikowski, 1993). Initially the yield was poor but after optimizing the reaction conditions, the yield was improved up to 89 %. Via column chromatography the product was purified.

The next step was the partial hydrolysis of compound 2 by using 1equivalent sodium hydroxide in ethanol. The initial low yield of the desired monoester was improved by increasing the time of reaction from 1-2 hours and by the sodium hydroxide addition drop wise. With 1M aqueous $KHSO_4$, the resultant salt was acidified. Column chromatography was carried out for the purification of the monoester from some unreacted starting materials and diacid. The monoester 3 was obtained as colorless oil, and the yield was 79 %.
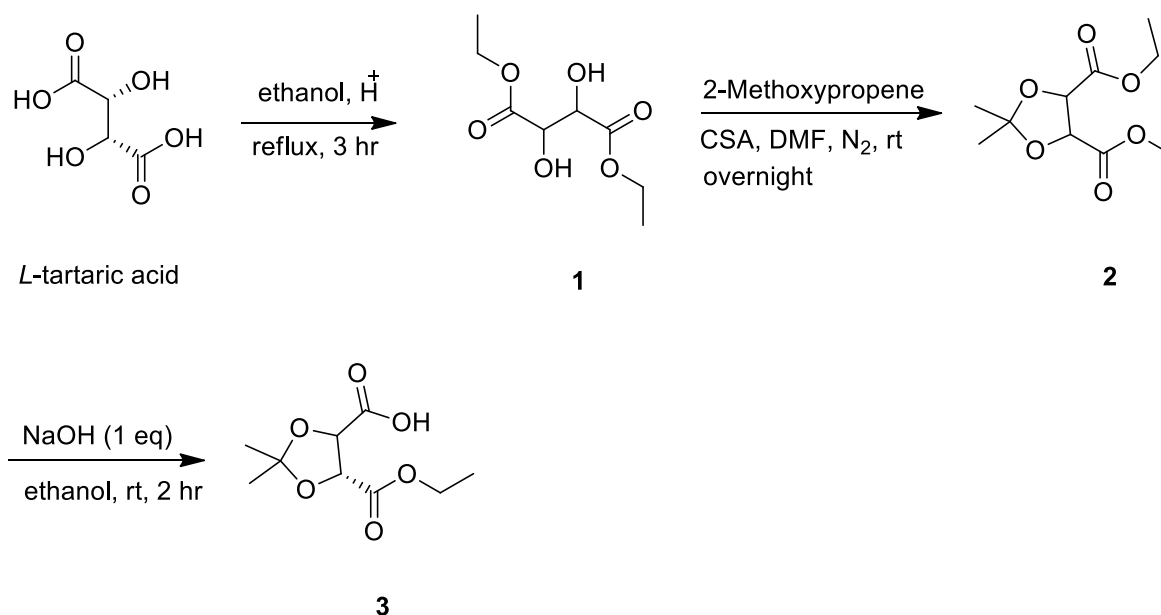
Scheme 1. Synthesis of diethyl 2,2-dimethyl-1,3-dioxolane-4,5-dicarboxylate 2 and monoester 3

The characterization of compound **2** and **3** was carried out via IR, ^{1}H NMR, ^{13}C NMR and EI-MS. Characteristic absorption frequencies for the compound **2** were shown at 1738 and 2968cm^{-1} in the IR absorption spectrum which was attributed to CO and C-H respectively. The spectrum of ^{1}H NMR showed a singlet at 4.76 ppm, with 2 protons integration, and was assigned to CH, 1,3-dioxolane's 2 methyl groups appeared as singlet at 1.74 ppm with six protons integration. In ^{13}C NMR spectrum, peak, at 113.7 ppm, was assigned to the quaternary carbon atom, while the two-methyl carbon of acetonide appeared at 14.4 ppm respectively. For compound **3**, characteristic stretching bands at 1708, 1730, 2972, and 3511 cm^{-1} were shown in the IR spectrum. The absorptions were allotted to CO (acid), CO (ester), C-H and OH respectively. ^{1}H NMR spectrum showed a broad singlet at 7.85 ppm, with 1 proton integration and was assigned to the OH group. Two doublets ranging from 4.90-4.80 ppm having coupling constants of 5.4 Hz appeared for the two methine protons. In the ^{13}C NMR spectrum, peak at 173.8 ppm was attributed to the CO (acid), which confirmed the hydrolysis.

Chiral amides **3a-e** was synthesized from monoester **3** and different substituted aromatic amines (scheme 2) by a method first reported by Vaughan and were based on the mixed anhydride (Vaughan, 1951, Vaughan, 1951).
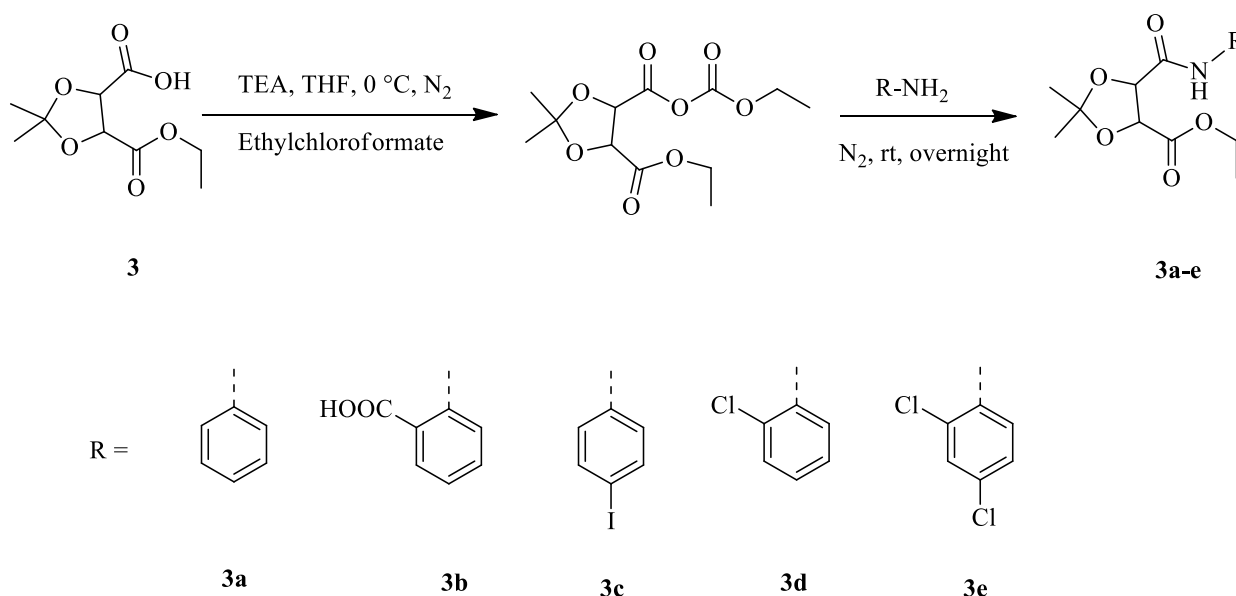
Scheme 2. Synthesis of chiral amides 3a-e

S. No.	Code	M.P (℃)	*R$_f$ values	Isolated Yield (%)	$^0(\alpha)_D^{25}$
1	3a	Oily	0.72	78	20.2
2	3b	112-115	0.71	77	28.4
3	3c	135-137	0.69	76	33.2
4	3d	129-131	0.70	69	27.0
5	3e	143-145	0.68	73	16.5

*Solvent System: Ethyl acetate: Hexane (2:8), ^{0}(c = 24 mg/2 ml CH$_2$Cl$_2$)

All the compounds **3a-e** were refined via column chromatography and characterized with the help of spectro analytical techniques.

3. Bioassays

All the synthetic amides (**3a**-**e**) were screened for their *in vitro* antibacterial and antifungal potential against different strains of bacteria (*Enterobacter sp*, *Vibrio Cholera* and *Klebsiella sp*) and fungi (*Fausarium solani*, *Helminthosporium sativum*, and *Aspergillus niger*). The results are listed in Table-1 and Table-2.

Antifungal activity

All the compounds showed good activity against *Fusarium solani, Helminthosporium sativum* and *Aspergillusn niger* at 1000μg/ml concentration. Compounds **3a**, (66%, 80%, 79%), **3b** (79%, 82%, 81%), **3c** (87%, 91%, 88%), **3d** (84%, 64%, 88%), **3e** (65%, 77%, 90%). However, as the concentration decrease to 800 and 600μg/ml these compounds showed a decline in activity. Compound **3a** showed (66%, 80% and 79%) inhibition at concentration of 1000μg/ml against all the three strains of fungi, respectively. As the concentration decreases to 800 and 600μg/ml there is a decrease in antifungal activity of all the compounds. The results reveal that the same way of inhibition is observed in the activity of compounds of **3a**-**e**. Compound **3c** with *para iodo* substitution was found to be the most active compound and showed inhibition of fungal strains as 87%, 91%, 88%, at 1000μg/ml concentration. Compound **3d** and **3e** with *ortho* and *para* chloro substitution showed 84%, 64%, 88% and 65%, 77%, 90% activity at 1000μg/ml concentration Table-1.

Table 1. Antifungal activities of the amides (3a-e) at three different concentrations against three fungal strains

S. No.	Compounds	Fausarium solani			Helminthosporium sativum			Aspergillus niger		
		1000μg/ml	800μg/ml	600μg/ml	1000μg/ml	800μg/ml	600μg/ml	1000μg/ml	800μg/ml	600μg/ml
1	3a	66	53	32	80	33	45	79	67	60
2	3b	79	44	41	82	42	33	81	42	34
3	3c	87	75	42	91	44	33	88	32	44
4	3d	84	56	51	64	20	36	88	43	42
5	3e	65	58	32	77	54	32	90	75	55
	+Ve control	-	-	-	-	-	-	-	-	-
	Terbinafine	94	94	94	98	98	98	98	98	98

Antibacterial activity

All the compounds (**3a**-**e**) were also assayed for their antibacterial activities and the results are shown in Table-2. All the compounds in a concentration of 1000μg/ml exhibited good activity against the tested bacterial strains (*Enterobacter sp, Vibrio cholera* and *Klebsiella sp.*). The test compounds show a decrease in their antibacterial activities at 800 and 600μg/ml concentrations. Compounds **3d** and **3e** with halogen substituents at ortho and *para* position show significant activity (zone of inhibition 29mm, 28mm for *Enterobacter sp*, 30mm, 27mm for *Vibrio cholerae,* and 31mm, 19 mm, for *Klebsiella sp,* respectively) against three bacterial strains at 1000μg/ml concentration. However, as the concentration decrease to 800 and 600μg/ml respectively, these compounds showed a drop in their activity.

Table 2. Antibacterial activity of the protected (3a-e) at three concentrations against bacterial strains. *Zone* of inhibition are shown in mm.

S. No.	Code	Enterobacter sp.			Vibrio cholerae			Klebsiella sp.		
		1000μg/ml	800μg/ml	600μg/ml	1000μg/ml	800μg/ml	600μg/ml	1000ug/ml	800μg/ml	600μg/ml
1	**3a**	22	11	11	22	13	09	20	11	13
2	**3b**	19	13	12	29	15	12	21	15	11
3	**3c**	28	14	12	27	22	12	19	17	15
4	**3d**	29	17	13	30	14	11	31	17	16
5	**3e**	28	14	15	27	10	11	19	11	12
	Chloramphenicol	32	25	34	32	25	34	32	25	34

[a]Zone of inhibition diameter in mm. (Activity): above 18 mm (significant activity), 16-18 mm 13-15 mm (low activity), (good activity), 9-12 mm (non-significant), Below 9 mm (no activity),

4. Conclusion

The amides derivative **3a-e** showed more antibacterial and antifungal activity against different bacterial and fungal isolates. The alarming rising resistance in bacteria, to currently available therapeutics is a threat to patients suffering from various diseases. There is need for new therapeutics to be evaluated as antibacterial agents. The synthesized amides *may serve as lead compounds for further research in drug designing and medicinal chemistry. These compounds may prove to be promising candidates against different pathogenic strains of fungi.*

5. Experimental

All chemicals used were of highest available purity and were used as supplied. 2-methoxypropene and L-tartaric acid were purchased from Sigma Aldrich. Through distillation over potassium hydroxide liquid amines were dried and stored in the nitrogen atmosphere over pellets of potassium hydroxide. By using standard procedures, dry solvents like methanol, ether, THF, chloroform, n-hexane and dichloromethane were obtained through distillation. Under anhydrous conditions, reactions were carried out in the atmosphere of nitrogen, rubber septa and three-way stopcocks were used.

All the glassware used were either flame dried instantly before use or were placed in an oven (at 150 ℃) for 2-3 hours and were allowed to cool under reduced pressure or in desiccators. Liquid solvents, solutions and reagents were added through rubber septa by means of cannula or syringe. Schlenk type adapters were used for adding solid reagents. Reactions were monitored via TLC on Kieselgel 60 F254 (Merck), methanol/chloroform and ethyl acetate/n-Hexane were used as eluent respectively. Chromatograms were detected under UV light (λ_{max} 254 and 365 nm) and by charring with 10% H_2SO_4 in EtOH, ninhydrine and vanillin respectively.

Column chromatography was carried out by using silica gel (Merck, 230−400 mesh (40−63 μm)). Different fractions were concentrated under low pressure by using both a Buchi rotary evaporator (up to 40 ℃ bath temperature) at a pressure of either 0.1 mm Hg (oil pump) or 15 mm Hg (diaphragm pump) as suitable and a high vacuum line (at room temperature). The melting points were determined, by using Gallenkamp digital melting point apparatus, in degree Celsius (℃). IR spectra were recorded on (ATR); Schimadzu Fourier Transform Infrared Spectrophotometer Model 270 and thermo scientific Nicolet 6700 FT IR. NaCl cell was used for oils while KBr pellets were used for solid samples. NMR Bruker apparatus was used for ^{1}H NMR and ^{13}C NMR spectra recording at 300 MHz and at 75 MHz respectively. Chemical shifts are given in parts per million (ppm) from residual solvent proton signals or $SiMe_4$ and coupling constants (*J*) are given in hertz (Hz). different abbreviations are used for multiplicities such as for broad (b), singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) or combinations. VG: 70 SE mass spectrometer JEOL MS Route instrument having direct probe inlet system was used for EI (Electron impact) mass spectra. For measuring optical rotations of the compounds ATAGO, AP-100 Automatic polarimeter was used.

General procedure for the synthesis of diethyl 2,2-dimethyl-1,3- dioxolane-4,5-dicarboxylate 2

10.3 ml or 60 mmol of diethyl-L-tartrate and camphor sulphonic acid (catalytic amount) were taken in a 250 ml round bottom flask and placed in nitrogen atmosphere. 100 ml DMF was added with the help of a syringe and stirred via magnetic stirrer. 7.5 ml or 60 mmol (1.3 eq.) of 2-methoxypropene was added dropwise in 30 min and kept overnight. The reaction was monitored by TLC and after completion; 300 ml of water was added to the reaction mixture and was

extracted three times with 100 ml ethyl acetate. The organic layer was washed two times with 100 ml of saturated sodium bicarbonate solution, water and three times with 100 ml of brine. The organic layer obtained was dried over anhydrous $MgSO_4$ and was purified via column chromatography (ethyl acetate: n-hexane (2:8) as eluent).

Light yellow oil. Yield: 89 %. IR (neat) cm^{-1}: 1738 (CO), 2968 (CH). ^{1}H NMR at 300 MHz (CDCl$_3$): δ (ppm): 1.74 (s, 6H, C(CH$_3$)$_2$), 4.76 (s, 2H, CH), 4.30-4.23 (q, J = 7.2 Hz, 4H, CH$_2$), 1.33-1.28 (t, J = 7.2 Hz, 6H, CH$_3$),. ^{13}C NMR at 75 MHz (CDCl$_3$): δ (ppm):14.4 C(CH$_3$)$_2$, 26.3 (CH$_3$), 61.9. (CH$_2$), 76.6 (CH), 113.7 (qt C), 169.6 (CO). EI-MS *m/z* (%): 172 (76), 188 (51), 230.0 (100), 246.0 M$^+$ (96.1).

General procedure for the synthesis of 5-(ethoxycarbonyl)-2,2-dimethyl-1, 3-dioxolane-4-carboxylic acid monoester 3

6.2 ml or 30 mmol of compound **2** solution in 30 ml ethanol was taken in a 250 ml round bottom flask. 1.2 g or 30 mmol solution of sodium hydroxide in 30 ml ethanol was added over 30 min. At 25 ^{o}C, the reaction mixture was stirred for 2 hour and under low pressure ethanol was evaporated to give a residue. 30 ml water was added and extracted three times with 30 ml dichloromethane (DCM) to recover the diester left unreacted. With 1 M KHSO$_4$, the aqueous layer was acidified and extracted three times with 30 ml DCM. The solvent was evaporated via rotary and, by column chromatography, the crude was purified (MeOH: DCM (2:8) as eluent).

Colorless oil. Yield: 79 %. IR (neat) cm^{-1}: 1708 (COOH), 1730 (COOEt), 2972 (CH), 3511 (OH). ^{1}H NMR at 300 MHz (CDCl$_3$): δ (ppm): 1.53 (s, 6H, C(CH$_3$)$_2$), 7.85 (bs, 1H, OH), 4.82-4.80 (d, *J* = 5.4 Hz, 1H, CH), 4.90-4.88 (d, *J* = 5.4 Hz, 1H, CH), 1.35-1.31 (t, J = 7.2 Hz, 3H, CH$_3$), 4.34-4.27 (q, J = 7.2 Hz 2H, CH$_2$). ^{13}C NMR at 75 MHz (CDCl$_3$): δ (ppm): 14.1 C(CH$_3$)$_2$, 26.3 (CH$_3$), 62.3 (CH$_2$), 76.5 (CH), 114.2 (qt C), 169.6 (COOCH$_3$), 174.4 (COOH).

General Procedure for the synthesis of chiral amides 3a-e

1.09 g or 5 mmol of compound **3** in 20 ml THF and 0.91 ml or 5 mmol (1.3 eq.) of triethylamine were placed in a 100 ml round bottom flask at 0$^°$C in the nitrogen atmosphere. 0.58 ml or 5 mmol (1.2 eq.) of ethyl chloroformate was added and was stirred for half an hour; 5 mmol of substituted aniline in 10 ml THF was then added and was allowed to warm to 25$^°$C and left overnight. The reaction was monitored by TLC and after completion of the reaction, solvent was removed via rotary and the crude was dissolved in 30 ml of ethyl acetate, washed with 30 ml of NaHCO$_3$ saturated solution, water and brine respectively. The organic layer obtained was dried over anhydrous $MgSO_4$ and purified via column chromatography (ethyl acetate: n-hexane (2:8) as eluent).

Ethyl 2,2-dimethyl-5-(phenylcarbamoyl)-1,3-dioxolane -4-carboxylate 3a

Yield: 78%. Oily. $(α)_D^{25}$ = 20.2 (c = 24 mg/2 ml CH$_2$Cl$_2$). (IR υ cm^{-1}): 3405 (NH), 2928 (CH), 1738 (COOEt), 1689 (CONH), 1535 (Ar). ^{1}H NMR (300 MHz, CDCl$_3$): δ (ppm): 8.29 (bs, 1H, NH), 7.64-7.57 (m, 5H, ArH), 4.92 (d, *J* = 5.4 Hz, 1H, CH), 4.82 (d, *J* = 5.4 Hz, 1H, CH), 4.33-4.17 (q, J = 7.2 Hz 2H, CH$_2$), 1.53 (s, 6H, C(CH$_3$)$_2$), 1.35-1.31 (t, J = 7.2 Hz, 3H, CH$_3$). ^{13}C NMR (75 MHz, CDCl$_3$): δ (ppm): 170.4 (COOEt), 167.6 (CONH), 114.2 (qt C), 76.5 (CH), 62.3 (CH$_2$), 26.3 (CH$_3$), 14.1 C(CH$_3$)$_2$. EI-MS *m/z* (%): 293.1 M$^+$ (23.4), 162.8 (46), 83.1 (95.0), 59.1 (100).

2-(4-(ethoxycarbonyl)-2,2-dimethyl-1,3-dioxolane-5-carboxamido) benzoic acid 3b

Yield: 77 %. White solid, m.p = 112-115 $°$C. $(α)_D^{25}$ = 28.4 (c = 24 mg /2 ml CH$_2$Cl$_2$). (IR υ cm^{-1}): 3520 (OH), 3403 (NH), 2908 (CH), 1731 (COOEt), 1701 (CONH), 1532 (Ar). ^{1}H NMR (300 MHz, CDCl$_3$): δ (ppm): 11.86 (bs, 1H, OH), 8.85 (bs, 1H, NH), 7.62-7.51 (m, 4H, ArH), 4.96 (d, *J* = 5.4 Hz, 1H, CH), 4.80 (d, *J* = 5.4 Hz, 1H, CH), 4.31-4.16 (q, J = 7.2 Hz 2H, CH$_2$), 1.63 (s, 6H, C(CH$_3$)$_2$), 1.38-1.31 (t, J = 7.2 Hz, 3H, CH$_3$). ^{13}C NMR (75 MHz, CDCl$_3$): δ (ppm): 172.4 (COOH), 170.4 (COOEt), 167.6 (CONH), 113.2 (qt C), 76.6 (CH), 62.4 (CH$_2$), 26.1 (CH$_3$), 14.2 C(CH$_3$)$_2$. EI-MS *m/z* (%): 337.1 M$^+$ (12.4), 322 (9.8), 146 (64), 83 (79), 59.1 (100).

Ethyl 5-(4-iodophenylcarbamoyl)-2,2-dimethyl-1,3-dioxolane -4-carboxylate 3c

Yield: 76 %. White crystalline solid, m.p. = 135-137 $°$C. $(α)_D^{25}$ = 33.2 (c = 24 mg/2 ml CH$_2$Cl$_2$). (IR υ cm^{-1}): 3388 (NH), 2925 (CH), 1732 (COOEt), 1678 (CONH), 1536 (Ar), 1216 (C-I). ^{1}H NMR (300 MHz, CDCl$_3$): δ (ppm): 8.29 (bs, 1H, NH), 7.56 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.34 (d, *J* = 8.6 Hz, 2H, Ar-H), 4.91 (d, *J* = 5.4 Hz, 1H, CH), 4.80 (d, *J* = 5.4 Hz, 1H, CH), 4.31-4.17 (q, J = 7.2 Hz 2H, CH$_2$), 1.52 (s, 6H, C(CH$_3$)$_2$), 1.33-1.31 (t, J = 7.2 Hz, 3H, CH$_3$). ^{13}C NMR (75 MHz, CDCl$_3$): δ (ppm): 170.4 (COOEt), 167.6 (CONH), 114.1 (qt C), 76.6 (CH), 62.5 (CH$_2$), 26.5 (CH$_3$), 14.2 C(CH$_3$)$_2$. EI-MS *m/z* (%): 419.1 M$^+$ (18.4), 345 (8.5), 219 (4.6), 85 (45.8), 59.1 (100).

Ethyl 5-(2-chlorophenylcarbamoyl)-2,2-dimethyl-1,3-dioxolane -4-carboxylate 3d

Yield: 69 %. White crystalline solid, m.p. = 129-131 $°$C. $(α)_D^{25}$ = 27.0 (c = 24 mg / 2 ml CH$_2$Cl$_2$). (IR υ cm^{-1}): 3399 (NH), 2922 (CH), 1737 (COOEt), 1676 (CONH), 1539 (Ar), 1123 (C-Cl). ^{1}H NMR (300 MHz, CDCl$_3$): δ (ppm): 8.29 (bs, 1H, NH), 7.64-7.57 (m, 4H, ArH), 4.92 (d, *J* = 5.4 Hz, 1H, CH), 4.82 (d, *J* = 5.4 Hz, 1H, CH), 4.33-4.17 (q, J = 7.2 Hz 2H, CH$_2$), 1.53 (s, 6H, C(CH$_3$)$_2$), 1.35-1.31 (t, J = 7.2 Hz, 3H, CH$_3$). ^{13}C NMR (75 MHz, CDCl$_3$): δ (ppm): 170.4

(COOEt), 167.6 (CONH), 114.2 (qt C), 76.5 (CH), 62.3 (CH$_2$), 26.3 (CH$_3$), 14.1 C(CH$_3$)$_2$ EI-MS *m/z* (%): 329 (6.5), 327.1 M$^+$ (19.4), 312 (15.0), 173 (42.4), 127 (81.1), 83.1 (98.0), 59.1 (100).

Ethyl 5-(2,4-dichlorophenylcarbamoyl)-2,2-dimethyl-1, 3-dioxolane-4-carboxylate 3e

Yield: 73 %. White solid. m.p = 143-145 ℃. (α) $_D^{25}$ = 16.5 (c = 24 mg/2 ml CH$_2$Cl$_2$). (IR υ cm^{-1}): 3401 (NH), 2932 (CH), 1732 (COOEt), 1677 (CONH), 1533 (Ar), 1103 (C-Cl). ^{1}H NMR (300 MHz, CDCl$_3$): δ (ppm): 8.29 (bs, 1H, NH), 7.64-7.57 (m, 4H, ArH), 4.92 (d, *J* = 5.4 Hz, 1H, CH), 4.82 (d, *J* = 5.4 Hz, 1H, CH), 4.33-4.17 (q, J = 7.2 Hz 2H, CH$_2$), 1.53 (s, 6H, C(CH$_3$)$_2$), 1.35-1.31 (t, J = 7.2 Hz, 3H, CH$_3$). ^{13}C NMR (75 MHz, CDCl$_3$): δ (ppm): 170.4 (COOEt), 167.6 (CONH), 114.2 (qt C), 76.5 (CH), 62.3 (CH$_2$), 26.3 (CH$_3$), 14.1 C(CH$_3$)$_2$ EI-MS *m/z* (%): 363.0 (15.0), 361.0 M$^+$ (23.4), 288.1 (9.0), 173 (47.3), 161 (62.1), 83.1 (100.0), 59.1 (79.0).

6. Material and Methods

Assay for Antifungal activity

In the present experiment three concentrations (1000, 800 and 600μg/ml) of synthetic compounds were used to study the inhibition potential against three fungal and three bacterial strains. for the antifungal activity determination of the compounds the agar tube dilution method was used (Washington, 1980). DMSO was used as a control solvent; terbinafine was used as the standard antifungal agent. The fungal strains were used in this study include *Fusarium solani, Helminthosporium sativum and Aspergillus niger*. Each fungal strain was maintained on Sabouraud dextrose agar (SDA) medium at 4 ℃.

Antibacterial Bioassay

Three bacterial strains were used in the study i.e *Enterobacter sp*, *Vibrio cholerae* and *Klebsiella sp*. Agar well diffusion method was used to check the antibacterial activities of the compounds .First nutrient broth medium was prepared by dissolving 0.4 g of nutrient broth in 50 mL of distilled water, pH was adjusted at 7.0 and was autoclaved. Then nutrient agar medium was prepared by dissolving 2.3 g nutrient agar medium in 100 mL of distilled water; pH was adjusted at 7.0 and was autoclaved at 121 ℃. After autoclaving the medium was poured in petri plates. A 0.5 McFarland standard was prepared by mixing 0.05 mL of 1.175 % Barium Chloride dihydrate (BaCl2•2H2O), with 9.95 mL of 1 % Sulfuric Acid (H$_2$SO$_4$). A 0.5 McFarland standard corresponds to approximately 1.5 x 108 cells per mL. Bacterial were cultured one day before the assay in nutrient broth medium suspension. The bacterial inoculum was adjusted according to 0.5 McFarland standard and added to nutrients agar plates. The test compounds in different concentrations were added to the wells of the petri plates and incubated at 37 ℃ for 24hrs.The zones of inhibition of the test compounds was measured after 24 hrs. The results were recorded and interpreted accordingly (Koneman, 1994).

Conflict of Interest

The author(s) confirm that this article content has no conflict of interest.

Acknowledgments

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